

Supplementary Information

Structural basis for the activation of a compact CRISPR-Cas13 nuclease

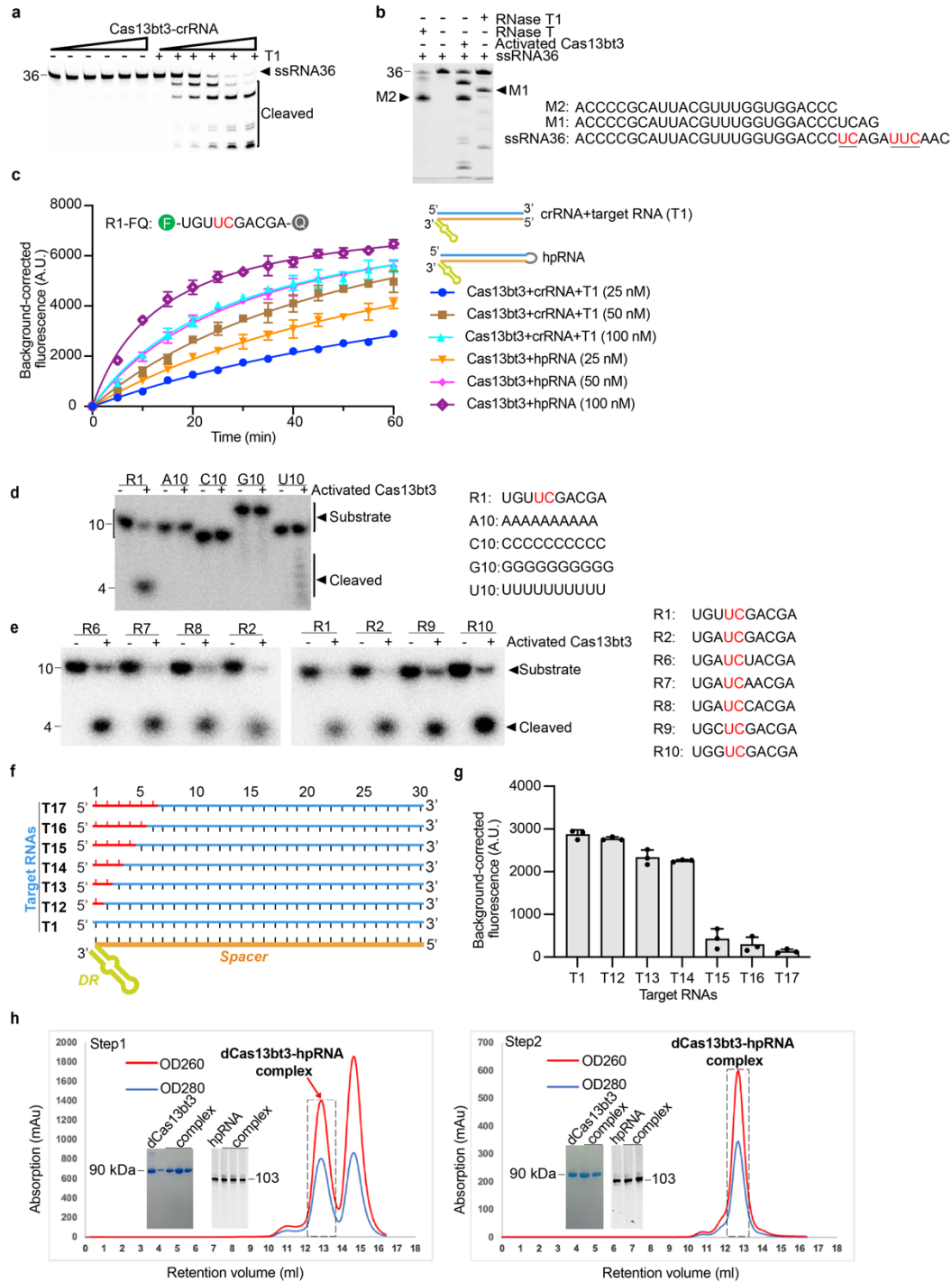
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This PDF file contains:

Supplementary Fig. 1-11

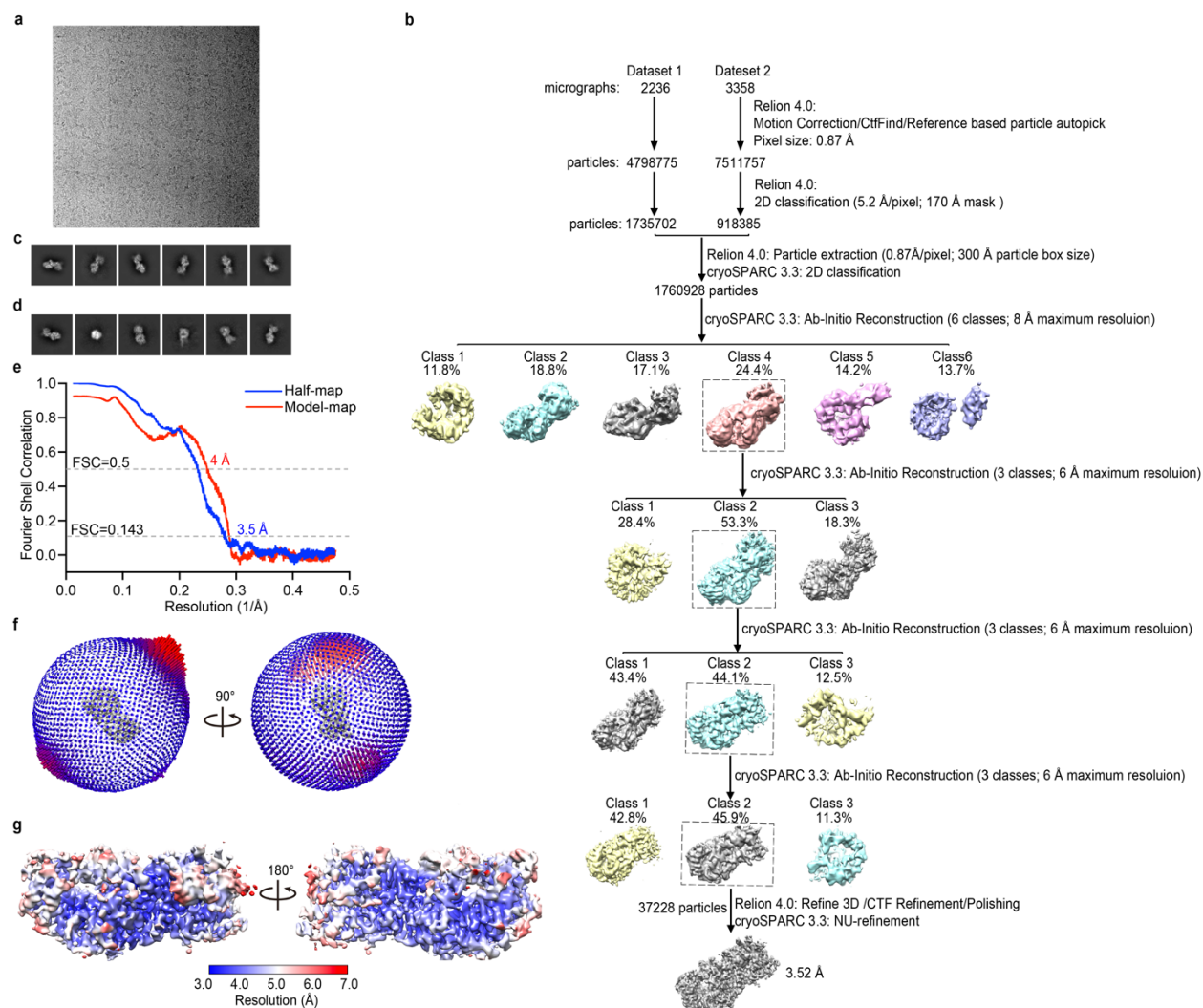
Supplementary Table 1-4



Supplementary Fig. 1 Biochemical characterization of Cas13bt3 cleavage and purification of dCas13bt3-hpRNA complex.

