Supplemental Figure Legends

Supplemental Figure 1: Effect of PPARγ antagonists on 15d-PGJ2 induced cytoplasmic vacuolation and cell death: HCT116 cells were pretreated with PPARγ antagonists GW9662 (20 μM) or T0070907 (20 μM) for 1h prior to the addition of 15d-PGJ2. Phase contrast image showing the failure of PPARγ antagonists in preventing 15d-PGJ2 induced cytoplasmic vacuolation (A) and cell death as measured by clonogenic assay (B).

Supplemental Figure 2: Quantitation of Bip and CHOP proteins in normal and 15d-PGJ2 treated cells.
Densitometric analysis was performed on immunoblots for Bip and CHOP proteins with dose response and time course of 15d-PGJ2 treatment. Protein levels were normalized to the levels of GAPDH expression. Fold increase in protein levels over control cells were plotted. Shown are the averages of three independent experiments ± SE for dose response and average of two experiments for time course.

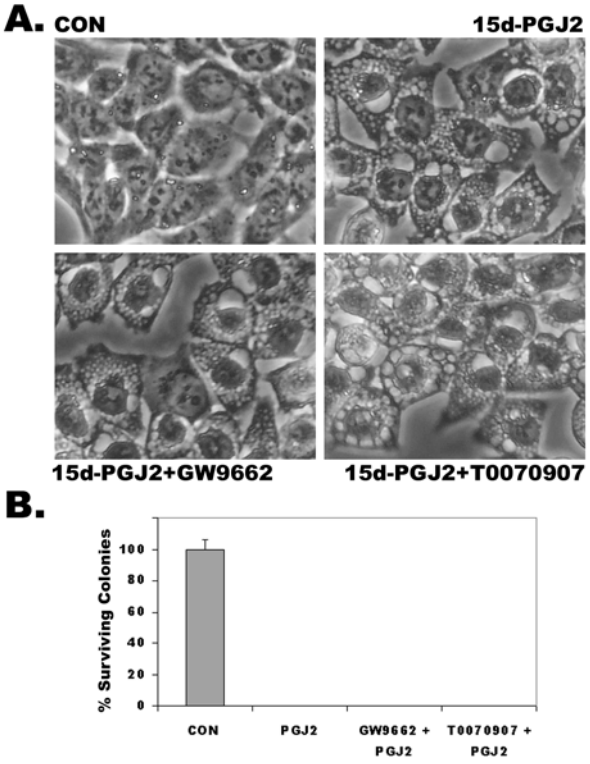
Supplemental Figure 3: Quantitation of LC3-I and II proteins in normal and 15d-PGJ2 treated cells. Quantitation of LC3 by Densitometry analysis was performed as in Supplemental Figure 2. Protein levels were normalized to the levels of GAPDH expression. Fold increase in LC3-I and LC3-II in 15d-PGJ2 treated cells over control cells was plotted. Shown are the averages of three independent experiments ± SE.

Supplemental Figure 4: Effect of cycloheximide and actinomycin D on 15d-PGJ2 induced ER stress and LC3 processing: Immunoblot showing the effect of CHX (25 or 50 μM) and Act D (8 or 16 μM) on the expression of ER stress markers Bip and CHOP, and induction and processing of LC3. Both the inhibitors blocked 15d-PGJ2 induced changes in Bip, CHOP and LC3. Inhibitors were added to cells 1h prior to the addition of 15d-PGJ2.

