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Supplementary Information for

# Cell phenotypic plasticity requires autophagic flux driven by YAP/TAZ-mechanotransduction

Antonio Totaro, Qiuyu Zhuang, Tito Panciera, Giusy Battilana, Luca Azzolin, Giulia Brumana, Alessandro Gandin, Giovanna Brusatin, Michelangelo Cordenonsi and Stefano Piccolo

Stefano Piccolo
Email: piccolo@bio.unipd.it

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Supplementary Material and Methods
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SI References



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## Supplementary Figures and their Legends

### Reagents, plasmids and transfections

Doxycycline hyclate, 3-methyladenine, chloroquine, collagen I, heparin, insulin, dexamethasone, SBTI (Soybean Trypsin Inhibitor), gastrin, N-acethylcysteine, nicotinamide, corn oil and tamoxifen were from Sigma. Human EGF, murine EGF, murine bFGF, human FGF10, human Noggin, were from Peprotech. HBSS (#14025 - calcium, magnesium, no phenol red), N2, B27, BPE and ITS-X (Insulin-Transferrin-Selenium-Ethanolamine) supplements were from Thermo Fischer. R-Spondin1 was from Sino Biological. Matrigel was from BD Biosciences (Corning). Rat tail collagen type I was from Cultrex. Fibronectin and lantrunculin A was from Santa Cruz Biotechnology.

pBABE-puro-GFP-LC3 was from Addgene (# 22405). pBABE-blasti-GFP-LC3 were obtained by subcloning GFP-LC3 sequence from pBABE-puro-GFP-LC3 into pBABE-blasti-MCS backbone. The pBABE-puro-mCherry-GFP-LC3 was obtained by subcloning the mCherry-GFP-LC3 construct from pcDNA3.1 mCherry-EGFP-LC3B (Ivan Dikic) into the corresponding backbone. For inducible expression of YAP cDNA constructs in MII-GFP-LC3 cells, siRNA-insensitive Flag-hYAP WT, S94A (TEAD-binding mutant (1)), 5SA (LATS-mutant sites (2)) and 5SA/S94A (1) were subcloned into pCW57.1 doxycycline-inducible lentiviral vectors (Addgene # 41393). pCW57.1-MCS (empty vector) was used as control. For inducible expression of Armus construct, the corresponding mouse cDNA was amplified by PCR with the oligos Mm Armus ATG HA For and Mm Armus STOP Rev and cloned into FUW-tetO-MCS (3) with the In-Fusion HD Cloning Kit (Takara Bio USA, # 121416). The pBABE-hygro (Empty) and pBABE-hygro-FLAG-mTAZ S89A constructs used for stable expression in MII cells, the FUdeltaGW-rtTA, FUW-tetO-MCS (Empty) and FUW-tetO-wtYAP for reprogramming of LD cells and the doxycycline-inducible mTAZ S89A constructs were previously described (4, 5). All constructs were confirmed by sequencing. The sequences of oligos used for cloning are provided in Table S1. GFP- and Cre-expressing adenoviruses were from University of Iowa, Gene Transfer Vector Core. The rtTA-expressing adenoviruses (Ad-TetOn) was from Vector Biolabs (#1720).

siRNA transfections were performed with Lipofectamine RNAi-MAX (Thermo Fischer) in antibiotics-free medium according to manufacturer instructions. The list and the sequences of siRNA oligonucleotides used in this study are provided in Table S2. siYAP/TAZ refers to siYAP/TAZ #1 and siArmus refers to siArmus #1 if without specific mentioning. DNA transfections were performed with TransitLT1 (Mirus Bio) according to manufacturer instructions.

**Microfabrications and experimental settings**

Fibronectin coated hydrogels of 2kPa (soft) and 40kPa (stiff) elastic modulus were synthesized as previously described (3, 6). For experiments, 5,000 cells/cm$^2$ were seeded in a drop of medium on top of hydrogel and let adhere for four hours; after attachment, the wells containing the hydrogels were filled with medium. All cells were plated at low confluency.

**3D Cell Culture**

Cells were embedded in a mixture of Rat Collagen-I and Growth Factor Reduced Matrigel. Collagen-I solution was neutralized on ice with 0.1M NaOH in PBS and adjusted with 0.1M HCl to bring the pH of the solution to 7.5. The Collagen-I solution was then mixed on ice with Matrigel to obtain 2X concentrated matrices of 0.5 mg/ml (for soft matrices) or 2 mg/ml (stiff matrices). Cells were trypsinized, counted, resuspended in growth medium; then, 1 volume of cells was mixed with 1 volume of the ECM mix to obtain 500 cells in 100 ml of 0.25 mg/ml and 1 mg/ml 3D matrices, respectively. 8-well chamber slide (Thermo Fisher) were precoated with 100 µl of cell-free 50% medium / 50% ECM, and left in the incubator until gelled; then, cells were seeded in drop on top of the pregelled ECM. After gelling, wells were supplemented with normal growth medium, which was changed every 2-3 days during the experiments.

**Virus preparation**

For lentivirus preparation, lentiviral particles were prepared by transiently transfecting HEK293T with lentiviral vectors together with packaging vectors pMD2-VSVG and pPAX2. Briefly, 60 µl of TransIT-LT1 reagent was diluted in 1.5 ml of Opti-MEM (Life

Technologies) for each 10 cm dish, incubated with DNA for 15 min at room temperature and gently distributed over to the cell medium. After 8 hours, cell medium was changed. 48 hours post-transfection supernatant was collected, filtered through 0.45 µm filter and directly stored at -20 °C. Retroviral particles were prepared by transiently transfecting HEK293GP cells with retroviral vectors together with an envelope-producing vector (pMD2-Env) using TransIT-LT1. Transfection and harvest procedure are the same as lentivirus preparation. Infections were carried out as previously described (4).

