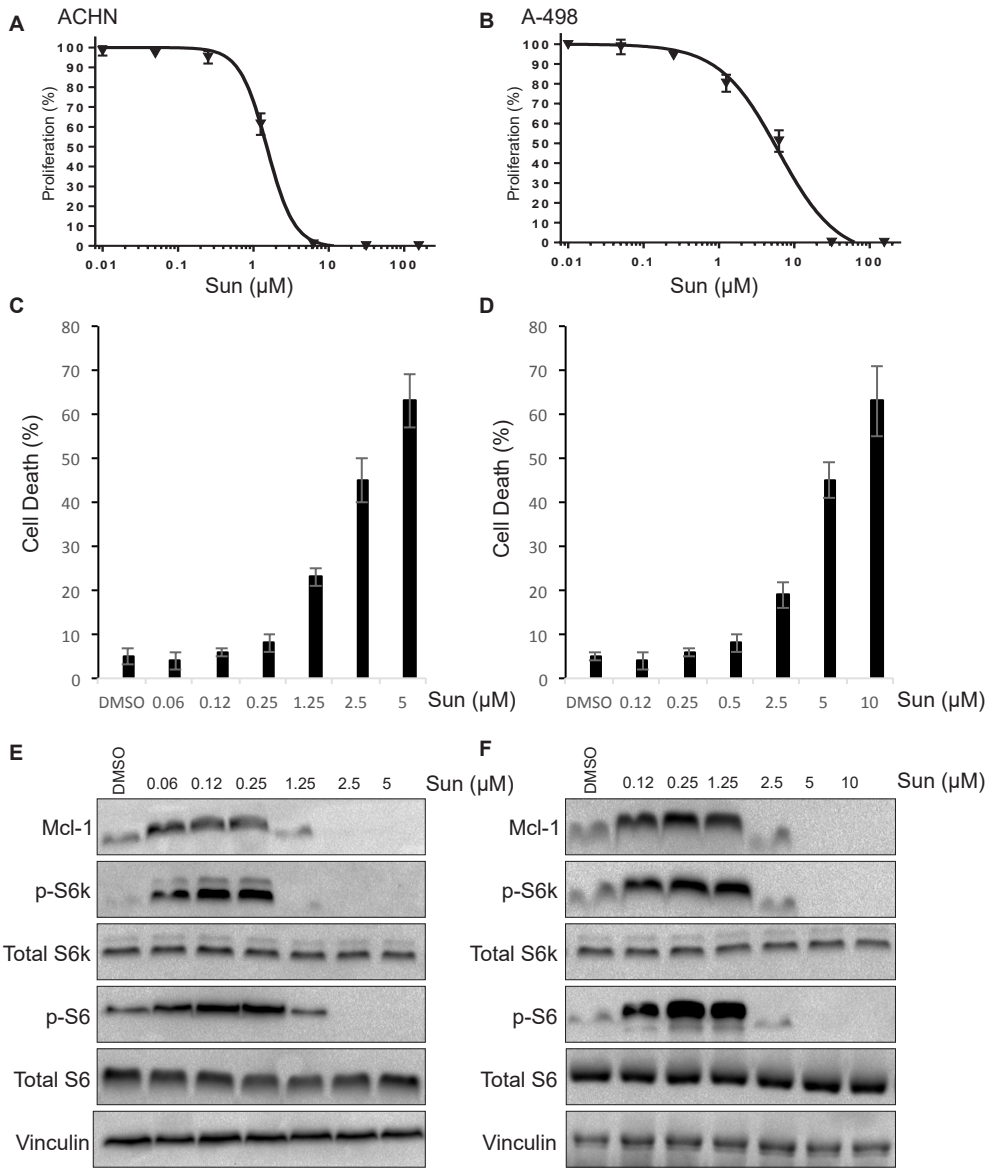
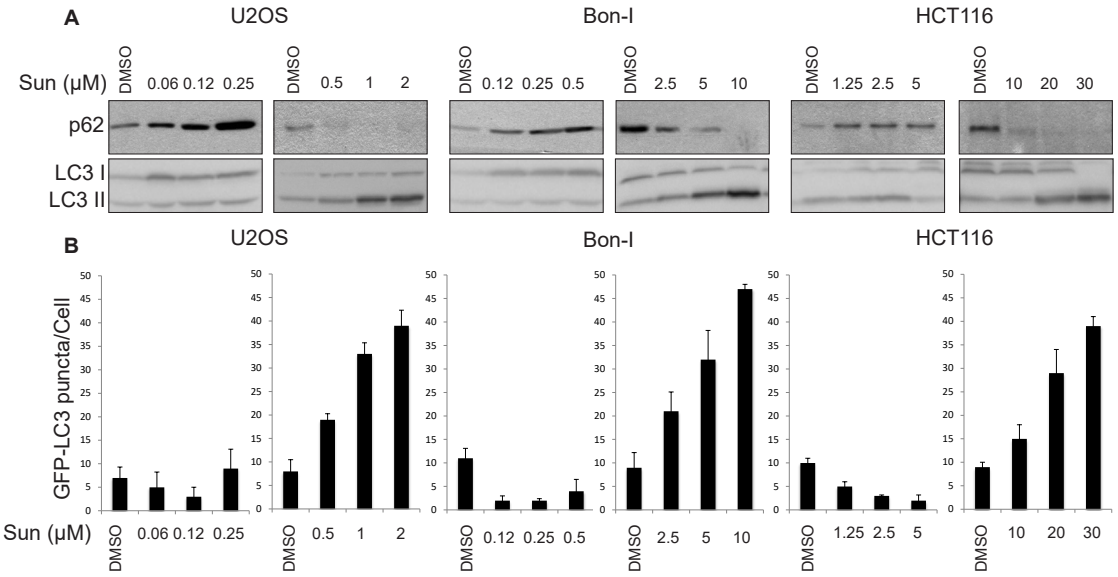
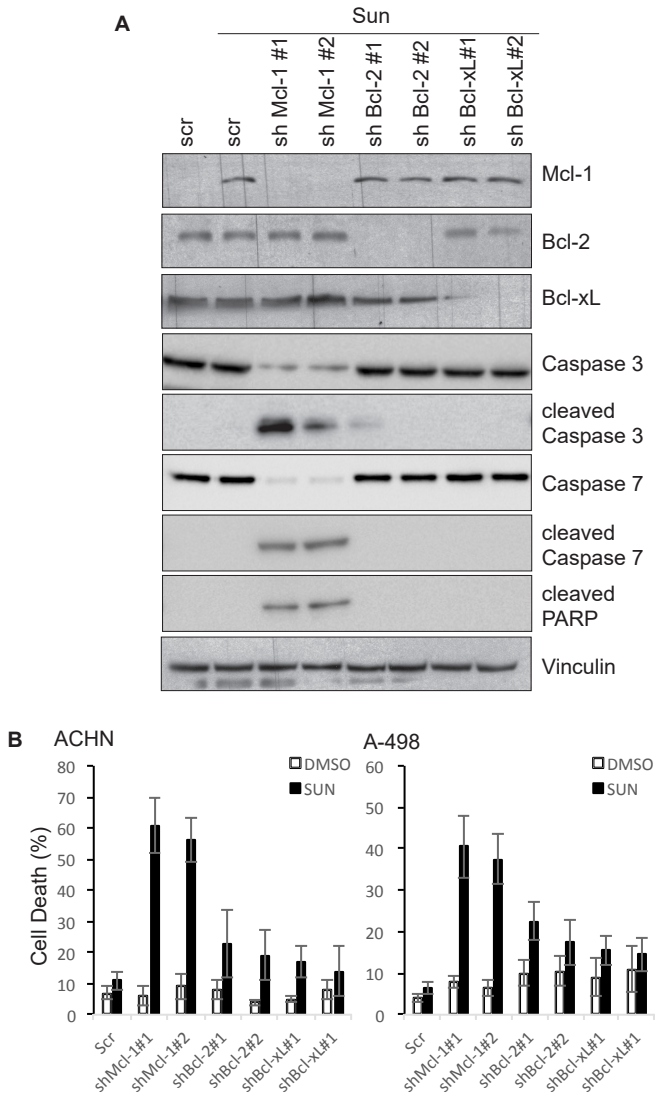


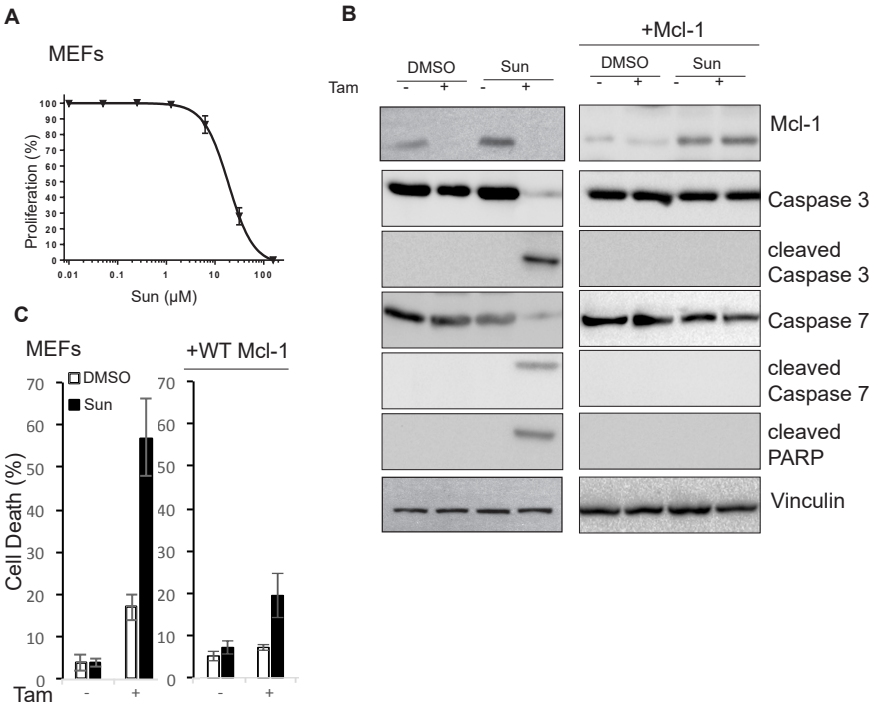
Supplemental Figure 1. Dual, concentration-range dependent effect of sunitinib on Mcl-1 levels and mTOR signaling in renal carcinoma cell lines. (**A** and **B**) Proliferation of ACHN (A) and A-498 (B) cells treated with increasing concentrations of sunitinib for 24h as assessed by CellTitre-Glo assay. (C and D) Percentage of cell death of ACHN (**C**) and A-498 (**D**) cells treated with the indicated concentrations of sunitinib for 24h. (**E** and **F**) Immunoblotting analysis of lysates prepared from ACHN (E) and A-498 (F) cells treated with the