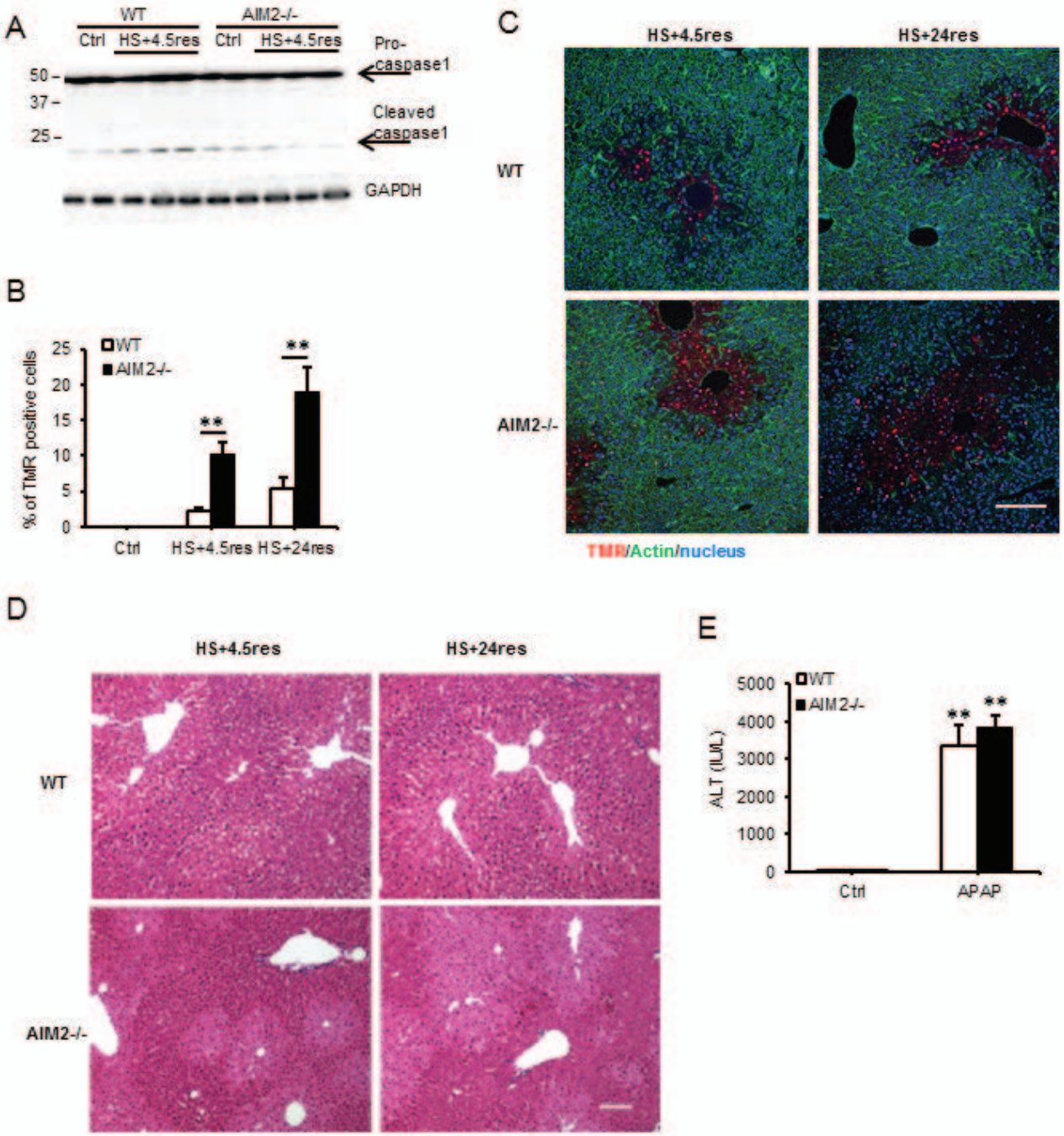
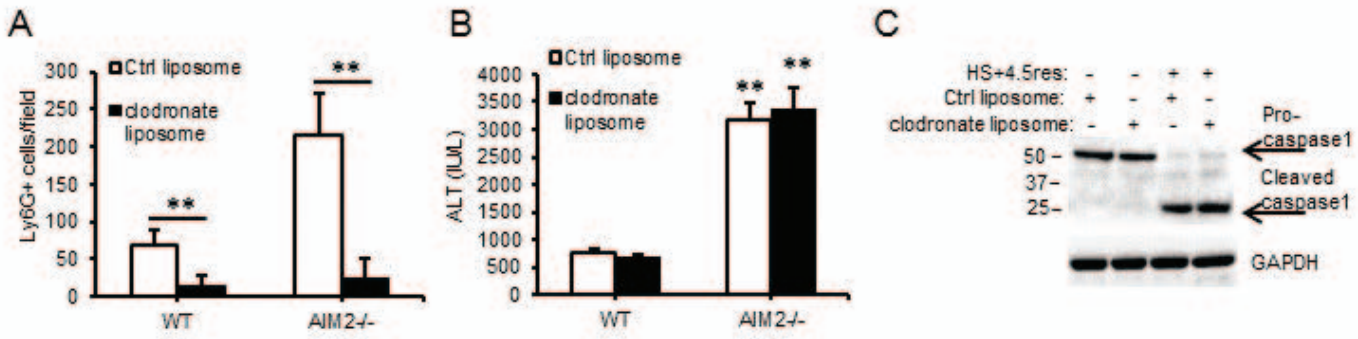
Supplemental Figure 1



