

Supplementary Material

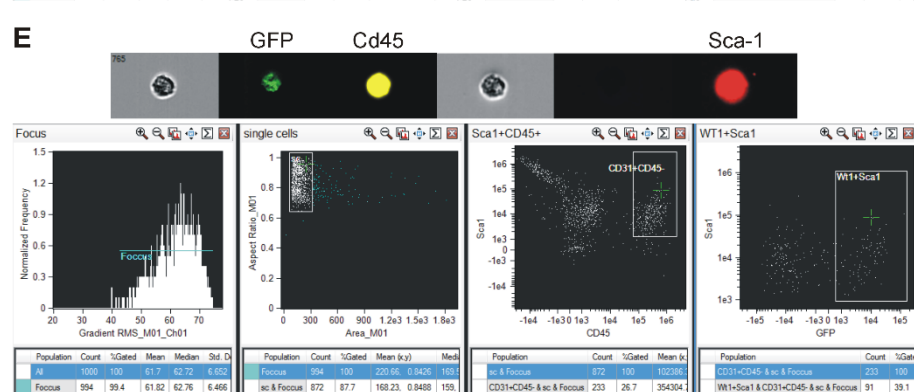
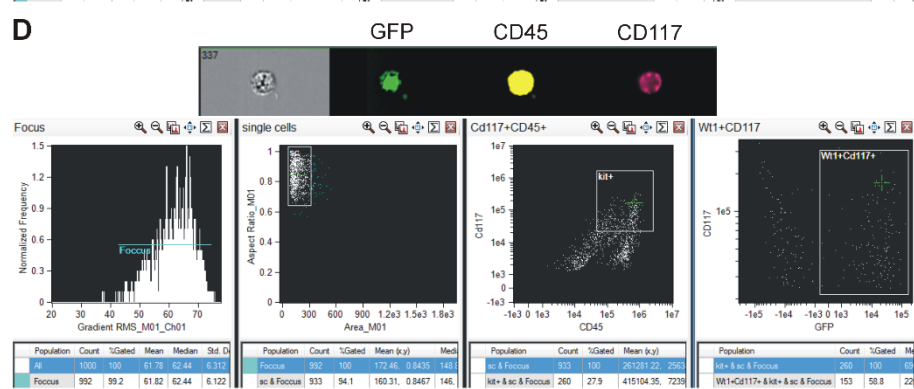
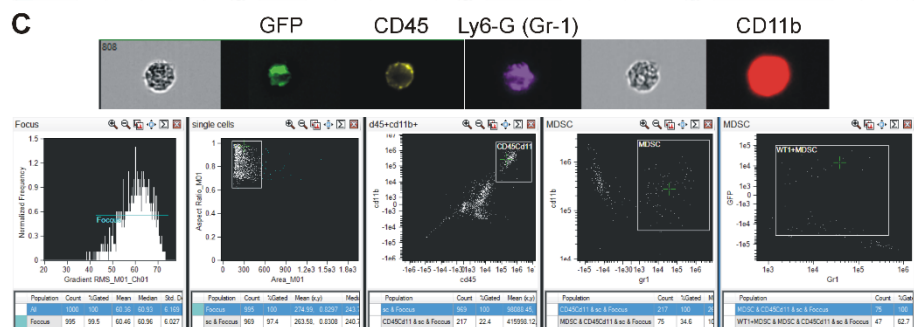
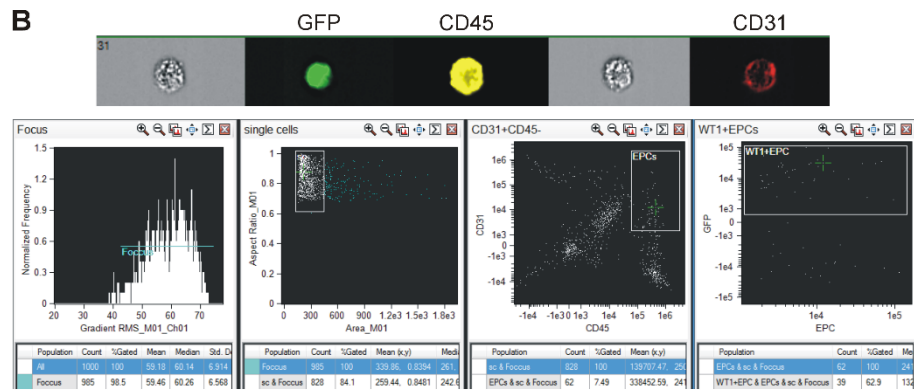
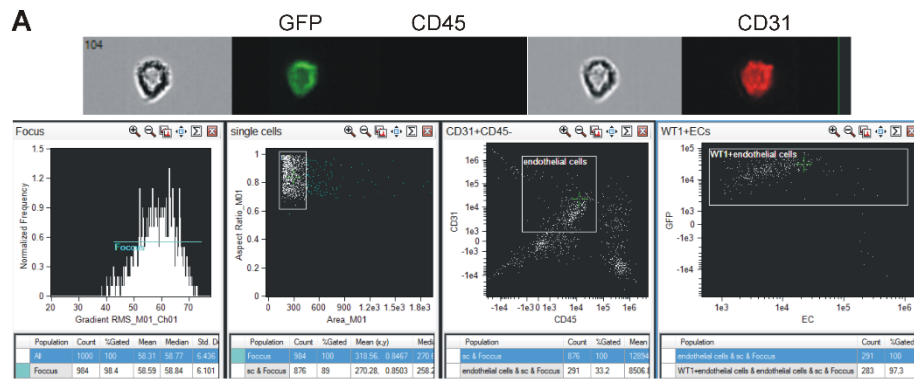


Figure S1: Representative images of ImageStream® multi-labelling cell analysis after myocardial infarction. (A) for GFP⁺CD45⁺CD31⁺ cells, (B) GFP⁺CD45⁺CD31⁺ cells, (C) GFP⁺CD45⁺Ly6-G (Gr-1)⁺CD11b⁺ cells, (D) GFP⁺CD45⁺CD117⁺ cells, and (E) GFP⁺CD45⁺Sca-1⁺ cells.

