**Supplementary Dataset.**

**Saha et al "Arginylation regulates intracellular actin polymer level by modulating actin properties and binding of capping and severing proteins"**

**Supplemental Table 1.**

**Percentages of actin present in the supernatant and pellet at different centrifugal steps**

| | WT | | KO | |
|---|---|---|---|---|
| | Pellet | Supernatant | Pellet | Supernatant |
| Total actin | | | | |
| 200 x g | 23.02 | 76.98 | 27.31 | 72.69 |
| 1,500 x g | 0.59 | 99.41 | 0.56 | 99.44 |
| 16,000 x g | 10.12 | 89.88 | 2.67 | 97.33 |
| 66,000 x g | 81.87 | 18.13 | 34.78 | 65.22 |
| β actin | | | | |
| 200 x g | 16.50 | 83.50 | 41.70 | 58.30 |
| 1,500 x g | 0.41 | 99.59 | 0.65 | 99.35 |
| 16,000 x g | 3.69 | 96.31 | 0.36 | 99.64 |
| 66,000 x g | 84.03 | 15.97 | 18.52 | 81.48 |
| γ actin | | | | |
| 200 x g | 16.43 | 83.57 | 18.17 | 81.83 |
| 1,500 x g | 0.64 | 99.36 | 3.30 | 96.70 |
| 16,000 x g | 6.75 | 93.25 | 0.51 | 99.49 |
| 66,000 x g | 49.06 | 50.94 | 22.34 | 77.66 |

**Figure S1: Fractionation of actin from total cell lysates by differential centrifugation. a.** Flow chart showing the centrifugation steps used during actin fractionation. **b.** Bar diagram showing the percentage of actin present in the supernatant and pellet at each centrifugation step.

**Figure S2: WT and KO actin have different elongation rates.** Pyrenyl actin polymerization curves of endogenous purified WT and KO actin in the presence of seeds, performed at higher actin concentrations compared to those shown in Fig. 2C of the main text.

**Figure S3: WT and KO actin have different nucleation rates.** Chart showing the average of the three polymerization curves used to calculate the lag time for spontaneous polymerization of WT and KO actin. The point (time --- T0, fluorescence --- F0) at the maximum slope (Sm), of the actin spontaneous polymerization curves at 1 μM WT or KO actin were determined according to the model of (Nishida and Sakai, 1983) averaged from three independently obtained curves. Error bars in the curves and calculated values below represent SEM, p value for the T0 = 0.0095. The derived values of T0 and Sm were as follows:

For WT:

T0 = 2.50 +- 0.13 (1000sec)

Sm = 2.31 +- 0.45 (a.u./1000sec)

For KO:

T0 = 1.83 +- 0.06 (1000sec)

Sm = 3.88 +- 0.38 (a.u./1000sec)

**Figure S4: SDS-PAGE of purified baculovirus-expressed M-beta and R-beta actin.** The doublet seen in R-actin represents the expressed R-actin constructs (lower band in the doublet) and the copurifying band of the endogenous actin from Sf9 cells (upper band in the doublet). Similar bands are likely present in the M-beta construct, but cannot be detected due to similar electrophoretic mobility.

**Figure S5: Characterization of baculovirus-expressed actin. A.** Time course polymerization curves at high actin concentrations. A. Arg-beta actin: 50 μM (closed circles), 40 μM (open circles), 30 μM (closed triangles) and 0.125 μM pyrene-labeled skeletal muscle actin control (open triangles). B. Met-beta actin: 50 μM (closed triangles), 40 μM (open circles), 30 μM (closed circles) and 0.75 μM pyrene-labeled skeletal actin control (open triangles). All beta actin samples contained 2.5% pyrene-labeled skeletal G-actin. B. Time course of DNase-I inhibition ($IC_{50}$ curves). A: Arg-beta actin. B: Met-beta actin. Met-beta actin $IC_{50}$s were 13.95-16.83 nM for the days assessed (day1- day15). Arg-beta actin $IC_{50}$s were comparable, i.e. 15.05-22.03 nM for the days assessed (day1- day18). For comparison, the values for normal alpha skeletal actin (wt) $IC_{50}$ is 12 nM and WT alpha cardiac actin is 19 nM.

**Figure S6: KO actin aggregates can serve as seeds for actin filament elongation.** Curves showing polymerization of 1 μM KO actin alone or in the presence of fraction 25 from the sucrose gradient similar to those shown in Fig. 3 of the main text. In the presence of fraction 25 actin polymerization is faster and the lag phase is absent, showing that this fraction can serve as seeds for elongating the actin filaments. The curves shown represent the average of 3 repeats.

**Figure S7: Comparisons of the endogenous protein levels** for drebrin (A), PDI (B) and gelsolin (C) in whole WT and *Ate* KO embryos (A and B), brain (A), heart (B), and mouse embryonic fibroblasts used in this study (C). Bars in C represent the average of 2 independent measurements, error bars represent SEM, p value 0.046.

