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Supplementary information

High-fidelity Cas13 variants for targeted RNA degradation with minimal collateral effects

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Supplementary information

High-fidelity Cas13 variants for targeted RNA degradation with minimal collateral effects

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Supplementary information

Supplementary Figure 1. Characteristics of Cas13-mediated mCherry degradation along with collateral effects in HEK293T cells.

Supplementary Figure 2. Characteristics collateral effects of Cas13-mediated endogenous transcripts degradation in HEK293T cells.

Supplementary Figure 3. Schematic of mutagenesis and screening approach for Cas13 with high on-target and reduced collateral degradation activity.

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Supplementary Figure 20. Comparison of on-target activity and collateral activity among different Cas13 proteins.

Supplementary Figure 21. Model of Cas13 on-target and collateral cleavage activity.

Supplementary Tables

Supplementary Table 1. The mutant sequences and corresponding nucleotide sequences used for Cas13d screening.

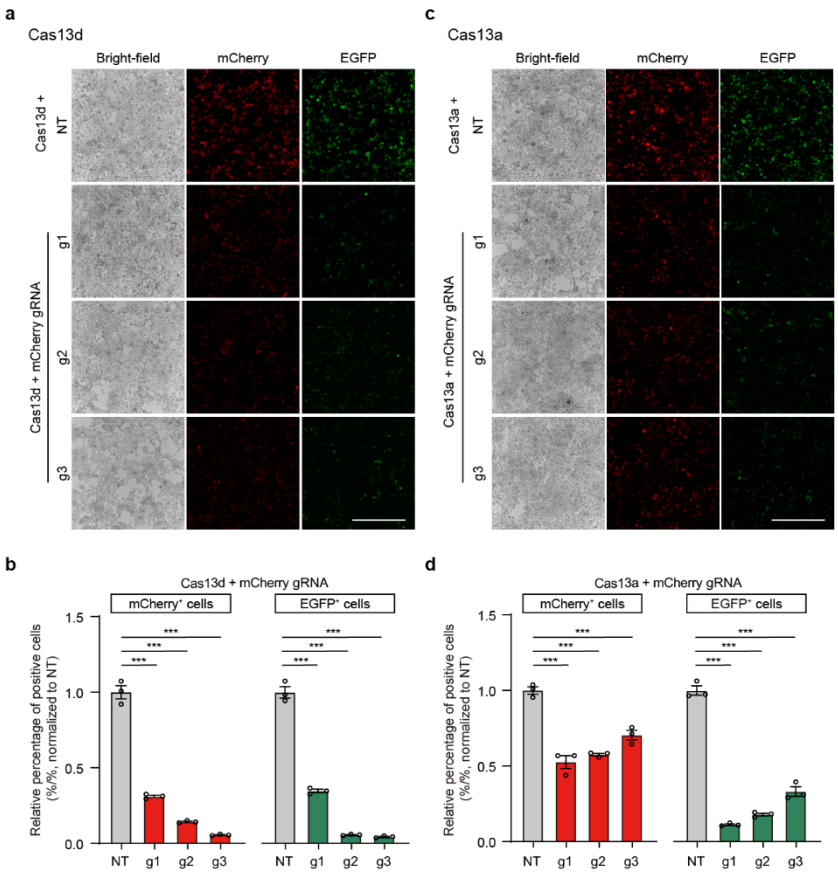
Supplementary Table 3. The mutant sequences and corresponding nucleotide sequences used for Cas13X screening.

Supplementary Table 4. gRNA sequences used in this study.

Supplementary Table 5. Primers used for qPCR reactions.

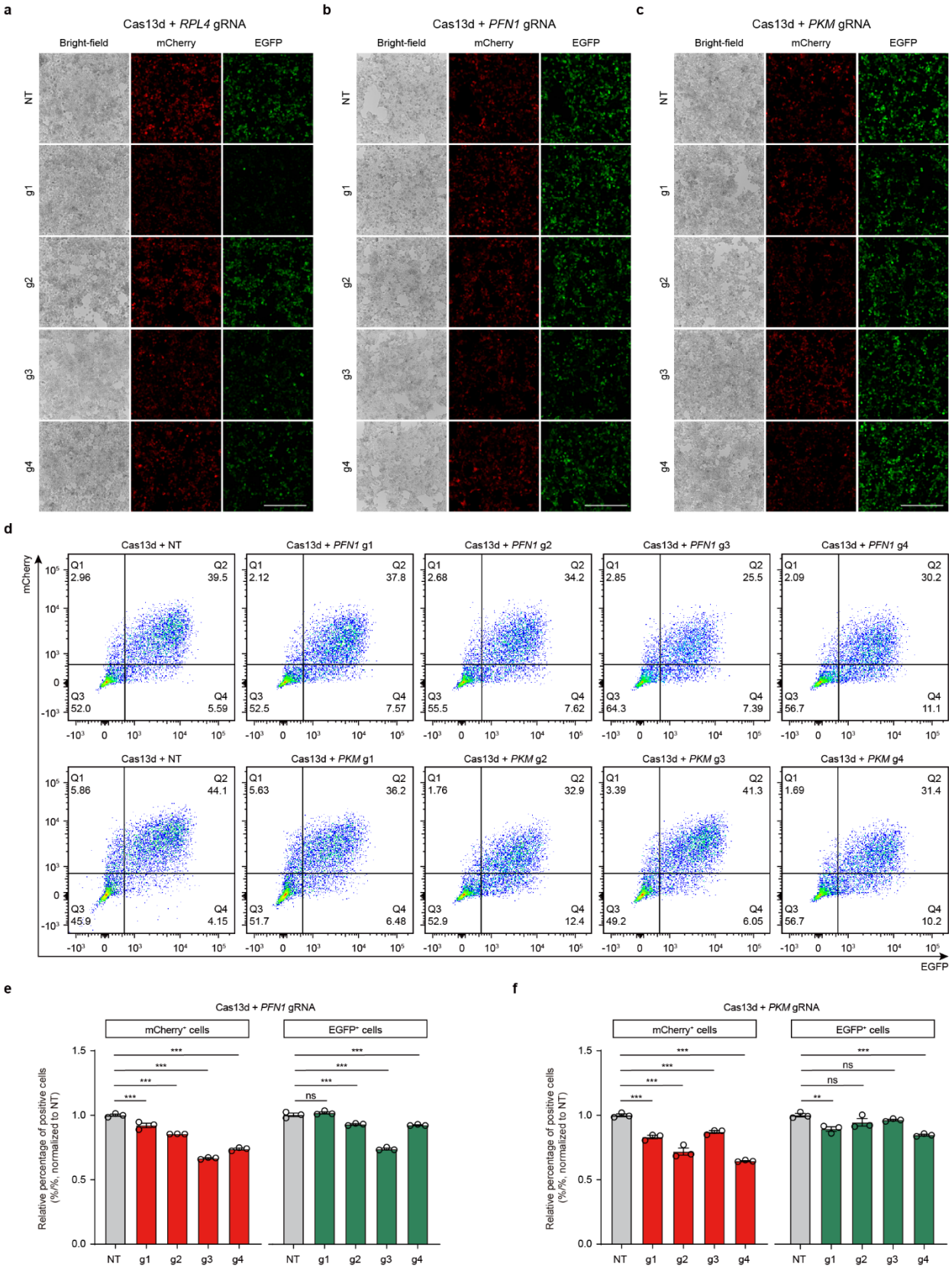
Supplementary Table 6. Sequences of target ssRNA, nontarget ssRNA, gRNAs and the corresponding primers used in *in vitro* cleavage assays.

Supplementary Note 1. Codes for predicting off-targets: offinder_cas13.sh



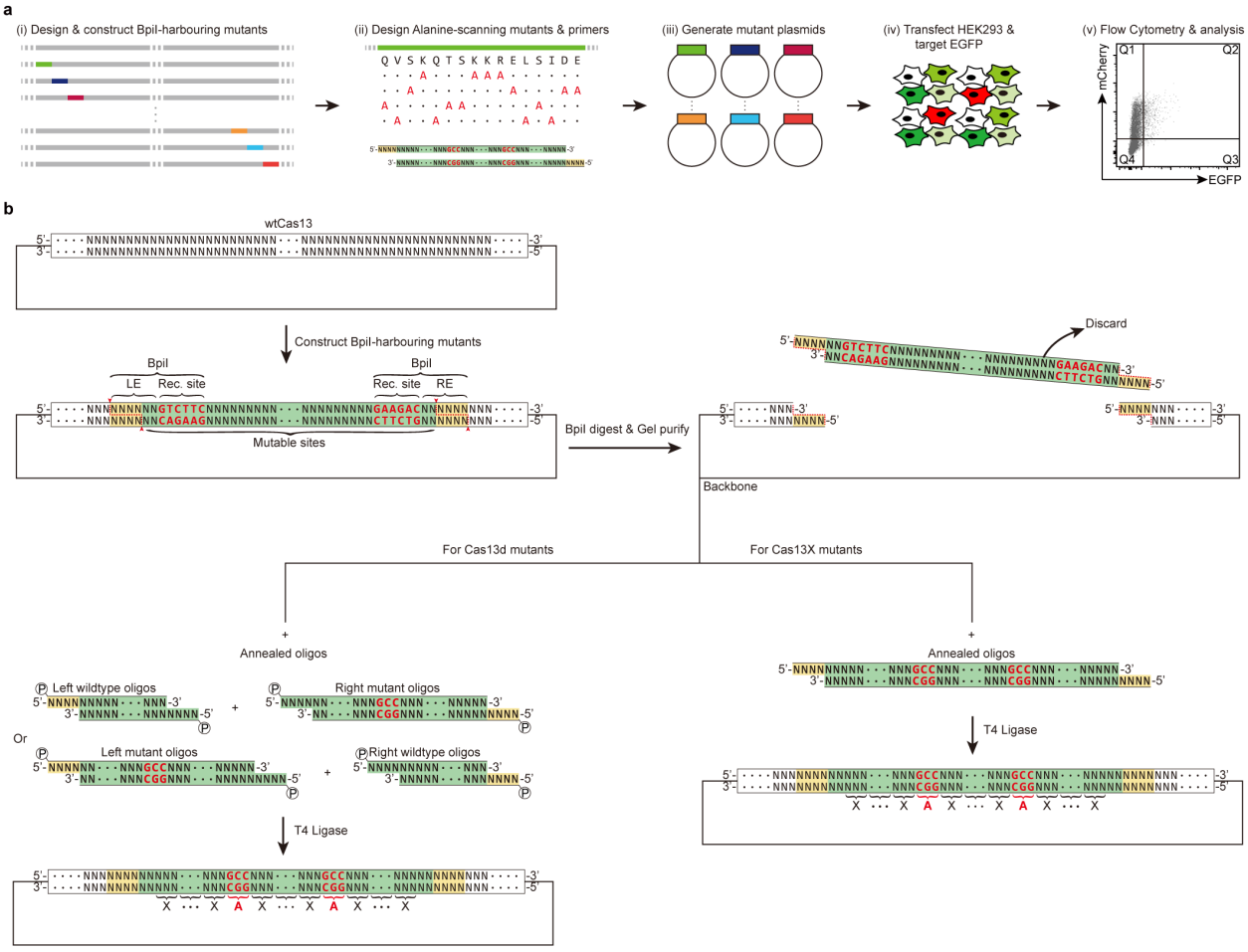
Supplementary Figure 1. Characteristics of Cas13-mediated mCherry degradation along with collateral effects in HEK293T cells.

a,**c**, Representative bright-field and fluorescence images of HEK293T cells with mCherry and EGFP RNA degradation by Cas13d (**a**) or Cas13a (**c**) with three different mCherry gRNAs. Scale bar, 300 μm. **b**,**d**, FACS quantitative analysis of relative percentage of EGFP or mCherry positive cells. NT: nontarget gRNA. All values are presented as mean ± s.e.m., n = 3, unless otherwise noted. In **b**, ***P<0.0001; In **d**, P=0.0003 for g3 vs. NT ($EGFP^+$ cells), otherwise ***P<0.0001. Dunnett's multiple comparisons test after one-way ANOVA.


a
Cas13d + RPL4 gRNA
Bright-field
mCherry
EGFP
NT
g1
g2
g3
g4
b
Cas13d + PFN1 gRNA
c
Cas13d + PKM gRNA
d
Cas13d + NT
Cas13d + PFN1 g1
Cas13d + PFN1 g2
Cas13d + PFN1 g3
Cas13d + PFN1 g4
Cas13d + PKM g1
Cas13d + PKM g2
Cas13d + PKM g3
Cas13d + PKM g4
mCherry
EGFP
Q1 2.96
Q2 39.5
Q3 52.0
Q4 5.59
Q1 2.12
Q2 37.8
Q3 52.5
Q4 7.57
Q1 2.68
Q2 34.2
Q3 55.5
Q4 7.62
Q1 2.85
Q2 25.5
Q3 64.3
Q4 7.39
Q1 2.09
Q2 30.2
Q3 56.7
Q4 11.1
Q1 5.86
Q2 44.1
Q3 45.9
Q4 4.15
Q1 5.63
Q2 36.2
Q3 51.7
Q4 6.48
Q1 1.76
Q2 32.9
Q3 52.9
Q4 12.4
Q1 3.39
Q2 41.3
Q3 49.2
Q4 6.05
Q1 1.69
Q2 31.4
Q3 56.7
Q4 10.2
e
Cas13d + PFN1 gRNA
mCherry+ cells
EGFP+ cells
Relative percentage of positive cells (%/%, normalized to NT)
***
ns
NT
g1
g2
g3
g4
f
Cas13d + PKM gRNA
**

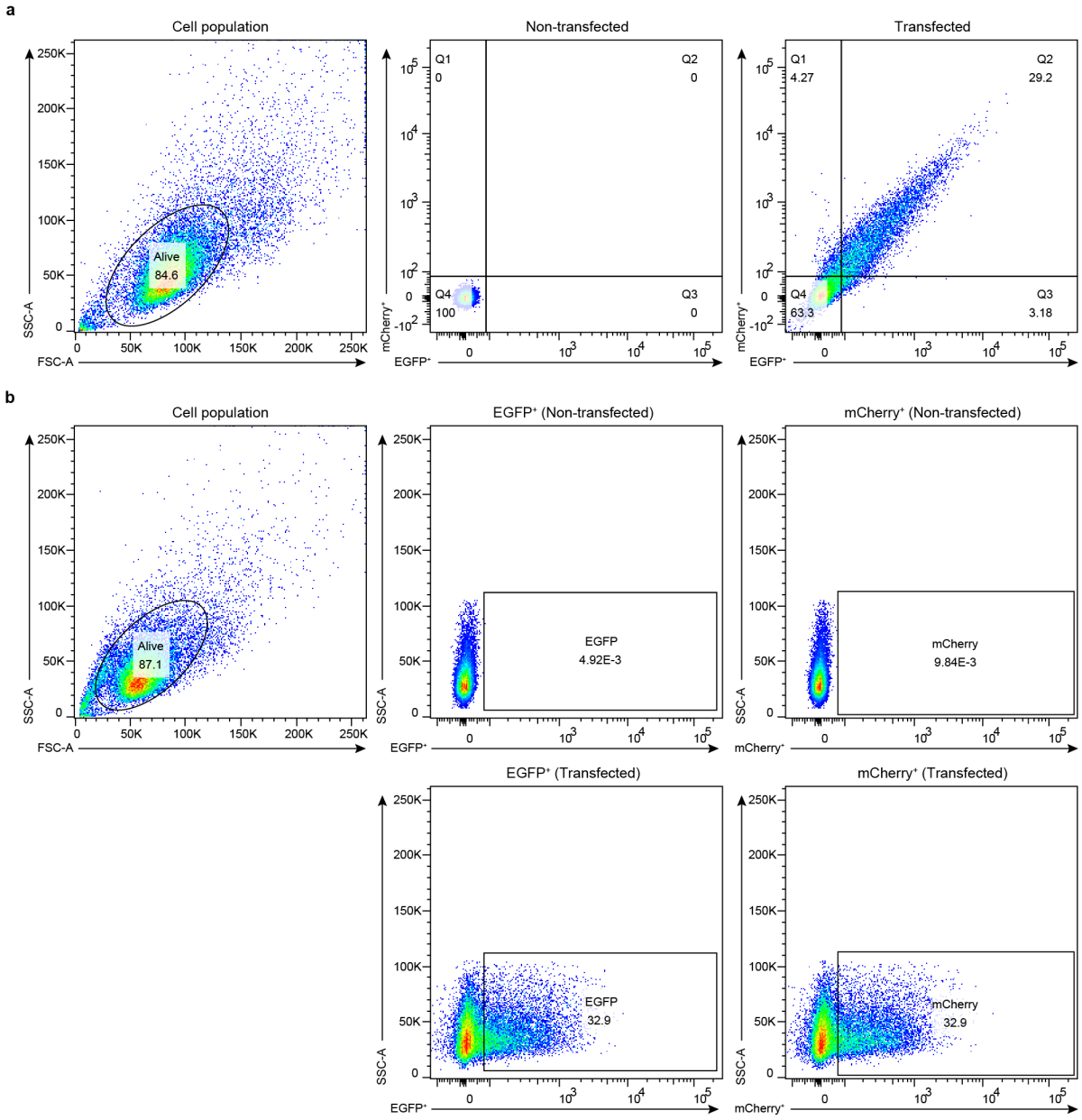
Supplementary Figure 2. Characteristics collateral effects of Cas13-mediated endogenous transcripts degradation in HEK293T cells.

a-**c**, Representative bright-field and fluorescence images of cells with reduced mCherry and EGFP fluorescence levels using Cas13d knockdown of three endogenous transcripts (*RPL4*, *PFN1*, or *PKM*), each with four gRNAs. Scale bar, 300 μm. **d**, Representative flow cytometry images of mCherry and EGFP fluorescence intensity from **b** and **c**. **e**,**f**, Differential decreases of relative percentage of EGFP or mCherry positive cells were induced by Cas13d targeting *PFN1* (**e**), *PKM* (**f**) transcript, with four gRNAs each transcript. NT: nontarget gRNA. All values are presented as mean ± s.e.m., n = 3, unless otherwise noted. In **e**, left-to-right, P=0.0004, P<0.0001, P<0.0001, P<0.0001, P=0.5022, P=0.0003, P<0.0001, and P=0.0002. In **f**, left-to-right, P<0.0001, P<0.0001, P=0.0004, P<0.0001, P=0.0036, P=0.1237, P=0.3777, and P=0.0002. Dunnett's multiple comparisons test after one-way ANOVA. $^{**}P < 0.01$, $^{***}P < 0.001$, ns, not significant.