**Quantitative real-time PCR (qRT-PCR)**

Total RNA was extracted using NucleoSpin RNA kit (Macherey-Nagel, 740465.4) with automated Freedom EVO workstastion (TECAN). cDNA synthesis was carried out with dT-primed or random hexamer-primed MuMLV Reverse Trascriptase (Thermo Fisher). qRT-PCR analyses were carried out with triplicate samplings on reverse-transcribed cDNAs with QuantStudio5 (Applied Biosystems, ThermoFisher Scientific) and analysed with QuantStudio Design & Analysis software (version 1.4.3). Expression levels are normalized to RPLP0, unless otherwise indicated. PCR oligonucleotide sequences are listed in Table S3.

**ChIP-qPCR**

Chromatin immunoprecipitation was performed as previously described (7). Briefly, cells were crosslinked with 1% formaldehyde in culture medium for 10 min at RT, and chromatin from lysed nuclei was sheared to 200-600 bp fragments using a Branson Sonifier 450A. For ChIP-qPCR, around 100µg of chromatin was incubated with 3-5µg of antibody overnight at 4°C. Antibody/antigen complexes were recovered with ProteinA-Dynabeads (Thermo Fisher) for 2 hours at 4°C. qRT-PCR was carried out as described before. ChIP-qPCR oligo sequences are listed in Table S4.

**Western blot**

Cells were washed in HBSS and harvested with lysis buffer (50 mM Hepes pH 7.8, 200 mM NaCl, 5mM EDTA, 1% NP40, 5% glycerol). In order to obtain protein lysates, extracts were exposed to ultrasound in a sonicator (Diagenode Bioruptor). Cellular

extracts were centrifuged for 10 min at 4°C to remove the insoluble fraction and total protein content was determined by Bradford quantification. Samples were boiled at 95 °C for 3 min in 1X Sample Buffer (50 mM Tris-HCl pH 6.8, 2% SDS, 0.1% Bromophenol Blue, 10% glycerol, 2% 2-mercaptoethanol). Proteins were run in 4-12% Nupage MOPS acrylamide gels (for LC3 used 12% acrylamide gels) and transferred onto PVDF membranes by wet electrophoretic transfer. Blots were blocked with non-fat dry milk and incubated overnight at 4°C with primary antibodies. Secondary antibodies were incubated for 1 hour at RT, and then blots were developed with chemiluminescent reagents. Images were acquired with Image Quant LAS 4000 (GE healthcare). The list of antibodies used in this study and the corresponding dilutions are provided in the Table S5.

**Immunofluorescence**

Cells were fixed 10 min at room temperature (RT) with 4% PFA solution and permeabilized for 10 min at RT with PBS 0.3% Triton X-100 (PBS-T 0,3%). Samples were processed for immunofluorescence according to the following conditions: blocking in 10% Goat Serum (GS) in PBS-T 0,1% for 1 hour followed by incubation with primary antibody diluted in 2% GS in PBST overnight at 4°C, four washes in PBS-T 0,1% and incubation with secondary antibodies diluted in 2% GS in PBS-T 0,1% for 1.5 hours at RT. After three washes in PBS, samples were counterstained with ProLong-DAPI (Thermo Fisher) to label cell nuclei. For LAMP1 staining, permeabilization was performed in ice cold methanol: acetone (80:20) solution for 10 min at -20° C; samples were processed by blocking in 5% BSA and diluting primary and secondary antibodies in 2% BSA. Confocal images were obtained with a Leica TCS SP5 equipped with a CCD camera. Bright field images were obtained with a Leica DM IRB inverted microscope equipped with a CCD camera (Leica DFC 450C). The list of primary and secondary antibodies used in this study and their corresponding dilutions are provided in the Table S5.

**Growth assays**

For soft-agar assay, 15,000 cells were suspended in complete growth medium with 0.3% agar and layered on top of 0.6% agar beds. After two weeks, colonies were fixed with 4%

PFA. For mammosphere assay, single cells were plated at 500 cells/cm$^2$ on ultra-low attachment plates (Costar) in mammosphere medium as previously described (7) and scored 5 days plating.

