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# Supplementary Materials for

## Cytoneme-Mediated Contact-Dependent Transport of the *Drosophila* Decapentaplegic Signaling Protein

Sougata Roy, Hai Huang, Songmei Liu, Thomas B. Kornberg*

*Corresponding author. E-mail: tkornberg@ucsf.edu

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**This PDF file includes:**



**Other supplementary material for this manuscript includes the following:**

**Supplemental Material**

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| Protein | Relative intensity (lower/upper layer) |
|---|---|
| Dpp:GFP | 2.9±1.16 |
| pMAD | 2.1±0.351 |
| *dad*-LacZ | 2.1±0.115 |
| *put*-LacZ | 1.13±0.117 |
| *tkv*-LacZ | 0.54±0.075 |

**Table S1. Relative levels of Dpp and Dpp signaling in the layers of ASP.** Intensities were measured using Image J, in a rectangle in the mid region of ASP that included 11-12 cells with the highest levels of Dpp or Dpp signaling; numbers represent ratio of lower/upper. The size and relative position of the rectangle was the same for both the upper and lower level optical sections. Five specimens were measured and the average relative values are presented.

| Mutants and transgene expression | Duplicated ASP | Abnormal TC* |
|---|---|---|
| *dpp*$^{ts}$ (18˚C) | 0% (0/20) | NS |
| *dpp*$^{ts}$ (29°C)** | 23% (8/35) | NS |
| *btl*>CD8:GFP***, *tub*-Gal80$^{ts}$ | 0% (0/30) | 0% (0/30) |
| *btl*>Tkv$^{DN}$, *tub*-Gal80$^{ts}$ | 17% (6/35) | 57% (20/35) |
| *btl*>CD8:GFP, >Put$^{DN}$, *tub*-Gal80$^{ts}$ | 9% (3/35) | 29% (10/35) |
| *btl*>CD8:GFP, >putRNAi, *tub*-Gal80$^{ts}$ | 80% (16/20) | NS |
| *btl*>CD8:GFP, >Dad, *tub*-Gal80$^{ts}$ | 20% (7/35) | 51% (18/35) |
| *ap*>, *tub*-Gal80$^{ts}$, *dad*-GFP | 0% (0/12) | NS |
| *ap*>*dppRNAi*, *dad*-GFP, *tub*-Gal80$^{ts}$ | 13% (6/50) | NS |
| *btl*>CD8:GFP, >Tkv$^{DN}$, >Dad, *tub*-Gal80$^{ts}$ | 67% (10/15) | NS |
| *dpp*$^{ts}$, *btl*>nGFP, >Tkv$^{DN}$ (29°C)** | 75% (9/12) | NS |
| *btl*>CD8:GFP, >Caps$^{DN}$/+ ; >Caps$^{DN}$ / *tub*-Gal80$^{ts}$ | 27% (8/30) | NS |
| *btl*>CD8:GFP, >*capsRNAi*, *tub*-Gal80$^{ts}$ | 13% (4/30) | NS |
| *btl*>CD8:GFP, *caps*$^{C28fs}$, *trn*$^{\Delta 17}$ / + | 0% (0 /30) | NS |
| *btl*>CD8:GFP, >Caps$^{DN}$/+ ; *caps*$^{C28fs}$, *trn*$^{\Delta 17}$ / + | 60% (9/15) | NS |
| *btl*>CD8:GFP, >*capsRNAi*; *caps*$^{C28fs}$, *trn*$^{\Delta 17}$ / + | 40% (6/15) | NS |
| *btl*>*trnRNAitub*-Gal80$^{ts}$ | 20% (3/15) | NS |
| *btl*>*nrgRNAitub*-Gal80$^{ts}$ | 59%(16/27)**<br>11% (2/19) | NS |

**Table S2. ASP and TC phenotypes associated with loss of Dpp signaling**. The two phenotypes associated with loss of Dpp function were not observed in any animals with normal Dpp signaling, either wild type (not listed), *dpp*$^{ts}$ at permissive temperature (18°C) or in the presence of *ap*-Gal4. Every genetic condition tested affected ASP morphology; TC morphology was also affected in every condition that was scored. Expression of these knock-down constructs was limited by Gal80 repression during growth at 18°C prior to the L3 period, and was strong only when the larvae were incubated at 29°C for various times (*dpp*$^{ts}$, 24 hr; *dppRNAi*, 12 hr; *nrgRNAi,* 12 and 24 hr; others, 6 hr) immediately prior to analysis. The increased penetrance with Tkv$^{DN}$ and Dad together (67%) compared to either alone (17% and 20%, respectively) or with Dpp$^{ts}$and Tkv$^{DN}$ together (75%) compared to either alone (23% and 20%, respectively) suggests that the knock-down conditions were partial and that the phenotypes that were scored were sensitive to the degree of knock-down under the conditions that were used. Therefore, the incomplete penetrance of the phenotypes is most likely attributable to the short window of knock-down or to incomplete inactivation of Dpp signaling and does not reflect the essential and major role of Dpp signaling in the TC and ASP. Caps$^{DN}$ genotypes had two copies of the *UAS*-Caps$^{DN}$ transgene.

