Supplemental Materials

for

A Dual-Function "TRE-Lox" System for Genetic Deletion or Reversible, Titratable, and Near-Complete Downregulation of Cathepsin D

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**Supplementary Figure S1. Targeted region of murine *CTSD* gene highlighting key elements.**



Promoter: lower-case gray

TATA Box: underlined

Transcript. start: ↱

EXON 1: UPPER-CASE BLACK

Introns: lower-case black

ATG site: OUTLINED

gRNA seqs: **BOLD BLACK**

gRNA orientation: horizontal arrows

PAM seqs: Yellow highlight

Cut site: ⇑

...acccgttgagggccaaacaagcgggtcagctgactccgcgggactgc

↱

ggcgtcatcctgcctATAAGCCGGCGACCTCTGGCTTTAAGCTTTGCTCT

CTTCGGG**CCGCCGCGACCATGAAGACTCCC**GGCGTCTTGCTGCTCATTCT ←----⇑---------------⤙

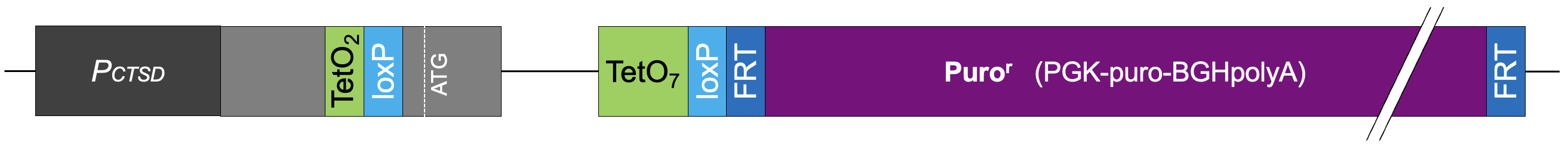
CGGCCTCCTGGCTTCGTCCTCCTTCGCGATTATCAGgtgaggacccgctc

⤚---------------⇓----⟶

tgggtccggagatgcggggctcgtc**acctggagtgccgcgtgctccgg**cc

gtgctggaatgcacctgtgcacccagcgcagccttcctcagggtccc...

**Supplementary Figure S2. Targeted region after insertion of TRE-Lox KI insert with Puror cassette.**

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Flanking seq: gray text

Homology Arms: purple italic

TATA Box: underlined

EXONS: UPPER CASE

Introns: lower case black

KI region: **bold**

ATG sites: OUTLINED

Stop codons: black box

Spacer seqs: gray highlight

TetO repeats: green highlight

LoxP sites: light blue highlight

FRT sites: blue highlight

PGK-Puror-pA: violet highlight

...gttcgggtcgccccgcccctcgcccgcgtctcacgtgacccgttgagggccaaacaagcgggtcagctgactccgcgggactgcggcgtcatcctgc*ctATAAGCCGGCGACCTCTGGCTTTAAGCTTTGCTCTCTTCGGGCCGCCG*

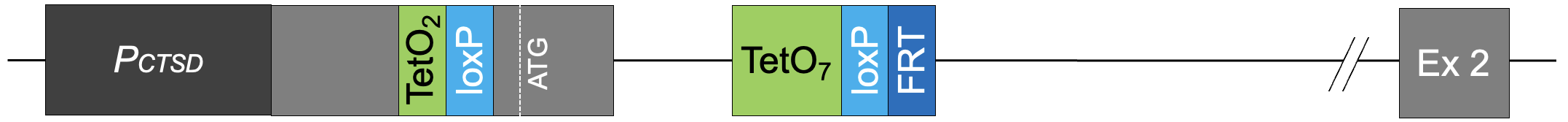
**CAAGCAGAGCTCtccctatcagtgatagagaTCtccctatcagtgatagagaTCGTCGATAACTTCGTATAAAGTATCCTATACGAAGTTATAGCCGCGACCATGAAGACTCCCGGCGTCTTGCTGCTCATTCTCGGCCTCCTGGCTTCGTCCTCCTTCGCGATTATCAGgtgaggacccgctctgggtccggagatgcggggctcgtcacctggagtgccgcgtcttcactcgagtttactccctatcagtgatagagaacgtatgaagagtttactccctatcagtgatagagaacgtatgcagactttactccctatcagtgatagagaacgtataaggagtttactccctatcagtgatagagaacgtatgaccagtttactccctatcagtgatagagaacgtatctacagtttactccctatcagtgatagagaacgtatatccagtttactccctatcagtgatagagaacgtatgtcgaggtagataacttcgtataaagtatcctatacgaagttatgtagtaataactagtcgaagttcctattctctagaaagtataggaacttcaggtctgaagaggagtttacgtccagccaagctagcttggctgcaggtcgtcgaaattctaccgggtaggggaggcgcttttcccaaggcagtctggagcatgcgctttagcagccccgctgggcacttggcgctacacaagtggcctctggcctcgcacacattccacatccaccggtaggcgccaaccggctccgttctttggtggccccttcgcgccacct**

