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Supplemental information

High-risk neuroblastoma with NF1 loss of function is targetable using SHP2 inhibition

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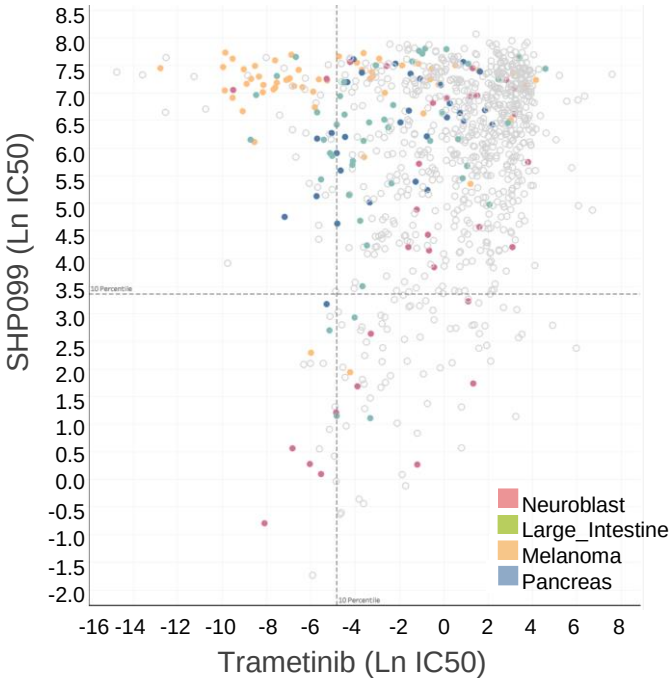
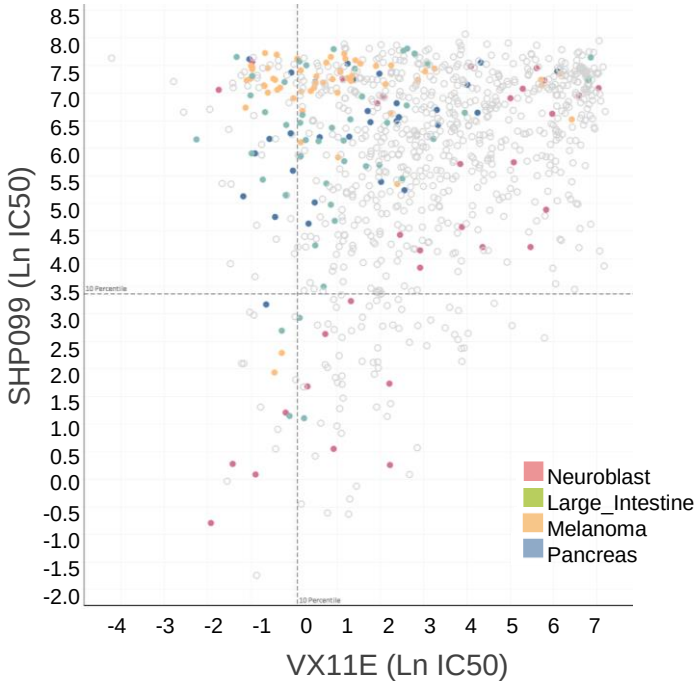
Sup. Fig. 1

A


SHP099 (Ln IC50)
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-16 -14 -12 -10 -8 -6 -4 -2 0 2 4 6 8
Trametinib (Ln IC50)
Neuroblast
Large_Intestine
Melanoma
Pancreas

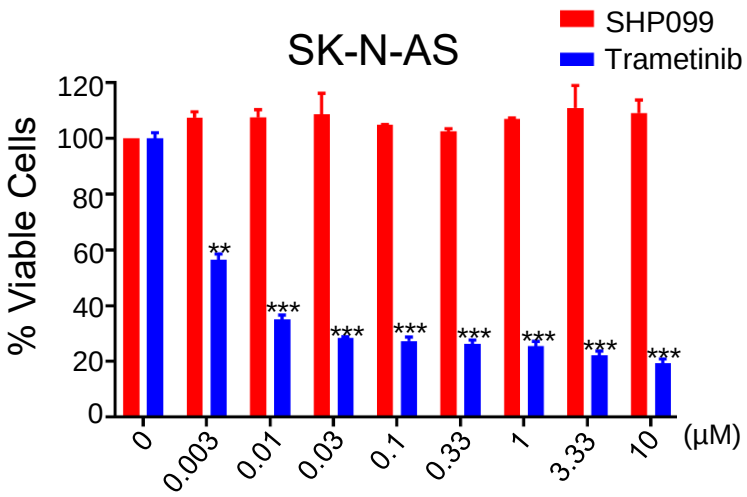
B


SHP099 (Ln IC50)
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-4 -3 -2 -1 0 1 2 3 4 5 6 7
VX11E (Ln IC50)
Neuroblast
Large_Intestine
Melanoma
Pancreas

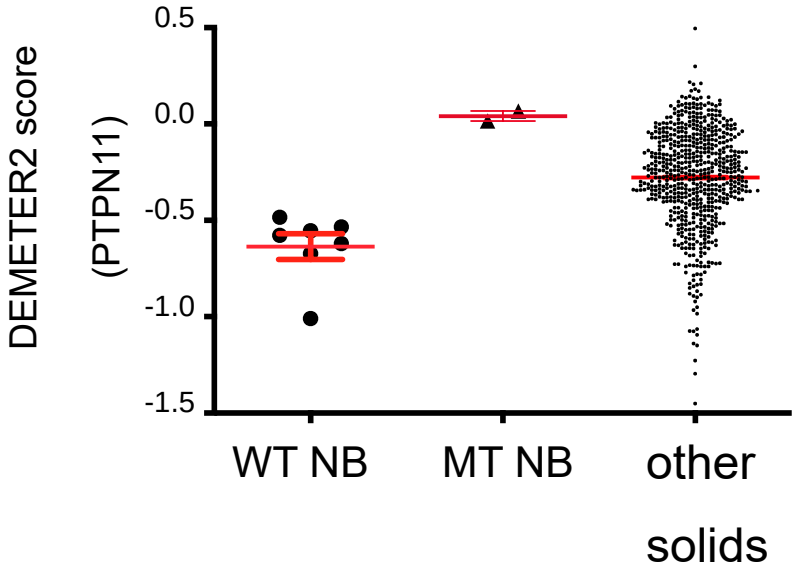
Sup Fig.1. Graphs represent differential activity of SHP099 (IC50) with respect to IC50 of trametinib (A) and VX11E (B) for each cell line (n=922; 32 NB cell lines). Dotted lines correspond to top 10% sensitive cell line for each drug. Related to Figure 1.

