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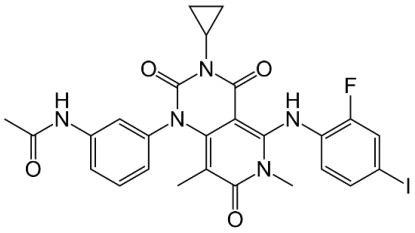
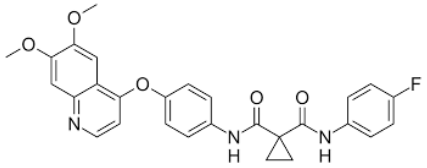
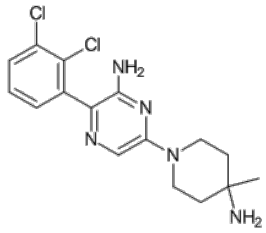
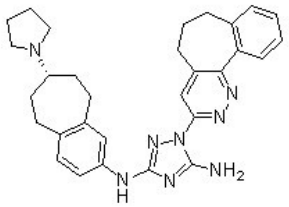
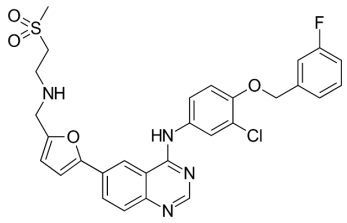
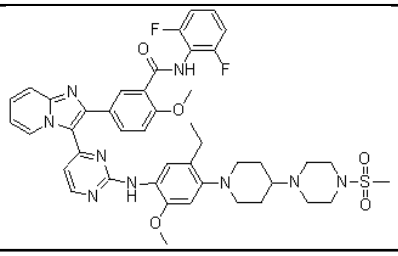
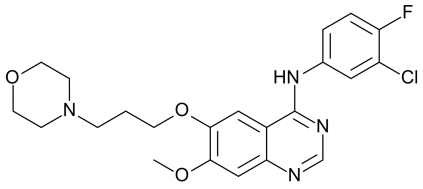
