

Online Supplement

MicroRNA-494-3p inhibits formation of fast oxidative muscle fibres by targeting E1A-binding protein p300 in human-induced pluripotent stem cells

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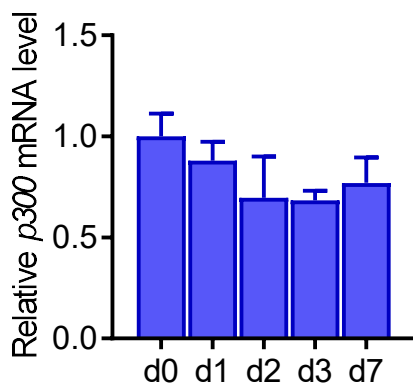
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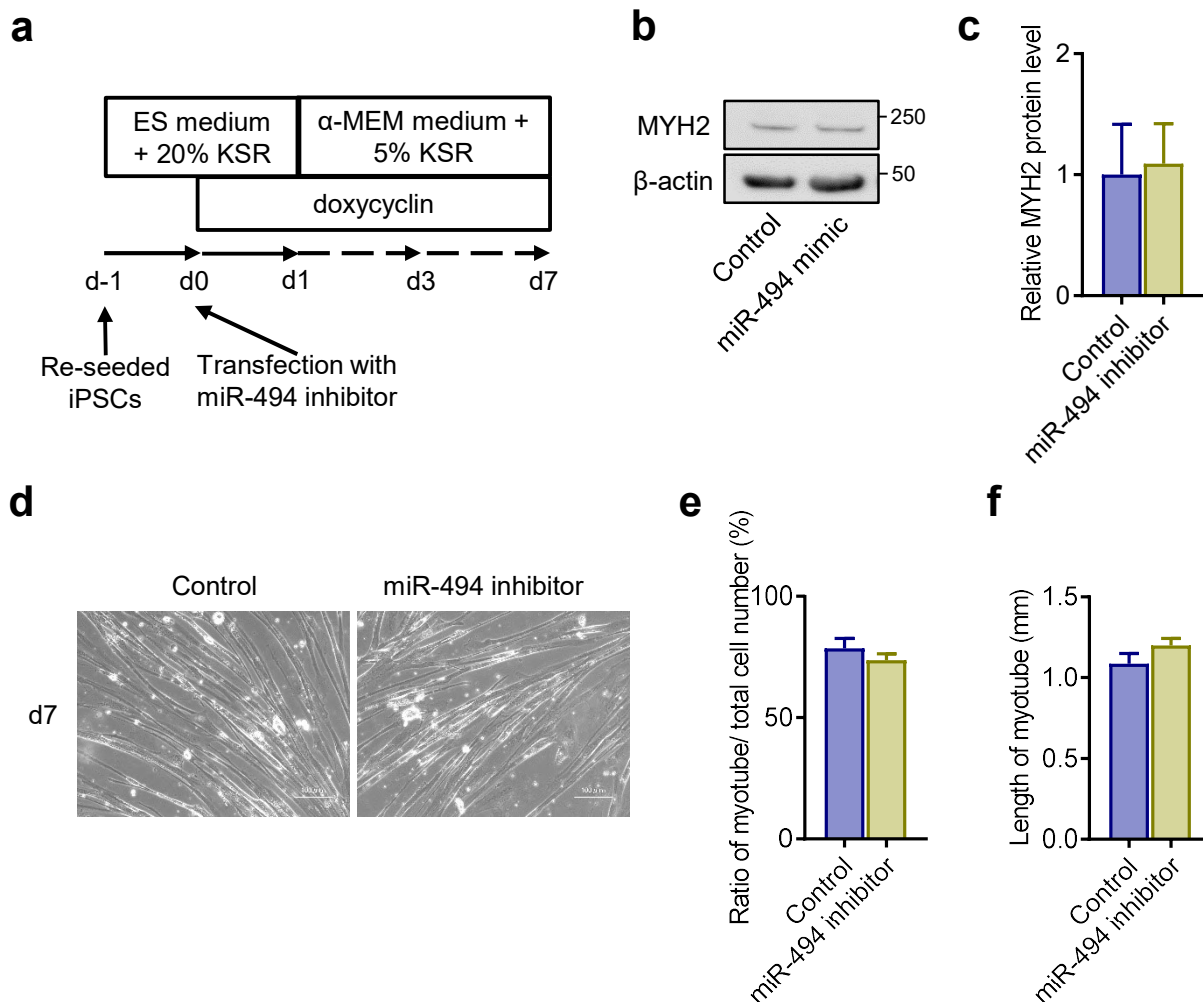
#Current affiliation: Department of Molecular and Cellular Biology, City of Hope, Los Angeles, USA

Supplementary Figure S1.



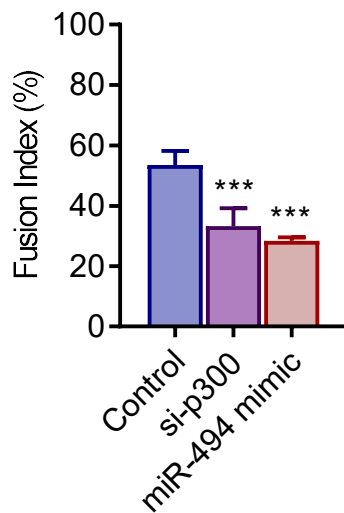
Supplementary Figure S1. Time course of *p300* expression during MyoD-hiPSCs differentiation. Time course of *p300* as analysed by RT-qPCR. Data were normalised with *β-actin*. n = 3.

Supplementary Figure S2.



