Supporting Information

Wang et al. 10.1073/pnas.0908924106

SI Text

ELISA of IL-18, IL-18BP, and VEGF. Mouse IL-18 and human IL-18BP were measured in the MSC supernatant and cell lysate after 24 h of incubation (R&D Systems Inc.). Culture supernatants from WT MSCs incubated with IL-18 (0, 1, 10, 100, and 500 ng/mL) for 24 h were measured for VEGF (R&D Systems).

MSCs from WT and IL-18BP Tg mice were also divided into the following groups: 1) control; 2) TNFα with or without IL-18; 3) LPS with or without IL-18; and 4) hypoxia (1% O_2) with or without IL-18. Hypoxia was achieved using an INVIVO$_2$ 300 hypoxic workstation (Ruskinn Life Sciences), which provides accurate control over O_2 (0.1–20.9% in 0.1% increments). After 24 h of incubation, supernatants were collected for VEGF measurement.

Western Blotting. Cells were lysed in cold RIPA buffer (Sigma) and centrifuged at 12,000 $\times$ *g* for 10 min. The protein extracts (5 μg/lane) were subjected to electrophoresis on a 4–12% Tris/HCL SDS PAGE and transferred to a nitrocellulose membrane. The membranes were incubated in 5% dry milk for 1 h and then incubated with antibodies for p38 MAPK, *p*-p38 MAPK, ERK1/2, *p*-ERK1/2, and GAPDH, followed by incubation with horseradish peroxidase-conjugated antibodies.

Isolated Rat Heart Experiments (Langendorff Model). Male Sprague-Dawley rats (275–300 g) from Harlan Laboratory were divided into three groups: I/R + Vehicle (n = 6), I/R + WT MSCs (n = 5) and I/R + IL-18BP MSCs (n = 6). MSCs from WT and IL-18BP Tg mice were suspended in a warm (37 °C), Krebs-Henseleit solution with a concentration of 1×10^6 cells/mL and 1 mL cell solution was infused through a port above the aortic root over 1 min immediately before inducing global ischemia. All isolated rat hearts were subjected to the same I/R protocol: 15 min of equilibration followed by 25 min of warm global ischemia (37 °C) and 40 min of reperfusion.

Myocardial Infarction. Male Sprague-Dawley rats (300–325 g) were randomized into four groups: 1) sham (n = 5); 2) Ischemia + vehicle (n = 13); 3) Ischemia + WT MSCs (n = 12); and 4) Ischemia + IL-18BP MSCs (n = 12). The sham group underwent the same procedure as the others with the exception of the LAD ligation and intramyocardial injection. Successful ligation of the LAD was verified by myocardial blanching and abnormal movement of the anterior wall. Ten minutes after ligation, a solution of 100 μL PBS (vehicle), WT MSCs or IL-18BP MSCs (1×10^7 cells/mL) was injected into the myocardium at two sites around the infarct border zone.