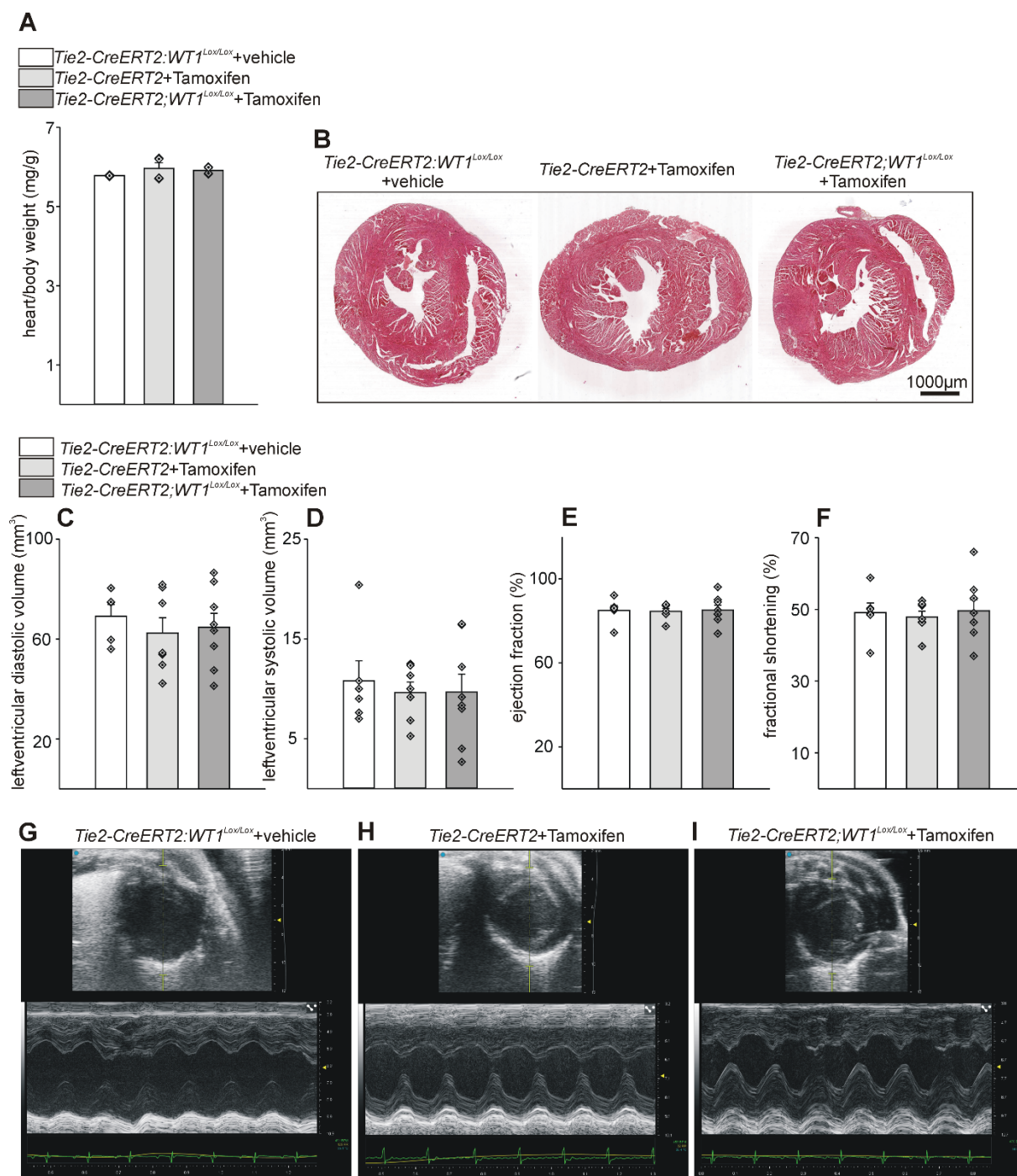


Figure S2: Conditional inducible knockout of WT1 using the Tie2-CreERT2 does not alter heart weights, sizes, or functional parameters. Respective heart-to-body weight ratios (A), and photomicrographs of HE-stained heart sections (B) of *Tie2-CreERT2;Wt1^{lox/lox}+Tamoxifen*, *n* = 4; *Tie2-CreERT2;Wt1^{lox/lox}+vehicle*, *n* = 4; *Tie2-CreERT2+Tamoxifen*, *n* = 4 animals without MI. Left-ventricular diastolic volume (C), left-ventricular systolic volume (D), ejection fraction (E), and fractional shortening (F) as echocardiographic parameters of *Tie2-CreERT2;Wt1^{lox/lox}+vehicle* (*n* = 6), *Tie2-CreERT2+Tamoxifen* (*n* = 7), and *Tie2-CreERT2;Wt1^{lox/lox}+Tamoxifen* (*n* = 8) animals without MI.

Representative echocardiographic images for (G) *Tie2-CreERT2;Wt1^{lox/lox}*+vehicle, (H) *Tie2-CreERT2*+Tamoxifen, and (I) *Tie2-CreERT2;Wt1^{lox/lox}*+Tamoxifen animals. Data are mean $\pm$ SEM.

