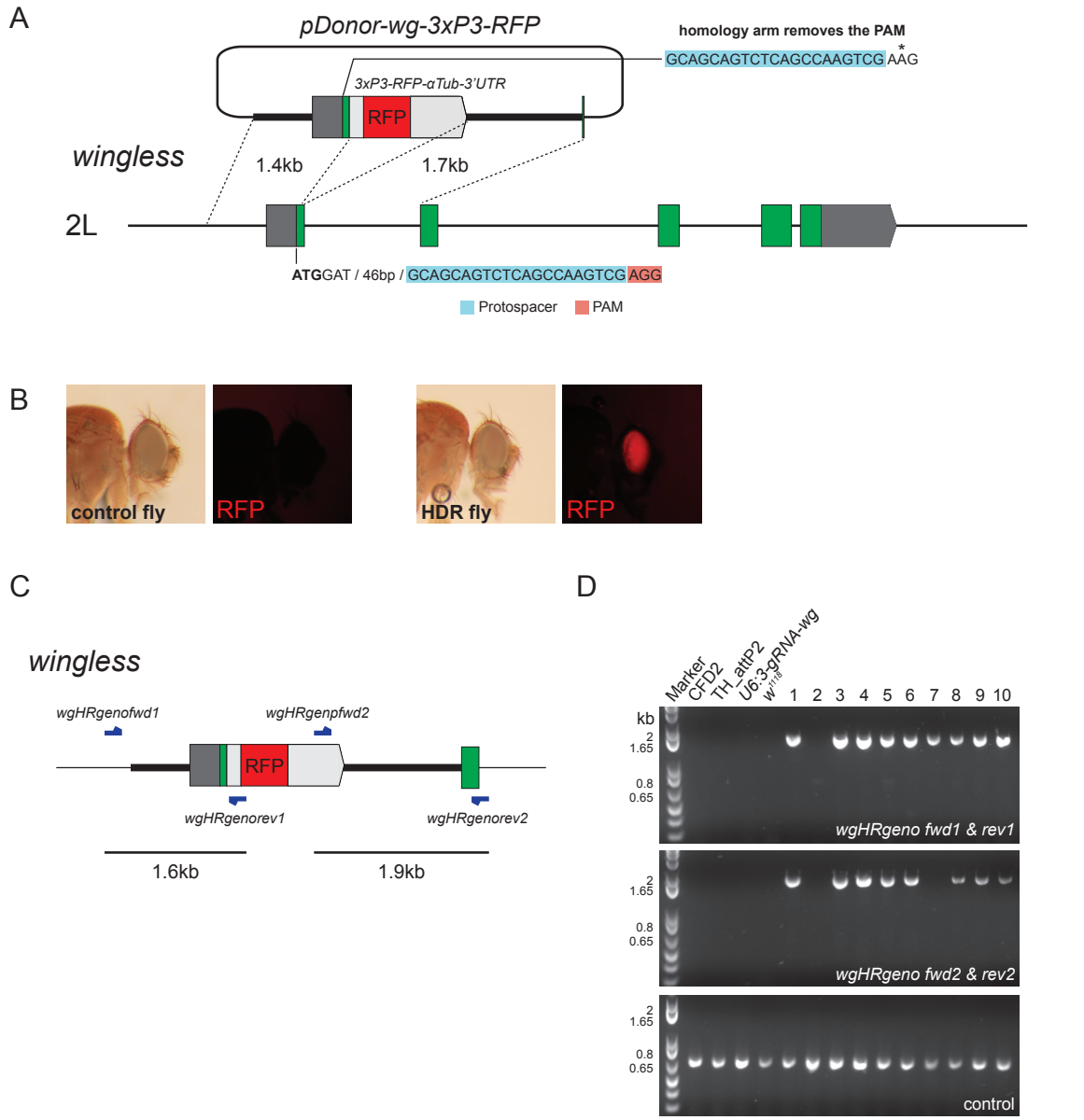

Figure S2. Targeted donor integration by HDR. (A) Schematic of the RFP donor vector in relation to the *wg* locus on chromosome arm 2L. The donor plasmid is based on a previous plasmid (Port *et al*., 2014), which was used to create a functional GFP knock-in in the *wg* locus. In the current plasmid, GFP was replaced by an autonomous RFP expression cassette. Integration of the donor plasmid disrupts *wg* gene function and removes the protospacer adjacent motif (PAM) that is essential for cleavage by *gRNA-wg*, thus preventing recutting of the targeted allele. (B) Flies with genomic insertions of the donor plasmid can be easily identified by strong red fluorescence in the eye compared to control animals. (C) Schematic showing the *wg* locus after successful ends-out integration of the donor sequence. The indicated primers were used to analyze whether donor integration in RFP positive flies occurred at the correct location. Note that primers *wgHRgenofwd1* and *wgHRgenorev2* anneal outside the homology arms that are present in the donor plasmid. (D) A representative example of diagnostic PCRs from 10 flies from experiments designed to integrate the donor plasmid at the *wg* locus. Eight out of 10 flies gave rise to a product of the expected size with both *wgHRgeno* primer pairs and were scored as positive for RFP integration in *wg*. Only one PCR product was amplified from fly number 7, suggesting a complex integration event. Genomic DNA from control genotypes did not give any PCR product with primers diagnostic for donor insertion in the *wg* locus (four lanes to the left of 1 – 10). Amplification of part of the *wls* locus was used as a positive control for DNA quality (bottom panel). A summary of all HDR genotyping experiments can be found in Table S1.