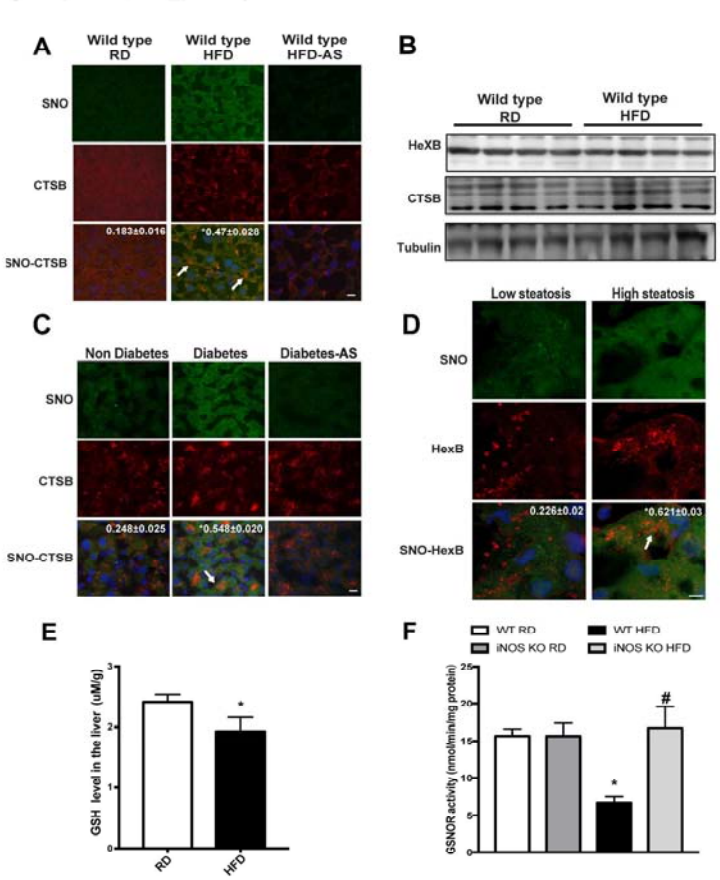
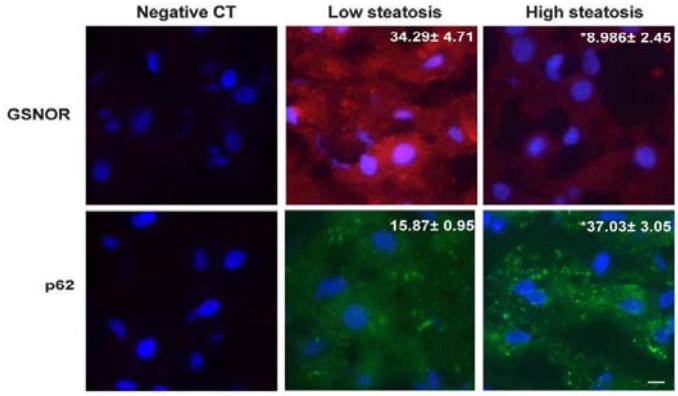
SUPPLEMENTARY DATA

