**SUPPLEMENTAL FIGURE LEGEND**

**Figure S1 | *In vivo* plasmid depletion assay for identifying PAM for FnCpf1. Related to Figure 1.**

(A) Transformation of *E.* coli harboring pFnCpf1 with a library of plasmids carrying randomized 5’ PAM sequences. A subset of plasmids were depleted. Plot shows depletion levels in ranked order. Depletion is measured as the negative log2 fold ratio of normalized abundance compared pACYC184 *E. coli* controls and PAMs above a threshold of 3.5 are used to generate sequence logos.

(B) Transformation of *E.* coli harboring pFnCpf1 with a library of plasmids carrying randomized 3’ PAM sequences. A subset of plasmids were depleted. Plot shows depletion levels in ranked order. Depletion is measured as the negative log2 fold ratio of normalized abundance compared pACYC184 *E. coli* controls and PAMs above a threshold of 3.5 are used to generate sequence logos.

(C) Input library of plasmids carrying randomized 5’ PAM sequences. Plot shows depletion levels in ranked order. Depletion is measured as the negative log2 fold ratio of normalized abundance compared pACYC184 *E. coli* controls and PAMs above a threshold of 3.5 are used to generate sequence logos.

(D) The number of unique PAMs passing significance threshold for pairwise combinations of bases at the 2 and 3 positions of the 5’ PAM.

**Figure S2 | FnCpf1 Protein Purification. Related to Figure 3.**

(A) Coomassie blue stained acrylamide gel of FnCpf1 stepwise purification. A band just above 160 kD eluted from the Ni-NTA column, consistent with the size of a MBP-FnCpf1 fusion (189.7 kD). Upon addition of TEV protease a lower molecular weight band appeared, consistent with the size of 147 kD free FnCpf1.

(B) Size exclusion gel filtration of FnCpf1. FnCpf1 eluted at a size approximately 300 kD (62.65 mL), suggesting Cpf1 may exist in solution as a dimer.

(C) Protein standards used to calibrate the Superdex 200 column. BDex = Blue Dextran (void volume), Ald = Aldolase (158 kD), Ov = Ovalbumin (44 kD), RibA = Ribonuclease A (13.7 kD), Apr = Aprotinin (6.5 kD).

(D) Calibration curve of the Superdex 200 column. Ka is calculated as (elution volume – void volume)/(geometric column volume – void volume). Standards were plotted and fit to a logarithmic curve.

**Figure S3 | Cleavage patterns of FnCpf1. Related to Figures 3 and 5.**

(A-D) Sanger sequencing traces from FnCpf1-digested DNA targets show staggered overhangs. The non-templated addition of an additional adenine, denoted as N, is an artifact of the polymerase used in sequencing ([Clark, 1988](#_ENREF_9)). Sanger traces are shown for different TTN PAMs with protospacer 1 (A), protospacer 2 (B), protospacer 3 (C), and targets DNMT1 and EMX1 (D). The (–) strand sequence is reverse-complemented to show the top strand sequence. Cleavage sides are indicated by red triangles. Smaller triangles indicate putative alternative cleavage side.

(E) Effect of PAM-distal crRNA-target DNA mismatch on FnCpf1 cleavage activity.

**Figure S4 | Protein alignment of FnCpf1, AsCpf1, LbCpf1. Related to Figure 4.**

Multiple sequence alignment of the amino acid sequences of FnCpf1, AsCpf1, and LbCpf1 shows many highly conserved residues. Residues that are conserved are highlighted with a red background and conserved mutations are highlighted with an outline and red font. Secondary structure prediction is highlighted above (FnCpf1) and below (LbCpf1) the alignment. Alpha helices are shown as a curly symbol and beta strands are shown as dashes. Putative catalytic residues are highlighted in yellow. Protein domains identified in Fig. 1A are also highlighted.

**Figure S5 | Phylogeny tree of non-redundant Cpf1-family proteins and candidate Cpf1 loci chosen for genome editing testing. Related to Figure 6.**

(A) Evolutionary relationships of the Cpf1 bacterial strains are shown. Numbered strains correspond to candidates that were synthesized for functional experimentation (red).

(B) Maps of bacterial genomic loci corresponding to the 16 Cpf1-family proteins selected for mammalian experimentation.

**Figure S6 | *In vitro* characterization of Cpf1-family proteins. Related to Figure 6.**

(A) Schematic for *in vitro* PAM screen using Cpf1-family proteins. A library of plasmids bearing randomized 5’ PAM sequences were cleaved by individual Cpf1-family proteins and their corresponding crRNAs. Uncleaved plasmid DNA was purified and sequenced to identify specific PAM motifs that were depleted.

(B) The number of unique sequences passing significance threshold for pairwise combinations of bases at the 2 and 3 positions of the 5’ PAM for 7 – AsCpf1.

(C) The number of unique PAMs passing significance threshold for triple combinations of bases at the 2, 3, and 4 positions of the 5’ PAM for 13 – LbCpf1.

(D) Western blot of Cpf1 orthologs showing stable expression of all orthologs tested.

(E, F) Sanger sequencing traces from 7 – AsCpf1-digested target (E) and 13 – LbCpf1-digested target (F) show staggered overhangs. The non-templated addition of an additional adenine, denoted as N, is an artifact of the polymerase used in sequencing ([Clark, 1988](#_ENREF_9)). Cleavage sites are indicated by red triangles. Smaller triangles indicate putative alternative cleavage side.

**Figure S7 | Human cell genome editing efficiency at additional loci. Related Figure 7.**

(A-E) Surveyor gels showing quantification of indel efficiency achieved by each Cpf1-family protein at DNMT1 target sites 1, 2, and 4, and EMX1 sites 1 and 2, respectively.

(F) Indel distributions for AsCpf1 and LbCpf1 and DNMT1 target sites 2, 3, and 4. Cyan bars represent total indel coverage; blue bars represent distribution of 3’ ends of indels. For each target, PAM sequence is in red and target sequence is in light blue.