Mariyappa et al. Generation of *Drosophila* attP containing cell lines using CRISPR-Cas9

Supplemental Figure Legends:

**Figure S1. PCR diagnosis of CRISPR homologous recombination in S2R+ cells.** (**A**) Homologous recombination after CRISPR was diagnosed with primer pairs 1+2 and 3+4 (Figure 2). (**B**) Initial screening for all the putative single cell clones was performed with the primer pair 1+4 (Figure 2). Clones positive (91, 141) for successful HR yielded a 5.1 kb amplicon in addition to the unmodified 1.5 kb amplicon, indicating insertion of the attP>>Act::GFP<<attP cassette.

**Figure S2. Recombination mediated cassette exchange using MT::GFP cassette in S2R+-attP-99F8-Clone 141. (A)** Bulk sorting the S2R+ 99F8 attP cells after transfecting with attB>>MT::GFP<<attB recombination mediated cassette exchange (RMCE) cassette byFACS revealed that 0.3% of the cells were positive for GFP alone indicating that all the copies of Act::dsRed were exchanged with MT::GFP in this fraction of cells. #Events is the number of dsRed only, GFP only or dsRed, GFP double positive cells detected by FACS with the respective percentage indicated in the %Parent column. **(B)** Successful RMCE in either orientation was diagnosed using the primer pairs 5+7 or 5+6 (Figure 3) that generated amplicons of 1.5 and 1.7 kb, respectively. The following fractions obtained from FACS were assessed for RMCE: dsRed positive (R, cells without successful RMCE), GFP positive (G, cells with all the copies of Act::dsRed replaced by RMCE with MT::GFP) and double positive (RG, cells with successful RMCE in at least one of the chromosomes at the 99F8 locus, but retaining the Act::dsRed in at least one other homolog).

**Figure S3. Sequence alignment of the genomic DNA from multiple Drosophila melanogaster cell lines derived from different genetic backgrounds at the 25C6 locus(2L:5,108,448..5,108,448).** Multiple sequence alignment of the 25C6 genomic locus for the following lines: ML-DmBG2-c2, ML-DmBG3-c2, ML-DmD17-c3, Kc167 and S2-DGRC. The sequences were conserved at the two gRNA cut sites. A zoomed in view of the multiple sequence alignment demonstrated the overall consensus.

**Figure S4**. **Generation of 25C6 attP site in *D. melanogaster* cell lines using CRISPR-Cas9.** (**A)** A schematic of the insertion at the 25C6 locus, indicating the regions outside the insert homology arms (black), 5' homology arm of the insert (5'H), attP site (yellow triangle), act5C promoter (gray arrow), dsRed (red bar) and the 3' homology arm of the insert (3'H). The numbered gray bars (1-3) correspond to the sizes of the PCR products for verifying the insertion. Bar 1 was amplified using Primer 8 + 9. Bar 2 is amplified with Primers 8 + 11. Bar 3 was amplified using primer 9 + 10. (**B**) PCR verification of S2-DGRC-attP-25C6-Clone 8, (-) control is the parental S2-DGRC line amplified with the primer pairs outside the homology arms. (**C**) PCR verification of ML-Dm-BG2-c2-attP-25C6-Clone 8, (-) control is the parental ML-Dm-BG2-c2 line amplified with the primer pairs outside the homology arms. (**D**) PCR verification of ML-Dm-BG3-c2 attP-25C6-Clone 74, (-) control is the parental ML-Dm-BG3-c2 line amplified with the primer pairs outside the homology arms.

**Figure S5**. **Generation of Kc167-attP-25C6 and Kc167-attP-99F8 lines and RMCE confirmation.** (**A**) Homologous recombination of the cassette after CRISPR was detected at 25C6 locus with primer pairs 8+9, 8+11 and 9+10 in clone 35 (Table 1). (-) control is the parental Kc167 line (**B**) Successful RMCE was diagnosed using either the primer pair 5+10 or 5+11, which indicated that the donor cassette was inserted in reverse (3' to 5') and forward (5' to 3') orientations, respectively. (-) control is the parental Kc167 amplified by primer pair 5+10 and primer pair 5+11, respectively. The following fractions obtained from FACS were assessed for RMCE: dsRed positive (R, cells without successful RMCE), GFP positive (G, cells with all the copies of Act::dsRed replaced by RMCE with Act::GFP) and double positive (RG, cells with successful RMCE in at least one of the chromosomes at the 25C6 locus, but retaining the Act::dsRed in the other homolog). (**C**) Homologous recombination after CRISPR was diagnosed at 99F8 locus with primer pairs 1+2, 2+7, 3+4 and 3+6 (Figures 2 and 3) in clone 71. (**D**) Successful RMCE was diagnosed using the primer pairs 5+7, 5+6, 1+5 or 4+5 (Figures 2 and 3), dsRed positive (R, cells without successful RMCE) and GFP positive (G, cells with all the copies of Act::dsRed replaced by RMCE with Act::GFP).

**Figure S6**. **Generation of ML-Dm-BG3-c2 -attP-99F8 line.** Homologous recombination after CRISPR editing was diagnosed at 99F8 locus with primer pairs 1+2, 2+7, 3+4 and 3+6 (Figures 2 and 3) in clone 28.