**Mice**

Animal experiments were performed adhering to our institutional guidelines as approved by OPBA (University of Padua) and the Italian Ministry of Health. For mammary gland experiments, we used exclusively 8 week-old virgin CD1 mice, purchased from Charles River (n = 10 females for each experiment). Transgenic lines used in the experiments were gently provided by: Duojia Pan (*Yap1*$^{fl/fl}$) (8); Fernando Camargo (*tetO-YAP*$^{S127A}$) (9); Marco Sandri (*Atg7*$^{fl/fl}$) (10); Dieter Saur and Jens Siveke (*R26*$^{CAG\text{-}FSF\text{-}CreER}$) (11); Paolo Bonaldo (*CMV-Flp*). *Taz*$^{fl/fl}$ and double *Yap*$^{fl/fl}$*; Taz*$^{fl/fl}$ conditional knock-out mice were as described in Ref.(12). *R26-rtTAM2* mice (stock #006965) were purchased from The Jackson Laboratory. To obtain *R26-rtTAM2/+; tetO-YAP*$^{S127A}$ mice, we crossed *R26-rtTAM2/+* mice with *tetO-YAP*$^{S127A}$ mice. *Atg7*$^{fl/fl}$*; tetO-YAP*$^{S127A}$ mice were obtained by intercrossing crossed *Atg7*$^{fl/fl}$ and *tetO-YAP*$^{S127A}$ mice. To obtain *R26*$^{CAG\text{-}CreER}$*; Yap*$^{fl/fl}$*; Taz*$^{fl/fl}$ mice, first we obtain the *R26*$^{CAG\text{-}CreER}$ line by crossing *R26*$^{CAG\text{-}FSF\text{-}CreER}$ mice with *CMV-Flp* mice: the offspring was analyzed by PCR to ensure the complete germline recombination of the Frt-Stop-Frt cassette. *R26*$^{CAG\text{-}CreER}$ mice were then intercrossed with *Yap*$^{fl/fl}$*; Taz*$^{fl/fl}$ mice. For the induction of the recombination, females *R26*$^{CAG\text{-}CreER}$*; Yap*$^{fl/fl}$*; Taz*$^{fl/fl}$ and control female *Yap*$^{fl/fl}$*; Taz*$^{fl/fl}$ littermates mice received Tamoxifen according to the following protocol: 1) at the age of 10 weeks, mice received 1 oral gavage per day of Tamoxifen (0.11 mg per g of body weight; dissolved in corn oil) during 2 consecutive days; 2) after one week (mice age: 11 weeks), mice received the same regimen of Tamoxifen. Organs were harvested for western blot analysis after one week from the last Tamoxifen injection. Animals were genotyped with standard procedures and with the recommended set of primers.

**Pancreatic acinar cell isolation and induction of yDucts**

Experimental procedure for isolation and reprogramming of pancreatic acini, the composition of the culture media and solutions required were as previously described (5,

13). In short, isolated acinar clusters from the pancreata of 6 week-old *Atg7$^{fl/fl}$; tetO-YAP$^{S127A}$* mice were transduced with the indicated adenoviral vectors and incubated at 37 °C in a cell culture incubator for 2 h. Acinar cells were, then, seeded in neutralized rat tail collagen type I/acinar culture medium (1:1), overlaid with the acinar culture medium once collagen gelled, and cultured as indicated for 4-5 days following ductal structures formation.

**Primary mammary epithelial cell isolation and induction of yMaSCs**

Experimental procedures for the purification of mammary epithelial cells by FACS and the induction of yMaSCs, together with the composition of the culture media and solutions required were as previously described (5, 13). Briefly, cells from dissected mammary glands were FACS-sorted into luminal LD cells, luminal progenitors (LP) cells, and MaSCs. Primary mouse mammary cells were gated according to forward and side scatter for live cells (P1); population P1 was then further gated according to its Lin profile: the subpopulation of Lineage-negative cells (P2) was selected, excluding Lineage-positive hematopoietic cells; population P2 was then separated into an EpCAM$^{high}$ (P3) and an EpCAM$^{low}$ (P6) subpopulations; P3 and P6 populations were then further gated according to their CD61/CD49f profile into three subpopulations: EpCAM$^{low}$CD49f$^{high}$CD61$^{-}$ Basal cells (Basal cells), EpCAM$^{high}$CD49f$^{low}$CD61$^{+}$ (LP cells) and EpCAM$^{high}$CD49f$^{low}$CD61$^{-}$ LD cells. For induction of yMaSCs, LD cells were transduced for 48 hours with FUW-tetO-YAP in combination with rtTA-encoding lentiviruses. LD cells transduced with FUW-tetO-MCS (Empty) were used as negative control. After infection, adherent cells cultured in mammary 2D culture medium and treated with 2μg/ml doxycycline for 7 days. Then cells were detached with trypsin and seeded in 24-well ultralow attachment plates at clonogenic density in three-dimensional 5% Matrigel mammary colony medium. The list of antibodies and their corresponding dilutions are provided in the Table S5.

**Fluorescence-Activated Cell Sorting**

Experimental procedures for the MII rtTA/TAZ-S89A cell sorting were as previously described (4). All the FACS-sorting procedure were performed on a BD FACS Aria cell sorter (BD Biosciences). The list of antibodies is provided in the Table S5.

**Imaging quantification**

All images were processed and analyzes using ImageJ software (NIH Image). To monitor cellular autophagic activity we referred to the guidelines indicated in ref. 17. For the quantification of the area of GFP-LC3 puncta per cells, confocal images were splitted into single-color channels to select the contribution of the green fluorescence. Then a single cell was defined as the region of interest (ROI) and GFP-LC3 puncta inside this ROI were refined by Threshold. Finally, the refined area by Threshold of this selected cell was measured as the area (measured as squared pixels) of GFP-LC3 puncta per cell and shown in arbitrary units (A.U.). If more than one cell per ROI was present, the area of GFP-LC3 puncta was normalized to the number of nuclei. The Threshold settings remained constant for the analysis of all the images from the same experiment. For the quantification in Fig. 3*A* and *B,* Fig. 3*E* and *F*, cells with more than 10 GFP-LC3 puncta were scored as positive and results were shown as percentage of GFP-puncta positive cells. For each experiment, at least 50 cells were scored for each condition. For the quantification of GFP colocalization with mCherry (Fig. 2*G*) or LAMP1 staining (Fig. 2*B* and *D*), each confocal image was splitted into single-color channels to select the contribution of the green and red fluorescence respectively. Background signal was excluded by setting a threshold in both colour channels, and the analysis of colocalization was manually performed. For each experiment, at least 10 cells were scored for each condition. For the quantification of the number of endogenous LC3 puncta (Fig. S2*D*) and WIPI2 puncta (Fig. S2*F*), confocal images were thresholded to remove background signal and manually scored. For each experiment, at least 30 cells were scored for each condition. Acinar reprogramming was quantified as percentage of pancreatic colonies converting to duct-like clusters; LD reprogramming was scored as number of yMaSC epithelial colonies over the number of single cells originally seeded in 5% Matrigel suspension culture.

