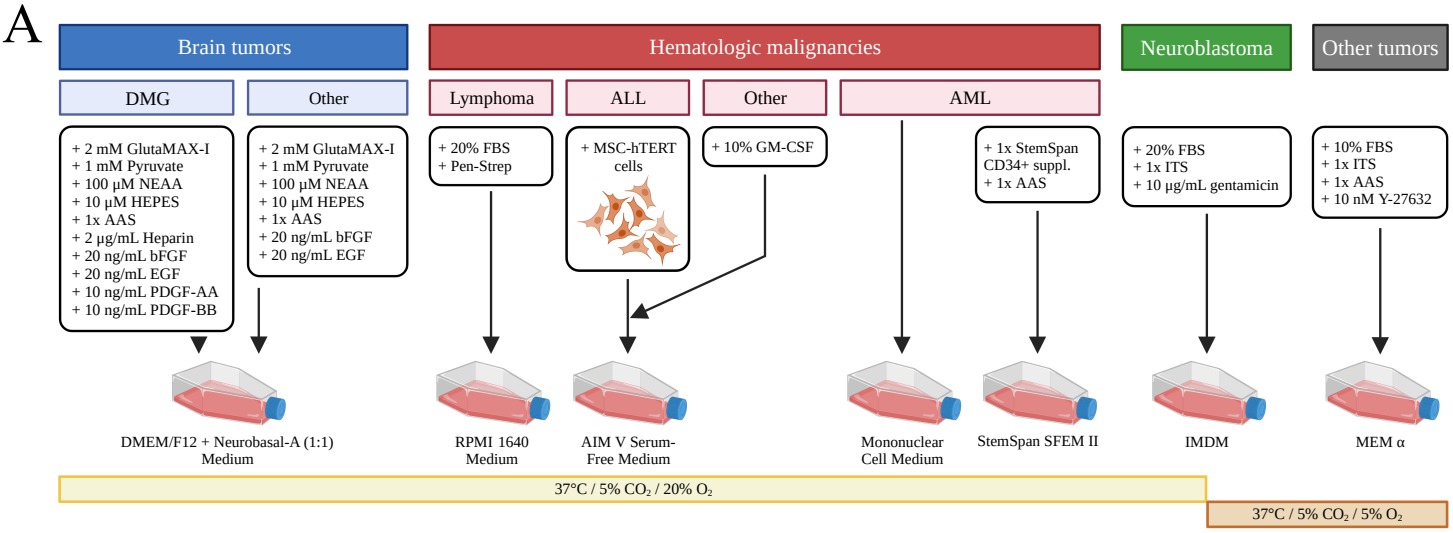
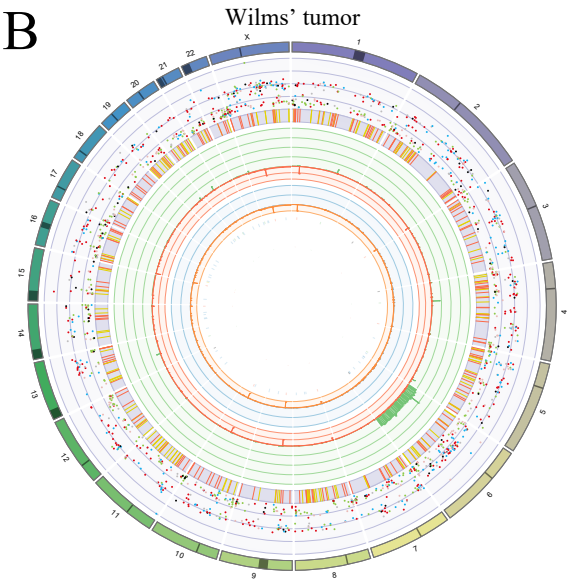
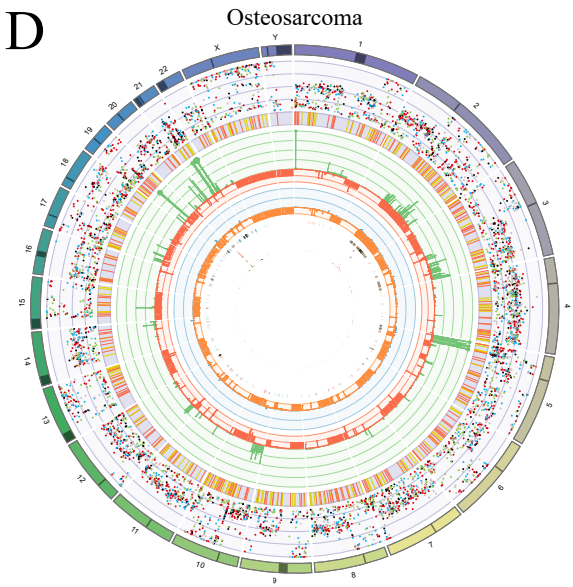
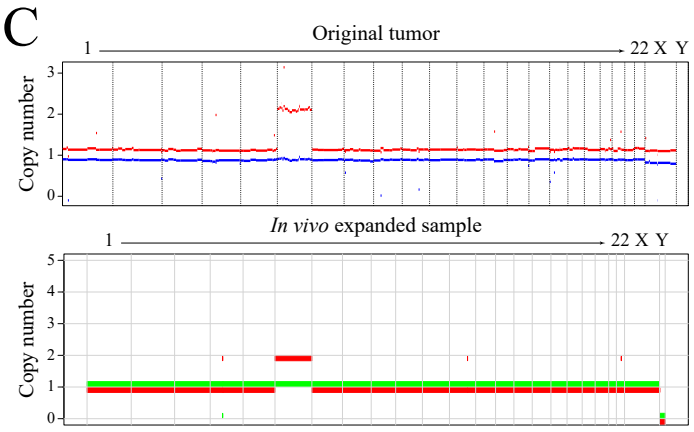
Supplementary Figure S1.

