

BRET-based RAS biosensors that show a novel small molecule is an inhibitor of RAS-effector protein-protein interactions

Nicolas Bery¹, Abimael Cruz-Migoni^{1,2}, Carole JR Bataille³, Camilo E Quevedo¹, Hanna Tulmin^{1†}, Ami Miller¹, Angela Russell³, Simon EV Phillips⁴, Stephen B Carr^{2,4}, Terence H Rabbitts^{1*}

¹MRC Molecular Haematology Unit, Weatherall Institute of Molecular Medicine, University of Oxford, Oxford, United Kingdom; ²Research Complex at Harwell, Rutherford Appleton Laboratory, Didcot, United Kingdom; ³Chemistry Research Laboratory, Oxford, United Kingdom; ⁴Department of Biochemistry, University of Oxford, Oxford, United Kingdom

Abstract The RAS family of proteins is amongst the most highly mutated in human cancers and has so far eluded drug therapy. Currently, much effort is being made to discover mutant RAS inhibitors and in vitro screening for RAS-binding drugs must be followed by cell-based assays. Here, we have developed a robust set of bioluminescence resonance energy transfer (BRET)-based RAS biosensors that enable monitoring of RAS-effector interaction inhibition in living cells. These include KRAS, HRAS and NRAS and a variety of different mutations that mirror those found in human cancers with the major RAS effectors such as CRAF, PI3K and RALGDS. We highlighted the utility of these RAS biosensors by showing a RAS-binding compound is a potent pan-RAS-effector interactions inhibitor in cells. The RAS biosensors represent a useful tool to investigate and characterize the potency of anti-RAS inhibitors in cells and more generally any RAS protein-protein interaction (PPI) in cells.

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***For correspondence:**

terence.rabbitts@imm.ox.ac.uk

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Introduction

RAS is the most prominent oncogene identified in cancer. Mutation in RAS proteins can be found in approximately 30% of all human tumors (*Downward, 2003; Prior et al., 2012*) (<http://cancer.sanger.ac.uk/cosmic>) prompting interest in the discovery of anti-RAS therapeutics. However, there are still no RAS-targeted drugs currently available in the clinic even though such molecules could prove widely efficacious in many human cancers as front-line drugs for therapy. Some forms of cancer, like pancreatic cancer, present late and are difficult therefore to treat (*Kleeff et al., 2016*) but these contain a high proportion of KRAS mutations and are thus potentially susceptible to RAS-binding drugs.

RAS has been regarded as undruggable partly because so far attempts to interfere with the protein have not been efficacious (*Cox et al., 2014*). RAS is a membrane-bound small GTPase switching between an inactive GDP-bound state and an active GTP-bound state. RAS signaling to the cell nucleus occurs after interaction of RAS-GTP with its effectors to trigger the activation of downstream signaling pathways. This activation thereby promotes cell survival and cell proliferation (*Wennerberg et al., 2005*) via gene modulation so that the blockade of mutant RAS signaling in tumors cells is an attractive therapeutic option. There are several ways in which this could be achieved (*Athuluri-Divakar et al., 2016; Burns et al., 2014; Spiegel et al., 2014; Zimmermann et al., 2013*) but methods such as implementing farnesylation inhibitors have limited

eLife digest A group of proteins known as the RAS family plays a critical role in controlling animal cell growth and division. RAS proteins are normally active only some of the time, but genetic mutations can create permanently active forms of the proteins. These constantly interact with other proteins called effectors. In response, cells multiply uncontrollably and give rise to cancers.

In an attempt to find new cancer treatments, researchers across the globe are trying to develop inhibitor drugs that prevent RAS and effector proteins from interacting. New drugs are often tested in laboratory experiments that directly apply the drugs to the proteins that they are designed to work on. But in some cases a drug may work well in the laboratory but fail to work when used in cells. Unfortunately, there are few ways to judge how well inhibitor drugs work inside living cells.

Bery et al. have now developed RAS biosensors – a collection of proteins that bind to RAS and produce light more brightly when RAS interacts with effector proteins in living cells. Tests on cells treated with an antibody that works inside cells and is known to prevent interactions between RAS and effector proteins confirmed that the RAS biosensors work well. Bery et al. then used the RAS biosensors to show that a new RAS inhibitor works in human cancer cells.

The RAS biosensors are available upon request to researchers across the globe. They should form an important tool for testing potential treatments for cancers that contain mutated RAS proteins.

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success due to side effects (Berndt et al., 2011; James et al., 1995; Whyte et al., 1997). One avenue that has largely been avoided in inhibiting RAS is the interaction with its effectors, such as RAF, RALGDS and PI3K. However, the effectiveness of the orthosteric RAS-effector PPI inhibition was shown using intracellular antibodies (Tanaka and Rabbitts, 2003; Tanaka et al., 2007) (herein called macrodrugs (Tanaka and Rabbitts, 2008) to distinguish them from conventional small molecule drugs) and a single domain intracellular antibody that blocks effector interaction sites of RAS-GTP. This PPI inhibition can prevent tumor growth in xenograft models and tumor initiation in a transgenic mouse model (Tanaka and Rabbitts, 2010; Tanaka et al., 2007). Other macrodrugs, such as DAR-Pins (Guillard et al., 2017), have also been shown to be effective in interfering with RAS PPIs. Moreover, for many years, RAS was regarded as a protein without any pockets suitable for small molecule interactions (McCormick, 2016) but recent studies have described compounds that are able to bind RAS-associated pockets (Gentile et al., 2017; Lito et al., 2016; Maurer et al., 2012; Ostrem et al., 2013; Patricelli et al., 2016; Shima et al., 2013; Sun et al., 2012; Waldmann et al., 2004; Welsch et al., 2017).

Most of the current RAS inhibitors have been selected and identified through in vitro techniques (Ostrem et al., 2013; Trinh et al., 2016; Upadhyaya et al., 2015; Welsch et al., 2017) but cell-based assay technologies are needed to assess initial hits for efficacy before hit to lead development is undertaken. Indeed, a robust cell-based assay is a mandatory step in any drug discovery programme, as it provides insights into the behavior of compounds in physiological conditions, including cell permeability, stability and potency in the cellular complexity of a whole cell. We now describe a toolbox of mutant and wild-type RAS BRET-based biosensors that can be used to assess PPI between activated, GTP-bound RAS (KRAS, HRAS or NRAS) and effectors such as CRAF, RALGDS or PI3K in living cells. We validate the toolbox using a published anti-RAS intracellular domain antibody (hereafter named iDAb RAS) (Tanaka et al., 2007), which is an inhibitor of RAS PPI to establish the RAS biosensor resource. We have further used this methodology to test a RAS-binding compound (herein referred to as 3344) that we have derived from an in vitro medicinal chemistry programme starting with an intracellular antibody fragment. By monitoring the change in BRET2-specific signal in transfected HEK293T cells expressing different RAS-effector donor-acceptor combinations, we have been able to characterize the pan-RAS-effector PPI inhibitor properties of 3344. This inhibitory mechanism shown using the BRET biosensor toolbox was supported by the crystal structure of KRAS with bound 3344, showing binding to a pocket close to the RAS switch. Therefore, the BRET2 toolbox we describe here is a critical resource and is available for all investigators in the international effort to produce anti-RAS drugs, that can be employed in the treatment of cancers with RAS mutations.

Results

Engineering and validation of mutant RAS biosensors

RAS biosensors were developed for use in the BRET2 method (*Bacart et al., 2008*) as a real-time system allowing the monitoring of protein-protein interactions and their inhibition in live cells. The scheme used is outlined in **Figure 1A**. The intracellular localization of BRET donor RAS proteins was recapitulated by expressing the full-length proteins including the CAAX box, which is the farnesylation site for trafficking to the plasma membrane. The CAAX sequences were fused to the carboxy terminal end of the *Renilla* Luciferase variant 8 (RLuc8) to act as the donor molecule in BRET2 (*De et al., 2007*) (for simplicity of the nomenclature, CAAX has been omitted from the RAS construct names). We used available structural data for RAS/effector and RAS/iDAb complexes to optimize the proximity of donor and acceptor moieties. Hence, RLuc8 was fused to the amino termini of full-length RAS family proteins and the GFP² (*Ramsay et al., 2002*) fused to the C-termini of the effectors (RALGDS, CRAF, PI3K) or of the iDABs. Other parameters can influence the BRET2 signal such as the linker length between RLuc8/RAS and effector-iDAb/GFP². For our study, we observed a higher BRET signal with a (GGGS)₃ linker between RLuc8-KRAS^{G12D} construct, a (GGGS)₃ linker between the CRAF RBD-GFP² molecule and a (GGGS)₂ linker between iDAb RAS-GFP² construct (**Figure 1—figure supplement 1A**). Therefore, we implemented these observations to all our BRET biosensors (**Supplementary file 1**). When donor and acceptor plasmids are transfected into HEK293T cells (although any cell line of choice would be suitable), the resultant cells are fluorescent and bioluminescent if treated with the luciferase substrate (coelenterazine 400a). If an interaction occurs between RAS and a partner-GFP² fusion, bringing the RLuc8 and GFP² within 100 Å, an energy transfer occurs from the RLuc8-RAS donor to the GFP² acceptor and a BRET2 signal is achieved (**Figure 1A**, middle panel). Inhibitors of the donor-acceptor molecule interaction will decrease the BRET signal whilst maintaining the RLuc8 bioluminescence and GFP² fluorescence signals (**Figure 1A**, right hand panel). The BRET signal (or BRET ratio) is calculated as the light emitted by the GFP² acceptor constructs (at 515 nm) upon addition of coelenterazine 400a, divided by the light emitted by the RLuc8 donor constructs (at 410 nm) (*Pfleger et al., 2006*). A background BRET signal is only observed with the donor-only construct where the RLuc8 plasmid is transfected alone into the cells (**Figure 1—figure supplement 1B**) and this signal is therefore subtracted from that BRET ratio. As shown in **Figure 1—figure supplement 1B**, un-transfected cells and those transfected with GFP²-only construct have a negligible auto-luminescence and emission at 515 nm upon addition of the BRET substrate and are not considered in the calculation of the BRET ratio.

BRET donor saturation assessments were first carried out with the RAS effector RAS binding domains (RBDs) to evaluate the optimal levels of expression plasmid transfection for the competition experiments (**Figure 1B**). All of the effector domains were found to interact specifically with KRAS^{G12D} since the BRET signal reached a donor saturation level (**Figure 1B**). Further, all the transfected plasmids expressed the proteins at equivalent levels as indicated by western blot analysis (**Figure 1C**) and their expression does not modify KRAS^{G12D} expression (**Figure 1—figure supplement 2A** shows the increase of acceptor protein level has little effect of donor protein levels). To further characterize this BRET2 system, we used the dominant negative mutant KRAS^{S17N}, which does not interact with the effectors (*Cool et al., 1999; Nassar et al., 2010; van den Berghe et al., 1997*), as a donor. We found that the BRET signal increased linearly with the concentration of acceptor for all the RAS binding domains. This result is typical of non-specific interactions (*Mercier et al., 2002*), confirming the S17N mutant does not interact with the effectors and supports the sensitivity of this system (**Figure 1—figure supplement 2B**).

We initially characterized the biosensor pairs with the iDAb RAS that is known to interact with mutant KRAS on the switch regions (*Tanaka et al., 2007*), compared with a non-relevant anti-LMO2 iDAb (*Sewell et al., 2014; Tanaka et al., 2011*) that was designated as iDAb control in this study (herein called iDAb Ctl). Introduction of mutations in the three CDRs of the iDAb RAS to generate a dematured iDAb RAS (iDAb_{dm} RAS), was shown to reduce its affinity towards RAS-GTP from 6.2 nM to ~1 μM affinity (*Assi et al., 2010*). While this did not alter the protein expression (**Figure 1—figure supplement 2C,D**), there was an expected BRET signal reduction (**Figure 1—figure supplement 2C**). Indeed, it significantly increased the BRET₅₀ (an approximation of the relative affinity of the acceptor fusion for the donor fusion proteins, corresponding to the acceptor/donor ratio necessary to reach 50% of the BRET_{max}) and significantly reduced the BRET_{max} (an approximation for the total

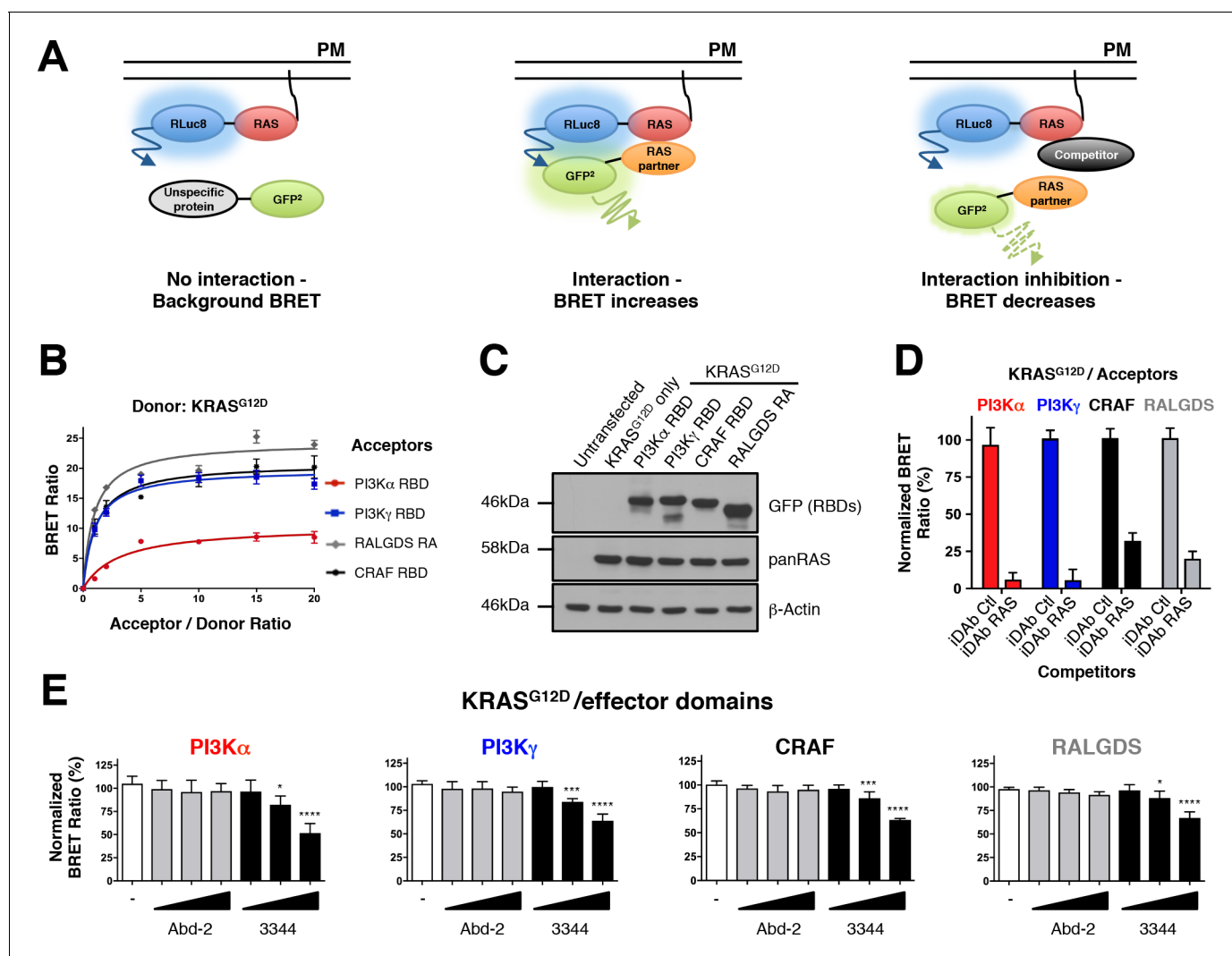


Figure 1. RAS-effector BRET biosensors and interference of KRAS-effector interactions by a RAS-binding compound. An outline of the BRET2-based RAS biosensor system is shown in **A**. RAS bound to the plasma membrane (PM) is fused at its amino terminal end to the RLuc8 moiety (donor). When a protein fused to the GFP² moiety (acceptor) does not bind to RAS, it only produces a background BRET signal. However, when an acceptor binds to RAS, it induces a BRET signal, if the luciferase and GFP domains are within 100 Å. The BRET signal can be decreased by addition of a competitor (either by a macrodrug or a small molecule inhibitor). The interaction titration of full-length KRAS^{G12D}-CAAX (for simplicity, the CAAX motif is omitted in all the RAS constructs described hereafter) with the four effector acceptor proteins and the effect on intracellular protein levels are shown in **B** and **C**. Competition assays show the specificity of the RAS biosensors in **D** (iDAb) and **E** (RAS-binding compounds). In **D**, the non-relevant anti-LMO2 iDAb (called hereafter iDAb control, Ctl) serves as a negative control and anti-RAS iDAb (herein named iDAb RAS) serves as a positive control. In **E**, 3344 (black bars) decreases KRAS^{G12D}/effector domain interactions in a dose-dependent manner showing its broad range of inhibition. Cells were treated with 5, 10 and 20 μM of 3344 (black bars), Abd-2 (grey bars) or DMSO alone (white bars) as the negative control. Statistical analysis was performed with a one-way ANOVA followed by Dunnett's post-hoc tests (**p*<0.05, ****p*<0.001, *****p*<0.0001). Each experiment was repeated three (**B**, **D**) or four times (**E**). Where error bars are presented, these correspond to mean values ± SD of biological repeats (**B**, **D**–**E**). See also **Figure 1—figure supplement 1**, **Figure 1—figure supplement 2**, **Figure 1—figure supplement 3** and **supplementary file 1**.

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The following figure supplements are available for figure 1:

Figure supplement 1. Optimization of the RAS biosensors.

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Figure supplement 2. Validation of the RAS biosensors with the anti-iDAb RAS.

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Figure supplement 3. 3344 inhibits RAS-RBD interactions.

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number of complex RAS/iDAb and the distance between the donor and the acceptor within the dimer), which together are consistent with a decreased affinity of this mutant iDAb toward RAS. Therefore, the results obtained with the iDAb RAS confirmed the sensitivity and accuracy of the RAS biosensors.

Finally, we tested the inhibition of interaction between RAS and its effector partners using BRET in a competition assay. HEK293T cells were transiently transfected with KRAS^{G12D}, each of the RAS-effector domain and a competitor (non-GFP²) version of the iDAb RAS or iDAb control. This competition showed that iDAb RAS, but not the control, drastically decreased the BRET ratio of all the interactions tested (**Figure 1D**). These results confirmed that the BRET2 biosensors enable monitoring of PPI inhibition of KRAS^{G12D} with each of the four effectors tested by the anti-RAS single domain antibody.

The BRET2 biosensors show that 3344 is an inhibitor of KRAS-effector interactions

Our major purpose in the development of the RAS BRET2 biosensors was to create a validation tool for compounds that bind to RAS and interfere with its PPI in living cells. We have identified compounds that bind to KRAS using *in vitro* screening and one compound 3344 (chemical structure and 1-D NMR characterization shown in **Figure 1—figure supplement 3A–C**) binds to KRAS^{G12V} with an affinity of 126 nM using ¹H Carr-Purcell-Meiboom-Gill (CPMG) NMR (**Baldwin and Kay, 2009**) (data are shown in **Figure 1—figure supplement 3D**). *In vitro* competition studies of 3344 binding to KRAS^{G12V} in waterLOGSY NMR show the anti-RAS scFv inhibits 3344 binding to KRAS (**Figure 1—figure supplement 3E**). *In vivo* of the *in vitro* inhibition by the anti-RAS scFv of 3344 binding to RAS and because the iDAb RAS interferes with BRET signal in cells (**Figure 1D**), 3344 was used for validation of the BRET2 toolbox for RAS-effector PPI inhibitors. In the subsequent experiments reported here, we compare 3344 with an initial compound (Abd-2) obtained through a SPR *in vitro* screening, which binds HRAS/KRAS with low affinity. It is the precursor of the 3344 compound and both share the same benzodioxane group (the structures of 3344 and Abd-2 are shown in **Figure 1—figure supplement 3A,F**). These compounds have been selected from a medicinal chemistry programme in order to validate the BRET-based RAS biosensors.

HEK293T cells were transiently transfected with BRET pairs and, after 24 hr to allow protein expression, the cells were seeded in 96-well plates. The compounds were added at different concentrations (5, 10 and 20 μM) and incubated on cells for a further 20 hr before the BRET reading. For each assay, the donor protein was RLuc8-KRAS^{G12D} and the acceptor proteins were PI3Kα RBD-GFP², PI3Kγ RBD-GFP², CRAF RBD-GFP² or RALGDS RA-GFP². We observed a dose response reduction in BRET signal for the assays with compound 3344 but not with the Abd-2 indicating that only 3344 interferes with the RAS-effector PPI (**Figure 1E**). To rule out the possibility of false positive compounds (for instance, that might interfere directly with the BRET signal), we included control BRET-based biosensors. We tested the RAS compounds with the iDAb RAS biosensors, either with RLuc8-LMO2 donor and iDAb_{dm} LMO2 (a dematured anti-LMO2 iDAb (**Sewell et al., 2014**)) acceptor (**Figure 1—figure supplement 3G**), RLuc8-KRAS^{G12D} donor with the iDAb RAS acceptor (**Figure 1—figure supplement 3H**), or RLuc8-KRAS^{G12D} donor with the iDAb_{dm} RAS acceptor (**Figure 1—figure supplement 3I**). Abd-2 has no effect on any of these assays while 3344 only interferes, in a dose response, with KRAS^{G12D}/iDAb_{dm} RAS-induced BRET without affecting the expression of the biosensors (**Figure 1—figure supplement 3J**). Hence, the inhibitory effects of 3344 on KRAS^{G12D}-effectors interactions are not simply due to interference with the BRET assay.

BRET2 reporter and associated RAS-CRAF signaling are affected by compound 3344

The RAS binding domain of the effector molecules lack some regulatory domains, which impedes a direct study of RAS inhibitors on pathways downstream of RAS. To reduce this limitation, we developed an optimized RAS biosensor of the full-length CRAF^{S257L} mutant (herein named CRAF^{FL}) since the S257L mutation increases ERK phosphorylation (**Razzaque et al., 2007**) and because we found that CRAF^{FL} interacts with KRAS^{G12D} but not with KRAS^{S17N} (**Figure 2—figure supplement 1A**). We performed a competition assay with the iDAb RAS confirming that it impedes the BRET2 signal due to the binding of CRAF^{FL} with KRAS^{G12D}, in a dose response mode, whereas the iDAb control had

no effect (**Figure 2A**). There was no alteration in CRAF^{FL} and KRAS^{G12D} protein expression due to the transfection of the iDABs, shown by western analysis (**Figure 2—figure supplement 1B**). In addition, iDAB RAS inhibition significantly decreased the phosphorylation of MEK1/2 and ERK1/2 kinases (**Figure 2B** shows western blot data, quantitated in **Figure 2C**), confirming results affecting endogenous ERK phosphorylation by iDAB RAS interaction with RAS (**Tanaka and Rabbitts, 2010**).

We further tested the ability of the small molecule 3344 to inhibit the KRAS^{G12D}/CRAF^{FL} biosensor and the downstream biomarker pathways with either a long incubation (20 hr, **Figure 2D–F**) or a short incubation (3 hr, **Figure 2—figure supplement 1D–F**) to further validate the specificity of our

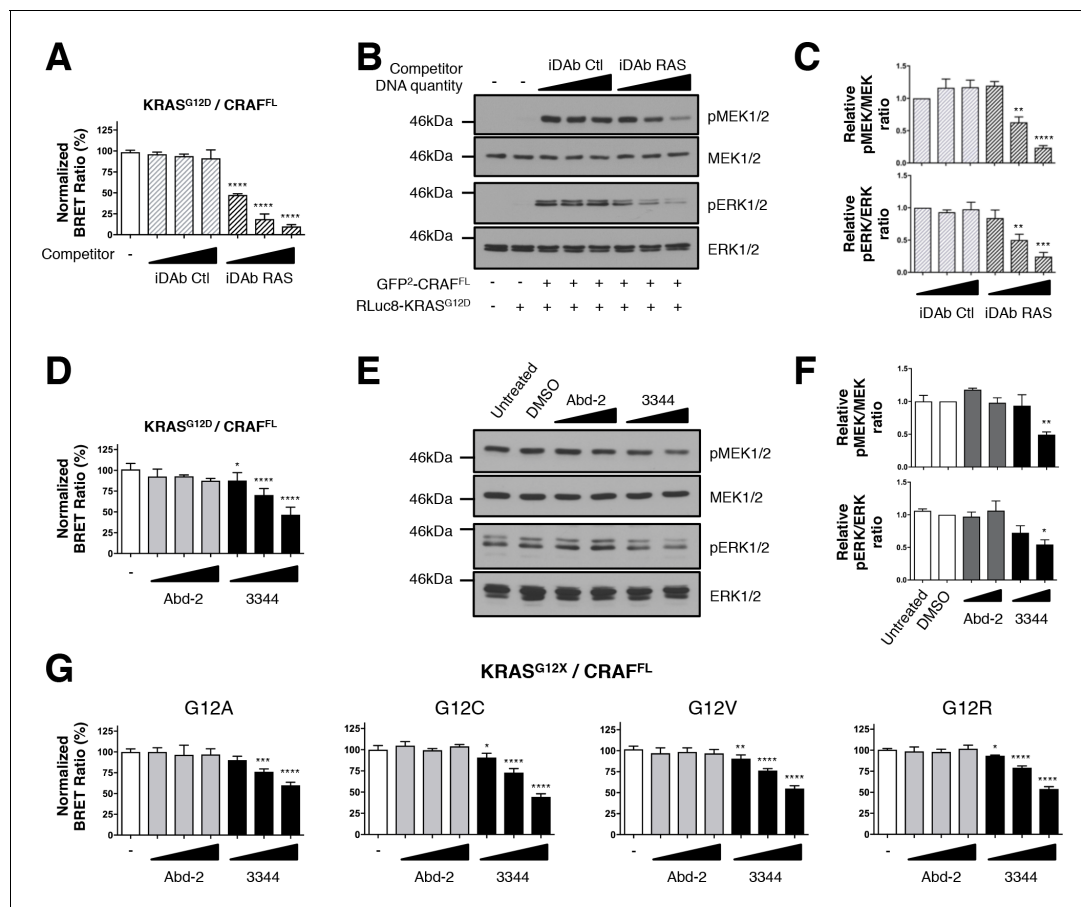


Figure 2. BRET biosensors of KRAS^{G12} mutants and full-length CRAF are inhibited by compound 3344. A biosensor for the full-length CRAF^{S257L} (CRAF^{FL}) protein was made and tested for interaction with mutants of KRAS glycine 12. For **A** and **B**, the plasmids expressing BRET pair KRAS^{G12D}/CRAF^{FL} was transfected into HEK293T cells and competed with iDAB expression as indicated; the BRET ratios are shown in **A** and western blot data in **B**. The iDAB RAS inhibition of phosphorylation of ERK and MEK signals are quantified in **C**. The β -actin loading control, iDABs and BRET pair expression controls are shown in **Figure 2—figure supplement 1**. In **D**, the BRET ratio of KRAS^{G12D}/CRAF^{FL} interaction was measured in the presence of an increasing dose of compound 3344. This induces a dose-dependent decrease of MEK and ERK kinase phosphorylation (**E**) after cells expressing the KRAS^{G12D}/CRAF^{FL} biosensor pair were treated 20 hr with DMSO, 10 and 20 μ M of Abd-2 and 3344 compounds or not treated (untreated lane). The β -actin loading control and BRET pair expression controls are shown in **Figure 2—figure supplement 1**. Quantification of the relative levels of pMEK1/2 and pERK1/2, normalized to total MEK1/2 and ERK1/2 respectively, are shown in **F**. The RAS biosensor toolkit includes KRAS G12A, G12C, G12V and G12R, in addition to KRAS G12D. In **G**, each was expressed with CRAF^{FL} and BRET ratios determined at 0, 5, 10 and 20 μ M Abd-2 or 3344. Statistical analyses in **C** were performed using a one-way ANOVA followed by Sidak's post-hoc tests and in **A**, **D**, **F** and **G** using a one-way ANOVA followed by Dunnett's post-tests (** $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$). Each experiment was repeated twice (**E–F**), three times (**B–D**), four times (**A**) or five times (**G**). Where error bars are presented, they correspond to mean values \pm SD of biological repeats (**A**, **D**, **G**) or correspond to mean \pm SEM of biological repeats (**C**, **F**). See also **Figure 2—figure supplement 1**.

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The following figure supplement is available for figure 2:

Figure supplement 1. Interactions of KRAS^{G12X} mutants and full-length CRAF are inhibited by 3344.

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inhibition. Indeed, long-term incubation with the compound may indirectly inhibit RAS downstream pathways by affecting autocrine mechanisms involved in secondary activation of RAS pathways (Arthur and Ley, 2013; Zhang et al., 2011). We compared the effect of Abd-2 and 3344 on the BRET pair and found a significant decrease in BRET signal with 3344 that occurred in a dose-dependent manner (Figure 2D and Figure 2—figure supplement 1D) without modifying RAS or CRAF expression (as shown by western analysis, Figure 2—figure supplement 1C,G). Western blots using anti-pMEK and anti-pERK showed that 3344 also significantly inhibited MEK1/2 and ERK1/2 phosphorylation whilst Abd-2 did not (Figure 2E, quantified in Figure 2F and Figure 2—figure supplement 1E–F). Therefore, these observations show a specific and functional effect of the inhibition of interaction between RAS and CRAF^{FL} by the 3344 with a long and short incubation.

Some compounds have been previously characterized that bind selectively on the cysteine of KRAS^{G12C} mutant (Lito et al., 2016; Ostrem et al., 2013; Patricelli et al., 2016). We assessed whether our compound 3344 was able to interfere with binding of a range of mutant KRAS Gly12 proteins, including G12C, with CRAF in BRET assays. Analysis of the BRET2 signals from interaction of KRAS^{G12A}, KRAS^{G12C}, KRAS^{G12V} and KRAS^{G12R} with CRAF^{FL} showed a dose response effect of compound 3344 but not Abd-2 (Figure 2G). The corresponding BRET biosensor acceptor and donor proteins are equally expressed after transfection as judged by western blot analysis (Figure 2—figure supplement 1H).

Therefore, using this new set of validated RAS biosensors, we show that the compound disrupts mutant KRAS/CRAF^{FL} interaction in cells. In turn, this leads to inhibition of the RAF/MEK/ERK downstream signaling pathway (that emanates from the transfected protein expression).

3344 inhibits the wild type KRAS-CRAF biosensor and its downstream signaling pathway

We extended the repertoire of biosensors by analyzing wild-type KRAS (KRAS^{WT}) donor molecule and also assessed if epidermal growth factor (EGF)-stimulated MEK/ERK phosphorylation (Burgering et al., 1993; Lange-Carter and Johnson, 1994) could be altered through the interaction of a KRAS^{WT}/CRAF^{FL} BRET2 biosensor protein pair. Although the iDab RAS binds weakly to RAS^{WT} in transfected mammalian two-hybrid reporter cells (Tanaka et al., 2007), we first established if the BRET2 signal from RLuc8-KRAS^{WT} and GFP²-CRAF^{FL} PPI could be inhibited by the iDab RAS in the BRET transfection assay. HEK293T cells were transfected with the BRET pair and serum was removed for 24 hr, stimulated for 5 min with EGF and the BRET ratio directly determined after the stimulation. EGF treatment brings KRAS^{WT} and CRAF^{FL} fusion proteins in a closer proximity and enhances the number of KRAS^{WT}/CRAF^{FL} dimers because the BRET_{max} value increases from 4.02 to 10.01 (Figure 3—figure supplement 1A). A dose response inhibition of the BRET2 signal was observed with iDab RAS, but not iDab control (Figure 3A), which correlated with the reduction of pMEK1/2 and pERK1/2 detected by western blots (Figure 3B and quantified in Figure 3C). This shows that the RAS BRET2 biosensors can be used to couple PPI effects and signaling effects.

We conducted parallel BRET2 dose response experiments with the 3344, compound compared to Abd-2, implementing EGF stimulation and using the KRAS^{WT}/CRAF^{FL} biosensor with short and long incubation times (3 hr and 20 hr, respectively). Compound 3344 inhibits this interaction in a dose-response manner (Figure 3D and Figure 3—figure supplement 1D) and prevents the phosphorylation of MEK1/2 and ERK1/2 kinases (Figure 3E, quantified in Figure 3F and Figure 3—figure supplement 1E–F). Protein levels per se were not affected by the BRET2 transfectants by either the iDab expression (Figure 3—figure supplement 1B) or Abd-2 or 3344 treatments (Figure 3—figure supplement 1C,G). In conclusion, use of the 3344 with the BRET2 RAS biosensors confirms this compound is a pan-KRAS-effector PPI inhibitor.

3344 inhibits the RAS-PI3K-AKT signaling pathway

We have also explored the second best-characterized RAS effector family, the RAS-PI3K α -AKT pathway (Castellano and Downward, 2011) by establishing a KRAS^{G12D}/full-length PI3K α (herein PI3K α ^{FL}) biosensor. In this case, we required a tripartite system as we observed that co-expression of the p85 α regulatory subunit with PI3K α ^{FL}-GFP² was required to obtain detectable, specific and optimized BRET signal from interaction of KRAS^{G12D} and PI3K α ^{FL} (Figure 4—figure supplement 1A). KRAS^{S17N} mutant showed no specific interaction with PI3K α ^{FL} further confirming the accuracy

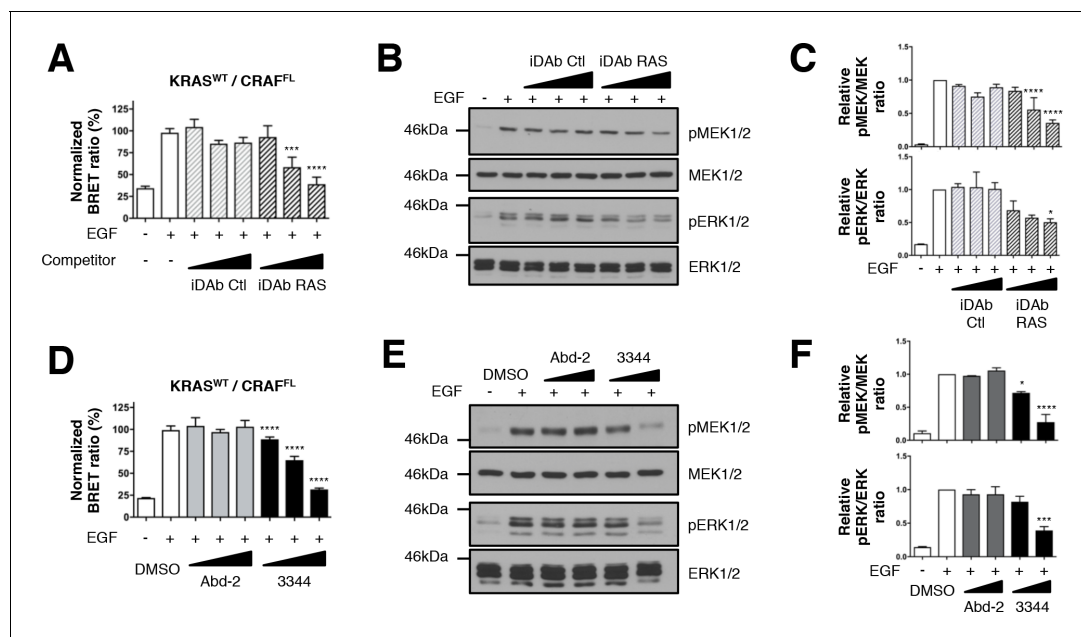


Figure 3. Wild-type KRAS and CRAF biosensor interaction-induced signaling is impaired by 3344. The BRET KRAS^{WT}/CRAF^{FL} pair was tested for interaction after EGF stimulation of HEK293T cells in presence of competitors. In **A**, cells were transfected with plasmids to express the KRAS^{WT} biosensor with or without iDABs and stimulated by EGF (50 ng/mL). iDab RAS shows an inhibition of KRAS^{WT}/CRAF^{FL} interaction after EGF treatment in a dose-dependent manner. **B** is a western blot of the transfected cells from panel **A** showing the effect of the iDABs on EGF-stimulated RAS-RAF-MEK-ERK signaling pathway (pMEK and pERK signals are quantified in **C**). β -actin loading control, iDABs and BRET pair expression controls are shown in **Figure 3—figure supplement 1**. The effect on BRET2 signal of compounds Abd-2 (grey bars) and 3344 (black bars) on KRAS^{WT}/CRAF^{FL} interaction after EGF treatment in a BRET competition experiment is shown in panel **D**. In panel **E**, HEK293T cells were transfected as in **D** with the plasmids expressing the BRET pair KRAS^{WT}/CRAF^{FL} for 24 hr and serum starved 20 hr in the presence of DMSO, 10 and 20 μ M of Abd-2 and 3344 compounds. Cells were treated 5 min with EGF (50 ng/mL), lysed and analyzed by western blot. The expression level of the BRET protein pair is shown in **Figure 3—figure supplement 1** as well as the loading control β -actin for the western blot. The western blot data are quantified in panel **F**. One-way ANOVA followed by Dunnett's post-hoc tests were used to determine the statistical significance of BRET, pERK and pMEK modulations induced by the compound or the iDAB (* p <0.05, *** p <0.001, **** p <0.0001). Each experiment was repeated twice (**B–C**) or three times (**A, D–F**). Where error bars are presented, they correspond to mean values \pm SD of biological repeats (**A, D**) or correspond to mean \pm SEM of biological repeats (**C, F**). See also **Figure 3—figure supplement 1**.

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Figure supplement 1. 3344 inhibits KRAS^{WT}/CRAF^{FL} interaction induced by EGF treatment.

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of this biosensor (**Figure 4—figure supplement 1A**). We validated the BRET biosensor by showing that the iDab RAS impaired that interaction in a dose-dependent manner, whereas the iDab control did not (**Figure 4A**). Western blot analysis showed some reduction in PI3K and RAS proteins, specifically concordant with expression of the iDab RAS (**Figure 4—figure supplement 1B**) and there was also a dose response reduction of phosphorylation of the downstream biomarker AKT at Ser473 (**Figure 4B** and quantified in **Figure 4C**).

Implementing the same biosensor assay treated with the compound 3344 for 3 or 20 hr, we confirmed this compound interferes with the KRAS^{G12D}/PI3K^{FL} interaction (**Figure 4D–F** and **Figure 4—figure supplement 1D–F**) without loss of protein (**Figure 4—figure supplement 1C,G**). Abd-2 has no effect on the phosphorylation of AKT that results from KRAS^{G12D}/PI3K^{FL} interaction. Conversely, 3344 does affect RAS-PI3K interaction and AKT phosphorylation. When increasing doses of either Abd-2 or 3344 were used in the BRET-transfected cells, we observed dose response reduction of BRET signal with 3344 but not Abd-2 (**Figure 4D** and **Figure 4—figure supplement 1D**). Associated with this inhibition, was a reduction in the downstream biomarker AKT Ser473 phosphorylation (**Figure 4E**, quantified in **Figure 4F** and **Figure 4—figure supplement 1E–F**). 3344 inhibits RAS-PI3K α PPI and thus signaling through AKT.

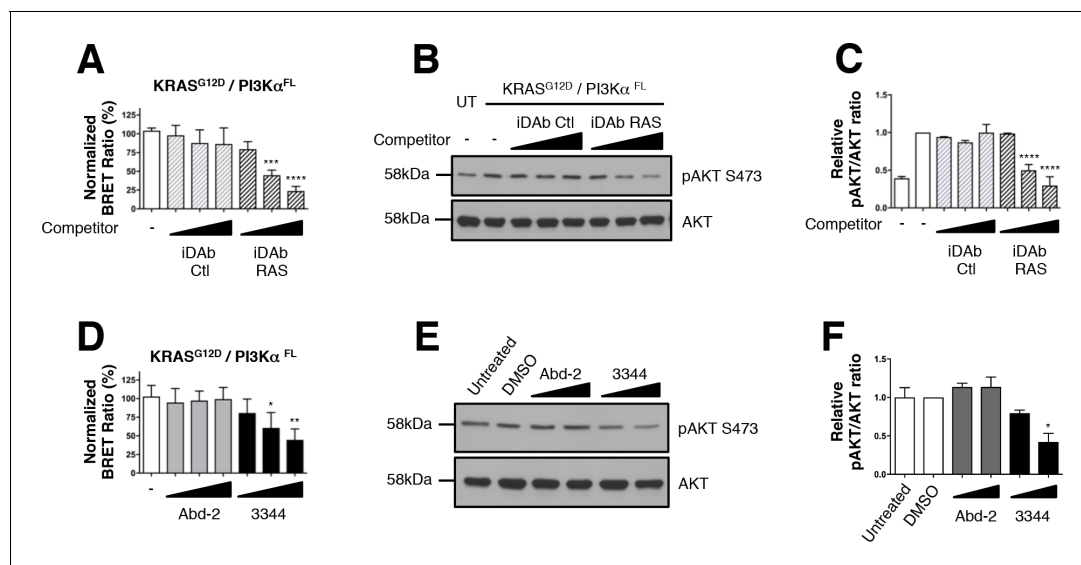


Figure 4. Interaction between mutant KRAS and full-length PI3K α BRET pair interaction is impeded by 3344. The BRET signal produced from the interaction of the KRAS^{G12D} and full-length PI3K α (PI3K α ^{FL}) was obtained by transfecting HEK293T cells with plasmids encoding this BRET pair. In **A**, cells were co-transfected with the biosensor and increasing levels of competitor plasmids encoding iDAb RAS (black striped bars) or iDAb control (grey striped bars) or biosensor alone (white bar). iDAb RAS impedes KRAS^{G12D}/PI3K α ^{FL} interaction and this inhibition causes a decrease of pAKT at serine 473 as shown by western blot in **B** and its quantification in **C**. UT is for untransfected cells. In **D**, HEK293T cells transfected with the BRET biosensor KRAS^{G12D}/PI3K α ^{FL} were treated for 20 hr with DMSO (white bar), 5, 10 and 20 μ M of Abd-2 (grey bars) and 3344 (black bars) compounds and the BRET signal of the biosensor was assessed. In panel **E**, the cells were transfected and treated as in **D** but with 10 and 20 μ M of Abd-2 and 3344 compounds. 20 hr after the treatment, cells were lysed and analysed by western blot using anti-pAKT (Ser 473) or anti-pan-AKT antibody. The signal in the western blot is quantitated in **F**. Related controls are shown on **Figure 4—figure supplement 1**. One-way ANOVA followed by Dunnett's post-hoc tests were used to determine the statistical significance of BRET and pAKT modulations induced by the compound or the iDAb (* p <0.05, ** p <0.01, *** p <0.001, **** p <0.0001). Each experiment was repeated twice (**E–F**) or three times (**A–D**). Where error bars are presented, they correspond to mean values \pm SD of biological repeats (**A, D**) or correspond to mean \pm SEM of biological repeats (**C, F**). See also **Figure 4—figure supplement 1**.

DOI: <https://doi.org/10.7554/eLife.37122.011>

The following figure supplement is available for figure 4:

Figure supplement 1. Interaction of KRAS^{G12D} with PI3K α ^{FL} is inhibited by 3344.

DOI: <https://doi.org/10.7554/eLife.37122.012>

The BRET2 biosensor toolbox includes NRAS and HRAS and shows 3344 inhibits PPI of the RAS family

The KRAS, NRAS and HRAS family members are conserved proteins that have an almost identical amino-acid domain (G domain) from residues 1–166 but a C-terminal hypervariable domain (Wennerberg *et al.*, 2005). We have extended the RAS biosensor toolbox to include NRAS and HRAS. We used full-length NRAS^{Q61H} and HRAS^{G12V} mutants to build these new RAS biosensors for use with the various effector RBDs. These mutants were used at the positions Q61 and G12, for NRAS and HRAS respectively, as these are the positions most frequently mutated in human cancer involving NRAS and HRAS mutants (Cox *et al.*, 2014). Titration of the RAS donor and CRAF^{FL} acceptor proteins show that the RLuc8-NRAS^{Q61H} and RLuc8-HRAS^{G12V} proteins interact and reach plateau BRET signals with GFP²-CRAF^{FL} (Figure 5—figure supplement 1A). Furthermore, the BRET2 signal is diminished by increasing levels of the iDAb RAS but not the iDAb control (Figure 5—figure supplement 1B–D) as expected from the analysis of the effects of the anti-RAS intracellular antibody (Tanaka and Rabbitts, 2010; Tanaka *et al.*, 2007).

We further evaluated the efficacy of the RAS-binding compounds Abd-2 and 3344 in binding to NRAS and HRAS using a BRET assay in which the RAS protein donors were co-expressed with either PI3K, CRAF or RALGDS acceptors (Figure 5A–D). While the low-affinity Abd-2 compound does not interfere with the BRET signal in any of the NRAS and HRAS BRET assays using either effector RBDs (Figure 5A,B) or full-length CRAF (Figure 5C,D), the compound 3344 disturbs the BRET2 signal in

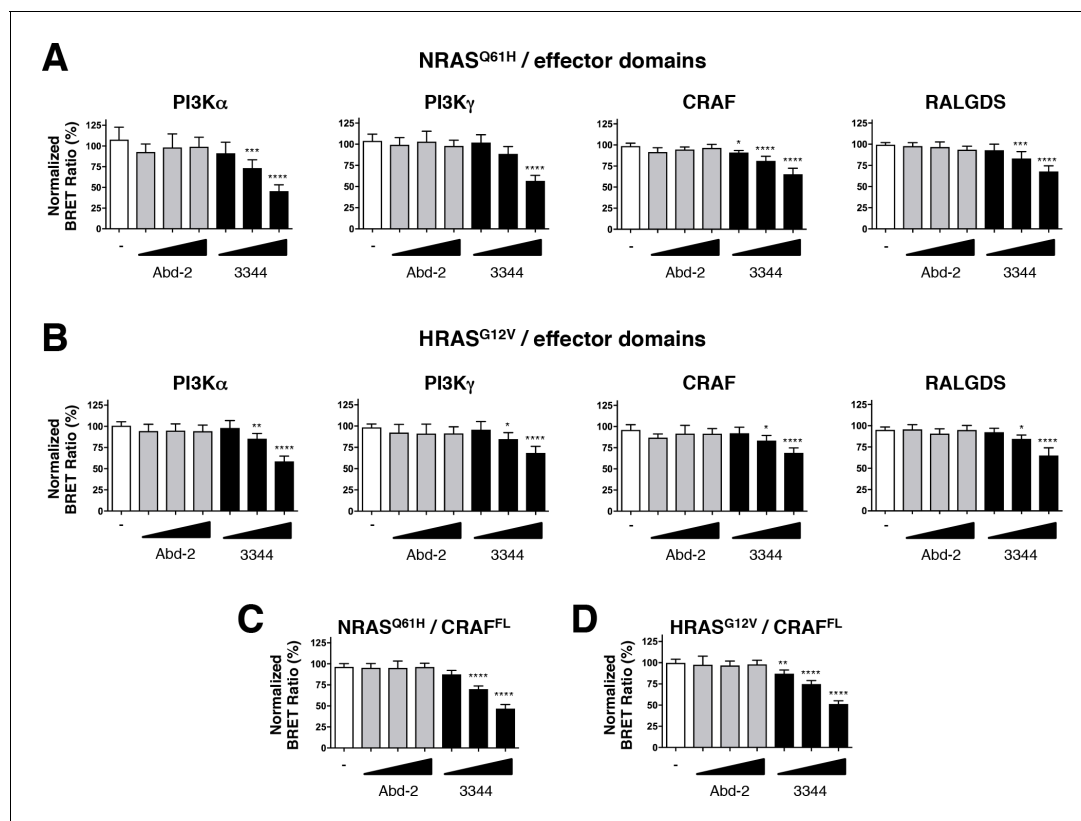


Figure 5. Compound 3344 inhibits NRAS and HRAS-effector BRET-based biosensors. HEK293T cells were transfected 24 hr with plasmids expressing the NRAS^{Q61H} (A, C) and HRAS^{G12V} (B, D) biosensors together with the indicated RBDs of PI3K, CRAF and RALGDS (A, B) or full-length CRAF (C, D). These were treated with 5, 10 and 20 μM of Abd-2 (grey bars) or 3344 (black bars) compounds for 20 hr. DMSO (white bar) was used as the negative control. Statistical analyses were performed using a one-way ANOVA followed by Dunnett's post-tests (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$). Each experiment was repeated at least four times. Where error bars are presented, they correspond to mean values \pm SD of biological repeats (A–D). See also **Figure 5—figure supplement 1**.

