

Supplemental Information

A Hepatic GAbp-AMPK Axis Links Inflammatory

Signaling to Systemic Vascular Damage

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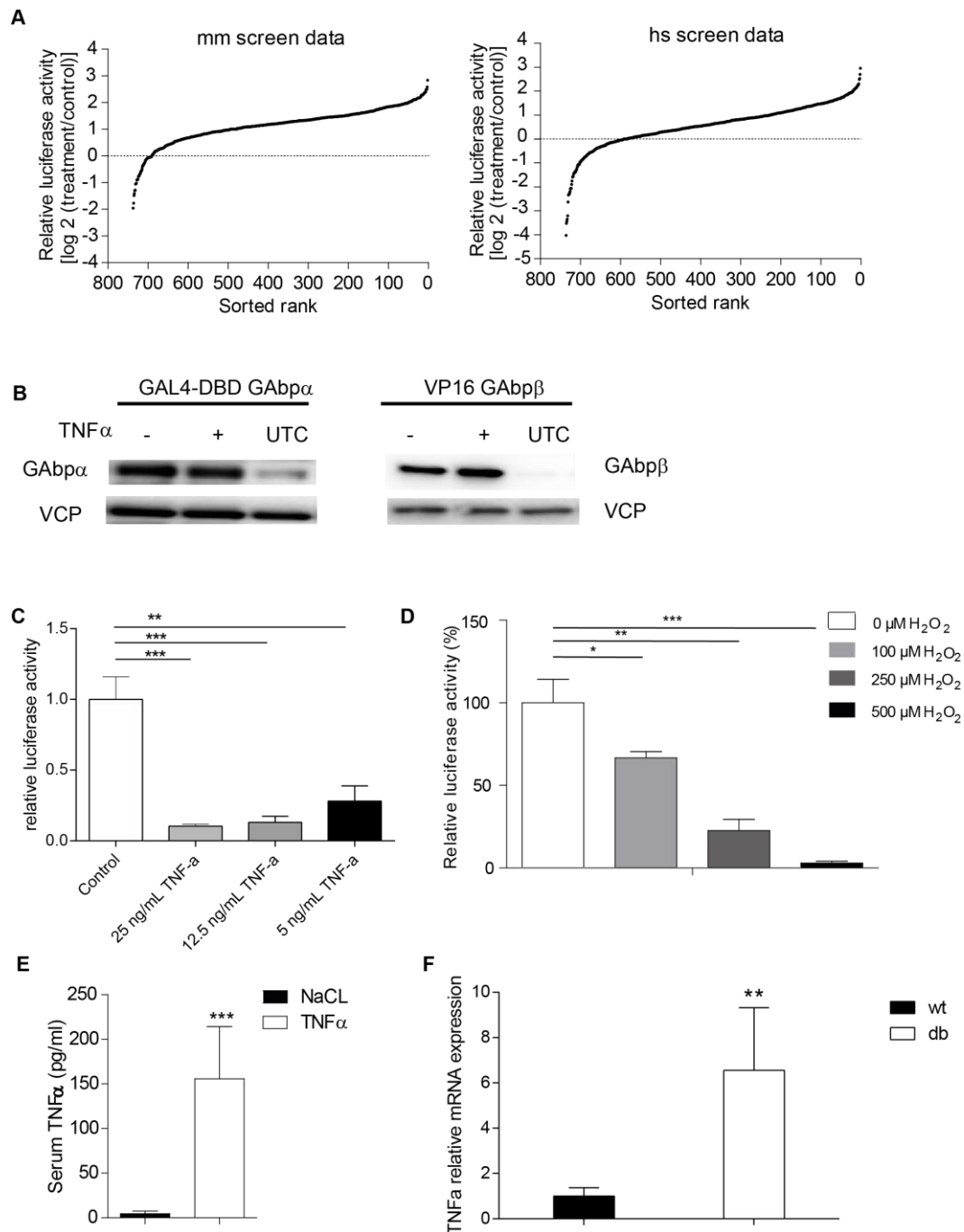


Figure S1 GABP activity is diminished by TNF α and ROS. Related to Figure 1. (A) High-throughput screen with a murine (mm) and human (hs) one-hybrid transcription factor library (Gal4). ~1500 transcriptional regulators in frame with a Gal4 DNA-binding domain (Gal) and a GAL4 UAS::luciferase reporter were transfected in HEK293T cells treated with 100 ng/ml TNF α for 24 h (n=6). Fold changes were plotted on a log₂ scale against a sorted rank of fusion constructs. **(B)** Immunoblots for GABP α and GABP β from Hepa1-6 cells transfected with the GAL4-DBD GABP α or VP16 GABP β 1 mammalian two-hybrid constructs, left untreated (-) or treated with TNF α (+). VCP served as loading control. **(C)** Mammalian two-hybrid assay