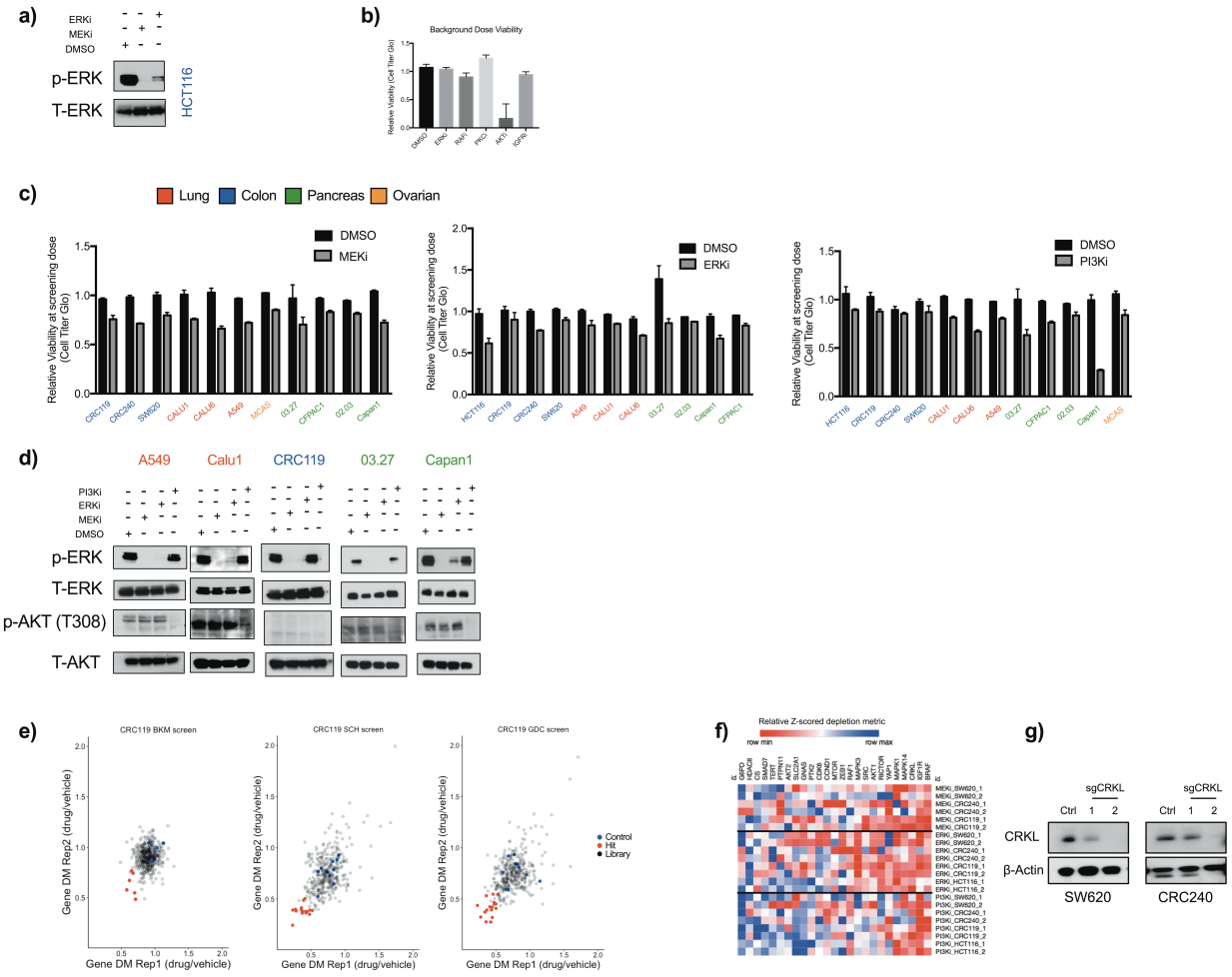
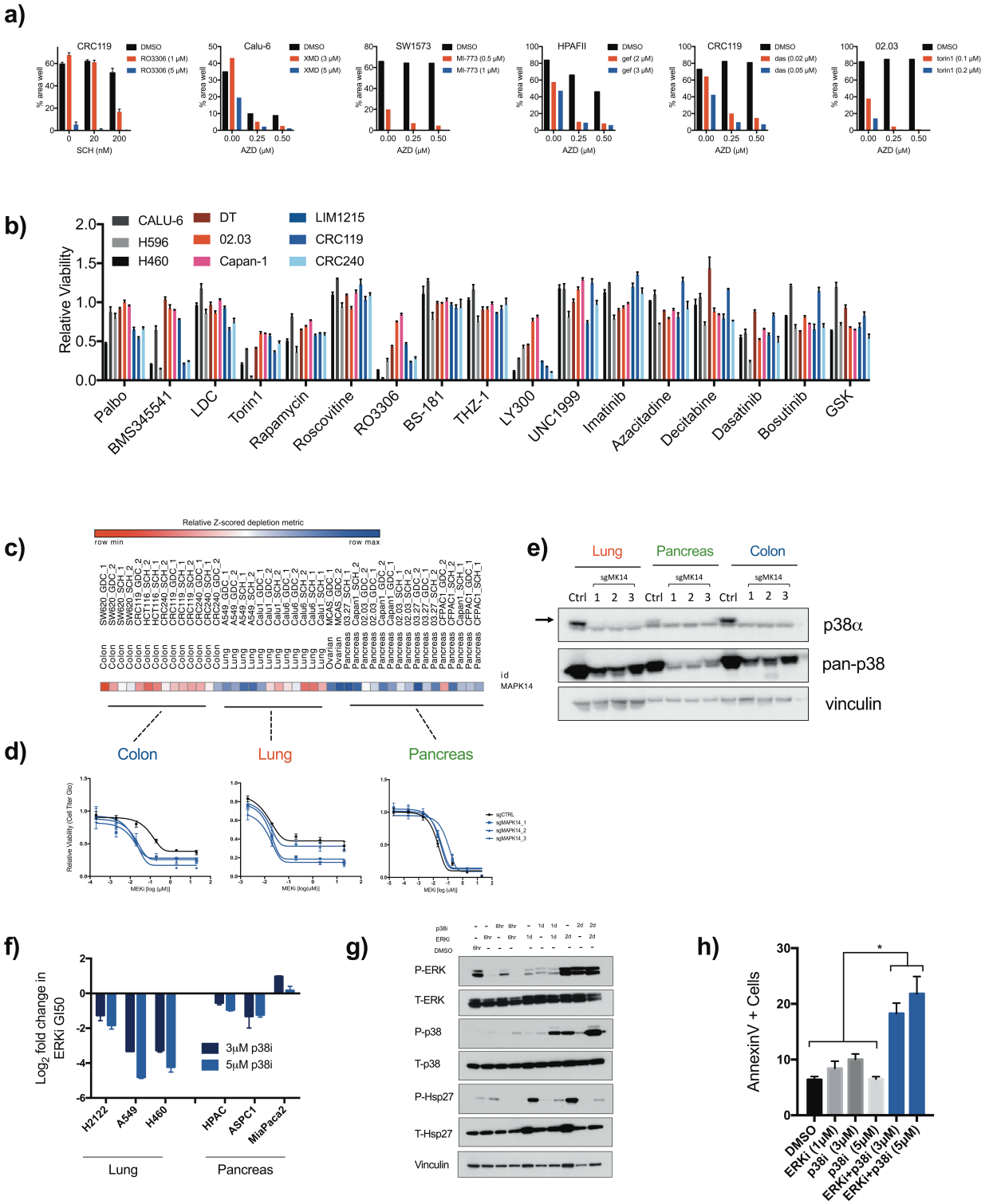
Supplemental Materials:



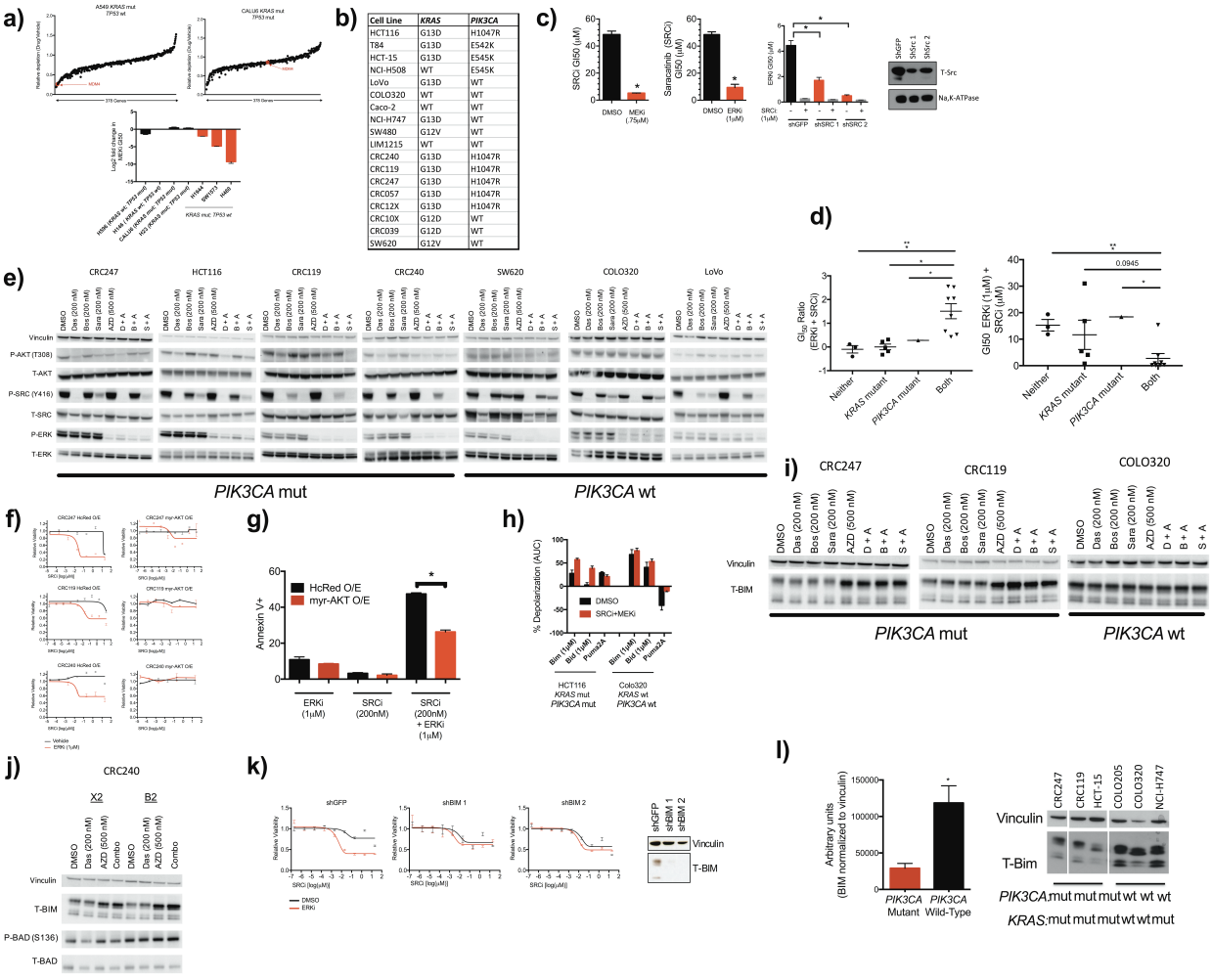
Supplemental Figure 1. Signaling and viability effects of drug doses used for screening alongside additional analyses, Related to Figures 1 and 2. a) Immunoblot in HCT116 cells treated with the indicated inhibitors at screened doses for 1hr (see Supplemental table 3 for screened doses; MEKi, AZD6244; ERKi, SCH772984). Images are cropped for clarity. **b)** Viability of cells at 72hrs in the presence of background does of the indicated inhibitors used in Figure 1C, inset. **c)** Relative viability at 72hrs at the dose used for screens for every cell line in our