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RESEARCH ARTICLE

Figures and figure supplements

Asymmetric recruitment and actin-dependent cortical flows drive the neuroblast polarity cycle

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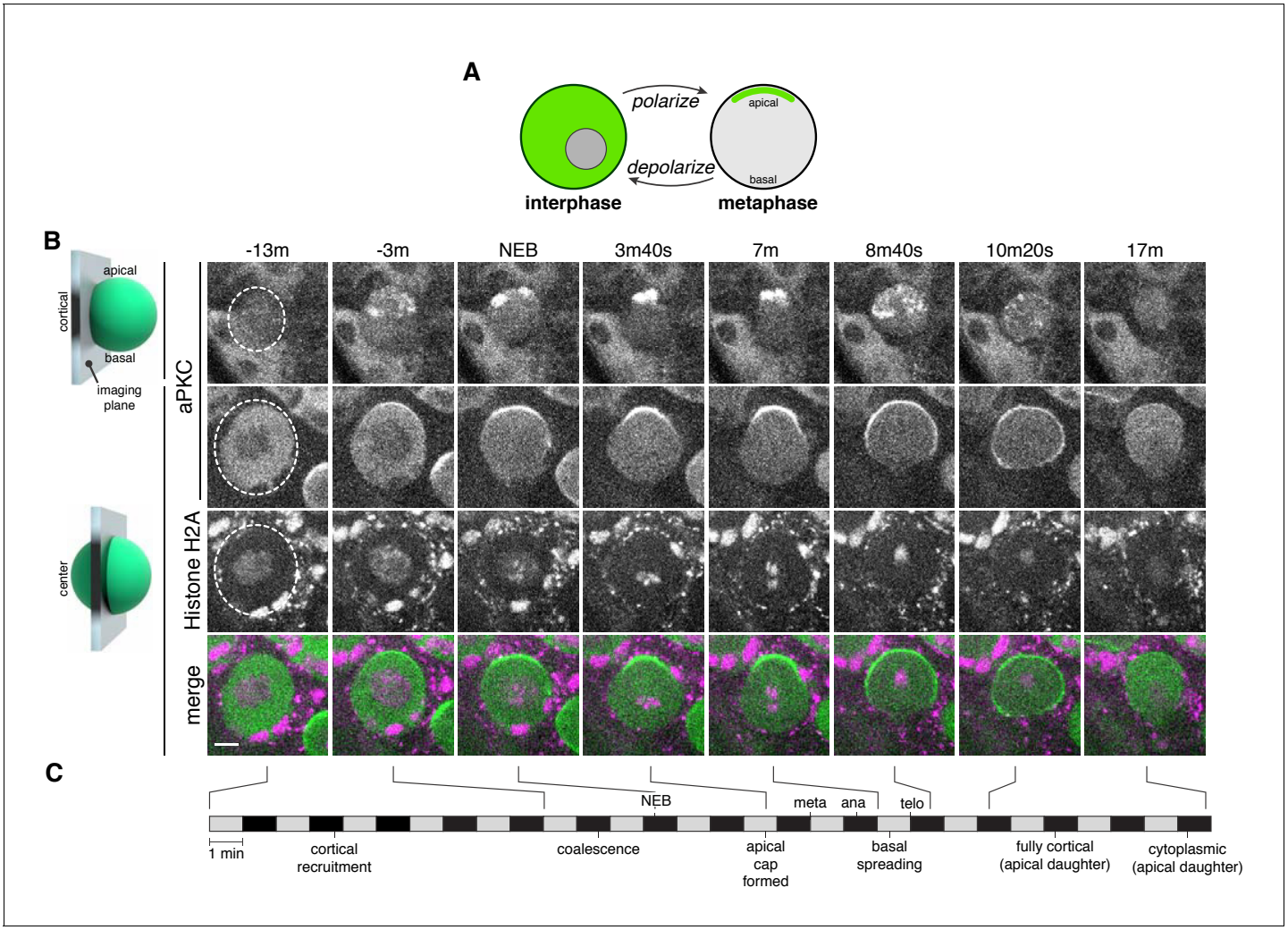

Figure 1. The neuroblast polarity cycle is a dynamic, multistep process. (**A**) Schematic of the neuroblast polarity cycle. Neuroblasts transition between unpolarized, cytoplasmic aPKC in interphase, to an apical cortical domain tightly focused around the apical pole in metaphase, the canonical neuroblast polarity state, during repeated asymmetric divisions. (**B**) Frames from ***Figure 1—video 1*** showing 1.5 µm maximum intensity projections of aPKC-GFP signal along the cortical edge ('cortical'; top row) and center ('center'; rows 2–4) of a neuroblast. A maximum intensity project of RFP-Histone H2A signal through the center of the cell, along with a merge of GFP and RFP central projections, are also shown. The outline of the neuroblast is highlighted with a dashed circle in the first column. Time is shown relative to nuclear envelope breakdown. (**C**) Timeline of the neuroblast polarity cycle with cell cycle hallmarks (NEB, nuclear envelope breakdown; meta, metaphase; ana, anaphase; telo, telophase) marked above the timeline and polarization events below.

