Supplemental Materials

Molecular Biology of the Cell

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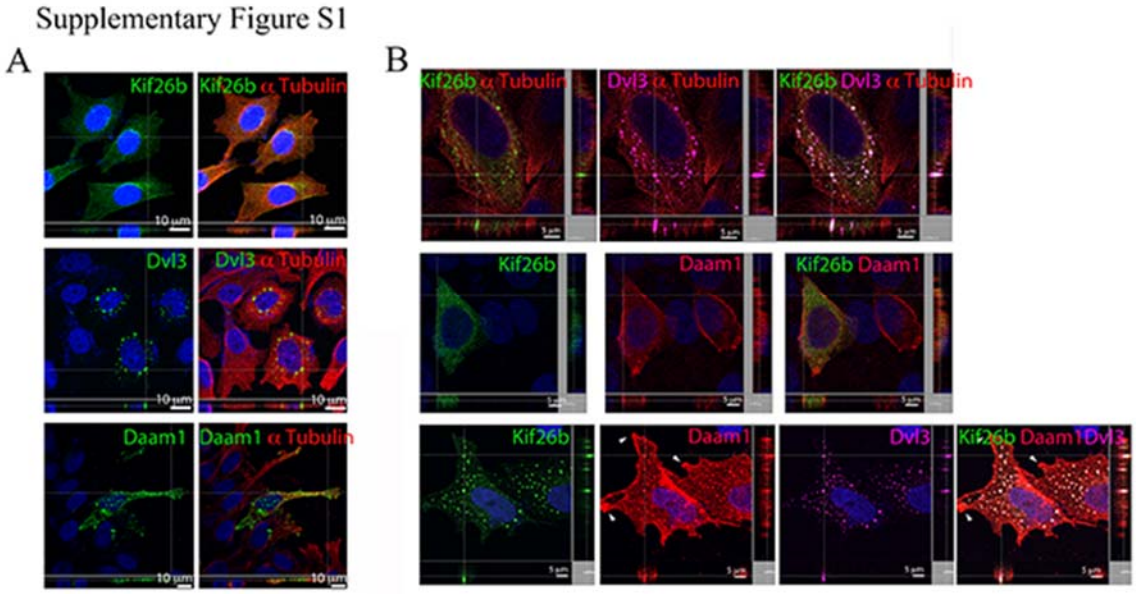
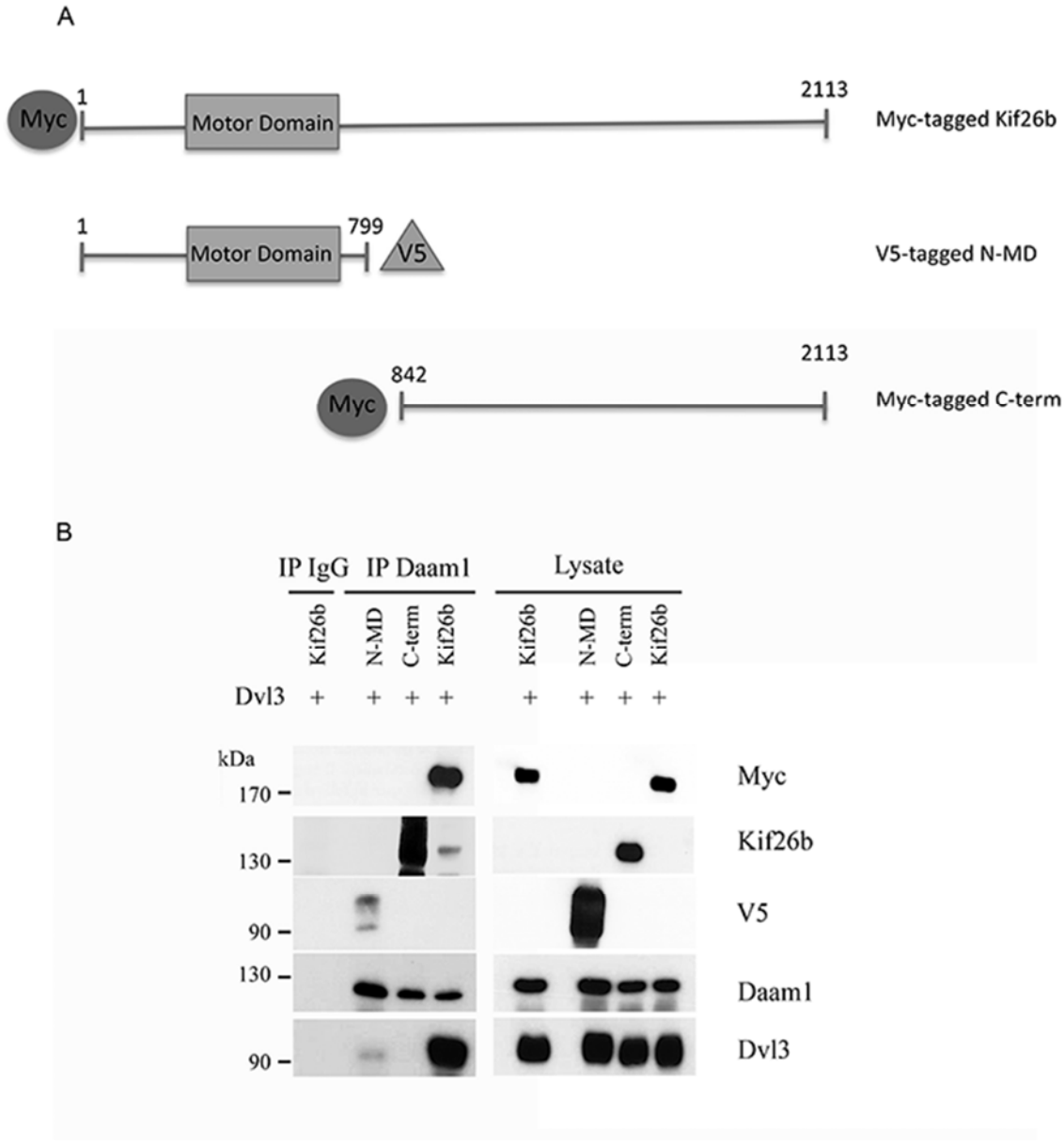
Supplementary Figures:



