A Flippase-mediated GAL80/GAL4 Intersectional Resource for Dissecting Appendage Development in *Drosophila*

Brittany N. Smith[*], Arash M. Ghazhanfari[*], Rudolf A. Bohm[*,§], William P. Welch[*], Bing Zhang[*,†], and John P. Masly[*, 1]
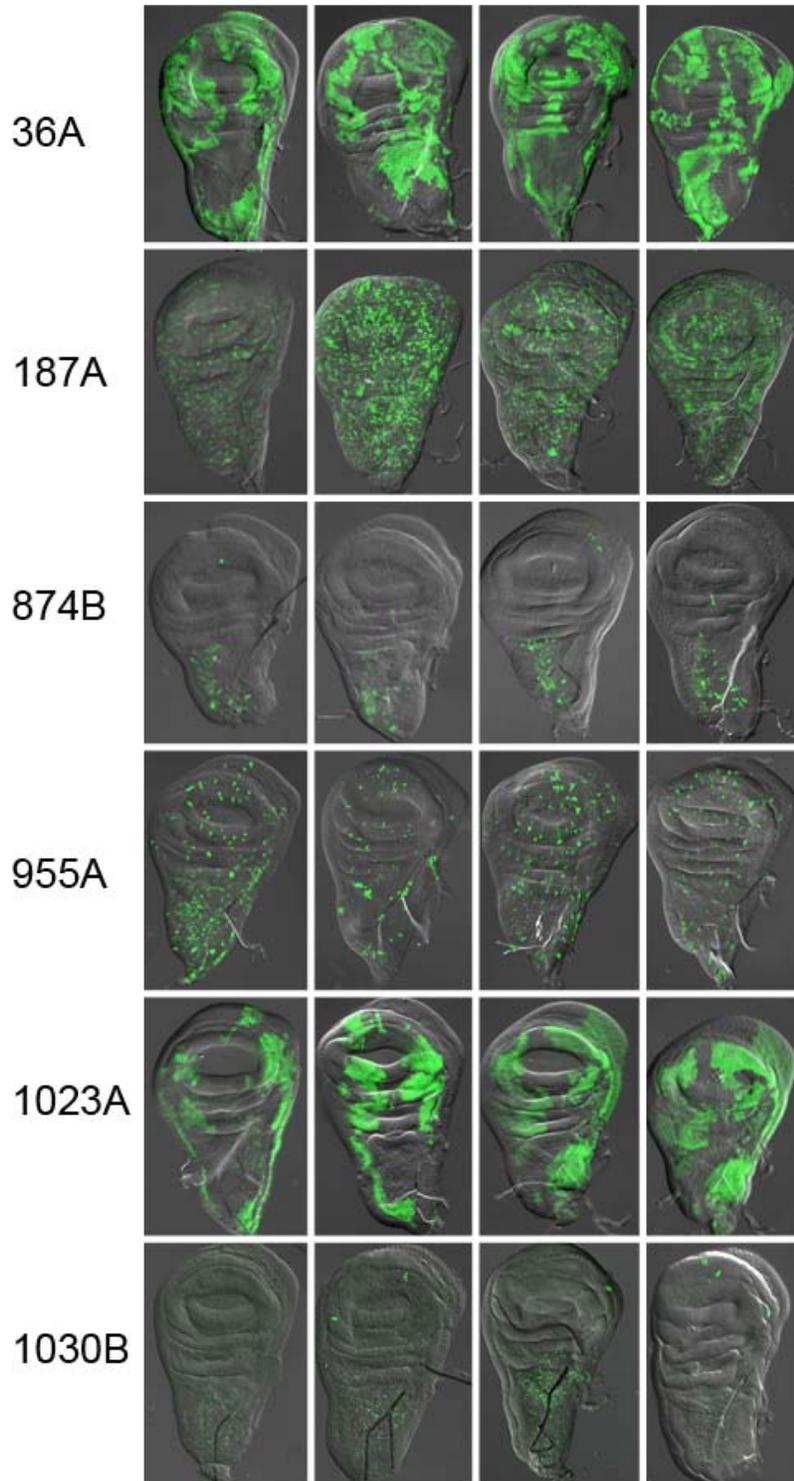
[*]Department of Biology, University of Oklahoma, Norman, OK 73019

[§]Department of Biological and Health Sciences, Texas A&M University-Kingsville, Kingsville, TX 78363

[†]Division of Biological Sciences, University of Missouri, Columbia, MO 65211

