**Supplementary Materials and Methods**

**RNA Isolation, cDNA Generation and qPCR**

RNA was extracted using TRIzol (Invitrogen). For 3D cultures, TRIzol was added directly to Matrigel. For tissue, snap frozen glands were pulverised on dry ice, and TRIzol was added to 30mg tissue powder. For cell lines, TRIzol was added directly to plate. For mammospheres, spheres were collected, pelleted and TRIzol was added. After 5 minutes at room temperature, 200μl of chloroform was added per 1ml of TRIzol and shook vigorously by hand for 15 seconds. Samples were then centrifuged at 12,000G for 15 minutes. The upper phase was transferred to a fresh Eppendorf tube, and 500μl of isopropanol was added, mixed and incubated at room temperature for 10 minutes. Samples were then centrifuged at 12,000G for 10 minutes. The RNA pellet was washed twice with 1ml of 70% EtOH. All steps were carried out at 4oC unless otherwise stated.

cDNA was generated using MMLV reverse transcriptase (Promega). Real time PCR was performed using Power Up SybR Green master mix (Thermo Scientific #A25741), see Supplementary Table for primer list. GAPDH or RPLO were used as housekeeping genes, to which values were normalised to. Bio-Rad CX manager was used to calculate Cq and analysed data.

**Histology and Immunostaining**

Mammary glands were fixed overnight at 4°C in 4% formaldehyde (Roth). Glands were then dehydrated and embedded in paraffin. For staining, tissue sections were de-paraffinised, rehydrated and stained with Hematoxylin and Eosin (H&E), dehydrated and mounted. Images were acquired with a bright-field Zeiss microscope. For immune-staining, antigen-retrieval was conducted on paraffin-embedded sections by boiling in Tris-EDTA buffer (10mmol/L Tris, 1mmol/L EDTA, pH 9.0) for 20 minutes. Cryo-sections were incubated for 5 minutes at room temperature and then fixed with 4% methanol-free paraformaldehyde for 15 minutes. Adherent cells were fixed with 4% methanol-free paraformaldehyde for 15mins. Stem cell-enriched spheres were collected, centrifuged and then fixed in 4% formaldehyde before being dehydrated and paraffin-embedded. All samples were blocked with 10% horse serum for 1 hour at room temperature prior to being incubated overnight with primary antibodies at 4oC (See Supplementary Table for antibody list). For fluorescent staining, samples were incubated with secondary antibodies and DAPI for 1 hour at room temperature before being mounted with Immu-mount (Thermo Scientific).

For immunohistochemistry, after antigen-retrieval, slides were washed and incubated with 3% H2O2 for 15 minutes at room temperature, washed 3 times for 5 minutes in PBS and incubated with primary antibodies overnight at 4oC. Horseradish Peroxidase-labelled secondary antibodies were diluted and incubated on slides for 1 hour at room temperature. Slides were then washed and developed using DAB chromogenic substrate (DAKO), dehydrated and mounted.

**Organotypic Stem Cell-enriched 3D Cultures**

Organotypic stem cell-enriched cultures were generated using previously published protocols35,56 with minor adjustments. Single cells from digested mammary glands were re-suspended and plated on Collagen I-coated plates (50μg/ml) in stem cell-enriching medium MEBM (Lonza Cat. #CC-3151), supplemented with 2% B27 (Invitrogen, Cat. # 17504044), 20 ng/ml bFGF (Invitrogen, Cat. # 13256029), 20 ng/ml EGF (Sigma, Cat. #SRP3196-500μg), 4 μg/ml heparin (Sigma, Cat. # H3149), 5 µg/ml insulin (Sigma, Cat. #I0516-5ml), 0.5µg/ml hydrocortisone (Sigma, Cat. #H0888-1G) and 1X Gentamicin (Sigma, Cat. # G1397-100ML) for 16-18 hours. Cells were washed twice with DPBS, washed with 0.25% trypsin-EDTA for 30 seconds and then incubated with 0.25% trypsin-EDTA at 37°C for 5 - 7 minutes until cells detached. Trypsin was inactivated with DMEM/F12 supplemented with 10% FBS, 1% HEPES and 1% Pen/Strep. Cells were re-suspended in stem-enriching medium and counted. Cells were then seeded in 25μl droplets containing 50% reduced growth factor Matrigel at a density of 100cells/μl. Plates were carefully flipped and Matrigel was let solidify at 37°C for 45mins - 1hour. 0.5ml of stem medium was added per well of 24-well plates containing a 25μl droplet. Medium was changed every second day. For secondary sphere formation, spheres were dissociated for 10mins in 0.25% trypsin-EDTA, and cells were filtered using 0.45μm filters. Single cell suspensions were then re-seeded as described for primary cells. All treatments with verteporfin were carried out under minimal lighting.

