Supplementary Information

aPKCi triggers basal extrusion of luminal mammary epithelial cells by tuning contractility and vinculin localization at cell junctions

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Movies S1 to S6

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Supplementary Information Experimental Procedures

DNA constructs and lentivirus production

For aPKCι/ζ knockdown, a pair of siRNAs targeting PKCζ and PKCι was used (aPKCζ sense sequence: 5'-CCAAAUUUACGCCAUGAAATT-3' and aPKCi-4 from Dharmacon D-004656-06). For Vinculin knockdown, two independent siRNAs targeting vinculin were used (sense sequence: VCL-9: 5'-GGUGUUUGUUCAUCUGUAATT-3' and VCL-10: 5'-CAGCAUUUAUUAAGGUUGATT63') from Qiagen. For RhoA knockdown, a pair of siRNAs targeting RhoA was used (sense sequence: 5'-CGACAGCCCUGAUAGUUUAUU-3' and 5'-GACCAAAGAUGGAGUGAGAUU-3') from Dharmacon. For E-cadherin knockdown, a pool of two siRNAs was used (Sense sequence: CDH-1_12: 5'-AGGUAUUGUCUACUCUGAATT-3' and CDH_1_13/ 5'-GGCCUGAAGUGACUCGUAATT-3') from Qiagen. Non-Targeting siRNAs (siNT) were purchased from Dharmacon. Cells were treated with specific siRNA (25 nM final concentrations) with Lullaby reagent (OZ Biosciences) and analyzed 72 h after treatment.

All the following used plasmids allow lentiviral production. The pLJM1 GFP vector was obtained from Addgene. The pLJM1 GFP-aPKCiota WT vector was obtained by insertion of GFP-aPKCi between the NheI and BamHI sites of the pLJM1 GFP vector. The pLJM1 mCherry vector and pLJM1 mCherry aPKCiota vector were generated by removing the GFP in pLJM1 GFP or pLJM1 GFP-aPKCiota and replacement with mCherry using the restriction sites AgeI-EcoRI. The lentiviral pSICO-MT1-MMP mCherry vector was a kind gift of Drs C. Albiges-Rizo and O. Destaing (IAB, Grenoble, France). The pLVX Tet ON GFP vector was obtained by the insertion of GFP between the EcoRI and BamHI sites of the pLVX Tet ON vector (Clontech). pLVX Tet ON GFP-aPKCiota WT was obtained by the insertion of GFP-aPKCiota between the BamHI and AgeI sites of the pLVX Tet ON vector. All constructs were checked by sequencing. Viral supernatants were generated by transient transfection into the Lenti-X 293T cell line (Clontech).

Cell culture, transfection, and stable cell lines

MCF-10A cells were maintained in DMEM-F12 (#11039-021, Gibco) containing 10% penicillin-glutamine, 10 µg/mL human insulin (#I9278, Sigma Aldrich), 100 ng/mL cholera toxin (#C8052, Sigma-Aldrich), 0.5 mg/mL hydrocortisone (Sigma Aldrich), 5% horse serum, and 20 ng/ml EGF (PeproTeck) at 5% CO2 in an incubator at 37°C. Stable MCF-10A-

expressing fluorescent reporter constructs were generated by lentiviral transduction followed by FACS sorting. The absence of mycoplasma contamination in cell cultures was routinely verified using a PCR test. When needed, doxycycline (Sigma-Aldrich) was used at a concentration of 1 µg/mL.

Immunostaining of MCF-10A cell monolayers on glass coverslips

To measure E-cadherin, vinculin, and P-MLC2 fluorescence intensity, MCF-10A-overexpressing doxycycline-inducible GFP or GFP-aPKCi were induced with doxycycline at 0.5 µg/mL 24 h before seeding. Cells were counted and mixed with MCF-10A WT cells at a ratio of 1:20 and seeded at a final concentration of 500,000 cells/mL on glass coverslips in MCF-10A medium. To measure E-cadherin and vinculin fluorescence intensity, cells were pre-permeabilized after 24 h in PBS-4% PFA-0.05% Triton for 90 s and fixed in 4% PFA for 20 min. Blocking was performed in PBS-10% FBS for 1 h. Primary antibody staining was performed in PBS-10% FBS at 4°C overnight. Secondary antibody incubations were performed for 45 min in PBS. Samples were mounted in Prolong-DAPI. To measure P-MLC2 fluorescence intensity, cells were first fixed after 24 h with PBS-4% PFA for 10 min, followed by permeabilization in PBS-0.5% Triton for 10 min. Blocking was performed in PBS-10% FBS for 1 h. Primary antibody incubation was performed in PBS-10% FBS for 3 h at room temperature. Secondary antibody incubation was performed for 45 min in PBS. Samples were mounted in Prolong-DAPI. For experiments presented Figure S7, MCF-10A WT or GFP cells were treated with specific siRNA siNon-Targeting or siVinculin (50 nM final concentration) in Lullaby reagent (OZ Biosciences) and mixed 48 h later with MCF-10A overexpressing doxycycline inducible GFP-aPKCi (induced 24 h before) or MCF-10A WT cells at a ratio of 19:20 or 1:20, respectively, at a final concentration of 500,000 cells/mL. Cells were then fixed 24 h later in PBS-4% PFA and immunofluorescence was performed as already described for P-MLC2 immunofluorescence.

Culture, infection, and immunostaining of MCF-10A acini

MCF-10A cells (400 µl, 20,000 cells/mL), cultured in MCF-10A media (DMEM-F12 (#11039-021, Gibco) containing 10% penicillin-glutamine, 10 µg/mL human insulin (#I9278, Sigma Aldrich), 100 ng/mL cholera toxin (Sigma-Aldrich), 0.5 mg/mL hydrocortisone (Sigma Aldrich), 5% horse serum) supplemented with EGF at 5 ng/mL and 1% matrigel, were seeded in eight-chamber culture slides (Corning, #354118) coated with 35 µl matrigel. Cells were maintained at 5% CO2 in an incubator at 37°C. The medium was replaced at four-day

intervals. At day 16, cells were infected with lentiviral particles concentrated to 50 times their initial concentration using a Lenti-X concentrator from Takara and incubated with Polybrene at 16 μg/mL (Sigma-Aldrich, #H9268) for 24 h. Analyses were performed at day 21. Immunostaining of MCF-10A acini was performed as described in (25). Samples were mounted in prolong-DAPI.

Isolation, infection, and 3D culture of primary mammary epithelial organoids

For organoid experiments, virgin FVB mice between 7 and 12 weeks of age were used. We used a combination of mechanical disruption, collagenase A (#10103578001, Sigma-Aldrich)/trypsin digestion, and differential centrifugation to purify fragments of primary mammary ducts (organoids), as previously described (23). Organoids were then infected in suspension in non-adherent plates using lentiviral particles concentrated to 50 times their initial concentration using a Lenti-X concentrator from Takara and fused with magnetic beads (Viromag R/L Transduction reagent, OZ Biosciences) for 12 h. Lentiviral particles were incubated with magnetic beads for 20 min prior to infection. Organoids were embedded in a 3D matrix composed of a mix of Matrigel (Corning) (4-5 mg/ml final concentration) – type I collagen (Corning) (1 mg/ml final concentration) and plated on MatTek dishes. Gels were allowed to polymerize for 30 min at 37°C before the addition of organoid medium: DMEM-F12 (#11039-021, Gibco) containing 1% insulin-transferrin-selenium (#I3146, Sigma-Aldrich) and 10% penicillin-streptomycin (P4333; Sigma). Medium was replaced every 48 h for the duration of culture. When needed, GM6001 (Millipore) diluted in ethanol was used at a concentration of 40 μM and Blebbistatin (Selleckchem) diluted in DMSO at a concentration of 10 μM.

