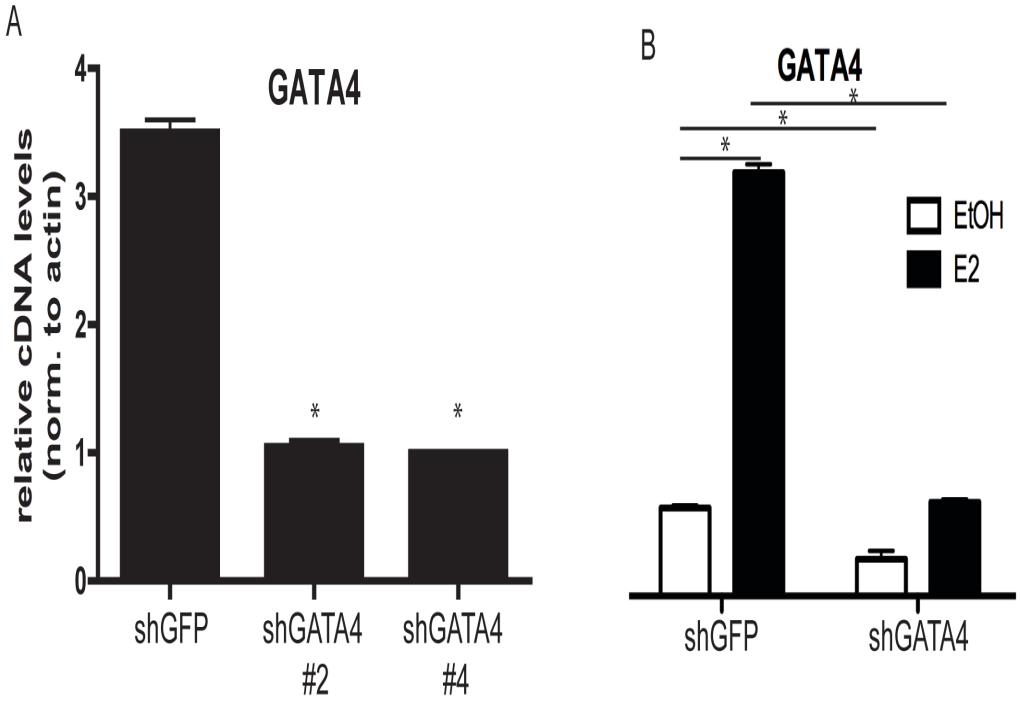
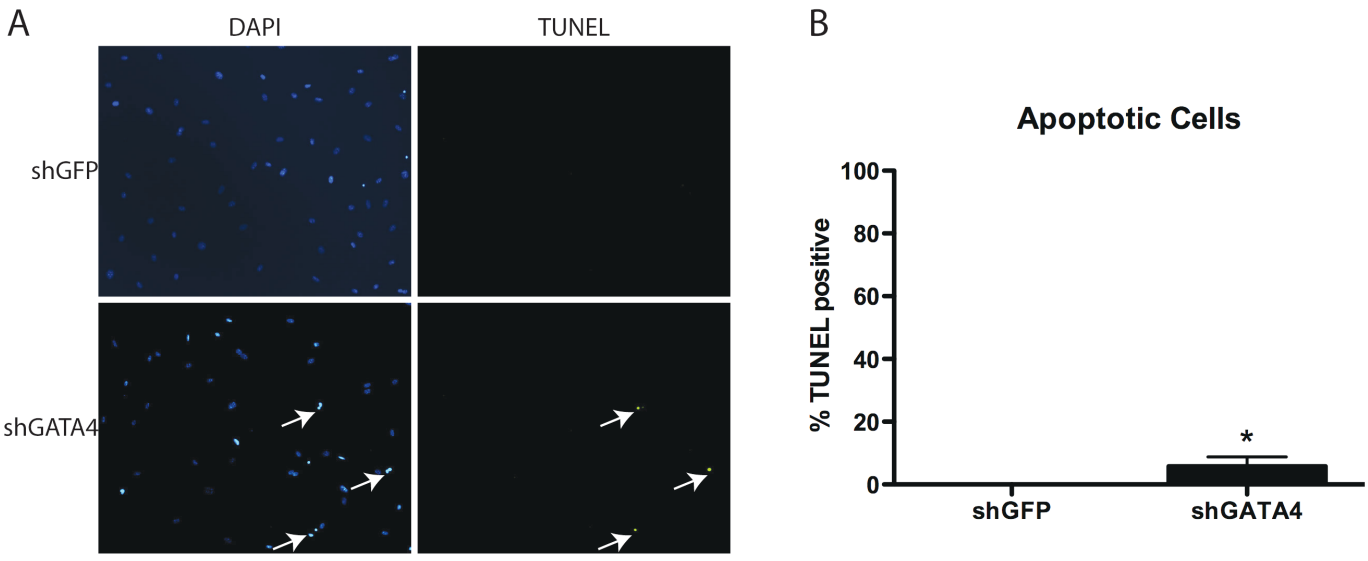
Supplemental Fig. 1



