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Supplementary Information for

Kinetochores protein Spindly controls microtubule polarity in *Drosophila* axons

Urko del Castillo*, Hans-Arno J. Müller, Vladimir I. Gelfand*

Urko del Castillo

urko@northwestern.edu

Vladimir I. Gelfand

vgelfand@northwestern.edu

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Figure S1

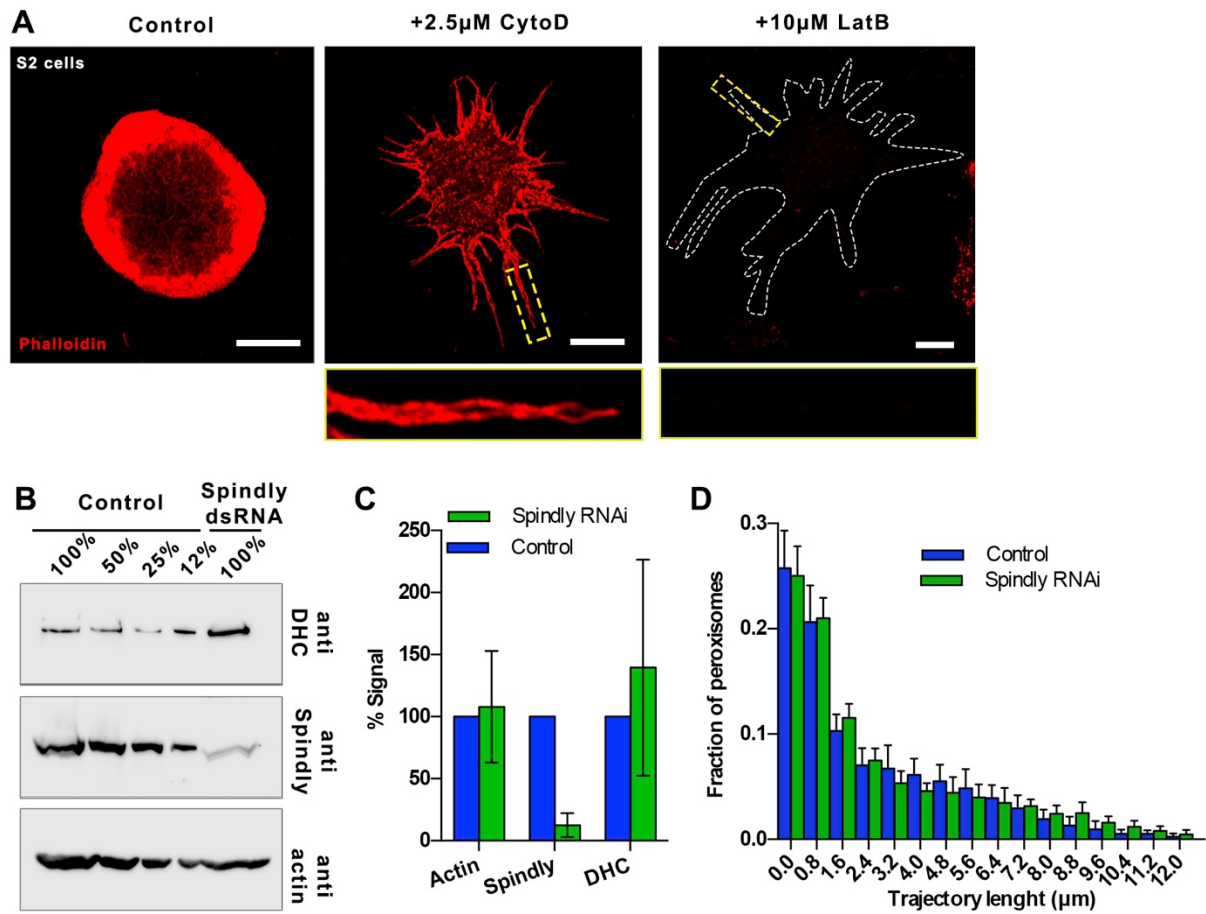


Figure S1. Spindly knockdown does not alter DHC levels.

