SUPPLEMENTARY MATERIAL

Supplementary Methods

Echocardiography

Mice were anaesthetised with isofluorane 2, 4, 7 and 28 days after surgery. Cardiac dimensions at systole and diastole were assessed by echocardiography (Diasus 10-22 MHz probe Dynamic Imaging, Livingstone, UK). The chest was shaved and the transducer was placed parasternally to obtain a 2D image of the myocardium. Images were saved and analysed offline using Diasus software (Dynamic Imaging). Left ventricle ejection fraction (%EF) was calculated as (left ventricle end diastole area – left ventricle end systolic area)/ left ventricle end diastolic area x 100 ((LVEDA-LVESA)/LVEDA x 100) in short axis images, as we have previously described (1, 2 below). The observer was blinded for the purpose of echocardiography measurements and all other analysis of tissue.

Immunohistochemistry

Paraffin embedded sections (5µm) were deparaffinised and rehydrated through ethanol for use in immunohistochemistry. After blocking, slides were incubated with rat anti-mouse monoclonal anti-mac 2 antibody (1:6000 clone M3/38 Cedarlane, UK) for 16 hours at 4°C or mouse anti-mouse α smooth muscle actin (1:400 clone 1A4 Sigma) for 1 hour at room temperature. Subsequent incubation in biotinylated rabbit anti-rat and goat anti-mouse secondary antibodies (1:200 Vector, Peterborough, UK) for 1 hour and 30 minutes respectively at room temperature was followed by extravidin peroxidase (1:200 Sigma).

Peroxidase activity was detected with the 3,3-diaminobenzidine kit (Vector). Sections were counterstained with haematoxylin, dehydrated and mounted in DPX resin (Fluka).

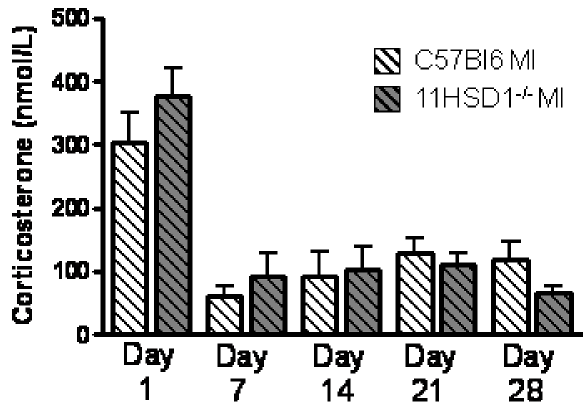
Antigens were retrieved with citrate buffer pH6.0, Proteinase K (20µgml Roche, Oxford, UK) or Trypsin and 2N HCl prior to blocking and primary antibody incubation with rabbit anti-mouse polyclonal anti-YM1 antibody (1:50 Stem cell Technologies, France) for 1 hour at room temperature, rat anti-mouse monoclonal anti-CD31 (PECAM-1) antibody (1:50 clone MEC 13.3 BD Pharmingen, UK) for 16 hours at 4°C or mouse anti-mouse monoclonal anti-BrdU antibody (1:1000 clone BU 33 Sigma) for 1 hour at 37°C. Biotinylated goat anti-rabbit, rabbit anti-rat or goat anti-mouse secondary antibodies (1/200 Vector) were incubated with the slides for 30 minutes at room temperature followed by extravidin peroxidase (1:200 Sigma). Peroxidase activity was detected with a 3,3-diaminobenzidine kit (Vector). Sections were counterstained with haematoxylin, dehydrated and mounted in DPX resin (Fluka).

References

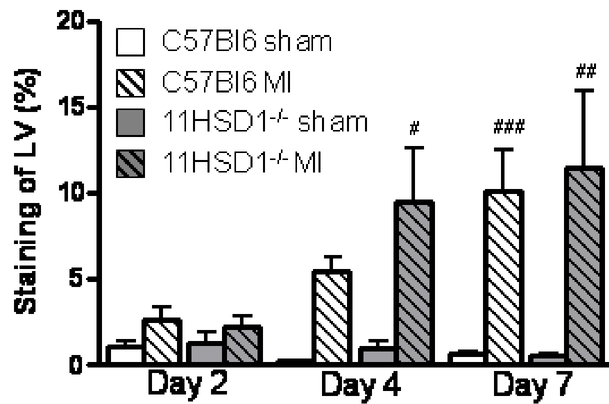
(1) Denvir, MA *et al.* (2005) Influence of scanning frequency and ultrasonic contrast agent on reproducibility of left ventricular measurements in the mouse. *J Am Soc Echo18,* 155-162

(2) Pepys MP *et al*. (2006) Targeting C-reactive protein for treatment of cardiovascular disease. *Nature* 440:1217-21

Supplementary Figures



Supplementary Figure 1. Circulating corticosterone up to 28 days after myocardial infarction (MI) surgery. The concentration of corticosterone in plasma collected at the morning nadir was evaluated by radioimmunoassay. Data are expressed as mean ± SEM (n=6 C57Bl6 (light bars) and 11βHSD1$^{-/-}$ (darker bars) at day 1 after MI; n=10 C57Bl6 and n=9 11βHSD1$^{-/-}$ at day 7, 14, 21 and 28 after MI).



Supplementary Figure 2: Myofibroblast activation during infarct healing. Activation was detected by αSMA immunoreactivity and quantified as percentage staining of the LV. Data are presented as the mean ± SEM (n=8 C57Bl6 sham; n=12 C57Bl6 MI; n=4 11βHSD1$^{-/-}$ sham; n=6 11βHSD1$^{-/-}$ MI. #P<0.05, ##P<0.01, ###P<0.001 compared to matched sham.

Supplementary Table 1: Ejection fractions pre-operatively (pre-op) and after sham-operation (sham) or myocardial infarction (MI).

	Pre-op	Day2		Day 4		Day 7	
		Sham	MI	Sham	MI	Sham	MI
C57BL/6	69.7 ± 2.7	63.1 ± 2.8	27.8 ± 2.4 #	69.6 ± 2.5	28.7 ± 1.6 #	63.6 ± 4.5	29.1 ± 2.7 #
11βHSD1$^{-/-}$	66.4 ± 3.9	68.8 ± 1.5	32.9 ± 3.5 #	67.0 ± 5.0	31.8 ± 1.6 #	65.4 ± 4.0	40.1 ± 1.6 #*

Ejection fraction was calculated from the LV end diastolic area (LVEDA) and LV end systolic area (LVESA) and is expressed as a percentage. Data is presented as mean ± SEM (pre-operative values n=8; sham-operated C57Bl/6 n=8; MI C57Bl/6 n=12, sham-operated 11HSD1$^{-/-}$ n=4; MI 11HSD1$^{-/-}$ n=6). # P<0.01 vs sham control; * P<0.05 vs MI in C57BL/6 group.