**Proteomics and Phospho-proteomics**

For proteomics and phospho-proteomics analysis, sample preparation was carried out essentially according to Mertins and collegues, 201857. Briefly, samples were lysed in urea lysis buffer containing protease and phosphatase inhibitors. From each sample, 500 µg was subjected to reduction with DTT and alkylation with iodoacetamide before performing digestion with LysC and trypsin. After desalting, peptides were labelled with TMT6 reagents (Thermo) and fractionated by basic reversed-phase separation into 24 fractions. 1.5 µg per fraction was injected into LC-MS for global proteome analysis, while the remaining majority of the samples were pooled into 12 fractions and enriched for phosphopeptides using robot-assisted iron-based IMAC on an AssayMap Bravo system (Agilent).

Proteomics samples were measured on an Exploris 480 orbitrap mass spectrometer (Thermo) connected to an EASY-nLC system (Thermo). HPLC-separation occurred on an in‐house prepared nano‐LC column (0.074 mm × 250 mm, 1.9 μm Reprosil C18, Dr Maisch GmbH) using a flow rate of 250 nl/min on a 110 min gradient with an acetonitrile concentration ramp from 4.7% to 55.2% (v/v) in 0.1% (v/v) formic acid. MS acquisition was performed at a resolution of 60,000 in the scan range from 375 to 1,500 m/z. Data-dependent MS2 scans were carried out at a resolution of 45,000 or 30,000 with an isolation window of 0.4 m/z and a maximum injection time of 86 ms (120 ms for phosphoproteomics) using the TopSpeed setting with a 1 sec cycle time. Dynamic exclusion was set to 20 s and the normalised collision energy was specified to 35.

For analysis, the MaxQuant software package version 1.6.10.4358,59 was used. TMT6 reporter ion quantitation was turned on using a PIF setting of 0.5. Variable modifications included Met-oxidation, acetylated N-termini and deamidation of Asn and Gln for proteomics analysis. For phosphoproteomics analysis, deamidation was replaced by phosphorylation on Ser, Thr and Tyr. An FDR of 0.01 was applied for peptides, sites and proteins, and the Andromeda search was performed using a mouse Uniprot database (July 2018, including isoforms). Further analysis was done using R and the Protigy package (https://github.com/broadinstitute/protigy). Protein groups were filtered for proteins that have been identified by at least one unique peptide and that had valid TMT reporter ion intensities across all samples. The phosphoproteome was also filtered for sites containing valid values for all samples. For both analyses, two-sample moderated t-tests (limma package60) comparing the two groups were calculated using the log2-transformed and median-MAD normalised TMT6 corrected reporter ion intensities, followed by a Benjamini-Hochberg (BH) p-value correction. Heatmaps of selected significant proteins or phosphosites according to BH-adjusted p-value were generated using the pheatmap package61 employing hierarchical clustering based on Euclidean distance. For annotation of gene lists derived from a human proteome, i.e. Zanconato and collegues (2016), genes were converted into mouse orthologs using biomaRt62 and manual curation.