indicated concentrations of sunitinib for 24 h using the indicated antibodies. Blots presented are derived from replicate samples run on parallel gels and controlled for even loading.



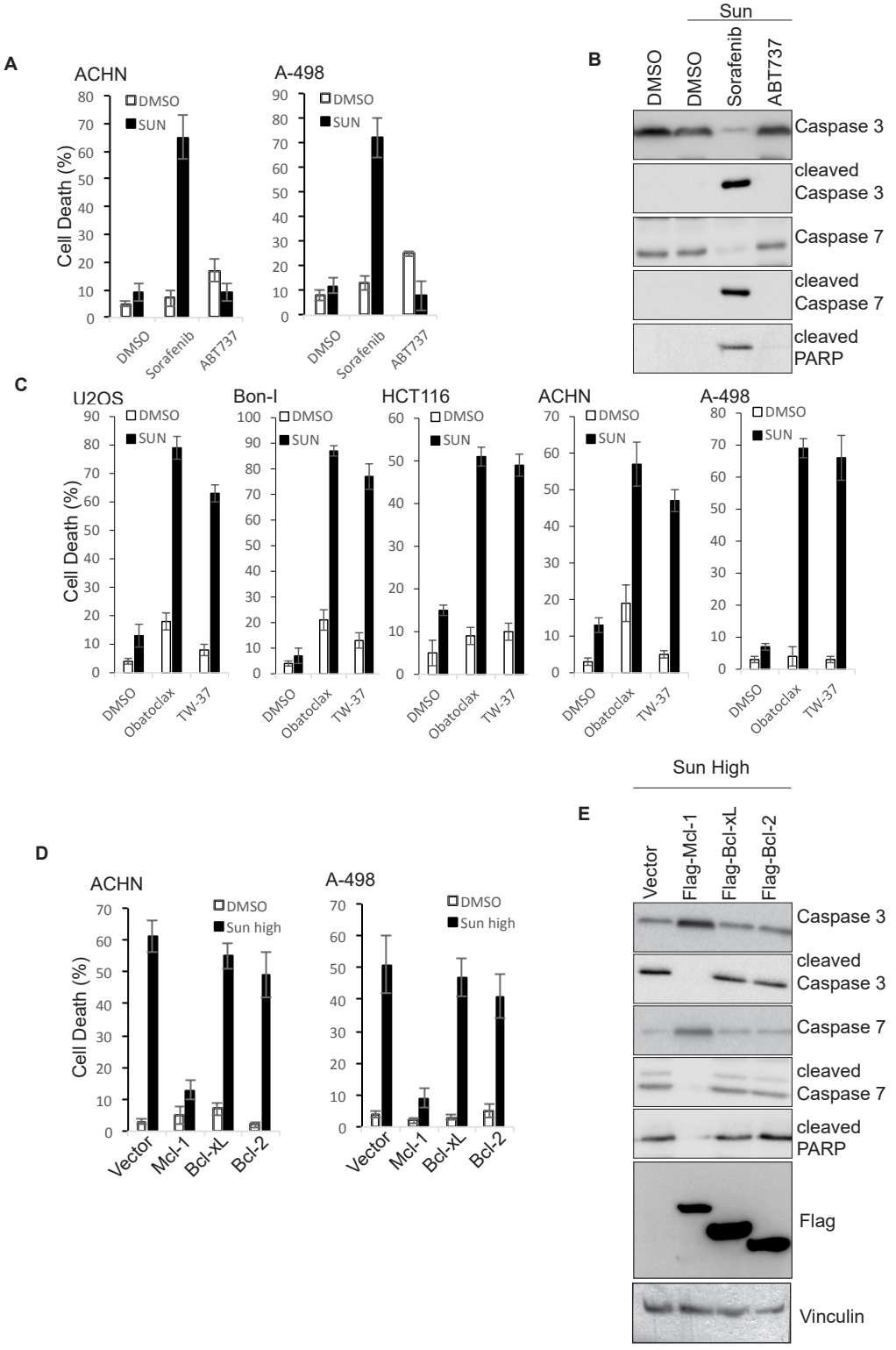
Supplemental Figure 2. Dual, concentration-range dependent effect of sunitinib on autophagy. (**A**) Immunoblotting analysis using p62 and LC3 antibodies of lysates used in Figure 1, G-I prepared from U2OS, Bon I and HCT116 cells treated with the indicated concentrations of sunitinib for 24 h. (**B**) Number of GFP-LC3 puncta in U2OS, Bon I and HCT116 cells transfected with the GFP-LC3 construct and treated with the indicated concentrations of sunitinib for 24h. Results are representative of three independent experiments. Error bars indicate s.e.m.



