The autophagy-senescence connection in chemotherapy; must tumor cells (self) eat before they sleep? Rachel W. Goehe, Xu Di, Khushboo Sharma, Molly L. Bristol, Scott C. Henderson, Kristoffer Valerie, Francis Rodier, Albert R. Davalos and David A. Gewirtz

Supplemental Figure 1. A) Representative histogram of a timecourse for ROS generation in MCF-7 cells exposed to 1μM ADR based on the mean intensity of 2', 7'-dichlorofluorescin (DCF) fluorescence. **B)** Cells were stained with CM-H_2DCFDA for 30 min and then observed by fluorescence microscopy. **C-D)** HCT-116 cells were pretreated with 20μM of glutathione (GSH) or 20μM N-acetyl cysteine (NAC) for 1h followed by 1μM of Adriamycin (ADR) for 2h. The drugs were removed and fresh media was restored. **C)** Senescence was evaluated by β-galactosidase staining 72 hrs post treatment. **D)** Acridine orange staining visualized by fluorescence microscopy.

Supplemental Figure 2. A) Images of p-ATM, γ-H2AX and 53BP-1 foci in MCF-7 cells exposed to 1μM ADR. **B)** MCF-7 cells were pretreated with 20μM KU55933 or 2mM caffeine for 1 h followed by 1μM of ADR for 2 h. 72h post treatment cells were imaged for p-ATM.

Supplemental Figure 3. A-B) HCT-116 p53-/- and HCT-116 p21-/- cells were treated with 1μM ADR for 2 h. β-galactosidase staining **(A)** and MDC staining **(B)** were imaged 72 h post treatment. **C)** MCF-7 WT and MCF-7 E6 cells were treated with 1μM ADR for 2 h. β-galactosidase staining (*Upper Panel*) and acridine orange staining (*Lower* Panel) were imaged 72 h post treatment.

Supplemental Figure 4. MCF-7 cells were pretreated with 10μM CQ or 200nM BAF or 1 h followed by 5μM of CPT for 2 h. **A)** Punctuate signal of RFP-LC3 imaging 72 h post treatment. **B)** β-galactosidase staining at the indicated timepoints.

Supplemental Figure 5. A model of Adriamycin or Campthothecin-induced autophagy and senescence. As MCF-7 or HCT-116 cancer cells are treated with chemotherapy the ATM-p53-p21 signaling mechanism is activated. Moreover, pRB is no longer able to by hyper-phosphorylated leading to both autophagy and senescence. Hence, chemotherapy-induced senescence and autophagy both have common signaling pathways. Activating and inhibitory relationships are denoted by → and ✖ respectively.