

Compact RNA-guided nucleases from nonpathogenic soil microbes provide new tools for efficient and robust editing in human cells

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Life Edit Background and Goals

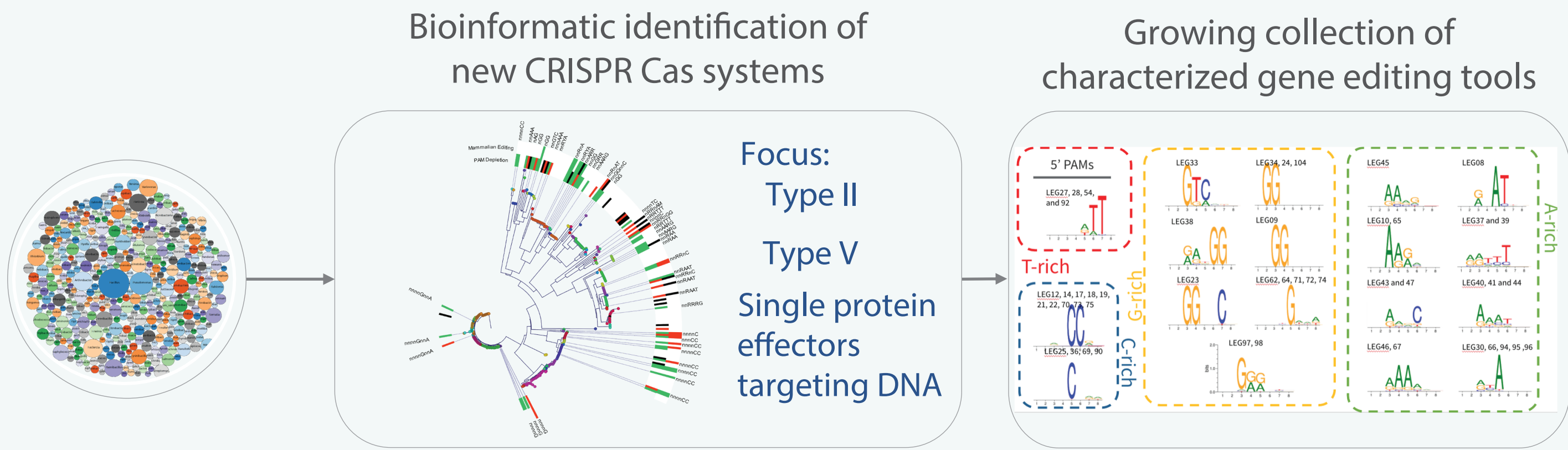
- 01

Unique and versatile platform of CRISPR-based enzymes
- 02

Curative in vivo therapeutics
- 03

Democratized access to editing systems
- Curated, diverse, modular panel of efficient RNA-guided nucleases and base editors
 - Leverage ongoing collaboration with AgBiome to tap an unparalleled microbial collection
 - Curative in vivo therapeutics enabled by small systems with diverse PAMs
 - Leverage existing AAV capabilities at ElevateBio; building delivery systems through key partnerships and acquisition
 - Early validation and tech-transfer readiness established with ElevateBio
 - Enabling broad partnering around R&D collaborations to support initial growth and incorporation into innovative therapies