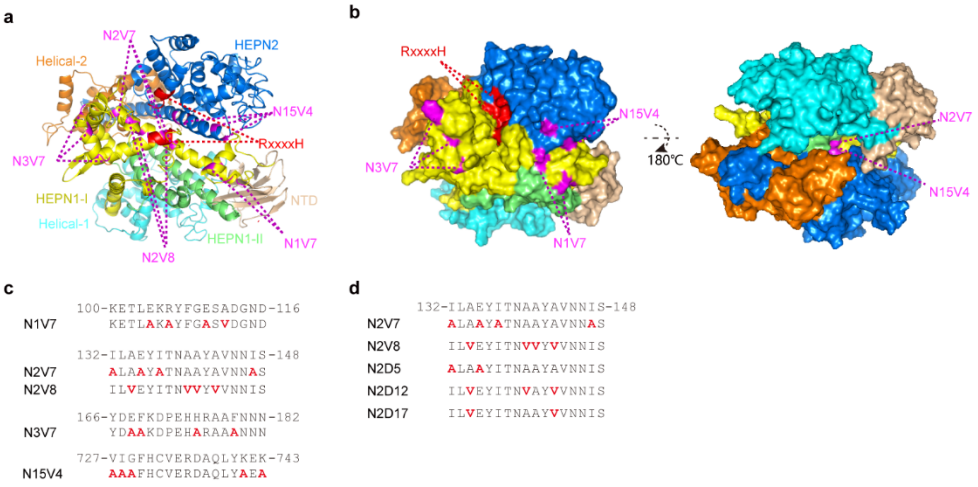
Supplementary Figure 3. Schematic of mutagenesis and screening approach for Cas13 with high on-target and reduced collateral degradation activity.

a, Diagram of mutagenesis and FACS screening for determining the efficiency and collateral effects of Cas13 proteins by analyzing dual-fluorescence intensity with EGFP gRNA. **b**, Cloning of Cas13 mutagenesis library for screening Cas13 mutant variants with reduced collateral degradation.



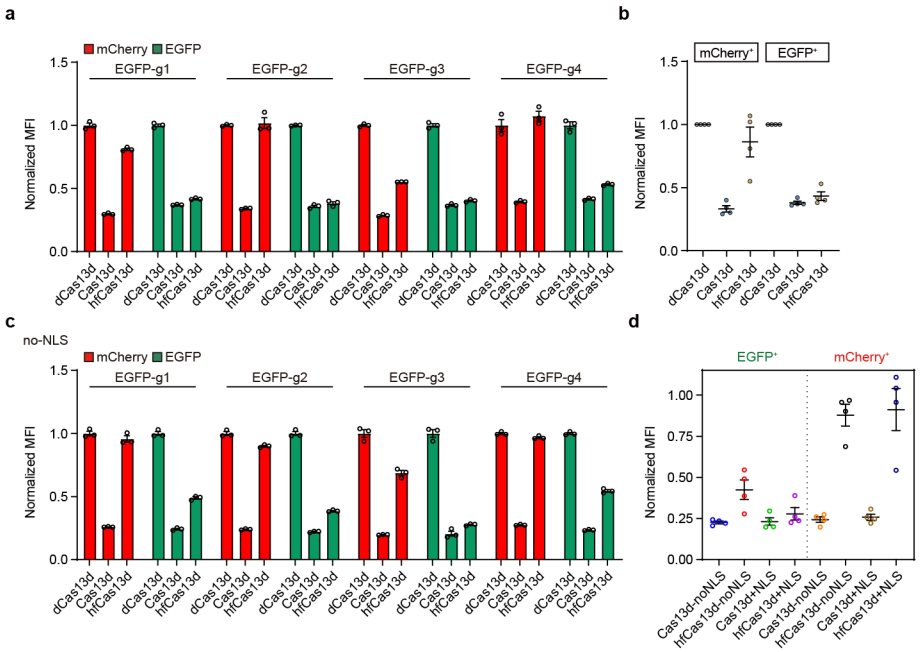
Supplementary Figure 4. Gating strategy for flow cytometry experiments.

Cells are gated on: **a**,**b**, FSC-A/SSC-A to exclude cell debris. Positive boundaries are determined by EGFP$^+$ or mCherry$^+$ cells, and negative boundaries are determined by non-transfected cells. **a**, The percentage of mCherry$^+$ fluorescence cells is sum of Q1 and Q2, and the percentage of EGFP$^+$ fluorescence cells is sum of Q2 and Q3. **b**, The mCherry$^+$ or EGFP$^+$ fluorescence cells gated by the rectangle are selected to calculate the MFI (mean fluorescence intensity).



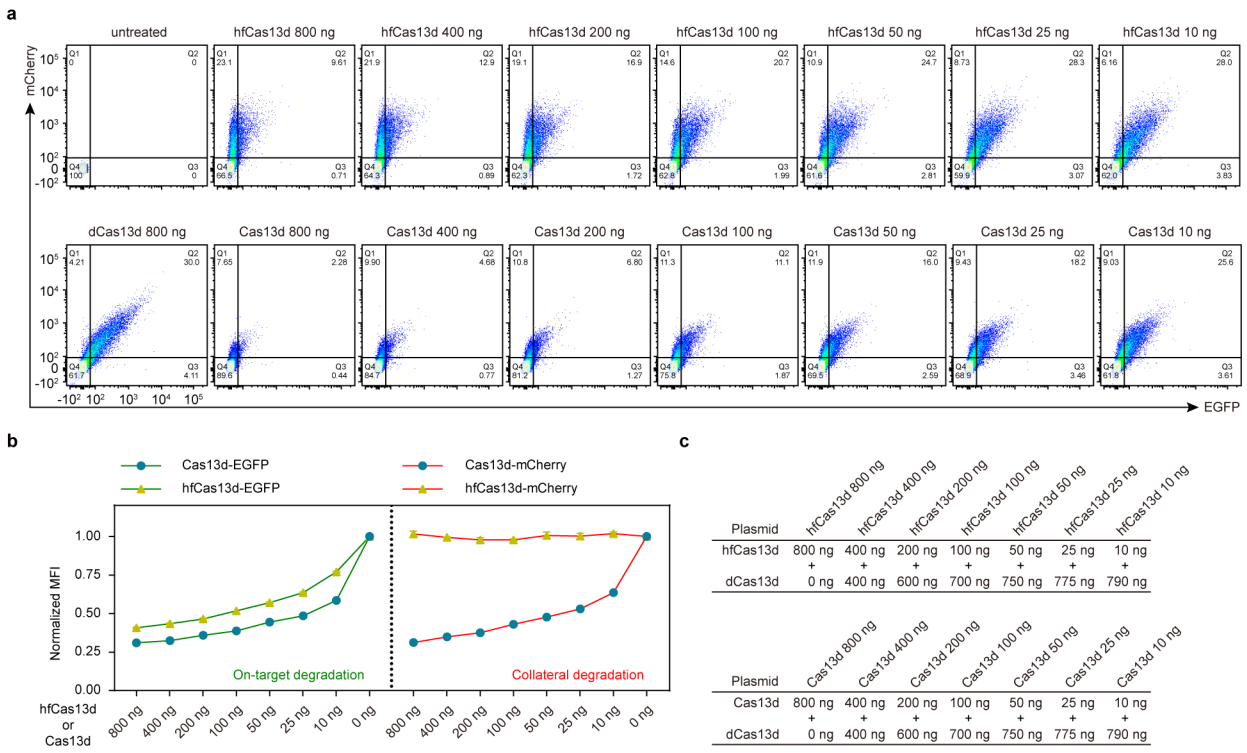
Supplementary Figure 5. Crystal structure of Cas13d variants.

a,**b**, Cartoon (**a**) and opposing surface (**b**) view of the predicted overall crystal structure of Cas13d (predicted by I-TASSER). Catalytic sites of the HEPN domains are shown in red, and effective mutant sites are shown in purple. **c**,**d**, Amino acid sequences of effective variants from Cas13d.



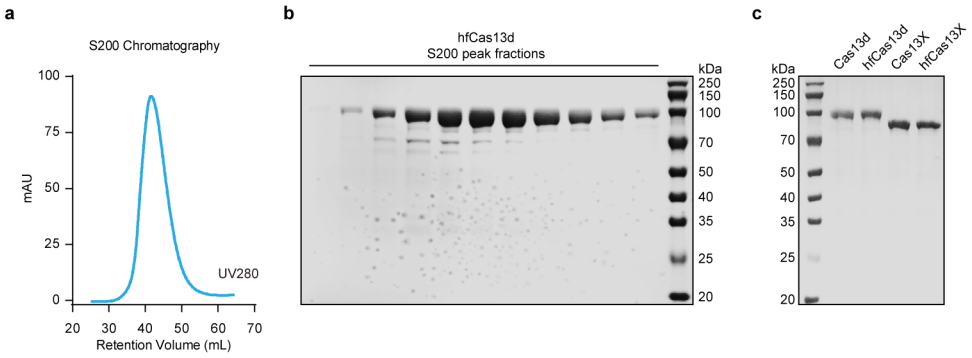
Supplementary Figure 6. Collateral degradation activity of Cas13d and hfCas13d with or without NLS.

a, Differential changes of normalized MFI were induced by Cas13d, hfCas13d, and dCas13d with EGFP-targeting gRNAs in HEK293 cell lines. n = 3. **b**, Statistical data analysis for relative degradation efficiency by Cas13d and hfCas13d from **a**, each dot represents the mean of three biological replicates for each gRNA. Normalized MFI, mean fluorescence intensity relative to the dCas13d condition, n = 3. **c**, Differential changes of normalized MFI were induced by Cas13d-noNLS, hfCas13d-noNLS, and dCas13d-noNLS with EGFP-targeting gRNAs in HEK293T cell lines. n = 3. **d**, Statistical data analysis for relative degradation efficiency by Cas13d-noNLS, hfCas13d-noNLS, Cas13d-NLS and hfCas13d-NLS, each dot represents the mean of three biological replicates for each gRNA. Normalized MFI, mean fluorescence intensity relative to the dCas13d-noNLS or dCas13d-NLS condition, n = 3. All values are presented as mean ± s.e.m.



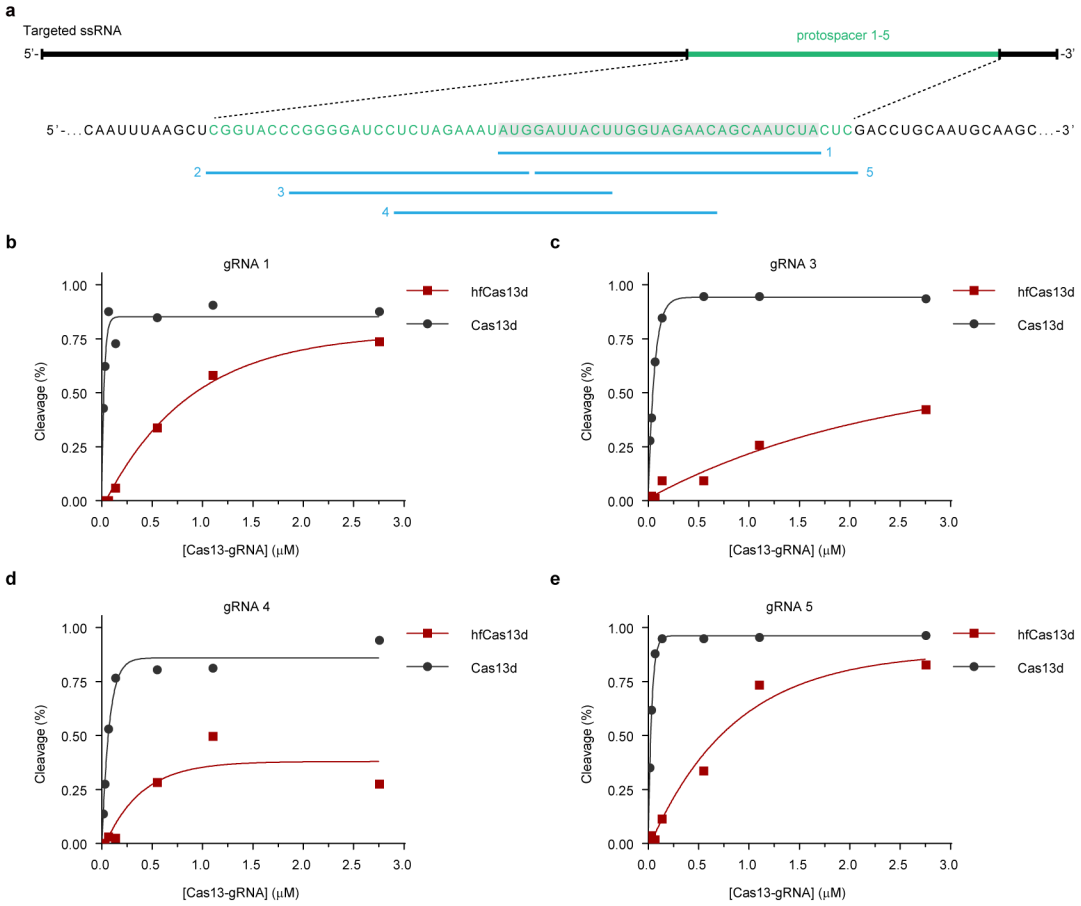
Supplementary Figure 7. Dose-dependent on-target and collateral degradation activity of Cas13d.

a, Representative FACS analysis of mCherry and EGFP degradation induced by transfecting different amount of hfCas13d or Cas13d constructs. The EGFP-targeting gRNA2 was used. n = 3. **b**, Normalized MFI analysis for **a**. **c**, A dilution series of Cas13d-expressing or hfCas13d-expressing plasmids mixed with dCas13d-expressing plasmids and a total of 800 ng plasmids were transfected per well in 24-well plates.



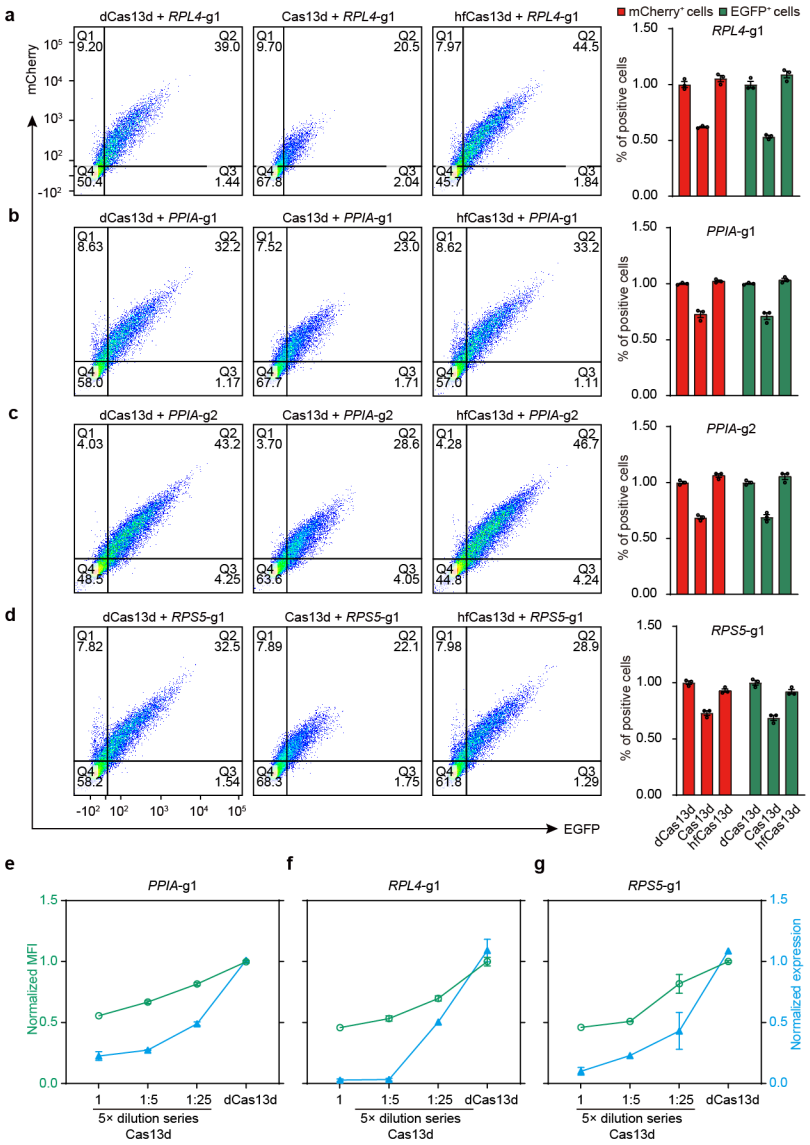
Supplementary Figure 8. Purification of recombinant Cas13 Protein.

a, Chromatogram from Sephacry 200 column for Cas13. The Cas13 fusion protein with N-terminal 6x His/Twinstrep-SUMO tag was purified by successive affinity and size exclusion chromatography. The SUMO-tag was removed by SUMO protease. **b**, SDS-PAGE gel of size exclusion chromatography fractions for Cas13. **c**, SDS-PAGE gel of purified Cas13 proteins. Data are representative of, at least, three independent experiments.



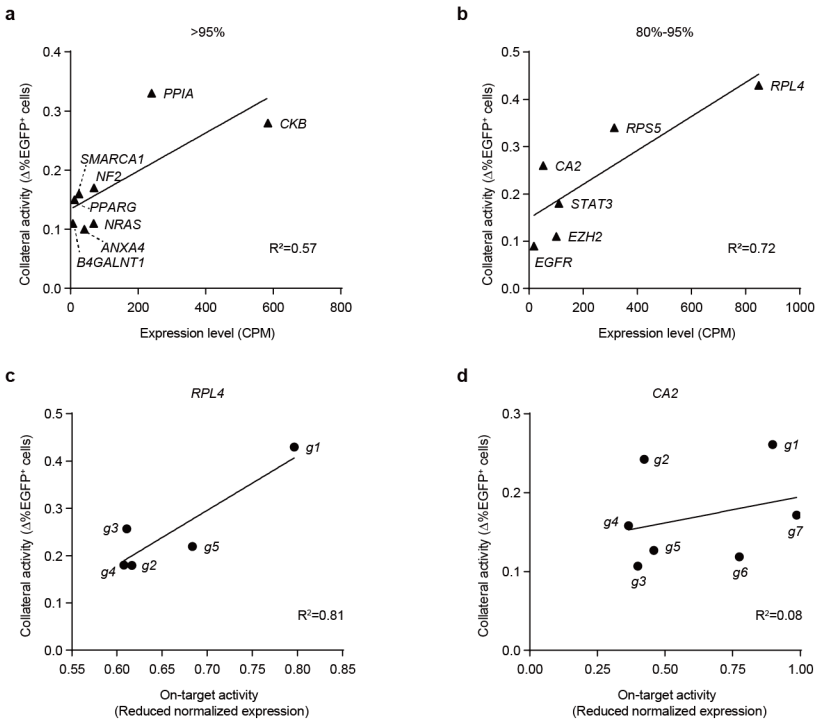
Supplementary Figure 9. *In Vitro* Characterization of Cas13d and hfCas13d.

a, Schematic showing the sequence of gRNA spacer and spacer position relative to the complementary ssRNA target. **b-e**, Quantification of collateral cleavage activity of Cas13d or hfCas13d with gRNA 1 (**b**), gRNA 3 (**c**), gRNA 4 (**d**) and gRNA 5 (**e**) in the presence of varying concentrations of Cas13–gRNA complex. Exponential fits are shown as solid lines.



