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Supplemental Information

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

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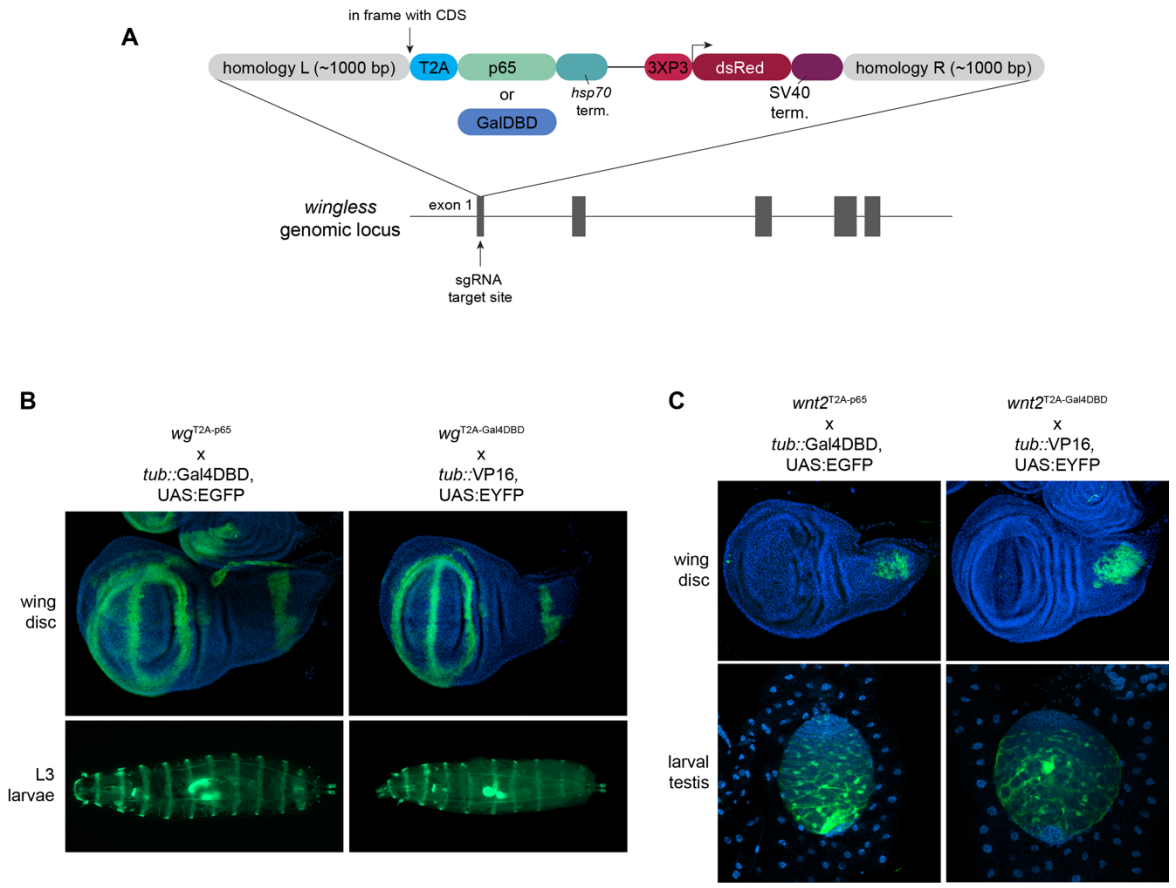

Figure S1. Related to Figure 1. Pilot characterization of split-gal4 knock-in reporters for *wg* and *wnt2*. (A) Schematic of the knock-in plasmid used to generate split-Gal4 reporters for Wnt genes, illustrated for *wg*. Not to scale. (B) *wg* split-Gal4 reporter expression in L3 larval wing disc (anterior 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.

