Supplemental Material

Supplemental Methods

Genotyping of *Mark3* global KO and *Mark3$^{f/f}$* mice. Mark3 global KO genotypes were determined based on the presence or absence of the KO allele and the WT allele using two separate genotyping reactions. The genotyping primers used to determine the presence of the KO allele were: F – GCTTCCTGTGCACTGGGGTTA, R – CCTTCATTCTTCCATGTGCAACC. The genotyping primers used to determine the presence of the WT allele were: F – GCTTCCTGTGCACTGGGGTTA, R – TGAGCCAATTCTCCAGCTCT. The genotyping primer set used for *Mark3$^{f/f}$* mice were: F –GCTTCCTGTGCACTGGGGTTA, R – TGAGCCAATTCTCCAGCTCT, with a PCR product of 220 bp WT and 438 bp transgene.

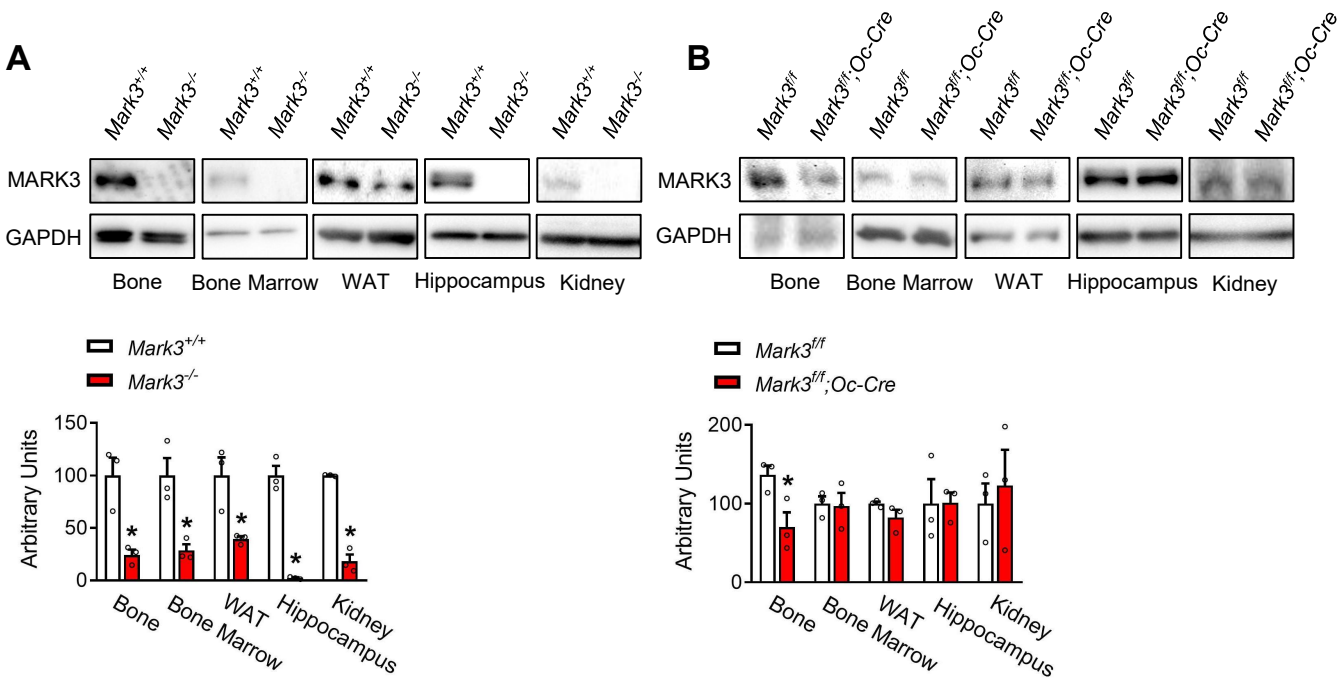
Supplemental Figure 1. Expression of MARK3 protein in *Mark3$^{-/-}$* mice, *Mark3$^{f/f}$;Oc-Cre* mice and their control littermates. (**A**) Immunoblot of MARK3 in femoral cortical bone, bone marrow, white adipose tissue (WAT), hippocampus and kidney of *Mark3$^{+/+}$* and *Mark3$^{-/-}$* mice. (**B**) Immunoblot of MARK3 in femoral cortical bone, bone marrow, WAT, hippocampus and kidney in *Mark3$^{f/f}$* and *Mark3$^{f/f}$;Oc-Cre* mice. Graphs represent mean ± SEM, and *p<0.05 by Student's t-test between groups.

