SUPPLEMENTARY DATA

Materials. Antibodies against ubiquitin and the GST-tag were obtained from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). MG132 was purchased from BioMol (Plymouth Meeting, PA). UIM-agarose was obtained from Boston Biochem Inc. (Cambridge, MA). Glutathione (GSH) beads were from Amersham Pharmacia Biotech (Uppsala, Sweden). Full-length human GTPCH1 cDNA (GCH1) and lipofectamine 2000 were obtained from Invitrogen (Carlsbad, CA) and QuikChange site-directed mutagenesis kits were obtained from Stratagene (La Jolla, CA). The restriction enzymes *EcoR*I and *Sal*I were from New England Biolabs (Ipswich, MA). Qiaquick DNA isolation kits were from Qiagen (Valencia, CA). The S-nitrosylation kits with the biotin-switch were from Cayman Chemicals (Ann Arbor, MI). All other chemicals, if not indicated, were purchased from Sigma-Aldrich.

Assays of zinc release. Release of zinc from GTPCH1 was measured by the 4-(2-pyridylazo)resorcinol (PAR) assay as described previously (1). All buffers used for the zinc assay were pretreated with Chelex 100 to remove background Zn^{2+}. To determine the total zinc content of untreated GTPCH1, maximal zinc release was determined by diluting GTPCH1 proteins in guanidine HCl (7 mol/L). The extinction coefficient for PAR_2Zn^{2+} was experimentally determined to be 70 $mM^{-1}cm^{-1}$ using a known concentration of zinc chloride.

Immunoprecipitation and immunoblotting. Cells were harvested and re-suspended in lysis buffer, and immunoprecipitated using anti-FLAG resin (Sigma). Western blotting was carried out using standard techniques with specific antibodies.

***In vitro* pull-down assay.** Tetraubiquitin (Ub4) was used in binding assays (2) with GST-GTPCH1 or mutant immobilized on GSH beads. Ub4 chains were also used in FLAG-GTPCH1 pull-down assays with recombinant FLAG-GTPCH1 immobilized on anti-FLAG resin.

Enrichments of ubiquitinated proteins. Ubiquitinated proteins were enriched by ubiquitin affinity beads (UIM-agarose). These beads are conjugated to an ubiquitin-associated domain to facilitate their binding to ubiquitinated proteins. Cell lysates or tissue homogenates were incubated with UIM-agarose overnight at 4°C.

Determination of GTPCH1 half-life assay. Cultured cells were treated with cycloheximide (CHX, 100 μg/mL), and lysed at the indicated time points in the presence of CHX. Total proteins were subjected to analysis by Western blotting.

Assays of the endothelium-dependent and –independent relaxation. Aortic rings isolated from the treated mice were subjected to organ chamber assay of endothelium-dependent and -independent vasodilation as described previously (3,4).

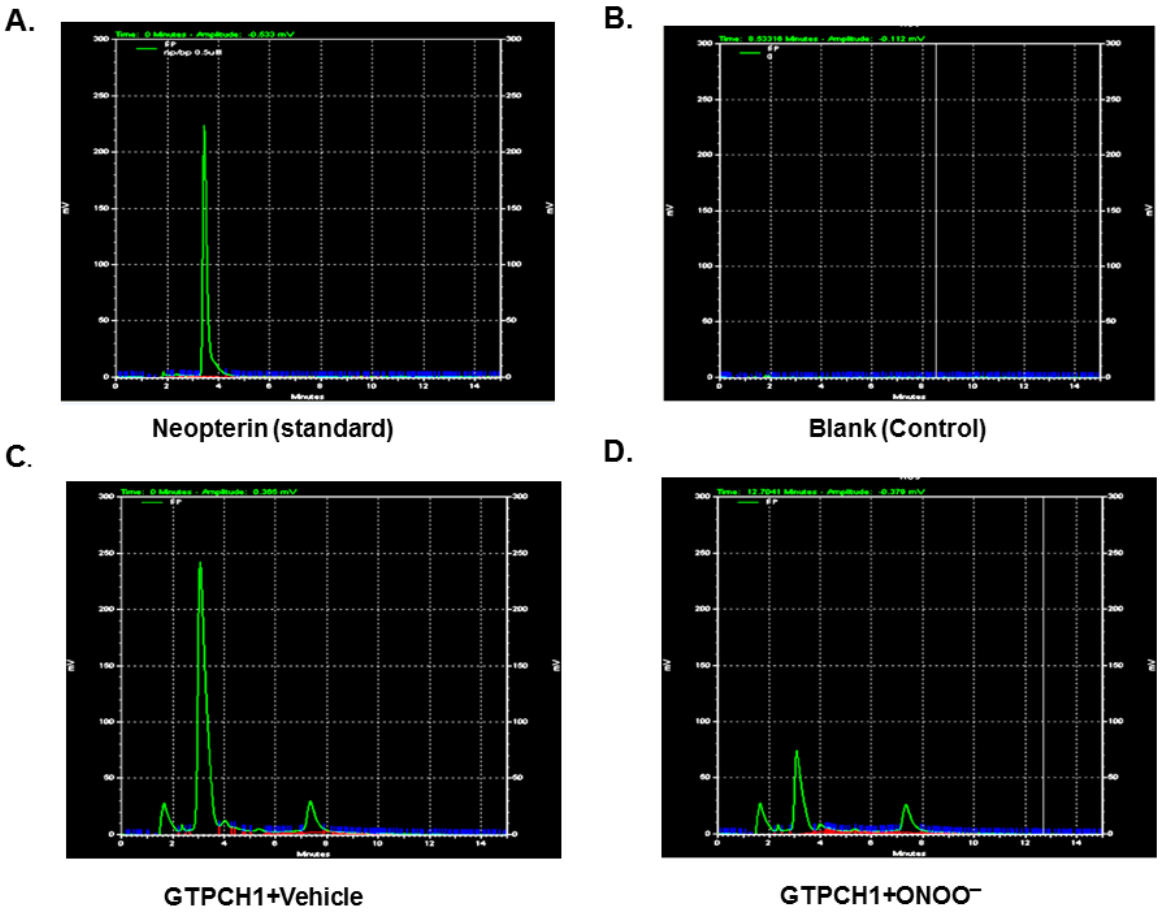
Detection of reactive oxygen species. Intracellular O_2^- was assessed by the DHE fluorescence/HPLC assays, as described previously (3,4).

