Supplementary information, Data S1

Materials and Methods

Protein expression and purification

The full-length gene encoding BzCas13b was synthesized and constructed into cloning sites between *Nde*I and *Xho*I of pET-30b vector with a C-terminal His-tag. CRISPR template was synthesized (Supplementary information, Table S2) and constructed into cloning sites between *Nde*I and *Xho*I of pCDFDuet-1 vector.

To obtain the binary complex assembled *in vivo*, vectors encoding the BzCas13b R1177A mutant and CRISPR template were firstly co-transformed into *E.coli* Rosetta (DE3) cells (Novagen). The cells were grown at 37°C for 3 hours until OD_{600} reached ~0.6, and then induced with 0.2 mM IPTG at 18°C for 12 hours. The cells were harvested and lysed in buffer-A (25 mM Tris-HCl (pH 7.5), 500 mM NaCl, 5 mM β-mercaptoethanol, 1 mM PMSF). The binary complex was firstly purified with a Ni-NTA Superflow (QIAGEN) column. Next, the target sample was collected and purified with the Heparin HP column (GE Healthcare) using a salt gradient between buffer-B1 (25 mM Tris-HCl (pH 7.5), 90 mM NaCl, 2 mM $MgCl_2$, 2 mM DTT) and buffer-B2 (25 mM Tris-HCl (pH 7.5), 1 M NaCl, 2 mM $MgCl_2$, 2 mM DTT). Then, the target sample was dialyzed in buffer-C (25 mM Tris-HCl (pH 7.5), 200 mM NaCl, 2 mM $MgCl_2$, 2 mM DTT) and further purified by using a Superdex 200 Increase (10/300 GL) column (GE Healthcare). Finally, fractions of the binary complex were concentrated and preserved at -80°C for later crystallization. The selenomethionine (SeMet)-labeled binary complex was expressed in *E.coli* Rosetta (DE3) cells grown in M9 minimal medium and supplemented with SeMet, Lys, Phe, Thr, Val, Leu and Ile. The SeMet BzCas13b binary complex was purified as described above.

To prepare the target protein alone, BzCas13b was expressed in *E.coli* Rosetta (DE3) cells (Novagen). In brief, the target protein was purified with the Ni-NTA Superflow (QIAGEN) column using buffer-A and further purified with the Heparin HP column (GE Healthcare) using a salt gradient between buffer-B3 (25 mM Tris-HCl (pH 7.5), 200 mM NaCl, 1 mM DTT) and buffer-B4 (25 mM Tris-HCl (pH 7.5), 1 M NaCl, 1 mM DTT). Fractions of

BzCas13b were dialyzed in buffer-B3, concentrated and preserved for later preparation of the binary complex with crRNA or the pre-crRNA cleavage assay.

To obtain the binary complex assembled *in vitro*, crRNA was heated to 75°C for 5 min and then cooled on ice. Protein was incubated with crRNA at a molar ratio of 1:1.2 on ice for 1 hour and subsequently purified in buffer-C by using a Superdex 200 (10/300 GL) column (GE Healthcare). Fractions of the binary complex were collected and concentrated.

Synthesis and purification of pre-crRNA, crRNA and target RNA in vitro

RNA fragments used in this study were synthesized by *in vitro* transcription utilizing T7 RNA polymerase. The synthetic DNA fragment encoding pre-crRNA, crRNA and target RNA sequences were cloned into sites between *Stu*I and *Hind*III of a modified pUC-119 vector.[16] The vector was amplified in *E.coli* DH5α competent cells (Novagen) and extracted. After linearization by *Hind*III, the vector was purified by phenol/chloroform extraction and ethanol precipitation. Transcription reactions were performed at 37°C for 4 hours and in buffer containing 100 mM HEPES-K (pH 7.9), 20 mM $MgCl_2$, 30 mM DTT, 3 mM NTPs, 2 mM spermidine, 30 ng/μl linearized template and 100 μg/ml home-made T7 RNA polymerase. The reaction sample was purified using the denaturing polyacrylamide gel containing 8 M urea. The RNA band was excised from the gel and eluted by using the Elutrap system (GE Healthcare). After the treatment of ethanol precipitation, RNA was dissolved in diethylpyrocarbonate (DEPC)-treated H_2O and preserved at -80°C.

Site-directed mutagenesis

Vectors encoding the wild-type BzCas13b, pre-crRNA, crRNA were used as templates for conducting site-directed mutagenesis. Briefly, oligonucleotides for site-directed mutagenesis were designed and PCRs were carried out to acquire the desired mutations. The successful mutagenesis was verified by sequencing.

Crystallization

The SeMet-labeled BzCas13b R1177A binary Complex assembled *in vivo* was crystallized by the hanging-drop vapor diffusion method at 16°C. Crystals were obtained by mixing 1μl of complex solution ($A_{280\ nm}$ = 12.7) and 1μl of reservoir solution (0.15 M Sodium chloride, 0.1 M Lithium sulfate, 0.1 M Sodium citrate (pH 5.5), 22% (w/v) Polyethylene glycol 1000).

Crystals grew to their full size within 10 days and cryoprotected in reservoir solution supplemented with 20% (v/v) glycerol.

Data collection and structure determination

X-ray diffraction data was collected on beamline BL-17U1 at Shanghai Synchrotron Radiation Facility (SSRF). Datasets were processed automatically by the program XIA2 integrated into data collection platform of beamline BL-17U1.[17] The crystal structures were solved by single-wavelength anomalous dispersion method with the program AutoSol in PHENIX[18] and twelve SeMet sites were identified. There is one binary complex contained in an asymmetric unit of the crystal structure. Model building was carried out in COOT.[19] Multiple rounds of refinement was performed using the program REFMAC in CCP4.[20, 21] All structural figures were prepared using PyMOL (http://pymol.org). Data collection and refinement statistics were listed (Supplementary information, Table S1).

Pre-crRNA cleavage assay

Pre-crRNA and its mutants were designed to contain two repeat regions separated by a spacer region (Supplementary information, Table S2). Reactions were performed by incubating 6.8 μM pre-crRNA with 8.2 μM purified BzCas13b in 20 μl assay buffer (25 mM Tris-HCl (pH 7.5), 200 mM NaCl, 2 mM $MgCl_2$, 1 mM DTT). The cleavage assays were allowed to proceed at 37°C for 45 minutes. Reactions were stopped synchronously in liquid nitrogen and further quenched at 75°C for 5 minutes by adding 2 x loading buffer (2 x TBE buffer, 12 M urea). Samples were analyzed by running a 20% PAGE TBE-urea denaturing gel. The cleavage assays were repeated three times to confirm the results. The cleavage products were visualized by using the toluidine blue staining.

