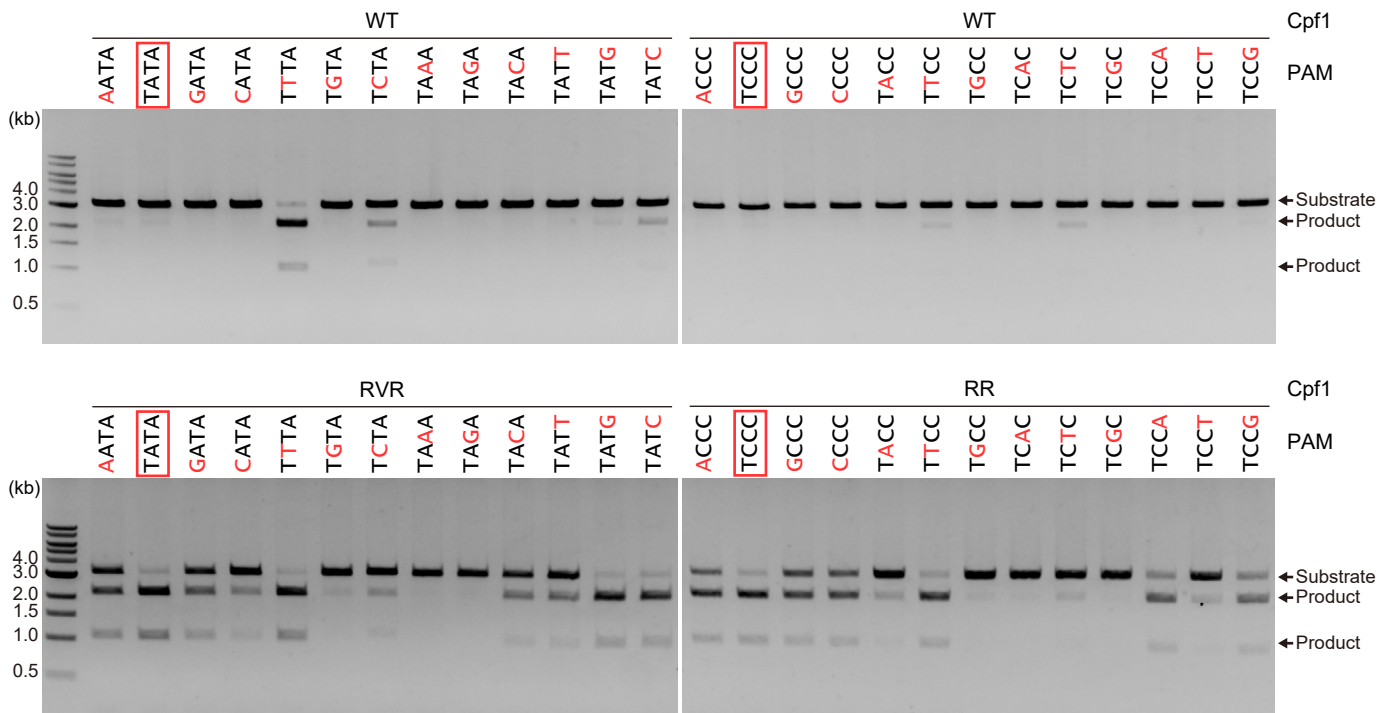**Figure S1. *In vitro* Cleavage Activities of WT AsCpf1 and AsCpf1 Variants, Related to Figure 1**

(A and B) PAM specificities of the RVR (A) and RR (B) variants. The AsCpf1-crRNA complex (100 nM) was incubated at 37°C for 10 min with a linearized plasmid target with the different PAMs.

(C) Fourth PAM nucleotide preferences of WT AsCpf1 and the RVR and RR variants. The AsCpf1-crRNA complex (100 nM) was incubated at 37°C for 10 min with a linearized plasmid target with the TTTN PAMs.