**Supplementary Figure S2. Targeted region after insertion of TRE-Lox KI insert with Puror cassette (cont'd).**

**tctactcctcccctagtcaggaagttcccccccgccccgcagctcgcgtcgtgcaggacgtgacaaatggaagtagcacgtctcactagtctcgtgcagatggacagcaccgctgagcaatggaagcgggtaggcctttggggcagcggccaatagcagctttgctccttcgctttctgggctcagaggctgggaagggg**

**tgggtccgggggcggggcgggcgcccgaaggtcctccggaggcccggcattctgcacgcttcaaaagcgcacgtctgccgcgctgttctcctcttcctcatctccgggcctttcgacctgcagcctgttgacaattaatcatcggcatagtatatcggcatagtataatacgacaaggtgaggaactaaaccatgaccgagtacaagcccacggtgcgcctcgccacccgcgacgacgtccccagggccgtacgcaccctcgccgccgcgttcgccgactaccccgccacgcgccacaccgtcgatccggaccgccacatcgagcgggtcaccgagctgcaagaactcttcctcacgcgcgtcgggctcgacatcggcaaggtgtgggtcgcggacgacggcgccgcggtggcggtctggaccacgccggagagcgtcgaagcgggggcggtgttcgccgagatcggcccgcgcatggccgagttgagcggttcccggctggccgcgcagcaacagatggaaggcctcctggcgccgcaccggcccaaggagcccgcgtggttcctggccaccgtcggcgtctcgcccgaccaccagggcaagggtctgggcagcgccgtcgtgctccccggagtggaggcggccgagcgcgccggggtgcccgccttcctggagacctccgcgccccgcaacctccccttctacgagcggctcggcttcaccgtcaccgccgacgtcgaggtgcccgaaggaccgcgcacctggtgcatgacccgcaagcccggtgcctgaggggatcaattctctagagctcgctgatcagcctcgactgtgccttctagttgccagccatctgttgtttgcccctcccccgtgccttccttgaccctggaaggtgccactcccactgtcctttcctaataaaatgaggaaattgcatcgcattgtctgagtaggtgtcattctattctggggggtggggtggggcaggacagcaagggggaggattgggaagacaatagcaggcatgctggggatgcggtgggctctatggcttctgaggcggaaagaaccagctggggctcgactagagcttgcggaacccttcgaagttcctattctctagaaagtataggaacttcggcgcgccattt***cggccgtgctggaatgcacctgtgcacccagcgcagccttcctcagggtc*ccacagactatggggcgagacactcaaaaggcaggggtctcctgggcccctctccactcctatgactcctatggtcgaacaggagggtacagt...