Target RNA cleavage assay

Two target RNAs (target-1 and target-2) were designed to contain different 5′-PFS. According to the previous study,[5] target-1 contains the nucleotide A of 5′-PFS, whereas target-2 contains the nucleotide C of 5′-PFS that greatly inhibiting the target RNA cleavage activity. Reactions were performed by incubating 10 μM target RNA with 1 μM BzCas13b-crRNA binary complex in 20 μl assay buffer (25 mM Tris-HCl (pH 7.5), 200 mM NaCl, 2 mM $MgCl_2$, 1 mM DTT). The cleavage assays were allowed to proceed at 37°C for

30 minutes. The cleavage assays were repeated three times. Reactions and samples were treated and visualized as described above.

RNA sequencing

RNA fragments from the pre-crRNA cleavage assay were purified using the denaturing polyacrylamide gel as described above. The purified sample was further treated by CIP (calf intestinal alkaline phosphatase). By using T4 RNA ligase-1, RNA sample was linked to a synthesized RNA connector containing a 3′-terminal dideoxynucleotide (GenePharma). After the reverse transcription by using ProtoScript II reverse transcriptase and PCR amplification, fragments were cloned into pUC-57 vector and sequenced.

References

16 Serganov, A. *et al.* Ribosomal protein S15 from Thermus thermophilus--cloning, sequencing, overexpression of the gene and RNA-binding properties of the protein. *European journal of biochemistry* **246**, 291-300 (1997).

17 Winter, G., Lobley, C. M. & Prince, S. M. Decision making in xia2. *Acta crystallographica. Section D, Biological crystallography* **69**, 1260-1273, doi:10.1107/S0907444913015308 (2013).

18 Adams, P. D. *et al.* PHENIX: a comprehensive Python-based system for macromolecular structure solution. *Acta crystallographica. Section D, Biological crystallography* **66**, 213-221, doi:10.1107/S0907444909052925 (2010).

19 Emsley, P. & Cowtan, K. Coot: model-building tools for molecular graphics. *Acta crystallographica. Section D, Biological crystallography* **60**, 2126-2132, doi:10.1107/S0907444904019158 (2004).

20 Vagin, A. A. *et al.* REFMAC5 dictionary: organization of prior chemical knowledge and guidelines for its use. *Acta crystallographica. Section D, Biological crystallography* **60**, 2184-2195, doi:10.1107/S0907444904023510 (2004).

21 Winn, M. D. *et al.* Overview of the CCP4 suite and current developments. *Acta crystallographica. Section D, Biological crystallography* **67**, 235-242, doi:10.1107/S0907444910045749 (2011).

Supplementary information, Fig. S1

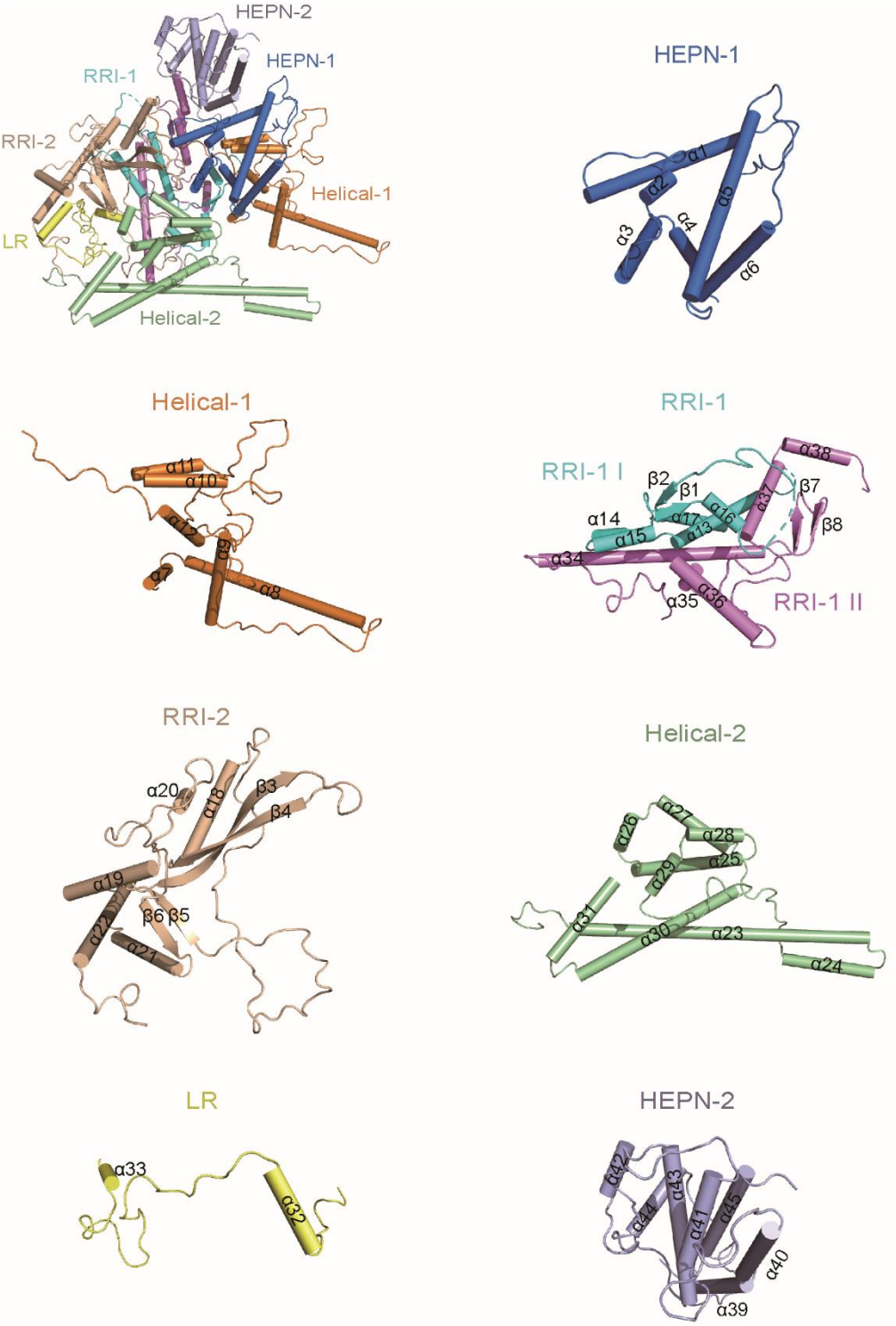

Fig. S1 Structures of individual domains and the linker region of BzCas13b in the BzCas13b-crRNA binary complex. Domains and the linker region (LR) are colored according to Fig. 1, except for RRI-1 II motif colored in violet.

