Supporting Information

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SI Materials and Methods

Assessment of LV Area at Risk. Mouse hearts were harvested and the area of the LV at risk for ischemic injury was determined by trypan blue staining of the myocardium via a cannula inserted into the proximal ascending aorta. Transverse sections of the heart were cut and digitally photographed and then counterstained with triphenyltetrazolium chloride (TTC) to delineate infarcted myocardium, as described previously (1).

Histological Evaluation of Infarct Size. Mice were euthanized 12 weeks after coronary artery ligation and hearts were arrested in diastole by injection of 300 μL saturated KCl into the right atrium. The hearts were harvested and the LV was cut at the level of the LAD suture. As previously described, myocardial segments were prepared for biochemical and histological assessment (2). The determination of infarct size was performed using computer-assisted image analysis software (ImageJ, National Institutes of Health).

Immunohistochemistry. Freshly harvested mouse hearts were saturated in 30% sucrose in phosphate buffered saline (PBS) solution at 4 °C for 2–4 h, placed in optimal cutting temperature medium (OCT, Miles Pharmaceuticals), and snap-frozen in liquid nitrogen. Frozen sections were cut on a cryostat and postfixed in 4% paraformaldehyde. Capillary density was measured by CD31 immunostaining, as described previously (3). Capillary density was quantified by counting $CD31^+$ endothelial cells in 6–10 random high-power fields (magnification, 400×) on an inverted light microscope. Photographs were taken using a PAXcam ARC digital camera interfaced with Adobe Photoshop CS, and images were analyzed using ImageJ image analysis software.

Miniosmotic Pump Implantation. Miniosmotic pumps (Alzet model 1007D; DURECT) were implanted 24 h before left coronary ligation. *S*-nitrosoglutathione (GSNO) was dissolved in PBS containing 100 μM DTPA, and pumps were programmed to infuse GSNO at a rate of 10 mg/kg/d over a period of 72 h. Control mice were implanted with pumps that delivered vehicle alone (PBS plus 100 μM DTPA).

Myocardial Oxygenation Measurements. Myocardial oxygen partial pressure was measured in WT and $GSNOR^{-/-}$ mice using the OxyLite oxygen sensors (Oxford Oxtronix). A loose suture was placed around the LAD artery as described above and a small track was made using a 24-gauge needle into the anterolateral aspect of the left ventricle distal to the LAD ligature. The OxyLite probe was inserted into the needle track and suspended in the myocardium for tissue oxygenation measurements at baseline and at 15 min after LAD ligation. Baseline oxygen measurements were recorded for 5 minutes after tissue oxygen readings had stabilized.

Coronary Anatomy Determination Using Silicone Casts. For structural elucidation of the coronary anatomy of WT and $GSNOR^{-/-}$ mice, casts of the coronary tree were created using Silastic sealant (Dow Corning) as described previously (4).

Immunoblotting. Immunoblotting was performed as described previously (3) using the indicated antibodies. Detection was carried out using ECL (Amersham Biosciences), and bands were quantified by densitometry using Bio-Rad Fluoro-S Multimage software.

VEGF ELISA. Myocardial tissue was homogenized at 4 °C with a Polytron tissue homogenizer in 2 mL of RIPA buffer (150 mM NaCl, 50 mM Tris pH 8.0, 5 mM EDTA, 1% Nonidet P-40, 0.5% deoxycholate, 10 mM NaF, 10 mM disodium pyrophosphate, 1 mM PMSF, 2 μg/mL aprotinin, 2 μg/mL leupeptin, 1 mM Na orthovanadate). Total protein concentration in each sample was determined using the Bradford method (BioRad), and VEGF concentration was analyzed in triplicate using the Quantikine VEGF ELISA kit (R&D Systems) as described previously (5).

