Djiane et al., http://www.jcb.org/cgi/content/full/jcb.201007023/DC1

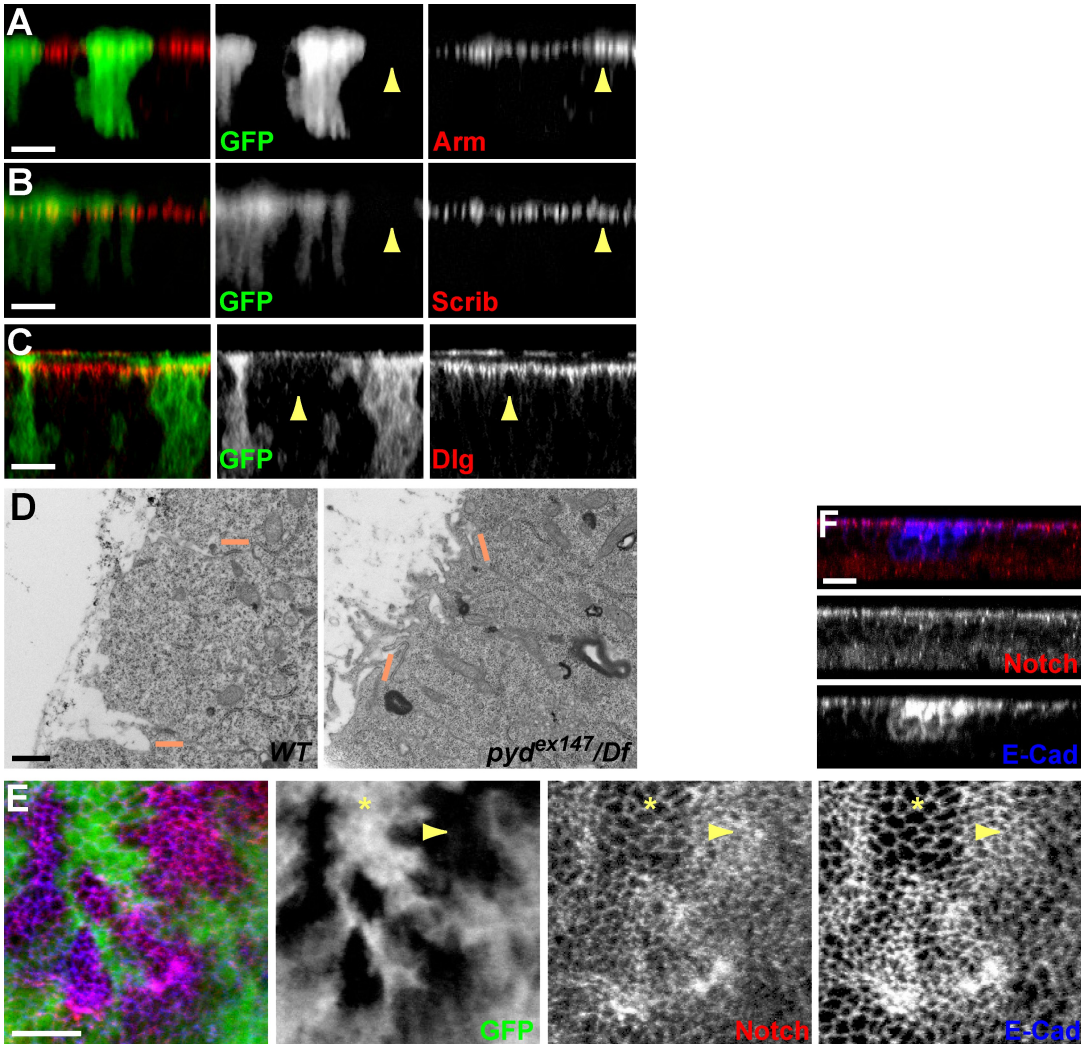

Figure S1. **Pyd is not required for epithelial polarity in *Drosophila*.** (A–C) In *pyd* mutant clones, marked by the absence of GFP and highlighted by arrowheads, levels of SJ proteins, such as Scrib or Dlg (B and C), are unaffected, whereas Arm (A) is enriched. Bars, 10 µm. (D) Transmission electron microscopic images of wing disc epithelial cells from wild-type (WT; left) and $pyd^{ex147}/Df(3R)p\text{-}XT103$ (right) larvae. AJs are highlighted with orange lines. *pyd* mutant cells have more apical processes (5.95 ± 1.58; $n = 20$) than wild type (1.80 ± 0.94; $n = 15$), reflecting a modest apical expansion. Bar, 500 nm. (E and F) E-Cad and Notch accumulate in *pyd* mutant cells. (E) More Notch and E-Cad are present at the cell surface in *pyd* mutant cells (arrowheads) compared with wild-type cells (asterisks). Confocal apical xy sections on unpermeabilized fixed wing discs containing *pyd* mutant clones (absence of GFP), where Notch extracellular and E-Cad were detected. (F) No up-regulation of Notch at the AJ was detected when E-Cad was overexpressed using the *scabrous-Gal4* driver. Confocal z section. Bars, 10 µm.

