99. Multiplexed gene editing and control with enhanced Cas12a

(Stanford University)

Asset Overview

Product Type	Gene Therapy
Disease Area	Others
Indication	Polygenic Diseases
Current Stage	HIT to Lead
Target	-
MoA	-
Brief Description	• Multiplexed modulation of endogenous genes is crucial for sophisticated gene therapy and cell engineering. CRISPR-Cas12a systems enable versatile multiple genomic loci targeting by processing numerous crRNAs from a single transcript, however, their low efficiency has hindered applications in vivo. • Through structure-guided protein engineering, inventors develop a hyper-efficient LbCas12a variant, termed hyperCas12a, with its catalytically dead version hyperdCas12a showing significantly enhanced efficacy for gene activation, particularly at low crRNA conditions. • Inventors demonstrate that hyperdCas12a has minimal off-target effects compared to the wildtype system and exhibits enhanced activity for gene editing and repression. • Delivery of the hyperdCas12a-activator and a single crRNA array simultaneously activating endogenous Oct4, Sox2, and Klf4 genes in the retina of postnatal mice alters the differentiation of retinal progenitor cells. • The hyperCas12a system offers a versatile in vivo tool for a broad range of gene modulation and gene therapy applications.
Intellectual Property	WO2022174108A1
Publication	Multiplexed genome regulation in vivo with hyper-efficient Cas12a. Nat Cell Biol. (2022)
Inventors	Lei S. QI, Lucie GUO, Hannah KEMPTON

Highlights

- Compared to Cas9:
  - ➢ Better multiplexed gene therapy
  - ➢ Targets multiple genes on one crRNA array
- Compared to wild-type Cas12a:
  - ➢ More efficient activation, repression, and editing
  - ➢ Activity remains high at low crRNA concentration

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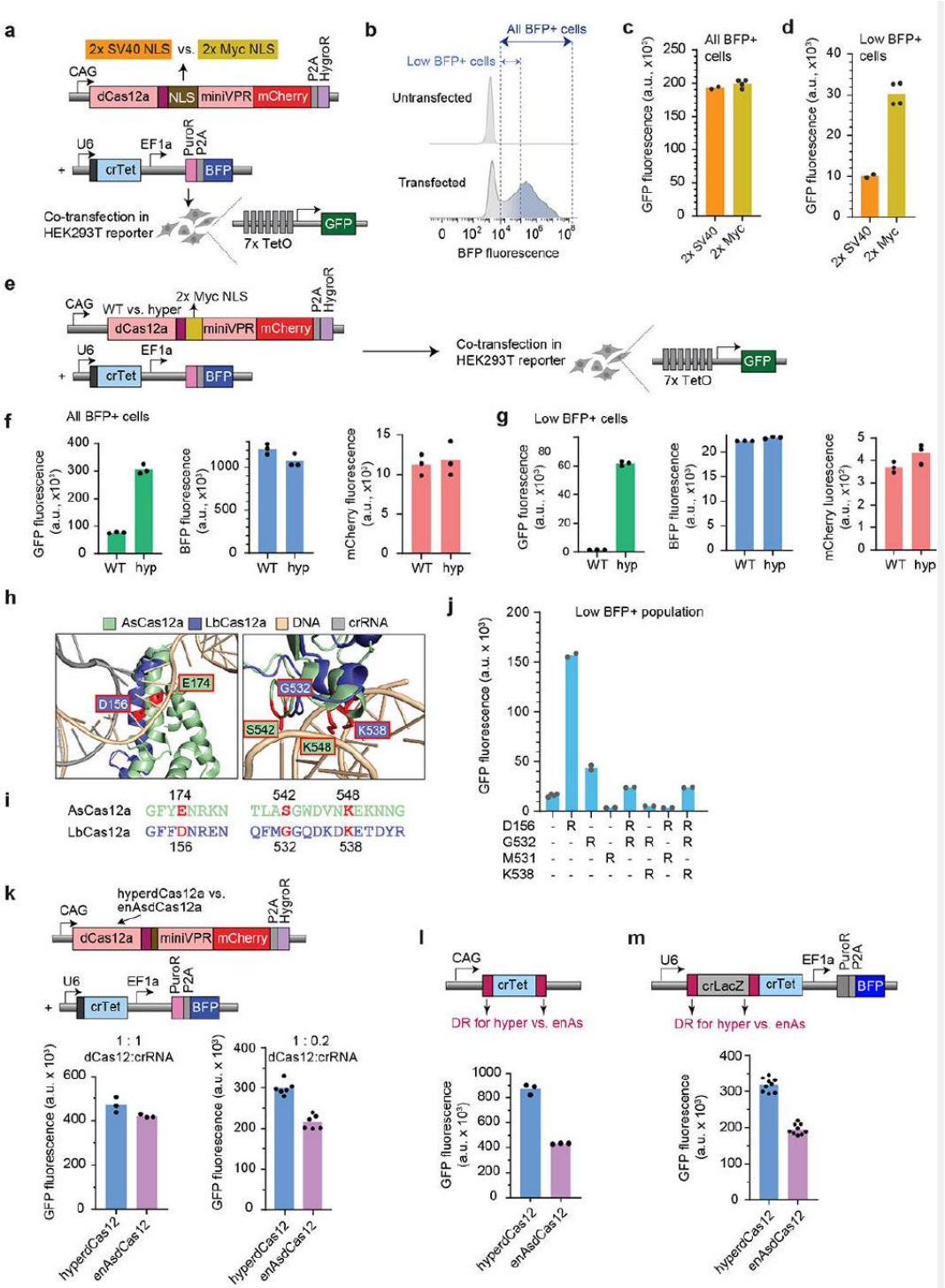
Key Data