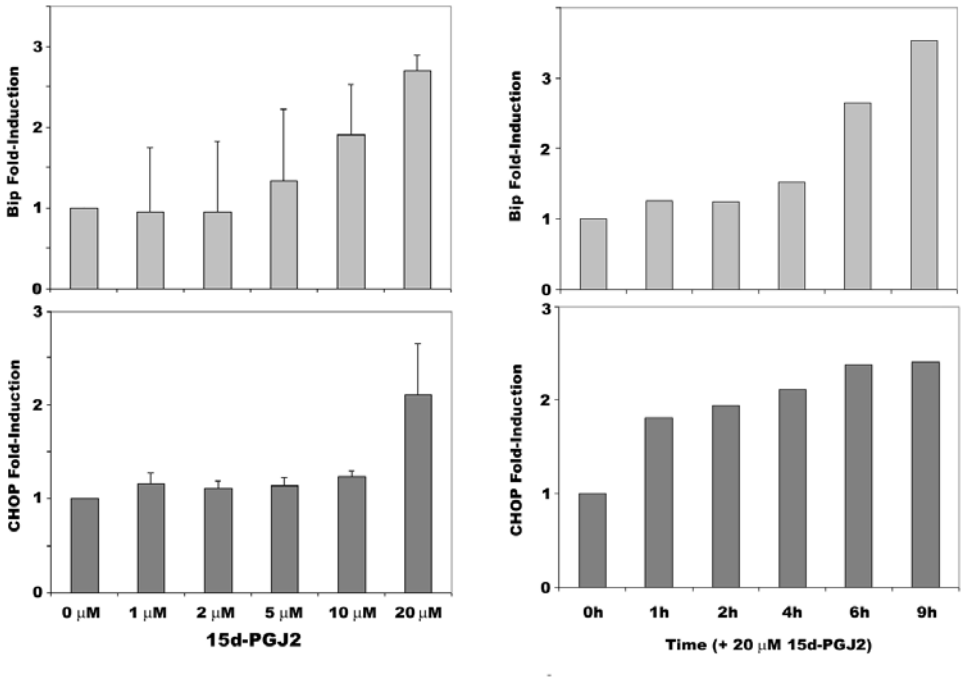
Supplemental Figure 5: Comparison of the effects of PGD2 and cyclopentenone prostaglandins PGA1, PGA2 and 15d-PGJ2 on HCT116 cells: A. Phase contrast image showing effect of 15d-PGJ2 (20 μM), PGA1 (20 μM), PGA2 (20 μM) and PGD2 (20 μM) on HCT116 cells treated for 9h. Unlike 15d-PGJ2, its precursor PGD2 or other cyclopentenone prostaglandins (PGA1 and PGA2) did not induce cytoplasmic vacuolation and these treated cells resemble untreated HCT116 cells (not shown). Even at 40 μM concentration, all three prostaglandins PGA1, PGA2 and PGD2 failed to induce cytoplasmic vacuolation in HCT116 cells (not shown). B. Western blot showing effect of 15d-PGJ2, PGA1, PGA2 and PGD2 on expression of Bip, CHOP and LC3. PGA1 and PGA2 were less potent than 15d-PGJ2 in inducing ER stress or LC3 expression.

Supplemental Figure 6: **Accumulation of p62 (SQSTM1) protein during cytoplasmic vacuolation but not in autophagy.** A. HCT116 cells were treated with 15d-PGJ2 for cytoplasmic vacuolation or serum starved for autophagy. Total extracts were collected and analyzed for p62 by immunoblotting. Note the increase in p62 levels upon 15d-PGJ2 treatment. The endosomal marker, EEA1 levels are nearly unchanged. B. Cellular distribution of p62 and EEA1, an endosomal marker, during vacuolation induced by 15d-PGJ2 (PGJ) or autophagy by serum starvation (STR) by immunofluorescence.

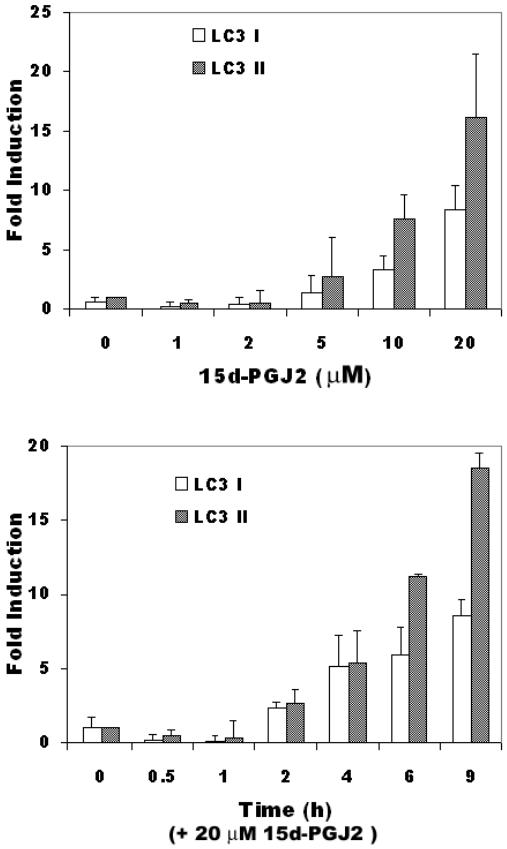
Supplemental Figure 7: **Lamp1 distribution is unaffected during 15d-PGJ2 induced cytoplasmic vacuolation.** Cellular distribution of ER marker Calnexin and lysosomal marker LAMP1 in normal (CON) and vacuolated (15d-PGJ2) cells was observed by immunofluorescence. Lysosomal distribution in the cells is unaffected by 15d-PGJ2 treatment.



