

Fig. S1. Lgl localization requires both PP1 and Scrib/Dlg

Compared to WT cells (A), *pp1*-depleted cells (B) exhibit mild polarity loss and occasional multilayering. Compared to WT cells (C), *pp1*-depleted cells display mild loss of cortical Lgl (D, also compare to *dlg*-depletion in F). (E) Quantification of Lgl localization. (F) *dlg*-depleted cells strongly mislocalize cortical Lgl and this is not rescued by overexpression of PP1 (G). (H) Quantification of Lgl localization. Scale bars, 10µm. White lines in (F-G) indicate flip-out GAL4 clones of given genotypes. (E) Two-tailed t-test with Welch's correction. (H) One-way ANOVA with Tukey's multiple comparisons test. Error bars indicate S.D. PM Index=cortical/cytoplasmic intensity. Data points are individual cell measurements. n.s. (not significant) P > 0.05, ****P < 0.0001.





(A) Cartoon showing the Scrib protein domain composition and location of the SILK and RVxF motifs. Below: alignment showing conservation of the SILK motif and RVxF motifs. Note that in vertebrates (right), the RVxF motif is located slightly C-terminal to its position in insects (left). Red boxes indicate residues mutated in myr-^{ScribTAAA/RAGA} construct. Compared to WT myr-Scrib (B), myr-Scrib^{TAAA/RAGA} (C) localizes less well to the cell cortex but is still enriched at the basolateral membrane, quantified in (D). Both constructs contain V5 epitope tags, used for detection. (E-H) Compared to WT wing discs (E), *scrib* mutant wing discs overgrow and form tumors (F). Overexpression of myr-Scrib largely rescues these phenotypes (G), while expression of myr-Scrib^{TAAA/RAGA} (J) provides less efficient rescue of *scrib* mutant: myr-Scrib shows complete restoration of the monolayered epithelium in 78.6% (n=14) of follicles, compared to complete restoration in 36.8% (n=19) of myr-Scrib^{TAAA/RAGA} -rescued follicles. Scale bars, 10μm, except E-H, 100μm. White lines in (I-J) indicate MARCM clones of given genotypes, (B-C) are flip-out GAL4 clones. (D) Two-tailed t-test with Welch's correction. Error bars indicate S.D. PM Index=cortical/cytoplasmic intensity. Data points are individual cell measurements. ****P < 0.0001.



Fig. S3. In depth examination of DIg SH3 and HOOK domains

(A) Schematic of the DIg domains used in the Ed-DIg^{PDZ3-SH3-HOOK-GUK} construct, with sequence alignments showing conservation of the SH3 and HOOK domain sequences chosen for study. Motifs targeted for mutation are indicated by red outlines. Arrows in cartoon indicate relative locations of targeted sequences in the protein. (B) Quantification of Scrib recruitment to the polarity site in S2 induced polarity assay. Compared to the WT Ed-DIg^{PDZ3-SH3-HOOK-GUK} construct, which does recruit Scrib, the SH3 mutant AAW construct has reduced ability to recruit Scrib. Similarly, all four constructs targeting single residues of the RVxF motif show equally impaired ability to recruit Scrib. However, the four constructs targeting conserved residues in the C-terminal HOOK domain do not impair Scrib recruitment. Red line indicates the average for the WT construct control. One-way ANOVA with Tukey's multiple comparisons test. Error bars indicate S.D. Data points are individual cell clusters. Statistical tests are comparisons to the Ed-DIg^{PDZ3-}SH3-HOOK-GUK</sup> control construct. Enrichment index = contact site/non-contact site intensity. n.s. (not significant) P > 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001.



Fig. S4. Validation of RNAi rescue approach for minimal DIg constructs

(A-D) Knocking down *dlg* in the posterior half of the wing disc using an RNAi construct targeting PDZ2-encoding sequences (B) causes polarity loss and disrupted epithelial architecture. These phenotypes are fully rescued by co-expression of Dlg^{SH3-HOOK-GUK} (C) and Dlg^{SH3-HOOK} (D), and neither constructs is targeted by the RNAi reagent used to deplete endogenous Dlg (C', D'). Scale bars, 100µm.



