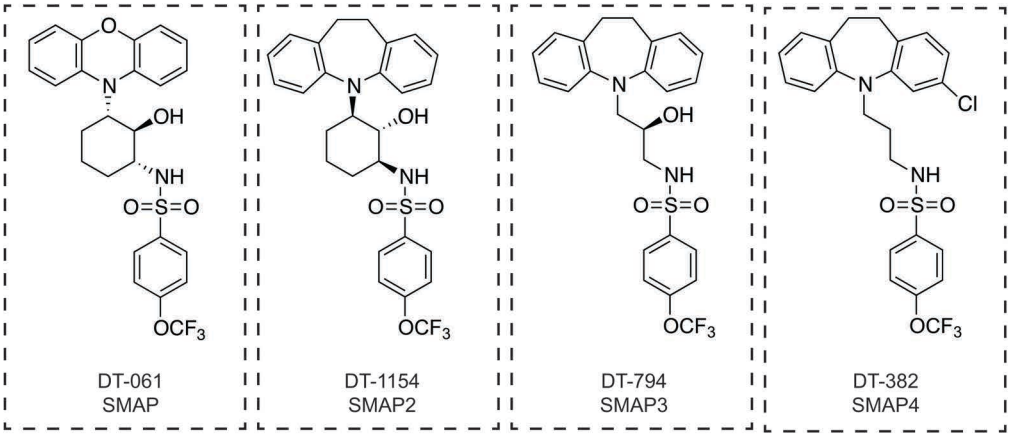
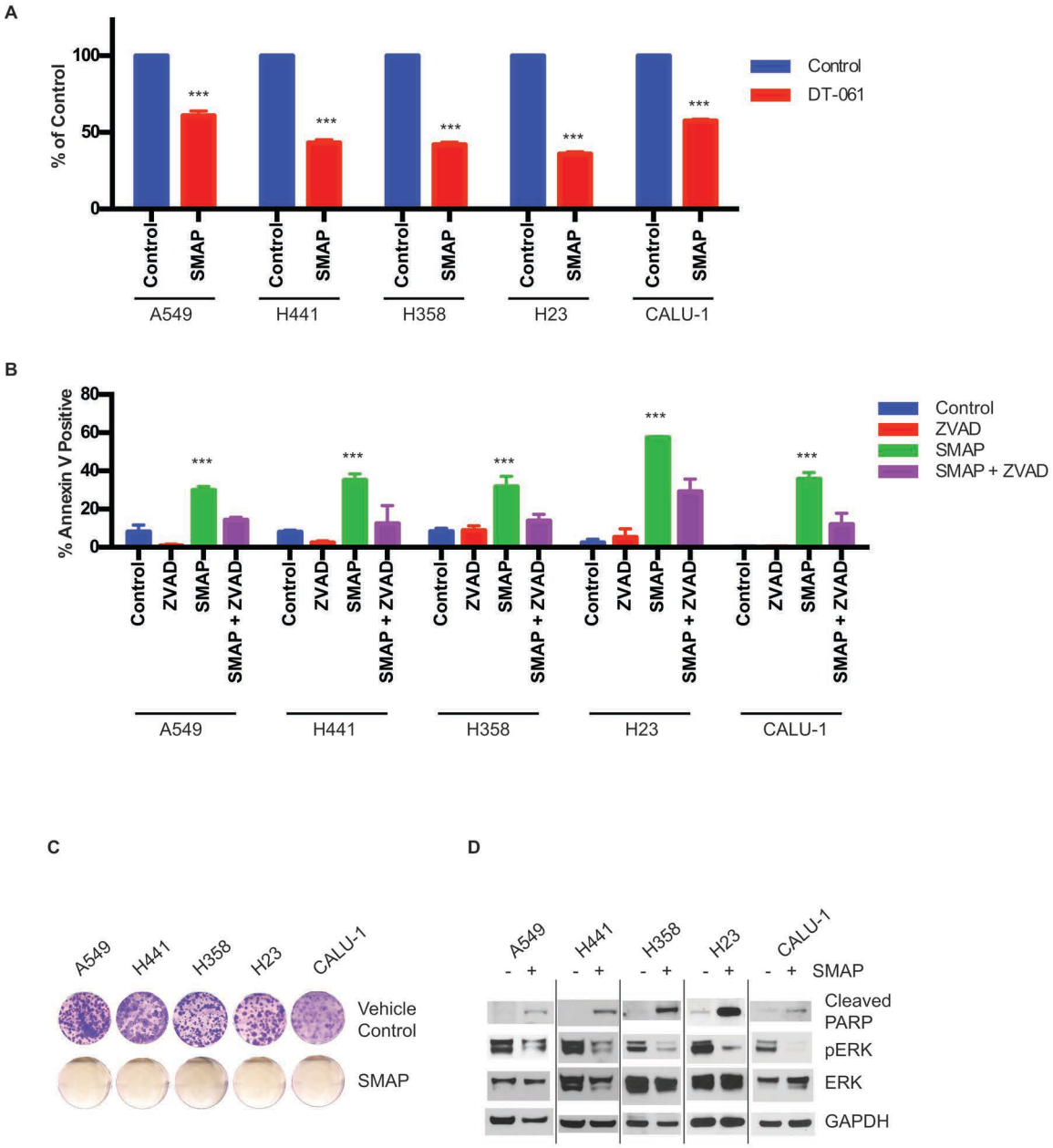
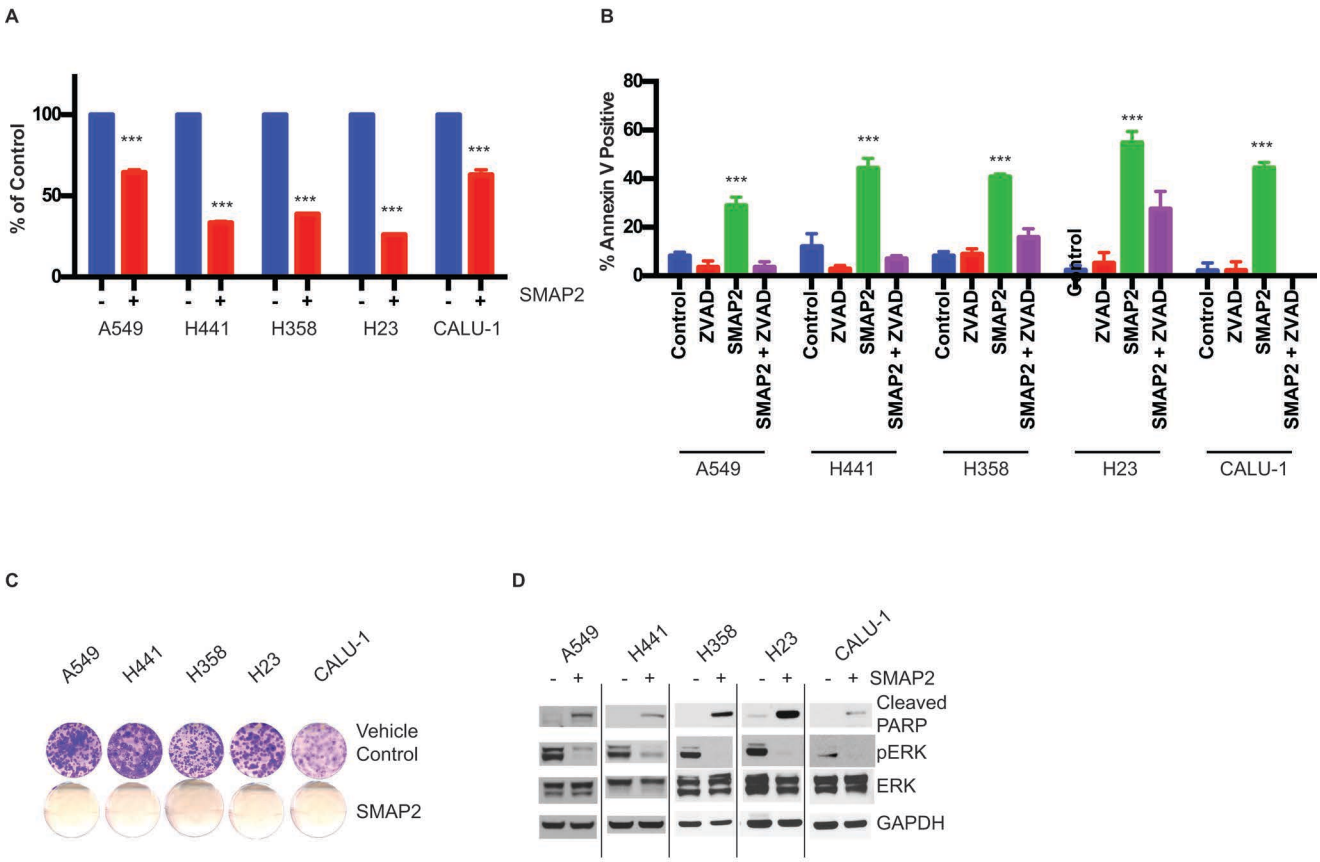


