Supplemental Material

Mutation Profiles in Glioblastoma 3D Oncospheres Modulate Drug Efficacy

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Supplemental Table 1. Additional Oncogene and Tumor Suppressor Mutation Status

Gene Symbol	Oncosphere Cell Line Mutational Status
APC	None
BRAF	None
BRCA1	None
BRCA2	None
CTNNB1	None
ERBB2	None
HRAS	None
FLT3	None
KIT	None
KRAS	JHH-520: WT/M75I
MAP2K4	None
MET	None
NRAS	JHH-68: WT/T74I
PDGFRA	None
RET	None
SMAD4	None
STK11	None
VHL	None

Supplemental Table 2. Comparison of Oncosphere Panel Mutations to Previous Studies

GBM CAN Genes	Parsons et al, 2008+	TCGA, 2008^	TCGA, 2013	Common 2D GBM cell lines§	Current Study*
CDKN2A	50%	52%	61%	75%	77%
TP53	40%	35%	28%	100%	44%
EGFR	37%	45%	57%	12.5%	22%
PTEN	30%	36%	41%	37.5%	66%
NF1	15%	18%	10%	25%	44%
CDK4	14%	18%	14%	0%	0%
RB1	12%	11%	7.6%	12.5%	22%
IDH1	11%	ND	6%	0%	0%
PIK3CA	10%	15%**	25%**	12.5%	0%
PIK3R1	8%	15%**	25%**	12.5%	11%

+ Only heterozygous and homozygous point mutations, homozygous deletions and amplifications greater than 12 copies were assessed.

^ Only heterozygous and homozygous point mutations, homozygous deletions and amplifications greater than 5.65 copies were assessed.

§ Cell lines assessed are U87MG, LN-229, and all 6 CNS lines present in the NCI-60 collection

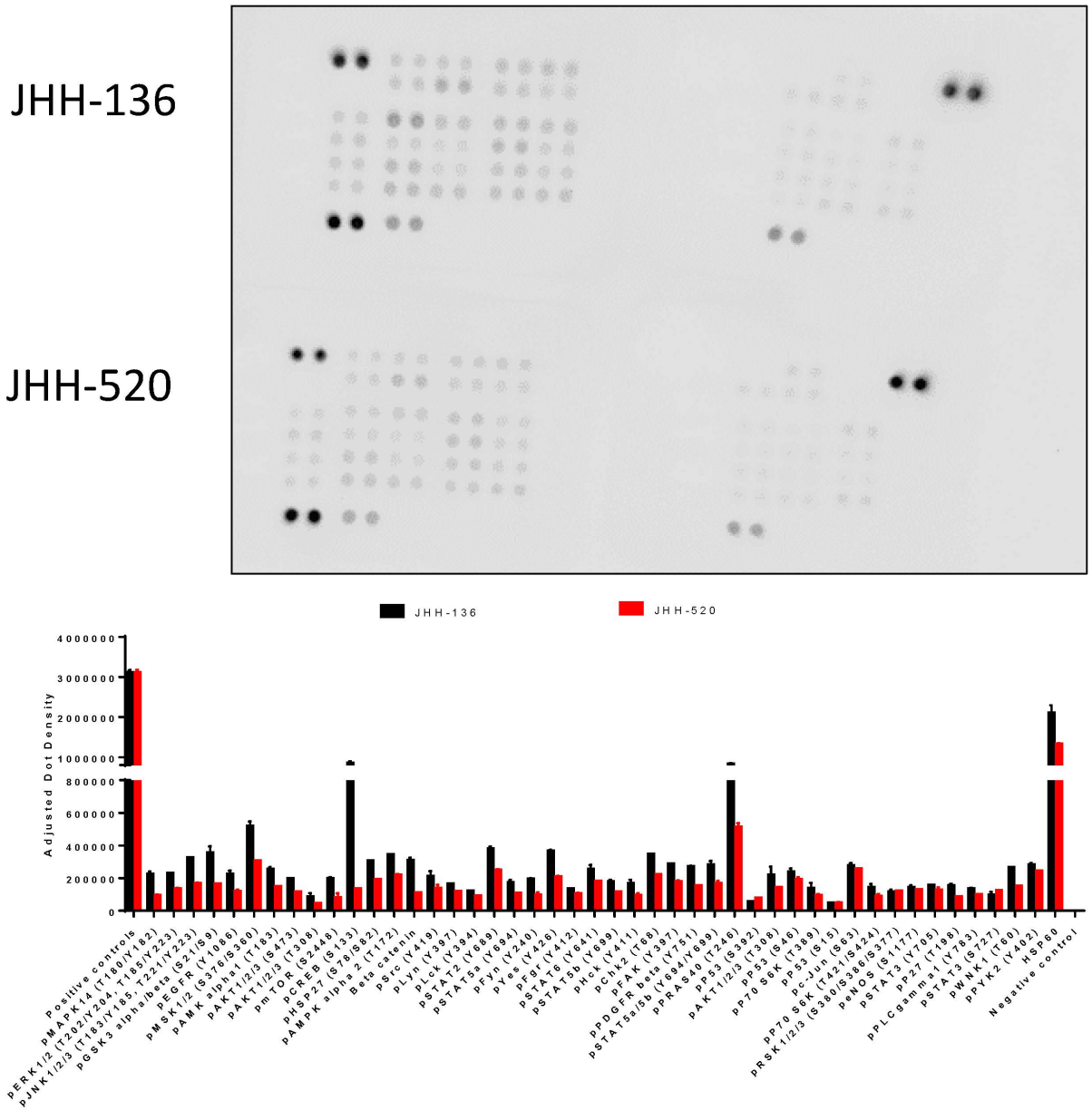
*Only heterozygous and homozygous point mutations, homozygous deletions, and amplifications greater than 10 copies were assessed.

