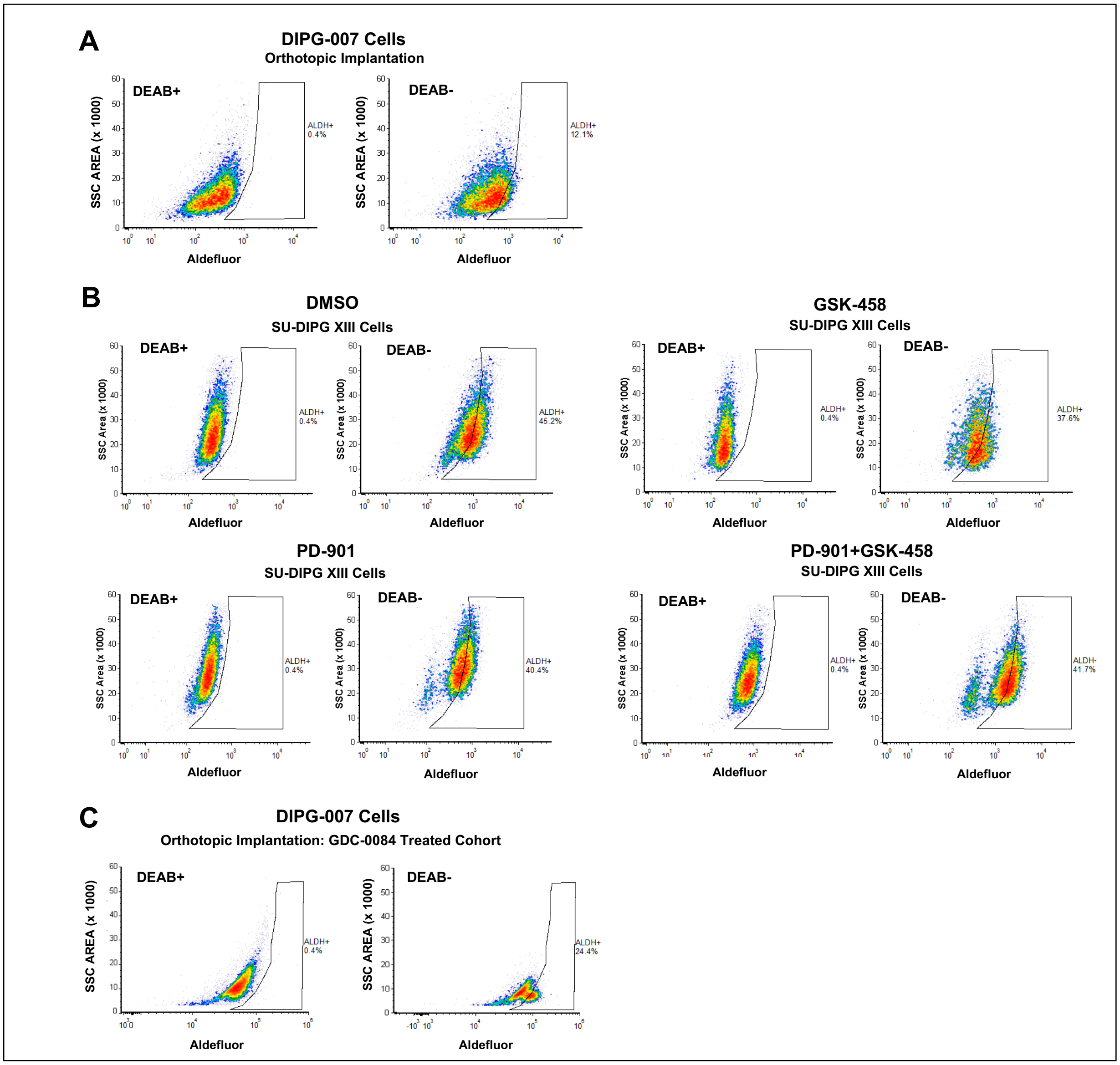
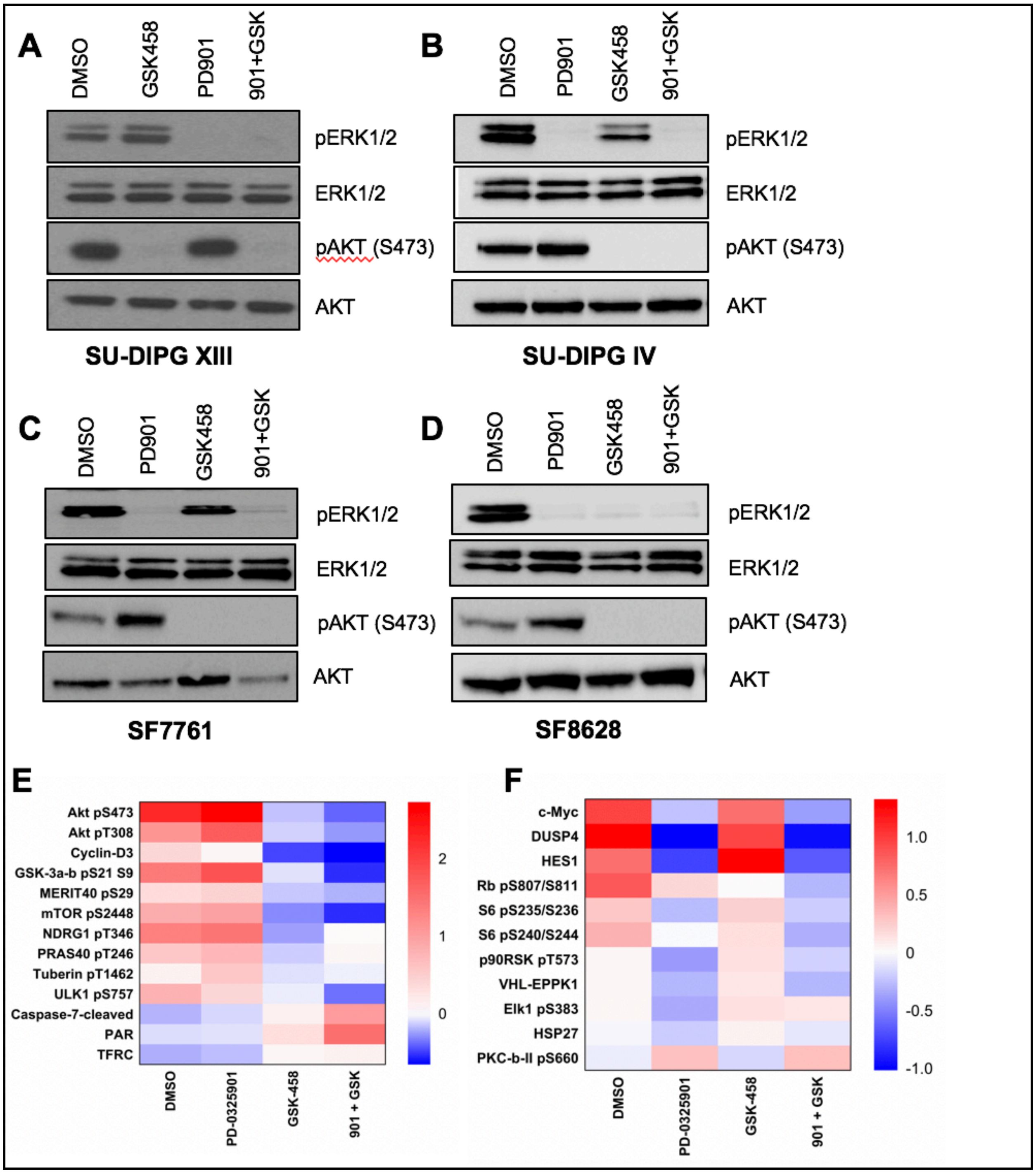
