Expanded View Figures

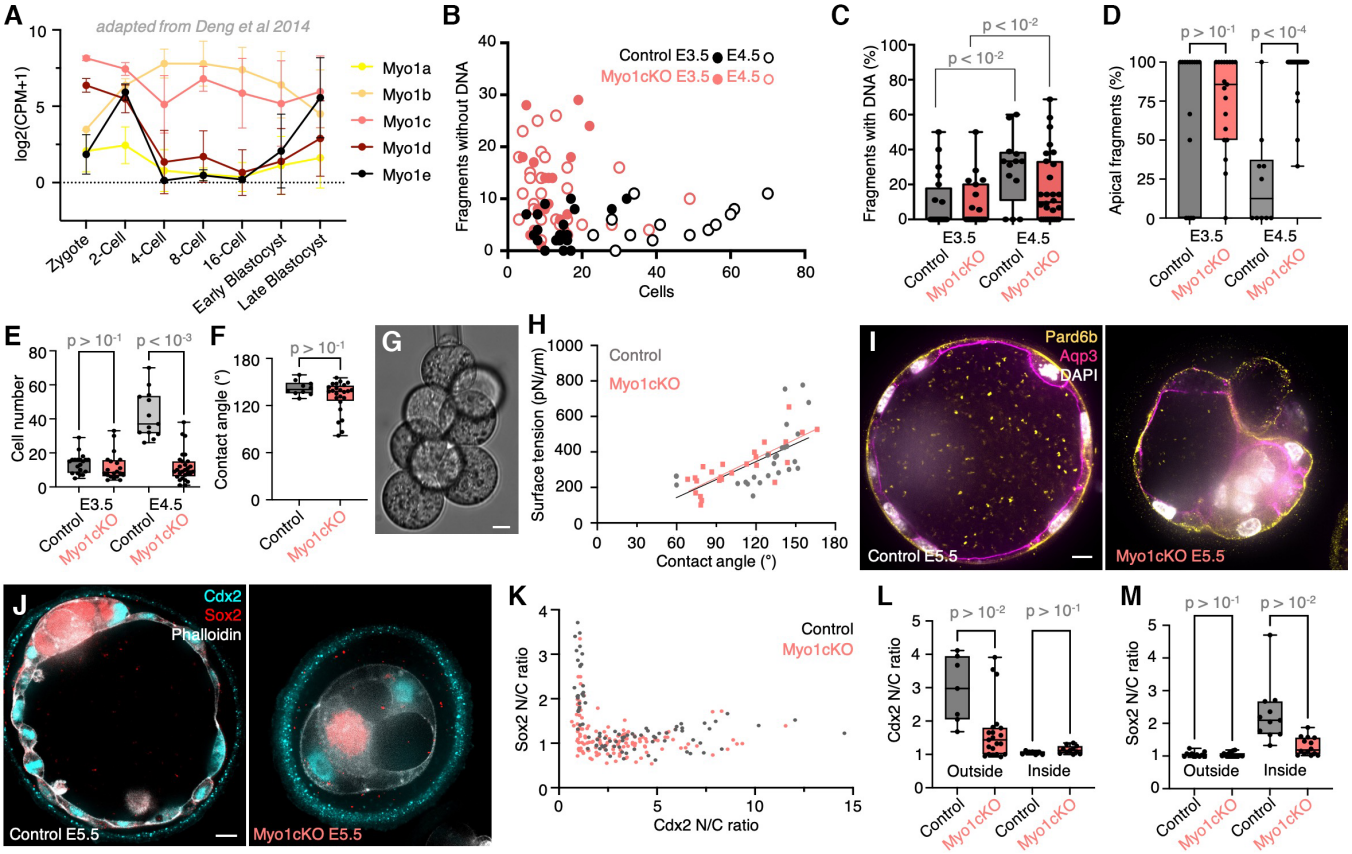

Figure EV1. Characterization of Myo1cKO embryos development.

A Mouse single cell RNA sequencing analysis adapted from Deng *et al* (2014), showing the expression levels of members for the Myosin 1 family at the zygote, 2-, 4-, 8-, 16-cell, early and late blastocyst stages. Data show mean ± SD of cells at the zygote (n = 4), 2- (n = 8), 4- (n = 13), 8- (n = 19), 16-cell (n = 57), early (n = 43), and late (n = 28) blastocyst stages.