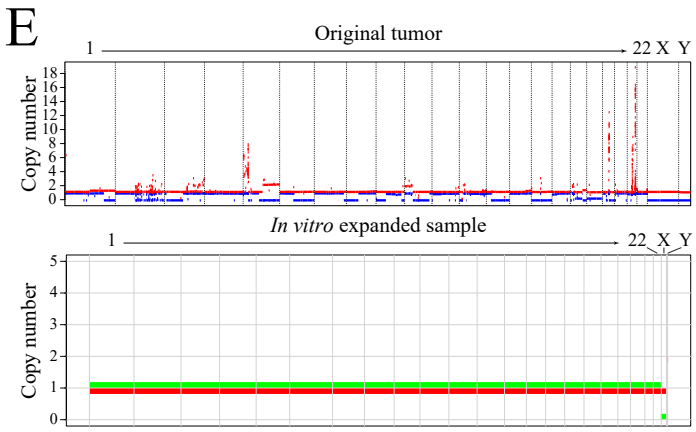
Validation and utilization of patient-derived tumor samples for *in vitro* HTS. **A,** Culture conditions for solid and hematologic tumor samples from ZERO patients. NEAA = non-essential amino acid; AAS = antibiotic antimycotic solution; bFGF = basic fibroblast growth factor; EGF = epidermal growth factor; PDGF = platelet-derived growth factor; FBS = fetal bovine serum; Pen-Strep = penicillin-streptomycin; MSC-hTERT cells = human telomerase reverse transcriptase-immortalized bone marrow mesenchymal stem cells; GM-CSF = granulocyte-macrophage colony-stimulating factor; ITS = insulin-transferrin-selenium; DMEM/F12 = Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12; RPMI 1640 = Roswell Park Memorial Institute 1640; StemSpan SFEM II = StemSpan Serum-Free Expansion Medium II; IMDM = Iscove Modified Dulbecco Medium; MEM α = Minimum Essential Medium α. (Created with BioRender.com). Examples of a successful validation of an *in vivo* expanded Wilms' tumor (**B** and **C**) and unsuccessful validation of an *in vitro* expanded osteosarcoma (**D** and **E**). **B,** Circos plot showing the genome-wide profile of the original Wilms' tumor detected by WGS. Inner areas show the structural rearrangements and inner to outer circles show the minor allele ploidy, copy number changes (red deletions, green amplifications), regions of loss of heterozygosity, and mutations. **C,** Copy number profiles of the original Wilms' tumor (detected by WGS; top) versus the matching *in vivo* expanded sample generated for *in vitro* HTS (detected by SNP array profiling; bottom). The copy number profile of the original Wilms' tumor was retained upon *in vivo* expansion. **D,** Circos plot showing the genome-wide profile of the original osteosarcoma detected by WGS. Inner areas show the structural rearrangements and inner to outer circles show the minor allele ploidy, copy number changes (red deletions, green amplifications), regions of loss of heterozygosity, and mutations. **E,** Copy number profiles of the original osteosarcoma (detected by WGS; top) versus the matching *in vitro* expanded sample generated for *in vitro* HTS (detected by SNP array profiling; bottom). The copy number variations present in the original osteosarcoma disappeared upon *in vitro* expansion due to the outgrowth of non-neoplastic cells. HTS of this sample was therefore considered failed. **F,** Time in days required to expand the patient-derived tumor cells for HTS, highlighted by cancer type.

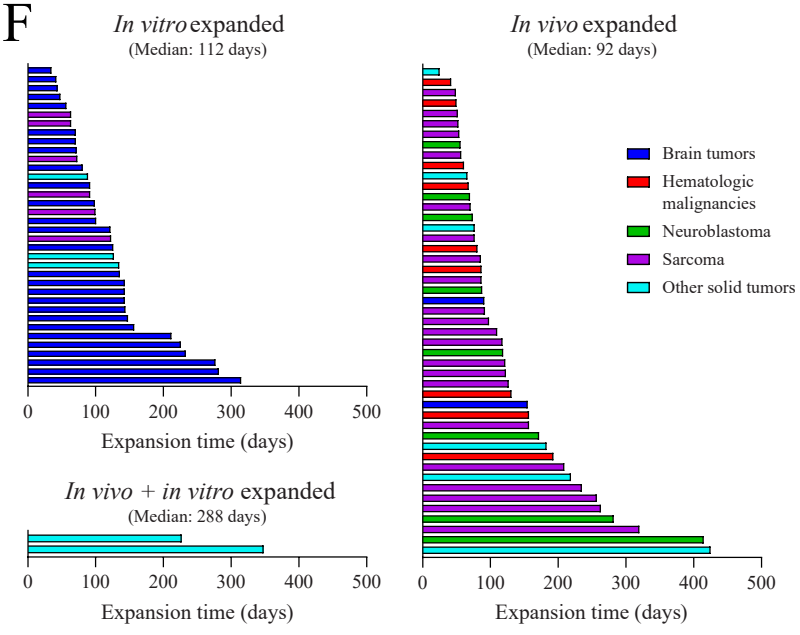

A
Brain tumors
Hematologic malignancies
Neuroblastoma
Other tumors
DMG
Other
Lymphoma
ALL
Other
AML
+ 2 mM GlutaMAX-I
+ 1 mM Pyruvate
+ 100 µM NEAA
+ 10 µM HEPES
+ 1x AAS
+ 2 µg/mL Heparin
+ 20 ng/mL bFGF
+ 20 ng/mL EGF
+ 10 ng/mL PDGF-AA
+ 10 ng/mL PDGF-BB
+ 2 mM GlutaMAX-I
+ 1 mM Pyruvate
+ 100 µM NEAA
+ 10 µM HEPES
+ 1x AAS
+ 20 ng/mL bFGF
+ 20 ng/mL EGF
+ 20% FBS
+ Pen-Strep
+ MSC-hTERT cells
+ 10% GM-CSF
+ 1x StemSpan CD34+ suppl.
+ 1x AAS
+ 20% FBS
+ 1x ITS
+ 10 µg/mL gentamicin
+ 10% FBS
+ 1x ITS
+ 1x AAS
+ 10 nM Y-27632
DMEM/F12 + Neurobasal-A (1:1) Medium
RPMI 1640 Medium
AIM V Serum-Free Medium
Mononuclear Cell Medium
StemSpan SFEM II
IMDM
MEM α
37°C / 5% CO2 / 20% O2
37°C / 5% CO2 / 5% O2


