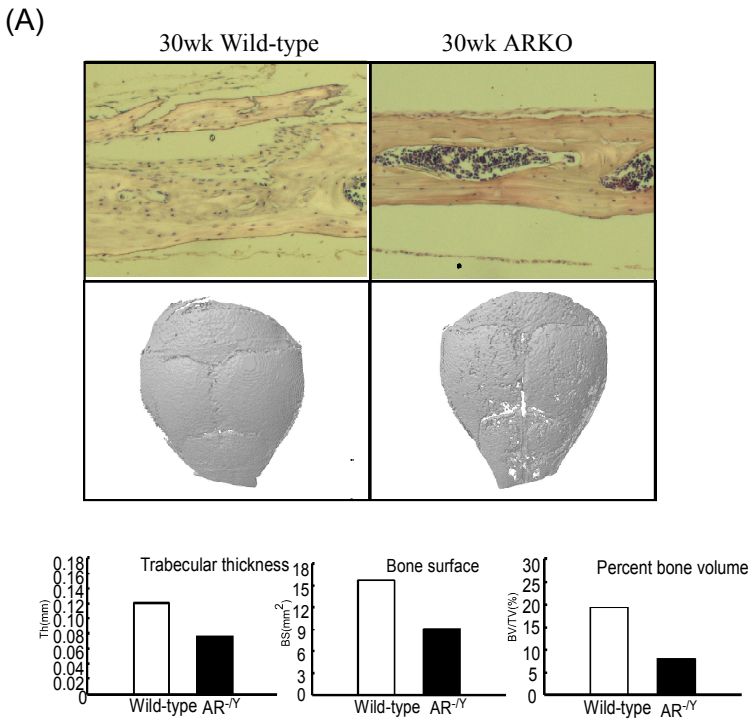
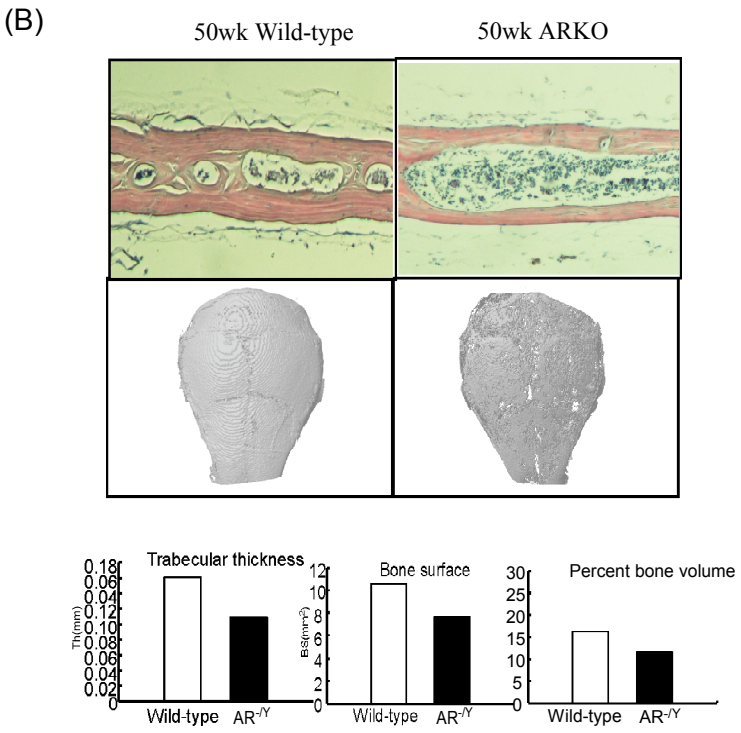
Supplemental Table 1

Histomorphometry of calvaria in ARKO mice

parameter	Males		Females	
	Wild-type	ARKO	Wild-type	ARKO
CWi (μm)	126±4.0	113.4±3.2[a]	124±5.1	103.2±10.1[**]
BAr (μm^2)	452,170±15,212	340,221±30,461[a,**]	532,125±20,336	407,268±16,439[a,**]
OS/BS (%)	16.21±1.73	14.31±1.51[*]	7.04±0.69	4.17±0.15[*]
N.Oy/BAr (#/mm^2)	720±54	419±38[**]	832±31	441±60[**]
N.Oc/BAr (#/mm^2)	5.2±2.1	20.4±3.2[**]	4.6±2.3	24±6.2[**]

8-week-old male and female ARKO mice and wild-type littermates (N=4-6). CWi, calvarial width; BAr, bone area; OS/BS, osteoid surface/bone surface; N.Oy/BAr, number of osteocytes/bone area, N.Oc/BAr, number of osteoclasts/bone area. Each value is the mean±SEM of the number of samples in parentheses. [a] Different from the respective wild type group with Bonferroni's adjustment, $P<0.01$. [*] Different from the respective wild type group, $P<0.05$. [**] Different from the respective wild type group, $P<0.01$.