Immunostaining of organoids cultivated in a 3D matrix

Organoids were fixed in PBS-4% paraformaldehyde for 20 min followed by permeabilization in PBS-0.5%Triton for 45 min. Organoids were incubated in blocking solution (PBS-10% FBS-1% BSA) for 2 h. Primary antibodies were incubated in PBS-1% FBS-1% BSA at 4°C overnight. Secondary antibody incubation was performed in 10% FBS-PBS for 2 h. Samples were mounted in Prolong-DAPI (Invitrogen).

Tissue immunofluorescence

Mammary gland number 4 was collected after sacrificing the mouse. Tissues were cut into small pieces and fixed in PBS-4% paraformaldehyde for 30 min, permeabilized in PBS-1% Triton for 1 h, and blocked in PBS-0.2% Triton-3% FBS-1% BSA for 1 h at room temperature. Primary antibodies were diluted in PBS-0.2% Triton (for dilution information, see Supplementary Table1). Three washes of 1 h in PBS-0.2% Triton were then performed. Secondary antibody solutions were diluted in PBS-0.2% Triton (for dilution information, see Supplementary Table 1) and incubated for 2 to 4 h. The samples were kept in PBS until confocal imaging. One day before acquisition, samples were mounted in Prolong-DAPI (life technologies).

Microscopy and image analysis

Confocal images of MCF-10A spheroids, mouse mammary organoids, and MCF-10A monolayers on coverslips were acquired with a Leica SP8 NLO microscope equipped with HyD hybrid detectors. Depending on the magnification, various objectives were used (10x dry 0.95 NA, 40x oil 1.30 NA, or 63x oil 1.40 NA). Images were processed using ImageJ software. To quantify E-cadherin, P-MLC2, and vinculin fluorescence intensity at cell-cell contacts, image stacks were acquired with the photon counting mode. Acquisition settings were identical (laser power, objectives, and magnification) for all images and conditions. Line-scan analysis was performed with Metamorph software (v 7.7.0.0) on a z-projection of five planes (z-step 0.3 µm) for each condition. Organoid live imaging was performed at 37°C and 5% CO2 with a spinning disk microscope (Gataca-System) based on a CSU-W1 Yokogawa head mounted on an inverted Ti-E Nikon microscope with a motorized XY stage (MadCity Lab). Actin was labelled with the dye SiR-Actin Cy5 from Spirochrome. Images were acquired through a 40x 1.15 NA with a Photometrics 95B-sCMOS camera.

Laser ablation

WT and GFP or GFP-aPKCi MCF-10A cells were mixed at a 1:25 ratio and plated on a glass coverslip 16 h before acquisition. GFP or GFP-aPKCi expression was induced with doxycycline 24 h prior to seeding. The laser ablation system was composed of a pulsed 355-nm-20kHz ultraviolet laser (Gataca System) interfaced with an iLas system running in parallel with Metamorph 7 Software. Experiments were performed at 37°C and 5% CO2 on a confocal spinning disk microscope (Yokagawa CSU-X1 spinning head on a Nikon Eclipse T*i*-

E inverted microscope) equipped with an EMCCD camera (Evolve, Photometrics) and a 100x oil immersion objective (Nikon S Fluor 100x 0.5-1.3 NA). The ablation region was drawn as a line perpendicularly crossing the middle of the junction at the interface between WT and GFP or GFP-aPKCi-overexpressing MCF-10A cells. Images were acquired at 5-s intervals for 15 s before ablation. For photoablation, the laser beam was focused on the region of interest during a pulse of 80 to 90 ms at 85% laser power. After photoablation, acquisition was performed at 1-s intervals for 20 s and then at 10-s intervals for another 2 min to ensure capture of the full displacement of the two vertices. The displacement between the two vertices $L(t)$, relative to their initial positions, was then tracked and the speed of retraction (termed "initial recoil velocity") was obtained after fitting the displacement curve $L(t)$ with a one-phase association exponential using GraphPad Prism (GraphPad Software).

Immunoprecipitation assay

MCF-10A-overexpressing doxycycline inducible GFP or GFP-aPKCi cells were induced with doxycycline at 0.5 µg/mL 24 h before the experiment. Cells were lysed in 50 mM Tris-HCl (pH 7.5), 150 mM NaCl, 10 mM $MgCl_2$, 1% Triton X-100, and 10% glycerol with anti-protease and anti-phosphatases. Anti-vinculin antibodies (Millipore, clone VIIF9) were then added (2.5 µg per immunoprecipitation). Vinculin and phosphorylated serine bands were determined by SDS-PAGE with anti-vinculin (Millipore, clone VIIF9) and anti-phospho-serine PKC substrate antibodies (Cell Signaling, #2261), respectively. Immunoprecipitation experiments were replicated two times.

Statistical analysis

Data were tested for a normal distribution using the D'Agostino-Pearson normality test. Otherwise, nonparametric tests were applied. All error bars represent the standard error of the mean (SEM). For mouse mammary organoid *ex vivo* experiments, the chi-squared test was performed for all cells quantified for each condition. For transplantation experiments, Fisher tests were performed. For segregation assays on spheroids, ANOVA with multiple comparisons were applied. All box plots represent the median, quartiles, and minimum and maximum values. For maximum fluorescence intensity, all quantified cells were considered and Mann-Whitney tests were performed. For photo-ablation experiments, Mann-Whitney tests were performed. For optical tweezer experiments, T tests were performed. Statistical significance was defined as *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$. All calculations were performed and plots generated using Microsoft Excel or GraphPad Prism software.

