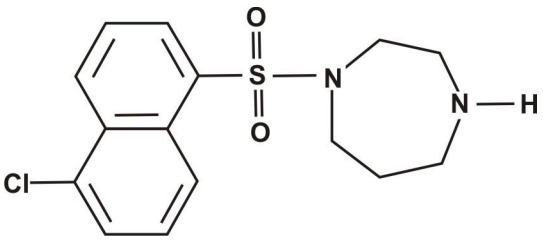
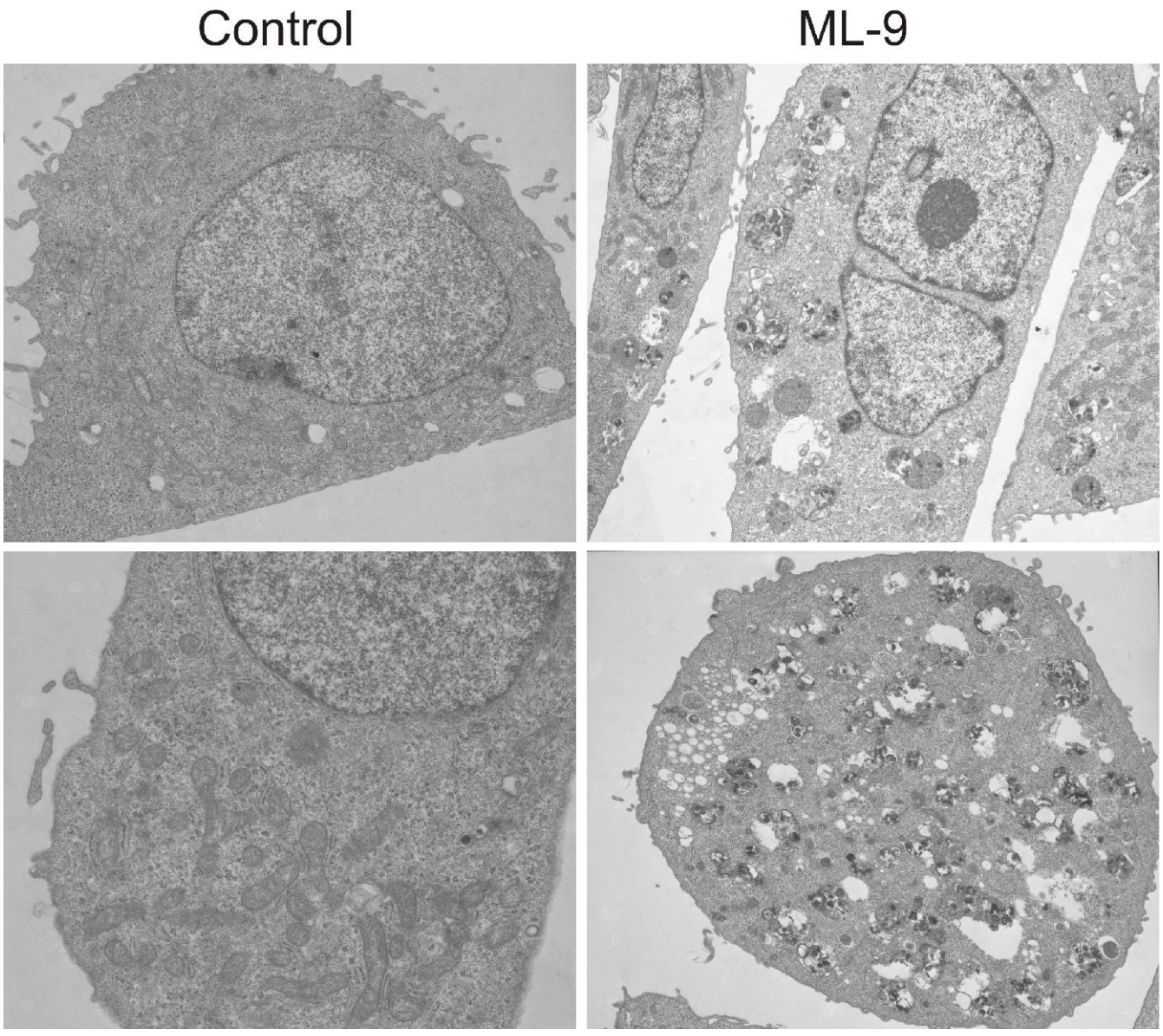
SUPPLEMENTARY FIGURES

