Targeting endothelin receptors in a murine model of myocardial infarction using a small molecular fluorescent probe

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Supplemental Material

Supplemental Figure S1: Cryo-images of hearts with pure IRDye800cw.
Supplemental Figure S2: Immunofluorescence staining for ET_AR, CD68 and α-SMA.
Supplemental Figure S3: Elastica van Gieson staining.
Supplemental Figure S4: Evaluation of expression levels of CD68 and collagen.
Supplemental Figure S5: ET_AR and CD31 staining.

Supplemental Figure S1

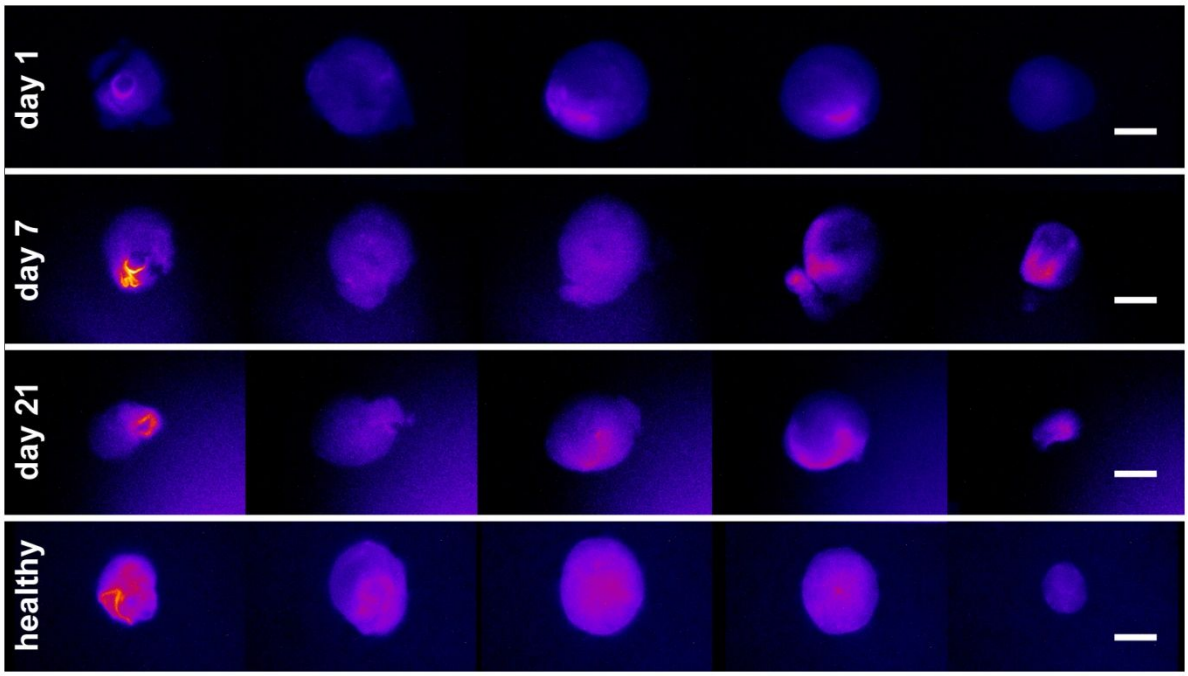

Figure S1: Series of cryo-CCD-images of hearts at day 1, day 7 and day 21 post MI surgery and of a healthy heart 4 hours after administration of 4.0 nmol of pure IRDye800cw. From left to right the series shows images from the heart base, with remains of superior vessels, towards the apex. Hearts with MI show a defined but low accumulation of the unspecific dye in the infarct region. In the healthy heart no distinct regions of probe accumulation can be depicted. Please note that the images at day 7 and 21 are brightness-enhanced compared to images at day 1 and images displayed in figure 1 in the main article. Also, please note that the images displayed here are not the same images that have been evaluated conc. signal intensity as shown in figure 2 in the main article (scale bar represents 3mm).