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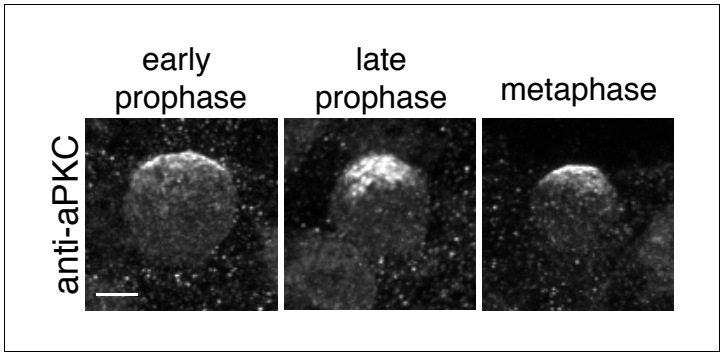

Figure 1—figure supplement 1. Cortical localization in fixed neuroblasts. Localization of aPKC in fixed neuroblasts. Cortical patches of aPKC are present in 12 μm maximum intensity projections of three different wild type neuroblasts stained with an anti-aPKC antibody. Cell cycle phases are from DAPI staining (early prophase, late prophase, metaphase; not shown). Scale bar is 5 μm.

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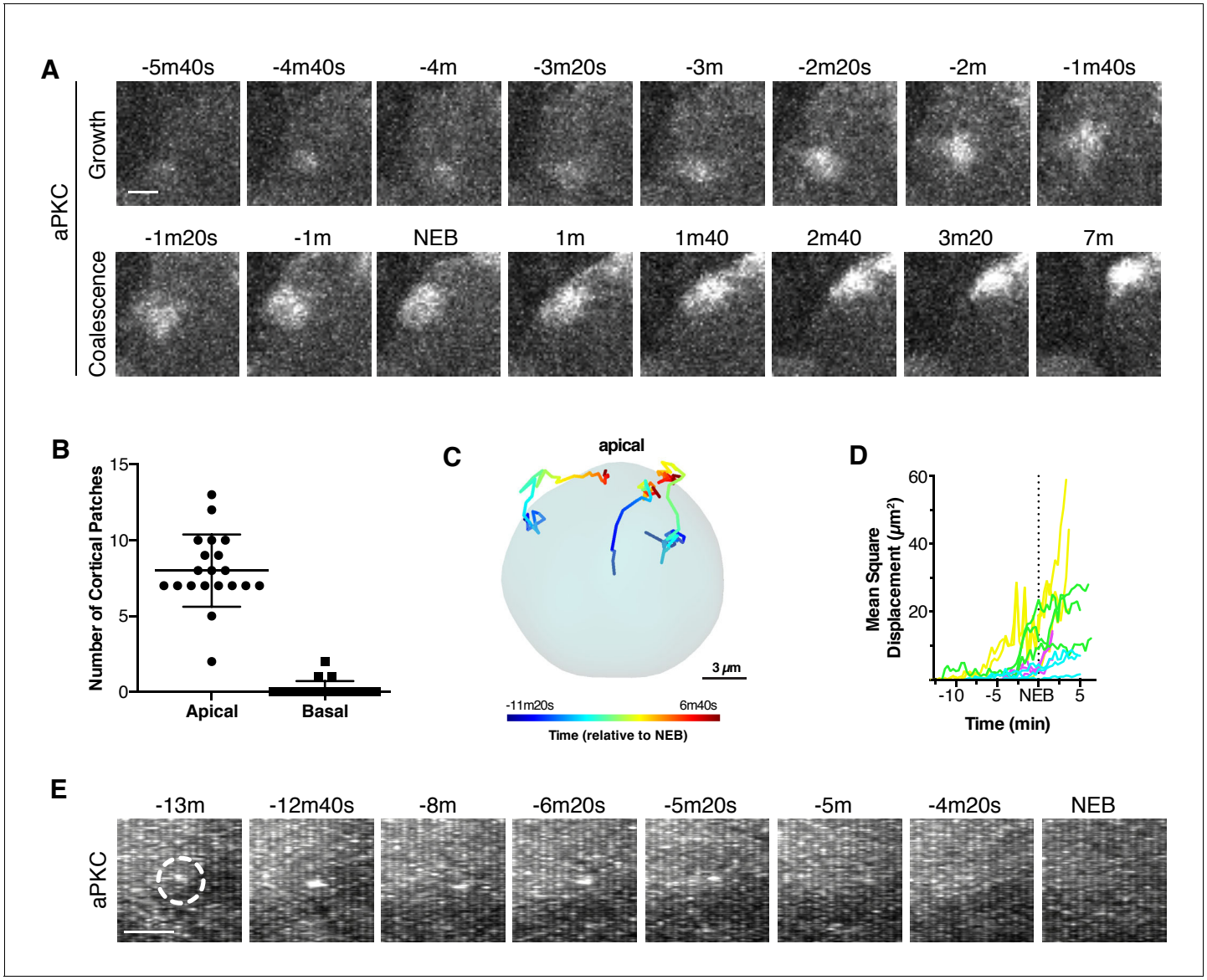

