Supplemental Materials

Molecular Biology of the Cell

Xu *et al*.

Figure S1. Tumor growth rate is not affected in paxillin KO animals. (A) Primary tumor volume. n = number of animals. (B) Representative tumor sections stained for ki67 and DAPI. (C) Quantification of the percentage of ki67 positive cells. n=3 animals per genotype. A student's t-test was performed for statistical analysis. Data represent mean ± s.e.m.

Figure S2. E-cadherin localization in normal mouse mammary gland. (A) Mammary gland sections stained for E-cadherin (green), EpCAM (red) and DAPI (blue). E-cadherin is primarily localized to cell-cell adherens junctions in paxillin$^{+/+}$cre (paxillin WT) mammary gland, whereas the paxillin$^{fl/fl}$cre (paxillin KO) mammary gland exhibits more diffuse E-cadherin localization. Asterisk indicates the mammary duct lumen. (B) Insets from panel A. Arrows point to apically localized E-cadherin and arrowheads point to delocalized (basolateral) E-cadherin. Asterisk indicates the mammary duct lumen.

Figure S3. Characterization of tumor organoids. (A) Representative Fuji-generated masks of paxillin WT and paxillin KO tumor organoids cultured in 3D collagen gels showing range of sizes and phenotypes. (B) Tumor organoids stained for paxillin (green) and vinculin (red). Arrows indicate potential sites of 3D matrix adhesions. The whole organoids are displayed as a max projection of 14 stacks of z-plane, each plane = 0.5 μm. The insets are displayed as a single z-plane. (C) Individual frames from time-lapse movies at times indicated, showing two examples of single cells invading into the collagen gel from the paxillin KO tumor organoids (arrows).

Figure S4. Adherens Junctions at the leading edge of migrating tumor cells. Vinculin, E-cadherin and F-actin greyscale images (A) in the cell monolayer and (B) at the cell migration leading edge. Arrows point to adherens junctions (see Figure 4D for merged color images). (C) Western blot showing the level of vinculin in paxillin WT and KO tumor cells. (D) Quantification of the relative expression of vinculin. n=3 animals per genotype. A student's t-test was performed for statistical analysis. Data represent mean ± s.e.m.

Figure S5. Adherens junctions (AJs) are perturbed in islands of paxillin KO tumor cells on 2D. (A) Cells plated on 2D fibronectin coverslips were stained for the AJ markers E-cadherin, p120 Catenin and F-actin. Zoomed images of the cells at periphery of the cell islands are

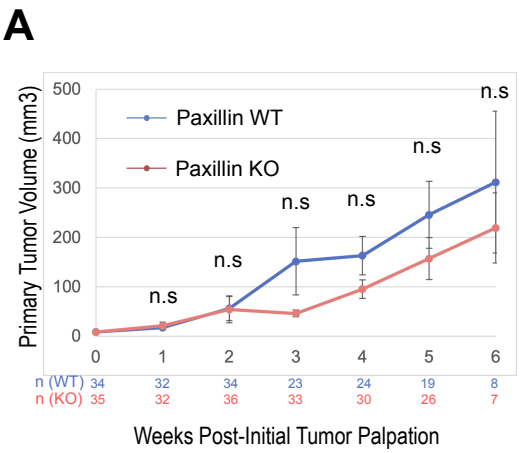
presented in the right panels. Arrows point to the mature AJ in paxillin WT cells (middle upper panel) or the disorganized AJ in the paxillin KO tumor cells (middle bottom panel). Arrowheads point to the F-actin. (B) Quantification of the AJ index. n=3 animals per genotype. Each dot represents one image. (C) Quantification of the percentage of mature AJs and disorganized AJs. n=3 animals per genotype. Shapiro-Wilk test was performed for the normality test. A student's t-test Statistical analysis was performed. Data represent mean ± s.e.m. *<0.05, **<0.01.

