

Supplemental Information

Maintaining bovine satellite cells stemness through p38 pathway

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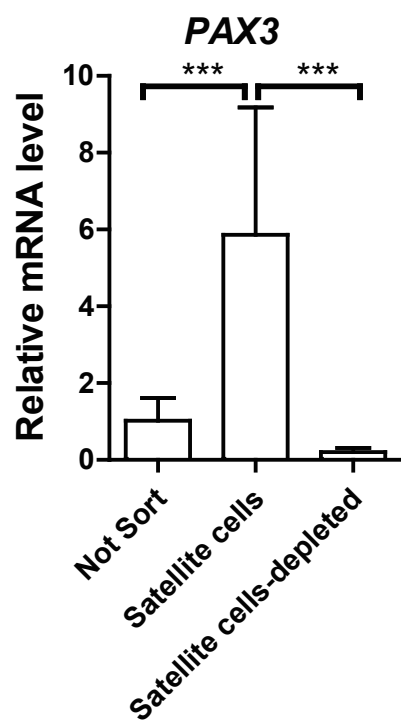
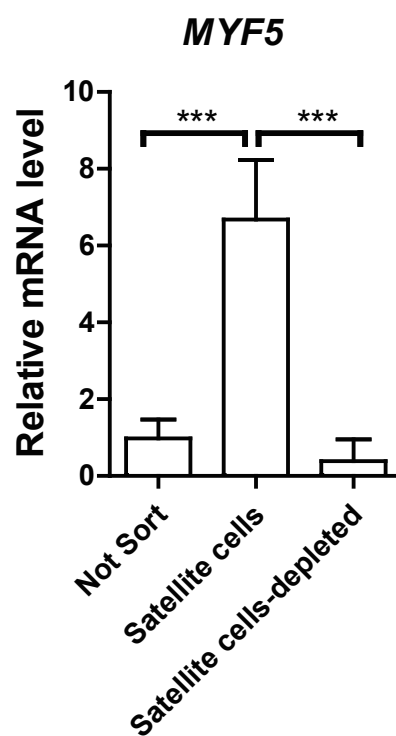
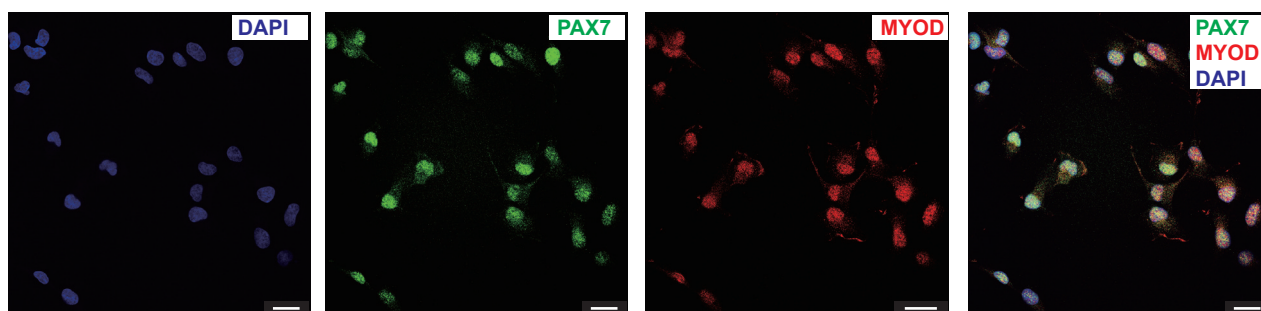
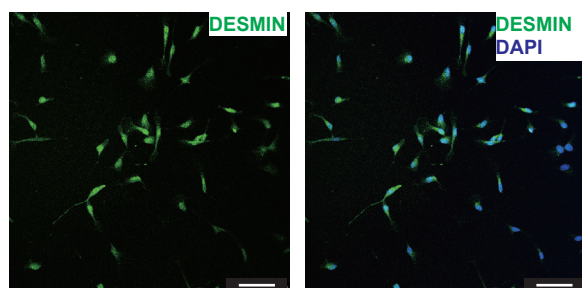
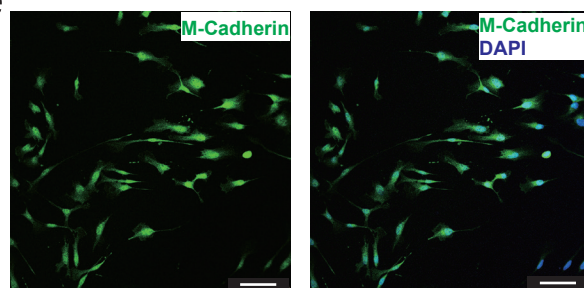
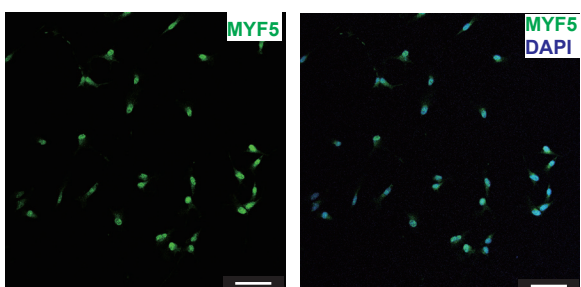
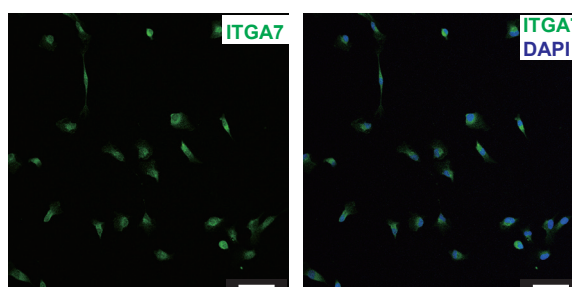
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Figure S1. Characterization of sorted bovine satellite cells. Related to Figure 1.