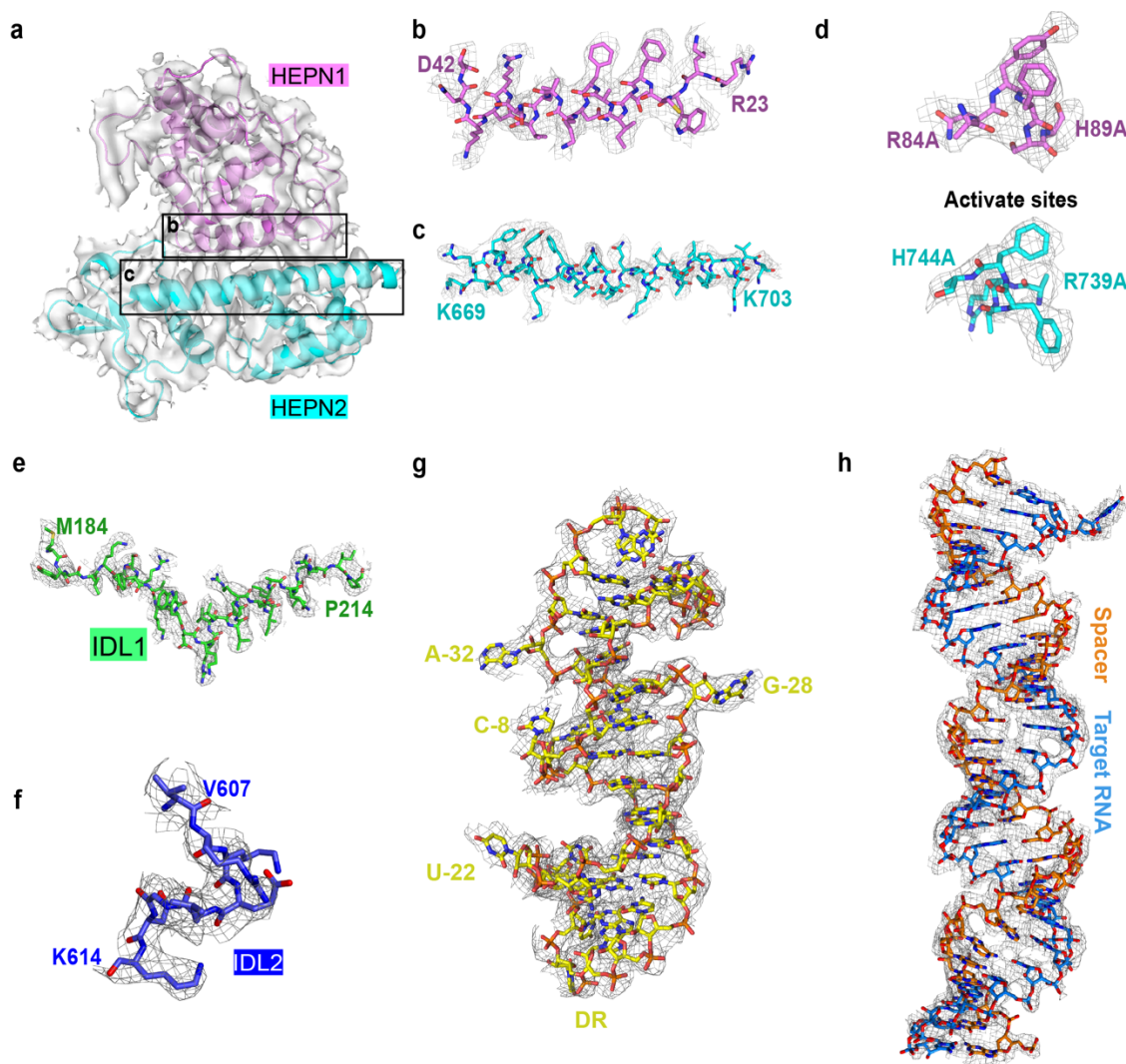
a-b Mapping the cleavage sites on ssRNA36. A representative gel image of ssRNA36 cleaved by 12.5-200 nM activated Cas13bt3 is shown in (a), and RNA size controls produced by RnaseT1 (M1) and RnaseT (M2) are in (b). The RnaseT1 specifically cleaves ssRNA36 at the G site to produce M1, whereas the RnaseT is a 3'-to-5' exonuclease that will stop at poly C motifs to produce M2. The possible cleavage sites on ssRNA 36 by Cas13bt3 according to the size comparison are indicated and colored in red. **c** Collateral activity of Cas13bt3 activated by crRNA-target RNA or hpRNA **d** Collateral activity of Cas13bt3 to ssRNA with poly-A10, poly-C10, poly-

G10, and poly-U10, R1 was used as a positive control. **e** Collateral activity of Cas13bt3 to ssRNA with UC cleavage site and various 5' or the 3' adjacent nucleotides. **f** Target RNAs designed for testing Cas13bt3 collateral activity with target RNAs 5' mismatches. The mismatched RNA segment is colored in red. **g** Collateral cleavage activities of Cas13bt3 with substrates in (f). (**c**, **g**) R1-FQ was used as reporter RNA. Data are present as means $\pm$ SDs (n=3). A.U. relative fluorescence intensity in arbitrary units. **h** The two-step purification of Cas13bt3-hpRNA complex for cryo-EM sample preparation (see methods for details). The purified complex indicated by bars in the chromatograms was checked with SDS-PAGE gel with protein control and Urea-PAGE gel with RNA control. Protein size (in kDa) and RNA size (in nt) are indicated. (**a**, **b**, **d**, **e**) RNA sizes (in nt) are indicated. At least three times each experiment was repeated independently with similar results. Source data are provided as Source Data File.

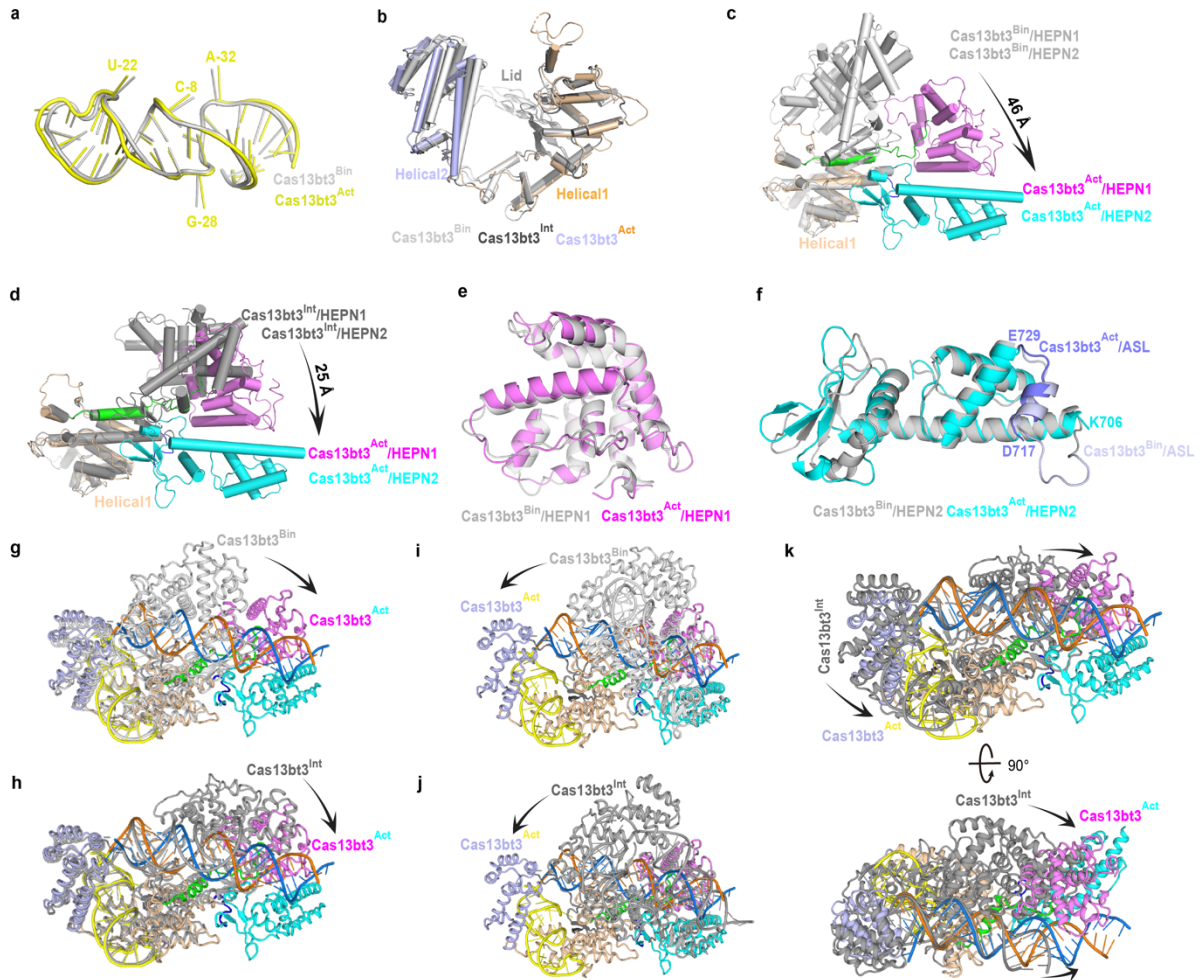


Supplementary Fig. 2 Cryo-EM reconstruction of Cas13bt3-hpRNA complex.