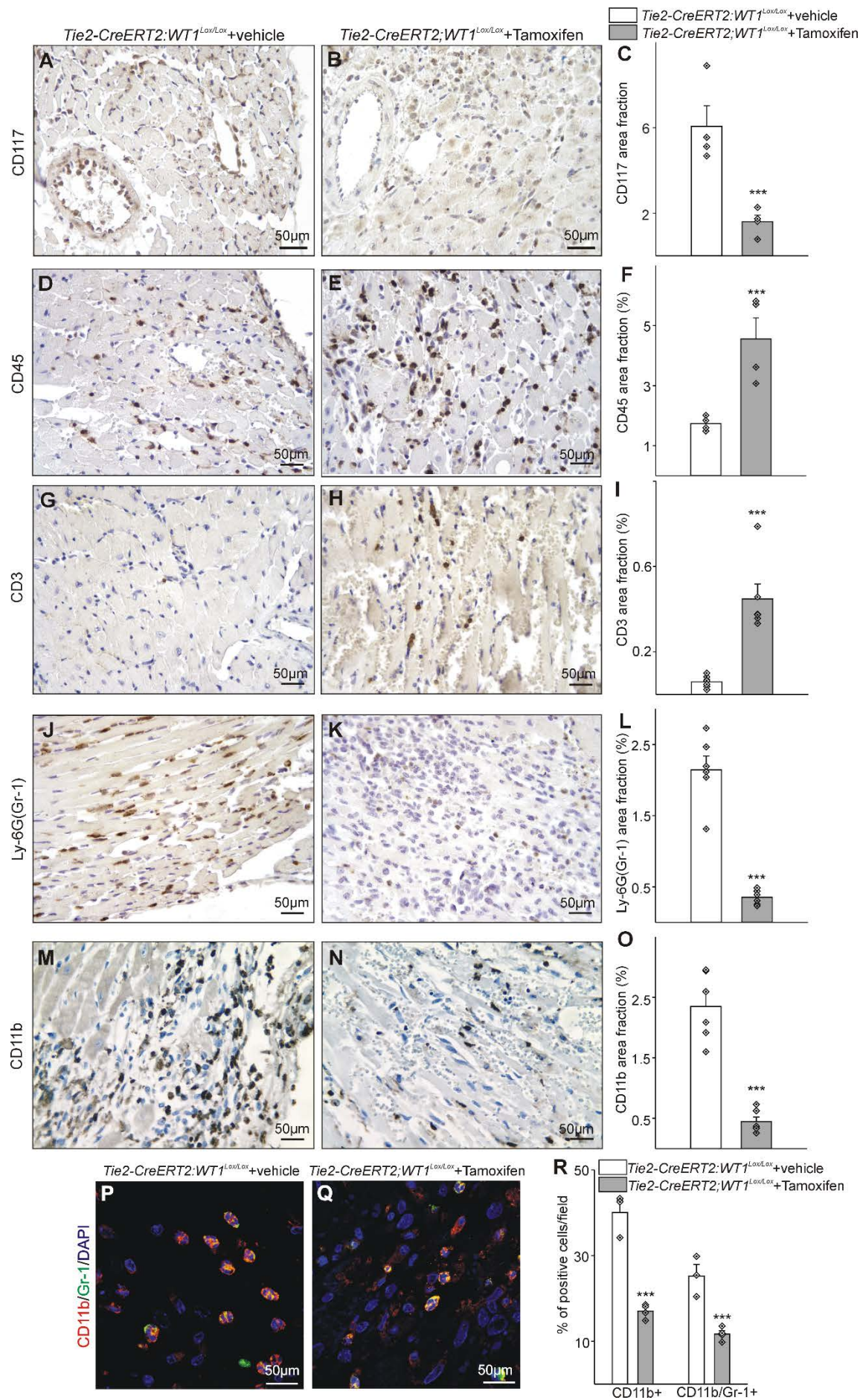


Figure S3: Less hematopoietic and immunosuppressive cell invasion and higher lymphocyte counts mark cardiac lesions of *Tie2-CreERT2;Wt1^{lox/lox}*+Tamoxifen animals in the acute phase after MI.

Photomicrographs of CD117 immunostained left ventricles of (A) *Tie2-CreERT2;Wt1^{lox/lox}*+vehicle and (B) *Tie2-CreERT2;Wt1^{lox/lox}*+Tamoxifen animals. (C) Quantification of CD117 signal area fractions of left ventricle sections from *Tie2-CreERT2;Wt1^{lox/lox}*+vehicle controls ($n = 4$) and *Tie2-CreERT2;Wt1^{lox/lox}*+Tamoxifen ($n = 6$) animals. Photomicrographs of CD45 immunostained left ventricles of (D) *Tie2-CreERT2;Wt1^{lox/lox}*+vehicle and (E) *Tie2-CreERT2;Wt1^{lox/lox}*+Tamoxifen animals. (F) Quantification of CD45 signal area fractions of left ventricle sections from *Tie2-CreERT2;Wt1^{lox/lox}*+vehicle controls ($n = 4$) and *Tie2-CreERT2;Wt1^{lox/lox}*+Tamoxifen ($n = 6$) animals. Photomicrographs of CD3 immunostained left ventricles of (G) *Tie2-CreERT2;Wt1^{lox/lox}*+vehicle and (H) *Tie2-CreERT2;Wt1^{lox/lox}*+Tamoxifen animals. (I) Quantification of CD3 signal area fractions of left ventricle sections from *Tie2-CreERT2;Wt1^{lox/lox}*+vehicle controls ($n = 4$) and *Tie2-CreERT2;Wt1^{lox/lox}*+Tamoxifen ($n = 6$) animals. Photomicrographs of Ly-6G(Gr-1) immunostained left ventricles of (J) *Tie2-CreERT2;Wt1^{lox/lox}*+vehicle and (K) *Tie2-CreERT2;Wt1^{lox/lox}*+Tamoxifen animals. (L) Quantification of Ly-6G(Gr-1) signal area fractions of left ventricle sections from *Tie2 - CreERT2;Wt1^{lox/lox}*+vehicle controls ($n = 4$) and *Tie2-CreERT2;Wt1^{lox/lox}*+Tamoxifen ($n = 6$) animals. Photomicrographs of CD11b immunostained left ventricles after acute MI of (M) *Tie2-CreERT2;Wt1^{lox/lox}*+vehicle and (N) *Tie2-CreERT2;Wt1^{lox/lox}*+Tamoxifen animals. (O) Quantification of CD11b signal area fractions of left ventricle sections from *Tie2-CreERT2;Wt1^{lox/lox}*+vehicle controls ($n = 4$) and *Tie2-CreERT2;Wt1^{lox/lox}*+Tamoxifen ($n = 6$) animals after acute MI. Representative confocal images of CD11b (red) and Ly-6G (Gr-1) (green) double-labelling in the left-ventricular infarct zone from (P) *Tie2-CreERT2;Wt1^{lox/lox}*+vehicle and (Q) *Tie2-CreERT2;Wt1^{lox/lox}*+Tamoxifen animals. DAPI (blue) served as counterstain. (R) Quantification of left-ventricular relative cell numbers positive for CD11b alone or double positive for CD11b and Ly-6G (Gr-1) of *Tie2-CreERT2;Wt1^{lox/lox}*+vehicle controls ($n=3$) and *Tie2-CreERT2;Wt1^{lox/lox}*+Tamoxifen ($n=4$) animals after acute MI. Data are mean $\pm$ SEM. *** $p < 0.001$.

