# Supplemental Materials

*Molecular Biology of the Cell*

Brooks *et al*.

**Supplementary Figure Legends**

**Figure S1: (a)** Immunofluorescence imaging of caveolin-1 (Cav-1) and cavin-1 in iso-osmotic conditions and after 5 mins of hypo-osmotic shock (150 mOsm $L^{-1}$). Scale bars 10 μm.
**(b)** Quantification of (a) shows individual junctional to cytoplasmic ratios of these proteins. Cav-1 levels at the PM are not significantly affected by hypo-osmotic shock ($p = 0.1900$), whereas cavin-1 levels show a decrease of ~17% ($p = <0.0001$), indicative of caveolae flattening and/or dissociation.
**(c)** Quantification of (a) showing the percentage change in junctional Cav-1 and cavin-1 after hypo-osmotic shock.
**(d, e)** Western Blot and analysis of cavin-1 before and after hypo-osmotic shock. Protein levels are unchanged ($p = 0.2057$; $N = 3$), indicating that cavin-1 is redistributed from the PM, rather than undergoing degradation.
**(f)** Fluorescence imaging of transient cavin-1-EGFP expression in MCF-10A epithelial monolayers under control and mechanically stretched conditions (scale bar = 10 μm).
**(g)** Quantification of (f) reveals that 5 m of cyclic stretching at 1 Hz to a strain of 10% promotes the dissociation of ~33% cavin-1 from the PM ($p = <0.0001$), indicative of caveolar disassembly.
**(h)** Quantification of (f) showing the percentage change in PM-associated cavin-1 following cyclic mechanical stretching of monolayers.
**(i)** Amino acid sequences of undecad cavin1 (UC1) repeats from *Mus musculus* (*Mm*Cavin1), *Danio rerio* (*Dr*Cavin1b), and a modified *Dr*Cavin1b sequence with the deletion of four UC1 repeats (Tillu *et al.*, 2018).

All data are means ± SD. All statistical analyses (with the exception of d, e) calculated from N=3 independent experiments with 60 junctions per experiment using unpaired t-tests. Statistical analysis of d,e calculated from N=3 independent western-blotting experiments. Points on graphs represent individual cell junctions. N.s, not significant; *p<0.05; **p<0.01, ***p<0.001; ****p<0.0001.

**Figure S2: (a)** Full dataset of F-actin changes in WT, Cav-1 KD, and Cav-1 KDR monolayers following hypo-osmotic shock (see figure 1a) showing data points from individual cell-cell junctions.
**(b)** Full dataset of F-actin changes in WT and Cav-1 KD monolayers following mechanical stretch (see figure 1d) showing data points from individual cell-cell junctions.

All data are means ± SD. All statistical analyses calculated from N=3 independent experiments (60 junctions per experiment) using unpaired t-tests. Points on graphs represent individual cell junctions. N.s, not significant; *p<0.05; **p<0.01, ***p<0.001; ****p<0.0001.

**Figure S3: (a**) Immunofluorescent imaging of PH-Akt-GFP, a fluorescent biosensor for PtdIns (3, 4, 5)$P_3$ in WT monolayers before (left) and after (right) hypo-osmotic shock. Scale bar = 10 μm.
**(b)** Quantification of (a) showing that there is no apparent change in the level of PtdIns (3, 4, 5)$P_3$ ($p = 0.1924$), the primary precursor of PtdIns(4, 5)$P_2$, following hypo-osmotic shock.
**(c)** Full dataset of junctional mCherry-PH intensity following hypo-osmotic shock (see figure 3a) showing data points from individual cell-cell junctions in WT, Cav-1 KD, and Cav-1 KDR monolayers.

All data are means ± SD. All statistical analyses calculated from N=3 independent experiments (60 junctions per experiment) using unpaired t-tests. Points on graphs represent individual cell junctions. N.s, not significant; *p<0.05; **p<0.01, ***p<0.001; ****p<0.0001.

