

# Trinucleotide Repeat-Targeting dCas9 as a Therapeutic Strategy for Fuchs' Endothelial Corneal Dystrophy

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## List of the material included:

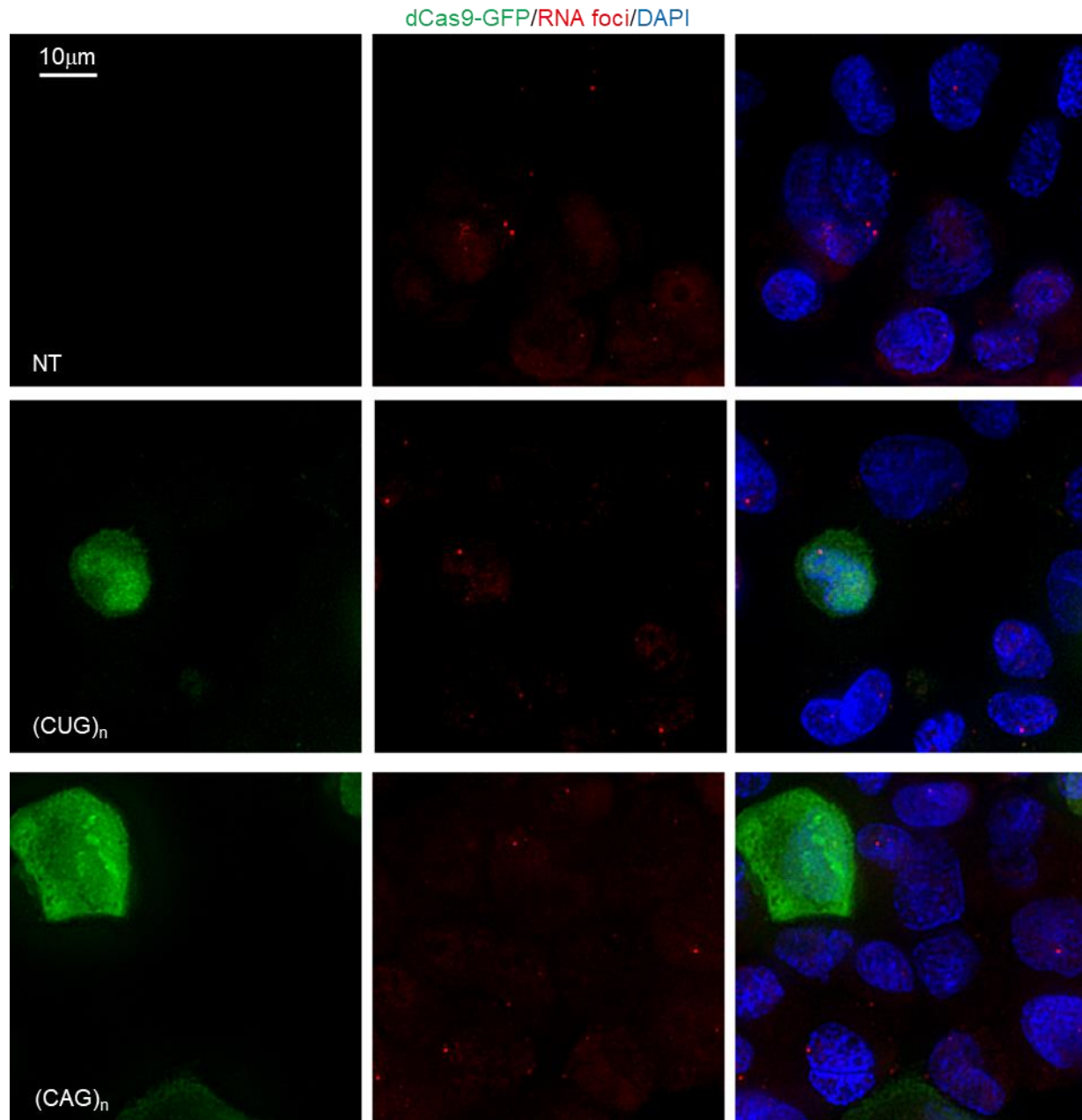
**FIGURE S1. Effects of transient expression of dCas9 and repeat targeting sgRNA on CUG<sup>exp</sup> nuclear foci in patient-derived FECD F45SV corneal cells.**

**FIGURE S2. Effects of transient expression of dCas9 and sgRNA on CUG<sup>exp</sup> foci in patient-derived F35T FECD corneal endothelial cell line.** A. Immunofluorescence images of dCas9-GFP and RNA foci in cells. DAPI staining (blue) defines cell nuclei. B. Transfection efficiency of plasmids was calculated. Results of 2 independent experiments are shown. NT, no plasmid treatment. C. Percentage of cells containing foci and number of foci per 100 cells are shown. In cells co-transfected with GFP-tagged dCas9 and (CAG)<sub>n</sub> sgRNA by Lipofectamine LTX, GFP negative and positive cells were analyzed separately. Results are shown as the mean ± SD, n=2 independent experiments. *P*-value was obtained by t tests analysis of NT compared with dCas9-(CAG)<sub>n</sub> sgRNA GFP+ cells. At least 100 cells were analyzed for each sample (GFP- and GFP+ cells).