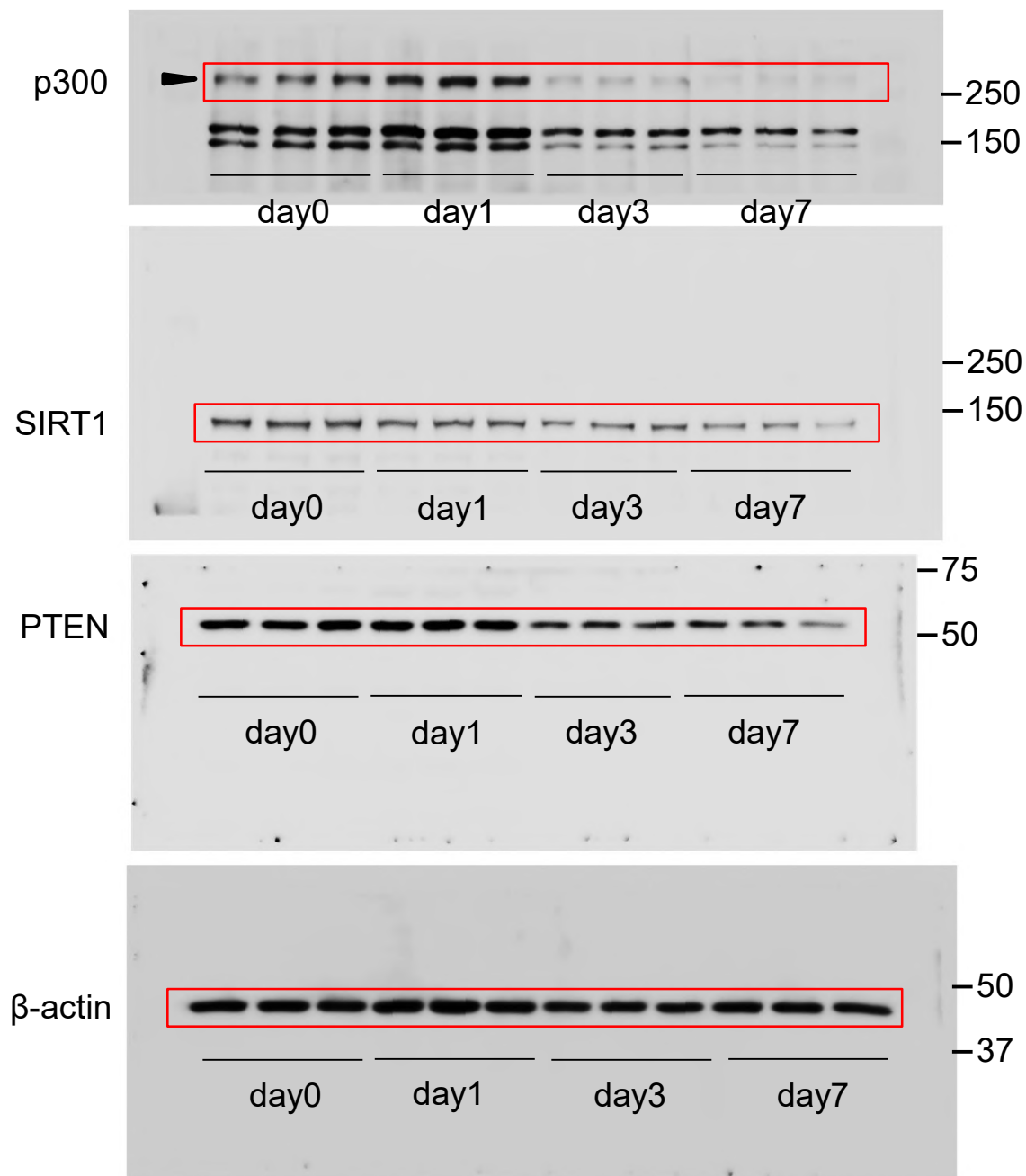
Supplementary Figure S2. Effects of day0 transfection with miR-494-3p inhibitor on human-skeletal myogenesis in MyoD-hiPSCs. **a)** Schematic diagram showing transfection of MyoD-hiPSCs with miR-494-3p inhibitor at day0. **b,c)** MyoD-hiPSCs were transfected with miR-494-3p inhibitor or negative control duplexes (Control). Whole-cell extracts were harvested at day 7 after myogenic induction, and MYH2 expression was evaluated using immunoblotting. Expression of MYH2 was quantified using Image J and normalised to β -actin expression. $n = 6$. Full-length blots are presented in Supplementary Figure 5. **d)** Representative images of MyoD-hiPSCs at day 7 post-transfection with Control and miR-494 inhibitor. **e,f)** The number (**e**) and length (**f**) of differentiated myotubes at day 7 was analysed in each microscopic field. $n=3$.

Supplementary Figure S3.



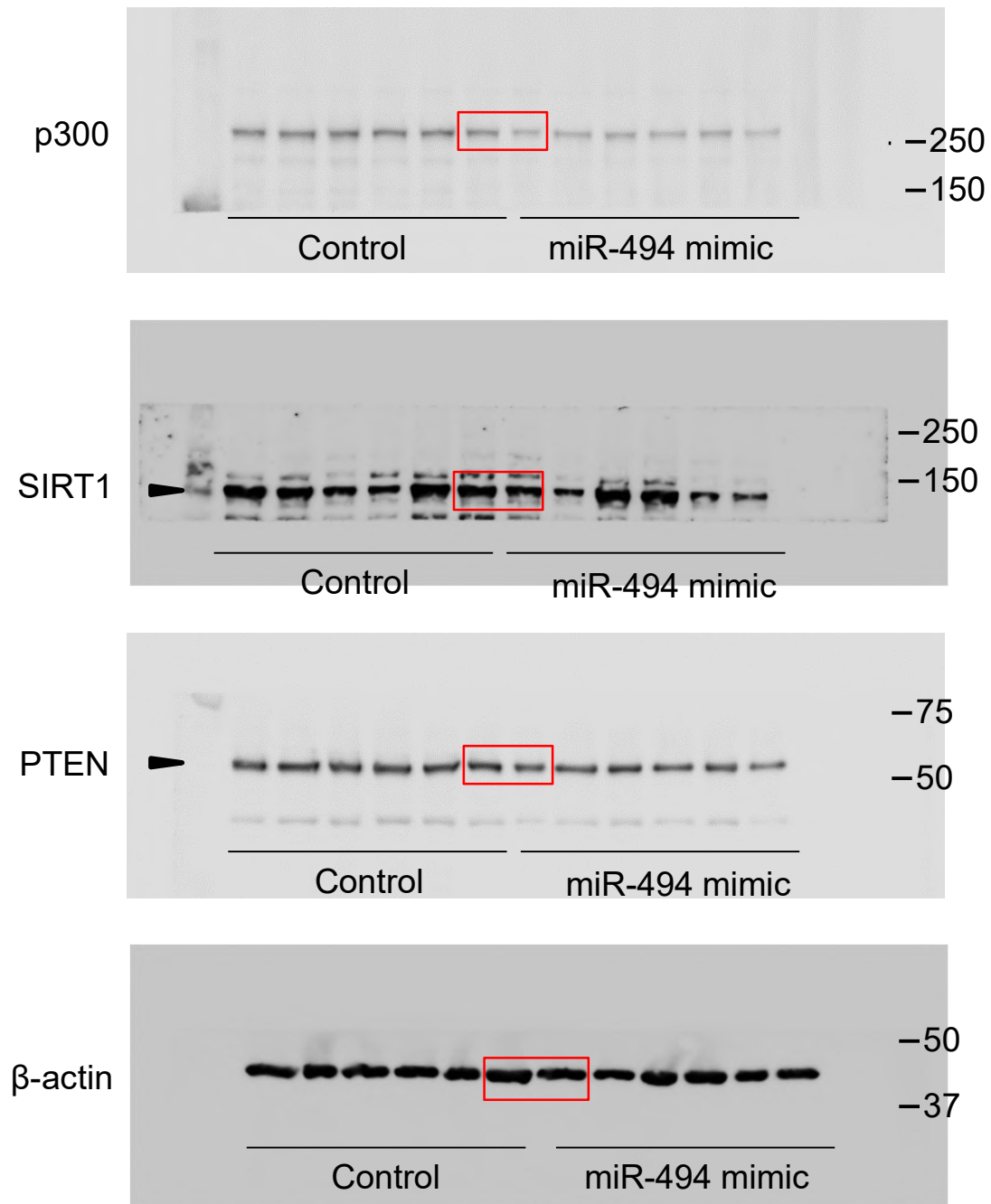
Supplementary Figure S3. Fusion index in MyoD-hiPSCs transfected with siRNA against *p300* or miR-494 mimic. Fusion index was calculated at day 7 as the ratio of the number of nuclei in the myotubes to the total number of nuclei in 5 random fields from the four independent experiments, *** $p < 0.001$.

Supplementary Figure S4.