Supplementary information, Fig. S2

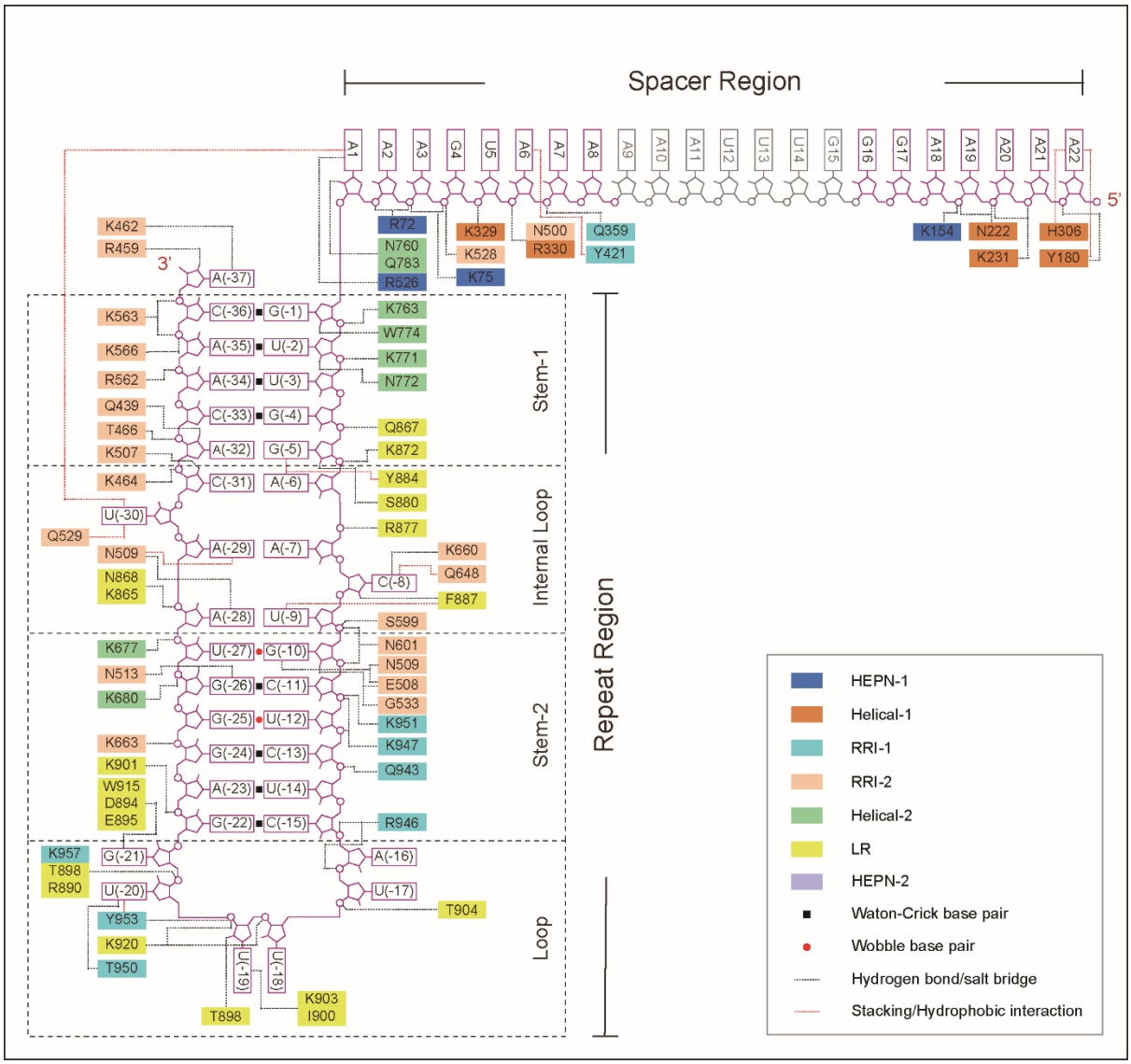

Fig. S2 Schematic of crRNA recognition by BzCas13b. Domains, the linker region (LR) and residues are colored according to Fig. 1. The disordered nucleotides are colored in grey. Hydrogen bonds and salt bridges are shown as black dashed lines. Stackings and hydrophobic interactions are shown as red dashed lines.

