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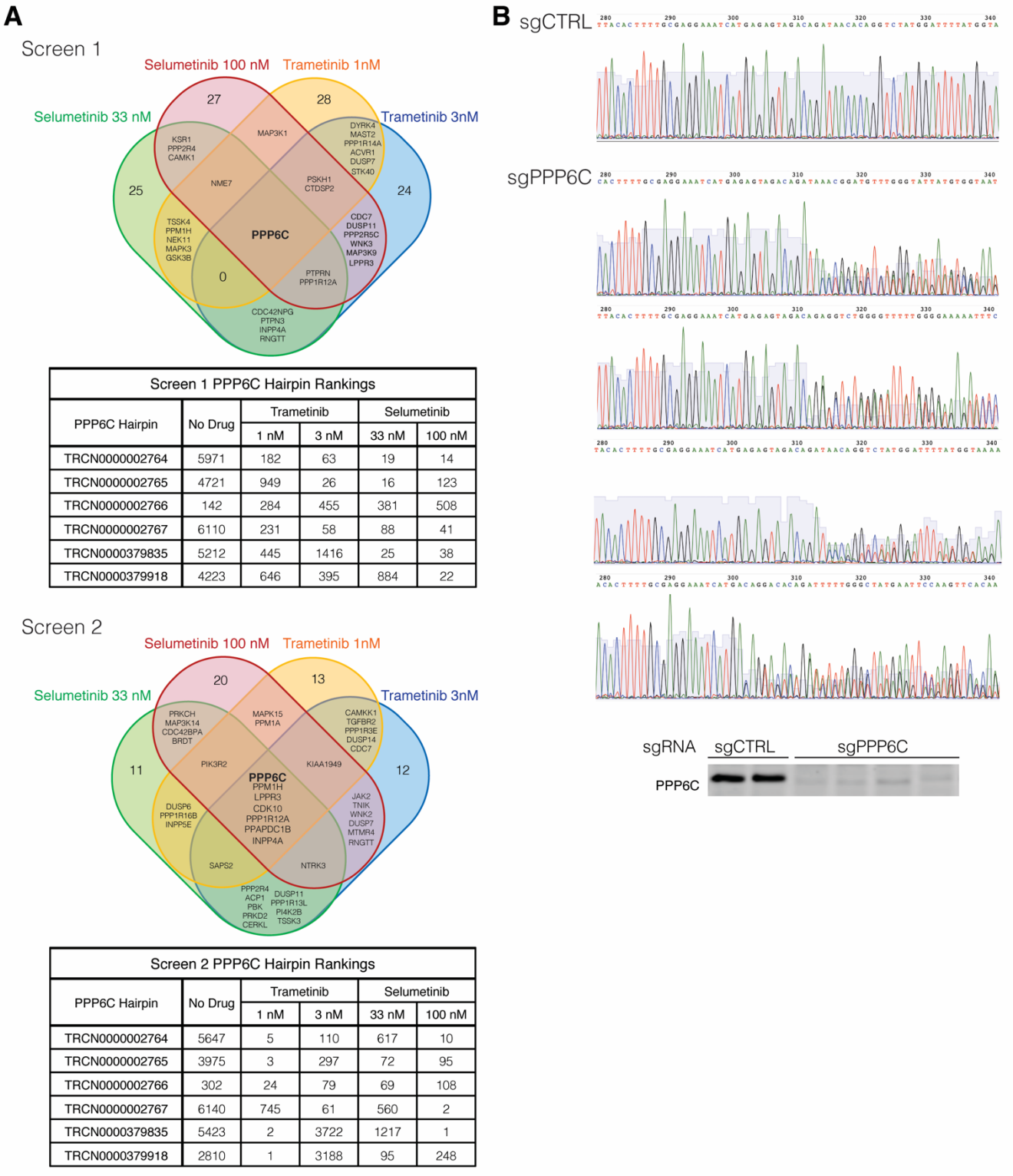
Supplemental information

