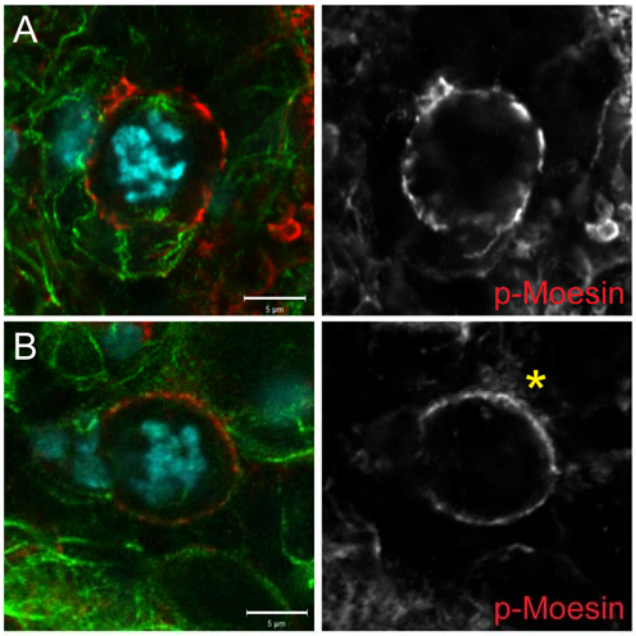
Supplemental Materials

Molecular Biology of the Cell

Abeysundara et al.



Supplementary Figure 1.
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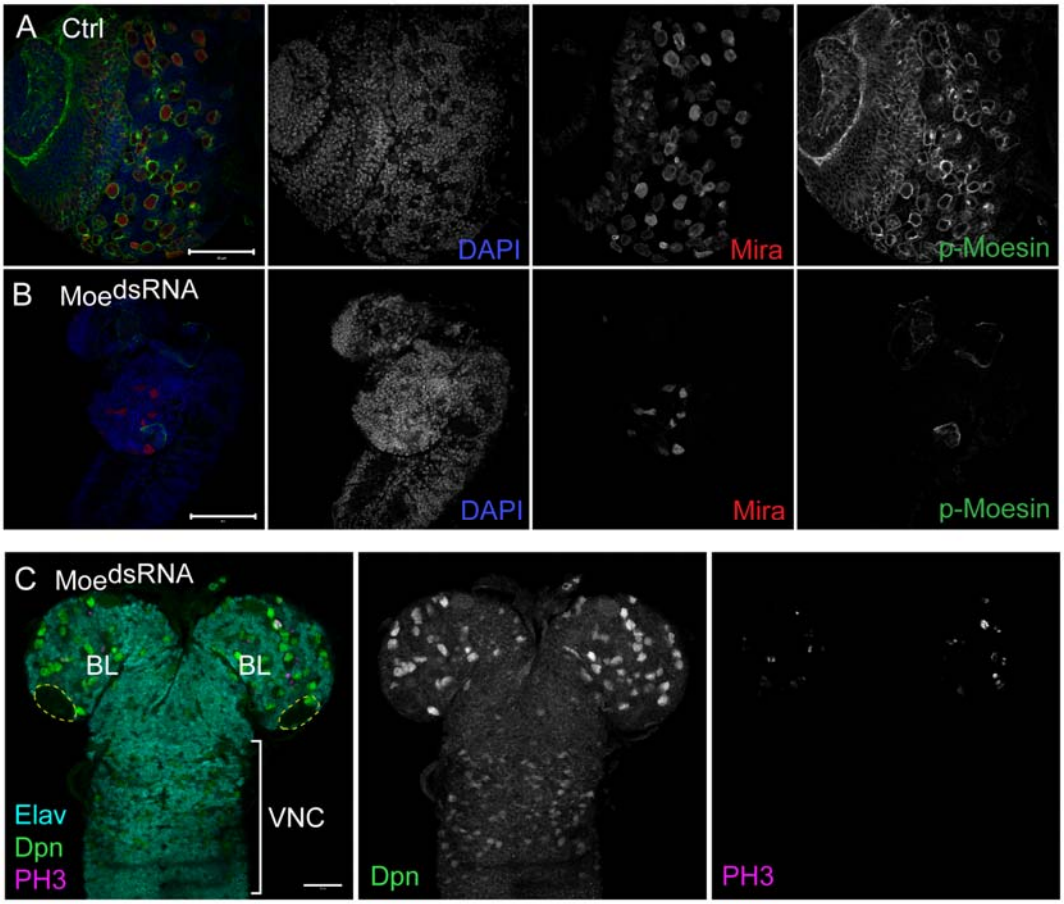
Supplementary Figure 1. Asymmetric distribution of phosphorylated Moesin is likely established at the prophase to metaphase transition. PhosphorylatedMoesin(p-Moesin) localization is (A) uniform and discontinuous at the cortex in 75% of w^{1118}prophase neuroblastsand (B) polar in 25% of w^{1118}prophase neuroblasts (n=20; yellow asterisk). Merged panels show anti-p-Moesin (red), anti-phospho-histone H3 (cyan), and anti-α-tubulin (green). All panels shown are single focal plane images. Scale bars represent 5 μm.