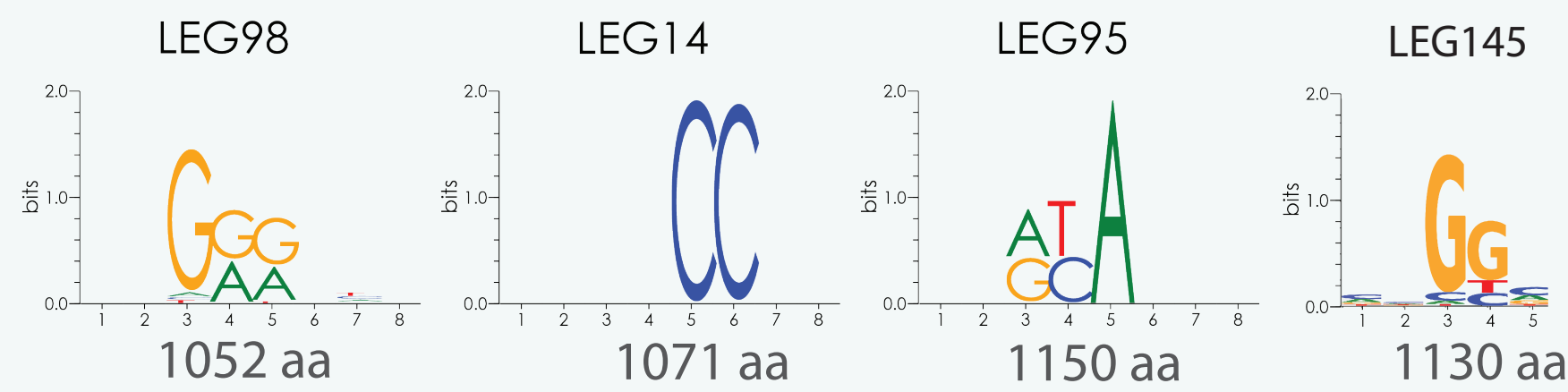
System Discovery



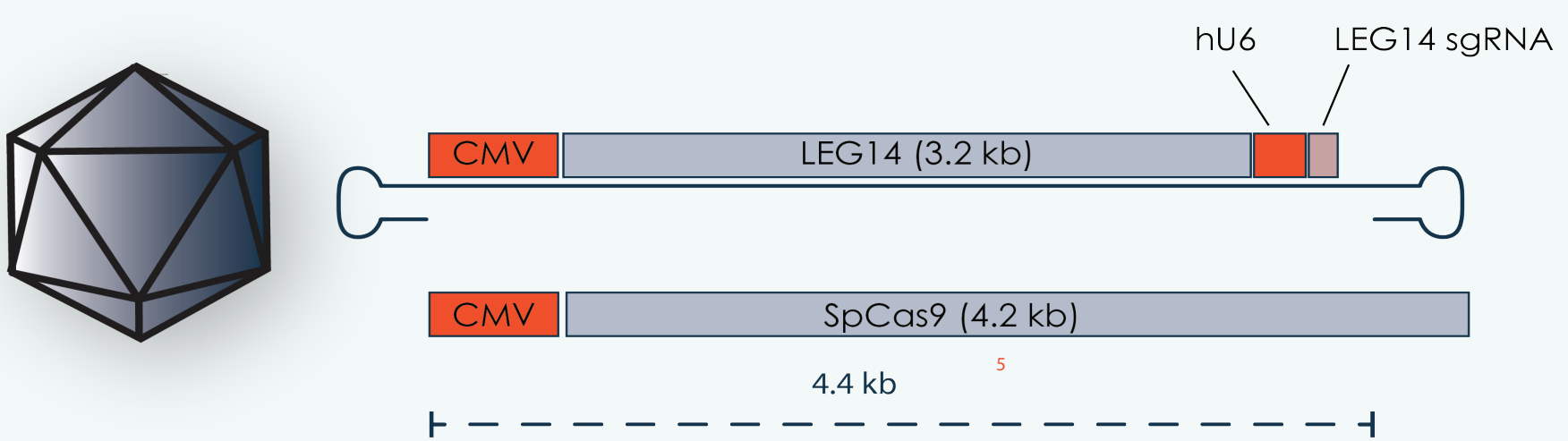
Diverse PAMs allow unprecedented access to genomic sequences of interest. Systems are sourced from nonpathogenic microbes.