PPP6C negatively regulates oncogenic ERK signaling through dephosphorylation of MEK

Eunice Cho, Hua Jane Lou, Leena Kuruvilla, David A. Calderwood, and Benjamin E. Turk



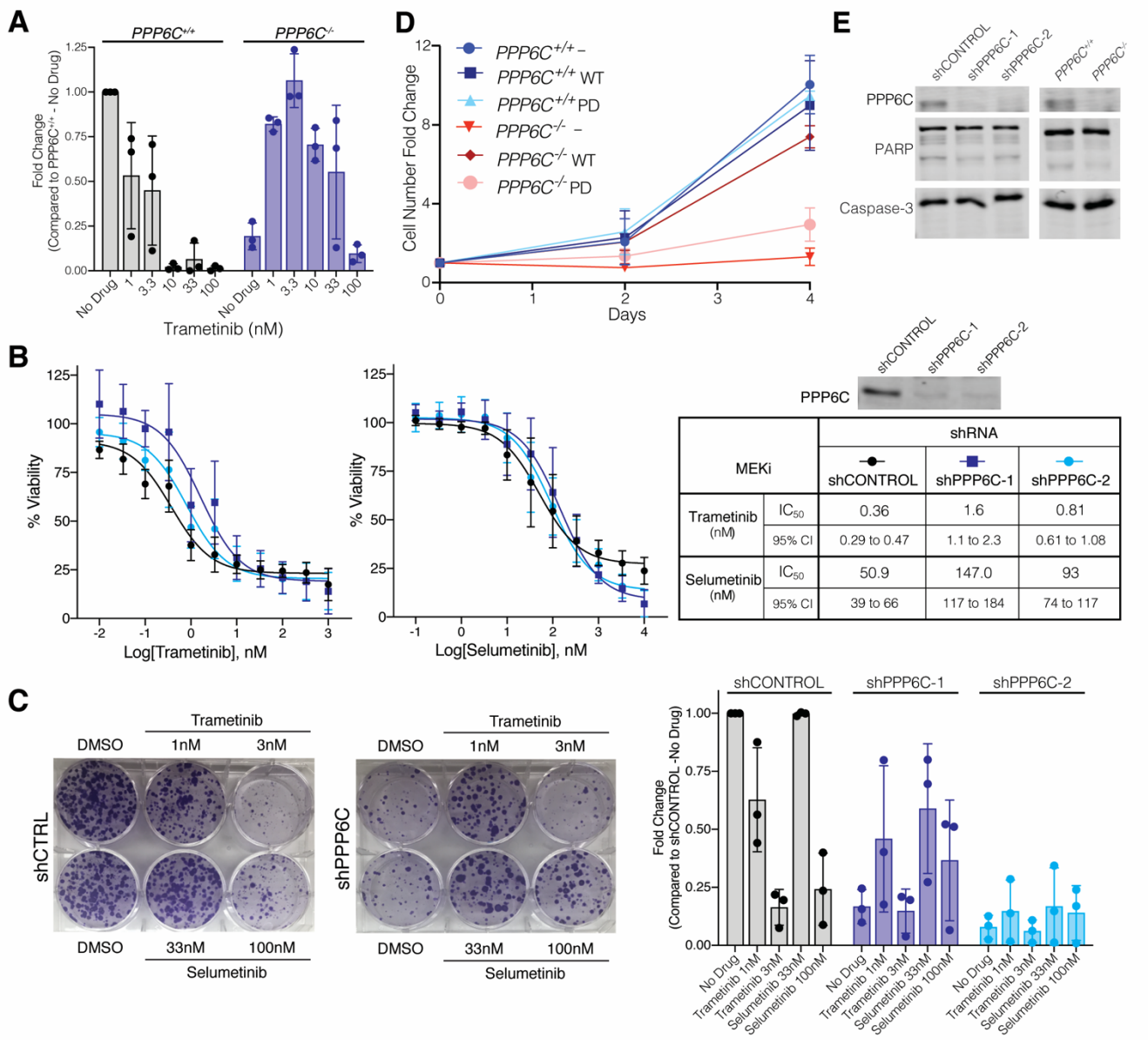
Screen 1 PPP6C Hairpin Rankings					
PPP6C Hairpin	No Drug	Trametinib		Selumetinib	
		1 nM	3 nM	33 nM	100 nM
TRCN0000002764	5971	182	63	19	14
TRCN0000002765	4721	949	26	16	123
TRCN0000002766	142	284	455	381	508
TRCN0000002767	6110	231	58	88	41
TRCN0000379835	5212	445	1416	25	38
TRCN0000379918	4223	646	395	884	22

