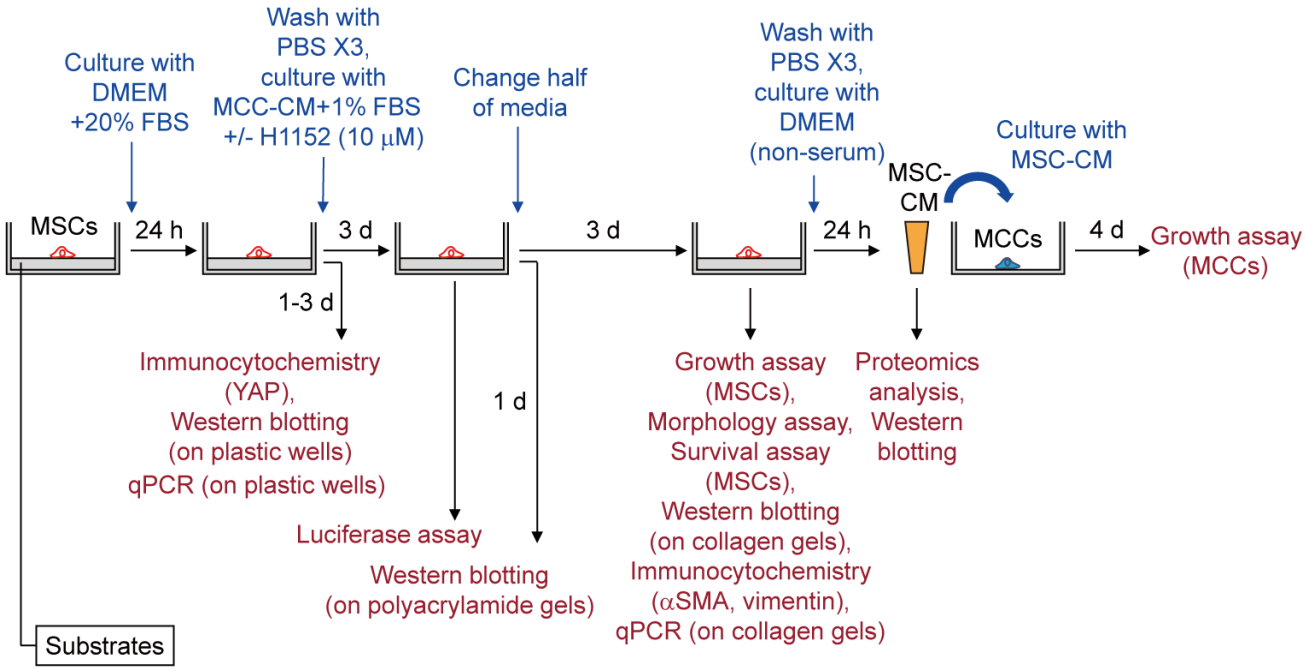
Supplementary Figures S1-S10, Materials and Methods

This file contains supplementary figures S1-S10 and supplementary materials and methods. Figure S1 shows experimental design of mesenchymal stem cells in this article. Figure S2 shows differentiation of transgenic mesenchymal stem cells. Figure S3 shows differentiation and gel contraction of mesenchymal stem cells. Figure S4 shows YAP activity in mesenchymal stem cells on a stiff matrix. Figure S5 shows expression and activation of YAP-related signaling molecules in mesenchymal stem cells. Figure S6 shows contribution of mesenchymal stem cells to tumor progression. Figure S7 shows prosaposin detection in mammary tumors. Figure S8 shows the role of prosaposin for ERK and caspase 3. Figure S9 shows contribution of prosaposin to late tumor growth and progression. Figure S10 shows the role of stiff matrix for mesenchymal stem cells from B6 mice.

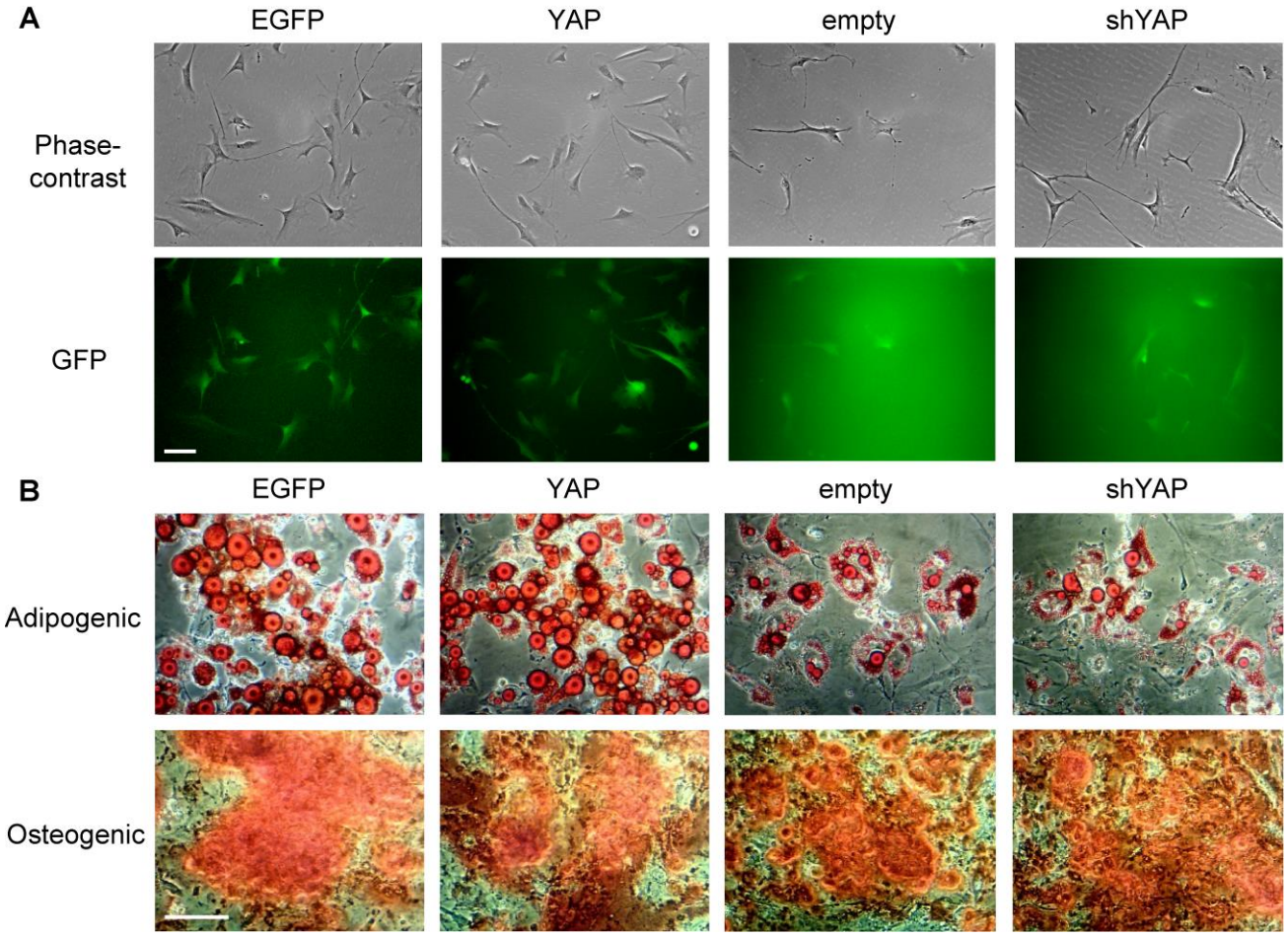
Figure S1



Supplementary Figure S1. Experimental design of mesenchymal stem cells cultured on different substrates.

