SUPPLEMENTARY METHODS

Materials

Gleevec was from Novartis. Rabbit c-kit (C-19), CD31 (H-300), SCF (H-189), GATA-4 (C-20), myosin heavy chain (Y-20), PCNA (FL-261), VEGF (147) antibodies and donkey and goat serum were from Santa Cruz Biotechnology Inc. Rat phycoerythrin (PE) c-kit (2B8), fluororisothiocyante (FITC) CD45 (30-F11), CD31, Sca-1 and isotype control antibodies were from Becton Dickinson. Rat anti-mouse Ki-67 (TEC-3) and rabbit anti-human vWF/VIII (A0082) were from DAKO. Alexa 647 donkey, Alexa 633 goat, and Alexa 488 goat anti-rabbit, 546 goat anti-rat, or 488 chicken anti-goat, and rabbit anti-GFP antibodies, and 488 phalloidin were from Molecular Probes. Biotinylated 2^{o} antibodies, straptavidin-horseradish peroxidase, Rhodamine conjugated avidin D, unconjugated avidin, and NovaRED reagents were from Vector labs. Rabbit angiopoietin-1 antibody was from US Biologicals. Rabbit angiopoietin-2 and SCF antibodies were from Cedarlane. ECL Western blot detection kit was from Amersham Biosciences. Lympholyte-M was from Cedarlane. Recombinant murine SCF was from R&D Systems. VEGF enzyme linked immunosorbent assay (ELISA) kits was from R&D System. iSCRIPT reverse transcriptase was from BIO RAD and *Taq* DNA polymerase was from Fermentas Life Sciences. The VEGF, Actin (20-33), and Cy3 conjugated mouse anti-human smooth muscle actin antibodies, Genomic DNA kit, and all other chemicals were from SIGMA.

Determination of the degree of bone marrow chimerism

In $Kit^{+/+} \rightarrow Kit^{W}/Kit^{W\text{-}v}$ group, reconstitution was assessed by polymerase chain reaction performed on genomic DNA prepared from bone marrow cells for the sex determining region "Y-chromosome" gene in a subset of animals in which the donor was male and the recipient was female. Greater than 70% reconstitution was obtained in every case. In C57Bl/6 mice reconstituted with GFP transgenic bone marrow cells, flow cytometry on blood mononuclear and bone marrow cells to assess degree of GFP chimerism was performed.

Determination of the extent of acute cardiac injury

To determine myocardial volume excluded from the circulation by coronary ligation (area at risk of infarction), 1% Evans Blue was retrogradely administered into the aortic root after coronary ligation until the perfused right ventricle became completely blue in color. The hearts were transversely cut into 1 mm thick sections. The myocardial rings were then incubated in 1% triphenyltetrazolium chloride (TTC) at 37^{o}C for 30 minutes. With this technique, perfused myocardium is visualized blue, viable myocardium is visualized red, and necrotic myocardium is visualized yellow in color. The areas were quantified by computerized planimetry using Scion Image software (NIH).

Pressure volume loop analysis

After the introduction of the pressure volume catheter, left ventricular pressures and volumes, maximal slope of systolic pressure increment (dp/dt max) and diastolic decrement (dp/dt min), ejection fraction, and the relaxation time constant (tau), an index of diastolic function (calculated by the Weiss method) were computed using the PVAN

software (Millar Instruments). Because some of the above measurements are influenced by cardiac preload, hemodynamic parameters were also determined under conditions of decreasing preload, elicited by transiently occluding the inferior vena cava just above the diaphragm. These measurements included preload-recruitable stroke work, which represents the slope of the relation between stroke work (area within the pressure volume loop) and end-diastolic volume and is independent of chamber size and mass, and the end-systolic PV relation (end-systolic elastance). The slope of the end-diastolic PV relation, an index of left ventricular stiffness, was also calculated using PVAN software.

