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Showcase Report

CLIENT: Showcase Client

WRITTEN BY

Claudius Mueller, PhD, Director of Bioinformatics

Brian A Corgiat, PhD, Senior Research Specialist

APPROVED BY

Justin B. Davis, PhD, Laboratory Director

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ABSTRACT: This is an example report created to demonstrate data analysis and reporting options. Individual statistics and graphing modules can be included/excluded based on client need. Data interpretation and summary can also be extended by client request.

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Cell Line Data

Cell Line A: Data Overview

Cell Line A is a breast cancer cell line. The effect of Drug A, a novel EGFR inhibitor, was tested in three concentrations: 0.1 μM , 1 μM , 10 μM . Treated cells were compared to a respective vehicle control and evaluated for signaling downstream of EGFR. Of particular interest in this model was the effect on CREB activation and whether CREB activation was regulated via MSK1 or RSK signaling.

Overall, 42 samples spread over 6 groups (three drug concentrations and three respective vehicle controls) were processed and 9 analytes chosen for analysis (see Table 1). No samples were missing from the analysis.

Table 1: Data Overview

Analyte	Groups	N	Missing	Mean	SD	Spread	Density
c-Raf S338	6	42	0	12,679.58	5,910.05		
CREB S133	6	42	0	5,108.98	3,216.53		
EGFR Y1068	6	42	0	21,393.49	12,153.66		
ERK1/2 T202 Y204	6	42	0	22,650.35	15,693.60		
MEK1/2 S217 S221	6	42	0	7,798.91	2,849.85		
MSK1 S360	6	42	0	66,319.91	41,116.89		
RSK1 T359 S363	6	42	0	1,654.72	962.18		
GTP-bound Ras	6	42	0	11,703.94	5,446.35		
RSK3 T356 S360	6	42	0	5,742.72	2,656.11		

Mean Comparison Statistics

Treatment with Drug A showed a significant inhibition of EGFR Y1068 phosphorylation (0.1 μM : 3.7-fold, 1 μM : 7.6-fold). This inhibition of EGFR was not significant at a concentration of 10 μM of Drug A due to the small sample size ($n = 2$).

In addition to the inhibition of EGFR, Drug A also significantly inhibited MEK 1/2 S217 S221, ERK 1/2 T202 Y204, MSK1 S360 and CREB S133 at concentrations of 0.1 and 1 μM . Again, the significance of the findings at 10 μM of Drug A could not be established due to the low sample size.

In each case, statistical significance was found with and without correction for multiple comparisons (see Table 2, column “p” vs. “p adj.”).

