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Supplementary information

Compact RNA editors with small Cas13 proteins

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Supplementary Information

Compact RNA editors with small Cas13 proteins

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SUPPLEMENTARY METHODS

Protein expression and purification of Cas13bt3

Wild-type and HEPN mutants were expressed from a pET28-based vector with an N-terminal TwinStrep-SUMO tag transformed into chemically competent Rosetta Competent Cells (Novagen/EMD Millipore). Cells transformed with the expression plasmid were grown in 1 L of Terrific Broth at 37°C until OD 0.6. Temperature was switched to 18°C and the cultures were induced with 0.2 mM IPTG. Cultures were grown for 16-18 h, then cells were harvested with centrifugation at 5000xg at 4°C. The pellets were resuspended in 150 mL lysis buffer (150 mM NaCl, 20 mM Tris-HCl pH 7.5, 1 mM DTT, 5% glycerol) and homogenized by mixing on a magnetic plate at 4°C for 30 min. Cells were lysed by two passes through a microfluidizer at 18,000 psi and soluble fraction was separated from cell debris by centrifugation at 9,000 RPM for 30 min at 4°C. The soluble fraction was passed through Strep-Tactin resin (Qiagen). Resin was washed with 8 column volumes of lysis buffer and eluted from the column in lysis buffer supplemented with 5 mM desthiobiotin (Millipore-Sigma). The tags were cleaved overnight at 4°C by addition of SUMO protease. After cleavage, the proteins were passed through a heparin column (GE Healthcare) and concentrated to approximately 500 uL. Concentrated proteins were then passed through a Superdex 200 increase column (GE Healthcare) equilibrated in storage buffer (500 mM NaCl, 20 mM Tris-HCl pH 7.5, 1 mM DTT, 5% glycerol). Peak fractions were pooled and concentrated.

In vitro cleavage assay

Target and crRNAs were ordered as oligonucleotides from IDT and in vitro transcribed using the HiScribe T7 Quick High Yield RNA Synthesis kit (NEB) and purified using the RNA Clean & Concentrator-25 kit (Zymo Research). To label target RNA, 2.5 mM aminoallyl-UTP (Thermo Fisher) was included in the in vitro transcription reaction. Purified aminallyl-labeled RNA was then incubated with 625 µg IR800 NHS Ester (LiCor) in 50 mM sodium bicarbonate for 1 hour at room temperature in the dark and purified again with the RNA Clean & Concentrator-25 kit (Zymo Research). Assays were carried out with 100 nM IR800-labeled target RNA and 1:4

molar ratio of crRNA to Cas13bt3 wild-type or HEPN mutant protein in cleavage assay buffer (50 mM NaCl, 20 mM Tris-HCl pH 7.5, 5 mM MgCl2) at 37°C for 30 min. Reactions were quenched with Novex TBE-Urea sample loading buffer 2X (Thermo Fisher) and denatured for 3 minutes at 70 C. Samples were run on 10% TBE-Urea gels (Thermo Fisher) at 200 V for 50 min and imaged on a LiCor Odyssey CLx imager.

Fluorescent collateral RNA cleavage assay

Target and crRNAs were ordered as oligonucleotides from IDT and in vitro transcribed using the HiScribe T7 Quick High Yield RNA Synthesis kit (NEB) and purified using the RNA Clean & Concentrator-25 kit (Zymo Research). Assays were carried out with 4 technical replicates with equimolar ratios of Cas13bt3 wild-type or HEPN mutant protein, crRNA and RNA target in cleavage assay buffer with 10 U murine RNase inhibitor (NEB) and 500 nM RNAse Alert v2 sensor (Thermo Fisher). Samples were incubated for 3 hours at 37°C on a fluorescent plate reader equipped with a FAM filter set. Measurements were taken at 5 minute intervals and data were normalized to the first time point.

Immunofluorescence

Transfection and transduction were performed as described in 96-well plates. After 48 hours, cells were fixed for 12 minutes at room temperature with 4% paraformaldehyde. Following 3 5 minute washes with PBS, cells were permeabilized for 5 minutes at room temperature with 0.1% Tween-20 in PBS. Blocking was performed for 1 hour at room temperature with 6% goat serum (Cell Signaling Technologies) supplemented with 0.3% Triton X-100 (Millipore Sigma) in PBS (blocking buffer). Cells were subsequently incubated overnight at 4°C with 1:100 dilution of rat Anti-HA High Affinity 3F10 antibody (Roche) in a 1:3 dilution of blocking buffer. Cells were then washed 3 times for 5 minutes each in PBS supplemented with 0.1% Tween-20, then incubated for 1 hour at room temperature with a goat anti-rat AlexaFluor 647 secondary antibody (Invitrogen) in 1:3 dilution of blocking buffer. Cells were then covered in PBS with 1X DAPI stain and imaged.

Design and cloning of yeast expression plasmid constructs

Yeast reporter constructs were cloned into a pYES3/CT backbone (Thermo Fisher). A previously described reporter containing a crRNA expression cassette under a pADH1 terminator[1] was digested with HindIII and MluI (Thermo Fisher). A *URA3* gene was amplified by PCR using the selection marker from a pRSII426 backbone[2] with the introduced stop codon added by site-directed mutagenesis (Supplementary Table 4) and cloned via Gibson assembly This backbone was digested with BcuI (Thermo Fisher) and an *ADE2* gene amplified from M3499 ura3::ADE2 Disruptor Converter, a gift from David Stillman (Addgene plasmid # 51674 ; http://n2t.net/addgene:51674 ; RRID:Addgene_51674)[3], with the introduced stop codon added by site-directed mutagenesis (Supplementary Table 4) and cloned via Gibson assembly. crRNA spacers were cloned into this backbone using Golden Gate assembly[4]. Final constructs are listed in Supplementary Table 10.

Yeast REPAIR expression plasmids were derived from a previously described pRSII426 backbone[2] with a pGAL promoter driving expression of the REPAIR fusion protein[1]. The *URA3* selection marker was replaced with a *LEU2* selection marker by digesting this backbone with Eco105I and KpnI (Thermo Fisher) and inserting a *LEU2* gene amplified from a synthesized gene (IDT) by Gibson assembly. ADAR2 mutants to create sequences that could be used as a basis for error-prone PCR for each subsequent evolution round were inserted by amplifying the analogous sequence from the previous round of evolution and adding the new mutation via the site-directed mutagenesis (Supplementary Table 4). Final constructs are listed in Supplementary Table 10.

Cloning of mutagenesis libraries for ADAR evolution

ADAR2dd mutant libraries were generated by performing 8 error-prone PCR reactions for 20 cycles using a GeneMorph II Random Mutagenesis Kit (Agilent) with titrated template concentrations. For each round of evolution, we used a yeast codon-optimized ADAR2dd gene containing the selected mutants from all prior rounds. Resulting PCR reactions were pooled, gel purified, subjected to DpnI (Thermo Fisher) treatment and cloned into a yeast RanCas13b-

REPAIR expression backbone (Supplementary Table 10) digested with KflI and Eco72I (Thermo Fisher) by Gibson assembly. Libraries were transformed into Endura Electrocompetent Cells (Lucigen) by electroporation and plated over one 22.7cmx22.7cm ampicillin LB agar plate. After 12-16 hours of growth, libraries were scraped from plates and DNA was extracted using the Macherey-Nagel Nucleobond Xtra Maxiprep Kit (Macherey-Nagel). Primers are listed in Supplementary Table 4.

Directed evolution of high-specificity ADAR mutants

We performed two rounds of evolution as follows. To select for highly specific and efficient ADAR variants, we engineered a yeast reporter based on simultaneous restoration of a TGA stop codon in *ADE2* and negative selection of restoration of a TAG stop codon in *URA3*. We transformed *Saccharomyces cerevisiae* Meyen ex E.C. Hansen (ATCC® 204681™) with this plasmid, which also included expression of a crRNA targeting *ADE2*. Yeast were transformed using the lithium acetate/single-stranded carrier DNA/PEG method[5].

Large scale transformations of mutagenesis libraries were performed as previously described[1,6]. Briefly, we picked a colony from the initial transformation of the reporter plasmid, inoculated 300 mL of 2% glucose minimal media -tryptophan (Trp) for selection and grew overnight in a baffled flask at 30C. After 12-16 hours of growth, we measured the optical density (OD) of the culture and used this measurement to seed 2.5E9 cells into 500 mL of pre-warmed 2xYPAD media in a non-baffled flask. Once this culture reached an OD of 2 (approximately 4 hours), cells were harvested by centrifugation at 3000xg for 5 min, followed by two washes with water. The resulting cell pellet was then resuspended in 36 mL of transformation mix consisting of 24mL of PEG 3350 (50% w/v), 3.6 mL of 1.0 M Lithium acetate, 5 mL of denatured single-stranded carrier salmon sperm DNA at 2.0 mg/mL (Thermo Fisher), 2.9 mL of water, and 500 µL of 1 µg/µL plasmid library. The mixture was incubated at 42°C for 60 minutes with agitation, then the cells were pelleted once more and resuspended in 750 mL of 2% glucose minimal media -Trp/-leucine (Leu) and grown overnight at 30°C in a baffled flask until OD reached between 6 and 8. 6.25 mL of the culture was then seeded into 250 mL of 2% raffinose -Trp/-Leu selection

media and grown until OD reached between 0.5 and 1. The culture was then induced by adding 27 mL of 30% galactose and incubated overnight at 30°C for 12-15 hours.

After overnight growth, cultures were plated across 20 22.7x22.7 cm selection plates of 2% raffinose/3% galactose -Trp/-Leu with 5 mg/L adenine (Ade) and 0.1% 5-fluoroorotic acid (5-FOA). After 2-3 days of selection, we picked white colonies corresponding to an on-target edit and restoration of *ADE2* and streaked these onto small selection plates of the same media base to ensure accurate colony picking. Plates were then allowed to grow again for up to 3 days. White streaks after this second selection were again picked.

To look for enriched single mutations, all picked streaks were pooled and the contained RanCas13b-REPAIR genes were amplified with NEBNext High-Fidelity 2X PCR Master Mix (NEB) for preparation of next generation sequencing libraries. Libraries were sequenced on an Illumina NextSeq. Primers for library amplification are found in Supplementary Table 5. Relative enrichment of mutations in the selected library was analyzed using a custom Python script. Identified enriched single mutants were introduced by site-directed mutagenesis to RanCas13b-REPAIR in mammalian expression vectors for validation (Supplementary Table 4).

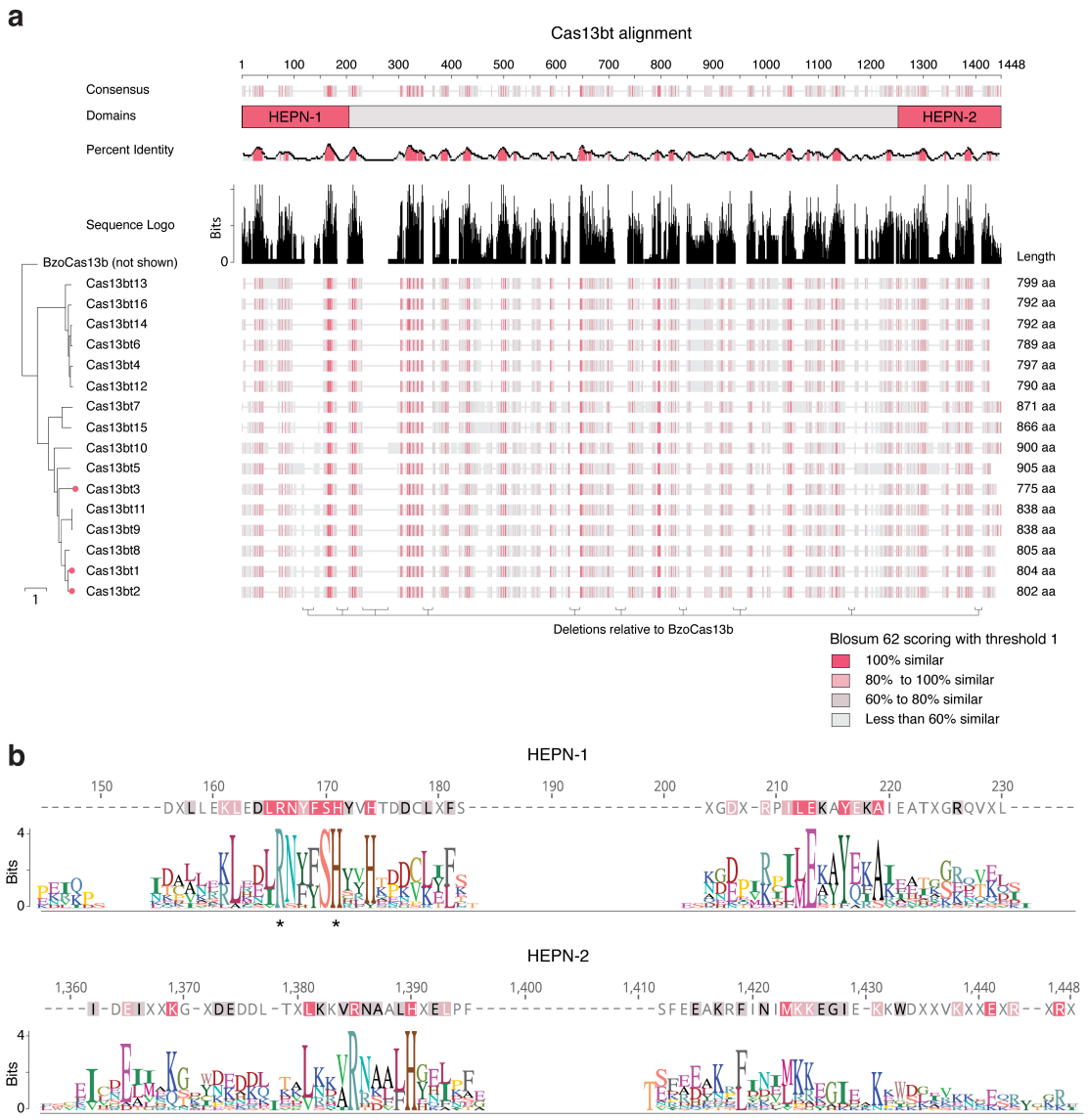
To test the candidate mutations, RNA editing assays using luciferase reporters in HEK293FT cells were performed as previously described. Specifically, after the first round of selection, RanCas13b-ADAR2dd mutants were targeted to either of 2 *Cypridina* luciferase reporters, one with a W85X mutation (TAG stop codon) and one with a W113X mutation (TGA stop codon) to evaluate the ability of the evolved ADAR2dd's to effectively edit at sites with both preferred and non-preferred 5' bases[7,8] (Supplementary Fig. 9a, b). After the second round of evolution, this initial screening was performed using the same *Cypridina luciferase* W85X reporter, along with a second *Cypridina* luciferase W85X (TGA stop codon) reporter and a *Gaussia* luciferase R93H reporter for which restoration of a CAT codon to CGT reverts a catalytically-inactivating mutation (Supplementary Fig. 10a-c). Luciferase activity of the *Cypridina* luciferase W85X TAG reporter in the non-targeting crRNA condition was also used as a proxy for measuring specificity, as previously described[9].

