provides a new approach for treating genetic disease or mimicking protective alleles, and establishes RNA editing as a useful tool for modifying genetic function.

Supplementary Materials

Materials and Methods

Figs. S1 to S20

Tables S1 to S9

References (*33-38*)

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Supplementary Materials

RNA Editing with CRISPR-Cas13

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SUPPLEMENTARY TEXT

As the knowledge of the protospacer flanking site (PFS) may be necessary for effective Cas13 targeting, we first sought to define (PFS) preferences for the recently described Cas13b family of RNases (*12, 13*). We heterologously expressed 15 Cas13b orthologs in *E. coli* and measured interference activity using an ampicillin resistance assay (fig. S1A, B). Sequencing of colonies revealed a mixture of PFS preferences, with Cas13b orthologs having either solely 5' PFS preferences or a dual 5' and 3' PFS (fig. S1C).

SUPPLEMENTARY METHODS

Design and cloning of bacterial constructs

Mammalian codon optimized Cas13b constructs were cloned into the chloramphenicol resistant pACYC184 vector under control of the Lac promoter. Two corresponding direct-repeat (DR) sequences separated by BsaI restriction sites were then inserted downstream of Cas13b, under control of the pJ23119 promoter. Last, oligos for targeting spacers were phosphorylated using T4 PNK (New England Biolabs), annealed and ligated into BsaI digested vectors using T7 ligase (Enzymatics) to generate targeting Cas13b vectors. Guide sequences used are in Supplementary Table 6.

Bacterial PFS screens

Ampicillin resistance plasmids for PFS screens were cloned by inserting PCR products containing Cas13b targets with two 5' randomized nucleotides and four 3' randomized nucleotides separated by a target site immediately downstream of the start codon of the ampicillin resistance gene *bla* using NEB Gibson Assembly (New England Biolabs). 100 ng of ampicillin-resistant target plasmids were then electroporated with 65-100 ng chloramphenicol-resistant Cas13b bacterial targeting plasmids into Endura Electrocompetent Cells (Lucigen). Plasmids were added to cells, incubated for 15 minutes on ice, electroporated using the manufacturer's recommended settings, and then 950 uL of recovery media was added to cells

before a one-hour outgrowth at 37° C. The outgrowth was plated onto chloramphenicol and ampicillin double selection plates. Serial dilutions of the outgrowth were used to estimate the cfu/ng DNA. 16 hours post plating, cells were scraped off plates and surviving plasmid DNA was harvested using the Qiagen Plasmid Plus Maxi Kit (Qiagen). Surviving Cas13b target sequences and their flanking regions were amplified by PCR and sequenced using an Illumina NextSeq. To assess PFS preferences, the positions containing randomized nucleotides in the original library were extracted, and sequences depleted relative to the vector only condition and that were present in both bioreplicates were extracted using custom python scripts. The $-\log_2$ of the ratio of PFS abundance in the Cas13b condition compared to the vector only control was then used to calculate preferred motifs. Specifically, all sequences having $-\log_2$(sample/vector) depletion ratios above a specific threshold were used to generate weblogos of sequence motifs (weblogo.berkeley.edu). The specific depletion ratio values used to generate weblogos for each Cas13b ortholog are listed in Supplementary table 2.

Design and cloning of mammalian constructs for RNA interference

To generate vectors for testing Cas13 orthologs in mammalian cells, mammalian codon optimized Cas13a, Cas13b, and Cas13c genes were PCR amplified and golden-gate cloned into a mammalian expression vector containing dual NLS sequences and a C-terminal msfGFP, under control of the EF1alpha promoter. For further optimization Cas13 orthologs were golden-gate cloned into destination vectors containing different C-terminal localization tags under control of the EF1alpha promoter.

The dual luciferase reporter was cloned by PCR amplifying *Gaussia* and *Cypridinia* luciferase coding DNA, the EF1alpha and CMV promoters and assembled using the NEB Gibson Assembly (New England Biolabs).

For expression of mammalian guide RNAs for Cas13a, Cas13b, or Cas13c orthologs, the corresponding direct repeat sequences were synthesized with golden-gate acceptor sites and cloned under U6 expression via restriction digest cloning. Individual guides were then cloned into the corresponding expression backbones for each ortholog by golden-gate cloning. All

Cas13 plasmids are listed in Supplementary Table 5. All Cas13 guide sequences for knockdown experiments are listed in Supplementary Tables 6-8.

Measurement of Cas13 expression in mammalian cells

Dual-NLS Cas13-msfGFP constructs were transfected into HEK293FT cells with targeting and non-targeting guides. GFP fluorescence was measured 48 hours post transfection in the non-targeting guide condition using a plate reader.

Cloning of pooled mismatch libraries for Cas13 interference specificity

Pooled mismatch library target sites were created by PCR using a forward primer containing the semi-degenerate target sequences and a constant reverse primer off of a *Gluc* template. The semi-degenerate forward oligo had at each position of the Cas13 target, plus the 5' and 3' three flanking bases, a nucleotide mixture containing 94% of the correct base and 2% of each incorrect base. The mismatch library amplicon was then cloned into the dual luciferase reporter in place of wild-type *Gluc* using NEB Gibson assembly (New England Biolabs).

Design and cloning of mammalian constructs for RNA editing

PspCas13b was made catalytically inactive (dPspCas13b) via two histidine to alanine mutations (H133A/H1058A) at the catalytic site of the HEPN domains. The deaminase domains of human ADAR1 and ADAR2 were synthesized and PCR amplified for Gibson cloning into pcDNA-CMV vector backbones and were fused to dPspCas13b at the C-terminus via GS or GSGGGGS linkers. For the experiment in which we tested different linkers we cloned the following additional linkers between dPspCas13b and $ADAR2_{DD}$: GGGGSGGGGSGGGGS, EAAAK, GGSGGSGGSGGSGGSGGS, and SGSETPGTSESATPES (XTEN). Specificity mutants were generated by Gibson cloning the appropriate mutants into the dPspCas13b-GSGGGGS backbone.

The luciferase reporter vector for measuring RNA editing activity was generated by creating a W85X mutation (TGG>TAG) in the luciferase reporter plasmid used for knockdown experiments. This reporter vector expresses functional *Gluc* as a normalization control, but a defective *Cluc* due to the addition of the W85X pretermination site. To test ADAR editing motif preferences, we cloned every possible motif around the adenosine at codon 85 (XAX) of *Cluc*. All plasmids are listed in Supplementary Table 5.

Testing PFS preferences for dCas13b

For testing PFS preference of REPAIR, we cloned a pooled plasmid library containing a 6 basepair degenerate PFS sequence upstream of a target region and adenosine editing site. The library was synthesized as an ultramer from Integrated DNA Technologies (IDT) and was made double stranded via annealing a primer and using the Klenow fragment of DNA polymerase I (New England Biolabs) to fill in the sequence. This dsDNA fragment containing the degenerate sequence was then Gibson cloned into the digested reporter vector and this was then isopropanol precipitated and purified. The cloned library was then electroporated into Endura competent *E. coli* cells (Lucigen) and plated on 245mm x 245mm square bioassay plates (Nunc). After 16 hours, colonies were harvested and midiprepped using endotoxin-free MACHEREY-NAGEL midiprep kits. Cloned libraries were verified by next-generation sequencing.

Cloning pathogenic G>A mutations for assaying REPAIR activity

For cloning disease-relevant mutations for testing REPAIR activity, 34 G>A mutations related to disease pathogenesis as defined in ClinVar were selected and 200-bp regions surrounding these mutations were golden-gate cloned between mScarlett and EGFP under a CMV promoter. Two additional G>A patient mutations in *AVPR2* and *FANCC* and their cDNA sequences were synthesized and Gibson cloned under expression of EF1alpha.

Guide cloning for REPAIR

For expression of mammalian guide RNAs for REPAIR, the PspCas13b direct repeat sequences were synthesized with golden-gate acceptor sites and cloned under U6 expression via restriction digest cloning. Individual guides were then cloned into this expression backbone by golden-gate cloning. Guide sequences for REPAIR experiments are listed in Supplementary Table 9.

Mammalian cell culture

Mammalian cell culture experiments were performed in the HEK293FT line (American Type Culture Collection (ATCC)), which was grown in Dulbecco's Modified Eagle Medium with high glucose, sodium pyruvate, and GlutaMAX (Thermo Fisher Scientific), additionally supplemented with 1× penicillin–streptomycin (Thermo Fisher Scientific) and 10% fetal bovine serum (VWR Seradigm). Cells were maintained at confluency below 80%. The U2OS specificity experiment was performed using the U2OS cell line from ATCC and cells were cultured in ATCC-formulated McCoy's 5a Medium Modified.

Unless otherwise noted, all transfections were performed with Lipofectamine 2000 (Thermo Fisher Scientific) in 96-well plates coated with poly-D-lysine (BD Biocoat). Cells were plated at approximately 20,000 cells/well 16 hours prior to transfection to ensure 90% confluency at the time of transfection. For each well on the plate, transfection plasmids were combined with Opti-MEM I Reduced Serum Medium (Thermo Fisher) to a total of 25 µl. Separately, 24.5 µl of Opti-MEM was combined with 0.5 µl of Lipofectamine 2000. Plasmid and Lipofectamine solutions were then combined and incubated for 5 minutes, after which they were pipetted onto cells. The U2OS transfections were performed using Lipofectamine 3000 according to the manufacturer's protocol.

Mammalian cell RNA knockdown assays

To assess RNA targeting in mammalian cells with reporter constructs, 150 ng of Cas13 construct was co-transfected with 300 ng of guide expression plasmid and 12.5 ng of the knockdown reporter construct. 48 hours post-transfection, media containing secreted luciferase was removed

from cells, diluted 1:5 in PBS, and measured for activity with BioLux Cypridinia and Biolux Gaussia luciferase assay kits (New England Biolabs) on a plate reader (Biotek Synergy Neo2) with an injection protocol. All replicates performed are biological replicates.

For targeting of endogenous genes, 150 ng of Cas13 construct was co-transfected with 300 ng of guide expression plasmid. 48 hours post-transfection, cells were lysed and RNA was harvested and reverse transcribed using a previously described(*33*) modification of the Cells-to-Ct kit (Thermo Fisher Scientific). cDNA expression was measured via qPCR using TaqMan qPCR probes for the *KRAS* transcript (Thermo Fisher Scientific), *GAPDH* control probes (Thermo Fisher Scientific), and Fast Advanced Master Mix (Thermo Fisher Scientific). qPCR reactions were read out on a LightCycler 480 Instrument II (Roche), with four 5 µl technical replicates in 384-well format.

Evaluation of RNA specificity using pooled libraries of mismatched targets

The ability of Cas13 to interfere with the mismatched target library was tested using HEK293FT cells seeded in 6-well plates. ~70% confluent cells were transfected using 2400 ng Cas13 vector, 4800 ng of guide, and 240 ng of mismatched target library. 48 hours post-transfection, cells were harvested and RNA was extracted using the QIAshredder (Qiagen) and the Qiagen RNeasy Mini Kit. 1 µg of extracted RNA was reverse transcribed using the qScript Flex cDNA synthesis kit (Quantabio) following the manufacturer's gene-specific priming protocol with a *Gluc* specific RT primer. cDNA was then amplified and sequenced on an Illumina NextSeq.

Sequencing was analyzed by counting reads per sequence and depletion scores were calculated by determining the $\log_2$(-read count ratio) value, where read count ratio is the ratio of read counts in the targeting guide condition versus the non-targeting guide condition. This score represents the level of Cas13 activity on the sequence, with higher values representing stronger depletion and thus higher Cas13 cleavage activity. Separate distributions for the single mismatch and double mismatch sequences were determined and plotted as heatmaps with a depletion score for each mismatch identity. For double mismatch sequences the average of all possible double mismatches at a given position were plotted.

Transcriptome-wide profiling of Cas13 in mammalian cells by RNA sequencing

For measurement of transcriptome-wide specificity, 150 ng of Cas13 construct, 300 ng of guide expression plasmid, and 15 ng of the knockdown reporter construct were co-transfected; for shRNA conditions, 300 ng of shRNA targeting plasmid, 15 ng of the knockdown reporter construct, and 150 ng of EF1-alpha driven mCherry (to balance reporter load) were co-transfected. 48 hours post-transfection, RNA was purified with the RNeasy Plus Mini kit (Qiagen), mRNA was isolated using NEBNext Poly(A) mRNA Magnetic Isolation Module (New England Biolabs), and prepared for sequencing with the NEBNext Ultra RNA Library Prep Kit for Illumina (New England Biolabs). RNA sequencing libraries were then sequenced on a NextSeq (Illumina).

To analyze transcriptome-wide sequencing data, reads were aligned to the RefSeq GRCh38 assembly using Bowtie and RSEM version 1.2.31 with default parameters(*34*). Transcript expression was quantified as $\log_2(\text{TPM} + 1)$, genes were filtered for $\log_2(\text{TPM} + 1) > 2.5$. For selection of differentially expressed genes, only genes with differential changes of >2 or <.75 were considered. Statistical significance of differential expression was evaluated using a Student's t-test on three targeting replicates versus non-targeting replicates, and filtered for a false discovery rate of <0.01% by the Benjamini-Hochberg procedure.

REPAIR editing in mammalian cells

To assess REPAIR activity in mammalian cells, we transfected 150 ng of REPAIR vector, 300 ng of guide expression plasmid, and 40 ng of the RNA editing reporter. After 48 hours, RNA from cells was harvested and reverse transcribed using a method previously described(*33*) with a gene specific reverse transcription primer. The extracted cDNA was then subjected to two rounds of PCR to add Illumina adaptors and sample barcodes using NEBNext High-Fidelity 2X PCR Master Mix (New England Biolabs). The library was then subjected to next generation sequencing on an Illumina NextSeq or MiSeq. RNA editing rates were then evaluated at all adenosines within the sequencing window.

In experiments where the luciferase reporter was targeted for RNA editing, we also harvested the media with secreted luciferase prior to RNA harvest. In this case, because corrected *Cluc* might be at low levels, we did not dilute the media. We measured luciferase activity with BioLux Cypridinia and Biolux Gaussia luciferase assay kits (New England Biolabs) on a plate reader (Biotek Synergy Neo2) with an injection protocol. All replicates performed are biological replicates.

PFS binding mammalian screen

To determine the contribution of the PFS to editing efficiency in mammalian cells, 625 ng of PFS target library, 4.7 μg of guide, and 2.35 μg of REPAIR were co-transfected in HEK293FT cells plated in 25 cm^2 flasks. Plasmids were mixed with 33 μl of PLUS reagent (Thermo Fisher Scientific), brought to 533 μl with Opti-MEM, incubated for 5 minutes, combined with 30 μl of Lipofectamine 2000 and 500 μl of Opti-MEM, incubated for an additional 5 minutes, and then pipetted onto cells. 48 hours post-transfection, RNA was harvested with the RNeasy Plus Mini kit (Qiagen), reverse transcribed with qScript Flex (Quantabio) using a gene specific primer, and amplified with two rounds of PCR using NEBNext High-Fidelity 2X PCR Master Mix (New England Biolabs) to add Illumina adaptors and sample barcodes. The library was sequenced on an Illumina NextSeq, and RNA editing rates at the target adenosine were mapped to PFS identity. To increase coverage, the PFS was computationally collapsed to 4 nucleotides adjacent to the 5' end of the target sequence. REPAIR editing rates were calculated for each PFS, averaged over biological replicates with non-targeting rates for the corresponding PFS subtracted.

Whole-transcriptome sequencing to evaluate ADAR editing specificity

For analyzing off-target RNA editing sites across the transcriptome, we harvested total RNA from cells 48 hours post-transfection using the RNeasy Plus Miniprep kit (Qiagen). The mRNA fraction was then enriched using a NEBNext Poly(A) mRNA Magnetic Isolation Module (NEB) and this RNA was then prepared for sequencing using an NEBNext Ultra RNA Library Prep Kit

for Illumina (NEB). The libraries were then sequenced on an Illumina NextSeq and loaded such that there were at least 5 million reads per sample.

RNA editing analysis for targeted and transcriptome-wide experiments

Analysis of the transcriptome-wide editing RNA sequencing data was performed on the FireCloud computational framework (https://software.broadinstitute.org/firecloud/) using a custom workflow we developed: https://portal.firecloud.org/#methods/m/rna_editing_final_workflow/rna_editing_final_workflow/1. For analysis, unless otherwise denoted, sequence files were randomly downsampled to 5 million reads. For the high-coverage sequencing analysis, samples were randomly downsampled to 5 million, 15 million, or 50 million reads. An index was generated using the RefSeq GRCh38 assembly with *Gluc* and *Cluc* sequences added, and reads were aligned and quantified using Bowtie/RSEM version 1.3.0. Alignment BAMs were then sorted and analyzed for RNA editing sites using REDitools (*35, 36*) with the following parameters: -t 8 -e -d -l -U [AG or TC] -p -u -m20 -T6-0 -W -v 1 -n 0.0. Any significant edits found in untransfected or EGFP-transfected conditions were considered to be SNPs or artifacts of the transfection and filtered out from the analysis of off-targets. Off-targets were considered significant if the Fisher's exact test yielded a p-value less than 0.05 after multiple hypothesis correction by Benjamini Hochberg correction and at least 2 of 3 biological replicates identified the edit site. Overlap of edits between samples was calculated relative to the maximum possible overlap, equivalent to the fewer number of edits between the two samples. The percentage of overlapping edit sites was calculated as the number of shared edit sites divided by minimum number of edits of the two samples, multiplied by 100. For the high-coverage sequencing analysis, an additional layer of filtering for known SNP positions was performed using the Kaviar (*37*) method for identifying SNPs.

For analyzing the predicted variant effects of each off-target, the list of off-target edit sites was analyzed using the variant annotation integrator (https://genome.ucsc.edu/cgi-bin/hgVai) as part of the UCSC genome browser suite of tools using the SIFT and PolyPhen-2 annotations. To predict whether the off-target genes are oncogenic, a database of oncogenic annotations from the

COSMIC catalogue of somatic mutations in cancer was used to characterize off-target genes (cancer.sanger.ac.uk).

For analyzing whether the REPAIR constructs perturbed RNA levels, the transcript per million (TPM) values output from the RSEM analysis were used for expression counts and transformed to log-space by taking the $\log_2$(TPM+1). To find differentially regulated genes, a Student's t-test was performed on three targeting guide replicates versus three non-targeting guide replicates. The statistical analysis was only performed on genes with $\log_2$(TPM+1) values greater than 2.5 and genes were only considered differentially regulated if they had a fold change greater than 2 or less than 0.8. Genes were reported if they had a false discovery rate (Benjamini Hochberg correction) of less than 0.01.

SUPPLEMENTARY FIGURES

Figure S1

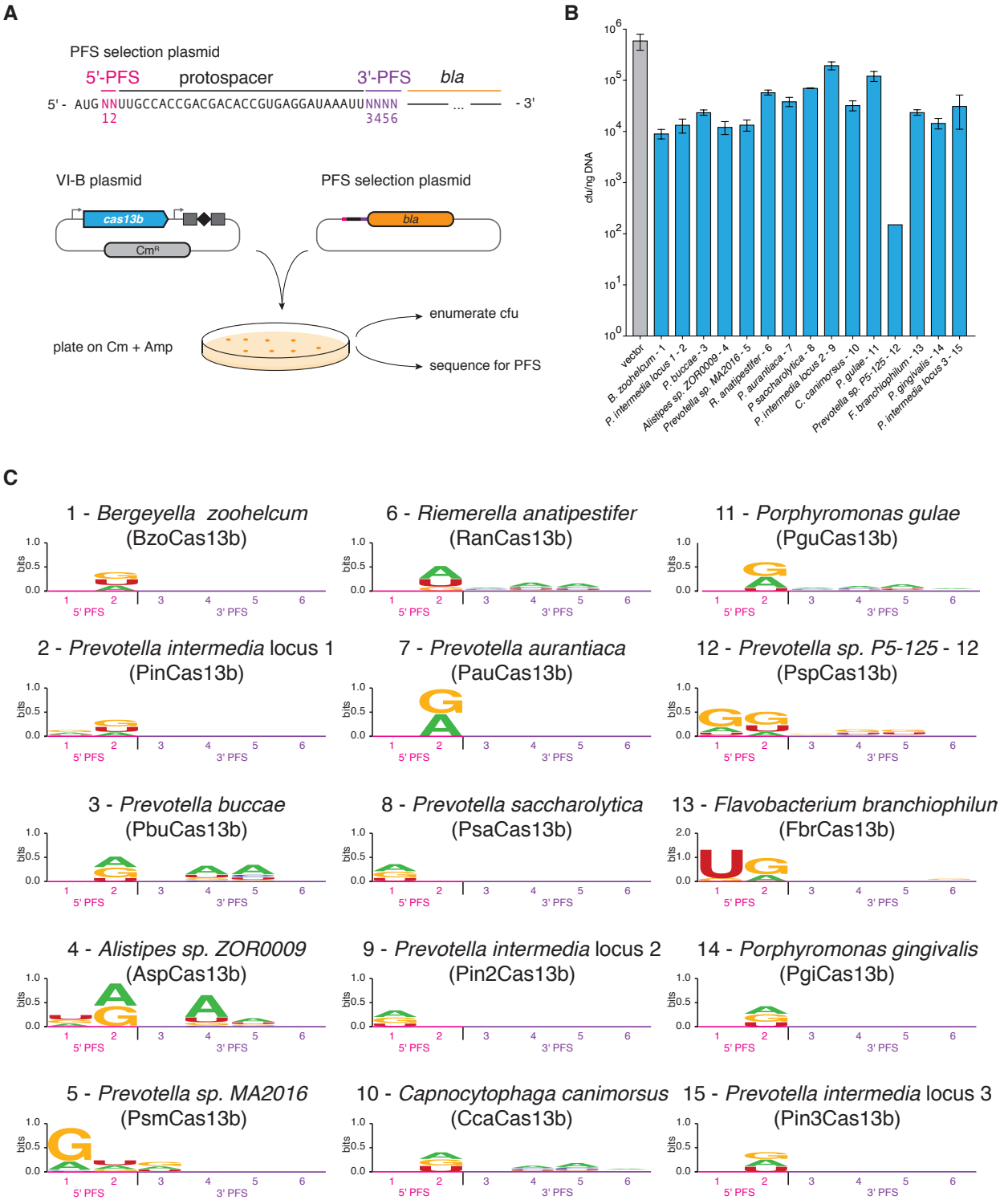

Figure S1: Bacterial screening of Cas13b orthologs for *in vivo* efficiency and PFS determination.