DOI: <https://doi.org/10.7554/eLife.37122.013>

The following figure supplement is available for figure 5:

Figure supplement 1. iDab RAS inhibits mutant NRAS and HRAS interaction with CRAF^{FL}.

DOI: <https://doi.org/10.7554/eLife.37122.014>

a dose-response manner in all these RAS interactions (**Figure 5** and **Figure 5—figure supplement 1E,F**). Therefore, the BRET-based RAS biosensors characterization of 3344 shows this compound as a pan-RAS-effector interactions inhibitor that binds KRAS, NRAS and HRAS.

Compound 3344 binds to a pocket close to the switch regions of mutant KRAS

The implementation of our RAS BRET2 toolbox showed that the compound 3344 is able to bind the transfected RAS protein products at the plasma membrane and interfere with their effector interaction. In addition, the downstream signaling was impeded. The mechanism of the interaction inhibition was corroborated by X-ray crystallography of KRAS^{Q61H} soaked with compound 3344. **Figure 6A** shows that 3344 binds to KRAS in a previously identified pocket (Maurer et al., 2012; Sun et al., 2012) close to the switch regions where the effectors interact with RAS (Table 1 has the refinement statistics for the X-ray data). The superimposition of the structures of three RAS-effector protein complexes with the structure of KRAS-3344 complex shows that parts of 3344 would overlap with the bound effector structures, suggesting that the competition effect of 3344 can be explained by straightforward steric hindrance (Figure 6B). We further confirmed that 3344 could interfere with the endogenous RAS-effector PPI in two human cancer cell lines (viz. colorectal adenocarcinoma DLD-1 cells expressing KRAS^{G13D} and non-small cell lung carcinoma H358 cells expressing

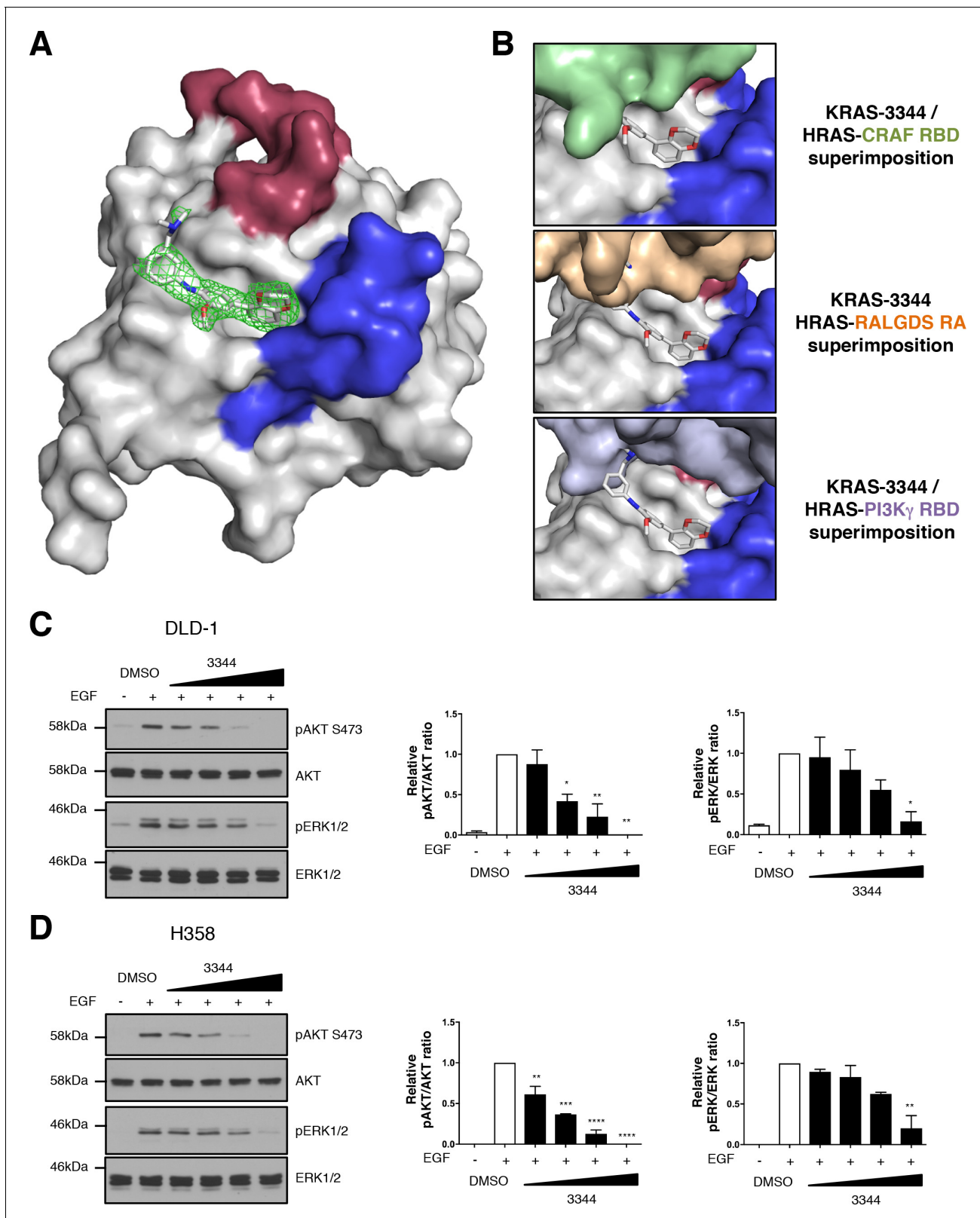


Figure 6. Compound 3344 interacts in a pocket close to the switch regions of KRAS. The interaction of mutant KRAS with compound 3344 was analyzed by X-ray crystallography. (A) KRAS^{Q61H} crystals were soaked with 3344 compound and crystal structures obtained from X-ray diffraction. The compound is shown binding in the hydrophobic pocket near switch I (shown in red) and switch II (shown in blue). The electron density map of the compound (2Fo-Fc) is shown as green mesh, and contoured at 1.0 rms. (B) We have modeled the potential interactions that could prevent 3344 and a

Figure 6 continued on next page

Figure 6 continued

RAS effector binding simultaneously to the same RAS molecule by overlaying our structure of the KRAS-3344 complex onto the published structures of top panel: HRAS-CRAF RBD (PDB 4G3X), middle panel: HRAS-RALGDS RA (PDB 1LFD), bottom panel: HRAS-PI3K γ RBD (PDB 1HE8). (C, D) Two human mutant KRAS expressing lines (C: DLD-1 and D: H358) were serum-starved for 24 hr and treated 3 hr with different concentrations of 3344 (2, 5, 10 and 20 μ M) before stimulation with EGF (50 ng/mL) for 10 min. Cells were harvested, proteins extracted and separated by SDS-PAGE for western blot analysis. Western membranes were treated with anti-pAKT S473; anti-pan AKT; anti-pERK1/2 and anti-ERK1/2 as indicated. Statistical analyses of pERK/ERK and pAKT/AKT quantifications were performed using a one-way ANOVA followed by Dunnett's post-tests (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$). Where error bars are presented, they correspond to mean values \pm SEM of biological repeats (C–D). Each experiment was performed twice (C–D).

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KRAS^{G12C}). The cells were serum starved 24 hr and stimulated 10 min with EGF in the presence of increasing amounts of 3344, followed by western blot protein analysis to detect phosphorylated AKT Ser473 or phosphorylated ERK (Figure 6C,D). 3344 decreases EGF-induced pAKT and pERK1/2 abundance in both cell types with an observed IC₅₀ of ~5–10 μ M without any change in the total levels of AKT or ERK1/2. Therefore, 3344 can interfere with endogenous RAS signaling in human cancer cell lines. As our BRET2 results show direct interference of RAS-effector PPI by 3344, we conclude that this is the mechanism of inhibition of the biomarkers in the tumor cell assay.

Discussion

BRET-based biosensors have been successfully used to discover and characterize small molecules inhibitors (Beutraut et al., 2017; Corbel et al., 2011; Lavoie et al., 2013; Mazars and Fähræus, 2010; Robinson et al., 2014). The development of such biosensors involves the optimization of multiple parameters such as the fusion position of the RLuc8 and GFP² moieties on their respective protein N- or C-terminus and the determination of the appropriate quantity of donor and acceptor plasmids for intracellular expression. Notably, the latest parameter has to be optimized in order to avoid the titration of active compounds if transient protein expression is used (Couturier and Deprez, 2012). In this study, we have engineered and optimized a complete set of RAS biosensors that includes several different mutant forms of KRAS and other family members (viz. mutant NRAS and HRAS). This toolbox allows the monitoring of RAS-effector interactions and the assessment of RAS PPI inhibition by a macrodrug (iDAb RAS) and 3344, a new anti-RAS small molecule derived from an intracellular antibody fragment, in living cells. Furthermore, when the full-length biosensors were used, we could couple the RAS PPI inhibition to the signaling effects, thereby providing additional insights into the behavior of RAS inhibitors.

The inhibition of RAS PPI by 3344 in cells was demonstrated by the RAS biosensors toolbox and validated by X-ray crystallography. 3344 binds to a hydrophobic pocket near to the effector-binding switch regions of RAS (Figure 6). Whereas 3344 does not make direct contact with the switch regions, the BRET data show that the binding geometry and potency of 3344 is sufficient to interfere with the interaction of RAS-effector molecules that bind close to the 3344 site.

While the RAS biosensors rely on transfection and expression of RAS with one of its partner proteins rather than observations of endogenous proteins, it nevertheless offers several advantages for the study of RAS-effector interactions inhibition. It provides a direct and quantitative measurement of the PPI interference with inhibitors (i.e. small molecules or macrodrugs), which could allow the comparison of different compounds (e.g. for structure-activity-relationship studies) or macrodrugs and therefore the selection of more potent inhibitors. It is also sensitive and consequently requires a small quantity of cells to study the inhibition of the interaction. Nonetheless, 3344 prevents endogenous RAS-dependent signaling in two different human tumor cell lines at a lower concentration (IC₅₀ around 5 μ M) (Figure 6C,D) than in the BRET assay with observed IC₅₀ around 20 μ M. This difference probably reflects the expression levels of the target proteins in the two assays, where the BRET2 assay relies on transient transfection. Indeed, the overexpression in HEK293T cells probably produces higher amount of mutant RAS/effector proteins than the endogenous counterparts in cancer cells. Therefore, it might be more difficult to quantitatively inhibit the exogenous RAS/effector interaction than the endogenous one with 3344 compound. Generating stable BRET2 cell lines could minimize this difference.

Table 1. Data processing and refinement statistics.

Structure	KRAS^{Q61H}-3344
Data collection	
PDB ID	6F76
Diffraction source	ID30A-1, ESRF
Temperature (K)	100
Wavelength (Å)	0.966
Rotation range per image (°)	0.05
Exposure time per image (s)	0.092
Space group	P 2 ₁ 2 ₁ 2 ₁
Molecules/asymmetric unit	6
Unit cell dimensions	
a, b, c (Å)	63.17, 118.19, 155.95
α, β, γ (°)	90, 90, 90
Resolution range (Å)	77.98–2.20 (2.16–2.20)*
Total no. of reflections	295785 (13854)
Unique reflections	65992 (2888)
Completeness (%)	99.2 (87.3)
Multiplicity	4.5 (4.8)
R _{meas} (I) [†]	0.193 (0.997)
R _{merge} [‡]	0.151 (0.780)
R _{pim} (I) [§]	0.119 (0.612)
I/σ	5 (1.8)
CC _{1/2} (%) [#]	0.985 (0.513)
Refinement	
No. of reflections, working set	62692 (2744)
No. of reflections, test set	3300 (144)
R _{work} /R _{free}	22.7/25.0
No. of atoms	
Protein	8400
Water	57
Average B factors (Å²)	
Protein	46.8
Ligand GTP	31.9
Water	30.1
RMSD	
Bond lengths (Å)	0.014
Bond angles (°)	1.67
Ramachandran plot	
Favoured regions (%)	97.1
Additionally allowed (%)	2.9
Outliers	0
MolProbity statistics	
Overall score	1.11
Clash score	1.22
Rotamer outliers (%)	1.4

a*Values in parentheses are for data in the highest resolution shell.

[†]Rmeas = $\sum_{hkl} \{N(hkl)/[N(hkl)-1]\}^{1/2} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of reflection hkl . \sum_i is the sum over all i measurements of reflection hkl and $N(hkl)$ is the multiplicity of reflection hkl .

[‡]Rmerge = $\sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of reflection hkl and \sum_i is the sum over all i measurements of reflection hkl .

[§]Rpim = $\sum_{hkl} \{1/[N(hkl)-1]\}^{1/2} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where $I_i(hkl)$ is the intensity of reflection hkl , \sum_i is the sum over all i measurements of reflection hkl and $N(hkl)$ is the multiplicity of reflection hkl .

[#]CC_{1/2} is Pearson's correlation coefficient between random half data sets.

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Another advantage of this toolbox has been shown by using the iDAb RAS as an acceptor within the RAS biosensors allowing a recapitulation of the published features of this intracellular single domain antibody. Therefore, the biosensors are also important tools to study RAS protein interactions in living cells and their effect on the RAS downstream pathways before being tested in cancer cell lines. RAS biosensors use should not be limited to the discovery and characterization of RAS inhibitors. Indeed, studies suggested that isoform and residue- or codon-specific RAS mutants show differences in their ability to engage effectors and signaling properties (Hunter *et al.*, 2015; Nakhaeizadeh *et al.*, 2016; Yan *et al.*, 1998). Accordingly, RAS biosensors could also be a methodology to decipher RAS isoform/mutant properties in cells. Our toolbox is an available resource for RAS-drug development programmes, and more generally for the RAS community, since our results demonstrate the possibility of using these RAS biosensors as a generic method to characterize cell-potent RAS-binding compounds or RAS-binding macrodrugs.

The BRET2 biosensor system could also be used for direct screens of PPI inhibitors with libraries of compounds. However, because initial compounds from a library are not expected to have high affinity for their target, relatively weak interactions between donor and acceptors should be involved in the generation of BRET2 signal. This provides a further use of intracellular domain antibodies where reduction of affinity (dematuration) from a tool initially used for target validation, can be achieved to make a screening tool. Thus, the method is an approach that is transferable to other PPI situations required for drug development programmes in cancer or any other clinical indication.

Materials and methods

Key resources table

Reagent type (species) or resource	Designation	Source or reference	Identifiers	Additional information
Cell line (human)	HEK293T	ATCC	Cat#CRL-3216 RRID:CVCL_0063	
Cell line (human)	DLD-1	ATCC	Cat#CCL-221 RRID:CVCL_0248	
Cell line (human)	H358	ATCC	Cat#CRL-5807 RRID:CVCL_1559	

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Reagent type (species) or resource	Designation	Source or reference	Identifiers	Additional information
Transfected construct (human)	pEF-RLuc8-(GGGS) ₃ -KRAS ^{G12D} -CAAX plasmid	This paper	N/A	DNA/protein sequences provided in the Supplementary file 1
Transfected construct (human)	pEF-RLuc8-(GGGS) ₃ -KRAS ^{G12A} -CAAX plasmid	This paper	N/A	
Transfected construct (human)	pEF-RLuc8-(GGGS) ₃ -KRAS ^{G12C} -CAAX plasmid	This paper	N/A	
Transfected construct (human)	pEF-RLuc8-(GGGS) ₃ -KRAS ^{G12V} -CAAX plasmid	This paper	N/A	
Transfected construct (human)	pEF-RLuc8-(GGGS) ₃ -KRAS ^{G12R} -CAAX plasmid	This paper	N/A	
Transfected construct (human)	pEF-RLuc8-(GGGS) ₃ -NRAS ^{Q61H} -CAAX plasmid	This paper	N/A	
Transfected construct (human)	pEF-RLuc8-(GGGS) ₃ -HRAS ^{G12V} -CAAX plasmid	This paper	N/A	
Transfected construct (human)	pEF-RLuc8-(GGGS) ₃ -KRAS ^{S17N} -CAAX plasmid	This paper	N/A	
Transfected construct (human)	pEF-RLuc8-(GGGS) ₃ -KRAS ^{WT} -CAAX plasmid	This paper	N/A	
Transfected construct (human)	pEF-GFP ² -(GGGS) ₃ -CRAF ^{S257LFL} plasmid	This paper	N/A	
Transfected construct (human)	pEF-PI3K α ^{FL} -(GGGS) ₃ -GFP ² plasmid	This paper	N/A	
Transfected construct (human)	pEF-CRAF RBD (aa 1–149)-(GGGS) ₃ -GFP ² plasmid	This paper	N/A	
Transfected construct (human)	pEF-PI3K α RBD (aa 161–315)-(GGGS) ₃ -GFP ² plasmid	This paper	N/A	
Transfected construct (human)	pEF-PI3K γ RBD (aa 190–315)-(GGGS) ₃ -GFP ² plasmid	This paper	N/A	DNA/protein sequences provided in the Supplementary file 1
Transfected construct (human)	pEF-iDAb RAS-(GGGS) ₂ -GFP ² plasmid	This paper	N/A	
Transfected construct (human)	pEF-iDAb _{dm} RAS-(GGGS) ₂ -GFP ² plasmid	This paper	N/A	
Transfected construct (human)	pEF-iDAb control-(GGGS) ₂ -GFP ² plasmid	This paper	N/A	
Transfected construct (human)	pEF-LMO2-(GGGS) ₂ -RLuc8 plasmid	This paper	N/A	
Transfected construct (human)	pEF-GFP ² -(GGGS) ₃ -iDAb _{dm} LMO2 plasmid	This paper	N/A	
Transfected construct (human)	pEF-memb-FLAG-iDAb RAS plasmid	This paper	N/A	
Transfected construct (human)	pEF-memb-FLAG-iDAb control plasmid	This paper	N/A	
Transfected construct (human)	pEF-iDAb RAS-myc plasmid	This paper	N/A	
Transfected construct (human)	pEF-iDAb control-myc plasmid	This paper	N/A	
Transfected construct (human)	pcDNA3.1-myc-p85 α ^{FL} plasmid	A gift from R. Williams and O. Perisic	N/A	
Transfected construct (mouse)	pEF-RALGDS RA (aa 788–884)-(GGGS) ₃ -GFP ² plasmid	This paper	N/A	The RALGDS RA domain corresponds to the mouse sequence
Antibody	Phospho-ERK 1/2 Rabbit antibody	Cell Signaling Technology	Cat#9101S RRID:AB_331646	

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Continued

Reagent type (species) or resource	Designation	Source or reference	Identifiers	Additional information
Antibody	Total ERK 1/2 Rabbit antibody	Cell Signaling Technology	Cat#9102S RRID:AB_330744	
Antibody	Phospho-MEK 1/2 Rabbit antibody	Cell Signaling Technology	Cat#9154S RRID:AB_2138017	
Antibody	Total MEK 1/2 Mouse antibody	Cell Signaling Technology	Cat#4694S RRID:AB_10695868	
Antibody	Phospho-AKT S473 Rabbit antibody	Cell Signaling Technology	Cat#4058S RRID:AB_331168	
Antibody	Total AKT Rabbit antibody	Cell Signaling Technology	Cat#9272S RRID:AB_329827	
Antibody	Pan-RAS Mouse antibody	Millipore	Cat#OP40 RRID:AB_213400	
Antibody	GFP Mouse antibody	Santa Cruz Biotechnology	Cat#sc-9996 RRID:AB_627695	
Antibody	β -Actin Mouse antibody	Sigma-Aldrich	Cat#A1978 RRID:AB_476692	
Antibody	CMYC HRP-linked Goat antibody	Novus Biologicals	Cat#NB600-341 RRID:AB_10000717	
Antibody	Anti-Mouse IgG HRP-linked antibody	Cell Signaling Technology	Cat#7076S RRID:AB_330924	
Antibody	Anti-Rabbit IgG HRP-linked antibody	Cell Signaling Technology	Cat#7074S RRID:AB_2099233	
Recombinant DNA reagent	pEF-myc-cyto vector	Invitrogen	Cat#V89120	
Recombinant DNA reagent	pRLuc8-N3 vector	A gift from J. Felce	Felce et al., 2017	
Recombinant DNA reagent	pGFP ² -N3 vector	A gift from J. Felce	Felce et al., 2017	
Recombinant DNA reagent	pBABEpuro-CRAF ^{S257L FL} plasmid	Addgene	Addgene#51125	
Peptide, recombinant protein	KRAS ^{Q61H}	This paper	N/A	
Peptide, recombinant protein	KRAS ^{G12V}	This paper	N/A	
Peptide, recombinant protein	Anti-RAS scFv	This paper	N/A	
Peptide, recombinant protein	Recombinant Human Epidermal Growth Factor (EGF)	Life Technologies	Cat#PHG0311	
Chemical compound, drug	Coelenterazine 400a	Cayman Chemical	Cat#16157	
Chemical compound, drug	2-bromo-6-methoxyphenol	This paper	N/A	
Chemical compound, drug	3-bromobenzene-1,2-diol	This paper	N/A	
Chemical compound, drug	5-bromo-2,3-dihydrobenzo[b][1,4]dioxine	This paper	N/A	
Chemical compound, drug	5-(4-chloro-3-methoxyphenyl)-2,3-dihydrobenzo[b][1,4]dioxine	This paper	N/A	
Chemical compound, drug	4-(2,3-dihydrobenzo[b][1,4]dioxin-5-yl)-N-(4-(dimethylamino)methyl)phenyl)-2-methoxyaniline	This paper	N/A	
Software, algorithm	Image J	National Institutes of Health	https://imagej.nih.gov/ij/download.html RRID:SCR_003070	
Software, algorithm	Prism 7.0 c	GraphPad	https://www.graphpad.com/scientific-software/prism/ RRID:SCR_002798	
Software, algorithm	PROCHECK	Laskowski et al. (1993a)	http://www.ccp4.ac.uk/html/procheck_man/index.html	
Software, algorithm	REFMAC	Murshudov et al. (1997)	http://www.ccp4.ac.uk/html/refmac5.html RRID:SCR_014225	

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Reagent type (species) or resource	Designation	Source or reference	Identifiers	Additional information
Software, algorithm	MolProbity	<i>Chen et al. (2010)</i>	http://molprobity.biochem.duke.edu/ RRID:SCR_014226	
Software, algorithm	Phenix	<i>Adams et al. (2010)</i>	https://www.phenix-online.org/ RRID:SCR_014224	
Software, algorithm	PyMOL	Schrodinger	https://pymol.org/2/ RRID:SCR_000305	
Other	Opti-MEM I Reduced Serum Medium, no phenol red	Thermo-Fisher	Cat#11058021	
Other	ViewPlate, White 96-well plate, clear bottom for tissue culture	PerkinElmer	Cat#6005181	
Other	BRET2 Dual Emission optical module	PerkinElmer	Cat#2100-8140	
Other	Envision instrument, Multilabel Reader	PerkinElmer	Cat#2103	

Cell culture

HEK293T human embryonic kidney cells, DLD-1 cells and H358 cells were grown in DMEM medium (Life Technologies) supplemented with 10% FBS (Sigma) and 1% Penicillin/Streptomycin (Life Technologies). Cells were grown at 37°C with 5% CO₂ and were tested using a MycoAlert Mycoplasma Detection Kit (Lonza) and found to be mycoplasma-free before use.

Mutation detection of RAS mutations using RT-PCR

RNA was extracted from 5×10^6 DLD-1 or H358 cells using the RNeasy Plus Mini Kit (Qiagen) according to the manufacturer's instructions. cDNA was synthesized from 1.5 to 2 µg RNA using SuperScript II Reverse Transcriptase (Invitrogen). Primers were designed to amplify KRAS DNA and incorporate HindIII and BamHI restriction sites for subcloning:

5'- TAAGCAAAGCTTATGACTGAATATAAACTGTGGTAG-3' and
3'-GAAAATTAATAAATGCATTATAATGTAAGGATCCTAAGCA-5'

DNA was amplified using Phusion High-Fidelity DNA Polymerase (New England Biolabs) and, following digestion with HindIII and BamHI, the DNA was cloned into pBlueScript II SK (+) (Stratagene). Plasmid DNA was prepared from individual DH5α transformants using a QIAprep Spin Miniprep Kit (QIAGEN). KRAS mutations were verified by Sanger sequencing (Source Bioscience) of at least six clones from each cell line. The KRAS mutations in the two human cancer cell lines were confirmed as KRAS^{G13D} in DLD-1 and KRAS^{G12C} in H358.

Cell treatment

For dose response experiments (BRET and western blot), drugs were prepared in 100% DMSO at 10 mM. Cells were treated with Abd-2 or 3344 compounds at concentration of 5, 10 or 20 µM for 3 hr (short-term incubation) or 20 hr (long-term incubation). The compounds were diluted in the BRET medium: OptiMEM no phenol red (Life Technologies) supplemented with 4% FBS and with a final concentration of 0.2% DMSO.

For serum starvation studies with the BRET assay, cells were grown 24 hr in the presence of OptiMEM no phenol red supplemented with 1% FBS and stimulated with 50 ng/mL EGF (Life Technologies) for 5 min at 37°C. For serum-starvation studies of cancer cell lines, cells were grown 24 hr in the presence of DMEM without FBS and stimulated 10 min with 50 ng/mL EGF. The compound was incubated for 3 hr before the EGF stimulation at 2, 5, 10 and 20 µM.

Molecular cloning

Generation of pEF-RLuc8 and pEF-GFP² plasmids

RLuc8 and GFP² cDNA was amplified by PCR from pRLuc8-N3 and pGFP²-N3 vectors respectively (Felce *et al.*, 2017). RLuc8 was cloned into the pEF-myc-cyto vector (Invitrogen) between BspHI/XhoI sites to produce a pEF-RLuc8-MCS plasmid or between NotI/XbaI sites to produce a pEF-MCS-RLuc8 plasmid. GFP² was inserted into the pEF-myc-cyto vector between NcoI/XhoI sites to produce the pEF-GFP²-MCS plasmid or between NotI/XbaI to produce the pEF-MCS-GFP² plasmid. A (GGGS)_n linker was introduced between XhoI/NotI of all the RLuc8 and GFP² plasmids.

Generation of KRAS mutants and BRET donor plasmids

The generation of the mutant and wild-type KRAS was PCR site-directed mutagenesis using pPGK-KRAS^{G12D}-CAAX-P2A-Puro as a template (a gift from Jennifer Chambers). The following full-length KRAS mutants have been produced: KRAS^{G12A}, KRAS^{G12C}, KRAS^{G12D}, KRAS^{G12V}, KRAS^{G12R}, KRAS^{S17N} and KRAS^{WT}, all with carboxy terminal CAAX. All RAS cDNAs (KRAS mutants, KRAS^{WT}, NRAS^{Q61H} and HRAS^{G12V}-CAAX) were cloned between NotI/XbaI of the pEF-RLuc8-MCS plasmid.

LMO2 was amplified by PCR and cloned between NcoI/XhoI sites of the pEF-MCS-RLuc8 plasmid.

Generation of effectors/iDAb BRET plasmids

CRAF RBD (1-149), PI3K α RBD (161-315), full-length PI3K α (a gift from Roger Williams and Olga Perisic), PI3K γ RBD (190-315), RALGDS RA (788-884), iDAb RAS, iDAb_{dm} RAS and iDAb LMO2 (iDAb control) were amplified by PCR and cloned between NcoI/XhoI sites of the pEF-MCS-GFP² plasmid. The full-length CRAF^{S257L} was cloned between NotI/XbaI sites of pEF-GFP²-MCS as well as the iDAb_{dm} LMO2.

All RAS and effectors are human sequences except RALGDS RA (mouse).

All the RAS BRET constructs DNA and protein sequences have been listed in the [supplementary file 1](#).

BRET2 titration curves and competition assays

The BRET experiment protocols have been adapted from previous studies (Lavoie *et al.*, 2013; Pflieger *et al.*, 2006). For all BRET experiments (titration curves and competition assays) 650,000 HEK293T were seeded in each well of a six well plates. After 24 hr at 37°C, cells were transfected with a total of 1.6 μ g of DNA mix, containing the donor + acceptor \pm competitor plasmids, using Lipofectamine 2000 transfection reagent (Thermo-Fisher). Cells were detached 24 hr later, washed with PBS and seeded in a white 96 well plate (clear bottom, PerkinElmer) in OptiMEM no phenol red medium complemented with 4% FBS. Cells were incubated for an additional 20–24 hr at 37°C before the BRET assay reading.

BRET2 measurements

BRET2 signal was determined immediately after addition of coelenterazine 400a substrate (10 μ M final) to cells (Cayman Chemicals), using an Envision instrument (2103 Multilabel Reader, PerkinElmer) with the BRET2 Dual Emission optical module (515 nm – 30 nm and 410 nm – 80 nm; PerkinElmer). Total GFP² fluorescence was detected with excitation and emission peaks set at 405 nm and 515 nm, respectively. Total RLuc8 luminescence was measured with the Luminescence 400–700 nm-wavelength filter.

The BRET signal or BRET ratio corresponds to the light emitted by the GFP² acceptor constructs (515 nm – 30 nm) upon addition of coelenterazine 400a divided by the light emitted by the RLuc8 donor constructs (410 nm – 80 nm). The background signal is subtracted from that BRET ratio using the donor-only negative control where only the RLuc8 plasmid is transfected into the cells. The normalized BRET ratio is the BRET ratio normalized to a negative control (DMSO, no competitor or iDAb control) during a competition assay. Total GFP² and RLuc8 signals were used to control the protein expression from each plasmid.

Western blot analysis

Cells were washed once with PBS and lysed in SDS-Tris buffer (1% SDS, 10 mM Tris-HCl pH 7.4) supplemented with protease inhibitors (Sigma) and phosphatase inhibitors (Thermo-Fisher). Cell lysates were sonicated with a Branson Sonifier and the protein concentrations determined by using the Pierce BCA protein assay kit (Thermo-Fisher). Equal amounts of protein (10 μ g) were resolved on 10 or 15% SDS-PAGE and subsequently transferred onto a PVDF membrane (GE). The membrane was blocked either with 10% non-fat milk (Sigma) or 10% BSA (Sigma) in TBS-0.1% Tween20 and incubated overnight with primary antibody at 4°C. After washing the membrane was incubated with HRP conjugated secondary antibody for 1 hr at room temperature (RT, 25°C). The membrane was washed with TBS-0.1% Tween and developed using Pierce ECL Western Blotting Substrate (Thermo-Fisher) and CL-XPosure films (Thermo-Fisher). Primary antibodies include anti-phospho-p44/22 MAPK (ERK1/2) (CST), anti-p44/42 MAPK (total ERK1/2) (CST), anti-phospho-MEK1/2 (CST), anti-MEK1/2 (CST), anti-phospho-AKT S473 (CST), anti-AKT (CST), anti-pan-RAS (Millipore), anti-GFP (Santa Cruz Biotechnologies), anti- β -actin (Sigma). Secondary antibodies include anti-CMYC HRP-linked (Novus Biologicals), anti-mouse IgG HRP-linked (CST) and anti-rabbit IgG HRP-linked (CST).

WaterLOGSY NMR

The waterLOGSY NMR method (Dalvit *et al.*, 2001) was used to measure RAS ligand interaction (Huang *et al.*, 2017). WaterLOGSY experiments were conducted at a ^1H frequency of 600 MHz using a Bruker Avance spectrometer equipped with a BBI probe. All experiments were conducted at RT, 25°C. 3 mm diameter NMR tubes with a sample volume of 200 μ L in all experiments. Solutions were buffered using an H_2O PBS buffer corrected to pH 7.4. The sample preparation is exemplified as follows; the compound (10 μ L of a 10 mM solution in $\text{DMSO-}d_6$) was added to an Eppendorf tube before sequential addition of the H_2O PBS buffer (163.6 μ L), D_2O (20 μ L), and protein (6.4 μ L, 311.8 μ M). The resulting solution was vortexed to mix and transferred to a 3 mm NMR tube prior to the NMR analysis.

For competition experiments using anti-RAS scFv, protein preparation for NMR was carried out in a similar manner; the compound (10 μ L of a 10 nM solution in $\text{DMSO-}d_6$) was added to an Eppendorf tube before sequential addition of the H_2O PBS buffer (146.4 μ L), D_2O (20 μ L), protein (6.4 μ L, 311.8 μ M) and anti-RAS scFv (17.2 μ L, 116.6 μ M). The resulting solution was vortexed to mix and transferred to a 3 mm NMR tube prior to the NMR analysis.

Negative controls (compound alone) were prepared in a similar manner, in order to obtain an end volume of 200 μ L.

Chemical synthesis procedures

All reactions involving moisture-sensitive reagents were carried out under a nitrogen atmosphere using standard vacuum line techniques and glassware that was flame-dried before use. Anhydrous solvents were prepared following the procedure outlined (Pangborn *et al.*, 1996). Water was purified by an Elix UV-10 system. All other solvents and reagents were used as supplied (analytical or HPLC grade) without prior purification. Brine refers to a sat. aq. solution of NaCl. *In vacuo* refers to the removal of solvent by the use of a rotary evaporator attached to a diaphragm pump.

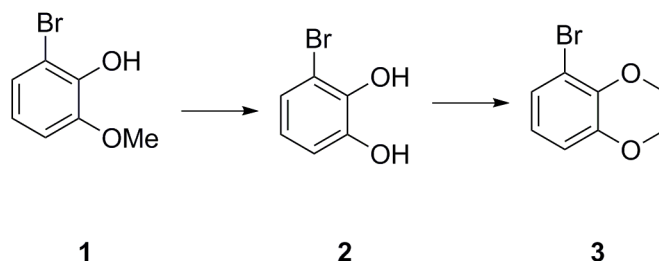
Thin layer chromatography was performed on normal phase Merck silical gel 60 F254 aluminum-supported thin layer chromatography sheets. Visualization of spots was either by absorption of ultra violet light (λ_{max} 254 nm), or by thermal development after staining with 1% aq. KMnO_4 . Flash column chromatography was performed on Kieselgel 60 silica in a glass column, under a positive pressure.

NMR spectra were recorded on Bruker Avance spectrometer (AVIII 600) in the deuterated solvent stated. The field was locked by external referencing to the relevant deuteron resonance. Chemical shifts (δ) are reported in parts per million (ppm). The multiplicity of each signal is indicated by: app. (apparent), s (singlet), br s (broad singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets) or m (multiplet). Coupling constants (J) are quoted in Hz and are reported to the nearest 0.1 Hz.

Low-resolution mass spectra were recorded on an Agilent 6120 spectrometer operating in positive or negative mode, from solutions of MeOH. Accurate mass measurements were run on either a Bruker MicroTOF internally calibrated with polyalanine, or a Micromass GCT instrument fitted with a

Scientific Glass Instruments BPX5 column (15 m x 0.25 mm) using amyl acetate as a lock mass, by the mass spectrometry department of the Chemistry Research Laboratory, University of Oxford, UK. m/z values are reported in Daltons.

5-bromo-2,3-dihydrobenzo[*b*][1,4]dioxine (3)



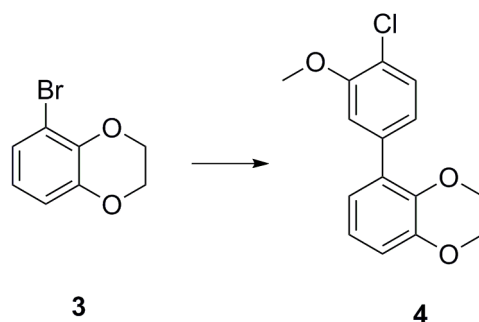
Chemical structure 1.

DOI: <https://doi.org/10.7554/eLife.37122.017>

A solution of 2-bromo-6-methoxyphenol **1** (2.50 g, 12.3 mmol) in CH_2Cl_2 (80 mL) was cooled to -78°C before dropwise addition of BBr_3 (1 M in heptane, 14.8 mL, 14.8 mmol). The resulting mixture was warmed to room temperature and stirred for 2 hr before being poured onto an ice/water (200 mL) and stirred for 30 min. The organic phase was separated, washed with water (100 mL) and brine (100 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo* to give the desired 3-bromobenzene-1,2-diol **two** as a brown oil (2.24 g, 11.9 mmol, 97%), which was used in the next step without further purification.

A solution of diol **2** (1.00 g, 5.35 mmol) in DMF (20 mL) was treated sequentially with K_2CO_3 (1.77 g, 12.8 mmol), and 1,2-dibromoethane (507 μL , 5.88 mmol) before being heated to 60°C for 18 hr. The reaction was then cooled down before addition of water and brine (1:1, 50 mL) and EtOAc (100 mL). The organic phase was washed further with water and brine (1:1, 4 \times 50 mL), dried (Na_2SO_4), filtered and concentrated *in vacuo* to give the crude material as a brown oil. Purification on silica gel (pentane/EtOAc, 4:1) afforded the desired 5-bromo-2,3-dihydrobenzo[*b*][1,4]dioxine **three** as a clear oil (1.11 g, 5.19 mmol, 97%).

5-(4-chloro-3-methoxyphenyl)–2,3-dihydrobenzo[*b*][1,4]dioxine (4)



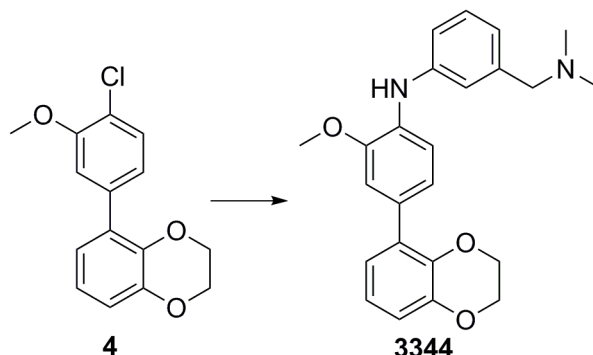
Chemical structure 2.

DOI: <https://doi.org/10.7554/eLife.37122.018>

Bromide **3** (600 mg, 2.79 mmol) was added to a vial before addition of 1,4-dioxane/water (5:1, 8 mL); the solution was degassed before sequential addition of K_2CO_3 (1.16 g, 8.37 mmol), 4-chloro-3-methoxyphenyl boronic acid (572 mg, 3.07 mmol), and $\text{Pd}(\text{dppf})\text{Cl}_2$ (100 mg, 0.140 mmol). The vial was sealed and the reaction heated to 100°C for 18 hr, cooled down and concentrated *in vacuo*. The residue was purified on silica gel (pentane/EtOAc, 9:1) to afford the desired 5-(4-chloro-3-methoxyphenyl)–2,3-dihydrobenzo[*b*][1,4]dioxine **four** as a clear oil (745 mg, 2.70 mmol, 97%). ^1H NMR (600 MHz, CDCl_3) δ 7.39 (1H, d, J 8.1 Hz), 7.11 (1H, s), 7.08 (1H, dd, J 8.2, 1.7 Hz), 6.91–6.89 (3H, m), 4.31–4.28 (4H, m), 3.94 (3H, s); ^{13}C NMR (150 MHz, CDCl_3) δ 154.5, 143.9, 140.6, 137.5,

130.0, 129.6, 122.6, 122.4, 121.4, 121.1, 117.0, 113.5, 64.4, 64.1, 56.2; m/z (ESI⁺) 277 ([M + H]⁺); HRMS (ESI⁺) [C₁₅H₁₄ClO₃] requires 277.0631, found 277.0591.

4-(2,3-dihydrobenzo[b][1,4]dioxin-5-yl)-N-(4-(dimethylamino)methyl)phenyl)-2-methoxyaniline (3344)



Chemical structure 3.

DOI: <https://doi.org/10.7554/eLife.37122.019>

Chloride **4** (75 mg, 0.272 mmol), Cs₂CO₃ (266 mg, 0.866 mmol), 3-((dimethylamino)methyl)aniline (61 mg, 0.408 mmol), XPhos (13 mg, 0.027 mmol) and Pd(OAc)₂ (3 mg, 0.014 mmol) were added sequentially to a vial and degassed with N₂ for 5 min. Degassed 1,4-dioxane (2 mL) was then added, the vial sealed and heated to 100°C for 18 hr. The mixture was cooled down, diluted with EtOAc (30 mL), and washed with a 50/50 solution of water and brine (2 × 30 mL). The organic phase was dried (Na₂SO₄) and concentrated *in vacuo*. Purification by column chromatography on silica gel (CH₂Cl₂/MeOH, 9:1) afforded the desired 4-(2,3-dihydrobenzo[b][1,4]dioxin-5-yl)-N-(3-((dimethylamino)methyl)phenyl)-2-methoxyaniline **3344** as a yellow oil (102 mg, 96%).

¹H NMR (400 MHz, MeOD) δ 7.26 (1H, d, *J* 8.3 Hz), 7.20 (1H, dd, *J* 7.6, 0.2 Hz), 7.12 (1H, d, *J* 2.0 Hz), 7.08–7.04 (2H, m), 7.00 (1H, dd, *J* 8.3, 2.0 Hz), 6.88 (1H, dd, *J* 7.6, 2 Hz), 6.83 (2H, *J* 7.8, 0.2 Hz), 6.78 (1H, dd, *J* 7.8, 2.0 Hz), 4.25–4.20 (4H, m), 3.87 (3H, s), 3.45 (2H, s), 2.27 (6H, s), NH was not observed; ¹³C NMR (125 MHz, CDCl₃) δ 150.2, 145.5, 145.3, 142.2, 139.4, 133.2, 132.4, 131.7, 130.3, 123.5, 123.0, 122.9, 122.0, 120.1, 118.3, 117.1, 116.7, 113.6, 65.8, 65.5, 65.1, 56.4, 45.3; m/z (ESI⁻) 38 ([M-H]⁻); HRMS (ESI⁻) [C₂₄H₂₅N₂O₃] requires 389.1865, found 389.1841.

¹H CPMG NMR experiments for compound Kd calculation

Typical experimental parameters for Carr-Purcell-Meiboom-Gill (CPMG) NMR spectroscopy were the following: total echo time, 40 ms; relaxation delay, 2 s; and number of transients, 264 (Abboud *et al.*, 2016). The PROJECT-CPMG sequence (90°x-[T-180°y-T-90°y-T-180°y-T]_n-acq) was applied. Water suppression was achieved by presaturation. Prior to Fourier transformation, the data were multiplied with an exponential function with 3 Hz line broadening. The CPMG experiments were conducted at a ¹H frequency of 700 MHz using a Bruker Avance with 5 mm inverse TCI 1 hr/13C/15N cryoprobe. All experiments were conducted at RT and lapsed 128 scans. 3 mm diameter NMR tubes with a sample volume of 200 μL were used in all experiments. Solutions were buffered using a D₂O PBS buffer corrected to pH 7.4. The sample preparation is exemplified as follows: for a 5 μM GST-KRAS^{G12V} sample: 55 μM of the 3344 compound (1.1 μL of a 10 mM solution in DMSO-*d*₆) was added to an Eppendorf before sequential addition of the D₂O PBS buffer (194.0 μL) and GST-KRAS^{G12V} (4.9 μL of a 205 μM solution, the protein is in an H₂O buffer for stability reason). The resulting solution was vortexed to be fully mixed and transferred to a 3 mm NMR tube before the run. Negative controls (compound alone, without the KRAS protein) were prepared in a similar manner, in order to obtain an end volume of 200 μL.

CPMG experiments were carried out at a fixed 3344 concentration (55 μM, optimal concentration for these CPMG NMR experiments) and a variable GST-KRAS^{G12V} concentration. The amount of GST-KRAS^{G12V} was increased from 0 μM until the signals of the compound completely disappear in the proton NMR at 20 μM. Seven measurements were done in total with 0 μM, 2.5 μM, 5 μM, 7.5

μM , 10 μM , 15 μM and 20 μM of GST-KRAS^{G12V}. The integrations of the protons acquired were all compared to the compound alone (with no KRAS) in order to obtain a percentage decrease for each concentration of KRAS. Three different proton signals were used and a mean was calculated for each run. KRAS concentration experiments were run in triplicate and a mean was also calculated for each concentration. Concentration and percentage of decrease were plotted and Kd fitting was run on the generated curve using Origin 2017 software with the following function: $A*(1/(2*C))^*((B+x+C)-\sqrt{((B+x+C)^2-(4*x*C))})$ where A is the maximum % of inhibition (i.e. 100), B is the Kd, C is the concentration of compound and x the concentration of KRAS protein necessary to reach 100% of signal reduction of the compound.

Recombinant protein expression for crystallography and NMR: KRAS^{G12V}, KRAS^{Q61H} and scFv

KRAS^{G12V} cDNA was cloned into the pGEX vector in-frame with an N-terminal Glutathione-S transferase (GST) tag. pGEX-GST-KRAS^{G12V} was transformed into *E.coli* BL21 (DE3) cells. Bacterial cells were cultured at 37°C to an OD₆₀₀ of 0.5 and induced with IPTG (isopropyl 1-thio-beta-D-galactopyranoside, final concentration 0.1 mM) at 16°C overnight. The bacteria cultures were harvested by centrifugation and the cell pellets re-suspended in 50 mM Tris-HCl pH8.0, 140 mM NaCl, 1 mM mercaptoethanol supplemented with complete protease inhibitor (Roche). The GST-fusion proteins were purified by glutathione-sepharose column chromatography (GE Healthcare) and eluted with 50 mM Tris-HCl pH8.0, 10 mM reduced glutathione, 1 mM mercaptoethanol, 5 mM MgCl₂.

KRAS^{Q61H} cDNA was cloned into the pRK-172 vector in-frame with an N-terminal 6xHis-tag and TEV protease recognition site. The plasmid containing KRAS^{Q61H} sequence was transformed into *E. coli* B834(DE3) pLysS cells, which were grown in 25 mL LB medium with 50 $\mu\text{g}/\text{mL}$ Carbenicillin and 34 $\mu\text{g}/\text{mL}$ Chloramphenicol for 16 hr, prior to inoculation of 1L LB medium. Protein expression was induced at OD₆₀₀ = 0.6 by addition of IPTG to a final concentration of 0.5 mM and cells grown overnight at 16°C. Bacteria were harvested by centrifugation and sonicated in 50 mM Tris-HCl, pH 7.5, 500 mM NaCl, 5 mM MgCl₂ and 10 mM imidazole and EDTA-free protease inhibitor cocktail (Roche Diagnostics). Proteins were purified using nickel agarose beads (Invitrogen) and bound proteins were eluted batch-wise in 50 mM Tris-HCl, pH 7.5, 500 mM NaCl, 5 mM MgCl₂ and 300 mM imidazole. RAS protein samples were concentrated using Vivapore 10/20 mL concentrator (7.5 kDa molecular weight cut-off; Sartorius Vivapore) to a final volume of approximately 1 mL. Nucleotide exchange for crystallographic samples was carried out following published procedures (Herrmann et al., 1996). RAS proteins were further purified by gel filtration on a HiLoad Superdex 75 10/300 GL column (GE Healthcare) in a buffer containing 20 mM HEPES pH 8.0, 150 mM NaCl, 5 mM MgCl₂ and 1 mM DTT at a flow rate of 0.5 mL/min. Fractions corresponding to the protein were pooled and concentrated to 45–75 mg/mL for crystallization trials. Protein concentration was determined by extinction coefficient ($\epsilon_{280} = 12045 \text{ L/mol/cm}$). Protein purity was analyzed by SDS-PAGE stained with Coomassie Brilliant Blue. scFv recombinant protein was expressed and purified as described elsewhere (Tanaka et al., 2007).