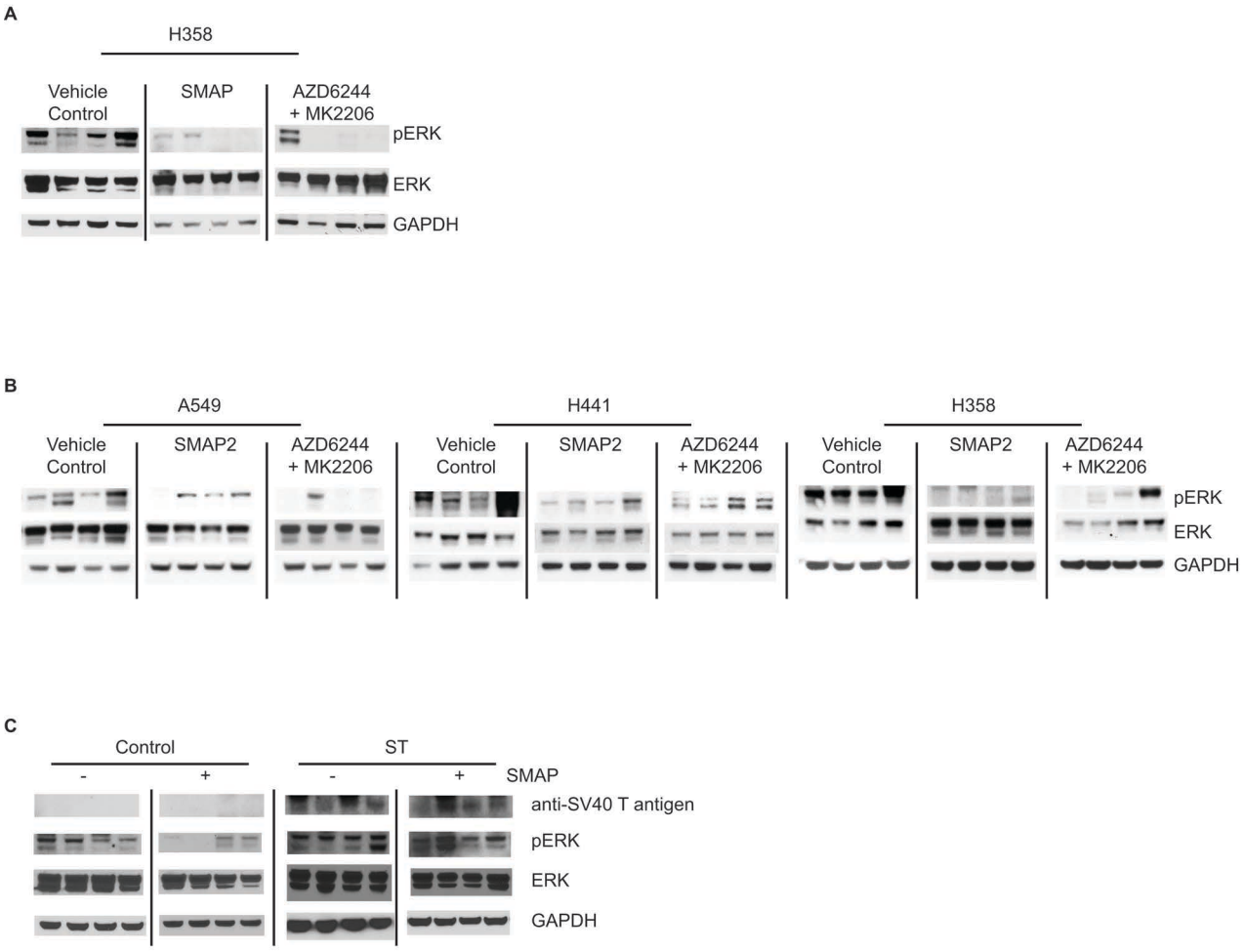
Supplemental Figure 1: Structures of SMAPs .