Fig. S5. Using S2 cell induced polarity to study Scrib-Dlg interaction

(A) Ed-GFP expression in S2 cells allows induction of a polarity domain where cells adhere. (B) Fusing the Scrib LRR+LAPSD domains to Ed creates a domain of polarized Scrib. (C) Schematic of the Scrib domains used in the Ed-Scrib^{LRR+LAPSD} construct. Quantification of Dlg enrichment shows that Scrib cannot recruit Dlg to the polarity site. (D) Co-IP assay of Scrib^{LRR+LAPSD} and Dlg^{PDZ3-SH3-HOOK-GUK} from S2 cells fails to detect interaction between these proteins. Scale bars, 10µm. (C) Two-tailed t-test with Welch's correction. Error bars indicate S.D. Data points are individual cell clusters. Enrichment index = contact site/non-contact site intensity. n.s. (not significant).



Fig. S6. Scrib and DIg protection of LgI is partly independent of phosphorylation

(A-B) Compared to its localization in WT cells, non-phosphorylatable Lgl^{S5A} exhibits a slight, but significant, reduction in cortical levels in *scrib* RNAi (A) and *dlg* RNAi expressing cells (B). (C) Quantification of LglS5A::GFP levels. *scrib* or *dlg* RNAi both significantly reduce Lgl^{S5A} cortical levels compared to WT cells. Scale bars, 10µm. White lines indicate flip-out GAL4 clones of given genotypes. PM Index=cortical/cytoplasmic intensity. Data points represent individual cell measurements. Error bars represent S.D. (C) One-way ANOVA with Dunnett's multiple comparisons test. ***P < 0.001, ****P < 0.0001.

Table S1. Key Resources

Reagent	Reference and Source
Drosophila stocks	
UAS-DIg ^{WT} ::HA	(Sharp et al., 2021)
UAS-DIg ^{ASAKA} ::HA	This study
UAS-DIg ^{SH3-HOOK} ::HA	This study
UAS-DIg ^{SH3-HOOK-GUK} ::HA	This study
UAS-myr-Scrib::V5	(Khoury and Bilder, 2020)
UAS-myr-Scrib ^{TAAA/RAGA} ::V5	This study
dlg ^{m52}	(Perrimon, 1988)
dlg ^{m30}	(Woods and Bryant, 1989)
dlg ^{40.2}	(Mendoza-Topaz et al., 2008)
scrib ¹	(Bilder and Perrimon, 2000)
scrib ²	(Zeitler et al., 2004)
UAS-dlg RNAi HMS01954	Bloomington Drosophila Stock Center (BDSC): 39035
UAS-scrib RNAi HMS01993	BDSC: 39073
UAS-Pp1-87B RNAi HMS00409	BDSC: 32414
UAS-Pp1-87B::HA	BSDC: 24098
UASp-Sds22::GFP	BDSC: 65851
UASp-Scrib::GFP	(Zeitler et al., 2004)
UAS-EGFP::Dlg	(Koh et al., 1999)
UAS-Lgl::GFP	(Wirtz-Peitz et al., 2008)
UAS-Lgl ^{KAFA} ::GFP	(Moreira et al., 2019), Generously provided by E.
	Morais de Sá.
Lgl::GFP	(Dong et al., 2015), Generously provided by Y. Hong.
Lgl ^{S5A} ::GFP	(Dong et al., 2015), Generously provided by Y. Hong
act>y+>GAL4 UAS-his::RFP	BDSC: 30558
tub-GAL80 FRT19A; act-GAL4 UAS-	(Lee and Luo, 1999), BDSC: 42726, 5134
GFP	
tj-GAL4	Kyoto Stock Center: 104055
hh-GAL4	(Tanimoto et al., 2000)
D174-GAL4	(Sharp et al., 2021)
Plasmids	
pMI-Ed-GFP	(Johnston et al., 2009), Generously provided by C. Johnston.
pMT-Ed-GFP-Dlg ^{PD23-SH3-HOOK-GUK}	(Garcia et al., 2014), Generously provided by C. Johnston.
pMT-Ed-GFP-Dlg ^{SH3-GUK,} ∆HOOK	Generously provided by C. Johnston.
pMT-Ed-GFP-DIg ^{SH3-GUK}	This study
pMT-Ed-GFP-DIg ^{PDZ3-SH3-HOOK}	This study
pMT-Ed-GFP-Dlg ^{SH3}	This study
рМТ-Ed-GFP-Dlg ^{HOOK}	This study
pMT-Ed-GFP-Dlg ^{SH3-HOOK}	This study
pMT-Ed-GFP-DIg ^{PDZ3-SH3-HOOK-GUK[m30]}	This study
pMT-Ed-GFP-Dlg ^{PDZ3-SH3-HOOK-} GUK[ASAKA]	This study
pMT-Ed-GFP-Dlg ^{PDZ3-SH3-HOOK-} GUK[ASVKF]	This study