A) Low concentration of CytoD (2.5µM) does not depolymerize cortical actin of S2 processes. S2 cells were plated in the absence of drugs (control) or 2.5µM CytoD or 10µM LatB. F-actin was stained with Rhodamine-Phalloidin. The concentration of CytoD used in the S2 candidate-based screen (2.5 µM) allowed the formation of microtubule-base processes containing cortical actin. In contrast, treatment with high concentration of LatB induced actin depolymerization. Images were taken using the same microscope settings. Scale bars, 10 µm.

B) Representative western blots of the S2 lysates of untreated (control) and treated with Spindly shRNA. Dilutions of untreated lysates were provided to estimate degree of knockdown.

C) Protein levels were quantified using the western-blot in (B). Data obtained from three independent assays.

D) Trajectory length of peroxisome transport in S2 cells untreated (control) and Spindly RNAi (see Video 2).

Figure S2

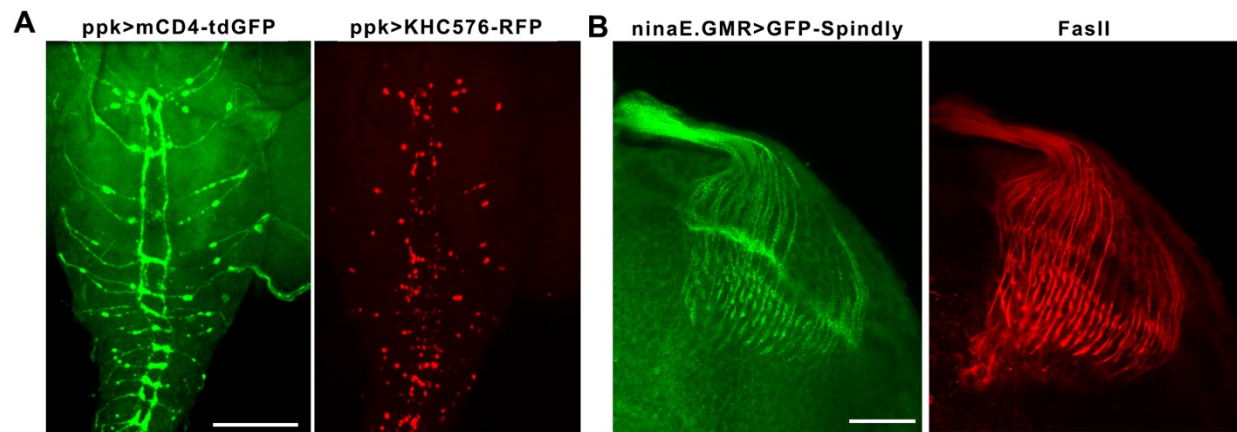


Figure S2. KHC(1-576)-RFP accumulated at the tips of axons in class IV *Da* neurons

A) Confocal z-projection of a fixed third-instar VNC expressing mCD4-tdGFP (left panel) and KHC(1-576)-RFP (red panel) driven by *ppk-gal4*. Note that KHC(1-576) accumulates at the tips of the axons in class IV neurons. Scale bar, 40 μm .

B) Confocal z-projection of a fixed third-instar optic lobe expressing *ninaE.GMR>GFP-SpindlyFL* (left panel) and immunostained with FasII (right panel). Scale bar, 20 μm .

