Cell Reports, Volume *29*

Supplemental Information

Synthetic Lethal Interaction of SHOC2 Depletion with MEK Inhibition in RAS-Driven Cancers

Rita Sulahian, Jason J. Kwon, Katherine H. Walsh, Emma Pailler, Timothy L. Bosse, Maneesha Thaker, Diego Almanza, Joshua M. Dempster, Joshua Pan, Federica Piccioni, Nancy Dumont, Alfredo Gonzalez, Jonathan Rennhack, Behnam Nabet, John A. Bachman, Amy Goodale, Yenarae Lee, Mukta Bagul, Rosy Liao, Adrija Navarro, Tina L. Yuan, Raymond W.S. Ng, Srivatsan Raghavan, Nathanael S. Gray, Aviad Tsherniak, Francisca Vazquez, David E. Root, Ari J. Firestone, Jeff Settleman, William C. Hahn, and Andrew J. Aguirre

SUPPLEMENTAL FIGURE 1

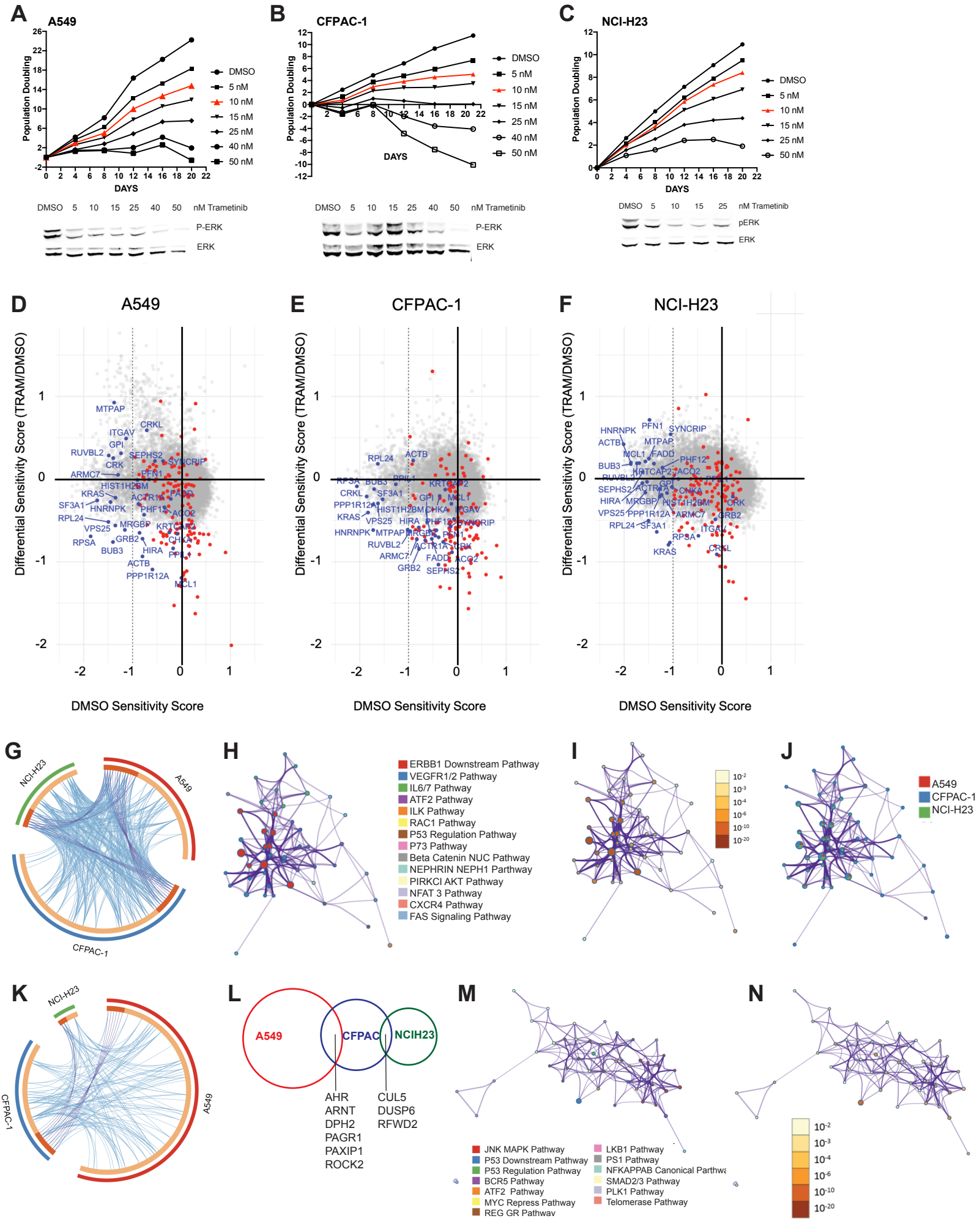
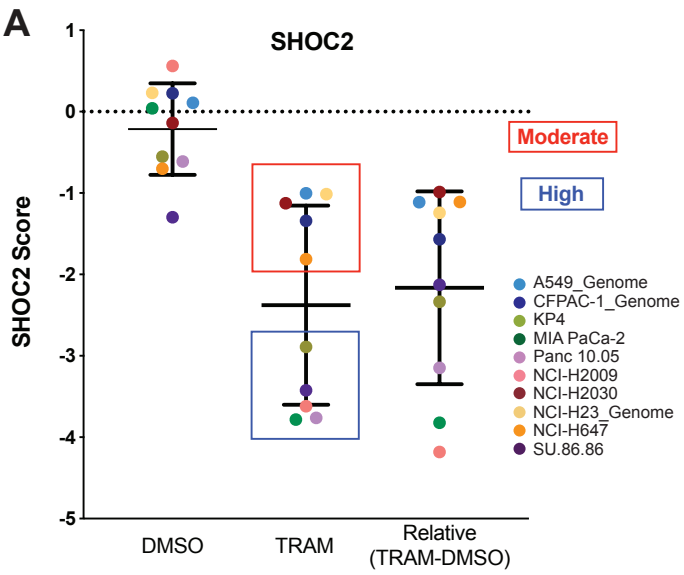
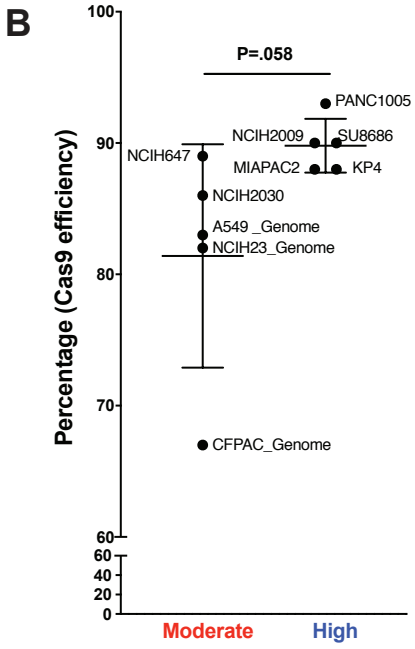
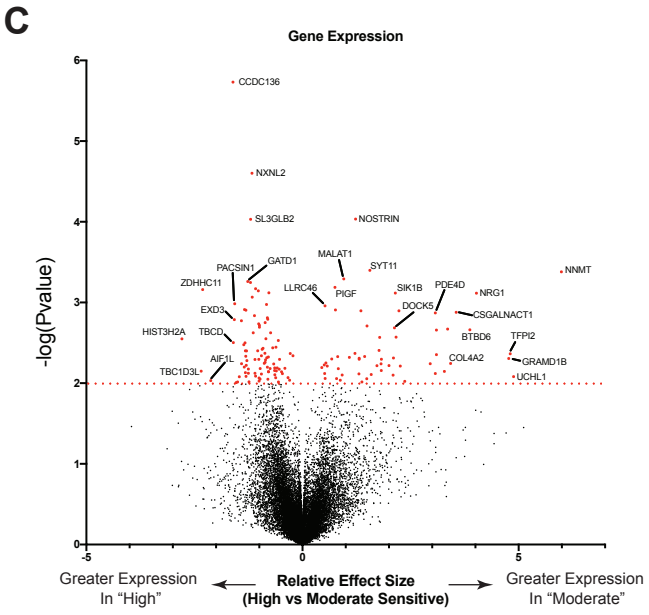


