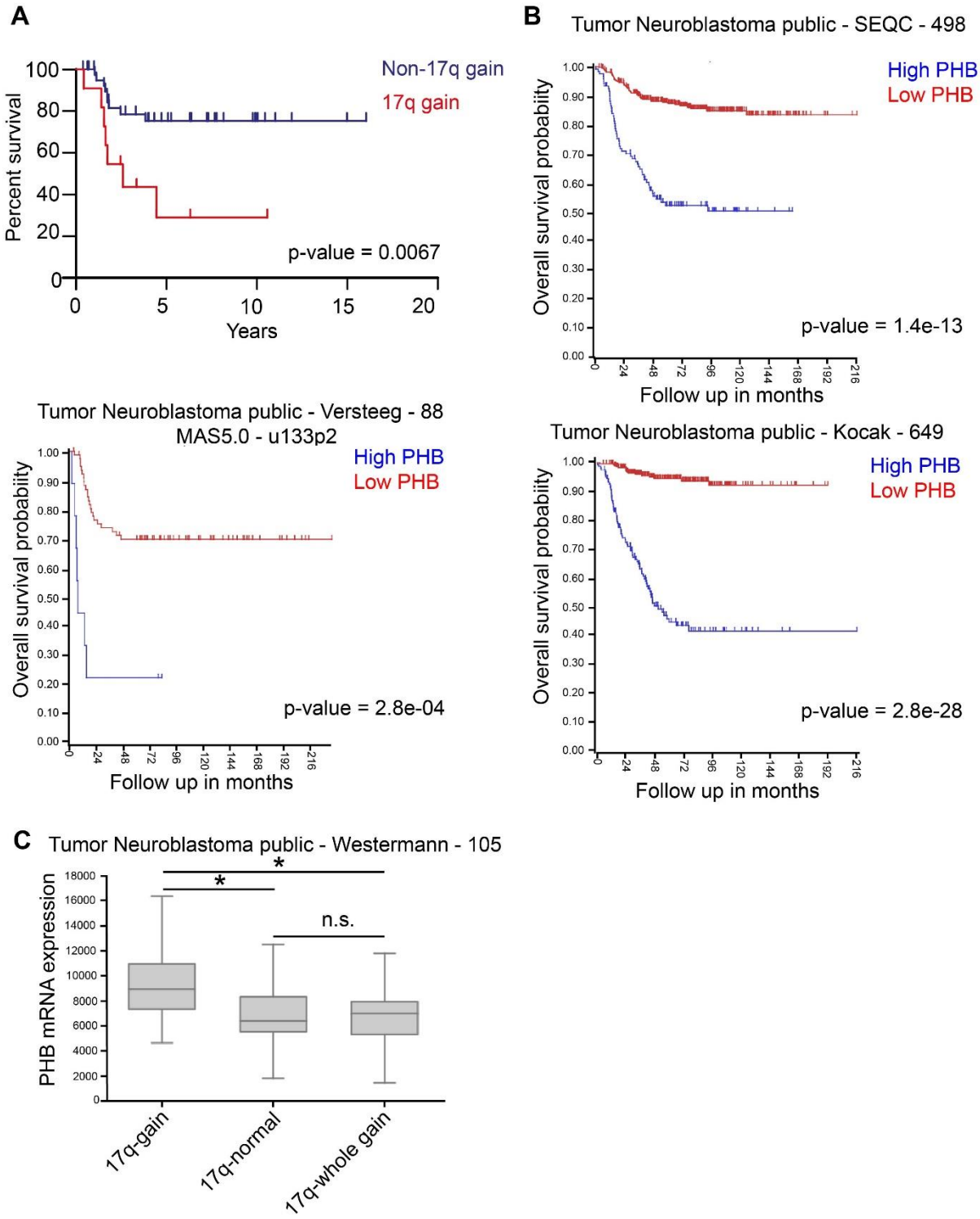
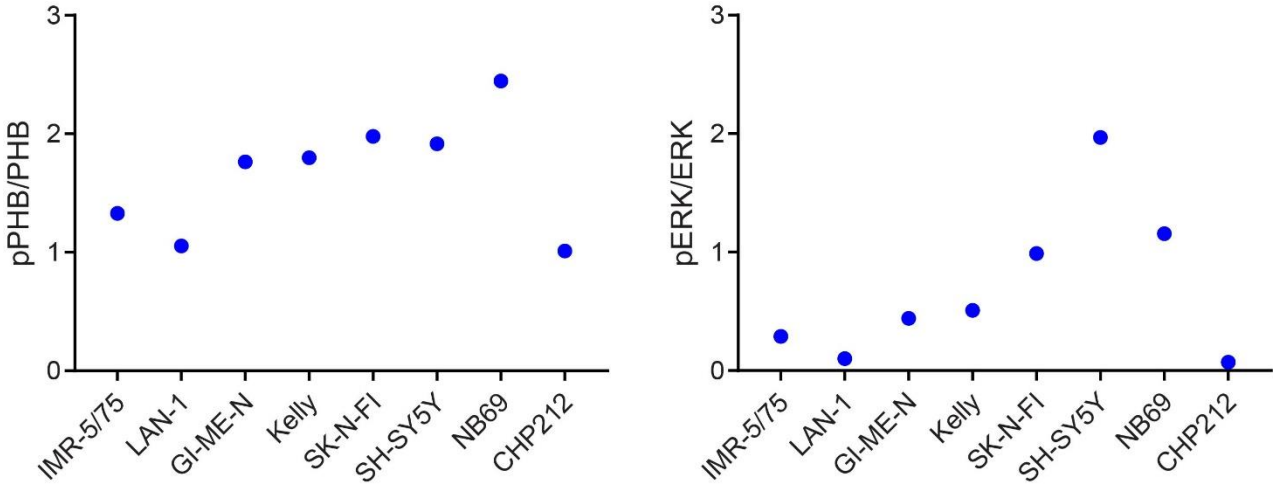
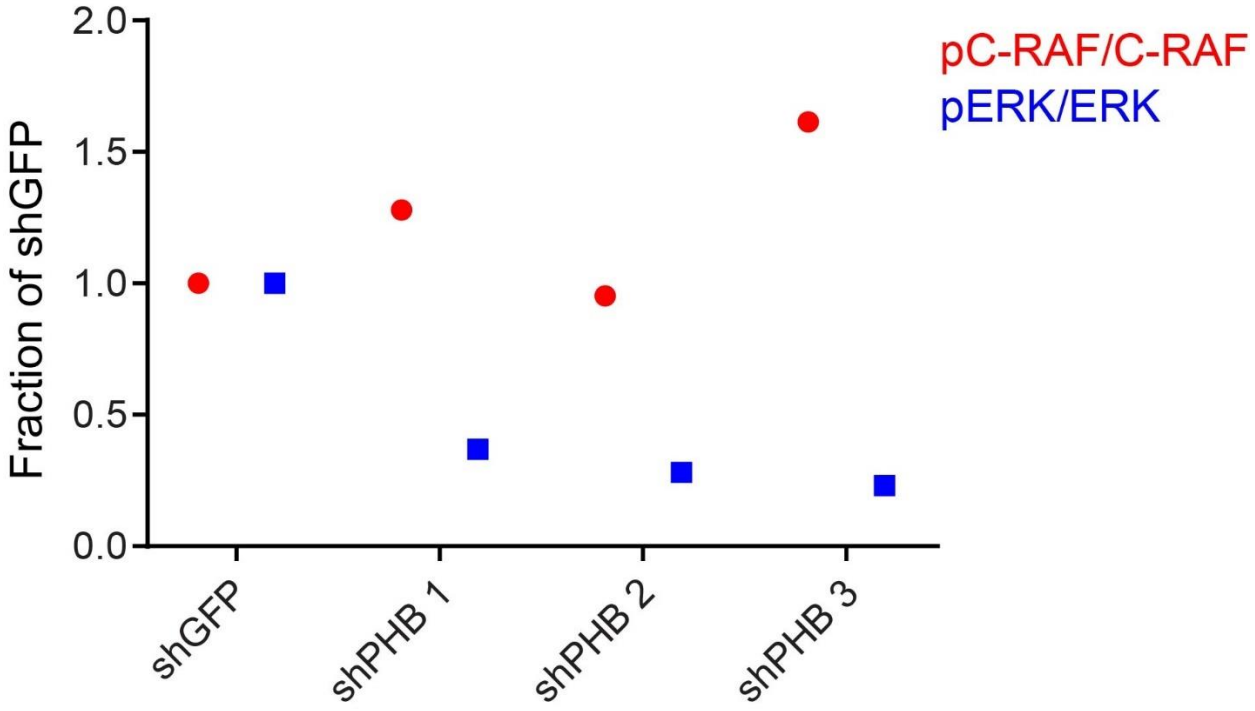


