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Supplementary information

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Discovery and engineering of retrons for precise genome editing

In the format provided by the authors and unedited

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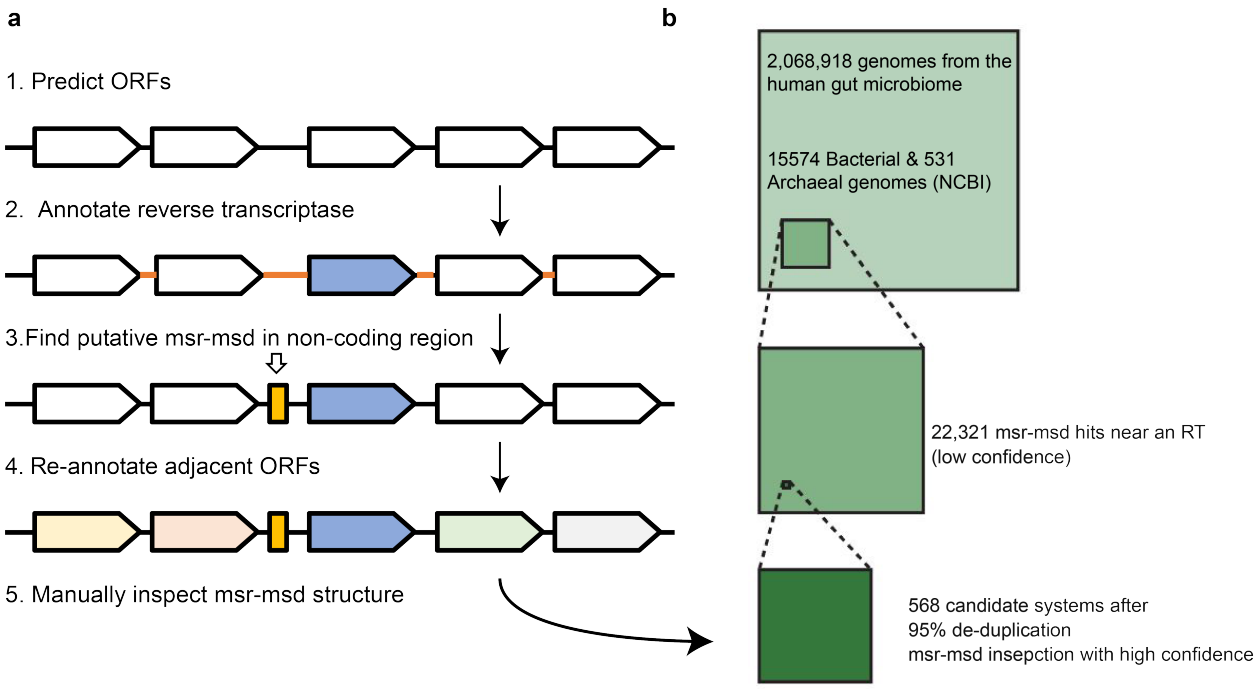
Supplemental Notes

Prioritization of retron-RTs for experimental characterization

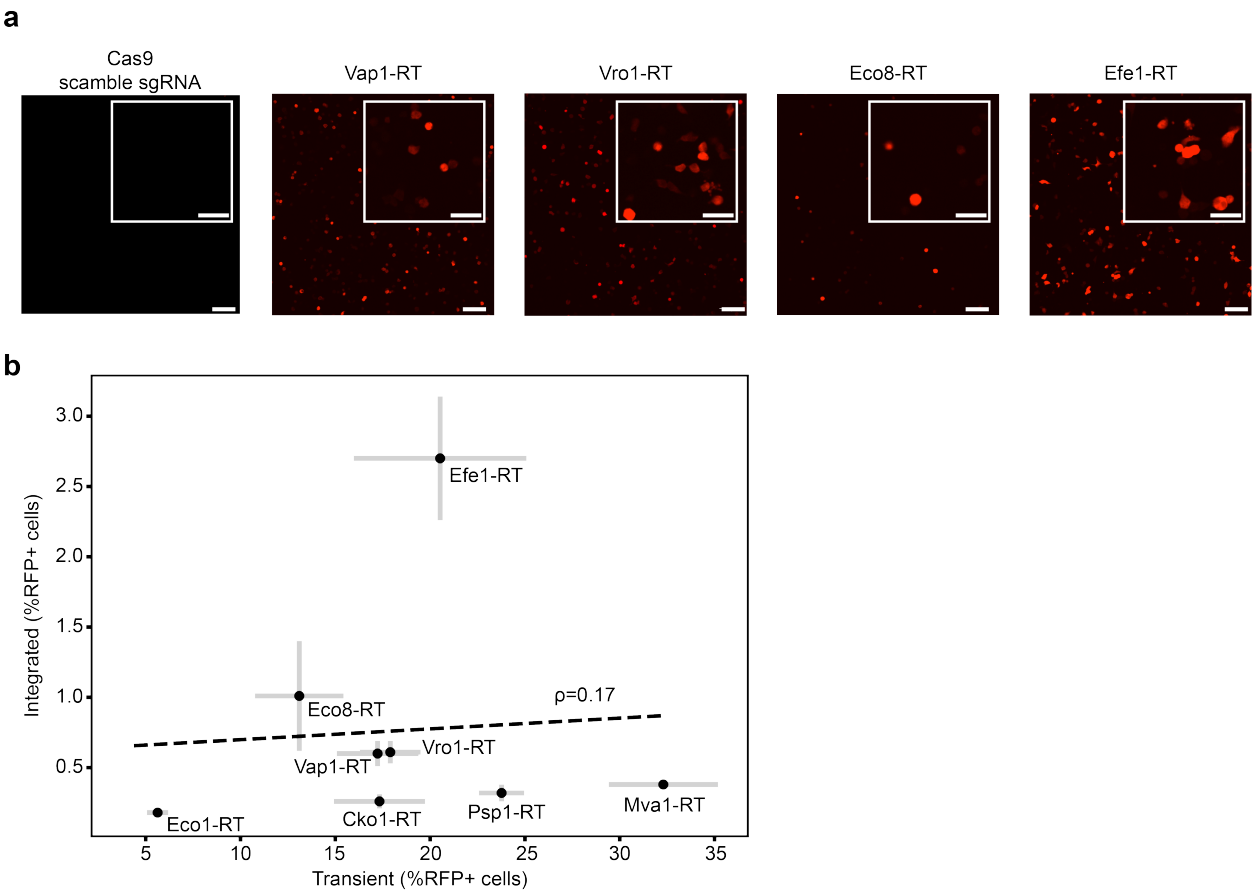
We selected high-confidence retron-RT systems based on our custom bioinformatics pipeline (**Supplementary Fig. 1a**). This pipeline consisted of the following key steps:

1. Predict open reading frames (ORFs) using Prodigal to identify candidate reverse transcriptase (RT) genes in bacterial genomes and metagenomic contigs.
2. Annotate RT genes via HMMER and a custom model that was trained on validated retron RTs.
3. Identify msr-msd ncRNAs in adjacent non-coding regions using CMfinder and Infernal (covariation/folding energy).
4. Re-annotate accessory and neighboring genes (e.g., toxin-antitoxin and other defense systems) with HMMER and Pfam.
5. Manually inspect msr-msd structures (ViennaRNA), align sequences (MAFFT), and refine models.
6. Cluster non-redundant systems (CD-HIT: 90% identity, 80% alignment overlap) and classify phylogenetically.
7. Prioritize high-confidence candidates—those with a clear msr-msd, a bacteria RT, and one of several prototypic operon organizations— across clades for experimental validation.

