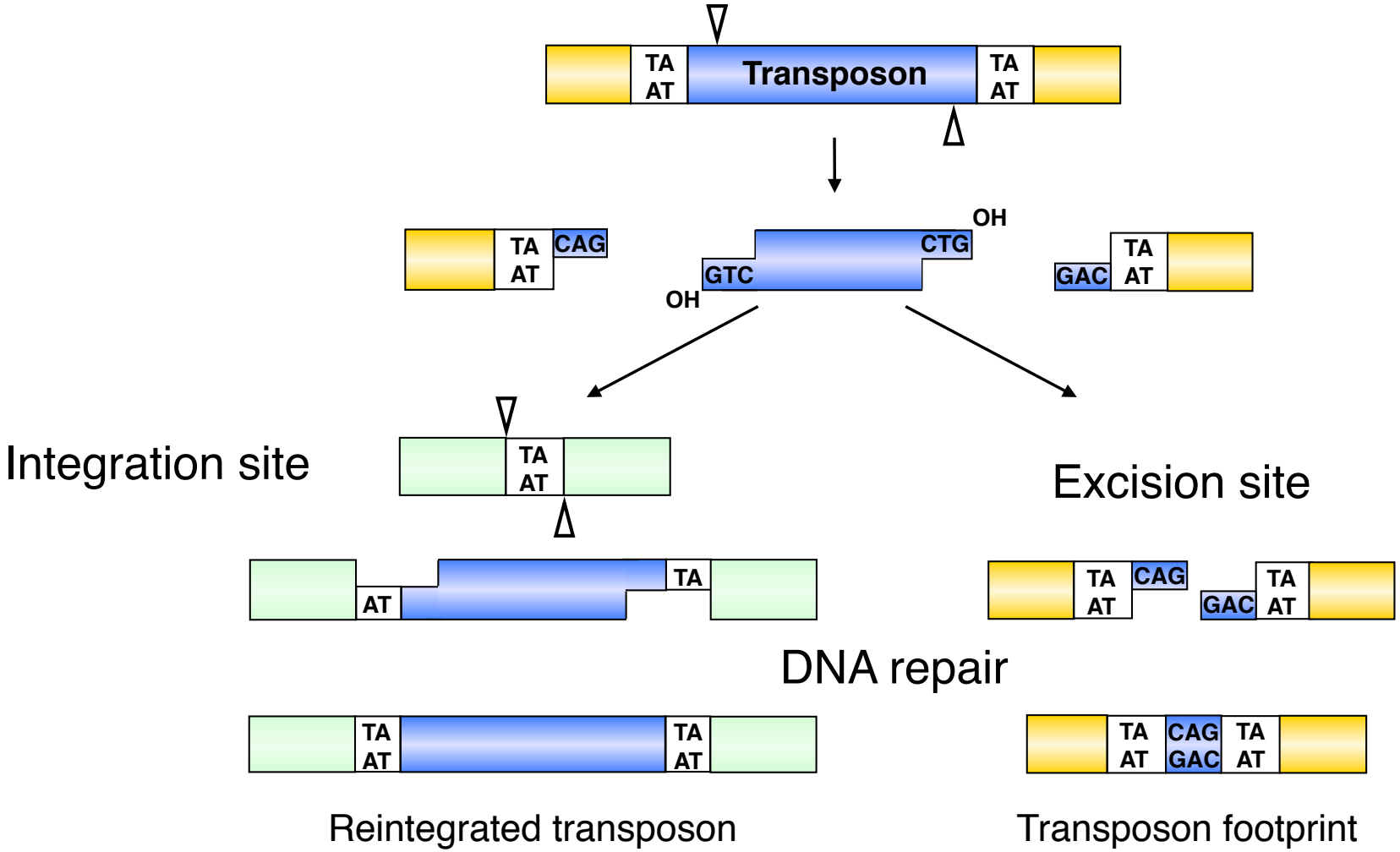
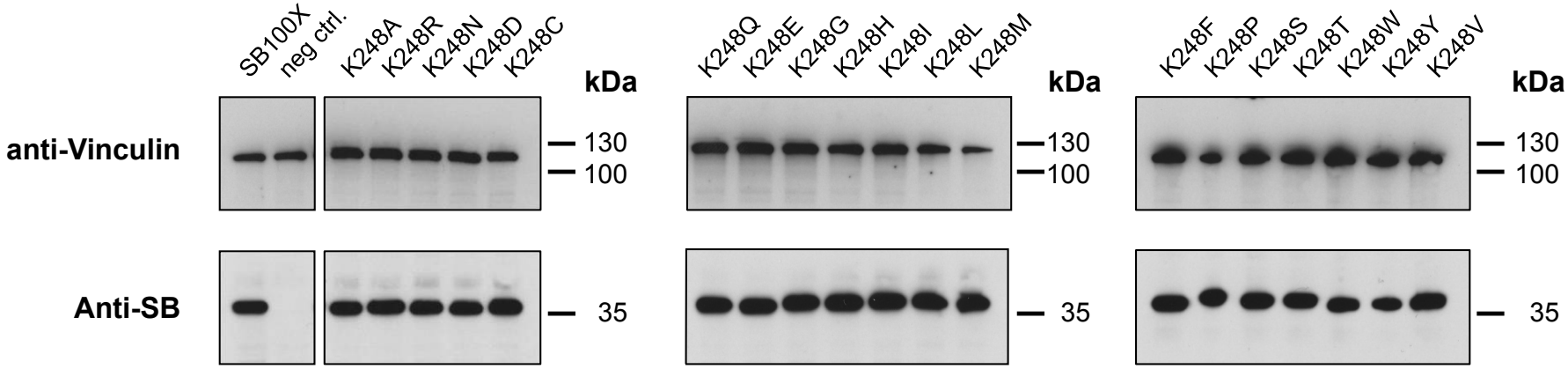
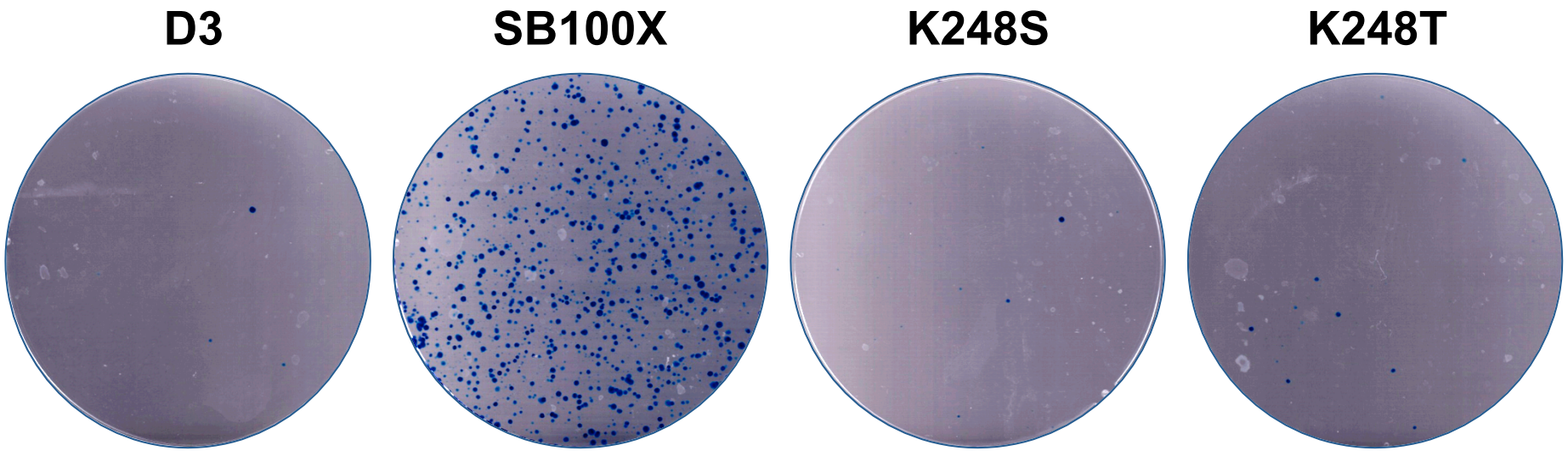


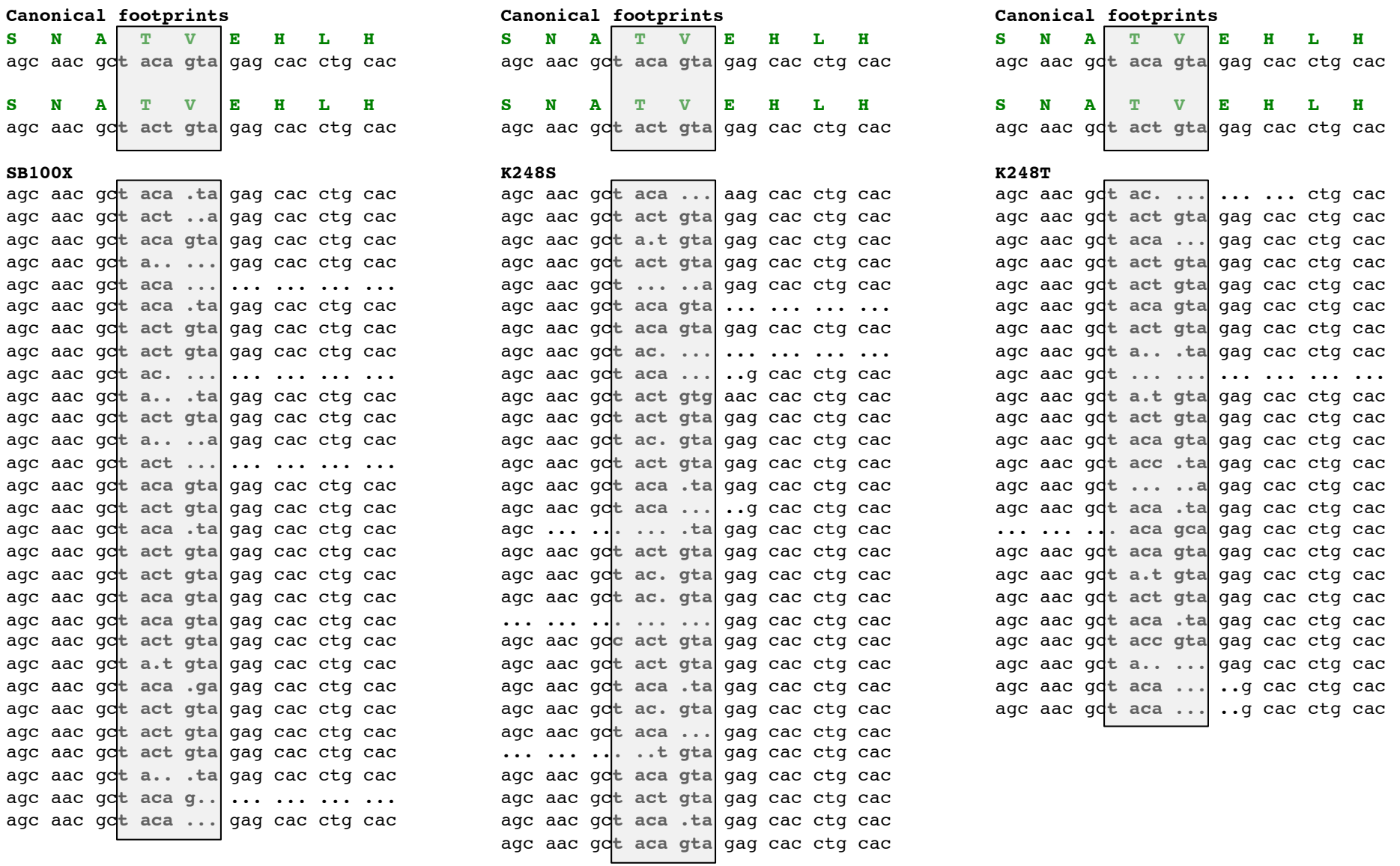
Supplementary Figure S1. Molecular events during cut-and-paste transposition. The model depicts the molecular events involved in Tc1/*mariner* transposition. The transposase initiates the excision of the transposon with staggered cuts and reintegrates it at a TA target dinucleotide. The single-stranded gaps at the integration site as well as the double-strand DNA breaks in the donor DNA are repaired by the host DNA repair machinery. After repair, the target TA is duplicated at the integration site, and a small footprint is left behind at the site of excision. The footprint is generated by the NHEJ pathway of DSB repair.