Crystal structure and 3344 soaking

For X-ray diffraction experiments, KRAS^{Q61H}-GppNHp crystals were grown by vapour diffusion at 4°C by mixing 1.5 + 1.5 volumes of KRAS solution at a concentration of 75 mg/mL KRAS^{Q61H}, with 8–15% w/v Polyethylene Glycol 3350 and 0.2 M lithium citrate pH 5.5. The resulting crystals are termed crystal form I hereafter. Prior to X-ray data collection, crystals were cryo-protected by addition of 20% glycerol to the crystallization buffer and flash-cooled in liquid nitrogen. 3344 was initially dissolved at 200 mM in 100% DMSO and sequentially mixed in a ratio of 1:1 with crystallization buffer (8–15% w/v Polyethylene Glycol 3350, 0.2 M lithium citrate 7.0 and 20 mM Tris-HCl pH 7.0) to give a final concentration of compound of 50 mM and 25% DMSO in a 5 μL drop. Soaked crystals were flash-cooled in liquid nitrogen prior to data collection using the final DMSO concentration on the soaking drop as cryo-protectant. X-ray diffraction data were collected at beamline ID30A-1 (Bowler et al., 2015; Bowler et al., 2016; Nurizzo et al., 2016; Svensson et al., 2015) at The European Synchrotron Radiation Facility (ESRF, Grenoble, France). The structure of KRAS^{Q61H} GppNHp-3344 was solved by molecular replacement using a KRAS169^{Q61H} GPPNHP-Abd-2, (PDB ID 5OCO) as a search model within the program Phaser (McCoy, 2007; McCoy et al., 2007). Structures were

manually adjusted using COOT (*Emsley et al., 2010*) and refined using REFMAC (*Murshudov et al., 1997*). Crystal Form I (KRAS^{Q61H}) has six KRAS molecules in the asymmetric unit, assembled as a hexamer. Electron density maps averaged with six-fold non-crystallographic symmetry (NCS) were used to improve the definition of the bound compounds. Refinements were also performed with the six fold NCS applied. The refined models were validated using PROCHECK (*Laskowski et al., 1993a*), MolProbity (*Chen et al., 2010*) and Phenix software packages (*Adams et al., 2010*; *Laskowski et al., 1993b*). Figures were created using PyMOL (Schrodinger). Data collection and refinement statistics are summarized in *Table 1*.

Quantification and statistical analysis

All quantifications were performed using ImageJ or Prism 7.0 c (GraphPad Software), BRET titration curves and statistical analysis were performed using Prism 7.0 c (GraphPad Software). Data are typically presented as mean \pm SD or SEM as specified in the figure legends. Statistical analyses were performed with a one-way ANOVA followed by Dunnett's post-hoc tests or Sidak's post-hoc tests unless otherwise indicated in the figure legends. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Data and software availability

Structure files and coordinates have been deposited to PDB under this accession number: 6F76.

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Additional information

Competing interests

Abimael Cruz-Migoni: Employed by Immunocore; no other competing financial interests to declare. Angela Russell: Founder of OxStem; no other competing financial interests to declare. The other authors declare that no competing interests exist.

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Author contributions

Nicolas Bery, Conceptualization, Methodology, Formal analysis, Investigation, Writing—original draft, Writing—review and editing; Abimael Cruz-Migoni, Formal analysis, Investigation, Writing—original draft; Carole JR Bataille, Hanna Tulmin, Formal analysis, Writing—original draft; Camilo E Quevedo, Formal analysis, Writing—original draft, Writing—review and editing; Ami Miller,

Investigation, Writing—review and editing; Angela Russell, Formal analysis, Supervision; Simon EV Phillips, Stephen B Carr, Formal analysis, Supervision, Writing—original draft, Writing—review and editing; Terence H Rabbitts, Conceptualization, Formal analysis, Supervision, Funding acquisition, Investigation, Methodology, Writing—original draft, Project administration, Writing—review and editing

Author ORCIDs

Nicolas Bery  <http://orcid.org/0000-0002-2643-3897>

Terence H Rabbitts  <http://orcid.org/0000-0002-4982-2609>

Decision letter and Author response

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Author response <https://doi.org/10.7554/eLife.37122.026>

Additional files

Supplementary files

- Supplementary file 1. DNA and protein sequences of BRET biosensors constructs. The list of the DNA and protein sequences from the different RAS BRET biosensor constructs used in this study.

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- Transparent reporting form

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Data availability

Diffraction data have been deposited in PDB ID 6F76.

The following dataset was generated:

Author(s)	Year	Dataset title	Dataset URL	Database, license, and accessibility information
Bery N, Cruz-Migoni A, Quevedo CE, Phillips SVE, Carr S, Rabbitts TH	2018	Antibody derived (Abd-8) small molecule binding to KRAS.	http://www.rcsb.org/pdb/results/results.do?tabtoshow=Unreleased&qrid=60BEFAFO	Publicly available at the RCSB Protein Data Bank (accession no. 6F76)

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Figures and figure supplements

BRET-based RAS biosensors that show a novel small molecule is an inhibitor of RAS-effector protein-protein interactions

Nicolas Bery *et al*

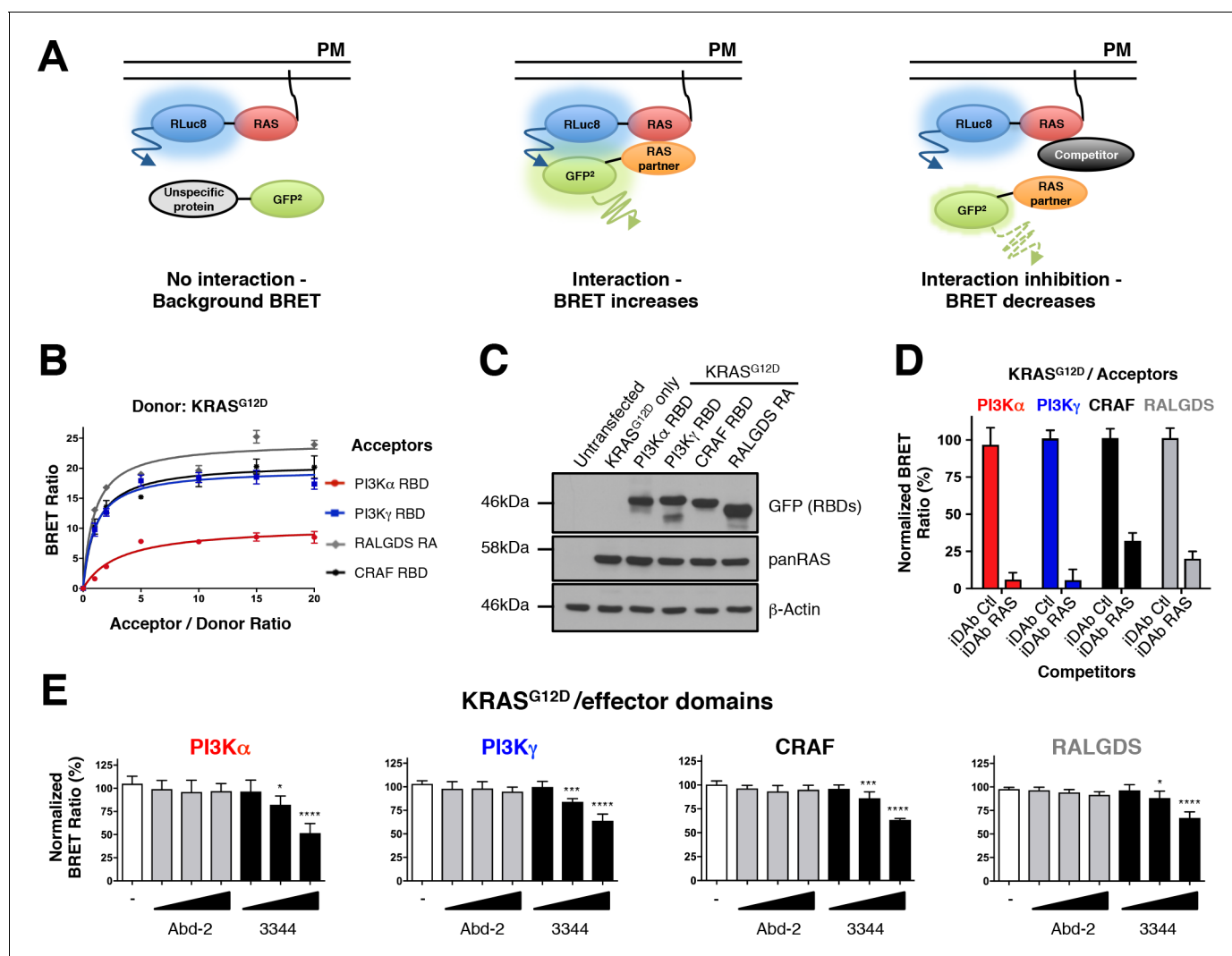


Figure 1. RAS-effector BRET biosensors and interference of KRAS-effector interactions by a RAS-binding compound. An outline of the BRET2-based RAS biosensor system is shown in **A**. RAS bound to the plasma membrane (PM) is fused at its amino terminal end to the RLuc8 moiety (donor). When a protein fused to the GFP² moiety (acceptor) does not bind to RAS, it only produces a background BRET signal. However, when an acceptor binds to RAS, it induces a BRET signal, if the luciferase and GFP domains are within 100 Å. The BRET signal can be decreased by addition of a competitor (either by a macrodrug or a small molecule inhibitor). The interaction titration of full-length KRAS^{G12D}-CAAX (for simplicity, the CAAX motif is omitted in all the RAS constructs described hereafter) with the four effector acceptor proteins and the effect on intracellular protein levels are shown in **B** and **C**. Competition assays show the specificity of the RAS biosensors in **D** (iDAb) and **E** (RAS-binding compounds). In **D**, the non-relevant anti-LMO2 iDAb (called hereafter iDAb control, Ctl) serves as a negative control and anti-RAS iDAb (herein named iDAb RAS) serves as a positive control. In **E**, 3344 (black bars) decreases KRAS^{G12D}/effector domain interactions in a dose-dependent manner showing its broad range of inhibition. Cells were treated with 5, 10 and 20 μM of 3344 (black bars), Abd-2 (grey bars) or DMSO alone (white bars) as the negative control. Statistical analysis was performed with a one-way ANOVA followed by Dunnett's post-hoc tests (*p<0.05, ***p<0.001, ****p<0.0001). Each experiment was repeated three (**B**, **D**) or four times (**E**). Where error bars are presented, these correspond to mean values ± SD of biological repeats (**B**, **D**–**E**). See also **Figure 1—figure supplement 1**, **Figure 1—figure supplement 2**, **Figure 1—figure supplement 3** and **supplementary file 1**.
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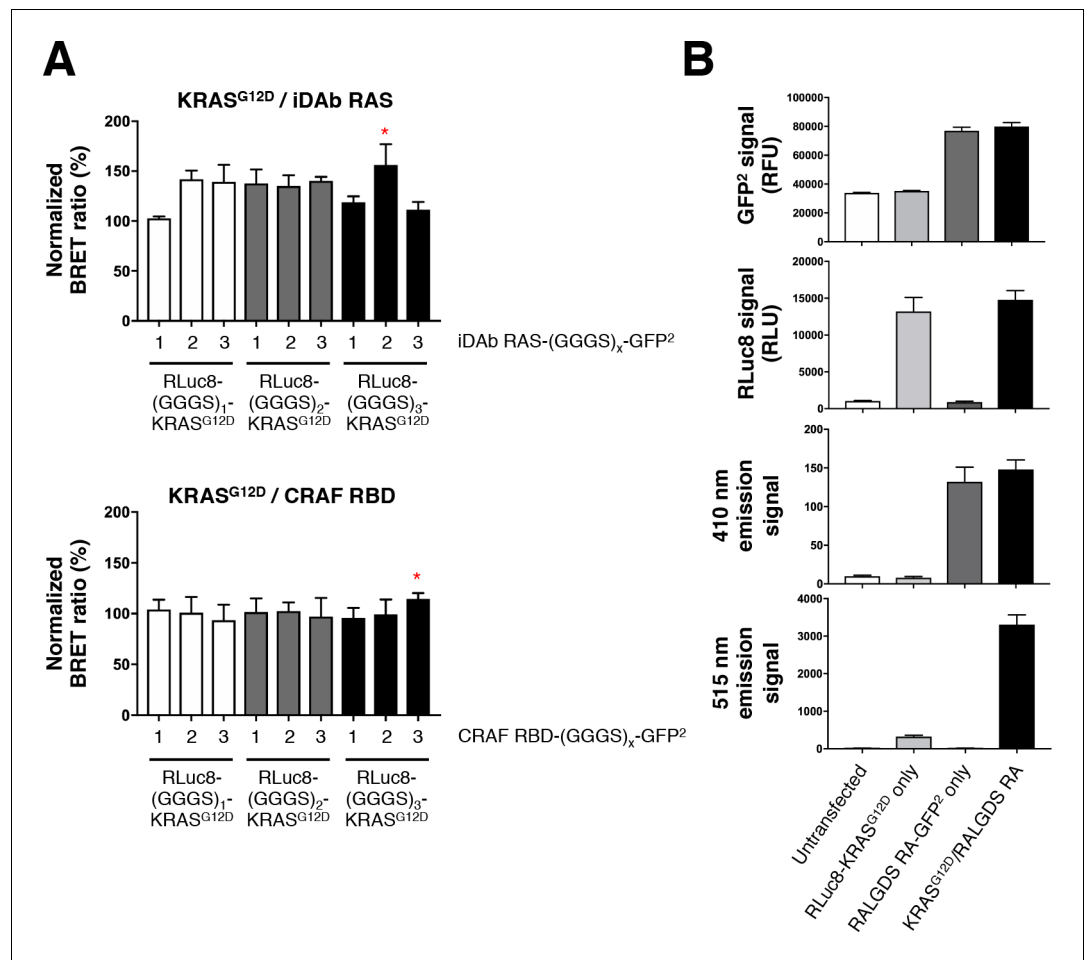


Figure 1—figure supplement 1. Optimization of the RAS biosensors. (A) Optimization of the donor and acceptor linker length. Top panel shows KRAS^{G12D}/iDAb RAS optimization and the bottom panel shows KRAS^{G12D}/CRAF RBD optimization. The red stars indicate the linker length chosen for the study: all RLuc8-RAS constructs bear a (GGGS)₃ linker, the iDAb-GFP² fusions a (GGGS)₂ linker and all effectors fused to the GFP² moiety a (GGGS)₃ linker. (B) Background analysis with total GFP² and RLuc8 levels, emission signal at 410 nm and at 515 nm upon coelenterazine 400a addition from untransfected cells, RLuc8-KRAS^{G12D} transfected cells only, RALGDS RA-GFP² transfected cells only and cells transfected with the BRET pair KRAS^{G12D}/RALGDS RA. Each experiment was repeated twice (A–B). Where error bars are presented, they correspond to mean values ± SEM of biological repeats.

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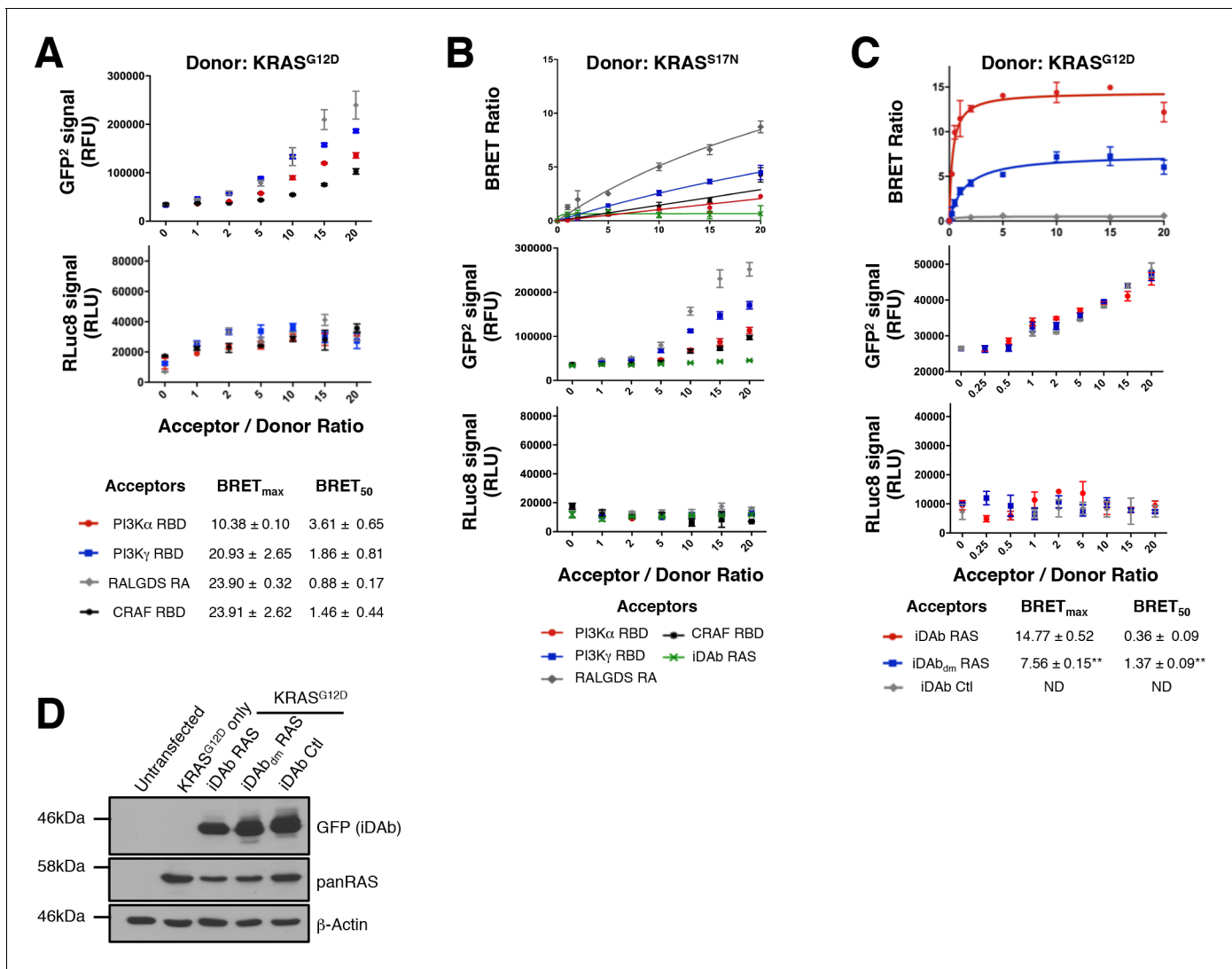


Figure 1—figure supplement 2. Validation of the RAS biosensors with the anti-iDAb RAS. (A) Total GFP² and RLuc8 levels from the BRET titration curves in **Figure 1B**. (B) Representative BRET titration curves of KRAS^{S17N} and RAS binders (RBDs and iDAb RAS) with total GFP² and RLuc8 controls. (C) BRET titration curves of KRAS^{G12D} and iDAb with total GFP² and RLuc8 controls. Statistical analyses were performed using an unpaired Student's test (**p<0.01). (D) Western blots for assessment of the expression levels of each KRAS^{G12D}-iDAb BRET pair. Each experiment was repeated three times (A–C). Where error bars are presented, they correspond to mean values \pm SD of biological repeats.

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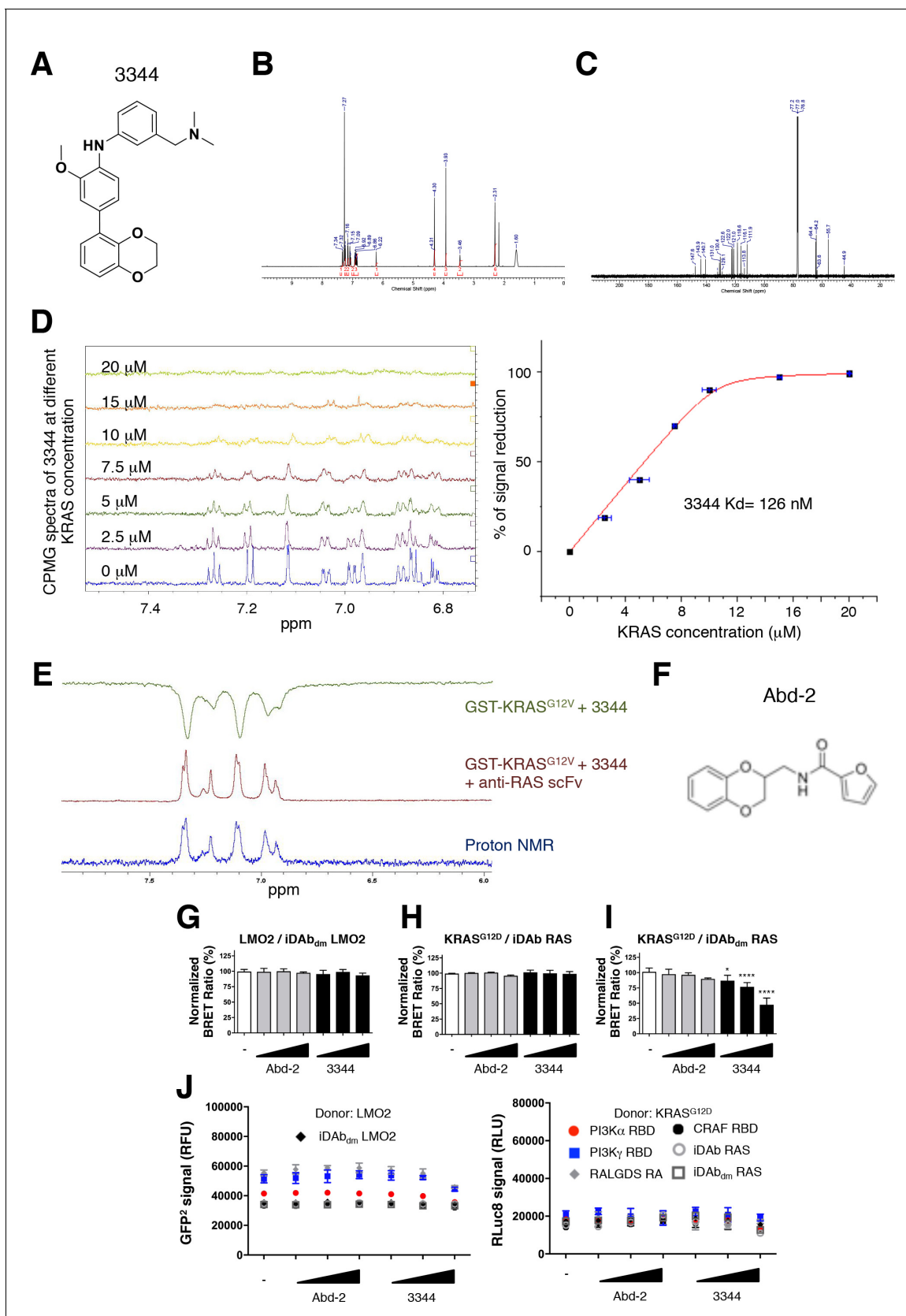


Figure 1—figure supplement 3. 3344 inhibits RAS-RBD interactions. (A) Chemical structure of 3344. (B) ¹H NMR and (C) ¹³C NMR spectra of 3344 were recorded on a Bruker Avance spectrometer (600 MHz) at room temperature in a solution of the deuterated solvent (CDCl₃). The field was locked by Figure 1—figure supplement 3 continued on next page

Figure 1—figure supplement 3 continued

external referencing to the relevant deuterium resonance. Chemical shifts are reported in parts per million (ppm). (D) NMR Carr-Purcell-Meiboom-Gill (CPMG) evaluation of 3344 Kd. Dose-dependent CPMG spectra of 3344 (at a fixed concentration of 55 μM) were recorded on a Bruker Avance spectrometer (700 MHz) at room temperature against an array of concentration of GST-KRAS^{G12V} (0 to 20 μM , left hand panel). The amount of protein was increased from 0 μM until the signals of the compound completely disappear in the proton NMR (here 20 μM). The integrations of the proton acquired were all compared to the compound alone (0 μM of protein) in order to obtain a percentage of decrease for each concentration of GST-KRAS^{G12V}. Concentration and percentage of decrease were plotted and Kd fitting was run on the generated binding curve using Origin[®] software (right hand panel, see Materials and methods for details). (E) WaterLOGSY spectra of 3344 interacting with GST-KRAS^{G12V}-GppNHp. The proton NMR of 3344 is the lower spectrum (blue), the spectrum of 3344 with KRAS is shown in the top (green) and the inhibitory effect of added anti-RAS scFv on 3344 binding to KRAS is shown in the middle spectrum (red). (F) Chemical structure of Abd-2. (G–I) 3344 decreases KRAS^{G12D}-iDAb_{dm} RAS interaction in a dose-dependent manner and not with iDAb RAS or with a negative BRET-biosensor LMO2-iDAb_{dm} LMO2. Statistical analyses were performed using a one-way ANOVA followed by Dunnett's post-tests (* $p < 0.05$, **** $p < 0.0001$). (J) Total GFP² and RLuc8 levels from the BRET competition assay shown in G–I and **Figure 1E**. Each experiment was repeated four times (G–I). Where error bars are presented, they correspond to mean values \pm SD of biological repeats.

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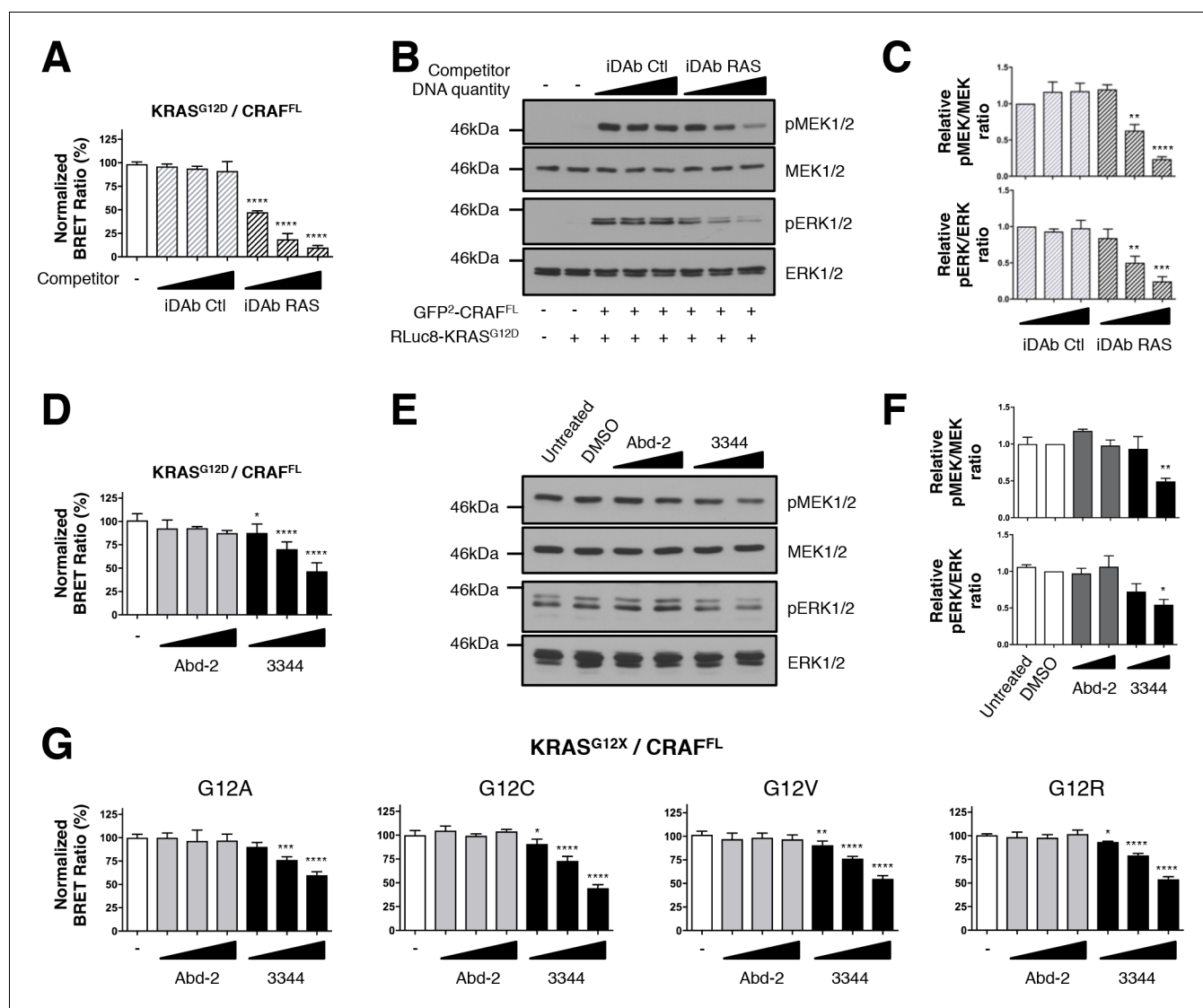


Figure 2. BRET biosensors of KRAS^{G12} mutants and full-length CRAF are inhibited by compound 3344. A biosensor for the full-length CRAF^{S257L} (CRAF^{FL}) protein was made and tested for interaction with mutants of KRAS glycine 12. For **A** and **B**, the plasmids expressing BRET pair KRAS^{G12D}/CRAF^{FL} was transfected into HEK293T cells and competed with iDAb expression as indicated; the BRET ratios are shown in **A** and western blot data in **B**. The iDAb RAS inhibition of phosphorylation of ERK and MEK signals are quantified in **C**. The β -actin loading control, iDABs and BRET pair expression controls are shown in **Figure 2—figure supplement 1**. In **D**, the BRET ratio of KRAS^{G12D}/CRAF^{FL} interaction was measured in the presence of an increasing dose of compound 3344. This induces a dose-dependent decrease of MEK and ERK kinase phosphorylation (**E**) after cells expressing the KRAS^{G12D}/CRAF^{FL} biosensor pair were treated 20 hr with DMSO, 10 and 20 μ M of Abd-2 and 3344 compounds or not treated (untreated lane). The β -actin loading control and BRET pair expression controls are shown in **Figure 2—figure supplement 1**. Quantification of the relative levels of pMEK1/2 and pERK1/2, normalized to total MEK1/2 and ERK1/2 respectively, are shown in **F**. The RAS biosensor toolkit includes KRAS G12A, G12C, G12V and G12R, in addition to KRAS G12D. In **G**, each was expressed with CRAF^{FL} and BRET ratios determined at 0, 5, 10 and 20 μ M Abd-2 or 3344. Statistical analyses in **C** were performed using a one-way ANOVA followed by Sidak's post-hoc tests and in **A**, **D**, **F** and **G** using a one-way ANOVA followed by Dunnett's post-tests (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$). Each experiment was repeated twice (**E–F**), three times (**B–D**), four times (**A**) or five times (**G**). Where error bars are presented, they correspond to mean values \pm SD of biological repeats (**A**, **D**, **G**) or correspond to mean \pm SEM of biological repeats (**C**, **F**). See also **Figure 2—figure supplement 1**.

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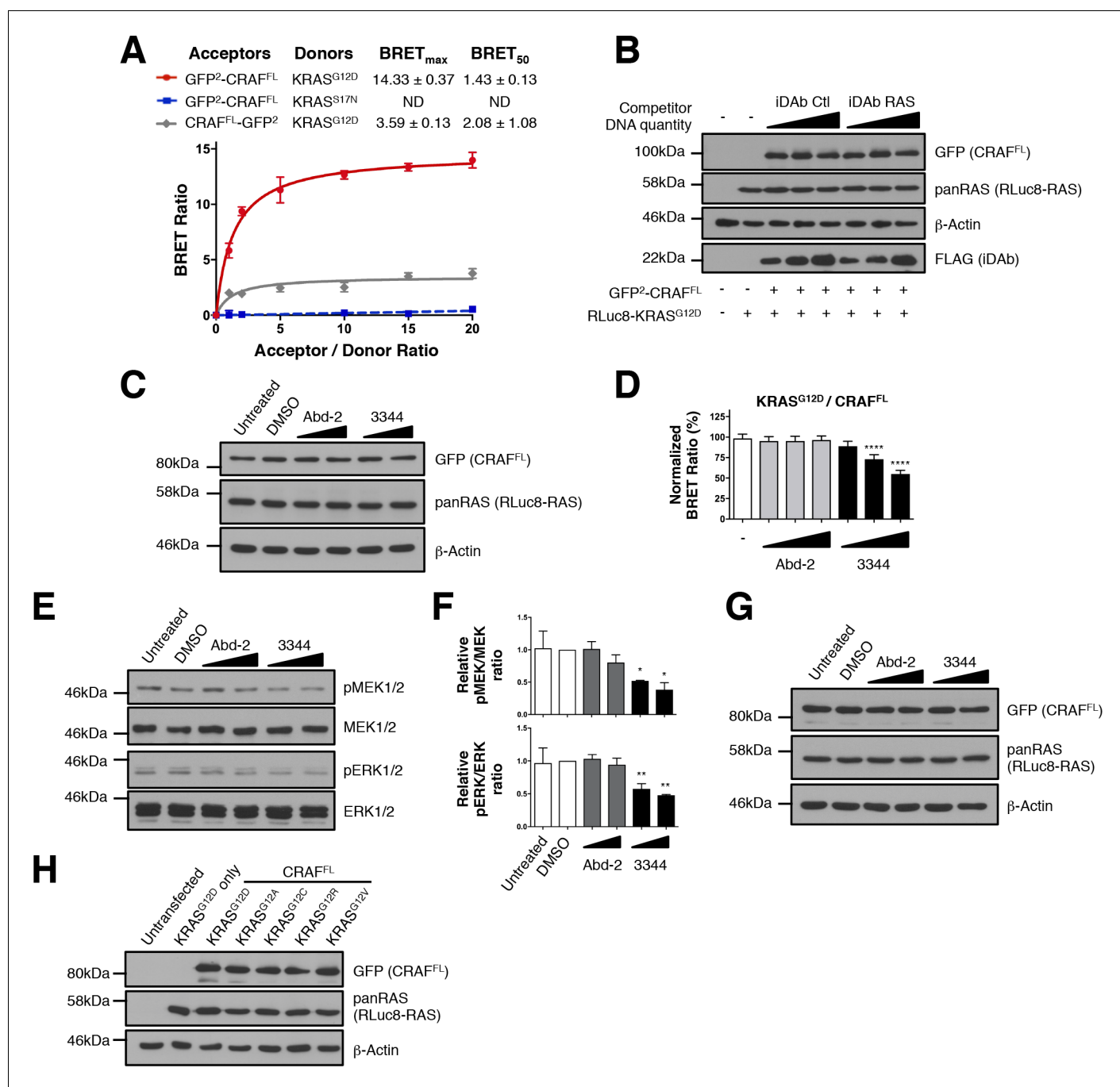


Figure 2—figure supplement 1. Interactions of KRAS^{G12X} mutants and full-length CRAF are inhibited by 3344. (A) BRET titration curves of KRAS mutants with full-length CRAF^{S257L} (CRAF^{FL}). KRAS^{G12D} interacts with GFP²-CRAF^{FL} while it gives a low BRET ratio with CRAF^{FL}-GFP². The dominant negative KRAS^{S17N} does not interact with GFP²-CRAF^{FL} showing the accuracy and optimization of this biosensor. (B) Controls from Figure 2B. The expression level of the BRET pair was assessed by western blot with the GFP (for CRAF^{FL}) and pan-RAS (for RLuc8-KRAS^{G12D}) antibodies. iDAb expression was revealed using anti-FLAG antibody; anti-β-actin binding was used as the loading control. (C) Controls from Figure 2E. The expression level of the BRET pair was assessed with the GFP (for CRAF^{FL}) and pan-RAS (for RLuc8-KRAS^{G12D}) antibodies, anti-β-actin binding was used as the loading control. (D–F) Short-term incubation of the compounds (3 hr) on cells transfected with the KRAS^{G12D}/CRAF^{FL} biosensor. The BRET ratio was measured in the presence of an increasing dose of compound 3344 (D). This induces a dose-dependent decrease of MEK and ERK kinase phosphorylation (E) after cells expressing the KRAS^{G12D}/CRAF^{FL} biosensor pair were treated 3 hr with DMSO, 10 and 20 μM of Abd-2 and 3344 compounds or not treated (untreated lane). Quantification of the relative levels of pMEK1/2 and pERK1/2, normalized to total MEK1/2 and ERK1/2 respectively, are shown in panel F. (G) Controls from panel E. (H) Controls from Figure 2G. The expression level of each BRET pair was assessed with the GFP (for CRAF^{FL}) and pan-RAS (for RLuc8-KRAS^{G12X}) antibodies. One-way ANOVA followed by Dunnett’s post-hoc tests were used to determine Figure 2—figure supplement 1 continued on next page

Figure 2—figure supplement 1 continued

the statistical significance of BRET, pERK and pMEK modulations induced by the compounds (* $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$). Each experiment was repeated twice (A, E–F) or three times (D). Where error bars are presented, they correspond to mean values \pm SD of biological repeats (A, D) or correspond to mean \pm SEM of biological repeats (F).

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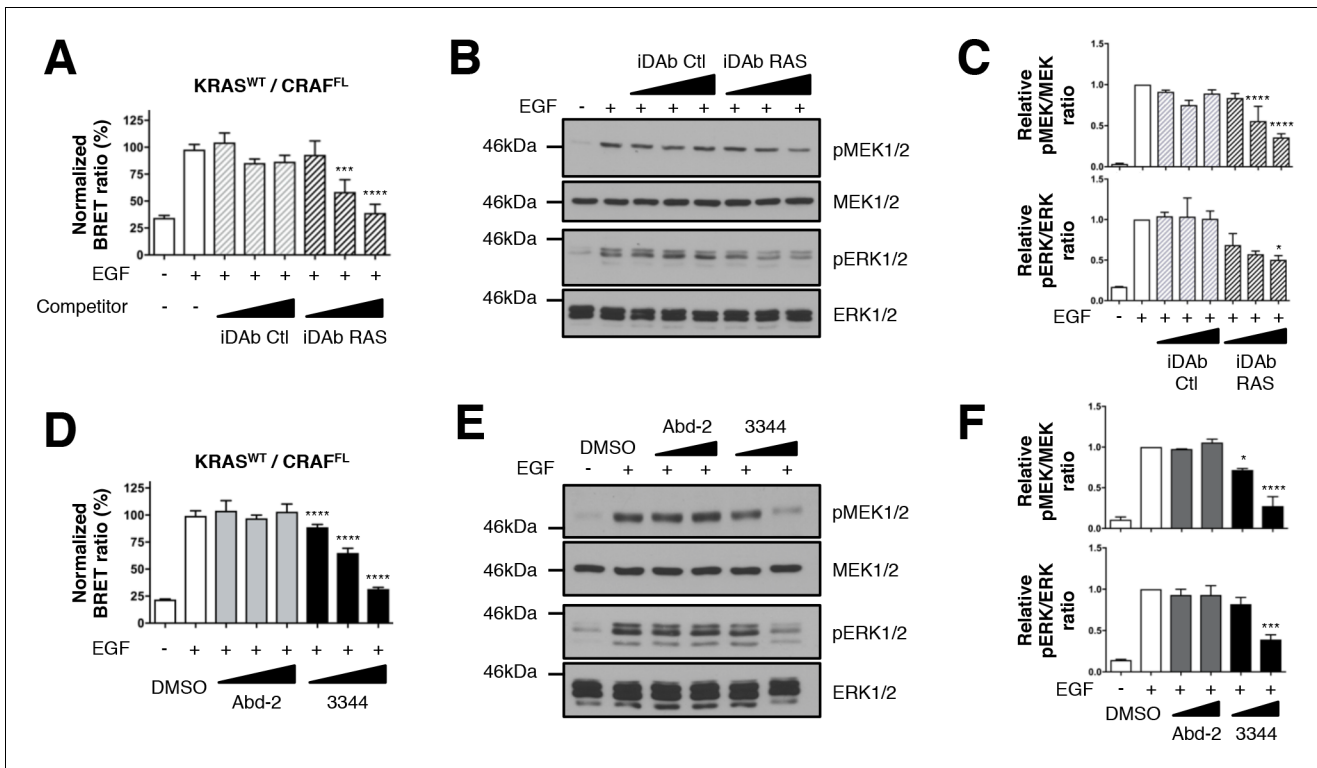


Figure 3. Wild-type KRAS and CRAF biosensor interaction-induced signaling is impaired by 3344. The BRET KRAS^{WT}/CRAF^{FL} pair was tested for interaction after EGF stimulation of HEK293T cells in presence of competitors. In **A**, cells were transfected with plasmids to express the KRAS^{WT} biosensor with or without iDABs and stimulated by EGF (50 ng/mL). iDAB RAS shows an inhibition of KRAS^{WT}/CRAF^{FL} interaction after EGF treatment in a dose-dependent manner. **B** is a western blot of the transfected cells from panel **A** showing the effect of the iDABs on EGF-stimulated RAS-RAF-MEK-ERK signaling pathway (pMEK and pERK signals are quantified in **C**). β -actin loading control, iDABs and BRET pair expression controls are shown in **Figure 3—figure supplement 1**. The effect on BRET2 signal of compounds Abd-2 (grey bars) and 3344 (black bars) on KRAS^{WT}/CRAF^{FL} interaction after EGF treatment in a BRET competition experiment is shown in panel **D**. In panel **E**, HEK293T cells were transfected as in **D** with the plasmids expressing the BRET pair KRAS^{WT}/CRAF^{FL} for 24 hr and serum starved 20 hr in the presence of DMSO, 10 and 20 μ M of Abd-2 and 3344 compounds. Cells were treated 5 min with EGF (50 ng/mL), lysed and analyzed by western blot. The expression level of the BRET protein pair is shown in **Figure 3—figure supplement 1** as well as the loading control β -actin for the western blot. The western blot data are quantified in panel **F**. One-way ANOVA followed by Dunnett’s post-hoc tests were used to determine the statistical significance of BRET, pERK and pMEK modulations induced by the compound or the iDAB (* p <0.05, *** p <0.001, **** p <0.0001). Each experiment was repeated twice (**B–C**) or three times (**A, D–F**). Where error bars are presented, they correspond to mean values \pm SD of biological repeats (**A, D**) or correspond to mean \pm SEM of biological repeats (**C, F**). See also **Figure 3—figure supplement 1**.

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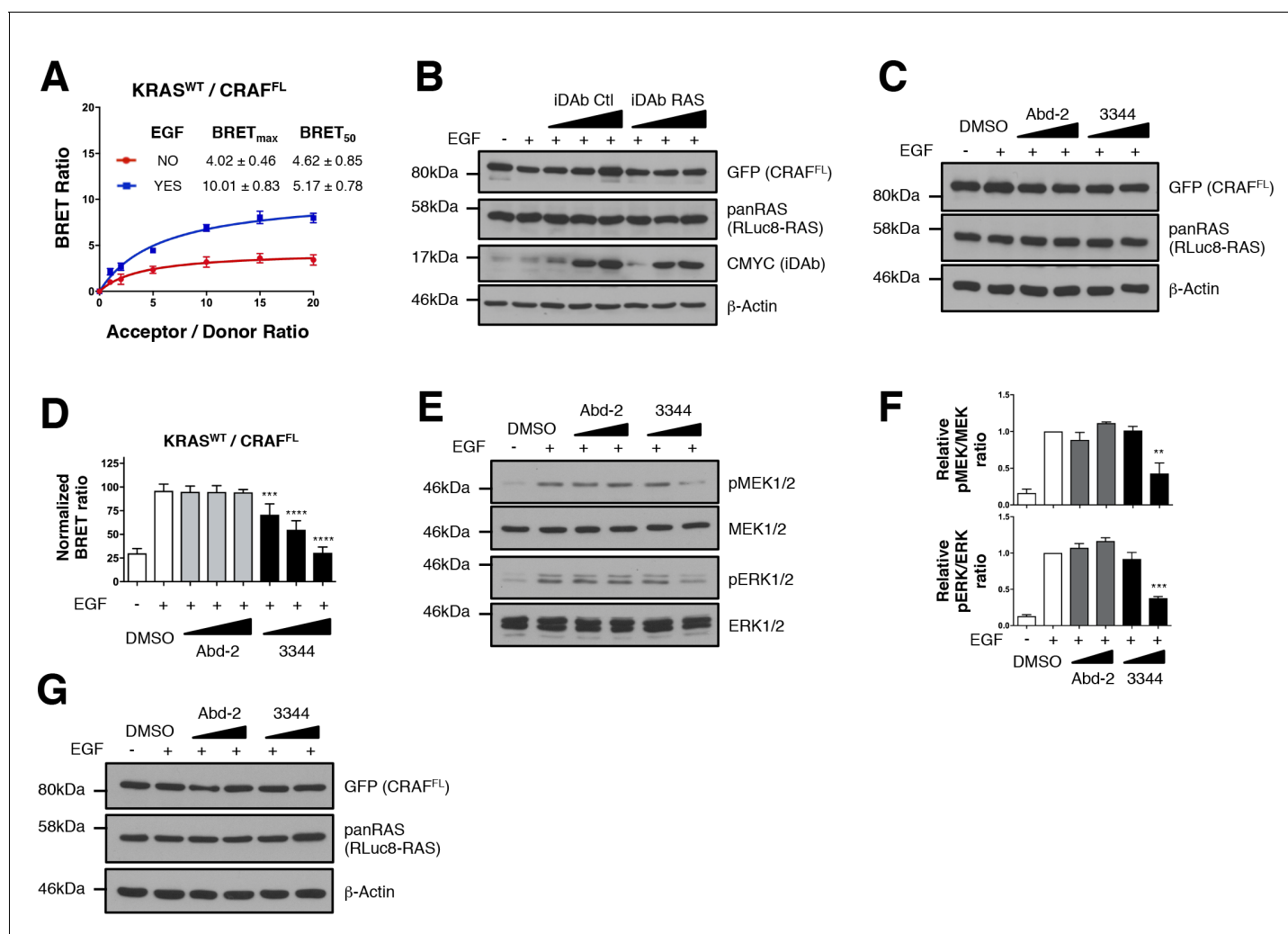


Figure 3—figure supplement 1. 3344 inhibits KRAS^{WT}/CRAF^{FL} interaction induced by EGF treatment. (A) BRET titration curves of KRAS^{WT} with CRAF^{FL}. After EGF stimulation (50 ng/mL), KRAS^{WT} contacts CRAF^{FL} as indicated by an increase of the BRET_{max} value. (B) Controls from **Figure 3B**. The expression level of the BRET pair was assessed with the GFP (for CRAF^{FL}) and pan-RAS (for RLuc8-KRAS^{WT}) antibodies. iDAb expression is revealed by the CMYC tag antibody; anti-β-actin binding was used as the loading control. (C) Controls from **Figure 3E**. The expression level of the BRET pair was assessed with the GFP (for CRAF^{FL}) and pan-RAS (for RLuc8-KRAS^{WT}) antibodies. Anti-β-actin binding was used as control. Panel D shows the short-term effect on BRET2 signal of compounds Abd-2 (grey bars) and 3344 (black bars) on KRAS^{WT}/CRAF^{FL} interaction after EGF treatment in a BRET competition experiment (3 hr incubation of the compounds). In panel E, HEK293T cells were transfected with the plasmids expressing the BRET pair KRAS^{WT}/CRAF^{FL} for 24 hr, serum starved 24 hr and then incubated for 3 hr with DMSO, 10 and 20 μM of Abd-2 and 3344 compounds. Cells were treated 5 min with EGF (50 ng/mL), lysed and analysed by western blot. Quantification of panel E is shown in panel F. (G) Controls from panel E. One-way ANOVA followed by Dunnett's post-hoc tests were used to determine the statistical significance of BRET, pERK and pMEK modulations induced by the compounds (**p<0.01, ***p<0.001, ****p<0.0001). Each experiment was repeated twice (A, E–F) or three times (D). Where error bars are presented, they correspond to mean values ± SD of biological repeats (A, D) or correspond to mean ± SEM of biological repeats (F).