pMT-Ed-GFP-DIg ^{PD23-SH3-HOOK-} GUK[RSAKF]	This study
pMT-Ed-GFP-Dlg ^{PDZ3-SH3-HOOK-} GUK[RSVKA]	This study
pMT-Ed-GFP-Dlg ^{PDZ3-SH3-HOOK-} GUK[RSAKA]	This study
pMT-Ed-GFP-Dlg ^{PDZ3-SH3-HOOK-} GUK[AEAV]	This study
pMT-Ed-GFP-Dlg ^{PDZ3-SH3-HOOK-} GUK[AEAA]	This study
pMT-Ed-GFP-Dlg ^{PDZ3-SH3-HOOK-} GUK[AAAA]	This study
pMT-Ed-GFP-DIg ^{PDZ3-SH3-HOOK-GUK[AAN]}	This study
pMT-Ed-GFP-Dlg ^{PDZ3-SH3-HOOK-} GUK[AAW]	This study
pMT-Ed-GFP-Scrib ^{LRR+LAPSD}	This study
pMT-Scrib ^{LRR+LAPSD} ::V5	This study
pMT-GFP::DIa ^{PDZ3-SH3-HOOK-GUK}	This study
Antibodies	
Mouse anti-Dlg (1:100 IHC)	Developmental Studies Hybridoma Bank (DSHB): 4F3
Rabbit anti-aPKC (1:200 IHC)	Santa Cruz Biotech: sc-216
Guinea pig anti-Scrib (1:500 IHC)	(Bilder and Perrimon, 2000)
Rabbit anti-HA tag C29F4 (1:500 IHC, 1:10,000 WB, 1:200 IP)	Cell Signaling Technologies (CST): #3724
Mouse anti-HA tag 6E2 (1:10,000 WB)	CST: #2367
Mouse anti-HA tag 2-2.2.14 (1:200 IP)	Invitrogen: 26183
Rabbit anti-GFP (1:5000 WB)	Origene: TP-401
Mouse anti-GFP JL-8 (1:10,000 WB)	Clontech: 632380
Mouse anti-V5 (1:500 IHC, 1:200 IP, 1:5000 WB)	Invitrogen: R960-25
Primers	
myrScrib_silk_F	tgaatagggaattggggtaccatgggtaactgcctcaccac
myrScrib_Silk_R	tctgatccaacgcggcagcagtcagtct
myrScrib_rvxf_F	tgctgccgcgttggatcagaatcgattgcagcggttgaacgatac
myrScrib_Rvxf_R	cctccacttgggcgccagcggcgcgatc
myrScrib_Agel_F	cgctggcgcccaagtggagggcgaagatg
myrScrib_Agel_R	ggtacgacggggagcgggcaccggttgacccgtggaactgtctatc
Nterm_Dlg_F	actctgaatagggaattggctcgagcaaaATGACAACGAGGAA AAAGAAGCGC
Nterm-Dlg-RVxF	tggGCcttaGcgctgGCgtccctagctcgcattttgcg
Dlg-RVxF-Cterm	gacGCCAGCgCtaagGCccagggacatgcggcag
Dlg_Cterm_R	ggttccttcacaaagatcctctagaatcTTAAGCGTAGTCTGGG AC
Dlg-SH3_for	Actctgaatagggaattggctcgagcaaaatgcagtaccgcccagagg ag