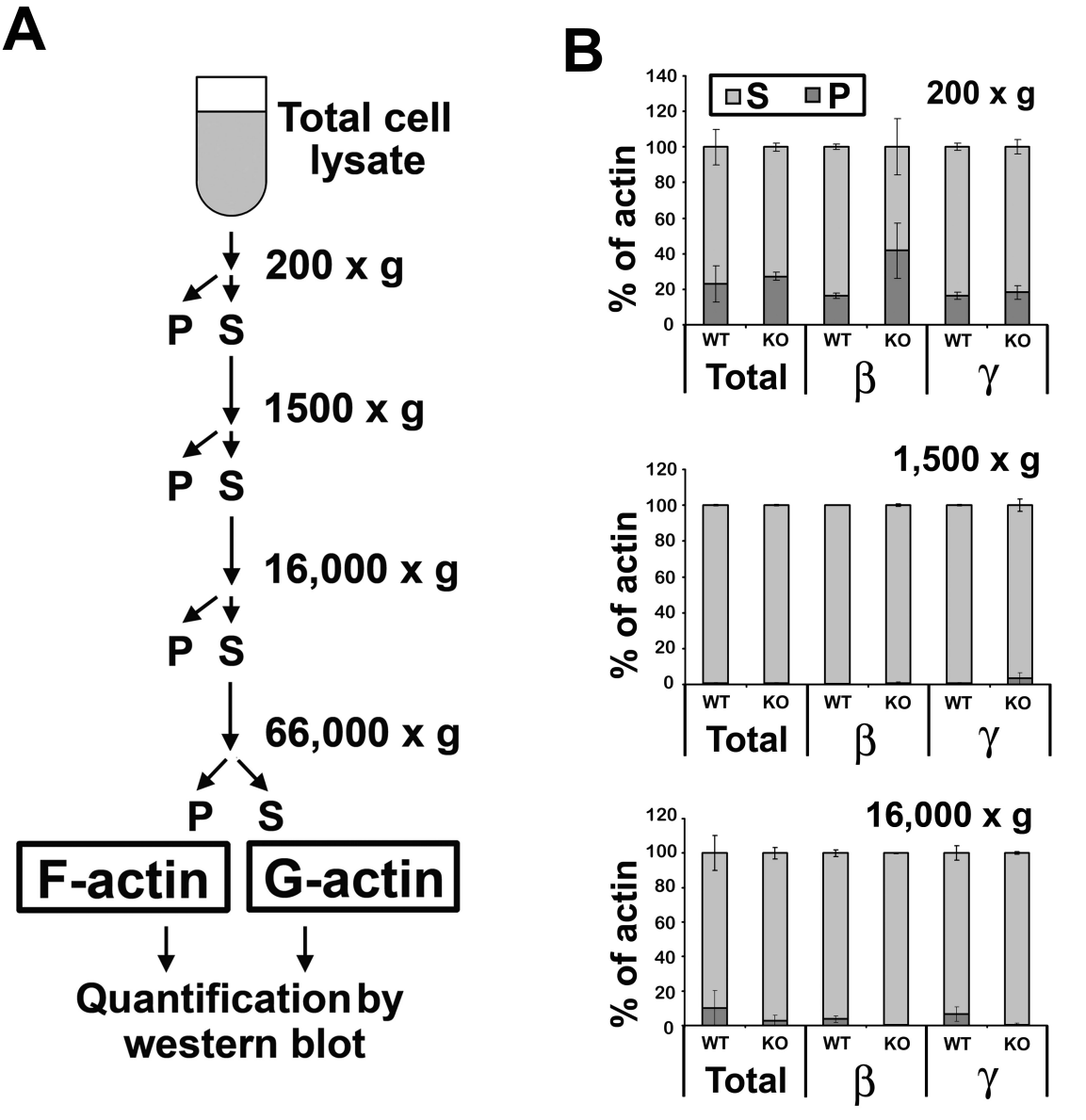
**Figure S8: Arginylation regulates actin network organization.** Electron micrographs of the platinum replicas of the cytoskeleton in ATE1 knockout fibroblasts. Regardless of the cell spreading and motile state, stress fibers (visible as prominent bundles of actin) extend all the way to leading edge. Top row, a less spread, more motile cell; bottom row, a more spread, stationary cell. Right-hand panels show high magnification images of the areas boxed in the corresponding left-hand panels. Bars, 10 μm (left panels) and 500 nm (right panels).

