Supporting Information

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SI Methods

Generation of Cys521Ser Soluble Epoxide Hydrolase Knockin Mice. Mice constitutively expressing the soluble epoxide hydrolase (sEH) Cys521Ser mutation were generated on a pure C57BL/6 background by Taconic Artemis. A gene-targeting vector was constructed using C57BL/6-derived genomic DNA of the *Ephx2* gene from the RP23 BAC library. The Cys521Ser substitution was introduced into exon 18 using site-directed mutagenesis to change the TGT codon (National Center for Biotechnology Information (NCBI) reference sequence mouse: 13850) to TCC. A positive selection marker (Puromycin resistance, PuroR) was flanked by F3 sites and inserted into intro 16. Transfection of germ-line–competent C57BL/6N Tac ES cells with linearized and purified targeting vector DNA was then carried out. G418-mediated enrichment of ES cell clones that had integrated the targeting vector into their genome and Southern analysis of genomic DNA from G418-resistant ES cells with an *Ephx2* locus-specific probe yielded a number of candidate clones. Candidate clones were tested for correct homologous recombination at the target locus and the absence of additional integrations of the targeting vector by Southern analysis with targeting-vector–specific (internal) and target-locus–specific (external) probes in combination with multiple restriction digests. Injection of cells from one correctly targeted ES cell clone into wild-type (WT) blastocysts yielded male mice with 50–100% chimerism, as judged from coat color. Chimeric males with a high contribution of targeted ES cells were directly bred with a transgenic mouse line [C57BL/6-Tg(CAG-Flpe)2 Arte] ubiquitously expressing Flp recombinase under the control of the chicken β-actin promoter and a hCMV immediate early enhancer for germ-line transmission of the targeted *sEH* knockin allele and in vivo deletion of the flippase recognition target-flanked selection marker. The presence of the C43S point mutation was confirmed in both ES cells and mice heterozygous for the targeted *sEH* allele by sequencing of genomic DNA. Mice were maintained as stated in the Principles of Laboratory Animal Care published by the National Institutes of Health (NIH Publication Number 85–23). Experiments were approved by the King's College London Animal Welfare and Ethical Review Body. The sEH Cys521Ser mice were characterized by comparing them to their WT littermate controls as reported in Figs. 1–4 of this study. Additional characterization data are provided in Fig. S1 *A*–*C*.

Blood Biochemistry. Venous blood from WT or Cys521Ser KI mice were analyzed using a hand-held iSTAT analyzer, with $EC8^+$ cartridges (Abbott Laboratories).

Myography. Vascular rings were isolated from the mesenteric (second order) arteries. The rings were mounted in a tension myograph (Danish Myo Technology), stretched to the optimal pretension conditions (using a Danish Myo Technology Normalization Module), bathed in Krebs solution maintained at 37 °C and gassed with 95% CO_2:5% O_2 (vol/vol). During the vessel "wake up" phase, if vessels did not generate greater than 1 mN of force, they were rejected and the myograph channel was switched off. No vessels were rejected during subsequent analyses of the responses. Vasotone measurements of mesenteric vessels were made by determining the responses of U46619-contracted (0.1 μM; Sigma) vessels to nitro-oleic acid (NO_2-OA) (0–10 μM). In some experiments, mesenteric vessels were incubated in 20 μM 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one (ODQ; Sigma) for 30 min before U46619 contraction and dose–response to NO_2-OA. In some studies acetylcholine (dose–response; Sigma), L-N^G-nitroarginine methyl ester (L-NAME) (300 μM; Sigma) and indomethacin (10 μM; Sigma) was used. Tension experiments were carried out using at least three vessels per treatment intervention derived from at least three different WT or knockin (KI) mice.

Echocardiography. Age- and weight-matched littermate WT or Cys521Ser KI mice (four per group) were anesthetized and examined by echocardiography using a high-resolution Vevo 770 echocardiography system (VisualSonics) with a RMV-707B transducer running at 30 MHz. High-resolution, 2D brightness mode and motion-mode images at the level of the papillary muscles were obtained. Wall thickness and ventricular dimensions were measured and then left ventricular mass, ejection fraction, stroke volume, and cardiac output were calculated with Vevo Software (VisualSonics).

