EXPANDED EXPERIMENTAL PROCEDURE

Cell culture. The patient-derived primary chordoma cell line, JHC7, was established from tissue obtained intraoperatively and processed for cell culture as described in *Hsu, Mohyeldin, Shah et al., 2011*. JHC7 Cells were cultured in MesenPRO basal medium with growth supplement (Invitrogen), glutamax (Invitrogen), and antibiotic/antimycotic (Invitrogen). The recurrent chordoma cell lines, UCH1 and UCH2, were obtained from The Chordoma Foundation and cultured on gelatin-coated (Invitrogen) plates using 4:1 IMDM (Invitrogen)/RPMI 1640(Sigma) media supplemented with 10% FBS (Invitrogen) and antibiotic/antimycotic (Invitrogen). Primary patient-derived glioblastoma tissue samples were obtained at the Johns Hopkins Hospital under the approval of the Institutional Review Board (IRB). All primary cell lines were established from excess tumor tissue from patients undergoing surgical resection for glioblastoma. GBM cell line derivation and culture protocols were performed as previously described (Pollard et al., 2009; Smith et al., 2015), without the addition of N2 supplement and cultured on laminin-coated (1μg/cm2) plates. Specifically, DMEM/F12 (Invitrogen) was supplemented with GEM21 Neuroplex (Invitrogen), antibiotic/antimycotic (Invitrogen), human EGF (Peprotech) and FGF (Peprotech). The lung cancer cell line, H460, was purchased from ATCC and maintained in DMEM/F12 (Invitrogen) supplemented with 10% FBS (Invitrogen) and antibiotic/antimycotic (Invitrogen) and cultured on non-laminin coated plates.

Lentiviral transduction. Glycerol stocks of Human Mission TRC1 sequence-verified shRNA lentiviral plasmid vectors were obtained from the Johns Hopkins University High Throughput Biology Center (Table below). Plasmids were isolated using a Spin Miniprep kit (Qiagen). Vesicular stomatitis virus glycoprotein-pseudotyped virus was produced by co-transfecting 293T cells using Lipofectamine 2000 (Invitrogen) with a shRNA transducing vector and 2 packaging

vectors, psPAX2 and pMD2.G. On days 3 and 4 post-transfection, virus was harvested and filtered through a 0.22-μm pore cellulose acetate filter before centrifugal concentration using a Centricon Plus-70 (Millipore). An empty TRC1 lentiviral construct was used as the control virus (shCtrl; from Sigma). The YAP overexpression lentiviral vector was prepared using pCMV-Flag YAP127/128/131/381A construct (Addgene) inserted into Duet011 lentiviral plasmid (Addgene). Cells were transduced with equal titers of virus in growth media supplemented with polybrene (Sigma) for 24 hours. After transduction, cells were cultured in normal media for 24 hours prior to selection in puromycin or hygromycin (Sigma) for a minimum of 7 days. Kill curves of non-transduced cells (data not shown) were used to determine the minimum effective antibiotic concentrations.

shRNA plasmids

Target Gene	TRC Number	TRC Version
Human brachyury (T) Clone 1	TRCN0000005484	TRC 1
Human brachyury (T) Clone 2	TRCN0000005482	TRC 1
Human YAP Clone 1	TRCN0000107267	TRC 1
Human YAP Clone 2	TRCN0000107265	TRC 1

siRNA. siRNA knockdown experiments were accomplished by seeding JHC7 cells into 6-well plates and transfecting them with 100nM of ON-TARGETplus SMARTpool siRNAs (GE Healthcare, Little Chalfont, UK) specific for brachyury or a non-targeting control using 6μL DharmaFECT2 transfection reagent (GE Healthcare) per transfection, according to the manufacturer's instructions.

Generation of H460-CRISPR clones. For generation of H460 clones expressing various levels of brachyury protein, the GeneArt Precision gRNA synthesis kit (Invitrogen) was used for preparation of brachyury-specific gRNA designed via the use of the GeneArt CRISPR Search and Design online tool (Invitrogen). H460 cells were co-transfected with brachyury-targeting gRNA and GeneArt Platinum Cas9 nuclease (Invitrogen) following the manufacturer's instructions, and subsequently grown and seeded for single cell sub-culture onto 96-well plates. Single clonal populations of H460-CRISPR cells were grown and evaluated for brachyury protein expression by western blot analysis with an anti-brachyury rabbit monoclonal antibody (MAb54-1). Three clones exhibiting high, intermediate and low levels of brachyury protein, respectively, were selected, expanded and banked for subsequent studies.

YAP-promoter luciferase assay. JHC7 cells were transfected with siRNAs as described above, incubated for 24hr, and then transfected with 1μg plasmid DNA encoding the promoter region of YAP (-106 to TSS or -608bp to TSS; kind gift from Drs. Jiayi Wang and Fenyong Sun) fused in frame to firefly luciferase or with a control GAPDH promoter fused in frame to renilla luciferase (SwitchGear Genomics, Menlo Park, CA) using 3μL FuGENE HD transfection reagent (Promega, Madison, WI) according to the manufacturer's instructions. Cells were incubated for 24hr and then seeded in triplicate into the wells of a white-walled 96-well plate. Firefly luciferase activity was measured using the ONE-Glo luciferase assay system (Promega, Madison, WI) according to the manufacturer's instructions, and Renilla luciferase activity was measured using the Renilla-Glo luciferase assay system (Promega, Madison, WI) according to the manufacturer's instructions. A duplicate plate for normalization was also seeded in triplicate, and cell viability was measured using the CellTiter-Glo assay (Promega, Madison, WI) according to the manufacturer's instructions. Luciferase activity measurements were corrected

by subtracting background measurements, and then relative luciferase activity was calculated by first normalizing corrected firefly luciferase counts to the corresponding average cell viability (CellTiter-Glo signal), and then dividing these normalized counts by the corresponding average renilla GAPDH signal.

Proliferation assay. MTT assay was conducted in 96-well plates, with each independent time point performed in triplicates to establish statistical significance. Wells were seeded at 3000-6000 cells/well. Monotetrazolium (MTT, Sigma) was suspended at a concentration of 5 mg/ml in PBS (MTT solution). 10% MTT solution was prepared in fresh cell culture media, and cells were incubated at 37°C for 4 hours. After 4 hours, each well was aspirated and the formazan salt was solubilized using 200 µl of isopropyl alcohol. Optical density measurements were read using a spectrophotometric plate reader (Epoch) at 570 nm. Measurements were taken at different time points for growth curve analysis. For experiments involving control shRNA and target gene shRNA transduced cells, growth curve studies were performed 10 days post-transduction and selection.

Immunoblotting and antibodies. All patient-derived tumor tissue specimens were obtained at the Johns Hopkins Hospital with appropriate patient consent in accordance to Institutional Review Board approval. Tissues from the operating room were washed in HBSS prior to freezing them in liquid nitrogen. Protein extraction from tissue specimen was performed by homogenizing tissues on ice using T-PER lysis buffer supplemented with protease and phosphatase inhibitor and collected upon removal of all debris. Cultured cells were lysed on ice with a radioimmunoprecipitation assay lysis buffer (Pierce) supplemented with protease and protease inhibitor cocktails (Pierce) and harvested using a cell scraper. Extracts were incubated for 30 minutes for complete lysis and centrifuged at 14,000G for 15 minutes at 4°C to pellet cell

debris. Supernatants containing protein were quantified for protein concentration using a BCA Protein Assay kit (Pierce) and a spectrophotometric plate reader (BioTek). Most protein lysates were separated by running 30-100 μg of lysate on 10% Bis-Tris NuPage gels (Invitrogen) and subsequently, transferred to 0.2μm pore polyvinylidene fluoride (PVDF) membranes (BioRad). Primary antibody incubations were performed following the manufacturer's recommendations in 0.1% Tween TBS supplemented with 5% non-fat dry milk or BSA. Protein bands were visualized using the appropriate horseradish peroxidase-conjugated anti-IgG antibodies (Pierce), and developed using chemiluminescence or prime detection reagent (GE Healthcare).

Antibodies

Target	Company	Note
YAP	Cell Signaling	Clone 4912
β-actin	Abcam	
Brachyury	Santa Cruz	Clones C-19 and, N-19
Brachyury	NCI	Clone 54-1 (Hamilton et al., 2015)
pLATS	Cell Signaling	Ser909; Clone 9157
LATS	Bethyl Laboratories	
GAPDH	Santa Cruz	
Cyclin D1	Abcam	
Survivin	Cell Signaling	

Immunohistochemistry. Paraffin-embedded patient-derived chordoma tissue blocks were deparaffinized for 15 minutes in xylene and rehydrated with graded alcohols and distilled water washes. Antigen retrieval was performed using a citrate 6.0 retrieval solution (Dako) in a steamer at 100°C for 25 minutes, and subsequently cooled for 25 minutes at room temperature, and subsequently washed in tap water followed by wash buffer (Dako). Next, 3% hydrogen peroxide was used to block endogenous peroxidase for 5 minutes at room temperature and rinsed in wash buffer (Dako). Next, a serum-free protein block (Dako) was conducted for 5 minutes at room

temperature. This was followed by incubation with primary antibody for 1 hour at room temperature, and rinsed twice with wash buffer (Dako). An anti-rabbit labeled polymer (Envision anti-Rabbit HRP antibody, Dako) or anti-mouse labeled polymer (Envision anti-mouse-HRP antibody, Dako) was used for 30 minutes at room temperature; and rinsed in wash buffer twice. Next, DAB+ (Dako) was used for 5 minutes at room temperature, and rinsed in distilled water. Samples were counterstained in Gills I hematoxylin for 30 seconds and rinsed in tap water, and subsequently dehydrated, cleared, mounted and imaged.