Supplementary Figure S1



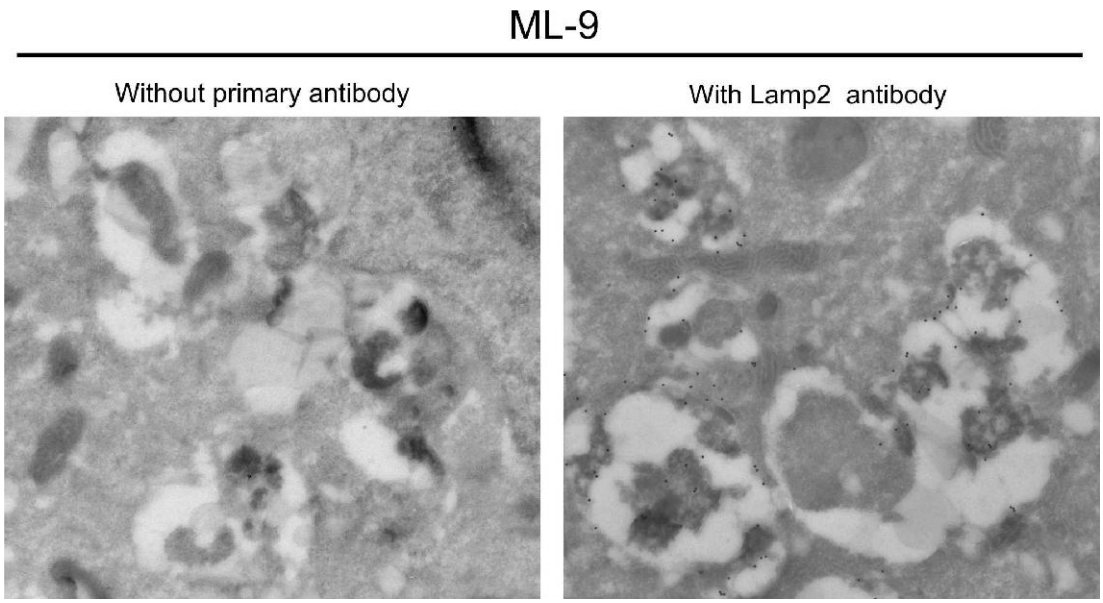
Supplementary Figure S1. Chemical structure of ML-9.