Based on this initial screening pass, top candidates were further validated for broad activity by testing again on the initial screen sites and additionally targeting the K19 and H36 codons in the endogenous *CTNNB1* transcript after the first round of selection (Supplementary Fig. 9c-f), and additionally on *Gaussia* luciferase reporters with G92R, R93K and R93Q catalytic mutations as well as the targeting of the T41 codon in *CTNNB1* (Supplementary Fig. 10d-j). Based on activity at all tested sites as measured by either next-generation sequencing and luciferase assays, as well as specificity measured as described, a single top candidate was identified and cloned into the RanCas13b-REPAIR yeast expression construct derived from the previous round of evolution to use as a basis for mutagenesis for the subsequent round.

After Round 1, we identified the E620G mutation and after Round 2, we identified the Q696L mutation. We additionally identified V505I as a mutation capable of enhancing editing at target sites with a 5'G (Supplementary Fig. 10).

SUPPLEMENTARY FIGURES

Supplementary Figure 1



c


BzoCas13b
Cas13bt1
Cas13bt2
Cas13bt3
19 aa
18 aa
18 aa
48 aa
13 aa
17 aa
19 aa
14 aa
11 aa
6 aa
20 aa
9 aa
6 aa
33 aa
19 aa
18 aa
29 aa
7 aa
18 aa
9 aa
6 aa
7 aa
11 aa
17 aa
6 aa
10 aa
Alpha helix
Beta sheet
Not present in Cas13bt1,2,3

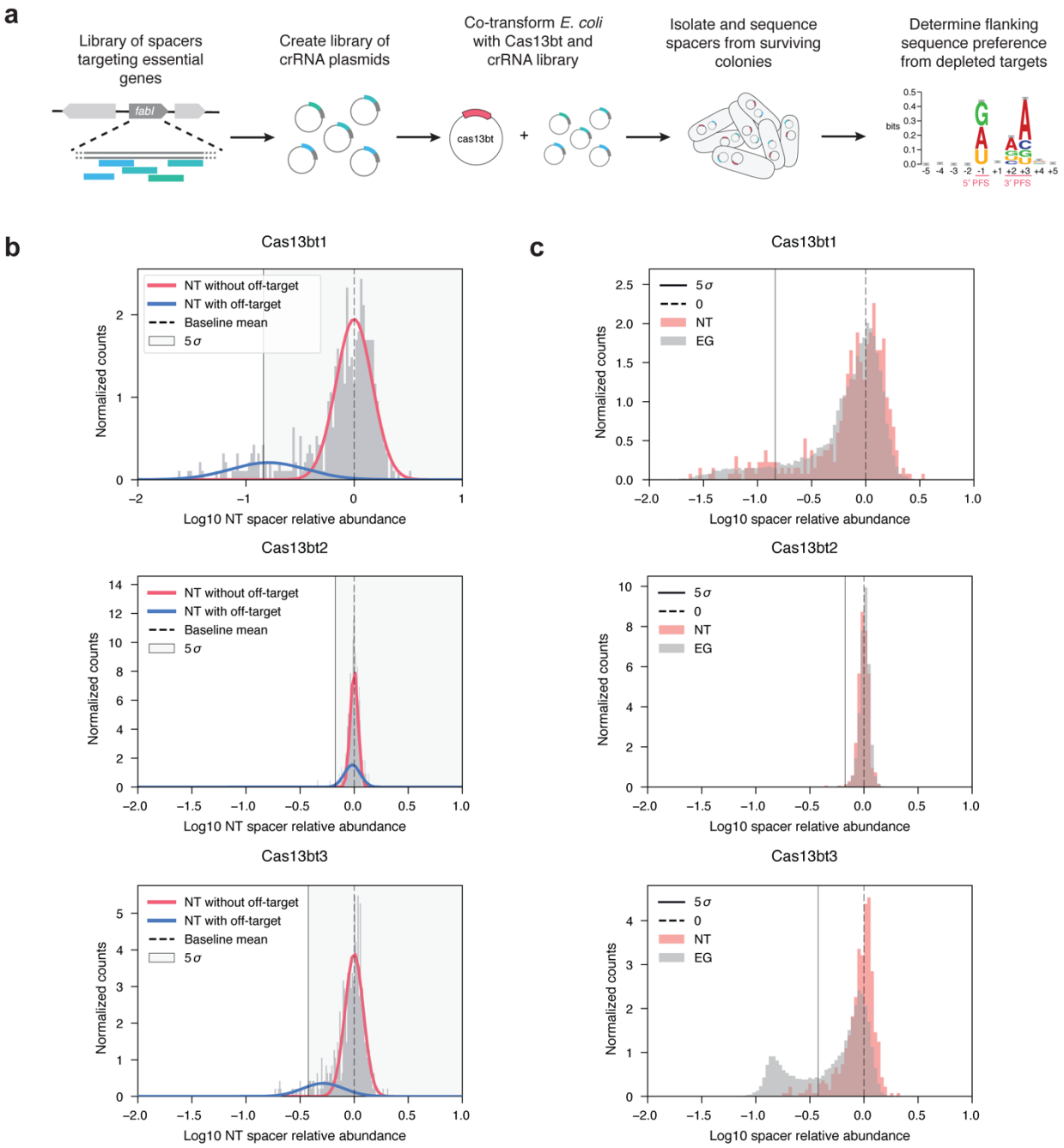
Supplementary Figure 1 | Cas13bt alignment and domain organization

a) Alignment of Cas13bt1 proteins juxtaposed to the phylogenetic tree of Cas13bt proteins. BzoCas13b is used as the outgroup but is not included in the alignment. Consensus sequence (at 25% or greater threshold) is shown at the top, followed by placement of HEPN domain locations as determined by alignment to BzoCas13b. Percent identity (smoothed over a 10bp sliding window, red ≥ 30%, grey < 30%) is displayed below, followed by the Cas13bt alignment, which is colored according to similarity as determined by a Blosum62 scoring. Amino acid deletions in Cas13bt relative to BzoCas13b are shown at the bottom. Such deletions are spread throughout the protein as opposed to concentrated in one location.

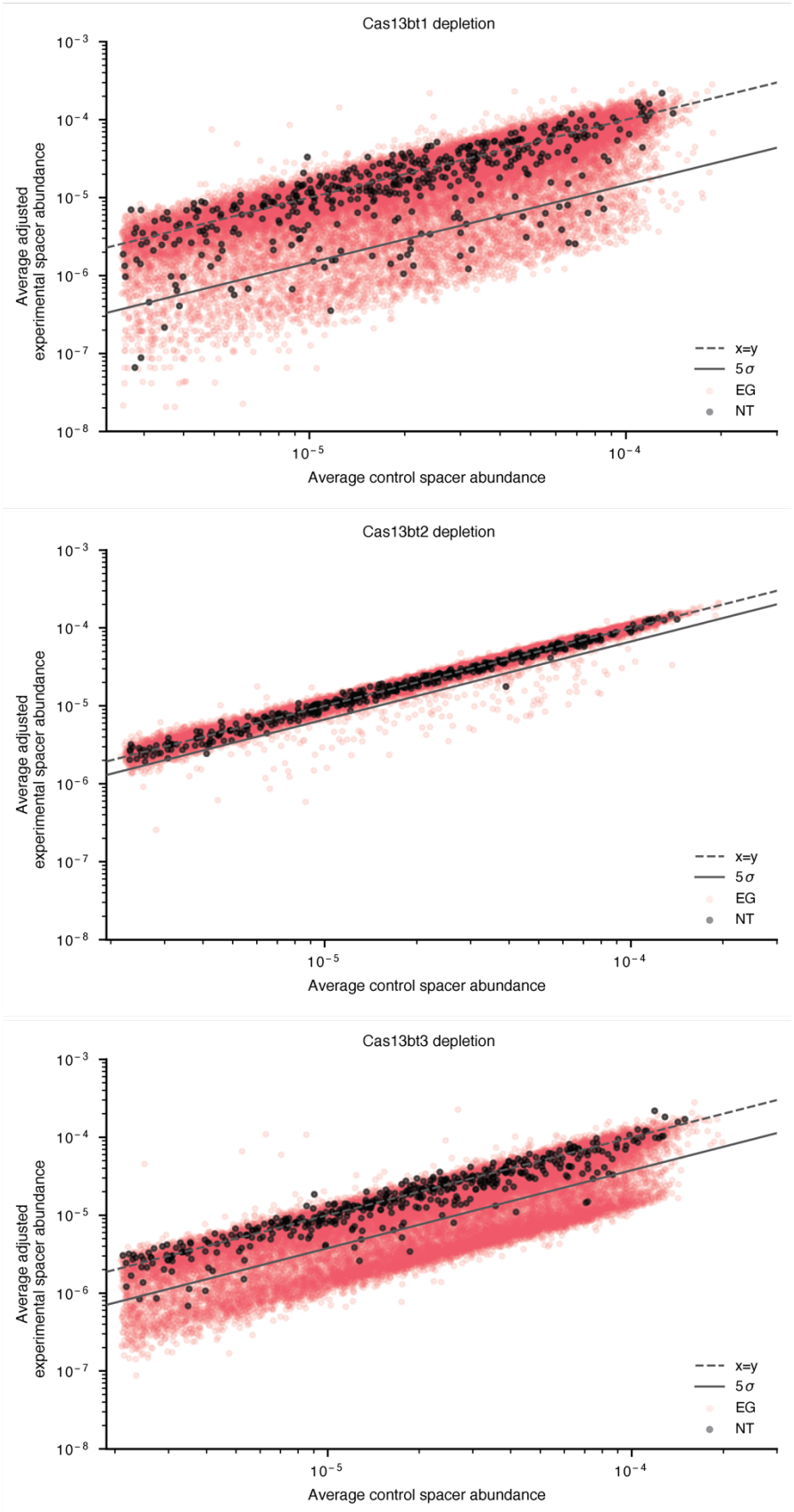
b) Consensus sequence (at 25% or greater threshold) and Weblogos of the core catalytic regions of the HEPN-1 and HEPN-2 domains of Cas13bt proteins.

c) Alignment of BzoCas13b, Cas13bt1, Cas13bt2, and Cas13bt3. Secondary structure for BzoCas13b was taken from the HHpred alignment to its pdb structure (6AAY)[10]. Deletions in Cas13bt1, Cas13bt2, and Cas13bt3 relative to BzoCas13b are shown. Amino acids are colored according to similarity as in (a).

