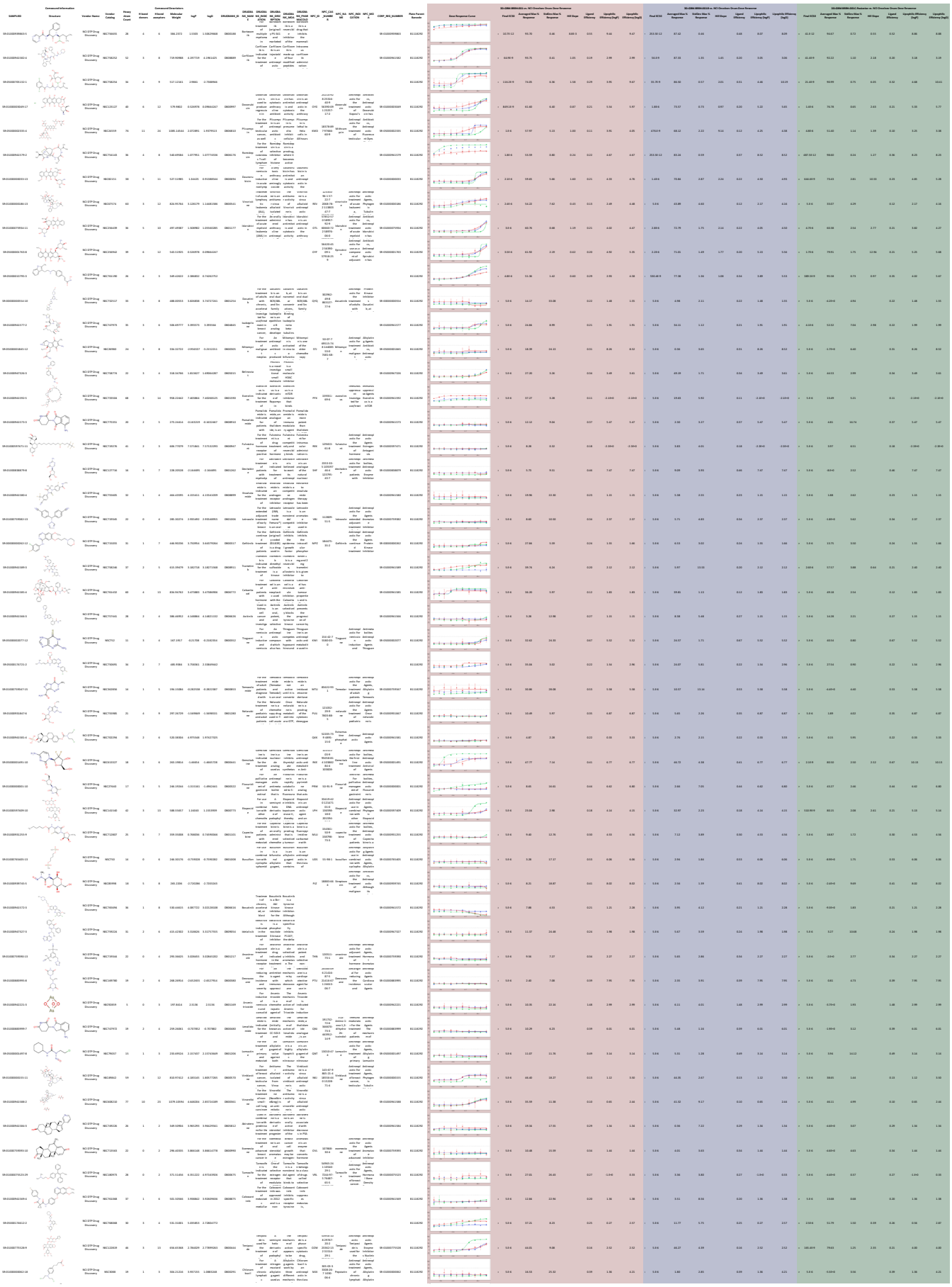
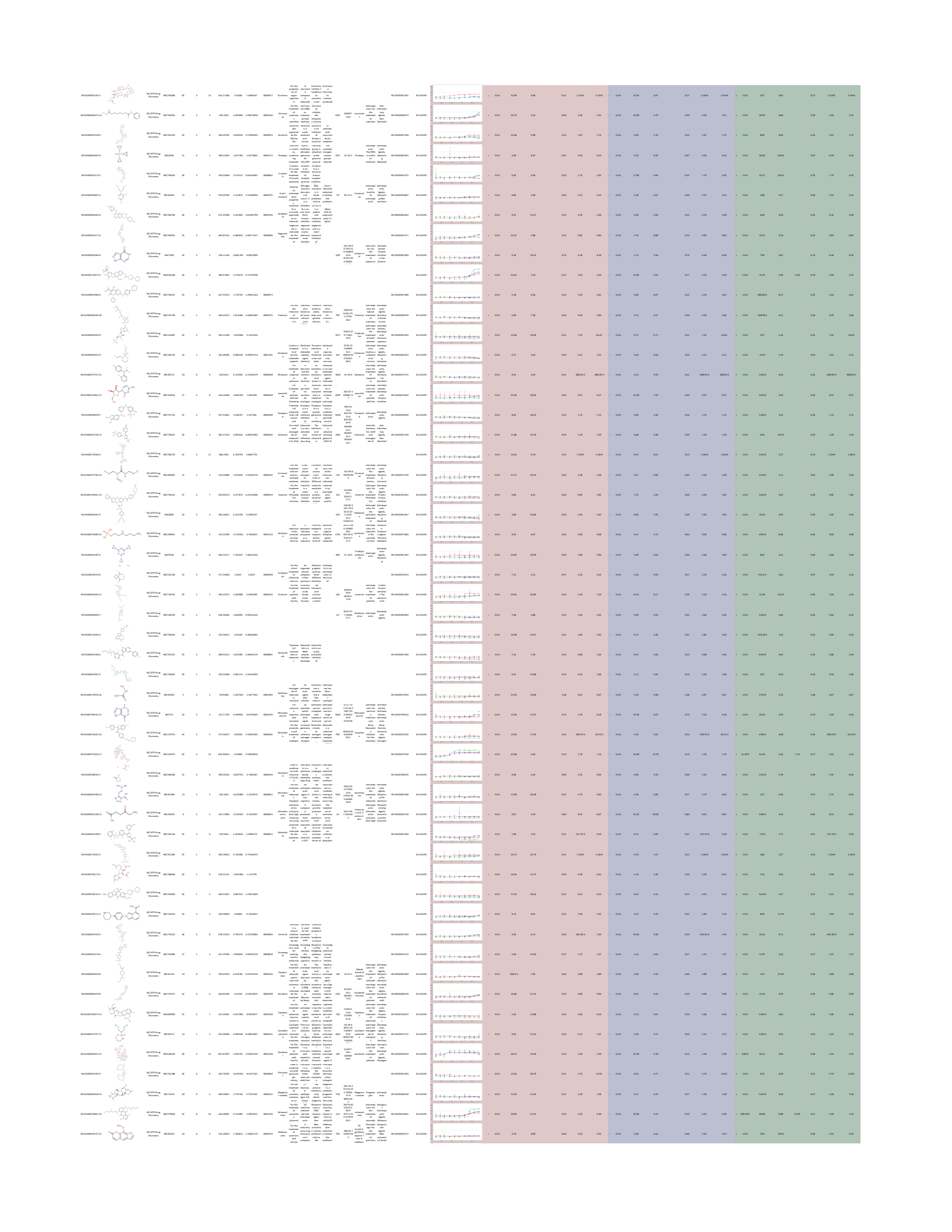
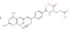
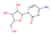
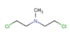
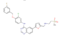
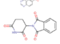
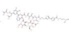
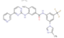
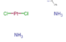
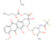
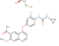
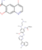
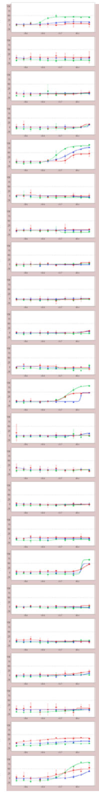
Supplemental figures