A) Schematic of bacterial assay for determining the PFS of Cas13b orthologs. Cas13b orthologs with beta-lactamase targeting spacers are co-transformed with beta-lactamase expression plasmids containing randomized PFS sequences and subjected to dual antibiotic selection. PFS sequences that are depleted during co-transformation with Cas13b suggest targeting activity and are used to infer PFS preferences.

B) Quantification of interference activity of Cas13b orthologs targeting beta-lactamase as measured by colony forming units (cfu). Values represent mean +/– S.D.

C) PFS weblogos for Cas13b orthologs as determined by depleted sequences from the bacterial assay. PFS preferences are derived from sequences depleted in the Cas13b condition relative to empty vector controls. Depletion values used to calculate PFS weblogos are listed in table S2.

Figure S2

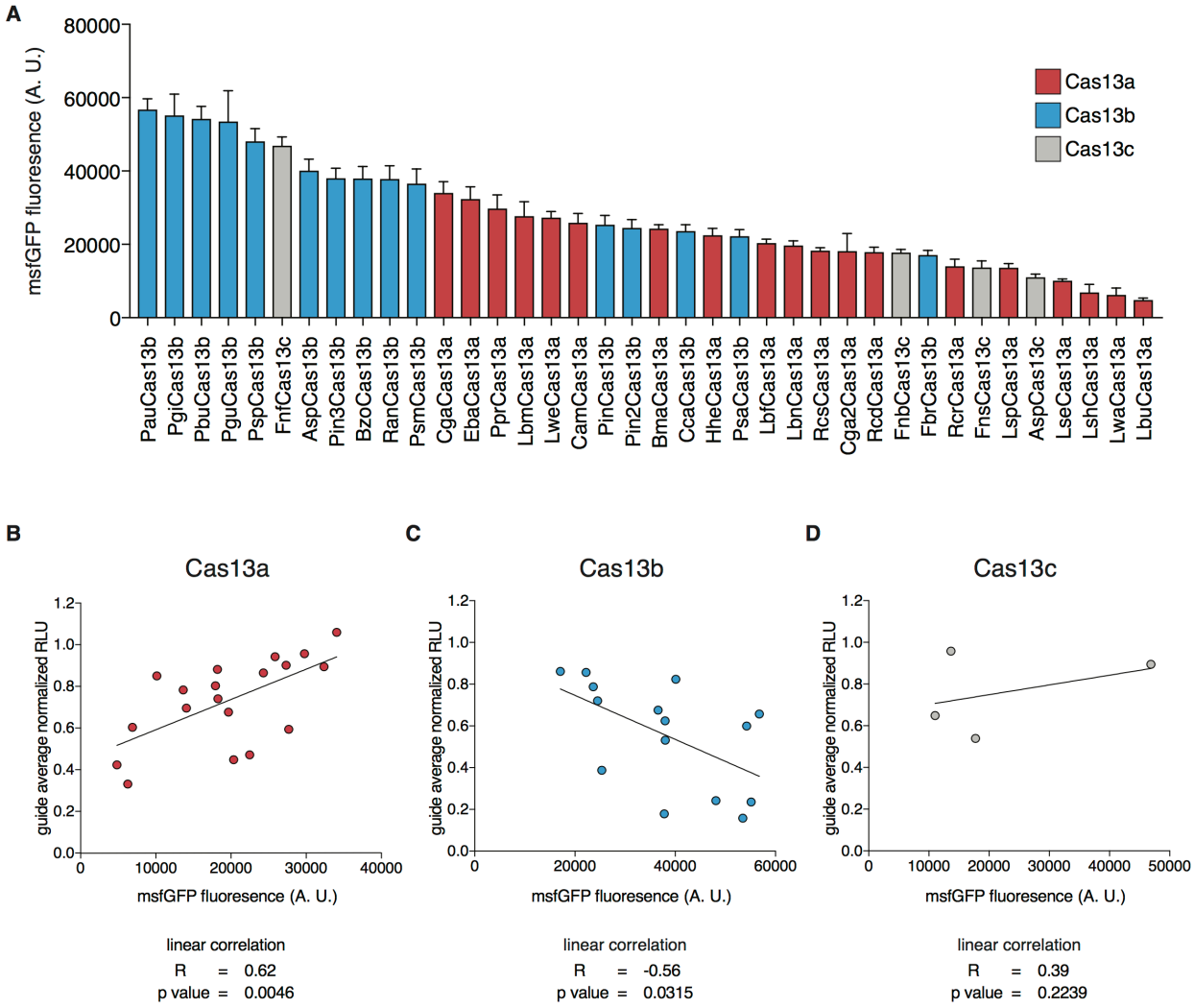

Figure S2: Relative expression of Cas13 orthologs in mammalian cells and correlation of expression with interference activity.

A) Expression of Cas13 orthologs as measured by msfGFP fluoresence. Cas13 orthologs C-terminally tagged with msfGFP were transfected into HEK293FT cells and their fluorescence measured 48 hours post transfection.

B) Correlation of Cas13 expression to interference activity. The average RLU of two *Gluc* targeting guides for Cas13 orthologs, separated by subfamily, is plotted versus expression as determined by msfGFP fluoresence. The RLU for targeting guides are normalized to RLU for a non-targeting guide, whose value is set to 1. The non-targeting guide is the same as in Figure 1B for Cas13b.

Figure S3

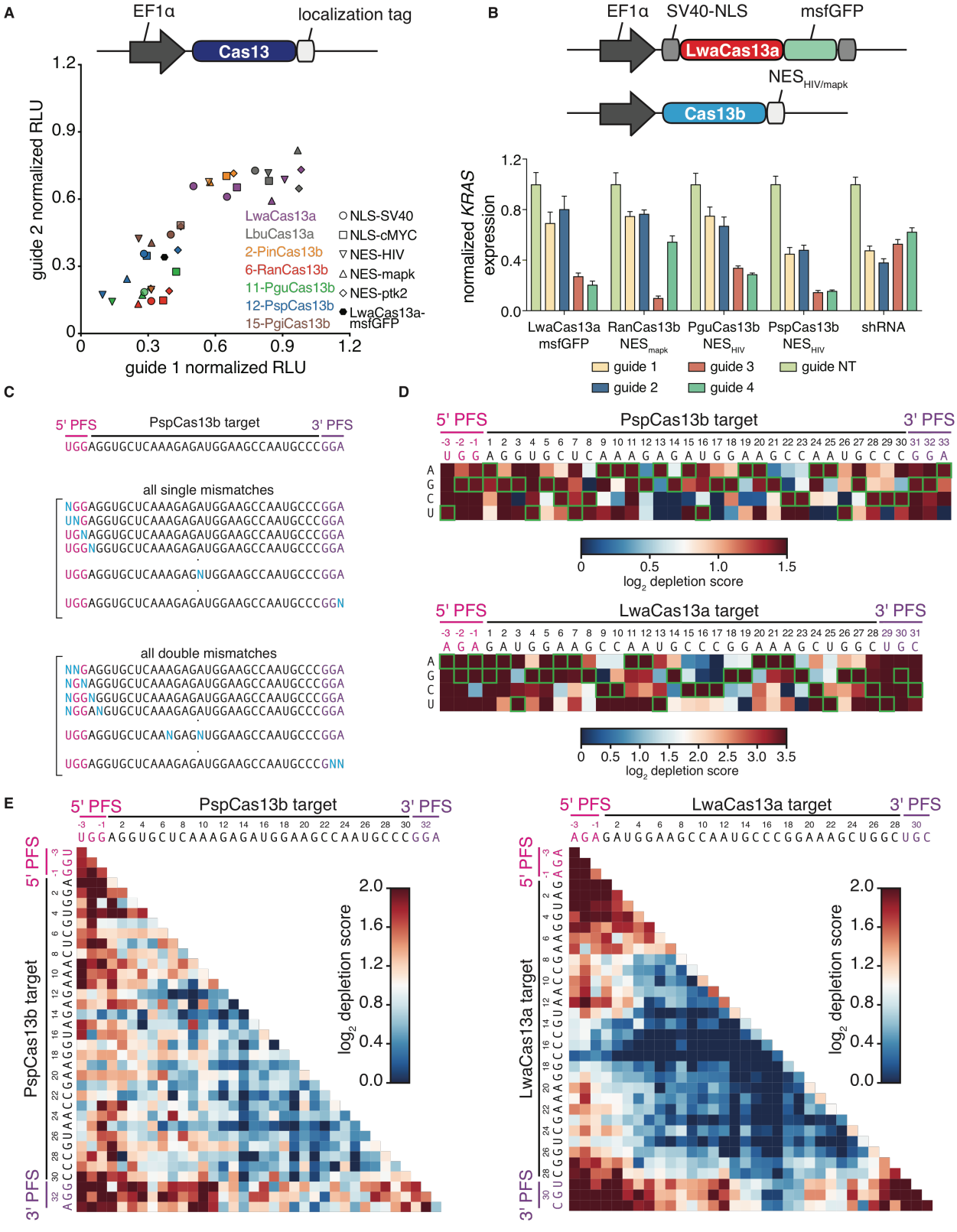
A
EF1α
localization tag
Cas13
guide 2 normalized RLU
guide 1 normalized RLU
LwaCas13a
LbuCas13a
2-PinCas13b
6-RanCas13b
11-PguCas13b
12-PspCas13b
15-PgiCas13b
NLS-SV40
NLS-cMYC
NES-HIV
NES-mapk
NES-ptk2
LwaCas13a-msfGFP
B
EF1α
SV40-NLS
msfGFP
LwaCas13a
NES HIV/mapk
Cas13b
normalized KRAS expression
LwaCas13a msfGFP
RanCas13b NES mapk
PguCas13b NES HIV
PspCas13b NES HIV
shRNA
guide 1
guide 2
guide 3
guide 4
guide NT
C
5' PFS
PspCas13b target
3' PFS
UGGAGGUGCUCAAAGAGAUGGAAGCCAAUGCCCGGA
all single mismatches
all double mismatches
D
PspCas13b target
LwaCas13a target
log2 depletion score
E
PspCas13b target
LwaCas13a target
log2 depletion score

Figure S3: Optimization of Cas13b knockdown and further characterization of mismatch specificity.

A) Gluc knockdown with two different guides is measured using the top two Cas13a and top four Cas13b orthologs fused to a variety of C-terminal nuclear localization and nuclear export tags.

B) Knockdown of *KRAS* is measured for LwaCas13a, RanCas13b, PguCas13b, PspCas13b and shRNA with four position-matched guides. Non-targeting guide is the same as in Figure 1B. shRNA non-targeting guide sequence is listed in table S6.

C) Schematic of the single and double mismatch plasmid libraries used for evaluating the specificity of LwaCas13a and PspCas13b knockdown. Every possible single and double mismatch is present in the target sequence as well as in three positions directly flanking the 5' and 3' ends of the target site.

D) The depletion levels of transcripts with the indicated single mismatches are plotted as a heatmap for both the LwaCas13a and PspCas13b conditions. The wildtype base is outlined by a green box.

E) The depletion levels of transcripts with the indicated double mismatches are plotted as a heatmap for both the LwaCas13a and PspCas13b conditions. Each box represents the average of all possible double mismatches for the indicated position.

Figure S4

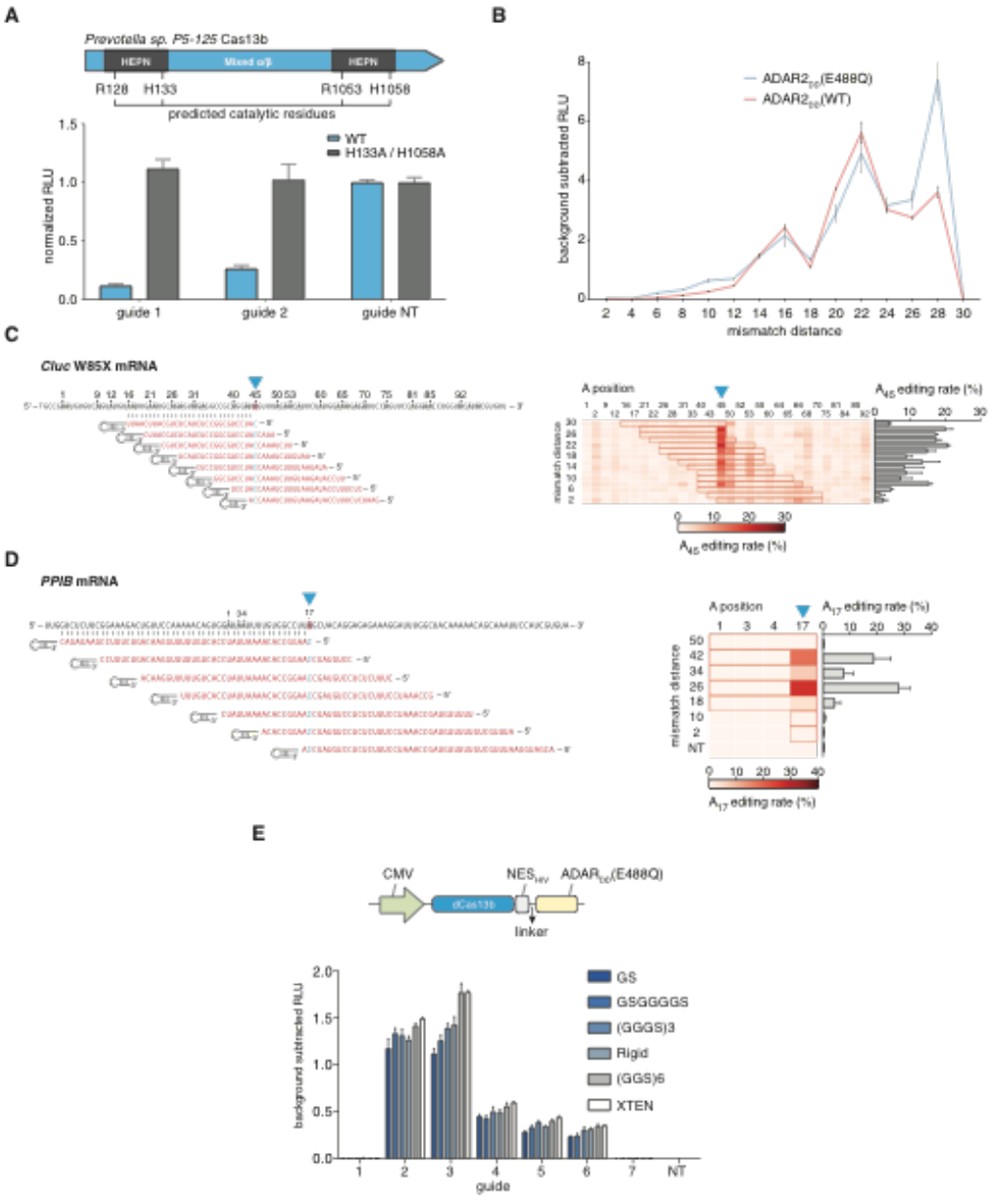

Figure S4: Characterization of design parameters for REPAIRv1.

A) Knockdown efficiency of Gluc with wild-type Cas13b or catalytically inactive H133A/H1058A Cas13b (dCas13b).

B) Quantification of luciferase activity restoration by dCas13b fused to either the wild-type

ADAR2 deaminase domain ($ADAR2_{DD)}$ or the hyperactive E488Q mutant $ADAR2_{DD}$(E488Q) deaminase domain, tested with tiling *Cluc* targeting guides.

C) Guide design and sequencing quantification of A to I editing for 30-nt guides targeting *Cluc* W85X.

D) Guide design and sequencing quantification of A to I editing for 50-nt guides targeting *PPIB*.

E) Influence of linker choice on luciferase activity restoration by REPAIRv1. Values represent mean +/– S.E.M.

Figure S5

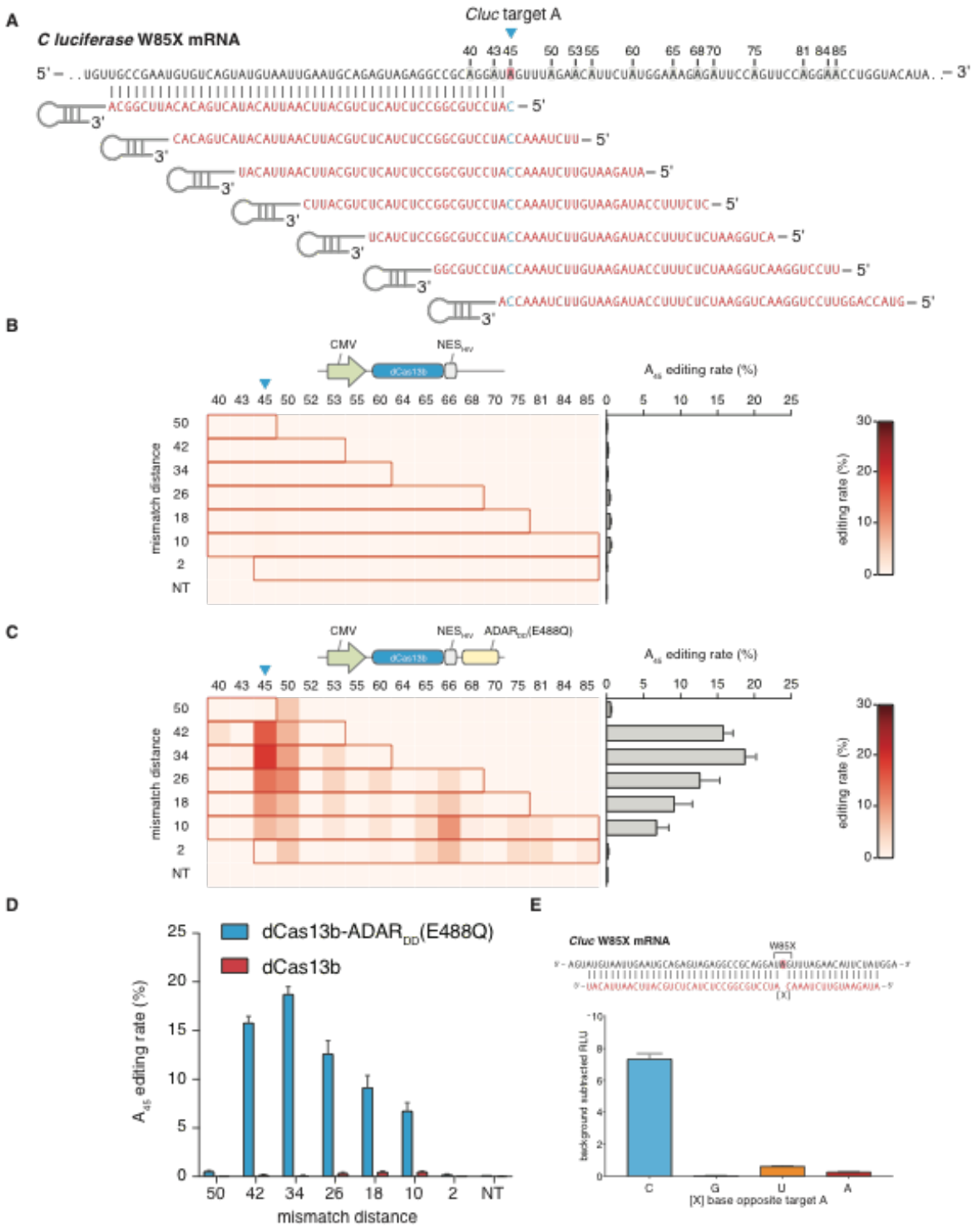

Figure S5: Comparison of RNA editing activity of dCas13b and REPAIRv1.

A) Schematic of guides used to target the W85X mutation in the *Cluc* reporter.

B) Sequencing quantification of A to I editing for indicated guides transfected with dCas13b. For each guide, the region of duplex RNA is outlined in red. Values represent mean +/– S.E.M. Non-targeting guide is the same as in Fig2C.

C) Sequencing quantification of A to I editing for indicated guides transfected with REPAIRv1. For each guide, the region of duplex RNA is outlined in red. Values represent mean +/– S.E.M. Non-targeting guide is the same as in Fig2C.

D) Comparison of on-target A to I editing rates for dCas13b and dCas13b-ADAR2$_{DD}$(E488Q) for guides tested in panel B and C.

E) Influence of base identify opposite the targeted adenosine on luciferase activity restoration by REPAIRv1. Values represent mean +/– S.E.M.