Supplementary Figure 2



d


Cas13bt1 depletion
Average adjusted experimental spacer abundance
Average control spacer abundance
x=y
5σ
EG
NT
Cas13bt2 depletion
Average adjusted experimental spacer abundance
Average control spacer abundance
x=y
5σ
EG
NT
Cas13bt3 depletion
Average adjusted experimental spacer abundance
Average control spacer abundance
x=y
5σ
EG
NT

Supplementary Figure 2 | PFS preferences of Cas13bt orthologs

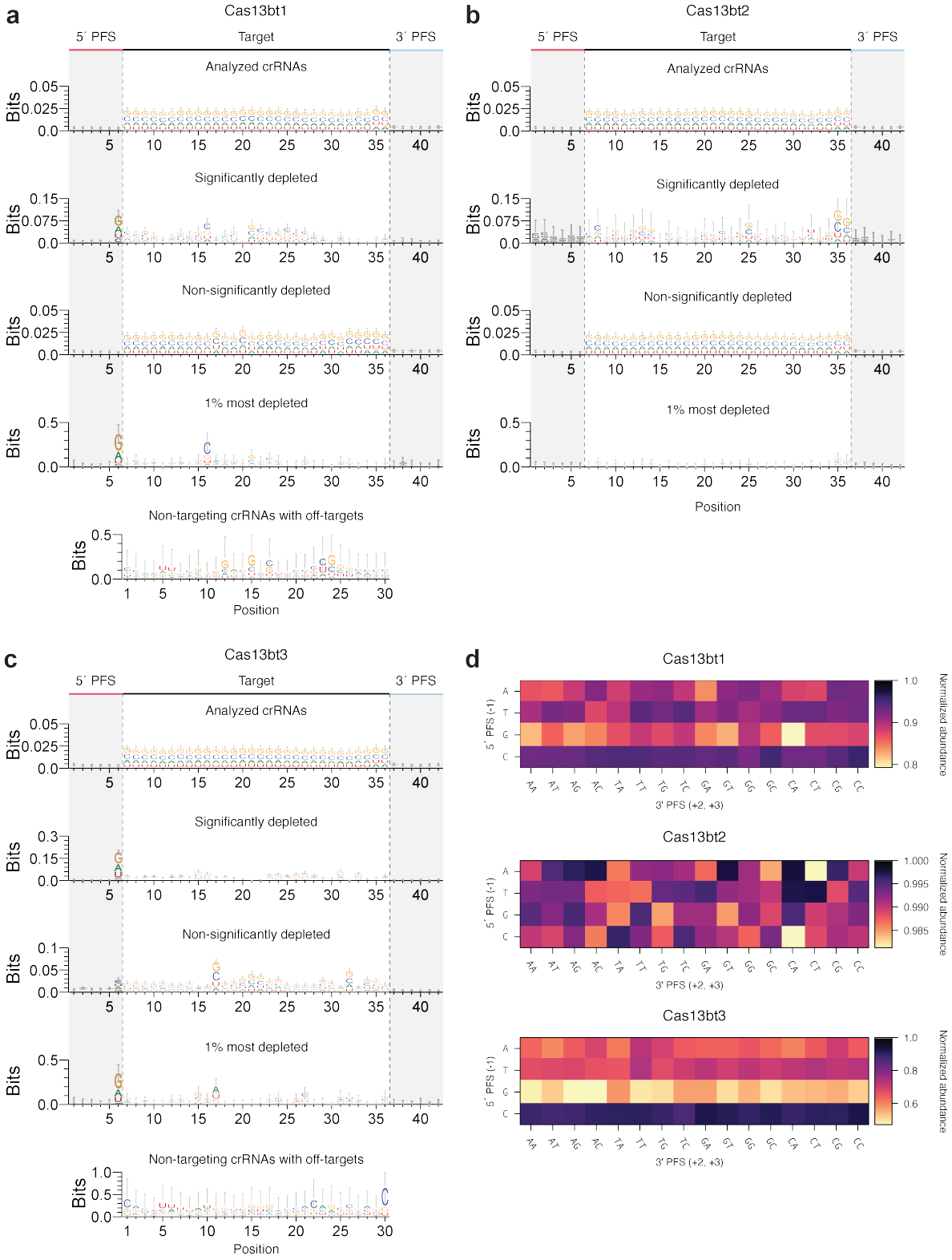
a) Workflow of *E. coli* essential gene screen for determining interference activity and PFS preference of Cas13bt orthologs.

b) Histogram of and two-component Gaussian mixture fit on log10 non-targeting spacer relative abundances on post-normalized data (centering the null distribution at zero). Blue line, first Gaussian component containing non-target spacers with off-target activity. Red line, second Gaussian component corresponding to the identified null distribution containing non-targeting spacers without depletion activity. 5σ significance threshold shown as black line with the rejection region shaded in light grey.

c) Histogram of post-normalized log10 spacer relative abundances for both non-targeting spacers (NT) and essential gene targeting spacers (EG), along with the 5σ significance threshold. Spacers to the left of the significance threshold are categorized as significantly depleted.

d) Spacer library with Cas13bt vs control library (with an empty vector) on post-normalized log10 spacer relative abundance for Cas13bt1, Cas13bt2, and Cas13bt3. For normalization, the log10 null distribution mean was subtracted from the log10 spacer library abundances to center non-targeting spacers with no off-targets at zero in log10 space. Black and red points are non-targeting (NT) spacers and essential gene targeting (EG) spacers respectively. The 5σ significance threshold is shown as a black line with the region below the line considered as significantly depleted.

Supplementary Figure 3



Supplementary Figure 3 | PFS screen of Cas13bt orthologs

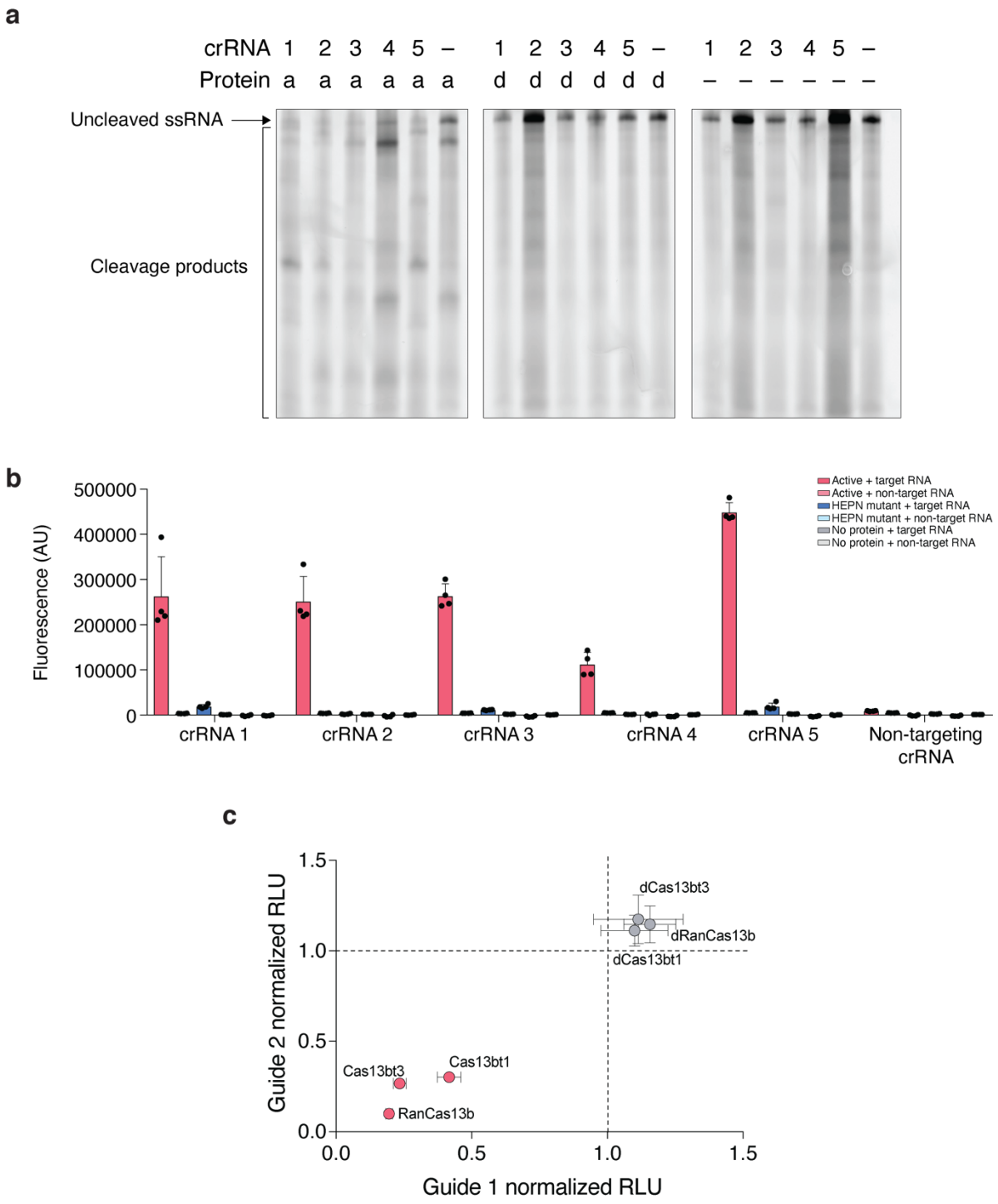
a) Cas13bt1 sequence preferences. From top to bottom: All analyzed targets of crRNAs in the input library for the Cas13bt1 screen, all targets of significantly depleted spacers, all targets of non-significantly depleted spacers, targets of the top 1% of most depleted spacers, and all non-targeting crRNAs with significant depletion (off-target activity). From top to bottom n = 30247, 2913, 27334, 304, 38.

b) Same as (A) but for Cas13bt2. Only two non-targeting spacers had off-target activity and are thus no Weblogo for non-targeting crRNAs is shown. From top to bottom, n = 30643, 265, 30378, 309.

c) Same as (A), but for Cas13bt3. From top to bottom n = 30822, 9319, 21503, 313, 15.

d) Heatmap of normalized relative spacer abundances across all spacers with specific 5′ and 3′ PFS triplet motifs.

Supplementary Figure 4



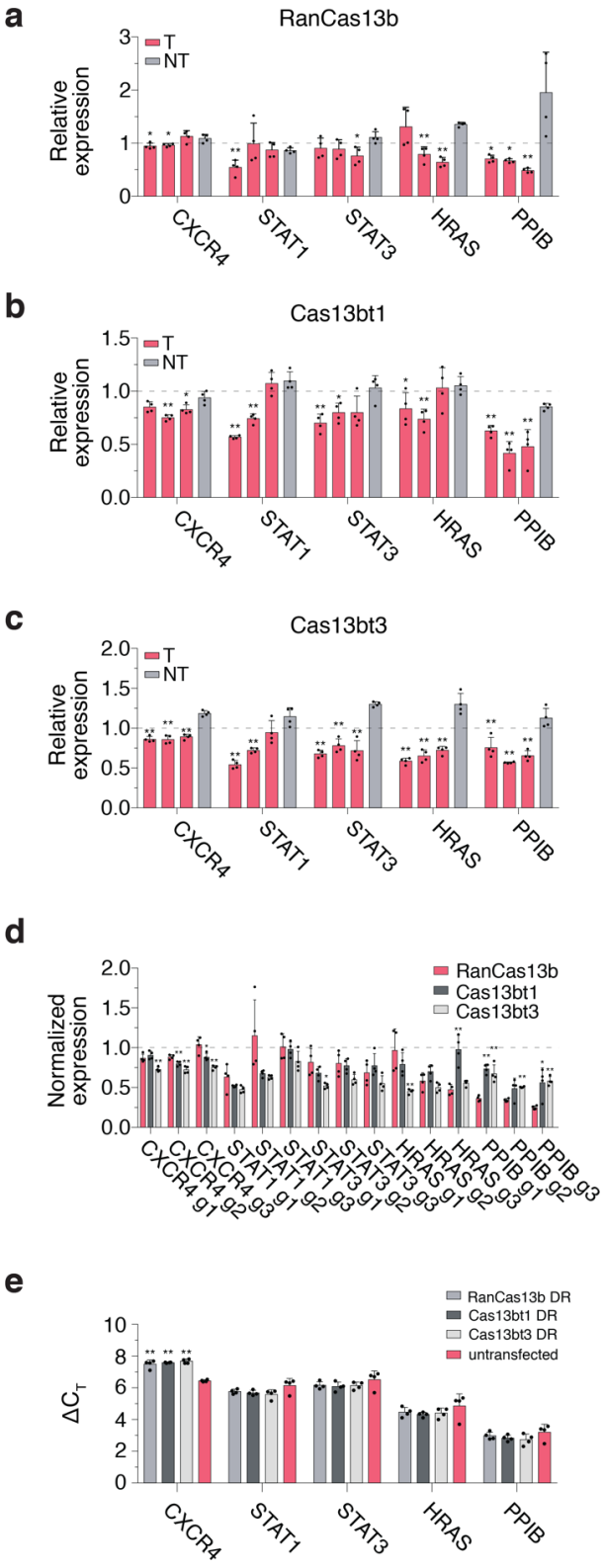
Supplementary Figure 4 | HEPN mutations abolish activity

a) Targeted in vitro cleavage of ssRNA targets by Cas13bt3. Mutation of catalytic residues in the HEPN domain abolishes cleavage activity. a: active HEPN domains; d: HEPN domains inactivated by alanine substitution of catalytic residues. This experiment was repeated once for a total of two experiments, with identical results.

b) Cas13bt3 exhibits collateral activity in a target- and HEPN domain-dependent manner. All values are average of $n = 4$ technical replicates, data are presented as mean ± standard deviation.

c) Wild-type sequence (red) and sequences with mutation of both the arginine and histidine residues to alanines in both HEPN domains of RanCas13b, Cas13bt1 and Cas13bt3 (gray) were targeted to a *Gaussia* luciferase transcript with two different targeting spacers. Knockdown, as indicated by decrease of luciferase activity, was abolished for HEPN-mutated proteins, with RanCas13b acting as a positive control. All values are normalized to a non-targeting spacer condition, points represent mean in both coordinates, and error bars represent standard deviation in both coordinates calculated by propagation of error, $n = 3$.

