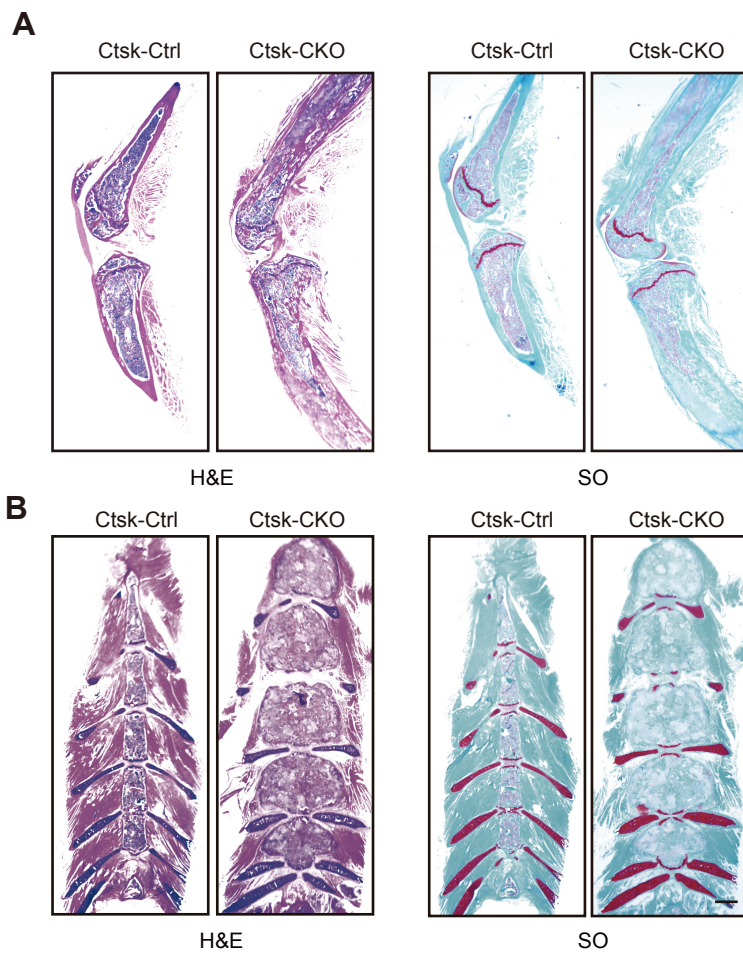
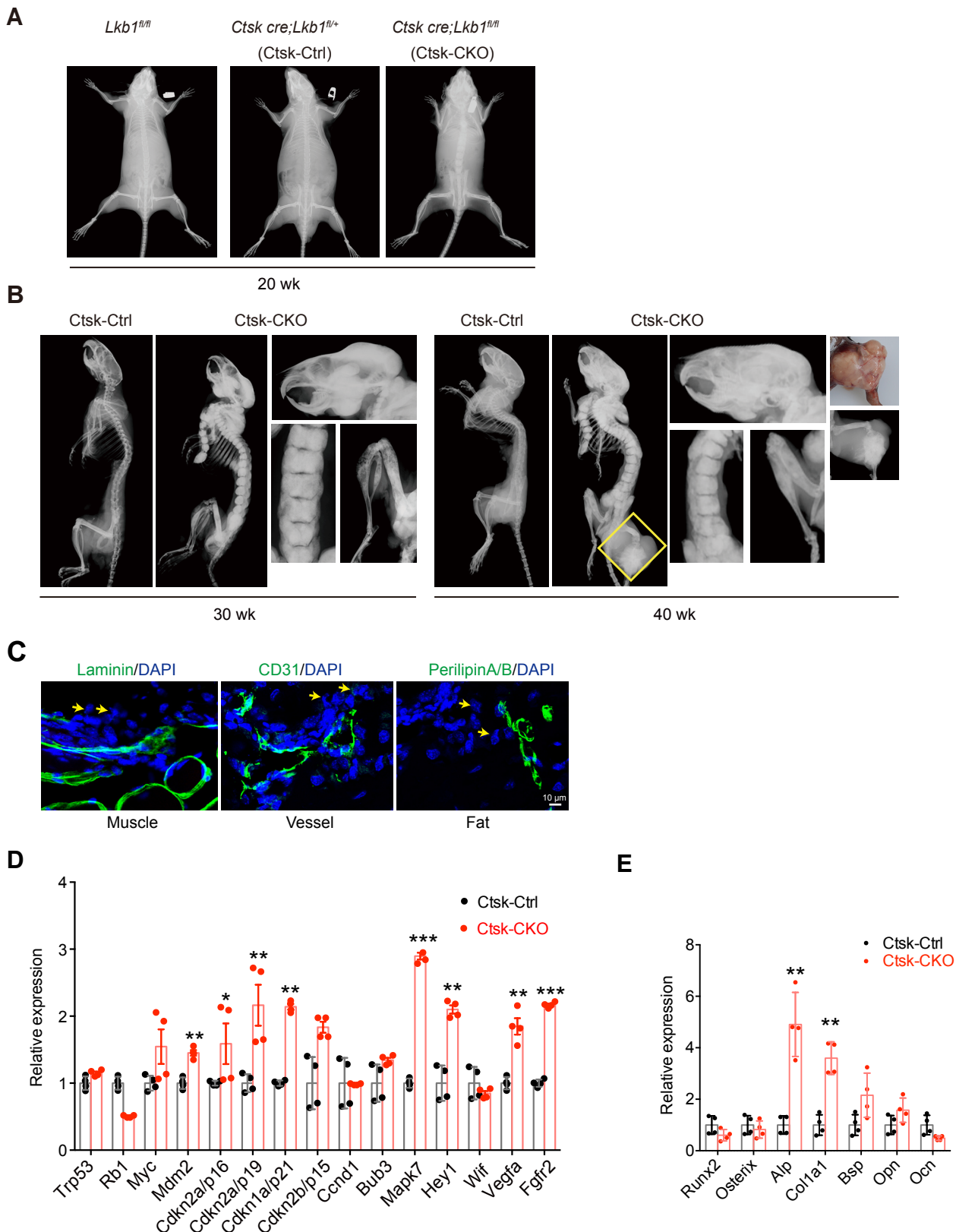




