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Supplemental information

Polarized SCAR and the Arp2/3 complex regulate apical cortical remodeling in asymmetrically dividing neuroblasts

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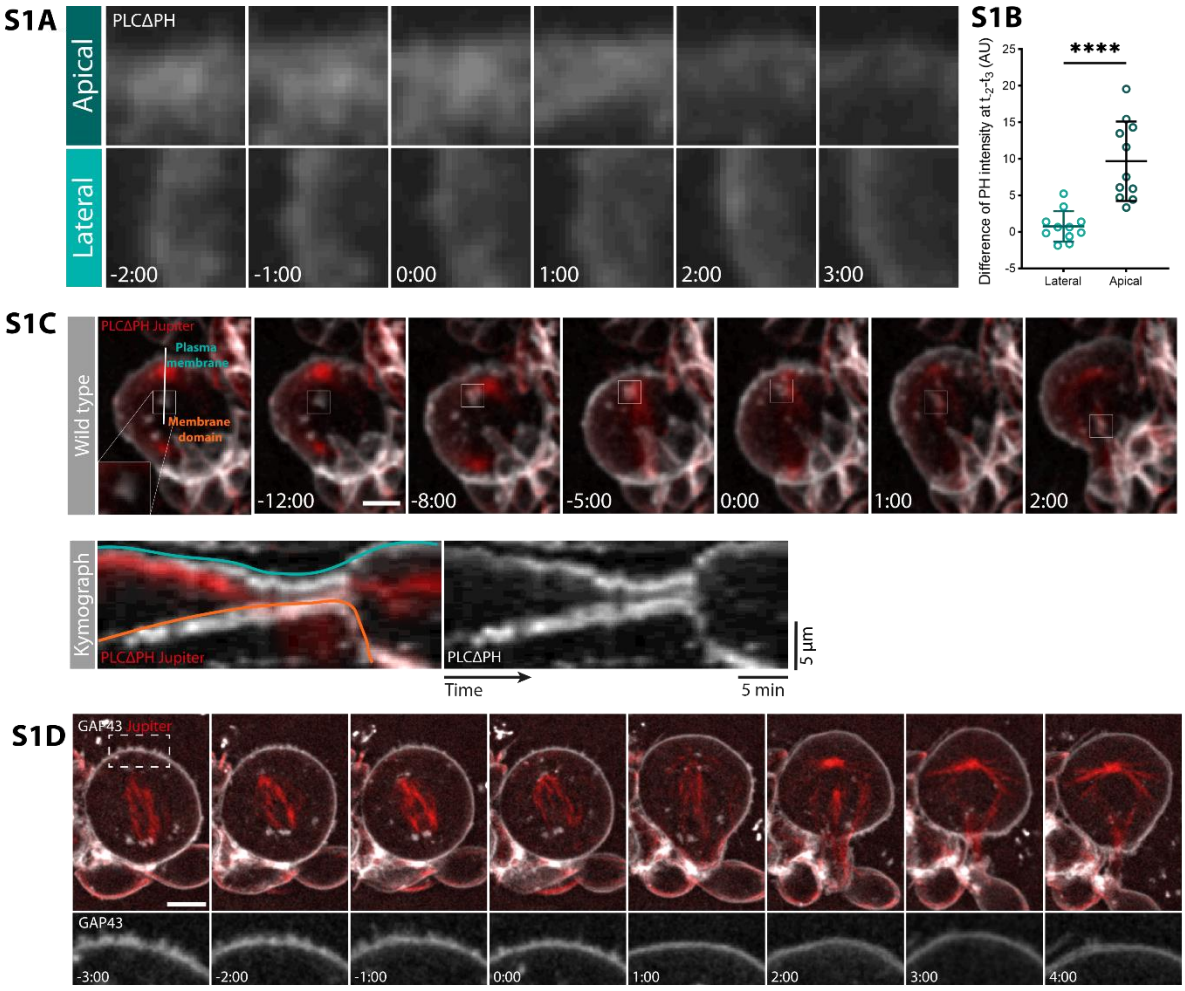

