

Orthogonal Inducible Cas13 Platform for Programmable RNA Regulation



Therapeutic Area	Oncology	Indications	CRISTAL [Control of RNA with Inducible SpliT CAs13 Orthologs and Exogenous Ligands] Platform
Modality	Gene therapy	Development Stage	Pre-clinical

Overview

Background

- The versatile Cas13, an RNA-guided ribonuclease, has exhibited remarkable potential in RNA manipulation, sensing, and editing across systems. It offers efficient RNA cleavage with minimal off-target effects, enhancing its safety profile compared to other regulatory machinery.
- Yet, its uncontrolled activity hinders cell engineering. To address this, Boston University researchers introduced the CRISTAL platform, featuring inducible split Cas13 orthologs, enabling precise RNA regulation for cell engineering and RNA biology exploration.

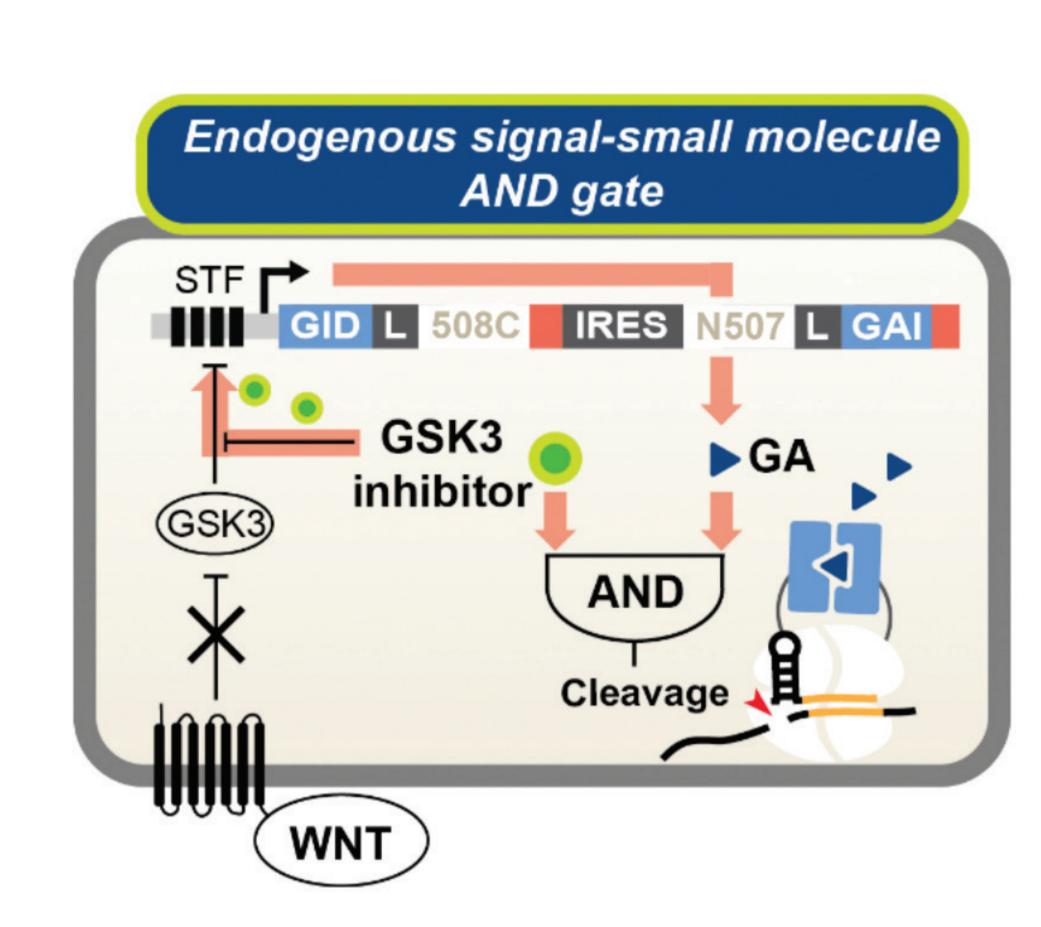
Technology Advantages

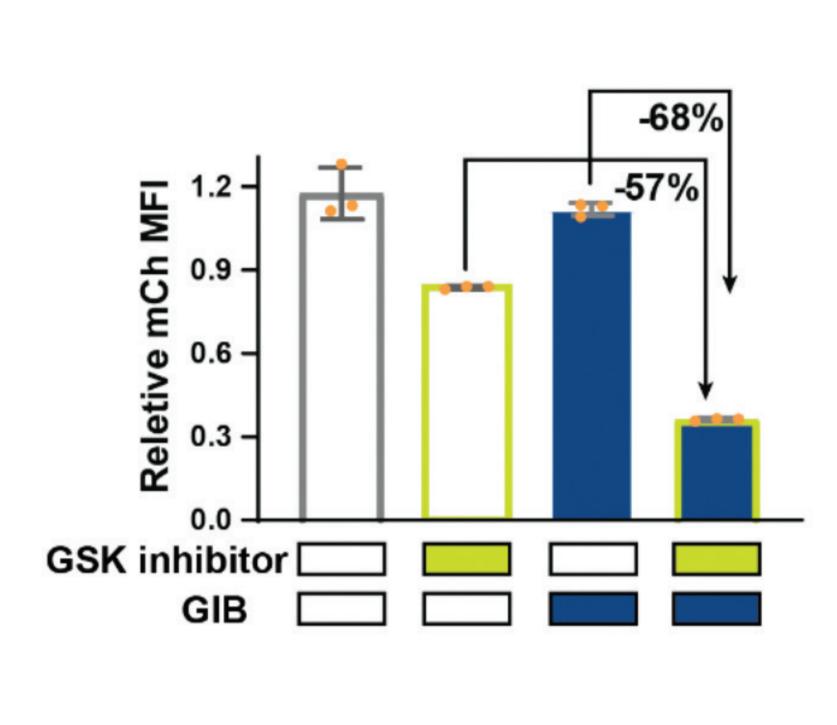
- Orthogonal, low leakiness, and high dynamic range of inducible Cas13d and Cas13b