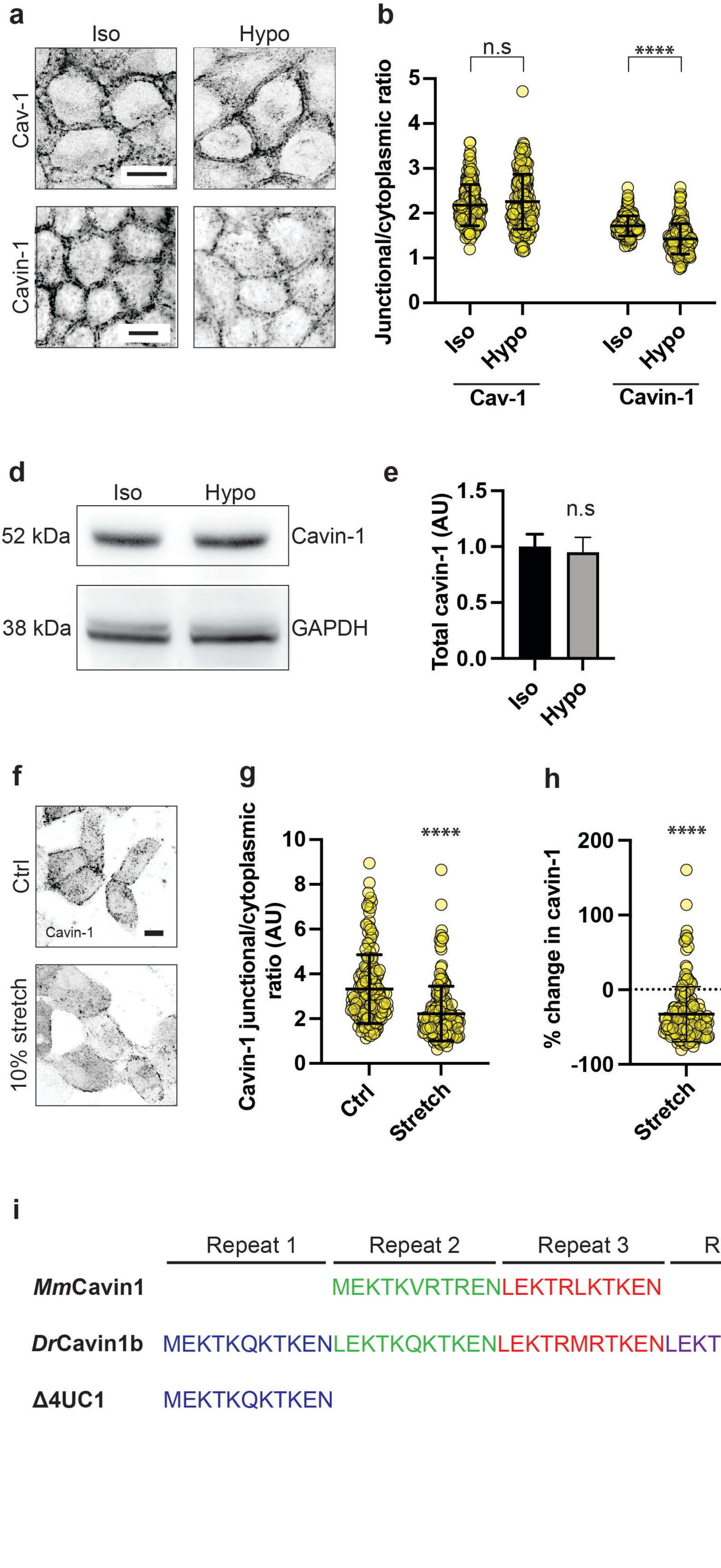
**Figure S4: (a)** Full dataset of cavin-1 variant dissociation following hypo-osmotic shock (see figure 4b) showing data points from individual cell-cell junctions.
**(b)** Full dataset of F-actin changes in monolayers expressing either the *Mm*Cavin1, *Dr*Cavin1b, or Δ4UC1

cavin-1 variant following hypo-osmotic shock (see figure 4d) showing data points from individual cell-cell junctions.
**(c)** Full dataset of cavin-1 variant dissociation following exposure to calyculin A (see figure 5a) showing data points from individual cell-cell junctions.
**(d)** Full dataset of F-actin changes in monolayers expressing either the *Mm*Cavin1, *Dr*Cavin1b, or Δ4UC1 cavin-1 variant following calyculin A exposure (see figure 5e) showing data points from individual cell-cell junctions.