(A) Two different lentiviruses successfully knockdown *Gata4* mRNA. Calvarial osteoblasts were infected with lentivirus expressing either shGFP or shGATA4 clone #2 or #4. Cells were then differentiated for two weeks and RNA was obtained. qPCR was performed for *Gata4* and normalized to actin mRNA.
(B) Calvarial osteoblasts were infected with lentivirus expressing either shGFP or shGATA4 clone #2. Cells were then differentiated for two weeks, treated with 10 nM E2 for 24 hours, and then RNA was obtained. qPCR was performed for *Gata4* and normalized to actin mRNA.

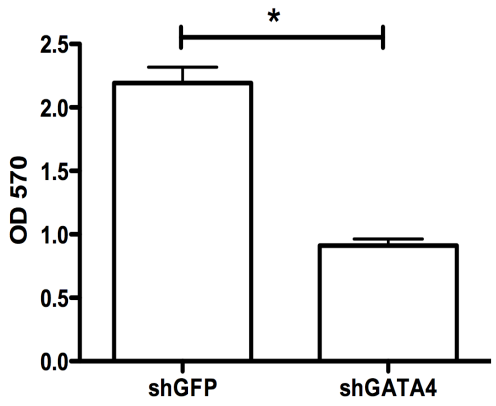
Supplemental Fig. 2



(A) Calvarial osteoblasts were infected with lentivirus expressing either shGFP or shGATA4. TUNEL was performed and cells were counterstained with DAPI.

(B) Multiple wells from part (A) were quantified.

