Supplemental Information

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EXTENDED EXPERIMENTAL PROCEDURES

Viral Vectors

For overexpression YAP or mutant YAP (2SA), Flag tagged- YAP or mutant YAP (2SA) was delivered by a retroviral vector containing a puromycin resistance cassette. Lentiviral vectors expressing shRNA to MST1 (hygromycin-resistant), MST2 (puromycin-resistant) or YAP (puromycin-resistant) were used for the generation of stable cell lines. The Sox2 lentivirus cloned in the RFP-expressing lentivirus FUCRW, the control GFP and GFP-Cre lentivirus were used as described (Basu-Roy et al., 2010; Seo et al., 2011). The c-MYC and YAP lentivirus were generated by PCR cloning into the FUCRW lentiviral backbone. All infections were carried out in the presence of 8μg/ml polybrene.

CHIP-Seq and Data analysis

Briefly, 10^7 immortalized osteoprogenitors (OB1) or OB1 cells transduced with a Sox2 lentivirus (OB1-Sox2) were cross-linked with 1% formaldehyde and quenched by 1M glycine. Cells were lysed in lysis buffer (1% SDS, 10mM EDTA, 50mM Tris-Hcl (pH8.0), protease inhibitors) followed by sonication with a Misonix 3000 Sonicator to shear chromatin. Thirty μg of chromatin fragments were immunoprecipitated with 5ug of Sox2 antibody purchased from R&D Systems (AF2018) or IgG control overnight. The next day, protein G-agarose beads were added to the chromatin – antibody solution. Elution and reverse-crosslinking were performed as described in (Seo et al., 2011). Construction of DNA library for ChIP-Seq and GA-IIX Illumina sequencing were performed at Genome Technology Center at the NYU School of Medicine. Each sequencing experiment yielded 40 million raw reads 3nt long; the reads were mapped onto the mouse genome UCSC mm9 using *bwa*, obtaining 23-25 million high-confidence mapped reads (Adhikari et al., 2010). High-resolutiongenome-wide maps were derived and visualized in UCSC Genome Browser. Finally, the *macs* peak calling package was used to identify Sox2 enriched regions (Crisan et al., 2008). Sequencing reads from IgG control were used as a negative control. For ChIP assay of Sox2 consensus site in the YAP 5′-UTR and the c-MYC promoter region, and YAP/Tead site on Dkk1 upstream region, antibody pull-down for Sox2-bound or flag-YAP-bound chromatin was performed similarly to the Sox2-ChIPSeq. Amplification of Sox2 or YAP-bound chromatin fragments was carried out with specific primers.

YAP exon I, F- A G A C T G G G C A T C A G C T C C T,, R-C T T C C C C G A C T A C C T G G A A
c-*myc*, F-A G C G A G A G A C A G A G G G A G T G, R- A A G C C C C T C T C A C T C C A G A
Dkk1, F-TTT AGC ACT GTG ATG GTT GGA, R-AAC AGA GGA CCT CCT GGA AAA

Primers for qRT-PCR Analysis

osteopontin F- TGC ACC CAG ATC CTA TAG CC, R- TCC GAG TCC ACA GAA TCC TC;
osteocalcin F- CTT GCA GGG CAG AGA GAG AG, R- CTT GCA GGG CAG AGA GAG AG;
Pparγ F- TTT TCA AGG GTG CCA GTT TC, R- AAT CCT TGG CCC TCT GAG AT;
Dkk1 F- TGG CCG TGT TTA CAA TGA TG, R- TAC TTG TTC CCG CCC TCA TA;
Sox2 F- CAC AAC TCG GAG ATC AGC AA, R- TC CGG GAA GCG TGT ACT TA
TAZ: - F- GAAGGTGATGAATCAGCCTCTG, R- GTTCTGAGTCGGGTGGTTCTG
YAP:- F- ACCCTCGTTTTGCCATGAAC, R- TTGTTTCAACCGCAGTCTCTC