Soft agar colony formation assay. A bottom agarose layer was prepared using 1% agarose base in complete culture media. A top layer 0.7% agarose in complete media was prepared with a single cell suspension of approximately 1000 cells. The cultures were supplemented with fresh media twice a week. Cells were fixed and stained using Crystal violet, imaged, and quantified for colony formation using Image J.

***In vitro* extreme limiting dilution assay (ELDA).** The assay was performed as previously described (Hu and Smyth, 2009). The following website from Walter and Eliza Hall Institute of Medical Research was used to generate experimental data: http://bioinf.wehi.edu.au/software/elda/. Briefly, 1, 5, 20, or 50 cells/well with complete culture media were plated in a 96-well plate using a flow-cytometry sorter. Sphere formation was monitored over a period of 14-18 days and each well is quantified and scored thereafter. A semilogarithmic plot is generated of the fraction of negative cultures also referred to as "nonresponding" (i.e. those wells lacking spheres) as a function of the dose of cells placed in each culture.

Cell Cycle Analysis. Briefly, cells were washed in PBS and fixed with 70% ethanol. Following treatment with ribonuclease I, cells were stained with PI and analyzed using a flow cytometer after gating for live single cells only. Quantification of results was conducted using FlowJo.

Aldefluor Assay. The ALDEFLUOR kit (StemCell Technologies, Durham, NC, USA) was used to identify the population of cancer stem cells with high ALDH enzymatic activity. Cells grown *in vitro* were cultured under different conditions as described in (Suppl. Fig. 3a) for 48 hours and then assayed per manufacturer's instructions. Briefly, cells were suspended in ALDEFLUOR assay buffer containing ALDH substrate (BAAA, 1 **μ** mol/l per $1x10^6$ cells) and incubated for 40 minutes at 37°C. As negative control, for each sample of cells, an aliquot was treated with 50mmol/L diethylaminobenzaldehyde (DEAB), a specific ALDH inhibitor. The sorting gates were established using negative controls (cells stained with PI only, for viability) and the ALDEFLUOR-stained cells treated with DEAB. Serum vs. non-serum conditions were compared to evaluate loss of the cancer stem population under high serum conditions.

Side population Analysis. Briefly, a cell suspension of $1x10^6$ was incubated for 2 hours at 37°C in serum-free media containing 5μg/ml Hoechst 33342 and gently mixed every 15 minutes. Parallel sample cell aliquots containing 50 μM verapamil to confirm ABCG2 inhibition were prepared. After complete washing with HBSS, cells were incubated in propidium iodide for 5 mins at a concentration of 5ug/ml in reduced serum-containing media and analyzed using flow cytometry after gating for live cells only.

Spheroid size assay. Spheroid cultures were established in non-adherent, low attachment cell culture flasks. Spheroids were then dissociated into single cells using accutase with gentle trituration. $1X10^6$ live cells from each group were plated and maintained for a week. After a week, spheres were counted using a bright field microscope from 10 different fields per flask.

Cellular structures consisting of 3 or more cells were considered spheres. Counting and image acquisition was performed independently by two individuals. Sphere area was determined using ImageJ.

RNA extraction and quantitative real-time PCR. Total RNA was extracted in TRIzol, phase separated using chloroform, and precipitated overnight at 4°C in isopropanol. Further extraction of total RNA was performed using the RNeasy Mini Kit (Qiagen) following the manufacturer's instructions. One μg purified RNA was reverse transcribed using the Superscript III First-Strand cDNA Synthesis Kit (Invitrogen). Quantitative real-time PCR using SYBR Green (Applied Biosystems) was conducted on a 7300 Cycler (Applied Biosystems) and the included data analysis software. GAPDH or β-actin primers were used as a loading control. Primer sequences were designed and verified using https://pga.mgh.harvard.edu/primerbank/.

For mRNA measurements from the H460 T-CRISPR clones, total RNA was prepared using the PureLink RNA Mini Kit (Thermo Fisher Scientific, Waltham, MA) and reverse transcribed with the XLAScript cDNA MasterMix (WordWide Life Sciences, Hamilton, NJ). The resulting cDNA (10ng) was amplified in triplicate with the following TaqMan human gene expression assays (Thermo Fisher Scientific): YAP1 (Hs00902712_g1) and GAPDH (4326317E). Expression of each target gene relative to GAPDH was calculated as $2^{-(Ct(GAPDH) - Ct(target\ gene)}$.

Cycloheximide assay. H460 T-CRISPR clones were treated in culture with 100μg/mL cycloheximide (Sigma-Aldrich) for indicated time points prior to obtaining protein lysates for immunoblot analysis with anti-YAP and β-actin primary antibodies. Western blots were imaged and quantified using the Odyssey infrared Imaging System (LI-COR Biotechnology). YAP protein levels were first normalized to β-actin levels for each sample, and subsequently

normalized to 0 hours (100%) for calculation of % protein expression remaining at each time point.

Proteasome inhibitor experiment. H460 T-CRISPR clones were seeded at 4.0 x 10^5 – 1.0 x 10^6 cells per 60mm dish, incubated overnight, and then treated with 20μM MG-132 (Sigma-Aldrich, St. Louis, MO) for 16hr prior to processing for immunoblot analysis**.**

***In vivo* experiments: subcutaneous xenografts**. Animal protocols were approved by the Johns Hopkins School of Medicine Animal Care and Use Committee. Subcutaneous tumor cell implantation into mice was conducted according to the protocol as described previously (Hsu et al., 2011). Briefly, non-obese diabetic/severe combined immunodeficient mice (NCI) and athymic nude mice (Jackson Laboratories) were manually immobilized in a prone position to perform cell injections. For the *in vivo* limiting dilution study, athymic nude 4-week old male mice were injected subcutaneously with cultured JHC7 chordoma cells in 25 μl of PBS at increasing cell counts of 10^3, 10^4, 10^5, and 10^6 cells using a 23-gauge syringe. Animals were given free access to water and rodent chow, and were monitored closely for development of tumor over a period of 1 year. All animals were treated in accordance with the policies and principles of laboratory animal care of the Johns Hopkins University School of Medicine Animal Care and Use Committee.

For the shCtrl versus shT tumor formation experiment using non-obese diabetic/severe combined immunodeficient mice (NCI) was conducted following the procedure as described previously in *Hsu et al., 2011*. Briefly, 4-week old male mice were injected subcutaneously with 10^6 cells of either JHC7 shCtrl or shT, and monitored periodically for tumor formation over a period of 1 year. All animals were treated according to the policies and principles of laboratory animal care of the Johns Hopkins University School of Medicine Animal Care and Use Committee.

ChIP Sequencing Analyses. Analysis of ChIP-Sequencing data was performed as previously described (Zanconato et al., 2015). Histone modification data tracks were downloaded and used as described in *Rhie et al., 2014* (Rhie et al., 2014).

Detection of brachyury by RNA-Seq. Pre-processed RNA-seq data was downloaded from The Cancer Genome Atlas (TCGA GBM dataset (Brennan et al., 2013). Samples with zero RPKM were considered negative, and the rest positive for brachyury.

Gene expression analyses from microarray data. Affymetrix microarray data was processed using the aroma.affymetrix package in R with updated probeset mappings from BrainArray v15.1. Differential expression analysis was performed using the siggenes package: genes with at least 50% change in expression and FDR < 0.05 were considered to be differentially expressed. These differentially expressed genes were used as up- or down-regulated gene expression signatures.

Gene set analyses. To assess significant co-expression of gene signatures in patient cohorts, a signature z-score was computed for each patient as previously described (Irizarry et al., 2009). From these scores, patients were categorized as high (score > 0.5), low (score < -0.5), or medium expression (score between -0.5 and 0.5).

Statistical enrichment between gene signatures was calculated by counting the genes found in both signatures (shared genes). This number was compared to random expectation with Fisher's exact test, which considers the size of each gene signature and the total number of genes in the genome.

