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Method for precisely tuning gene expression in mammalian cells

Asset Overview

Product Type	Nucleic Acid
Indication	Oncology
Current Stage	Discovery
Target (MoA)	Use of synthetic microRNA response elements (MREs) to control the levels of user-defined target genes
Brief Description	Researchers at Oxford have developed a new platform to precisely modulate gene expression that is applicable to to a wide range of therapeutic applications.
Organization	Oxford University

Differentiation

□ **A new paradigm to precisely modulate gene expression**

- Researchers at Oxford have developed a new platform to precisely modulate gene expression that is applicable to a wide range of therapeutic applications. This approach relies on the engineering of synthetic microRNA response elements (MREs), which can harness the repressive potential of endogenous microRNAs to control the levels of user-defined target genes. By introducing defined
- mismatches in these synthetic MREs the team was able to tune the strength of endogenous miRNA-mediated repression and consequently gene expression output to within 0.02% of any desired level. This strategy could provide an ideal solution for preventing tumor-induced exhaustion of engineered T-cells while mitigating the risk of autoimmune reactions.

□ **Benefits of this method**

- Intergration into existing manufacturing protocols for engineered T-cells
- Precise tuning of gene expression
- No exogenous interaction once the system is integrated into native genes or therapeutic
- Transgenes
- Reduced probability of off-target effects.

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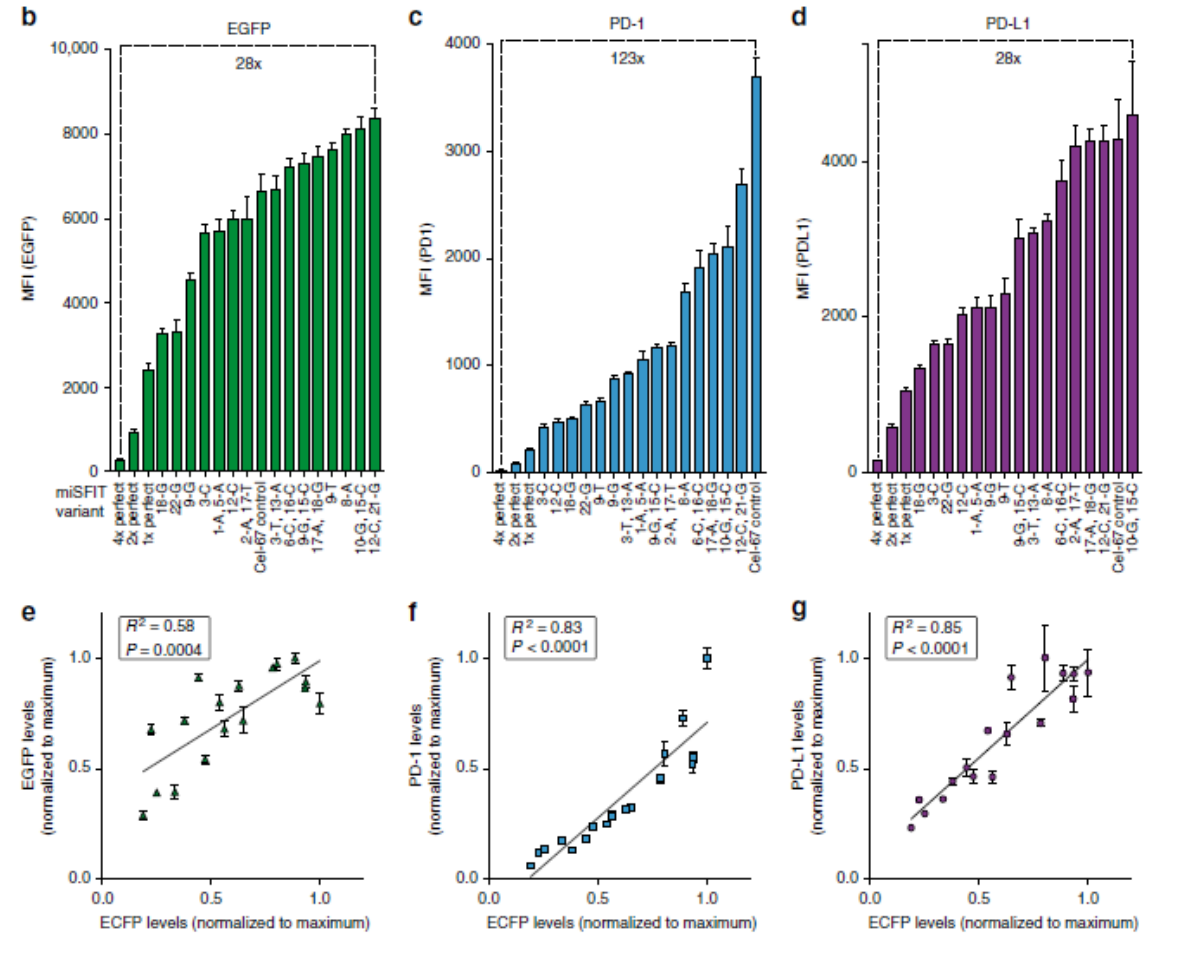
Key Data