B–E Number of fragments without DNA as a function of the number of cells (B), percentage of fragments containing DNA per embryo (C), percentage of fragments located at the embryo apical surface (D), cell number per embryo (E) in Control (gray) and Myo1cKO (salmon) embryos at E3.5 (Control n = 20, Myo1cKO n = 20) and E4.5 (Control n = 13, Myo1cKO n = 28).

F Mean contact angle at maximal compaction (Control n = 11, Myo1cKO n = 39).

G Representative image of surface tension measurement.

H Surface tension of control (gray, n = 6 embryos, 27 contacts) and Myo1cKO (salmon, n = 6 embryos, 53 contacts) blastomeres as a function of their contact angle. Representative image of tension measurement on a control 8-cell stage embryo.

I Representative images of Control (left) and Myo1cKO (right) embryos at E5.5 stained for Pard6b (yellow), Aqp3 (magenta) and DAPI (gray).

J Representative images of Control (left) and Myo1cKO (right) embryos at E5.5 stained for Cdx2 (cyan) Sox2 (red) and Phalloidin (gray).

K Sox2 nuclear to cytoplasmic (N/C) ratio as a function of Cdx2 N/C ratio of individual cells in Control (gray, n = 7) and Myo1cKO (salmon, n = 11) embryos.

L Cdx2 N/C ratio of cells located at the surface (left) or inside (right) the embryo in Control (n = 8) and Myo1cKO (n = 21) embryos.

M Sox2 N/C ratio of cells located at the surface (left) or inside (right) the embryo in Control (n = 11) and Myo1cKO (n = 16) embryos.

Data information: the Mann–Whitney test (F) or the Kruskal–Wallis test (C, D, E, L, M) P-values are indicated. Boxplots show median, upper and lower quartiles, min and max values. Scale bars, 10 µm.

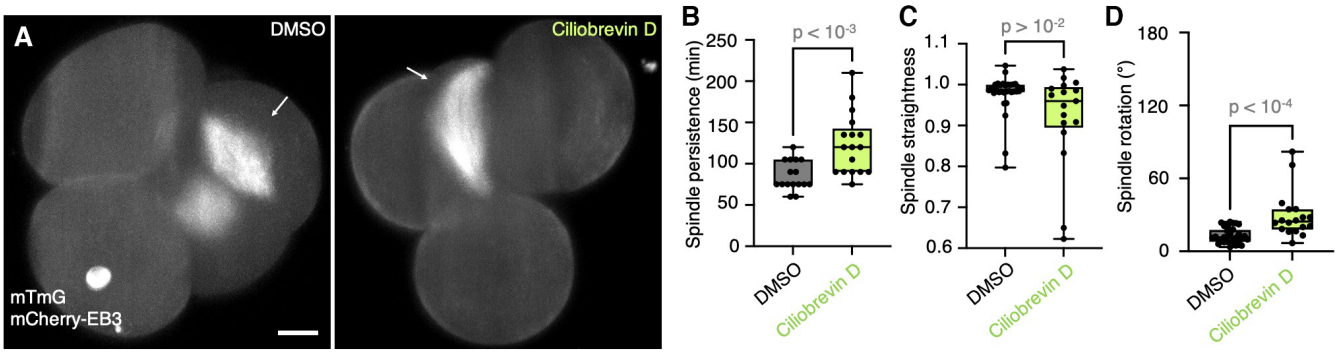

Figure EV2. Mitotic spindle movement in Ciliobrevin D treated embryos.

A Representative images of DMSO (left) and Ciliobrevin D (right) treated embryos expressing mTmG and mCherry–EB3 (gray) shown as max projections. White arrows indicate the mitotic spindle. Scale bar, 10 μm.

B–D Spindle persistence (B), straightness (C) and rotation (D) in DMSO (n = 11) and Ciliobrevin D (n = 11) treated embryos. Persistence measures the time from appearance to disassembly of the spindle. Straightness reports the pole to pole distance of the spindle divided by its continuous length. Rotation describes the angle between the initial orientation of the spindle pole and its maximal deviation in 2D.