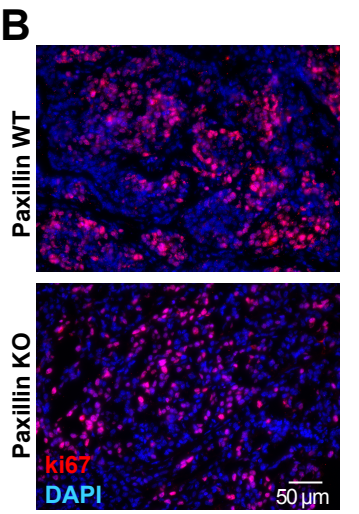
Figure S6. Paxillin KO tumor cells have irregular cell morphology in 2D. (A) Representative images of Imaris analysis of tumor cell islands. Heatmap shows total number of voxels per cell. (B) Quantitation of cell aspect ratio (major:minor). n=3 animals per genotype. Each dot represents one cell. A minimum number of 27 cells per condition were quantitated. A Mann-Whitney U test was performed for statistical analysis. (C) Quantification of average cell area. n=3 animals per genotype. Each dot represents one cell. A minimum number of 27 cells per condition were quantitated. A student's t-test was performed for statistical analysis. (D) Histogram of the cell area distribution of tumor cells. Straight line indicates gaussian fit of the data. n=3 animals per genotype. Data represent mean ± s.e.m. *<0.05.

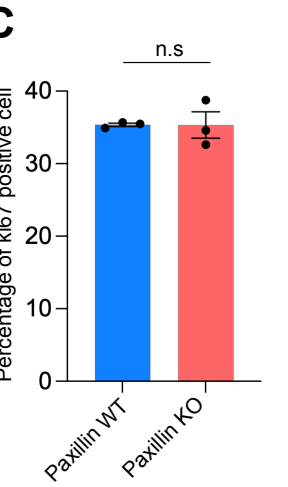