Synthetic miSFIT variants enable fine-tuning of gene expression in human cells.



b–d Flow cytometry analysis of HEK-293T cells transfected with a panel of 19 miR-17 miSFIT variants placed in the 3'UTR of EGFP (b), PD-1 (c), and PD-L1 (d) (n = 3 biological replicates, mean+ s.d.). Fold-change between maximum and minimum expression is noted for each transgene. e–g Linear regression analysis of miSFIT strength correlation between ECFP-EGFP (e), - PD-1 (f), and - PD-L1 (g) in HEK-293T cells (n = 3 biological replicates, mean+ s.d.).

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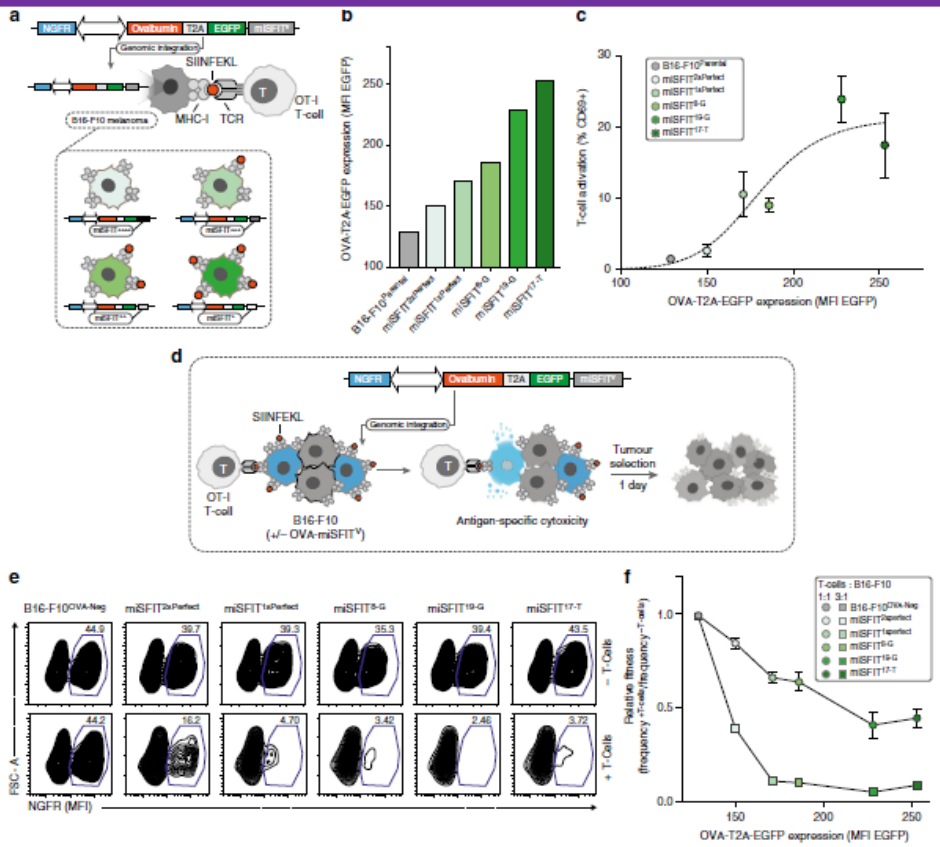
Key Data

Fine-tuning antigen expression levels and T-cell activity



a Strategy for tuning Ovalbumin (OVA) expression in B16-F10 melanoma cells using lentivirally integrated miSFITs. Red circles represent SIINFEKL, a peptide antigen derived from ovalbumin. OT-I T-cells express a TCR specific for SIINFEKL presented on MHC-I. b Flow cytometry analysis of OVA-T2A-EGFP expression in B16-F10 cell lines transduced with six different miSFIT variant (miSFITV) lentiviruses (see Supplementary Figure 6 for the gating strategy and distribution of fluorescence intensity) c CD8+, OT-I T-cell activation by OVA-misfit B16-F10 cell lines. CD69 expression was quantified by flow-cytometry (n = 5 biological replicates, mean +/− s.d.). d Schematic representation of mixedculture experimental design. OVA-negative (NGFR-) are mixed with OVA-miSFIT (NGFR+) B16-F10 cells and are challenged overnight with OT-I T-cells. e Representative flow cytometry plots of mixed culture experiments. The percentage of NGFR+ (OVA-miSFIT) cells (blue polygon gate) surviving after overnight selection in the presence or absence of CD8+, OT-I T-cells(at a ratio of 3:1 T-cells to B16-F10 cells) is indicated for each condition. f Relative fitness of B16-F10 cell lines as a function of OVA expression. Relative fitness was calculated by dividing the frequency of NGFR+ cells with T-cells by the frequency of NGFR+ cells without T-cells (n = 3 biological replicates, mean +/− s.d.). Source data are provided as a Source Data file

