

SUPPLEMENTAL MATERIAL

Brd4 is required for chondrocyte differentiation and endochondral ossification

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Running Title: Brd4 and endochondral ossification

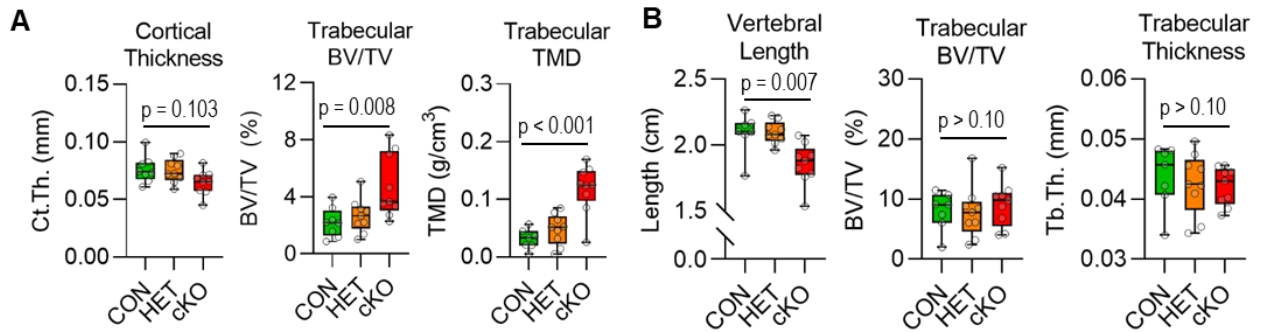
Supplemental Table 1: Primers used in this study.

Genotyping Primer Pairs		
Gene	Forward Sequence	Reverse Sequence
Brd4	CCTGTGTGCACTTGCTCCCGAGGAGAGA	GGAACCTCGCTATGTGTAACCA
Cre	TCCAATTTACTGACCGTACACCAA	GGACTAGAAACCTCCCAAATGTCTACAA
DNA Quantification Primer Pairs		
Gene	Forward Sequence	Reverse Sequence
Brd4 exon 3 v1	CTGCCAGTAATGGGGGATGG	TGCAGTTGGTTTGTCTGTCTCT
Brd4 exon 3 v2	GGACACTGGTGGTTAAGAGTTCA	CCTAGCCATCCTGACCAGTT
Brd4 exon 6	AACTCACCCCTTTCCTGCTG	CAATGATGGGCGGGTGACT
RT-qPCR Primer Pairs		
Gene	Forward Sequence	Reverse Sequence
Acan	CCGCTTGCCAGGGGGAGTTG	GATGATGGGCGCACGCCGTA
Bglap	GCAATAAGGTAGTGAACAGACTCC	CCATAGATGCGTTTGTAGGCGG
Brd4	GGAGGAAAGAAACAGGGGCA	GAGTCTGAAGTGGCTGAGGG
Col10a1	GGGATGAAGTATTGTGTCTTGGG	TTCTGCTGCTAATGTTCTTGACC
Col1a1	CCTCAGGGTATTGCTGGACAAC	CAGAAGGACCTTGTGGCCAGG
Col2a1	GCTGGTGAAGAAGGCAAACGAG	CCATCTTGACCTGGGAATCCAC
Comp	TGCGAGAACTTCAGGAGACT	CTGCATTCCGCAAGCATCA
Dcn	GCTCACGCAGTAAAACCTTAG	TTTCACGACCTTTTAATCCGGG
Gapdh	CATCACTGCCACCCAGAAGACTG	ATGCCAGTGAGCTTCCCCTTCAG
Hist2h4	AAGTTTCTCCGCGACAACATCC	GTCGCGGATCACATTCTCAAGG
Ibsp	GAATGGCCTGTGCTTTCTCG	CCGGTACTTAAAGACCCCGTT
Ihh	GCTTTCCTGCCGGAGCCCAG	GGTGGGGTCCCATCCTCCC
Mki67	CAGAGCTAACTTGCGCTGAC	ACTACAGGCAGCTGGATACG
Mmp13	CTTCTGGCACACGCTTTTCC	TGGCTTTTGCCAGTGTAGGT
Runx2	CCTGAACTCTGCACCAAGTCCT	TCATCTGGCTCAGATAGGAGGG
Sox5	CGCCAGATGAAAGAGCAACTCAG	TGAGTCAGGCTCTCCAGTGTTG
Sox6	GCATAAGTGACCGTTTTGGCAGG	GGCATCTTTGCTCCAGGTGACA
Sox9	CACACGTCAAGCGACCCATGAA	TCTTCTCGCTCTCGTTCAGCAG
Sp7	GGCTTTTCTGCGGCAAGAGGTT	CGCTGATGTTTGCTCAAGTGGTC
Vegfa	AGAGGCTTGGGGCAGCCGAG	ACTCCCGGGCTGGTGAGTCC

