

Supplementary Information

Responses of primary osteoblasts and osteoclasts from hemizygous β -globin knockout thalassemic mice with elevated plasma glucose to 1,25-dihydroxyvitamin D₃

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Table S1. *Mus musculus* primers used in real-time PCR

Gene	Accession no.	Primer (Forward/Reverse)	Product size (bp)	Annealing temperature (°C)	Bone related-function
<i>Osteoblast-derived osteoclastogenic factors</i>					
RANKL	NM_011613	5' -CGCTCTGTTTCCTGTACTTTCG-3' 5' -CTCTCCAGAGTCGAGTCCTGC-3'	126	57	• Inducing osteoclastogenesis
M-CSF	NM_007778	5' -GACCCCTCGAGTCAACAGAGC-3' 5' -TGTCAGTCTCTGCCTGGATG-3'	236	58	
MCP-1	NM_011333	5' -AGGTCCCTGTCTATGCTTCTG-3' 5' -TCTGGACCCATTCCTTCTTG-3'	249	55	• Recruitment of osteoclast precursors
IL-1 β	NM_008361	5' -GGGCCTCAAAGGAAAGAATC-3' 5' -TACCAGTTGGGGAACCTCTGC-3'	183	54	• Promoting osteoclastogenesis and osteoclast activity
IL-6	NM_031168	5' -AGTTGCCTTCTTGGGACTGA-3' 5' -TCCACGATTTCCAGAGAAC-3'	159	56	
<i>Osteoblast differentiation markers</i>					
ALP	NM_001287172	5' -TCTCCAGACCTGCAACCTC-3' 5' -CATCCTGAGCAGACCTGGTC-3'	150	58	• Marker of early osteoblast differentiation
Osteocalcin	NM_001305448	5' -ACGGTATCACTATTTAGGACCTGTG-3' 5' -ACTTTATTTTGGAGCTGCTGTGAC-3'	141	57	• Marker of late osteoblast differentiation
Runx2	NM_001146038	5' -AGGGACTATGGCGTCAAACA-3' 5' -GGCTCACGTCGCTCATCTT-3'	137	57	• Transcription factor inducing osteoblast differentiation
<i>Osteoclast-related markers</i>					
RANK	NM_009399	5' -CATCTTCGGCGTTTACTACAGG-3' 5' -TCCACTTAGACTACTGCAAGCA-3'	91	57	• Receptor for RANKL
Cathepsin K	NM_007802	5' -GAACGAGAAAGCCCTGAAGAGA-3' 5' -TATCGAGTGCTTGCTTCCCTTC-3'	190	57	• Protease enzyme involved in bone resorption
Calcr	NM_001355192	5' -CGGACTTTGACACAGCAGAA-3' 5' -GTCACCTCTGGCAGCTAAG-3'	247	55	• Marker of osteoclast differentiation
NFATc1	NM_001164112	5' -CCGTTGCTTCCAGAAAATAACA-3' 5' -TGTGGGATGTGAACTCGGAA-3'	152	54	• Stimulating osteoclast differentiation
c-Fos	NM_010234	5' -CCAGTCAAGAGCATCAGCAA-3' 5' -AAGTAGTGCAGCCCGGAGTA-3'	247	55	• Transcription factor activating osteoclast differentiation
<i>Iron transporter</i>					
DMT1	NM_001146161	5' -TCAGAGCTCCACCATGACTG-3' 5' -TGTGAACGTGAGGATGGGTA-3'	235	57	• Initiating iron uptake
<i>Insulin receptor</i>					
INSR	NM_010568	5' -TCCTGGATTCTGTGGAGGAC-3' 5' -ATGGTTGGGCAAACTTTCTG-3'	178	54	
<i>Housekeeping gene</i>					
β -actin	NM_007393	5' -CCAGGTCATCACTATTGGCA-3' 5' -ACCACCAGACAGCACTGTGTT-3'	172	54	

RANKL, receptor activator of nuclear factor- κ B ligand; M-CSF, macrophage colony-stimulating factor; MCP-1, monocyte chemoattractant protein-1; IL-1 β , interleukin-1beta; IL-6, interleukin-6; ALP, alkaline phosphatase; Runx2, *runt*-related transcription factor-2; RANK, receptor for receptor activator of nuclear factor- κ B ligand; Calcr, calcitonin receptor; NFATc1, nuclear factor of activated T-cells c1; DMT1, divalent metal ion transporter-1; INSR, insulin receptor