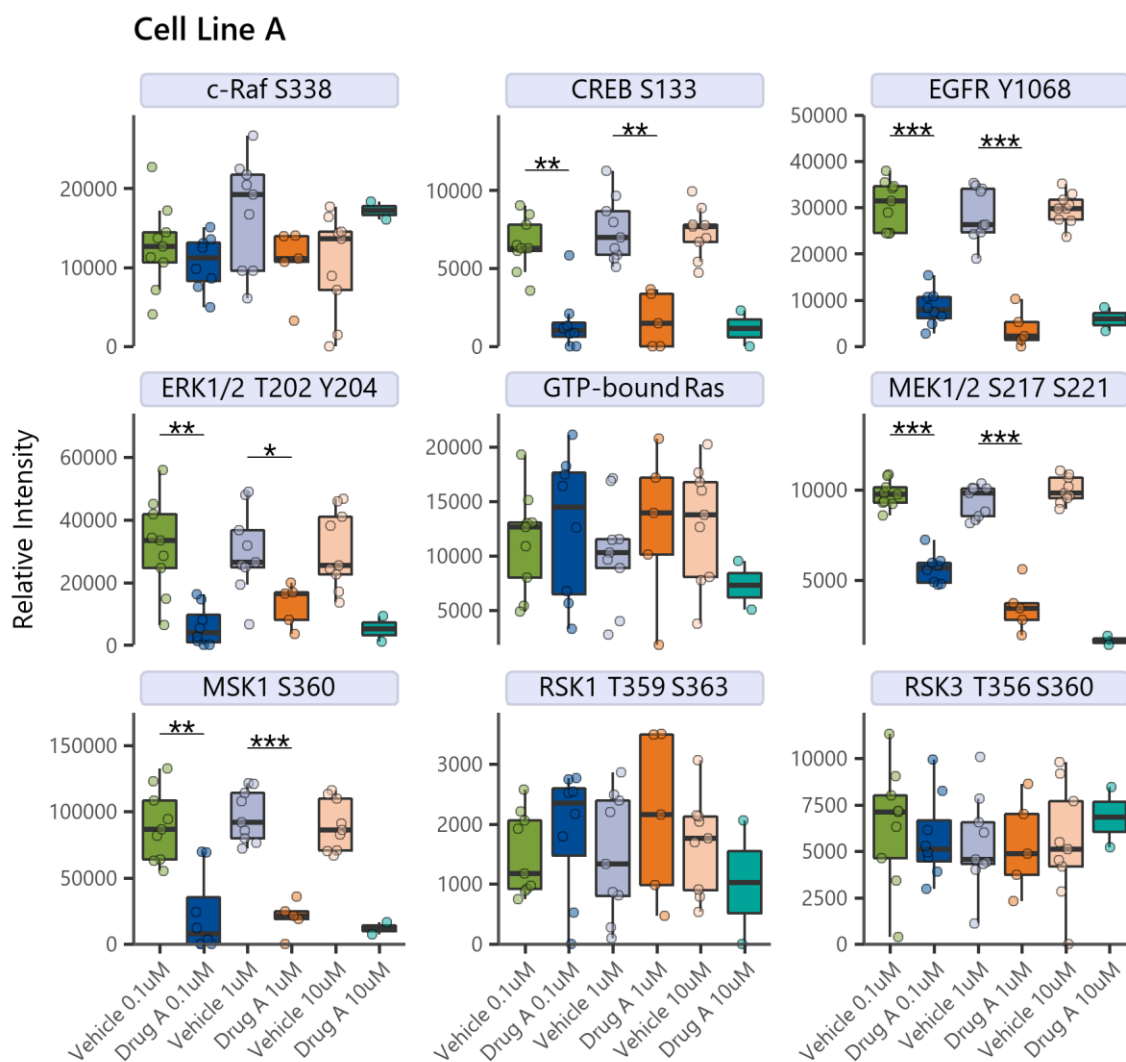
Table 2: Mean Comparison Statistics

Var ₁	Var ₂	N ₁	N ₂	log2FD	Mean ₁ ^a	SD ₁ ^a	Mean ₂ ^a	SD ₂ ^a	p	p _{adj}
EGFR Y1068										
Drug A 0.1uM	Vehicle 0.1uM	8	9	-1.88	8.30	3.92	30.63	5.30	< 0.01	< 0.01
Drug A 1uM	Vehicle 1uM	5	9	-2.92	3.79	4.04	28.64	5.88	< 0.01	< 0.01
Drug A 10uM	Vehicle 10uM	2	9	-2.34	5.89	3.65	29.77	3.42	N/A ^b	N/A ^b
GTP-bound Ras										
Drug A 0.1uM	Vehicle 0.1uM	8	9	0.16	12.69	6.67	11.36	4.65	0.67	0.88
Drug A 1uM	Vehicle 1uM	5	9	0.31	12.77	7.27	10.30	4.89	0.44	0.88
Drug A 10uM	Vehicle 10uM	2	9	-0.83	7.30	3.16	12.96	5.41	N/A ^b	N/A ^b
c-Raf S338										
Drug A 0.1uM	Vehicle 0.1uM	8	9	-0.25	10.62	3.48	12.63	5.46	0.48	0.48
Drug A 1uM	Vehicle 1uM	5	9	-0.68	10.60	4.39	16.94	7.01	0.19	0.38
Drug A 10uM	Vehicle 10uM	2	9	0.72	17.22	1.60	10.45	6.44	N/A ^b	N/A ^b
MEK1/2 S217 S221										
Drug A 0.1uM	Vehicle 0.1uM	8	9	-0.79	5.62	0.84	9.75	0.75	< 0.01	< 0.01
Drug A 1uM	Vehicle 1uM	5	9	-1.42	3.50	1.36	9.35	0.89	< 0.01	< 0.01
Drug A 10uM	Vehicle 10uM	2	9	-2.60	1.65	0.36	9.99	0.73	N/A ^b	N/A ^b
ERK1/2 T202 Y204										
Drug A 0.1uM	Vehicle 0.1uM	8	9	-2.39	6.04	6.42	31.65	15.26	< 0.01	< 0.01
Drug A 1uM	Vehicle 1uM	5	9	-1.20	12.99	6.83	29.78	13.43	0.02	0.02
Drug A 10uM	Vehicle 10uM	2	9	-2.54	5.25	5.77	30.53	12.55	N/A ^b	N/A ^b
MSK1 S360										
Drug A 0.1uM	Vehicle 0.1uM	8	9	-2.01	22.32	30.25	89.66	27.28	< 0.01	< 0.01
Drug A 1uM	Vehicle 1uM	5	9	-2.27	20.07	12.98	96.68	19.65	< 0.01	< 0.01
Drug A 10uM	Vehicle 10uM	2	9	-2.91	11.91	6.28	89.51	19.40	N/A ^b	N/A ^b
RSK1 T359 S363										
Drug A 0.1uM	Vehicle 0.1uM	8	9	0.33	1.88	1.06	1.50	0.69	0.37	0.74
Drug A 1uM	Vehicle 1uM	5	9	0.52	2.12	1.40	1.48	1.03	0.44	0.74
Drug A 10uM	Vehicle 10uM	2	9	-0.70	1.03	1.46	1.67	0.80	N/A ^b	N/A ^b
RSK3 T356 S360										
Drug A 0.1uM	Vehicle 0.1uM	8	9	-0.15	5.75	2.31	6.37	3.22	0.61	1.00
Drug A 1uM	Vehicle 1uM	5	9	-0.03	5.30	2.53	5.43	2.57	1.00	1.00
Drug A 10uM	Vehicle 10uM	2	9	0.34	6.84	2.29	5.42	3.10	N/A ^b	N/A ^b
CREB S133										
Drug A 0.1uM	Vehicle 0.1uM	8	9	-2.12	1.50	1.87	6.52	1.73	< 0.01	< 0.01
Drug A 1uM	Vehicle 1uM	5	9	-2.14	1.69	1.76	7.48	2.06	< 0.01	< 0.01
Drug A 10uM	Vehicle 10uM	2	9	-2.67	1.15	1.62	7.31	1.61	N/A ^b	N/A ^b

^ax * 1000^bSample size too low

Boxplots

MEK 1/2 S217 S221 demonstrates a dose-response pattern in response to treatment with Drug A, with increasing inhibition when drug concentrations are increased. EGFR Y1068, ERK 1/2 T202 Y204, MSK1 S360, and CREB S133 don't show this pattern, indicating that evaluations of lower concentrations of Drug A may be warranted in future experiments since maximal inhibition is already reached at 0.1 μ M.



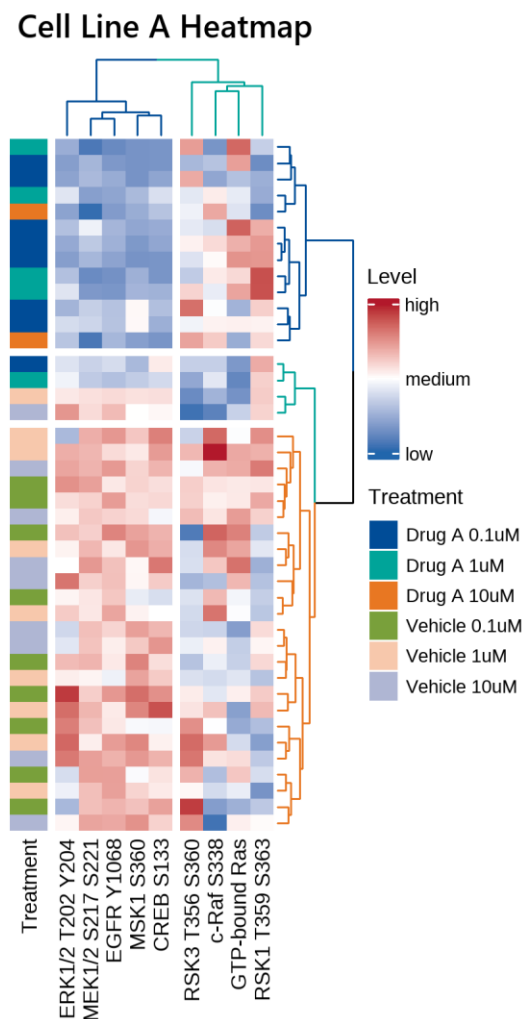
Source: Example Report



Figure 1: Boxplots by Analyte

Heatmap

Unsupervised, 2-way hierarchical clustering separates Vehicle control from Drug A treated samples (Figure 2). This clustering is driven by the attenuated activation of ERK 1/2, MEK 1/2, EGFR, MSK1, and CREB. In addition, these analytes cluster together, further supporting that this subset of analytes is affected by Drug A treatment, whereas the other analytes evaluated are not.