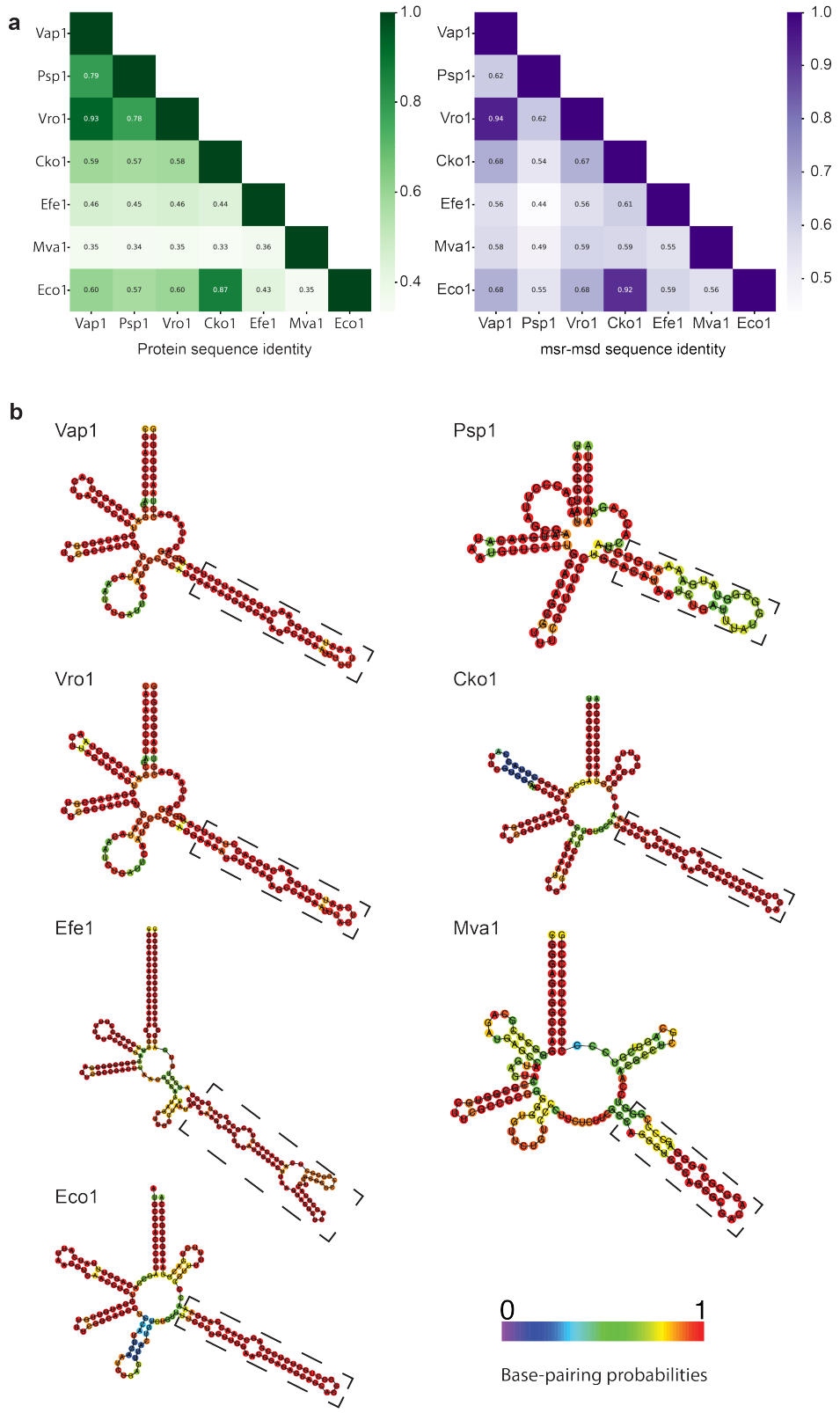
This bioinformatics approach yielded $\sim$500 high confidence non-redundant retron systems (**Supplementary Fig. 1b**). These systems were classified into 11 phylogenetic clades (**Fig. 1f**). We noticed that clade 9 showed higher activity (experiments were conducted in batches of 20 RTs). Thus, we focused our selection on sampling across multiple clades with additional samples from clade 9.



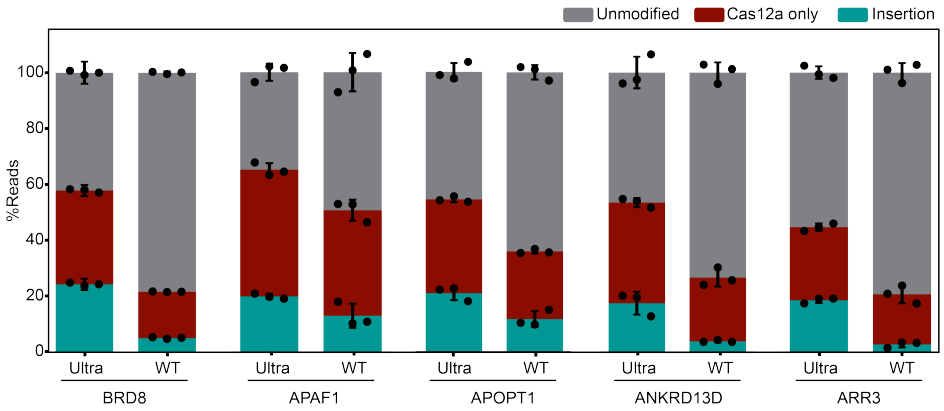
Supplementary Figure 1: Overview of the bioinformatic retron discovery pipeline. a, The pipeline involves five steps: 1. Predict open reading frames (ORFs) with Prodigal [1]; 2. Annotate reverse transcriptase (RT) genes using HMMER [2]; 3. Identify putative msr-msd sequences in non-coding regions using cmfinder and infernal [3, 4]; 4. Re-annotate adjacent ORFs with HMMER; and 5. Manually inspect msr-msd structures with ViennaRNA. b, The analysis was conducted on 2,068,918 reference genomes from the human gut microbiome [5], along with 15,574 bacterial and 531 archaeal genomes from the NCBI database [6]. After a 95% de-duplication at the amino acid sequence level and annotating msr-msds, we identified 568 new candidate systems.