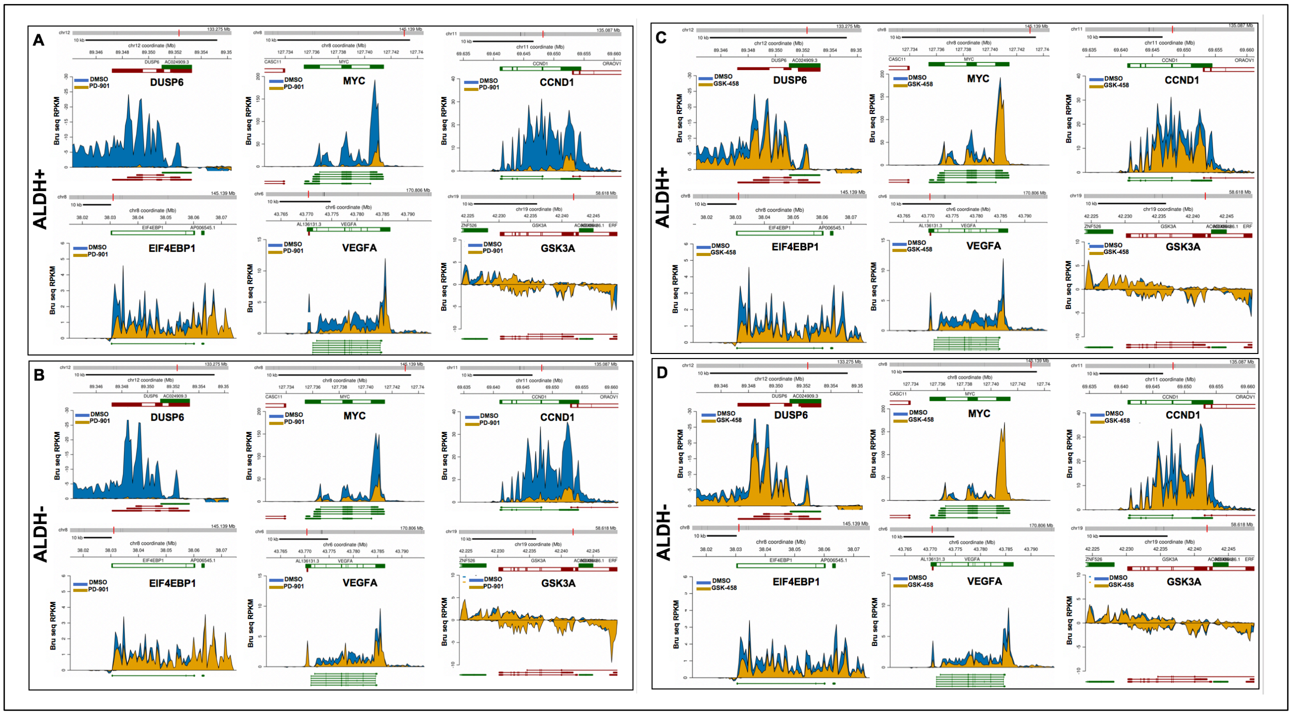