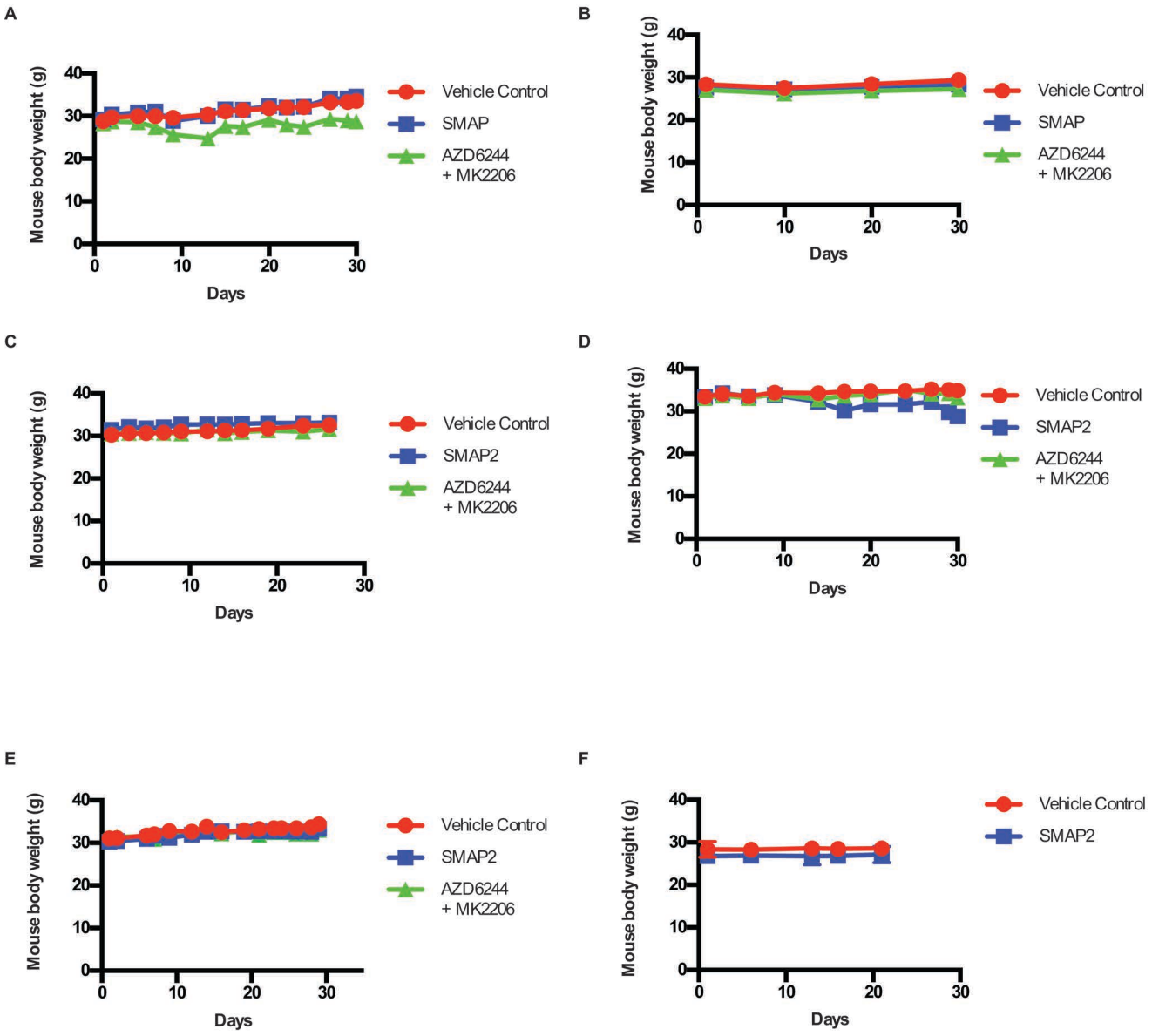
Supplemental Figure 2: SMAPs decrease cell viability and induce apoptosis in KRAS mutant lung cancer cells. **A,** Cell proliferation assay (CyQuant) in A549, H441, H358, H23, and CALU-1 treated with 20μM SMAP for 24 hours. **B,** Annexin V staining of lung cancer cell lines treated with SMAP (20μM), ZVAD (100μM), and combination of ZVAD with SMAP for 24 hours. **C,** Clonogenic assay of KRAS mutant cell lines (A549, H441, H358, H23, CALU-1) treated with 10μM SMAP for three weeks. **D,** Western blots for cleaved PARP, pERK, and ERK normalized to GAPDH in KRAS mutant cell lines treated with 20μM of SMAP for 24 hours. Data are means ± SD of three experiments. *P < 0.05, **P < 0.01, ***P < 0.001 by Student's *t* test.



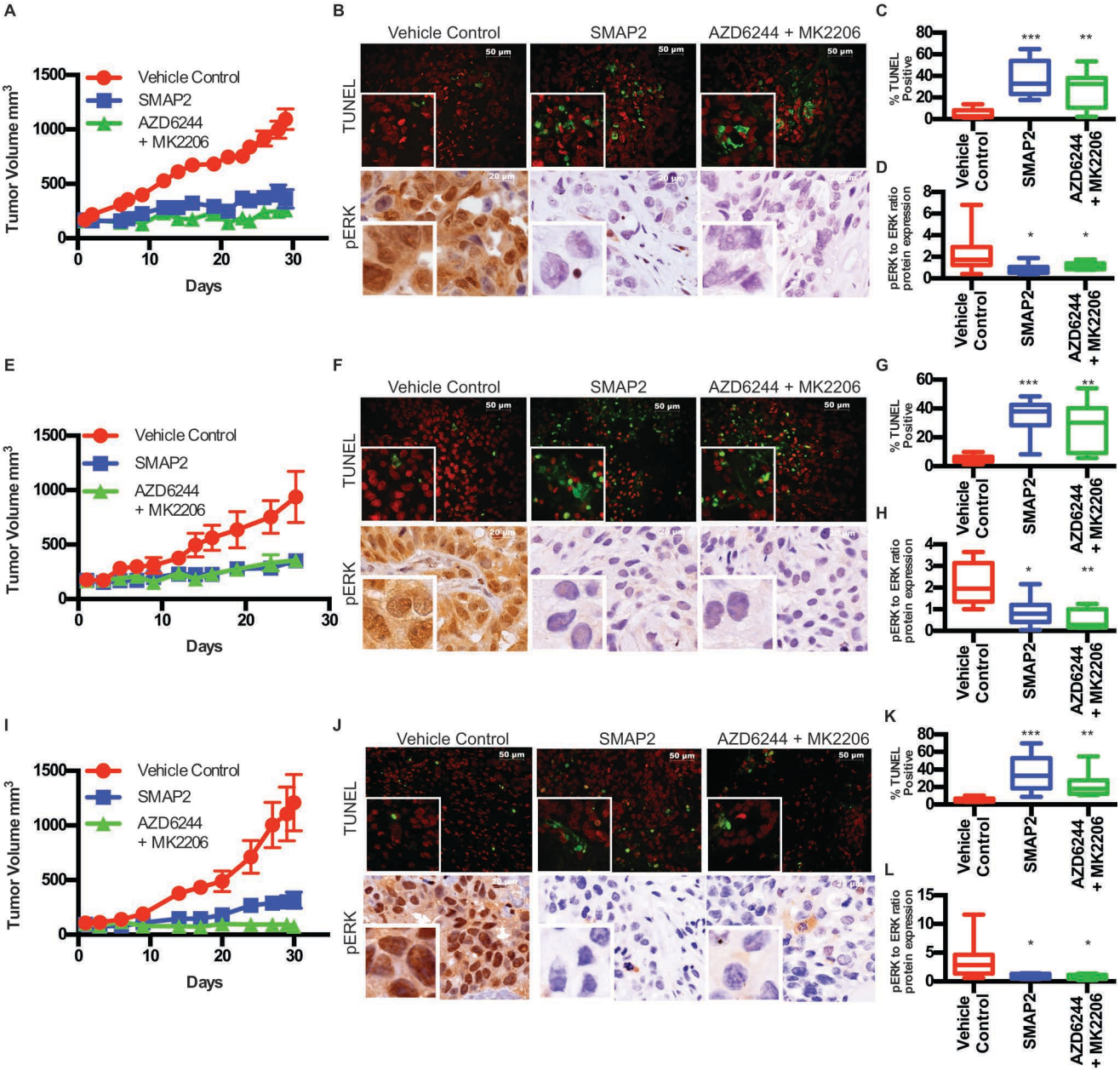
Supplemental Figure 3: SMAPs decrease cell viability, induce apoptosis, and inhibits downstream signaling in KRAS mutant lung cancer cells. **A,** Cell proliferation assay in A549, H441, H358, H23, and CALU-1 treated with 20μM SMAP2 for 24 hours. **B,** Annexin V staining of lung cancer cell lines treated with SMAP2 (20μM), ZVAD (100μM), and combination of ZVAD with SMAP2 for 24 hours. **C,** Clonogenic assay of KRAS mutant cell lines (A549, H441, H358, H23, CALU-1) treated with 10μM SMAP2 for three weeks. **D,** Western blots for cleaved PARP, pERK, and ERK normalized to GAPDH in KRAS mutant cell lines treated with 20μM of SMAP2 for 24 hours. Data are means ± SEM of three experiments. *P < 0.05, **P < 0.01, ***P < 0.001 by Student's *t* test.



