Supplemental Data

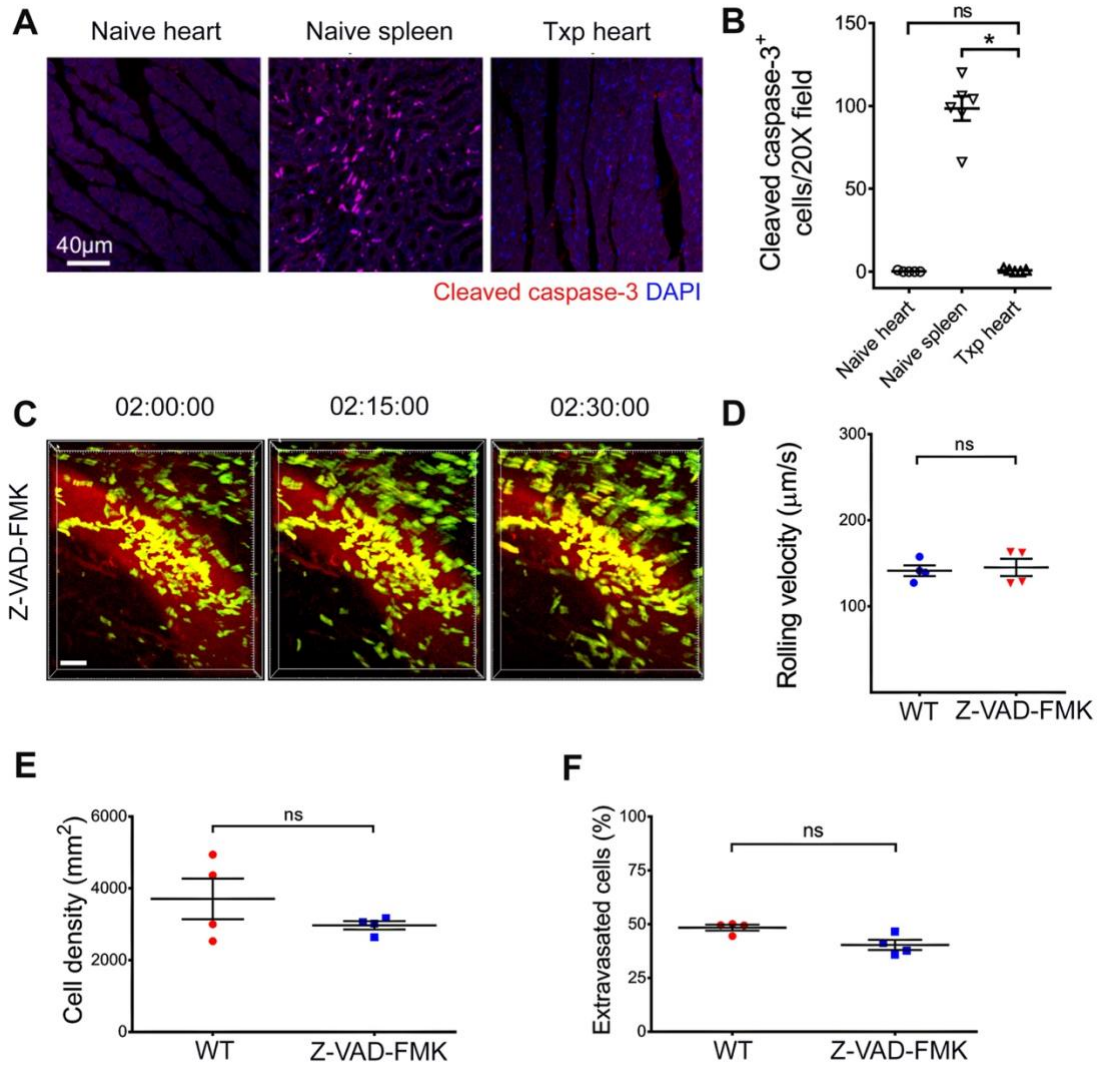

Figure S1: Early neutrophil recruitment after heart transplantation is not sensitive to caspase inhibition. (**A**) Immunostaining and (**B**) quantification of cleaved caspase 3 staining in naïve B6 hearts, naïve B6 spleens and B6 heart grafts 2 hours after transplantation into B6 recipients. n≥5 per experimental condition. (**C**) Intravital two-photon imaging of neutrophil (green) behavior in wildtype grafts that have been treated

with Z-VAD-FMK. (**D**) Intravascular rolling velocities of neutrophils, (**E**) density of neutrophils and (**F**) percentage of extravasated neutrophils in Z-VAD-FMK-treated mice compared to wildtype (WT) controls (n=4 per experimental group). Data in (**B**), (**D**)-(**F**) represent the mean ± s.e.m. ns = not significant, *p<0.05 by Mann-Whitney U test.