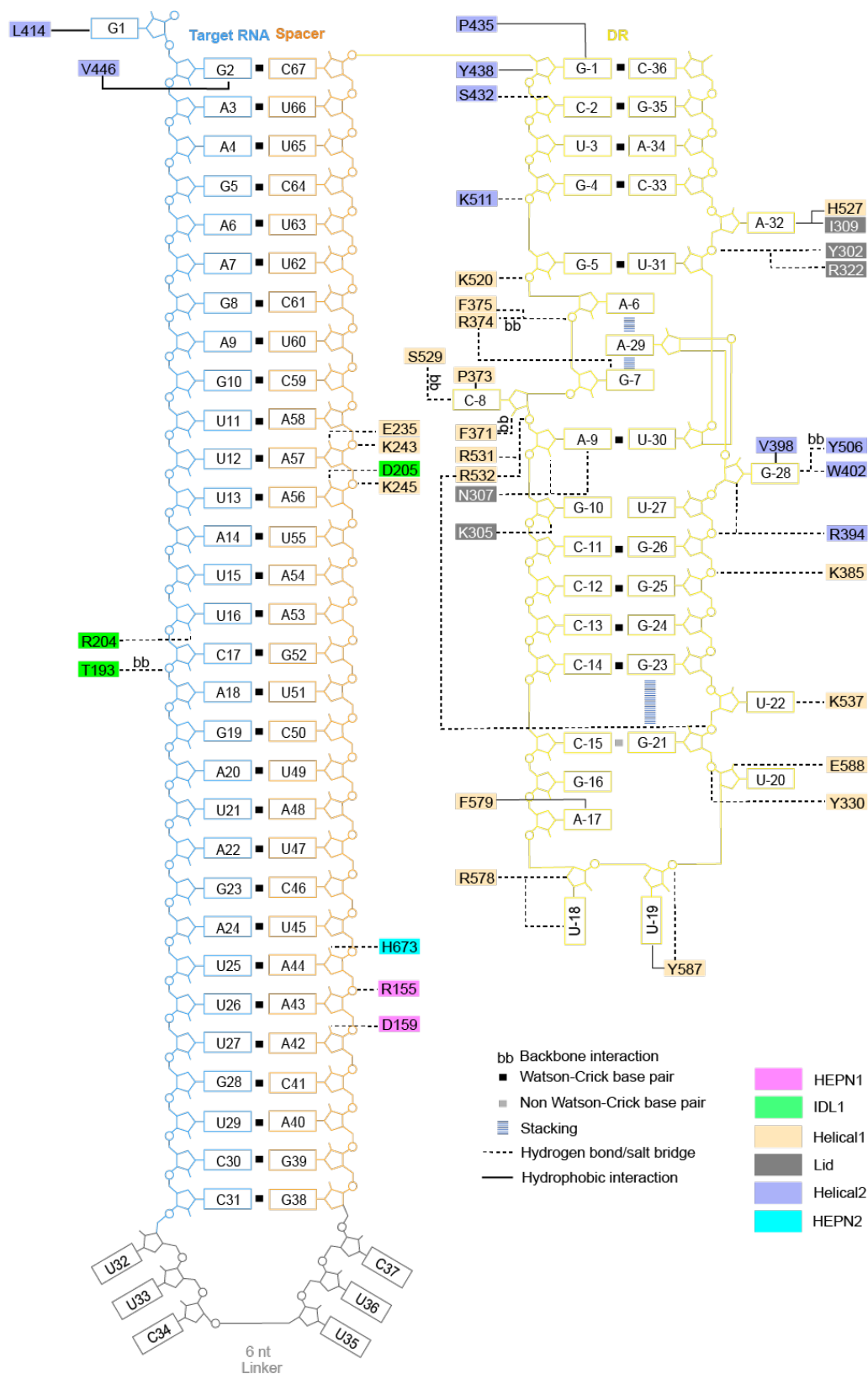
a representative cryo-EM micrograph. **b** Overview of image processing and refinement strategy. **c** Representative 2D class averages of Dataset 1 from QuantiFoil grid. **d** Representative 2D class averages of Dataset 2 from UltraAu grid. **e** Gold-standard FSC curves between the two half maps with indicated resolution at FSC = 0.143 are in blue. FSC curves between the refined model and the cryo-EM map with indicated resolution at FSC = 0.5 are in red. **f** The angular distribution of particles used in the final 3D reconstruction. **g** Local resolution estimation of the cryo-EM density map of Cas13bt3-hpRNA complex.



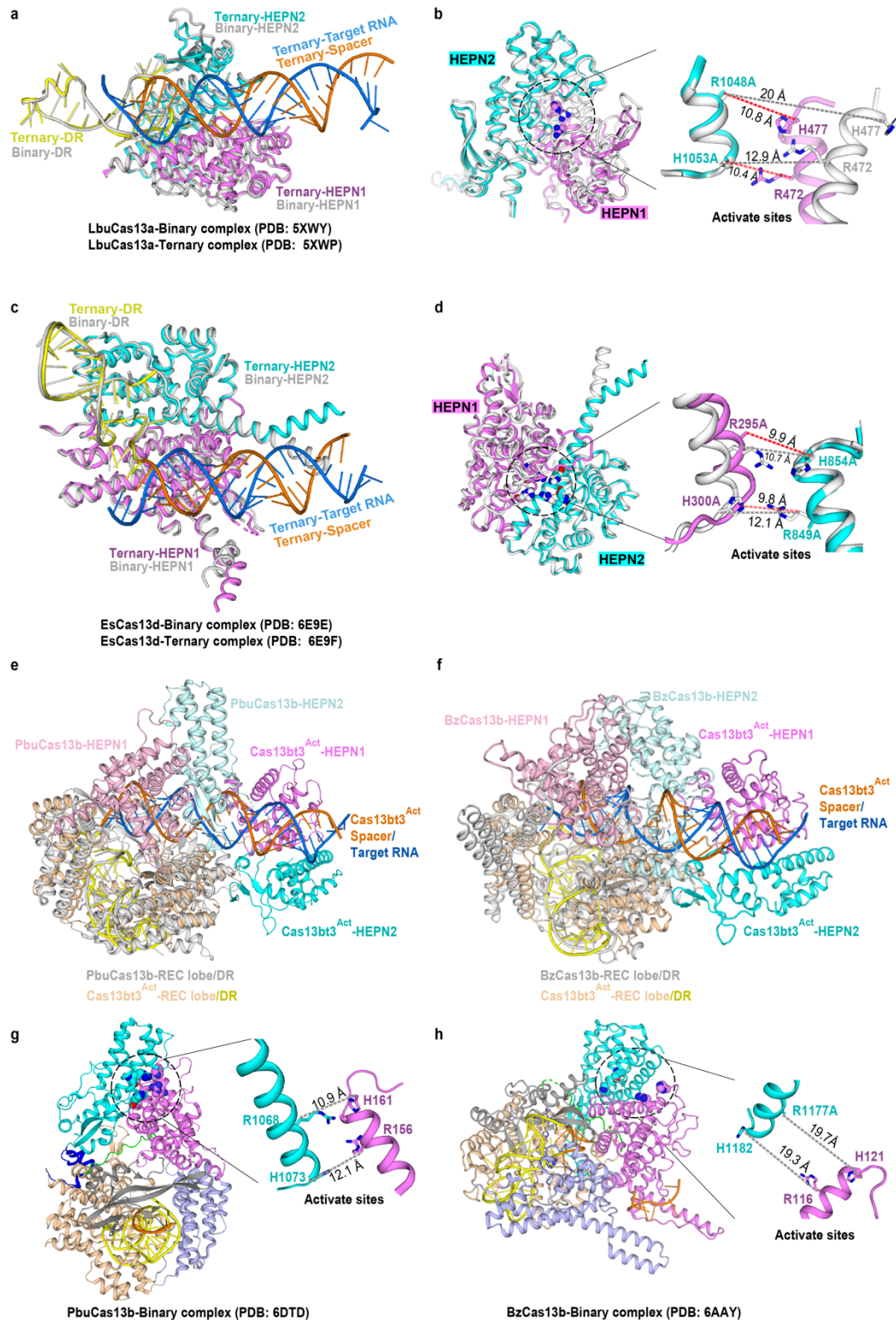
Supplementary Fig. 3 Density map to the structure models of Cas13bt3-hpRNA complex. **a** Cryo-EM density maps of the HEPN1 and HEPN2 domains in Cas13bt3^{Act}. **b-c**. Zoom in view of the local density map in (a). **d-h**. Cryo-EM density maps of activate sites (the RXXXXH motif) on HEPN domains, IDL1, IDL2, DR, and spacer-target RNA duplex in Cas13bt3^{Act}. Color codes are defined as in Fig. 1c and 1d, respectively.