Supplemental Figure 4: A, Western blots for p-ERK, ERK, and GAPDH in H358 xenograft treated with SMAP. **B,** Western blots for p-ERK, ERK, and GAPDH in A549, H441, H358 xenograft models treated with SMAP2. **C,** Western blot for anti-SV40 T antigen, pERK, ERK, and GAPDH in H358 Control and H358 ST xenograft model. Each lane corresponds to different animal.

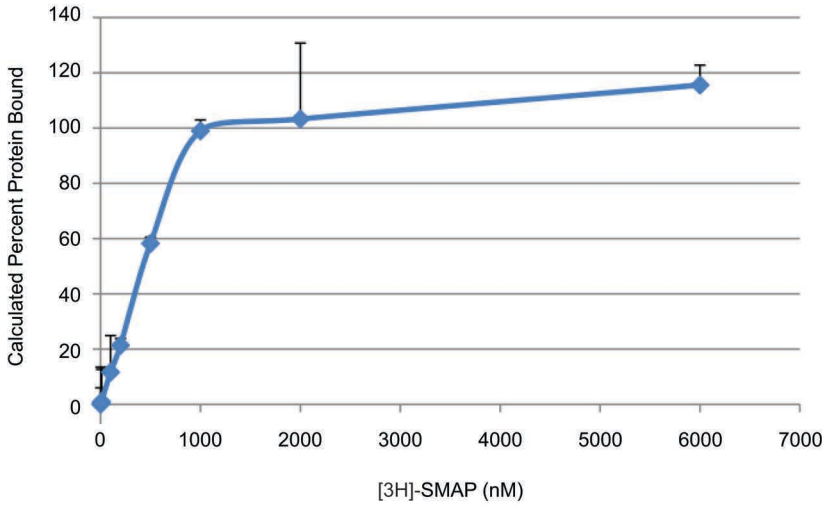


Supplemental Figure 5: Mouse body weights over period of three to four weeks in **A,** H358 xenograft **B,** KRAS PDX **C,** A549 xenograft model **D,** H441 xenograft model **E,** H358 xenograft model **F,** $KRAS^{LA2}$ transgenic mouse model. Data are means ± SEM.



Supplemental Figure 6: SMAPs promote tumor growth inhibition and inhibits MAPK signaling in vivo in xenograft models. **A,** 1x10^7 H358 cells were subcutaneously injected into nude mice and grew to an average of 100 mm^3. Mice were treated with vehicle control (n=10), combination of 6mg/kg MK2206 and 24mg/kg AZD6244 (n=9), or 100mg/kg SMAP2 (n=9) BID for four weeks. Tumor volume over course of treatment. . **B,** Tumors were evaluated by sacrificing the mice two hours after final treatment. Representative TUNEL staining (Scale bar: 50µM) and pERK IHC (Scale bar: 20µM) of treated-tumors. **C,** Quantification of the TUNEL positivity in tumors treated. D, Quantification of the pERK level in the xenograft tumors as performed by immunoblotting and densitometry. **E,** 5x10^6 A549 cells were subcutaneously injected into nude mice and grew to an average of 100 mm^3. Mice were treated with vehicle control (n=6), combination of 6mg/kg MK2206 and 24mg/kg AZD6244 (n=8), or 100mg/kg SMAP2 (n=8) BID for four weeks. Tumor volume over course of treatment. **F,** Tumors were evaluated by sacrificing the mice two hours after final treatment. Representative TUNEL staining (Scale bar: 50µM) and pERK IHC (Scale bar: 20µM) of treated-tumors. **G,** Quantification of the TUNEL positivity in tumors treated. **H**, Quantification of the pERK levels in the xenograft tumors as performed by immunoblotting and densitometry. **I,** 5x10^6 H441 cells were subcutaneously injected into nude mice and allowed to grow to an average of 100 mm^3. Mice were treated with vehicle control (n=10), combination of 6mg/kg MK2206 and 24mg/kg AZD6244 (n=9), or 100mg/kg SMAP2 (n=10) BID for our weeks. Tumor volume over course of treatment. **J,** Tumors were evaluated by sacrificing the mice two hours after final treatment. Representative TUNEL staining (Scale bar: 50µM) and pERK IHC (Scale bar: 20µM) of treated-tumors. **K,** Quantification of the TUNEL positivity in tumors treated. **L**, Quantification of the pERK levels in the xenograft tumors as performed by immunoblotting and densitometry. Data are means ± SEM *P < 0.05, **P < 0.01, ***P < 0.001 by Student's *t* test.

