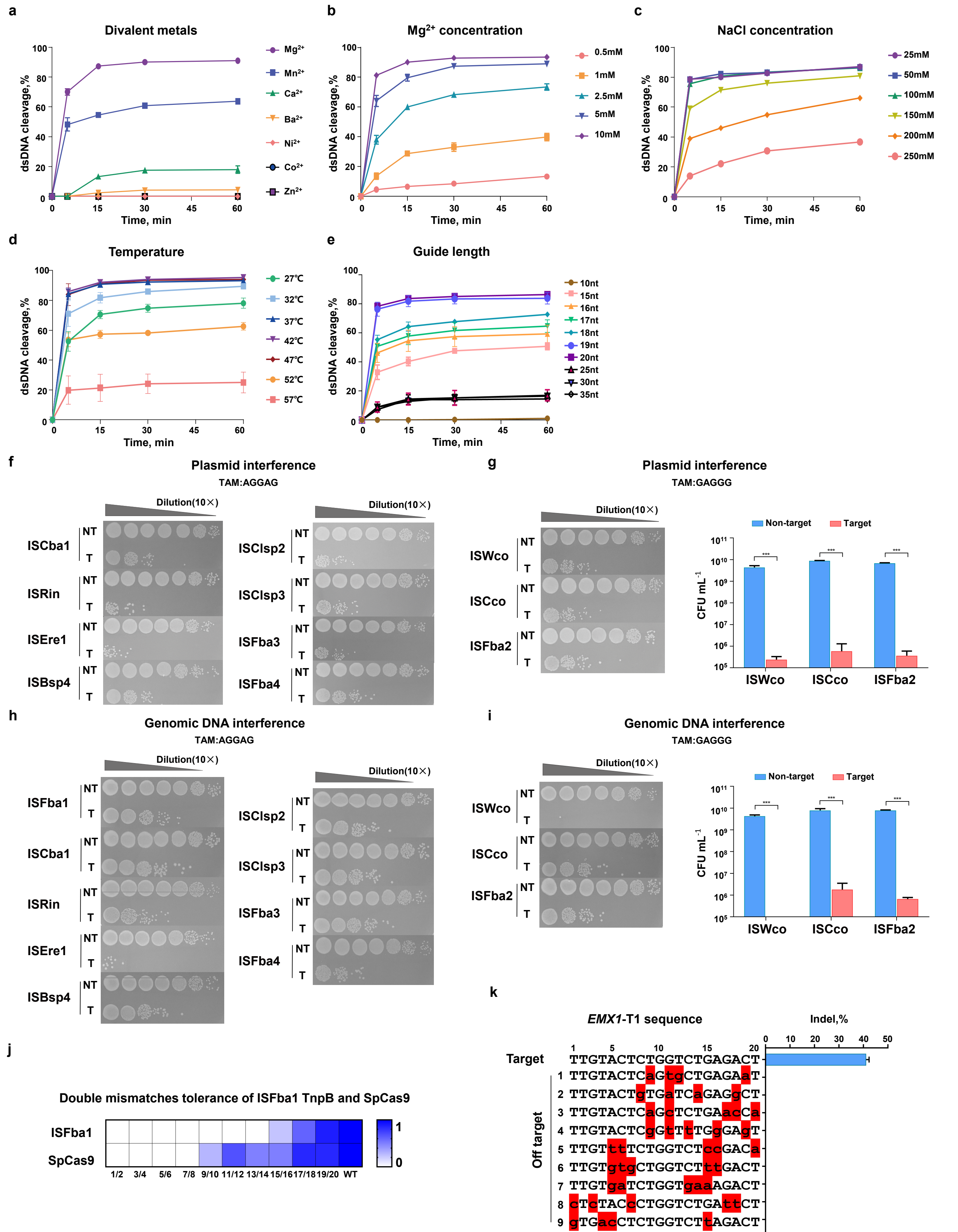




Supplementary information, Fig.S3: Biochemical features of ISFba1 TnpB-mediated cleavage, DNA interference activity of IS607 TnpB systems in *E. coli* and the specificity of ISFba1 TnpB in 293F.

Tests of different reaction conditions on the dsDNA cleavage activity of ISFba1 TnpB.

a Divalent metal types. **b** Mg^{2+} concentrations. **c** NaCl concentrations. **d** Temperatures. **e** Guide length. Data represent mean $\pm$ SD of three biological replicates.

Culture samples of IS607 TnpB-mediated plasmid interference using **f** 5'-AGGAG TAM and **g** 5'-GAGGG TAM (left panel) in *E. coli* were serially diluted ($10\times$) and selected by the media supplemented with Kan and Spe.

g Quantification of the plasmid interference assay mediated by ISWco, ISCco and ISFba2 TnpB using 5'-GAGGG TAM (right panel). Data represent mean $\pm$ SD of three biological replicates. Two-tailed unpaired t-test: *** $P < 0.001$.

The culture samples of genomic DNA interference assay mediated by IS607 TnpB family proteins using **h** 5'-AGGAG TAM and **i**, 5'-GAGGG TAM (left panel) in *E. coli* were serially diluted ($10\times$) and selected.

i Quantification of the genomic DNA interference assay mediated by ISWco, ISCco and ISFba2 TnpB using 5'-GAGGG TAM (right panel). NT, non-target control group. T, target treated group. Data represent mean $\pm$ SD of three biological replicates. Two-tailed unpaired t-test: *** $P < 0.001$.

j Heatmap of the double mismatch tolerance of ISFba1 and SpCas9 on the *EMX1* target sites. 1-20, mismatch position 1-20. WT, original guide sequence. Data shown are representative of three independent experiments.

k ISFba1 TnpB-mediated indel frequencies at predicted genomic off-target loci. Mismatched bases were highlighted by the red squares. Data shown are representative of three independent experiments. The guide sequences are provided in Supplementary information, Table S5.