Supplementary information, Fig. S3

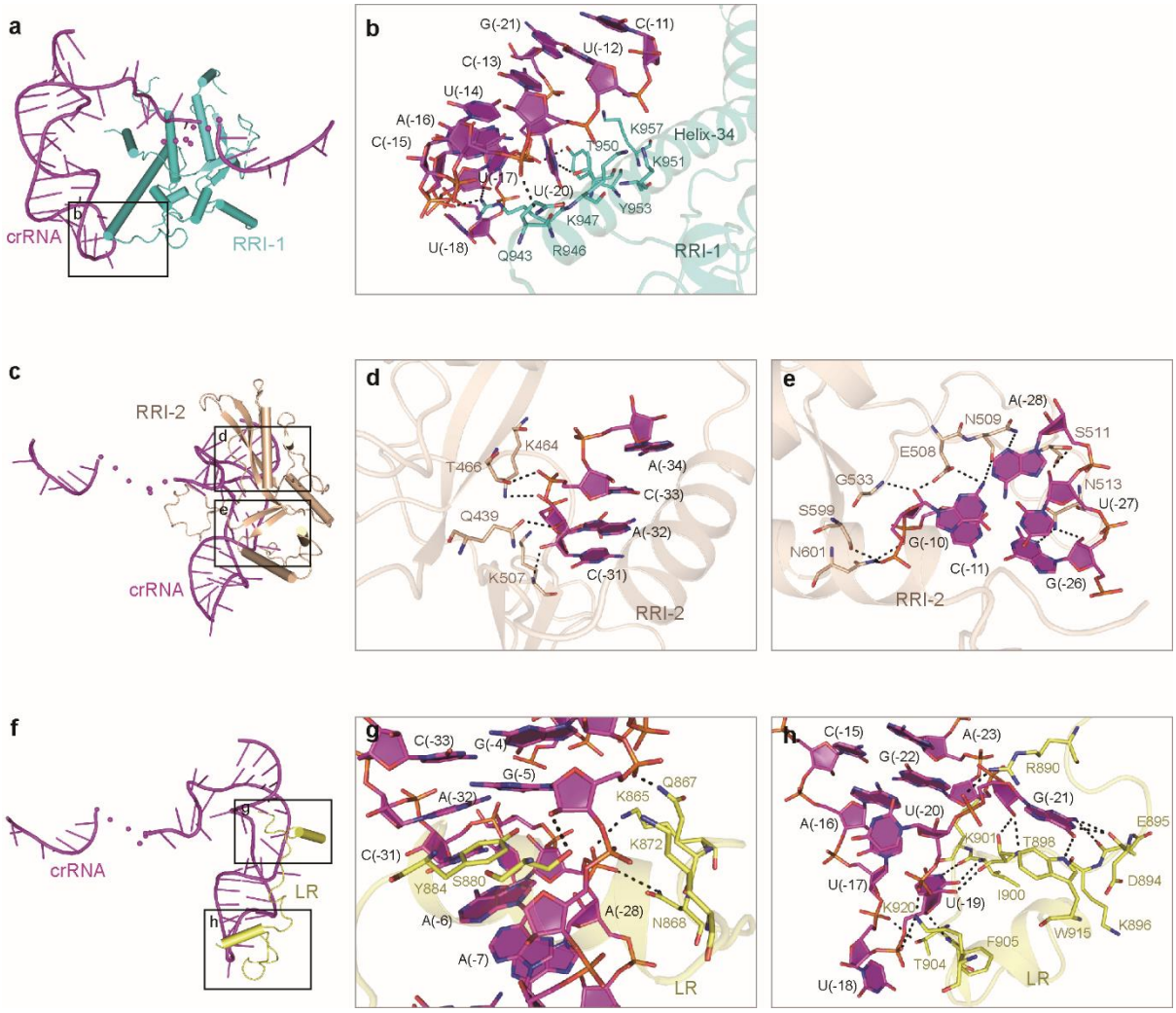

Fig. S3 Detailed interactions of crRNA with different domains and the linker region of BzCas13b. **a**, **b** Detailed interactions of crRNA with RRI-1 domain. Hydrogen bonds are shown as dashed lines. Domain and residues are colored according to Fig. 1. **c**-**e** Detailed interactions of crRNA with RRI-2 domain. **f**-**h** Detailed interactions of crRNA with the linker region (LR). The linker region and residues are colored according to Fig. 1.

Supplementary information, Fig. S4

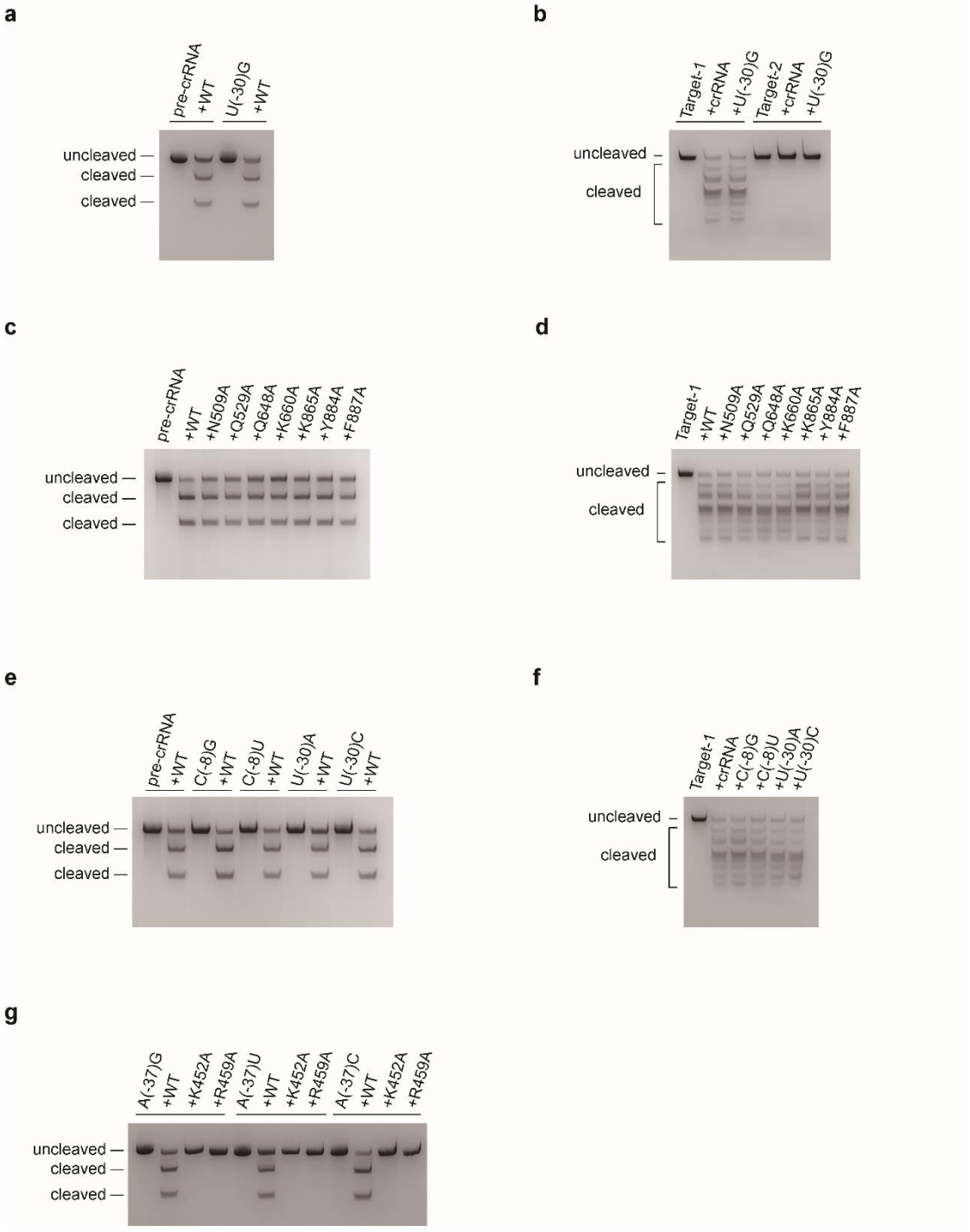

Fig. S4 The pre-crRNA processing and target RNA cleavage by BzCas13b. **a** Denaturing gel demonstrating the cleavage of pre-crRNA and the U(-30)G pre-crRNA mutant by wild-type BzCas13b. **b** Denaturing gel demonstrating the cleavage of target-1 and target-2 RNAs by wild-type BzCas13b in complex with crRNA and the U(-30)G crRNA mutant, respectively. **c** Denaturing gel demonstrating the cleavage of pre-crRNA by