[1]Corresponding author: University of Oklahoma, Department of Biology, 730 Van Vleet Oval, Norman, OK 73019. Email: masly@ou.edu

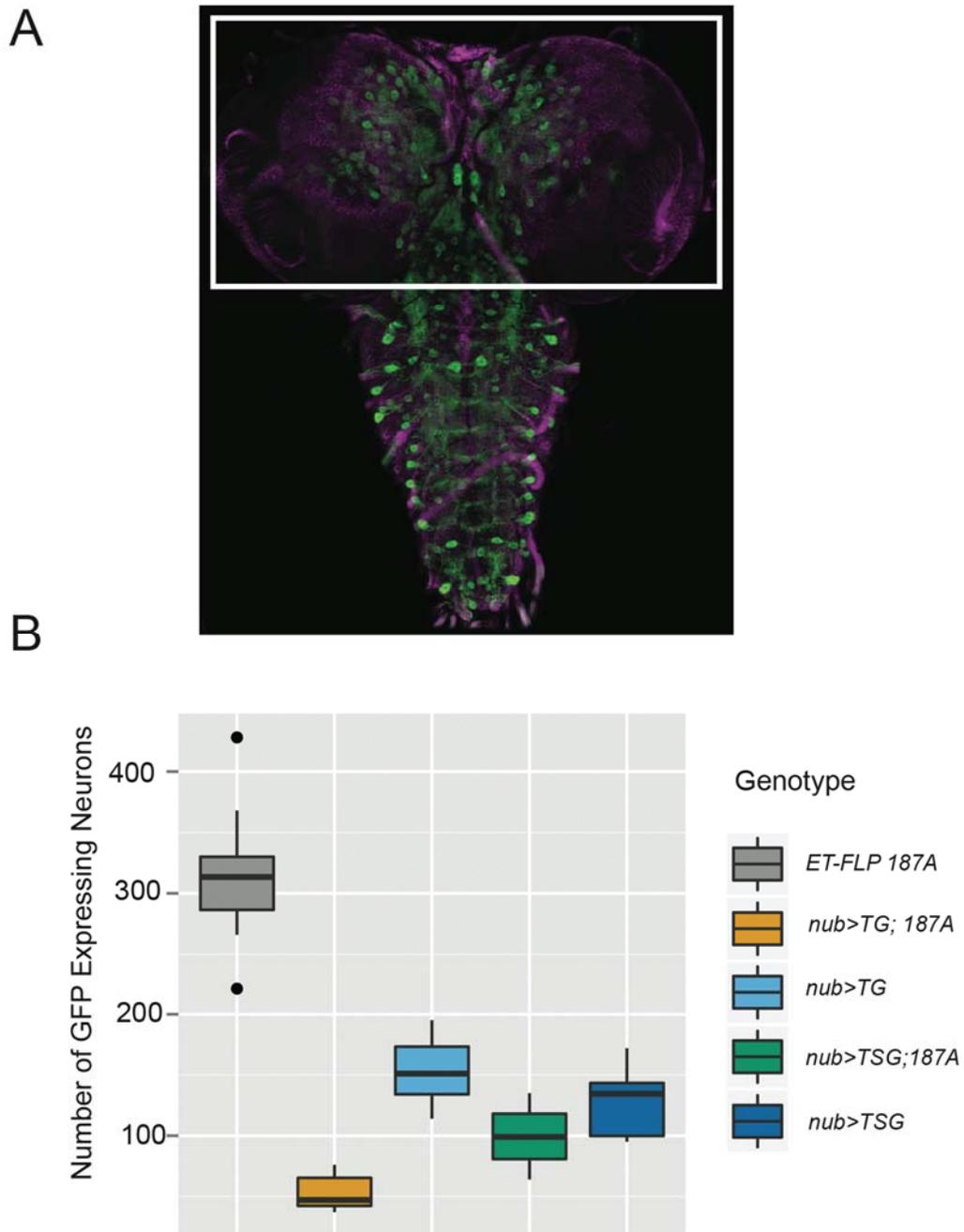**Figure S1. Variation in expression patterns that result from Flippase recombination events in developing imaginal discs.** Evidence of Flp recombination events was visualized by crossing each ET-FLPx2 line to *actin^P^>CD2>GAL4; UAS-GFP*. Cells that express Flp catalyze the removal of the stop cassette *CD2* to allow GFP expression. Each of the four panels shown for a particular ET-FLPx2 line shows wing disc expression from four different individuals.