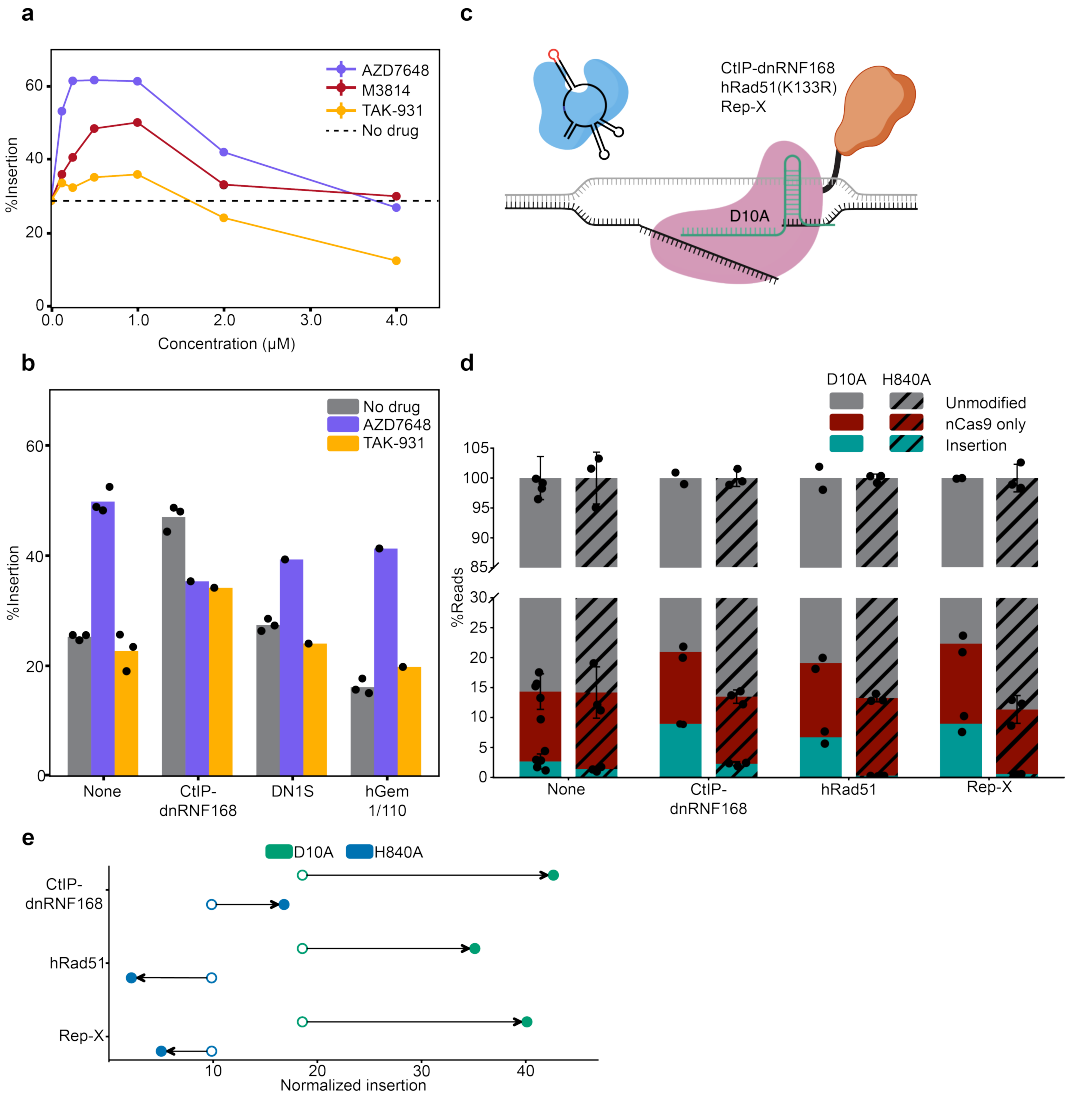
Supplementary Figure 2: Comparison of top RTs in transient and genomically-integrated RFP reporter assays. a, Confocal images of the indicated RTs, or Cas9 with a scrambled sgRNA. Scale bar: 100 µm; inset: 50 µm. b, Correlation between retron editing activity in a genomic vs. transient RFP reporter system. The genomic RFP reporter was integrated into the AAVS1 locus. Error bars represent standard deviation from n=3 biological replicates.



Supplementary Figure 3: Sequence identity and structural analysis of highly active retron-RTs. a, Sequence identity heatmap of RT's amino acid sequences and ncRNA sequences for retron candidates. b, Structure of representative msr-msd transcripts encoded by retron candidates. The structures are predicted by ViennaRNA. The msd region is highlighted by a dashed rectangle.



Supplementary Figure 4: AsCas12a-based editing outcomes at the indicated loci. AsCas12a Ultra cleaves more efficiently at all loci that we tested in this study. Error bars represent standard deviation from n=3 biological replicates.

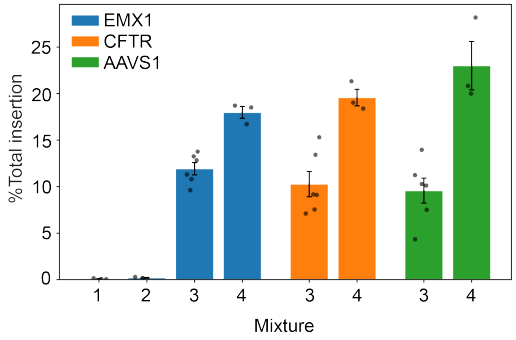


Supplementary Figure 5: Optimizing inhibitor and DNA repair fusions improves gene editing with nickase Cas9 (nCas9). a, Optimization of inhibitor concentrations for optimal retron editing at the EMX1 locus. Dashed line: gene editing without inhibitors. Experiment performed with n=1 biological replicate. b, The effect of combining DNA repair inhibitors with Cas9-DNA repair domain fusions. Combining the most active inhibitor, AZD7468, with Cas9-CtIP-dnRNF168 reduces overall insertion activity. In other cases, AZD7468 improves retron editing, likely due to the limited improvement observed with Cas9-DN1S and Cas9-hGem1/100 fusions. Individual dots represent one biological replicate. c, Illustration of the nickase Cas9(D10A) and Cas9 (H840A)-DNA repair fusions, and a nickase-based retron editor. d) Breakdown of editing outcomes between Cas9(D10A) (solid) and Cas9(H840A) (striped), as reported by NGS read counts. Cyan: retron editor-mediated insertion; Red: Cas9(D10A)-only edits without any insert, Gray: unmodified reads. Error bars represent standard deviation from n=3 biological replicates. Individual dots represent one biological replicate. e, Fusing Cas9(D10A) with CtIP-dnRNF168, hRad51(K133R), and Rep-X helicase increases the relative rates of templated repair at EMX1. Open circles: editing with no repair factor fusion. Closed circle: editing with the indicated repair factor fusion. H840A circles indicate the mean across n=3 biological replicates. D10A circles indicate the mean across n=2 biological replicates. Arrow: change in editing efficiency with the indicated Cas9-DNA repair protein fusion. Normalized insertion: sum of Efe1-RT insertions divided by total editing. Schematic created with BioRender.com