Source: Example Report

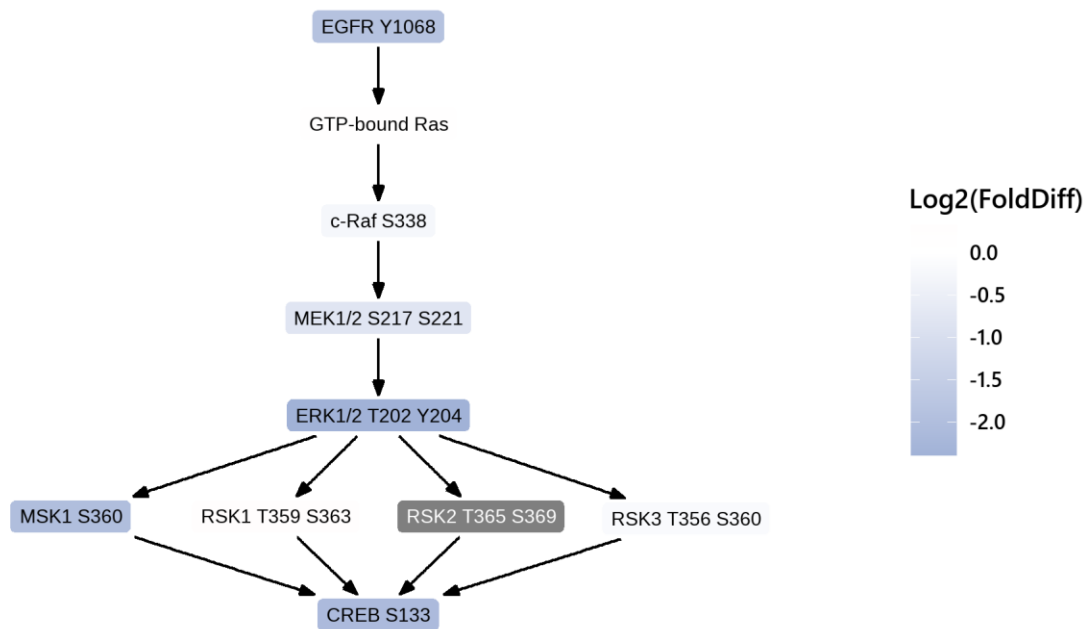


Figure 2: Heatmap Cell Line A

Pathmaps

The fold-change between each respective treatment-vehicle control pair was mapped onto cell signaling transduction modules (Figures 3 - 5). This provides a signaling context to the protein phosphorylation and protein level changes described above. Overall, a general decrease in pathway activation can be seen across all Drug A treatment concentrations. Furthermore, the data indicate that the inhibition of CREB S133 via this particular EGFR inhibitor is a result of an EGFR-MEK-ERK-MSK1-CREB signaling pathway, at the exclusion of RSK signaling.

Drug A 0.1uM vs. Vehicle 0.1uM

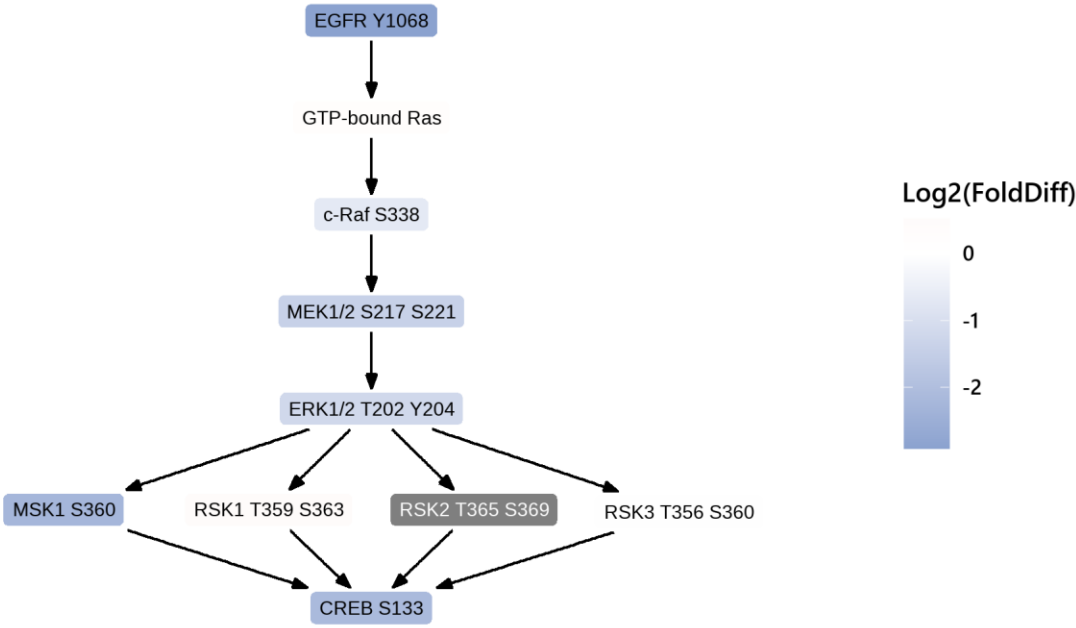


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Figure 3: Pathmap for Drug A 0.1uM vs. Vehicle 0.1uM

Drug A 1uM vs. Vehicle 1uM

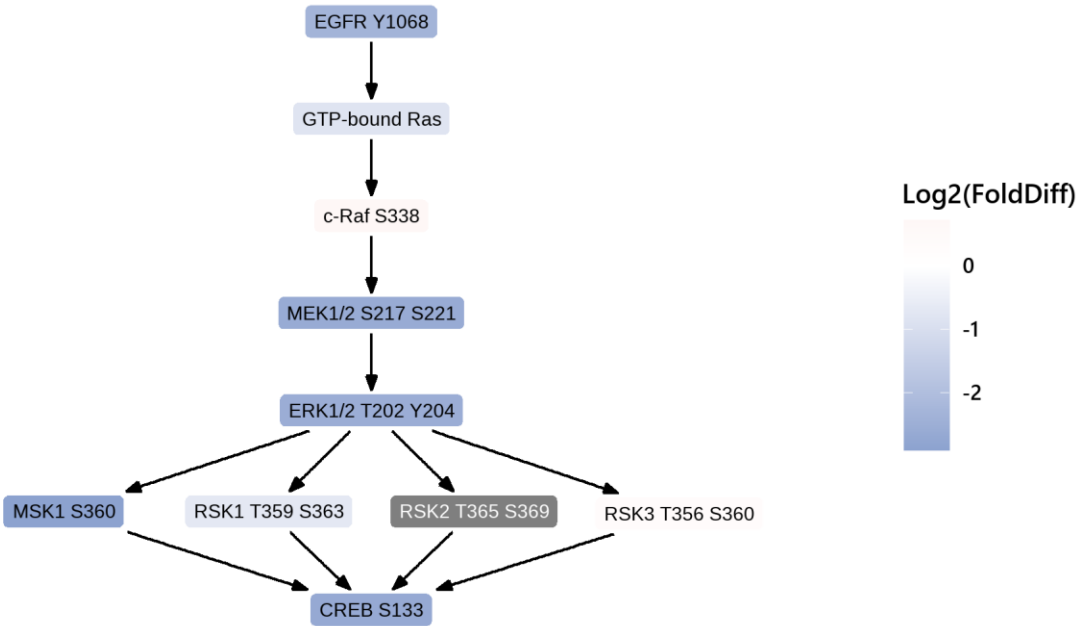


Source: Example Report



Figure 4: Pathmap for Drug A 1uM vs. Vehicle 1uM

Drug A 10uM vs. Vehicle 10uM



Source: Example Report



Figure 5: Pathmap for Drug A 10uM vs. Vehicle 10uM

Correlations

We further performed a correlation analysis, evaluating correlation patterns between analytes within each particular sample group.

Correlations Sample Size Overview

Overall, sample size was limited, which reduces the impact of these results somewhat. Future experiments with larger sample sizes are warranted to support the results described below. No correlation analysis was possible for the treatment group at 10 μ M due to a sample size of 2 (Table 3).