Figure S1 128 drugs used in this study



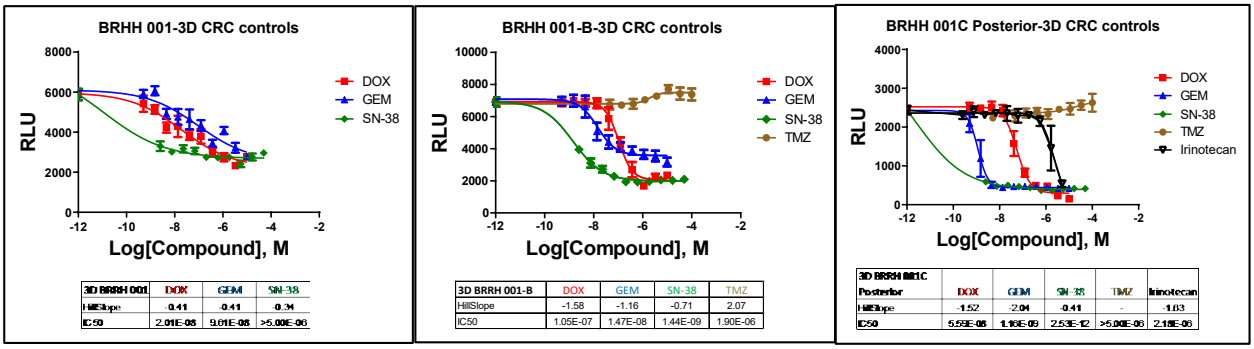


SR-01000941578-4		NCI DTP Drug Discovery	NSC754230	35	5	11	477.47262	0.615166	-5.7050032	DB06813	Pralatrexate	Treatment of relapsed or refractory	Pralatrexate is an antimetabolite for the treatment	The selectivity of pralatrexate for cancer	Pralatrexate is a 10-deazaaminopterin analogue						SR-01000941578	B1118292	>	5.E-6	7.49	4.22		0.21	4.69	11.01	>	5.E-6	17.76	11.46		0.21	4.69	11.01	>	5.E-6	47.92	8.11		0.21	4.69	11.01
SR-01000075881-13		NCI DTP Drug Discovery	NSC19893	9	2	2	130.07722	-0.655848	-0.810272	DB00544	Fluorouracil	For the topical treatment of multiple actinic or	A pyrimidine analog that is an antineopl	The precise mechanism of action has not	Fluorouracil is an antineoplastic antimetabolite. Anti-	UUX	51-21-8 10318-20 4 57050-04-1 57172-36 8 68021-	Fluorouracil	Antineoplastic For the topical treatment of	Antimetabolites Antimetabolites, Antineoplastic	SR-01000075881	B1118292	>	5.E-6	15.91	18.48		0.82	5.96	6.11	>	5.E-6	6.58	2.57		0.82	5.96	6.11	>	5.E-6	-3.2E+0	2.01		0.82	5.96	6.11
SR-01000000117-18		NCI DTP Drug Discovery	NSC13875	15	0	6	210.27941	2.216222	1.71631938	DB00488	Altretamine	For use as a single agent in the palliative	An alkylating agent proposed as an antineopl	The precise mechanism by which altretami	Altretamine is a novel antineoplastic agent.	RKT	645-05-6 15468-34 5 2975-00-0	Altretaminum	Antineoplastic For use as a single agent in the	Antineoplastic Agents, Alkylating The precise	SR-01000000117	B1118292	>	5.E-6	3.87	7.95		0.49	3.09	3.59	>	5.E-6	2.96	1.08		0.49	3.09	3.59	>	5.E-6	11.10	34.70		0.49	3.09	3.59
SR-01000597029-4		NCI DTP Drug Discovery	NSC63878	17	4	7	243.21662	-2.797517	-2.7975203													B1118292	>	5.E-6	16.33	4.56		0.44	8.10	8.10	>	5.E-6	14.72	3.52		0.44	8.10	8.10	>	5.E-6	15.05	1.46		0.44	8.10	8.10
SR-01000597562-4		NCI DTP Drug Discovery	NSC758253	39	2	10	545.62125	1.884671	-0.1241306	DB04865	Homoharringtonine	Used in patients who are intolerant and/or resistant	Homoharringtonine, AKA HHT or omacetaxine	Homoharringtonine inhibits protein synthesis by not	The pharmacodynamics of homoharringtonine	SMW	26833-87 4 88179-67-3	4'-Methyl 2'r-hydroxy-2'-(4-hydroxy-4-	Antineoplastic	Antineoplastic Agents, Phytogenic Angiogen	SR-01000597562	B1118292	>	5.E-6	43.86	15.72		0.19	3.42	5.43	=	486.2E-9	78.42	1.37	1.07	0.23	4.43	6.44	=	92.1E-9	91.80	1.89	1.48	0.25	5.15	7.16
SR-05000001664-7		NCI DTP Drug Discovery	NSC762	8	0	1	156.05354	1.519149	1.49906237	DB00888	Mechlorethamine	For the palliative treatment of Hodgkin's disease	A vesicant and necrotizing irritant	Alkylating agents work by three different mechanis	Mechlorethamine also known as mustine, nitrogen	TPK	51-75-2 126-85-2 302-70-5 55-86-7 159923-90-7	Chlormethine	Antineoplastic For the palliative treatment of	Alkylating Agents Antineoplastic Agents, Alkylatin	SR-05000001664	B1118292	>	5.E-6	16.16	17.58		0.93	3.78	3.80	>	5.E-6	0.52	2.54		0.93	3.78	3.80	>	5.E-6	4.00	21.15		0.93	3.78	3.80
SR-05000174588-2		NCI DTP Drug Discovery	NSC778909	32	2	7	434.53729	2.379943	0.89095841													B1118292	>	5.E-6	10.37	21.65		0.23	2.92	4.41	>	5.E-6	3.02	6.78		0.23	2.92	4.41	>	5.E-6	0.12	18.75		0.23	2.92	4.41
SR-05000001472-9		NCI DTP Drug Discovery	NSC745750	40	2	7	581.05754	4.638509	4.42501139	DB01259	Lapatinib	Indicated in combination with capecitabine for	Lapatinib is an anticancer drug developed by	Lapatinib is a 4-anilinoquinazoline kinase inhibitor	Lapatinib is a small molecule and a member	NSK	388082-78-8 231277-92-2	lapatinib	Antineoplastic Indicated in combination with	Tyrosine kinase inhibitors Antineoplastic	SR-05000001472	B1118292	>	5.E-6	8.90	5.28		0.19	0.66	0.88	>	5.E-6	1.84	3.25		0.19	0.66	0.88	>	5.E-6	4.41	2.06		0.19	0.66	0.88
