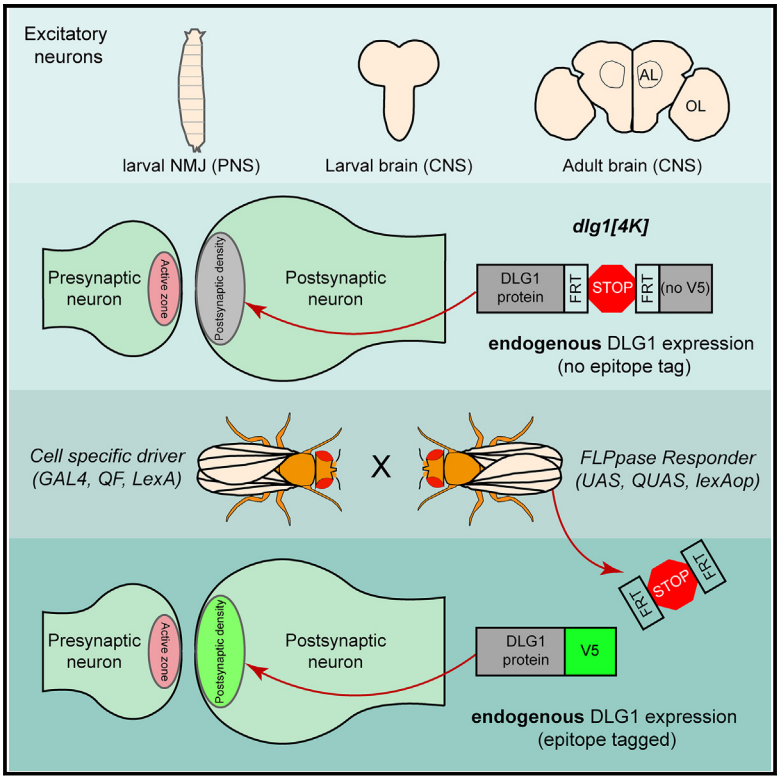
Cell Reports
Methods

Article

A conditional strategy for cell-type-specific labeling of endogenous excitatory synapses in *Drosophila*

Graphical abstract



Highlights

- *dlg1[4K]* is an *in vivo* cell-type-specific endogenous excitatory postsynapse label
- *dlg1[4K]* labels multiple subtypes of endogenous central and peripheral postsynapses
- The *dlg1[4K]* label permits quantitative synaptic organization/apposition analyses
- Use with GAL4/QF/lexA systems enables concurrent pre- and postsynaptic labeling

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In brief

Studying neuronal circuits requires concurrent pre- and postsynaptic labeling in identified subsets of neurons. Parisi et al. create *dlg1[4K]*, a CRISPR-modified *dlg1* locus that conditionally labels DLG1 at excitatory postsynapses *in vivo* using cell-type-specific binary expression. The *dlg1[4K]* label enables previously unavailable postsynaptic labeling and enhances quantitative circuit analysis.

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Supplemental information

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SUPPLEMENTAL TABLES

Table S1. Complete genotypes referenced by figure panel, related to all Figures.