study (see Supplemental table 3 for doses; MEKi, GDC-0623; ERKi, SCH772984; PI3Ki, BKM-120). **d)** Immunoblot analysis of signaling effects at screening doses for representative cell lines from each tissue (see Supplemental table 3 for doses; MEKi, GDC-0623; ERKi, SCH772984; PI3Ki, BKM-120). Images are cropped for clarity. **e)** Depletion metric (DM) for each replicate screen in CRC119 cells across the three different inhibitors screened (DM is calculated using the three score). **f)** Heatmap for the hits identified in the 4 CRC cell lines (those hits scoring in ≥ 2

lines) for each drug. Data are the Z-scored average for the 3 most active sgRNAs per gene. **g)** Representative immunoblots for CRKL knockout in CRC240 and SW620 cells. Images are cropped for clarity. Data are n = 3.



Supplemental Figure 2. Screen validations and tissue specificity of p38α, Related to Figure 2. a) Quantification of the Figure panel in Figure 6 D-F clonogenic assays. **b)** Viability effects of inhibitors used to test sensitizers for each cell line in Figure 2G-I (see Figure 2 legend for dose information). **c)** Relative depletion of MAPK14 (p38α)

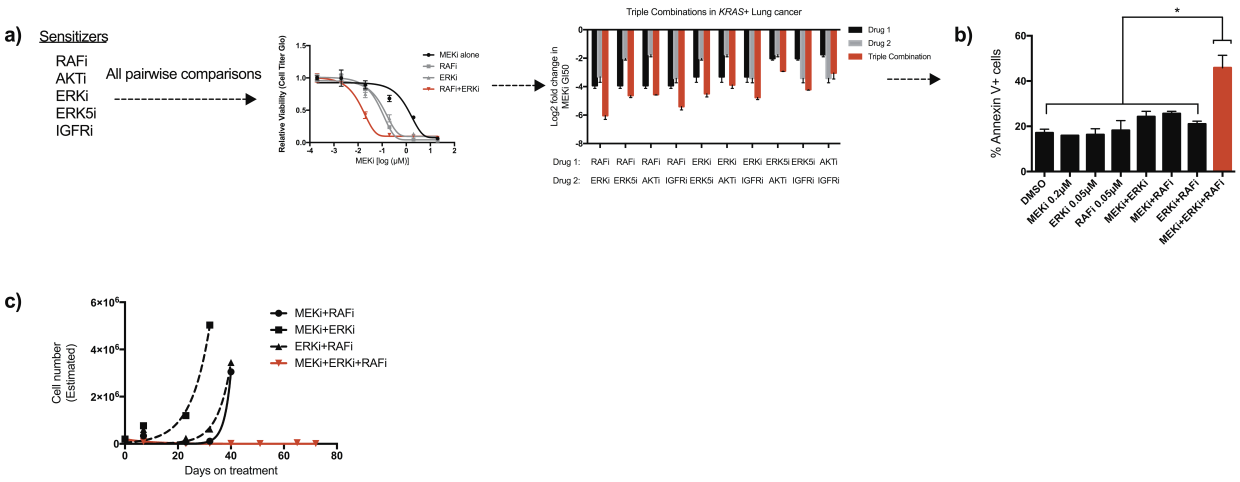
across all MAPKi screens. **d)** GI50 to MEK inhibitors in cells edited for p38α in lung, colon, and pancreas cell lines (Colon and Pancreas: AZD6244, Lung: GDC-0623). CRISPRs are top 3 guides for p38α from our library, sgCTRL is scrambled control B5. **e)** Immunoblot for knockout of p38α. Images are cropped for clarity. **f)** Log2 transformed GI50s to the ERK inhibitor SCH772984 in the presence of the p38 inhibitor LY2228820 at the indicated constant background dose in a panel of lung and PDAC lines. **g)** Immunoblot in A549 lung cancer cells treated with the ERK inhibitor SCH772984 (2μM), the p38 inhibitor LY2228820 (3μM), or the combination for the indicated times and probed for the indicated targets. Images are cropped for clarity. **h)** Apoptosis measurements reported as percentage of annexin V positive cells after 48 hours of treatment with the indicated inhibitors. (p38i, LY2228820; ERKi, SCH772984) *$p<0.05$, data are n = 3.