Dlg-SH3-HOOK_rev	Ccgactgggagtagttgatggacaaacgctgtac	
Dlg-Cterm_for	catcaactactcccagtcgggaccaacc	
Ed-PDZ3-SH3-HOOK-GUK m30 F	TGTGCGCGCCCCGTTTGACTACG	
Ed-PDZ3-SH3-HOOK-GUK m30 R	TACAGCGATCGCTTTTGCG	
Ed-PDZ3-SH3-HOOK-GUK ASAKA	TAAGGCCCAGGGACATGCGGCAGCT	
F		
Ed-PDZ3-SH3-HOOK-GUK ASAKA	GCGCTGGCGTCCCTAGCTCGCATTTTGC	
	GAGGAGTACAATCGCTTCG	
delPDZ3 F		
Ed- PDZ3-SH3-HOOK-GUK	GCGCGGTTCTCTGGTTAT	
delPDZ3 R		
Ed- PDZ3-SH3-HOOK-GUK delGUK	TCCCAGTCGGGACCAACC	
Fd- PD73-SH3-HOOK-GUK delGUK	GTAGTTGATGGACAAACGCTGTAC	
R		
Ed- PDZ3-SH3-HOOK-GUK	CGAGCTAGGGACCGCAGC	
deltaSH3_F	TT000T00T000040040	
Ed- PDZ3-SH3-HOOK-GUK		
Ed- PDZ3-SH3-HOOK-GUK	TAAGTTCCAGGGACATGCGGCAGCT	
ASVKF_F		
Ed- PDZ3-SH3-HOOK-GUK	ACGCTGGCGTCCCTAGCTCGCATTTTGC	
ASVKF_R		
Ed- PDZ3-SH3-HOOK-GUK		
Fd- PDZ3-SH3-HOOK-GUK	GCGCTGCGGTCCCTAGCTCGCATTTTGC	
RSAKF_R		
Ed- PDZ3-SH3-HOOK-GUK	TAAGGCCCAGGGACATGCGGCAGCT	
RSVKA_F		
Ed- PDZ3-SH3-HOOK-GUK		
Ed- PDZ3-SH3-HOOK-GUK	TAAGGCCCAGGGACATGCGGCAGCT	
RSAKA F		
Ed- PDZ3-SH3-HOOK-GUK	GCGCTGCGGTCCCTAGCTCGCATTTTGC	
RSAKA_R		
Ed- PDZ3-SH3-HOOK-GUK	GTGGCAGGCACGACGAGTTCTC	
Fd- PD73-SH3-HOOK-GUK	GCTGCATCGTCGGAGGCATTGGT	
AAW661/2 R		
Ed- PDZ3-SH3-HOOK-GUK	GAACGTGTTGTCCTACGAGGCC	
EEN768_F		
Ed- PDZ3-SH3-HOOK-GUK	GCCGCGGAAGCTCCTTCAGCATTG	
	CGTGTTGTCCGCAGAGGCCGTACAGC	
AEAV F	CGTGTTGTCCGCAGAGGCCGTACAGC	
Ed- PDZ3-SH3-HOOK-GUK	TTCTCCTCGGAAGCTCCT	
AEAV_R		