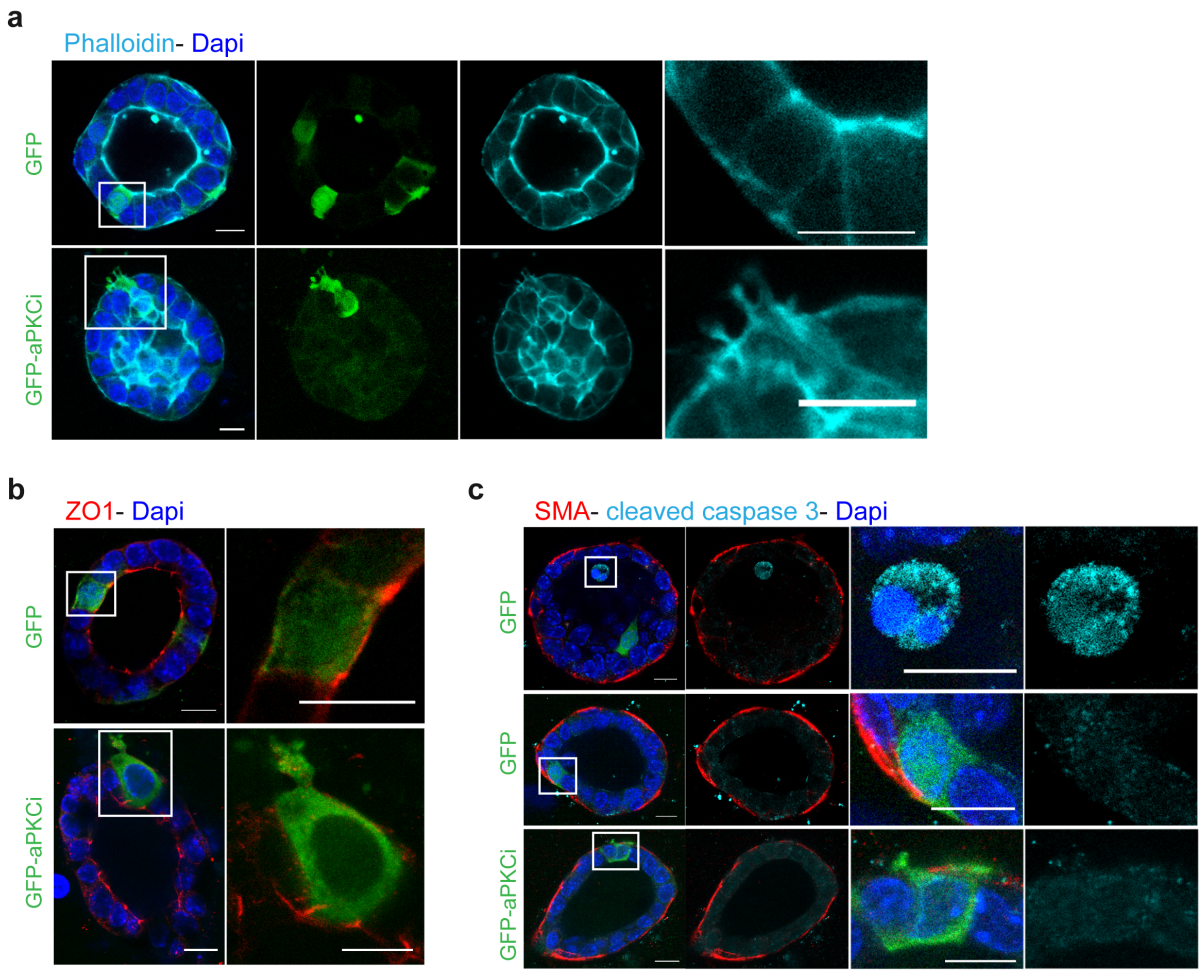


Fig. S1. Characterization of GFP-aPKCi^{+}-, mCherry-aPKCi^{+}-, and MT1-MMP-mCherry-infected mouse mammary organoids. (a-c) Representative confocal immunofluorescence images of mouse mammary organoids stained for actin (a, actin in cyan) and ZO-1 (b, ZO-1 in red). **(c)** Myoepithelial cells and apoptotic cells were stained with an antibody against SMA (red) and cleaved caspase 3 (cyan), respectively. Nuclei were stained with DAPI (blue). Scale bars, 10 µm for all images.