Figure S1. The apical plasma membrane is remodelled as neuroblasts progress through mitosis, related to Figure 1. A. Images show a zoom in of the apical and lateral membrane of a wild-type neuroblast expressing UAS-PLCΔPH::GFP. **B.** Plot showing difference of PH intensity between t = -2min and t = 3min in both lateral and apical membrane domains. Asterisk (****) denote statistical significance. P ≤ 0.0001 (paired t-test). Central and error bars: mean and SD. **C.** Maximum intensity z-projection of dividing neuroblast expressing membrane marker, UAS-PLCΔPH::GFP and a microtubule marker UAS-mCherry::Jupiter, both expressed via Wor-GAL4/UAS. The white line indicates the position used to generate the kymograph. Insert shows an example of a membrane domain. Kymograph shows the movement of the plasma membrane (blue) and of a more basal PH::GFP-rich membrane domain (orange). **D.** High resolution imaging of neuroblast expressing membrane marker, mCherry::GAP43, driven by sqh promoter. Insert shows membrane protrusions at the apical side of the cell in metaphase (-3:00 to -1:00 min). Apical protrusions begin to disappear as cells enter anaphase. Scale bars = 5 µm.

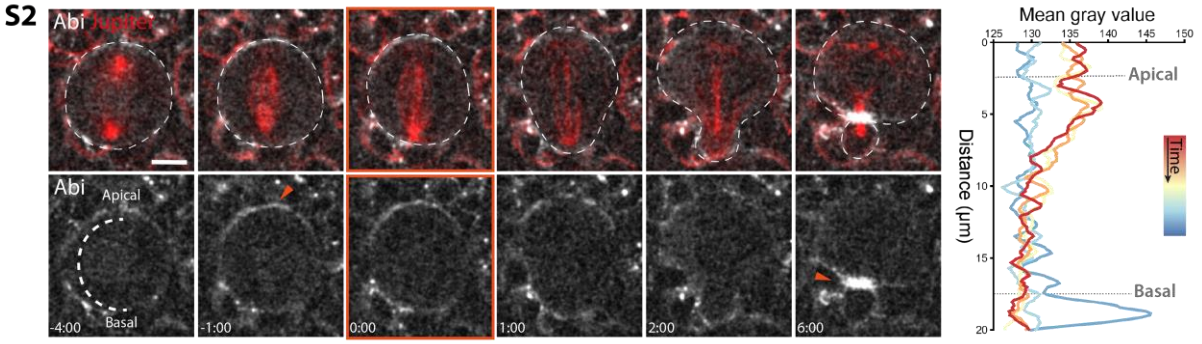

Figure S2. A second component of the SCAR complex, Abi, localizes at the apical side of the neuroblast at metaphase and the furrow at cytokinesis, related to Figure 2. Representative super-resolution images of neuroblast expressing *ubi*-mCherry::Abi. Arrowheads point to Abi localization at the apical cortex in metaphase and at the furrow at cytokinesis. Mean Abi signal intensity at each timepoint was acquired by drawing a line around the cortex from the apical to the basal side, as depicted at time -4:00 relative to anaphase onset. These values were then plotted in graphs on the right. Scale bar = 5 µm.