SR-01000076184-22		NCI DTP Drug Discovery	NSC66847	19	1	4	258.22949	0.015707	0.01567983	DB01041	Thalidomide	For the acute treatment of the cutaneous	A piperidinyl isoindole originally	In patients with erythema nodosum leprosum	Thalidomide is an immunomodulatory agent with a	MDT	50-35-1 731-40-8 841-67-8 14088-68 7 2614-06-4	N-(2,6-dioxo-3-piperidyl)phthalimide	Immunomodulator For the acute treatment of the	Angiogenesis Inhibitors Immunosuppressi	SR-01000076184	B1118292	>	5.E-6	-243.8E-3	9.34		0.39	5.29	5.29	>	5.E-6	2.47	8.04		0.39	5.29	5.29	>	5.E-6	-2.E+0	7.34		0.39	5.29	5.29
SR-01000941586-5		NCI DTP Drug Discovery	NSC125066	96	20	29	1415.55175	-9.710223	-8.9322067							MNH	9041-93-4 11056-06-7 1400-95-9 37208-04-1	Bleomycin	Antineoplastic For palliative treatment in the managem	Antineoplastic Agents Although the exact mechanis	SR-01000941586	B1118292	>	5.E-6	9.81	3.11		0.08	15.01	14.23	>	5.E-6	8.86	5.08		0.08	15.01	14.23	>	5.E-6	-1.5E+0	8.57		0.08	15.01	14.23
SR-01000721911-6		NCI DTP Drug Discovery	NSC75520	20	3	5	296.19994	-0.754626	-0.9644963	DB00432	Trifluridine	Ophthalmic solution for the treatment of primary	An antiviral derivative of thymidine used	The mechanism of action of trifluridine has	Trifluridine is a fluorinated pyrimidine	UTN	70-00-8	Trifluridine	Antiviral Ophthalmic solution for the treatment	Antimetabolites Antiviral Agents The mechanis	SR-01000721911	B1118292	>	5.E-6	14.97	2.65		0.37	6.06	6.27	>	5.E-6	7.10	2.10		0.37	6.06	6.27	>	5.E-6	8.30	6.29		0.37	6.06	6.27
SR-01000930565-7		NCI DTP Drug Discovery	NSC606869	20	3	7	303.67744	-0.291322	-0.291324	DB00631	Clofarabine	For the treatment of pediatric patients 1 to 21	Clofarabine is a purine nucleoside antimeta	Clofarabine is metabolized intracellularly to	Clofarabine is a purine nucleoside antimeta	PYV	123318-82-1	clofarabine	Antineoplastic For the treatment of pediatric	Antineoplastic Agents Antimetabolites Clofarabi	SR-01000930565	B1118292	>	5.E-6	48.03	0.87		0.37	5.59	5.59	>	5.E-6	46.63	1.53		0.37	5.59	5.59	=	397.9E-9	83.50	0.62	1.40	0.45	6.69	6.69
SR-01000597410-4		NCI DTP Drug Discovery	NSC3053	90	5	18	1255.41704	-0.096882	-0.0971764	DB00970	Dactinomycin	For the treatment of Wilms' tumor, childhood	A compound composed of a two	Good evidence exists that this drug binds strongly,	Generally, the actinomycins exert an inhibitory						SR-01000597410	B1118292	>	5.E-6	15.00	44.49		0.08	5.40	5.40	>	5.E-6	11.82	4.20		0.08	5.40	5.40	>	5.E-6	8.60	24.82		0.08	5.40	5.40
SR-01000931150-7		NCI DTP Drug Discovery	NSC747599	39	2	6	529.51578	5.36154	5.33148059	DB04868	Nilotinib	For the potential treatment of various leukemia	Nilotinib, also known as AMN107, is a tyrosine	Chronic myelogenous leukaemia (CML) is caused	Nilotinib is a transduction inhibitor that						SR-01000931150	B1118292	>	5.E-6	10.72	22.13		0.19	-59.6E-3	-29.6E-3	>	5.E-6	11.81	5.22		0.19	-59.6E-3	-29.6E-3	>	5.E-6	0.33	4.13		0.19	-59.6E-3	-29.6E-3
SR-01000076254-7		NCI DTP Drug Discovery	NSC119875	5	2	2	300.04504	1.299	1.299	DB00515	Cisplatin	For the treatment of metastatic testicular	Cisplatin cisplatinum or cis diamminedichloro platinum	Alkylating agents work by three different mechanis	Cisplatin is an antineoplastic in the class of						SR-01000076254	B1118292	>	5.E-6	5.82	0.72		1.48	4.00	4.00	>	5.E-6	3.69	2.77		1.48	4.00	4.00	>	5.E-6	-2.1E+0	3.62		1.48	4.00	4.00