Schematic chart of experimental procedures is shown here. MSCs: mesenchymal stem cells, MCCs: mammary cancer cells. CM: conditioned media, BIS: bisacrylamide.

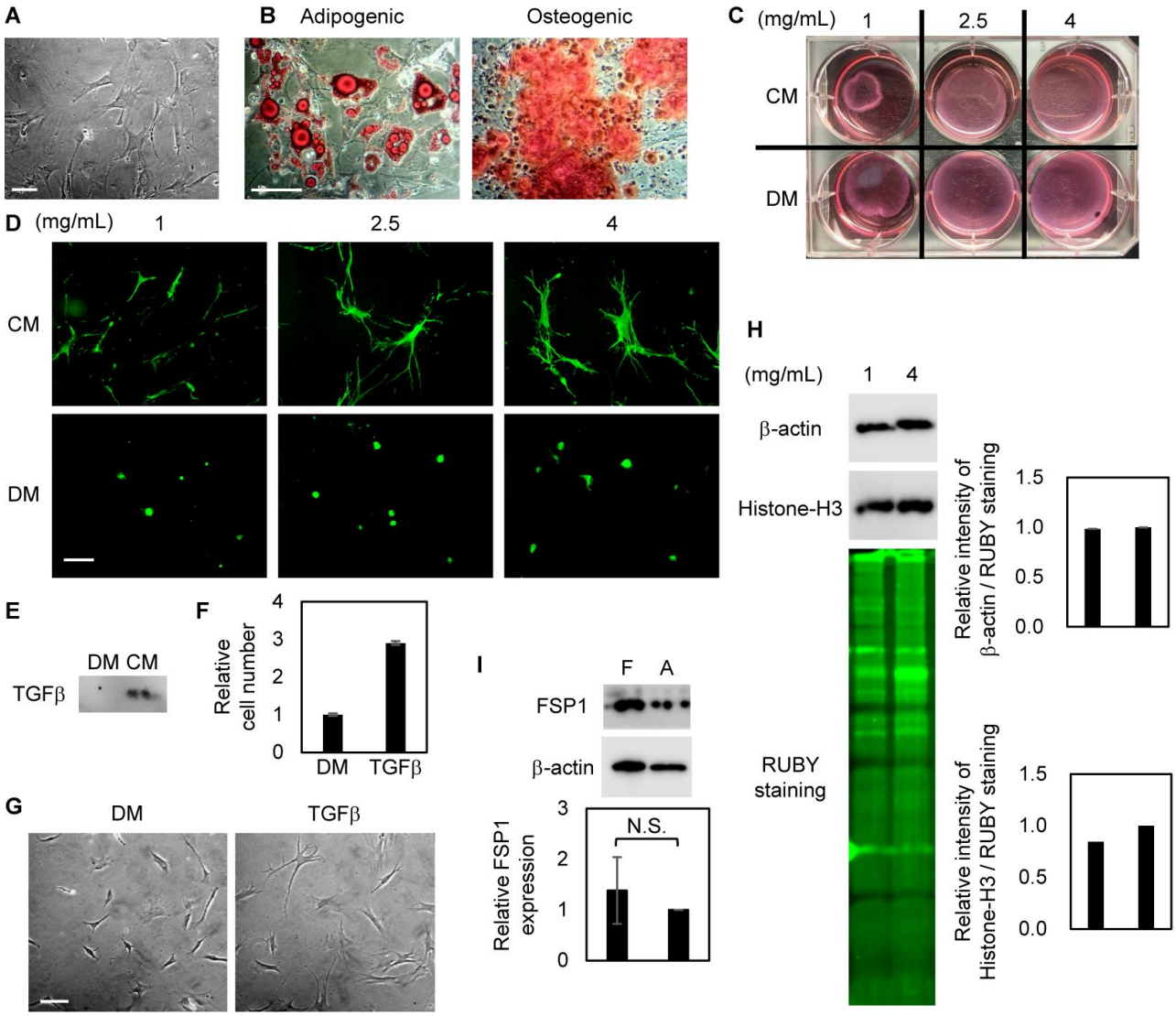
Figure S2



Supplementary Figure S2. Transgenic mesenchymal stem cells (MSCs) differentiated to adipocytes and osteocytes.

A, A phase-contrast and fluorescence images (GFP) of MSCs on a non-coated plastic dish. **B,** Differentiation assay of MSCs to adipocytes and osteocytes. MSCs were cultured with adipogenic or osteogenic media. Adipocytes and osteocytes were detected by Oil Red O and Alizarin Red S staining, respectively. EGFP: EGFP-IRES-overexpressed MSCs. YAP: EGFP-hYAP-overexpressed MSCs. empty: empty pGIPZ-transgenic MSCs. shYAP: shYAP-pGIPZ-transgenic MSCs. Bar = 100 μm.

Figure S3

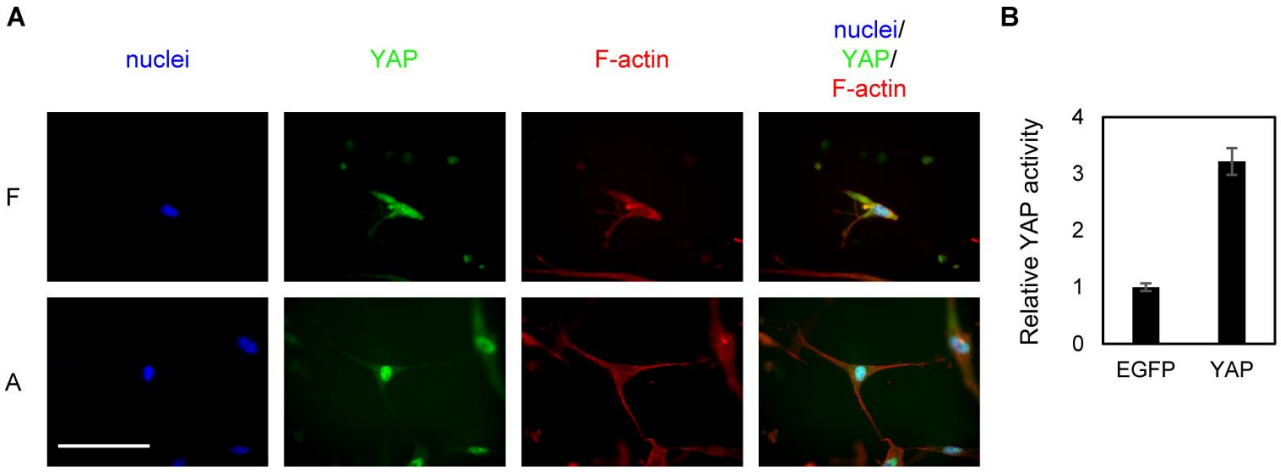


Supplementary Figure S3. Mesenchymal stem cells (MSCs) from BALBc mice are pluripotent and contract 1 mg/mL collagen gels.

