



**Figure S1** PCR scheme to verify the correct HDR event.

(A) Scheme for the generation of *salm*[1<sup>st</sup> exon-dsRed] by HDR. Possible results of “ends-out” and “ends-in” homologous recombination are shown, including the positions of the homology arms and the primers used for PCR. Note that only “ends-in” homologous recombination results in the pBS-backbone in the genome, which can be detected by PCR with primers T7 / XZ85. (B) PCR verification of the “ends-out” insertion of the dsRed-STOP cassette in the 1<sup>st</sup> intron of *salm*. Left and right arms amplify only from DNA isolated from the *salm*[1<sup>st</sup> exon-dsRed] flies. Primers XZ144 and XZ109 prime outside of the used homology arms and thus show that homologous recombination has occurred at the correct location. As T7 / XZ85 primers only amplify the correct fragment from the donor plasmid source but not from *salm*[1<sup>st</sup> exon-dsRed] genomic DNA the insertion occurred by “ends-out” homologous recombination.