Sup. Fig. 2

A

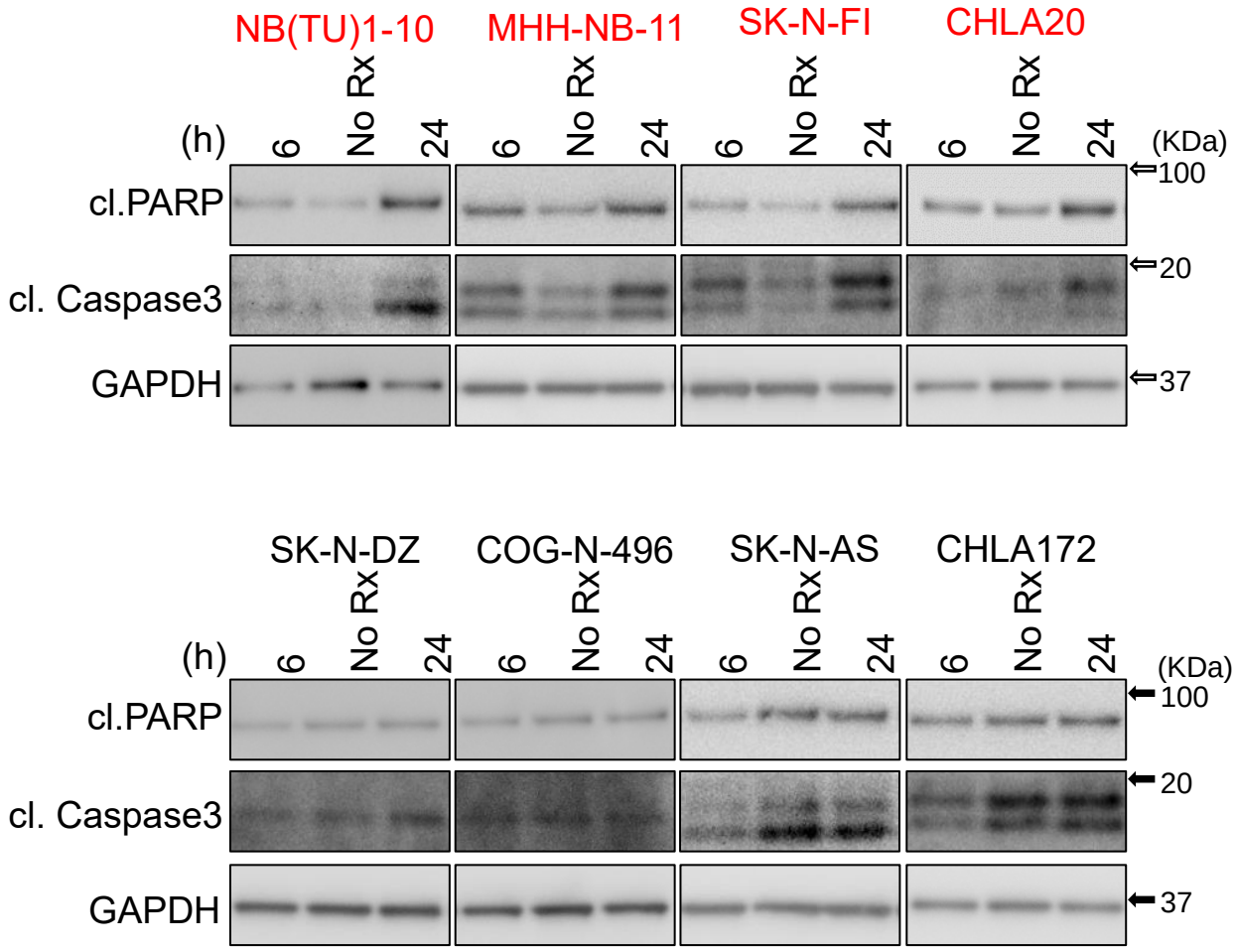

SK-N-AS
SHP099
Trametinib
% Viable Cells
120
100
80
60
40
20
0
**
***
***
***
***
***
***
***
0
0.003
0.01
0.03
0.1
0.33
1
3.33
10
(μM)

B


DEMETER2 score
(PTPN11)
0.5
0.0
-0.5
-1.0
-1.5
WT NB
MT NB
other
solids

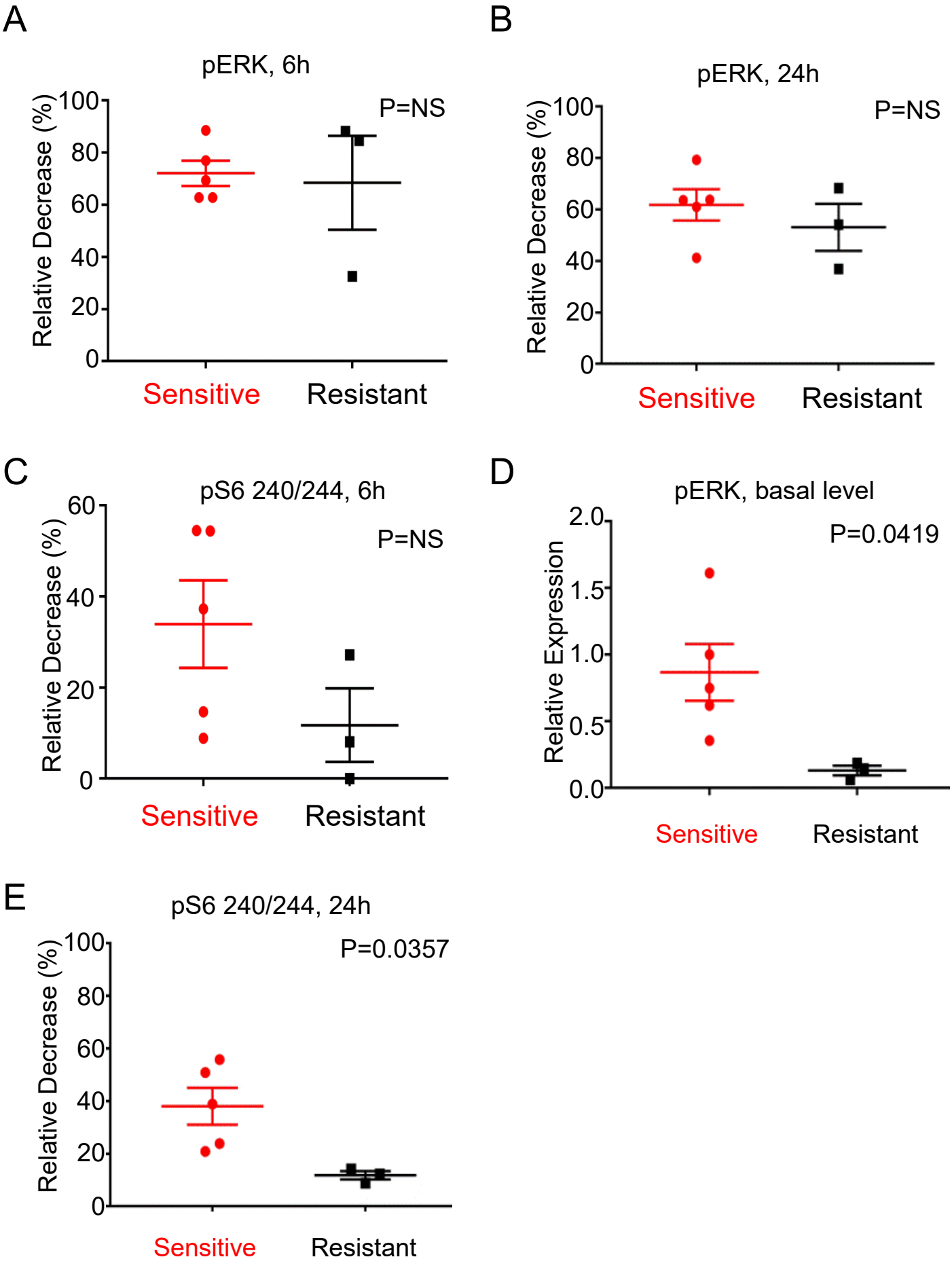
Sup Fig.2. Trametinib sensitivity in an *NRAS* MT NB cell line. A) Graph represents percent viable cells assessed by Cell Titer-Glo in the *NRAS* MT SK-N-AS cell line following 7 days treatment with increasing concentrations (0.003-10 μM) of trametinib or SHP099. Error bars are ±SEM. Three individual sets of experiments were performed. B) PTPN11 (SHP2) RNAi screens (Achilles+DRIVE+Marcotte) DEMETER2 scores from DepMap. NB RAS/RAF WT n=7, NB RAS/RAF MT n=2, other tumors, n= 639. WT NB versus other tumors, $p<0.0001$ by Mann-Whitney. Related to Figure 1.

Sup. Fig. 3


NB(TU)1-10
MHH-NB-11
SK-N-FI
CHLA20
(h)
6
No Rx
24
(KDa)
cl.PARP
cl. Caspase3
GAPDH
100
20
37
SK-N-DZ
COG-N-496
SK-N-AS
CHLA172

Sup Fig.3. Viability effects were in part due to cell death. Immunoblot of the NB cell lines indicated, treated with SHP099 (5 μM) for 6h and 24h, respectively, or untreated (No Rx), and assessed with the indicated antibodies. Related to Figure 1.

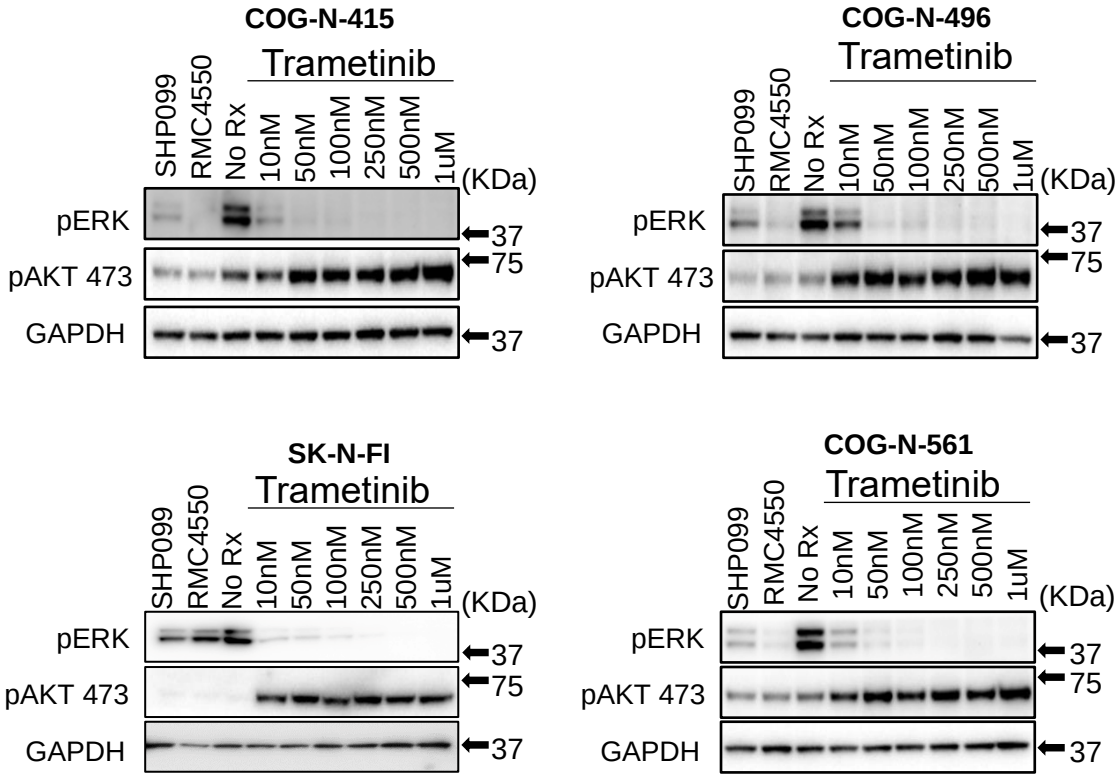
Sup .Fig. 4


A
pERK, 6h
P=NS
Relative Decrease (%)
100
80
60
40
20
0
Sensitive
Resistant
B
pERK, 24h
P=NS
Relative Decrease (%)
100
80
60
40
20
0
Sensitive
Resistant
C
pS6 240/244, 6h
P=NS
Relative Decrease (%)
60
40
20
0
Sensitive
Resistant
D
pERK, basal level
P=0.0419
Relative Expression
2.0
1.5
1.0
0.5
0.0
Sensitive
Resistant
E
pS6 240/244, 24h
P=0.0357
Relative Decrease (%)
100
80
60
40
20
0
Sensitive
Resistant