Supplemental Figure S2

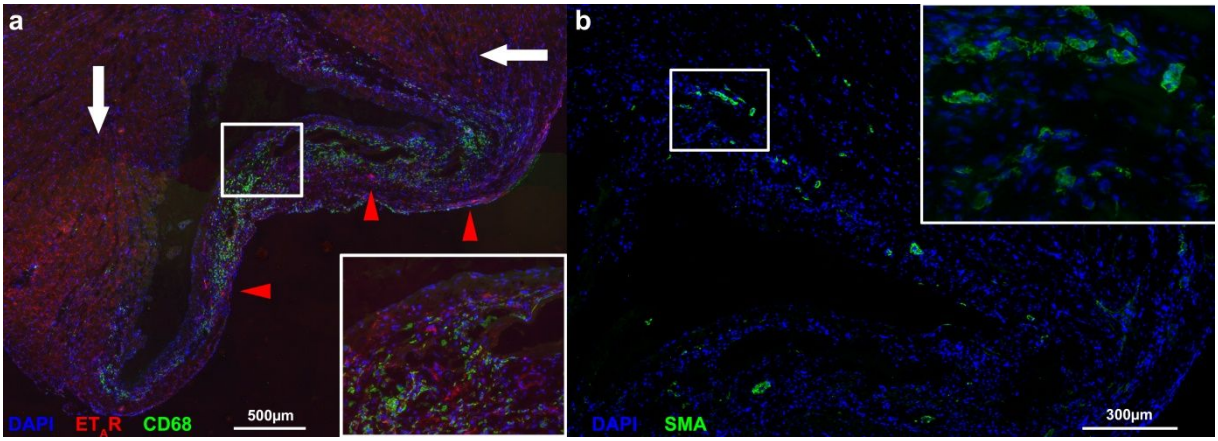

Figure S2: Immunofluorescence for ET_AR (red), macrophage infiltration (green) (**a**) and smooth muscle actin (green) (**b**) in infarcted hearts 7 days post surgery. A high number of macrophages are located in the left ventricular wall, indicating enhanced inflammatory activity within the infarcted regions (magnification). (**a**) High and extensive ET_AR abundance can be perceived in the infarct-adjacent regions (white arrows), but also more localized in the left ventricular wall (red arrowheads and magnification). (**b**) Activated myofibroblasts expressing high amounts of SMA can be identified by their typical spindle-like structure (magnification) and are responsible for collagen deposition in the infarcted area.

