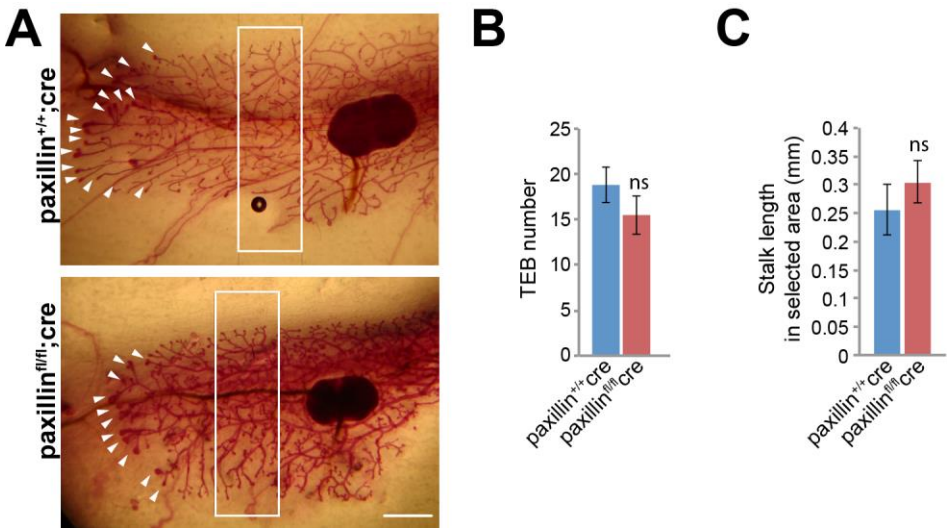


Fig S1. Paxillin$^{fl/fl}$cre mammary glands do not have altered stalk length or number of TEBs. (**A**) Whole mount staining of 6 week old mammary gland. Arrowheads indicate terminal end buds (TEBs). Stalk length was measured in the indicated white boxed area. The box was drawn 1mm from lymph node with a 2 mm width. Scale bar: 2 mm. (**B**) Quantification of TEB number, n=9. (**C**) Quantification of stalk length, n=3. A Student's T-test was performed. Data represent mean ± s.e.m. ns=not significant.