SR-01000611320-11		NCI DTP Drug Discovery	NSC369100	18	1	3	240.30364	2.650669	2.64643826	DB00724	Imiquimod	For the topical treatment of clinically typical,	Imiquimod is an immune response modifier that acts	Imiquimod's mechanism of action is via	Imiquimod is an immune response modifier that acts	TOU	99011-02-6	imiquimod	Antiviral For the topical treatment of clinically	Adjuvants, Immunologic Antineoplastic	SR-01000611320	B1118292	>	5.E-6	10.40	5.73		0.41	2.65	2.66	>	5.E-6	2.82	1.09		0.41	2.65	2.66	>	5.E-6	-3.2E+0	6.49		0.41	2.65	2.66
SR-01000879991-4		NCI DTP Drug Discovery	NSC246131	51	5	13	723.64374	4.486147	3.50592857	DB00385	Valrubicin	For the treatment of cancer of the bladder.	Valrubicin (N-trifluoroacetyladriamycin-14-	Valrubicin is an anthracycline that affects a variety of	Valrubicin is a semisynthetic analog of the	ROD	56124-62-0	Valrubicin	Antineoplastic For the treatment of cancer of the	Antineoplastic Agents Valrubicin is an anthracy	SR-01000879991	B1118292	>	5.E-6	44.60	3.82		0.15	0.82	1.80	>	5.E-6	47.24	4.18		0.15	0.82	1.80	=	1.9E-6	68.93	1.46	11.71	0.16	1.24	2.22
SR-01000883935-5		NCI DTP Drug Discovery	NSC218321	19	4	7	268.26914	-2.016391	-2.9345256	DB00552	Pentostatin	For the treatment of hairy cell leukaemia	A potent inhibitor of adenosine deamina	Pentostatin is a potent transition state inhibitor	Pentostatin is an antineoplastic antimetabolite used in	OPS	53910-25 1 63677-95-2 59979-24 7 69196-00-5	pentostatin	Antineoplastic For the treatment of hairy cell	Antibiotics, Antineoplastic Enzyme Inhibitor	SR-01000883935	B1118292	>	5.E-6	6.25	7.11		0.39	7.32	8.24	>	5.E-6	2.61	1.66		0.39	7.32	8.24	>	5.E-6	0.60	5.68		0.39	7.32	8.24
SR-05000174531-2		NCI DTP Drug Discovery	NSC755980	30	3	5	426.85295	2.521607	2.51743845													B1118292	>	5.E-6	11.60	12.77		0.25	2.78	2.78	>	5.E-6	1.25	1.96		0.25	2.78	2.78	>	5.E-6	5.34	3.25		0.25	2.78	2.78
SR-05000174592-2		NCI DTP Drug Discovery	NSC779217	37	2	7	499.60731	4.486981	3.00856316													B1118292	>	5.E-6	21.00	7.82		0.20	0.81	2.29	>	5.E-6	12.32	2.90		0.20	0.81	2.29	>	5.E-6	4.07	2.66		0.20	0.81	2.29
SR-01000763375-11		NCI DTP Drug Discovery	NSC77213	16	3	3	221.29876	0.985734	0.97892093	DB01168	Procarbazine	For use with other anticancer drugs for the	An antineoplastic agent used primarily	The precise mode of cytotoxic action of procarba	Procarbazine is an antineoplastic in the class of	OBW	366-70-1 671-16-9 13309-74 5 18969-59-0 98600-80	Procarbazine hydrochloride	Antineoplastic For use with other anticancer drugs	Antineoplastic Agents The precise mode of	SR-01000763375	B1118292	>	5.E-6	15.23	13.07		0.46	4.32	4.32	>	5.E-6	1.55	4.14		0.46	4.32	4.32	>	5.E-6	-554.8E-3	2.15		0.46	4.32	4.32
SR-01000898799-5		NCI DTP Drug Discovery	NSC683864	73	4	16	1030.28708	7.129959	7.12877626	DB06287	Temsirolimus	For the treatment of renal cell carcinoma (RCC).	Temsirolimus is an intravenous drug for the treatment	Temsirolimus is an inhibitor of mTOR (mammalian target		VRX	162635-04-3	temsirolimus	Antineoplastic	Antineoplastic Agents Protein Kinase Inhibitor	SR-01000898799	B1118292	>	5.E-6	33.78	3.05		0.10	-1.8E+0	-1.8E+0	>	5.E-6	15.47	3.32		0.10	-1.8E+0	-1.8E+0	>	5.E-6	5.20	3.14		0.10	-1.8E+0	-1.8E+0
SR-01000003063-17		NCI DTP Drug Discovery	NSC105014	19	3	7	285.68698	-0.281775	-0.2817757	DB00242	Cladribine	For the treatment of active hairy cell leukemia (leukemic	An antineoplastic agent used in the	Cladribine is structurally related to fludarabi	Cladribine is a synthetic purine nucleoside that						SR-01000003063	B1118292	=	97.5E-6	55.62	16.61	0.99	0.30	4.29	4.29	>	5.E-6	37.84	2.93		0.39	5.58	5.58	=	336.7E-9	84.29	2.06	1.26	0.48	6.75	6.75