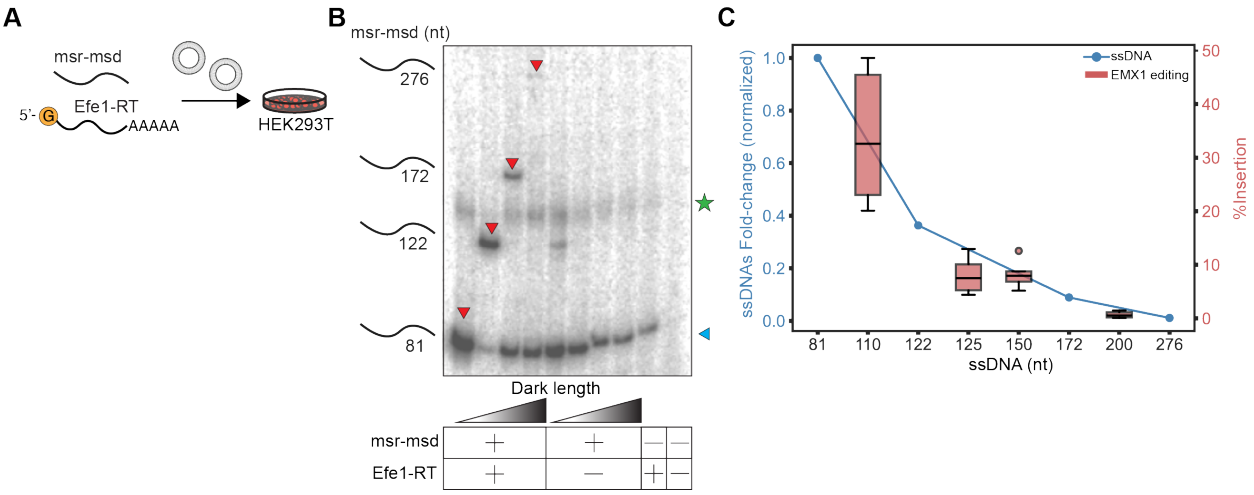
a

Mixture	Cas9 (ng)	sgRNA (ng)	RT(ng)	msr-msd(ng)
1	15	7.5	1.5	6
2	15	7.5	3	12
3	15	7.5	15	60
4	15	7.5	150	600

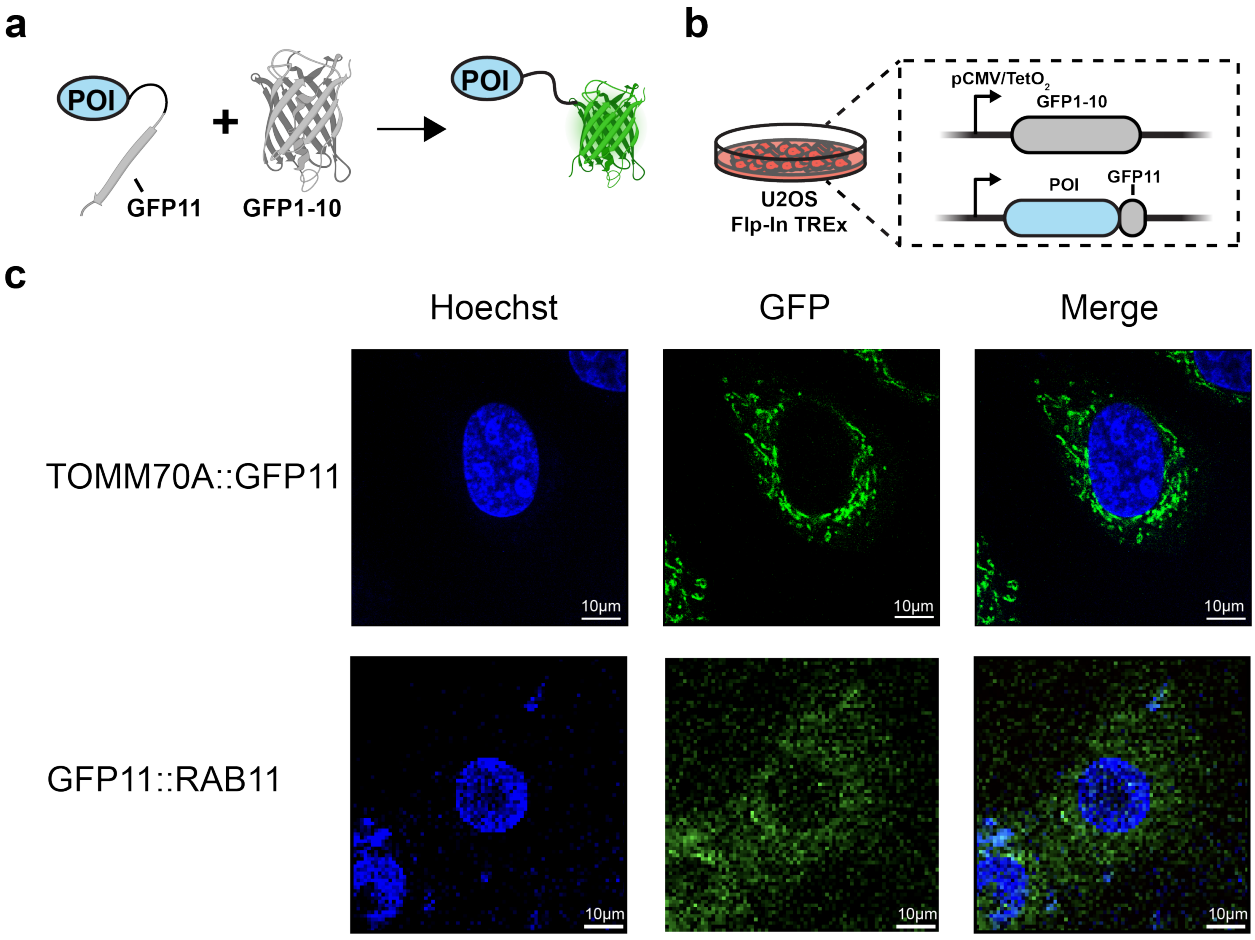
b



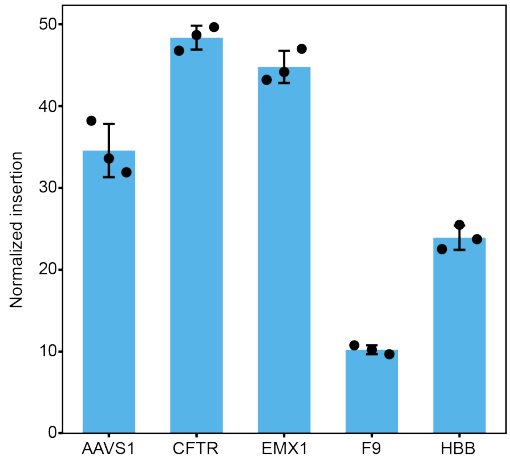
Supplementary Figure 6: Retron editor RNA delivery optimization. a, We kept the sgRNA:Cas9 mRNA ratio constant and varied the msr-msd:retron-RT RNA ratio. b, Cas9 mRNA, Efe1-RT mRNA, msr-msd, and sgRNA were transfected into HEK293Ts at the ratios indicated in (A). Error bars represent standard deviation from at least three biological replicates. Individual dots represent one biological replicate.