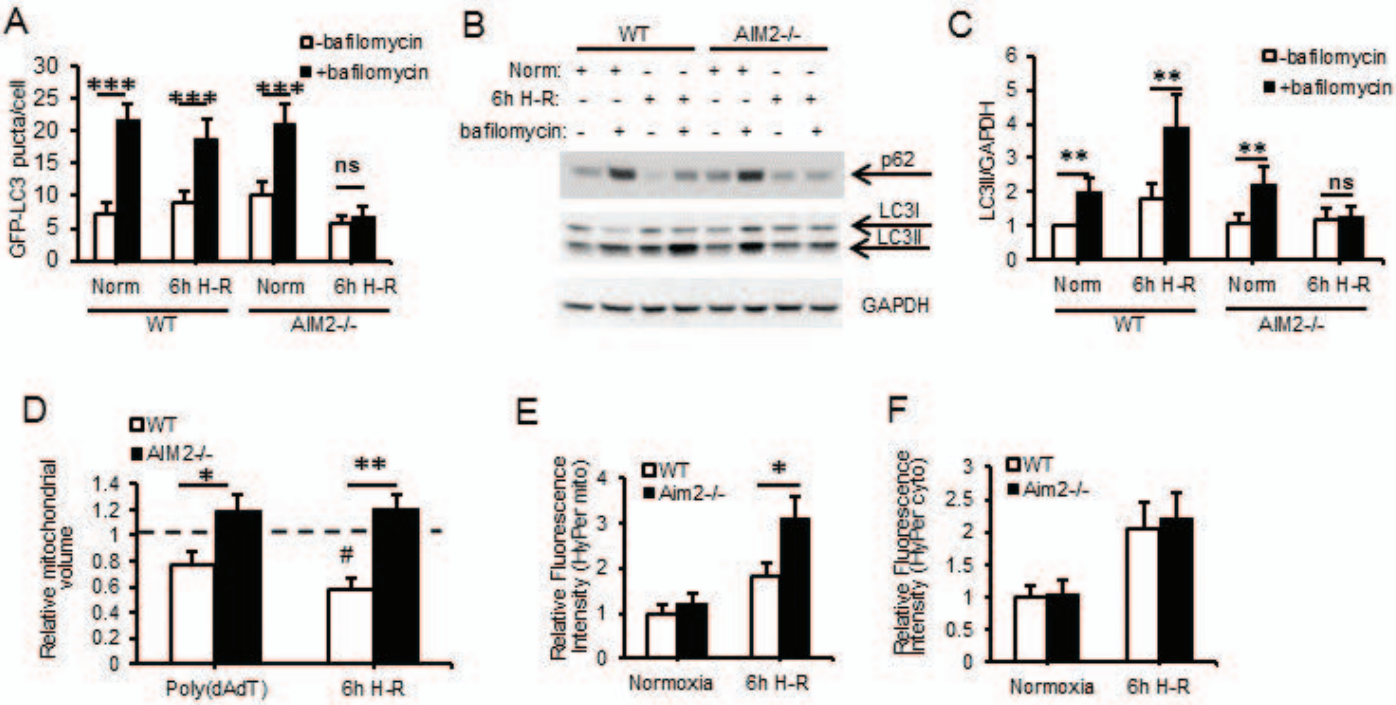
Supplemental Figure 1: AIM2 is liver protective during hemorrhagic shock (A) Western blot of caspase-1 in WT and AIM2-/- liver lysates from control (Ctrl) mice and mice after HS+4.5h of resuscitation (res). (B) Cell death and (C) Representative staining (scale bar=100μm) in livers of Control/AIM2-/- mice after HS/R as measured by TMR staining (n=3/gp control; n=7/gp HS/R; **P<0.01); (D) Representative H&E staining (scale bar=100μm) in livers of Control/AIM2-/- mice after HS/R; (E) Plasma ALT in Control/AIM2-/- mice treated with APAP (400 mg/kg) for 12h (n=3/gp control; n=6/gp HS/R; **P<0.01).