Supplemental Figure 1. 17q gain and prohibitin expression correlates with poor prognosis in neuroblastoma. A. Kaplan-Meier curve displaying overall survival of patients with 17q gain vs. whole 17 or no gain. p-value calculated by log-rank test.

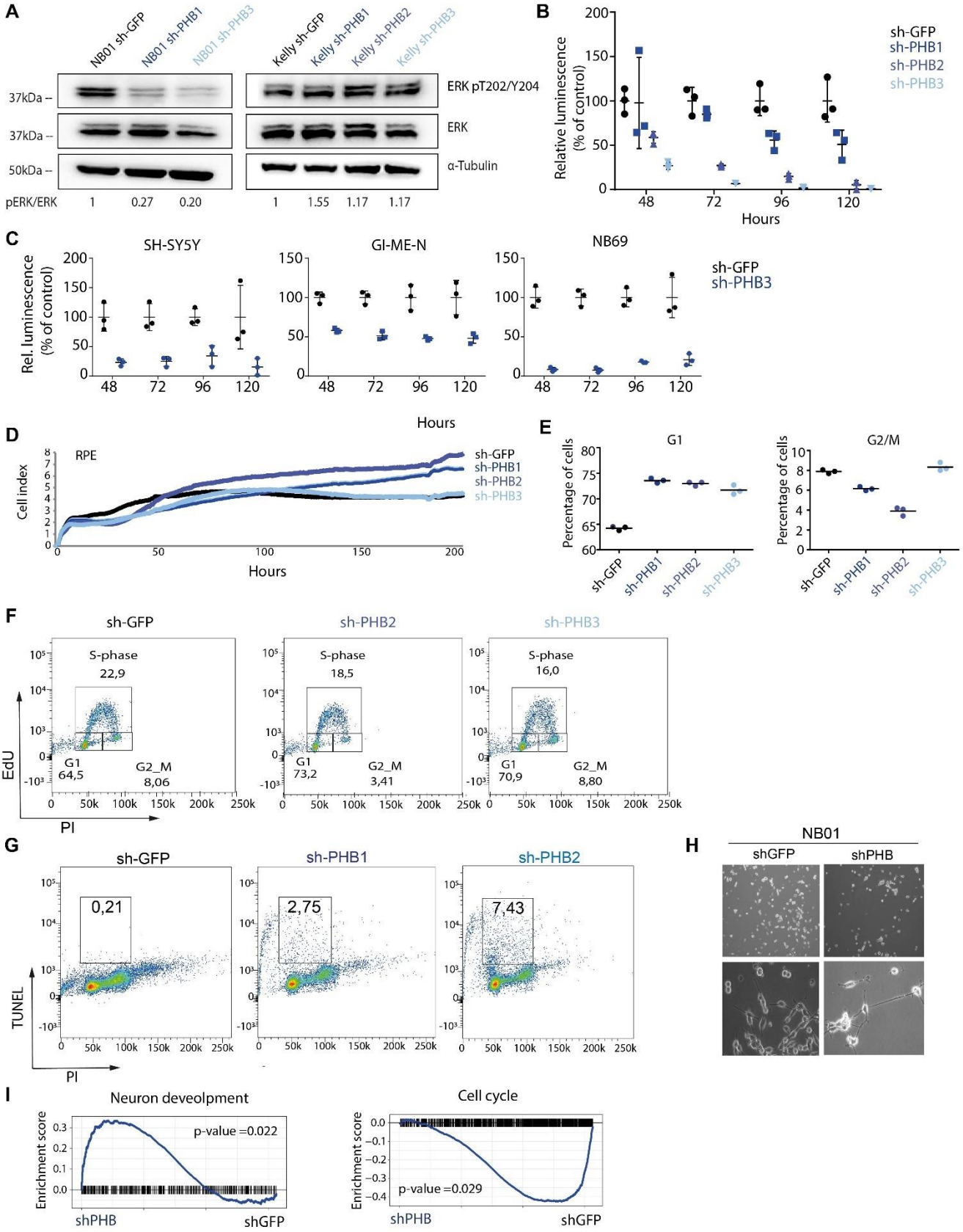
B. Kaplan-Meier curves of overall neuroblastoma survival probability generated from publicly-available neuroblastoma data sets accessible at the R2 Genomics Analysis and Visualization Platform. Survival cutoffs determined by KaplanScan. p-values calculated by log-rank test. **C**. *PHB* expression in patients with 17q gain, whole 17 gain, or no gain. *p < 0.001, one-way analysis of variance.



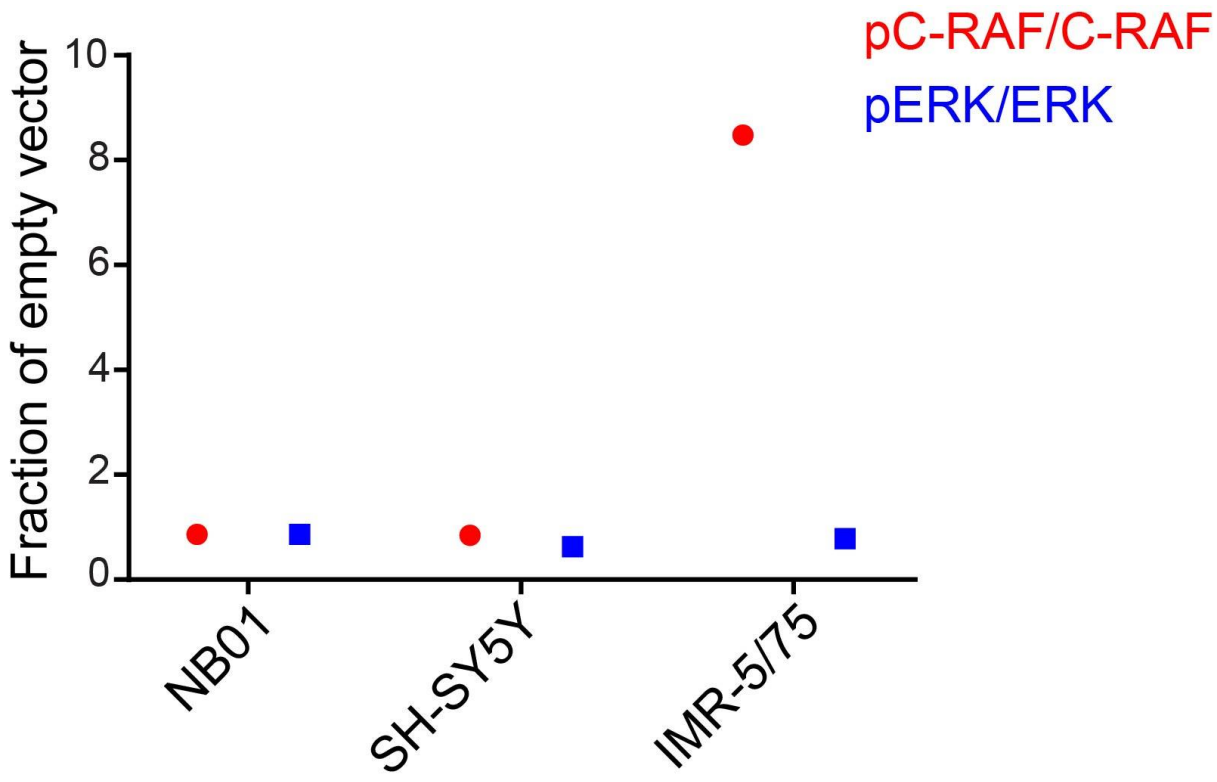
Supplemental Figure 2. Prohibitin and ERK are phosphorylated in neuroblastoma cell lines. Quantification of western blot displayed in Figure 2C showing ratios of phosphorylated to unphosphorylated PHB and ERK1/2.