in Hepa1-6 cells treated with increasing concentrations of TNF α (n=3, mean \pm SEM, significance is indicated relative to untreated control, * p \leq 0.05, **p \leq 0.01, ***p \leq 0.001). **(D)** Mammalian two-hybrid assay in Hepa1-6 cells treated with increasing concentrations of H₂O₂ (n=3, mean \pm SEM, significance is indicated relative to untreated control, * p \leq 0.05, **p \leq 0.01, ***p \leq 0.001). **(E)** Levels of TNF α in the serum of wt mice injected i.v. with 5 μ g/kg human recombinant TNF α (n=3, ***p \leq 0.001). **(F)** Relative TNF α mRNA expression in the liver of fasted wild-type and db/db mice (n=5; **p \leq 0.01).

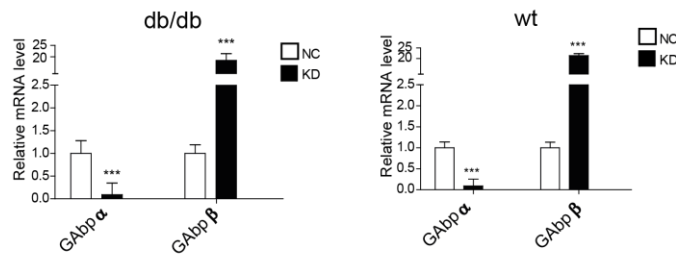
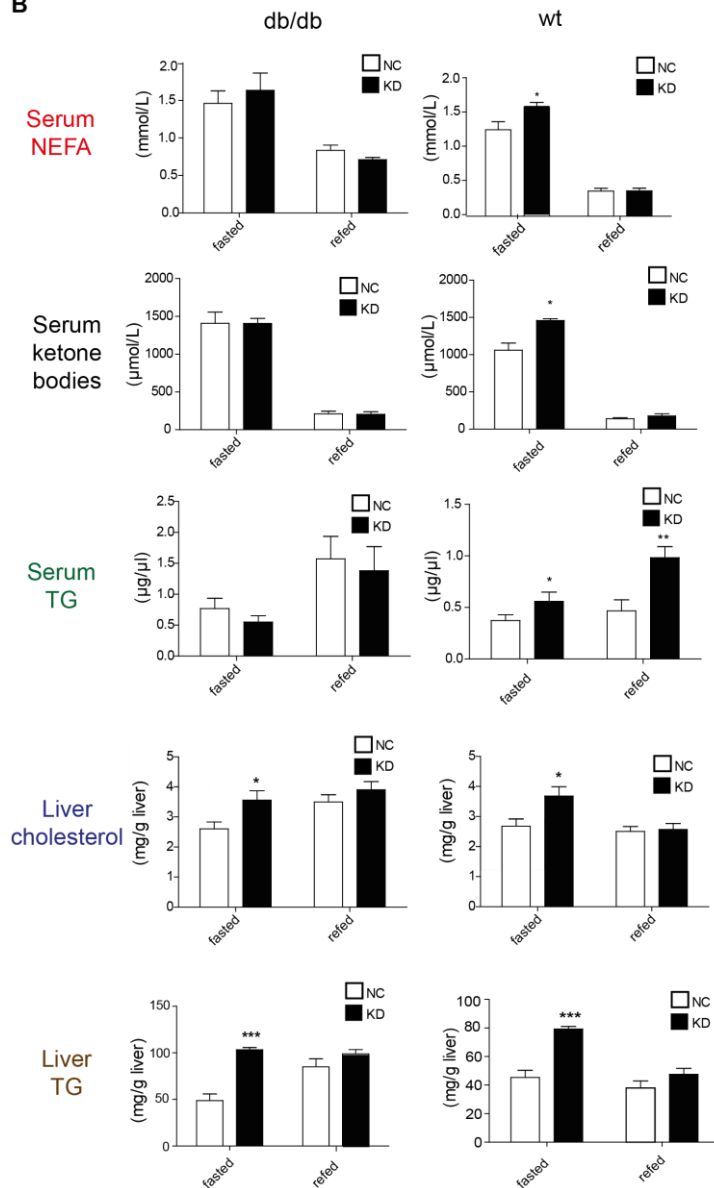
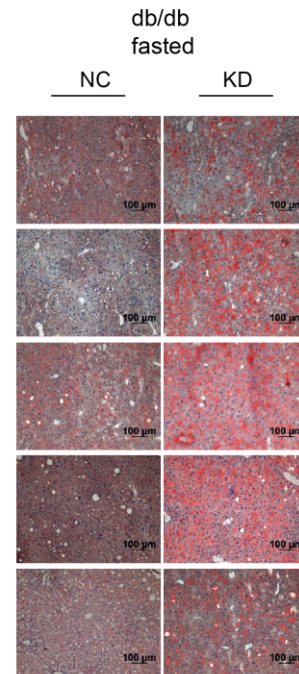
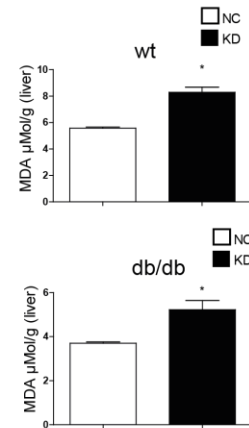
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Figure S2 Hepatic GABPα knockdown leads to metabolic changes in serum and liver of mice. Related to Figure 2. (A) GABPα and GABPβ expression levels in wt and db/db mice treated with control (NC) or GABPα-knockdown AAV (KD) (expression data represent combined refed and fasted groups, n=5, mean ± SEM, significance relative to the respective untreated control (NC): *** p ≤ 0.001). **(B)** Serum and liver metabolite overview in serum and liver of wt and db/db mice upon GABPα knockdown in hepatocytes. (n=5, mean ± SEM, significance is indicated relative to the respective control NC, * p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001). **(C)** Oil red O staining of liver tissue from fasted db/db mice upon hepatic GABPα knockdown (representative images are shown). **(D)** Levels of Malondialdehyde in liver samples of wild-type and db/db mice were measured using a colorimetric plate assay (n=5, mean ± SEM, significance is indicated relative to the respective control NC, * p ≤ 0.05).