Selected Nucleases

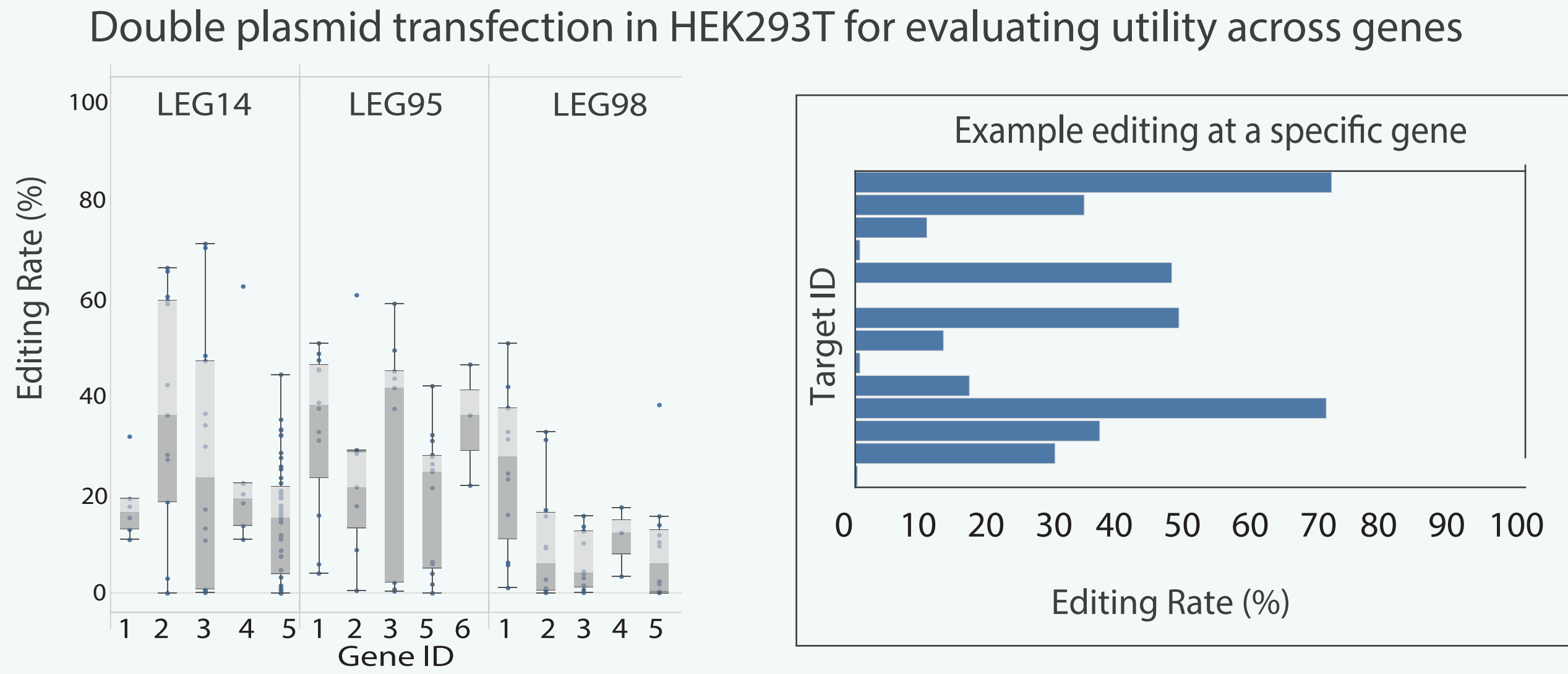
Complementary PAMs enable targeting across genome