All data are means ± SD. All statistical analyses calculated from N=3 independent experiments (60 junctions per experiment) using unpaired t-tests. Points on graphs represent individual cell junctions. N.s, not significant; *p<0.05; **p<0.01, ***p<0.001; ****p<0.0001.

**Figure S5: (a)** Representative DIC imaging of WT MCF-10A epithelial monolayers treated with 50 nM calyculin A in the absence/presence of blebbistatin.

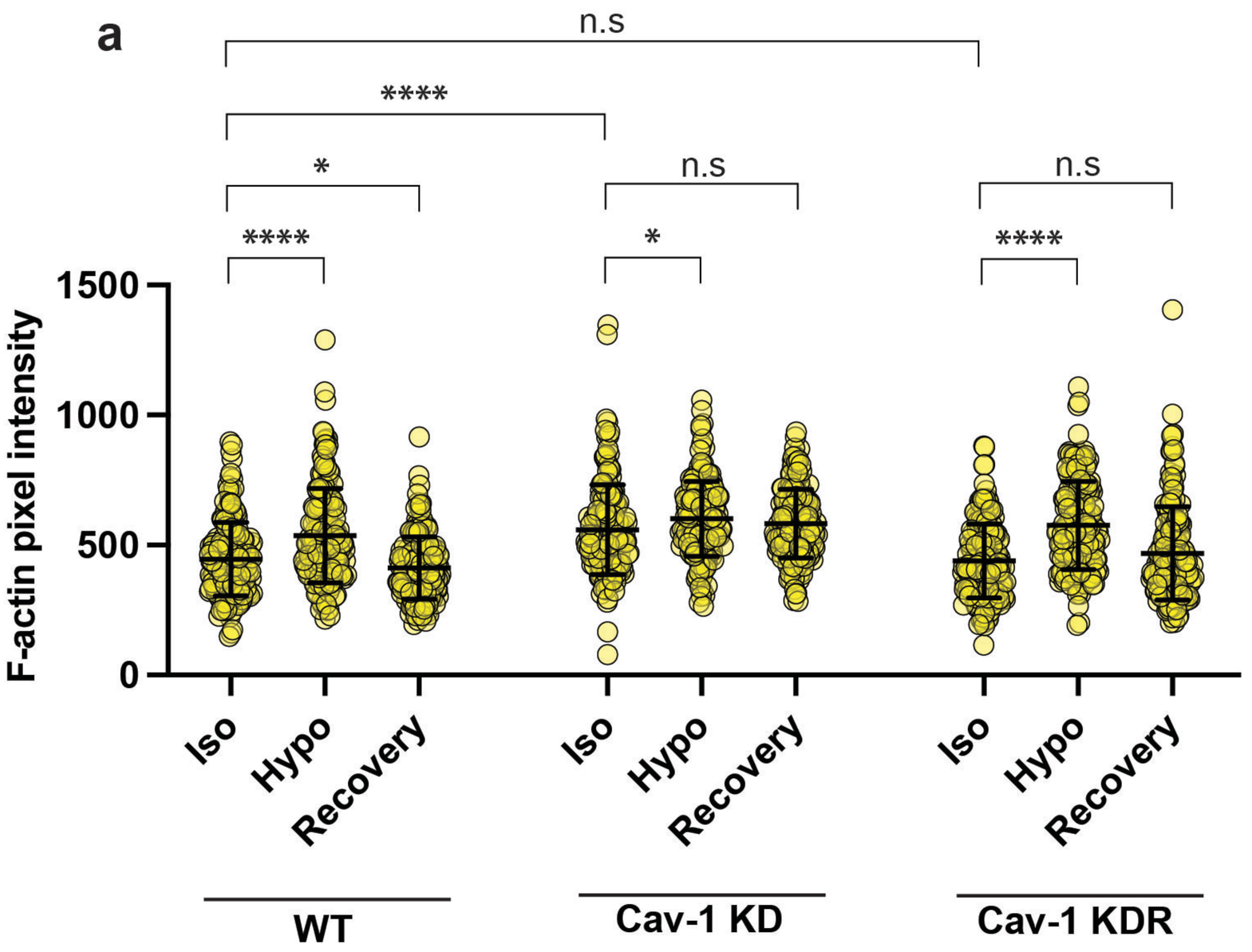
# Fig S1

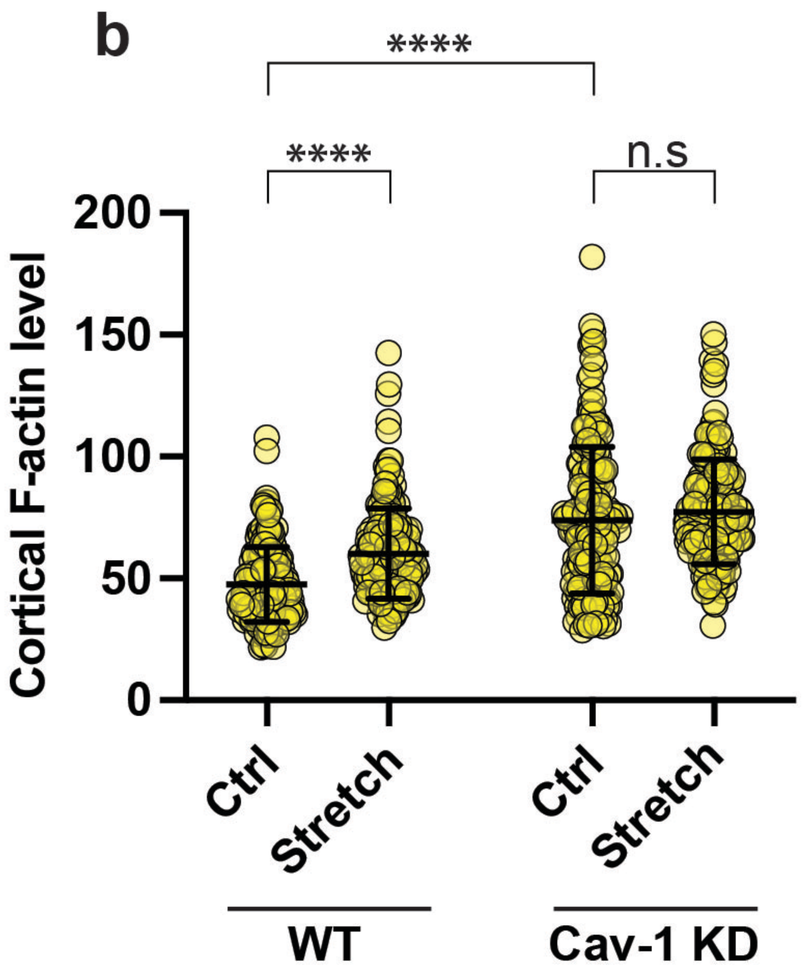



i

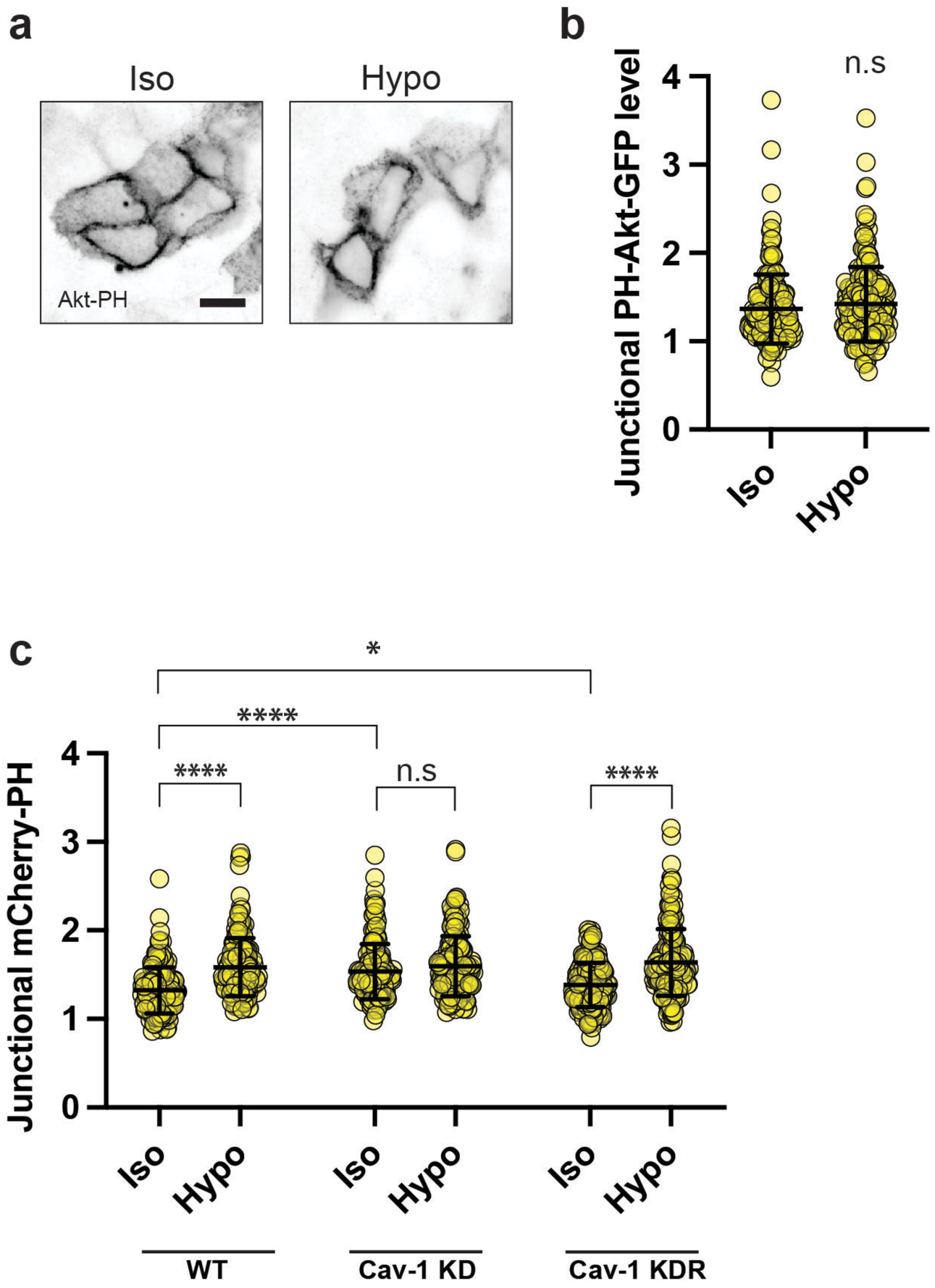
| | Repeat 1 | Repeat 2 | Repeat 3 | Repeat 4 | Repeat 5 |
|---|---|---|---|---|---|
| ***Mm*Cavin1** | | MEKTKVRTREN | LEKTRLKTKEN | | |
| ***Dr*Cavin1b** | MEKTKQKTKEN | LEKTKQKTKEN | LEKTRMRTKEN | LEKTRQKTKEN | LEKTKQKTREN |
| **Δ4UC1** | MEKTKQKTKEN | | | | |