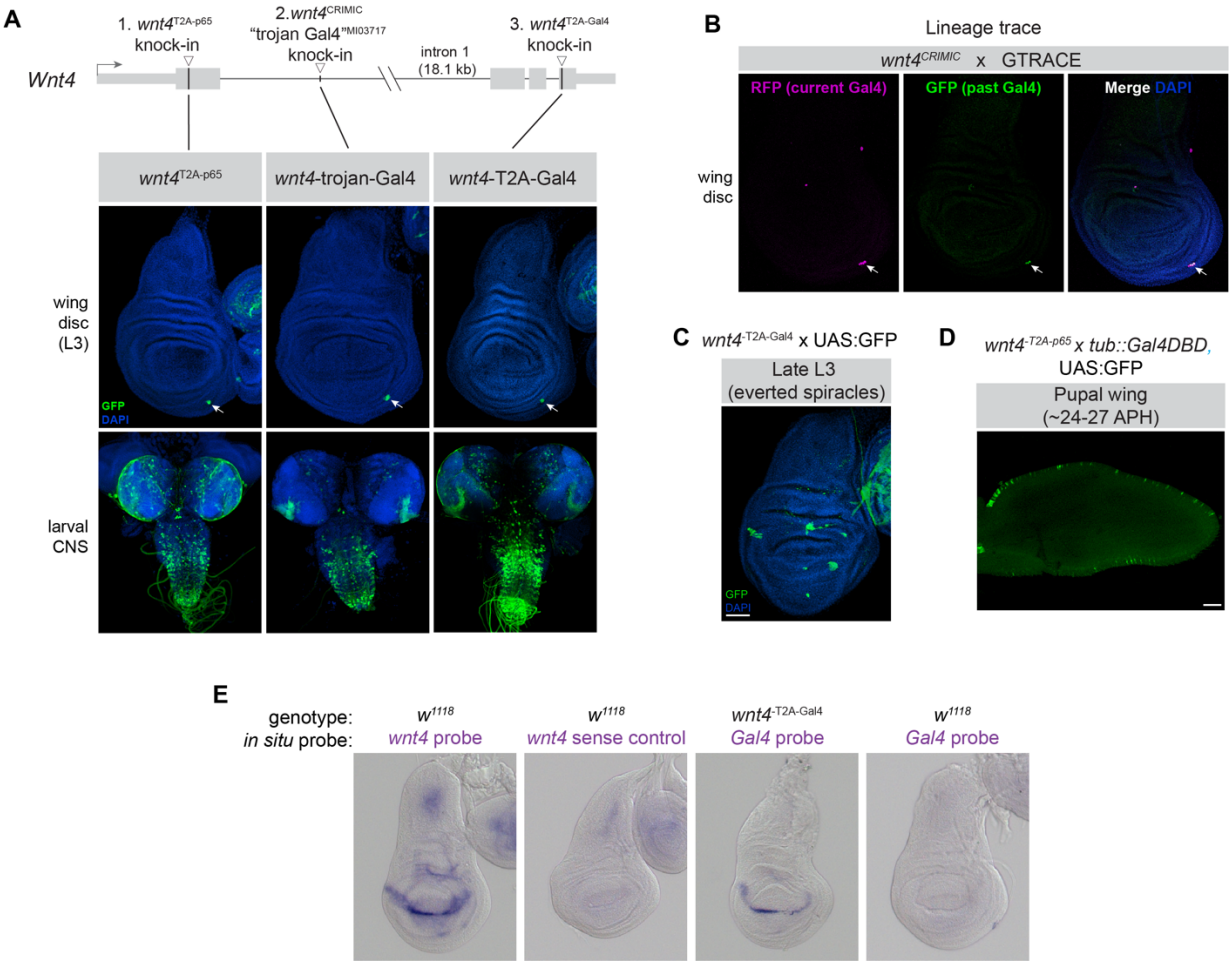

Figure S2. Related to Figure 1. Three independent Gal4-based *wnt4* knock-in reporters are largely consistent with one another and do not drive reporter expression in the L3 wing margin, despite Gal4 transcription. (A) Schematic of three independent knock-in reporters in the *wnt4* locus, in the first 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.