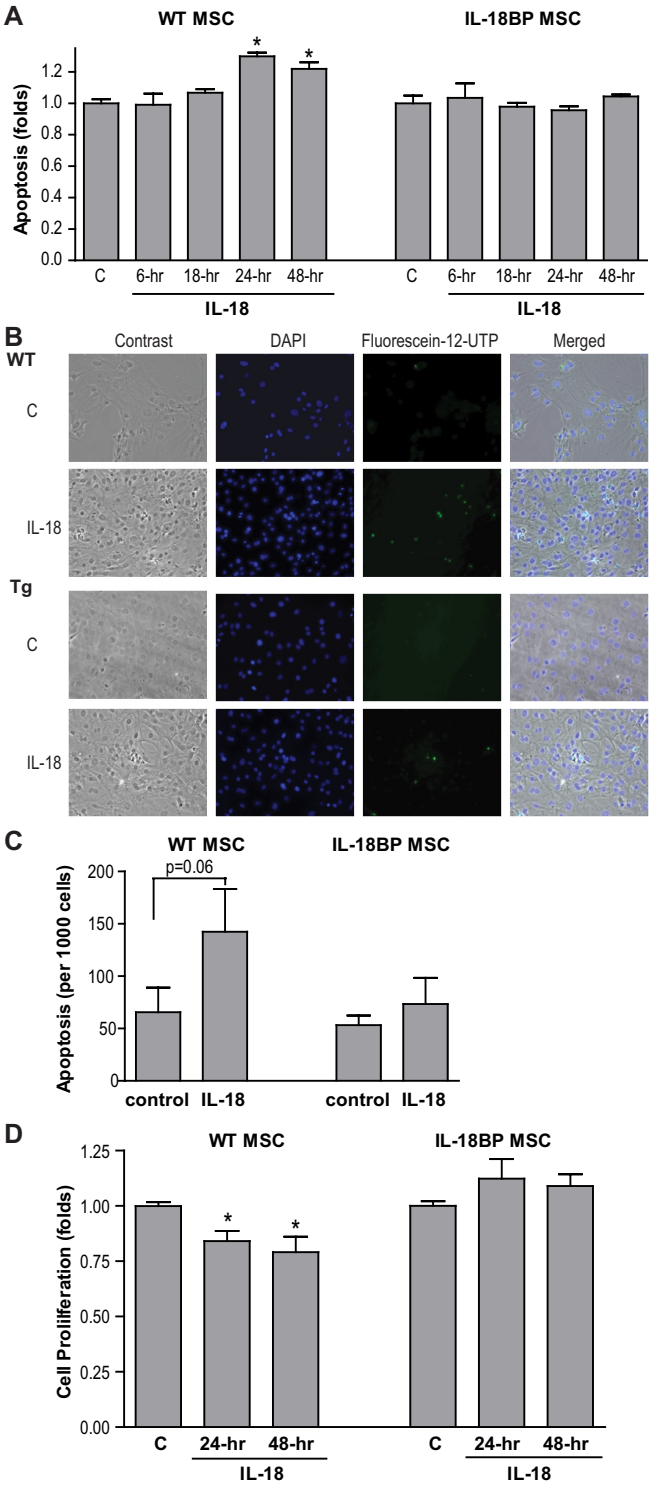

Fig. S1. Effects of IL-18 on apoptosis and cell proliferation in mesenchymal stem cells (MSCs) from wild-type (WT) and IL-18-binding protein (IL-18BP) overexpressing transgenic mice. (*A*) Levels of mono- and oligonucleosomes in the cytoplasmic fraction were measured using a cell death detection ELISA kit in WT and IL-18BP MSCs following 6 h, 18 h, 24 h, and 48 h of IL-18 treatment. The results are depicted as a ratio to the control. (*B*) Photomicrographs (magnification, ×200) demonstrating stem cell apoptosis with or without 100 ng/mL IL-18 in WT and IL-18BP MSCs. Nuclear staining with DAPI (blue) denotes nonapoptotic cells while TUNEL assay staining with fluorescein-12-dUTP (green) shows apoptotic cells. (*C*) The bar graph depicts the number of apoptotic cell/1,000 cells. (*D*) Cell proliferation in WT and IL-18BP MSCs after 24 and 48 h of treatment with IL-18 is also depicted. (Mean ± SEM, $n = 3$, *, $P < 0.05$ vs. control, hr: hour).