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SUPPLEMENTAL FIGURES

Figure S1, related to Figure 1 | Brachyury regulates cancer stemness and proliferation in chordoma. a, Representative immunoblot of brachyury expression in shCtrl, shT-1 (shT), or shT-2 JHC7 cells. **b,** mRNA expression of cell cycle regulator genes in shCtrl or shT UCH1 cells. **c, d,** Representative MTT proliferation assay of shCtrl or shT1 (shT) JHC7 and UCH2 cells. **e,** Representative phase contrast and immunofluorescence staining images of JHC7 cells. **f,** Representative flow cytometry plots of CD90, CD105, CD31, CD45 expression in JHC7 cells. Human mesenchymal stem cells were used as a positive control; data not shown. **g,** Left: Representative images of athymic nude mice bearing JHC7-derived flank subcutaneous tumor. Right: Representative immunohistochemical images of Oct, vimentin, and nestin staining in chordoma xenografts established from JHC7 cells. **h,** Top: Representative phase contrast images of JHC7 sarcospheres. Bottom: Representative images of Oil Red staining after adipogenic differentiation of JHC7 cells. Human mesenchymal stem cells were used as a positive control; data not shown. **i,** Top: Representative phase contrast images of JHC7 sarcospheres. Bottom: immunofluorescence staining images of GFAP and Tuj1 in JHC7 exposed to 10% FBS. (human mesenchymal stem cells were used as a positive control, data not shown). All error bars are s.e.m. *= $P<0.05$.

Figure S2, related to Fig 1| Chordoma xenografts harbor a putative stem cell population. a, Representative flow cytometry plots of aldefluor staining using adherent and spheroid cultures of JHC7 cells. **b,** Representative images of NOD/SCID mice bearing JHC7-derived flank subcutaneous tumor upon serial passaging and implantation for three subsequent generations from left to right. **c,** Flow cytometry plots of Hoechst staining with or without verapamil in JHC7 cells. Only live cells gated using propidium iodide were analyzed. **d,** Side population analysis of shCtrl and shT JHC7 cells. **e,** ABCG2 mRNA expression in shCtrl or shT JHC7 cells. **f,** mRNA expression of stemness-related genes in shCtrl or shT JHC7 cells. All error bars are s.e.m. *= $P<0.05$.

Figure S3, related to Fig 2| Patient-derived primary GBM cells express Brachyury. a, Kaplan-Meier graph of cumulative progression-free survival in patient groups defined by high or low T-transcript expression in TCGA dataset. **b,** Representative immunoblot of brachyury expression in primary glioblastoma patient cell lines. **c,** mRNA expression of stemness-related genes in adherent and spheroid cultures of GBM1A cells. **d, e,** mRNA expression of T and nestin (NES) in shCtrl vs shT GBM1A and GBM1049 cells. **f,** Spheroid size of shCtrl and shT GBM1A cells. **g,** Representative immunoblot of brachyury and YAP expression in shCtrl, shT1, and shT2 GBM1A cells. **h,** YAP mRNA expression in shCtrl vs shT GBM1A cells. **i,** Representative immunoblot of brachyury and YAP expression in shCtrl, shT1, and shT2 GBM1049 cells. j, k, T and YAP mRNA expression in shCtrl vs shT GBM1049 and GBM612 cells. All error bars are s.e.m. *= $P<0.05$.

Figure S4, related to Fig 3 and 4| Brachyury regulates YAP. a, Representative immunoblot of brachyury and YAP expression in shCtrl, shT1, and shT-2 JHC7 cells. **b,** YAP mRNA expression in shCtrl vs shT-2 JHC7, UCH1, UCH2 cells. **c,** mRNA expression of YAP downstream target genes in shCtrl and shT JHC7 cells. **d,** Representative immunofluorescence image of DAPI, brachyury, and YAP staining in JHC7 cells. **e,** Representative immunoblot of brachyury, YAP, and TAZ expression in siCtrl vs siT JHC7 cells. **f,** Brachyury binding to the proximal promoter region of TAZ as observed in a publicly available ChIP-sequencing dataset using UCH1 cells. **g, h,** Representative immunoblot of brachyury and YAP expression in siCtrl vs siT JHC7 cells untreated or treated with MG132 (20μM, 16hr). All error bars are s.e.m. *= $P<0.05$.

Figure S5, related to Fig 3| Histological images of patient-derived chordoma tissues. Representative images of immunohistochemical staining of Brachyury, YAP, and H&E in patient-derived chordoma tissues. Images shown at low and high magnification where the scale bars are 2mm or 200μm, respectively,

Figure S6, related to Figs 3 and 5| Expression levels of T and YAP-targets correlate in patient-derived chordoma tissues. a-h, Correlation plot of relative brachyury or YAP mRNA expression and YAP target genes (normalized to β-actin) in a subset of patient-derived chordoma tissues (n=10). **i,** Representative immunoblot of

YAP expression in shCtrl, shYAP-1, and shYAP-2 JHC7 cells. **j,** Side population analysis of shCtrl and shYAP JHC7 cells. **k,** ABCG2 mRNA expression in shCtrl or shYAP JHC7 cells. **l,** Representative long-term MTT proliferation assay of shCtrl or shYAP-2 JHC7 and UCH2 cells. **e,** mRNA expression of cell cycle regulator genes in CONT or YAP-OE JHC7 cells. All error bars are s.e.m. *= P<0.05.

Figure S7| Brachyury regulates YAP in carcinomas by enhancing protein stability. a, Left: Immunoblots of brachyury and YAP expression in patient samples of metastases to the brain. Right: Densitometric quantification of brachyury and YAP expression (normalized to β-actin). **b,** YAP gene signature expression in T-low, T-intermediate, and T-high patient groups in the TCGA lung cancer dataset. One sample t-test. **c,** Representative immunoblot of brachyury and YAP expression in 3 different T-CRISPR clones of H460 cells. **d,** mRNA expression of YAP in 3 different T-CRISPR clones of H460 cells. **e,** Densitometric quantification of YAP expression (normalized to β -actin) in T-high and T-intermediate (T-int) CRISPR clones (1 and 2, respectively) of H460 cells treated with cycloheximide (100μg/mL) for the indicated time points. **f,** Representative immunoblot of brachyury and YAP expression in T-high and T-intermediate CRISPR clones (1 and 2, respectively) of H460 cells untreated or treated with MG132 (20μM, 16hr). **g,** Stemness-related gene signature expression in T-low, T-intermediate, or T-high patient groups in the TCGA lung cancer dataset. ES1/2, embryonic stem cell signatures. NOS, Nanog, Oct4, and Sox2 signature. One sample t-test. **h,** Top: Representative images of soft agar colony formation assay using shCtrl or shT H460 cells. Bottom: Quantification of the soft agar colony formation assay. **i,** T-signature gene expression in lung cancer patient groups in NCIDC dataset stratified by differentiation status of the primary tumor (i.e. well, intermediate, or poor). One sample t-test. All error bars are s.e.m. * = P<0.05.

Figure S8| T-YAP signaling in lung cancer. a-c, Correlation plot of relative brachyury and YAP protein expression (normalized to β-actin) based on densitometric quantification of immunoblots in **Figure 6a**; a.u. = arbitrary units. **d,** Representative immunoblot of TAZ expression in T-CRISPR clones 1-3 of H460 cells. **e,** Representative immunoblot of expression of Hippo pathway kinases in T-CRISPR clones 1-3 of H460 cells. **f,** mRNA expression of YAP downstream target genes in T-high and low CRISPR clones of H460 cells. **g, h,** Representative immunoblot of brachyury, YAP, and TAZ expression in pCMV or pT T-CRISPR clone 3 of H460 cells. **i,** Quantification of the soft agar colony formation assay using shCtrl or shYAP H460 cells. **j,** mRNA expression of stemness-related genes in T-CRISPR clones of H460 cells. k, Representative immunoblot of brachyury and YAP expression in pCMV or pT H1299 cells. All error bars are s.e.m. *= P<0.05.

Figure S9| T-YAP signaling in carcinomas. a, Representative immunoblot of brachyury and YAP expression in pCMV vs pT PANC-1 and ONYCAP23 cells; SW480 and SW620 cells, and siCtrl vs siT SW620 cells. **b,** Densitometric quantification of brachyury and YAP expression (normalized to β-actin) from the immunoblots shown in a. a.u. = arbitrary units. **c,** Correlation plot of relative brachyury and YAP protein expression (normalized to β-actin) based on densitometric quantification of immunoblots in a; a.u. = arbitrary units. **d-g,** mRNA expression of brachyury and YAP expression in pCMV vs pT PANC-1 and ONYCAP23 cells; SW480 and SW620 cells, and siCtrl vs siT SW620 cells. All error bars are s.e.m. *= P<0.05.