Supplemental figures

Figure S2 Controls used for dose-response test of the tumor organoids against the NCI library (128 compounds)



3D BRRH 001	DOX	GEM	SN-38
HillSlope	-0.41	-0.41	-0.34
IC50	2.01E-08	9.61E-08	>5.00E-06

3D BRRH 001-B	DOX	GEM	SN-38	TMZ
HillSlope	-1.58	-1.16	-0.71	2.07
IC50	1.05E-07	1.47E-08	1.44E-09	1.90E-06

3D BRRH 001C Posterior	DOX	GEM	SN-38	TMZ	Irinotecan
HillSlope	-1.52	-2.04	-0.41	-	-1.03
IC50	5.55E-08	1.93E-09	2.53E-12	>5.00E-06	2.18E-08

BRHH 001 Run Statistics (n=3 plates)
- **128 compounds tested starting at ~5uM* in 10 point 3 fold dilution in triplicate.**
- *** Most common starting concentration**
- **Ave Z' = 0.69 ± 0.10**
- **Ave S:B = 98.25 ± 44.39**

BRRH 001-B Run Statistics (n=3 plates)
- **128 compounds tested starting at ~5uM* in 10 point 3 fold dilution in triplicate.**
- *** Most common starting concentration**
- **Ave Z' = 0.86 ± 0.01**
- **Ave S:B = 135.44 ± 1.07**

BRRH 001-C Run Statistics (n=3 plates)
- **128 compounds tested starting at ~5uM* in 10 point 3 fold dilution in triplicate.**
- *** Most common starting concentration**
- **Ave Z' = 0.84 ± 0.04**
- **Ave S:B = 155.08 ± 20.12**

Figure S2: Controls used for dose-response analysis of the 3 tumor organoids against the 128-compound library

Concentration response curves of the controls run each time the individual 3D organoids were tested. Hillslope and CC50 results are shown below each graph which are color coded to match their individual curves. Analysis was done use GraphPad Prism non-linear curve fit with variable slope. Each concentration tested in each curve represents an N of 16 and error bars are shown as SD. Runs statistics are shown for each of the experiments for the individual organoids when tested vs. 128 separate NCI FDA approved oncologic drugs. The average Z's where derived from the 3 separate plates tested in each assay which included 24 control wells per plate.

Figure S3: Control for synergy combinations (Dabrafenib and Trametinib)