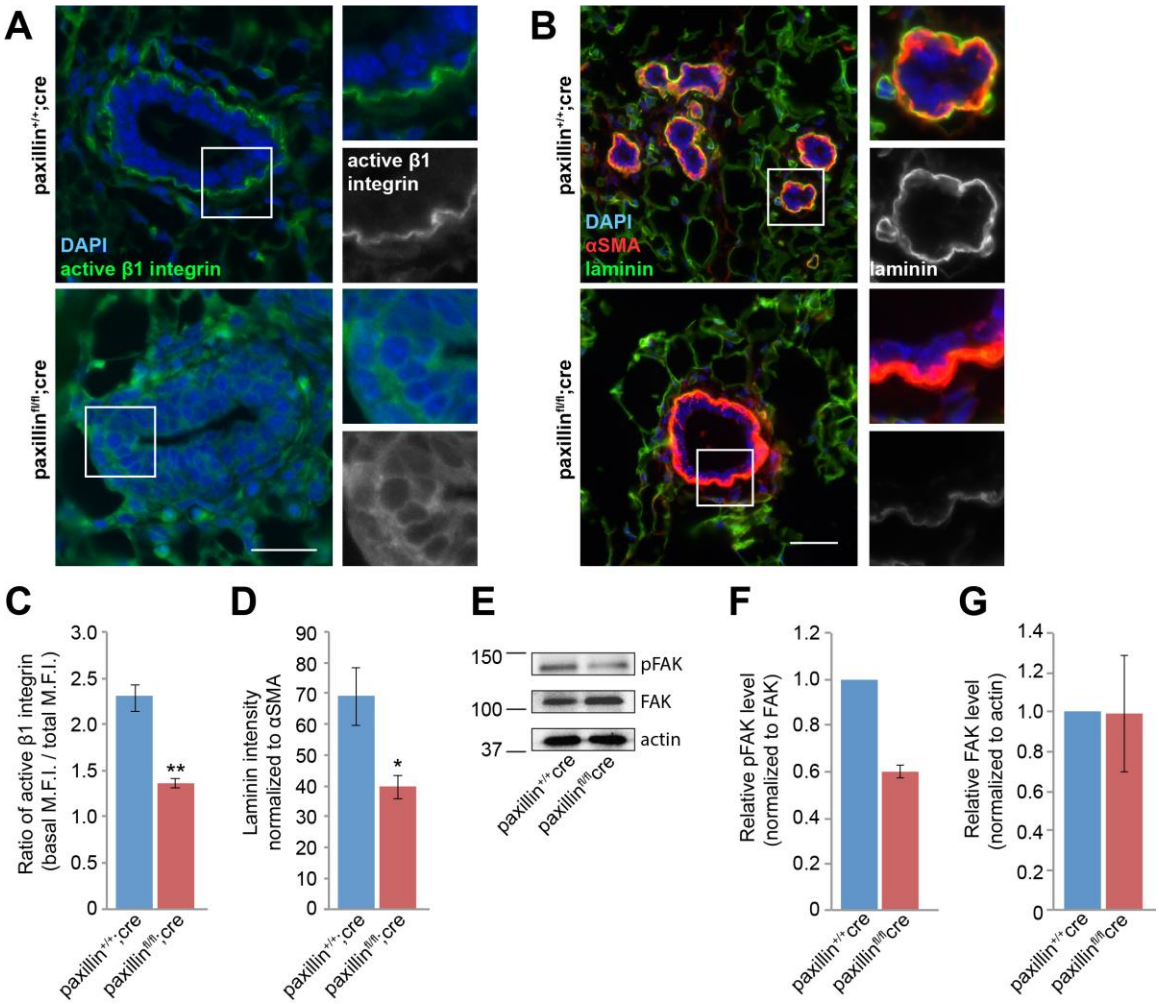


Fig S2. Paxillin$^{fl/fl}$cre mammary glands have mislocalized active β1 integrin and reduced laminin deposition. (**A**) 6 week old mammary gland sections stained for active β1 integrin. Scale bar: 50 μm. (**B**) Quantification of basal membrane versus total duct active β1 integrin staining, n=4. (**C**) Mammary gland sections stained for laminin and α-smooth muscle actin (α-SMA). Scale bar: 50 μm. (**D**) Quantification of laminin intensity normalized to α-SMA, n=3. (**E**) Mammary epithelial cell lysates blotted for phosphor-FAK and total FAK. (**F**) Quantification of pFAK level (normalized to total FAK), n=2. (**G**) Quantification of total FAK level (normalized to total actin), n=2. A Student's T-test was performed. Data represent mean ± s.e.m. *<0.05, **<0.01.

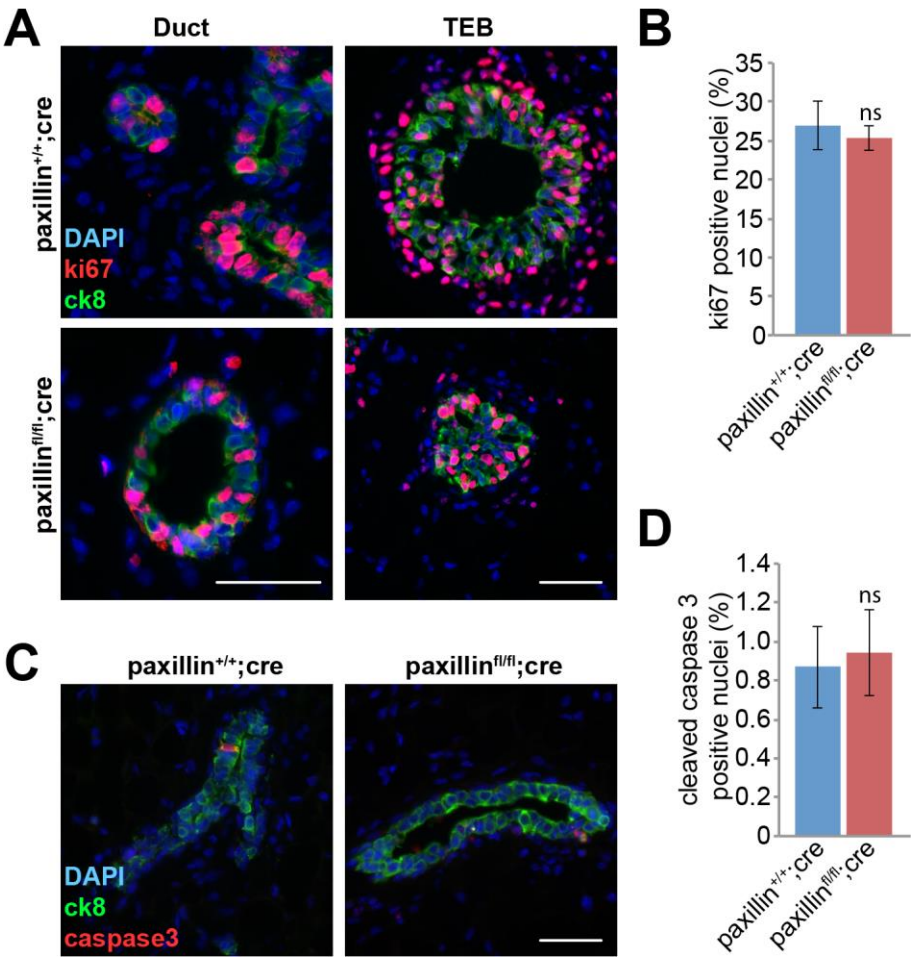


Fig S3. Paxillin$^{fl/fl}$cre mammary glands do not have altered cell proliferation or apoptosis. (**A**) Paxillin$^{+/+}$cre and paxillin$^{fl/fl}$cre ducts and terminal end buds (TEBs) stained with Ki67 and for cytokeratin-8 (CK8). Scale bar: 50 μm. (**B**) Quantification of Ki67-positive cells, n=3 (**C**) Paxillin$^{+/+}$cre and paxillin$^{fl/fl}$cre ducts and TEBs stained for cleaved-caspase 3 and CK8. Scale bar: 50 μm. (**D**) Quantification of cleaved-caspase 3-positive cells, n=3. A Student's T-test was performed. Data represent mean ± s.e.m. ns=not significant.