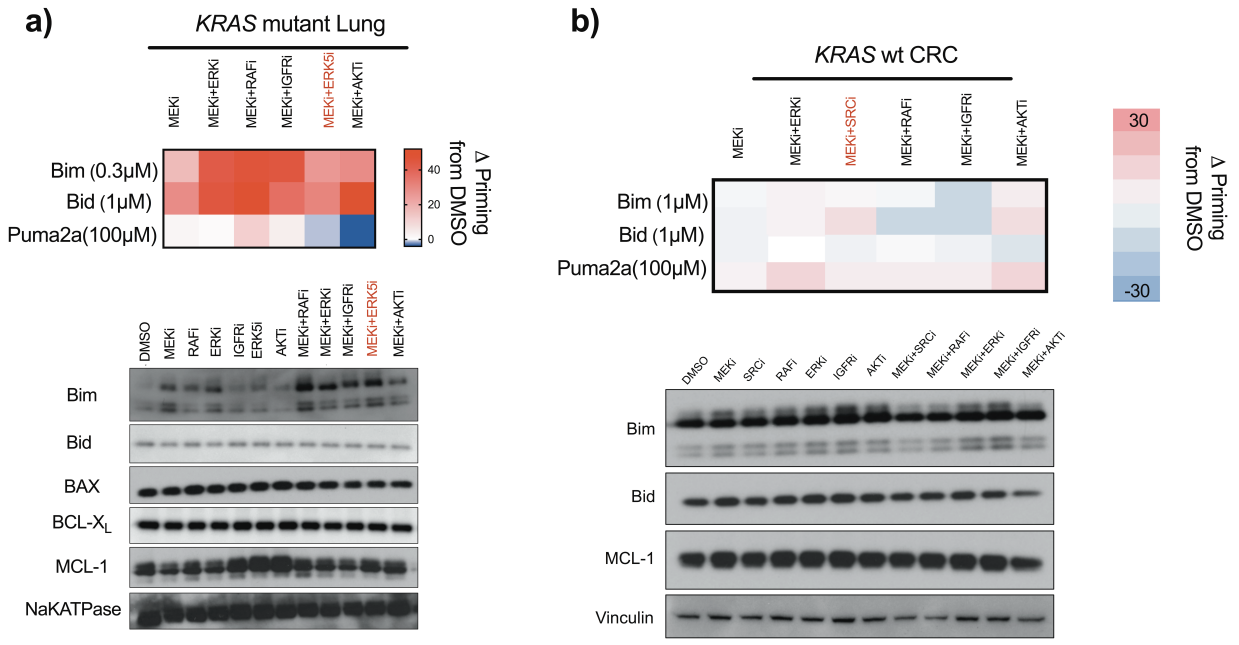
Cell Line	*KRAS*	*PIK3CA*
HCT116	G13D	H1047R
T84	G13D	E542K
HCT-15	G13D	E545K
NCI-H508	WT	E545K
LoVo	G13D	WT
COLO320	WT	WT
Caco-2	WT	WT
NCI-H747	G13D	WT
SW480	G12V	WT
LIM1215	WT	WT
CRC240	G13D	H1047R
CRC119	G13D	H1047R
CRC247	G13D	H1047R
CRC057	G13D	H1047R
CRC12X	G13D	H1047R
CRC10X	G12D	WT
CRC039	G12D	WT
SW620	G12V	WT

Supplemental Figure 3. Understanding secondary modifiers of sensitivity, Related to Figure 3. a) Top: The rank ordered relative depletion scores (three-score) plotted for all 378 genes in p53 wild-type and p53 mutant lung cancer cell lines. Bottom: The log2 transformed GI50 values for the MEKi AZD6244 in the presence of a constant background dose of MI-773 (MDM2/4i, 1μM) in seven lung cell lines. **b)** Table of all CRC cell lines and their mutational status for *KRAS* and *PIK3CA*. **c)** GI50 to SRCi (dasatinib) with a constant dose of MEKi (AZD6244) in the background for CRC240 cells (left). GI50 value for an additional SRCi (saracatinib) with a constant dose of ERKi (VX-11e) in the background for CRC240 cells (center). GI50 value to an ERKi (VX-11e) in combination with either vehicle or a constant background dose of SRCi (dasatinib) in CRC240 transduced with either shGFP or two independent hairpins targeting SRC (right). An immunoblot