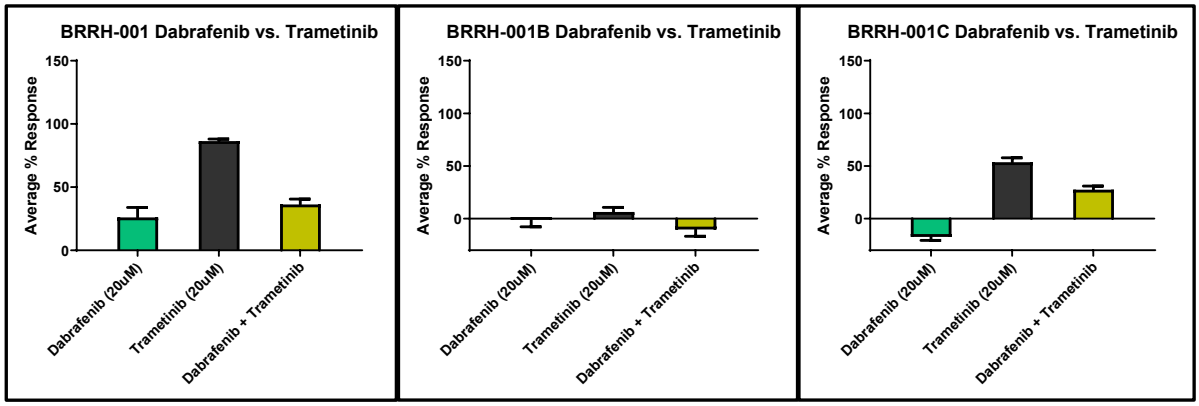
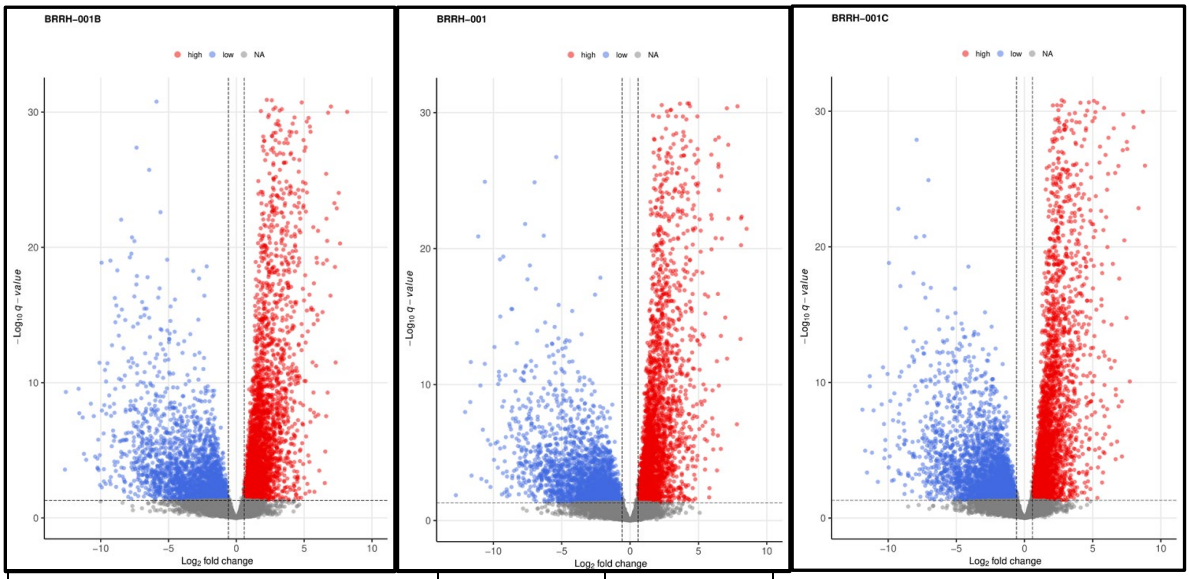


Figure S3: Controls used for synergy drug testing

In vitro drug response, derived from the 3D viability assay of patient derived GBM spheroids treated with sub IC-50 concentration (20uM) of Debrafenib, Trametinin or a combination of both shown as avg % cytotoxicity (Response). Dabrafenib and Trametinib clearly show no synergistic effects on the tumor growth. Data presented as mean±SD. Measures repeated with 6 replicate wells for individual drugs and 9 replicate wells for the combinations.

Figure S4 KEGG enrichment P-values and Volcano plots showing overview of differential expression of all the genes tested by RNAseq analysis



Pathway Enrichment P-Values	BRRH-001	BRRH-001B	BRRH-001C
EGFR tyrosine kinase	0.117	0.112	0.045
ErbB signaling pathway	0.045	0.046	0.018
Circadian rhythm	0.496	0.890	0.012
Glioma	0.045	0.206	0.021
FoxO signaling pathway	0.049	0.263	0.026
MAPK signaling pathway	0.654	0.031	0.465
Rap1 signaling pathway	0.148	0.003	0.169
Pathways in cancer	0.046	0.008	0.238
Cell cycle	5.0E-16	3.1E-12	1.1E-08
p53 signaling pathway	8.9E-06	2.5E-04	1.3E-04
DNA replication	2.3E-12	1.9E-06	4.8E-09

Figure S4: Overview of differential expression of genes in Tumor organoids BRRH-001, BRRH-001B, BRRH-001C

The threshold in the volcano plot was FDR q-value < 0.05 and |Fold Change| > 1.5. The unbalanced distribution is typical when comparing single samples against many (i.e. GTEX normal brain samples) in edgeR. KEGG Pathway Enrichment p-values between GBM organoids and GTEx healthy brains. Pathways involved in DNA replication, cell cycle, and p53 signaling are dysregulated in all three samples while the MAPK and Rap1 signaling pathways are enriched in only the first recurrence but the EGFR, ErbB, and FoxO signaling pathways are more dysregulated in the second recurrence.