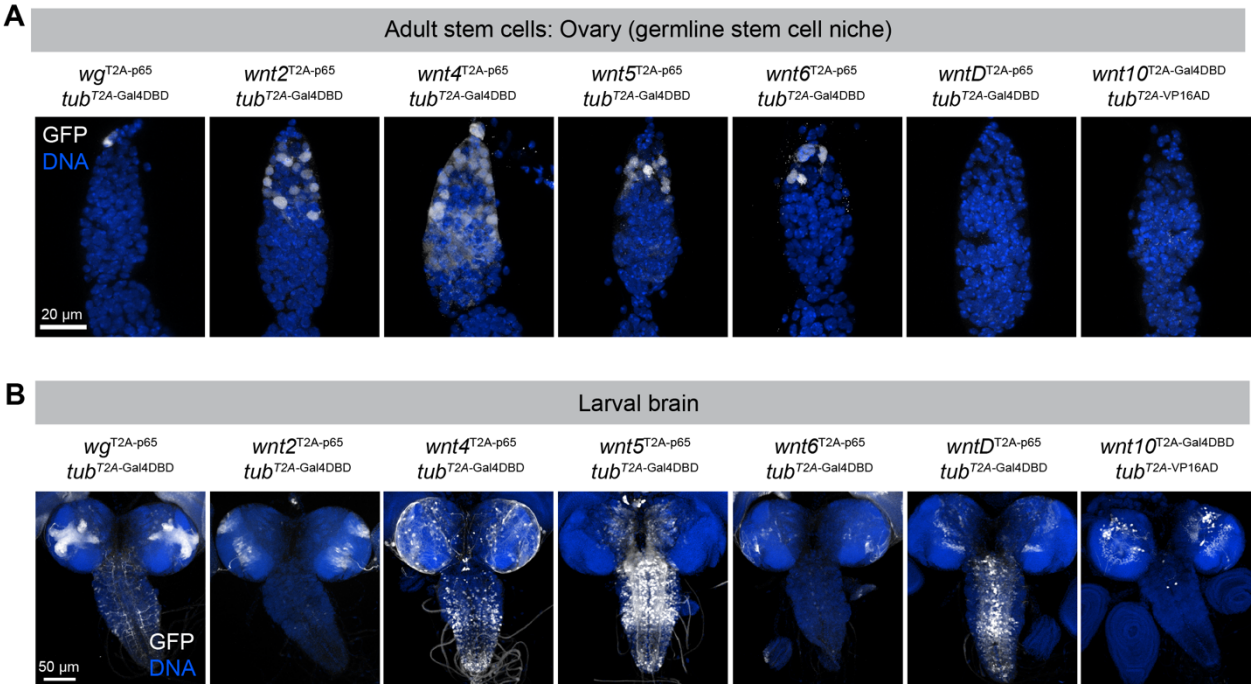

Figure S3. Related to Figure 1. Additional characterization of split-Gal4 Wnt reporters in larval and adult tissues. Wnt split-gal4 reporter expression in (A) the ovarian germline stem cells and surrounding tissues of the adult germarium, and (B) the larval CNS.