Figure S2. Comparison of the PAM Specificities of WT AsCpf1 and AsCpf1 Variants, Related to Figure 1

The AsCpf1-crRNA complex (100 nM) was incubated at 37°C for 5 min with a linearized plasmid target with the different PAMs. For comparison, the cleavage data for the RVR (Figure 1A) and RR (Figure 1B) variants are shown below those for WT AsCpf1.
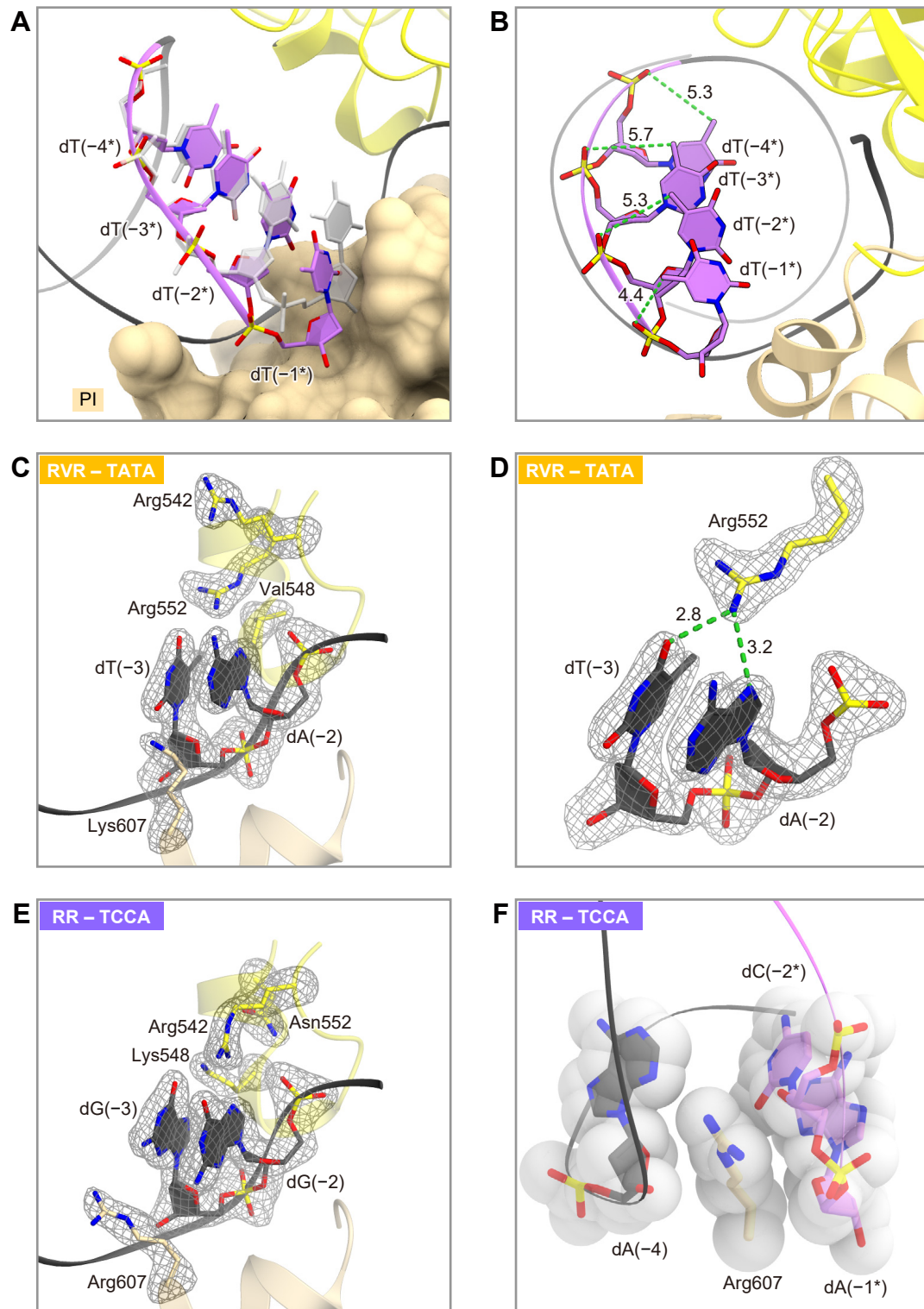
Figure S3. PAM Recognition by the AsCpf1 Variants, Related to Figure 3

(A) Conformational differences between the PAM nucleotides. The dT(−1*) nucleotide was modeled into the WT AsCpf1 structure (Yamano et al., 2016) (PDB: 5B43). Superimposition of the nucleotides −5* to −2* (gray) onto the nucleotides −4* to −1* (purple) highlights the displacement of the fourth PAM nucleotide (at −1* position), due to the interaction with the PI domain (shown as a surface representation).