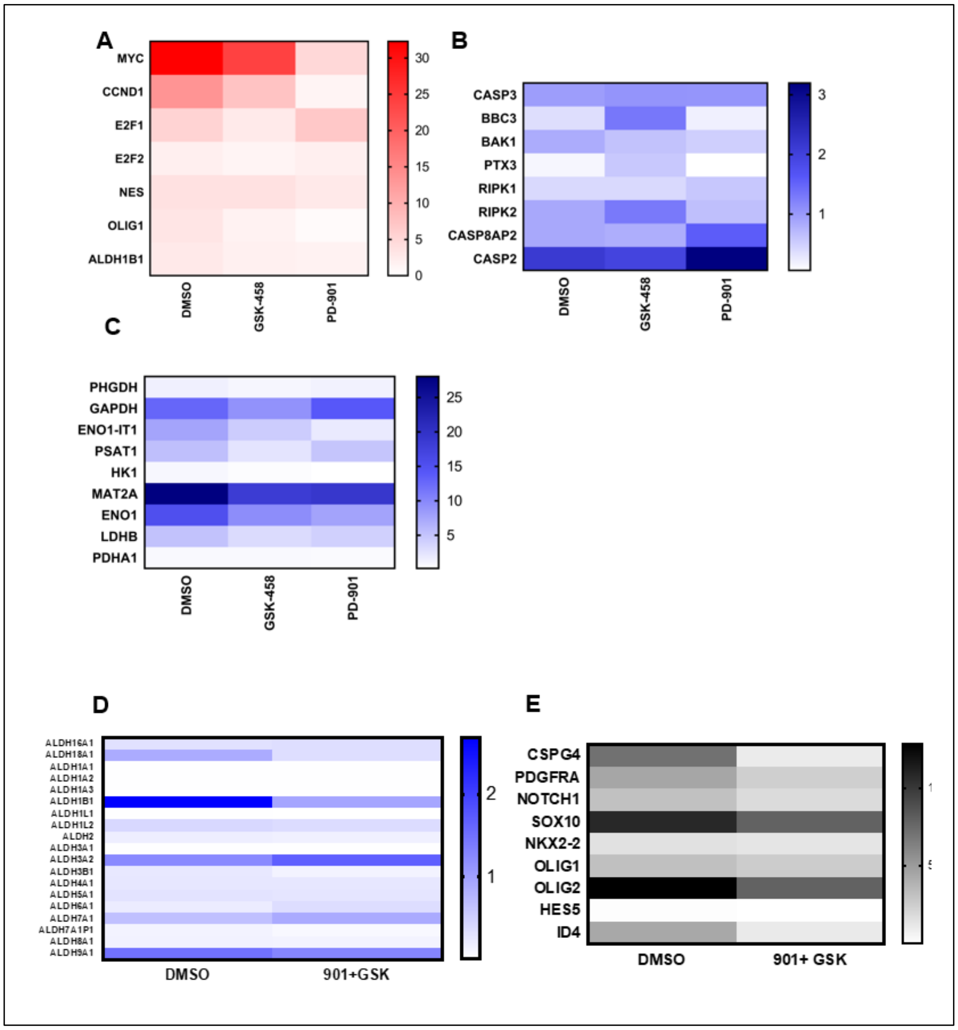
Supplemental Figure S1. FACS analysis of Aldefluor stained SU-DIPG XIII cells. Representative dot blots are shown for A-B. **A**. Cells were treated 2 hours with respective drug or equimolar DMSO wherein bromouridine was added in last 30 min prior to Aldefluor assay. **B**. FACS of DIPG-007 Luc cells used for implantation in the orthotopic model described in Figure 4. **C**. FACS of DIPG-007 Luc cells used for implantation in the orthotopic model. Mice were randomized into two treatment groups (vehicle control, GDC-0084) as described for Figure 7.