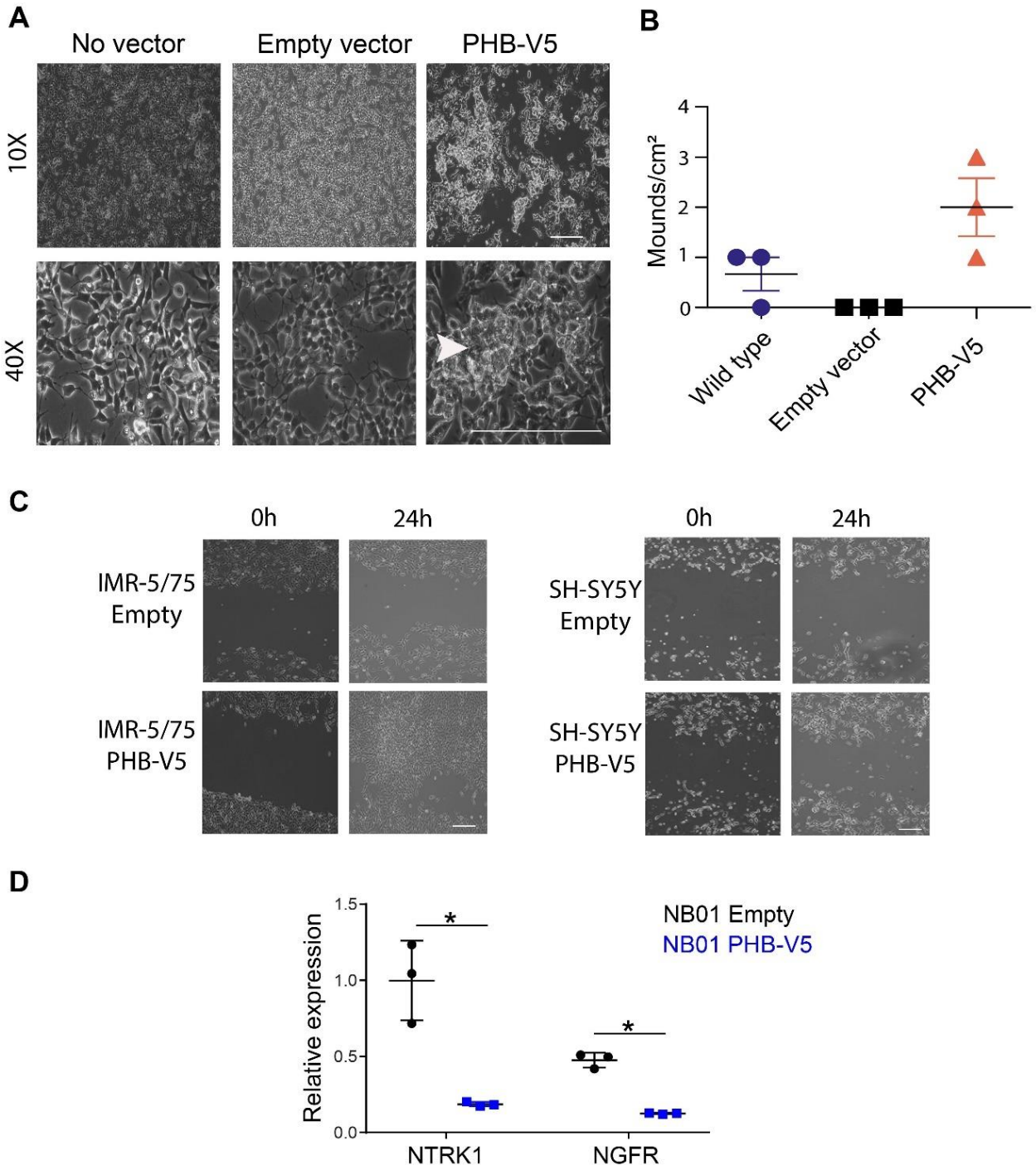
Supplemental Figure 3. *PHB* knockdown impairs ERK activation. Quantification of western blot displayed in Figure 3A showing ratios of phosphorylated to total c-RAF and ERK1/2.


A
NB01 sh-GFP
NB01 sh-PHB1
NB01 sh-PHB3
Kelly sh-GFP
Kelly sh-PHB1
Kelly sh-PHB2
Kelly sh-PHB3
37kDa
37kDa
50kDa
ERK pT202/Y204
ERK
α-Tubulin
pERK/ERK 1 0.27 0.20 1 1.55 1.17 1.17
B
Relative luminescence (% of control)
Hours
sh-GFP
sh-PHB1
sh-PHB2
sh-PHB3
C
SH-SY5Y
GI-ME-N
NB69
Rel. luminescence (% of control)
sh-GFP
sh-PHB3
D
RPE
Cell index
Hours
sh-GFP
sh-PHB1
sh-PHB2
sh-PHB3
E
G1
G2/M
Percentage of cells
F
sh-GFP
sh-PHB2
sh-PHB3
S-phase 22,9
G1 64,5
G2_M 8,06
S-phase 18,5
G1 73,2
G2_M 3,41
S-phase 16,0
G1 70,9
G2_M 8,80
EdU
PI
G
sh-GFP
sh-PHB1
sh-PHB2
0,21
2,75
7,43
TUNEL
PI
H
NB01
shGFP
shPHB
I
Neuron deveolpment
p-value =0.022
Cell cycle
p-value =0.029
Enrichment score
shPHB
shGFP