B
Wilms' tumor


D
Osteosarcoma

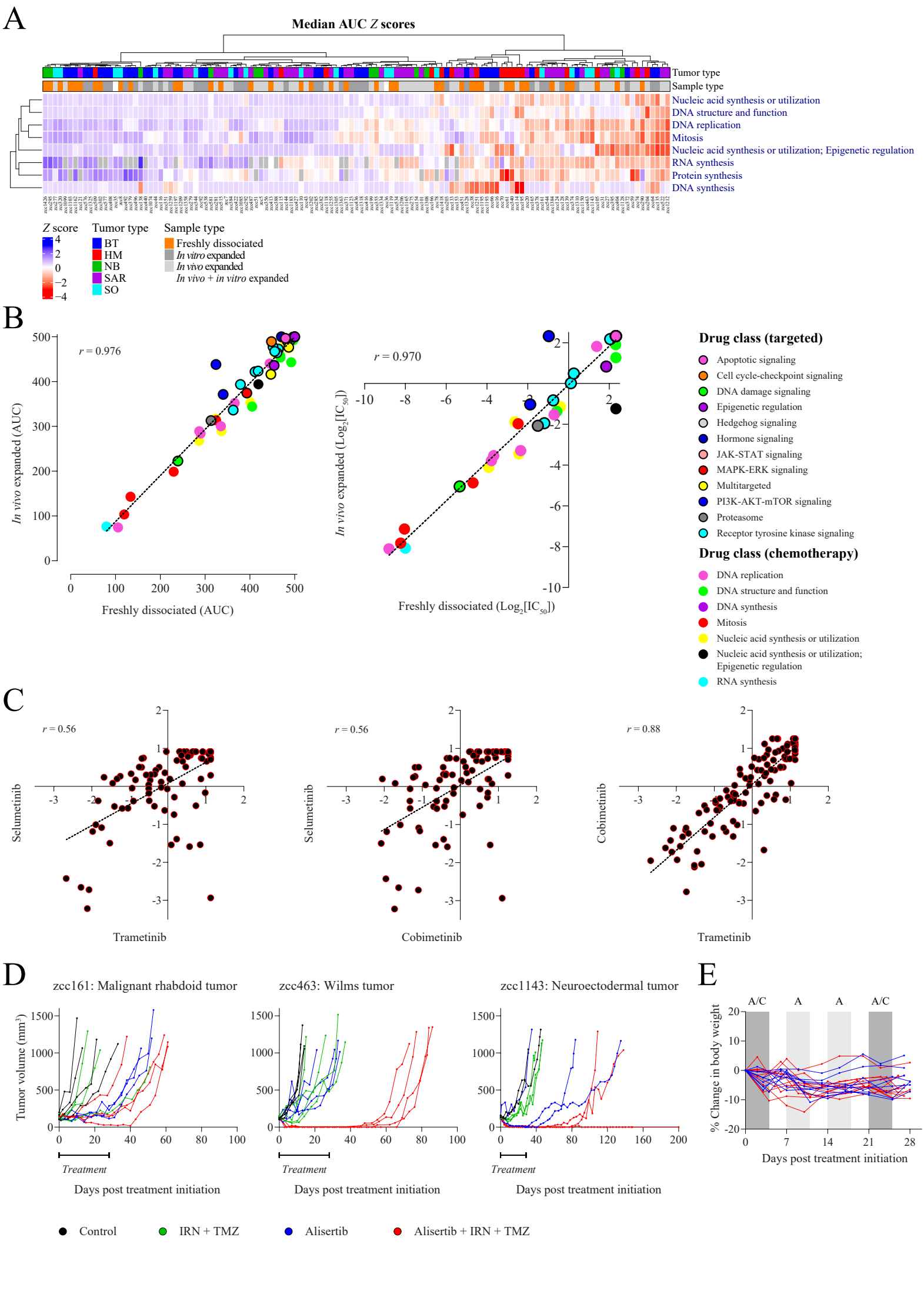

C
Original tumor
1
22 X Y
Copy number
In vivo expanded sample


E
Original tumor
1
22 X Y
Copy number
In vitro expanded sample


F
In vitro expanded
(Median: 112 days)
In vivo expanded
(Median: 92 days)
In vivo + in vitro expanded
(Median: 288 days)
Expansion time (days)
Brain tumors
Hematologic malignancies
Neuroblastoma
Sarcoma
Other solid tumors

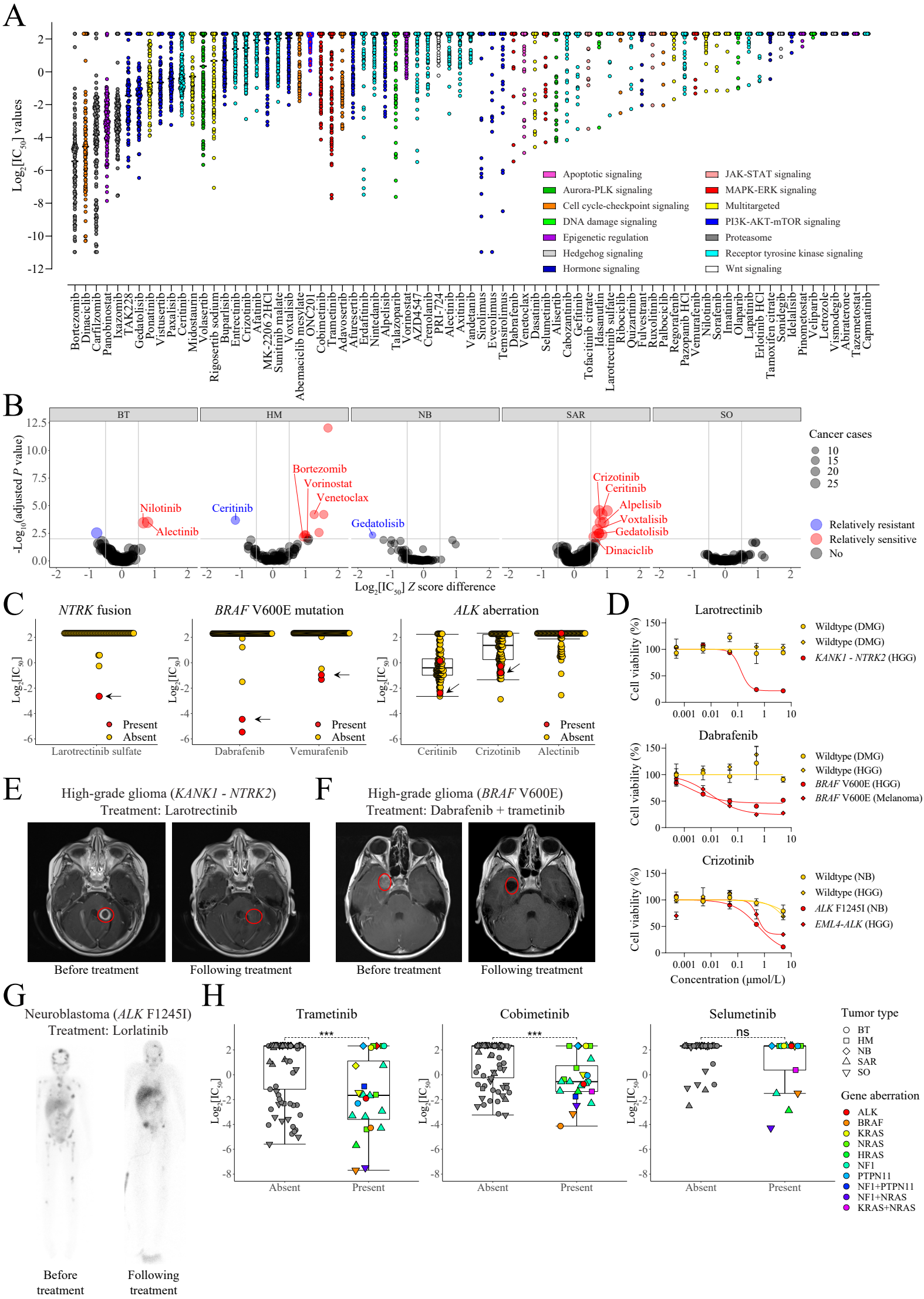
Supplementary Figure S2.

Drug response profiles are consistent across freshly dissociated and expanded samples and drug-drug correlations identify effective combination therapies. **A,** Heatmap of the chemotherapeutic drug response profiles across the 125-sample cohort. Drug response profiles were established by calculating the median AUC *Z* scores for chemotherapeutic drugs grouped according to the same MOA. Median AUC *Z* scores are represented by a color scale from blue (resistant) to red (sensitive). MOAs and tumor samples are ordered by unsupervised hierarchal clustering. Top annotations indicate tumor type and type of sample. **B,** Correlation analysis of drug AUC (left) and $\log_2[IC_{50}]$ (right) values comparing freshly dissociated cells and *in vivo* expanded cells derived from the same rhabdomyosarcoma tumor. Each dot represents a drug and colors indicate their MOA. **C,** Pearson's correlation between MEK inhibitor AUC *Z* scores across the sample cohort: trametinib versus selumetinib (left), cobimetinib versus selumetinib (middle) and trametinib versus cobimetinib (right). **D,** *In vivo* effects of vehicle control (black), irinotecan plus temozolomide (IRN + TMZ; green), alisertib monotherapy (blue) and alisertib in combination with irinotecan plus temozolomide (red) on tumor volume in patient-derived xenograft (PDX) models for a malignant rhabdoid tumor (MRT; left), Wilms' tumor (WT; middle), and neuroectodermal tumor (NET; right). Effects on tumor volume were established during and after a 28-day treatment period. Each line represents an individual mouse. **E,** Percentages change in body weight for each individual mouse treated with alisertib monotherapy (= A, blue) or alisertib in combination with irinotecan plus temozolomide (= C, red). Grey areas indicate treatment schedules for A and C. Tumor type key: brain tumors (BT), hematologic malignancies (HM), neuroblastoma (NB), sarcoma tumors (SAR), solid other (SO).