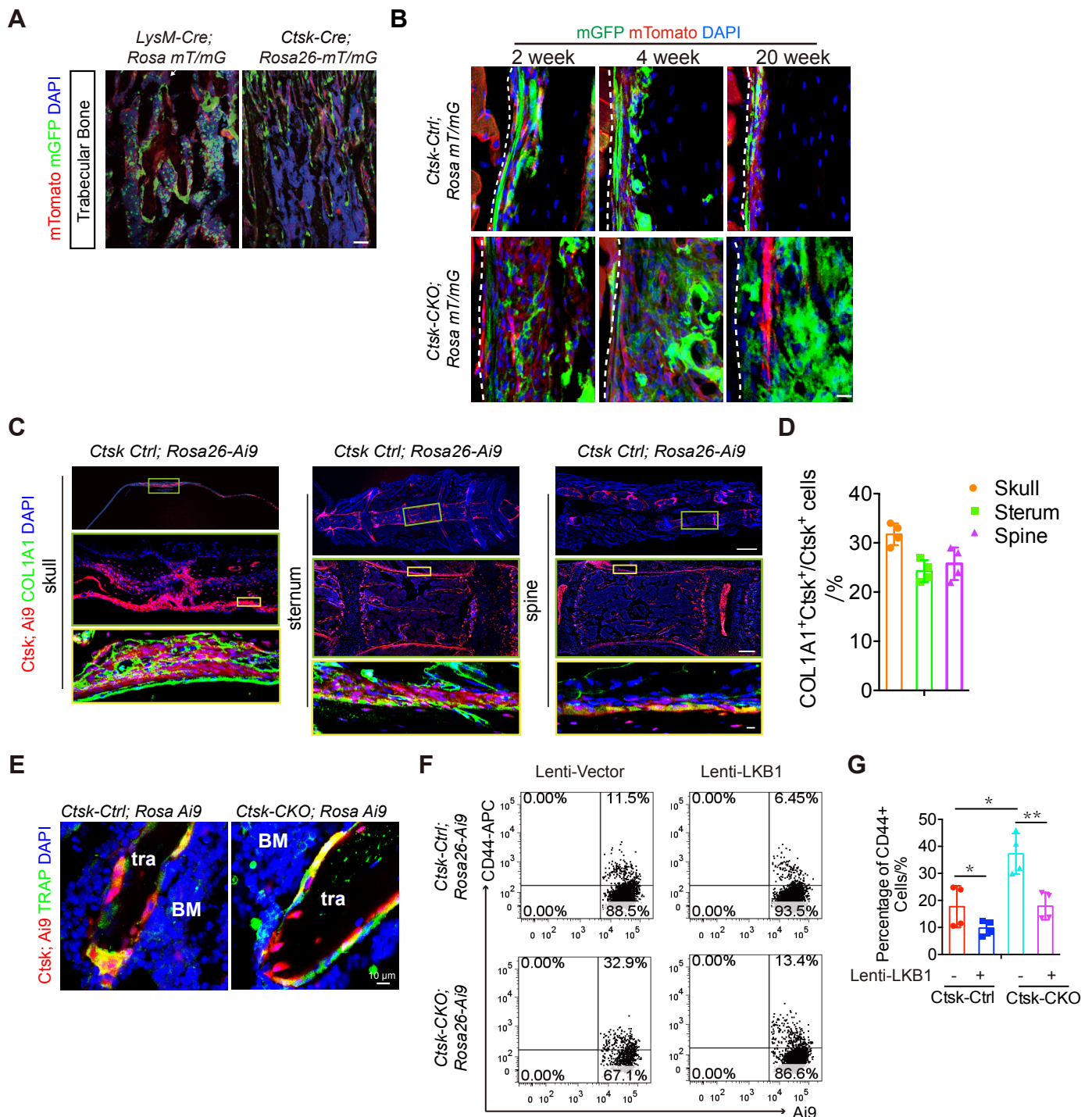
Supplemental Figure 1. *Lkb1* deletion in *Ctsk-Cre* expressing cells dose not show cartilage tumors.