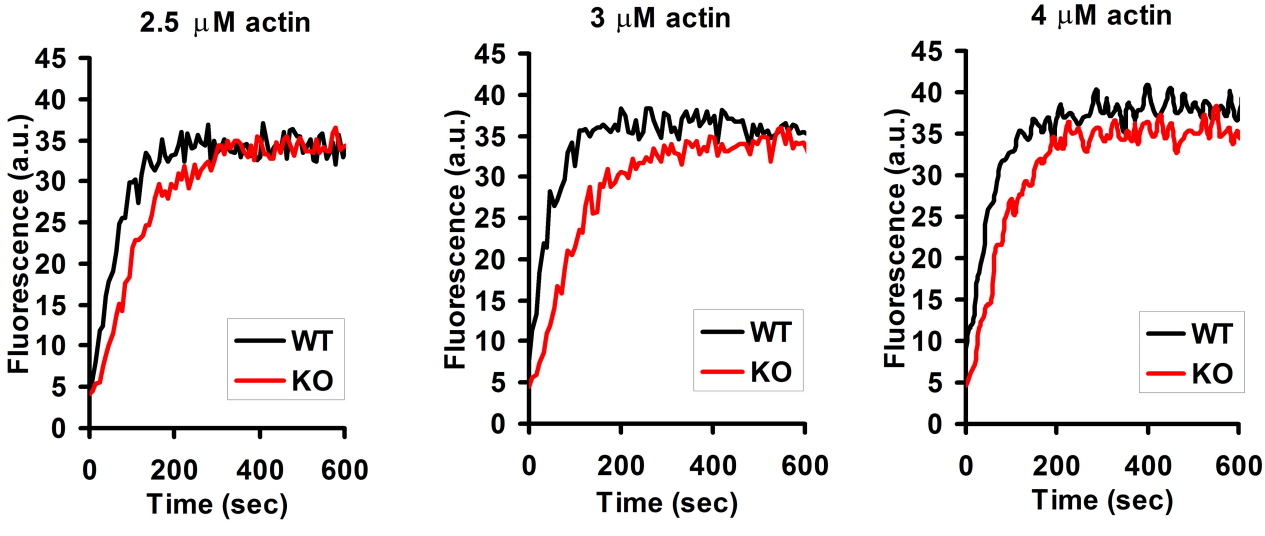
**Figure S9: Arginylation regulates the number and distribution of filament ends in the leading edge actin network.** Areas of the leading edge used to quantify the number of filament ends shown in Fig. 7 of the main text.

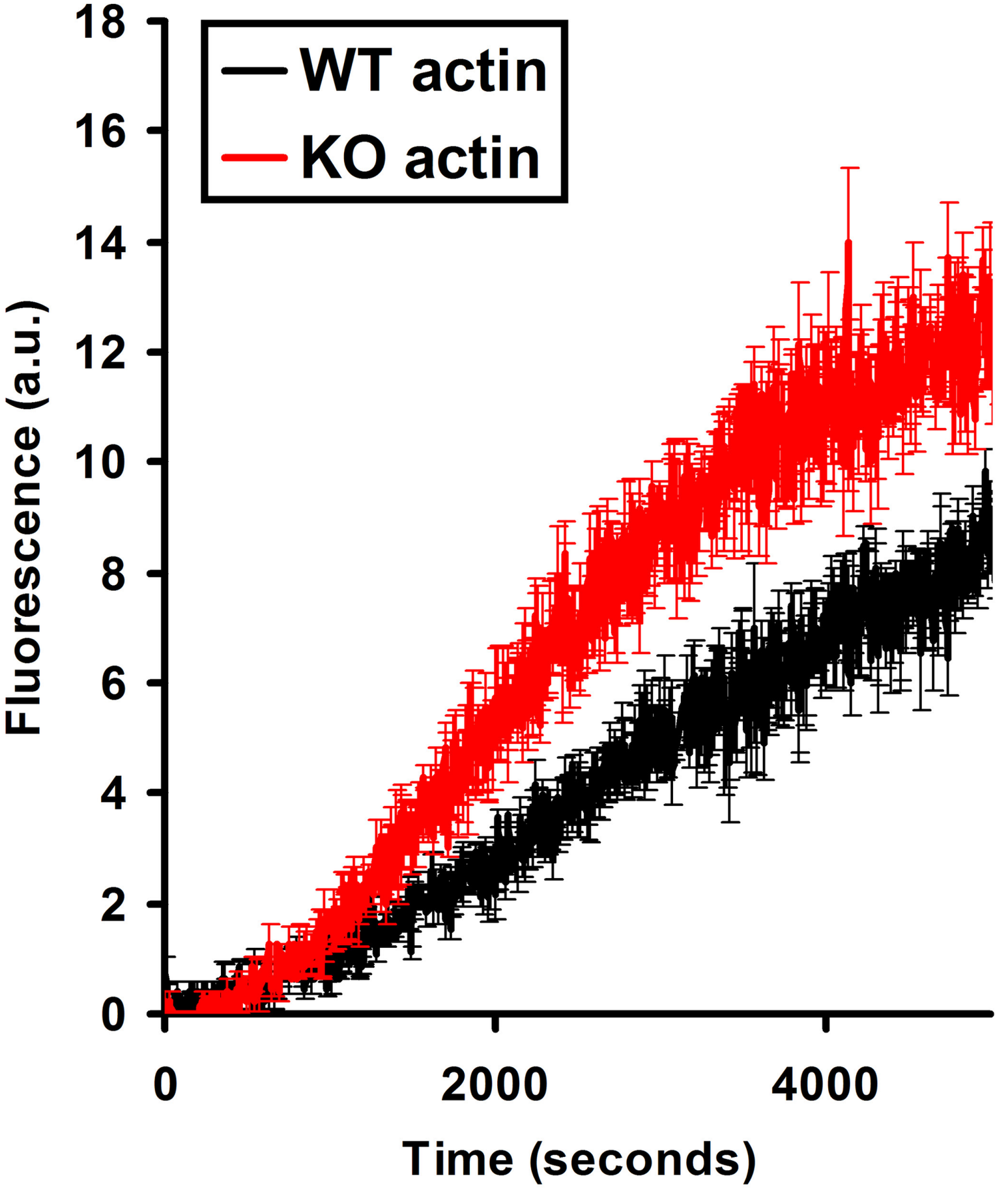
**Figure S10: Arginylation regulates filopodia formantion.** Electron micrographs of the platinum replicas of the leading edge actin network representing filopodial structures in WT (top panels) and *Ate1* knockout (bottom panels) fibroblasts. Filopodia in KO cells are greatly reduced in number, terminate prematurely and are not properly anchored in the leading edge network. Scale bars, 500 nm.