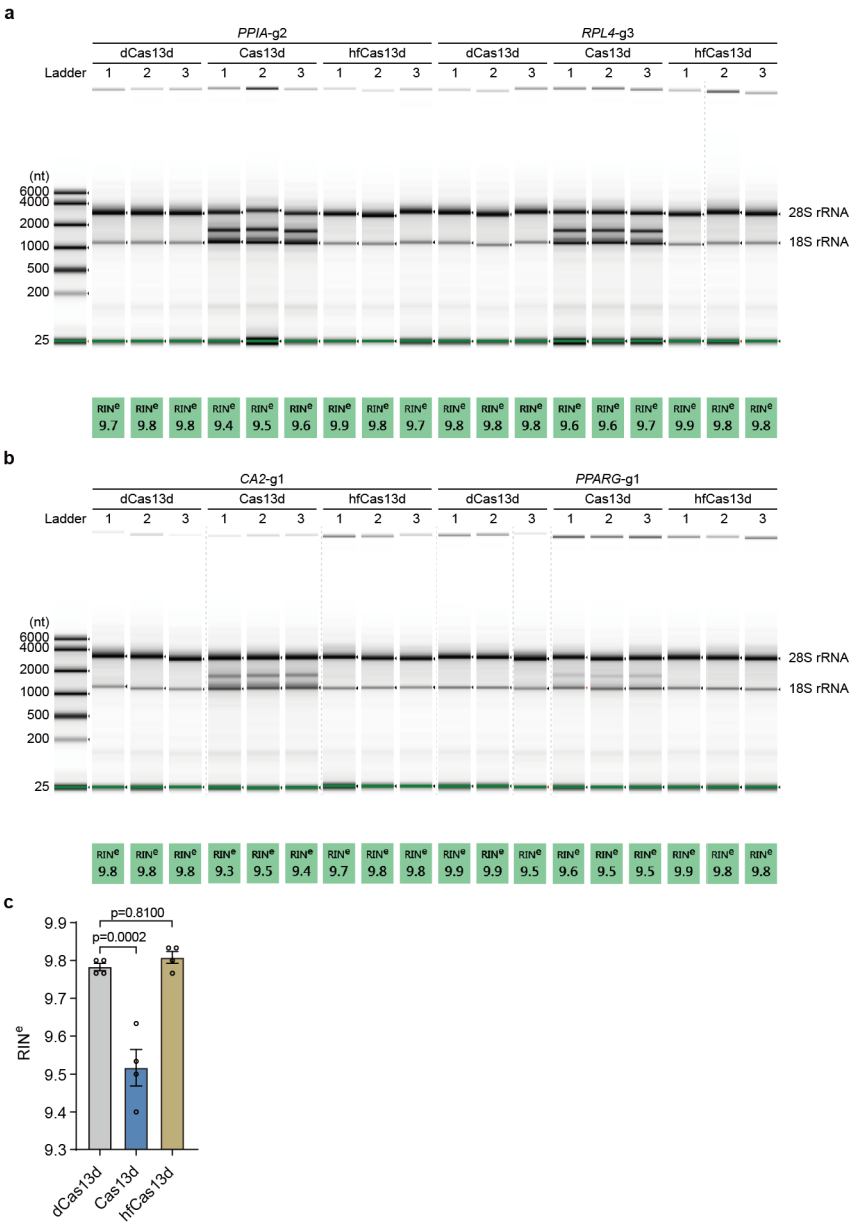
Supplementary Figure 10. FACS analysis of mCherry and EGFP degradation induced by targeting different endogenous transcripts.

a-d, Representative FACS analysis of mCherry and EGFP degradation induced by dCas13d, Cas13d, or hfCas13d with gRNAs targeting *RPL4* (**a**), *PPIA* (**b**,**c**), and *RPS5* (**d**), respectively, n = 3. **e-g**, Differential changes of normalized MFI of EGFP and normalized expression levels of *PPIA* (**e**), *RPL4* (**f**), and *RPS5* (**g**) induced by Cas13d when a 5-fold dilution series were used, respectively. A dilution series of Cas13d-expressing plasmids mixed with dCas13d-expressing plasmids were used. A total of 2 μg plasmids were transfected per well in 12-well plates. n = 2, for normalized expression levels of dCas13d (**e**, **g**), 1:25 (**f**), and 1:5 (**g**). n = 3, unless otherwise noted. Data are presented as mean values ± s.e.m.



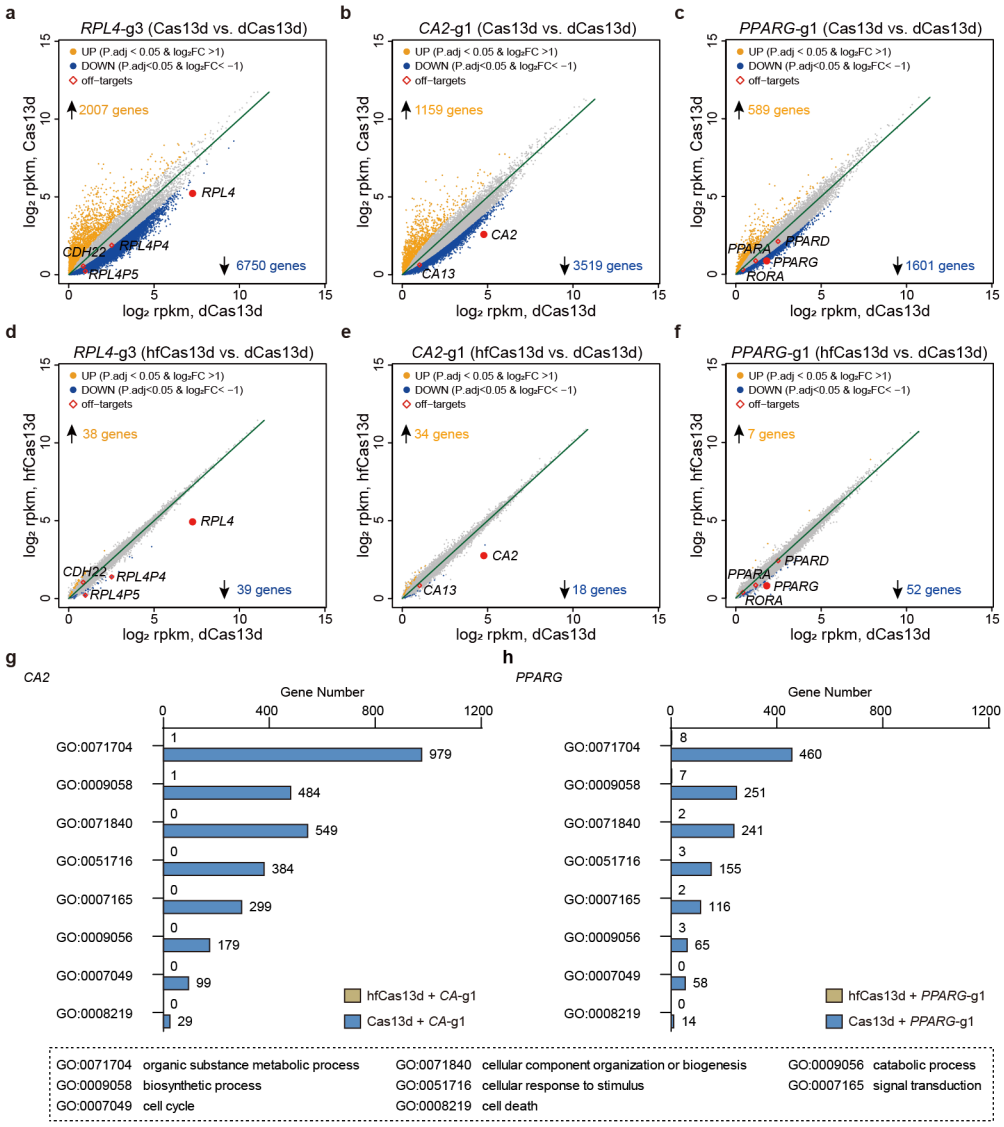
Supplementary Figure 11. Relationship between collateral activity and expression level of targeted genes or on-target activity of Cas13d.

a,**b**, Correlation of collateral activity to expression levels of targeted genes with over 95% (**a**) or 80-95% knockdown (**b**) from Fig. 3. **c**,**d**, Correlation of collateral activity to on-target activity. The on-target activity of Cas13d activated by corresponding gRNA is calculated as reduced normalized expression levels from Fig. 3f (*RPL4*) or Fig. 3g (*CA2*). The collateral activity of Cas13d activated by corresponding gRNA is calculated as relative percentage changes of EGFP positive cells from Fig. 3b. Linear fits are shown as solid lines. Each dot represents the mean of biological replicates for each gRNA.



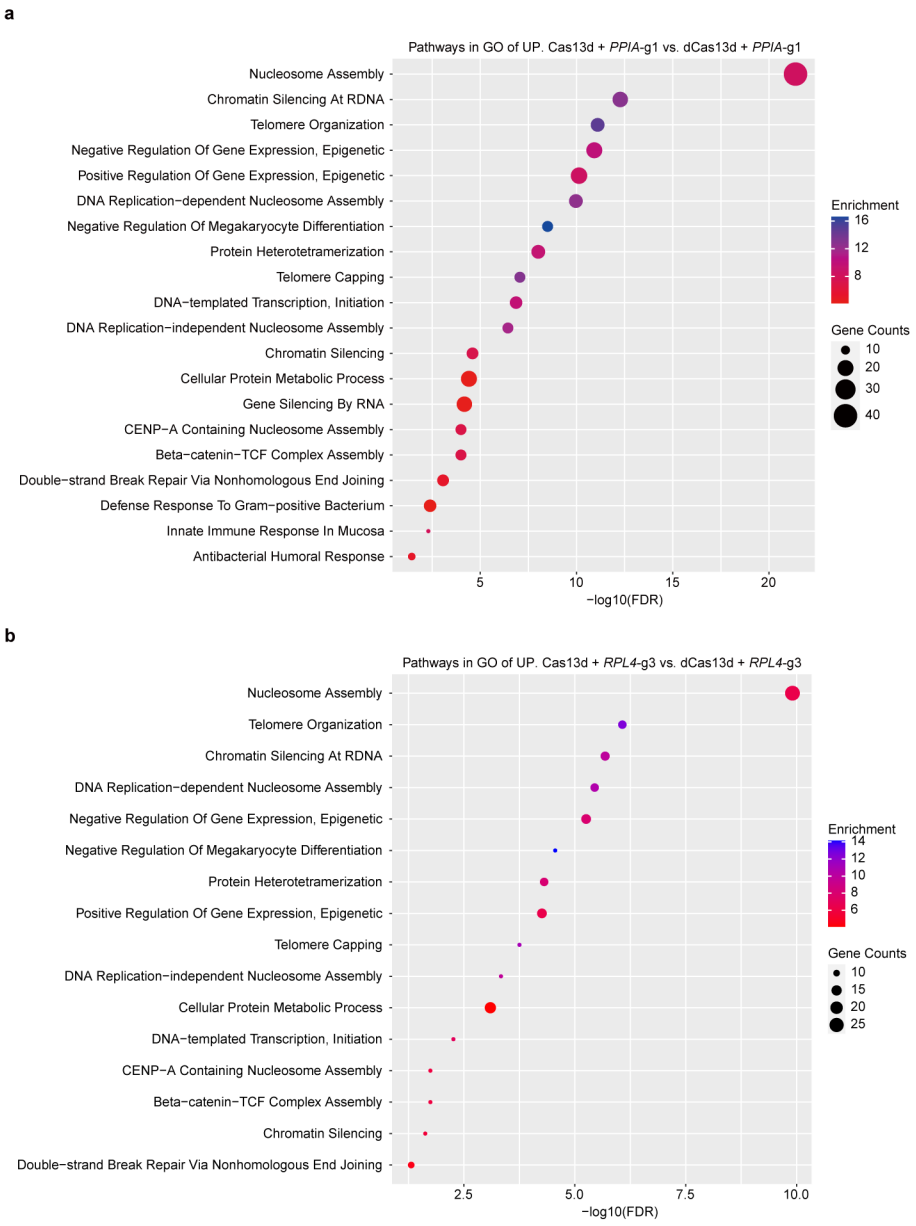
Supplementary Figure 12. Total RNA integrity analysis.

a,**b,** Total RNA integrity analysis quantified by Agilent 2100 Bioanalyzer. The RIN^e (RNA integrity number equivalent), 28S rRNA, and 18S rRNA bands for each corresponding sample are shown, n = 3. **c**, Statistical data analysis for RIN^e from **a** and **b**, each dot represents the mean of three biological replicates for each gRNA. All values are presented as mean ± s.e.m. Dunnett's multiple comparisons test after one-way ANOVA.



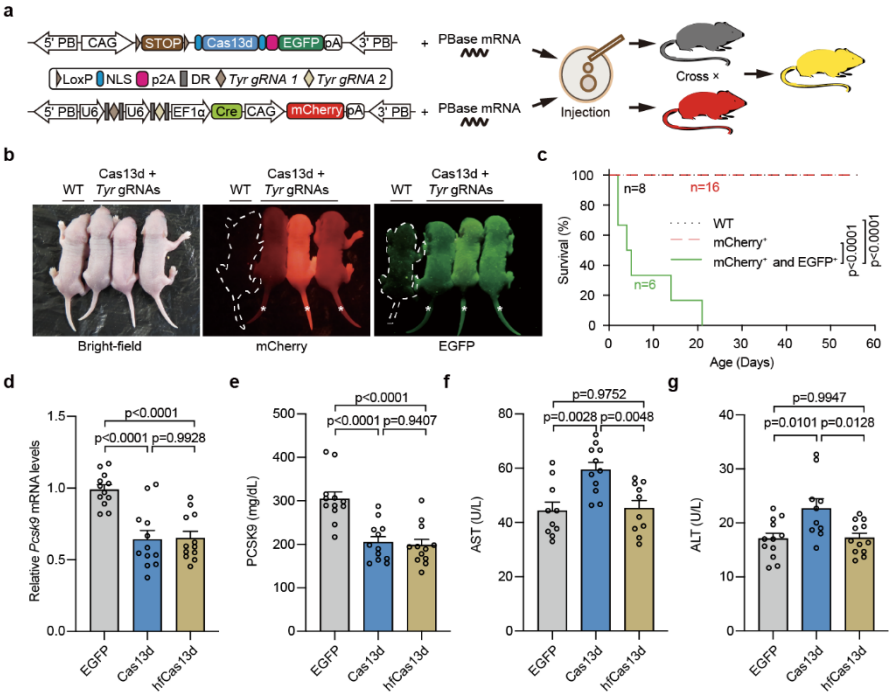
Supplementary Figure 13. Transcriptome-wide collateral effect analysis for Cas13d and hfCas13d.

a-c, Scatter plot of differential expression levels between Cas13d-mediated and dCas13d-mediated RNA degradation using *RPL4*-g3 (**a**), *CA2*-g1 (**b**), or *PPARG*-g1 (**c**). **d-f**, Scatter plot of differential expression levels between hfCas13d-mediated and dCas13d-mediated RNA degradation using *RPL4*-g3 (**d**), *CA2*-g1 (**e**), or *PPARG*-g1 (**f**). P_{adj}, adjusted P value. **g**,**h**, Biological process of genes significantly downregulated by Cas13d-mediated or hfCas13d-mediated *CA2* (**g**) or *PPARG* (**h**) degradation. n = 3.



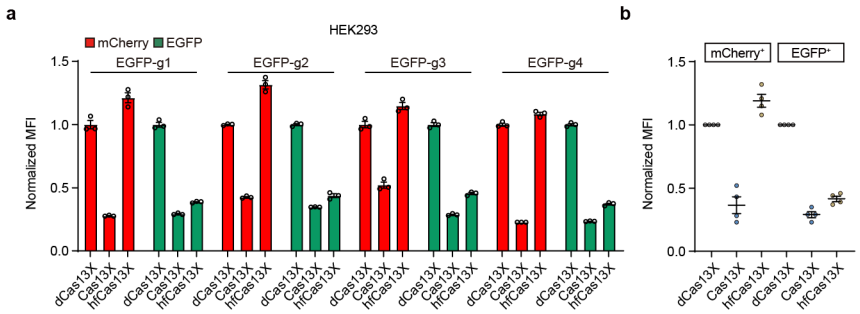
Supplementary Figure 14. Bulk RNA-seq analysis of genes with differential expression level.

Clustering analysis of genes upregulated by Cas13d targeting *PPIA* (**a**) or *RPL4* (**b**).



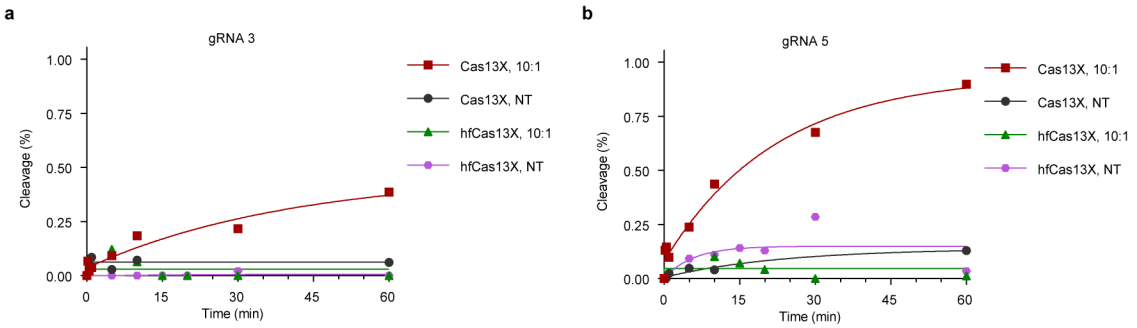
Supplementary Figure 15. Collateral effects of Cas13d-mediated *Tyr* reduction in mice and *Pcsk9* reduction in the liver.

a, Schematic diagram of Cas13d-mediated *Tyr* reduction in mice using the *Cre*-LoxP system based on *piggyBac* transposons. **b**, Representative F1 generation mice exhibited EGFP positive along with Cas13d overexpression and mCherry positive along with *Tyr* gRNAs and Cre transposase. White * represents significant expression of EGFP and mCherry. **c**, Survival curve of F1 generation of WT mice, mCherry$^+$ transgenic mice, and EGFP$^+$ and mCherry$^+$ transgenic mice. Statistical survival differences were evaluated by two-sided log-rank test. **d**,**e**, The levels of *Pcsk9* mRNA in hepatocytes (**d**) and PCSK9 protein in serum (**e**) were quantified at 4 weeks post-injection of AAV, n = 12. **f**,**g**, The levels of serum aspartate aminotransferase (AST) (**f**) and alanine aminotransferase (ALT) (**g**) were quantified in EGFP-injected, Cas13d-*Pcsk9*-gRNAs-injected, and hfCas13d-*Pcsk9*-gRNAs-injected mice, n = 12. All values are presented as the mean ± s.e.m. Dunnett's multiple comparisons test after one-way ANOVA.