Supplementary Figure 2.
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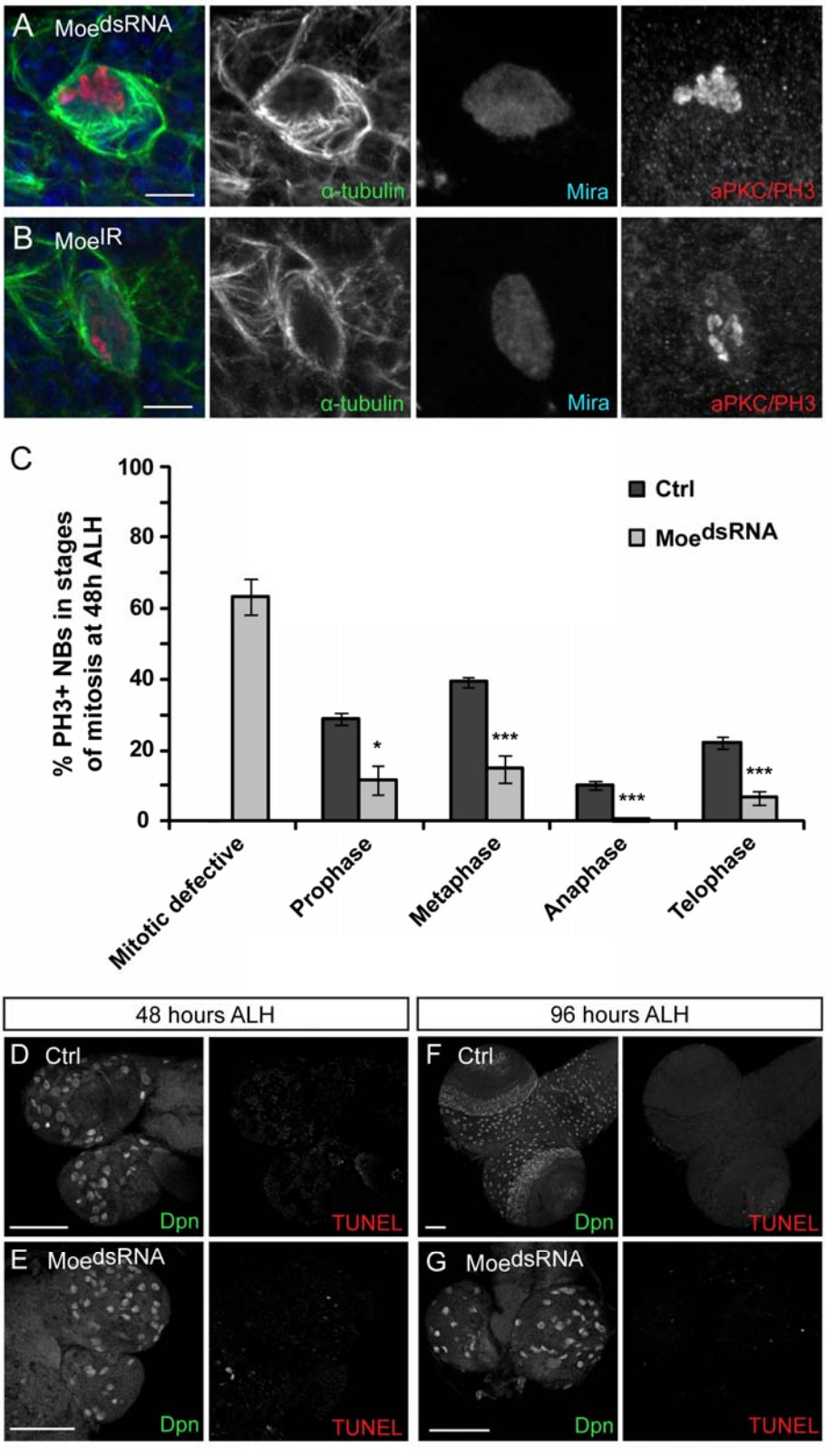
Supplementary Figure 2. Reduced Moesin levels using *Insc-GAL4*display defects in neuroblast proliferation and optic lobe development. LarvalCNS isolated from progeny of *UAS-Dicer*;;*UAS-Moe*^{dsRNA} crossed to *w*^{1118}(Ctrl) and *Insc-GAL4* (Moe^{dsRNA}). (A) A single control larval brain lobe is shown. (B) Two larval brain lobes and the ventral nerve cord of the Moe^{dsRNA} are shown. The p-Moesin signal was largely reduced in the Moe^{dsRNA} larval neuroblasts. (A-B) Merged panels show DAPI (blue), anti-Miranda (Mira; red), and anti-p-Moesin (green). (C) Larval brain lobes (BL) and the thoracic region of the ventral nerve cord (VNC) from Moe^{dsRNA} larvae at 96 hours ALH. Deadpan (Dpn)-positive and phospho-histone H3 (PH3)-positive neuroblasts are present within the brain lobes, however no mitotic neuroblasts are observed in the thoracic region (87%, *n*=30 larvae) at 96 hours ALH. Yellow dashed line outlines optic lobe primordium. (C)