*, abnormal branching in ventral TC of Tr2
**, 24 hr heat shock
***, >denotes Gal4 dependent expression mediated by UAS
NS, not scored.

| | Condition | *Dad*>GFP | *Dad*>LacZ | pMAD |
|---|---|---|---|---|
| **Tracheal expression** | Dad | -2.2 | -4 | N |
| | *putRNAi* | -4 | -4.5 | N |
| | $Tkv^{DN}$ | -2 | -3.6 | -1.5 |
| | $Tkv^{DN}$,Dad | -6 | N | N |
| | *diaRNAi* | -2.4 | N | -1.3 |
| | *nrgRNAi* | -2.8 | N | -2.2 |
| | $Caps^{DN}$ | -2 | -3.6 | N |
| | $caps^{DN}$, $caps^{C28fs}$, $trn^{D17}$ | -4 | N | N |
| | *capsRNAi*, $caps^{C28fs}$, $trn^{D17}$ | -3 | N | N |
| **Disc expression** | *ap>dppRNAi* | -2.3 | N | -1.6 |
| | *dpp>nrgRNAi* | -1.5 | N | N |
| | | | | |

**Table S3. Levels of Dpp signaling in ASP.** *Dad*-driven GFP and LacZ expression and pMAD staining were measured in the mutant genotypes indicated, and fold changes were calculated based on appropriate control. Five samples were examined for each genotype. Heterozygous mutant constructs (as in Table S2) were examined in the following genetic backgrounds: for tracheal expression, *btl*-Gal4, UAS-CD8:Cherry, *Dad*-GFP, *tub*-$Gal80^{ts}$, or *btl*-Gal4, UAS-CD8:GFP, *Dad*-LacZ, *tub*-$Gal80^{ts}$; for disc expression, *ap*-Gal4 or *dpp*-Gal4, *Dad*-GFP, *tub*-$Gal80^{ts}$; for pMAD staining, *btl*-CD8:GFP; *tub*-$Gal80^{ts}$. Controls lacked mutant constructs. N, not performed.

| | # cytonemes/μm perimeter | | ASP pMAD / wing disc pMAD | |
|---|---|---|---|---|
| **Induction (hr)** | **<25μm** | **>25μm** | **Upper layer** | **Lower layer** |
| 0 | 0.438±0.084 | 0.122±0.056 | 0.73±0.062 | 0.88±0.026 |
| 1/2 | 0.318±0.054 | 0.012±0.011 | 0.71±0.05 | 0.83±0.06 |
| 1 | 0.2426±0.056 | 0.0048±0.004 | 0.49±0.05 | 0.54±0.07 |
| 2 | 0.1546±0.022 | 0.003±0.004 | 0.375±0.04 | 0.41±0.07 |
| 3 | 0.0896±0.013 | 0.0024±0.005 | 0.33±0.02 | 0.37±0.02 |

**Table S4. Dependence of cytoneme number and Dpp signaling on Shi function.** Third instar larvae that expressed *shi*$^{ts1}$ in trachea (*btl*>Gal4 *UAS*-Shi$^{ts1}$) and that had been raised at 18°C were transferred to 30°C for the indicated times. Cytoneme numbers and levels of pMAD were measured and calculated as described in SOM. Based on ANOVA followed by Tukey HSD test, the numbers of cytonemes that were <25 μm or>25 μm significantly declined (compared to 0 hr) within ½ hr after shift to 30°C ($p<0.01$ for all changes to cytoneme numbers, except for <25 μm cytonemes at ½ hr, $p<0.05$). In contrast, pMAD levels did not change significantly between 0 and ½ hr in either upper or lower layers, but longer incubations reduced pMAD levels significantly compared to 0 hr ($p<0.01$) in both layers.

| Genotype | # cytonemes/μm perimeter | |
|---|---|---|
| | **<25 μm** | **>25 μm** |
| *btl>Gal4*, >CD8:GFP/+; *tub*-Gal80$^{ts}$/+ | 0.54±0.15 | 0.129±.9 |
| *btl>Gal4*, >CD8:GFP/>*nrgRNAi*; *tub*-Gal80$^{ts}$/+ | 0.247±0.07 | 0.103±0.02 |
| *btl>Gal4*, >CD8:Cherry/*tub*-Gal80$^{ts}$ ; +/+ | 0.396 ± 0.054 | 0.101±0.023 |
| *btl>Gal4*, >CD8:Cherry/*tub*-Gal80$^{ts}$ ; *diaRNAi*/+ | 0.191±0.24 | 0.0152±0.012 |

**Table S5. Dependence of cytoneme number on *neuroglian* and *diaphanous* function.** Counts were made of cytonemes in 10 ASP preparations from control (*btl-Gal4, UAS-CD8:GFP/+; Gal80$^{ts}$/+*) and *nrgRNAi*-expressing (*btl-Gal4, UAS-CD8:GFP/UAS-nrgRNAi; Gal80$^{ts}$/+*) animals. Larvae were reared at 18°C and shifted to 29°C 12 hours prior to dissection. Cytonemes on the circumference of the ASP were counted and categorized into two size groups (<25 μm and >25 μm). Flies expressing *nrgRNAi* ($p < 0.0001$ for both types of cytonemes) or *diaRNAi* ($p=0.0339$ for <25 μm and $p<0.0001$ for >25 μm) significantly reduced number of cytonemes compared to the control.

