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Supplemental Material to:

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MAPK14/p38α confers irinotecan resistance to TP53-defective cells by inducing survival autophagy

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Figure S1

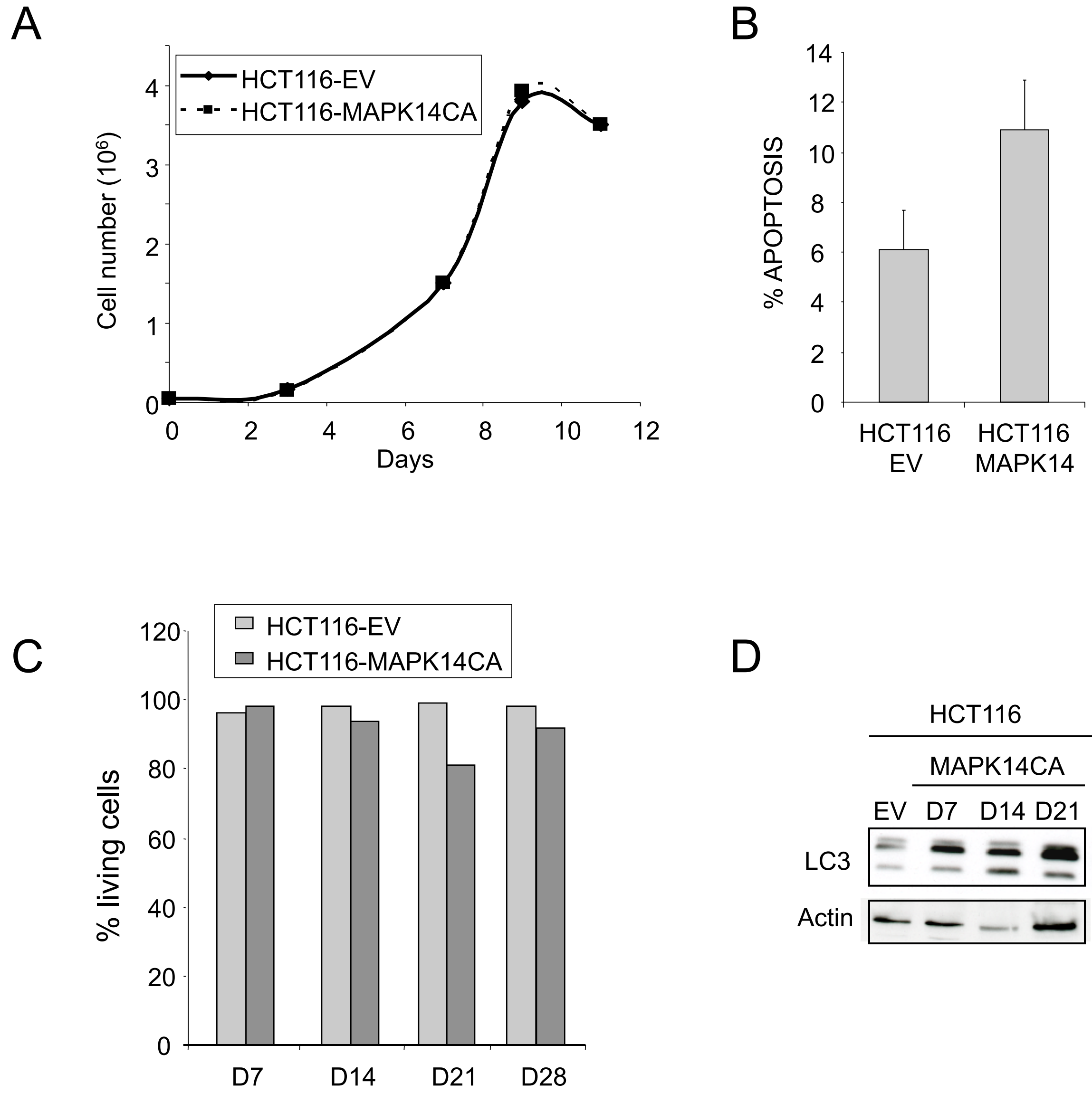

Figure S2

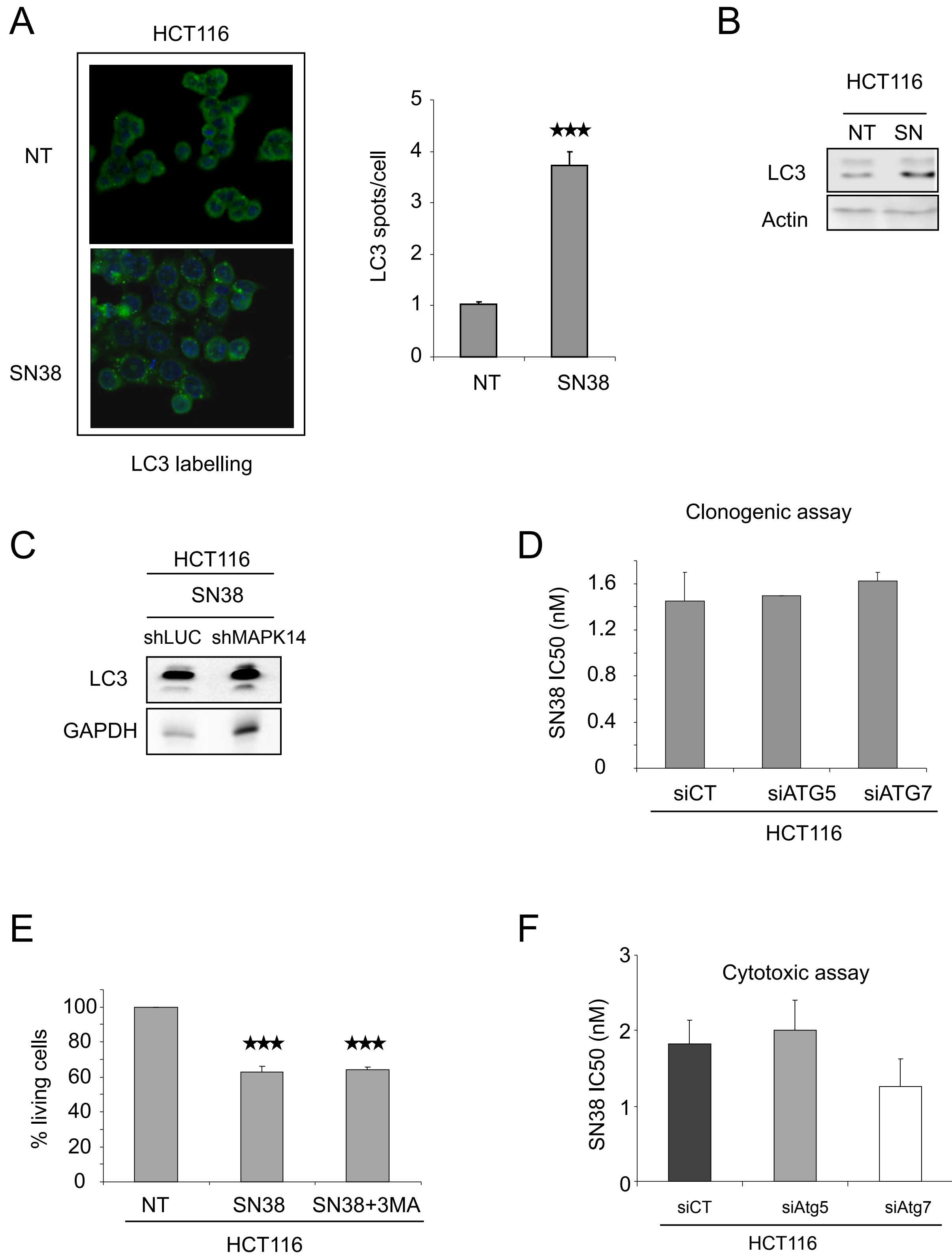

Figure S3

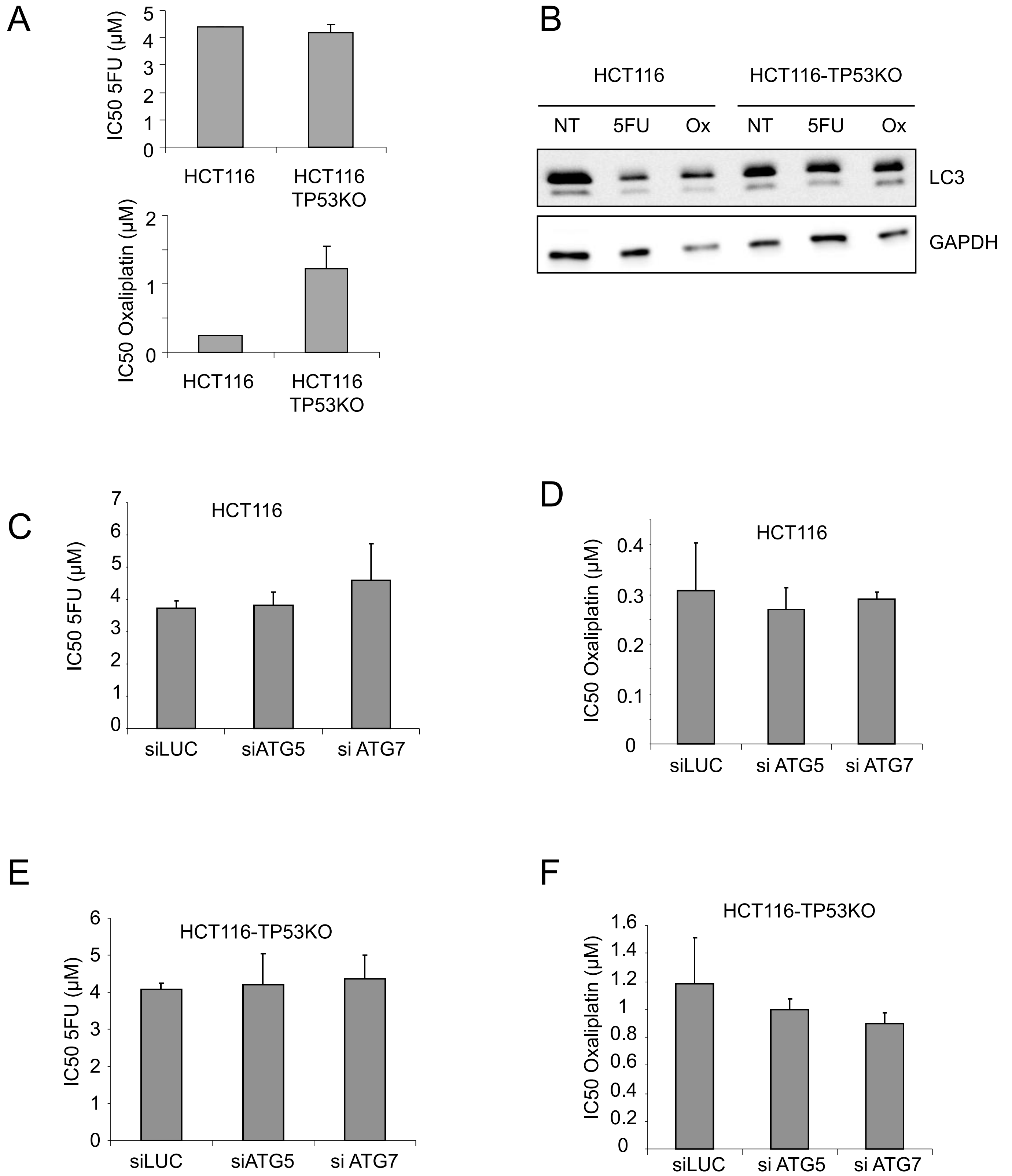

Figure S1. MAPK14 overexpression in HCT116 cells does not lead to cell growth arrest and survival-promoting autophagy. (A) Growth curve of HCT116 cells transduced with retroviruses expressing a constitutively active variant of MAPK14 (HCT116-MAPK14-CA) or pMSCV empty vector (HCT116-EV) cultured in complete medium (10% FCS) (B) Quantification of apoptosis in HCT116-EV and HCT116-MAPK14-CA cells at day 14 after retroviral transduction. Apoptosis was determined by 7AAD and Annexin V-FLUOS staining