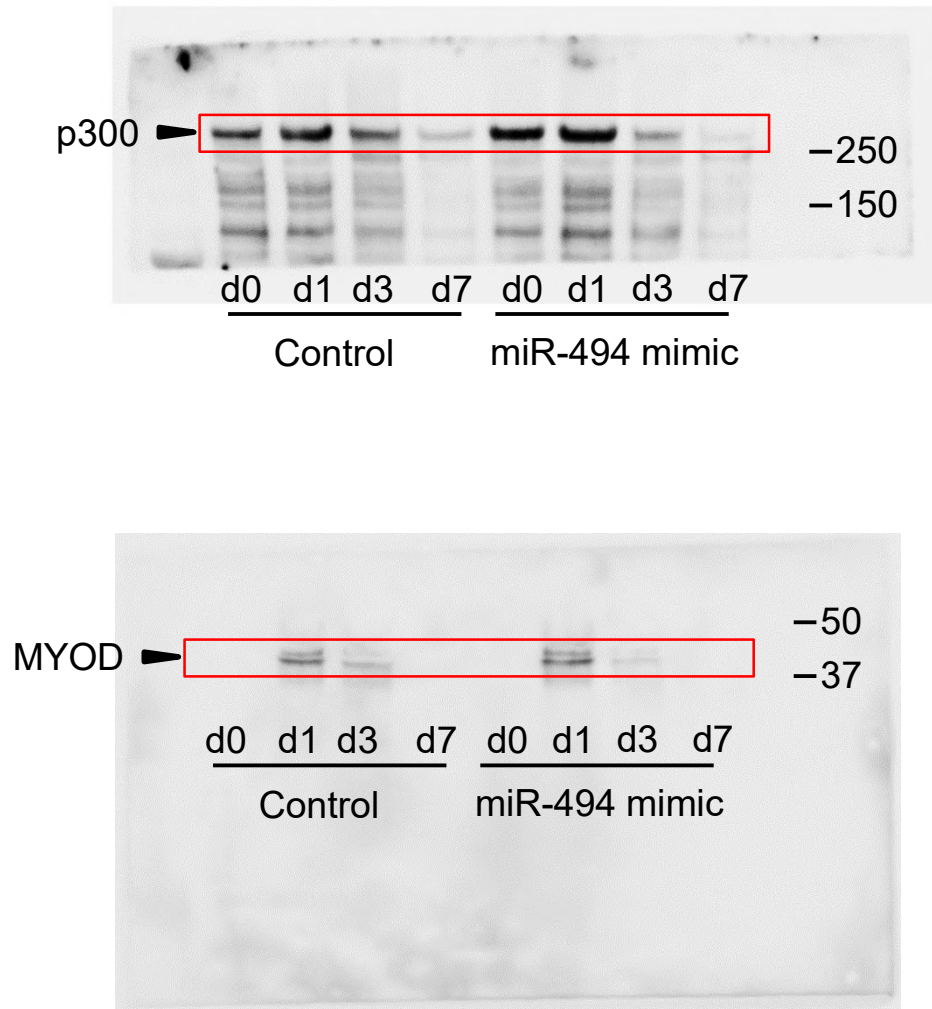
Original western blots for the images shown in Figure 1b. The regions of original blots used in main figures were encircled with red boxes.

Supplementary Figure S5.



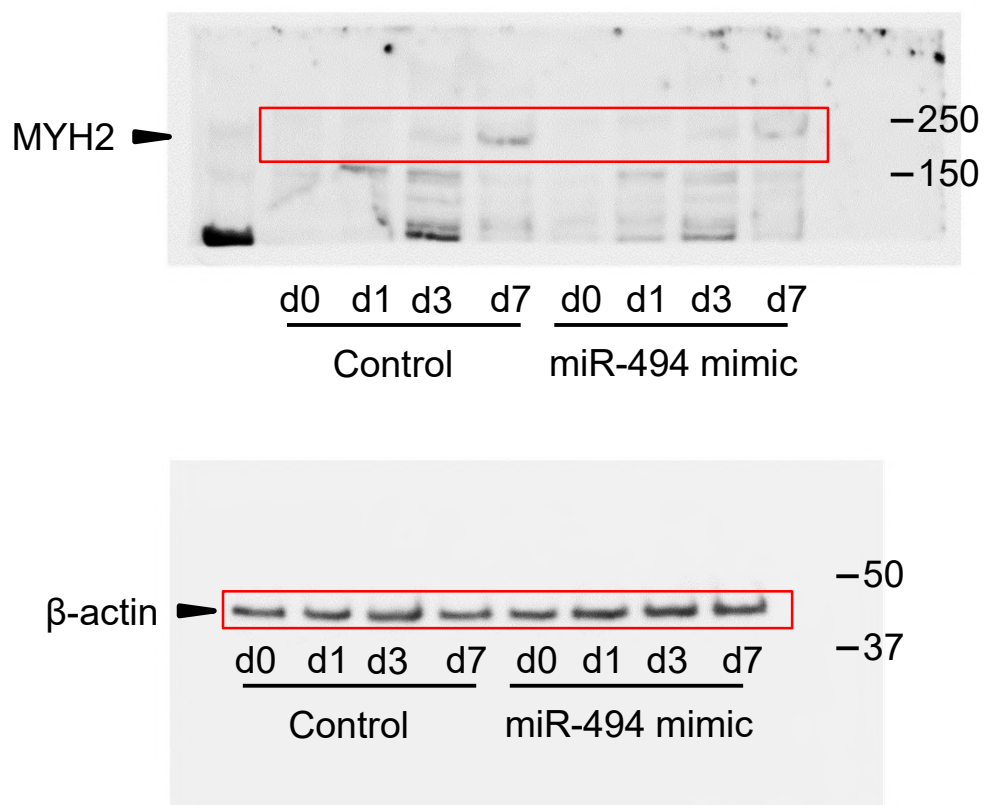
Original western blots for the images shown in Figure 1f. The regions of original blots used in main figures were encircled with red boxes.

Supplementary Figure S6 (to be continued).



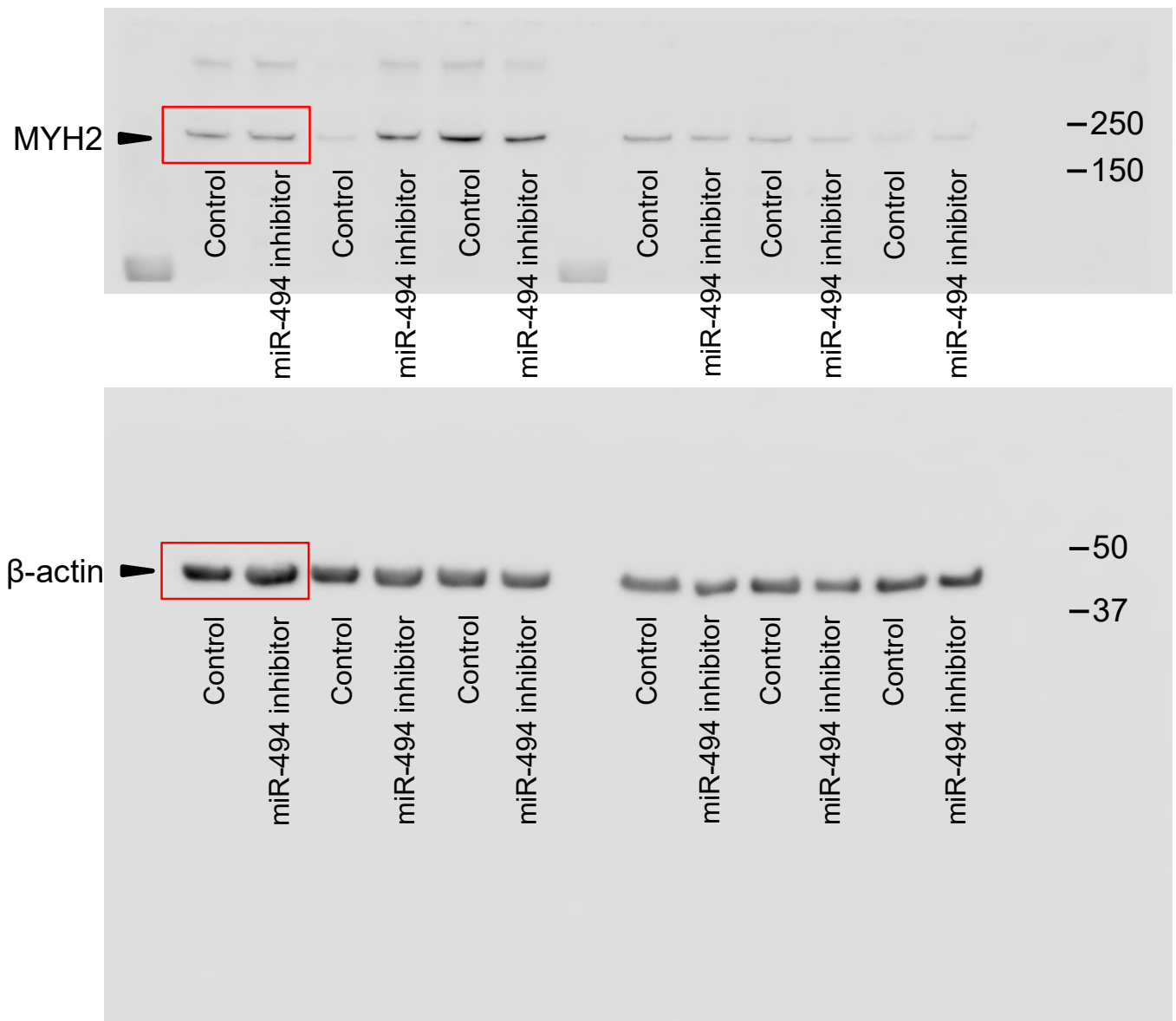
Original western blots for the images shown in Figure 1h. The regions of original blots used in main figures were encircled with red boxes.