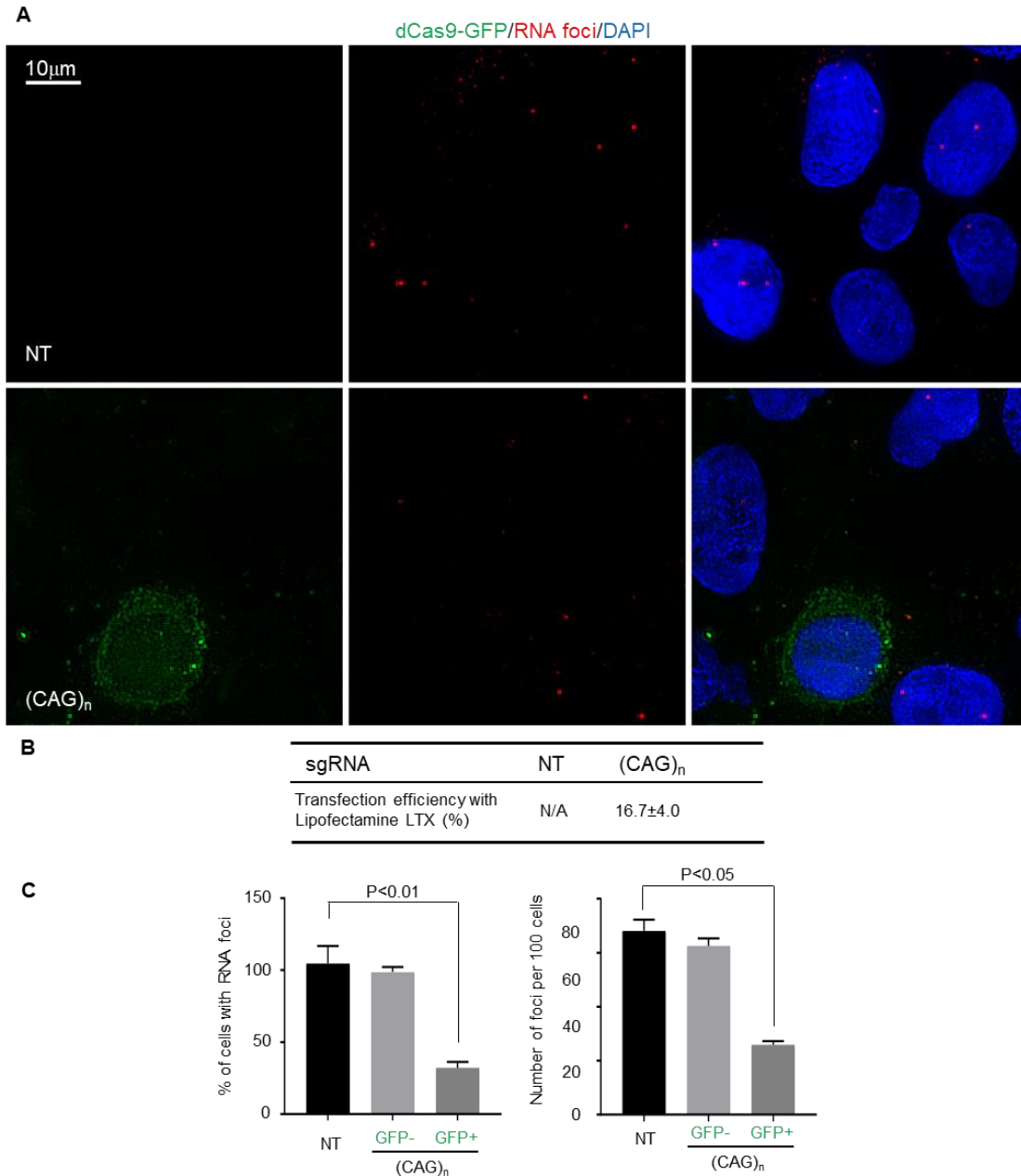
**FIGURE S3. Generation of FECD cell lines stably expressing dCas9 and repeat targeting sgRNAs.** A. Scheme describing generation of stable FECD F45SV cell lines with dCas9-sgRNA or sgRNA alone using lentivirus. Stable cell lines expressing dCas9-sgRNA or sgRNA alone were selected by hygromycin B. B. A GFP-containing positive control lentivirus was used to estimate a 36% transduction efficiency of F45SV cells transduced with lentivirus prior to selection. BF, bright field. C. Hygromycin B resistant gene expression was measured by PCR to confirm the establishment of stable cell line. PCR product was used to run in a DNA gel.

