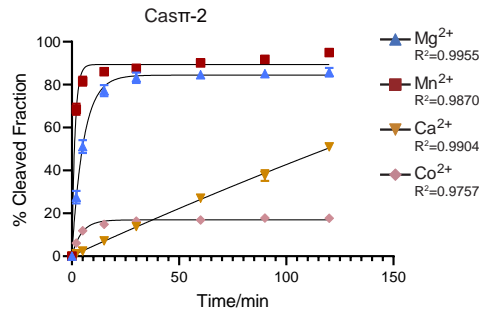
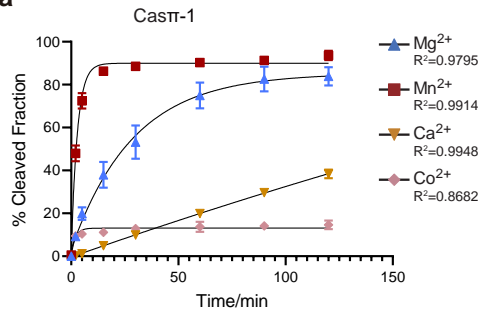
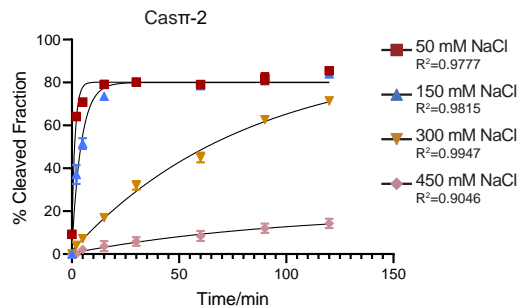
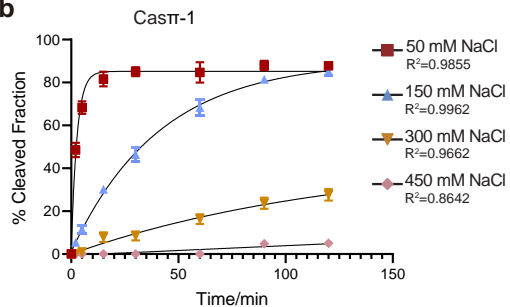
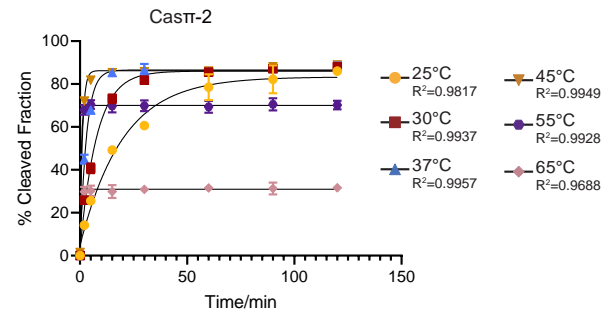
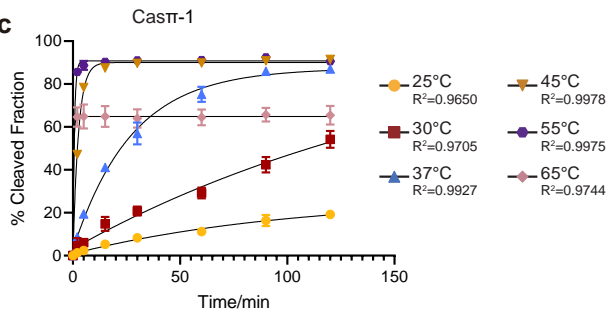


**a****b****c**

**Fig. S3 Cleavage efficiency of Cas $\pi$  nuclease in various biochemical conditions.**

(a) The plot for cleavage efficiency based on three replicates of the dsDNA cleavage by Cas $\pi$  effectors using different divalent ions. NTS denotes the non-target strand DNA which is cy5-labeled at 5' end. Bottom, P means products (n=3 each; mean  $\pm$  SD). The rate constant k values for Cas $\pi$ -1 in Mg<sup>2+</sup>, Mn<sup>2+</sup>, Ca<sup>2+</sup> and Co<sup>2+</sup> are 0.03460, 0.3497, 0.0015 and 0.5170 (fraction/minute), respectively. The rate constant k values for Cas $\pi$ -2 in Mg<sup>2+</sup>, Mn<sup>2+</sup>, Ca<sup>2+</sup> and Co<sup>2+</sup> are 0.1833, 0.6899, 0.0015 and 0.2227 (fraction/minute), respectively.

(b) The plot for cleavage efficiency based on three replicates of the dsDNA cleavage by Cas $\pi$  effectors using different salt concentrations. (n=3 each; mean  $\pm$  SD). The rate constant k values for Cas $\pi$ -1 in 50mM NaCl, 150mM NaCl, 300mM NaCl and 450mM NaCl are 0.3730, 0.02434, 0.0079 and 4.717e<sup>-6</sup> (fraction/minute), respectively. The rate constant k values for Cas $\pi$ -2 in 50mM NaCl, 150mM NaCl, 300mM NaCl and 450mM NaCl are 0.6748, 0.2285, 0.01228 and 0.01027 (fraction/minute), respectively.

(c) The plot for cleavage efficiency based on three replicates of the dsDNA cleavage by Cas $\pi$  effectors in different temperatures. (n=3 each; mean  $\pm$  SD). The rate constant k values for Cas $\pi$ -1 in 25 °C, 30 °C, 37 °C, 45 °C, 55 °C and 65 °C are 0.00987, 0.005353, 0.03663, 0.3849, 1.426 and 2.781 (fraction/minute), respectively. The rate constant k values for Cas $\pi$ -2 in 25 °C, 30 °C, 37 °C, 45 °C, 55 °C and 65 °C are 0.05026, 0.1285, 0.3340, 0.8713, 1.754 and 1.640 (fraction/minute), respectively.