Supplementary Fig. 4 Structural comparisons of Cas13bt3^{Bin}, Cas13bt3^{Int} and Cas13bt3^{Act}.
a Structural alignment of DR in Cas13bt3^{Bin} (grey) and Cas13bt3^{Act} (yellow). **b** Structural alignment of REC lobe in Cas13bt3^{Bin} (grey), Cas13bt3^{Int} (black), and Cas13bt3^{Act} (colored). **c-d** The conformational change of HEPN domains in Cas13bt3^{Act} (colored), compare with Cas13bt3^{Bin} (grey) (**c**) and Cas13bt3^{Int} (black) (**d**), the distance of the NUC lobe's mass center movement was indicated. **e-f** Structural alignment of HEPN1 (**e**) and HEPN2 (**f**) in Cas13bt3^{Bin} (grey) and Cas13bt3^{Act} (colored). **g-k** Overall structural comparison of Cas13bt3^{Bin} (grey), Cas13bt3^{Int} (black) with Cas13bt3^{Act} (colored) by fixing the REC lobe (**g-h**) and NUC lobe (**i-j**), or duplex RNA (**k**). The movements of the domains and RNAs were indicated.



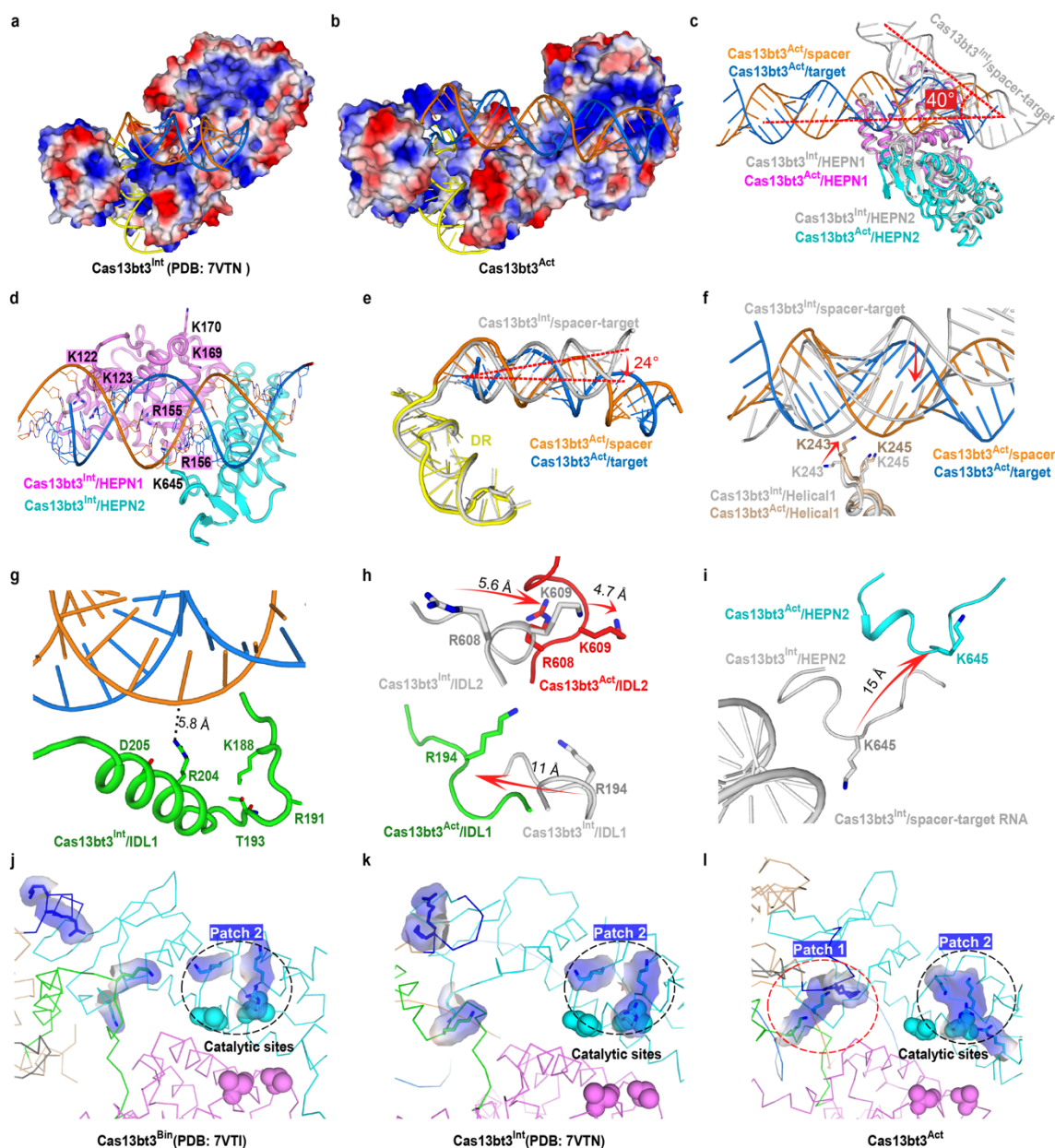
Supplementary Fig. 5 Schematics of protein-RNA interactions in Cas13bt3^{Act}. The 6 nt linker between spacer and target is disorder and colored grey. bb, backbone interaction.



Supplementary Fig. 6 Structural comparisons of LbuCas13a, EsCas13d, PbuCas13b and BzCas13b.

a Structural alignment of binary and ternary complexes of LbuCas13a. **b** Zoom-in views of the HEPN domains in binary and ternary complexes of LbuCas13a. **c** Structural alignment of binary

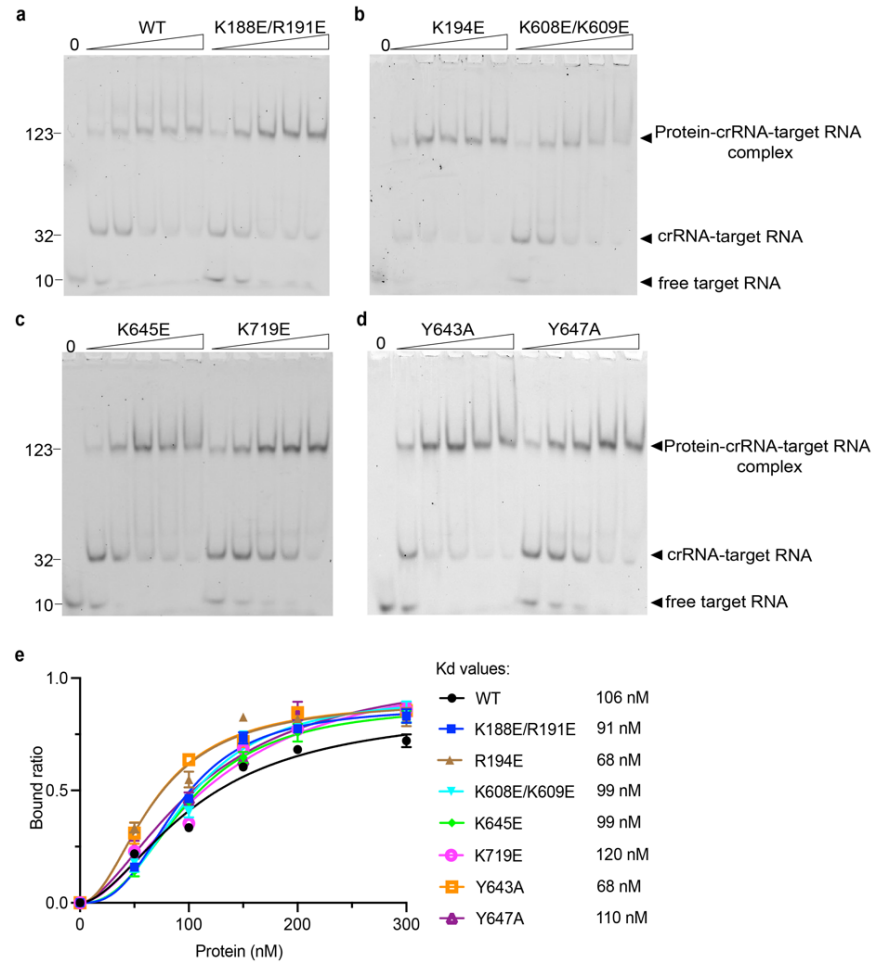
and ternary complexes of EsCas13d. **d** Zoom-in views of the HEPN domains in binary and ternary complexes of EsCas13d. (**a, c**) The DR RNA was aligned and only NUC lobe was shown for clarity. **e-f** Structural alignment of PbuCas13b (**e**) or BzCas13b (**f**) with Cas13bt3^{Act}, the REC lobes were aligned in both (**e**) and (**f**). **g-h** The binary structure of PbuCas13a (**g**) and BzCas13b (**h**), The active sites' distance between Cα-atoms of the in both structures are indicated.