Figure S1. Titration of trametinib, evaluation of heterogeneity in intrinsic dependencies, and pathway enrichment for genome scale screens. Related to Figure 1. A, B, and C) Proliferation assay assessed by cell counting and calculation of population doubling (y-axis) in the presence of increasing concentrations of trametinib for five passages. Each point represents the mean of two independent replicates. The red line indicates the concentration of trametinib that was used to perform the pooled genome scale CRISPR-Cas9 screen. D, E, and F) Genome-scale screen results for A549, CFPAC-1, and NCI-H23 respectively. Mean DMSO Sensitivity (y-axis) and Mean Differential Sensitivity Score (x-axis) for each gene is plotted. Red points indicate genes with FDR < 0.25 based on the STARS algorithm (Methods) and blue points indicate genes with FDR < 0.25 and that are strong synthetic lethal interactors with MEKi in at least one cell line (Differential Sensitivity Score < -0.5) and that is also intrinsically dependent at baseline in at least one other line (DMSO Sensitivity Score < -1). Among the significant genes that were found to have Differential Sensitivity Score < -0.5, we identified 51 genes that were intrinsically dependent in at least one other cell line (DMSO Sensitivity Score < -1), suggesting that this biological heterogeneity may in part determine which genes score as synthetic lethal interactions with MEK inhibition in each cell line. G-N) The metascape suite of tools (http://metascape.org) was used to perform a meta-analysis of gene targets undergoing negative or positive selection in three genome scale CRISPR-MEKi screens. Genes were included in the analysis if they scored with a STARS FDR $\leq$ 0.25 and a Trametinib Sensitivity Score of -0.5 (negative selection) or 0.5 (positive selection) in one of the screens. G-J) MEKi sensitizers or synthetic lethal interactors from genome scale screens (negative selection). G) Circos plot showing genes recurrently scoring as MEKi sensitizers with differentially dependency in the context of MEK inhibition across multiple screens. On the outside, each arc represents the identity of each gene list, with a unique color for each cell line screened. On the inside, each arc represents a gene list, where each gene has a spot on the arc. Dark orange color represents the genes that appear in multiple lists and light orange color represents genes that are unique to that gene list. Purple lines link the same gene that are shared by multiple gene lists. H) Network representation of pathway enrichment within the MSigDB Canonical Pathways database. A subset of representative terms was selected from the full cluster and converted into a network layout. Each term is represented by a circle node, where its size is proportional to the number of input genes fall into that term, and its color representing its cluster identity. Terms with a similarity score > 0.3 are linked by an edge (the thickness of the edge represents the similarity score). The network is visualized with Cytoscape (v3.1.2) with "force-directed" layout and with edge bundled for clarity. One term from each cluster is selected to have its term description shown as label. I) Network with nodes colored by p-value, where terms containing more genes tend to have a more significant p-value. J) Network of enriched terms represented as pie charts, where pies are color-coded based on the identities of the gene lists with the size of a pie proportional to the total number of hits that fall into that specific term and the size of a slice representative the percentage of genes under the term that originated from the corresponding gene list. K-N) Analysis of MEKi resistance factors that were positively selected for in the CRISPR-MEKi screens. K) Circos plot, as defined above. L) Venn diagram of overlapping hits scoring as resistance factors across all three screens. M) Network representation of pathway enrichment for MEKi resistance factors within the MSIGDB Canonical Pathways database. N) Network representation with nodes colored by p-value.