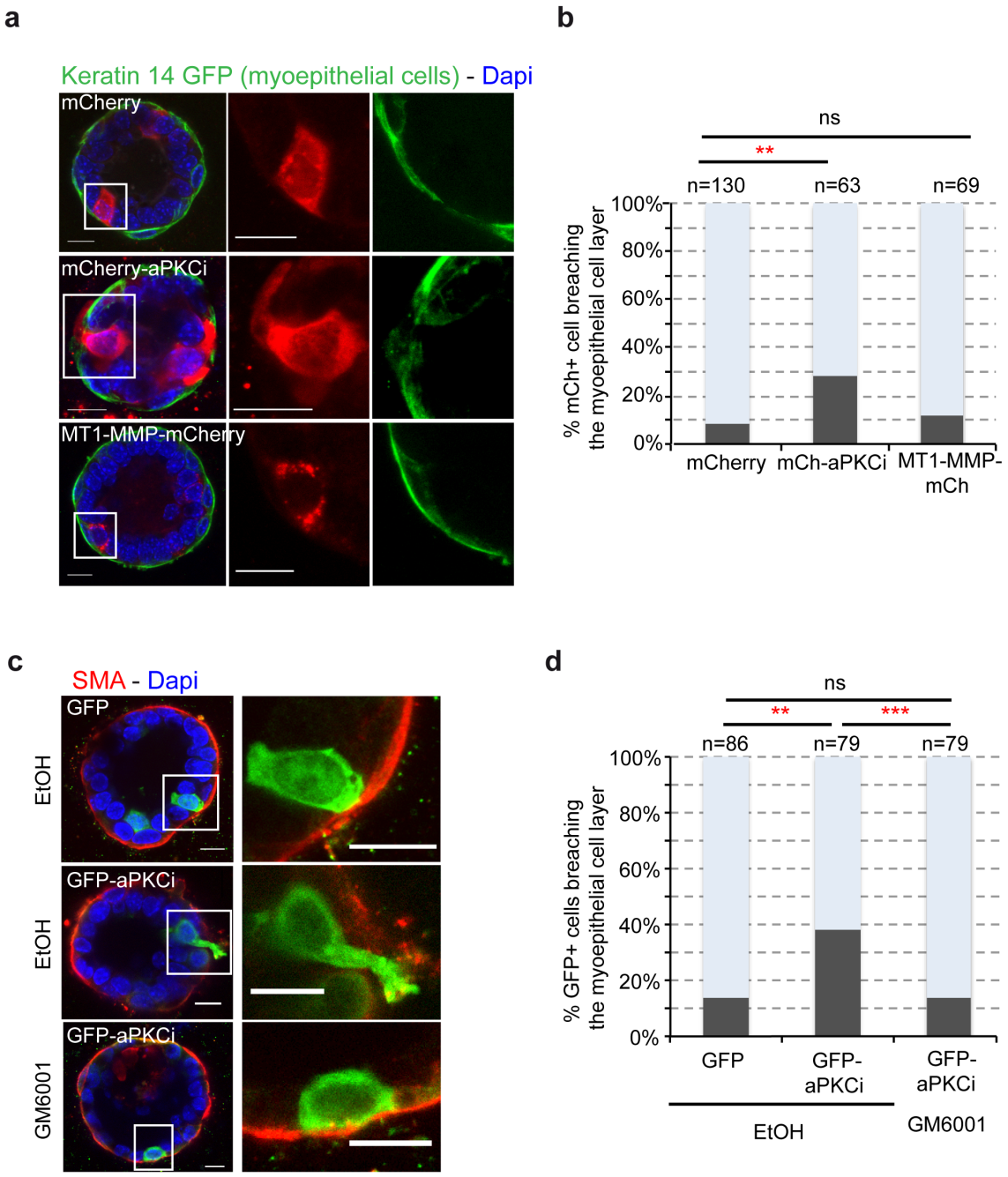


Fig. S2. MMPs are required to form basal protrusions dependent on aPKCi but MT1-MMP overexpression is not sufficient. (a) Representative confocal immunofluorescence images of mouse mammary organoids expressing transgenic cytokeratin-14 (K14) promoter-driven GFP (K14: Actin-GFP) to facilitate the visualization of myoepithelial cells. **(b)** Quantification of the number of mCherry^{+}, mCherry-aPKCi^{+}, or MT1-MMP-mCherry^{+} epithelial cells breaching the myoepithelial cell layer (*$p < 0.05$, **$p < 0.01$, ns, not significant). **(c)** Effect of a Pan MMP inhibitor, GM6001 (40 µM), on the breaching of the myoepithelial cell layer, labelled with an antibody against SMA (red). **(d)** Quantification of the total number of GFP^{+} cells breaching the myoepithelial cell layer (**$p < 0.01$, ***$p < 0.001$). Four independent experiments were performed. Nuclei were stained with DAPI (blue). Quantifications of all GFP^{+} cells were pooled from the independent experiments and Chi^{2} tests performed for all data. Scale bars, 10 µm. n = the number of cells analyzed.

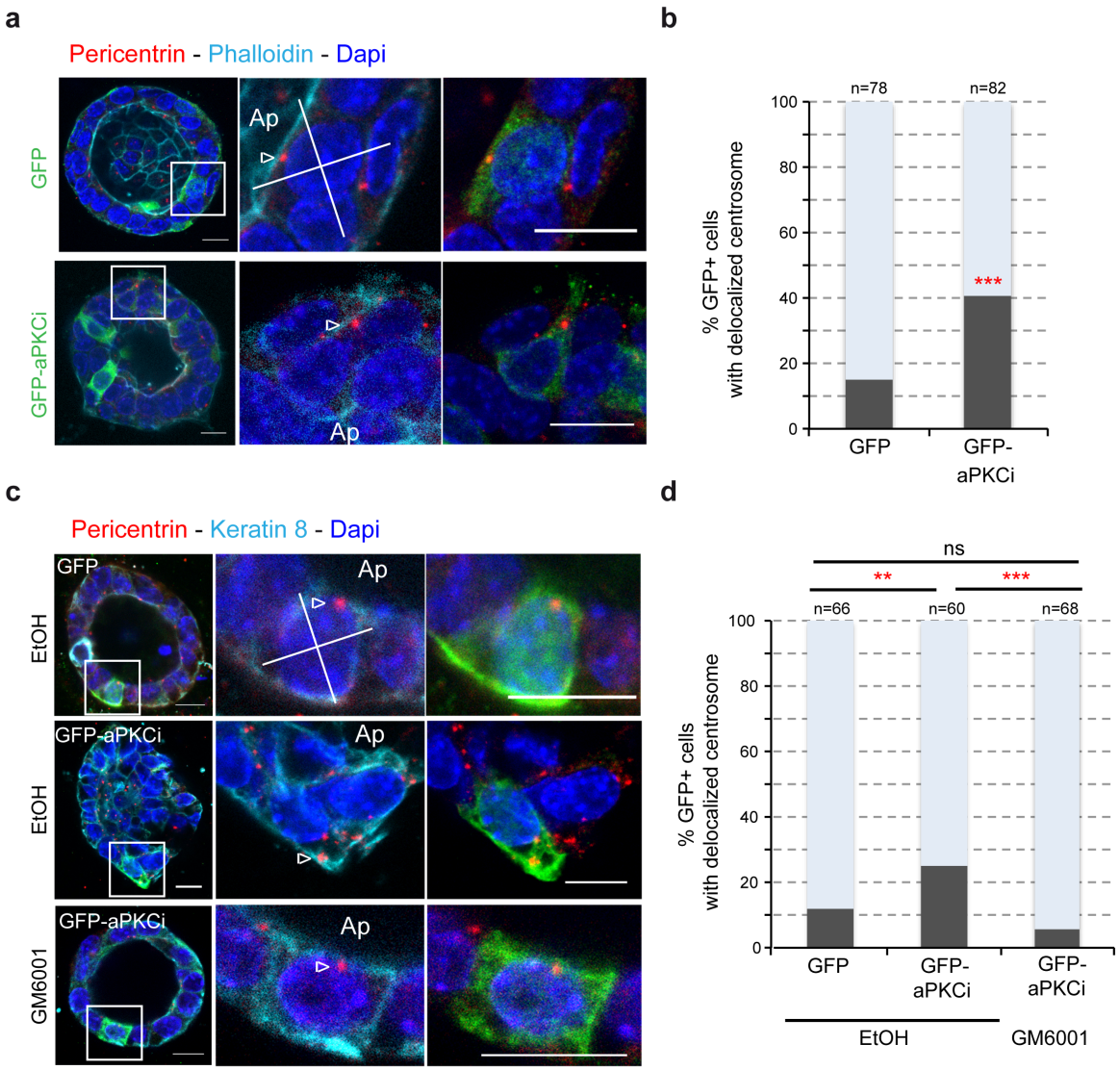


Fig. S3. aPKCi overexpression triggers the loss of apical polarity of the centrosome dependent on protrusion formation. (a) Representative confocal immunofluorescence images of mouse mammary organoids in which few cells overexpress GFP or GFP-aPKCi. Centrosomes were detected with an antibody against pericentrin (red). Actin was stained with phalloidin (cyan). **(b)** Quantification of centrosome localization in GFP^+ epithelial cells. Each GFP^+ cell is separated into quarters. Only centrosomes localized in the apical pole (Ap) are considered to be correctly localized. The quantifications of all the GFP^+ cells were pooled from the independent experiments and a Fisher test was performed (***$p < 0.001$). Five independent experiments were performed. **(c)** Effect of GM6001 (40 µM) on centrosome localization on mouse mammary organoids. Centrosomes are represented in red with an antibody against pericentrin (red). Luminal cells were stained with an anti-cytokeratin-8 antibody (cyan). **(d)** Quantification of centrosome localization in GFP^+ cells as in (b). The quantifications of all GFP^+ cells were pooled from the independent experiments and a Chi^2 test performed (**$p < 0.01$, ***$p < 0.001$). Nuclei are stained with DAPI (blue). Scale bars, 10 µm. n = the number of cells analyzed.