**Statistics**

The number of biological and technical replicates are indicated in figure legends. For qRT–PCR, at least three independent experiments (each with at least two biological replicates and three technical replicas for each biological replicate) were performed, if not otherwise indicated. All immunofluorescence experiments were performed at least in biological triplicate. If not otherwise indicated, at least three independent replicates were performed for reprogramming experiments with consistent results. Western blot analysis were performed at least three times from independent experiments, with similar results. All the experiments were performed without methods of randomization; sample size was chosen based on previous studies to ensure adequate statistical power. Statistical significance was tested using two-tailed *t*-test for simple comparisons, whereas group-level *P* values were estimated with ANOVA analysis of variance. All statistics were conducted using GraphPad Prism 7.0. Differences at $P \leq 0.05$ were considered statistically significant.

**Data availability**

The data that support the findings of this study are available from the corresponding author on request.

## Supplementary Figures and their Legends

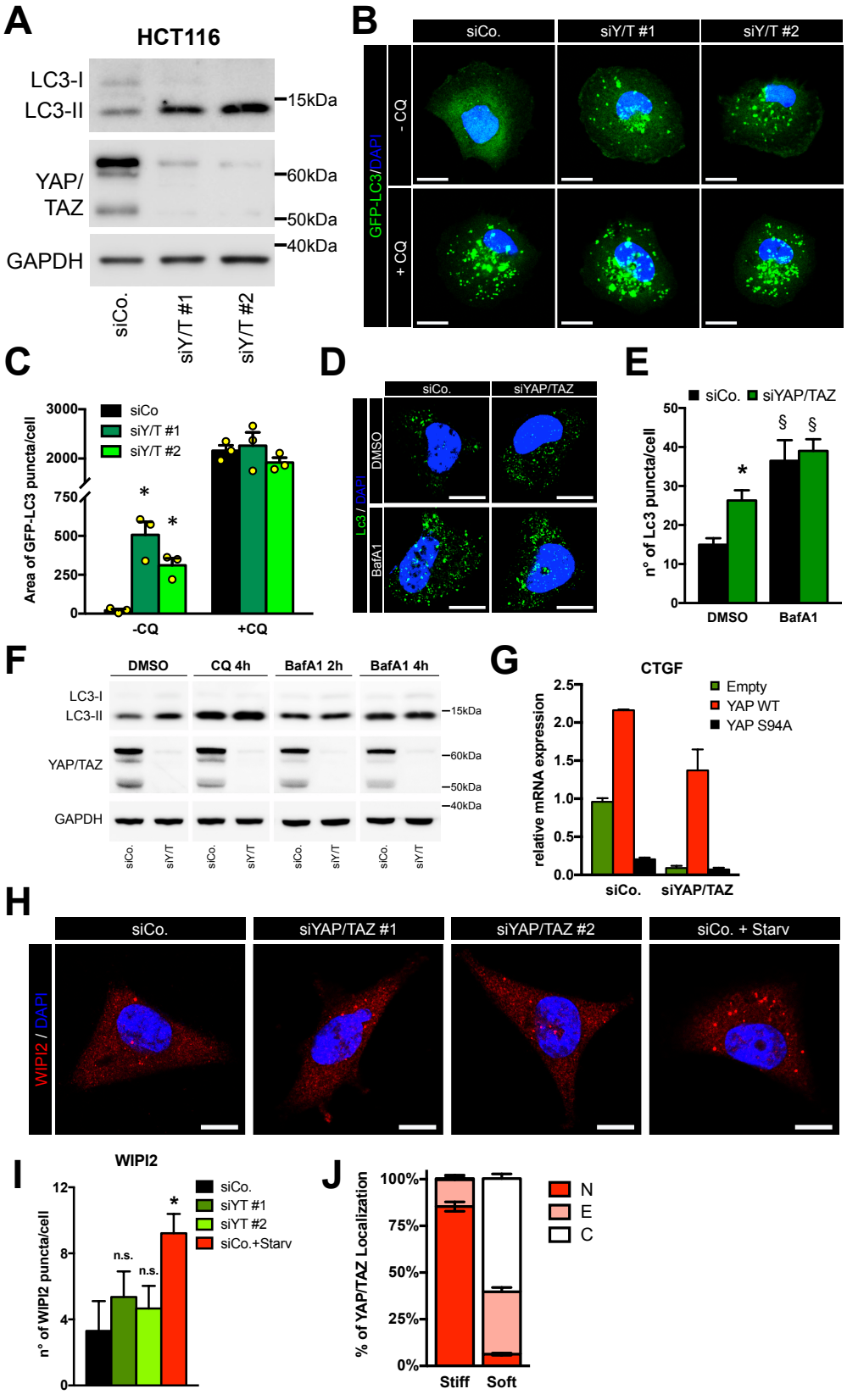

**Fig. S1 YAP/TAZ control autophagic flux by regulating autophagosome degradation.** (*A*) Immunoblot analysis for YAP/TAZ and LC3 in HCT116 cells transfected with control siRNA (siCo.) or two independent YAP/TAZ siRNA mixes (siY/T #1 or siY/T #2) for 48 hours. GAPDH serves as loading control. (*B-C*) MII cells stably expressing GFP-LC3 construct (MII-GFP-LC3) were transfected with control siRNA (siCo.) or two independent YAP/TAZ siRNA mixes (siY/T #1, siY/T #2) for 48 hours. Cells were treated with medium (-CQ) or 50 μM CQ (+CQ) for the last 4 hours. (*B*) Panels are representative confocal images. Scale bar: 20μm. (*C*) Quantification of GFP-LC3 puncta of MII-GFP-LC3 cells treated as in (*B*). Bars represent mean + SEM from three independent experiments (**P*<0.05 compared to -CQ siCo; one-way ANOVA). (*D-E*) MDA-MB-231 cells transfected with control (siCo.) or YAP/TAZ (siYAP/TAZ) siRNA mixes for 48 hours and treated with vehicle (DMSO) or 400 nM Bafilomycin A1 (BafA1) for the last 4 hours. Cells were stained for endogenous LC3B and scored for the number of LC3 puncta. (*D*) Panels are representative confocal images. Endogenous LC3B (green), DAPI nuclear counterstain (blue). Scale bar: 10μm. (*E*) Quantification of the number of endogenous LC3 puncta per cells in MDA-MB-231 cells treated as above. Bars represent mean + SEM from three independent experiments (**P*<0.05, §*P*<0.0001 compared to DMSO siCo.; one-way ANOVA). (*F*) Immunoblot analysis for LC3 and YAP/TAZ in MDA-MB-231 cells transfected with control (siCo.) or YAP/TAZ (siY/T) siRNA mixes for 48 hours. Cells were treated with vehicle (DMSO), 50 μM CQ (+CQ) or 400 nM BafA1 (BafA1) as indicated before lysis. GAPDH serves as loading control. (*G*) Validation of YAP/TAZ transcriptional activity for the experimental setting described in Fig. 1*I* and *J*. qRT-PCR analysis of mRNA levels for the YAP/TAZ-target gene CTGF, normalized to the Empty-infected cells transfected with control siRNA (siCo., green bar). Bars represent mean + SD (*N*=3 biological replicates) from one of three independent experiments of Fig. 1*I* and *J*, providing similar results. (*H-I*) MDA-MB-231 cells were transfected with control (siCo.) or two independent YAP/TAZ siRNA mixes (siYAP/TAZ #1 or siYAP/TAZ #2) for 48 hours. As positive control of autophagy induction control siRNA-transfected cells were subjected to nutrients starvation in HBSS (siCo.+Starv) for the last 4 hours before fixation. Cells were stained for WIPI2 and scored for the number of WIPI2 puncta. (*H*)

Panels are representative confocal images. WIPI2 (red), DAPI nuclear counterstain (blue). Scale bar: 10μm. (*I*) Quantification of the number of WIPI2 puncta per cells in MDA-MB-231 cells treated as above. Bars represent mean + SD from three independent experiments (*$P$<0.01, n.s.: not significant, compared to siCo.; one-way ANOVA). (*J*) Proportion of MII-GFP-LC3 displaying preferential nuclear YAP/TAZ localization (N, red), even distribution of YAP/TAZ in nucleus and cytoplasm (E, pink) or cytoplasmic YAP/TAZ (C, white) in Stiff vs. Soft plated cells, according to Fig. 3*A* and *B*. More than 150 cells from three independent experiments were scored for each condition. Bars represent mean + SD