**siRNA**

siRNAs were purchased from Thermo Scientific (see Supplementary Table for list of specific siRNAs). 75pmol/well (6-well) of siRNA was transfected using Lipofectamine 2000 following manufacturer’s instructions. Cells were harvested 48 hours post transfection.

**shRNA**

PLKO.1 shRNA plasmids were a gift from Yaron Fuchs18. Lentiviral packaging plasmids, pMD2.G and psPAX2 were co-transfected along with shscramble and shYAP1 into HEK293 cells. Viruses were collected and concentrated 24 - 48 hours post-transfection. Concentrated viruses were used to transduce Wnt-Met primary mammary cells before being seeded as stem cell-enriched spheres.

**Cell Culture**

Breast cancer cell lines were purchased from ATCC (MCF10A, MCF7, BT474, T47D, MDA-MB-231 and BT-549) and Asterand Bioscience (SUM1315 and SUM149). MCF10A cells were maintained in DMEM/F12 Glutamax, 5% horse serum, 20ng/ml EGF, 0.5mg/ml hydrocortisone, 100ng/ml cholera toxin, 10μg/ml insulin and 1% pen/strep. MCF7, BT474 and T47D were maintained in DMEM, 10% FBS, 5μg/ml insulin and 1% pen/strep. MDA-MB-231 and BT-549 were maintained in DMEM, 10% FBS, 1% NEAAs and 1% pen/strep. SUM1315 were maintained in DMEM/F12 HAM-Glutamax, 1% Hepes, 5% FBS, 10ng/ml EGF, 5μg/ml insulin and 1% pen/strep. SUM149 were grown in DMEM/F12 HAM-Glutamax, 1% Hepes, 5% FBS, 1μg/ml hydrocortisone, 5μg/ml insulin and 1% pen/strep. For mammosphere assays, cells were seeded at 5,000cells/ml on poly-HEMA-coated non-adherent 10cm plates in DMEMF12, FGF 10ng/ml, EGF 20ng/ml, ITS 1x and B27 1x. Spheres were grown for up to 10 days with medium supplemented every second day.

**CellTitre-Glo assay**

3D cultures were seeded into 96-well opaque-walled plates. Control and treated spheres with 100μl of medium per well were incubated for 30mins at room temperature. 100μl of CellTiter-Glo reagent was added to each well and incubated for 2 minutes shaking to induce cell lysis. Plates were then rested for 10 minutes at room temperature to stabilise the luminescent signal. Luminescence was recorded on a luminometer.

**Crystal Violet staining**

Primary mammary cells were seeded at 2x105 cells/well of a collagen I coated 6-well plate for 16-18hours. Cells were then treated with indicated concentrations of verteporfin under minimal lighting for 48hours. Cells were then treated with CV

***In vivo* Inhibitor Studies**

For simvastatin treatments, Wnt-Met mice received 100mg/kg of simvastatin or vehicle control via oral gavage daily. Simvastatin was diluted in 2% DMSO, 30% PEG 300, 5% Tween80 and ddH2O. For verteporfin treatments, mice were treated with 2.5mg/kg daily via subcutaneous injection. Verteporfin was diluted in DMSO and then brought to 2.5% DMSO in PBS. Tumour volumes and body weight were determined several times per week.

**PDX models**

PDX models were established from triple-negative breast cancer patients with their informed

consent as previously described45. The experimental protocol and animal housing were in accordance with institutional guidelines as proposed by the French Ethics Committee (Agreement N° B75-05-18).

**ChIP-Atlas data analysis**

ChIP-seq tracks for CTNNB1 (SRX833403), TEAD4 (SRX190301) and YAP1 (SRX2844314) in H1-hESC were downloaded from chip-atlas.org41,63. Functional annotation for genome regions was taken from ChromHMM64. Data were loaded into UCSC genome browser65 to make the plots.

**Kaplan-Meier and GOBO analysis**

Relapse-free survival of breast cancer patients was analysed using Kaplan-Meier Plotter online software ([http://kmplot.com](http://kmplot.com/)). Trichotomization was set to Q1 vs Q4. Expression analysis of YAP in breast cancer cell lines and human datasets were generated using GOBO online software (<http://co.bmc.lu.se/gobo>).