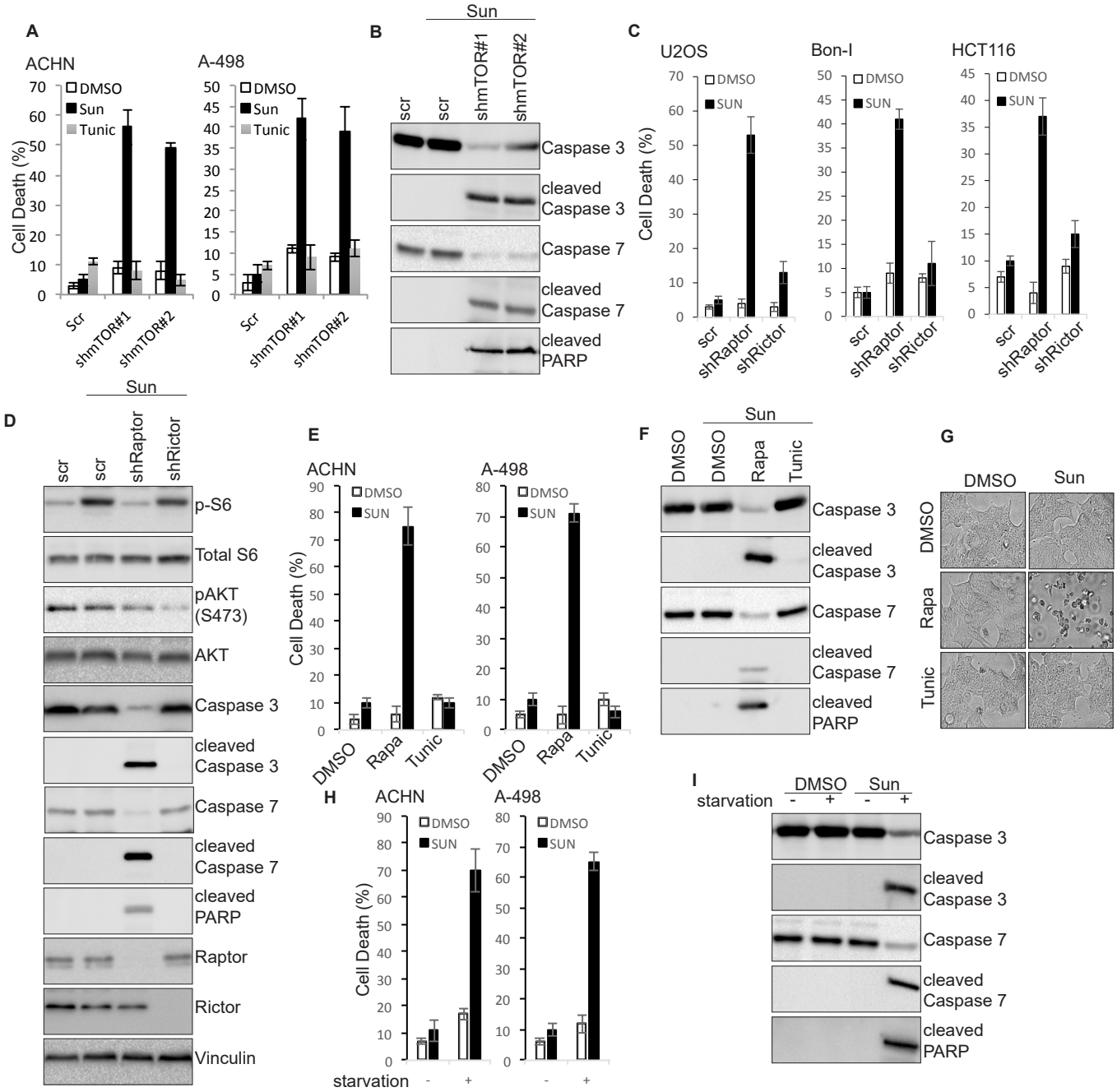
Supplemental Figure 3. Depletion of Mcl-1 levels sensitizes tumor cell response to sunitinib. (**A**) Immunoblotting analysis of lysates prepared from HCT116 cells transduced with the indicated shRNAs and treated with 1.25 μM sunitinib for 24h. Blots presented are derived from replicate samples run on parallel gels and controlled for even loading. (**B**) Percentage of cell death of ACHN and A 498 cells transduced with the indicated shRNAs and treated with either DMSO or sunitinib at 0.0625 μM (ACHN) or 0.125 μM (A-498) for 24h. Results are representative of three independent experiments. Error bars indicate s.e.m.