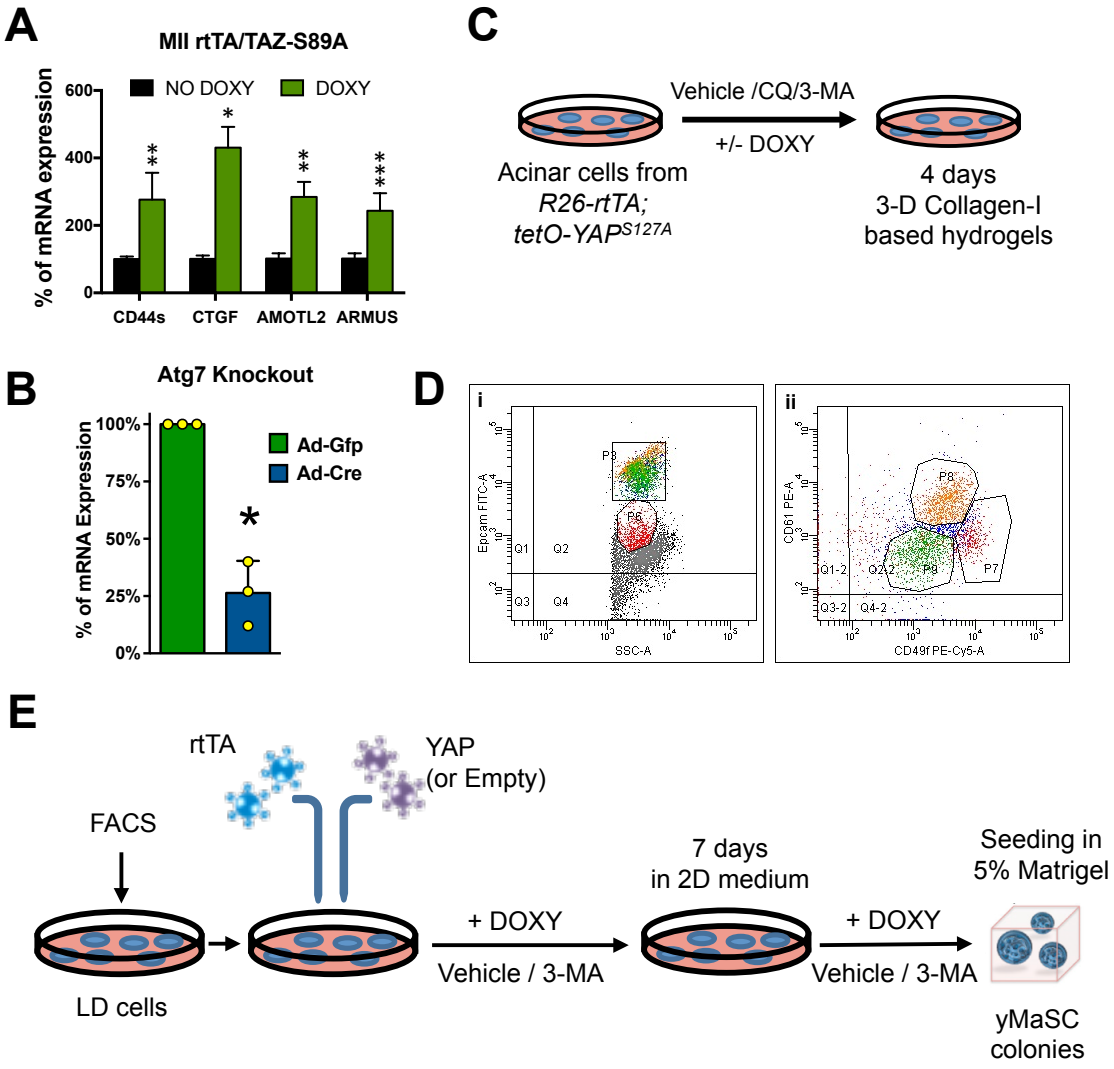


**Fig. S2 YAP/TAZ require efficient autophagy flux to sustain their biological responses.** (*A*) MII cells were transduced with viral vectors encoding for the reverse tetracycline-dependent transactivator (rtTA) and a doxycycline-inducible TAZ S89A construct (MII rtTA/TAZ-S89A), and FACS-sorted to isolate the $CD44^{low}/CD24^{low}$ subpopulation. MII rtTA/TAZ-S89A $CD44^{low}/CD24^{low}$ cells were cultured either in the absence (NO DOXY) or presence of doxycycline (DOXY), to turn on TAZ S89A expression and convert them into CSC-like cells, and analyzed by qRT–PCR. Doxycicline-dependent TAZ activation induced upregulation of the breast CSC-associated CD44 standard form (CD44s), of established YAP/TAZ targets (CTGF and AMOTL2) and of ARMUS. Values were normalized the corresponding NO DOXY

condition. Bars represent mean + SD from three independent experiments (**P*<0.0001, ***P*<0.001, ****P*<0.01 compared to the corresponding NO DOXY condition; one-way ANOVA). (*B*) Validation of Atg7 knockout in *Atg7fl/fl* acini transduced with either GFP- (Ad-Gfp) or Cre-encoding adenoviral vector (Ad-Cre), as in Fig. 4*G* and *H*. qRT-PCR analysis of mRNA levels for the *Atg7* gene relative to *18S* and normalized to the Ad-Gfp-infected control cells. Bars represent mean + SD (*N*=3 technical replicates) from one of three independent experiments, providing similar results. (**P*<0.0001, compared to Empty; two-tailed Student's *t*-test). (*C*) Schematic of the experimental setting for pancreatic reprogramming of acinar cells isolated from *R26-rtTA; tetO-YAP*$^{S127A}$ transgenic mice shown in Fig. 4*I* and *J*. (*D*) Representative FACS-plots illustrating the sorting procedure to purify LD cells. From dissociated cells gated according to forward and side scatter for live cells, the subpopulation of Lineage-negative cells (P2) was selected. i) P2 population was then separated into an EpCAM$^{high}$ (P3; yellow + green) and an EpCAM$^{low}$ (P6; red) subpopulations; ii) P3 and P6 were then further gated according to their CD61/CD49f profile into three subpopulations: EpCAM$^{low}$CD49f$^{high}$CD61$^{-}$ Basal cells (P7; red), EpCAM$^{high}$CD49f$^{low}$CD61$^{+}$ LP cells (P8; yellow) and EpCAM$^{high}$CD49fl$^{ow}$CD61$^{-}$ LD cells (P9, green). The latter LD population was used for the reprogramming experiments described in this study. (*E*) Schematic of the experiments performed with LD cells shown in Fig. 4*K* and *L*. LD cells were plated on collagen I-coated supports and transduced with rtTA-encoding lentiviruses (rtTA) in combination with an empty vector (Empty) or a doxycycline-inducible lentiviral YAP construct (YAP). After infection, cells were cultured with 2µg/ml doxycycline to induce YAP expression and treated either with vehicle or 3-MA 10 µM. After 7 days, cells were replated as indicated at clonogenic density in three-dimensional 5% Matrigel and growth as mammary colonies.