Figure S1 | Brachyury regulates cancer stemness and proliferation in chordoma

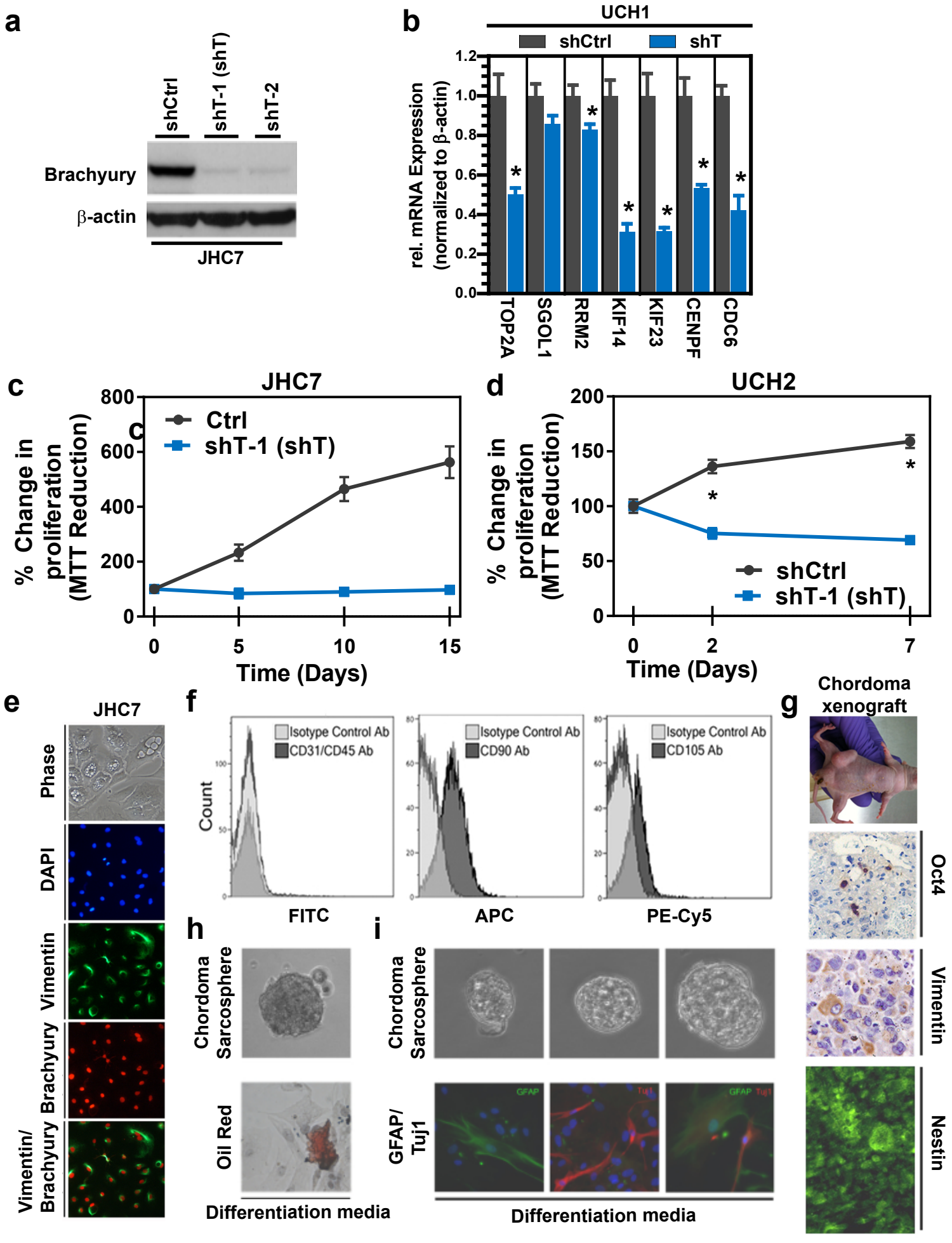
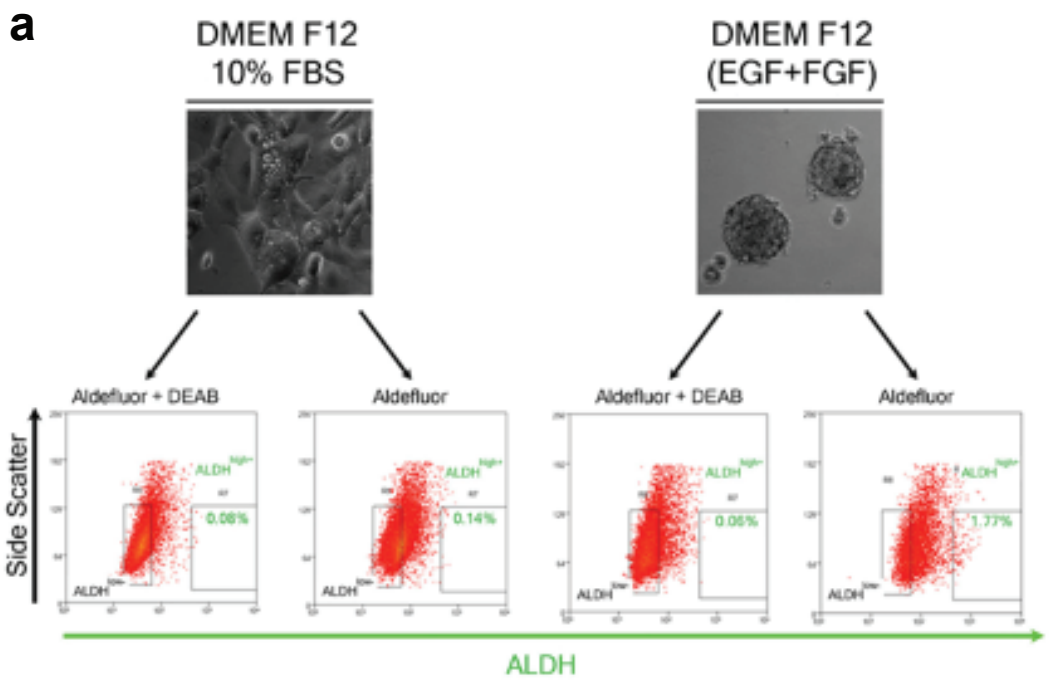
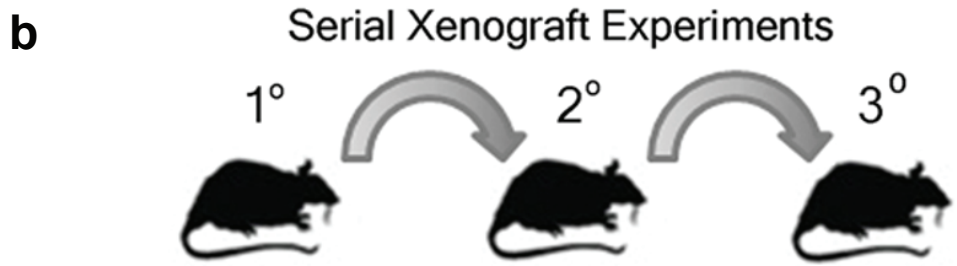
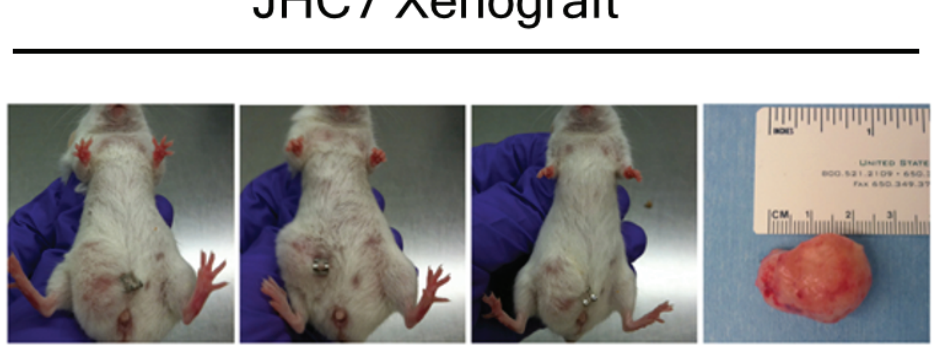
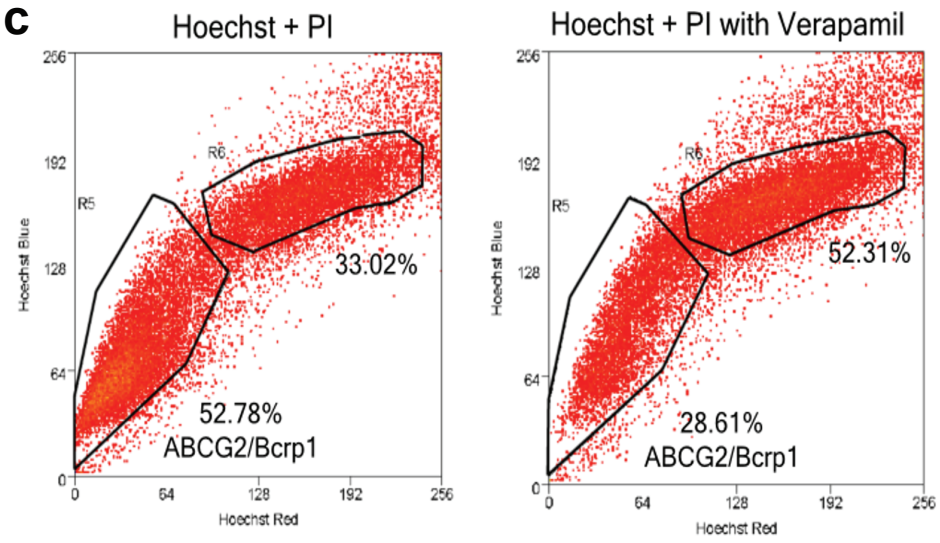