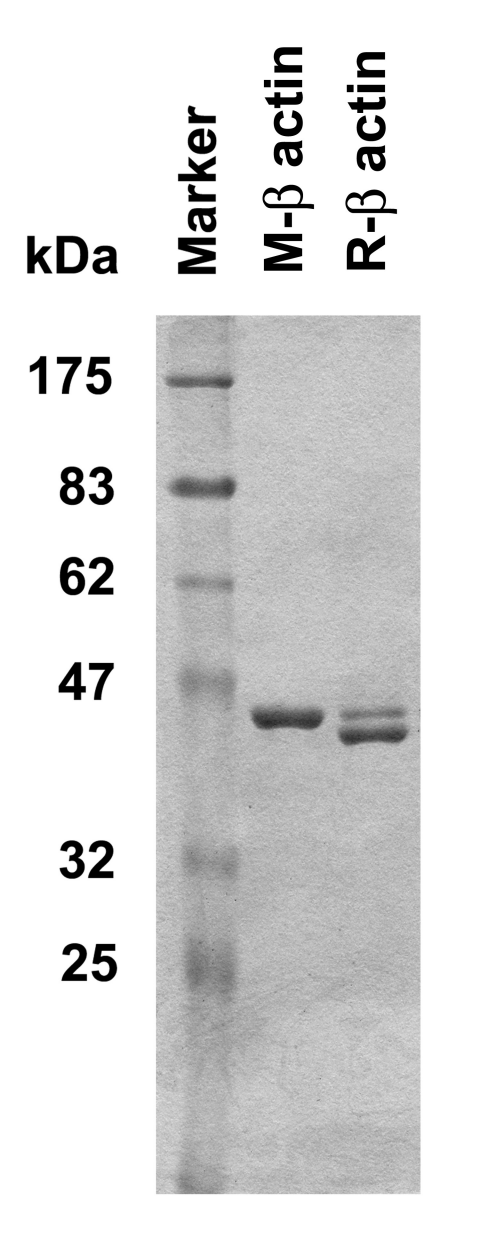
**Reference.**

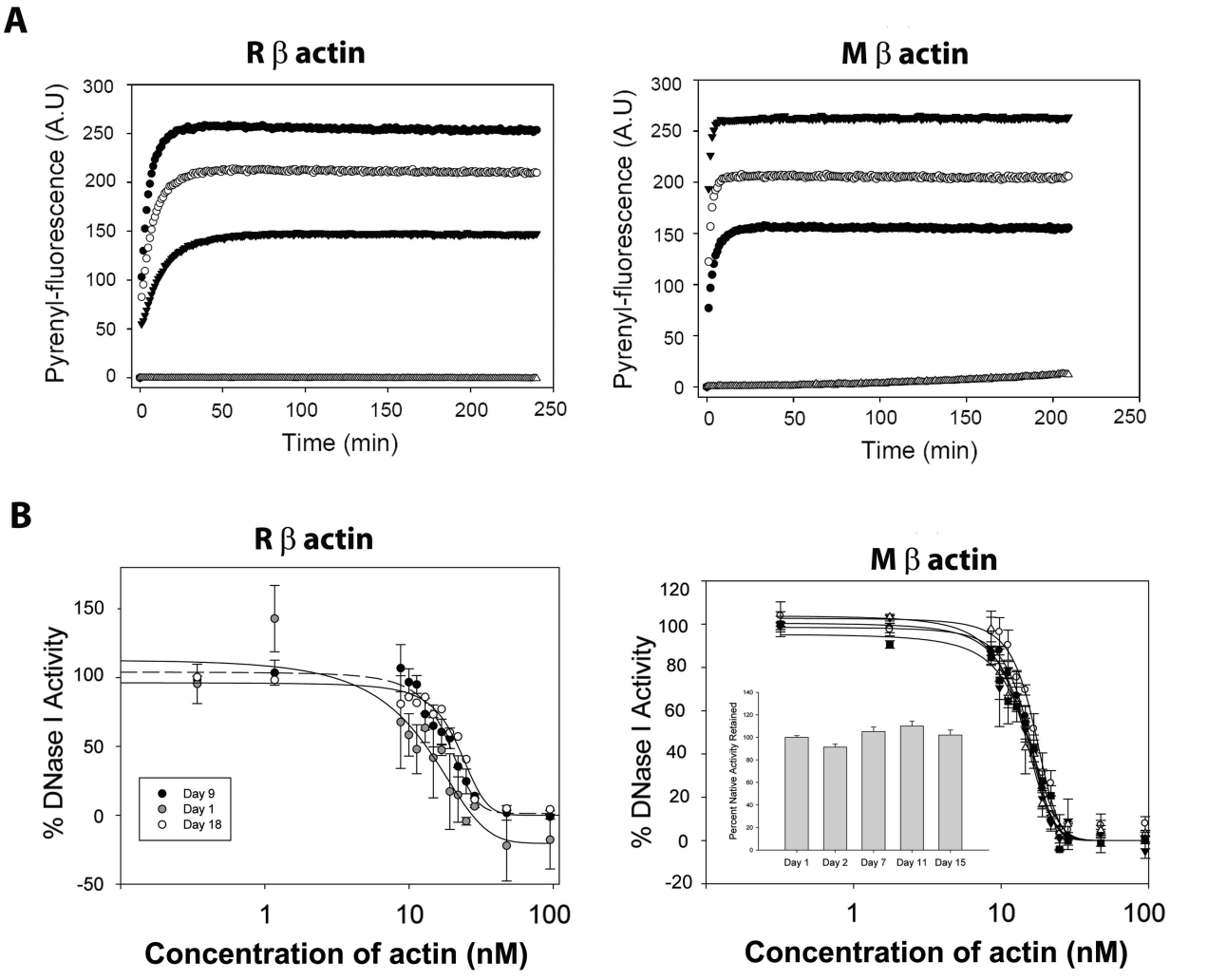
Nishida, E., and Sakai, H. (1983). Kinetic analysis of actin polymerization. J Biochem *93*, 1011-1020.