# Fig S2

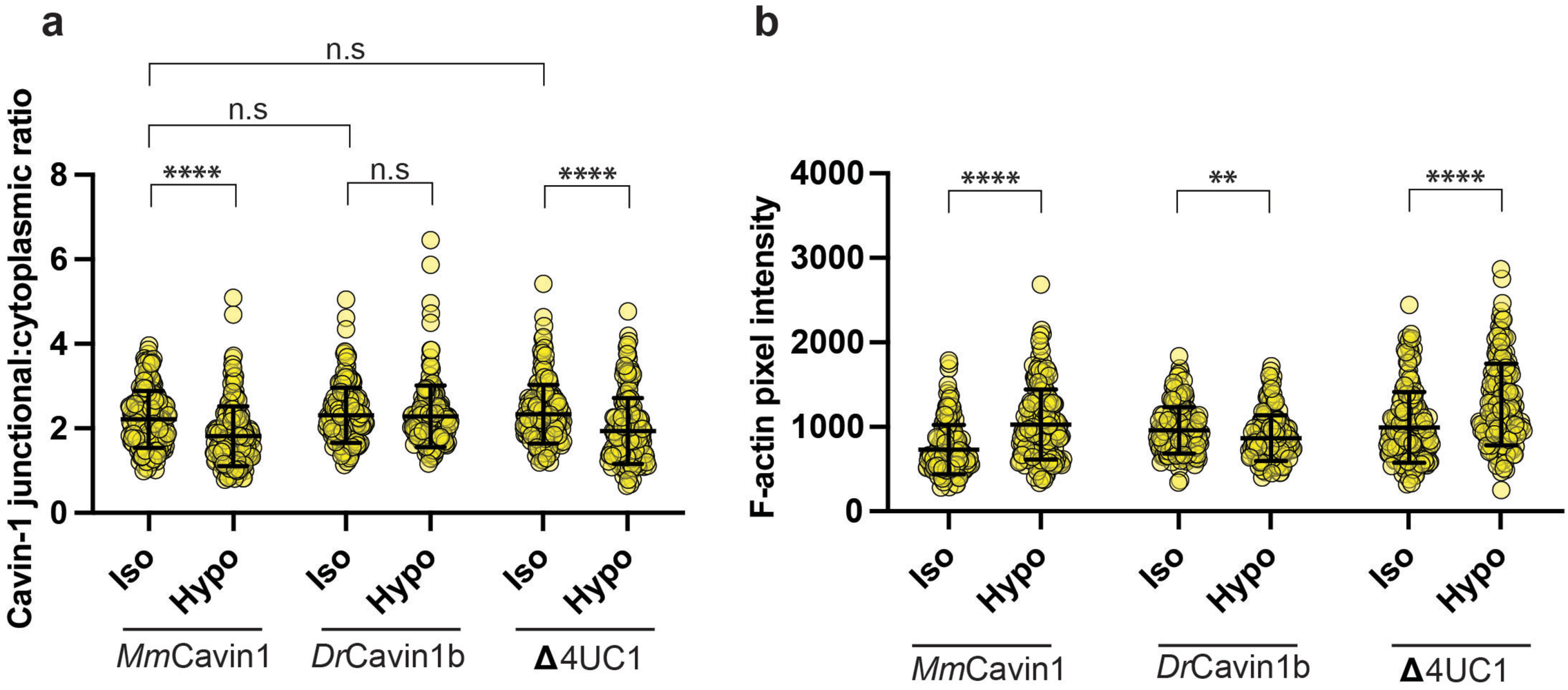

a
n.s
****
*
n.s
n.s
****
*
****
1500
1000
500
0
F-actin pixel intensity
Iso
Hypo
Recovery
Iso
Hypo
Recovery
Iso
Hypo
Recovery
WT
Cav-1 KD
Cav-1 KDR