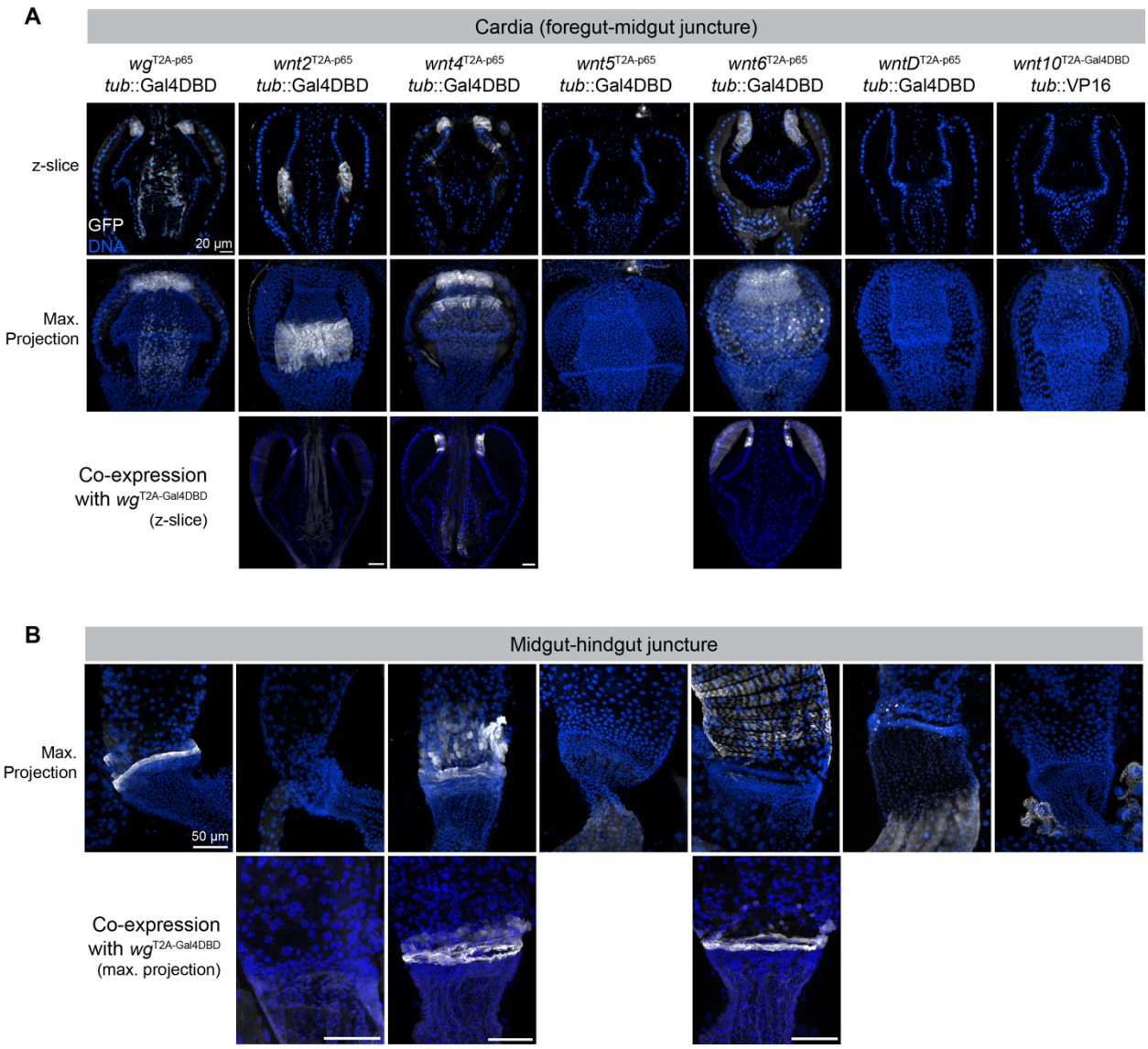

Figure S4. Related to Figure 1. Multiple Wnt genes are expressed in populations of adult stem cells located at regional junctions of the gut. (A) Wnt split-gal4 reporter expression in stem cells and surrounding tissue of the adult cardia (foregut-midgut juncture) and (B) in a band of cells at the midgut-hindgut junction.