Supplementary Fig. 7 The RNA binding interface comparisons of Cas13bt3^{Bin}, Cas13bt3^{Int} and Cas13bt3^{Act}.

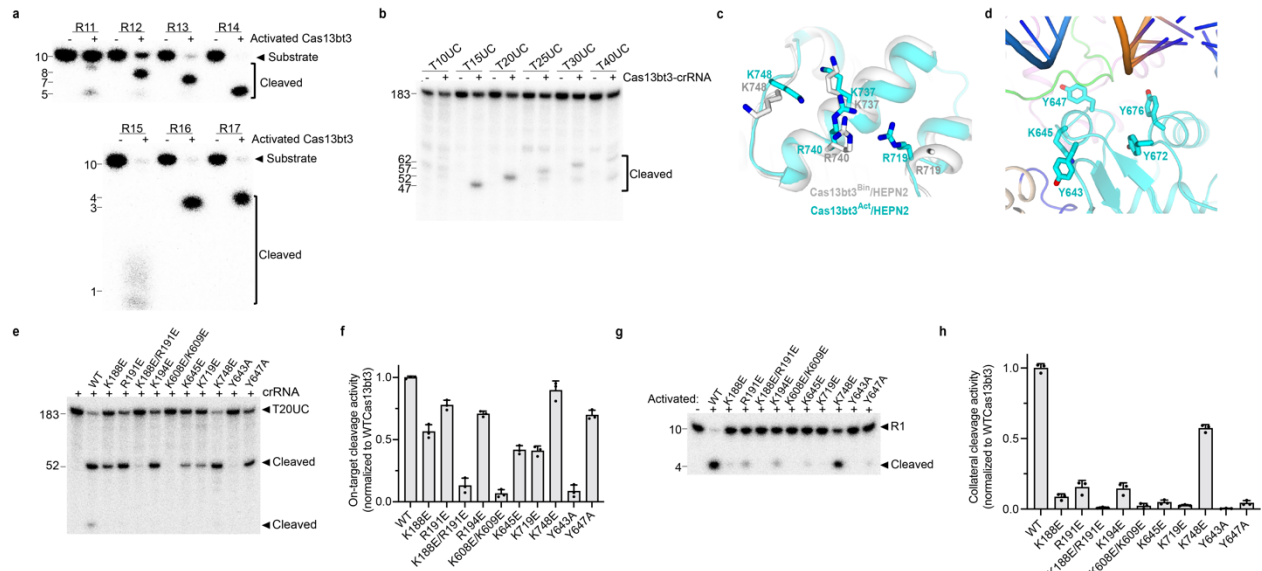
a-b The electrostatic potential surface of Cas13bt3^{Int} and Cas13bt3^{Act}. **c** Structural alignment of HEPN domains and spacer/target RNAs in both Cas13bt3^{Int} (grey) and Cas13bt3^{Act} (colored). The dsRNA tilted 40 degrees from Cas13bt3^{Int} to Cas13bt3^{Act} when the HEPN domains are aligned. **d** The possible RNA interacting residues in Cas13bt3^{Int}. **e** Structural alignment of spacer/target duplex RNA in Cas13bt3^{Int} and Cas13bt3^{Act} with the DR aligned. **f** The structural comparison of Helical 1 loop in Cas13bt3^{Int} and Cas13bt3^{Act}, the conformational change of K243 and the spacer/target duplex are indicated with red arrow. **g** Zoom-in view of IDL1 and the spacer/target duplex in Cas13bt3^{Int}, the distance between R204 and dsRNA is indicated. **h-i** The structural comparison of R608, K609 on IDL2, R194 on IDL1, and K645 on HEPN2 in Cas13bt3^{Int} and Cas13bt3^{Act}, the movement of each residue is indicated with red arrow. **j-l** The structure difference of Patch 1 composed residues in Cas13bt3^{Bin}, Cas13bt3^{Int}, and Cas13bt3^{Act},

Patch 1 only formed upon the long target RNA binding in the active state. Color codes are defined as in Fig. 1c and 1d, respectively.



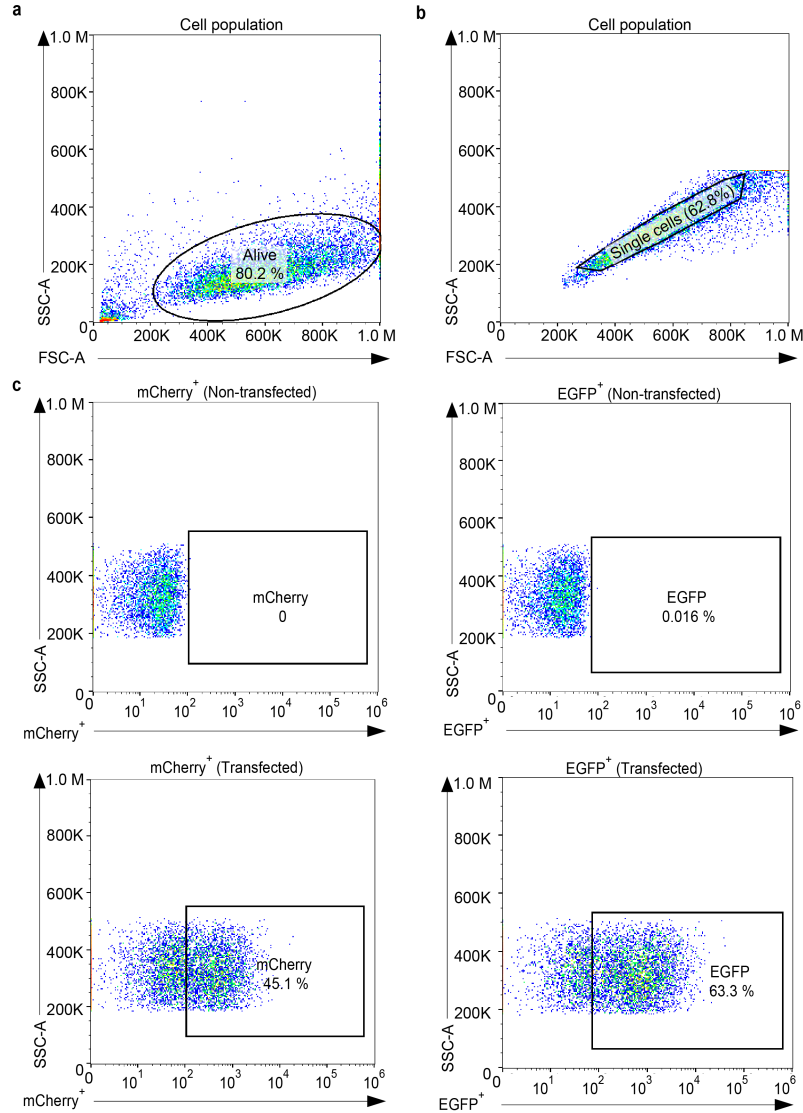
Supplementary Fig. 8 Quantification of target RNA binding affinity with WT and mutated Cas13bt3.

a-d Representative EMSA gel images of WT and mutant Cas13bt3 complex with crRNA binding to 10 nM of 5'-FAM-T1. The protein-crRNA complex at 1:1 molar ratio was serially diluted to 300, 200, 150, 100, and 50 nM. **e** Calculation of binding affinity between protein-crRNA and target RNA. Bound and unbound fractions were quantified by densitometry and fitted. (**a-d**) RNA or protein-RNA complex sizes (in kDa) are indicated. At least two times each experiment was repeated independently with similar results. (**e**) Data are present as means $\pm$ SDs (n=2). Source data are provided as Source Data File.



