Perdiguero et al., http://www.jcb.org/cgi/content/full/jcb.201104053/DC1

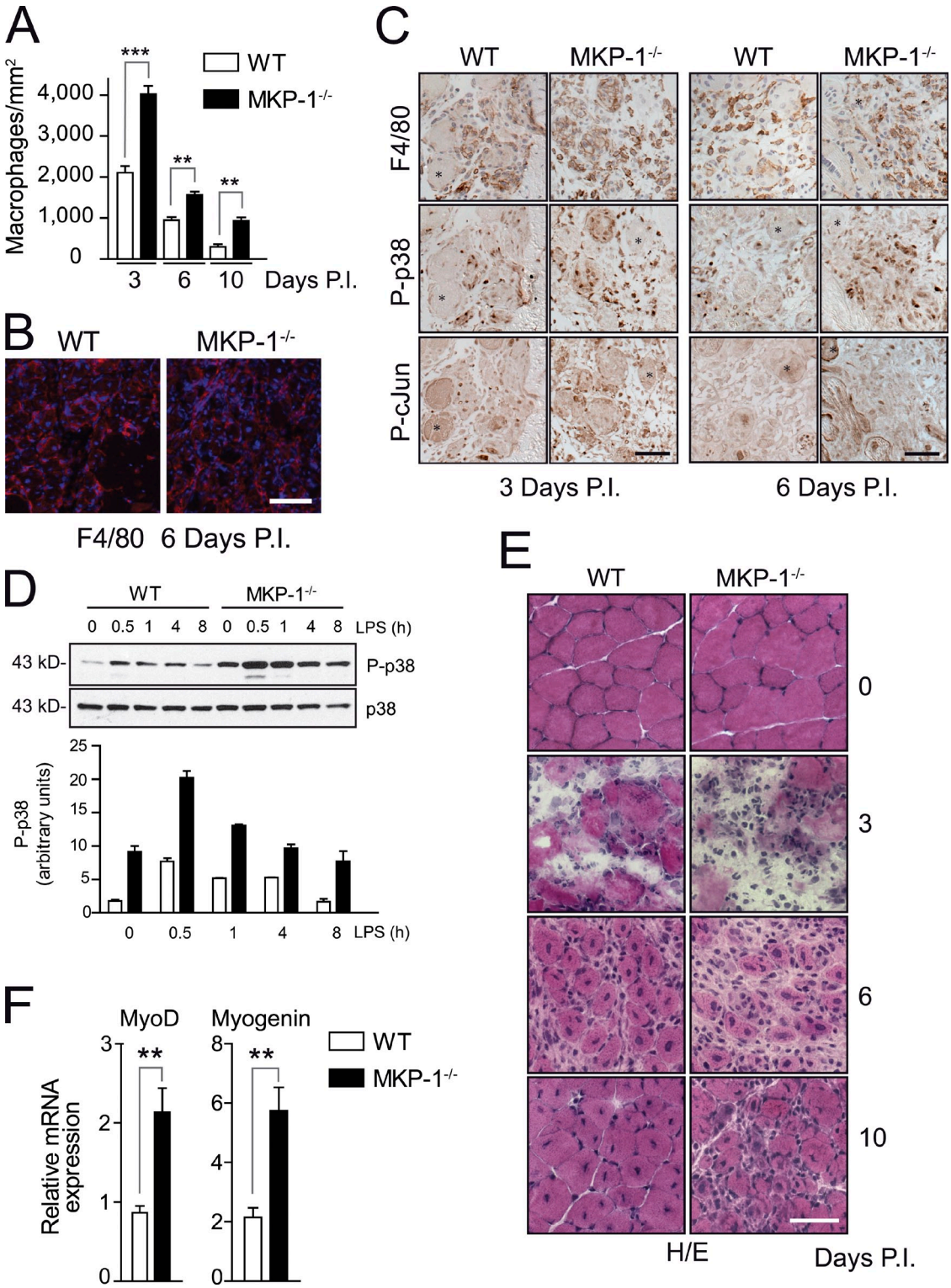

Figure S1. **Increased presence of inflammatory cells in regenerating MKP-1$^{-/-}$ muscles.** (A) Number of infiltrated macrophages in WT and MKP-1$^{-/-}$ muscle at the indicated times postinjury (P.I) were counted from immunostained cryosections with the F480 antibody. (B) Representative pictures of cryosections of 6-d CTX-injured gastrocnemius muscle from WT and MKP-1$^{-/-}$ mice stained with the F480 antibody (macrophages; red). Nuclei were stained with DAPI (blue). (C) Activation of p38 and JNK (indirectly addressed by phospho–c-Jun [P-cJun]) pathways in macrophages during CTX-induced muscle regeneration. Representative pictures of consecutive sections of gastrocnemius muscles obtained from WT and MKP-1$^{-/-}$ mice at 3 and 6 d after injury immunostained with antibodies against F4/80, phospho-p38 (P-p38), or phospho–c-Jun. Asterisks indentify the same fibers. (D) BMDMs isolated from WT and MKP-1$^{-/-}$ mice were cultured in vitro and stimulated with LPS at the indicated times. The activation of p38 was analyzed by Western blotting using antiphospho-specific and total antibodies. A quantification of the phospho-p38 immunoblots by scanning densitometry (corrected by p38 expression) is shown. (E) Cryosections from gastrocnemius muscles of WT and MKP-1$^{-/-}$ mice, obtained from uninjured