Supplementary Figure 5



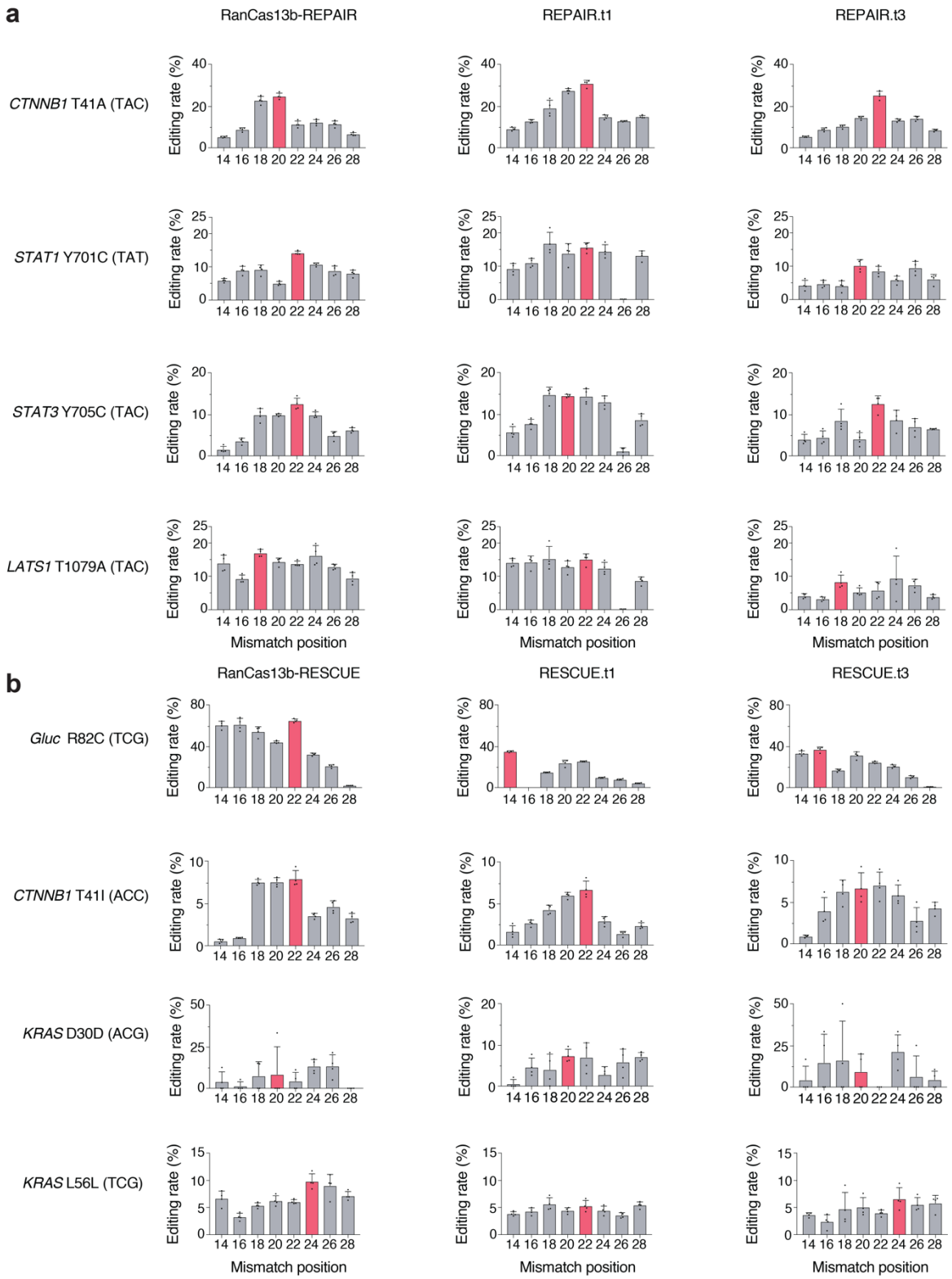
Supplementary Figure 5 | Comparison of RNA knockdown by RanCas13b and Cas13bt proteins

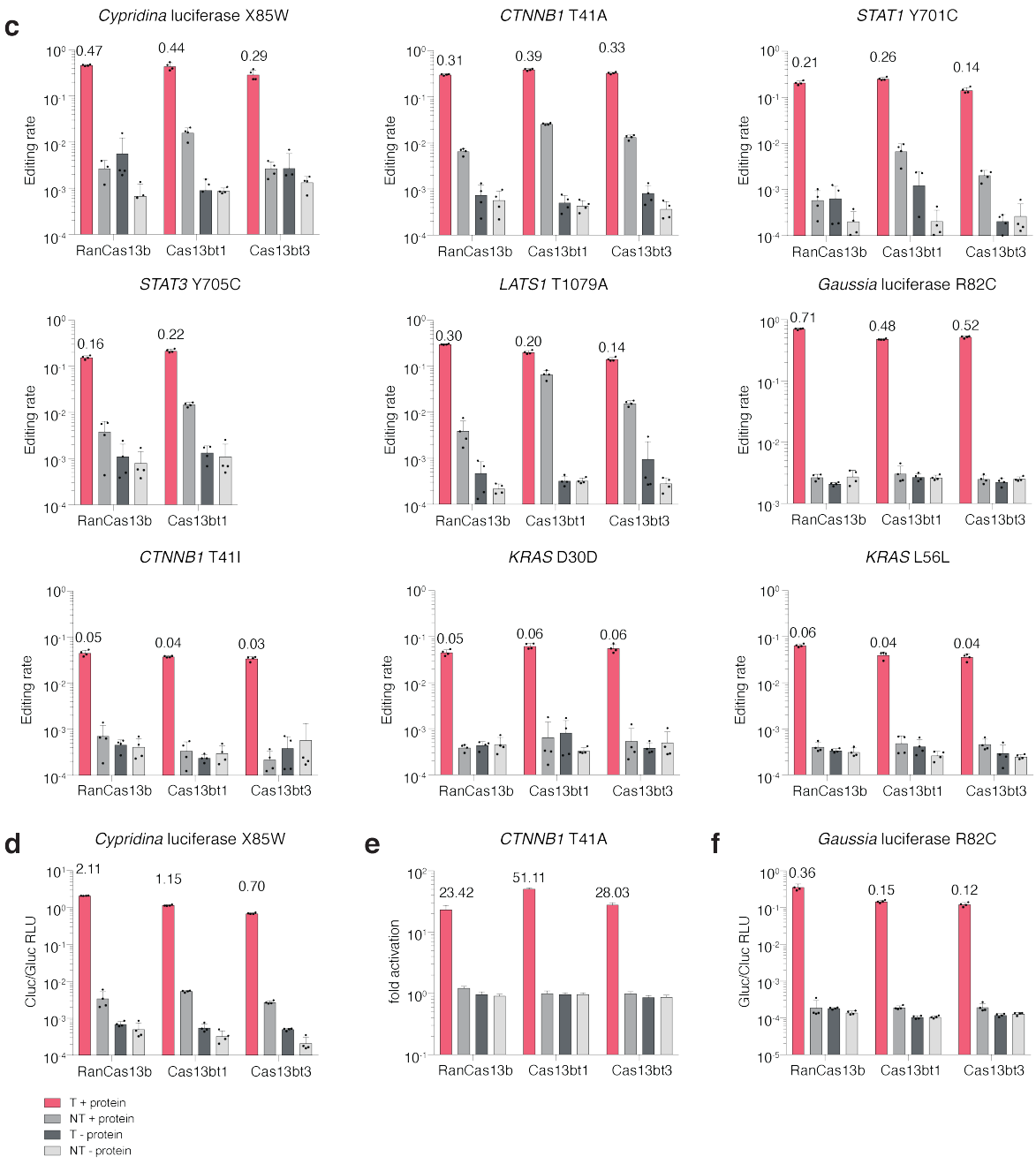
a) – d) Evaluation of RanCas13b, Cas13bt1 and Cas13bt3 knockdown of endogenous transcripts in HEK293FT cells. Cas13bt1 and Cas13bt3 data is the same as shown in Fig. 1h, shown here for direct comparison to RanCas13b. All values are normalized to a transfection control containing the corresponding crRNA without Cas13bt expression. Data are presented as mean ± standard deviation, $n = 4$. T: targeting crRNA, NT: non-targeting crRNA. Statistical significance compared to NT condition was assessed by a two-tailed t-test, with $*P < 0.05$ and $**P < 0.01$.

e) Comparison of expression of target genes in NT crRNA with GFP transfection condition for RanCas13b, Cas13bt1 and Cas13bt3 with untransfected cells. ΔC_T is relative to GAPDH control. Data are presented as mean ± standard deviation, $n = 4$. T: targeting crRNA, NT: non-targeting crRNA. Statistical significance compared to untransfected control was assessed by a two-tailed t-test, with $*P < 0.05$ and $**P < 0.01$.

Exact p-values listed in Table S11.

Supplementary Figure 6





Supplementary Figure 6 | RNA editing with Cas13bt

a) – b) Determination of optimal mismatch distance in RNA editing crRNA spacers. Quantitative evaluation of optimal mismatch distance for (a) RanCas13b-REPAIR, REPAIR.t1, REPAIR.t1 and (b) RanCas13b-RESCUE, RESCUE.t1, RESCUEt.3 targeting the indicated site by next-generation sequencing. In all panels, data are presented as mean ± standard deviation, $n = 4$. Red bars represent optimal mismatch

distance selected for each target/ortholog for all further experiments. The target adenosine or cytosine and flanking base on each side is shown in parentheses.

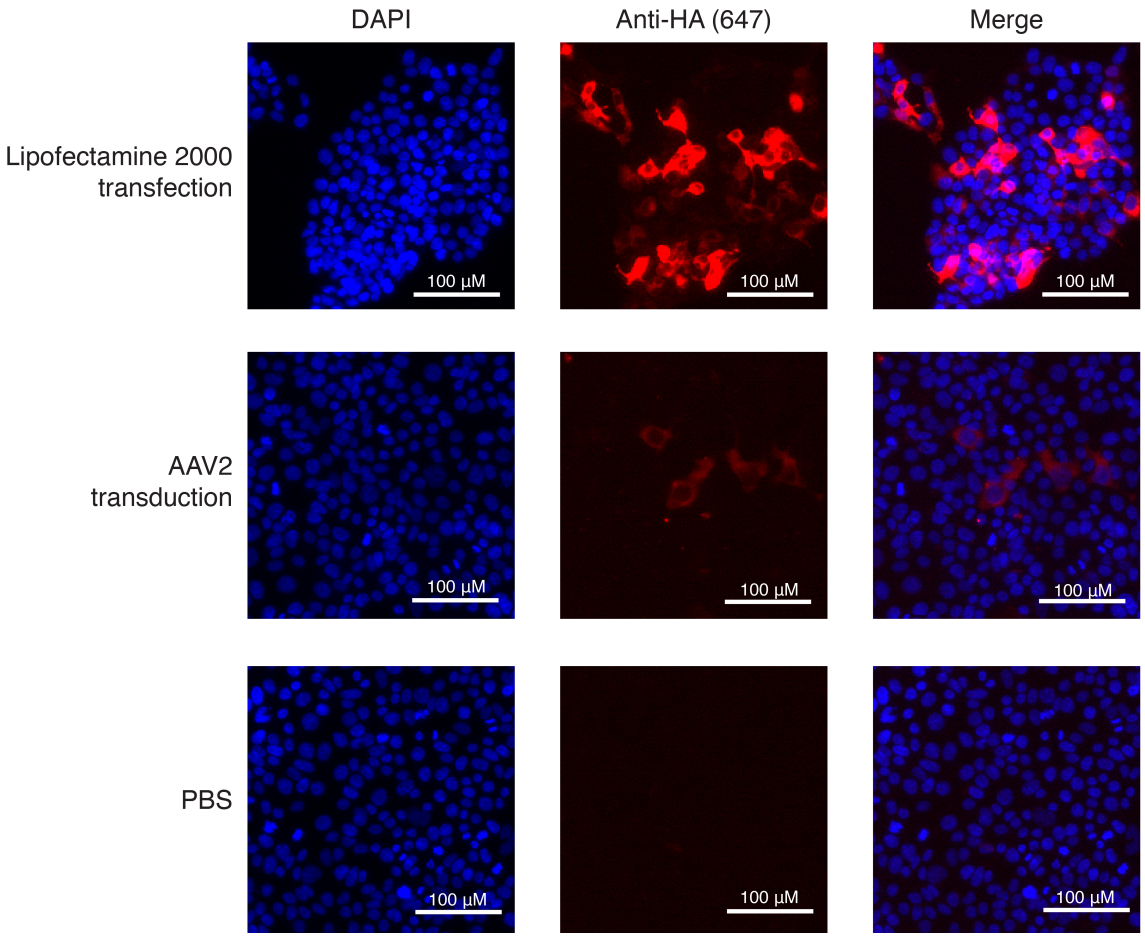
c) Comparison of RNA editing by RanCas13b, Cas13bt1, and Cas13bt3 at selected sites by quantification of editing rate by deep sequencing. In all panels, data are presented as mean ± standard deviation, $n = 4$. Mean value for targeting crRNA with REPAIR/RESCUE protein expression condition (red) is shown above the corresponding bar. Measurement of editing rate by deep sequencing at indicated target sites.

d) Restoration of luciferase activity by A-to-I RNA editing of a W85X *Cypridina* luciferase reporter. Data are presented as mean ± standard deviation, $n = 4$.

e) Fold activation of beta-catenin signaling by A-to-I RNA editing of the *CTNNB1* T41 codon as measured by normalized luciferase activity of a beta-catenin driven (TCF/LEF) luciferase reporter. Data are presented as mean ± standard deviation calculated by propagation of error, $n = 4$.

f) Restoration of luciferase activity by C-to-U RNA editing of a C82R *Gaussia* luciferase reporter. Data are presented as mean ± standard deviation, $n = 4$.

