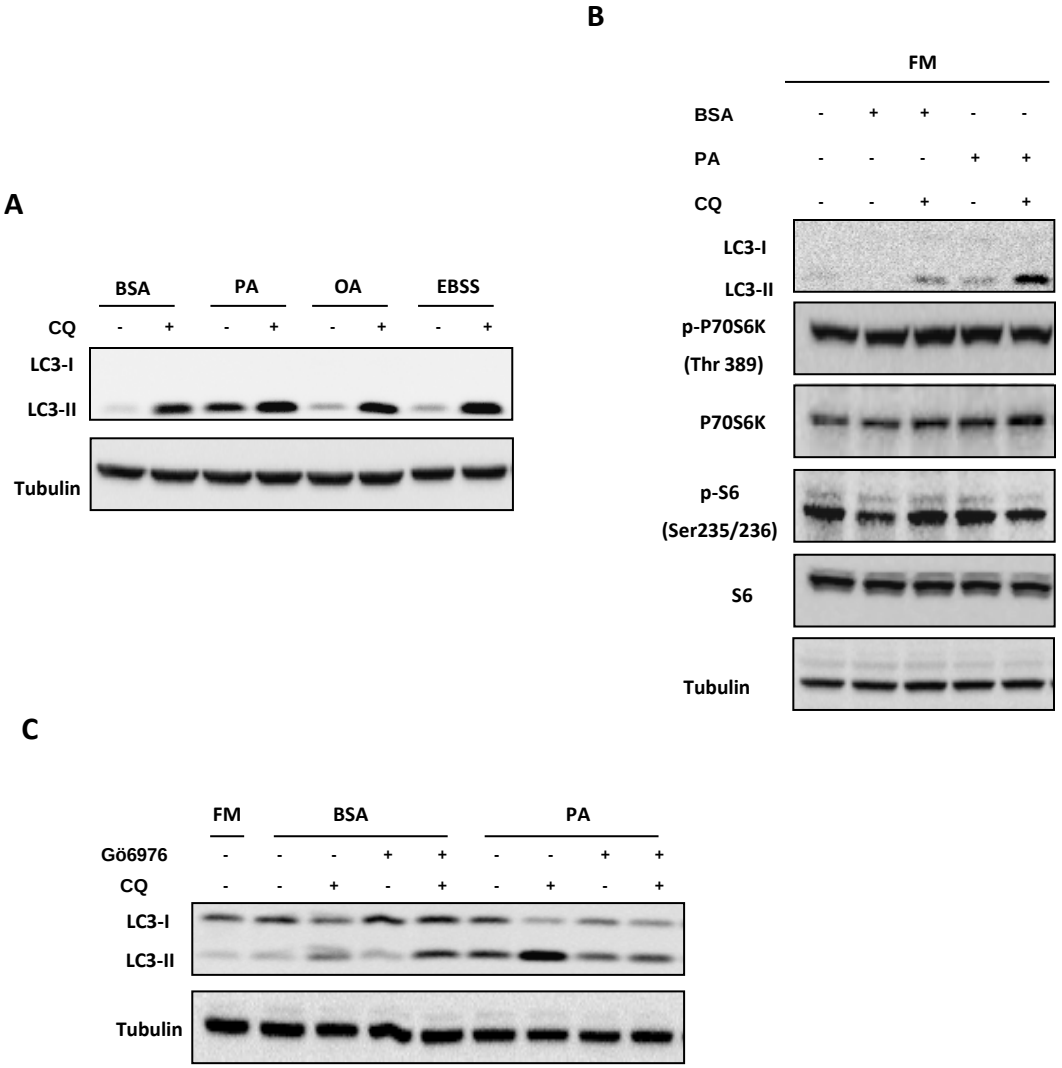
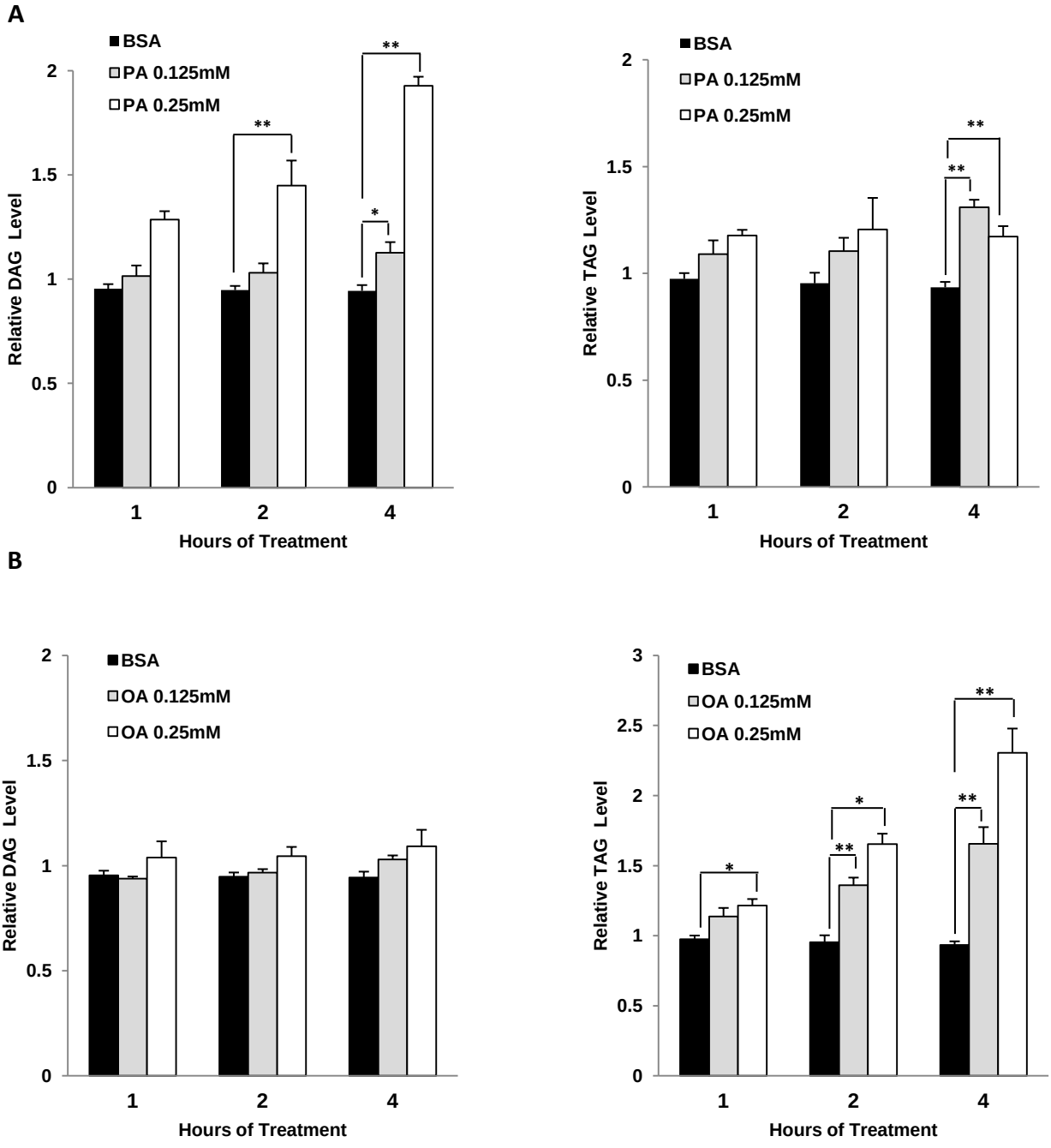
Supplementary Figure 1. PA also induces autophagy in HepG2 cells which is independent of mTOR signaling pathway but dependent on the activation of the classical PKC family A. HepG2 cells were treated with BSA control, PA (0.25mM) or OA (0.25mM) for 4 hours with or without the addition of chloroquine (CQ) (10μM). Treatment with EBSS was used as a positive control for autophagy induction in the HepG2 cells. B. HepG2 cells were treated with BSA control or PA (0.25mM) for 4 hours in full medium (FM). CQ (10μM) was added to the treated cells to determine the level of autophagic flux in the cells. C. HepG2 cells were treated with BSA control or PA (0.25mM) for 4 hours with or without the presence of the classical PKC inhibitor Gö6976 (1μM). CQ (10μM) was added to the treated cells to determine the levels of autophagic flux.

