SUPPLEMENTARY FIGURE LEGENDS

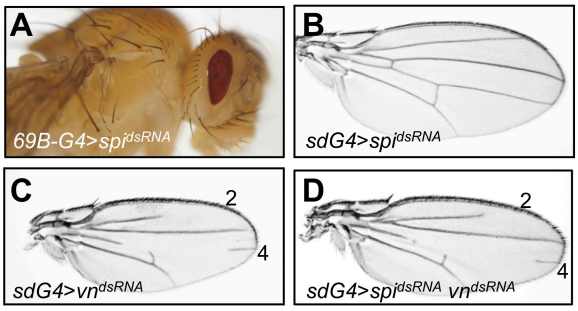

Fig. S1. Reduction of *spi* expression does not enhance *vn* mutant wing phenotypes. (A) *69B-Gal4; UAS-spi*^{dsRNA}. The fly has small eyes indicating effective RNAi. (B) *scalloped (sd)-Gal4; UAS-spi*^{dsRNA} (29°C). The wing is normal. (C) *sd-Gal4; UAS-vn*^{dsRNA} (29°C) and (D) *sd-Gal4; UAS-spi*^{dsRNA}*; UAS-vn*^{dsRNA} (29°C). The wings have similar phenotypes with loss of parts of L4, L2 and the ACV. Two dsRNA lines for *spi* were tested (TRiP 28387 and 34645) with multiple drivers (29°C) that are expressed in the wing *(71B-Gal4, 69B-Gal4, Act5C-Gal4,* and *sd-Gal4).* None resulted in wing defects, but all gave a small eye phenotype (except *71B-Gal4).* Two dsRNA lines for *vn* were tested (VDRC 109437 and 50358) and both gave a vein-loss phenotype with *69B-Gal4* and *sd-Gal4*. The vein loss with *sd-Gal4* was more extreme and is shown here.

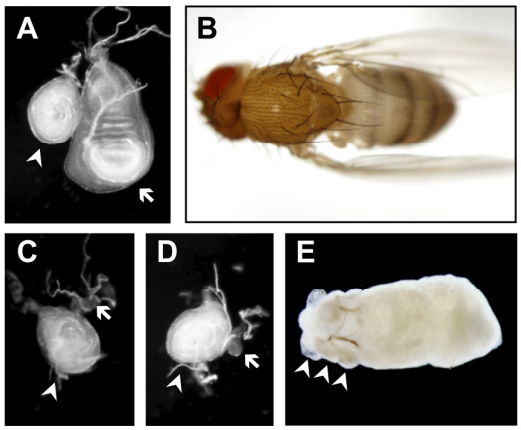

Fig. S2. *vn-Gal4* is an amorphic allele of *vn*. (A) Wild-type third instar wing disk (arrow) and leg disk (arrowhead). (B) Wild-type adult. (C) $vn^{L6/RG}$ wing disk (arrow) and leg disk (arrowhead). (D) vn^{L6}*/vn-Gal4* wing disk (arrow) and leg disk (arrowhead). In both (C) and (D) the wing disk fails to grow beyond a rudimentary size. vn^{L6} and vn^{RG} are known molecular nulls (Donaldson et al., 2004) and the similar phenotype associated with the *vn-Gal4* allele shows it functions as a amorph. (E) Terminal vn^{L6}*/vnGal4* animal dissected from pupal case, lacking any wing disk derived structures and without pigmented adult cuticle. Partially everted leg structures are present (arrowheads).

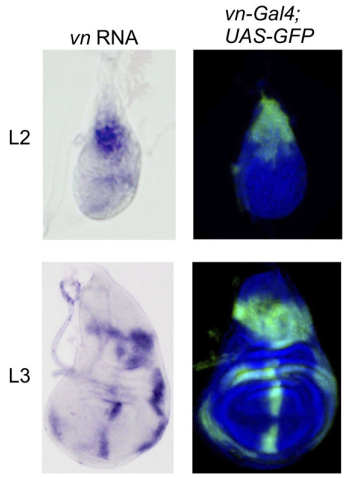

Fig. S3. *vn-Gal4* is expressed in a similar pattern to *vn*. *vn* RNA detected using *in situ* hybridization *(vn* RNA) is expressed in a similar pattern as GFP *(UAS-CD8::GFP; vn-Gal4)* in early (second larval instar, L2) and late (third instar, L3) wing disks.