Figure S5. Body weight as a measure of toxicity during tumor growth and treatment.

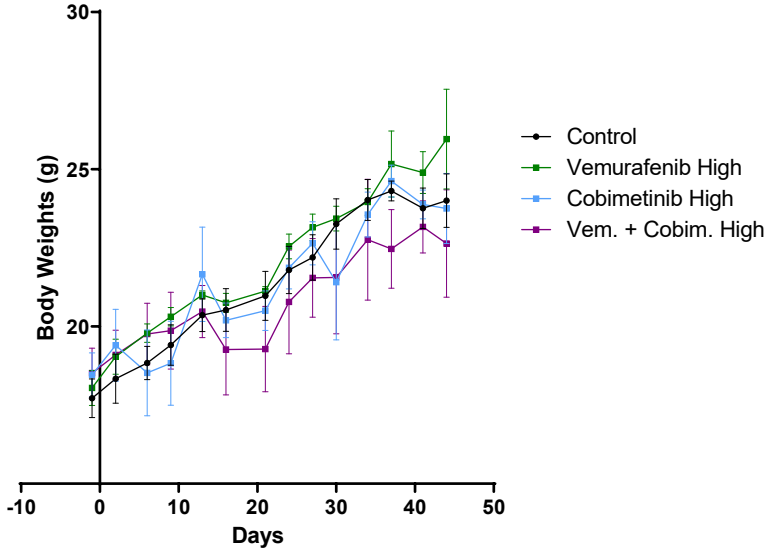
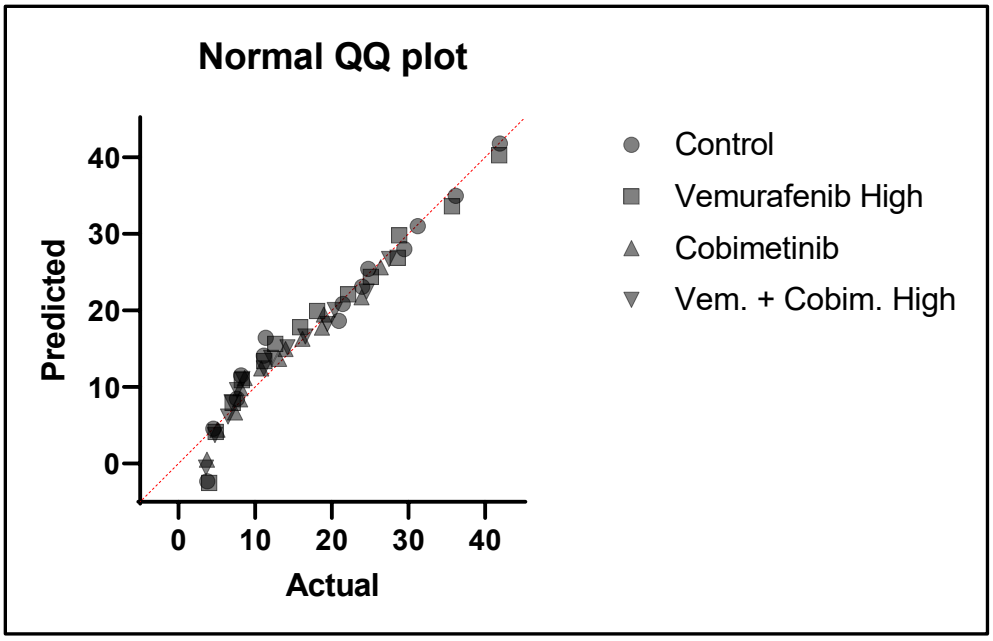


Figure S5: Body weight in mice with tumor growth and treatment.

Mice subcutaneously implanted with a patient derived spheroids cultured from a primary tumor (BRRH-001), were treated with control placebo or Cobimetinib or Vemurafenib or a combination of both). Body weights were monitored at regular intervals starting 48 hours prior to treatment. (N=4 mice in each group, all data are mean ± SEM; 2-way ANOVA and multiple comparison against control group with Bonferroni correction: Not significantly different)

Figure S6 Normality testing of tumor growth data



Test for normal distribution D'Agostino & Pearson test				
K2	1.360	1.131	1.207	1.481
P value	0.5067	0.5681	0.5468	0.4768
Passed normality test (alpha=0.05)?	Yes	Yes	Yes	Yes
P value summary	ns	ns	ns	ns
Number of values	14	14	14	14