Supplementary Figure S2



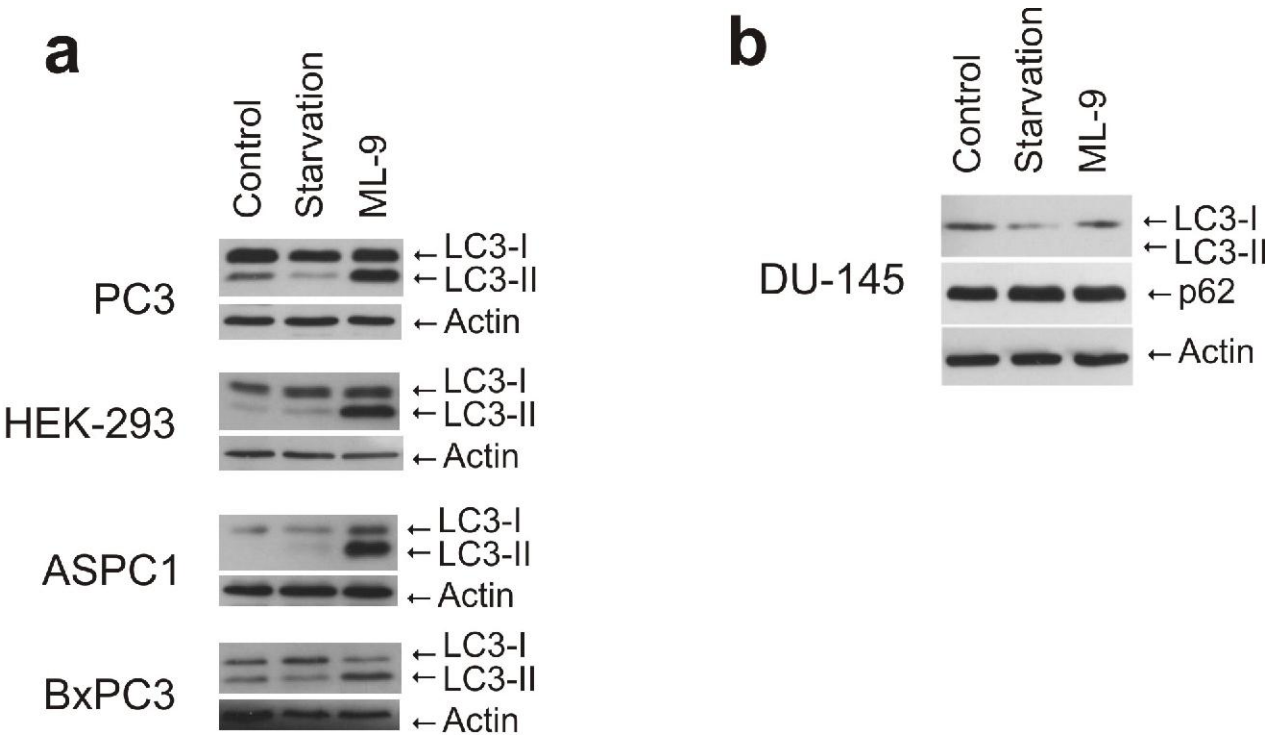
Supplementary Figure S2. ML-9 induces accumulation of autophagic vacuoles. TEM images of LNCaP cells untreated and treated with 30 μM ML-9 for 12 h. The numerous autophagosomes and autolysosomes/amphisomes are clearly visible upon ML-9 treatment. Accumulation of multi-lamellar membrane structures, small vesicles, cytoplasmic material and organelles at different stages of degradation is evident suggesting induction of phospholipodosis.

Supplementary Figure S3



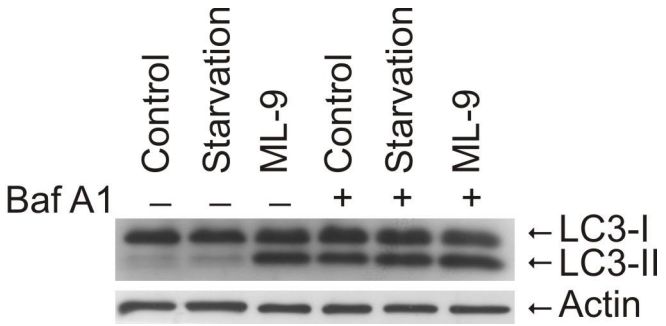
Supplementary Figure S3. ML-9 induces accumulation of Lamp2-positive autophagic vacuoles. To the right - immuno-TEM image of LNCaP cell treated with 30 μM ML-9 for 12 h shows the presence of immunogold-labeled lysosomal protein Lamp2 in large vesicles with degraded content. To the left – control of specificity (secondary antibodies without primary antibodies).