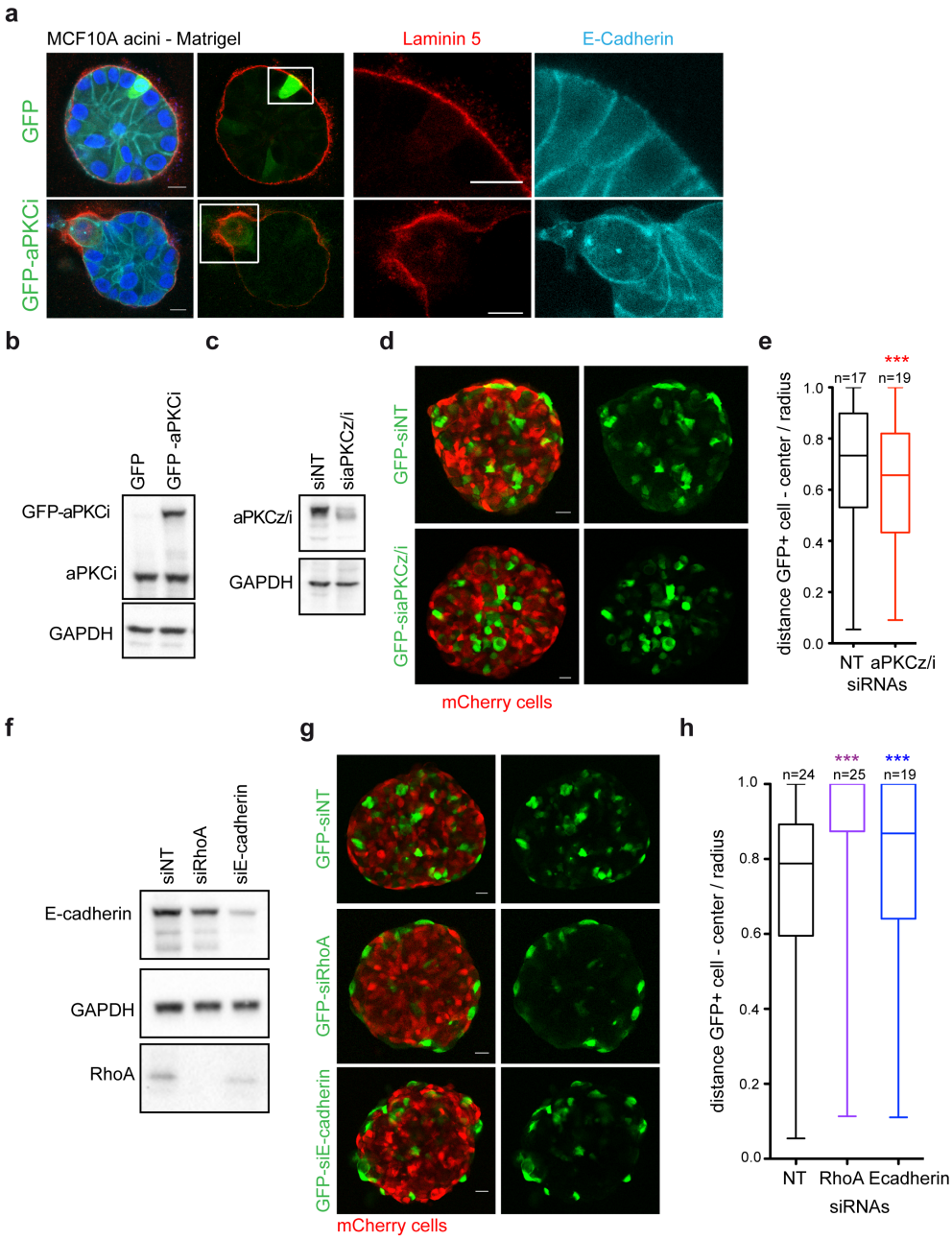
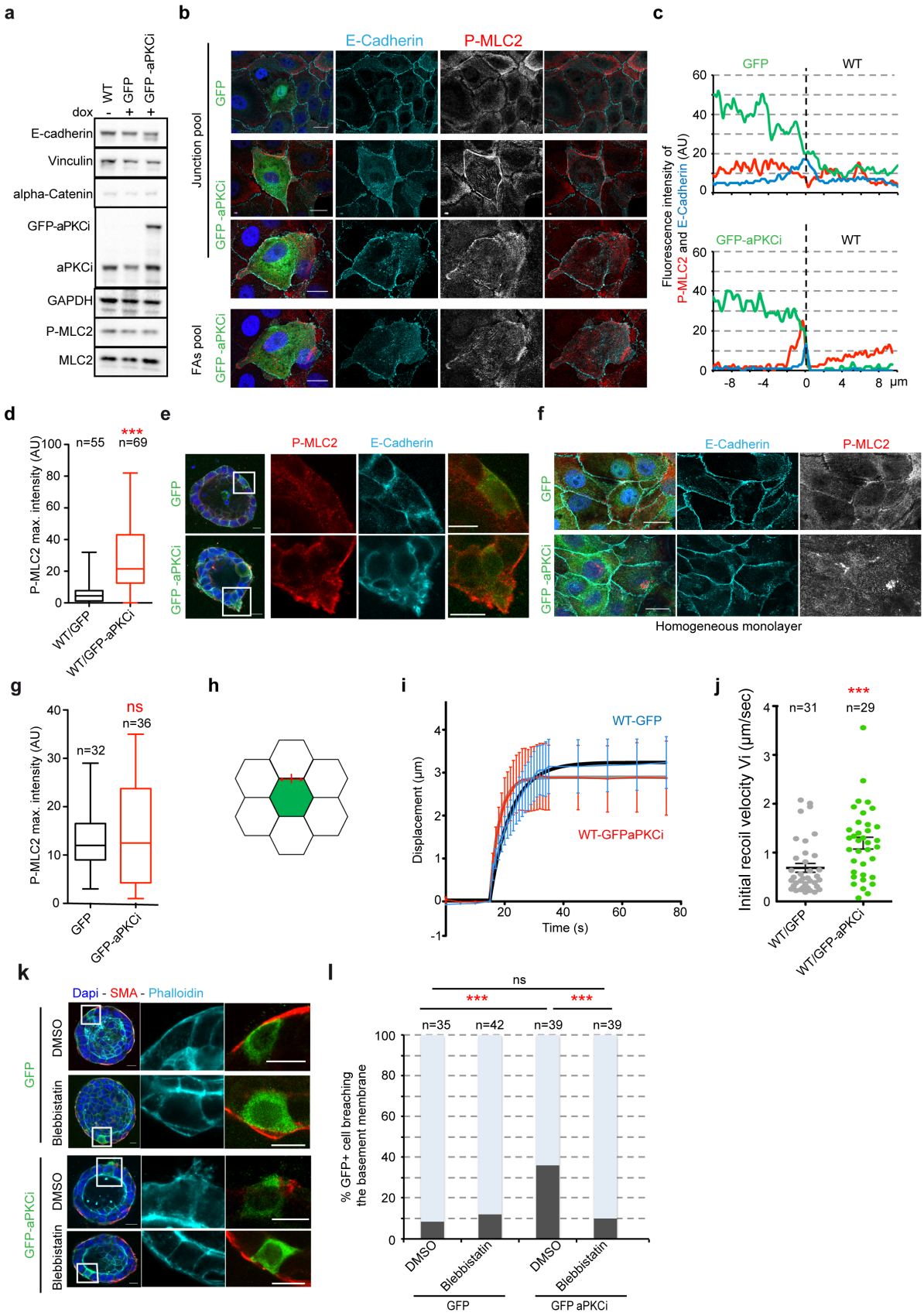