Figure S6

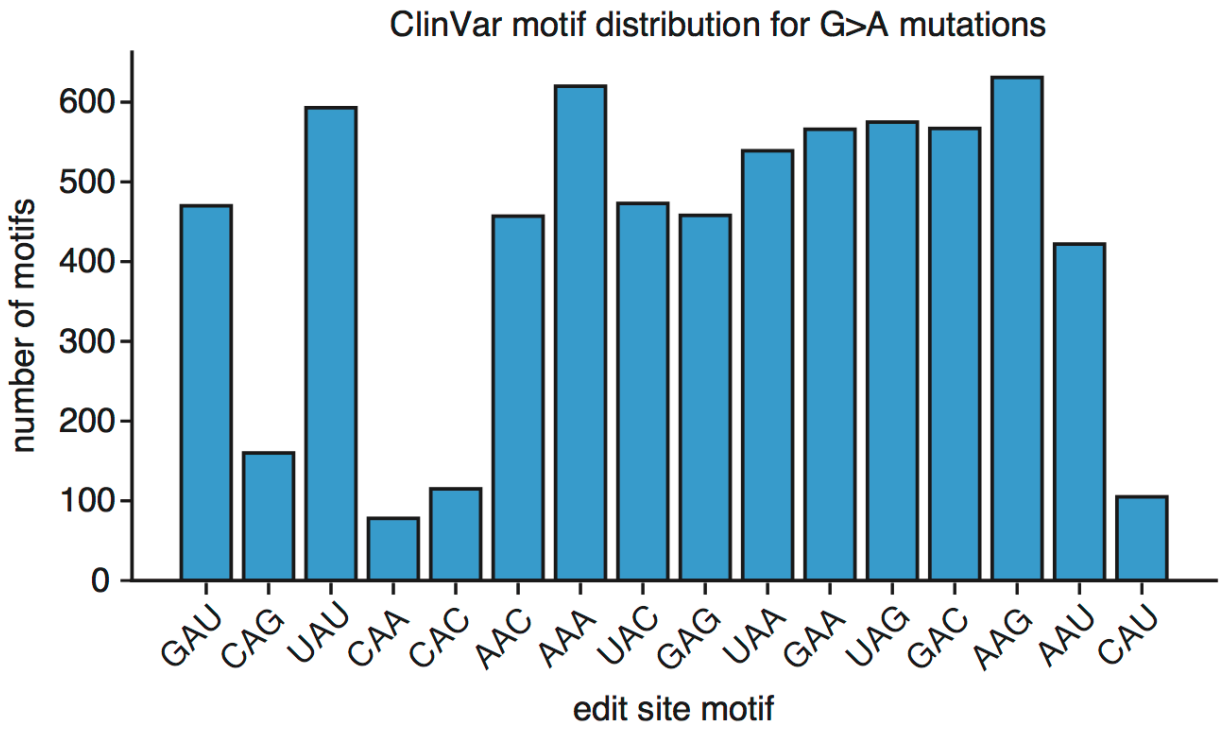

Figure S6: ClinVar motif distribution for G>A mutations.

The number of each possible triplet motif observed in the ClinVar database for all G>A mutations.

Figure S7

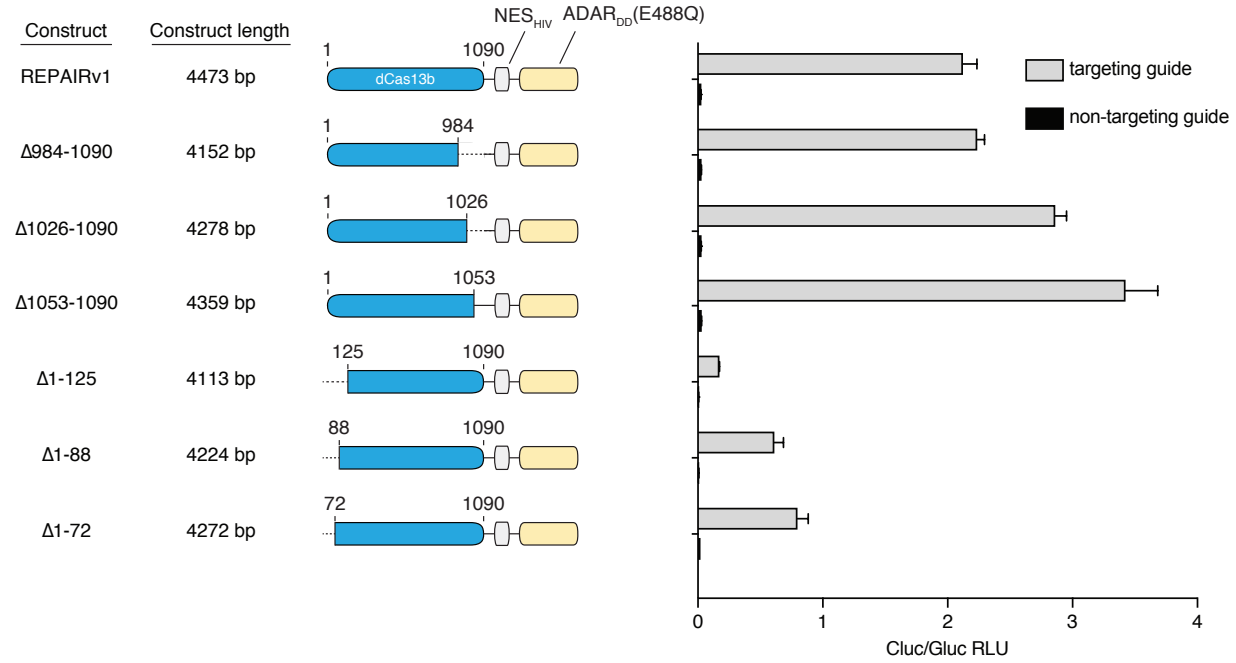

Figure S7: Truncations of dCas13b support functional RNA editing.

N-terminal and C-terminal truncations of dCas13b allow for RNA editing as measured by restoration of luciferase signal for the *Cluc* W85X reporter. Values represent mean +/– S.E.M. The construct length refers to the coding sequence of the REPAIR constructs.

Figure S8

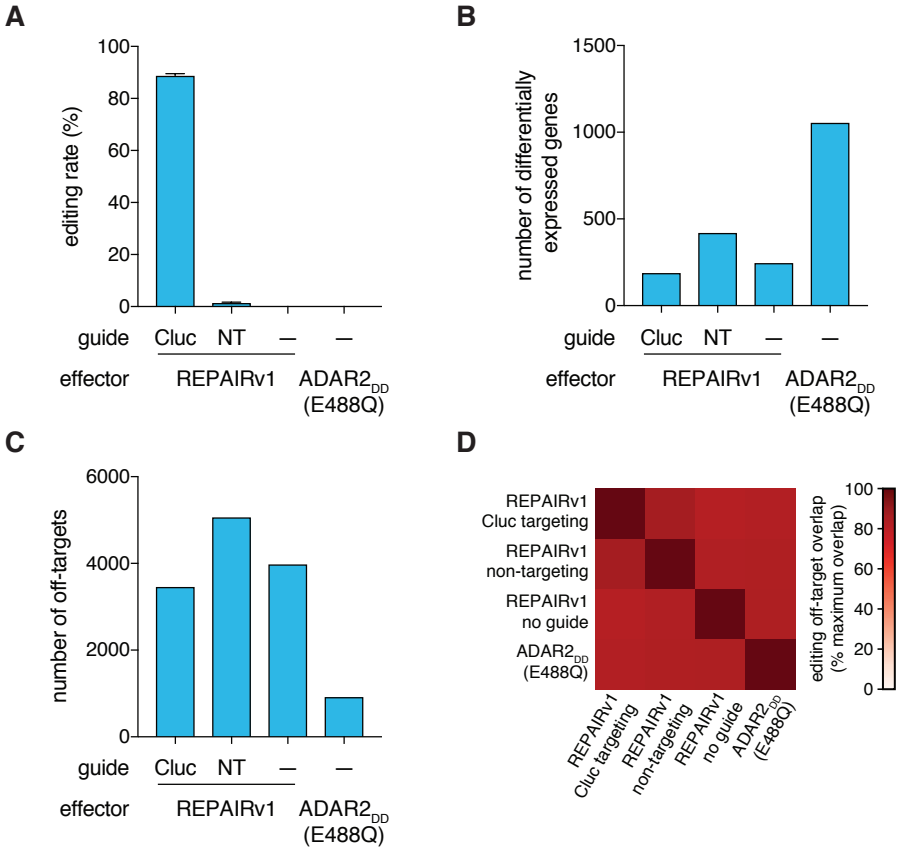

Figure S8: REPAIRv1 editing activity evaluated without a guide and in comparison to ADAR2 deaminase domain alone.

A) Quantification of A to I editing of the *Cluc* W85X mutation by REPAIRv1 with and without guide as well as the ADAR2 deaminase domain only without guide. Values represent mean +/– S.E.M. Non-targeting guide is the same as in Fig2C.

B) Number of differentially expressed genes in the REPAIRv1 and ADAR2$_{DD}$ conditions from panel A.

C) The number of significant off-targets from the REPAIRv1 and ADAR2$_{DD}$ conditions from panel A.

D) Overlap of off-target A to I editing events between the REPAIRv1 and ADAR2$_{DD}$ conditions from panel A. The values plotted are the percent of the maximum possible intersection of the two off-target data sets.

Figure S9

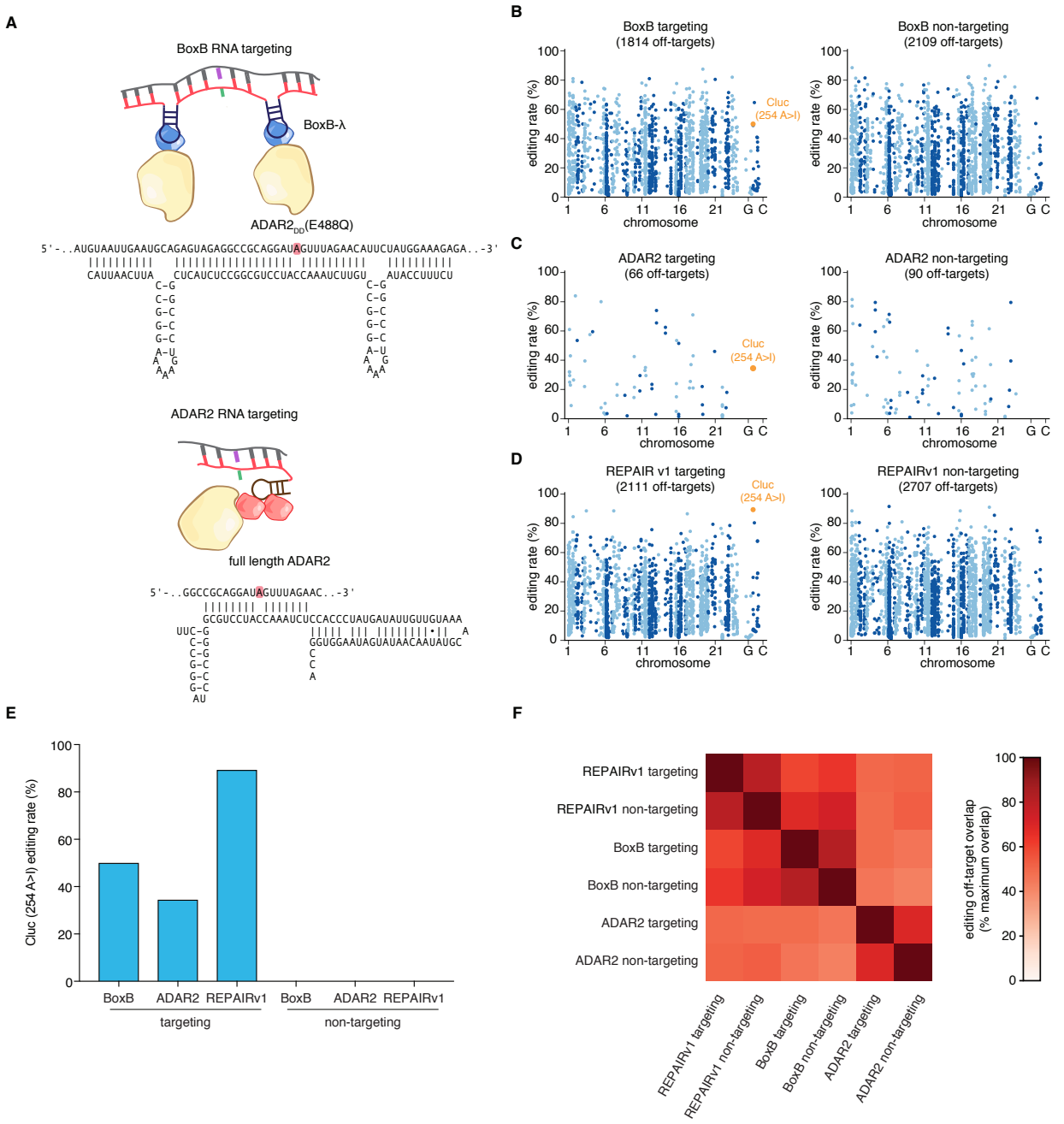

Figure S9: Comparison of REPAIRv1 to other programmable ADAR systems.

A) Schematic of two programmable ADAR schemes: BoxB-based targeting (top) and full length ADAR2 targeting (bottom). For BoxB-based targeting, $ADAR_{DD}$(E488Q) is fused to the viral protein lambda N (BoxB-ƛ), and the fusion protein is recruited to target adenosines by a guide RNA containing homology to the target site and hairpins that BoxB-ƛ binds to. Full length ADAR2 targeting utilizes a guide RNA with homology to the target site and a motif recognized

by the double strand RNA binding domains of ADAR2.

B) Transcriptome-wide sites of significant RNA editing by BoxB-ADAR2 $_{DD}$(E488Q) with a guide targeting *Cluc* and a non-targeting guide. The on-target *Cluc* site (254 A>I) is highlighted in orange.

C) Transcriptome-wide sites of significant RNA editing by full length ADAR2 with a guide targeting *Cluc* and a non-targeting guide. The on-target *Cluc* site (254 A>I) is highlighted in orange.

D) Transcriptome-wide sites of significant RNA editing by REPAIRv1 with a guide targeting *Cluc* and a non-targeting guide. The on-target *Cluc* site (254 A>I) is highlighted in orange. The non-targeting guide is the same as in Fig2C.

E) Quantification of on-target editing rate percentage for BoxB-ADAR2 $_{DD}$(E488Q), ADAR2, and REPAIRv1 for targeting guides against *Cluc.*

F) Overlap of off-target sites between different targeting and non-targeting conditions for programmable ADAR systems. The values plotted are the percent of the maximum possible intersection of the two off-target data sets.

Figure S10

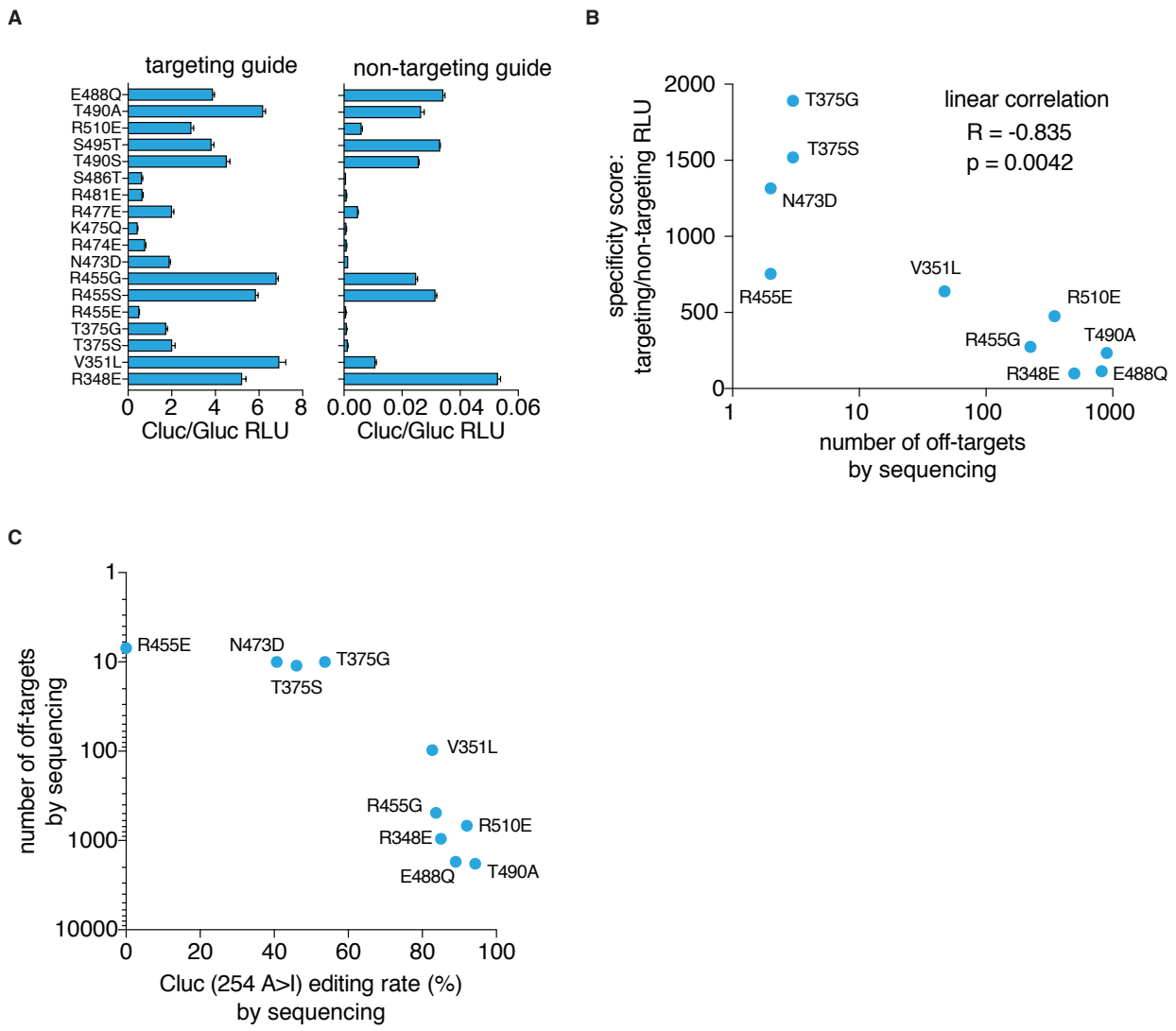

Figure S10: Efficiency and specificity of dCas13b-ADAR2$_{DD}$(E488Q) mutants.

A) Quantification of luciferase activity restoration by dCas13b-ADAR2$_{DD}$(E488Q) mutants for *Cluc*-targeting and non-targeting guides. Non-targeting guide is the same as in Fig2C.

B) Relationship between the ratio of targeting and non-targeting guide RLU and the number of RNA-editing off-targets as quantified by transcriptome-wide sequencing

C) Quantification of transcriptome-wide off-target RNA editing sites versus on-target *Cluc* editing efficiency for dCas13b-ADAR2$_{DD}$(E488Q) mutants.

Figure S11

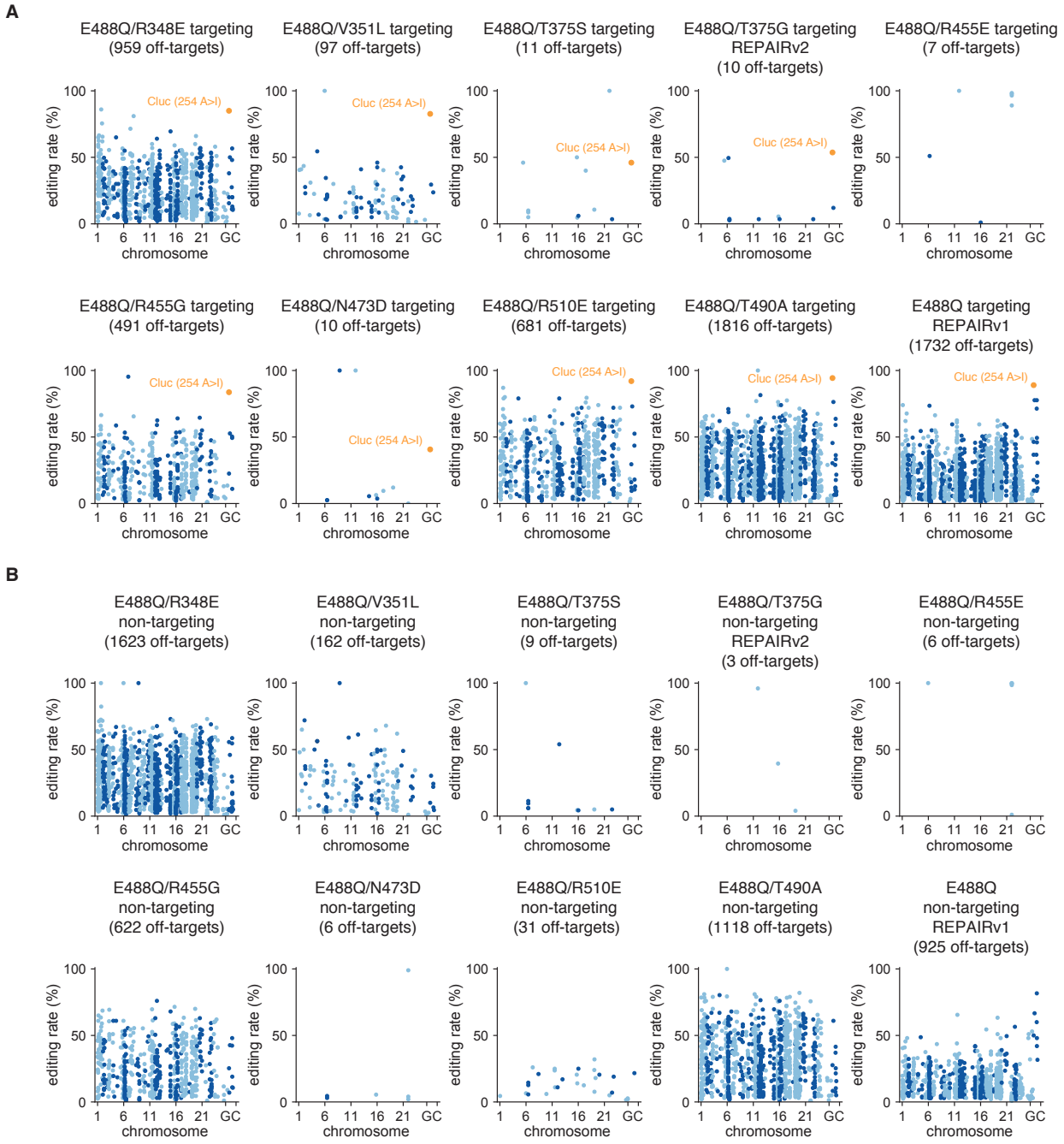

Figure S11: Transcriptome-wide specificity of RNA editing by dCas13b-ADAR2$_{DD}$(E488Q).