Optimizing the nuclear localizing signal and comparing to enAsdCas12a



To be continued

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Optimizing the nuclear localizing signal and comparing to enAsdCas12a

a. Schematic to test two different nuclear localization signals. Constructs containing either 2×SV40 or 2×Myc NLS fused with WT dCas12a are co-transfected with Tet crRNA in TRE3-GFP HEK293T reporter cells. **b**, Representative flow cytometry histogram of BFP intensity, showing threshold for BFP+ cells, and subset of "low BFP+" cells (similar to Fig. 1d). **c-d,** GFP fluorescence in BFP+ (c) or "low BFP+" cells (d). **e-g,** GFP fluorescence in BFP+ (f) or low BFP+ cells (g) to compare WT vs. hyperdCas12a (hyp) with 2×Myc NLS, as well as BFP and mCherry average fluorescence in each gated BFP group. **h-i,** Alignment of the structure of LbCas12a vs. AsCas12a proteins (h) and alignment of peptide sequences (i) encompassing mutations harbored by enAsCas12a, a reported enhanced variant of Cas12a from Acidaminococcus with the E174R/S542R/K548R mutations5 corresponding to homologous residues (D156R/G532R/K538R) mutations in LbCas12a. **j,** Comparison of variants containing mutations of homologous residues in LbCas12a in "low BFP+" cells. Interestingly, D156R combined with G532R and/or K538R did not achieve activation higher than the single D156R mutant, in contrast to results with homologous residues in AsCas12a5. **k,** Comparison of hyperdCas12a vs. enAsdCas12a with a single crRNA driven by U6 promoter in 1:1 vs. 1:0.2 ratio of dCas12:crRNA, in TRE3G-GFP HEK293T cells. **l,** Comparison of hyperdCas12a vs. enAsdCas12a with single crRNA driven by CAG promoter flanked by direct repeats (DR) specific to LbCas12a vs. AsCas12a. m, Comparison of hyperdCas12a vs. enAsdCas12a with dual crRNAs containing crTet on the second position and non-targeting crLacZ on the first position flanked by As or Lb direct repeats (DR). All transfections in this figure were carried out in TRE3G-GFP HEK293T reporter cells. Bar graph in f, g and k-m shows the mean of n≥3 independent experiments; bar graph in j shows the mean of n≥2 independent experiments; each data point represents value of an independent experiment.