Fig. S4. MCF-10A cell segregation is dependent on contractility, cell-cell adhesion, and the level of aPKC. (a) Confocal images of MCF-10A acini in matrigel infected for GFP (top) or GFP-aPKCi (bottom). Actin was detected with phalloidin (cyan) and the basement membrane was labelled with an anti-laminin 5 antibody (red). Scale bars, 10 µm. **(b)** Level of endogenous aPKCi in MCF-10A GFP cells and GFP-aPKCi in MCF-10A GFP-aPKCi cells. **(c)** Levels of aPKCz/i in MCF-10A GFP cells treated with siNT (Non-Targeting) or siaPKCz/i. **(d)** Representative confocal immunofluorescence images of MCF-10A spheroids. MCF-10A mCherry cells were mixed with MCF-10A GFP cells depleted of aPKCz/i or control MCF-10A GFP cells (siNT). **(e)** Quantification of GFP^+ cell segregation in three independent experiments. n = the number of spheroids analyzed. A Mann Whitney test was performed (***$p < 0.001$). **(f)** Levels of E-cadherin and RhoA in MCF-10A GFP cells treated with an siNT, siRhoA, or siEcadherin. **(g)** Representative confocal immunofluorescence images of MCF-10A spheroids. MCF-10A mCherry cells were mixed with MCF-10A GFP cells depleted of RhoA or E-cadherin or control MCF-10A GFP cells (siNT). **(h)** Quantification of GFP^+ cell segregation from three independent experiments. n = the number of spheroids analyzed. A Kruskal-Wallis test was performed (***$p < 0.001$). Scale bars, 20 µm.


a
WT
GFP
GFP -aPKCi
dox
-
+
+
E-cadherin
Vinculin
alpha-Catenin
GFP-aPKCi
aPKCi
GAPDH
P-MLC2
MLC2
b
E-Cadherin
P-MLC2
Junction pool
FAs pool
GFP
GFP -aPKCi
c
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WT
GFP-aPKCi
WT
Fluorescence intensity of
P-MLC2 and E-Cadherin (AU)
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40
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WT/GFP
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n=31
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Fig. S5. A specific increase of myosin activity at the WT/aPKCi interface is required for basal protrusion formation. (a) Levels of E-cadherin, vinculin, alpha-catenin, aPKCi, P-MLC2, and total MLC2 in GFP^+ and GFP-$aPKCi^+$ cells. **(b)** Images of a monolayer of MCF-10A WT cells mixed with MCF-10A GFP or GFP-aPKCi cells seeded on glass, stained for nuclei (DAPI in blue), E-cadherin (cyan), and P-MLC2 (red or gray). The z-plane of focal adhesions (FAs) is 0.9 µm from the z-plane of the junction (E-cadherin) plane. Scale bar, 20 µm. **(c)** Representative line-scans of P-MLC2, E-cadherin, and GFP fluorescence intensity, in arbitrary units (AU), at the interface between a WT cell and a GFP^+ cell (top) or between an MCF-10A WT cell and a GFP-$aPKCi^+$ cell (bottom). **(d)** Quantification of the maximum intensity of P-MLC2 staining at the interface between MCF-10A WT/GFP^+ or MCF-10A WT/GFP-$aPKCi^+$ cells from three experiments. A Mann Whitney test was performed (***$p< 0.001$). n, number of boundaries. **(e)** Confocal images of mouse mammary organoids stained for E-cadherin (cyan) and P-MLC2 (red). A zoom of the boxed area is shown in the three images on the right. Scale bars, 10 µm. **(f)** Representative images of a homogeneous monolayer of MCF-10A GFP or MCF-10A GFP-aPKCi cells on glass coverslips. Cells were stained for E-cadherin (cyan) and P-MLC2 (red). Nuclei were stained with DAPI (blue). Scale bars, 20 µm. **(g)** Quantification of the maximum intensity of P-MLC2 staining at the interface of GFP^+/GFP^+ or GFP-$aPKCi^+$/GFP-$aPKCi^+$ cells from two experiments. A Mann Whitney test was performed (ns, non-significant). n = the number of boundaries. **(h)** Scheme of the laser ablation experiment at the junction between a WT cell and a GFP^+ cell. **(i)** Vertex displacement curve after ablation of the junction between WT and GFP^+ cells (blue curve) or between WT and GFP-$aPKCi^+$ MCF-10A cells (red curve). Ablation occurs at t = 15 s. **(j)** Quantification of initial recoil velocity of vertices of ablated cell junctions in MCF-10A cells (mean ± SEM). Laser ablation was performed either between an MCF-10A WT cell and a GFP^+ cell or between an MCF-10A WT cell and a GFP-$aPKCi^+$ cell. A Mann Whitney test was performed (**$p < 0.01$). Three independent experiments were performed. n = the number of cell junctions. **(k)** Effect of inhibiting cell contractility with Blebbistatin (10 µM) on the breaching of the myoepithelial cell layer (SMA, red) by GFP-$aPKCi^+$ cells. Actin was stained with phalloidin (cyan) and nuclei were stained with DAPI (blue). Scale bars, 10 µm. **(l)** Quantification of the number of epithelial GFP^+ cells breaching the myoepithelial cell layer. A Chi^2 test was performed (***$p < 0.001$, ns, non-significant). Three independent experiments were performed. n = the number of cells analyzed.