Compact size for AAV packaging

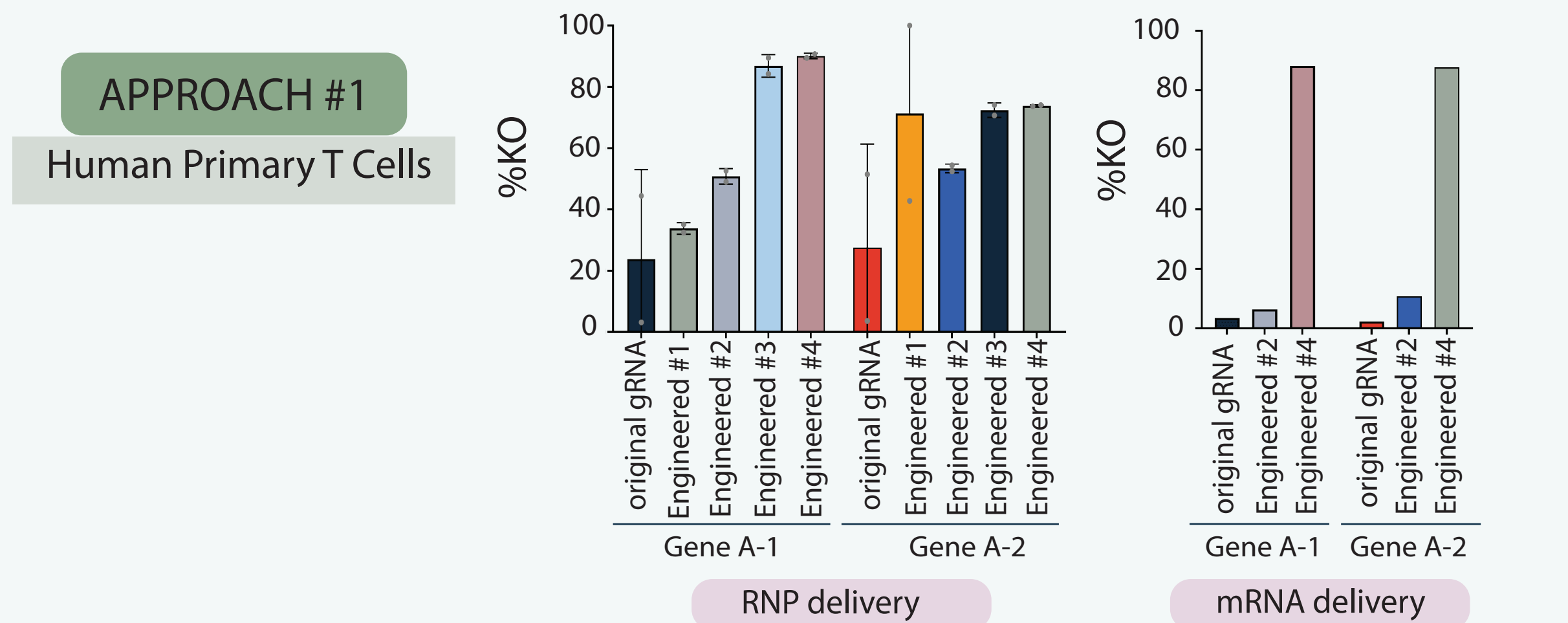


Editing in Mammalian Cells