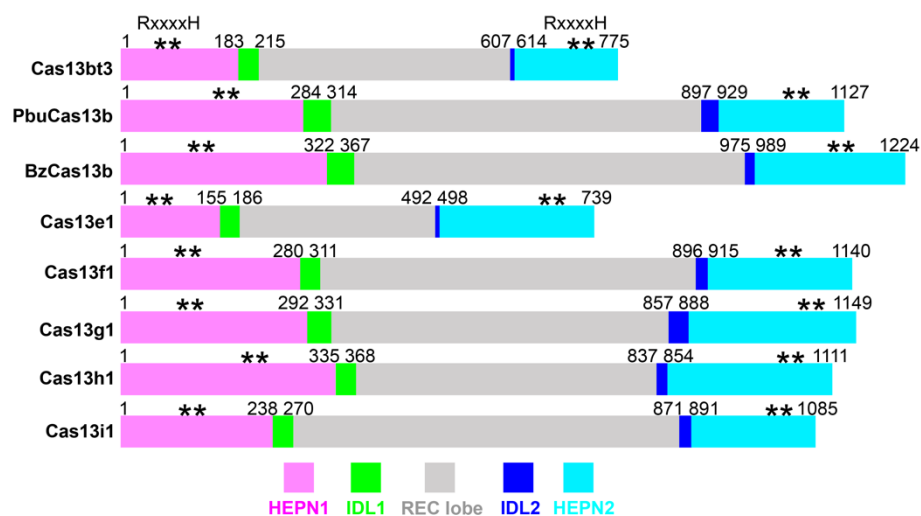
Supplementary Fig. 9 Substrate length preference of Cas13bt3 and engineering of Cas13bt3 for high-fidelity cleavage.

a Representative gels of the collateral cleavage activities of Cas13bt3 with UC site at different positions on ssRNA substrates. **b** Representative gels of the on-target RNA cleavage activities of Cas13bt3 with UC site at the different positions on target RNA. **c** Structural alignment of positively charged residues on Pacth2 in Cas13bt3^{Bin} and Cas13bt3^{Act}. **d** The relative positions of Y643, K645, Y647, and Y672/Y676A in Cas13bt3^{Act}. Color codes are defined as in Fig. 1c and 1d. **e** Representative gels of on-target cleavage activities of WT and mutated Cas13bt3. **f** Normalized on-target cleavage activity as shown in (e). **g** Representative gels of collateral cleavage activities of WT and mutated Cas13bt3. **h** Normalized collateral cleavage activity as shown in (g). The target RNA (T20UC) was used as the substrate for on-target cleavage assay and the reporter RNA (R1) was used as the substrate for collateral cleavage assay. (a, b, e, g) RNA sizes (in nt) are indicated. At least three times each experiment was repeated independently with similar results. (f, h) Data are present as means $\pm$ SDs (n=3). Source data are provided as Source Data File.



Supplementary Fig. 10 Gating strategy for flow cytometry experiments.

a-b An example well is gated by collecting the live cell population (**a**) and then stratifying the resulting population by FSC-A and FSC-H to only keep single cells (**b**). **c** The proportion of mCherry⁺ or EGFP⁺ is captured for a non-transfected well, and a transfected well. Negative cells are those captured outside of their respective boxes in the non-transfected condition, while positive is within the bounds.



Supplementary Fig. 11 Domain organization of newly identified Cas13 effectors.
The domains assignments of Cas13e1-Cas13i1 are based on sequence alignment with Cas13bt3 or PbuCas13b and Alpha-fold predicted structures. RxxxxH: the activate sites motif of HEPN domains.

Supplementary Table 1**Cryo-EM data collection, model refinement, and validation for Cas13bt3^{Act}**

Data collection and processing	
EMDB ID	EMD-29433
PDB ID	8FTI
Microscope	Titan Krios G3
Detector	Gatan K2 camera
Magnification	165000
Voltage (kV)	300
Electron exposure (e-/Å ²)	49
Defocus range (μM)	-0.6 to -2.2 μM
Pixel size (Å)	0.435
Number of movies	5594
Initial particles (no.)	12310532
Final particles (no.)	37228
Map resolution (Å)	3.5
FSC threshold	0.143
Map sharpening B factor (Å ²)	-101.4
Refinement	
No. of atoms	8126
No. of residues	
Protein	737
Nucleic acid	97
R.m.s. deviations	
Bond lengths (Å)	0.002
Bond angles (°)	0.558
Validation	
Clashscore	9.2
Rotamer outlier (%)	4.55
Ramachandran plot (%)	
Favored	95.47
Allowed	4.53
Outliers	0

Supplementary Table 2

Oligonucleotides for Cas13bt3 cloning

Mutation (for in vitro studies)	Forward primer	Reverse primer
A84RA89H	CGCAACTACTTTTCCCACTACAGGCACAGCCCTGGCTGC	CAGGGCCTCGGCTTTGGCATTG
A739RA744H	CGCAGAGCCTTTTCCACCACCACCTGAAATTTGTGATT	CACCTTATTCACGGCGGTCTTC
K54A	ACACGAGGCATATTCCAAGCACGACTGGTAC	GGAATATGCCTCGTGTCTCAGCAGG GACTCC
K54E	CACGAGGAATATTCCAAGCACGACTGGTACGAT	GGAATATTCCTCGTGTCTCAGCAGGG ACT
K57A	ATATTCCGCGCACGACTGGTACGATGAGGAT	GTCGTGCGCGGAATTTCTCGTGTC TCAGC
R155A/R156A	TGGAGGCGGCGGTGCTGGACAGGCTGTATGG	AGCACCGCCGCTCCACGAAGAAGG ACACGA
R179A	ACTGACCGCAAAGGCCCTGTCCATGTACTGT	GGCCTTTGCGGTCAGTTTGTACTGG CCCTCG
R179E/K180E	ACCGAAGAAGCCCTGTCCATGTACTGTCTGA	CAGGGCTTCTTCGGTCAGTTTGTACT GGCCCTC
K188E	CTGTCTGGAAGACAGCAGGTTTACCAAGGCC	TGCTGTCTTCAGACAGTACATGGAC AGGGCC
R191E	GGACAGCGAGTTTACCAAGGCCTGGGATAAGAGG	TGGTAAACTCGCTGTCTTCAGACAG TACATGGA
K188E/R191E	CTGGAAGACAGCGAATTTACCAAGGCCTGGGATAAGAGG	AAATTCGCTGTCTTCAGACAGTACA TGGACAGGGCC
K194E	GTTTACCGAAGCCTGGGATAAGAGGGTGCTG	CCCAGGCTTCGGTAAACCTGCTGTC CTTCAGACA
K243E/K245E	ACGAGGATGAATTTATCGAGTTTGCCCTGCACTAC	TAAATTCATCTCGTGTCTCTTAGGAT TGGTGCCCT
R204E	CTGTTTGAAGACATCCTGGCCCAGCTG	GATGTCTTCAAACAGCAGCACCCCTCT TATCC
R608E/K609E	GCGTGGAAGAGGAGCTGGGCAATAAGATTGTGTGG	GCTCCTCTTCCAGCTGCCCAGAAA ATACTGG
Y643A	GTCCGACGCCGGCAAAGCTGTACGTGCTGGAC	GTTTGCCGGCGTCGGACACGCTGAA CACAAAT
K645E	CTATGGCGAGCTGTACGTGCTGGACGACG	CGTACAGCTCGCCATAGTCGGACAC GCTG
Y647A	CAAAGCTGGCCGTGCTGGACGACGCCG	CCAGCACGGCCAGTTTGCCATAGTC GGACACG
R681A	GGGCTTTGCAGCCTACAACGACCTGCAGAAG	GTAGGCTGCAAAGCCCTTCTCGTACA CGGTG
R719A	GACTTCGCAGAGATCCTGGCCCAAACCATGTG	GATCTCTGCGAAGTCGATGTAGTGG GCGCC
R719E	CGACTTCGAGGAGATCCTGGCCCAAACCATGT	GGATCTCCTCGAAGTCGATGTAGTGG GCGC
K748E	CCACCTGGAGTTTGTGATTGACGAGTTTGGCCTG	TCACAAACTCCAGGTGGTGGTGGAA AAAGGC
Mutation (for in vivo studies)	Forward primer	Reverse primer
R84A/H89A	GCCAACTACTTCAGTGCCTACCGGCATAGCCCTGGCTG	GGCACTGAAGTAGTTGGCCAGGGCT TCAGCCTTGCG
R739A/H744A	GCTCGCGCCTTCTTCGCTCACCACCTGAAGTTCGTGATT GACG	GAAGAAGGCGCGAGCCACCTTGTTT CTGCGGTCTTTTCG
Y643A	TCTGACGCTGGCAAGCTGTACGTGCTGGAC	CTTGCCAGCGTCAGACACGGGAGAAC ACGATGC
Y647A	CAAGCTGGCCGTGCTGGACGATGCCGAATTCC	AGCACGGCCAGCTTGCCGTAGTCAG ACACGG
Cas13bt3_	TTCAGGTTGGACCGGTGCCACCATGCCCAAGAAGAAGC	
AgeI_F	GGAAGGTGGCCAGGTGAGCAAGCAGA	
Cas13bt3_	TAAACAAGTTAAGCTTCTACACCTTCCGCTTCTTCTTGGG	
HindIII_R	CTTGACAGGGAATTCC	