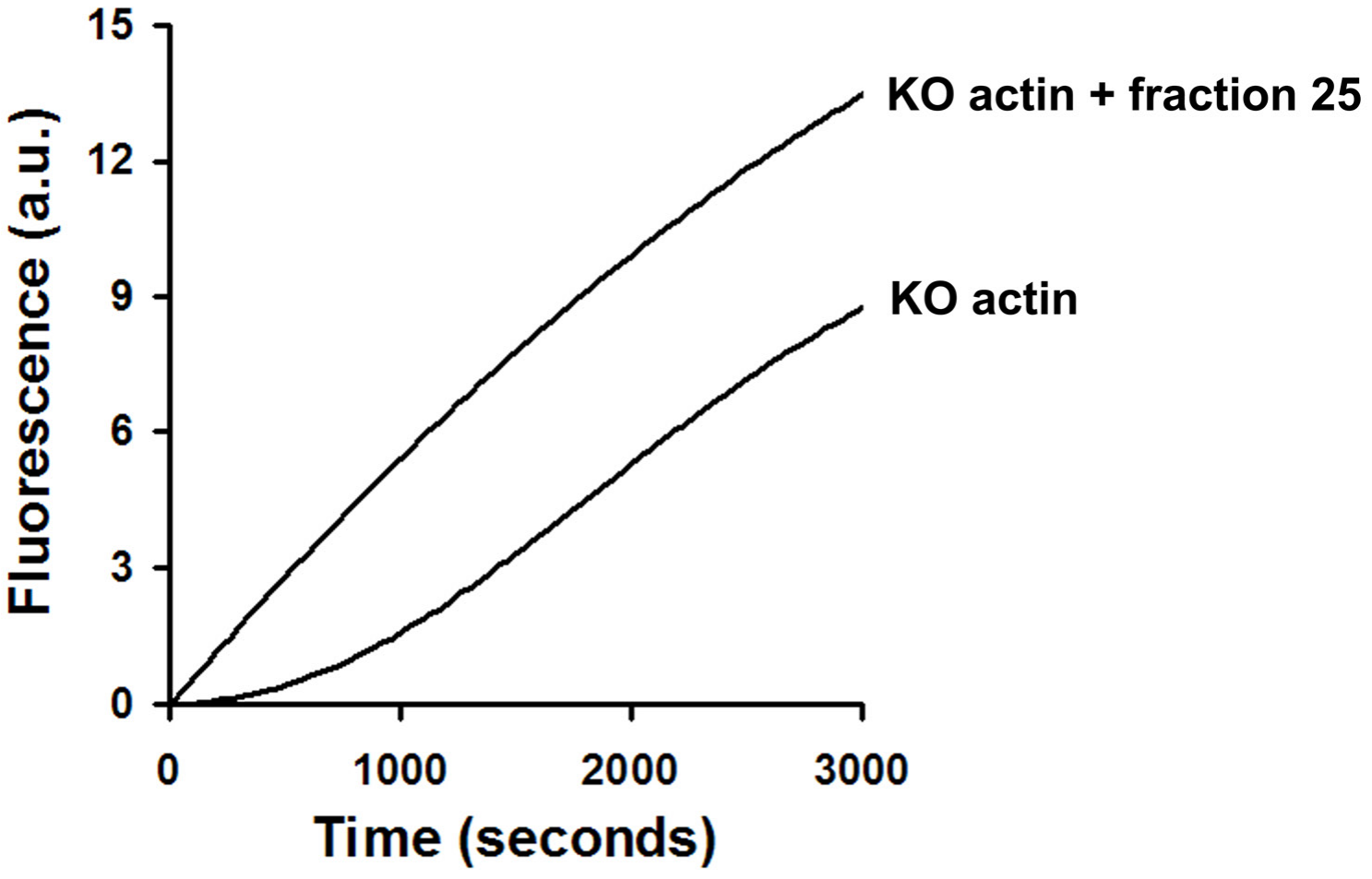

A
Total cell lysate
200 x g
P S
1500 x g
P S
16,000 x g
P S
66,000 x g
P S
F-actin
G-actin
Quantification by western blot
B
S
P
200 x g
% of actin
140
120
100
80
60
40
20
0
WT KO WT KO WT KO
Total β γ
1,500 x g
16,000 x g