Supplemental Figure 2







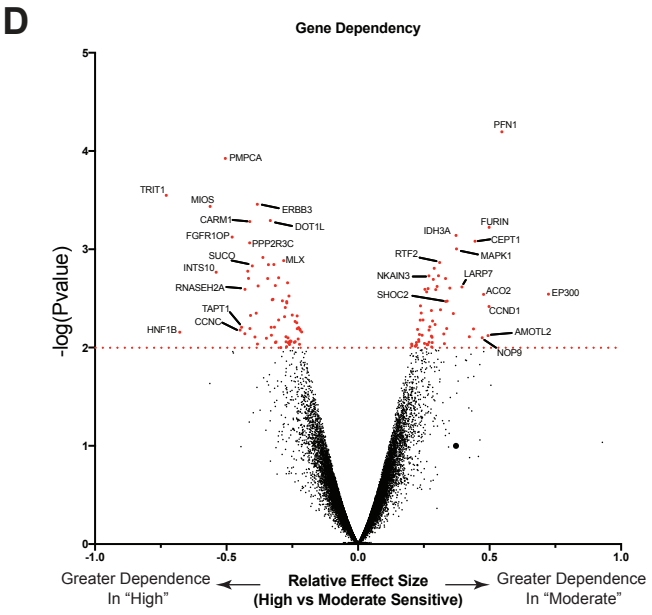


Figure S2. Evaluating bimodal distribution of SHOC2 Trametinib Sensitivity Score. Related to Figure 2. A) Summary of Mean Sensitivity Score in DMSO, or trametinib, or differential (DMSO-TRAM) is shown for SHOC2 in all cell lines screened. Each dot represents the sensitivity score for that gene in a given cell line, indicated by the color legend. "Moderately" dependent cell lines are demarcated in red and "Highly" dependent cell lines in blue. The apparent biomodality in SHOC2 dependence in the trametinib screens is not observed in the differential sensitivity between TRAM-DMSO. B) Cas9 efficiency determined as a decrease in green fluorescent protein expression (GFP, 100 - %positive) following 12-14 days infected with gRNA targeting GFP. Notably, genome-scale screens are represented in the "moderate" class and used a two-vector system with stable Cas9 expression whereas the focused CRISPR-MEKi screens used a single-vector Cas9+sgRNA system as described in the methods. C and D) 2-class comparison analysis comparing "High" against "Moderate" cell lines for C) RNA-seq gene expression and D) intrinsic gene dependency within the CCLE/DepMap database (www.depmap.org). CRISPR-Cas9 screening data were from version 19Q2. Red dots indicate genes -log(Pvalue) > 2.0. Directionality of the features is labeled on each plot for C and D.