Figure 2. Apically directed cortical recruitment and patch coalescence. (**A**) Example of aPKC-GFP cortical patches during growth and coalescence phases. Scale bar 2 μm. (**B**) The number of aPKC-GFP cortical patches in the apical and basal hemispheres immediately before cortical flow begins. Each point represents a distinct neuroblast (taken from four larvae). Bars represent one standard deviation from the mean. Data are included in ***Figure 2—source data 1***. (**C**) Example patch trajectories during coalescence from particle tracking. Cell outline is shown in light blue. (**D**) Mean square displacement of several different patches identified by particle tracking as a function of time. (**E**) Frames (3 μm maximum intensity projection) from a time series showing the example fate of an aPKC-GFP cortical focus (dashed circle) that appeared in the basal cortical hemisphere and dissipated before NEB. Scale bar 2 μm.

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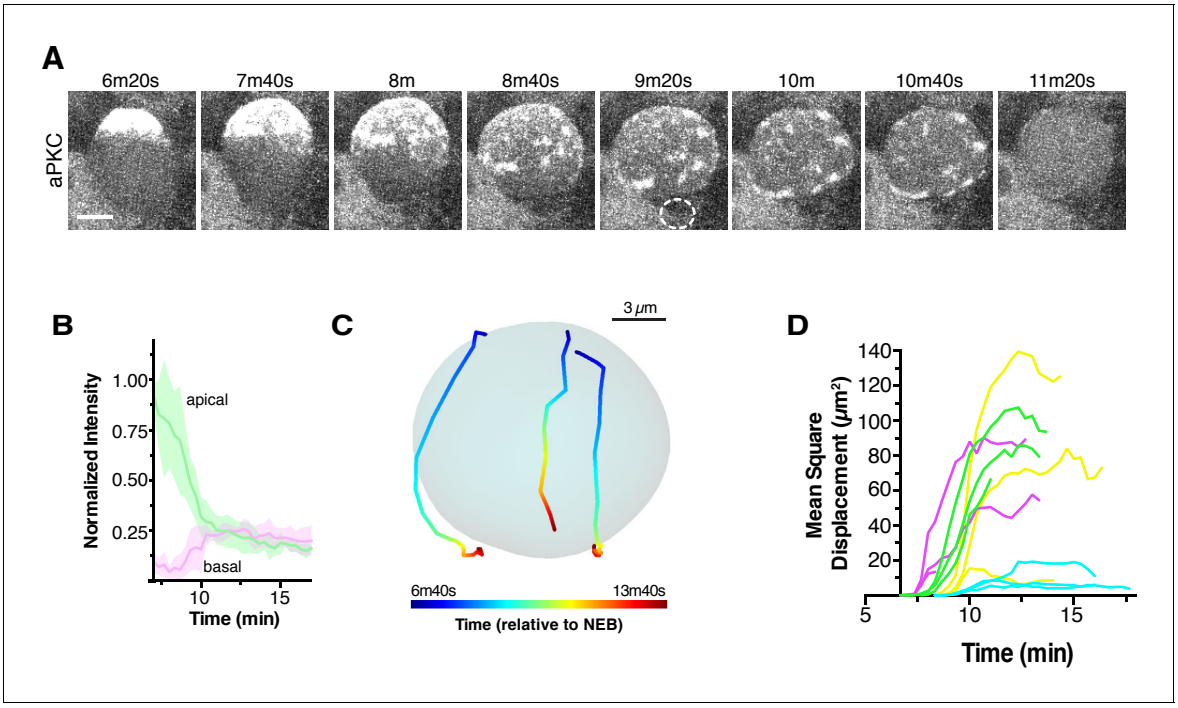