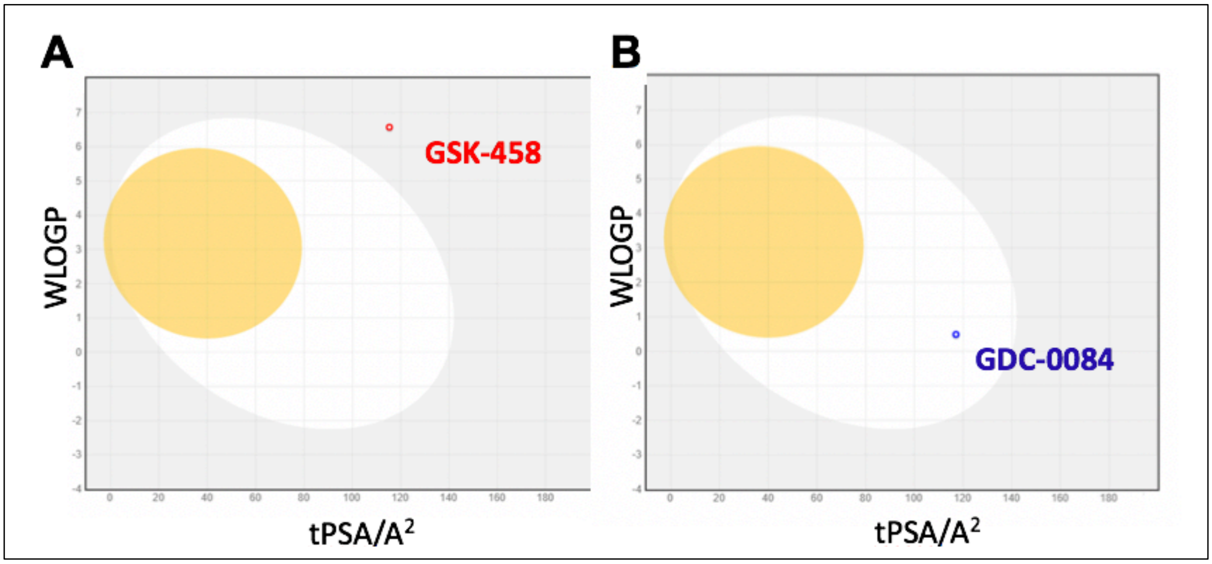
Supplemental Figure S2: Downstream signaling changes by inhibition of MAPK and PI3K/mTOR in DIPG cells. A.-D. SU-DIPG XIII, SU-DIPG IV, SF7761 and SF8628 cells were treated with 10 µM PD-901, GSK-458 or combination (901+GSK; 10µM each) for 2 hours and immunoblots of pERK 1/2 and pAKT (S473) were performed, respectively. **E.** and **F.** Lysates obtained from cells treated as described above for 6 hrs were analyzed by reverse phase protein array (RPPA). Heatmap of RPPA data, MAPK and PI3K/mTOR induced protein expression, respectively. Relative protein expression changes represented as norm log2 median centered.