Figure	Panel	Genotype	
2	**A**	*dlg1[4K], UAS-Flp[8208] / + ; +; +; +*	
	B	*dlg1[4K], UAS-Flp[8208] / +; + ; Mef2-Gal4 / + ; +*	
	C	*dlg1[4K] / +; LexA-op-Flp / +; +; +*	
	D	*dlg1[4K] / +; LexA-op-Flp / Mef2-LexA; +; +*	
	E	*dlg1[4K] / +; QUAS-Flp[32107] / +; +; +*	
	F	*dlg1[4K] / +; QUAS-Flp[32107 / +; Mef2-QF / +; +*	
	G	*dlg1[4K], UAS-Flp[8208] / +; BG487-Gal4 / + ;+;+*	
	H	*dlg1[4K]*	*dlg1[4K] / +; +; +; +*
		dlg1[4K], Flp	*dlg1[4K], UAS-Flp[8208] / +; +; +; +*
		dlg1[4K], Flp+Gal4	*dlg1[4K], UAS-Flp[8208] / +; Mef2-Gal4 / +; +; +*
	I	*dlg1[4K], UAS-Flp[8208] / +; synj-QF / +; QUAS-Brp-Short-mStraw / +; +*	
	J	*dlg1[4K], UAS-Flp[8208] / +; synj-QF / +; QUAS-Brp-Short-mStraw / 24B-Gal4; /+*	
3	**A**	*dlg1[4K], UAS-Flp[8208] / +; +; +; +*	
	B	*dlg1[4K], UAS-Flp[8208] / elav[C155]-Gal4 ; +; +; +*	
	C	*dlg1[4K] / +; +; QUAS-Flp[30008] / +; +*	
	D	*dlg1[4K] / +; Synj-QF / +; QUAS-Flp[30008] / 24B-Gal4; +*	
4	**A**	*dlg1[4K], UAS-Flp[8208] / +; +; +; +*	
	B	*dlg1[4K], UAS-Flp[8208] / +; GH146-Gal4 / +; +; +*	
	C	*dlg1[4K], UAS-Flp[8208] / +; GH146-Gal4 / +; +; +*	
	D	*dlg1[4K] / +; GH146-Gal4 / +; UAS-Flp[55804]*	
	E	*dlg1[4K], UAS-Flp[8208] / Or67d-QF; +; QUAS-Brp-Short-mStraw / +; +*	
	F	*dlg1[4K], UAS-Flp[8208]/ Or67d-QF; Mz19-Gal4 / +; QUAS-Brp-Short-mStraw / +; +*	
5	**B-C**	*dlg1[4K], UAS-Flp[8208] / Or67d-QF; Mz19-Gal4 / +; QUAS-Brp-Short-mStraw / +; +*	
	G-H	*dlg1[4K], UAS-Flp / Or67d-QF; +; NP3056-Gal4 / QUAS-Brp-Short-mStraw; +*	
	L-M	*dlg1[4K], UAS-Flp / w; Mz19-QF / +; NP3056-Gal4 / QUAS-Brp-Short-mStraw; +*	
6	**A**	*dlg1[4K], UAS-Flp[8208] / +; +; +*	
	B	*dlg1[4K], UAS-Flp[8208] / +; +; VT032906-Gal4; +*	
	C	*dlg1[4K], UAS-Flp[8208] / +; +; DIPγ-Gal4; +*	
	E	*dlg1[4K], UAS-Flp[8208] / +; +; +; +*	

	F	*dlg1[4K], UAS-Flp[8208] / +; +; DIPγ-Gal4; +*
7	**A**	*dlg1[4K], UAS-Flp[8208] / +; +; +; +*
	B	*dlg1[4K], UAS-Flp[8208] / elav[C155]-Gal4; +; +; +*
	C	*dlg1[4K], UAS-Flp[8208] / elav[C155]-Gal4; +; +; +*
	D	*dlg1[4K], UAS-Flp[8208] / elav[C155]-Gal4; +; +; +*
S2	**A**	*Canton S*
	B	*dlg1[4K] / +; +; +; +*
	C	*dlg1[4K], UAS-Flp[8208]; +; +; +*
	D	*dlg1[4K], UAS-Flp[8208] /+; Dmef2-Gal4; +*
	E	*dlg1[4K], UAS-Flp[8208] / C155-Gal4; +; +; +*
S3	**A**	*dlg1[4K] / +; +; +; +*
	B	*dlg1[4K], UAS-Flp[8208] / +; +; +; +*
	C	*dlg1[4K], UAS-Flp[8208] / + ; +; 24B-Gal4 / +; +*
S4	**B-F**	*dlg1[4K], UAS-Flp / Or67d-QF; Mz19-Gal4 / +; QUAS-Brp-Short-mStraw / +; +*
S5	**A**	*dlg1[4K], UAS-Flp[8208] / + ; +; +; +*
	B	*dlg1[4K], UAS-Flp[8208] / + ; +; GR1-Gal4 / +; +*

Table S2, related to Figure 2. Quantitation of semi-lethality in DMef-QF2 driving QUAS-Flp scored against the Tubby (Tb) marker on TM6. * imaged in Figure 2.