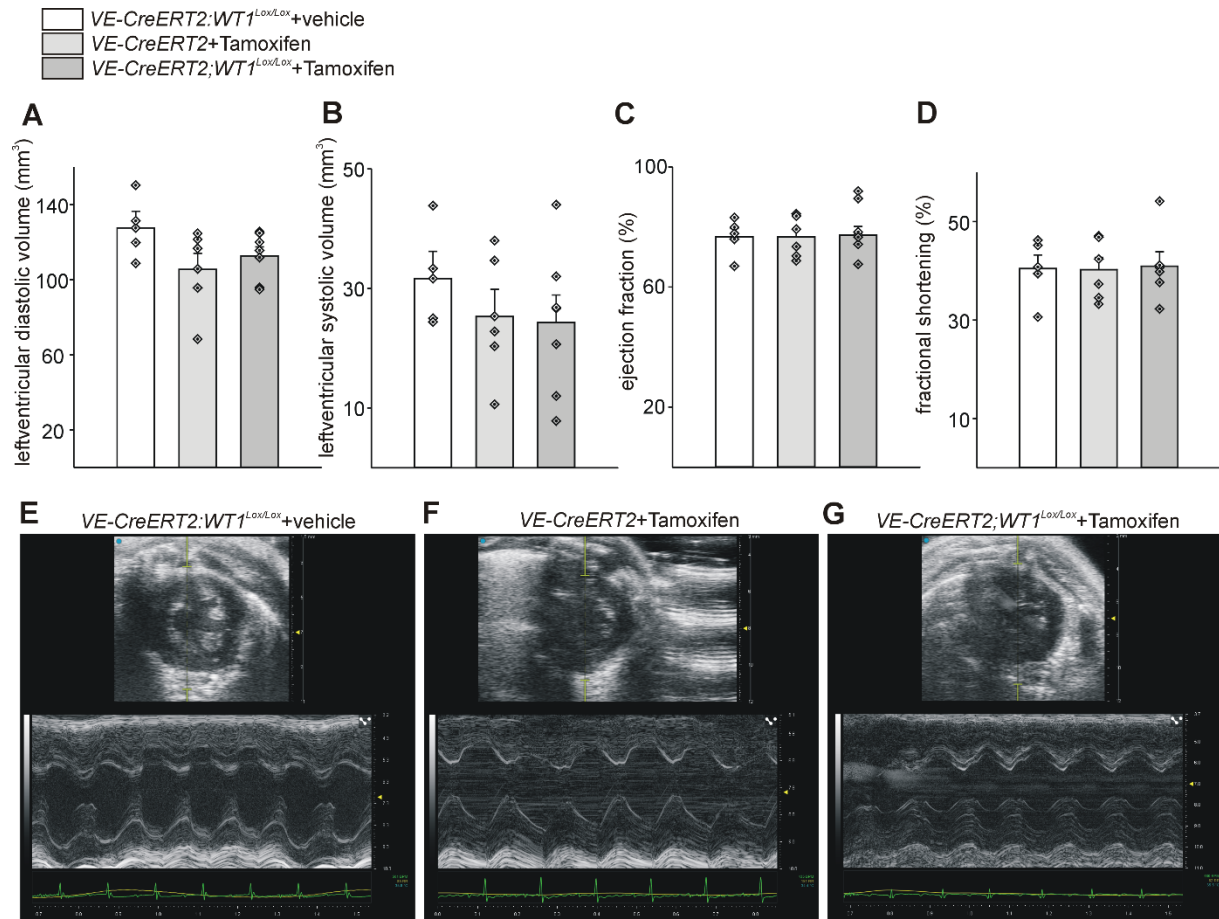


Figure S4: Baseline echocardiographic parameters of animals with restricted conditional inducible knockout of WT1 in endothelial cells without myocardial infarction. Left-ventricular diastolic volume (A), left-ventricular systolic volume (B), ejection fraction (C), and fractional shortening (D) as echocardiographic parameters of VE-CreERT2;Wt1^{lox/lox}+vehicle ($n = 5$); VE-CreERT2+Tamoxifen ($n = 6$), and VE-CreERT2;Wt1^{lox/lox}+Tamoxifen ($n = 6$) animals without MI. Representative echocardiographic images for VE-CreERT2;Wt1^{lox/lox}+vehicle (E), VE-CreERT2+Tamoxifen (F), and VE-CreERT2;Wt1^{lox/lox}+Tamoxifen (G) animals. Data are mean $\pm$ SEM.