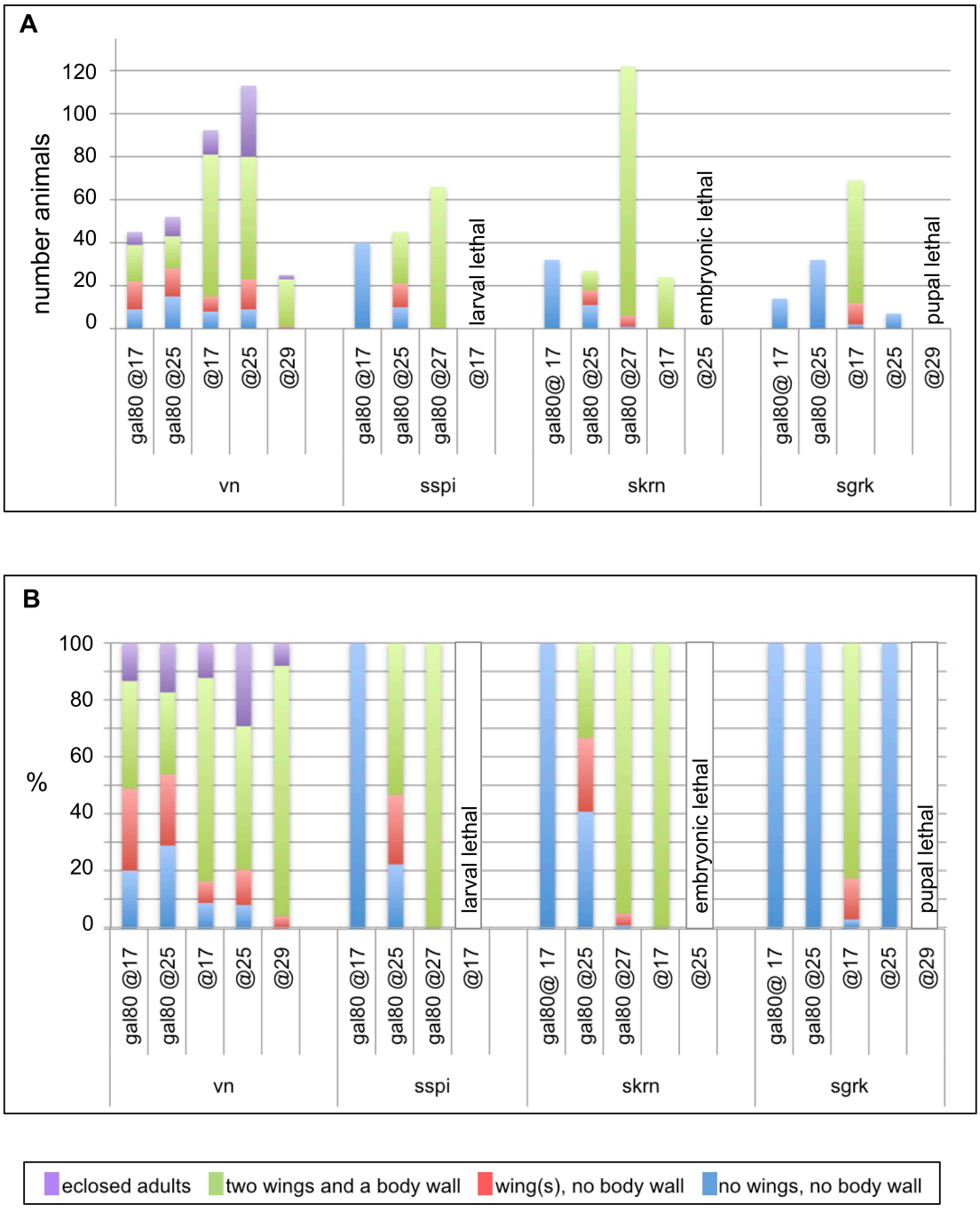

Fig. S4. Extent of rescue of *vn* mutants by expression of TGF-α ligands is dependent on expression level. Each *UAS-ligand* transgene was expressed using the *vn-Gal4* allele. The animals were classified according to the extent of rescue using the color-coded key shown in the figure. Only adults and pharate adults (differentiated adults that fail to eclose) were