Ed- AEAA F	PDZ3-SH3-HOOK-(GUK	GCCGCACAGCGTTTGTCCATCAAC
Ed-	PDZ3-SH3-HOOK-0	GUK	CTCTGCGGACAACACGTTCTCCTC
AEAA_R			
Ed-	PDZ3-SH3-HOOK-0	GUK	GCCGCACAGCGTTTGTCCATCAAC
AAAA_F			
Ed-	PDZ3-SH3-HOOK-(GUK	CGCTGCGGACAACACGTTCTCCTC
AAAA_R			
Ed_scrib_G	Bibson_F		gcatggacgagctgtacaagctatgttcaagtgcattcccatcttc
Ed_scrib_G	Bibson_R		ccttcgaagggccctctagagtcggtgctagcctctgc
GFP_fwd			ctactagtccagtgtggtggatggtgagcaagggcgag
GFP_rev			tgctgacagcggatctcttgtacagctcgtc
PDZ3-SH3-	-HOOK-GUK_fwd	(for	caagagatccgctgtcagcaccgaggatataac
cytosolic D	g)		
PDZ3-SH3-	-HOOK-GUK_rev	(for	aatggtgatggtgatgatgatcatagagattccttggaaggtac
cytosolic D	g)	•	
scrib_fwd (for cytosolic Scrib)		ctactagtccagtgtggtggatgttcaagtgcattcccatcttcaag
scrib_rev (f	or cytosolic Dlg)		cttcgaagggccctctagacgcgtcggtgctagcctct

Table S2. Scrib and Dlg transgenic constructs

Construct	Description
Dlg ^{ASAKA}	RSVKF 675-679 to ASAKA
DIgSH3-HOOK	Fragment encompassing aa564-784+961-975
DIgSH3-HOOK-GUK	Fragment encompassing aa564-975
myr-Scrib ^{TAAA/RAGA}	TILK 286-289 to TAAA and RVGF 621-624 to
	RAGA
pMT-Ed-GFP-DIg ^{PDZ3-SH3-HOOK-GUK}	Fragment encompassing aa473-975
pMT-Ed-GFP-Dlg ^{SH3-GUK,} ∆HOOK	Fragment encompassing aa597-678+771-975
pMT-Ed-GFP-DIg ^{SH3-GUK}	Fragment encompassing aa568-975
pMT-Ed-GFP-Dlg ^{PDZ3-SH3-HOOK}	Fragment encompassing aa473-784+961-975
pMT-Ed-GFP-Dlg ^{SH3}	Fragment encompassing aa597-678+766-
	783+961-975
pMT-Ed-GFP-Dlg ^{HOOK}	Fragment encompassing aa473-485+671-
	784+961-975
pMT-Ed-GFP-Dlg ^{SH3-HOOK}	Fragment encompassing aa473-485+564-
	784+961-975
pMT-Ed-GFP-Dlg ^{PDZ3-SH3-HOOK-GUK[m30]}	L608→P
pMT-Ed-GFP-Dlg ^{PDZ3-SH3-HOOK-GUK[ASAKA]}	RSVKF 675-679 to ASAKA
pMT-Ed-GFP-Dlg ^{PDZ3-SH3-HOOK-GUK[ASVKF]}	RSVKF 675-679 to ASVKF
pMT-Ed-GFP-Dlg ^{PDZ3-SH3-HOOK-GUK[RSAKF]}	RSVKF 675-679 to RSAKF
pMT-Ed-GFP-Dlg ^{PDZ3-SH3-HOOK-GUK[RSVKA]}	RSVKF 675-679 to RSVKA
pMT-Ed-GFP-Dlg ^{PDZ3-SH3-HOOK-GUK[RSAKA]}	RSVKF 675-679 to RSAKA
pMT-Ed-GFP-Dlg ^{PDZ3-SH3-HOOK-GUK[AEAV]}	YEAV774-777 to AEAV
pMT-Ed-GFP-Dlg ^{PDZ3-SH3-HOOK-GUK[AEAA]}	YEAV774-777 to AEAA
pMT-Ed-GFP-Dlg ^{PDZ3-SH3-HOOK-GUK[AAAA]}	YEAV774-777 to AAAA
pMT-Ed-GFP-DIg ^{PDZ3-SH3-HOOK-GUK[AAN]}	EEN768-770 to AAN
pMT-Ed-GFP-DIg ^{PDZ3-SH3-HOOK-GUK[AAW]}	EWW641-643 to AAW
pMT-Ed-GFP-Scrib ^{LRR+LAPSD}	Fragment encompassing aa1-715