Screen 2 PPP6C Hairpin Rankings					
PPP6C Hairpin	No Drug	Trametinib		Selumetinib	
		1 nM	3 nM	33 nM	100 nM
TRCN0000002764	5647	5	110	617	10
TRCN0000002765	3975	3	297	72	95
TRCN0000002766	302	24	79	69	108
TRCN0000002767	6140	745	61	560	2
TRCN0000379835	5423	2	3722	1217	1
TRCN0000379918	2810	1	3188	95	248

Figure S1. Hits and PPP6C hairpin rankings from MEKi sensitivity shRNA screens. Related to Figure 1 and 2.

(A) Venn diagrams of top 50 enriched genes for each drug condition. Rankings for each of the 6 PPP6C hairpins out of the 7,649 shRNAs in the library are listed for each drug condition.

(B) Sanger sequencing chromatograms for *PPP6C*$^{+/+}$ and *PPP6C*$^{-/-}$ clonal 501mel cell lines generated by CRISPR/Cas9. Immunoblot confirming PPP6C loss in *PPP6C*$^{-/-}$ 501mel cell lines is shown.



MEKi		shRNA shCONTROL	shPPP6C-1	shPPP6C-2
Trametinib (nM)	IC_{50}	0.36	1.6	0.81
	95% CI	0.29 to 0.47	1.1 to 2.3	0.61 to 1.08
Selumetinib (nM)	IC_{50}	50.9	147.0	93
	95% CI	39 to 66	117 to 184	74 to 117

Figure S2. PPP6C mediates cell growth and response to MEKi. Related to Figures 1 and 2.

(A) Quantification of colony forming assays with *PPP6C*$^{+/+}$ and *PPP6C*$^{-/-}$ cells in Figure 1F. Clonogenic growth was analyzed by ColonyArea in ImageJ and normalized to *PPP6C*$^{+/+}$, no drug. Mean values ± SD are shown, *n* = 3.

(B) 501mel cell lines stably expressing control shRNA (shCTRL) or PPP6C-targeting shRNA (shPPP6C-1, shPPP6C-2) were treated for 72 hours with increasing concentrations of trametinib or selumetinib. Cell viability was detected by alamarBlue reagent and normalized to a no drug control for each cell line. Dose response curves for shCTRL (black), shPPP6C-1 (dark blue), and shPPP6C-2 (light blue) are shown. MEKi IC_{50} values and 95% confidence intervals are listed in the table.

(C) shCTRL, shPPP6C-1, and shPPP6C-2 expressing 501mel cells were cultured in DMSO or the indicated concentration of trametinib or selumetinib for 2 weeks in colony forming assays. Colonies were stained with crystal violet. Clonogenic growth was analyzed by ColonyArea in ImageJ. Quantification was normalized to *PPP6C*$^{+/+}$, no drug. Mean values ± SD are shown, *n* = 3.

(D) Cell proliferation was measured by cell counting for *PPP6C*$^{+/+}$ and *PPP6C*$^{-/-}$ cells expressing GFP, WT PPP6C, or PD PPP6C. Mean values ± SD are shown, *n* = 2.