controls (0) and at the indicated times after CTX injury, were stained with H/E. Representative pictures are shown. (F) MyoD and Myogenin mRNA expression levels from gastrocnemius muscles of WT and MKP-1$^{-/-}$ mice obtained 10 d after CTX injury were analyzed by quantitative RT-PCR. Results represent the means of at least three experiments. ***, $P < 0.001$; **, $P < 0.01$. Error bars indicate SEM. Bars, 50 µm.

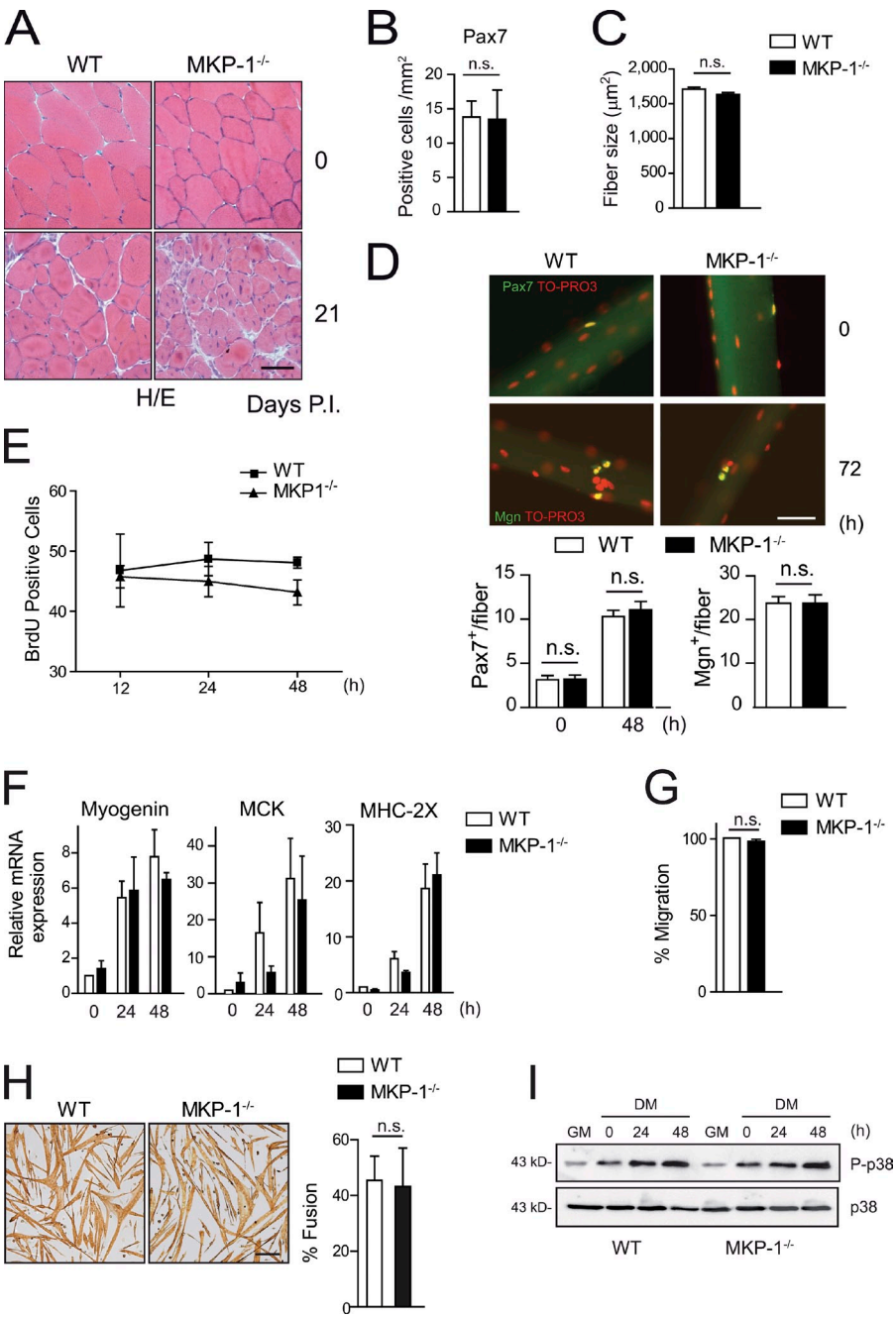

Figure S2. **Laceration pictures, basal Pax7 and CSA numbers, single fiber experiments, and muscle-intrinsic in vitro paradigms, which demonstrate the dispensability of MKP-1.** (A) Representative pictures of cryosections from tibialis muscles of WT and MKP-1$^{-/-}$ mice obtained from uninjured controls (0) and at 21 d after laceration stained with H/E. P.I., postinjury. (B) Muscle cryosections from uninjured (basal) muscles were immunostained with antibodies against Pax7 to identify satellite cells, and the number of positive cells was quantified. (C) Cryosections from gastrocnemius muscles of WT and MKP-1$^{-/-}$ mice, obtained from uninjured (basal) controls, were stained with H/E, and the mean area of myofibers was calculated. Results represent the means of at least three experiments. (D) Batches of single fibers with associated satellite cells from the same mouse were cultured ex vivo in GM medium for the indicated times; satellite cells were