Supplementary Figure S6.



Original western blots for the images shown in Figure 1h. The regions of original blots used in main figures were encircled with red boxes.

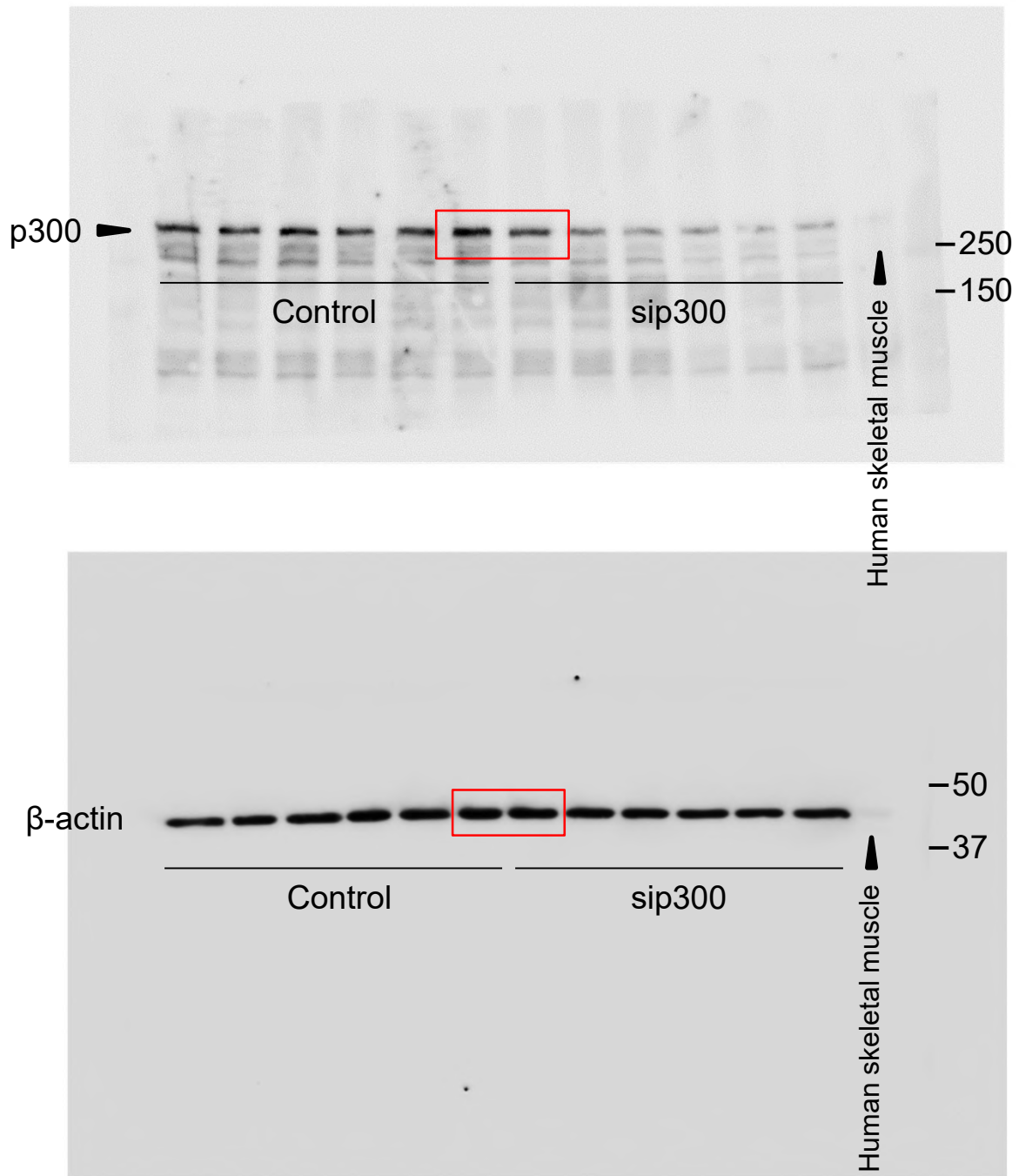
Supplementary Figure S7.



Original western blots for the images shown in Supplementary Figure 1b. The regions of original blots used in main figures were encircled with red boxes.

Supplementary Figure S8 (to be continued).

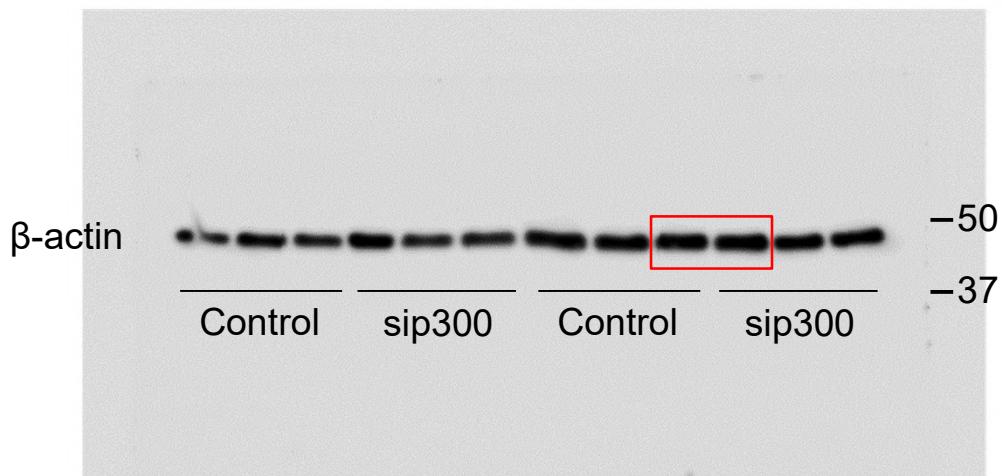
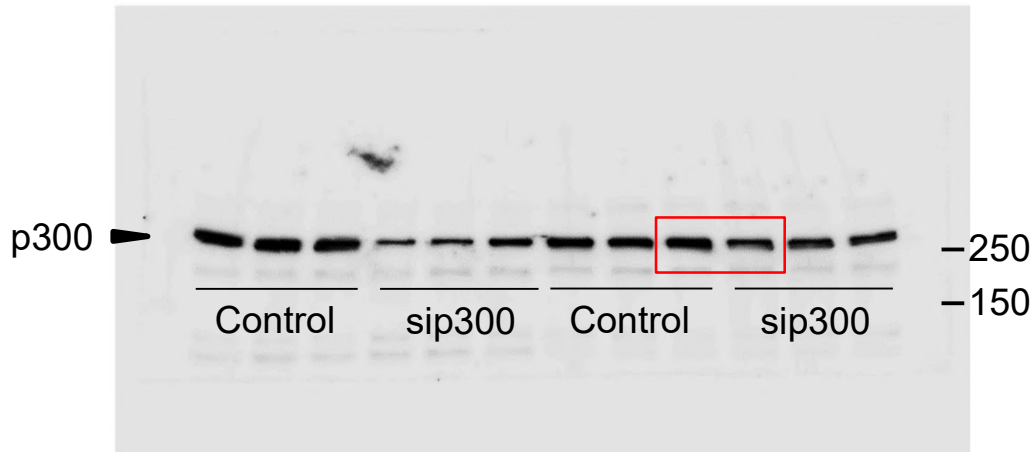
day4



Original western blots for the images shown in Figure 2b. The regions of original blots used in main figures were encircled with red boxes.