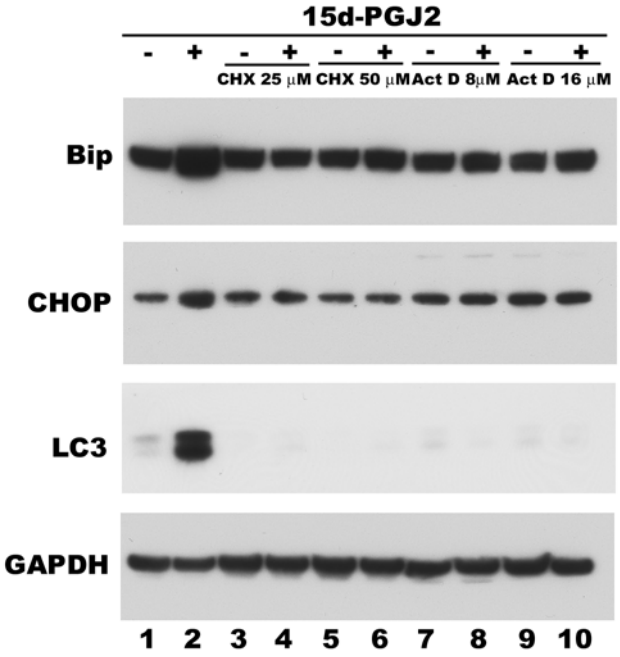
Kar et al Supplemental Figure 1



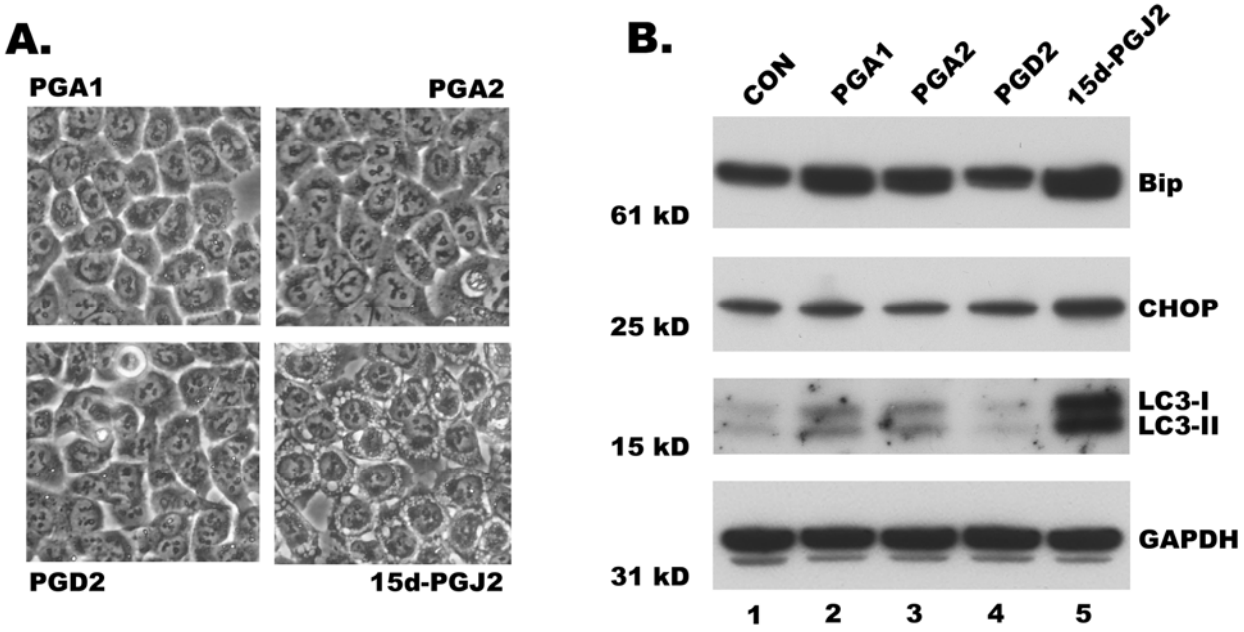
Kar et al Supplemental Figure 2



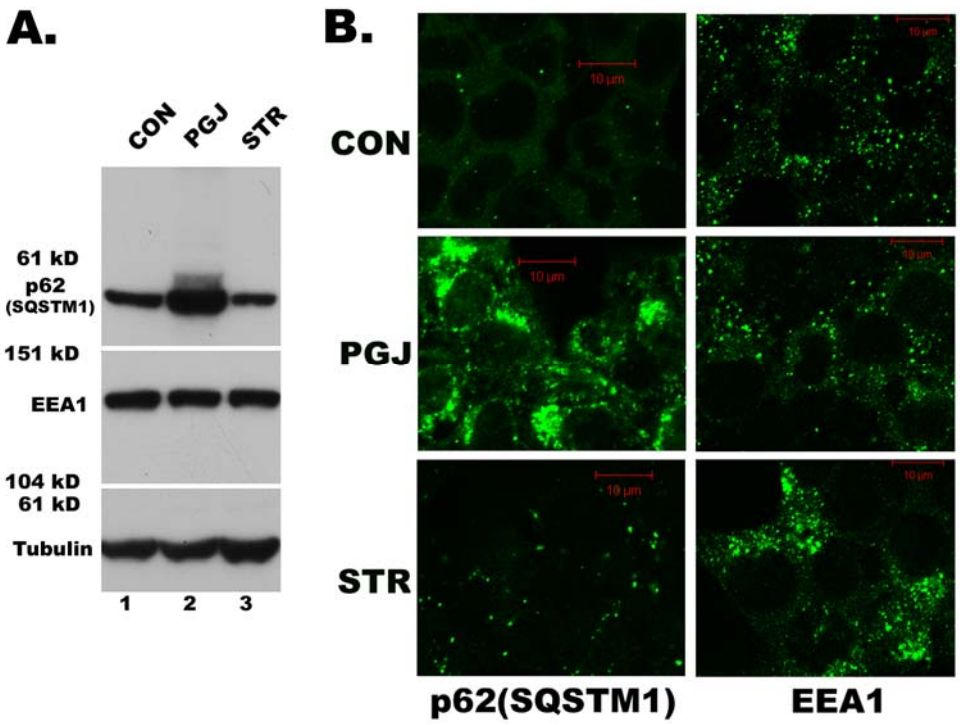