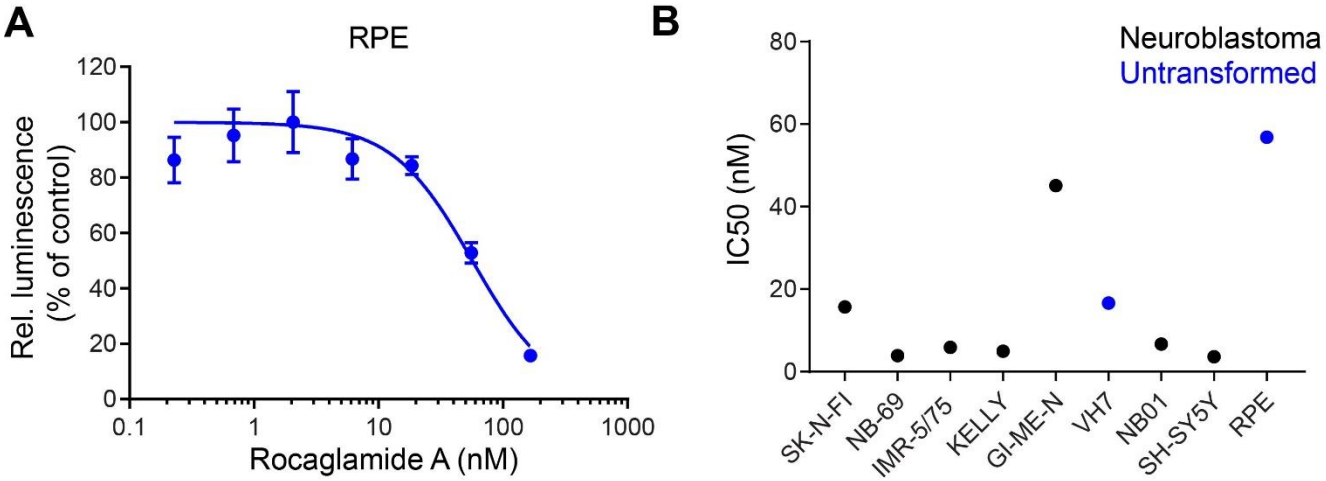
Supplemental Figure 4. *PHB* knockdown impairs ERK activation, reduces cell viability, slows cell cycle progression, induces apoptosis, and promotes differentiation of neuroblastoma cells. A. Western blot analysis of NB01 and Kelly cells after *PHB* knockdown. Data represent a single experiment. **B.** Relative viability of IMR-5/75 cells after knockdown of *PHB* using three independent hairpins as measured with the CellTiter-Glo luminescent viability assay over the course of 120 hours. Data represent mean +/- SEM. Three technical replicates shown. **C.** Relative viability of SH-SY5Y, GI-ME-N, and NB69 cells after knockdown of *PHB* using one hairpin as measured with the CellTiter-Glo luminescent viability assay over the course of 120 hours. Data represent mean +/- SEM. Three technical replicates shown. **D**. Proliferation of RPE cells stably expressing shRNAs targeting *PHB* compared to cells expressing an shRNA targeting *GFP* as measured with the RTCA iCelligence system. **E**. Quantification of IMR-5/75 cells in G2/M phase (left) and G1 phase (right) after *PHB* knockdown. **F**. FACS plots showing cell cycle distribution of IMR-5/75 cells after *PHB* knockdown with two independent hairpins. Three technical replicates shown. **G**. Representative FACS plots of TUNEL-stained IMR-5/75 neuroblastoma cells after *PHB* knockdown. Three technical replicates shown. **H**. Photomicrographs of NB01 cells after transduction with shRNAs against either *PHB* or *GFP*. Photomicrographs representative of three independent samples. **I**. GSEA plots of neuron development and cell cycle-related genes after *PHB* knockdown. n = 3, Fisher's exact test. Stated n-values indicate number of biological replicates.



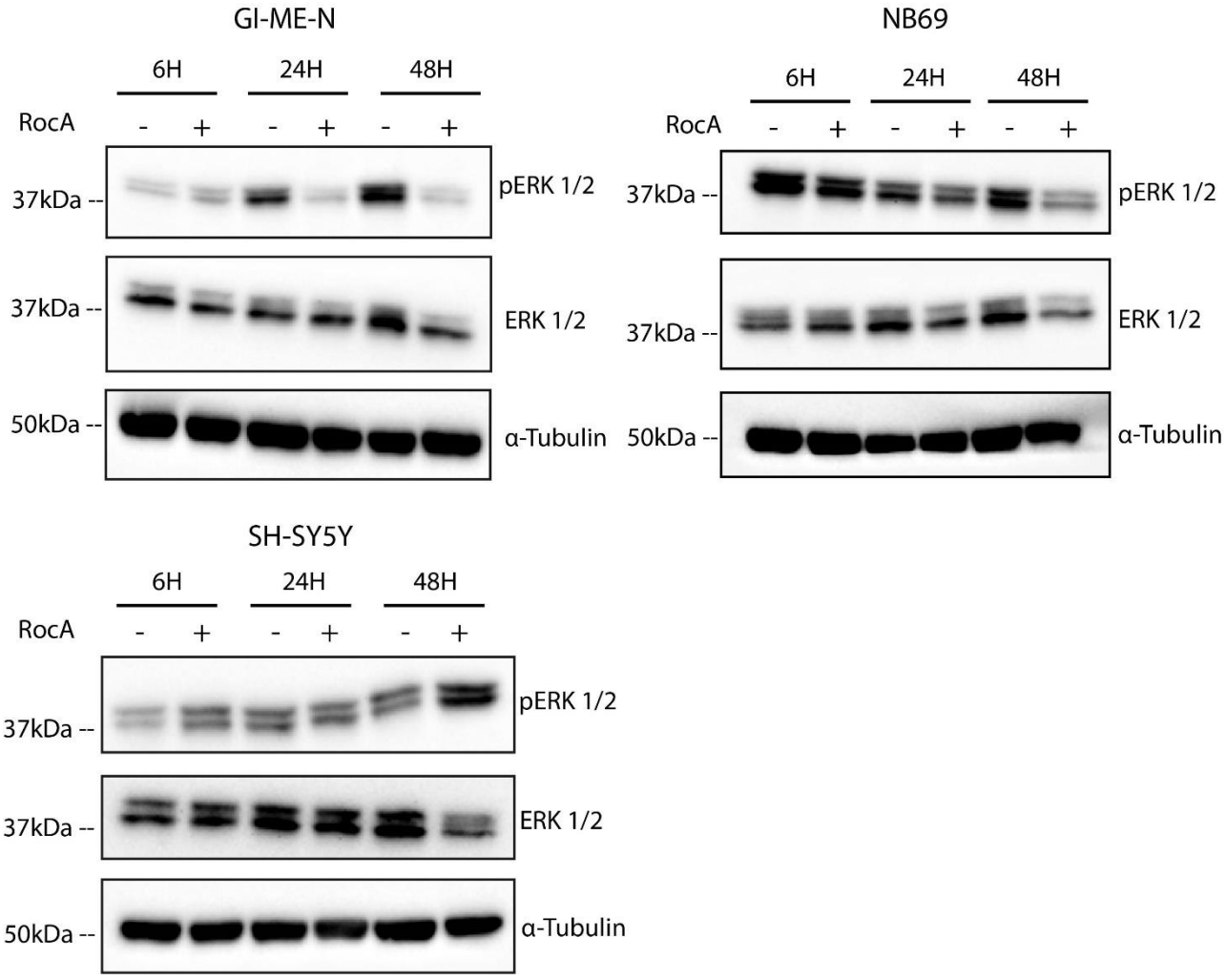
Supplemental Figure 5. Expression of *PHB-V5* is not sufficient to increase phosphorylation of c-RAF or ERK1/2. Quantification of western blot displayed in Figure 5A showing ratios of phosphorylated to total c-RAF and ERK1/2.