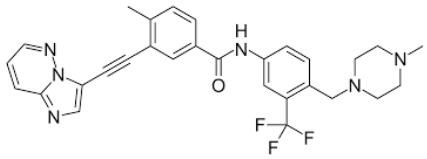
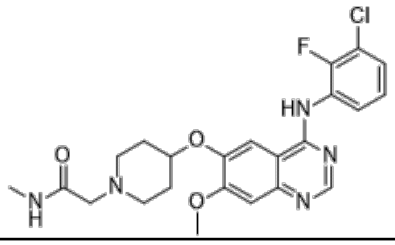
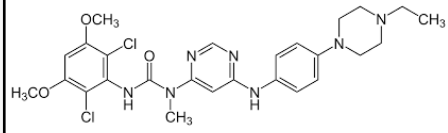
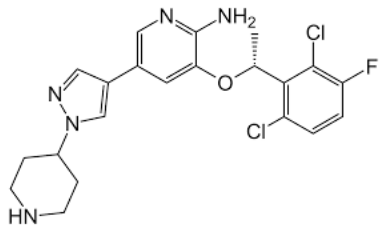
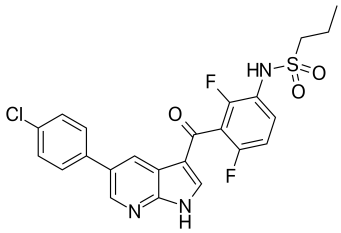
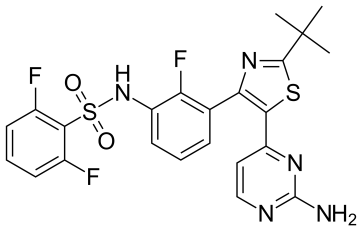
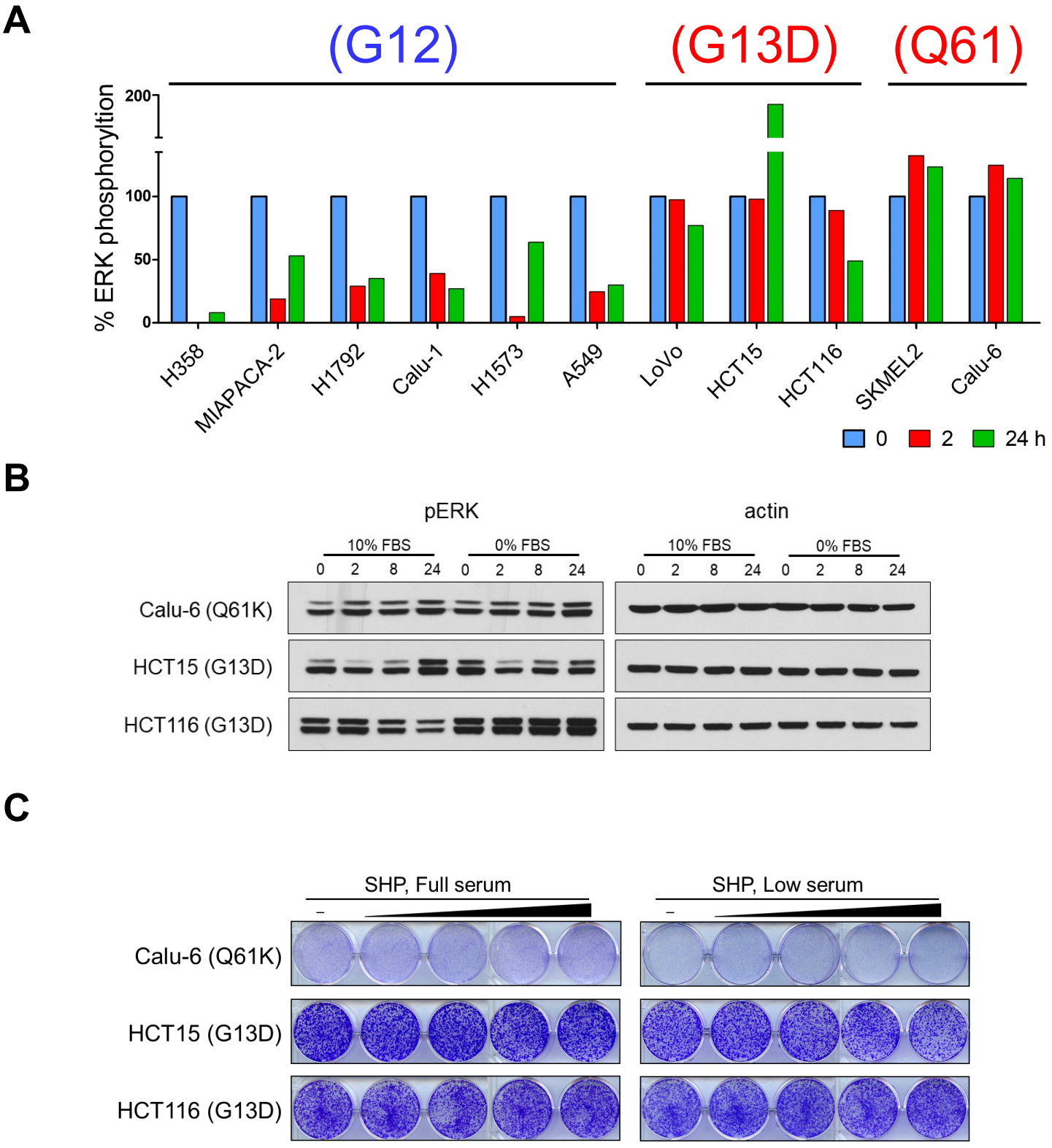
Supplemental Information