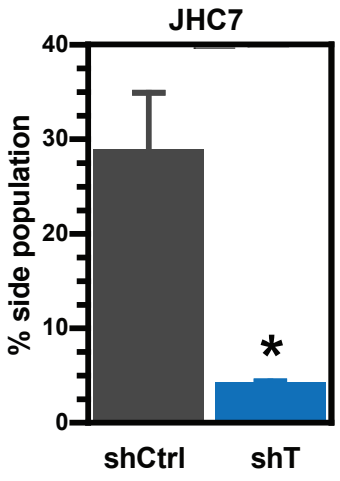
Figure S2 | Chordoma xenografts harbor a putative stem cell population

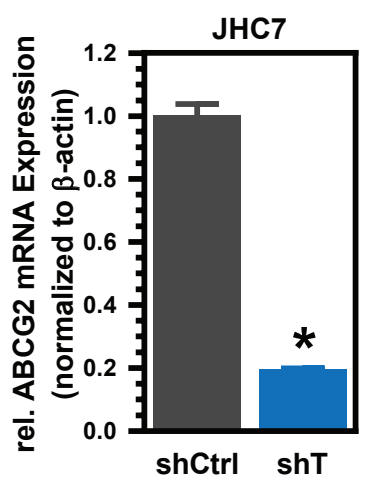












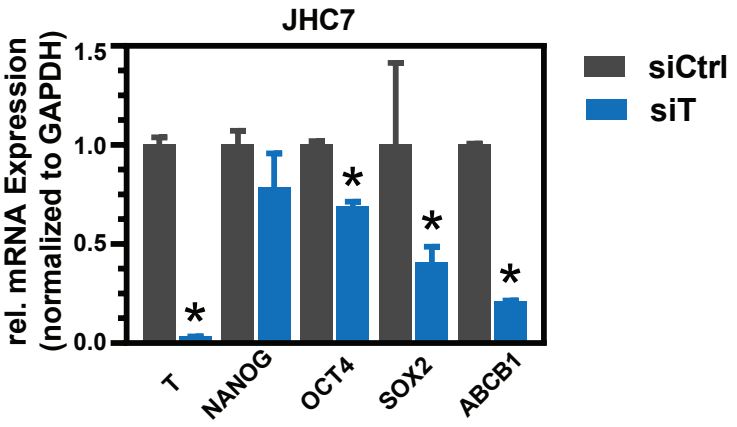


Figure S3 | Patient-derived primary GBM cells express Brachyury

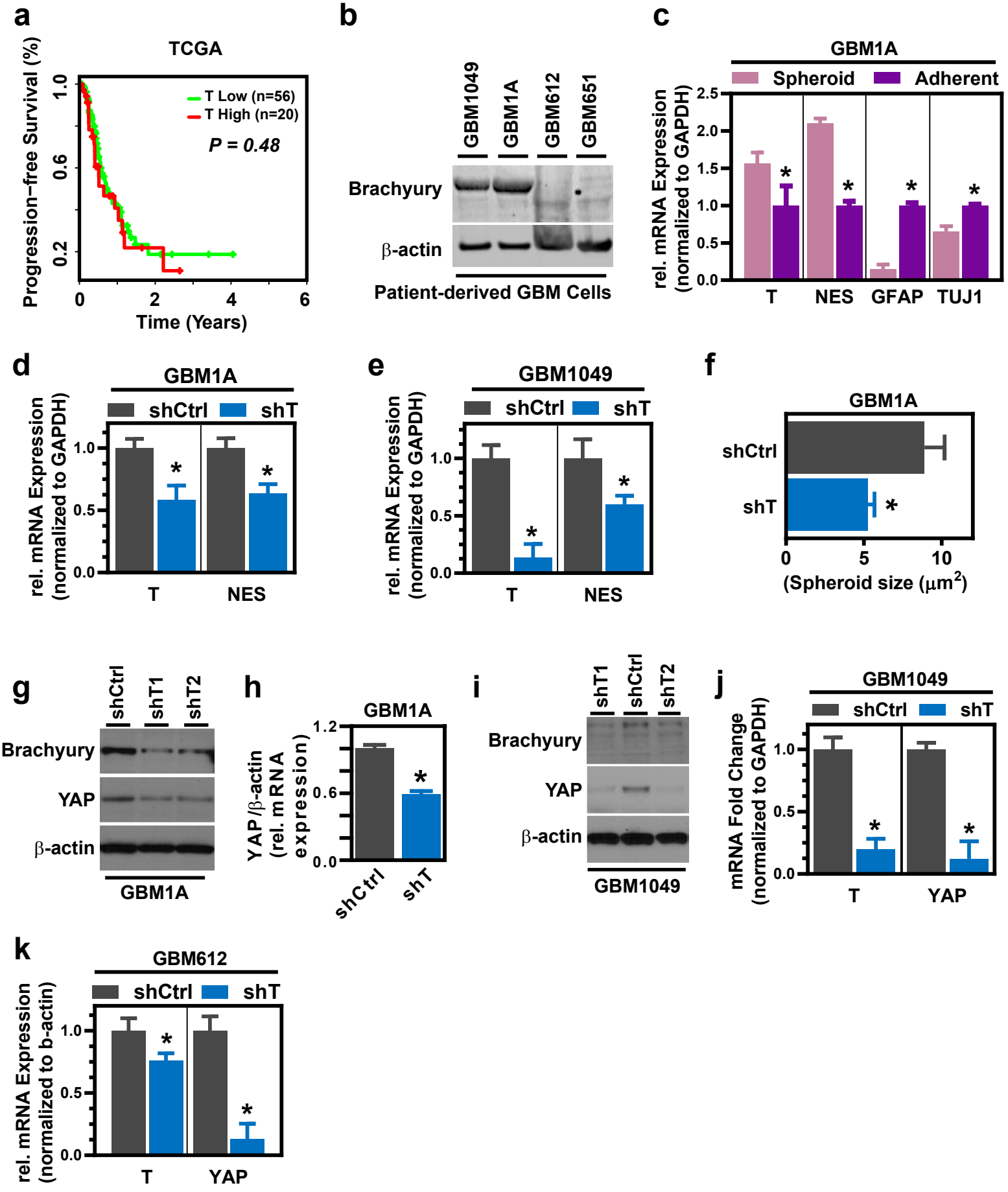


Figure S4 | Brachyury regulates YAP

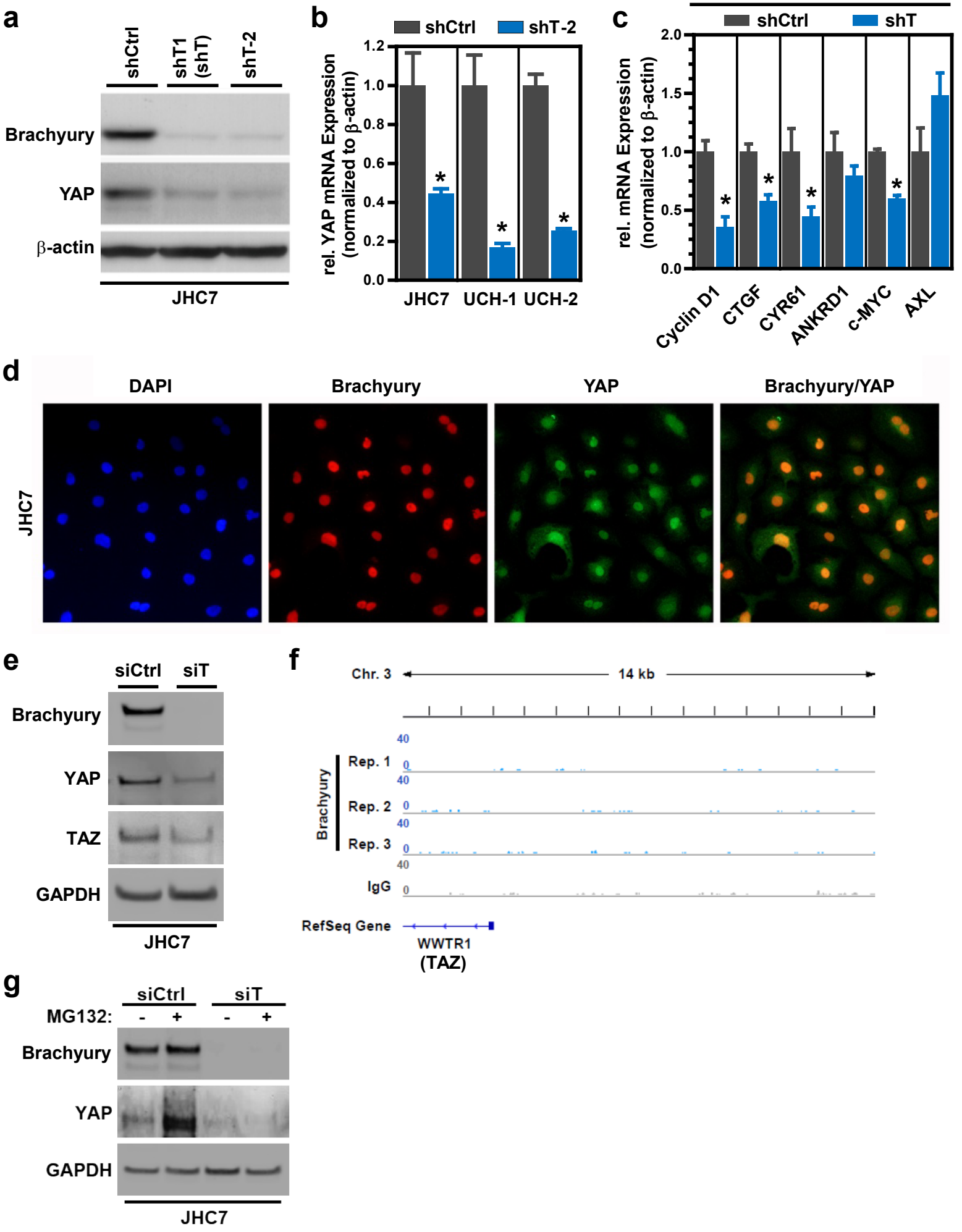


Figure S5 | Histological images of patient-derived chordoma tissues

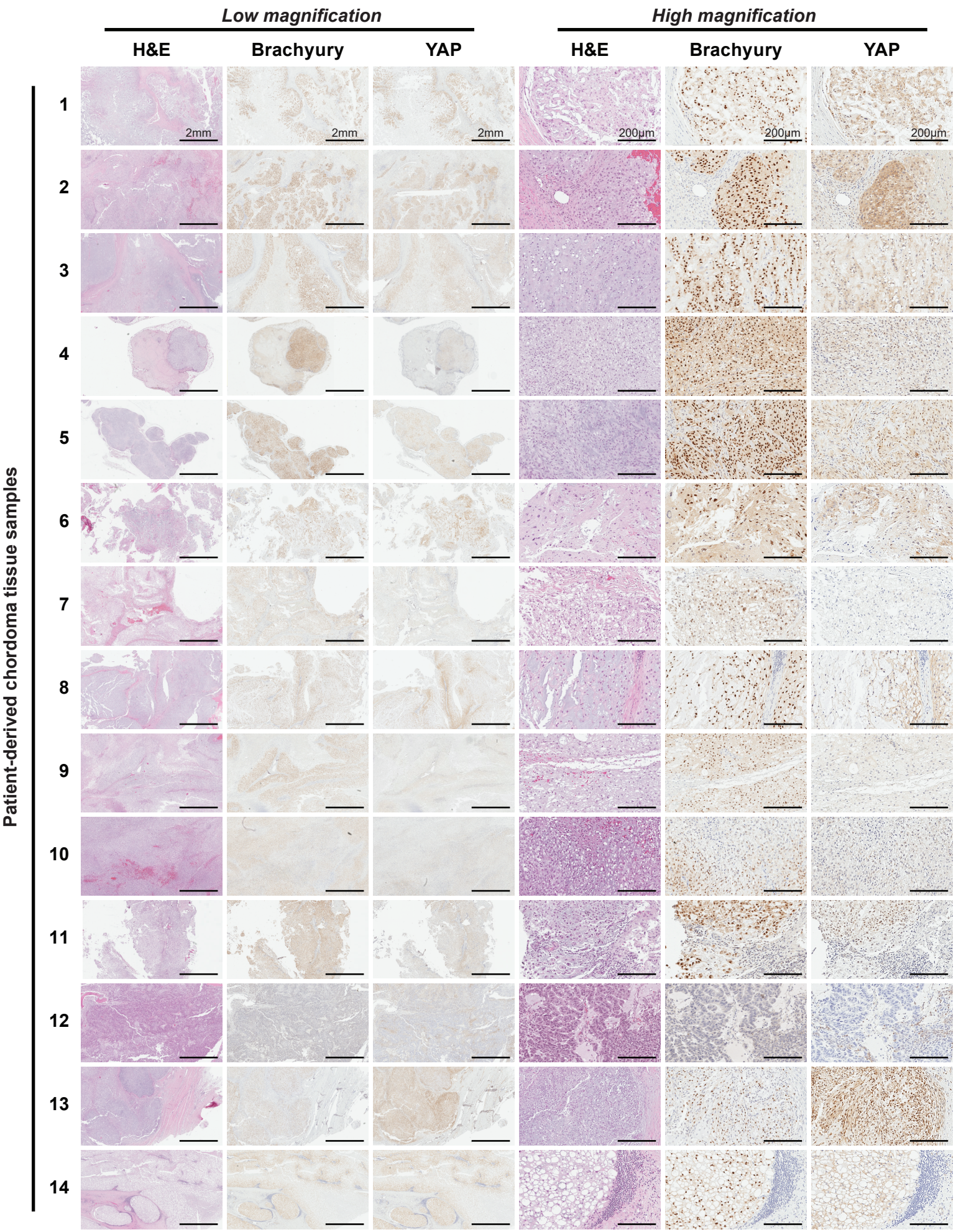


Figure S6 | T-YAP signaling in chordoma

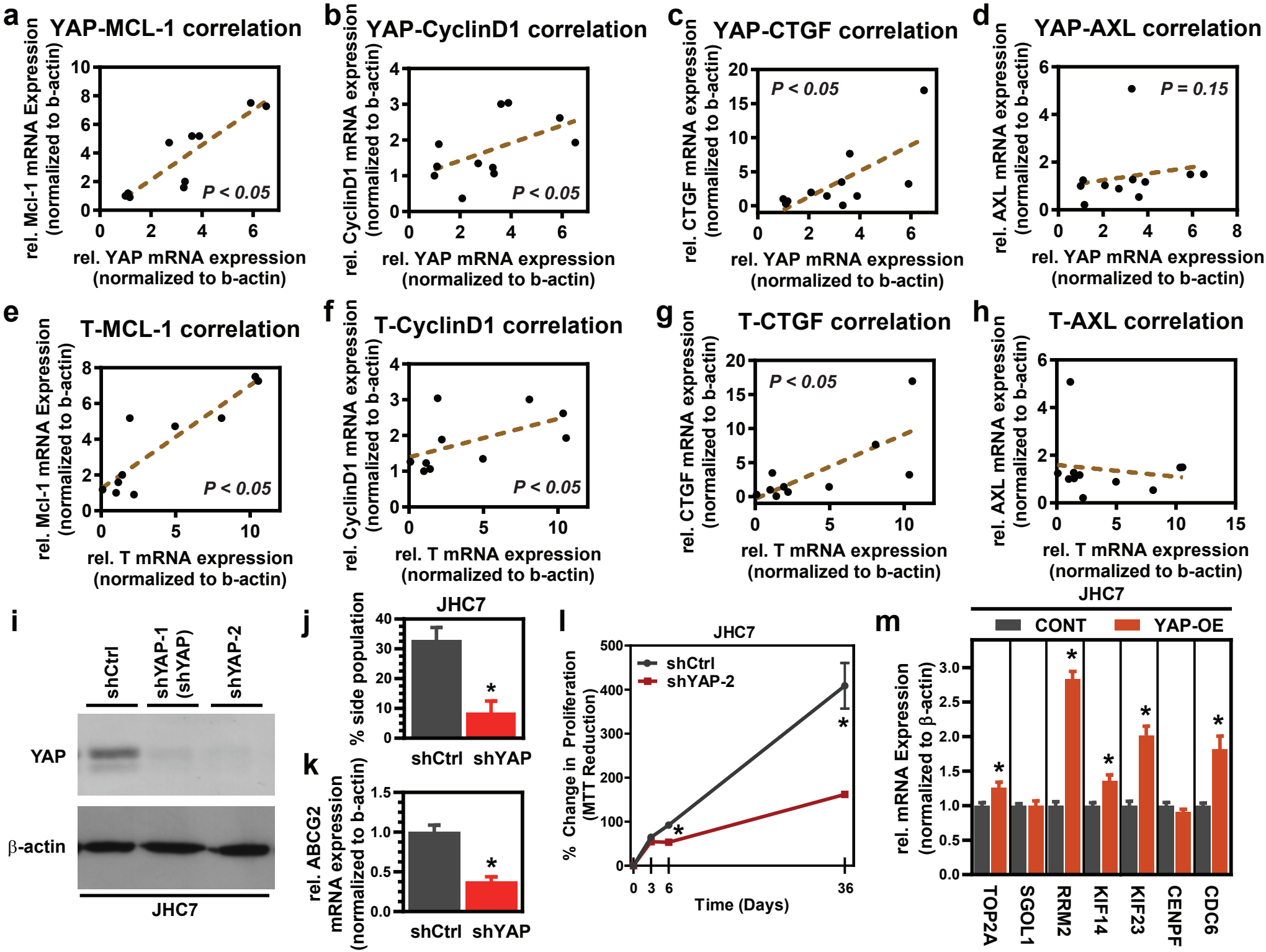


Figure S7 | Brachyury regulates YAP in carcinomas by enhancing protein stability

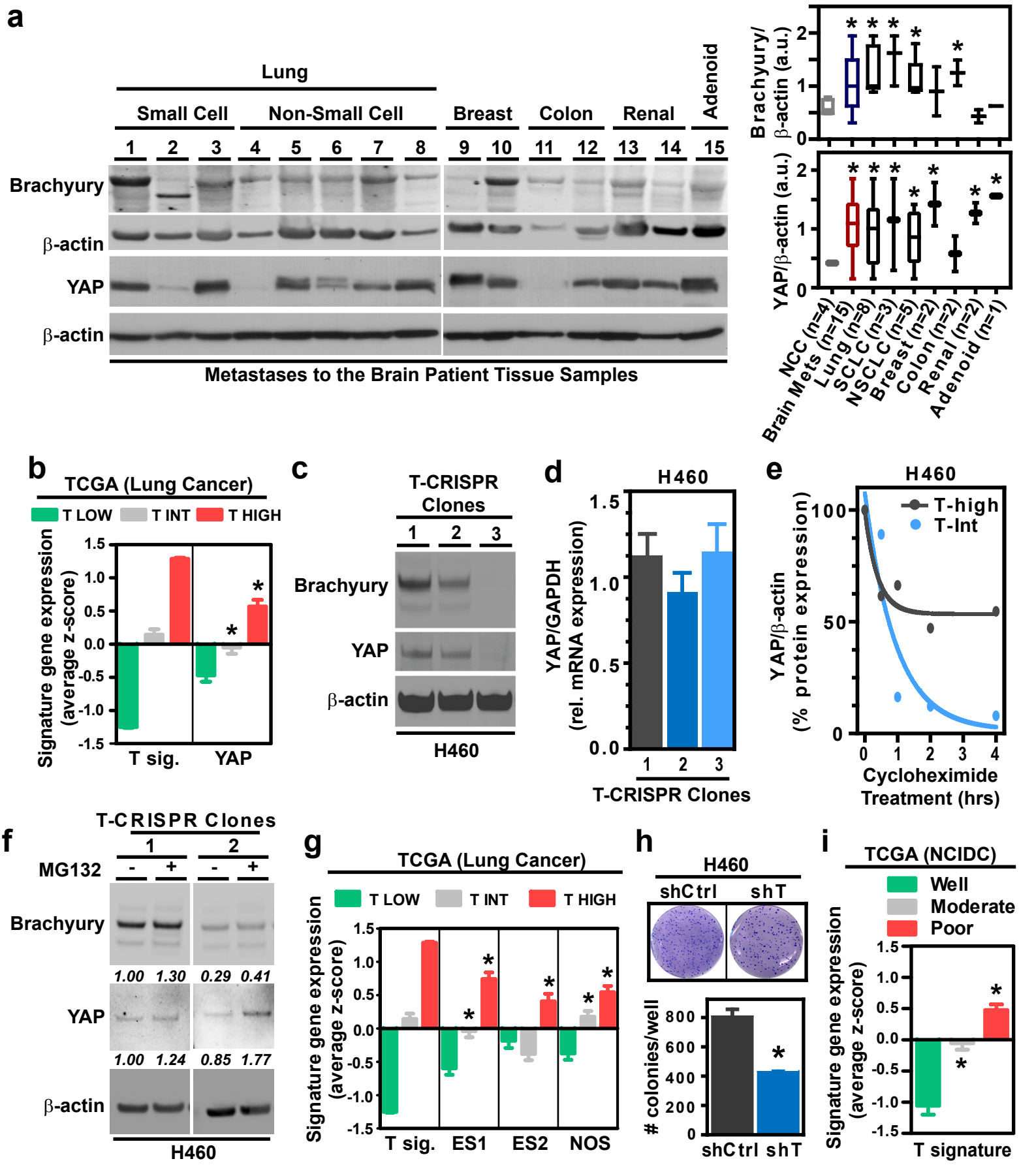


Figure S8 | T-YAP signaling in lung cancer

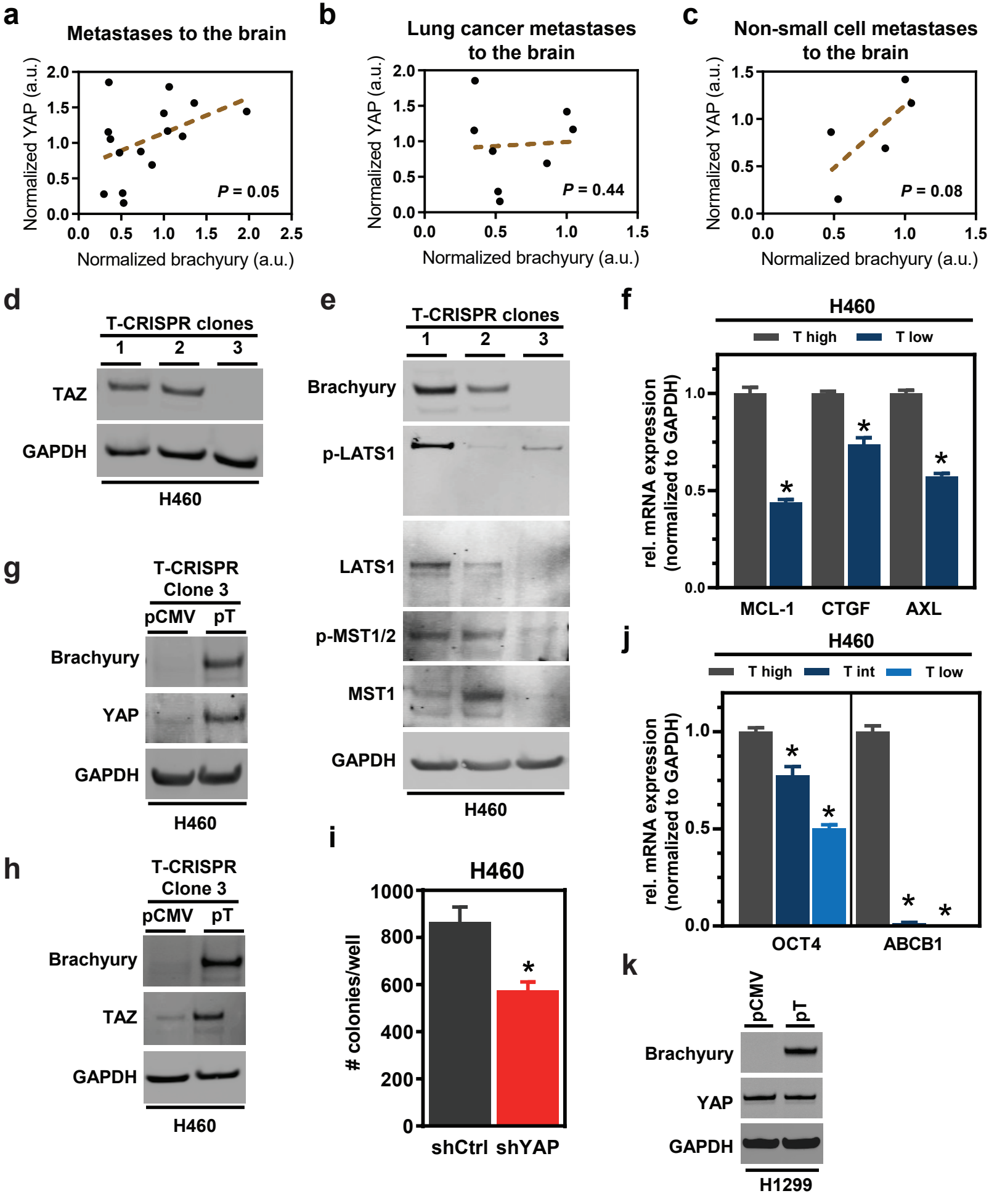


Figure S9 | T-YAP signaling in carcinomas

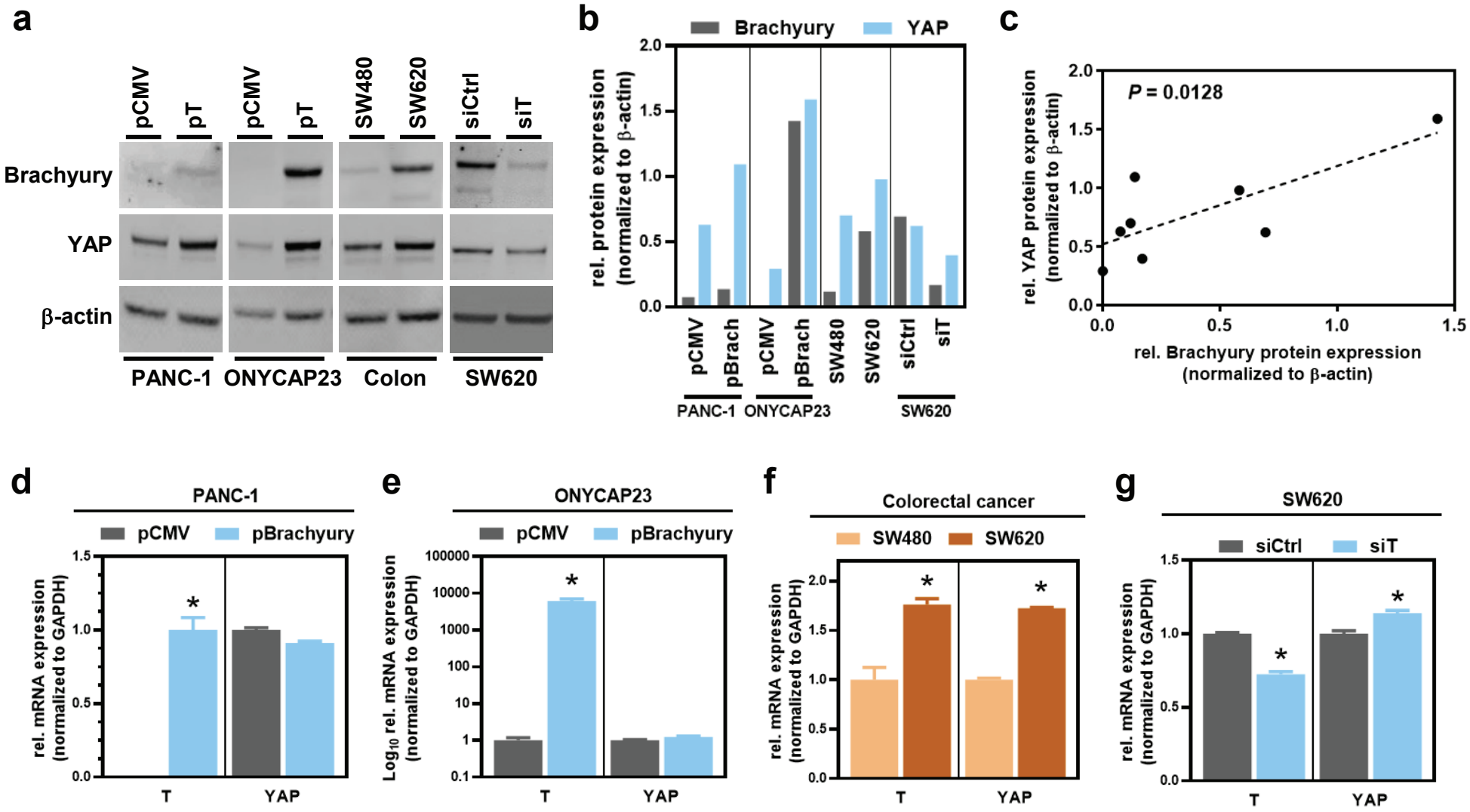


Table S1, related to Fig. 1 | T gene signature.

EntrezID	Symbol	C7_shT_foldchange	UCH1_shT_foldchange	Name
27063	**ANKRD1**	9.603	2.808	ankyrin repeat domain 1 (cardiac muscle)
256691	**MAMDC2**	7.569	1.582	MAM domain containing 2
51200	**CPA4**	6.053	1.866	carboxypeptidase A4
114907	**FBXO32**	5.813	2.081	F-box protein 32
5266	**PI3**	3.517	4.018	peptidase inhibitor 3, skin-derived
11031	**RAB31**	3.438	1.577	RAB31, member RAS oncogene family