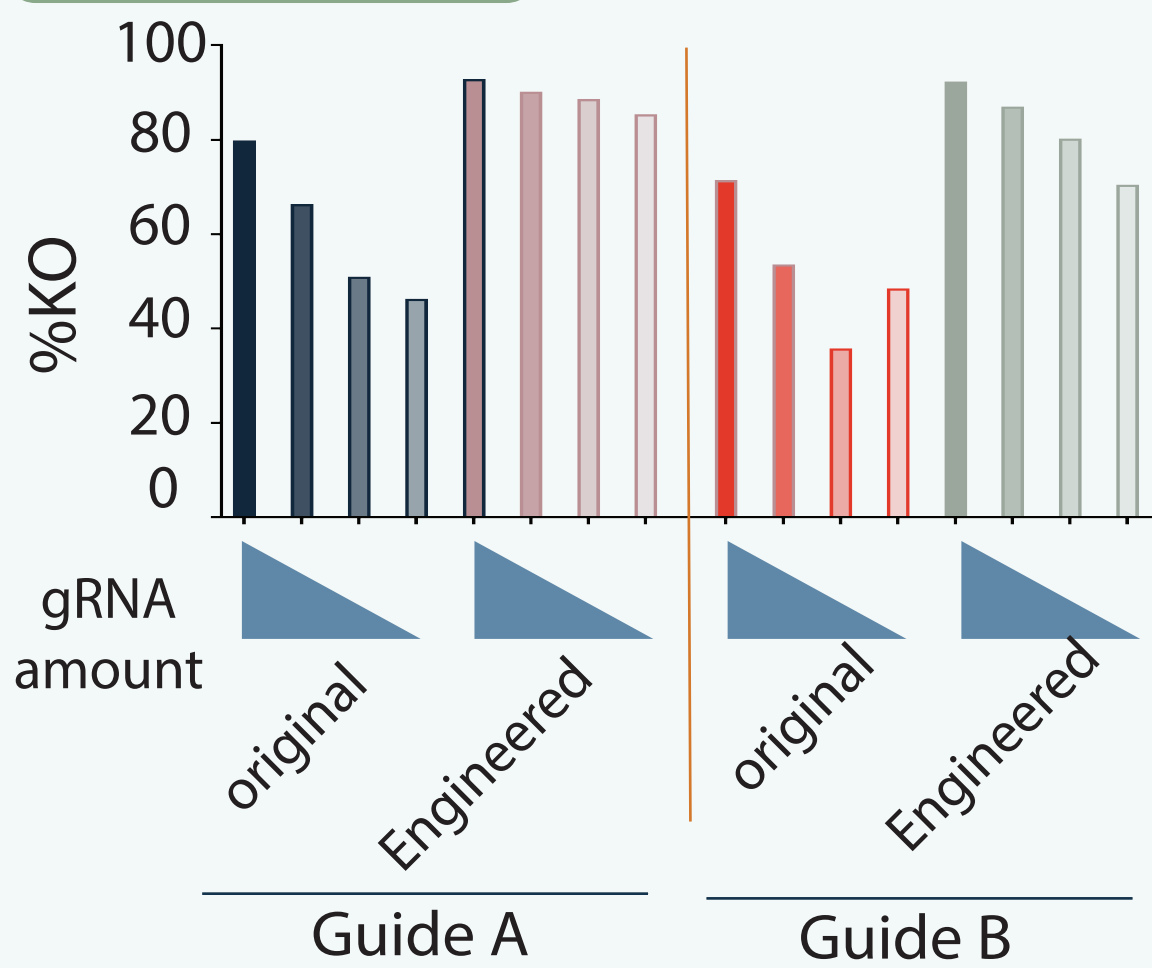


Robust activity across multiple human genes.