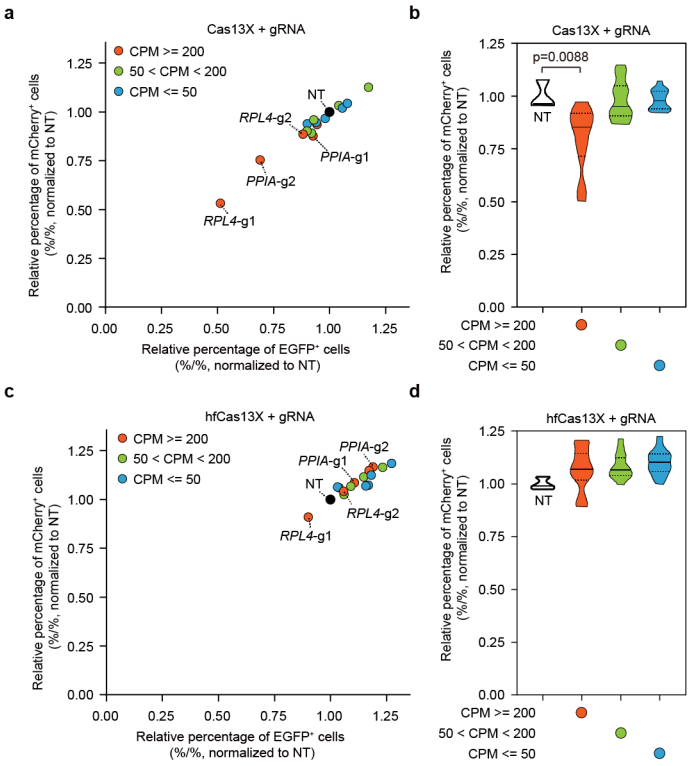


Supplementary Figure 16. Collateral degradation activity of Cas13X and hfCas13X in HEK293 cells.

a, Differential changes of normalized MFI were induced by Cas13X, hfCas13X, and dCas13X in HEK293 cell lines. **b**, Statistical data analysis for relative degradation efficiency by Cas13X and hfCas13X from **a**, each dot represents the mean of three biological replicates for each gRNA. Normalized MFI, mean fluorescence intensity relative to the dCas13X condition, n = 3. All values are presented as mean ± s.e.m.

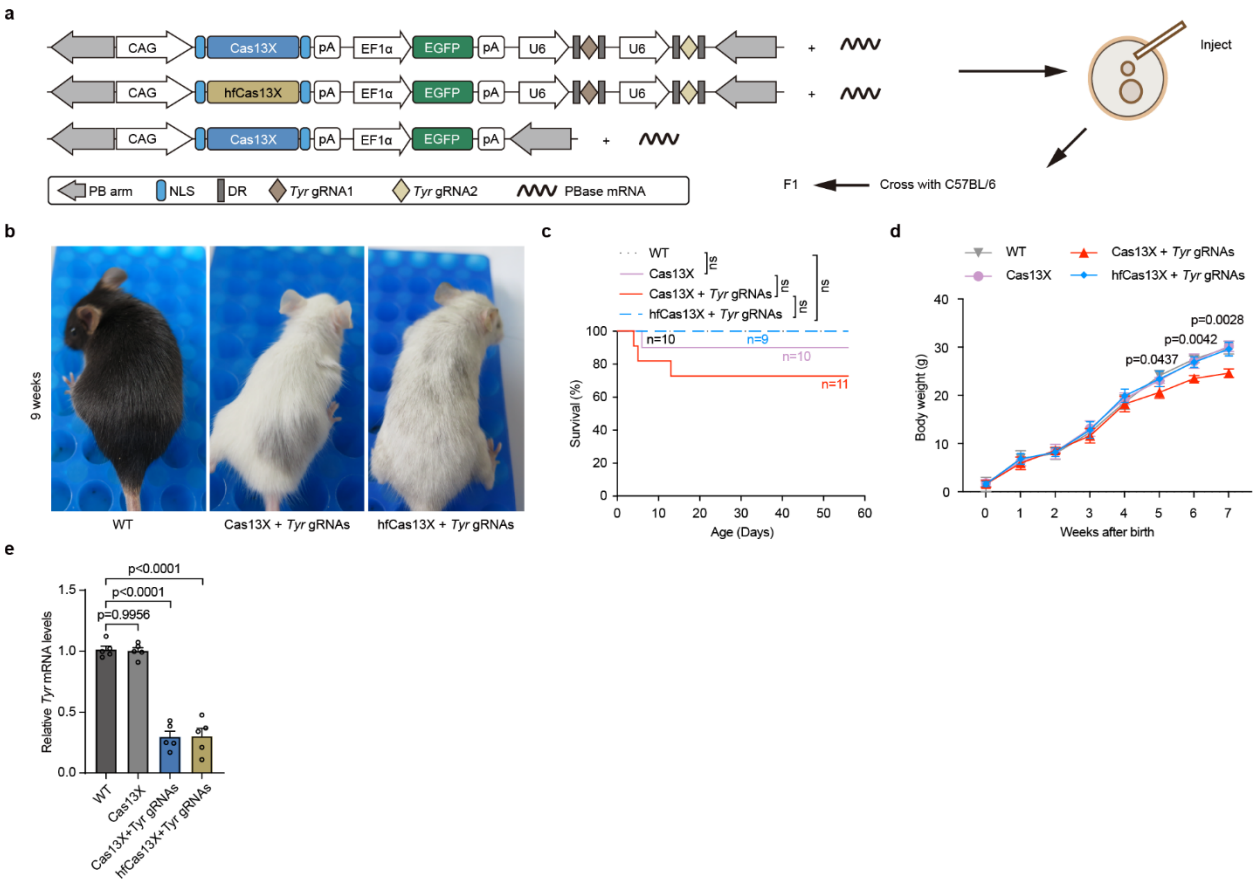


Supplementary Figure 17. *In Vitro* Characterization of Cas13X and hfCas13X.
Quantified time-course data of collateral cleavage by Cas13X or hfCas13X with gRNA 3 (**a**), and gRNA 5 (**b**). Exponential fits are shown as solid lines with 10:1 molar ratio of nontarget (NT) to target (T) RNA or only with NT RNA.



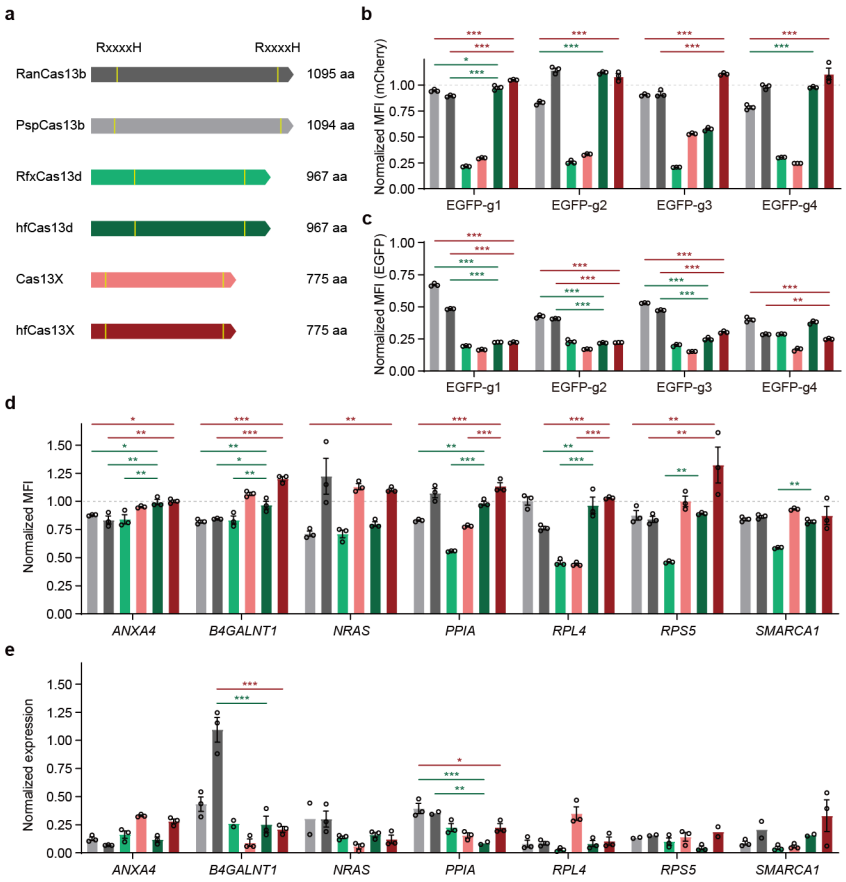
Supplementary Figure 18. Efficiency and specific interference activity of hfCas13X in HEK293 cells.

a, Differential reduction of relative percentages of EGFP and/or mCherry positive cells was induced by Cas13X targeting endogenous genes with 15 gRNAs, compared with NT. Each dot represents the mean of three biological replicates for each gRNA targeting endogenous gene with Cas13X. NT: nontarget gRNA. **b**, Statistical quantification from **a**. **c**, Differential reduction of relative percentages of EGFP and/or mCherry positive cells was induced by hfCas13X. **d**, Statistical quantification from **c**. 5 (3 genes, CPM>=200), 5 (5 genes, 50<CPM<200), and 5 (5 genes, CPM<=50) gRNAs were used respectively. n = 3, unless otherwise noted. In **b** and **d**, violin plots are centered around the median (black line) with quartiles (lower and upper dashed lines). Minima and maxima are shown as the bottom and top of the violin plots. Dunnett's multiple comparisons test after one-way ANOVA.



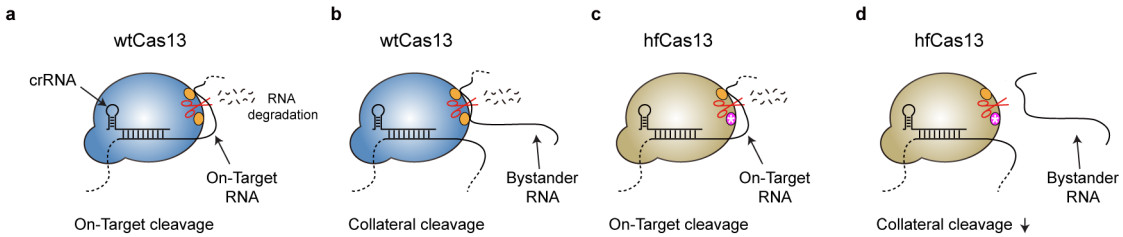
Supplementary Figure 19. The in vivo consequences of collateral cleavage by Cas13X and their elimination.

a, Schematic diagram of Cas13X-mediated or hfCas13X-mediated *Tyr* transcript reduction in mice using the *piggyBac* system. **b**, Representative F1 generation albino mice resulting from Cas13X-mediated or hfCas13X-mediated *Tyr* knockdown compared to WT mice at 9 weeks. **c**, Kaplan–Meier curve of F1 generation of WT mice (n = 10) and mice expressing Cas13X (n = 10), Cas13X with *Tyr* gRNAs (n = 11), or hfCas13X with *Tyr* gRNAs (n = 9). Statistical survival differences were evaluated by two-sided log-rank test. **d**, Body weight for mice from **c**. *P* values of Cas13X with *Tyr* gRNAs vs. WT mice are showed at 3 time points. Two-tailed unpaired two-sample *t*-test. **e**, The levels of *Tyr* mRNA from 36-week-old mice, n = 5. Dunnett's multiple comparisons test after one-way ANOVA. Data are presented as mean values ± s.e.m.



Supplementary Figure 20. Comparison of on-target activity and collateral activity among different Cas13 proteins.

a, Schematics describing protein-size and HEPN motif position of well-charactered Cas13b/d, Cas13X, hfCas13d and hfCas13X proteins used in this study. RxxxxH motifs define the catalytic site, shown as yellow lines. **b**,**c**, Differential changes of normalized MFI of mCherry (**b**) and EGFP (**c**) induced by PspCas13b, RanCas13b, Cas13d, Cas13X, hfCas13d and hfCas13X in HEK293T cell lines. n = 3. In **b**, dark green asterisks, left-to-right, P=0.0470, P<0.0001, P<0.0001, and P=0.0006; dark red asterisks, ***P<0.0001. In **c**, dark green asterisks, ***P<0.0001; dark red asterisks, ***P<0.0001 and **P=0.0068. **d**, Differential changes of normalized MFI of EGFP induced by different Cas13 proteins. n = 3, dark green asterisks, left-to-right, P=0.0219, P=0.0025, P=0.0034, P=0.0031, P=0.0107, P=0.0063, P=0.0021, P<0.0001, P=0.0072, P<0.0001, P=0.0043, and P=0.0017; dark red asterisks, left-to-right, P=0.0156, P=0.0018, P<0.0001, P<0.0001, P=0.0074, P<0.0001, P<0.0001, P=0.0008, P<0.0001, P=0.0033, and P=0.0017. **e**, Relative expression level of targeted endogenous transcripts by different Cas13 proteins. Normalized MFI and normalized expression levels were relative to corresponding dCas13 condition. n = 2, for *B4GALNT1* (Cas13d), *NRAS* (PspCas13b), *PPIA* (RanCas13b, hfCas13d), *RPS5* (PspCas13b, RanCas13b, hfCas13X), and *SMARCA1* (RanCas13b, hfCas13d). n = 3, unless otherwise noted. Dark green asterisks, left-to-right, P<0.0001, P=0.0004, and P=0.0023; dark red asterisks, left-to-right, P<0.0001 and P=0.0144, All values are presented as mean ± s.e.m. Selected results of Dunnett's multiple comparisons test after one-way ANOVA are indicated by asterisks. *P < 0.05, **P < 0.01, ***P < 0.001.



Supplementary Figure 21. Model of Cas13 on-target and collateral cleavage activity.

Once activated by targeted RNA binding, wtCas13 exhibits both on-target cleavage activity and collateral cleavage activity (**a**,**b**), while hfCas13 (e.g., hfCas13d and hfCas13X) with mutant sites maintains undiminished on-target cleavage activity (**c**) but eliminates collateral cleavage activity (**d**).

Supplementary Tables

Supplementary Table 1. The mutant sequences and corresponding nucleotide sequences used for Cas13d screening.

Variants	Amino Acids	Nucleotide sequence
N1L	KGYAVVANNPLYTGPVQ	AAGGGCTACGCCGTGGTGGCTAACAACCCACTGTACACCGGACCAGTGCAG
N1-Y79A	KGAAVVANNPLYTGPVQ	AAGGGCgccGCCGTGGTGGCTAACAACCCACTGTACACCGGACCAGTGCAG
N1R	QDMLGLKETLEKRYFGESA	CAGGACATGCTGGGACTGAAGGAGACACTGGAGAAGAGGTACTTCGGCGAGTCCGCC
N1V5	QDMAAAKEALEKRYFAESA	CAGGACATGgccgccgccAAGGAGgccCTGGAGAAGAGGTACTTCgccGAGTCCGCC
N1V6	QDALGLKATAEKRYAGESA	CAGGACgccCTGGGACTGAAGgccACAgccGAGAAGAGGTACgccGGCGAGTCCGCC
N1V7	QDMLGLKETLAKAYFGASV	CAGGACATGCTGGGACTGAAGGAGACACTGgccAAGgccTACTTCGGCgccTCCgtg
N1-Y107A	QDMLGLKETLEKRAFGESA	CAGGACATGCTGGGACTGAAGGAGACACTGGAGAAGAGGgccTTCGGCGAGTCCGCC
N2L	DGNDNICIQVIHNILDI	GACGGAAACGATAACATCTGCATCCAGGTCATCCACAACATCCTGGATATC
N2V1	AAAANICIQVIHNILAI	gccgccgccgccAACATCTGCATCCAGGTCATCCACAACATCCTGgccATC
N2V4	DGNDNICAQVAHNALDA	GACGGAAACGATAACATCTGCgccCAGGTCgccCACAACgccCTGGATgcc
N2R	EKILAEYITNAAYAVNNIS	GAGAAGATCCTGGCTGAGTACATCACAAACGCCGCTTACGCCGTGAACAACATCTCC
N2V5	AAILAEYIAAAAYAVNNIA	gccgccATCCTGGCTGAGTACATCgccgccGCCGCTTACGCCGTGAACAACATCgcc
N2V6	EKIAAEYITNAAYAAAAIS	GAGAAGATCgccGCTGAGTACATCACAAACGCCGCTTACGCCgccgccgccATCTCC
N2V7	EKALAAYATNAAYAVNNAS	GAGAAGgccCTGGCTgccTACgccACAAACGCCGCTTACGCCGTGAACAACgccTCC
N2V8	EKILVEYITNVVYVVNNIS	GAGAAGATCCTGgtgGAGTACATCACAAACgtggtgTACgtgGTGAACAACATCTCC
N2-Y136A	EKILAEAITNAAYAVNNIS	GAGAAGATCCTGGCTGAGgccATCACAAACGCCGCTTACGCCGTGAACAACATCTCC
N2-Y142A	EKILAEYITNAAAAVNNIS	GAGAAGATCCTGGCTGAGTACATCACAAACGCCGCTgccGCCGTGAACAACATCTCC
N3L	GLDKDIIGFGKFSTVYT	GGCCTGGACAAGGATATCATCGGCTTCGGAAAGTTTTCTACCGTGTACACA
N3-Y164A	GLDKDIIGFGKFSTVAT	GGCCTGGACAAGGATATCATCGGCTTCGGAAAGTTTTCTACCGTGgccACA