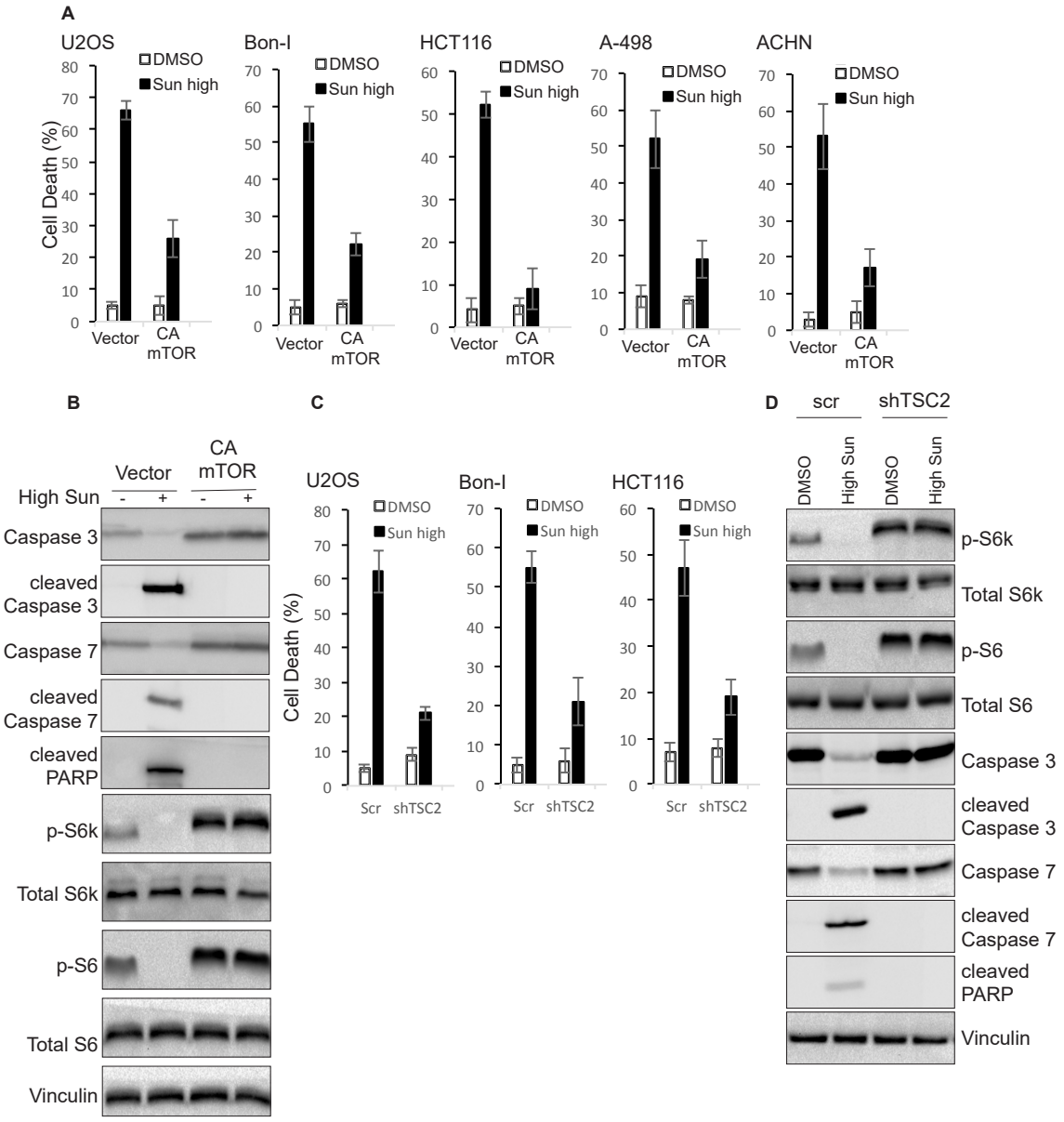
Supplemental Figure 4. Deletion of MCL1 renders MEFs sensitive to sunitinib. (**A**) Proliferation of Mcl-1f/f Rosa-ERCreT2 MEFs treated with increasing concentrations of sunitinib for 24 h as assessed by CellTiter-Glo assay. (**B**) Percentage of cell death of Mcl-1f/f Rosa-ERCreT2 MEFs treated for 48h with either DMSO or sunitinib (0.5 μM) in the absence or presence of tamoxifen (100nM) to induce the deletion of MCL-1. Results are representative of three independent experiments. Error bars indicate s.e.m. (**C**) Immunoblotting analysis of lysates derived from Mcl-1f/f Rosa-ERCreT2 MEFs transduced with or without a construct encoding wild type mouse Mcl-1 and treated as in (B). Blots presented are derived from replicate samples run on parallel gels and controlled for even loading.



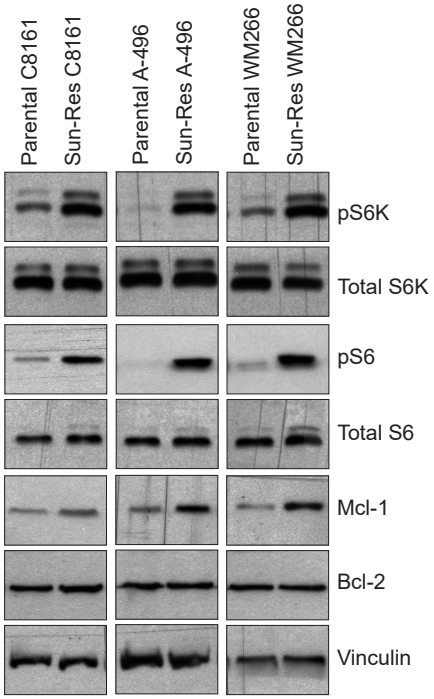
Supplemental Figure 5. Modulation of Mcl-1 levels determines the response to sunitinib.
(**A**) Percentage of cell death of ACHN and A-498 cells treated with either DMSO or sunitinib at 0.0625 μM (ACHN) or 0.125 μM (A-498) for 24h in the absence or presence of sorafenib (2.5 μM) or ABT737 (2.5 μM). (**B**) Immunoblotting analysis of lysates used in Figure 3F derived from HCT116 cells treated for 24h with either DMSO or sunitinib (1.25 μM) in the absence or presence of sorafenib (2.5 μM) or ABT737 (2.5 μM). (**C**) Percentage of death of cells treated with either DMSO or sunitinib at 0.0625 μM (U2OS), 0.125 μM (Bon I) or 1.25 μM (HCT116) 0.0625 μM (ACHN) or 0.125 μM (A-498) for 24h in combination with DMSO, Obatoclax (50 nM) or TW-37 (200nM). Results are representative of three independent experiments. Error bars indicate s.e.m. (**D**) Percentage of cell death of ACHN and A-498 cells transfected with the indicated constructs and treated with either DMSO or sunitinib at 2.5 μM (ACHN) or 5 μM (A-498) for 24h. Results are representative of three independent experiments. Error bars indicate s.e.m. (**E**) Immunoblotting analysis of lysates derived from HCT116 cells transfected with vector or Flag-tagged constructs of either Mcl-1, Bcl-2 or Bcl-xL and treated with sunitinib (10 μM) for 24h. Blots presented are derived from replicate samples run on parallel gels and controlled for even loading.