Western Analysis and Immunoprecipitation

Western analysis was performed as previously described (Seo et al., 2011). For the preparation of whole cell protein lysates, cells were lysed in RIPA (radio-Immunoprecipitation Assay buffer). For co-immunoprecipitation experiments, cells were lysed with lysis buffer (0.5% Triton X-100, 20mM Tris.Cl pH7.5, 150mM NaCl, 50mM NaF, 2mM EDTA and protease inhibitor) and incubated with anti-Flag antibody (purchased from Wako, Richmond, VA). For preparation of cytoplasmic and nuclear fractions, cells were lysed in a pre-chilled Dounce homogenizer in hypotonic lysis buffer (10mM Tris-HCl pH 7.4, 140mM NaCl, 5mM EDTA, 2mM DTT, 0.5mM PMSF,2ug/ml aprotinin, 1ug/ml leupeptin). The lysates were centrifuged at 500X g for 10 min and collected separately as supernatant (cytoplasmic) and pellet (nuclear), which was solubilized in RIPA buffer.

Antibodies

Sox2 (#2748), YAP1 (#4912), phospho-YAP1 (#4911), TAZ (#2149), PPARγ (#2430) (all from Cell Signaling, Danvers, MA); c-MYC (ab32072) and Runx2 (ab76956) (both from Abcam); β-catenin (Cat: 61053 from BD Transduction, San Jose, CA); actin (A2228) and tubulin (T5168) (both from Sigma, St. Louis, MO); CRE (MAB 3120 from Millipore, Billerica, MA); and GAPDH (sc-32233), RNA Polymerase II (sc-899) and Lamin B (sc-374015) (all from SCBT, Santacruz, CA).

Reporter Constructs and Luciferase Activity

For expression of Venus (eGFP) or luciferase (pGL3) driven by Sox2 consensus site in YAP exon I (Figure 1A), the regions around YAP exon I were amplified by PCR using a primer pairs including both Mlu I recognition sites F- GAC GCG TAA CTC GGA CCA GTT CAA CC and R- GAC GCG TCT GGG GGT TGT TTT CGC GGA GTT. PCR products were directly ligated to pTOP V2 (TOPcloner #EZ002S, Enzynomics) that was digested with Mlu I. The YAP DNA fragments including Sox2 consensus sites were inserted into pCS2-Venus or

Fgf4 minimal promoter linked to lucifease vector (provided by Dr. Lisa Dailey). To obtain Dkk1 promoter that includes the YAP/Tead binding site, Dkk1 upstream regions were generated by PCR using primers including Mlu I restriction sites F- GAC GCG TCT CAG ATT TAT CGA TCT GTT C and R- GAC GCG TTT GCA CAT CAA ATG AGG ATG C and ligated into the pGL3 vector directly. For mutagenesis of Dkk1 promoter, site-directed mutagenesis subcloning was followed using standard protocols, mutation 1, F- CTA ATT TAT AAG CAC GGC GGG CTT ATG TCC ATG TTG TTT and mutation 2 F- CGA AAC CCC TTC AAA TTC GTG CTC TGA TTT AGG TTC AGC A. For luciferase assay, reporter constructs (YAP- Fgf4 or Dkk1-Fgf4 minimal promoter reporter) and pTk-Renilla (transfection control) were co-transfected into C3H10T1/2 cells expressing Sox2 or YAP constructs and luciferase activity was measured using the Promega luciferase assay reagents as described by the manufacturer. CHIR 99021 treatment (Stemgent, Cambridge, MA), was added to cells 10 hr after transfection and luciferase activity was measured the next day.

Immunofluorescence Analysis

C3H10T1/2 cells were washed with PBS, fixed with 4% paraformaldehyde at 4°C, permeabilized with 0.1% saponin in PBS, blocked with 2%BSA-PBS, and then incubated with mouse anti-YAP antibody (Novus # H00010413-M01, 1:200) for 1 hr. Cells were washed with 1X PBS five times and treated with secondary antibody (Alexa Fluor 488 goat anti-mouse (LifeTechnologies A-11001) for 1 hr. Cells were washed with 1X PBS four times. Nuclei were counterstained with DAPI and cells were mounted in DAKO mounting medium.