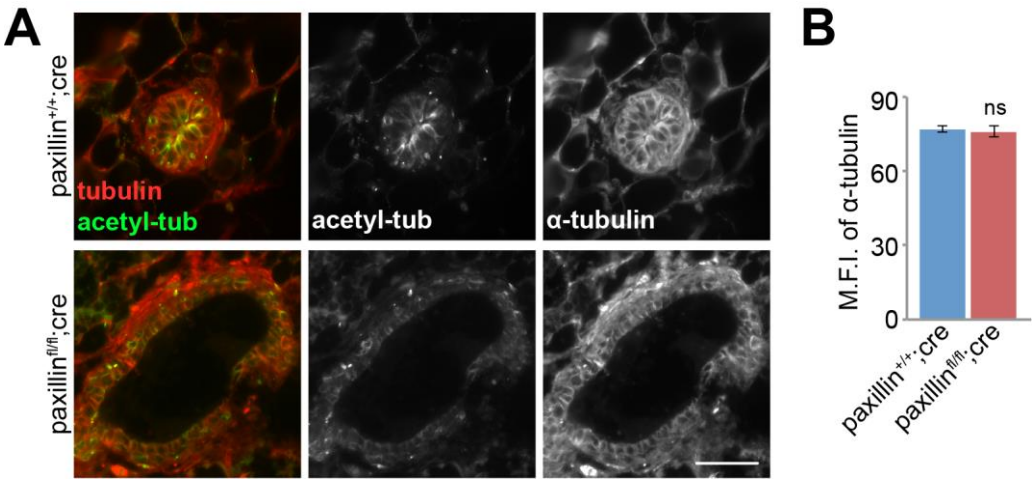


Fig S4. Total microtubule content is not noticeably perturbed in the paxillin$^{fl/fl}$cre mammary gland. (**A**) 6 week old mammary glands co-stained with acetylated tubulin and α-tubulin. Scale bar: 50 µm. (**B**) Quantification of mean fluorescence intensity of α-tubulin, n=3 (at least 5 ducts per animal). A Student's T-test was performed. Data represent mean ± s.e.m. ns=not significant.

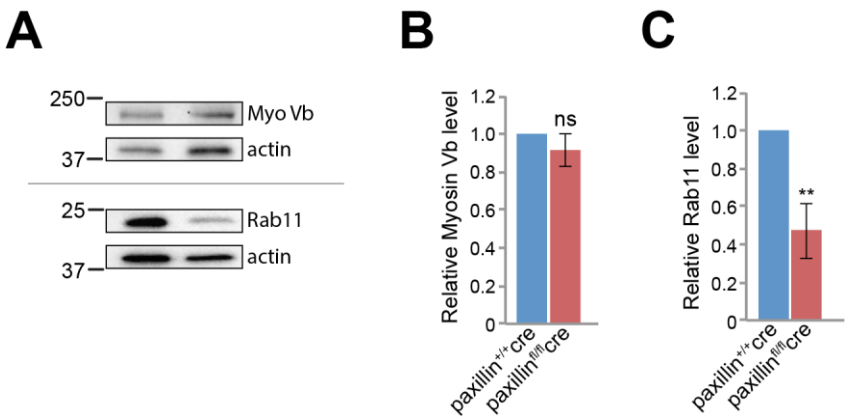


Fig S5. MyoVb and Rab11protein levels in the paxillin$^{+/+}$cre and paxillin$^{fl/fl}$cre mammary gland. (**A**) Mammary epithelial cell lysates blotted for MyoVb and Rab11. (**B**) Quantification of MyoVb level (adjusted to actin), n=2. (**B**) Quantification of Rab11 level (adjusted to actin), n=3. A Student T-test was performed. Data represent mean ± s.e.m. *<0.05, **<0.01.

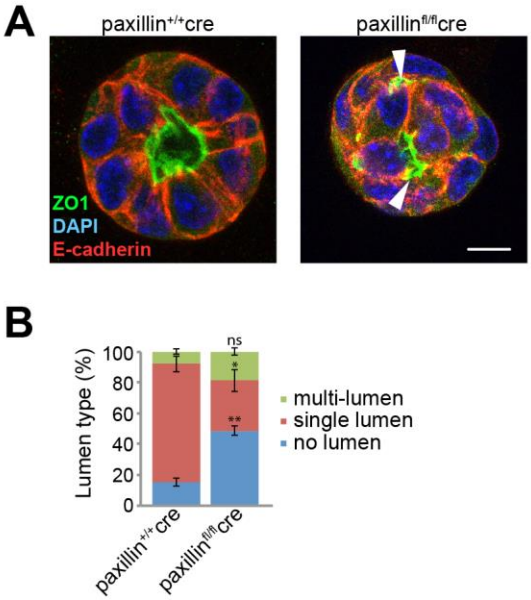


Fig S6. Paxillin$^{fl/fl}$cre acini lack a central lumen. (**A**) Day 6 acini stained for ZO1 (green), E-cadherin (red) and DAPI (blue). Arrowheads indicate small lumens. Scale bar: 5 µm. (**B**) Quantification of different types of lumen, n=2 (total of 85-126 acini per genotype were counted in each experiment). One-way ANOVA with Tukey's multiple comparisons test was performed for statistical analysis. Data represent mean ± s.e.m. *<0.05, **<0.01.