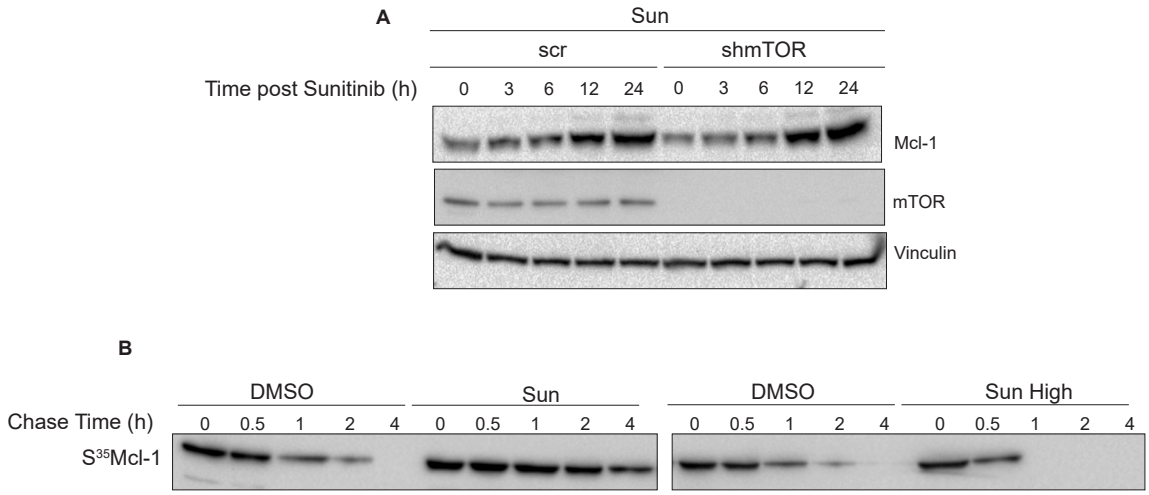
Supplemental Figure 6. Dual modulation of mTOR signaling at different dose ranges contributes to determine the response of tumor cells to sunitinib. (**A**) Percentage of cell death of ACHN and A-498 cells transduced with the indicated shRNAs and treated with either DMSO or sunitinib at 0.0625 μM (ACHN) or 0.125 μM (A-498) for 24h. (**B**) Immunoblotting analysis of lysates used in Figure 4A derived from HCT116 cells transduced with the indicated shRNAs and treated for 24h with either DMSO or sunitinib (1.25 μM). (**C**) Percentage of cell death of U2OS, Bon I and HCT116 cells transduced with the indicated shRNAs and treated with either DMSO or sunitinib at 0.0625 μM (U2OS), 0.125 μM (Bon I) or 1.25 μM (HCT116) for 24h. (**D**) Immunoblotting analysis of HCT116 cells transduced with the indicated shRNAs and treated for 24h with sunitinib (1.25 μM). (**E**) Percentage of cell death of ACHN and A-498 cells treated with either DMSO or sunitinib at 0.0625 μM (ACHN) or 0.125 μM (A-498) for 24h in the absence or presence of rapamycin (2.5 μM) or tunicamycin (2.5 μM). (**F**) Immunoblotting analysis of lysates used in Figure 4C derived from HCT116 cells treated for 24h with either DMSO or sunitinib (1.25 μM) in the absence or presence of rapamycin (2.5 μM) or tunicamycin (2.5 μM). (**G**) Representative images of wash-out experiment of HCT116 cells treated as in Figure 4D for 24 h followed by washing in PBS and replating equal numbers of viable cells in fresh medium without drugs for the ensuing 48h. (**H**) Percentage of cell death of ACHN and A-498 cells plated in either complete DMEM medium or starved in HBSS and treated with either DMSO or sunitinib at 0.0625 μM (ACHN) or 0.125 μM (A-498) for 8 h. (**I**) Immunoblotting analysis of lysates used in Figure 