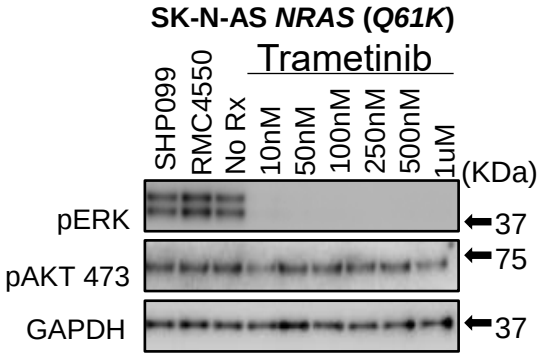
Sup Fig.4. Relative levels of pERK and pS6 in SHP099 sensitive and insensitive NB cell lines. (A) Relative levels of pERK in SHP099 sensitive and insensitive lines normalized to GAPDH. (B) Relative percent decrease of pERK expression in SHP099 sensitive and resistant NB cell lines treated with SHP099 for 24h. (C) Relative percent decrease of pS6 in SHP099 sensitive and resistant NB cell lines treated with SHP099 for 6h. (D) Relative expression of pERK basal level in SHP099 sensitive (red) and resistant (black) NB cell lines compared to untreated (No Rx) cells. (E) Relative percentage decrease of pS6 (240/244) in SHP099 sensitive (red) and resistant (black) NB cell lines compared to untreated (No Rx), quantified from figure 2A. Related to Figure 2.

Sup. Fig. 5

A


COG-N-415
Trametinib
SHP099
RMC4550
No Rx
10nM
50nM
100nM
250nM
500nM
1uM
(KDa)
pERK
pAKT 473
GAPDH
37
75
37
COG-N-496
Trametinib
SHP099
RMC4550
No Rx
10nM
50nM
100nM
250nM
500nM
1uM
(KDa)
pERK
pAKT 473
GAPDH
37
75
37
SK-N-FI
Trametinib
SHP099
RMC4550
No Rx
10nM
50nM
100nM
250nM
500nM
1uM
(KDa)
pERK
pAKT 473
GAPDH
37
75
37
COG-N-561
Trametinib
SHP099
RMC4550
No Rx
10nM
50nM
100nM
250nM
500nM
1uM
(KDa)
pERK
pAKT 473
GAPDH
37
75
37

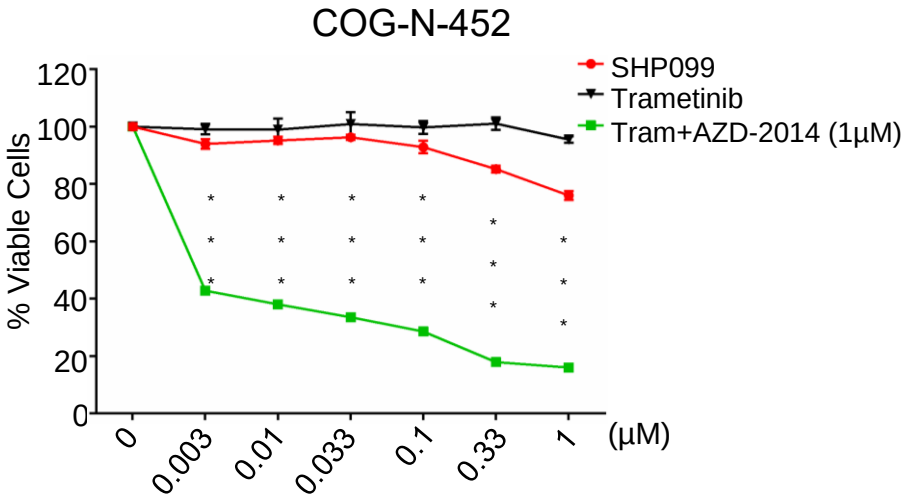
B


SK-N-AS NRAS (Q61K)
Trametinib
SHP099
RMC4550
No Rx
10nM
50nM
100nM
250nM
500nM
1uM
(KDa)
pERK
pAKT 473
GAPDH
37
75
37

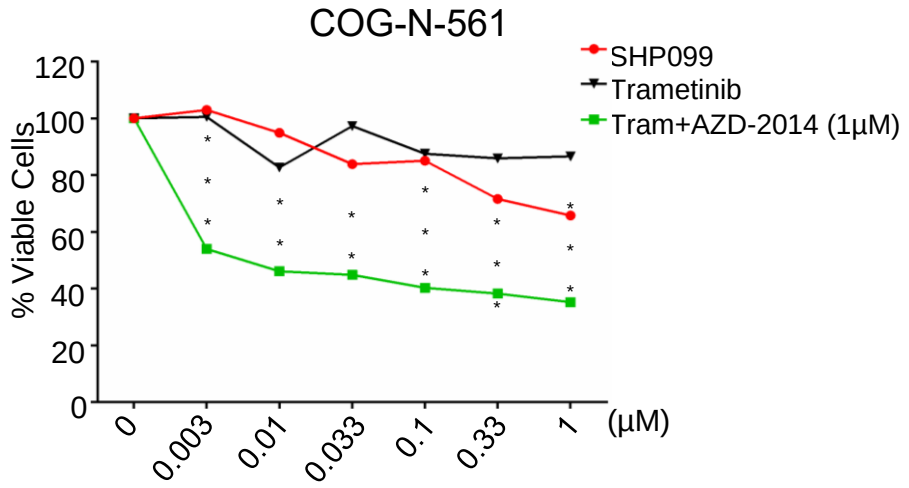
Sup Fig.5. Immunoblot analysis of NRAS WT and MT NB cell lines treated with SHP099 (5 µM) or RMC-4550 (10 µM) or trametinib. A) Immunoblot analysis of NB PDX lines COG-N-415, COG-N-496, COG-N-561 and SK-N-FI treated with SHP099 (5 µM) or RMC-4550 (10 µM) or trametinib (0.01 µM-1 µM) for 24h and untreated control (No Rx) cells assessed with the indicated antibodies. GAPDH was used as loading control. B) Immunoblot analysis of NRAS mutant SK-N-AS cells treated with SHP099 (5 µM) or RMC-4550 (10 µM) or trametinib (0.01 µM-1 µM) for 24h and untreated control (No Rx) cells were assessed with the indicated antibodies. GAPDH was used as loading control. Related to Figure 2.

Sup Fig. 6

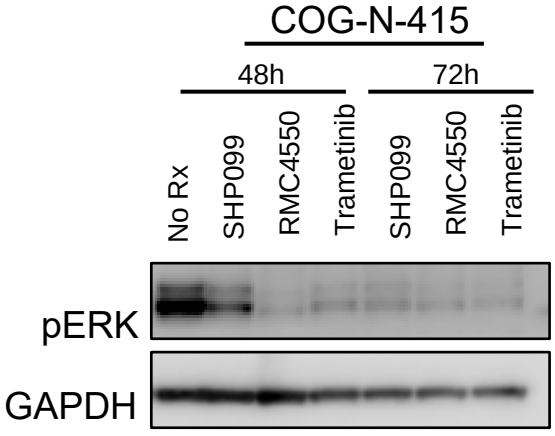
A


COG-N-452
SHP099
Trametinib
Tram+AZD-2014 (1μM)
% Viable Cells
120
100
80
60
40
20
0
0
0.003
0.01
0.033
0.1
0.33
1
(μM)