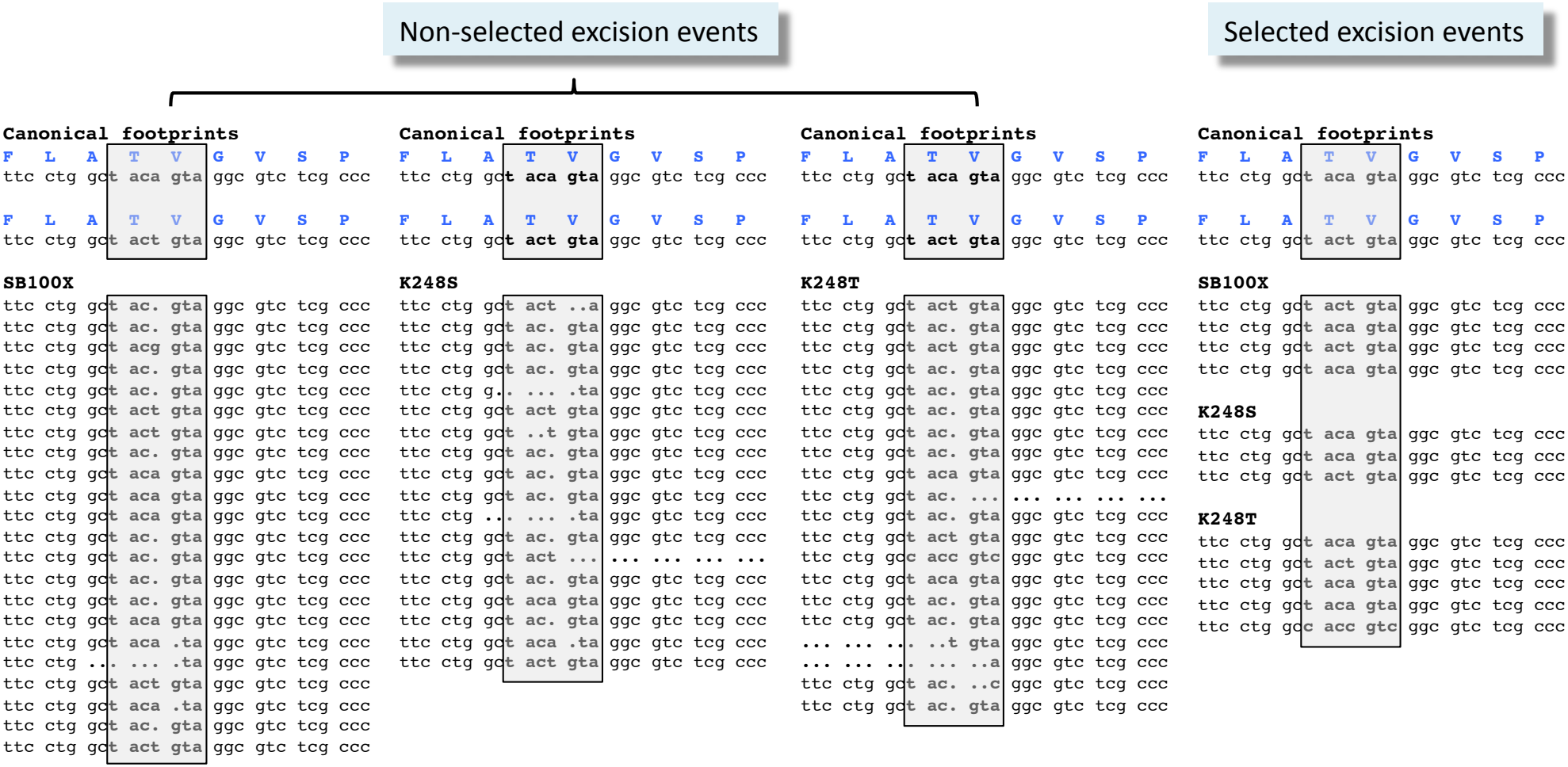
Supplementary Figure S2. Western blot analysis of single amino acid replacement mutants derived from the SB100X transposase protein detected with a polyclonal antibody against the SB transposase (anti-SB). Anti-Vinculin served as loading control. The positions of molecular size markers are indicated on the right of each panel. The two boxes on the left show separate lanes from the same blot.