SUPPLEMENTAL FIGURE 3

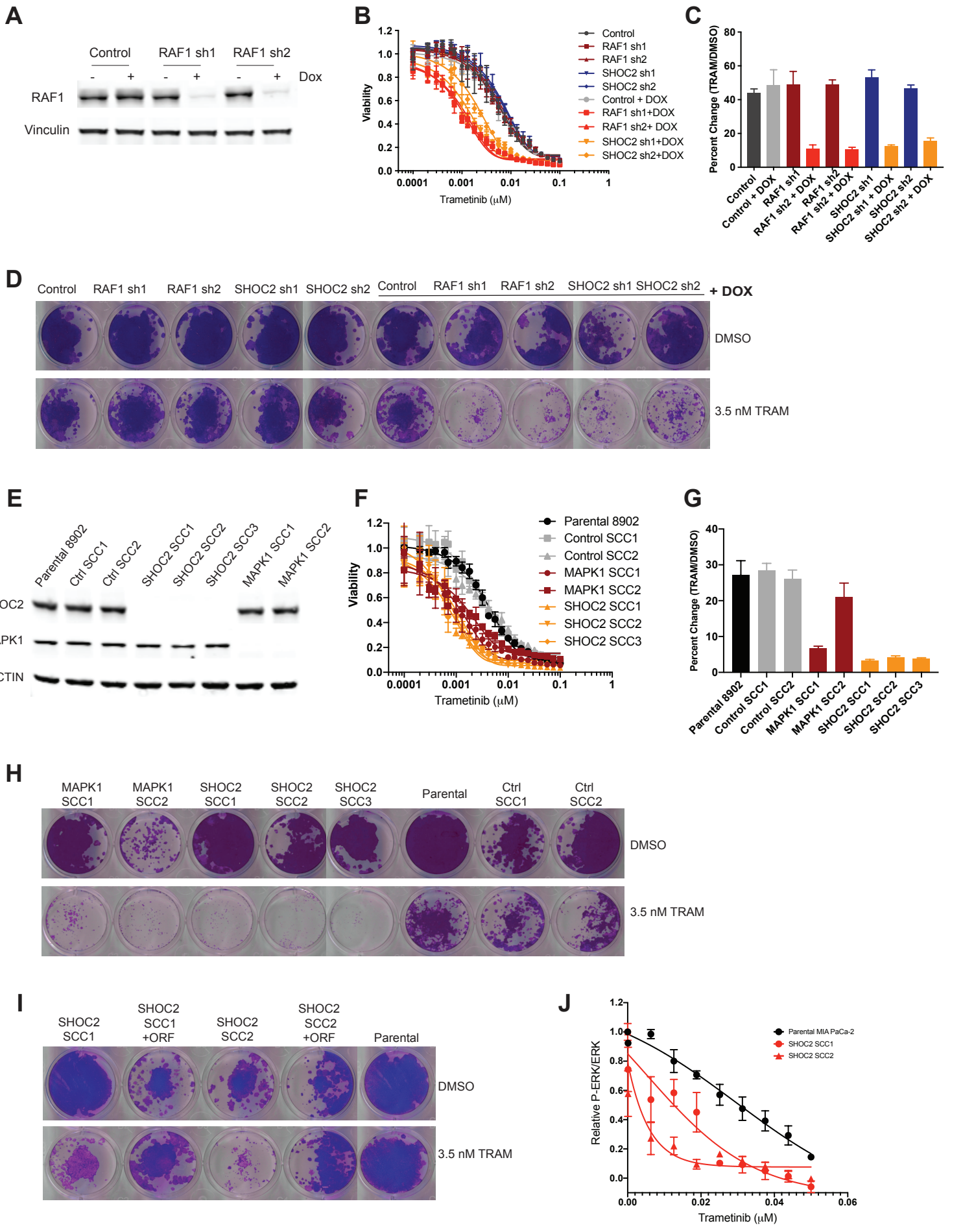


Figure S3. Validation of SHOC2 dependency including positive controls and impact of SHOC2 loss on MIA PaCa-2. Related to Figure 3. A) RAF1 levels in PA-TU-8902 stable cell lines for SHOC2 inducible or control shRNA after 72 hours of DOX treatment. SHOC2 knockdown levels are shown in Figure 3A. B) Proliferation assay in PA-TU-8902 cells after 6 days of trametinib treatment by TG viability assay and C) Quantification of clonogenic assay (9 days of treatment). D) Effects of SHOC2 and RAF1 knockdown on sensitivity to trametinib in PA-TU-8902 KRAS mutant line as shown by crystal violet staining (9 days assay). E) Immunoblot for protein expression of SHOC2 and MAPK1 in CRISPR KO Single Cell Clones (SCC). F) Proliferation assay in PA-TU-8902 cells after 6 days of trametinib treatment by CTG assay and G) Quantification of clonogenic assay (9 days of treatment). H) Effects of SHOC2 and MAPK1 knockout on sensitivity to trametinib in PA-TU-8902 KRAS mutant line as shown by crystal violet staining. I) Crystal violet images corresponding to SHOC2 rescue experiment shown in Figure 3I. Data in panels B, C, F, and G represent the average of three independent replicates ± standard deviation. J) In-cell western quantification of relative p-ERK to total ERK in MIA PaCa-2 at various doses of trametinib, 48 hours post-treatment, normalized to DMSO control. Points indicate the mean of three replicates ± standard deviation.