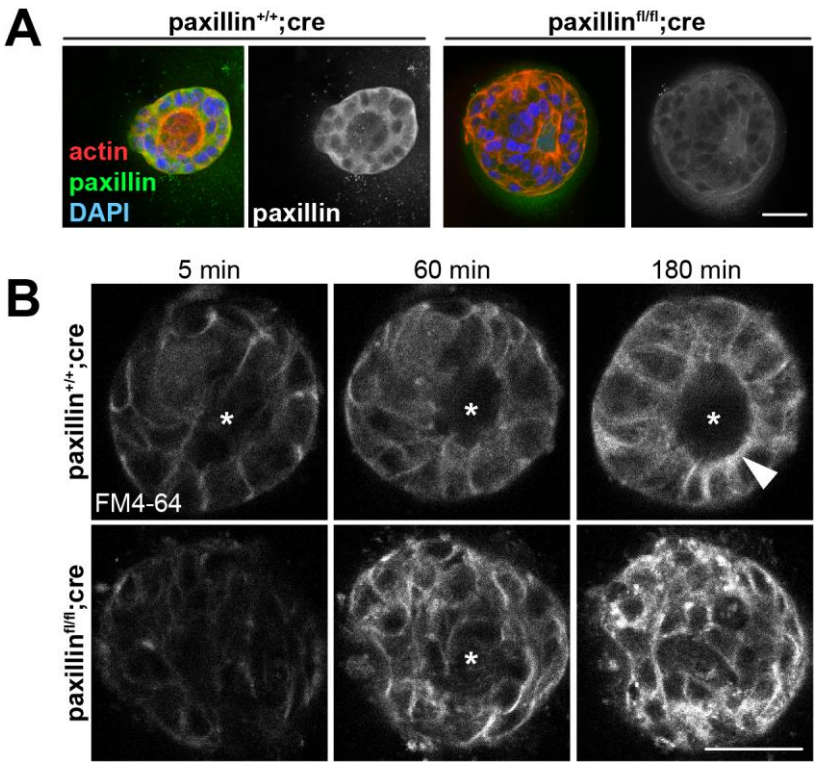


Fig S7. Paxillin$^{fl/fl}$cre fail to accumulate apical FM4-64. (**A**) Paxillin expression pattern in acini. Scale bar: 10 µm. (**B**) Montage images of FM4-64 dye uptake experiments for paxillin$^{+/+}$;cre and paxillin$^{fl/fl}$;cre acini. Asterisk indicates the lumen, arrowhead points to apically accumulated FM4-64 dye. Scale bar: 10 µm.

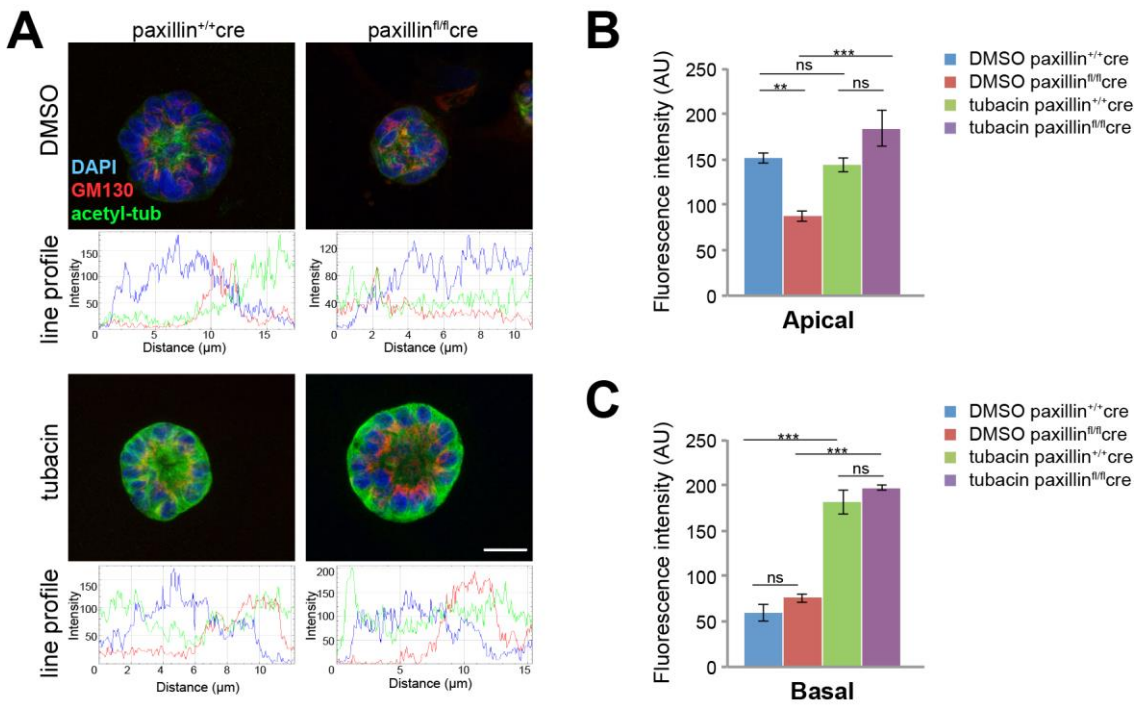


Fig S8. Tubacin treatment rescues apical MT acetylation in paxillin$^{fl/fl}$cre acini. (**A**) Confocal images of acini taken at the same laser power. The bottom of each confocal image shows a representative line profile graph through a single cell. Scale bar: 10 μm. (**B**) Quantification of fluorescence intensity of acetylated-tubulin at the apical surface (or the center of paxillin$^{fl/fl}$;cre acini). (**C**) Quantification of fluorescence intensity of acetylated-tubulin at the basal surface. 5 acini for each condition, at least 5 cells per acinus were included in line profile analysis. One-way ANOVA with Tukey's multiple comparisons test was performed for statistical analysis. Data represent mean ± s.e.m. *<0.05, **<0.01.