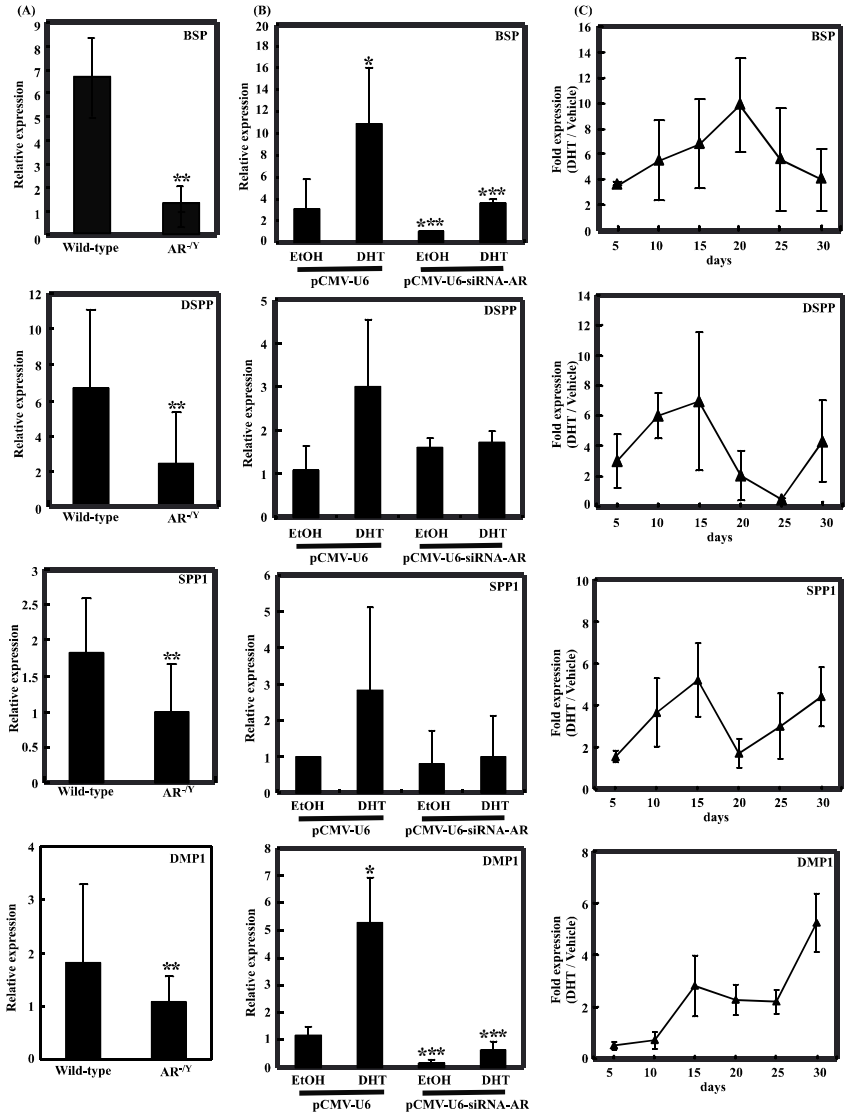
Supplemental Figure 1. Progression of altered calvarial morphology and defective mineralization observed in $AR^{-/Y}$ mice. Three-dimensional computer tomography images, H & E staining and bone histomorphometric measurements of the calvaria from representative (A) 30-week-old and (B) 50-week-old male wild-type and $AR^{-/Y}$ mice. Bone histomorphometric analysis of calvarial bone volume, thickness, and bone surface values are measured.