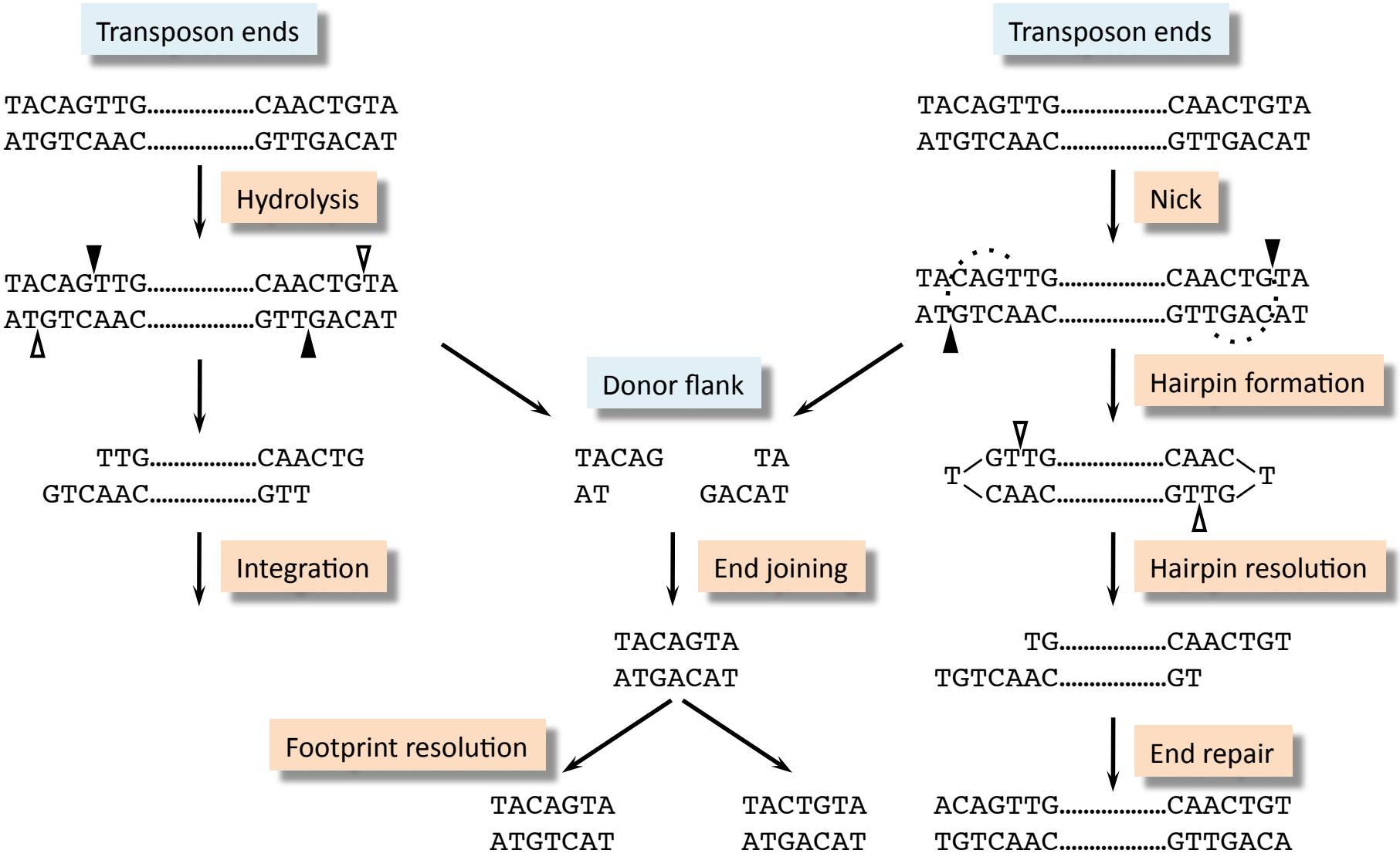
Supplementary Figure S3. Relative transposition activities of the K248S and K248T mutants. HeLa cells were transiently cotransfected with a transposon donor plasmid (pT2Bpuro) and plasmids expressing the mutants, inactive SB transposase (D3, negative control) or SB100X (positive control). Cells were selected for puromycin resistance and stained with methylene blue to identify viable cell colonies.