Generation of Replication-deficient Recombinant Adenovirus. Recombinant adenoviruses were produced using the AdEasy system (Qbiogene) as described previously (6). Briefly, the firefly luciferase cDNA was subcloned into the pShuttle vector downstream of 4 tandem HIF response elements (HRE-luc), following site-directed mutagenesis of its internal PacI restriction site. Recombination between pShuttle-HRE-luc and pAdEasy-1 was carried out in BJ5183 *E. coli* (Qbiogene), and recombinant adenoviral plasmid DNA was linearized with PacI and transfected into HEK293 cells. High titer viral stocks ($\approx 1 \times 10^{10}$ plaque forming units/mL) were obtained after amplification in HEK293 cells and purification by CsCl gradient ultracentrifugation. AdHRE-luc was verified by infecting HeLa cells that had been transiently transfected with a vector expressing stabilized HIF-1α (pcDNA3-HIF-$1\alpha^{P402A,\ P564G}$) or empty vector as a control. After 24 h, luciferase assays were performed to assess HIF-1α-mediated luciferase activity.

In Vivo Bioluminescence Imaging. A total of 1×10^5 viral particles of AdHRE-luc or control empty adenoviral vectors (AdEV) were injected into the apex of the myocardium of $GSNOR^{-/-}$ and WT littermate control mice. On days 3 and 6 postinfection, luciferase expression was measured in vivo following i.p. injection of luciferin (150 mg/kg; Xenogen) 10 min before imaging. Animals were placed into the Xenogen IVIS system, consisting of a light-tight chamber connected to a charge-coupled device camera system. Luciferase-mediated photon emission was quantified using Living Image software (Xenogen). The animals were then euthanized, and hearts were extracted, immediately flash frozen in liquid nitrogen, and stored at -80 °C for subsequent PCR and luciferase assay analyses.

Luciferase PCR Protocol. Frozen heart samples were ground into a fine powder with a mortar and pestle and then divided in half for protein and DNA analysis. Ground tissue samples were processed using the QIAamp Mini Kit from Qiagen, according to the manufacturer's instructions. DNA isolated from each sample was quantified using a BioRad SmartSpec Plus and 1 ng of DNA was used for amplification by PCR with the following primers: 5′-AGA CGC CAA AAA CAT AAA GAA AGG CCC GGC-3′; 5′-TAT AAA TGT CGT TCG CGG GCG CAA CTG CAA-3′.

Luminometry. Ground tissues were suspended in 200 μL Passive Lysis Buffer (Promega), and protein concentrations were determined using the BioRad RcDc protein assay kit. Triplicate 10 μL samples were mixed with 100 μL of Luciferase Assay Reagent (Promega) in wells of a 96-well plate. Luciferase activity was measured using a Veritas luminometer (Turner Biosystems) with

an integration time of 10 sec. Luminescence in each sample was normalized per microgram of protein.

Densitrometry. All Western blotting signals were quantitated by ImageJ (National Institutes of Health) and expressed as the relative ratio of protein to its histone loading control. The relative ratio of protein to histone in WT mice was arbitrarily set at 1. Data are expressed as mean ± SEM.

Biotin Switch Assay for Protein S-nitrosylation. The Biotin Switch assay was performed on nuclear extracts of mouse hearts as previously described (7). The samples were then analyzed by SDS/PAGE, followed by immunoblotting with anti-HIF-1α (1:1,000) antibodies to determine *S*-nitrosylation of HIF-1α.

Chromatin Immunoprecipitation Assay (ChIP). W293 cells were treated with increasing concentrations of GSNO for 5 h and then harvested for ChIP analysis, as previously described (8). Chromatin was immunoprecipitated with an anti-HIF1a antibody (Upstate Biotechnology, Cat. No. 07–628). PCR amplification of the immunoenriched DNA samples was performed using primers for the VEGF promoter (CCTCAGTTCCCTGGCAACATCTG and GAAGAATTTGGCACCAAGTTTGT) and detected by agarose gel electrophoresis.