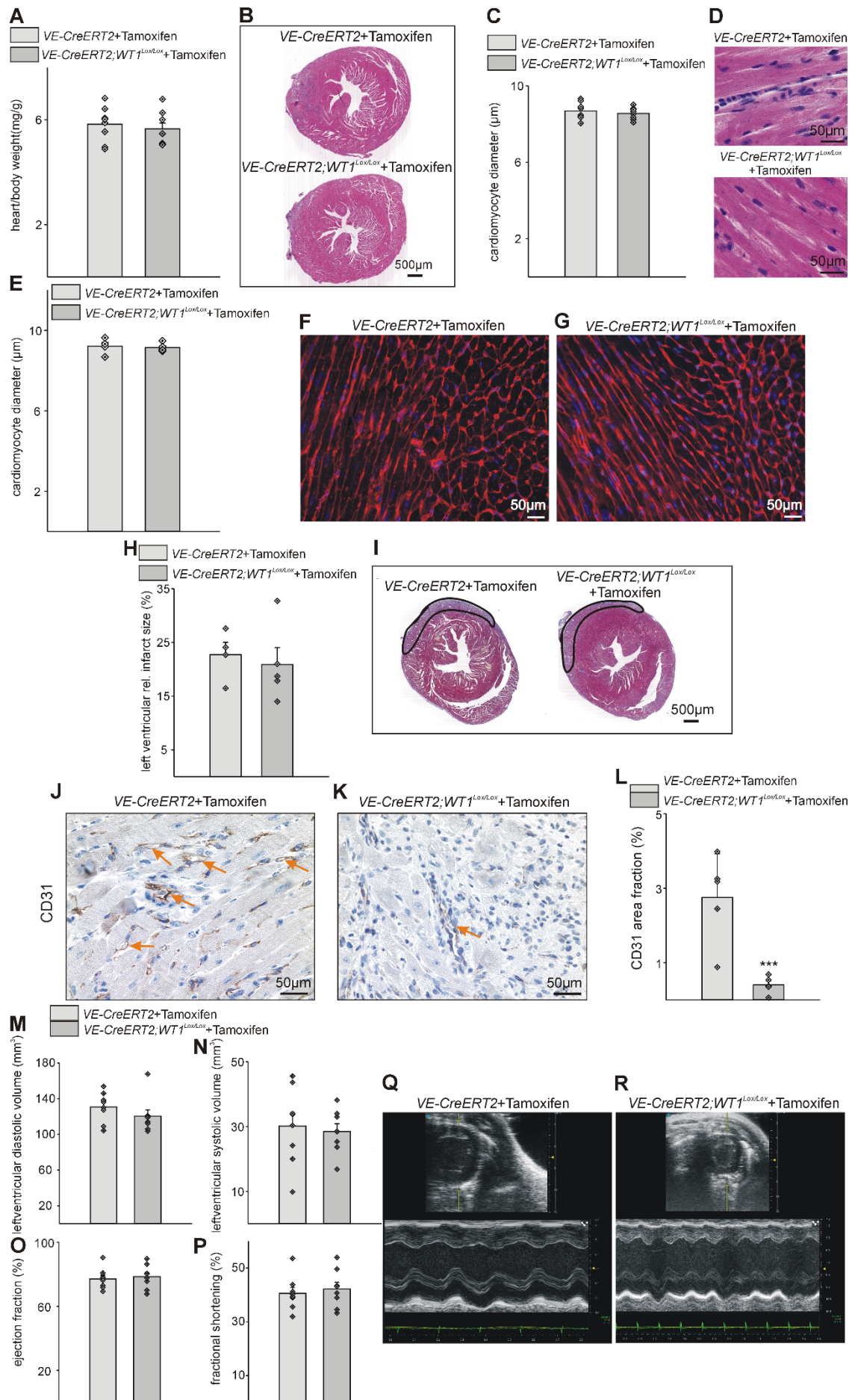


Figure S5: Restricted conditional inducible knockout of WT1 in endothelial cells diminishes vessel density but does not significantly influence morphological and functional cardiac parameters following MI. Respective heart-to-body weight ratios (A), photomicrographs of HE-stained heart sections (B), quantification of cardiomyocyte diameters based on measurements from HE-stained heart sections (C), and high power photomicrographs of HE-stained heart sections showing individual cardiomyocytes (D) from *VE-CreERT2*+Tamoxifen controls, ($n = 8$); and *VE-CreERT2;Wt1^{lox/lox}*+Tamoxifen, ($n = 8$) animals, 72 hours after MI. (E) Quantification of cardiomyocyte diameters based on measurements from WGA-stained heart sections, and high-power photomicrographs of WGA-stained heart sections showing individual cardiomyocytes (F) from *VE-CreERT2*+Tamoxifen controls ($n = 4$) and (G) *VE-CreERT2;Wt1^{lox/lox}*+Tamoxifen mice ($n = 5$). (H) Left ventricular infarct sizes of *VE-CreERT2*+Tamoxifen controls ($n = 4$) and *VE-CreERT2;Wt1^{lox/lox}*+Tamoxifen mice ($n = 5$). (I) Representative photomicrographs of Trichrome Masson-stained heart sections indicating the relative infarct size. CD31 immunostaining of heart sections from (J) *VE-CreERT2*+Tamoxifen controls ($n = 5$) and (K) *VE-CreERT2;Wt1^{lox/lox}*+Tamoxifen animals ($n = 5$) after MI. Orange arrows indicate some of the visible vessels in the respective photomicrographs. (L) Quantification of CD31 signal area fraction (*VE-CreERT2*+Tamoxifen controls ($n = 5$) and *VE-CreERT2;Wt1^{lox/lox}*+Tamoxifen animals ($n = 5$)). Left-ventricular diastolic volume (M), left-ventricular systolic volume (N), ejection fraction (O), and fractional shortening (P) as echocardiographic parameters of *VE-CreERT2;Wt1^{lox/lox}*+Tamoxifen, ($n = 8$); *VE-CreERT2;Wt1^{lox/lox}*+vehicle, ($n = 8$); *VE-CreERT2*+Tamoxifen, ($n = 8$) animals 72hrs after MI. Representative echocardiographic images for *VE-CreERT2*+Tamoxifen (Q), and *VE-CreERT2;Wt1^{lox/lox}*+Tamoxifen (R) animals after MI. Data are mean $\pm$ SEM. *** $p < 0.001$.