A) Transcriptome-wide sites of significant RNA editing by dCas13b-ADAR2$_{DD}$(E488Q) mutants with a guide targeting *Cluc*. The on-target *Cluc* site (254 A>I) is highlighted in orange.

B) Transcriptome-wide sites of significant RNA editing by dCas13b-ADAR2$_{DD}$(E488Q) mutants with a non-targeting guide.

Figure S12

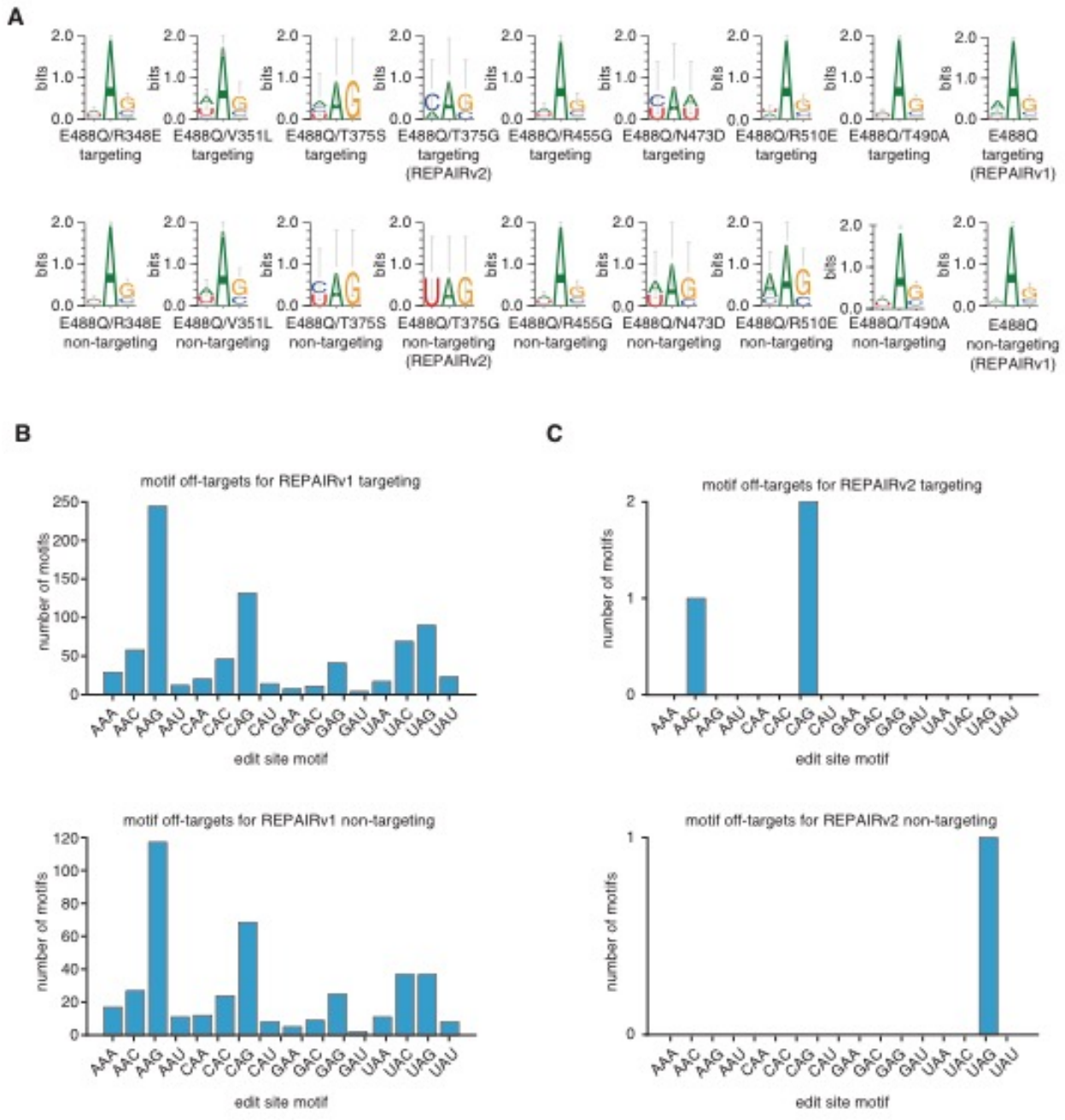

Figure S12: Characterization of motif biases in the off-targets of dCas13b-ADAR2 $_{DD}$(E488Q) editing.

A) For each dCas13b-ADAR2 $_{DD}$(E488Q) mutant, the motif present across all A>I off-target edits in the transcriptome is shown.

B) The distribution of off-target A>I edits per motif identity is shown for REPAIRv1 with targeting

and non-targeting guide.

C) The distribution of off-target A>I edits per motif identity is shown for REPAIRv2 with targeting and non-targeting guide.

Figure S13

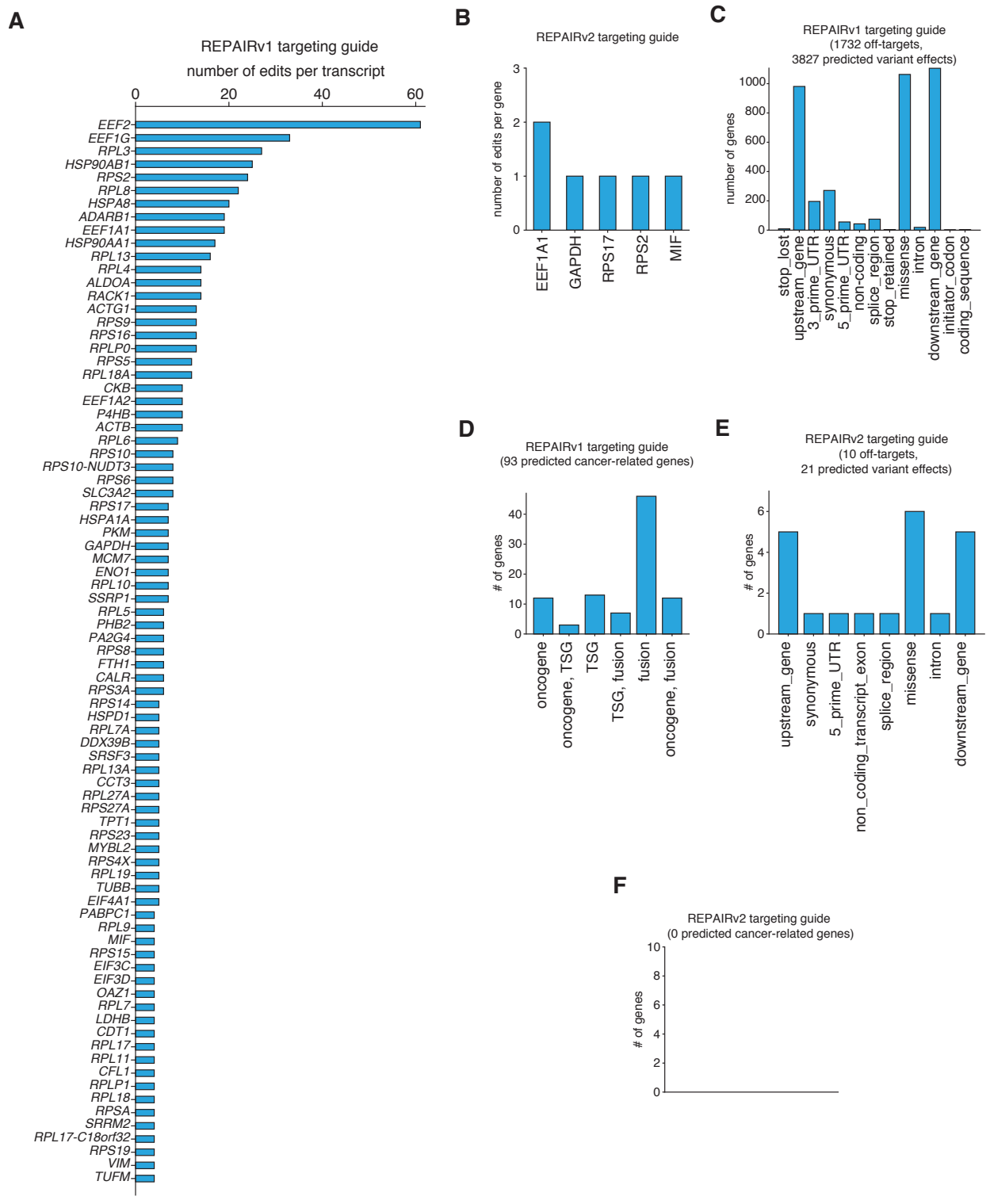

Figure S13: Further characterization of REPAIRv1 and REPAIRv2 off-targets.

A) Histogram of the number of off-targets per transcript for REPAIRv1.

B) Histogram of the number of off-targets per transcript for REPAIRv2.

C) Variant effect prediction of REPAIRv1 off targets.

D) Distribution of REPAIRv1 off targets in cancer-related genes. TSG, tumor suppressor gene.

E) Variant effect prediction of REPAIRv2 off targets.

F) Distribution of REPAIRv2 off targets in cancer-related genes.

Figure S14

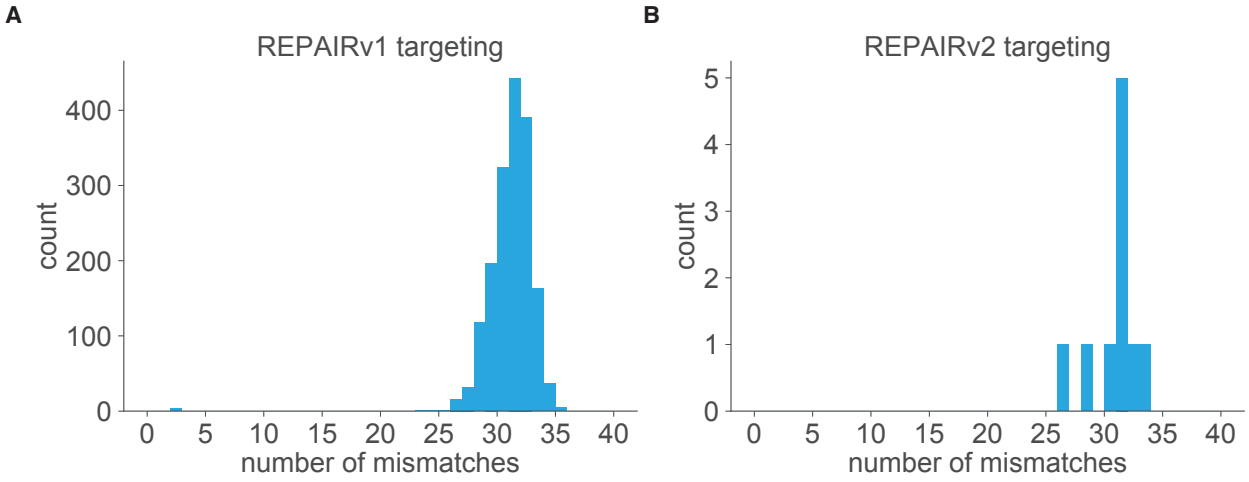

Figure S14: Evaluation of off-target sequence similarity to the guide sequence.

A) Distribution of the number of mismatches (hamming distance) between the targeting guide sequence and the off-target editing sites for REPAIRv1 with a Cluc targeting guide.

B) Distribution of the number of mismatches (hamming distance) between the targeting guide sequence and the off-target editing sites for REPAIRv2 with a Cluc targeting guide.

Figure S15

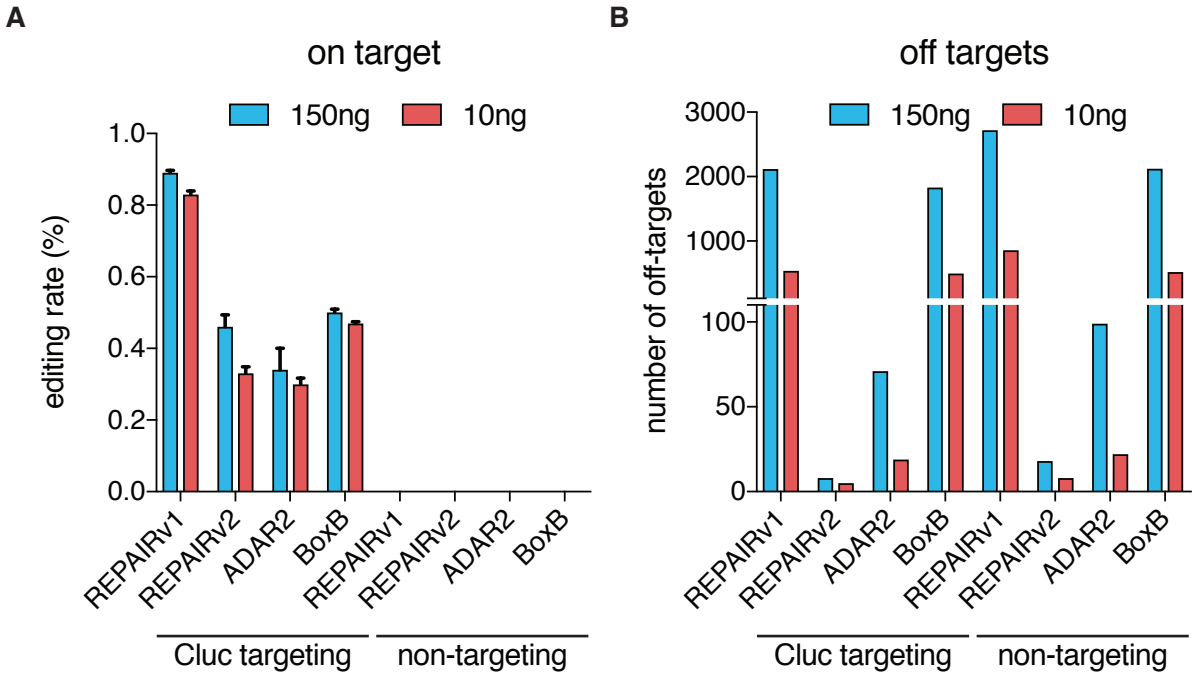

Figure S15: Comparison of REPAIRv1, REPAIRv2, ADAR2 RNA targeting, and BoxB RNA targeting at two different doses of vector (150ng and 10ng effector).

A) Quantification of RNA editing activity at the *Cluc* W85X (254 A>I) on-target editing site by REPAIRv1, REPAIRv2, ADAR2 RNA targeting, and BoxB RNA targeting approaches. Each of the four methods were tested with a targeting or non-targeting guide. Values shown are the mean of the three replicates.

B) Quantification of RNA editing off-targets by REPAIRv1, REPAIRv2, ADAR2 RNA targeting, and BoxB RNA targeting approaches. Each of the four methods were tested with a targeting guide for the *Cluc* W85X (254 A>I) site or non-targeting guide. For REPAIR constructs, non-targeting guide is the same as in Fig. 2C.

Figure S16

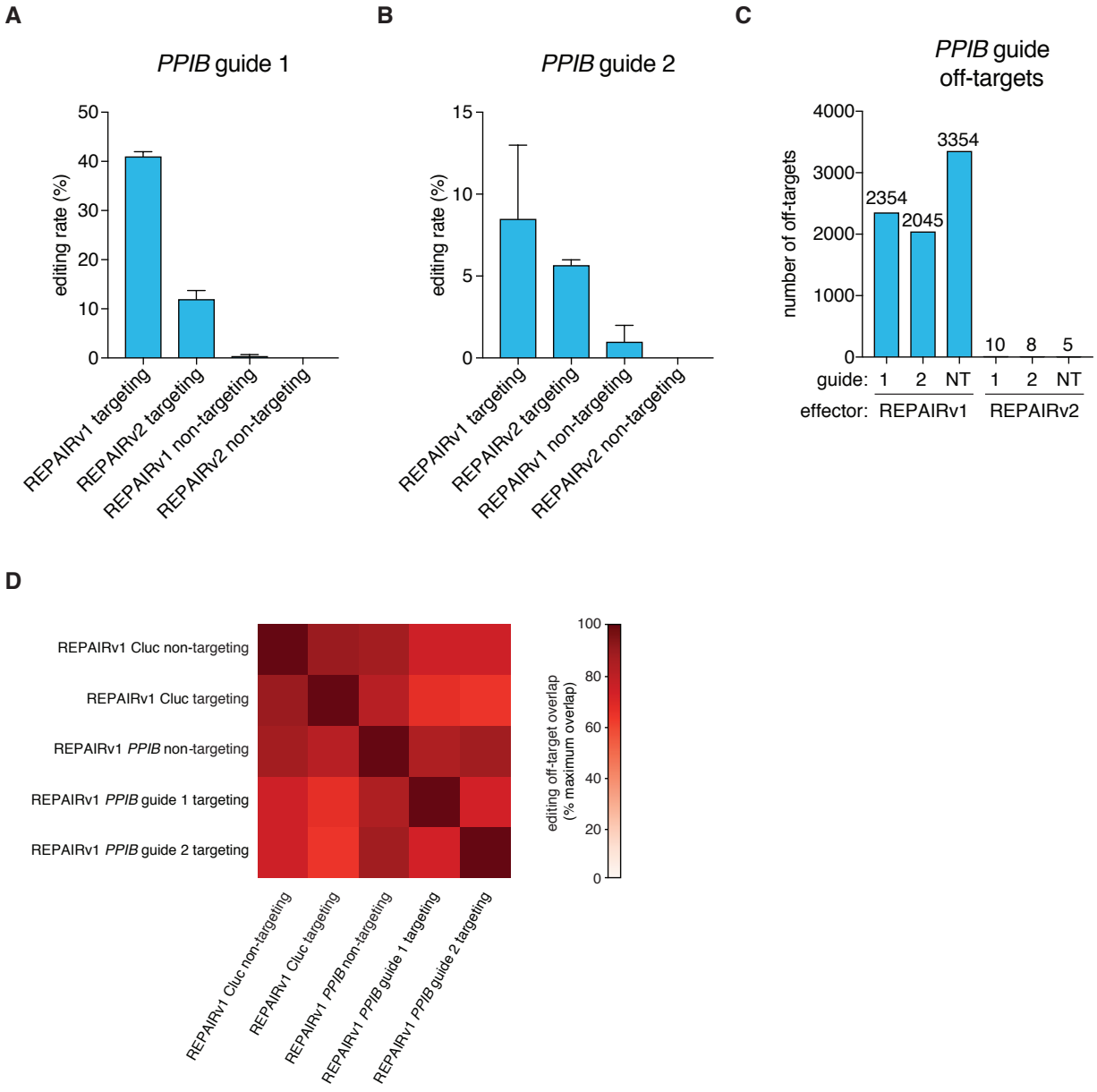

Figure S16: RNA editing efficiency and genome-wide specificity of REPAIRv1 and REPAIRv2.

A) Quantification of RNA editing activity at the *PPIB* guide 1 on-target editing site by REPAIRv1, REPAIRv2 with targeting and non-targeting guides. Values represent mean +/– S.E.M.

B) Quantification of RNA editing activity at the *PPIB* guide 2 on-target editing site by REPAIRv1, REPAIRv2 with targeting and non-targeting guides. Values represent mean +/– S.E.M.

C) Quantification of RNA editing off-targets by REPAIRv1 or REPAIRv2 with *PPIB* guide 1, *PPIB*

guide 2, or non-targeting guide.

D) Overlap of off-targets between REPAIRv1 for *PPIB* targeting, Cluc targeting, and non-targeting guides. The values plotted are the percent of the maximum possible intersection of the two off-target data sets.

