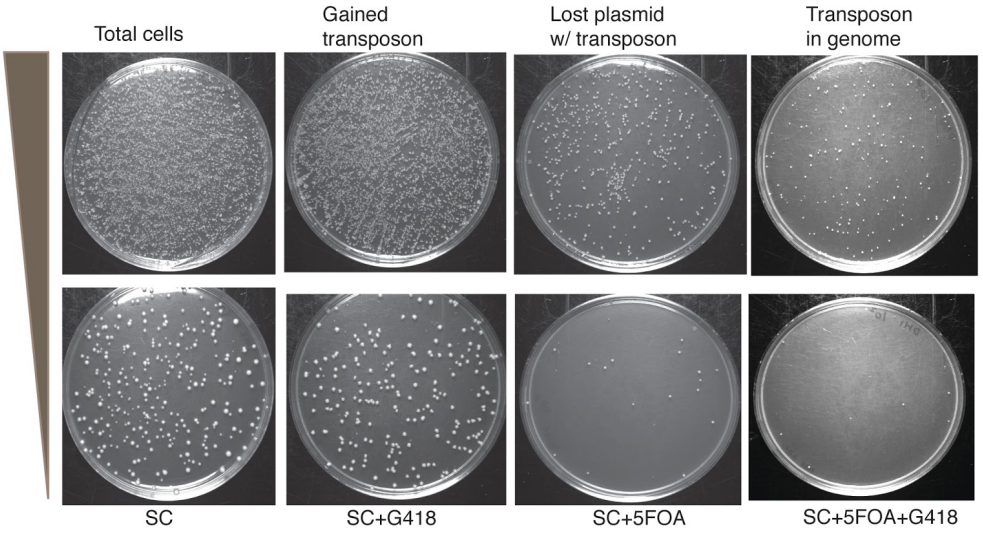

Figure S1: Transposition efficiency for a PiggyBac transposon-based calling card method. Transposition efficiency is calculated as the number of cells that survive the selection medium (SC+5FOA+G418) divided by the total number of cells plated on rich medium(SC). Two dilutions are shown.