wild-type BzCas13b and the mutants. **d** Denaturing gel demonstrating the cleavage of target-1 RNA by wild-type BzCas13b and the mutants in complex with crRNA. **e** Denaturing gel demonstrating the cleavage of pre-crRNA, the C(-8)G, C(-8)U, U(-30)A and U(-30)C pre-crRNA mutants by wild-type BzCas13b. **f** Denaturing gel demonstrating the cleavage of target-1 RNA by wild-type BzCas13b in complex with crRNA, the C(-8)G, C(-8)U, U(-30)A and U(-30)C crRNA mutants, respectively. **g** Denaturing gel demonstrating the cleavage of A(-37)G, A(-37)U and A(-37)C pre-crRNA mutants by wild-type BzCas13b, the K452A and R459A mutants, respectively.

Supplementary information, Fig. S5

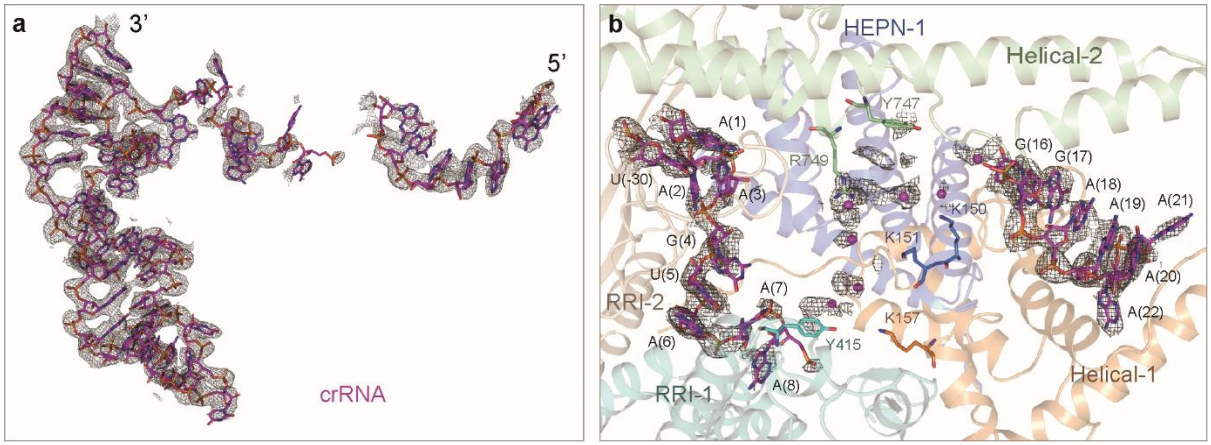

Fig. S5 Electron density map of crRNA in the BzCas13b-crRNA binary complex. **a** The electron density map of crRNA in the binary complex. The 2Fo-Fc omit map is contoured at 1.0 σ level. **b** The discontinuous electron density of G(15)-A(9) located between Helical-1 and HEPN-1 domains. The 2Fo-Fc omit map is contoured at 1.0 σ level. Domains and residues are colored according to Fig. 1. Dots represent possible positions of the disordered nucleotides within the spacer region. Residues speculated to interact with the disordered nucleotides are shown.