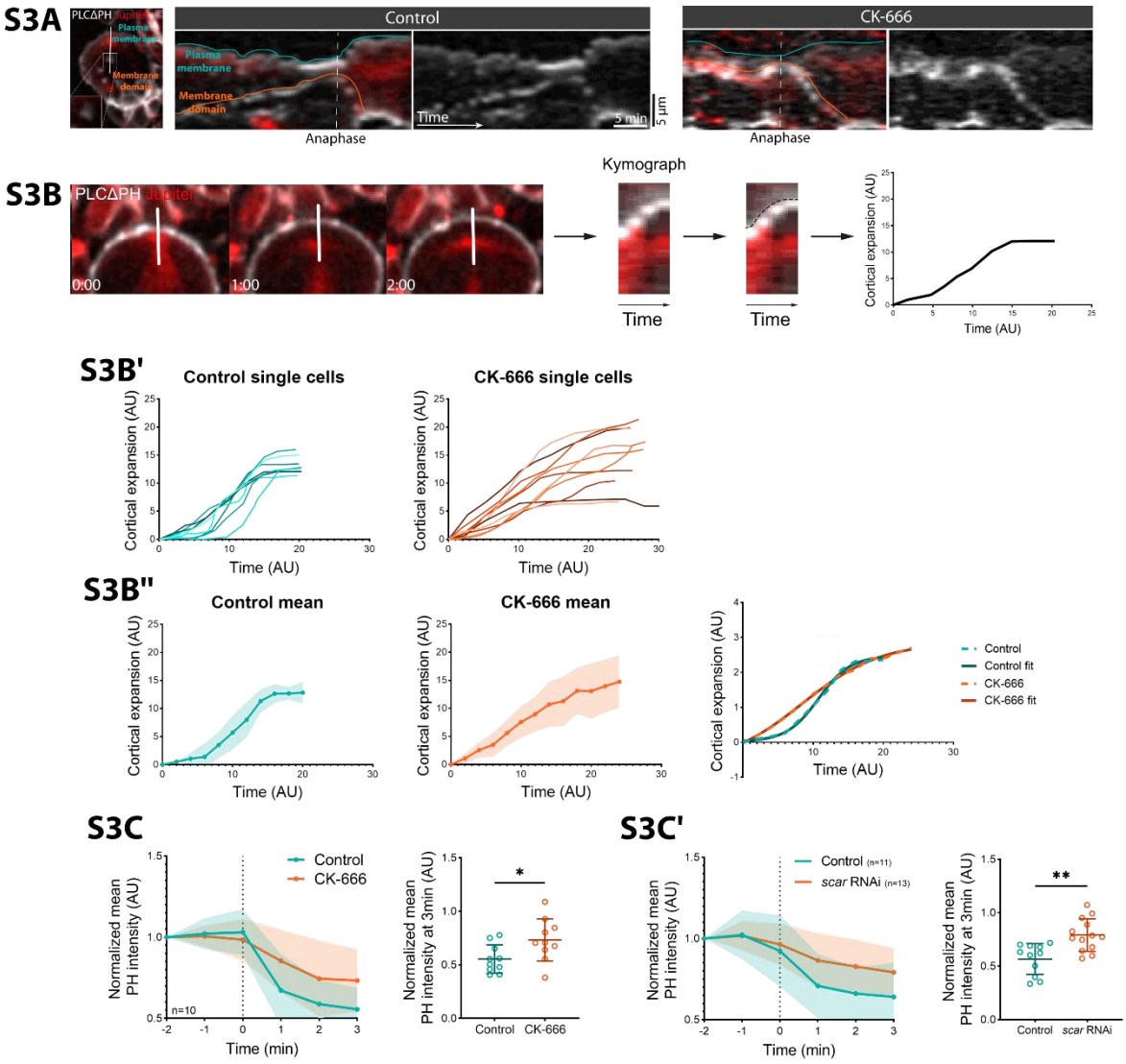

Figure S3. Arp2/3 and SCAR are required for the normal remodelling of the apical membrane in neuroblasts at the onset of anaphase, related to Figure 3. A. Representative kymographs of control (CK-689 treatment) and CK-666 treated cells expressing PLCΔPH::GFP and mCherry::Jupiter as they progress from prophase to cytokinesis. Lines indicate the movement of the apical plasma membrane (blue lines) and the movement of PLCΔPH::GFP punctae (orange lines). The cell shown on the left is an example of a maximum intensity z-projection of a dividing neuroblast in which the white line indicates the position used to generate the kymograph. **B.** Schematics showing how the kymograph and plots in B'-B" were obtained. In brief, a line drawn on the movie was used to generate a kymograph from which coordinates were manually tracked, exported and plotted to follow the movement of the membrane (graph on the right). **B'-B".** Graphs show single cells tracks (**B'**) and mean path (**B"**) of membrane expansion during anaphase for control (CK-689) and CK-666 treated cells. Coordinates were centred to start at (x=0, y=0), and a linear interpolation of the x set of coordinates was performed. Graphs on the right show curves for mean cortical expansion for control and CK666 treated cells with their fitted curves: the control curve is fitted best by a linear regression, and the treatment curve is fitted best by a sigmoid. n=10. **C.** Plots show changes in PLCΔPH::GFP intensity during metaphase-anaphase transition in control (CK-689) and CK-666 treated cells, and statistically significant difference between PH intensity at the last time point (3 min). n=10. t-test: *P ≤ 0.0001. **C'.** Plot showing changes in PLCΔPH::GFP intensity during metaphase-anaphase transition in Control and Scar RNAi cells, and statistically significant difference between PH intensity at the last time point (3 min). n control=11, n RNAi=13. T-test: **P ≤ 0.01. Central and error bars = mean and standard deviation.