A



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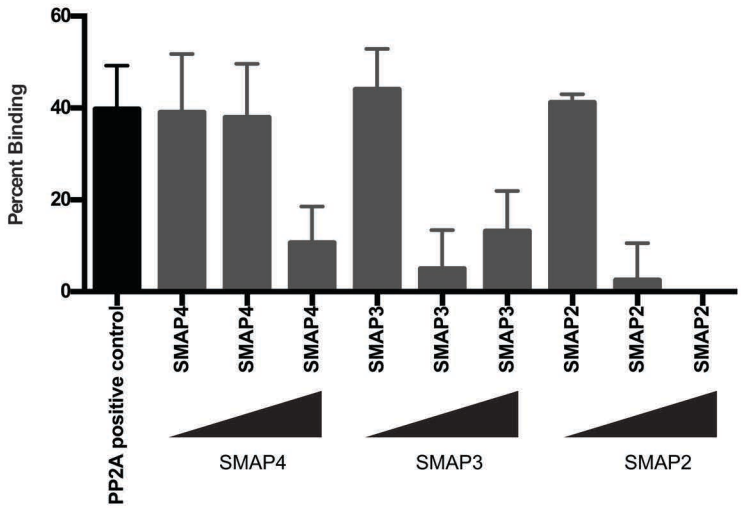
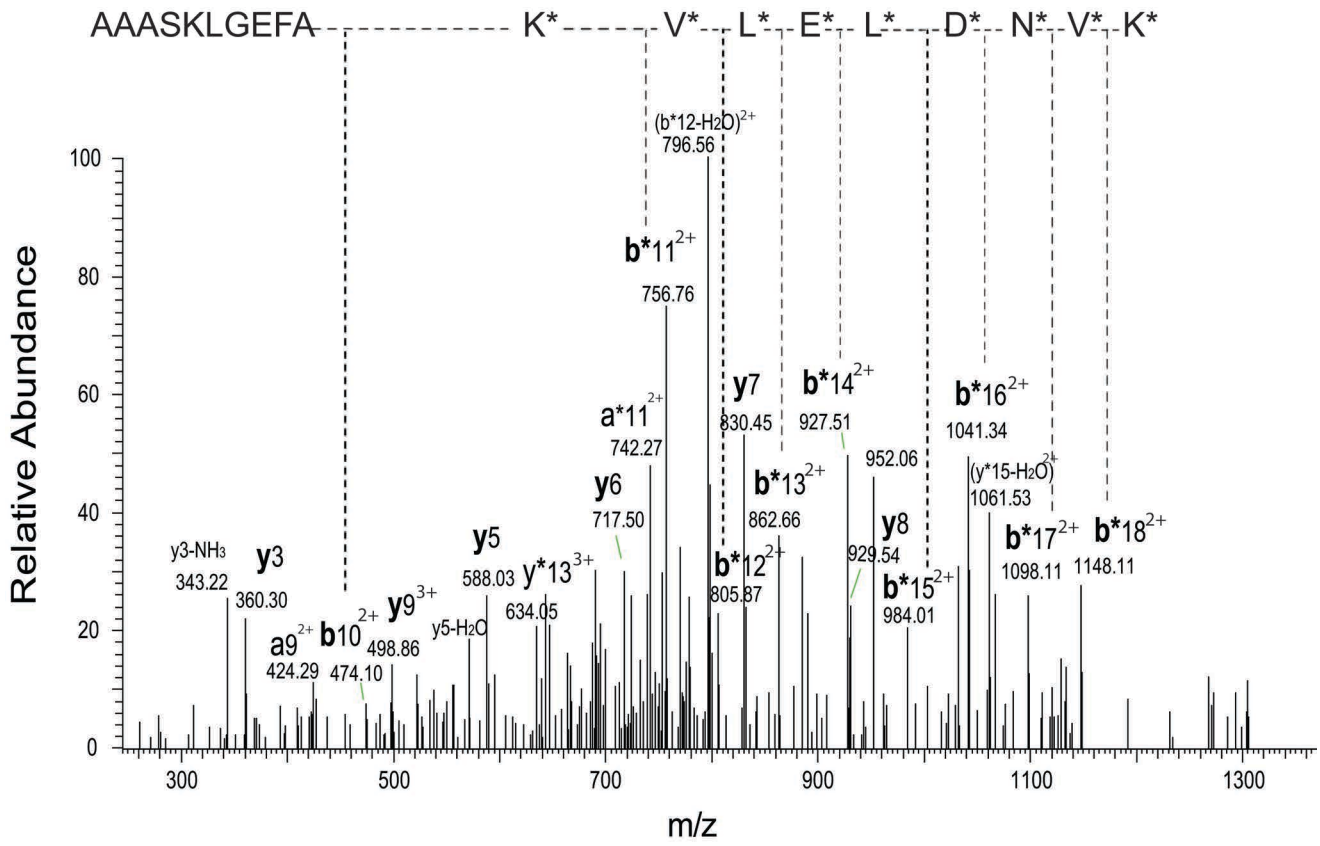
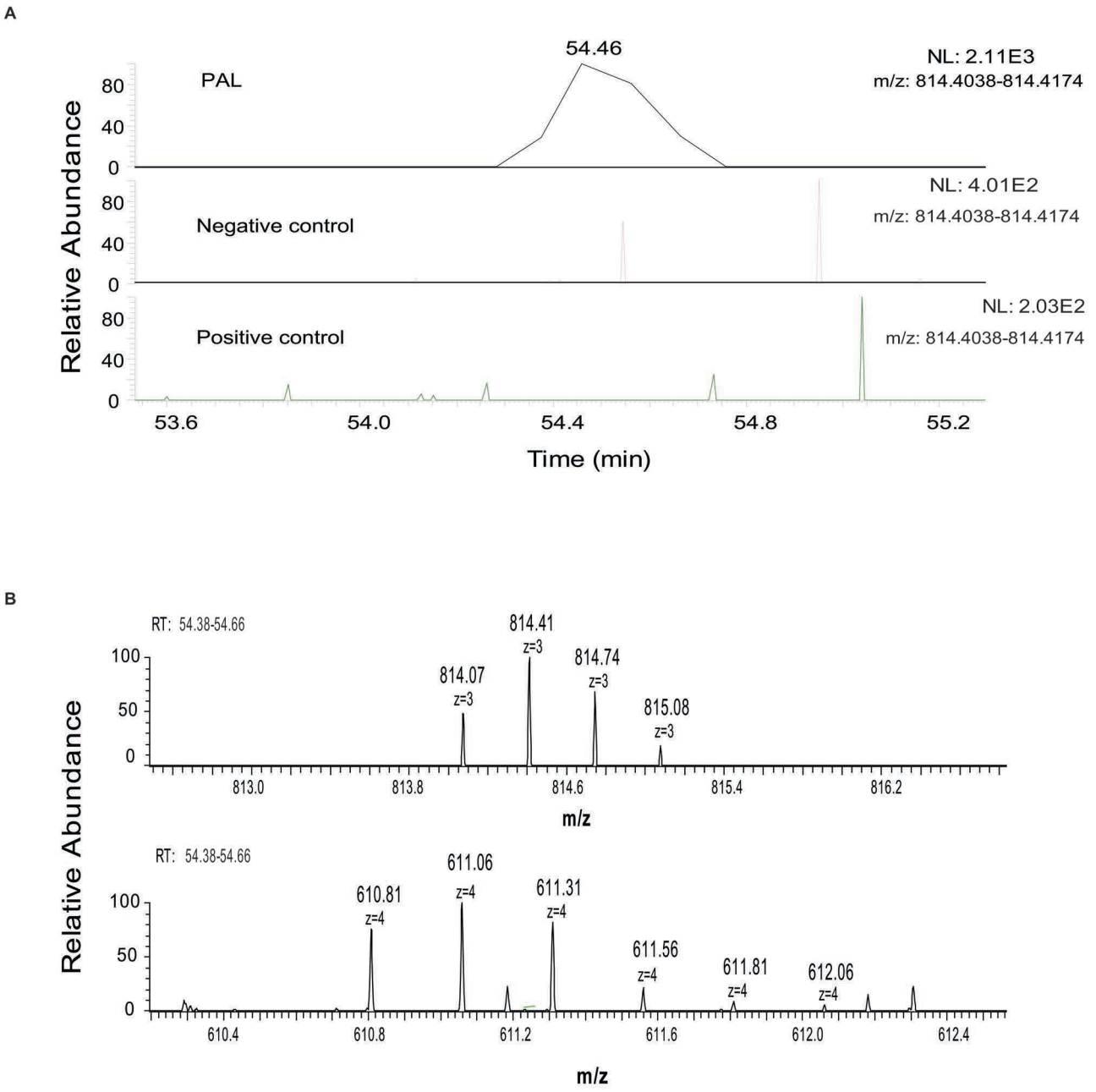