Supplementary Table 3**The in-vitro RNA transcription template used in this study**

Transcription template	Forward primer	Reverse primer
hplVT-S1	AGCTTAATACGACTCACTATAGGAAGAAGA G	TAAACTCTTCTTCTCTATAGTGAGTCGTATTA
hplVT-S2	TTTATTCAGATAGATTTGTCTTCTTCGGA	TTTGTCCGAAGAAGGACAAATCTATCTGAA
hplVT-S3	CAAATCTATCTGAATAAACTCTTCTTCGCT GGAGCAGCCCCGATTTGTGGGGTGATTA	CTCCAGCGAAGAAGAGTTTATTTCAGATAGA CTAGGCTGTAATCACCCCACAAATCGGGG GCTG
hplVT-S4	CAGC	
M13F	GAGCGGATAACAATTTACACAGGAAAC	
hplVT-R		GCTGTAATCACCCCACAAAT
T0-S1	AGCTTAATACGACTCACTATAGGAAGAAGA GTTTAT	CTGAATAAACTCTTCTTCTCTATAGTGAGTC GTATTA
T0-S2	TCAGATAGATTTGTCCGCTCGACCTTTTTA GGTCGA	CGACTCGACCTAAAAAGGTCGAGCGGACA AATCTAT
T0-S3	GTCGACGGAGTCTAGACTCCGTCCTGATG AGTCCGT	CCTCACGGACTCATCAGGACGGAGTCTAG ACTCCGT
T0-S4	GAGGACGAAAGACTCGACCTGATGTATAG AGTAGA	CTAGTCTACTCTATACATCAGGTCGAGTCT TTCGT
T0-R		TCTACTCTATACATCAGGTCGAG
T10UC/T15UC/T 20UC/T25UC/T3 0UC/T40UC-S1	AGCTTAATACGACTCACTATAGGAAGAAGA GTTTATTCAGATAGATTTGTCC GCACGACCAATCTAAAAACAAGTACACACA AGAGACTAGACACACACACTG	GTGCGGACAAATCTATCTGAATAAACTCTT CTTCCTATAGTGAGTCGTATTA TCATCAGTGTGTGTGTGTCTAGTCTCTTGT GTGTACTTGTTTTAGATTGGTC
T10UC-S2		
T10UC/T15UC/T 20/T25UC/T30U C/T40UC/-S3	ATGAGACCACCAGAGACAGAAAGACTACA GACAATAGATAGATATAGACAGA	ATTTTCTGTCTATATCTATCTATTGTCTGTAG TCTTTCTGTCTCTGGTGGTC
T10UC/T15UC/T 20UC/T25UC/T3 0UC/T40UC-S4	AAATGAAGCACATGTAAATAAGATGCAACA ACAGTAAGCATATAAATG GCACGACCAATAAAATCCAAGTACACACAA GAGACTAGACACACACACTG	CTAGCATTATATGCTTACTGTTGTTGCATC TTATTTACATGTGCTTC TCATCAGTGTGTGTGTGTCTAGTCTCTTGT GTGTACTTGGATTTTATTGGTC
T15UC-S2	GCACGACCAATAAAACAAGTTCACACACA AGAGACTAGACACACACACTG	TCATCAGTGTGTGTGTGTCTAGTCTCTTGT GTGTGAACCTGTTTATTGGTC
T20UC-S2	GCACGACCAATAAAACAAGTACACATCCAA GAGACTAGACACACACACTG	TCATCAGTGTGTGTGTGTCTAGTCTCTTGG ATGTGTACTTGTTTTATTGGTC
T25UC-S2	GCACGACCAATAAAACAAGTACACACAAG ATCGACTAGACACACACACTG	TCATCAGTGTGTGTGTGTCTAGTCGATCTT GTGTGTACTTGTTTTATTGGTC
T30UC-S2	GCACGACCAATAAAACAAGTACACACAAG AGACTAGACACTCACACACACTG	TCATCAGTGTGTGTGTGTCTAGTCGATCTT GTGTGTACTTGTTTTATTGGTC
T40UC-S2		CATTATATGCTTACTGTTGTTGCATCTTATT TACATGT
TUC-R		GCTGTAATCACCCCACAAATCGGGGGCTG CTCCAGCGAAGAAGAGTTTATTTCAGATAGA TTTGTCTATAGTGAGTCGTATTA
crIVT	TAATACGACTCACTATAGGACAAATCTATCT GAATAAACTCTTCTTCGCTGGAGCAGCCC CCGATTTGTGGGGTGATTACAGC	CGGACAAATCTATCTGAATAAACTCTTCTT CCTATAGTGAGTCGTATTA
T1IVT	TAATACGACTCACTATAGGAAGAAGAGTTT ATTCAGATAGATTTGTCCG	CCAAATCTATCTGAATAAACTCTTCTTCCTA TAGTGAGTCGTATTA
T2IVT	TAATACGACTCACTATAGGAAGAAGAGTTT ATTCAGATAGATTTGG	CATCTATCTGAATAAACTCTTCTTCCTATAG TGAGTCGTATTA
T3IVT	TAATACGACTCACTATAGGAAGAAGAGTTT ATTCAGATAGATG	GTATCTGAATAAACTCTTCTTCCTATAGTGA GTCGTATTA
T4IVT	TAATACGACTCACTATAGGAAGAAGAGTTT ATTCAGATAC	

T5IVT	TAATACGACTCACTATAGGAAGAAGAGTTT ATTCAGATAGATTTGTCAA	TTGACAAATCTATCTGAATAAACTCTTCTTC CTATAGTGAGTCGTATTA
T6IVT	TAATACGACTCACTATAGGAAGAAGAGTTT ATTCAGATAGATTTGTAAA	TTTACAAATCTATCTGAATAAACTCTTCTTC CTATAGTGAGTCGTATTA
T7IVT	TAATACGACTCACTATAGGAAGAAGAGTTT ATTCAGATAGATTTGAAAA	TTTTCAAATCTATCTGAATAAACTCTTCTTC CTATAGTGAGTCGTATTA
T8IVT	TAATACGACTCACTATAGGAAGAAGAGTTT ATTCAGATAGATTTAAAAA	TTTTTAAATCTATCTGAATAAACTCTTCTTC CTATAGTGAGTCGTATTA
T9IVT	TAATACGACTCACTATAGGAAGAAGAGTTT ATTCAGATAGATTTAAAAA	TTTTTTAATCTATCTGAATAAACTCTTCTTC CTATAGTGAGTCGTATTA
T10IVT	TAATACGACTCACTATAGGAAGAAGAGTTT ATTCAGATAGATAAAAAA	TTTTTTTATCTATCTGAATAAACTCTTCTTC TATAGTGAGTCGTATTA
T11IVT	TAATACGACTCACTATAGGAAGAAGAGTTT ATTCAGATAGAAAAA	TTTTTTTTCTATCTGAATAAACTCTTCTTC CTATAGTGAGTCGTATTA
T12IVT	TAATACGACTCACTATAGGTAAGAAGAGTT TATTCAGATAGATTTGTCCG	CGGACAAATCTATCTGAATAAACTCTTCTTA CCTATAGTGAGTCGTATTA
T13IVT	TAATACGACTCACTATAGGTTAGAAGAGTT TATTCAGATAGATTTGTCCG	CGGACAAATCTATCTGAATAAACTCTTCTAA CCTATAGTGAGTCGTATTA
T14IVT	TAATACGACTCACTATAGGTTTGAAGAGTT TATTCAGATAGATTTGTCCG	CGGACAAATCTATCTGAATAAACTCTTCAA ACCTATAGTGAGTCGTATTA
T15IVT	TAATACGACTCACTATAGGTTTTAAGAGTTT ATTCAGATAGATTTGTCCG	CGGACAAATCTATCTGAATAAACTCTTAAAA CCTATAGTGAGTCGTATTA
T16IVT	TAATACGACTCACTATAGGTTTTTAGAGTTT ATTCAGATAGATTTGTCCG	CGGACAAATCTATCTGAATAAACTCTAAAA ACCTATAGTGAGTCGTATTA
T17IVT	TAATACGACTCACTATAGGTTTTTTGAGTTT ATTCAGATAGATTTGTCCG	CGGACAAATCTATCTGAATAAACTCAAAAA ACCTATAGTGAGTCGTATTA
T18IVT	TAATACGACTCACTATAGGAAGAAGAGTTT ATTCAGATAGATTTCACCG	CGGTGAAATCTATCTGAATAAACTCTTCTT CCATAGTGAGTCGTATTA
T19IVT	TAATACGACTCACTATAGGAAGAAGAGTTT ATTCAGATAGTATTGTCCG	CGGACAATACTATCTGAATAAACTCTTCTTC CATAGTGAGTCGTATTA
T20IVT	TAATACGACTCACTATAGGAAGAAGAGTTT ATTCAGTAAGATTTGTCCG	CGGACAAATCTTACTGAATAAACTCTTCTT CCATAGTGAGTCGTATTA
T21IVT	TAATACGACTCACTATAGGAAGAAGAGTTT ATAGAGATAGATTTGTCCG	CGGACAAATCTATCTATAAACTCTTCTTC CATAGTGAGTCGTATTA
T22IVT	TAATACGACTCACTATAGGAAGAAGAGTAA ATTCAGATAGATTTGTCCG	CGGACAAATCTATCTGAATTTACTCTTCTTC CTATAGTGAGTCGTATTA
T23IVT	TAATACGACTCACTATAGGAAGAATTGTTTA TTCAGATAGATTTGTCCG	CGGACAAATCTATCTGAATAAACAAATTCTTC CTATAGTGAGTCGTATTA