The volume calibration of this system was performed to allow conversion of relative volume units to real volumes. Briefly, seven cylindrical holes in a block 1 cm deep and with known diameter ranging from 1.4 to 5 mm were filled with fresh heparinized whole murine blood. An inter-electrode distance of 4.5 mm was used to calculate the absolute volume in each cylinder. In this calibration, the linear regression between the absolute volumes in each cylinder versus the raw signal acquired by the conductance catheter was used as the volume calibration formula. At the end of each experiment, 10 uL of 15% saline was injected intravenously. From the shift of PV relation, parallel conductance volume was calculated by PVAN software and used for correction for the cardiac mass volume.

Echocardiography

Left ventricular end-diastolic and end-systolic dimensions were determined in M-mode imaging. Fractional shortening was calculated as [(end-diastolic dimension – end-systolic dimension)/ends-diastolic dimension] $\times 100$. In 2D imaging, the left ventricular end-

diastolic area was determined as the largest and the left ventricular end-systolic area as the smallest cavity size. Fractional area contraction was used to evaluate the systolic function and was calculated as [(end-diastolic area-end-systolic area)/end-diastolic area]×100.

Tissue preparations

Bone marrow was flushed from the long bones using 0.2 mL of phosphate-buffered saline, and disaggregated by gentle aspiration. Bone marrow cells were prepared by centrifugation at 1000 g. Peripheral blood was aspirated from the heart into a heparinized syringe. Spleens, lungs, livers, and kidneys were collected 7 days after coronary ligation and stored on ice until use. Hearts were collected at 0, 1, 3, and 7 days post-MI, and cut into the injured and non-injured segments prior to digestion using 0.2% collagenase at 37^{o}C for 30 minutes. Spleen, kidney, lungs, and liver tissue were minced and expressed through a 70 μm wire mesh. The cells were then resuspended in phosphate-buffered saline. Splenocytes and blood cells were loaded on top of a column of Lympholyte-M solution and spun at 3500 rpm for 30 minutes to allow separation of mononuclear cells on top of the column. After washing, the cells were stained for flow cytometry. For protein and RNA extraction, tissues were obtained, submerged immediately in liquid nitrogen, and stored in -80^{o}C freezer until use.

Functional in vitro progenitor cell assays

Technique of EPC quantification from mice has been previously described (49). Briefly, spleens were removed on days 0 and 1 after MI, expressed through a 70 μm wire mesh,

and centrifuged across Lympholyte-M. 1.0×10^6 splenic mononuclear cells were plated in duplicates on fibronectin-coated plates in EGM medium. After 48 hours in culture, non-adherent cells were washed and adherent cells were incubated with 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine-labeled acetylated low density lipoprotein (LDL) for 4 hours. After washing with PBS (3x), cells were fixed with 4% paraformaldehyde for 10 mins at room temperature and incubated with fluoroisothiocyanate conjugated *Ulex europaeus* lectin for 1 hr in the dark at room temperature. The cells were then washed with PBS (3x) and taken to microscopy. EPC were quantified as adherent cells that up took acetylated LDL and stained with lectin. Total number of EPC was counted in 5 random fields under 400× magnification in a blinded fashion by 2 investigators.

We also confirmed absent hematopoietic progenitor cell mobilization in response to MI by semi-solid methylcellulose CFU assays. Briefly, on days 0 and 1 $1x10^6$ blood mononuclear cells harvested by spinning through Lympholyte-M solution were then plated in duplicates in 1mL Methocult medium (Stem Cell Technologies) in small plates as per the manufacturer's instructions without the addition of antibiotics and antifungal agents. Cells were incubated in humidified 37^oC incubators with 5% CO_2 and supplemental O_2. After 7-10 days, number of colonies with >50 cells per colony in each plate was counted in a blinded fashion by 2 investigators using an Olympus phase-contrast microscope.