Figure S17

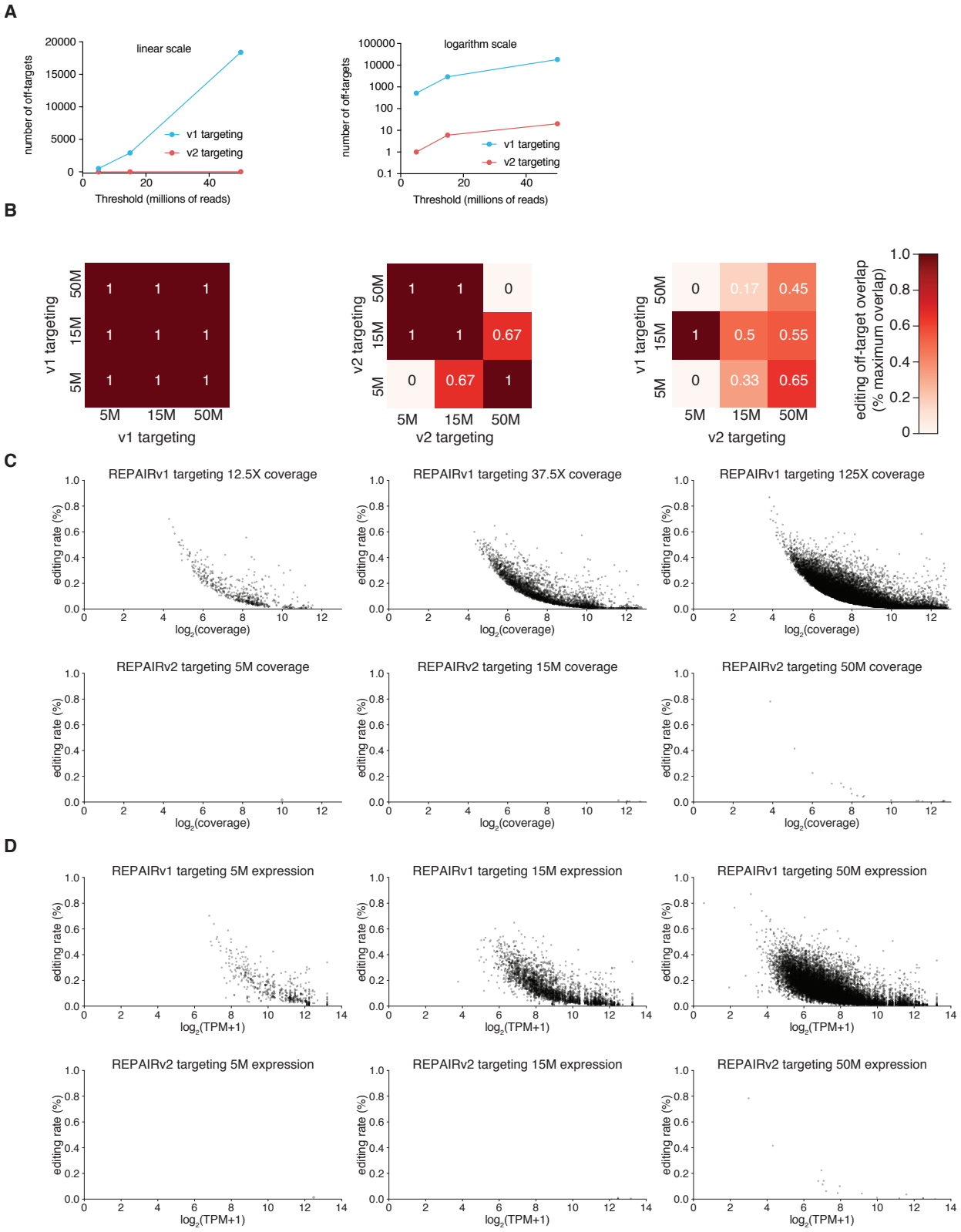

Figure S17: High coverage sequencing of REPAIRv1 and REPAIRv2 off-targets.

A) Quantitation of off-target edits for REPAIRv1 and REPAIRv2 as a function of read depth with a total of 5 million reads (12.5x coverage), 15 million reads (37.5x coverage) and 50 million reads (125x coverage) per condition.
B) Overlap of off-target sites at different read depths of the following conditions: REPAIRv1 versus REPAIRv1 (left), REPAIRv2 versus REPAIRv2 (middle), and REPAIRv1 versus REPAIRv2 (right). The values plotted are the percent of the maximum possible intersection of the two off-target data sets.
C) Editing rate of off-target sites compared to the coverage (log2(number of reads)) of the off-target for REPAIRv1 and REPAIRv2 targeting conditions at different read depths.
D) Editing rate of off-target sites compared to the log2(TPM+1) of the off-target gene expression for REPAIRv1 and REPAIRv2 targeting conditions at different read depths.

Figure S18

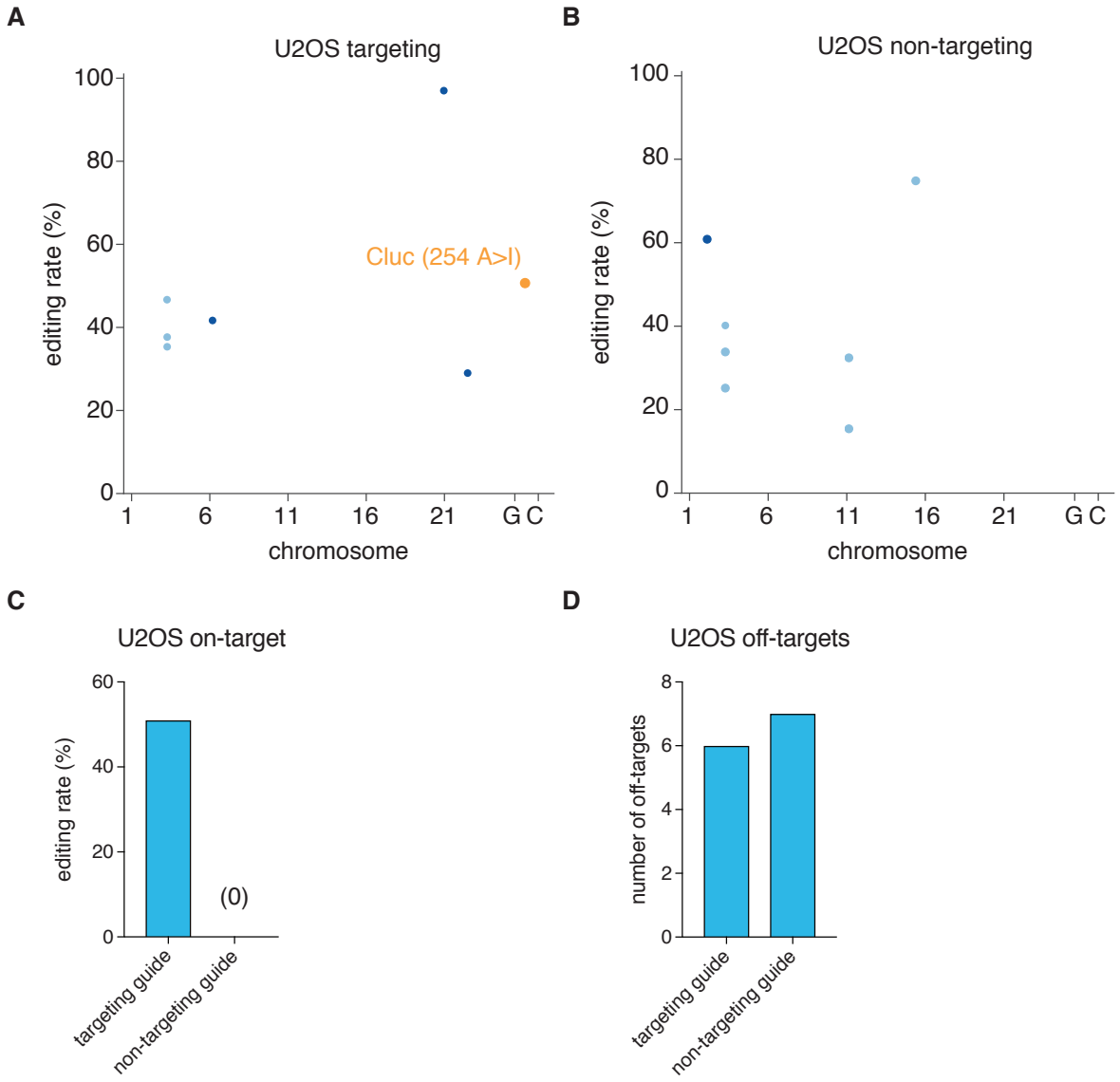

Figure S18: Quantification of REPAIRv2 activity and off-targets in the U2OS cell line.

A) Transcriptome-wide sites of significant RNA editing by REPAIRv2 with a guide targeting *Cluc* in the U2OS cell line. The on-target *Cluc* site (254 A>I) is highlighted in orange.

B) Transcriptome-wide sites of significant RNA editing by REPAIRv2 with a non-targeting guide in the U2OS cell line.

C) The on-target editing rate at the *Cluc* W85X (254 A>I) by REPAIRv2 with a targeting guide or non-targeting guide in the U2OS cell line.

D) Quantification of off-targets by REPAIRv2 with a guide targeting *Cluc* or non-targeting guide in the U2OS cell line.

Figure S19

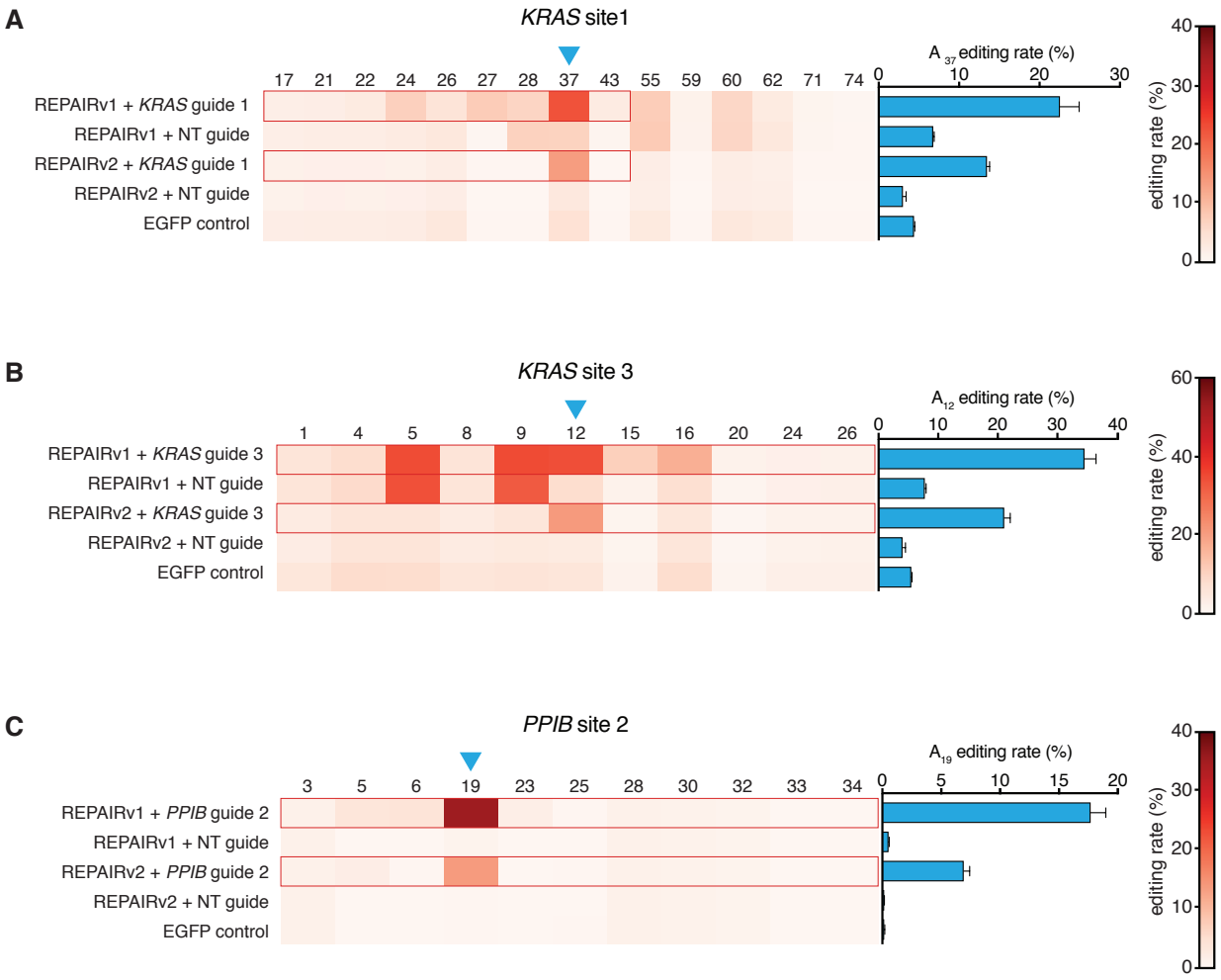

Figure S19: RNA editing efficiency and specificity of REPAIRv1 and REPAIRv2.

A) Quantification of percent editing of *KRAS* with *KRAS*-targeting guide 1 at the targeted adenosine (blue triangle) and neighboring sites for REPAIRv1 and REPAIRv2. For each guide, the region of duplex RNA is outlined in red. Values represent mean +/– S.E.M. Non-targeting guide is the same as in Fig. 2C.

B) Quantification of percent editing of *KRAS* with *KRAS*-targeting guide 3 at the targeted adenosine and neighboring sites for REPAIRv1 and REPAIRv2. Non-targeting guide is the same as in Fig. 2C.

C) Quantification of percent editing of *PPIB* with *PPIB*-targeting guide 2 at the targeted adenosine and neighboring sites for REPAIRv1 and REPAIRv2. Non-targeting guide is the same as in Fig. 2C.

Figure S20

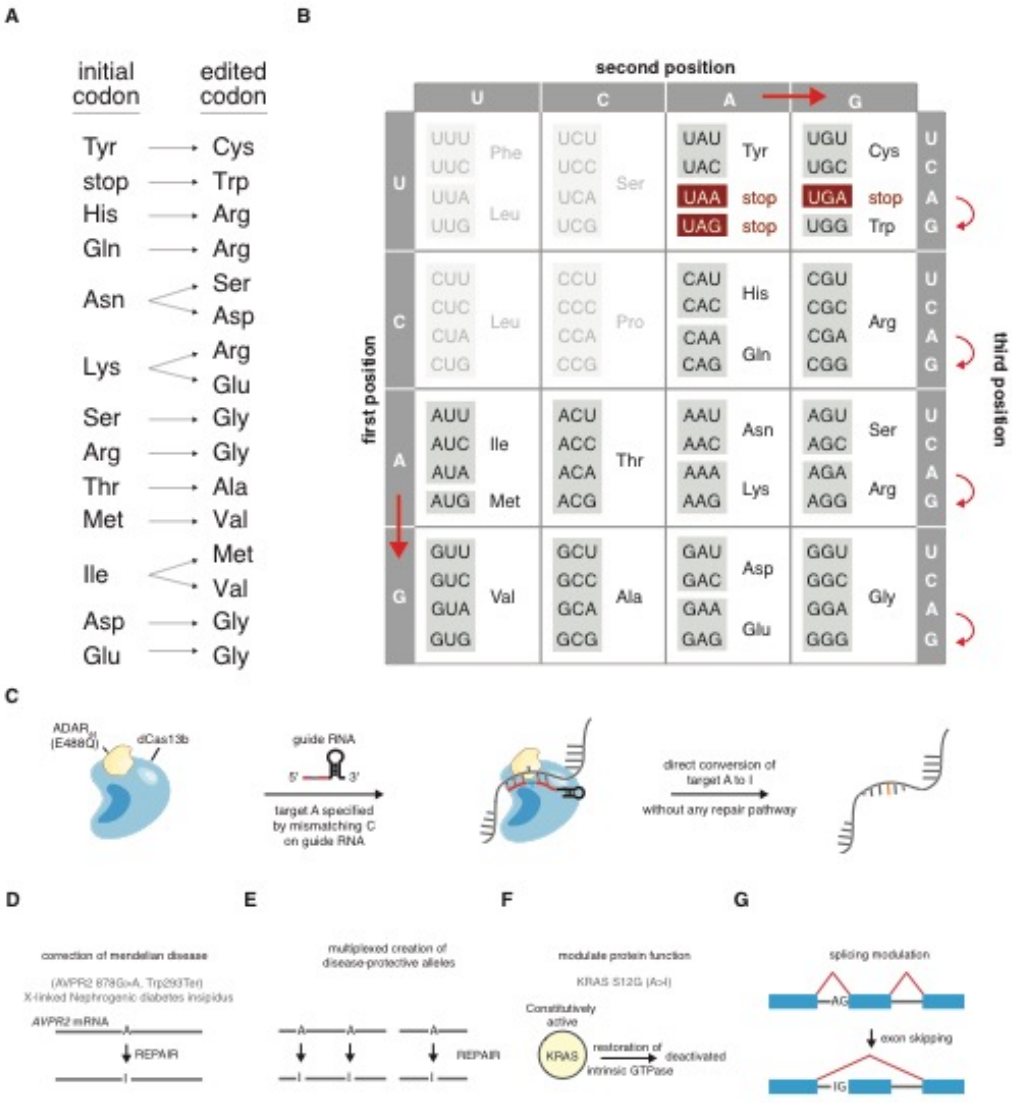

Figure S20: Demonstration of all potential codon changes with an A>I RNA editor.

A) Table of all potential codon transitions enabled by A>I editing.

B) A codon table demonstrating all the potential codon transitions enabled by A>I editing. Adapted and modified based on (*38*).

C) Model of REPAIR A to I editing of a precisely encoded nucleotide via a mismatch in the guide sequence. The A to I transition is mediated by the catalytic activity of the ADAR2 deaminase

domain and will be read as a guanosine by translational machinery. The base change does not rely on endogenous repair machinery and is permanent for as long as the RNA molecule exists in the cell.

D) REPAIR can be used for correction of Mendelian disease mutations.

E) REPAIR can be used for multiplexed A to I editing of multiple variants for engineering pathways or modifying disease. Multiplexed guide delivery can be achieved by delivering a single CRISPR array expression cassette since the Cas13b enzyme processes its own array.

F) REPAIR can be used for modifying protein function through amino acid changes that affect enzyme domains, such as kinases.

G) REPAIR can modulate splicing of transcripts by modifying the splice acceptor site.

Supplementary Table 1: Cas13 Orthologs used in this study.

Cas13 ID	Cas13 abbreviation	Host Organism	Protein Accession
Cas13a1	LshCas13a	*Leptotrichia shahii*	WP_018451595.1
Cas13a2	LwaCas13a	*Leptotrichia wadei* (Lw2)	WP_021746774.1
Cas13a3	LseCas13a	*Listeria seeligeri*	WP_012985477.1
Cas13a4	LbmCas13a	*Lachnospiraceae* bacterium MA2020	WP_044921188.1
Cas13a5	LbnCas13a	*Lachnospiraceae* bacterium NK4A179	WP_022785443.1
Cas13a6	CamCas13a	[*Clostridium*] *aminophilum* DSM 10710	WP_031473346.1
Cas13a7	CgaCas13a	*Carnobacterium gallinarum* DSM 4847	WP_034560163.1
Cas13a8	Cga2Cas13a	*Carnobacterium gallinarum* DSM 4847	WP_034563842.1
Cas13a9	Pprcas13a	*Paludibacter propionicigenes* WB4	WP_013443710.1
Cas13a10	LweCas13a	*Listeria weihenstephanensis* FSL R9-0317	WP_036059185.1
Cas13a11	LbfCas13a	*Listeriaceae* bacterium FSL M6-0635	WP_036091002.1
Cas13a12	Lwa2cas13a	*Leptotrichia wadei* F0279	WP_021746774.1
Cas13a13	RcsCas13a	*Rhodobacter capsulatus* SB 1003	WP_013067728.1
Cas13a14	RcrCas13a	*Rhodobacter capsulatus* R121	WP_023911507.1
Cas13a15	RcdCas13a	*Rhodobacter capsulatus* DE442	WP_023911507.1
Cas13a16	LbuCas13a	*Leptotrichia buccalis* C-1013-b	WP_015770004.1
Cas13a17	HheCas13a	*Herbinix hemicellulosilytica*	CRZ35554.1
Cas13a18	EreCas13a	[*Eubacterium*] *rectale*	WP_055061018.1
Cas13a19	EbaCas13a	*Eubacteriaceae* bacterium CHKCI004	WP_090127496.1
Cas13a20	BmaCas13a	*Blautia* sp. Marseille-P2398	WP_062808098.1
Cas13a21	LspCas13a	*Leptotrichia* sp. oral taxon 879 str. F0557	WP_021744063.1
Cas13b1	BzoCas13b	*Bergeyella zoohelcum*	WP_002664492
Cas13b2	PinCas13b	*Prevotella intermedia*	WP_036860899
Cas13b3	PbuCas13b	*Prevotella buccae*	WP_004343973
Cas13b4	AspCas13b	*Alistipes* sp. ZOR0009	WP_047447901
Cas13b5	PsmCas13b	*Prevotella* sp. MA2016	WP_036929175
Cas13b6	RanCas13b	*Riemerella anatipestifer*	WP_004919755
Cas13b7	PauCas13b	*Prevotella aurantiaca*	WP_025000926
Cas13b8	PsaCas13b	*Prevotella saccharolytica*	WP_051522484
Cas13b9	Pin2Cas13b	*Prevotella intermedia*	WP_061868553
Cas13b10	CcaCas13b	*Capnocytophaga canimorsus*	WP_013997271

Cas13b11	PguCas13b	*Porphyromonas gulae*	WP_039434803
Cas13b12	PspCas13b	*Prevotella* sp. P5-125	WP_044065294
Cas13b13	FbrCas13b	*Flavobacterium branchiophilum*	WP_014084666
Cas13b14	PgiCas13b	*Porphyromonas gingivalis*	WP_053444417
Cas13b15	Pin3Cas13b	*Prevotella intermedia*	WP_050955369
Cas13c1	FnsCas13c	*Fusobacterium necrophorum* subsp. funduliforme ATCC 51357 contig00003	WP_005959231.1
Cas13c2	FndCas13c	F*usobacterium necrophorum* DJ-2 contig0065, whole genome shotgun sequence	WP_035906563.1
Cas13c3	FnbCas13c	*Fusobacterium necrophorum* BFTR-1 contig0068	WP_035935671.1
Cas13c4	FnfCas13c	*Fusobacterium necrophorum* subsp. funduliforme 1_1_36S cont1.14	EHO19081.1
Cas13c5	FpeCas13c	*Fusobacterium perfoetens* ATCC 29250 T364DRAFT_scaffold00009.9_C	WP_027128616.1
Cas13c6	FulCas13c	*Fusobacterium ulcerans* ATCC 49185 cont2.38	WP_040490876.1
Cas13c7	AspCas13c	*Anaerosalibacter* sp. ND1 genome assembly Anaerosalibacter massiliensis ND1	WP_042678931.1

Supplementary Table 2: PFS cutoffs in bacterial screens

Cas13b ortholog	Key	-Log_2 depletion score used to generate PFS motif
Bergeyella zoohelcum	1	2
Prevotella intermedia locus 1	2	1
Prevotella buccae	3	3
Alistipes sp. ZOR0009	4	1
Prevotella sp. MA2016	5	2
Riemerella anatipestifer	6	4
Prevotella aurantiaca	7	1
Prevotella saccharolytica	8	0
Prevotella intermedia locus 2	9	0
Capnocytophaga canimorsus	10	3
Porphyromonas gulae	11	4
Prevotella sp. P5-125	12	2.1
Flavobacterium branchiophilum	13	1
Porphyromonas gingivalis	14	3
Prevotella intermedia locus 2	15	4

Supplementary Table 3: dCas13b-ADAR linker sequences used in this study for RNA editing in mammalian cells.