- Engineered Cas13 logic circuits for endogenous signaling and exogenous inputs
- Safer reversible gene expression regulation at the RNA level
- Simultaneous multiplexed control of multiple genes with combinatory Cas13s
- Validated high induced activity in mammalian cells and mice
- Orthogonal regulatory mechanism, no genome modification needed

Key Data

Multiplex control of split Cas13d ribonucleases

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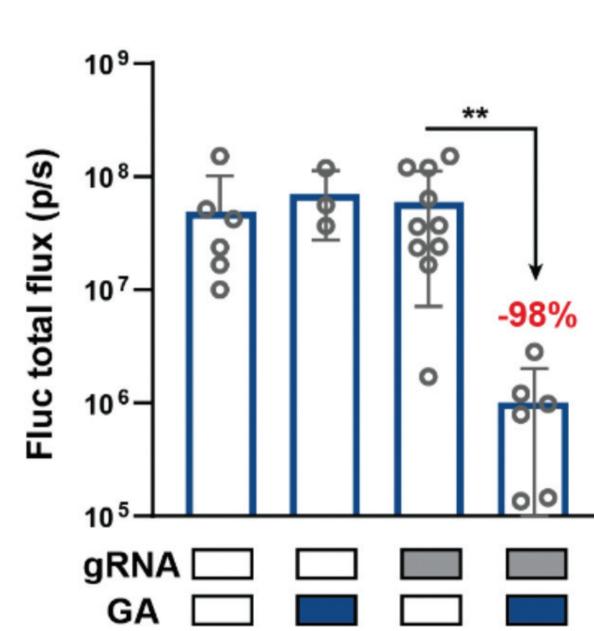




Schematic of the small molecule-endogenous signal AND gate. A GSK inhibitor (green circles), mimicking the endogenous WNT signaling pathway, activates the WNT-responsive SuperTOP Flash (STF) promoter and activates the transcription of the GA-inducible split Cas13d. This Cascading transcriptional and post-translational control of split Cas13d achieved ~70% mCh-specific knockdown only in the presence of both the GSK inhibitor and GA (blue triangle)