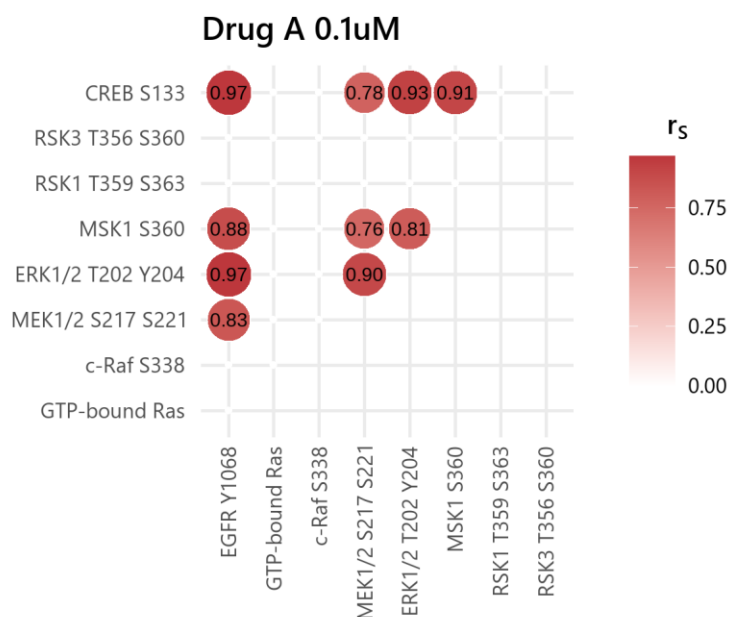
Table 3: Correlations Sample Size

Treatment	N _{minimal}
Drug A 0.1uM	8
Drug A 10uM	2 ^a
Drug A 1uM	5
Vehicle 0.1uM	9
Vehicle 10uM	9
Vehicle 1uM	9

^aSample size too low

Correlation Analysis per Treatment

We found a significant increase in the number of analyte-analyte correlations for drug treated samples compared to vehicle controls (Figures 6 - 10, Tables 4 - 8). This highlights the direct response to Drug A, which drives cell behavior. **Most of the correlations were found between the pathway core analytes (MEK, ERK, MSK1) and either EGFR Y1068 or CREB S133.** Interestingly, inverse correlations were found between GTP-bound Ras and EGFR Y1068, MEK 1/2 S217 S221, ERK 1/2 T202 Y204, and MSK1 S360.



Source: Example Report



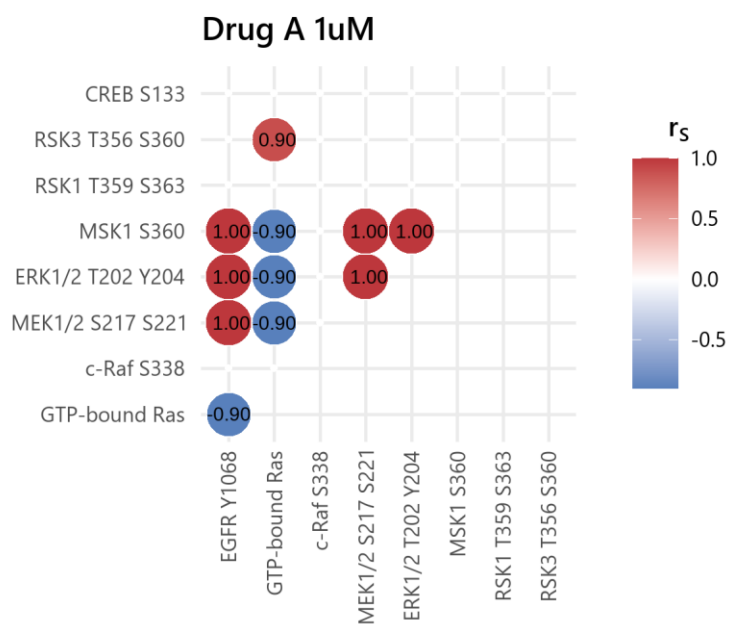
Figure 6: Spearman's rho correlogram for Drug A 0.1uM

Table 4: Correlations for Drug A 0.1uM

Analyte 1	Analyte 2	Pearson's r^a	p_P	Spearman's rho	p_s
EGFR Y1068	CREB S133	0.91	< 0.01	0.97	< 0.01
EGFR Y1068	ERK1/2 T202 Y204	0.89	< 0.01	0.97	< 0.01
ERK1/2 T202 Y204	CREB S133	0.77	< 0.01	0.93	< 0.01
GTP-bound Ras	CREB S133	-0.70	0.08	-0.66	0.08
c-Raf S338	RSK1 T359 S363	0.70	0.12	0.60	0.12
EGFR Y1068	GTP-bound Ras	-0.66	0.12	-0.60	0.12
MEK1/2 S217 S221	ERK1/2 T202 Y204	0.64	< 0.01	0.90	< 0.01
EGFR Y1068	MSK1 S360	0.60	< 0.01	0.88	< 0.01
ERK1/2 T202 Y204	MSK1 S360	0.59	0.01	0.81	0.01
EGFR Y1068	MEK1/2 S217 S221	0.56	0.01	0.83	0.01
MEK1/2 S217 S221	RSK1 T359 S363	0.50	0.74	0.14	0.74
EGFR Y1068	RSK1 T359 S363	0.49	0.69	0.17	0.69
GTP-bound Ras	RSK3 T356 S360	-0.48	0.42	-0.33	0.42
GTP-bound Ras	MSK1 S360	-0.43	0.22	-0.49	0.22
MSK1 S360	RSK3 T356 S360	0.42	0.37	0.37	0.37
GTP-bound Ras	ERK1/2 T202 Y204	-0.40	0.24	-0.47	0.24
MEK1/2 S217 S221	MSK1 S360	0.39	0.03	0.76	0.03
c-Raf S338	MSK1 S360	0.39	0.37	0.37	0.37
MEK1/2 S217 S221	CREB S133	0.36	0.02	0.78	0.02
ERK1/2 T202 Y204	RSK1 T359 S363	0.35	0.80	0.11	0.80

Analyte 1	Analyte 2	Pearson's r ^a	p _P	Spearman's rho	p _s
RSK1 T359 S363	CREB S133	0.34	0.80	0.11	0.80
MSK1 S360	CREB S133	0.32	< 0.01	0.91	< 0.01
GTP-bound Ras	c-Raf S338	0.30	0.65	0.19	0.65
c-Raf S338	MEK1/2 S217 S221	0.21	0.53	0.26	0.53
MSK1 S360	RSK1 T359 S363	0.18	0.91	0.05	0.91
ERK1/2 T202 Y204	RSK3 T356 S360	-0.16	0.82	0.10	0.82
EGFR Y1068	RSK3 T356 S360	0.16	0.61	0.21	0.61
EGFR Y1068	c-Raf S338	0.13	0.57	0.24	0.57
c-Raf S338	CREB S133	-0.13	0.73	0.14	0.73
c-Raf S338	ERK1/2 T202 Y204	0.11	0.69	0.17	0.69
GTP-bound Ras	MEK1/2 S217 S221	0.11	0.78	-0.12	0.78
GTP-bound Ras	RSK1 T359 S363	0.09	0.69	0.17	0.69
c-Raf S338	RSK3 T356 S360	0.08	0.57	0.24	0.57
RSK1 T359 S363	RSK3 T356 S360	0.04	0.91	-0.05	0.91
RSK3 T356 S360	CREB S133	-0.04	0.53	0.26	0.53
MEK1/2 S217 S221	RSK3 T356 S360	0.01	0.61	0.21	0.61

^aSorted by absolute value of Pearson's r



Source: Example Report



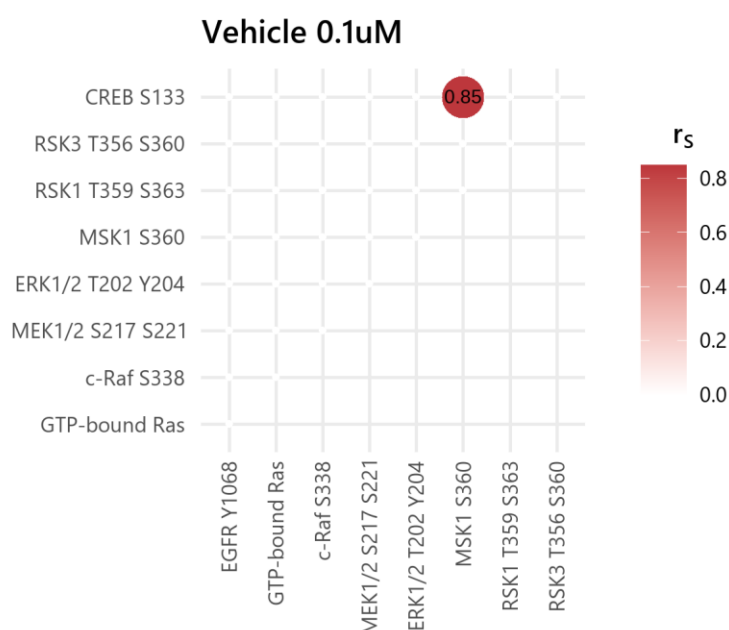
Figure 7: Spearman's rho correlogram for Drug A 1uM