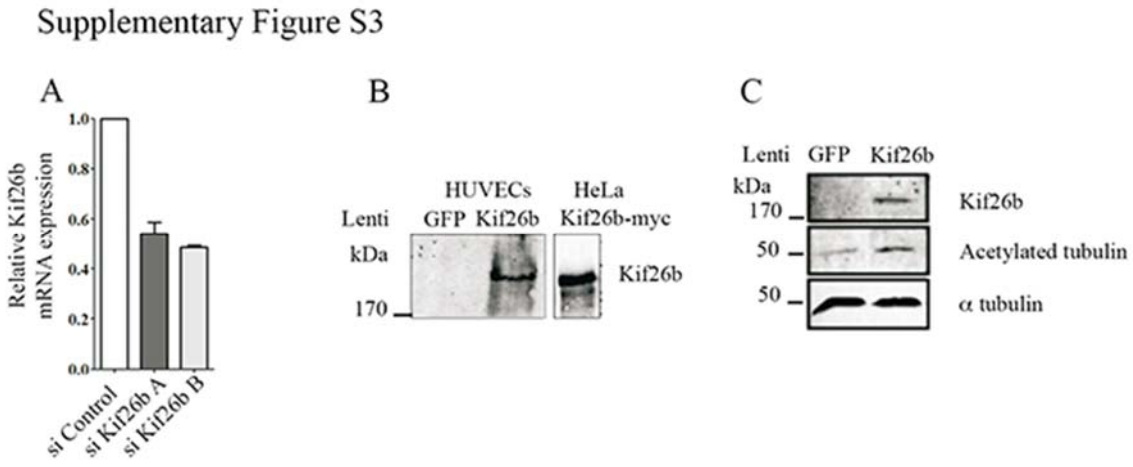
Suppl Figure S1. (A) Confocal analysis of HeLa cells transfected with plasmids coding for either Kif26b, Dvl3-tag (green), Daam1 and immunostained either with anti Kif26b (green), anti Daam1 (green), or anti α tubulin (red) respectively. (B) Confocal analysis of HeLa co-transfected with plasmids coding for either Kif26b, Dvl3-tag (purple), Daam1 and immunostained either with anti Kif26b (green), anti Daam1 (red), or anti α tubulin (red), respectively. Upon Kif26b, Dvl3 and Daam1 triple co-expression, Daam1 is redistributed and co-localized with Kif26b and Dvl3 in large puncta in the cytoplasm and at the leading edge (white arrows). In A, Scale bars, 10 μm; B, Scale bars, 5 μm.