with a FACScan flow cytometer. (C) Cell viability in HCT116-EV and HCT116-MAPK14-CA cells at day 7, 14, 21 and 28 after transduction. The number of viable cells was determined by counting the cells not stained by Trypan blue. Data are representative of three independent experiments. (D) Western blot analysis of LC3-I and II expression in HCT116-MAPK14-CA cells at day 7, 14 and 21 following retroviral transduction (for HCT116-EV only day 14 is shown). Equal loading is shown by actin expression.

Figure S2. Autophagy is not involved in resistance to SN38 in HCT116 cells. (A, left panel) LC3 staining analyzed by immunofluorescence of HCT116 cells treated or not (NT) with 1 μM SN38 for 24 h. The pattern of LC3 expression in the cytosol changed from diffuse to punctate/vesicular. (A, right panel) The number of vesicular LC3 spots/cell in HCT116 cells treated or not with 1 μM SN38 for 24 h was scored (100 cells were counted for each cell type). The results are represented as the mean ± S.D. (B) Western blot analysis of LC3-I and II expression in HCT116 cells treated or not with 1 μM SN38 for 24 hours. Equal loading is shown by actin expression. (C) Western blot analysis of LC3-I and II expression in HCT116-ShLuc and HCT116-ShMAPK14 cells treated or not with SN38. Equal loading is shown by GAPDH expression. (D) Clonogenic assay to assess SN38 cytotoxicity in HCT116 cells transfected with control siRNA or anti-*ATG5* or -*ATG7* siRNAs. (E) Percentage of living cells in HCT116 treated with SN38 or SN38 + 3MA. The number of viable cells was determined by counting the number of cells not stained by the

Trypan blue dye. (F) SRB assay to assess SN38 cytotoxicity in HCT116 cells in which *ATG5* or *ATG7* or *Luciferase* (Ctrl) were downregulated by siRNA. The difference between siATG7 and siCtrl is not significant (p value = 0.052).

Figure S3. Autophagy is not responsible for resistance to 5-FU and Oxaliplatin. (A) SRB assay to determine 5-FU and oxaliplatin IC50 HCT116 and HCT116-TP53KO cells (B) Western blot analysis of LC3-I and II expression in HCT116 and HCT116-TP53KO cells treated or not with 4 µM of 5-FU or oxaliplatin (0.4 µM for HCT116 and 1.4 µM for HCT116-TP53KO). Equal loading is shown by GAPDH expression. (C) SRB assay to assess 5-FU cytotoxicity in HCT116 cells in which *ATG5* or *ATG7* or *Luciferase* (Ctrl) were downregulated by siRNA. (D) SRB assay to assess oxaliplatin cytotoxicity in HCT116 cells in which *ATG5* or *ATG7* or *Luciferase* (Ctrl) were downregulated by siRNA. (E) SRB assay to assess 5-FU cytotoxicity in HCT116-TP53KO cells in which *ATG5* or *ATG7* or *Luciferase* (Ctrl) were downregulated by siRNA. F: SRB assay to assess oxaliplatin cytotoxicity in HCT116-TP53KO cells in which *ATG5* or *ATG7* or *Luciferase* (Ctrl) were downregulated by siRNA.