Supplemental Information

Supplemental Results

To determine the effect of hepatocyte cPLA$_2\alpha$ in LPS/D-GalN-induced liver injury, the cPLA$_2\alpha$ transgenic mice and wild type mice at 6–8 weeks of age (all male) were administered intraperitoneally 60 ng/g body weight of LPS (*Escherichia coli* O55: B6, Sigma) in combination with 800 μg/g body weight of D-galactosamine (GalN) (Sigma) (the reagents were dissolved in sterile nonpyrogenic saline solution). The animals were sacrificed 5 hours after injection to obtain blood samples for liver enzyme assay and liver tissues for histological evaluation. The cPLA$_2\alpha$ transgenic mice showed significantly higher serum alanine transaminase (ALT) and aspartate transaminase (AST) levels than wild type mice (**Figure S2**). More prominent liver damage was observed in the cPLA$_2\alpha$ transgenic than in wild type mice (**Figure S3**). In the cPLA$_2\alpha$ transgenic group, massive hemorrhagic necrosis and hepatocyte apoptosis were observed, with prominent vascular congestion and neutrophil infiltration; only residual areas of surviving hepatocytes were present, showing vacuolar degeneration and cytoplasmic swelling. In contrast, only mild scattered necrosis and apoptosis were observed in the wild type mice. The number of TUNEL-positive hepatocytes in the cPLA$_2\alpha$ transgenic mice is significantly higher than in the wild type mice (58.83$\pm$1.26% vs 16.50$\pm$1.01%, $p<0.01$). These findings suggest that hepatic overexpression of cPLA$_2\alpha$ accelerates LPS-induced liver failure.

Supplemental Figure Legends

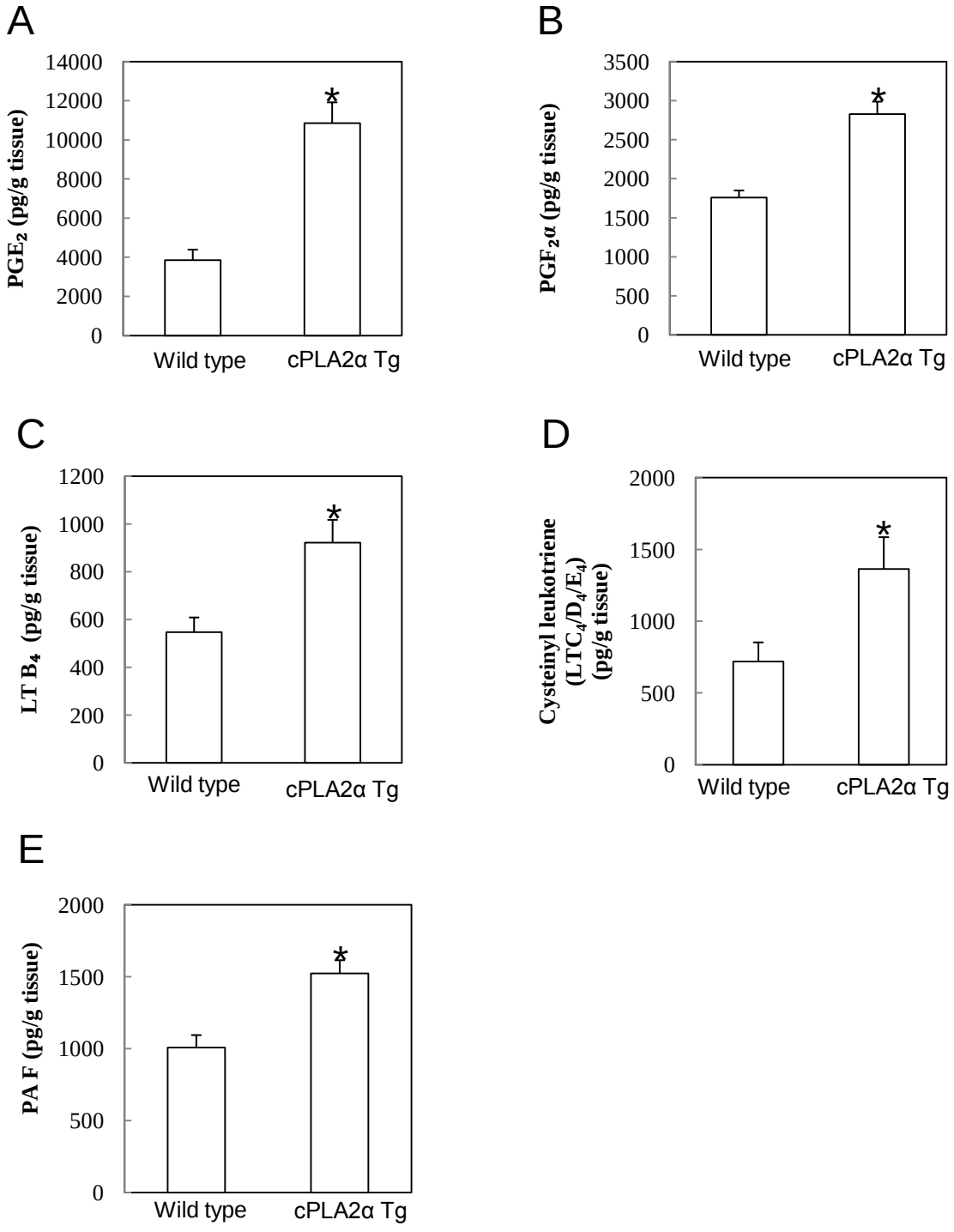
Figure S1. The levels of PGE_2, $PGF_{2\alpha}$, LTB_4, $LTC_4/D_4/E_4$ and PAF in the liver tissues from wild type and cPLA$_2\alpha$ transgenic mice. Liver tissue samples were snap-frozen in liquid nitrogen immediately upon collection and then stored at –80°C. 1g of liver tissue was placed in 5ml buffer (0.1M phosphate, pH7.4, containing 1mM EDTA and 10μM indomethacin) for homogenization. The tissue homogenates were then spun at 8,000×g for 10 min. The supernatants were collected, extracted and analyzed by EIA

