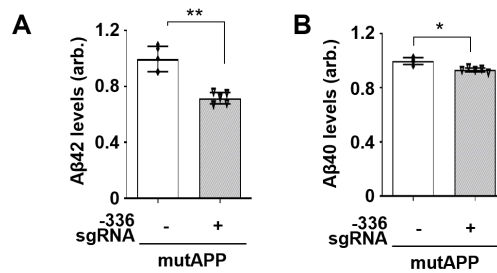


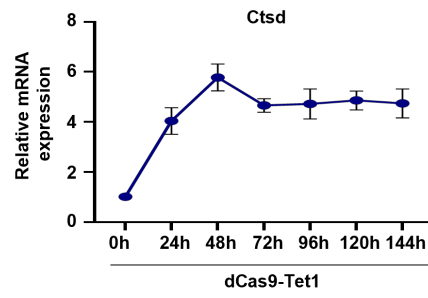
## Supplementary Figure 1



### Supplementary Figure 1. Quantification of Aβ42 and Aβ40 levels in mutAPP primary neurons.

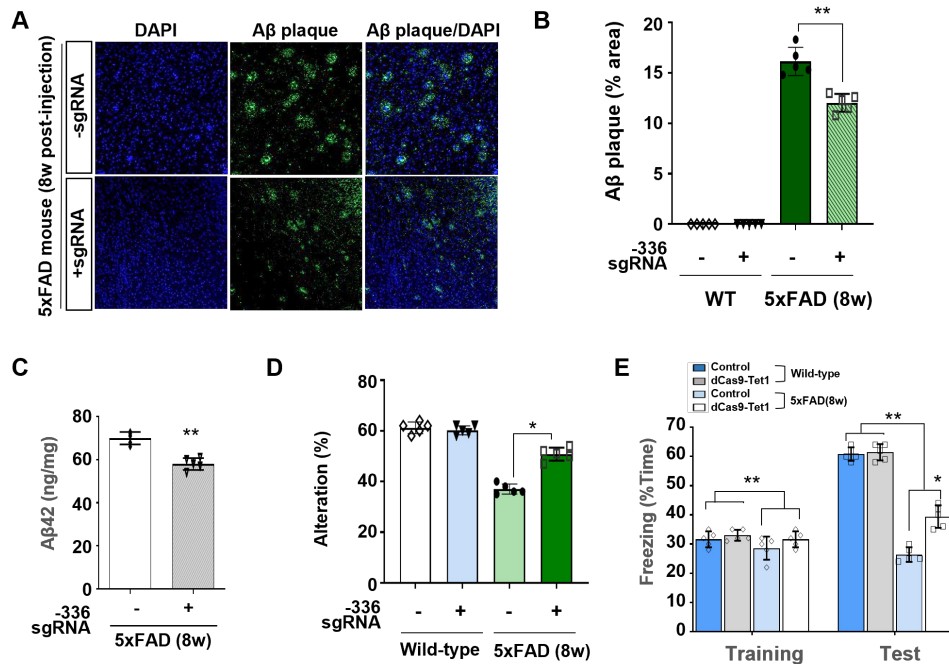
(A) ELISA analysis of Aβ42 levels in mutAPP mouse primary neurons transduced with -336 sgRNA and dCas9-Tet1. Data are presented as mean ± SEM (n = 5). \*\* $p < 0.01$ , two-tailed unpaired t-tests. (B) ELISA analysis of Aβ40 levels in mutAPP mouse primary neurons transduced with -336 sgRNA and dCas9-Tet1. Data are expressed as mean ± SEM (n = 5). \* $p < 0.05$ , two-tailed unpaired t-tests.

## Supplementary Figure 2



**Supplementary Figure 2. Verification of sustained gene activation in mouse brain following dCas9-Tet1 injection.** Samples were analyzed at 24, 48, 72, 96, 120, and 144 hours post-injection.

### Supplementary Figure 3



**Supplementary Figure 3. Longitudinal effects of dCas9-Tet1-injected 5xFAD mouse model.** (A) Immunostaining for ThT in -336 sgRNA and dCas9-Tet1 injected 5xFAD mice on 8 weeks post-injection. (B) Percentage of Aβ plaque in -336 sgRNA and dCas9-Tet1 injected 5xFAD mouse on 8 weeks post-injection. Data are shown as mean ± SEM (n = 5). \*\**p* < 0.01, two-tailed unpaired t-tests. (C) Levels of Aβ42 measured by ELISA in -336 sgRNA and dCas9-Tet1 injected 5xFAD mice on 8 weeks post-injection. Data are shown as mean ± SEM (n = 5). \*\*\**p* < 0.001, two-tailed unpaired t-tests. (D) Y-maze test of -336 sgRNA and dCas9-Tet1 injected 5xFAD on 8 weeks post-injection. Data are shown as mean ± SEM. \*\**p* < 0.01, two-way ANOVA with Tukey's multiple comparisons test. (E) Fear conditioning test of -336 sgRNA and dCas9-Tet1 injected 5xFAD mice on 8 weeks post-injection. Data are shown as mean ± SEM. \*\**p* < 0.01, two-way ANOVA with Tukey's multiple comparisons test. Image in panel A is representative of three or more similar experiments.

### Supplementary Table 1

Target	Sequence	Chromosome	Position
-336 sgRNA	TATGCAAAAAAGACAGATTAAGG	Chr.7	142387747
-239 sgRNA	ACCTGTATCCAGTCATTCTCCGG	Chr.7	142387758
-151 sgRNA	GCCCGGGCAGTGTGGAGGCGGG	Chr.7	142387745

**Supplementary Table 1. sgRNA sequences targeting Ctsd.**

## Supplementary Table 2

No.	Target	Chromosome	Position	Direction	Gene
OF1	TATGTCAAAAcAGAAAGATTATGG	Chr8	22288595	-	Tpte
OF2	TATGCAAATAAAcACAGATaAAGG	ChrX	56197821	+	Gm773
OF3	TATGtAAAAAAGACAAGAgTATGG	Chr10	109862984	-	Nav3
OF4	aCATiCAAAAAGACAGATTAAGG	Chr18	89741195	+	Dok6
OF5	gATGCAAcAAAGACAGACTTAAGG	Chr19	34118103	-	Lipm

**Supplementary Table 2. Predicted Ctsd off-target sites for -336 sgRNA.** Off-target sites are those with no more than two mismatches compared to on-target sequences. OF, off-target.