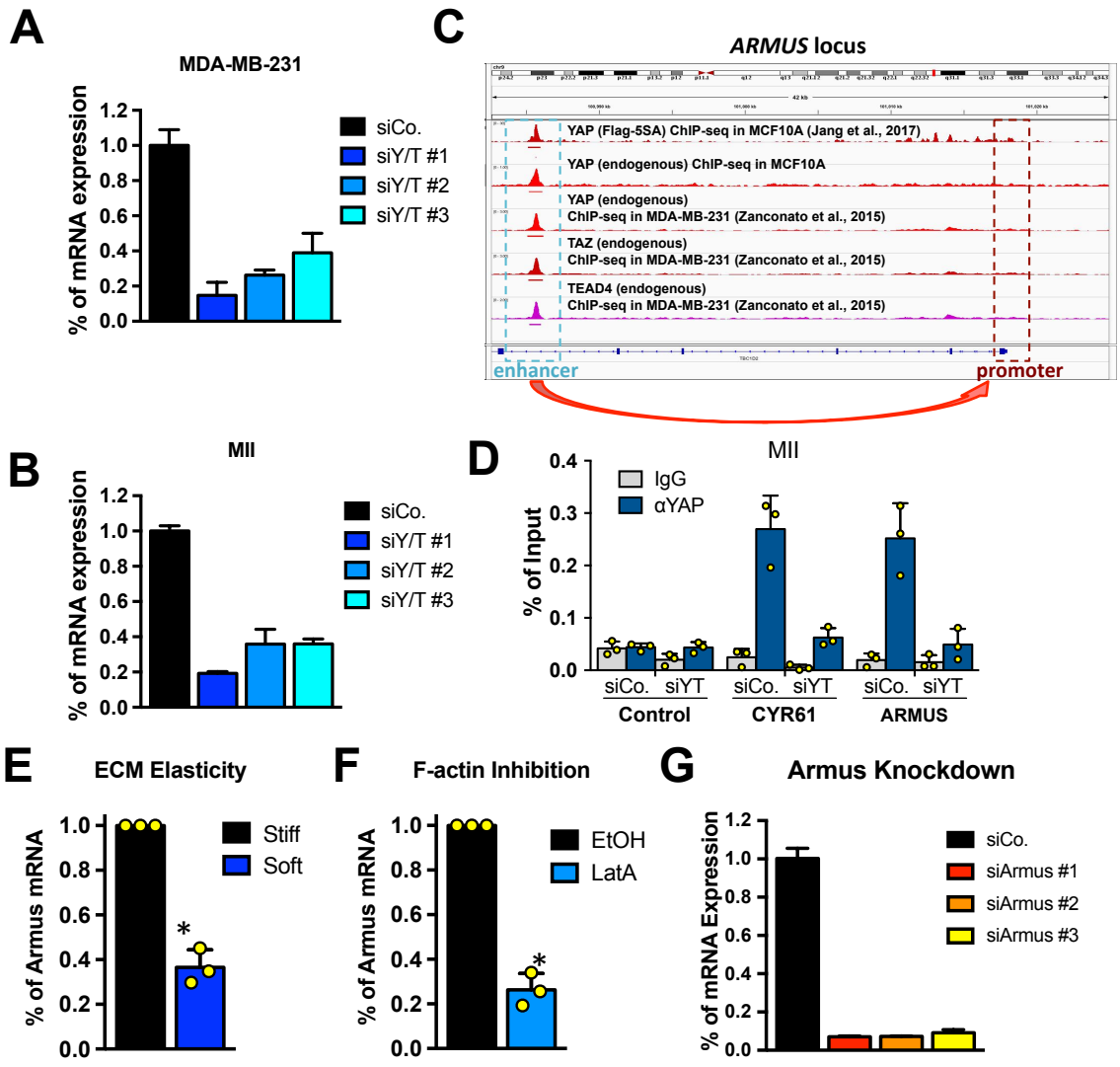


**Fig. S3 YAP/TAZ control autophagic flux through their direct target Armus.** (*A-D*) Mechano-YAP/TAZ-dependent regulation of Armus. MDA-MB-231 (*A*) or MII cells (*B*) were transfected with control siRNA (siCo.) or with three independent YAP/TAZ siRNA mixes (siY/T #1, siY/T #2 and siY/T #3) and analyzed by qRT–PCR for Armus mRNA levels. Values were normalized siCo. treated cells. Bars represent mean + SD (*N*=3 biological replicates) from one of three independent experiments, providing similar results. (*C*) YAP/TAZ regulate Armus expression through their association to a distal enhancers. Genome browser view of YAP-, TAZ- and TEAD4-binding profiles on the ARMUS promoter (right) and the YAP/TAZ-occupied enhancer (chr9:100,985,454-100,986,091, left) in MDA-MB-231 and MCF10A cells from the corresponding

YAP/TAZ/TEAD ChIP-seq datasets (7, 14). (*D*) Validation of the YAP/TAZ-binding site on the Armus-associated enhancer by ChIP-qPCR in a different cell lines when compared to Fig 5*B* (MII cells). The Armus enhancer is enriched in YAP-immunoprecipitated chromatin from control siRNA transfected cells (siCo.), but not in negative control IP (IgG) or in chromatin obtained from YAP/TAZ-depleted cells (siYT). ChIP-qPCR positive and negative controls are as in Fig. 5*B*. (*E-F*) MII cells were plated on stiff vs soft substrates (*E*) or treated either with EtOH or latrunculin A (LatA) to inhibit the F-actin cytoskeleton (*F*). After 24 hours, cells were analyzed by qRT–PCR for Armus mRNA levels. Values were normalized and statistically compared to Stiff-plated (*E*) or EtOH-treated cells (*F*). Bars represent mean + SD from three independent experiments (**P*<0.001 in *E*; **P*<0.0001 in *F*; two-tailed Student's *t*-test). (*G*) Validation of siRNA molecules targeting Armus, as in Fig. 5*C*. qRT-PCR analysis of MDA-MB-231 cells transfected with either control siRNA (siCo.) or three independent Armus siRNAs (siArmus #1, siArmus #2 and siArmus #3). Data were normalized to control siRNA condition (siCo, Black bar). Bars represent mean + SD (*N*=3 biological replicates) from one of three independent experiments, providing similar results.