indicating shRNA-mediated knockdown is shown. Images are cropped for clarity. **d)** Fold change in GI50 for the SRC inhibitor in the presence of either DMSO or a constant dose of ERKi across a panel of CRC cell lines with indicated alterations in *KRAS* and *PIK3CA*. GI50 Ratio is calculated as the $\log_{10}$(SRCi $GI50_{Veh}$/SRCi $GI50_{ERKi\ constant\ background\ dose}$). Cell lines are stratified by their mutational background. To the right, raw GI50 value for overall sensitivity to a SRCi in the presence of a constant background dose of ERKi in CRC cell lines stratified by mutational background (Drug identities and concentrations are the same

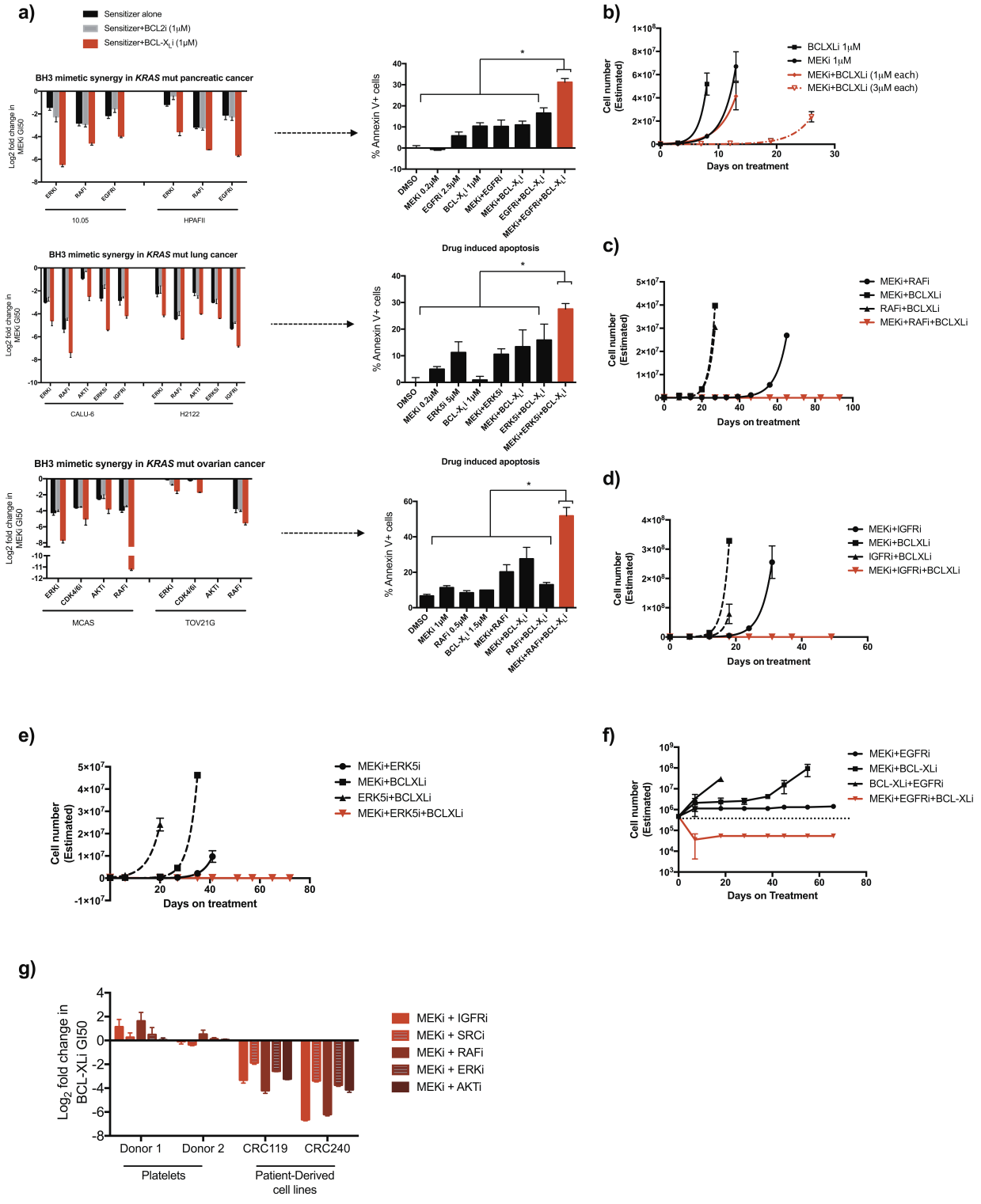
as in Figure 3C). **e)** Immunoblot of P-AKT (T308), T-AKT, P-SRC, T-SRC, P-ERK, T-ERK in CRC cell lines across different mutational backgrounds treated for 24hrs with vehicle, one of three SRC inhibitors (dasatinib, bosutinib, saracatinib), a MEK inhibitor (AZD6244), or the combination of one SRC inhibitor and the MEK inhibitor. **f)** GI50 value for a SRCi (dasatinib) in three CRC lines in combination with a constant background dose of ERKi (VX-11e) transduced with either HcRed or myr-AKT. Images are cropped for clarity. **g)** Apoptosis measurements reported as percent annexin V+ in CRC247 with HcRed or myr-AKT