B

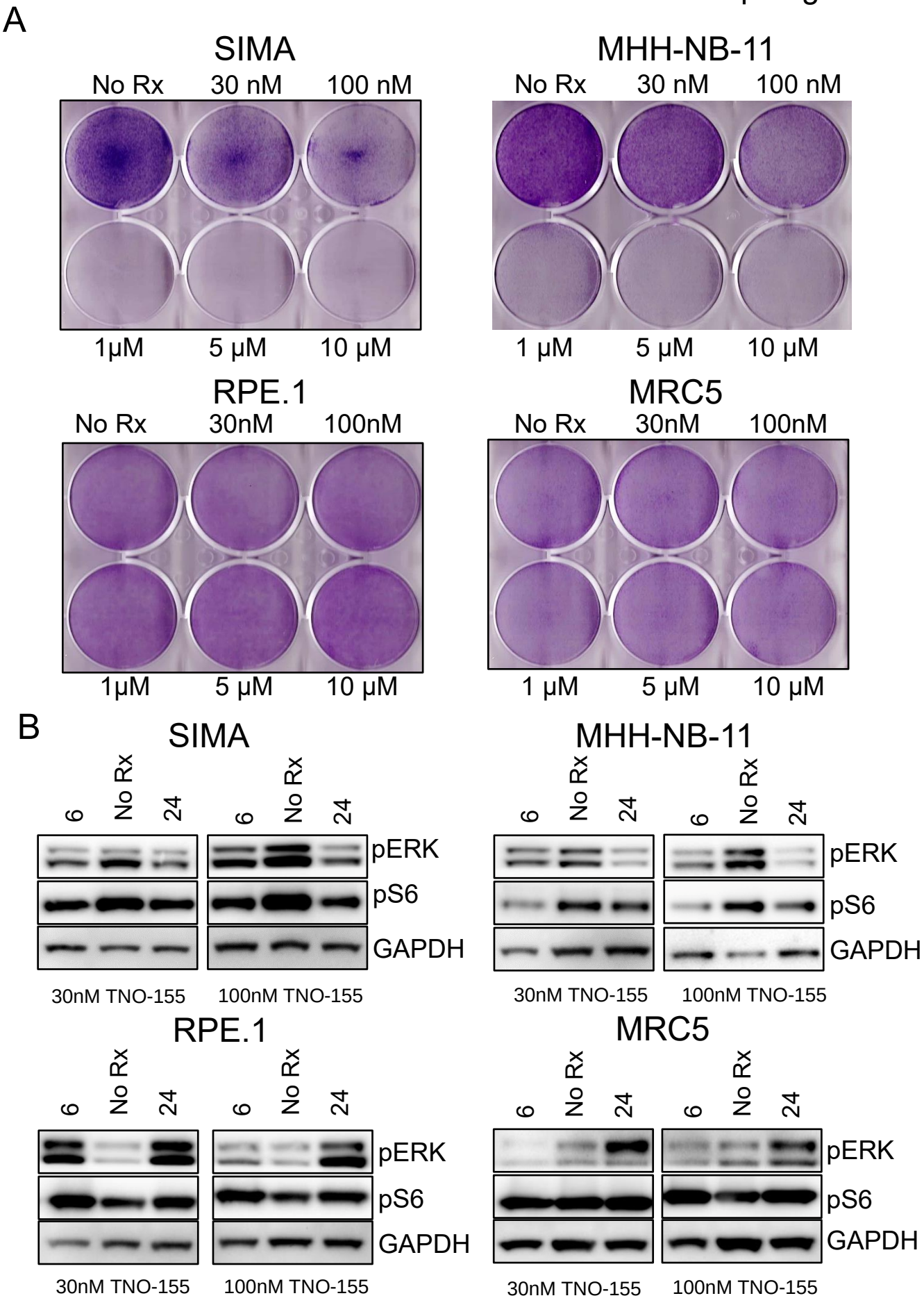

COG-N-561
SHP099
Trametinib
Tram+AZD-2014 (1μM)
% Viable Cells
120
100
80
60
40
20
0
0
0.003
0.01
0.033
0.1
0.33
1
(μM)

C


COG-N-415
48h
72h
No Rx
SHP099
RMC4550
Trametinib
SHP099
RMC4550
Trametinib
pERK
GAPDH

Sup Fig.6. mTOR inhibitor AZD-2014 sensitizes NB lines to trametinib. Percent viable cells assessed by Cell Titer-Glo in the (A) COG-N-452 and (B) COG-N-561 cell lines following 72h treatment with increasing concentrations (0.003-10 µM) of SHP099, trametinib, AZD-2014 and trametinib in combination with 1 µM AZD-2014. Error bars are ±SEM. Three individual sets of experiments were performed. (C) Immunoblot analysis of pERK in the COG-N-415 cell line treated with SHP099 (5 µM), RMC-4550 (10 µM) or trametinib (1 µM) for 48h and 72h. Related to Figure 2.

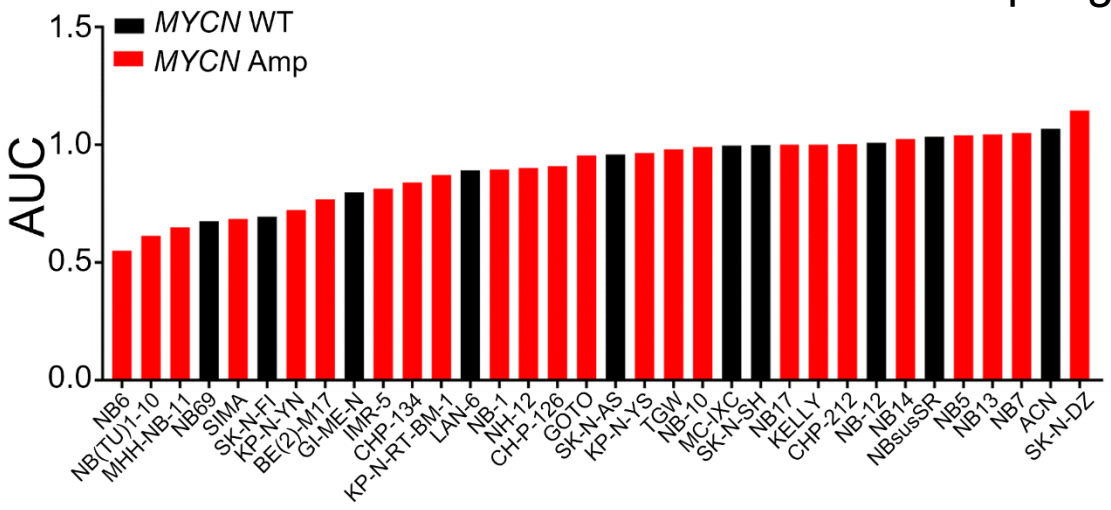
Sup. Fig. 7


A
SIMA
No Rx
30 nM
100 nM
1μM
5 μM
10 μM
MHH-NB-11
No Rx
30 nM
100 nM
1 μM
5 μM
10 μM
RPE.1
No Rx
30nM
100nM
1μM
5 μM
10 μM
MRC5
No Rx
30nM
100nM
1 μM
5 μM
10 μM
B
SIMA
6
No Rx
24
6
No Rx
24
pERK
pS6
GAPDH
30nM TNO-155
100nM TNO-155
MHH-NB-11
6
No Rx
24
6
No Rx
24
pERK
pS6
GAPDH
30nM TNO-155
100nM TNO-155
RPE.1
6
No Rx
24
6
No Rx
24
pERK
pS6
GAPDH
30nM TNO-155
100nM TNO-155
MRC5
6
No Rx
24
6
No Rx
24
pERK
pS6
GAPDH
30nM TNO-155
100nM TNO-155

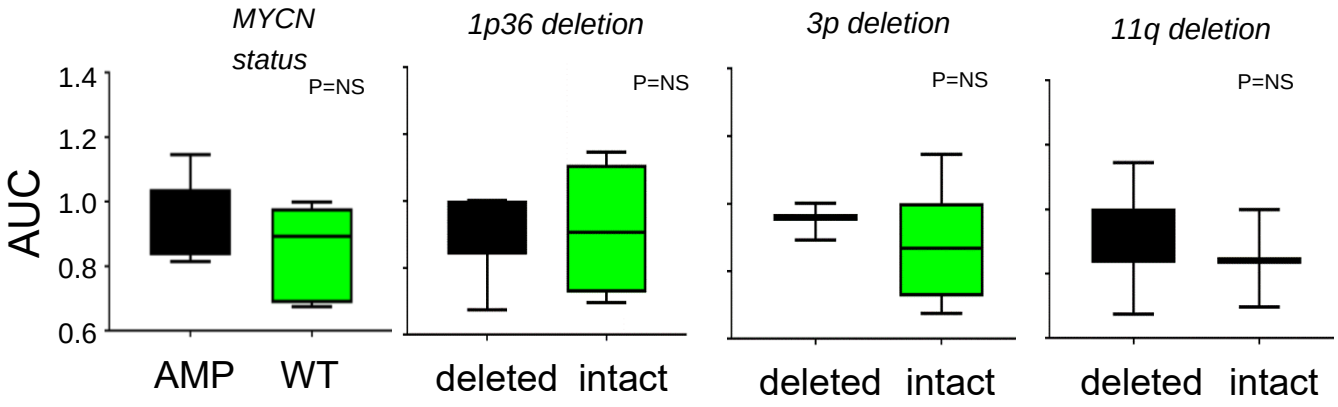
Sup Fig.7. TNO-155 show similar sensitivity to SHP099 sensitive in both NB cell lines and normal tissue-derived cell lines. (A) Crystal violet assays or (B) immunoblot analysis of SIMA and MHH-NB-11 NB cell lines and RPE.1 and MRC5 normal tissue-derived cell lines treated with vehicle (No Rx), and for (A), increasing TNO-155 from 30 nM to 10 µM until the untreated (No Rx) cells reached confluency. Experiments were performed in triplicates. (B) Immunoblot analysis of NB cell lines SIMA, MHH-NB-11 and the normal tissue-derived cell lines RPE.1and MRC5 treated with TNO-155 (30 nM or 100 nM) for 6h or 24h and untreated control (No Rx) cells assessed with the indicated antibodies. GAPDH was used as loading control. For (A), experiments were performed in triplicates. Related to Figure 2.