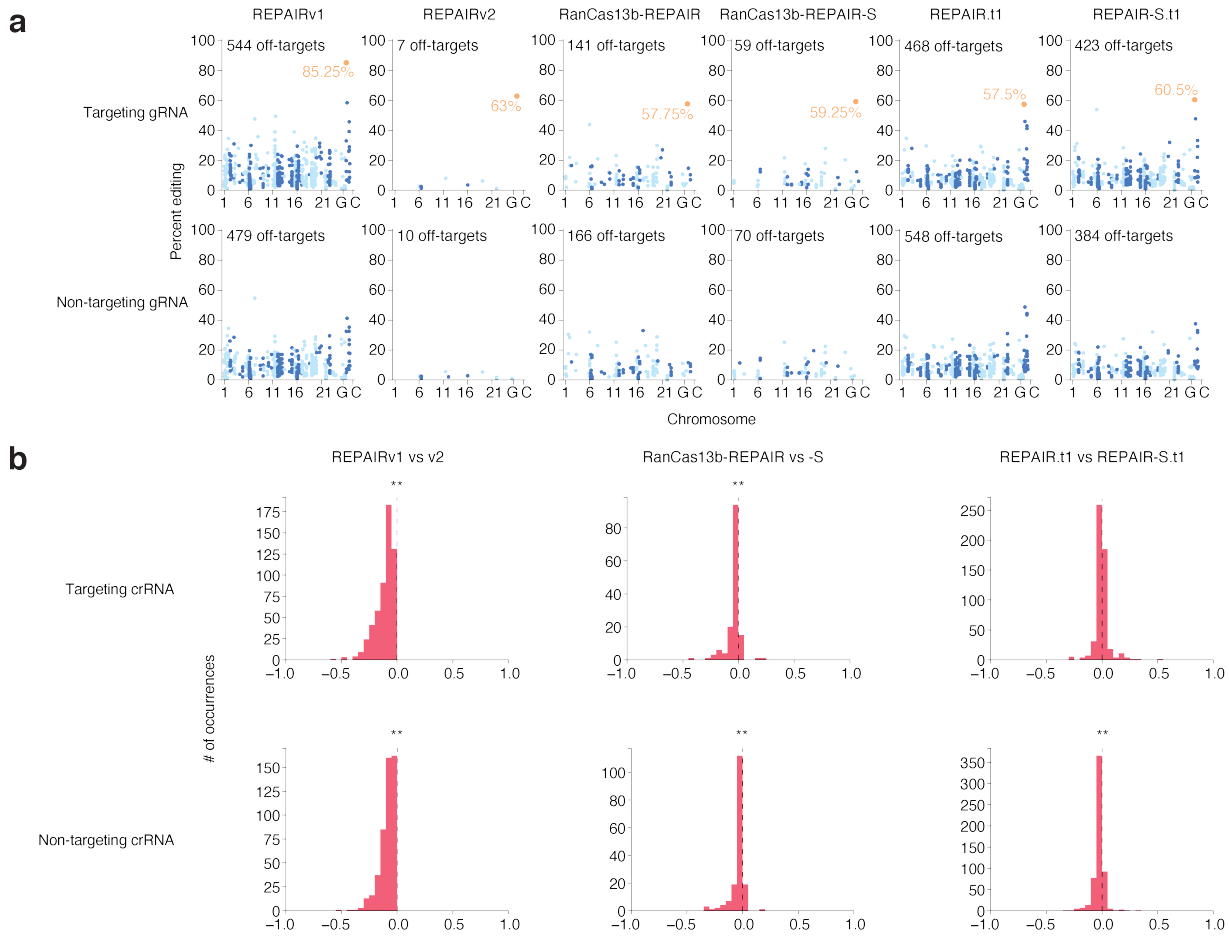
Supplementary Figure 7



Supplementary Figure 7 | Localization of Cas13bt-REPAIR

Representative images of immunofluorescence staining of 3xHA-tagged REPAIR.t1 delivered by transfection or recombinant AAV2 in HEK293FT cells. A PBS only control is included for comparison. This experiment was repeated once for a total of two experiments, with identical results.

Supplementary Figure 8

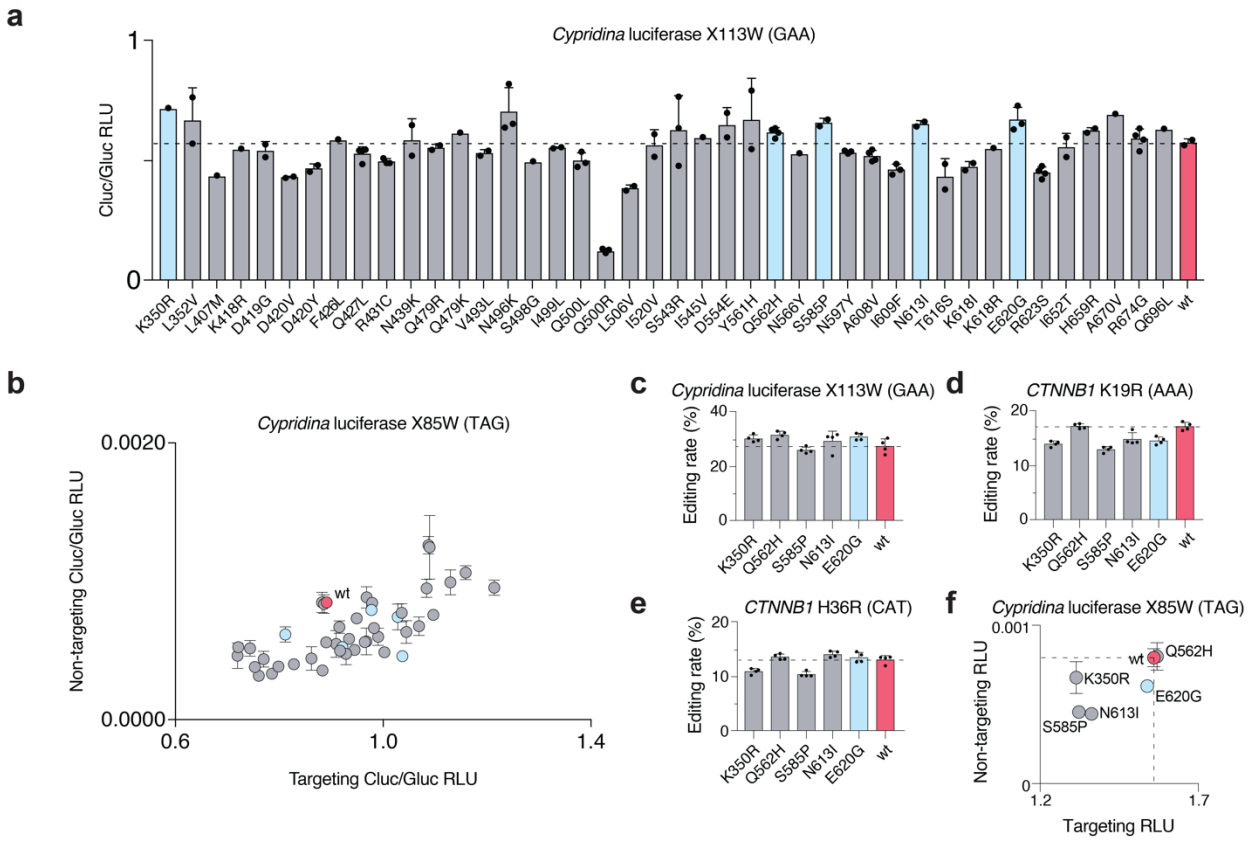


Supplementary Figure 8 | Comparison of off-target edits between REPAIR variants

a) Quantitative comparison of off-target editing between REPAIR variants in targeting and non-targeting crRNA conditions. Gold point marks the on-target edit. REPAIR-S refers to addition of E620G and Q696L specificity-enhancing mutants in ADAR2dd. G: *Gaussia* luciferase mRNA, C: *Cypridina* luciferase mRNA.

b) Histograms of change in editing rate at each off-target site between original and specific version of each REPAIR editing system as indicated. Statistical significance was assessed by a two-tailed paired t-test, and results are included in Supplementary Table 11.

Supplementary Figure 9



Supplementary Figure 9 | Evaluation of ADAR2dd mutants after Round 1 of evolution

In all panels, error bars represent standard deviation, $n = 4$. Wt refers to RanCas13b-ADAR2dd(E488Q). All amino acid changes refer to position in ADAR2dd and are on top of an ADAR2dd(E488Q) background. The nucleotide triplet containing the target adenosine is shown in parentheses. For (a)-(b), blue bars or points indicate mutations selected for further analysis. For (c)-(f), the blue bar or point indicates the final mutation selected from this round of evolution.

a) Evaluation of candidate mutants targeting a W113X *Cypridina* luciferase reporter as measured by restoration of luciferase activity. Data are presented as mean ± standard deviation, $n = 3$.

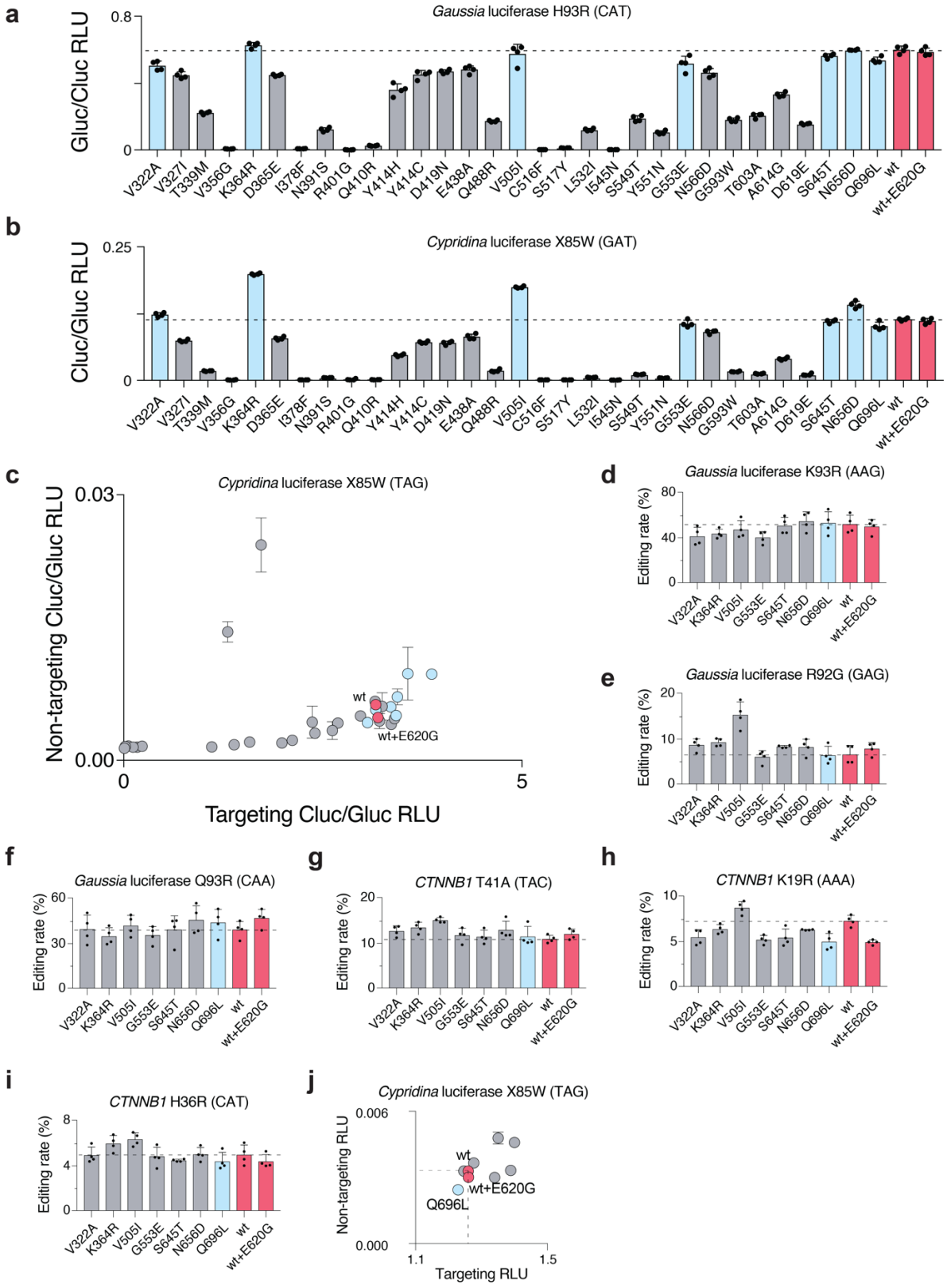
b) Evaluation of candidate mutants targeting a W85X *Cypridina* luciferase reporter as measured by restoration of luciferase activity. Non-targeting RLU refers to restoration of

luciferase activity in a non-targeting spacer condition and is used as a proxy for off-target editing. Data are presented as mean ± standard deviation of non-targeting RLU, $n = 3$.

c) – e) Evaluation of selected mutants targeting indicated sites as measured by next generation sequencing. Data are presented as mean ± standard deviation, $n = 4$.

f) Evaluation of candidate mutants targeting a W85X *Cypridina* luciferase reporter as measured by restoration of luciferase activity. Non-targeting RLU refers to restoration of luciferase activity in a non-targeting spacer condition and is used as a proxy for off-target editing. Data are presented as mean ± standard deviation of non-targeting RLU, $n = 4$.