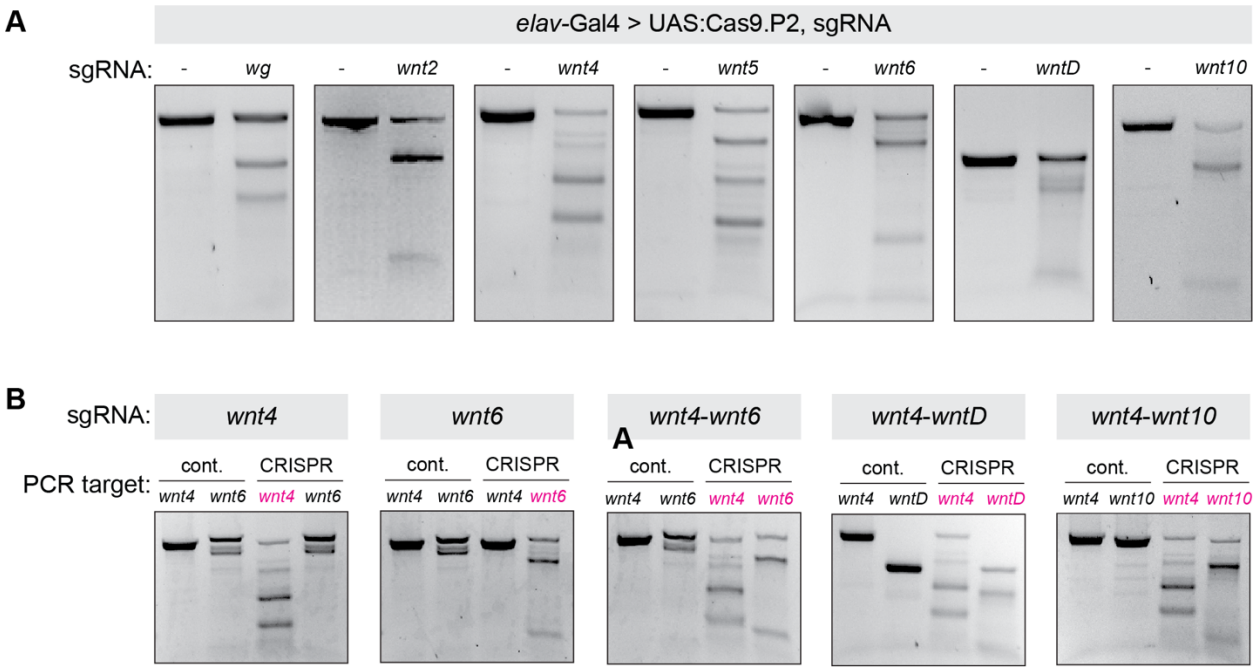

Figure S5. Related to Figure 2. Effective cleavage of Wnt genes, both singly and in pairs, using multiplexed *in vivo* somatic CRISPR. Two sgRNAS per target Wnt gene were cloned into the pCFD6 backbone, which expresses multiple sgRNAs under UAS^{t} control, each separated by tRNAs. *UAS:Cas9.P2* and *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.