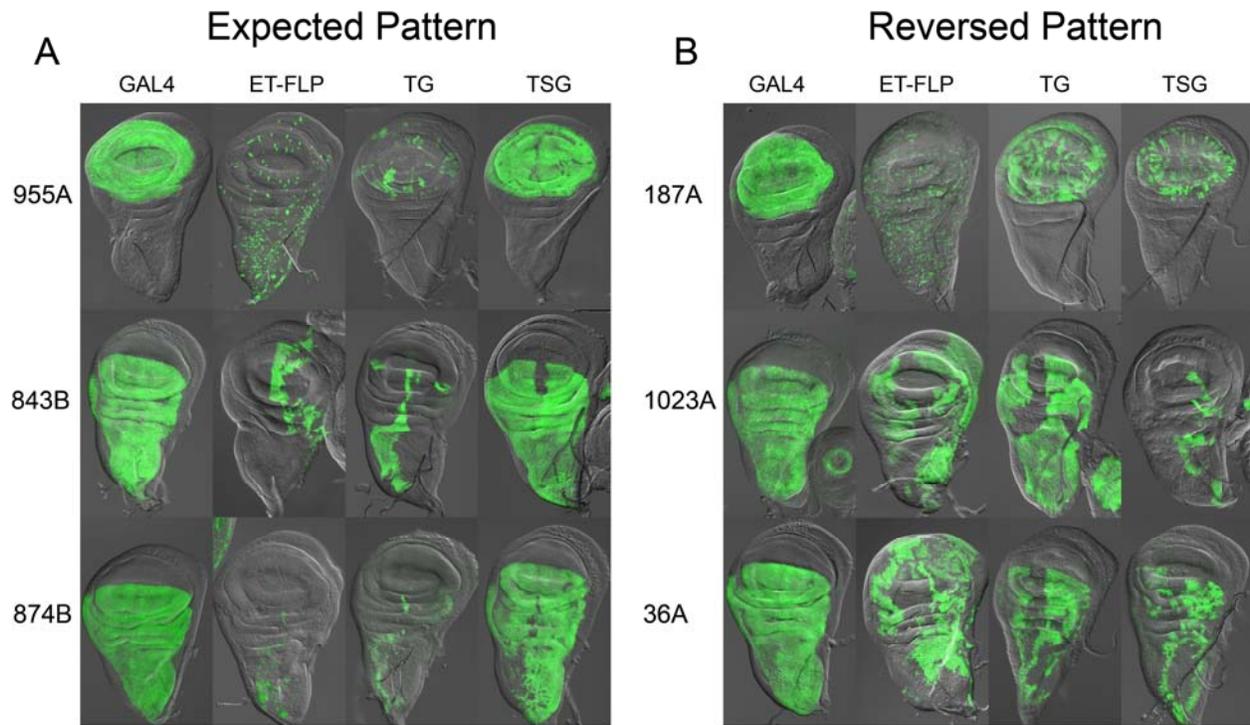
Figure S2. Three ET-FLPx2 lines show patterns opposite of those expected from TG and TSG crosses. GAL4 expression was visualized by crossing each ET-FLPx2 line to *TG; UAS-GFP* or *TSG; UAS-GFP*. Each of the three "reversed" ET-FLPx2 lines was tested with three different wing imaginal disc GAL4 drivers and produced similar results. **A)** GAL4 expression patterns consistent with the expectation from TG and TSG crosses. **B)** GAL4 expression patterns that are reversed compared to the expectation in TG and TSG crosses. GAL4 driver used for 955A and 187A is *nubGAL4*. GAL4 driver used for 843B, 874A, 1023A, and 36A is *apGAL4*.

Figure S3. "Reversed" imaginal disc ET-FLPx2 lines show the expected GAL4 expression patterns in larval CNS tissues. **A)** Larval brain and ventral nerve cord from a *nubGAL4; UAS-GFP* individual. White box shows the area of neuron quantification in TG and TSG crosses using ET-FLPx2 187A. **B)** Cell counts of GAL4-expressing cells in the larval brain. "*nub>*" denotes the *nubGAL4* driver. TSG crosses in the larval brain using 187A show broader GAL4 expression compared to TG crosses as expected from the FINGR method. Boxplots show interquartile range (IQR), black horizontal lines show the median of each sample, whiskers show either the extreme values or ±1.5*IQR, black dots represent outlier values.