(B) Differences in the distances between the 5-methyl group of the T nucleotide and its adjacent phosphate group at each PAM position. The dT(−1*) nucleotide was modeled into the WT AsCpf1 structure (Yamano et al., 2016) (PDB: 5B43). The distances are given in Å.

(C) $mF_O − DF_C$ omit electron density map for the key residues and nucleotides in the RVR variant (contoured at 4σ).

(D) Hydrogen-bonding interactions between Arg552 and the PAM duplex. The $mF_O − DF_C$ omit electron density map is shown as a gray mesh (contoured at 5σ). Hydrogen bonds are shown as dashed lines, and the distances are given in Å.

(E) $mF_O − DF_C$ omit electron density map for the key residues and nucleotides in the RR variant (contoured at 4σ).

(F) Hydrophobic interactions between Arg607 and the PAM duplex.
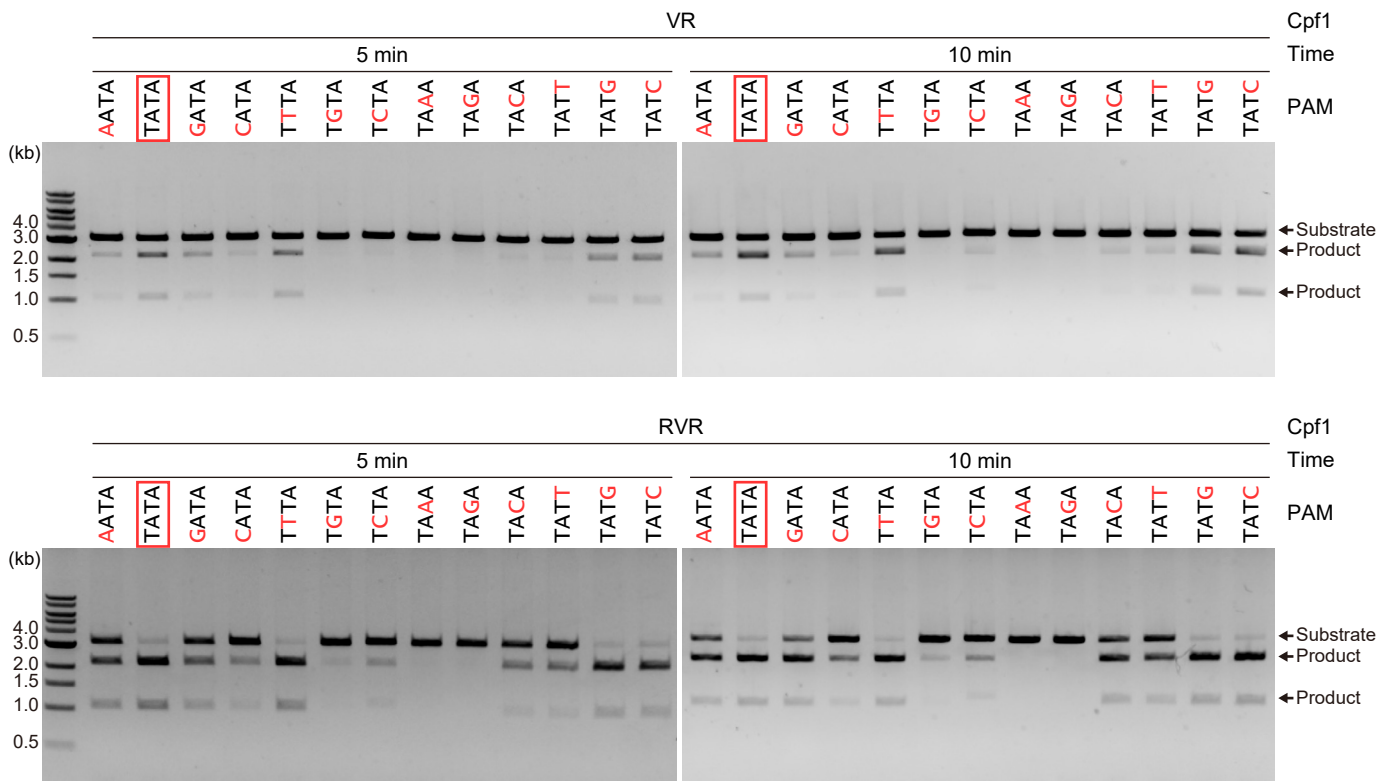
Figure S4. *In vitro* **Cleavage Activity of the VR Variant, Related to Figure 4**
The AsCpf1-crRNA complex (100 nM) was incubated at 37°C for 5 or 10 min with a linearized plasmid target with the different PAMs. For comparison, the cleavage data for the RVR variant (Figures 1A and S1A) are shown below those for the VR variant.