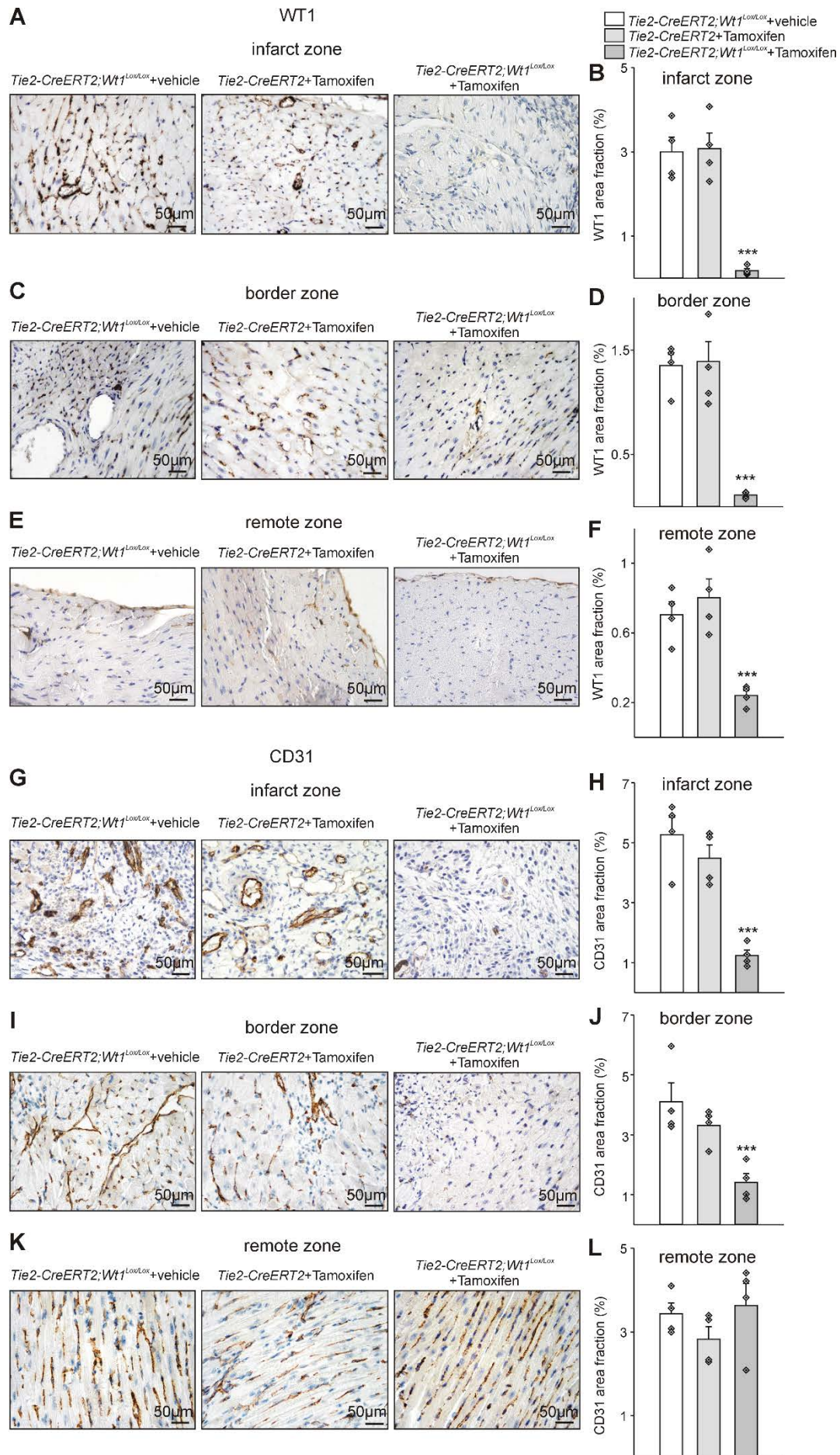


Figure S6: Tie2-CreERT2-mediated WT1 loss impairs cardiac revascularization in the repair process following MI. Photomicrographs of WT1 immunostained left ventricles 3 weeks after MI in the infarct, border, and remote zone (A,C,E) of *Tie2-CreERT2;Wt1^{lox/lox}*+vehicle, *Tie2-CreERT2* +Tamoxifen and of *Tie2-CreERT2;Wt1^{lox/lox}*+Tamoxifen animals. Quantification of WT1 signal area fractions in the infarct (B), border (D), and remote (F) zone of hearts from *Tie2-CreERT2;Wt1^{lox/lox}*+vehicle controls ($n = 4$), *Tie2-CreERT2*+Tamoxifen ($n = 4$), and *Tie2-CreERT2;Wt1^{lox/lox}*+Tamoxifen ($n = 4$) animals 3 weeks after MI. Photomicrographs of CD31 immunostained left ventricles 3 weeks after MI in the infarct, border, and remote zone (G, I, K) of *Tie2-CreERT2;Wt1^{lox/lox}*+vehicle, *Tie2-CreERT2* +Tamoxifen and of *Tie2-CreERT2;Wt1^{lox/lox}*+Tamoxifen animals. Quantification of CD31 signal area fractions in the infarct (H), border (J), and remote (L) zone of hearts from *Tie2-CreERT2;Wt1^{lox/lox}*+vehicle controls ($n = 4$), *Tie2-CreERT2*+Tamoxifen ($n = 4$), and *Tie2-CreERT2;Wt1^{lox/lox}*+Tamoxifen ($n = 4$) animals 3 weeks after MI. Data are mean $\pm$ SEM. *** $p < 0.001$.

