



**Fig. S5 Reconstitution of Cas $\pi$ -sgRNA-dsDNA R-loop complex.**

(a) Purification of deactivated Cas $\pi$ -1 (D537A, E643A) protein. The peak fractions were analyzed by SDS-PAGE.

(b) Plasmid cleavage by wild type Cas $\pi$ -1 (wtCas $\pi$ -1) and deactivated Cas $\pi$ -1(dCas $\pi$ -1). (SC, supercoiled plasmids; L, linearized plasmids; OC, open-circle plasmids).

(c) Reconstitution and purification of dCas $\pi$ -1-sgRNA-dsDNA R-loop complex by size-exclusion chromatography. The reconstituted complex was analyzed by SDS-PAGE (top) and urea-PAGE (bottom). The sample labeled with 5 from the peak fraction was used for cryo-EM study.