284344	**LOC284344**	3.352	2.105	uncharacterized LOC284344
5205	**ATP8B1**	3.214	1.633	ATPase, aminophospholipid transporter, class I, type 8B, member 1
677	**ZFP36L1**	3.057	1.636	zinc finger protein 36, C3H type-like 1
9245	**GCNT3**	2.676	1.966	glucosaminyl (N-acetyl) transferase 3, mucin type
23397	**NCAPH**	-2.076	-1.644	non-SMC condensin I complex, subunit H
11113	**CIT**	-2.091	-1.608	citron (rho-interacting, serine/threonine kinase 21)
10164	**CHST4**	-2.168	-2.246	carbohydrate (N-acetylglucosamine 6-O) sulfotransferase 4
5163	**PDK1**	-2.176	-1.588	pyruvate dehydrogenase kinase, isozyme 1
81796	**SLCO5A1**	-2.184	-1.837	solute carrier organic anion transporter family, member 5A1
10615	**SPAG5**	-2.279	-1.564	sperm associated antigen 5
3832	**KIF11**	-2.279	-2.378	kinesin family member 11
79750	**ZNF385D**	-2.346	-2.178	zinc finger protein 385D
10635	**RAD51AP1**	-2.356	-1.904	RAD51 associated protein 1
10886	**NPFFR2**	-2.36	-1.632	neuropeptide FF receptor 2
9771	**RAPGEF5**	-2.373	-2.757	Rap guanine nucleotide exchange factor (GEF) 5
79733	**E2F8**	-2.417	-1.884	E2F transcription factor 8
9833	**MELK**	-2.492	-1.659	maternal embryonic leucine zipper kinase
55355	**HJURP**	-2.5	-1.502	Holliday junction recognition protein
7103	**TSPAN8**	-2.5	-1.744	tetraspanin 8
286133	**SCARA5**	-2.534	-2.21	scavenger receptor class A, member 5 (putative)