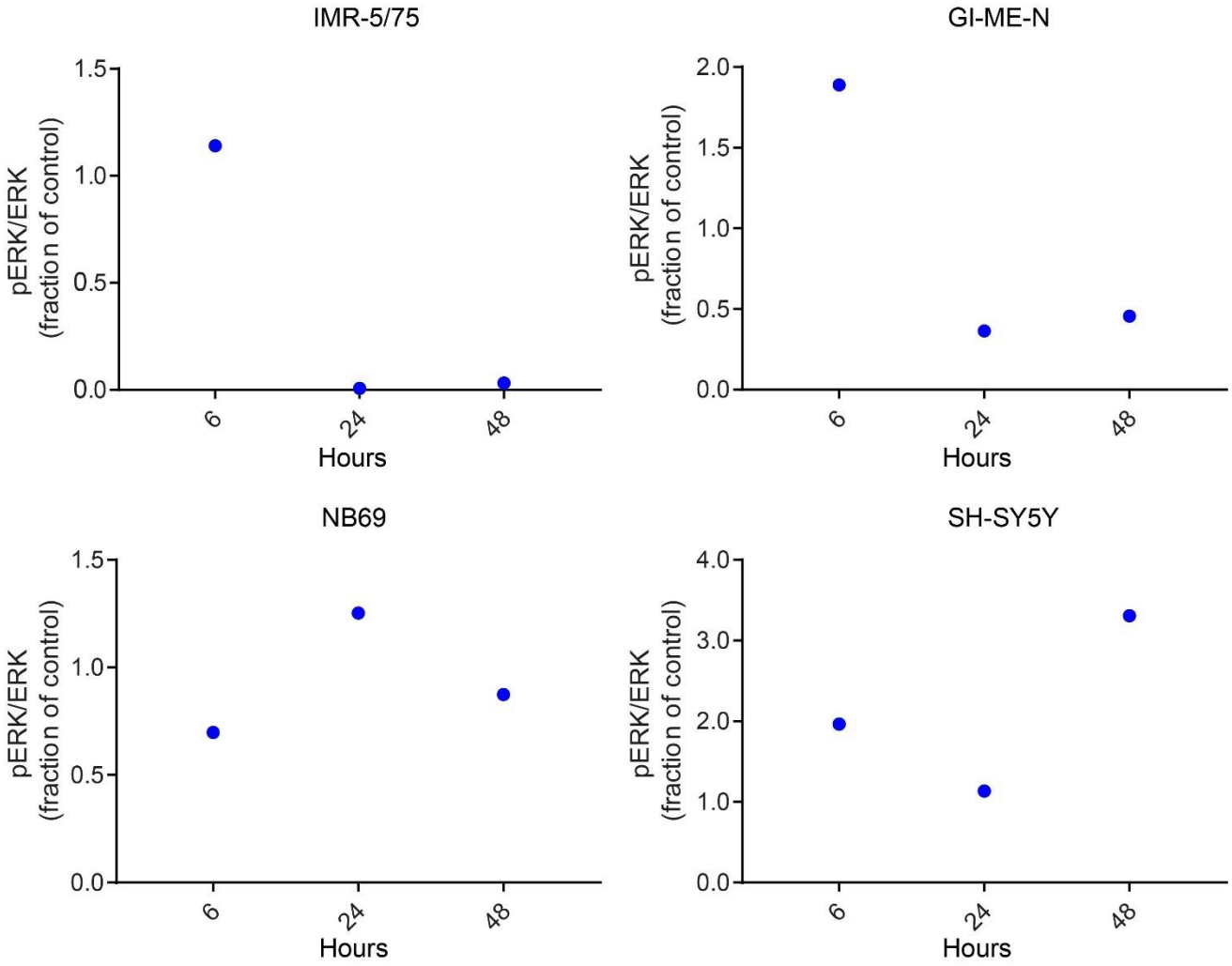
Supplemental Figure 6. Ectopic expression of *PHB-V5* promotes mound growth and migration and suppresses the expression of favorable prognostic markers in neuroblastoma cells. A. Photomicrographs of SH-SY5Y cells stably expressing *PHB-V5* compared to cells expressing an empty vector control. White arrowhead indicates exemplary mound. Scale bars indicate 100 μm. Photomicrographs are representative of three independent samples. **B**. Quantification of mounds formed by NB01 cells stably expressing *PHB-V5* compared to cells expressing an empty vector control. $p > 0.05$, two-tailed t-test. **C**. Representative photomicrographs of the scratch migration assay of IMR-5/75 (n = 3) and SH-SY5Y (n = 1). cells stably expressing *PHB-V5* or empty vector. Scale bars indicate 100 μm. **D**. mRNA expression of *NTRK1* and *NGFR* measured by qRT-PCR in NB01 cells stably expressing *PHB-V5* compared to cells expressing an empty vector control. * $p < 0.05$, two-tailed t-test. Data represent mean +/- SD. Data represents three technical replicates.



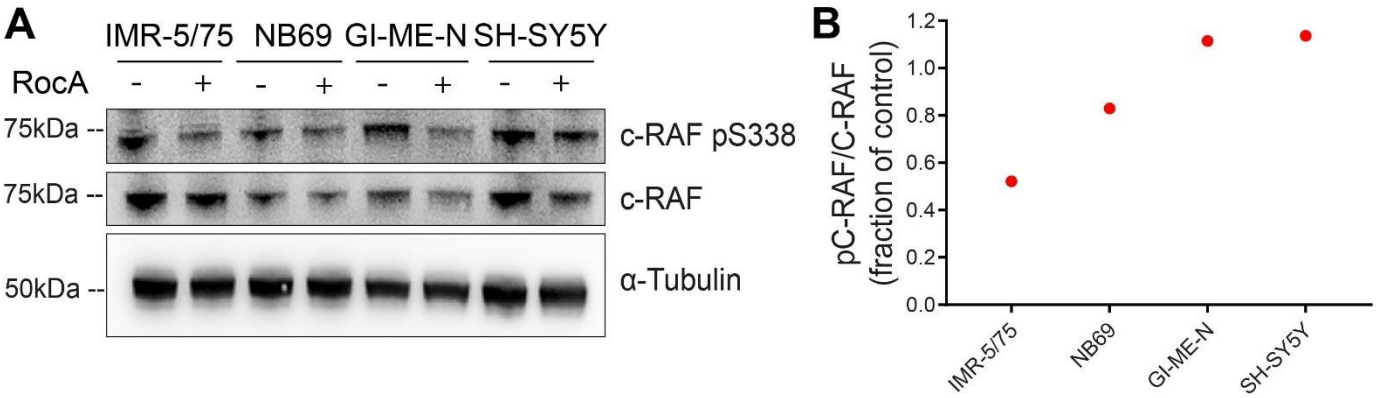
Supplemental Figure 7. Neuroblastoma cell lines exhibit enhanced sensitivity to rocaglamide A treatment. A. Dose response curve of RPE cells treated with RocA as measured with CellTiter-Glo luminescent viability assay 72 hours after treatment. Data represent three technical replicates. **B**. IC50 values of RocA in neuroblastoma cell lines (black) and untransformed RPE and VH7 fibroblast cells (blue).