Immunohistochemistry

Sections were treated with 3% hydrogen peroxide to block endogenous peroxidase activity, blocked with normal serum from animals in which the secondary antibody was

raised, incubated with primary antibodies followed by biotinylated secondary antibodies, labeled with streptdavidin-horseradish peroxidase, developed with NovaRED, and counterstained with Mayer's haematoxylin when indicated. TUNEL staining was performed as described (50). For mast cell identification, a 1% Toludine Blue stock solution in 70% alcohol was diluted in (1:5) in a 1% sodium chloride solution. Tissue sections were hydrated in distilled water, stained for 90s, rinsed three times in distilled water, and dehydrated. Slides were cover-slipped in a non-aqueous mounting medium. Hematoxylin and Eosin and Masson trichrome stains were performed as per routine protocol.

Laser scanning confocal microscopy

Frozen OCT-embedded (Sakura-Finetek Inc., Japan) blocks were cut into 10 μm sections, fixed with -20^{o}C acetone, blocked with 5% normal serum, incubated with 1^{o} antibodies overnight, and detected with conjugated 2^{o} antibodies. Nuclear counter-stain was performed with 50 μg/mL propidium iodide solution containing RNase A (100 U/mL). For evaluation of the collagen matrix, picrosirius red staining was used. Briefly, paraffin-embedded sections were treated with Xylene for 6 minutes, followed by incubation in graded ethanol solutions to allow rehydration. After washing with copious amounts of water, the slides were stained with filtered 0.1% Sirius red and 1% Fast Green solution. The slides were then dehydrated in graded ethanol solutions, washed with Xyline, and mounted. For the visualization of GFP^{+} cells, hearts were excised and immersion fixed in 2% parafomaldehyde for 24 hours at 4°C. They were then cryoprotected through incubation in 10%, 20%, and 30% sucrose in PBS. Hearts were

then cut in half, embedded in OCT and frozen with liquid nitrogen. From these samples 5-8μm sections were cut and stored at –80^{o}C until used. Sections were then post fixed in acetone at –20^{o}C for 10 minutes. Sections were blocked using the appropriate sera to make a blocking solution containing 5% of the appropriate sera and 2% BSA. Sections were incubated with primary antibodies overnight at 4C or for 1 hour at RT. Sections were then incubated with the appropriate secondary antibodies. Nuclei were counterstained with Hoechst 33342 or propidium iodide. In sections were avidin staining was employed, an additional incubation of rhodamine cnjugated avidin in PBS (1:100) was applied to the tissue sections. Unstained controls and non-conjugated avidin was used to set scanning levels. Slides were examined using an MRC 1024 (BIO RAD), LSM 510 META (Zeiss), or a FluoView1000 (Olympus) confocal microscope. Negative controls where the primary antibodies were omitted were used to set the laser power and detector gains prior to scanning.

Immunoblotting and reverse transcriptase polymerase chain reaction

Hearts were pulverized in liquid nitrogen prior to the addition of the cell lysis buffer (protein) or TriZol reagent (RNA). Cell lysis buffer contained 4M urea, 0.15M NaCl, 5mM EDTA, 1% Triton X100, 10 mM Tris-Cl (pH 7.4), 5mM dithiothreitol, 0.1 mM phenylmethylsolfonyl fluoride, 5mM ε-aminocaproic acid, 10 ug/mL aprotinin, 10 ug/mL leupeptin, and 1 ug/mL pepstatin. Proteins from cell supernatants were concentrated using the Centricon-30 system. Protein quantification was performed with DC protein assay from BIO RAD using bovine serum albumin as standard. Protein samples (100-300 ug) were subjected to SDS-PAGE on separating gel containing 10-15% acrylamide. The