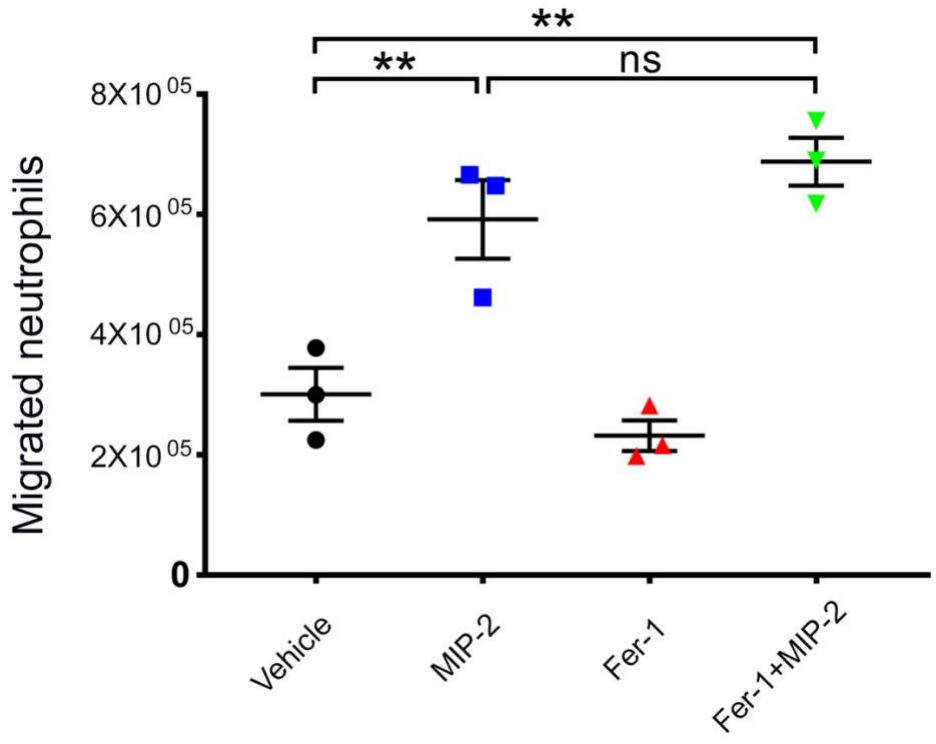

Figure S2: Ferrostatin-1 does not inhibit neutrophil chemotaxis. In vitro chemotactic behavior of B6 neutrophils in response to vehicle (circles), recombinant MIP-2 (squares), ferrostatin-1 (Fer-1) (triangles) as well as recombinant MIP-2 in the presence of Fer-1 (inverted triangles). Graphs depict mean of migrated neutrophils derived from three individual experiments. Data represent the mean ± s.e.m. ns = not significant, **p<0.01 by one-way analysis of variance with post-hoc Dunnett's test.