SHP2 Drives Adaptive Resistance to ERK Signaling Inhibition in Molecularly Defined Subsets of ERK-Dependent Tumors

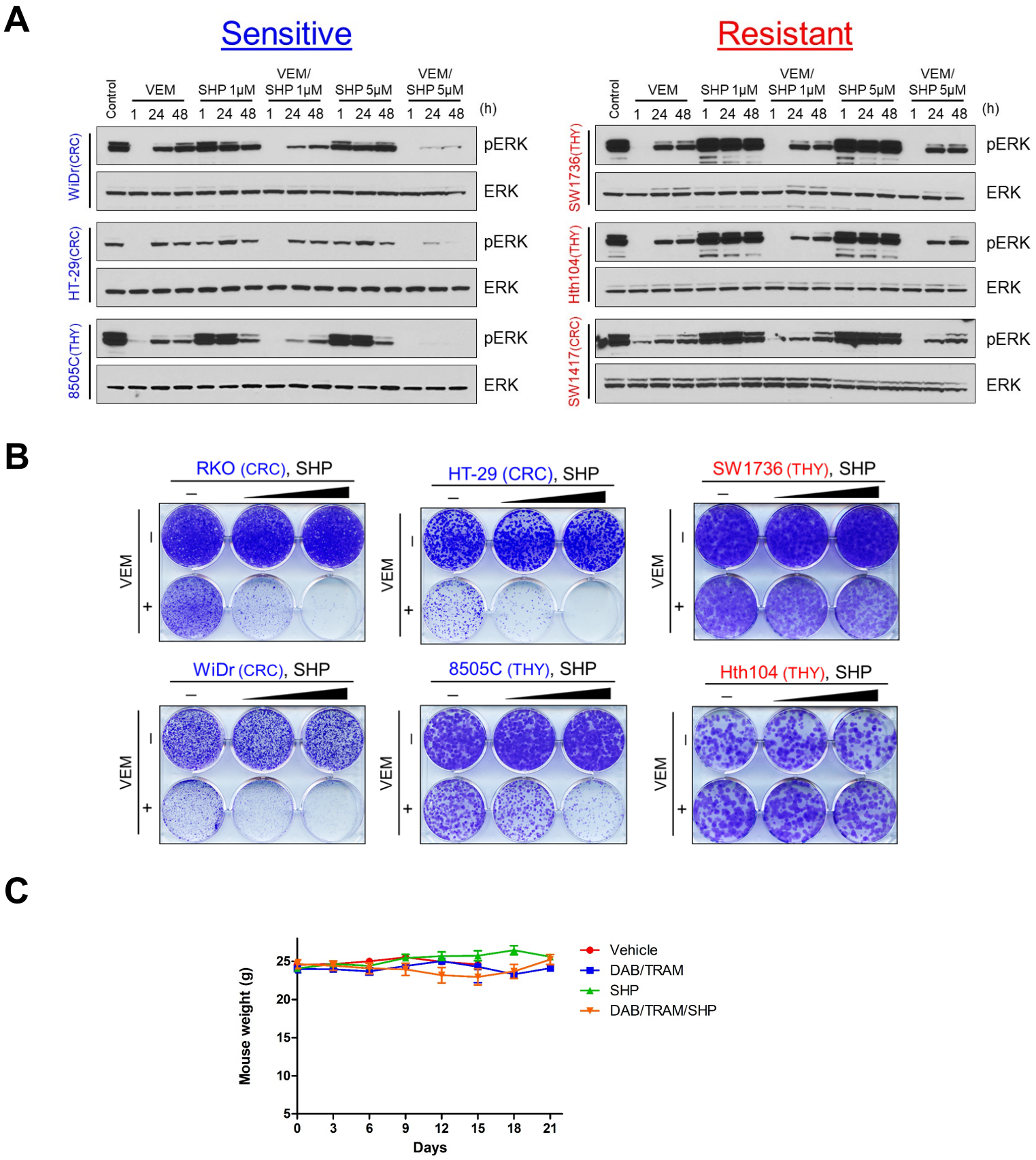
Tamer A. Ahmed, Christos Adamopoulos, Zoi Karoulia, Xuewei Wu, Ravi Sachidanandam, Stuart A. Aaronson, and Poulikos I. Poulikakos

Compound (Selectivity)	Chemical structure	Compound (Selectivity)	Chemical structure
Trametinib (MEK1/2)		Cabozantinib (VEGFR2, c-Met, Ret, Kit, Flt-1/3/4, Tie2, and AXL)	
SHP099 (SHP2)		R428 (AXL)	
Lapatinib (EGFR and HER2)		GSK 1904529A (IGF-1R and IR)	
Gefitinib (EGFR)		Ponatinib (Abl, PDGFRα, VEGFR2, Src and FGFR1/2/3)	
AZD 8931 (EGFR, HER2, and HER3)		BGJ398 (FGFR1/2/3)	
Crizotinib (C-Met and ALK)		Vemurafenib (RAF)	
Dabrafenib (RAF)			