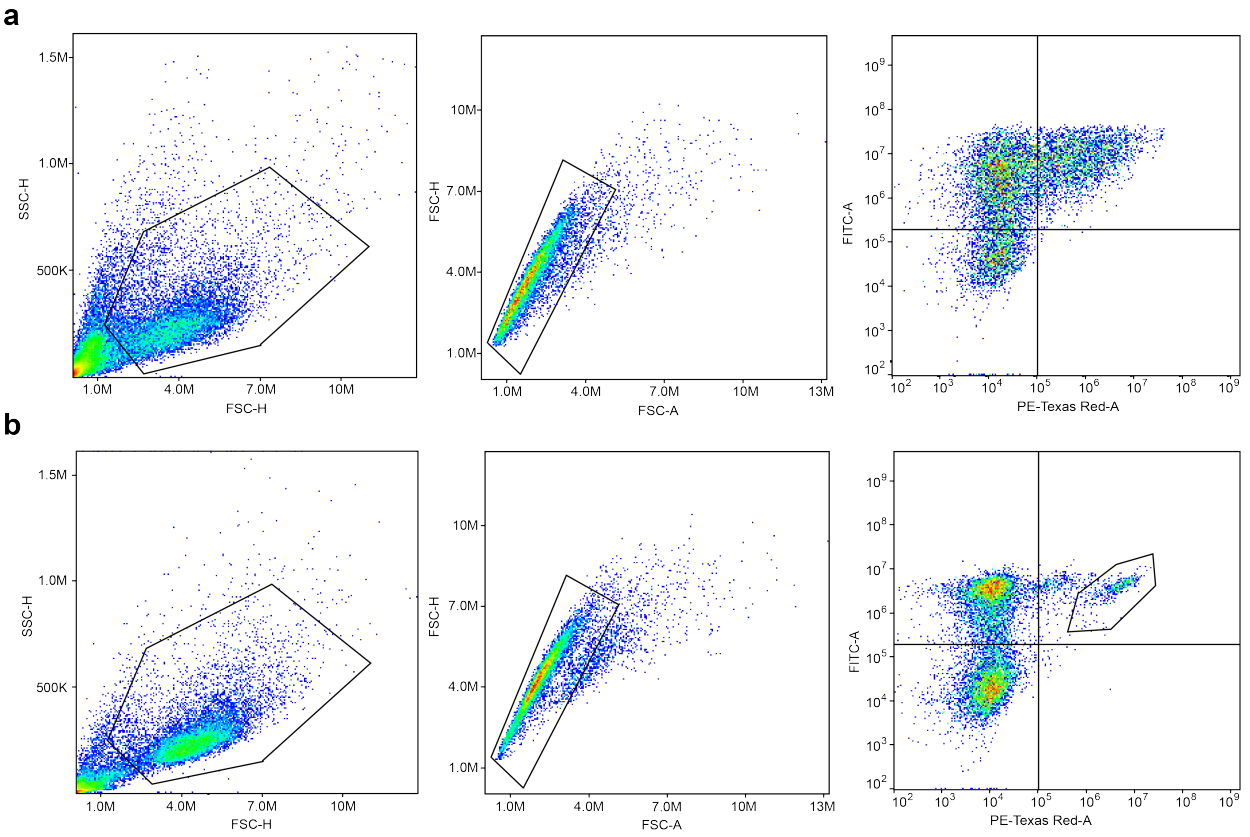
Supplementary Figure 7: Longer ssDNAs are produced at lower concentrations *in vivo*. a, Schematic of the experiment. An mRNA encoding the Efe1-RT and msr-msd with increasing msd lengths are transfected into HEK293T cells. This permits direct amplification and quantification of the resulting ssDNA. b, Radiolabeled gel showing reduced ssDNA signal as the msd length increases. See Methods for details. c, ssDNA synthesis (left y-axis) is correlated with insertion rates, as measured by deep sequencing (right y-axis). Error bars represent standard deviation from n=3 biological replicates. Schematic created with BioRender.com



Supplementary Figure 8: In-frame precise epitope insertion with retron editors a, Schematic of the split super folder GFP system. The 11th GFP β-strand (GFP11) is expressed as a fusion to the protein of interest (POI, blue). Reconstitution of GFP11 with GFP1-10 restores fluorescence. b, GFP1-10 is expressed from a genomically-integrated inducible promoter. GFP11 is inserted in-frame into the native genomic locus. c, Confocal images of two GFP11-protein fusions. Scale bars: 10 µm. Schematic created with BioRender.com



Supplementary Figure 9: A plasmid-based system for rapid retron editor assembly. SpCas9 and Efe1-RT are driven by two CAG promoters while the guide RNA (gRNA) and retron non-coding RNA (ncRNA) are driven off of U6 and H1 promoters, respectively. BsaI Golden gate sites flank the gRNA-H1-ncRNA region for rapid one-step cloning (Addgene #237445). Error bars represent standard deviation from n=3 biological replicates. Normalized insertion: sum of Efe1-RT insertions divided by total editing.



Supplementary Figure 10: Flow cytometry gating stategy, a, Gating scheme for transient RFP(Δ9)-EGFP reporter analysis showing: (left) SSC-H vs FSC-H plot for initial live cell population selection (red gate); (middle) FSC-H vs FSC-A plot for singlet discrimination (green gate); (right) FITC-A vs PE-Texas Red-A quadrant plot showing reporter expression. Editing efficiency was determined by cells in the upper right quadrant ($EGFP^+/RFP^+$). b, Gating scheme for RFP(Δ9)-EGFP integrated reporter analysis showing: (left) SSC-H vs FSC-H plot for initial live cell population selection (red gate); (middle) FSC-H vs FSC-A plot for singlet discrimination (green gate); (right) FITC-A vs PE-Texas Red-A plot showing reporter expression in the integrated system with reporter-positive cells identified by the bivariate gate (red outline).