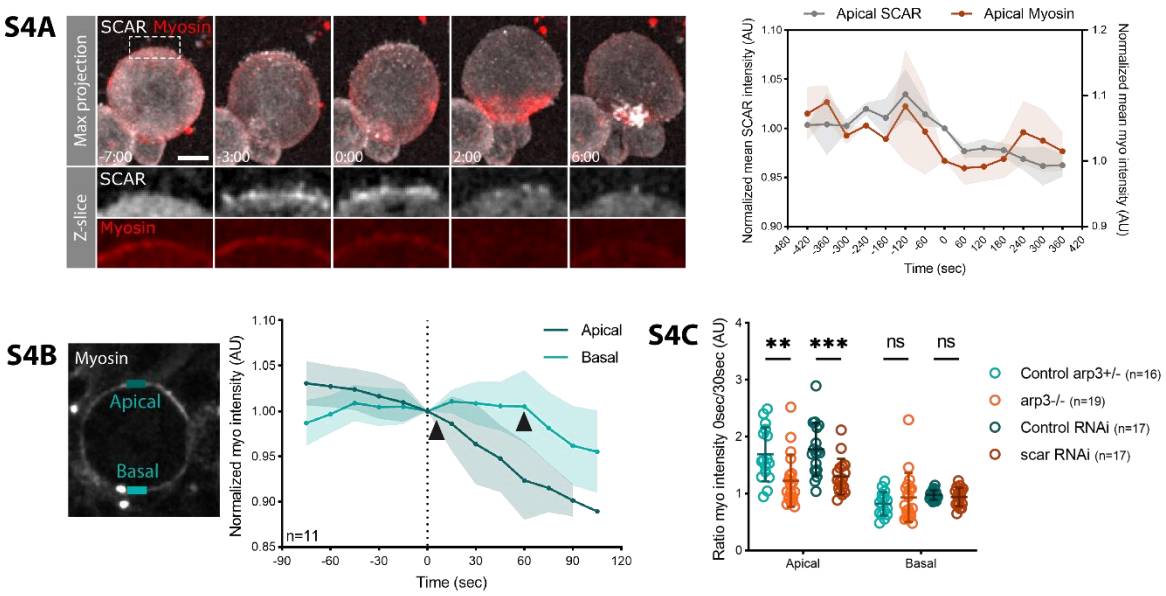

Figure S4. Apical Myosin dynamics at the metaphase-anaphase transition, related to Figure 4. A. Representative images of dissociated neuroblast cells expressing both UAS-SCAR::GFP and the non-muscle Myosin II marker, UAS-Sqh::cherry, under the control of Wor-GAL4. Inserts show apical SCAR and Myosin signals separately. Graph on the right shows SCAR and Myosin apical intensities during neuroblast division, with anaphase onset indicated by t=0. **B.** Graph shows changes in apical and basal Myosin intensity before and after anaphase onset (t=0) measured in cells expressing the Myosin marker, Sqh::GFP. Arrowheads mark the approximate times at which Myosin starts to be cleared. **C.** Graph shows the ratio of Myosin intensity at anaphase onset (0 sec) versus 30 seconds later in both apical and basal domains in CK-666 treated cells, and SCAR RNAi cells and in their respective controls (Tukey's multiple comparison test). ns, not significant, $P > 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$. Scale bar = 5 µm. Central and error bars = mean and standard deviation.