Figure	**linker**
2C	GSGGGGS
2E	GS
S3B	GSGGGGS
S3C	GS
S3D	GS
S3E: GS	GS
S3E: GSGGGGS	GSGGGGS
S3E: (GGGS)3	GGGGSGGGGSGGGGS
S3E: Rigid	EAAAK
S3E: (GGS)6	GGSGGSGGSGGSGGSGGS
S3E: XTEN	SGSETPGTSESATPES
3B	GS
S3F	GS
3C	GS
4B	GS
4D	GS
4E	GS
S5A: Δ984-1090, Δ1026-1090, Δ1053-1090	GS
S5A: Δ1-125, Δ1-88, Δ1-72	GSGGGGS
5B	GS
5C	GS
5D	GS
S6A	GS
S6C	GS
S6D	GS
S7D	GS
6A	GS
S8A	GS
6B	GS
S8B	GS
S8C	GS
S9A	GS
S9B	GS
6C	GS
6D	GS
6E	GS
6F	GS

S13A	GS
S13B	GS

Supplementary Table 4: Disease information for disease-relevant mutations

Full length candidates	**Gene**	**Disease**
NM_000054.4(AVPR2):c.878G>A (p.Trp293Ter)	*AVPR2*	Nephrogenic diabetes insipidus, X-linked
NM_000136.2(FANCC):c.1517G>A (p.Trp506Ter)	*FANCC*	Fanconi anemia, complementation group C
Additional simulated candidates		
Candidate	**Gene**	**Disease**
NM_000206.2(IL2RG):c.710G>A (p.Trp237Ter)	*IL2RG*	X-linked severe combined immunodeficiency
NM_000132.3(F8):c.3144G>A (p.Trp1048Ter)	*F8*	Hereditary factor VIII deficiency disease
NM_000527.4(LDLR):c.1449G>A (p.Trp483Ter)	*LDLR*	Familial hypercholesterolemia
NM_000071.2(CBS):c.162G>A (p.Trp54Ter)	*CBS*	Homocystinuria due to CBS deficiency
NM_000518.4(HBB):c.114G>A (p.Trp38Ter)	*HBB*	betaThalassemia
NM_000035.3(ALDOB):c.888G>A (p.Trp296Ter)	*ALDOB*	Hereditary fructosuria
NM_004006.2(DMD):c.3747G>A (p.Trp1249Ter)	*DMD*	Duchenne muscular dystrophy
NM_005359.5(SMAD4):c.906G>A (p.Trp302Ter)	*SMAD4*	Juvenile polyposis syndrome
NM_000059.3(BRCA2):c.582G>A (p.Trp194Ter)	*BRCA2*	Familial cancer of breast|Breast-ovarian cancer, familial 2
NM_000833.4(GRIN2A):c.3813G>A (p.Trp1271Ter)	*GRIN2A*	Epilepsy, focal, with speech disorder and with or without mental retardation
NM_002977.3(SCN9A):c.2691G>A (p.Trp897Ter)	*SCN9A*	Indifference to pain, congenital, autosomal recessive
NM_007375.3(TARDBP):c.943G>A (p.Ala315Thr)	*TARDBP*	Amyotrophic lateral sclerosis type 10
NM_000492.3(CFTR):c.3846G>A (p.Trp1282Ter)	*CFTR*	Cystic fibrosis|Hereditary pancreatitis|not provided|ataluren response - Efficacy
NM_130838.1(UBE3A):c.2304G>A (p.Trp768Ter)	*UBE3A*	Angelman syndrome
NM_000543.4(SMPD1):c.168G>A (p.Trp56Ter)	*SMPD1*	Niemann-Pick disease, type A
NM_206933.2(USH2A):c.9390G>A (p.Trp3130Ter)	*USH2A*	Usher syndrome, type 2A
NM_130799.2(MEN1):c.1269G>A (p.Trp423Ter)	*MEN1*	Hereditary cancer-predisposing syndrome
NM_177965.3(C8orf37):c.555G>A (p.Trp185Ter)	*C8orf37*	Retinitis pigmentosa 64
NM_000249.3(MLH1):c.1998G>A (p.Trp666Ter)	*MLH1*	Lynch syndrome
NM_000548.4(TSC2):c.2108G>A (p.Trp703Ter)	*TSC2*	Tuberous sclerosis 2|Tuberous sclerosis syndrome

NM_000267.3(NF1):c.7044G>A (p.Trp2348Ter)	*NF1*	Neurofibromatosis, type 1
NM_000179.2(MSH6):c.3020G>A (p.Trp1007Ter)	*MSH6*	Lynch syndrome
NM_000344.3(SMN1):c.305G>A (p.Trp102Ter)	*SMN1*	Spinal muscular atrophy, type II|Kugelberg-Welander disease
NM_024577.3(SH3TC2):c.920G>A (p.Trp307Ter)	*SH3TC2*	Charcot-Marie-Tooth disease, type 4C
NM_001369.2(DNAH5):c.8465G>A (p.Trp2822Ter)	*DNAH5*	Primary ciliary dyskinesia
NM_004992.3(MECP2):c.311G>A (p.Trp104Ter)	*MECP2*	Rett syndrome
NM_032119.3(ADGRV1):c.7406G>A (p.Trp2469Ter)	*ADGRV1*	Usher syndrome, type 2C
NM_017651.4(AHI1):c.2174G>A (p.Trp725Ter)	*AHI1*	Joubert syndrome 3
NM_004562.2(PRKN):c.1358G>A (p.Trp453Ter)	*PRKN*	Parkinson disease 2
NM_000090.3(COL3A1):c.3833G>A (p.Trp1278Ter)	*COL3A1*	Ehlers-Danlos syndrome, type 4
NM_007294.3(BRCA1):c.5511G>A (p.Trp1837Ter)	*BRCA1*	Familial cancer of breast|Breast-ovarian cancer, familial 1
NM_000256.3(MYBPC3):c.3293G>A (p.Trp1098Ter)	*MYBPC3*	Primary familial hypertrophic cardiomyopathy
NM_000038.5(APC):c.1262G>A (p.Trp421Ter)	*APC*	Familial adenomatous polyposis 1
NM_001204.6(BMPR2):c.893G>A (p.W298*)	*BMPR2*	Primary pulmonary hypertension

Supplementary Table 5: Key plasmids used in this study

Plasmid	Description	**Benchling link**
pC0037	CMV-Cluciferase-polyA EF1a-G-luciferase-polyA	https://benchling.com/s/seq-GMa3RAbt0JkjT8kX9aRa
pC0038	CMV-Cluciferase(W85X)-polyA EF1a-G-luciferase-polyA	https://benchling.com/s/seq-W2n4wX4vSUuslGzYgYO5
pC0039	CMV-dCas13b12-GS-ADAR2DD(E488Q)	https://benchling.com/s/seq-arzpsupZEzGu3ghBDhtv
pC0040	LwaCas13a crRNA backbone	https://benchling.com/s/seq-0SqKieU2CWyd3RRawuKp
pC0041	RanCas13b crRNA backbone	https://benchling.com/s/seq-yKHvxw5C84w9inEx3XaU
pC0042	PguCas13b crRNA backbone	https://benchling.com/s/seq-ZLKtRrNkhNw0BOzcgdW5
pC0043	PspCas13b crRNA backbone	https://benchling.com/s/seq-OH6nMmZCZn930BWqcFNa
pC0044	EF1a-BsiWI-Cas13b6-NES-mapk	https://benchling.com/s/seq-hxOBIW6sDZE1o4DMz6lZ
pC0045	EF1a-BsiWI-Cas13b11-NES-HIV	https://benchling.com/s/seq-GYuyzloHGlD8CNO4TCSy
pC0046	EF1a-BsiWI-Cas13b12-NES-HIV	https://benchling.com/s/seq-g62SIhluOlRdD8aArJaC
pC0047	CMV-dCas13b12-ADAR1DD(E1008Q)	https://benchling.com/s/seq-R3zRpb4whgEiZBoTvpgM
pC0048	CMV-dCas13b12-longlinker-ADAR2DD(E488Q)	https://benchling.com/s/seq-Y92Xyc1WxOlZDLMNv8K8
pC0049	EF1a-BsiWI-Cas13-B12-NES-HIV, H133A/H1058A	https://benchling.com/s/seq-lK5ZoHDkOCTPV0SwG7VD

pC0050	CMV-dCas13b12-longlinker-ADAR2DD(wt)	https://benchling.com/s/seq-YuFM6m06znFKA9txLrrw
pC0051	W85X REPAIR targeting guide	https://benchling.com/s/seq-pJjKdbYG6YdpAMKAyXE0
pC0052	REPAIR non-targeting guide	https://benchling.com/s/seq-U9gHnOW41C1DVUBGQypw
pC0053	CMV-dCas13b12-GS-ADAR2DD(E488Q)-delta-984-1090	https://benchling.com/s/seq-HASFia3255bkdC9iUtxu
pC0054	T375G specificity mutant	https://benchling.com/s/seq-lWXqpjFVHeqkLlHVFZ4t
pC0055	T375G Cas13b C-term delta 984-1090	https://benchling.com/s/seq-1KNBN52nxWXZgwekbbiO

Supplementary Table 6: Guide/shRNA sequences used in this study for knockdown in mammalian cells

Name	Spacer sequence	Interference Mechanism	Notes	First figure
Bacterial PFS guide	GCCAGCUUUCCGGGCAUUGGCUUCCAUC	Cas13b	Used for all orthologs	
Cas13a-Gluc guide 1	GCCAGCUUUCCGGGCAUUGGCUUCCAUC	Cas13a	Used for all Cas13a orthologs	Figure 1B
Cas13a-Gluc guide 2	ACCCAGGAAUCUCAGGAAUGUCGACGAU	Cas13a	Used for all Cas13a orthologs	Figure 1B
Cas13a-non-targeting guide (LacZ)	AGGGUUUUCCCAGUCACGACGUUGUAAA	Cas13a	Used for all Cas13a orthologs	Figure 1B
Cas13b-Gluc guide 1.1	GGGCAUUGGCUUCCAUCUCUUUGAGCACCU	Cas13b	Used for orthologs 1-3, 6, 7, 10, 11, 12, 14, 15	Figure 1B
Cas13b-Gluc guide 1.2	GUGCAGCCAGCUUUCCGGGCAUUGGCUUCC	Cas13b	Used for ortholog 4	Figure 1B
Cas13b-Gluc guide 1.3	GCAGCCAGCUUUCCGGGCAUUGGCUUCCAU	Cas13b	Used for ortholog 5	Figure 1B
Cas13b-Gluc guide 1.4	GGCUUCCAUCUCUUUGAGCACCUCCAGCGG	Cas13b	Used for ortholog 8, 9	Figure 1B
Cas13b-Gluc guide 1.5	GGAAUGUCGACGAUCGCCUCGCCUAUGCCG	Cas13b	Used for ortholog 13	Figure 1B
Cas13b-	GAAUGUCGACGAUCGCCUCGCCUAUGCCGC	Cas13b	Used for	Figure 1B

Gluc guide 2.1			orthologs 1-3, 6, 7, 10, 11, 14, 15	
Cas13b-Gluc guide 2.2	GACCUGUGCGAUGAACUGCUCCAUGGGCUC	Cas13b	Used for ortholog 12	Figure 1B
Cas13b-Gluc guide 2.2	GUGUGGCAGCGUCCUGGGAUGAACUUCUUC	Cas13b	Used for ortholog 4	Figure 1B
Cas13b-Gluc guide 2.3	GUGGCAGCGUCCUGGGAUGAACUUCUUCAU	Cas13b	Used for ortholog 5	Figure 1B
Cas13b-Gluc guide 2.4	GCUUCUUGCCGGGCAACUUCCCGCGGUCAG	Cas13b	Used for ortholog 8, 9	Figure 1B
Cas13b-Gluc guide 2.6	GCAGGGUUUUCCCAGUCACGACGUUGUAAAA	Cas13b	Used for ortholog 13	Figure 1B
Cas13b-non targeting guide	GCAGGGUUUUCCCAGUCACGACGUUGUAAAA	Cas13b	Used for all orthologs	Figure 1B
Cas13a-Gluc guide-RNASeq	ACCCAGGAAUCUCAGGAAUGUCGACGAU	Cas13a		Figure 1E
shRNA-Gluc guide	CAGCUUUCCGGGCAUUGGCUU	shRNA		Figure 1F
Cas13b-Gluc guide-RNASeq	CCGCUGGAGGUGCUCAAAGAGAUGGAAGCC	Cas13b		Figure 1F
Cas13a-Gluc-guide-1	GCCAGCUUUCCGGGCAUUGGCUUCCAUC	Cas13a		Figure S2A
Cas13a-Gluc-guide-2	ACCCAGGAAUCUCAGGAAUGUCGACGAU	Cas13a		Figure S2A
Cas13b-Gluc-opt-guide-1	GGGCAUUGGCUUCCAUCUCUUUGAGCACCU	Cas13b		Figure S2A
Cas13b-Gluc-opt-guide-2	GAAUGUCGACGAUCGCCUCGCCUAUGCCGC	Cas13b		Figure S2A
Cas13a KRAS guide 1	CAAGGCACUCUUGCCUACGCCACCAGCU	Cas13a		Figure S2B

Cas13a KRAS guide 2	UCAUAUUCGUCCACAAAAUGAUUCUGAA	Cas13a		Figure S2B
Cas13a KRAS guide 3	AUUAUUUAUGGCAAAUACACAAAGAAAG	Cas13a		Figure S2B
Cas13a KRAS guide 4	GAAUAUCUUCAAAUGAUUUAGUAUUAUU	Cas13a		Figure S2B
Cas13a KRAS guide 5	ACCAUAGGUACAUCUUCAGAGUCCUUAA	Cas13a		Figure S2B
Cas13b KRAS guide 1	GUCAAGGCACUCUUGCCUACGCCACCAG CU	Cas13b		Figure S2B
Cas13b KRAS guide 2	GAUCAUAUUCGUCCACAAAAUGAUUCUG AA	Cas13b		Figure S2B
Cas13b KRAS guide 3	GUAUUAUUUAUGGCAAAUACACAAAGAA AG	Cas13b		Figure S2B
Cas13b KRAS guide 4	GUGAAUAUCUUCAAAUGAUUUAGUAUUA UU	Cas13b		Figure S2B
Cas13b KRAS guide 5	GGACCAUAGGUACAUCUUCAGAGUCCUU AA	Cas13b		Figure S2B
shRNA KRAS guide 1	aagagugccuugacgauacagcCUCGAG gcuguaucgucaaggcacucuu	shRNA		Figure S2B
shRNA KRAS guide 2	aaucauuuuguggacgaauauCUCGAGa uauucguccacaaaaugauu	shRNA		Figure S2B
shRNA KRAS guide 3	aaauaauacuaaaucauuugaCUCGAGu caaaugauuuaguauuauuu	shRNA		Figure S2B
shRNA KRAS guide 4	aauaauacuaaaucauuugaaCUCGAGu ucaaaugauuuaguauuauu	shRNA		Figure S2B
shRNA KRAS non-targeting guide	aaggacucugaagauguaccuCUCGAGa gguacaucuucagaguccuu	shRNA		Figure S2B

Supplementary Table 7: Guide sequences used for *Gluc* knockdown

Name	Spacer sequence	Position	Notes	First figure
Gluc tiling guide 1	GAGAUCAGGGCAAACAGAACUUUGACUCCC	2	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 2	GGAUGCAGAUCAGGGCAAACAGAACUUUGA	7	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 3	GCACAGCGAUGCAGAUCAGGGCAAACAGAA	13	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling	GCUCGGCCACAGCGAUGCAGAUCAGGGCAA	19	Note that the Cas13a spacers are	1C

guide 4			truncated by two nucleotides at the 5' end	
Gluc tiling guide 5	GGGGCUUGGCCUCGGCCACAGCGAUGCAGA	28	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 6	GUGGGCUUGGCCUCGGCCACAGCGAUGCAG	29	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 7	GUCUCGGUGGGCUUGGCCUCGGCCACAGCG	35	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 8	GUUCGUUGUUCUCGGUGGGCUUGGCCUCGG	43	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 9	GGAAGUCUUCGUUGUUCUCGGUGGGCUUGG	49	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 10	GAUGUUGAAGUCUUCGUUGUUCUCGGUGGG	54	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 11	GCGGCCACGAUGUUGAAGUCUUCGUUGUUC	62	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 12	GUGGCCACGGCCACGAUGUUGAAGUCUUCG	68	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 13	GGUUGCUGGCCACGGCCACGAUGUUGAAGU	73	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 14	GUCGCGAAGUUGCUGGCCACGGCCACGAUG	80	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 15	GCCGUGGUCGCGAAGUUGCUGGCCACGGCC	86	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 16	GCGAGAUCCGUGGUCGCGAAGUUGCUGGCC	92	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 17	GCAGCAUCGAGAUCCGUGGUCGCGAAGUUG	98	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 18	GGGUCAGCAUCGAGAUCCGUGGUCGCGAAG	101	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 19	GCUUCCCGCGGUCAGCAUCGAGAUCCGUGG	109	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 20	GGGGCAACUUCCCGCGGUCAGCAUCGAGAU	115	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 21	GUCUUGCCGGGCAACUUCCCGCGGUCAGCA	122	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 22	GGCAGCUUCUUGCCGGGCAACUUCCCGCGG	128	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 23	GCCAGCGGCAGCUUCUUGCCGGGCAACUUC	134	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 24	GCACCUCCAGCGGCAGCUUCUUGCCGGGCA	139	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 25	GCUUUGAGCACCUCCAGCGGCAGCUUCUUG	146	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 26	GCAUCUCUUUGAGCACCUCCAGCGGCAGCU	151	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 27	GUCCAUCUCUUUGAGCACCUCCAGCGGCAG	153	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 28	GGGCAUUGGCUUCCAUCUCUUUGAGCACCU	163	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 29	GUCCGGGCAUUGGCUUCCAUCUCUUUGAGC	167	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 30	GGCCAGCUUUCCGGGCAUUGGCUUCCAUCU	175	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 31	GGGUGCAGCCAGCUUUCCGGGCAUUGGCUU	181	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 32	GAGCCCCUGGUGCAGCCAGCUUUCCGGGCA	188	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 33	GAUCAGACAGCCCCUGGUGCAGCCAGCUUU	195	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 34	GGCAGAUCAGACAGCCCCUGGUGCAGCCAG	199	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 35	GACAGGCAGAUCAGACAGCCCCUGGUGCAG	203	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 36	GUGAUGUGGGACAGGCAGAUCAGACAGCCC	212	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 37	GACUUGAUGUGGGACAGGCAGAUCAGACAG	215	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C