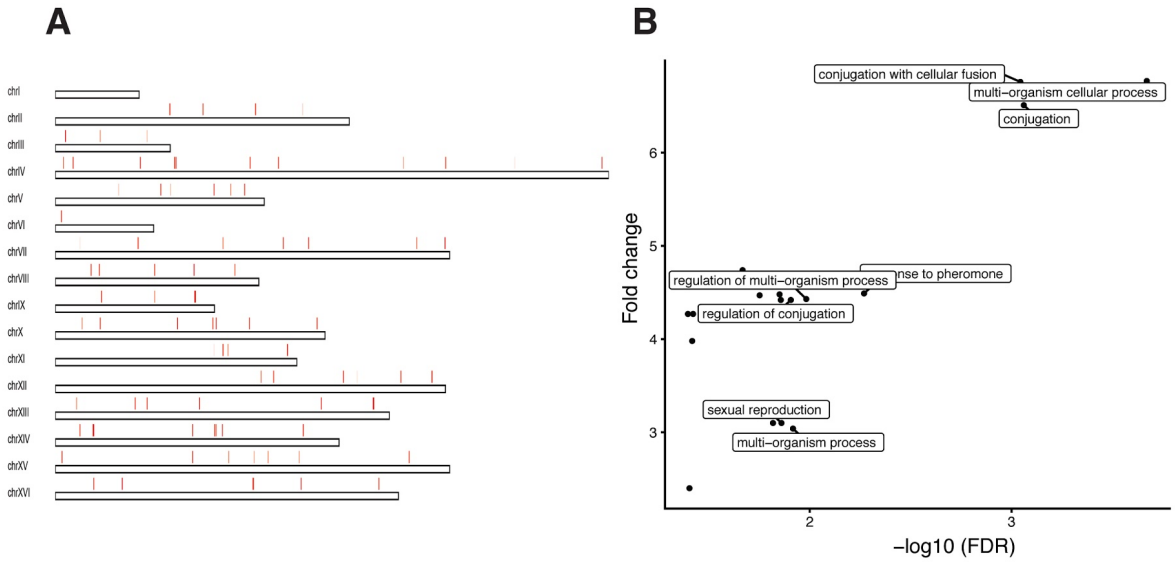

Figure S2: Overview and quality control for the insertion peaks detected in wild type Ste12 (A) The genome-wide PiggyBac insertion patterns of wild type Ste12 is shown along the 16 *S. cerevisiae* chromosomes. (B) Biological processes enriched through Gene Ontology (GO) annotations for genes assigned by insertion peaks.