## Supplementary Tables

**Table S1. Primers used for Cloning**

| Primer | Sequence |
|---|---|
| Mm Armus ATG HA For | CAAGGGCCATGAGTTACCATGGAGTACCCATACGATGTTCC AGATTACGCTGAGGACGCCCCCGAGCGC |
| Mm Armus STOP Rev | CATCTAGAACTGGTTATCAGCTGTCGCCTTCTCCTT |

**Table S2. siRNA Sequences**

| Name | Sequence (5' to 3') |
|---|---|
| siCo. | AllStars Negative Control siRNA 1027280 (Qiagen) |
| siYAP/TAZ #1 | GACAUCUUCUGGUCAGAGA[dT][dT] (YAP) |
| | ACGUUGACUUAGGAACUU[dT][dT] (TAZ) |
| siYAP/TAZ #2 | GGUGAUACUAUCAACCAAA[dT][dT] (YAP) |
| | AGGUACUUCCUCAAUCACA[dT][dT] (TAZ) |
| siYAP/TAZ #3 | GGUGAUACUAUCAACCAAA [dT] [dT] (YAP) |
| | AGGUACUUCCUCAAUCACA [dT][dT] (TAZ) |
| siArmus #1 | CGGACAGUCUCAUUAGCAA [dT][dT] |
| siArmus #2 | GCAAGUACCUGGCCGGUCU [dT][dT] |
| siArmus #3 | GCAACACGCUGACGGCAUC [dT][dT] |
| siATG7 | CGAGUAUCGGCUGGAUGAA [dT][dT] |

**Table S3. Primers used for RT-qPCR**

| Target gene | Forward primer | Reverse primer |
|---|---|---|
| *Hs RPLP0* | CGGATTACACCTTCCCACTTG | CCGACTCTTCCTTGGCTTCA |
| *Hs CTGF* | AGGAGTGGGTGTGTGACGA | CCAGGCAGTTGGCTCTAATC |
| *Hs Armus* | TTGCCCTGCTGGTCCTAGAG | GGTGACGAGGGAGAGATCCA |
| *Hs CD44s* | CATCTACCCCAGCAACCCTA | TGGGTCTCTTCTTCCACCTG |
| *Hs AMOTL2* | GCAAGGGCTCGTATCCAGTG | CGTCTCTGCTGCCATGTTTG |
| *Mm Atg7* | GCTGTTGGCTGGGAGAAGAA | AGTCCAAGGTGGGGACCAAT |
| *Mm 18S* | TGTCTCAAAGATTAAGCCATGC | GCGACCAAAGGAACCATAAC |

**Table S4. Primers used for ChIP-qPCR**

| Target gene | YAP/TAZ peak | Sequence |
|---|---|---|
| hBG | negative control locus | For: GCTTCTGACACAACTGTGTTCACTAGC |
| | | Rev: CACCAACTTCATCCACGTTCACC |
| CYR61 | positive control locus | For: CACACACAAAGGTGCAATGGAG |
| | | Rev: TGAGCTGAATGGAGTCCTACACA |
| Armus | chr9:100,985,454-100,986,091 | For: AAGTCAGCTTCTCAGGGCTCA |
| | | Rev: TTGAGGGAAAGACACCCACTG |

**Table S5. List of antibodies**

| Antibody | Company | Catalog Number | Working dilution |
|---|---|---|---|
| anti-GAPDH | Millipore | MAB374 | 1:50000 (WB) |
| anti-YAP 63.7 | Santa Cruz Biotechnology | sc-101199 | 1:1000 (WB)<br>1:200 (IF) |
| anti-YAP | Proteintech | 13584-1-AP | 1:2000 (WB) |
| anti-TAZ (anti-WWTR1) | Sigma Aldrich | HPA007415 | 1:500 (WB) |
| anti-HA tag | Santa Cruz Biotechnology | sc-7932 | 1:500 (IF) |
| anti-Flag tag | Sigma | A8592 | 1:1000 (IF) |
| anti-LC3B | Novus | NB100-2220 | 1:2000 (WB) |
| anti-LC3B | Thermo Fisher | PA1-16930 | 1:100 (IF) |
| anti-LAMP1 | DSHB | H4A3 | 2µg/ml (IF) |
| anti-CD49f PE-Cy5 | BD Biosciences | 551129 | 25 ng/mL (FACS) |
| anti-CD29 PE-Cy7 | BioLegend | 102222 | 2.5 ng/mL (FACS) |
| anti-CD61 | BD Biosciences | 55334 | 10 ng/mL (FACS) |
| anti-EpCAM Fitc | BioLegend | 118208 | 30 ng/mL (FACS) |
| APC Mouse Lineage Ab Cocktail | BD Biosciences | 51-9003632 | 1:25 (FACS) |
| anti-CD44 FITC | BD Biosciences | 555478 | 10µl/Test (FACS) |
| anti-CD24 PE-conjugated | BD Biosciences | 555428 | 10µl/Test (FACS) |
| normal rabbit IgG | Sigma Aldrich | I5006 | 3-5µg (ChIP) |
| anti-YAP1 | Abcam | ab52771 | 3-5µg (ChIP) |
| Goat anti-mouse Alexa-Fluor 568 | Thermo Fisher Scientific | A-11004 | 1:300 (IF) |
| Goat anti-rabbit Alexa-Fluor 488 | Thermo Fisher Scientific | A-11034 | 1:300 (IF) |
| Phalloidin Alexa-Fluor 568 | Thermo Fisher Scientific | A12380 | 1:300 (IF) |
| Prolong Diamond Antifade Mountant with DAPI | Thermo Fisher Scientific | P36971 | |

## References to Supplementary Information


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