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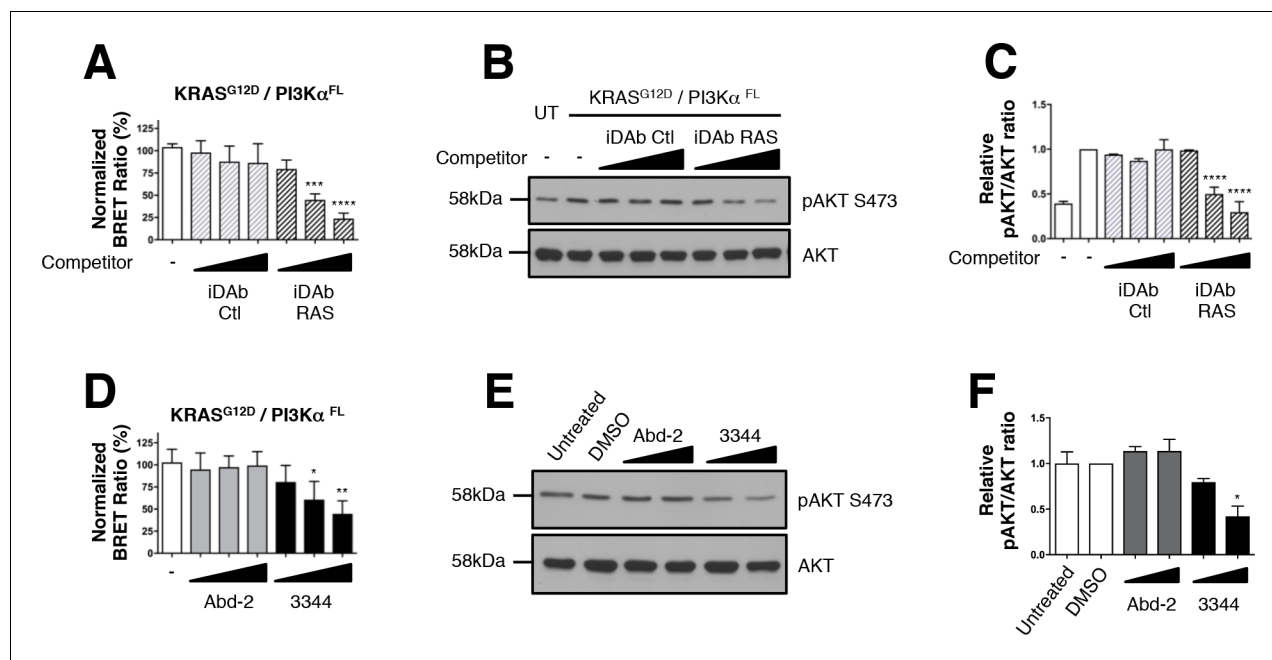


Figure 4. Interaction between mutant KRAS and full-length PI3K α BRET pair interaction is impeded by 3344. The BRET signal produced from the interaction of the KRAS^{G12D} and full-length PI3K α (PI3K α ^{FL}) was obtained by transfecting HEK293T cells with plasmids encoding this BRET pair. In **A**, cells were co-transfected with the biosensor and increasing levels of competitor plasmids encoding iDABs RAS (black striped bars) or iDAB control (grey striped bars) or biosensor alone (white bar). iDAB RAS impedes KRAS^{G12D}/PI3K α ^{FL} interaction and this inhibition causes a decrease of pAKT at serine 473 as shown by western blot in **B** and its quantification in **C**. UT is for untransfected cells. In **D**, HEK293T cells transfected with the BRET biosensor KRAS^{G12D}/PI3K α ^{FL} were treated for 20 hr with DMSO (white bar), 5, 10 and 20 μ M of Abd-2 (grey bars) and 3344 (black bars) compounds and the BRET signal of the biosensor was assessed. In panel **E**, the cells were transfected and treated as in **D** but with 10 and 20 μ M of Abd-2 and 3344 compounds. 20 hr after the treatment, cells were lysed and analysed by western blot using anti-pAKT (Ser 473) or anti-pan-AKT antibody. The signal in the western blot is quantitated in **F**. Related controls are shown on **Figure 4—figure supplement 1**. One-way ANOVA followed by Dunnett's post-hoc tests were used to determine the statistical significance of BRET and pAKT modulations induced by the compound or the iDAB (* p <0.05, ** p <0.01, *** p <0.001, **** p <0.0001). Each experiment was repeated twice (**E–F**) or three times (**A–D**). Where error bars are presented, they correspond to mean values \pm SD of biological repeats (**A, D**) or correspond to mean \pm SEM of biological repeats (**C, F**). See also **Figure 4—figure supplement 1**.

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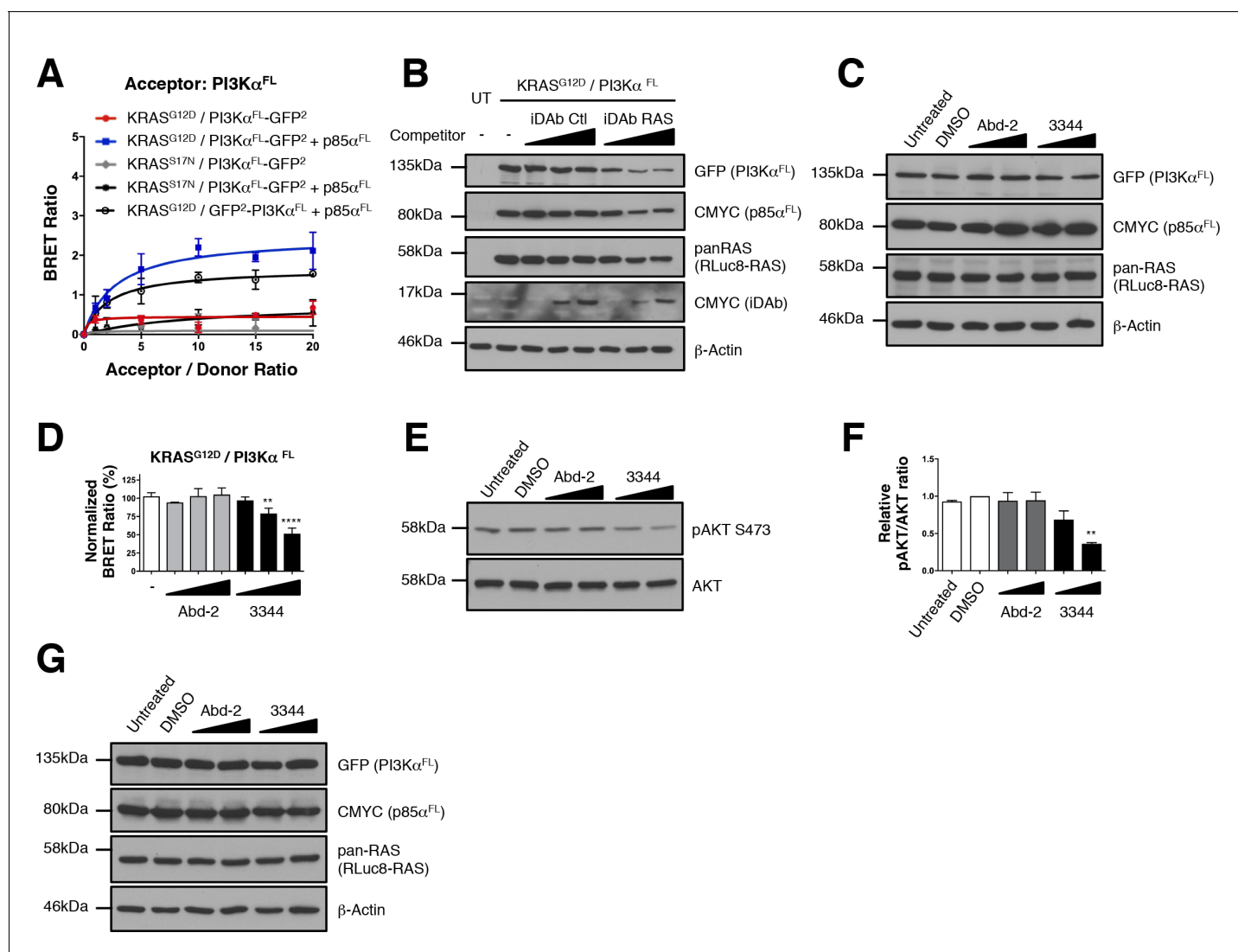


Figure 4—figure supplement 1. Interaction of KRAS^{G12D} with PI3K α^{FL} is inhibited by 3344. (A) BRET titration curves of KRAS^{G12D} and KRAS^{S17N} mutants with full-length PI3K α (PI3K α^{FL}). KRAS^{G12D} interacts with PI3K α^{FL} when the full-length regulatory subunit p85 α is co-expressed along with the BRET pair but not KRAS^{S17N}. The optimal BRET signal is obtained with the following pair: RLuc8-KRAS^{G12D}/PI3K α^{FL} -GFP². (B) Controls from **Figure 4B**. The expression level of the BRET pair was assessed with the GFP (for PI3K α^{FL}) and pan-RAS (for RLuc8-KRAS^{G12D}) antibodies. iDab and p85 α^{FL} expression was revealed by the CMYC tag antibody, β -actin was used as the loading control. (C) Controls from **Figure 4E**. The expression level of the BRET pair was assessed with the GFP (PI3K α^{FL}) and pan-RAS (RLuc8-KRAS^{G12D}) and CMYC (p85 α^{FL}) antibodies. Anti- β -actin was used as the loading control. In panel D, HEK293T cells transfected with the BRET biosensor KRAS^{G12D}/PI3K α^{FL} were treated for 3 hr with DMSO (white bar), 5, 10 and 20 μ M of Abd-2 (grey bars) and 3344 (black bars) compounds and the BRET signal of the biosensor was assessed. In panel E, the cells were transfected and treated as in panel D but with 10 and 20 μ M of Abd-2 and 3344 compounds. 3 hr after the treatment, cells were lysed and analyzed by western blot using anti-pAKT (Ser 473) or anti-pan-AKT antibody. The signal in the western blot is quantitated in panel F. (G) Controls from panel E. One-way ANOVA followed by Dunnett's post-hoc tests were used to determine the statistical significance of BRET and pAKT modulations induced by the compounds (** $p < 0.01$, **** $p < 0.0001$). Each experiment was repeated twice (A, E–F) or three times (D). Where error bars are presented, they correspond to mean values \pm SD of biological repeats (A, D) or correspond to mean \pm SEM of biological repeats (F).

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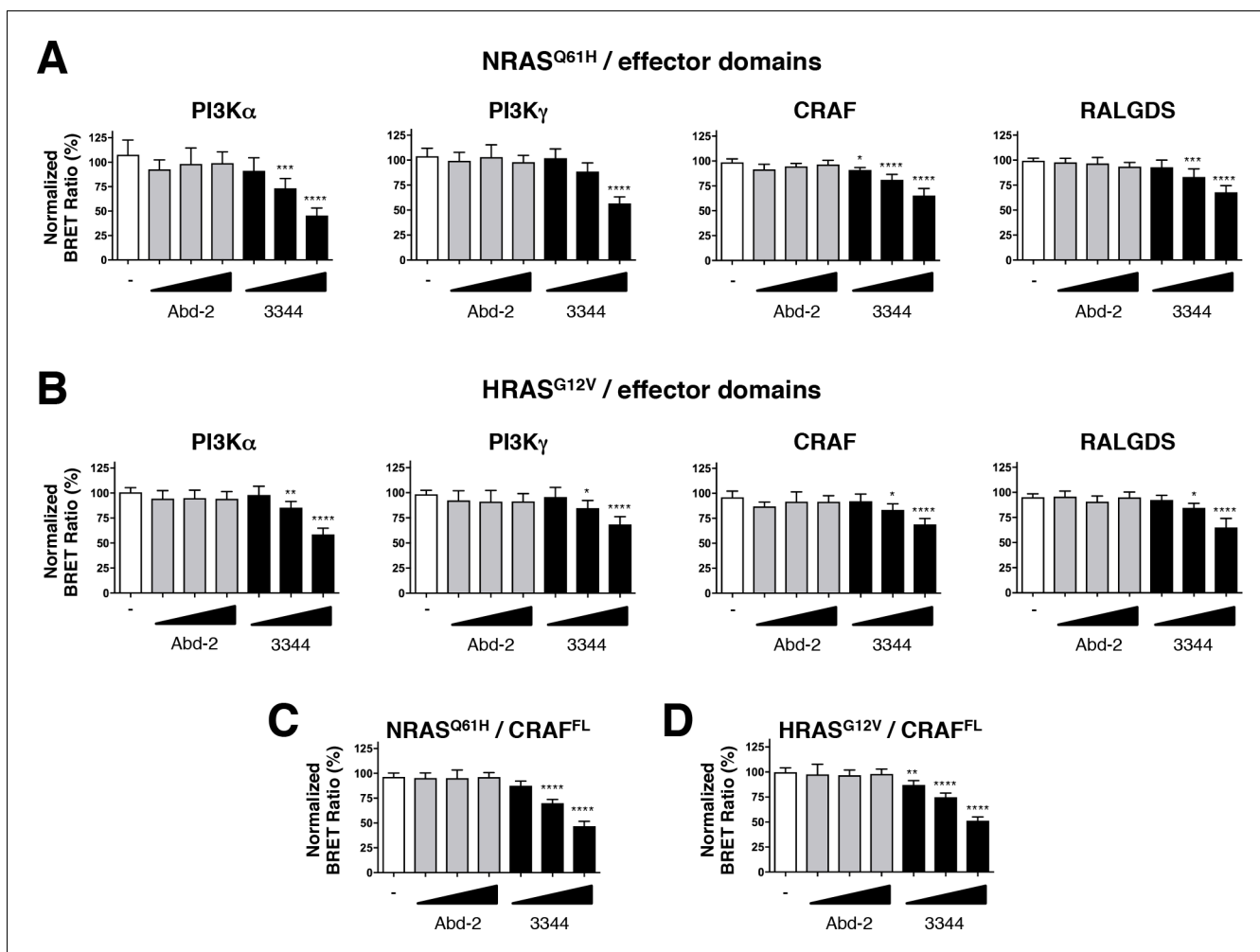


Figure 5. Compound 3344 inhibits NRAS and HRAS-effector BRET-based biosensors. HEK293T cells were transfected 24 hr with plasmids expressing the NRAS^{Q61H} (A, C) and HRAS^{G12V} (B, D) biosensors together with the indicated RBDs of PI3K, CRAF and RALGDS (A, B) or full-length CRAF (C, D). These were treated with 5, 10 and 20 μ M of Abd-2 (grey bars) or 3344 (black bars) compounds for 20 hr. DMSO (white bar) was used as the negative control. Statistical analyses were performed using a one-way ANOVA followed by Dunnett's post-tests (* p <0.05, ** p <0.01, *** p <0.001, **** p <0.0001). Each experiment was repeated at least four times. Where error bars are presented, they correspond to mean values \pm SD of biological repeats (A–D). See also **Figure 5—figure supplement 1**.

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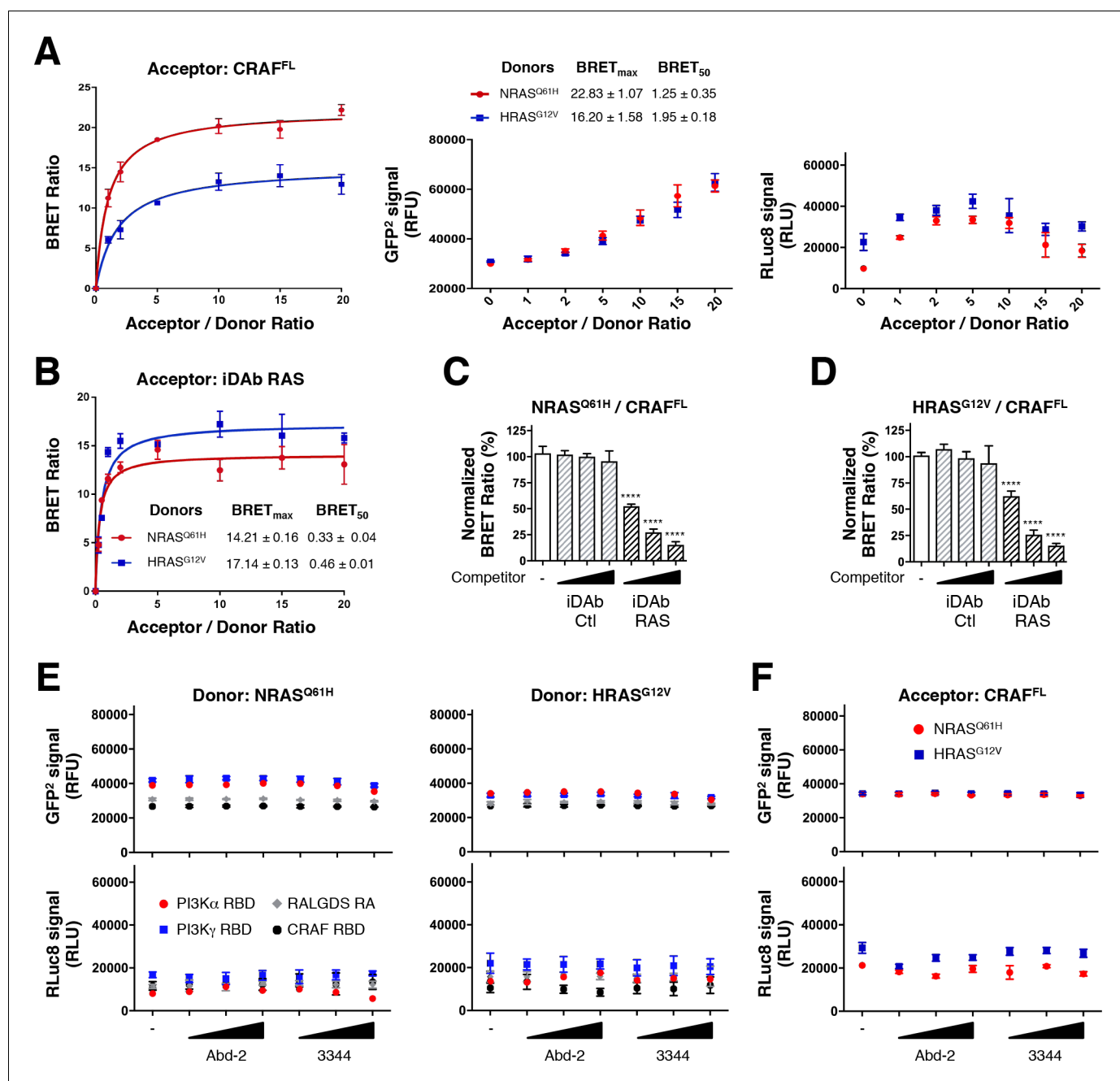


Figure 5—figure supplement 1. iDab RAS inhibits mutant NRAS and HRAS interaction with CRAF^{FL}. (A) BRET titration curves of NRAS^{Q61H} and HRAS^{G12V} with CRAF^{FL} with total GFP² and RLuc8 controls. (B) BRET titration curves of NRAS^{Q61H} and HRAS^{G12V} with iDab RAS. (C, D) Competition assays show the inhibition of NRAS^{Q61H}/CRAF^{FL} interaction (C) and HRAS^{G12V}/CRAF^{FL} interaction (D) by iDab RAS (black striped bars) in a dose-dependent manner compared to the non-relevant iDab control (grey striped bars) and the no competitor control (-, white bar). (E, F) Total GFP² and RLuc8 levels from the BRET competition assay shown in **Figure 5A–D**. Statistical analyses in C and D were performed using a one-way ANOVA followed by Dunnett’s post-hoc tests (****p<0.0001). Each experiment was repeated twice (A, B) or four times (C, D). Where error bars are presented, they correspond to mean values ± SD biological repeats.

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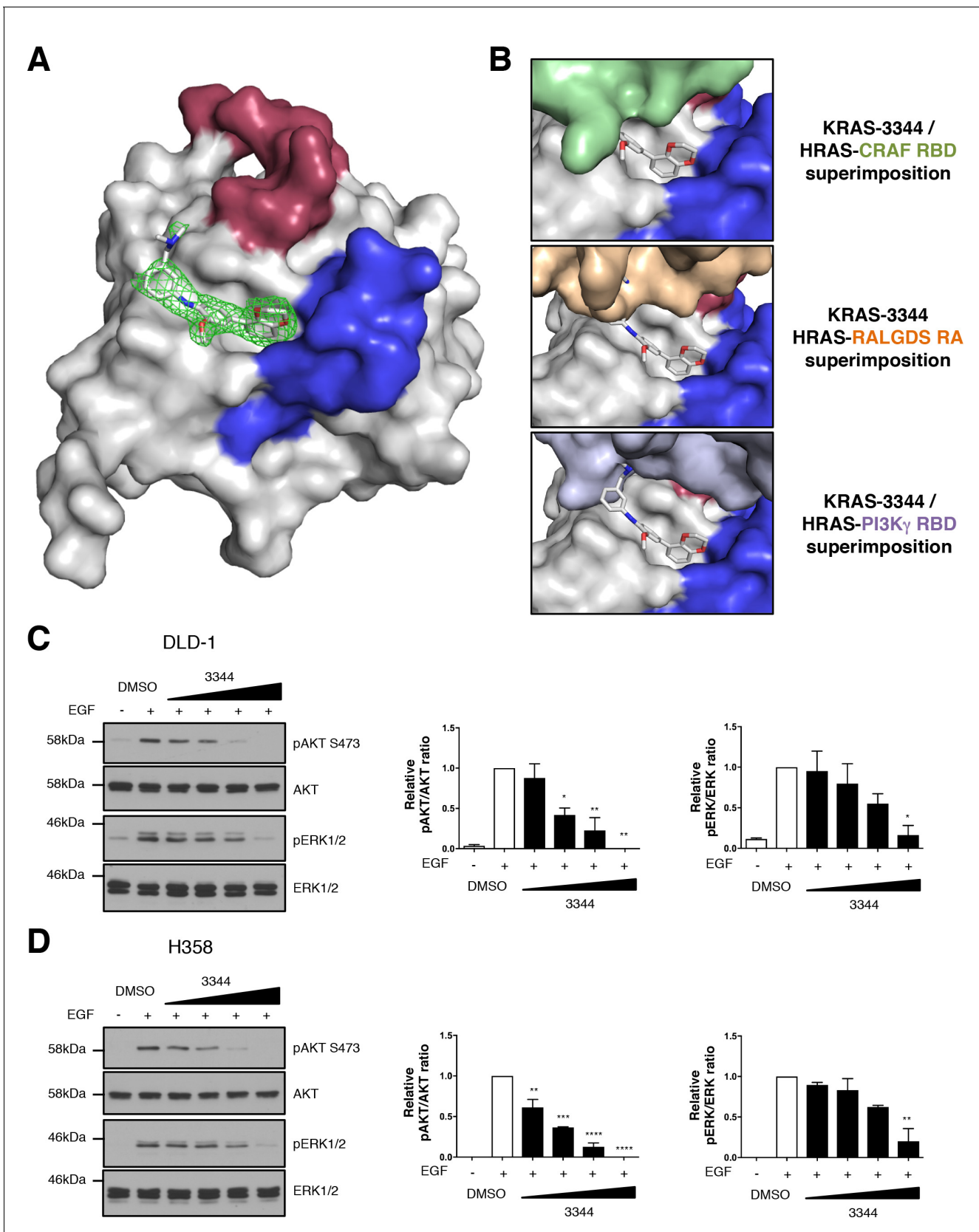
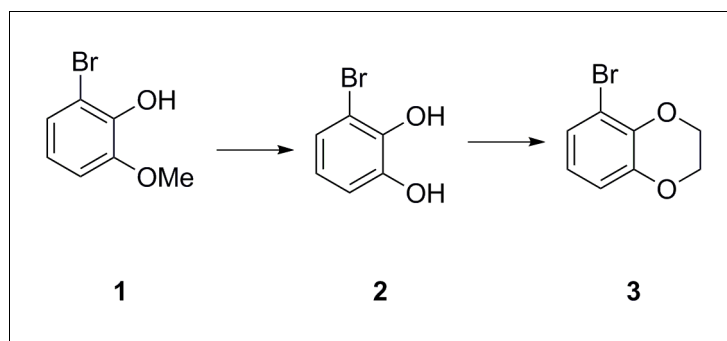


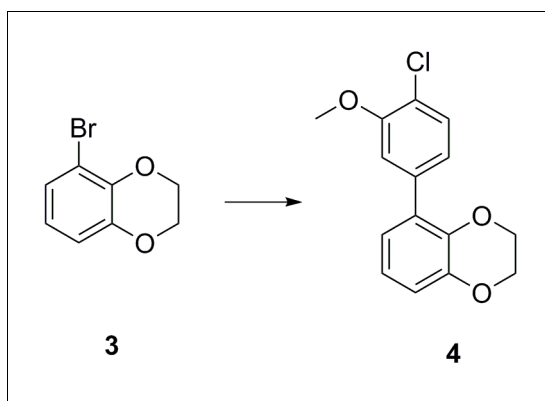
Figure 6. Compound 3344 interacts in a pocket close to the switch regions of KRAS. The interaction of mutant KRAS with compound 3344 was analyzed by X-ray crystallography. (A) KRAS^{Q61H} crystals were soaked with 3344 compound and crystal structures obtained from X-ray diffraction. The Figure 6 continued on next page

Figure 6 continued

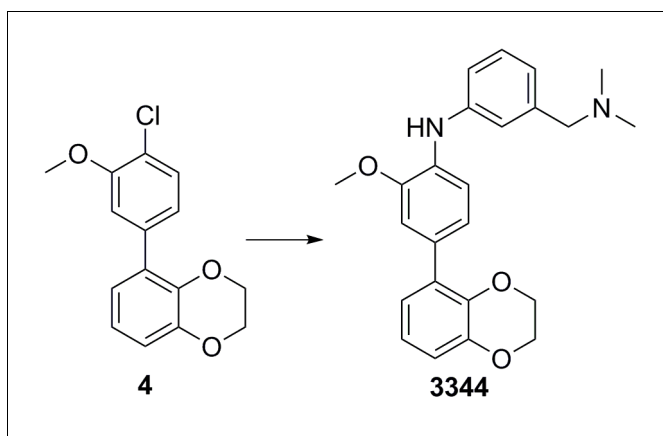
compound is shown binding in the hydrophobic pocket near switch I (shown in red) and switch II (shown in blue). The electron density map of the compound (2Fo-Fc) is shown as green mesh, and contoured at 1.0 rms. (B) We have modeled the potential interactions that could prevent 3344 and a RAS effector binding simultaneously to the same RAS molecule by overlaying our structure of the KRAS-3344 complex onto the published structures of top panel: HRAS-CRAF RBD (PDB 4G3X), middle panel: HRAS-RALGDS RA (PDB 1LFD), bottom panel: HRAS-PI3K γ RBD (PDB 1HE8). (C, D) Two human mutant KRAS expressing lines (C: DLD-1 and D: H358) were serum-starved for 24 hr and treated 3 hr with different concentrations of 3344 (2, 5, 10 and 20 μ M) before stimulation with EGF (50 ng/mL) for 10 min. Cells were harvested, proteins extracted and separated by SDS-PAGE for western blot analysis. Western membranes were treated with anti-pAKT S473; anti-pan AKT; anti-pERK1/2 and anti-ERK1/2 as indicated. Statistical analyses of pERK/ERK and pAKT/AKT quantifications were performed using a one-way ANOVA followed by Dunnett's post-tests (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$). Where error bars are presented, they correspond to mean values \pm SEM of biological repeats (C–D). Each experiment was performed twice (C–D).

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**Chemical structure 1.**DOI: <https://doi.org/10.7554/eLife.37122.017>

**Chemical structure 2.**

DOI: <https://doi.org/10.7554/eLife.37122.018>

**Chemical structure 3.**

DOI: <https://doi.org/10.7554/eLife.37122.019>

Supplementary file 1: DNA and protein sequences of BRET biosensors constructs

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M V S K G E E L F T G V V P I L V E L D G D V N G H K F S V S G E>
TRANSLATION OF GFP2-IDABDM CONTROL [A] >

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CGCTCCCGCTACGGTGGATGCCGATTCGACTGGGACTTCAAGTAGACGTGGTGGCCGTTTCGACGGGACCGGGACCGGGTGGGAGCAGTGGTGGGACTCGAT
G E G D A T Y G K L T L K F I C T T G K L P V P W P T L V T T L S Y>
TRANSLATION OF GFP2-IDABDM CONTROL [A] >

210     220     230     240     250     260     270     280     290     300
CGGCGTGCAGTGTTCAGCCGCTACCCCGACACATGAAGCAGCAGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCACCATCTTC
GCCGACGTCACGAAGTCCGGATGGGGCTGGTGTACTTCTGTCTGTGAAGAAGTTCAGCGGGTACGGGCTCCGATGCAGGTCCTCGCGTGTGAAG
G V Q C F S R Y P D H M K Q H D F F K S A M P E G Y V Q E R T I F>
TRANSLATION OF GFP2-IDABDM CONTROL [A] >

310     320     330     340     350     360     370     380     390     400
TTCGAAGGACGAGGCAACTACAAGACCCCGCGAGGTGAAGTTCGAGGGCGACACCCCTGGTGAACCGCATCGAGCTGAAGGCAATCGACTTCAAGGAGG
AAGTTCCTGTGCGCTGTGATGTTCTGGGCGGGCTCCACTTCAAGTCCCGCTGTGGGACCACTGGCGTAGCTCGACTTCCGTAAGTTCAGTTCCTCC
F K D D G N Y K T R A E V K F E G D T L V N R I E L K G I D F K E>
TRANSLATION OF GFP2-IDABDM CONTROL [A] >

410     420     430     440     450     460     470     480     490     500
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TGCCCTGTAGGACCCCGTTCGACCTCATGTGATGTTGTCTGGTGGTGGCAGATATAGTACCGGCTGTTCTGCTTCTTGGCGTAGTTCACCTTGAAGTT
D G N I L G H K L E Y N Y N S H N V Y I M A D K Q K N G I K V N F K>
TRANSLATION OF GFP2-IDABDM CONTROL [A] >

510     520     530     540     550     560     570     580     590     600
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I R H N I E D G S V Q L A D H Y Q Q N T P I G D G P V L L P D N H>
TRANSLATION OF GFP2-IDABDM CONTROL [A] >

610     620     630     640     650     660     670     680     690     700
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Y L S T Q S A L S K D P N E K R D H M V L L E F V T A A G I T L S>
TRANSLATION OF GFP2-IDABDM CONTROL [A] >

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M D E L Y K L E G G G S G G G S G G G S A A R M A E V Q L L E>
TRANSLATION OF GFP2-IDABDM CONTROL [A] >

810     820     830     840     850     860     870     880     890     900
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S G G G L V Q P G G S L R L S C A A S G F S F S H S P M N W V R Q>
TRANSLATION OF GFP2-IDABDM CONTROL [A] >

910     920     930     940     950     960     970     980     990     1000
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CGAGTCCCTTCCCGACCTCACCCAAAGTATGTAATCAATATTACGAAGCTCATATATGATACGCTGAGACACTTCCCGCTAAGTGGTAGAGGCTCT
A P G K G L E W V S Y I S Y N A S S I Y Y A D S V K G R F T I S R>
TRANSLATION OF GFP2-IDABDM CONTROL [A] >

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TRANSLATION OF GFP2-IDABDM CONTROL [A] >

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A A D W F D Y W G Q G T L V T V S S A A A E Q K L I S E E D L N G>
TRANSLATION OF GFP2-IDABDM CONTROL [A] >

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A A *>
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      20      30      40      50      60      70      80      90     100
ATGCCGAGGTGACGTGGAGTGGGGAGGCTGGTACAGCTGGGGGGTCCCTGAGACTCTCTGTGCAGCTTGGATTGAGTTCAGTTCAGTTCATA
TACGGCTTCCAGTGCAGCAACTCAGACCCCTCCGAACCATGTCGGACCCCGAGGACTCTGAGAGGACACAGTCCGAGACCTAAGTCGAAGTCAGTAT
M A E V Q L L E S G G G L V Q P G G S L R L S C A A S G F S F S H>
      _____
      TRANSLATION OF IDAB CONTROL-GFP2 [A] >
      _____

      110     120     130     140     150     160     170     180     190     200
GTCCATGAATTGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTTTCATACATAGTTATAAATTCCTGAGTATATACTATGCAGACTCTGTGAA
CAGGATACTTAACCCAGGCGTCCGAGTCCCTCCCGACCTCACCCAAAGTATGTAATCAATATTAAGAACTCATATATGATACGCTGAGACACTT
S P M N W V R Q A P G K G L E W V S Y I S Y N S S S I Y Y A D S V K>
      _____
      TRANSLATION OF IDAB CONTROL-GFP2 [A] >
      _____

      210     220     230     240     250     260     270     280     290     300
GGCCGATTACCATCTCCAGAGACAATCCAAGAACACACTGTATCTGCAAAATGAACAGCCTGAGAGCCGAGGACACGGCTGTCTATTACTGTGCGAGA
CCCGCTAAGTGTAGAGGCTCTGTTAAGGTTCTTGTGTGACATAGACGTTTACTTGTGGACTCTCGGCTCCTGTGCCAGAGATAATGACACGCTCT
G R F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A R>
      _____
      TRANSLATION OF IDAB CONTROL-GFP2 [A] >
      _____

      310     320     330     340     350     360     370     380     390     400
GGTTGAGCGAGTCTTTGAGTTGACGGGATTTGATTAATCTGGGCGCAGGGAACCTGGTCAACCTTAGTTCTCTCGAGGGCGAGGGCGGATCTG
CCCACTCCCTCAGAGAACCAACAGCCGCTAACCAAACTAATGACCCCGCTTGGGACCACTGGCAATCAAGAGACTCCCGCTCCCGCTAGAC
G L T E S L E L T A D W F D Y W G Q G T L V T V S S L E G G G G S>
      _____
      TRANSLATION OF IDAB CONTROL-GFP2 [A] >
      _____

      410     420     430     440     450     460     470     480     490     500
GGGGGAGGATCTCGCCGCGCAGGAGTGGTATGGTGGAGCAAGGGCGAGGAGCTGTCACCGGGTGGTCCCATCTCTGGTTCGAGCTGGACGGCGGACGT
CCCGCTCCTAGAGCCGCGCTCCCTCACATACCCTCGTTCCCGCTCCTCGACAAGTGGCCCAACCGGTTAGGACCACTCGACCTGCCGCTGCA
G G G G S A A A G S G M V S K G E E L F T G V V P I L V E L D G D V>
      _____
      TRANSLATION OF IDAB CONTROL-GFP2 [A] >
      _____

      510     520     530     540     550     560     570     580     590     600
AAACGGCCACAAGTTACGCTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCCTGAAGTTCATCTGCACCACGGCAAGCTGCCCGTG
TTTGCCGGTTCAGTGCACAGCCGCTCCCGCTCCCGCTACCGTGGATGCCGTTGACTGGGACTTCAAGTAGAGCTGGTGGCCGTTGACGGGGCAC
N G H K F S V S G E G D A T Y G K L T L K F I C T T G K L P V>
      _____
      TRANSLATION OF IDAB CONTROL-GFP2 [A] >
      _____

      610     620     630     640     650     660     670     680     690     700
CCCTGGCCACCTCGTACCACCTGAGCTACGGGCTGAGTCTTCCGCTACCCCGACCATGAAGCAGCAGCACTTCTTCAAGTCCGCCATGCG
GGGACCGGTTGGGACACTGGTGGACTCGATGCCGACCTCAGCAAGTCCGCGATGGGGTGGTGTACTTCTGCTGCTGAGAGAGTTTACGGGCTAGC
P W P T L V T T L S Y G V Q C F S R Y P D H M K Q H D F F K S A M>
      _____
      TRANSLATION OF IDAB CONTROL-GFP2 [A] >
      _____

      710     720     730     740     750     760     770     780     790     800
CCGAAGGTCAGTCCAGGACCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCGAGGTGAAGTTCGAGGGCGACACCCCTGGTGAACCG
GGCTTCCGATGCAGTCCCGCTGAGTGAAGAAGTTCTGCTGCCGTTGATGTTCTGGGCGCGGCTCCACTTCAAGCTCCCGCTGTGGGACCACTTGGC
P E G Y V Q E R T I F F K D D G N Y K T R A E V K F E G D T L V N R>
      _____
      TRANSLATION OF IDAB CONTROL-GFP2 [A] >
      _____

      810     820     830     840     850     860     870     880     890     900
CATCGAGCTGAAGGCATCGACTTCAAGGAGGACGGCAACATCTGGGGCACAAGCTGGAGTACAACACAGCCACACCTCTATATATGGCCGAC
GTAGCTCGACTTCCGCTAGCTGAAGTCTTCCGCTGAGGACCCCTGTCGACCTCATGTTGATGTTGTCGGTGTTCAGATATAGTACCGGCTG
I E L K G I D F K E D G N I L G H K L E Y N Y N S H N V Y I M A D>
      _____
      TRANSLATION OF IDAB CONTROL-GFP2 [A] >
      _____

      910     920     930     940     950     960     970     980     990     1000
AAGCAGAAGAAGGCACTAAGTGAAGTCAAGATCCGCCACAACATCGAGGACGGCAGCTGACGCTCGCCGACCACTACCAGCAGAACACCCCATCG
TTCGCTTCTTCCGCTAGTCCACTTGAAGTCTAGGCGGTTGAGTCTTCCGCTCGCACGCTCGAGCGGCTGGTGTGCTGTTGTGGGGTAGC
K Q K N G I K V N F K I R H N I E D G S V Q L A D H Y Q Q N T P I>
      _____
      TRANSLATION OF IDAB CONTROL-GFP2 [A] >
      _____

      1010    1020    1030    1040    1050    1060    1070    1080    1090    1100
GCGAGGCCCCGTGCTGCTGCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAGACCCCAACGAGAAGCGCGATCACATGGTCTGTGGA
CGCTGCCGGGGCACGACGACGGGCTGTTGGTATGGACTCGTGGTTCAGGCGGGACTCGTTTCTGGGGTGTCTTCCGCTAGTGTACCGAGGACGACT
G D G P V L L P D N H Y L S T Q S A L S K D P N E K R D H M V L L E>
      _____
      TRANSLATION OF IDAB CONTROL-GFP2 [A] >
      _____

      1110    1120    1130    1140    1150
GTTGCTGACCCCGCGGGATCACTCTCAGCATGGACGAGCTGTACAAGTAA
CAAGCACTGGCGGGCCCTAGTGAAGTCTGACCTGCTGACATGTTCATT
F V T A A G I T L S M D E L Y K *>
      _____
      TRANSLATION OF IDAB CONTROL-GFP2 [A] >
      _____
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Sequence: iDab RAS-GFP2 Range: 1 to 1125

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10      20      30      40      50      60      70      80      90     100
ATGCCCGAGGTGCAGCTGTGGAGTCTGGGGGAGGCTTGGTACAGCTTGGGGGTCCTGAGACTCTCTGTGCAGCCTTGGATTACCTTTAGTACCT
TACCGTCTCCAGTGCACCACTCAGACCCCTCCGAACCATGTCCGACCCCGGAGGACTCTGAGAGGACACAGTCCGAGACCTAAGTGGAAATCATGGA
M A E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F S T>
TRANSLATION OF IDAB RAS-GFP2 [A] >

110     120     130     140     150     160     170     180     190     200
TTAGCATGAAGTGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTTTCATACATAGTAGGACGTCGAAGACGATATACTATGCAGACTCTGTGAA
AATCGTACTTGACCCAGGCGGTCCGAGTCCCTCCCGACCTCACCCAAGTATGTAATCATCTGCAGCTTCTGTATATGATACGCTGAGACACT
F S M N W V R Q A P G K G L E W V S Y I S R T S K T I Y Y A D S V K>
TRANSLATION OF IDAB RAS-GFP2 [A] >

210     220     230     240     250     260     270     280     290     300
GGGCCGATTACCATCTCCAGAGACAATCCAAGAACACACTGTATCTGCAAAATGAACAGCCTGAGAGCCGAGGACACGGCTGTCTATTACTGTGCGAGA
CCCGCTAAGTGTAGAGGCTCTGTTAAGGTTCTTGTGTGACATAGACGTTTACTTGTGCGACTCTCGGCTCCTGTGCCGACAGATAATGACACGCTCT
G R F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A R>
TRANSLATION OF IDAB RAS-GFP2 [A] >

310     320     330     340     350     360     370     380     390     400
GGGAGATTTTGGAGTGGGGGAGGAAACCTGGGTACCGTGTAGTCTCTCGAGGGGGAGGGCGGATCTGGCGGGGAGGATCTGGCCCGCAGGGA
CCCTTAAGAACTGATGACCCCGTCCCTTGGGACAGTGGCAATCAAGAGAGCTCCCGCTCCGCTAGACCCGCGCTCCCTAGACCCGCGCTCCCT
G R F F D Y W G Q G T L V T V S S L E G G G S G G G G S A A A G>
TRANSLATION OF IDAB RAS-GFP2 [A] >

410     420     430     440     450     460     470     480     490     500
GTGGTATGGTGGAGCAAGGGGAGGAGCTGTTCACCGGGGTGGTCCCATCTGGTCCGACTGGACGGGACGTAACAGGGCCACAAGTTCAGCGTGTCCGG
CACCATACCACTCGTTCGCGCTCCCGACCAAGTGGCCACCAAGTGGAGGACGCTCGACCTGCGGCTGCATTTGCCGCTGTCAAGTCCGACAGGCC
S G M V S K G E E L F T G V V P I L V E L D G D V N G H K F S V S G>
TRANSLATION OF IDAB RAS-GFP2 [A] >

510     520     530     540     550     560     570     580     590     600
CGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCTGAAGTTCATCTGCACCACGGCAAGCTGCCGCTGCCCTGGCCACCCCTCGTGACCACCCCTG
GCTCCCGCTCCCGTACGGTGGATGCCGTCGACTGGGACTTCAAGTAGAGCTGGTGGCGGTCGACGGGGCACGGGACCGGGTGGGAGGACTGGTGGGAC
E G E G D A T Y G K L T L K F I C T T G K L P V P W P T L V T T L>
TRANSLATION OF IDAB RAS-GFP2 [A] >

610     620     630     640     650     660     670     680     690     700
AGCTACGGCGTGCAGTGTTCAGCCGCTACCCCGACCAATGAAGCAGCAGACTTCTCAAGTCCGCAATGCCGAGGCTACGTCAGGAGCGCACCA
TCGATCCCGCAGCTCACGAAGTCCGCGATGGGGTGGTGTACTTCTGCTGTGCTGAAGAAAGTTCAGCGGTCAGGGCTCCGATGCAGGTCCTCGCGTGT
S Y G V Q C F S R Y P D H M K Q H D F F K S A M P E G Y V Q E R T>
TRANSLATION OF IDAB RAS-GFP2 [A] >

710     720     730     740     750     760     770     780     790     800
TCTTCTCAAGGACGAGGGCAACTACAAGACCCCGCGCGAGGTTGAAGTTCGAGGGCGACACCCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAA
AGAAGAAGTTCCTGCTGCCGTTGATGTTCTGGGCGCGGCTCCACTTCAAGCTCCCGCTGCGGGACCACTTGGCGTAGCTCGACTTCCCGTAGCTGAAGTT
I F F K D D G N Y K T R A E V K F E G D T L V N R I E L K G I D F K>
TRANSLATION OF IDAB RAS-GFP2 [A] >

810     820     830     840     850     860     870     880     890     900
GGAGGCGGCAACATCTTGGGGCACAAGCTGGAGTACAACTACAACACGCCACAACCTCTATATCATGGCCGACAAGCAGAAGACGGCATCAAGGTGAAC
CTTCTGCGCTGTAGGACCCGCTGTTGACCTCATGTTGATGTTGTCGCTGTCGAGATATAGTACCGGCTGTTCTGCTTCTTGGCGTAGTCCACTTG
E D G N I L G H K L E Y N Y N S H N V Y I M A D K Q K N G I K V N>
TRANSLATION OF IDAB RAS-GFP2 [A] >

910     920     930     940     950     960     970     980     990     1000
TTCAAGATCCGCCACAACATCGAGGACGGCAGCTGCGGACCACTACCAGCAGAACACCCCACTCGGCGACGGCCCGTGTGCTGCCCGACA
AAGTTCTAGGCGTGTGTAGCTCCTCGCGCTCGACGCTCGAGCGGCTGGTGTGCTGCTTGTGGGGTAGCCGCTGCCGGGCGACGACGAGGGCTGT
F K I R H N I E D G S V Q L A D H Y Q Q N T P I G D G P V L L P D>
TRANSLATION OF IDAB RAS-GFP2 [A] >

1010    1020    1030    1040    1050    1060    1070    1080    1090    1100
ACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAAGACCCCAACGAGAAGCGGATCACATGGTCTGCTGGAGTTCGTGACCCCGCGCGGGATCACTCT
TGGTGTAGGACTCGTGGTCCAGGCGGACTCGTTTCTGGGGTGGCTCTTCCGCTAGTGTACCAGGACGACCTCAAGCACTGGCGGCGGCGCTAGTGAGA
N H Y L S T Q S A L S K D P N E K R D H M V L L E F V T A A G I T L>
TRANSLATION OF IDAB RAS-GFP2 [A] >

1110    1120
CAGCATGGACGAGCTGTACAAGTAA
GTCGTACTGCTCGACATGTTTATT
S M D E L Y K *>
TRANSLATION OF IDA >
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Sequence: iDabdm RAS-GFP2 Range: 1 to 1125

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10      20      30      40      50      60      70      80      90     100
ATGCCCGAGGTGACGCTGTGGAGTCTGGGGGAGGCTTGGTACAGCTGGGGGTCCCTGAGACTCTCTGTGCAGCTCTGGATTGCGCTTTGCTGCT
TACCGCTCCACGTGACCAACCTCAGACCCCTCCGAACCATGTGGACCCCGGAGGACTCTGAGAGGACACAGTCCGAGACCTAAGCGGAAACGACGGA
M A E V Q L L E S G G L V Q P G G S L R L S C A A S G F A F A A >
TRANSLATION OF IDABDM RAS-GFP2 [A] >

110     120     130     140     150     160     170     180     190     200
TTAGCATGAAGTGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTTTCATACATAGTAGGACGTCGAGAGCGATATACTATGCAGACTCTGTGAA
AATCGTACTTGACCCAGGCGGTCCGAGTCCCTCCCGACCTCACCCAAGTATGTAATCATCTGAGCTCTCTGTATATGATACGCTGAGACACT
F S M N W V R Q A P G K G L E W V S Y I S R T S K T I Y Y A D S V K >
TRANSLATION OF IDABDM RAS-GFP2 [A] >

210     220     230     240     250     260     270     280     290     300
GGGCCGATTACCATCTCCAGAGACAATCCAAGAACACACTGTATCTGCAAAATGAACAGCCTGAGAGCCGAGGACACGGCTGTCTATTACTGTGCGAGA
CCCGCTAAGTGTAGAGGCTCTGTTAAGGTTCTTGTGTGACATAGACGTTTACTTGTGCGACTCTGGGCTCCTGTGCCGACAGATAATGACACGCTCT
G R F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A R >
TRANSLATION OF IDABDM RAS-GFP2 [A] >

310     320     330     340     350     360     370     380     390     400
GGGGAGGCTTGGTCCCGGAGGCAACCTGGGTCACCGTGTAGTCTCTCGAGGGCGGAGGCGGATCTGGCGGCGGAGGATCTGGCGCCGACGGA
CCCGCTCCGAACTGATGACCCCGTCCCTGGGACCAAGTGAAGAGCTCCCGCTCCGCTAGACCCGCGCTCCAGACCCGCGCTCCCTGAGACCCGCGCTCCCT
G G G F D Y W G Q G T L V T V S S L E G G G S G G G G S A A A >
TRANSLATION OF IDABDM RAS-GFP2 [A] >

410     420     430     440     450     460     470     480     490     500
GTGGTATGGTGGAGCAAGGGGAGGCTGTTCACCGGGTGGTCCCATCTGGTCCGACTGGACGGGACGTAACAGCCGACAAAGTTCAGCGTGTCCGG
CACCATACCACTCGTTCGCGCTCCGACCAAGTGGCCCAAGTAGGACCAAGTCCGACTCCGCTGCATTTGCCGCTGTCAAGTCCGACAGGCC
S G M V S K G E E L F T G V P I L V E L D G D V N G H K F S V S G >
TRANSLATION OF IDABDM RAS-GFP2 [A] >

510     520     530     540     550     560     570     580     590     600
CGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCTGAAGTTCATCTGCACCACCGGCAAGCTGCCGCTGCCCTGGCCACCCCTCGTGACCACCCCTG
GCTCCCGCTCCGCTACGGTGGATGCCGTCGACTGGGACTTCAAGTAGAGCTGGTGGCGGCTCGACGGGACGGGACCGGGTGGGAGGACTGGTGGGAC
E G E G D A T Y G K L T L K F I C T T G K L P V P W P T L V T T L >
TRANSLATION OF IDABDM RAS-GFP2 [A] >