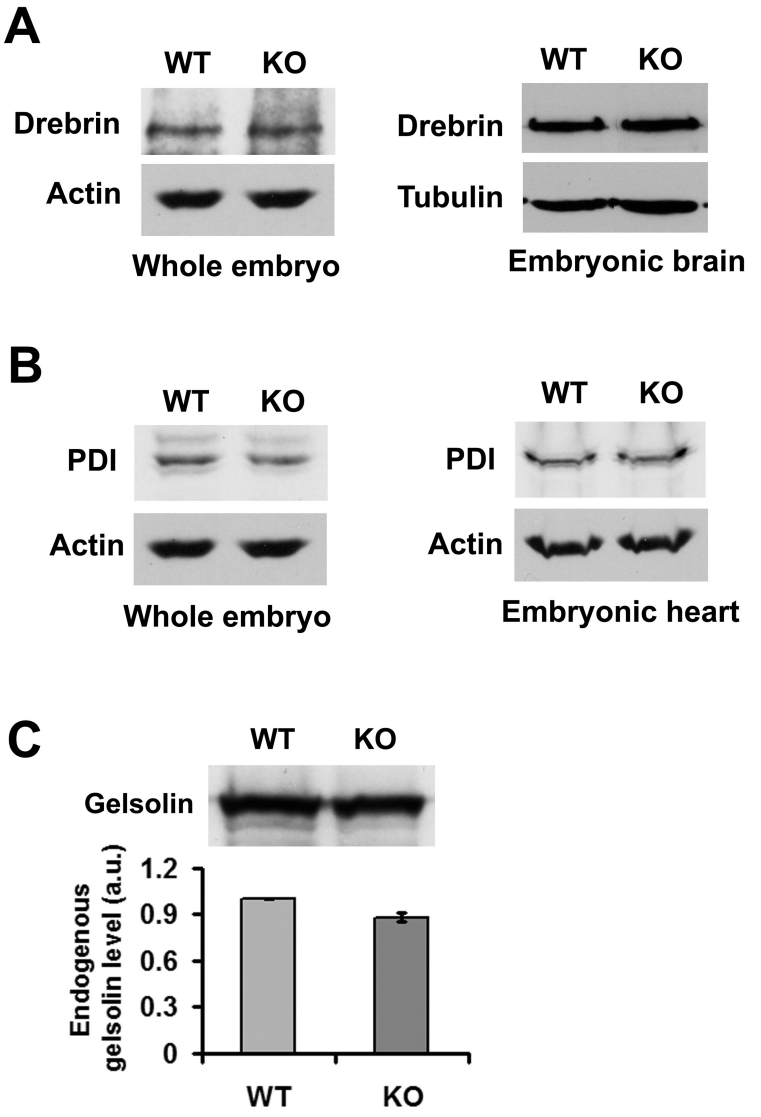

2.5 μM actin
3 μM actin
4 μM actin
Fluorescence (a.u.)
Time (sec)
WT
KO
0
5
10
15
20
25
30
35
40
45
0
200
400
600

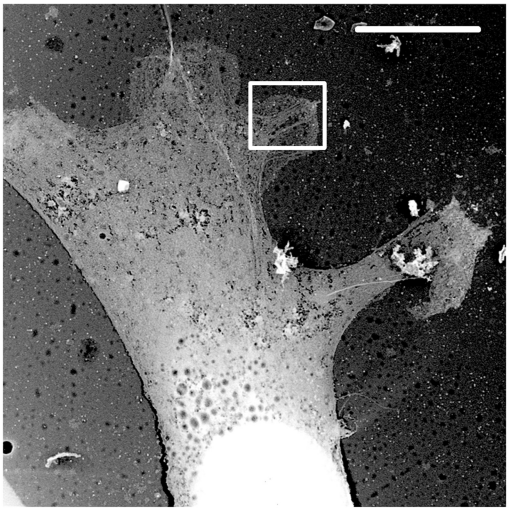

WT actin
KO actin
Fluorescence (a.u.)
18
16
14
12
10
8
6
4
2
0
0
2000
4000
Time (seconds)