Supplemental Figure 2



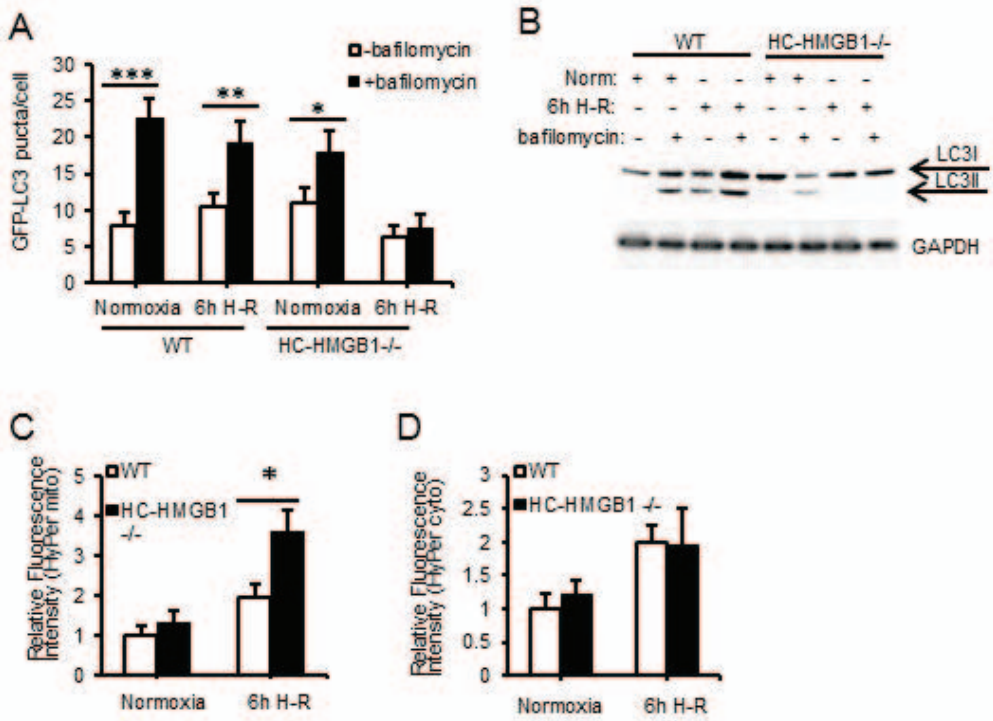
Supplemental Figure 2: AIM2-mediated caspase-1 activation and hepatoprotection is not dependent on liver macrophages during hemorrhagic shock (A) Quantification of immunofluorescent staining of liver macrophages (Ly6G+ cells) from control liposome-treated and clodronate liposome-treated (macrophage-depleted) mice after HS+4.5h of resuscitation (n=10/gp; **P<0.01); (B) Plasma ALT in Control/AIM2-/- mice treated with Control liposome/clodronate liposome after HS/R (n=3/gp control; n=6/gp HS/R; **P<0.01); (C) Representative western blot of caspase-1 in WT liver lysates from control/clodronate liposome-treated mice after HS+4.5h of resuscitation (res).