Figure 3. Apical cap disassembly. (**A**) Spreading of aPKC-GFP during cap disassembly and patch dissipation. A 6 μm maximum intensity projection (one hemisphere along the apical-basal axis) is shown in each panel. The time is relative to nuclear envelope breakdown. The position of the budding GMC is shown by a dotted circle as identified from the Histone H2A channel (not shown). Scale bar 5 μm. (**B**) Cortical and cytoplasmic intensity of aPKC-GFP in the apical and basal hemispheres during cap disassembly measured from four neuroblasts (error bars represent one standard deviation). Time is shown relative to NEB. (**C**) Example patch trajectories during cap disassembly from particle tracking. Cell outline is shown in light blue. (**D**) Particle tracking of independent patches reveals their mean square displacement as a function of time (relative to nuclear envelope breakdown).
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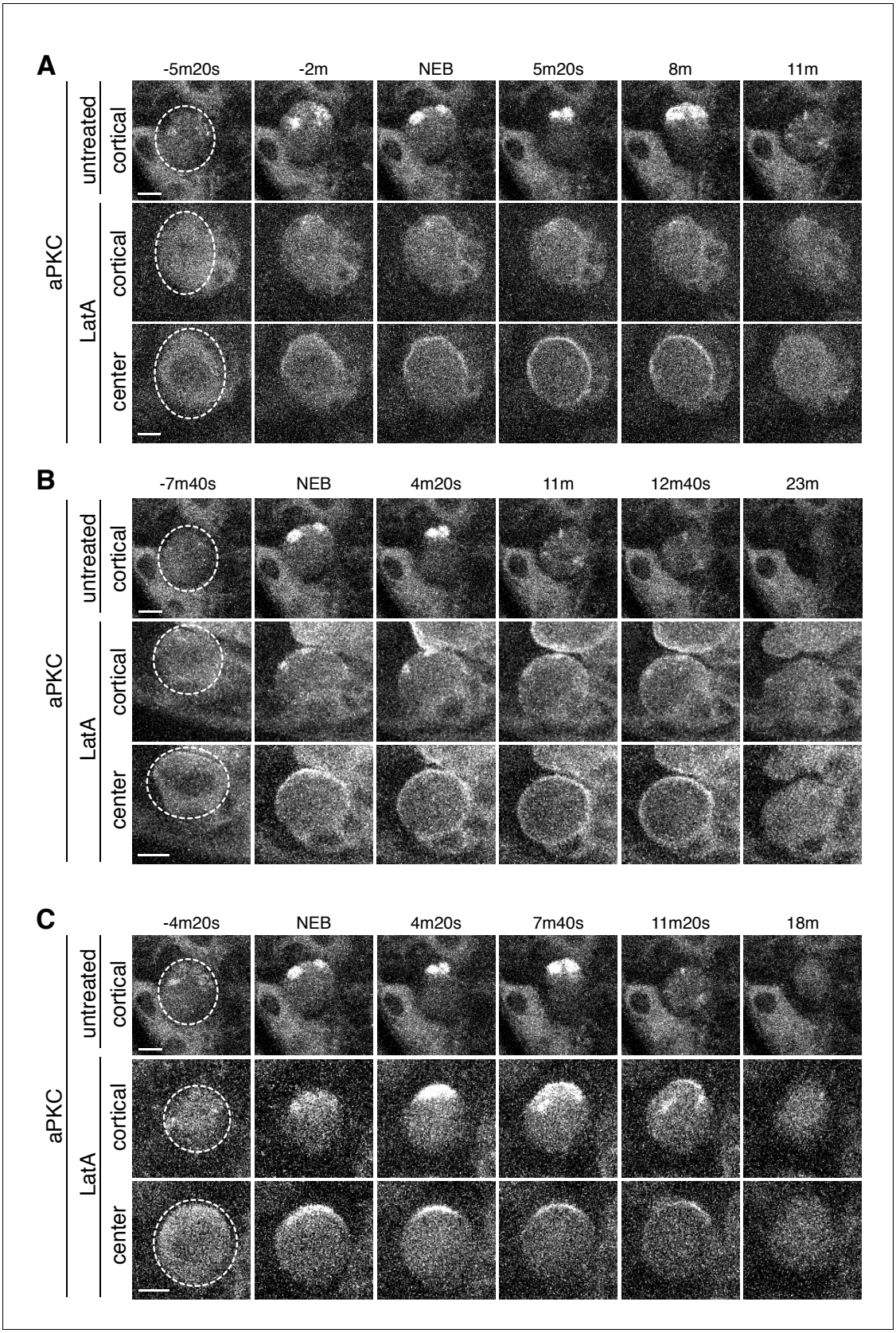

Figure 4. aPKC cortical dynamics following disruption of the actin cytoskeleton. (**A**) Effect of treating a neuroblast with LatA beginning in interphase (24m20s prior to NEB) on aPKC localization dynamics. Frames from ***Figure 4—video 1*** are shown as 4 µm maximum intensity projections along the

Figure 4 continued on next page

Figure 4 continued

cortical edge and center of aPKC-GFP taken from ***Figure 4—video 1***. The cortical projections from an untreated neuroblast at equivalent time points are shown for reference in the top row. The neuroblast is highlighted by a dashed circle in the first column. Time is shown relative to nuclear envelope breakdown (NEB). Scale bar 5 μm. (**B**) Effect of treating a neuroblast with LatA following the initial cortical recruitment events (7m20s prior to NEB) on aPKC localization dynamics. Frames from ***Figure 4—video 2*** are shown as in panel A. (**C**) Effect of treating a neuroblast with LatA following cap coalescence (4 m prior to NEB) on aPKC localization dynamics. Frames from ***Figure 4—video 3*** are shown as in panel A.