A
Median AUC Z scores
Tumor type
Sample type
Nucleic acid synthesis or utilization
DNA structure and function
DNA replication
Mitosis
Nucleic acid synthesis or utilization; Epigenetic regulation
RNA synthesis
Protein synthesis
DNA synthesis
Z score
4
2
0
−2
−4
Tumor type
BT
HM
NB
SAR
SO
Sample type
Freshly dissociated
In vitro expanded
In vivo expanded
In vivo + in vitro expanded
B
r = 0.976
In vivo expanded (AUC)
Freshly dissociated (AUC)
r = 0.970
In vivo expanded (Log2[IC50])
Freshly dissociated (Log2[IC50])
Drug class (targeted)
Apoptotic signaling
Cell cycle-checkpoint signaling
DNA damage signaling
Epigenetic regulation
Hedgehog signaling
Hormone signaling
JAK-STAT signaling
MAPK-ERK signaling
Multitargeted
PI3K-AKT-mTOR signaling
Proteasome
Receptor tyrosine kinase signaling
Drug class (chemotherapy)
DNA replication
DNA structure and function
DNA synthesis
Mitosis
Nucleic acid synthesis or utilization
Nucleic acid synthesis or utilization; Epigenetic regulation
RNA synthesis
C
r = 0.56
Selumetinib
Trametinib
r = 0.56
Selumetinib
Cobimetinib
r = 0.88
Cobimetinib
Trametinib
D
zcc161: Malignant rhabdoid tumor
zcc463: Wilms tumor
zcc1143: Neuroectodermal tumor
Tumor volume (mm3)
Treatment
Days post treatment initiation
Control
IRN + TMZ
Alisertib
Alisertib + IRN + TMZ
E
A/C
A
A
A/C
% Change in body weight
Days post treatment initiation

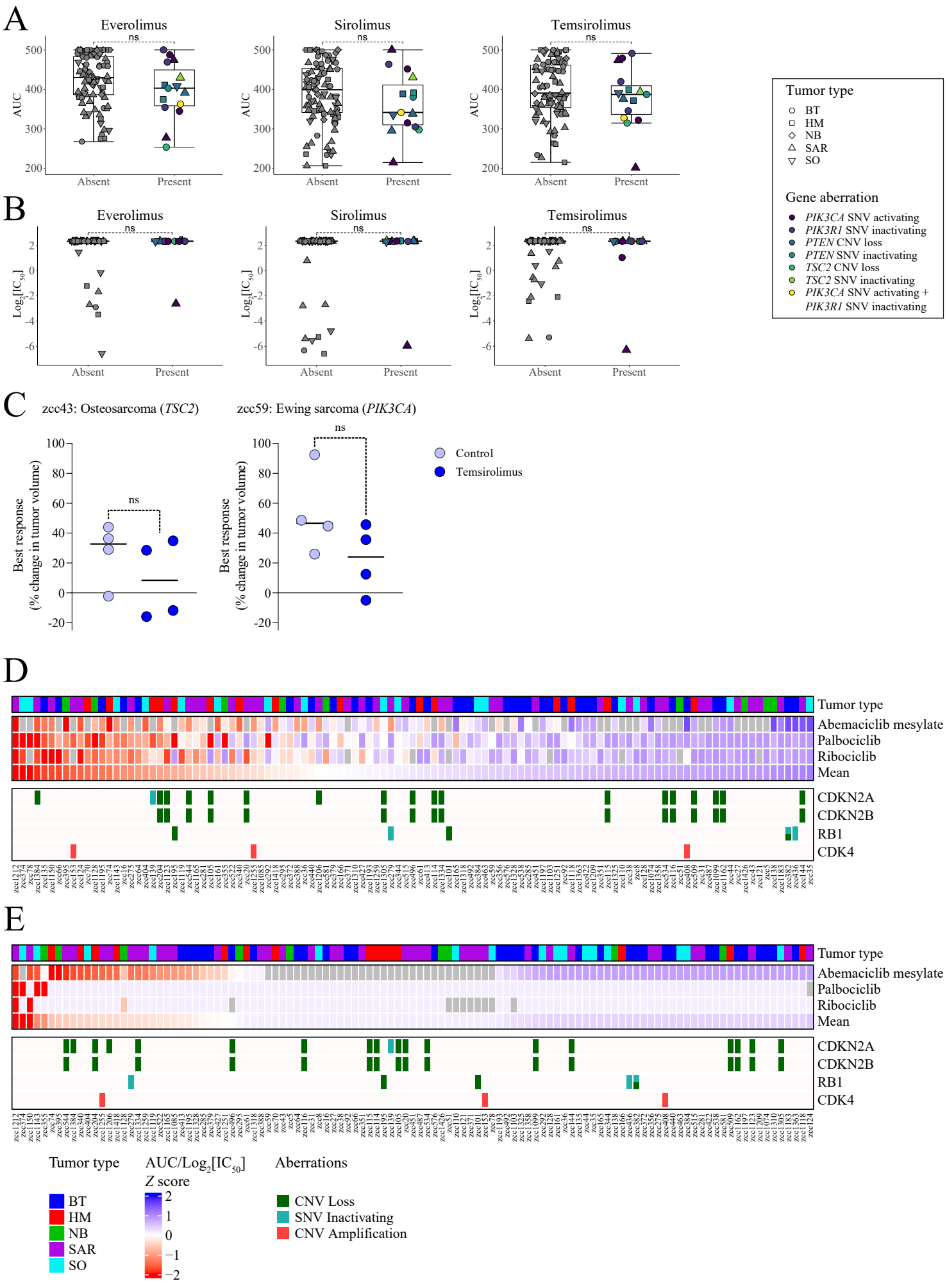
Supplementary Figure S3.

In vitro HTS on patient-derived tumor samples confirms known associations between drug sensitivity and driver aberrations. **A,** $Log_2[IC_{50}]$ distribution across all samples for targeted drugs. Drugs are ordered from most (left) to least (right) effective based on lowest median $log_2[IC_{50}]$ followed by lowest quartile $log_2[IC_{50}]$ and lowest detected $log_2[IC_{50}]$, respectively. Colors indicate drug MOAs. **B,** Volcano plots of tumor type-specific sensitivity and resistance to drugs based on the $log_2[IC_{50}]$ *Z* scores for each drug, using the following formula: [average $log_2[IC_{50}]$ *Z* score for tumor type of interest] - [average $log_2[IC_{50}]$ *Z* score for the remaining cohort]. A difference in average $log_2[IC_{50}]$ *Z* score < -0.5 and adjusted *P* value < 0.01 is considered relatively resistant (blue) and a difference in average $log_2[IC_{50}]$ *Z* score > 0.5 and adjusted *P* value < 0.01 is considered relatively sensitive (red). Only significant targeted drugs are named in the figure. Circle size represents the number of samples within the given tumor type on which the indicated drug has been tested. **C,** $Log_2[IC_{50}]$ values for TRK inhibitor Larotrectinib sulfate, BRAF inhibitors dabrafenib and vemurafenib, and ALK inhibitors ceritinib, crizotinib, and alectinib with samples harboring an *NTRK* fusion, *BRAF* V600E mutation, or *ALK* aberration (i.e., *ALK* F1245I mutation or *EML4-ALK* fusion) highlighted in red, respectively. Arrows indicate the *NTRK* fusion-positive HGG, *BRAF* V600E-mutated HGG and *ALK* F1245I-mutated NB sample with available clinical data shown in E, F, and G, respectively. **D,** Cell viability curves of larotrectinib, dabrafenib, and crizotinib for two resistant wildtype samples versus samples harboring genomic alterations in *NTRK*, *BRAF*, and *ALK*, respectively. **E,** Post-contrast T1-weighted MRI scans demonstrating the *NTRK* fusion-positive high-grade glioma prior to treatment (left) and a complete remission following treatment (right) with larotrectinib. **F,** Post-contrast T1-weighted MRI scans demonstrating the *BRAF* V600E-mutated high-grade glioma prior to treatment (left) and a complete remission following treatment (right) with dabrafenib plus trametinib. **G,** I-123 MIBG scans demonstrating widespread metastatic bone disease in the *ALK* F1245I-mutated neuroblastoma patient prior to treatment (left) and a partial response following treatment with the ALK inhibitor lorlatinib (right). **H,** $Log_2[IC_{50}]$ values of MEK inhibitors trametinib (left), cobimetinib (middle), and selumetinib (right) in samples with (colored) versus without (grey) bona fide aberrations in RAS-MAPK signaling. Shapes of the symbols indicate tumor type. Tumor type key: brain tumors (BT), hematologic malignancies (HM), neuroblastoma (NB), sarcoma tumors (SAR), solid other (SO). ***$P < 0.001$, ns=not significant.