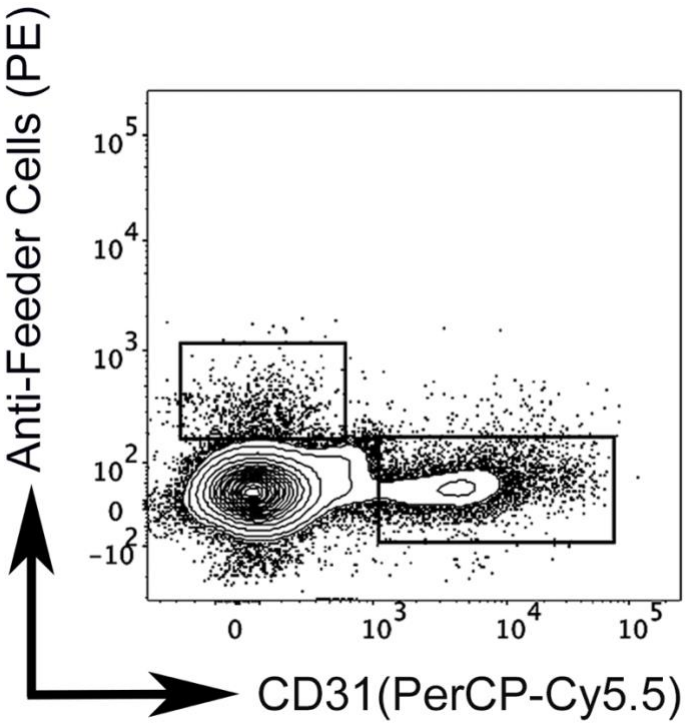

Figure S3: Gating strategy for endothelial cells and fibroblasts in cardiac grafts. Contour plot depicts gating strategy for endothelial cells (CD31) and fibroblasts (anti-feeder cells) in B6 cardiac grafts, prepared for flow cytometric analysis 2 hours after transplantation. Plot is gated on $CD45^-$ cells in cardiac grafts.