SUPPLEMENTAL REFERENCES

Adhikari, A.S., Agarwal, N., Wood, B.M., Porretta, C., Ruiz, B., Pochampally, R.R., and Iwakuma, T. (2010). CD117 and Stro-1 identify osteosarcoma tumor-initiating cells associated with metastasis and drug resistance. Cancer Res. *70*, 4602–4612.

Crisan, M., Yap, S., Casteilla, L., Chen, C.W., Corselli, M., Park, T.S., Andriolo, G., Sun, B., Zheng, B., Zhang, L., et al. (2008). A perivascular origin for mesenchymal stem cells in multiple human organs. Cell Stem Cell *3*, 301–313.

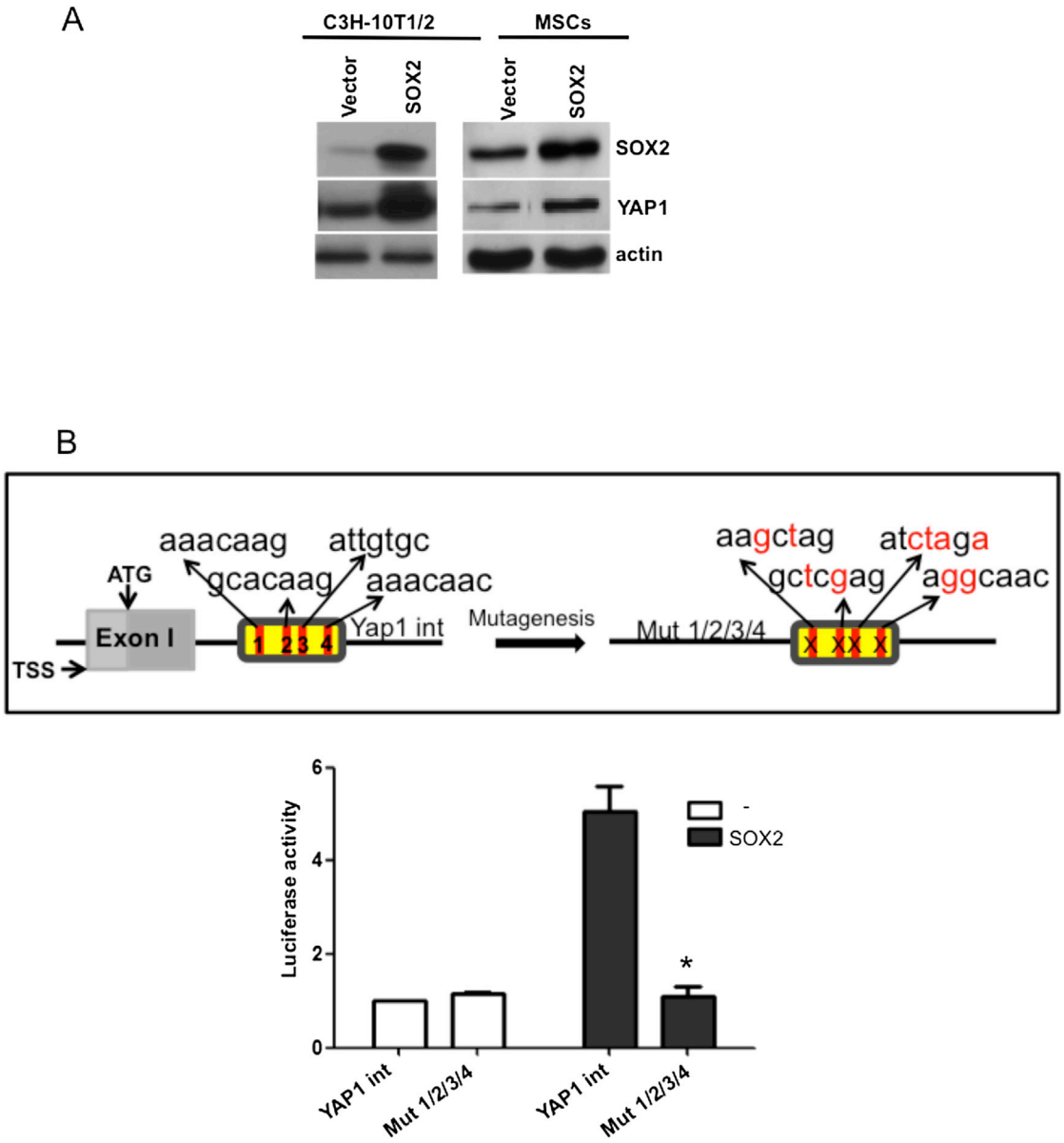


Figure S1. YAP1 Induction Requires SOX2 Binding Sites, Related to Figure 1

(A) YAP1 expression is induced in in C3H-10T1/2 and in primary MSCs overexpressing SOX2. Western analysis of SOX2 and YAP1expression in C3H10T1/2 cells or primary MSCs transduced with empty vector or SOX2 lentiviral vector.