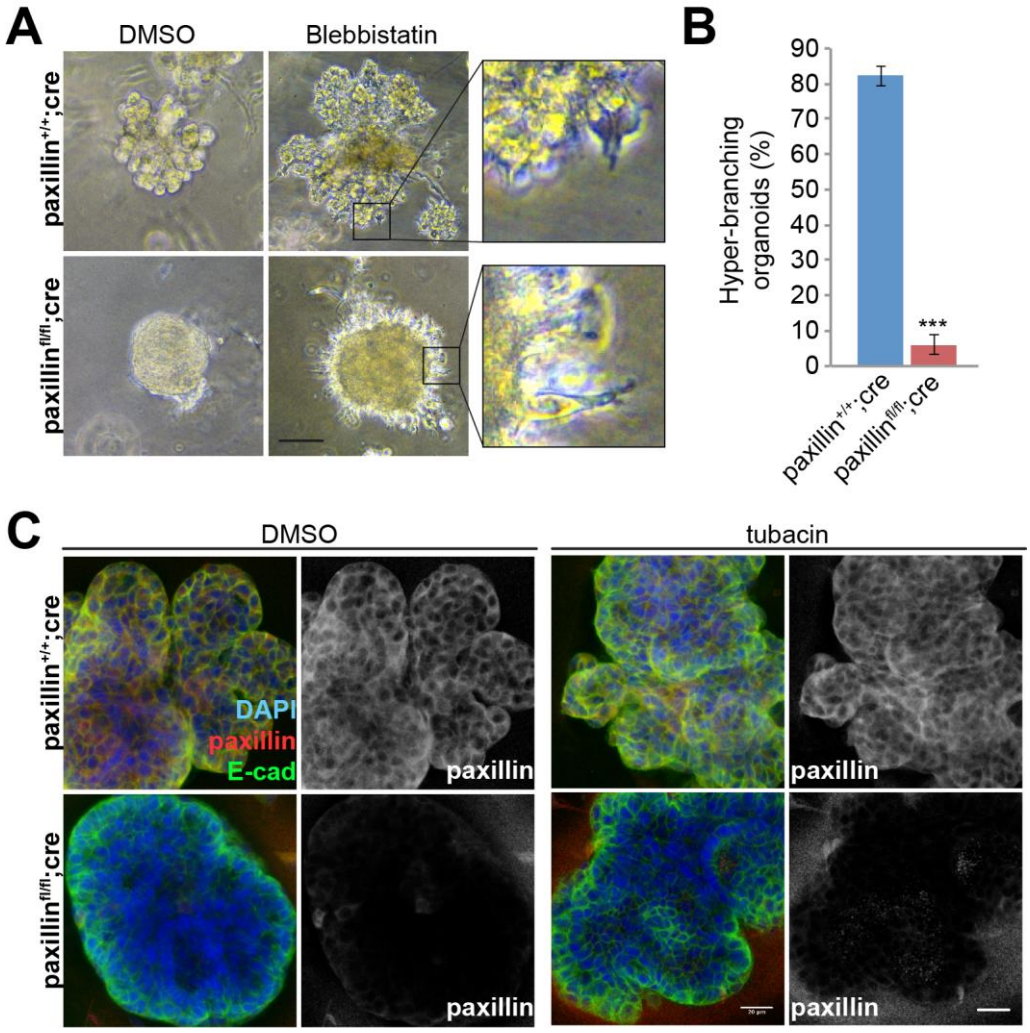


Fig S9. Tubacin and blebbistatin treatment of paxillin$^{+/+}$cre and paxillin$^{fl/fl}$cre organoids. (**A**) Phase images of blebbistatin-treated paxillin$^{+/+}$cre and paxillin$^{fl/fl}$cre organoids. Scale bar: 50 µm. (**B**) Quantification of "hyper-branching" in blebbistatin-treated organoids, n=4. A Student's T-test was performed. Data represent mean ± s.e.m. *<0.05, **<0.01, ***<0.001. (**C**) DMSO and tubacin-treated organoids stained for paxillin. Scale bar: 20 µm.