(E) shCTRL, shPPP6C-1, and shPPP6C-2 expressing 501mel cells and *PPP6C*$^{+/+}$ and *PPP6C*$^{-/-}$ 501mel cells were lysed and assessed by immunoblot for full length and cleaved Caspase-3 and PARP. Representative blots shown, *n* = 3.

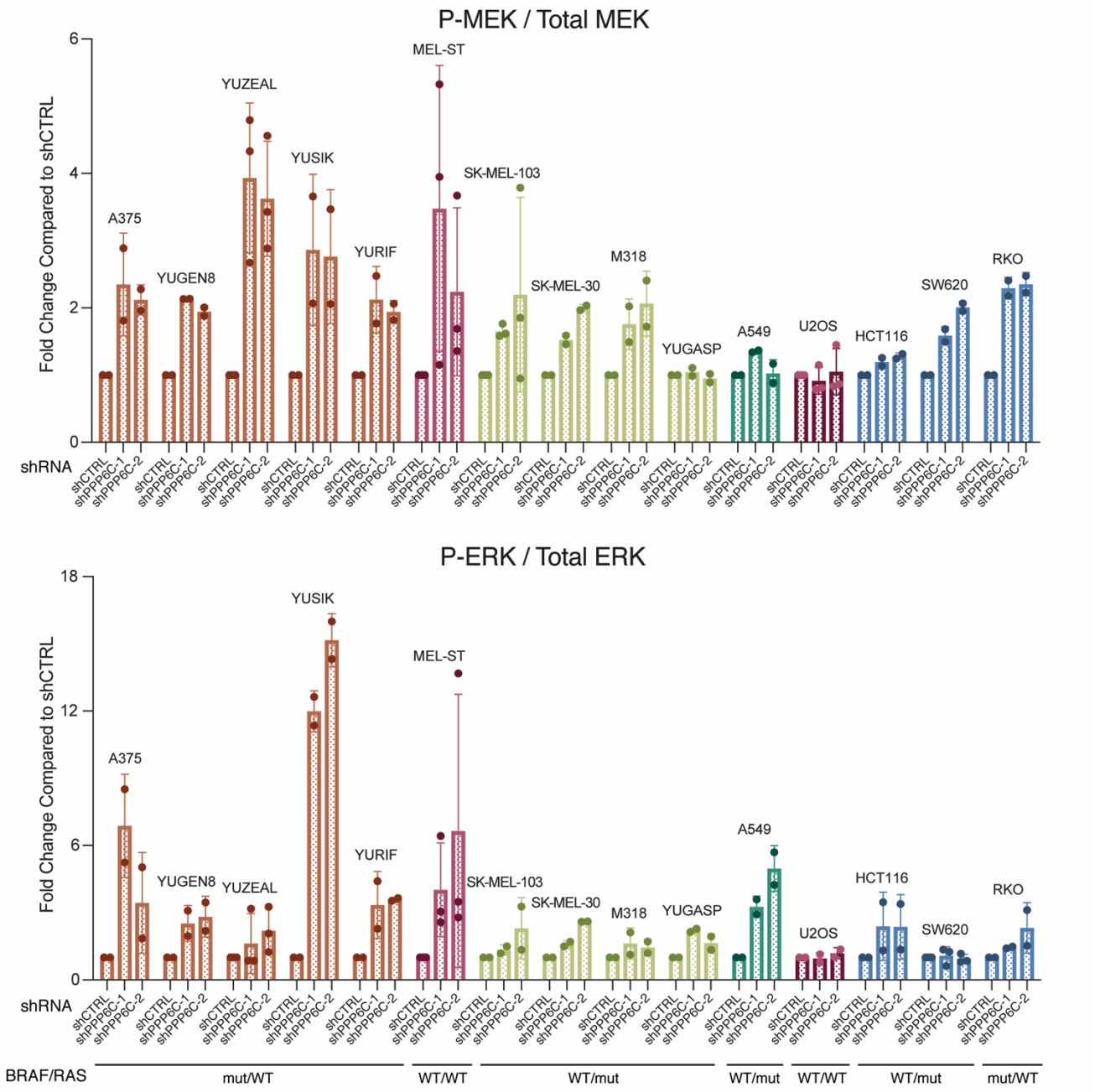


Figure S3. PPP6C regulation of ERK signaling in cancer cell lines. Related to Figure 3. Quantification of the relative levels of Phospho/Total MEK and ERK from Figure 3A was normalized to shCTRL for each cell line. Data are represented as mean ± SD, $n \geq 2$.