Figure	Genotype
Fig. 1B	hsFLP[122]/+;+/UAS-Dlg[WT]::HA;act>y+>GAL4,UAS-his2av::mRFP/+
Fig. 1C	hsFLP[122]/+;+/UAS-Dlg[ASAKA]::HA;act>y+>GAL4,UAS-his2av::mRFP/+
Fig. 1D	hsFLP[122]/+;UAS-scrib RNAi 39073/UAS-Dlg[WT]::HA;act>y+>GAL4,UAS- his2av::mRFP/+
Fig. 1E	hsFLP[122]/+;UAS-scrib RNAi 39073/UAS-
5	Dlg[ASAKA]::HA;act>y+>GAL4,UAS-his2av::mRFP/+
Fig. 1G	d174-GAL4,dlg[40.2]/+
Fig. 1H	d174-GAL4,dlg[40.2]/Y
Fig. 1I	d174-GAL4,dlg[40.2]/Y;+/UAS-Dlg[WT]::HA
Fig. 1J	d174-GAL4,dlg[40.2]/Y;+/UAS-Dlg[ASAKA]::HA
Fig. 1K	hsFLP[1],FRT19A tub-GAL80/dlg[m52],FRT19A;act-GAL4,UAS-GFP/+
Fig. 1L	hsFLP[1],FRT19A tub-GAL80/dlg[m52],FRT19A;act-GAL4,UAS-GFP/UAS- Dlg[WT]::HA
Fig. 1M	hsFLP[1],FRT19A tub-GAL80/dlg[m52],FRT19A;act-GAL4,UAS-GFP/UAS- Dlg[ASAKA]::HA
Fig. 2A	
Fig. 2C	hsFLP[122]/+;+/UAS-Lgl::GFP;act>y+>GAL4,UAS-his2av::mRFP/+
Fig. 2D	hsFLP[122]/+;UAS-dlg RNAi 39035/UAS-Lgl::GFP;act>y+>GAL4,UAS- his2av::mRFP/+
Fig. 2E	hsFLP[122]/+;+/UAS-Lgl[KAFA]::GFP;act>y+>GAL4,UAS-his2av::mRFP/+
Fig. 2F	hsFLP[122]/+;UAS-dlg RNAi 39035/UAS-Lgl[KAFA]::GFP;act>y+>GAL4,UAS-
	his2av::mRFP/+
Fig. 4A	TJ-GAL4/UAS-Dlg[WT]::HA
Fig. 4B	TJ-GAL4/UAS-Dlg[SH3-HOOK-GUK]::HA
Fig. 4C	TJ-GAL4/UAS-Dlg[SH3-HOOK]::HA
Fig. 4E	d174-GAL4,dlg[40.2]/+
Fig. 4F	d174-GAL4,dlg[40.2]/Y
Fig. 4G	d174-GAL4,dlg[40.2]/Y;+/UAS-Dlg[SH3-HOOK-GUK]::HA
Fig. 4H	d174-GAL4,dlg[40.2]/Y;+/UAS-Dlg[SH3-HOOK]::HA
Fig. 4I	hsFLP[122]/+;UAS-dlg RNAi 39035/+;act>y+>GAL4,UAS-his2av::mRFP/+
Fig. 4J	hsFLP[122]/+;UAS-dlg RNAi 39035/UAS-Dlg[SH3-HOOK-
	GUK]::HA;act>y+>GAL4,UAS-his2av::mRFP/+
Fig. 4K	hsFLP[122]/+;UAS-dlg RNAi 39035/UAS-Dlg[SH3-HOOK-
	GUK]::HA;act>y+>GAL4,UAS-his2av::mRFP/+
Fig. 4M	hsFLP[122]/+;UAS-dlg RNAi 39035/+;act>y+>GAL4,UAS-his2av::mRFP/+
Fig. 4N	hsFLP[122]/+;UAS-dlg RNAi 39035/UAS-Dlg[SH3-HOOK-
	GUK]::HA;act>y+>GAL4,UAS-his2av::mRFP/+
Fig. 4O	hsFLP[122]/+;UAS-dlg RNAi 39035/UAS-Dlg[SH3-HOOK-
	GUK]::HA;act>y+>GAL4,UAS-his2av::mRFP/+
Fig. 5A	hsFLP[1],FRT19A tub-GAL80/dlg[m52],FRT19A;act-GAL4,UAS-GFP/+
Fig. 5B	hsFLP[1],FRT19A tub-GAL80/dlg[m52],FRT19A;act-GAL4,UAS-GFP/UAS- Dlg[WT]::HA
Fig. 5C	hsFLP[1],FRT19A tub-GAL80/dlg[m52],FRT19A;act-GAL4,UAS-GFP/UAS-
	Dlg[ASAKA]::HA
Fig. 5E	hsFLP[1],FRT19A tub-GAL80/dlg[m52],FRT19A;act-GAL4,UAS-GFP/+
Fig. 5F	hsFLP[1],FRT19A tub-GAL80/dlg[m30],FRT19A;act-GAL4,UAS-GFP/+