Supplemental FIGURE 4

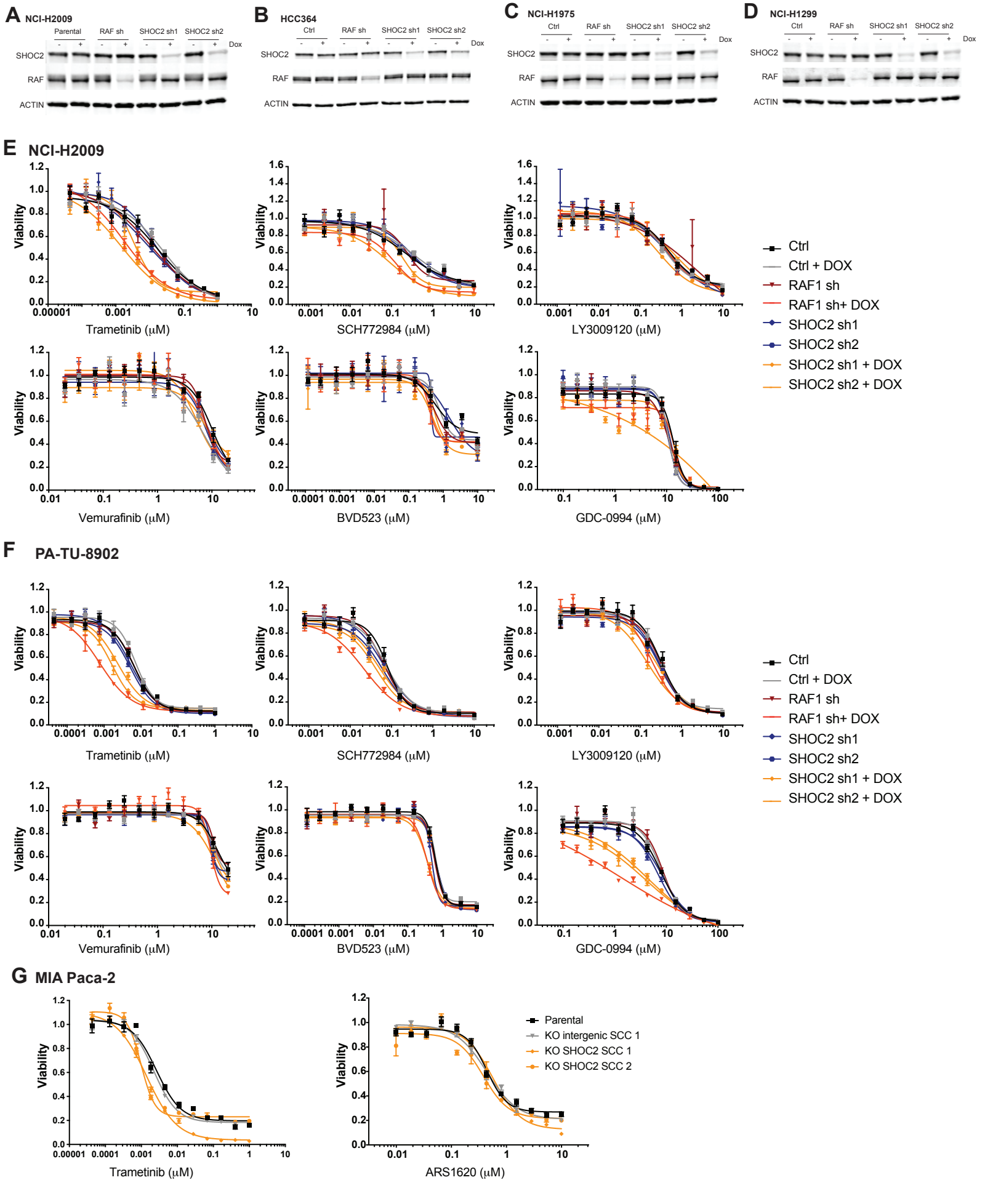


Figure S4. SHOC2 suppression potently sensitizes RAS-driven cancer cells specifically to MEK inhibition. Related to Figure 4. A-D) Supporting Western blots for plots in Figure 4. Immunoblots for protein expression after shRNA knockdown in the presence of doxycycline (Dox) for SHOC2 and RAF is demonstrated in all tested lines, including A) NCI-H2009, B) HCC364, C) NCI-H1975 and D) NCI-H1299. E-F) 6 day CTG proliferation assay with stable cell lines infected with inducible non-targeting control, RAF1, and SHOC2 shRNA. E) NCI-H2009 and F) PA-TU-8902 KRAS mutant cell lines treated with the MEK1/2 allosteric inhibitor trametinib, BRAF inhibitor (Vemurafenib), pan-RAF inhibitor (LY3009120) and ERK inhibitors (SCH772984, BVD523, GDC-0994). G) CTG proliferation assay of MIA PaCa-2 $KRAS^{G12C}$ mutant parental, intergenic control, and SHOC2 KO single cell clones (SCC) treated with trametinib or KRASG12C inhibitor, ARS1620.