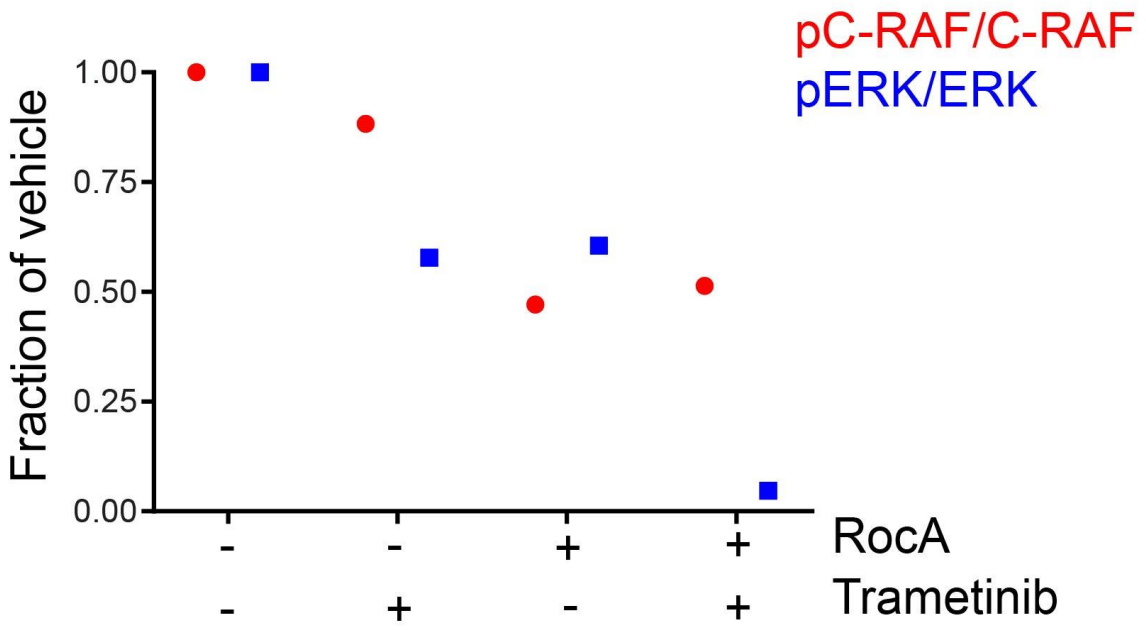
Supplemental Figure 8. RocA treatment impairs ERK activation in neuroblastoma cells. Western blot analysis of GI-ME-N, NB69, and SH-SY5Y cells 6, 24, and 48 hours after treatment with 50 nM RocA compared to DMSO-treated cells. Data represent a single experiment.



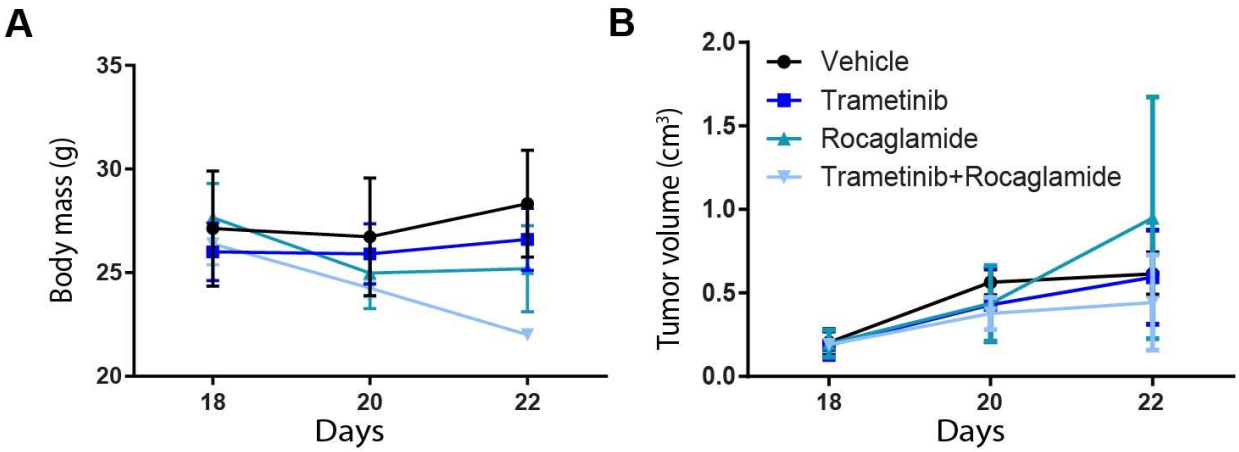
Supplemental Figure 9. RocA treatment impairs ERK activation in neuroblastoma cells. Quantification of western blots displayed in Figure 6B and Supplemental Figure 9 showing ratio of phosphorylated ERK1/2 to total ERK1/2.



Supplemental Figure 10. **RocA treatment does not observably reduce phosphorylation of c-RAF pS338 in neuroblastoma cell lines. A**. Western blot analysis of neuroblastoma cells 24 hours after treatment with 50 nM RocA compared to DMSO-treated cells. Data represent a single experiment. **B**. Quantification of western blot displayed in panel A showing ratio of phosphorylated c-RAF to total c-RAF.



Supplemental Figure 11. Rocaglamide A treatment impairs ERK activation in an *ALK*-mutant patient-derived xenograft in vivo. Quantification of western blot displayed in Figure 7C showing ratios of phosphorylated to total c-RAF and ERK1/2.



Supplemental Figure 12. Effect of combination rocaglamide A and trametinib treatment on mouse body weight and patient-derived xenograft tumor volume. A. Growth curve of mouse body weight over the course of 4 days. n = 3-4. **B**. Tumor volume of an *ALK*-mutant patient-derived xenograft over the course of 4 days. n = 3-4. Data represents mean +/- SEM. Stated n-values indicate number of biological replicates.

335 **Supplemental Table 1**. Clinical characteristics of neuroblastoma patient-derived xenograft

Age	2 years
INSS[A]	IV
INRG[B]	High
Primary tumor site	Abdomen
Biopsy site	Abdomen
Genetics	*MYCN* amplified, *ALK* mutant

336 [A]International Neuroblastoma Staging System

337 [B]International Neuroblastoma Risk Group

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Supplemental Table 2. Sequences of oligonucleotide primers used for qRT-PCR

Name	Description	Sequence (5'-3')
NTRK1_qPCR_F	qRT-PCR of human NTRK1	CACTAACAGCACATCTGGAGACC
NTRK1_qPCR_R	qRT-PCR of human NTRK1	TGAGCACAAGGAGCAGCGTAGA
NGFR_qPCR_F	qRT-PCR of human NGFR	CCTCATCCCTGTCTATTGCTCC
NGFR_qPCR_R	qRT-PCR of human NGFR	GTTGGCTCCTTGCTTGTTCTGC
HPRT1_qPCR_F	qRT-PCR of human HPRT1	TGACACTGGCAAAACAATGCA
HPRT1_qPCR_R	qRT-PCR of human HPRT1	GGTCCTTTTCACCAGCAAGCT

Supplemental Table 3. Plasmid vectors

Name	Clone	Source
pLKO.1-shGFP	TRCN0000559399	Dhmarmacon Inc., Lafayette, CO, USA
pLKO.1-shPHB-1	TRCN0000029204	Dhmarmacon Inc.
pLKO.1-shPHB-2	TRCN0000029206	Dhmarmacon Inc.
pLKO.1-shPHB-3	TRCN0000029208	Dhmarmacon Inc.
pLX304-Empty	N.A.	David Root
pLX304-PHB-V5	N.A.	Alex Kentsis

Supplemental Table 4. Antibodies

Name	Clone	Catalogue #	Source
Mouse anti-Prohibitin	E-5	sc-377037	Santa Cruz Biotechnology, Inc., Dallas, TX, USA
Rabbit anti-Prohibitin	Polyclonal	sc-28259	Santa Cruz Biotechnology, Inc.
Mouse anti-Prohibitin pT258	Polyclonal	GTX55299	GeneTex, Inc., Irvine, CA, USA
Mouse anti-α-Tubulin	DM1A	3873	Cell Signaling Technology, Danvers, MA, USA
Rabbit anti-ERK	137F5	4695	Cell Signaling Technology
Rabbit anti-ERK pT202/Y204	D13.14.4E	4370	Cell Signaling Technology
Rabbit anti-c-RAF	Polyclonal	9422	Cell Signaling Technology
Rabbit anti-c-RAF pS338	56A6	9427	Cell Signaling Technology
Rabbit anti-Cox IV	Polyclonal	4844	Cell Signaling Technology
Mouse anti-V5	SV5-PK1	ab27671	Abcam, Cambridge, UK
Mouse anti-β-Actin	8H10D10	3700	Cell Signaling Technology