Table S3. List of genotypes used in Figures

Fig. 5G	hsFLP[1],FRT19A tub-GAL80/dlg[m52],FRT19A;act-GAL4,UAS-GFP/UAS-myr- Scrib::V5
Fig. 5H	hsFLP[1],FRT19A tub-GAL80/dlg[m52],FRT19A;act-GAL4,UAS-GFP/UAS-myr-
0	Scrib::V5
Fig. S1A,C	TJ-GAL4/+
Fig. S1B,D	TJ-GAL4/+;+/UAS-Pp1-87B RNAi 32414
Fig. S1F	hsFLP[122]/+;UAS-dlg RNAi 39035/+;act>y+>GAL4,UAS-his2av::mRFP/+
Fig. S1G	hsFLP[122]/+;UAS-dlg RNAi 39035/+;act>y+>GAL4,UAS-his2av::mRFP/UAS-
	Pp1-87B::HA
Fig. S2B	hsFLP[122]/+;+/UAS-myr-Scrib::V5;act>y+>GAL4,UAS-his2av::mRFP/+
Fig. S2C	hsFLP[122]/+;+/UAS-myr-Scrib[TAAA/RAGA]::V5;act>y+>GAL4,UAS-
	his2av::mRFP/+
Fig. S2E	d174-GAL4/+;scrib[2]/+
Fig. S2F	d174-GAL4/+;scrib[2]/scrib[1]
Fig. S2G	d174-GAL4/+;scrib[2]/scrib[1];+/UAS-myr-Scrib::V5
Fig. S2H	d174-GAL4/+;scrib[2]/scrib[1];+/UAS-myr-Scrib[TAAA/RAGA]::V5
Fig. S2I	hsFLP/+;act-GAL4,UAS-GFP/UAS-myr-Scrib::V5;tub-
	GAL80,FRT82B/scrib[1],FRT82B
Fig. S2J	hsFLP/+;act-GAL4,UAS-GFP/UAS-myr-Scrib[TAAA/RAGA]::V5;tub-
	GAL80,FRT82B/scrib[1],FRT82B
Fig. S4A	hh-GAL4/+
Fig. S4B	+/UAS-dlg RNAi 39035;hh-GAL4/+
Fig. S4C	UAS-dlg RNAi 39035/UAS-Dlg[SH3-HOOK-GUK]::HA;hh-GAL4/+
Fig. S4D	UAS-dlg RNAi 39035/UAS-Dlg[SH3-HOOK]::HA;hh-GAL4/+
Fig. S6A	hsFLP[122]/+;UAS-scrib RNAi 39073/lgl[S5A]::GFP;act>y+>GAL4,UAS-
	his2av::mRFP/+
Fig. S6B	hsFLP[122]/+;UAS-dlg RNAi 39035/lgl[S5A]::GFP;act>y+>GAL4,UAS-
	his2av::mRFP/+