Supplementary Figure S4



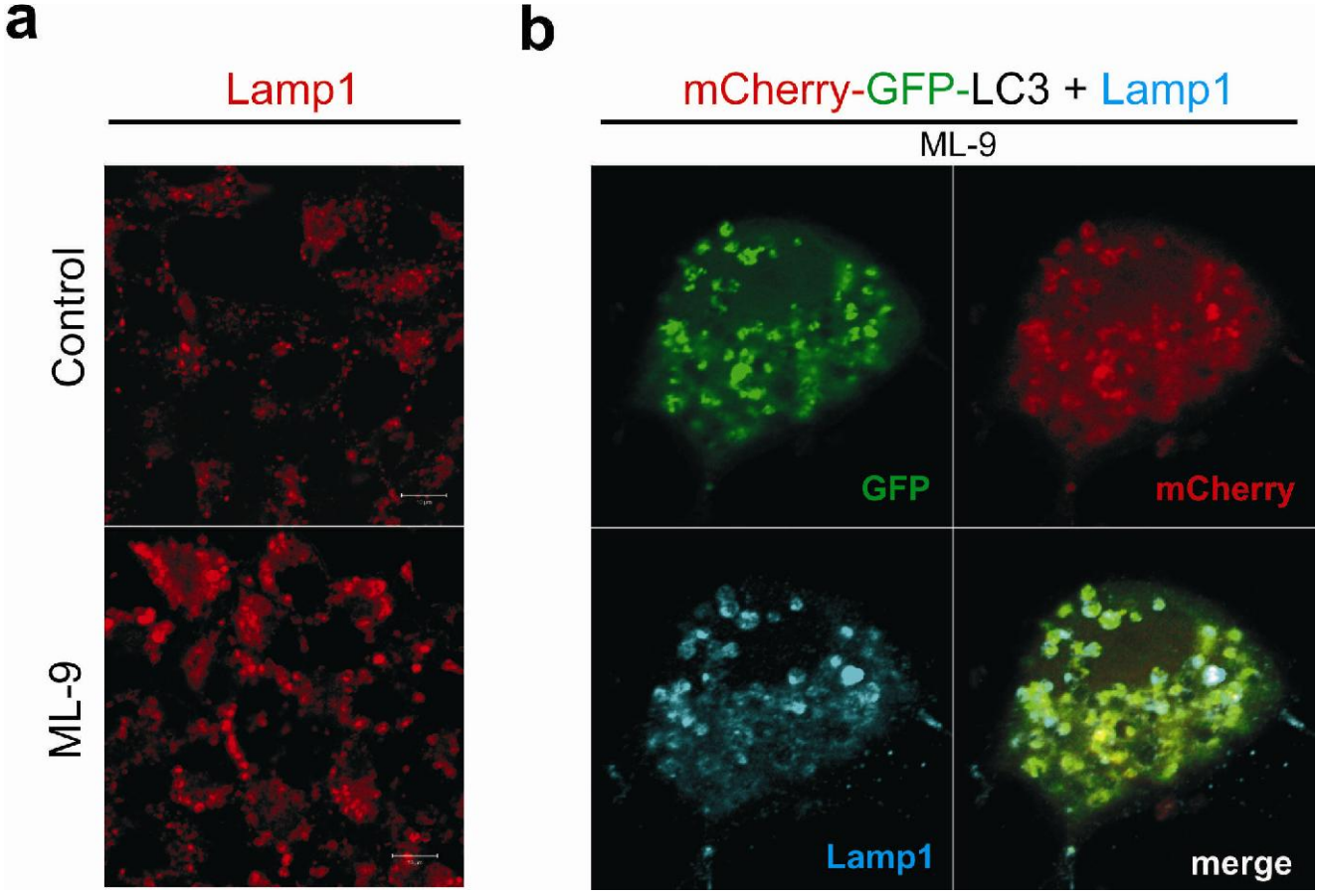
Supplementary Figure S4. (a) ML-9 caused an increase in LC3-II levels in a range of cell lines. PC-3, HEK-293, ASPC1 and BxPC3 cells were treated with full, serum-starved or 30 μM ML-9-containing media for 12 h. Increase in LC3-II levels is obvious in all the cell lines. (b) ML-9 failed to increase LC3-II and p62 levels in DU145 cells. DU145 cells were treated with full, serum-starved or 30 μM ML-9-containing media for 12 h.