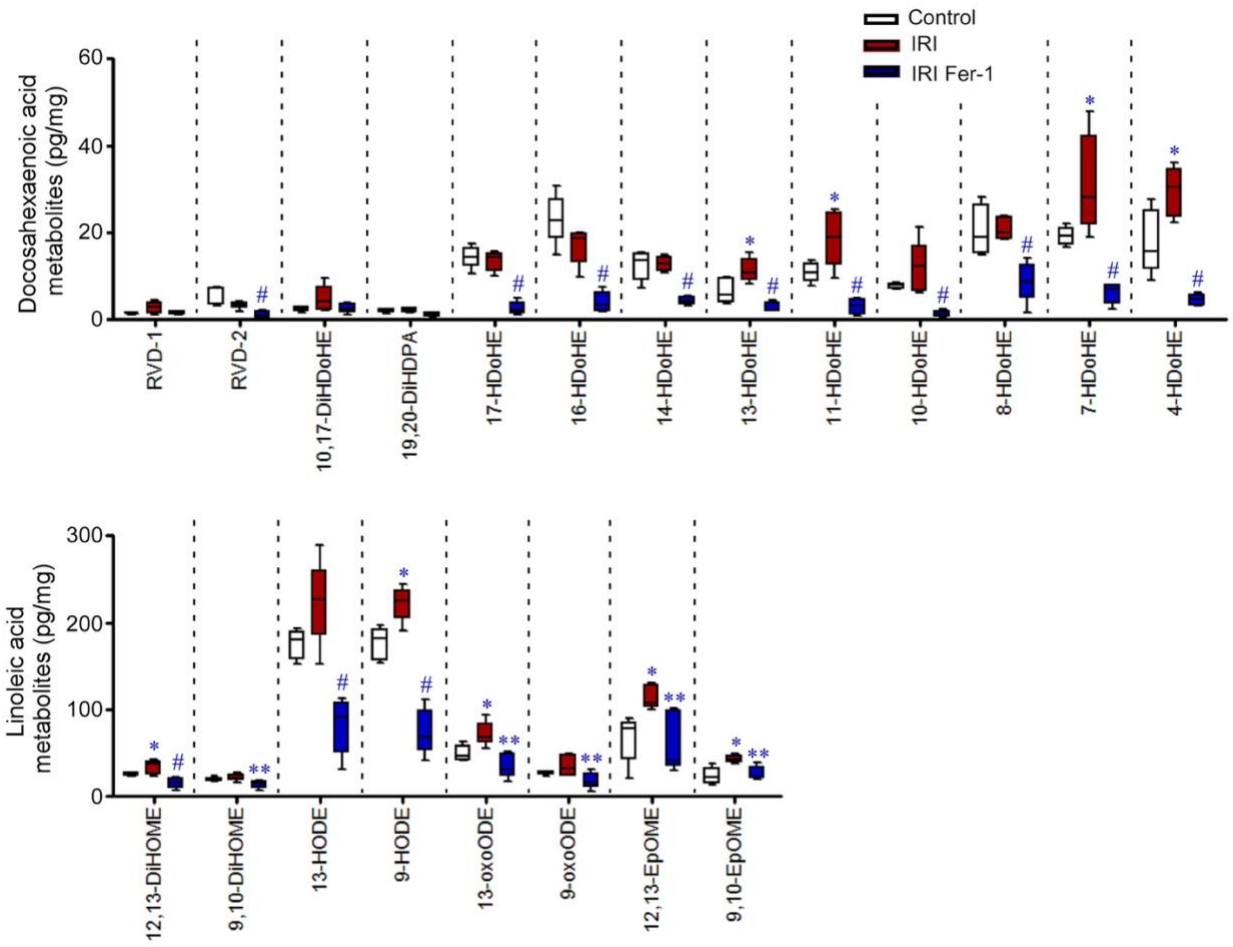

Figure S4: Ferrostatin-1 treatment reduces expression of docosahexaenoic acid and linoleic acid metabolites in injured hearts. Mass spectrometry (LC-MS/MS) of docosanoids and oxidized linoleic acid species in control hearts, vehicle-treated hearts subjected to IRI, and Fer-1-treated hearts subjected to IRI. Data are displayed as box and whisker plots. Line represents the mean. ns = not significant, *p<0.05 compared to control, **p<0.05 compared to other groups, $^{\#}$p<0.05 compared to vehicle IRI group (two-sided Mann-Whitney U test). Fer-1: ferrostatin-1.

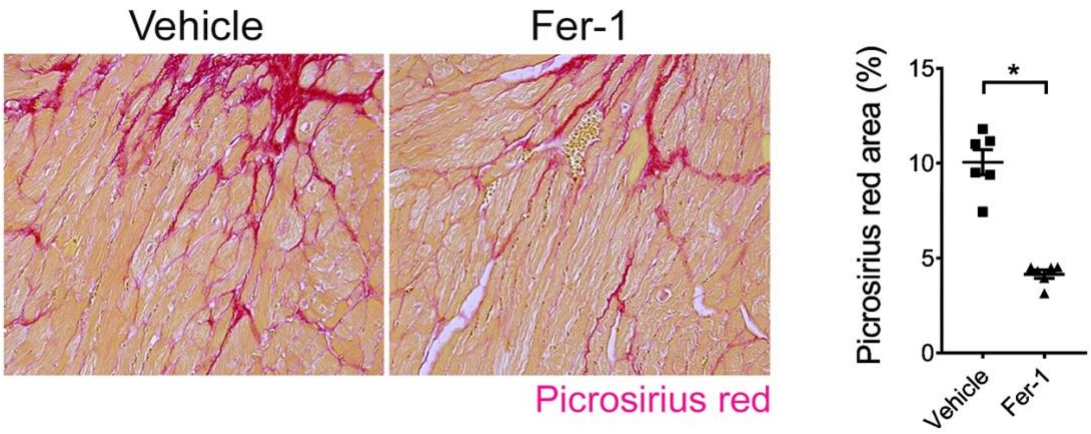

Figure S5: Pathological adverse remodeling following myocardial infarction. Picrosirius red staining demonstrating that Fer-1-treated hearts have reduced interstitial fibrosis compared to vehicle-treated hearts 4 weeks after 90 minutes of IRI. n=6 per experimental group. 100X magnification. Data represent the mean ± s.e.m. *p<0.05 compared to control (two-sided Mann-Whitney U test). Fer-1: ferrostatin-1.