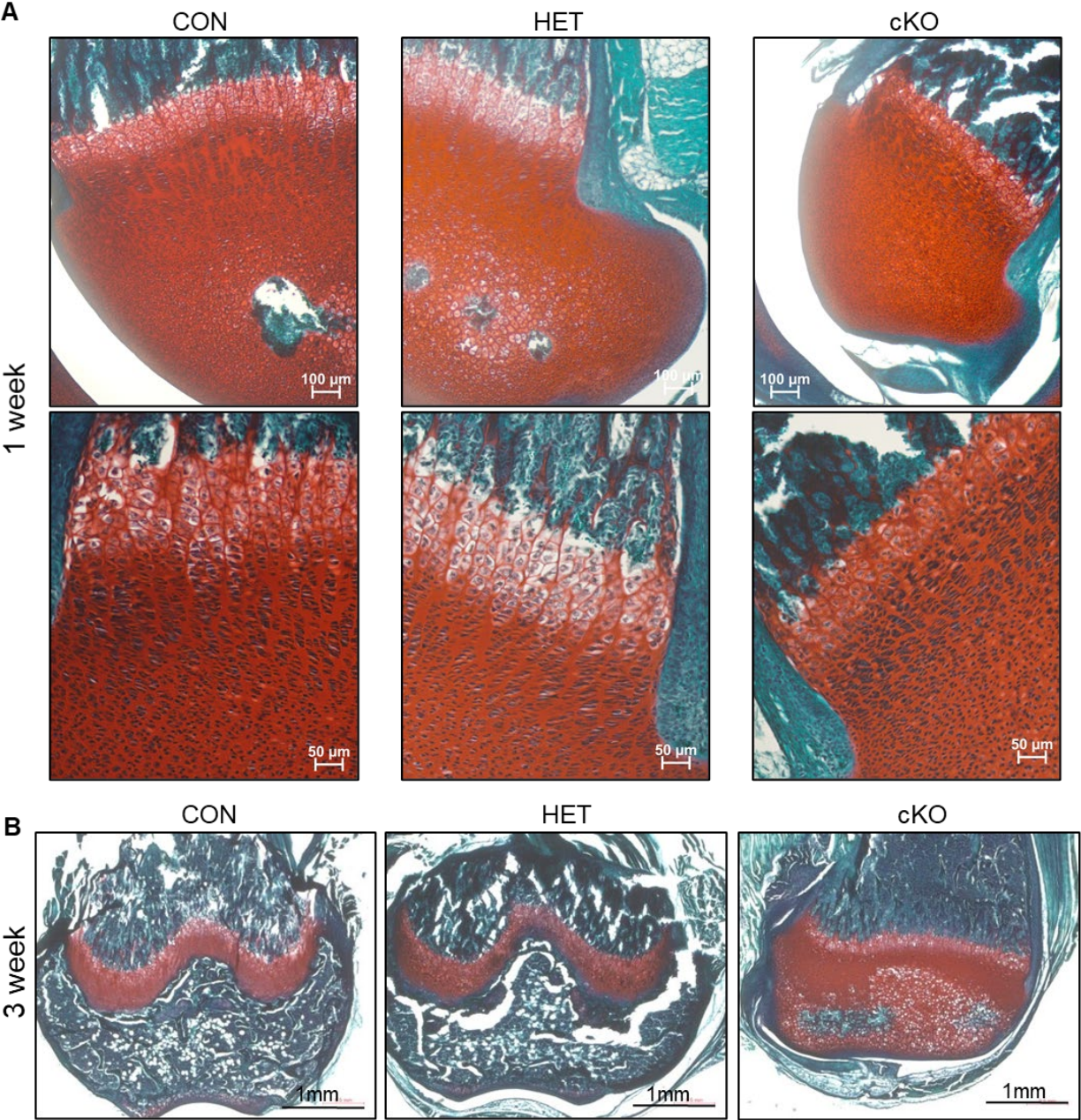
Supplemental Table 2. Statistical evaluation of chondrogenic differentiation. For statistical evaluation of the impact of +JQ1 treatment throughout the chondrogenic differentiation time course, a mixed-effects model (REML) analysis was performed for each graph (alpha = 0.05). The p-value reported for the fixed effects was then assessed. For all datasets, the time variable had a statistically significant impact with a p-value < 0.001. The p-value calculated for the “treatment group” variable is reported on each graph. A follow-up multiple comparisons test was performed using the Tukey method (correct for multiple comparisons) to compare the means of each group.