Condition	**Cross to Produce F_1 Progeny**	**Tb+**	**Tb-**
no dlg1[4K]	+; +; QUAS-Flp[30008]; + x + / Y; +; DMef-QF2 / TM6, Tb; +	1	141
no dlg1[4K]	+; +; QUAS-Flp[30126]; + x + / Y ; +; DMef-QF2 / TM6,Tb; +	1	354
no dlg1[4K]	+; +; QUAS-Flp[30127]; + x + / Y ; +; DMef-QF2 / TM6, Tb; +	2	122
three components	*dlg1[4K] / +; +; QUAS-Flp[30127];* + x + / Y ; +; DMef-QF2/TM6, Tb; +	2*	224
three components	*dlg1[4K] / +; +; QUAS-Flp[30008];* + *x* + / Y ; +; DMef-QF2/TM6, Tb; +	0	156
No QUAS-Flp	*dlg1[4K] / +; +; +;* + *x* + / Y; +; DMef-QF2 / TM6, Tb; +	136	128

Table S3, related to Figure 1. Viability test of *dlg1[4K]* flies.

genotype	embryos	males	females	total	% survival
dlg[4K]/w[1118]	133	41	51	92	69.2
dlg[4K], UAS-Flp/w[1118]	139	55	53	108	77.7
dlg[4K], UAS-Flp;DMef2-Gal4	125	38	43	81	64.8
dlg[4K], UAS-Flp/C155-Gal4	120	44	52	96	80