(B) SOX2 binding sites are required for YAP1 reporter induction. Yap1 genomic region (243bp) near exon I has four SOX2-binding consensus sites. Each putative SOX2 binding site was mutated in the Fgf4-Yap1 243bp-pro (Figure 1F) construct (indicated by red nucleotides) and assayed for luciferase activity. YAP1 int = WT 243 bp region.

*$p < 0.05$; error bars represent the average + SD.

A

TEAD-dependent YAP1 target genes down-regulated by SOX2 knockout

Gene		Entrez ID
ABHD2	abhydrolase domain containing2	54608
BDNF	brain-derived neurotrophic factor	12064
FRY	furry homolog- like	72313
GGH	gamma glutamyl hydrolase	14590
LIFR	leukemia inhibitory factor receptor	16880
OLR1	oxidized low density lipoprotein	108078
PARVA	parvin, alpha	57342
PTGS2	prostaglandin-endoperoxidase synthase 2	19225
RASAL2	RAS protein activator like 2	226525
SPRED1	sprouty-related EVH1 domain-containing 1	114715
TGFB2	transforming growth factor, beta 2	21808
DNM3	dynamin 3	474332
LOX	lysyl oxidase	16948
NDRG1	N-myc downstream regulated gene 1	17988
REEP1	receptor accessory protein 1	225362
VLDLR	very low density lipoprotein receptor	22359