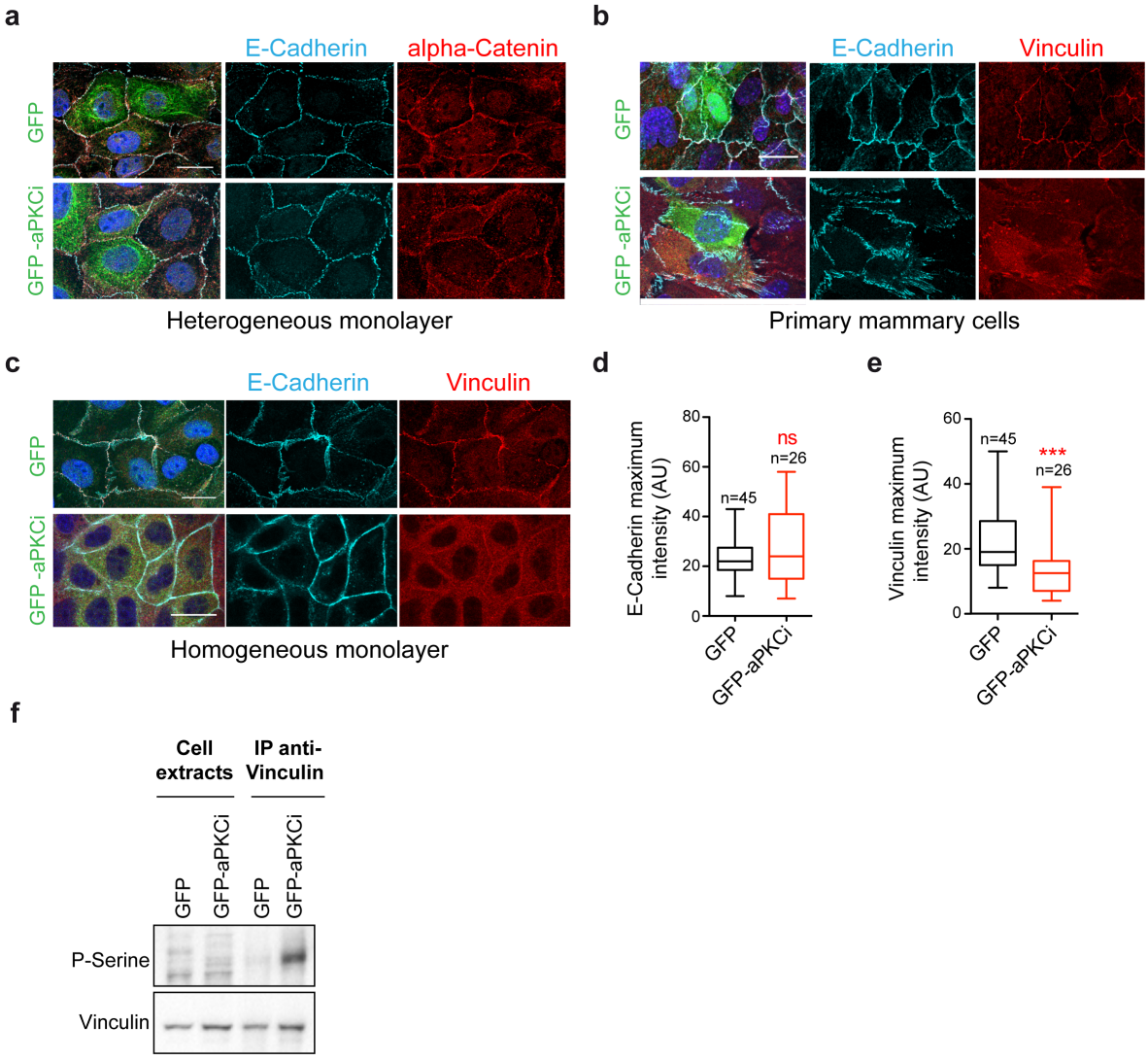

Fig. S6. Expression and localization of junctional proteins in GFP-aPKCi$^+$ cells. (a) Representative images of a monolayer of MCF-10A WT cells mixed with MCF-10A GFP or GFP-aPKCi cells seeded on glass coverslips. Cells were stained with DAPI (blue) and anti-E-cadherin (cyan) and anti-alpha-catenin (red) antibodies. **(b)** Representative images of a single Z-stack in a monolayer of primary mouse mammary cells on glass coverslips. Cells were stained with DAPI (blue) and anti-E-cadherin (cyan) and anti-vinculin (red) antibodies. Scale bars, 20 µm. (c) Representative images of a homogeneous monolayer of MCF-10A GFP or MCF-10A GFP-aPKCi cells on glass coverslips. Cells were stained with DAPI (blue) and anti-E-cadherin (cyan) and anti-vinculin (red) antibodies. **(d-e)** Quantification of the maximum intensity of E-cadherin **(d)** and vinculin **(e)** staining at the interface of MCF-10A GFP$^+$/GFP$^+$ or GFP-aPKCi$^+$/GFP-aPKCi$^+$ cells from three experiments. A Mann Whitney test was performed (***p < 0.001, ns, non-significant). n = the number of boundaries. **(f)** Immunodetection of the phosphorylation on serine specific for PKC substrates in vinculin immunoprecipitates in MCF-10A cells overexpressing doxycycline inducible GFP or GFP-aPKCi. Bound proteins were analyzed by immunoblotting with antibodies against vinculin and the phospho-serine PKC substrate. Input lysates (1%) were loaded as a control.