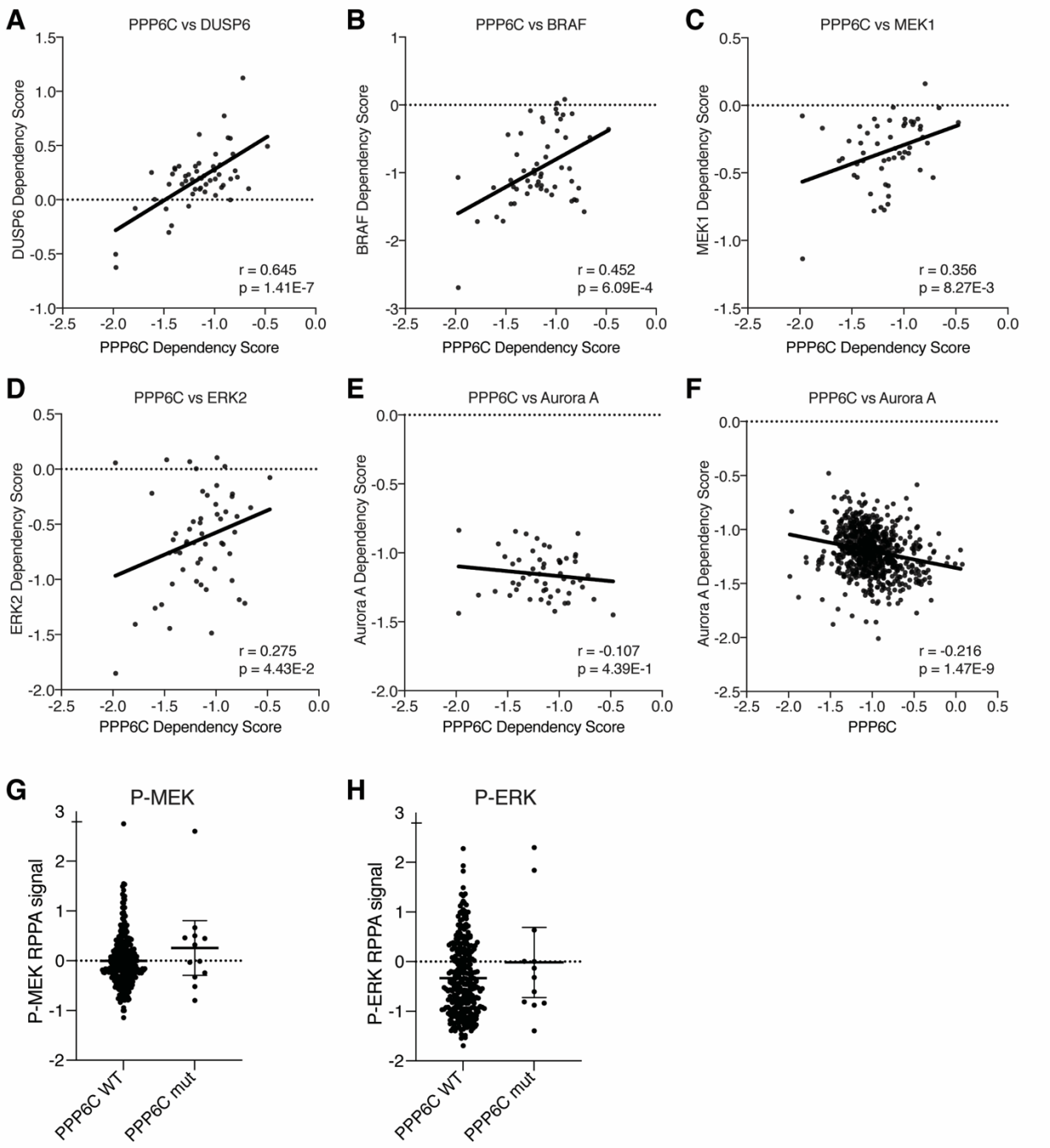


Figure S4. Correlations of PPP6C dependency and ERK pathway dependency. Related to Figure 3.

(A-E) CERES scores for PPP6C (x-axis) plotted against CERES scores for (A) DUSP6, (B) BRAF, (C) MEK1, (D) ERK2, and (E) Aurora A (y-axis). CERES scores are for all skin cancer cell lines from the Cancer Dependency MAP Project. Pearson's correlation coefficients (r) and associated *p*-values from linear regression analysis are indicated.

(F) CERES scores for PPP6C (x-axis) plotted against CERES scores for Aurora A (y-axis). CERES scores are for all cancer cell lines from the Cancer Dependency MAP Project. Pearson's correlation coefficient (r) and associated p-value from linear regression analysis are indicated.

(G) MEK phosphorylation levels (MEK1 pSer221, pThr217) for TCGA tumor samples with WT PPP6C or recurrent/truncating PPP6C mutations. Data were obtained from cBioPortal and are represented as mean ± SD.

(H) As in (G), but showing levels of ERK phosphorylation (ERK2 pThr202, pTyr204) Data are represented as mean ± SD.