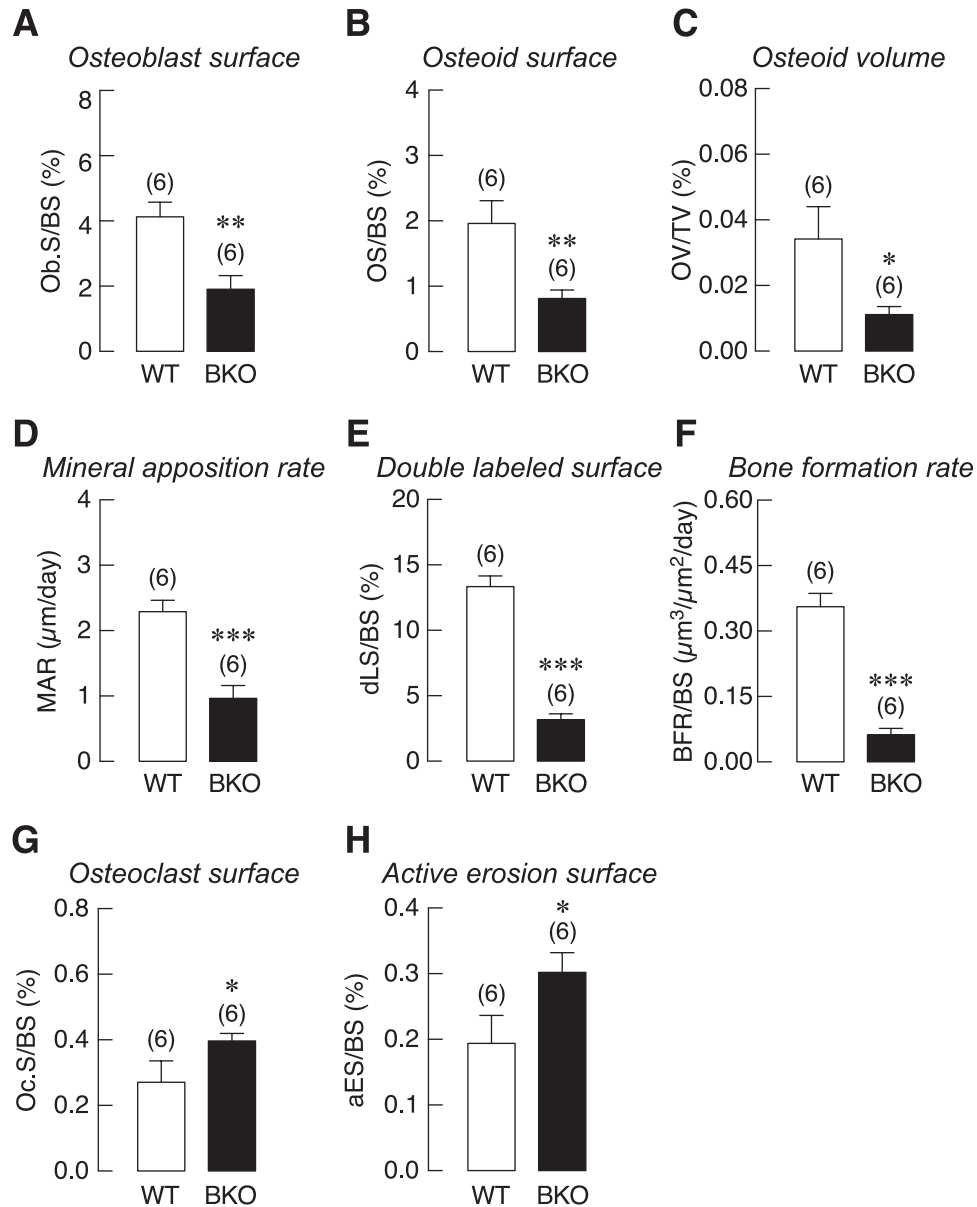


Figure S1: Bone formation- and resorption-related parameters in the proximal tibial metaphyses of 2-month-old male BKO mice. *A*: osteoblast surface (Ob.S) normalized by bone surface (BS), *B*: osteoid surface (OS) normalized by BS, *C*: osteoid volume (OV) normalized by tissue volume (TV), *D*: mineral apposition rate (MAR), *E*: double labeled surface (dLS) normalized by BS, *F*: bone formation rate (BFR) normalized by BS, *G*: osteoclast surface (Oc.S) normalized by BS, *H*: active erosion surface (aES) normalized by BS. Numbers of animals are shown in the parentheses. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ vs. age-matched WT group.