A
Log2[IC50] values
Apoptotic signaling
Aurora-PLK signaling
Cell cycle-checkpoint signaling
DNA damage signaling
Epigenetic regulation
Hedgehog signaling
Hormone signaling
JAK-STAT signaling
MAPK-ERK signaling
Multitargeted
PI3K-AKT-mTOR signaling
Proteasome
Receptor tyrosine kinase signaling
Wnt signaling
Bortezomib
Dinaciclib
Carfilzomib
Panobinostat
Ixazomib
TAK228
Gedatolisib
Ponatinib
Vistusertib
Paxalisib
Ceritinib
Midostaurin
Volasertib
Rigosertib sodium
Buparlisib
Entrectinib
Crizotinib
Afatinib
MK-2206 2HCl
Sunitinib malate
Voxtalisib
Abemaciclib mesylate
ONC201
Cobimetinib
Trametinib
Adavosertib
Alfuresertib
Erdafitinib
Nintedanib
Alpelisib
Talazoparib
Vorinostat
AZD4547
Crenolanib
PRI-724
Alectinib
Axitinib
Vandetanib
Sirolimus
Everolimus
Temsirolimus
Dabrafenib
Venetoclax
Dasatinib
Selumetinib
Alisertib
Cabozantinib
Gefitinib
Tofacitinib citrate
Idasanutlin
Larotrectinib sulfate
Ribociclib
Quizartinib
Fulvestrant
Ruxolitinib
Palbociclib
Regorafenib
Pazopanib HCl
Vemurafenib
Nilotinib
Sorafenib
Imatinib
Olaparib
Lapatinib
Erlotinib HCl
Tamoxifen citrate
Sonidegib
Idelalisib
Pinometostat
Veliparib
Letrozole
Vismodegib
Abiraterone
Tazemetostat
Capmatinib
B
BT
HM
NB
SAR
SO
-Log10(adjusted P value)
Log2[IC50] Z score difference
Nilotinib
Alectinib
Ceritinib
Bortezomib
Vorinostat
Venetoclax
Gedatolisib
Crizotinib
Ceritinib
Alpelisib
Voxtalisib
Gedatolisib
Dinaciclib
Cancer cases
10
15
20
25
Relatively resistant
Relatively sensitive
No
C
NTRK fusion
BRAF V600E mutation
ALK aberration
Log2[IC50]
Present
Absent
Larotrectinib sulfate
Dabrafenib
Vemurafenib
Ceritinib
Crizotinib
Alectinib
D
Larotrectinib
Dabrafenib
Crizotinib
Cell viability (%)
Concentration (μmol/L)
Wildtype (DMG)
KANK1 - NTRK2 (HGG)
Wildtype (HGG)
BRAF V600E (HGG)
BRAF V600E (Melanoma)
Wildtype (NB)
ALK F1245I (NB)
EML4-ALK (HGG)
E
High-grade glioma (KANK1 - NTRK2)
Treatment: Larotrectinib
Before treatment
Following treatment
F
High-grade glioma (BRAF V600E)
Treatment: Dabrafenib + trametinib
Before treatment
Following treatment
G
Neuroblastoma (ALK F1245I)
Treatment: Lorlatinib
Before treatment
Following treatment
H
Trametinib
Cobimetinib
Selumetinib
***
***
ns
Log2[IC50]
Absent
Present
Tumor type
BT
HM
NB
SAR
SO
Gene aberration
ALK
BRAF
KRAS
NRAS
HRAS
NF1
PTPN11
NF1+PTPN11
NF1+NRAS
KRAS+NRAS

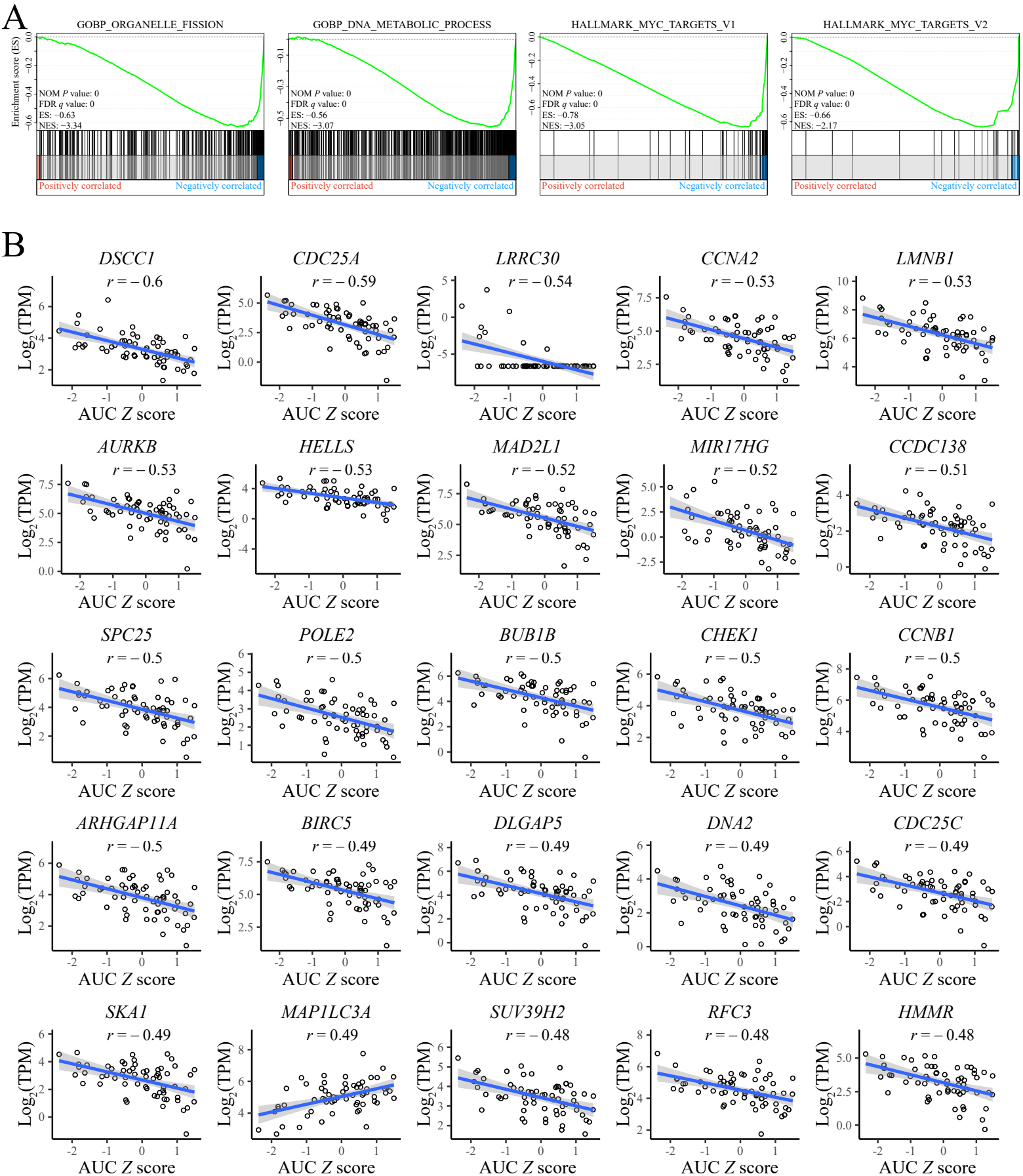
Supplementary Figure S4.