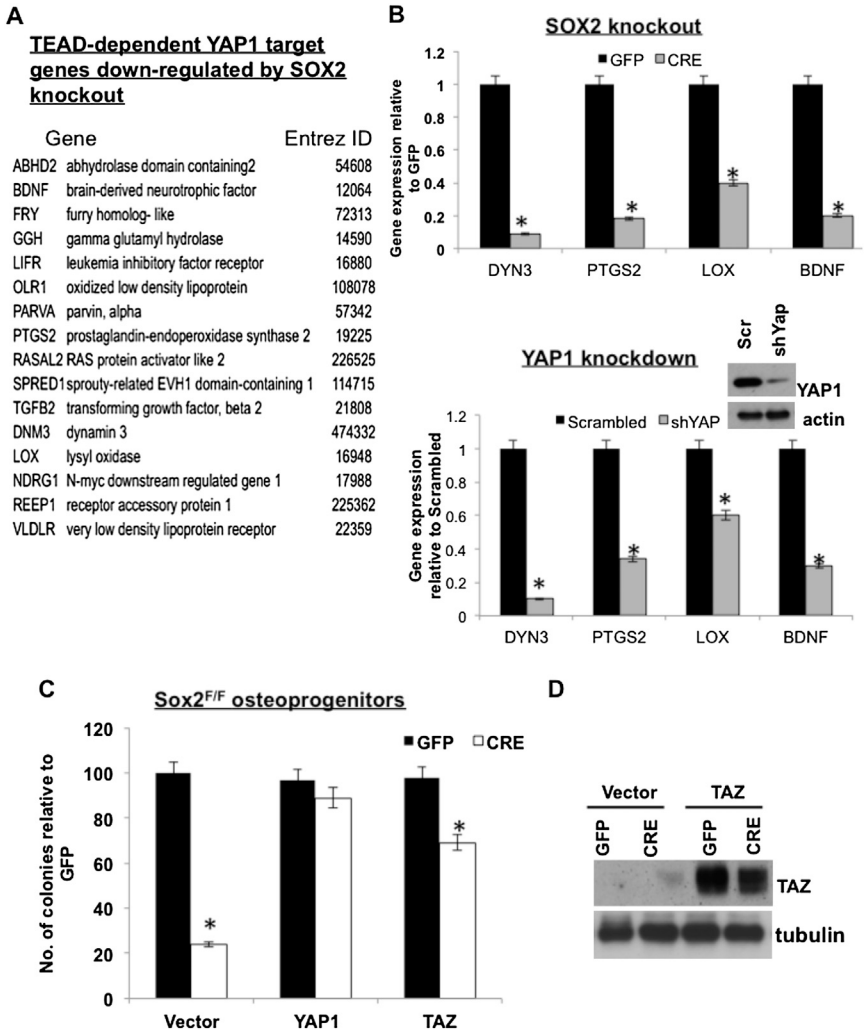


Figure S2. SOX2 Regulates YAP1 Targets, and Unlike YAP1, TAZ Overexpression Partially Compensates for SOX2 Function in Osteoprogenitor Cells, Related to Figure 2

(A) YAP1 target genes from Zhang et al. that are downregulated in SOX2 knockout osteoprogenitor cells.

(B) qRT-PCR validation of YAP1 target genes in SOX2 knockout (top) and YAP1 knockdown (bottom) osteoprogenitor cells.

(C) Colony assay of $Sox2^{F/F}$ osteoprogenitor cells infected with GFP or CRE in cells expressing vector, YAP1 or TAZ lentivirus. YAP1 rescue is shown for comparison. Note the partial rescue in TAZ-expressing osteoprogenitors.

(D) TAZ expression in vector and TAZ overexpressing GFP or CRE–virus infected cells $Sox2^{F/F}$ osteoprogenitor cells.

*$p < 0.05$; error bars represent the average + SD.

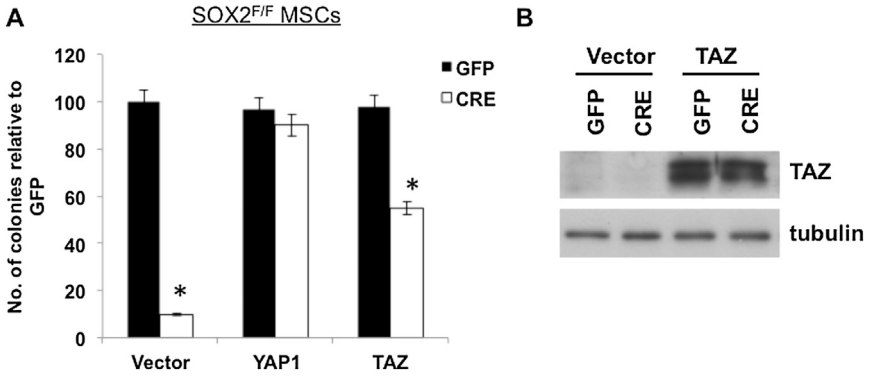


Figure S3. TAZ Overexpression Partially Rescues the Self-Renewal Defect due to SOX2 Inactivation in Primary MSCs, Related to Figure 3

(A) Colony assay of Sox2^{-}deleted MSCs expressing transgenic YAP1 and TAZ. YAP1 rescue is shown for comparison. Note the partial rescue in TAZ-expressing MSCs.

(B) Expression of TAZ protein in SOX2 $^{F/F}$ MSCs before (GFP) and after (CRE) SOX2 deletion in control or TAZ overexpressing cells (right panel).

*$p < 0.05$; error bars represent the average + SD.