Supplemental Figure 2. Immunoblot of β-catenin in Mark3 deleted primary osteoblasts. *Mark3$^{f/f}$* primary osteoblasts were transfected with Ad-GFP and Ad-CRE. 48h later, p-β-catenin and t-β-catenin was measured (n=6). p-β-catenin was normalized by t-β-catenin. Graphs represent mean ± SEM, and *p<0.05 by Student's t-test between groups.

Supplemental Figure 3. Schematic of R26-LSL-JAG1 overexpression mouse model. Schematic of R26-LSL-JAG1 vector is showing before and after Cre-mediated excision. The floxed mice have loxP-flanked STOP cassette (Neo with 3x polyA) upstream of the *Jag1* locus.

Supplemental Table 2. Gene symbols, fold changes in expression, and adjusted p-values of Notch pathway in pathway analyses of RNA-seq.

Gene	fold change	p-value
Kat2a	0.559561	0.00283
Dvl2	0.394093	0.000846
Dvl3	0.377788	0.000589
Ctbp1	0.40716	0.003426
Dtx2	0.369867	0.000791
Dtx3	0.526604	0.014069
Rfng	0.39808	0.000559
Dvl1	0.154124	2.21E-07
Numbl	0.239531	0.000873
Notch3	0.389655	0.00173
Notch2	0.716462	0.017649
Notch1	0.377058	0.0009
Psen2	0.428345	0.005485
Lfng	0.288177	0.000732
Ncor2	0.202379	9.09E-05


A
Mark3+/+
Mark3-/-
MARK3
GAPDH
Bone
Bone Marrow
WAT
Hippocampus
Kidney
B
Mark3f/f
Mark3f/f;Oc-Cre
MARK3
GAPDH
Bone
Bone Marrow
WAT
Hippocampus
Kidney

Mark3+/+
Mark3-/-
Arbitrary Units
150
100
50
0
Bone
Bone Marrow
WAT
Hippocampus
Kidney

Mark3f/f
Mark3f/f;Oc-Cre
Arbitrary Units
200
100
0
Bone
Bone Marrow
WAT
Hippocampus
Kidney

Figure S1

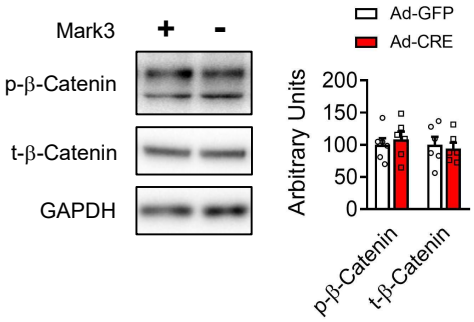

Mark3
+
-
p-β-Catenin
t-β-Catenin
GAPDH
Ad-GFP
Ad-CRE
Arbitrary Units
200
150
100
50
0
p-β-Catenin
t-β-Catenin

Figure S2

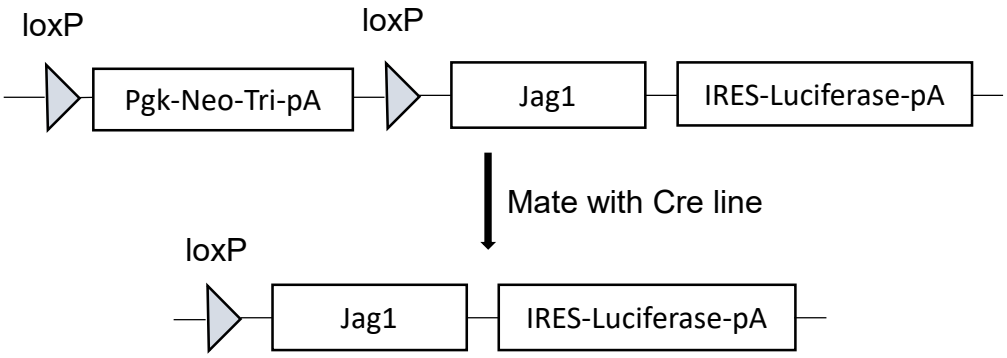

loxP
loxP
Pgk-Neo-Tri-pA
Jag1
IRES-Luciferase-pA
Mate with Cre line
loxP
Jag1
IRES-Luciferase-pA

Figure S3