

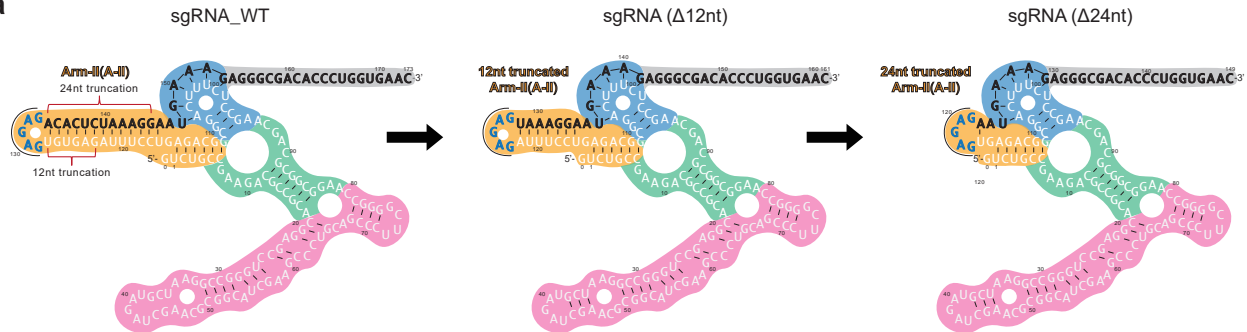
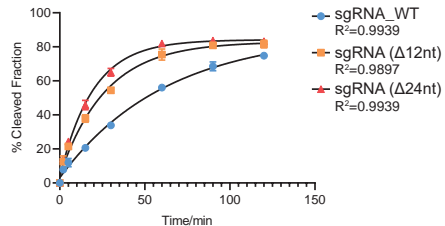
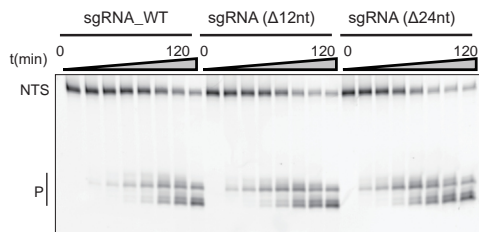
a**b**

Fig. S10 sgRNA engineering on the A-II region.

(a) Left, the naive version of the sgRNA (sgRNA_WT); Middle, the secondary structure of sgRNA (Δ 12nt) with 12-nt truncation on the A-II region; Right, the secondary structure of sgRNA (Δ 24nt) with 24-nt truncation on the A-II region.

(b) Left, *In vitro* dsDNA cleavage by Cas π -1 effector using different sgRNAs; Right, the plot of cleavage efficiency (n=3; mean \pm SD), The rate constant k values for Cas π -1 with sgRNA_WT, sgRNA (Δ 12nt) and sgRNA (Δ 24nt) are 0.0165, 0.03702 and 0.05125 (fraction/minute).