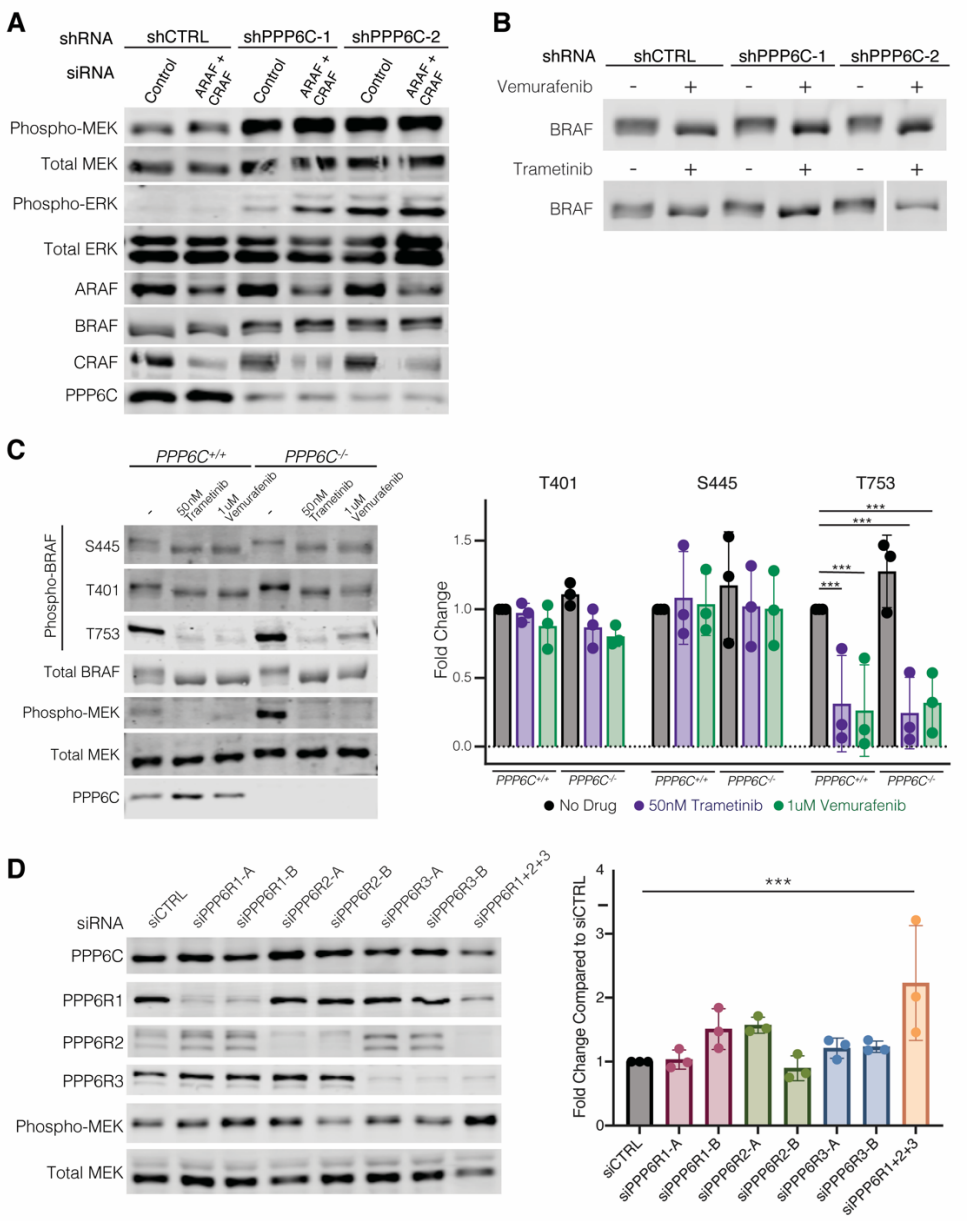


Figure S5. Involvement of RAF isoforms and PPP6R subunits in ERK pathway regulation by PPP6C. Related to Figure 4 and 5

(A) 501mel cells expressing shCTRL, shPPP6C-1, or shPPP6C-2 were transfected with non-targeting control siRNA or siRNA targeting both ARAF and CRAF. Cells were lysed and assessed by immunoblot for phosphorylated and total MEK and ERK. Knockdown of ARAF, CRAF, and PPP6C was also confirmed via immunoblot.

(B) shCTRL, shPPP6C-1, and shPPP6C-2 501mel cells were treated with 1uM vemurafenib or 50nM trametinib for 24 hours as indicated. Cells were lysed and assessed by immunoblot for BRAF electrophoretic mobility shifts indicative of changes in phosphorylation.

(C) *PPP6C*$^{+/+}$ and *PPP6C*$^{-/-}$ 501mel cells were treated with 1uM vemurafenib or 50nM trametinib for 24 hours as indicated. Cells were lysed and assessed by immunoblot for phosphorylation at regulatory sites on BRAF. Quantification of the relative levels of Phospho/Total BRAF was normalized to *PPP6C*$^{+/+}$. Data are represented as mean ± SD, *n* = 3. ***p<0.001, unpaired t-test.

(D) 501mel cells were transfected with non-targeting control siRNA or siRNA targeting PPP6R1, PPP6R2, and/or PPP6R3. Cells were lysed and assessed by immunoblot for phosphorylated and total MEK. Knockdown of PPP6Rs was also confirmed via immunoblot. Quantification of the relative levels of Phospho/Total MEK was normalized to siCTRL. Data are represented as mean ± SD, *n* = 3. ***p<0.001, unpaired t-test.