Supplementary Figure S5



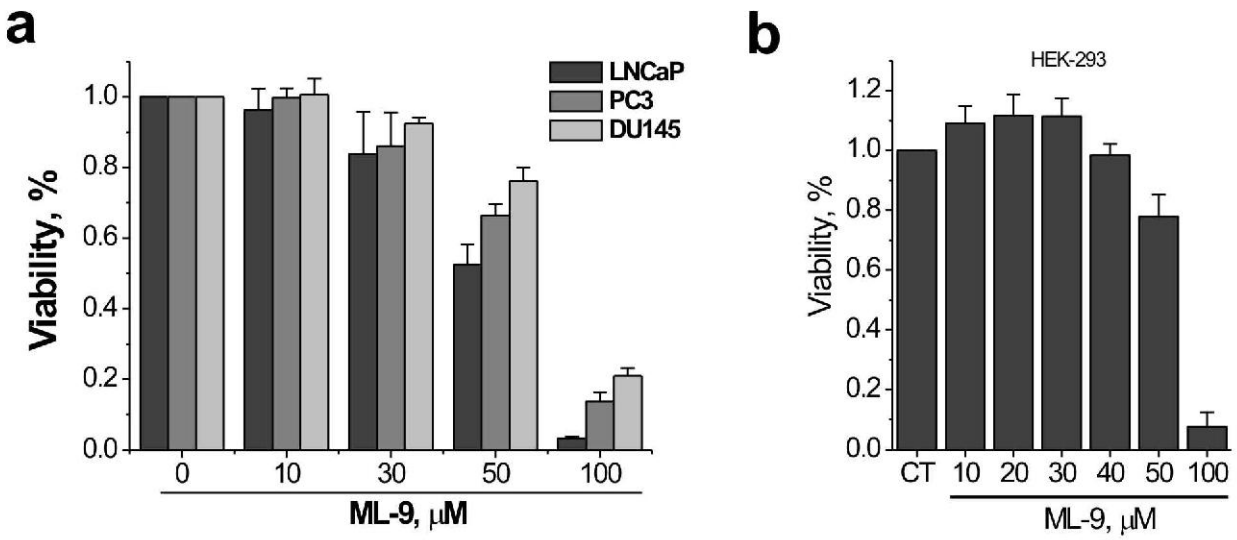
Supplementary Figure S5. ML-9 blocks autophagic flux. Bafilomycin A1 (100 nM) does not increase LC3-II levels induced by elevated concentrations of ML-9. LNCaP cells were incubated in full, serum-starved or 30 μM ML-9-containing media for 6 h in the absence or presence of 100 nM bafilimycin A1 for the last hour.

