Supplemental Materials
for

5' DREDGE: Direct Repeat-Enabled Downregulation of Gene Expression via the 5' UTR of Target Genes

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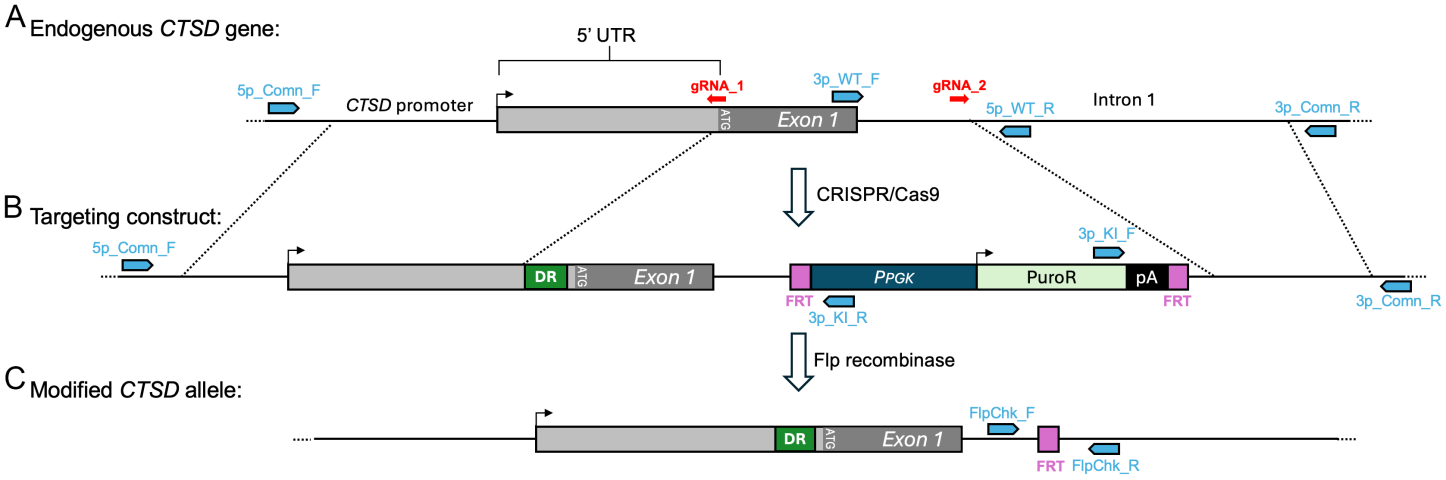
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Supplementary Figure S1.



D gRNA design and alterations to knock-in insert:

	gRNA_1	gRNA_2
gRNA sequence	**GGGAGTCTTCATGGTCGCGG**	**ACCTGGAGTGCCGCGTGCTC**
Endog. Sequence (top strand)	...CCGCCGCGACCATGAAGACTCCC...	...ACCTGGAGTGCCGCGTGCTCCGG...
A.A. sequence	MetLysThrPro…	N/A
KI insert sequence	...**GAAA**GCCGCGACCATGAAGACTCCC...	deleted and replaced w/ FRT site

E Genotyping primers:

Primer Name	Primer Sequence
5p_Comn_F	CCTTGTATCAATAGATGCATAGTC
5p_WT_R	CCTACTGTACCCTCCTGTTC
5p_KI_R	GAATAGGAACTTCGAACGCG
3p_Comn_R	CTCTACTCCCGCAGCCTACG
3p_WT_F	GTCCTCCTTCGCGATTATCAGGTG
3p_KI_F	GCTCGACTAGAGCTTGCGGAAC
FlpChk_F	GTCTTGCTGCTCATTCTCG
FlpChk_R	CTGTTCGACCATAGGAGTCATAGG

F Genotyping results:

Amplicon	Forward Primer	Position in WT	Position in KI, pre-Flp	Position in KI, post-Flp	Reverse Primer	Position in WT	Position in KI, pre-Flp	Position in KI, post-Flp	Size of Correct Amplicon (bp)	Result
5' WT	5p_Comn_F	397	397	397	5p_WT_R	1326	-	-	930	no amplicon
5' KI	5p_Comn_F	397	397	397	5p_KI_R	-	1222	-	826	100% match by Sanger sequencing
3' WT	3p_WT_F	1101	-	-	3p_Comn_R	1844	-	-	744	no amplicon
3' KI	3p_KI_F	-	2749	-	3p_Comn_R	-	3477	-	729	100% match by Sanger sequencing
Flp Check	FlpChk_F	-	-	1100	FlpChk_R	-	-	1378	279	100% match by Sanger sequencing

Figure S1. Overview of genotyping results confirming the successful introduction of one Csy4 DR into the 5' UTR of murine *CTSD* via CRISPR-Cas. **A**, Genomic structure of the mouse *CTSD* gene prior to modification, showing the locations of the gRNAs used for CRISPR-Cas (red). **B**, Design of the modified allele after integration of the targeting construct. Note the presence of a Puro^r resistance cassette flanked by FRT sites (purple). **C**, Final structure of the modified CTSD allele after removal of the Puro^r resistance cassette with Flp-recombinase. The approximate positions of genotyping primers are indicated in **A-C** (blue arrows). **D**, Table showing the gRNA sequences using for CRISPR-Cas, the targeted endogenous sequence (with PAM sequence in red), the amino-acid sequences encoded by the sequence targeted by gRNA_1, and the modifications to the corresponding targeted region within the KI targeting construct. Note that the top strand of the endogenous sequence is shown to highlight the translated region. **E**, Table showing the sequences for the DNA primers used for genotyping, with relative positions indicated in **A-C** (blue arrows). **F**, Summary of genotyping results, showing the relative positions of individual primers, the predicted amplicon sizes for primer pairs, and the outcome of PCR amplification and subsequent Sanger sequencing. These results apply to a single clonal cell line chosen for use in downstream experiments. Note that no amplification was obtained for the 5' or 3' WT amplicons.