(**A** and **B**) H&E staining and Safranin O (SO) staining of the tibiae (**A**) and sternums (**B**) of 20-week-old Ctsk-Ctrl and Ctsk-CKO mice showed that Ctsk-CKO mice did not display cartilage tumor. Scale bars, 300 μ m. Similar results were obtained from analyses of at least 3 mice of each genotype.



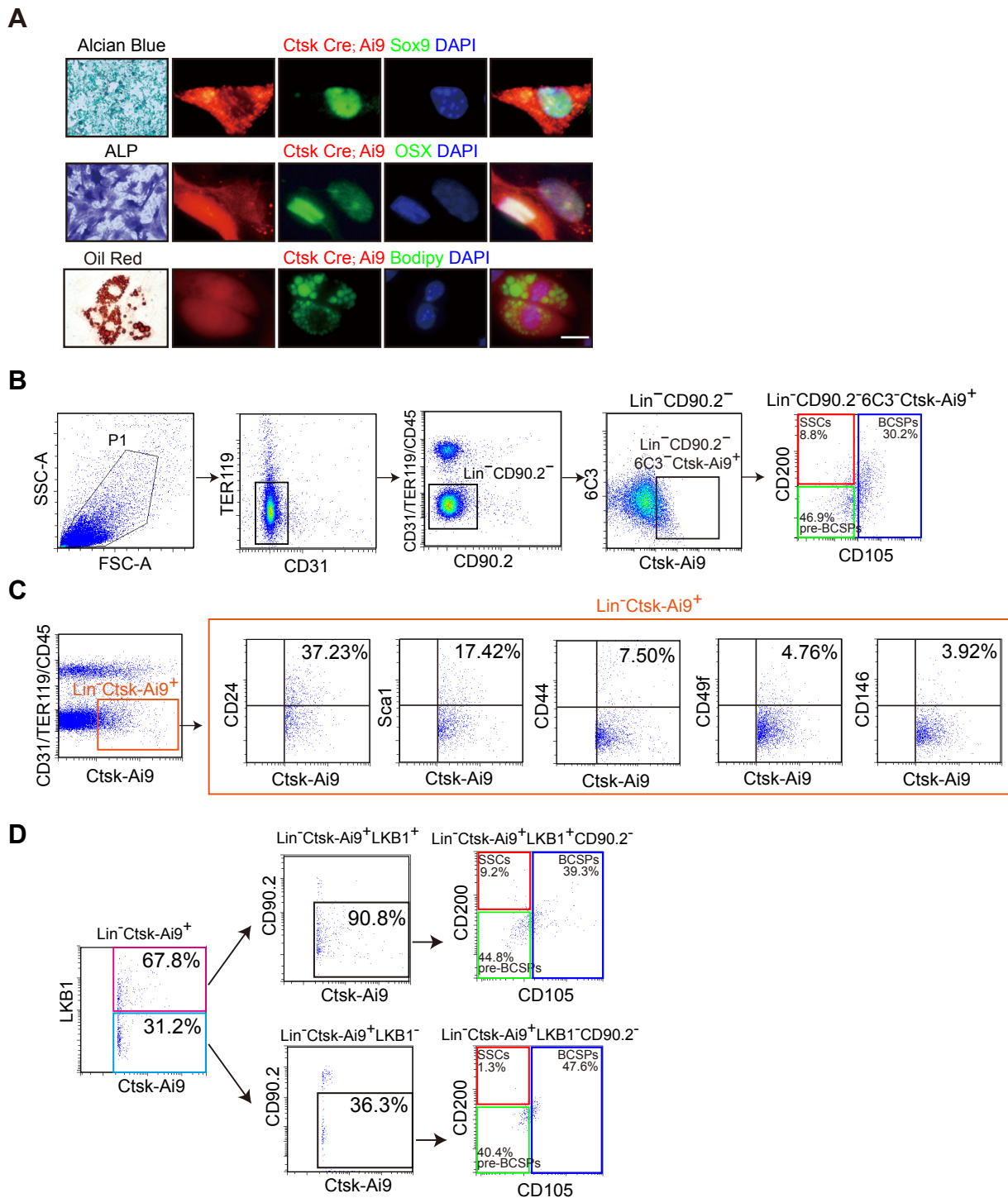
Supplemental Figure 2. *Lkb1* deletion in *Ctsk-Cre* expressing cells causes osteogenic tumor-like phenotype.

(A) X-ray images of the whole-body of 20-week-old *Lkb1^{fl/fl}*, *Ctsk Cre; Lkb1^{fl/+}* and *Ctsk Cre; Lkb1^{fl/fl}* mice. (B) X-ray images of the whole-body skeletons, spines, tibiae, and skulls of the 30-week-old and 40-week-old Ctsk-Ctrl and Ctsk-CKO mice. (C) Immunostaining of markers of fat (Perilipin A/B), vessels (CD31) and muscles (Laminin) in the tumor region of 40-week-old mice. (D and E) qPCR analysis of osteosarcoma-related genes at 2-week-old (D) and osteoblast markers at 2-week-old (E). Data represent means $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$, by unpaired Student's *t* test (D and E).