Data information: the Mann–Whitney test P-values are indicated. Boxplots show median, upper and lower quartiles, min and max values.

Figure EV3. Characterization of chromosome movements and nuclear envelope dynamics during mitosis of Myo1cKO embryos.

A, B Measured distance between the DNA visualized with SiRDNA or H2B-mCherry and the cell cortex (A) and standard deviation of this distance throughout division (B) for Control (n = 10) and Myo1cKO embryos (n = 9).

C Representative images of Control (left) and Myo1cKO (right) embryos expressing mTmG (magenta) and Lap2b–GFP (cyan).

D Comparison of the time from nuclear envelope breakdown to its reassembly between Control (n = 8) and Myo1cKO embryos (n = 15).

Data information: Mann–Whitney test (A) or Student's t-test (B and D) P values are indicated. Boxplots show median, upper and lower quartiles, min and max values. Scale bar, 10 μm.

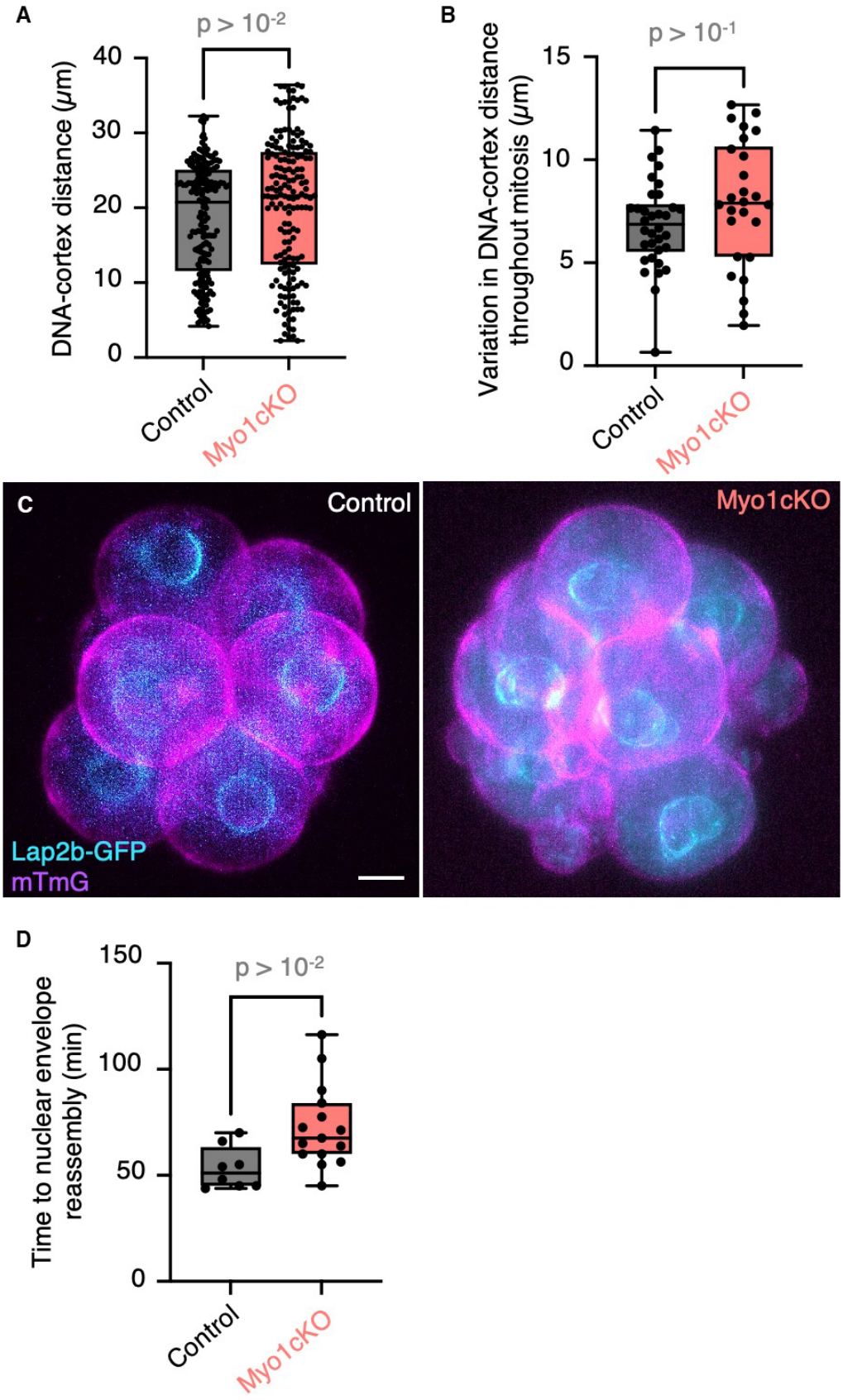
A
p > 10-2
DNA-cortex distance (μm)
40
30
20
10
0
Control
Myo1cKO
B
p > 10-1
Variation in DNA-cortex distance throughout mitosis (μm)
15
10
5
0
Control
Myo1cKO
C
Control
Myo1cKO
Lap2b-GFP
mTmG
D
p > 10-2
Time to nuclear envelope reassembly (min)
150
100
50
0
Control
Myo1cKO