scored. The classes range from pharate adults lacking any derivatives of the wing disk (wing and body wall) to normal flies that eclosed. Transgene expression was manipulated by temperature and/or co-expression of the Gal80^{TS} inhibitor (Fig. S2). (A) The histogram shows the numbers of animals scored in each phenotypic class. Survival varied by genotype and so different total numbers were examined for each genotype. In optimal conditions, approximately 30% of the expected number of *vn* mutants survived to adults when rescued by *UAS-vn*. In optimal conditions, rescue by the TGF-α ligands allowed a fraction of the expected progeny to reach the pharate adult stage, approximately 10% with *UAS-sspi,* 7% with *UAS-sKrn* and 5% with *UAS-sgrk.* (B) The same data are shown as a percentage of total animals scored. The results suggest that expression of *UAS-vn* was relatively insensitive to dose and showed a similar extent of rescue across the range of conditions tested. In contrast, each of the transgenes encoding TGF-α ligands were sensitive to the level of expression and optimal rescue of wing-disk derived structures occurred within a narrow expression range. A single UAS-transgene was tested for *grk* and *Krn*. Five different *UAS-vn* transgenes were tested and all gave similar results (*UAS-vn1.1, UAS-vn1.2, UAS-vn2.8, UAS-vnGFP*^{58} and *UAS-vnGFP*^{12}*)*. Four different *UAS-spi* transgenes were tested and gave similar results *(UAS-sspi* on X, *UAS-sspi* at 49A, *UAS-sspiGFP*^{M36} and *UAS-sspiGFP*^{F5N}*).*

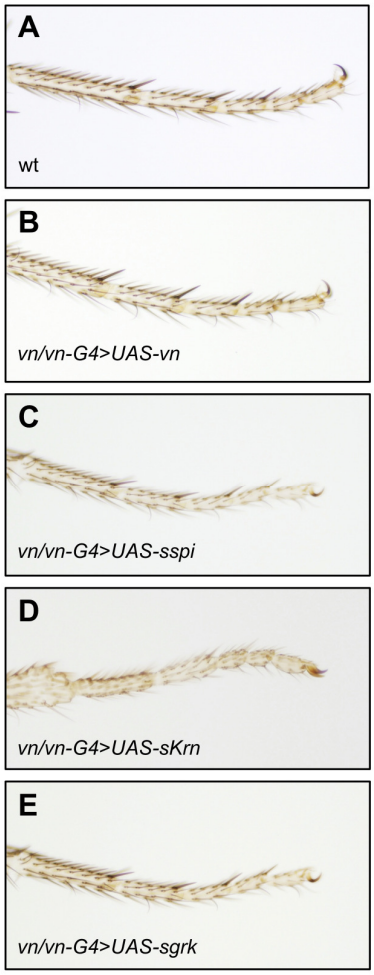

Fig. S5. Expression of the TGF-α ligands in the correct expression domain rescues leg development in *vn* mutants. Tarsal segments of the second leg and the terminal claw of the indicated genotypes are shown. (A) wild type. (B) *UAS-vn; vn^{L6}/vn-Gal4* (17°C). (C) *UAS-sspi; $Gal80^{TS}$; vn^{L6}/vn-Gal4* (27°C). (D) *UAS-sKrn, vn^{L6}/vn-Gal4* (17°C). (E) *UAS-sgrk, vn^{L6}/vn-Gal4* (17°C). All genotypes have five tarsal segments and claws. The flies shown in (C-E) are pharate adults and have shorter legs because they are not fully extended.