Figure S1

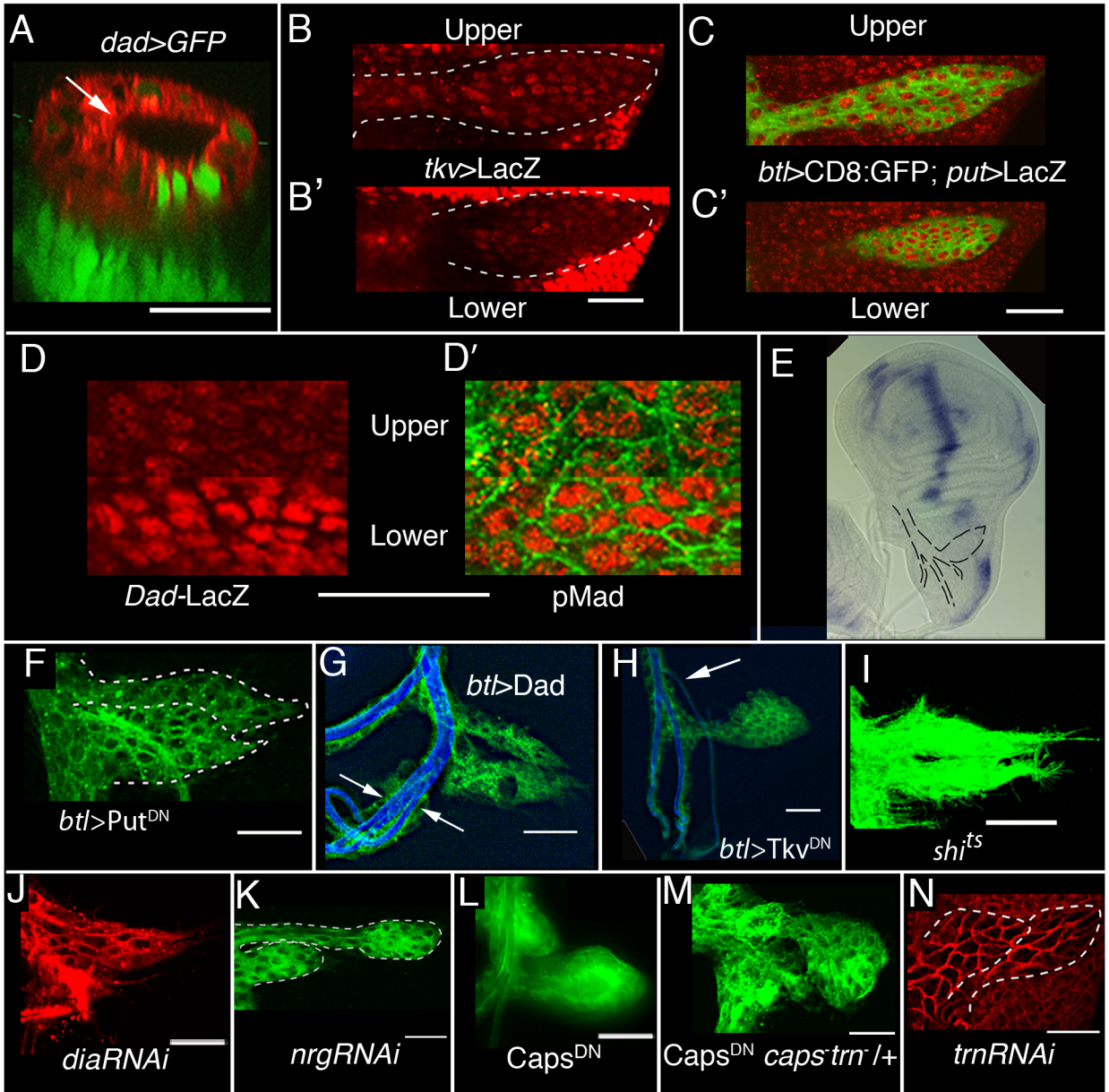
A
dad>GFP
B
Upper
tkv>LacZ
B'
Lower
C
Upper
btl>CD8:GFP; put>LacZ
C'
Lower
D
Dad-LacZ
D'
Upper
Lower
pMad
E
F
btl>Put^DN
G
btl>Dad
H
btl>Tkv^DN
I
shi^ts
J
diaRNAi
K
nrgRNAi
L
Caps^DN
M
Caps^DN caps-trn-/+
N
trnRNAi

