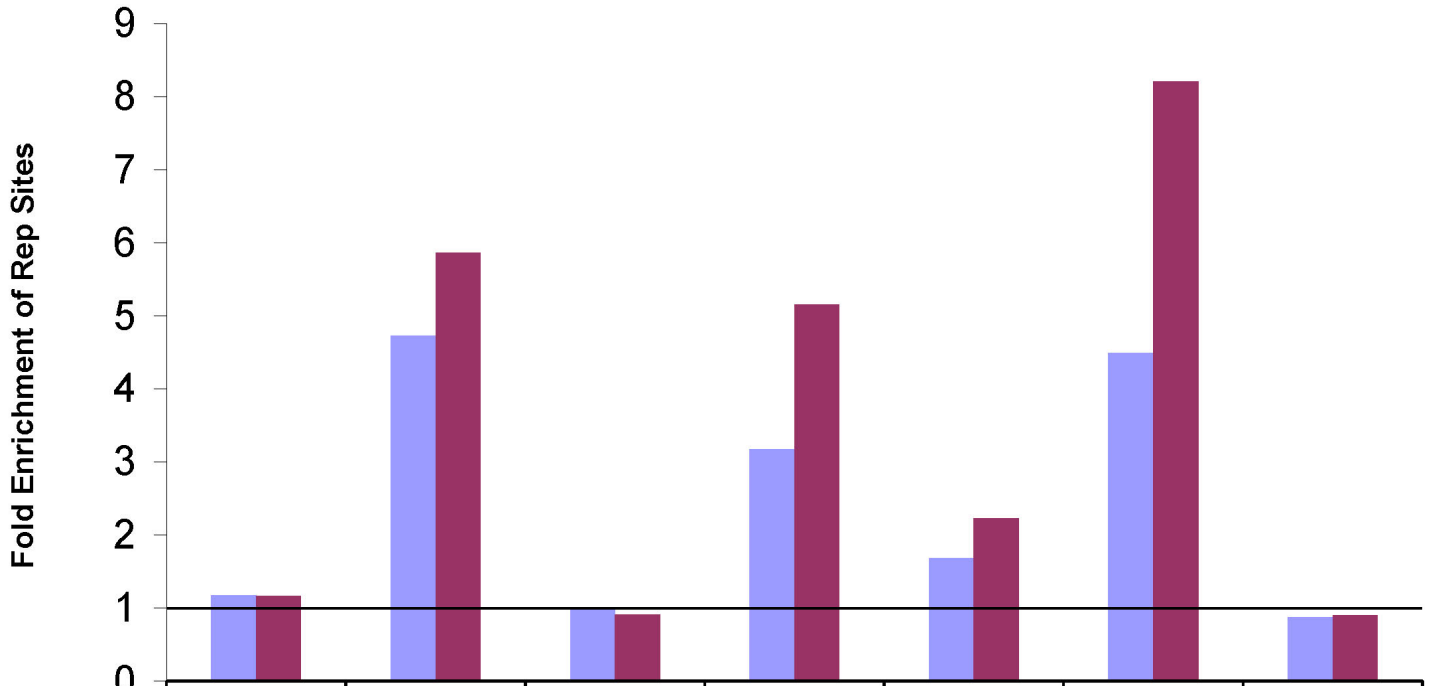
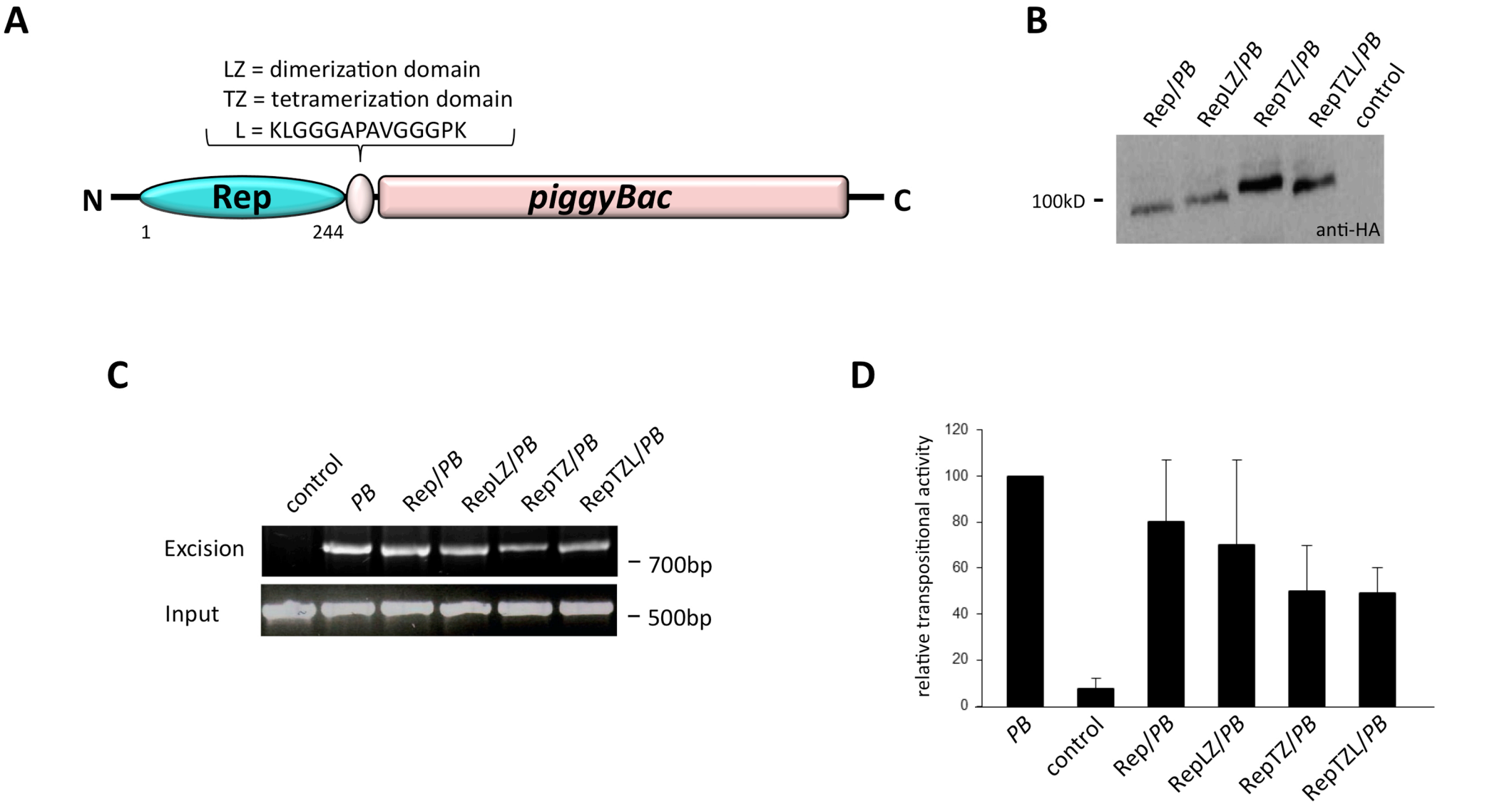
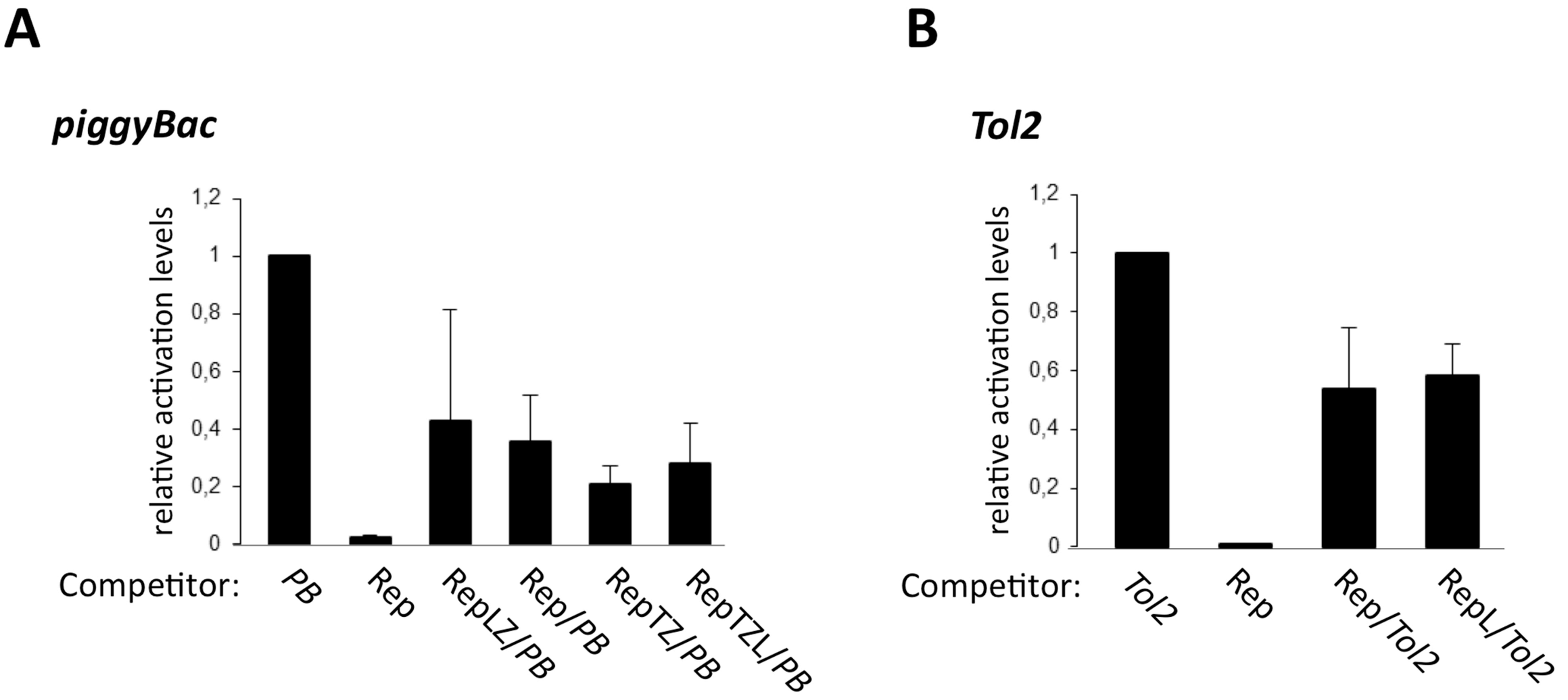


	Gene	Exon	Intron	TSS ± 5kb	H3K4me1	H3K4me3	H3K27me3
GAGC GAGC, 0 errors	1.18	4.73	0.98	3.17	1.69	4.49	0.87
GAGC GAGC GAGC GAGC, 2 errors	1.16	5.87	0.91	5.16	2.23	8.21	0.90

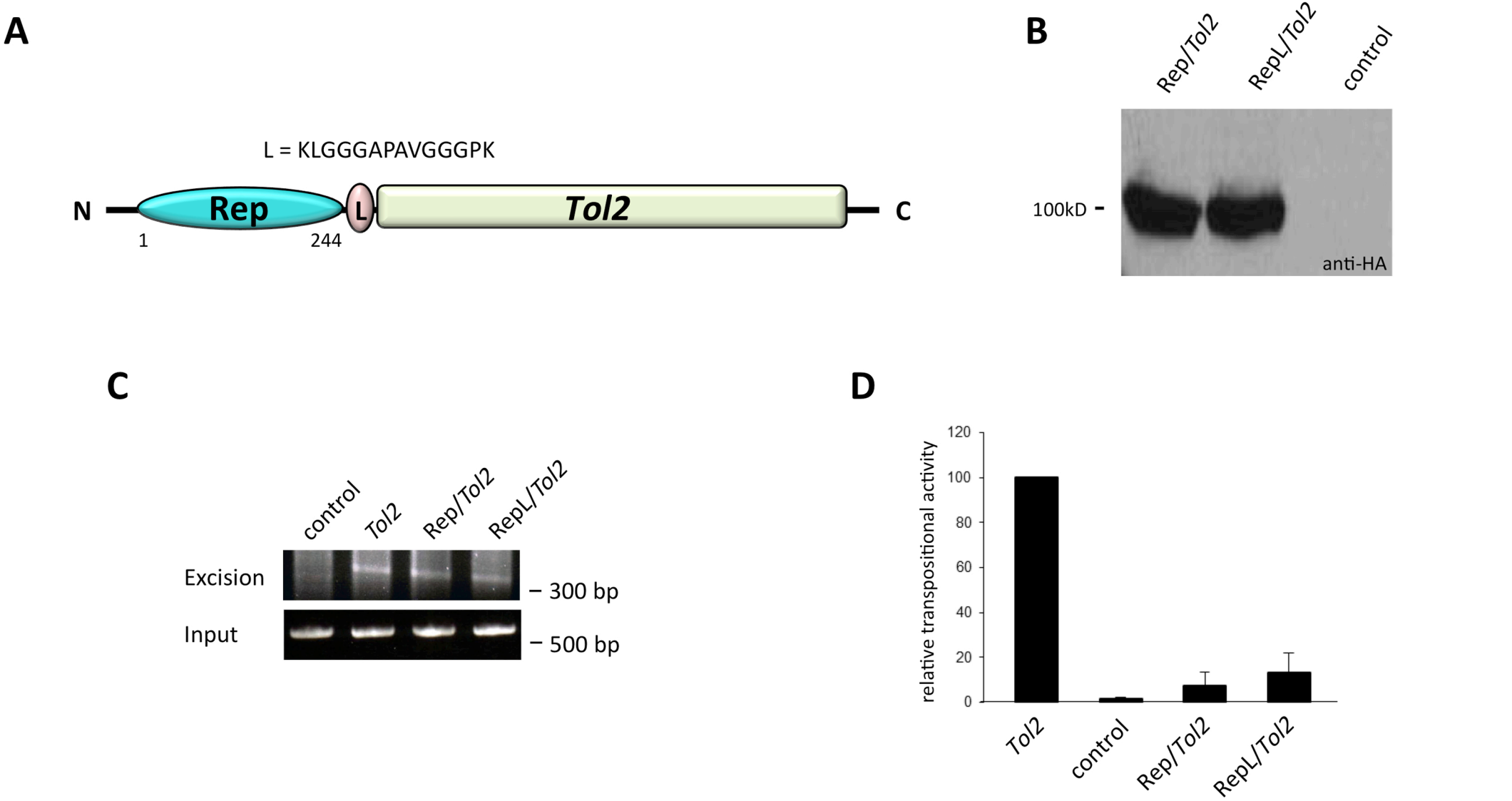
Supplementary Figure 1. Enrichment of RRS sites in different genomic features as compared to random sites. RRS sites were mapped with respect to