Supplementary Figure 10



Supplementary Figure 10 | Evaluation of ADAR2dd mutants after Round 2 of evolution
In all panels, error bars represent standard deviation, $n = 4$. Wt refers to RanCas13b-ADAR2dd(E488Q) and wt+E620G refers to RanCas13b-ADAR2dd(E488Q/E620G). All amino acid changes refer to position in ADAR2dd and all mutations are on top of an ADAR2dd(E488Q/E620G) background. The target adenosine along with the flanking base each side is shown in parentheses. For (a)-(c), blue bars or points indicate mutations selected for further analysis. For (d)-(j), the blue bar or point indicates the final mutation selected from this round of evolution.

a) Evaluation of candidate mutants targeting a R93H *Gaussia* luciferase reporter as measured by restoration of luciferase activity. Data are presented as mean ± standard deviation, $n = 3$.
b) Evaluation of candidate mutants targeting a W85X (TGA stop codon) *Cypridina* luciferase reporter as measured by restoration of luciferase activity. Data are presented as mean ± standard deviation, $n = 3$.
c) Evaluation of candidate mutants targeting a W85X (TAG stop codon) *Cypridina* luciferase reporter as measured by restoration of luciferase activity. Non-targeting RLU refers to restoration of luciferase activity in a non-targeting spacer condition and is used as a proxy for off-target editing. Data are presented as mean ± standard deviation of non-targeting RLU, $n = 4$.
d) – (i) Evaluation of selected candidate mutants targeting indicated sites as measured by next generation sequencing. Data are presented as mean ± standard deviation, $n = 4$.
j) Evaluation of candidate mutants targeting a W85X (TAG stop codon) *Cypridina* luciferase reporter as measured by restoration of luciferase activity. Non-targeting RLU refers to restoration of luciferase activity in a non-targeting spacer condition and is used as a proxy for off-target editing. Data are presented as mean ± standard deviation of non-targeting RLU, $n = 4$.

SUPPLEMENTARY TABLES

Supplementary Table 1 | Accessions of contigs containing Cas13bt orthologs
JGI: Joint Genome Institute
NCBI WGS: National Center for Biotechnology Information Whole Genome Shotgun

Source database	Contig accession	Ortholog name (if applicable)	Source habitat/organism	(past JGI embargo)
JGI	Ga0246100_107590	Cas13bt7	Groundwater	(yes, published)
JGI	Ga0315552_1002530	Cas13bt15	Salt marsh sediment	(yes)
JGI	Ga0315552_1001799	Cas13bt13	Salt marsh sediment	(yes)
JGI	Ga0315532_1010951	Cas13bt16	Salt marsh sediment	(yes)
JGI	Ga0307431_1000754	Cas13bt14	Salt marsh sediment	(yes)
JGI	Ga0315541_1003536	Cas13bt6	Salt marsh sediment	(yes)
JGI	Ga0315296_10033793	Cas13bt4	Freshwater lake sediment	(yes)
JGI	Ga0307443_1009138	Cas13bt12	Salt marsh sediment	(yes)
JGI	Ga0315532_1006943	Cas13bt5	Salt marsh sediment	(yes)
JGI	Ga0315554_1005387	Cas13bt10	Salt marsh sediment	(yes)
NCBI WGS	QNBS01000103.1	Cas13bt3	Planctomycetes bacterium isolate B28_G16 (marine sediment)	N/A
JGI	Ga0315285_10018775	Cas13bt11	Freshwater lake sediment	(yes)
JGI	Ga0315294_10038294	Cas13bt9	Freshwater lake sediment	(yes)
JGI	Ga0315533_1000464	Cas13bt8	Salt marsh sediment	(yes)
JGI	Ga0209427_10000033	Cas13bt2	Marine sediment	(yes, published)
JGI	Ga0114919_10002421	Cas13bt1	Atlantic deep subsurface	(yes, obtained permission)

Supplementary Table 2 | Direct repeat sequences of Cas13 orthologs used in this study

Organism	Abbreviation key	DR sequence
Riemerella anatipestifer	Ran	GTTGGGACTGCTCTCACTTTGAAGGGTATTCACAAC
Prevotella sp. P5-125	Psp	GTTGTGGAAGGTCCAGTTTTGAGGGGCTATTACAAC
	bt1	GCTGTAATCACCCCACAAATCGGAGGCTTCTTCAGC
	bt2	GCTGTAATCACCCCACAAATCGGGGGCTTCTCCAGC
	bt3	GCTGTAATCACCCCACAAATCGGGGGCTGCTCCAGC

Supplementary Table 3 | Cas13 orthologs used in this study

Abbreviation key	Protein sequence
Ran	MEKPLLPNVYTLKHKFFWGAFLNIARHNAFITICHINEQLGLKTPSNDDKIVDVVC ETWNNILNNDHDLLKKSQLTELILKHFPFLTAMCYHPPKKEGKKKGHQKEQQKEKE SEAQSQAEALNPSKLIEALEILVNQLHSLRNYYSHYKHKKPDAEKDIFKHLYKAFD ASLRMVKEDYKAHFTVNLTRDFAHLNRKGKNKQDNPDFNRYRFEKDGFFTESGLLF FTNLFLDKRDAYWMLKKVSGFKASHKQREKMTTEVFCRSRILLPKLRLESRYDHNQ MLLDMLSELSRCPKLLYEKLSEENKKHFQVEADGFLDEIEEEQNPFKDTLIRHQDR FPYFALRYLDLNESFKSIRFQVDLGTYHYCIYDKKIGDEQEKRHLTRTLLSFGRLQ DFTEINRPQEWKALTKDLDYKETSNQPFISKTTPHYHITDNKIGFRLGTSKELYPS LEIKDGANRIAKYPYNSGFVAHAFISVHELLPLMFYQHLTGKSEDLLKETVRHIQR IYKDFEEERINTIEDLEKANQGRLPLGAFPKQMLGLLQNKQPDLSEKAKIKIEKLI AETKLLSHRLNTKLKSSPKLGKRREKLIKTGVLADWLVKDFMRFQPVAYDAQNQPI KSSKANSTEFWFIRRALALYGGEKNRLEGYFKQTNLIGNTNPHPFLNKFNWKACRN LVDFYQQYLEQREKFLEAIKNQPWEPYQYCLLLKIPKENRKNLVKGWEQGGISLPR GLFTEAIRETLSEDLMLSKPIRKEIKKHGRVGFISRAITLYFKEKYQDKHQSFYNL SYKLEAKAPLLKREEHYEYWQQNKPQSPTESQRLELHTSDRWKDYLLYKRWQHLEK KLRLYRNQDVMLWLMTLELTKNHFKELNLNYHQLKLENLAVNVQEADAKLNPLNQT LPMVLPVKVYPATAFGEVQYHKTPIRTVYIREEHTKALKMGNFKALVKDRRLNGLF SFIKEENDTQKHPISQLRLRRELEIYQSLRVDAFKETLSLEEKLLNKHTSLSSLEN EFRALLEEWKKEYAASSMVTDEHIAFIASVRNAFCHNQYPFYKEALHAPIPLFTVA QPTTEEKDGLGIAEALLKVLREYCEIVKSQI
Psp	MNIPALVENQKKYFGTYSVMAMLNAQTVLDHIQKVADIEGEQNENNENLWFHPVMS HLYNAKNGYDKQPEKTMFIIERLQSYFPFLKIMAENQREYSNGKYKQNRVEVNSND IFEVLKRAFGVLKMYRDLTNHYKTYEEKLNDGCEFLTSTEQPLSGMINNYYTVALR

	NMNERYGYKTEDLAFIQDKRFKFVKDAYGKKKSQVNTGFFLSLQDYNGDTQKKLHL SGVGIALLICLFLDKQYINIFLSRLPIFSSYNAQSEERRIIIRSFGINSIKLPKDR IHSEKSNKSVAMDMLNEVKRCPDELFTTLSAEKQSRFRIISDDHNEVLMKRSSDRF VPLLLQYIDYGKLFDHIRFHVNMGKLRYLLKADKTCIDGQTRVRVIEQPLNGFGRL EEAETMRKQENGTFGNSGIRIRDFENMKRDDANPANYPYIVDTYTHYILENNKVEM FINDKEDSAPLLPVIEDDRYVVKTIPSCRMSTLEIPAMAFHMFLFGSKKTEKLIVD VHNRYKRLFQAMQKEEVTAENIASFGIAESDLPQKILDLISGNAHGKDVDAFIRLT VDDMLTDTERRIKRFKDDRKSIRSADNKMGKRGFKQISTGKLADFLAKDIVLFQPS VNDGENKITGLNYRIMQSAIAVYDSGDDYEAKQQFKLMFEKARLIGKGTTEPHPFL YKVFARSIPANAVEFYERYLIERKFYLTGLSNEIKKGNRVDVPFIRRDQNKWKTPA MKTLGRIYSEDLPVELPRQMFDNEIKSHLKSLPQMEGIDFNNANVTYLIAEYMKRV LDDDFQTFYQWNRNYRYMDMLKGEYDRKGSLQHCFTSVEEREGLWKERASRTERYR KQASNKIRSNRQMRNASSEEIETILDKRLSNSRNEYQKSEKVIRRYRVQDALLFLL AKKTLTELADFDGERFKLKEIMPDAEKGILSEIMPMSFTFEKGGKKYTITSEGMKL KNYGDFFVLASDKRIGNLLELVGSDIVSKEDIMEEFNKYDQCRPEISSIVFNLEKW AFDTYPELSARVDREEKVDFKSILKILLNNKNINKEQSDILRKIRNAFDHNNYPDK GVVEIKALPEIAMSIKKAFGEYAIMK
bt1	MEFENIKKTSNKEVYSIEQYEGEKKWCFAIVLNRAQTNLEENPKLFEQTLTRFEKIM KQDWFNEETKKLIYEKEEENKVKEEIQIAASERLKNLANYFSAYLHAPDCLIFNRND TIRIIMEKAYEKSRFEAKKKQQEDISIEFPELFEEEDKITSAGVVFFVSFFIERRFL NRLMGYVQGFRKTEGEYNITRQVFSKYCLKDSYSVQAQDHDAVMFRDILGYLSRVPT EIYQHIKLTRKRSQDQLSERKTDKFILFALKYLEDYGLKDLADYTACFARSKIKREN EDTKETDGNKHKFHREKPVVEIHFDKEKQDQFYIKRNNVILKAQKKGGQSNVFRMGV YELKYLVLLSLLGKAEEAIQRIDRYISSLKKQLPYLDKISNEEIQKSINFLPRFVRS RLGLLQVDDEKRLKTRLEYVKAKWTDKKEGSRKLELHRKGRDILRYINERCDRPLSR KEYNNILKFIVNKDFAGFYNELEELKRTRRLDKNIIQKLSGHTTLNALHERVCDLVL QELGSLQSENLKEYIGLIPKEEKEVTFREKVDRILEQPVVYKGFLRYEFFKEDKKSF ARLVEEAIKTKWSDFDIPLGEEYYNIPSLDRFDRTNKKLYETLAMDRLCLMMARQYY LRLNEKLAEKAQHIYWKKEDGREVIIFKFQNPKEQKKSFSIRFSILDYTKMYVMDDP EFLSRLWEYFIPKEAKEIDYHKHYARAFDKYTNLQKEGIDAILKLEGRIIERRKIKP AKNYIEFQEIMNRSGYNNDQQVALKRVANALLAYNLNFEREHLKRFYGVVKREGIEK KWSLIV
bt2	MQVENIKKGSSQGMYSIEQYEGAKKWCFAIVLNRAQTNLQGNPKLFEETLTRFERIR KEDWFDQETKKLIYAKQEQNEVEEEIQKAADEKLRDLRNYFSHYFHTPDCLIFTQND PVRIIMEKAYEKARFEQAKKEQEDISIEFGELFEENGRITSAGVVFFASFFAERRFL NRLMGYVQGFTRTEGEYKITRDVFSTYCLRDSYSVKTPDHDAVMFRDILGYLSRVPS ESYQRIKESQMRSETQLSERKTDKFILFALNYLEDYGLEDLADYTACFARTRIKREQ DENTDGKEQKPHRKKPRVEIHFERAEGDPFYIKHNNVILRTQKKGAQTYIFRMGVYE