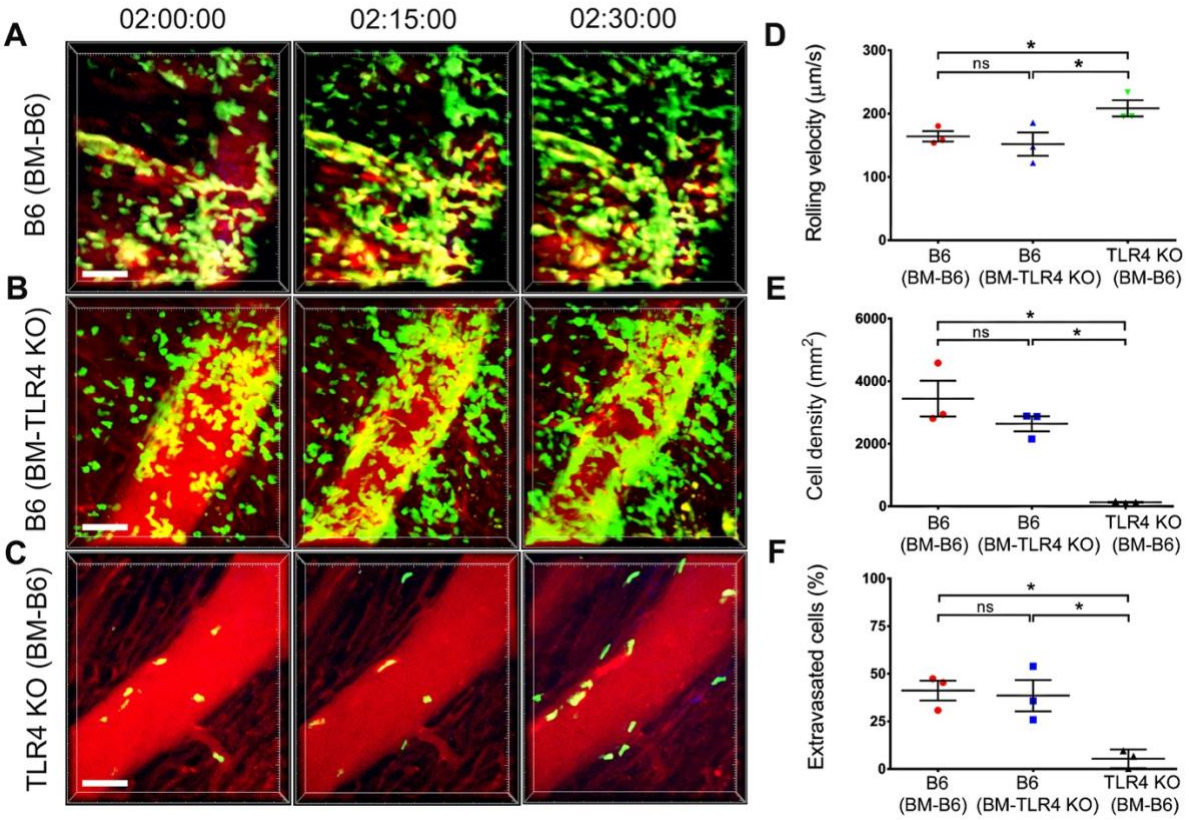

Figure S6: TLR4 expression on non-hematopoietic cells regulates neutrophil recruitment to injured hearts. Intravital two-photon imaging of neutrophil (green) behavior in (**A**) control wildtype grafts that have been reconstituted with wildtype bone marrow (BM), (**B**) wildtype grafts that have been reconstituted with TLR4-deficient (TLR4 KO) bone marrow and (**C**) TLR4-deficient hearts that have been reconstituted with wildtype bone marrow. Vessels are labeled red after injection of quantum dots. (**D**) Intravascular rolling velocities of neutrophils, (**E**) density of neutrophils and (**F**) percentage of extravasated neutrophils in experimental conditions displayed in **A-C**. n=3 per experimental group. Data in (**D**)-(**F**) represent the mean ± s.e.m. ns = not significant, *p<0.05, **p<0.01 by one-way analysis of variance followed by post-hoc Tukey's multiple comparison test.

