



## Supplementary Information for

Behavior of homing endonuclease gene drives targeting genes required for viability or female fertility with multiplexed guide RNAs

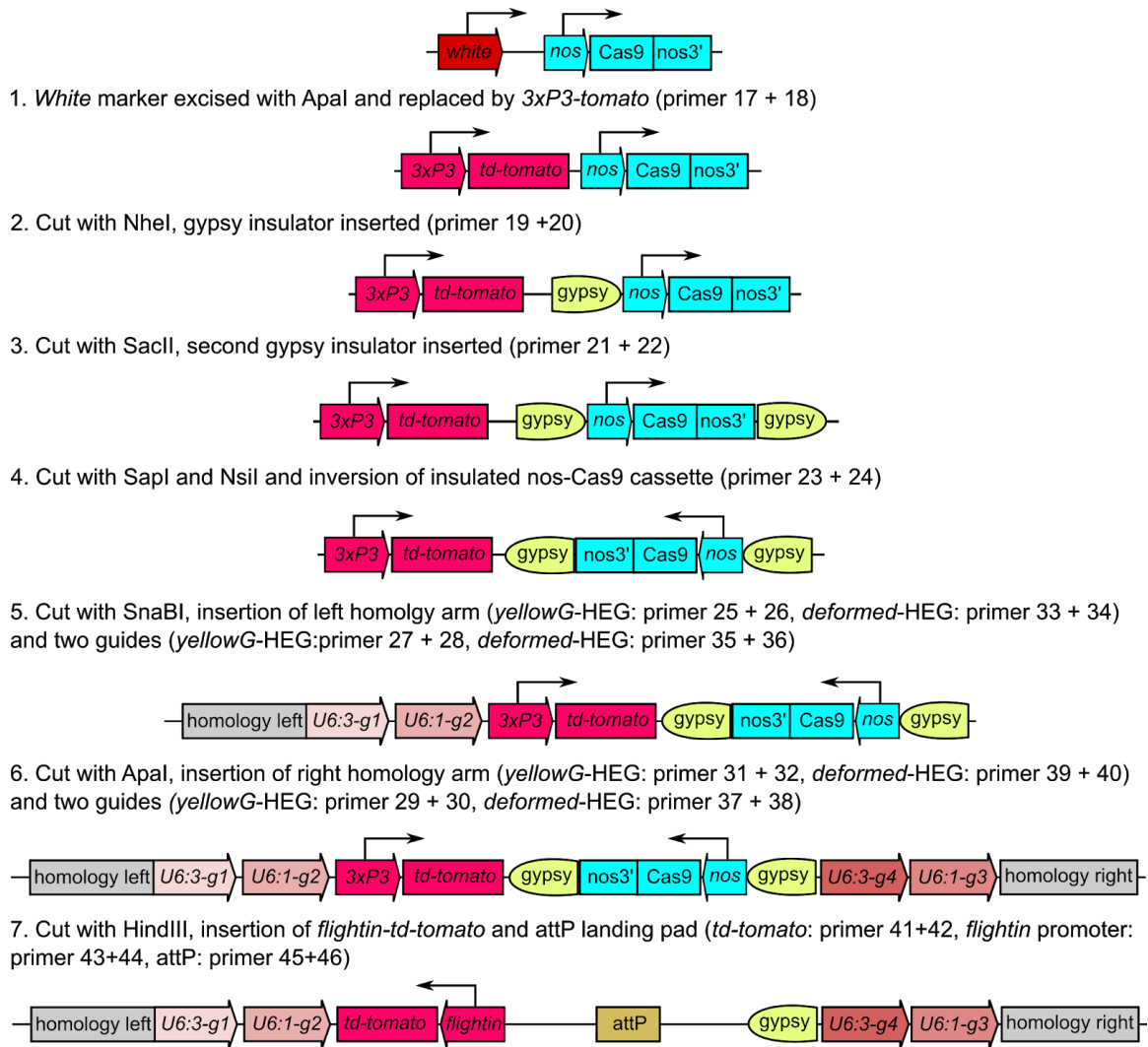