610     620     630     640     650     660     670     680     690     700
AGCTACGGCGTGCAGTGTTCAGCCGCTACCCCGACCACTGAAGCAGCAGACTTCTCAAGTCCGCGATGCCGGAAGGCTACGTCAGGAGCGCACCA
TCGATCCCGCAGCTCACGAAGTCCGCGATGGGCTGGTGTACTTCGTCGTGCTGAAGAAAGTTCAGCGGTCACGGGCTCCGATGCAGGTCCTCGCGTGT
S Y G V Q C F S R Y P D H M K Q H D F F K S A M P E G Y V Q E R T >
TRANSLATION OF IDABDM RAS-GFP2 [A] >

710     720     730     740     750     760     770     780     790     800
TCTTCTCAAGGACGAGGCAACTACAAGACCCGCGCGAGGTTGAAGTTCGAGGGCGACACCCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAA
AGAAGAAGTTCCTGCTGCCGTTGATGTTCTGGGCGCGGCTCCACTTCAAGCTCCCGCTGTCGGGACCACTTGGCGTAGCTCGACTTCCCGTAGCTGAAGT
I F F K D D G N Y K T R A E V K F E G D T L V N R I E L K G I D F K >
TRANSLATION OF IDABDM RAS-GFP2 [A] >

810     820     830     840     850     860     870     880     890     900
GGAGGCGGCAACATCTCGGGGCAAGCTGGAGTACAACTACAACACCCACCAAGCTTATATCATGGCCGACAAAGCAGAAGACGGCATCAAGGTGAAC
CTCTCGCGCTGTAGGACCCGCTGTCGACTCATGTTGATGTTGTCGCTGTCGAGATATAGTACCGGCTGTTCTGCTCTCTTGGCGTAGTCCACTTG
E D G N I L G H K L E Y N Y N S H N V Y I M A D K Q K N G I K V N >
TRANSLATION OF IDABDM RAS-GFP2 [A] >

910     920     930     940     950     960     970     980     990     1000
TTCAGATCCGCCACAACATCGAGGACGGCAGCTGCGACTCGCCGACCACTACCAGCAGAACACCCCACTCGGCGACGGCCCGTGTGTCGCCGACA
AAGTTCAGGCGTGTGTAGCTCCTCGCGCTCGACGCTCGAGCGGCTGGTGTGCTGCTTGTGGGGTAGCCGCTGCGGGGACGACGACGGGCTGT
F K I R H N I E D G S V Q L A D H Y Q Q N T P I G D G P V L L P D >
TRANSLATION OF IDABDM RAS-GFP2 [A] >

1010    1020    1030    1040    1050    1060    1070    1080    1090    1100
ACCACTACCTGAGCACCAGTCCGCCCTGAGCAAAAGCCCAACGAGAAGCGGATCACATGGTCTGCTGGAGTTCGTGACCCGCGCGGGATCACTCT
TGGTGTAGGACTCGTGGTCCAGGCGGACTCGTTTCTGGGGTGGCTCTTCGCGTAGTGTACCAGGACGACCTCAAGCACTGGCGGCGGCTTAGTGAGA
N H Y L S T Q S A L S K D P N E K R D H M V L L E F V T A A G I T L >
TRANSLATION OF IDABDM RAS-GFP2 [A] >

1110    1120
CAGCATGGACGAGCTGTACAAGTAA
GTCGTACCTGCTCGACATGTTTATT
S M D E L Y K * >
TRANSLATION OF IDA >
```

Sequence: membrane bound FLAG-IDAb control-myc competitor Range: 1 to 528

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10      20      30      40      50      60      70      80      90     100
ATGCTGTCTGTATGAGAAGAACCAACAGGTTGAAAAGAAATGATGAGGACCAAAAAGATCCTCGACATGGACTACAAGGACGACGATGACAGGCCCATGG
TACGACACGACATACTCTTCTTGGTTTGTCCAACCTTTCTTACTACTCCTGGTTTCTTAGCAGCTGTACCTGATGTTCTGTCTGCTACTGTCCGGGTACC
M L C C M R R T K Q V E K N D E D Q K I V D M D Y K D D D D R P M>
-----
TRANSLATION OF MEMBRANE BOUND FLAG-IDAB CONTROL-MYC COMPETITOR [A] ----->

110     120     130     140     150     160     170     180     190     200
CCGAGGTGACGCTGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGTCCTGAGACTCTCCTGTGACGCTCTGGATTCAGCTTCAGTCATAGTCC
GGCTCCACGTCGACAACCTCAGACCCCTCCGAACCATGTCGGACCCCCAGGGACTCTGAGAGGACACGTCGGAGACCTAAGTCGAAGTCAGTATCAGG
A E V Q L L E S G G G L V Q P G G S L R L S C A A S G F S F S H S P>
-----
TRANSLATION OF MEMBRANE BOUND FLAG-IDAB CONTROL-MYC COMPETITOR [A] ----->

210     220     230     240     250     260     270     280     290     300
TATGAATTGGGTCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTTTCATACATTAGTTATAATTCTTCGAGTATATACTATGCAGACTCTGTGAAGGGC
ATACTTAAACCCAGGCGGTCCGAGGTCCTTCCCGACCTCACCCAAAGTATGTAATCAATATTAAGAAGCTCATATATGATACCTCTGAGACACTTCCCG
M N W V R Q A P G K G L E W V S Y I S Y N S S S I Y Y A D S V K G>
-----
TRANSLATION OF MEMBRANE BOUND FLAG-IDAB CONTROL-MYC COMPETITOR [A] ----->

310     320     330     340     350     360     370     380     390     400
CGATTACCATCTCCAGAGACAATTCCAAGAACACACTGTATCTGCAAAATGAACAGCCTGAGAGCCGAGGACACGGGCTGTCTATTACTGCGAGAGGGT
GCTAAGTGGTAGAGTCTCTGTGTTAAGGTTCTTGTGTGACATAGACGTTTACTTGTGCGACTCTCGGCTCCGTGCGCCGACAGATAATGACACGCTCTCCCA
R F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A R G>
-----
TRANSLATION OF MEMBRANE BOUND FLAG-IDAB CONTROL-MYC COMPETITOR [A] ----->

410     420     430     440     450     460     470     480     490     500
TGACGGAGTCTCTGAGTTGACGGCGGATTGGTTTGATTACTGGGGCCAGGGAACCTGGTCACCGTCTCGAGCGCGGCCGAGAACAAAACTCATCTC
ACTGCCTCAGAGAACTCAACTGCCCTAACCAAACTAATGACCCCGTCCCTTGGGACCAAGTGGCAGAGCTCGCGCCGCGCTTGTGTTTGTAGTAGAG
L T E S L E L T A D W F D Y W G Q G T L V T V S S A A A E Q K L I S>
-----
TRANSLATION OF MEMBRANE BOUND FLAG-IDAB CONTROL-MYC COMPETITOR [A] ----->

510     520
AGAAGAGGATCTGAATGGGGCCCATAG
TCTTCTCCTAGACTTACCCCGCGTATC
E E D L N G A A *>
-----
TRANSLATION OF MEMBRA ----->
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Sequence: membrane bound FLAG-IDAb RAS-myc competitor Range: 1 to 501

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10      20      30      40      50      60      70      80      90     100
ATGCTGTGCTGTATGAGAAGAACCAACAGGTTGAAAAGAAATGATGAGGACCAAAAGATCGTCGACATGGACTACAAAGACGACGATGACAGGCCCATGG
TACGACACGACATACTCTTTGGTTTGTCCAACCTTTCTTACTACTCTGGTTTCTAGCAGCTGTACCTGATGTTCTGTGCTACTGTCCGGGTACC
M L C C M R R T K Q V E K N D E D Q K I V D M D Y K D D D D R P M>
-----
          TRANSLATION OF MEMBRANE BOUND FLAG-IDAB RAS-MYC COMPETITOR [A]----->

110     120     130     140     150     160     170     180     190     200
CCGAGGTGCAGCTGTGGAGTCTGGGGGAGGCTTGGTACAGCCTGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTCACCTTTAGTACCTTTAG
GGCTCCACGTCGACAACCTCAGACCCCTCCGAACCATGTCGGACCCCCAGGGACTCTGAGAGGACACGTCGGAGACCTAAGTGGAAATCATGGAAATC
A E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F S T F S>
-----
          TRANSLATION OF MEMBRANE BOUND FLAG-IDAB RAS-MYC COMPETITOR [A]----->

210     220     230     240     250     260     270     280     290     300
CATGAACTGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTTTCATACATTTAGTAGGACGTCGAAGACGATATACTATGCAGACTCTGTGAAGGGC
GTACTTGACCCAGGCGGTCCGAGGTCCCTTCCCGACCTCACCCAAAGTATGTAATCATCTGCAGCTTCTGCTATATGATACGCTCTGAGACACTTCCCG
M N W V R Q A P G K G L E W V S Y I S R T S K T I Y Y A D S V K G>
-----
          TRANSLATION OF MEMBRANE BOUND FLAG-IDAB RAS-MYC COMPETITOR [A]----->

310     320     330     340     350     360     370     380     390     400
CGATTCCACATCTCCAGAGACAATCCAAAGAACACACTGTATCTGCAATGAACAGCCTGAGAGCCGAGGACACGGGTCTTATCTGCGAGAGGGA
GCTAAGTGGTAGAGTCTCTGTGTTAAGGTTCTTGTGTGACATAGACGTTTACTTGTGCGACTCTCGGCTCCGTGTCGACAGATAATGACACGCTCTCCCT
R F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A R G>
-----
          TRANSLATION OF MEMBRANE BOUND FLAG-IDAB RAS-MYC COMPETITOR [A]----->

410     420     430     440     450     460     470     480     490     500
GATTTCTTGACTACTGGGGCCAGGAAACCTGGTCAACGCTCTCGAGCGCGCCGACAGAACAAAACTCATCTCAGAAGAGGATCTGAATGGGGCCGCATA
CTAAGAACTGATGACCCCGTCCCTTGGGACCAAGTGGCAGAGCTCGCGCCGGCTCTTGTGTTTGGAGTAGAGTCTTCTCCTAGACTTACCCGGCGTAT
R F F D Y W G Q G T L V T V S S A A A E Q K L I S E E D L N G A A >
-----
          TRANSLATION OF MEMBRANE BOUND FLAG-IDAB RAS-MYC COMPETITOR [A]----->
```

G
C
_>

Sequence: iDab control-myc competitor Range: 1 to 432

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      10      20      30      40      50      60      70      80      90     100
ATGGCCGAGGTGCAGCTGTGGAGTCTGGGGAGGGCTGGGTACAGCCTGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTTCAGTCAGTCATA
TACCGGCTCCACGTCGACAACCTCAGACCCCTCCGAACCATGTCGGACCCCGGAGGACTCTGAGAGGACACGTCGGAGACCTAAGTCGAAGTCAGTAT
M A E V Q L L E S G G G L V Q P G G S L R L S C A A S G F S F S H>
      _____
                TRANSLATION OF IDAB CONTROL-MYC COMPETITOR [A] _____>

      110     120     130     140     150     160     170     180     190     200
GTCCTATGAATTGGTCCGCCAGGCTCCAGGGAAGGGGTGGAGTGGGTTTCATACATTAGTTATAAATTCCTCGAGTATATACTATGCAGACTCTGTGAA
CAGGATACTTAACCCAGGCGGTCCGAGTCCCTTCCCGACCTCACCCAAAGTATGTAATCAATATTAAGAAGCTCATATATGATACGTCGAGACACTT
S P M N W V R Q A P G K G L E W V S Y I S Y N S S S I Y Y A D S V K>
      _____
                TRANSLATION OF IDAB CONTROL-MYC COMPETITOR [A] _____>

      210     220     230     240     250     260     270     280     290     300
GGGCCGATTACCATCTCCAGAGACAATCCAAGAACACACTGTATCTGCAAAATGAACAGCCTGAGAGCCGAGGACACGGCTGTCTATTACTGTGCGAGA
CCCGGTAAGTGTAGAGGTCTCTGTTAAGGTTCTTGTGTGACATAGACGTTTACTTGTGGACTCTCGGCTCCTGTGCCGACAGATAATGACACGCTCT
G R F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A R>
      _____
                TRANSLATION OF IDAB CONTROL-MYC COMPETITOR [A] _____>

      310     320     330     340     350     360     370     380     390     400
GGGTTGACGGAGTCTCTTGAGTTGACGGCGGATTGGTTTGATTCTGGGGCCAGGGAACCTGGTCACCGTCTCGAGCGCGCGCAGAACAAAACCTCA
CCCACTGCCTCAGAGAACTCAACTGCCGCCTAACCAACTAATGACCCCGTCCCTGGGACCAGTGGCAGAGCTCGCGCCGCGCTCTTTTTTGAGT
G L T E S L E L T A D W F D Y W G Q G T L V T V S S A A A E Q K L>
      _____
                TRANSLATION OF IDAB CONTROL-MYC COMPETITOR [A] _____>

      410     420     430
TCTCAGAAGAGGATCTGAATGGGGCCGCATAG
AGAGTCTCTCCTAGACTTACCCCGCGGTATC
I S E E D L N G A A *>
      _____
                TRANSLATION OF IDAB CONTR_____>

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Sequence: iDab RAS-myc competitor Range: 1 to 405

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10      20      30      40      50      60      70      80      90     100
ATGGCCGAGGTGCAGCTGTGGAGTCTGGGGAGGGTTGGTACAGCCTGGGGGGTCCCTGAGACTCTCCTGTGCAGCCTCTGGATTACACCTTTAGTACCT
TACCGGCTCCACGTGACAACCTCAGACCCCTCCGAACCATGTCGGACCCCGGAGGACTCTGAGAGGACACGTGCGAGACCTAAGTGGAAATCATGGA
M A E V Q L L E S G G G L V Q P G G S L R L S C A A S G F T F S T>
```

TRANSLATION OF IDAB RAS-MYC COMPETITOR [A] >

```
110     120     130     140     150     160     170     180     190     200
TTAGCATGAAGTGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTTTCATACATTAGTAGGACGTCGAAGACGATATACTATGCAGACTCTGTGAA
AATCGTACTTGACCCAGGCGGTCCGAGGTCCCTTCCCGACCTCACCCAAAGTATGTAATCATCTGCAGCTTCTGTATATGATACGCTGAGACACTT
F S M N W V R Q A P G K G L E W V S Y I S R T S K T I Y Y A D S V K>
```

TRANSLATION OF IDAB RAS-MYC COMPETITOR [A] >

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210     220     230     240     250     260     270     280     290     300
GGGCCGATTACCATCTCCAGAGACAATCCAAGAACACACTGTATCTGCAAAATGAACAGCCTGAGAGCCGAGGACACGGCTGTCTATTACTGTGCGAGA
CCCGGTAAGTGTAGAGGTCTCTGTTAAGGTTCTTGTGTGACATAGACGTTTACTTGTGGACTCTCGGCTCCTGTGCCGACAGATAATGACACGCTT
G R F T I S R D N S K N T L Y L Q M N S L R A E D T A V Y Y C A R>
```