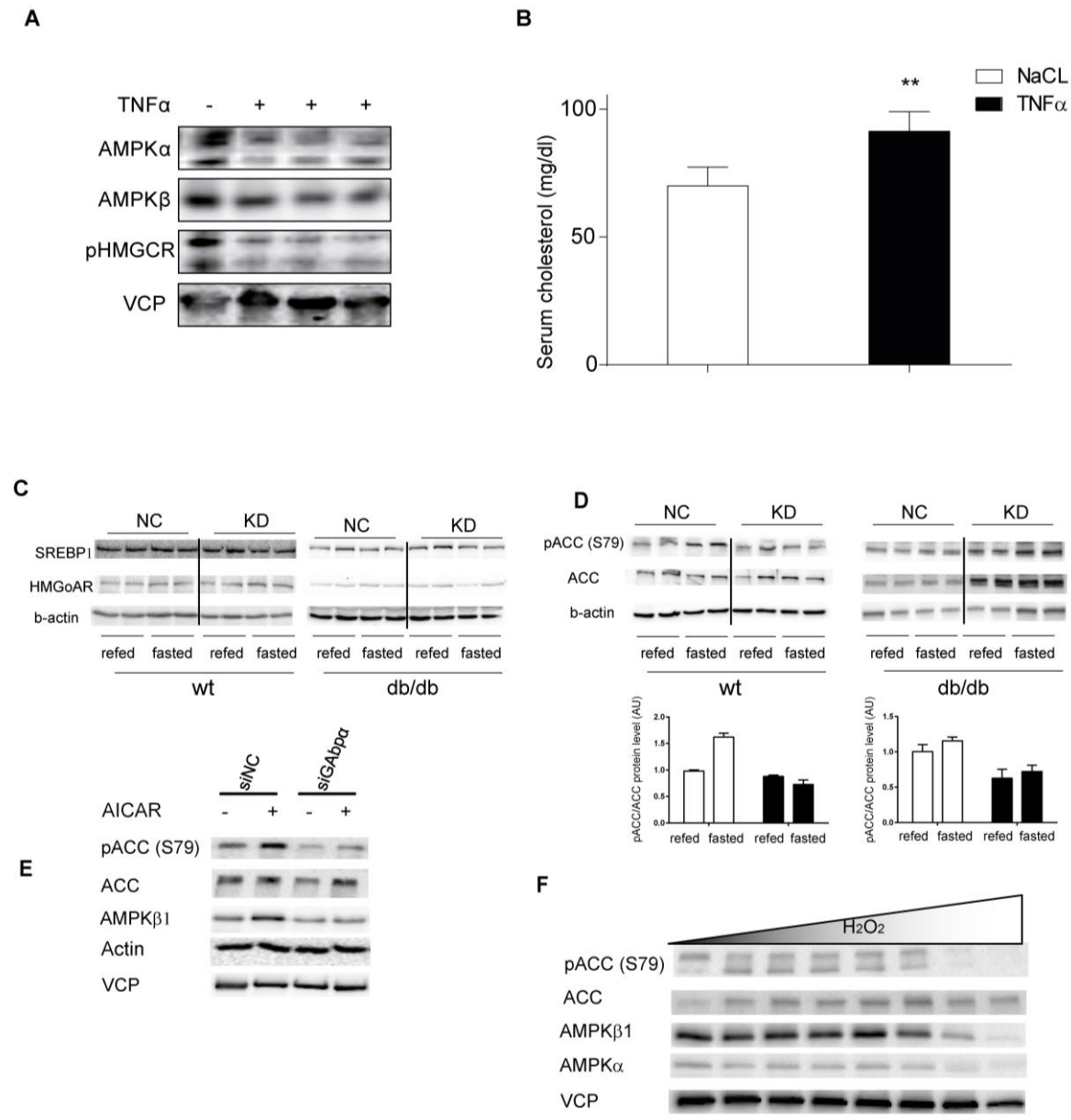


Figure S3. Depletion of GAbpa affects AMPK signaling. Related to Figure 3. (A) Immunoblots for AMPKα, AMPKβ, and pHMGCRCR from livers of wt mice injected i.v. with 5 μg/kg human recombinant TNFα. Samples were harvested 16 h after injection. VCP was used as a loading control. **(B)** Levels of cholesterol in the serum of wt mice injected with 5 μg/kg human recombinant TNFα (n=3, **p ≤ 0.01). **(C)** Immunoblots for total SREBP1 and total HMGCoAR protein in liver protein lysates from refed and fasted wild-type and db/db mice treated with AAV-miRNC (NC) or AAV-miRGAbpa (KD). Actin was used as loading control. **(D)** Immunoblots of phospho-ACC (Ser79) and total ACC in liver protein lysates from refed and fasted wild-type and db/db mice treated with AAV-miRNC (NC) or AAV-miRGAbpa (KD). Actin was used as loading control. **(E)** HEK293T cells were transfected with non-targeting control siRNA (NC) or siRNA targeting GAbpa and treated for 3 h with 1 mM 5-Aminoimidazole-4-carboxamide ribonucleoside (AICAR) or vehicle (DMSO) and components of the AMPK pathway were visualized using specific primary antibody detecting the indicated total or phospho-proteins (n=1). **(F)** Immunoblot analysis of components of the AMPK signaling pathway in primary hepatocytes from male C57Bl6/J mice treated with increasing concentrations of H₂O₂ (0, 10, 25, 50, 100, 250, 500 to 1000 μM) for 8 h. Proteins were detected using specific primary antibodies. VCP was used as a loading control.