7102	**TSPAN7**	-2.541	-2.216	tetraspanin 7
1368	**CPM**	-2.542	-2.153	carboxypeptidase M
2591	**GALNT3**	-2.544	-2.025	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 3 (GalNAc-T3)
84329	**HVCN1**	-2.583	-2.605	hydrogen voltage-gated channel 1
2491	**CENPI**	-2.693	-2.233	centromere protein I
25928	**SOSTDC1**	-2.712	-3.774	sclerostin domain containing 1
55247	**NEIL3**	-2.733	-1.849	nei endonuclease VIII-like 3 (E. coli)
157570	**ESCO2**	-2.841	-2.28	establishment of cohesion 1 homolog 2 (S. cerevisiae)
701	**BUB1B**	-2.842	-1.751	budding uninhibited by benzimidazoles 1 homolog beta (yeast)
57016	**AKR1B10**	-2.868	-3.215	aldo-keto reductase family 1, member B10 (aldose reductase)
9108	**MTMR7**	-2.875	-2.958	myotubularin related protein 7
81617	**CAB39L**	-2.917	-1.792	calcium binding protein 39-like
56243	**KIAA1217**	-2.926	-1.617	KIAA1217
57488	**ESYT2**	-2.942	-1.826	extended synaptotagmin-like protein 2
4306	**NR3C2**	-2.949	-1.615	nuclear receptor subfamily 3, group C, member 2
3702	**ITK**	-3.001	-1.562	IL2-inducible T-cell kinase
9156	**EXO1**	-3.104	-1.766	exonuclease 1
285605	**DTWD2**	-3.162	-1.886	DTW domain containing 2
285966	**FAM115C**	-3.18	-2.265	family with sequence similarity 115, member C
387103	**CENPW**	-3.183	-1.503	centromere protein W
5243	**ABCB1**	-3.39	-1.755	ATP-binding cassette, sub-family B (MDR/TAP), member 1