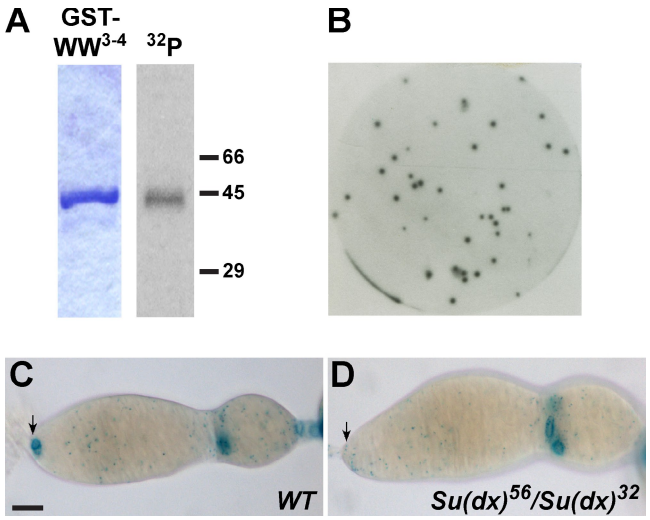

Figure S2. **Interaction between Su(dx) and Pyd.** (A and B) A peptide-binding screen for Su(dx) partners identifies Pyd. (A, left) SDS-PAGE of purified GST-WW$^{3–4}$ protein detected by Coomassie blue staining. (right) ^{32}P-labeled and purified GST-WW$^{3–4}$ detected by autoradiography. The protein fusion containing GST, a protein kinase recognition site, and the Su(dx) WW$^{3–4}$ (GST-WW$^{3–4}$) was purified on glutathione-Sepharose (GE Healthcare) and radioactively labeled using cAMP-dependent bovine heart muscle kinase (Sigma-Aldrich) and γ-^{32}P–labeled ATP (PerkinElmer). Molecular masses are given in kilodaltons. (B) Fourth round of screening showing binding of ^{32}P-labeled GST-WW$^{3–4}$ to phage plaques containing Pyd cDNA, which were detected by autoradiography. Approximately 600,000 colonies of a *Drosophila* third instar larval λGT11 cDNA expression library (5′ Stretch; Takara Bio Inc.) were screened after transfer for 3.5 h at 37°C to nitrocellulose filters saturated in 10 mM IPTG. To detect binding, filters were incubated with ^{32}P-labeled GST-WW$^{3–4}$ for 3 h at room temperature, and bound WW domain was detected by autoradiography after extensive rinsing with PBT (PBS and 0.1% Tween 20). Positives were subjected to four further rounds of rescreening. (C and D) Expression of the Notch reporter *E(spl)m7–lacZ* is reduced in *Su(dx)* mutant germaria carrying one copy of the Notch reporter *E(spl)m7–lacZ*. (C) 3–4-d-old wild-type germaria stained for β-galactosidase activity normally show clear staining in cap cells (arrow; 74.9%, *n* = 339). (D) Trans-heterozygous *Su(dx)* mutant combination *Su(dx)^{32}/ Su(dx)^{56}* has no apparent staining in cap cells (arrow; 92.7%, *n* = 205). Other combinations of *Su(dx)* mutants show similar effects: *Su(dx)^{SP}/ Su(dx)^{32}* and *Su(dx)^{SP}/ Su(dx)^{56}* have no apparent *E(spl)m7–lacZ* staining in 92.4% (*n* = 158) and 76% (*n* = 271) of cases, respectively. Bar, 10 µm.

Table S1. **Dissociation constants (K_ds) for Su(dx) WW domain–Pyd peptide interactions**

WW domain	Peptide ligands	
	Pyd-EGLPPPYTV	Pyd-APPPQSYPQ
WW[1]	23.2 ± 1.5	–
WW[2]	20.4 ± 0.9	–
WW[1–2]	28.0 ± 4.1	0.7 ± 0.06
WW[3–4]	89.3 ± 5.0	–

The equilibrium K_ds given in micromolars were calculated using fluorescence data. All errors are SDs from triplicate measurements (smaller errors result from curve fitting). – indicate no observable binding.

Table S2. **Su(dx) overexpression does not modify the extra cells found between the L3 and L4 veins in *pyd^{ex147}/+* or *pyd RNAi***

Genotype	L3/L4 width at posterior cv			
	Total	*n*	Mean	SD
WT	1,130	70	16.14	0.98
dppG4/+	795	48	16.56	0.82
dppG4 UAS Su(dx)	643	42	15.31	1.11
dppG4/+ pyd^{ex147}/+	1,792	98	18.29	0.93
dppG4 UAS Su(dx) pyd^{ex147}/+	479	27	17.74	1.02
dppG4 UAS pyd RNAi	1,760	99	17.78	1.01
dppG4 UAS Su(dx) UAS pyd RNAi	1,183	67	17.66	1.25

The number of cells marked by trichome was counted in defined segments adjacent to the intersection of the posterior cross vein with L4. Comparisons were made primarily between *dppG4/+ pyd^{ex147}/+* and *dppG4 UAS Su(dx) pyd^{ex147}/+* or *dppG4 UAS pyd RNAi* and *dppG4 UAS Su(dx) UAS pyd RNAi*. All other genotypes are controls. cv, cross vein. WT, wild type.

Table S3. ***Su(dx)* loss-of-function combination does not modify the extra cells found between the L3 and L4 veins in *pyd^{ex147}/+***

Genotype	L3/L4 width at posterior cv			
	Total	*n*	Mean	SD
WT	1,130	70	16.14	0.98
Su(dx)^{32}/Su(dx)^{56}	936	56	16.71	1.23
pyd^{ex147}/+	1,513	83	18.23	0.93
Su(dx)^{32}/Su(dx)^{56} pyd ^{ex147}/+	909	48	18.94	0.91
pyd^{ex147}/pyd^{ex147}	1,386	63	22	1.51

The number of cells marked by trichome was counted in defined segments adjacent to the intersection of the posterior cross vein with L4. Comparisons were made primarily between *pyd^{ex147}/+* and *Su(dx)^{32}/Su(dx)^{56} pyd^{ex147}/+*. All other genotypes are controls. cv, cross vein. WT, wild type.