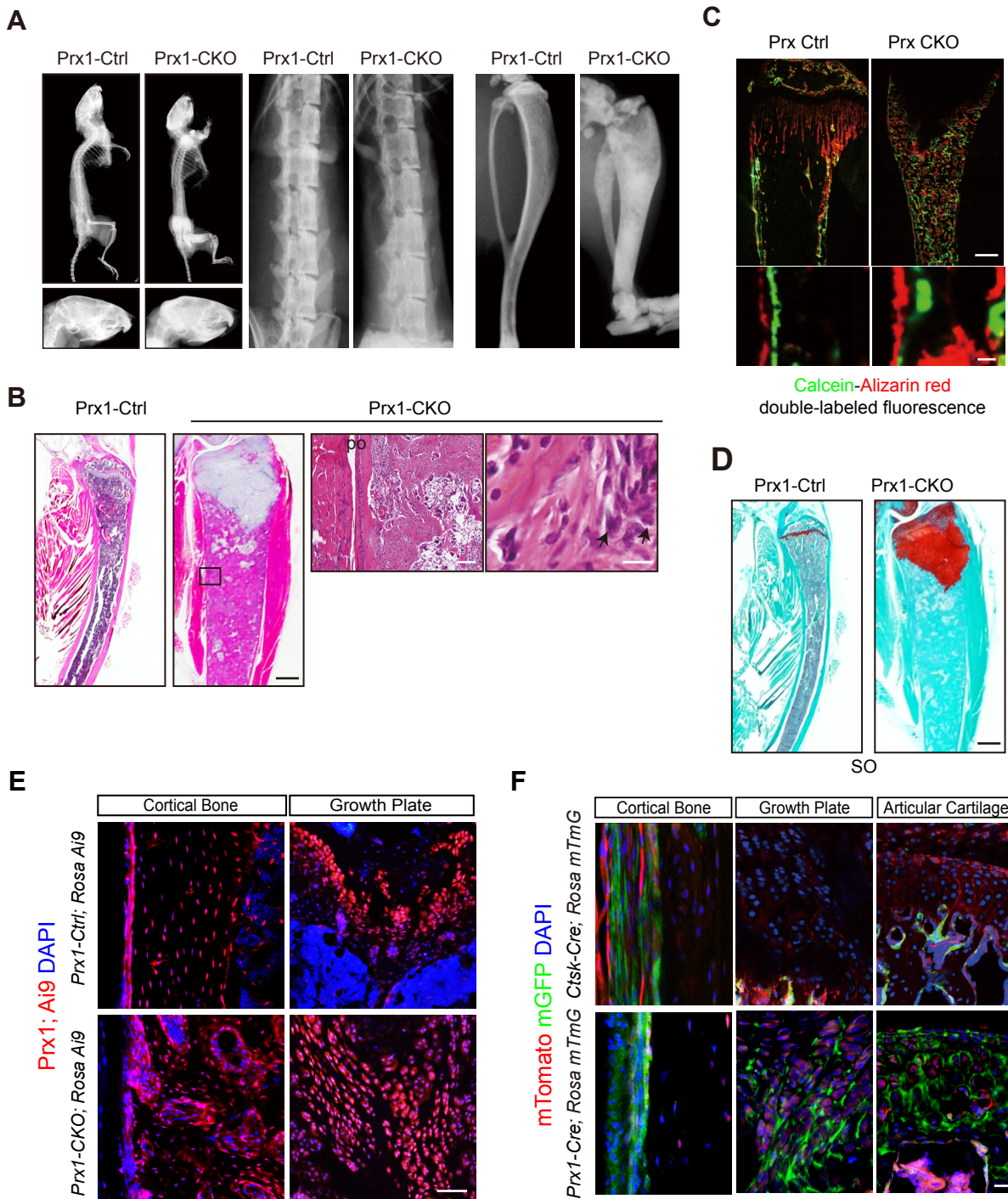
Supplemental Figure 3. *Ctsk*⁺ periosteal mesenchymal stem cells are novel potential sources for osteogenic tumor.

(A) Confocal images of the tibiae of 4-week-old male *LysM-Cre; Rosa26-mT/mG* and *Ctsk-Cre; Rosa26-mT/mG* mice showing both *Ctsk-Cre* and *LysM-Cre* were expressed in osteoclasts on the surface of trabecular bone of 4-week-old mice, and *LysM-Cre* were expressed in macrophages in the bone marrow. Scale bar, 20 μ m. (B) *Ctsk*⁺ cells in the periosteum of 2-, 4-, 20-week-old male *Ctsk-Ctrl; Rosa mT/mG* and *Ctsk-CKO; Rosa mT/mG* mice. Scale bar, 20 μ m. (C and D) Images of the skull, sternum and spine of *Ctsk-Cre; Rosa26-Ai9* mice showing *Ctsk-Cre* cells (red) in the periosteum expressed osteoblast marker COL1A1. Scale bar, 1000 μ m (up), 50 μ m (middle), 10 μ m (below). (E) Immunostaining of TRAP in trabecular bone showing that most of *Ctsk*⁺ cells in trabecular bone are TRAP⁺ osteoclasts. Scale bar, 10 μ m. (F and G) Flow cytometry analysis showing that *Ctsk-Ai9* cells isolated from the cortical bone of *Ctsk-CKO; Rosa26-Ai9* tibiae displayed increased CD44 expression and re-expression of LKB1 using a lentivirus reduced number of CD44 positive cells in *Ctsk-Ai9* cells (n = 4). Data represent means $\pm$ SEM. **P* < 0.05, ***P* < 0.01, and ****P* < 0.001, by two-way ANOVA (G).