**Figure S1. Dpp signaling in the ASP**. **(A)** Transverse section of the medial region of an unfixed ASP (*btl*-CD8:Cherry *Dad*-nlsGFP) showing GFP fluorescence in cells of lower layer and in the disc cells below. **(B, B')** *tkv* expression (red, *tkv*-lacZ detected by anti-ß-galactosidase staining) is higher in the upper **(B)** than lower **(B')** level of the ASP (outlined by dashed white line). **(C, C')** Expression of *put* (red, *put*-lacZ detected by anti-ß-galactosidase staining) is similar in upper **(C)** and lower **(C')** levels of the ASP (green, *btl*-Gal4 *UAS*-CD8:GFP). **(D, D')** Optical sections showing *Dad* (**D**) and pMad (**D'**) assays of Dpp signaling in medial region of the upper and lower layers of ASP: (*Dad*-lacZ (red)**,** α-pMAD staining (red), α-Dlg (green)). (**E**) RNA *in-situ* hybridization detects *dpp* expression in the disc but not in the trachea. Black dashed line marks position of ASP and TC. (**F**-**N**) Functional knockdown conditions in the ASP that induce morphogenetic malformations in the ASP (bifurcations and abnormal shapes) and tracheal duplications (arrows in (**G, H**). Genotypes: *btl*-Gal4, *UAS*-CD8:GFP; *tub*-Gal80$^{ts}$ X *UAS*-Put$^{DN}$ (**F**), X *UAS*-Dad (**G**), X *UAS*-Tkv$^{DN}$ (**H**), X *UAS-shi$^{ts}$* (**I**), X *UAS-diaRNAi* (**J**), X *UAS-nrgRNAi* (**K**), and X *UAS-trnRNAi* (**N**). *shi$^{ts}$* ASP in (**J**) was incubated at 30°C for 1 hr followed by 20°C for 24 hrs. **(L)** Genotype: *btl*-Gal4 *UAS*-CD8:GFP / *UAS*-Caps$^{DN}$ *; UAS*-Caps$^{DN}$ and (**M**) *btl*-Gal4 *UAS*-CD8:GFP / *UAS*-Caps$^{DN}$ ; *caps$^{C28fs}$ trn$^{\Delta 17}$* / *UAS*-Caps$^{DN}$. Blue fluorescence in (**G**) and (**H**) is autofluorescence of lumen at 405 nm. (**N**) α-Dlg (red). Scale bar, 30 μm.

# Figure S2

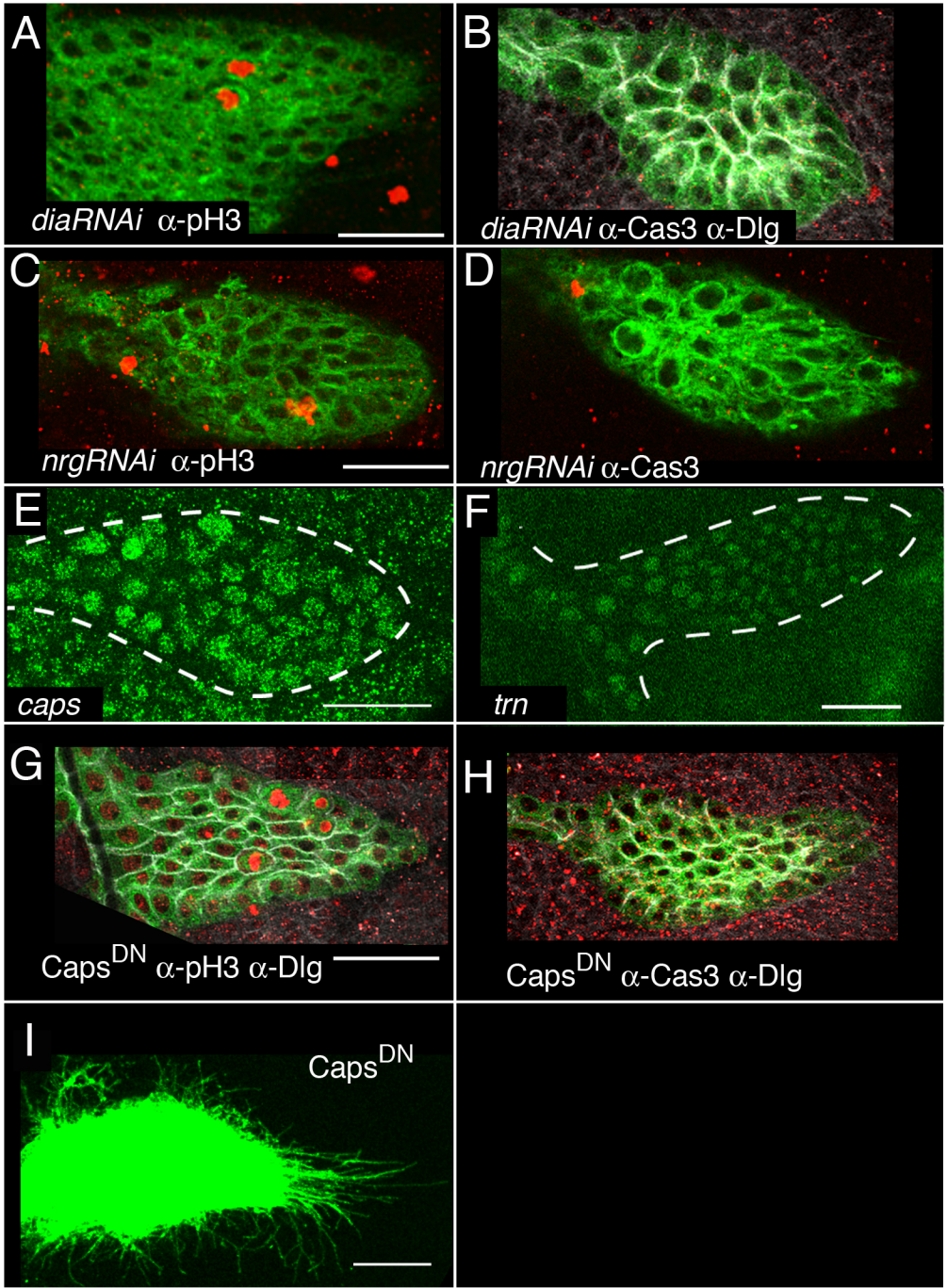
A
diaRNAi α-pH3
B
diaRNAi α-Cas3 α-Dlg
C
nrgRNAi α-pH3
D
nrgRNAi α-Cas3
E
caps
F
trn
G
Caps^DN α-pH3 α-Dlg
H
Caps^DN α-Cas3 α-Dlg
I
Caps^DN