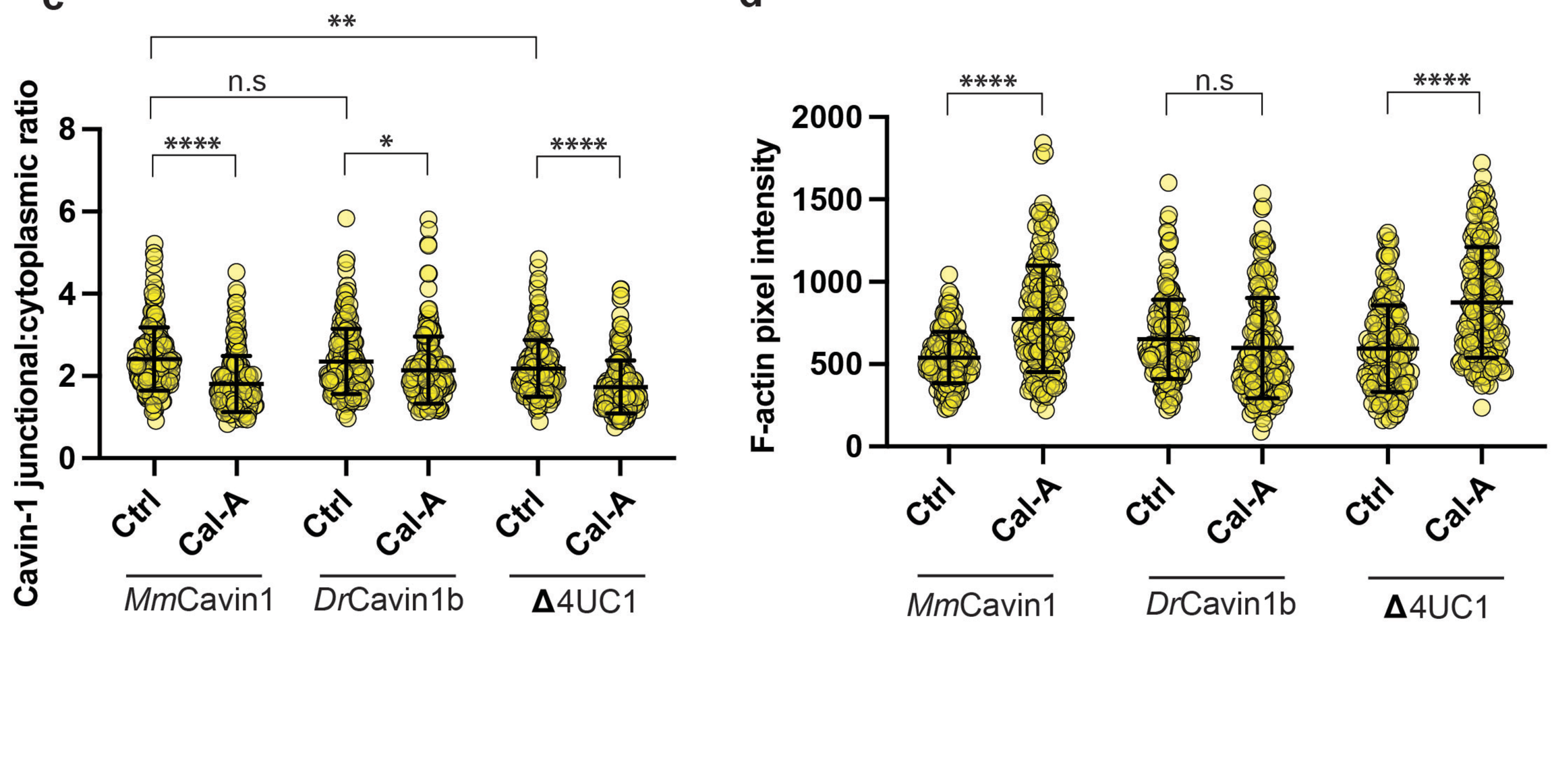

b
****
****
n.s
200
150
100
50
0
Cortical F-actin level
Ctrl
Stretch
Ctrl
Stretch
WT
Cav-1 KD