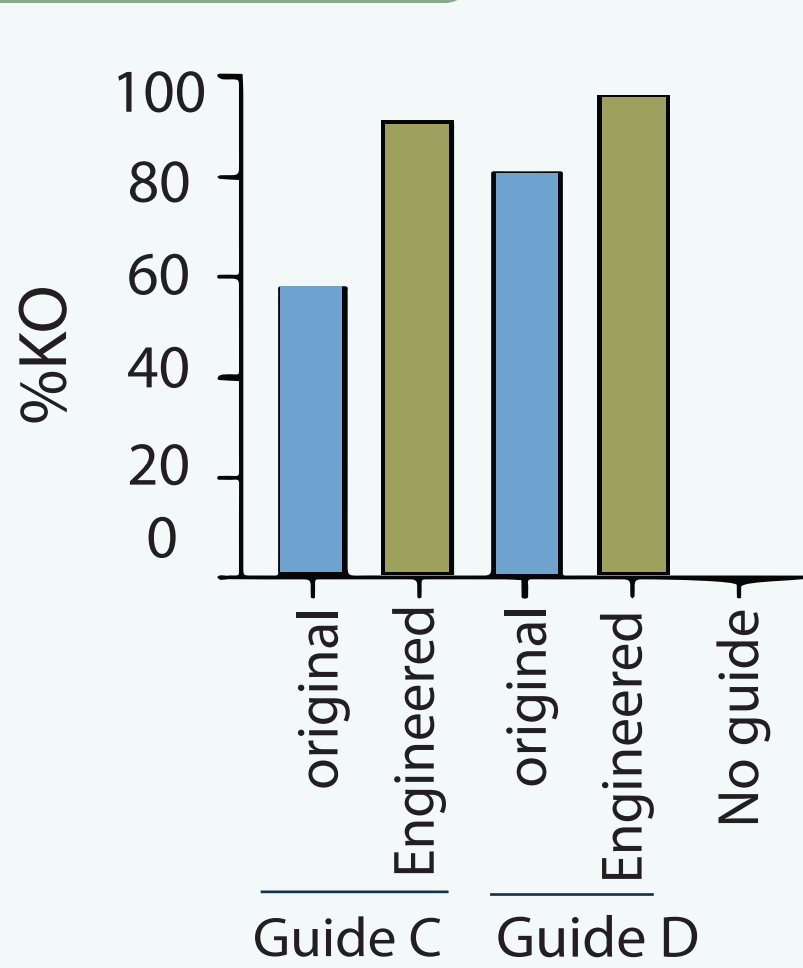
Guide RNA Engineering Improves Editing



APPROACH #2 Human Primary T Cells

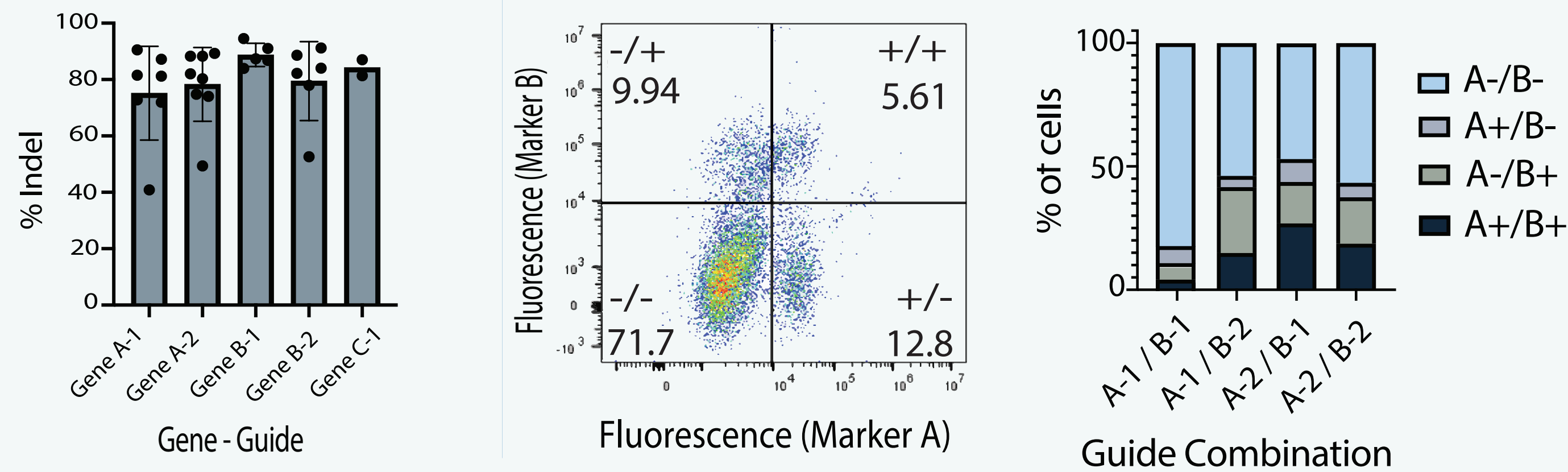


APPROACH #3 iPSC Cells



Engineered guide RNA improves editing functions.