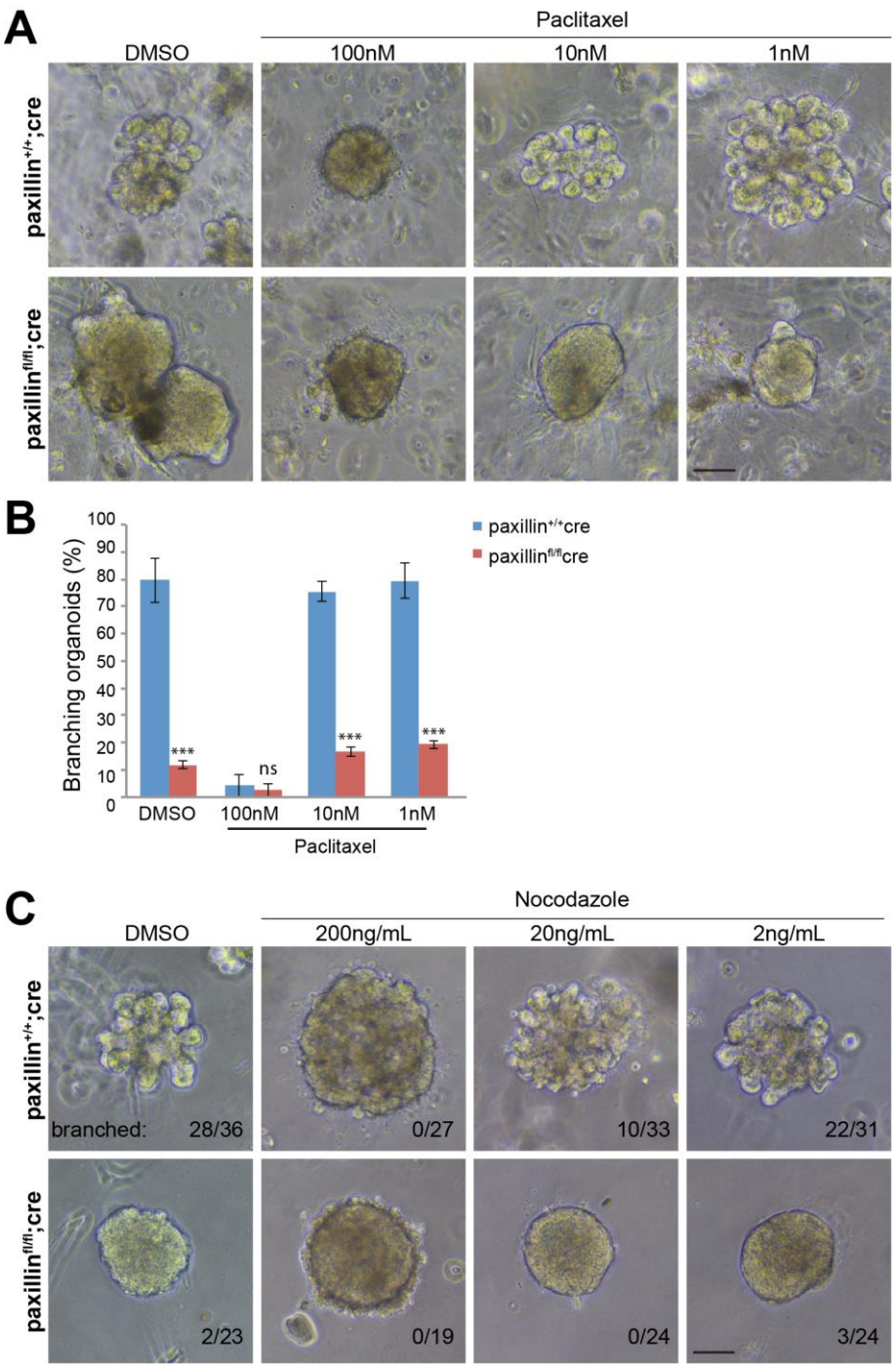
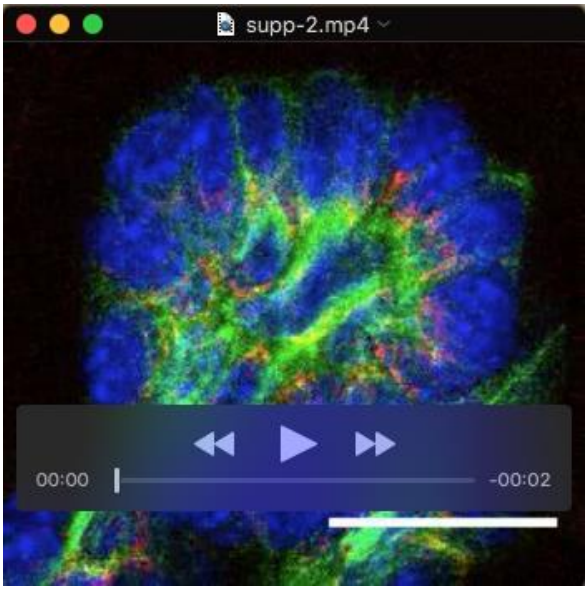
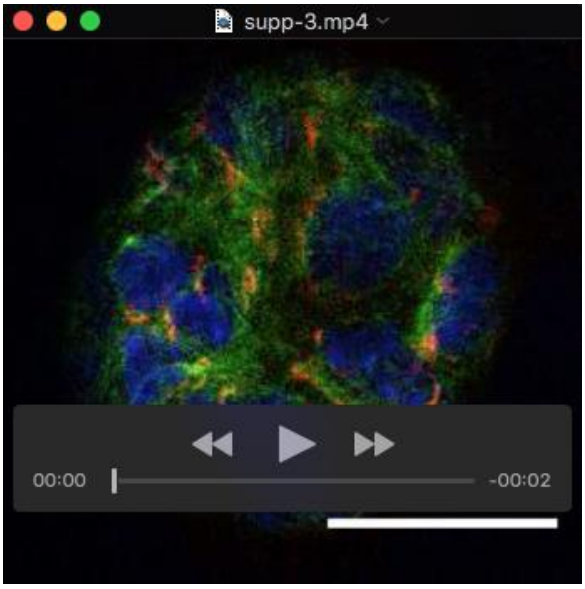