Supplementary Table 4

The RNAs used in this study

RNA	Sequence (the guide/target sequences are underlined, the UC cleavage site are colored red, the mismatch are indicated with lower case letters)
crRNA	<u>GGACAAAU</u> CUAUCUGAAUAAACUCUUCUUCGCUUGGAGCAGCCCCGAUUUGUGGGGUGAUUACAGC
hpRNA	<u>GGAAGAAGAGUUU</u> AUUCAGAUAGAUUUUGUCCUUCUUCGGACAAAUUCUAUCUGAAUAAACUCUUCUU <u>CGCUGGAGCAGCCCCGAUUUGUGGGGUGAUUACAGC</u>
T0	<u>GGAAGAAGAGUUU</u> AUUCAGAUAGAUUUUGUCCGCUUGGAGCAGCCCCGAUUUGUGGGGUGAUUACAGC GACUCCGUCCUGAUGAGUCCGUGAGGACGAAAGACUCGACCUGAUGUAUAGAGUAGA
T10UC	<u>GGAAGAAGAGUUU</u> AUUCAGAUAGAUUUUGUCCGCGACGACCAAUAAAAUCACAGUACACACAAGAGAC UAGACACACACACACUGAUGAGACCACCAGAGACAGAAAGACUACAGACAAUAGAUAGAUUAGACA GAAAAUGAAGCACAUGUAAAUAGAUGCAACAACAGUAAGCAUUAUAAUUG
T15UC	<u>GGAAGAAGAGUUU</u> AUUCAGAUAGAUUUUGUCCGCGACGACCAAUAAAAUCACAGUACACACAAGAGAC UAGACACACACACACUGAUGAGACCACCAGAGACAGAAAGACUACAGACAAUAGAUAGAUUAGACA GAAAAUGAAGCACAUGUAAAUAGAUGCAACAACAGUAAGCAUUAUAAUUG
T20UC	<u>GGAAGAAGAGUUU</u> AUUCAGAUAGAUUUUGUCCGCGACGACCAAUAAAAUCACAGUACACACAAGAGAC UAGACACACACACACUGAUGAGACCACCAGAGACAGAAAGACUACAGACAAUAGAUAGAUUAGACA GAAAAUGAAGCACAUGUAAAUAGAUGCAACAACAGUAAGCAUUAUAAUUG
T25UC	<u>GGAAGAAGAGUUU</u> AUUCAGAUAGAUUUUGUCCGCGACGACCAAUAAAAUCACAGUACACACAAGAGAC UAGACACACACACACUGAUGAGACCACCAGAGACAGAAAGACUACAGACAAUAGAUAGAUUAGACA GAAAAUGAAGCACAUGUAAAUAGAUGCAACAACAGUAAGCAUUAUAAUUG
T30UC	<u>GGAAGAAGAGUUU</u> AUUCAGAUAGAUUUUGUCCGCGACGACCAAUAAAAUCACAGUACACACAAGAGAC UAGACACACACACACUGAUGAGACCACCAGAGACAGAAAGACUACAGACAAUAGAUAGAUUAGACA GAAAAUGAAGCACAUGUAAAUAGAUGCAACAACAGUAAGCAUUAUAAUUG
T40UC	<u>GGAAGAAGAGUUU</u> AUUCAGAUAGAUUUUGUCCGCGACGACCAAUAAAAUCACAGUACACACAAGAGACUA GACACUACACACACACUGAUGAGACCACCAGAGACAGAAAGACUACAGACAAUAGAUAGAUUAGACA GAAAAUGAAGCACAUGUAAAUAGAUGCAACAACAGUAAGCAUUAUAAUUG
T1	<u>GGAAGAAGAGUUU</u> AUUCAGAUAGAUUUUGUCCG
T2	<u>GGAAGAAGAGUUU</u> AUUCAGAUAGAUUUUGG
T3	<u>GGAAGAAGAGUUU</u> AUUCAGAUAGAUUG
T4	<u>GGAAGAAGAGUUU</u> AUUCAGAUAC
T5	<u>GGAAGAAGAGUUU</u> AUUCAGAUAGAUUUUGUCAA
T6	<u>GGAAGAAGAGUUU</u> AUUCAGAUAGAUUUUGUAAA
T7	<u>GGAAGAAGAGUUU</u> AUUCAGAUAGAUUUUGAAAA
T8	<u>GGAAGAAGAGUUU</u> AUUCAGAUAGAUUUAAAAA
T9	<u>GGAAGAAGAGUUU</u> AUUCAGAUAGAUUUAAAAA
T10	<u>GGAAGAAGAGUUU</u> AUUCAGAUAGAUUUAAAAA
T11	<u>GGAAGAAGAGUUU</u> AUUCAGAUAGAUUUAAAAA
T12	<u>GGUAGAAGAGUUU</u> AUUCAGAUAGAUUUUGUCCG
T13	<u>GGUUAGAAGAGUUU</u> AUUCAGAUAGAUUUUGUCCG
T14	<u>GGUUUAGAAGAGUUU</u> AUUCAGAUAGAUUUUGUCCG
T15	<u>GGUUUUAGAAGAGUUU</u> AUUCAGAUAGAUUUUGUCCG
T16	<u>GGUUUUUAGAAGAGUUU</u> AUUCAGAUAGAUUUUGUCCG
T17	<u>GGUUUUUUAGAAGAGUUU</u> AUUCAGAUAGAUUUUGUCCG
T18	<u>GGAAGAAGAGUUU</u> AUUCAGAUAGAUUUcaCCG
T19	<u>GGAAGAAGAGUUU</u> AUUCAGAUAGAUUUUUGUCCG
T20	<u>GGAAGAAGAGUUU</u> AUUCAGUaAGAUUUUGUCCG
T21	<u>GGAAGAAGAGUUU</u> AUagAGAUAGAUUUUGUCCG
T22	<u>GGAAGAAGAGU</u> aaAUUCAGAUAGAUUUUGUCCG
T23	<u>GGAAGAA</u> uuGUUUAUUCAGAUAGAUUUUGUCCG
5'-FAM-ssRNA36	5'-6-FAM-ACCCCCGAUUACGUUUGGUGGACCCUCAGAUUCAAC
5'-FAM-U20	5'-6-FAM-UUUUUUUUUUUUUUUUUUUUU
R1	UGUUCGACGA
5'-FAM-R1	5'-6-FAM-UGUUCGACGA

R1-FQ	5'-6-FAM-UGUUCGACGA-IABkFQ-3'
R2	UGAUCGACGA
R3	UGUACGACGA
R4	UGUUAGACGA
R5	UGACUGACGA
R6	UGAUCUACGA
R7	UGAUCAACGA
R8	UGAUCCACGA
R9	UGCUCGACGA
R10	UGGUCGACGA
R11	UUUUUUUGAUC
R12	UUUUUUGAUCG
R13	UUUUUGAUCGA
R14	UUGAUCGACG
R15	UCAGUUUUUU
R16	GAUCAGUUUU
R17	GCGAUCAGUU
A10/AA10	AAAAAAAAAA
C10	CCCCCCCCC
G10	GGGGGGGGG
U10	UUUUUUUUU
AC10	AAAACAAAA
AG10	AAAAGAAAA
AU10	AAAAUAAAA
CA10	AAACAAAAA
CC10	AAACCAAAAA
CG10	AAACGAAAA
CU10	AAACUAAAA
GA10	AAAGAAAAA
GC10	AAAGCAAAAA
GG10	AAAGGAAAA
GU10	AAAGUAAAA
UA10	AAAUAAAAA
UC10	AAAUCAAAAA
UG10	AAAUGAAAA
UU10	AAAUUAAAA