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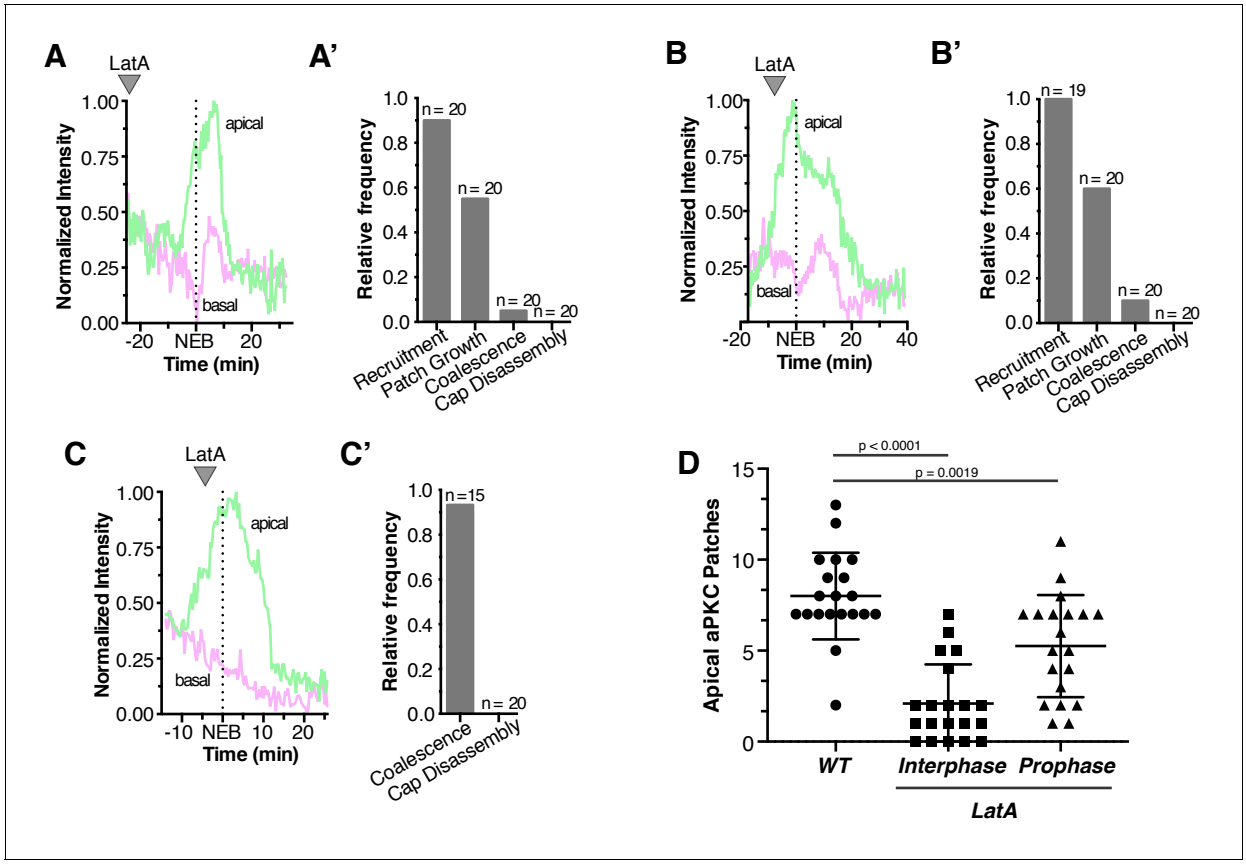

Figure 4—figure supplement 1. Quantification of Latrunculin A effects on aPKC localization dynamics. (**A**) Effect of treating an interphase neuroblast with LatA on aPKC localization. Normalized apical and basal cortical intensity is shown from ***Figure 4—video 1***. (**A'**) The frequency of neuroblasts treated with LatA in interphase that exhibit any aPKC recruitment to the cortex ('Recruitment'), growth of foci into patches ('Patch Growth'), coalescence of patches into an apical cap ('Coalescence'), and cap disassembly, are shown. Frequency is relative to wild type neuroblasts (wild type neuroblasts exhibit each effect with a frequency of 1.0; n = 20). (**B**) Effect of treating a neuroblast with LatA following the initial cortical recruitment events on normalized apical and basal cortical aPKC intensity (from ***Figure 4—video 2***). (**B'**) The frequency of neuroblasts treated with LatA following the initial cortical recruitment events that exhibit characteristics of the neuroblast polarity cycle, as in panel A'. (**C**) Effect of treating a neuroblast with LatA near cap coalescence on normalized apical and basal cortical aPKC intensity (from ***Figure 4—video 3***). (**C'**) The frequency of neuroblasts treated with LatA near cap coalescence that exhibit characteristics of the neuroblast polarity cycle, as in panel A' ('Recruitment' and 'Patch Growth' phases are not shown because they are completed by metaphase). (**D**) Number of apical aPKC patches in wild type neuroblasts and those treated with LatA either in interphase or prophase. Error bars represent one standard deviation from the mean. Statistical significance was calculated using a two-tailed t-test. Data are included in ***Figure 4—figure supplement 1—source data 1***.