Merged panel shows anti-Elav (cyan), anti-Dpn (green), and anti-PH3 (magenta). Scale bars represent (A-B) 50 μm and (C) 20 μm.



Supplementary Figure 3.
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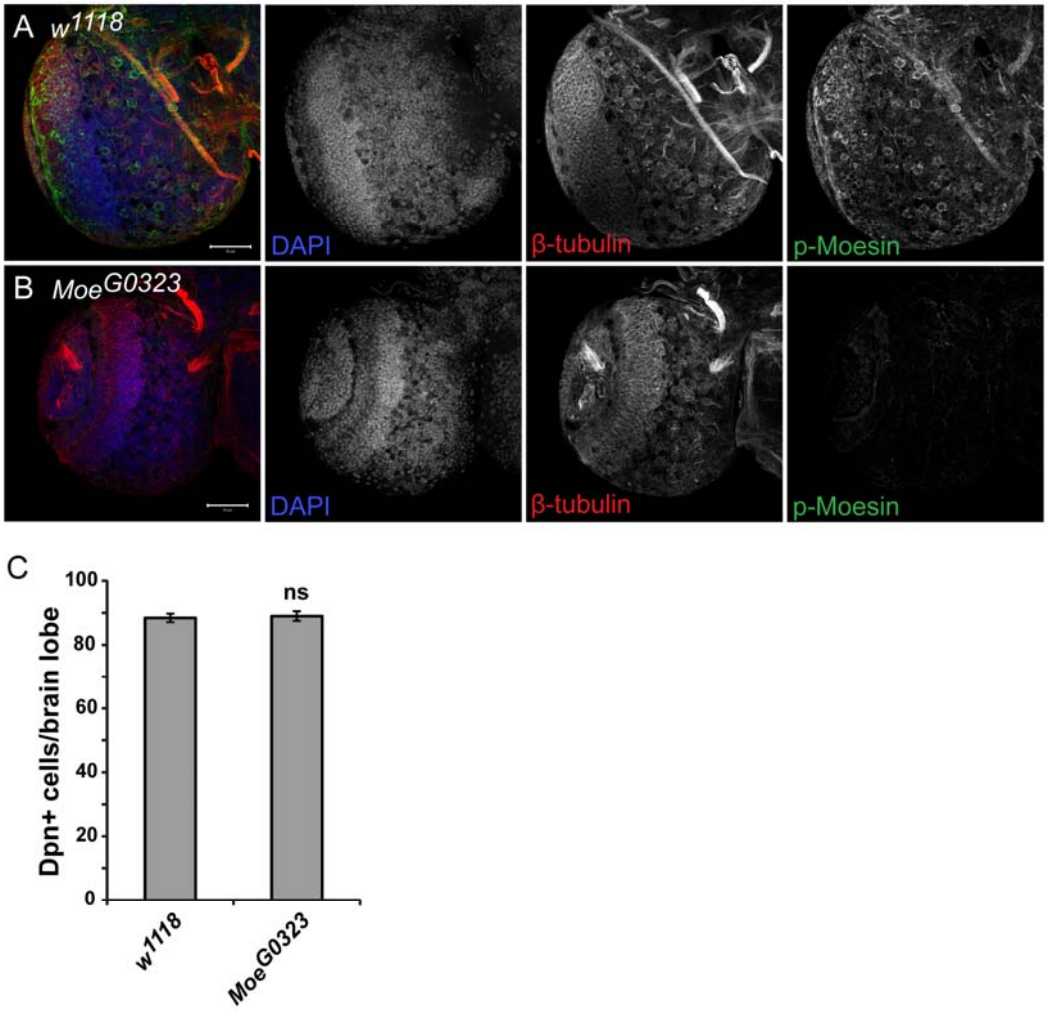
Supplementary Figure 3. Mitotic defective neuroblasts are observed with reduced Moesin levels. Mitotic defective neuroblasts observed in larval brains isolated from *Insc-GAL4* crossed to (A) *UAS-Dicer*;;*UAS-Moe*^{dsRNA} (Moe^{dsRNA}) and (B) *UAS-Dicer*;;*UAS-Moe.IR.327-775*(Moe^{IR}). (A-B) Mitotic defective neuroblasts are PH3-positive but lack spindle poles (α-tubulin panel), even though the nuclear envelope appears broken down (Mira panels). Merged panels show DAPI (blue), anti-α-tubulin (green), anti-aPKC/PH3 (red), and anti-Miranda (Mira; cyan). Gray-scale panels of Mira and aPKC/PH3 are maximum intensity projections and the α-tubulin panels are single focal plane images. (C) The mean proportion of PH3-positive, Dpn-positive cells undergoing the specific stages of mitosis per central brain lobe of Control (*n*=22) and Moe^{dsRNA} (*n*=34) at 48 hours ALH. In control brain lobes, the mean proportions (± standard error) of mitotic neuroblasts in