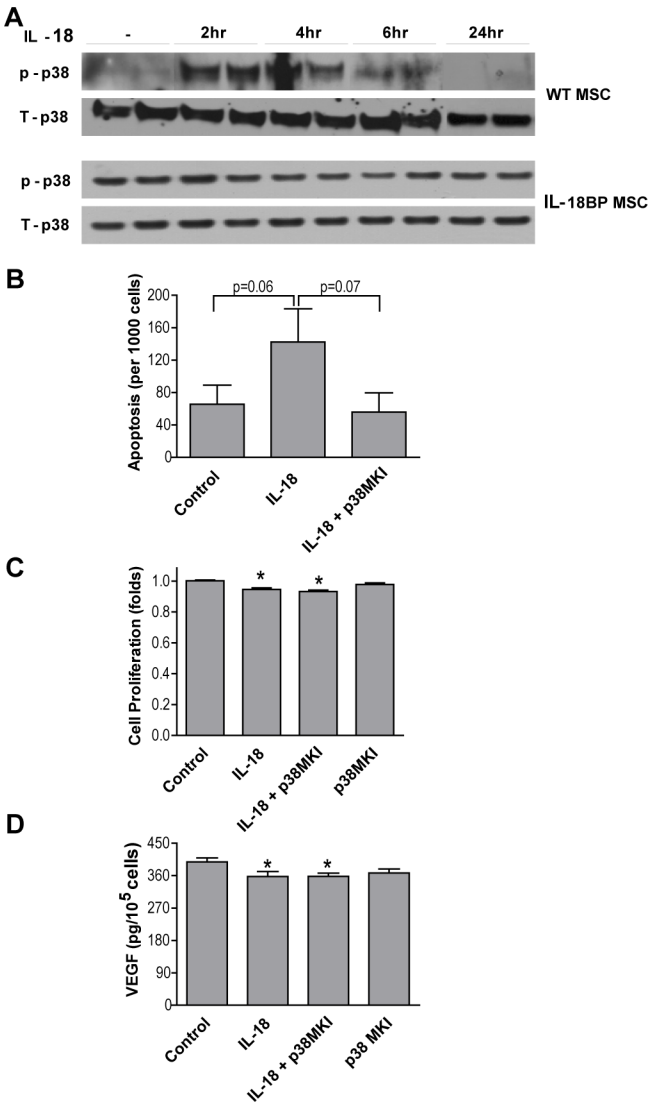

Fig. S2. (*A*) Representative immunoblots (two lanes/group) of phosphorylated-p38 MAPK (*p*-p38) and total-p38 MAPK (T-p38) are shown in WT and IL-18BP MSCs after 2 h, 4 h, 6 h, and 24 h with or without IL-18 (100 ng/mL). (*B*) WT MSC apoptosis was measured by TUNEL assay and is represented by the number of apoptotic cells/1,000 cells after 24 h of incubation in with: 1) control, 2) IL-18 treatment, or 3) IL-18 plus p38 MAPK inhibitor (p38MKI: 10 μM SB 203580). Cell proliferation was also measured in the same groups (*C*). Panel *D* depicts the effects of p38 MKI on VEGF production in WT MSCs in the presence of IL-18. (Mean $\pm$ SEM, $n = 3$, *, $P < 0.05$ vs. control).