buffer system was 1× Tris/glycine buffer containing 1% SDS. Separated proteins were transblotted onto a nitrocellulose membrane in 1× Tris/glycine buffer containing 20% methanol at 60 V for 2 hours in a cold room. The membrane was blocked in TBST (10 mM Tris-Cl (pH 8.0), 150 mM NaCl, and 0.05% Tween 20) containing 10% nonfat dry milk powder (TBSTM) for 30 minutes at room temperature to saturate nonspecific binding sites on the membrane. The membrane was then incubated overnight at 4°C with primary antibodies. The membranes were washed with TBST (3 × 15 minute washes) and then incubated for 2 hours with horseradish peroxidase-conjugated goat anti-Rabbit IgG antibodies (1:500 dilution) in TBSTM. After washing as described above, the bound antibodies were visualized by chemiluminescence (ECL kit). Total RNA was extracted from bone marrow cells by TriZol reagent as per manufacturer's instructions. RNA quantity and quality was determined by spectrophotometry at A260 and A280. One μg of RNA was used for the reverse transcription as per manufacturer's instruction in a total volume of 25 μL. One μL of final cDNA preparation was used for the PCR reaction. PCR parameters were set to ensure final amplification was within the linear range. Primer sequences are available upon request. The products were then size fractionated using 1-3% agarose gels.

Supplementary Table 1. Invasive hemodynamic measurements

Invasive hemodynamics	Baseline		Day 14 after MI	
	***Kit*$^{+/+}$**	***Kit*^{W}/*Kit*$^{W\text{-}v}$**	***Kit*$^{+/+}$**	***Kit*^{W}/*Kit*$^{W\text{-}v}$**
Heart rate (bpm)	454+/-6	443+/-15	528+/-12[a]	404+/-6[c]
End-systolic Volume (uL)	7.2+/-0.9	10.6+/-0.4	19.1+/-1.0[a]	30.5+/-0.7[b,c]
End-diastolic Volume (uL)	18.2+/-0.7	21.5+/-1.3	25.6+/-1.0[a]	37.6+/-0.9[b,c]
End-systolic Pressure (mmHg)	93.8+/-3.1	89.8+/-1.3	91.2+/-2	78.2+/-0.5[b,c]
End-diastolic Pressure (mmHg)	8.9+/-0.7	11.6+/-0.6	15.7+/-0.9[a]	19.6+/-1.0[b]
Ejection Fraction (%)	66.6+/-3.9	54.1+/-1.2	31.3+/-1.3[a]	22.4+/-0.9[b,c]
dPdt max (mmHg/sec)	8421+/-150	7473+/-144	6339+/-139[a]	4272+/-46[b,c]
dPdt min (- mmHg/sec)	6855+-228	5660+/-212	5110/-300[a]	3268+/-61[b,c]
Tau (msec)	7.4+/-0.4	9.2+/-0.4	11.1+/-0.3[a]	14.5+/-0.4[b,c]

[a], $P<0.05$ versus *Kit*$^{+/+}$ Baseline; [b], $P<0.05$ versus *Kit*^{W}/*Kit*$^{W\text{-}v}$ Baseline; [c], $P<0.05$ versus *Kit*$^{+/+}$ Day 14.

Supplementary Table 2. Histomorphometric measurements

Histomorphometrics	Day 42 after MI	
	***Kit*$^{+/+}$**	***Kit*^{W}/*Kit*$^{W\text{-}v}$**
Mouse weight (g)	23.5+/-0.6	24.4+/-1.0
Heart weight (mg)	150.6+/-5.6	183+/-6.8*
Heart/mouse weight (mg/g)	6.4+/-0.4	7.6+/-0.3*
Left ventricular diameter (mm)	4.6+/-0.4	6.4+/-0.2*
Left ventricular volume (mm^3)	49.2+/-8.1	87.9+/-8.0*
Spared myocardium (mm^3)	103.4+/-3.3	107.5+/-5.1
Scar area (mm^2)	8.9+/-3.2	25.2+/-4.9*
Thickness of septum (mm)	0.46+/-0.05	0.45+/-0.03
Cardiomyocyte diameter (AU)	1.5+/-0.05	1.9+/-0.2*
Cardiomyocyte nuclei/hpf (#)	8.8+/-0.3	6.0+/-0.5*
Total collagen (% area)	1.7+/-1.8	1.6+/-0.3
Collagen fibril diameter (AU)	8.0+/-1.4	7.0+/-2.3
Collagen fibril length (AU)	239.0+/-71.1	145.5+/-37.6*