Sup Fig.8

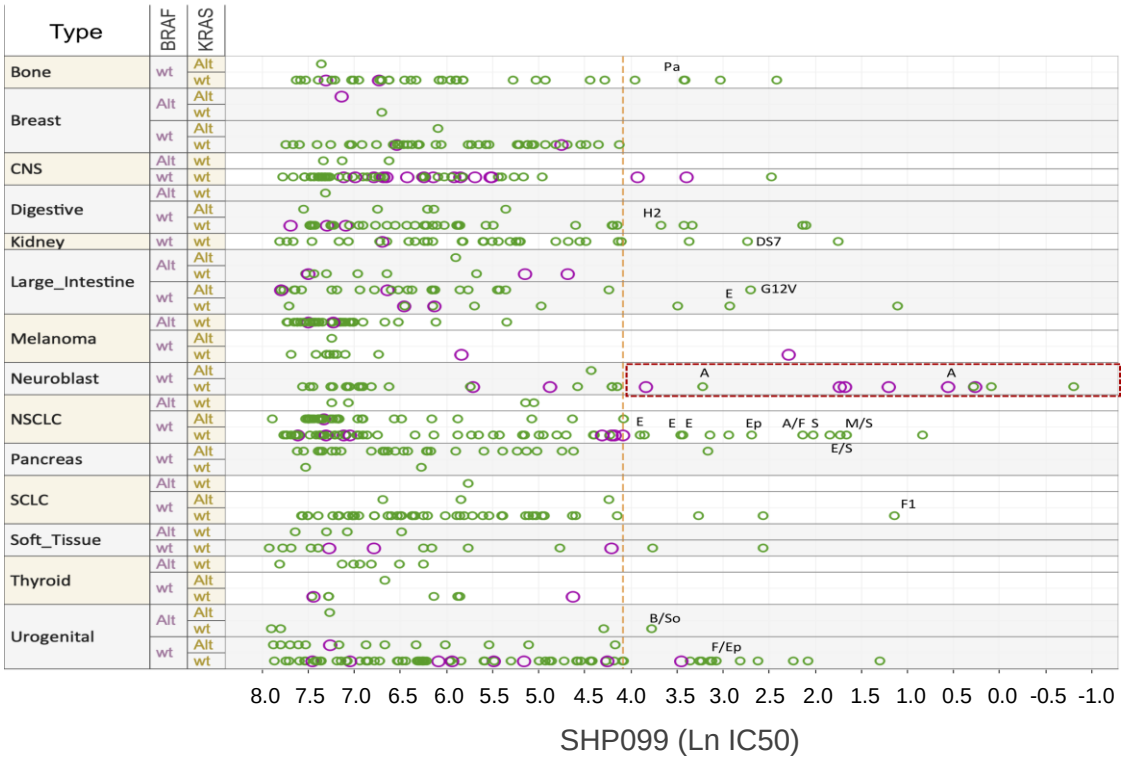
A


MYCN WT
MYCN Amp
AUC
1.5
1.0
0.5
0.0
NB6
NB(TU)1-10
MHH-NB-11
NB69
SIMA
SK-N-FI
KP-N-YN
BE(2)-M17
GI-ME-N
IMR-5
CHP-134
KP-N-RT-BM-1
LAN-6
NB-1
NH-12
CH-P-126
GOTO
SK-N-AS
KP-N-YS
TGW
NB-10
MC-IXC
SK-N-SH
NB17
KELLY
CHP-212
NB-12
NB14
NBsusSR
NB5
NB13
NB7
ACN
SK-N-DZ

B

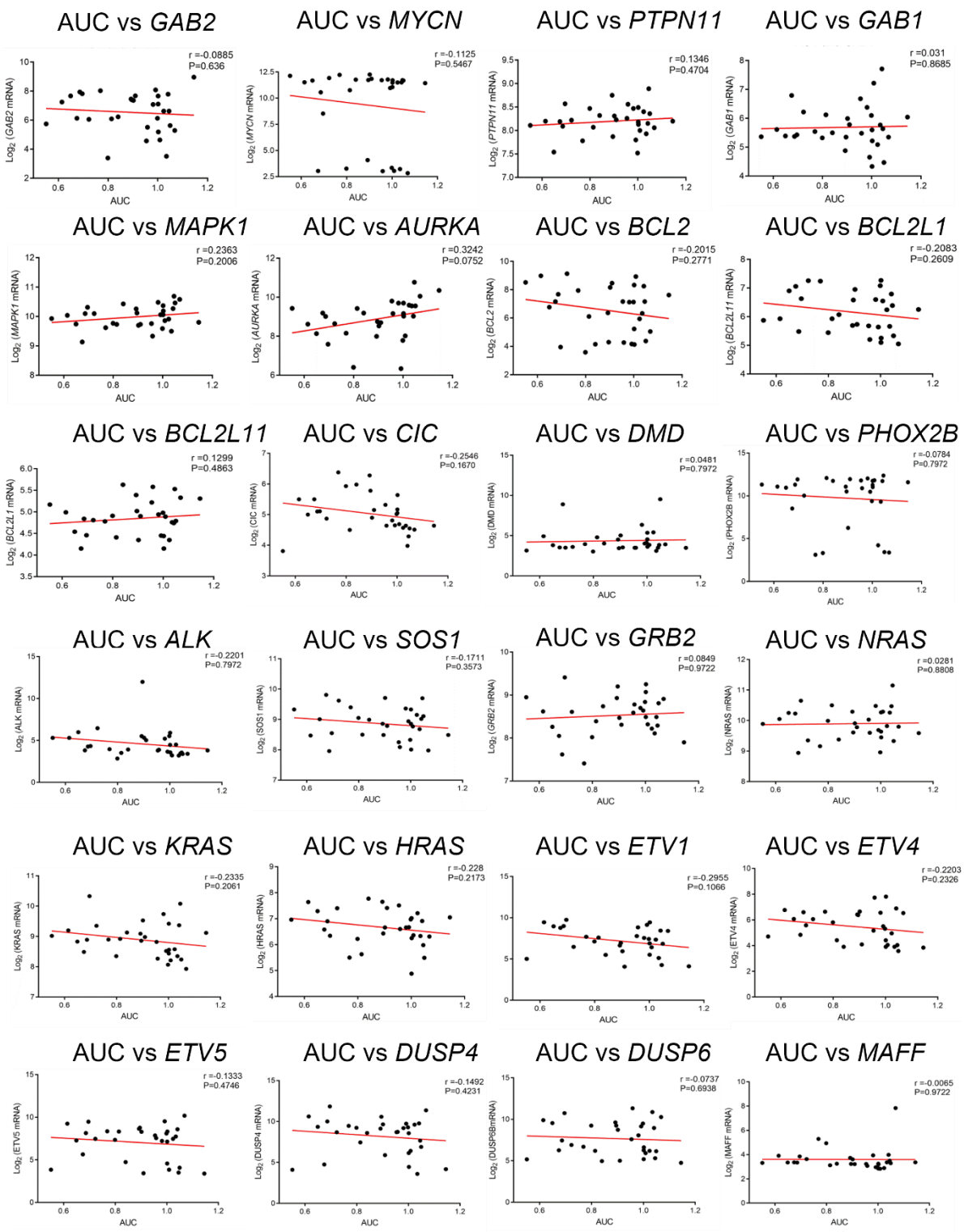

MYCN status
1p36 deletion
3p deletion
11q deletion
AUC
1.4
1.2
1.0
0.8
0.6
P=NS
P=NS
P=NS
P=NS
AMP
WT
deleted
intact
deleted
intact
deleted
intact

C


Type
BRAF
KRAS
Bone
Breast
CNS
Digestive
Kidney
Large_Intestine
Melanoma
Neuroblast
NSCLC
Pancreas
SCLC
Soft_Tissue
Thyroid
Urogenital
Alt
wt
Pa
H2
DS7
G12V
A
E
E E
Ep
A/F S
M/S
E/S
F1
B/So
F/Ep
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
SHP099 (Ln IC50)

Sup Fig 8. SHP099 sensitivity does not correlate with typical genomic alterations in NB. A) Area under curve (AUC) of SHP099 in NB cell lines assayed in HTS. Amplification status of MYCN are indicated as MYCN amplified (red bars) and MYCN-WT (black bars). B) AUC of SHP099 compared to MYCN status (WT and amplified) and various deletions in NB cell lines. Mann-Whitney test was performed to calculate statistical significance between the groups (NS- nonsignificant). C) SHP099 sensitivity correlates with NF1 mutational status in NB. Activity of SHP099 across different tissue types including 32 NB cell lines. The orange dotted line corresponds to the top 10% sensitive cell lines. Mutational status of selected lines are shown: Pa- PDGFRA; H2- HER2 amplified; DS7- DUSP7; E- EGFR; G12V- KRASG12V; A- ALK; Ep- EPHA2; F- FGFR2; S- STK11; M- MET; F1- FGFR1; B- BRAF; So- SOS1. Related to Figure 4.