Figure S7. Paxillin KO tumor organoids have decreased levels of acetylated-tubulin. (A) Examples of paxillin WT and paxillin KO tumor organoids stained for acetylated-tubulin and F-actin. (B) Mean fluorescence intensity of acetylated-tubulin. One animal per genotype, each dot represents one organoid. n (paxillin WT)=14, n (paxillin KO)=14.

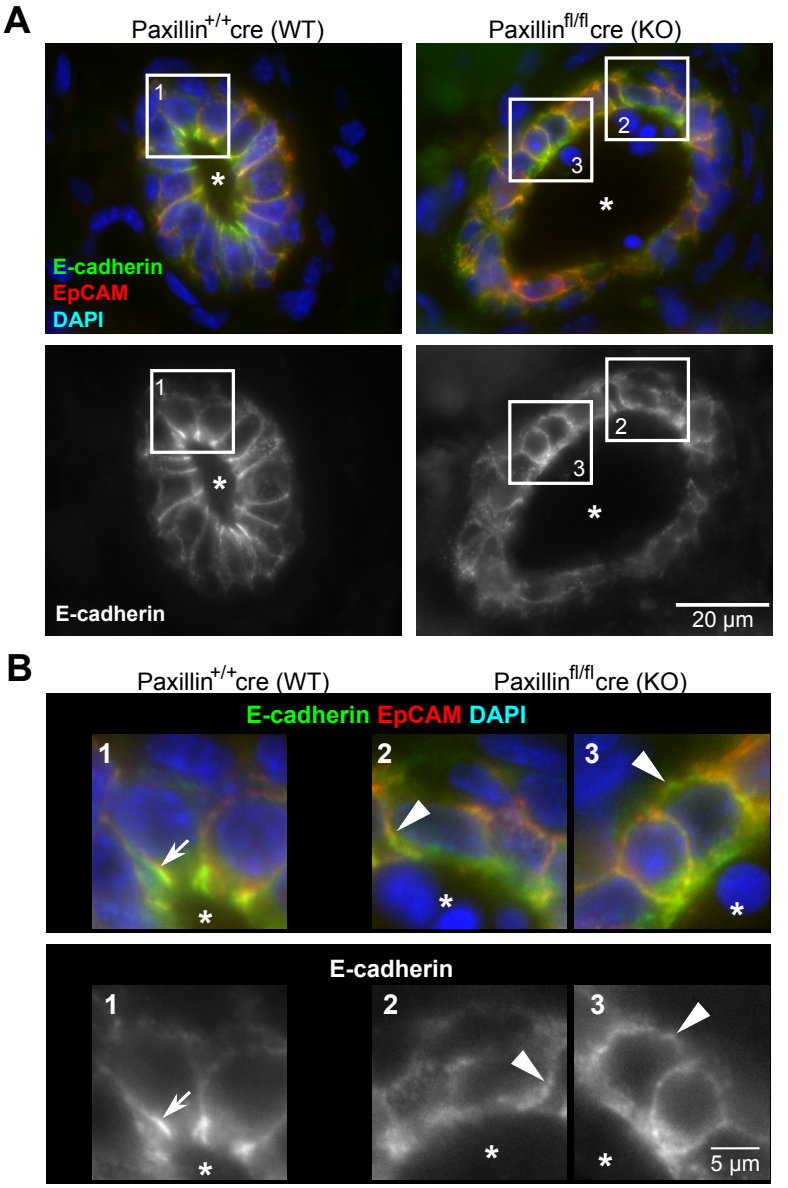
Figure S8. Paxillin KO tumor cells have dysregulated Rho GTPase activity during AJ recovery in calcium switch assay. (A) Rac1 activity in tumor cell lysates, at times indicated following calcium re-addition was detected using GST-PBD pulldown assay. Average normalized active Rac1 levels from 3 independent experiments. (B) RhoA activity in tumor cell lysates, at times indicated following calcium re-addition was determined using a G-LISA RhoA activation assay. The average normalized active RhoA levels from 2 independent experiments. Individual experiments are represented by the same shaped dots.