A, A phase-contrast image of MSCs cultured on a non-coated plastic culture dish. **B,** Differentiation assay of MSCs to adipocytes and osteocytes. MSCs were cultured with adipogenic or osteogenic media. Adipocytes and osteocytes were detected by Oil Red O and Alizarin Red S staining, respectively. **C,** Floating collagen gels (1, 2.5 or 4 mg/mL collagen) cultured with mesenchymal stem cells with 4T1 conditioned media (CM) or control DMEM (DM). **D,** Morphology of MSCs cultured with CM or DM on floating collagen gels of 1, 2.5, or 4 mg/mL collagen. Fluorescent images of F-actin with a x10 objective demonstrate representative cell phenotypes. **E,** Western blot of TGFβ in DM and CM. **F,** Growth assay of MSCs cultured with DM or DM + TGFβ (TGFβ, 20 ng/mL) on attached gels of 1 mg/mL collagen. n = 2 technical duplicates. **G,** Phase contrast images of MSCs

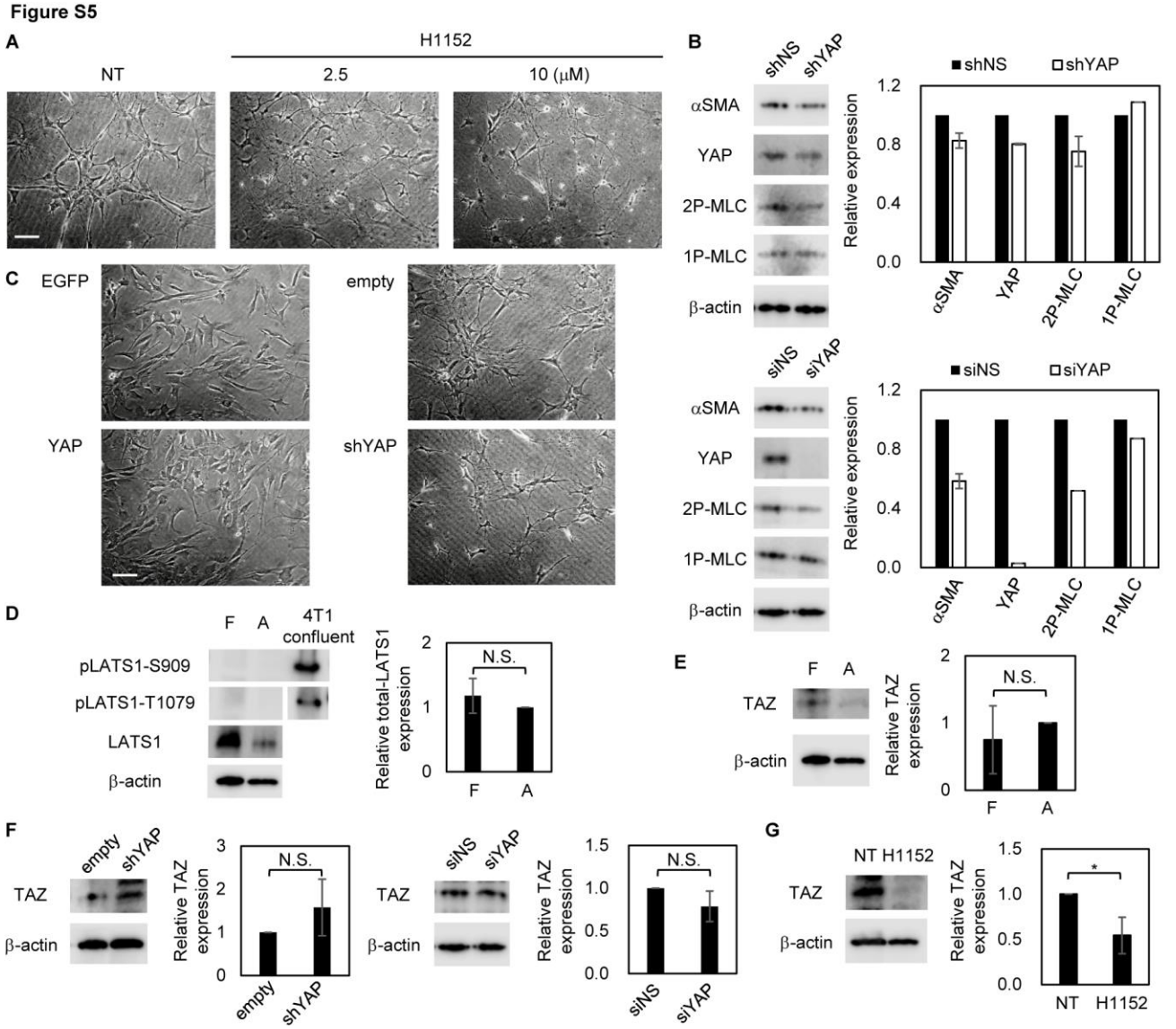
cultured with DM or TGFβ on attached gels of 1 mg/mL collagen for 3 days. **H,** Western blotting of β-actin, Histone-H3, and RUBY staining in MSCs cultured with CM on floating collagen gels of 1 or 4 mg/mL collagen. Relative intensity of β-actin or Histone-H3 divided with total protein detected by RUBY staining is shown together. **I,** Western blot of FSP1 and β-actin in MSCs cultured with CM on floating (F) or attached (A) collagen gels of 1 mg/mL collagen. A representative blot is shown, and quantitation of n = 3 experiments. N.S.: no significance with 95% confidence interval. Mean±S.E. are shown. Bar = 100 μm. MSCs were established from BALBc mice.

Figure S4



Supplementary Figure S4. YAP is activated in MSCs on a stiff matrix.

A, Fluorescence images of nuclei, YAP, and F-actin in MSCs cultured with CM on floating or attached collagen gels of 1 mg/mL collagen. **B,** Luciferase assay of a YAP reporter construct in MSCs cultured with CM on attached collagen gels of 1 mg/mL collagen. EGFP: EGFP-IRES-overexpressed MSCs. YAP: EGFP-hYAP-overexpressed MSCs. n = 2 technical replicates. Mean±S.D. are shown. Bar = 100 μm. MSCs were established from BALBc mice.