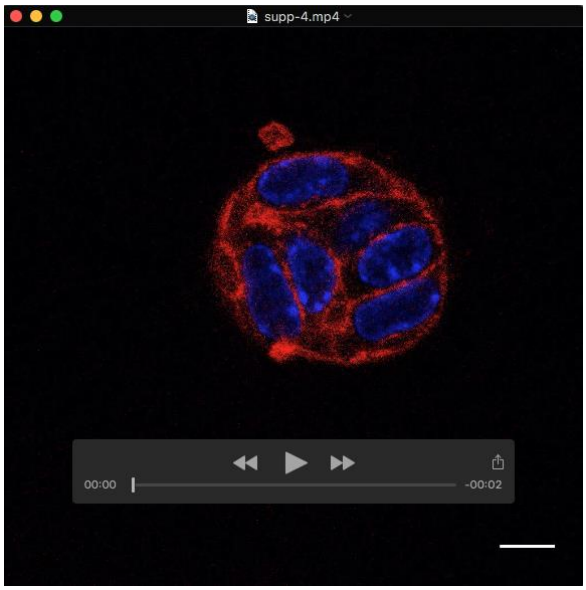
Fig S10. Titration of paclitaxel and nocodazole treatment for organoid branching morphogenesis. (**A**) Phase images of paclitaxel-treated paxillin$^{+/+}$cre and paxillin$^{fl/fl}$cre organoids. Scale bar: 50 µm. (**B**) Quantification of branching, n=2 (total of 19-49 organoids per treatment were counted). A Student's T-test was performed. Data represent mean ± s.e.m. *<0.05, **<0.01, ***<0.001. (**C**) Phase images of nocodazole-treated paxillin$^{+/+}$cre and paxillin$^{fl/fl}$cre organoids. Scale bar: 50 µm.



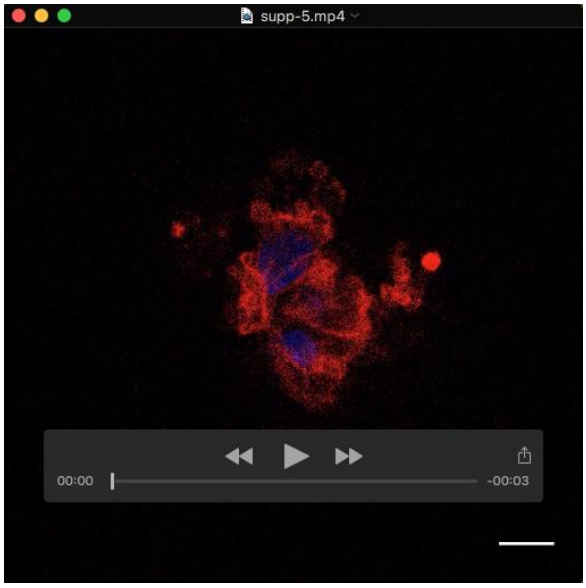
Movie 1. Z-stack movie of paxillin$^{+/+}$;cre acini. Acini stained for GM130 (red), acetylated-tubulin (green) and DAPI (blue). Scale bar: 10 µm.



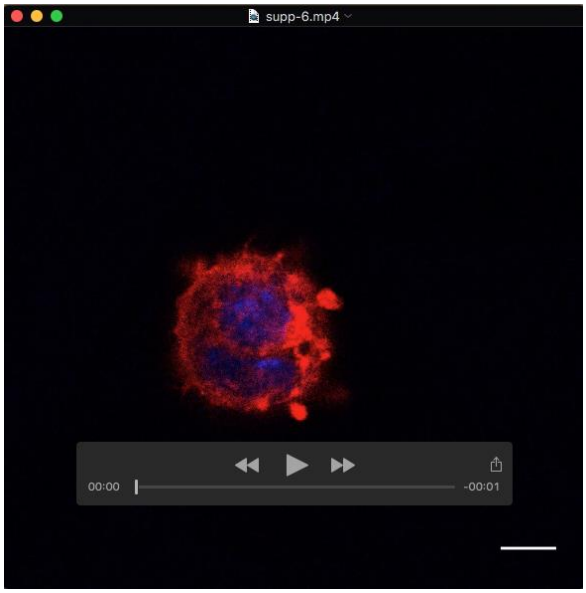
Movie 2. Z-stack movie of paxillin$^{fl/fl}$;cre acini. Acini stained for GM130 (red), acetylated-tubulin (green) and DAPI (blue). Scale bar: 10 µm.



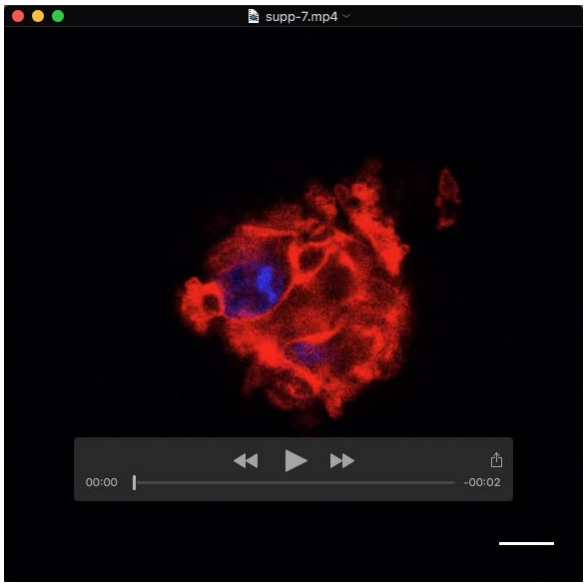
Movie 3. Z-stack movie of paxillin$^{+/+}$;cre acini. Acini stained with phalloidin (red) and DAPI (blue). Scale bar: 10 μm.