Sensitivity to mTORC1 and CDK4/6 inhibitors is not predicted by clinically applied predictive biomarkers. **A,** AUC and **B,** $\log_2[IC_{50}]$ values of mTORC1 inhibitors everolimus (left), sirolimus (middle) and temsirolimus (right) in samples without (grey) versus with (colored) genomic alterations in PI3K-AKT-mTOR signaling. Shapes of the symbols indicate tumor type. **C,** *In vivo* best responses to temsirolimus for an osteosarcoma PDX model harboring a *TSC2* mutation (left) and an Ewing sarcoma PDX model harboring a *PIK3CA* mutation (right) demonstrating average and high *in vitro* sensitivity to temsirolimus, respectively. Heatmaps of **D,** AUC and **E,** $\log_2[IC_{50}]$ *Z* scores for CDK4/6 inhibitors abemaciclib mesylate, palbociclib, and ribociclib for the 111-sample cohort with matching drug efficacy and WGS data. AUC and $\log_2[IC_{50}]$ *Z* scores are represented by a color scale from red (low *Z* score; sensitive) to blue (high *Z* score; resistance). Tumor samples are ordered by mean AUC and $\log_2[IC_{50}]$ *Z* score values, respectively, with lowest values (most sensitive) shown on the left and highest values (most resistant) shown on the right. Top annotation indicates the tumor type. Loss-of-function events in *CDKN2A*, *CDKN2B*, and *RB1* and gain-of-function events in *CDK4* are shown at the bottom. Tumor type key: brain tumors (BT), hematologic malignancies (HM), neuroblastoma (NB), sarcoma tumors (SAR), solid other (SO). ns=not significant.


A
Everolimus
Sirolimus
Temsirolimus
ns
AUC
Absent
Present
B
Log2[IC50]
C
zcc43: Osteosarcoma (TSC2)
zcc59: Ewing sarcoma (PIK3CA)
Best response
(% change in tumor volume)
Control
Temsirolimus
D
E
Tumor type
Abemaciclib mesylate
Palbociclib
Ribociclib
Mean
CDKN2A
CDKN2B
RB1
CDK4
Tumor type
BT
HM
NB
SAR
SO
AUC/Log2[IC50]
Z score
Aberrations
CNV Loss
SNV Inactivating
CNV Amplification
Gene aberration
PIK3CA SNV activating
PIK3R1 SNV inactivating
PTEN CNV loss
PTEN SNV inactivating
TSC2 CNV loss
TSC2 SNV inactivating
PIK3CA SNV activating +
PIK3R1 SNV inactivating

Supplementary Figure S5.

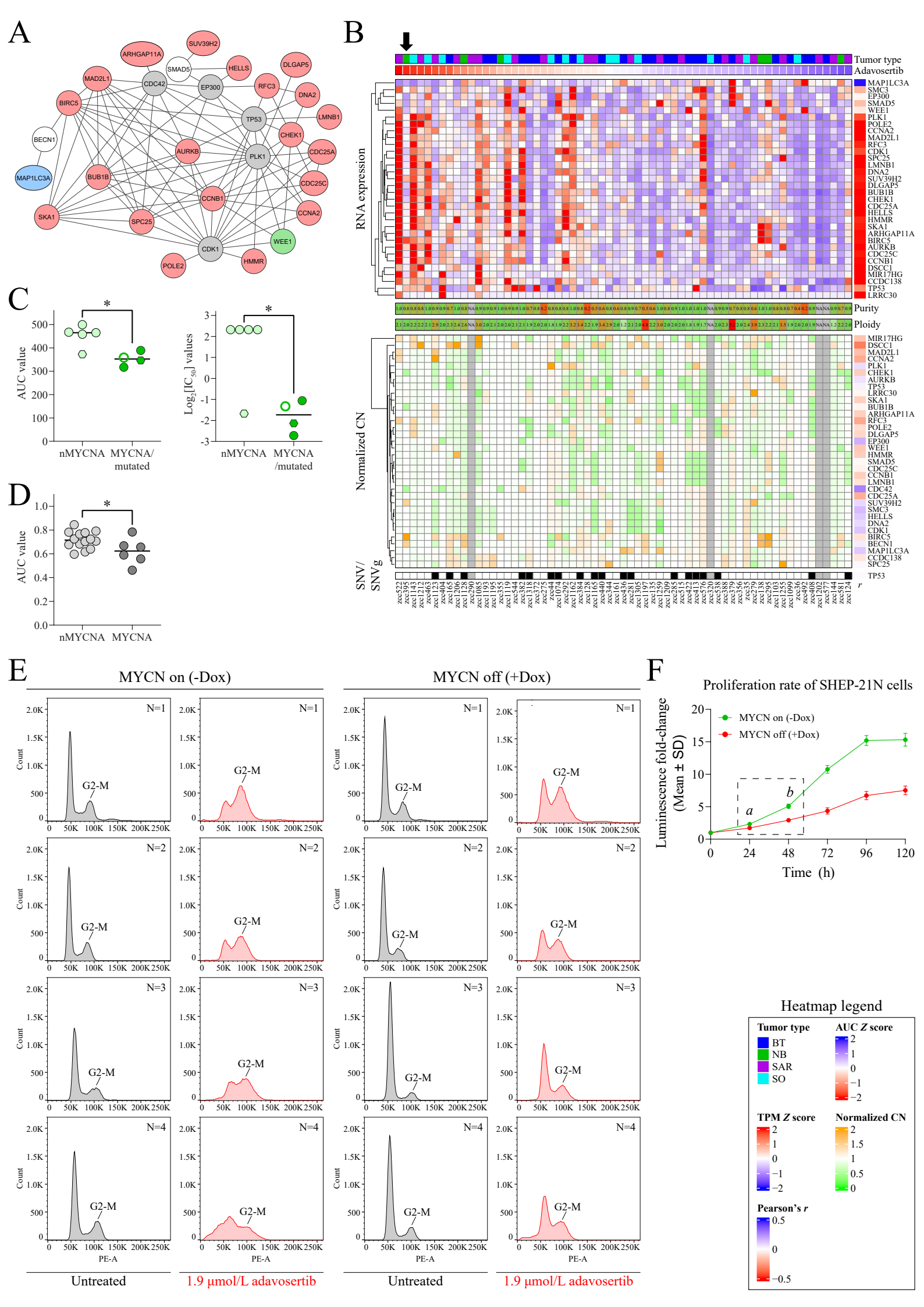
Integrative analysis links adavosertib efficacy to aberrant cell cycle phase transition and DNA replication. **A,** GSEA enrichment plots for top enriched GO biological processes and hallmark gene sets for the positively correlating genes with adavosertib efficacy. Gene sets enriched among the genes for which $\log_2$[TPM] values negatively correlated with adavosertib AUC *Z* scores (high expression correlates with increased efficacy) with FDR *q* value < 0.01, list > 10%, and NES ≤ -2 were selected as most relevant. Genes with $|r| \geq 0.3$ are highlighted in the barcode regions. **B,** Correlation between gene $\log_2$[TPM] values and adavosertib AUC *Z* scores for the 25 top correlating genes to adavosertib response. Blue lines are the regression lines of best fit and the grey shadings represent the 95% confidence intervals.