Supplementary Figure S2

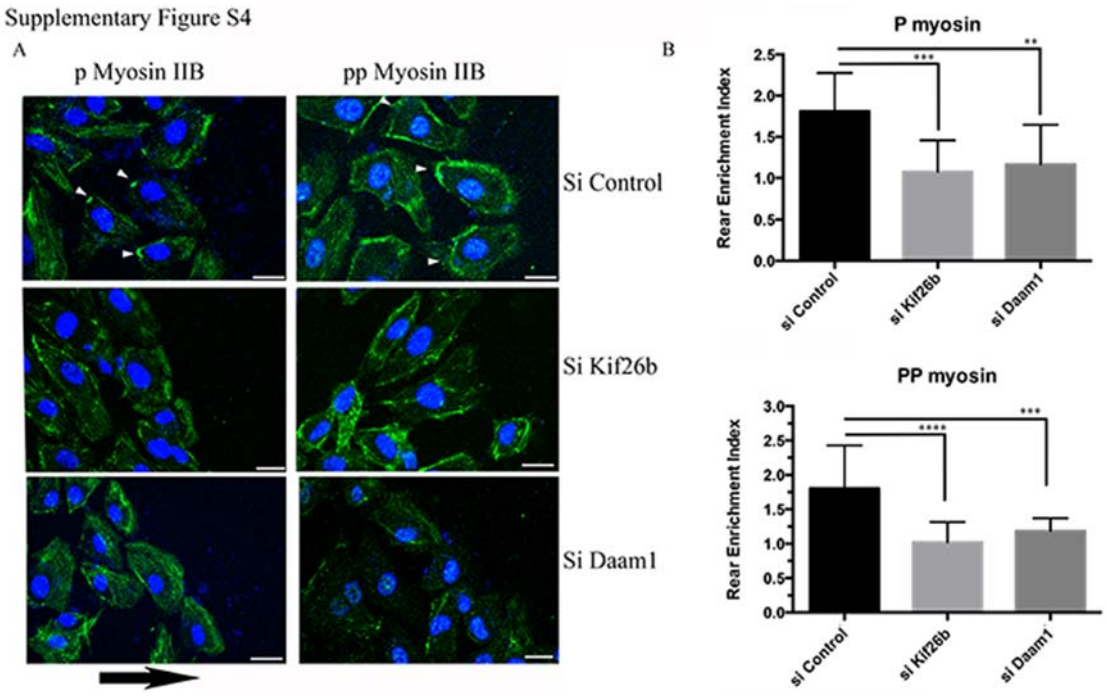


Suppl Figure S2. A. Schematic representation of Kif26b full length (Kif26b with a Myc Tag), of N-Motor domain Kif26b mutant deleted of the C-terminal portion (N-MD with V5 Tag) and of C-term Kif26b mutants deleted of the Motor Domain (C-term Kif26b with a Myc Tag). B. Extracts from HeLa cells transfected with each indicated vector in combination with Dvl3 were immunoprecipitated (IP) either with antibody against non specific IgG or Daam1. IP and lysates were then immunoblotted with anti-myc (for Myc

tagged Kif26b), anti Kif26b, anti V5 (for V5 tagged N-MD), anti-Daam1 and anti-Dvl3 antibodies.