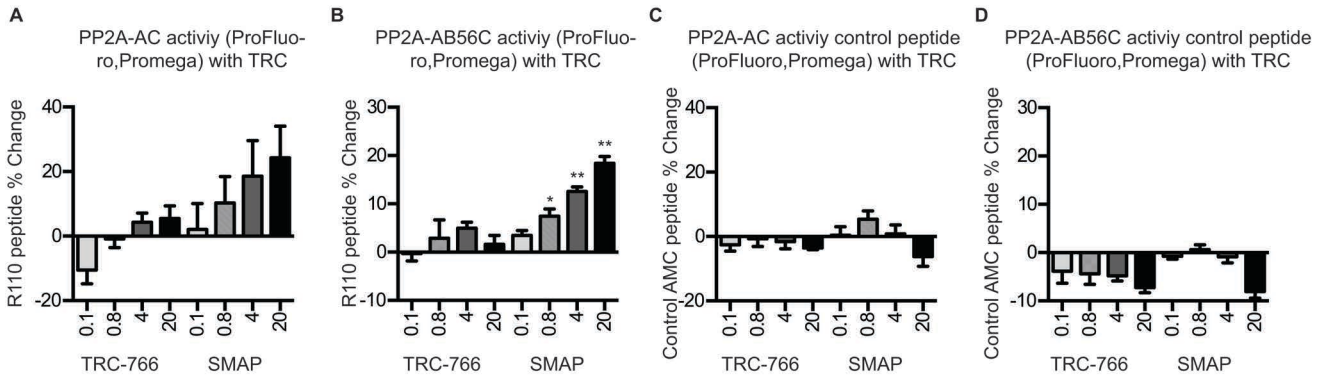
Supplemental Figure 7: Equilibrium dialysis for SMAP binding. **A,** Saturation curve for [3H]-SMAP. PP2A-Aα subunit was titrated with increasing concentration of drug to determine the Kd and binding max of [3H]-SMAP. Values corrected for background and plotted against drug concentration. **B,** Competition of SMAPs with [3H]-SMAP. Pre-incubation of the Aα subunit with [3H]-SMAP was followed by subsequent addition of increasing amounts of different SMAPs (0.5, 2.5 and 5 μM) to test the ability to reduce the amount of drug bound.. The specific binding is plotted, determined by subtracting the residual binding at the highest concentration of SMAP2. These results show that different SMAPs can compete the interaction of [3H]-SMAP with PP2A.Data are means ± SD for triplicate experiments.



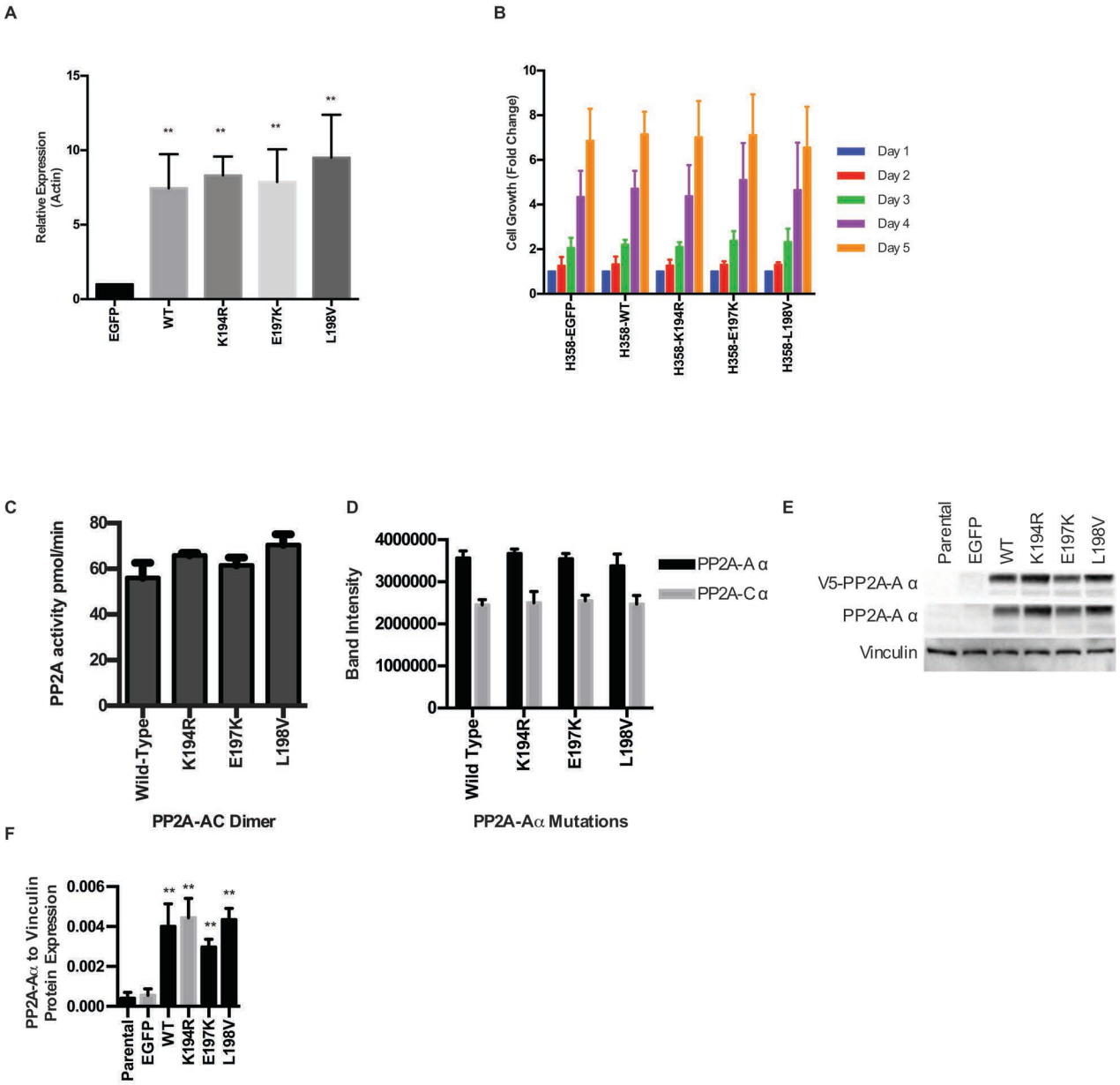
Supplemental Figure 8: MS/MS spectrum of tryptic peptide 184-AAASKLGEFAKVLELDNVK-202 photo cross-linked to a drug compound TRC-453 that contains a photoactivatable nitrene (C23H22ClN3O2S). The triply protonated ion (*m/z* 814.41) was selected as a precursor. The b- and y- ions labeled as b*n and y*n ions, respectively, have a TRC-453 tag (439.11 Da) attached.