Supplementary Figure S6



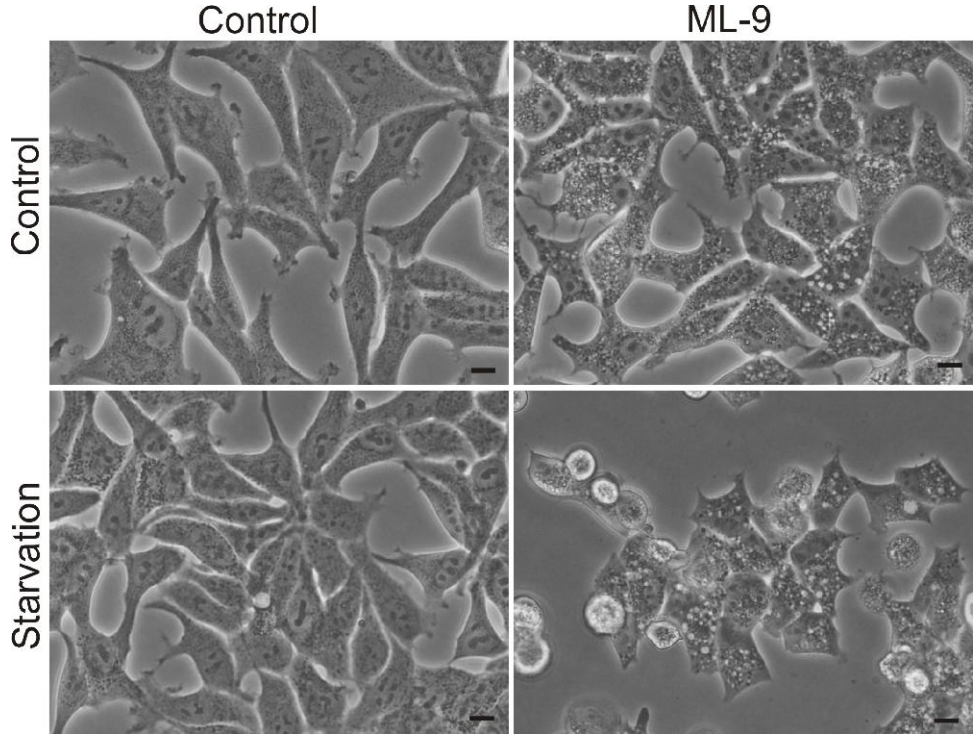
Supplementary Figure S6. ML-9 affects maturation of autophagosomes and induces lysosome swelling. (a) ML-9 visibly increased the size of Lamp1-positive vesicles/puncta. LNCaP cells were treated with full medium or ML-9 (30 μM) containing medium for 12 h. (b) ML-9 induces accumulation of mCherry-GFP-LC3B puncta, which are largely colocalized with Lamp1-positive vesicles/puncta. LNCaP cells were transiently transfected with mCherry-GFP-LC3B and treated with ML-9 (30 μM) containing medium for 12 h.

Supplementary Figure S7



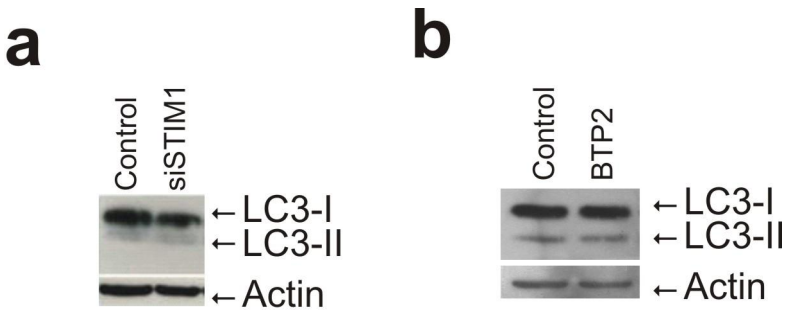
Supplementary Figure S7. ML-9 induces cell death in normal and cancer cells. (a) ML-9 reduced cell viability in a concentration-dependent manner. LNCaP, PC3 and DU145 cells, plated on 96-well plate, were treated with different doses of ML-9 in full media for 24 h. Cell viability was monitored using the CellTiter 96 Aqueous One Solution cell proliferation assay (MTS assay). Following treatment the cells were incubated with reagent solution and absorbance was recorded at 490 nm wavelength using an ELISA plate reader. Values represent means±sem. n=3. (b) ML-9 reduced HEK-293 cell viability in a concentration-dependent manner. HEK-293 cells were treated as in (a). Cell viability was monitored as in (a). Values represent means±sem. n=3.

Supplementary Figure S8



Supplementary Figure S8. ML-9 induces vacuolization. LNCaP cells were treated with 30 μM ML-9 in full or serum-starved media for 12 h. Scale bars represent 20 μm.

Supplementary Figure S9



Supplementary Figure S9. ML-9 stimulates autophagy independently of Stim1 and SOCE inhibition. (a) LNCaP cells were untransfected or transfected with siSTIM1 and the LC3-II levels were analyzed. n=3. (b) LNCaP cells were treated with full medium or 10 μM BTP2-containing medium for 6h and the LC3-II levels were analyzed. n=2.