Appendix A. Supplementary data

S1. Supplemental Materials and Methods

S1.1. Reagents

Lipopolysaccharide (LPS) from *Escherichia coli* 0111:B4 was purchased from Sigma. Polyclonal antibodies to HM74 was from Novus Biologicals; monoclonal antibodies to β-actin were from Santa Cruz Biotechnology, HRP-conjugated anti-goat, anti-rabbit, were from Amersham.

S1.2. Animal studies

All animal procedures were conducted in accordance with applicable regulation on animal experimentation and welfare. These procedures were approved by the University of Pennsylvania Institutional Animal Care and Use Committee. Six- to eight-week-old female C57BL/6J mice were obtained from the Jackson Laboratory or and maintained on a chow diet. Mice received an intraperitoneal (IP) injection of a dose of 625 μg/kg LPS in PBS. At various time intervals, mice were anesthetized or euthanized by IP administration of ketamine/xylazine, then bled retroorbitally. For extraction of tissues, the heart was perfused with ice-cold PBS and organs excised and stored in RNAlater solution (Ambion) at 4°C.

S1.3. RNA isolation and gene expression analysis

Total RNA was isolated using EZ1 RNA Mini Kit (QIAGEN) according to the manufacturer's instructions. One μg of total RNA was reverse transcribed using the High Capacity cDNA Reverse Transcription Kit (ABI). Real-time quantitative PCR was performed on an Applied Biosystems 7300 sequence detector. Primer and probe sequences are available on request.

Supplemental Figure Legends:

Supplemental Figure. GPR109A expression in mouse liver stimulated by LPS *in vivo* and *ex vivo*. Mice received an IP injection of a dose of 625 μg/kg LPS in PBS. (A) Time course for changes in mouse liver GPR109A expression following LPS stimulation. Liver GPR109A mRNA was measured by quantitative real time PCR (QRT-PCR). Data are expressed as mean ± SEM, n=4 for each time point. ***P <0.01. (B) Western blotting demonstrating the substantial increase expression of GPR109A protein in liver response to LPS. Representative blot for 3 separate experiments. (C) LPS increases hepatocyte GPR109A expression in vivo. Mouse hepatocytes and liver tissue were isolated at 0 and 2 hours after in vivo LPS injection, followed immediately by total RNA preparation for GPR109A mRNA analysis by QRT-PCR. Data are expressed as mean ± SEM, n=3, ***$P<0.001$. (D) Time course of GPR109A expression in primary murine hepatocytes after LPS treatment. Hepatocytes were treated with 100 ng/ml LPS for different times, and GPR109A mRNA was measured by quantitative PCR. Data are expressed as mean ± SEM, n=3, ***$P < 0.01$, *$P <0.05$.