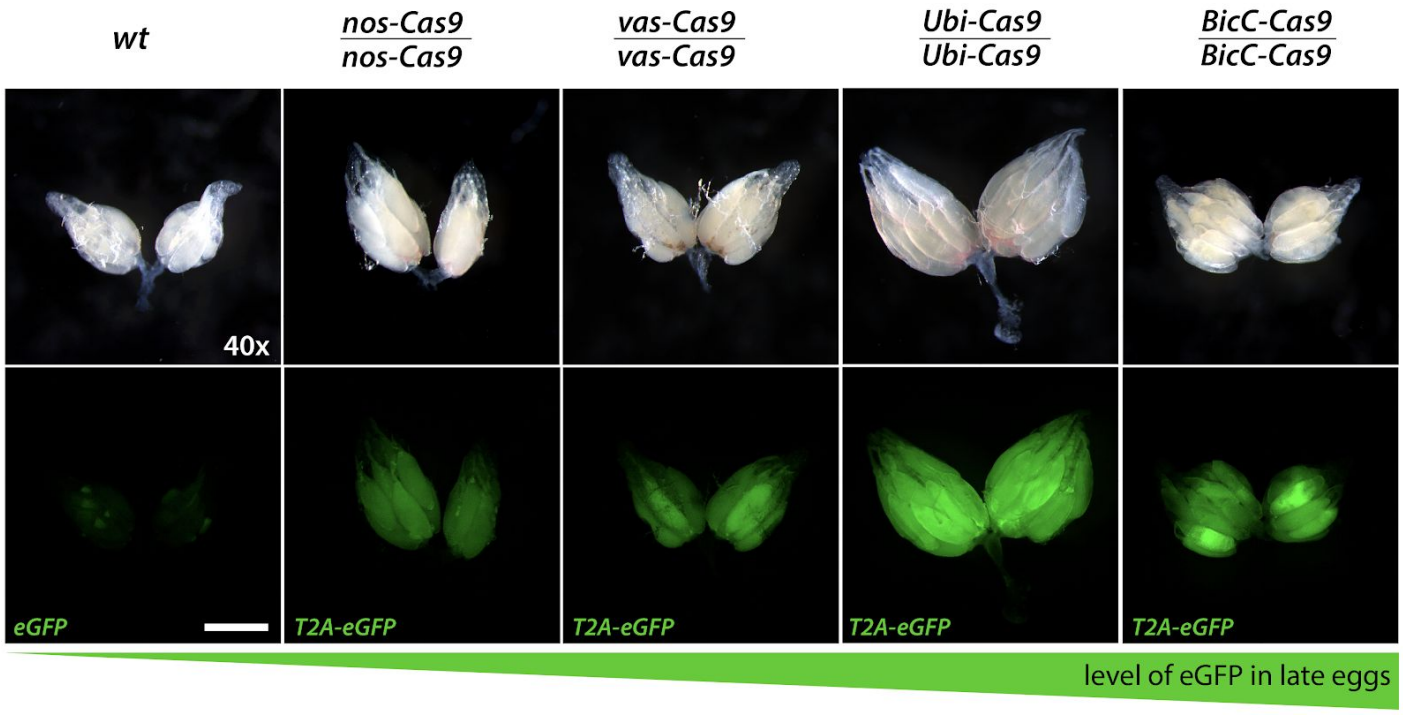
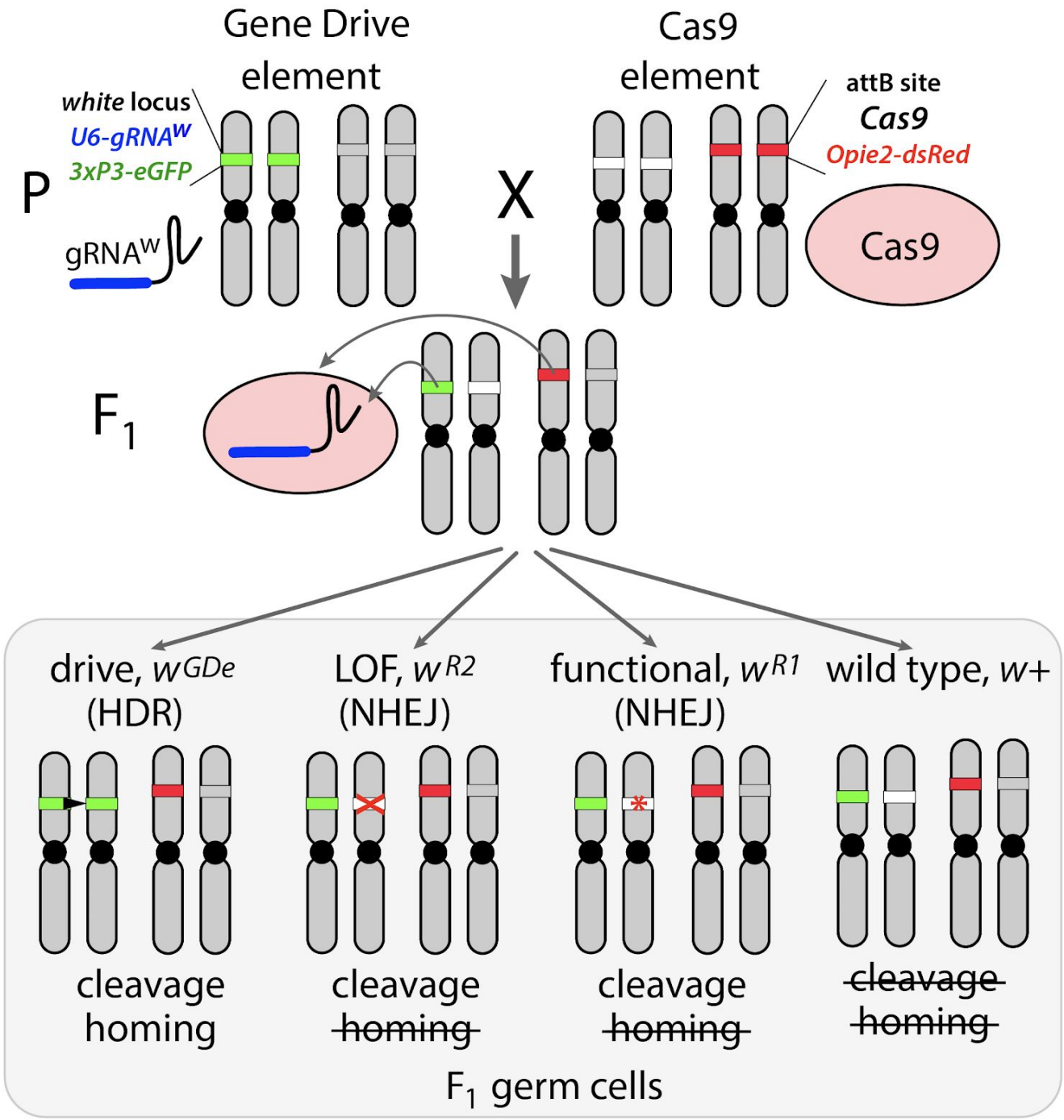
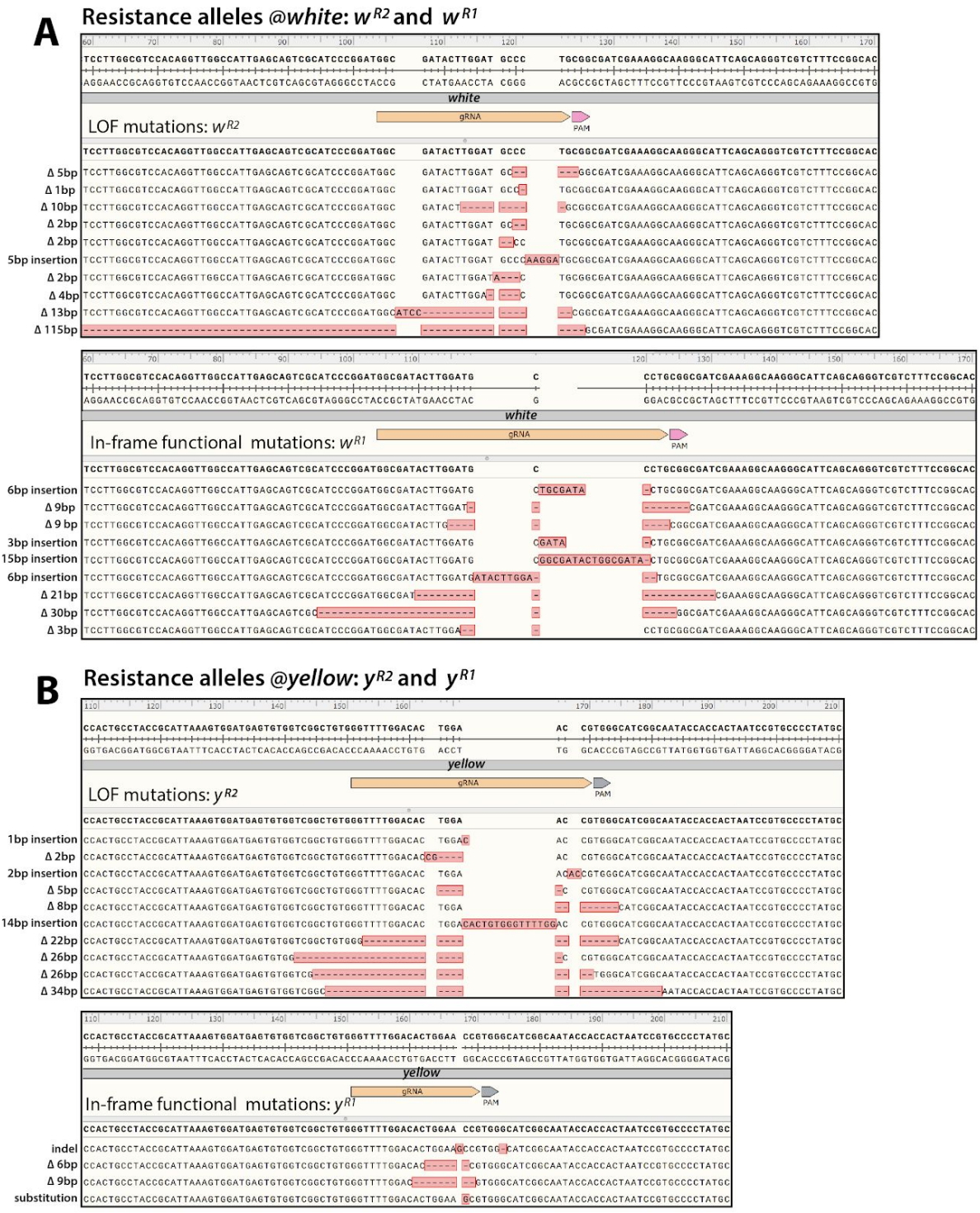


Supplementary Figure S1. Genetics of the split-drive design and possible repair events. Each element (Gene Drive and Cas9) is inactive on its own and can be maintained as a homozygous parental line (P). The cross between the homozygous lines results in 100% trans-heterozygous F_1 progeny that carry both elements. gRNA^{w} expressed by *GDe* in the germline and soma and directs cleavage at *white* locus by Cas9, which can be repaired in three different ways: via HDR using w^{GDe} as a repair template and result in homing of *GDe*; and via Non-Homologous End Joining (NHEJ) and lead to *white* loss-of-function resistance (w^{R2}) allele or an in-frame functional resistance (w^{R1}) allele.