4E derived from HCT116 cells plated in either complete DMEM medium or starved in Hank's balanced salt solution (HBSS) and treated with either DMSO or 1.25 μM sunitinib for 8h. Blots presented are derived from replicate samples run on parallel gels and controlled for even loading. Results are representative of three independent experiments. Error bars indicate s.e.m.



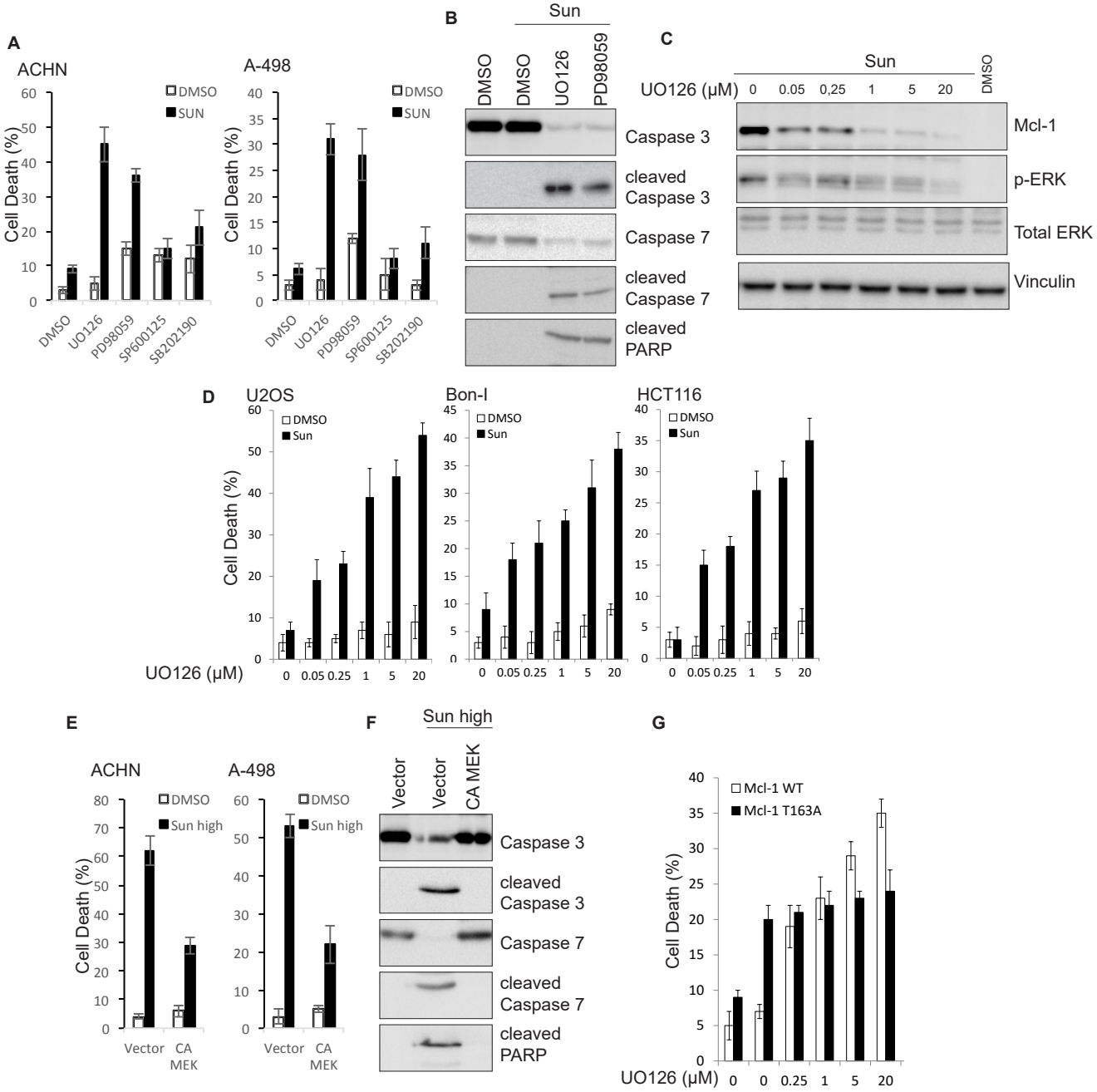
Supplemental Figure 7. Activation of mTOR renders cancer cells resistant to high doses of sunitinib. (**A**) Percentage of death of cells transfected with the indicated constructs and then treated with either DMSO or sunitinib at at 0.0625 μM (U2OS), 0.125 μM (Bon I) or 1.25 μM (HCT116) 0.0625 μM (ACHN) or 0.125 μM (A-498) for 24h. (**B**) Immunoblotting analysis of lysates derived from HCT116 cells transfected with the indicated constructs and treated with either DMSO or 10 μM sunitinib for 24h. (**C**) Percentage of death of cells transfected with the indicated shRNAs and treated with either DMSO or sunitinib at 1 μM (U2OS), 5 μM (Bon I), 10 μM (HCT116) 24h. Results are representative of three independent experiments. Error bars indicate s.e.m. (**D**) Immunoblotting analysis of lysates derived from HCT116 cells transfected with the indicated shRNAs and treated with either DMSO or 10 μM sunitinib for 24h. Blots presented are derived from replicate samples run on parallel gels and controlled for even loading.



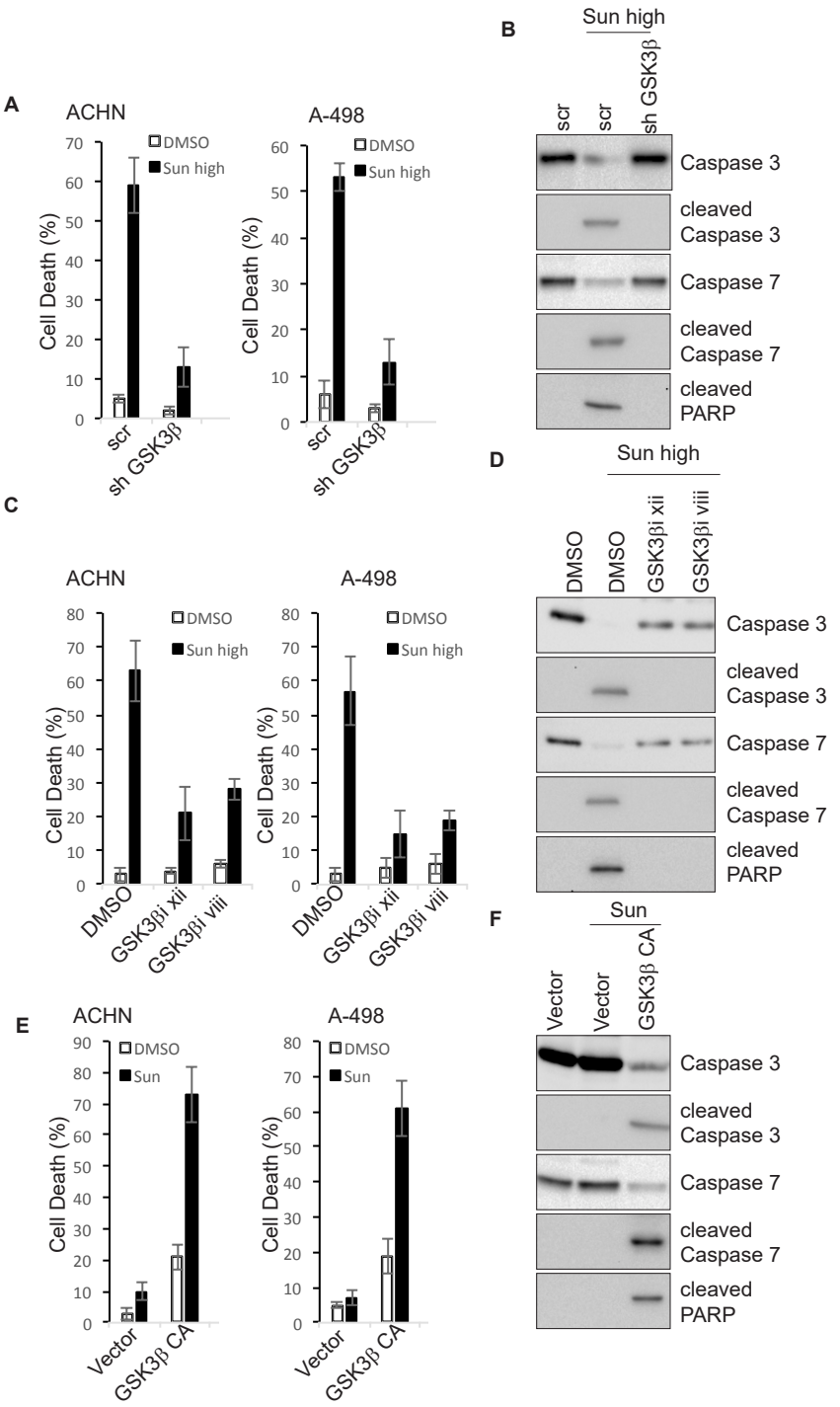
Supplemental Figure 8. Enhanced Mcl-1 levels and mTOR activation in sunitinib-desensitized melanoma cells. Immunoblotting analysis with the indicated antibodies of lysates prepared from either parental or sunitinib-desensitized cells. Blots presented are derived from replicate samples run on parallel gels and controlled for even loading.