Bone Marrow Harvest and Progenitor Cell Quantification. Bone marrow (BM) was collected from the femurs of 10-week-old male GSNOR$^{-/-}$ and WT mice, as described previously (9). BM contents were washed with Hanks' Balanced Salt Solution, filtered, and blocked using an anti-mouse CD16/32 FcR blocker (eBioscience). BM cells were labeled with the dead cell discriminator ethidium monoazide and a mixture of lineage-specific antibodies against CD3, CD5, CD8, B220/CD45RA, Mac1/CD11b, Gr1, Ter119 and thy-1 (Miltenyi Biotech); APC-conjugated c-Kit, (BD PharMingen); FITC-conjugated Sca-1 (BD PharMingen); and PE-conjugated VEGFR-2 (eBioscience) monoclonal antibodies. Hematopoietic stem cells were characterized as lineage-depleted cells that expressed c-Kit and Sca-1; endothelial progenitor cells were defined as HSCs that also expressed VEGFR-2.

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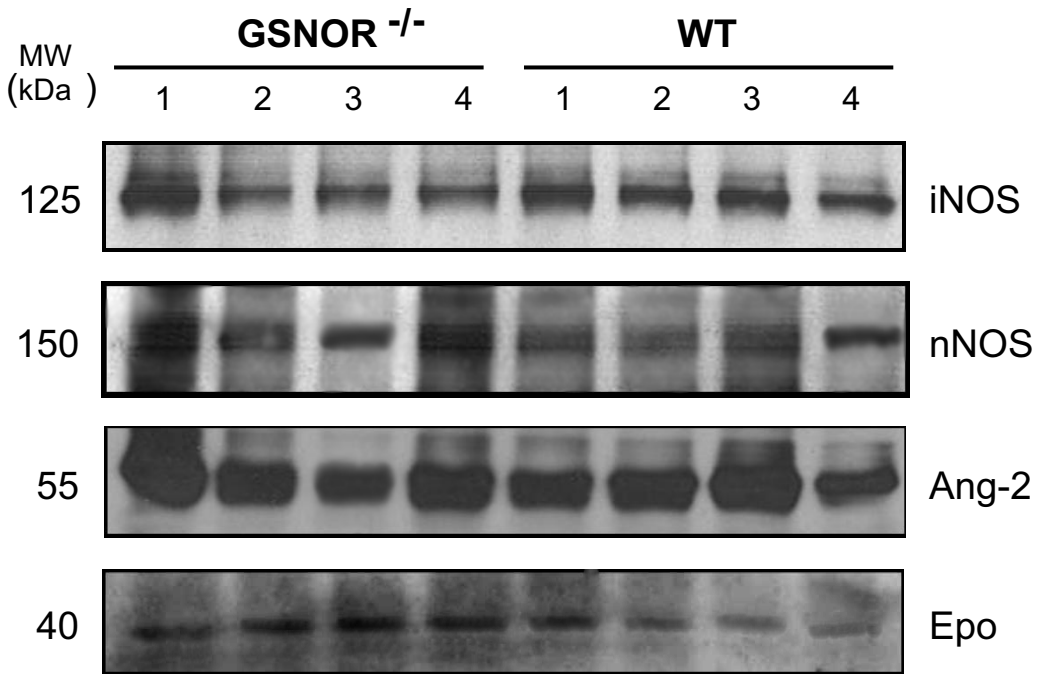

Fig. S1. Baseline levels of NOS isoforms, Epo, and angiogenic factors in WT and GSNOR$^{-/-}$ mice. Western blot analysis did not demonstrate a significant difference in the baseline levels of iNOS, nNOS, Ang-2, or Epo expression in WT and GSNOR$^{-/-}$ mice. (iNOS, inducible nitric oxide synthase; nNOS, neuronal nitric oxide synthase; Ang-2, angiopoietin-2; Epo, erythropoietin.)