Table 5: Correlations for Drug A 1uM

Analyte 1	Analyte 2	Pearson's r ^a	p _P	Spearman's rho	p _s
EGFR Y1068	MEK1/2 S217 S221	0.98	< 0.01	1.00	< 0.01

Analyte 1	Analyte 2	Pearson's r ^a	p _P	Spearman's rho	p _S
EGFR Y1068	GTP-bound Ras	-0.97	0.04	-0.90	0.04
GTP-bound Ras	MEK1/2 S217 S221	-0.94	0.04	-0.90	0.04
MEK1/2 S217 S221	MSK1 S360	0.94	< 0.01	1.00	< 0.01
ERK1/2 T202 Y204	MSK1 S360	0.91	< 0.01	1.00	< 0.01
GTP-bound Ras	MSK1 S360	-0.90	0.04	-0.90	0.04
GTP-bound Ras	RSK3 T356 S360	0.90	0.04	0.90	0.04
MEK1/2 S217 S221	ERK1/2 T202 Y204	0.89	< 0.01	1.00	< 0.01
ERK1/2 T202 Y204	CREB S133	0.88	0.05	0.87	0.05
EGFR Y1068	MSK1 S360	0.88	< 0.01	1.00	< 0.01
EGFR Y1068	CREB S133	0.85	0.05	0.87	0.05
MSK1 S360	RSK3 T356 S360	-0.85	0.19	-0.70	0.19
EGFR Y1068	ERK1/2 T202 Y204	0.82	< 0.01	1.00	< 0.01
MEK1/2 S217 S221	CREB S133	0.81	0.05	0.87	0.05
GTP-bound Ras	CREB S133	-0.78	0.17	-0.72	0.17
MSK1 S360	CREB S133	0.78	0.05	0.87	0.05
MEK1/2 S217 S221	RSK3 T356 S360	-0.77	0.19	-0.70	0.19
EGFR Y1068	RSK3 T356 S360	-0.77	0.19	-0.70	0.19
GTP-bound Ras	ERK1/2 T202 Y204	-0.76	0.04	-0.90	0.04
c-Raf S338	MSK1 S360	0.70	0.87	0.10	0.87
c-Raf S338	RSK3 T356 S360	-0.69	0.50	-0.40	0.50
ERK1/2 T202 Y204	RSK3 T356 S360	-0.57	0.19	-0.70	0.19
c-Raf S338	ERK1/2 T202 Y204	0.56	0.87	0.10	0.87
RSK3 T356 S360	CREB S133	-0.52	0.55	-0.36	0.55
GTP-bound Ras	c-Raf S338	-0.49	0.62	-0.30	0.62
c-Raf S338	CREB S133	0.42	0.87	0.10	0.87
c-Raf S338	MEK1/2 S217 S221	0.41	0.87	0.10	0.87
RSK1 T359 S363	CREB S133	-0.40	0.32	-0.56	0.32
c-Raf S338	RSK1 T359 S363	0.34	0.50	0.40	0.50
EGFR Y1068	c-Raf S338	0.34	0.87	0.10	0.87
MSK1 S360	RSK1 T359 S363	0.22	0.75	-0.20	0.75
RSK1 T359 S363	RSK3 T356 S360	-0.21	0.62	-0.30	0.62
EGFR Y1068	RSK1 T359 S363	-0.15	0.75	-0.20	0.75
ERK1/2 T202 Y204	RSK1 T359 S363	0.07	0.75	-0.20	0.75
GTP-bound Ras	RSK1 T359 S363	0.06	0.87	0.10	0.87
MEK1/2 S217 S221	RSK1 T359 S363	0.05	0.75	-0.20	0.75

^aSorted by absolute value of Pearson's r



Source: Example Report



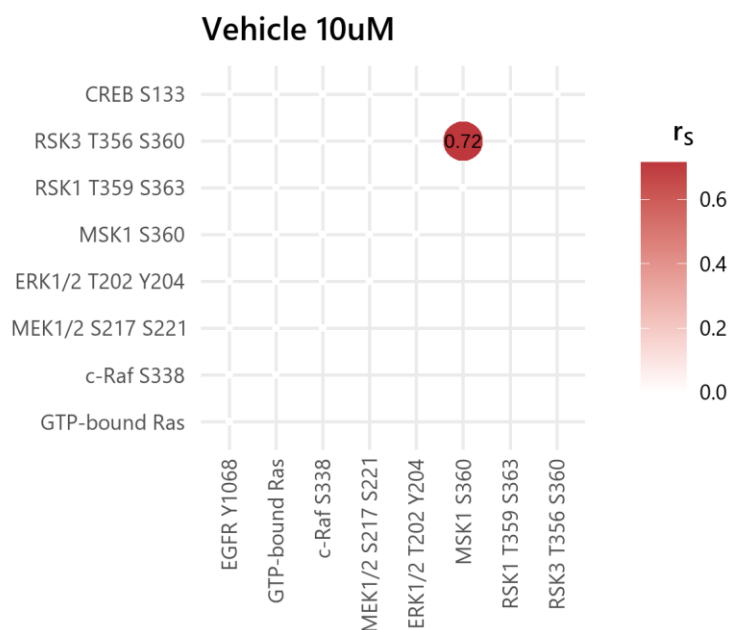
Figure 8: Spearman's rho correlogram for Vehicle 0.1uM

Table 6: Correlations for Vehicle 0.1uM

Analyte 1	Analyte 2	Pearson's r^a	p_P	Spearman's rho	p_s
c-Raf S338	RSK3 T356 S360	-0.81	0.08	-0.62	0.08
MSK1 S360	CREB S133	0.78	< 0.01	0.85	< 0.01
EGFR Y1068	GTP-bound Ras	0.66	0.07	0.63	0.07
GTP-bound Ras	RSK3 T356 S360	-0.63	0.29	-0.40	0.29
GTP-bound Ras	c-Raf S338	0.57	0.31	0.38	0.31
EGFR Y1068	CREB S133	0.55	0.10	0.58	0.10
MSK1 S360	RSK1 T359 S363	0.48	0.31	0.38	0.31
c-Raf S338	MEK1/2 S217 S221	-0.46	0.12	-0.55	0.12
ERK1/2 T202 Y204	MSK1 S360	0.41	0.36	0.35	0.36
ERK1/2 T202 Y204	RSK1 T359 S363	0.39	0.55	0.23	0.55
c-Raf S338	ERK1/2 T202 Y204	0.39	0.58	0.22	0.58
MSK1 S360	RSK3 T356 S360	-0.36	0.33	-0.37	0.33
EGFR Y1068	MEK1/2 S217 S221	-0.33	0.31	-0.38	0.31
MEK1/2 S217 S221	RSK3 T356 S360	0.32	0.24	0.43	0.24
ERK1/2 T202 Y204	RSK3 T356 S360	-0.30	0.55	-0.23	0.55
c-Raf S338	CREB S133	-0.27	0.41	-0.32	0.41
RSK1 T359 S363	CREB S133	0.27	0.43	0.30	0.43
EGFR Y1068	RSK3 T356 S360	-0.23	0.55	-0.23	0.55
EGFR Y1068	MSK1 S360	0.22	0.46	0.28	0.46
GTP-bound Ras	CREB S133	0.21	0.73	0.13	0.73