Supplemental Figure 4. *Ctsk-Cre* expressing cells display mesenchymal stem cell markers and differentiation potential.

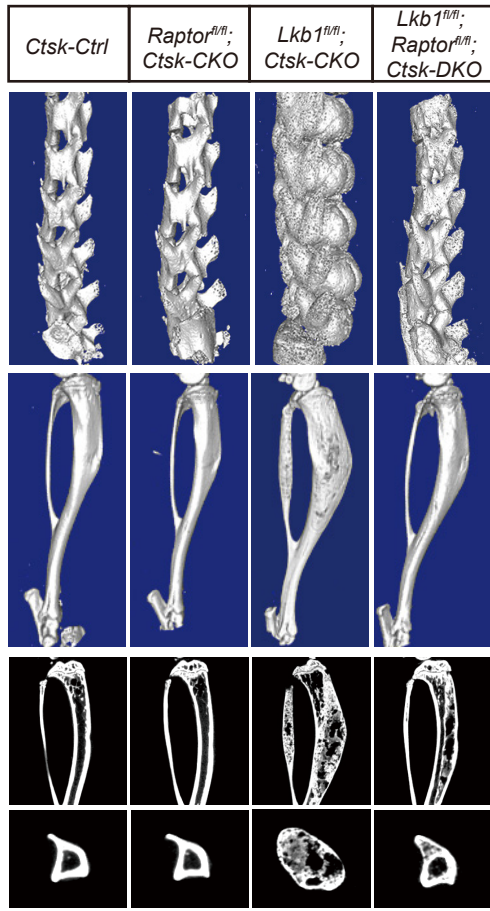
(A) *Ctsk-Ai9* cells were able to differentiate into chondrocyte (Alcian Blue staining and Sox9 immunostaining), osteoblast (ALP staining and OSX immunostaining) and adipocyte (Oil Red O and Bodipy staining). Scale bar, 10 μ m. (B and C) Flow cytometry analysis of *Ctsk-Ai9* cells from the periosteum of cortical bones of 4-week-old *Ctsk-Ctrl*; *Rosa26-Ai9* mice. (D) Flow cytometry analysis of periosteum-derived *Ctsk*⁺*Lkb1*⁺ and *Ctsk*⁺*Lkb1*⁻ cells.



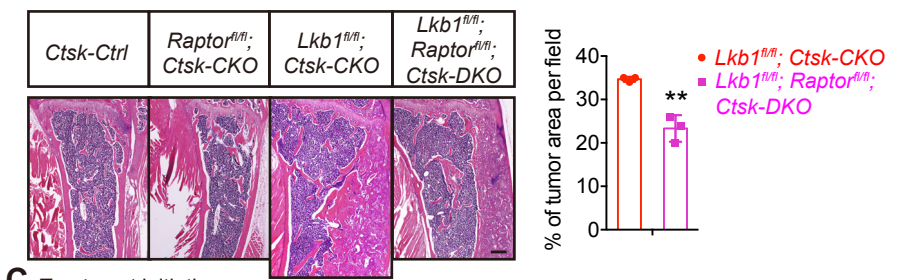
Supplemental Figure 5. Loss of *Lkb1* in *Prx1-Cre* expressing cells causes both osteogenic tumor and cartilage tumor.

(A) X-ray images of the whole-body skeletons, skulls, spines and long bones of 20-week-old *Prx1-Ctrl* and *Prx1-CKO* mice. (B) Representative images of H&E staining of the tibiae from 20-week-old *Prx1-Ctrl* and *Prx1-CKO* mice showing both osteogenic tumor and cartilage tumor formation. Scale bar, 1 mm (left), 100µm (middle), 20 µm (right). (C) Calcein-alizarin red double-labeled fluorescence showing increased bone formation in 5-week-old *Prx1-CKO* mice. Scale bars, 1 mm (up) 100µm (below). (D) SO staining of the tibiae from 20-week-old *Prx1-Ctrl* and *Prx1-CKO* mice. Scale bars, 1 mm. (E) Confocal imaging of *Prx1-Ai9* cells showing howing *Prx1*⁺ cells were expanded from the cortex to the marrow cavity in the periosteum and also expanded to form a mass of cartilage in the growth plate of *Prx1-CKO* mice. Scale bar, 50 µm. (F) Confocal images of the tibiae of 4-week-old *Ctsk-Ctrl*; *Rosa26-mT/mG* reporter mice and *Prx1-Ctrl*; *Rosa26-mT/mG* reporter mice including cortical bone, growth plate and articular cartilage. Scale bar, 20 µm.