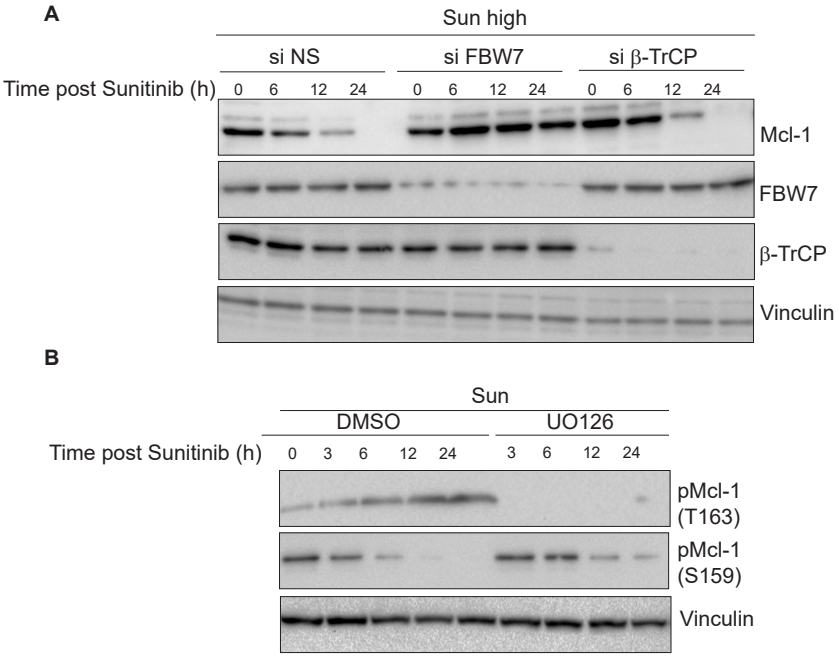
Supplemental Figure 9. Sunitinib modulates Mcl-1 stability. (**A**) Immunoblotting analysis of lysates derived from HCT116 cells transduced with scrambled shRNA or shRNA against mTOR and then treated with sunitinib (1.25 μM) for the indicated time points. (**B**) HCT116 cells washed twice with PBS and then incubated in methionine starvation DMEM supplemented with 10% serum for 30 min before adding [S35] methionine to pulse for 2 h. Cells were then chased by complete medium containing either DMSO or sunitinib (1.25 μM) for the indicated time points and then lysed. Mcl-1 was immunoprecipitated from the lysates and analyzed by autoradiography.



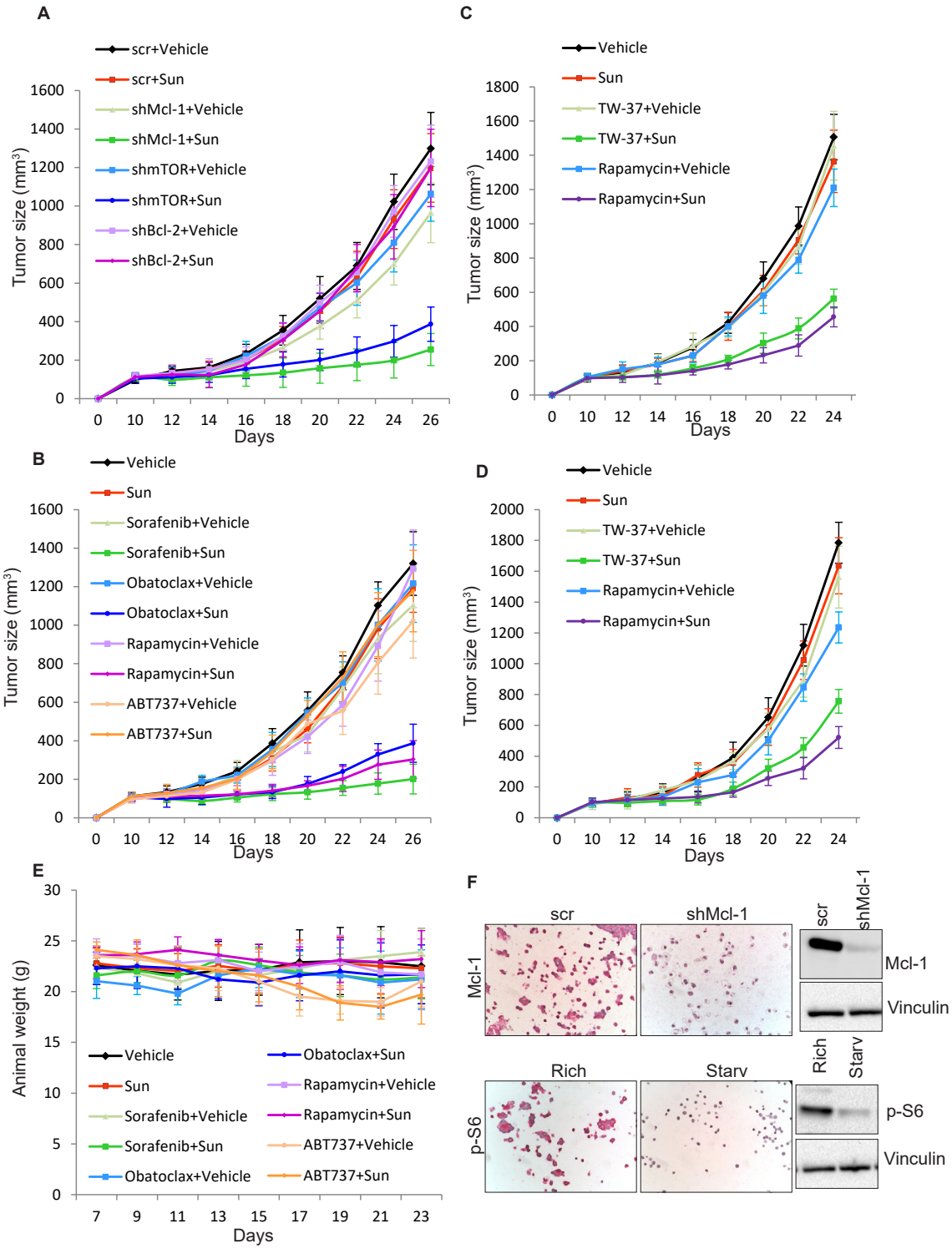
Supplemental Figure 10. Modulation of ERK activity contributes to determining the response of tumor cells to sunitinib. (**A**) Percentage of death of ACHN and A-498 cells treated with either DMSO or sunitinib at 0.0625 μM (ACHN) or 0.125 μM (A-498) for 24h in the absence or presence of the compounds UO126 (20 μM), PD98059 (50 μM), SP600125 (20 μM) or SB202190 (20 μM). (**B**) Immunoblotting analysis of lysates used in Figure 7A derived from HCT116 cells treated for 24h with either DMSO or sunitinib (1.25 μM) in the absence or presence of MEK inhibitors UO126 (20 μM) or PD98059 (50 μM). (**C**) Immunoblotting analysis of lysates derived from HCT116 cells treated for 24h with either DMSO or sunitinib (1.25 μM) in the absence or presence of the indicated concentrations of MEK inhibitors UO126. (**D**) Per¬centage of cell death of cells treated for 24h with either DMSO or sunitinib at 0.0625 μM (U2OS), 0.125 μM (Bon I) or 1.25 μM (HCT116) 0.0625 μM (ACHN) or 0.125 μM (A-498) for 24h in combination with the indicated concentrations of UO126. (E) Percentage of cell death of ACHN and A-498 cells transfected with the indicated constructs and treated with either DMSO or sunitinib at 2.5 μM (ACHN) or 5 μM (A-498) for 24h. (F) Immunoblotting analysis of lysates used in Figure 7D derived from HCT116 cells transfected with the indicated constructs and treated for 24h with sunitinib (10 μM). (G) Percentage of cell death of MCL1 knockout HCT116 cells transduced with the indicated constructs and treated for 24h with with either DMSO or sunitinib (1.25 μM) in combination with the indicated concentrations of UO126. Results are representative of three independent experiments. Error bars indicate s.e.m.