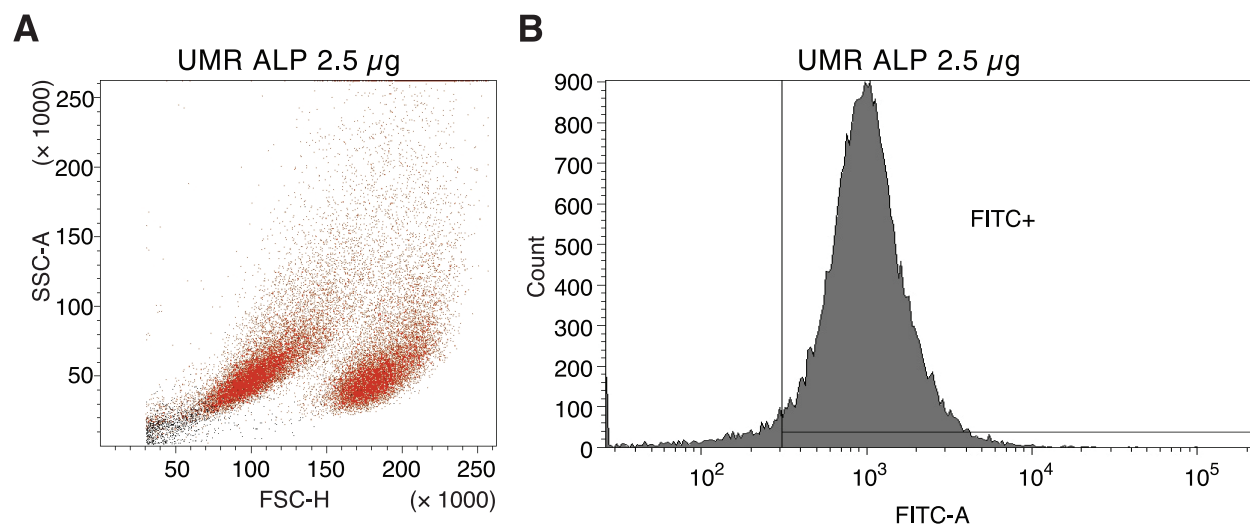


Figure S2: (A) Forward scatter (FSC) and side scatter (SSC), and (B) histogram of fluorescein (FITC)-conjugated alkaline phosphatase (ALP) in UMR-106 cells as analyzed by flow cytometry.

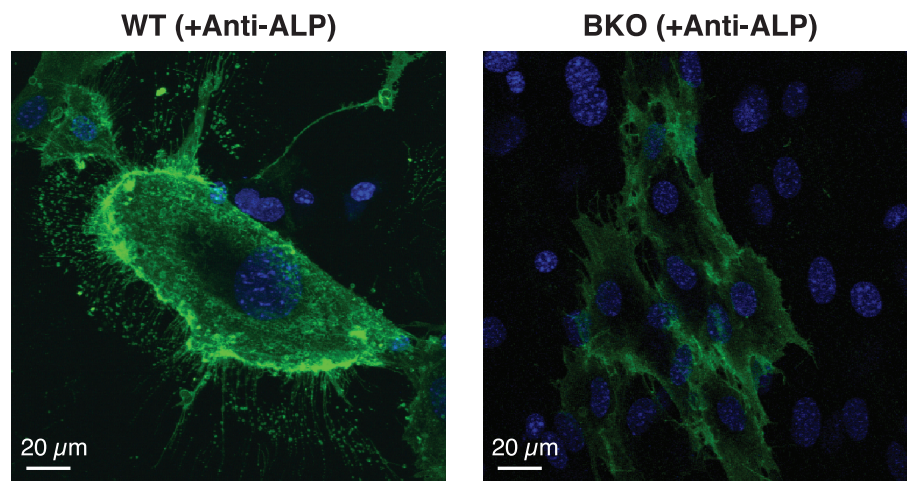


Figure S3: Representative immunofluorescent photomicrographs show the expression and localization of ALP protein in primary osteoblasts isolated from wild-type (WT) and BKO mice (scale bars, 20 μm). The primary osteoblasts were plated on a coverslip and incubated with primary antibody against ALP (+Anti-ALP). The cells were stained for ALP (green) and nuclei (blue).