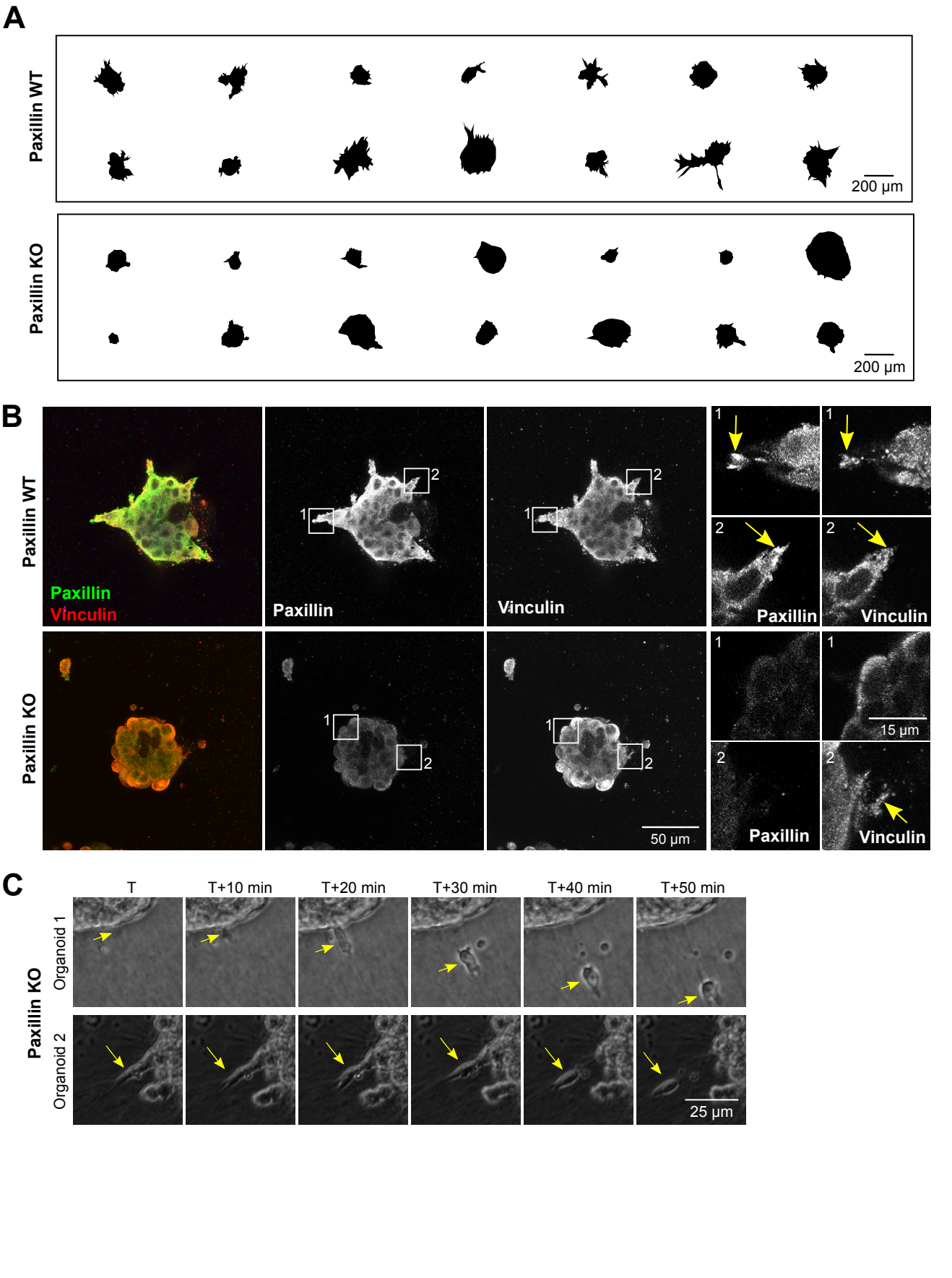
Figure S9. Analysis of FAK phosphorylation. (A) Western blot showing the levels of FAK and pFAK in paxillin WT and KO tumor cells. (B) Quantification of the relative level of FAK phosphorylation. n=3 animals per genotype. A student's t-test was performed for statistical analysis. Data represent mean ± s.e.m.