SUPPLEMENTARY DATA

Supplementary Table 1. Effects of Tempo on body weights and blood glucose in control and STZ-injected mice

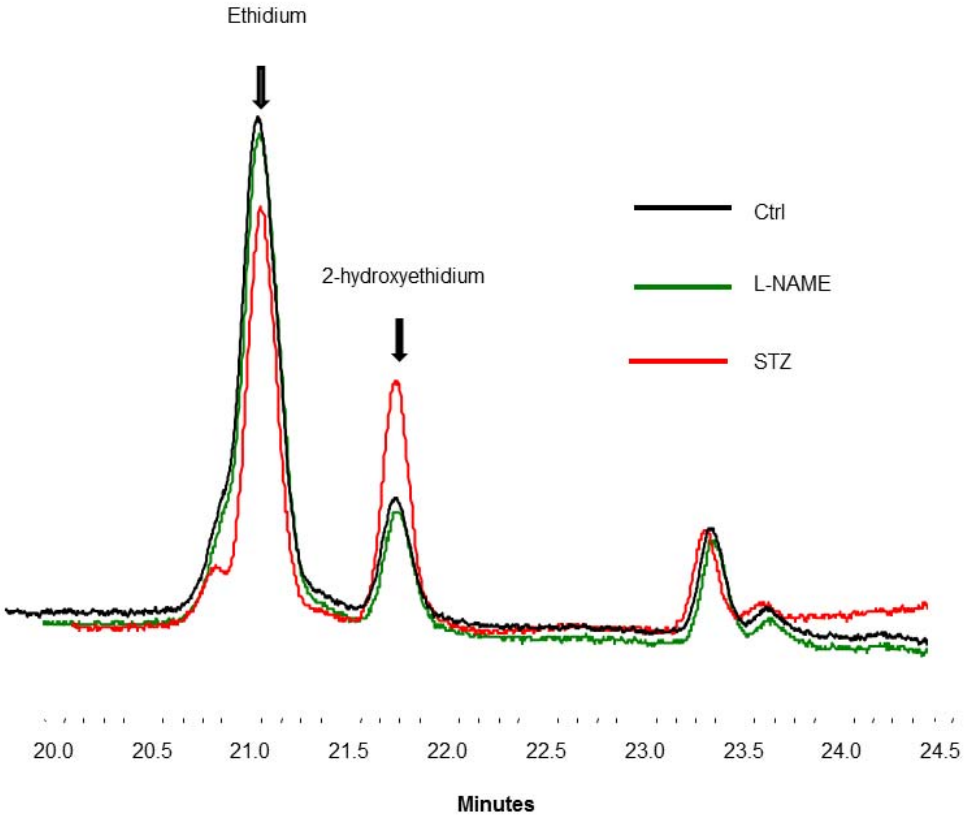
	Control		STZ	
	w/o Tempo	with Tempo	w/o Tempo	with Tempo
Body weight (g/dl)	23.9 ± 0.2	23.3 ± 0.2	21.0 ± 0.6	20.9 ± 0.7
Glucose (mg/dl)	153 ± 29	169 ± 25	523 ± 49	549 ± 46

Supplementary Figure 1. Representative chromatograms for HPLC measurement of GTPCH1 activity. A. Neopterin (Sigma, 1 μM) was used for standard for HPLC measurement of GTPCH1 activity. B. Buffer only as blank for negative control. C. GTPCH1 activity. 0.4 μM recombinant GTPCH1 plus 2 mM GTP was incubated in 37 ℃ for 2 hours. D. 0.4 μM recombinant GTPCH1 was pretreated 50 μM ONOO– on ice for 5 minutes and then plus 2 mM GTP was incubated in 37 ℃ for 2 hours.



SUPPLEMENTARY DATA

Supplementary Figure 2. Representative chromatograms for HPLC measurement of superoxide production (2-hydroxyethidium and ethidium) in diabetes.



SUPPLEMENTARY DATA

Supplementary Figure 3. $ONOO^-$-induced GTPCH1 modification and ubiquitination in diabetic mouse hearts *in vivo* Control and STZ-induced diabetic mice were given Tempo as described in Experimental Procedures. Mouse heats from control and diabetic mice were isolated and assayed for A (right panel) to E. ***A***. 3-NT-positive proteins; ***B*.** GTPCH1 activity; ***C,D,E*.** Effects of Tempol on GTPCH1 and ubiquitinated GTPCH1 in isolated mouse aortas from non-diabetic and diabetic mice *in vivo*. The blot is a representative of three blots obtained from 5 mice. Results were obtained from five mice. **P*<0.05 vs. control. $^{\#}P$<0.05 vs. STZ. NS indicates *P*>0.05 vs. control.