**FIGURE S4. (CAG)<sub>n</sub> sgRNA cannot inhibit CUG<sup>exp</sup> foci without dCas9.** A. FISH images of CUG repeat RNA foci in F45SV corneal endothelial cells transduced with the indicated sgRNAs. B. Percentage of cells containing foci and number of foci per 100 cells are analyzed. Results are shown as the mean ± SD, n=2 independent experiments. At least 100 cells were analyzed for each sample. NT, no treatment. NTC, non-targeting control.

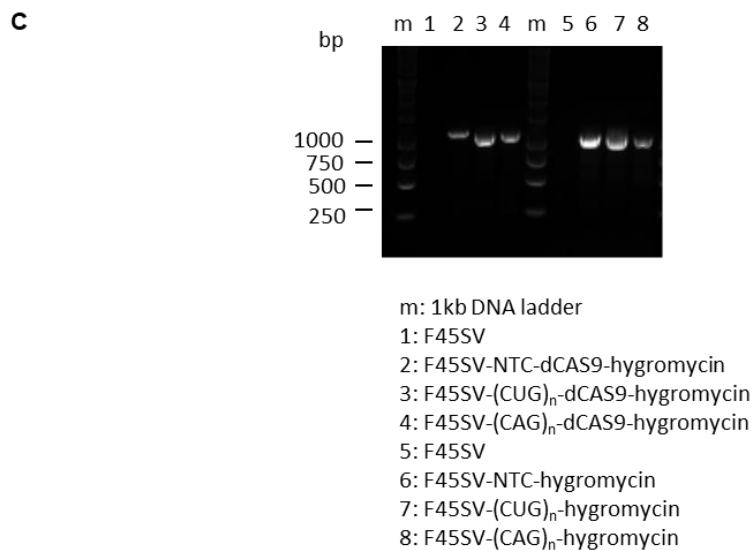
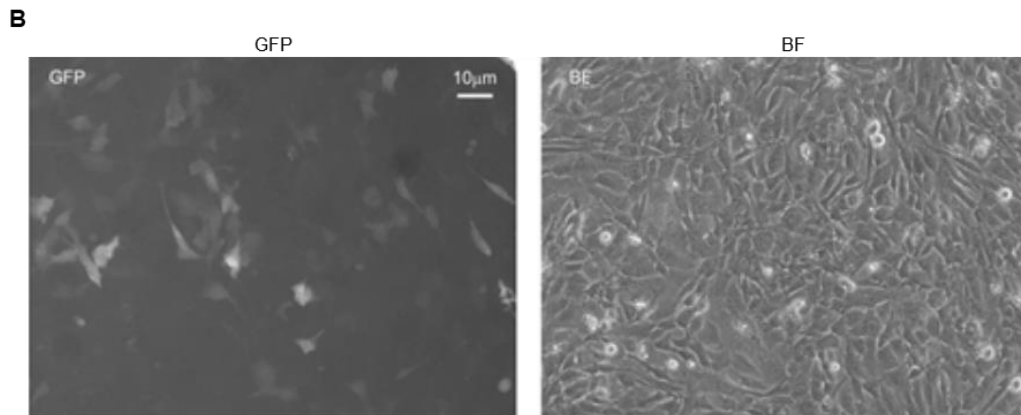
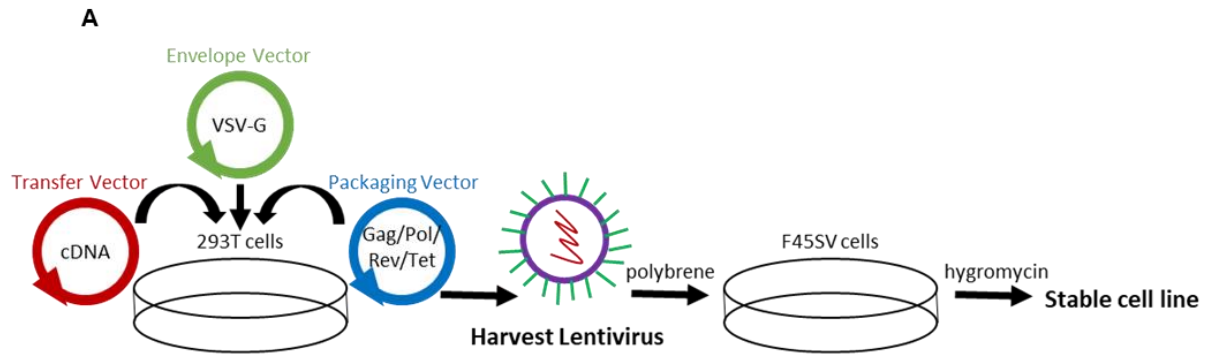
**TABLE S1. Summary of sgRNA targets and sequences**