(a) qRT-PCR analysis of *PAX3*, *MYF5* mRNA levels in total cells (not sorted), sorted satellite cells and satellite cells depleted populations after a period of 4 days in culture (n=3). (b) Immunofluorescent staining of PAX7 and MYOD in sorted bovine satellite cells cultured for 4 days. Scale bars: 25µm. (c) Immunofluorescent staining of DESMIN, M-Cadherin, MYF5, ITGA7 in sorted bovine satellite cells cultured for 4 days. Scale bars: 75µm. Data are represented as mean ± SEM. Significance was analyzed by One-way ANOVA with Bonferroni's Multiple Comparison Test. Asterisks: *** indicates $P < 0.001$.

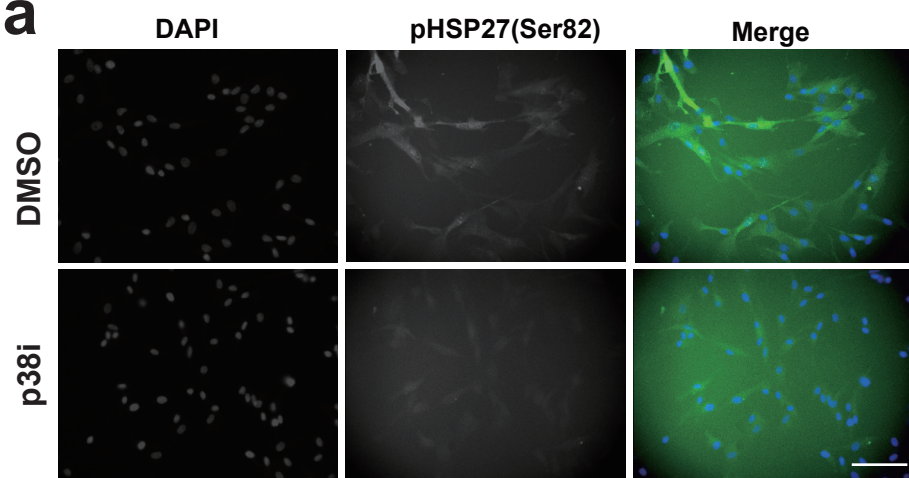
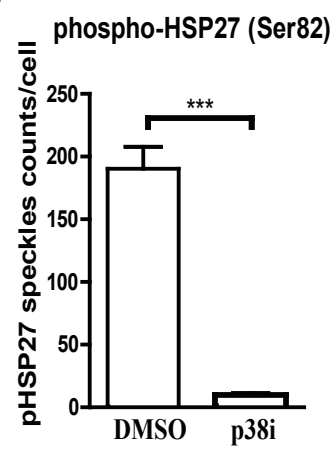
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Figure S2. p38i treatment repressed phosphorylation of the p38 substrate HSP27 (Ser82). Related to Figure 2.