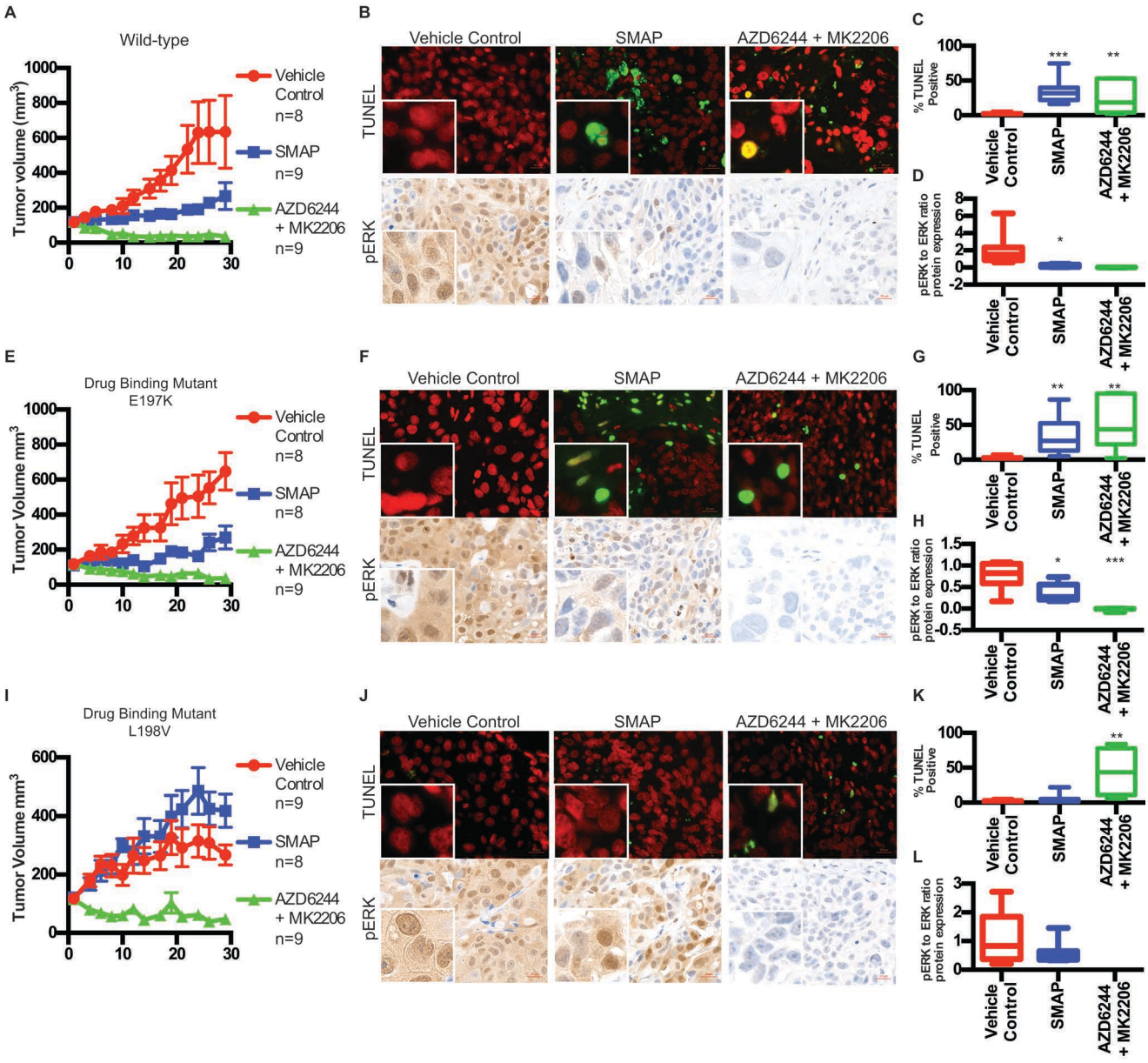
Supplemental Figure 9: Nano- LC-MS analysis of tryptic digest of PP2A and its cross-linked product to TRC-453 drug compound that contains photoactivabale nitrene.**A**, Selected ion chromatogram of the triply protonated ion (m/z 814.41) of the tryptic peptide 184-AAASKLGEFAKVLELDN-VK-202 photo cross-linked to a TRC-453 derived from PAL (+drug/+UV), the negative control (-drug/-UV) and the positive control (+drug/-UV) experiments. **B**, Isotopic distribution of the 3+ and 4+ -charged ions of the tryptic peptide 184-202 photo cross-linked to TRC-453 drug.