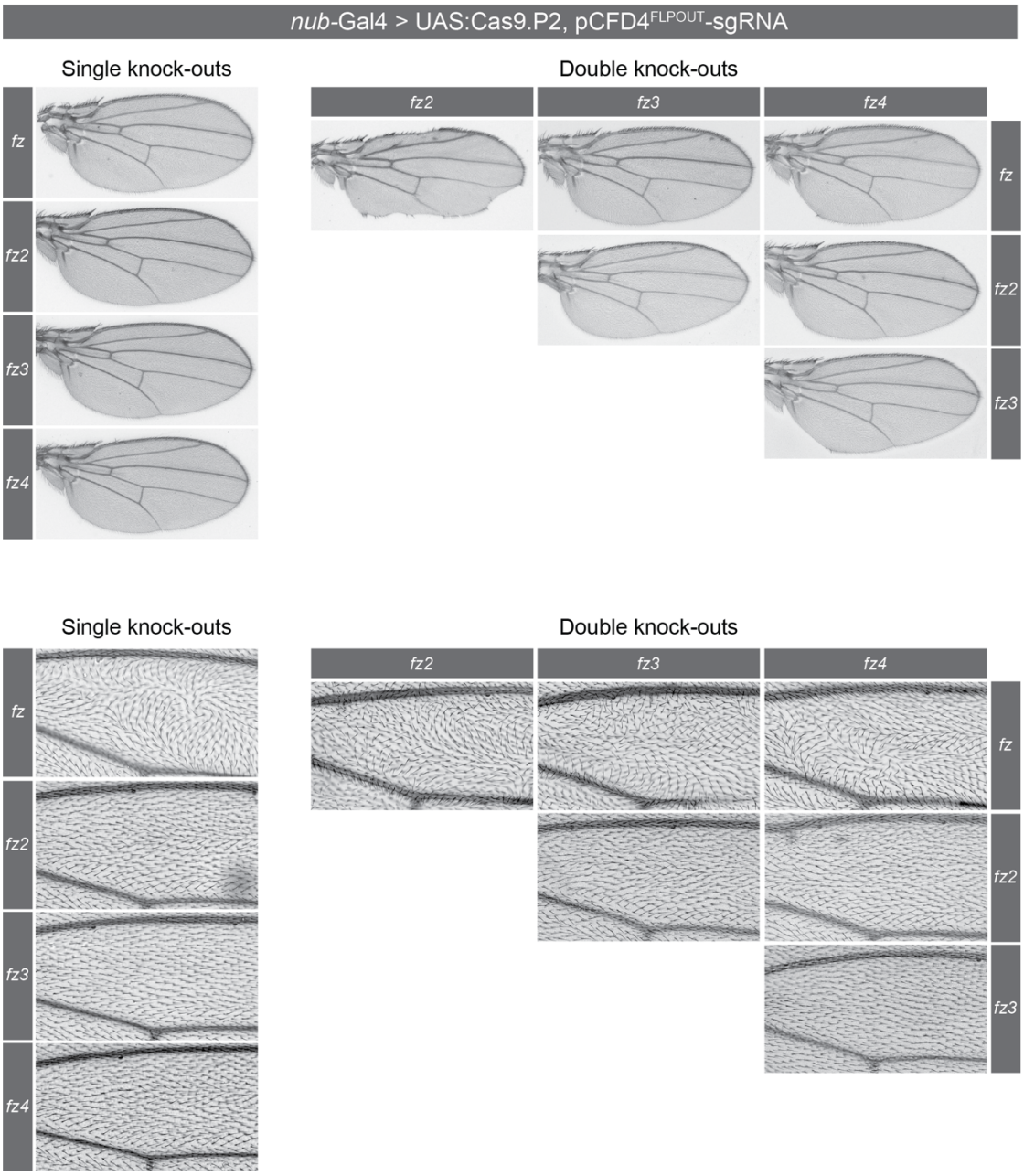

Figure S6. Related to Figure 2. Somatic CRISPR of *fz*, but not *fz2*, *fz3*, or *fz4* nor any combination thereof, produces planar cell polarity defects in the adult wing. Each of the four *fz* paralogs was targeted (one sgRNA per target gene, in a modified pCFD4 backbone [see Methods]) in the wing 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.