Supplementary information, Fig. S6

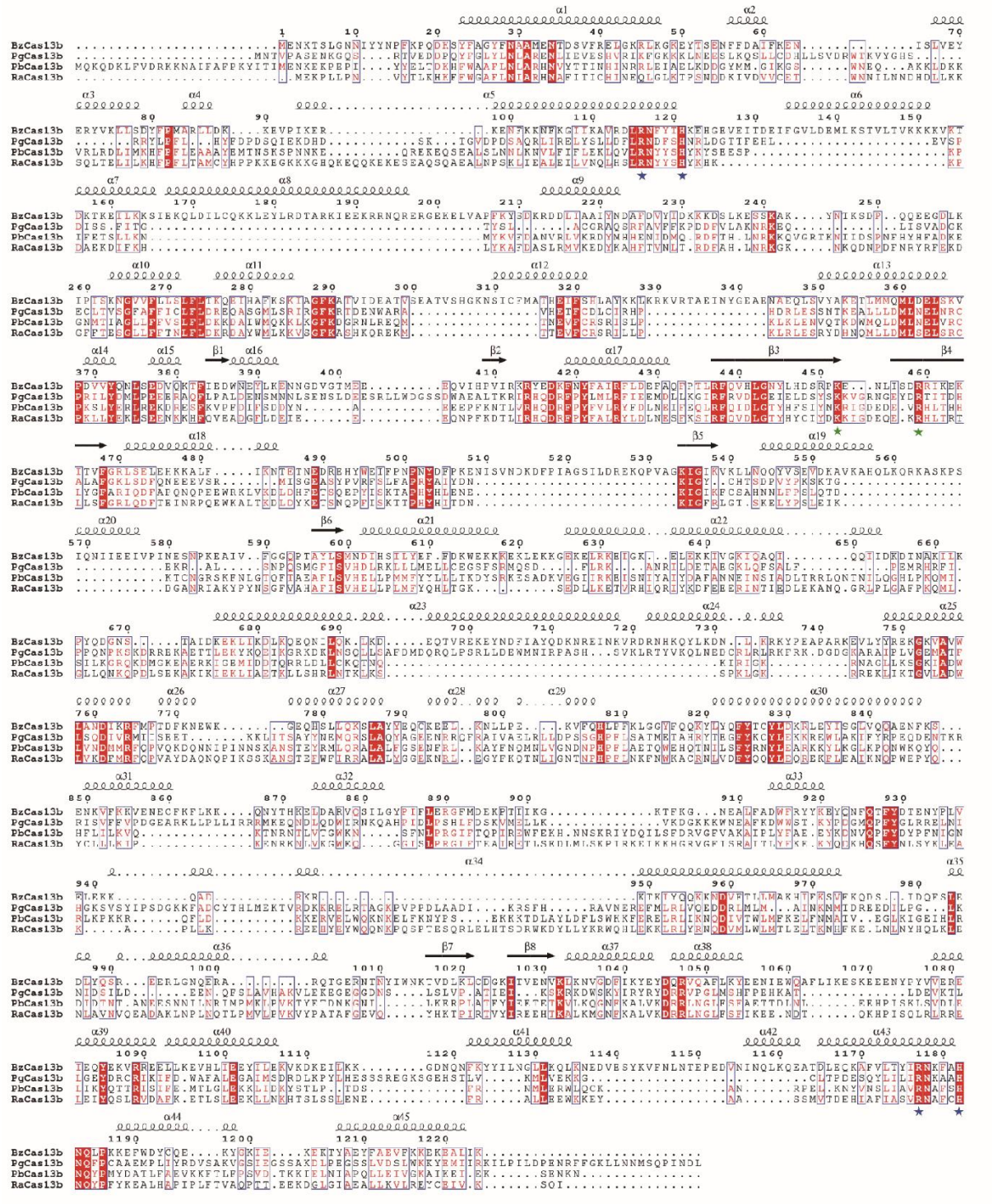

Fig. S6 Multiple sequence alignment of Cas13b proteins from different species. Sequence alignment of BzCas13b (*Bergeyella zoohelcum* Cas13b), PgCas13b (*Porphyromonas gingivalis* Cas13b), PbCas13b (*Prevotella buccae* Cas13b) and RaCas13b (*Riemerella anatipestifer* Cas13b) was generated using Clustal

Omega and the figure was prepared using ESPript (http://espript.ibcp.fr). The secondary structure of BzCas13b is shown above the sequence. Identical and similar residues are highlighted in red and white boxes, respectively. Catalytic residues involved in the pre-crRNA cleavage and target RNA cleavage are marked with green and blue stars, respectively.

Supplementary information, Fig. S7

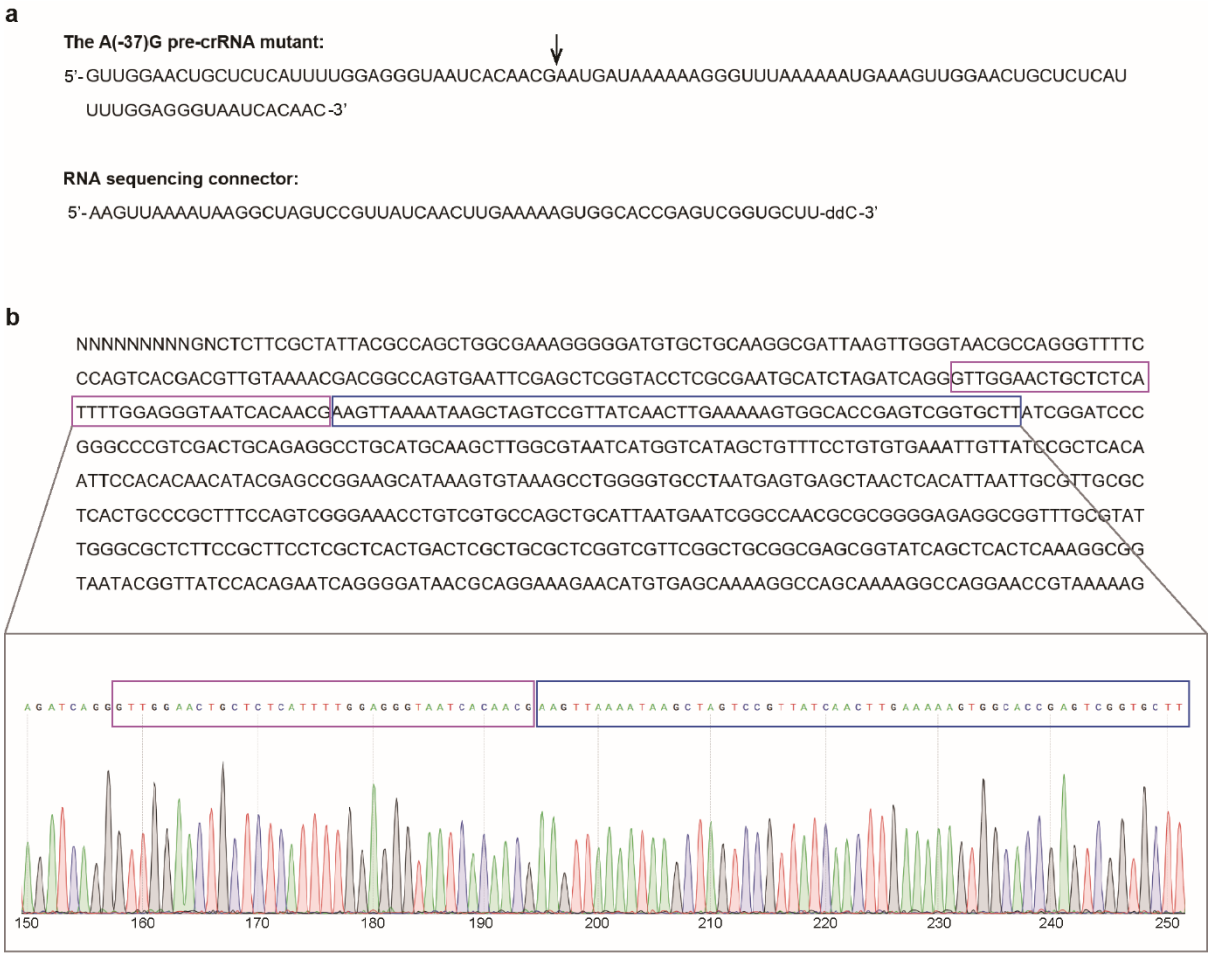

Fig. S7 Determination of the pre-crRNA cleavage site by RNA sequencing. **a** The A(-37)G pre-crRNA mutant was processed by BzCas13b, after which the short 5′-RNA fragment from the cleavage assay was retrieved as described in Materials and Methods. The black arrow indicates the putative cleavage site. The short 5′-RNA fragment was linked to a synthesized RNA connector containing a 3′-terminal dideoxynucleotide (GenePharma) by using T4 RNA ligase-1. **b** After the reverse transcription and PCR amplification, fragments were cloned into pUC-57 vector and sequenced. The result indicates that BzCas13b processes pre-crRNA one nucleotide 3′-downstream of the crRNA repeat region.