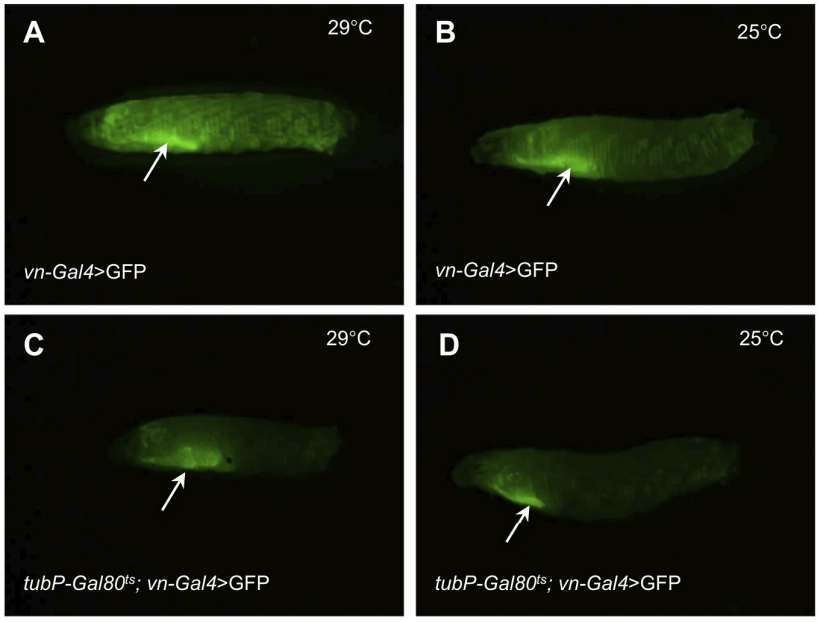

Fig. S6. Production of a range of *vn-Gal4*-driven transgene expression levels. The level of transgene expression was manipulated by temperature (Gal4 is more active at higher temperature) and/or co-expression of the $Gal80^{TS}$ inhibitor and temperature ($Gal80^{TS}$ is less active at higher temperature). The figures show expression of *UAS-GFP* in the indicated genotypes at two different temperatures. (A) *vn-Gal4; UAS-GFP* at 29°C shows the strongest expression of GFP. Expression is most prominent in the salivary glands (arrow) and the muscles (lattice throughout the body). (B) *vn-Gal4; UAS-GFP* at 25°C shows expression of GFP in the salivary glands (arrow) and the muscles (lattice throughout the body) although the level of expression is lower. (C) *Gal80; vn-Gal4; UAS-GFP* at 29°C shows expression of GFP in the salivary gland (arrow). (D) *Gal80; vn-Gal4; UAS-GFP* at 25°C shows expression of GFP in the salivary gland (arrow). Expression in (D) appears weaker than in (C). All images were taken with the same exposure.

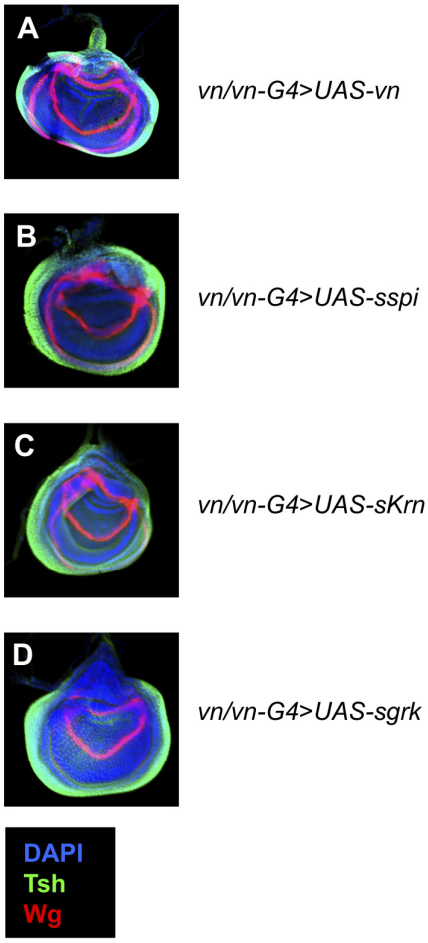

Fig. S7. Partial rescue of *vn* mutant wing disks. (A) *UAS-vn; vn^{L6}/vn-Gal4* (25°C). (B) *UAS-sspi; $Gal80^{TS}$; vn^{L6}/vn-Gal4* (25°C). (C) *UAS-sKrn, vn^{L6}/vn-Gal4* (17°C). (D) *UAS-sgrk, vn^{L6}/vn-Gal4* (17°C). All the wing disks have a round shape corresponding to the wing region only. These develop into the thorax of pharate adults with wings but no body wall (for example, fly shown in Fig. 5A, II). Wing disks were stained for Tsh (green), Wg (red) and DAPI (DNA, blue).

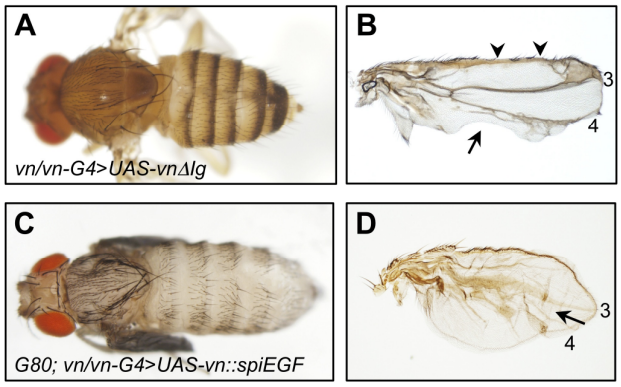

Fig. S8. An Ig-domain is not essential for rescuing *vn* mutants. (A) Expression of *vn* transgene lacking the Ig-domain *(UAS-vnΔIg; vn^{L6}/vn-Gal4)* rescues *vn* mutants to adults. The wing (B) had deletions of margin bristles (arrowheads) and notches (arrow). (C) Expression of transgene encoding a chimera in which the Vn EGF is replaced by the Spi EGF domain *($Gal80^{TS}$/UAS-vn*::spiEGF*; vn^{L6}/vn-Gal4)* recues *vn* mutants to the pharate adult stage. The wing (D) had extra veins, characteristic of Egfr overactivity (arrow).