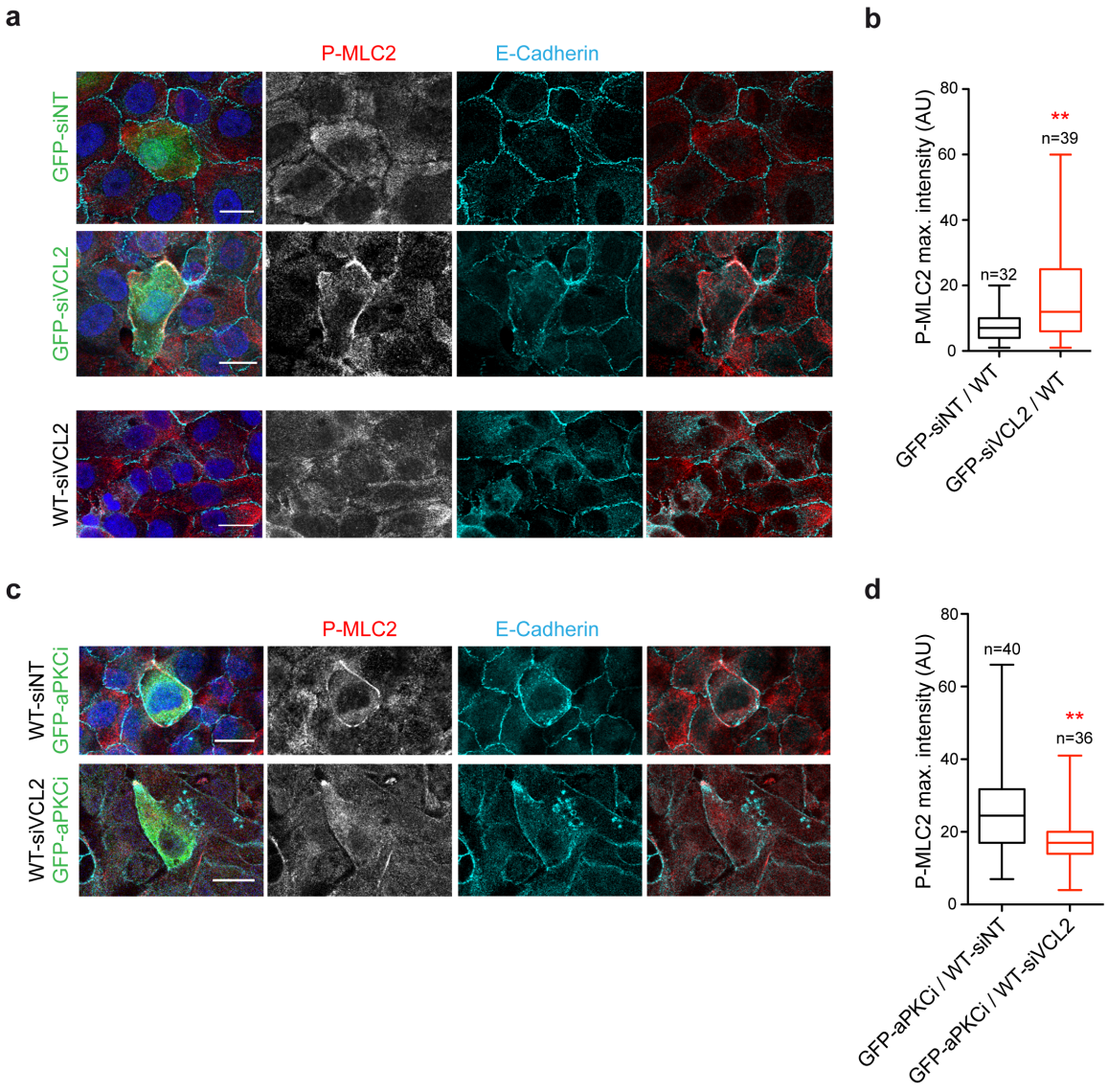

Fig.S7. Increase of P-MLC2 at the junction between a vinculin depleted cell and a normal cell. **(a)** Representative images of a monolayer of MCF-10A WT cells mixed with MCF-10A GFP cells transfected with siNon-Targeting (siNT, upper panels line), siVinculin (siVCL, middle panels line) or homogeneous monolayer of MCF-10A WT cells transfected with siVinculin (siVCL, lower panels line) seeded on glass coverslips. Cells were stained with DAPI (blue) and anti-E-cadherin (cyan) and anti-P-MLC2 (red) antibodies. Scale bars, 20 µm. **(b)** Quantification of the maximum intensity of P-MLC2 staining at the interface of MCF-10A GFP-siNT/MCF-10A WT or GFP-siVCL/MCF-10A WT cells from two experiments. A Mann Whitney test was performed (**$p < 0.01$). n = the number of boundaries. **(c)** Representative images of a monolayer of MCF-10A GFP-aPKCi cells mixed with MCF-10A WT cells transfected with siNT or siVCL, seeded on glass coverslips. Cells were stained with DAPI (blue) and anti-E-cadherin (cyan) and anti-P-MLC2 (red) antibodies. Scale bars, 20 µm. Quantification of the maximum intensity of P-MLC2 staining at the interface of MCF-10A GFP-aPKCi/MCF-10A siNT or GFP-aPKCi/MCF-10A siVCL from two experiments. A Mann Whitney test was performed (**$p < 0.01$). n = the number of boundaries.

Table S1. Commercial antibodies and dye used for this study

Primary Antibodies	**Company**	**Reference**	**Assay**	**Dilution**
Alpha-catenin	BD Biosciences	610193	IF-WB	1/100-1/1000
aPKCi	BD Biosciences	610175	WB	1/1000
aPKci/z	Santa Cruz	SC-216	WB	1/1000
Cleaved caspase 3	Cell signalling	9664	IF	1/400
E-cadherin (ECCD-2)	Life technologies	13-1900	IF	1/100
E-cadherin	BD Biosciences	610181	WB	1/1000
GAPDH	Santa Cruz	SC-25778	WB	1/10000
GFP	Abcam	Ab13970	IF	1/400
GFP	BioLegend	902601	WB	1/5000
Keratin 5	Ozyme	905501	IF	1/200
Keratin 8	DSHB- Iowa	TROMA-I	IF	1/200
Keratin 14	Biolegend	905301	IF	1/1000
MHCII	BioLegend	909801	WB	1/1000
MLC	Cell signalling	3672	WB	1/1000
P-MLCII (Ser-19)	Cell signalling	3671S	IF-WB	1/50-1/1000
Phospho (-Ser) PKC substrate	Cell signalling	2261S	WB	1/1000
Pericentrin	Abcam	Ab4448	IF	1/50
RFP	Rockland	600-401-379	IF	1/300
RhoA	Cell signalling	21175	WB	1/1000
Smooth Muscle actin	Dako	M0851	IF	1/200
Tubulin	Sigma	T-9026	WB	1/5000
Vinculin	Millipore	MAB3574-C	IF	1/50
ZO-1	Millipore	MABT11	IF	1/100
Secondary Antibodies	**Company**	**Reference**	**Assay**	**Dilution**
IgG-chicken 488	Life technologies	A11039	IF	1/400
IgG-mouse-Alexa Fluor 647	Invitrogen	A31571	IF	1/200
IgG-mouse-Cy3	Jackson ImmunoResearch	715-165-151	IF	1/200
IgG-mouse-Hrp	Jackson ImmunoResearch	115-035-062	WB	1/10000
IgG-rabbit-Alexa647	Invitrogen	A31573	IF	1/200
IgG-rabbit-Cy3	Jackson ImmunoResearch	711-165-152	IF	1/200
IgG-rabbit-Hrp	Jackson ImmunoResearch	111-035-045	WB	1/10000
IgG-rat Alexa Fluor 647	Molecular Probes	A21247	IF	1/200
Reagents	**Company**	**Reference**	**Assay**	**Dilution**
Alexa Fluor 633 phalloidin	Molecular Probes	A22284	IF	1/200
CellMask Deep red	Thermofisher	C10046	Live Imaging	1/1000
Sir Actin	Spirochrome.	SC001	Live Imaging	1/10000

Movie S1. Behavior of GFP-aPKCi-expressing cells in mouse mammary organoids in a 3D matrix. GFP-aPKCi$^+$ cells form highly dynamic cell protrusions towards the stroma. Actin is visualized with Sir-Actin (in red) and collagen with labelled cy3 collagen (in blue). Time-lapse images were captured every 90 min for 24 h.

Movie S2. Inducible MCF-10A GFP-aPKCi-expressing cells surrounded by MCF-10A WT cells seeded on a glass coverslip. The movie corresponds to the z-stack shown in Fig. 3a - GFP- aPKCi, showing the localization of P-MLC2 (in red) and E-cadherin (in cyan) from the apical to basal pole. P-MLC2 is enriched at the apical-lateral part at the interface between GFP-aPKCi$^+$ and WT cells and on the basal pole of GFP-aPKCi$^+$ cells on structures similar to focal adhesions. Z-stack images were captured at 0.3-µm intervals.

Movie S3. Laser ablation of a junction between an MCF-10A WT cell and a GFP$^+$ cell or between an MCF-10A WT cell and a GFP-aPKCi$^+$ cell. MCF-10A WT cell monolayers on glass mixed with cells expressing GFP (left panel) or GFP-aPKCi (right panel) analyzed by confocal spinning-disk microscopy before, during, and after photoablation. Cell membranes were stained with the CellMask Deep Red dye (in grey) and photo-ablations were performed along regions shown in magenta. Images were captured every 5 s before ablation and every second after ablation. Ablation occurred at t = 15 seconds. (time is in hr:min:s). Scale bars, 10 µm.

Movie S4. Inducible MCF-10A GFP-aPKCi-expressing cells surrounded by MCF-10A WT cells seeded on a glass coverslip. The movie corresponds to the z-stack shown in Fig. 4a - GFP- aPKCi, showing the localization of vinculin (red) and E-cadherin (cyan) from the apical to basal pole. Vinculin is enriched at the apical-lateral part of the interface between GFP-aPKCi$^+$ cells and WT cells and at the basal pole of GFP-aPKCi$^+$ cells on structures similar to focal adhesions. Z-stack images were captured at 0.3-µm intervals.

Movie S5. Vinculin-mCherry dynamics at focal adhesion sites in inducible MCF-10A GFP-expressing cells surrounded by MCF-10 WT cells seeded on a glass coverslip. Images were captured every minute for 90 min.

Movie S6. Vinculin-mCherry dynamics at focal adhesion sites in inducible MCF-10A GFP-aPKCi-expressing cells surrounded by MCF-10 WT cells seeded on a glass coverslip. Images were captured every minute for 90 min.

1. Nguyen-Ngoc KV, *et al.* (2015) 3D culture assays of murine mammary branching morphogenesis and epithelial invasion. *Methods Mol Biol* 1189:135-162.