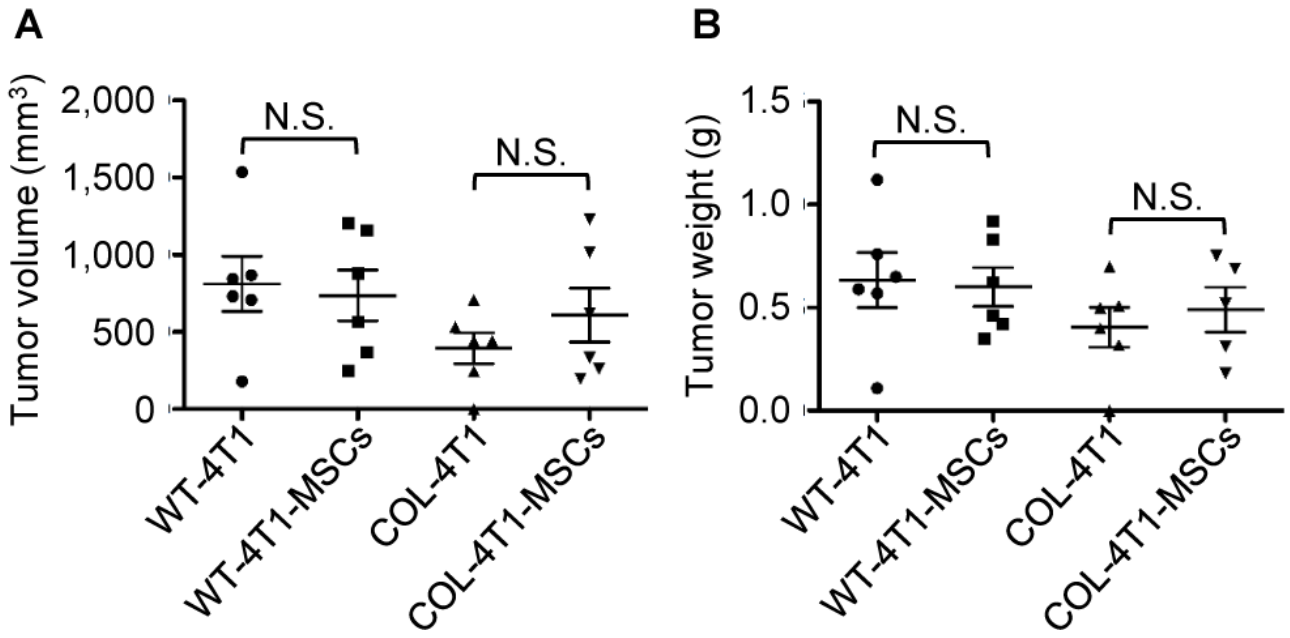


Supplementary Figure S5. YAP-related signaling molecules were not regulated by matrix stiffness in mesenchymal stem cells (MSCs).

A, Phase-contrast images of non-treated (NT) or H1152 (5, 10 μM)-treated MSCs cultured with 4T1-conditioned media (CM) on attached collagen gels of 1 mg/mL collagen. **B,** Western blot of αSMA, YAP, 2P-MLC, 1P-MLC, and β-actin in non-silencing RNA-pGIPZ-transgenic MSCs (shNS) or shYAP-pGIPZ-transgenic MSCs (shYAP) with CM on a non-coated plastic well plate for 2 days and non-silencing siRNA-transfected MSCs (siNS) or siYAP transfected-MSCs (siYAP) cultured with CM on a collagen-coated plastic well plate for 3 days. Quantitation of n = 2 technical replicates (αSMA, YAP, and 2P-MLC in shNS and shYAP; αSMA in siNS and siYAP) or one experiment (1P-MLC in shNS and shYAP; YAP, 2P-MLC, and 1P-MLC in siNS and siYAP). **C,** Phase-contrast images of MSCs cultured with CM on a non-coated plastic well plate (EGFP,

YAP) or attached collagen gels of 1 mg/mL collagen (empty, shYAP). EGFP: EGFP-IRES-overexpressed MSCs. YAP: EGFP-hYAP-overexpressed MSCs. empty: empty pGIPZ-transgenic MSCs. **D,** Western blot of phosphorylated LATS1 at S909 (pLATS1-S909), phosphorylated LATS1 at T1079 (pLATS1-T1079), total-LATS1 (LATS1), and β-actin in MSCs cultured with CM on floating or attached collagen gels of 1 mg/mL collagen. Results of confluent 4T1 cells are shown together as a positive control of pLATS1-S909 and pLATS1-T1079. A representative blot is shown, and quantitation of n = 2 experiments. **E,** Western blot of TAZ and β-actin in MSCs cultured with CM on floating or attached collagen gels of 1 mg/mL collagen. A representative blot is shown, and quantitation of n = 3 experiments. **F,** Western blot of TAZ and β-actin in empty or shYAP cultured with CM on attached collagen gels of 1 mg/mL collagen and siNS or siYAP cultured with CM on a collagen-coated plastic well plate for 3 days. A representative blot is shown, and quantitation of n = 3 experiments (empty and shYAP) or 2 technical replicates (siNS and siYAP). **G,** Western blot of TAZ and β-actin in NT or 10 μM H1152-treated MSCs cultured with CM on attached collagen gels of 1 mg/mL collagen. A representative blot is shown, and quantitation of n = 3 experiments. For all data sets: *statistical significance determined with 95% confidence interval. N.S.: no significance with 95% confidence interval. Mean±S.E. are shown. Bar = 100 μm. MSCs were established from BALBc mice. F: floating collagen gels, A: attached collagen gels.

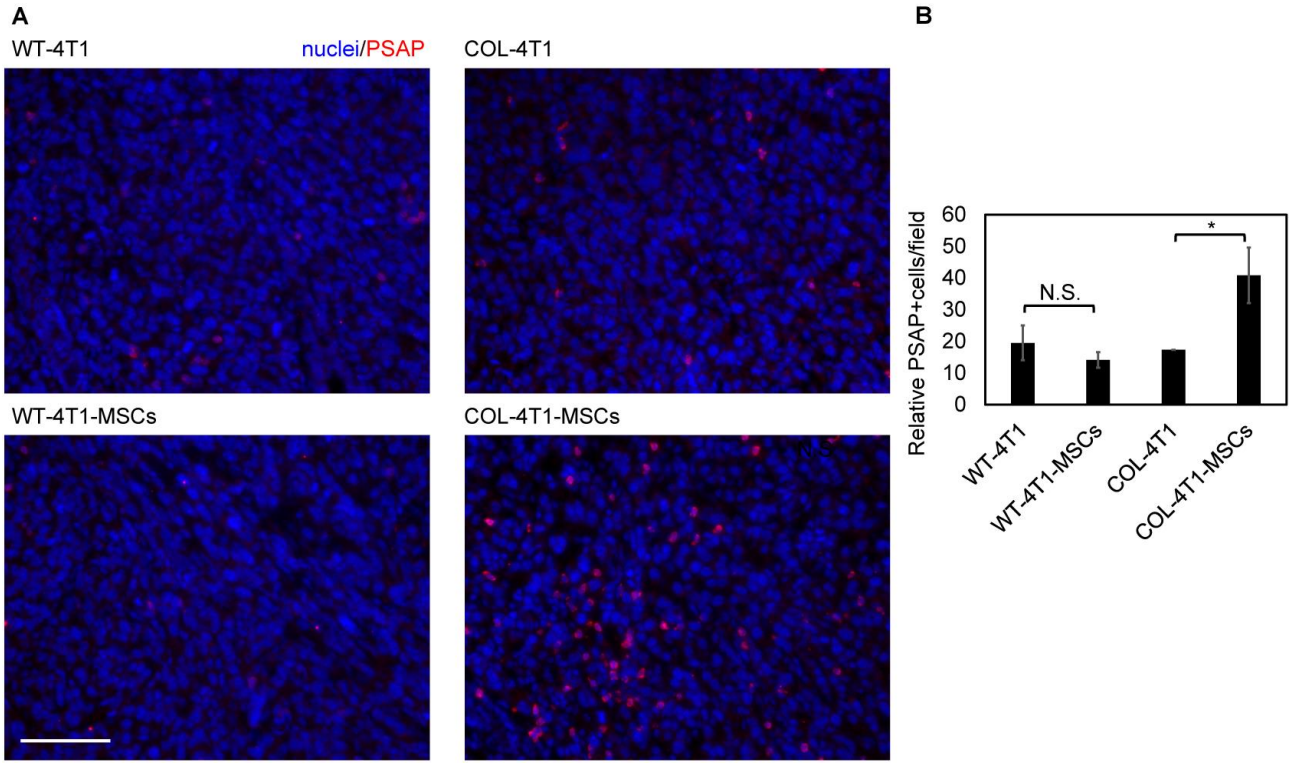
Figure S6



Supplementary Figure S6. Mesenchymal stem cells (MSCs) in stiff tissues do not promote late tumor growth.