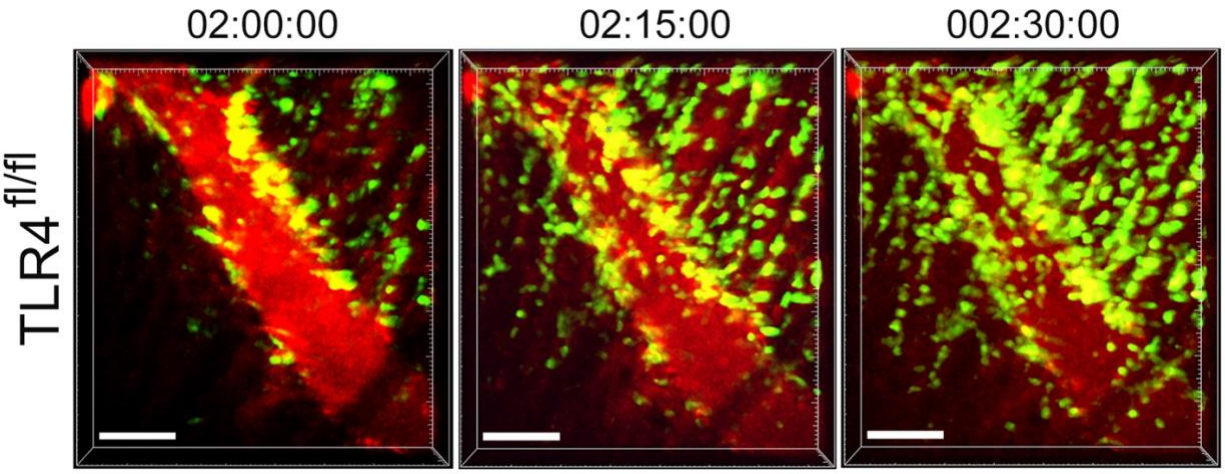

Figure S7: Intravital two-photon imaging of neutrophil (green) behavior in in B6 TLR4$^{fl/fl}$ heart grafts after transplantation into syngeneic B6 LysM-GFP mice. Vessels are labeled red after injection of quantum dots (n=3).

Video S1: Time-lapse intravital two-photon imaging of neutrophil behavior in wildtype heart grafts. Neutrophils (green) roll, adhere to the vessel wall, display intraluminal crawling and extravasate into myocardial tissue. Blood vessels (red) were labeled by intravenous injection of non-targeted 655-nm quantum dots. Scale bar: 50 µm. Relative time is displayed in hrs:min:sec.

Video S2: Time-lapse intravital two-photon imaging of neutrophil behavior in wildtype heart grafts after administration of Z-VAD-FMK to recipient. Neutrophils (green) roll, adhere to the vessel wall, display intraluminal crawling and extravasate into myocardial tissue. Blood vessels (red) were labeled by intravenous injection of non-targeted 655-nm quantum dots. Scale bar: 50 µm. Relative time is displayed in hrs:min:sec.

Video S3: Time-lapse intravital two-photon imaging of neutrophil behavior in wildtype heart grafts after administration of Nec-1 to recipient. Neutrophils (green) fail to adhere to vessel wall. Blood vessels (red) were labeled by intravenous injection of non-targeted 655-nm quantum dots. Scale bar: 50 µm. Relative time is displayed in hrs:min:sec.

Video S4: Time-lapse intravital two-photon imaging of neutrophil behavior in Rip3-deficient heart grafts. Neutrophils (green) roll, adhere to the vessel wall, display intraluminal crawling and extravasate into myocardial tissue. Blood vessels (red) were labeled by intravenous injection of non-targeted 655-nm quantum dots. Scale bar: 50 µm. Relative time is displayed in hrs:min:sec.

Video S5: Time-lapse intravital two-photon imaging of neutrophil behavior in

wildtype heart grafts after administration of Fer-1 to recipient. Neutrophils (green) do not adhere to vessel wall. Blood vessels (red) were labeled by intravenous injection of non-targeted 655-nm quantum dots. Scale bar: 50 µm. Relative time is displayed in hrs:min:sec.

Video S6: Time-lapse intravital two-photon imaging of neutrophil behavior in TLR4-deficient heart grafts. Neutrophils (green) do not adhere to vessel wall. Blood vessels (red) were labeled by intravenous injection of non-targeted 655-nm quantum dots. Scale bar: 50 µm. Relative time is displayed in hrs:min:sec.

Video S7: Time-lapse intravital two-photon imaging of neutrophil behavior in CD14-deficient heart grafts. Neutrophils (green) do not adhere to vessel wall. Blood vessels (red) were labeled by intravenous injection of non-targeted 655-nm quantum dots. Scale bar: 50 µm. Relative time is displayed in hrs:min:sec.