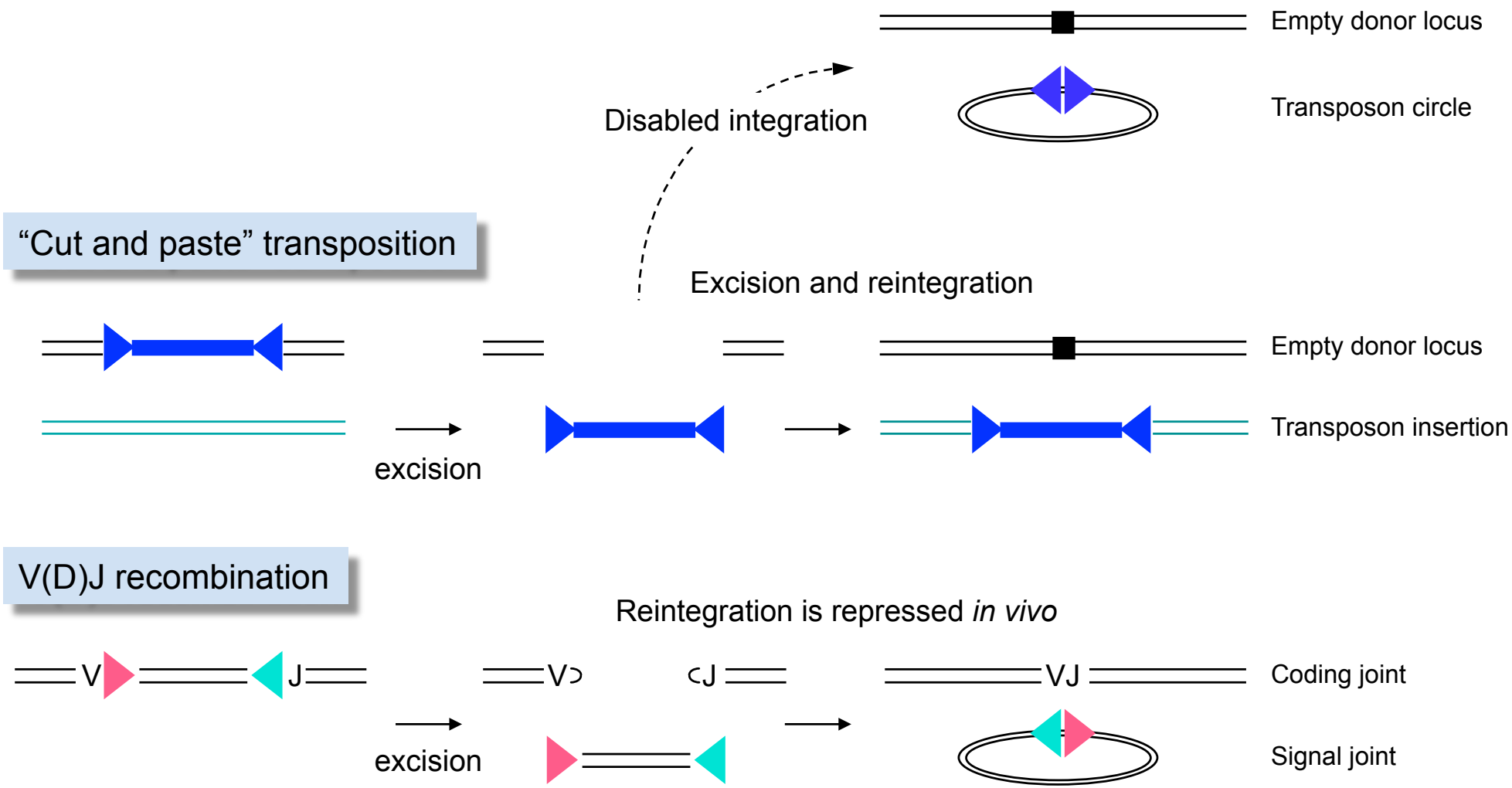
Supplementary Figure S4. Transposon footprint analysis following excision from plasmids. SB transposon excision followed by direct rejoining of DNA ends restores the open reading frame of GFP, thereby producing a scorable report. A segment of GFP amino acid sequence directly flanking the SB transposon is displayed in green capital letters. The two canonical SB footprints, TACAGTA and TACTGTA, reconstitute ACA and ACT codons encoding threonine, and thus both preserve authentic GFP sequence. The experimental footprints were recovered from transiently transfected HeLa cells.



Supplementary Figure S5. Transposon footprint analysis following excision from the genome. SB transposon excision followed by direct rejoining of DNA ends restores the open reading frame of puro, thereby producing a scorable report. A segment of puro amino acid sequence directly flanking the SB transposon is displayed in blue capital letters. The two canonical SB footprints, TACAGTA and TACTGTA, reconstitute ACA and ACT codons encoding threonine, and thus both preserve authentic puro sequence. The experimental footprints were recovered either from transgenic human HepG2 cells containing a single genomic donor element without applying puro selection (non-selected excision events) or from transgenic human HeLa cells containing a single genomic donor element following puro selection (selected excision events).