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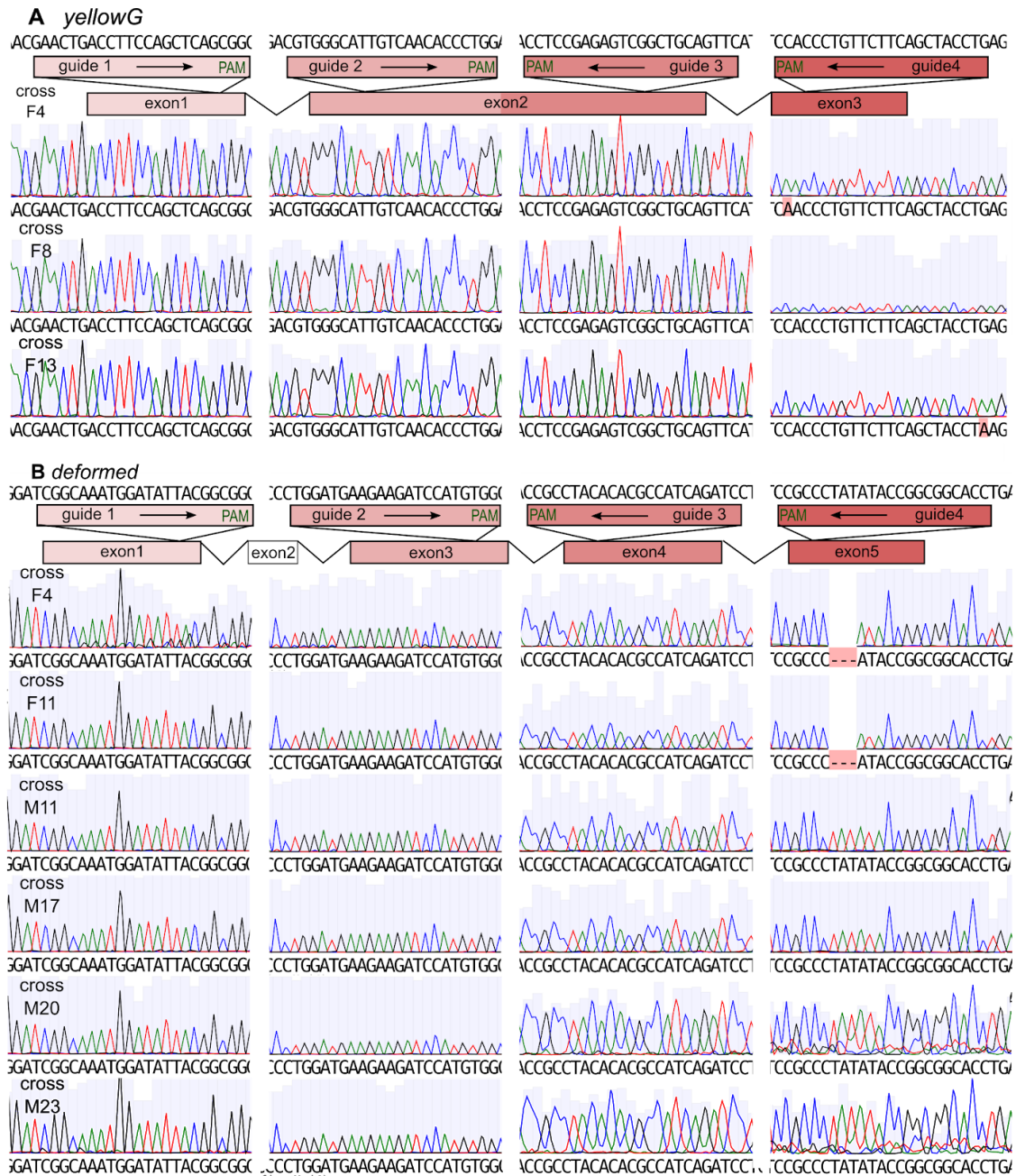
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### **This PDF file includes:**