Figure 2

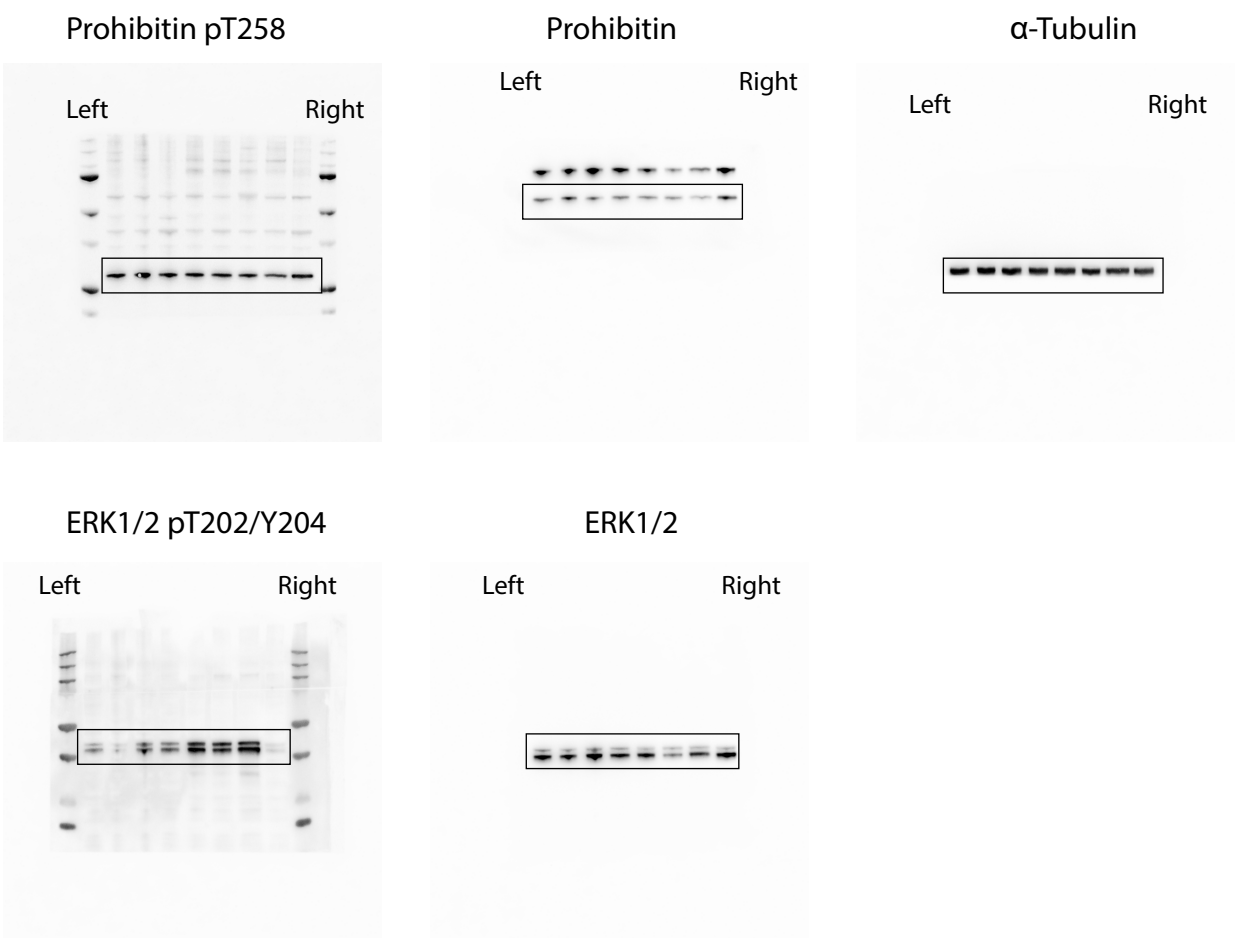
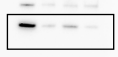

Prohibitin pT258
Left
Right
Prohibitin
Left
Right
α-Tubulin
Left
Right
ERK1/2 pT202/Y204
Left
Right
ERK1/2
Left
Right

Figure 3

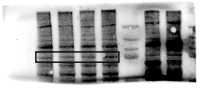
Prohibitin

Left Right

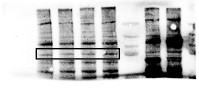


c-RAF pS338

Left Right

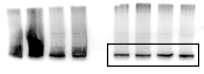


Different brightness in entire blot due to high background



c-RAF

Left Right

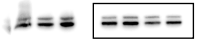


ERK1/2 pT202/Y204

Left Right

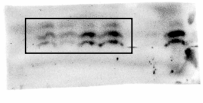
ERK1/2

Left Right



Cleaved caspase 3

Left Right



α-Tubulin

Left Right

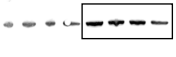
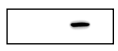