Supplementary information, Fig. S8

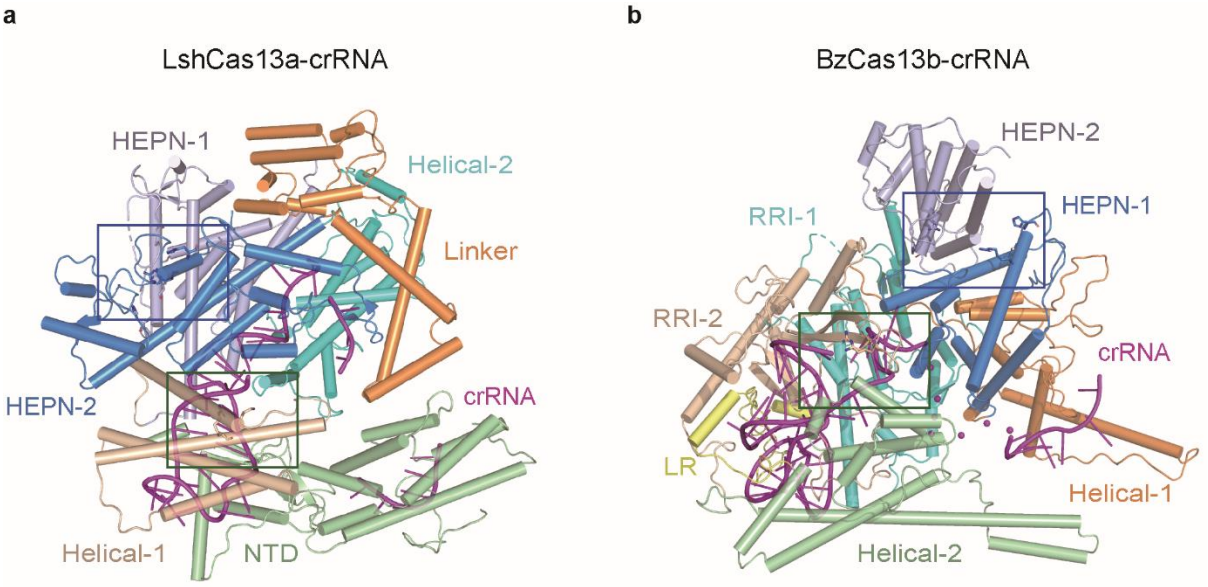

Fig. S8 Comparison of Cas13a and Cas13b. **a**, **b** Comparison of domain architectures of the LshCas13a-crRNA (*Leptotrichia shahii* Cas13a-crRNA, PDB code: 5WTK) and BzCas13b-crRNA binary complexes. The catalytic sites for the cleavages of pre-crRNA and target RNA are marked with green and blue boxes, respectively.

Supplementary information, Fig. S9

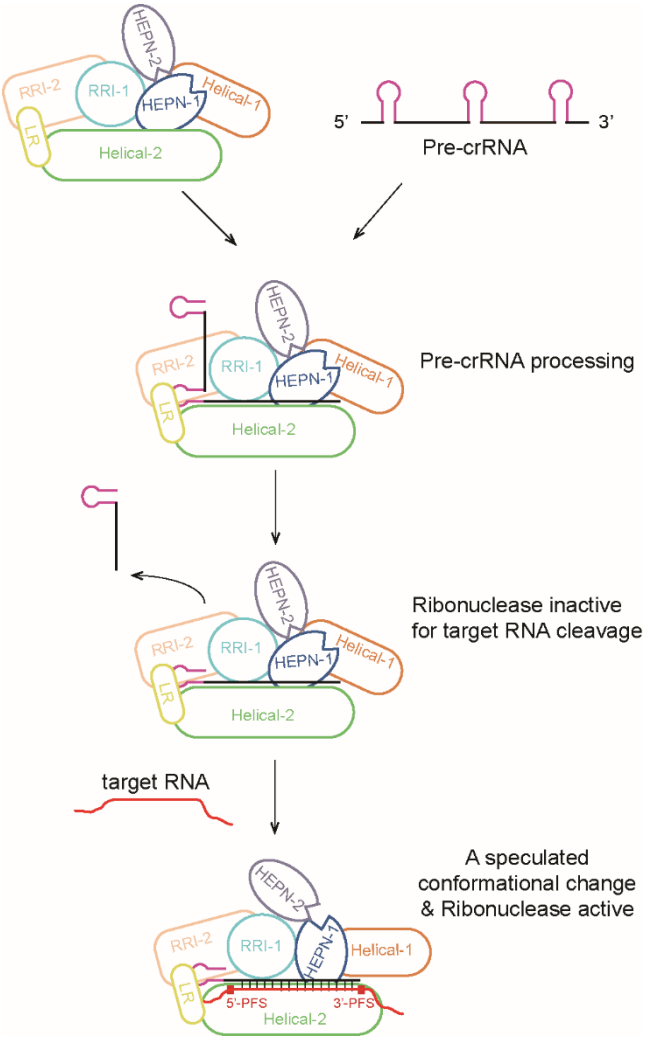

Fig. S9 Model of crRNA-guided RNA cleavage by Cas13b. Cas13b recognizes the repeat region of pre-crRNA and uses RRI-2 domain to process pre-crRNA into a mature crRNA. Upon recurrent infection, Cas13b utilizes crRNA to interrogate the invading RNA. The target RNA binding is speculated to rearrange the conformation of Cas13b, and the ribonuclease is fully activated by a double-sided PFS dependent manner to cleave the target and collateral RNAs.

Supplementary information, Table S1

Table S1. X-ray crystallography data collection and refinement statistics.

Dataset	SeMet-BzCas13b-R1177A-crRNA
Data collection	
Beamline	BL-17U1, SSRF
Wavelength (Å)	0.9792
Space group	$P4_12_12$
Cell dimensions	
a, *b*, *c* (Å)	196.74, 196.74, 96.23
α, β, γ (°)	90, 90, 90
Resolution range (Å)*	62.21-2.79 (2.89-2.79)
Completeness (%)	99.88 (100.00)
$I/\sigma(I)$	12.67 (2.04)
R_{merge}	0.053 (0.316)
Multiplicity	25.0 (28.0)
CC half	0.994 (0.660)
Anomalous completeness (%)	100.0 (100.0)
Anomalous multiplicity	13.2 (14.6)
Refinement	
Resolution (Å)	2.79
No. reflections	47,452
R_{work}/ R_{free} (%)	22.2/ 28.6
No. atoms	
Protein	10,107
Nucleic acid	1,118
Solvent	37
B-factors (Å^2)	
Protein	72.19
Nucleic acid	74.68
solvent	55.69
R.m.s deviations	
Bond length (Å)	0.012
Bond angles (°)	1.73
Ramachandran plot (%)	
Favored region	96.73
Allowed region	3.11
Outliers region	0.17

*Highest resolution shell is shown in parentheses.