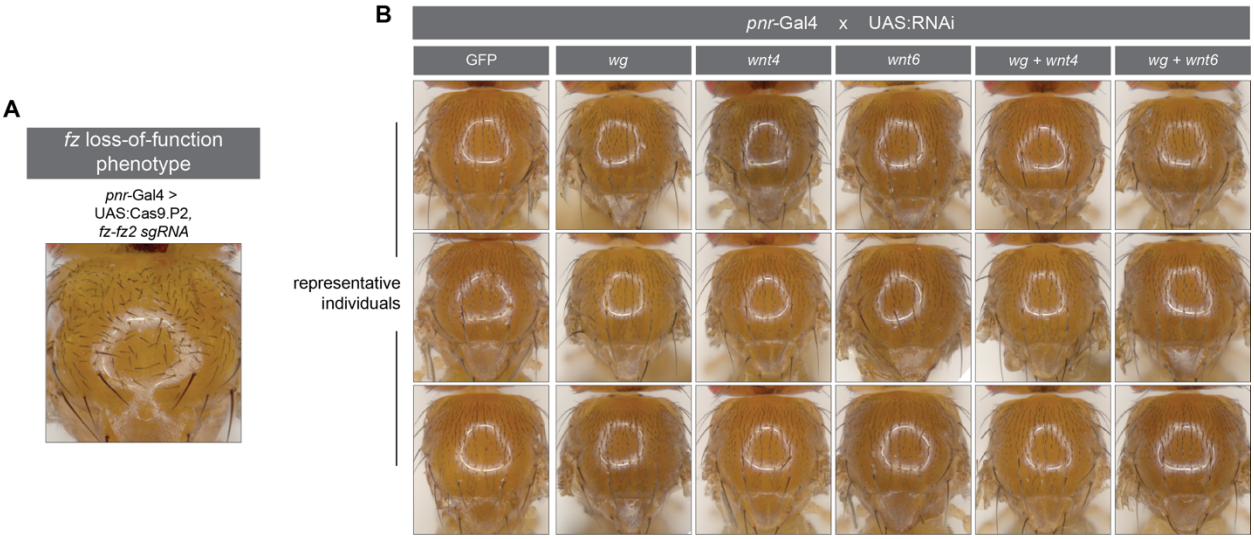

Figure S7. Related to Figure 3. Pairwise double RNAi against *wg* in combination with *wnt4* or *wnt6* in the notum does not disrupt PCP patterning. (A) Example of a characteristic PCP phenotype (misoriented bristles) in the adult notum caused *fz* loss-of-function (*pnr-Gal4 > UAS:Cas9.P2, sgRNA-fz-fz2*). (B) *UAS:RNAi* constructs targeting the indicated genes were expressed in the developing notum using *pnr-Gal4*, 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*	GGGGCCGGGGCTCCATGTGGTGG	Also used for knock-in
pCFD6	*wg*	CGATCCACTCTACGTTGAGAAGG	
pCFD6	*wnt2*	CGCTGGCCCGGGGTCAGGCCCGG	
pCFD6	*wnt2*	AATCTACATACTCTGGATTATGG	Also used for knock-in
pCFD6	*wnt4*	TGTTCAGATTGTGATTGCTCTGG	
pCFD6	*wnt4*	TCGAGTGTACCCTCGGTGATTGG	
pCFD6	*wnt5*	GCCAGTGGACCTGCGGGACTCGG	
pCFD6	*wnt5*	GCCAGCCAGTCTCACCGACGAGG	
pCFD6	*wnt6*	ATTTGGATCGAGAAGGATGTTGG	
pCFD6	*wnt6*	TGCCAATGACTGGATTCGGCTGG	Also used for knock-in
pCFD6	*wntD*	GTGTACTGGTAGTAGCTCATGGG	Also used for knock-in
pCFD6	*wntD*	CATGGGTATTACGAGCACTCTGG	
pCFD6	*wnt10*	ATGGCGCGATGTGGCCGGTGTGG	
pCFD6	*wnt10*	CAGAAGCAGCAGCAAGAAGCAGG	Also used for knock-in
pCFD6	*wnt4*	CTCCTACTGCGCGGTCACCAAGG	For knock-in Gal4 in last exon
pCFD6	*wnt5*	TGAACGTACCGAGCTTGCGTAGG	Used for knock-in, not knock-out
pCFD6	*fz*	ATGTGGCAGTCCATCTAGTTCGG	Used in Figure 2
pCFD6	*fz*	GGCATAATGGTCATGTTATATGG	Used in Figure 2
pCFD6	intergenic	GCAGACAACTGTGATGGCTCCGG	
pCFD6	intergenic	CAGGACTTTATCGCCCAGCAGGG	
pCFD4[flpOUT]	*fz*	AGCGCTGGACCCCCTGTATCAGG	Used in Figure S6
pCFD4[flpOUT]	*fz2*	TATGACTGGCACTCCGTAACCGG	Used in Figure S6
pCFD4[flpOUT]	*fz3*	CATAATGGCCCGGTGGCATCCGG	Used in Figure S6
pCFD4[flpOUT]	*fz4*	TGAGACCTCCATGCCAAATCTGG	Used in Figure S6