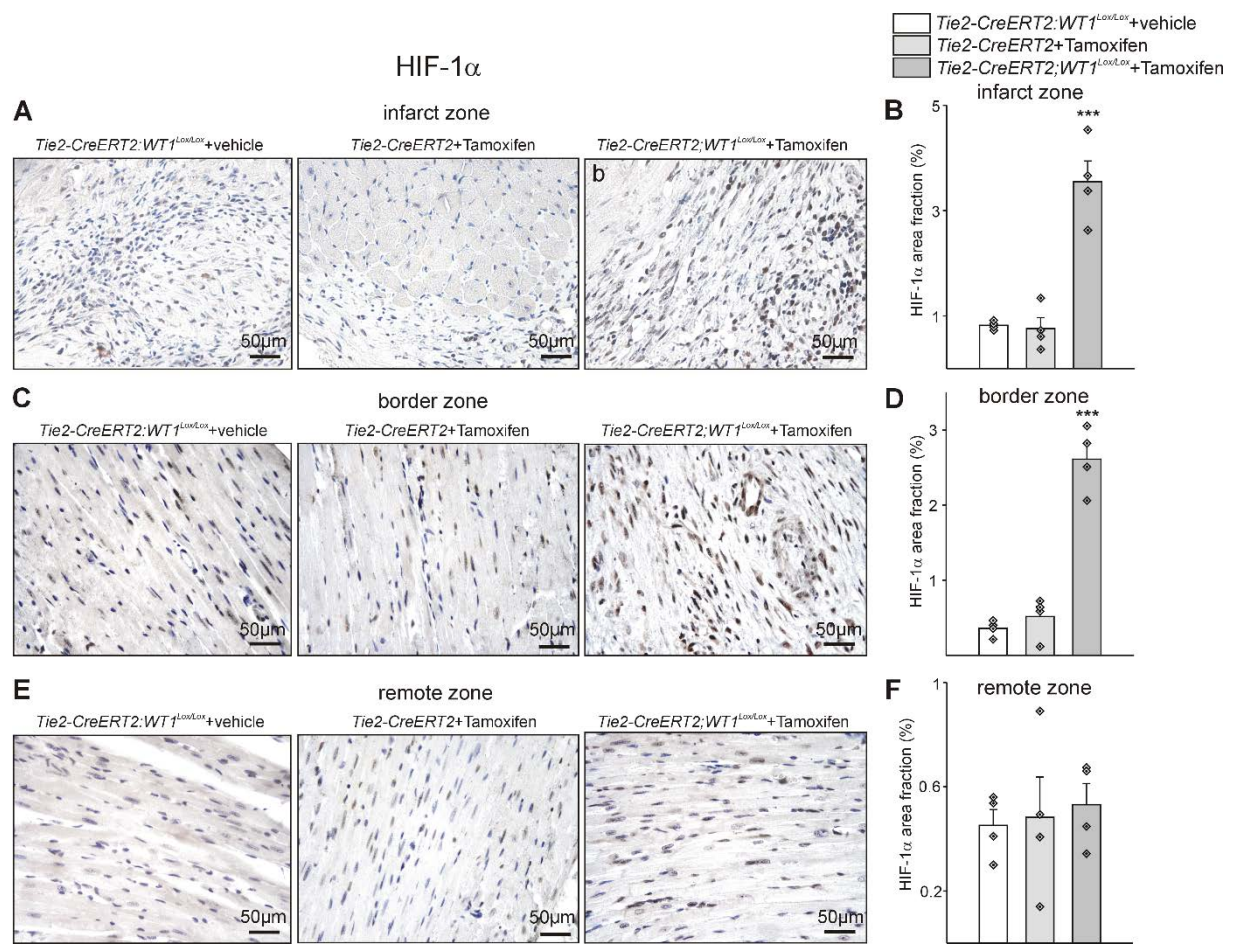


Figure S7: High levels of HIF-1 α protein correlate to diminished vascularization in cardiac repair due to loss of WT1. Photomicrographs of HIF-1 α immunostained left ventricles 3 weeks after MI in the infarct, border, and remote zone (A, C, E) of *Tie2-CreERT2;Wt1^{lox/lox}*+vehicle, *Tie2-CreERT2*+Tamoxifen and of *Tie2-CreERT2;Wt1^{lox/lox}*+Tamoxifen animals. Quantification of HIF-1 α signal area fractions in the infarct (B), border (D) and remote (F) zone of hearts from *Tie2-CreERT2;Wt1^{lox/lox}*+vehicle controls ($n = 4$), *Tie2-CreERT2*+Tamoxifen ($n = 4$), and *Tie2-CreERT2;Wt1^{lox/lox}*+Tamoxifen ($n = 4$) animals 3 weeks after MI. Data are mean $\pm$ SEM. *** $p < 0.001$.

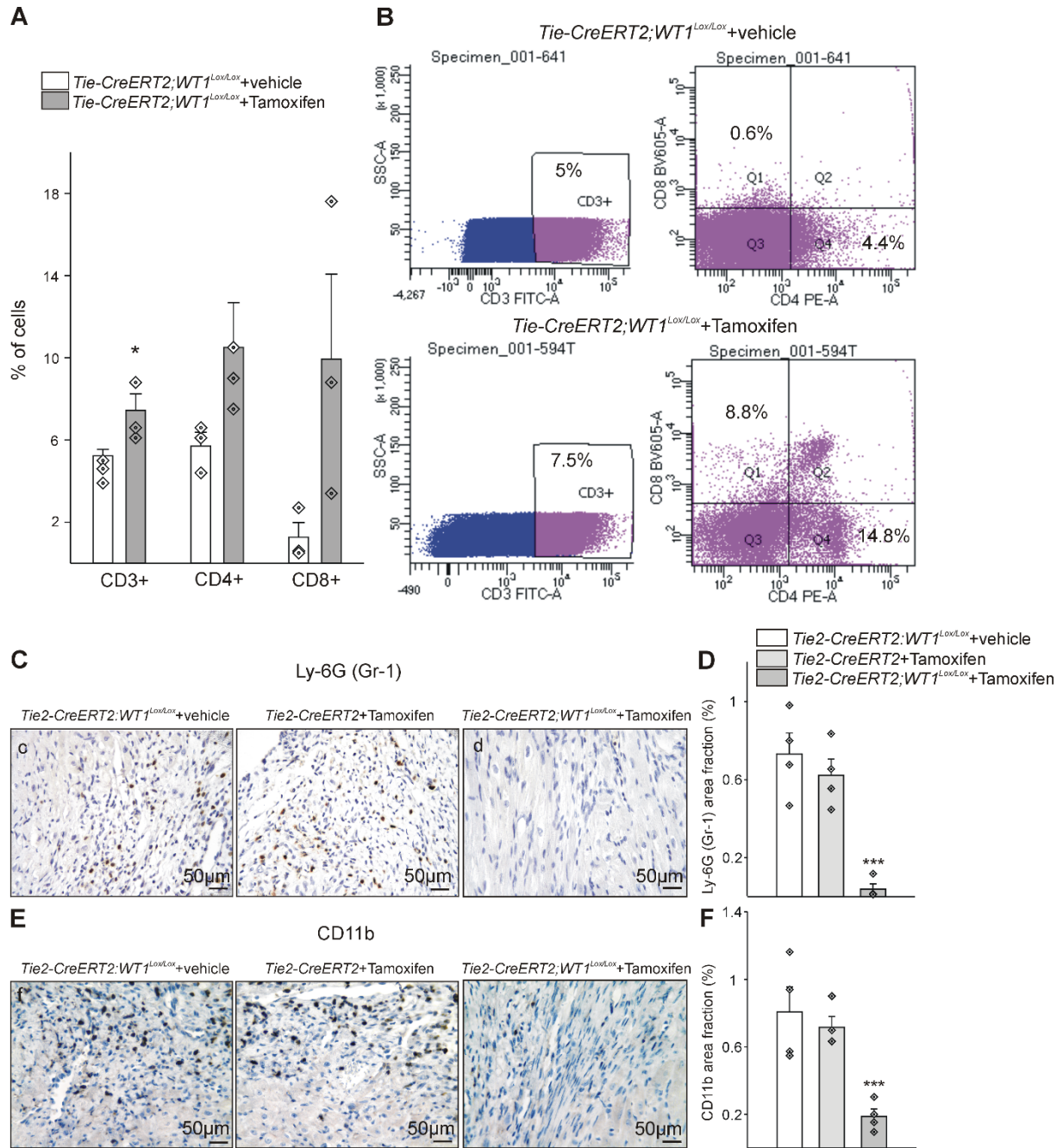


Figure S8: Higher lymphocyte counts and decreased numbers of immunosuppressive cells in *Tie2-CreERT2;Wt1^{lox/lox}*+Tamoxifen hearts after MI. (A) Quantification of FACS analysis of the fraction of CD3⁺, CD3⁺CD4⁺, and CD3⁺CD8⁺ lymphocytes from hearts of *Tie2-CreERT2;Wt1^{lox/lox}*+vehicle controls ($n=3$) and *Tie2-CreERT2;Wt1^{lox/lox}*+Tamoxifen ($n = 3$) animals 3 weeks after MI. Representative flow cytometry data (B) of a *Tie2-CreERT2;Wt1^{lox/lox}*+vehicle control heart (upper panel) and a *Tie2-CreERT2;Wt1^{lox/lox}*+Tamoxifen heart (lower panel) for lymphocyte quantification. Photomicrographs of Ly-6G (Gr-1) immunostained left ventricles 3 weeks after MI (C) of *Tie2-CreERT2;Wt1^{lox/lox}*+vehicle, *Tie2-CreERT2*+Tamoxifen, and *Tie2-CreERT2;Wt1^{lox/lox}*+Tamoxifen animals. (D) Quantification of Ly-6G(Gr-1) signal area fractions of left ventricle sections from *Tie2-CreERT2;Wt1^{lox/lox}*+vehicle controls ($n = 4$), *Tie2-CreERT2*+Tamoxifen ($n = 4$), and *Tie2-CreERT2;Wt1^{lox/lox}*+Tamoxifen ($n = 4$) animals. Photomicrographs of CD11b immunostained left ventricles 3 weeks after MI (E) of *Tie2-*

CreERT2;Wt1^{lox/lox}+vehicle, *Tie2-CreERT2* +Tamoxifen, and *Tie2-CreERT2;Wt1^{lox/lox}*+Tamoxifen animals.