Supplementary Figure S6. Pathways of SB transposition. Canonical SB transposition catalyzed by wild-type SB transposase (left) is initiated by a hydrolysis reaction at the non-transferred strands (filled arrowheads), followed by a second hydrolysis at the transferred strands (empty arrowheads), thereby liberating the transposon from its donor. In an alternative mode of SB transposon excision by K248S and K248T (right), excision initiates with nicks at the transferred strands (filled arrowheads) of the transposon, exposing 3'-OHs. These 3'-OHs then attack the complementary strands 3 nt into the transposon DNA, thereby forming hairpins on the transposon. Transposon end hairpins are resolved by the transposase (second hydrolysis), generating 5-nt overhangs on the ends of the excised transposon that undergo end repair before circularization. Both canonical SB excision and the alternative pathway result in identical overhangs at the transposon donor site, which undergo end joining and the formation of footprints.



Supplementary Figure S7. Parallels between cut-and-paste DNA transposition and V(D)J recombination. During transposition, the transposon is excised from the donor DNA and integrates into the target DNA. During V(D)J recombination, excision readily occurs, but integration is severely impaired *in vivo*. Excised, unintegrated DNA molecules form extrachromosomal circles. The K248S and K248T SB transposase mutants do not support transposon integration and generate transposon circles in a V(D)J recombination-like process.

Supplementary Information

Oligonucleotide sequences. All sequences are specified in a 5' to 3' direction.

PUC2	GCGAAAGGGGGATGTGCTGCAAGG
PUC5	TCTTTCCTGCGTTATCCCCTGATTC
PUC3	CGATTAAGTTGGGTAACGCCAGGG
PUC4	GCTGGCACGACAGGTTTCCCG
SB_IRDR_R_FW	GCTGAAATGAATCATTCTCTCTACTATTATTCTGA
SB_IRDR_L_RV	GTGTCATGCACAAAGTAGATGTCCTA
Linker Primer	GTAATACGACTCACTATAGGGC
T-Bal Rev	GAATTGTGATACAGTGAATTATAAGTG
Nested Primer	AGGGCTCCGCTTAAGGGAC
T-Bal	CTTGTGTCATGCACAAAGTAGATGTCCTAACTGACT
GFP fw1	ACAACGGCGGCTACACCAAC
GFP fw2	TCGAGAAGTACGAGGACGGCGG
GFP rev1	GCTCGAGATCTGGCGAAGGC
GFP rev2	AGCTGCCCACCAGCACGTTA
puroFw1	ATCCGGACCGCCACATCG
puroRev0.5	TGCGGGTCATGCACCAGGT
puroFw1.5	GTCACCGAGCTGCAAGAAC
puroRev1.5	GAGCCGCTCGTAGAAGGG
pJet1.2Fw	AGCACAAAGAAACGGGCAAC
pJet1.2Rev	GGCGAACAAACTGCCTTCTG
K248A	P-CAATGACCCCgccCATACTTCCAAAGTTGTG
K248R	P-CAATGACCCCagaCATACTTCCAAAGTTG
K248N	P-CAATGACCCCaacCATACTTCCAAAG
K248D	P-CAATGACCCCgacCATACTTCCAAAGTTG
K248C	P-CAATGACCCCtgcCATACTTCCAAAGTTGTG
K248Q	P-CAATGACCCCcagCATACTTCCAAAG
K248E	P-CAATGACCCCgagCATACTTCCA
K248G	P-CAATGACCCCggcCATACTTCCAAAGTTGTG
K248H	P-CAATGACCCCcacCATACTTCCAAAGTTG
K248I	P-CAATGACCCCatcCATACTTCCAAAGTTG
K248L	P-CAATGACCCCctgCATACTTCCAAAG
K248M	P-CAATGACCCCatgCATACTTCCAAAG
K248F	P-CAATGACCCCttcCATACTTCCAAAGTTGTG
K248P	P-CAATGACCCCcccCATACTTCCAAAGTTGTG
K248S	P-CAATGACCCCagcCATACTTCCAAAGTTG
K248T	P-CAATGACCCCaccCATACTTCCAAAGTTGTG
K248W	P-CAATGACCCCtggCATACTTCCAAAG
K248Y	P-CAATGACCCCtacCATACTTCCAAAGTTG