**Figure S2. Characterizations of ASP in normal and mutant conditions**. **(A-D)** Expression of *diaRNAi* **(A, B)** or *nrgRNAi* (**C, D**) did not change the number of α-phosphohistone-3 or α-Caspase-3 staining (red). (**E**, **F**) Expression of *caps* and *trn* detected by α-LacZ antibody ((**E**), *caps-LacZ* (P{PZ}*caps*$^{02937}$; green) and GFP fluorescence ((**F**), *trn*-GFP). (**G**, **H**) Expression of Caps$^{DN}$ did not change the number of α-phosphohistone-3 or α-Caspase-3 staining (red). (**I**) Number and distribution of ASP cytonemes were not significantly changed by Caps$^{DN}$ over-expression. (**B**, **G**, **H**), α-Dlg (white); (**A-D, G-I**) CD8:GFP. Scale bar, 30 μm.

Figure S3

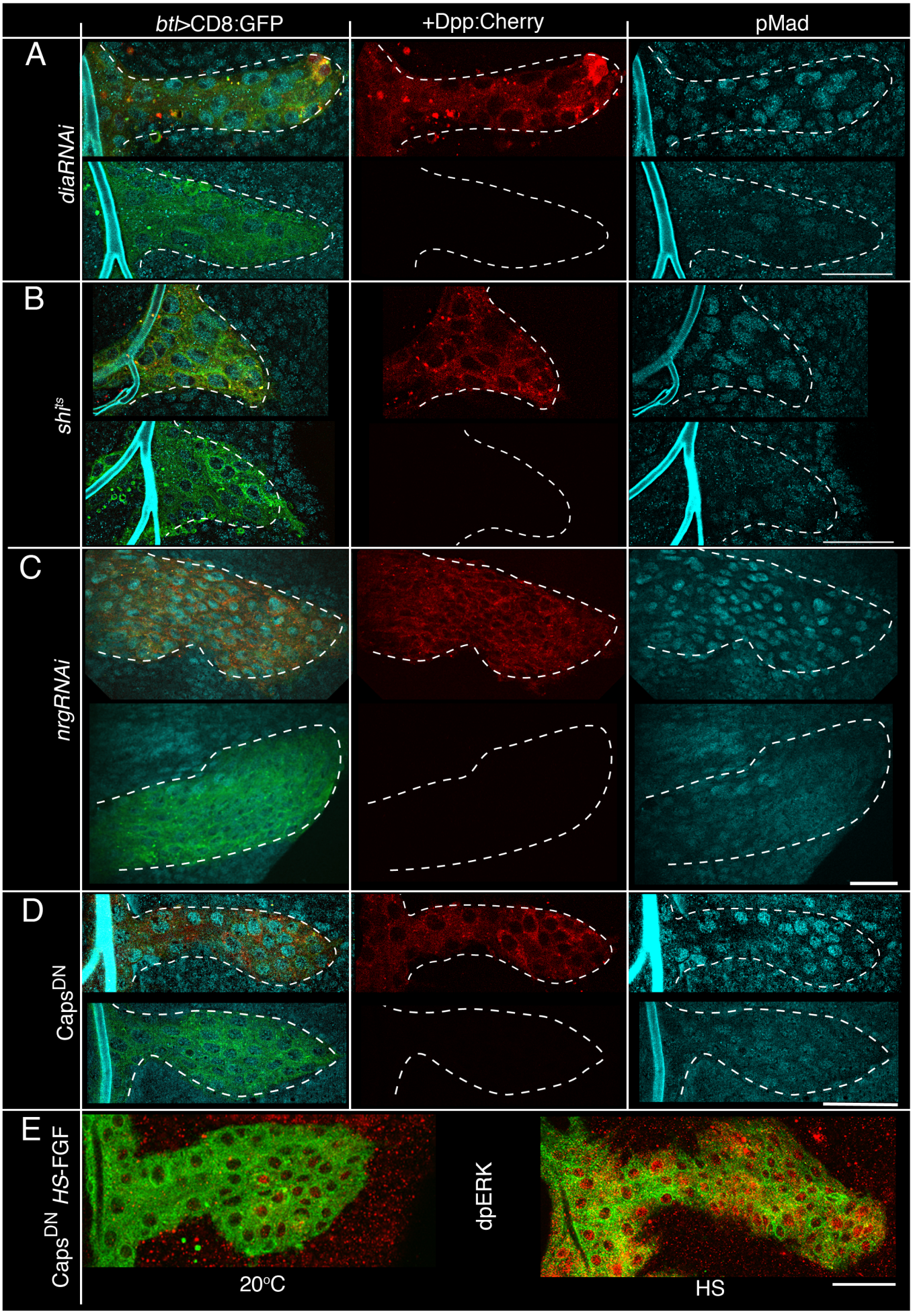
btl>CD8:GFP
+Dpp:Cherry
pMad
A
diaRNAi
B
shi^ts
C
nrgRNAi
D
Caps^DN
E
Caps^DN HS-FGF
dpERK
20°C
HS