**Statistical analyses**

For all statistical analyses ≥ 3 biological replicates were used per genotype. All data were reproduced ≥ 2 times in independent experiments. Statistical tests used are outlined in figure legends. Images were analysed and processed using Image J, ZEN and LAS X software. Densitometry was performed using Image J or Image Studio software.

**Supplementary Fig. Legends**

***Supplementary Fig. 1. Met signalling regulates YAP and β-catenin.* A.** Western blot for active YAP in triplicates of Wnt and Wnt-Met mammary glands at 1 week PP. **B.** Heatmap of proteomics analysis showing YAP and TAZ (Wwtr1) phosphorylation sites that were significant in a two-sample moderated t-test (adj. p-value ≤ 0.1) revealing significant increase in YAP phosphorylation at S46 and T48 (row-scaling was applied). The heatmap shows median-MAD-normalised input data across all proteins and row-scaling across all samples as was clustered based on Euclidian distance. **C.** Western blot for CTGF in Wnt and Wnt-Met mammary glands at 1 week PP. Right, quantification right. Data are mean ±SEM, n=3 biological replicates, \*p<0.05, by Student’s t test. **D.** Western blot of p-YAP and active-YAP in nuclear and cytoplasmic fractionations from MDA-MB-231 cells. **E.** Immunofluorescence of YFP (green) and β-catenin (red) in WAPicre; β-catGOF; ROSA26EYFP (Wnt) and WAPicre; Wap-HGF; β-catGOF ; ROSA26EYFP (Wnt-Met) at 1-week PP, scale bar, 20μm. **F.** Quantification of β-catenin-positive nuclei (β-catenin+ nuclei/number of nuclei per field x 100) in Wnt vs. Wnt-Met tissues. Data are mean ±SEM, n=3 biological replicates, \*\*p<0.01, by Student’s t test. **G.** RT-qPCR of YAP target genes in PHA665752-treated mammospheres. Data shown are mean ±SEM, n=2 biological replicates.

***Supplementary Fig. 2. Pharmacological evidence of YAP dependency in Wnt-Met tumours.* A.** Immunohistochemistry staining of YAP in Wnt-Met-YAPKO mammary glands, scale bar, 20μm. **B.** qRT-PCR of *Wap* in Wnt-Met-YAPCtrl and Wnt-Met-YAPKO mammary glands. Data are mean ±SEM, n=3 biological replicates, by Student’s t-test. **C.** Immunohistochemistry of YAP in Wapicre;YAPCtrl and Wapicre;YAPKO post-partum mammary glands, scale bar, 100μm. **D.** Tumour volume curve of Wnt-Met tumours treated with vehicle control or 100mg/kg simvastatin (SIM). Data are mean ±SEM, n=3 biological replicates, \*\*\*\*p<0.0001, Two-way ANOVA, Sidak’s multiple comparisons test. **E.** Average tumour weight of vehicle control and SIM-treated mice. Data shown are mean ±SEM, n=3 biological replicates, \*p<0.05, by Student’s t test. **F.** Tumour volume curve of Wnt-Met tumours treated with vehicle control or 2.5mg/kg verteporfin (VP). Data are mean ±SEM, n=3 biological replicates, \*\*p<0.01, Two-way ANOVA, Sidak’s multiple comparisons test. **G.** Average tumour weight of vehicle control and VP treated mice. Data shown are mean ±SEM, n=3 biological replicates, \*p<0.05, by Student’s t test. **H.** H&E staining of vehicle control and SIM treated Wnt-Met tumours, scale bar, 50μm. **I.** Immunohistochemistry of Ki-67 in vehicle control and SIM-treated tumours, scale bar, 20μm. **J.** H&E staining of vehicle control and VP-treated Wnt-Met tumours, scale bar, 50μm. **K.** Immunohistochemistry of Ki-67 in vehicle control and VP-treated tumours, scale bar, 20μm. **L.** Quantification of Ki-67-positive cells/area in vehicle control and SIM-treated tumours. Data are mean ±SEM, n=3 biological replicates, \*\*p<0.01, by Student’s t test. **M.** Quantification of Ki-67-positive cells/area in vehicle control and VP-treated tumours. Data shown are mean ±SEM, n=3 biological replicates, \*\*p<0.01, by Student’s t test. **N.** RT-qPCR of *Ctgf* in vehicle control and SIM-treated tumours. Data shown are mean ±SEM, n=3 biological replicates, \*p<0.05, by Student’s t-test. **O.** RT-qPCR of *Ctgf* in vehicle control and VP-treated tumours. Data shown are mean ±SEM, n=3 biological replicates, \*\*p<0.01, by Student’s t-test. **P.** Immunohistochemistry of TAZ in Wnt-Met-YAPCtrl and Wnt-Met-YAPKO tumours at 2.4 weeks PP, scale bar, 50μm. **Q.** RT-qPCR of *Wwtr1/Taz in* Wnt-Met-YAPCtrl and Wnt-Met-YAPKO tumours at 2.4 weeks PP. Data shown are mean ±SEM, n=3 biological replicates, n.s. p>0.05, by Student’s t-test.