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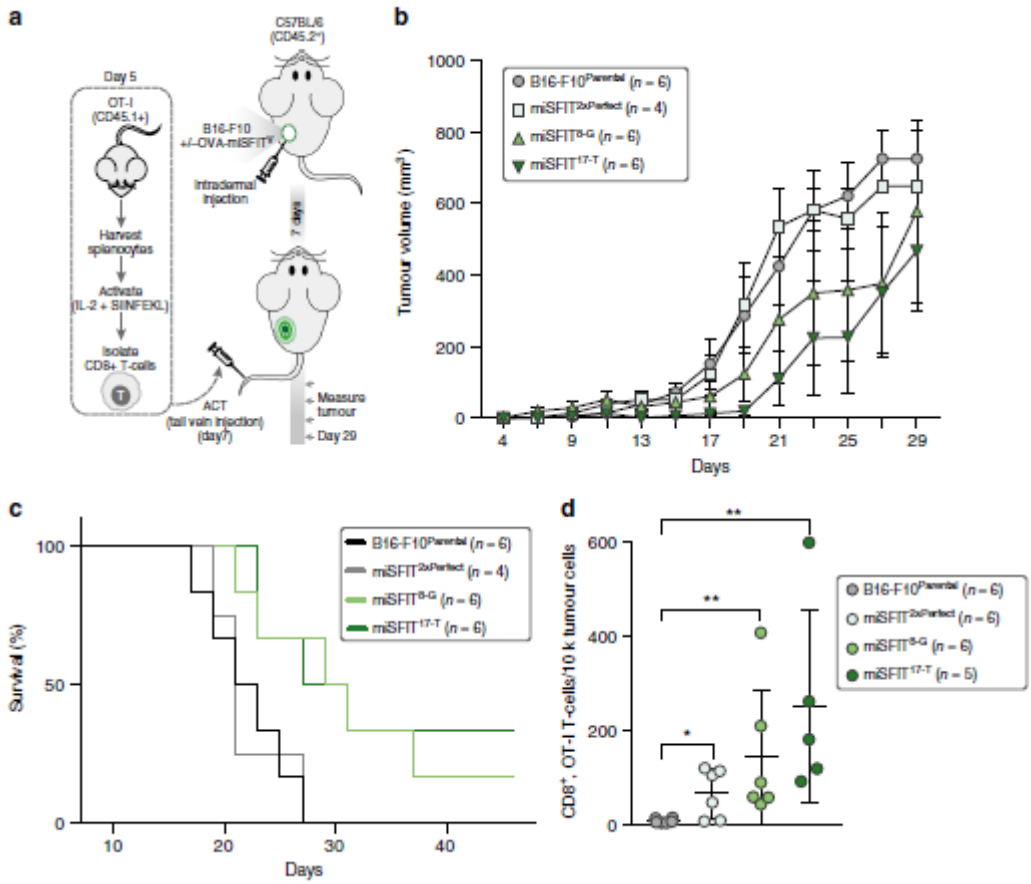
Key Data

Antigen expression levels determine the anti-tumour immune response in vivo.



a Experimental design for in vivo OVA-miSFIT B16-F10 tumour growth experiments.

b Analysis of tumour volume over time for four B16-F10 cell lines (3 OVA-miSFIT variant lines and one B16-F10 parental control line) challenged with OT-I CD8+ T-cells (x-axis= number of days from tumour cells injection; mean +/− s.e.m.)

c Survival curves for mice injected with B16-F10 lines following the same experimental setup as in a.

d Frequency of CD8+, OT-I TILs per tumour (mean +/− s.d., Mann–Whitney U-test, *P < 0.05, **P < 0.01).

For experimental setup see Supplementary Figure 8. Source data are provided as a Source Data file

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Intellectual Property

Patent No.	PCT-GB2017-052068
Application Date	2017.07.13
Status	Application Pending
Country	US, EP

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