N3R	YDEFKDPEHHRA AFNNNDK	TACGACGAGTTCAAGGATCCAGAGCACCACCGGGC CGCTTTTAACAACAACGACAAG
N3V5	AAEFAAPEHHRA AFNNNDA	gccgccGAGTTCgccgccCCAGAGCACCACCGGGCCGCTT TTAACAACAACGACgcc
N3V6	YDEFKDPEAHRA AFAAAAK	TACGACGAGTTCAAGGATCCAGAGgccCACCGGGCC GCTTTTgccgccgccgccAAG
N3V7	YDAAKDPEHARA AANNNDK	TACGACgccgccAAGGATCCAGAGCACgccCGGGCCGC TgccAACAACAACGACAAG
N3V8	YDEFKDPAHHAV VFNNNDK	TACGACGAGTTCAAGGATCCAgccCACCACgccgtggtgT TTAACAACAACGACAAG
N3-Y166A	ADEFKDPEHHRA AFNNNDK	gccGACGAGTTCAAGGATCCAGAGCACCACCGGGCC GCTTTTAACAACAACGACAAG
N4L	LINAIKAQYDEFD NFLD	CTGATCAACGCCATCAAGGCTCAGTACGACGAGTTC GATAACTTTCTGGAT
N4V1	LINAIAAQYAEFA NFLA	CTGATCAACGCCATCgccGCTCAGTACgccGAGTTCgcc AACTTTCTGgcc
N4V2	AIAAIKAAYDEFD AFLD	gccATCgccGCCATCAAGGCTgccTACGACGAGTTCGAT gccTTTCTGGAT
N4V3	LANAAKAQYDE ADNFAD	CTGgccAACGCCgccAAGGCTCAGTACGACGAGgccGA TAACTTTgccGAT
N4V4	LINVIKVQYDAFD NALD	CTGATCAACgtgATCAAGgtgCAGTACGACgccTTCGAT AACgccCTGGAT
N4-Y193A	LINAIKAQADEFD NFLD	CTGATCAACGCCATCAAGGCTCAGgccGACGAGTTCG ATAACTTTCTGGAT
N4R	NPRLGYFGQAFFS KEGRNY	AACCCCAGGCTGGGCTACTTCGGACAGGCTTTCTTT TCTAAGGAGGGCAGAAACTAC
N4V5	AARLAYFGQAFF AAEGRNY	gccgccAGGCTGgccTACTTCGGACAGGCTTTCTTTgccgc cGAGGGCAGAAACTAC
N4V6	NPRLGYFAAAFFS KEARAY	AACCCCAGGCTGGGCTACTTCgccgccGCTTTCTTTTCT AAGGAGgccAGAgccTAC
N4V7	NPRAGYAGQAA ASKEGRNY	AACCCCAGGgccGGCTACgccGGACAGGCTgccgccTCT AAGGAGGGCAGAAACTAC
N4V8	NPALGYFGQVFF SKAGANY	AACCCCgccCTGGGCTACTTCGGACAGgtgTTCTTTTCT AAGgccGGCgccAACTAC
N4-Y207A	NPRLGAFGQAFFS KEGRNY	AACCCCAGGCTGGGCgccTTCGGACAGGCTTTCTTTT CTAAGGAGGGCAGAAACTAC
N4-Y220A	NPRLGYFGQAFFS KEGRNA	AACCCCAGGCTGGGCTACTTCGGACAGGCTTTCTTT TCTAAGGAGGGCAGAAACgcc
N5L	IINYGNECYDILA LLSG	ATCATCAACTACGGAAACGAGTGTTACGACATCCTG GCCCTGCTGAGCGGA
N5V2	IINYGAEAYDIAA AASG	ATCATCAACTACGGAgccGAGgccTACGACATCgccGC CgccgccAGCGGA
N5V3	AANYGNACYDA LVLLSG	gccgccAACTACGGAAACgccTGTTACGACgccCTGgtgCT GCTGAGCGGA
N5R	LRHWVVHNNEEE SRISRTW	CTGAGGCACTGGGTGGTGCACAACAACGAGGAGGA GTCTCGGATCAGCCGCACCTGG

N5V4	AAHWVVHNNEEEARIARAW	gccgccCACTGGGTGGTGCACAACAACGAGGAGGAGgccCGGATCgccCGCgccTGG
N5V5	LRHWVVHAAAAESRASRTW	CTGAGGCACTGGGTGGTGCACgccgccgccgccGAGTCTCGGgccAGCCGCACCTGG
N5V6	LRHWVVHNNEEASAISATA	CTGAGGCACTGGGTGGTGCACAACAACGAGGAGgccTCTgccATCAGCgccACCgcc
N6L	LYNLDKNLDNEYISTLN	CTGTACAACCTGGACAAGAACCTGGATAACGAGTACATCTCCACACTGAAC
N6V1	LYNLAANLANEYIAALN	CTGTACAACCTGgccgccAACCTGgccAACGAGTACATCgccgccCTGAAC
N6V2	AYALDKALDAEYISTLA	gccTACgccCTGGACAAGgccCTGGATgccGAGTACATCTCCACACTGgcc
N6V3	LYNADKNADNAYASTAN	CTGTACAACgccGACAAGAACgccGATAACgccTACgccTCCACAgccAAC
N6-Y258A	LANLDKNLDNEYISTLN	CTGgccAACCTGGACAAGAACCTGGATAACGAGTACATCTCCACACTGAAC
N6-Y268A	LYNLDKNLDNEAISTLN	CTGTACAACCTGGACAAGAACCTGGATAACGAGgccATCTCCACACTGAAC
N6R	YLYDRITNELTNSFSKNSA	TACCTGTACGACAGGATCACCAACGAGCTGACAAACAGCTTCTCCAAGAACTCTGCC
N6V4	AAYDRITNELTNAFAANSA	gccgccTACGACAGGATCACCAACGAGCTGACAAACgccTTCgccgccAACTCTGCC
N6V5	YLYARIAAELANSFSKNAA	TACCTGTACgccAGGATCgccgccGAGCTGgccAACAGCTTCTCCAAGAACgccGCC
N6V6	YLYDRATNEATASFSKASA	TACCTGTACGACAGGgccACCAACGAGgccACAgccAGCTTCTCCAAGgccTCTGCC
N6-Y274A	ALYDRITNELTNSFSKNSA	gccCTGTACGACAGGATCACCAACGAGCTGACAAACAGCTTCTCCAAGAACTCTGCC
N6-Y276A	YLADRITNELTNSFSKNSA	TACCTGgccGACAGGATCACCAACGAGCTGACAAACAGCTTCTCCAAGAACTCTGCC
N7L	ANVNYIAETLGINPAEF	GCTAACGTGAACTACATCGCTGAGACCCTGGGCATCAACCCAGCTGAGTTC
N7V1	AAVAYIAEALAIAPAEF	GCTgccGTGgccTACATCGCTGAGgccCTGgccATCgccCCAGCTGAGTTC
N7V2	ANANYAAETAGANPAEA	GCTAACgccAACTACgccGCTGAGACCgccGGCgccAACCCAGCTGAGgcc
N7V3	VNVNYIVATLGINPVAF	gtgAACGTGAACTACATCgtggccACCCTGGGCATCAACCCAgtggccTTC
N7R	AEQYFRFSIMKEQKNLGFN	GCTGAGCAGTACTTCAGATTTTCCATCATGAAGGAGCAGAAGAACCTGGGCTTCAAC
N7V5	AEAYFRFSIMKEAKALAFA	GCTGAGgccTACTTCAGATTTTCCATCATGAAGGAGgccAAGgccCTGgccTTCgcc
N7V6	AEQYARFSAAKEQKNAGFN	GCTGAGCAGTACgccAGATTTTCCgccgccAAGGAGCAGAAGAACgccGGCTTCAAC
N7V7	AEQYFAASIMKAQKNLGAN	GCTGAGCAGTACTTCgccgccTCCATCATGAAGgccCAGAAGAACCTGGGCgccAAC

N7-Y313A	AEQAFRFSIMKEQKNLGFN	GCTGAGCAGgccTTCAGATTTTCCATCATGAAGGAGCAGAAGAACCTGGGCTTCAAC
N8L	AGRDVSAFSKLMYALTM	GCTGGAAGGGACGTGAGCGCCTTCAGCAAGCTGATGTACGCCCTGACAATG
N8-Y470A	AGRDVSAFSKLMAALTM	GCTGGAAGGGACGTGAGCGCCTTCAGCAAGCTGATGgccGCCCTGACAATG
N8R	FLDGKEINDLLTTLINKFD	TTTCTGGACGGAAAGGAGATCAACGATCTGCTGACCACACTGATCAACAAGTTCGAC
N8V5	AADAAEINDLLTTLINAFD	gccgccGACgccgccGAGATCAACGATCTGCTGACCACACTGATCAACgccTTCGAC
N8V6	FLAGKEINALLAALINKFA	TTTCTGgccGGAAAGGAGATCAACgccCTGCTGgccgccCTGATCAACAAGTTCgcc
N8V7	FLDGKEIADAATTAIAKFD	TTTCTGGACGGAAAGGAGATCgccGATgccgccACCACAgccATCgccAAGTTCGAC
N8V8	FLDGKAANDLLTTLANKAD	TTTCTGGACGGAAAGgccgccAACGATCTGCTGACCACACTGgccAACAAGgccGAC
N10L	LIKSFARMGEPIADARR	CTGATCAAGTCCTTTGCCAGGATGGGAGAGCCAATCGCTGACGCTAGGAGA
N10V1	LIAAFARMAEPIAAARR	CTGATCgccgccTTTGCCAGGATGgccGAGCCAATCGCTgccGCTAGGAGA
N10V2	AAKSFARAGEPAADARR	gccgccAAGTCCTTTGCCAGGgccGGAGAGCCAgccGCTGACGCTAGGAGA
N10V3	LIKSAVRMGAPIVDARR	CTGATCAAGTCCgccgtgAGGATGGGAgccCCAATCgtgGACGCTAGGAGA
N10R	AMYIDAIRILGTNLSYDEL	GCTATGTACATCGATGCCATCCGGATCCTGGGAACCAACCTGTCTTACGACGAGCTG
N10V5	VAYIDAIRILAANLAYDEL	gtggccTACATCGATGCCATCCGGATCCTGgccgccAACCTGgccTACGACGAGCTG
N10V6	AMYIAAIRIAGTALSYAEL	GCTATGTACATCgccGCCATCCGGATCgccGGAACCgccCTGTCTTACgccGAGCTG
N10V7	AMYADAARILGTNASYDEA	GCTATGTACgccGATGCCgccCGGATCCTGGGAACCAACgccTCTTACGACGAGgcc
N10V8	AMYIDVIAALGTNLSYDAL	GCTATGTACATCGATgtgATCgccgccCTGGGAACCAACCTGTCTTACGACgccCTG
N10-Y549A	AMAIDAIRILGTNLSYDEL	GCTATGgccATCGATGCCATCCGGATCCTGGGAACCAACCTGTCTTACGACGAGCTG
N10-Y562A	AMYIDAIRILGTNLSADEL	GCTATGTACATCGATGCCATCCGGATCCTGGGAACCAACCTGTCTgccGACGAGCTG
N12L	FHYLIRYGDPAHLHEIA	TTTCACTACCTGATCAGATACGGCGACCCAGCTCACCTGCACGAGATCGCT
N12-Y604A	FHALIRYGDPAHLHEIA	TTTCACgccCTGATCAGATACGGCGACCCAGCTCACCTGCACGAGATCGCT
N12-Y608A	FHYLIRAGDPAHLHEIA	TTTCACTACCTGATCAGAgccGGCGACCCAGCTCACCTGCACGAGATCGCT
N12R	KNEAVVKFVLGRIADIQKK	AAGAACGAGGCCGTGGTGAAGTTCGTGCTGGGACGGATCGCCGATATCCAGAAGAAG

N12V4	AAEAVVAFVLGR IADIQAA	gccgccGAGGCCGTGGTGgccTTCGTGCTGGGACGGATC GCCGATATCCAGgccgcc
N12V5	KNEAAAKFALAR IAAIQKK	AAGAACGAGGCCgccgccAAGTTCgccCTGgccCGGATC GCCgccATCCAGAAGAAG
N12V6	KNEAVVKAVAG RAADAAKK	AAGAACGAGGCCGTGGTGAAGgccGTGgccGGACGGg ccGCCGATgccgccAAGAAG
N12V7	KNAVVVKFVLGA IVDIQKK	AAGAACgccgtgGTGGTGAAGTTCGTGCTGGGAgccATC gtgGATATCCAGAAGAAG
N14L	TGMNYDQFDKK RSVIED	ACAGGCATGAACTACGACCAGTTCGATAAGAAGAG ATCTGTGATCGAGGAC
N14-Y678A	TGMNADQFDKK RSVIED	ACAGGCATGAACgccGACCAGTTCGATAAGAAGAGA TCTGTGATCGAGGAC
N14R	TGRENAEREKFK KIISLYL	ACCGGAAGGGAGAACGCCGAGAGAGAGAAGTTTAA GAAGATCATCAGCCTGTACCTG
N14V5	AARENAEREAFA AIISLYL	gccgccAGGGAGAACGCCGAGAGAGAGgccTTTgccgccA TCATCAGCCTGTACCTG
N14V6	TGREAAEREKFK KAIAAYA	ACCGGAAGGGAGgccGCCGAGAGAGAGAAGTTTAAG AAGgccATCgccgccTACgcc
N14V7	TGRANAAREKAK KIASLYL	ACCGGAAGGgccAACGCCgccAGAGAGAAGgccAAGA AGATCgccAGCCTGTACCTG
N14V8	TGAENVEAAKFK KIISLYL	ACCGGAgccGAGAACgtgGAGgccgccAAGTTTAAGAAG ATCATCAGCCTGTACCTG
N14-Y708A	TGRENAEREKFK KIISLAL	ACCGGAAGGGAGAACGCCGAGAGAGAGAAGTTTAA GAAGATCATCAGCCTGgccCTG
N15L	TVIYHILKNIVNIN ARY	ACAGTGATCTACCACATCCTGAAGAACATCGTGAAC ATCAACGCTAGATAC
N15V1	AVIYHILAAIVAI AARY	gccGTGATCTACCACATCCTGgccgccATCGTGgccATCgc cGCTAGATAC
N15V2	TAAYAIAKNIANI NARY	ACAgccgccTACgccATCgccAAGAACATCgccAACATCA ACGCTAGATAC
N15R	VIGFHCVERDAQ LYKEKGY	GTGATCGGCTTCCACTGCGTGGAGCGCGATGCCCAG CTGTACAAGGAGAAGGGATAC
N15V4	AAAFHCVERDAQ LYAEAGY	gccgccgccTTCCACTGCGTGGAGCGCGATGCCCAGCTG TACgccGAGgccGGATAC
N15V5	VIGFHCAERAAA LYKEKAY	GTGATCGGCTTCCACTGCgccGAGCGCgccGCCgccCTG TACAAGGAGAAGgccTAC
N15V6	VIGAAAVERDAQ AYKEKGY	GTGATCGGCgccgccgccGTGGAGCGCGATGCCCAGgcc TACAAGGAGAAGGGATAC
N15V7	VIGFHCVAADVQ LYKAKGY	GTGATCGGCTTCCACTGCGTGgccgccGATgtgCAGCTG TACAAGgccAAGGGATAC
N16L	DINLKKLEEKGFS SVTK	GACATCAACCTGAAGAAGCTGGAGGAGAAGGGCTT TAGCTCCGTGACCAAG
N16V1	DINLAALEEAGFA SVTA	GACATCAACCTGgccgccCTGGAGGAGgccGGCTTTgccT CCGTGACCgcc
N16V2	AINLKKLEEKAFS AVAK	gccATCAACCTGAAGAAGCTGGAGGAGAAGgccTTTA GCgccGTGgccAAG

N16V3	DIAAKKAEEKGF SSATK	GACATCgccgccAAGAAGgccGAGGAGAAGGGCTTTAG CTCCgccACCAAG
N16V4	DANLKKLAAKG ASSVTK	GACgccAACCTGAAGAAGCTGgccgccAAGGGCgccAGC TCCGTGACCAAG
N16R	LCAGIDETAPDKR KDVEKE	CTGTGCGCTGGAATCGACGAGACAGCCCCCGACAA GAGGAAGGATGTGGAGAAGGAG
N16V5	AAAGIDETAPDA RADVEAE	gccgccGCTGGAATCGACGAGACAGCCCCCGACgccAG GgccGATGTGGAGgccGAG
N16V6	LCAAIAEAAPAK RKAVEKE	CTGTGCGCTgccATCgccGAGgccGCCCCCgccAAGAGG AAGgccGTGGAGAAGGAG
N16V7	LCAGADATAPDK RKDAAKE	CTGTGCGCTGGAgccGACgccACAGCCCCCGACAAGA GGAAGGATgccgccAAGGAG
N16V8	LCVGIDETVPDK AKDVEKA	CTGTGCgtgGGAATCGACGAGACAgtgCCCGACAAGgc cAAGGATGTGGAGAAGgcc
N17L	MAERAKESIDSLE SANP	ATGGCCGAGAGAGCTAAGGAGAGCATCGACTCCCT GGAGTCTGCTAACCCT
N17V4	MVEAVKESIDSLE SVNP	ATGgtgGAGgccgtgAAGGAGAGCATCGACTCCCTGGA GTCTgtgAACCCT
N17R	KLYANYIKYSDE KKAEEFT	AAGCTGTACGCCAACTACATCAAGTACTCCGATGAG AAGAAGGCCGAGGAGTTCACC
N17V6	KLYANYIKYAAE KAAEEFA	AAGCTGTACGCCAACTACATCAAGTACgccgccGAGA AGgccGCCGAGGAGTTCgcc
N17V7	KLYAAYAKYSDA KKAEEAT	AAGCTGTACGCCgccTACgccAAGTACTCCGATgccAAG AAGGCCGAGGAGgccACC
N17V8	KLYVNYIKYSDE KKVAAFT	AAGCTGTACgtgAACTACATCAAGTACTCCGATGAGA AGAAGgtggccgccTTCACC
N18L	RQINREKAKTAL NAYLR	AGGCAGATCAACAGAGAGAAGGCCAAGACCGCTCT GAACGCCTACCTGAGG
N18V1	RQIAREAAAAAL NAYLR	AGGCAGATCgccAGAGAGgccGCCgccgccGCTCTGAAC GCCTACCTGAGG
N18V2	RAINREKAKTAA AAYAR	AGGgccATCAACAGAGAGAAGGCCAAGACCGCTgccg ccGCCTACgccAGG
N18V3	RQANRAKVKTVL NAYLR	AGGCAGgccAACAGAgccAAGgtgAAGACCgtgCTGAAC GCCTACCTGAGG
N18V4	AQINAEKAKTAL NVYLA	gccCAGATCAACgccGAGAAGGCCAAGACCGCTCTGA ACgtgTACCTGgcc
N18R	NTKWNVIIREDLL RIDNKT	AACACAAAGTGGAACGTGATCATCCGGGAGGACCT GCTGCGCATCGATAACAAGACC
N18V6	NTKWAAIIREALL RIAAKT	AACACAAAGTGGgccgccATCATCCGGGAGgccCTGCT GCGCATCgccgccAAGACC
N18V7	NTKWNVAARED AARADNKT	AACACAAAGTGGAACGTGgccgccCGGGAGGACgccgcc CGCgccGATAACAAGACC
N18V8	NTKANVIIAADLL AIDNKT	AACACAAAGgccAACGTGATCATCgccgccGACCTGCT GgccATCGATAACAAGACC
N19L	CTLFRNKAVHLE VARYV	TGTACACTGTTCCGGAACAAGGCTGTGCACCTGGAG GTGGCTCGCTACGTG