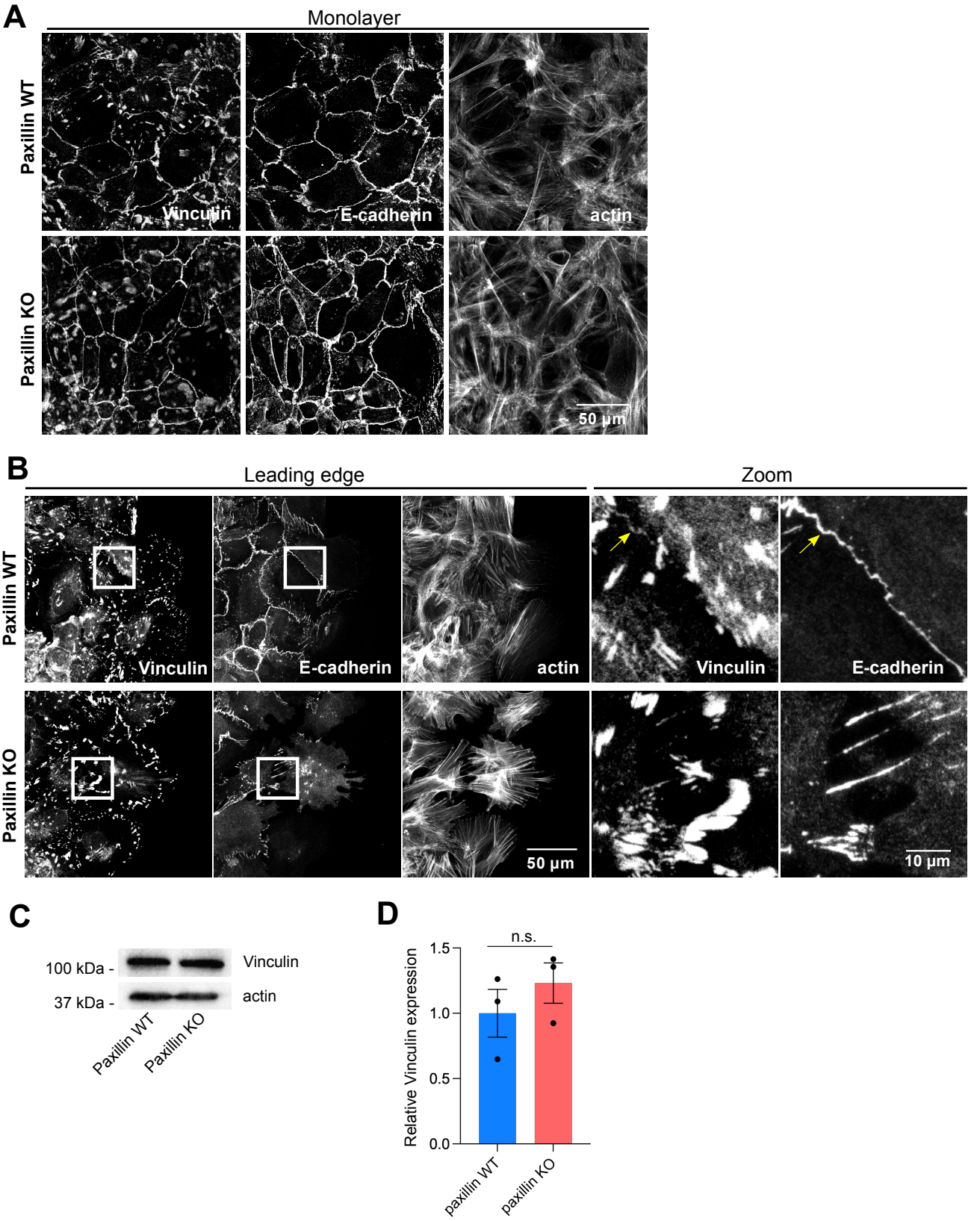

A
Primary Tumor Volume (mm3)
500
400
300
200
100
0
Paxillin WT
Paxillin KO
n.s
n.s
n.s
n.s
n.s
0 1 2 3 4 5 6
n (WT) 34 32 34 23 24 19 8
n (KO) 35 32 36 33 30 26 7
Weeks Post-Initial Tumor Palpation