stained for either Pax7 at 0 and 48 h in culture or Myogenin after 72 h in culture. (top) Representative pictures. Values are population means ± SEM of the number of satellite cells immunostained per myofiber (isolated from at least three age-matched mice). (E–I) Proliferation, differentiation, migration, and fusion are not significantly altered in MKP-1–deficient satellite cells. (E) WT and MKP-1–deficient satellite cells were cultured in GM for the indicated times and incubated for 1 h with BrdU. Cells were fixed and immunostained against BrdU, and positive cells were quantified. (F) WT and MKP-1–deficient satellite cells were cultured in GM until subconfluence and then shifted to DM for the indicated times (hours). Comparative qPCR mRNA analysis of Myogenin, MCK, and MHC-2×. (G) Relative migration capacity of WT MKP-1–deficient myoblasts using transwells coated with Matrigel. (H) Cells were immunostained for eMHC to define nuclei inside myotubes after 72 h in DM. Fusion index was calculated as the percentage of plurinucleated eMHC-positive cells. (I) As in H, activation of p38 was analyzed by Western blotting in WT and MKP-1$^{-/-}$ satellite cells cultured in GM until subconfluence and then shifted to DM for the indicated times (hours) using an antiphospho-p38 (P-p38)–specific antibody. Results represent the means of at least three experiments. Error bars indicate SEM. Bars: (A and H) 50 μm; (D) 100 μm.