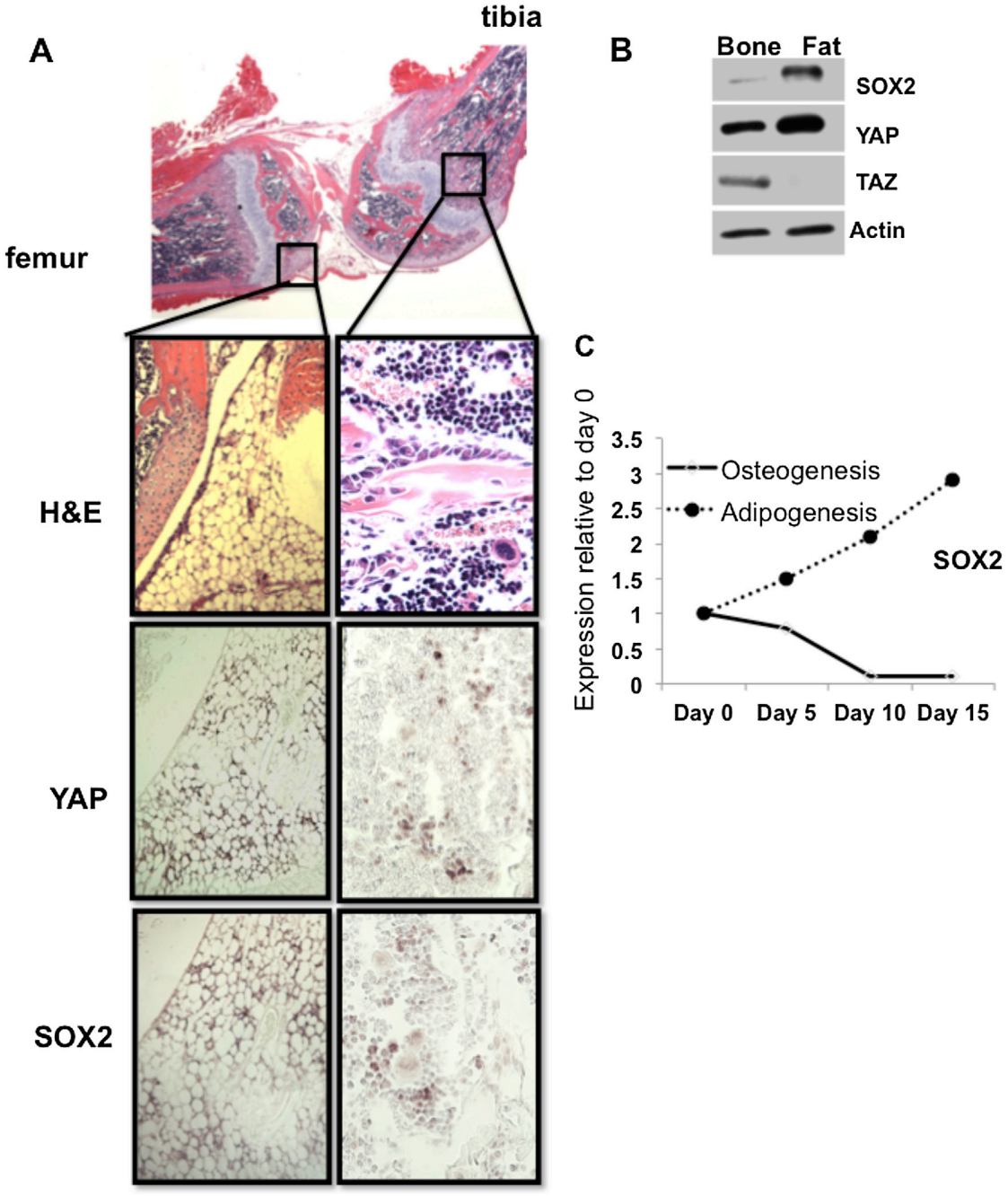
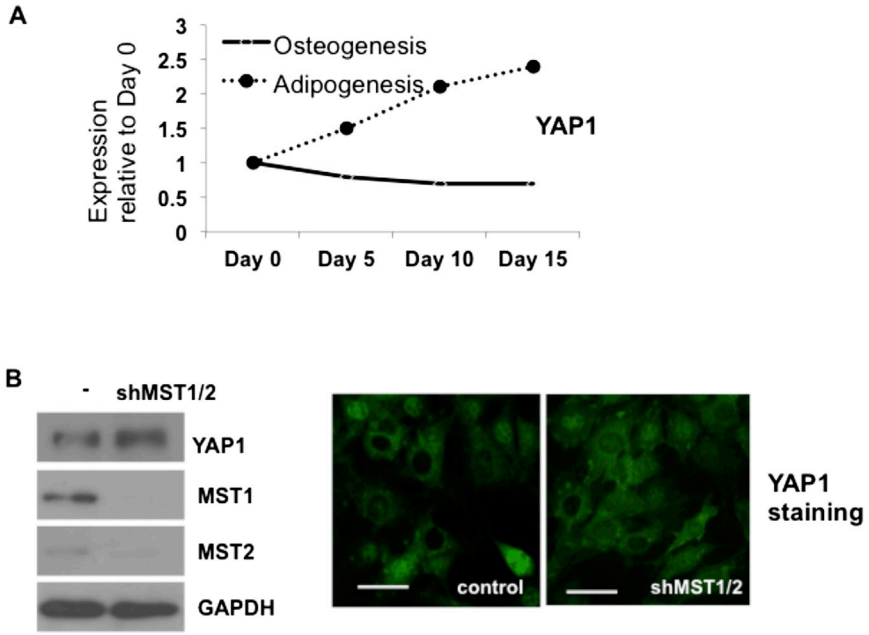