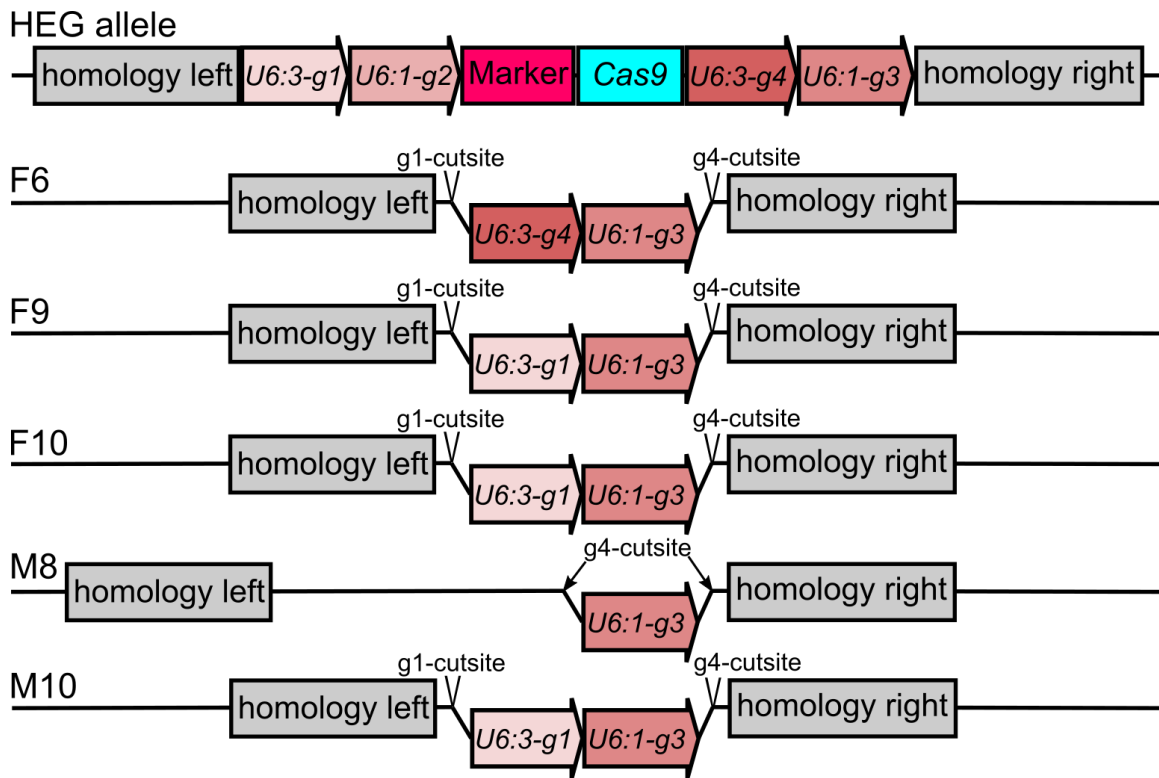
Figures. S1 to S3



**Fig. S1. Cloning strategy for HEG and split-HEG constructs.** Step by step assembly guide listing used enzymes and primers. See Material and Methods section for details.



**Fig. S2. gRNA target site sequences of escapers.** Chromatograms from one fly of each cross containing escapers is shown. All flies were of the +/Df genotype **(A) yg-HEG Escapers.** gRNA4 target sequence had a mutated PAM site in progeny of cross F4. Progeny from cross F13 had a mutation in the distal most base of the PAM. All other target sites did not show mutations. **(B) Dfd-HEG Escapers.** Progeny coming from cross F4 and F11 had a 3bp deletion in the target sequence. All other target sites were intact.



**Fig. S3: Incomplete homing events.** Shown are details of the incomplete homing events observed in Fig. 3. In all cases a likely recombination event between repetitive sequences within the left and right gRNA cassettes eliminated Cas9 and the marker from the construct.