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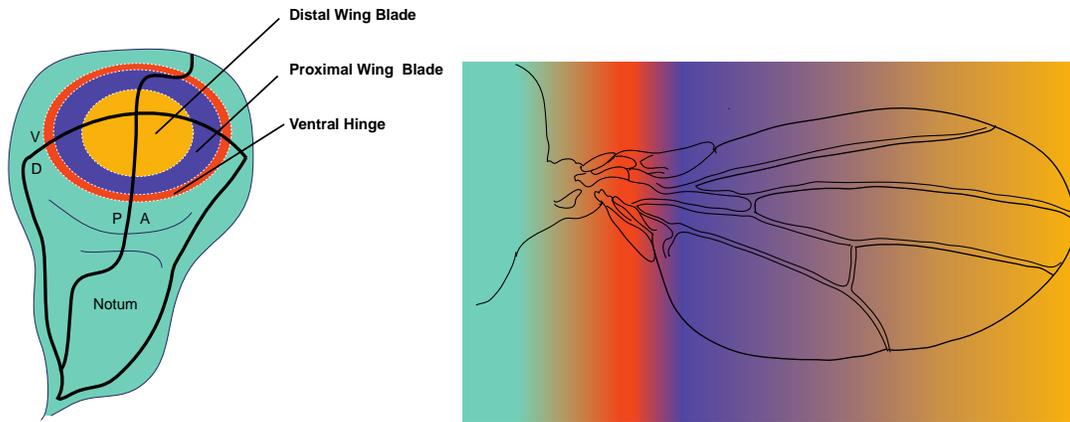
Figure S4. Wing imaginal disc fate map after Bryant (1975). Colors show the regions of the disc that give rise to the corresponding adult wing regions. Dark black lines on the diagram of the wing disc denote axes boundaries between dorsal (D), ventral (V), anterior (A), and posterior (P).
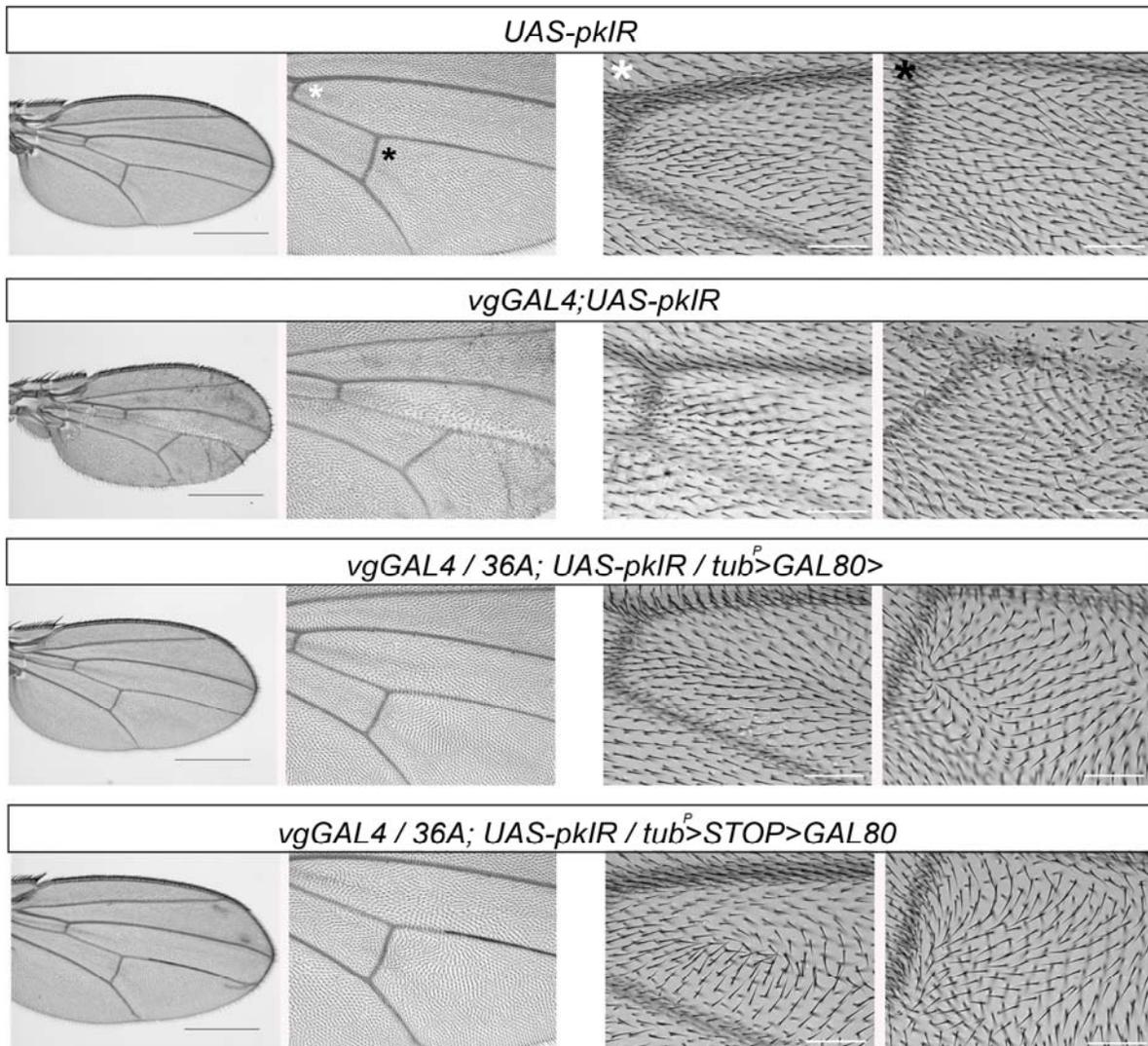
Figure S5. Knockdown of *pk* expression in the developing wing using *vgGAL4* in TG and TSG crosses. From left to right: First panel shows an entire adult wing at 25X magnification. Scale bar is 500μm. Second panel shows the same wing at 50X magnification. Final two panels show wing