Supplementary Information

Involvement of autophagy in the outcome of mitotic catastrophe

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Supplementary Figures 1-4

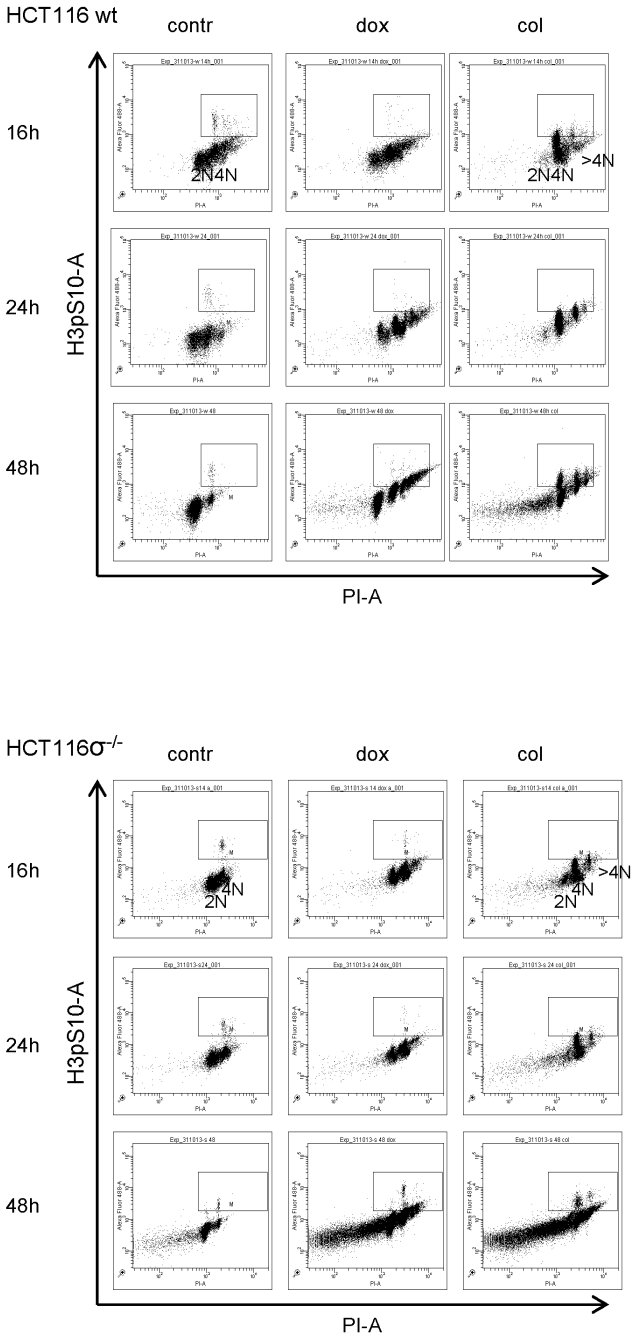


Figure S1.

Polyploidization occurs during MC. Representative images of the time-dependent polyploidy appearance (>4N cells) after treatment with doxorubicin or colcemid. Assay for the simultaneous detection of diploid and tetraploid cells by PI and cells in mitosis by an antibody against H3Sp10 (specific marker Ser-10- phosphorylated histone H3).