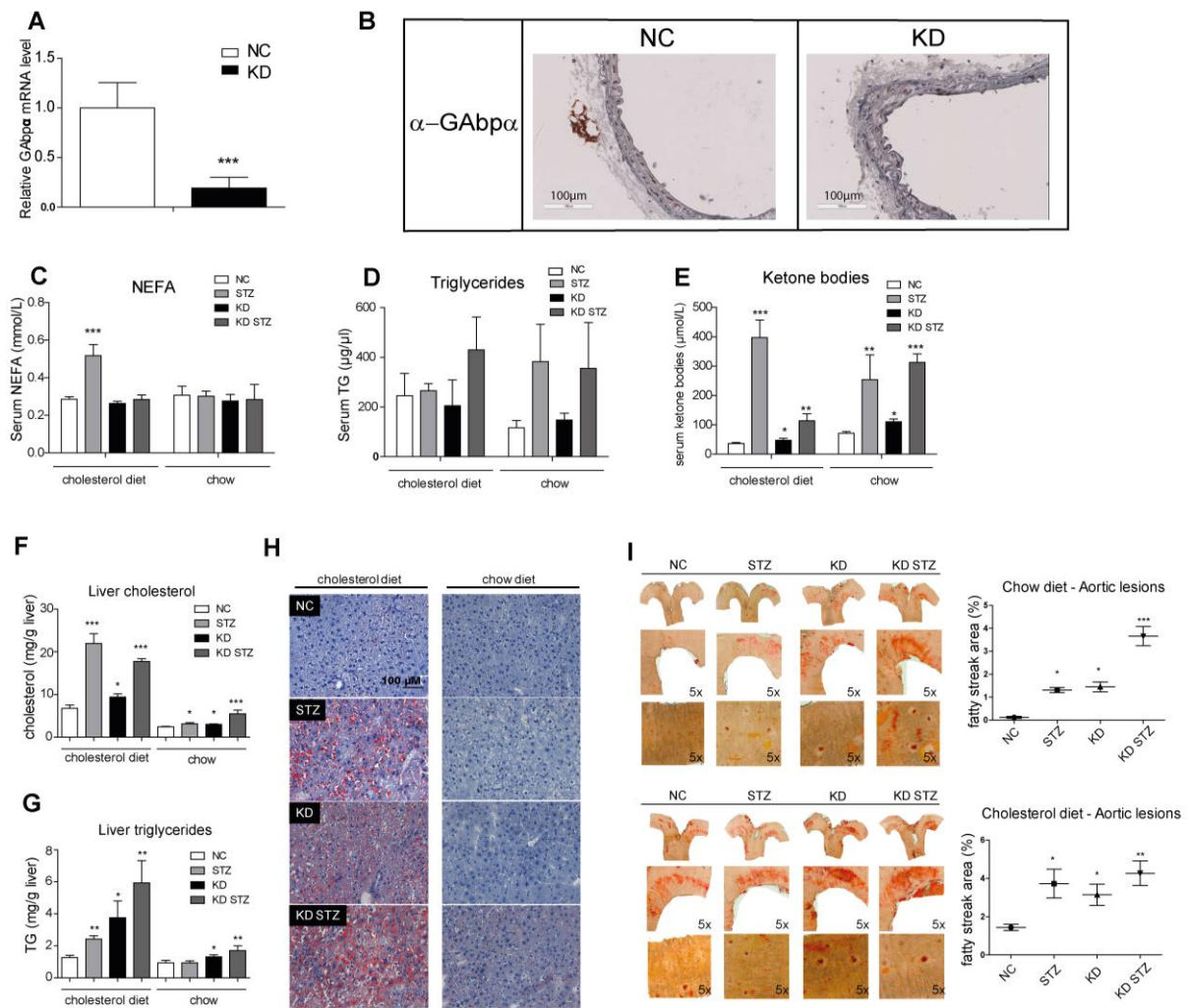


Figure S4. Hepatic GABPα is crucial for maintaining metabolic homeostasis. Related to Figure 4. (A) Relative GABPα mRNA levels in male LDLR-KO mice treated with control (NC) or GABPα knockdown AAV (KD) and fed a chow or an 0.15 % cholesterol diet (pooled data for STZ and Ctrl samples, n=10, mean ± SEM, significance is indicated relative to the respective control NC, *** p ≤ 0.001). (B) Immunostaining with a GABPα-specific antibody in cryo-fixed aorta of LDLR-KO mice on a chow diet treated with control (NC) or GABPα knockdown AAV (KD), counterstained with hematoxylin (representative images are shown). (C) Levels of non-esterified fatty acids in serum of LDLR-KO mice measured using a colorimetric plate assay (n=5, mean ± SEM, significance is indicated relative to the respective control NC, *** p ≤ 0.001). (D) Levels of triglycerides in serum of LDLR-KO mice treated with control (NC) or GABPα knockdown AAV (KD) and fed a chow or an 0.15 % cholesterol diet measured using a colorimetric plate assay (n=5, mean ± SEM). (E) Levels of ketone bodies in serum of LDLR-KO mice treated with control (NC) or GABPα knockdown AAV (KD) and fed a chow or an 0.15 % cholesterol diet measured using a colorimetric plate assay (n=5, mean ± SEM, significance is indicated relative to the respective control NC, * p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001). (F) Levels