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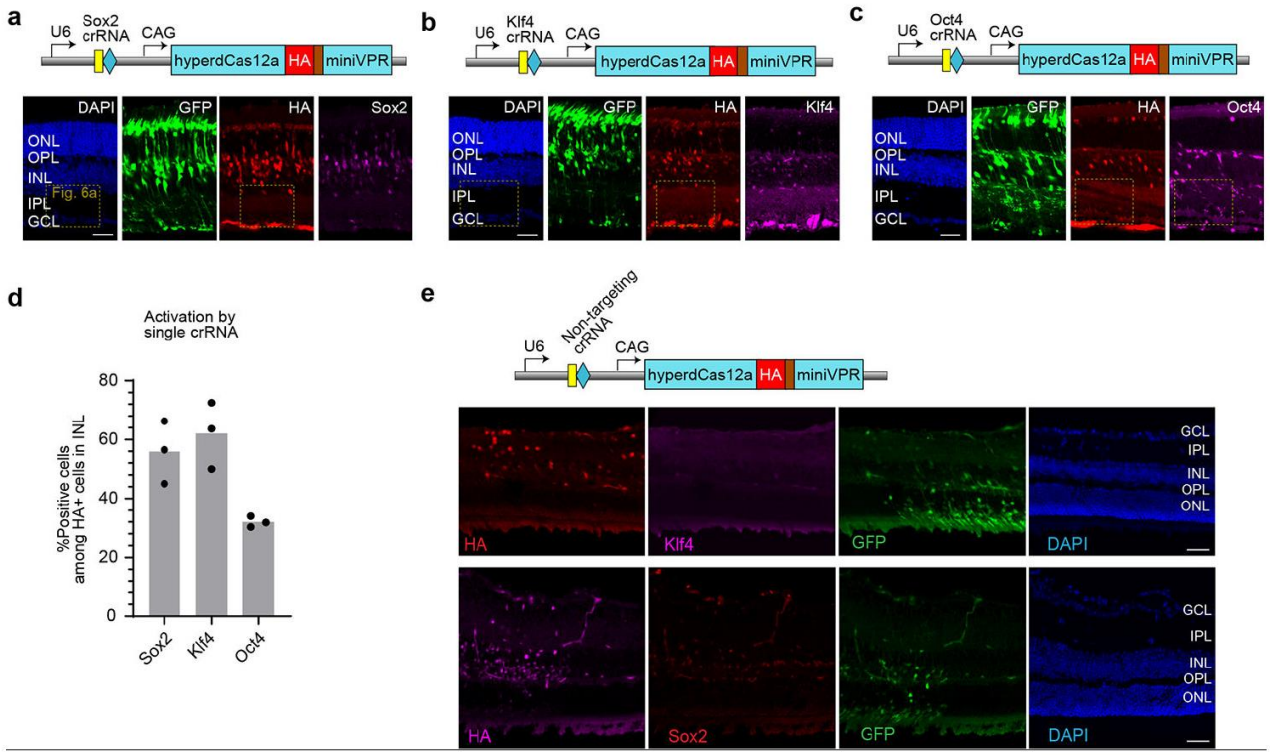
In vivo single crRNA activation by hyperdCas12a



a-c, Constructs containing hyperdCas12a and single crRNA to Sox2 (a), Klf4 (b) or Oct4 (c) for in vivo electroporation in postnatal mouse retina and representative immunofluorescence images. CAG-GFP is used to mark the electroporated patch. Scale bar, 50 μm. **d,** Quantification of percentages of Oct4+, Sox2+ or Klf4+ cells among HA+ cells in INL. Bar graph shows the mean of 3 independent experiments, and each data point represents value of an independent experiment. **e**, Immunofluorescence images of in vivo electroporation in mouse retina with hyperdCas12a with non-targeting LacZ crRNA. Scale bar, 50 μm.