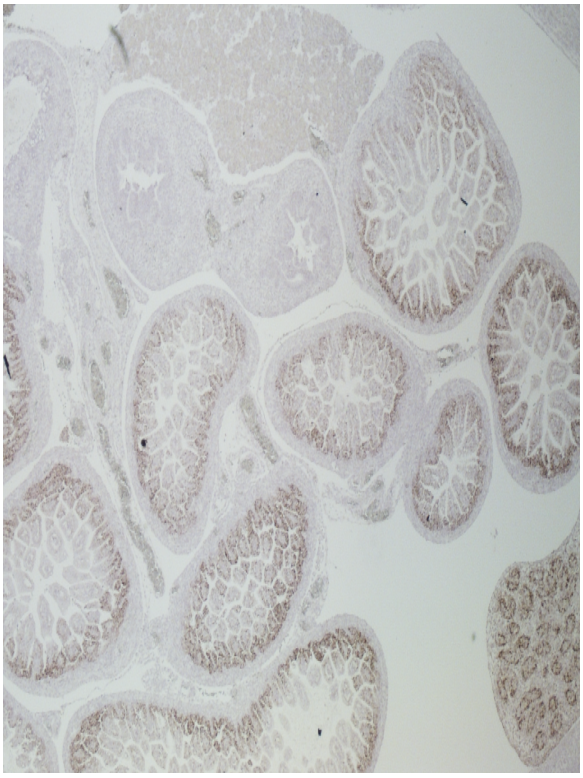
Supplemental Fig. 3



An independent shRNA targeting of *Gata4 (*shGATA4 #4) also yielded a significant reduction in mineralization . Calvarial osteoblasts were infected with lentivirus expressing either shGFP or shGATA4 clone #4. Cells were then differentiated for two weeks. Cells were then fixed and stained using alizarin red. Alizarin red was eluted and the mineral content was measured at an OD of 570. Silencing was performed in three wells and the average OD is displayed. Experiments were then performed in triplicate.

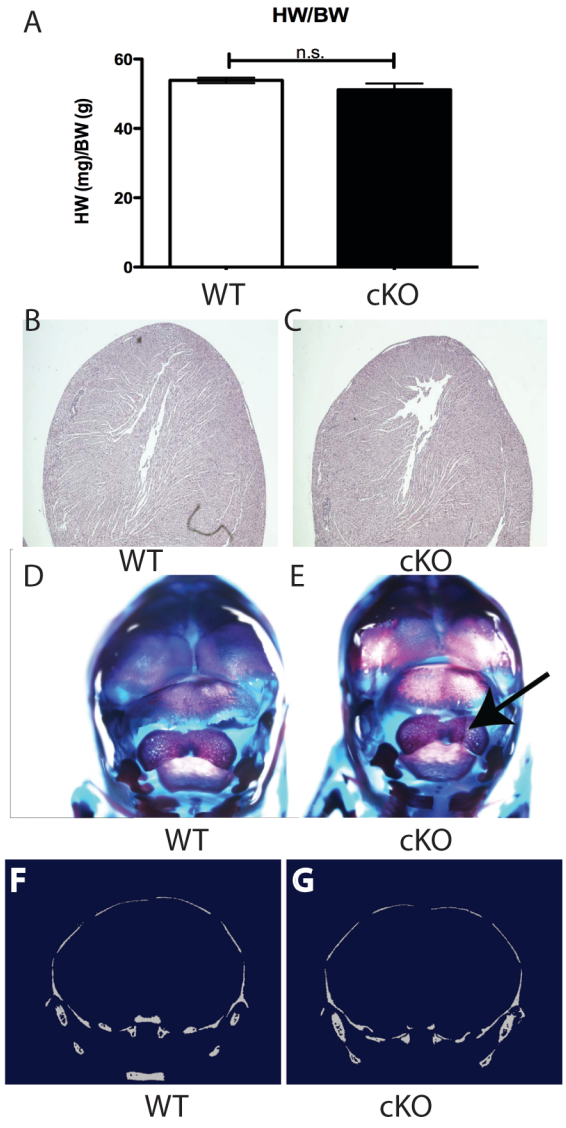
* $P < .05$.

Supplemental Fig. 4



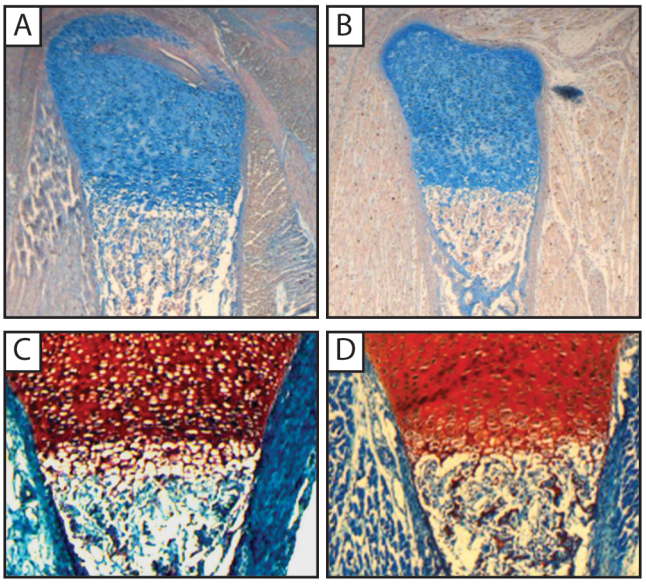
GATA4 *in situ* analysis. E18.5 embryos were probed for *Gata4* expression. The highest level of staining was seen in the intestines shown here.