N19V1	CAAFRNKAVHAEAARYA	TGTgccgccTTCcggaacaaggctgtgcacgccGAGgccGCTCGCTACgcc
N19R	HAYINDIAEVNSYFQLYHY	CACGCCTACATCAACGACATCGCCGAGGTGAACTCCTACTTTCAGCTGTACCACTAC
N19V4	HAYINDIAEAASYFAAYAY	CACGCCTACATCAACGACATCGCCGAGgccgccTCCTACTTTgccgccTACgccTAC
N19V5	HAYANDAVAVNSYAQLYHY	CACGCCTACgccAACGACgccgtggccGTGAACTCCTACgccCAGCTGTACCACTAC
N20L	IMQRIIMNERYEKSSGK	ATCATGCAGAGGATCATCATGAACGAGAGATACGAGAAGTCTAGCGGCAAG
N20V4	IMQAIIMNAAYAKSSGK	ATCATGCAGgccATCATCATGAACgccgccTACgccAAGTCTAGCGGCAAG
N20-Y900A	IMQRIIMNERAEKSSGK	ATCATGCAGAGGATCATCATGAACGAGAGAgccGAGAAGTCTAGCGGCAAG
N20R	VSEYFDAVNDEKKYNDRLL	GTGTCTGAGTACTTCGACGCCGTGAACGATGAGAAGAAGTACAACGATAGACTGCTG
N20V6	VSEYFDAVAAEKKYAARLL	GTGTCTGAGTACTTCGACGCCGTGgccgccGAGAAGAAGTACgccgccAGACTGCTG
N20V7	VSEYADAANDEKKYNDRAA	GTGTCTGAGTACgccGACGCCgccAACGATGAGAAGAAGTACAACGATAGAgccgcc
N20-Y910A	VSEAFDAVNDEKKYNDRLL	GTGTCTGAGgccTTCGACGCCGTGAACGATGAGAAGAAGTACAACGATAGACTGCTG
N20-Y920A	VSEYFDAVNDEKKANDRLL	GTGTCTGAGTACTTCGACGCCGTGAACGATGAGAAGAAGgccAACGATAGACTGCTG
N21L	KLLCVPFGYCIPRFKNL	AAGCTGCTGTGCGTGCCTTTCGGATACTGTATCCCACGGTTTAAGAACCTG
N21V1	ALLCAPFAYCIPRFAAL	gccCTGCTGTGCgccCCTTTCgccTACTGTATCCCACGGTTTgccgccCTG
N21-Y934A	KLLCVPFGACIPRFKNL	AAGCTGCTGTGCGTGCCTTTCGGAgccTGTATCCCACGGTTTAAGAACCTG
N21R	SIEALFDRNEAAKFDKEKK	AGCATCGAGGCCCTGTTCGACCGCAACGAGGCTGCCAAGTTTGATAAGGAGAAGAAG
N21V4	AAEALFDRNEAAAFDAEAK	gccgccGAGGCCCTGTTCGACCGCAACGAGGCTGCCgccTTTGATgccGAGgccAAG
N21V5	SIEAAFARAEAAKFAKEKA	AGCATCGAGGCCgccTTCgccCGCgccGAGGCTGCCAAGTTTgccAAGGAGAAGgcc
N21V6	SIAALADRNAAAKADKAKK	AGCATCgccGCCCTGgccGACCGCAACgccGCTGCCAAGgccGATAAGgccAAGAAG
N21V7	SIEVLFDANEVVKFDKEKK	AGCATCGAGgtgCTGTTCGACgccAACGAGgtggtgAAGTTTGATAAGGAGAAGAAG
N2C	ILAEYITNAAYAVNNIS	ATCCTGGCTGAGTACATCACAAACGCCGCTTACGCCGTGAACAACATCTCC
I132A	ALAEYITNAAYAVNNIS	gccCTGGCTGAGTACATCACAAACGCCGCTTACGCCGTGAACAACATCTCC
L133A	IAAEYITNAAYAVNNIS	ATCgccGCTGAGTACATCACAAACGCCGCTTACGCCGTGAACAACATCTCC

A134V	ILVEYITNAAYAV NNIS	ATCCTGgccGAGTACATCACAAACGCCGCTTACGCCG TGAACAACATCTCC
E135A	ILAAYITNAAYAV NNIS	ATCCTGGCTgccTACATCACAAACGCCGCTTACGCCG TGAACAACATCTCC
I137A	ILAEYATNAAYA VNNIS	ATCCTGGCTGAGTACgccACAAACGCCGCTTACGCCG TGAACAACATCTCC
T138A	ILAEYIANAAYAV NNIS	ATCCTGGCTGAGTACATCgccAACGCCGCTTACGCCG TGAACAACATCTCC
N139A	ILAEYITAAAYAV NNIS	ATCCTGGCTGAGTACATCACAgccGCCGCTTACGCCG TGAACAACATCTCC
A140V	ILAEYITNVAYAV NNIS	ATCCTGGCTGAGTACATCACAAACgtgGCTTACGCCG TGAACAACATCTCC
A141V	ILAEYITNAVYAV NNIS	ATCCTGGCTGAGTACATCACAAACGCCgtgTACGCCG TGAACAACATCTCC
A143V	ILAEYITNAAYVV NNIS	ATCCTGGCTGAGTACATCACAAACGCCGCTTACgtgG TGAACAACATCTCC
V144A	ILAEYITNAAYAA NNIS	ATCCTGGCTGAGTACATCACAAACGCCGCTTACGCC gccAACAACATCTCC
N145A	ILAEYITNAAYAV ANIS	ATCCTGGCTGAGTACATCACAAACGCCGCTTACGCC GTGgccAACATCTCC
N146A	ILAEYITNAAYAV NAIS	ATCCTGGCTGAGTACATCACAAACGCCGCTTACGCC GTGAACgccATCTCC
I147A	ILAEYITNAAYAV NNAS	ATCCTGGCTGAGTACATCACAAACGCCGCTTACGCC GTGAACAACgccTCC
S148A	ILAEYITNAAYAV NNIA	ATCCTGGCTGAGTACATCACAAACGCCGCTTACGCC GTGAACAACATCgcc
N2D1	ALAAYATNAAY AVNNIS	gccCTGGCTgccTACgccACAAACGCCGCTTACGCCGTG AACAACATCTCC
N2D2	ALAAYITNAAYA VNNAS	gccCTGGCTgccTACATCACAAACGCCGCTTACGCCGT GAACAACgccTCC
N2D3	ALAEYATNAAYA VNNAS	gccCTGGCTGAGTACgccACAAACGCCGCTTACGCCGT GAACAACgccTCC
N2D4	ILAAYATNAAYA VNNAS	ATCCTGGCTgccTACgccACAAACGCCGCTTACGCCGT GAACAACgccTCC
N2D5	ALAAYITNAAYA VNNIS	gccCTGGCTgccTACATCACAAACGCCGCTTACGCCGT GAACAACATCTCC
N2D6	ALAEYATNAAYA VNNIS	gccCTGGCTGAGTACgccACAAACGCCGCTTACGCCGT GAACAACATCTCC
N2D7	ALAEYITNAAYA VNNAS	gccCTGGCTGAGTACATCACAAACGCCGCTTACGCCG TGAACAACgccTCC
N2D8	ILAAYATNAAYA VNNIS	ATCCTGGCTgccTACgccACAAACGCCGCTTACGCCGT GAACAACATCTCC
N2D9	ILAAYITNAAYAV NNAS	ATCCTGGCTgccTACATCACAAACGCCGCTTACGCCG TGAACAACgccTCC
N2D10	ILAEYATNAAYA VNNAS	ATCCTGGCTGAGTACgccACAAACGCCGCTTACGCCG TGAACAACgccTCC

N2D11	ILVEYITNVVYAVNNIS	ATCCTGgtgGAGTACATCACAAACgtggtgTACGCCGTGAACAACATCTCC
N2D12	ILVEYITNVAYVVNNIS	ATCCTGgtgGAGTACATCACAAACgtgGCTTACgtgGTGAACAACATCTCC
N2D13	ILVEYITNAVYVVNNIS	ATCCTGgtgGAGTACATCACAAACGCCgtgTACgtgGTGAACAACATCTCC
N2D14	ILAEYITNVVYVVNNIS	ATCCTGGCTGAGTACATCACAAACgtggtgTACgtgGTGAACAACATCTCC
N2D15	ILVEYITNVAYAVNNIS	ATCCTGgtgGAGTACATCACAAACgtgGCTTACGCCGTGAACAACATCTCC
N2D16	ILVEYITNAVYAVNNIS	ATCCTGgtgGAGTACATCACAAACGCCgtgTACGCCGTGAACAACATCTCC
N2D17	ILVEYITNAAYVVNNIS	ATCCTGgtgGAGTACATCACAAACGCCGCTTACgtgGTGAACAACATCTCC
N2D18	ILAEYITNVVYAVNNIS	ATCCTGGCTGAGTACATCACAAACgtggtgTACGCCGTGAACAACATCTCC
N2D19	ILAEYITNVAYVVNNIS	ATCCTGGCTGAGTACATCACAAACgtgGCTTACgtgGTGAACAACATCTCC
N2D20	ILAEYITNAVYVVNNIS	ATCCTGGCTGAGTACATCACAAACGCCgtgTACgtgGTGAACAACATCTCC
N2T	ALAAYITNAAYVVNNIS	gccCTGGCTgccTACATCACAAACGCCGCTTACgtgGTGAACAACATCTCC
N2Q	ALVAYITNAAYVVNNIS	gccCTGgtggccTACATCACAAACGCCGCTTACgtgGTGAACAACATCTCC

Supplementary Table 3. The mutant sequences and corresponding nucleotide sequences used for Cas13X screening.

Variants	Amino Acids	Nucleotide sequence
M1-WT	RTIMERAYERAI FECRRR	CGGACCATCATGGAGAGAGCCTATGAGCGGGCCAT CTTCGAGTGCAGAAGAAGA
M1	KTIMDKAYDKA IFDCKKT	aaaACCATCATGgacaagGCCTATgacaagGCCATCTTCgac TGCaagaagacc
M2-WT	AFEEKVVKAKK MSEKE	GCTTTCGAAGAGAAGGTGGTGAAGGCCAAGAAGAT GAGCGAGAAGGAA
M2	VFDEQVVQAQQ MSEKT	gtcTTCGAcGAGcAGGTGGTGcAGGCCcAGcAGATGAG CGAGAAGacc
M3-WT	VMKKYGIEKEW KFPVK	GTGATGAAGAAGTACGGCATCGAGAAGGAATGGAA GTTCCCTGTCAAG
M3	VFMQKYGIDKD WQFPVK	GTcttcATGcAGAAGTACGGCATCGACAAGGACTGGCA GTTCCCTGTGAAG
M4-WT	QVSKQTSKKRE LSIDE	CAGGTGAGCAAGCAGACCTCCAAGAAGAGGGAGCT GAGCATCGACGAG
M4	VFSAATSAAAA LSIED	GTcttctcggccgctACCTCCgctgccgccgctCTGAGCATCGAaG Ac
M5-WT	YQGARKWCFTI AFNKA	TACCAGGGCGCCCGGAAGTGGTGCTTCACCATTGCC TTCAACAAGGCC
M5	VFGAAAWCFTI AFAED	GTcttcGGCGCCgccgccTGGTGCTTCACCATTGCCTTCgc cgaagac
M6-WT	LVNRDKNDGLF VESLLR	CTGGTGAACCGGGACAAGAACGACGGCCTGTTCGTG GAAAGCCTGCTGAGA
M6	VFAAAAAAGLF VASLED	gtcttcgcCgccGcCgccgccGcCGGCCTGTTCGTGGccAGCCT Ggaagac
M7-WT	HEKYSKHDWYD EDTRA	CACGAGAAGTACAGCAAGCACGACTGGTACGACGA AGATACCCGGGCC
M7	VFAYSAAAWYA AATED	gtcttcgccTACAGCgccgccgccTGGTACgccgccgccACCgaaga c
M8-WT	LIKCSTQAANAK AEAL	CTGATCAAGTGCAGCACCCAGGCCGCCAACGCCAA GGCTGAAGCCCTG
M8	VFACSTQAAAA AAAED	gtcttcgccTGCAGCACCCAGGCCGCCgccGCCgccGCTgccg aagac
M9-WT	YRHSPGCLTFTA EDEL	TACCGGCATAGCCCTGGCTGCCTGACCTTCACCGCC GAGGACGAACTG
M9	VFASPGCLAFTA ADED	gtcttcgccAGCCCTGGCTGCCTGgCCTTCACCGCCgccGA Cgaagac
M10-WT	ETEVIIEFPSLFE GDR	GAGACAGAGGTGATCATCGAGTTTCCCAGCCTGTTC GAGGGCGACCGG
M10	VFAVIIAFPALFA GED	gtcttcGccGTGATCATCGccTTTCCCgccCTGTTCGccGGCg aagac
M11-WT	ITTAGVVFFVSF FVER	ATCACCACCGCCGGCGTGGTGTTTTTCGTGAGCTTTT TCGTGGAAAGA

M11	VFAAGVAFAVAFAVED	gtcttcgCCGCCGGCGTGgccTTTgccGTGgccTTTgccGTGgaagac
M12-WT	RVLDRLYGAVSGLKKN	AGAGTGCTGGATCGGCTGTATGGAGCCGTGTCCGGCCTGAAGAAGAAT
M12	VFLAALAGAVAGLAED	gtcttcCTGGccgccCTGgccGGAGCCGTGgCCGGCCTGgccgaagac
M13-WT	EGQYKLTRKALSMYCL	GAGGGACAGTACAAGCTGACCCGGAAGGCCCTGAGCATGTACTGCCTG
M13	VFQAALTAAALSAAED	gtcttcCAGgccgccCTGACCgccgccGCCCTGAGCgccgccgaagac
M14-WT	DKKRANDNEGTNPKRH	GATAAGAAGAGAGCTAACGACAATGAGGGCACAAATCCCAAGCGGCAC
M14	VFAAAAAAAGTAPAED	gtcttcgccgccGCTgccgccgccgccGGCACAgccCCCgccgaagac
M15-WT	KSIVFSVSDYGKLYVL	AAGAGCATCGTGTTCTCCGTGTCTGACTACGGCAAGCTGTACGTGCTG
M15	VFIVFAVAAAGALAED	gtcttcATCGTGTTCgCCGTGgccGcCgccGGCgccCTGgcCgaagac
M16-WT	DDAEFLGRICEYFMPH	GACGATGCCGAATTCCTGGGCCGGATCTGCGAATACTTCATGCCCCAC
M16	VFAAFLGAICAAFAED	gtcttcGCCGccTTCCTGGGCgccATCTGCGccgccTTCgccgaagac
M17-WT	EKGKIRYHTVYEKGFR	GAAAAGGGCAAGATCCGGTACCACACAGTGTACGAAAAGGGCTTTAGA
M17	VFGAIAAATVYAAGED	gtcttcGGCgccATCgccgccgccACAGTGTACgccgccGGCgaagac
M17V1	EAAAIRYHTVYEAAFR	GAAgccgccgccATCCGGTACCACACAGTGTACGAAgccgccTTTAGA
M17V2	EKGKARYAAAYEKGAR	GAAAAGGGCAAGgccCGGTACgccgccgccTACGAAAAGGGCgccAGA
M17V3	AKGKIAYHTVYAKGFA	gccAAGGGCAAGATCgccTACCACACAGTGTACgccAAGGGCTTTgcc
M17-Y672A	EKGKIRAHTVYEKGFR	GAAAAGGGCAAGATCCGGgccCACACAGTGTACGAAAAGGGCTTTAGA
M17-Y676A	EKGKIRYHTVAEKGFR	GAAAAGGGCAAGATCCGGTACCACACAGTGgccGAAAAGGGCTTTAGA
M17RR	EKGKIAYHTVYEKGFA	GAAAAGGGCAAGATCgccTACCACACAGTGTACGAAAAGGGCTTTgcc
M17KKK	EAGAIRYHTVYEAGFR	GAAgccGGCgccATCCGGTACCACACAGTGTACGAAgccGGCTTTAGA
M17EE	AKGKIRYHTVYAKGFR	GccAAGGGCAAGATCCGGTACCACACAGTGTACGccAAGGGCTTTAGA
M17YYF	EKGKIRAHTVAEKGAR	GAAAAGGGCAAGATCCGGgcCCACACAGTGgcCGAAAAGGGCgccAGA
M17GITG	EKAKARYHAVYEKAFR	GAAAAGGcCAAGgcCCGGTACCACgCgGTGTACGAAAAGGcCTTTAGA

M17-F680A	EKGKIRYHTVYEKGAR	GAAAAGGGCAAGATCCGGTACCACACAGTGTACGAAAAGGGCgccAGA
M17GG	EKAKIRYHTVYEKAFR	GAAAAGGcCAAGATCCGGTACCACACAGTGTACGAAAAGGcCTTTAGA
M17-I670A	EKGKARYHTVYEKGFR	GAAAAGGGCAAGgcCGGTACCACACAGTGTACGAAAAGGGCTTTAGA
M17-T674A	EKGKTRYHAVYEKGFR	GAAAAGGGCAAGACCCGGTACCACgCgGTGTACGAAAAGGGCTTTAGA
M17YY	EKGKIRAHTVAEKGFR	GAAAAGGGCAAGATCCGGgcCCACACAGTGgcCGAAAAGGGCTTTAGA
M18-WT	AYNDLQKKCVEAVLAF	GCATACAACGACCTGCAGAAGAAGTGCGTGGAGGCCGTGCTGGCTTTC
M18	VFAALAAACVAAVLED	gtcttcgcCGcCCTGgccgccgccTGCGTGgccGCCGTGCTGgaagac
M18V1	AYAALQAACAEAVLAF	GCATACgccgccCTGCAGgccgccTGCgccGAGGCCGTGCTGGCTTTC
M18V2	AYNDAAKKAVEAAAAF	GCATACAACGACgccgccAAGAAGgccGTGGAGGCCgccgccGCTTTC
M18V3	VYNDLQKKCVAVVLVA	gtgTACAACGACCTGCAGAAGAAGTGCGTGgccgtgGTGCTGgtggcc
M18-Y683A	AANDLQKKCVEAVLAF	GCAgccAACGACCTGCAGAAGAAGTGCGTGGAGGCCGTGCTGGCTTTC
M19-WT	GAHYIDFREILAQTMC	GGCGCCCACTACATCGACTTCCGGGAGATCCTGGCCCAGACCATGTGC
M19	VFAAIAFAAILAQAED	GtcttcgcCgcCATCGcCTTCgccGccATCCTGGCCCAGgCCgaagaC
M20-WT	KEAEKTAVNKVRRAFF	AAGGAGGCCGAAAAGACCGCAGTGAACAAGGTGAGACGCGCCTTCTTC
M20	VFAAAAAVAAVRRAED	gtcttcGCCgccgccgCCGCAGTGgccgccGTGAGACGCGCCgaagac
M21-WT	HHLKFVIDEFGLFSD	CACCACCTGAAGTTCGTGATTGACGAGTTCGGCCTGTTCAGCGAC
M21	VFLAFVIAAAGLAED	gtcttcCTGgccTTCGTGATTgccgccgccGGCCTGgcCgaagac

Supplementary Table 4. gRNA sequences used in this study.