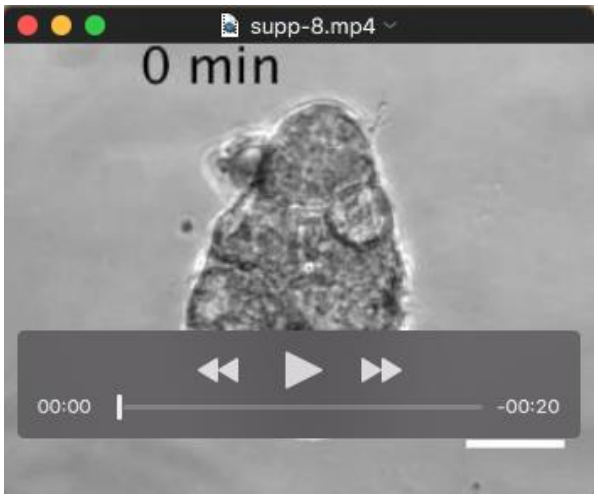
Movie 4. Z-stack movie of paxillin$^{fl/fl}$;cre acini. Acini stained with phalloidin (red) and DAPI (blue). Scale bar: 10 μm.



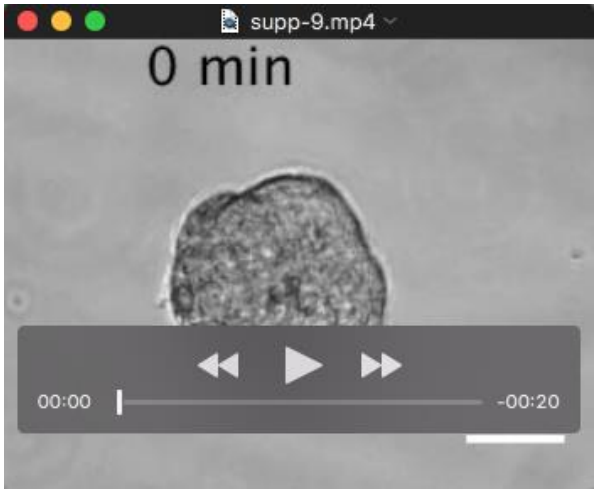
Movie 5. Z-stack movie of paxillin$^{+/+}$;cre early stage acini. Acini stained with phalloidin (red) and DAPI (blue). Scale bar: 10 μm.



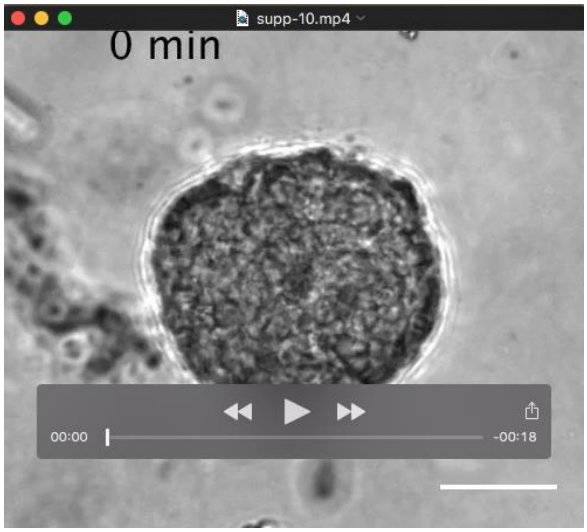
Movie 6. Z-stack movie of paxillin$^{fl/fl}$;cre early stage acini. Acini stained with phalloidin (red) and DAPI (blue). Scale bar: 10 μm.



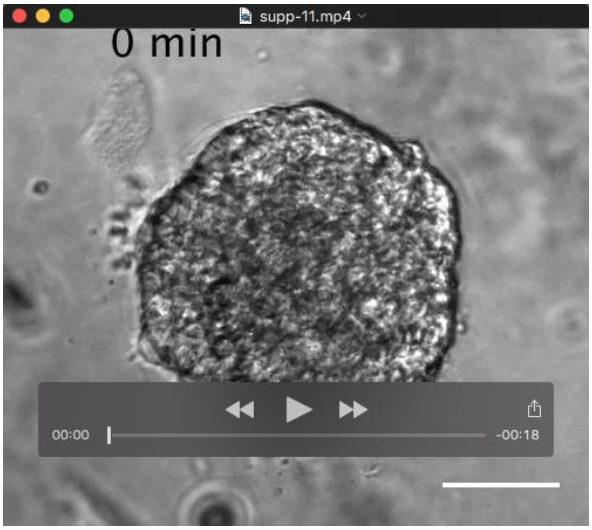
Movie 7. Branching morphogenesis assay. Paxillin$^{+/+}$;cre organoid embedded in Matrigel. Imaged for 22 hours. Scale bar: 50 μm. Timescale: min.



Movie 8. Branching morphogenesis assay. Paxillin$^{fl/fl}$;cre organoid embedded in Matrigel. Imaged for 22 hours. Scale bar: 50μm. Timescale: min.



Movie 9. Branching morphogenesis assay. Paxillin$^{fl/fl}$;cre organoid embedded in Matrigel and treated with DMSO. Imaged for 20 hours. Scale bar: 100 μm. Timescale: min.



Movie 10. Branching morphogenesis assay. Paxillin$^{fl/fl}$;cre organoid embedded in Matrigel and treated with 2 μM tubacin. Imaged for 20 hours. Scale bar: 100 μm. Timescale: min.