



Fig. S8 Local analysis of OBD, HNC and PCT domains in Cas π protein.

(a) Left, purification of three Cas π mutants (R390A, R392A and R390A/R392A). The SEC curves were aligned referring to the injection volume. Right, the peak fractions were analyzed by SDS-PAGE.

(b) Left, *in vitro* dsDNA cleavage by wild type Cas π (WT) and Cas π mutants revealed by denaturing PAGE. Right, the plot of dsDNA substrate cleavage efficiency by WT and mutants of Cas π (n=3 each; mean \pm SD).

(c) Structural comparison between Cas π HNC domain and the NTSB to Helical-I region of CasX.

(d) Comparison between Cas π PCT domain and CasX TSL domain. Both the primary and 3D location of each domain in the entire protein were presented.