(F) Quantification of CD11b signal area fractions of left ventricle sections from *Tie2 - CreERT2;Wt1^{lox/lox}*+vehicle controls ($n = 4$), *Tie2-CreERT2*+Tamoxifen ($n = 4$), and *Tie2-CreERT2;Wt1^{lox/lox}*+Tamoxifen ($n = 4$) animals 3 weeks after MI. Data are mean $\pm$ SEM. * $p < 0.05$; *** $p < 0.001$.

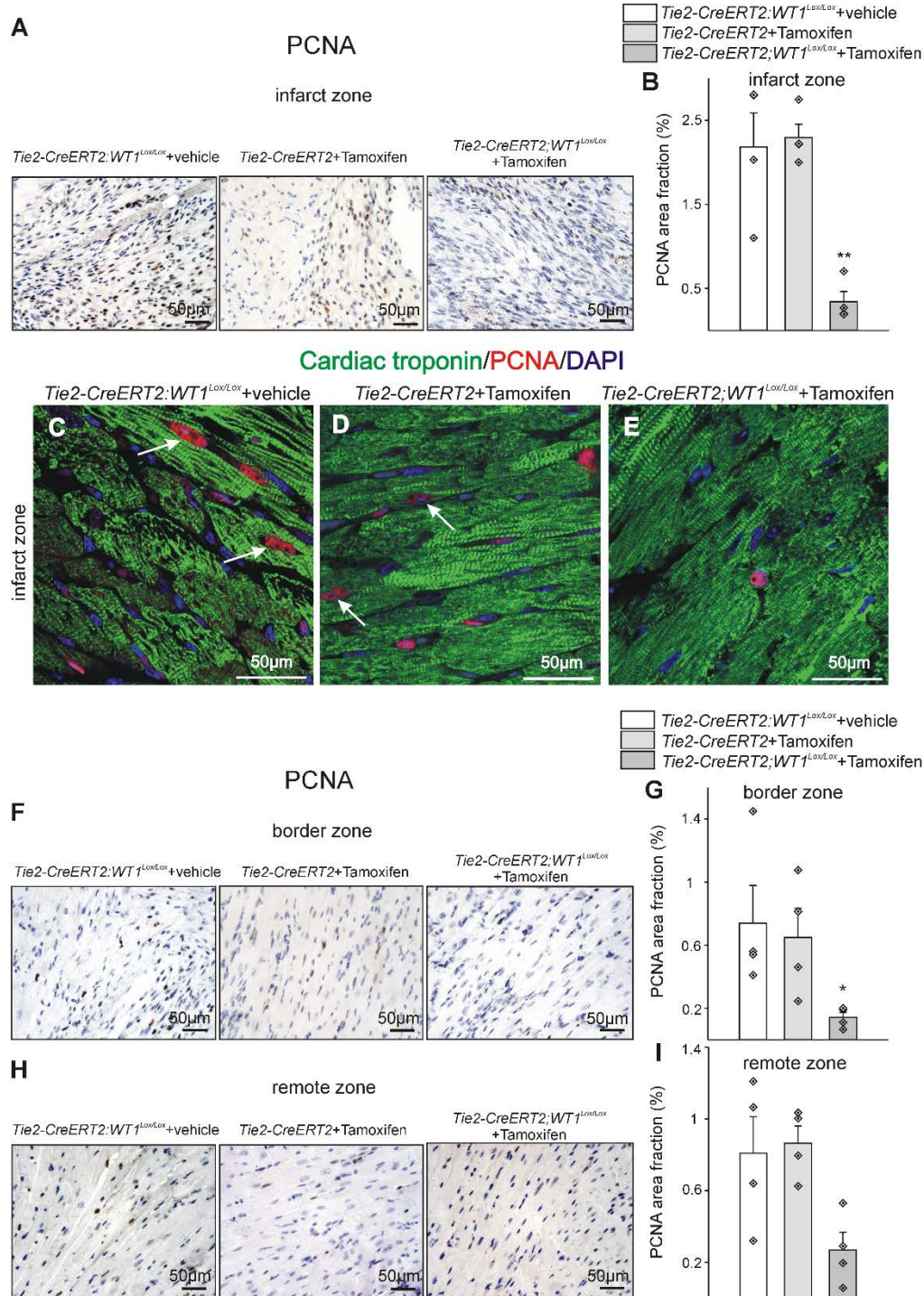


Figure S9: Diminished proliferation in *Tie2-CreERT2;Wt1^{lox/lox}*+Tamoxifen hearts after MI. Photomicrographs of PCNA immunostained left ventricles 3 weeks after MI in the infarct, border, and remote zone (A, F, H) of *Tie2-CreERT2;Wt1^{lox/lox}*+vehicle, *Tie2-CreERT2* +Tamoxifen, and *Tie2-CreERT2;Wt1^{lox/lox}*+Tamoxifen animals. Quantification of PCNA signal area fractions in the infarct (B), border (G), and remote (I) zone of hearts from *Tie2-CreERT2;Wt1^{lox/lox}*+vehicle controls ($n = 4$), *Tie2-CreERT2*+Tamoxifen ($n = 4$), and *Tie2-CreERT2;Wt1^{lox/lox}*+Tamoxifen ($n = 4$) animals 3 weeks after MI. Representative confocal images of cardiac troponin (green) and PCNA (red) double-labelling of the infarct zone from heart sections from *Tie2-CreERT2;Wt1^{lox/lox}*+vehicle (C), *Tie2-CreERT2*+Tamoxifen (D), and *Tie2-CreERT2;Wt1^{lox/lox}*+Tamoxifen (E) animals. DAPI (blue) served as nuclear counterstain. White arrows indicate PCNA positive cardiomyocytes. Data are mean $\pm$ SEM. *** $p < 0.001$.