*, $P<0.05$ versus *Kit*$^{+/+}$

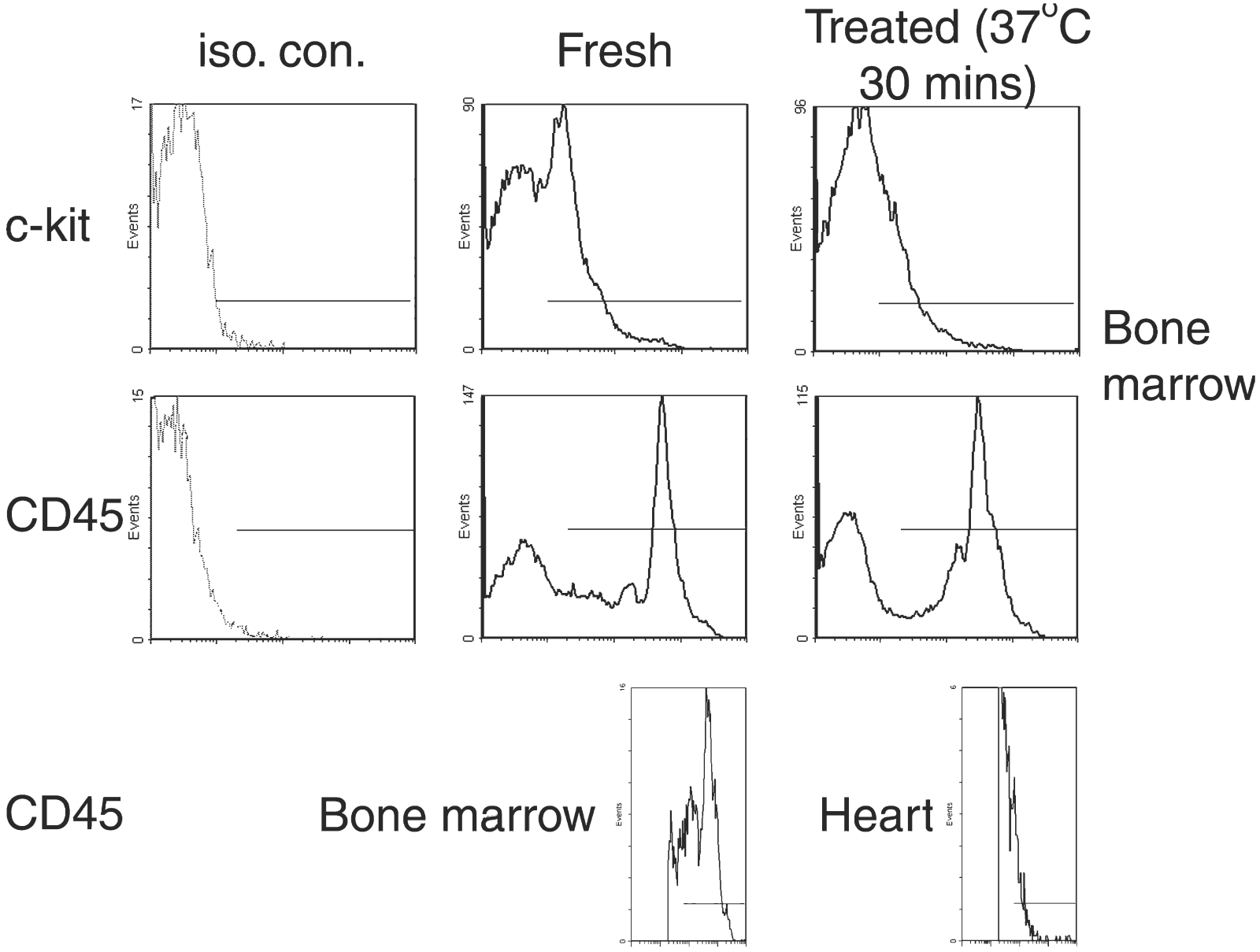
Supplementary Figure 1. CD45 expression is lower on bone marrow cells that traffic to the infarcted heart. Impact of collagenase digestion for 30 minutes at 37^{o}C on c-kit (*upper* panel) and CD45 (*middle* panel) on bone marrow cells. Treatment reduces cell surface expression of c-kit but not of CD45. CD45 expression is reduced on c-kit^{+} cells in collagenase digested heart tissue (*lower* panel).