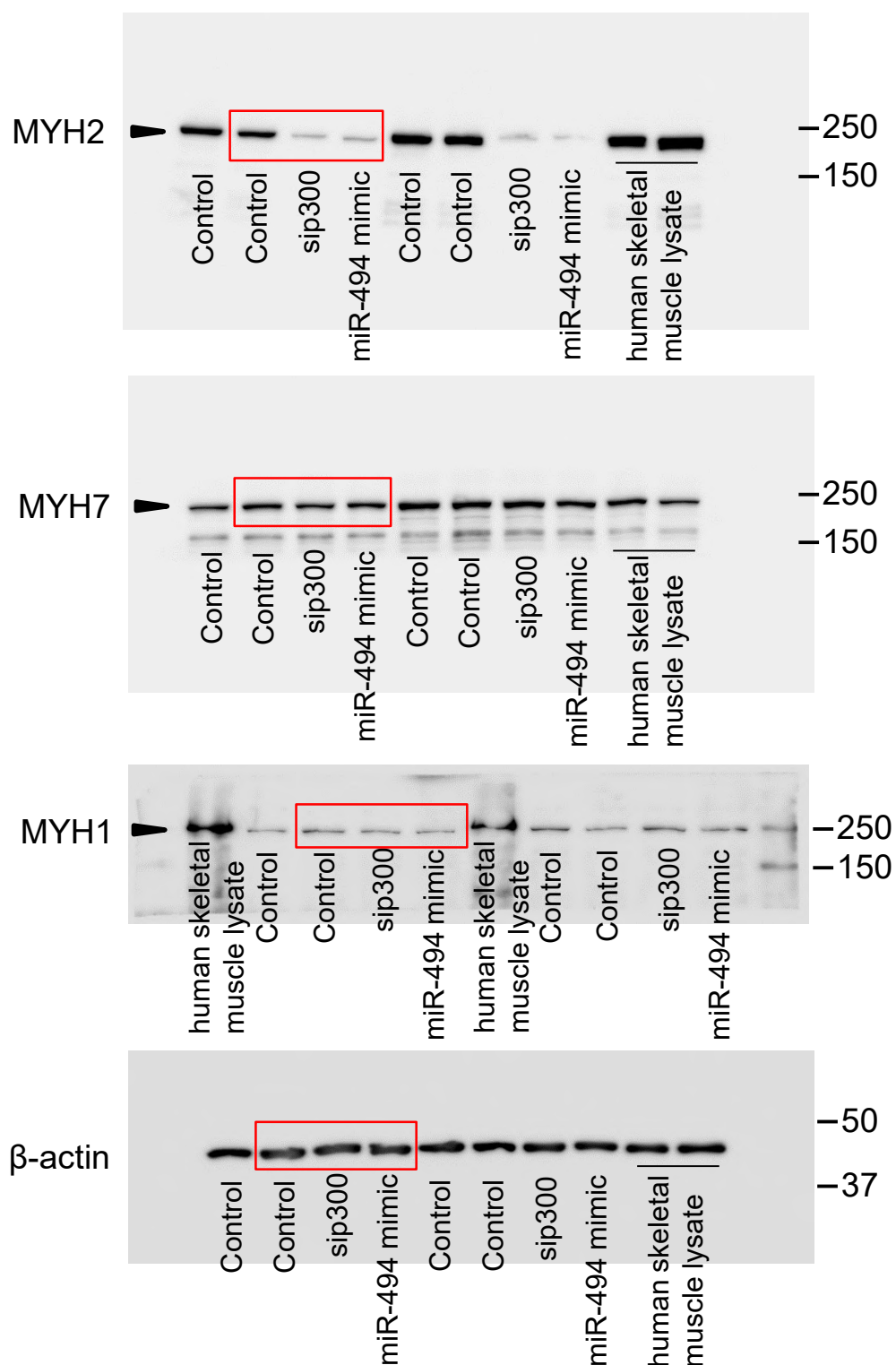
Supplementary Figure S8.

day7



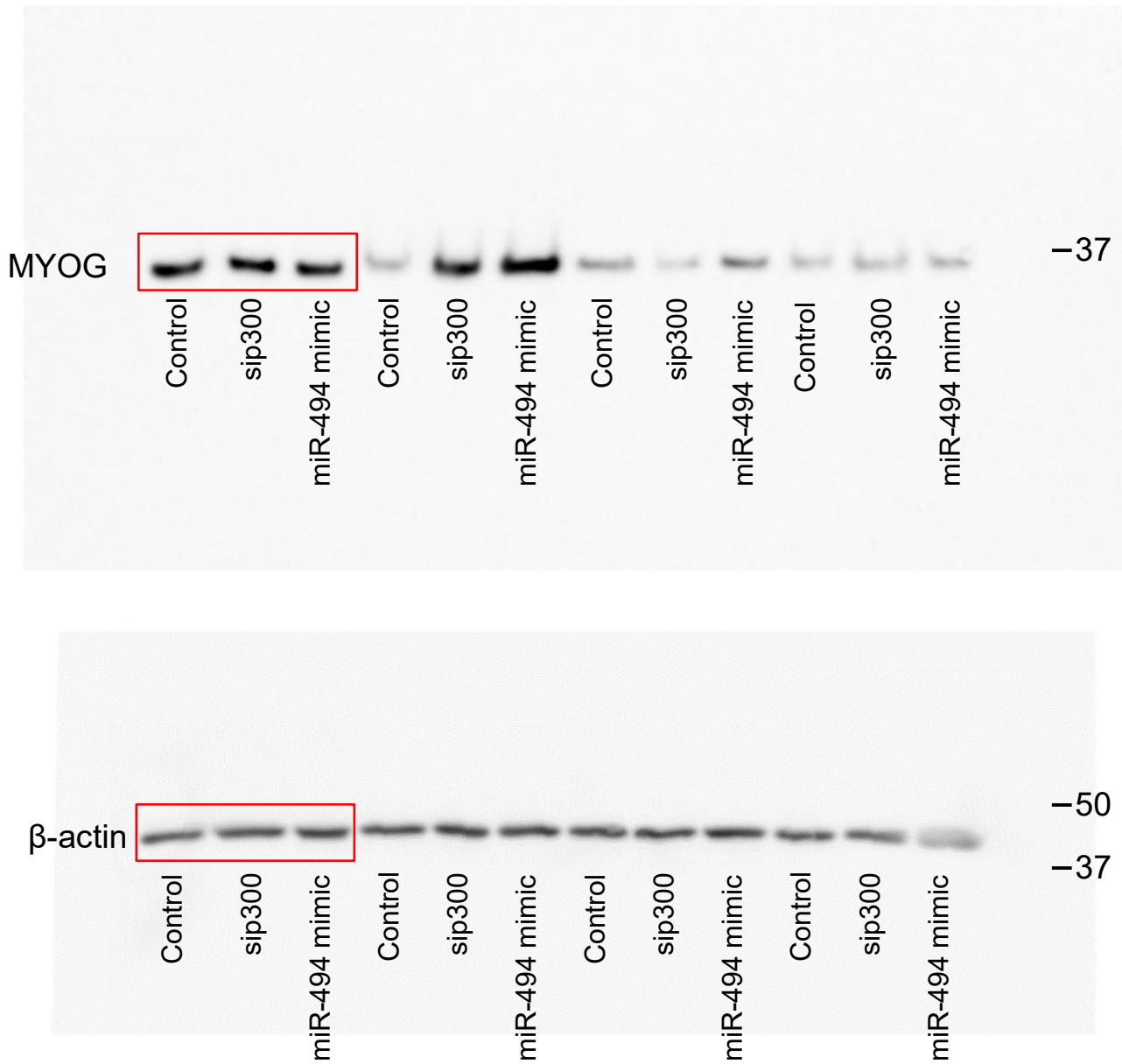
Original western blots for the images shown in Figure 2b. The regions of original blots used in main figures were encircled with red boxes.