Supplementary Figure 1. Diabetes and steatosis elevates S-nitrosylation of lysosomal enzymes in the liver A. Representative images (40X) of staining for S-nitroyslated CTSB in the livers from mice fed with RD or HFD (16 weeks on HFD). The S-nitrosylation staining was performed by a modified *in situ* Biotin switch method. Red: HexB; green: S-nitrosylation; blue: DAPI. Arrows point to S-nitrosylated CTSB, and –AS is ascorbate omitted (negative control for S-nitrosylation). Quantified colocalizations of S-nitrosylated CTSB are shown on the top of each image. Data are shown as Pearson's correlation coefficient as means ± SEM. * indicates statistically significant difference relative to lean condition determined by Student's *t*-test ($p<0.05$). **B.** Representative western blottings for HexB and CTSB in livers of WT mice fed with HFD or RD. Each lane is a sample from an individual mouse. **C.** Representative images (40X) of staining for S-nitroyslated CTSB in the livers from diabetic or non-diabetic patients. Quantified colocalizations of S-nitrosylated CTSB are shown on the top of each image. Data are shown as Pearson's correlation coefficient as means ± SEM. * indicates statistically significant difference relative to non-diabetic condition determined by Student's *t*-test ($p<0.05$). **D**. Representative images (63X) of staining for S-nitroyslated HexB in the livers from high or low steatosis patients. Scale bar: 10µm. Quantified colocalizations of S-nitrosylated HexB are shown on the top of each image. Data are shown as Pearson's correlation coefficient as means ± SEM. * indicates statistically significant difference relative to low steatosis condition determined by Student's *t*-test ($p<0.05$). **E.** GSH levels measured in liver tissue of mice on the RD or HFD. GSH levels were detected at 405 nm and normalized to weight of liver tissue. Statistical significance was determined by Student's t-test (*$p<0.05$), n=4. **F.** GSNOR activity in livers from WT and iNOS KO mice. 10 µg liver lysate was used to measure the kinetic of GSNO-dependent NADH consumption in the absence or presence of 100µM of GSNO. All data are presented as means ± SEM, *indicates statistical significance compared to WT RD, #indicates statistical significance between HFD groups determined by ANOVA followed by post-hoc test ($p<0.05$).