Kar et al Supplemental Figure 3



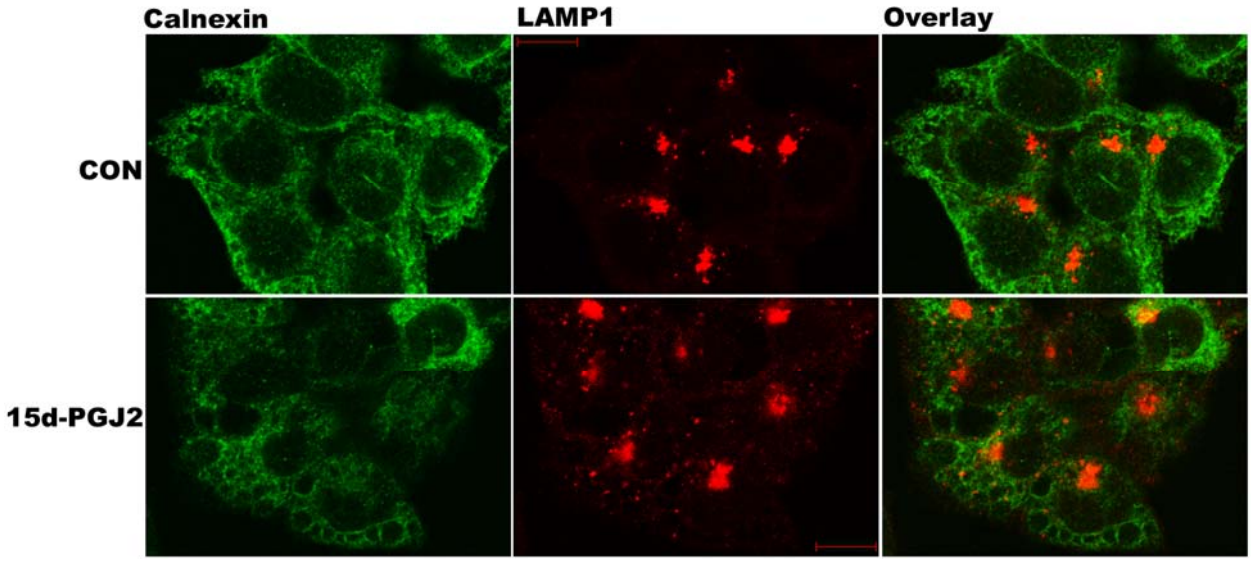
Kar et al Supplemental Figure 4



Kar et al Supplemental Figure 5



Kar et al Supplemental Figure 6



Kar et al Supplemental Figure 7