Figure S3

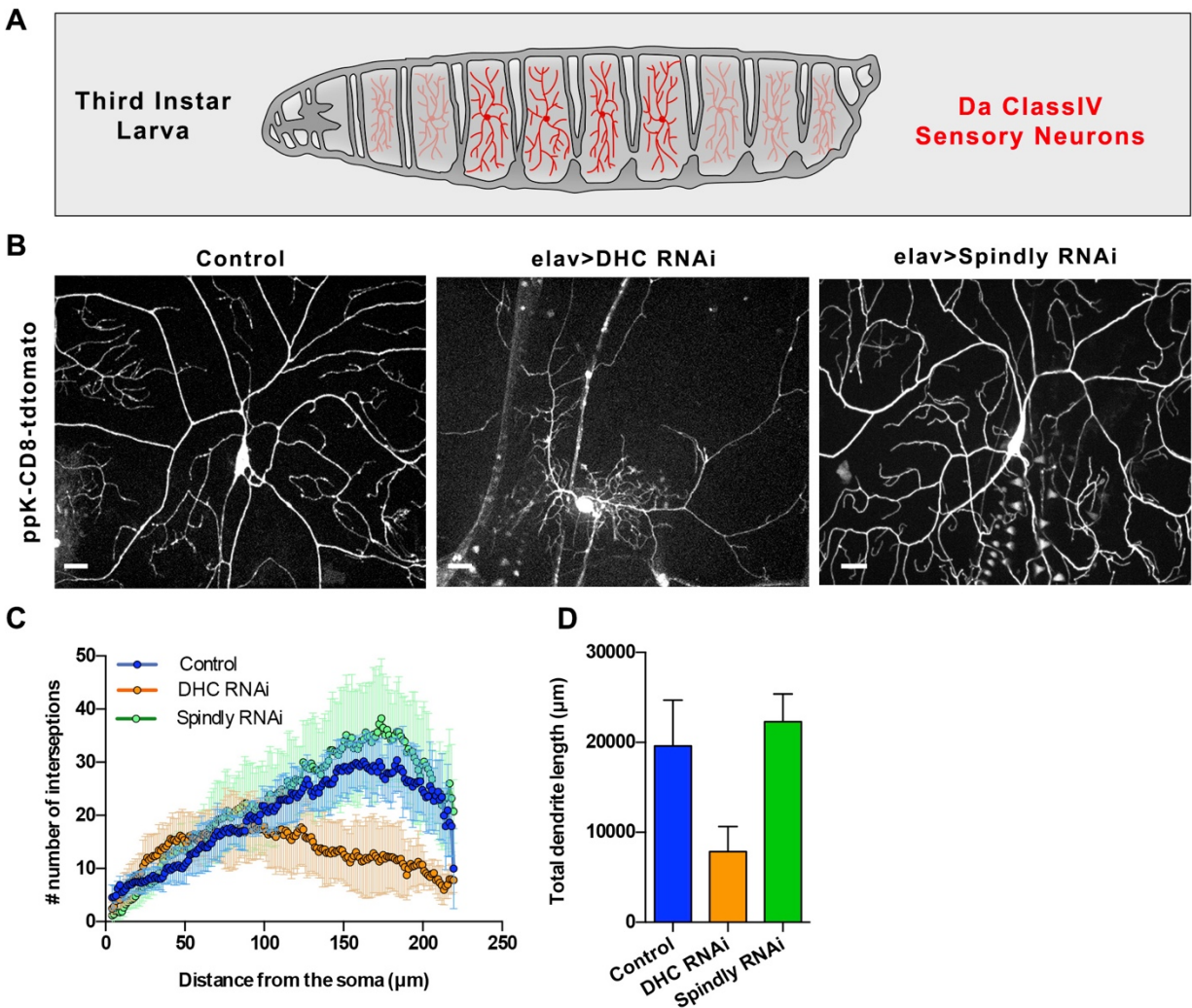


Figure S3. Spindly does not affect development of dendrites

A) Diagram of third-instar larva showing the class IV Da sensory neurons in red.

B) Representative max-projection images showing DA neurons labelled with ppk::tdTomato in 3rd instar larvae under control conditions (left panel), DHC RNAi (middle panel) or Spindly RNAi (Left panel). Scale bars, 20 μm .

C) Sholl analysis of dendritic arborization of class IV sensory neurons. Data obtained from 12, 13 and 10 animals for control, DHC RNAi and Spindly RNAi, respectively.

D) Quantification of the total dendritic length of Da neurons from data obtained in (C).

Video legends

Video 1. Time-lapse of control (untreated cell) or Spindly RNAi S2 cells expressing EB1-GFP. Scale bar, 10 μm . Related to Fig. 1D.

Video 2. Time-lapse of control (untreated cell) or Spindly RNAi S2 cells expressing SKL-GFP. Scale bar, 10 μm . Related to Fig. S1D.

Video 3. Time-lapse of primary neurons expressing Ubi-EB1-mCherry of three different genotypes. Control (elav-Gal4), DHC RNAi (elav>DHC shRNA) and Spindly RNAi (elav>Spindly shRNA). Scale bar, 10 μm . Related to Fig. 3A.

Video 4. Motility assay of control (elav-Gal4) and elav>Spindly RNAi adult flies in a 35 mm dish. Related to Fig. 4G and 4I.