Gene	Time variable p-value	Treatment variable p-value	Veh vs. JQ1 1X p-value	Veh vs JQ1 cont. p-value	Veh vs. JQ1 late p-value
Sox5	< 0.0001	0.1506	0.4431	0.1226	0.7881
Sox6	< 0.0001	0.5107	0.8800	0.4383	0.8612
Sox9	< 0.0001	0.6933	0.9876	0.6567	0.9781
Col2a1	< 0.0001	0.9957	>0.9999	0.9992	0.9981
Acan	< 0.0001	0.9940	0.9976	0.9945	> 0.9999
Comp	< 0.0001	0.1099	0.7155	0.9931	0.4859
Runx2	< 0.0001	0.0001	0.4311	0.0003	0.9984
Sp7	< 0.0001	< 0.0001	0.4325	0.0004	>0.9999
Col10a1	< 0.0001	0.0459	0.3330	0.6484	0.5791
Mmp13	< 0.0001	.0107	0.9967	0.0148	0.1873
Ihh	< 0.0001	<0.0001	0.2356	<0.0001	0.1945
Bglap	< 0.0001	0.3301	0.8996	0.9439	0.6232
Ibsp	< 0.0001	<0.0001	0.9262	< 0.0001	0.2160

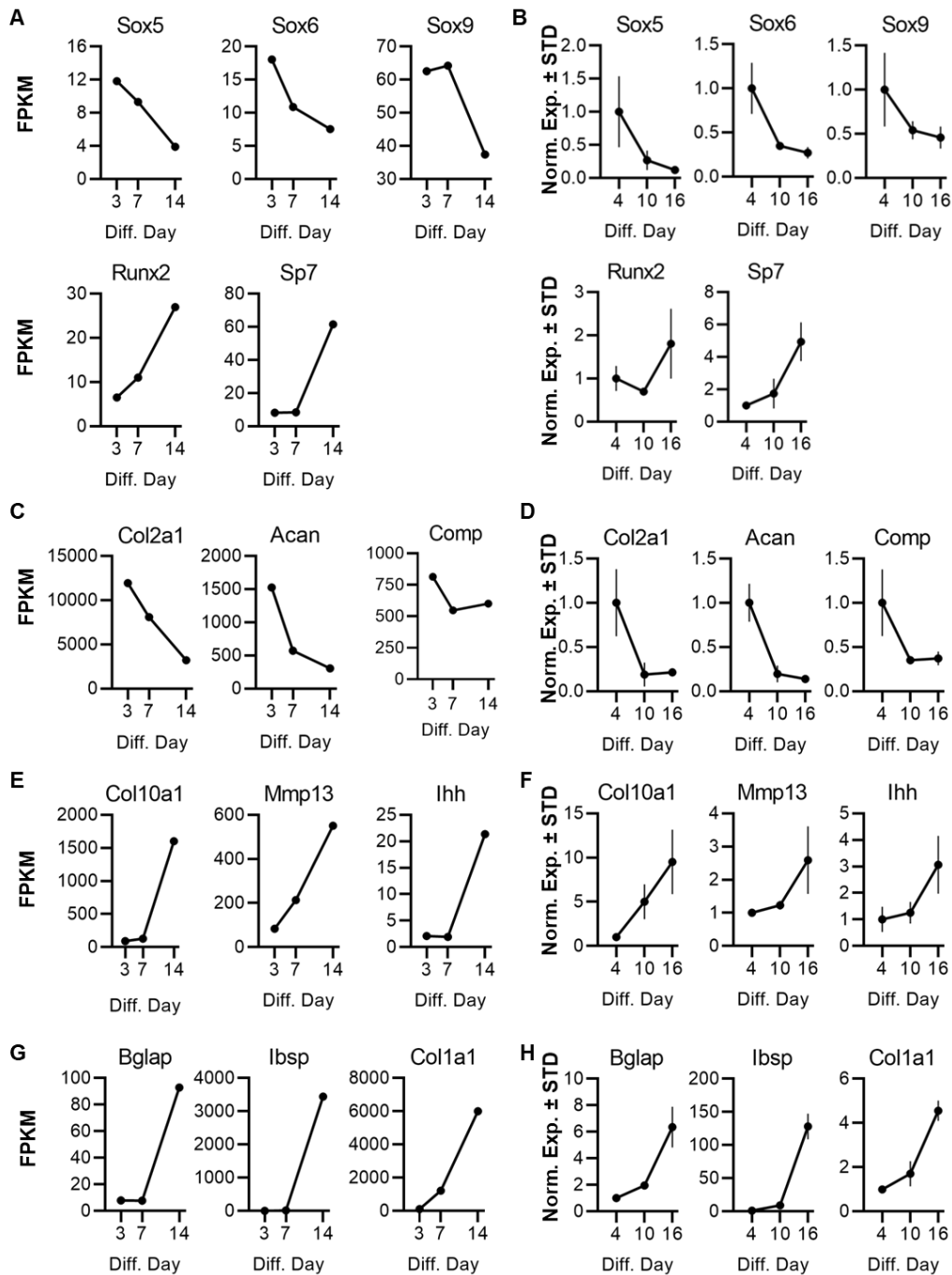
Supplemental Figure 1. Assessment of bone parameters in 3 week old cKO mice. Bone parameters were assessed by microcomputed tomography (μ CT) analysis of three week old CON ($Brd4^{wt/wt}; Prrx1-Cre$), HET ($Brd4^{wt/fl}; Prrx1-Cre$), and cKO ($Brd4^{fl/fl}; Prrx1-Cre$) female and male mice. The cortical thickness (Ct.Th), trabecular bone volume fraction of total volume (BV/TV), and trabecular total mineral density (TMD) were calculated in the femora of female mice (n = 8 to 9) (**A**). The length, trabecular bone volume to total volume (BV/TV), and trabecular thickness (Tb.Th) of L5 vertebrae of male mice (n = 7 to 9) (**B**). Boxplots indicate the median, interquartile range, and the minimum and maximum value in each dataset. Individual mice are represented by a single point on the graph. P-values shown on the graphs represent the results of a one-way ANOVA followed by a multiple comparisons test performed using the Tukey method (correct for multiple comparisons) to compare the means of the CON and cKO groups.