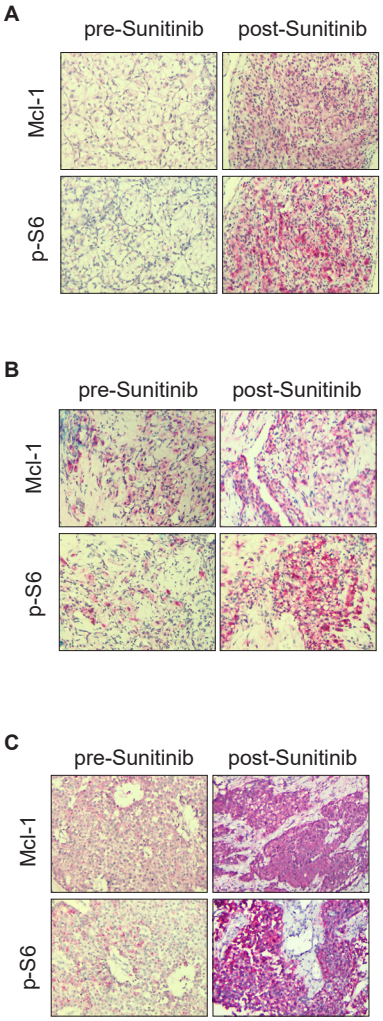
Supplemental Figure 11. Dual modulation of GSK3β phosphorylation contributes to determining the response of tumor cells to sunitinib. (**A**) Percentage of death of ACHN and A-498 cells transfected with the indicated shRNAs and treated with either DMSO or sunitinib at 2.5 μM (ACHN) or 5 μM (A-498) for 24h. (**B**) Immunoblotting analysis of lysates used in Figure 8A derived from HCT116 cells transfected with the indicated shRNAs and treated with either DMSO or 10 μM sunitinib for 24h. (**C**) Percentage of cell death of ACHN and A-498 pre-treated for 1h with GSK3β inhibitor xii (20 μM) or GSK3b inhibitor viii (25 μM) followed by treatment with either DMSO or sunitinib at 2.5 μM (ACHN) or 5 μM (A-498) for 24h. (**D**) Immunoblotting analysis of lysates used in Figure 8B derived from HCT116 cells pre-treated for 1h with GSK3β inhibitor xii (20 μM) or GSK3β inhibitor viii (25 μM) (B) followed by treatment with either DMSO or 10 μM sunitinib for 24h. (**E**) Percentage of cell death of ACHN and A-498 cells transduced with the indicated constructs and treated with either DMSO or sunitinib at 0.0625 μM (ACHN) or 0.125 μM (A-498) for 24h. Results are representative of three independent experiments. Error bars indicate s.e.m. (**F**) Immunoblotting analysis of lysates used in Figure 8C derived from HCT116 cells transfected with the indicated constructs and treated with 1.25 μM sunitinib for 24h.



Supplemental Figure 12. Mcl-1 phosphorylation by GSK3β is independent of ERK and triggers FBW7-mediated Mcl-1 degradation. (**A**). Immunoblotting analysis of lysates derived from HCT116 cells transfected with the indicated siRNA and treated for the indicated time points with sunitinib (10 μM). (**B**) Immunoblotting analysis of lysates derived from HCT116 cells treated for the indicated time points with sunitinib (1.25 μM) in the absence or presence of UO126 (0.25 μM).



Supplemental Figure 13. Synergism between sunitinib and Mcl-1 or mTOR inhibition in Patient-derived and ACHN tumor xenografts. (**A**) In vivo growth of xenograft tumors derived from ACHN cells transduced with the indicated shRNA. After establishment of xenografts, mice were kept on 1mg/ml Doxycycline supplemented in the drinking water to induce shRNA expression and were treated daily with either 10mg/kg sunitinib or vehicle by oral gavage for the indicated time. Error bars indicate s.e.m. (n=6 per group). (**B**) In vivo growth of ACHN tumor xenografts in mice treated daily with vehicle or sunitinib (10mg/kg) either alone or in combination with sorafenib (15mg/kg administered by oral gavage), obatoclax (2mg/kg administered by IV injection), rapamycin (0.5 mg/kg administered by IP injection) or ABT737 (75 mg/kg administered by IP injection). Error bars indicate s.e.m. (n=6 per group). (**C** and **D**) In vivo growth of xenografts derived from tumor samples isolated from two melanoma patients. After establishment of the xenografts, mice were treated daily with vehicle or sunitinib (10mg/kg) either alone or in combination with TW-37 (5mg/kg administered by IV injection) or rapamycin (0.5 mg/kg administered by IP injection). Error bars indicate s.e.m. (n=6 per group). (**E**) Body weight of mice used in B. (**F**) Validation of Mcl-1 and pS6 antibodies for immunohistochemistry application. 293T cells either depleted of Mcl-1 using shRNA or starved for 6 hours were used as control for Mcl-1 and pS6 respectively. Cells were split into two halves; one was pelleted and used for IHC procedures while the other half was lysed and analyzed by immunoblotting to confirm the reduction in Mcl-1 or pS6 levels.



Supplemental Figure S14. Representative images of immunohistochemical analysis of tumor tissues isolated from RCC (**A** and **B**) and NET (**C**) patients before and after treatment with sunitinib (original magnification is 20x).