compartments at 100X magnification. Scale bar is 50μm. White asterisks denote the region at the intersection of wing vein L3 and the anterior crossvein. Black asterisks denote the region at the intersection of wing vein L4 and the posterior crossvein.

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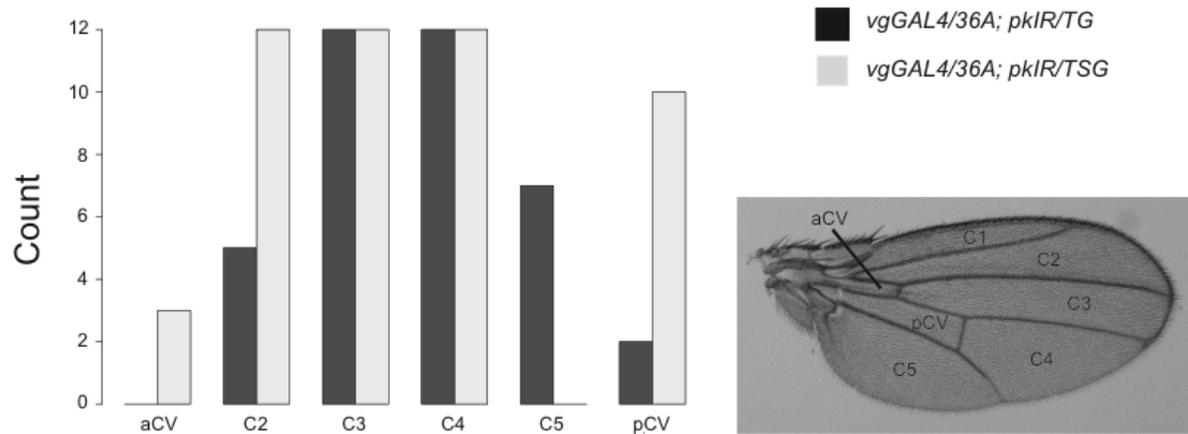
Figure S6. Quantification of compartments that show bristle polarity defects in TG and TSG crosses. Bar plot shows the frequency of compartments with polarity defects in TG and TSG individuals. Wing image shows the inter-vein compartment designations within the adult wing. A single wing was chosen at random from each of 12 individuals for both the TG and TSG crosses.