Table S2.
PCR primers used in this study, related to Figures 1, Figure S2, and Figure S5

Target region & Purpose	F primer	R primer
wg - left homology arm	TACTTTCATAGCCAAAAGCTAGAAGTTAAAAGTAGTAA TAC	CCGGCCCCTTCCGGATTT
wg - right homology arm	TCCATGTGGTTGTAAGTTC	AAGTGTAACATCTGTGGG
wnt2 - left homology arm	GAACGATTCACATCCATC	CCAGAGTATGTAGATTAAGAG
wnt2 - right homology arm	AAGGTATAGTAGCCCCATAAAC	CCACTGACAGGAAGGGAATG
wnt4 (first exon) - left homology arm	GAAAACCCTCAAGCCCAAG	CTGATGCTGCTGGTGTTG
wnt4 (first exon) - right homology arm	AATCACAATCTGAACAACG	TTTTGCATCTCGTACTTC
wnt5 - left homology arm	GATGGAATCGGTCGTCGC	GCCCTCTTGATGCATATTGG
wnt5 - right homology arm	GTACGTTCATAAAGCCAG	TTAATCGTCTGTATGATCG
wnt6 - left homology arm	GAATGTGTGCGTCTTGTG	TCCAGTCATTGGCATTGC
wnt6 - right homology arm	AGTGGCATTTAAATACCTATTATC	GCAAATATTTTGGCAAAGTG
wntD - left homology arm	AAGCCCTTTGTATCGCATTTTTG	CTCCAAAACTGCTGCCAG
wntD - right homology arm	CTACTACCAGTACACCCAG	ACGTATTTATTGATAGCATTTGTTG
wnt10 - left homology arm	TAAAACCCTCCTAAAACCG	TTCTTGCTGCTGCTTCTG
wnt10 - right homology arm	CAGCAGCAGCAGCAACAG	TTGACAAGCGACCCAGGC
wg - T7 assay	CGCATGCTAATGATGATTATGCCT	TCACCCAAAACCCCATTCATATCT
wnt2 - T7 assay	GAAACGGCAAAGGAAGGGAAATTA	CTGTCTTTCTGTCAGTTTTGTGCT
wnt4 - T7 assay	GTATCCCAGATTCCCAGGTAATCC	GCATCCACAATATATCACGCCAAA
wnt5 - T7 assay	GTGCAAGAAGAGTATGTGCTTCTT	AATAGTAAGAGGAACAGGAATAGGAA
wnt6 - T7 assay	TCTGAAAGGGATTAGTCAGAGCAG	CAACAGCTTTGTAGGCTCATTTCA
wntD - T7 assay	CAGGGTACATTCAGAGCATTTTCC	CGATCTAACGACATCGCAGAGATA
wnt10 - T7 assay	AACGAAAATGCCGTGCAATTAAAC	CATGCATGAAGGTGAAAACAGTCT
wnt4 - in situ probe (5' linker for appending T7)	ggccgcggTGGACCCTGCAGATACATGC	cccggggcGTCCATTGTTGAACCGGCAC
Gal4 - in situ probe (5' linker for appending T7)	ggccgcggAAGAAAAACCGAAGTGCGCC	cccggggcATCACTGAAGGACGAGGGGA