Figure EV3.

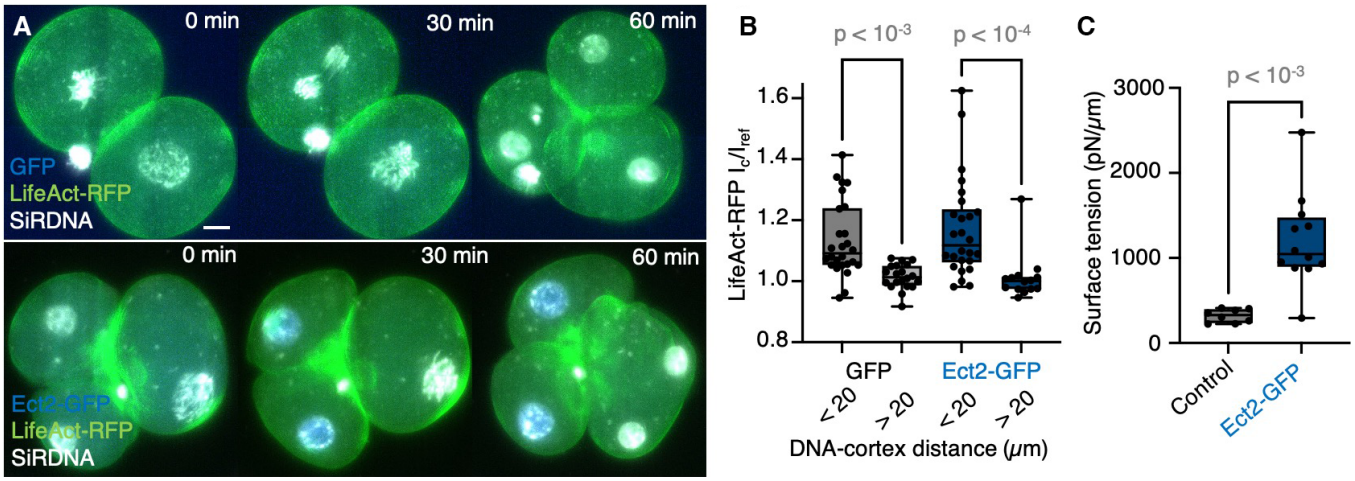

Figure EV4. Characterization of Ect2–GFP expressing embryos.

A Representative images of GFP (top, blue) and Ect2–GFP (bottom, blue) expressing embryos during mitosis labeled with LifeAct-RFP (green) and SiRDNA (white) shown as max projections. Scale bar, 10 μm.

B LifeAct–RFP I_c/I_{ref} ratio compared between DNA-cortex distances < 20 and > 20 μm for GFP (gray) and Ect2–GFP (blue) embryos (GFP embryos $n = 7$, cells $n = 26$; Ect2-GFP embryos $n = 9$, cells $n = 26$).

C Surface tension of 4-cell stage embryos in Control (gray) and Ect2–GFP (blue) embryos (Control $n = 8$; Ect2–GFP $n = 12$).

Data information: the Kruskal–Wallis and pairwise Mann–Whitney tests (B) or unpaired Student's *t*-test (C) *P*-values are indicated. Boxplots show median, upper and lower quartiles, min and max values.