Supplementary Figure 2. Mast cell identification. (**A**) The acidic mast cell granules cause metachromasia when incubated with toluidine blue. A mast cell is visualized by the purplish granular appearance within the cell cytoplasm. (**B**) The heprin-rich granules of mast cells bind rhodamine-conjugated avidin. A mast cell is visualized by the red cytoplasmic stain.

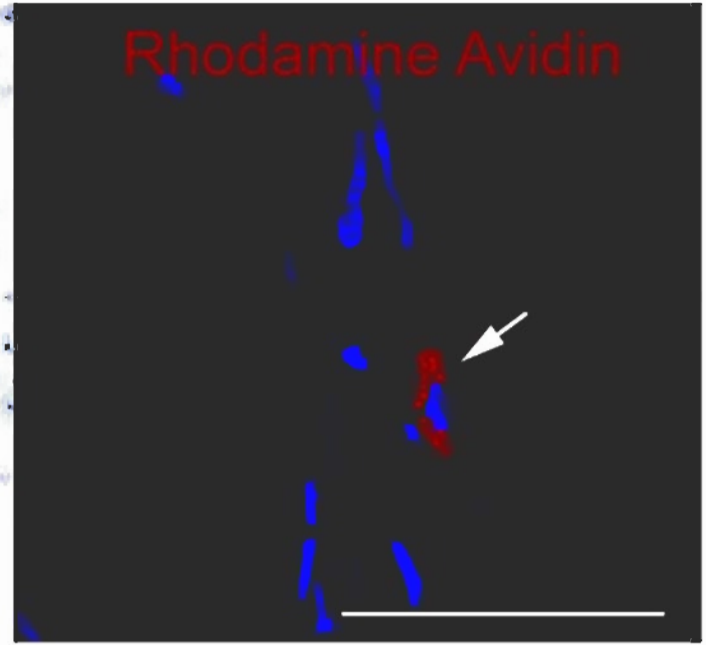
Supplementary Figure 3. Non-ischemic wound healing in the ear is not affected by the c-kit mutation. Contraction of a circular ear wound over 21 days is similar in C57Bl/6, $Kit^{+/+}$, and $Kit^{W}/Kit^{W\text{-}v}$ mice (n=5 per group). AU, arbitrary units; *, P<0.05.

Supplementary Figure 4. Pharmacological inhibition of c-kit phosphorylation impairs cardiac repair after myocardial infarction. (**A**) Gleevec inhibits c-kit phosphorylation induced by SCF in a dose-dependant fashion in fresh bone marrow cultures. (**B**) Administration of Gleevec by oral gavage to mice also inhibits c-kit phosphorylation. (**C**) Invasive hemodynamic measures showing that treatment of mice with Gleevec after coronary ligation (MI +Gleevec) results in lower ejection fraction, higher ventricular volumes, and lower dP/dt max compared to mice only having coronary