Supplemental Figure S3

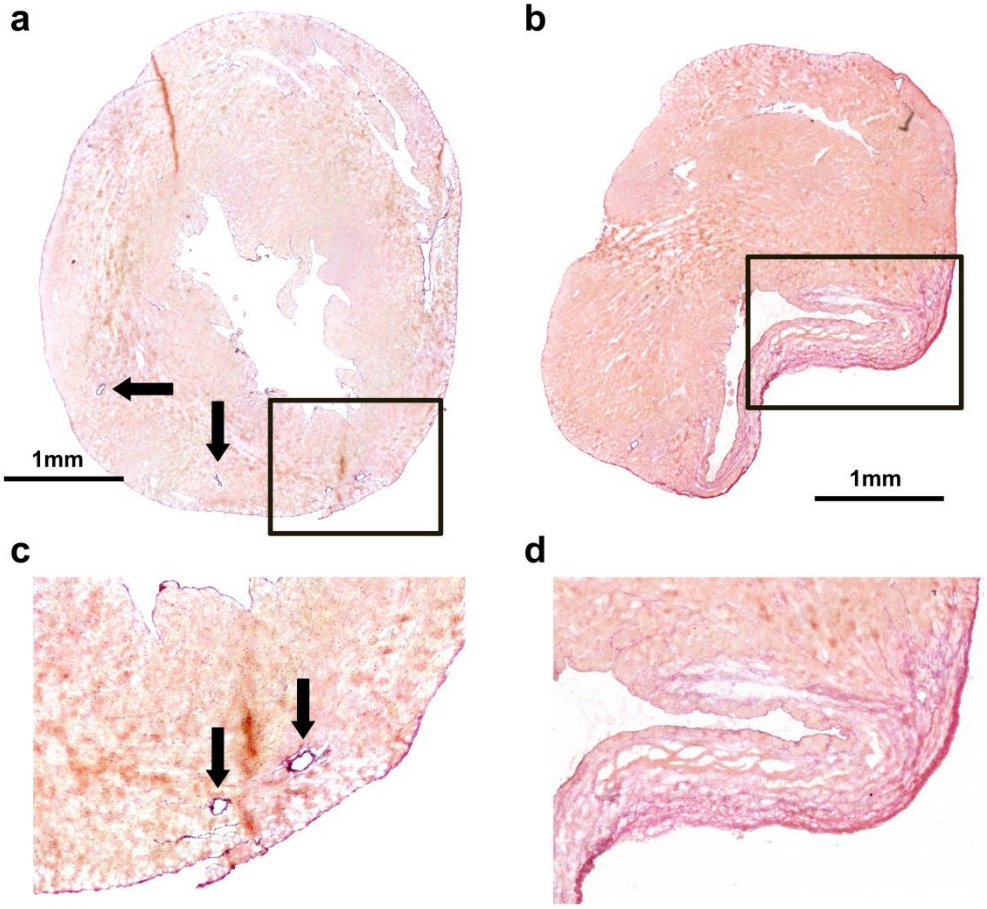

Figure S3: Elastica van Gieson staining of a healthy (**a**) and infarcted heart 7 days post surgery (**b**). In healthy hearts functional epithelium with vessels (black arrows) can be found. (**c**) Magnification of indicated area in (**a**). Infarcted hearts show tissue remodeling within the affected left ventricular wall, identified by high amount of collagenous fibers (pink color). (**d**) Magnification of indicated area in (**b**).

Supplemental Figure S4

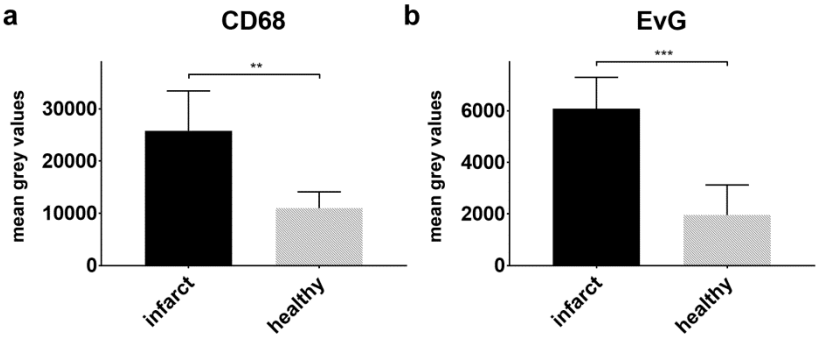

Figure S4: Evaluation of expression levels of CD68 and collagen deposition inside infarcted regions compared to healthy myocardium from immunohistochemistry (CD68) and Elastica van Gieson (EvG) staining (collagen), as shown in figures **S2** and **S3**. Both show a significant increase in expression seven days after surgery in infarcted myocardium.

Supplemental Figure S5

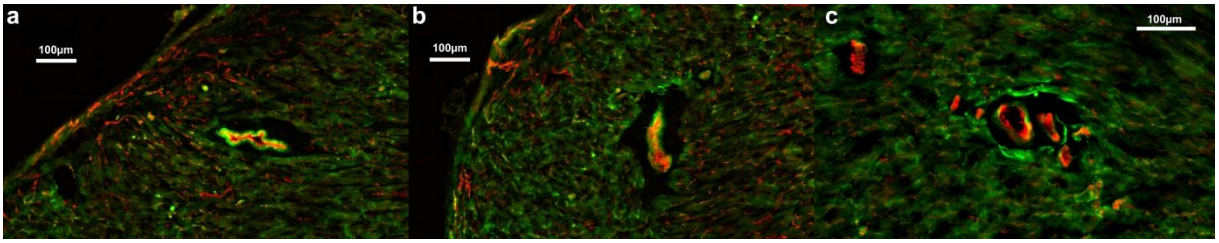

Figure S5: ET_AR (green) and CD31 (red) staining of infarcted murine hearts day 7 (**a,b**) and day 1 post surgery (**c**), showing the co-localization of ET_AR and CD31 in vascular structures inside infarcted myocardium.