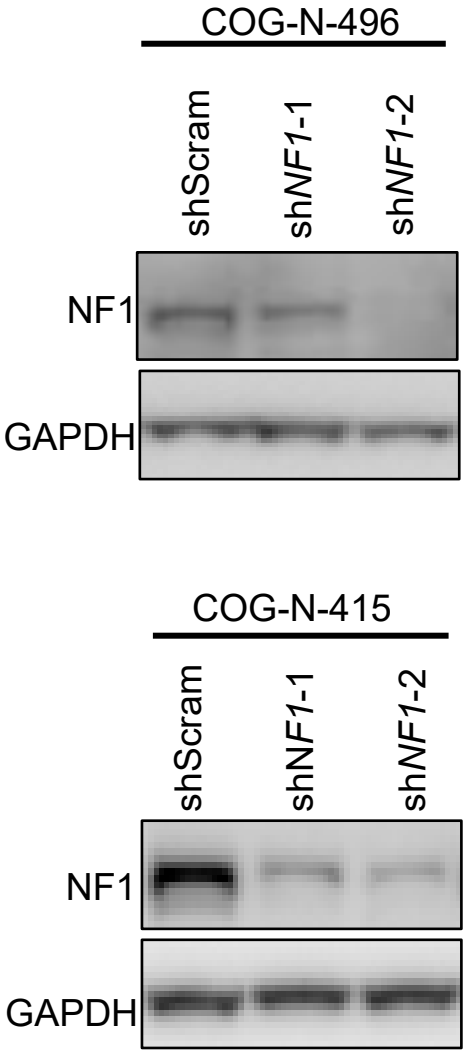
Sup Fig.9


AUC vs GAB2
r =-0.0885
P=0.636
Log2 (GAB2 mRNA)
AUC
AUC vs MYCN
r =-0.1125
P=0.5467
Log2 (MYCN mRNA)
AUC
AUC vs PTPN11
r =0.1346
P=0.4704
Log2 (PTPN11 mRNA)
AUC
AUC vs GAB1
r =0.031
P=0.8685
Log2 (GAB1 mRNA)
AUC
AUC vs MAPK1
r =0.2363
P=0.2006
Log2 (MAPK1 mRNA)
AUC
AUC vs AURKA
r =0.3242
P=0.0752
Log2 (AURKA mRNA)
AUC
AUC vs BCL2
r =-0.2015
P=0.2771
Log2 (BCL2 mRNA)
AUC
AUC vs BCL2L1
r =-0.2083
P=0.2609
Log2 (BCL2L11 mRNA)
AUC
AUC vs BCL2L11
r =0.1299
P=0.4863
Log2 (BCL2L1 mRNA)
AUC
AUC vs CIC
r =-0.2546
P=0.1670
Log2 (CIC mRNA)
AUC
AUC vs DMD
r =0.0481
P=0.7972
Log2 (DMD mRNA)
AUC
AUC vs PHOX2B
r =-0.0784
P=0.7972
Log2 (PHOX2B mRNA)
AUC
AUC vs ALK
r =-0.2201
P=0.7972
Log2 (ALK mRNA)
AUC
AUC vs SOS1
r =-0.1711
P=0.3573
Log2 (SOS1 mRNA)
AUC
AUC vs GRB2
r =0.0849
P=0.9722
Log2 (GRB2 mRNA)
AUC
AUC vs NRAS
r =0.0281
P=0.8808
Log2 (NRAS mRNA)
AUC
AUC vs KRAS
r =-0.2335
P=0.2061
Log2 (KRAS mRNA)
AUC
AUC vs HRAS
r =-0.228
P=0.2173
Log2 (HRAS mRNA)
AUC
AUC vs ETV1
r =-0.2955
P=0.1066
AUC
AUC vs ETV4
r =-0.2203
P=0.2326
Log2 (ETV4 mRNA)
AUC
AUC vs ETV5
r =-0.1333
P=0.4746
Log2 (ETV5 mRNA)
AUC
AUC vs DUSP4
r =-0.1492
P=0.4231
Log2 (DUSP4 mRNA)
AUC
AUC vs DUSP6
r =-0.0737
P=0.6938
Log2 (DUSP6mRNA)
AUC
AUC vs MAFF
r =-0.0065
P=0.9722
Log2 (MAFF mRNA)
AUC

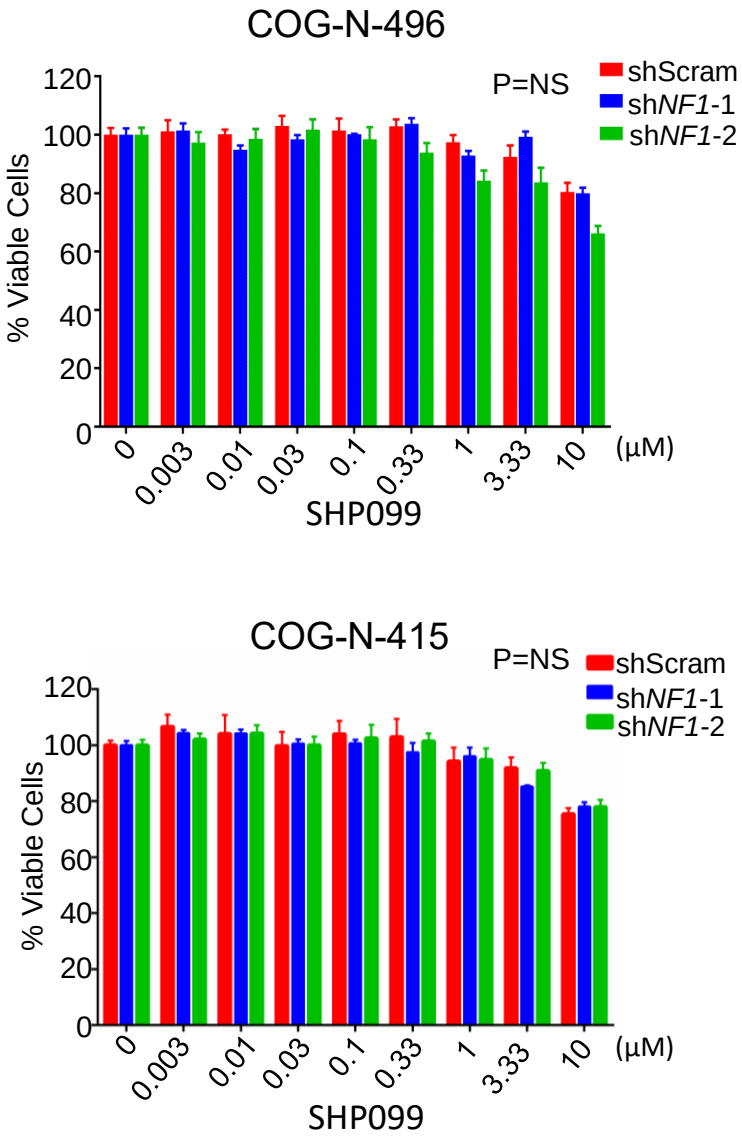
Sup Fig.9. SHP099 sensitivity is not correlated with MEK/ERK expression in NB cell lines. AUC of SHP099 compared to RNA levels of various genes associated with the MEK/ERK pathway. Data obtained from R2 Genomic (Sanger) Database (http://hgserver1.amc.nl). Related to Figure 4.