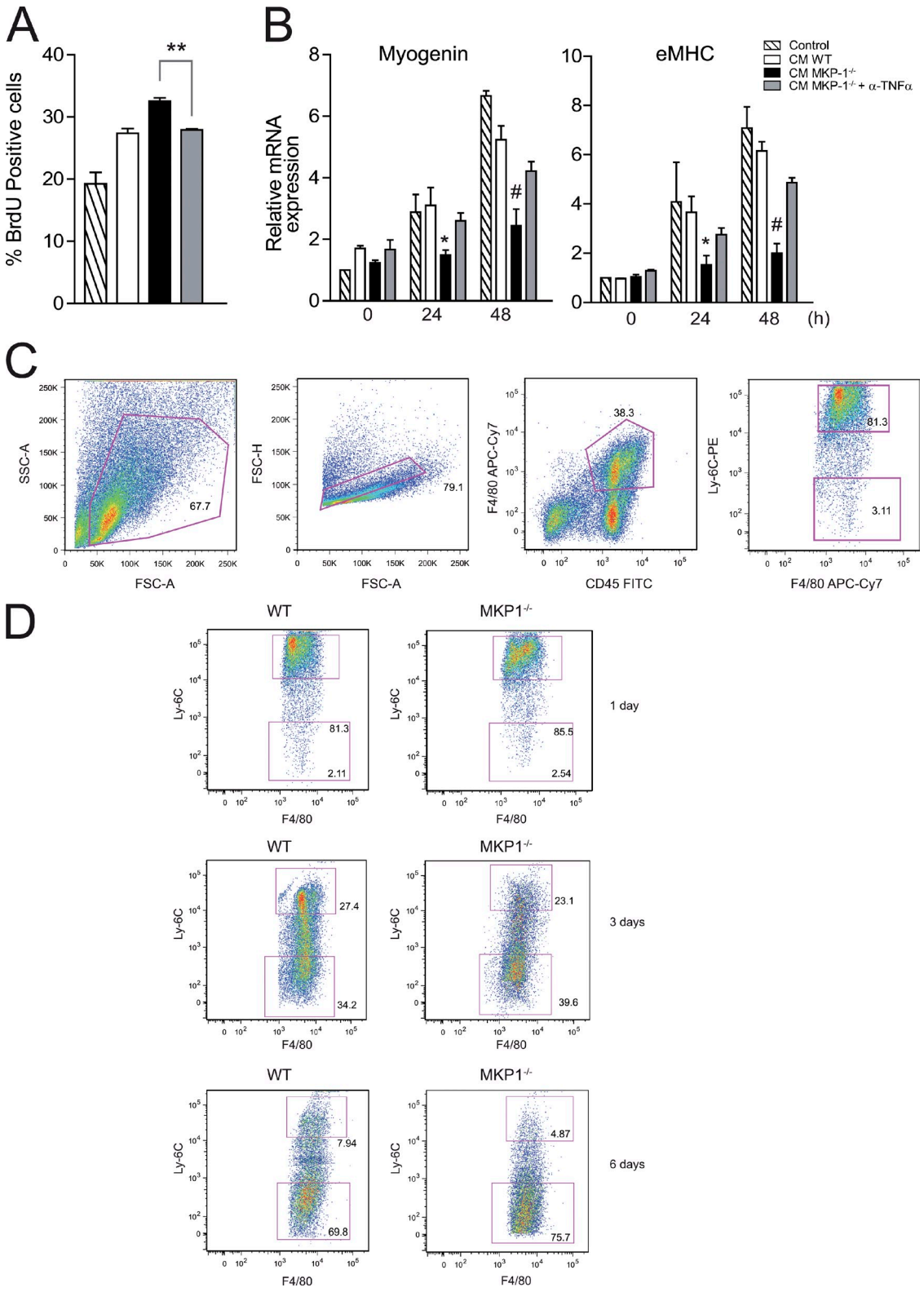

Figure S3. **Macrophage CM experiments and FACS gating scheme and profiles.** (A) Proliferation of WT satellite cells treated with conditioned media (CM) from WT and MKP1$^{-/-}$ macrophages transiently stimulated with LPS, plus 50 ng/ml anti-TNF antibody for 24 h and incubated for 1 h with BrdU. Cells were fixed and immunostained against BrdU, and positive cells were quantified. (B) Differentiation-specific gene expression in WT satellite cells treated with WT and MKP1$^{-/-}$ CM. WT satellite cells were cultured in GM and then shifted to DM or supplemented with macrophage CM with or without anti-TNF antibody for the indicated times. Comparative qPCR analysis of Myogenin and MCK mRNA. (C) FACS sorting strategy. Representative example of cell sorting strategy and gating scheme. (D) FACS profiles of the selected macrophage populations. F4/80^{+} cells present in injured muscle were analyzed for Ly-6C expression by flow cytometry. Representative examples of FACS analysis at 1, 3, and 6 d after injury. The two rectangles represent the two gated populations. Percentage of cells in each gate is indicated. Error bars indicate SEM. #, $P < 0.01$; *, $P < 0.05$. APC, allophycocyanin; PE, phycoerythrin; FSC-A, forward scatter area; SSC-A, side scatter area.