TRANSLATION OF IDAB RAS-MYC COMPETITOR [A] >

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310     320     330     340     350     360     370     380     390     400
GGGAGATTCCTTGGACTACTGGGGCCAGGGAACCCCTGGTCAACGCTCTCGAGCGCGGCCGAGAACAACAACTCATCTCAGAAGAGGATCTGAATGGGCGG
CCCTTAAGAACTGATGACCCCGTCCCTTGGGACAGTGGCAGAGCTCCGCGCGGCTTGTGTTTGGAGTAGAGTCTTCTCTAGACTTACCCCGG
G R F F D Y W G Q G T L V T V S S A A A E Q K L I S E E D L N G A>
```

TRANSLATION OF IDAB RAS-MYC COMPETITOR [A] >

CATAG
GTATC
A *>
____>

Sequence: myc-p85alpha Range: 1 to 2217

ATGGAGCAGAAACATCTCTGAGAGGACTTGGGGGATCCATGAGTGTGAGGGGTACCAGTACAGAGCGCTGTATGATTATAAAAAGGAAAGAGAGA
TACCCTCGCTTTGAGTAGAGACTTCTCTAGACCCGCTAGGTACTACGACTCCCATGGTGTATGCTCGGCACATACTAATATTTCTCTTCTCTTC
M E Q K L I S E E D L G G S M S A E G Y Q Y R A L Y D Y K K E R E >
TRANSLATION OF MYC-P85ALPHA [A] >

110 120 130 140 150 160 170 180 190 200
AAGATATTGACTTGCCTTGGGTGACATATTGACTGTGAATAAAGGGTCTTAGTAGTCTTGGATTGATGATGGACAGGAAAGCCAGGCTGAAGAAAT
TTCTATACTGAACGTGAACCCACTGTATACTGACACTTATTTCCAGGAATCATCGAGAACCTAAGTCACTACCTGCTCTCGTCCGGACTTCTTTA
E D I D L H L G D I L T V N K G S L V A L G F S D G Q E A R P E E I >
TRANSLATION OF MYC-P85ALPHA [A] >

210 220 230 240 250 260 270 280 290 300
TGGCTGGTTAAATGGCTATAATGAAACCCAGGGGAAAGGGGACTTCCGGGAACCTACGTAGAATATATTGGAAGGAAAAAATCTCGCCTCCACACA
ACCCACCAATTTACCGATATTACTTTGGTGTCCCTTTCCCGGCTGAAAGCCCTTGAATGCATCTTATAACCTTCTTTTTTTAGAGCGGAGGGTGT
G W L N G Y N E T T G E R G D F P G T Y V E Y I G R K K I S P P T >
TRANSLATION OF MYC-P85ALPHA [A] >

310 320 330 340 350 360 370 380 390 400
CCAAGCCCGGCGCCCTTCCGCTTTCGACCGAGTTCCTCGAAACTGAAGCAGATGTTGAACAACAAGCTTTGACTCTCCCGGATCTGCGAC
GTTTCCGGGCGGCGGAGCGGAGGACAACCTGGTCCAGAAGCTTTTACTGCTTCAACTTGTGTTTGAAGCTGAGAGGGCTTAGAACCTC
P K P R P P R P L P V A P G S S K T E A D V E Q Q A L T L P D L A >
TRANSLATION OF MYC-P85ALPHA [A] >

410 420 430 440 450 460 470 480 490 500
AGCAGTTTGGCCCTCCTGACATTCGCCCGCTTCTTATCAAGCTCGTGGAGCCATTGAAAGAAAGGGTGGAAATGTTCAACTCTATACAGAACACA
TCGTCAACCGGGAGGACTTAACCGGGCGGAGAAGAAATAGTTCGAGCACCTTCGTAACCTTTCTTCCAGACCTTACAAGTTGAGATATGCTTGTGT
E Q F A P P D I A P P L L I K L V E A I E K K G L E C S T L Y R T Q >
TRANSLATION OF MYC-P85ALPHA [A] >

510 520 530 540 550 560 570 580 590 600
GAGCTCCAGCAACCTGGCAGAATTACGACAGCTTCTGTGATACACCCCTCCGTTGACTTGGAAATGATCGATGTGCACGTTTTGGTGCAGCTTTC
CTCGAGGTCTGGACGCTTAATGCTGTGCAAGAACTAACACTATGTTGGAGGGCACCTGAACCTTTACTAGCTACACGTGCAAAACCCGACTGCGAAG
S S S N L A E R Q L L D C D T P S V D L E M I D V H V L A D A F >
TRANSLATION OF MYC-P85ALPHA [A] >

610 620 630 640 650 660 670 680 690 700
AAACGCTATCTCCGACTTACCAATCTGTCAATTCAGCAGCGTTTACAGTGAATGATTCTTTAGCTCAGAAGTACAAGCTCCGAAGAATATA
TTTTCGATAGAGGAGCTGAATGGTTTAGGACAGTAAGTGTGGCAGAAATGCTACTTAAAGAAATCGAGGTCTTCAATGTTTCGAGGCTTCTATAT
K R Y L L D L P N P V I P A A V Y S E M I S L A P E V Q S S E E Y >
TRANSLATION OF MYC-P85ALPHA [A] >

710 720 730 740 750 760 770 780 790 800
TTCAGCTATTGAAGAAGCTTATAGTTCGCTAGCATACTCATCAGTATTGGCTTACGTTTTCAGTATTGTTAAACATTTCTCAAGCTCTCTCAAA
AAGTCGATAACTCTTTCGAATAATCCAGCGGATCGTATGGAGTAGTCATAACCGAATCGGAAGTCATAAAACAATTTTGAAGAAAGTTTCGAGAGATTTG
I Q L L K K L I R S P S I P H Q Y W L T L Q Y L L K H F F K L S Q T >
TRANSLATION OF MYC-P85ALPHA [A] >

810 820 830 840 850 860 870 880 890 900
CTCAGCAAAAATCTGTTGAATCGAAGTACTCTCTGAAATTTTACGCCCTATGCTTTTTCAGATTCTCAGCAGCGACTGTGATAATCTGAAAACCTC
GAGGCTGTTTTTAGACAACCTTACCTTCTCATGAGAGACTTTAAAAGTCGGGATACGAAAAGCTAAGAGTCTCGGTCGAGACTATTATGACTTTTGGAG
S S K N L L N A R V L S E I F S P M L F R F S A A S S D N T E N L >
TRANSLATION OF MYC-P85ALPHA [A] >

910 920 930 940 950 960 970 980 990 1000
ATAAAAGTTATAGAAATTTTAACTCAACTGAATGGAATGAACGACGACTGCACCAGCCTGCCTTAAACCAACCAAACTACTACTGTAGCCAAACA
TATTTTCAATATCTTAAATTAAGAGTTGACTTACCTTACTTGTCTCGGACGTGGTGTGACGGAGGATTTGGTGGTTTTGGATGATGACATCGTGTGT
I K V I E I L I S T E W N E R Q P A P A L P P K P P K P T T V A N >
TRANSLATION OF MYC-P85ALPHA [A] >

1010 1020 1030 1040 1050 1060 1070 1080 1090 1100
ACGGTATGAATAACAATATGCTTACAGATGCTGAATGGTACTGGGGAGATATCTCGAGGAAGAAGTGAATGAAAACTTCGAGATACAGCAGACGG
TGCCATATCTATTGTTATACAGGAATGTTCTACGACTTACCATGACCCCTTATAGAGCTCCCTTCTTCACTACTTTTTGAGGCTTATGCTGCTGCC
N G M N N N M S L Q D A E W Y W G D I S R E E V N E K L R D T A D G >
TRANSLATION OF MYC-P85ALPHA [A] >

1110 1120 1130 1140 1150 1160 1170 1180 1190 1200
GACCTTTTGGTACGAGATCGCTTACTAAAATGATGGTATTACTTCTTACTAAGGAAAGGGGAAATAACAATTAATCAAAAATTTTCTATCGA
CTGGAATAACCATGCTTACGACAGATGTTTACGTACCACTAATATGAGAAATGTTGATTCCTTCCCGCTTTATTGTTAATAGTTTATAAAGTAGCT
T F L V R D A S T K M H G D Y T L T L R K G G N N K L I K I F H R >
TRANSLATION OF MYC-P85ALPHA [A] >

1210 1220 1230 1240 1250 1260 1270 1280 1290 1300
GATGGGAAATATGGTCTCTGACCCATTAACTTCACTTCTGTGGTTGAATTAATAAACCACTACCGGAATGAATCTTCTAGCTCAGTATAATCCAAAT
CTACCCCTTATACCGAAGAGACTGGGTAATGGAAGTCAAGACACCAACTTAATATTGGTGTGATGGCTTACTTAGAGATCGAGTCATATTAGGGTTTA
D G K Y G F S D P L T F S S V V E L I N H Y R N E S L A Q Y N P K >
TRANSLATION OF MYC-P85ALPHA [A] >

1310 1320 1330 1340 1350 1360 1370 1380 1390 1400
TGGATGTGAAATTTACTTTTCCAGTATCCAAATACCAACAGGATCAAGTGTCAAAGAAGATAATATGAAGCTGTAGGGAAAAAATACATGAATATATA
ACCTACACTTTAATGAATAGGTCATAGGTTTATGGTTGCTTACTTCAACAGTTTCTTCTATTATAACTTCGACATCCCTTTTTTAATGTACTTATATT
L D V K L L Y P V S K Y Q Q D Q V V K E D N I E A V G K K L H E Y N >
TRANSLATION OF MYC-P85ALPHA [A] >

1410 1420 1430 1440 1450 1460 1470 1480 1490 1500
CACTCAGTTTCAAGAAAAAGTCGAGAATATGATAGATTATGAAAGATATACCCGCACATCCAGGAAATCAAATGAAAGGACAGCTATTGAAGCA
GTGATAACAAGTTCTTTTTTCCAGTCTTACTATCTAATACTTCTTATATGGGCGTGTAGGGTCTTTAGGTTTACTTTTCTTCTGATACCTCTGT
T Q F Q E K S R E Y D R L Y E E Y T R T S Q E I Q M K R T A I E A >
TRANSLATION OF MYC-P85ALPHA [A] >

1510 1520 1530 1540 1550 1560 1570 1580 1590 1600
TTTAATGAAACCAATAAAAATTTTGAAGAACAGTCCAGACCCCAAGAGCGGTACAGCAAGAATAACATAGAAAAGTTTAAACGTGAAGGCAATGAGAAAG
AAATTAATTTGGTATTTTATAAATCTTGTGTCAGGCTGCGGTCTCGCATGCTGTTCTTATGATCTTTTCAAAATTTGCACTTCCGTTACTCTTTC
F N E T I K I F E E Q C Q T Q E R Y S K E Y I E K F K R E G N E K >
TRANSLATION OF MYC-P85ALPHA [A] >

1610 1620 1630 1640 1650 1660 1670 1680 1690 1700
AAATCAAAAGGATTATGCATAAATGATAAGTTGAAGTCTCGAATCAGTGAATATTGACAGTGAAGAAGATTGGAAGAAGACTTGAAGAAGCAGG
TTTATGTTTCTAATACGATTAATTAATTAATCACTTCAAGCTTCAAGCTTCACTTCACTTCACTTCTTCAACTTCTTCACTTCTTCTGCTCCTG
E I Q R I M H N Y D K L K S R I S E I I D S R R L E E D L K K Q A >
TRANSLATION OF MYC-P85ALPHA [A] >

1710 1720 1730 1740 1750 1760 1770 1780 1790 1800

AGCTGAGTATCGAGAAATGACAAACGTATGAACAGCATTAACCAGACCTTATCCAGCTGAGAAAGACGAGAGACCAATACTTGATGTGGTTGACTCAA
TCGACTCATAGCTCTTTAACTGTTGCATACTTGTGTAATTTGGTCTGGAATAGTGTGACTCTTTCTGCTCTCTGGTTATGAACCTACACCAACTGAGTT
A E Y R E I D K R M N S I K P D L I Q L R K T R D Q Y L M W L T Q >
TRANSLATION OF MYC-P85ALPHA [A] >

1810 1820 1830 1840 1850 1860 1870 1880 1890 1900
AAAGGTGTTGGCAAAGAGTTGAACGAGTGGTTGGGCAATGAAACACTGAAGACCAATATTCACCTGGTGGAGATGATGAAGATTTGCCCATCATG
TTCCACAAGCCGTTTCTCAACTTGTCCACCAACCCGTTACTTTTGTGACTTCTGGTTATAAGTGACCACCTTCTACTACTTCTAAACGGGTAGTAC
K G V R Q K K L N E W L G N E N T E D Q Y S L V E D D E D L P H >
TRANSLATION OF MYC-P85ALPHA [A] >

1910 1920 1930 1940 1950 1960 1970 1980 1990 2000
ATGAGAAGACATGGAATGTTGGAACGAGCAACCGAAACAAGCTGAAACCTGTTGCGAGGGAAGCGAGATGGCACTTTTCTTGTCCGGGAGAGCAGTAA
TACTCTTCTACCTTACACCTTGTGCTGGCTTTGTTTCGACTTTTGGACAACGCTCCCTTCGCTCTACCGTAAAAGAACAGGCCCTCTCGCATT
D E K T W N V G S S N R N K A E N L L R G K R D G T F L V R E S S K >
TRANSLATION OF MYC-P85ALPHA [A] >

2010 2020 2030 2040 2050 2060 2070 2080 2090 2100
ACAGGGCTGCTATGCCCTGCTCTAGTGGTGGACGGCGAAGTAAAGCATTGTGTCATAAAACAAAACAGCAACTGGCTATGGCTTTGCCGAGCCCTATAAC
TGTCCCGACGATACGGACGAGACATACCACCTGCCGCTTCATTTGTAACACAGTATTTGTTTGTGCTGACCGATACCGAAACGGCTCGGGATATG
Q G C Y A C S V V V D G E V K H C V I N K T A T G Y G F A E P Y N >
TRANSLATION OF MYC-P85ALPHA [A] >

2110 2120 2130 2140 2150 2160 2170 2180 2190 2200
TTGTACAGCTCTGAAAGACTGTTGCTACATTACCAACACACCTCCCTTGTGCAGCACAAAGACTCCCTCAATGTCACACTAGCTACCCAGTATATG
AACATGTCGAGAGACTTCTTGACCACGATGTAATGGTTGTGTTGGAGGGAACACGTCGTTGCTGAGGGAGTTACAGTGTATCGGATGGGTCATATAC
L Y S S L K E L V L H Y Q H T S L V Q H N D S L N V T L A Y P V Y >
TRANSLATION OF MYC-P85ALPHA [A] >

2210
CACAGCAGAGCGATGA
GTGTCGTCTCCGCTACT
A Q Q R R * >
TRANSLATIO >

Sequence: PI3Kalpha RBD-GFP2 Range: 1 to 1257

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10      20      30      40      50      60      70      80      90      100
ATGAGTAGAGCAATGTATGTTTATCTCCAAATGTAGAACTTCCACCAGAAGTCCCAAAGCACATATATAATAAAATGGATAAAGGGCAAATAATAGTGG
TACTCATCTCGTTACATACAAAATAGGAGGTTTACATCTTAGAAGTGGTTCGACGGTTCGTGTATATATTTAACTATTCCCGTTATTATACACC
M S R A M Y V Y P P N V E S S P E L P K H I Y N K L D K G Q I I V>
TRANSLATION OF PI3KALPHA RBD-GFP2 [A] >

110     120     130     140     150     160     170     180     190     200
TGATTTGGGTAATAGTTTCTCCAAATAATGACAAACAGAAGTATACTCTGAAAATCAACCATGACTGTGTGCCAGAACAAAGTAATTGCTGAAGCAATCAG
ACTAAACCCATTATCAAGAGGTTTACTGTCTTTCATATGAGACTTTTAGTTGGTACTGACACACGGTCTTGTTCATTAACGACTTCGTTAGTCC
V I W V I V S P N N D K Q K Y T L K I N H D C V P E Q V I A E A I R>
TRANSLATION OF PI3KALPHA RBD-GFP2 [A] >

210     220     230     240     250     260     270     280     290     300
GAAAAAACTCGAAGTATGTGTATCATCTGAACAACTAAAACCTGTGTTTGAATAATCAGGGCAAGTATATTTTAAAAGTGTGGATGTGATGAA
CMTTTTTGAGCTTCATACAAAGTAGTAGACTTTGTGATTTTGGAGACAAAATCTTATAGTCCCGTTCATATAAAATTTTCACACACCTACACTACTT
K K T R S M L L S S E Q L K L C V L E Y Q G K Y I L K V C G C D E>
TRANSLATION OF PI3KALPHA RBD-GFP2 [A] >

310     320     330     340     350     360     370     380     390     400
TACTTCTAGAAAATATCTCTGAGTCAAGTATAAGTATAAAGAGCTGTATAATGCTTGGGAGGATGCCCAATTGATGCTGATGATGATGATGATGATG
ATGAAGGATCTTTTATAGGAGACTCAGTCAATATTCATATATCTTCGACATATTCAGAACCCCTCCTACGGGTAAACTACGACTACCGATTCTTCCGG
Y F L E K Y P L S Q Y K Y I R S C I M L G R H M P N L M L M A K E S>
TRANSLATION OF PI3KALPHA RBD-GFP2 [A] >

410     420     430     440     450     460     470     480     490     500
TCTATTTCAACTGCCAATGGACTGTTTACAATGCCATCATATCCAGACGCATTTCCACAGCTACACTCGAGGGCGGGGAGGATCTGGGGCGGGAG
AGATAAGAGTTGACGGTTACTGACAAAATGTTACGGTAGTATAAGGTTGCGTAAAGGTGTCGATGTGAGCTCCCGCCGCTCCTAGACCCCGCTCC
L Y S Q L P M D C F T M P S Y S R R I S T A T L E G G G G S G G G G>
TRANSLATION OF PI3KALPHA RBD-GFP2 [A] >

510     520     530     540     550     560     570     580     590     600
AAGTGGGGGAGGGGCTCTCGGCGCGAGGAGTGGTATGGTGAGCAAGGCGAGGAGCTGTTCCCGGGTGGTGGCCATCCTGGTCCGAGCTGGACGGC
TTCACCCCTCCCGGAGAGCGCGGGCTCCCTCACCATACCACTCGTTCGCGTCCGACAAAGTGGCCACCACCGGGTAGGACCACTCGACTCGCCG
S G G G G S A A A G S G M V S K G E E L F T G V V P I L V E L D G>
TRANSLATION OF PI3KALPHA RBD-GFP2 [A] >

610     620     630     640     650     660     670     680     690     700
GACGTAACCGCCACAAGTTACGCGTGTCCGGCGAGGGCGAGGGCAGTCCACCTACGGCAAGCTGACCCCTGAAGTTTCATCTGCACCACCGCAAGCTGC
CTGCATTTGCGGTGTTCAAGTGCACAGCGGCTCCCGTCCCGTACGGTGGATGCGCTTCGACTGGGACTTCAAGTAGAGCTGGTGGCGGCTCGAGG
D V N G H K F S V S G E G E G D A T Y G K L T L K F I C T T G K L>
TRANSLATION OF PI3KALPHA RBD-GFP2 [A] >

710     720     730     740     750     760     770     780     790     800
CCGTGCCCTGGCCACCCTCGTACCACCTGAGCTACGGCGTGCAGTGTTCAGCCGCTACCCCGACCATGAAGCAGCAGACTTCTTCAAGTCCCG
GGCACGGGACCGGGTGGGAGCACTGGTGGACTCCGATCCCGCACCTCACGAAAGTCCGGGATGGGGCTGGTGTACTTCCGCTGCTGAAGAAGTTACGGCG
P V P W P T L V T T L S Y G V Q C F S R Y P D H M K Q H D F F K S A>
TRANSLATION OF PI3KALPHA RBD-GFP2 [A] >

810     820     830     840     850     860     870     880     890     900
CATGCCCGAAGGCTACCTCCAGGAGCCGACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCCGCGGAGGTGAAGTTCCGAGGGCGACACCCCTGGTG
GTACGGGCTCCGATGACAGGCTCCGCTGGTGAAGAAGTTCTCTGCTGGCGGCTCCACTTCAAGCTCCCGCTGCGGACCCAC
M P E G Y V Q E R T I F F K D D G N Y K T R A E V K F E G D T L V>
TRANSLATION OF PI3KALPHA RBD-GFP2 [A] >

910     920     930     940     950     960     970     980     990     1000
AACCAGTCCGAGCTGAAGGCATCGACTTCAAGGAGGACGGCAACATCTGGGGCACAAAGCTGGAGTACAACACTACAACAGCCACAACCTTATATCATGG
TTGGCTAGCTCGACTCCCGTAGTGAAGTCTCTCTGCGGTTGAGGACCCCGTGTTCGACCTCATGTTGATGTTGCTCGGTGTTGACATATAGTACC
N R I E L K G I D F K E D G N I L G H K L E Y N Y N S H N V Y I M>
TRANSLATION OF PI3KALPHA RBD-GFP2 [A] >

1010    1020    1030    1040    1050    1060    1070    1080    1090    1100
CCGACAAGCAGAAGAACGGCATCAAGTGAACCTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCCGCGACCACTACCAGCAGAACACCC
GGCTGTTGCTTCTTTCGCTTTCGCGTAGTCCACTTGAAGTCTAGGGCGTGTGTAGTCTCCTGCGTCCGACGTCGAGCGGCTGGTGTGTTGCTTGTGGGG
A D K Q K N G I K V N F K I R H N I E D G S V Q L A D H Y Q Q N T P>
TRANSLATION OF PI3KALPHA RBD-GFP2 [A] >

1110    1120    1130    1140    1150    1160    1170    1180    1190    1200
CATCGGCGACGGCCCGTGTGTGCTGCCGACAACCACTACCTGAGCACCCAGTCCGCGCTGAGCAAAGACCCCAACGAGAAGCGGATCACATGGTCTGTG
GTAGCCGCTCCCGGGCACGACGAGCGGCTTGTGTGATGGACTCGTGGTTCAGGGGGACTCGTTTCTGGGTTGCTTTCGCGCTAGTGTACAGGAC
I G D G P V L L P D N H Y L S T Q S A L S K D P N E K R D H M V L>
TRANSLATION OF PI3KALPHA RBD-GFP2 [A] >

1210    1220    1230    1240    1250
CTGGAGTTCGTGACCGCGCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAA
GACCTCAAGCACTGGCGGGCCCTAGTAGAGCCGCTACCTGCTCGACATGTTTATT
L E F V T A A G I T L G M D E L Y K *>
TRANSLATION OF PI3KALPHA RBD-GFP2 [A] >
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Sequence: PI3Kalpha full-length-GFP2 Range: 1 to 3948

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10      20      30      40      50      60      70      80      90      100
ATGCCCCCAAGAATCTAGTAGAATGTTTACTACCAATGGAATGATGACTTTAGAAATGCCTCCGTGAGGCTACATTAAACCAATAAAGCATGAAC
TACGGGGGTCTTAGAGATCATCTTACAAATGATGGTTACTCTACTATCACTGAAATCTTACGGAGGCACTCCGATGTAATATTGGTATTTCGTAAGT
M P P R I L V E C L L P N G M I V T L E C L R E A T L I T I K H E >
TRANSLATION OF PI3KALPHA FULL-LENGTH-GFP2 [A] >

110     120     130     140     150     160     170     180     190     200
TATTTAAGAAGCAAGAAAATACCCCTCCATCAACTCTTCAAGATGAATCTTCTACATTTTCGTAAGTGTACTCAAGAAGCAGAAAGGGAAGAATT
ATAAATTTCTTCTTTTATGGGGAGGTAGTTGAAGAAGTCTACTTAGAAGAATGAAAAGCATTACAAATGAGTCTTCCGCTTTCCCTCTTAA
L F K E A R K Y P L H Q L L Q D E S S Y I F V S V T Q E A E R E E F >
TRANSLATION OF PI3KALPHA FULL-LENGTH-GFP2 [A] >

210     220     230     240     250     260     270     280     290     300
TTTTGATGAAACAAGCAGACTTTGTGACCTTCGGCTTTTCAACCTTTTAAAGTAATTGAACCAGTAGGCAACCGTGAAGAAAAGATCCTCAATCGA
AAAACACTTTGTTCTGCTGAAACACTGGAAGCCGAAAAGTTGGGAAAATTTTCATTAACCTTGCTCATCCGTTGGCAGCTTTTCTTAGGAGTTAGCT
F D E T R R L C D L R L F Q P F L K V I E P V G N R E E K I L N R >
TRANSLATION OF PI3KALPHA FULL-LENGTH-GFP2 [A] >

310     320     330     340     350     360     370     380     390     400
GAAATTTGTTTTCGCTATCGCATGCCAGTGTGTAATTTGATATGGTTAAAGATCCAGAAGTACAGGACTCCGAGAATAATTTCTGAAGCTTTTGAAG
CTTTAACCAAACGATAGCCGTACCGTACACACTTAACTATACCAATTTCTAGCTTCTATGCTCCGTAAGGCTCTTTTAAAGACTTGCAAAACATTC
E I G F A I G M P V C E F D M V K D P E V Q D F R R N I L N V C K >
TRANSLATION OF PI3KALPHA FULL-LENGTH-GFP2 [A] >

410     420     430     440     450     460     470     480     490     500
AAGCTGTGGATCTTAGGGCACTCAATTCACCTCATAGTAGAGCAATGTATGTCTATCCCTCAAATGAGAATCTCACCAGAATTGCCAAAGCACATATA
TCATCAACCTTAGAATCCCTGGAGTTAAGTGGAGTATCATCTCGTTACATACAGATAGGAGGTTACATCTTGAAGTGGTCTTAACGGTTTCGTGTATAT
E A V D L R D L N S P H S R A M Y V Y P P N V E S S P E L P K H I Y >
TRANSLATION OF PI3KALPHA FULL-LENGTH-GFP2 [A] >

510     520     530     540     550     560     570     580     590     600
TAATAAATTAGATAAAGGGCAATAATAGTGGTATCTGGGTAATAGTTTCTCAAATAATGACAAGCAGAAGTATACTCTGAAAATCAACCATGACTGT
ATTATTTAATCTATTTCCCGTTTATATCACCAGTAGACCATATCAAGAGGTTTATTACTGTTCTGCTTTCATATGAGACTTTTAGTTGGTACTGACA
N K L D K G Q I I V I W V I V S P N N D K Q K Y T L K I N H D C >
TRANSLATION OF PI3KALPHA FULL-LENGTH-GFP2 [A] >

610     620     630     640     650     660     670     680     690     700
GTACCAGAACAGTAATTTGCTGAACCAATCAGGAAAAAAGTGAAGTATGTTGCTATCCCTGTAACAACTAAAACCTGTTGTTTGAAGATATCAGGGCA
CATGTTCTTCTTCAATCAACACTCGTTAGTCTCTTTTGGAGCTTCATACACAGATAGGAGACTTGTGATTTTGGAGACAAAATCTTATAGTCCCGT
V P E Q V I A E A I R K K T R S M L L S S E Q L K L C V L E Y Q G >
TRANSLATION OF PI3KALPHA FULL-LENGTH-GFP2 [A] >

710     720     730     740     750     760     770     780     790     800
AGTATATTTAAAAGTGTGGATGTGATGAATACTTCTAGAAAAATATCCTCTGAGTCAAGTATAAGTATAAAGAAGCTGTATAAGCTTGGGAGGAT
TCATATAAAATTTTACACACTTACTACTTATGAAGACTTTTATAGGAGACTCAGTCAATTTTATATATTTCTCGACATATTCAGCAACCTCCCTA
K Y I L K V C G C D E Y F L E K Y P L S Q Y K Y I R S C I M L G R M >
TRANSLATION OF PI3KALPHA FULL-LENGTH-GFP2 [A] >

810     820     830     840     850     860     870     880     890     900
GCCAAATTTGATGTTGATGCTAAGAAAGCCTTTATTTCTCACTGCCATGGACTGTTTACAATGCCATCTTATTTCCAGACGCATTTCCACAGCTACA
CGGTTAAACTACAACACTACCGATTCTTTCGAAAATAGAGTTGACGGTTACCTGACAAAATTTACGGTAGAATAAGGTCTCGCTAAAGGCTGTCGATGT
P N L M L M A K E S L Y S Q L P M D C F T M P S Y S R R I S T A T >
TRANSLATION OF PI3KALPHA FULL-LENGTH-GFP2 [A] >

910     920     930     940     950     960     970     980     990     1000
CCATATATGAATGGAGAAACATCTACAAAATCCCTTTGGGTTATAAATAGTGCACACTCAGAATAAAAAATCTTTGTGCAACCTACGTTGAATGTAATTC
GGTATATACCTTACTCTTTAGTAGATGTTTGGGAAACCAATATTTTACAGTCACTTATTTTAAAGAACACGTTGGATGCACTTACATTTATAAG
P Y M N G E T S T K S L W V I N S A L R I K I L C A T Y V N V N I >
TRANSLATION OF PI3KALPHA FULL-LENGTH-GFP2 [A] >

1010    1020    1030    1040    1050    1060    1070    1080    1090    1100
GAGACATTTAAGATCTATGTTGCAACAGGTATCTACCATGGAGGAGAACCCTTATGTGCAATGTGAACACTCAAAGAGTACCTTTGTTCCAATCCCAG
CTCTGTAATTTCTAGATACAAGCTTGTCCATAGATGTTACCTCTCTTGGGAATACACTGTTACACTTGTGAGTTTCTCATGGAACAAGGTTAGGGTC
R D I D K I Y V R T G I Y H G G E P L C D N V N T Q R V P C S N P R >
TRANSLATION OF PI3KALPHA FULL-LENGTH-GFP2 [A] >

1110    1120    1130    1140    1150    1160    1170    1180    1190    1200
GTGGAATGAATGGCTGAATATGATATATACATTTCTGATCTTCTCGTGTGCTCGACTTTGCCCTTCCATTTGCTCTGTTAAAGGCCGAAAGGTTGGT
CACCTTACTTACGACTTATATACTATATGTAAGGACTAGAAGGACGACGAGCTGAAAACGGAAGGTAACGAGACAAATTTCCGGCTTTCCACGGA
W N E W L N Y D I Y I P D L P R A A R L C L S I C S V K G R K G A >
TRANSLATION OF PI3KALPHA FULL-LENGTH-GFP2 [A] >

1210    1220    1230    1240    1250    1260    1270    1280    1290    1300
AAAGAGAACTGTCATTTGGCAGTGGGAAATATAAACTTTGTTGATACACAGACTCTAGTATCTGAAAAATGGCTTTGAATCTTTGGCCAGTAC
TTTCTCCTTGTGACAGGTAACCGTACCCCTTATATTTGAACAACTAATGTGCTGTGAGATCATAGACTTTTACCAGAACTTAGAAAACCGGTCATG
K E E H C P L A W G N I N L F D Y T D T L V S G K M A L N L W P V >
TRANSLATION OF PI3KALPHA FULL-LENGTH-GFP2 [A] >

1310    1320    1330    1340    1350    1360    1370    1380    1390    1400
CTCATGGATTAGAAGATTGCTGAACCTATTTGGTGTACTGGATCAAATCAAATAAAGAACTCCATGCTTAGAGTTGGAGTTTGAAGCTGTTGACTGGTTCAGCAG
GAGTACCTAATCTTAAACGACTTGGGATAACCACAAAGACTAGTTAGGTTTATTTCTTTGAGGTACGAATCTCAACCTCAAAGTACCAAGTCCGTC
P H G L E D L L N P I G V T G S N P N K E T P C L E L E F D W F S >
TRANSLATION OF PI3KALPHA FULL-LENGTH-GFP2 [A] >

1410    1420    1430    1440    1450    1460    1470    1480    1490    1500
TGTGGTAAAGTTCCAGATATGTCAGTGTGTAAGGAGCATGCCAATTTGGTCTGATCCCGAGAAGCAGGATTTAGCTATTTCCACGAGGACTGAGTAAC
ACACCTTCAAGGCTTATACAGTCACTAATCTCTGACGGTTAACAGACATAGGCTCTTCTGCTCAATCGATAAGGTTGGCTGCTGACTCATG
V V K F P D M S V I E E H A N W S V S R E A G F S Y S H A G L S N >
TRANSLATION OF PI3KALPHA FULL-LENGTH-GFP2 [A] >

1510    1520    1530    1540    1550    1560    1570    1580    1590    1600
AGACTAGCTAGAGACAATTAAGGGAAAATGACAAAGAACAGCTCAAAGCAATTTCTACACAGATCTCTCTCTGAAAATCACTGAGCAGGAGAAAG
TCTGATCGATCTCTGTTACTTAATTTCCCTTTTACTGTTTCTGTGAGTTTCTGTTAAAGATGTGCTTAGGAGAGAGACTTTAGTACTCTCTCTCTTC
R L A R D N E L R E N D K E Q L K A I S T R D P L S E I T E Q E K >
TRANSLATION OF PI3KALPHA FULL-LENGTH-GFP2 [A] >

1610    1620    1630    1640    1650    1660    1670    1680    1690    1700
ATTTCTATAGGACTCACAGACTATTGTAAGTATCCCGAAATTTACCCAAATTTCTGCTGTTAAATGGAATTTAGAGATGAAGTAGCCCA
TAAAGATACCTCACTGTCTGTGATTAACATTTGATAGGGGCTTAAAGTGGGTTTAAAGAACAGACAGCAATTTACCTTAAAGTCTTACTTCACTCGGGT
D F L W S H R H Y C V T I P E I L P K L L S V K W N S R D E V A Q >
TRANSLATION OF PI3KALPHA FULL-LENGTH-GFP2 [A] >

1710    1720    1730    1740    1750    1760    1770    1780    1790    1800
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GATGTATGCTTGGTAAAAGATTGGCTCCAATCAAACCTGAACAGGCTATGGAACCTTGGACTGTAATACCCAGATCCTATGGTTCGAGGTTTGGCT
CTACATAACGAACCATTTTCTAACCGGAGGTTAGTTTGGACTTGTCCGATACCTTGAAGACCTGACATTAATGGGTCTAGGATACCAAGCTCCAAACGGA
M Y C L V K D W P P I K P E Q A M E L L D C N Y P D P M V R G F A >
TRANSLATION OF PI3KALPHA FULL-LENGTH-GFP2 [A] >

1810 1820 1830 1840 1850 1860 1870 1880 1890 1900
GTTGCGGCTTGGAAAAATATTTAACAGATGACAAACTTCTCAGTATTTAATTCAGCTAGTACAGGTCCTAAAAATGAACAATATTTGGATAACTTGC
CAAGCCAGCAACCTTTTATAAAATGCTACTGTTTGAAGAGTATATAAATTAAGTCGATCATGTCAGGATTTTATACTTGTATAAACCTATTTGAAGC
V R C T L E K Y L T D D K L S Q Y L I Q L V Q V L K Y E Q Y L D N L >
TRANSLATION OF PI3KALPHA FULL-LENGTH-GFP2 [A] >

1910 1920 1930 1940 1950 1960 1970 1980 1990 2000
TTGTGAGATTTTACTGAAGAAAGCATTGACTAATCAAAGGATTGGGCACCTTTTCTTTGGCATTAAAATCTGAGATGCACAATAAAACAGTTAGCCA
AACACTTAAAATGACTTTCTCCTAAGTATGATTTCTAACCCTGAAAAGAAAACCTAAATTTTGAACCTACGCTGTATTTTGTCAATCGGT
L V R F L L K K A L T N Q R I G H F F F W H L K S E M H N K T V S Q >
TRANSLATION OF PI3KALPHA FULL-LENGTH-GFP2 [A] >

2010 2020 2030 2040 2050 2060 2070 2080 2090 2100
GAGGTTGGGCTGCTTTTGGAGTCTATTTGCTGCATGTGGGATGATTTGAAGCACTGAATAGGCAAGTCGAGGCAATGGAAAAGCTCATAACTTA
TCCAAACCTGGGACGAAAACCTCAGGATAACAGCAGTACACCTACATAAATTCGTGGACTTATCCGTTCACTCCGTTTACCTTTTTCGAGTAAATGAAT
R F G L L L E S Y C R A C G M Y L K H L N R Q V E A M E K L I N L >
TRANSLATION OF PI3KALPHA FULL-LENGTH-GFP2 [A] >

2110 2120 2130 2140 2150 2160 2170 2180 2190 2200
ACTGACATTTCAACAGGAGAAGGATGAAACACAAAAGTACAGATGAAGTTTCTACTGAGCAAAATGAGGCGACAGATTTTATGATGCTCTAC
TGACTGTAAGAGTTTGTCCCTCTCTCTACTTTGTGTTTCCATGTCTACTTCAAAAATCAACTCGTTTACTCCGCTGGTCTAAAAGTACCTACGAGATG
T D I L K Q E K K D E T Q K V Q M K F L V E Q M R R P D F M D A L >
TRANSLATION OF PI3KALPHA FULL-LENGTH-GFP2 [A] >

2210 2220 2230 2240 2250 2260 2270 2280 2290 2300
AGGGTTCTGCTCTCTAAACCTGCTCATCAACTAGGAAACCTCAGGCTTGAAGAGTGTGCAATATGTCCTCTGCAAAAAGGCCACTGTGGTTGAA
TCCCGAAGCAGAGGAGATTTGGCAGGATGTTGATCTTTGGAGTCCGAATCTCAGACCTTAATACAGGAGAGCTTTTCCGTTGACACCAACT
Q G F L S P L N P A H Q L G N L R L E E C R I M S S A K R P L W L N >
TRANSLATION OF PI3KALPHA FULL-LENGTH-GFP2 [A] >

2310 2320 2330 2340 2350 2360 2370 2380 2390 2400
TTGGGAGAACCCAGACATCATGTCAGAGTACTGTTTCAAGCAATGAGATCATCTTTAAAATGGGGATGATTTACCGCAAGATATGCTAACACTTCAA
AACCTCTGGGCTGCTAGTACAGTCAATGACAAAAGTCTTGTACTCTAGTAAATTTTACCCTACTAAATGCCCTCTATACGATTTGAAAGT
W E N P D I M S E L L F Q N N E I I F K N G D D L R Q D M L T L Q >
TRANSLATION OF PI3KALPHA FULL-LENGTH-GFP2 [A] >

2410 2420 2430 2440 2450 2460 2470 2480 2490 2500
ATTATTCGATTTATGAAAATATCTGGCAAAATCAAGGCTTGTATCTTCAAGTGTACCTTATGTTGTCTGTCAATCGGTGACTGTGTGGACTTATTTG
TAATAAGCATAAATACCTTTTATAGACGCTTTTATGTTCCAGAACTAGAAGCTTACAAATGGAATACCAACAGACAGTTAGCCACTGACACACCTGAATAAC
I I R M E N I W Q N Q G L D L R M L P Y G C L S I G D C V G L I A >
TRANSLATION OF PI3KALPHA FULL-LENGTH-GFP2 [A] >

2510 2520 2530 2540 2550 2560 2570 2580 2590 2600
AGGTGGTCCGAAATCTCACACTATTTATGCAAAATCAAGTGCAAAAGCGGCTTGAAGAGTGCAGTCAAGTCAACAGCCACACACTACATCAGTGGCTCAA
TCCACCACGCTTAAAGAGTGTGATAATACGTTTAAAGTCAAGTTCGCGGCAACTTCCACAGTCAAGTCAAGTGTGTGGTGTGATGATGACACCGAGT
E V V R N S H T I M Q I O C K G G L K G A L Q F N S H T L H Q W L K >
TRANSLATION OF PI3KALPHA FULL-LENGTH-GFP2 [A] >

2610 2620 2630 2640 2650 2660 2670 2680 2690 2700
AGACAAGAACAAAGGAGAAATATATGATGACGACCTGTTTACAGTTCATGTGTGATGACTGTGTAGCTACTTCTTTGGGAATTTGGAGAT
TCTGTTCTGTTCTCTTTATATACTAGTCCGTAACCTGGACAAATGTGCAAGTACAGCAATGACACATCGAATGGAAGTAAAACCTTAACTCTTA
D K N K G E I Y D A A I D L F T R S C A G Y C V A T F I L G I G D >
TRANSLATION OF PI3KALPHA FULL-LENGTH-GFP2 [A] >

2710 2720 2730 2740 2750 2760 2770 2780 2790 2800
CGTCAAAATAGTAACTCATGTTGAAAGACGATGGCAACTGTTTCATATAGATTTTGGACACTTTTGGATCACAAGAAAGAAAAATTTGGTTATAAAC
GCAGTGTATACGATGATGAAAGCTTTCTGCTACTGTTGACAAAGTATATCTAAAACCTGTGAAAACCTAGTGTCTTCTTTTAACTACGTTAGT
R H N S N I M V K D D G Q L F H I D F G H F L D H K K K K F G Y K >
TRANSLATION OF PI3KALPHA FULL-LENGTH-GFP2 [A] >

2810 2820 2830 2840 2850 2860 2870 2880 2890 2900
GAGAAGTGTGCCATTGTTTGGACAGGATTTCTTAATAGTATTAGTAAAGGAGCCCAAGAAATGACAAAAGCAAGAGAAATTTGAGAGGTTTCAGGA
CTCTTGCACACGGAACAAACCTGCTCCTAAAGAAATATCACTAATTTCTCCGCTTCTTACGTTGTTCTTCTTCTTAACTCTCAAACTCTCAAACTCT
R E R V P F V L T Q D F L I V I S K G A Q E C T K T R E F E R F Q E >
TRANSLATION OF PI3KALPHA FULL-LENGTH-GFP2 [A] >

2910 2920 2930 2940 2950 2960 2970 2980 2990 3000
GATGTGTTCAAGGCTTATAGCTATTCGACAGCATGCCAATCTCTCATAAATCTTTCTCAATGATGCTGGGCTCTGGAATGCCAGCACTACAATCT
CTACAAATGTTCCGAATAGATCGATAAGCTGCTGACGTTAGAGAAGTATTAGAAAAGAGTTACTACGACCCGAGACCTTACGCTTCTGATGTAGA
M C Y K A Y L A I R Q H A N L F I N L F S M M L G S G M P E L Q S >
TRANSLATION OF PI3KALPHA FULL-LENGTH-GFP2 [A] >

3010 3020 3030 3040 3050 3060 3070 3080 3090 3100
TTTGTATGACATTCGATACATTCGAAAGACCTAGCTTAGATAAAAATGAGCAAGAGGCTTTGGAGTATTTCAATGAAAACAAATGAATGATGACATCATG
AAACTACTGTAAGCTATGTAAGCTTTTGGATCGGAATCTATTTGACTCGTTCCGAAAACCTATAAAGTACTTTGTTTACTACTACGTTAGTATAC
F D D I A Y I R K T L A L D K T E Q E A L E Y F M K Q M N D A H H >
TRANSLATION OF PI3KALPHA FULL-LENGTH-GFP2 [A] >

3110 3120 3130 3140 3150 3160 3170 3180 3190 3200
GTGGCTGGACAAACAAATGGATGGATCTCCACACAAATTAACAGCATGCATTGAACCTCGAGGGCGGGAGGATCTGGGGCGGAGGAAGTGGGGG
CACCACCTGTTGTTTACTTAACTAGAAGGTGTTAATTTGTCGACTGAACTTGGAGCTCCCGCCGCTCCTAGACCCCGCCCTCTTACCGCC
G G W T T K M D W I F H T I K Q H A L N L E G G G G S G G G S G >
TRANSLATION OF PI3KALPHA FULL-LENGTH-GFP2 [A] >

3210 3220 3230 3240 3250 3260 3270 3280 3290 3300
AGGGGCTTCCGCGCCGAGGAGTGGTATGGTGGAGCAAGGGGAGGAGCTTTCACCGGGTGGTGGCCATCCTGTTGAGCTGGAGGGGAGCAGTAAAC
TCCCGGAGACCCGCGCTCCCTACCATACCCTGTTCCCGCTCCTCGACAGTGGCCACCACCGGTTAGGACAGCTTACGCTGCGCTGCATTTG
G G S A A A G S G M V S K G E E L F T G V V P I L V E L D G D V N >
TRANSLATION OF PI3KALPHA FULL-LENGTH-GFP2 [A] >

3310 3320 3330 3340 3350 3360 3370 3380 3390 3400
GGCCCAAGTTTCAAGCTGTCCGCGGAGGGGAGGGCGATGCCACCTACGGCAAGCTGACCTGAAGTTCATCTGCACCAACCGGCAAGCTGGCCGCTCCCT
CCGTTGTTCAAGTCCGACAGGCGCTCCCGCTCCCGTACGCTGGATCCCGTTTCAAGTGGGACTTCAAGTACAGCGGTTGGCCGCTTCCGCGGGGAGGGA
G H K F S V S G E G E G D A T Y G K L T L K F I C T T G K L P V P >
TRANSLATION OF PI3KALPHA FULL-LENGTH-GFP2 [A] >

3410 3420 3430 3440 3450 3460 3470 3480 3490 3500
GGCCACCTCTGTGACCACTGACTACGGCTGCAGTGTCTCAGCGCTTACCCGACCAATGAAAGCAGCAGACTTCTTCAAGTCCGCCATGCCCGA
CCGGTGGGAGCAGTGCAGTCCGCGCAGTCCGAGTGGGCTGGTGTACTTGTCTGTGTAAGAAGTTCCAGCGGTACCGGCT
W P T L V T T L S Y G V Q C F S R Y P D H M K Q H D F F K S G M P E >

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TRANSLATION OF PI3KALPHA FULL-LENGTH-GFP2 [A]
3510 3520 3530 3540 3550 3560 3570 3580 3590 3600
AGGCTACGTCCAGGAGCGCACCATTTCTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCCGATC
TCCGATGACGGTCCCTCGCGTGGTAGAAGAAGTTCCTGCTGCCCTTGATGTTCTGGGGCGCGCTCCACTTCAAGCTCCCGCTGTGGGACCCTTGGCGTAG
G Y V Q E R T I F F K D D G N Y K T R A E V K F E G D T L V N R I>
TRANSLATION OF PI3KALPHA FULL-LENGTH-GFP2 [A]
3610 3620 3630 3640 3650 3660 3670 3680 3690 3700
GAGCTGAAGGCATCGACTTCAAGGAGGACGGCAACATCCCTGGGGCACAAGCTGGAGTACAACATAACAAGCCACAACGCTCTATATCATGGCCGACAAGC
CTCGACTTCCCGTAGCTGAAGTTCCTCCTGCGGTGTAGGACCCCGTTCGACCTCATGTTGATGTTGTCGGTGTTCAGATATAGTACCGGTGTTTCG
E L K G I D F K E D G N I L G H K L E Y N Y N S H N V Y I M A D K>
TRANSLATION OF PI3KALPHA FULL-LENGTH-GFP2 [A]
3710 3720 3730 3740 3750 3760 3770 3780 3790 3800
AGAAGAACCGCATCAAGTGAACCTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCATCGGGCA
TCTTCTTCCCGTAGTTCACCTTGAAGTTCAGGCGGTGTGTAGCTCCTGCGCTCGACGTCGAGCGGTGTTGATGGTCTCTTGTGGGGTAGCCGCT
Q K N G I K V N F K I R H N I E D G S V Q L A D H Y Q Q N T P I G D>
TRANSLATION OF PI3KALPHA FULL-LENGTH-GFP2 [A]
3810 3820 3830 3840 3850 3860 3870 3880 3890 3900
CGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCGCTGAGCAAAGACCCCAACGAGAAGCGGATCACATGGTCTCTGGAGTTC
GCGGGGACGACGACGGGTGTGGTGTGACTCGTGGGTGAGCGGGACTCGTTCTGGGGTGTCTCTCGCGTAGTGTACCAGGACGACCTCAAG
G P V L L P D N H Y L S T Q S A L S K D P N E K R D H M V L L E F>
TRANSLATION OF PI3KALPHA FULL-LENGTH-GFP2 [A]
3910 3920 3930 3940
GTGACCCCGCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAA
CACTGGCGGGCGCCCTAGTGAGAGCCGTACCTGCTCGACATGTTTATT
V T A A G I T L G M D E L Y K *>
TRANSLATION OF PI3KALPHA FULL-LENGTH-GFP2 [A]

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Sequence: PI3Kgamma RBD-GFP2 Range: 1 to 1167

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10      20      30      40      50      60      70      80      90      100
ATGAGCCGCGACCCCAAGCTCTACGCCATGCACCCGTGGGTGACGTCCCAAGCCCTCCCGGAGTACCTGTGGAAGAAGATTGCCAACARCTGCATCTTCA
TACTCGGGCTGGGGTTCGAGATGCGGTACGTGGGCACCCACTGCAGGTTCCGGGAGGGCCCTCATGGACACCTTCTTCAACGGTTGTTGACGTAGAAGT
M S R D P K L Y A M H P W V T S K P L P E Y L W K K I A N N C I F>
TRANSLATION OF PI3KGAMMA RBD-GFP2 [A] >

110     120     130     140     150     160     170     180     190     200
TCGTCAATCACCGCAGCACCACCAGCCAGACCATTAAGGTCTCACCCGACGACACCCCGGCGCCATCCTGCAGAGCTTCTTCAACAAGATGGCCAAGAA
AGCAGTAAGTGGCGTTCGAGTGGGTGCTGTAATCCAGAGTGGGTGCTGTGGGGCCCGGTAGGACGCTCGAAGAAGTGGTCTACCGGTCTT
I V I H R S T T S Q T I K V S P D D T P G A I L Q S F F T K M A K K>
TRANSLATION OF PI3KGAMMA RBD-GFP2 [A] >

210     220     230     240     250     260     270     280     290     300
GAAATCTCTGATGGATATTCGCCAAGCCAAAGCCAAAGCAGGATTTGTGCTGCGCGTCTGTGGCGGGATGAGTACCTGGTGGCGAAACGCCCATCAAA
CCTTAGAGACTACTATAAGGGCTTCGGTTTCGCTTCTCTAAACACGACGCGCAGACACCGGCCCTACTCATGGACACCCGCTTTCGGGTAGTTT
K S L M D I P E S Q S E Q D F V L R V C G R D E Y L V G E T P I K>
TRANSLATION OF PI3KGAMMA RBD-GFP2 [A] >

310     320     330     340     350     360     370     380     390     400
AACTCCAGTGGGTGAGGACTGCCTCAAGAACGGAGAAGATTACGCTGACTGACACGCTCCAGACCCCGCCCTCGAGGGCGGGGAGGATCTG
TTGAAGTCCACCACTCCGTGACGAGTCTTCTGCTCTTCTTAAGTGCACCATGACCTGTGCGGAGTCTGGGCGGGACTCCCGCCGCTCTTAGAC
N F Q W V R H C L K N G E E I H V V L D T P P D P A L E G G G G S>
TRANSLATION OF PI3KGAMMA RBD-GFP2 [A] >

410     420     430     440     450     460     470     480     490     500
GGGGCGGAGGAGTGGGGAGGGGCTCTCGCGCCGACGGGAGTGGTATGGTGGAGCAAGGGCGAGGAGCTGTTACCGGGTGGTCCCATCTCTGGTCA
CCCGCCTCCTTCCACCCCTCCCGGAGACGCGCGTCCCTACCATACCACCTCGTTCCCGCTCCTCGACAAGTGGCCACCACCGGTAGGACCACT
G G G G S G G G S A A A G S G M V S K G E E L F T G V V P I L V E>
TRANSLATION OF PI3KGAMMA RBD-GFP2 [A] >

510     520     530     540     550     560     570     580     590     600
GCTGGAGCGGACGTAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGGATGCCACCTACGGCAAGCTGACCTGAAGTTCATCTGCACCACC
CGACCTCGCCGTCATTTCCCGGTGTTCAAGTCCGACAGGCGCTCCCGTCCCGTACGGTGGATCCCGTTCGACTGGGACTTCAAGTAGACGTGGTGG
L D G D V N G H K F S V S G E G E D A T Y G K L T L K F I C T T>
TRANSLATION OF PI3KGAMMA RBD-GFP2 [A] >

610     620     630     640     650     660     670     680     690     700
GGCAAGTGGCGTCCCTGGCCACCCCTCGTGACACCCCTGAGCTACGGCGTGCAGTCTCAGCCGCTACCCCGACCATGAAGCAGCACGACTTCT
CCGTTCCAGCGGACGGGACCGGGTGGGACACTGGTGGACTCGATGCCGACCTCAGCAAGTCCGGATGGGGTGGTACTTCTGCTGCTGAAGA
G K L P V P W P T L V T T L S Y G V Q C F S R Y P D H M K Q H D F>
TRANSLATION OF PI3KGAMMA RBD-GFP2 [A] >

710     720     730     740     750     760     770     780     790     800
TCAAGTCCGCGATCCCGAAGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGCGCAACTACAAGACCCCGCGAGGTGAAGTTCGAGGGCGA
AGTTCAGCGGTACGGGCTCCGATGCAGTCTCCGCTGGTAGAAGAAGTCTCTGCTGCGCTGATGTTCTGGGCGGGCTCCACTTCAAGCTCCCGCT
F K S A M P E G Y V Q E R T I F F K D D G N Y K T R A E V K F E G D>
TRANSLATION OF PI3KGAMMA RBD-GFP2 [A] >

810     820     830     840     850     860     870     880     890     900
CACCTGGTGAACCCATCAGAGTGAAGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGACCAAGTGGAGTACAACACTACAACAGCCACACCTC
GTGGGACCACTTGGCGTAGCTCCGTTCCGTTAGTTCCTCTGCGGTTGTTAGGACCCCGTTCGACTCATGTTGATGTTGCTGGTGTGTCAG
T L V N R I E L K G I D F K E D G N I L G H K L E Y N Y N S H N V>
TRANSLATION OF PI3KGAMMA RBD-GFP2 [A] >

910     920     930     940     950     960     970     980     990     1000
TATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACCTCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGC
ATATAGTACCGGCTGTTGCTTCTTCCGCTAGTTCCTACTTGAAGTTCAGGCGGTGTTGTTAGCTCCTGCGCTCGCAGCTCGAGCGGCTGGTGTGGT
Y I M A D K Q K N G I K V N F K I R H N I E D G S V Q L A D H Y Q>
TRANSLATION OF PI3KGAMMA RBD-GFP2 [A] >

1010    1020    1030    1040    1050    1060    1070    1080    1090    1100
AGAACACCCCATCCGGCAGCGCCCGTGTGCTGCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCA
TCTTGTGGGGTAGCCGCTCCGGGGCAGCAGCGGGCTGTTGGTATGGACTCGTGGGTCAGGGGGGACTCGTTTCTGGGGTGTCTTCCGCGTAT
Q N T P I G D G P V L L P D N H Y L S T Q S A L S K D P N E K R D H>
TRANSLATION OF PI3KGAMMA RBD-GFP2 [A] >

1110    1120    1130    1140    1150    1160
CATGCTCTGCTGGAGTTCGTGACCCGCGCGGGATCACTCTCGGCATGGACGAGGTGTACAAGTAA
GTACCAGGACGACCTCAAGCACTGGCGGGCCCTAGTAGAGCCGTACTGCTCGACATGTTCAAT
M V L L E F V T A A G I T L G M D E L Y K *>
TRANSLATION OF PI3KGAMMA RBD-GFP2 [A] >
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Sequence: CRAF RBD-GFP2 Range: 1 to 1245

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10      20      30      40      50      60      70      80      90      100
ATGGAGCATAACAGGAGCTTGAAGACGATCAGCAATGGTTTTGGATTCAAAGATGCCGTGTTGATGGCTCCAGCTGCATCTCTCTACAATAGTTTC
TACCTCGTATGTCCCTCGAACCTTCTGCTAGTGGTTACCAAAACCTAAGTTTCTACGGCACAACCTACCGAGGTCGACGTAGAGAGGATGTTTATCAAG
M E H I Q G A W K T I S N G F G F K D A V F D G S S C I S P T I V>
TRANSLATION OF CRAF RBD-GFP2 [A] >

110     120     130     140     150     160     170     180     190     200
AGCAGTTTGGCTATCAGCGCCGGGCATCAGATGATGGCAAACCTACAGATCCTTCTAAGACAAGCAACACTATCCGTGTTTTCTTCCGCAACAAGCAAG
TCGTCAAACCGATAGTCCGGCCCTAGTCTACTACCGTTTGGAGTGTCTAGGAAGATTCTGTTGTTGTGATAGGCACAAAAGACGGCTTGTTCGTTTC
Q Q F G Y Q R R A S D D G K L T D P S K T S N T I R V F L P N K Q R>
TRANSLATION OF CRAF RBD-GFP2 [A] >

210     220     230     240     250     260     270     280     290     300
AACAGTGGTCAATGTGCGAAATGGAATGAGCTTGCATGACTGCCTTATGAAAGCACTCAAGGTGAGGGGCCTGCAACCAGAGTGCCTGTCAGTGTTCAGA
TTGTCACAGTTTACACGCTTACTCTCGAACCTACTGACGGAATACTTTCTGAGTTCCTACTCCCGGACGTTGGCTCTCACGACAGCTCACAGTCT
T V V N V R N G M S L H D C L M K A L K V R G L Q P E C C A V F R>
TRANSLATION OF CRAF RBD-GFP2 [A] >

310     320     330     340     350     360     370     380     390     400
CTTCTCCACGAACAAAAGTAAAAAGCAGCTTAGATTTGAAATCTGATGCTGGCTTGTGATTGGAGAGAAGTTCAGTAGATTTCCTGGGATCATG
GAAGAGTGTCTGTTTTCATTTTCTGTCGCAATCTAACCTTATGACTACGACGACGAACTAACCTTCTTGAAGTTCATCTAAAGACCTAGTAC
L L H E H K G K K A R L D W N T D A A S L I G E E L Q V D F L D H>
TRANSLATION OF CRAF RBD-GFP2 [A] >

410     420     430     440     450     460     470     480     490     500
TTCCTCCACAACACAACTTTGCTCGAAGACGCTTCTGAAGCTTAATTCATCGCTCGAGGGCGGGGAGGATCGGGGGCGGAGGAAGTGGGGGAGG
AAGGGAGTGTGTGTGTTGAAACGAGCTTCTGCAAGGACTTCAATTAAGTAGCGAGCTCCCGCCCTCTAGACCCCGCTTCTCACCCCTTCTCACCCCTCC
V P L T T H N F A R K T F L K L N S S L E G G G G S G G G G S G G G >
TRANSLATION OF CRAF RBD-GFP2 [A] >

510     520     530     540     550     560     570     580     590     600
GGGCTCTCGGGCCGAGGAGTGGTATGGTGGAGCAAGGGCGAGGAGTGTTCACCGGGTGGTCCCATCTGGTCGAGCTGGACGGCGAGCTAAACGGC
CCCGAGAGCCCGGCTCCCTCACCATACACTCGTTCCCGCTCTCGCAAGTGGCCCCACCGGTTAGGACAGCTCGACCTGGCCGTGCATTTGGCG
G S A A A G S G M V S K G E E L F T G V V P I L V E L D G D V N G >
TRANSLATION OF CRAF RBD-GFP2 [A] >

610     620     630     640     650     660     670     680     690     700
CACAAGTTTCAAGGTGTCCGGCAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCCTGAAGTTCATCTGCACCACGGCAAGCTGGCCCTGGC
GTGTTCAAGTCGACAGGCGCTCCCGCTCCCGCTACGGTGGATGCCGTTGACTGGGACTTCAAGTAGAGCTGGTGGCCGTTGACGGGACGGGACCG
H K F S V S G E G E G D A T Y G K L T L K F I C T T G K L P V P W>
TRANSLATION OF CRAF RBD-GFP2 [A] >

710     720     730     740     750     760     770     780     790     800
CCACCTCGTACCCCTGAGCTACGGCGTGCAGTGTCTACCGCTTACCCCGACCATGAAGCAGCAGACTTCTTCAAGTCCGCCATGCCCGAAGG
GGTGGGAGCAGTGGTGGGACTCGATGCCGACCTCACGAAGTCGGCGATGGGGCTGGTACTTCTGCTGCTGAAGAAGTTCAGGCGGTACGGGCTTCC
P T L V T T L S Y G V Q C F S R Y P D H M K Q H D F F K S A M P E G>
TRANSLATION OF CRAF RBD-GFP2 [A] >

810     820     830     840     850     860     870     880     890     900
CTACGCTCAGGAGCCACCTTCTTCAAGGACGACGGCAACTACAAGACCCCGCCGAGGTGAAGTTCGAGGGCGACCCCTGGTGAACCCGATCGAG
GATGACAGTCTCCGCTGGTAGAAGAAGTCTGCTGCCGTTGATGTTTGGGGCGGCTCCACTCAAGCTCCCGCTGTGGACCACTTGGCGTAGCTC
Y V Q E R T I F F K D D G N Y K T R A E V K F E G D T L V N R I E>
TRANSLATION OF CRAF RBD-GFP2 [A] >

910     920     930     940     950     960     970     980     990     1000
CTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCAAGCTGGAGTACAACACTACAACAGCCACAACGCTCTATATCATGGCCGACAAGCAGA
GACTTCCCGTAGCTGAAGTTCCTCTCCGCTTGTAGGACCCCGTGTGACCTCATGTTGATGTTGCTGGTGTGCAGATATAGTACCGGCTGTTGCTCT
L K G I D F K E D G N I L G H K L E Y N Y N S H N V Y I M A D K Q>
TRANSLATION OF CRAF RBD-GFP2 [A] >

1010    1020    1030    1040    1050    1060    1070    1080    1090    1100
AGAACGGCATCAAGGTGAACCTCAAGATCCGCCACAACATCGAGGACGGCAGGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCATCGCGGACGG
TCTTCCGCTAGTTCCACTTGAAGTTCTAGGCGGTGTGTAGCTCCTGCCGTCGACGTCGAGCGGCTGGTATGGTCTGTTGGGGGTAGCCGCTGCC
K N G I K V N F K I R H N I E D G S V Q L A D H Y Q Q N T P I G D G>
TRANSLATION OF CRAF RBD-GFP2 [A] >

1110    1120    1130    1140    1150    1160    1170    1180    1190    1200
CCCGTGTGCTGCCGACAACCACTACCTGAGCACCAGTCCCGCTGAGCAAAGACCCCAACGAGAAGCGGATCACATGGTCTGCTGGAGTTCGTG
GGGACGACGACGGCTGTTGGTATGGACTCGTGGGTGAGCGGACTCGTTTGGGGTGGCTTCCCGCTAGTGTACAGGACGACTCAAGCAC
P V L L P D N H Y L S T Q S A L S K D P N E K R D H M V L L E F V>
TRANSLATION OF CRAF RBD-GFP2 [A] >

1210    1220    1230    1240
ACCGCCCGGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAA
TGGCGCGGCCCTAGTGAGAGCCGACTACCTGCTCGACATGTTTCAAT
T A A G I T L G M D E L Y K *>
TRANSLATION OF CRAF RBD-GFP2 [A] >
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Sequence: GFP2- CRAF full-length S257L Range: 1 to 2724

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100
ATGGTGAAGGGCGAGGAGTGTTCCACCGGGTGGTCCCATCTCGTGGTGGAGCTGGACGGCGACGTAACCGCCACAAGTTCAGCGTGTCCCGCGAGG
TACCACCTGTTCCCGCTCTCGACAAAGTGGCCCGCCAGGGTAGGACCACTCGACCTGCGCTGCATTTGCGGGTGTCAAGTCGACAGGCGCCCTCC
M V S K G E E L F T G V V P I L V E L D G D V N G H K F S V S G E>
TRANSLATION OF GFP2- CRAF FULL-LENGTH S257L [A] >

110 120 130 140 150 160 170 180 190 200
GCGAGGCGATGACACCTACGGCAAGCTGACCCGAAAGTTCATCTGCACCACCGCAAGCTGCGCGTCCCTGGCCACCCCTCGTGACCACCCCTGAGCTA
CGCTCCCGCTACGGTGGATGCGCTTCGACTGGGACTTCAAGTAGACGTTGGTGGCGCTTCGACGGGCACGGGACCGGGTGGGAGCACTGGTGGGACTCGAT
G E G D A T Y G K L T L K F I C T T G K L P V P W P T L V T T L S Y>
TRANSLATION OF GFP2- CRAF FULL-LENGTH S257L [A] >

210 220 230 240 250 260 270 280 290 300
CGCGGTGCGAGTCTCAGCCGCTACCCGACCATGAAGCAGCAGACTTCTTCAAGTCCGCGATGCCGGAAGGCTACGTCCAGGAGCGCACCATCTTC
GCCGCAAGTACGAAAGTCCGCGATGGGCTGGTGTACTTCTGCTGCTGAAGAAGTTCAGCGGGTACCGGCTCCCGATGCAGGTCCTCGCTGGTGAAGA
G V Q C F S R Y P D H M K Q H D F F K S A M P E G Y V Q E R T I F>
TRANSLATION OF GFP2- CRAF FULL-LENGTH S257L [A] >

310 320 330 340 350 360 370 380 390 400
TTCAGGACACGCGCAACTACAAGCCCGCGCGAGGTAAGTTCGAGGGCGACACCCCTGGTGAACCCGATCGAGCTGAAGGGCATCGACTTCAAGGG
AAGTTCCTGCTGCCGTTGATGTTTCGGGCGCGCTCCACTTCAAGCTCCCGCTGGTGGGACCACTTGGCGTAGCTCGACTTCCGTAAGTTCCTCC
F K D D G N Y K T R A E V K F E G D T L V N R I E L K G I D F K E>
TRANSLATION OF GFP2- CRAF FULL-LENGTH S257L [A] >

410 420 430 440 450 460 470 480 490 500
ACGGCAACATCTGGGGCACAAGCTGGAGTACAACATAACAAGCCACAACCTATATATCATGGCCGACAAGCAGAAGACGGCATCAAGGTGAACCTCAA
TGCCGTTGAGGACCCCGCTTCGACCTCATGTTGATGTTGCGGTGTTGCGAGATATAGTACCGGCTGTTCTGCTTCTTGGCGTATGTTCCACTGAAGTT
D G N I L G H K L E Y N Y N S H N V Y I M A D K Q K N G I K V N F K>
TRANSLATION OF GFP2- CRAF FULL-LENGTH S257L [A] >

510 520 530 540 550 560 570 580 590 600
GATCCGCGACAACATCGAGGACGCGGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCATCGGCGACGGCCCGTGTGCTGCCGACAACCAC
CTAGGCGGTTGTTAGTCTCTGCGCTCGACGCTCGAGCGGCTGGTGTGCTTGTGGGGTAGCCGCTGCCGGGGCAGGACGAGGGCTGTGGTG
I R H N I E D G S V Q L A D H Y Q N T P I G D G P V L L P D N H>
TRANSLATION OF GFP2- CRAF FULL-LENGTH S257L [A] >

610 620 630 640 650 660 670 680 690 700
TACCTGAGCACCCAGTCCGCGCTGAGCAAGACCCCAACGAGAAGCGGATCACATGGTCTGCTGGAGTTCGTGACCGCGCGGGTACTCTCAGCA
ATGACTCGTGGGTCAGCGGGACTCGTTCGCGGTTGCTTTCGCGCTAGTGTACCAGGACGACCTCAAGCACTGGCGGGCCCTAGTGAGAGTCGT
Y L S T Q S A L S K D P N E K R D H M V L L E F V T A A G I T L S>
TRANSLATION OF GFP2- CRAF FULL-LENGTH S257L [A] >

710 720 730 740 750 760 770 780 790 800
TGGACGAGCTGTACAAGCTCGAGGGCGCGGAGGATCTGGGGCGGAGGAAGTGGGGGAGGGGCTCTGCGGCGCCATGGAGCACATACAGGGAGCTTG
ACCTGCTGACATGTTGAGCTCCGCGCTCTTAGACCCCGCTCCTTACCCCTCCCGGAGACGCGGGCGGTACCTCGTGTATGTTCCCTCGAAC
M D E L Y K L E G G G G S G G G S G G G S A A A M E H I Q G A W>
TRANSLATION OF GFP2- CRAF FULL-LENGTH S257L [A] >

810 820 830 840 850 860 870 880 890 900
GAAGCAGTACGCAATGTTTGGATCAAAGATGCCGTTGTTGATGGCTCCAGCTGCATCTCTCACAATAGTTTCCAGGTTTGGCTATCAGCGCGG
CTTCTGCTAGCTTACCAAACTAAGTTTTCAGGCAACAACCTACCGAGGTCGAGTACAGAGGATGTTATCAAGTCTCAAAACGATAGTCCGCGCC
K T I S N G F G F K D A V F D G S S C I S P T I V Q Q F G Y Q R R>
TRANSLATION OF GFP2- CRAF FULL-LENGTH S257L [A] >

910 920 930 940 950 960 970 980 990 1000
GCATCAGATGATGGCAACTCACAGATCCTTCTAAGACAAGCAACACTATCCGTTGTTTCTTGGCAACAAGCAAGAACAGTGGTCAATGTGCGAAATG
CGTAGTCTACTACCGTTTGAAGTGTAGGAAATCTGTTGTTGATAGGACAAAAGACCGGCTGTTGCTTCTGTCACCACTTACACGCTTTAC
A S D D G K L T D P S K T S N T I R V F L P N K Q R T V V N V R N>
TRANSLATION OF GFP2- CRAF FULL-LENGTH S257L [A] >

1010 1020 1030 1040 1050 1060 1070 1080 1090 1100
GAATGAGCTGTCATGCTGCTTATGAAAGCACTCAAGGTCAGGGGCTGCAACCAGAGTGTGTGCAAGTTCAGACTTCTCCAGCAACAAGGTTAA
CTTACTCGAAGTACTGACGGAATCTTCGTGAGTTCACCTCCCGGAGCTGGTCTCACGACACGTCACAAGTCTGAAGAGGTTGTTGTTCCATT
G M S L H D C L M K A L K V R G L Q P E C C A V F R L L H E H K G K>
TRANSLATION OF GFP2- CRAF FULL-LENGTH S257L [A] >

1110 1120 1130 1140 1150 1160 1170 1180 1190 1200
AAAAGCAGCTTAGATGGAACTAGTGTGCGTCTTGTGATGGAGAAAGTTCAGTATGATTTCTGGATCATGTTCCCTCACAAACACAACTTAA
TTTTGTCGAACTTAACCTTATGACTACGACGCAAGAACTAACCTCTTCTTGAAGTTCATCTAAAGACCTAGTCAAGGGGAGTGTGTTGTTGAAA
K A R L D W N T D A A S L I G E E L Q V D F L D H V P L T T H N F>
TRANSLATION OF GFP2- CRAF FULL-LENGTH S257L [A] >

1210 1220 1230 1240 1250 1260 1270 1280 1290 1300
GCTCGGAAGAGCTTCTGAAAGTTCGCTTGTGACATCTGTCAGAAATTCCTGCTCAATGGATTTTCATGATGTCAGACTTGTGGTACAAAATTTATGAGC
CGAGCTTCTGCAAGGACTTCGAAAGGACACTGTAGACAGTCTTAAAGGACGAGTACCTAAAGTACAGCTGTAACACCCGATGTTTAAAGTACTCG
A R K T F L K L A F C D I C Q K F L L N G F R C Q T C G Y K F H E>
TRANSLATION OF GFP2- CRAF FULL-LENGTH S257L [A] >

1310 1320 1330 1340 1350 1360 1370 1380 1390 1400
ACTGTAGCACCAAAGTACTACTATGTTGTTGAGTGGAGTAAACATCAGACAACTCTATTGTTTCCAAATTCACATATTGGTATAGTGGAGTCCAGC
TGACATCGTGGTTTTCATGGATGATACACACACTGACCTCATTTGATGCTGTTGAGAATAACAAGGTTTAAAGTGATAACCACTATCACCTCAGGGTCG
H C S T K V P T M C V D W S N I R Q L L L F P N S T I G D S G V P A>
TRANSLATION OF GFP2- CRAF FULL-LENGTH S257L [A] >

1410 1420 1430 1440 1450 1460 1470 1480 1490 1500
ACTACCTTCTTTGATATGCGTGTATGCGAGTCTGTTTCCAGGATGCCTGTTAGTTCCTCAGCACAGATATTTACACCTCACGCTTACCTTTAAC
TGATGGAAGAACTGACTACCGACTACGCTCTCAGACAAAGTCTTACGGACAATCAAGAGTCTGTTGTTATAGATGTTGAGTGGGAGTGGAAATG
L P S L T M R R M R E S V S R M P V S S Q H R Y S T P H A F T F N>
TRANSLATION OF GFP2- CRAF FULL-LENGTH S257L [A] >

1510 1520 1530 1540 1550 1560 1570 1580 1590 1600
ACCTCCAGTCCCTCATCTGAAGGTTCCCTCTCCAGGAGGAGGTTGACATCCACACCTAATGTCCACATGGTCAGCACCCCTGCTGTGGACAGCA
TGGAGGTCAGGAGTAGACTTCCAAAGGAGGAGGTTCCGCTTCCAACCTGAGGTTGGATTACAGGTTACAGTTCGTTGGTGGGACCGACCACTGCTGT
T S S P S S E G S L S Q R Q R L T S T P N V H M V S T T L P V D S>
TRANSLATION OF GFP2- CRAF FULL-LENGTH S257L [A] >

1610 1620 1630 1640 1650 1660 1670 1680 1690 1700
GGATGATGAGGATGCAATTCGAAGTTCAGCGAATCAGCCTCACCTCAGCCCTGTCAGTACGCCCAACAATCTGAGCCCAACAGGCTGGTACAGCC
CCTACTAACCTCAGTAAAGCTTACGTTGCTGCTTACTGCGGAGTGAAGTCCGACAGGTCATCGGGTGTGTTAGACTCGGGTGTCCGACCACTGCGG
R M I E D A I R S H S E S A S P S A L S S P N N L S P T G W S Q P>
TRANSLATION OF GFP2- CRAF FULL-LENGTH S257L [A] >

1710 1720 1730 1740 1750 1760 1770 1780 1790 1800
```

GAAAAACCCCGTCCAGCACAAAGAGAGCGGGCACCAGTATCTGGGACCCAGGAGAAAAACAAAATTAGGCCTCGTGGACAGAGAGATCAAGCTATTAT
CTTTTGGGGCACCGTCTGTGTTTCTCTCGCCCGTGGTCTAGACCCCTGGTCTCTTTTGGTTTAAATCCGGAGCACCTGTCTCTAAGTTCGATAATA
K T P V P A Q R E R A P V S G T Q E K N K I R P R G Q R D S S Y V >
TRANSLATION OF GFP2- CRAF FULL-LENGTH S257L [A] >

1810 1820 1830 1840 1850 1860 1870 1880 1890 1900
TGGGAAATAGAAGCCAGTGAAGTGATGCTGCCACTCGGATTTGGGTCAGGCTCTTTTGGAACTGTTTATAAGGGTAAATGGCAGGAGATGTTGCAGTAA
ACCCCTTATCTTCGGTCACTTCACTACGACAGGTGAGGCTAACCCAGTCCGAGAAAACCTTGACAAAATATCCCATTTACCGTGCCTCTACAACGCTATT
W E I E A S E V M L S T R I G S G S F G T V Y K G K W H G D V A V >
TRANSLATION OF GFP2- CRAF FULL-LENGTH S257L [A] >

1910 1920 1930 1940 1950 1960 1970 1980 1990 2000
AGATCCTAAGGTTGTGACCCAAACCCAGAGCAATCCAGGCTTCAGGAATGAGGTGGTGTCTGCGCAAAACACGGCATGTGAACATTTCTGCTTTT
TCTAGGATTTCCAAAGCTGGTGGGCTCTGCTTAAGTCCGGAGTCTTACTCCACGACAAGACGGCTTTTGTGCCGTACACTTGTAAAGACGAAAA
K I L K V V D P T P E Q F Q A F R N E V A V L R K T R H V N I L L F >
TRANSLATION OF GFP2- CRAF FULL-LENGTH S257L [A] >

2010 2020 2030 2040 2050 2060 2070 2080 2090 2100
CATGGGTACATGACAAAGGACAACTGGCAATTTGACCCAGTGGTCCGAGGGCAGCAGCTCTACAAAACCTGCATGTCAGGAGACCAAGTTTCAG
GTACCCCATGTACTGTTTCTGTTGACCCGTTAACACTGGTCCACAGCTCCCGTCCGCGAGATGTTTGTGGACGTACAGGTCCTCTGGTTCAAAGTC
M G Y M T K D N L A I V T Q W C E G S S L Y K H L H V Q E T K F Q >
TRANSLATION OF GFP2- CRAF FULL-LENGTH S257L [A] >

2110 2120 2130 2140 2150 2160 2170 2180 2190 2200
ATGTCCAGCTAATGACATGCGCCGAGACGGCTCAGGGAATGGACTATTGTCATGCAAAAGACATATCCATAGAGACATGAAATCCCAATATAT
TACAAGTCCGATTAAGTGAACGGGCGCTGCGGAGTCCCTTACCTGATAAACGTCAGCTTTCTGTAGTAGTATCTCTGTACTTTAGGTTGTTATATA
M F Q L I D I A R Q T A Q G M D Y L H A K N I I H R D M K S N N I >
TRANSLATION OF GFP2- CRAF FULL-LENGTH S257L [A] >

2210 2220 2230 2240 2250 2260 2270 2280 2290 2300
TTCTCCATGAAGGCTTAACAGTGAATAATGGAGATTTGGTTTGGCAACAGTAAAGTCAACGCTGGAGTGGTCTCAGCAGGTTGAACAACCTACTGGCTC
AAGAGGTACTTCCGAAATGTCACCTTTAACTTAAACCAACCGTTGTCATTTGAGTGGACCTCACCAGAGTCCCAACTTGTGATGACCCGAG
F L H E G L T V K I G D F G L A T V K S R W S G S Q Q V E Q P T G S >
TRANSLATION OF GFP2- CRAF FULL-LENGTH S257L [A] >

2310 2320 2330 2340 2350 2360 2370 2380 2390 2400
TGTCTCTGATGGCCCGAGGATGATCCGAATGACAGGATAACAACCCATTCAGTTTCCAGTCCGATGCTACTCTATGCGATCGTATTGTATGAACG
ACAGGAGACCTACCGGGTCTCCACTAGGCTTACGTCCTATTGTTGGTAAAGTCAAAGGTCAGCCTACAGATGAGGATACCGTAGCATAACATACTTGAC
V L W M A P E V I R M Q D N N P F S F Q S D V Y S Y G I V L Y E L >
TRANSLATION OF GFP2- CRAF FULL-LENGTH S257L [A] >

2410 2420 2430 2440 2450 2460 2470 2480 2490 2500
ATGACGGGGAGCTTCCATTCTCACATCAACAACCGAGATCAGATCATCTTCATGGTGGCCGAGGATATGCCCTCCAGATCTTAGTAAGCTATATA
TACTCCCCCTCGAAGAAATAGAGTGTAGTTTGGCTCTAGTCTAGTAGAAGTACCACCCGGCTCTATACGGAGGGGCTAGAAATCATTCGATATAT
M T G E L P Y S H I N N R D Q I I F M V G R G Y A S P D L S K L Y >
TRANSLATION OF GFP2- CRAF FULL-LENGTH S257L [A] >

2510 2520 2530 2540 2550 2560 2570 2580 2590 2600
AGAAGTCCCCAAAGCAATGAAGAGGCTGGTAGTGACTGTGTGAAGAAAGTAAAGGAAGAGAGGCTCTTTTCCCCAGATCCTGTCTCCATTGAGCT
TCTTGACGGGTTTCTGTTACTTCTCCGACCATCGACTGACACTTCTTTCATTTCTCTCGGAGAAAAAGGGTCTAGGACAGAAGGTAACATCGA
K N C P K A M K R L V A D C V K K V K E E R P L F P Q I L S S I E L >
TRANSLATION OF GFP2- CRAF FULL-LENGTH S257L [A] >

2610 2620 2630 2640 2650 2660 2670 2680 2690 2700
GCTCCAACTCTCTACCGAAGATCAACCGAGCGCTTCCGAGCCATCTTGCATCGGGCAGCCACACTGAGGATATCAATGCTTGCACGCTGACCCAG
CGAGTTGTGAGAGATGGCTTCTAGTTGGCTCGCAAGGCTCGTAGGAACGTAGCCCGTCCGGTGTGACTCTATAGTTACGAACGTGCGACTGGTGC
L Q H S L P K I N R S A S E P S L H R A A H T E D I N A C T L T T >
TRANSLATION OF GFP2- CRAF FULL-LENGTH S257L [A] >

2710 2720
TCCCGAGGCTGCCTGCTTCTAG
AGGGCTCCGACGACAGAAGATC
S P R L P V F * >
TRANSLATION OF GF >

Sequence: RALGDS RA-GFP2 Range: 1 to 1083

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10      20      30      40      50      60      70      80      90      100
ATGGCGCTGCCGCTCTACAACCCAGCAGTGGGCGACTGCTGCATCATCAGGCTCAGCGTGGATGTGGACAACGGCAACATGTACAAGAGCATCCTGGTGA
TACCCGCGACGGCGAGATGTTGGTCGTCACCCCGCTGACGACGCTAGTAGTCCAGTCCGACCTACACCTGTTGCCGTTGTACATGTTCTCGTAGGACCATT
M A L P L Y N Q Q V G D C C I I R V S L D V D N G N M Y K S I L V>
TRANSLATION OF RALGDS RA-GFP2 [A] >

110     120     130     140     150     160     170     180     190     200
CCAGCCAGGATAAGGCTCCGACTGTCATCCGCAAGGCTATGGACAAACACAACCTAGATGAGGACGAGCCGAGGATTATGAGCTGGTGCAGATCATCTC
GGTCGGTCCCTATTCCGAGGCTGACAGTAGGCGTTCGATACCTGTTTGTGGATCTACTCTGCTCGGCCCTTAATACTCGACCACGCTAGTAGAG
T S Q D K A P T V I R K A M D K H N L D E D E P E D Y E L V Q I I S>
TRANSLATION OF RALGDS RA-GFP2 [A] >