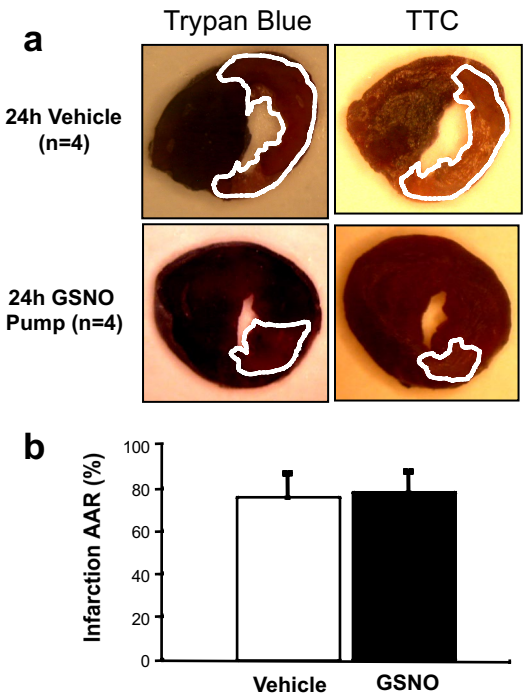

Fig. S2. Short-term GSNO infusion does not reduce myocardial infarct size in WT mice. (*a*) Miniosmotic pumps were implanted in WT mice and GSNO or a placebo control was continuously administered beginning 24 h before and ending 48 h after LAD ligation. Representative WT and GSNO treated hearts are shown. The ischemic area at risk (AAR) and infarction are demarcated (outlined in white). (*b*) No difference in infarct size is noted between WT and GSNO treated hearts as a percent of ischemic AAR.

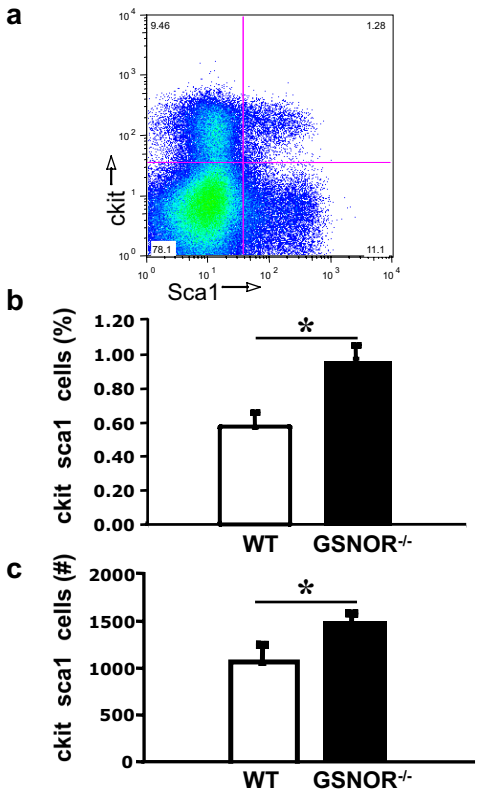

Fig. S3. Baseline levels of hematopoietic progenitor cells (HSCs) are elevated in GSNOR$^{-/-}$ mice. Bone marrow from WT and GSNOR$^{-/-}$ mice was harvested for calculation of baseline levels of HSCs. After exclusion of clumped or dead cells, lineage (Lin)^{lo}–Lin^{negative} cells were isolated from whole bone marrow and immunophenotyped by flow cytometry for co-expression of the cell surface markers, c-kit and sca1 (*a*). Double positive (c-kit^{+}/sca1^{+}) cells were classified as HSCs. (*b*) At baseline, GSNOR$^{-/-}$ mice exhibited a higher percentage composition (*, $P = 0.009$) and (*c*) numerical quantity ($^{\dagger}P = 0.048$) of bone marrow-derived HSCs compared to WT animals ($n = 10$ per group). Endothelial progenitor cells (EPCs) were defined as a subset of HSCs that co-expressed VEGFR2. The actual numbers of bone marrow-derived EPCs detected by flow cytometric analysis were too small to ascribe significance in both WT and knockout mice (data not shown).