**Figure S3. Cells with defective cytonemes do not activate signal transduction but are signaling competent.** ASPs (outlined by dashed lines) that expressed under *btl-Gal4*: GFP (**A-D**), RNAi directed against *dia* (**A**), inactive $Shi^{ts}$ (**B**), RNAi directed against *nrg* (**C**), or $Caps^{DN}$ (**D**). α-pMad antibody stained ASP cells that also expressed Dpp:Cherry ((driven by *btl-Gal4*; upper panels) but did stain ASP cells that did not express Dpp:Cherry (lower panels). Right panels: pMad staining; middle panels Dpp:Cherry fluorescence; left panels: merge with CD8:GFP fluorescence. (**E**) ASPs that expressed GFP and $Caps^{DN}$ under *btl-Gal4* stained for dpERK in the presence of ectopic FGF (driven by heat shock; right panel) but not in the absence of ectopic FGF (left panel). Scale bar: 30 mm.

**Movie S1. Movement of co-localized Dpp:GFP and Tkv:Cherry puncta in cytonemes.** Tkv:Cherry was expressed in trachea (*btl*-Tkv:Cherry); Dpp:GFP was expressed in the *dpp* domain of the wing disc (*dpp*-Dpp:GFP). Each frame of the movie is a maximum Z-projection that was compiled with ImageJ, and is shown at two frames per second. Each Z-stack (0.4 μm steps) was captured at intervals of two time points per minute at 488 nm and 561 nm using an inverted spinning disc confocal microscope. Scale bar, 40 μm.

**References**


1. W. M. Gelbart, The decapentaplegic gene: A TGF-β homologue controlling pattern formation in *Drosophila*. *Development* **107** (suppl.), 65–74 (1989).
2. O. Wartlick *et al*., Dynamics of Dpp signaling and proliferation control. *Science* **331**, 1154–1159 (2011). doi: 10.1126/science.1200037
3. F. A. Ramírez-Weber, T. B. Kornberg, Cytonemes: Cellular processes that project to the principal signaling center in *Drosophila* imaginal discs. *Cell* **97**, 599–607 (1999). doi: 10.1016/S0092-8674(00)80771-0
4. F. Hsiung, F. A. Ramirez-Weber, D. D. Iwaki, T. B. Kornberg, Dependence of *Drosophila* wing imaginal disc cytonemes on Decapentaplegic. *Nature* **437**, 560–563 (2005). doi: 10.1038/nature03951
5. S. Roy, F. Hsiung, T. B. Kornberg, Specificity of *Drosophila* cytonemes for distinct signaling pathways. *Science* **332**, 354–358 (2011). doi: 10.1126/science.1198949
6. A. Bilioni *et al*., Balancing Hedgehog, a retention and release equilibrium given by Dally, Ihog, Boi and shifted/DmWif. *Dev. Biol.* **376**, 198–212 (2013). doi: 10.1016/j.ydbio.2012.12.013
7. A. Callejo *et al*., Dispatched mediates Hedgehog basolateral release to form the long-range morphogenetic gradient in the *Drosophila* wing disk epithelium. *Proc. Natl. Acad. Sci. U.S.A.* **108**, 12591–12598 (2011). doi: 10.1073/pnas.1106881108
8. P. Rojas-Ríos, I. Guerrero, A. González-Reyes, Cytoneme-mediated delivery of Hedgehog regulates the expression of bone morphogenetic proteins to maintain germline stem cells in *Drosophila*. *PLOS Biol.* **10**, e1001298 (2012). doi: 10.1371/journal.pbio.1001298
9. M. Sato, T. B. Kornberg, FGF is an essential mitogen and chemoattractant for the air sacs of the *Drosophila* tracheal system. *Dev. Cell* **3**, 195–207 (2002). doi: 10.1016/S1534-5807(02)00202-2
10. K. Tsuneizumi *et al*., Daughters against dpp modulates dpp organizing activity in *Drosophila* wing development. *Nature* **389**, 627–631 (1997). doi: 10.1038/39362
11. H. Tanimoto, S. Itoh, P. ten Dijke, T. Tabata, Hedgehog creates a gradient of DPP activity in *Drosophila* wing imaginal discs. *Mol. Cell* **5**, 59–71 (2000). doi: 10.1016/S1097-2765(00)80403-7