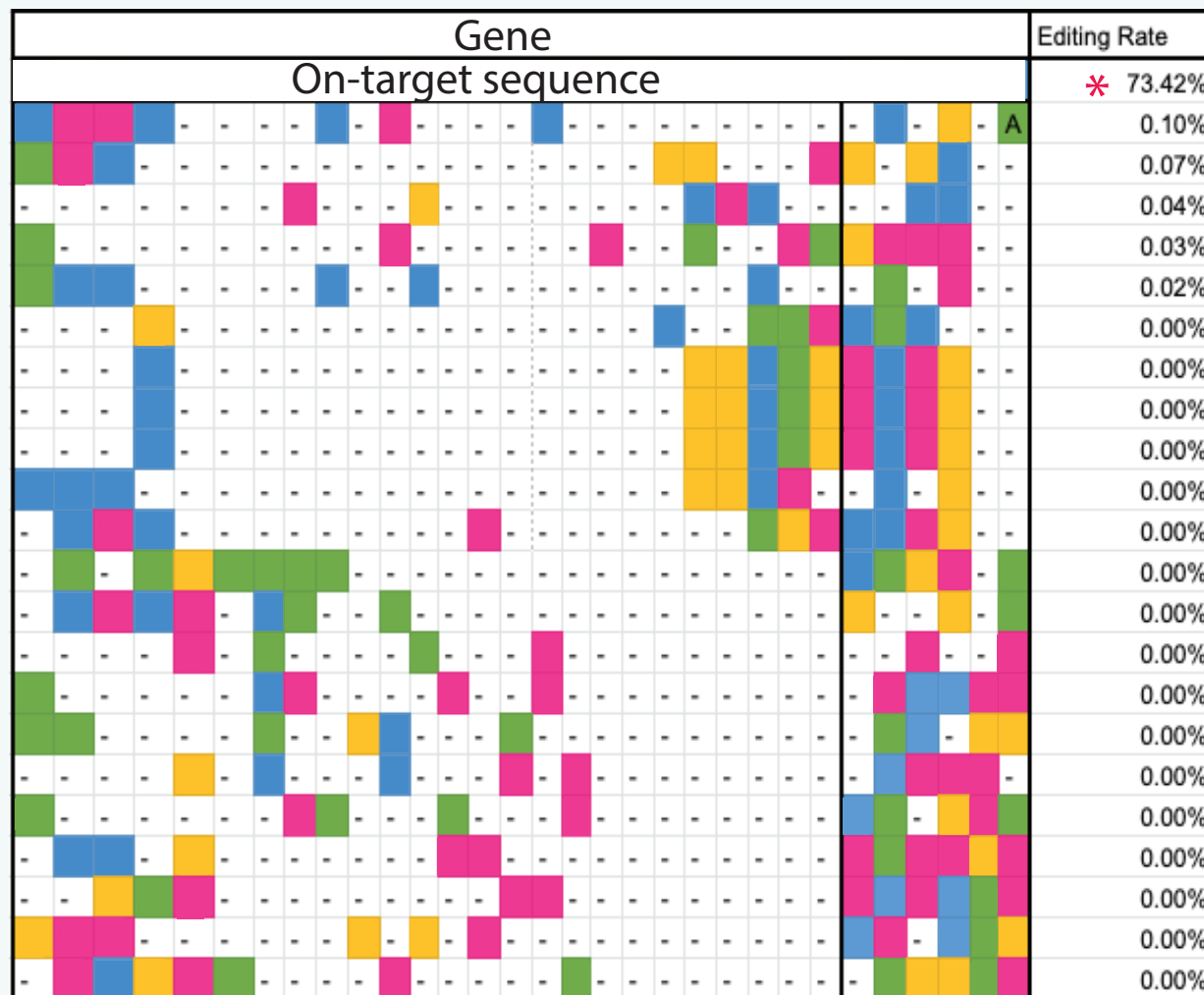
Efficient Single and Multiplexed Editing in T Cells



Negligible Off-target Editing

- Predicted sites of higher off-target risk using homology searches with the human genome
- Performed targeted amplicon sequencing at selected loci

Delivery	Guide	On-target Efficiency	Number of loci tested	Maximum observed editing
mRNA	Gene A - Guide 1	82	12	0.06%
mRNA	Gene A - Guide 2	73	22	0.10%
mRNA	Gene B - Guide 1	75	16	0.06%
mRNA	Gene B - Guide 2	73	6	0.03%



Conclusions

Discovered a panel of new RNA-guided nucleases

- Diverse PAM sequences
- Compact size - AAV delivery enabled
- Efficient and multiplexable editing with multiple delivery methods
- Negligible off-target editing

References

- Crawley, A. B., Henriksen, J. R. & Barrangou, R. CRISPRdisco: An Automated Pipeline for the Discovery and Analysis of CRISPR-Cas Systems. *Crispr J* 1, 171–181 (2018).
- Pinello, L. et al. Analyzing CRISPR genome-editing experiments with CRISPResso. *Nat Biotechnol* 34, 695–697 (2016).