according to the manufacture's instructions. The data are expressed as mean + SD ($^{*}p<0.05$, n = 6).

Figure S2. Hepatic expression of cPLA$_2\alpha$ enhances serum transaminase levels induced by LPS. The cPLA$_2\alpha$ transgenic mice and the age/sex-matched wild type mice were administered intraperitoneally 60 ng/g body weight of LPS in combination with 800 μg/g body weight of D-galactosamine (D-GalN). The animals were sacrificed 5 hours after injection. Blood samples were collected and sera were separated for transaminase analysis. The cPLA$_2\alpha$ transgenic mice show significantly higher serum ALT and AST levels than the wild type mice after LPS/D-GalN treatment. The data are expressed as mean ± SD from 6 mice (*$p<0.01$ vs. corresponding wild type mice, Student's t test).

Figure S3. Hepatic expression of cPLA$_2\alpha$ aggravates LPS-induced liver injury. The cPLA$_2\alpha$ transgenic mice and the age/sex-matched wild type mice were administered intraperitoneally 60 ng/g body weight of LPS in combination with 800 μg/g body weight of D-GalN. The animals were sacrificed 5 hours after injection and the liver tissues were harvested for histological evaluation. Formalin-fixed and paraffin-embedded sections (5 μm thick) were stained with hematoxylin and eosin (H&E), terminal deoxynucleotidy1-transferase-mediated deoxyuridine triphosphate-digoxigenin nick-end labeling (TUNEL). Histopathological characteristics of the liver tissues are shown. H&E stain (upper) at a magnification of ×200. The livers of cPLA$_2\alpha$ transgenic mice (right panel) exhibit more prominent hemorrhage necrosis, hepatocyte apoptosis and degeneration when compared to the livers of wild type mice (left panel). TUNEL stain (lower) at a magnification of × 200 in liver tissues of LPS-treated mice. The number of TUNEL-positive hepatocytes in cPLA$_2\alpha$ transgenic mice is significantly higher than in wild type mice.

Figure S1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2000
1500
1000
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Wild type
cPLA2α Tg
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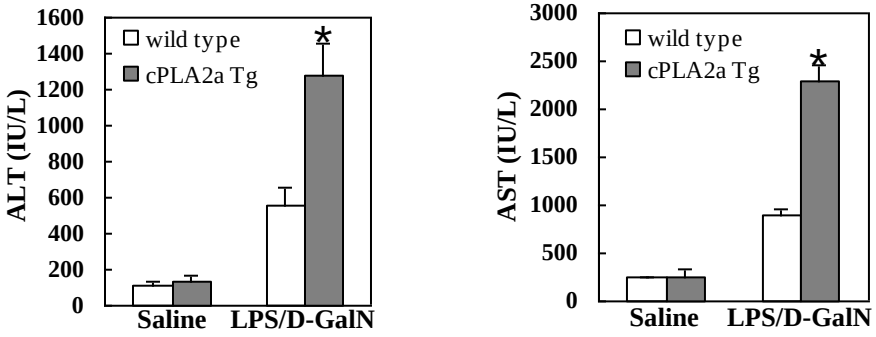
Wild type
cPLA2α Tg

Figure S2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wild type
cPLA2a Tg
*
Saline
LPS/D-GalN
AST (IU/L)
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2500
2000
1500
1000
500
0
wild type
cPLA2a Tg
*
Saline
LPS/D-GalN

Figure S3