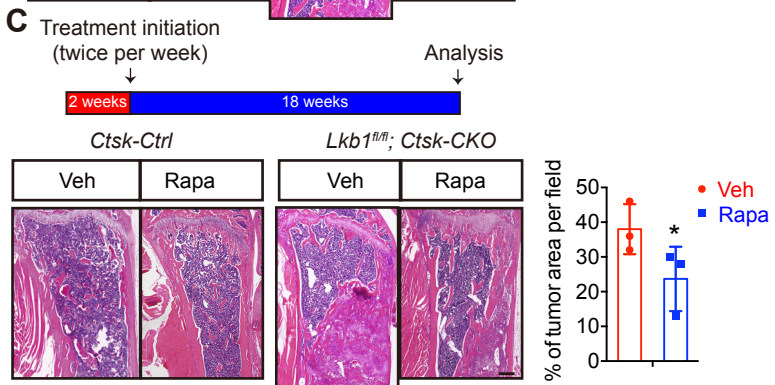
A



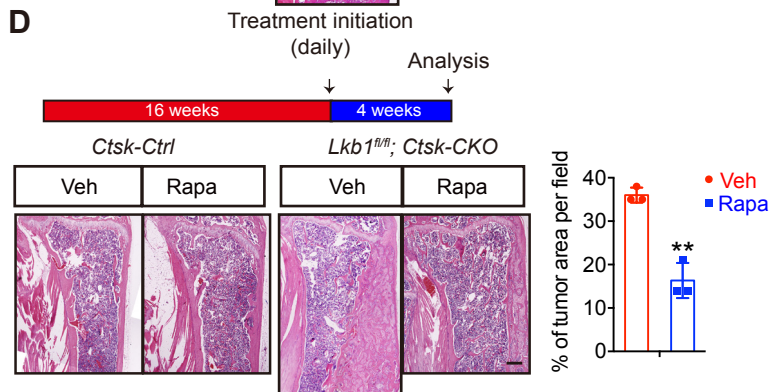
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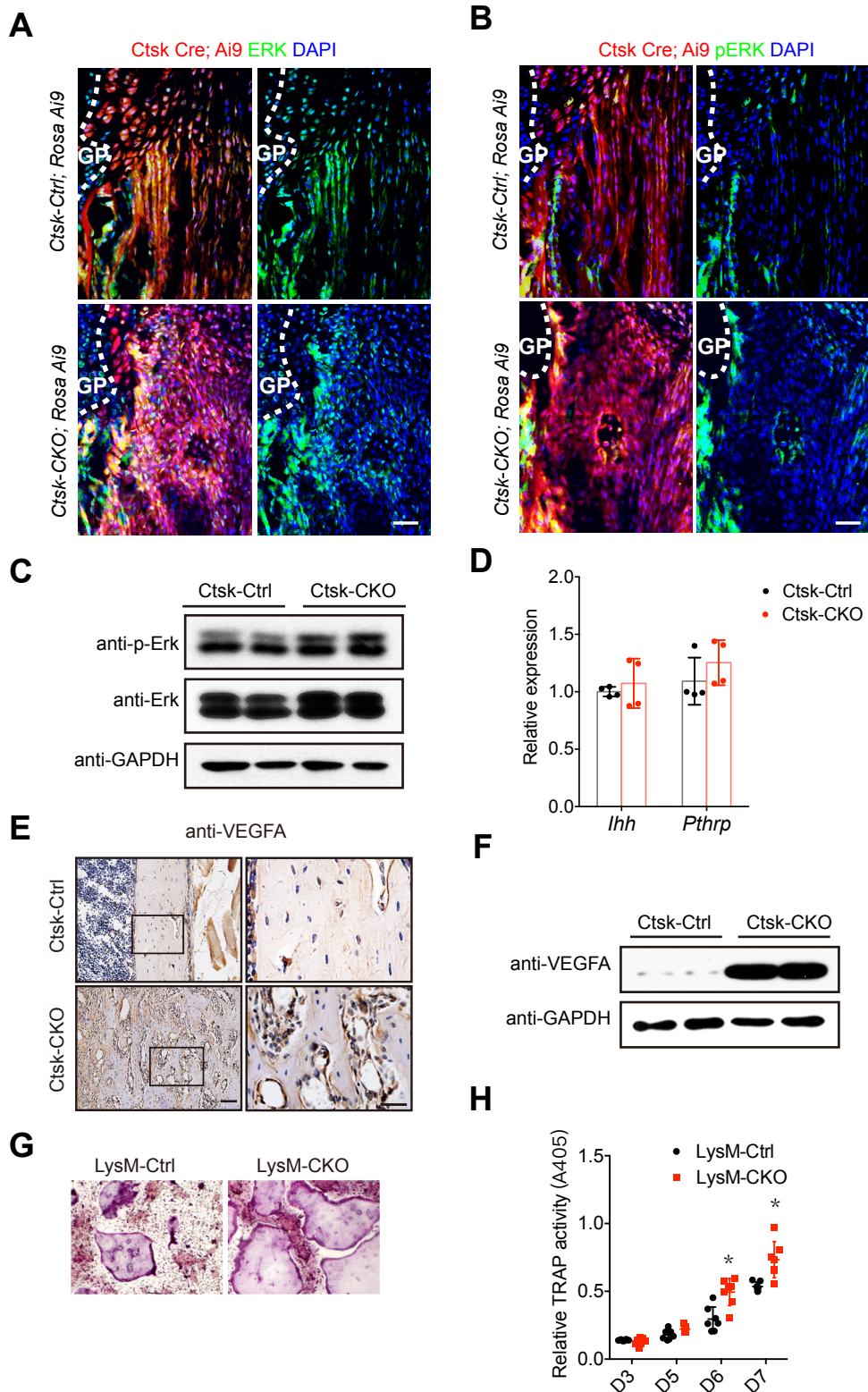
C