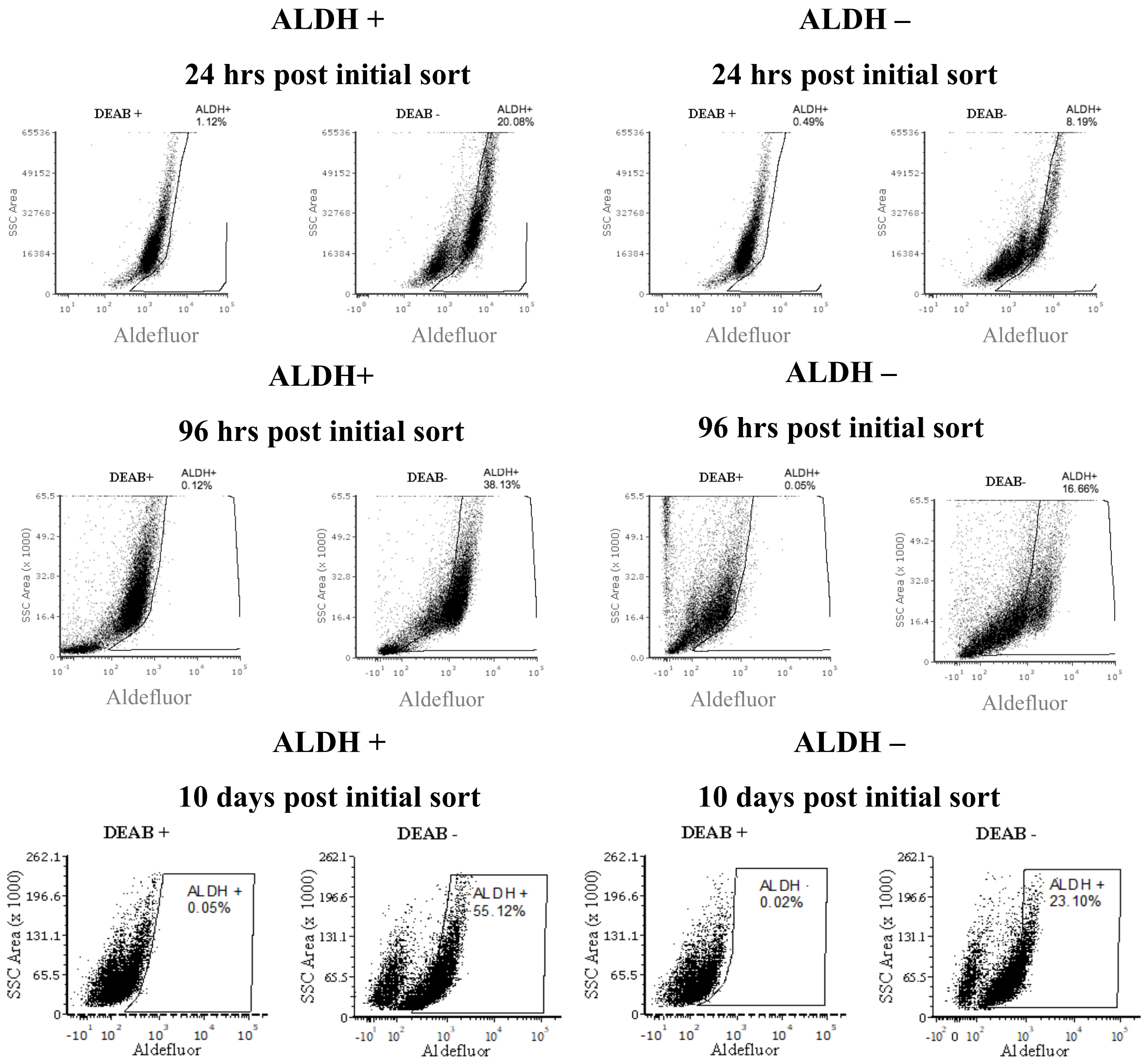
Supplemental Figure S3. Bru-sequencing transcriptome analysis identifies gene sets regulated by MAPK and PI3K/mTOR inhibition in ALDH positive SU-DIPG XIII cells. **A -D**. Bru-seq was performed on single agent treated (yellow) PD-901 (**A, B**), GSK-458 (**C, D**) or DMSO (blue) treated SU-DIPG XIII ALDH +/- cells. Sequencing reads from nascent RNA expressed as reads per thousand base pairs per 1 million reads (RPKM) and mapped to target genes. Expression changes of MAPK (*DUSP6, MYC, CCND1*) and PI3K (*VEGFA, 4EBP1, GSK3A*) target genes are presented.