Supplemental Table 2

Symbol	Fold	Accession number	Gene description
Bone Mineralization			
AKP2	**0.2**	**NM_007431**	***Mus musculus*** **alkaline phosphatase 2, liver / Bone / kindney**
DMP1	**0.5**	**NM_016779**	***Mus musculus*** **dentin matrix protein 1**
DSPP	**0.6**	**NM_010080**	***Mus musculus*** **dentin sialophosphoprotein**
MEPE	**0.5**	**NM_053172**	***Mus musculus*** **matrix extracellular phosphoglycoprotein with ASARM motif (bone)**
OPN	**0.5**	**NM_009263**	***Mus musculus*** **secreted phosphoprotein 1 (Spp1)**
Pit-1	**0.3**	**NM_015747**	***Mus musculus*** **solute carrier family 20, member 1**
Pit-2	**0.7**	**NM_011394**	***Mus musculus*** **solute carrier family 20, member 2 (Slc20a2)**
BSP	**0.6**	**XM_001475269**	***Mus musculus*** **similar to Integrin binding sialoprotein**
Skeletal Development			
Ahsg	**3.8**	**NM_013465**	***Mus musculus*** **alpha-2-HS-glycoprotein**
Anxa5	**0.9**	**NM_009673**	***Mus musculus*** **annexin A5**
Fgfr2	**16**	**NM_201601**	***Mus musculus*** **fibroblast growth factor receptor 2**
Smad1	**0.9**	**NM_008539**	***Mus musculus*** **MAD homolog 1 (Drosophila)**
Tuft1	**4.2**	**NM_011656**	***Mus musculus*** **tuftelin 1**
Vdr	**5**	**NM_009504**	***Mus musculus*** **vitamin D receptor**
Extracellular Matrix (ECM) Proteins			
BMP1	**1.3**	**NM_009755**	***Mus musculus*** **bone morphogenetic protein 1**
Ctsk	**0.7**	**NM_007802**	***Mus musculus*** **cathepsin K**
MMP2	**1.1**	**NM_008610**	***Mus musculus*** **matrix metallopeptidase 2**
MMP8	**0.7**	**NM_008611**	***Mus musculus*** **matrix metallopeptidase 8**
MMP9	**1.1**	**NM_013599**	***Mus musculus*** **matrix metallopeptidase 9**
Phex	**1.3**	**NM_011077**	***Mus musculus*** **phosphate regulating gene with homologies to endopeptidases on the X chromosome (hypophosphatemia, vitamin D resistant rickets)**
Phosphate Transport			
Bmp5	**6.1**	**NM_007555**	***Mus musculus*** **bone morphogenetic protein 5**
Col1a1	**0.6**	**NM_007742**	***Mus musculus*** **collagen, type I, alpha 1**
Col1a2	**0.8**	**NM_007743**	***Mus musculus*** **collagen, type I, alpha 2**
Col2a1	**2**	**NM_031163**	***Mus musculus*** **collagen, type II, alpha 1**
Col4a1	**1.9**	**NM_009931**	***Mus musculus*** **collagen, type IV, alpha 1**
Col4a2	**3.7**	**NM_009932**	***Mus musculus*** **collagen, type IV, alpha 2**

Col5a1	**2.7**	**NM_015734**	***Mus musculus* procollagen, type V, alpha 1**
Col6a1	**3.9**	**NM_009933**	***Mus musculus* collagen, type VI, alpha 1**
Regulation of Cell Cycle			
Fgf1	**0.7**	**NM_010197**	***Mus musculus* fibroblast growth factor 1**
Itgb1	**0.9**	**NM_010578**	***Mus musculus* integrin beta 1 (fibronectin receptor beta)**
Pdgfa	**1.4**	**NM_008808**	***Mus musculus* platelet derived growth factor, alpha**
Tgfb1	**0.6**	**NM_011577**	***Mus musculus* transforming growth factor, beta 1**
Tgfb2	**1.2**	**NM_009367**	***Mus musculus* transforming growth factor, beta 2**
Tgfb3	**2.6**	**NM_009368**	***Mus musculus* transforming growth factor, beta 3**
Vegfa	**0.8**	**NM_001025257**	***Mus musculus* vascular endothelial growth factor A, transcript variant 3**
Vegfb	**0.5**	**NM_011697**	***Mus musculus* vascular endothelial growth factor B**

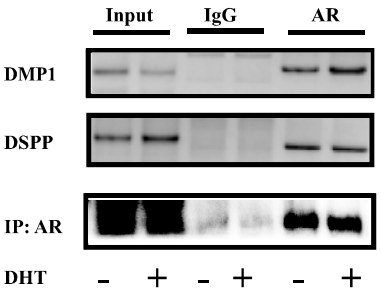
Comparative microarray analysis of genes expressed in the primary mouse osteoblasts from wildtype and ARKO mice. The upregulated values represent the fold increase in expression of a particular gene in the primary wildtype osteoblasts when compared to the primary ARKO osteoblasts, and the downregulated values represent the inverse reciprocal fold decrease in expression of candidate genes in the primary wildtype osteoblasts when compared to the primary ARKO osteoblasts.