12. E. V. Entchev, A. Schwabedissen, M. González-Gaitán, Gradient formation of the TGF-β homolog Dpp. *Cell* **103**, 981–992 (2000). doi: 10.1016/S0092-8674(00)00200-2
13. A. A. Teleman, S. M. Cohen, Dpp gradient formation in the *Drosophila* wing imaginal disc. *Cell* **103**, 971–980 (2000). doi: 10.1016/S0092-8674(00)00199-9
14. See supplementary materials on *Science* Online.
15. M. Michel, I. Raabe, A. P. Kupinski, R. Pérez-Palencia, C. Bökel, Local BMP receptor activation at adherens junctions in the *Drosophila* germline stem cell niche. *Nat. Commun.* **2**, 415 (2011). doi: 10.1038/ncomms1426
16. E. H. Feinberg *et al*., GFP Reconstitution Across Synaptic Partners (GRASP) defines cell contacts and synapses in living nervous systems. *Neuron* **57**, 353–363 (2008). doi: 10.1016/j.neuron.2007.11.030
17. M. D. Gordon, K. Scott, Motor control in a *Drosophila* taste circuit. *Neuron* **61**, 373–384 (2009). doi: 10.1016/j.neuron.2008.12.033
18. S. E. Ryu *et al*., Crystal structure of an HIV-binding recombinant fragment of human CD4. *Nature* **348**, 419–426 (1990). doi: 10.1038/348419a0
19. J. H. Wang *et al*., Atomic structure of a fragment of human CD4 containing two immunoglobulin-like domains. *Nature* **348**, 411–418 (1990). doi: 10.1038/348411a0
20. S. Cabantous, T. C. Terwilliger, G. S. Waldo, Protein tagging and detection with engineered self-assembling fragments of green fluorescent protein. *Nat. Biotechnol.* **23**, 102–107 (2005). doi: 10.1038/nbt1044
21. D. H. Castrillon, S. A. Wasserman, diaphanous is required for cytokinesis in *Drosophila* and shares domains of similarity with the products of the limb deformity gene. *Development* **120**, 3367–3377 (1994).
22. C. C. Homem, M. Peifer, Exploring the roles of diaphanous and enabled activity in shaping the balance between filopodia and lamellipodia. *Mol. Biol. Cell* **20**, 5138–5155 (2009). doi: 10.1091/mbc.E09-02-0144
23. T. Rousso, A. M. Shewan, K. E. Mostov, E. D. Schejter, B.-Z. Shilo, Apical targeting of the formin Diaphanous in *Drosophila* tubular epithelia. *eLife* **2**, e00666 (2013). doi: 10.7554/eLife.00666

24. T. Kitamoto, Conditional modification of behavior in *Drosophila* by targeted expression of a temperaturesensitive shibire allele in defined neurons. *J. Neurobiol.* **47**, 81–92 (2001). doi: 10.1002/neu.1018

25. A. B. Muhlberg, D. E. Warnock, S. L. Schmid, Domain structure and intramolecular regulation of dynamin GTPase. *EMBO J.* **16**, 6676–6683 (1997). doi: 10.1093/emboj/16.22.6676

26. J. E. Hinshaw, Dynamin spirals. *Curr. Opin. Struct. Biol.* **9**, 260–267 (1999). doi: 10.1016/S0959-440X(99)80036-0

27. E. M. Enneking *et al*., Transsynaptic coordination of synaptic growth, function, and stability by the L1-type CAM Neuroglian. *PLOS Biol.* **11**, e1001537 (2013). doi: 10.1371/journal.pbio.1001537

28. M. Milán, U. Weihe, L. Pérez, S. M. Cohen, The LRR proteins Capricious and Tartan mediate cell interactions during DV boundary formation in the *Drosophila* wing. *Cell* **106**, 785–794 (2001). doi: 10.1016/S0092-8674(01)00489-5

29. W. Hong *et al*., Leucine-rich repeat transmembrane proteins instruct discrete dendrite targeting in an olfactory map. *Nat. Neurosci.* **12**, 1542–1550 (2009). doi: 10.1038/nn.2442

30. H. Kohsaka, A. Nose, Target recognition at the tips of postsynaptic filopodia: Accumulation and function of Capricious. *Development* **136**, 1127–1135 (2009). doi: 10.1242/dev.027920

31. E. Shishido, M. Takeichi, A. Nose, *Drosophila* synapse formation: Regulation by transmembrane protein with Leu-rich repeats, CAPRICIOUS. *Science* **280**, 2118–2121 (1998). doi: 10.1126/science.280.5372.2118

32. H. Taniguchi, E. Shishido, M. Takeichi, A. Nose, Functional dissection of *Drosophila* capricious: Its novel roles in neuronal pathfinding and selective synapse formation. *J. Neurobiol.* **42**, 104–116 (2000). doi: 10.1002/(SICI)1097-4695(200001)42:1<104::AID-NEU10>3.0.CO;2-V

33. D. S. Lidke, K. A. Lidke, B. Rieger, T.M. Jovin, D. J. Arndt-Jovin, Reaching out for signals: Filopodia sense EGF and respond by directed retrograde transport of activated receptors. *J. Cell Biol.* **170**, 619–626 (2005). doi: 10.1083/jcb.200503140