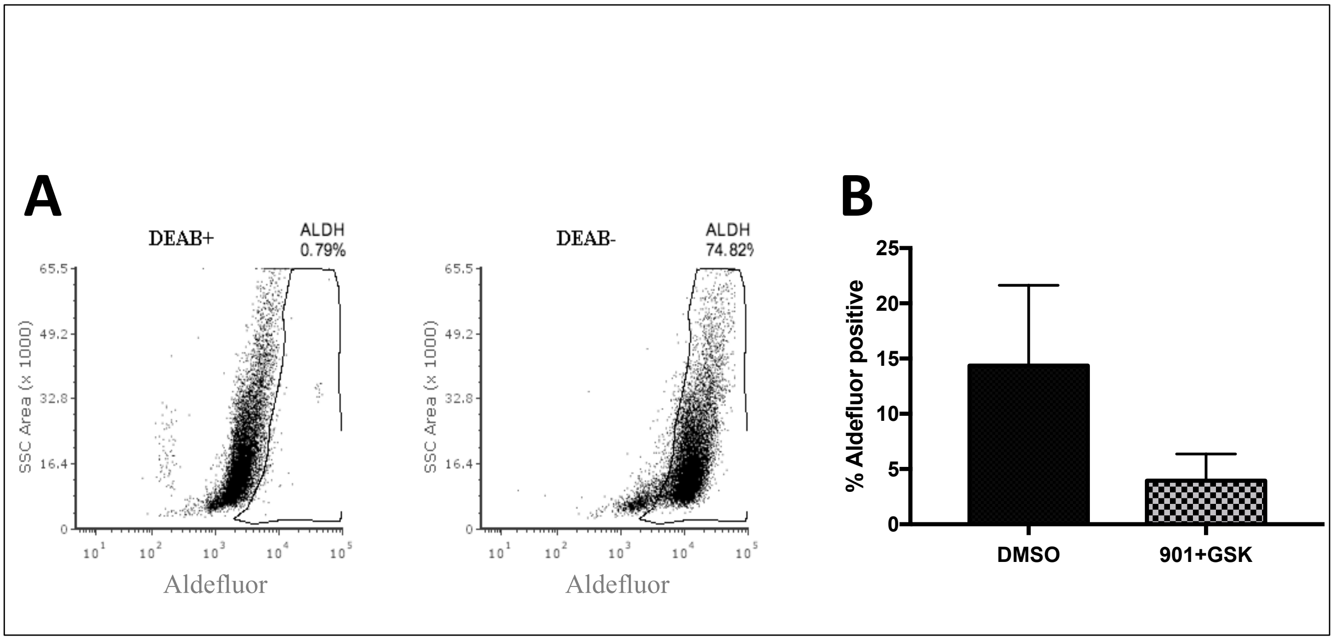
Supplemental Figure S4. A-C. Heatmaps of Bru-seq RPKM values in single agent treated (901 or GSK) ALDH+ SU-DIPG XII cells highlight treatment effects on “stemness” and DDR genes (**A**) apoptotic genes **(B)** and metabolic genes (**C**). **D-E.** Heatmaps of Bru-seq RPKM values in combination treated (901+GSK) ALDH+ SU-DIPG XII cells on ALDH family genes (**D**) and markers of OPC differentiation (**E**).



