Supplemental Information

No Evidence that Wnt Ligands Are Required for Planar Cell Polarity in *Drosophila*

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Figure S1. Related to Figure 1. Pilot characterization of split-gal4 knock-in reporters for wg and wnt2. (A) Schematic of the knock-in plasmid used to generate split-Gal4 reporters for Wnt genes, illustrated for wg. Not to scale. (B) wg split-Gal4 reporter expression in L3 larval wing disc (anterior is down), and in L3 larvae (anterior is left), revealed for both p65 and Gal4DBD knock-ins by crossing to a ubiquitous reciprocal split-Gal4 reporter. (C) wnt2 is expressed in the presumptive notum at the base of the wing disc, and in the larval testis, consistent with its described roles in thoracic muscle and testis development. Note that reporter expression in the wing pouch begins at later stages of L3 development, as shown in Fig. 1. UAS:EGFP/EYFP expression is shown in green, and DAPI is shown in blue.
Figure S2. Related to Figure 1. Three independent Gal4-based wnt4 knock-in reporters are largely consistent with one another and do not drive reporter expression in the L3 wing margin, despite Gal4 transcription. (A) Schematic of three independent knock-in reporters in the wnt4 locus, in the first exon, first intron, and last exon, and their expression pattern in the L3 wing disc and in the larval CNS. (B) Lineage tracing of a "trojan Gal4" Wnt4 insertion using GTRACE indicates that wnt4-T2A-Gal4 is not expressed during wing development prior to L3. White arrows in (A) and (B) indicate a consistently labeled population of cells on the antero-ventral margin of the wing pouch. (C) wnt4 reporter expression expands in late larval stage (everted spiracles stage) and (D) is visible in the pupal wing margin by ~24 hours after pupal formation. (E) in situ hybridization demonstrates that both endogenous wnt4 and wnt4-T2A-Gal4 transcripts are present in the DV wing margin in L3 wing discs.
Figure S3. Related to Figure 1. Additional characterization of split-Gal4 Wnt reporters in larval and adult tissues. Wnt split-gal4 reporter expression in (A) the ovarian germline stem cells and surrounding tissues of the adult germarium, and (B) the larval CNS.
Figure S4. Related to Figure 1. Multiple Wnt genes are expressed in populations of adult stem cells located at regional junctions of the gut. (A) Wnt split-gal4 reporter expression in stem cells and surrounding tissue of the adult cardia (foregut-midgut juncture) and (B) in a band of cells at the midgut-hindgut junction.
Figure S5. Related to Figure 2. Effective cleavage of Wnt genes, both singly and in pairs, using multiplexed in vivo somatic CRISPR. Two sgRNAs per target Wnt gene were cloned into the pCFD6 backbone, which expresses multiple sgRNAs under UAS\(^i\) control, each separated by tRNAs. UAS:Cas9.P2 and UAS:sgRNAs were expressed in the adult nervous system using elav-Gal4, and T7 assays were conducted on adult heads. (A) In vivo CRISPR-mediated cleavage of each Wnt gene visualized by T7 endonuclease activity. (B) Simultaneous in vivo CRISPR cleavage of pairs of Wnt genes. Target gene is indicated in magenta. Note that each gene is targeted by two sgRNAs, and that in some cases the PCR-amplified fragment used for the T7 endonuclease assay only includes one such target site. Also note that these tissue samples include non-neuronal tissue from whole heads, and thus do not directly reflect cutting efficiency.
Figure S6. Related to Figure 2. Somatic CRISPR of fz, but not fz2, fz3, or fz4 nor any combination thereof, produces planar cell polarity defects in the adult wing. Each of the four fz paralogs was targeted (one sgRNA per target gene, in a modified pCFD4 backbone [see Methods]) in the wing using nub-Gal4 > UAS:Cas9.P2, either singly or in each pairwise combination, and morphology and PCP was assayed in the adult wing. Top panels show whole phenotype (note that wing margin defects are specific to double knock-out of fz + fz2), and bottom panels show higher magnification of wing hair orientation in the of the L2-L3 intervein region. Note PCP phenotypes were exclusively observed when fz was targeted.
Figure S7. Related to Figure 3. Pairwise double RNAi against wg in combination with wnt4 or wnt6 in the notum does not disrupt PCP patterning. (A) Example of a characteristic PCP phenotype (misoriented bristles) in the adult notum caused fz loss-of-function (pnrgal4 > UAS:Cas9.P2, sgRNA-fz-fz2). (B) UAS:RNAi constructs targeting the indicated genes were expressed in the developing notum using pnrgal4, and PCP was analyzed by observing bristle polarity.
### Table S1. sgRNAs used in this study. Related to Figures 1-3.