Gluc tiling guide 38	GGGGCGUGCACUUGAUGUGGGACAGGCAGA	223	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 39	GCUUCAUCUUGGGCGUGCACUUGAUGUGGG	232	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 40	GUGAACUUCUUCAUCUUGGGCGUGCACUUG	239	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 41	GGGAUGAACUUCUUCAUCUUGGGCGUGCAC	242	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 42	GUGGGAUGAACUUCUUCAUCUUGGGCGUGC	244	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 43	GGGCAGCGUCCUGGGAUGAACUUCUUCAUC	254	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 44	GGGUGUGGCAGCGUCCUGGGAUGAACUUCU	259	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 45	GUUCGUAGGUGUGGCAGCGUCCUGGGAUGA	265	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 46	GCGCCUUCGUAGGUGUGGCAGCGUCCUGGG	269	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 47	GUCUUUGUCGCCUUCGUAGGUGUGGCAGCG	276	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 48	GCUUUGUCGCCUUCGUAGGUGUGGCAGCGU	275	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 49	GUGCCGCCCUGUGCGGACUCUUUGUCGCCU	293	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 50	GUAUGCCGCCCUGUGCGGACUCUUUGUCGC	295	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 51	GCCUCGCCUAUGCCGCCCUGUGCGGACUCU	302	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 52	GGAUCGCCUCGCCUAUGCCGCCCUGUGCGG	307	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 53	GAUGUCGACGAUCGCCUCGCCUAUGCCGCC	315	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 54	GCAGGAAUGUCGACGAUCGCCUCGCCUAUG	320	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 55	GAAUCUCAGGAAUGUCGACGAUCGCCUCGC	325	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 56	GCCCAGGAAUCUCAGGAAUGUCGACGAUCG	331	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 57	GCCUUGAACCCAGGAAUCUCAGGAAUGUCG	338	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 58	GCCAAGUCCUUGAACCCAGGAAUCUCAGGA	344	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 59	GUGGGCUCCAAGUCCUUGAACCCAGGAAUC	350	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 60	GCCAUGGGCUCCAAGUCCUUGAACCCAGGA	353	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 61	GGAACUGCUCCAUGGGCUCCAAGUCCUUGA	361	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 62	GUGCGAUGAACUGCUCCAUGGGCUCCAAGU	367	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 63	GGACCUGUGCGAUGAACUGCUCCAUGGGCU	373	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 64	GACAGAUCGACCUGUGCGAUGAACUGCUCC	380	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 65	GACACACAGAUCGACCUGUGCGAUGAACUG	384	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 66	GUGCAGUCCACACACAGAUCGACCUGUGCG	392	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 67	GCCAGUUGUGCAGUCCACACACAGAUCGAC	399	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 68	GGGCAGCCAGUUGUGCAGUCCACACACAGA	404	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 69	GUUUGAGGCAGCCAGUUGUGCAGUCCACAC	409	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 70	GAAGCCCUUUGAGGCAGCCAGUUGUGCAGU	415	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 71	GCACGUUGGCAAGCCCUUUGAGGCAGCCAG	424	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C

Gluc tiling guide 72	GACUGCACGUUGGCAAGCCCUUUGAGGCAG	428	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 73	GGGUCAGAACACUGCACGUUGGCAAGCCCU	437	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 74	GCAGGUCAGAACACUGCACGUUGGCAAGCC	439	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 75	GAGCAGGUCAGAACACUGCACGUUGGCAAG	441	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 76	GGCCACUUCUUGAGCAGGUCAGAACACUGC	452	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 77	GCGGCAGCCACUUCUUGAGCAGGUCAGAAC	457	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 78	GUGCGGCAGCCACUUCUUGAGCAGGUCAGA	459	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 79	GAGCGUUGCGGCAGCCACUUCUUGAGCAGG	464	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 80	GAAAGGUCGCACAGCGUUGCGGCAGCCACU	475	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 81	GCUGGCAAAGGUCGCACAGCGUUGCGGCAG	480	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 82	GGGCAAAGGUCGCACAGCGUUGCGGCAGCC	478	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 83	GUGGAUCUUGCUGGCAAAGGUCGCACAGCG	489	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 84	GCACCUGGCCCUGGAUCUUGCUGGCAAAGG	499	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 85	GUGGCCCUGGAUCUUGCUGGCAAAGGUCGC	495	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 86	GUGAUCUUGUCCACCUGGCCCUGGAUCUUG	509	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 87	GCCCCUUGAUCUUGUCCACCUGGCCCUGGA	514	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 88	GCCCUUGAUCUUGUCCACCUGGCCCUGGAU	513	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 89	GCCUUGAUCUUGUCCACCUGGCCCUGGAUC	512	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 90	GGCAAAGGUCGCACAGCGUUGCGGCAGCCA	477	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 91	GCAAAGGUCGCACAGCGUUGCGGCAGCCAC	476	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 92	GAAGGUCGCACAGCGUUGCGGCAGCCACUU	474	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Gluc tiling guide 93	GAGGUCGCACAGCGUUGCGGCAGCCACUUC	473	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Non-targeting guide 1	GGUAAUGCCUGGCUUGUCGACGCAUAGUCUG	N/A	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Non-targeting guide 2	GGGAACCUUGGCCGUUAUAAAGUCUGACCAG	N/A	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C
Non-targeting guide 3	GGAGGGUGAGAAUUUAGAACCAAGAUUGUUG	N/A	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1C

Supplementary Table 8: Guide sequences used for *Cluc* knockdown

Name	Spacer sequence	Position	Notes	First figure
Cluc tiling guide 1	GAGUCCUGGCAAUGAACAGUGGCGCAGUAG	32	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1D
Cluc tiling guide 2	GGGUGCCACAGCUGCUAUCAAUACAUUCUC	118	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1D
Cluc tiling guide 3	GUUACAUACUGACACAUUCGGCAACAUGUU	197	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1D
Cluc tiling guide 4	GUAUGUACCAGGUUCCUGGAACUGGAAUCU	276	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1D
Cluc tiling guide 5	GCCUUGGUUCCAUCCAGGUUCUCCAGGGUG	350	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1D
Cluc tiling guide 6	GCAGUGAUGGGAUUCUCAGUAGCUUGAGCG	431	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1D

Cluc tiling guide 7	GAGCCUGGCAUCUCAACAACAGCGAUGGUG	512	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1D
Cluc tiling guide 8	GUGUCUGGGGCGAUUCUUACAGAUCUUCCU	593	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1D
Cluc tiling guide 9	GCUGGAUCUGAAGUGAAGUCUGUAUCUUCC	671	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1D
Cluc tiling guide 10	GGCAACGUCAUCAGGAUUUCCAUAGAGUGG	747	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1D
Cluc tiling guide 11	GAGGCGCAGGAGAUGGUGUAGUAGUAGAAG	830	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1D
Cluc tiling guide 13	GAGGGACCCUGGAAUUGGUAUCUUGCUUUG	986	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1D
Cluc tiling guide 14	GGUAAGAGUCAACAUUCCUGUGUGAAACCU	1066	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1D
Cluc tiling guide 15	GACCAGAAUCUGUUUUCCAUCAACAAUGAG	1143	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1D
Cluc tiling guide 16	GAUGGCUGUAGUCAGUAUGUCACCAUCUUG	1227	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1D
Cluc tiling guide 17	GUACCAUCGAAUGGAUCUCUAAUAUGUACG	1304	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1D
Cluc tiling guide 18	GAGAUCACAGGCUCCUUCAGCAUCAAAAGA	1380	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1D
Cluc tiling guide 19	GCUUUGACCGGCGAAGAGACUAUUGCAGAG	1461	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1D
Cluc tiling guide 20	GCCCCUCAGGCAAUACUCGUACAUGCAUCG	1539	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1D
Cluc tiling guide 21	GCUGGUACUUCUAGGGUGUCUCCAUGCUUU	1619	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1D
Non-targeting guide 1	GGUAAUGCCUGGCUUGUCGACGCAUAGUCUG	N/A	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1D
Non-targeting guide 2	GGGAACCUUGGCCGUUAUAAAGUCUGACCAG	N/A	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1D
Non-targeting guide 3	GGAGGGUGAGAAUUUAGAACCAAGAUUGUUG	N/A	Note that the Cas13a spacers are truncated by two nucleotides at the 5' end	1D

Supplementary Table 9: Guide sequences used in this study for RNA editing in mammalian cells. Mismatched base flips are capitalized

Name	Spacer sequence	Notes	First figure
Tiling 30 nt 30 mismatch distance	gCauccugcggccucuacucugcauucaauu	Has a 5' G for U6 expression	2C
Tiling 30 nt 28 mismatch distance	gacCauccugcggccucuacucugcauucaa	Has a 5' G for U6 expression	2C
Tiling 30 nt 26 mismatch distance	gaaacCauccugcggccucuacucugcauuc	Has a 5' G for U6 expression	2C
Tiling 30 nt 24 mismatch distance	gcuaaacCauccugcggccucuacucugcau	Has a 5' G for U6 expression	2C
Tiling 30 nt 22 mismatch distance	guucuaaacCauccugcggccucuacucugc	Has a 5' G for U6 expression	2C
Tiling 30 nt 20 mismatch distance	guguucuaaacCauccugcggccucuacucu	Has a 5' G for U6 expression	2C
Tiling 30 nt 18	gaauguucuaaacCauccugcggccucuacu	Has a 5' G for U6 expression	2C

mismatch distance			
Tiling 30 nt 16 mismatch distance	gagaauguucuaaacCauccugcggccucua	Has a 5' G for U6 expression	2C
Tiling 30 nt 14 mismatch distance	gauagaauguucuaaacCauccugcggccuc	Has a 5' G for U6 expression	2C
Tiling 30 nt 12 mismatch distance	gccauagaauguucuaaacCauccugcggcc	Has a 5' G for U6 expression	2C
Tiling 30 nt 10 mismatch distance	guuccauagaauguucuaaacCauccugcgg	Has a 5' G for U6 expression	2C
Tiling 30 nt 8 mismatch distance	gcuuuccauagaauguucuaaacCauccugc	Has a 5' G for U6 expression	2C
Tiling 30 nt 6 mismatch distance	gcucuuuccauagaauguucuaaacCauccu	Has a 5' G for U6 expression	2C
Tiling 30 nt 4 mismatch distance	gaucucuuuccauagaauguucuaaacCauc	Has a 5' G for U6 expression	2C
Tiling 30 nt 2 mismatch distance	ggaaucucuuuccauagaauguucuaaacCa	Has a 5' G for U6 expression	2C
Tiling 50 nt 50 mismatch distance	gCauccugcggccucuacucugcauucaauuacauacugacacauucggca	Has a 5' G for U6 expression	2C
Tiling 50 nt 48 mismatch distance	gacCauccugcggccucuacucugcauucaauuacauacugacacauucgg	Has a 5' G for U6 expression	2C
Tiling 50 nt 46 mismatch distance	gaaacCauccugcggccucuacucugcauucaauuacauacugacacauuc	Has a 5' G for U6 expression	2C
Tiling 50 nt 44 mismatch distance	gcuaaacCauccugcggccucuacucugcauucaauuacauacugacacau	Has a 5' G for U6 expression	2C
Tiling 50 nt 42 mismatch distance	guucuaaacCauccugcggccucuacucugcauucaauuacauacugacac	Has a 5' G for U6 expression	2C
Tiling 50 nt 40 mismatch distance	guguucuaaacCauccugcggccucuacucugcauucaauuacauacugac	Has a 5' G for U6 expression	2C
Tiling 50 nt 38 mismatch distance	gaauguucuaaacCauccugcggccucuacucugcauucaauuacauacug	Has a 5' G for U6 expression	2C
Tiling 50 nt 36 mismatch distance	gagaauguucuaaacCauccugcggccucuacucugcauucaauuacauac	Has a 5' G for U6 expression	2C
Tiling 50 nt 34 mismatch	gauagaauguucuaaacCauccugcggccucuacucugcauucaauuacau	Has a 5' G for U6 expression	2C

distance			
Tiling 50 nt 32 mismatch distance	gccauagaauguucuaaacCauccugcggccucuacucug cauucaauuac	Has a 5' G for U6 expression	2C
Tiling 50 nt 30 mismatch distance	guuccauagaauguucuaaacCauccugcggccucuacuc ugcauucaauu	Has a 5' G for U6 expression	2C
Tiling 50 nt 28 mismatch distance	gcuuuccauagaauguucuaaacCauccugcggccucuac ucugcauucaa	Has a 5' G for U6 expression	2C
Tiling 50 nt 26 mismatch distance	gcucuuuccauagaauguucuaaacCauccugcggccucu acucugcauuc	Has a 5' G for U6 expression	2C
Tiling 50 nt 24 mismatch distance	gaucucuuuccauagaauguucuaaacCauccugcggccu cuacucugcau	Has a 5' G for U6 expression	2C
Tiling 50 nt 22 mismatch distance	ggaaucucuuuccauagaauguucuaaacCauccugcggc cucuacucugc	Has a 5' G for U6 expression	2C
Tiling 50 nt 20 mismatch distance	guggaaucucuuuccauagaauguucuaaacCauccugcg gccucuacucu	Has a 5' G for U6 expression	2C
Tiling 50 nt 18 mismatch distance	gacuggaaucucuuuccauagaauguucuaaacCauccug cggccucuacu	Has a 5' G for U6 expression	2C
Tiling 50 nt 16 mismatch distance	ggaacuggaaucucuuuccauagaauguucuaaacCaucc ugcggccucua	Has a 5' G for U6 expression	2C
Tiling 50 nt 14 mismatch distance	guggaacuggaaucucuuuccauagaauguucuaaacCau ccugcggccuc	Has a 5' G for U6 expression	2C
Tiling 50 nt 12 mismatch distance	gccuggaacuggaaucucuuuccauagaauguucuaaacC auccugcggcc	Has a 5' G for U6 expression	2C
Tiling 50 nt 10 mismatch distance	guuccuggaacuggaaucucuuuccauagaauguucuaaa cCauccugcgg	Has a 5' G for U6 expression	2C
Tiling 50 nt 8 mismatch distance	ggguuccuggaacuggaaucucuuuccauagaauguucua aacCauccugc	Has a 5' G for U6 expression	2C
Tiling 50 nt 6 mismatch distance	gcagguuccuggaacuggaaucucuuuccauagaauguuc uaaacCauccu	Has a 5' G for U6 expression	2C
Tiling 50 nt 4 mismatch distance	gaccagguuccuggaacuggaaucucuuuccauagaaugu ucuaaacCauc	Has a 5' G for U6 expression	2C
Tiling 50 nt 2 mismatch distance	gguaccagguuccuggaacuggaaucucuuuccauagaau guucuaaacCa	Has a 5' G for U6 expression	2C
Tiling 70 nt 70 mismatch	gCauccugcggccucuacucugcauucaauuacauacuga cacauucggcaacauguuuuuccugguuuau	Has a 5' G for U6 expression	2C

distance			
Tiling 70 nt 68 mismatch distance	gacCauccugcggccucuacucugcauucaauuacauacu gacacauucggcaacauguuuuuccugguuu	Has a 5' G for U6 expression	2C
Tiling 70 nt 66 mismatch distance	gaaacCauccugcggccucuacucugcauucaauuacaua cugacacauucggcaacauguuuuuccuggu	Has a 5' G for U6 expression	2C
Tiling 70 nt 64 mismatch distance	gcuaaacCauccugcggccucuacucugcauucaauuaca uacugacacauucggcaacauguuuuuccug	Has a 5' G for U6 expression	2C
Tiling 70 nt 62 mismatch distance	guucuaaacCauccugcggccucuacucugcauucaauua cauacugacacauucggcaacauguuuuucc	Has a 5' G for U6 expression	2C
Tiling 70 nt 60 mismatch distance	guguucuaaacCauccugcggccucuacucugcauucaau uacauacugacacauucggcaacauguuuuu	Has a 5' G for U6 expression	2C
Tiling 70 nt 58 mismatch distance	gaauguucuaaacCauccugcggccucuacucugcauuca auuacauacugacacauucggcaacauguuu	Has a 5' G for U6 expression	2C
Tiling 70 nt 56 mismatch distance	gagaauguucuaaacCauccugcggccucuacucugcauu caauuacauacugacacauucggcaacaugu	Has a 5' G for U6 expression	2C
Tiling 70 nt 54 mismatch distance	gauagaauguucuaaacCauccugcggccucuacucugca uucaauuacauacugacacauucggcaacau	Has a 5' G for U6 expression	2C
Tiling 70 nt 52 mismatch distance	gccauagaauguucuaaacCauccugcggccucuacucug cauucaauuacauacugacacauucggcaac	Has a 5' G for U6 expression	2C
Tiling 70 nt 50 mismatch distance	guuccauagaauguucuaaacCauccugcggccucuacuc ugcauucaauuacauacugacacauucggca	Has a 5' G for U6 expression	2C
Tiling 70 nt 48 mismatch distance	gcuuuccauagaauguucuaaacCauccugcggccucuac ucugcauucaauuacauacugacacauucgg	Has a 5' G for U6 expression	2C
Tiling 70 nt 46 mismatch distance	gcucuuuccauagaauguucuaaacCauccugcggccucu acucugcauucaauuacauacugacacauuc	Has a 5' G for U6 expression	2C
Tiling 70 nt 44 mismatch distance	gaucucuuuccauagaauguucuaaacCauccugcggccu cuacucugcauucaauuacauacugacacau	Has a 5' G for U6 expression	2C
Tiling 70 nt 42 mismatch distance	ggaaucucuuuccauagaauguucuaaacCauccugcggc cucuacucugcauucaauuacauacugacac	Has a 5' G for U6 expression	2C
Tiling 70 nt 40 mismatch distance	guggaaucucuuuccauagaauguucuaaacCauccugcg gccucuacucugcauucaauuacauacugac	Has a 5' G for U6 expression	2C
Tiling 70 nt 38 mismatch distance	gacuggaaucucuuuccauagaauguucuaaacCauccug cggccucuacucugcauucaauuacauacug	Has a 5' G for U6 expression	2C
Tiling 70 nt 36 mismatch	ggaacuggaaucucuuuccauagaauguucuaaacCaucc ugcggccucuacucugcauucaauuacauac	Has a 5' G for U6 expression	2C

distance			
Tiling 70 nt 34 mismatch distance	guggaacuggaaucucuuuccauagaauguucuaaacCauccugcggccucuacucugcauucaauuacau	Has a 5' G for U6 expression	2C
Tiling 70 nt 32 mismatch distance	gccuggaacuggaaucucuuuccauagaauguucuaaacCauccugcggccucuacucugcauucaauuac	Has a 5' G for U6 expression	2C
Tiling 70 nt 30 mismatch distance	guuccuggaacuggaaucucuuuccauagaauguucuaaacCauccugcggccucuacucugcauucaauu	Has a 5' G for U6 expression	2C
Tiling 70 nt 28 mismatch distance	ggguuccuggaacuggaaucucuuuccauagaauguucuaaacCauccugcggccucuacucugcauucaa	Has a 5' G for U6 expression	2C
Tiling 70 nt 26 mismatch distance	gcagguuccuggaacuggaaucucuuuccauagaauguucuaaacCauccugcggccucuacucugcauuc	Has a 5' G for U6 expression	2C
Tiling 70 nt 24 mismatch distance	gaccagguuccuggaacuggaaucucuuuccauagaauguucuaaacCauccugcggccucuacucugcau	Has a 5' G for U6 expression	2C
Tiling 70 nt 22 mismatch distance	gguaccagguuccuggaacuggaaucucuuuccauagaauguucuaaacCauccugcggccucuacucugc	Has a 5' G for U6 expression	2C
Tiling 70 nt 20 mismatch distance	gauguaccagguuccuggaacuggaaucucuuuccauagaauguucuaaacCauccugcggccucuacucu	Has a 5' G for U6 expression	2C
Tiling 70 nt 18 mismatch distance	gguauguaccagguuccuggaacuggaaucucuuuccauagaauguucuaaacCauccugcggccucuacu	Has a 5' G for U6 expression	2C
Tiling 70 nt 16 mismatch distance	gacguauguaccagguuccuggaacuggaaucucuuuccauagaauguucuaaacCauccugcggccucua	Has a 5' G for U6 expression	2C
Tiling 70 nt 14 mismatch distance	gacacguauguaccagguuccuggaacuggaaucucuuuccauagaauguucuaaacCauccugcggccuc	Has a 5' G for U6 expression	2C
Tiling 70 nt 12 mismatch distance	gcaacacguauguaccagguuccuggaacuggaaucucuuuccauagaauguucuaaacCauccugcggcc	Has a 5' G for U6 expression	2C
Tiling 70 nt 10 mismatch distance	gcccaacacguauguaccagguuccuggaacuggaaucucuuuccauagaauguucuaaacCauccugcgg	Has a 5' G for U6 expression	2C
Tiling 70 nt 8 mismatch distance	ggacccaacacguauguaccagguuccuggaacuggaaucucuuuccauagaauguucuaaacCauccugc	Has a 5' G for U6 expression	2C
Tiling 70 nt 6 mismatch distance	guugacccaacacguauguaccagguuccuggaacuggaaucucuuuccauagaauguucuaaacCauccu	Has a 5' G for U6 expression	2C
Tiling 70 nt 4 mismatch distance	gccuugacccaacacguauguaccagguuccuggaacuggaaucucuuuccauagaauguucuaaacCauc	Has a 5' G for U6 expression	2C
Tiling 70 nt 2 mismatch	guuccuugacccaacacguauguaccagguuccuggaacuggaaucucuuuccauagaauguucuaaacCa	Has a 5' G for U6 expression	2C

