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SUPPLEMENTAL DATA

MAPK3/1 (ERK1/2) and Myosin Light Chain Kinase in mammalian eggs affect myosin-II function and regulate the metaphase II state in a calcium- and zinc-dependent manner

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LEGENDS FOR SUPPLEMENTAL FIGURES

Figure S1

Panel A-C: Eggs were treated with 0.5% DMSO (lane 1) and 15 μM ML-7 (lane 2) for 90 min. Panel A shows a representative blot of egg lysates (35 cells per lane), probed with anti-pMAPK and anti-MAPK, with Panel B showing quantification of band intensities of anti-pMAPK levels, and Panel C showing quantification of band intensities of anti-MAPK levels. The pMAPK blots and MAPK blots were performed three times each. Values were normalized to DMSO-treated eggs. Error bars represent standard error of the mean. Panel D-I: Line-scan analysis around the circumference of the cortical region to assess relative intensities of the pMRLC cortical signal in DMSO-, ML-7-and U0126 treated eggs. This graph shows data from individual eggs, complementing the pooled data from multiple eggs shown in Fig. 1J. Panels D, F, and H are line scans of representative ML-7-treated eggs; Panel D is normal metaphase II egg, Panel F is a metaphase II with a drifted spindle, and Panel H is an egg that exited from metaphase II arrest. Panels E, G, and I are line scans of representative U0126-treated eggs, where Panel E is normal metaphase II egg, Panel G is a metaphase II with a drifted spindle, and Panel I is an egg that exited from metaphase II arrest.

Figure S2

Metaphase II eggs were treated with 0.5% DMSO (Panel A), 15 μM ML-7 (Panel B), or 50 μM U0126 (Panel C) for 1.5 h, then fixed and stained with DAPI to label DNA and anti-β-tubulin

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to label the meiotic spindle. Eggs were classified as metaphase II (Met II; normal metaphase II spindle morphology and cortical localization), Met II drifted spindle (i.e., the DNA was aligned along the metaphase II plate and the spindle was greater than 12 μm from the cortex; shown in Fig. 2), anaphase II, or telophase II (Ana II and Telo II, respectively; i.e., progressing out of metaphase II arrest). For Panels D-F eggs were cultured in calcium-deficient medium and treated with 0.5% DMSO (Panel D), 15 μM ML-7 (Panel E), 50 μM U0126 (Panel F) for 1.5 h. The numbers in or above bars indicates numbers of eggs analyzed. The extent of progression out of metaphase II arrest (combined numbers of eggs in anaphase II and telophase II states) in U0126-treated eggs and ML-7-treated eggs is statistically significant as compared to DMSO-treated eggs (χ^2 analysis, $p < 0.0001$, indicated with one asterisk). The extent of drifted spindle incidences in ML-7-treated eggs is statistically significant as compared to DMSO-treated eggs (χ^2 analysis, $p < 0.0001$, indicated with two asterisks). The extent of drifted spindle incidences in ML-7-treated eggs in calcium-deficient medium is statistically significant as compared to DMSO-treated eggs in calcium-deficient medium (χ^2 analysis, $p < 0.0001$, indicated with two asterisks).

Figure S3

Metaphase II eggs were treated with 0.5% DMSO (Panel A), 15 μM ML-7 (Panel B), or 50 μM U0126 (Panel C) for 8 h, then fixed and stained with DAPI to label DNA and anti-β-tubulin to label the meiotic spindle. Eggs were classified as metaphase II (Met II; normal metaphase II spindle morphology and cortical localization), Met II drifted spindle (i.e., the DNA was aligned along the metaphase II plate and the spindle was greater than 12 μm from the cortex; shown in Fig. 2), anaphase II, telophase II, or pronuclear (Ana II, Telo II, PN, respectively; i.e., progressing out of metaphase II arrest). For Panels D-F, eggs were cultured in calcium-deficient medium and treated with 0.5% DMSO (Panel D), 15 μM ML-7 (Panel E) and 50 μM U0126 (Panel F) for 8 h. Panel G (Fertilized) shows control eggs, not loaded with BAPTA-AM and inseminated for 8 h. For Panels H-J, eggs were pre-loaded with 5 μM of the calcium