**Reported values are combined for PIK3CA and PIK3R1 so the individual gene percentage is not known.

Supplemental Table 3. Genetic alterations present in both Br23X (xenograft) and Br23C (cell line)

Gene Name	Amino Acid Change
GPR85	T352P
HIVEP1	F1388L
DIMT1L	E85K
LMX1A	C26Y
MPZ	Y187C
NF1	I526S
PDIA2	R303C
SLC9A2	R160H
SNRPA	G99D
TACC3	E680K
TNMD	D167Y
TP53	C176F
PTEN	Homozygous Deletion

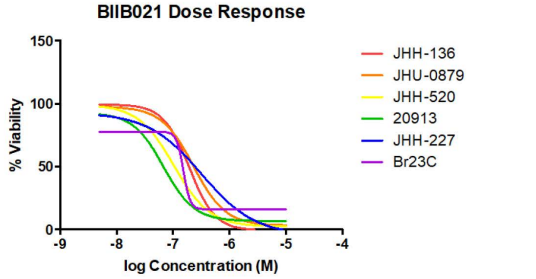
Supplemental Figure 1. Antibody arrays of phosphokinase protein expression and dot quantitation in JHH-136 and JHH-520



Phosphokinase array were done for JHH-136 and JHH-520. The top image shows the signal after a 10 second exposure. Dot density was quantitated and normalized to the positive controls and background. Results are shown here in bar graph.