Figure S4. SOX2 and YAP1 Are Expressed in Bone and Fat, and SOX2 Expression Is Regulated in MSCs during Osteogenesis and Adipogenesis, Related to Figure 4

(A) SOX2 and YAP1 protein expression was detected by immunohistochemistry in femur-tibia sections of wild-type 4 weeks old mice. Insets show expression in cells in trabecular bone area surrounding the areas of bone formation, which is consistent with the position of immature osteoprogenitor cells (right), and in adipocytes adjacent to the femur and tibia(left). H&E, hematoxylin-eosin stain.

(B) Western analysis in compact bone and inguinal fat tissue.

(C) SOX2 mRNA expression by qRT-PCR in MSCs undergoing osteogenic or adipogenic differentiation for the indicated days.



C

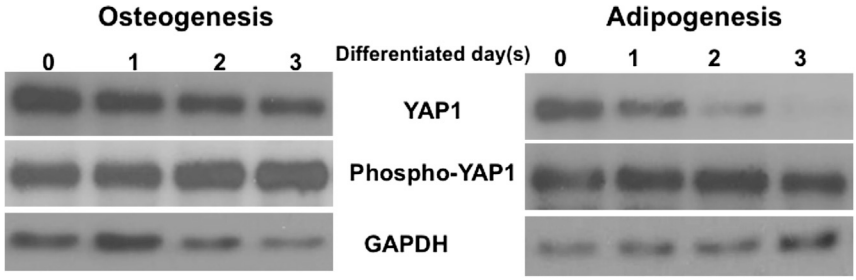
	Basal YAP1 nuclear stain (%)	YAP1 nuclear stain adipo (%)
Control	60	-
YAP1	85	15
YAP1(2SA)	100	86
shMST1/2	87	20

Figure S5. Regulation of YAP1 Expression, Related to Figure 5

(A) qRT-PCR analysis of YAP1 during osteogenic and adipogenic differentiation.

(B) Western blot of C3H10T1/2 cells expressing shMST1/2 shows efficient knockdown of MST1/2 and increased YAP1 expression. Right panel shows immunostaining with anti-YAP1 antibody in control and shMST1/2 C3H10T1/2 cells. Scalebar = 50 μM.

(C) Immunostaining with anti-YAP1 antibody in C3H10T1/2 cells expressing the indicated constructs under undifferentiated (basal) and adipogenic (adipo) conditions for 3 days. Cells positive for nuclear staining are increased in YAP1, YAP1(2SA) and shMST1/2 infected cells. Endogenous YAP1 is decreased during adipogenesis in control cells but some nuclear staining persists in cells expressing shMST1/2 or YAP1. Cells positive for nuclear YAP1 staining were counted in approximately 200 cells and average number of positive cells is shown.



The expression of YAP1 and Phospho-YAP1 during osteogenic and adipogenic differentiation.
C3H10T1/2 cells were maintained in osteogenic- or adipogenic media and collected at indicated day(s). YAP1 and phospho-YAP1 were detected by Western analysis.

Figure S6. Expression of YAP1 and Phospho-YAP1 during Osteogenic and Adipogenic Differentiation, Related to Figures 5 and 6
C3H10T1/2 cells were maintained in osteogenic- or adipogenic media and collected at the indicated day(s). YAP1 and phospho-YAP1 were detected by Western analysis.