**Supplementary Figure S3. Targeted region after Flp recombination to remove Puror cassette.**



Flanking seq: gray text

Homology Arms: purple italic

TATA Box: underlined

EXONS: UPPER CASE

Introns: lower case black

KI region: **bold**

ATG sites: OUTLINED

Stop codons: black box

Spacer seqs: gray highlight

TetO repeats: green highlight

LoxP sites: light blue highlight

FRT sites: blue highlight

...gttcgggtcgccccgcccctcgcccgcgtctcacgtgacccgttgagggccaaacaagcgggtcagctgactccgcgggactgcggcgtcatcctgc*ctATAAGCCGGCGACCTCTGGCTTTAAGCTTTGCTCTCTTCGGGCCGCCG***CAAGCAGAGCTCtccctatcagtgatagagaTCtccctatcagtgatagagaTCGTCGATAACTTCGTATAAAGTATCCTATACGAAGTTATAGCCGCGACCATGAAGACTCCCGGCGTCTTGCTGCTCATTCTCGGCCTCCTGGCTTCGTCCTCCTTCGCGATTATCAGgtgaggacccgctctgggtccggagatgcggggctcgtcacctggagtgccgcgtcttcactcgagtttactccctatcagtgatagagaacgtatgaagagtttactccctatcagtgatagagaacgtatgcagactttactccctatcagtgatagagaacgtataaggagtttactccctatcagtgatagagaacgtatgaccagtttactccctatcagtgatagagaacgtatctacagtttactccctatcagtgatagagaacgtatatccagtttactccctatcagtgatagagaacgtatgtcgaggtagataacttcgtataaagtatcctatacgaagttatgtagtaataactagtcgaagttcctattctctagaaagtataggaacttcgaagttcctattctctagaaagtataggaacttcggcgcgccattt***cggccgtgctggaatgcacctgtgcacccagcgcagccttcctcagggtc*ccacagactatggggcgagacactcaaaaggcaggggtctcctgggcccctctccactcctatgactcctatggtcgaacaggagggtacagt...

**Supplementary Figure S4. Targeted region after genetic deletion (recombination w/ Flp and Cre).**

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Flanking seq: gray

Homology Arms: purple italic

TATA Box: underlined

KI region: **bold black**

ATG sites OUTLINED UPPER CASE

Stop codons: black box

Spacer seqs: gray highlight

TetO repeats: green highlight

LoxP site: light blue highlight

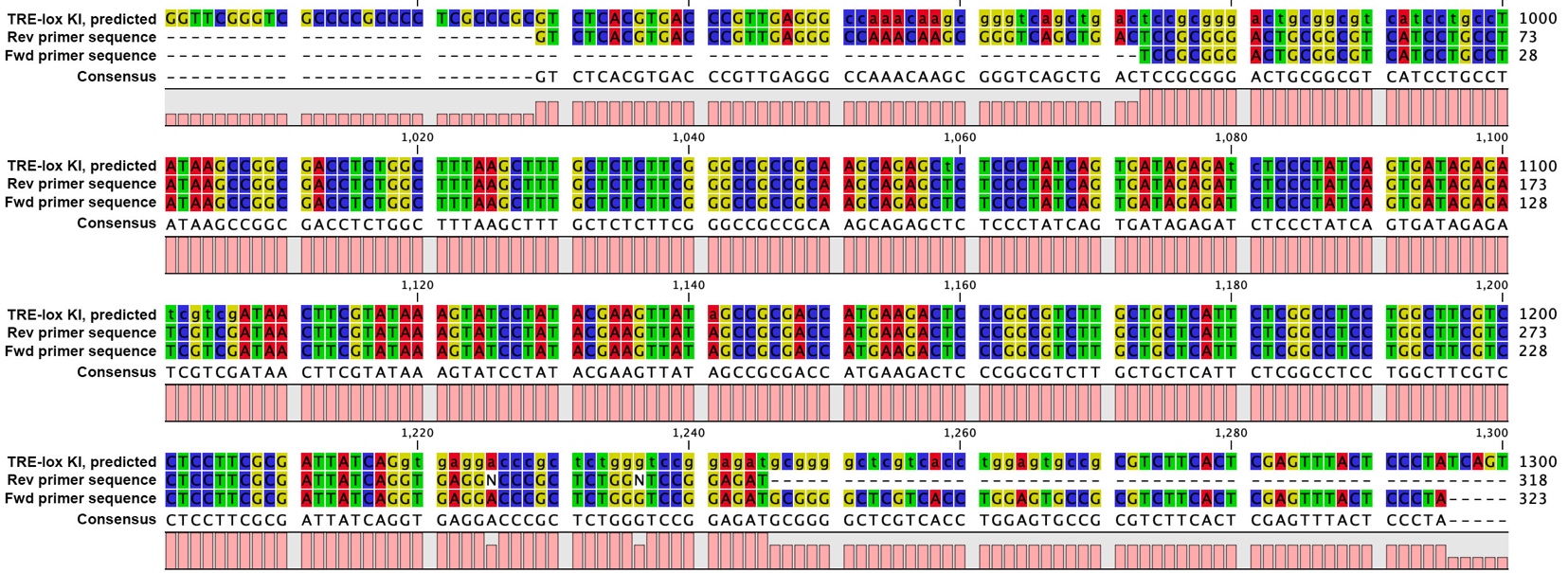
FRT site: blue highlight

...gttcgggtcgccccgcccctcgcccgcgtctcacgtgacccgttgag

ggccaaacaagcgggtcagctgactccgcgggactgcggcgtcatcctgcct*ataagccggcgacctctggctttaagctttgctctcttcgggccgccgcg***gtggaattgctctccctatcagtgatagagatctccctatcagtgatagagatcgtcgataacttcgtataaagtatcctatacgaagttatgtagtaataactagtcgaagttcctattctctagaaagtataggaacttcggcgcgccattt***ctccggccgtgctggaatgcacctgtgcacccagcgcagccttcctcagg*gtcccacagactatggggcgagacactcaaaaggcaggggtctcctgggcccctctccactcctatgactcctatggtcgaacaggagggt...