frequencies within genes, exons, introns, in 5-kb windows surrounding transcription start sites (TSS ± 5-kb) and chromosomal regions characterized by H3K4me1, H3K4me3 and H3K27me3 histone modifications. The values are presented as fold-change as compared to random sample, which has a value of 1 and is marked by a horizontal line. The table below the diagram lists the actual values of fold-change compared to random control.



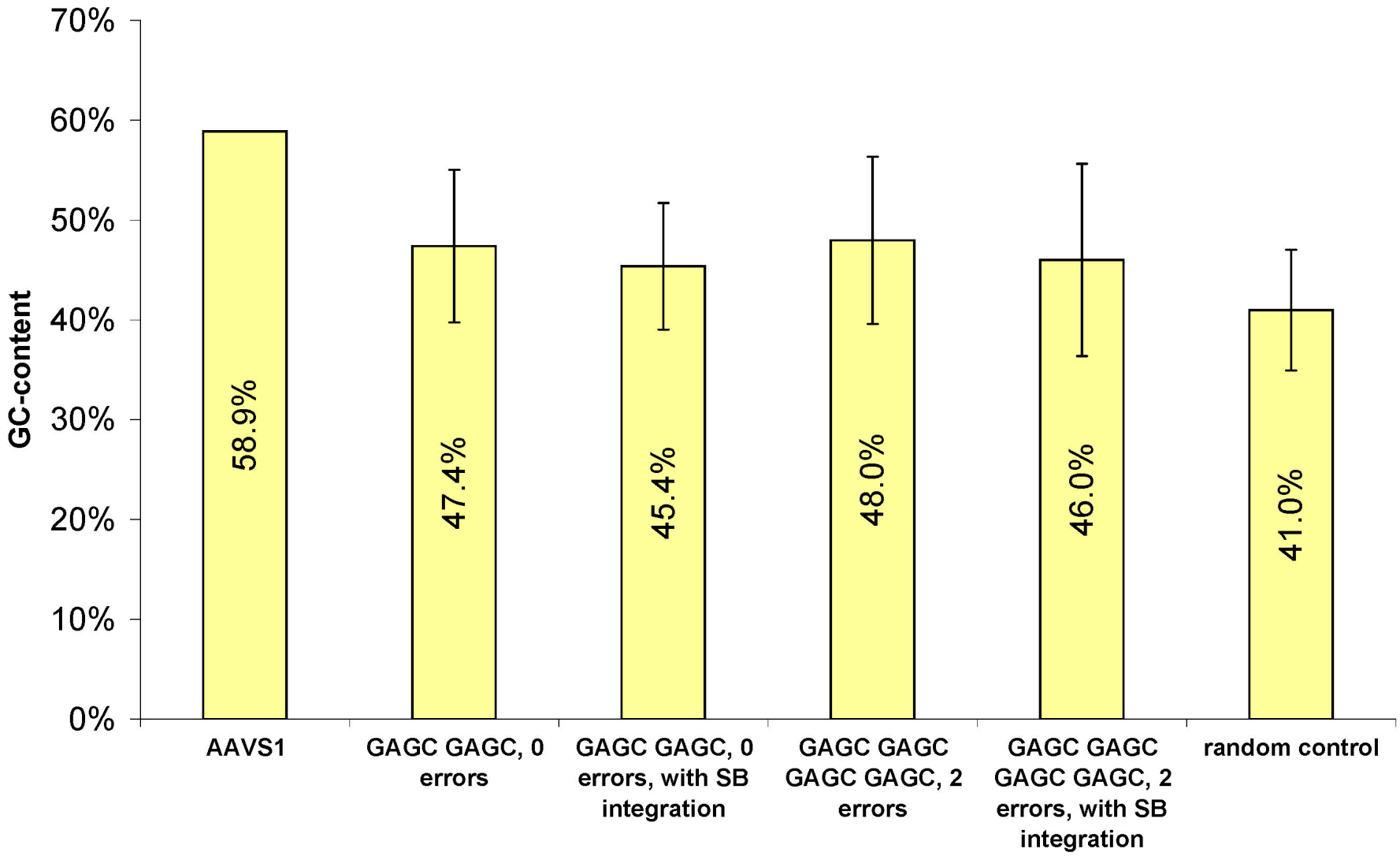