Sup Fig.10

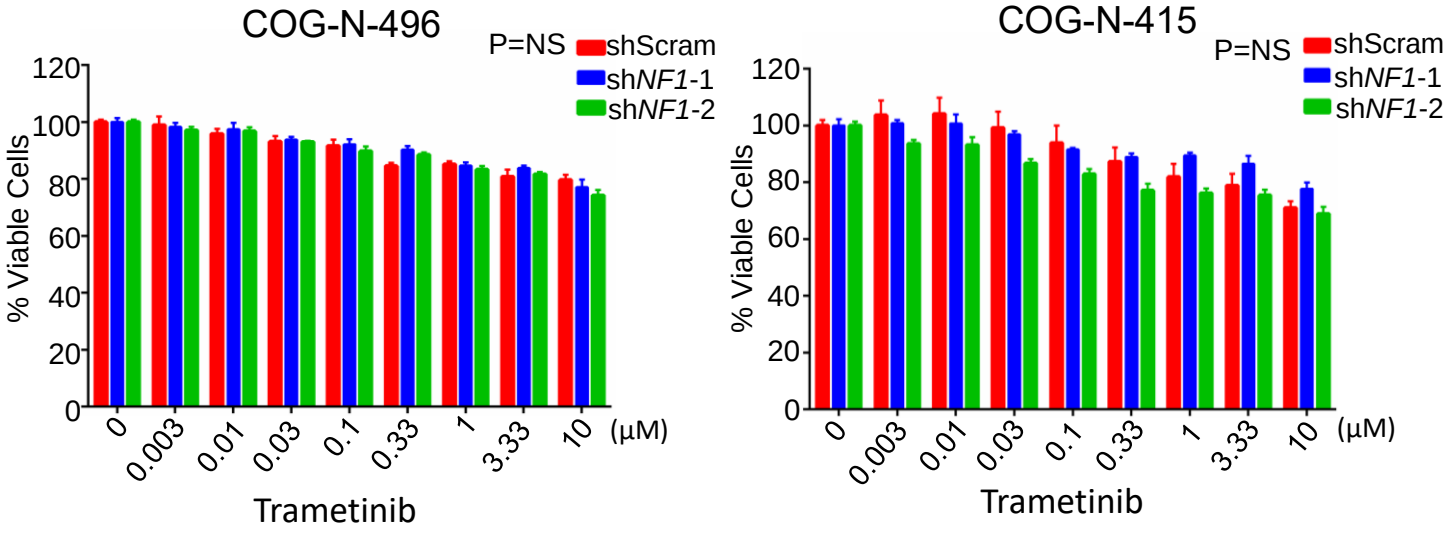
A



B

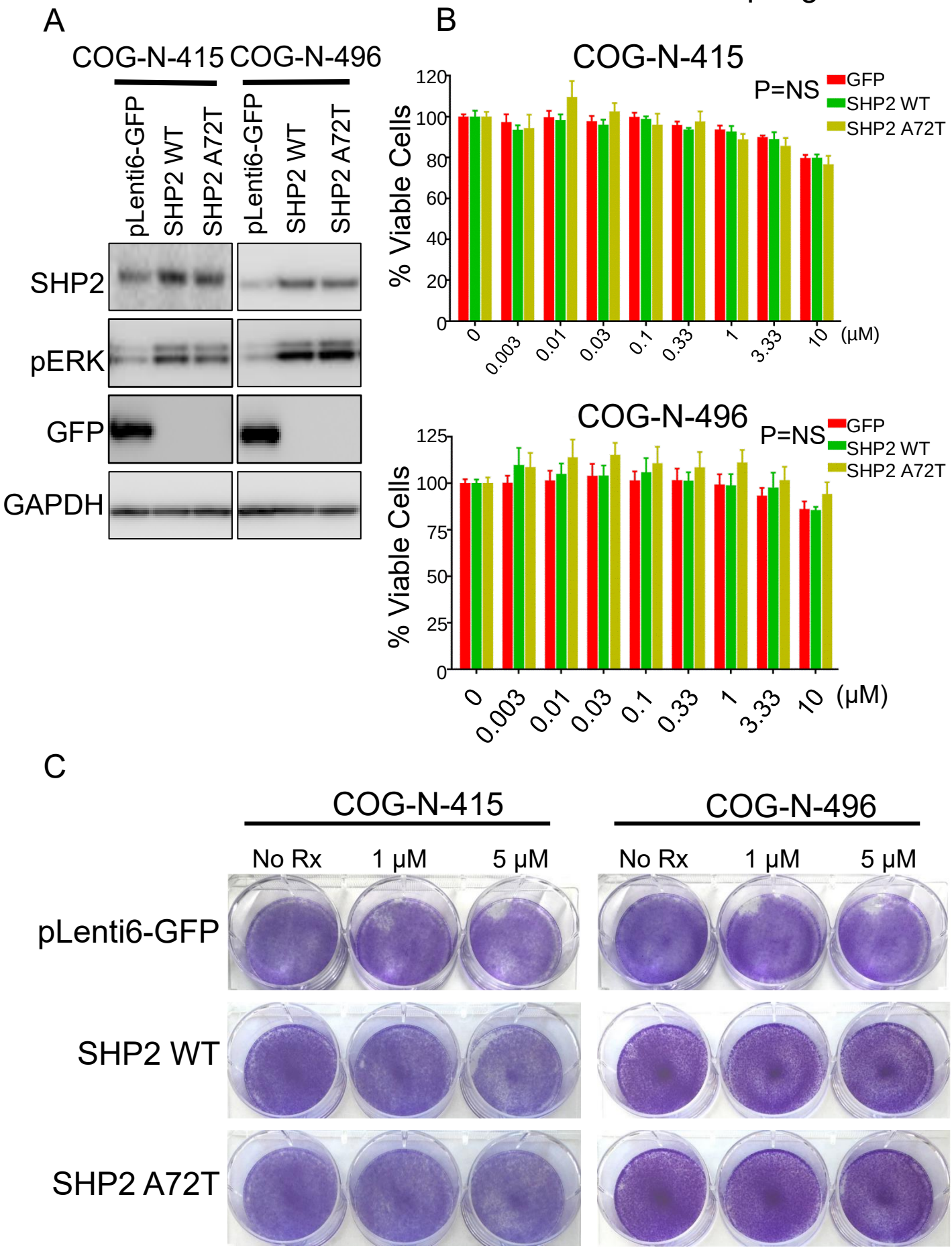


C



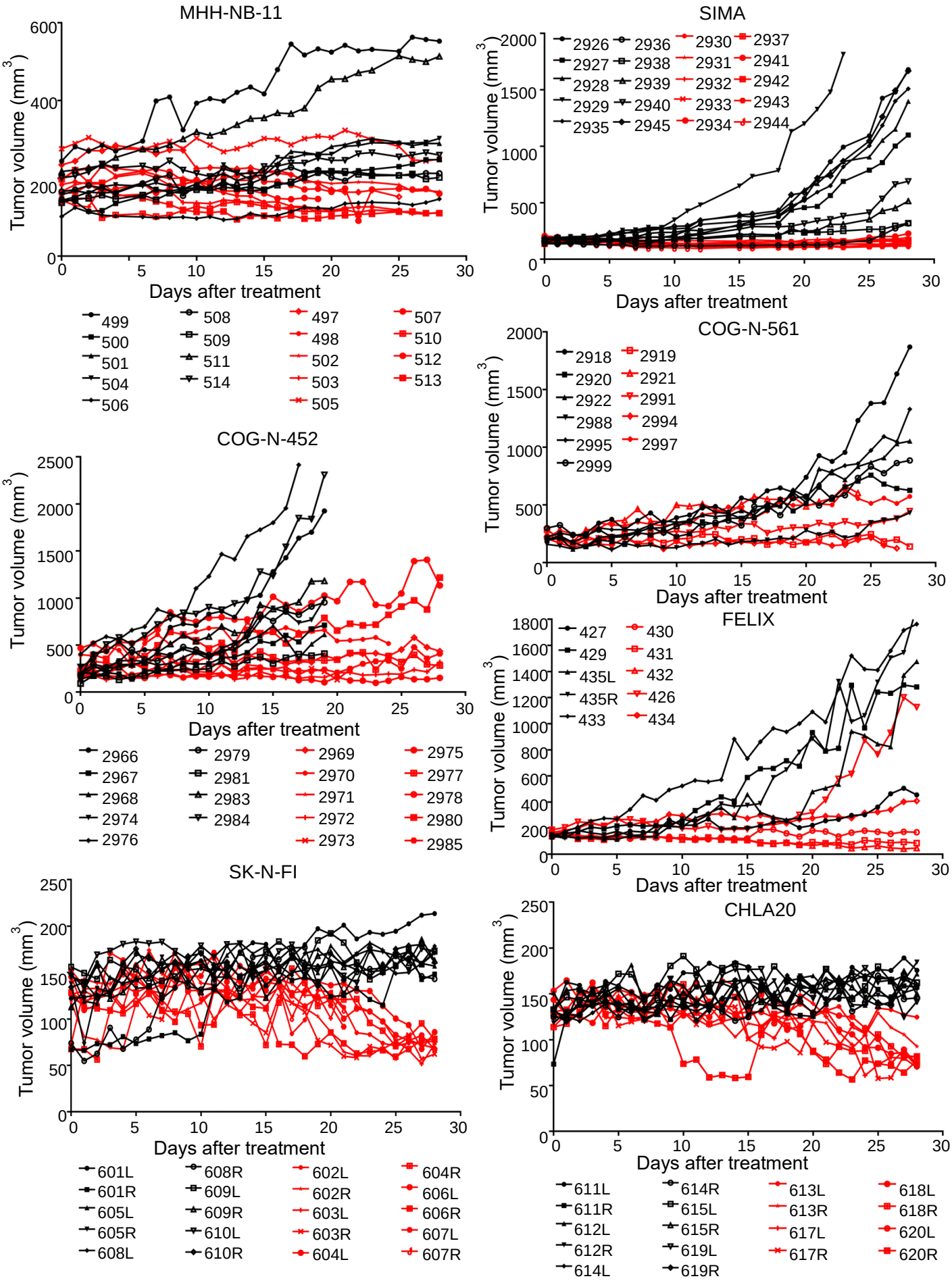
Sup Fig.10. NF1 knockdown is not sufficient to confer SHP099 sensitivity in SHP099-insensitive NB cell lines. A) Immunoblot analysis of NF1 knockdown using NF1shRNA in SHP099 insensitive lines: COG-N-496 and COG-N-415. Scrambled shRNA was used for an experimental control and GAPDH as the loading control. (B and C) Percent viable cells assessed by Cell Titer-Glo in NF1 shRNA knockdown and scrambled shRNA (control) COG-N-496 and COG-N-415 cells treated with increasing concentrations (0.003-10 μM) of SHP099 (B) and Trametinib (C) for 72 h. Error bars are ±SEM. Three individual sets of experiments were performed. Related to Figure 4.