Video S8: Time-lapse intravital two-photon imaging of neutrophil behavior in Trif-deficient heart grafts. Neutrophils (green) do not adhere to vessel wall. Blood vessels (red) were labeled by intravenous injection of non-targeted 655-nm quantum dots. Scale bar: 50 µm. Relative time is displayed in hrs:min:sec.

Video S9: Time-lapse intravital two-photon imaging of neutrophil behavior in heart grafts procured from B6 wildtype donors that had been reconstituted with B6 wildtype bone marrow (B6 WT (B6 WT)). Neutrophils (green) roll, adhere to the vessel wall, display intraluminal crawling and extravasate into myocardial tissue. Blood vessels (red) were labeled by intravenous injection of non-targeted 655-nm quantum dots. Scale bar: 50 µm. Relative time is displayed in hrs:min:sec.

Video S10: Time-lapse intravital two-photon imaging of neutrophil behavior in heart grafts procured from B6 wildtype donors that had been reconstituted with B6 TLR4-deficient bone marrow (B6 WT (B6 TLR4 KO)). Neutrophils (green) roll, adhere to the vessel wall, display intraluminal crawling and extravasate into myocardial tissue. Blood vessels (red) were labeled by intravenous injection of non-targeted 655-nm quantum dots. Scale bar: 50 µm. Relative time is displayed in hrs:min:sec.

Video S11: Time-lapse intravital two-photon imaging of neutrophil behavior in heart grafts procured from B6 TLR4-deficient donors that had been reconstituted with B6 wildtype bone marrow (B6 TLR4 KO (B6 WT)). Neutrophils (green) do not adhere to vessel wall. Blood vessels (red) were labeled by intravenous injection of non-targeted 655-nm quantum dots. Scale bar: 50 µm. Relative time is displayed in hrs:min:sec.

Video S12: Time-lapse intravital two-photon imaging of neutrophil behavior in heart grafts that lack TLR4 expression on vascular endothelial cells (Tie2-Cre-TLR4$^{fl/fl}$). Neutrophils (green) do not adhere to vessel wall. Blood vessels (red) were labeled by intravenous injection of non-targeted 655-nm quantum dots. Scale bar: 50 µm. Relative time is displayed in hrs:min:sec.

Video S13: Time-lapse intravital two-photon imaging of neutrophil behavior in heart grafts that lack TLR4 expression on cardiomyocytes (Myh6-Cre-TLR4$^{fl/fl}$). Neutrophils (green) roll, adhere to the vessel wall, display intraluminal crawling and extravasate into myocardial tissue. Blood vessels (red) were labeled by intravenous injection of non-targeted 655-nm quantum dots. Scale bar: 50 µm. Relative time is displayed in hrs:min:sec.

Video S14: Time-lapse intravital two-photon imaging of neutrophil behavior in TLR4 floxed heart grafts. Neutrophils (green) roll, adhere to the vessel wall, display intraluminal crawling and extravasate into myocardial tissue. Blood vessels (red) were labeled by intravenous injection of non-targeted 655-nm quantum dots. Scale bar: 50 µm. Relative time is displayed in hrs:min:sec.

Video S15: Time-lapse intravital two-photon imaging of neutrophil behavior in IFNAR-deficient heart grafts. Neutrophils (green) do not adhere to vessel wall. Blood vessels (red) were labeled by intravenous injection of non-targeted 655-nm quantum dots. Scale bar: 50 µm. Relative time is displayed in hrs:min:sec.

Video S16: Time-lapse intravital two-photon imaging of neutrophil behavior in IRF 3/5/7-deficient heart grafts. Neutrophils (green) do not adhere to vessel wall. Blood vessels (red) were labeled by intravenous injection of non-targeted 655-nm quantum dots. Scale bar: 50 µm. Relative time is displayed in hrs:min:sec.

Video S17: Time-lapse intravital two-photon imaging of neutrophil behavior in wildtype heart grafts after administration of ferrostatin-1 and recombinant type I interferon to recipient. Neutrophils (green) roll, adhere to the vessel wall, display intraluminal crawling and extravasate into myocardial tissue. Blood vessels (red) were labeled by intravenous injection of non-targeted 655-nm quantum dots. Scale bar: 50 µm. Relative time is displayed in hrs:min:sec.