SUPPLEMENTAL FIGURES

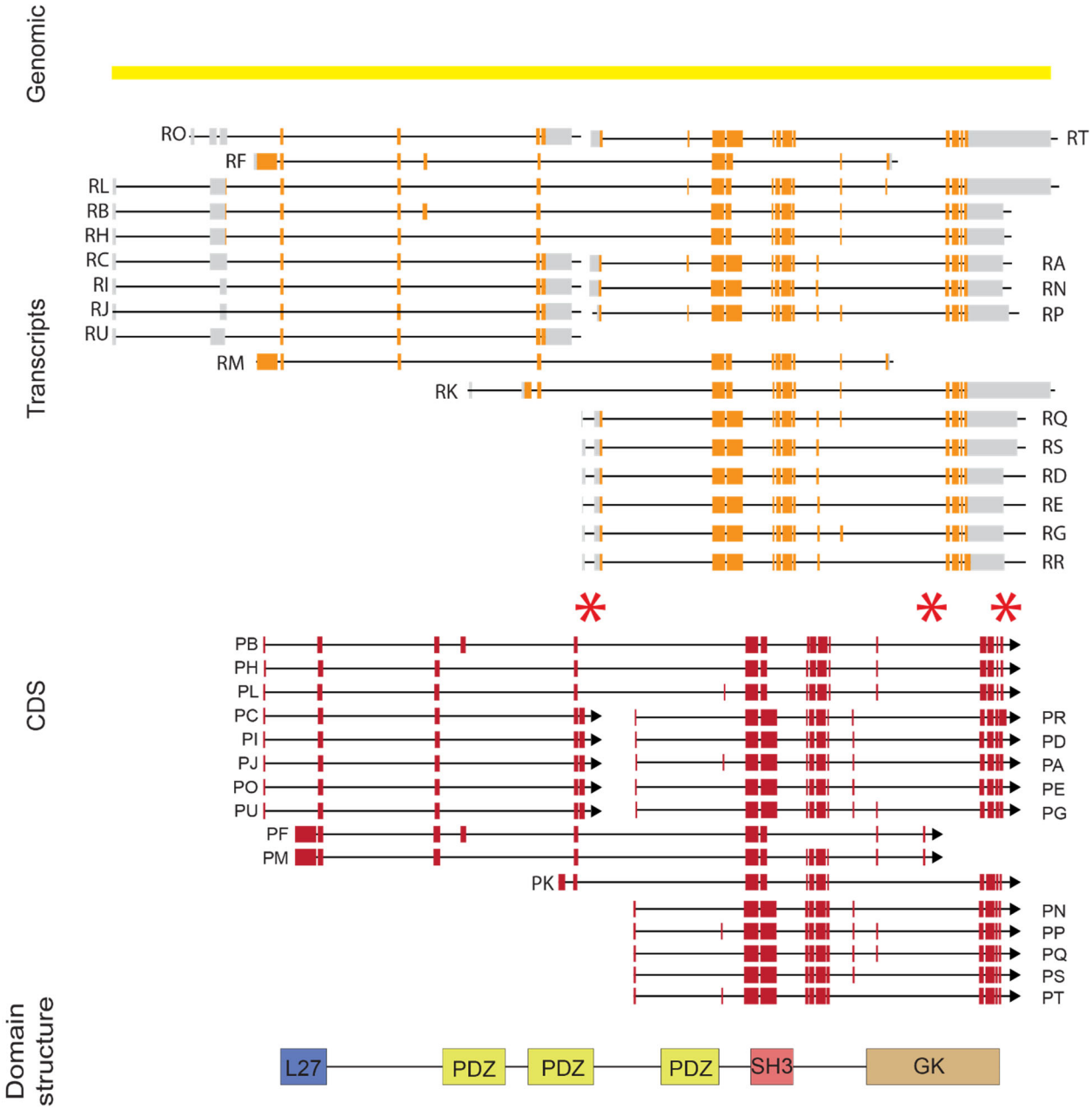

Figure S1, related to Figure 1. Isoform complexity at the *dlg1* locus.
dlg1 transcripts and CDS isoforms (adapted from FlyBase using version FB2022_04, released 8 August 2022). Asterisks indicate three alternative stop codons utilized by different protein isoforms. Bottom is a schematic showing protein interaction domains.