K248V	P-CAATGACCCCgtgCATACTTCCAAAGTTG
Universal reverse primer for site-directed mutagenesis	P-TCGTGTTGGAAGACCCATTTG

Additional information for Figure 1a

Names and host species of the Tc1 transposons ("Mar" in the transposon names shown in the figure is an abbreviation of "Mariner", excluding SMAR19): SB, Sleeping Beauty - fish; Tdr1, Tc1-1_DR, Tc1-4_DR – *Danio rerio*, Tc1-1_GA and Tc1-2_GA - *Gasterosteus aculeatus*; Frog_Prince - *Rana pipiens*; Tc1-1_PM - *Petromyzon marinus* lamprey; Tc1-1_ACar - *Anolis carolinensis* lizard; Passport –*Pleuronectes platessa* fish (GenBank CAB51371); Mariner-16_SSa, Mariner-20_SSa and Tc1-2_SSa - *Salmo salar*; Tc1-7_Xt and Tc1-10Xt - *Xenopus tropicalis*; Tc1-1_HVu – *Hydra vulgaris*, consensus from several 99% copies; Quetzal - *Anopheles albimanus*; Mariner-12_RPr - *Rhodnius prolixus* (bloodsucking bug); Mariner-7_LMi and Mariner-17_LMi - *Locusta migratoria*; Mariner-9_DBp and Mariner-10_DBp - *Drosophila bipectinata*; Tc1-1_DPs - *Drosophila pseudoobscura* (GenBank AAO25747); S, Bari-1, TC1_DM, TC1-2_DM - *Drosophila melanogaster*; Paris - *Drosophila virilis*; Tc1-1_FCa and Tc1-2_FCa - *Folsomia candida* hexapoda (GenBank OXA48087 and OXA45461); Mariner-15_CFl - *Camponotus floridanus* ant; AeTango2 and Mariner-3_AAe - *Aedes aegypti*; Tc1-1_RVa - *Ramazzottius varieornatus* tardigrade (GenBank GAV01586); SMAR19 - *Schmidtea mediterranea* planarian; Mariner-7_BM - *Bombyx mori*; Tc1-1_AG and Mariner2_AG – *Anopheles gambiae*; Mariner-10_Sin and Mariner-17_Sin - *Solenopsis invicta* ant; Tc1 - *Caenorhabditis elegans*; Mariner1_PPa, Mariner2_PPa, Mariner-3_PPa - *Physcomitrella patens* moss; Tc1-1_RDe and Tc1-2_RDe - *Rhizopus delemar RA 99-880* fungus; Mariner-1_SLL - *Serpula lacrymans* fungus; Tc1-1_EGl - *Exidia glandulosa HHB12029* fungus (KZW01351); Tc1-1_TCa - *Tilletia caries* fungus (OAJ23253); Tc1-1_COA - *Capsaspora owczarzaki ATCC 30864* protist (GenBank XP_011270969; Tc1_2_Tn - Tetraodon nigroviridis; Tc1_6_Ol - *Oryzias latipes*; Tc1-1_LNi - *Lasius niger* ant (KMQ82797), Tc1-1_SMi - *Stegodyphus mimosarum* spider (KFM73601); Minos - *Drosophila hydei*; Tc1-2_Gm - *Gadus morhua* cod; Tc1_4_On - *Oreochromis niloticus*; Mariner-24_EL - *Esox lucius* pike; Tc3 - *Caenorhabditis elegans*; Mariner-1_PI - *Phytophthora infestans*; Mariner-5_PH - *Parhyale hawaiensis* crustacea; Mariner-58_CCri and Mariner-92_CCri - *Chondrus crispus* red seaweed; Mariner-5_DF - *Drosophila ficusphila*;

Mariner-9_Del - *Drosophila elegans*; Mariner-6_DBi - *Drosophila biarmipes*; Impala - *Fusarium oxysporum* fungus; Mariner-1_AN - *Aspergillus nidulans* fungus; Mariner5_AO - *Aspergillus oryzae* fungus; Tc1-1_PCa - *Penicillium camemberti* fungus (GenBank CRL31137). *Mariner* transposons: Hsmar1 – *Homo sapiens*; Mos1 - *D. melanogaster*; Famar1 – *Forficula auricularia* earwig. Transposons sequences not accompanied by the GenBank accession numbers are collected in Repbase.