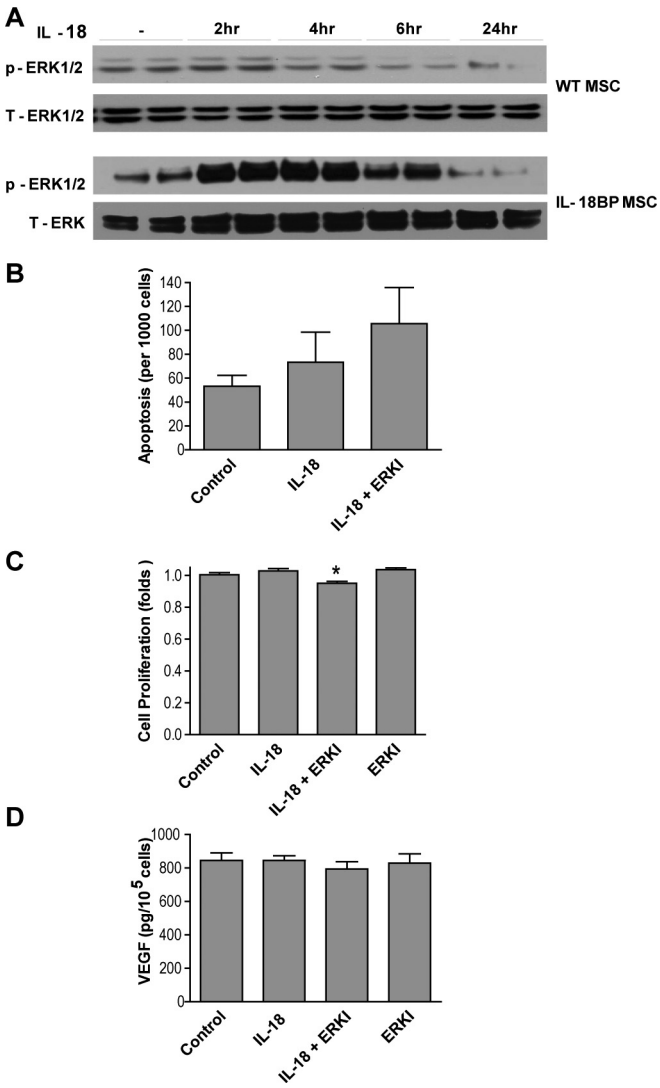

Fig. S3. (*A*) Representative immunoblots (two lanes/group) of phosphorylated-ERK1/2 (*p*-ERK1/2) and total-ERK1/2 (T-ERK1/2) are shown in WT and IL-18BP MSCs after 2 h, 4 h, 6 h, and 24 h incubation with or without IL-18 (100 ng/mL). (*B*) IL-18BP MSC apoptosis was measured by TUNEL assay and presented as the number of apoptotic IL-18BP cells/1,000 cells with or without ERK1/2 inhibitor (ERKI: 25 μM 328006) in the presence of IL-18. (*C*) IL-18BP MSC proliferation was measured after 24 h of incubation without and with 1) IL-18, 2) IL-18 + ERKI, and 3) ERKI alone. (*D*) Effects of ERKI after 24 h of incubation on IL-18BP MSC VEGF production with and without the addition of IL-18. (Mean $\pm$ SEM, $n = 3$, *, $P < 0.05$ vs. control).

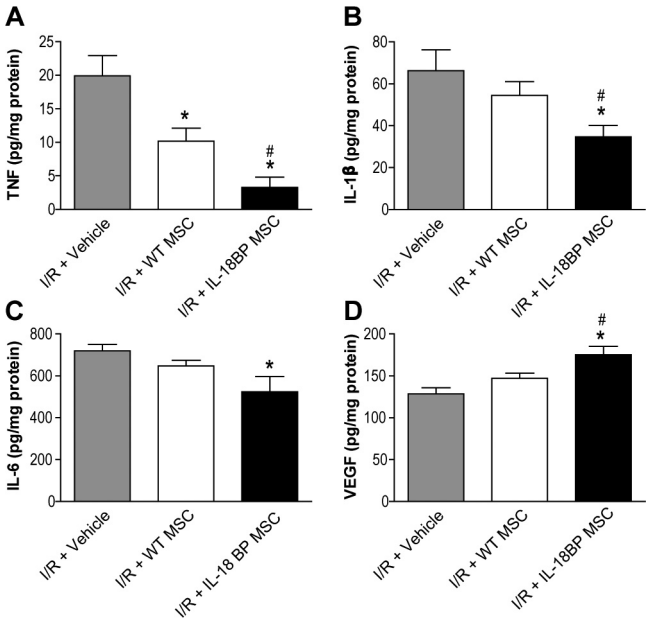

Fig. S4. Myocardial production of TNF (*A*), IL-1β (*B*), IL-6 (*C*), and VEGF (*D*) after acute I/R in I/R + vehicle, I/R + WT MSC and I/R + IL-18BP MSC groups. Cytokine production was measured in the myocardial tissue subjected to acute I/R using ELISA. (Mean ± SEM, $n = 5–6$/group, *, $P < 0.05$ vs. I/R + vehicle; #, $P < 0.05$ vs. I/R + WT MSC).

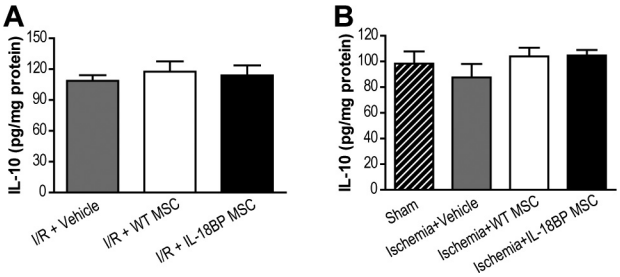

Fig. S5. Effects of IL-18BP MSCs on myocardial IL-10 levels. (*A*) IL-10 production was measured in I/R + vehicle ($n = 6$), I/R + WT MSC ($n = 5$) and I/R + IL-18BP MSC ($n = 6$) groups after global I/R by ELISA. (*B*) IL-10 levels in the at-risk area of heart tissue 28 days post-LAD ligation in the sham ($n = 3$), ischemia + vehicle ($n = 6$), ischemia + WT MSC ($n = 3$) and ischemia + IL-18BP MSC groups ($n = 4$).

Table S1. Effect of IL-18 on mesenchymal stem cell VEGF production (pg/10^5 cells)

	IL-18 , ng/mL				
	0	1	10	100	500
Wild type	501.0 ± 5.15	488.5 ± 33.4	460.4 ± 5.45*	440.2 ± 2.28*	438.7 ± 17.3*
IL-18BP MSC	535.6 ± 11.6	552.5 ± 13.4	517.6 ± 8.75	538.5 ± 6.84	519 ± 14.2

Mean ± SEM, $n = 3$ individual experiments, * $P < 0.05$ vs. 0 (control).