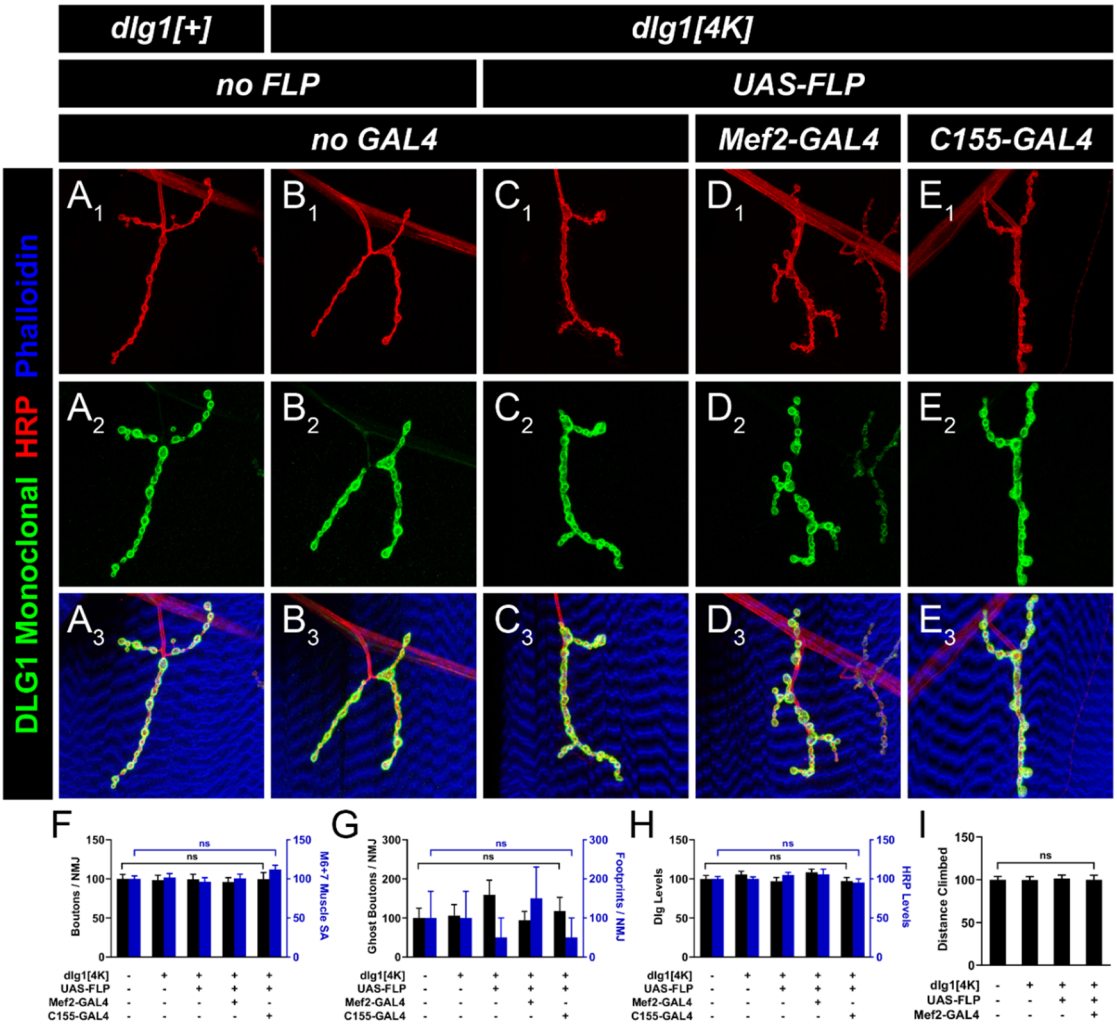

Figure S2, related to Figure 2. *dlg1[4K]* expression does not alter NMJ morphology or motor function.

(A – E) Representative confocal images of NMJs at muscle 4 stained with phalloidin (blue) and antibodies against endogenous DLG1 (green) and HRP (red) in larvae of various genotypes. In all cases, neither the presence of *dlg1[4K]* knock-in allele, UAS-FLP, or GAL4-driven FLP expression resulting in DLG1 labeling influences gross NMJ or muscle morphology. (F – H) Quantification of multiple NMJ parameters including bouton number and muscle 6/7 surface area (F), ghost boutons / NMJ and footprint boutons / NMJ (G), and HRP and DLG immunofluorescence (H) and expressed as a percent of wild-type control values. In all cases, there are no significant changes to multiple NMJ parameters in any examined genotypes, suggesting no influence of *dlg1[4K]* on synaptic development. (I) Quantification of distance climbed in cm by adult flies expressed as a percent of wild-type control values in a negative geotaxis assay to test locomotor behavior. No differences were observed, suggesting *dlg1[4K]* does not impair motor function.