Supplementary Figure S9.



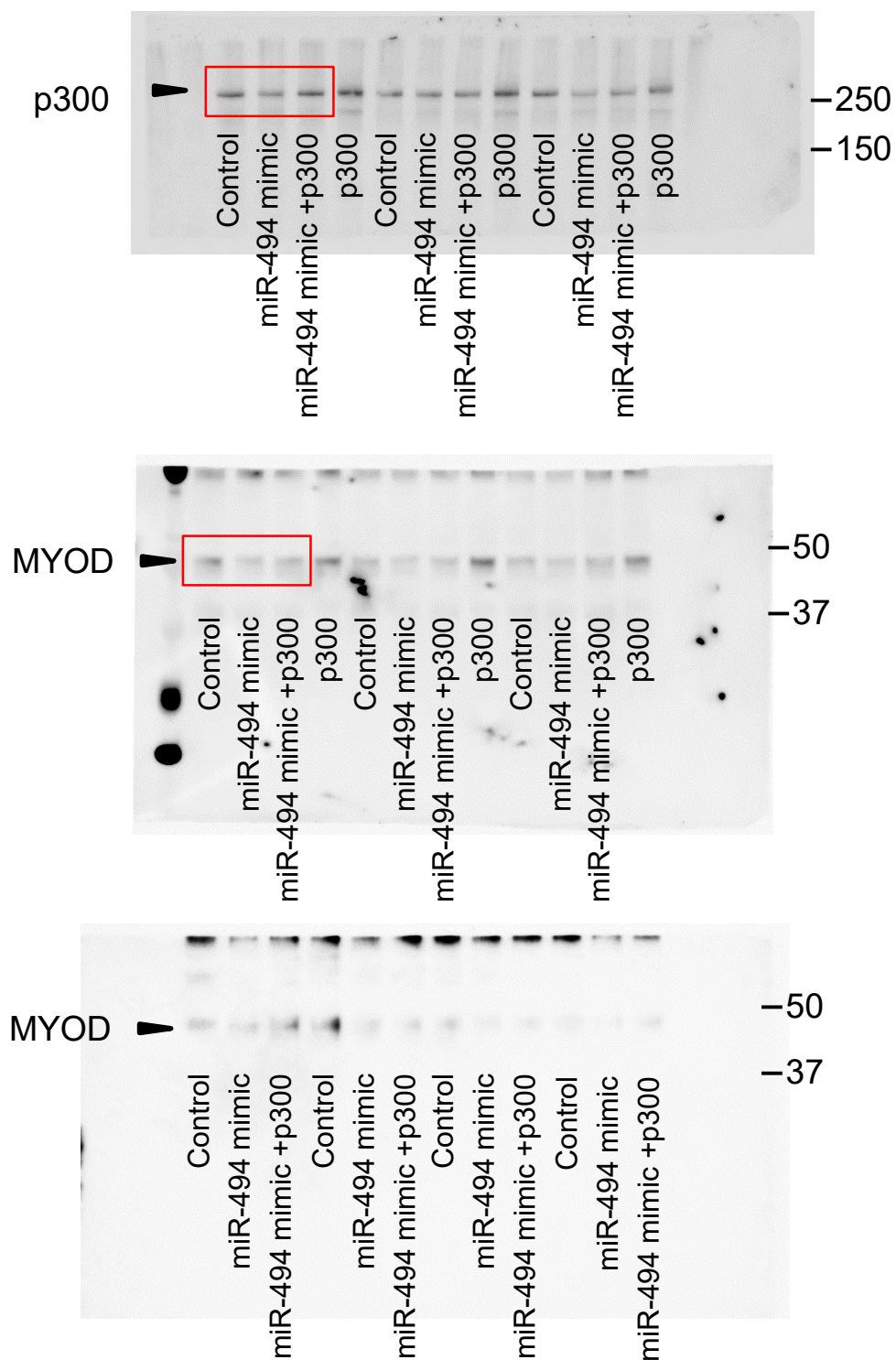
Original western blots for the images shown in Figure 2g. The regions of original blots used in main figures were encircled with red boxes.

Supplementary Figure S10.



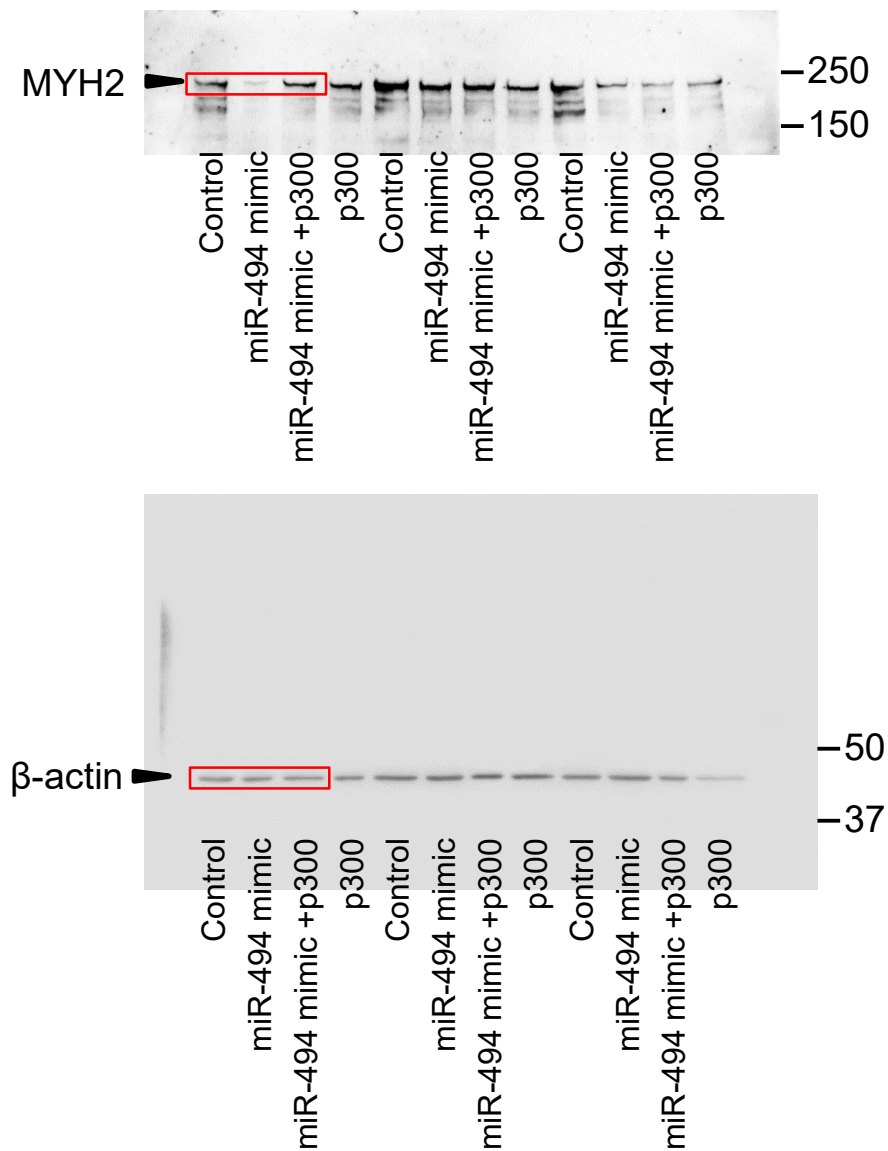
Original western blots for the images shown in Figure 2i. The regions of original blots used in main figures were encircled with red boxes.