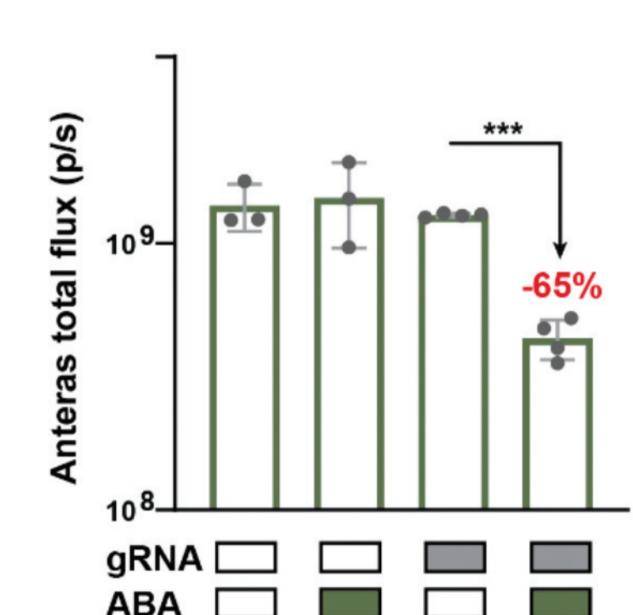
Simultaneous and orthogonal regulated gene knockdown in mice

B GIB INDUCED FLUC KNOCKDOWN



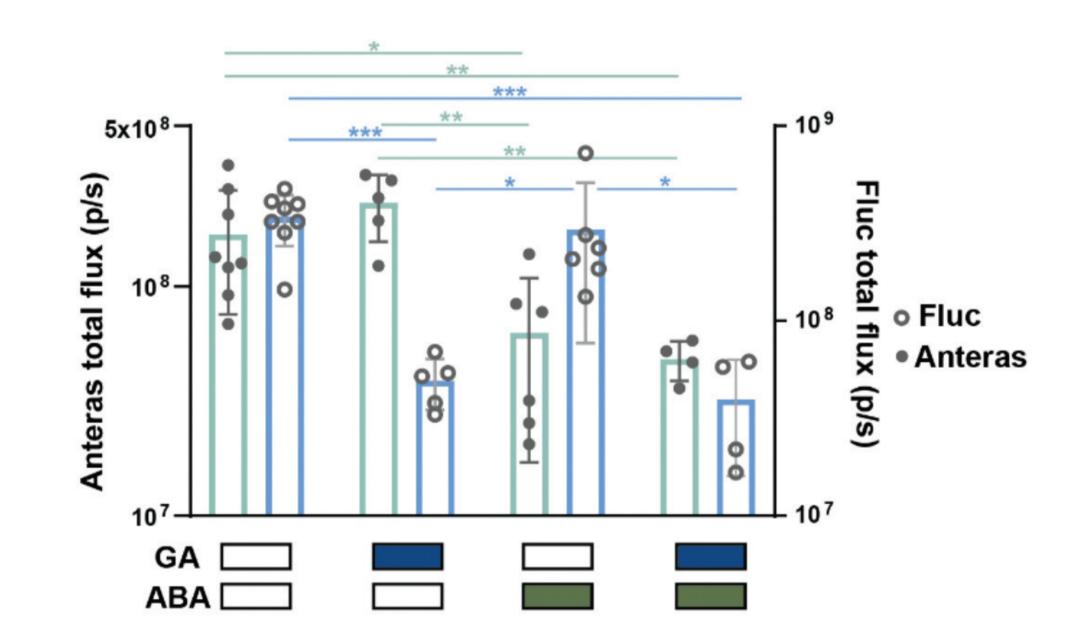
The GA-inducible Cas13d generated 98% reduction in Antares luminescence in the GA-inducible targeted group compared to uninduced and or untargeted groups in vivo. Left to right: n=6, n=3, n=10, n=6; **P<.01

C ABA INDUCED ANTERAS KNOCKDOWN



The ABA-inducible Cas13b generated 65% reduction in Fluc luminescence in the ABA-inducible targeted group compared to uninduced and or untargeted groups in vivo. Sample replicates left to right: n=3, n=3, n=4, n=4; ***P<.001

D ORTHOGONAL AND MULTIPLEXED KNOCKDOWN



Mice were transfected with both Fluc targeting GA-inducible Cas13d system and Antares targeting ABA-inducible Cas13b system, randomly injected with either vehicle control, GA, ABA, or GA and ABA. Luminescence imaging shows that Fluc and Antares were orthogonally regulated by GA and ABA in vivo. Sample replicates left to right: n=8, n=5, n=6, n=4; *P<.05, **P<.01, ***P<.001. P-values were calculated by two-tailed t-test.

IP Status & Publication(s)

Intellectual Property

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US 11572565 B2 (2023.02.07)

Patent Family
PCT, US

Publication(s)

 Ding at al. (2023). Orthogonal inducible control of Cas13 circuits enables programmable RNA regulation in mammalian cells. bioRxiv (Cold Spring Harbor Laboratory)