Supplemental Figure S5: A.-B. Illustrative result of BOILED-Egg predictive model for GSK-458 and GDC-0084, respectively.



Supplemental Figure S6: A-C. SU-DIPG-XIII cells were Aldefluor stained and sorted into an ALDH+ and ALDH- population. After sorting cells were plated into neurosphere TSM media and cultured for either 24 (**A**), 96 hrs (**B**) or 10 days (**C**) before cells in each cell population (ALDH +/-) were Aldefluor stained and analyzed by flow cytometry for ALDH expression.



Supplemental Figure S7: A. Aldefluor stain and FACS of SU-DIPG XIII cells. **B.** ALDH+ cells were treated with 50 nM 901+GSK each and 24 hrs later Aldefluor re-stained and analyzed by flow cytometry.

Supplemental Table 1: Characteristics of Patient-Derived Diffuse Intrinsic Pontine Glioma (DIPG) Cells.

Cell line	Mutations	Tissue Origin	Prior Therapy	Source/Ref.	*ALDH+ (Aldefluor)	*CD133+ (APC)	*ALDH/ CD133+
SU-DIPG IV	H3.1K27M, MDM4,ACVR1 G328V	Early postmortem autopsy	Radiotherapy, cetuximab, irinotecan	Grasso et al. *Nature Medicine (2015)*	31.41 ± 3.17	0.16 ± 0.14	0.08 ± 0.083
SU-DIPG XIII	H3.3K27M	Early postmortem autopsy	Radiotherapy	Grasso et al. *Nature Medicine (2015)*	24.54 ± 7.80	98.42 ± 0.25	18.21 ± 2.28
SF7761	H3.3K27M	Surgical biopsy	None	Millipore	0.01 ± 0.01	46.3 ± 22.75	0.14 ± 0.090
SF8628	H3.3K27M	Surgical biopsy	None	Millipore	8.27 ± 4.29	0.35 ± 0.35	0.027 ± 0.027
HSJD-DIPG 007	H3.3K27M; ACVR1	Early postmortem autopsy	Irinotecan-cisplatin	Vinci et al. *Nature Medicine (2018)*	55.15 ± 4.26	2.75 ± 0.87	0.613 ± 0.32
SU-DIPG 29	H3.3K27M	Early postmortem autopsy	Radiotherapy; panobinostat; avastin; THC oil	Qin et al. *Cell (2017)*	3.11 ± 0.52	20.12 ± 2.48	0.65 ± 0.26

*Data presented with mean values ± SEM of percentages from three independent flow cytometric analyses**.**

Supplemental Table 2. Selected Genes Upregulated in ALDH+ DIPG Cells.