**Supplementary Figure S5. Genotyping of TL1C8 line by sequencing.**

**a. Alignment of sequencing of upper, TRE-Lox KI PCR amplicon from TL1C8**

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**b. Alignment of sequencing of lower, NHEJ PCR amplicon from TL1C8 with WT sequence.**

**A picture containing timeline

Description automatically generated**

**c. Alignment of sequencing of lower, NHEJ PCR amplicon from TL1C8 with predicted NHEJ sequence.**

**A screenshot of a computer

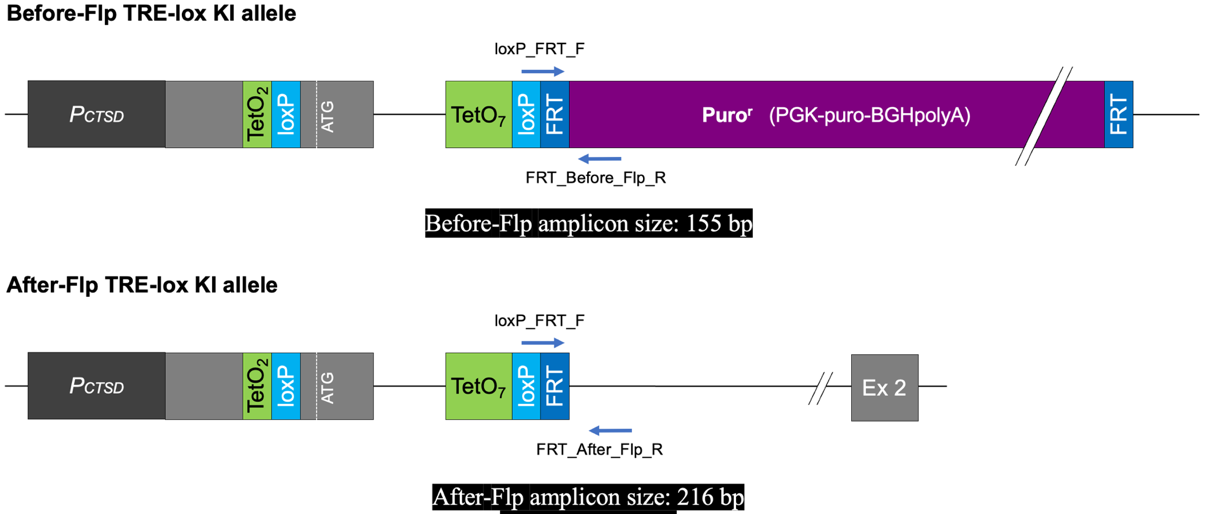
Description automatically generated with low confidence**

**d. Sanger sequencing of lower, NHEJ PCR amplicon from TL1C8 stable clone.Timeline

Description automatically generated with medium confidence**

**Supplementary Figure S6. PCR verification before vs. after Flp-mediated recombination.**

**a**



**Graphical user interface, text

Description automatically generated**

**b**

Note: The "After-Flp" amplicon is larger than the "Before-Flp" amplicon because the latter uses a different reverse primer located exclusively within the PuroR cassette.

**Supplementary Figure S7. Dox dose-response curves for stable clones TFR1B11 and TFR2E9.**

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**Supplementary Figure S8. Time courses of CatD downregulation in clones TFR1B11 and TFR2E9 after addition of Dox (100 ng/mL).**

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**Supplementary Figure S9. Effect of rtTR on CatD activity in the presence and absence of Dox (1000 ng/mL) in transient pools and stable clones of TL1C8-Flp cells.**

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**Supplementary Figure S10. Steady-state CatD protein levels in MEFs vs. TFR1D7 cells and in response to Dox addition and withdrawal, using CatD-KO line C1C5 as a control.**