55304	**SPTLC3**	-3.596	-1.983	serine palmitoyltransferase, long chain base subunit 3
2690	**GHR**	-3.601	-1.825	growth hormone receptor
285025	**CCDC141**	-3.671	-2.112	coiled-coil domain containing 141
3776	**KCNK2**	-3.677	-2.289	potassium channel, subfamily K, member 2
3751	**KCND2**	-3.938	-3.741	potassium voltage-gated channel, Shal-related subfamily, member 2
2823	**GPM6A**	-4.009	-1.69	glycoprotein M6A
29091	**STXBP6**	-4.227	-1.606	syntaxin binding protein 6 (amisyn)
51059	**FAM135B**	-4.72	-2.161	family with sequence similarity 135, member B
22986	**SORCS3**	-4.864	-1.891	sortilin-related VPS10 domain containing receptor 3
115701	**ALPK2**	-4.96	-1.817	alpha-kinase 2
1008	**CDH10**	-5.102	-1.871	cadherin 10, type 2 (T2-cadherin)
6862	**T**	-5.34	-2.349	T, brachyury homolog (mouse)
3880	**KRT19**	-5.5	-2.364	keratin 19
30811	**HUNK**	-5.879	-2.269	hormonally up-regulated Neu-associated kinase
117195	**MRGPRX3**	-6.412	-2.386	MAS-related GPR, member X3
729025	**SLC15A5**	-6.896	-3.45	solute carrier family 15, member 5
8358	**HIST1H3B**	-8.18	-1.719	histone cluster 1, H3b
27306	**HPGDS**	-11.278	-4.689	hematopoietic prostaglandin D synthase

Genes with greater than 2-fold decrease in RNA abundance relative to shCtrl in JHC7 and UCH1 cells were considered to be potential targets of brachyury and together defined the “T gene signature”