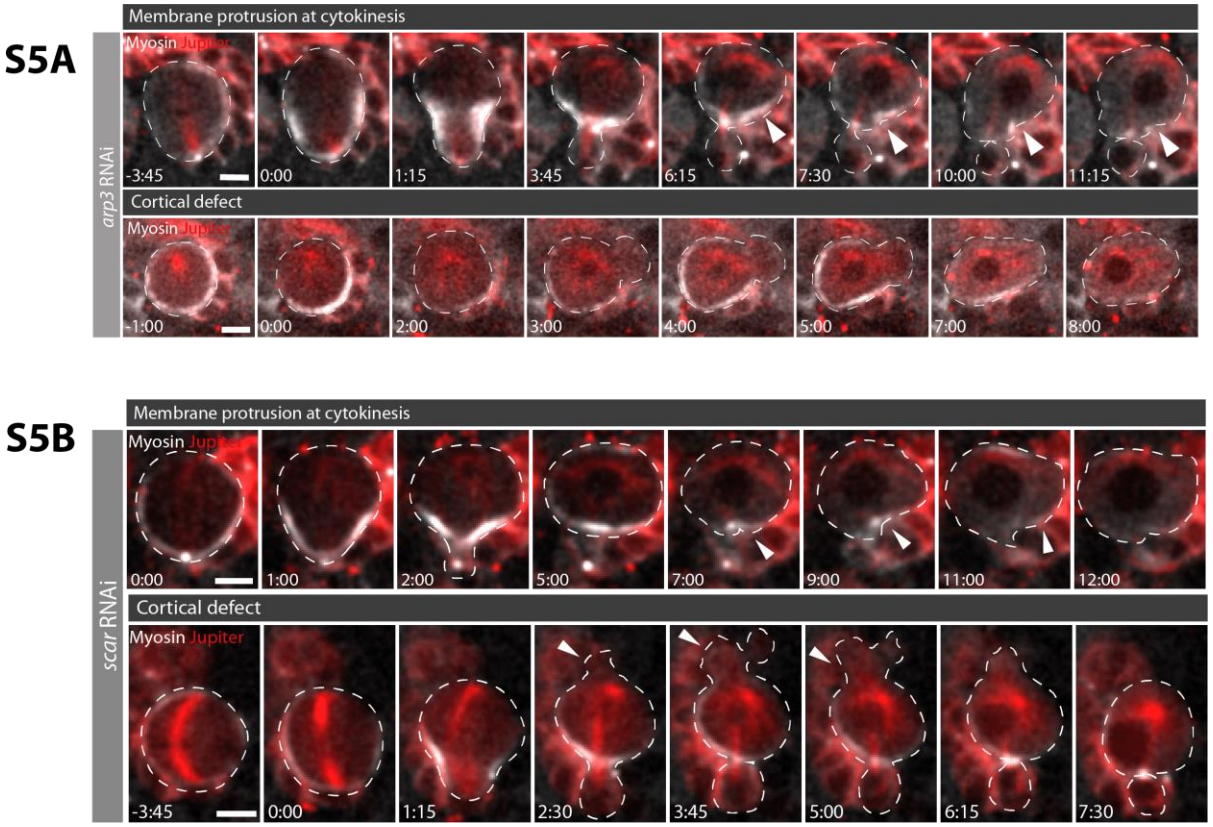

Figure S5. RNAi mediated silencing of either *arp3* or *scar* leads to cortical defects and membrane instability in neuroblasts undergoing cytokinesis, related to Figure 5. Time-lapse image of representative neuroblasts expressing the Myosin marker Sqh::GFP along with the microtubule marker cherry::Jupiter. **A.** Cells expressing dsRNA targeting the *arp3* subunit of the Arp2/3 complex. **B.** Cells expressing dsRNAs targeting *scar*. Panels on top show an example of a membrane protrusion phenotype at cytokinesis. Panels on the bottom show example of cortical defects, including blebbing. Scale bar = 5 µm.