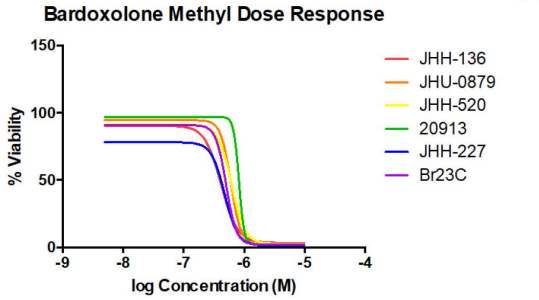
Supplemental Figure 2. Activity of individual compounds across multiple GBM cell lines

A



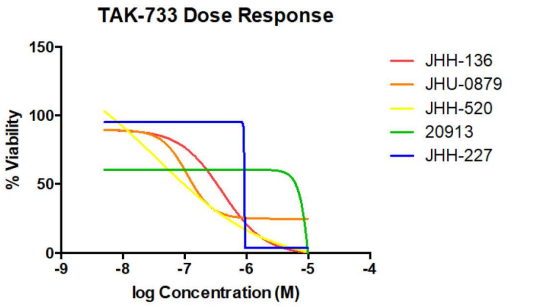
JHH-136	JHU-0879	JHH-520	20913	JHH-227	Br23C
1.928e-007	2.288e-007	1.037e-007	6.772e-008	3.186e-007	1.516e-007

B



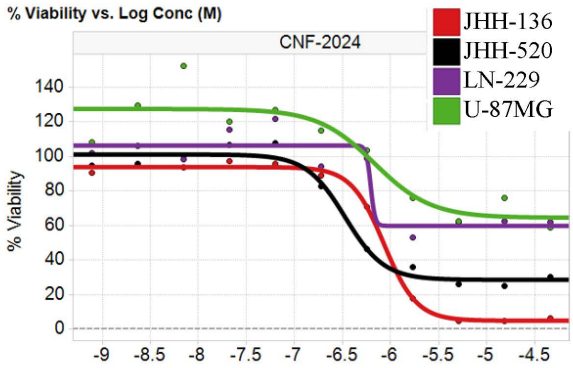
JHH-136	JHU-0879	JHH-520	20913	JHH-227	Br23C
4.169e-007	6.011e-007	6.174e-007	8.191e-007	4.755e-007	5.049e-007

C



JHH-136	JHU-0879	JHH-520	20913	JHH-227
4.078e-007	1.096e-007	3.641e-008	~ 1.600e-005	~ 9.088e-007

D



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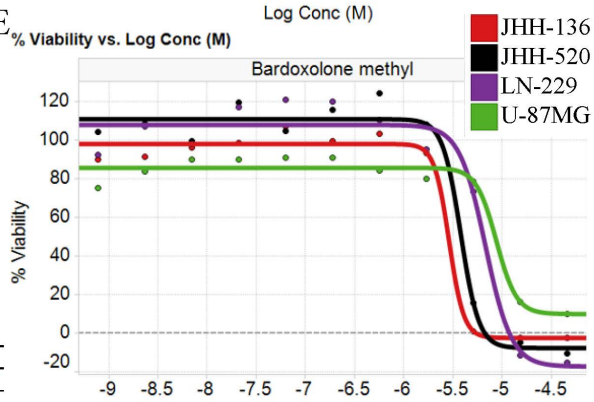
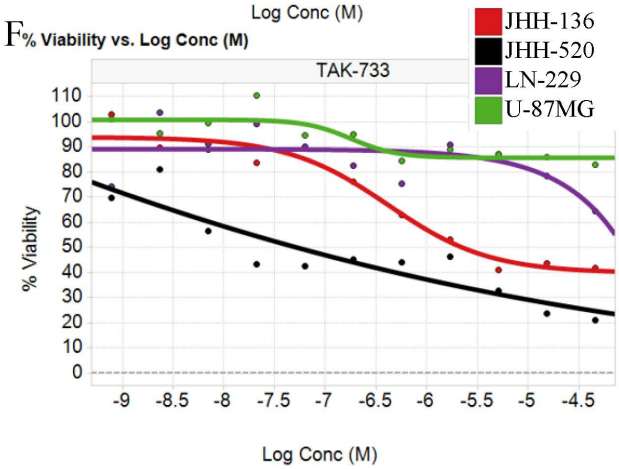


F



Dose response curves of cell viability across multiple GBM cell lines. 6 GBM oncosphere cell lines were tested against BIIB021 (CNF-2024), Bardoxolone methyl and TAK-733 in Figure 2A-C using an alamarBlue assay. The same three compounds were included in the qHTS screen as shown in Figure 2D-F using a CellTiter-Glo assay.