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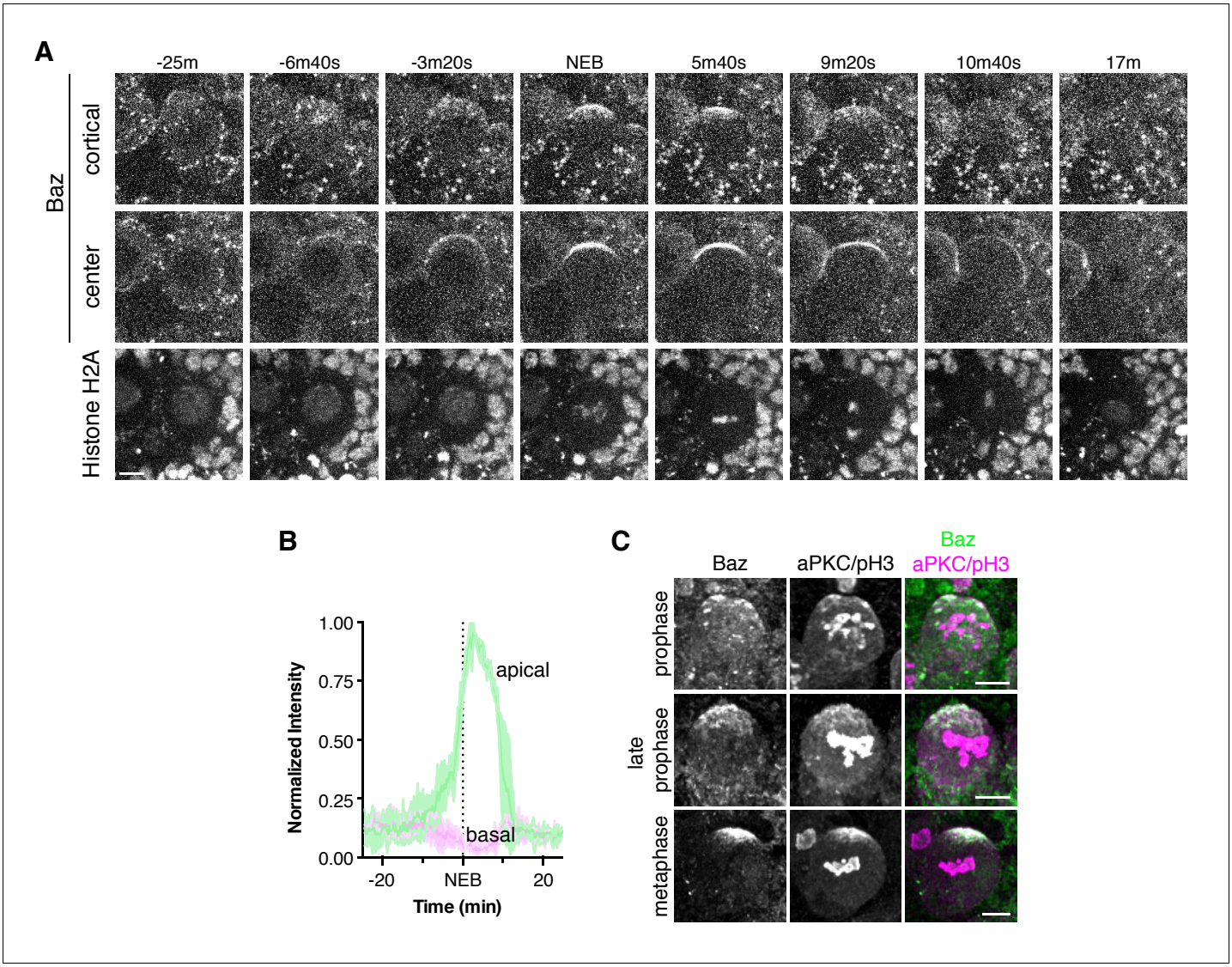

Figure 5. Bazooka dynamics during the neuroblast polarity cycle. (**A**) Frames from ***Figure 5—video 1*** showing 4 µm maximum intensity projections through the cortical edge and center of a larval brain neuroblast expressing Baz-GFP. A central projection of Histone H2A fusion to RFP is shown in the bottom row. The time relative to nuclear envelope breakdown ('NEB') is shown. Scale bar 5 µm. (**B**) Normalized apical and basal cortical intensity (see Materials and methods) of Baz-GFP as a function of time relative to NEB from the divisions of three different neuroblasts with the mean and standard deviation of the signal shown. (**C**) Localization of Baz and aPKC in fixed neuroblasts at early stages of mitosis (pH3 = phospho histone H3).