Sup Fig.11


A
COG-N-415
COG-N-496
pLenti6-GFP
SHP2 WT
SHP2 A72T
pLenti6-GFP
SHP2 WT
SHP2 A72T
SHP2
pERK
GFP
GAPDH
B
COG-N-415
P=NS
GFP
SHP2 WT
SHP2 A72T
% Viable Cells
120
100
80
60
40
20
0
0
0.003
0.01
0.03
0.1
0.33
1
3.33
10
(μM)
COG-N-496
P=NS
GFP
SHP2 WT
SHP2 A72T
% Viable Cells
125
100
75
50
25
0
0
0.003
0.01
0.03
0.1
0.33
1
3.33
10
(μM)
C
COG-N-415
COG-N-496
No Rx
1 μM
5 μM
No Rx
1 μM
5 μM
pLenti6-GFP
SHP2 WT
SHP2 A72T

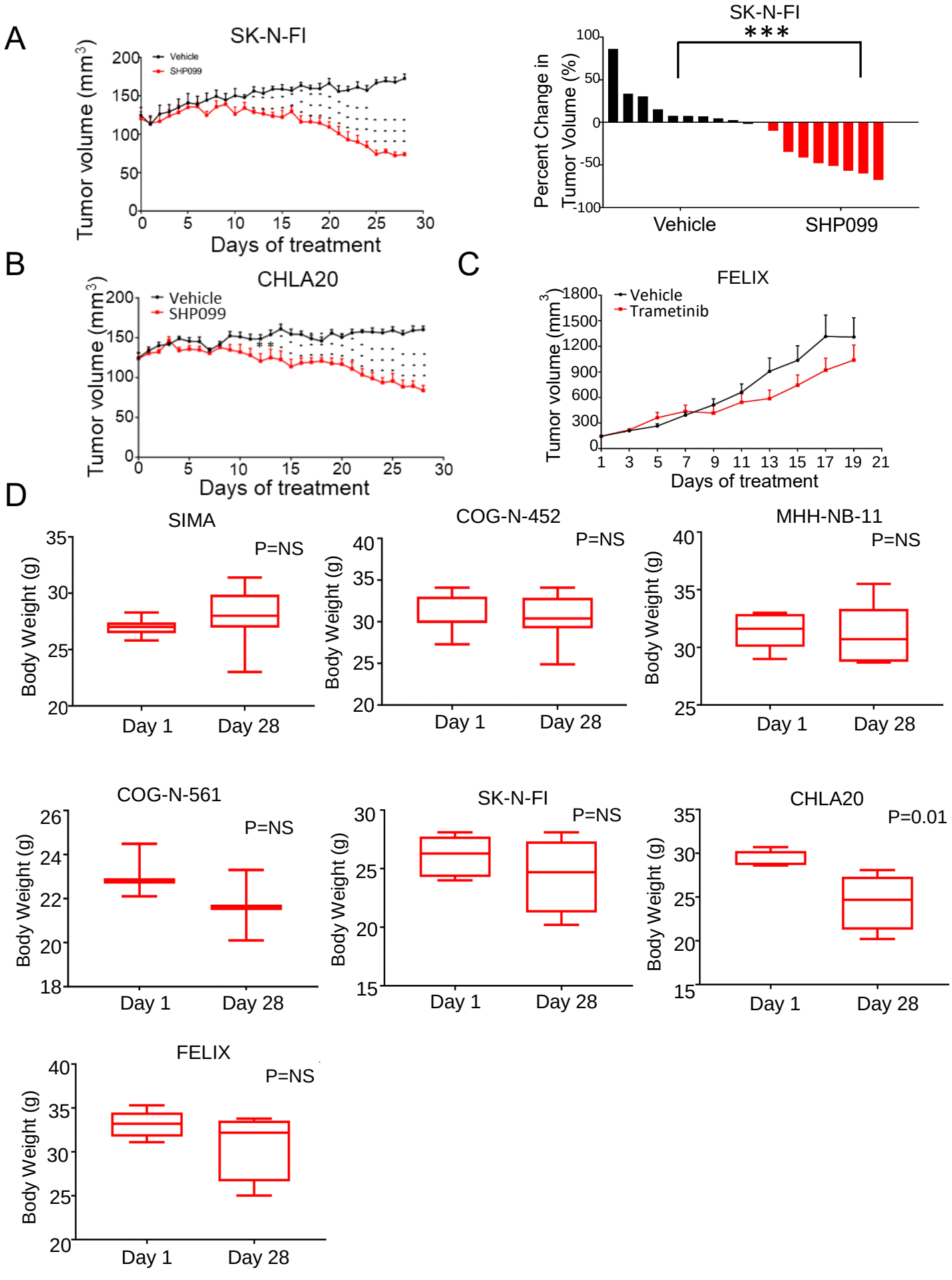
Sup Fig 11. SHP2 mutation (A72T) in NB does not affect SHP099 sensitivity. A) Immunoblot of pLentiGFP, SHP2 WT or SHP2 A72T mutant expressing CO-N-415 and COG-N-496 NB cell lines assessed for SHP2, pERK and GFP expression. GAPDH was used as loading control. B) Graph represents percent viable cells assessed by Cell Titer-Glo in the pLentiGFP, SHP2 WT or SHP2 A72T mutant expressing COG-N-415 and COG-N-496 NB cell lines following 72h treatment with increasing concentrations (0.003-10 µM) of SHP099. Error bars are ±SEM. Three individual sets of experiments were performed. C) Crystal violet assay of pLentiGFP, SHP2 WT or SHP2 A72T mutant expressing COG-N-415 and COG-N-496 NB lines treated with vehicle (No Rx) and SHP099 at 1 µM and 5 µM until the untreated cells (No Rx) reached confluency. Experiments were performed in triplicates. Related to Figure 4.

Sup Fig. 12


MHH-NB-11
Tumor volume (mm3)
Days after treatment
499 500 501 504 506 508 509 511 514 497 498 502 503 505 507 510 512 513
SIMA
Tumor volume (mm3)
Days after treatment
2926 2927 2928 2929 2935 2936 2938 2939 2940 2945 2930 2931 2932 2933 2934 2937 2941 2942 2943 2944
COG-N-561
Tumor volume (mm3)
Days after treatment
2918 2920 2922 2988 2995 2999 2919 2921 2991 2994 2997
COG-N-452
Tumor volume (mm3)
Days after treatment
2966 2967 2968 2974 2976 2979 2981 2983 2984 2969 2970 2971 2972 2973 2975 2977 2978 2980 2985
FELIX
Tumor volume (mm3)
Days after treatment
427 429 435L 435R 433 430 431 432 426 434
SK-N-FI
Tumor volume (mm3)
Days after treatment
601L 601R 605L 605R 608L 608R 609L 609R 610L 610R 602L 602R 603L 603R 604L 604R 606L 606R 607L 607R
CHLA20
Tumor volume (mm3)
Days after treatment
611L 611R 612L 612R 614L 614R 615L 615R 619L 619R 613L 613R 617L 617R 618L 618R 620L 620R

Sup Fig.12. Tumor measurements of cell line xenograft and patient derived xenograft models of NB treated with SHP099. Graph represents tumor volume of 7 individual cell line xenograft and patient derived xenograft models measured during SHP099 (75 mg/kg) treatment for 28 days. Related to Figure 6.

Sup Fig. 13


A
SK-N-FI
Tumor volume (mm3)
Vehicle
SHP099
Days of treatment
SK-N-FI
***
Percent Change in Tumor Volume (%)
Vehicle
SHP099
B
CHLA20
Tumor volume (mm3)
Vehicle
SHP099
**
Days of treatment
C
FELIX
Tumor volume (mm3)
Vehicle
Trametinib
Days of treatment
D
SIMA
P=NS
COG-N-452
P=NS
MHH-NB-11
P=NS
COG-N-561
P=NS
SK-N-FI
P=NS
CHLA20
P=0.01
FELIX
P=NS
Body Weight (g)
Day 1
Day 28

Sup Fig.13. SHP099 is effective *in vivo* and trametinib is ineffective *in vivo*. (A and B) Anti-tumor activity of SHP099 was assessed in mice bearing the indicated tumors. Mice were dosed with SHP099 (75 mg/kg) daily or vehicle (control) for 28 days. Waterfall plot represents change in tumor volume percent of each tumor to their initial tumor size (right panel) in the control and treated group. Error bars are ±SEM. Student t-test was used to calculate significance (*$p<0.05$, **$p<0.01$, ***$p<0.001$). C) FELIX PDX model treated with 1 mg/kg/qd trametinib. D) SHP099 treatment does not induce toxicity in mice. Body weight of SHP099 (75 mg/kg) treated *MYCN*-amplified or wild type xenograft and PDX mouse models of NB measured at day1 and day28 of treatment, NS= Non-significant by student's t test. Related to Figure 6.