Supplemental Figure 3



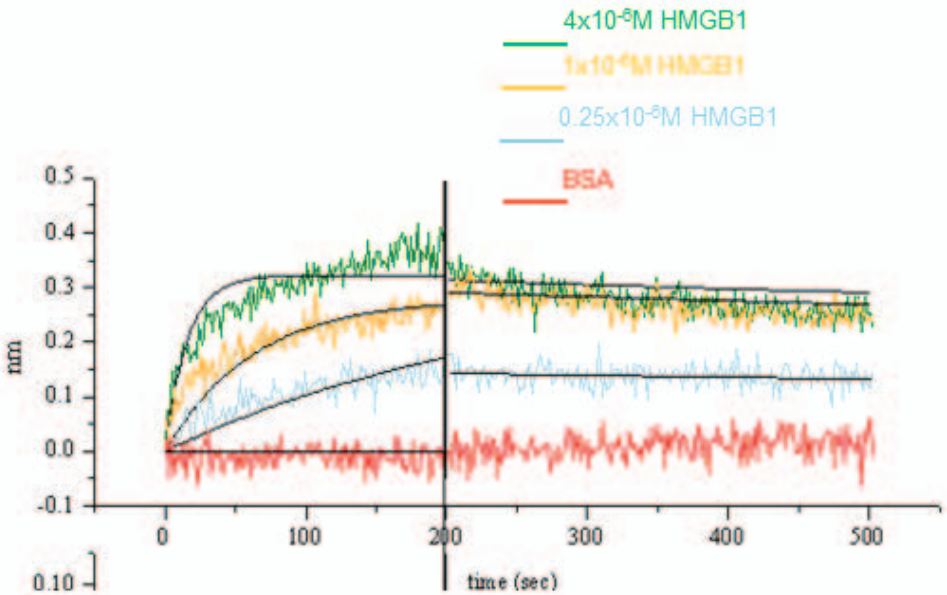
Supplemental Figure 3: **AIM2 is essential for up-regulation of mitochondrial autophagy in hepatocytes after redox stress.** (A) Autophagosomes (GFP-LC3 puncta) in WT/AIM2$^{-/-}$ hepatocytes after 6h hypoxia/1h reoxygenation+/-bafilomycin (1h; 50nM) (***P<0.001); (B) LC3 and p62 levels in WT/AIM2-/- hepatocytes after 6h hypoxia/1h reoxygenation +/- bafilomycin (1h; 50nM); data representative of at least 3 separate repeats; (C) Quantification of LC3II levels from 3 separate repeats, normalized by GAPDH expression; (D) Mitochondrial volume in WT and AIM2-/- hepatocytes cultured under normoxia or treated with 6h hypoxia/1h reoxygenation. Data are shown as percentage of normoxic levels (#P<0.05, Normoxia vs Hypoxia-reoxygenation. *P<0.05, **P<0.01, WT vs AIM2 -/-).(E) Mitochondrial and (F) cytosolic ROS measured by HyperMito or HyperCyto fluorescence respectively in hepatocytes after 6h hypoxia/1h reoxygenation (*P<0.05). Data are presented as mean±SEM. Data shown are representative of 3 independent experiments.

Supplemental Figure 4



Supplemental Figure 4: **HMGB1 is essential for up-regulation of mitochondrial autophagy in hepatocytes after redox stress.** (A) Autophagosomes (GFP-LC3 puncta) in WT/HC-HMGB1-/- hepatocytes after 6h hypoxia/1h reoxygenation+/-bafilomycin (1h; 50nM) (*P<0.05;**P<0.01;***P<0.001); (B) LC3 levels in WT/HMGB1-/- hepatocytes after 6h hypoxia/1h reoxygenation +/- bafilomycin (1h; 50nM); data representative of at least 3 separate repeats; (C) Mitochondrial and (D) cytosolic ROS measured by HyperMito or HyperCyto fluorescence respectively in hepatocytes after 6h hypoxia/1h reoxygenation (*P<0.05). Data shown are representative of 3 independent experiments. Data shown are representative of 3 independent experiments.

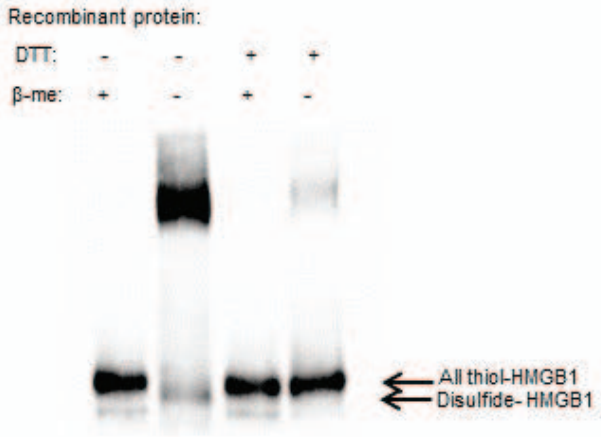
Supplemental Figure 5



Supplemental Figure 5: HMGB1 binds AIM2 in a concentration-dependent manner. Representative associate/disociation curves of AIM2 with increasing concentrations of HMGB1. GST biosensors loaded with 250nM GST-AIM2 and introduced into solutions containing 0.25, 1 or $4x10^{6}$ M HMGB1 or bovine serum albumin (BSA-negative control). Experiments repeated 3 times.

Supplemental Figure 6

a



Supplemental Figure 6: Recombinant HMGB1 is fully reduced after 5mM DTT. Mobility shift assay of 1μg Myc-HMGB1 recombinant protein under oxidizing (350 mM β-ME) or reducing (5mM DTT) conditions for 10min. Data shown are representative of 3 independent experiments.