34. K. Koizumi *et al*., RhoD activated by fibroblast growth factor induces cytoneme-like cellular protrusions through mDia3C. *Mol. Biol. Cell* **23**, 4647–4661 (2012). doi: 10.1091/mbc.E12-04-0315

35. Z. Huang, S. Kunes, Hedgehog, transmitted along retinal axons, triggers neurogenesis in the developing visual centers of the *Drosophila* brain. *Cell* **86**, 411–422 (1996). doi: 10.1016/S0092-8674(00)80114-2

36. C. Korkut *et al*., Trans-synaptic transmission of vesicular Wnt signals through Evi/Wntless. *Cell* **139**, 393–404 (2009). doi: 10.1016/j.cell.2009.07.051

37. M. Cohen, M. Georgiou, N. L. Stevenson, M. Miodownik, B. Baum, Dynamic filopodia transmit intermittent Delta-Notch signaling to drive pattern refinement during lateral inhibition. *Dev. Cell* **19**, 78–89 (2010). doi: 10.1016/j.devcel.2010.06.006

38. C. de Joussineau *et al*., Delta-promoted filopodia mediate long-range lateral inhibition in *Drosophila*. *Nature* **426**, 555–559 (2003). doi: 10.1038/nature02157

39. O. Renaud, P. Simpson, scabrous modifies epithelial cell adhesion and extends the range of lateral signalling during development of the spaced bristle pattern in *Drosophila*. *Dev. Biol.* **240**, 361–376 (2001). doi: 10.1006/dbio.2001.0482

40. Y. Peng, C. Han, J. D. Axelrod, Planar polarized protrusions break the symmetry of EGFR signaling during *Drosophila* bract cell fate induction. *Dev. Cell* **23**, 507–518 (2012). doi: 10.1016/j.devcel.2012.07.016

41. T. A. Sanders, E. Llagostera, M. Barna, Specialized filopodia direct long-range transport of SHH during vertebrate tissue patterning. *Nature* **497**, 628–632 (2013). doi: 10.1038/nature12157

42. N. Ninov *et al*., Dpp signaling directs cell motility and invasiveness during epithelial morphogenesis. *Curr. Biol.* **20**, 513–520 (2010). doi: 10.1016/j.cub.2010.01.063

43. M. Reichman-Fried, B.-Z. Shilo, Breathless, a *Drosophila* FGF receptor homolog, is required for the onset of tracheal cell migration and tracheole formation. *Mech. Dev.* **52**, 265–273 (1995). doi: 10.1016/0925-4773(95)00407-R

44. R. Yagi, F. Mayer, K. Basler, Refined LexA transactivators and their use in combination with the *Drosophila* Gal4 system. *Proc. Natl. Acad. Sci. U.S.A.* **107**, 16166–16171 (2010). doi: 10.1073/pnas.1005957107

45. D. Nellen, R. Burke, G. Struhl, K. Basler, Direct and long-range action of a DPP morphogen gradient. *Cell* **85**, 357–368 (1996). doi: 10.1016/S0092-8674(00)81114-9

46. T. E. Haerry, O. Khalsa, M. B. O'Connor, K. A. Wharton, Synergistic signaling by two BMP ligands through the SAX and TKV receptors controls wing growth and patterning in *Drosophila*. *Development* **125**, 3977–3987 (1998).

47. R. Massarwa, E. D. Schejter, B.-Z. Shilo, Apical secretion in epithelial tubes of the *Drosophila* embryo is directed by the Formin-family protein Diaphanous. *Dev. Cell* **16**, 877–888 (2009). doi: 10.1016/j.devcel.2009.04.010

48. T. Ohshiro, Y. Emori, K. Saigo, Ligand-dependent activation of breathless FGF receptor gene in *Drosophila* developing trachea. *Mech. Dev.* **114**, 3–11 (2002). doi: 10.1016/S0925-4773(02)00042-4

49. C. Ribeiro, A. Ebner, M. Affolter, In vivo imaging reveals different cellular functions for FGF and Dpp signaling in tracheal branching morphogenesis. *Dev. Cell* **2**, 677–683 (2002). doi: 10.1016/S1534-5807(02)00171-5

50. A. Klebes, B. Biehs, F. Cifuentes, T. B. Kornberg, Expression profiling of *Drosophila* imaginal discs. *Genome Biol.* **3**, RESEARCH0038 (2002). doi: 10.1186/gb-2002-3-8-research0038

51. U. Persson *et al*., The L45 loop in type I receptors for TGF-β family members is a critical determinant in specifying Smad isoform activation. *FEBS Lett.* **434**, 83–87 (1998). doi: 10.1016/S0014-5793(98)00954-5

52. K. G. Eulenberg, R. Schuh, The tracheae defective gene encodes a bZIP protein that controls tracheal cell movement during *Drosophila* embryogenesis. *EMBO J.* **16**, 7156–7165 (1997). doi: 10.1093/emboj/16.23.7156

53. A. Guha, L. Lin, T. B. Kornberg, Organ renewal and cell divisions by differentiated cells in *Drosophila*. *Proc. Natl. Acad. Sci. U.S.A.* **105**, 10832–10836 (2008). doi: 10.1073/pnas.0805111105