D



Supplemental Figure 6. Inhibition of mTOR signaling ameliorates the tumor progression of Ctsk-CKO mice. (A) MicroCT scans of spines and tibiae from 20-week-old Ctsk-Ctrl, *Raptor^{fl/fl}; Ctsk-CKO*, *Lkb1^{fl/fl}; Ctsk-CKO* and *Lkb1^{fl/fl}; Raptor^{fl/fl}; Ctsk-DKO* mice. (B) H&E staining of the tibiae from Ctsk-Ctrl, *Raptor^{fl/fl}; Ctsk-CKO*, *Lkb1^{fl/fl}; Ctsk-CKO* and *Lkb1^{fl/fl}; Raptor^{fl/fl}; Ctsk-DKO* mice. Scale bar, 300 μ m. (C and D) H&E staining showing the treatment of Ctsk-Ctrl and Ctsk-CKO mice with rapamycin twice per week from 2-week-old to 20-week-old (C) or daily from 16-week-old to 20-week-old (D) delayed the tumor growth of Ctsk-CKO mice. Scale bars, 300 μ m. Data represent means $\pm$ SEM. * P < 0.05, ** P < 0.01, and *** P < 0.001, by unpaired Student's t test (B, C and D).



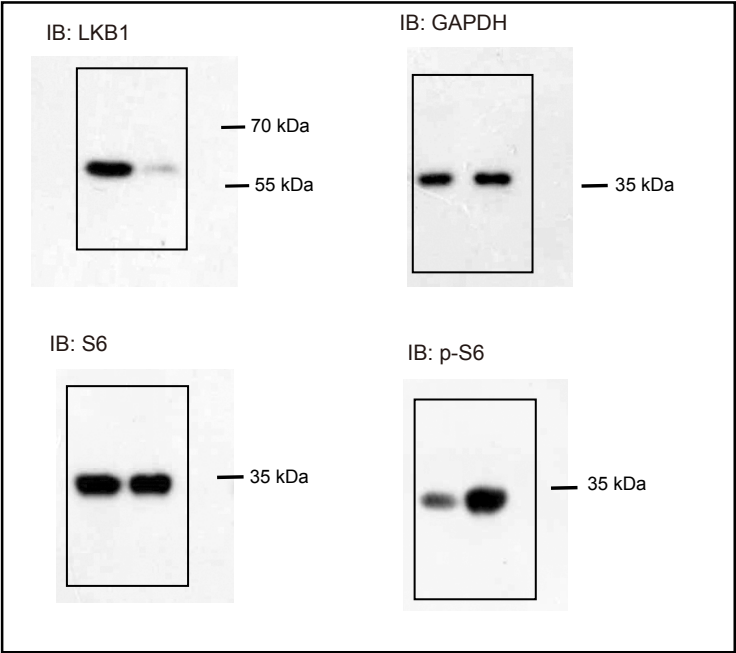
Supplemental Figure 7. Effect of *Lkb1* loss on *Ihh*, VEGF and osteoclastogenesis.

(A and B) Immunostaining of ERK (green) (A) and pERK (green) (B) in Ctsk⁺ (red) cells. (C) Western blot showing expression of ERK and pERK in the tumor region of Ctsk-CKO mice and the cortical bones of control mice. Scale bar, 50 μ m. (D) qPCR showing *Ihh* and *Pthrp* production in Ctsk-CKO and control mice. GP: growth plate. Immunostaining (E) and western blot (F) of VEGFA in the tumor region of Ctsk-CKO mice and the cortical bones of control mice. Scale bar, 100 μ m (left), 100 μ m (right). (G and H) TRAP staining (G) and quantification of TRAP activity (H) of cells differentiated from LysM-Ctrl and LysM-CKO bone marrow (BM) cells in the presence of M-CSF and RANKL. Data represent means $\pm$ SEM. * P < 0.05, ** P < 0.01, and *** P < 0.001, by unpaired Student's *t* test (H).