***Supplementary Fig. 3.* Wnt-Met signalling induces YAP-dependent luminal-to-basal transition A.** Immunofluorescent images of Wap-cre;YFP mammary glands for YFP (green), CK8 (red, upper) and CK14 (red, lower) at virgin, 12 days pregnancy and 0 days PP, scale bar, 50μm. **B.** FACS plots of Wap-cre;YFP mammary glands at 10 days pregnancy and 0 days PP showing luminal and basal cell populations. **C.** Immunofluorescent images of DMSO vehicle control and SIM-treated Wnt-Met tumours for CK8 (red), CK14 (white) and YFP (green), scale bar, 20μm.

***Supplementary Fig. 4. YAP is activated in CSCs of Wnt-Met-driven mammary gland tumours.* A.** Confocal analysis of YAP (green) in Wap-cre;YFP and Wnt-Met tumours, scale bar, 10μm. **B.** qRT-PCR of stem cell genes in Wap-cre;YFP and Wnt-Met mammary glands at 2 weeks PP. Data are mean ±SEM, n=3 biological replicates, \*\*p<0.01, \*\*\*p<0.001, by Student’s t-test. **C.** RT-qPCR of *Cd29* and *Cd49f* in TECs and CSCs. Data shown are mean ±SEM, n=3 biological replicates, \*p<0.05, by Student’s t-test. **D.** KEGG pathway analysis of genes upregulated in CSCs vs. TECs. **E.** Heatmap of YAP target gene expression on CSCs vs. TECs.