Supplemental Figure 5

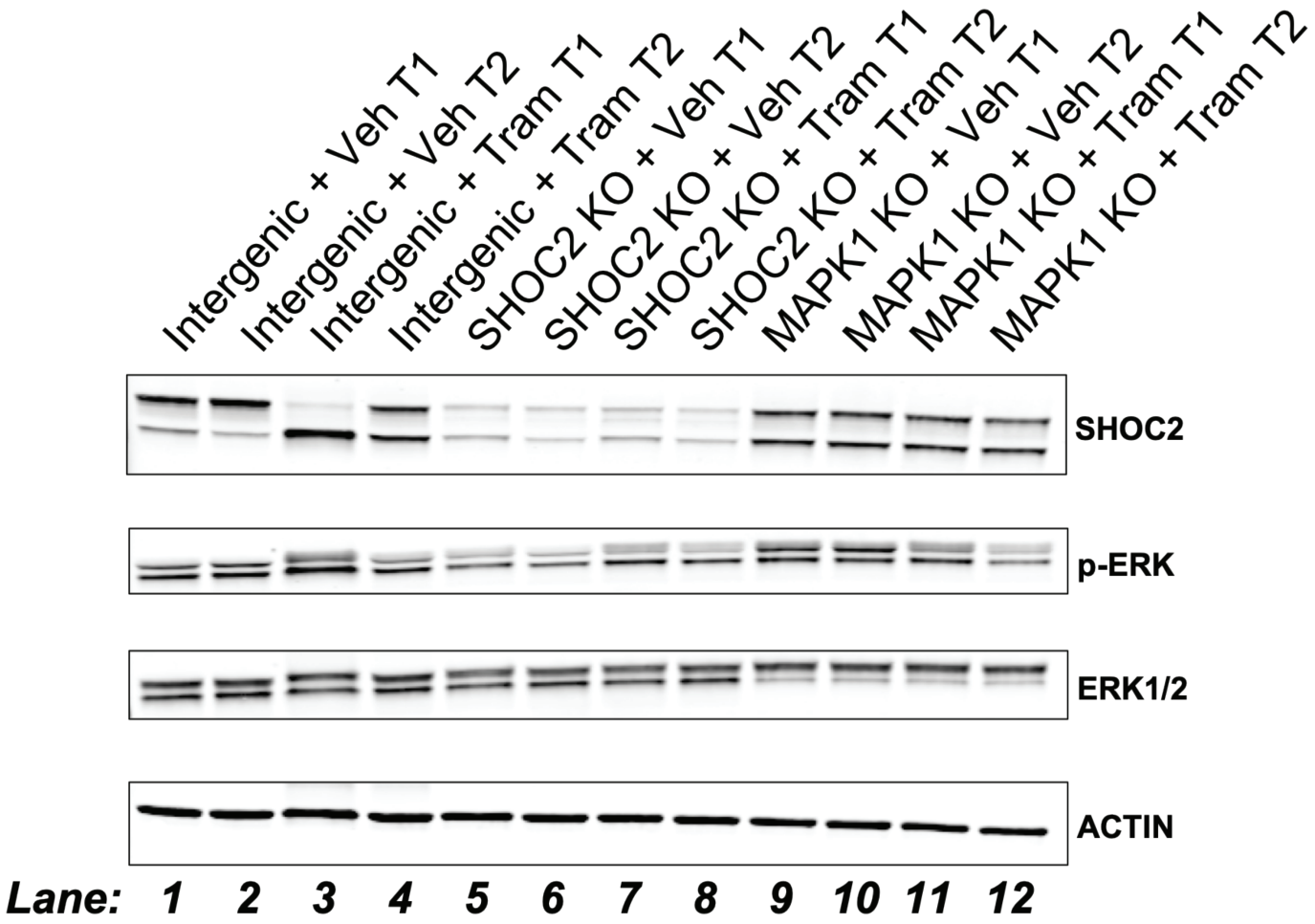


Figure S5. SHOC2 loss impacts baseline p-ERK levels *in vivo*. Related to Figure 5. Immunoblot of PA-TU-8902 control, *SHOC2*, or *MAPK1* knockout or control subcutaneous xenograft tumors treated with control trametinib (1 mg/kg) or vehicle treatment harvested after the 4th dose of trametinib (1 mg/kg) or vehicle treatment at approximately 20 days post-implantation. We observed that loss of SHOC2 alone caused a modest relative decrease (30-50%) in p-ERK level (Figure S8). Residual SHOC2 expression observed within SHOC2 KO tumors, potentially due to contaminating murine stromal cells (expressing wild-type SHOC2) or the positive selection for SHOC2 wild-type cells from a small and initially undetectable clone within the implanted polyclonal knock-out population.