Cas13 orthologs	Name	gRNA sequence	Figure
RfxCas13d	Cas13d mCherry gRNA-g1	ACTTGATGTTGACGTTGTAGGCGCCGGGCA	1b, d; S1a-b
RfxCas13d	Cas13d mCherry gRNA-g2	CACGTAGGCCTTGGAGCCGTACATGAACTG	1b, d; S1a-b
RfxCas13d	Cas13d mCherry gRNA-g3	GCAGCTTCACCTTGTAGATGAACTCGCCGT	1b, d; S1a-b
RfxCas13d	Cas13d nontarget gRNA-NT	CGTCTGGCCTTCCTGTAGCCAGCTTTCATC	1b, d, f; S1a-b; S2a-f; 3b-e
LwaCas13a	Cas13a mCherry gRNA-g1	ACTTGATGTTGACGTTGTAGGCGCCGGGCA	1b, e; S1c-d
LwaCas13a	Cas13a mCherry gRNA-g2	CACGTAGGCCTTGGAGCCGTACATGAACTG	1b, e; S1c-d
LwaCas13a	Cas13a mCherry gRNA-g3	GCAGCTTCACCTTGTAGATGAACTCGCCGT	1b, e; S1c-d
LwaCas13a	Cas13a nontarget gRNA-NT	CGTCTGGCCTTCCTGTAGCCAGCTTTCATC	1b, e; S1c-d
RfxCas13d	Human RPL4 Cas13d gRNA-g1	GTTGTGTTCACTCTACGATGCCAACGGCGC	1c, f; S2a; 3b-f, h-i; 5b-f; S20d,e; S10a,f
RfxCas13d	Human RPL4 Cas13d gRNA-g2	CTTTAGACATGACCAGTGCTGGTAGGGCTG	1c, f; S2a; 3b-f, i
RfxCas13d	Human RPL4 Cas13d gRNA-g3	GAAGTTCAGGAACTTCCTCAATACGATGAC	1c, f; S2a; 3b-f, i; 4e-h, j; S12; S13a,d; S14b
RfxCas13d	Human RPL4 Cas13d gRNA-g4	GGTTTCTCATTTTGCCTTTGCCAGCTCTCA	1c, f; S2a
RfxCas13d	Human PKM Cas13d gRNA-g1	GGCTCCCTTCTTCAGCTCCACCTCTGCAGT	S2b, d-e
RfxCas13d	Human PKM Cas13d gRNA-g2	GTAGGCGTTATCCAGCGTGATTTTGAGAGT	S2b, d-e
RfxCas13d	Human PKM Cas13d gRNA-g3	GTTCTTGTAGTCCAGCCACAGGATGTTCTC	S2b, d-e
RfxCas13d	Human PKM Cas13d gRNA-g4	GTAGATCTTGCTGCCCACTTCCACCACCTT	S2b, d-e

RfxCas13d	Human PFN1 Cas13d gRNA-g1	GGTCTTTGCCAACCAGGACACCCACCTCAG	S2c-d, f
RfxCas13d	Human PFN1 Cas13d gRNA-g2	GCAGTGAGTCCCGGATCACCGAACATTTCT	S2c-d, f
RfxCas13d	Human PFN1 Cas13d gRNA-g3	GTGCTCTTGGTACGAAGATCCATGCTAAAT	S2c-d, f
RfxCas13d	Human PFN1 Cas13d gRNA-g4	GTCAGTCTTGGTGACAGTGACATTGAAGGT	S2c-d, f
RfxCas13d	Cas13d EGFP gRNA-g1	GTCCTCCTTGAAGTCGATGCCCTTCAGCTC	2d-f; 3b; S20b,c; S6
RfxCas13d	Cas13d EGFP gRNA-g2	AGCACTGCACGCCGTAGGTCAGGGTGGTCA	S20b,c; S6; S7
RfxCas13d	Cas13d EGFP gRNA-g3	GCAGGACCATGTGATCGCGCTTCTCGTTGG	S20b,c; S6
RfxCas13d	Cas13d EGFP gRNA-g4	GAACTTCAGGGTCAGCTTGCCGTAGGTGGC	S20b,c; S6
RfxCas13d	Human BSG Cas13d gRNA-g1	ACTCGTAAGTGCCCGTGTCCTCCTCCACGA	3b-e
RfxCas13d	Human BSG Cas13d gRNA-g2	GTCATTCAAGGAGCAGGTGAGGAGTATCTT	3b-e
RfxCas13d	Human BSG Cas13d gRNA-g3	TCTGACGACTTCACAGCCTTCACTCTGGGA	3b-e
RfxCas13d	Human BSG Cas13d gRNA-g4	CTTGTCCTCAGAGTCAGTGATCTTGTACCA	3b-e
RfxCas13d	Human BSG Cas13d gRNA-g5	GTGCAGAGCCGGCGTCGTCATCATCCAGGA	3b-e
RfxCas13d	Human CA2 Cas13d gRNA-g1	AGCACAATCCAGGTCACACATTCCAGAAGA	3b-e, g, i; 4e-h; S12; S13b,e,g
RfxCas13d	Human CA2 Cas13d gRNA-g2	TATGCCAGTGCTCAGGTCCGTTGTGTTTGC	3b-e, g, i
RfxCas13d	Human CA2 Cas13d gRNA-g3	CAGGGAAGTTGCTTGATCATAGGAAACAGA	3b-e, g, i
RfxCas13d	Human CA2 Cas13d gRNA-g4	AAACTGAATCAATCTGTAAGTGCCATCCAG	3b-e, g, i
RfxCas13d	Human CA2 Cas13d gRNA-g5	TTTATCCACAGTATGCTCTGAACCTTGTCC	3b-e, g, i
RfxCas13d	Human CA2 Cas13d gRNA-g6	GAATCCAGCACATCAACAACTTTCTGAAGG	3b-e, g, i
RfxCas13d	Human CA2 Cas13d gRNA-g7	GCGCCAGTTGTCCACCATCAGTTCTTCGGG	3b-e, g, i
RfxCas13d	Human CKB Cas13d gRNA-g1	GCAGCCGCTTAAGCACCTCCGAGAACTTCT	3b-e; h-i

RfxCas13d	Human CKB Cas13d gRNA-g2	TGGCCCGGGTTGTCCACGCCTGTCTGGATG	3b-e
RfxCas13d	Human CKB Cas13d gRNA-g3	CGCCCGCCACGCAGCCCACGGTCATGATGT	3b-e
RfxCas13d	Human CKB Cas13d gRNA-g4	CTGCTGCTGCTCCGCCTCCGTCATGCTCTT	3b-e
RfxCas13d	Human ENO1 Cas13d gRNA-g1	ATATAGCGAGTCTTATCATTGTCCCGGAGC	3b-e
RfxCas13d	Human ENO1 Cas13d gRNA-g2	ATCATCAGTTTGTCAATCTTCTCTTGTTCT	3b-e
RfxCas13d	Human ENO1 Cas13d gRNA-g3	CGCCATTGATGACATTGAACGCCGGGACTG	3b-e
RfxCas13d	Human ENO1 Cas13d gRNA-g4	TCTTTCCCATATTTCTCCTTGATGACATTC	3b-e
RfxCas13d	Human ENO1 Cas13d gRNA-g5	CCTTATCAGTGTAGCCAGCTTTCCCAATAG	3b-e
RfxCas13d	Human ENO1 Cas13d gRNA-g6	ACTACCTGGATTCCTGCACTGGCTGTGAAC	3b-e
RfxCas13d	Human LENG8 Cas13d gRNA-g1	CCACCATGCTGTACTGAGAAGACCAATCTG	3b-e
RfxCas13d	Human LENG8 Cas13d gRNA-g2	GCAGGTTCGGTGTAGGTGTGTGGCCCATAG	3b-e
RfxCas13d	Human LENG8 Cas13d gRNA-g3	TGCGGTCCTTGTCCTCCTCCGACTCACAGG	3b-e
RfxCas13d	Human LENG8 Cas13d gRNA-g4	TTGTCCTTCATGAAGACGTTGCGGTTGCCA	3b-e
RfxCas13d	Human LENG8 Cas13d gRNA-g5	CCTCACACTCCAGCGCCGCCATCTTCTTTC	3b-e
RfxCas13d	Human LENG8 Cas13d gRNA-g6	AACGCGTAGTCCTGCTTCTCTTTCCAGTGG	3b-e
RfxCas13d	Human NEFM Cas13d gRNA-g1	GACCACGACTGCGAGCGGAAGCCACTGGAC	3b-e
RfxCas13d	Human NEFM Cas13d gRNA-g2	TTATAGGAGGAGGACACGGTGCTGGGCGAG	3b-e
RfxCas13d	Human NEFM Cas13d gRNA-g3	ATCTCCGCCTCAATCTCCTTATTCTGCTGC	3b-e
RfxCas13d	Human NEFM Cas13d gRNA-g4	CTCCACCTTGACCAGCGACGCCTCCTCGAT	3b-e
RfxCas13d	Human NEFM Cas13d gRNA-g5	CTTGGCGTAGCGGCATTTGAACCACTCTTC	3b-e
RfxCas13d	Human NEFM Cas13d gRNA-g6	TCCTGCAAATGTGCTAAATCTAGTCTCTTC	3b-e
RfxCas13d	Human PEBP1 Cas13d gRNA-g1	TCAGCACTTTGCCCAGCTCGTCCACCGCCG	3b-e
RfxCas13d	Human PEBP1 Cas13d gRNA-g2	TATTCTTAACCTGGGTGGGCGTCAGCACTT	3b-e

RfxCas13d	Human PEBP1 Cas13d gRNA-g3	CTTCCCTGAATCAAGACCATCCCACGAAAT	3b-e
RfxCas13d	Human PEBP1 Cas13d gRNA-g4	ATGCCATTCTCTGTATTTGGGATCCTTCCT	3b-e
RfxCas13d	Human PEBP1 Cas13d gRNA-g5	ATGTTGACCACCAGGAAATGATGCCATTCT	3b-e
RfxCas13d	Human PEBP1 Cas13d gRNA-g6	CCACTGCTGATGTCATTGCCCTTCATGTTG	3b-e
RfxCas13d	Human PPIA Cas13d gRNA-g1	AAACACCACATGCTTGCCATCCAACCACTC	3b-e, h-i; 4a, c, e-i; S20d,e; S10b,e; S14a
RfxCas13d	Human PPIA Cas13d gRNA-g2	ATGCCAGGACCCGTATGCTTTAGGATGAAG	3b-e, h-i; 4b, d, e-i; S10c; S12
RfxCas13d	Human PPIA Cas13d gRNA-g3	CAAACAGCTCAAAGGAGACGCGGCCCAAGG	3b-e
RfxCas13d	Human PPIA Cas13d gRNA-g4	AACCCTTATAACCAAATCCTTTCTCTCCAG	3b-e
RfxCas13d	Human PPIA Cas13d gRNA-g5	CACCCTGACACATAAACCCTGGAATAATTC	3b-e
RfxCas13d	Human PPIA Cas13d gRNA-g6	CCTCCACAATATTCATGCCTTCTTTCACTT	3b-e
RfxCas13d	Human RPS5 Cas13d gRNA-g1	ACACATCCACAGCCTGTCGTCTCACAGTCC	3b-e, h-i; S20d,e; S10d,g
RfxCas13d	Human RPS5 Cas13d gRNA-g2	ATACTTCTCCTTCACTGCAATGTAATCCTG	3b-e
RfxCas13d	Human RPS5 Cas13d gRNA-g3	GAGTTAGTGAGGCGCTCCACAATGGGACAC	3b-e
RfxCas13d	Human ANXA4 Cas13d gRNA-g1	TTAGGCAGCCCTCATCAGTGCCGGCTCCCT	3b-e, h-i; S20d,e
RfxCas13d	Human B4GALNT1 Cas13d gRNA-g1	CCTCCTGACCAGAAGCTGCCTGAAGGCTCA	3b-e, h-i; S20d,e
RfxCas13d	Human EGFR Cas13d gRNA-g1	GTTTCTGGCAGTTCTCCTCTCCTGCACCCC	3b-e, h-i
RfxCas13d	Human EZH2 Cas13d gRNA-g1	CAAATGCTGGTAACACTGTGGTCCACAAGG	3b-e, h-i
RfxCas13d	Human NF2 Cas13d gRNA-g1	CTTGGCCTGGACGGCGTAAGAAGCCAGGAG	3b-e, h-i
RfxCas13d	Human NFKB1 Cas13d gRNA-g1	CTCATAGTTGTCCATAAGTGTTTTGGAAGG	3b-e

RfxCas13d	Human NRAS Cas13d gRNA-g1	CTGTCTGGTCTTGGCTGAGGTTTCAATGAA	3b-e, h-i; S20d,e
RfxCas13d	Human PPARG Cas13d gRNA-g1	CATTATGAGACATCCCCACTGCAAGGCATT	3b-e, h-i; 4e-h; S12; S13c,f,h
RfxCas13d	Human PPIB Cas13d gRNA-g1	GGCCCGTAGTGCTTCAGTTTGAAGTTCTCA	3b-e
RfxCas13d	Human RAF1 Cas13d gRNA-g1	CTCAATCATCCTGCTGTCCACAGGCAGGGT	3b-e
RfxCas13d	Human SMARCA1 Cas13d gRNA-g1	CTGGTGAGGATTCCAGTCGCTGTCAAAAAT	3b-e, h-i; S20d,e
RfxCas13d	Human STAT3 Cas13d gRNA-g1	ATCACAATTGGCTCGGCCCCCATTCCCACA	3b-e, h-i
RfxCas13d	Human RPL4 Cas13d gRNA-g5	TCAGTCCAAATGCAGAAACGTCCCACATGC	3b-f, h-i (as g4 in 3f)
RfxCas13d	Human RPL4 Cas13d gRNA-g6	CAATACGATGACCTTTAGACATGACCAGTG	3b-f, h-i (as g5 in 3f)
RfxCas13d	Mouse Tyr Cas13d gRNA-g1	GGAACCTCTGCCTGAAAGCTGGCCGCAGGG	5h-k; S15b-c
RfxCas13d	Mouse Tyr Cas13d gRNA-g2	GGGGAACTGAGGTCCAGATGGTGCACTGGA	5h-k; S15b-c
RfxCas13d	Mouse Pcsk9 Cas13d gRNA-g1	GTGGGTGCCGTGGCTGTCACACTTGCTCGC	S15d-g
RfxCas13d	Mouse Pcsk9 Cas13d gRNA-g2	CAGCATCCCGGCCGCTGACCACACCTGCCA	S15d-g
Cas13X	Cas13X EGFP gRNA-g1	AGCACTGCACGCCGTAGGTCAGGGTGGTCA	6c-d; S16
Cas13X	Cas13X EGFP gRNA-g2	GTCCTCCTTGAAGTCGATGCCCTTCAGCTC	S16
Cas13X	Cas13X EGFP gRNA-g3	TCGCCGTCCAGCTCGACCAGGATGGGCACC	S16
Cas13X	Cas13X EGFP gRNA-g4	TTCGGGCATGGCGGACTTGAAGAAGTCGTG	S16
Cas13X	Human ANXA4 Cas13X gRNA-g1	TTAGGCAGCCCTCATCAGTGCCGGCTCCCT	6h; S20d,e; S18
Cas13X	Human B4GALNT1 Cas13X gRNA-g1	CCTCCTGACCAGAAGCTGCCTGAAGGCTCA	6h; S20d,e; S18

Cas13X	Human EGFR Cas13X gRNA-g1	GTTTCTGGCAGTTCTCCTCTCCTGCACCCC	6h; S18
Cas13X	Human EZH2 Cas13X gRNA-g1	CAAATGCTGGTAACACTGTGGTCCACAAGG	6h; S18
Cas13X	Human NF2 Cas13X gRNA-g1	CTTGGCCTGGACGGCGTAAGAAGCCAGGAG	6h; S18
Cas13X	Human NRAS Cas13X gRNA-g1	CTGTCTGGTCTTGGCTGAGGTTTCAATGAA	6h; S20d,e; S18
Cas13X	Human PPARG Cas13X gRNA-g1	CATTATGAGACATCCCCACTGCAAGGCATT	6h; S18
Cas13X	Human PPIB Cas13X gRNA-g1	GGCCCGTAGTGCTTCAGTTTGAAGTTCTCA	S18
Cas13X	Human SMARCA1 Cas13X gRNA-g1	CTGGTGAGGATTCCAGTCGCTGTCAAAAAT	6h; S20d,e
Cas13X	Human STAT3 Cas13X gRNA-g1	ATCACAATTGGCTCGGCCCCCATTCCCACA	6h; S18
Cas13X	Human RPL4 Cas13X gRNA-g1	GTTGTGTTCACTCTACGATGCCAACGGCGC	6h-l; s20d,e; S18
Cas13X	Human RPL4 Cas13X gRNA-g2	GAAGTTCAGGAACTTCCTCAATACGATGAC	S18
Cas13X	Human PPIA Cas13X gRNA-g1	AAACACCACATGCTTGCCATCCAACCACTC	6h; S20d,e; S18
Cas13X	Human PPIA Cas13X gRNA-g2	ATGCCAGGACCCGTATGCTTTAGGATGAAG	S18
Cas13X	Human RPS5 Cas13X gRNA-g1	ACACATCCACAGCCTGTCGTCTCACAGTCC	6h; S20d,e; S18
Cas13X	Mouse Tyr Cas13X gRNA-g1	GGAACCTCTGCCTGAAAGCTGGCCGCAGGG	S19
Cas13X	Mouse Tyr Cas13X gRNA-g2	GGGGAACTGAGGTCCAGATGGTGCACTGGA	S19

Cas13X	Cas13X EGFP g1	GTCCTCCTTGAAGTCGATGCCCTTCAGCTC	S20b,c
Cas13X	Cas13X EGFP g2	AGCACTGCACGCCGTAGGTCAGGGTGGTCA	S20b,c
Cas13X	Cas13X EGFP g3	GCAGGACCATGTGATCGCGCTTCTCGTTGG	S20b,c
Cas13X	Cas13X EGFP g4	GAACTTCAGGGTCAGCTTGCCGTAGGTGGC	S20b,c
PspCas13b	PspCas13b EGFP g1	GTCCTCCTTGAAGTCGATGCCCTTCAGCTC	S20b,c
PspCas13b	PspCas13b EGFP g2	AGCACTGCACGCCGTAGGTCAGGGTGGTCA	S20b,c
PspCas13b	PspCas13b EGFP g3	GCAGGACCATGTGATCGCGCTTCTCGTTGG	S20b,c
PspCas13b	PspCas13b EGFP g4	GAACTTCAGGGTCAGCTTGCCGTAGGTGGC	S20b,c
PspCas13b	Human ANXA4 PspCas13b gRNA-g1	TTAGGCAGCCCTCATCAGTGCCGGCTCCCT	S20d,e
PspCas13b	Human B4GALNT1 PspCas13b gRNA-g1	CCTCCTGACCAGAAGCTGCCTGAAGGCTCA	S20d,e
PspCas13b	Human NRAS PspCas13b gRNA-g1	CTGTCTGGTCTTGGCTGAGGTTTCAATGAA	S20d,e
PspCas13b	Human SMARCA1 PspCas13b gRNA-g1	CTGGTGAGGATTCCAGTCGCTGTCAAAAAT	S20d,e
PspCas13b	Human RPL4 PspCas13b gRNA-g1	GTTGTGTTCACTCTACGATGCCAACGGCGC	S20d,e
PspCas13b	Human PPIA PspCas13b gRNA-g1	AAACACCACATGCTTGCCATCCAACCACTC	S20d,e
PspCas13b	Human RPS5 PspCas13b gRNA-g1	ACACATCCACAGCCTGTCGTCTCACAGTCC	S20d,e
RanCas13b	RanCas13b EGFP g1	GTCCTCCTTGAAGTCGATGCCCTTCAGCTC	S20b,c
RanCas13b	RanCas13b EGFP g2	AGCACTGCACGCCGTAGGTCAGGGTGGTCA	S20b,c
RanCas13b	RanCas13b EGFP g3	GCAGGACCATGTGATCGCGCTTCTCGTTGG	S20b,c

RanCas13b	RanCas13b EGFP g4	GAACTTCAGGGTCAGCTTGCCGTAGGTGGC	S20b,c
RanCas13b	Human ANXA4 RanCas13b gRNA-g1	TTAGGCAGCCCTCATCAGTGCCGGCTCCCT	S20d,e
RanCas13b	Human B4GALNT1 RanCas13b gRNA-g1	CCTCCTGACCAGAAGCTGCCTGAAGGCTCA	S20d,e
RanCas13b	Human NRAS RanCas13b gRNA-g1	CTGTCTGGTCTTGGCTGAGGTTTCAATGAA	S20d,e
RanCas13b	Human SMARCA1 RanCas13b gRNA-g1	CTGGTGAGGATTCCAGTCGCTGTCAAAAAT	S20d,e
RanCas13b	Human RPL4 RanCas13b gRNA-g1	GTTGTGTTCACTCTACGATGCCAACGGCGC	S20d,e
RanCas13b	Human PPIA RanCas13b gRNA-g1	AAACACCACATGCTTGCCATCCAACCACTC	S20d,e
RanCas13b	Human RPS5 RanCas13b gRNA-g1	ACACATCCACAGCCTGTCGTCTCACAGTCC	S20d,e

Supplementary Table 5. Primers used for qPCR reactions.