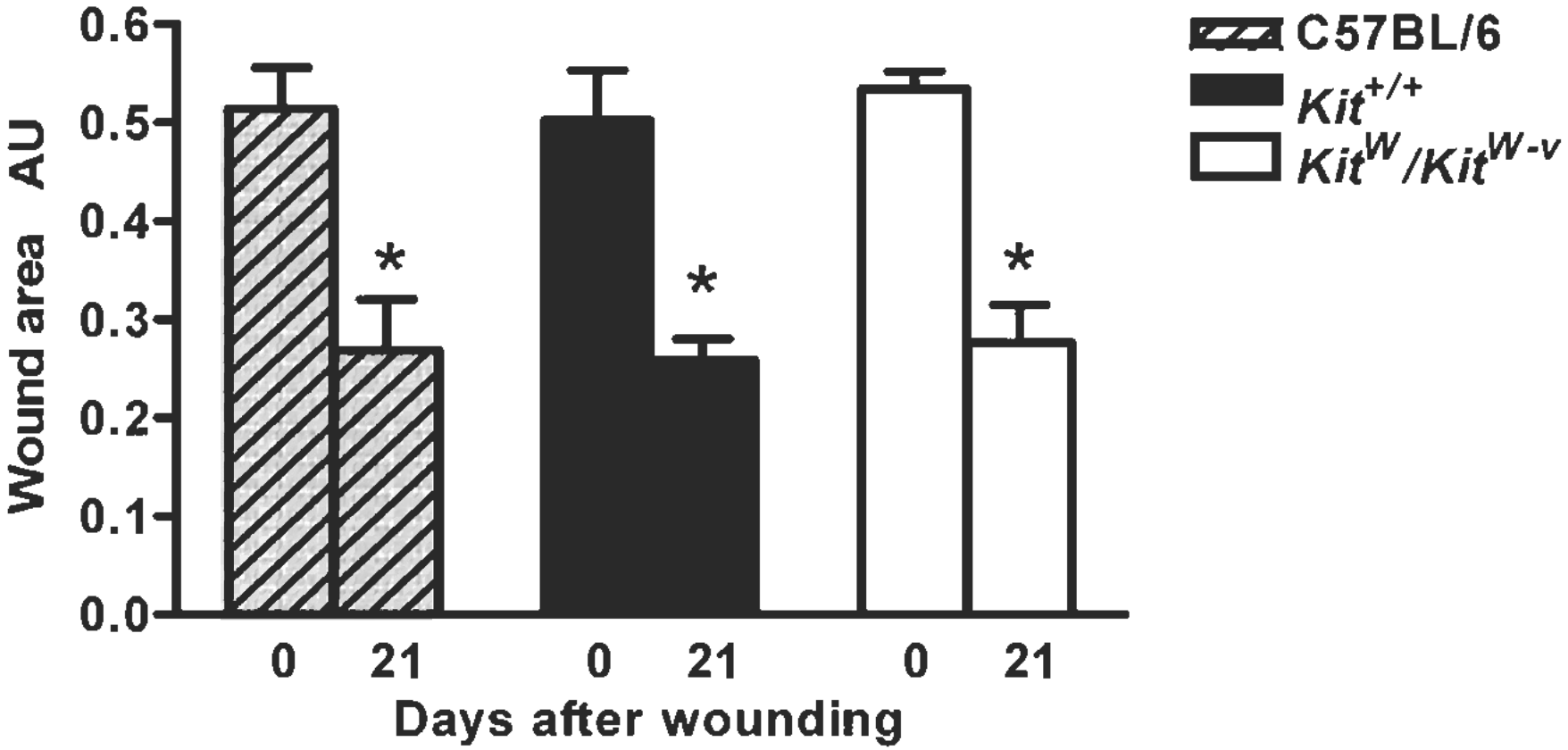
ligation (MI). Induction of acute anemia by hemodilution (MI + Hem) did not affect ventricular ejection fraction, end diastolic volumes or *d*P/*d*t max (*n*=4-7 per group). *, *P*<0.05 versus Sham; **, *P*<0.05 versus MI. (D) Adminstration of Gleevec reduced myocardial vascular endothelial growth factor (VEGF) and proliferating cell nuclear antigen (PCNA) levels. Representative immunoblot from 3-4 independent experiments is shown.