***Supplementary Fig. 5. Stem cell-enriched spheres recapitulate in vivo cell phenotypes.* A.** Scheme showing the method used to isolate and generate stem cell-enriched spheres. **B.** Bright-field and fluorescent images of stem cell-enriched spheres generated from Wap-cre;YFP or Wnt-Met mammary glands at 2 weeks PP, scale bar, 50μm. **C.** FACS histogram showing YFP-positive cells in Wap-cre;YFP (control) or Wnt-Met derived stem-enriched spheres. **D.** RT-qPCR of transgene-associated genes in Wap-cre;YFP and Wnt-Met stem cell-enriched spheres. Data are mean ±SEM, n=3 biological replicates, \*p<0.05, \*\*p<0.01, by Student’s t test. **E.** Confocal images of CK8 (red), CK14 (white) and YFP (green) in Wap-cre;YFP (control) and Wnt-Met stem cell-enriched spheres, scale bar, 50μm. **F.** Crystal violet staining of Wap-cre;YFP mammary epithelial cells treated with DMSO and VP. **G.** Fluorescent images showing YFP in stem cell-enriched spheres generated from FACS-isolated TECs and CSCs. Quantification of sphere numbers (right). Data are mean ±SEM, n=3 replicates, \*\*p<0.01, by Student’s t-test. **H.** Confocal images of YAP (green) in Wnt-Met stem cell-enriched spheres, scale bar, 50μm. **I.** RT-qPCR YAP target genes in Wap-cre;YFP and Wnt-Met stem cell-enriched spheres. Data are mean ±SEM, n=3 biological replicates, \*p<0.05, \*\*p<0.01, by Student’s t-test. **J.** Confocal images of Ki-67 (green) in DMSO vehicle control and 2.5μM simvastatin (SIM)-treated stem cell-enriched spheres, scale bar, 50μm. **K.** Fluorescent images of the H2B-RFP reporter in Wnt-Met stem cell-enriched spheres, scale bar, 50μm. Quantification of sphere numbers on the right. Data are mean ±SEM, n=3 biological replicates, \*p<0.05, by Student’s t test. **L.** RT-qPCR of *Yap* in sh-treated scramble (Ctrl) and shYAP Wnt-Met spheres**. M.** Western Blot for YAP in siSramble (control) and siYAP treated MDA-231 cells, GAPDH was used as a loading control. **N.** Bright-field images of spheres generated from siSramble (control) and siYAP treated MDA-231 cells. Quantification of sphere numbers on the right. Data are mean ±SEM, n=3 replicates \*\*\*p<0.001 by Student’s t test. **O.** RT-qPCR of *Yap* in Wnt-Met-YAPCtrl and Wnt-Met-YAPKO spheres.

***Supplementary Fig. 6. YAP controls β-catenin activity.*** *­***A.** RT-qPCR of YAP and β-catenin target genes in Wnt-Met mammary gland spheres treated with DMSO vehicle control or 2µM verteporfin (VP) for 48Hrs. Data are mean ±SEM, n=3 biological replicates, \*p<0.05, \*\*p<0.01, by Student’s t-test. **B.** Confocal images of β-catenin (green) in DMSO vehicle control and SIM-treated stem cell-enriched spheres, scale bar, 50μm. **C.** Confocal images of YAP (green) and β-catenin (red) in siScramble (siCtrl) and siYAP treated MDA-MD-231 cells, scale bar, 20μm. **D.** Quantification of mean nuclear YAP fluorescence intensity of siScramble (siCtrl) and siYAP treated MDA-MD-231 cells. Data are mean ±SEM, n=3 biological replicates, \*\*\*p<0.005, by Student’s t-test. **E.** Quantification of mean nuclear β-catenin fluorescence intensity of siScramble (siCtrl) and siYAP treated MDA-MD-231 cells. Data are mean ±SEM, n=3 biological replicates, \*p<0.05, by Student’s t-test. **F.** RT-qPCR of YAP target genes *ANKRD1* and *CTGF* in siScramble (siCtrl) and siYAP treated MDA-MD-231 cells. Data are mean ±SEM, n=3 biological replicates, \*p<0.05, by Student’s t-test. **G.** RT-qPCR of β-catenin target genes *CCND1* and *CLDN1* in siScramble (siCtrl) and siYAP treated MDA-MD-231 cells. Data are mean ±SEM, n=3 biological replicates, \*p<0.05, by Student’s t-test. **H.** Western blot showing co-immunoprecipitation of p-YAP (cytoplasmic) with β-catenin and LATS1 in 4T1 cells. **I.** Venn diagram showing overlapping β-catenin, YAP and TEAD4 chip-seq peaks in hESC H1 cells.

***Supplementary Fig. 7. YAP is active in human basal-like breast tumours and predicts patient outcome in a subtype-dependent manner.* A.** Bright-field images of spheres generated from basal-like (lower) and luminal (upper) cell lines. Scale bar, 100µm. **B.** Box-plot showing *YAP1* expression in basal-like and luminal breast tumours. **C.** Immunohistochemistry of β-catenin in PDX sections, scale bar, 100μm.