(a) Representative IF analysis of phospho-HSP27 (Ser82) in passage 2 bovine cells cultured for 2 days in the presence or absence of p38i. Scale bar: 100 μ m (b) Quantification of IF for phospho-HSP27 (Ser82) per cell from (a). (n=3). Data are represented as mean $\pm$ SEM. Significance was analyzed by Student's t-test. Asterisks: *** indicates $P < 0.001$.

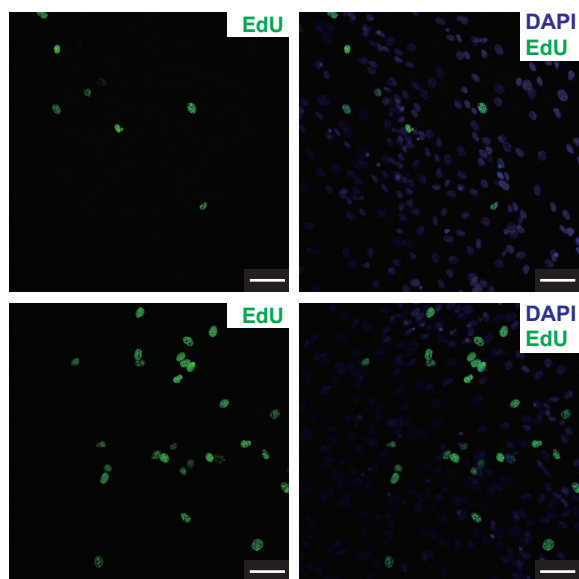
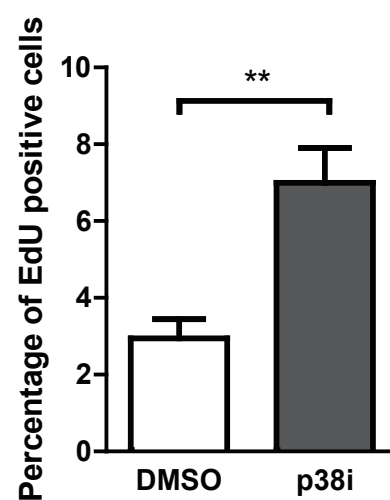
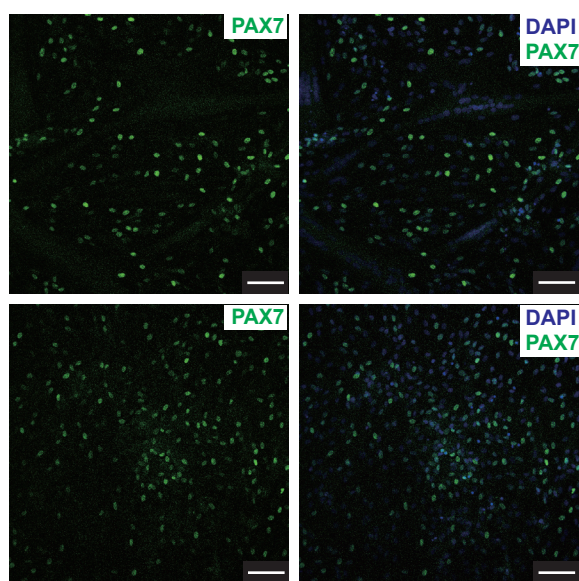
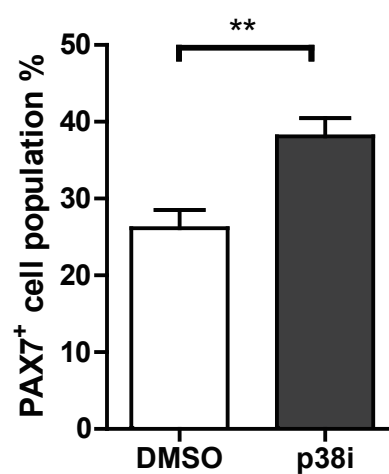
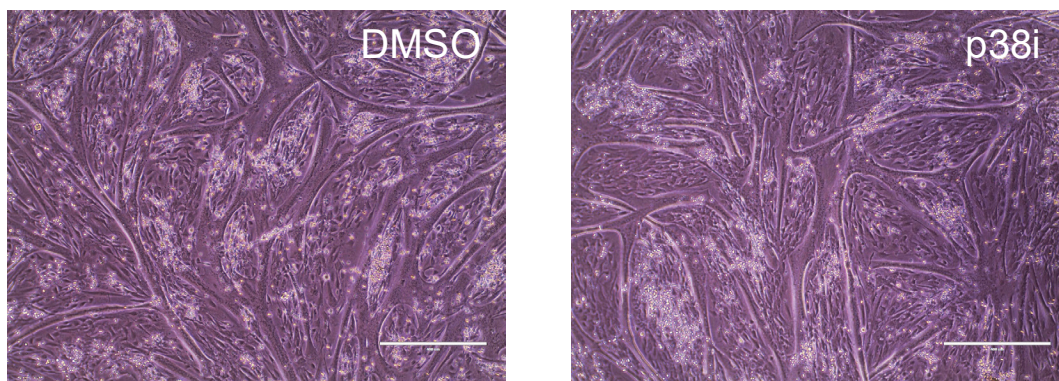
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Figure S3. p38i treatment maintains the proliferation and PAX7 population of satellite cells. Related to Figure 3.