Supplemental Figure 6

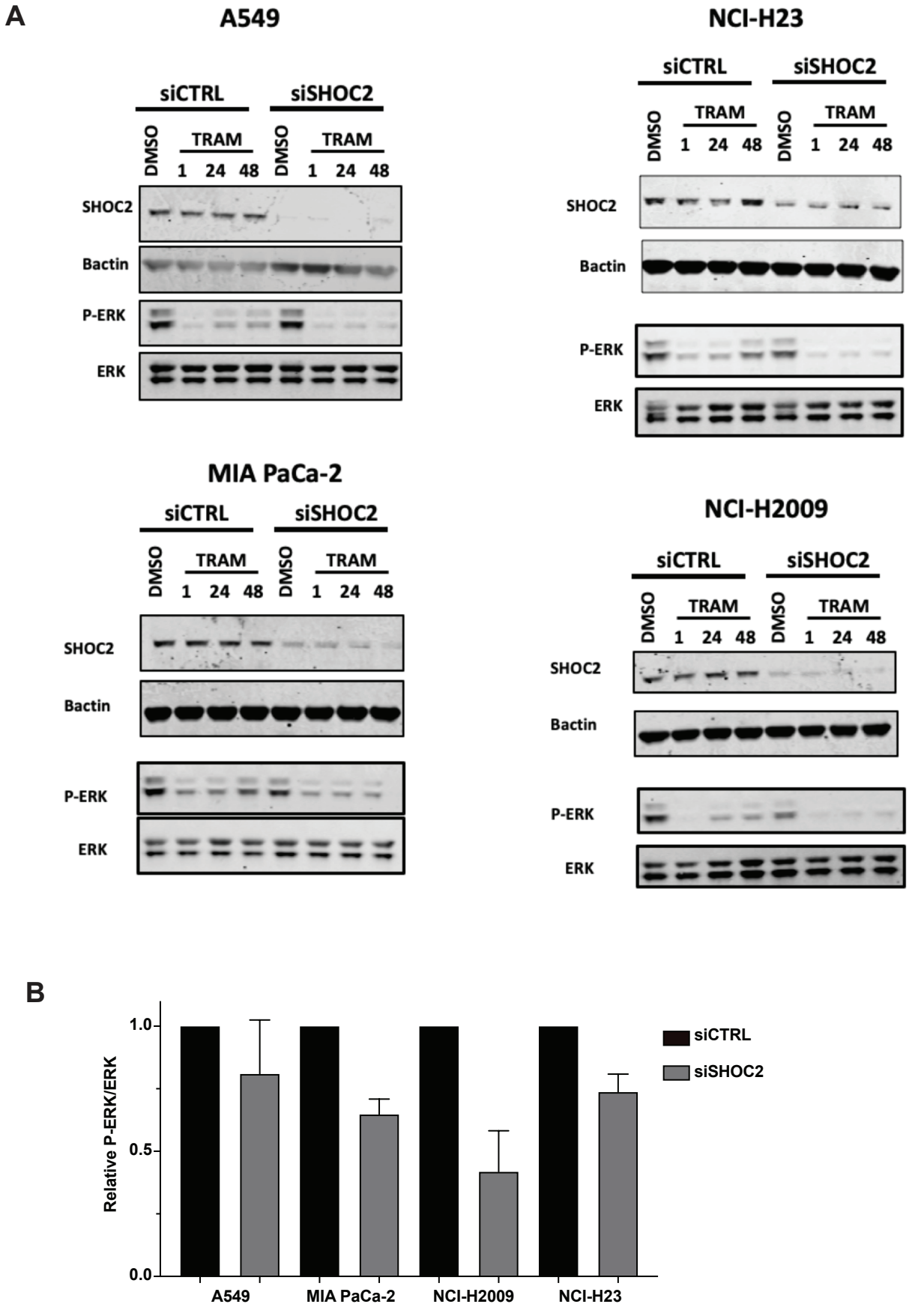


Figure S6. Loss of SHOC2 blunts MEKi adaptive reactivation of MAPK signaling and variably impacts p-ERK levels at baseline. Related to Figure 6. A) Representative immunoblot of A549, NCI-H23, MIA PaCa-2 and NCI-H2009 transfected with siCTRL or siSHOC2 and subjected to a trametinib timecourse treatment. B) Bar plot representing the average ± standard deviation for the relative p-ERK normalized to ERK at baseline (DMSO) of three independent replicate siRNA experiments per cell line, with one representative example illustrated in panel A.