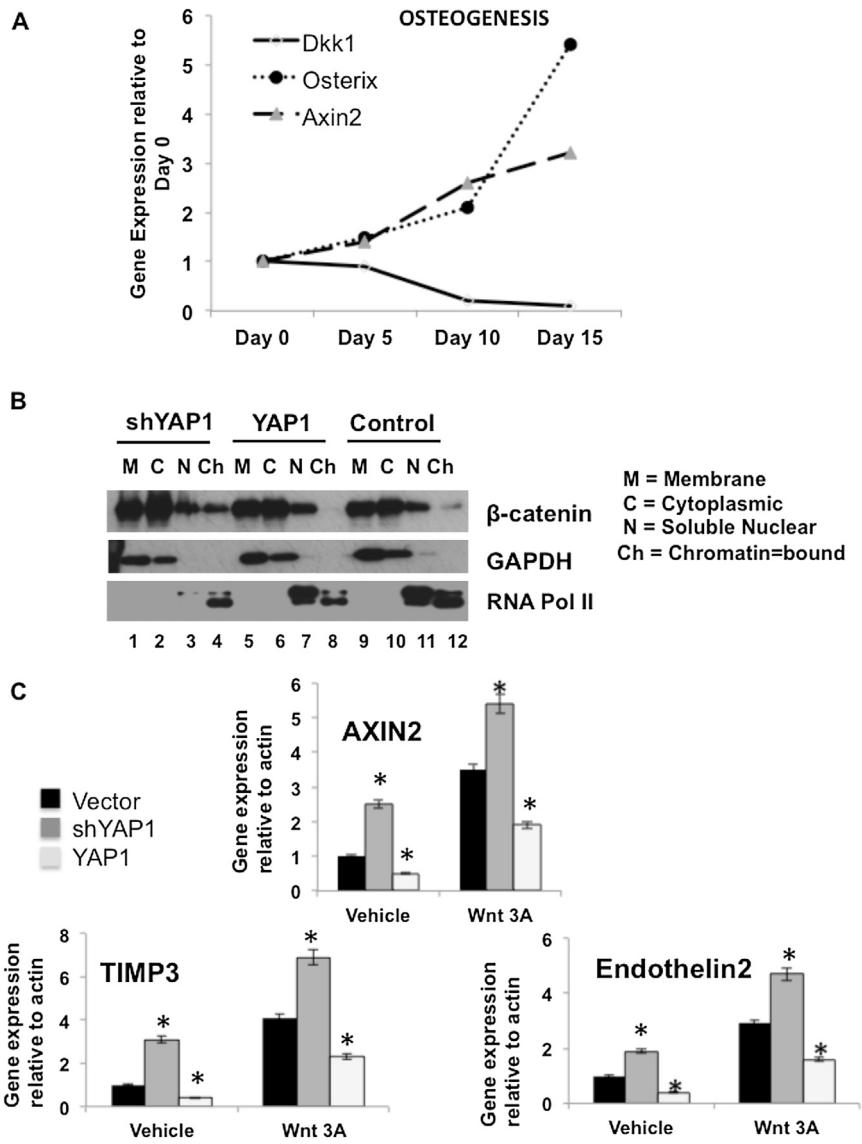


Figure S7. Wnt signaling Is Inhibited by YAP1 and Derepressed when YAP1 Is Depleted, Related to Figure 7

(A) Expression of Dkk1, Axin 2 (Wnt target gene) and Osterix (Osteoblast differentiation marker, during osteogenic differentiation of C3H10T1/2 cells.

(B) Whole chromatin bound b-catenin in cells expressing YAP1 or shYAP1. Chromatin–bound b-catenin is reduced in YAP1-overexpressing cells and increased in YAP1 knockdown cells (compare lanes 4, 8 and 12).

(C) Expression of Wnt target genes Axin2, Timp3 and Endothelin2 were analyzed by qRT-PCR in C3H10T1/2 cells expressing shYAP1 or overexpressing YAP1 in vehicle treated or Wnt3A treated cells.

*$p < 0.05$; error bars represent the average + SD.