(D)

Gene	ARE Sequence	Position	%Match
AKP2	AGAACGaacAGCTCT	-2284	75
BSP	ATAAAAaaaTCT-	-2479	75
	TCTtACActtTGGTTT	-1460	75
	AGAAAGcacTGTTCC	-868	75
DMP1	AGAAAAtgaTATCCT	-1797	75
	AGATCAgtaAGAATT	-541	75
	AGAACCacaAGAGCT	-178	75
SPP1	AGAAGCaatTGTACT	-1363	75
	ATAAGAgacTGTACT	-268	75
DSPP	TGTATAcagTGTTCT	-1931	75
	AGAGTAgccAGTGCT	-1813	75
	GAAACAggtAGAACT	-1495	75
	AGGACAagcAGGGCT	-1440	75
	AGCACAgccTGTTTT	-746	83
Experimental Consensus	AGAACAnnnAGTACT		
Putative Consensus	AGAACAnnnTGTTCT		

(E)



Supplemental Figure 2. SIBLING family genes are downstream target genes in androgen signal transduction pathways in osteoblasts. Real-time PCR shows a decrease in SIBLING family gene expression in primary $AR^{-/Y}$ osteoblasts and MC3T3-E1 cells transfected with siRNA-AR, compared with control cells. (A) Primary osteoblasts from neonatal calvaria of Wild-type and $AR^{-/Y}$ mice and (B) MC3T3-E1 cells stably transfected with siRNA-control (pCMV-U6), siRNA-AR, were stimulated with or without 10^{-8} M of DHT in differentiation medium. The relative mRNA expression patterns of SPP1, DSPP, BSP and DMP1 were determined by real-time PCR. (C) The fold expression profiles of SPP1, DSPP, BSP and DMP1 mRNA in MC3T3-E1 cells treated with 10^{-8} M of DHT were analyzed by real-time PCR, compared with the control (ethanol) at each time point as indicated. Data are representative of three independent experiments and are shown as mean ± SEM. *$p < 0.05$, as compared with control. (D) AREs verified through the identifying gene promoters in the NCBI database are shown with positions relative to the transcriptional start site. Light gray background represents the sequence to be the same as experimental consensus sequence and the heavy gray background represents the sequence to be same as putative consensus sequence. A consensus sequence indicates the relative frequency and importance of nucleotides in the motif. (E) ChIP assay for AR binding to DMP1 and DSPP promoter. Immunoprecipitations were performed with AR antibody. AR regulates the SIBILING family genes through binding to these gene promoters that contains androgen response elements (AREs).

Supplemental Table 3

ALP activity in newborn, 7-week and 12-week old male mice

	wild-type	$AR^{-/Y}$
Calvaria (newborn)	23384 ± 670	18753 ± 1223*
Plasma (7 weeks)	102 ± 7.38	110 ± 4.71
Plasma (12 weeks)	70 ± 15.9	57 ± 10.3

(Unit = n mole *p*-nitrophenol/mg protein/minute)

Relative to age-matched wild-type littlermates, ALP activity is significantly elevated in newborn calvaria (N=6) but not in plasma (N>7) from 7- and 12-week-old $AR^{-/Y}$ mice.*$p<0.05$ for ANOVA test.

Supplemental Table 4

Primer sequences used for Real-time PCR

Gene	Primer Sequence
AKP2	Forward: CAC CTG CCT TAC CAA CTC TTT TG Reverse: GGC TAC ATT GGT GTT GAG CTT TT
DMP1	Forward: GAG AAC TTC GCT GAG GTT TTG AC Reverse: CCC AAA GGA ACA CAA GGA GAA T
DSPP	Forward: TGT GGC TGT GCC TCT TCT AAC A Reverse: TCG CTA AGT ACC TGC TCT CCT ATC TC
BSP	Forward: CAG AGG AGG CAA GCG TCA CT Reverse: CTG TCT GGG TGC CAA CAC TG
SPP1	Forward: GAT GCC ACA GAT GAG GAC CTC Reverse: CTG GGC AAC AGG GAT GAC AT
β-actin	Forward: AGG CCA ACC GTG AAA AGA TG Reverse: TGT GGT ACG ACC AGA GGC ATA C