Supplemental Figure 2. Histologic assessment of Brd4 cKO femora. Trichrome staining of distal femora derived from one (A) and three (B) week old CON ($Brd4^{wt/wt}; Prrx1-Cre$), HET ($Brd4^{wt/fl}; Prrx1-Cre$), and cKO ($Brd4^{fl/fl}; Prrx1-Cre$) male mice. Data presented in panel A are higher magnification images of data presented in Fig 4F.



Supplemental Figure 3. Transcriptional profile of differentiating iMACs. iMACs were collected from five day-old wild-type C57BL/6J mice and cultured in three-dimensional micromass (3D- μ mass) conditions in chondrogenic differentiation media. Gene expression was assessed using RNA-sequencing (n = 1) and RT-qPCR (n = 3). RT-qPCR values (mean \pm standard deviation) were normalized to day four (set at 1). Expression levels of key chondrogenic transcription factors (**A** and **B**), early chondrogenic markers (**C** and **D**), hypertrophic markers (**E** and **F**), and osteogenic markers (**G** and **H**).



Original Western Blot Images:

Fig. 2B

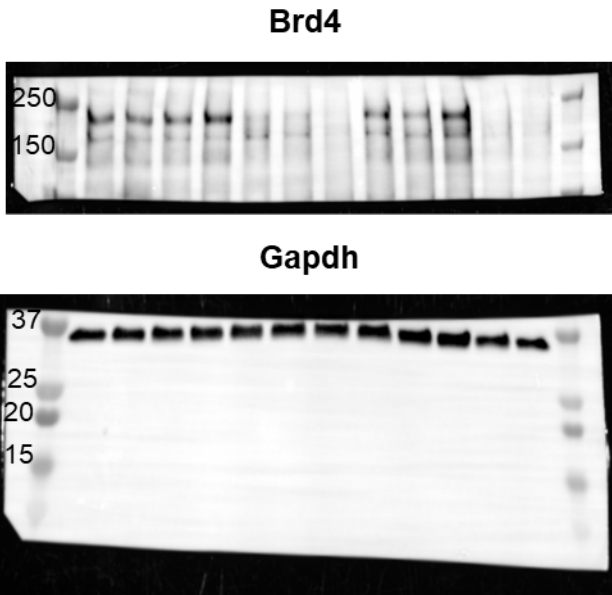


Figure 6B