Supplementary Figure 2. PA and OA treatment induce differential accumulation of intracellular DAG and TAG which is both time and dose dependent in HepG2 cells. A. Cells were treated with BSA control and PA (0.125mM and 0.25mM) for 1, 2 and 4 hours. The total DAG (Left panel) and TAG (Right panel) levels were then quantified using LC-MS as described in Materials and Methods. The relative DAG and TAG levels were calculated by normalizing their respective levels in each treatment at different timepoints to the levels present in untreated cells. B. Cells were treated with BSA control and OA (0.125mM and 0.25mM) for 1, 2 and 4 hours. The total DAG (Left panel) and TAG (Right panel) levels were then quantified using LC-MS. The relative DAG and TAG levels were calculated as described in A. Data were presented as means ± S.D. of three independent experiments and Student's T-test were calculated between the BSA treated cells and either PA or OA treated cells at each respective timepoint. (*$p<0.05$, **$p<0.01$, student's t-test).

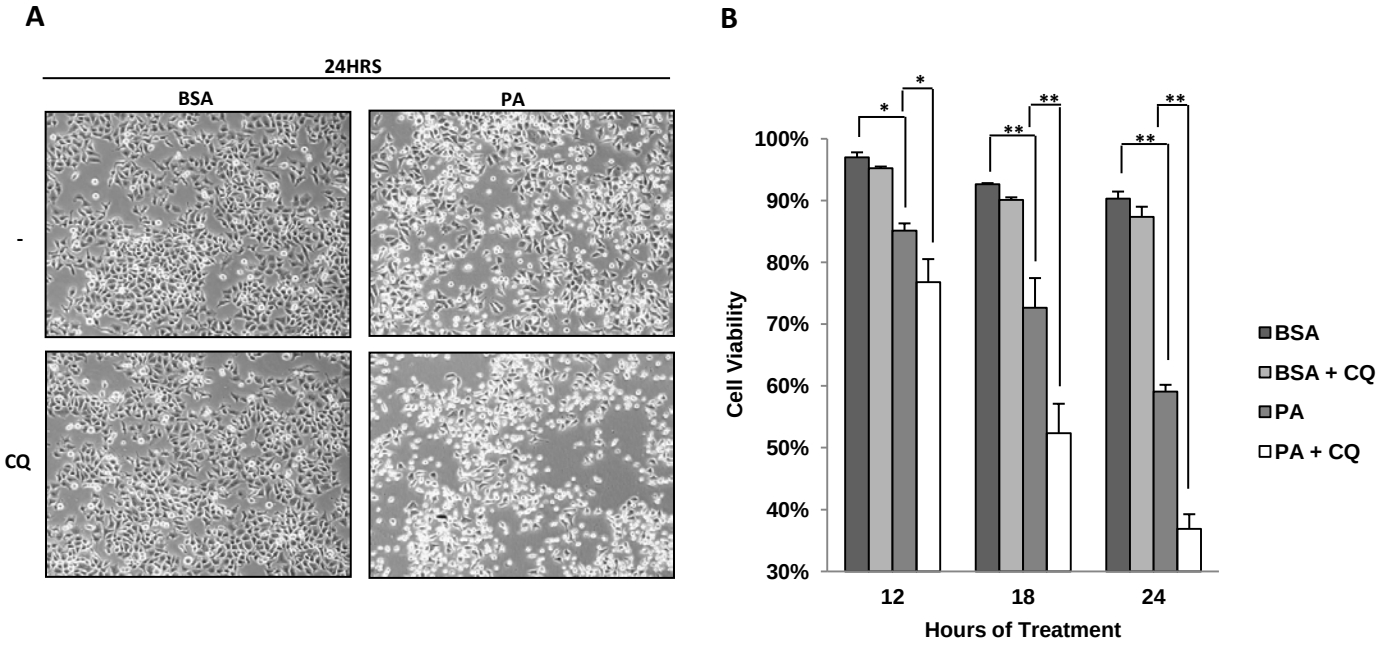
Supplementary Figure 3. Autophagic process protects HepG2 cells against lipotoxicity caused by PA. A. Cells were treated with either BSA control or PA (0.25mM) for 24 hours with or without the presence of CQ (10μM) and the cell morphology was observed under a phase contrast microscopy (×200). B. Cells were treated with BSA control or PA (0.25mM) with or without the addition of CQ (10μM) for the time points indicated and then subjected to PI staining. Quantification of the cell viability data are presented as means ± S.D. of three independent experiments. (*$p<0.05$, **$p<0.01$, student's t-test). C. Atg7 was knocked-down with Atg7 siRNA and the cells were treated with either BSA control or PA (0.25mM) for the indicated time points.

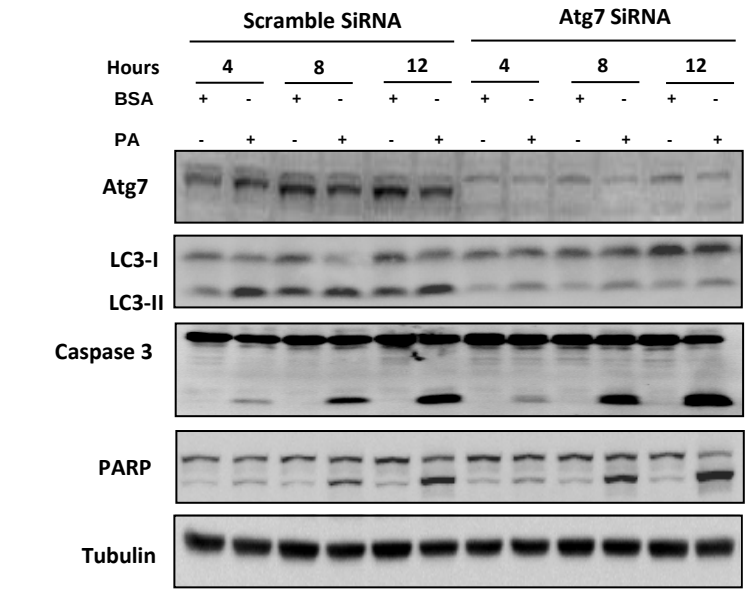


Supplementary Figure 1



Supplementary Figure 2





Supplementary Figure 3