the specific stages are 28.7 ± 1.6% (prophase), 39.2 ± 1.6% (metaphase), 10.1 ± 1.3% (anaphase), and 22.0 ± 1.7% (telophase). In Moe^{dsRNA} brain lobes,the mean proportions (± standard error) of mitotic neuroblasts are 63.5 ± 5.1% (mitotic defective), 11.6 ± 3.9% (prophase), 14.8 ± 3.8% (metaphase), 0.4 ± 0.4% (anaphase), and 6.7 ± 2.0% (telophase). (D-G) Control and Moe^{dsRNA}larval brains fluorescently labelled with anti-Dpn and TUNEL at (D-E) 48 hours and (F-G) 96 hours ALH. Scale bars represent (A-B) 5 μm and (D-G) 50 μm. *represents $p<0.05$ and ***represents $p<0.0001$ using unpaired *t* test.



Supplementary Figure 4.
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Supplementary Figure 4. *Moesin* hypomorphic mutant larval brains display reduced phosphorylated Moesin signal and normal brain morphology. (A) w^{1118} and (B) Moe^{G0323}third instar larval brains labelled with DAPI (blue), anti-β-tubulin (red), and anti-p-Moesin(green) show normal brain morphology in the Moe^{G0323} mutant, compared to controls. (C) The mean number of Dpn-positive cells per central brain lobe in w^{1118} (n=27) and Moe^{G0323} (n=26). Scale bars represent 50 μm. ns=not significant using unpaired t test.