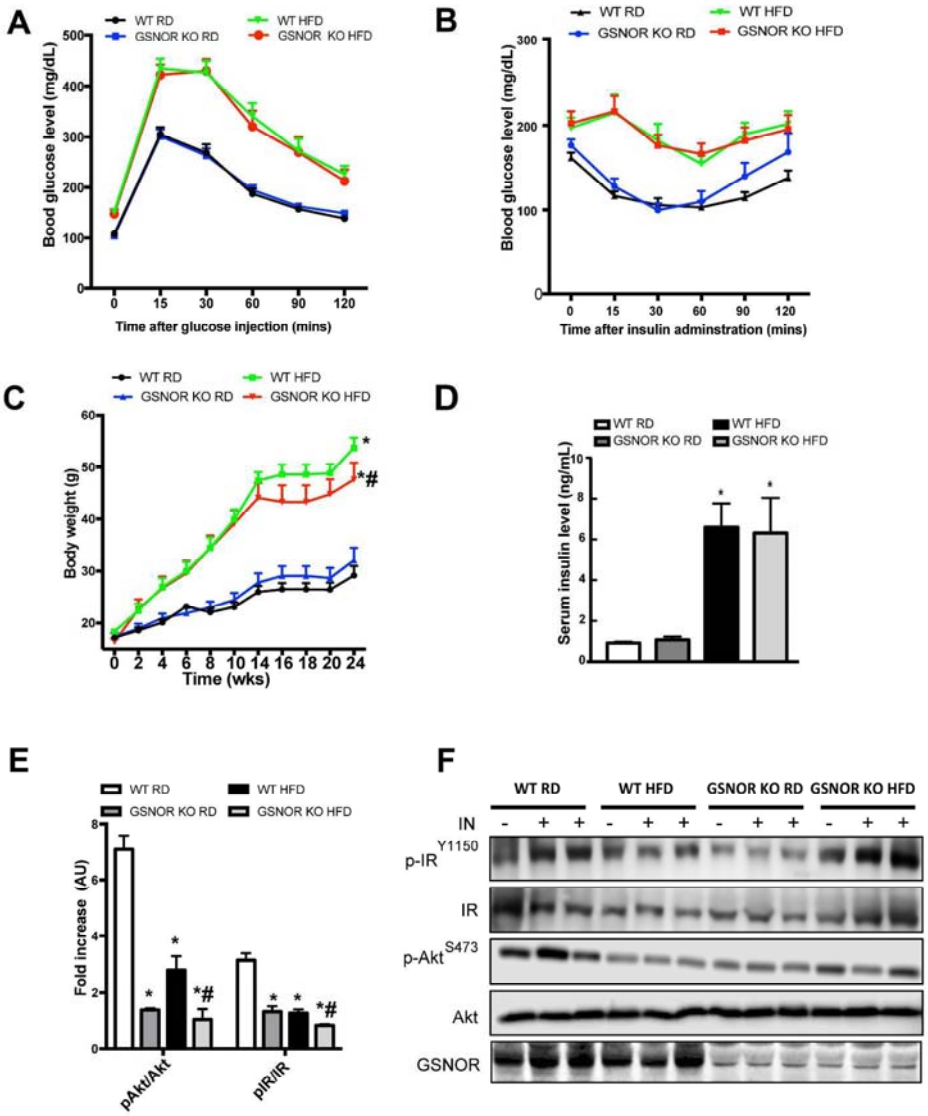
SUPPLEMENTARY DATA

Supplementary Figure 2. Downregulation of GSNOR in the liver from steatosis patient. Representative confocal images (63X) of expression of GSNOR and p62 in livers from low steatosis, high steatosis, and negative controls (primary antibody omitted). Scale bar: 10μm. Quantification of fluoresce intensity is shown at top of each image. * indicates statistically significant difference relative to low steatosis condition determined by Student's *t* test ($p<0.05$).



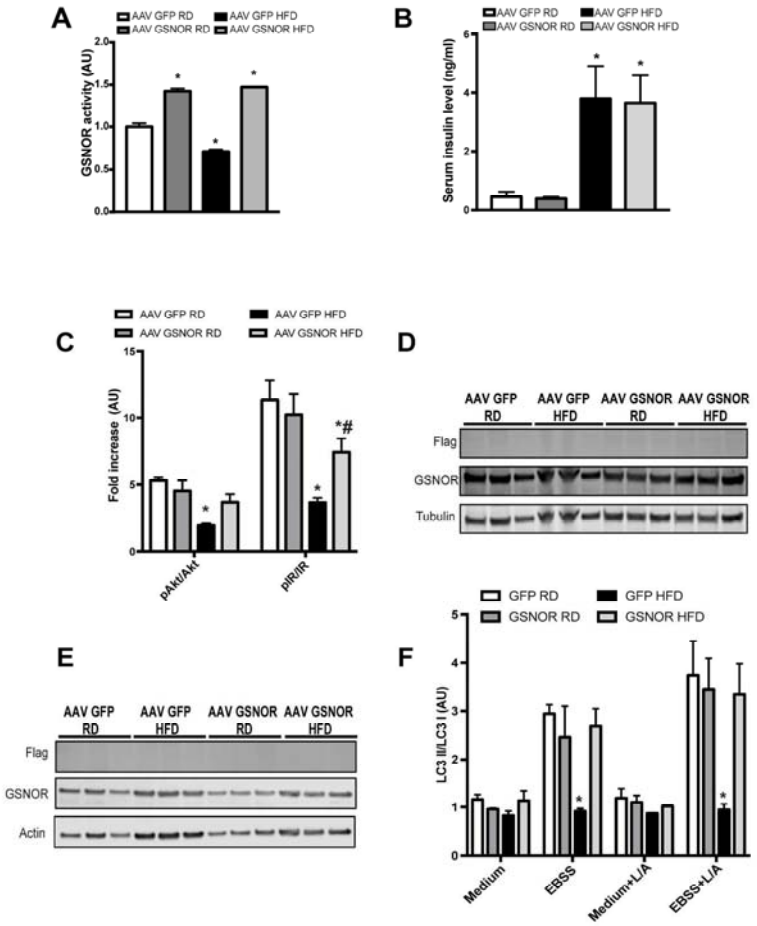
SUPPLEMENTARY DATA

Supplementary Figure 3. Metabolic profiles of mice with loss of GSNOR function. A. Glucose tolerance, **B.** insulin tolerance tests in WT and GSNOR KO mice (n=8-10, 12 weeks on HFD), and **C.** Body weight of mice in A&B. Data are presented as mean±SEM. * statistical analysis of AUC between HFD groups, # statistical analysis of AUC between HFD groups performed by two-way ANOVA with post-hoc test ($p<0.05$). Data are representative of two individual cohorts of mice. **D.** Serum insulin levels in the same mice (n=6, 6 hrs after food withdrawal). All data are presented as means ± SEM. * statistically significant difference relative to WT RD. **E.** Densitometry analysis of hepatic insulin action in the livers of WT and GSNOR KO mice (Fig. 5A). *indicates statistical significance compared to WT RD, #indicates statistical significance between HFD groups determined by ANOVA followed by post-hoc test ($p<0.05$). **F.** Hepatic insulin action in the skeletal muscle from WT and GSNOR KO mice (16 weeks on HFD) with portal vein insulin stimulation. Ins: insulin, 0.75IU/kg for 3 mins; p-IR: $IR^{tyr1150/1151}$; p-AKT: Akt^{ser473}.