(a) Representative DAPI and EdU immunofluorescent staining of 1 h window EdU incorporation after 6 days of culture in the absence (top panels) or presence (bottom panels) of p38i. Scale bars: 50µm. (b) Relative percentage of EdU from (a). (c) Representative immunofluorescent staining of DAPI and PAX7 after 6 days of culture in the absence (top panels) or presence (bottom panels) of p38i. Scale bars: 75µm. (d) Quantification of PAX7 immunofluorescent staining from (c). (e) Representative phase contrast images of differentiated bovine satellite cells. Passage 2 satellite cells are originally cultured in F-10 medium with DMSO or 10µM p38i for 4 days before differentiation. Scale bars: 400µm. Data are represented as mean ± SEM. Significance was analyzed by Student's t-test. Asterisks: ** indicates $P < 0.01$.

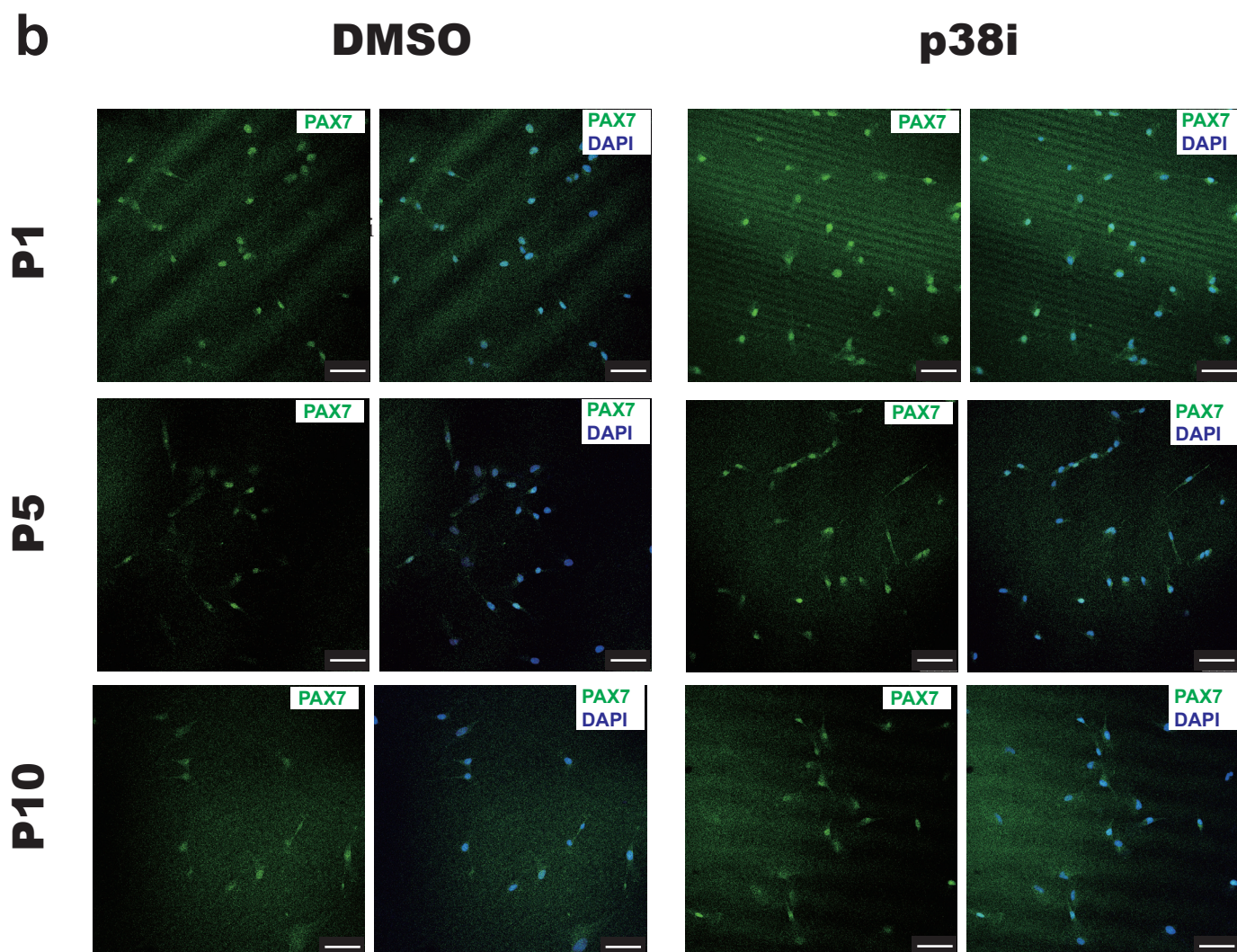
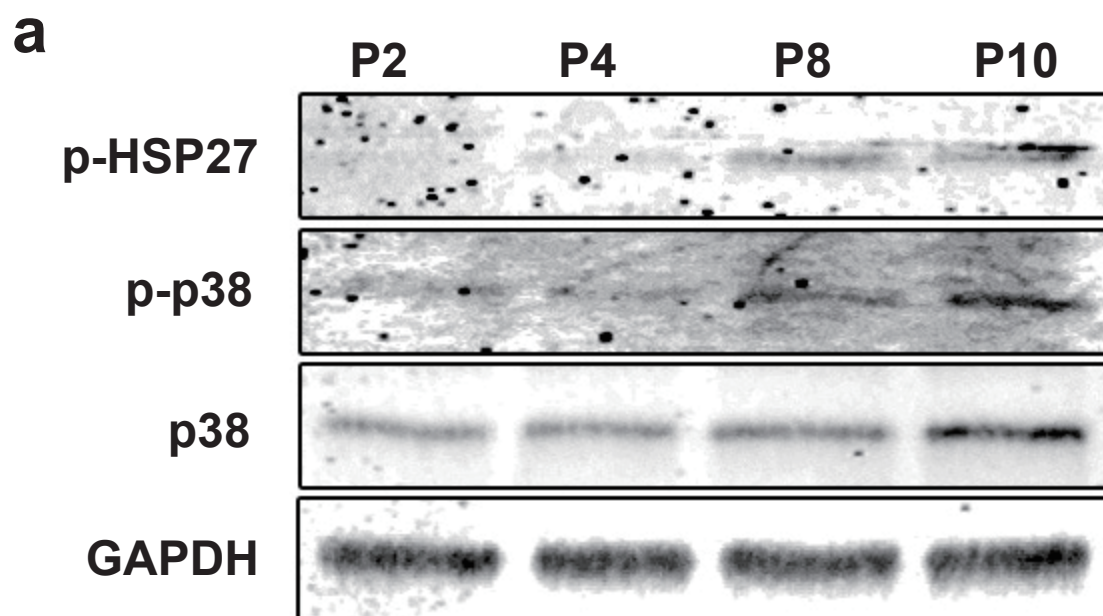


Figure S4. p38-MAPK signal is up-regulated during long-term culturing and p38i treatment maintains PAX7 population. Related to Figure 4.