	LKYLVLLSLLGKGAEAVKRIDRYVHSLRNQLPHIEKKSTEEIEGYVRFLPRFVRSHL GLLGVDDEKKIKARVDYVKAKWLEKKEKSRELQLHRKGRDILRYINERCERPLNIDE YNRILELLVTKHLDGFYRELEELKKTRRIDKNIVCNLSRHKSVNALHEKVCDLVVQE LESLGREELKEYVGLIPKEEKEVSFEEKTDRVVKQPVIYKGFLRNEFFRESRKSFAR LVEEAVREKGEVYDVPLGGEYYEIVSLDTFDKDNKRLYETLAMDRLLLMIARQYHLS LNKELAKRAQQIEWKKEDGEEVIIFTLKNPAQPEQSCSVRFSLRDYTKLYVMDDAEF LARLCDYFLPKDEEQIDYHRLYTQGMNRYTNLQREGIEAILELEKKTIGPEQPRPPK NYIPFSEIMDKSAYNEDDQKALRRVRNALLHHNLNFARADFKRFCGIMKREGIEKRW SLAV
bt3	MAQVSKQTSKKRELSIDEYQGARKWCFTIAFNKALVNRDKNDGLFVESLLRHEKYSK HDWYDEDTRALIKCSTQAANAKAEALANYFSAYRHSPGCLTFTAEDELRTIMERAYE RAIFECRRRETEVIIEFPSLFEGDRITTAGVVFFVSFFVERRVLDRLYGAVSGLKKN EGQYKLTRKALSMYCLKDSRFTKAWDKRVLLFRDILAQLGRIPAEAYEYYHGEQGDK KRANDNEGTNPKRHKDKFIEFALHYLEAQHSEICFGRRHIVREEAGAGDEHKKHRTK GKVVVDFSKKDEDQSYYISKNNVIVRIDKNAGPRSYRMGLNELKYLVLLSLQGKGDD AIAKLYRYRQHVENILDVVKVTDKDNHVFLPRFVLEQHGIGRKAFKQRIDGRVKHVR GVWEKKKAATNEMTLHEKARDILQYVNENCTRSFNPGEYNRLLVCLVGKDVENFQAG LKRLQLAERIDGRVYSIFAQTSTINEMHQVVCDQILNRLCRIGDQKLYDYVGLGKKD EIDYKQKVAWFKEHISIRRGFLRKKFWYDSKKGFAKLVEEHLESGGGQRDVGLDKKY YHIDAIGRFEGANPALYETLARDRLCLMMAQYFLGSVRKELGNKIVWSNDSIELPVE GSVGNEKSIVFSVSDYGKLYVLDDAEFLGRICEYFMPHEKGKIRYHTVYEKGFRAYN DLQKKCVEAVLAFEEKVVKAKKMSEKEGAHYIDFREILAQTMCKEAEKTAVNKVARA FFAHHLKFVIDEFGLFSDVMKKYGIEKEWKFPVK

Supplementary Table 4 | Primers used for plasmid cloning in this study (attached file)

Supplementary Table 5 | Next-generation sequencing library preparation first round PCR primers (attached file)

Supplementary Table 6 | crRNA spacer sequences and reported mean knockdown efficiency for RNA knockdown experiments (attached file)

Supplementary Table 7 | TaqMan probes used for qPCR

Gene	**TaqMan assay ID**
CXCR4	Hs00607978_s1

STAT1	Hs01013996_m1
STAT3	Hs00374280_m1
HRAS	Hs00978050_g1
PPIB	Hs00168719_m1
GAPDH	Hs99999905_m1

Supplementary Table 8 | crRNA spacer sequences used in RNA editing experiments and reported mean editing rates (attached file)

Supplementary Table 9 | Gene-specific reverse transcription primers

Gene	**RT primer sequence**
Cypridina luciferase	TTTGCATTCATCTGGTACTTCTAGGGTGTC
STAT1	TTCATCATACTGTCGAATTCTACAGAGCCC
CTNNB1	TTACAGGTCAGTATCAAACCAGGCCAG
STAT3	TTTCTGCAGCTTCCGTTCTCAGCTCCTCAC
LATS1	TACTAGATCGCGATTTTTAATCTCTGAGCC
Gaussia luciferase	TTGTCCACCTGGCCCTGGATC
KRAS	TCATCAACACCCTGTCTTGTCTTTGCT

Supplementary Table 10 | Plasmids used in this study

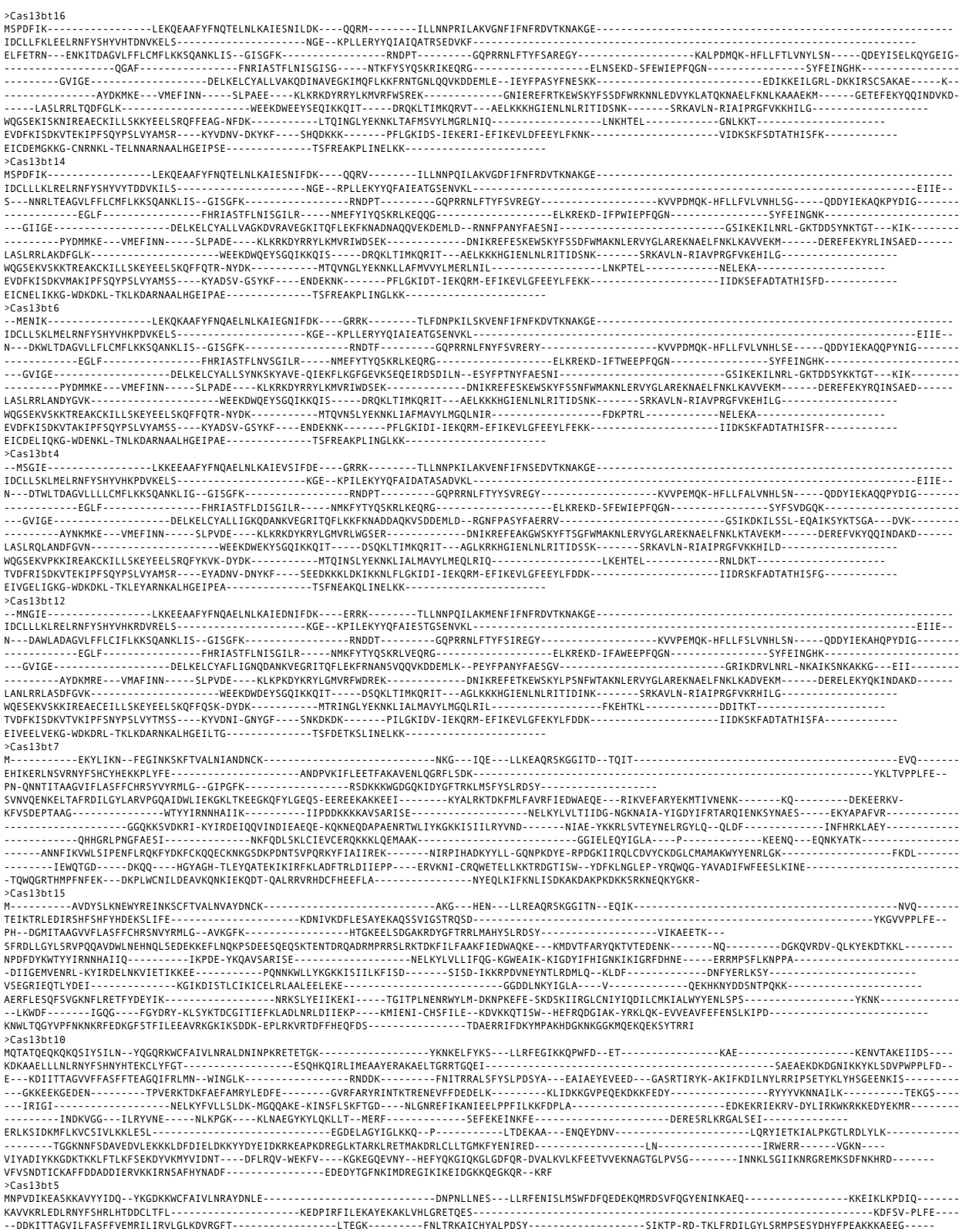
Name	**Description**	**Expression system**	**Link to map**
pAB1865	pACYC184 pJ23119-BsmbI-Bt1 DR	Bacterial	https://benchling.com/s/seq-jdXHoC8o1gHQOAsKMDgt
pAB1866	pACYC184 pJ23119-BsmbI-Bt2 DR	Bacterial	https://benchling.com/s/seq-RsjPyVdN3mzWutGY3own

pAB1867	pACYC184 pJ23119-BsmbI-Bt3 DR	Bacterial	https://benchling.com/s/seq-WTEP5cSlRvVSrad9ABV9
pAB1898	pBR322 pLac-Cas13bt1	Bacterial	https://benchling.com/s/seq-EVVXT0kZN2ggPrEIx2Q8
pAB1899	pBR322 pLac-Cas13bt2	Bacterial	https://benchling.com/s/seq-OIpN9ZAueFhUnGnt7Vbv
pAB1900	pBR322 pLac-Cas13bt3	Bacterial	https://benchling.com/s/seq-8EKdfcC22W4xaaKOdY4Z
pAB1619	U6-BpiI-Cas13bt1-DR	Mammalian	https://benchling.com/s/seq-74LcGCcwVgbjcobwvpOF
pAB1620	U6-BpiI-Cas13bt3-DR	Mammalian	https://benchling.com/s/seq-jOItmmKUMFC4p0XkQn87
pAB1678	CMV-HIVNES-GS-Cas13bt1	Mammalian	https://benchling.com/s/seq-1GbngY39NHrnQx1WyjR1
pAB1679	CMV-HIVNES-GS-Cas13bt3	Mammalian	https://benchling.com/s/seq-lzOtR98Znh5xe5Fsj3q2
pAB1680	CMV-HIVNES-GS-dCas13bt1	Mammalian	https://benchling.com/s/seq-jOBIs4seR2lEQgAwyY2R
pAB1681	CMV-HIVNES-GS-dCas13bt3	Mammalian	https://benchling.com/s/seq-LhKabcsXDZtjApamjRo4
pAB1676	CMV-HIVNES-GS-dCas13bt1-(GGS)2-huADAR2dd(E488Q)	Mammalian	https://benchling.com/s/seq-F8hF1Xw8nRBb7lnOf1S1
pAB1677	CMV-HIVNES-GS-dCas13bt3-(GGS)2-huADAR2dd(E488Q)	Mammalian	https://benchling.com/s/seq-RUlMPZLeMyBUsQ8tiJVP
pAB1322	CMV-HIVNES-GS-dCas13b6-(GGS)2-huADAR2dd(E488Q)	Mammalian	https://benchling.com/s/seq-yV5vnqB5ryKne078EIsp
pAB1659	CMV-HIVNES-GS-dCas13b6-(GGS)2-huADAR2dd(E488Q/E620G)	Mammalian	https://benchling.com/s/seq-PsZGBlhVj9w5NgYuK19S
pAB1810	CMV-HIVNES-GS-dCas13b6-	Mammalian	https://benchling.com/s/seq-

	(GGS)2-huADAR2dd(E488Q/E620G/Q696L)		XLOzXjEsqGoAasELWepp
pAB1923	CMV-HIVNES-GS-dCas13bt1-(GGS)2-huADAR2dd(E488Q/E620G/Q696L)	Mammalian	https://benchling.com/s/seq-uV9joNAPLNBZFgYRxFOw
pAB0040	CMV-Cluciferase(STOP85)-polyA EF1a-G-luciferase-polyA	Mammalian	Previously described[9]
pAB1424	CMV-Cluciferase(W113X TGA)-polyA EF1a-G-luciferase-polyA	Mammalian	https://benchling.com/s/seq-acYfrnZ9yJIXY04xl2C1
pAB1230	pYES3/CT pADH1-HH-BsmBI-B6-DR-HDV-ADH1-term TGA-ADE2 TAG-URA3	Yeast	https://benchling.com/s/seq-CYf3c9t6y8Eq1IgeJsBK
pAB1417	pGAL-dCas13b6-(GGS)2-dADAR2(E488Q)	Yeast	https://benchling.com/s/seq-9kuxRwYrqoyS6yB8O75P
pAB1773	pGAL-dCas13b6-(GGS)2-dADAR2(E488Q/E620G)	Yeast	https://benchling.com/s/seq-WQzBpL605i52mv5w2ahL
pAB2076	pAAV EFS-HIVNES-dCas13bt1-(GGS)2-huADAR2(E488Q)-3xHA BGHpolyA::U6-BpiI-Cas13bt1 DR	AAV (Mammalian)	https://benchling.com/s/seq-1J450xvOJ2vfgPa3FcB5

Supplementary Table 11 | Results from statistical analyses including test statistics and p-values for tests performed in main figures and Supplemental Figs. 8 and 14.