Table S1 Oligonucleotides

Oligonucleotides used to generate the AsCpf1 variant		
Mutation	Forward primer	Reverse primer
RR_S542R	AGAGGCTGGGACGTGAATAAGGAGAAGA	GGCCAGTGTAGGCATCTGAAAGTTC
RR_K607R	ATCCCAAGATGCAGCACCCAGCTGAAG	GCTGCATCTTGGGATCATCTTGGCGGC
RVR_S542R	AGAGGCTGGGACGTGAATGTTGAGAAGA	GGCCAGTGTAGGCATCTGAAAGTTC
RVR_K548V_N552R	GAACAGAGGCGCCATCCTGTTTGTGAAGAAC	TTCTCAACATTCACGTCCCAGCCAGAGGC

DNA oligonucleotides used for crystallization		
PAM sequence	Target DNA strand	Non-target DNA strand
TCCA	GGTTGCCAAGCGCACCTAATTTCCTGGAGGACTG	CAGTCCTCCA
TATA	GGTTGCCAAGCGCACCTAATTTCCTATAGGACTG	CAGTCCTATA

crRNA	
AsCpf1 crRNA	AAUUUCUACUCUUGUAGAUGGAAAUUAGGUGCGCUUGGCAACC

Oligonucleotides used to generate the target plasmids with the different PAMs		
PAM sequence	Forward primer	Reverse primer
TTTA	TTTAGGAAATTAGGTGCGCTTGGCAACC	GTATTTAGAAAAATAAACAAATAGGG
TTTT	TTTTGGAAATTAGGTGCGCTTGGCAACC	
TTTG	TTTGGGAAATTAGGTGCGCTTGGCAACC	
TTTC	TTTCGGAAATTAGGTGCGCTTGGCAACC	
TCCC	TCCCGGAAATTAGGTGCGCTTGGCAACC	
ACCC	ACCCGGAAATTAGGTGCGCTTGGCAACC	
GCCC	GCCCGGAAATTAGGTGCGCTTGGCAACC	
CCCC	CCCCGGAAATTAGGTGCGCTTGGCAACC	
TACC	TACCGGAAATTAGGTGCGCTTGGCAACC	
TTCC	TTCCGGAAATTAGGTGCGCTTGGCAACC	
TGCC	TGCCGGAAATTAGGTGCGCTTGGCAACC	
TCAC	TCACGGAAATTAGGTGCGCTTGGCAACC	
TCTC	TCTCGGAAATTAGGTGCGCTTGGCAACC	
TCGC	TCGCGGAAATTAGGTGCGCTTGGCAACC	
TCCA	TCCAGGAAATTAGGTGCGCTTGGCAACC	
TCCT	TCCTGGAAATTAGGTGCGCTTGGCAACC	
TCCG	TCCGGGAAATTAGGTGCGCTTGGCAACC	
TATA	TATAGGAAATTAGGTGCGCTTGGCAACC	
AATA	AATAGGAAATTAGGTGCGCTTGGCAACC	
GATA	GATAGGAAATTAGGTGCGCTTGGCAACC	
CATA	CATAGGAAATTAGGTGCGCTTGGCAACC	
TGTA	TGTAGGAAATTAGGTGCGCTTGGCAACC	
TCTA	TCTAGGAAATTAGGTGCGCTTGGCAACC	
TAAA	TAAAGGAAATTAGGTGCGCTTGGCAACC	
TAGA	TAGAGGAAATTAGGTGCGCTTGGCAACC	
TACA	TACAGGAAATTAGGTGCGCTTGGCAACC	
TATT	TATTGGAAATTAGGTGCGCTTGGCAACC	
TATG	TATGGGAAATTAGGTGCGCTTGGCAACC	
TATC	TATCGGAAATTAGGTGCGCTTGGCAACC	