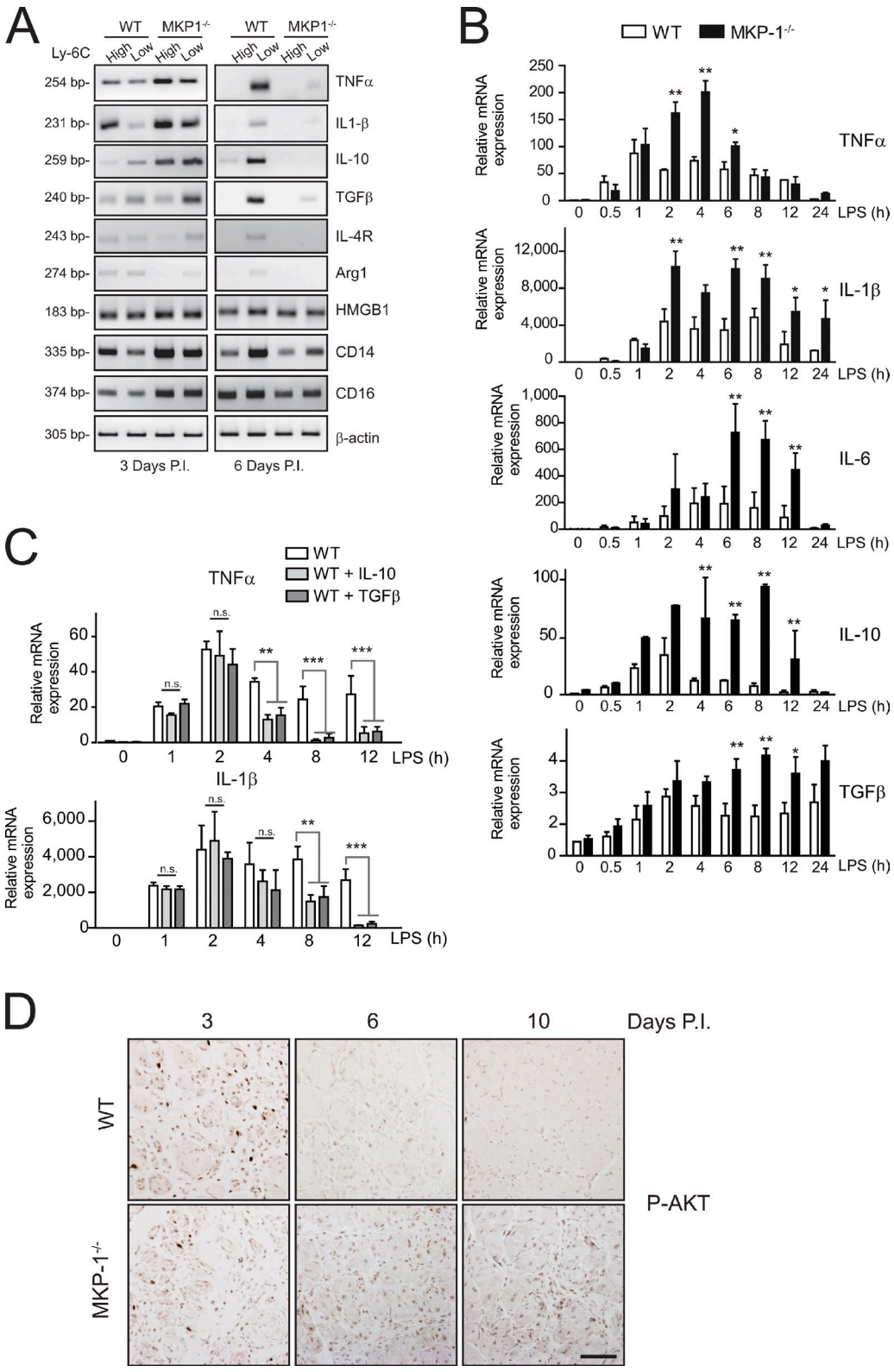

Figure S4. **Molecular characterization of WT and MKP-1$^{-/-}$ macrophage population subsets at early and late stages of muscle tissue repair.** (A) Expression of TNF, IL-1β, IL-10, TGFβ, IL4R, Arg1, HMGB1, CD14, CD16, and β-actin as a loading control was analyzed by RT-PCR in isolated macrophage populations. P.I., postinjury. (B) BMDMs isolated from WT and MKP-1$^{-/-}$ mice were cultured in vitro and stimulated with LPS at the indicated times. Comparative qPCR analysis of TNF, IL-1β, IL-6, IL-10, and TGFβ mRNA. (C) BMDMs from WT and MKP-1$^{-/-}$ mice were cultured in vitro and stimulated with LPS or LPS plus IL-10 or TGFβ at the indicated times. Comparative qPCR mRNA analysis of TNF and IL-1β. (D) Activation of AKT in macrophages during CTX-induced muscle repair. Gastrocnemius muscles from uninjured and injured WT and MKP-1$^{-/-}$ mice at the indicated times after CTX injection. Sections of gastrocnemius muscles obtained from WT and MKP-1$^{-/-}$ mice at 3, 6, and 10 d after injury were immunostained