Supplementary Figure 2. Design, expression and activities of chimeric Rep-*PB* transposases in human cells. (A) Schematic overview of Rep/*PB* chimeras. Each construct contains the Rep DNA-binding domain fused to the *PB* transposase. The purple oval denotes either a flexible linker (KLGGGAPAVGGGPK), a GCN4 wild-type (LZ) or modified leucine zipper (TZ) domain. **(B)** Western blot analysis of Rep/*PB* fusion expression. Transfected HeLa cells were harvested 48h post-transfection, lysed and subjected to immunoblot analysis using an antibody against the hemagglutinin epitope (anti-HA). **(C)** Excision activity of chimeric Rep/*PB* transposases. Assay was done as described in **Fig. 3C**. The upper gel picture shows the amplification of a 750-bp footprint product diagnostic of transposon excision. The lower gel photograph shows PCR amplification products of a segment of the neo-marked transposon plasmids that served as input control. The positions of the molecular size markers are indicated on the right. **(D)** *PB* transposition assay. Rep-*PB* fusion transposase were tested for their transpositional activity in HeLa cells. The assay was done as described in **Fig. 3D**.



Supplementary Figure 3. DNA-binding activities of Rep/*PB* and Rep/*Tol2* transposase fusion proteins. Competition assay to monitor the DNA-binding activities of full-length Rep/*PB* **(A)** and Rep/*Tol2* **(B)** fusion proteins within in human HeLa cells. The assay was done as described in **Fig. 4**.