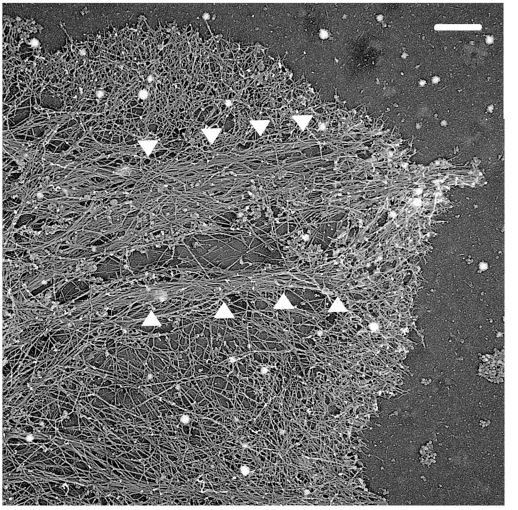

kDa
Marker
M-β actin
R-β actin
175
83
62
47
32
25

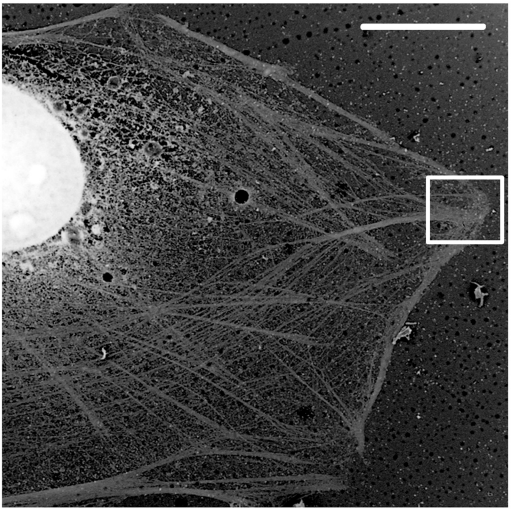

A
R β actin
M β actin
Pyrenyl-fluorescence (A.U)
Time (min)
B
% DNase I Activity
Concentration of actin (nM)
Day 9
Day 1
Day 18
Percent Native Activity Retained
Day 1
Day 2
Day 7
Day 11
Day 15

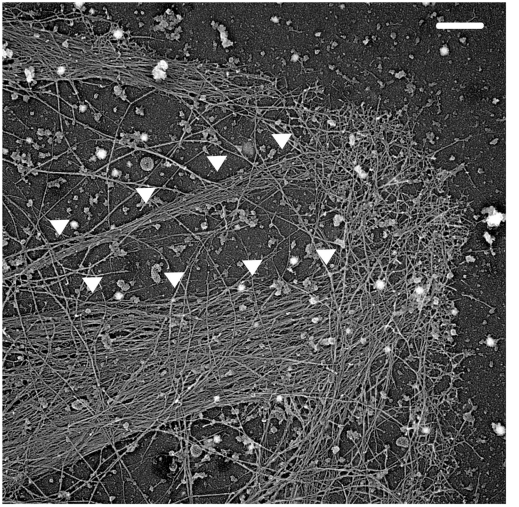

15
12
9
6
3
0
Fluorescence (a.u.)
0
1000
2000
3000
Time (seconds)
KO actin + fraction 25
KO actin