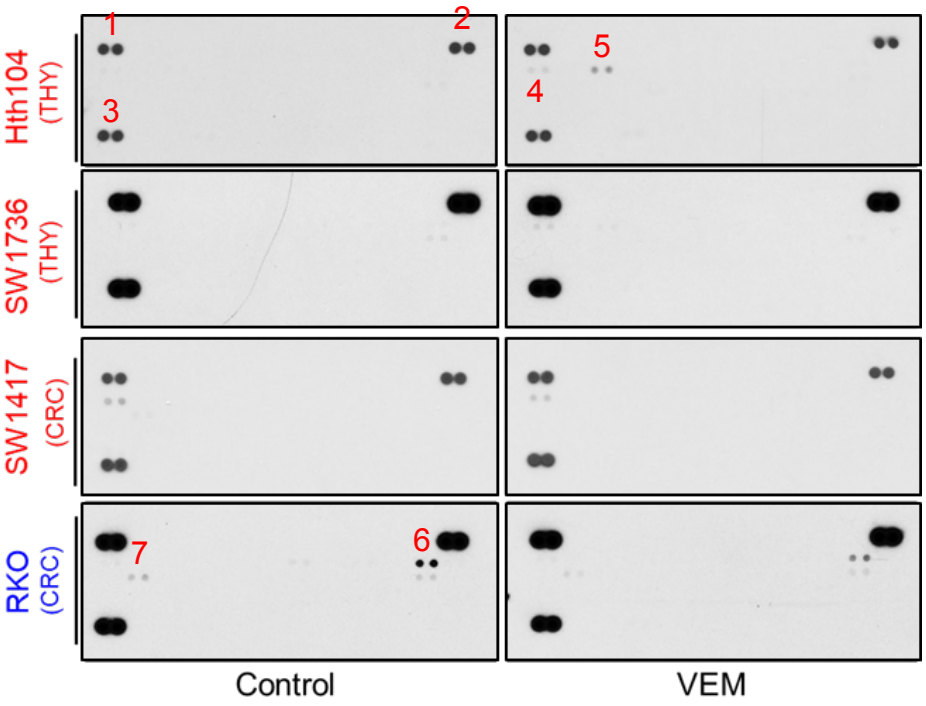
Supplemental Figure 1. Chemical structure and reported selectivity of each compound used in the study. Related to Figures 1-6.



Supplemental Figure 2. Effect of SHP099 on RAS-mutant cell lines. Related to Figure 3. (A) Quantitation of ERK phosphorylation in RAS (G12X), RAS (G13D) and RAS (Q61X) treated with SHP099 (10 μM) for 2 and 24 hours (From **Figure 3B**). pERK expression was normalized to actin and calculated as a percentage change relative to the control (0 h). (B) KRAS (G13D) and KRAS (Q61K)-mutant cell lines were treated with SHP099 (10 μM) for the indicated time points under either serum-free or full (10% v/v FBS) serum conditions. ERK activity was monitored for 24 hours by immunoblotting with the indicated antibodies. (C) Crystal violet cell growth assays of the indicated KRAS (G13D) and KRAS (Q61K)-mutant cell lines treated with SHP099 (0.5 ,1 ,2 and 5 μM), under either serum-free or full (10% v/v FBS) serum conditions.