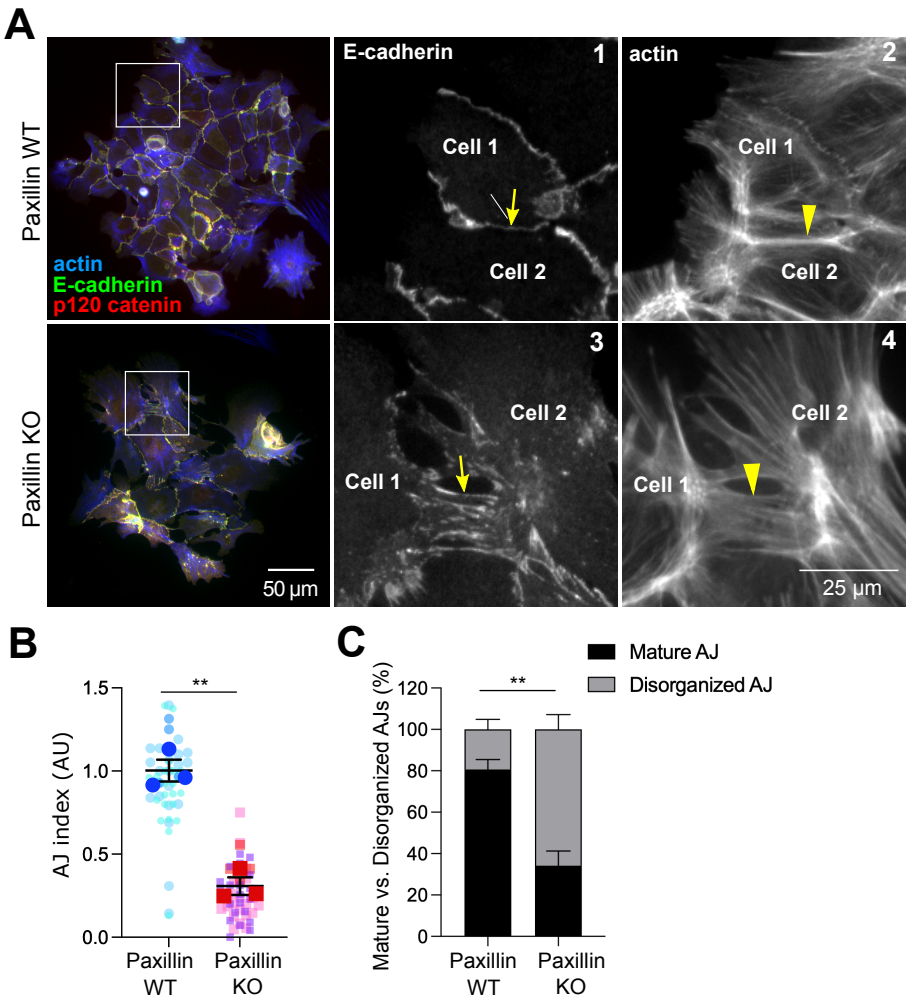

B
Paxillin WT
Paxillin KO
ki67
DAPI
50 µm

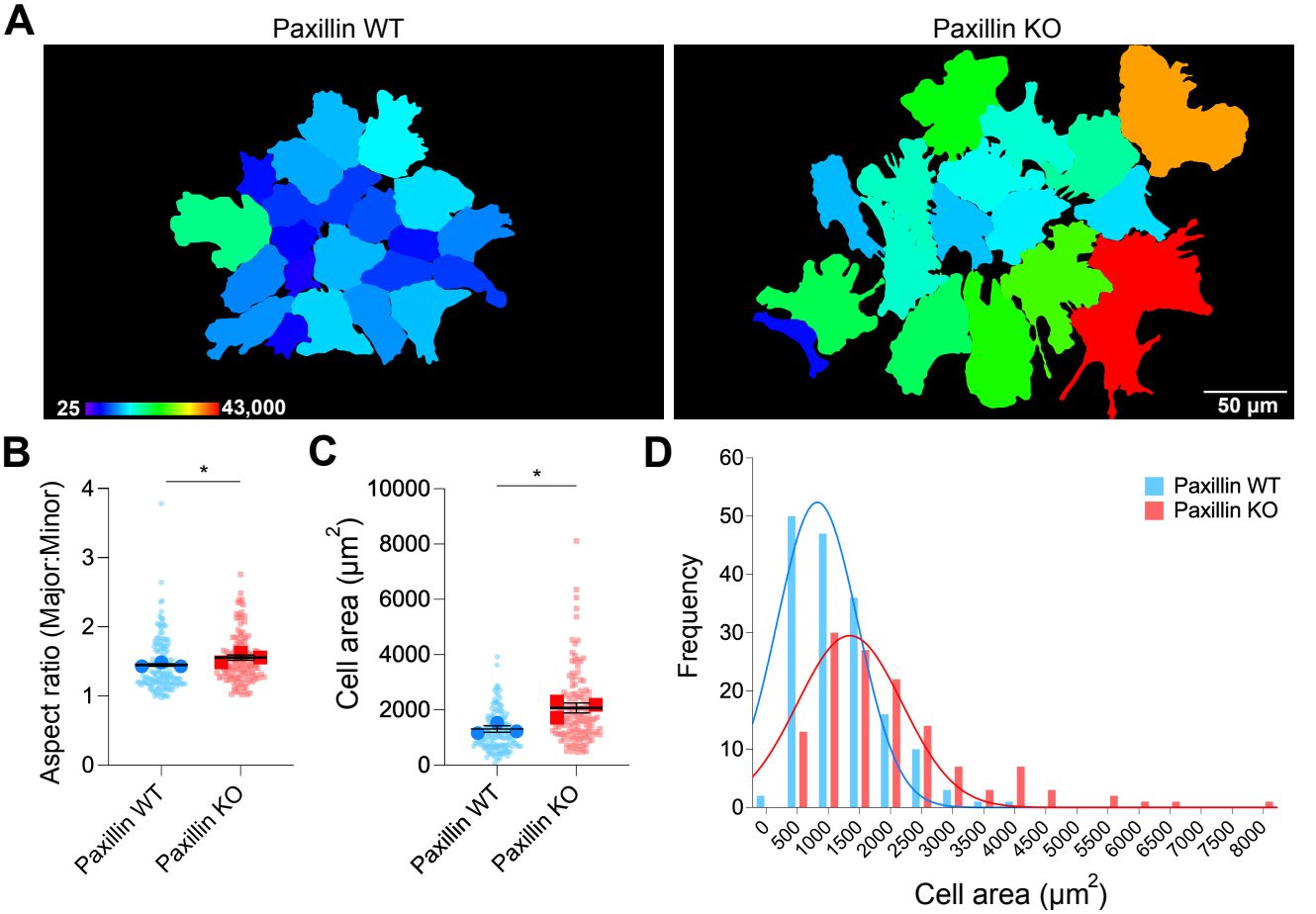

C
n.s
Percentage of ki67 positive cell
40
30
20
10
0
Paxillin WT
Paxillin KO