A
GOBP_ORGANELLE_FISSION
NOM P value: 0
FDR q value: 0
ES: −0.63
NES: −3.34
GOBP_DNA_METABOLIC_PROCESS
NOM P value: 0
FDR q value: 0
ES: −0.56
NES: −3.07
HALLMARK_MYC_TARGETS_V1
NOM P value: 0
FDR q value: 0
ES: −0.78
NES: −3.05
HALLMARK_MYC_TARGETS_V2
NOM P value: 0
FDR q value: 0
ES: −0.66
NES: −2.17
Enrichment score (ES)
Positively correlated
Negatively correlated
B
DSCC1 r = −0.6
CDC25A r = −0.59
LRRC30 r = −0.54
CCNA2 r = −0.53
LMNB1 r = −0.53
AURKB r = −0.53
HELLS r = −0.53
MAD2L1 r = −0.52
MIR17HG r = −0.52
CCDC138 r = −0.51
SPC25 r = −0.5
POLE2 r = −0.5
BUB1B r = −0.5
CHEK1 r = −0.5
CCNB1 r = −0.5
ARHGAP11A r = −0.5
BIRC5 r = −0.49
DLGAP5 r = −0.49
DNA2 r = −0.49
CDC25C r = −0.49
SKA1 r = −0.49
MAP1LC3A r = 0.49
SUV39H2 r = −0.48
RFC3 r = −0.48
HMMR r = −0.48
Log2(TPM)
AUC Z score

Supplementary Figure S6.

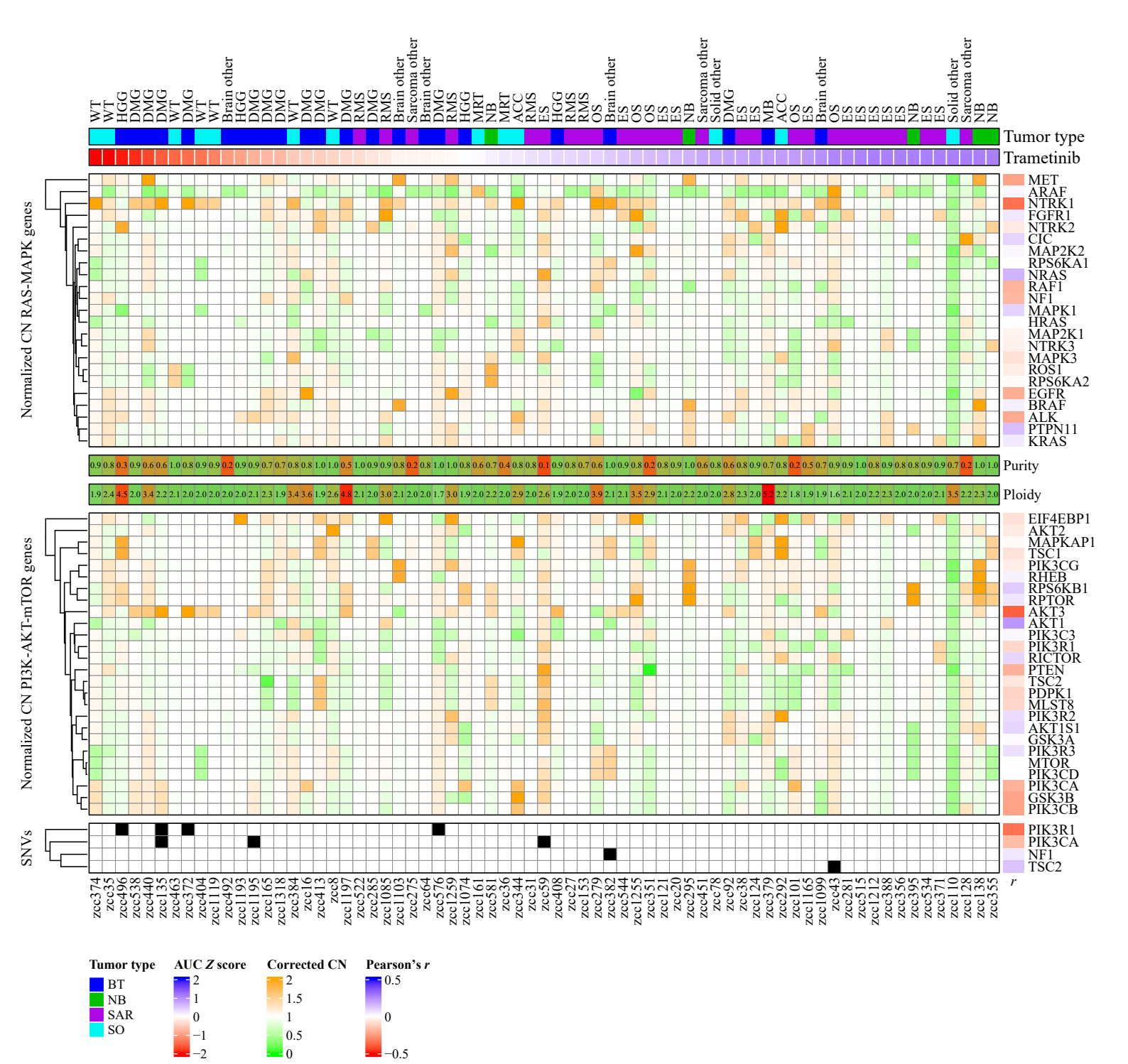
Neuroblastoma sensitivity to adavosertib is linked to *MYCN* status. **A,** Reactome functional interaction (FI) network of the adavosertib target gene *WEE1* with the 25 top correlating genes to adavosertib response. *WEE1* is highlighted in green, positively correlated genes in blue and negatively correlated genes in red. Genes linking at least 4 out of the 26 genes are indicated in grey, other linker genes in white. *CCDC138*, *MIR17HG*, *LRRC30*, and *DSCC1* are not included in the FI network as they are not covered in the reactome database. **B,** Heatmap of the expression profiles (top), copy number (CN) (middle), and SNV (bottom) for the 25-gene set associated with adavosertib efficacy plus *WEE1* and the linker genes identified by network analysis in the 63 pediatric solid tumor cohort with matching RNA-seq and adavosertib efficacy data. Adavosertib AUC *Z* scores are represented by a color scale from red (low *Z* score; sensitive) to blue (high *Z* score; resistance); Gene TPM *Z* scores are represented by a color scale from blue (low *Z* score; low expression) to red (high *Z* score; high expression); Gene CN are represented by a color scale from green (low normalized CN) to orange (high normalized CN). Middle annotations indicate the tumor purity and ploidy as estimated by WGS. For four samples, no matching WGS data was available (tumor purity, tumor ploidy, CN and SNV/SNVg indicated in gray). Right annotation represents the Pearson's correlation coefficient between advosertib AUC *Z* scores and gene $\log_2$[TPM], corrected copy number for ploidy or SNV/SNVg presence (red; negatively correlated, blue; positively correlated). Tumor samples are ordered by adavosertib AUC *Z* score values, with lowest values (most sensitive) on the left and highest values (most resistant) on the right. Top annotation indicates the tumor type, and the black arrow indicates the adavosertib-sensitive neuroblastoma sample with a deviated expression of the 25-gene set. **C,** Adavosertib AUC (left) and $\log_2$[IC_{50}] (right) values in patient-derived neuroblastoma samples with normal *MYCN* (nMYCN) versus amplified or mutated *MYCN* (MYCNA/mutated). The *MYCN* status of two neuroblastoma samples was confirmed by WGS of an earlier received biopsy (hexagon symbols) and the neuroblastoma sample harboring a *MYCN* activating mutation (P44L) is indicated by the open circle. Horizontal lines indicate median values. **D**, Higher potency of adavosertib in neuroblastoma cells with normal versus amplified *MYCN* was confirmed using the publicly available datasets Genomics of Drug Sensitivity in Cancer 2 (GDSC2) and DepMap Public 22Q4 to retrieve matching adavosertib AUC values and *MYCN* statuses for classical neuroblastoma cell lines. Horizontal lines indicate median values. **E,** Effects of 24 hours treatment with 1.9 µmol/L adavosertib on G2-M transition in SHEP-21N neuroblastoma cells with MYCN on (-