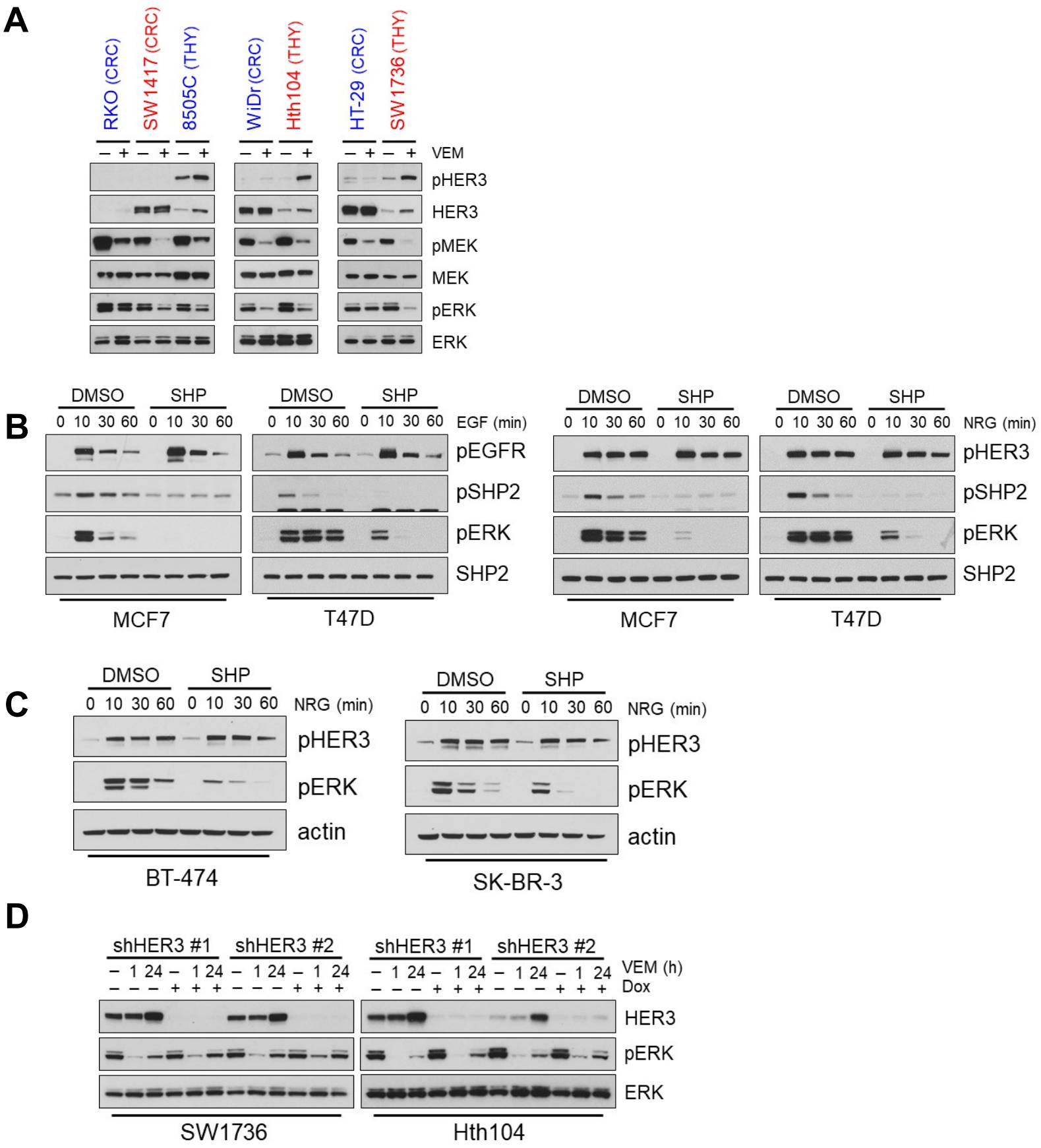


Supplemental Figure 3. Differential sensitivity to SHP2 inhibition in colorectal or thyroid BRAF (V600E) cells when combined with RAF inhibitor. Related to Figure 4. (A) The indicated BRAF(V600E) tumor lines were treated with vemurafenib (2 μM), SHP099 (1 and 5 μM) or the combination, and reactivation of ERK signaling was monitored for 48 hours by immunoblotting with the indicated antibodies. 8505C and HT-29 cells were treated with 1 or 3 μM vemurafenib, respectively. (B) Crystal violet cell growth assays assessing the effect of vemurafenib (2 μM), SHP099 (1 and 5 μM) and the combination in the indicated BRAF(V600E) cell lines. (C) Body weight of mice treated with indicated inhibitors was measured every three days. Data are represented as mean +/- SEM.