distance			
Tiling 84 nt 84 mismatch distance	gCauccugcggccucuacucugcauucaauuacauacugacacauucggcaacauguuuuuccugguuuauuuucacacagucca	Has a 5' G for U6 expression	2C
Tiling 84 nt 82 mismatch distance	gacCauccugcggccucuacucugcauucaauuacauacugacacauucggcaacauguuuuuccugguuuauuuucacacaguc	Has a 5' G for U6 expression	2C
Tiling 84 nt 80 mismatch distance	gaaacCauccugcggccucuacucugcauucaauuacauacugacacauucggcaacauguuuuuccugguuuauuuucacacag	Has a 5' G for U6 expression	2C
Tiling 84 nt 78 mismatch distance	gcuaaacCauccugcggccucuacucugcauucaauuacauacugacacauucggcaacauguuuuuccugguuuauuuucacac	Has a 5' G for U6 expression	2C
Tiling 84 nt 76 mismatch distance	guucuaaacCauccugcggccucuacucugcauucaauuacauacugacacauucggcaacauguuuuuccugguuuauuuucac	Has a 5' G for U6 expression	2C
Tiling 84 nt 74 mismatch distance	guguucuaaacCauccugcggccucuacucugcauucaauuacauacugacacauucggcaacauguuuuuccugguuuauuuuc	Has a 5' G for U6 expression	2C
Tiling 84 nt 72 mismatch distance	gaauguucuaaacCauccugcggccucuacucugcauucaauuacauacugacacauucggcaacauguuuuuccugguuuauuu	Has a 5' G for U6 expression	2C
Tiling 84 nt 70 mismatch distance	gagaauguucuaaacCauccugcggccucuacucugcauucaauuacauacugacacauucggcaacauguuuuuccugguuuau	Has a 5' G for U6 expression	2C
Tiling 84 nt 68 mismatch distance	gauagaauguucuaaacCauccugcggccucuacucugcauucaauuacauacugacacauucggcaacauguuuuuccuggguuu	Has a 5' G for U6 expression	2C
Tiling 84 nt 66 mismatch distance	gccauagaauguucuaaacCauccugcggccucuacucugcauucaauuacauacugacacauucggcaacauguuuuuccuggu	Has a 5' G for U6 expression	2C
Tiling 84 nt 64 mismatch distance	guuccauagaauguucuaaacCauccugcggccucuacucugcauucaauuacauacugacacauucggcaacauguuuuuccug	Has a 5' G for U6 expression	2C
Tiling 84 nt 62 mismatch distance	gcuuuccauagaauguucuaaacCauccugcggccucuacucugcauucaauuacauacugacacauucggcaacauguuuuucc	Has a 5' G for U6 expression	2C
Tiling 84 nt 60 mismatch distance	gcucuuuccauagaauguucuaaacCauccugcggccucuacucugcauucaauuacauacugacacauucggcaacauguuuuu	Has a 5' G for U6 expression	2C
Tiling 84 nt 58 mismatch distance	gaucucuuuccauagaauguucuaaacCauccugcggccucuacucugcauucaauuacauacugacacauucggcaacauguuu	Has a 5' G for U6 expression	2C
Tiling 84 nt 56 mismatch distance	ggaaucucuuuccauagaauguucuaaacCauccugcggccucuacucugcauucaauuacauacugacacauucggcaacaugu	Has a 5' G for U6 expression	2C
Tiling 84 nt 54 mismatch distance	guggaaucucuuuccauagaauguucuaaacCauccugcggccucuacucugcauucaauuacauacugacacauucggcaacau	Has a 5' G for U6 expression	2C
Tiling 84 nt 52 mismatch	gacuggaaucucuuuccauagaauguucuaaacCauccugcggccucuacucugcauucaauuacauacugacacauucggcaac	Has a 5' G for U6 expression	2C

distance			
Tiling 84 nt 50 mismatch distance	ggaacuggaaucucuuuccauagaauguucuaaacCaucc ugcggccucuacucugcauucaauuacauacugacacauu cggca	Has a 5' G for U6 expression	2C
Tiling 84 nt 48 mismatch distance	guggaacuggaaucucuuuccauagaauguucuaaacCau ccugcggccucuacucugcauucaauuacauacugacaca uucgg	Has a 5' G for U6 expression	2C
Tiling 84 nt 46 mismatch distance	gccuggaacuggaaucucuuuccauagaauguucuaaacC auccugcggccucuacucugcauucaauuacauacugaca cauuc	Has a 5' G for U6 expression	2C
Tiling 84 nt 44 mismatch distance	guuccuggaacuggaaucucuuuccauagaauguucuaaa cCauccugcggccucuacucugcauucaauuacauacuga cacau	Has a 5' G for U6 expression	2C
Tiling 84 nt 42 mismatch distance	ggguuccuggaacuggaaucucuuuccauagaauguucua aacCauccugcggccucuacucugcauucaauuacauacu gacac	Has a 5' G for U6 expression	2C
Tiling 84 nt 40 mismatch distance	gcagguuccuggaacuggaaucucuuuccauagaauguuc uaaacCauccugcggccucuacucugcauucaauuacaua cugac	Has a 5' G for U6 expression	2C
Tiling 84 nt 38 mismatch distance	gaccagguuccuggaacuggaaucucuuuccauagaaugu ucuaaacCauccugcggccucuacucugcauucaauuaca uacug	Has a 5' G for U6 expression	2C
Tiling 84 nt 36 mismatch distance	gguaccagguuccuggaacuggaaucucuuuccauagaau guucuaaacCauccugcggccucuacucugcauucaauua cauac	Has a 5' G for U6 expression	2C
Tiling 84 nt 34 mismatch distance	gauguaccagguuccuggaacuggaaucucuuuccauaga auguucuaaacCauccugcggccucuacucugcauucaau uacau	Has a 5' G for U6 expression	2C
Tiling 84 nt 32 mismatch distance	gguauguaccagguuccuggaacuggaaucucuuuccaua gaauguucuaaacCauccugcggccucuacucugcauuca auuac	Has a 5' G for U6 expression	2C
Tiling 84 nt 30 mismatch distance	gacguauguaccagguuccuggaacuggaaucucuuucca uagaauguucuaaacCauccugcggccucuacucugcauu caauu	Has a 5' G for U6 expression	2C
Tiling 84 nt 28 mismatch distance	gacacguauguaccagguuccuggaacuggaaucucuuuc cauagaauguucuaaacCauccugcggccucuacucugca uucaa	Has a 5' G for U6 expression	2C
Tiling 84 nt 26 mismatch distance	gcaacacguauguaccagguuccuggaacuggaaucucuu uccauagaauguucuaaacCauccugcggccucuacucug cauuc	Has a 5' G for U6 expression	2C
Tiling 84 nt 24 mismatch distance	gcccaacacguauguaccagguuccuggaacuggaaucuc uuuccauagaauguucuaaacCauccugcggccucuacuc ugcau	Has a 5' G for U6 expression	2C
Tiling 84 nt 22 mismatch distance	ggacccaacacguauguaccagguuccuggaacuggaauc ucuuuccauagaauguucuaaacCauccugcggccucuac ucugc	Has a 5' G for U6 expression	2C
Tiling 84 nt 20 mismatch distance	guugacccaacacguauguaccagguuccuggaacuggaa ucucuuuccauagaauguucuaaacCauccugcggccucu acucu	Has a 5' G for U6 expression	2C
Tiling 84 nt 18 mismatch	gccuugacccaacacguauguaccagguuccuggaacugg aaucucuuuccauagaauguucuaaacCauccugcggccu cuacu	Has a 5' G for U6 expression	2C

distance			
Tiling 84 nt 16 mismatch distance	guuccuugacccaacacguauguaccagguuccuggaacuggaaucucuuuccauagaauguucuaaacCauccugcggccucua	Has a 5' G for U6 expression	2C
Tiling 84 nt 14 mismatch distance	ggguuccuugacccaacacguauguaccagguuccuggaacuggaaucucuuuccauagaauguucuaaacCauccugcggccuc	Has a 5' G for U6 expression	2C
Tiling 84 nt 12 mismatch distance	guugguuccuugacccaacacguauguaccagguuccuggaacuggaaucucuuuccauagaauguucuaaacCauccuggcc	Has a 5' G for U6 expression	2C
Tiling 84 nt 10 mismatch distance	gccuugguuccuugacccaacacguauguaccagguuccuggaacuggaaucucuuuccauagaauguucuaaacCauccugcgg	Has a 5' G for U6 expression	2C
Tiling 84 nt 8 mismatch distance	ggcccuugguuccuugacccaacacguauguaccagguuccuggaacuggaaucucuuuccauagaauguucuaaacCauccugc	Has a 5' G for U6 expression	2C
Tiling 84 nt 6 mismatch distance	gccgcccuugguuccuugacccaacacguauguaccaggguccuggaacuggaaucucuuuccauagaauguucuaaacCauccu	Has a 5' G for U6 expression	2C
Tiling 84 nt 4 mismatch distance	gcgccgcccuugguuccuugacccaacacguauguaccaggguuccuggaacuggaaucucuuuccauagaauguucuaaacCauc	Has a 5' G for U6 expression	2C
Tiling 84 nt 2 mismatch distance	ggucgccgcccuugguuccuugacccaacacguauguaccagguuccuggaacuggaaucucuuuccauagaauguucuaaacCa	Has a 5' G for U6 expression	2C
ADAR non-targeting guide	GUAAUGCCUGGCUUGUCGACGCAUAGUCUG	Has a 5' G for U6 expression	2C
PFS binding screen guide for TAG motif	gaaaacgcagguuccucCaguuucgggagcagcgcacgucucccuguaguc	Has a 5' G for U6 expression	3B
PFS binding screen guide for AAC motif	gacgcagguuccucuagCuucgggagcagcgcacgucuccccuguagucaag	Has a 5' G for U6 expression	3B
PFS binding screen non-targeting	GUAAUGCCUGGCUUGUCGACGCAUAGUCUG	Has a 5' G for U6 expression	3B
Motif preference targeting guide	gauagaauguucuaaacCauccugcggccucuacucugcauucaauuacau	Has a 5' G for U6 expression	3C
Motif preference non-targeting guide	GUAAUGCCUGGCUUGUCGACGCAUAGUCUG	Has a 5' G for U6 expression	3C
PPIB tiling guide 50 mismatch	gCaaggccacaaaauuauccacuguuuuuggaacagucuuuccgaagagac	Has a 5' G for U6 expression	S3D

distance			
PPIB tiling guide 42 mismatch distance	gccuguagcCaaggccacaaaauuauccacuguuuuuggaacagucuuucc	Has a 5' G for U6 expression	S3D
PPIB tiling guide 34 mismatch distance	gcuuucucuccuguagcCaaggccacaaaauuauccacuguuuuuggaaca	Has a 5' G for U6 expression	S3D
PPIB tiling guide 26 mismatch distance	ggccaaauccuuucucuccuguagcCaaggccacaaaauuauccacuguuu	Has a 5' G for U6 expression	S3D
PPIB tiling guide 18 mismatch distance	guuuuuguagccaaauccuuucucuccuguagcCaaggccacaaaauuauc	Has a 5' G for U6 expression	S3D
PPIB tiling guide 10 mismatch distance	gauuugcuguuuuuguagccaaauccuuucucuccuguagcCaaggccaca	Has a 5' G for U6 expression	S3D
PPIB tiling guide 2 mismatch distance	gacgauggaauuugcuguuuuuguagccaaauccuuucuccuccuguagcCa	Has a 5' G for U6 expression	S3D
Targeting guide, opposite base G	gauagaauguucuaaacGauccugcggccucuacucugcauucaauuacau	Has a 5' G for U6 expression	S3D
Targeting guide, opposite base A	gauagaauguucuaaacAauccugcggccucuacucugcauucaauuacau	Has a 5' G for U6 expression	S3D
Targeting guide, opposite base C	gauagaauguucuaaacUauccugcggccucuacucugcauucaauuacau	Has a 5' G for U6 expression	S3D
AVPR2 guide 37 mismatch distance	ggucccacgcggccCacagcugcaccaggaagaagggugccagcacagca	Has a 5' G for U6 expression	4A
AVPR2 guide 35 mismatch distance	ggggucccacgcggccCacagcugcaccaggaagaagggugcccagcacag	Has a 5' G for U6 expression	4A
AVPR2 guide 33 mismatch distance	gccgggucccacgcggccCacagcugcaccaggaagaagggugcccagcac	Has a 5' G for U6 expression	4A
FANCC guide 37 mismatch distance	gggugaugacauccCaggcgaucguguggccuccaggagccagagcagga	Has a 5' G for U6 expression	4B
FANCC guide 35 mismatch distance	gagggugaugacauccCaggcgaucguguggccuccaggagcccagagcag	Has a 5' G for U6 expression	4B
FANCC guide 32 mismatch distance	gaucagggugaugacauccCaggcgaucguguggccuccaggagcccagag	Has a 5' G for U6 expression	4B
Synthetic	gguggcuccauucacucCaaugcugagcacuuccacagag	Has a 5' G for U6 expression	4E

disease gene target IL2RG	uggguuaaagc		
Synthetic disease gene target F8	guuucuaauauauuuugCcagacugauggacuauucucaauuaauaaugau	Has a 5' G for U6 expression	4E
Synthetic disease gene target LDLR	gagauguugcuguggauCcaguccacagccagcccgucgggggccuggaug	Has a 5' G for U6 expression	4E
Synthetic disease gene target CBS	gcaggccggcccagcugCcaggugcaccugcucggagcaucgggccggauc	Has a 5' G for U6 expression	4E
Synthetic disease gene target HBB	gcaaagaaccucuggguCcaaggguagaccaccagcagccugcccagggcc	Has a 5' G for U6 expression	4E
Synthetic disease gene target ALDOB	gaagagaaacuuaguuuCcagggcuuugguagagggcaaagguugauagca	Has a 5' G for U6 expression	4E
Synthetic disease gene target DMD	gucagccuagugcagagCcacugguaguugguggguuagaguuucaaguucc	Has a 5' G for U6 expression	4E
Synthetic disease gene target SMAD4	ggcucauugugaacaggCcaguaauguccgggaugggggcggcauaggcggg	Has a 5' G for U6 expression	4E
Synthetic disease gene target BRCA2	guagcuaaagaacuugaCcaagacauaucaggauccaccucagcuccuaga	Has a 5' G for U6 expression	4E
Synthetic disease gene target GRIN2A	ggggcauuguucugugcCcaguccugcugguagaccugcucccgguggcu	Has a 5' G for U6 expression	4E
Synthetic disease gene target SCN9A	gagaagucguucaugugCcaccgugggagcguacagucaucauugaucuug	Has a 5' G for U6 expression	4E
Synthetic disease gene target TARDBP	gggauuaaugcugaacgCaccaaaguucaucccaccacccauauuacuacc	Has a 5' G for U6 expression	4E
Synthetic disease gene target CFTR	gcuccaaaggcuuuccuCcacuguugcaaaguuauugaaucccaagacaca	Has a 5' G for U6 expression	4E
Synthetic disease gene target UBE3A	gaugaaugaacgauuucCcagaacucccuaaucagaacagagucccuggua	Has a 5' G for U6 expression	4E

Synthetic disease gene target SMPD1	ggagccucugccggagcCcagagaacccgagagucagacagagccagcgcc	Has a 5' G for U6 expression	4E
Synthetic disease gene target USH2A	ggcuuccguggagacacCcaaucaauuugaagagaucuugaagugaugcca	Has a 5' G for U6 expression	4E
Synthetic disease gene target MEN1	gugggacugcccuccucCcauuugcagaugccgucguagaaucgcagcagg	Has a 5' G for U6 expression	4E
Synthetic disease gene target C8orf37	gcuucuucaauaguucuCcagcuacacuggcaggcauaugcccguguuccu	Has a 5' G for U6 expression	4E
Synthetic disease gene target MLH1	gauuccuuuucuucgucCcaauucaccucaguggcuagucgaagaaugaag	Has a 5' G for U6 expression	4E
Synthetic disease gene target TSC2	gcagcuucagcaccuucCagucagacuccugcuucaagcacugcagcagga	Has a 5' G for U6 expression	4E
Synthetic disease gene target NF1	gccauuugcuugcagugCcacuccagaggauuccggauugccauaaauacu	Has a 5' G for U6 expression	4E
Synthetic disease gene target MSH6	guucaauaguuuuggucCaguaucguuuacagcccuucuugguagauuuca	Has a 5' G for U6 expression	4E
Synthetic disease gene target SMN1	ggcaaccgucuucugacCaaauggcagaacauuuguccccaacuuuccacu	Has a 5' G for U6 expression	4E
Synthetic disease gene target SH3TC2	gcgacuuuccaaugaacCacugaagcccagguaugacaaagccgaugaucu	Has a 5' G for U6 expression	4E
Synthetic disease gene target DNAH5	guuuacacucaugcuucCacagcuuuaacagaucauuugguuccuugauga	Has a 5' G for U6 expression	4E
Synthetic disease gene target MECP2	gcuuaagcuuccgugucCagccuucaggcaggguggggucaucauacaugg	Has a 5' G for U6 expression	4E
Synthetic disease gene target ADGRV1	ggacagcugggcugaucCaugaugucauccagaaacacuggggacccucag	Has a 5' G for U6 expression	4E
Synthetic disease gene target	gucucaucucaacuuucCauauccguaucauggaaucauagcauccuguaa	Has a 5' G for U6 expression	4E

AHI1			
Synthetic disease gene target PRKN	gcaugcagacgcgguucCacucgcagccacaguuccagcaccacucgagcc	Has a 5' G for U6 expression	4E
Synthetic disease gene target COL3A1	guugguuagggucaaccCaguauucuccacucuugaguucaggauggcaga	Has a 5' G for U6 expression	4E
Synthetic disease gene target BRCA1	gcuacacuguccaacacCcacucucgggucaccacaggugccucacacauc	Has a 5' G for U6 expression	4E
Synthetic disease gene target MYBPC3	gcugcacuguguaccccCagagcuccguguugccgacauccuggggguggcu	Has a 5' G for U6 expression	4E
Synthetic disease gene target APC	gagcuuccugccacuccCaacagguuucacaguaagcgcguaucuguucca	Has a 5' G for U6 expression	4E
Synthetic disease gene target BMPR2	gacggcaagagcuuaccCagucacuuguguggagacuuaaauacuugcaua	Has a 5' G for U6 expression	4E
KRAS tiling guide 50 mismatch distance	gCaaggccacaaaauuauccacuguuuuuggaacagucuuuccgaagagac	Has a 5' G for U6 expression	5A
KRAS tiling guide 42 mismatch distance	gccuguagcCaaggccacaaaauuauccacuguuuuuggaacagucuuucc	Has a 5' G for U6 expression	5A
KRAS tiling guide 34 mismatch distance	gcuuucucuccuguagcCaaggccacaaaauuauccacuguuuuuggaaca	Has a 5' G for U6 expression	5A
KRAS tiling guide 26 mismatch distance	ggccaaauccuuucucuccuguagcCaaggccacaaaauuauccacuguuu	Has a 5' G for U6 expression	5A
KRAS tiling guide 18 mismatch distance	guuuuuguagccaaauccuuucucuccuguagcCaaggccacaaaauuauc	Has a 5' G for U6 expression	5A
KRAS tiling guide 10 mismatch distance	gauuugcuguuuuuguagccaaauccuuucucuccuguagcCaaggccaca	Has a 5' G for U6 expression	5A
KRAS tiling guide 2 mismatch distance	gacgauggaauuugcuguuuuuguagccaaauccuuucuccuccuguagcCa	Has a 5' G for U6 expression	5A
KRAS tiling	GUAAUGCCUGGCUUGUCGACGCAUAGUCUG	Has a 5' G for U6 expression	5A

non-targeting guide			
Luciferase W85X targeting guide for transcriptome specificity	gauagaauguucuaaacCauccugcggccucuacucugcauucaauuacau	Has a 5' G for U6 expression	5B
Non-targeting guide for transcriptome specificity	GCAGGGUUUUCCCAGUCACGACGUUGUAAAGUUG	Has a 5' G for U6 expression	5C
endogenous KRAS guide 2	gucaaggcacucuugccCacgccaccagcuccaacuaccacaaguuuauau	Has a 5' G for U6 expression	6F
endogenous PPIB guide 1	gcaaagaucacccggccCacaucuucaucuccaauucguaggucaaaauac	Has a 5' G for U6 expression	6G
endogenous KRAS guide 1	GcgccaccagcuccaacCaccacaaguuuauauucaguucauuuucagcagg	Has a 5' G for U6 expression	S13A
endogenous KRAS guide 3	GuuucuccaucaauuacCacuugcuuccuguaggaauccucuauuGUugga	Has a 5' G for U6 expression	S13B
endogenous PPIB guide 2	GcuuucucuccuguagcCaaggccacaaaauuauccacuguuuuuggaaca	Has a 5' G for U6 expression	S13C
endogenous non-targeting guide	GUAAUGCCUGGCUUGUCGACGCAUAGUCUG	Has a 5' G for U6 expression	6F
BoxB Cluc guide	ucuuuccauaGGCCCUGAAAAAGGGCCuguucuaaacCauccugcggccucuacucGGCCCUGAAAAAGGGCCauucaauuac	Has a 5' G for U6 expression	S8B
BoxB non-targeting guide	cagcuggcgaGGCCCUGAAAAAGGGCCggggaugugcCgcaaggcgauuaaguuggGGCCCUGAAAAAGGGCCacgccaggu	Has a 5' G for U6 expression	S8B
Stafforst full length ADAR2 guide 1	GUGGAAUAGUAUAACAAUAUGCUAAAUGUUGUUAUAGUAUCCCACucuaaaCCAuccugcgGGGCCCUCUUCAGGGCCC	Has a 5' G for U6 expression	S8C
Stafforst full length ADAR2 non-targeting guide	GUGGAAUAGUAUAACAAUAUGCUAAAUGUUGUUAUAGUAUCCCACacccuggcguuacccaGGGCCCUCUUCAGGGCCC	Has a 5' G for U6 expression	S8C

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