Analyte 1	Analyte 2	Pearson's r ^a	p _P	Spearman's rho	p _S
EGFR Y1068	ERK1/2 T202 Y204	-0.18	0.73	-0.13	0.73
GTP-bound Ras	RSK1 T359 S363	0.17	0.38	0.33	0.38
GTP-bound Ras	MSK1 S360	0.14	0.73	0.13	0.73
EGFR Y1068	c-Raf S338	0.13	0.80	0.10	0.80
MEK1/2 S217 S221	CREB S133	0.12	0.90	-0.05	0.90
RSK1 T359 S363	RSK3 T356 S360	-0.10	0.33	-0.37	0.33
MEK1/2 S217 S221	ERK1/2 T202 Y204	-0.10	0.97	-0.02	0.97
GTP-bound Ras	ERK1/2 T202 Y204	0.09	0.80	-0.10	0.80
ERK1/2 T202 Y204	CREB S133	0.07	0.80	0.10	0.80
MEK1/2 S217 S221	RSK1 T359 S363	0.06	0.90	-0.05	0.90
c-Raf S338	RSK1 T359 S363	-0.05	0.97	-0.02	0.97
RSK3 T356 S360	CREB S133	0.05	0.80	-0.10	0.80
EGFR Y1068	RSK1 T359 S363	0.04	0.61	0.20	0.61
GTP-bound Ras	MEK1/2 S217 S221	-0.02	0.86	0.07	0.86
c-Raf S338	MSK1 S360	-0.01	0.55	-0.23	0.55
MEK1/2 S217 S221	MSK1 S360	0.01	0.67	0.17	0.67

^aSorted by absolute value of Pearson's r



Source: Example Report



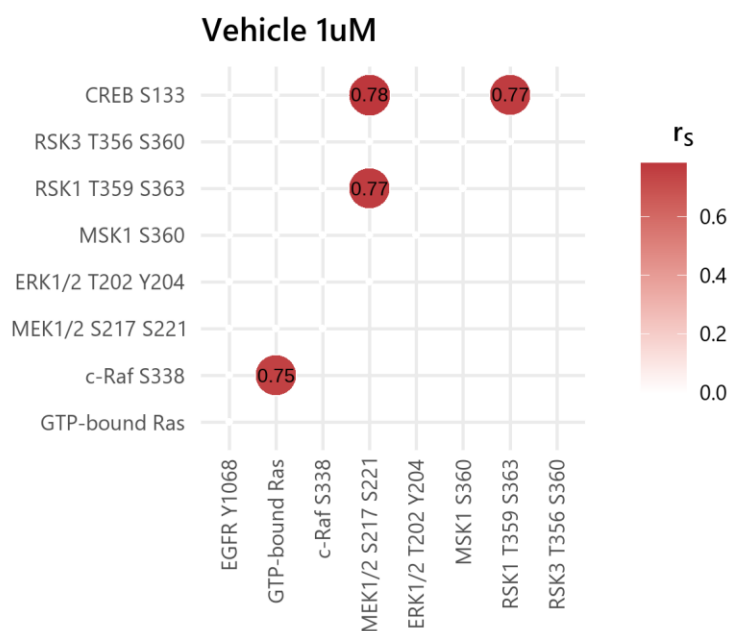
Figure 9: Spearman's rho correlogram for Vehicle 10uM

Table 7: Correlations for Vehicle 10uM

Analyte 1	Analyte 2	Pearson's r ^a	p _P	Spearman's rho	p _S
MSK1 S360	RSK3 T356 S360	0.73	0.03	0.72	0.03

Analyte 1	Analyte 2	Pearson's r ^a	p _P	Spearman's rho	p _S
MEK1/2 S217 S221	RSK3 T356 S360	0.67	0.09	0.60	0.09
MEK1/2 S217 S221	CREB S133	0.62	0.06	0.65	0.06
EGFR Y1068	ERK1/2 T202 Y204	0.58	0.10	0.58	0.10
c-Raf S338	CREB S133	0.48	0.10	0.58	0.10
MEK1/2 S217 S221	MSK1 S360	0.48	0.21	0.47	0.21
GTP-bound Ras	c-Raf S338	0.46	0.10	0.58	0.10
EGFR Y1068	GTP-bound Ras	0.41	0.46	0.28	0.46
RSK1 T359 S363	CREB S133	-0.41	0.19	-0.48	0.19
GTP-bound Ras	MEK1/2 S217 S221	0.41	0.43	0.30	0.43
GTP-bound Ras	MSK1 S360	-0.38	0.29	-0.40	0.29
EGFR Y1068	MEK1/2 S217 S221	0.37	0.49	0.27	0.49
MEK1/2 S217 S221	RSK1 T359 S363	-0.36	0.26	-0.42	0.26
c-Raf S338	MEK1/2 S217 S221	0.36	0.17	0.50	0.17
GTP-bound Ras	ERK1/2 T202 Y204	0.34	0.38	0.33	0.38
GTP-bound Ras	RSK1 T359 S363	-0.29	0.70	-0.15	0.70
GTP-bound Ras	RSK3 T356 S360	0.29	0.55	0.23	0.55
ERK1/2 T202 Y204	MSK1 S360	-0.28	0.49	-0.27	0.49
ERK1/2 T202 Y204	CREB S133	-0.28	0.43	-0.30	0.43
c-Raf S338	RSK3 T356 S360	0.25	0.41	0.32	0.41
EGFR Y1068	RSK3 T356 S360	0.20	0.38	0.33	0.38
EGFR Y1068	RSK1 T359 S363	0.17	0.80	0.10	0.80
MEK1/2 S217 S221	ERK1/2 T202 Y204	-0.15	0.46	-0.28	0.46
ERK1/2 T202 Y204	RSK1 T359 S363	-0.14	0.83	-0.08	0.83
EGFR Y1068	c-Raf S338	-0.11	0.90	0.05	0.90
ERK1/2 T202 Y204	RSK3 T356 S360	-0.11	1.00	0.00	1.00
c-Raf S338	MSK1 S360	-0.11	0.80	-0.10	0.80
EGFR Y1068	CREB S133	-0.10	0.70	-0.15	0.70
EGFR Y1068	MSK1 S360	0.09	0.70	0.15	0.70
GTP-bound Ras	CREB S133	0.09	0.86	0.07	0.86
RSK1 T359 S363	RSK3 T356 S360	-0.08	0.93	0.03	0.93
MSK1 S360	RSK1 T359 S363	0.08	0.97	-0.02	0.97
MSK1 S360	CREB S133	0.08	0.77	0.12	0.77
c-Raf S338	ERK1/2 T202 Y204	-0.07	0.93	-0.03	0.93
RSK3 T356 S360	CREB S133	0.05	0.80	0.10	0.80
c-Raf S338	RSK1 T359 S363	0.01	0.80	0.10	0.80