Telemetric Blood Pressure Monitoring in Vivo. Blood pressure (BP) was assessed by remote radiotelemetry in conscious freely moving mice. Briefly, mice were anesthetized with 2% isoflurane (Centaur Services) in 1 L of oxygen per minute with pre- and postoperative analgesia (buprenorphine, 0.1 mg per kg of body weight; Abbot Laboratories). A radiotelemetry probe catheter (TA11PA-C10, outside diameter of 0.4 mm; Data Science International) was implanted into the aortic arch of each mouse through the left carotid artery. After 1 wk of recovery, mice housed individually in cages were placed above the telemetric receivers with an output to a computer. Blood pressure was recorded by scheduled sampling for 10 s every 5 min (Dataquest LabPRO Acquisition system version 3.01; Data Sciences International). Baseline measurements were initiated after 1 wk postsurgery recovery and subsequent NO_2-OA, angiotensin (Ang) II, and saline delivery (below). A total of 7–12 WT or KI mice were studied per group to produce the average blood pressure data.

Ang II-Induced Hypertension and NO_2-OA Delivery. After baseline recordings (3 d) mice were again anesthetized and subjected to s.c. implantation of osmotic minipumps (model 1002; Alzet) for delivery of Ang II (Sigma) at an infusion rate of 1 mg/kg/day. After 3 d, a second osmotic minipump (model 1007D) containing either NO_2-OA at a concentration of 5 mg/kg/day or saline, was implanted s.c. A total of 7–12 WT or knockin mice were studied per group to produce the average blood pressure data. In some experiments, mice had only one osmotic minipump implanted with NO_2-OA or saline.

Soluble Epoxide Hydrolase Activity Assay. Mouse hearts from telemetered animals were rapidly isolated and frozen in liquid N_2 at the end of the BP monitoring protocol. Hearts were powdered under and stored in liquid nitrogen until ready for analysis. They were homogenized (1 mL of buffer per 100 mg of cardiac tissue) on ice in 100 mM Tri·HCl, pH 7.4 using a Polytron tissue grinder. Cytosolic fractions were prepared from the hearts by centrifugation at 25,000 × *g* for 5 min at 4 °C. A total of 20 μL of the cytosolic fraction was then used for the activity assay, adding it to 175 μL of 25 mM Tris·HCl, pH 7.4. After equilibration at room temperature, the reaction was initiated by the addition of 5 μL of 10 μM (3-phenyl-oxiranyl)-acetic acid cyano-(6-methoxy-naphthalen-2-yl)-methyl ester (PHOME; Cayman) dissolved in DMSO. The reaction mixture was left for 15 min, after which the fluorescence was monitored at 330/465 nm. In an additional reaction mixture, an sEH inhibitor [5 μL of 10-μM

12-(3-adamantan-1-yl-ureido)dodecanoic acid; Cayman] was added to ascertain the baseline reading for sEH activity for each sample.