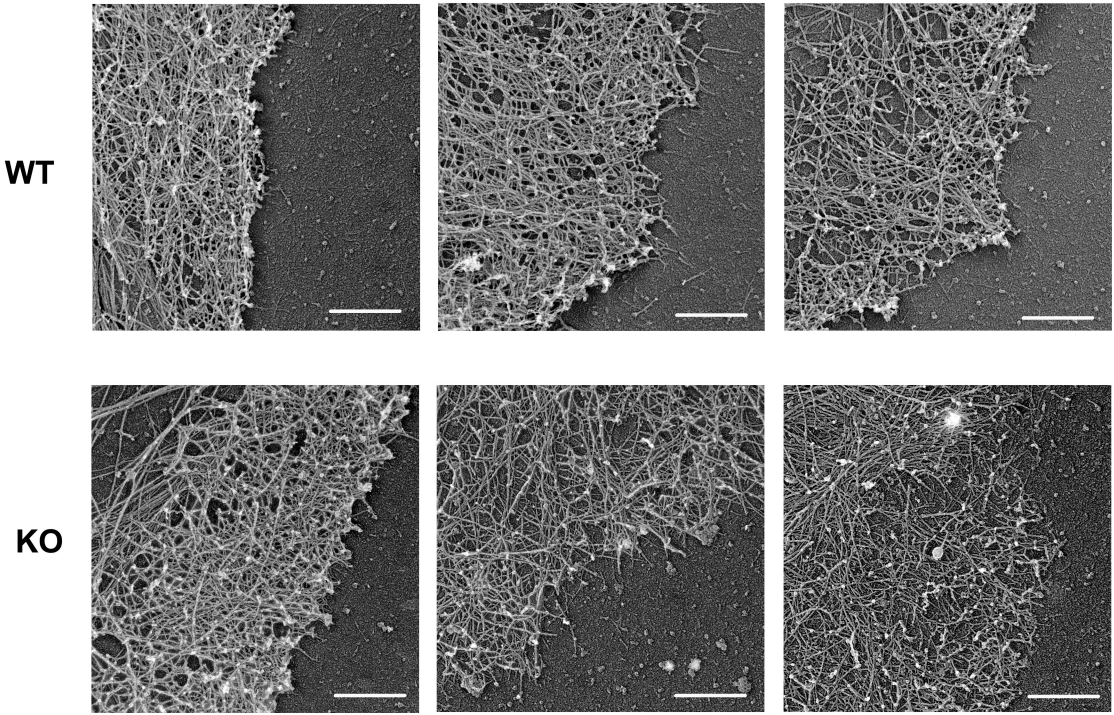

A
WT
KO
Drebrin
Actin
Whole embryo
WT
KO
Drebrin
Tubulin
Embryonic brain
B
WT
KO
PDI
Actin
Whole embryo
WT
KO
PDI
Actin
Embryonic heart
C
WT
KO
Gelsolin
Endogenous gelsolin level (a.u.)
1.2
0.9
0.6
0.3
0
WT
KO

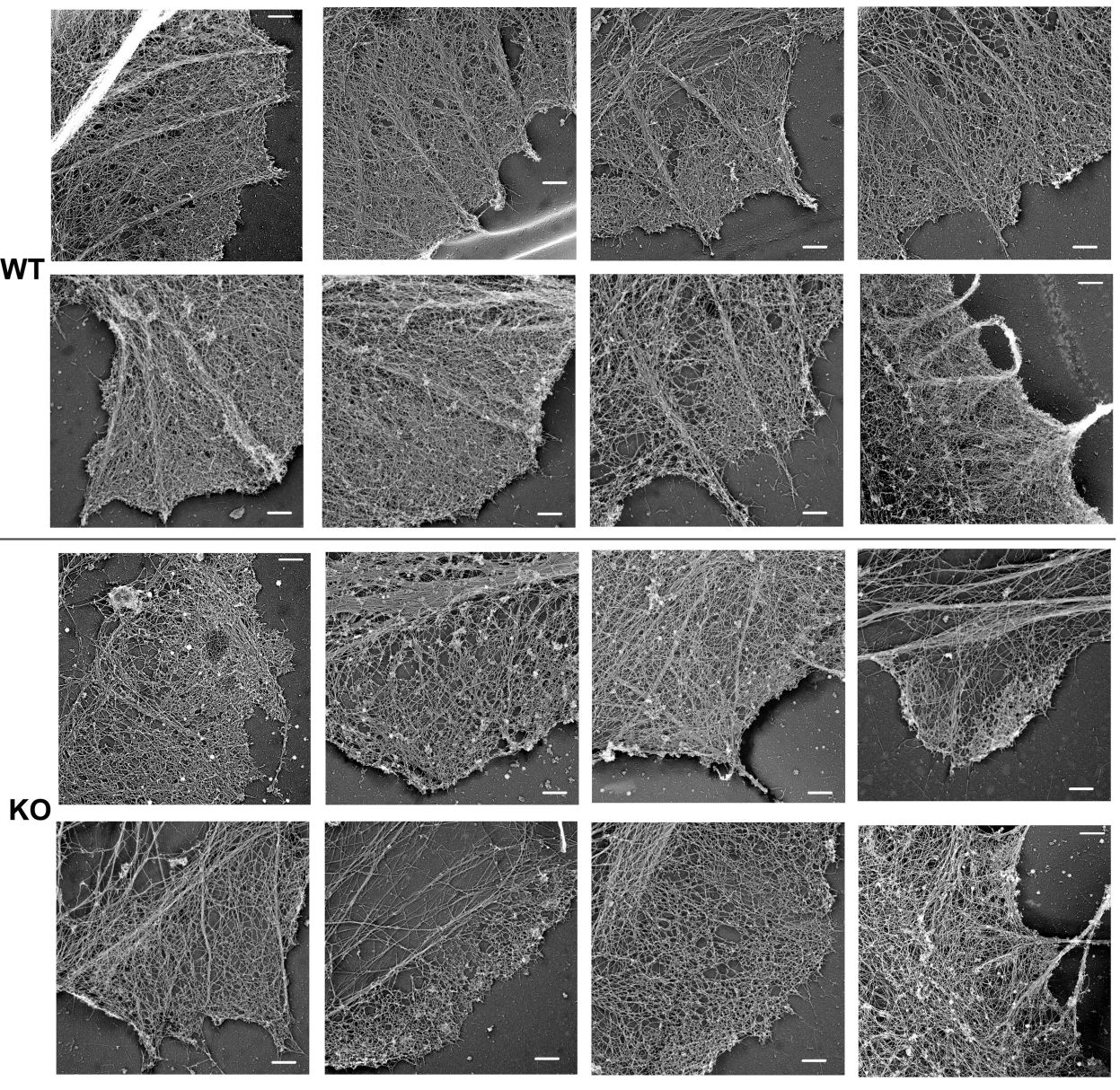









WT
KO


WT
KO