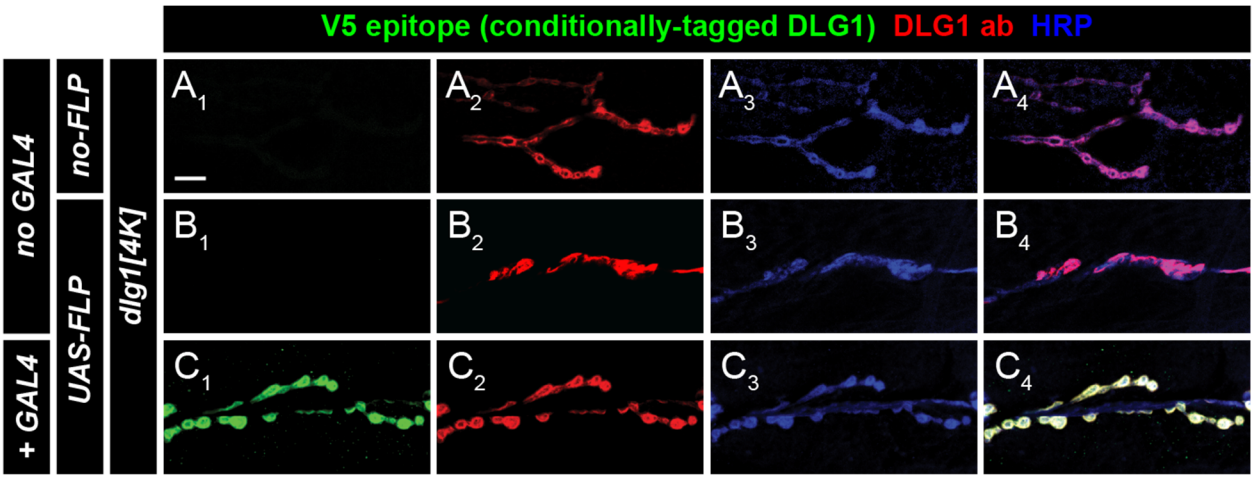

Figure S3, related to Figure 2. *dlg1[4K]* shows tight coupling of DLG1-V5 expression to the presence of GAL4-driven FLP.

Representative confocal images of NMJs in multiple genotypes and stained with antibodies to DLG1-V5 (green), endogenous DLG1 (red), and HRP. In the absence of both GAL4 and FLP (A) or only FLP but no GAL4 (B), only endogenous DLG1 is evident. When muscle-specific 24B-GAL4 and UAS-FLP are present (C), DLG1-V5 expression is robustly observed. This indicates that there is little “leak” expression associated with *dlg1[4K]* V5 labeling. Scale bar, 10 μm.