of cholesterol content in the liver of LDLR-KO mice treated with control (NC) or GABPα knockdown AAV (KD) and fed a chow or an 0.15 % cholesterol diet measured using a colorimetric plate assay (n=5, mean ± SEM, significance is indicated relative to the respective control NC, * p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001). (G) Levels of triglycerides in the liver of LDLR-KO mice treated with control (NC) or GABPα knockdown AAV (KD) and fed a chow or an 0.15 % cholesterol diet measured using a colorimetric plate assay (n=5, mean ± SEM, significance is indicated relative to the respective control NC, * p ≤ 0.05, ** p ≤ 0.01, *** p ≤ 0.001). (H) Oil red O staining for neutral lipids in cryosection of liver tissue from LDLR-KO mice treated with control (NC) or GABPα knockdown AAV (KD) and fed a chow or an 0.15 % cholesterol diet and treated with vehicle or STZ (representative images are shown). (I) Bright field microscopy images of the aortic arch (upper part) and of vessels branching away from the aorta in chow (left) and 0.15 % cholesterol diet (right) fed LDLR-KO mice treated with STZ or vehicle and control (NC) or GABPα knockdown AAV (KD). Oil red O staining and quantification of aortic plaque area in paraffin fixed aorta (n=4-5, mean ± SEM, significance is indicated relative to NC, * p ≤ 0.05, *** p ≤ 0.001).