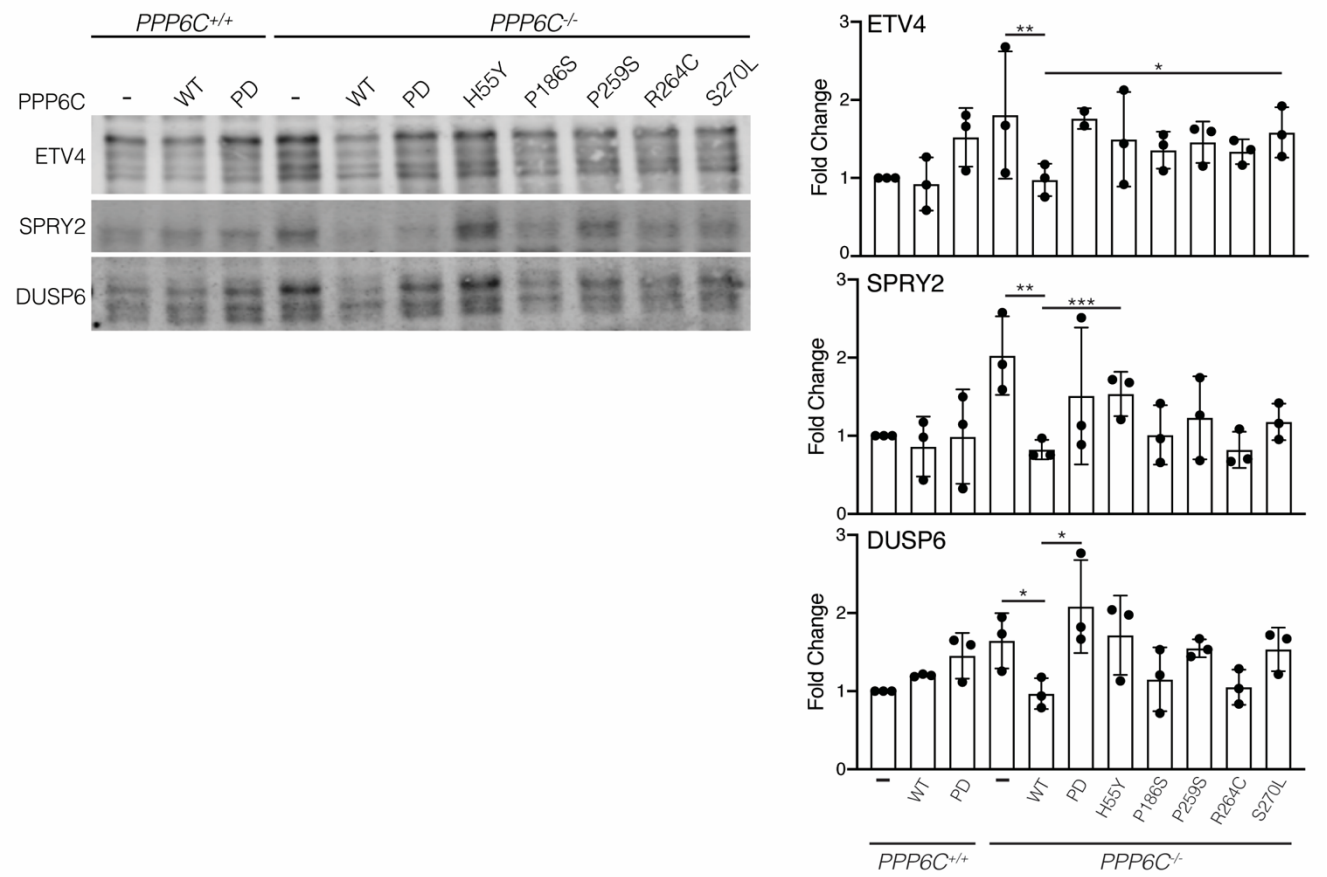


Figure S6. Expression of transcriptional targets of ERK with cancer-associated PPP6C mutations. Related to Figure 6.

Cells from Figure 6C were lysed and assessed by immunoblot for ETV4, SPRY2, and DUSP6. Quantifications were normalized to the GFP-expressing *PPP6C*$^{+/+}$ (-) samples. Mean values ± SD are shown, n = 3. Significance is shown in comparison to *PPP6C*$^{+/+}$ cells expressing GFP. *p < 0.05, **p < 0.01, ***p<0.001, paired t test.