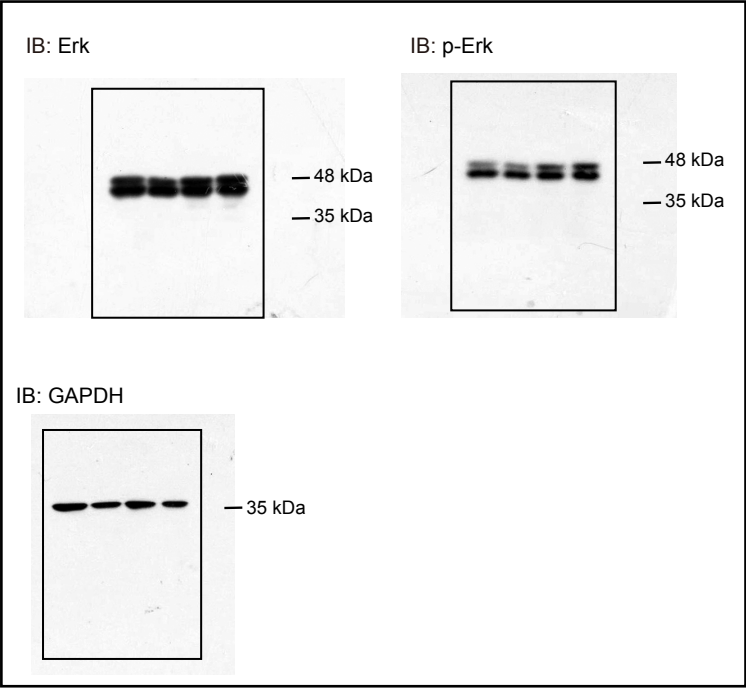
TableS1- qPCR Primer Sequence

NO.	PrimerName	Sequence(5'-3')
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	mTrp53-qR	CGGAACATCTCGAAGCGTTTA
2	mRb1-qF	TGCATCTTTATCGCAGCAGTT
	mRb1-qR	GTTACACAGTCCGTTCTAATTTG
3	mMyc-qF	ATGCCCCCTCAACGTGAAC TTC
	mMyc-qR	CGCAACATAGGATGGAGAGCA
4	mMdm2-qR	TGTCTGTGTCTACCGAGGGTG
	mMdm2-qF	TCCAACGGACTTTAACAAC TTCA
5	mCdkn2a/p16-qF	AACTCTTTCGGTCGTACCCC
	mCdkn2a/p16-qR	GCGTGCTTGAGCTGAAGCTA
6	mCdkn2a/p19-qF	CGCAGGTTCTTGGTCACTGT
	mCdkn2a/p19-qR	TGTTACGAAAGCCAGAGCG
7	mCdkn1a/p21-qR	CCTGGTGATGTCCGACCTG
	mCdkn1a/p21-qF	CCATGAGCGCATCGCAATC
8	mCdkn2b/p15-qR	CCCTGCCACCCTTACCAGA
	mCdkn2b/p15-qF	CAGATACCTCGCAATGTCACG
9	mCcn1-qF	GCGTACCCTGACACCAATCTC
	mCcn1-qR	CTCCTCTTCGCACTTCTGCTC
10	mBub3-qF	GATGGCATCTCCTCGGTTAAG
	mBub3-qR	AATTGGCGGGCACATCGTAG
11	mMapk7-qF	GTGGGGGACGAGTACGAGAT
	mMapk7-qR	TGGTCACCACATCAAAAGCAT
12	mHey1-qF	GCGCGGACGAGAATGGAAA
	mHey1-qR	TCAGGTGATCCACAGTCATCTG
13	mWif1-qF	TCTGGAGCATCCTACCTTGC
	mWif1-qR	ATGAGCACTCTAGCCTGATGG
14	mVegfa-qF	T TACTGCTGTACCTCCACC
	mVegfa-qR	ACAGGACGGCTTGAAGATG
15	mFgfr2-qR	GCCTCTCGAACAGTATTCTCCT
	mFgfr2-qF	ACAGGGTTCATAAGGCATGGG
16	mHprt-qF	GTTAAGCAGTACAGCCCCAAA
	mHprt-qR	AGGGCATATCCAACAACAACTT
17	mRunx2-qF	CCAACCGAGTCATTTAAGGCT
	mRunx2-qR	GCTCACGTCGCTCATCTTG
18	mOsx-qF	ATGGCGTCCTCTCTGCTTGA
	mOsx-qR	GAAGGGTGGGTAGTCATTTG
19	mAlp-qF	CGGGACTGGTACTCGGATAA
	mAlp-qR	ATTCCACGTCGGTTCTGTTC
20	mCol1a1-qF	GCTCCTCTTAGGGGCCACT
	mCol1a1-qR	CCACGTCTCACCATTGGGG
21	mBsp-qF	GACTTTTGAGTTAGCGGCACT
	mBsp-qR	CCGCCAGCTCGTTTTTCATC
22	mOpn-qF	TCAAGTCAGCTGGATGAACC
	mOpn-qR	CTTGTCCTTGTGGCTGTGAA
23	mOcn-qF	GCAGCACAGGTCCTAAATAG
	mOcn-qR	GGGCAATAAGGTAGTGAACAG
24	mLkb1-qF	GTGTGCCAAGCTCATGGGTA
	mLkb1-qR	ATGTCCTTGTGAACAATGCCC
25	mIhh-qF	CTCAGACCGTGACCGAAATAAG
	mIhh-qR	CCTTGGA CTGTAATACACCCAG
26	mPthrp-qF	CATCAGCTACTGCATGACAAGG
	mPthrp-qR	GGTGGTTTTTGGTGTTGGGAG

Full unedited gel for Figure 7A



Full unedited gel for Supplemental Figure 7C



Full unedited gel for Supplemental Figure 7F