Figure 5

NB01 V5

Left Right



SH-SY5Y V5

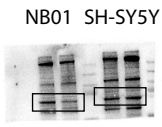
Left Right

IMR-5/75 V5

Left Right

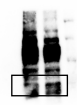
NB01 and SH-SY5Y c-RAF pS338

Left Right



IMR-5/75 c-RAF pS338

Left Right

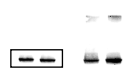


NB01 c-RAF

Left Right

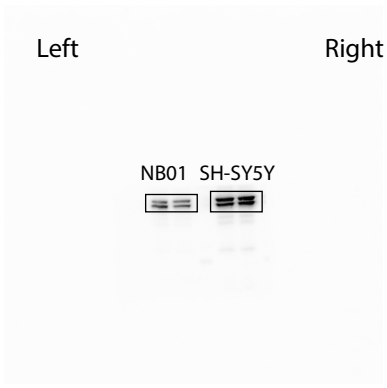
SH-SY5Y and IMR-5/75 c-RAF

Left Right



NB01 and SH-SY5Y ERK1/2 pT202/Y204

Left Right



IMR-5/75 ERK1/2 pT202/Y204

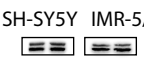
Left Right

NB01 ERK1/2

Left Right

SH-SY5Y and IMR-5/75 ERK1/2

Left Right



NB01 α-Tubulin

Left Right

SH-SY5Y α-Tubulin

Left Right

IMR-5/75 α-Tubulin

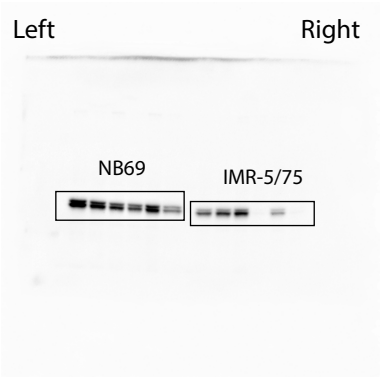
Left Right

IMR-5/75 α-Tubulin for c-RAF pS338

Left Right

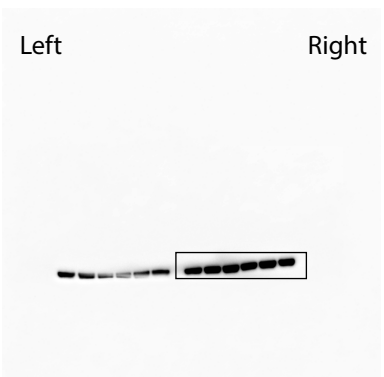
Figure 6 and Supplemental Figure 9

IMR-5/75 and NB69 ERK1/2 pT202/Y204



IMR-5/75 and NB69 ERK1/2

IMR-5/75 α-Tubulin



NB69 α-Tubulin

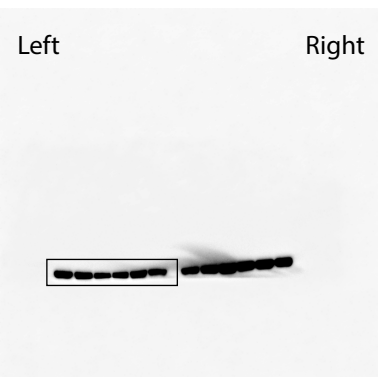
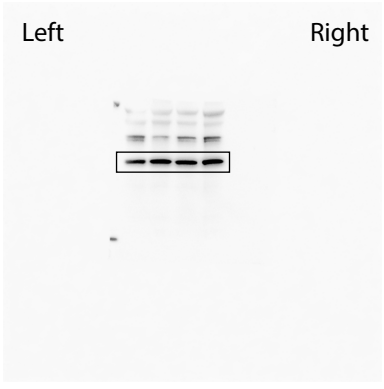
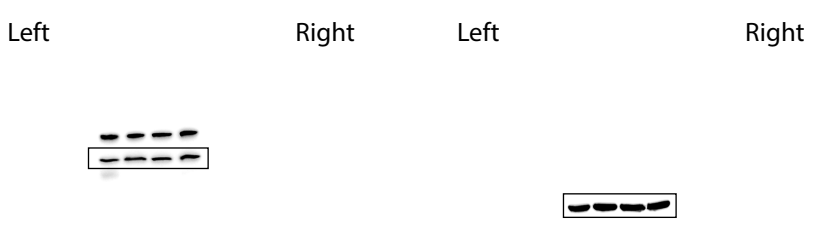


Figure 7

Prohibitin pT258

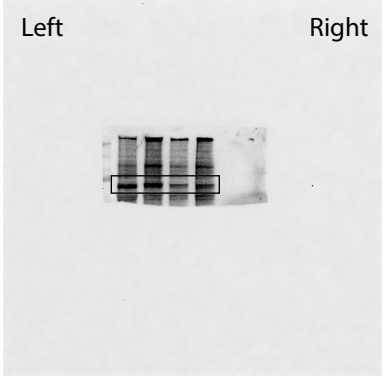


Prohibitin

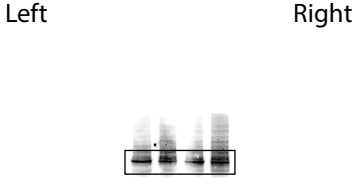


α-Tubulin

c-RAF pS338



c-RAF

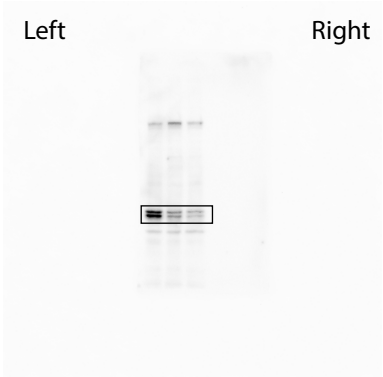


ERK1/2 pT202/Y204

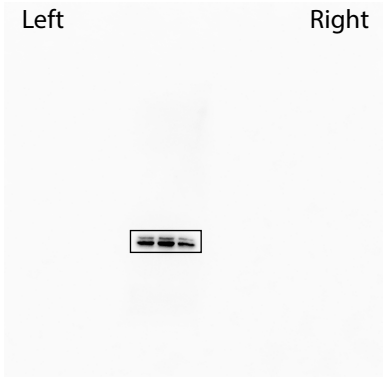
ERK1/2

Supplemental Figure 5

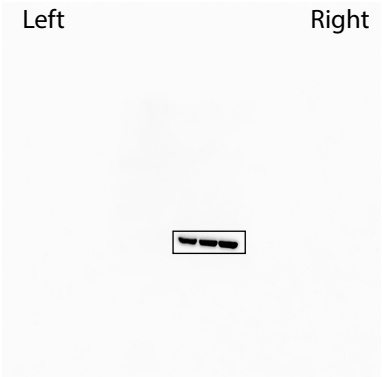
NB01 ERK1/2 pT202/Y204



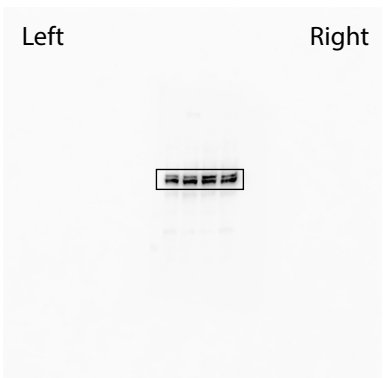
NB01 ERK1/2



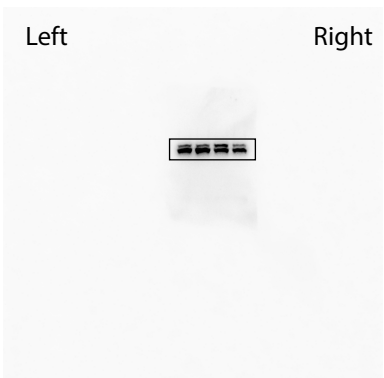
NB01 α-Tubulin



Kelly ERK1/2 pT202/Y204



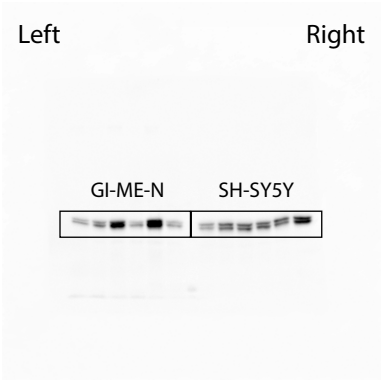
Kelly ERK1/2



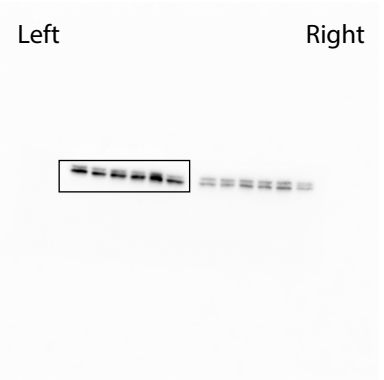
Kelly α-Tubulin

Supplemental Figure 9

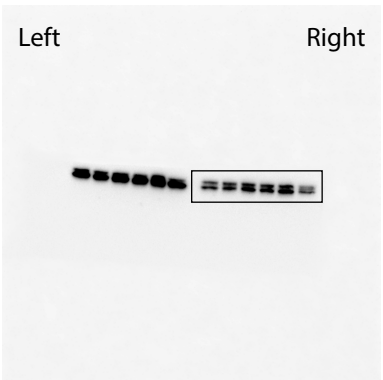
GI-ME-N and SH-SY5Y ERK1/2 pT202/Y204



GI-ME-N ERK1/2



SH-SY5Y ERK1/2



GI-ME-N and SH-SY5Y α-Tubulin