| Plasmid   | Target gene | sgRNA                        | Note                                      |
|-----------|-------------|------------------------------|-------------------------------------------|
| pCFD6     | wg          | GGGGCGGCGGCTCCATGTGTGG       | Also used for knock-in                    |
| pCFD6     | wg          | CGATCCACCTCTACGTGGAGAAGG     |                                           |
| pCFD6     | wnt2        | CGCTGGGCCCCGGTCAAGGCCCGG     |                                           |
| pCFD6     | wnt2        | AATCTACATCTCTGGATTATGG       | Also used for knock-in                    |
| pCFD6     | wnt4        | TGTTCAGATTGTAGTCTCTTGG       |                                           |
| pCFD6     | wnt4        | TCGAGTGTACCCTGGTGATTGG       |                                           |
| pCFD6     | wnt5        | GCCAGTGACCTGGGACTCGG         |                                           |
| pCFD6     | wnt5        | GCCAGCCAGTCCTACCGGAGG        |                                           |
| pCFD6     | wnt6        | ATTTAGCAGAGAAGGATTGG         |                                           |
| pCFD6     | wnt6        | TGCCCAATAGACTGGATTCCGTGG     | Also used for knock-in                    |
| pCFD6     | wntD        | GTGTACATGCTAGTCCTAGGG        | Also used for knock-in                    |
| pCFD6     | wntD        | CATGAGTATACGACGACTCTGG       |                                           |
| pCFD6     | wnt10       | ATGGGCGGATGTCGCGGTGGTGG      |                                           |
| pCFD6     | wnt10       | CAGAAGCAGACGACAGAAGCAGG      | Also used for knock-in                    |
| pCFD6     | wnt4        | CTCCTACTGCGCGTCACCAAGG       | For knock-in Gal4 in last exon            |
| pCFD6     | wnt5        | TGAACGTACCGAGCTCGGTAGG       | Used for knock-in, not knock-out          |
| pCFD6     | fz          | ATGGGCGGATGTCGCGGTGGTGG      | Used in Figure 2                          |
| pCFD6     | fz          | GCCATGAGAAGGATTGGATGTTATGG   | Used in Figure 2                          |
| pCFD6     | intergenic  | GCCAGACAAGGATGTCGCGGTGG      |                                           |
| pCFD6     | intergenic  | CAGGACTTTATGCACGAGG          |                                           |
| pCFD4[flpOUT] | fz      | AGCGCTGGACCCCGCTGCAGG        | Used in Figure S6                         |
| pCFD4[flpOUT] | fz2     | TATGAGCAGACCCGCTTGAGG        | Used in Figure S6                         |
| pCFD4[flpOUT] | fz3     | CATGACTGCGACTCCGATTGCAGG     | Used in Figure S6                         |
| pCFD4[flpOUT] | fz4     | TGAGACCTCACGCAATCGTCAGGG     | Used in Figure S6                         |
| Target region & Purpose                        | F primer                                      | R primer                                      |
|----------------------------------------------|----------------------------------------------|----------------------------------------------|
| wg - left homology arm                       | TACTTTCATAGCCAAAAAGCTGAGTTAAAAAGTAA          | CCGGCCCCCTCCGGATTT                           |
| wg - right homology arm                      | TTCATGTTGTGTAAGTTC                           | AAGTGTAAACATCTGTGGG                           |
| wnt2 - left homology arm                     | GAACGGATTCCACATCCATC                        | CCAGAGTATGAGATTAAAGG                        |
| wnt2 - right homology arm                    | AAGGTATAGTACCCCCATAAAC                      | CCACTGACAGGAAGGAAATG                        |
| wnt4 (first exon) - left homology arm        | GAAAACCCCTCAAGCCCAAG                        | CTGTGTGCTGCTGTTT                            |
| wnt4 (first exon) - right homology arm       | AATCACAATCTGAAACAG                         | TTTCGATCTCCTGTTT                            |
| wnt5 - left homology arm                     | GATGGAAATCGGTCGTCGC                        | GCCCTCTTGTGACTATGGG                          |
| wnt5 - right homology arm                    | GTACGGTCTAAAGCCCAAG                        | TTAATCTGCTGATGATCG                           |
| wnt6 - left homology arm                     | GAATGTGTGGCTTGTGT                            | TCTGATCATTGGCCATGG                           |
| wnt6 - right homology arm                    | AGTGGCATTTAATACCCTATTAC                     | GCAAATATTGTGCAAAGTG                           |
| wntD - left homology arm                     | AAGCCCTTTTGTATCGCTTCTTGT                    | CTCCAAACCTGCTGCCAG                           |
| wntD - right homology arm                    | CTACTACCATGACACCAG                          | ACGTATTTATGATGCGTTGTG                        |
| wnt10 - left homology arm                    | TAAACCCCTCTAAACCCCA                        | TCTGCTGCTGCTTCTG                            |
| wnt10 - right homology arm                   | CAGCAGACAGCAACAGAC                        | TGACAAGGCCAGCAACAG                           |
| wg - T7 assay                                | CGCATGCTAATGATGATGATTGCT                    | TCCCCAAAAACCCCATTTACCTATC                     |
| wnt2 - T7 assay                              | GAAACGGCAGAAAGGGAAAGGAAAATTA                | CTGCTTTTCTGCTGCTTCTTGT                     |
| wnt4 - T7 assay                              | GTATCCCAGATTCAGGATCTCC                      | GCATCCCAATATATCAGGCCAAA                      |
| wnt5 - T7 assay                              | GTGCAAGAAGATGATGCTTCTTGT                    | AATAGTAAGAGAAAGGAATAGGAA                     |
| wnt6 - T7 assay                              | TCTGAAAGGGATTAGTCAGAGCAG                    | CACAGCTTTTATGCCATTCTTA                      |
| wnt7 - T7 assay                              | CAGGCTACATTAGCAGAAGCAGTTTTCC                | CGATCATTAGCAGAAGCAGAGATA                     |
| wnt10 - T7 assay                             | AACGCAAATGCGTCAATTAAC                      | CATGCGATGAAATGGAAACAGTCT                     |
| wnt4 - in situ probe (S' linker for appending T7) | ggcggcgaggTGAGCTGAGATCTG                   | cccggggcGTTCATTGTTGTAACAGGGCAC               |
| Gal4 - in situ probe (S' linker for appending T7) | ggcggcgaggAAGAAAAACCGAGATGGGCC            | cccggggcATCAGAGGACAGAGGGG                   |