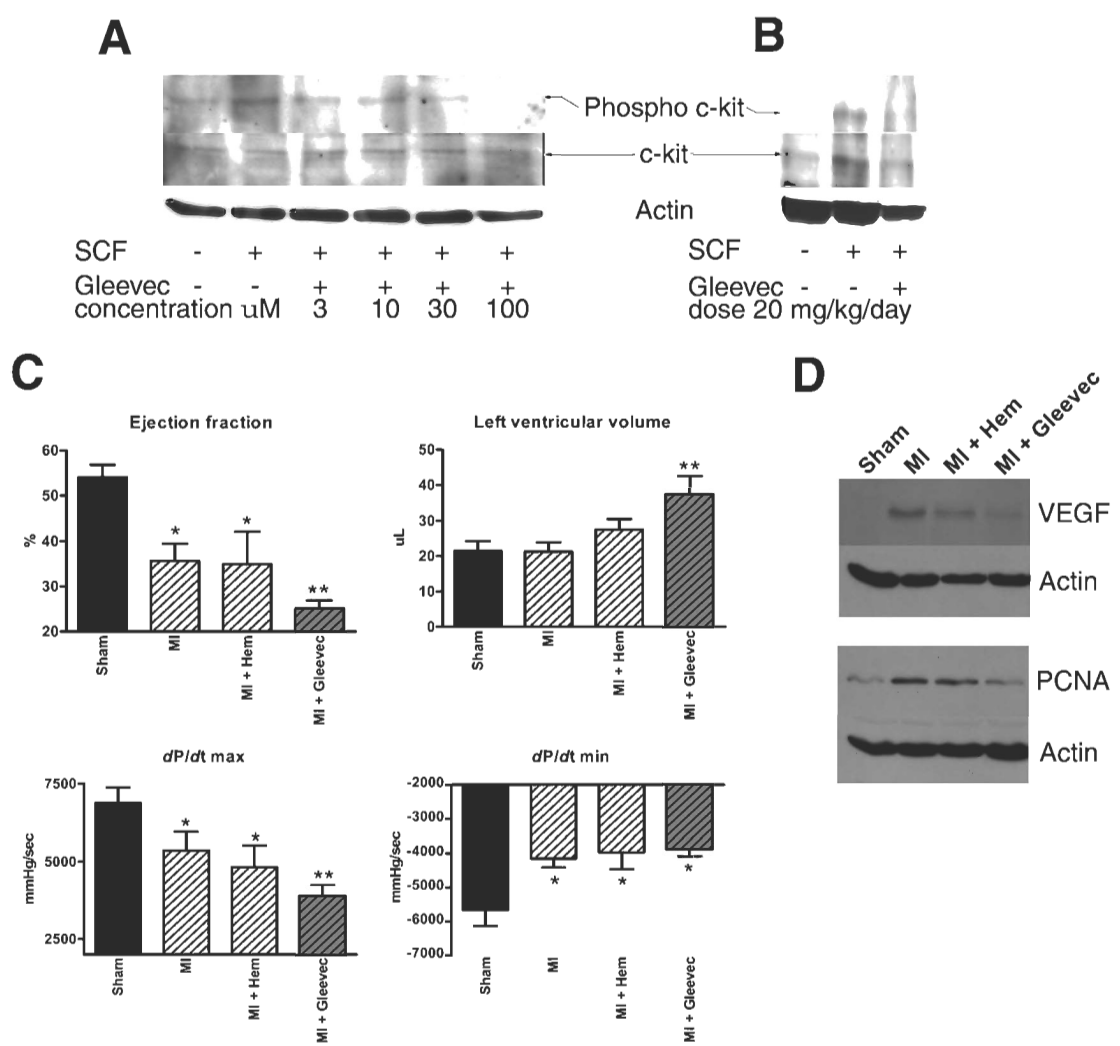
Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3



Supplementary Figure 4