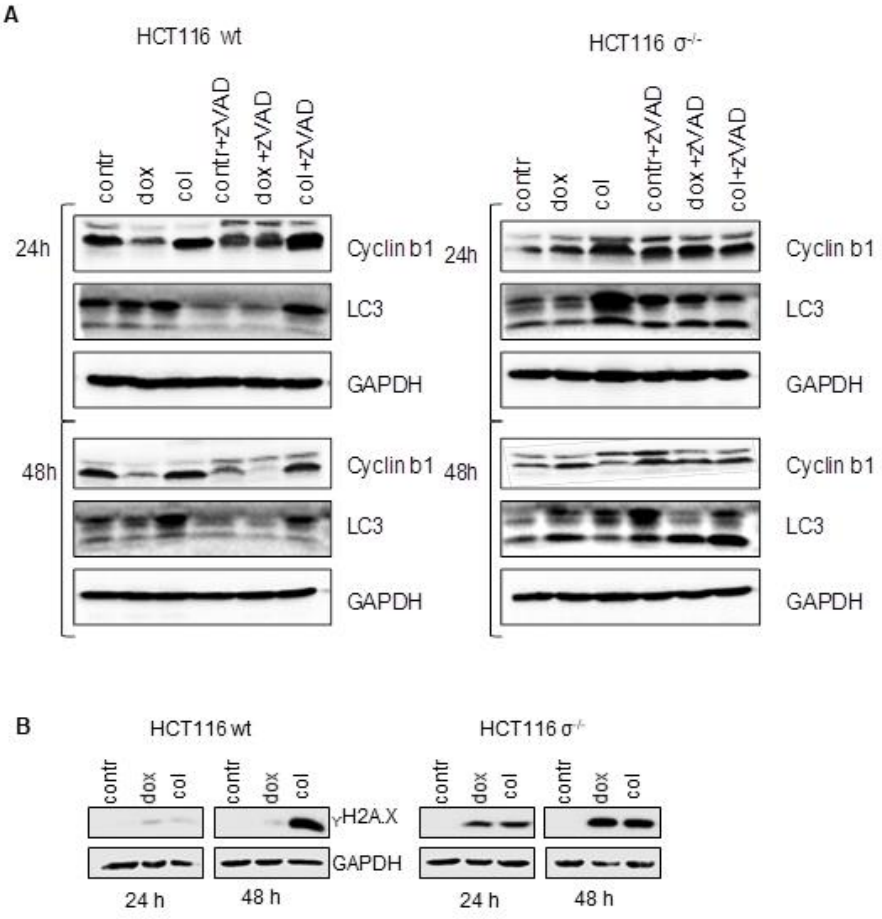


Figure S2.

Crosstalk between MC, autophagy and apoptosis. A – HCT116 and HCT116 $\sigma^{-/-}$ cells were treated with zVAD-FMK (40 μM), 1h after doxorubicin (600 nM) or colcemid (0.1 μg/ml) was added. Cell lysates were probed by western for cleavage of LC3 and level of cyclin B1. GAPDH was used as a loading control. **B -** Assessment of γH2A.X accumulation in HCT116 and HCT116 $\sigma^{-/-}$ after MC stimulation. GAPDH was used as a loading control.

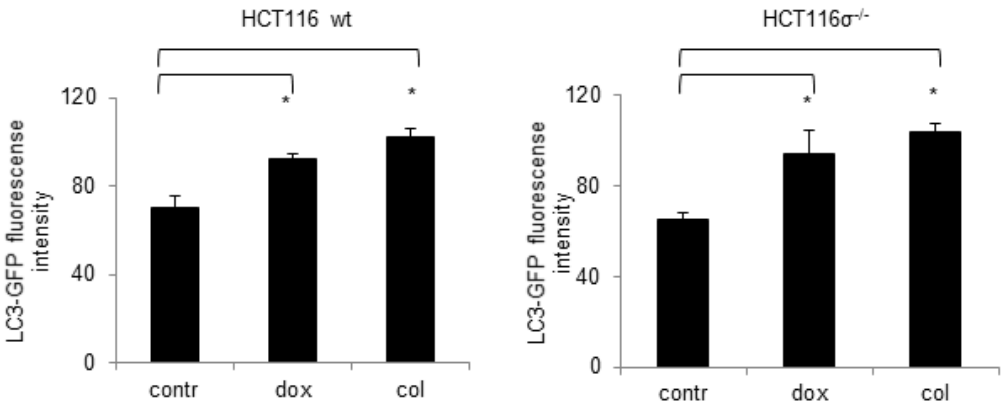
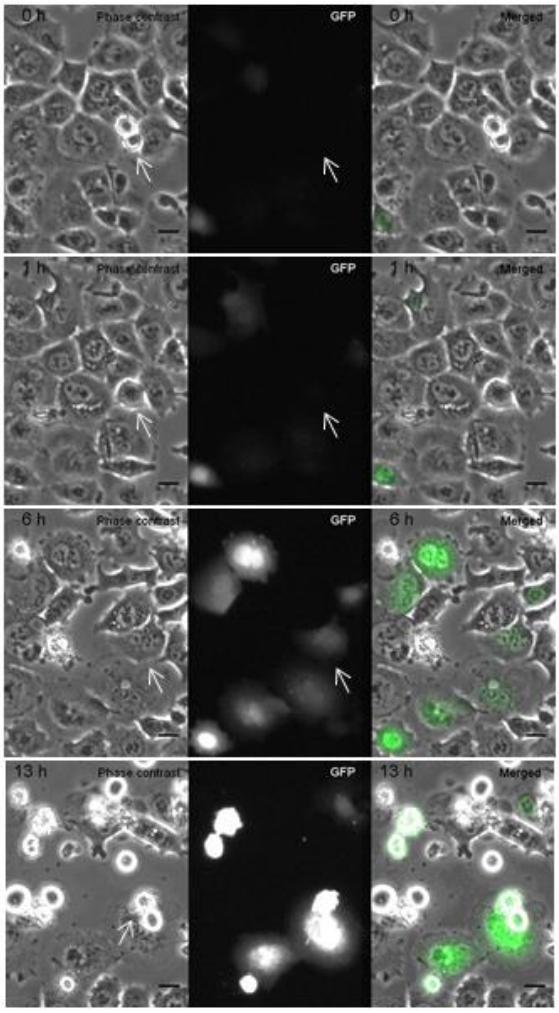