210     220     230     240     250     260     270     280     290     300
AGAGGATCACAAGCTGAAGATTCCAGAAAACGCCAATGTGTTCTATGCCATGAACTCTACGCCAACTATGACTTTGCTCAAGAAGCGGACCCCTCGAG
TCTCCTAGTCTTCTAAGGCTTTTGGCGTTTACACAAGATACGGTACTTGAGATGGCGGTTGATACTGAAACAGGAGTCTTCCGCTGGGAGCTC
E D H K L K I P E N A N V F Y A M N S T A N Y D F V L K K R T L E>
TRANSLATION OF RALGDS RA-GFP2 [A] >

310     320     330     340     350     360     370     380     390     400
GGCGCGGAGATCTGGGGCGGAGGAAGTGGGGGAGGGGGCTCTCGGCCGCGAGGAGTGGTATGGTGAGCAAGGGCGAGGAGCTGTTCCACCGGGTGG
CGCGCGCTCTAGACCCCGCTCTTCCACCCCTCCCCCGAGACGCGCGCTCCCTCACCATACCCTCGTTCCCGCTCTCGACAAGTGGCCACC
G G G G S G G G S G G G S A A A G S G M V S K G E E L F T G V>
TRANSLATION OF RALGDS RA-GFP2 [A] >

410     420     430     440     450     460     470     480     490     500
TGCCCATCTGGTGCAGCTGGACGGCGACCTAAACGGCCACAAGTTCAGCGTGTCCGCGAGGGCGAGGGCGATGCCACCTACGGCAAGCTGACCCCTGAA
ACGGTAGGACCAAGCTCGACTCGCGCTGCATTTCCGCGTGTTCAGTCCGACAGGCGCTCCCGCTCCCGCTACGGTGGATGCCCTTCGACTGGGACTT
V P I L V E L D G D V N G H K F S V S G E G E G D A T Y G K L T L K>
TRANSLATION OF RALGDS RA-GFP2 [A] >

510     520     530     540     550     560     570     580     590     600
GTTTCATGTCACCACCCGCAAGCTGCCCGTGGCCACCCTCGTGACCACCCCTGAGCTACGGCGTGCATGCTTCAGCCGCTACCCCGACCCACATG
CAAGTAGACCTGGTGGCGTTCGACGGGACCGGGTGGGAGCACTGGTGGGACTCGATGCCGCGACGTCACGAAGTCGGCGATGGGGCTGGTGTAC
F I C T T G K L P V P W P T L V T T L S Y G V Q C F S R Y P D H M>
TRANSLATION OF RALGDS RA-GFP2 [A] >

610     620     630     640     650     660     670     680     690     700
AAGCAGCAGACTTCTTCAAGTCCGCCATGCCGCAAGGCTACGTCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCCGCCGAGG
TTCGTCGTGTAAGAAGTTCAGGCGGTACGGGCTTCCGATGCAGTCTCCGCGTGTAGAAGAAGTTCCTGCTGCCGTTGATGTTCTGGGCGGGCTCC
K Q H D F F K S A M P E G Y V Q E R T I F F K D D G N Y K T R A E>
TRANSLATION OF RALGDS RA-GFP2 [A] >

710     720     730     740     750     760     770     780     790     800
TGAAGTTCGAGGGCGACACCCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAATA
ACTTCAAGCTCCCGCTGTGGGACCACTTGGCGTAGCTCGACTTCCCGTAGCTGAAGTTCCTCCTGCCGTTGTAGGACCCCGTTCGACCTCATGTTGAT
V K F E G D T L V N R I E L K G I D F K E D G N I L G H K L E Y N Y>
TRANSLATION OF RALGDS RA-GFP2 [A] >

810     820     830     840     850     860     870     880     890     900
CAACAGCCACAACGCTATATATCATGGCCGACAGCAGAAAGACGGCATCAAGGTGAACCTTCAAGATCCGCGCACAAACATCGAGGACGGCAGCGTGCAGCTC
GTTCTGGTGTTCGATATAGTACCGGCTGTTCCGCTTCTTCCGCTAGTTCACACTTGAAGTTCAGGCGGTGTTGTAGCTCCTGCCGTCGACGTCGAG
N S H N V Y I M A D K Q K N G I K V N F K I R H N I E D G S V Q L>
TRANSLATION OF RALGDS RA-GFP2 [A] >

910     920     930     940     950     960     970     980     990     1000
GCCGACCACTACCAGCAGAACCCCCATCGGGCAGCGGCCCGTGTCTGCTGCCGACAAACCACTACCTGAGCACCCAGTCCGCGCTGAGCAAAGACCCCA
CGGCTGGTATGTTGCTTGTGGGGTAGCCGCTGCGCGGGCACGACGACGGGCTGTTGGTGTGATGACTCGTGGGTGAGGCGGACTCGTTTCTGGGGT
A D H Y Q Q N T P I G D G P V L L P D N H Y L S T Q S A L S K D P>
TRANSLATION OF RALGDS RA-GFP2 [A] >

1010    1020    1030    1040    1050    1060    1070    1080
ACGAGAAGCGCATCACATGGTCTGCTGGAGTTCGTGACCCGCGCGGGATCACTCTCCGCTGGACGAGCTGTACAAGTAA
TGCTCTTCGCGCTAGTGTACCAGGACGACCTCAAGCACTGGCGCGGGCCCTAGTGAGAGCCGTACCTGCTCGACATGTTTCATT
N E K R D H M V L L E F V T A A G I T L G M D E L Y K *>
TRANSLATION OF RALGDS RA-GFP2 [A] >
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Sequence: RLuc8-KRASG12A full-length Range: 1 to 1560

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10      20      30      40      50      60      70      80      90      100
ATGACCAGCAAGGTGTACGACCCCGAGCAGAGGAAGGATGATCACCGCCCGCCAGTGGTGGCCAGGTGCAAGCAGATGAACGTGCTGGACAGCTTCA
TACTGGTCGTTCACATGCTGGGGCTCGTCTCCTCTCTACTAGTGGCCGGGGTACACCCCGTCCAGCTTCGTCTACTTGCACGACCTGTCGAAGT
M T S K V Y D P E Q R K R M I T G P Q W W A R C K Q M N V L D S F>
TRANSLATION OF RLuc8-KRASG12A FULL-LENGTH [A] >

110     120     130     140     150     160     170     180     190     200
TCAACTACTACGACAGCAGAGCAGCCGAGAACCCGCTGATCTTCCTGCACGGCAACGCCACTAGCAGTACCTGTGGAGGCACGTGGTCCCCACAT
AGTTGATGATGCTGTGCTCTTCGTCGGCTCTTGGGCACTAGAGGACGTGCCGTTGCGGTGATCGTCGATGGACACCTCCGTGCACCCAGGGGTGTA
I N Y Y D S E K H A E N A V I F L H G N A T S S Y L W R H V V P H I>
TRANSLATION OF RLuc8-KRASG12A FULL-LENGTH [A] >

210     220     230     240     250     260     270     280     290     300
CGAGCCCGTGGCCAGGTGCATCATCCCGATCTGATCGGCATGGGCAAGAGCGGCAAGAGCGGCAACGCCAGTACAGGCTGTGGACACTACAAGTAC
GCTCGGGCCACCGCTCCAGCTAGTAGGGGCTAGACTAGCCGTACCCGTTCTCGCCGTTCTCGCCGTTGCGGTCGATCCGACGACCTGGTGTATCTCATG
E P V A R C I I P D L I G M G K S G K S G N G S Y R L L D H Y K Y>
TRANSLATION OF RLuc8-KRASG12A FULL-LENGTH [A] >

310     320     330     340     350     360     370     380     390     400
CTGACCCGCTGGTTCGAGCTCCGAACTGCCAAGAATCATCTTCGTGGCCACGACTGGGGCGCCCGCTGGCCTTCCACTACGCCCTACGACACC
GACTGGCGGACCAAGCTCGAGGACTTGGACGGTTCCTCTAGTAGAAGCACCCGGTGTGACCCCGGGCGGACCGAAGGTGATGGCGGATGCTCGTGG
L T A W F E L L N L P K K I I F V G H D W G A A L A F H Y A Y E H>
TRANSLATION OF RLuc8-KRASG12A FULL-LENGTH [A] >

410     420     430     440     450     460     470     480     490     500
AGGACAGGATCAAGCCATCGTGCACATGGAGAGCGTGGTGGACCTGATCGAGAGCTGGGACGAGTGGCCAGACATCGAGAGGAGCATCGCCCTGATCAA
TCCTGTCTAGTTCGGTTCAGCAGTGTACTCTCGCACCCACTGACTAGCTCTCGACCCGCTCACCCTGCTGAGCTCCTCTGTAGCTCCTGTAGCTAGT
Q D R I K A I V H M E S V V D V I E S W D E W P D I E E D I A L I K>
TRANSLATION OF RLuc8-KRASG12A FULL-LENGTH [A] >

510     520     530     540     550     560     570     580     590     600
GAGCGAGGAGGGCGAGAAGATGGTGTGGAGAACAATCTTCGTGGAGACCGTGTGCCAGCAAGATCATGAGAAAAGTGGAGCCCGAGGAGTTCGCC
CTCGCTCCTCCGCTCTTCTACACGACCTCTTGTGTAAGAAGCACCTCTGGCACGACGGGTGCTTCTAGTACTCTTTCGACCTCGGGCTCCTCAAGCGG
S E E G E K M V L E N N F F V E T V L P S K I M R K L E P E E F A>
TRANSLATION OF RLuc8-KRASG12A FULL-LENGTH [A] >

610     620     630     640     650     660     670     680     690     700
GCCTACTGGAGCCCTCAAGGAGAAGGGGAGGTGAGAAGACCACCTGAGCTGGCCAGAGAGATCCCCCTGGTGAAGGGCGGCAAGCCGAGCTGG
CGGATGGACCTCGGAAGTTCCTTCCCGCTCCACTCTTCTGGTGGGACTCGACCCGGTCTCTAGGGGGACCACTCCCGCGTTCGGGCTGCACC
A Y L E P F K E K G E V R R P T L S W P R E I P L V K G G K P D V>
TRANSLATION OF RLuc8-KRASG12A FULL-LENGTH [A] >

710     720     730     740     750     760     770     780     790     800
TGCAGATCGTGAGAACTACAACCGCTACCTGAGAGCCAGCGACCTGCCAAGCTGTTTCATCGAGAGCGACCCCGGCTTTCAGCAACGCCATCGT
ACGCTAGCACTCTTTGATTTCCGGATGGACTCTCGGTGCTGCTGGACGGTTCGACAAGTAGCTCTCGTGGGGCCGAAAGTCTGTCGGGTAGCA
V Q I V R N Y N A Y L R A S D D L P K L F I E S D P G F F S N A I V>
TRANSLATION OF RLuc8-KRASG12A FULL-LENGTH [A] >

810     820     830     840     850     860     870     880     890     900
GGAGGGCCCAAGAAGTTCGCCAACCCGAGTTCGTGAAGGTGAAGGCCCTGCACCTTCTCCAGGAGGACGCCCGCAGAGATGGGCAAGTACATCAAG
CTCCCGCGTTCCTCAAGGGTGTGGCTCAAGCACTTCCACTTCCCGGACGTGAAGGAGTCTCTCGGGGGCTGTCTACCCGTTTCATGTATGTC
E G A K K F P N T E F V K V K G L H F L Q E D A P D E M G K Y I K>
TRANSLATION OF RLuc8-KRASG12A FULL-LENGTH [A] >

910     920     930     940     950     960     970     980     990     1000
AGCTTCGTGGAGAGAGTCTGAAGAACGAGCAGCTCGAGGGCGGGAGGATCTGGGGGGGAGGAAGTGGGGGAGGGGGCTTGCGGCCGCTATGACCG
TCGAGACACCTCTCTCAGCACTCTTGTGCTGCTGAGCTCCCGCCGCTCTTAGACCCCGCCCTTCCACCCCTCCCGGAGACCGCGGCTATGAGC
S F V E R V L K N E Q L E G G G G S G G G G S G G G S A A A M T>
TRANSLATION OF RLuc8-KRASG12A FULL-LENGTH [A] >

1010    1020    1030    1040    1050    1060    1070    1080    1090    1100
AATATAAACTTGTGAGTGTGGAGCTGCTGGCTAGGCAAGAGTGCCTTGCAGTACAGCTAATTCAGAATCATTTTGTGGACGAATATGATCCAACAAT
TTATATTTGAACACCACTCAACTCCGACCGCATCCGTTCTCAGGAACCTGCTATGTCGATTAAGTCTTAGTAAAACACCTGCTTACTAGTTGTTA
E Y K L V V V G A A G V G K S A L T I Q L I Q N H F V D E Y D P T I>
TRANSLATION OF RLuc8-KRASG12A FULL-LENGTH [A] >

1110    1120    1130    1140    1150    1160    1170    1180    1190    1200
AGAGGATTCCTACAGGAAGCAAGTATGATGGAGAAACCTGCTCTTGGATATTCCTGACACAGCAGGTCAAGAGGAGTACAGTGAATGAGGGAC
TATCCTAAGATGCTCTTCGTTTCATTAACCTCTTGGACAGAACTATAAGAGTGTGTGCTCCAGTTCCTCTCATGTCACGTTACTCCCTG
E D S A Y R K Q V V I D G E T C L L D I L D T A G Q E E Y S A M R D>
TRANSLATION OF RLuc8-KRASG12A FULL-LENGTH [A] >

1210    1220    1230    1240    1250    1260    1270    1280    1290    1300
CAGTACATGAGGACTGGGGAGGGCTTTCTTTGTGATTTGGCATAAATAACTAAATCAATTTGAAGATATTCCACATTATAGAGAAACAAATTAAGAG
GTCATGTACTCTGACCCCTCCCGAAAGAAACACATAAACCGTATTTATATGATTTAGTAACTTCTATAAGTGGTAATATCTCTTCTTTAATTTCTC
Q Y M R T G E G F L C V F A I N N T K S F E D I H H Y R E Q I K R>
TRANSLATION OF RLuc8-KRASG12A FULL-LENGTH [A] >

1310    1320    1330    1340    1350    1360    1370    1380    1390    1400
TTAAGACTCTGAAGATGACCTATGGTCTAGTAGGAAATAAATGATTTGCCTTCCAGAACAGTACAGACAAAACAGGCTCAGGACTTAGCAAGAG
AATTCCTGAGACTTCTACATGATACAGGATCATCTTTATTTACACTAAACGGAAGTCTTGTCTATGTTTGTCCGAGTCTGAATCGTTCTCTC
V K D S E D V P M V L V G N K C D L P S R T V D T K Q A Q D L A R S>
TRANSLATION OF RLuc8-KRASG12A FULL-LENGTH [A] >

1410    1420    1430    1440    1450    1460    1470    1480    1490    1500
TTATGGAATTCCTTTTATGAAACATCAGCAAGACAAGACAGGGTGTGATGATGCTTCTATACATAGTTCGAGAAATTCGAAACATAAAGAAAAG
AATACCTTAAGGAAAATAACTTTGAGTCTTCTGTTCTGCCAAGTACTACGGAAGATATGATTAAGTCTTTAAGCTTTTGTATTTCTTTTC
Y G I P F I E T S A K T R Q G V D D A F Y T L V R E I R K H K E K>
TRANSLATION OF RLuc8-KRASG12A FULL-LENGTH [A] >

1510    1520    1530    1540    1550    1560
ATGAGCAAGAGTGGTAAAAAGAAAAGAAAGTCAAGACAAAGTGTGTAATATGATAA
TACTCGTTTCTACCAATTTTCTTCTTTTCTTCTCAGTTTCTGTTTTCACACATTAATACAT
M S K D G K K K K K K S K T K C V I M *>
TRANSLATION OF RLuc8-KRASG12A FULL-LENGTH [A] >
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Sequence: RLUC8-KRASG12C full-length Range: 1 to 1560

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10      20      30      40      50      60      70      80      90      100
ATGACCAGCAAGGTGTACGACCCCGAGCAGAGGAAGGATGATCACCGCCCGCCAGTGGTGGCCAGGTGCAAGCAGATGAACGTGCTGGACAGCTTCA
TACTGGTCGTTCCACATGCTGGGGCTCGTCTCCTCTCTACTAGTGGCCGGGGTACACCCCGTCCAGCTTCGCTACTTGCACGACCTGTCGAAGT
M T S K V Y D P E Q R K R M I T G P Q W W A R C K Q M N V L D S F>
TRANSLATION OF RLUC8-KRASG12C FULL-LENGTH [A] >

110     120     130     140     150     160     170     180     190     200
TCAACTACTACGACAGCAGAGCAGCCGAGAACCCGCTGATCTTCTGACGGCAACGCCACTAGCAGTACCTGTGGAGGCACGTGGTCCCCACAT
AGTTGATGATGCTGTCGCTCTTCGTCGGCTCTGCGCACTAGAGGACGTGCCGTTGCGGTGATCGTCGATGGACACCTCCGTGCACCCAGGGGTGTA
I N Y Y D S E K H A E N A V I F L H G N A T S S Y L W R H V V P H I>
TRANSLATION OF RLUC8-KRASG12C FULL-LENGTH [A] >

210     220     230     240     250     260     270     280     290     300
CGAGCCCGTGGCCAGGTGCATCATCCCGATCTGATCGGCATGGGCAAGAGCGGCAAGAGCGGCAACGCCAGTACAGGCTGTGGACACTACAAGTAC
GCTCGGGCCACCGCTCCAGCTAGTAGGGGTAGACTAGCCGTACCCGTTCTCGCCGTTCTCGCCGTTGCGGTCGATCCGACGACCTGGTGTATTCATG
E P V A R C I I P D L I G M G K S G K S G N G S Y R L L D H Y K Y>
TRANSLATION OF RLUC8-KRASG12C FULL-LENGTH [A] >

310     320     330     340     350     360     370     380     390     400
CTGACCCGCTGGTTCGAGCTCCGAACTGCCAAGAATCATCTTCTGGGCCACGACTGGGGCGCCCGCTGGCCTTCCACTACGCCACTACGACACC
GACTGGCGGACCAAGCTCGAGGACTTGGACGGTTCCTCTAGTAGAAGCACCCGCTGCTGACCCCGGGCGGACCGAAGGTGATGGCGGATGCTCGTGG
L T A W F E L L N L P K K I I F V G H D W G A A L A F H Y A Y E H>
TRANSLATION OF RLUC8-KRASG12C FULL-LENGTH [A] >

410     420     430     440     450     460     470     480     490     500
AGGACAGGATCAAGCCATCGTGCACATGGAGAGCGTGGTGGACCTGATCGAGAGCTGGGACGAGTGGCCAGACATCGAGAGGAGCATCGCCCTGATCAA
TCCTGTCTAGTTCGCGTAGCAGCTGTACCTTCGACCCACCTGCACTAGCTCTCGACCCGCTCACCAGGCTGTAGCTCCTCTGTAGCTGAGTGTAGT
Q D R I K A I V H M E S V V D V I E S W D E W P D I E E D I A L I K>
TRANSLATION OF RLUC8-KRASG12C FULL-LENGTH [A] >

510     520     530     540     550     560     570     580     590     600
GAGCGAGGAGGGCGAGAAGATGGTGTGGAGAACAATCTTCTGGAGAGCCGCTGCCAGCAAGATCATGAGAAAAGTGGAGCCCGAGGAGTTCGCC
CTCGCTCCTCCGCTCTTCTACCAAGCTCTTGTGTAAGAAGCACTTGGCACGACGGGTGCTTGTAGTACTTCTTCGACCTCGGGCTCCTCAAGCGG
S E E G E K M V L E N N F F V E T V L P S K I M R K L E P E E F A>
TRANSLATION OF RLUC8-KRASG12C FULL-LENGTH [A] >

610     620     630     640     650     660     670     680     690     700
GCCTACTGGAGCCCTCAAGGAGAAGGGGAGGTGAGAAGACCACCTGAGCTGGCCAGAGAGATCCCCCTGGTGAAGGGCGGCAAGCCGAGCTGG
CGGATGGACCTCGGAAGTTCCTTCCGCTCCACTTCTGGTGGGACTCGACCCGCTCTTAGGGGACCACTCCCGCGTTCGGGCTGCACC
A Y L E P F K E K G E V R R P T L S W P R E I P L V K G G K P D V>
TRANSLATION OF RLUC8-KRASG12C FULL-LENGTH [A] >

710     720     730     740     750     760     770     780     790     800
TGCAGATCGTGAGAACTACAACCGCTACCTGAGAGCCAGCGACCTGCCAAGCTGTTTCATCGAGAGCGACCCCGGCTTTCAGCAACGCCATCGT
ACGCTAGCACTCTTTGATTTCCGGATGGACTTCCGCTCGCTGCTGGAGGGTTCGACAAGTAGCTCTCGTGGGGCGGAAAGTCTTGGCGGTAGCA
V Q I V R N Y N A Y L R A S D D L P K L F I E S D P G F F S N A I V>
TRANSLATION OF RLUC8-KRASG12C FULL-LENGTH [A] >

810     820     830     840     850     860     870     880     890     900
GGAGGGCCCAAGAAGTTCGCCAACCCAGTTCGTGAAGGTGAAGGCCCTGCACCTTCTCCAGGAGGACGCCCGCAGAGATGGGCAAGTACATCAAG
CTCCCGCGTTCCTCAAGGGTGGTCAAGCACTTCCACTTCCCGGACGTGAAGGAGTCTCCGCTGGGGGCTGCTACCCGTTTCATGTATGTC
E G A K K F P N T E F V K V K G L H F L Q E D A P D E M G K Y I K>
TRANSLATION OF RLUC8-KRASG12C FULL-LENGTH [A] >

910     920     930     940     950     960     970     980     990     1000
AGCTTCTGGAGAGAGTCTGAAGAACGAGCAGCTCGAGGGCGGGAGGATCTGGGGCGGAGGAAGTGGGGAGGGGGCTTGCAGCCGCTATGACCG
TCGAGACACCTCTCTCAGCACTCTTGTGCTCGTCCGCTCCCGCCGCTCTAGACCCCGCTCTTCCACCCCTCCCGGACGACCCGGGATATGGC
S F V E R V L K N E Q L E G G G G S G G G G S G G G S A A A M T>
TRANSLATION OF RLUC8-KRASG12C FULL-LENGTH [A] >

1010    1020    1030    1040    1050    1060    1070    1080    1090    1100
AATATAAACTTGTGAGTGGAGCTTGGGCTAGGCAAGAGTGCCTTGCAGATACAGCTAATTCAGAATCATTTTGTGGACGAATATGATCCAACAAT
TTATATTTGAACACCACTCAACTCGAACCCGCATCCGTTCTCAGGAACCTGCTATGTCGATTAAGTCTTAGTAAAACACCTGCTTACTAGTTGTTA
E Y K L V V V G A C G V G K S A L T I Q L I Q N H F V D E Y D P T I>
TRANSLATION OF RLUC8-KRASG12C FULL-LENGTH [A] >

1110    1120    1130    1140    1150    1160    1170    1180    1190    1200
AGAGGATTCCTACAGGAAGCAAGTATGATGGAGAAACCTGCTCTTGGATATTTCTCGACACAGCAGGTCAAGAGGAGTACAGTGAATGAGGGAC
TATCCTAAGATGCTCTTCGTTTATCACTAATTAACCTCTTGGACAGAACTATAAGAGTGTGTGTCAGTTCCTCTCATGTCACGTTACTCCCTG
E D S A Y R K Q V V I D G E T C L L D I L D T A G Q E E Y S A M R D>
TRANSLATION OF RLUC8-KRASG12C FULL-LENGTH [A] >

1210    1220    1230    1240    1250    1260    1270    1280    1290    1300
CAGTACATGAGGACTGGGGAGGGCTTTCTTGTGATTTGGCCATAAATACTAAATCAATTTGAAGATATTCCACCATATAGAGAAACAAATTAAGAG
GTCATGTACTCTGACCCCTCCGAAAGAAACACATAAACCGTATTTATATGATTTAGTAACTTCTATAAGTGGTAATATCTTCTTTTATTTTCTC
Q Y M R T G E G F L C V F A I N N T K S F E D I H H Y R E Q I K R>
TRANSLATION OF RLUC8-KRASG12C FULL-LENGTH [A] >

1310    1320    1330    1340    1350    1360    1370    1380    1390    1400
TTAAGACTCTGAAGATGACCTATGGTCTAGTAGGAAATAAATGTGATTTGCCTTCCAGAACAGTACAGACAAAACAGGCTCAGGACTTAGCAAGAG
AATTCCTGAGACTTCTACATGATACAGGATCATCTTTATTTACACTAAACGGAAGTCTTGTCTGTGTTTGTCCGAGTCTGAATCGTTCTTC
V K D S E D V P M V L V G N K C D L P S R T V D T K Q A Q D L A R S>
TRANSLATION OF RLUC8-KRASG12C FULL-LENGTH [A] >

1410    1420    1430    1440    1450    1460    1470    1480    1490    1500
TTATGGAATTCCTTTTATTTGAAACATCAGCAAGACAAGAGGGTGTGATGATGCTTCTATACATAGTTCGAGAAATTCGAAACATAAAGAAAAG
AATACCTTAAGGAAAATAAATTTAGTCTTCTGTTCTGTCACCACTACTACGGAAGATATGATTAAGCTCTTTAAGCTTTTGTATTTCTTTTC
Y G I P F I E T S A K T R Q G V D D A F Y T L V R E I R K H K E K>
TRANSLATION OF RLUC8-KRASG12C FULL-LENGTH [A] >

1510    1520    1530    1540    1550    1560
ATGAGCAAGAGTGGTAAAAAGAAAAGAAAGTCAAGACAAAGTGTGTAATATGTA
TACTCGTTTCTACCAATTTTCTTCTTTTCTTCTCAGTTTCTGTTTTCACACATTAATACAT
M S K D G K K K K K K S K T K C V I M *>
TRANSLATION OF RLUC8-KRASG12C FULL-LENGTH [A] >
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Sequence: RLUC8-KRASG12D full-length Range: 1 to 1560

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10      20      30      40      50      60      70      80      90      100
ATGACCAGCAAGGTGTACGACCCCGAGCAGAGGAAGGATGATCACCGCCCGCCAGTGGTGGCCAGGTGCAAGCAGATGAACGTGCTGGACAGCTTCA
TACTGGTCGTTCACATGCTGGGGCTCGTCTCCTCTCTACTAGTGGCCGGGGTACACCCCGTCCAGCTTCGTCTACTTGCACGACCTGTCGAAAT
M T S K V Y D P E Q R K R M I T G P Q W W A R C K Q M N V L D S F>
TRANSLATION OF RLUC8-KRASG12D FULL-LENGTH [A] >

110     120     130     140     150     160     170     180     190     200
TCAACTACTACGACAGCAGAAGCAGCCGAGAACCCGCTGATCTTCTGACGGCAACGCCACTAGCAGTACCTGTGGAGGCACGTGGTCCCCACAT
AGTTGATGATGCTGTCGCTCTTCGTCGGCTCTGCGCACTAGAGGACGTGCCGTTGCGGTGATCGTCGATGGACACCTCCGTGCACCCAGGGGTGTA
I N Y Y D S E K H A E N A V I F L H G N A T S S Y L W R H V V P H I>
TRANSLATION OF RLUC8-KRASG12D FULL-LENGTH [A] >

210     220     230     240     250     260     270     280     290     300
CGAGCCCGTGGCCAGGTGCATCATCCCGATCTGATCGGCATGGGCAAGAGCGGCAAGAGCGGCAACGCCAGTACAGGCTGTGGACACTACAAGTAC
GCTCGGGCCACCGCTCCAGCTAGTAGGGCTAGACTAGCCGTACCCGTTCTCGCCGTTCTCGCCGTTGCGGTCGATCCGACGACCTGGTGTATCTCATG
E P V A R C I I P D L I G M G K S G K S G N G S Y R L L D H Y K Y>
TRANSLATION OF RLUC8-KRASG12D FULL-LENGTH [A] >

310     320     330     340     350     360     370     380     390     400
CTGACCCGCTGGTTCGAGCTTCAAGCTGCCAAGAAGATCATCTTCTGGGCCACGACTGGGGCGCCCGCTGGCCTTCCACTACGCCCTACGACACC
GACTGGCGGACCAAGCTCGAGGACTTGGACGGTTCCTCTAGTAGAAGCACCAGTGTGACCCCGGGCGGACCGAAGGTGATGGCGGATGCTCGTGG
L T A W F E L L N L P K K I I F V G H D W G A A L A F H Y A Y E H>
TRANSLATION OF RLUC8-KRASG12D FULL-LENGTH [A] >

410     420     430     440     450     460     470     480     490     500
AGGACAGGATCAAGCCATCGTGCACATGGAGAGCGTGGTGGACCTGATCGAGAGCTGGGACGAGTGGCCAGACATCGAGAGGAGCATCGCCCTGATCAA
TCCTGTCTAGTTCGCGTAGCAGCTGTACCTTCGACCCACCTGCACTAGCTCTCGACCCGCTCACCAGTGTAGCTCCTCTGTAGCTCCTGTAGCTAGT
Q D R I K A I V H M E S V V D V I E S W D E W P D I E E D I A L I K>
TRANSLATION OF RLUC8-KRASG12D FULL-LENGTH [A] >

510     520     530     540     550     560     570     580     590     600
GAGCGAGGAGGGCGAGAAGATGGTGTGGAGAACAATCTTCTGAGAGCCGCTGCTGCCAGCAAGATCATGAGAAAGCTGGAGCCCGAGGAGTTCGCC
CTCGCTCCTCCGCTCTTCTACACGACCTCTTGTGTAAGAAGCACCCTGGCACGACGGGTGCTTAGTACTCTTTCGACCTCGGGCTCCTCAAGCGG
S E E G E K M V L E N N F F V E T V L P S K I M R K L E P E E F A>
TRANSLATION OF RLUC8-KRASG12D FULL-LENGTH [A] >

610     620     630     640     650     660     670     680     690     700
GCCTACTGGAGCCCTCAAGGAGAAGGGGAGGTGAGAAGACCACCTGAGCTGGCCAGAGAGATCCCCCTGGTGAAGGGCGGCAAGCCGAGCTGG
CGGATGGACCTCGGAAGTTCCTTCCCGCTCCACTTCTGGTGGGACTCGACCCGGTCTCTAGGGGACCACTTCCCGCGTTCGGGCTGCACC
A Y L E P F K E K G E V R R P T L S W P R E I P L V K G G K P D V>
TRANSLATION OF RLUC8-KRASG12D FULL-LENGTH [A] >

710     720     730     740     750     760     770     780     790     800
TGCAGATCGTGAGAACTACAACCGCTACCTGAGAGCCAGCGACCTGCCAAGCTGTTTCATCGAGAGCGACCCCGGCTTTCAGCAACGCCATCGT
ACGCTAGCACTCTTTGATTTCCGGATGGACTTCCGCTCGCTGCTGGACGGTTCGACAAGTAGCTCTCGTGGGGCGGAAAGTCTTGGCGGTAGCA
V Q I V R N Y N A Y L R A S D D L P K L F I E S D P G F F S N A I V>
TRANSLATION OF RLUC8-KRASG12D FULL-LENGTH [A] >

810     820     830     840     850     860     870     880     890     900
GGAGGGCCCAAGAAGTTCACCAACCCGAGTTCGTGAAGGTGAAGGCCCTGCACCTTCTCCAGGAGGACGCCCGCAGAGATGGGCAAGTACATCAAG
CTCCCGCGTTCCTCAAGGGTGTGGCTCAAGCACTTCCACTTCCCGGACGTGAAGGAGTCTCTCGGGGGCTGTCTACCCGTTTCATGTATTC
E G A K K F P N T E F V K V K G L H F L Q E D A P D E M G K Y I K>
TRANSLATION OF RLUC8-KRASG12D FULL-LENGTH [A] >

910     920     930     940     950     960     970     980     990     1000
AGCTTCTGGAGAGAGTCTGAAGAACGAGCAGCTCGAGGGCGGGAGGATCTGGGGCGGAGGAAGTGGGGAGGGGGCTTGCAGCCGCTATGACCG
TCGAGACACCTCTCTCAGCACTTCTGCTCGTGCAGCTCCCGCCGCTCTTAGACCCCGCTTCTTACCCCTCCCGGAGACCGCGGCTATCTGGC
S F V E R V L K N E Q L E G G G G S G G G G S G G G S A A A M T>
TRANSLATION OF RLUC8-KRASG12D FULL-LENGTH [A] >

1010    1020    1030    1040    1050    1060    1070    1080    1090    1100
AATATAAACTTGTGAGTGGAGCTGACGGCTAGGCAAGAGTGCCTTACGATACAGCTAATTCAGAATCATTTTGTGGACGAATATGATCCAACAAT
TTATATTTGAACACCATCAACTCCGATCCGCTTCTCAGGAAGTCTATGTCGATTAAGTCTTAGTAAACACCTGCTTACTAGTTGGTTGA
E Y K L V V V G A D G V G K S A L T I Q L I Q N H F V D E Y D P T I>
TRANSLATION OF RLUC8-KRASG12D FULL-LENGTH [A] >

1110    1120    1130    1140    1150    1160    1170    1180    1190    1200
AGAGGATTCCTACAGGAAGCAAGTATGATGGAGAAACCTGCTCTTGGATATTTCTCGACACAGCAGGTCAAGAGGAGTACAGTGAATGAGGGAC
TATCCTAAGATGCTCTTCGTTTCATTAACCTCTTGGACAGAACTATAAGAGTGTGTGTCAGTTCCTCTCATGTCACGTTACTCCCTG
E D S Y R K Q V V I D G E T C L L D I L D T A G Q E E Y S A M R D>
TRANSLATION OF RLUC8-KRASG12D FULL-LENGTH [A] >

1210    1220    1230    1240    1250    1260    1270    1280    1290    1300
CAGTACATGAGGACTGGGGAGGGCTTTCTTGTGATTTGGCATAAATAACTAAATCAATTTGAAGATATTCACCATTATAGAGAAACAAATTAAGAG
GTCATGTACTCTGACCCCTCCGAAAGAAACACATAAACCGGTATTTATATGATTTAGTAACTTCTATAAGTGGTAATATCTTCTTTTATTTTCTC
Q Y M R T G E G F L C V F A I N N T K S F E D I H H Y R E Q I K R>
TRANSLATION OF RLUC8-KRASG12D FULL-LENGTH [A] >

1310    1320    1330    1340    1350    1360    1370    1380    1390    1400
TTAAGGACTTGAAGATGACCTATGGTCTAGTAGGAAATAAATGATTTGCCTTCCAGAACAGTACAGACAAAACAGGCTCAGGACTTAGCAAGAG
AATTCCTGAGACTTCTACATGATACAGGATCATCTTTATTTACACTAAACGGAAGTCTTGTCTATGTTTGTCCGAGTCTGAATCGTTCTTC
V K D S E D V P M V L V G N K C D L P S R T V D T K Q A Q D L A R S>
TRANSLATION OF RLUC8-KRASG12D FULL-LENGTH [A] >

1410    1420    1430    1440    1450    1460    1470    1480    1490    1500
TTATGGAATTCCTTTTATGAAACATCAGCAAGACAAGCAGGGTGTGATGATGCTTCTATACATAGTTCGAGAAATTCGAAACATAAAGAAAAG
AATACCTTAAGGAAAATAACTTTGATGCTTCTGTTCTCCCAACTACTACGGAAGATATGATTAAGCTCTTTAAGCTTTGATTTCTTTTC
Y G I P F I E T S A K T R Q G V D D A F Y T L V R E I R K H K E K>
TRANSLATION OF RLUC8-KRASG12D FULL-LENGTH [A] >

1510    1520    1530    1540    1550    1560
ATGAGCAAGAGTGGTAAAAAGAAAAGAAAGTCAAGACAAAGTGTGTAATATGTA
TACTCGTTTCTACCAATTTTCTTCTTTTCTTCTCAGTTTCTGTTTTCACACATTAATACAT
M S K D G K K K K K K S K T K C V I M *>
TRANSLATION OF RLUC8-KRASG12D FULL-LENGTH [A] >
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Sequence: RLUC8-KRASG12R full-length Range: 1 to 1560

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10      20      30      40      50      60      70      80      90      100
ATGACCAGCAAGGTGTACGACCCCGAGCAGAGGAAGGATGATCACCAGCCCGCCAGTGGTGGCCAGGTGCAAGCAGATGAACGTGCTGGACAGCTTCA
TACTGGTCGTTCCACATCGTGGGGCTCGTCTCCTCTCTACTAGTGGCCGGGGTACCACCCGGTCCAGCTTCGCTACTTGCACGACCTGTCGAAGT
M T S K V Y D P E Q R K R M I T G P Q W W A R C K Q M N V L D S F>
TRANSLATION OF RLUC8-KRASG12R FULL-LENGTH [A] >

110     120     130     140     150     160     170     180     190     200
TCAACTACTACGACAGCAGAAAGCAGCCGAGAACCCGCTGATCTTCTGCACGGCAACGCCACTAGCAGTACCTGTGGAGGCACGTGGTCCCCACAT
AGTTGATGATGCTGTGCTCTTCGTCGGCTCTTGGGCACTAGAGGACGTGCCGTTGCGGTGATCGTCGATGGACACCTCCGTGCACCCAGGGGTGTA
I N Y Y D S E K H A E N A V I F L H G N A T S S Y L W R H V V P H I>
TRANSLATION OF RLUC8-KRASG12R FULL-LENGTH [A] >

210     220     230     240     250     260     270     280     290     300
CGAGCCCGTGGCCAGGTGCATCATCCCGATCTGATCGGCATGGGCAAGAGCGGCAAGAGCGGCAACGCCAGTACAGGCTGTGGACCTACAAGTAC
GCTCGGGCACCCTCCACTAGTAGGGCTAGACTAGCCGTACCCGTTCTCGCCGTTCTCGCCGTTGCGGTCGATCCGACGACCTGGTGTATTCATG
E P V A R C I I P D L I G M G K S G K S G N G S Y R L L D H Y K Y>
TRANSLATION OF RLUC8-KRASG12R FULL-LENGTH [A] >

310     320     330     340     350     360     370     380     390     400
CTGACCCGCTGGTTCGAGCTCCGAACTGCCAAGAATCATCTTCGTGGCCACGACTGGGGCGCCGCTGGCCTTCCACTACGCCCTACGACACC
GACTGGCGGACCAAGCTCGAGGACTTGGACGGTTCTTCTAGTAGAAGCACCAGGTCGTGACCCCGGGGGGACCGGAAGGTGATGGCGGATGCTCGTGG
L T A W F E L L N L P K K I I F V G H D W G A A L A F H Y A Y E H>
TRANSLATION OF RLUC8-KRASG12R FULL-LENGTH [A] >

410     420     430     440     450     460     470     480     490     500
AGGACAGGATCAAGCCATCGTGCACATGGAGAGCGTGGTGGACCTGATCGAGAGCTGGGACGAGTGGCCAGACATCGAGGAGGACATCGCCCTGATCAA
TCCTGTCTAGTTCGCGTAGCAGCTGTACCTTCGACCCACCTGCAGCTAGCTCTCGACCCGCTCACCAGGTCGTAGCTCCTCTGATGGGACTAGT
Q D R I K A I V H M E S V V D V I E S W D E W P D I E E D I A L I K>
TRANSLATION OF RLUC8-KRASG12R FULL-LENGTH [A] >

510     520     530     540     550     560     570     580     590     600
GAGCGAGGAGGGGCGAGAAGTGGTGTGGAGAACAATCTTCTGTTGAGAGCCGTCGTCGCCAGCAAGATCATGAGAAAAGTGGAGCCCGAGGAGTTCGCC
CTCGCTCCTCCGCTCTTCTACACGACCTCTTGTGTAAGAAGCACCCTGGCACGACGGGTGCTTAGTACTCTTTCGACCTCGGGCTCCTCAAGCGG
S E E G E K M V L E N N F F V E T V L P S K I M R K L E P E E F A>
TRANSLATION OF RLUC8-KRASG12R FULL-LENGTH [A] >

610     620     630     640     650     660     670     680     690     700
GCCTACTGGAGCCCTCAAGGAGAAGGGGAGGTGAGAAGACCACCTGAGCTGGCCAGAGAGATCCCCCTGGTGAAGGGCGGCAAGCCGAGCTGG
CGGATGGACCTCGGAAGTTCCTTCCCGCTCCACTCTTCTGGTGGGACTCGACCCGGTCTCTAGGGGGACCACTCCCGCGTTCGGGTGCACC
A Y L E P F K E K G E V R R P T L S W P R E I P L V K G G K P D V>
TRANSLATION OF RLUC8-KRASG12R FULL-LENGTH [A] >

710     720     730     740     750     760     770     780     790     800
TGCAGATCGTGAGAACTACAACCGCTACCTGAGAGCCAGCGACCTGCCAAGCTGTTTCATCGAGAGCGACCCCGGCTTTCAGCAACGCCATCGT
ACGCTAGCACTCTTGTATGTTGCGGATGGACTTCGCTCGCTGCTGGAGGGTTCGACAAGTAGCTCTCGTGGGGCGGAAGAAGTCTGGCGGTAGCA
V Q I V R N Y N A Y L R A S D D L P K L F I E S D P G F F S N A I V>
TRANSLATION OF RLUC8-KRASG12R FULL-LENGTH [A] >

810     820     830     840     850     860     870     880     890     900
GGAGGGCCCAAGAAGTTCGCCAACCCAGTTCGTTGAAGGTGAAGGCCCTGCACCTTCTCCAGGAGGACGCCCGCAGAGATGGGCAAGTACATCAAG
CTCCCGCGTTCCTCAAGGGTGTGGCTCAAGCACTTCCACTTCCCGGACGTGAAGGAGTCTTCCGCGGGGCTGCTACCCGTTTCATGTATGTC
E G A K K F P N T E F V K V K G L H F L Q E D A P D E M G K Y I K>
TRANSLATION OF RLUC8-KRASG12R FULL-LENGTH [A] >

910     920     930     940     950     960     970     980     990     1000
AGCTTCGTGGAGAGTCTGAAGAACGAGCAGCTCGAGGGCGGGAGGATCTGGGGGGGAGGAAGTGGGGGAGGGGGCTTGCGGCCGCTATGACCG
TCGAGACACCTCTCTCAGCACTCTTGTGCTCGTGCAGCTCCCGCCGCTCTTAGACCCCGCCCTTCCACCCCTCCCGGAGACCGCGGCTATGAGC
S F V E R V L K N E Q L E G G G G S G G G G S G G G S A A A M T>
TRANSLATION OF RLUC8-KRASG12R FULL-LENGTH [A] >

1010    1020    1030    1040    1050    1060    1070    1080    1090    1100
AATATAAACTTGTGTAGTGGAGCTCGTGGCTAGGCAAGAGTGCCTTGACGATACAGCTAATTCAGAATCATTTCGTTGGACGAATATGATCCAACAAT
TTATATTTGAACACCATCAACTCGAGCAGCCGATCCGTTCTCAGGAACCTGCTATGTCGATTAGTCTTAGTAAACACCTGCTTACTAGTGGTGTGA
E Y K L V V V G A R G V G K S A L T I Q L I Q N H F V D E Y D P T I>
TRANSLATION OF RLUC8-KRASG12R FULL-LENGTH [A] >

1110    1120    1130    1140    1150    1160    1170    1180    1190    1200
AGAGGATTCCTACAGGAAGCAAGTATGATGGAGAAACCTGCTCTTGGATATTCCTGACACAGCAGGTCAAGAGGAGTACAGTGAATGAGGGAC
TATCCTAAGATGCTCTTCGTTTATCACTAATACTACTCTTGGACAGAACTATAAGAGTGTGTGCTCCAGTTCCTCTCATGTCACGTTACTCCCTG
E D S A Y R K Q V V I D G E T C L L D I L D T A G Q E E Y S A M R D>
TRANSLATION OF RLUC8-KRASG12R FULL-LENGTH [A] >

1210    1220    1230    1240    1250    1260    1270    1280    1290    1300
CAGTACATGAGGACTGGGGAGGGCTTTCTTGTGTAATTTGCCATAAATAACTAAATCAATTTGAAGATATTCACCATTATAGAGAAACAAATAAAAGAG
GTCATGTACTCTGACCCCTCCCGAAAGAAACACATAAACCAGTATTTATATGATTTAGTAACTTCTATAAGTGGTAATATCTCTTCTTAAATTTCTC
Q Y M R T G E G F L C V F A I N N T K S F E D I H H Y R E Q I K R>
TRANSLATION OF RLUC8-KRASG12R FULL-LENGTH [A] >

1310    1320    1330    1340    1350    1360    1370    1380    1390    1400
TTAAGACTCTGAAGATGACCTATGGTCTAGTAGGAATAAATGATGATTTGCCTTCAGAACAGTACAGACAAAAACAGGCTCAGGACTTAGCAAGAG
AATTCCTGAGACTTCTACATGATACAGGATCATCTTTATTTACACTAAACGGAAGTCTTGTCTATGTTTGTCCGAGTCTGAATCGTTCTCTC
V K D S E D V P M V L V G N K C D L P S R T V D T K Q A Q D L A R S>
TRANSLATION OF RLUC8-KRASG12R FULL-LENGTH [A] >

1410    1420    1430    1440    1450    1460    1470    1480    1490    1500
TTATGGAATTCCTTTTATGAAACATCAGCAAGACAAGACAGGGTGTGATGATGCTTCTATACATAGTTCGAGAAATTCGAAAACATAAAGAAAAG
AATACCTTAAGGAAAATAACTTTGATGCTTCTGTTCTGTCACCAACTACTACGGAAGATATGATTAAGCTTCTTAAAGTTTGTATTTCTTTTC
Y G I P F I E T S A K T R Q G V D D A F Y T L V R E I R K H K E K>
TRANSLATION OF RLUC8-KRASG12R FULL-LENGTH [A] >

1510    1520    1530    1540    1550    1560
ATGAGCAAGAGTGGTAAAAAGAAGAAAAGAGTCAAGACAAAGTGTGTAATATGTAAT
TACTCGTTTCTACCAATTTTCTTCTTTTCTTCTCAGTTTCTGTTTTCACACATTAATACAT
M S K D G K K K K K K S K T K C V I M *>
TRANSLATION OF RLUC8-KRASG12R FULL-LENGTH [A] >
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Sequence: RLUC8-KRASG12V full-length Range: 1 to 1560

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10 20 30 40 50 60 70 80 90 100
ATGACCAGCAAGGTGTACGACCCCGAGCAGAGGAAGAGGATGATCACCGCCCGCCAGTGGTGGCCAGGTGCAAGCAGATGAACGTGCTGGACAGCTTCA
TACTGGTGGTCCACATGCTGGGGCTCGTCTCCTCTCTACTAGTGGCCGGGGTACCACCCGGTCCAGCTTCGTCTACTTGCACGACCTGTCGAAGT
M T S K V Y D P E Q R K R M I T G P Q W W A R C K Q M N V L D S F>
TRANSLATION OF RLUC8-KRASG12V FULL-LENGTH [A] >

110 120 130 140 150 160 170 180 190 200
TCAACTACTACGACAGCAGAGCAGCCGAGAACCCGCTGATCTTCCTGCACGGCAACGCCACTAGCAGTACCTGTGGAGGCACGTGGTCCCCACAT
AGTTGATGATGCTGTGCTGCTTTCGTCGGCTCTTGGGCACTAGAGGACGTGCCGTTGGCGTATCGTTCGATGGACACCTCCGTGCACCCAGGGGTGTA
I N Y Y D S E K H A E N A V I F L H G N A T S S Y L W R H V V P H I>
TRANSLATION OF RLUC8-KRASG12V FULL-LENGTH [A] >

210 220 230 240 250 260 270 280 290 300
CGAGCCCGTGGCCAGGTGCATCATCCCGATCTGATCGGCATGGGCAAGAGCGGCAAGAGCGGCAACGCCAGTACAGGCTGTGGACCACTACAAGTAC
GCTCGGGCCACCGCTCCAGCTAGTAGGGGTAGACTAGCCGTACCCGTTCTCGCCGTTCTCGCCGTTGGCGTTCGATCCGACGACCTGGTGTATTCATG
E P V A R C I I P D L I G M G K S G K S G N G S Y R L L D H Y K Y>
TRANSLATION OF RLUC8-KRASG12V FULL-LENGTH [A] >