Doxycycline (Dox); left) versus MYCN off (+Dox; right) in 4 independent experiments. **F,** Proliferation rate of SHEP-21N neuroblastoma cells with MYCN on (-Dox; green) versus MYCN off (+Dox; red). Viable cells were measured every 24 hours up to 120 hours with 30 technical replicates for each timepoint (56 for t = 0 h). Proliferation rates were established by calculating the fold-change in luminescence value at each timepoint using the following formula: [luminescence value at timepoint of interest] / [luminescence value at 0 h]. Dots indicate the average fold-increases ± SD. The black dotted line box indicates the time window used to study adavosertib effects on cell viability and G2-M cell cycle arrest, with *a* = start of treatment and *b* = end of treatment. Tumor type key: brain tumors (BT), neuroblastoma (NB), sarcoma tumors (SAR), solid other (SO). SNVg = germline SNV. **P* < 0.05.


A
SUV39H2
ARHGAP11A
SMAD5
HELLS
DLGAP5
MAD2L1
CDC42
EP300
RFC3
DNA2
BIRC5
TP53
LMNB1
BECN1
CHEK1
AURKB
PLK1
CDC25A
MAP1LC3A
BUB1B
CDC25C
CCNB1
SKA1
SPC25
CCNA2
WEE1
CDK1
HMMR
POLE2
B
Tumor type
Adavosertib
RNA expression
MAP1LC3A
SMC3
EP300
SMAD5
WEE1
PLK1
POLE2
CCNA2
MAD2L1
RFC3
CDK1
SPC25
LMNB1
DNA2
SUV39H2
DLGAP5
BUB1B
CHEK1
CDC25A
HELLS
HMMR
SKA1
ARHGAP11A
BIRC5
AURKB
CDC25C
CCNB1
DSCC1
MIR17HG
CCDC138
TP53
LRRC30
Purity
Ploidy
Normalized CN
MIR17HG
DSCC1
MAD2L1
CCNA2
PLK1
CHEK1
AURKB
TP53
LRRC30
SKA1
BUB1B
ARHGAP11A
RFC3
POLE2
DLGAP5
EP300
HMMR
SMAD5
CDC25C
CCNB1
LMNB1
CDC42
CDC25A
SUV39H2
SMC3
HELLS
DNA2
CDK1
BIRC5
BECN1
MAP1LC3A
CCDC138
SPC25
SNV/
SNVg
TP53
r
C
AUC value
nMYCNA
MYCNA/
mutated
Log2[IC50] values
nMYCNA
MYCNA
/mutated
D
AUC value
nMYCNA
MYCNA
E
MYCN on (-Dox)
MYCN off (+Dox)
N=1
N=2
N=3
N=4
G2-M
Count
PE-A
Untreated
1.9 μmol/L adavosertib
F
Proliferation rate of SHEP-21N cells
MYCN on (-Dox)
MYCN off (+Dox)
Luminescence fold-change
(Mean ± SD)
Time (h)
a
b
Heatmap legend
Tumor type
BT
NB
SAR
SO
AUC Z score
TPM Z score
Normalized CN
Pearson's r

Supplementary Figure S7.

Glioma samples with *PIK3R1* mutations are sensitive to trametinib. Heatmap of normalized copy number (CN) values for RAS-MAPK (top) and PI3K-AKT-mTOR genes (middle) and somatic SNVs in RAS-MAPK and PI3K-AKT-mTOR genes (bottom) in pediatric solid tumor samples without bona fide driver mutations in RAS-MAPK signaling (N=69). Trametinib AUC *Z* scores are represented by a color scale from red (low *Z* score; sensitive) to blue (high *Z* score; resistance); Normalized CN values of the genes are represented by a color scale from green (low normalized CN) to orange (high normalized CN). Middle annotations indicate the tumor purity and ploidy as estimated by WGS. Right annotations are the Pearson's correlation coefficient between trametinib AUC *Z* score and normalized copy number values or SNV presence (red; negatively correlated, blue; positively correlated). Tumor samples are ordered by trametinib AUC *Z* score value, with lowest values (most sensitive) on the left and highest values (most resistant) on the right. Top annotation indicates the tumor type. Tumor type key: brain tumors (BT), neuroblastoma (NB), sarcoma tumors (SAR), solid other (SO). Cancer subtype key: diffuse midline glioma (DMG), high-grade glioma (HGG), medulloblastoma (MB), Ewing's sarcoma (ES), osteosarcoma (OS), rhabdomyosarcoma (RMS), adrenocortical carcinoma (ACC), malignant rhabdoid tumor (MRT), and Wilms' tumor (WT).


Tumor type
Trametinib
Normalized CN RAS-MAPK genes
MET
ARAF
NTRK1
FGFR1
NTRK2
CIC
MAP2K2
RPS6KA1
NRAS
RAF1
NF1
MAPK1
HRAS
MAP2K1
NTRK3
MAPK3
ROS1
RPS6KA2
EGFR
BRAF
ALK
PTPN11
KRAS
Purity
Ploidy
Normalized CN PI3K-AKT-mTOR genes
EIF4EBP1
AKT2
MAPKAP1
TSC1
PIK3CG
RHEB
RPS6KB1
RPTOR
AKT3
AKT1
PIK3C3
PIK3R1
RICTOR
PTEN
TSC2
PDPK1
MLST8
PIK3R2
AKT1S1
GSK3A
PIK3R3
MTOR
PIK3CD
PIK3CA
GSK3B
PIK3CB
SNVs
PIK3R1
PIK3CA
NF1
TSC2
r
Tumor type
BT
NB
SAR
SO
AUC Z score
2
1
0
−1
−2
Corrected CN
2
1.5
1
0.5
0
Pearson's r
0.5
0
−0.5