Supplemental References

- **Bilder, D. and Perrimon, N.** (2000). Localization of apical epithelial determinants by the basolateral PDZ protein Scribble. *Nature* **403**, 676–680.
- Dong, W., Zhang, X., Liu, W., Chen, Y. jiun, Huang, J., Austin, E., Celotto, A. M., Jiang, W. Z., Palladino, M. J., Jiang, Y., et al. (2015). A conserved polybasic domain mediates plasma membrane targeting of Lgl and its regulation by hypoxia. J. Cell Biol. 211, 273–286.
- Garcia, J. D., Dewey, E. B. and Johnston, C. A. (2014). Dishevelled binds the Discs large "Hook" domain to activate GukHolder-dependent spindle positioning in Drosophila. *PLoS One* 9, 1–17.
- Johnston, C. A., Hirono, K., Prehoda, K. E. and Doe, C. Q. (2009). Identification of an Aurora-A/PinsLINKER/ Dlg Spindle Orientation Pathway using Induced Cell Polarity in S2 Cells. *Cell* 138, 1150–1163.
- Khoury, M. J. and Bilder, D. (2020). Distinct activities of Scrib module proteins organize epithelial polarity. *Proc. Natl. Acad. Sci.* **117**, 11531–11540.
- Koh, Y. H., Popova, E., Thomas, U., Griffith, L. C. and Budnik, V. (1999). Regulation of DLG localization at synapses by CaMKII-dependent phosphorylation. *Cell* **98**, 353–363.
- Lee, T. and Luo, L. (1999). Mosaic analysis with a repressible cell marker for studies of gene function in neuronal morphogenesis. *Neuron* 22, 451–61.
- Mendoza-Topaz, C., Urra, F., Barría, R., Albornoz, V., Ugalde, D., Thomas, U., Gundelfinger, E.
 D., Delgado, R., Kukuljan, M., Sanxaridis, P. D., et al. (2008). DLGS97/SAP97 is developmentally upregulated and is required for complex adult behaviors and synapse morphology and function. J. Neurosci. 28, 304–314.
- Moreira, S., Osswald, M., Ventura, G., Gonçalves, M., Sunkel, C. E. and Morais-de-Sá, E. (2019). PP1-Mediated Dephosphorylation of Lgl Controls Apical-basal Polarity. *Cell Rep.* 26, 293–301.
- Perrimon, N. (1988). The maternal effect of lethal(1)discs-large-1: A recessive oncogene of Drosophila melanogaster. *Dev. Biol.* **127**, 392–407.
- Sharp, K. A., Khoury, M. J., Wirtz-Peitz, F. and Bilder, D. (2021). Evidence for a nuclear role for Drosophila Dlg as a regulator of the NURF complex. *Mol. Biol. Cell* **32**,.
- Tanimoto, H., Itoh, S., Ten Dijke, P. and Tabata, T. (2000). Hedgehog creates a gradient of DPP activity in Drosophila wing imaginal discs. *Mol. Cell* 5, 59–71.
- Wirtz-Peitz, F., Nishimura, T. and Knoblich, J. A. (2008). Linking Cell Cycle to Asymmetric Division: Aurora-A Phosphorylates the Par Complex to Regulate Numb Localization. *Cell* 135, 161–173.
- Woods, D. F. and Bryant, P. J. (1989). Molecular cloning of the lethal(1)discs large-1 oncogene of Drosophila. *Dev. Biol.* **134**, 222–235.
- Zeitler, J., Hsu, C. P., Dionne, H. and Bilder, D. (2004). Domains controlling cell polarity and proliferation in the Drosophila tumor suppressor scribble. *J. Cell Biol.* **167**, 1137–1146.