Supplemental Tables

Plasmid	Description	Source
pIF1056	RFP(△9)-EGFP reporter	This paper
pIF1057	Mammalian expression ccdB dropout	This paper
pIF1058	sgRNA and msr-msd entry vectors	This paper
pI1F059	SpCas9 entry vector	This paper
pIF1060	Cas9(D10A) entry vector	This paper
pIF1061	AsCas12a entry vector	This paper
pIF1062	AsCas12a Ultra entry vector	This paper
pIF1063	Efe1-RT entry vector	This paper
pIF1064	Cex1-RT entry vector	This paper
pIF1065	Eco8-RT entry vector	This paper
pIF1066	Vap1-RT entry vector	This paper
pIF1067	Vro1-RT entry vector	This paper
pIF1068	SpCas9-CtIP-dnRNF168 entry vector	This paper
pIF1069	SpCas9-DN1S entry vector	This paper
pIF1070	hRad51(K133R) entry vector	This paper
pIF1071	Rep-X entry vector	This paper
pIF1072	SpCas9-SGGSx2-XTEN-SGGSx2-Efe1-RT sgRNA and msr-msd entry vector	This paper
pIF1073	SpCas9 T2A Efe1-RT entry vector	This paper
pIF1074	SpCas9 and Efe1-RT sgRNA and msr-msd entry vector	This paper
pIF1079	SpCas9 and Efe1-RT sgRNA and msr-msd user-friendly plasmid	This paper

Supplementary Table 1: Plasmids used in this study.

Name	Sequence, 5'→3'	Description
EMX1-outer-F	GACCCGCTTCCTCCCTGTCCTT	Outer PCR for EMX1
EMX1-outer-R	GTCTCAACCTGCCCAGCGGTTC	Outer PCR for EMX1
EMX1-NGS-F	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTGGCCTCCTGAGTTTCTCATC	NGS primer for EMX1
EMX1-NGS-R	CAAGCAGAAGACGGCATACGAGAT[BC]GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCTCTGCCCTCGTGGG	NGS primer for EMX1
AAVS1-outer-F	GTTTCTTAGGATGGCCTTCTCC	Outer PCR for AAVS1
AAVS1-outer-R	TCTGGGCGGAGGAATATGT	Outer PCR for AAVS1
AAVS1-NGS-F	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTGAAATGGGGGTGTGTCACC	NGS primer for AAVS1
AAVS1-NGS-R	CAAGCAGAAGACGGCATACGAGAT[BC]GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGCTGCCTCCAGGGATC	NGS primer for AAVS1
CFTR-outer-F	GTTTACTAGATTTCAGCCAGTTTTCAG	Outer PCR for CFTR
CFTR-outer-R	GATTAAACAAGTCTTGAATCTGTCTCC	Outer PCR for CFTR
CFTR-NGS-F	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTCTATGTTAAGGGAAATAGGACAAC	NGS primer for CFTR
CFTR-NGS-R	CAAGCAGAAGACGGCATACGAGAT[BC]GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTACAAATGAGATCCTTACCCC	NGS primer for CFTR
HBB-outer-F	TGTTCCCCCAGACACTCTTGCA	Outer PCR for HBB
HBB-outer-R	AGAATGGTGCAAAGAGGCATGA	Outer PCR for HBB
HBB-NGS-F	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTGCCAGGGCTGGGCATA	NGS primer for HBB
HBB-NGS-R	CAAGCAGAAGACGGCATACGAGAT[BC]GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCACATGCCCAGTTTCTATTGG	NGS primer for HBB
F9-outer-F	GTGGAAGAGTTCTAAATGTGATCC	Outer PCR for F9
F9-outer-R	GAAAAAACAAAGTGATATATGTTGCAT	Outer PCR for F9

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Supplementary Table 2: (continued)

Name	Sequence, 5'→3'	Description
F9-NGS-F	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTGTCAAATCATGTAATCAAAATTTAGTGAAG	NGS primer for F9
F9-NGS-R	CAAGCAGAAGACGGCATACGAGAT[BC]GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTACTTTGGTACAACTAATCGACC	NGS primer for F9
Innpl1a-NGS-F	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTCTTCCTGTCATGTGACCTTCAG	NGS primer for Innpl1a
Innpl1a-NGS-R	CAAGCAGAAGACGGCATACGAGAT[BC]GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTTTGCATGTGTGTGTGTTC	NGS primer for Innpl1a
ARR3-NGS-F	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTGGAGAGGTCTGTGGGT	NGS primer for ARR3
ARR3-NGS-R	CAAGCAGAAGACGGCATACGAGAT[BC]GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTGGGGGTCACAAACC	NGS primer for ARR3
APAF1-NGS-F	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTAGAATGCGGAGACGGTC	NGS primer for APAF1
APAF1-NGS-R	CAAGCAGAAGACGGCATACGAGAT[BC]GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTCCATTTAAAGGAGTACTCCTGT	NGS primer for APAF1
ANKRD13D-NGS-F	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTGCCCCTTGTCCTCTG	NGS primer for ANKRD13D
ANKRD13D-NGS-R	CAAGCAGAAGACGGCATACGAGAT[BC]GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTCCCTGCCTTGCCC	NGS primer for ANKRD13D
BRD8-NGS-F	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTGGAGTCAAGTCCCCAAG	NGS primer for BRD8
BRD8-NGS-R	CAAGCAGAAGACGGCATACGAGAT[BC]GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGCTTTAGTAATGCAACATACCT	NGS primer for BRD8

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Supplementary Table 2: (continued)

Name	Sequence, 5'→3'	Description
APOPT1-NGS-F	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTGTTAATCCAATTTACTTTGTTAAAGG	NGS primer for APOPT1
APOPT1-NGS-R	CAAGCAGAAGACGGCATACGAGAT[BC]GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTGTCAAATTCTGGTTTGCCCA	NGS primer for APOPT1
KIF6_F	AATGATACGGCGACCACCGAGATCTACACTCTTTCCCTACACGACGCTCTTCCGATCTGCTACGTTTCCAGAATGAGCTT	Forward primer for genomic DNA amplification from the KIF6 locus for NGS
KIF6_R	CAAGCAGAAGACGGCATACGAGAT[BC]GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCTTGTACACCGATGTTCTTCTTCTC	Reverse primer for genomic DNA amplification from the KIF6 locus for NGS

Supplementary Table 2: Oligonucleotides used to amplify genomic loci in this study. [BC] indicates a unique Illumina index.