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**Table S1. Primers used in this study.**

|  |  |  |
| --- | --- | --- |
| **Primer name** | **Sequence** | **Purpose** |
| gRNA1\_top | CACCGGGGAGTCTTCATGGTCGCGG | gRNA1 cloned into px330A-1x2 |
| gRNA1\_bottom | AAACCCGCGACCATGAAGACTCCCC | " |
| gRNA2\_top | CACCGACCTGGAGTGCCGCGTGCTC | gRNA2 cloned into px330S-2, then px330A-1x2 |
| gRNA2\_bottom | AAACGAGCACGCGGCACTCCAGGTC | " |
| Vector\_TetO2\_F | GTCCAGTGTGGTGGAATTCAAGCAGAGCtCTCCCTATC | cloning of TetO2 for TRE-Lox KI template |
| TetO2\_R | CGTATAGGATACTTTATACGAAGTTATCGACGATCTCTATCACTGAT | cloning of TetO2/LoxP sequence for KI template |
| mCTSD\_loxP-Ex1\_F | CGTATAAAGTATCCTATACGAAGTTATaGCCGCGACCATGAAGACTCC | cloning of LoxP/mCTSD region for KI template |
| mCTSD\_3G\_In1-2\_R | GGAGTAAACTCGAGTGAAGACGCGGCACTCCAGGTGACGAG | cloning of mCTSD region/TRE3G for KI template |
| TRE3G\_F | CGTCTTCACTCGAGTTTACTCC | cloning of TRE3G for KI template |
| TRE3G\_R | CGACTAGTTATTACTACATAACTTCGTATAGGATACTTTATACGAAGTTATCTACCTCGACATACGTTCTC | cloning of TRE3G/LoxP for KI template |
| LoxP\_FRT\_F | CGAAGTTATGTAGTAATAACTAGTCGAAGTTCCTATTC | cloning of LoxP/FRT-flanked Puror for KI template/verification of Flp recombination |
| Vector\_FRT\_R | CCGCCACTGTGCTGGATATCAAATGGCGCGCCGAAGTTCC | cloning of FRT-flanked Puror for KI template |
| 5'HA\_mCTSD\_TRE-Lox\_KI\_F | C\*T\*ATAAGCCGGCGACCTCTGGCTTTAAGCTTTGCTCTCTTCGGGCCGCCGCAAGCAGAGCTCTCCCTATCAGTG | PCR amplifying TRE-Lox KI insert from template  (asterisks denote phosphorothioate bonds) |
| 3'HA\_mCTSD\_TRE-Lox\_KI\_R | G\*A\*CCCTGAGGAAGGCTGCGCTGGGTGCACAGGTGCATTCCAGCACGGCCGAAATGGCGCGCCGAAGTTCC | " |
| mCTSD\_WT\_prom\_F | GCGTCATCCTGCCTATAAGCCGGCG | genotyping of WT vs KI vs NHEJ mCTSD alleles/ verification of Cre recombination |
| mCTSD\_WT\_2R | GTCTCACGTGACCCGTTGAGG | " |
| mCTSD\_TRE-Lox\_2R | AACTCGAGTGAAGACGCGGCAC | " |
| FRT\_Before\_Flp\_R | AGCGCATGCTCCAGACTGCC | verification of Flp recombination |
| FRT\_After\_Flp\_R | CCTACTGTACCCTCCTGTTCG | " |
| mCTSD\_KI\_Cre\_verification\_R | CTTCGACTAGTTATTACTACATAACTTCG | verification of Cre recombination |
| pICN\_KOZ\_rtTR(V16)\_F | CTTAATACGACTCACTATAGGCTAGCCGCCACCATGTCTAGACTGGACAAGAGC | cloning of rtTR(V9I)/KRAB into pICherryNeo |
| KRAB\_rtTR(V16)\_R | CGAGCCGCTTTCGCACTTTAGCTG | " |
| rtTR(V16)\_KRAB\_F | CAGCTAAAGTGCGAAAGCGGCTCGCCAAAAAAGAAGAGAAAGGTCG | " |
| IRES\_KRAB\_only\_R | CTCTAGAGGTACCACGCGTGAATTACACCAGCCAGGGCTCTTCTCC | " |
| DEL\_KRAB\_69oC\_F | TAATTCACGCGTGGTACCTCTAGAG | deletion of KRAB from rtTRKRAB in pICherryNeo |
| DEL\_KRAB\_69oC\_R | GCCGCTTTCGCACTTTAGCTGTTTC | " |