Supplementary Note 1 | Alignment of Cas13bt orthologs



```
KKRQTVNDLHQRVCNLMIRKLEDLQQS-------------------------GDLEQLKCYIGLA----PAWEYRDEDQ--TGKEQ-----KEQRFENK----------------------VKNL---KPMLYRGFLRERFFAEY----------
------RSKENKRNFADLVEDVRQKKG-EGDVPVDLIYYQIEGDTQE-------IQVANKKLTETLARDRLCLLIGREYLEQLNRT-----------------LSQNAIEERHGPKRKYFIKTEWSKEP-
VQMGDGKERMRDVIICRIRENPEDENPLCSIRFAVKHWTKLYVMDDP----FFLSDV-HSYFL----S--KSNEIDY--HTLNQKGICQ-YTNLQA-DCMVNILKLEKKVFERVTKRDIDKEKDIKKLINEINNKL-QHLPAKKEDNRVPFL---
----------LVCEAALEKGILKEKSEIDVVSMVRNSAFHYQLYF----------------SKSEKETFDRIMRREGI-------------------
>Cas13bt3
M-AQVSKQTSKKRELSIDE--YQGARKWCFTIAFNKALVNRD---------------------------KNDGLFVES---LLRHEKYSKHDWYD--EDTRAL----------IKCST-----------------------------------
QAANAKAEALRNYFSHYRHSPGCLTFT---------------------AEDELRTIMERAYERAIFECRRRETEVI-----------------------------------------------------------------------IEFPSLFE----
--GDRITTAGVVFFVSFFVERRVLDRLYG--AVSGLK----------------KNEGQ---------YKLTRKALSMYCLKDSR-----------------FTKAWDKR-VLLFRDILAQLGRIPAEAYEYYHGEQ----------------
-GDKKRANDNEG---------TNPKRHKDKFIEFALHYLEAQ-------HSEICFGRRHIVREEAG-----AGDEHK---------KHRTKGKV-VVDFSKKDEDQ----------------SYYISKNNVIVR----------IDKNA---
GPRSYRMGL-------------------NELKYLVLLSLQG-KGDDAIA-KLYRYRQHVENILDVVKVTDKDN----HVFLPRFVLEQHGIGR-------------------------KAFKQRIDGRV-KHVRGVWEKKKAATNEMT-------
----------LHEKARD---ILQYVNE-------NCTR----SFNPGEYNRLLVCLV--GKDV-------------ENFQAGLKRL----------------------QLAERIDGRVYSIF--------------
AQTSTINEMHQVVCDQILNRLCRI-----------------------------GDQKLYDYVGLGKK-------------------------DEIDYKQK----------------------VAWFKE-HISIRRGFLRKKFWY------------
---------DSKKGFAKLVEEHLESGGGQRDVGLDKKYYHID-AIGR------FEGANPALYETLARDRLCLMMAQYFLGSVRKE-------------------LGNK---------------IVWSND------SIEL-----------
PVEGSVGNEKSIVFSVSDYGKLYVLDDA----EFLGRI-CEYFM----P-HEKGKIRY--HTVYEKGFRA-YNDLQK-KCVEAVLAFEEKVVKAK----------------------KMSEKEGAHYIDF----------------
REILAQTMCKEAEK-TAVNKVRRAFFHHHLKF----------------VIDEFGLFSDVMKKYGIE-KEWKFPVK----------
>Cas13bt11
MQFENIKDTGQKPIYSIDQ--YEGAKKWCFAIVLNRACDNYE---------------------------DNPQLFSES---LLRFEEVNRRDWFD--KDIRDL----------IKKAD------------------TEDQIEPKRKP-----
NTPVNRRLHDIRNYFSHSRHQDDCLYFK---------------------NDDPMRCIMEAAYEKAKIHIKGRQTEQS-------------------------------------------------------------DIPLPELFD--
A---NNKITSAGVLFLASFFVERGILHRLMG--NIGGFK----------------DNRGK---------YGLTHDIFTTYCLKDSY-------------------SIHASDPK-VVLFRDIAGYLSLVACEYYPTYLSKIPK------------
---ENAGEKSSDEE---------KYAERKTDKFILFALKYLEEFVLPSLKDDYLVDIGRIDIIREESKET-EEKDEQYK---------PHPNQGKV-KVVFDSINKEL----------------PYYINHNTVILR----------IQKNG--
VMAYSCKIGV-------------------NDLKYLLLLCLQG-KTDKALD-AIYNYLHSMQDPPEVVKIGATDKL---FQGLPEFILKQSGIKV-----------------------QDKNKEKAARI-KYIRDKWEKKKSESADME------
-----------LHRKGRD---ILRYVNW-------HCET----PLGTEKYDQLLVLLV--NKNF-------------VVFGDELNQL-----------------------KRTEIISKDILEKL--------------
SGFQTINTLHQKVCNLVLEELSSLEKN--------------------------DPGKLAEHIGLVRKPAP-------------ENN-----PPPEYKEK----------------------VRRFVE-QPMIYKGFLRDQFFVNKDQ---------
----DGKKLKEQKTFAKLVEETL---GQNADVPLGKDFYYVP-NIEKDEKK-NRFHKDNAVLYETLALDRLCAMMARKCLTQINKN-----------------LAEKSEE------------IDWRNE------DGKD----FIYLKL-
VKSDRPQETFKIRFKVNDFAKLYVMDDP----DFLGGL-MKHFF----P--QEHSIEY--HKLYRNGIER-YTDRQK-DGIEAILRLEDSVIRQK----------------------GMKPKPAKNYISF----------------
SEIMAQTDYPEHDQ-KVLNKVRRAVLHYHLKF----------------EPADYNRFVDIMKKN----KFWDGERKNKESR--GR-
>Cas13bt9
MQFENIKDTGQKPIYSIDQ--YEGAKKWCFAIVLNRACDNYE---------------------------DNPQLFSES---LLRFEEVNRRDWFD--KDIRDL----------IKKAD------------------TEDQIEPKRKP-----
NTPVNRRLHDIRNYFSHSRHQDDCLYFK---------------------NDDPMRCIMEAAYEKAKIHIKGRQTEQS-------------------------------------------------------------DIPLPELFD--
A---NNKITSAGVLFLASFFVERGILHRLMG--NIGGFK----------------DNRGK---------YGLTHDIFTTYCLKDSY-------------------SIHASDPK-VVLFRDIAGYLSLVACEYYPTYLSKIPK------------
---ENAGGKSSDEE---------KYAERKTDKFILFALKYLEEFVLPSLKDDYLVDIGRIDIIREESKET-EEKDEQYK---------PHPNQGKV-KVVFDSINKEL----------------PYYINHNTVILR----------IQKNG--
VMAYSCKIGV-------------------NDLKYLLLLCLQG-KTDKALD-AIYNYLHSMQDPPEVVKIGATDKL---FQGLPEFILKQSGIKV-----------------------QDKNKEKAARI-KYIRDKWEKKKSESADIE------
-----------LHRKGRD---ILRYVNW-------HCET----PLGTEKYDQLLVLLV--NKNF-------------AGFGDELNQL-----------------------KRTEIISKDIFEKL--------------
SGFKTINTLHQKVCNLVLEELSFFEKS----------------------NPEKLEEYIGLIRKPAP-------------ENN-----PPPEYKEK----------------------VRRFVE-QPMIYKGFLRDQFFVNKDQ---------
----DGKKLKEQKTFAKLVEETL---GQNADVPLGKDFYYVP-NIEKDEKK-NRFHKDNAVLYETLALDRLCAMMARKCLTQINKN-----------------LAEKSEE------------IDWRNE------DGKD----FIYLKL-
VKSDRPQETFKIRFKVNDFAKLYVMDDP----DFLGGL-MKHFF----P--QEHSIEY--HKLYRNGIER-YTDRQK-DGIEAILRLEDSVIRQK----------------------GMKPKPAKNYISF----------------
SEIMAQTDYPEHDQ-KVLNKVRRALLHYHLKF----------------EPADYNRFVDIMKKD----KFWDGERKNEESR--GK-
>Cas13bt8
MKVENIKEKSKKAMYLINH--YEGPKKWCFAIVLNRACDNYE---------------------------DNPHLFSKS---LLEFEKTSRKDWFD--EETREL----------VEQAD------------------TEIQPNPNLKP-----
NTTANRKLKDIRNYFSHHYHKNECLYFK---------------------NDDPIRCIMEAAYEKSKIYIKGKQIEQS-------------------------------------------------------------DIPLPELFE--
S---SGWITPAGILLLASFFVERGILHRLMG--NIGGFK----------------DNRGE---------YGLTHDIFTTYCLKGSY-------------------SIRAQDHD-AVMFRDILGYLSRVPTESFQRI------------------
-----KQPQIRKEG---------QLSERKTDKFITFALNYLEDYGLKDLE-GCKACFARSKIVREQENVE-SINDKEYK---------PHENKKKV-EIHFDQSKED----------------RFYINRNNVILK----------IQKKD--
GHSNIVRMGV-------------------YELKYLVLMSLVG-KAKEAVE-KIDNYIQDLRDQLPYIEGKNKEEIKEYVRFFPRFIRSHLGLLQ-----------------------INDEEKIKARL-DYVKTKWLDKKEKSKELE------
-----------LHKKGRD---ILRYINE-------RCDR----ELNRNVYNRILELLV--SKDL-------------TGFYRELEEL-----------------------KRTRRIDKNIVQNL--------------
SGQKTINALHEKVCDLVLKEIESL-----------------------------DTENLRKYLGLI----P-------------KEE-----KEVTFKEK----------------------VDRILK-QPVIYKGFLRYQFFK------------
---------DDKKSFVLLVEDALKEKGGGCDVPLGKEYYKIV-SLDK------YDKENKTLCETLAMDRLCLMMARQYYLSLNAK-----------------LAQEAQQ------------IEWKKE------DSIE----LIIFTL-
KNPDQSKQSFSIRFSVRDFTKLYVTDDP----EFLARL-CSYFF----P--VEKEIEY--HKLYSEGINK-YTNLQK-EGIEAILELEKKLIERN-------------------------RIQSAKNYLSF----------------
NEIMNKSGYNKDEQ-DDLKKVRNSLLHYKLIF----------------EKEHLKKFYEVMRGEGIE-KKWSLIV-----------
>Cas13bt1
MEFENIKKTSNKEVYSIEQ--YEGEKKWCFAIVLNRAQTNLE---------------------------ENPKLFEQT---LTRFEKIMKQDWFN--EETKKL----------IYEKE------------------EENKVKEEIQ-------
IAASERLKNLRNYFSHYLHAPDCLIFN---------------------RNDTIRIIMEKAYEKSRFEAKKKQQEDI-----------------------------------------------------------------SIEFPELFE--E-
--EDKITSAGVVFFVSFFIERRFLNRLMG--YVQGFR----------------KTEGE---------YNITRQVFSKYCLKDSY-------------------SVQAQDHD-AVMFRDILGYLSRVPTEIYQHI--------------------
---KLTRKRSQD---------QLSERKTDKFILFALKYLEDYGLKDLA-DYTACFARSKIKRENEDTK-ETDGNKHK---------FHREKPVV-EIHFDKEKQD----------------QFYIKRNNVILK----------AQKKG--
GQSNVFRMGV-------------------YELKYLVLLSLLG-KAEEAIQ-RIDRYISSLKKQLPYLDKISNEEIQKSINFLPRFVRSRLGLLQ-----------------------VDDEKRLKTRL-EYVKAKWTDKKEGSRKLE------
-----------LHRKGRD---ILRYINE-------RCDR----PLSRKEYNNILKFIV--NKDF-------------AGFYNELEEL-----------------------KRTRRLDKNIIQKL--------------
SGHTTLNALHERVCDLVLQELGSL-----------------------------QSENLKEYIGLI----P-------------KEE-----KEVTFREK----------------------VDRILE-QPVVYKGFLRYEFFK------------
---------EDKKSFARLVEEAIKTKWSDFDIPLGEEYYNIP-SLDR------FDRTNKKLYETLAMDRLCLMMARQYYLRLNEK-----------------LAEKAQH------------IYWKKE------DGRE----VIIFKF-
QNPKEQKKSFSIRFSILDYTKMYVMDDP----EFLSRL-WEYFI----P-KEAKEIDY--HKHYARAFDK-YTNLQK-EGIDAILKLEGRIIERR-------------------------KIKPAKNYIEF----------------
QEIMNRSGYNNDQQ-VALKRVRNALLHYNLNF----------------EREHLKRFYGVVKREGIE-KKWSLIV-----------
>Cas13bt2
MQVENIKKGSSQGMYSIEQ--YEGAKKWCFAIVLNRAQTNLQ---------------------------GNPKLFEET---LTRFERIRKEDWFD--QETKKL----------IYAKQ------------------EQNEVEEEIQ-------
KAADEKLRDLRNYFSHYFHTPDCLIFT---------------------QNDPVRIIMEKAYEKARFEQAKKEQEDI-----------------------------------------------------------------SIEFGELFE--E-
--NGRITSAGVVFFASFFAERRFLNRLMG--YVQGFT----------------RTEGE---------YKITRDVFSTYCLRDSY-------------------SVKTPDHD-AVMFRDILGYLSRVPSESYQRI--------------------
---KESQMRSET---------QLSERKTDKFILFALNYLEDYGLEDLA-DYTACFARTRIKREQDEN---TDGKEQK---------PHRKKPRV-EIHFERAEGD----------------PFYIKHNNVILR----------TQKKG--
AQTYIFRMGV-------------------YELKYLVLLSLLG-KGAEAVK-RIDRYVHSLRNQLPHIEKKSTEEIEGYVRFLPRFVRSHLGLLG-----------------------VDDEKKIKARV-DYVKAKWLEKKEKSRELQ------
-----------LHRKGRD---ILRYINE-------RCER----PLNIDEYNRILELLV--TKHL-------------DGFYRELEEL-----------------------KKTRRIDKNIVCNL--------------
SRHKSVNALHEKVCDLVVQELESL-----------------------------GREELKEYVGLI----P-------------KEE-----KEVSFEEK----------------------TDRVVK-QPVIYKGFLRNEFFR------------
---------ESRKSFARLVEEAVREKGEVYDVPLGGEYYEIV-SLDT-------FDKDNKRLYETLAMDRLLLMIARQYHLSLNKE-----------------LAKRAQQ------------IEWKKE------DGEE----VIIFTL-
KNPAQPEQSCSVRFSLRDYTKLYVMDDA----EFLARL-CDYFL----P-KDEEQIDY--HRLYTQGMNR-YTNLQR-EGIEAILELEKKTIGPE-------------------------QPRPPKNYIPF----------------
SEIMDKSAYNEDDQ-KALRRVRNALLHHNLNF----------------ARADFKRFCGIMKREGIE-KRWSLAV-----------
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