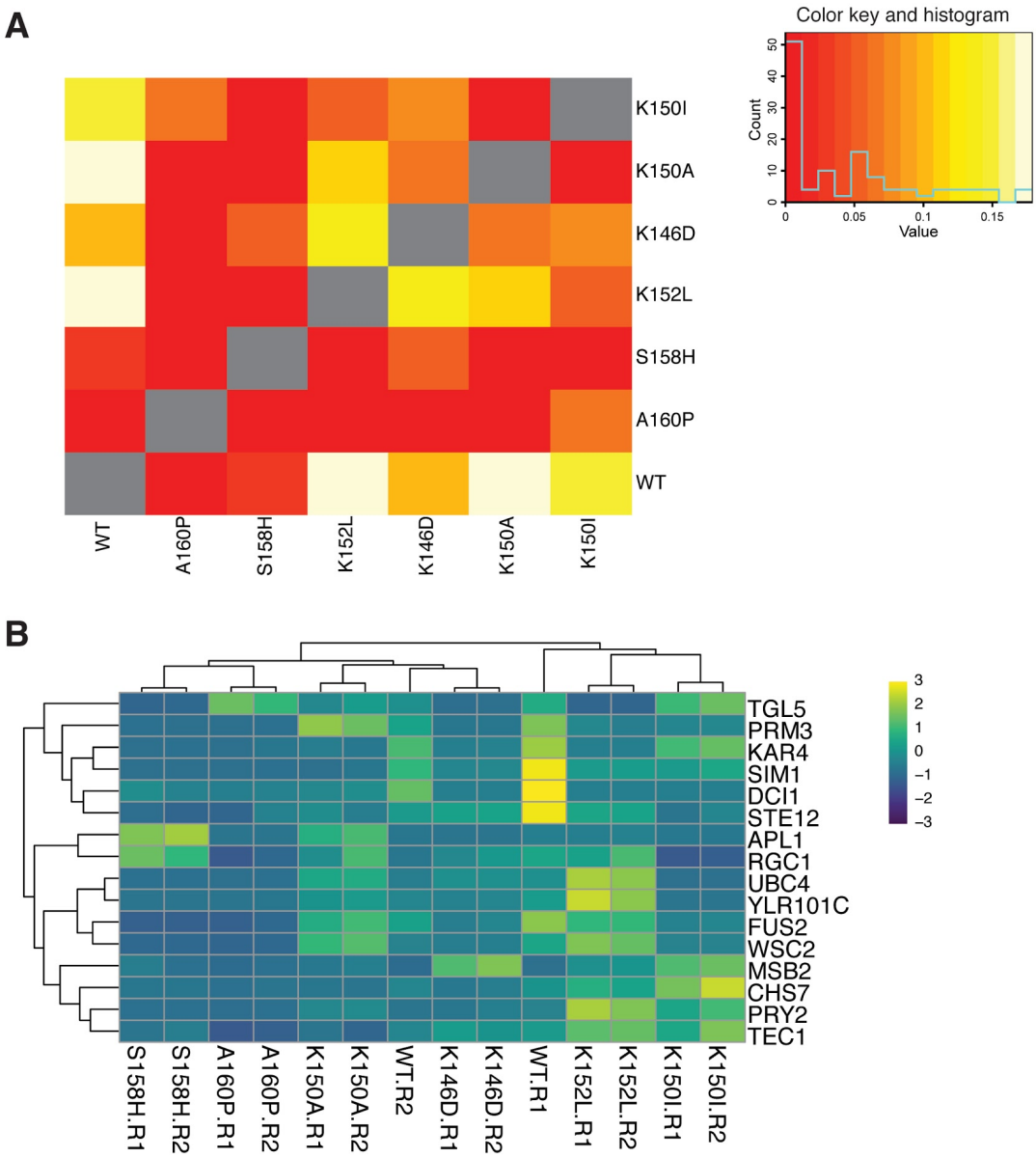

Figure S3: Overview of overlapping binding sites and genes among all variants (A) A heatmap showing proportions of overlapping binding sites among variants. The overlap proportion represents the similarity between binding profiles between pairs of variants; low

overlap proportion values are shown in red (0-5% overlapping sites) while higher overlap proportion values are shown in yellow (10-20%). Gray color indicates no data. (B) A heatmap showing genes that overlapped among variants and that are nearby sites of calling card insertions.

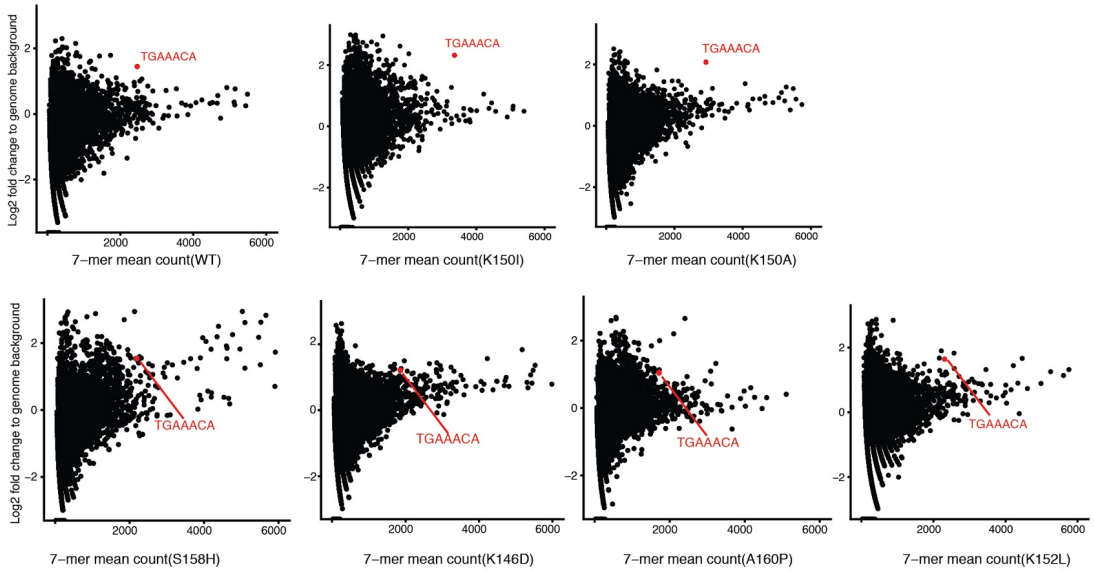

Figure S4: 7-mer analysis of the region 100 bp around each insertion peak for wild type Ste12 and six variants. Each point represents a unique 7-mer sequence; the x-axis shows the total count of each 7-mer while the y-axis shows the relative enrichment of each 7-mer over the genome background. The canonical Ste12 binding site TGAAACA is indicated in red in each plot.