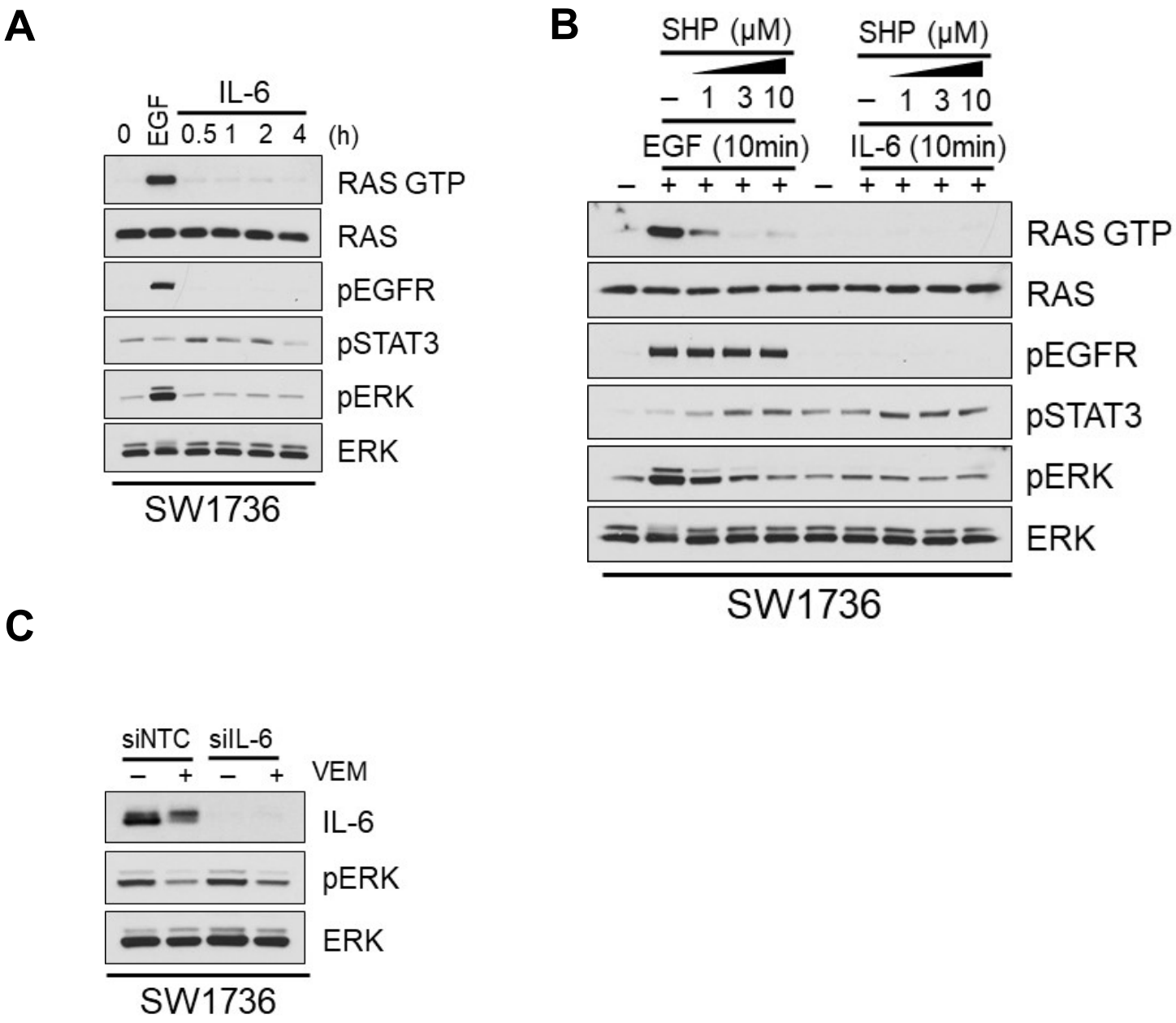


Number	Receptor
1,2, 3	Reference
4	EGFR
5	HER3
6	AXL
7	HGFR (Met)