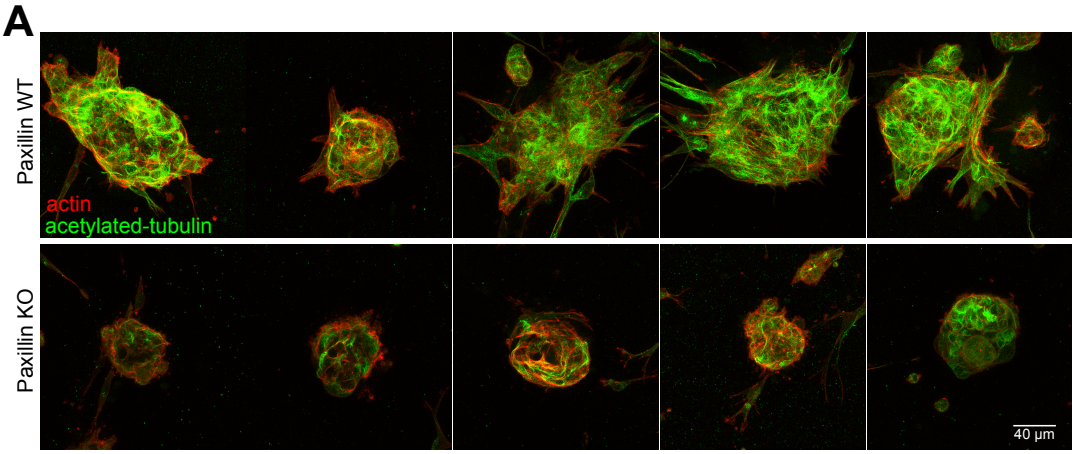

A
Paxillin+/+cre (WT)
Paxillinfl/flcre (KO)
1
2
3
*
E-cadherin
EpCAM
DAPI
E-cadherin
20 µm
B
Paxillin+/+cre (WT)
Paxillinfl/flcre (KO)
E-cadherin EpCAM DAPI
1
2
3
*
E-cadherin
5 µm