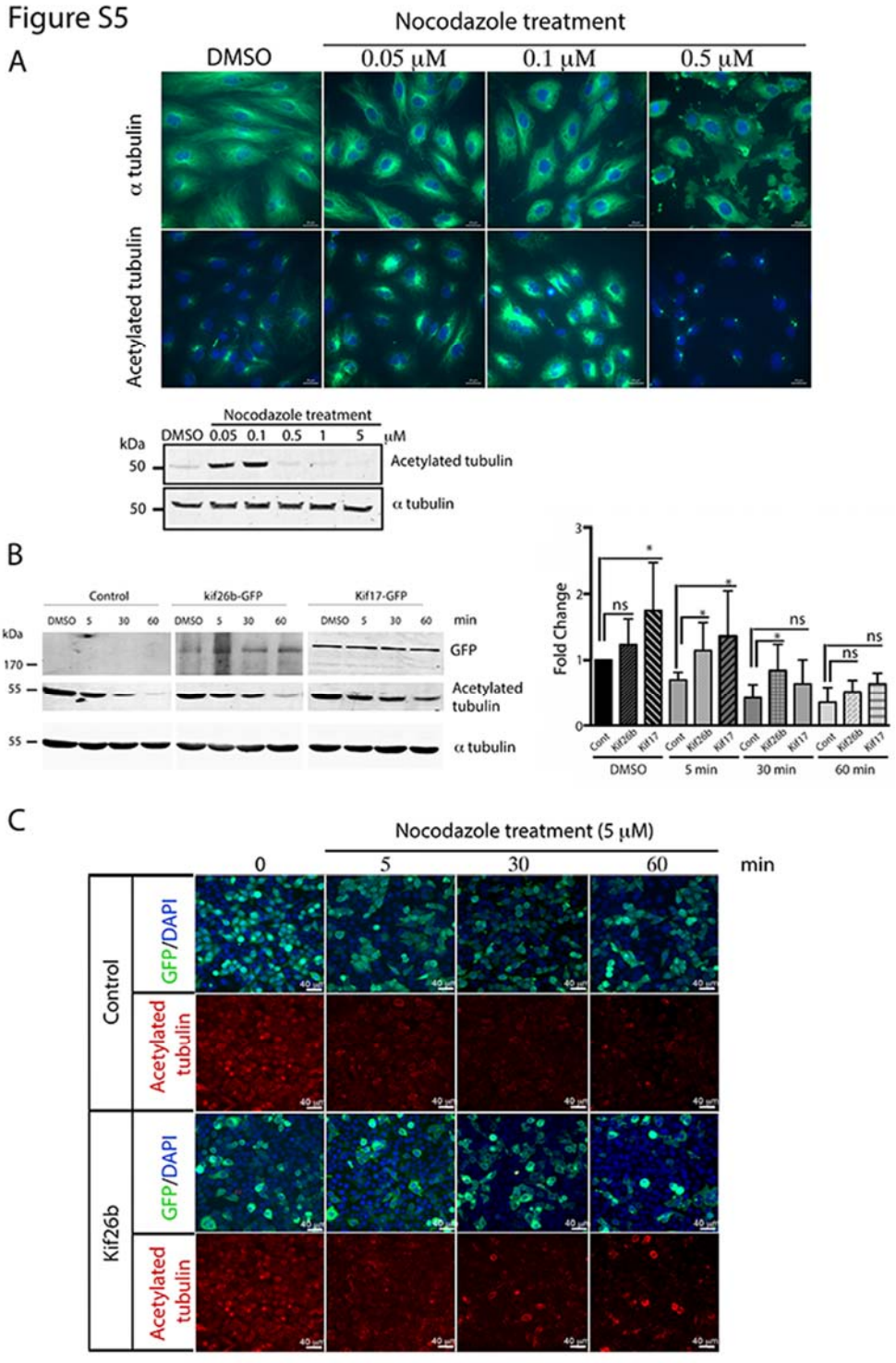
Suppl Figure S3. A. qPCR of the inhibition of human Kif26b transcript expression in si Kif26b (A or B) HUVECs compared to si Control conditions. **B.** Immunoblot of GFP or Kif26b lentiviral-transduced HUVEC lysates with anti-Kif26b antibody. Kif26b-transfected HeLa cells were loaded as a positive control. **C.** Kif26b ectopic expression induced an increase in acetylated tubulin expression level. Immunoblotting of either Control or Kif26b lentiviral-transduced HUVEC lysates stained with anti Kif26b, anti-acetylated tubulin and anti-α tubulin antibodies.



Suppl Figure S4. Knockdown of Kif26b and Daam1 impaired phosphorylated Myosin II cell rear enrichment in migrating cells.

A. Si Control, si Kif26b or si Daam1 HUVECs were fixed and stained with p (Ser19) or pp (Thr18/Ser19) myosin light chain II (green) and with DAPI (blue) one hour after wounding a confluent monolayer in the presence of Wnt3a to induce cell migration and imaged. Scale bars, 50 µm. Black arrow indicates direction of migration.