(a) Representative images of immunoblotting against p-HSP27, p-p38, p38 and GAPDH of myoblast cells from P2, P4, P8, P10 cultured in F-10 medium. Full-length blots are presented in Supplementary Figure S5.

(b) Representative Immunofluorescent staining of PAX7 in different passage myoblast cells cultured in the presence or absence of p38i. Scale bars: 75 μ m.

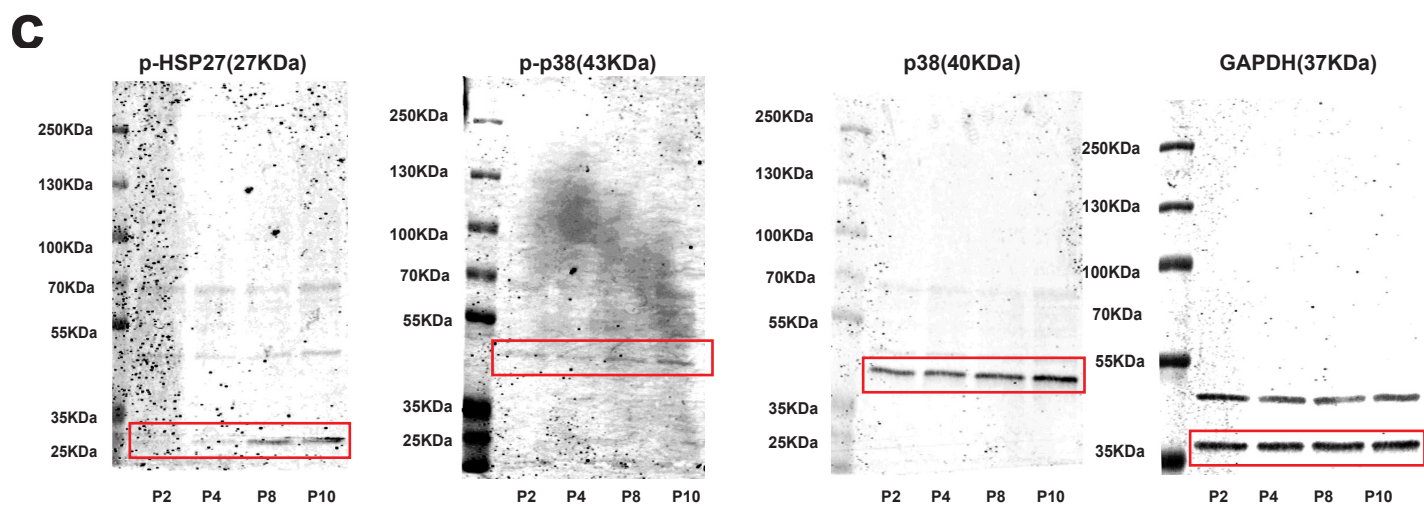
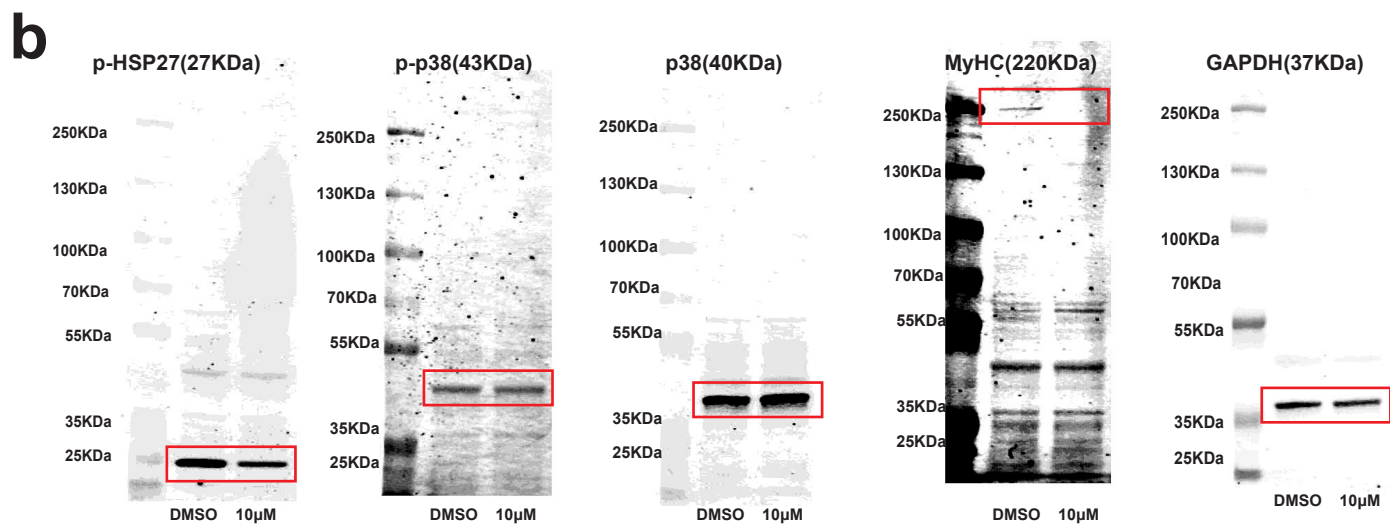
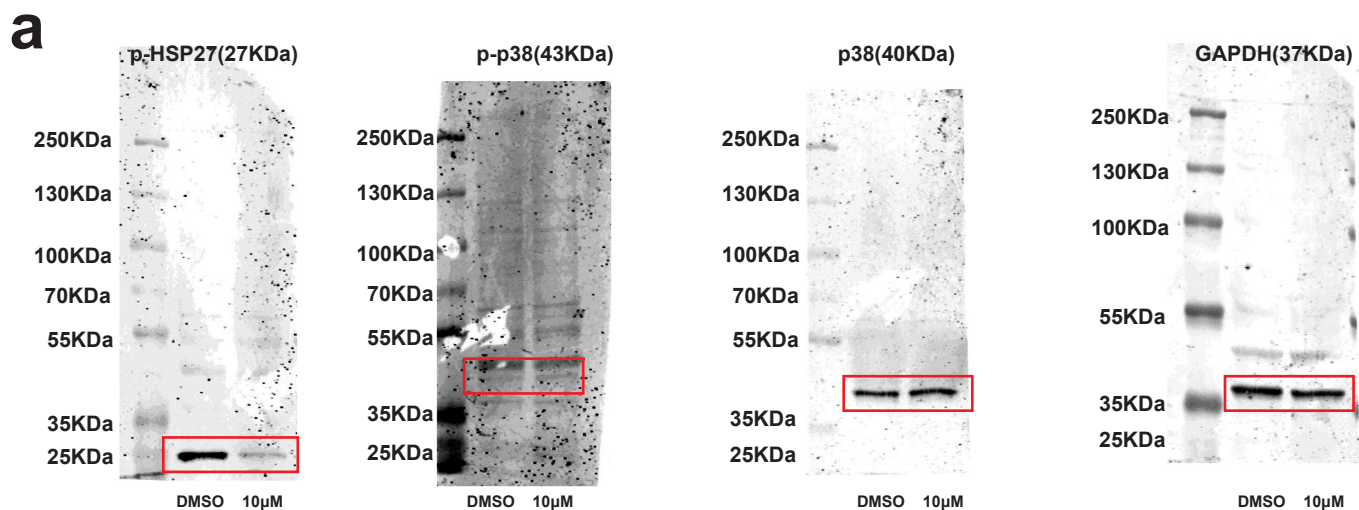


Figure S5. Full-length western blots. Related to Figure 2, Figure3, Figure S4.

(a) Representative full-length immunoblotting blots against p-HSP27, p-p38, p38 and GAPDH from cell lysates of cells cultured 4 days to 90% confluent in the presence or absence of 10 μ M p38i. (b) Representative full-length immunoblotting blots against p-HSP27, p-p38, p38, MyHC and GAPDH from cell lysates of cells cultured 6 days in the presence or absence of 10 μ M p38i. (c) Representative full-length immunoblotting blots against p-HSP27, p-p38, p38 and GAPDH of myoblast cells from P2, P4, P8, P10 cultured in F-10 medium.