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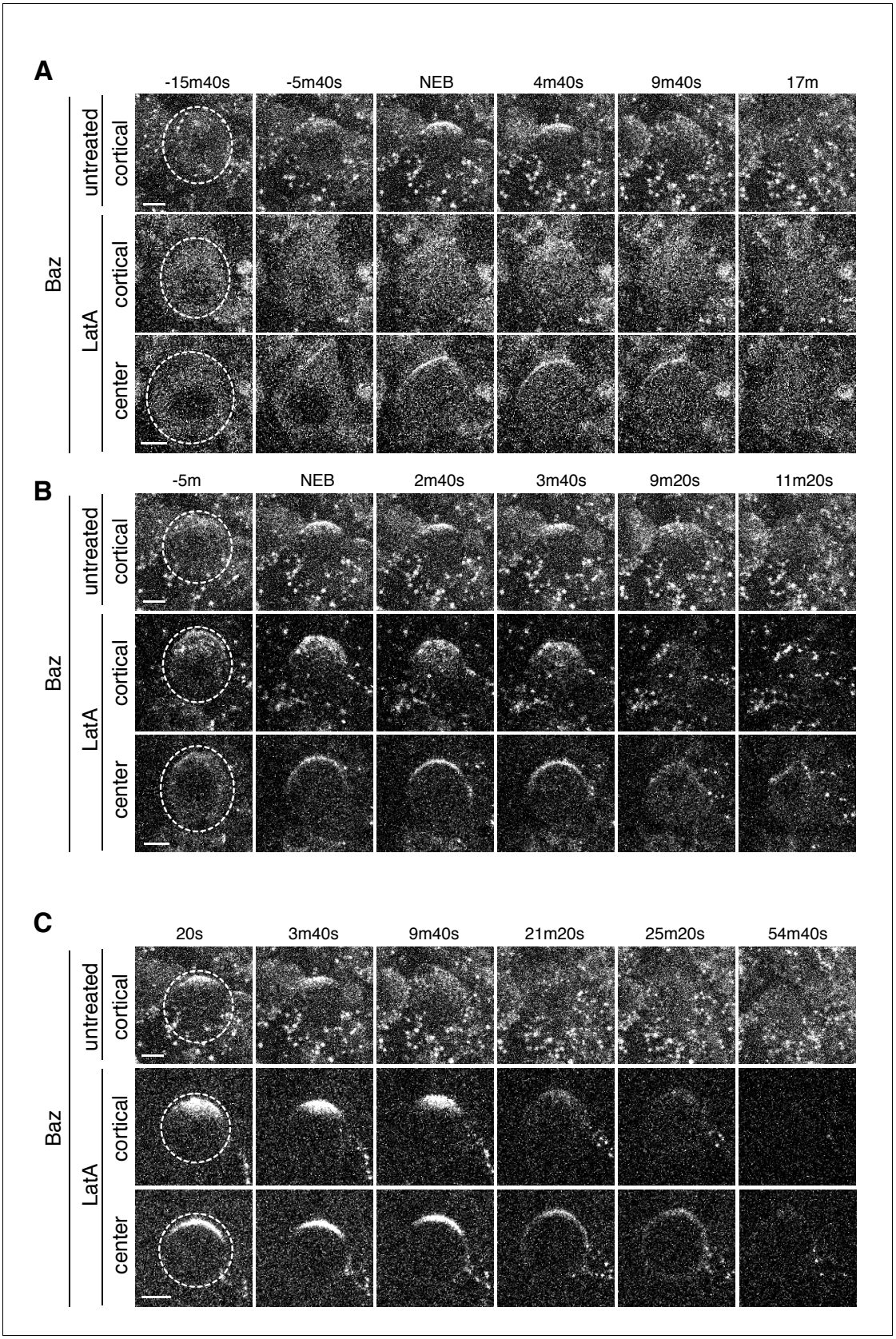

Figure 6. Baz cortical dynamics following disruption of the actin cytoskeleton. (**A**) Effect of treating a neuroblast with LatA beginning in interphase (83m20s before NEB) on Baz localization dynamics. Frames from ***Figure 6—video 1*** are shown as 4 µm maximum intensity projections along the

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Figure 6 continued

cortical edge and center of Baz-GFP taken from ***Figure 4—video 1***. The cortical projections from an untreated neuroblast at equivalent time points are shown for reference in the top row. The neuroblast is highlighted by a dashed circle in the first column. Time is shown relative to nuclear envelope breakdown (NEB). Scale bar 5 μm. (**B**) Effect of treating a neuroblast with LatA following the initial cortical recruitment events (7m40s prior to NEB) on Baz localization dynamics. Frames from ***Figure 6—video 2*** are shown as in panel A. (**C**) Effect of treating a neuroblast with LatA following cap coalescence (30 s prior to NEB) on Baz localization dynamics. Frames from ***Figure 6—video 3*** are shown as in panel A.