Supplementary Figure S11 (to be continued).



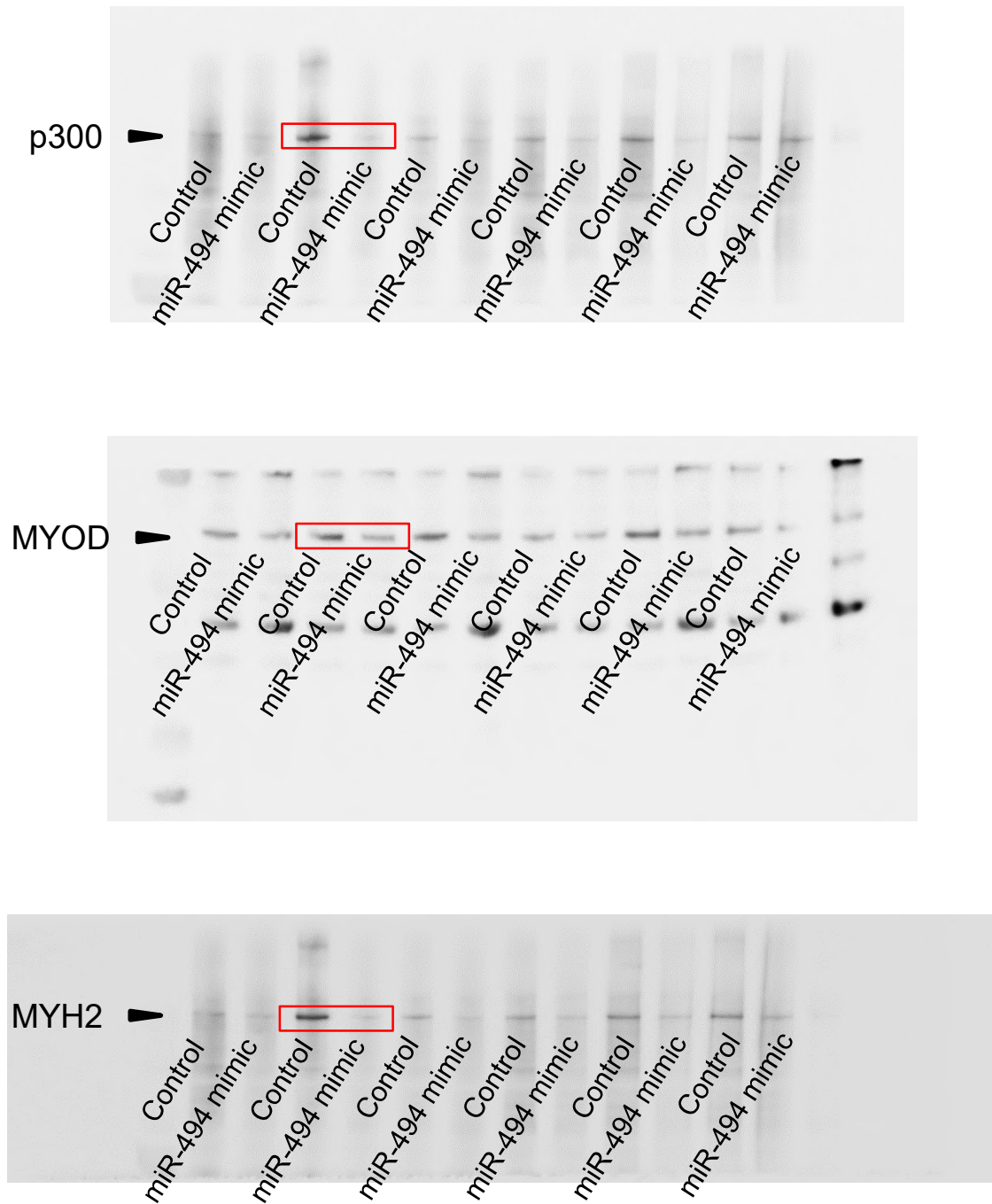
Original western blots for the images shown in Figure 4b. The regions of original blots used in main figures were encircled with red boxes.

Supplementary Figure S11.



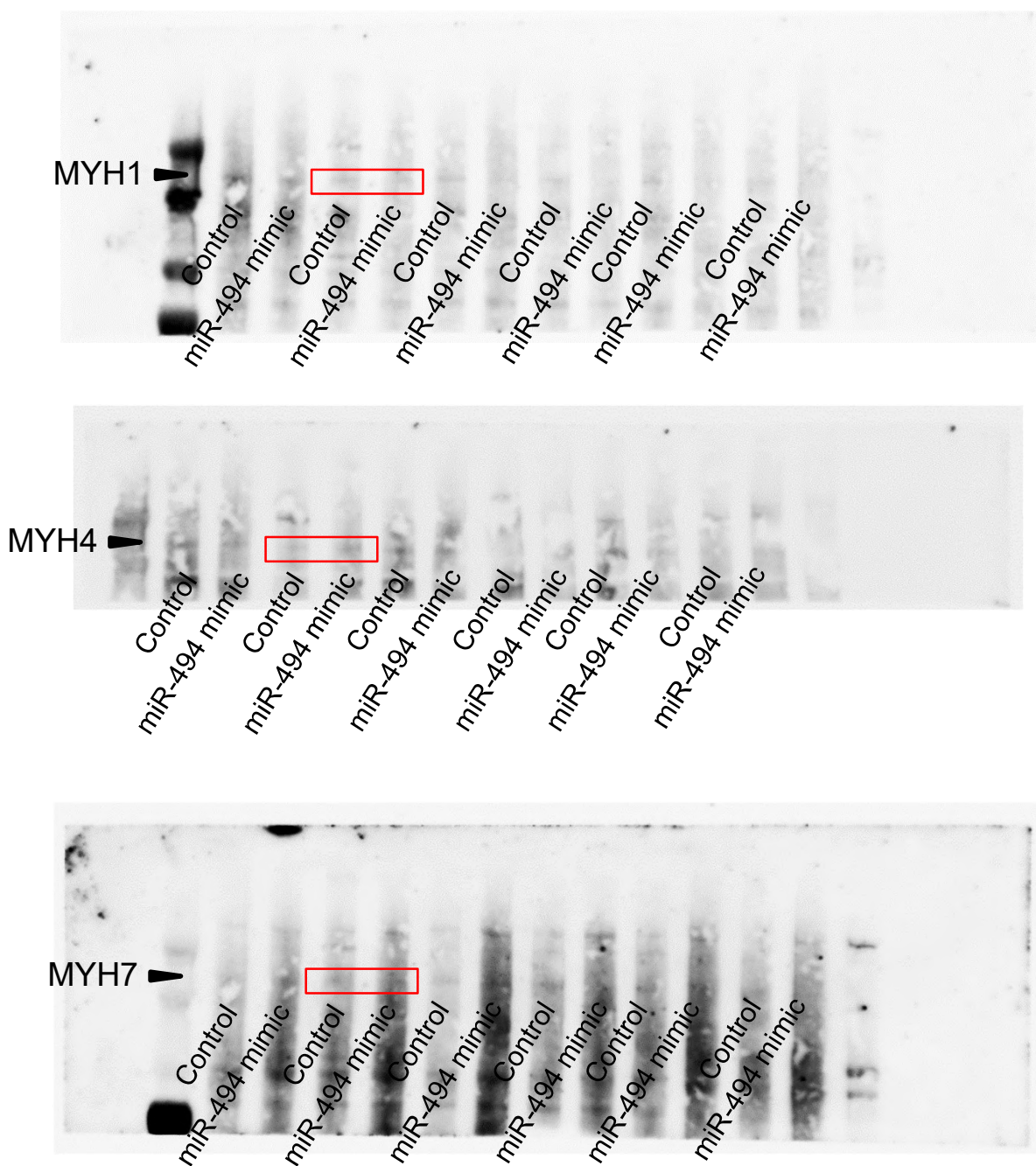
Original western blots for the images shown in Figure 4b. The regions of original blots used in main figures were encircled with red boxes.