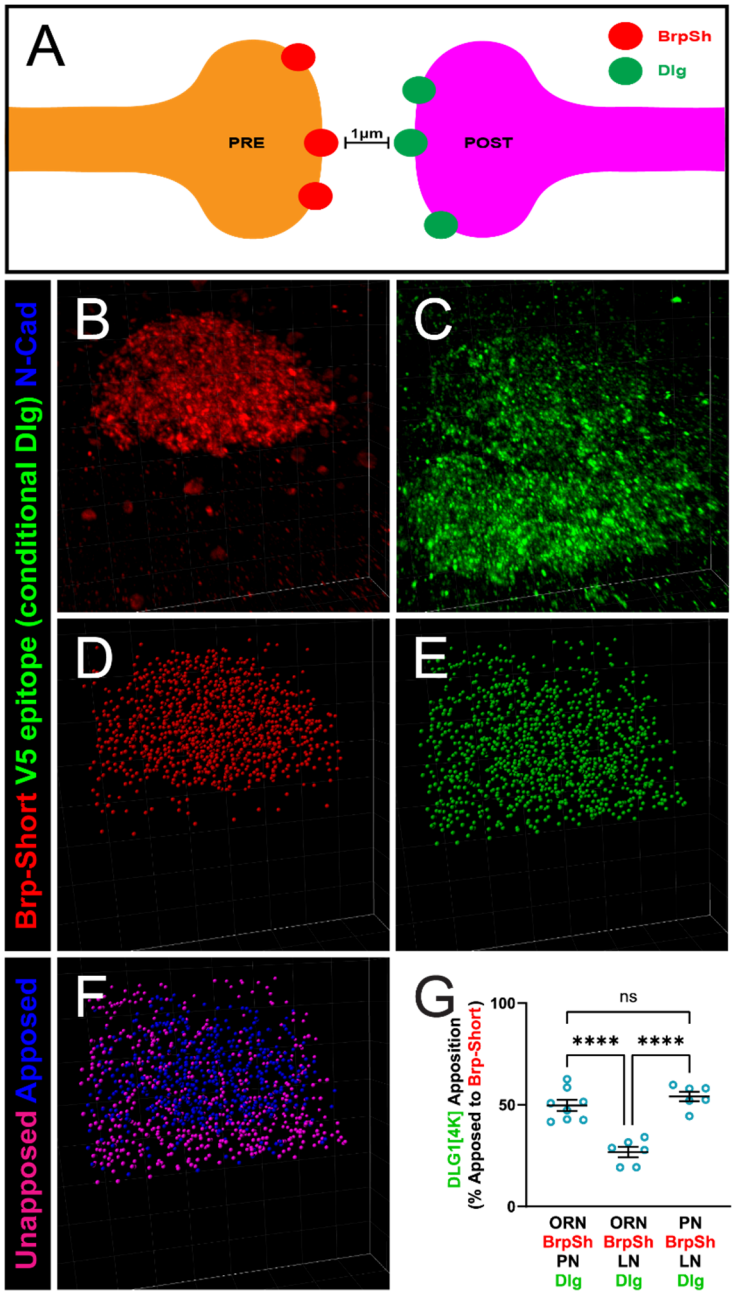

Figure S4, related to Figure 5. A quantitative analysis of postsynaptic DLG1 puncta for PNs and LNs in the DA1 glomerulus.

(A) Schematic of apposition between presynaptic Brp-Short puncta and postsynaptic DLG1-V5 puncta. For apposition analysis, Brp-Short puncta (red) at the presynaptic membrane (orange) are considered apposed to DLG1-V5 puncta (green) at the postsynaptic membrane (magenta) if they are less than or equal to 1 µm away from each other. (B – C) Screenshots from Imaris of the DA1 and VA1d glomeruli of the *Drosophila* antennal lobes showing Brp-Short puncta from presynaptic ORNs (B) and DLG1-V5 puncta from postsynaptic PNs (C) stained with antibodies against mStraw (red) and V5

(green). (D – E) Screenshots of three-dimensional renderings in Imaris software showing the conversion of Brp-Short puncta (D) and DLG1-V5 puncta (E) into “Spots” overlaid on a merge of the images from (B-C). (F) Screenshot of DLG1-V5 “Spots” from Imaris after applying the “Shortest Distance to Spots” function to determine apposition to Brp-Short “Spots” with apposition defined as 1 μm or lower in distance between puncta. Blue spots represent DLG1-V5 puncta apposed to Brp-Short puncta while magenta spots represent unapposed puncta. (G) Quantification of the percent of DLG1-V5 puncta apposed to Brp-Short puncta for the three pairs of cell types from Figure 5 (ORN Brp to PN Dlg; ORN Brp to LN Dlg; PN Brp to LN Dlg). Apposition between ORN Brp-Short puncta and LN DLG1-V5 puncta is significantly lower than apposition between the other two cell type pairings. ****, $p < 0.0001$.