Locus	Spacer sequence, 5'→3'	Type	Description
RFP	TGGCTACCAGCTTCATGCT	sgRNA	Targets RFP(Δ9) in the plasmid and integrated reporters
EMX1	GAGTCCGAGCAGAAGAAGAA	sgRNA	Target EMX1 locus
AAVS1	GGGGCCACTAGGGACAGGAT	sgRNA	Targets AAVS1 locus
CFTR	CTAAACTCATTAATGCCCTT	sgRNA	Targets CFTR locus
HBB	AGTCTGCCGTTACTGCCCTG	sgRNA	Targets HBB locus
F9	CACTGAGTAGATATCCTAAA	sgRNA	Targets F9 locus
BRD8	CTGCTTGACGAGCTAGAACAT	crRNA	Target BRD8 locus
APAF1	ATCAGAAGCCCAGATTTGTCT	crRNA	Target APAF1 locus
APOPT1	AGGTATGTAAAAGTGAACAGG	crRNA	Target APOPT1 locus
ANKRD13D	CCAGAGACACGGCCAGCTCCA	crRNA	Target ANKRD13D locus
ARR3	CACCACCGGAGGCAGGCCCTG	crRNA	Target ARR3 locus
TOMM70A	GCACCAACATTATAAAACAG	sgRNA	Targets TOMM70A locus in U2OS
RAB11	GGTAGTCGTACTCGTCGTCG	sgRNA	Targets RAB11 locus in U2OS
KIF6	ATAGGTCTCACATCACGTAT	sgRNA	Targets Kif6 locus in zebrafish

Supplementary Table 3: Spacers for single guide RNAs (sgRNAs) and CRISPR RNAs (crRNAs) used in this study.

Name	Amino acid sequence
C-myc	PAAKRVKLD
SV40	PKKKRKV
BPSV40	KRTADGSEFEPKKKRKV
vBPSV40	KRTADGSE
NLP	AVKRPAATKKAGQAKKKKLD
3xHA	YPYDVPDYAYPYDVPDYAYPYDVPDYA
Short NLS linker	EFE

Supplementary Table 4: Nuclear localization sequences used in this study.

N-terminal NLS	C-terminal NLS	Rel. editing efficiency (mean ±S.D.)
SV40	NLP	1.00 ±0.09
Cmyc-GG-BPSV40	BPSV40-SV40	1.00 ±0.05
Cmyc-GG-BPSV40	BPSV40-SV40-EFE-NLP	0.75 ±0.05
Cmyc-GG-BPSV40	BPSV40-vBPSV40-EFE-NLP	0.76 ±0.11
Cmyc-GG-BPSV40	BPSV40-vBPSV40	0.73 ±0.02
Cmyc-GG-BPSV40	BPSV40-vBPSV40-EFE-BPSV40	0.70 ±0.07
Cmyc-GG-BPSV40	BPSV40-NLP-3xHA-SV40	0.49 ±0.02
Cmyc-GG-vBPSV40	BPSV40-SV40	0.97 ±0.08
Cmyc-GG-vBPSV40	BPSV40-SV40-EFE-NLP	0.69 ±0.03
Cmyc-GG-vBPSV40	BPSV40-vBPSV40-EFE-NLP	0.85 ±0.08
Cmyc-GG-vBPSV40	BPSV40-vBPSV40	0.82 ±0.04
Cmyc-GG-vBPSV40	BPSV40-vBPSV40-EFE-BPSV40	0.63 ±0.01
Cmyc-GG-vBPSV40	BPSV40-NLP-3xHA-SV40	0.53 ±0.01
BPSV40	BPSV40-SV40	0.73 ±0.07
BPSV40	BPSV40-SV40-EFE-NLP	0.81 ±0.09
BPSV40	BPSV40-vBPSV40-EFE-NLP	0.74 ±0.01
BPSV40	BPSV40-vBPSV40	0.69 ±0.05
BPSV40	BPSV40-vBPSV40-EFE-BPSV40	0.70 ±0.01
BPSV40	BPSV40-NLP-3xHA-SV40	0.37 ±0.05
vBPSV40	BPSV40-SV40	1.02 ±0.11
vBPSV40	BPSV40-SV40-EFE-NLP	0.64 ±0.04
vBPSV40	BPSV40-vBPSV40-EFE-NLP	0.85 ±0.10
vBPSV40	BPSV40-vBPSV40	0.66 ±0.08
vBPSV40	BPSV40-vBPSV40-EFE-BPSV40	0.76 ±0.03
vBPSV40	BPSV40-NLP-3xHA-SV40	0.54 ±0.07

Supplementary Table 5: Nuclear localization signals used in this study.

Name	Type	Amino acid sequence	Rel. editing (mean ±S.D.)
SGGS-XTEN-SGGS	Flexible	SGGSSGSETPGTSESATPESSGGS	1.0 ±0.09
GGS x 4	Flexible	GGSGGSGGSGGSS	1.09 ±0.06
GGS x 5	Flexible	GGSGGSGGSGGSGGSS	1.07 ±0.06
GGS x 7	Flexible	GGSGGSGGSGGSGGSGGSGGSS	0.91 ±0.02
GGS x 8	Flexible	GGSGGSGGSGGSGGSGGSGGSGGSS	0.92 ±0.02
Long 1	Flexible	SGGSSGGSSGSETPGTSESATPESSGGSSGGSSKSGGSSGGSS	1.08 ±0.1
Long 2	Flexible	SGGSSGGSSGSETPGTSESATPESSGGSSGGSSKSGGSSGGSSK SGGSSGGSS	1.04 ±0.11
Long 3	Flexible	SGGSSGGSSGSETPGTSESATPESSGGSSGGSSKSGGSSGGSSK SGGSSGGSSKSGGSSGGSS	1.10 ±0.04
Long 4	Flexible	SGGSSGGSSGGSSGGSSKSGSETPGTSESATPESSGGSSGGSSK SGGSSGGSSKSGGSSGGSSKSGGSSGGSS	1.07 ±0.04
X-ten	Rigid	SGSETPGTSESATPES	0.78 ±0.01
Linker A	Rigid	GSAGSAAGSGEF	1.13 ±0.04
KL(A/E)AA	Rigid	KLAEAA	1.11 ±0.06
KL(A/E)AA x 2	Rigid	KLAEAAAKEAAAKAAA	1.04 ±0.01
KL(A/E)AA x 3	Rigid	KLAEAAAKEAAAKEAAAKAAA	1.04 ±0.03
KL(A/E)AA x 4	Rigid	KLAEAAAKEAAAKEAAAKEAAAKAAA	1.05 ±0.07
KL(A/E)AA x 5	Rigid	KLAEAAAKEAAAKEAAAKEAAAKEAAAKAAA	1.03 ±0.06
SpyTag	Multimer	GGGSGSGLQAHIVMVDAYKPTKGGSGSGLQAHIVMVDAYKPTK	0.16 ±0.02
SunTag	Multimer	LLPKNYHLENEVARLKKLVGERGGSGGLLPKNYHLENEVARLKK LVGERGGSGGLLPKNYHLENEVARLKKLVGERGGSGGLLPKNYH LENEVARLKKLVGERGGSGGLLPKNYHLENEVARLKKLVGERGG SGGLLPKNYHLENEVARLKKLVGERGGSGGLLPKNYHLENEVAR LKKLVGERGGSGGLLPKNYHLENEVARLKKLVGERGGSGGLLPK NYHLENEVARLKKLVGERGSGEGRGSLLTCGDVEENPGPEDPPV ATMVMGPDIVMTQSPSSLSASVGDRVTITCRSSTGAVTTSNYAS WVQEKPGKLFKGLIGGTNNRAPGVPSRFSGSLIGDKATLTISSL QPEDFATYFCALWYSNHWVFGQGTKVELKRGGGGSGGGGSGGGG SSGGGSEVKLLESGGGLVQPGGSLKLSCAVSGFSLTDYGVNWVR QAPGRGLEWIGVIWGDGITDYNSALKDRFIISKDNGKNTVYLQM SKVRSDDTALYYCVTGLFDYWGQGTLVTVSS	0.10 ±0.01
T2A	split	EGRGSLLTCGDVEENPGPEDPPVATMV	0.85 ±0.04

Supplementary Table 6: Linker sequences used in this study.

