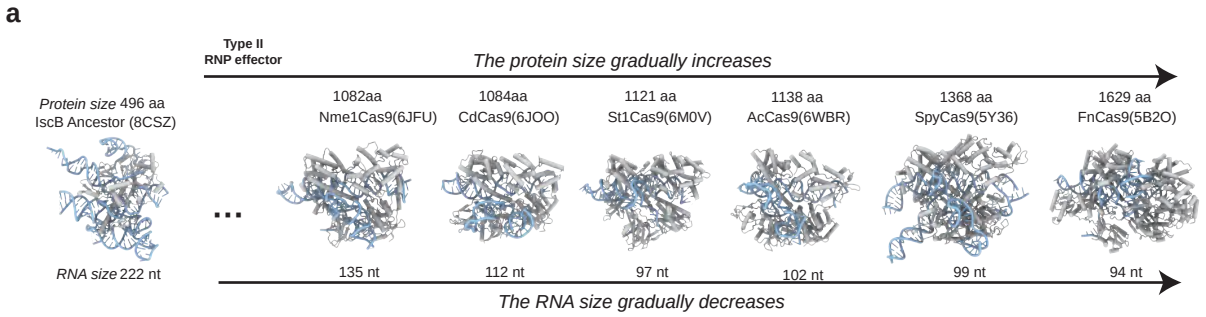
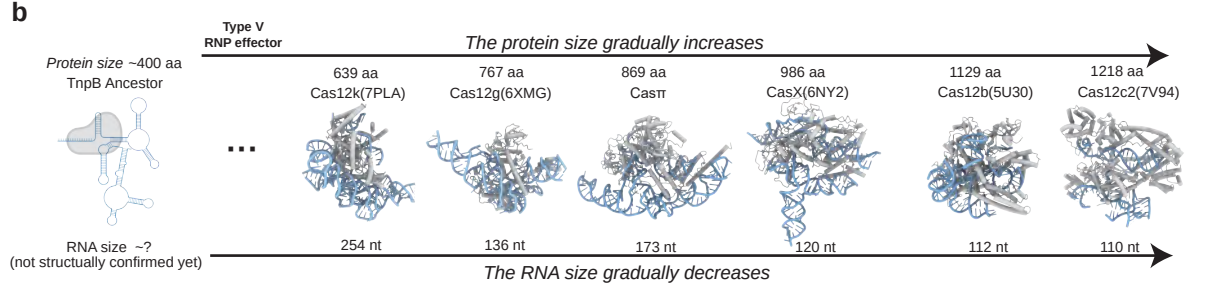

a
Type II RNP effector
The protein size gradually increases
Protein size 496 aa IscB Ancestor (8CSZ)
1082aa Nme1Cas9(6JFU)
1084aa CdCas9(6JOO)
1121 aa St1Cas9(6M0V)
1138 aa AcCas9(6WBR)
1368 aa SpyCas9(5Y36)
1629 aa FnCas9(5B2O)
RNA size 222 nt
135 nt
112 nt
97 nt
102 nt
99 nt
94 nt
The RNA size gradually decreases


b
Type V RNP effector
The protein size gradually increases
Protein size ~400 aa TnpB Ancestor
639 aa Cas12k(7PLA)
767 aa Cas12g(6XMG)
869 aa Casπ
986 aa CasX(6NY2)
1129 aa Cas12b(5U30)
1218 aa Cas12c2(7V94)
RNA size ~? (not structually confirmed yet)
254 nt
136 nt
173 nt
120 nt
112 nt
110 nt
The RNA size gradually decreases

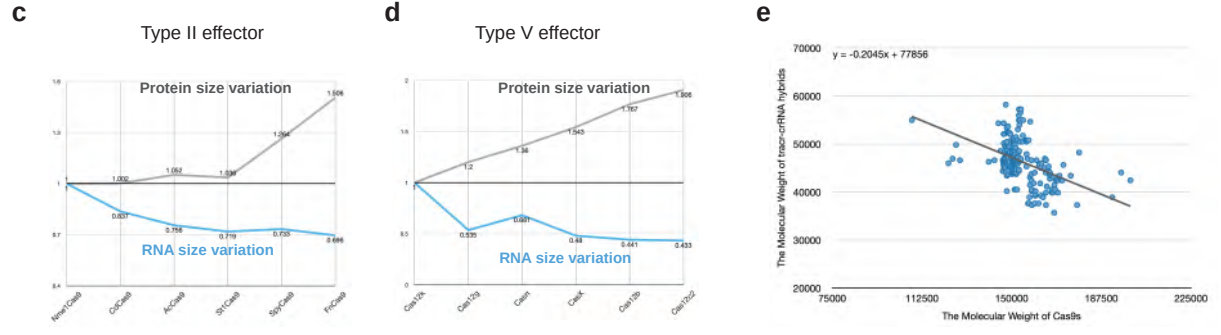

c
Type II effector
Protein size variation
RNA size variation
d
Type V effector
Protein size variation
RNA size variation
e
y = -0.2045x + 77856
The Molecular Weight of trans-crRNA hybrids
The Molecular Weight of Cas9s

Fig. S11 Molecular size analysis for the gRNA and protein in CRISPR effectors.

(**a**) The summary of the RNA and protein size in type II CRISPR effectors comprising of a protein monomer and tracr-crRNA hybrid. The atomic model each effector was shown in the same size scale. The protein part was colored in grey and RNA part in cyan. The number of amino acids (aa) in each protein, the length of each RNA (nt), and the PDB code for each structure were labeled, accordingly. The structure of ancestral IscB effector was shown in the left panel.

(**b**) The summary of the RNA and protein size in type V CRISPR effectors comprising of a protein monomer and tracr-crRNA hybrid. The cartoon of ancestral TnpB effector was presented in the left panel.

(**c**) The RNA- and protein-size variation trend for type II effectors referring to Nme1Cas9 effector (pdb 6JFU). The variation value (y axis) for the protein in each effector equals its protein size divided by Nme1Cas9 protein size. The variation value for the RNA in each effector equals its RNA size divided by NmeCas9 RNA size. The variation value >1 suggests a size increase compared to NmeCas9, and variation value >1 suggests a size decrease.

(**d**) The RNA- and protein-size variation trend for type V effectors referring to Cas12k effector (pdb 7PLA).

(**e**) The correlation analysis of the protein length and the tracr-crRNA molecular weights within 383 bioinformatically identified Cas9 effectors（Cas9's molecular weight is between 100,000-200,000 Da and tracr-crRNA's molecular weight is between 30,000-60,000 Da）. The Y axis represents the tracr-crRNA molecular weights and the X axis indicates the molecular weight of Cas9 proteins in each effector.