chelator BAPTA-AM and then inseminated (Panel H, Fertilized + BAPTA-AM), or treated with 15 μM ML-7 (Panel I), or 50 μM U0126 (Panel J) for 8 h. The numbers in or above bars indicates numbers of eggs analyzed. The extent of progression out of metaphase II arrest (combined numbers of eggs in anaphase II and telophase II states) in U0126-treated eggs and ML-7-treated eggs is statistically significant as compared to DMSO-treated eggs in calcium-containing medium, calcium-deficient medium, and BAPTA-AM-loaded eggs (χ^2 analysis, $p < 0.0001$, indicated with one asterisk). The extent of drifted spindle incidences in ML-7-treated eggs is statistically significant as compared to DMSO-treated eggs (χ^2 analysis, $p < 0.0001$, indicated with two asterisks).

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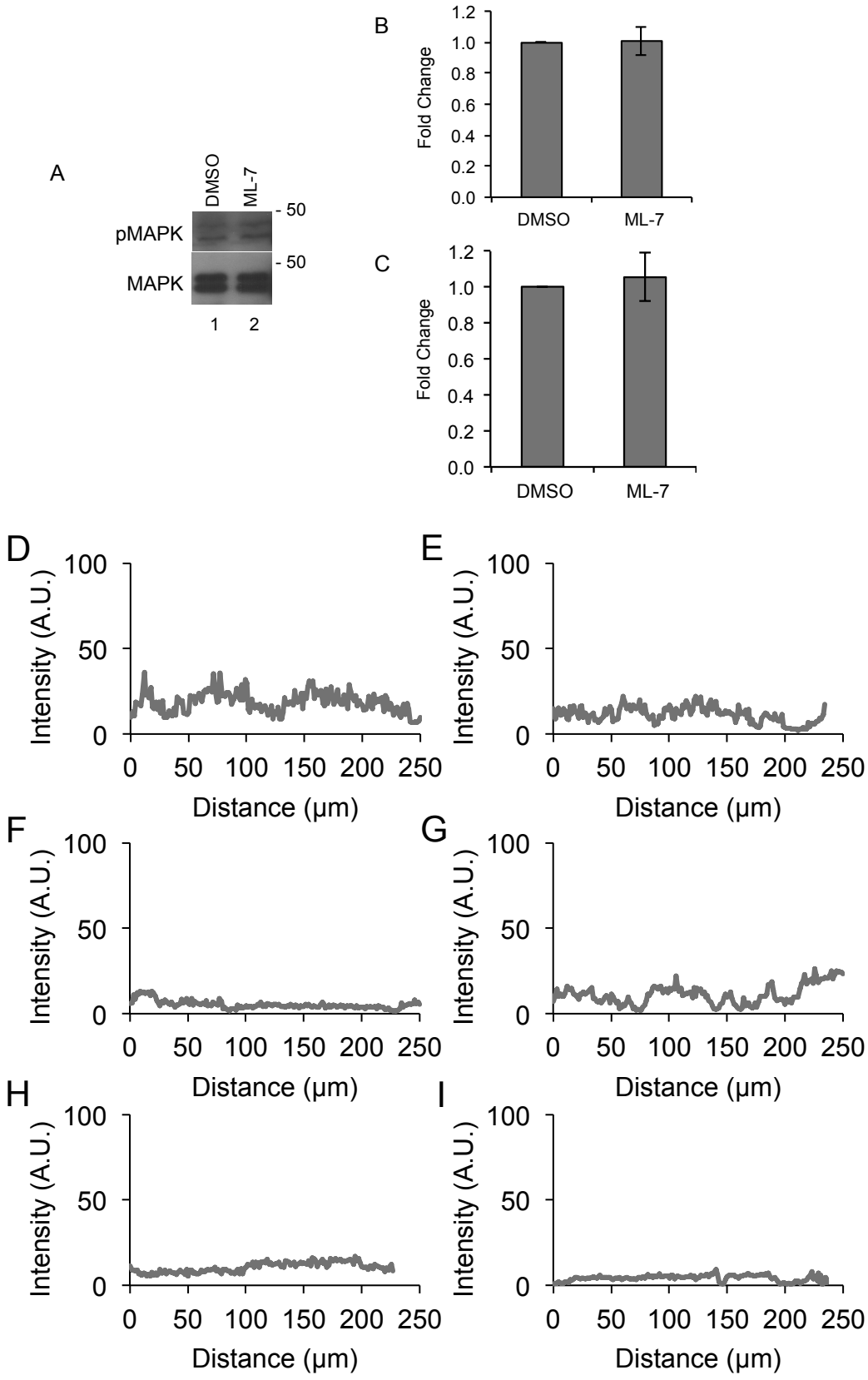
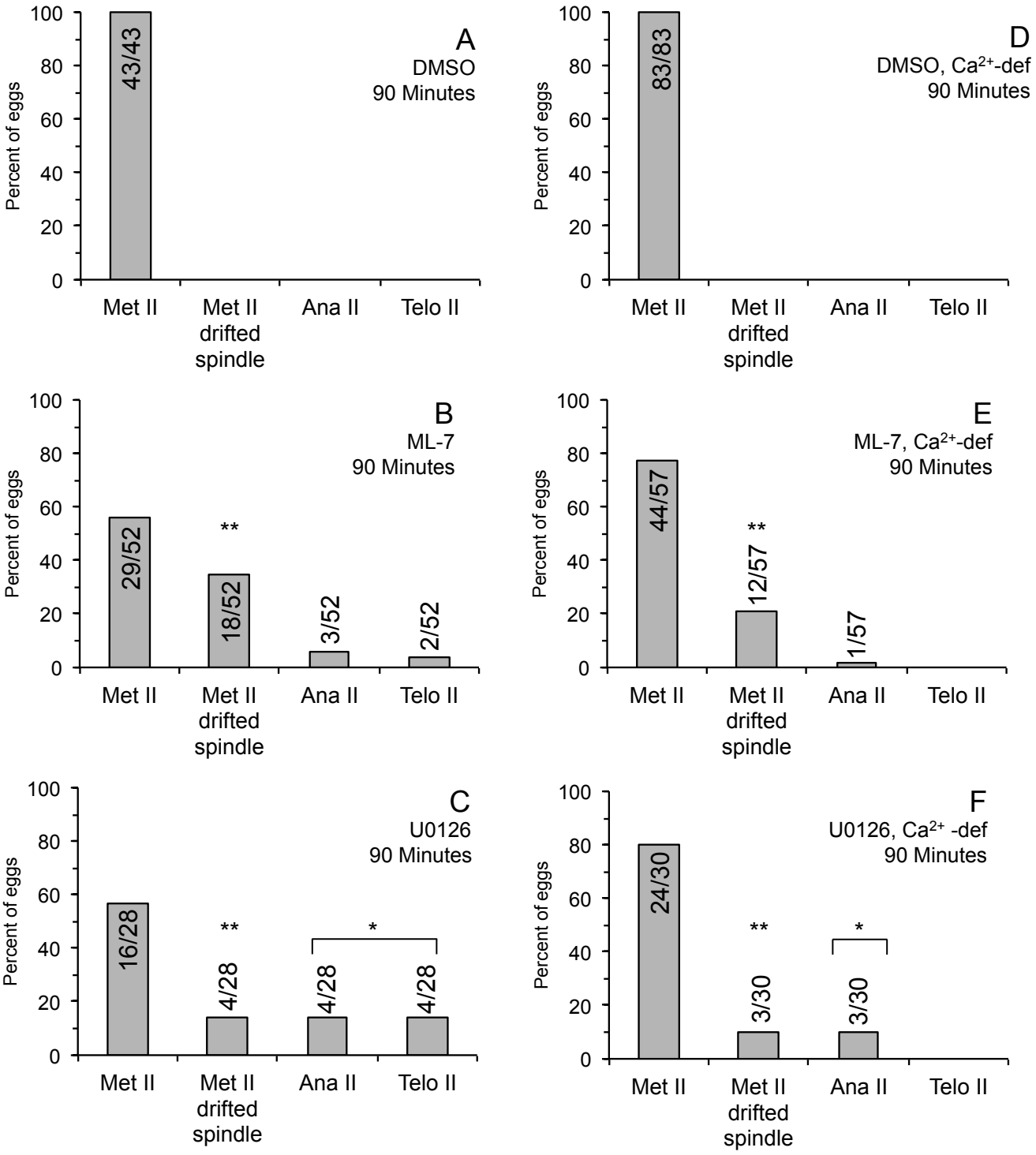
Supplemental Figure S1



Supplemental Figure S2



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Supplementary Figure 3