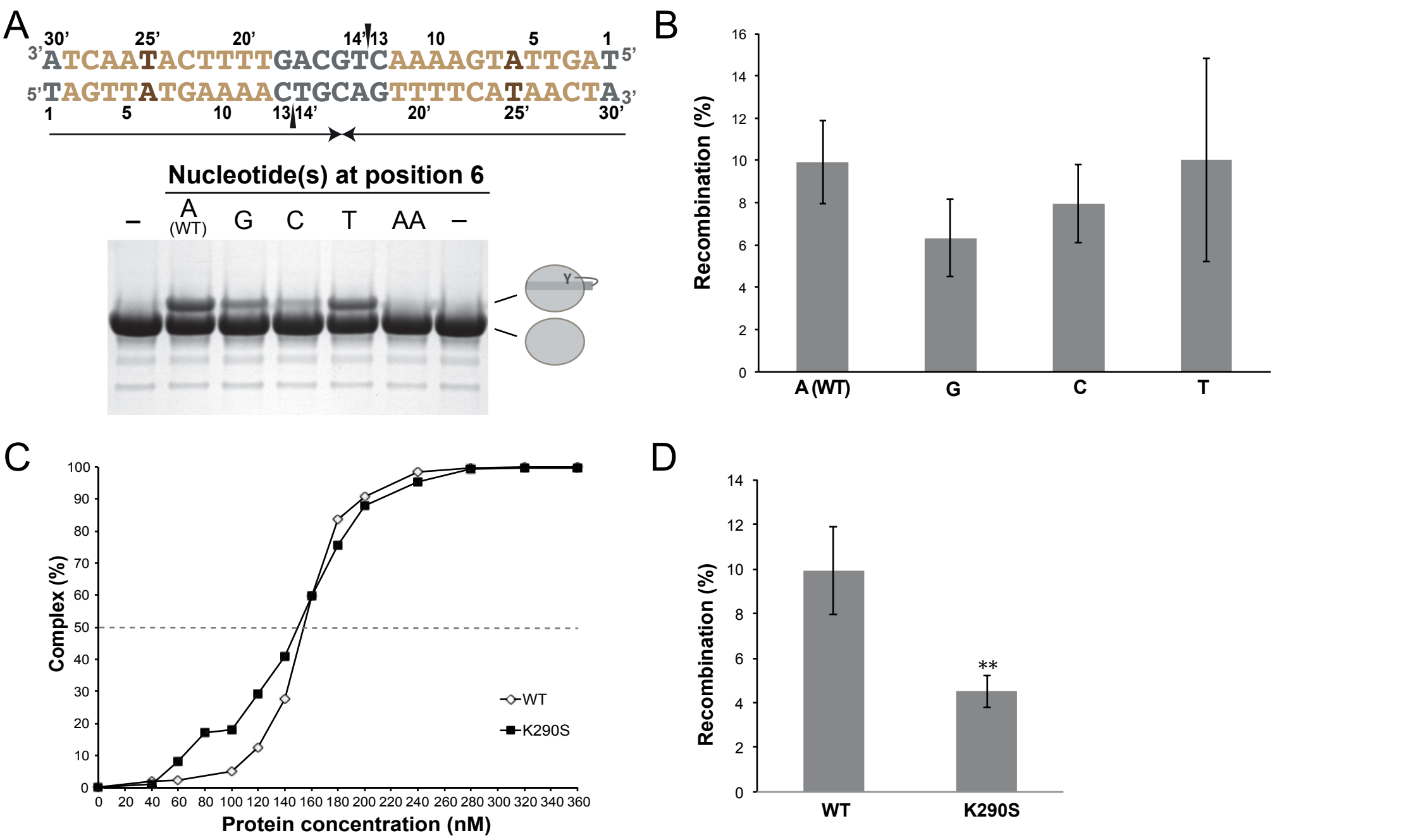

Figure 3–figure supplement 2: Functional characterization of the role of the specific features of the left *dif*$_H$ arm.

A) Cleavage assays with *dif*$_H$LP derivatives modified at the inserted base-pair (positions 6/25', brown). The figure illustrates the unmodified substrate (written in the 3' to 5' direction), nicked at the two positions marked by black wedges. Upon cleavage, XerH becomes covalently attached to DNA via a phosphotyrosyl bond, which is separated from unmodified XerH on SDS-PAGE (see also Figure 3C).

B) Mutations of the inserted base in the left arm do not affect XerH-mediated recombination in *E. coli*. *galK* reporter plasmids contained two wild-type *dif*$_H$ sites (A-WT) or two *dif*$_H$ sites with the left arm mutated at position 6/25' (A6 mutated to G, C, or T as indicated). Bars indicate standard deviation (n=3). None of the mutations showed significant reduction of recombination ($p > 0.05$; Student's test). Exact colony counts, recombination rates, and their statistical analysis are shown in Figure 3–source data 2.

C) K290S mutation does not affect *dif*$_H$ binding (WT: wild-type XerH control). Binding curves were quantified from EMSA (see numerical data in Figure 3–source data 1).

D) K290S mutation reduces XerH recombination in *E. coli* by 50% (WT: control assay with wild-type XerH). Reporter plasmids contained two wild-type *dif*$_H$ sites. Statistical analysis is as in (B). **$p < 0.05$ (Student's test). Colony counts, recombination rates, and statistical analysis are included in Figure 3–source data 2.