Supplemental Fig. 5



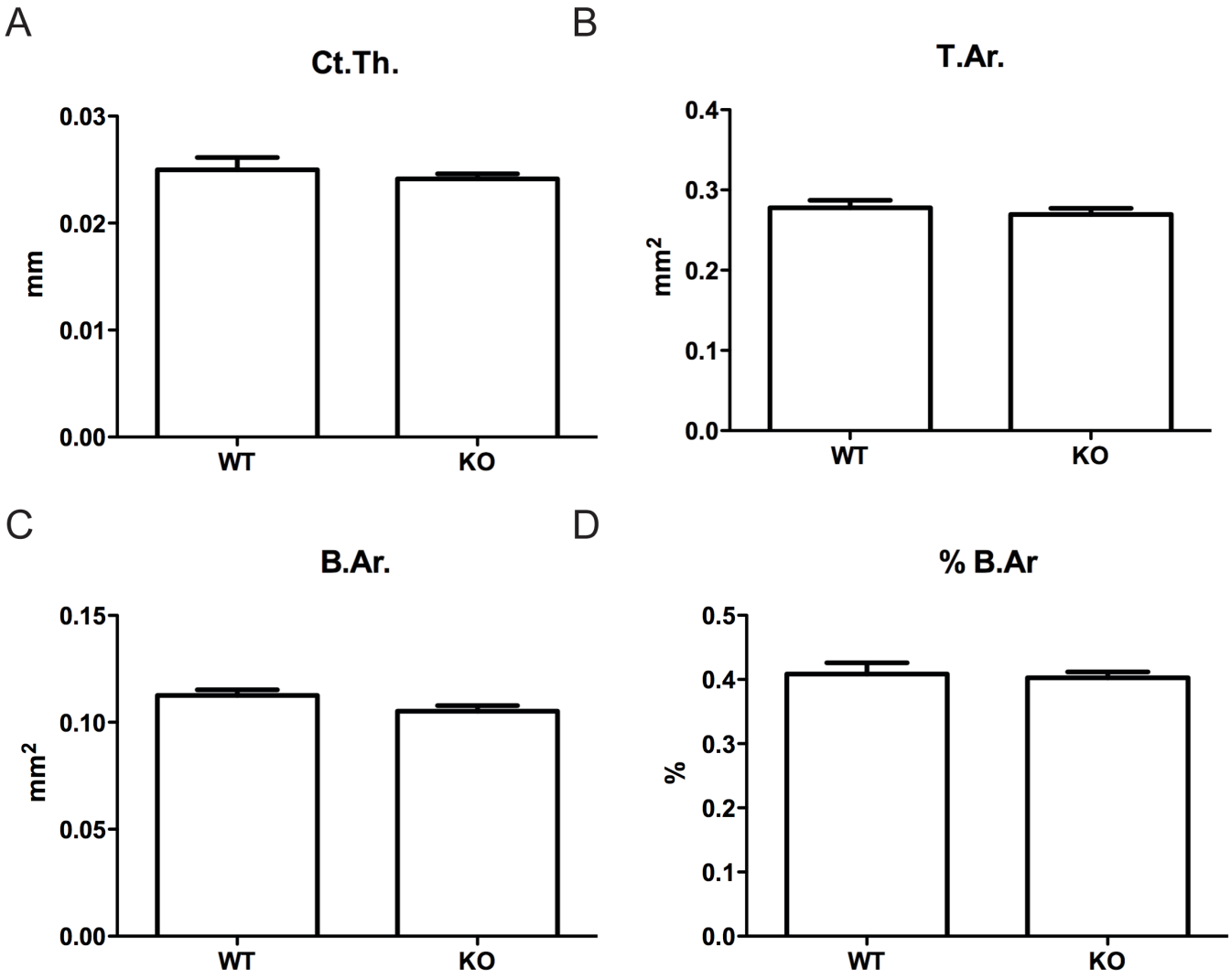
(A) Heart weight/body weight (HW/BW) in wildtype (WT) and cKO mice.
(B and C) H&E staining of hearts from WT and cKO mice.
(D and E) Skeletal preparations of E18.5 mice reveal skull defects (arrow) in cKO mice.
(F and G) microCT images of WT (F) and cKO (G) skulls.