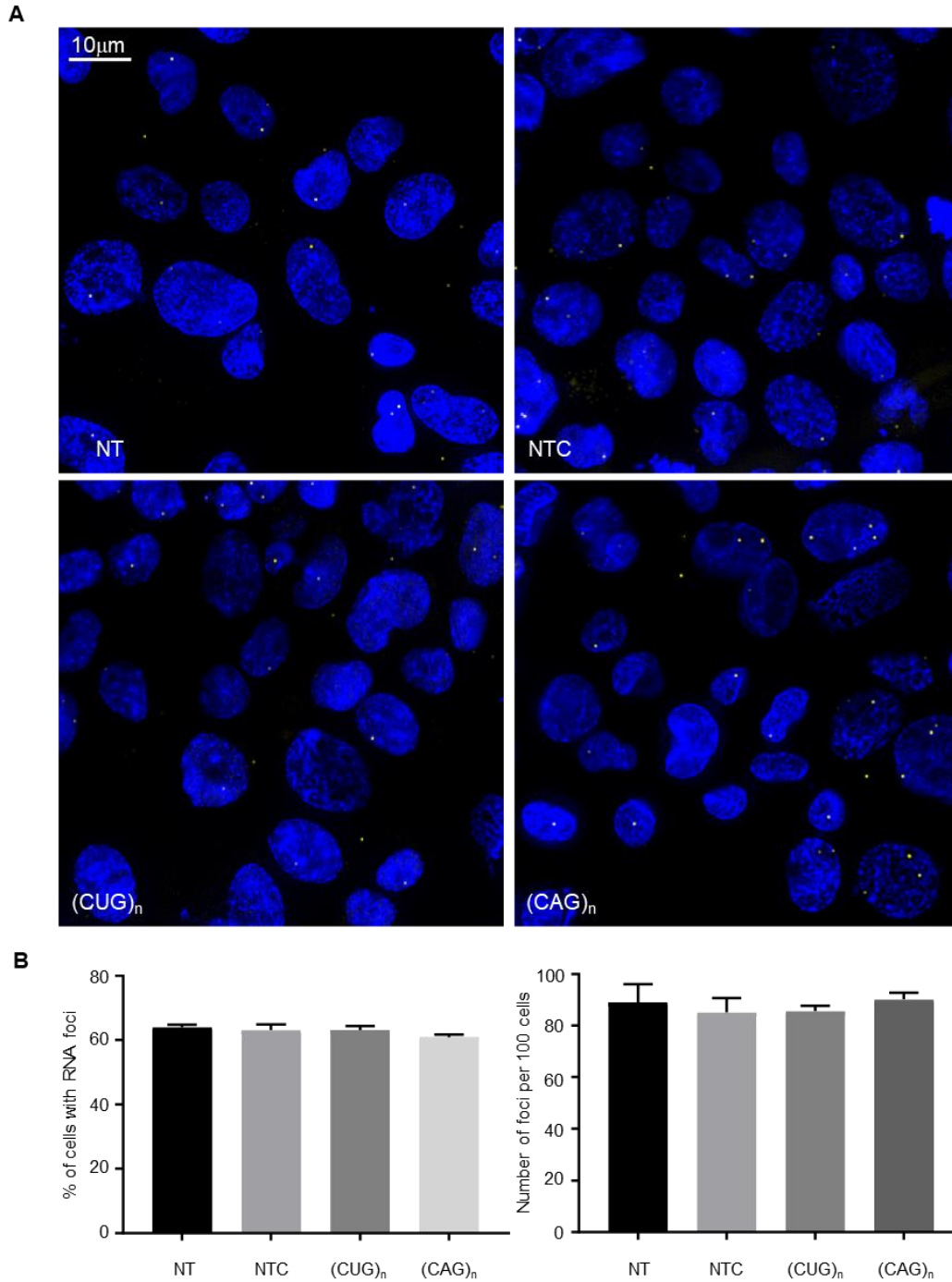
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<b>sgRNA</b>	<b>sgRNA spacer sequence</b>
Non-targeting control	GTGATAAGTGAATGCCATG
(CUG) <sub>n</sub>	CTGCTGCTGCTGCTGCTGCT
(CAG) <sub>n</sub>	AGCAGCAGCAGCAGCAGCAG

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