Sample Preparation for Oxylipid Profiling Analysis. Plasma samples were spiked with 10 μL 500 nM internal standard I [d4-6-keto-prostaglandin F1a, d4-prostaglandin E2, d4-thromboxane2, d4-leukotriene B4, d11-14,15-dihydroxy-eicosatrienoic acid, d6-20-hydroxy-eicosatetraenoic acid (HETE), d4-9-hydroxy-octadecadienoic acid, d8-12-HETE, d8-5-HETE, d11-11(12)-epoxy-eicosatrienoic acid, d4-9(10)-epoxy-octadecenoic acid, d8-AA] and then were extracted by solid phase extraction using Oasis hydrophilic-lipophilic-balanced (HLB) cartridges (3 cc 60 mg; Waters). The HLB cartridges were first washed with 2 mL ethyl acetate, 2 mL methanol twice, and 2 mL 95:5 vol/vol water/methanol with 0.1% acetic acid. The 6-mL plasma samples were then loaded onto the cartridges with 3-mL samples each time. The samples were then washed with 6 mL 95:5 vol/vol water/methanol with 0.1% acetic acid and dried for 20 min with low vacuum. The target analytes were then eluted with 0.5 mL methanol followed by 2 mL of ethyl acetate into the tubes with 6 μL 30% glycerol in methanol as the trap solution. The volatile solvents were evaporated by using vacuum centrifugation (SpeedVac) until 2 μL of trap solution remained in the tube. The residues were dissolved in 50 μL of methanol containing 200 nM internal standard II (1-cyclohexyl-dodecanoic acid urea, CUDA). The samples were mixed with a vortex mixer for 2 min, centrifuged at 14,000 × *g* for 5 min, and then transferred to autosampler vials with 150 μL inserts for liquid chromatography tandem MS (LC/MS/MS) analysis. LC/MS/MS analysis of oxylipids was performed using an Agilent 1200 SL liquid chromatography series (Agilent) with an Agilent Eclipse Plus C18 2.1 × 150 mm, 1.8-μm column for the oxylipins separation. The mobile phase A was water with 0.1% acetic acid, whereas the mobile phase B was composed of acetonitrile/methanol (80/15, vol/vol) and 0.1% acetic acid. Gradient elution was performed at a flow rate of 250 mL/min. The injection volume was 10 μL and the samples were kept at 4 °C in the autosampler. Analytes were detected by negative multiple reaction monitoring mode using a 4000 QTrap tandem mass spectrometer (Applied Biosystems) equipped with an electrospray ionization source (Turbo V). Calibration curves were generated by 10-μL injections of seven standards containing each analyte, internal standard I, and internal standard II for quantification purposes.

Assay for Quantifying the Stoichiometry of NO_2-OA Adduction to sEH. This is based on a modified "PEG-switch" assay that allows the stoichiometry of reversible thiol oxidations to be determined using a Western blotting-based approach and an antibody to the protein of interest, namely sEH in these studies. The method, adapted to use β-mercaptoethanol (β-Me), as it allows reductive labeling of proteins that adduct NO_2-OA. Briefly, hearts from mice that had been subjected to 7 d of saline or NO_2-OA treatment from a minipump were rapidly isolated and frozen in nitrogen. Tissue samples were powdered under and stored in liquid nitrogen until subsequent analysis, when they were homogenized (1 mL of buffer per 100 mg of cardiac tissue) on ice in 100 mM Tris·HCl, pH 7.4, 100 mM maleimide, and 1% SDS using a Polytron tissue grinder. The samples were heated at 50 °C for 25 min to alkylate-free cysteine thiols. The samples were desalted using spin columns (Pierce) and then 500 mM β-Me was added. The samples were heated at 37 °C for 60 min to reverse NO_2-OA adducts. The samples were desalted with a spin column again and 10 mM PEG-maleimide (Sigma) and 0.5% SDS were added to the sample, which was left rotating for 2 h at room temperature. A 2× SDS sample buffer [100 mM Tris·HCl, pH 6.8, 4% SDS (vol/vol), 20% glycerol (vol/vol), and 0.01% bromophenol blue] containing 100 mM maleimide was added to each sample. The samples were then resolved on a standard SDS/PAGE gel, Western blotted, and probed with an antibody for sEH (H-215, sEH antibody; Santa Cruz Biotechnology). GelPro Analyzer 3.1 was used to quantify the proportion of PEG-modified sEH to that of the reduced sEH.

Feeding Studies to Generate Endogenous Nitro Fatty Acids. Mice were gavaged daily for 5 d with conjugated linoleic acid (CLA, 10 mg/kg) and sodium nitrite (20 mg/kg) in 200 μL PEG 400. This protocol models concentrations of key components of the Mediterranean diet. Animals were killed 2 h after the last dose and tissues were rapidly isolated and frozen in liquid nitrogen. Control mice were gavaged with 200 μL PEG 400.