Supplemental Figure 7

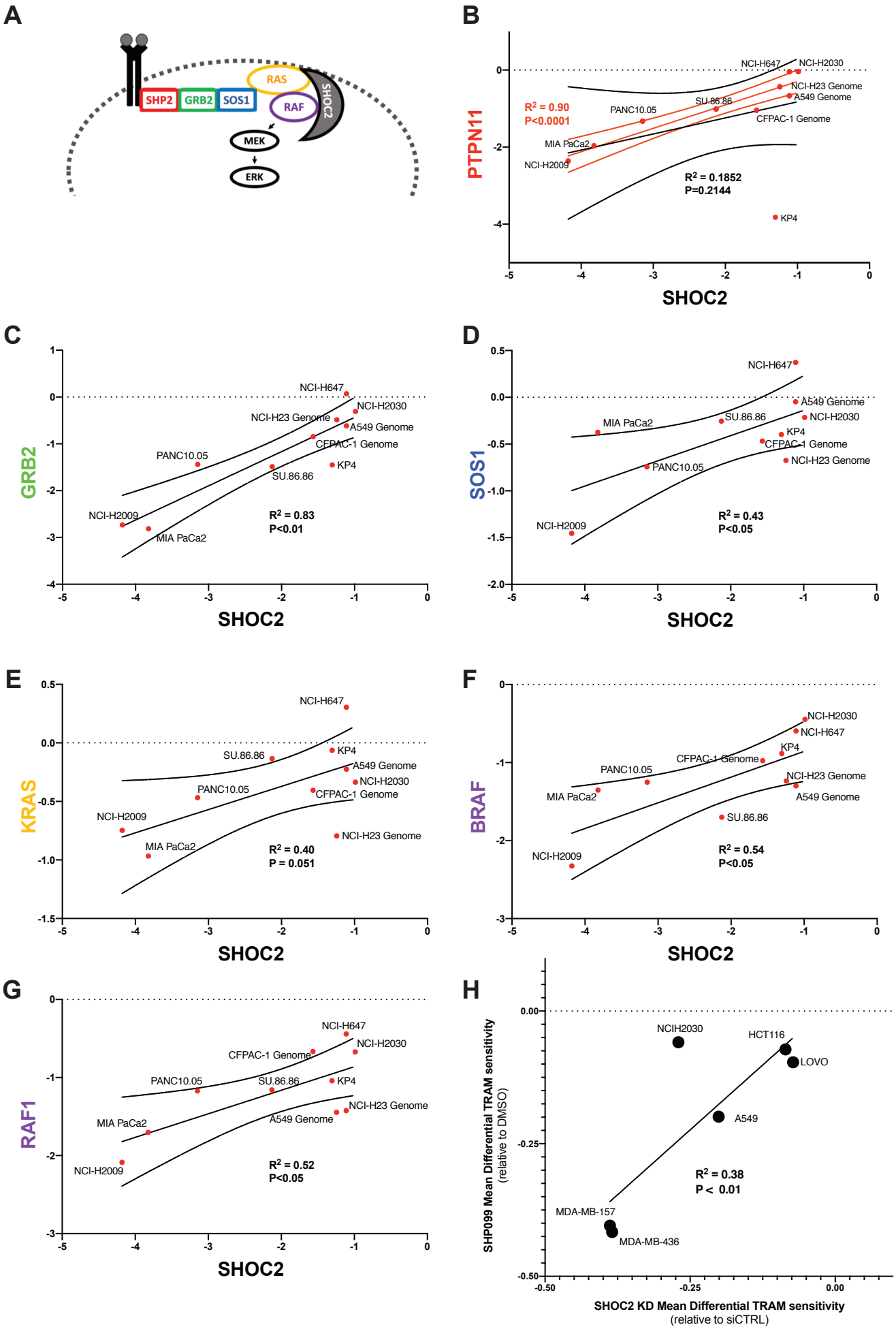


Figure S7. SHOC2 gene essentiality profile strongly correlates with RTK-MAPK pathway members and phenocopies SHP2 inhibition. Related to Figures 1 and 6. A) Schematic representation of RTK signaling pathway and contextualization of genes that positively correlate with SHOC2 gene essentiality profile. B-G) Each point represents respective cell line Differential Sensitivity Scores of indicated gene (y-axis) against SHOC2 Differential Sensitivity Score (x-axis). Solid line indicates linear best fit curve with dotted lines reflecting 95% confidence interval and (B) orange linear fit curve of PTPN11 represents correlation excluding outlier cell line KP4 (which has intrinsic baseline dependency on PTPN11). H) Differential impact on viability (DMSO-TRAM) correlated in an expanded panel of cell lines between SHOC2 knockdown (x-axis) or SHP2 inhibitor (SHP099) treatment (y-axis) after 6 days as assessed by CTG viability assay. Each point represents the average of three biological replicates of indicated cell lines.

	Cell Line	A549	CFPAC-1	KP4	MiaPaCa-2	NCI-H2009	NCI-H2030	NCI-H23	NCI-H647	Panc 10.05	SU.86.86
	Source	ATCC	ATCC	ATCC	ATCC	ATCC	ATCC	ATCC	ATCC	ATCC	ATCC
	Screen Scale	Genome	Genome	Focused	Focused	Genome	Focused	Focused	Focused	Focused	Focused
	Lineage	Lung	Pancreas	Pancreas	Pancreas	Lung	Lung	Lung	Lung	Pancreas	Pancreas
Gene Mutation Status	KRAS	G12S	G12V/AMP	G12D/AMP	G12C	G12A	G12C	G12C/AMP	G13D	G12D	G12D/AMP
	NRAS	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
	HRAS	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
	BRAF	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
	RAF1	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
	EGFR	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
	ERBB2	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
	ERBB3	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
	ERBB4	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
	FGFR1	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
	MET	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
	NF1	WT	WT	WT	WT	WT	WT	WT	DEL	WT	WT
	SMAD4	WT	DEL	DEL	WT	WT	WT	WT	WT	WT	DEL
	CDKN2A	DEL	WT	DEL	DEL	WT	WT	DEL	DEL	WT	DEL
	TP53	WT	C242R	WT	R248W	R273L	G262V	M246I	WT	PI255N	PG245S and PG360V
	ARID1A	WT	WT	WT	P1240L and Q321*	WT	WT	WT	WT	WT	WT
	KEAP1	G333C	WT	WT	WT	WT	V568F	Q193H	G523W/DEL	WT	WT
	NFE2L2	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
	SHOC2	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
	MCL1	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
	BCL2L1	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT
	YAP1	WT	WT	WT	WT	WT	WT	WT	WT	WT	WT

Supplemental Table S1: Cell lines used for CRISPR-MEKi screening. Related to Figure 1.
Mutation status of select genes for all cell lines screened are indicated: wildtype (WT), amplified (AMP), and deleted (DEL).