with phospho-AKT (P-AKT) antibody. Results represent the means of at least three experiments. ***, P < 0.001; **, P < 0.01; *, P < 0.05. Error bars indicate SEM. Bar, 50 µm.

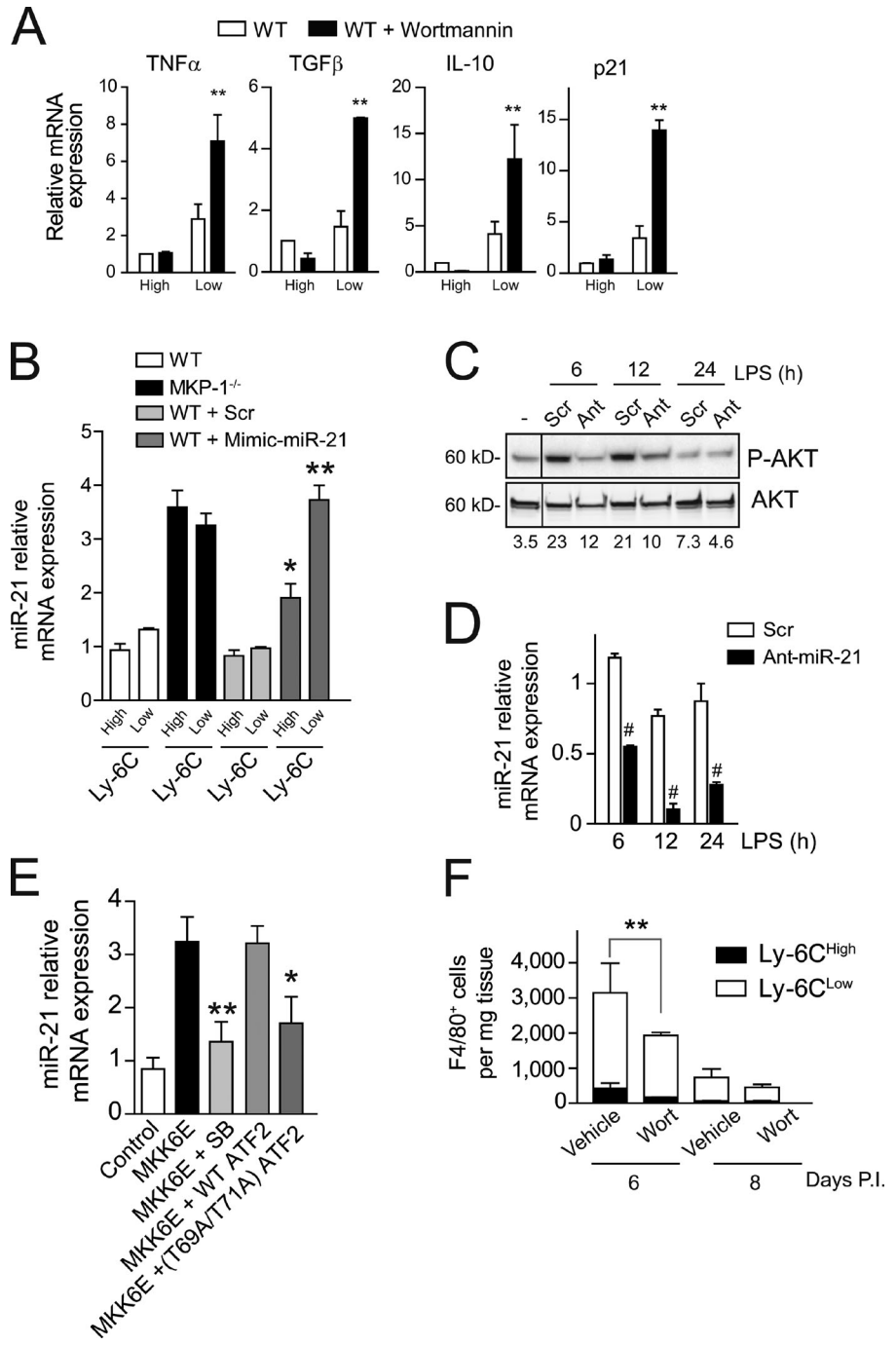

Figure S5. **qPCR controls from wortmannin treatments and miR-21 expression, AKT activity, and number of cells after interference of miR-21–AKT in vivo and in vitro.** (A) Expression of TNF, IL-10, TGFβ, and p21 was analyzed by qPCR in FACS-isolated macrophage populations at 6 d after injury from mice treated with wortmannin or vehicle for the preceding 3 d. (B) Comparative qPCR mRNA analysis of miR-21 in macrophages isolated by FACS from the muscle of WT and MKP-$1^{-/-}$ mice and from WT muscle overexpressing miR-21 by injection of an miR-21 mimic or a scrambled oligo-miR (Scr) as a control. (C) Primary macrophages were transfected with scramble oligo-miR or ant–miR-21 (Ant) oligonucleotides and stimulated with LPS for the indicated times. Activation of AKT was analyzed by Western blotting using antiphospho-specific and total antibodies. Quantification values of phospho-AKT (P-AKT) immunoblots by scanning densitometry (corrected by AKT total protein) is shown below. Black lines indicate that intervening lanes have been spliced out. The minus sign refers to nonstimulated macrophages. (D) Comparative qPCR mRNA analysis of miR-21 in primary macrophages transfected with scramble or ant–miR-21 oligos and stimulated with LPS for the indicated times. (E) Comparative qPCR mRNA analysis of miR-21 in primary macrophages infected with a retrovirus expressing MKK6E (a constitutively active form of MKK6, which leads to downstream p38 activation) or an empty retrovirus (control), in the absence or presence of SB203580 (SB), or the WT or dominant-negative forms of c-Jun and ATF2 transcription factor. (F) Total number of macrophages per milligram of muscle present at the indicated times after injury from mice treated with vehicle or wortmannin (Wort). P.I., postinjury. Results represent the means of at least three experiments. #, $P < 001$; **, $P < 0.01$; *, $P < 0.05$. Error bars indicate SEM.