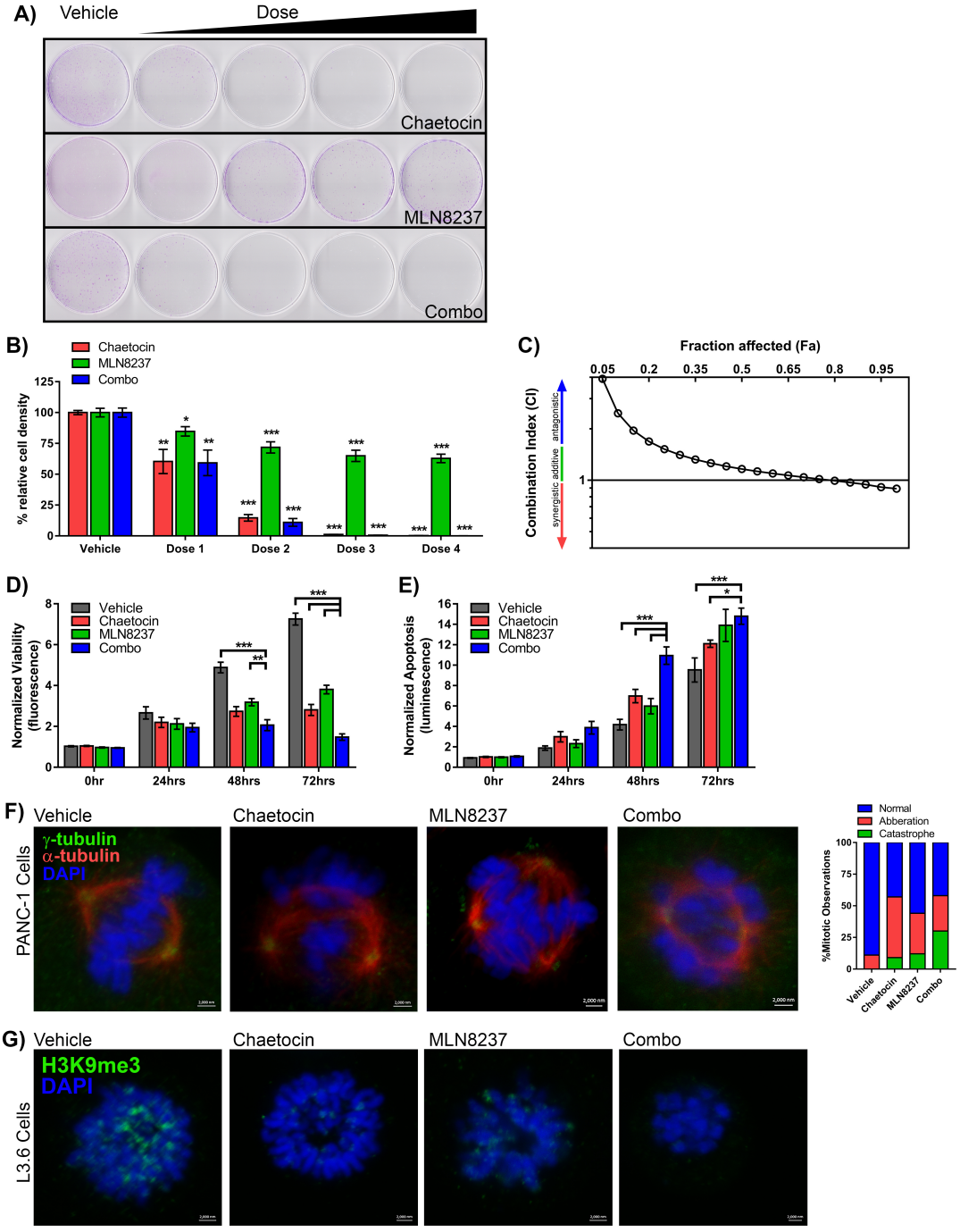
Supplementary Figure 1



Supplementary Figure 1

PANC-1 cells were plated for a clonogenic cell survival assay and stained for cell density after 7 days of treatment with chaetocin or MLN8237 alone or in combination (Dose 1: 7.5nM chaetocin, 22.5nM MLN8237; Dose 2: 15nM chaetocin, 45nM MLN8237; Dose 3: 22.5nM chaetocin, 67.5nM MLN8237; Dose 4: 30nM chaetocin, 90nM MLN8237). A) Representative images of clonogenic staining for the vehicle (DMSO) treated cells and dose escalation (left to right). B) Cell density (mean±SEM, n=3) was measured and normalized to vehicle for each treatment. Statistical significance was calculated by 1-way ANOVA with multiple comparisons (* indicates p-value ≤0.05, ** indicates p-value ≤0.005, *** indicates p-value ≤0.0005). C) The CI is indicated at various Fa values. Levels of D) cell viability and E) apoptosis were observed in L3.6 cells treated for 0, 24, 48 and 72hrs with vehicle, chaetocin (30nM), MLN8237 (90nM) or combination (30 + 90nM, CH + MLN8237 respectively). Values were normalized to 0hr vehicle for each treatment and statistical significance calculated by 2-way ANOVA with Tukey's multiple comparisons test. * indicates p- value ≤0.05, ** indicates p-value ≤0.005, *** indicates p-value ≤0.0005. F) PANC-1 cells were treated with vehicle, chaetocin, MLN8237 or combination for 48hrs, fixed and stained antibodies to γ-tubulin (green), α-tubulin (red), and DNA stained with DAPI (blue) to consider mitotic progression. Representative images of normal mitosis (vehicle), multiple spindle poles (chaetocin and MLN8237 alone), and total disruption of the spindle apparatus in mitotic catastrophe (combination) are shown. Scale = 2000nm. Over 150 mitotic cells were observed and categorized as normal, aberration, or catastrophe and resultant graph indicates the percentage of the total number of cells counted. G) Representative images of H3K9me3 immunofluorescence staining of PANC-1 cells demonstrate a global decrease of H3K9me3 upon treatment with the combination of drugs. Scale = 2000nm.