Supplementary Figure 4. Design, expression and activity of chimeric Rep/*Tol2* transposases in human cells. (A) Schematic overview of Rep/*Tol2* chimeras. Each construct contains the Rep DNA-binding domain fused either directly or together with a flexible linker domain (KLGGGAPAVGGGPK) to the *Tol2* transposase. **(B)** Western blot analysis of Rep/*Tol2* fusion expression. Transfected HeLa cells were harvested 48h post-transfection, lysed and subjected to immunoblot analysis using an antibody against the hemagglutinin epitope (anti-HA). **(C)** Excision activity of chimeric Rep-*Tol2* transposases. Assay was done as described in **Fig. 3C.** The upper gel picture shows the amplification of a 300-bp footprint product diagnostic of transposon excision. The lower gel photograph shows PCR amplification products of a segment of the neo-marked transposon plasmids that served as input control. The positions of the molecular size markers are indicated on the right. **(D)** *Tol2* transposition assay. Rep-*Tol2* fusion transposase were tested for their transpositional activity in HeLa cells. The assay was done as described in **Fig. 3D**.



Supplementary Figure 5. GC-content around RSS sites in the human genome. Average GC-content ± standard deviation in 5-kb windows centered around (1) RSS in *AAVS1*, (2) all "GAGC GAGC" sites in the genome, (3) "GAGC GAGC" sites with Rep/*SB* +pTneo integration within the 5-kb window, (4) all "GAGC GAGC GAGC GAGC" sites with up to 2 mismatches in the genome, (4) "GAGC GAGC GAGC GAGC" sites with up to 2 mismatches and Rep/N57+pTneoDR3 integration within the 5-kb window, and (5) random control sites.

SUPPLEMENTARY METHODS

Primer Name	Sequence (5'-3'), caps stand for barcodes
LAM-SB/L-50/Bio	Biotin-agttttaatgactccaacttaagtg
LAM-PB-R53-Bio	Biotin-agcaatatttcaagaatgcatgcgt
LAM-Tol2-L79-Bio	Biotin-tcaagtaaagtaaaaatccccaaaa
LAM-SB/L-20hmr	acttaagtgtatgtaaacttccgact
LAM-PB-R35	catgcgtcaattttacgcagacta
LAM-Tol2-L-48	cttaagtacagtaatcaagtaaaattac
SBIII-AAAA-OVH	acactctttccctacacgacgctcttccgatctAAAAgtaaacttccgacttcaactgta
SBIII-CCCA-OVH	acactctttccctacacgacgctcttccgatctCCCAgtaaacttccgacttcaactgta
SBIII-GGGA-OVH	acactctttccctacacgacgctcttccgatctGGGAgtaaacttccgacttcaactgta
SBIII-TTTA-OVH	acactctttccctacacgacgctcttccgatctTTTAgtaaacttccgacttcaactgta
SBIII-GCAG-OVH	acactctttccctacacgacgctcttccgatctGCAGgtaaacttccgacttcaactgta
SBIII-TACC-OVH	acactctttccctacacgacgctcttccgatctTACCgtaaacttccgacttcaactgta
SBIII-ATGC-OVH	acactctttccctacacgacgctcttccgatctATGCgtaaacttccgacttcaactgta
SBIII-CGTC-OVH	acactctttccctacacgacgctcttccgatctCGTCgtaaacttccgacttcaactgta
Tol2-III-AAAA-OVH	acactctttccctacacgacgctcttccgatctAAAAactcaagtactttacacctctg
Tol2-III-CCCA-OVH	acactctttccctacacgacgctcttccgatctCCCAactcaagtactttacacctctg
Tol2-III-GGGA-OVH	acactctttccctacacgacgctcttccgatctGGGAactcaagtactttacacctctg
PB-III-AAAA-OVH	acactctttccctacacgacgctcttccgatctAAAAcgcagactatctttctagggttaa
PB-III-CCCA-OVH	acactctttccctacacgacgctcttccgatctCCCAcgcagactatctttctagggttaa
PB-III-GGGA-OVH	acactctttccctacacgacgctcttccgatctGGGAcgcagactatctttctagggttaa
PB-III-TTTA-OVH	acactctttccctacacgacgctcttccgatctTTTAcgcagactatctttctagggttaa
Illumina-Primer1	aatgatacggcgaccaccgagatctacactctttccctacacgacgctcttccgatct*
Illumina-Primer2	caagcagaagacggcatacgagctcttccgatct*
Sonic TA Link (+)	gtaatacgactcactatagggctccgcttaagggaccatacgagctcttccgatct
Sonic Link (-) amino	gatcggaagagctcgtatg-Amino
Linker-Primer	gtaatacgactcactatagggc
Nested-Primer	agggctccgcttaagggac