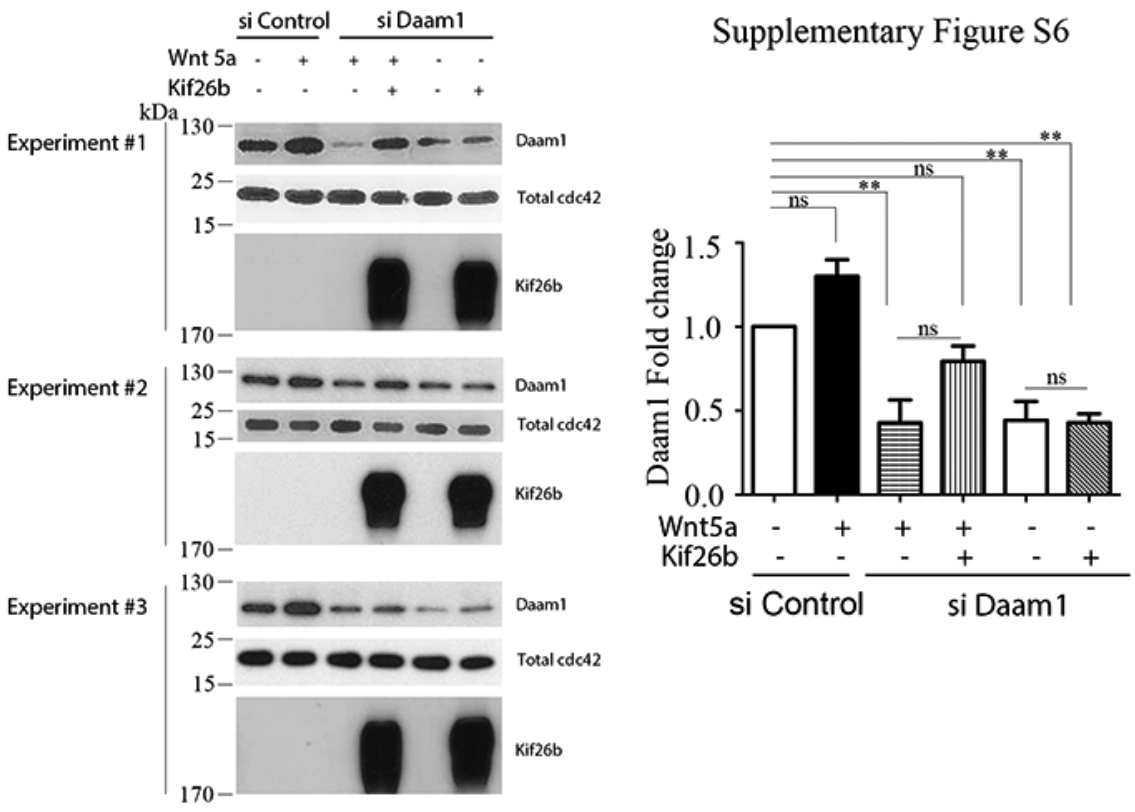
B. Asymmetric rear enrichment of Myosin IIB was assessed by analyzing the intensity values along the line drawn across the nucleus long axis and cell extension processes perpendicular to the wound line. Intensity profile curve per cell was then plotted; area under curve in the rear (A_R) and front (A_F) of the nucleus was calculated and a rear enrichment index determined ($i=A_R/A_F$). Bars denote mean ± s.e.m, n ≥ 12 cells from n=3 experiments. Statistical comparison between groups was performed using one-way ANOVA followed by Tukey's test ($P<0.05$) to detect differences between all groups. ** $P<0.01$, *** $P<0.001$, **** $P<0.0001$ by one-way ANOVA. ns=not significant.



Suppl Figure S5. A. Sensitivity of MT to nocodazole treatment in HUVECs. HUVECs were treated with DMSO or different concentrations of nocodazole (0.05, 0.1, 0.5 μM). Cells were then either immunostained, with anti α tubulin (Green) or anti acetylated-

tubulin (Green) and DAPI (Blue), or lysed. Western blots were revealed with anti-acetylated tubulin and anti-α tubulin antibodies. Scale bars, 10 μm.

Kif26b over-expression induced MT stabilization in HeLa cells. **B.** Control, Kif26b-GFP or Kif17-GFP transfected HeLa cells were treated with either DMSO or nocodazole (5 μM) during 5, 30 or 60 min and lysates were subjected to SDS-Page. Western blots were revealed with anti-GFP, anti-acetylated tubulin and anti-α tubulin antibodies. Fold change quantification was calculated in relation to the acetylated tubulin level in HeLa under basal control conditions at different time points. Error bars denote mean ± s.e.m, n=3. ns=not significant and * $P<0.05$ by one-way ANOVA followed by Tukey's test. **C.** GFP-control or Kif26b-GFP transfected HeLa cells were treated with nocodazole during 5, 30 or 60 min and then stained with either GFP (green) or acetylated tubulin (red) and dapi (blue) and imaged. Scale bars, 40 μm.



Suppl Figure S6. Kif26b ectopic expression did not significantly modify Daam1 expression.

HeLa were treated with si Control or si Daam1 and then transfected with a control plasmid or a plasmid coding for Kif26b. Lysates were blotted with Daam1, Myc and total cdc42. Fold change in Daam1 expression levels from 3 independent experiments was calculated in relation to Daam1 level under si Control basal condition and are displayed graphically (right panel). We could note that the expression level of Daam1 is not significantly reduced in si Daam1 condition under Kif26b ectopic expression plus Wnt5a activation, suggesting that activation of PCP signaling may increase Daam1 expression. Error bars denote mean ± s.e.m, n=3 experiments. ns = not significant and ** $P<0.01$ by One-way ANOVA followed by Tukey's test. ns=not significant.