A, Volume of tumors 28 days after injection. n = 6 tumors in 3 mice. wt/wt (WT) or wt/*Col1a1*^{jaetm1} mutation (COL) mice were injected with 4T1 cells only (4T1) or 4T1 cells with empty pGIPZ-transgenic MSCs (4T1-MSCs). **B,** Weight of tumors 28 days after injection. n = 6 tumors (WT-4T1, WT-4T1-MSCs, COL-4T1) or 5 tumors (COL-4T1-MSCs) in 3 mice. For all data sets: N.S.: no significance (P>0.05) with Wilcoxon rant sum test. Mean±S.E. are shown. MSCs were established from BALBc mice.

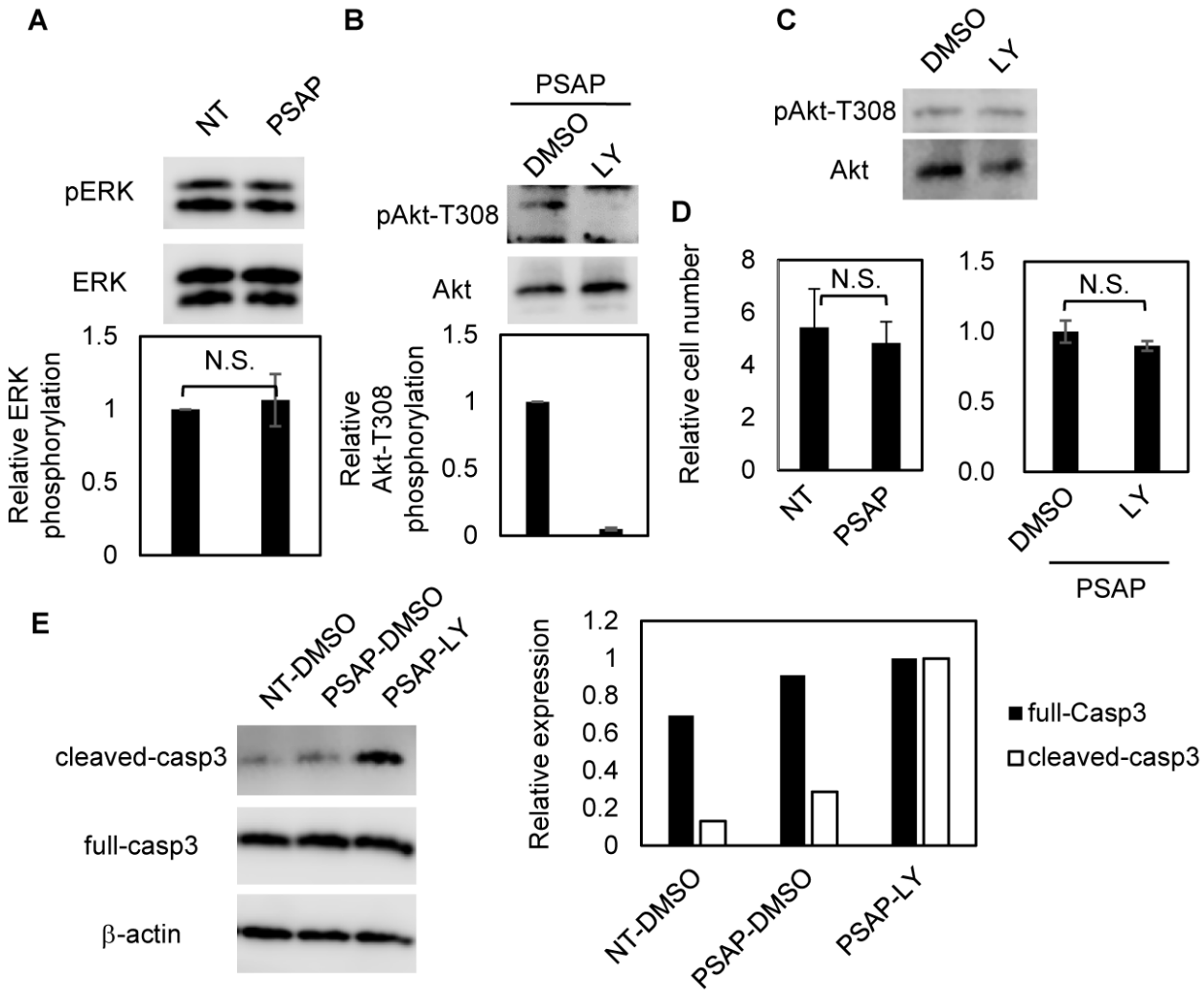
Figure S7



Supplementary Figure S7. Prosaposin (PSAP) is highly detected in mammary tumors with mesenchymal stem cells (MSCs) in stiff tissue.

A, nuclei and PSAP staining in tumors 7 days after injection. wt/wt (WT) or wt/*Col1a1*^{jaetm1} mutation (COL) mice were injected with 4T1 cells only (4T1) or 4T1 cells with empty pGIPZ-transgenic MSCs (4T1-MSCs). **B,** Relative PSAP positive cells per field of tumors in **A**. n = 4 tumors (WT-4T1-MSCs and COL-4T1-MSCs) in 2 mice or 2 tumors (WT-4T1 and COL-4T1) in 2 mice. *statistical significance determined with 95% confidence interval. N.S.: no significance with Student's *t*-test (P>0.05). Mean ± S.E. are shown. Bar = 100 μm. MSCs were established from BALBc mice.