SUPPLEMENTARY DATA

Supplementary Figure 4. Metabolic profiles of mice with gain of GSNOR function. A. GSNOR activity in livers of (n=3) WT mice transduced with AAV8-TBG-eGFP (AAV GFP) or AAV8-TBG-GSNOR (AAV GSNOR). Mice were fed the RD or HFD for 12 weeks. The data were normalized to the AAV GFP RD group, and are presented as means ± SEM, *indicates statistical significance compared to AAV GFP RD determined by one-way ANOVA with post-hoc test ($p<0.05$). **B.** Serum insulin levels in these mice (n=4-6, after 6 hrs of food withdrawal). All data are presented as means ± SEM. *indicates statistical significance compared to AAV GFP RD group determined by one-way ANOVA with post-hoc test ($p<0.05$). **C.** Densitometry analysis of hepatic insulin action in the livers from WT mice transduced with AAV8-TBG eGFP or AAV8-TBG GSNOR and on the RD or HFD. *indicates statistical significance compared to AAV GFP RD, #indicates statistical significance between HFD groups determined by ANOVA followed by post-hoc test ($p<0.05$). **D-E.** AAV8-TBG-mediated GSNOR expression in the skeletal muscle (D) and epididymal white adipose tissue (E) of WT mice with the RD or HFD. **F**. Densitometry analysis of LC3 conversion in the primary hepatocytes from livers from WT mice with GSNOR overexpression (protein lysates were from 6 mice). *indicates statistical significance compared to GFP RD group in each treatment determined by ANOVA ($p<0.05$).



SUPPLEMENTARY DATA

Supplementary Figure 5. GSNOR-mediated lysosomal nitrosative stress contributes to impaired hepatic autophagy. A-B. CTSB activity (A) and autophagic vacuoles (B) in primary hepatocytes from WT and GSNOR KO mice (n=3; 8wk on RD) with EBSS treatment (4 hrs). CTSB-R: S-nitrosylation resistant CTSB; pcDNA: control plasmid. Data are presented as means ± SEM, *indicates statistical significance compared to pcDNA in same mouse line, #indicates statistical significance between WT and GSNOR KO groups in same treatment, and &indicates statistical significance between CTSB and CTSB-R groups in the same mouse line, determined by ANOVA followed by post-hoc test ($p<0.05$). **C.** Autophagic vacuoles in primary hepatocytes from WT and GSNOR KO mice (n=3; 8wk on RD) with EBSS treatment (4 hrs), with or without pretreatment of trehalose (100mM, 16hrs). Data are presented as means ± SEM, *indicates statistical significance compared to WT medium in same mouse line, #indicates statistical significance within vehicle and trehalose groups, and &indicates statistical significance between EBSS and medium in GSNOR-/- line, determined by ANOVA followed by post-hoc test ($p<0.05$). **D.** Densitometry analysis of insulin-stimulated Akt phosphorylation in the primary hepatocytes from WT and GSNOR KO mice transduced with Ad-Atg7 or control virus (Ad-lacZ). *indicates statistical significance compared to control virus in the WT cells, #indicates statistical significance between control virus and Ad-Atg7 in the same type of cells determined by ANOVA followed by post-hoc test ($p<0.05$, n=3). **E.** Densitometry analysis of insulin action in the primary hepatocytes from WT and GSNOR KO mice with or without trehalose treatment. *indicates statistical significance compared to WT, #indicates statistical significance between vehicle and trehalose in the same type of cells determined by ANOVA followed by post-hoc test ($p<0.05$, n=3). **F.** Densitometry analysis of insulin signaling in the livers from WT and GSNOR KO mice with or without trehalose administration. *indicates statistical significance compared to WT with vehicle administration, #indicates statistical significance between vehicle and trehalose in the same mouse line determined by ANOVA followed by post-hoc test ($p<0.05$, n=5).

SUPPLEMENTARY DATA