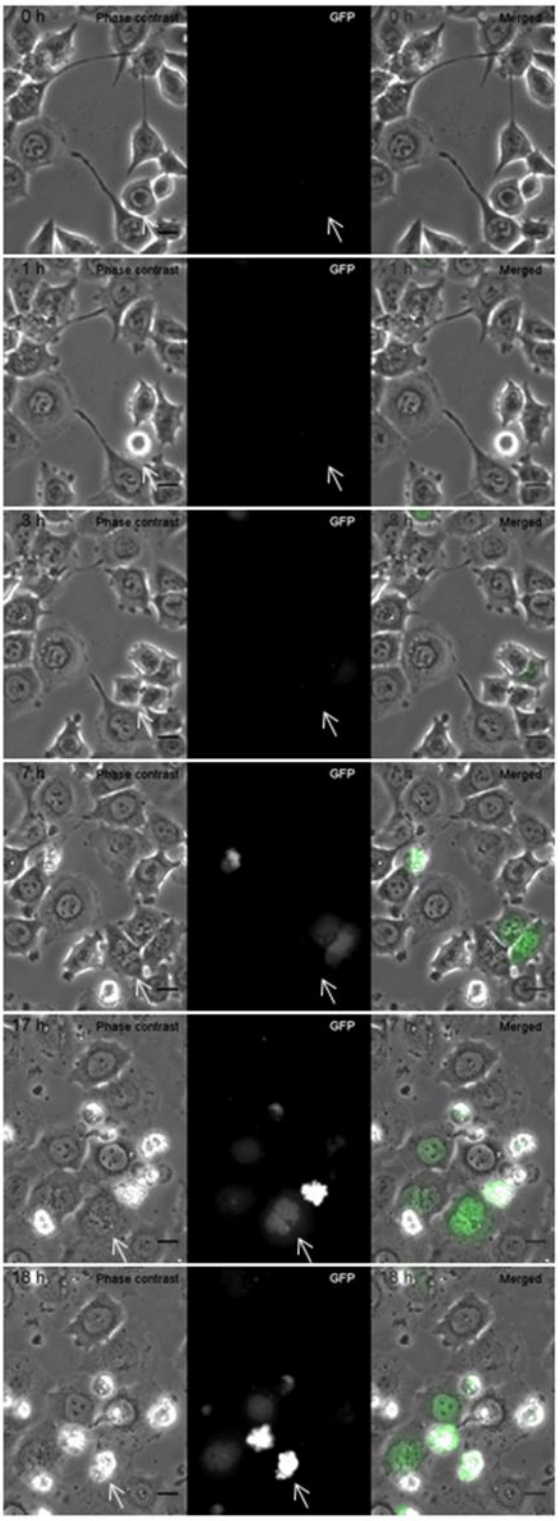
Figure S3.

MC stimulation promotes autophagy flux in HCT116 cells. HCT116 wt and HCT116 $\sigma^{-/-}$ cells were transfected with pET28a-LC3-GFP plasmid, after 6h they were co-treated with pepstatin A (5 μg/ml) and E64D (2 μg/ml). After 1h, doxorubicin (600 nM) or colcemid (0.1 μg/ml) were added. The cells were analyzed by live-imaging for 3 days at 10× magnification; scale bar, 20 μm. Each cell population calculated in the nine different fields of view. Bar chart represents the quantitation of autophagic cells with of maximum LC3-GFP fluorescence ranges. *$p<0.05$ (U test)