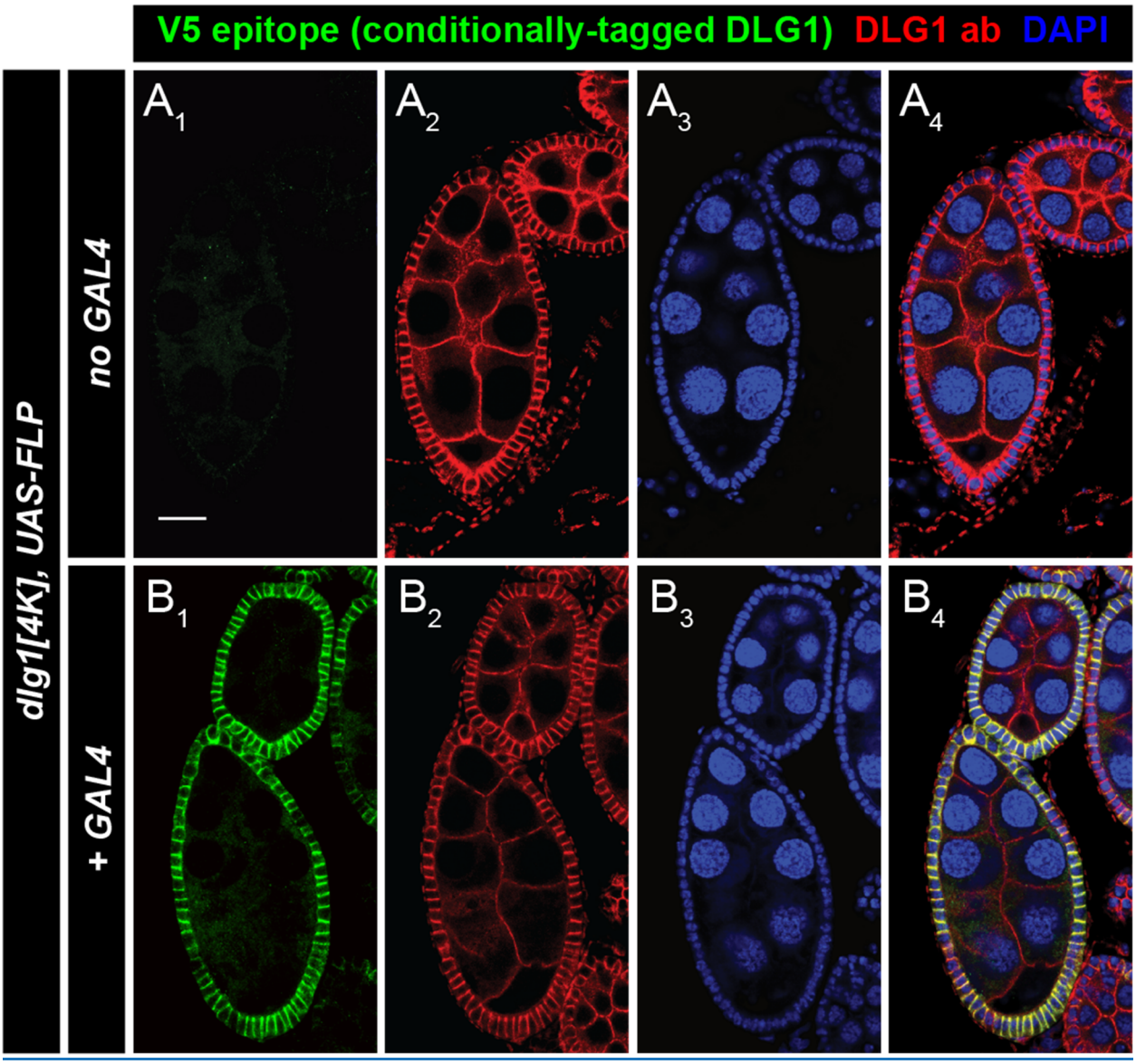

Figure S5. Related to Star Methods. Expression of DLG1-V5 via *dlg1[4K]* in a non-neuronal (epithelial) cell type.

(A-B) Representative confocal images of three-day old, early to mid-stage egg chambers of *dlg1[4k]* flies with a UAS-FLP transgene in either the absence (A) or presence (B) of GR1-GAL4 in the ovarian follicular epithelia and stained with DAPI (blue) and antibodies to DLG1-V5 (green) and endogenous DLG1 (red). In the absence of GAL4 (A), no DLG1-V5 is evident but when GAL4 is present (B) DLG1-V5 expression is seen in the follicular epithelia and precisely overlaps endogenous DLG1 staining. Note that GR1-GAL4 is not expressed in the germline so DLG1-V5 signal is not observed with endogenous DLG1 in oocyte and nurse cell membranes. Scale bar, 20 μm.