Supplemental Fig. 6



(A-B) Alcian Blue staining of femurs from WT (A) and cKO (B) P0 mice.
(C-D) Safranin O staining of femurs from WT (C) and cKO (D) P0 mice.

Supplemental Fig. 7



Comparison of cortical bone structure in P0 WT and cKO mice assessed by μCT

(A) Cortical thickness (Ct.Th.), (B) Total Area (T. Ar.), (C) Bone Area (B.Ar.) and (D) percent bone area (% B.Ar.). N=9

Supplemental Fig. 8

Most decreased after shGATA4

Gene	Gene Name	DiffScore
Ibsp	integrin binding sialoprotein	-347.0
Akp2	alkaline phosphatase, liver/bone/kidney (Alpl)	-346.4
Prss35	protease, serine, 35	-346.4
Bglap	bone gamma carboxyglutamate protein (osteocalcin)	-265.0
Phex	Phosphate regulating gene with homologies to endopeptidases on the X chromosome	-200.9
Bmp3	bone morphogenetic protein 3	-194.8
Igfbp3	insulin-like growth factor binding protein 3	-126.2
Igfbp5	insulin-like growth factor binding protein 5	-93.0
Dlk1	delta-like 1 homolog	-65.4
Chrd	chordin	-59.1
Sox9	SRY-box containing gene 9	-57.1
Foxc1	forkhead box C1	-47.7
Rbp4	retinol binding protein 4	-43.9
Fgfr2	fibroblast growth factor receptor 2	-35.3
Kazald1	Kazal-type serine peptidase inhibitor domain 1	-31.2
Col11a1	collagen, type XI, alpha 1	-27.1
Sort1	sortilin 1	-27.0
Insig2	insulin induced gene 2	-26.7
BmpR1a	bone morphogenetic protein receptor, type 1A	-20.4
Smad5	MAD homolog 5	-17.3
Mmp13	matrix metallopeptidase 13	-15.4
Thra	thyroid hormone receptor alpha	-14.7
Sp7	Sp7 transcription factor 7 (osterix)	-13.1
Ror2	receptor tyrosine kinase-like orphan receptor 2	-11.4
Bmp4	bone morphogenetic protein 4	-11.3
Smad3	MAD homolog 3	-10.3
Insig1	insulin induced gene 1	-10.3
Smo	smoothened homolog	-10.2
Tgfbr1	transforming growth factor, beta receptor I	-9.6
Msx1	homeobox, msh-like 1	-9.3
Cbfb	core binding factor beta	-8.9
Mef2c	myocyte enhancer factor 2C	-8.4
Hspg2	perlecan (heparan sulfate proteoglycan 2)	-7.0
Tgfbr2	transforming growth factor, beta receptor II	-6.4
Ltbp3	latent transforming growth factor beta binding protein 3	-6.0
Osr2	odd-skipped related 2	-6.0
Bmp6	bone morphogenetic protein 6	-5.9
Fgfr1	fibroblast growth factor receptor 1	-5.7
Foxc2	forkhead box C2	-5.6
Znrd1	zinc ribbon domain containing, 1	-5.2
Col9a1	collagen, type IX, alpha 1	-3.2
Bcl2	B-cell leukemia/lymphoma 2	-3.2