Retron msr-msd sequences used in this study

Legend: homology arms; insert; msr; msd ; native msd

Efe1-RT

Native msr-msd sequence

Efe1:

```
  1 CGCCAGCAGT GGCAATAGCG TTTCCGGCCT TTTGTGCCGG GAGGGTCGGC GAGTCGCCGA CTTAACGCCA
 71 GTAGTTTGTC TATATACCCA AAGCCGCTTC ATTGTACTTA AGTACGCTTT GCGTACGTCG CGCTGACGCG
141 CTCAGTACAG TTACGCGCCT TCGGGATGGT TTGATGGTAT TGCCGCTGTT GGCG
```

Eco8:

```
  1 GCCAGCAGTG GCAATAGCGT TTCCGGCCTT TGTGCCGGGA GGGTCGGCGA GTCGCCGACT TAACGCCAGT
 71 AGTATGTCCA TATACCCAAA GTCGCTTCAT TGTACCTGAG TACGCTTCGC GTACGTCGCG CTGACGCGCT
141 CAGTACAGTT ACGCGCCTTC GGGATGGTTT AATGGTATTG CCGCTGTTGG C
```

Vap1:

```
  1 CGCACCCTTA GCGAATGAGC TTACTTAGTT CATTGGATAG CGTTTCGCTA TCCTGCATAC AATCTGATTC
 71 AATGCCGCAT GAAAATGTGC AGAGCCAGAA TACAGTAGTT TCTGGAACTG CACATTTTCA TCCGCGACTT
141 AAGACGTAAG GGTGTG
```

Cex1:

```
  1 AGTGGTCGAG AGAGGTCTGG ACCGCATCAG CCTTAACGCC TCGAGCGTAG GAACGGCGTT GCGCCGTTCT
 71 GGTTGAAATG CTGGACACTC TCCGCAAGGT AGCCTGTTCT TGGCTCTCTC CCTCCCGAGC ACTACCGTCG
141 GGGTGGGAAG CGGAACCAAC GACGCAGCCG CCGTTTTCCC ACCCCGACGG TAGTGCTCGG GAGGGGAGAG
211 CCGGTGAGGC TACCGTGCCC CAGGTGAGCT GGTGGTGCCT TCCTGGCCTC CCTCGACCGC TCGC
```

Vro1:

```
  1 CACACCCTTA GCGAATGAGC TAACTTAGTT CATTGGATAG CGTTTCGCTA TCCTGCATAC AATCTGATTC
 71 AATGCCGCAT GAAAATGTGC AGAGCCAGAA TACAGTCGTT TCTGGAACTG CACCTTTTCA TCCGCGACTT
141 AAGACGTAAG GGTGTG
```

RFP reporter

```
  1 TCGGGGATGC CCTGGGTGTG GTTGATGAAG GCTTTGCTGC CGTACATGAA GCTGGTAGCC AGGATGTCGA
 71 AGGCGAAGGG G
```

Genomic loci in HEK293T cells

EMX1:

```
  1 TGGCCTGCTT CGTGGCAATG CGCCACCGGT TGATGTGATG GGAGCCCTTC GCTACATGCT TTCTTCTGCT
 71 CGGACTCAGG CCCTTCCTCC TCCAGCTTCT GCCGTTTGTA
```

AAVS1:

```
  1 GGAGACTAGG AAGGAGGAGG CCTAAGGATG GGGCTTTTCT GTCACCAATC GCTACATGCT CTGTCCCTAG
 71 TGGCCCCACT GTGGGGTGGA GGGGACAGAT AAAAGTACCC
```

HBB:

```
  1 CTGCCCAGGG CCTCACCACC AACTTCATCC ACGTTCACCT TGCCCCACAG GCTACATGCT GGCAGTAACG
 71 GCAGACTTCT CCTCAGGAGT CAGATGCACC ATGGTGTCTG
```

CFTR:

```
  1 TATAAAAAGA TTCCATAGAA CATAAATCTC CAGAAAAAAC ATCGCCGAAG GCTACATGCT GGCATTAATG
 71 AGTTTAGGAT TTTTCTTTGA AGCCAGCTCT CTATCCCATT
```

F9:

```
  1 GTGAACATGA TCATGGCAGA ATCACCAGGC CTCATCACCA TCTGCCTTTT GCTACATGCT AGGATATCTA
 71 CTCAGTGCTG AATGTACAGG TTTGTTTCCT TTTTTAAAAT
```

BRD8:

```
  1 GGAGACTAGG AAGGAGGAGG CCTAAGGATG GGGCTTTTCT GTCACCAATC GCTACATGCT CTGTCCCTAG
 71 TGGCCCCACT GTGGGGTGGA GGGGACAGAT AAAAGTACCC
```

Genomic loci in U2OS

TOMM70A:

```
  1 AGTGTCTTTA GGGTTCAGTT GAAGAGGGGG TAAACTTTTA AAAAGAGGGT CAGTCTGCTT TCCCCCTGTT
 71 TTATGTAATC CCAGCAGCAT TTACATACTC ATGAAGGACC ATGTGGTCAC GGCCGCCACC TAATGTTGGT
141 GGTTTTAATC CGTATTTCTT TGCAACTTCT GTCTGGGCAT GGGCGGCATC GCAAAGTGAA
```

RAB11:

```
  1 TAGAGTGCGA GAGCCCATGG CCTCACCTTT AAAGAGGTAG TCGTACTCGT CGTCCCGTGT GCCGCCGCCA
 71 CCTGTAATCC CAGCAGCATT TACATACTCA TGAAGGACCA TGTGGTCACG CATTGCGCGG CCGAGGAGCG
141 AAAGGGCGGG AGCAGCAGTG GTATCTGTGG GACCAGGGGG CGTCGCTGCA GGGGTAACCC
```

Correcting Kif6 mutations

```
  1 TTACCGCCCA AACTGTCTCT GAGTACAGAG GTCATCATTG AATTCCTATA GGGGATGTGG GACCTATCCT
 71 TCTCTGACAG GGCTATGATG ACCTAAACCA
```

Supplementary References

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