



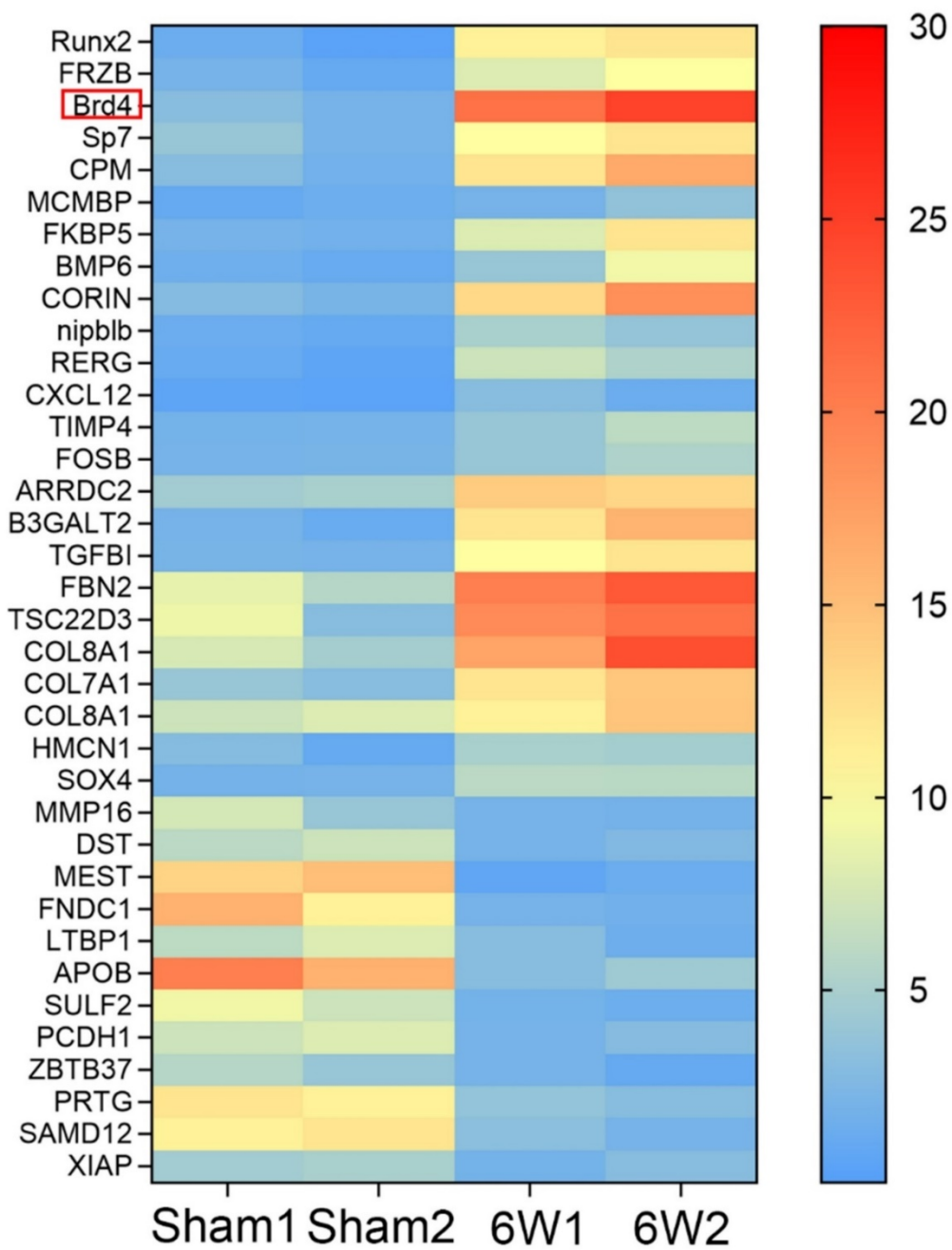
## Supplementary Material

10.1302/2046-3758.1010.BJR-2020-0454.R1

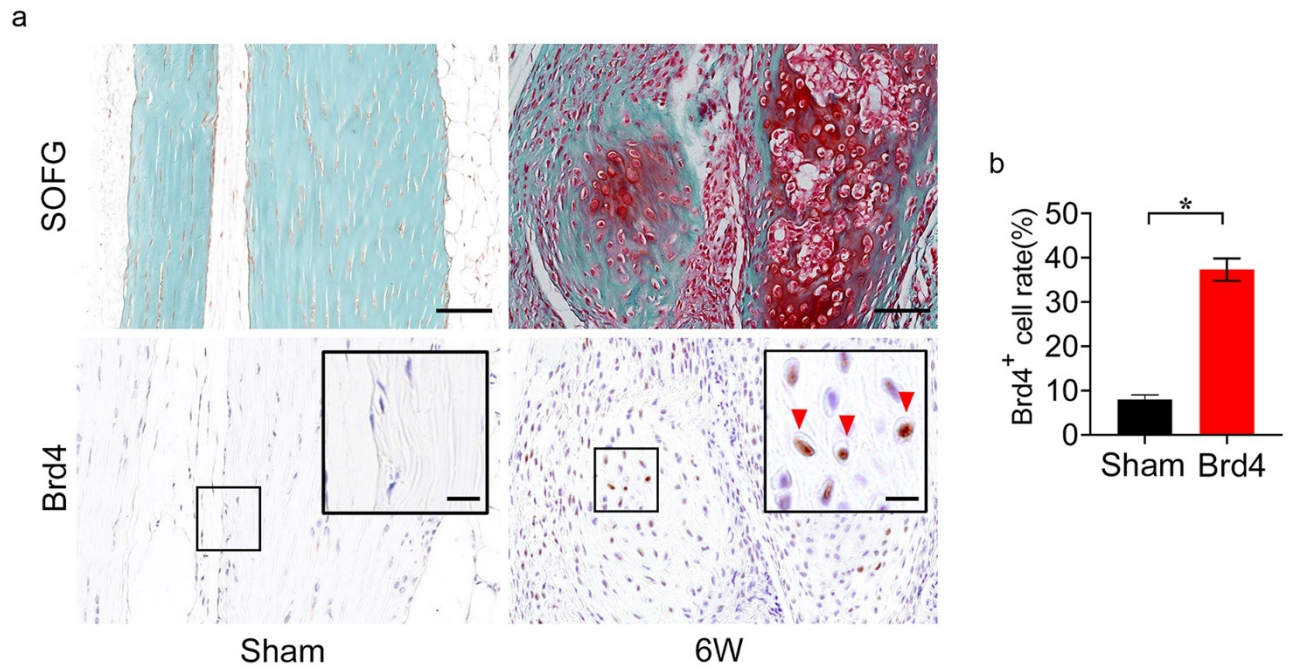
**Table i.** Primers for real-time quantitative polymerase chain reaction analysis of gene expression.

Primer	5' Forward 3'	5' Reverse 3'
$\beta$ -actin	AGGCTCTTTTCCAGCCTT CC	AATGCCAGGGTACATG GTGG