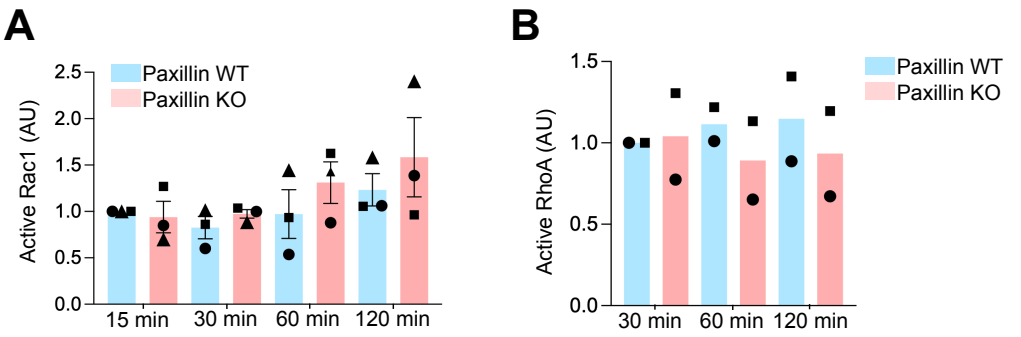

A
Paxillin WT
200 µm
Paxillin KO
200 µm
B
Paxillin WT
Paxillin
Vinculin
Paxillin
Vinculin
Paxillin
Vinculin
Paxillin KO
50 µm
15 µm
Paxillin
Vinculin
C
T
T+10 min
T+20 min
T+30 min
T+40 min
T+50 min
Paxillin KO
Organoid 1
Organoid 2
25 µm

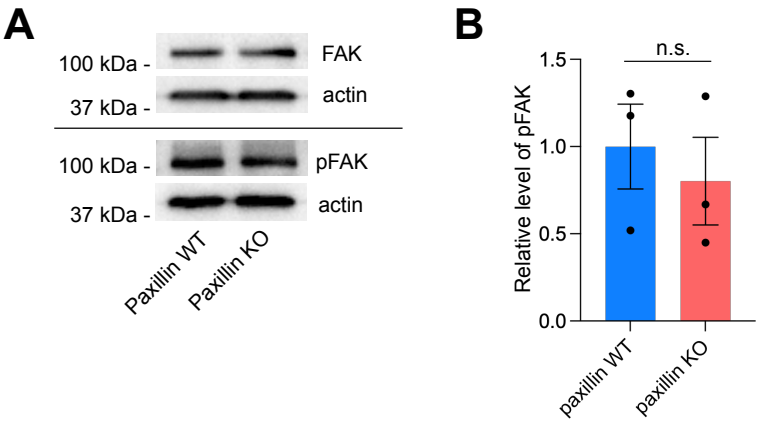

A
Monolayer
Paxillin WT
Paxillin KO
Vinculin
E-cadherin
actin
50 µm
B
Leading edge
Zoom
Paxillin WT
Paxillin KO
Vinculin
E-cadherin
actin
Vinculin
E-cadherin
50 µm
10 µm
C
100 kDa -
37 kDa -
Vinculin
actin
Paxillin WT
Paxillin KO
D
n.s.
Relative Vinculin expression
1.5
1.0
0.5
0.0
paxillin WT
paxillin KO


A
Paxillin WT
Paxillin KO
actin
E-cadherin
p120 catenin
50 µm
E-cadherin
1
Cell 1
Cell 2
actin
2
Cell 1
Cell 2
3
Cell 2
Cell 1
4
Cell 2
Cell 1
25 µm
B
AJ index (AU)
1.5
1.0
0.5
0.0
**
Paxillin WT
Paxillin KO
C
Mature AJ
Disorganized AJ
Mature vs. Disorganized AJs (%)
120
100
80
60
40
20
0
**
Paxillin WT
Paxillin KO


A
Paxillin WT
Paxillin KO
25
43,000
50 µm
B
Aspect ratio (Major:Minor)
Paxillin WT
Paxillin KO
C
Cell area (µm2)
Paxillin WT
Paxillin KO
D
Frequency
Paxillin WT
Paxillin KO
Cell area (µm2)


A
Paxillin WT
Paxillin KO
actin
acetylated-tubulin
40 µm
B
acetylated-tubulin M.F.I. (AU)
200
150
100
50
0
paxillin WT
paxillin KO


A
Active Rac1 (AU)
2.5
2.0
1.5
1.0
0.5
0.0
Paxillin WT
Paxillin KO
15 min
30 min
60 min
120 min
B
Active RhoA (AU)
1.5
1.0
0.5
0.0
Paxillin WT
Paxillin KO
30 min
60 min
120 min


A
100 kDa -
FAK
37 kDa -
actin
100 kDa -
pFAK
37 kDa -
actin
Paxillin WT
Paxillin KO
B
n.s.
Relative level of pFAK
1.5
1.0
0.5
0.0
paxillin WT
paxillin KO