Supplemental Table 4. Compounds selected for 6x6 Drug combination screening

Drug Name	Gene Target	Activity profile
Cladribine	*ADA*	JHH-136 specific
SR-3306	*MAPK8*	Pan-active
AZD-8055	*MTOR*	Inactive
Pelitinib	*EGFR*	Inactive
OSI-027	*MTOR*	Inactive
TAK-733	*MAP2K1*	JHH-520 specific
NCGC00161703	*NFKB1*	Pan-active
CNF-2024	*HSP90AB1*	Pan-active
Carfilzomib	*PSMD*	JHH-136 specific
ABT-263	*BCL2L1*	Inactive
Trametinib	*MAP2K1*	Pan-active
GSK-2126458	*PIK3CA*	Pan-active
Topotecan	*TOP1*	JHH-136 specific
Combrestatin A-4	*TUBB*	Inactive
Plinabulin	*TUBB*	Inactive
Doxorubicin	*TOP2A*	Pan-active
GDC-0980	*MTOR*	Pan-active
Podofilox	*IGFR1*	Inactive
JK 184	*ADH7*	Inactive
Mebendazole	*TUBB*	Inactive
Flavopiridol	*CDK1*	Pan-active
Rotenone	*NDUFAF1*	JHH-520 selective
Panobinostat	*HDAC1*	Pan-active
Fenretinide	*RARB*	Pan-active
Obatoclax	*BCL2L1*	Pan-active
GDC-0941	*PIK3CA*	Pan-active
Artenimol	*N/A*	Pan-active
Bardoxolone methyl	*NFKB1*	Pan-active
PAC-1	*CASP3*	Inactive
MLN-2238	*PSMD1*	Pan-active

Supplementary Table 5. Combinations selected for 10x10 Drug combination screening

Drug Name A	Drug Name B
OSI-027	Mebendazole
Bardoxolone methyl	Carfilzomib
Bardoxolone methyl	GSK-2126458
CNF-2024	Bardoxolone methyl
SR-3306	Carfilzomib
SR-3306	Trametinib
SR-3306	PAC-1
Marizomib	Cladribine
Marizomib	Trametinib
GSK-2126458	Marizomib
Pictilisib	Obatoclax
Pictilisib	Mebendazole
CNF-2024	Pictilisib
Pelitinib	Cladribine
Pelitinib	Trametinib
Pelitinib	Obatoclax
Pelitinib	Mebendazole
AZD-8055	Cladribine
AZD-8055	Trametinib
AZD-8055	Obatoclax
AZD-8055	Mebendazole
Navitoclax (ABT-263)	Cladribine
Navitoclax (ABT-263)	Trametinib
Navitoclax (ABT-263)	GSK-2126458
Navitoclax (ABT-263)	Mebendazole
Cladribine	Carfilzomib
GSK-2126458	Carfilzomib
Carfilzomib	PAC-1
GSK-2126458	PAC-1
Cladribine	PAC-1

Drug Name A	Drug Name B
CNF-2024	PAC-1
Trametinib	PAC-1
Obatoclax	Cladribine
CNF-2024	Obatoclax
Mebendazole	Obatoclax
Obatoclax	Obatoclax
CNF-2024	Cladribine
CNF-2024	Trametinib
CNF-2024	GSK-2126458
Mebendazole	Cladribine
Mebendazole	Mebendazole
GSK-2126458	Cladribine
GSK-2126458	Trametinib
GSK-2126458	GSK-2126458
Trametinib	Cladribine
Trametinib	Trametinib
Cladribine	Cladribine