Supplementary Figure S2. Fluorescent microscopy imaging of relative amount of maternally deposited Cas9-T2A-eGFP protein in four homozygous lines expressing *Cas9* under different promoters. *Nanos* (*nos-Cas9*), *vasa* (*vas-Cas9*), *Ubiquitin-63E* (*Ubi-Cas9*), and *Bicaudal C* (*BicC-Cas9*) constructs (Figure 1A) were inserted at the same site on the 3rd chromosome using φC31-mediated integration. A self-cleaving *T2A-eGFP* sequence, which was attached to the 3'-end of *Cas9* coding sequence, provided an indicator of *Cas9* expression. Expression levels of *eGFP* in ovaries of a homozygous female from each *Cas9* line were compared to that in wild type (*wt*) ovaries. Both *nos-Cas9* and *vas-Cas9* supported weak maternal deposition, while *Ubi-Cas9* and especially *BicC-Cas9* resulted in strong maternal deposition in developing eggs. Out of four tested Cas9 promoters, *nanos* and *Bicaudal C* supported the weakest and the strongest, respectively, maternal deposition into developing late eggs. Scale bars correspond to 500 μm.



Supplementary Figure S3. Examples of *white* and *yellow* resistance alleles generated by Cas9/gRNA-mediated DNA cleavage. Not every DSB induced by Cas9/gRNA in germ cells is repaired by HDR resulting in homing. NHEJ pathway also ligates DSBs and can lead to base insertions or deletions (*indels*) incorporated at the ligated DSBs. These *indels* change recognition sequence for gRNA and can result in mutations that are resistant to further cleavages by the same Cas9/gRNA system. We identified both types of resistance alleles – loss-of-function (LOF) (R2) and in-frame functional (R1) mutations – generated at both *white* and *yellow* loci. Diversities of w^{R2} and w^{R1} (A), and y^{R2} and y^{R1} (B) found at *white* and *yellow* loci, respectively. Homozygous LOF mutations of both *white* and *yellow* genes are viable and fertile in *Drosophila*, and thus frequencies of resistance alleles increased between F_2 and F_3 generations (Figure 3B).