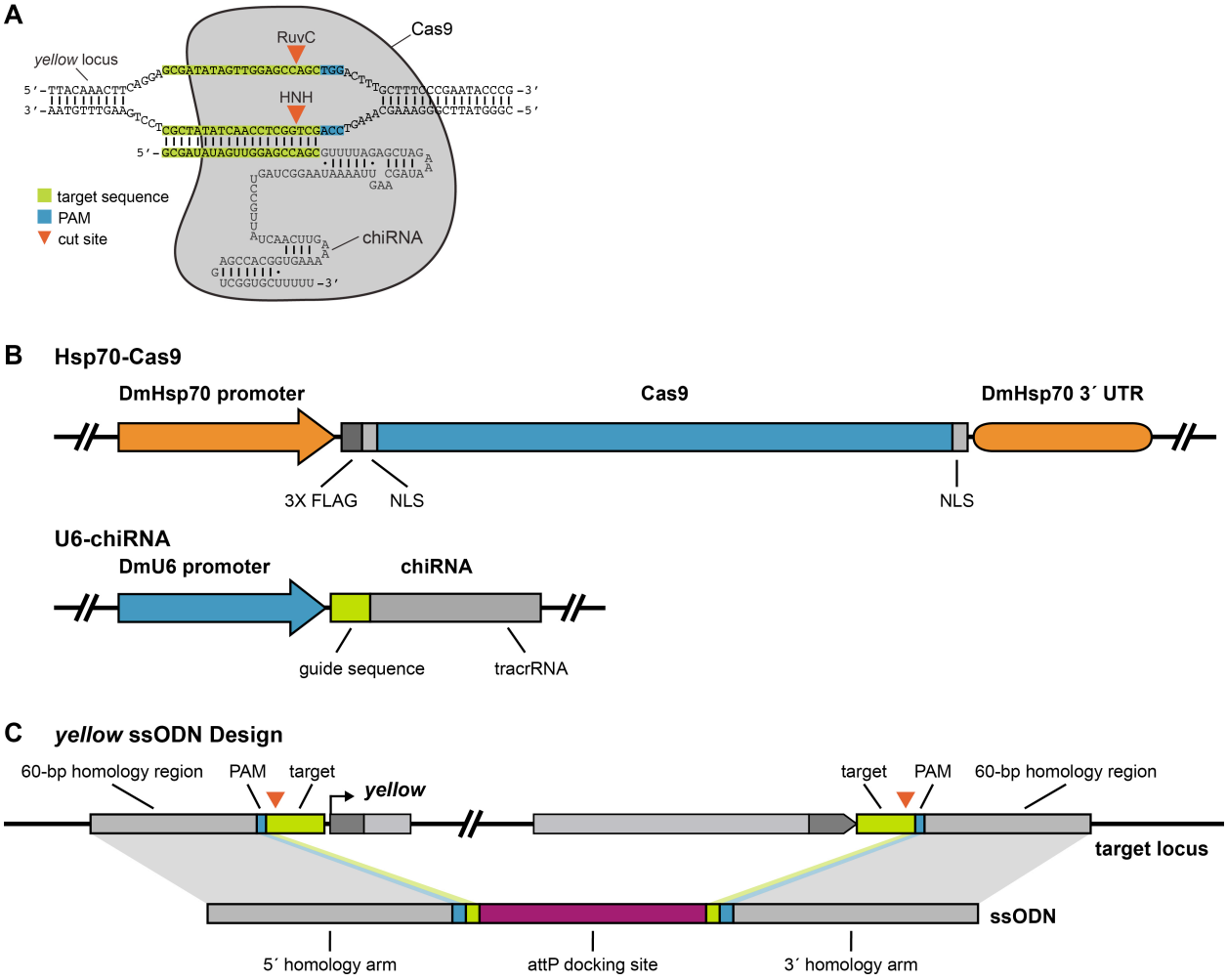

Figure S1 CRISPR RNA/Cas9 molecular reagents used in this study

(A) Schematic of the modified CRISPR RNA/Cas9 system (based on Jinek et al., 2012) used in this study (YE1 is shown as an example). A chiRNA guides Cas9 to complementary target DNA. Target recognition requires 20-nt of homology and a 3-bp PAM sequence, NGG, at the 3′ end of the genomic target sequence. Cas9 cleaves the complementary DNA strand via its HNH endonuclease domain, while its RuvC-like endonuclease domain cleaves the noncomplementary strand (red arrowheads), resulting in a DSB at the targeted site. (B) Cas9, derived from *Streptococcus pyogenes* and codon optimized for eukaryotic expression (Cong *et al.* 2013), was expressed under the control of the Drosophila *hsp70* promoter and 3′ UTR. The chiRNAs utilized in this study consist of 20-nt of target-specific guide sequence followed by 76-nt of tracrRNA sequence as this chiRNA exhibited maximal activity in mammalian cells (Patrick Hsu and Feng Zhang, personal communication). The chiRNA is transcribed from the Drosophila snRNA:U6:96Ab promoter (Wakiyama *et al.* 2005). (C) ssODN donor templates contain 60-nt homology arms matching genomic sequence immediately adjacent to each site-specific cut flanking a 50-nt attP docking site for efficient subsequent manipulation of the targeted locus. Red arrowheads mark the predicted Cas9 cleavage sites. Note that the ssODN is designed such that neither it nor the modified locus will be CRISPR targets. Not to scale. More detailed information and protocols can be found at FlyCRISPR.molbio.wisc.edu.