overexpression treated with an ERKi (VX-11e), a SRCi (dasatinib), or the combination of both. **h)** BH3 profiling of two CRC cell lines treated with either vehicle or the combination of a SRCi (dasatinib, 0.5μM) and a MEKi (AZD6244, 1μM). **i)** Immunoblot of T-BIM in CRC cell lines of different mutational background treated for 24hrs with vehicle, one of three SRC inhibitors (dasatinib, bosutinib, saracatinib), a MEK inhibitor (AZD6244), or the combination of one SRC inhibitor and the MEK inhibitor. Images are cropped for clarity. **j)** Immunoblot of T-BIM, P-BAD(Ser136), and T-BAD in CRC240 cells transduced with either control (X2) or myristolated-AKT (B2) then treated for 24hrs with vehicle, a SRC inhibitor (dasatinib), a MEK inhibitor (AZD6244), or the combination of a SRC inhibitor and the MEK inhibitor. Images are cropped for clarity. **k)** CRC119 GI50 for a SRCi (dasatinib) in combination with a constant background dose of ERKi (VX-11e, 1μM) transduced with either shGFP or two independent hairpins targeting BIM. Immunoblots indicating knockdown shown at right. **l)** Immunoblot of T-BIM in a panel of six CRC cell lines. BIM expression is quantified to the left. Images are cropped from the same immunoblot at the same exposure level. Error bars show data ± SEM. *$p<0.05$, data are n = 3.