310 320 330 340 350 360 370 380 390 400
CTGACCCGCTGGTTCGAGCTTCAAGCTGCCCCAAGAATCATCTTCGTGGCCACGACTGGGGCGCCCGCTGGCCTTCCACTACGCCACTACGACACC
GACTGGCGGACCAAGCTCGAGGACTTGGACGGTTCCTCTAGTAGAAGCACCCGGTGTGACCCCGGGCGGACCGAAGGTGATGGCGGATGCTCGTGG
L T A W F E L L N L P K K I I F V G H D W G A A L A F H Y A Y E H>
TRANSLATION OF RLUC8-KRASG12V FULL-LENGTH [A] >

410 420 430 440 450 460 470 480 490 500
AGGACAGGATCAAGCCATCGTGCACATGGAGAGCGTGGTGGACCTGATCGAGAGCTGGGACGAGTGGCCAGACATCGAGAGGACATCGCCCTGATCAA
TCCTGTCTAGTTCGGTTCAGCAGTGTACTCTCGCACCCACTGACTAGCTCTCGACCCGCTCACCCTGCTAGCTCCTCTGTAGCTCCTGTAGCTAGT
Q D R I K A I V H M E S V V D V I E S W D E W P D I E E D I A L I K>
TRANSLATION OF RLUC8-KRASG12V FULL-LENGTH [A] >

510 520 530 540 550 560 570 580 590 600
GAGCGAGGAGGGCGAGAAGTGTGCTGGAGAACAATCTTCGTGGAGACCGTGTGCCAGCAAGATCATGAGAAAGCTGGAGCCCGAGGAGTTCGCC
CTCGCTCCTCCGCTCTTCTACCAAGCTCTTGTGTAAGAAGCACTCTGGCAGGACGGGTGCTTAGTACTCTTTCGACCTCGGGCTCCTCAAGCGG
S E E G E K M V L E N N F F V E T V L P S K I M R K L E P E E F A>
TRANSLATION OF RLUC8-KRASG12V FULL-LENGTH [A] >

610 620 630 640 650 660 670 680 690 700
GCCTACTGGAGCCCTCAAGGAGAAGGGGAGGTGAGAAGCCACCCTGAGCTGGCCAGAGAGATCCCCCTGGTGAAGGGCGGCAAGCCGAGCTGG
CGGATGGACCTCGGAAGTTCCTTCCCGCTCCACTTCTGGTGGGACTCGACCCGGTCTCTAGGGGACCACTTCCCGCGTTCGGGTGCACC
A Y L E P F K E K G E V R R P T L S W P R E I P L V K G G K P D V>
TRANSLATION OF RLUC8-KRASG12V FULL-LENGTH [A] >

710 720 730 740 750 760 770 780 790 800
TGCAGATCGTGAGAACTACAACCGCTACCTGAGAGCCAGCGACCTGCCAAGCTGTTTCATCGAGAGCGACCCCGGCTTTCAGCAACGCCATCGT
ACGCTAGCACTCTTTGATTTCCGGATGGACTTCCGCTCGCTGGTGGAGGGTTCGACAAGTAGCTCTCGTGGGGCCGAAAGTCTTGGCGGTAGCA
V Q I V R N Y N A Y L R A S D D L P K L F I E S D P G F F S N A I V>
TRANSLATION OF RLUC8-KRASG12V FULL-LENGTH [A] >

810 820 830 840 850 860 870 880 890 900
GGAGGGCCCAAGAAGTTCACCAACCCAGTTCCTGAAAGTGAAGGCTGCACTTCTCCAGGAGGACGCCCGCAGAGATGGGCAAGTACATCAAG
CTCCCGCGTTCCTCAAGGGTGGTCAAGCACTTCCACTTCCCGGACGTGAAGGAGTCTCCTCGGGGGCTGTCTACCCGTTTCATGTATGTC
E G A K K F P N T E F V K V K G L H F L Q E D A P D E M G K Y I K>
TRANSLATION OF RLUC8-KRASG12V FULL-LENGTH [A] >

910 920 930 940 950 960 970 980 990 1000
AGCTTCGTGGAGAGTGTGGAAGCAAGCAGCTCGAGGGCGGGAGGATCTGGGGCGGAGGAAGTGGGGAGGGGGCTTGCGGCCGCTATGACCC
TCGAGACACCTCTCTCAGCACTTCTGCTCGTGCAGCTCCCGCCGCTCTTAGACCCCGCTCTTCAACCCCTCCCGGAGACCGGGGATATGGC
S F V E R V L K N E Q L E G G G G S G G G G S G G G S A A A M T>
TRANSLATION OF RLUC8-KRASG12V FULL-LENGTH [A] >

1010 1020 1030 1040 1050 1060 1070 1080 1090 1100
AATATAAACTTGTGAGTGGAGCTGTGGCTAGGCAAGAGTGCCTTGCAGATACAGCTAATTCAGAATCATTTTGTGGACGAATATGATCCAACAAT
TTATATTTGAACACCACTCAACTCCGACACCGCATCCGTTCTCAGGAACCTGCTATGTCGATTAACTTCTAGTAAACACCTGCTTACTAGTGGTTGTA
E Y K L V V V G A V G V G K S A L T I Q L I Q N H F V D E Y D P T I>
TRANSLATION OF RLUC8-KRASG12V FULL-LENGTH [A] >

1110 1120 1130 1140 1150 1160 1170 1180 1190 1200
AGAGGATTCCTACAGGAAGCAAGTATGATGGAGAAACCTGCTCTTGGATATTCCTGACACAGCAGGTCAAGAGGAGTACAGTGAATGAGGGAC
TATCCTAAGATGCTCTTCGTTTCATTAACCTCTTGGCAGAGAACCTATAAGAGTGTGTCGTCAGTTCCTCTCATGTCACGTTACTCCCTG
E D S Y R K Q V V I D G E T C L L D I L D T A G Q E E Y S A M R D>
TRANSLATION OF RLUC8-KRASG12V FULL-LENGTH [A] >

1210 1220 1230 1240 1250 1260 1270 1280 1290 1300
CAGTACATGAGGACTGGGGAGGGCTTTCTTTGTGATTTGGCATAAATAACTAAATCAATTTGAAGATATTTCATCATTATAGAGAAACAAATTAAGAG
GTCATGTACTTCTGACCCCTCCGAAAGAAACACATAAACCGTATTTATATGATTTAGTAACTTCTATAAGTAGTAATATCTCTTTTAAATTTTCTC
Q Y M R T G E G F L C V F A I N N T K S F E D I H H Y R E Q I K R>
TRANSLATION OF RLUC8-KRASG12V FULL-LENGTH [A] >

1310 1320 1330 1340 1350 1360 1370 1380 1390 1400
TTAAGGACTTGAAGATGACCTATGGTCTAGTAGGAAATAAATGTGATTTGCCTTCAGAACAGTACAGACAAAACAGGCTCAGGACTTAGCAAGAG
AATTCCTGAGACTTCTACATGATACCAGGATCATCTTTATTTACACTAAACGGAAGTCTTGTCTGTGTTTGTCCGAGTCTGAATCGTTCTCTC
V K D S E D V P M V L V G N K C D L P S R T V D T K Q A Q D L A R S>
TRANSLATION OF RLUC8-KRASG12V FULL-LENGTH [A] >

1410 1420 1430 1440 1450 1460 1470 1480 1490 1500
TTATGGAATTCCTTTTATGAAACATCAGCAAGACAAGCAGGGTGTGATGATGCTTCTATACATAGTTCGAGAAATTCGAAACATAAAGAAAAG
AATACCTTAAGAAAATAACTTTAGTCTTCTGTTCTGCCAAGTACTACGGAAGATATGATTAAGCTCTTAAAGCTTTTAAAGTTTGTATTTCTTTTC
Y G I P F I E T S A K T R Q G V D D A F Y T L V R E I R K H K E K>
TRANSLATION OF RLUC8-KRASG12V FULL-LENGTH [A] >

1510 1520 1530 1540 1550 1560
ATGAGCAAGAGTGTAAAAAGAAAAGAAAGTCAAGACAAAAGTGTAAATATGTAA
TACTCGTTTCTACCAATTTTCTTCTTCTTCTCAGTTTCTGTTTTCACACATTAATACATTT
M S K D G K K K K K K S K T K C V I M *>
TRANSLATION OF RLUC8-KRASG12V FULL-LENGTH [A] >
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Sequence: RLuc8-KRASWT full-length Range: 1 to 1560

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10      20      30      40      50      60      70      80      90      100
ATGACCAGCAAGGTGTACGACCCCGAGCAGAGGAAGAGGATGATCACCGCCCGCCAGTGGTGGCCAGGTGCAARGCAGATGAACGTGCTGGACAGCTTCA
TACTGGTCGTTCCACATGCTGGGGCTCGTCTCCTCTCTACTAGTGGCCGGGGTACACCACCGGTCCAGTTCTGCTACTTGCACGACCTGTCGGAAGT
M T S K V Y D P E Q R K R M I T G P Q W W A R C K Q M N V L D S F>
TRANSLATION OF RLuc8-KRASWT FULL-LENGTH [A] >

110     120     130     140     150     160     170     180     190     200
TCAACTACTACGACAGCAGAGAAGCAGCCGAGAACCCGCTGATCTTCTGACGGCAACGCCACTAGCAGTACCTGTGGAGGCACGTGGTCCGCCACAT
AGTTGATGATGCTGTGCTGCTTCGTCGGCTCTTGGGCACTAGAGGACGTCCGCTTGGCGGTATCGTTCGATGGACACCTCCGTGCACCACGGGGTGTGA
I N Y Y D S E K H A E N A V I F L H G N A T S S Y L W R H V V P H I>
TRANSLATION OF RLuc8-KRASWT FULL-LENGTH [A] >

210     220     230     240     250     260     270     280     290     300
CGAGCCCGTGGCCAGGTGCATCATCCCGATCTGATCGGCATGGCAAGAGCGGCAAGAGCGGCAACGCCAGTACAGGCTGTGGACACTACAAGTAC
GCTCGGGCCACCGCTCCACTAGTAGGGCTAGACTAGCCGTACCCGTTCTCGCCGTTCTCGCCGTTGCGGTCGATGCCGACACCTGGTGATGTTCAATG
E P V A R C I I P D L I G M G K S G K S G N G S Y R L L D H Y K Y>
TRANSLATION OF RLuc8-KRASWT FULL-LENGTH [A] >

310     320     330     340     350     360     370     380     390     400
CTGACCCGCTGGTTCGAGCTCCGAACTGCCCCAAGAATCATCTTCTGGGCCACGACTGGGGGCGCCCTGGCCTTCCACTACGCCCTACGAGCACC
GACTGGCGGACCAAGCTCGAGGACTTGGACGGTCTTCTAGTAGAAGCACCAGGTGCTGACCCCGGGCGGACCGAAGGTGATGGCGGATGCTCGTGG
L T A W F E L L N L P K K I I F V G H D W G A A L A F H Y A Y E H>
TRANSLATION OF RLuc8-KRASWT FULL-LENGTH [A] >

410     420     430     440     450     460     470     480     490     500
AGGACAGGATCAAGGCCATCGTGCACATGGAGAGCGTGGTGGACCTGATCGAGAGTGGGACGAGTGGCCAGACATCGAGGAGGACATCGCCCTGATCAA
TCCTGTCTAGTTCCGGTAGCAGCTGTACCTTCGACACCACTGCACCTAGCTCTCGACCCGCTCACCAGGTCTGTAGCTCCTCTGTAGCGGACTAGTT
Q D R I K A I V H M E S V V D V I E S W D E W P D I E E D I A L I K>
TRANSLATION OF RLuc8-KRASWT FULL-LENGTH [A] >

510     520     530     540     550     560     570     580     590     600
GAGCGAGGAGGGGCGAGAAGATGGTGTGGAGAACAACCTTCTTCTGGAGACCGTGTGCCAGCAAGATCATGAGAAAGCTGGAGCCCGAGGAGTTCGCC
CTCGCTCCTCCGCTCTTCTACACGACCTCTTGTGTAAGAAGCACCCTTGGCAGCAGCGGTCTTCTAGTACTCTTCTGACCTCGGGCTCCTCAAGCGG
S E E G E K M V L E N N F F V E T V L P S K I M R K L E P E E F A>
TRANSLATION OF RLuc8-KRASWT FULL-LENGTH [A] >

610     620     630     640     650     660     670     680     690     700
GCCTACTGGAGCCCTCAAGGAGAAGGGCGAGGTGAGAAGACCACCTGAGCTGGCCAGAGAGATCCCCCTGGTGAAGGGCGGCAAGCCGAGCTGG
CGGATGGACCTCGGAAGTTCCTTCCCGCTCCACTTCTGGTGGGACTCGACCGGTCTCTAGGGGGACCACTTCCCGCGTTCGGGCTGCACC
A Y L E P F K E K G E V R R P T L S W P R E I P L V K G G K P D V>
TRANSLATION OF RLuc8-KRASWT FULL-LENGTH [A] >

710     720     730     740     750     760     770     780     790     800
TGCAGATCGTGAGAACTACAACCGCTACCTGAGAGCCAGCGACCTGCCAAGCTGTTTCATCGAGAGCGACCCCGGCTTCTCAGCAACGCCATCGT
ACGCTAGACTCTTTGATGTTCCGGATGGACTTCCGCTCGCTGCTGGACGGTTCGACAAGTAGCTCTCGTGGGGCGGAAAGTCTTGGCGGACTAGTA
V Q I V R N Y N A Y L R A S D D L P K L F I E S D P G F F S N A I V>
TRANSLATION OF RLuc8-KRASWT FULL-LENGTH [A] >

810     820     830     840     850     860     870     880     890     900
GGAGGGCCCAAGAAGTTCGCCAACCCGAGTTCGTAAGGTGAAGGCTGCACCTTCTCCAGGAGGACGCCCGCAGAGATGGGCAAGTACATCAAG
CTCCCGCGTTCCTCAAGGGTGTGGCTCAAGCACTTCCACTTCCCGGACGTGAAGGAGTCTCTCGGGGGCTGTCTACCCGTTTATGATGTC
E G A K K F P N T E F V K V K G L H F L Q E D A P D E M G K Y I K>
TRANSLATION OF RLuc8-KRASWT FULL-LENGTH [A] >

910     920     930     940     950     960     970     980     990     1000
AGCTTCTGGAGAGAGTGTGAAGAACGAGCAGCTCGAGGGCGGGAGGATCTGGGGGGGAGGAAGTGGGGGGGGGCTTGGCGCCGCTATGACCG
TCGAGACACCTCTCTCAGACTTCTTGTCTGCTCGAGTCCCGCCGCTCTAGACCCCGCCTCTTACCCCTCCCGGAGACCGCGGATATCGGC
S F V E R V L K N E Q L E G G G G S G G G G S G G G S A A A M T>
TRANSLATION OF RLuc8-KRASWT FULL-LENGTH [A] >

1010    1020    1030    1040    1050    1060    1070    1080    1090    1100
AATATAAACTTGTGAGTGTGGAGCTGGTGGCTAGGCAAGAGTGCCTTGACGATACAGCTAATTCAGAATCATTTTGTGGACGAATATGATCCAACAAT
TTATATTTGAACACCACTCAACTCCGACCCGATCCGTTCTCAGGAAGTGTATGTCGATTAAGTCTTAGTAAACACCTGCTTACTAGTGGTGTGA
E Y K L V V V G A G G V G K S A L T I Q L I Q N H F V D E Y D P T I>
TRANSLATION OF RLuc8-KRASWT FULL-LENGTH [A] >

1110    1120    1130    1140    1150    1160    1170    1180    1190    1200
AGAGGATTCCTACAGGAAGCAAGTATGATGGAGAAACCTGTCCTTGGATATTTCTCGACACAGCAGGTCAAGAGGAGTACAGTGCATGAGGGAC
TCTCCTAAGATGCTCTTCTGTTATCATTAACCTCTTGGACAGAACTATAAGAGTGTGTGCTCCAGTCTTCTCTCATGTCACGTTACTCCCTG
E D S A Y R K Q V V I D G E T C L L D I L D T A G Q E E Y S A M R D>
TRANSLATION OF RLuc8-KRASWT FULL-LENGTH [A] >

1210    1220    1230    1240    1250    1260    1270    1280    1290    1300
CAGTACATGAGGACTGGGGAGGGCTTTCTTGTGATTTGCCATAAATAACTAAATCAATTTGAAGATATTCACCATTATAGAGAAACAAATTAAGAG
GTCATGTACTCTGACCCCTCCGAAAGAAACACATAAACCGGTATTTATATGATTTAGTAACTTCTATAAGTGGTAATATCTTCTTTTATTTTCTC
Q Y M R T G E G F L C V F A I N N T K S F E D I H H Y R E Q I K R>
TRANSLATION OF RLuc8-KRASWT FULL-LENGTH [A] >

1310    1320    1330    1340    1350    1360    1370    1380    1390    1400
TTAAGGACTGAAGATGACCTTGGTCTAGTAGGAAATAAATGTGATTTGCCTTCCAGAACAGTAGACACAAAACAGGCTCAGGACTTAGCAAGAG
AATTCCTGAGACTTCTACATGATACAGGATCATCTTTATTTACACTAAACGGAAGTCTTGTCTGTGTTTGTCCGAGTCTGATCGTTCTCTC
V K D S E D V P M V L V G N K C D L P S R T V D T K Q A Q D L A R S>
TRANSLATION OF RLuc8-KRASWT FULL-LENGTH [A] >

1410    1420    1430    1440    1450    1460    1470    1480    1490    1500
TTATGGAATTCCTTTTATGAAACATCAGCAAAGACAAGACAGGGTGTGATGATGCTTCTATACATAGTTCGAGAAATTCGAAACATAAAGAAAAG
AATACCTTAAGGAAAATAACTTTGATGCTTCTGTTCTTCCCAACTACTACGGAAGATATGATTAAGCTTCTTAAAGTTTGTATTTCTTTTC
Y G I P F I E T S A K T R Q G V D D A F Y T L V R E I R K H K E K>
TRANSLATION OF RLuc8-KRASWT FULL-LENGTH [A] >

1510    1520    1530    1540    1550    1560
ATGAGCAAGAGTGGTAAAAAGAAAAGAAAGTCAAGACAAAGTGTGTAATTATGTAA
TACTCGTTTCTACCAATTTTTCTTCTTTTCTTCTCAGTTTCTGTTTTCACACATTAATACAT
M S K D G K K K K K K S K T K C V I M *>
TRANSLATION OF RLuc8-KRASWT FULL-LENGTH [A] >
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Sequence: RLUC8-HRASG12V full-length Range: 1 to 1563

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10      20      30      40      50      60      70      80      90      100
ATGACCAGCAAGGTGTACGACCCCGAGCAGAGGAAGGATGATCACCGCCCGCCAGTGGTGGCCAGGTGCAAGCAGATGAACGTGCTGGACAGCTTCA
TACTGGTCGTTCACATGCTGGGGCTCGTCTCCTCTCTACTAGTGGCCGGGGTACACCCCGTCCACGTTTGGTCTACTTGCACGACCTGTCGAAGT
M T S K V Y D P E Q R K R M I T G P Q W W A R C K Q M N V L D S F>
TRANSLATION OF RLUC8-HRASG12V FULL-LENGTH [A] >

110     120     130     140     150     160     170     180     190     200
TCAACTACTACGACAGCAGAGCAGCCGAGAACCCGCTGATCTTCTGCACGGCAACGCCACTAGCAGTACCTGTGGAGGCACGTGGTCCCCACAT
AGTTGATGATGCTGTCTCGTCTTTCGTCGGCTCTGCGCACTAGAGGACGTGCCGTTGCGGTGATCGTTCGATGGACACCTCCGTGCACCCAGGGGTGTA
I N Y Y D S E K H A E N A V I F L H G N A T S S Y L W R H V V P H I>
TRANSLATION OF RLUC8-HRASG12V FULL-LENGTH [A] >

210     220     230     240     250     260     270     280     290     300
CGAGCCCGTGGCCAGGTGCATCATCCCGATCTGATCGGCATGGGCAAGAGCGGCAAGAGCGGCAACGCCAGTACAGGCTGTGGACACTACAAGTAC
GCTCGGGCCACCGCTCCACTAGTAGGGCTAGACTAGCCGTACCCGTTCTCGCCGTTCTCGCCGTTGCGGTCGATCCGACGACCTGGTGTATGTCATG
E P V A R C I I P D L I G M G K S G K S G N G S Y R L L D H Y K Y>
TRANSLATION OF RLUC8-HRASG12V FULL-LENGTH [A] >

310     320     330     340     350     360     370     380     390     400
CTGACCCGCTGGTTCGAGCTTCAAGCTGCCAAGAAGATCATCTTCTGGGCCACGACTGGGGCGCCCGCTGGCCTTCCACTACGCCACTACGACACC
GACTGGCGGACCAAGCTCGAGGACTTGGACGGTTCCTCTAGTAGAAGCACCCGGTGTGACCCCGGGCGGACCGGAAGGTGATGGCGGATGCTCGTGG
L T A W F E L L N L P K K I I F V G H D W G A A L A F H Y A Y E H>
TRANSLATION OF RLUC8-HRASG12V FULL-LENGTH [A] >

410     420     430     440     450     460     470     480     490     500
AGGACAGGATCAAGCCATCGTGCACATGGAGAGCGTGGTGGACCTGATCGAGAGCTGGGACGAGTGGCCAGACATCGAGGAGGACATCGCCCTGATCAA
TCCTGTCTAGTTCGGTAGCAGCTGTACCTCTCGCACCCACTGCAGTCTCGACCCGCTCACCAGTGTAGCTCCTCTGATGCTGATGCTGATGCTGATG
Q D R I K A I V H M E S V V D V I E S W D E W P D I E E D I A L I K>
TRANSLATION OF RLUC8-HRASG12V FULL-LENGTH [A] >

510     520     530     540     550     560     570     580     590     600
GAGCGAGGAGGGCGAGAAGATGGTGTGGAGAACAATCTTCTGTTGAGAGCCGCTGCCAGCAAGATCATGAGAAAAGTGGAGCCCGAGGAGTTCGGC
TTCGCTCCTCCGCTCTTCTACACGACCTCTTGTGTAAGAAGCACCTTGGCACGACGGGTGCTTGTAGTACTTCTTCCGACCTCGGGCTCCTCAAGCGG
S E E G E K M V L E N N F F V E T V L P S K I M R K L E P E E F A>
TRANSLATION OF RLUC8-HRASG12V FULL-LENGTH [A] >

610     620     630     640     650     660     670     680     690     700
GCCTACTGGAGCCCTCAAGGAGAAGGGGAGGTGAGAAGACCACCTGAGCTGGCCAGAGAGATCCCCCTGGTGAAGGGCGGCAAGCCGAGCTGG
CGGATGGACCTCGGAAGTTCCTTCCCGCTCCACTTCTGGTGGGACTCGACCCGGTCTCTAGGGGGACCACTCCCGCGTTCGGGTGCACC
A Y L E P F K E K G E V R R P T L S W P R E I P L V K G G K P D V>
TRANSLATION OF RLUC8-HRASG12V FULL-LENGTH [A] >

710     720     730     740     750     760     770     780     790     800
TGCAGATCGTGAGAACTACAACCGCTACCTGAGAGCCAGCGACCTGCCAAGCTGTTTCATCGAGAGCGACCCCGGCTTTCAGCAACGCCATCGT
ACGCTAGCACTCTTTGATGTTCCGGATGGACTTCCGCTCGCTGCTGGACGGTTCGACAAAGTAGTCTCGTGGGGCCGAAAGTCTGCTGGCGTAGCA
V Q I V R N Y N A Y L R A S D D L P K L F I E S D P G F F S N A I V>
TRANSLATION OF RLUC8-HRASG12V FULL-LENGTH [A] >

810     820     830     840     850     860     870     880     890     900
GGAGGGCCCAAGAAGTTCACCAACCCGAGTTCGTGAAGGTGAAGGCCCTGCACCTTCTCCAGGAGGACGCCCGGACGAGATGGGCAAGTACATCAAG
CTCCCGCGTTCCTCAAGGGTGTGGCTCAAGCACTTCCACTTCCCGGACGTGAAGGAGTCTCTCGGGGGCTGCTACCCGTTTATGATGTC
E G A K K F P N T E F V K V K G L H F L Q E D A P D E M G K Y I K>
TRANSLATION OF RLUC8-HRASG12V FULL-LENGTH [A] >

910     920     930     940     950     960     970     980     990     1000
AGCTTCTGGAGAGAGTCTGAAGAACGAGCAGCTCGAGGGCGGGAGGATCTGGGGCGGAGGAAGTGGGGAGGGGGCTTGCGGCCGCTATGACCG
TCGAGACCTCTCTCAGCACTTCTGTGCTCGTGCAGCTCCCGCCGCTTCTAGACCCCGCTTCTACCCCTCCCGGACGACCCGGGATATGGC
S F V E R V L K N E Q L E G G G G S G G G G S G G G S A A A M T>
TRANSLATION OF RLUC8-HRASG12V FULL-LENGTH [A] >

1010    1020    1030    1040    1050    1060    1070    1080    1090    1100
AATACAAGCTTGTGTTGGCGCCGTCGGTGTGGGCAAGAGTGCCTGACCATCCAGCTGATCCAGAACCATTTTGTGGACGAATACGACCCCACTAT
TTATGTTGAAACAACAACCCCGGACCCACCCGTTCTCAGCGACTGGTAGGTCGACTAGTCTTGGTAAACACCTGCTTATGCTGGGGTGATA
E Y K L V V V G A V G V G K S A L T I Q L I Q N H F V D E Y D P T I>
TRANSLATION OF RLUC8-HRASG12V FULL-LENGTH [A] >

1110    1120    1130    1140    1150    1160    1170    1180    1190    1200
AGAGGATTCCTACCGAAGCAGGTGGTCAATGATGGGAGACGTGCTGTTGGACATCCTGGATACCCCGCCGAGGAGGTACAGCCGATGCGGGAC
TCTCCTAAGGATGGCTTCTGTCACCACTAACCCTCTGCAGGACAACTGTAGGACCTATGGCGGGCTCTCTCTCATGTCGGGTACGCCCCTG
E D S Y R K Q V V I D G E T C L L D I L D T A G Q E E Y S A M R D>
TRANSLATION OF RLUC8-HRASG12V FULL-LENGTH [A] >

1210    1220    1230    1240    1250    1260    1270    1280    1290    1300
CAGTACATGCGCACCGGGGAGGGCTTCTGTGTGTTGTCATCAACAACCAAGTCTTTGAGGACATCCACCAGTACAGGGAGCAGATCAACCGGG
GTCATGTACGCTGCGCCCTCCGAGGACACACAAACGGTAGTGTGTTGGTTCAGAAAACCTCTGTAGTGGTGTATGCTCCCTGCTAGTTTGGCCC
Q Y M R T G E G F L C V F A I N N T K S F E D I H Q Y R E Q I K R>
TRANSLATION OF RLUC8-HRASG12V FULL-LENGTH [A] >

1310    1320    1330    1340    1350    1360    1370    1380    1390    1400
TGAAGGACTCGGATGACCTCCCATGGTGTGCTGGGGAACAAGTGTGACCTGGCTGCACGCACTGTGGAATCTCGGAGGCTCAGGACCTCCCGCAAG
ACTTCTGAGCTTACTGCACGGTACCACGACCCCTTGTTCACACTGGACCGACGTGCGTGACACCTTAGAGCCGTCGAGTCTGGAGCGGGCTTC
V K D S D D V P M V L V G N K C D L A A R T V E S R Q A Q D L A R S>
TRANSLATION OF RLUC8-HRASG12V FULL-LENGTH [A] >

1410    1420    1430    1440    1450    1460    1470    1480    1490    1500
CTACGGCATCCCTACATCGAGACCTCGGCCAAGCCCGGAGGATGGAGGATGCTTCTACACGTTGGTGGTGGATCCCGGACGACAAAGCTGGCG
GATGCCCTAGGATGCTGCTGACCACTTCTGGGCGCTTCTACCTTCTACGGAAGATGTCAACACGCACTTAGGCCCTGCTTTCGACGCC
Y G I P Y I E T S A K T R Q G V E D A F Y T L V R E I R Q H K L R>
TRANSLATION OF RLUC8-HRASG12V FULL-LENGTH [A] >

1510    1520    1530    1540    1550    1560
AAGCTGAACCCCTCGTATGAGAGTGGCCCGGCTGCATGAGCTGCAAGTGTGTCTCTCTGA
TTCGACTTGGGAGGACTACTCTCACCAGGGCCGACGACTCGACGTTACACACGAGGAGACT
K L N P P D E S G P G C M S C K C V L S *>
TRANSLATION OF RLUC8-HRASG12V FULL-LENGTH [A] >
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Sequence: RLUC8-NRASQ61H full-length Range: 1 to 1563

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10      20      30      40      50      60      70      80      90      100
ATGACCAGCAAGGTGTACGACCCCGAGCAGAGGAAGGATGATCACCGCCCGCCAGTGGTGGCCAGGTGCAAGCAGATGAACGTGCTGGACAGCTTCA
TACTGGTCGTTCACATGCTGGGGCTCGTCTCCTCTCTACTAGTGGCCGGGGTACACACCCGGTCCACGTTCTGCTACTTGCACGACCTGTCGAAAT
M T S K V Y D P E Q R K R M I T G P Q W W A R C K Q M N V L D S P>
TRANSLATION OF RLUC8-NRASQ61H FULL-LENGTH [A] >

110     120     130     140     150     160     170     180     190     200
TCAACTACTACGACAGCAGAGCAGCCGAGAACCCGCTGATCTTCTGACGGCAACGCCACTAGCAGTACCTGTGGAGGCACGTGGTCCCCACAT
AGTTGATGATGCTGTCTCGTCTTTCGCGGCTCTTGGGCACTAGAGGACGTGCCGTTGCGGTGATCGTTCGATGGACACCTCCGTGCACCCAGGGGTGTA
I N Y Y D S E K H A E N A V I F L H G N A T S S Y L W R H V V P H I>
TRANSLATION OF RLUC8-NRASQ61H FULL-LENGTH [A] >

210     220     230     240     250     260     270     280     290     300
CGAGCCCGTGGCCAGGTGCATCATCCCGATCTGATCGGCATGGCAAGAGCGGCAAGAGCGGCAACGCCAGTACAGGCTGTGGACACTACAAGTAC
GCTCGGGCCACCGCTCCACTAGTAGGGCTAGACTAGCCGTACCCTTCTCGCCGTTCTCGCCGTTGCGGTCGATCCGACGACCTGGTGTATGTCATG
E P V A R C I I P D L I G M G K S G K S G N G S Y R L L D H Y K Y>
TRANSLATION OF RLUC8-NRASQ61H FULL-LENGTH [A] >

310     320     330     340     350     360     370     380     390     400
CTGACCCGCTGGTTCGAGCTTCAAGTCCGCAAGAGATCATCTTCTGGGCCACGACTGGGGCGCCCGCTGGCCTTCCACTACGCCACTACGACACC
GACTGGCGGACCAAGCTCGAGGACTTGGACGGTCTTCTAGTAGAAGCACCAGTGTGACCCCGGGCGGACCGAAGGTGATGGCGGATGCTCGTGG
L T A W F E L L N L P K K I I F V G H D W G A A L A F H Y A Y E H>
TRANSLATION OF RLUC8-NRASQ61H FULL-LENGTH [A] >

410     420     430     440     450     460     470     480     490     500
AGGACAGGATCAAGCCATCGTGCACATGGAGAGCGTGGTGGACCTGATCGAGAGTGGGACGAGTGGCCAGACATCGAGGAGGACATCGCCCTGATCAA
TCCTGTCTAGTTCGGTAGCAGCTGTACCTTCGACCCACCTGCACCTAGCTCTCGACCCGCTCACCAGTGTAGCTCCTCTGTAGCTCCTGTAGCTAGT
Q D R I K A I V H M E S V V D V I E S W D E W P D I E E D I A L I K>
TRANSLATION OF RLUC8-NRASQ61H FULL-LENGTH [A] >

510     520     530     540     550     560     570     580     590     600
GAGCGAGGAGGGGAGAGATGGTGTGGAGAACAACCTTCTGTTGAGAGCCGTGCTGCCAGCAAGATCATGAGAAAAGTGGAGCCCGAGGAGTTCGGC
TTCGCTCCTCCGCTCTTCTACACGACCTCTTGTGTAAGAAGCACCCTTGGCACGACGGGTGCTTAGTACTCTTTCGACCTCGGGCTCCTCAAGCGG
S E E G E K M V L E N N F F V E T V L P S K I M R K L E P E E F A>
TRANSLATION OF RLUC8-NRASQ61H FULL-LENGTH [A] >

610     620     630     640     650     660     670     680     690     700
GCCTACTGGAGCCCTCAAGGAGAAGGGGAGGTGAGAAGACCACCTGAGCTGGCCAGAGAGATCCCCCTGGTGAAGGGCGGCAAGCCGAGCTGG
CGGATGGACCTCGGGAAGTTCCTTCCGCTCCACTTCTGGTGGGACTCGACCCGGTCTCTAGGGGACCACTCCCGCGTTCGGGCTGCACC
A Y L E P F K E K G E V R R P T L S W P R E I P L V K G G K P D V>
TRANSLATION OF RLUC8-NRASQ61H FULL-LENGTH [A] >

710     720     730     740     750     760     770     780     790     800
TGCAGATCGTGAGAACTACAACCGCTACCTGAGAGCCAGCGACCTGCCAAGCTGTTTCATCGAGAGCGACCCCGGCTTCTCAGCAACGCCATCGT
ACGCTAGCACTCTTTGATTTCCGGATGGACTTCCGCTCGCTGCTGGACGGTTCGACAAGTAGCTCTCGTGGGGCCGAAAGTCTGTTGGCGGTAGTA
V Q I V R N Y N A Y L R A S D D L P K L F I E S D P G F F S N A I V>
TRANSLATION OF RLUC8-NRASQ61H FULL-LENGTH [A] >

810     820     830     840     850     860     870     880     890     900
GGAGGGCCCAAGAGTTCACCAACCCAGTTCGTTGAAGGTGAAGGCCCTGCACCTTCTCCAGGAGGACGCCCGCAGAGATGGGCAAGTACATCAAG
CTCCCGCGTTCCTCAAGGGTGGTGGCTCAAGCACTTCCACTTCCCGGACGTGAAGGAGTCTCTCGGGGGCTGCTACCCGTTTATGATGTC
E G A K K F P N T E F V K V K G L H F L Q E D A P D E M G K Y I K>
TRANSLATION OF RLUC8-NRASQ61H FULL-LENGTH [A] >

910     920     930     940     950     960     970     980     990     1000
AGCTTCTGGAGAGAGTCTGAAGAACGAGCAGCTCGAGGGCGGGAGGATCTGGGGGGGAGGAAAGTGGGGAGGGGGCTTGGCGCCGCTATGACTG
TCGAGACACCTCTCTCAGCACTTCTGCTCGTGCAGCTCCCGCCGCTCTTAGACCCCGCCCTTCCACCCCTCCCGGACGACCCGGGATGACTGAC
S F V E R V L K N E Q L E G G G G S G G G G S G G G S A A A M T>
TRANSLATION OF RLUC8-NRASQ61H FULL-LENGTH [A] >

1010    1020    1030    1040    1050    1060    1070    1080    1090    1100
AGTACAACCTGGTGGTGGAGCAGTGGTGTGGGAAAAGCCGACTGACAATCCAGCTGATCCAGAACCACTTTGTAGATGAATATGATCCCACT
TCATGTTTGACCAACCAACCTTCCGCTGACTTTCGCGTACTGTTAGTTCGACTAGTCTTGGTGAACATCTACTTACTAGGGTGGTA
E Y K L V V V G A G G V G K S A L T I Q L I Q N H F V D E Y D P T I>
TRANSLATION OF RLUC8-NRASQ61H FULL-LENGTH [A] >

1110    1120    1130    1140    1150    1160    1170    1180    1190    1200
AGAGGATCTTACAGAAAACAAGTGGTATAGATGGTGAACCTGTTTGGTGGACACTGGATACAGCTGGACATGAAGAGTACAGTGCATGAGAGAC
TATCCCTAAGAAATGTTTTTGTTCACCAATATCTACCCTTTGGCAACAACCTGTATGACCTATGTGACCTGTGACCTTCTCATGTCACGGTACTCTCTG
E D S Y R K Q V V I D G E T C L L D I L D T A G H E E Y S A M R D>
TRANSLATION OF RLUC8-NRASQ61H FULL-LENGTH [A] >

1210    1220    1230    1240    1250    1260    1270    1280    1290    1300
CAATACATGAGGACAGGCGAAGGCTTCTGTGATTTGCCATCAATAATAGCAAGTCAATTTGCGGATATTAACCTCTACAGGAGCAGATTAAGCGAG
GTTATGTACTCTCTCGGCTCCGAGGAGACACATAAACCGGTAGTTATATCGTTCAGTAAACGCTATAAATGGAGATGTCCTCTCTAATTCGCTC
Q Y M R T G E G F L C V F A I N N S K S F A D I N L Y R E Q I K R>
TRANSLATION OF RLUC8-NRASQ61H FULL-LENGTH [A] >

1310    1320    1330    1340    1350    1360    1370    1380    1390    1400
TAAAGACTCGGATGATGACTACCTATGGTGTACTGGGAAACAAGTGTGATTTGCCAACAAGGACAGTGGATACAAAACAAGCCACGAACTGGCCAAAG
ATTTTCTGAGCTACTACATGATACACGATCACCTTTGTTACACTAAACGGTGTGTTCTCTCACTATGTTTGTTCGGGTGCTTACCCGGTCTC
V K D S D D V P M V L V G N K C D L P T R T V D T K Q A H E L A K S>
TRANSLATION OF RLUC8-NRASQ61H FULL-LENGTH [A] >

1410    1420    1430    1440    1450    1460    1470    1480    1490    1500
TTACGGGATTCATTCATTTGAAACCTCAGCCAAGCAGAGGGTGTGAAGATGCTTTTACACACTGGTAAGAGAAATACCCAGTACCGAATGAAA
AATGCCCTAAGTAACTTGGAGTCCGTTCTGGTCTGTCACAACTTCTACGAAAAATGTGACCACTTCTTTTATGCGGTGCTTACTCTT
Y G I P F I E T S A K T R Q G V E D A F Y T L V R E I R Q Y R M K>
TRANSLATION OF RLUC8-NRASQ61H FULL-LENGTH [A] >

1510    1520    1530    1540    1550    1560
AAACTAACAGCAGTGTATGGGACTCAGGGTGTATGGGATGCCATGTGTGGTGTATGATA
TTTGAGTTGCTGCTACTACTACCTGAGTCCCAACATACCTAACGGTACACACCACTACTATT
K L N S S D D G T Q G C M G L P C V V M *>
TRANSLATION OF RLUC8-NRASQ61H FULL-LENGTH [A] >
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Sequence: LMO2-RLuc8 Range: 1 to 1464

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10      20      30      40      50      60      70      80      90      100
ATGAGTTCGCCATCGAAAGAGAGACCTGGACCCGCTCGAGGAACCCGTTGGATGAGGTGCTGCAGATACCCCATCCCTGCTGACATGTGGTGGCTGCC
TACTCAAGCCGGTACTTTCCTTCCTGGGACCTGGGCAGACTCCTTGGGCACCTACTCCACGAGCTATGGGGGTAGGGACGACTGTACACCACCGACGG
M S S A I E R K S L D P S E E P V D E V L Q I P P S L L T C G G C >
TRANSLATION OF LMO2-RLUC8 [A] >

110     120     130     140     150     160     170     180     190     200
AGCAGAACATAGGGGACCGCTACTTCCGAAAGCCATCGACCAGTACTGGCATGAGGATTCCTCAGCTGAGTCTGTGGGTGTCGGCTGGGAGAGGT
TCGCTTGTGATCCCTGGCGATGAAGACTTTCGGTAGCTGGTCATGACCGTACTCCTAACGGAGTCGACACTGGAGACACCCACAGCCGACCTCTCCA
Q Q N I G D R Y F L K A I D Q Y W H E D C L S C D L C G C R L G E V >
TRANSLATION OF LMO2-RLUC8 [A] >

210     220     230     240     250     260     270     280     290     300
GGGAGGCGCCTCTACTACAAGTGGGACGAAATTTGTCAGGAGAGACTATCTCAGGCTTTTGGTTCAGGATGGTCTCTGTGCATCCTGTGACAAGCGG
CCCTCCCGGAGATGATGTTCCGACCTGCCTTAAACACCTCTCTGTATAGTTCGAAAAACCACTCTACCAGAGACACTGAGGACACTGTTCCGCC
G R R R L Y Y K L G R K L C R R D Y L R L F G Q D G L C A S C D K R >
TRANSLATION OF LMO2-RLUC8 [A] >

310     320     330     340     350     360     370     380     390     400
ATCCGTGCCATGAGATGAGTGGGGTGAAGACAAAGTGTATCACTGGAGTGTTCAAATGGCCCGCTGTCAGAAGCATTTCTGTGTAGGTGACA
TAGGCACGGTACTCTACTCTACGCCCACTTTCGTGTTACATAGTGGACTCCACAAGTTCACGGCGGACACTCTCGTAAGACACATCCACTGT
I R A Y E M T M R V K D K V Y H L E C F K C A A C Q K H F C V G D >
TRANSLATION OF LMO2-RLUC8 [A] >

410     420     430     440     450     460     470     480     490     500
GATACCTCTCATCAACTCCGACATAGTGTGTAACAAGACATCTACGAGTGGACTAAGATCAATGGGATGATACTCGAGGGCGGTGGCGGATCGGGCGG
CTATGGAAAGAGTAGTTGAGGCTGATCACACACTGTTCTGTAGATGCTCACCTGATTTCTAGTTACCCCTACTATGAGCTCCCGCACCGCCTAGCCCGC
R Y L L I N S D I V C E Q D I Y E W T K I N G M I L E G G G G S G G >
TRANSLATION OF LMO2-RLUC8 [A] >

510     520     530     540     550     560     570     580     590     600
AGGTGGCAGTGGCGCCGAGGGAGTGGTATGACACAGCAAGGTGTACGACCCCGAGCAGAGGAAGGATGATCACCGGCCCCAGTGGTGGCCAGGTGC
TCCACCTGACCGCCCGCTCCCTACCATACTGTCGTTCCACATGCTGGGGTCTGCTCTCTCTCTACTAGTGGCCGGGGTACCACCCCGTCCAGC
G G S A A A G S G M T S K V Y D P E Q R K R M I T G P Q W W A R C >
TRANSLATION OF LMO2-RLUC8 [A] >

610     620     630     640     650     660     670     680     690     700
AAGCAGATGAACGCTGGACAGCTTCACTCAACTACTACGACAGCAGAGCAGCAGGAGAACCGCGTACTTCTCTGACGGCAACGCCACTAGCAGCT
TTCGCTACTGACGACCTGTGCAAGTAGTTGATGATGCTGTCGCTCTCTGTCGGCTCTCTGCGCACTAGAAGGAGCTGCCGTTGCGGTGATGCTCGA
K Q M N V L D S F I N Y Y D S E K H A E N A V I F L H G N A T S S >
TRANSLATION OF LMO2-RLUC8 [A] >

710     720     730     740     750     760     770     780     790     800
ACCTGTGGAGGCACGTGGTCCCCACATCGAGCCCGTGGCCAGGTGCATCATCCCGATCTGATCGGCATGGGCAAGAGCGGCAAGAGCGGCAACGGCAG
TGGACACCTCCGTGACACCGGGGTAGCTCGGGCACCGGTCCACGTAGTAGGGGCTAGACTAGCCGTAACCGTTCCTCGCCCTCTCGCCCTGTCGCTC
Y L W R H V V P H I E P V A R C I I P D L I G M G K S G K S G N G S >
TRANSLATION OF LMO2-RLUC8 [A] >

810     820     830     840     850     860     870     880     890     900
CTACAGGCTGCTGGACCACTACAAGTACCTGACCCGCTGGTTCGAGCTCTGAACTGCCCAAGAAGATCATCTCTGTTGGCCACGACTGGGGCGCCGC
GATCTCCGACGACCTGGTGTATGTTCACTGACTGGGGACCAAGCTCGAGGACTGGACGGGTTCTCTAGTAGAAGCACCAGGCTGACCCCGGGGGG
Y R L L D H Y K Y L T A W F E L N L P K K I I F V G H D W G A A >
TRANSLATION OF LMO2-RLUC8 [A] >

910     920     930     940     950     960     970     980     990     1000
CTGGCCCTTCACCTACGCCCTACGACACAGGACAGGATCAAGGCCATCGTGCACATGGAGAGCGTGGTGGACGTGATCGAGAGCTGGGACGAGTGGCCAG
GACCGGAAGTGTATGCGGATGCTCGTGGTCTCTCTAGTTCGCTGACGAGTACCTCTCGCACCACTGCACTAGCTCTCGACCTGCTCACCGGTC
L A F H Y A Y E H Q D R I K A I V H M E S V V D V I E S W D E W P >
TRANSLATION OF LMO2-RLUC8 [A] >

1010    1020    1030    1040    1050    1060    1070    1080    1090    1100
ACATCGAGGAGGACATCGCCCTGATCAAGAGCGAGGAGGGCGAGAAGATGGTGTGGGAGAACAACTTCTCTGTTGAGACCGTGTGCCAGCAAGATCAT
TGTAGCTCCTCTGTAGCGGGACTAGTTCTCGCTCCTCCCGCTCTTCTACACGACCTCTGTTGAAAGAAGCACCCTGGGCACGAGGGTCTGTAGTA
D I E E D I A L I K S E E G E K M V L E N N F F V E T V L P S K I M >
TRANSLATION OF LMO2-RLUC8 [A] >

1110    1120    1130    1140    1150    1160    1170    1180    1190    1200
GAGAAAGCTGGAGCCGAGGATTCGCGCCTACCTGGAGCCCTTCAAGGAGAAGGGCGAGGTGAGAAGACCCACCTGAGCTGGCCAGAGAGATCCCC
CTCTTCGACTCCGGCTCCTCAAGCGGGATGGACTCGGGAAGTCTCTCTCCCGCTCCACTTCTTGGGTGGGACTCGACCGGTTCTCTTAGGGG
R K L E P E E F A A Y L E P F K E K G E V R R P T L S W P R E I P >
TRANSLATION OF LMO2-RLUC8 [A] >

1210    1220    1230    1240    1250    1260    1270    1280    1290    1300
CTGGTGAAGGGCGGCAAGCCGACGTCGTCAGATCGTGAGAACTACAACGCCTACCTGAGAGCCAGCGACCTGCCAAGCTGTTTCATCGAGAGCG
GACCACTTCCCGCGCTCGGGTGCACACAGTCTAGCACTCTTGTATGTTGCGGATGGACTCTCGTCTGCTGGAGCGGTTCCGCAAGTAGCTCTGCC
L V K G G K P D V V Q I V R N Y N A Y L R A S D D L P K L F I E S >
TRANSLATION OF LMO2-RLUC8 [A] >

1310    1320    1330    1340    1350    1360    1370    1380    1390    1400
ACCCGCTTCTTACGCAACGCCATCGTGGAGGGCGCAAGAGTTCCCAACACCGAGTTCGTGAAGGTGAAGGGCCTGCACTTCTCTCAGGAGGACGC
TGGGGCCGAAGAAGTCTGTCGGTAGCACCTCCCGCGTCTCTCAAGGGGTTGTGGCTCAAGCACTTCCACTTCCCGGACGTAAGGAGTCTCTCTGCG
D P G F F S N A I V E G A K K F P N T E F V K V K G L H F L Q E D A >
TRANSLATION OF LMO2-RLUC8 [A] >

1410    1420    1430    1440    1450    1460
CCCCGACGAGATGGCAAGTACATCAAGAGCTTCGTGGAGAGAGTCTGAAGAACGAGCAGTAA
GGGGCTGCTACCCGTTCTATGATGTTCTCGAAGCACCTCTCTCAGACTTCTGTGCTGCTATT
P D E M G K Y I K S F V E R V L K N E Q * >
TRANSLATION OF LMO2-RLUC8 [A] >
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