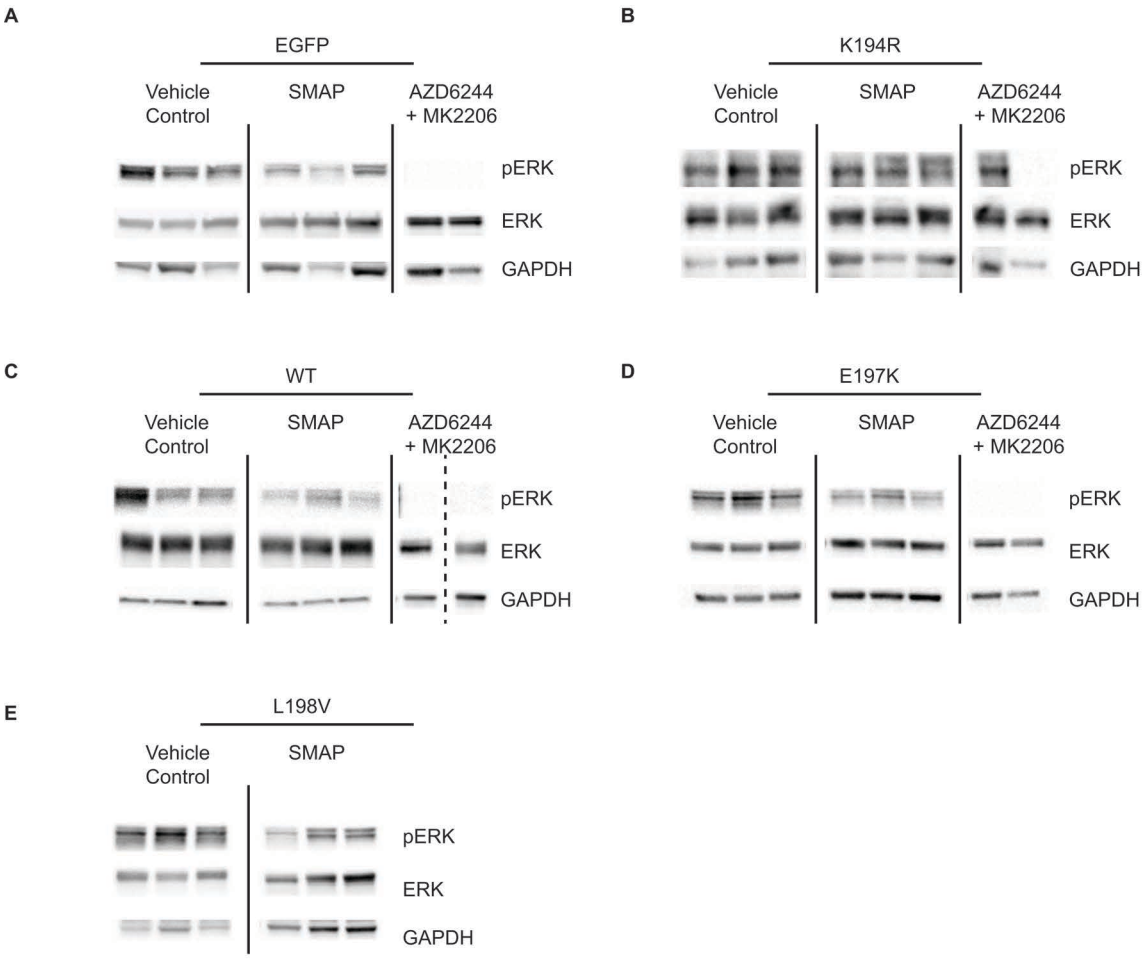
Supplemental Figure 10: Assay of PP2A with peptide substrate. **A**) and **C**) PP2A AC dimer and **B**) and **D**) recombinant AB56C trimer assembled from purified recombinant subunits were assayed for phosphatase activity using the ProFluor Ser/Thr R110 substrate system. The PP2A were assayed over a range of 0 to 100 ng to demonstrate a linear response range and 15 ng of AC and 10 ng AB56C were used to test for effects of compounds that were included at the indicated final concentrations (0.1 to 20 μM). Activities are plotted as the percent change to control. TRC-766 is an inactive compound, SMAP is the lead compound. **A**),**B**) The R110 phosphopeptide reactions show dose-dependent increase in phosphatase activity, **C**),**D**) compared to no change in reaction with the control AMC substrate peptide. Assays were completed in triplicate with calculation of the mean and SE, shown by the error bars. *P < 0.05, **P < 0.01, ***P < 0.001.



Supplemental Figure 11: Mutations at the drug-binding site of PP2A decrease sensitivity to SMAPs. **A** Real-time PCR analysis validates the overexpression of PP2A-Aα in the isogenic H358 cell lines. **B** MTT assay over five days shows that the different isogenic cell lines overexpressing PP2A-A α preserve their cell growth potential at a comparable level to the parental cell line (EGFP control). **C** Phosphatase activity assay of PP2A-AC containing PP2A-Aα wt or mutations at the putative drug-binding site. Assay buffer was 50 mM MOPS, pH 7.5, 100 mM NaCl, 0.1% 2-ME, 10 mM MgSO4 and 1 mM MnCl2. DiFMUP (6,8-Difluoro-4-Methylumbelliferyl Phosphate) was added to a final concentration of 100 μM to 1 nM PP2A at 25 °C and activity was calculated from fluorescence measured at 358/455 nm with plate reader. **D** Pull down of regulatory subunit B56 shows that the different mutants PP2A-Aα retain their ability to form holoenzymes. **E** Western blot analysis confirms the overexpression of V5-tagged wild type and mutant PP2A- Aα at a translational level. **F**, The densitometry results depict the averages of three independent experiments ± SD. *P < 0.05, **P < 0.01, ***P < 0.001 by Student's *t* test.



Supplemental Figure 12: SMAPs activate the protein phosphatase PP2A in tumors. Male nude mice were subcutaneously injected ($1x10^7$ cells per injection) in the right flank with the different isogenic cell lines (control WT, putative drug binding mutant E197K, and putative drug binding mutant L198V). Once the tumors reached a volume of 100 mm^3, the mice were randomly enrolled with vehicle control (n=8 for WT, n=8 for E197K, n=9 for L198V), combination of 6mg/kg MK2206 and 24mg/kg AZD6244 (n=9 for WT, n=9 for E197K, n=9 for L198V), or 5mg/kg SMAP (n=9 for WT, n=8 for E197K, n=8 for 198V) BID for four weeks. **A,** Mouse tumor volume for control WT expressing H358 xenograft over course of treatment. Tumor volume over course of treatment. **B,** Tumors were evaluated by sacrificing the mice two hours after final treatment. Representative TUNEL staining and pERK IHC of treated-tumors. Scale bar: 20μM. **C,** Quantification of the TUNEL positivity in tumors treated. **D,** Quantification of the pERK levels in the xenograft tumors as performed by immunoblotting and densitometry. **E,** Mouse tumor volume for drug binding mutant E197K expressing H358 xenograft over course of treatment. Tumor volume over course of treatment. **F,** Tumors were evaluated by sacrificing the mice two hours after final treatment. Representative TUNEL staining and pERK IHC of treated-tumors. Scale bar: 20μM. **G,** Quantification of the TUNEL positivity in tumors treated. **H,** Quantification of the pERK levels in the xenograft tumors as performed by immunoblotting and densitometry. **I,** Mouse tumor volume for drug binding mutant L198V expressing H358 xenograft over course of treatment. Tumor volume over course of treatment. **J,** Tumors were evaluated by sacrificing the mice two hours after final treatment. Representative TUNEL staining and pERK IHC of treated-tumors. Scale bar: 20μM. **K,** Quantification of the TUNEL positivity in tumors treated. **L**, Quantification of the pERK levels in the xenograft tumors as performed by immunoblotting and densitometry. Data are means ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001 by Student's *t* test.



Supplemental Figure 13: Western blots for pERK, ERK, and GAPDH in **A**, control EGFP **B**, putative drug binding mutant K194R **C**, control WT **D**, putative drug binding mutant E197K and **E**, putative drug bindng mutant L198V expressing H358 xenograft treated with vehicle control, combination of MK2206 (6mg/kg) and AZD6244 (24mg/kg), or SMAP (5mg/kg). Each lane corresponds to different animal.