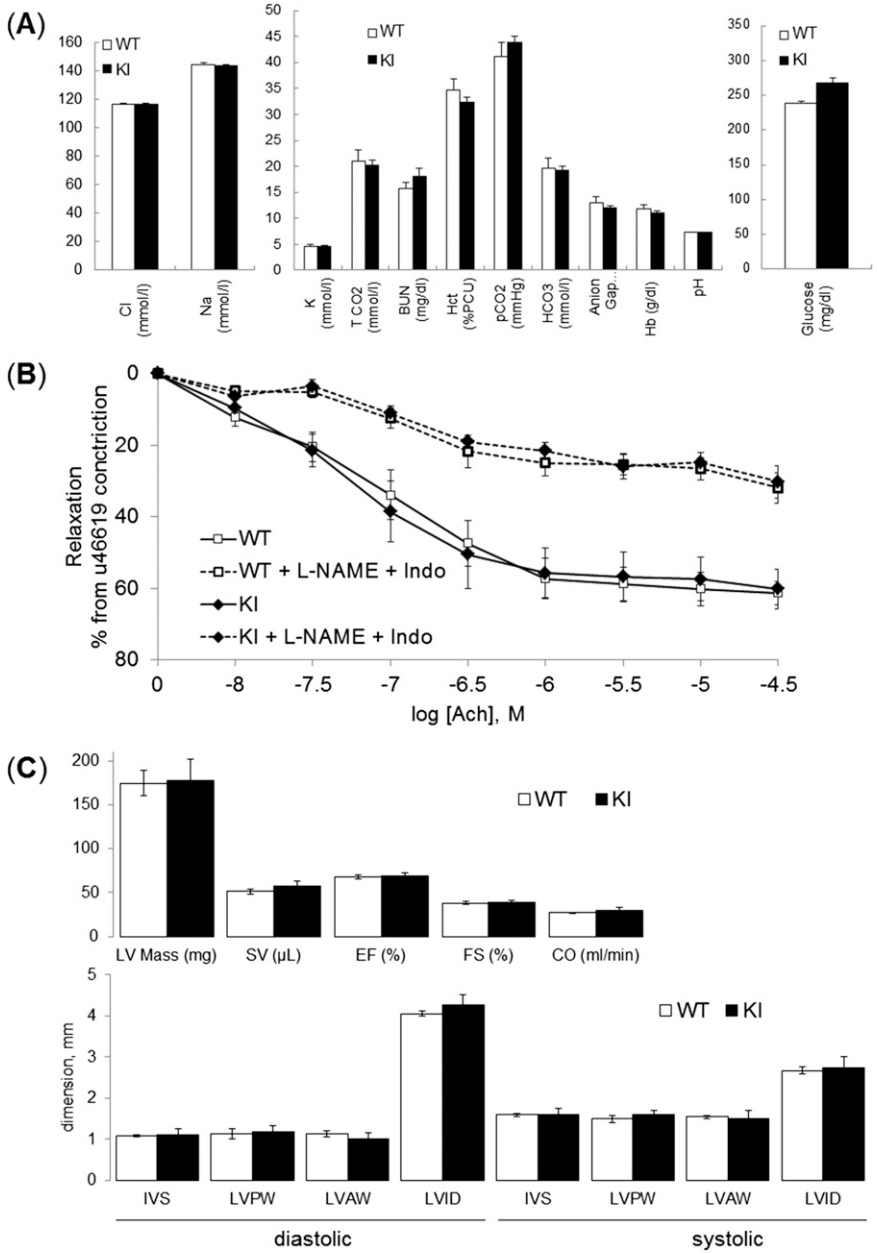

Fig. S1. Comparison of basal phenotype of WT and sEH KI mice. (*A*) Hematological analysis of WT and KI showed there were no differences in terms of WT and KI blood biochemical indices. (*B*) Myograph analysis showed there was no difference between either genotype in their vasodilatory responses to acetylcholine or acetylcholine in the presence of L-N^G-nitroarginine methyl ester (L-NAME) and indomethacin. (*C*) Echocardiographic analysis of cardiac function showed there were no differences in basal heart function parameters between WT and KI mice. LV mass, left ventricular mass; SV, stroke volume; EF, ejection fraction; FS, fractional shortening; CO, cardiac output; IVS, interventricular septum thickness; LVPW, left ventricular posterior wall thickness; LVAW, left ventricular anterior wall thickness; LVID, left ventricular internal dimension.