MANC R	TGTTGGAGGATACCTGT GCAT	TGCCATTCCCAGATTGT GGAG
BRD4	ACACCTGCACCTACCAG ACTC	CATCGGCTACAGTCTAG GCC
Runx2	TACTGTCATGGCGGGTA ACG	CACCTGCCTGGCTCTTC TTA
OSX	GAGTGGAACAGGAGTG GAGC	TGCCCCATATCCACCA CTA
ALP	CTGAAGCCTCCGTGGAA CAT	TGACCACAAAGACTCA GCTC

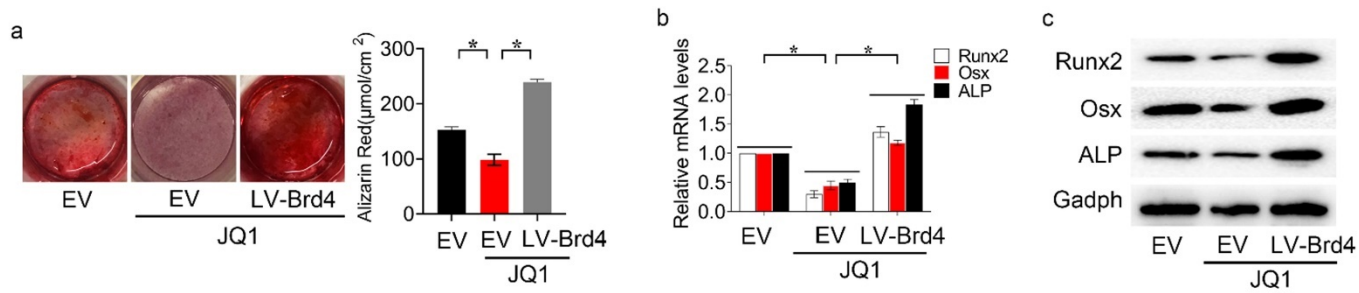
ALP, alkaline phosphatase; MANCR, mitochondrially associated long non-coding RNA.