Supplementary Figure S12 (to be continued).



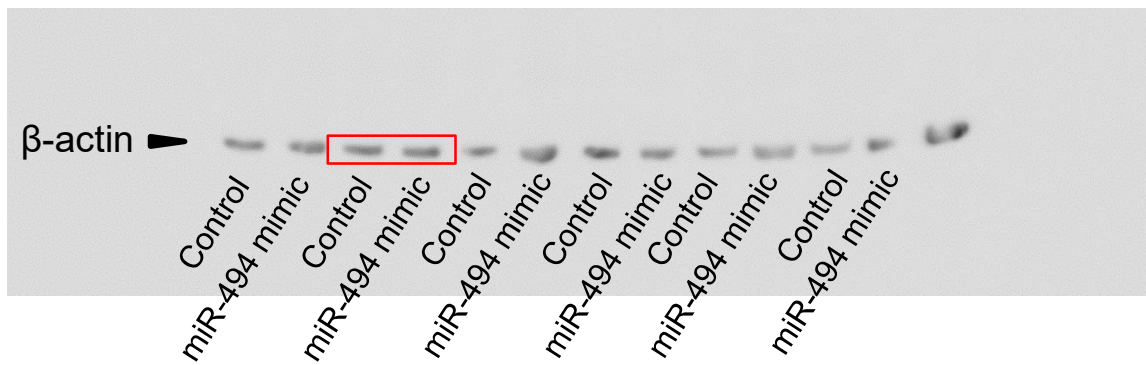
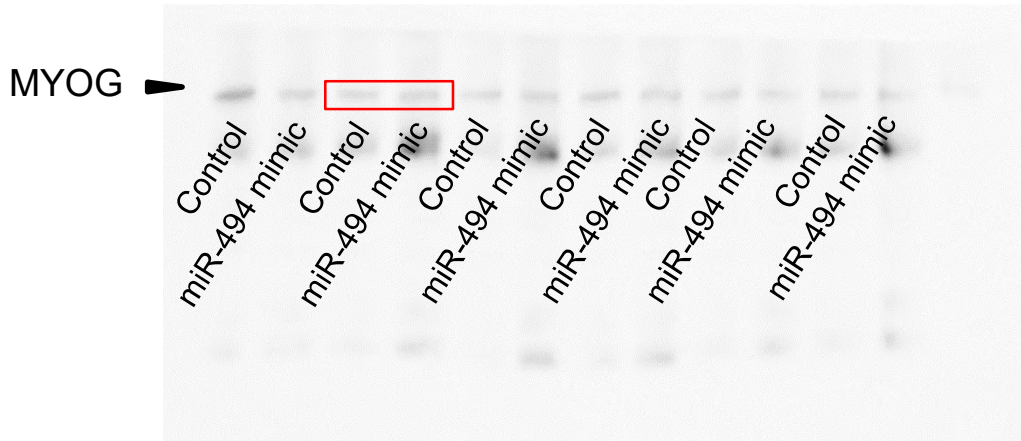
Original western blots for the images shown in Figure 6a. The regions of original blots used in main figures were encircled with red boxes.

Supplementary Figure S12 (to be continued).



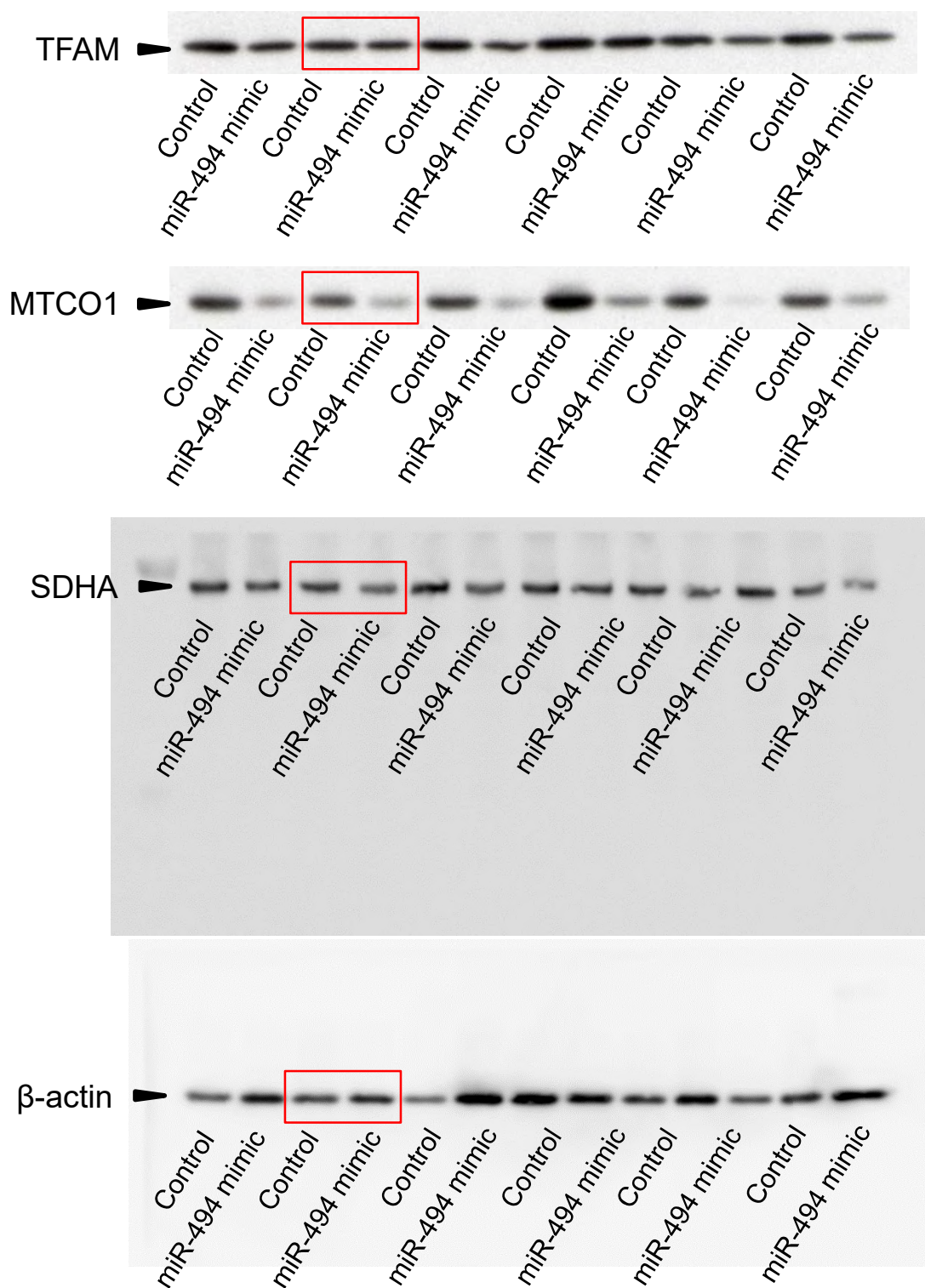
Original western blots for the images shown in Figure 6a. The regions of original blots used in main figures were encircled with red boxes.

Supplementary Figure S12.



Original western blots for the images shown in Figure 6a. The regions of original blots used in main figures were encircled with red boxes.

Supplementary Figure S13.



Original western blots for the images shown in Figure 6c. The regions of original blots used in main figures were encircled with red boxes.

Supplementary Table S1.

Gene	Forward (5' -> 3')	Reverse (5' -> 3')
<i>p300</i>	TTCAAACGCCGAGTCTTCTT	GTTGAGCTGCTGTTGGCATA
<i>β-actin</i>	CTCTTCCAGCCTTCCTTCC	CACCTTCACCGTTCCAGTTT

Supplementary Table S1. Primers for qRT-PCR analysis