Less decreased after shGATA4

Supplemental Fig. 9

Gene	Gene Name	DiffScore
Bmp3	bone morphogenetic protein 3	-194.8
BmpR1a	bone morphogenetic protein receptor, type 1A	-20.4
Smad5	MAD homolog 5	-17.3
Tgfbr3	transforming growth factor, beta receptor II	-16.3
Bmp4	bone morphogenetic protein 4	-11.3
Smad3	MAD homolog 3	-10.3
Tgfbr1	transforming growth factor, beta receptor I	-9.6
Tgfbr2	transforming growth factor, beta receptor II	-6.4
Bmp6	bone morphogenetic protein 6	-5.9
Bmp8a	bone morphogenetic protein 8a	-2.0
TGFB1I1	Transforming growth factor beta 1 induced transcript 1	3.4
Bmp1	bone morphogenetic protein 1	3.6
Tgfb1	Transforming growth factor, beta 1	4.3
BMPER	BMP binding endothelial regulator	10.8
Tgfb2	Transforming growth factor, beta 2	10.9
Bmpr1b	bone morphogenetic protein receptor, type 1B	11.2
Tgfb3	Transforming growth factor, beta 3	69.4

Supplemental Fig. 10

HUMAN mRNA PRIMERS

SEQUENCE	FORWARD PRIMER 5′ TO 3′	REVERSE PRIMER 5′ TO 3′
B-ACTIN	GGACTTCGAGCAAGAGATGG	AGCACTGTGTTGGCGTACAG
GATA4	TCCCTCTTCCCTCCTCAAAT	TCAGCGTGTAAAGGCATCTG
RUNX2	TTTGCACTGGGTCATGTGTT	TGGCTGCATTGAAAAGACTG
COL1A1	ACGTCCTGGTGAAGTTGGTC	ACCAGGGAAGCCTCTCTCTC
Alkaline phosphatase	CCACGTCTTCACATTTGGTG	AGACTGCGCCTGGTAGTTGT

MOUSE mRNA PRIMERS

SEQUENCE	FORWARD PRIMER 5′ TO 3′	REVERSE PRIMER 5′ TO 3′
B-ACTIN	AGCCATGTACGTAGCCATCC	CTCTCAGCTGTGGTGGTGAA
GATA4	GTTGTGGTGGTGGGTTTTTC	CCCCAGGAAGCATTCAGTAA
COL1A1	ACGTCCTGGTGAAGTTGGTC	CAGGGAAGCCTCTTTCTCCT
ALP1	GTTGCCAAGCTGGGAAGAACAC	CCCACCCCGCTATTCCAAAC
RUNX2	CACGGTGACTCCCGTTACTT	ATACGTGTGACCCAGTGCAA
ERα	GCCAAGGAGACTCGCTACTG	CTCCGGTTCTTGTCAATGGT
FASL	CTGGGTTGTACTTCGTGTATTCC	TGTCCAGTAGTGCAGTAGTTCAA
EAP	GACGCAGAGTCCCTTCAGAC	CACCCCTACTCCCCATACCT
BSP	AAAGTGAAGGAAAGCGACGA	GTTCCTTCTGCACCTGCTTC
OCN	GGCCCTGAGTCTGACAAAGC	GCGCCGGAGTCTGTTCACT
BMP4	GCGGGACTTCGAGGCGACAC	CGGGACGCTCCGGGTACTCA
BMP6	GCGGTGACGGCTGCTGAGTT	GCACGGGGGTTGACGTGGAG
BMPR1A	CACCGAAAGCCCAGCTACGCA	GGGGGCAGTGTAGGCTGCAA
SMAD5	TCACCTGCGAGCCACGCTTT	TGCTTGGCTGTCCTGGGCTG
TGFB1	AGGGCTACCATGCCAACTTC	CCACGTAGTAGACGATGGGC
TGFB2	CTGCCTTCGCCCTCTTTACA	CCCCAGCACAGAAGTTAGCA
TGFB3	ATGACCCACGTCCCCTATCA	ACTCAGACTCCGAGGTCTCC