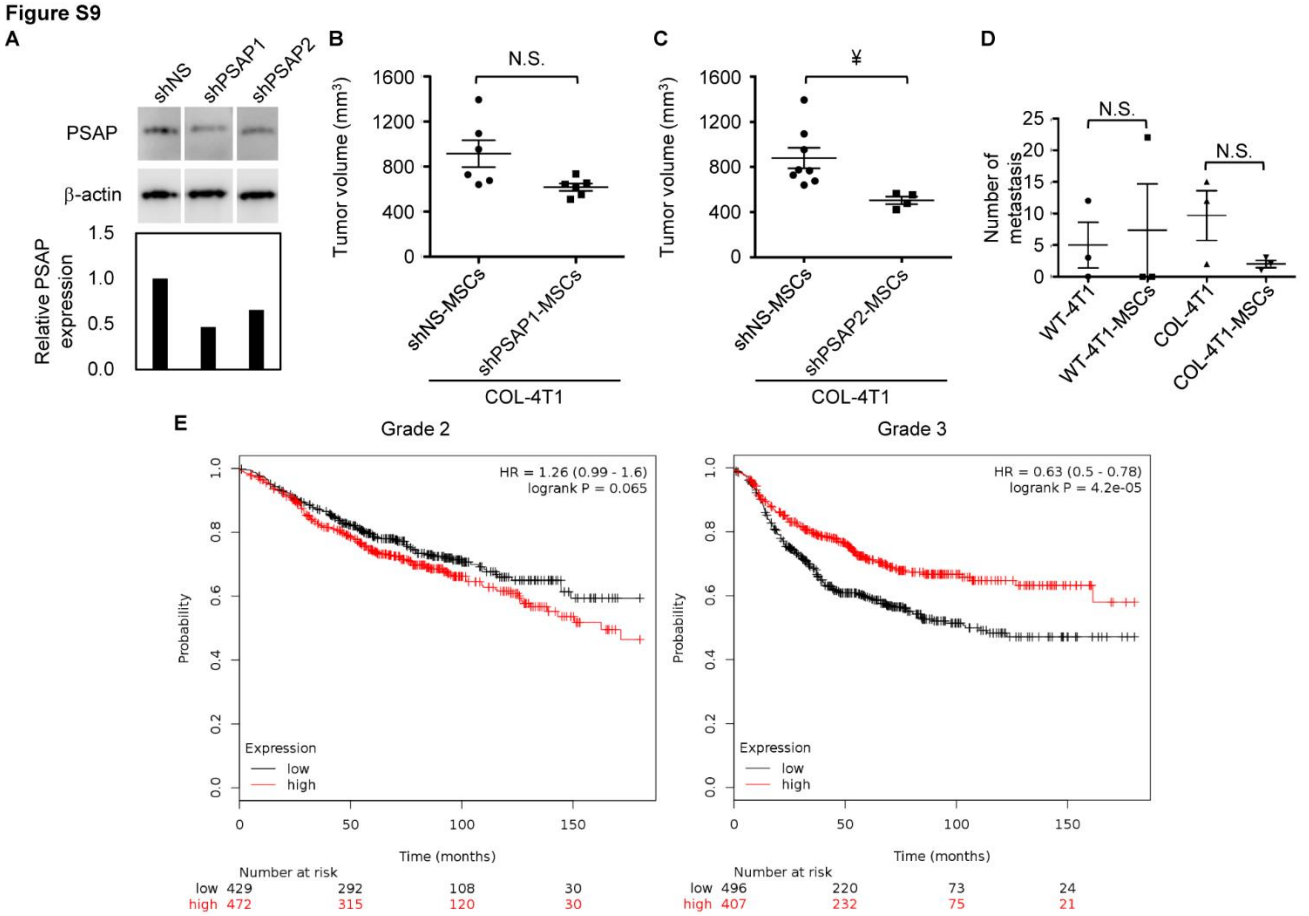
Figure S8



Supplementary Figure S8. Prosaposin (PSAP) regulates cell proliferation and survival independent of ERK and caspase 3.

A, Western blot of phosphorylated ERK (pERK) and total-ERK (ERK) in 4T1 cells cultured with DMEM (NT), or DMEM with PSAP (PSAP) for 15 min. A representative blot is shown, and quantitation of n = 3 experiments. **B,** Western blot of phosphorylated Akt at T308 (pAkt-T308) and total-Akt (Akt) in 4T1 cells cultured with DMEM with PSAP+DMSO (DMSO) or +LY294002 (LY) for 15 min. A representative blot is shown, and quantitation of n = 3 experiments. **C,** Western blot of pAkt-T308 and Akt in 4T1 cells cultured with DMEM with DMSO (DMSO) or LY294002 (LY) without PSAP for 15 min. **D,** Growth assay of 4T1 cells cultured with DMEM (NT), DMEM with PSAP (PSAP), DMEM with PSAP+DMSO (DMSO), or DMEM with PSAP+LY294002 (LY) for 1 day. n = 3 technical replicates (NT vs PSAP). n = 9 samples in 3 experiments (DMSO vs LY with PSAP). **E,** Western blot of cleaved caspase 3 (cleaved-casp3), full length caspase 3 (full-

caps3), and β-actin in 4T1 cells cultured with DMEM with DMSO (NT-DMSO), DMEM with PSAP+DMSO (PSAP-DMSO), or DMEM with PSAP+LY294002 (PSAP-LY) for 3 hours. For all data sets: N.S.: no significance with 95% confidence interval (**A**), Student's *t*-test (P>0.05, **C** (NT vs PSAP)), or Welch's *t*-test (P>0.05, **C** (DMSO vs LY with PSAP)). Mean±S.E. (**A, B,** and **C** (DMSO vs LY with PSAP)) or S.D. (**C** (NT vs PSAP)) are shown. MSCs were established from BALBc mice. F: floating collagen gels, A: attached collagen gels.

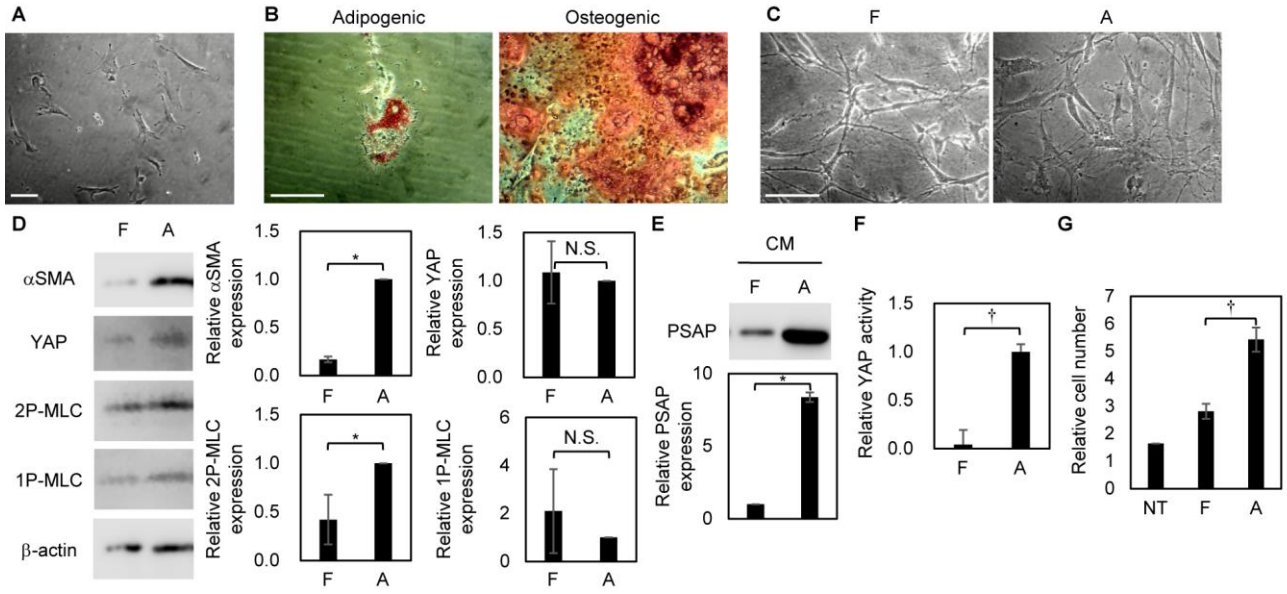


Supplementary Figure S9. Prosaposin (PSAP) do not promote late tumor growth and high grade tumor progression in breast cancer.

A, Western blot of PSAP and β-actin in non-silencing RNA-pGIPZ-transgenic (shNS), shPSAP1-pGIPZ-transgenic (shPSAP1)-MSCs, or shPSAP2-pGIPZ-transgenic (shPSAP2)-MSCs cultured on a non-coated plastic well plate for 1 day. **B,** Volume of tumors 21 days after injection. wt/*Col1a1*^{jaetm1} mutation (COL) mice were injected with 4T1 cells with shNS-MSCs or shPSAP1-MSCs. n = 6 tumors in 3 mice. **C,** Volume of tumors 21 days after injection. wt/*Col1a1*^{jaetm1} mutation (COL) mice were injected with 4T1 cells with shNS-MSCs or shPSAP2-MSCs. n = 8 tumors in 4 mice (shNS-MSCs), or n = 4 tumors in 2 mice (shPSAP2-MSCs). **D,** Number of metastatic lung lesions 28 days after injection. wt/wt (WT) or wt/*Col1a1*^{jaetm1} mutation (COL) mice were injected with 4T1 cells only (4T1) or 4T1 cells with empty pGIPZ-transgenic MSCs (4T1-MSCs). n = 3 mice. **E,** Kaplan-Meier curves of relapse free survival in relation to the gene expression of PSAP in grade 2 and 3 breast cancer patients. For all data sets: N.S.: no significance (P>0.05) with Student's *t*-test (**B**) or Wilcoxon

rant sum test (**D**). ¥statistical significance ($P<0.05$) determined with Wilcoxon rant sum test. Mean±S.E. are shown. MSCs were established from BALBc mice.