Video 5. Climbing assay of adult flies with different genotypes. From left to right: Control (elav-Gal4), Spindly RNAi (elav>Spindly RNAi). Related to Fig. 4H.

Table S1.

			T7 promoter sequence (TAATACGACTCACTATAGGG) were added to 5' end of each primer.	
	CG	Expression levels in S2 cells	Primer 1 (5'-3')	Primer 2 (5'-3')
Axon Initial Segment				
α -spectrin	CG1977	Very high	AGCGCGATGCCGACGAGTTGGAGTC	ACGCGATACTCTGGGAGGCCATG
β -spectrin	CG5870	Moderate-high	AGCGCTTGAGAAATGCCGTCGAGTAC	ACTCAATGTAGAAGGTCTGGACGCCATG
Ankyrin	CG1651	Moderate	AGTGCCCCAGGTCAAAGCTATATTTCCG	TCAAGGGTCTTATCCTCTTTATCATCAGTCATGCA
Asymmetrical Cell Division				
Inscutable	CG11312	Very low	GATTCGGGCTTCTCGGACAAT	CGGGCGTTTGCTGCTTATC
Pins	CG5692	Moderate-high	TGTCTCGCTCTCTGCGTCCG	AGGTGCCTCTCGCAACAGATG
Gi-alpha	CG10060	Very high	GAAGATCATCCATGACACGGGCTAC	AGTTCCGGTTGCGCGATCCTATCCAG
Mud	CG12047	Moderate	TCATCGAATCGAACTACATTACCTTG	TCATCGAATCGAACTACATTACCTTG
Numb	CG3779	Very low	TCATCGAATCGAACTACATTACCTTG	GTTGTTGAAGTTTAGATCCTCGCCGGCG
Bazooka	CG5055	Very low	AGCGAGGTCAACGAGGAGGTGGAAG	AGCACTCGACAAATTGATAGCGCTTTACGG
Cdc42	CG12530	Very high	AGGCCGTCAAAGTACGTGGAGTGCTC	GACACTACTGACACAGATACGCGG
Khc73	CG8183	Moderate	CTAACCGACCGCCTGAAGA	CTCCAAAGCTGTGGGACTT
Cell Adhesion				
Abl	CG4032	Moderate high	AAGAGAGAGAGGTGAAGCCAGAGGCG	TTCTCTCTTCTCTCGGCTCCTGCTG
Aplip1	CG1200	Very low	GAGTTCGCCAACGGTGGTGGTGG	GGATCCGAGGTAGCCACAGATAGC
Armadillo	CG11579	Very high	CGACTCCGGCATCCACTCGGGTG	ATCTGAGGGCTGTTCCATAATGGCATGTC
Shaggy	CG2621	Low	AATACAGCCCAGCCTAGTGCCG	GTTGACGAGACGCTGGTGGAGCC
Kinetochores				
Spindly	CG15415	Moderate	GACACCCTTAGCGTGGATGACATCGTG	TCAGCCACCGCCAGAGTGTTTGC
Rod1	CG1569	Moderate	TGTCAACGCACTGCAGGATCTTTCTG	TAGTGATCAGGCCAGGGCACAGG
Apical/Basal polarity				
Yurt	CG9764	Moderate	ACGCATCGTGGTCAACAAGAACAATCG	ATGCTCCTGATTGTCATAGTCGCCAG
Tao	CG14217	Moderate-high	AGCCAGACATGAAGATCAACCTCCGTATG G	ACCAGTCAGCACTCCATCACGCACATAC
Actin-related proteins				
Short Stop	CG18076	Low	CAAATCCGTCAGATCGTGCTC	CTTGCTCTCAAAGTGCCTC
Arpc4 (Arp2/3 complex sub4)	CG5972	Very High	GATTGAGCGCATACTGTGCC	CTCCGTGTGGAAGTTGGTGA
Arp5	CG7940	Moderate	TCGAGCGAATGTGATAACCA	TTGTCTGTCCATCGAATCCC

Table S1. Genes tested in the candidate-based RNAi screen in S2 cells. Expression levels of these genes in S2 cells were obtained from (1).

SI Reference

1. L. Cherbas *et al.*, The transcriptional diversity of 25 *Drosophila* cell lines. *Genome Res* **21**, 301-314 (2011).