Name	primer sequence	Figure
Human GAPDH-F	GTGGACCTGACCTGCCGTCT	1d-f; 3f-j; 5c; 6h-l; S20d,e; S10e-g
Human GAPDH-R	GGAGGAGTGGGTGTCGCTGT	1d-f; 3f-j; 5c; 6h-l; S20d,e; S10e-g
mCherry-F	TCATCAAGGAGTTCATGCGC	1d-e
mCherry-R	CCACCCTTGGTCACCTTCAG	1d-e
EGFP-F	AAACGGCCACAAGTTCAGCG	1d-f
EGFP-R	GAAGTCGTGCTGCTTCATGTG	1d-f
Human RPL4-F	GCCTGCTGTATTCAAGGCTC	1f; 3f, i; 5c; 6h-l; S20d,e; S10f
Human RPL4-R	GGTTGGTGCAAACATTCGGC	1f; 3f, i; 5c; 6h-l; S20d,e; S10f
Human ANXA4-F	GATCAGGACAGCCTACAAGAG	3h-i; 6h; S20d,e
Human ANXA4-R	GCTTATGCGCCGGATCTCCT	3h-i; 6h; S20d,e
Human B4GALNT1-F	GTTCCAGGCCTTTCTGTCGA	3h-i; 6h; S20d,e
Human B4GALNT1-R	CAGTCAGGTTCACCTGGTAT	3h-i; 6h; S20d,e
Human EGFR-F	ACTTCCAGAACCACCTGGGCA	3h-i; 6h
Human EGFR-R	CTGGTTGTGGCAGCAGTCACT	3h-i; 6h
Human EZH2-F	GCGGAAGAACACAGAAACAGC	3h-i; 6h
Human EZH2-R	TTCTGCGGCCTCCTGGACGTT	3h-i; 6h
Human NF2-F	GCTGAAGAGGAGCTGGTTCAG	3h-i; 6h
Human NF2-R	TCCTCCCACATTTCCGGAGTC	3h-i; 6h
Human NRAS-F	GTACCTATGGTGCTAGTGGG	3h-i; 6h; S20d,e
Human NRAS-R	CTGAGTCCCATCATCACTGC	3h-i; 6h; S20d,e
Human PPARG-F	GTGATCTTAACTGTCGGATCC	3h-i; 6h
Human PPARG-R	ACAAATGTTTTGCCAGGGCC	3h-i; 6h
Human SMARCA1-F	CATCTTCCTGCTCAGCACCC	3h-i; 6h; S20d,e
Human SMARCA1-R	GGATCTTCTCCTCCACGCTG	3h-i; 6h; S20d,e
Human STAT3-F	GAATCCAACAACGGCAGCCT	3h-i; 6h
Human STAT3-R	GGAGTGGGTCTCTAGGTCAA	3h-i; 6h
Human PPIA-F	TCTGCACTGCCAAGACTGAG	3h-i; 6h; S20d,e; S10e
Human PPIA-R	TCGAGTTGTCCACAGTCAGC	3h-i; 6h; S20d,e; S10e
Human CKB-F	GCTGCGACTTCAGAAGCGA	3h-i
Human CKB-R	GGCATGAGGTCGTCGATGG	3h-i
Human RPS5-F	GAAGTGGAGCACCGATGATG	3h-i; 6h; S20d,e; S10g
Human RPS5-R	GCACAGTCATGAGCTTCTTGC	3h-i; 6h; S20d,e; S10g
Human CA2-F	GTGGCCTCCTTCCTGAATCC	3g-i
Human CA2-R	TTCTTCAGTGGCTGAGCTGG	3g-i
Mouse En2SA-F	TACTGGTTGCTGGAGTCTAGCTACTT	5k
Mouse En2SA-R	CCCCAGTATCTGCAACCTCAA	5k
Mouse Actb-F	TTCAACACCCCAGCCATGTA	5k
Mouse Actb-R	TGTGGTACGACCAGAGGCATAC	5k
Cas13d/hfCas13d-F	GGACAGCGCCAAGATCGCTG	5k

Cas13d/hfCas13d-R	AGCGATTGGCTCTCCCATCC	5k
Mouse Pcsk9-F	GCCCATCGGGAGATTGAGG	S15d
Mouse Pcsk9-R	TTCCCTTGACAGTTGAGCACA	S15d
Mouse Gapdh-F	CCGTAGACAAAATGGTGAAGGT	5j,S15d; S19e
Mouse Gapdh-R	CGTGAGTGGAGTCATACTGGAA	5j,S15d; S19e
Mouse Tyr-F	CAAAGGGGTGGATGACCGTG	5j,S19e
Mouse Tyr-R	AACTTACAGTTTCCGCAGTTGA	5j,S19e

Supplementary Table 6. Sequences of target ssRNA, nontarget ssRNA, gRNAs and the corresponding primers used in *in vitro* cleavage assays.

Name	Sequence	Figure
target ssRNA	GGGUUACAGUUAAGGCUCUUUAUUAAAAGAGCCUUAAUU GUAAAACGCCUAUACGGUGAGGGUAUGUACGUUUGGGUU UGUCCAGCCUAAACCUCUACGCCAGAAAUGGCACCUUCA UUGUGGGUUAGGACAAUUUAAGCUCGGUACCCGGGGAUC CUCUAGAAAUAUGGAUUACUUGGUAGAACAGCAAUCUAC UCGACCUGCAGGCAUGCAAGC	2g-i; 6e-g; S9; S17
nontarget ssRNA	GGACAACUUCGACGAGCUCUACAGUUACAGUUAAGGCUC UUUAUUAAAAGAGCCUUAAUUGUAAAACGCCUAUACGGU GAGGGUAUGUACGUUUGGGUUUGUCCAGCCUAAACCUCU ACGCCAGAAAUGGCACCUUCAUUGUGGGUUAGGACAAUU UAA	2g-i; 6e-g; S9; S17
Cas13d-gRNA 1 (**spacer**)	GGGGG CAAGUAAACCCCUACCAACUGGUCGGGGUUUGAAAC **UAGAUUGCUGUUCUACCAAGUAAUCCAU**	S9b
Cas13d-gRNA 2 (**spacer**)	GGGGG CAAGUAAACCCCUACCAACUGGUCGGGGUUUGAAAC **CAUAUUUCUAGAGGAUCCCCGGGUACCG**	2g-i
Cas13d-gRNA 3 (**spacer**)	GGGGG CAAGUAAACCCCUACCAACUGGUCGGGGUUUGAAAC **AGUAAUCCAUAUUUCUAGAGGAUCCCCG**	S9c
Cas13d-gRNA 4 (**spacer**)	GGGGG CAAGUAAACCCCUACCAACUGGUCGGGGUUUGAAAC **GUUCUACCAAGUAAUCCAUAUUUCUAGA**	S9d
Cas13d-gRNA 5 (**spacer**)	GGGGG CAAGUAAACCCCUACCAACUGGUCGGGGUUUGAAAC **GAGUAGAUUGCUGUUCUACCAAGUAAUC**	S9e
Cas13X-gRNA 1 (**spacer**)	GGGGG **UAGAUUGCUGUUCUACCAAGUAAUCCAU** GCUGGAGCAGCCCCCGAUUUGUGGGGUGAUUACAGC	6e-g
Cas13X-gRNA 3 (**spacer**)	GGGGG **AGUAAUCCAUAUUUCUAGAGGAUCCCCG** GCUGGAGCAGCCCCCGAUUUGUGGGGUGAUUACAGC	S17a
Cas13X-gRNA 5 (**spacer**)	GGGGG **GAGUAGAUUGCUGUUCUACCAAGUAAUC** GCUGGAGCAGCCCCCGAUUUGUGGGGUGAUUACAGC	S17b
Primers for target RNA templates		
Target-primer-1	taatacgactcactataggGTTACAGTTAAGGCTCTTTATTAAAAGAG C	
Target-primer-2	GTGGGTTAGGACAATTTAAGCTCGGTACCCGGGGATCCTCT AGAAATATGGATTACTTG	
Target-primer-3	GCTTGCATGCCTGCAGGTCGAGTAGATTGCTGTTCTACCAA GTAATCCATATTTCTAG	
Target-primer-4	GTTAAGGCTCTTTATTAAAAGAGCCTTAATTGTAAAACGCC TATACGGTGAGGGTATG	
Target-primer-5	CGCCTATACGGTGAGGGTATGTACGTTTGGGTTTGTCCAGC CTAAACCTCTACG	
Target-primer-6	CCAGCCTAAACCTCTACGCCAGAAATGGCACCTTCATTGTG GGTTAGGACAATTTAAGC	
Primers for nontarget RNA templates		

Nontarget-primer-1	taatacgactcactataggACAACTTCGACGAGCTCTACAGTTACAGT TAAGGCTCTTTA	
Nontarget-primer-2	TTAAATTGTCCTAACCCACAATGAAGGTGC	
Primers for Cas13d gRNA templates		
Cas13d-T7-F	taatacgactcactataggGGGcaagtaaacccctaccaac	
Cas13d-gRNA1-R	ATGGATTACTTGGTAGAACAGCAATCTA gtttcaaaccccgaccagttggtaggggtttacttgCCCcctatagtgagtcgtatta	
Cas13d-gRNA2-R	CGGTACCCGGGGATCCTCTAGAAATATG gtttcaaaccccgaccagttggtaggggtttacttgCCCcctatagtgagtcgtatta	
Cas13d-gRNA3-R	CGGGGATCCTCTAGAAATATGGATTACT gtttcaaaccccgaccagttggtaggggtttacttgCCCcctatagtgagtcgtatta	
Cas13d-gRNA4-R	TCTAGAAATATGGATTACTTGGTAGAAC gtttcaaaccccgaccagttggtaggggtttacttgCCCcctatagtgagtcgtatta	
Cas13d-gRNA5-R	GATTACTTGGTAGAACAGCAATCTACTC gtttcaaaccccgaccagttggtaggggtttacttgCCCcctatagtgagtcgtatta	
Primers for Cas13X gRNA templates		
Cas13X-T7-F	taatacgactcactatagg	
Cas13X-gRNA1-R	GCTGTAATCACCCCACAAATCGGGGGCTGCTCCAGC ATGGATTACTTGGTAGAACAGCAATCTACCCcctatagtgagtcgtatta	
Cas13X-gRNA3-R	GCTGTAATCACCCCACAAATCGGGGGCTGCTCCAGC CGGGGATCCTCTAGAAATATGGATTACTCCCcctatagtgagtcgtatta	
Cas13X-gRNA5-R	GCTGTAATCACCCCACAAATCGGGGGCTGCTCCAGC GATTACTTGGTAGAACAGCAATCTACTCCCCcctatagtgagtcgtatta	

Supplementary Note 1. Codes for predicting off-targets: offinder_cas13.sh

```
#! /usr/bin/sh
####input#####
ref=$1 ###label of reference, hg38 in our case
threads=$2 ### threads used for blastn
max_mismatch=$3 ###maximum mismatch, up to 10 in our test
db=$4 #feature considered for offtarget search, might be pre-mRNA, cdna or dna(whole genome), cdna and pre-mRNA are considered in our case
path_db=$5 #directory for blast index of the reference
path_refs=$path_db/${ref}
file_input=crRNAs.fasta ##reverse and complementary of sgRNAs

######output directory#####
path=`pwd`
path_out=$path/off_targets


####alignments of the crRNA sequence to references using blastn
mkdir -p $path/tmps
cd $path/tmps

len_crRNA=30
temp=$(echo "$len_crRNA-1"|bc)
targets_max=100000
evalue=100000
word_size=$(echo "$temp/$max_mismatch"|bc)
if (( $word_size < 4 ));then
        word_size=4
fi
identity=$(echo "100*($len_crRNA-$max_mismatch)/$len_crRNA"|bc)
penalty_mm=$(echo "-$max_mismatch"|bc)
reward=$max_mismatch

blastn -evalue $evalue -perc_identity $identity -penalty $penalty_mm -reward $reward -strand plus -num_threads $threads -word_size $word_size  -max_target_seqs $targets_max -ungapped  -query $file_input -db ${path_refs}/index_blast/$db/$ref -outfmt ' 6 qseqid sseqid pident length mismatch gapopen qstart qend sstart send qseq sseq evalue bitscore'|awk -v len=$len_crRNA -v m=$max_mismatch '{a=len-$4+$5; if(a<=m)print $0"\t"a}' >temp.blast.out

### reform the blast output ######
awk -v len=$len_crRNA '$4==len{print $1"\t"$2"\t"$3"\t"$4"\t"$5"\t"$9"\t"$10"\t"toupper($11)"\t"toupper($12)"\tID_"NR}' temp.blast.out >temp.blast.full.out
awk -v len=$len_crRNA '$4<len{sstart=$9-$7+1;send=$10+len-$8; print $1"\t"$2"\t"$3"\t"$4"\t"$15"\t"sstart"\t"send"\t"$11"\t"$12"\tID_"NR}' temp.blast.out >temp.blast.part.out

awk '{print $2"\t"$6-1"\t"$7"\t"$1"\t.\t"$10}'  temp.blast.part.out >temp.blast.part.bed
bedtools getfasta -name -s -fi ${path_refs}/$file_ref -tab -bed temp.blast.part.bed|awk -F ':' '{split($4,a,"");split(a[1],b,"(");split(b[1],c,"-");print $1"\t"$3"\t"c[1]+1"-"c[2]"\t"toupper(a[2]) }' >temp.blast.part.seq

awk '{print $1}' temp.blast.part.out >temp.names.list.part.forcrRNAs

seqtk subseq $file_input temp.names.list.part.forcrRNAs|awk 'BEGIN{RS=">"}{print $1"\t"$2;}' | tail -n+2 >temp.part.id-seq
awk -v OFS='\t' 'FILENAME==ARGV[1]{a[$1]=$2}FILENAME==ARGV[2]{$8=a[$1];print $0}' temp.part.id-seq temp.blast.part.out >temp.blast.part1.out
awk -v OFS='\t' 'FILENAME==ARGV[1]{a[$1":"$2":"$3]=$4}FILENAME==ARGV[2]{$9=a[$1":"$2":"$6"-"$7];print $0}' temp.blast.part.seq temp.blast.part1.out >temp.blast.part.reformed.out
```

```
cat temp.blast.full.out temp.blast.part.reformed.out|awk '{print $1"\t"$2":"$6"-"$7"\t"$5"\t"$8"\t"$9"\t"$10}'|awk
'{qseq=$4;sseq=$5;split(qseq,a,"");split(sseq,b,"");bb="";m=0;for(i=1;i<=length(a);i++){if(a[i]!=b[i]){b[i]=tolower(b[i]);m=
m+1};bb=bb""b[i]};print $1"\t"$2"\t"m"\t"$4"\t"bb"\t"$6}'|sort -k1,1 -k3n,3 >blast.reformed.out

echo -e "sgRNA_Source\tPos_Target\tMismatch\tcrRNA\tTargets" >temp.title
cat temp.title blast.reformed.out >${path_out}/crRNAs_offtargets_MM-${max_mismatch}_${db}_${ref}.txt

awk -F '\t' '{split($2,a,":");split(a[2],b,"-");print "chr"a[1]"\t"b[1]"\t"b[2]"\t.\t.\t"$6}'
blast.reformed.out >blast.reformed.out.bed

###annotating the off-targets using annotatePeaks.pl from HOMER#######
annotatePeaks.pl blast.reformed.out.bed hg38 |awk -F '\t' '{print
$2"\t"$3"\t"$4"\t"$5"\t"$8"\t"$9"\t"$10"\t"$15"\t"$16}' >temp.annos.txt
awk -F '\t' 'FILENAME==ARGV[1]{id=$1":"$2-1"-
"$3":"$4;a1[id]=$5;a2[id]=$6;a3[id]=$7;a4[id]=$8;a5[id]=$9}FILENAME==ARGV[2]{id1="chr"$2":"$6;print
"ID_"NR"\t"$0"\t"a1[id1]"\t"a2[id1]"\t"a3[id1]"\t"a4[id1]"\t"a5[id1]}' temp.annos.txt
blast.reformed.out >temp.blast.reformed.out.withanno
echo -e
"Aln_ID\tsgRNA_Source\tPos_Target\tMismatch\tcrRNA\tTargets\tAnno\tDetailedAnno\tDist2TSS\tNearestEnsembl\t
GeneName" >temp.title
cat temp.title  temp.blast.reformed.out.withanno >${path_out}/crRNAs_offtargets_MM-
${max_mismatch}_${db}_${ref}_withAnno.txt
cd $path
rm -r $path/tmps
```