Figure S10



Supplementary Figure S10. Mesenchymal stem cells from B6 mice respond to matrix stiffness and promotes mammary cancer progression.

A, A phase-contrast image of MSCs cultured on a non-coated plastic culture dish. **B,** Differentiation assay of MSCs to adipocytes and osteocytes. MSCs were cultured with adipogenic or osteogenic media. Adipocytes and osteocytes were detected by Oil Red O and Alizarin Red S staining, respectively. **C,** Phase-contrast images of MSCs cultured with E0771-conditioned media (CM) on floating or attached collagen gels of 1 mg/mL collagen. **D,** Western blot of αSMA, YAP, di-phosphorylated myosin light chain (2P-MLC), mono-phosphorylated myosin light chain (1P-MLC), and β-actin in MSCs cultured with CM on floating or attached collagen gels of 1 mg/mL collagen. A representative blot is shown, and quantitation of n = 3 experiments. **E,** Western blot of PSAP in CM of MSCs cultured with E0771-CM on floating or attached collagen gels of 1 mg/mL collagen. A representative blot is shown, and quantitation of n = 3 technical replicates. **F,** Luciferase assay of YAP in MSCs cultured with E0771-CM on floating or attached collagen gels of 1 mg/mL collagen. n = 4 samples in 2 experiments. **G,** Growth assay of E0771 cells cultured with DMEM (NT), conditioned media of MSCs cultured with E0771-CM on floating collagen gels of 1 mg/mL collagen, or attached collagen gels of 1 mg/mL collagen, n = 1 (NT) or 3 (F and A) technical replicates. For all data sets: *statistical significance determined with 95% confidence interval. † statistical significance ($P<0.05$) determined with Student's *t*-test. N.S.: no significance

with 95% confidence interval. Mean±S.E. (**D, E, F**) or S.D. (**G**) are shown. Bar = 100 μm. MSCs were established from B6 mice. F: floating collagen gels, A: attached collagen gels.

Supplementary Materials and Methods

Antibodies and reagents

We used Hoechst 33258 (1:500, Sigma-Aldrich, B2883) for nucleus staining. We also used Alexa Fluor 488 phalloidin (1:500, Invitrogen, A12379) and TRITC phalloidin (100 ng/mL, Sigma-Aldrich, P1951) for F-actin staining. Antibodies of α-smooth muscle actin (1:1,000 for western blot and 1:500 for fluorescence staining, Abcam, ab5694), vimentin (1:1,000 for western blot, Abcam, ab92547 and 1:500 for fluorescence staining, R and D Systems, MAB2105), β-actin (1:5,000 for western blot, Sigma-Aldrich, A5316), YAP (1:1,000 for western blot and 1:100 for fluorescence staining, Cell Signaling Technology, 14074), di-phosphorylated myosin light chain (1:1,000 for western blot, Cell Signaling Technology, 3674), mono-phosphorylated myosin light chain (1:1,000 for western blot, Cell Signaling Technology, 3671), prosaposin (1:1,000 for western blot and 1:100 for staining of tumors, Abcam, ab68466), phosphorylated Akt at T308 (1:1,000 for western blot, Cell Signaling Technology, 2965), phosphorylated Akt at S473 (1:1,000 for western blot, Cell Signaling Technology, 9271), total-Akt (1:1,000 for western blot, Cell Signaling Technology, 9272), transforming growth factor-β (1:1000 for western blot, Cell Signaling Technology, 3711), fibroblast specific protein-1 (1:1,000 for western blot, Cell Signaling Technology, 13018), Histone-H3 (1:1000 for western blot, Cell Signaling Technology, 3638), phosphorylated LATS1 at S909 (1:1,000 for western blot, Cell Signaling Technology, 9157), phosphorylated LATS1 at T1079 (1:1,000 for western blot, Cell Signaling Technology, 8654), total-LATS1 (1:1,000 for western blot, Cell Signaling Technology, 9153), TAZ (1:1,000 for western blot, Sigma-Aldrich, SAB2102718), phosphorylated ERK (1:2,000 for western blot, Cell Signaling Technology, 4370), total-ERK (1:1,000 for western blot, Cell Signaling Technology, 9102), p-histone-H3 (1:1600 for fluorescence staining, Cell Signaling Technology, 3377), and caspase 3 (1:1,000 for western blot, Cell Signaling Technology, 9662) were used for western blot, fluorescence staining, and/or staining of tumors. As secondary antibodies of western blot, Peroxidase-AffiniPure Donkey Anti-Rabbit IgG (Jackson ImmunoResearch, 711-035-152) and Peroxidase-AffiniPure Donkey Anti-Mouse IgG (1:5,000, Jackson ImmunoResearch, 715-035-150) were used. As secondary antibodies of fluorescence staining, Alexa Fluor 488 donkey anti-rabbit IgG (Life Technologies, A21206) and Alexa Fluor 594 donkey anti-rat IgG (Invitrogen, 56629A) were used. As secondary antibody of

staining of tumors, Alexa Fluor 594 donkey anti-rabbit IgG (Life Technologies, A21207) was used. Non-silencing siRNA (ON-TARGETplus Non-targeting Pool, Dharmacon, D-001810-10-05) or siYAP (SMARTpool: ON-TARGETplus Yap1 siRNA, Dharmacon, L-046247-01-0005) were used for siRNA transfection with Lipofectamine RNAiMAX reagent (Invitrogen, 13778-075). H1152 (Millipore, cat#:555550) and LY294002 (Cell Signaling Technology, 9901) reagents were used for inhibition of phosphorylation of myosin light chain and Akt, respectively. Recombinant human prosaposin (20 ng/mL, Abcam, ab167924) and recombinant human TGFβ1 (20 ng/mL, R and D Systems, 240-B) was used for prosaposin and TGFβ stimulation, respectively.

Growth and morphology assays

For the growth and morphology assay of MSCs, we seeded $5x10^3$ MSCs onto a 0.5 mL of 1, 2.5, or 4 mg/ml collagen gel in a 12 well culture plate as described above and in Supplementary Fig. S1. After 6 days, the cells were fixed with 4% paraformaldehyde in PBS, washed with 0.15 M glycine in PBS, and permeabilized with 0.02% Triton X100 in PBS. Then, the cells were treated with blocking solution (1% bovine serum albumin (BSA) and 1% donkey serum (DS) in PBS) for 1 hour at room temperature. The cells were stained with Hoechst 33258 and 488-phalloidin in PBS with 1% DS for 1 hour at room temperature. After three washes with PBS and two with distilled water, we put the gel onto a slide glass and covered with a cover slip and ProLong Gold Antifade Mountant (Thermo Fisher Scientific, P36930). After capturing fluorescence images of the nucleus and F-actin, we analyzed cell number by calculating the number of nuclei from the Hoechst images and determining cell area from the images of F-actin with ImageJ software (National Institute of Health). In Supplementary Fig. S3F, the relative cell number was analyzed by calcein AM (3.4 μM, Life Technologies, C3100MP) treatment for 1 hour at 37 ^oC and Fluoroskan Ascent FL.