Upregulated Gene	log2 Fold Change	Function
PLK1	1.834	G2 to M transition, Cell Division
GTSE1	1.691	DNA Damage Response
AURKA	1.685	Proliferation, Cell Cycle
INCENP	1.680	Cell Division, Cell Cycle
TACC3	1.676	Cell Division, Cell Cycle
IQGAP3	1.599	Proliferation, Metastasis
FAM83D	1.571	Proliferation, Cell Cycle
RECQL4	1.263	Homologous Recombination
PKMYT1	0.870	G2 to M Transition
CHEK2	0.827	DNA Repair, Cell Cycle Arrest
E2F2	0.737	Stem Cell, Cell Cycle
MYC	0.381	stem cell, proliferation
PCNA	0.269	DNA Damage Response
BRCA1	0.224	Homologous Recombination
NES	0.218	Stem Cell Marker
OLIG1	0.196	Stem Cell Marker
E2F1	0.123	Stem Cell, Cell Cycle
RAD51	0.111	Homologous Recombination
RFC2	0.101	DNA Damage Response

Suplemental Table 3. Selected Metabolome Genes Upregulated in ALDH+ DIPG Cells.

Upregulated Gene	log2 Fold Change	Gene Name
PHGDH	0.405	Phoshoglycerate Dehydrogenase
GAPDH	0.432	Glyceraldehyde-3-Phosphate Dehydrogenase
ENO1-IT1	0.315	Enolase One Ionic Transcript 1
PSAT1	0.256	Phosphoserine Aminotransferase 1
HK1	0.209	Hexokinase 1
MAT2A	0.046	Methionine Adenosyltransferase 2A
ENO1	0.011	Enolase 1
LDHB	-0.034	Lactate Dehydrogenase B
PDHA1	-0.055	Pyruvate Dehydrogenase

Supplementary Table 4: RT-qPCR primer sequences.

Gene name	**Sequence 5'-3'**
GAPDH	F: CGCTGAGTACGTCGTGGAGTC R: GCAGGAGGCATTGCTGATGA
MYC	F: CAGCTGCTTAGACGCTGGATT R: GTAGAAATACGGCTGCACCGA
E2F1	F: CAGCTGCTTAGACGCTGGATT R: GTAGAAATACGGCTGCACCGA
EIF4EBP 1	F: CTATGACCGGAAATTCCTGATGG R:CCCGCTTATCTTCTGGGCTA
E2F2	F: CGTCCCTGAGTTCCCAACC R: GCGAAGTGTCATACCGAGTCTT
GSK3A	F: GGAAAGGCATCTGTCGGGG R: GAGTGGCTACGACTGTGGTC
DUSP6	F: GTTTTTCCCTGAGGCCATTT R: TAGGCATCGTTCATCGACAG
VEGF	F: TGCCAAGTGGTCCAAG R: GTGAGGTCTTGATCCG
CCND1	F: ACGAAGGTCTGCGCGTGTT R: CCGCTGGCCATGAACTACCT
OLIG1	F: TACTATGCGGTTTCCCAGGC R: GCTGAGTAGGGCAGGATGA
ENO1	F: TTACCACAACCTGAAGAATG R:TCCAGGCCTTCTTTATTCTC
MAT2A	F:CTTGTTCAGGTCTCTTATGC R:TGCAGTCCTCTGATAAATTG
PSAT1	F: GAGTTTGACTTTATACCCGATG R:CTGTTCCCATGAACTTCTTC