Supplementary information, Table S2

Table S2. RNA coding sequences used in the study.

Description	RNA coding sequences
CRISPR RNA	GTTGGAACTGCTCTCATTTTGGAGGGTAATCACAACAAATGATAAAA AAGGGTTTAAAAAATGAAAGTTGGAACTGCTCTCATTTTGGAGGGTA ATCACAACAAATGATAAAAAAGGGTTTAAAAAATGAAAGTTGGAACT GCTCTCATTTTGGAGGGTAATCACAACAAATGATAAAAAAGGGTTTA AAAAATGAAAGTTGGAACTGCTCTCATTTTGGAGGGTAATCACAACA AATGATAAAAAAGGGTTTAAAAAATGAAAGTTGGAACTGCTCTCATT TTGGAGGGTAATCACAACAAATGATAAAAAAGGGTTTAAAAAATGA AAGTTGGAACTGCTCTCATTTTGGAGGGTAATCACAACAAATGATAA AAAAGGGTTTAAAAAATGAAAGTTGGAACTGCTCTCATTTTGGAGGG TAATCACAACAAATGATAAAAAAGGGTTTAAAAAATGAAAGTTGGA ACTGCTCTCATTTTGGAGGGTAATCACAAC
Pre-crRNA	GTTGGAACTGCTCTCATTTTGGAGGGTAATCACAACAAATGATAAAA AAGGGTTTAAAAAATGAAAGTTGGAACTGCTCTCATTTTGGAGGGTA ATCACAAC
The △C(-8) pre-crRNA mutant	GTTGGAATGCTCTCATTTTGGAGGGTAATCACAACAAATGATAAAAA AGGGTTTAAAAAATGAAAGTTGGAATGCTCTCATTTTGGAGGGTAAT CACAAC
The △U(-30) pre-crRNA mutant	GTTGGAACTGCTCTCATTTTGGAGGGTAACACAACAAATGATAAAAA AGGGTTTAAAAAATGAAAGTTGGAACTGCTCTCATTTTGGAGGGTAA CACAAC
The A(-37)G pre-crRNA mutant	GTTGGAACTGCTCTCATTTTGGAGGGTAATCACAACGAATGATAAAA AAGGGTTTAAAAAATGAAAGTTGGAACTGCTCTCATTTTGGAGGGTA ATCACAAC
The A(-37)U pre-crRNA mutant	GTTGGAACTGCTCTCATTTTGGAGGGTAATCACAACTAATGATAAAA AAGGGTTTAAAAAATGAAAGTTGGAACTGCTCTCATTTTGGAGGGTA ATCACAAC
The A(-37)C pre-crRNA mutant	GTTGGAACTGCTCTCATTTTGGAGGGTAATCACAACCAATGATAAAA AAGGGTTTAAAAAATGAAAGTTGGAACTGCTCTCATTTTGGAGGGTA ATCACAAC
The U(-30)G pre-crRNA mutant	GTTGGAACTGCTCTCATTTTGGAGGGTAAGCACAACAAATGATAAAA AAGGGTTTAAAAAATGAAAGTTGGAACTGCTCTCATTTTGGAGGGTA AGCACAAC
The U(-30)A pre-crRNA mutant	GTTGGAACTGCTCTCATTTTGGAGGGTAAACACAACAAATGATAAAA AAGGGTTTAAAAAATGAAAGTTGGAACTGCTCTCATTTTGGAGGGTA AACACAAC
The U(-30)C pre-crRNA mutant	GTTGGAACTGCTCTCATTTTGGAGGGTAACCACAACAAATGATAAAA AAGGGTTTAAAAAATGAAAGTTGGAACTGCTCTCATTTTGGAGGGTA ACCACAAC
The C(-8)G pre-crRNA mutant	GTTGGAAGTGCTCTCATTTTGGAGGGTAATCACAACAAATGATAAAA AAGGGTTTAAAAAATGAAAGTTGGAAGTGCTCTCATTTTGGAGGGTA ATCACAAC
The C(-8)U pre-crRNA mutant	GTTGGAATTGCTCTCATTTTGGAGGGTAATCACAACAAATGATAAAA AAGGGTTTAAAAAATGAAAGTTGGAATTGCTCTCATTTTGGAGGGTA ATCACAAC
crRNA	AAATGATAAAAAAGGGTTTAAAAAATGAAAGTTGGAACTGCTCTCAT TTTGGAGGGTAATCACAAC
The △C(-8) crRNA mutant	AAATGATAAAAAAGGGTTTAAAAAATGAAAGTTGGAATGCTCTCATT TTGGAGGGTAATCACAAC
The △U(-30) crRNA mutant	AAATGATAAAAAAGGGTTTAAAAAATGAAAGTTGGAACTGCTCTCAT TTTGGAGGGTAACACAAC
The U(-30)G crRNA mutant	AAATGATAAAAAAGGGTTTAAAAAATGAAAGTTGGAACTGCTCTCAT TTTGGAGGGTAAGCACAAC

The U(-30)A crRNA mutant	AAATGATAAAAAAGGGTTTAAAAAATGAAAGTTGGAACTGCTCTCAT TTTGGAGGGTAAACACAAC
The U(-30)C crRNA mutant	AAATGATAAAAAAGGGTTTAAAAAATGAAAGTTGGAACTGCTCTCAT TTTGGAGGGTAACCACAAC
The C(-8)G crRNA mutant	AAATGATAAAAAAGGGTTTAAAAAATGAAAGTTGGAAGTGCTCTCAT TTTGGAGGGTAATCACAAC
The C(-8)U crRNA mutant	AAATGATAAAAAAGGGTTTAAAAAATGAAAGTTGGAATTGCTCTCAT TTTGGAGGGTAATCACAAC
Target-1 RNA	CAATTTCATTTTTTAAACCCTTTTTTATCATTTAAGAAGATCAACTCTT TATGTATTGATCTTCCT
Target-2 RNA	CACTTTCATTTTTTAAACCCTTTTTTATCATTTAAGAAGATCAACTCTT TATGTATTGATCTTCCT

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Supplementary information, Movie S1

Movie S1

Surface representations, domain architecture and electrostatic potential surface of the BzCas13b-crRNA binary complex.