For the growth assay of 4T1 cells, we seeded $5x10^2$ 4T1 cells onto a collagen-coated 96 well plate and cultured for 24 hours. After two washes with PBS, the cells were cultured with each media for 1 or 4 days. Relative cell number was analyzed with CyQUANT Cell Proliferation Assay (Life Technologies, C35007) and Fluoroskan Ascent FL (Thermo Fisher Scientific).

For the growth assay of E0771 cells, we prepared 45 μL of 1 mg/mL collagen gels with $5x10^2$ E0771

cells and seeded it into a 96 well plate. The cells were cultured with appropriate media for 3 days. Then, the relative cell number was analyzed by calcein AM (3.4 μM) treatment for 1 hour at 37 ºC and Fluoroskan Ascent FL.

Survival assay

For the survival assay of MSCs, we seeded $5x10^3$ MSCs onto 0.5 mL of 1 or 4 mg/ml collagen gels in a 12 well culture plate as described above and in Supplementary Fig. S1. After 6 days, we treated the cells with calcein AM (3.4 μM) for 1 hour at 37 ºC and captured phase-contrast images and green fluorescence images. Then, the ratio of dead cells (green fluorescence negative cells / total cells) was calculated.

For the survival assay of 4T1 cells, we seeded $5x10^2$ 4T1 cells onto a collagen-coated 96 well plate and cultured for 24 hours. After two washes with PBS, the cells were cultured with appropriate media for 1 day. We treated the cells with calcein AM (3.4 μM) for 1 hour at 37 ºC and detected survival cells (green fluorescence positive cells) with Fluoroskan Ascent FL.

Fluorescence staining

For staining MSCs, $5x10^3$ cells were cultured onto 0.5 mL collagen gels as described above and in Supplementary Fig. S1. For antibodies against α-smooth muscle actin and vimentin, the cells were cultured for 6 days, while cells were cultured for 1 day when using antibodies against YAP. For 4T1 cell staining, we seeded 100 μL media drop with $3x10^4$ cells onto a 50 mm glass bottom dish. After 15 min, we added 4 mL culturing media. After 24 hours, we washed the cells with PBS twice and cultured the cells with each media for 1 day. In either cell type, processing for immunofluroescense analysis was completed by fixing cells with 4% paraformaldehyde in PBS and permeabilized with 0.02% Triton X100 in PBS. Then, the cells were treated with blocking solution (1% BSA and 1% DS in PBS) for 1 hour at room temperature. The cells were incubated in primary antibodies in PBS with 1% DS overnight at 4 ºC or 1 hour at room temperature, followed by three wash with Tris buffered saline with 0.3% Tween (TBS-T) or PBS. Next, the cells were incubated with secondary antibodies, Hoechst 33258, 488 phalloidin, and/or TRITC-phalloidin in PBS with 1% DS for 1 hour at room temperature, followed by three wash with TBS-T or PBS and two washed with distilled water. Finally, the gel

or coverslip was transferred onto a slide glass and mounted with ProLong Gold reagents. The relative ratio of p-histone-H3 positive cells or YAP in the nuclei was analyzed by ImageJ software.

Proteomics analysis

Conditioned media (3 mL) of MSCs was concentrated and water exchanged once using 3kDa MWCO spin filters, then protein concentration in the final volume (~200 µL per sample) was determined using Pierce™ 660 nm Protein Assay kit (Thermo Fisher Scientific). TCA/acetone precipitation for 40 µg protein extraction followed. Pellets were re-solubilized and denatured in 15 µL of 8 M Urea / 50 mM NH_4HCO_3 (pH = 8.5) / 1 mM Tris-HCl then diluted to 60 µL for reduction step with: 2.5 µL of 25 mM DTT, 5 µL MeOH, 37.5 µL 25 mM NH_4HCO_3 (pH = 8.5). Incubated at 52°C for 15 min, cooled on ice to room temperature then 3 µL of 55 mM IAA was added for alkylation and incubated in darkness at room temperature for 15 min. Reaction was quenched by adding 8 µL of 25 mM DTT. Subsequently 7 µL of Trypsin/LysC solution [100 ng/µL Trypsin/LysC Mix from PROMEGA Corp. in 25 mM NH_4HCO_3] and 22 µL of 25 mM NH_4HCO_3 (pH = 8.5) was added to 100 µL final volume. Digestion was conducted for 2 hours at 42°C then additional 4 µL of trypsin/LysC solution added and digestion proceeded o/n at 37°C. Reaction was terminated by acidification with 2.5% Trifluoroacetic Acid to 0.3% final. Then, proteins were analyzed via LC-MS/MS on Orbitrap Elite at the UW-Madison Biotech Center.

qPCR

We seeded $7x10^4$ MSCs onto a collagen gel for 6 days as previously described and in Supplementary Fig. S1 or a non-coated 6 well plate and cultured the cells for 24 hours. RNA was extracted with TRIzol (Thermo Fisher Scientific, 15596-026) and then cDNA was generated with iScript cDNA Synthesis Kit (BioRad, 1708891). qPCR was performed with SsoAdvanced Universal SYBR Green Supermix (Bio-Rad, 172-5271) and CFX Connect Real-Time System (BioRad). Relative expression of each mRNA was calculated by normalizing the target value with the value of β-actin. We used following primers: PSAP (forward: 5'-ggacatgattaagggcgaga-3', reverse: 5'-gggactggcagaggttgag-3'), MMP13 (forward: 5'-gccagaacttcccaaccat-3', reverse: 5'-tcagagcccagaattttctcc-3'), MMP3 (forward: 5'-ttgttctttgatgcagtcagc-3', reverse: 5'-gatttgcgccaaaagtgc-3'), and

β-actin (forward: 5’-ctaaggccaaccgtgaaaag-3’, reverse: 5’-accagaggcatacagggaca-3’).

Statistics

The statistical significance was determined as follows. All the experiments except mice experiments, for the comparison of the value with variance to another value with no variance, we determined significance by 95% confidence interval. For the comparison between the two values both with variance, we first confirmed the both data sets met the normal distribution by Kolmogorov-Smirnov test, in which $P > 0.05$ indicates the normal distribution of the data set. Then, we determined whether the variance of two data sets was significantly different by using *F*-test, in which $P < 0.05$ indicates the variance was significantly different. For data sets without statistically different variances, we analyzed significance with two-sided Student’s *t*-test. In contrast, for data sets with statistically different variances, we used two-sided Welch’s *t*-test for analyzing statistical significance. In Student’s *t*-test and Welch’s *t*-test, we considered that $P < 0.05$ indicates statistical significance. For multiple comparisons, we analyzed the significance with Bonferroni correction. In mice experiments, statistical significance was determined with Wilcoxon rank sum test or Student’s *t*-test. These analysis were performed with Excel software (for confidence interval, *F*-test, Student’s *t*-test, and Welch’s *t*-test) and R software (for Kolmogorov-Smirnov test, Wilcoxon rank sum test). No statistical methods were performed for pre-determining sufficient sample size.

For all figures, we showed *statistical significance determined with 95% confidence interval, † statistical significance ($P<0.05$) determined with Student’s *t*-test, ‡ statistical significance ($P<0.05$) determined with Welch’s *t*-test, and ¥statistical significance ($P<0.05$) determined with Wilcoxon rank sum test.