Supplemental Figure 4. Synergistic triple combinations in lung cancer, Related to Figure 4. a) Similar to Figure 4D but in the *KRAS* mutant lung cancer line Calu6, novel sensitizer is ERK5 (drug identities and concentrations same as Figure 4D except ERK5i; XMD8-92, 5μM). **b)** Same as Figure 4E but in Calu6 cells with the indicated inhibitors (drug identities same as Figure 4D). **c)** TTP assay for candidate triple combination in Calu6 cells (RAFi, 0.05μM; ERKi 0.05μM; MEKi 0.2μM; drug identities are the same as Figure 4D). *p<0.05, data are n=3.



Supplemental Figure 5. Two-body combinations prime *KRAS* mutant lung cancer, and two-body combinations do not prime WT CRC, Related to Figure 5. a) BH3 profiling in the *KRAS* mutant lung cell line Calu6 treated with the indicated combinations (MEKi 1μM; RAFi 0.5μM; IGFRi 3μM; ERK5i, XMD8-92 5μM; ERKi 0.25; AKTi 5μM; drug identities are the same as Figure 5A). (below) Immunoblot of indicated proteins in the *KRAS* mutant lung cell line Calu6 treated with the indicated combinations (MEKi 0.2μM; RAFi 0.1μM; IGFRi 1μM; ERK5i 5μM; ERKi 0.1μM; AKTi 5μM. Images are cropped for clarity. **b)** BH3 profiling in the *KRAS* wild-type CRC cell line Colo-320 treated with the indicated combinations (drugs and doses are same as in Figure 5A). (below) Immunoblot of indicated proteins in the *KRAS* wild-type CRC cell line Colo-320 treated with the indicated combinations (drugs and doses are same as in Figure 5B). Images are cropped for clarity. Data are n=3.



Supplemental Figure 6. Targeting BCL-X_L in *KRAS* mutant lung, pancreatic, and ovarian cancers and addressing concerns of toxicity, Related to Figure 6. a) Log2 transformed GI50 values for a MEKi (AZD6244) in combination with a constant background dose of one of the sensitizers indicated as well as a constant background

dose of either a BCL-X_L inhibitor (WEHI-539, 1μM) or a BCL2 inhibitor (ABT-199, 1μM). PDAC doses: RAFi LY3009120, 0.2μM, EGFRi Gefitinib, 3μM, ERKi SCH772984 0.1μM. Lung doses: RAFi 0.2μM, ERKi 0.1μM, ERK5i XMD8-92 5μM, IGF1Ri GSK1838705A 1μM, AKTi MK2206 5μM (RAFi and ERKi same as PDAC). Ovarian doses: RAFi 0.2μM, ERKi 0.1μM, AKTi 2μM, CDK4/6i Palbociclib 1μM (all other drug identities are same as PDAC and Lung). **b-f)** TTP assay for cell lines treated with the indicated combinations. Data are means ± SD of 2 replicate experiments. **b)** CRC119 cells treated with the indicated combinations. **c)** HCT116 cells treated with MEKi 0.2μM, RAFi 0.1μM, and BCL-XLi 2μM. **d)** CRC119 cells treated with MEKi 0.5μM, IGFRi 2μM, and BCL-XLi 2μM. **e)** Calu6 cells treated with MEKi 0.5μM, ERK5i 5μM, and BCL-XLi 2μM. **f)** HPAFII cells treated with MEKi 0.2μM, EGFRi 3μM, and BCL-XLi 2μM (drug identities for b-f same as in panel a). **g)** Log2 transformed GI50 values for a BCL-X_L inhibitor (WEHI-539) in platelets freshly isolated from two human donors and in two early passage patient-derived CRC cell lines treated with a constant background dose of each of the indicated drugs. MEKi (500nM), IGFRi (1μM), SRCi (dasatinib, 200nM), RAFi (100nM), ERKi (100nM), AKTi (5μM). Drug identities same as in panel a. Error bars show data ± SEM. *$p<0.05$, data are n=3.