^aSorted by absolute value of Pearsons' r



Source: Example Report



Figure 10: Spearman's rho correlogram for Vehicle 1uM

Table 8: Correlations for Vehicle 1uM

Analyte 1	Analyte 2	Pearson's r^a	p_P	Spearman's rho	p_S
RSK1 T359 S363	CREB S133	0.76	0.02	0.77	0.02
MEK1/2 S217 S221	CREB S133	0.73	0.01	0.78	0.01
GTP-bound Ras	c-Raf S338	0.68	0.02	0.75	0.02
ERK1/2 T202 Y204	RSK3 T356 S360	0.63	0.08	0.62	0.08
MEK1/2 S217 S221	RSK1 T359 S363	0.57	0.02	0.77	0.02
c-Raf S338	RSK3 T356 S360	0.46	0.70	0.15	0.70
MSK1 S360	RSK3 T356 S360	0.41	0.29	0.40	0.29
ERK1/2 T202 Y204	MSK1 S360	0.41	0.61	0.20	0.61
c-Raf S338	MEK1/2 S217 S221	0.40	0.24	0.43	0.24
MSK1 S360	CREB S133	0.35	0.31	0.38	0.31
GTP-bound Ras	MEK1/2 S217 S221	0.33	0.36	0.35	0.36
c-Raf S338	CREB S133	0.32	0.49	0.27	0.49
EGFR Y1068	CREB S133	-0.32	0.36	-0.35	0.36
c-Raf S338	RSK1 T359 S363	0.31	0.14	0.53	0.14
RSK1 T359 S363	RSK3 T356 S360	-0.31	0.46	-0.28	0.46
EGFR Y1068	MSK1 S360	-0.28	0.55	-0.23	0.55
EGFR Y1068	c-Raf S338	0.28	0.58	0.22	0.58
MEK1/2 S217 S221	ERK1/2 T202 Y204	-0.28	0.52	-0.25	0.52
GTP-bound Ras	RSK3 T356 S360	0.25	0.97	-0.02	0.97
GTP-bound Ras	ERK1/2 T202 Y204	-0.25	0.26	-0.42	0.26

Analyte 1	Analyte 2	Pearson's r ^a	p _P	Spearman's rho	p _S
EGFR Y1068	RSK3 T356 S360	0.23	0.77	-0.12	0.77
EGFR Y1068	RSK1 T359 S363	-0.20	0.90	-0.05	0.90
EGFR Y1068	ERK1/2 T202 Y204	-0.18	0.61	-0.20	0.61
GTP-bound Ras	MSK1 S360	-0.16	0.49	-0.27	0.49
MSK1 S360	RSK1 T359 S363	-0.16	0.80	-0.10	0.80
ERK1/2 T202 Y204	RSK1 T359 S363	-0.16	0.90	-0.05	0.90
c-Raf S338	ERK1/2 T202 Y204	0.15	0.86	0.07	0.86
EGFR Y1068	MEK1/2 S217 S221	0.10	0.73	0.13	0.73
EGFR Y1068	GTP-bound Ras	0.06	0.83	0.08	0.83
GTP-bound Ras	RSK1 T359 S363	-0.06	0.55	0.23	0.55
RSK3 T356 S360	CREB S133	0.06	0.93	0.03	0.93
c-Raf S338	MSK1 S360	-0.05	0.58	-0.22	0.58
ERK1/2 T202 Y204	CREB S133	0.03	0.73	-0.13	0.73
MEK1/2 S217 S221	RSK3 T356 S360	0.02	0.83	-0.08	0.83
MEK1/2 S217 S221	MSK1 S360	-0.02	0.83	0.08	0.83
GTP-bound Ras	CREB S133	-0.01	0.67	0.17	0.67

^aSorted by absolute value of Pearson's r

PDX Model Data - Tumor

PDX Tumor: Data Overview

For an example data overview interpretation see p. 4.

Table 9: Data Overview

Analyte	Groups	N	Missing	Mean	SD	Spread	Density
c-Raf S338	6	26	0	10,008.94	5,599.31		
CREB S133	6	26	0	5,001.02	3,607.43		
EGFR Y1068	6	26	0	19,070.87	13,188.21		
ERK1/2 T202 Y204	6	26	0	25,210.61	13,300.08		
MEK1/2 S217 S221	6	26	0	8,354.40	1,831.90		
MSK1 S360	6	26	0	49,861.57	36,155.55		
RSK1 T359 S363	6	26	0	1,207.68	1,016.20		
GTP-bound Ras	6	26	0	13,976.61	6,753.51		
RSK3 T356 S360	6	26	0	7,167.86	2,435.01		

Mean Comparison Statistics

For an example mean comparison interpretation see p. 4.

Table 10: Mean Comparison Statistics

Var ₁	Var ₂	N ₁	N ₂	log2FD	Mean ₁ ^a	SD ₁ ^a	Mean ₂ ^a	SD ₂ ^a	p	p _{adj}
EGFR Y1068										
Drug A 0.1uM	Vehicle 0.1uM	6	6	-1.61	9.87	3.78	30.16	7.49	< 0.01	< 0.01
Drug A 1uM	Vehicle 1uM	5	5	-3.09	3.82	4.06	32.59	7.70	0.01	0.01
Drug A 10uM	Vehicle 10uM	2	2	-1.63	9.00	2.81	27.83	2.48	N/A ^b	N/A ^b
GTP-bound Ras										
Drug A 0.1uM	Vehicle 0.1uM	6	6	-0.04	14.51	5.40	14.96	8.72	0.82	1.00
Drug A 1uM	Vehicle 1uM	5	5	0.07	13.68	3.45	13.00	6.42	1.00	1.00
Drug A 10uM	Vehicle 10uM	2	2	1.54	19.79	14.72	6.79	0.98	N/A ^b	N/A ^b
c-Raf S338										
Drug A 0.1uM	Vehicle 0.1uM	6	6	-0.23	9.67	4.19	11.33	5.66	1.00	1.00
Drug A 1uM	Vehicle 1uM	5	5	0.92	10.74	6.66	5.68	5.14	0.42	0.84
Drug A 10uM	Vehicle 10uM	2	2	-1.12	8.22	3.91	17.88	0.19	N/A ^b	N/A ^b