# Fig S3

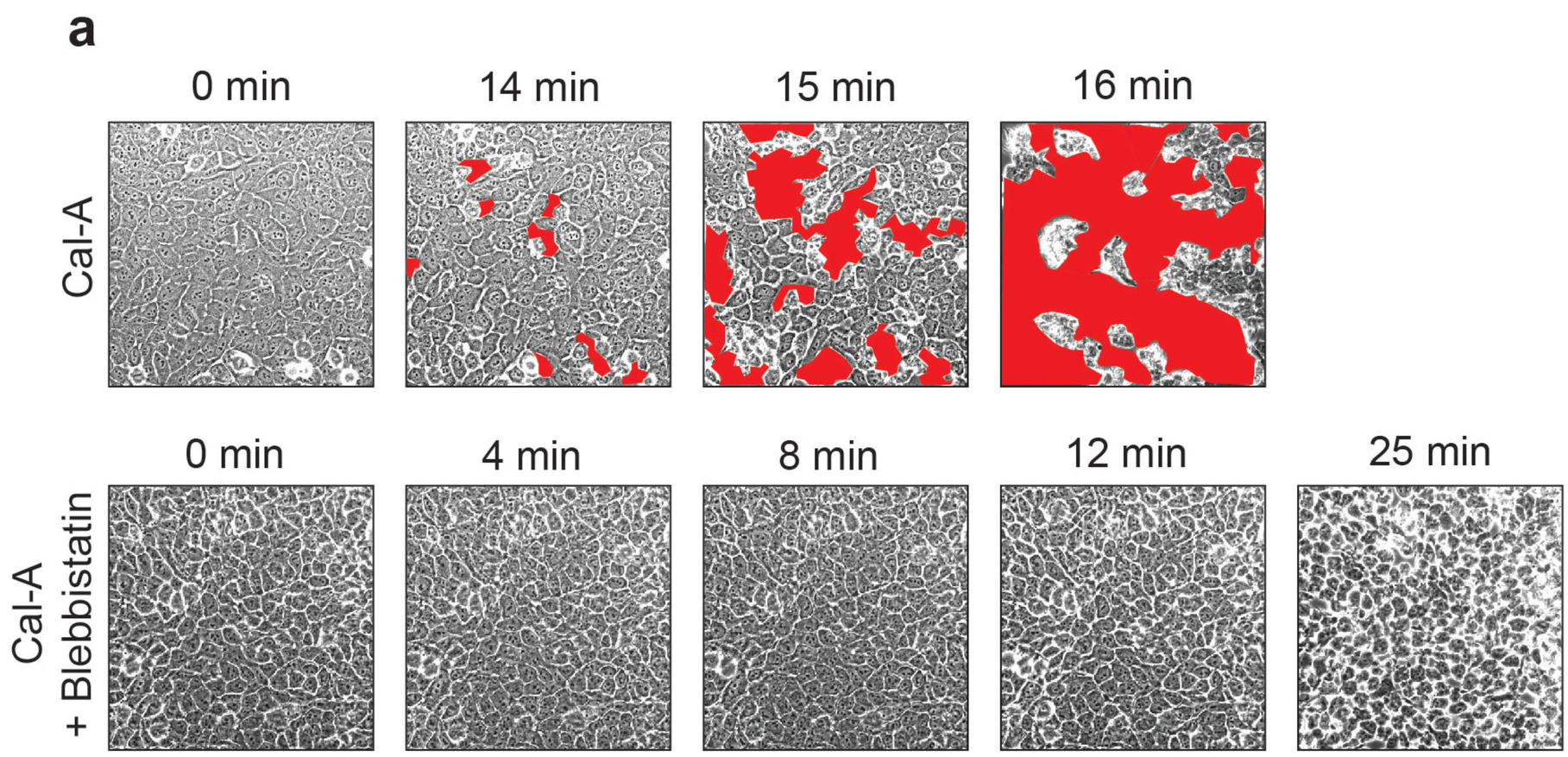

a
Iso
Hypo
Akt-PH
b
n.s
Junctional PH-Akt-GFP level
4
3
2
1
0
Iso
Hypo
c
*
****
****
n.s
****
Junctional mCherry-PH
4
3
2
1
0
Iso
Hypo
Iso
Hypo
Iso
Hypo
WT
Cav-1 KD
Cav-1 KDR

# Fig S4


a
n.s
n.s
****
n.s
****
Cavin-1 junctional:cytoplasmic ratio
0
2
4
6
8
Iso
Hypo
Iso
Hypo
Iso
Hypo
MmCavin1
DrCavin1b
Δ4UC1
b
****
**
****
F-actin pixel intensity
0
1000
2000
3000
4000
Iso
Hypo
Iso
Hypo
Iso
Hypo
MmCavin1
DrCavin1b
Δ4UC1


c
**
n.s
****
*
****
Cavin-1 junctional:cytoplasmic ratio
0
2
4
6
8
Ctrl
Cal-A
Ctrl
Cal-A
Ctrl
Cal-A
MmCavin1
DrCavin1b
Δ4UC1
d
****
n.s
****
F-actin pixel intensity
0
500
1000
1500
2000
Ctrl
Cal-A
Ctrl
Cal-A
Ctrl
Cal-A
MmCavin1
DrCavin1b
Δ4UC1

# Fig S5


a
0 min
14 min
15 min
16 min
Cal-A
0 min
4 min
8 min
12 min
25 min
Cal-A
+ Blebbistatin