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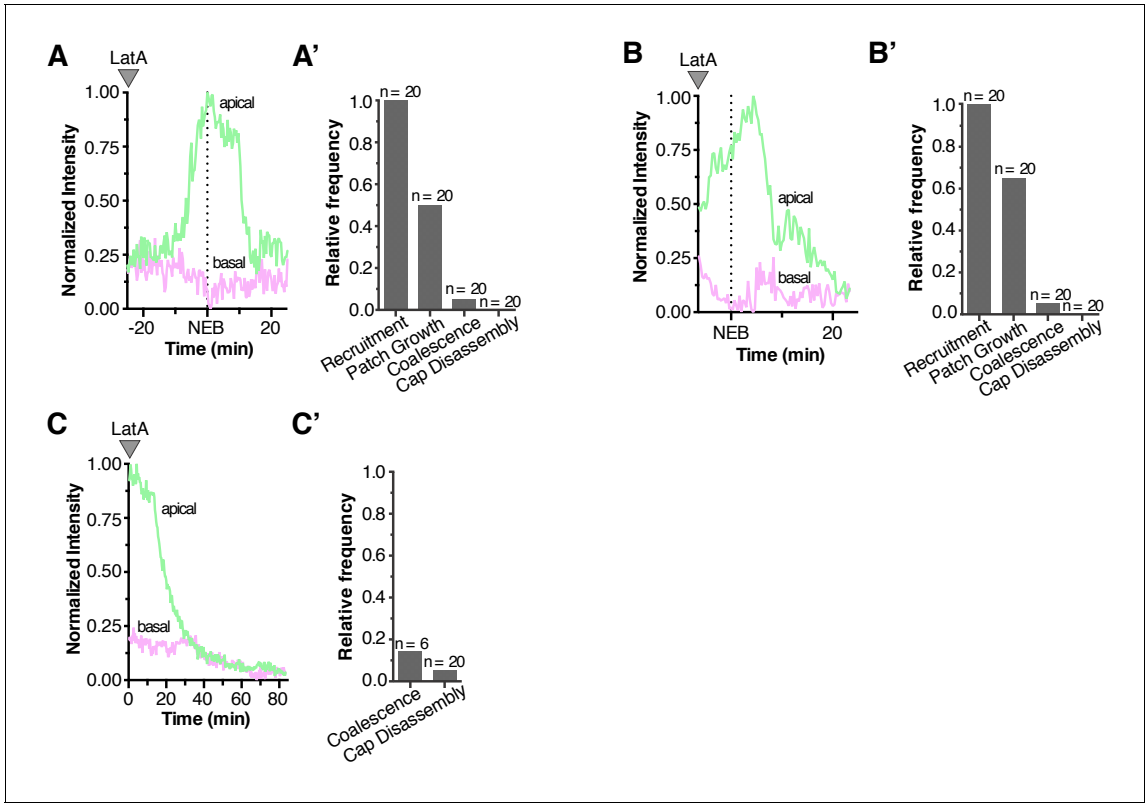

Figure 6—figure supplement 1. Quantification of Latrunculin A effects on Baz localization dynamics. (**A**) Effect of treating an interphase neuroblast with LatA on Baz localization. Normalized apical and basal cortical intensity is shown from ***Figure 6—video 1***. (**A'**) The frequency of neuroblasts treated with LatA in interphase that exhibit any Baz recruitment to the cortex ('Recruitment'), growth of foci into patches ('Patch Growth'), coalescence of patches into an apical cap ('Coalescence'), and cap disassembly, are shown. Frequency is relative to wild type neuroblasts. (**B**) Effect of treating a neuroblast with LatA following the initial cortical recruitment events on normalized apical and basal cortical Baz intensity (from ***Figure 6—video 2***). (**B'**) The frequency of neuroblasts treated with LatA following the initial cortical recruitment events that exhibit Baz cortical dynamics, as in panel A'. (**C**) Effect of treating a neuroblast with LatA following cap coalescence on normalized apical and basal cortical Baz intensity (from ***Figure 6—video 3***). (**C'**) The frequency of neuroblasts treated with LatA following cap coalescence that exhibit Baz cortical dynamics, as in panel A' ('Recruitment' and 'Patch Growth' phases are not shown because they are completed by metaphase).

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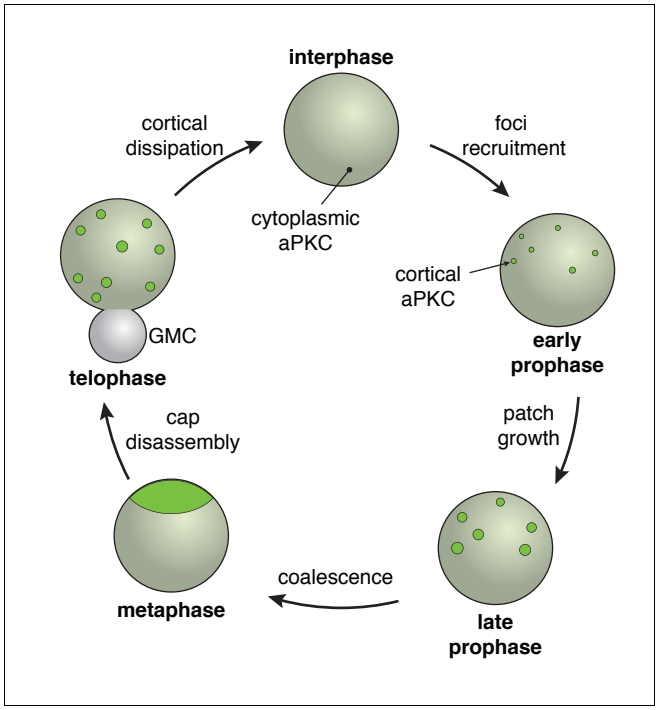

Figure 7. The neuroblast polarity cycle. The cycle begins with discontinuous patches of aPKC forming in the apical hemisphere via recruitment to the cortex from the cytoplasm. The aPKC cap observed in metaphase neuroblasts is formed from coordinated cortical flows that lead to coalescence of the discontinuous patches into a uniform structure tightly localized around the apical pole. During anaphase, the cap is disassembled leading to discontinuous spreading that extends to the cleavage furrow, followed by cortical dissipation back into the cytoplasm.

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