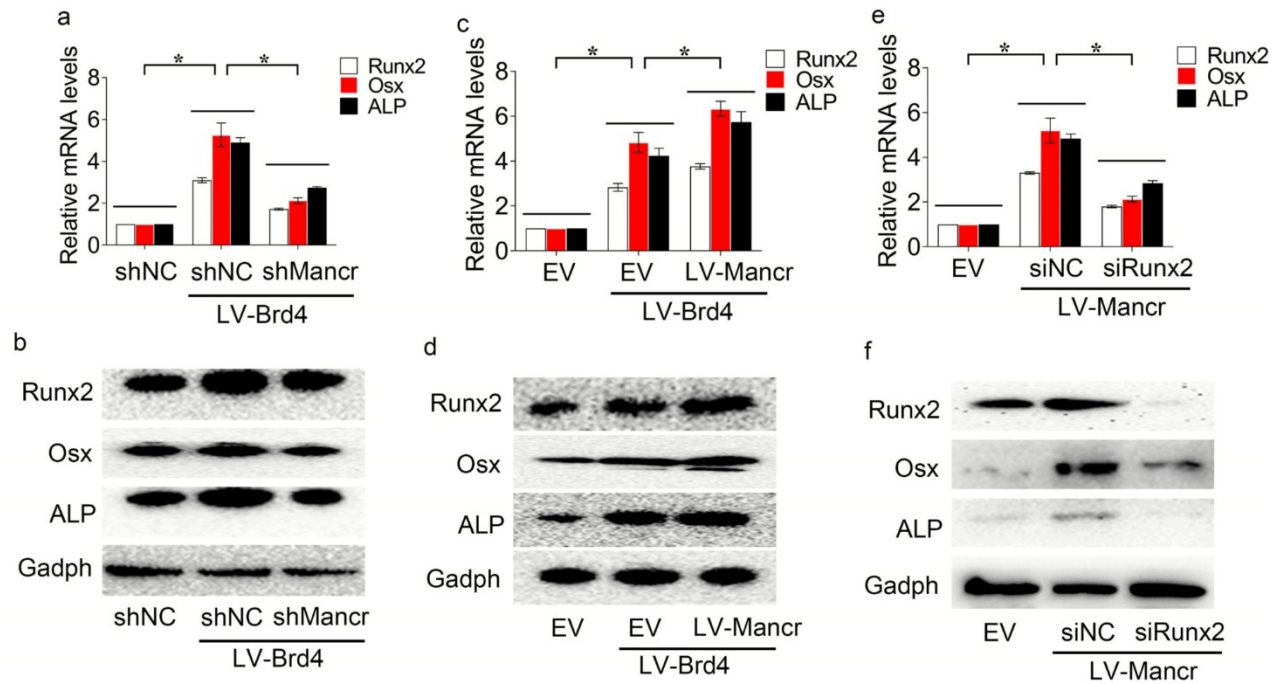
**Fig. a.** Increased expression of Brd4 in heterotopic ossification (HO) model. Heat map of RNA-seq profiling results of differentially expressed messenger RNA (mRNA) from tendon samples in HO model and sham-operated group. N = 10.



**Fig. b.** Increased Brd4<sup>+</sup> cells were observed at the site of pathological bone formation.  
a) Safranin O Fast Green (SOFG) and immunohistochemical staining of tendon tissues.  
b) Quantification analysis of Brd4<sup>+</sup> cells in heterotopic ossification (HO) model and sham-operated group, n = 10. Data are presented as means and standard deviations.  
\*p < 0.05. scale bar = 200  $\mu$ m, scale bar = 80  $\mu$ m (zoomed in image on the black square).



**Fig. c.** Increased expression of Brd4 in heterotopic ossification model. a) Alizarin red staining and quantification of human bone marrow mesenchymal stem cells (hBMSCs) transfected with empty vector (EV) or LV-Brd4 under osteogenic induction against JQ1 treatment. b) Real-time quantitative polymerase chain reaction (RT-qPCR) analysis of Runx2, Osx, and alkaline phosphatase (ALP) in hBMSCs transfected with EV or LV-Brd4 under osteogenic induction against JQ1 treatment. c) Western blot analysis of Runx2, Osx, and ALP in hBMSCs transfected with EV or LV-Brd4 under osteogenic induction against JQ1 treatment. Data are presented as means and standard deviations. \* $p < 0.05$ . GAPDH, glyceraldehyde 3-phosphate dehydrogenase.



**Fig. d.** Increased expression of Brd4 in heterotopic ossification (HO) model. a) Real-time quantitative polymerase chain reaction (RT-qPCR) analysis of osteogenic markers in Brd4-overexpressed human bone marrow mesenchymal stem cells (hBMSCs) treated with shNC or shMancr under osteogenic induction. b) Western blot analysis of osteogenic markers in Brd4-overexpressed hBMSCs treated with shNC or shMancr under osteogenic induction. c) RT-qPCR analysis of osteogenic markers in Brd4-overexpressed hBMSCs treated with empty vector (EV) or LV-Mancr. d) Western blot analysis of osteogenic markers in Brd4-overexpressed hBMSCs treated with EV or LV-Mancr. e) RT-qPCR analysis of osteogenic markers in hBMSCs transfected with siNC or siRunx2. f) Western blot analysis of osteogenic markers in hBMSCs transfected with siNC or siRunx2. Data are presented as means and standard deviations. \* $p < 0.05$ . Mancr, mitotically associated lncRNA; mRNA, messenger RNA.