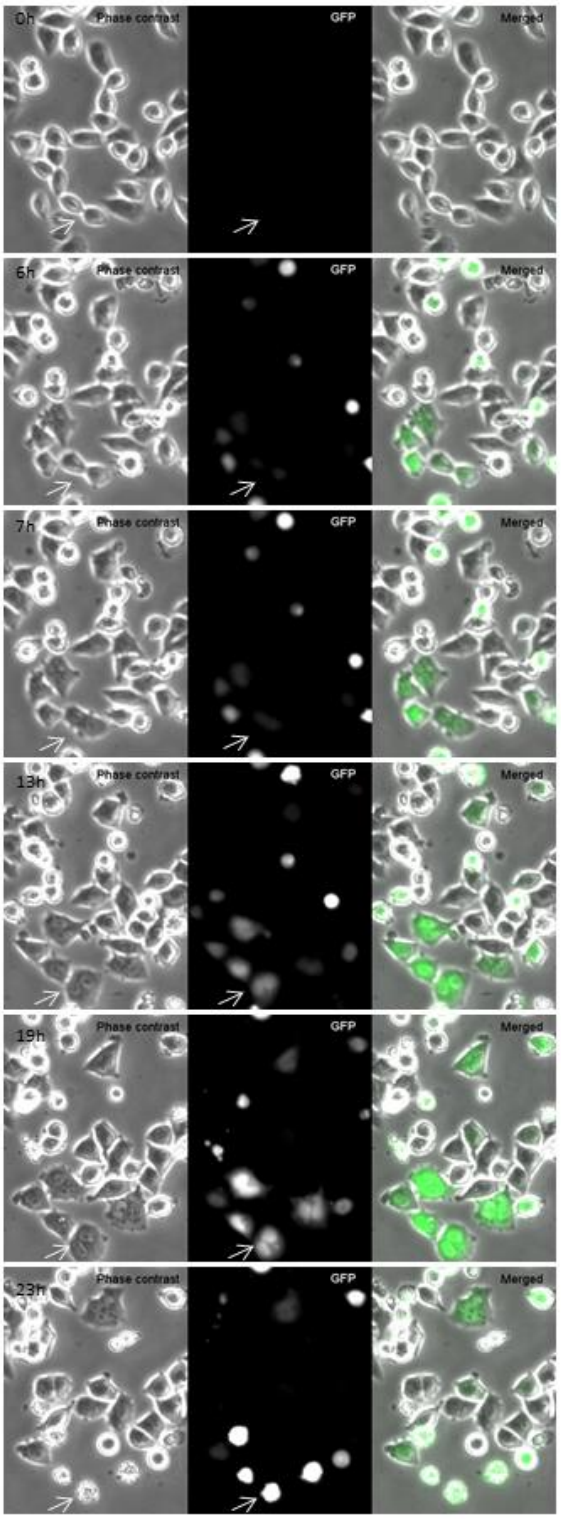
HCT116σ$^{-/-}$dox

S4A

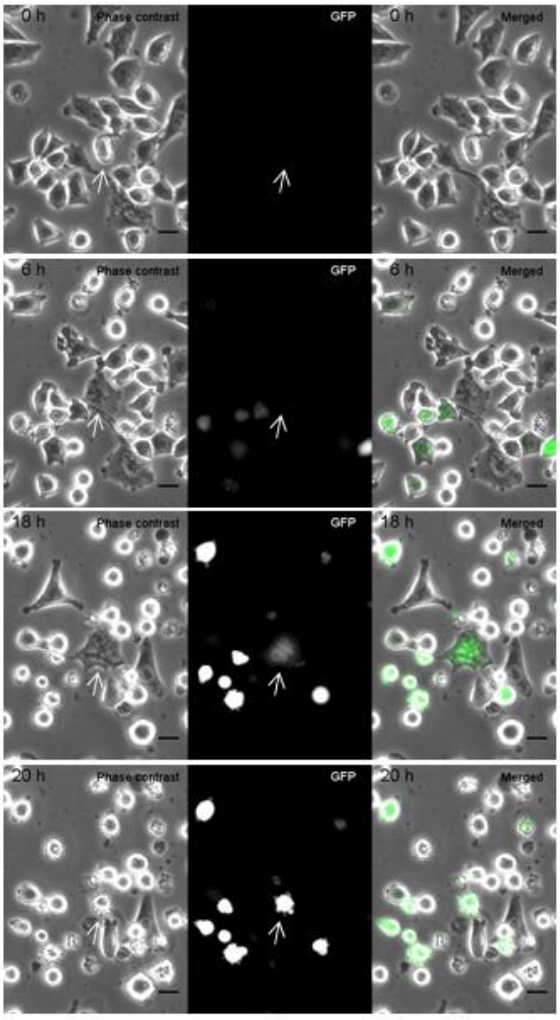


S4B

HCT116σ$^{-/-}$col



S4C HCT116 wt dox



S4D HCT116 wt col

Figure S4.

Storyboard of live-imaging movies of cells undergoing MC followed by autophagy and apoptosis. HCT116 $\sigma^{-/-}$ (**A, B**) and HCT116 wt cells (**C, D**) were transfected with pET28a-LC3-GFP plasmid. After 6h they were co-treated with lysosomal inhibitors pepstatin A (5 μg/ml) and E64D (2 μg/ml). One hour later, cells were treated with doxorubicin (600 nM) (**A, C**) or colcemid (0.1 μg/ml) (**B, D**). White arrows point to cells undergoing division followed by MC. The MC stimulation promotes appearance of LC3-GFP puncta formation. MC progression and subsequent autophagy resulted in stimulation of apoptotic cell death. Live imaging of cultured cells was performed at 10× magnification; scale bar, 20 μm.