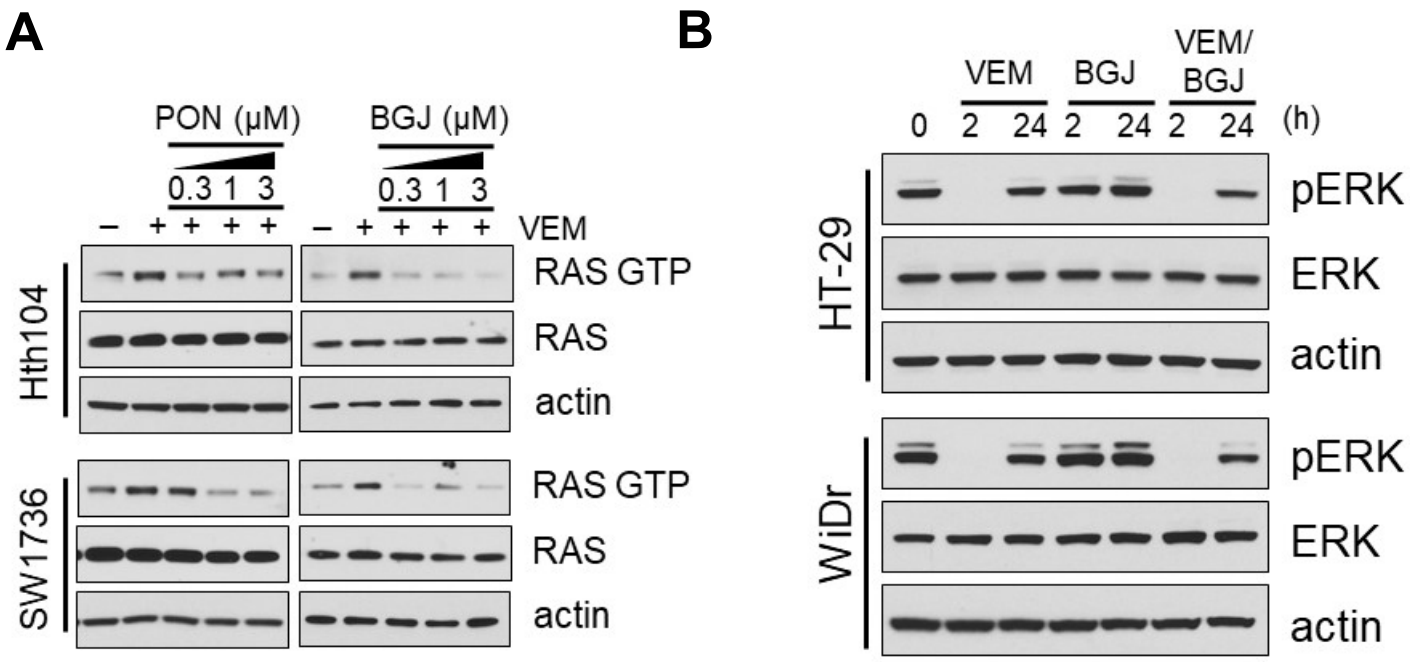
Supplemental Figure 4. Lighter exposure of RTK arrays using lysates from the indicated cell lines treated with VEM for 24 hours. Related to Figure 5.



Supplemental Figure 5. Adaptive resistance to RAF inhibitors in SHP2-negative BRAF(V600E) cancer cells is independent of HER3. Related to Figure 5. (A) The indicated BRAF(V600E)-expressing tumor cells treated with or without vemurafenib (2 μM) for 24 hours. HER3 expression and phosphorylation were detected by immunoblotting. (B) MCF7 or T47D cells pretreated with DMSO or SHP099 (10 μM) for 1 hour, then stimulated with either epidermal growth factor (EGF, 10 ng/mL) or neuregulin (NRG, 5 ng/mL) for the indicated time points. (C) BT474 or SK-BR-3 cells pretreated with either DMSO or SHP099 (10 μM) for 1 hour then neuregulin (NRG, 5 ng/mL) were added for the indicated time points. (D) SW1736 or Hth104 cells transduced with doxycycline-inducible shRNAs targeting HER3 were treated with vemurafenib (2 μM) and doxycycline for the times indicated. HER3 expression and ERK rebound were assessed by immunoblotting.



Supplemental Figure 6. Autocrine IL-6 does not drive feedback-induced RAS activity in SW1736 cells. Related to Figure 6. (A) IL-6 (50 ng/mL) was added at the indicated time points in SW1736 cells, then lysates were either subjected to RAS-GTP pull-down assay or immunoblotted with the indicated antibodies. (B) SW1736 cells were pretreated with SHP099 (10 μM) for 1 hour then EGF or IL-6 were added for the indicated time points. Cell lysates were either subjected to RAS-GTP pull-down assay or immunoblotted with the indicated antibodies. (C) SW1736 cells were transfected with non-targeting control or IL-6 siRNAs (100 nM) for 24 hours then treated for additional 24 hours with vemurafenib (2 μM). IL-6 expression and ERK phosphorylation were assessed by immunoblotting.



Supplemental Figure 7. Effect of VEM and BGJ combination in BRAF(V600E) colorectal and thyroid tumor cells. Related to Figure 6. (A) Hth104 or SW1736 cells were treated with VEM (2 μM) for 48 hours followed by PON or BGJ for 2 hours at the indicated concentrations, then subjected to RAS-GTP pull-down assay. (B) HT-29 or WiDr cells were treated with 2 μM VEM combined with BGJ (5 μM) for 24 hours. ERK phosphorylation was assessed by immunoblotting.