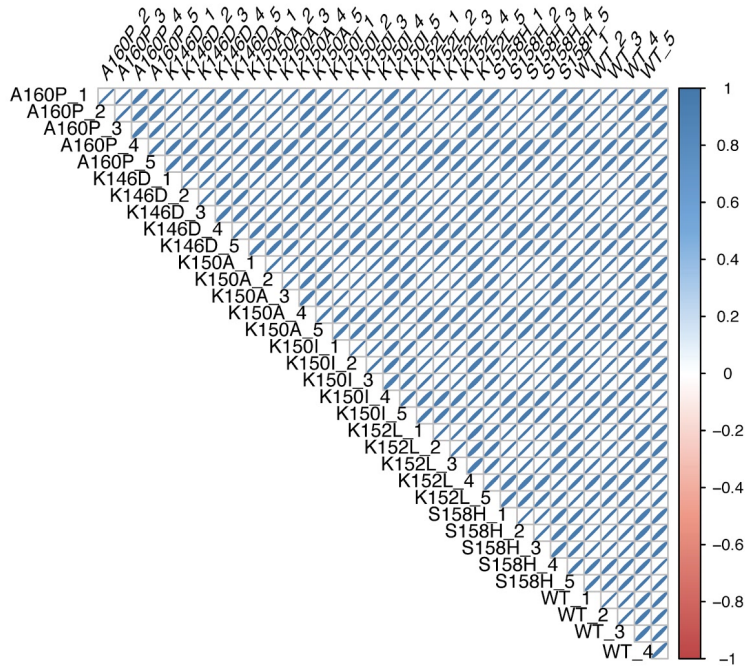

Figure S5: Correlation plot showing the Pearson's correlation (r) of the transcriptome across all replicates and variants in the RNA-seq experiment. High correlations are shown as blue and low correlations as red.

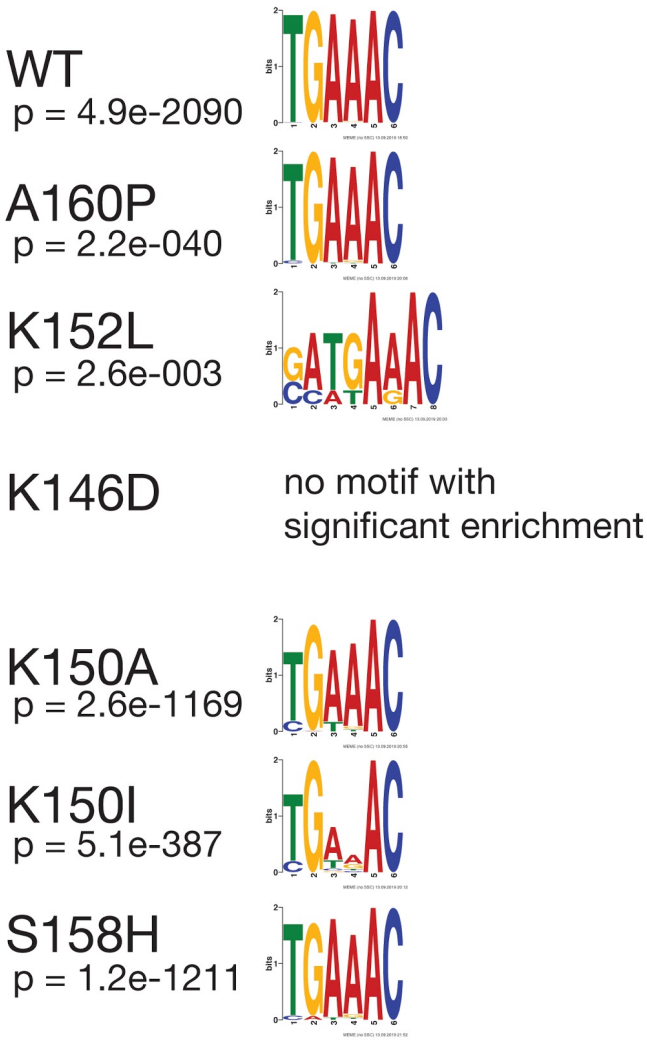

Figure S6: Motif Logos shows highly enriched 10mers sequence for the wild type Ste12 and each variant. (P value is generated by the MEME suite.)