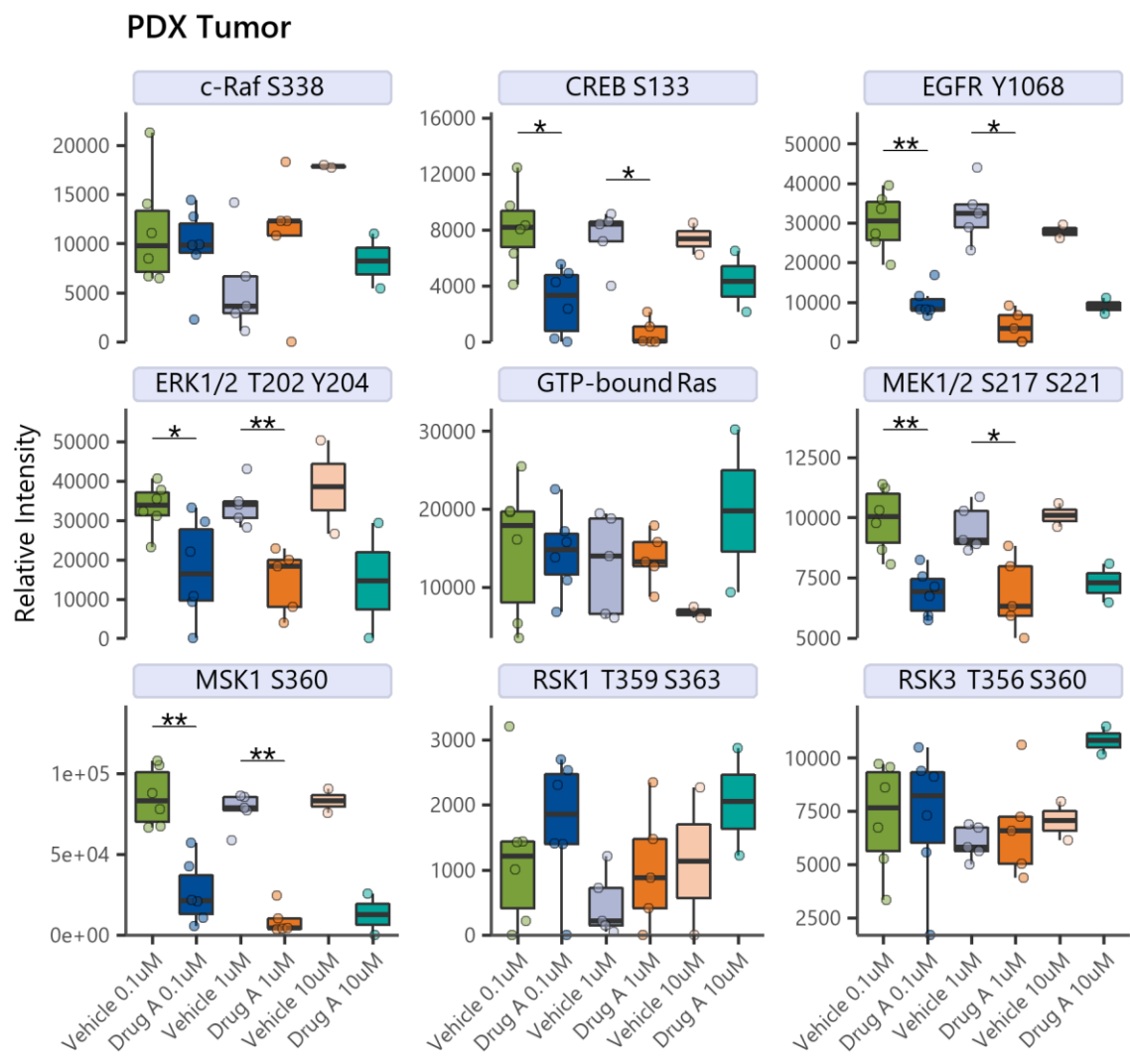
Var ₁	Var ₂	N ₁	N ₂	log2FD	Mean ₁ ^a	SD ₁ ^a	Mean ₂ ^a	SD ₂ ^a	p	p _{adj}
MEK1/2 S217 S221										
Drug A 0.1uM	Vehicle 0.1uM	6	6	-0.52	6.89	0.96	9.91	1.34	< 0.01	< 0.01
Drug A 1uM	Vehicle 1uM	5	5	-0.49	6.80	1.57	9.54	0.97	0.02	0.02
Drug A 10uM	Vehicle 10uM	2	2	-0.47	7.28	1.13	10.10	0.70	N/A ^b	N/A ^b
ERK1/2 T202 Y204										
Drug A 0.1uM	Vehicle 0.1uM	6	6	-0.93	17.48	12.92	33.40	6.11	0.03	0.03
Drug A 1uM	Vehicle 1uM	5	5	-1.22	14.65	8.20	34.15	5.61	< 0.01	0.02
Drug A 10uM	Vehicle 10uM	2	2	-1.39	14.64	20.70	38.46	16.87	N/A ^b	N/A ^b
MSK1 S360										
Drug A 0.1uM	Vehicle 0.1uM	6	6	-1.70	26.35	19.69	85.52	18.00	< 0.01	< 0.01
Drug A 1uM	Vehicle 1uM	5	5	-3.04	9.38	9.01	77.30	11.13	< 0.01	< 0.01
Drug A 10uM	Vehicle 10uM	2	2	-2.70	12.78	18.07	83.11	10.44	N/A ^b	N/A ^b
RSK1 T359 S363										
Drug A 0.1uM	Vehicle 0.1uM	6	6	0.50	1.72	1.01	1.21	1.15	0.63	0.84
Drug A 1uM	Vehicle 1uM	5	5	1.12	1.02	0.92	0.47	0.49	0.42	0.84
Drug A 10uM	Vehicle 10uM	2	2	0.85	2.05	1.17	1.13	1.60	N/A ^b	N/A ^b
RSK3 T356 S360										
Drug A 0.1uM	Vehicle 0.1uM	6	6	0.01	7.26	3.24	7.20	2.56	0.94	1.00
Drug A 1uM	Vehicle 1uM	5	5	0.17	6.76	2.44	6.02	0.79	0.84	1.00
Drug A 10uM	Vehicle 10uM	2	2	0.62	10.82	0.94	7.04	1.29	N/A ^b	N/A ^b
CREB S133										
Drug A 0.1uM	Vehicle 0.1uM	6	6	-1.50	2.88	2.40	8.16	2.85	0.01	0.02
Drug A 1uM	Vehicle 1uM	5	5	-3.52	0.65	0.96	7.45	2.06	0.01	0.02
Drug A 10uM	Vehicle 10uM	2	2	-0.77	4.31	3.08	7.35	1.59	N/A ^b	N/A ^b

^ax * 1000

^bSample size too low

Boxplots

For example boxplot interpretations see p. 6.



Source: Example Report

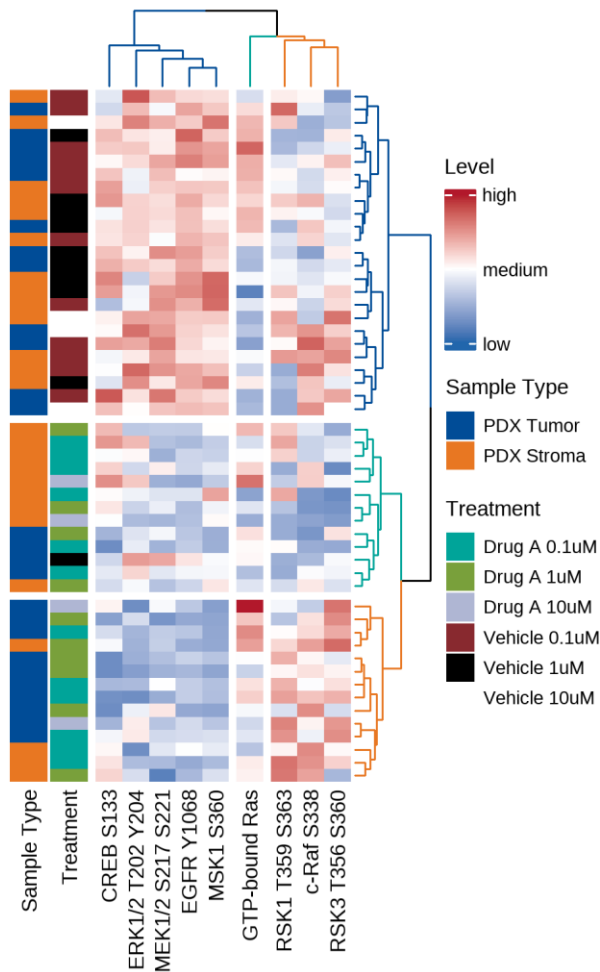


Figure 11: Boxplots by Analyte

Heatmap

For an example heatmap interpretation see p. 7.

PDX Tumor/Stroma Heatmap



Source: Example Report

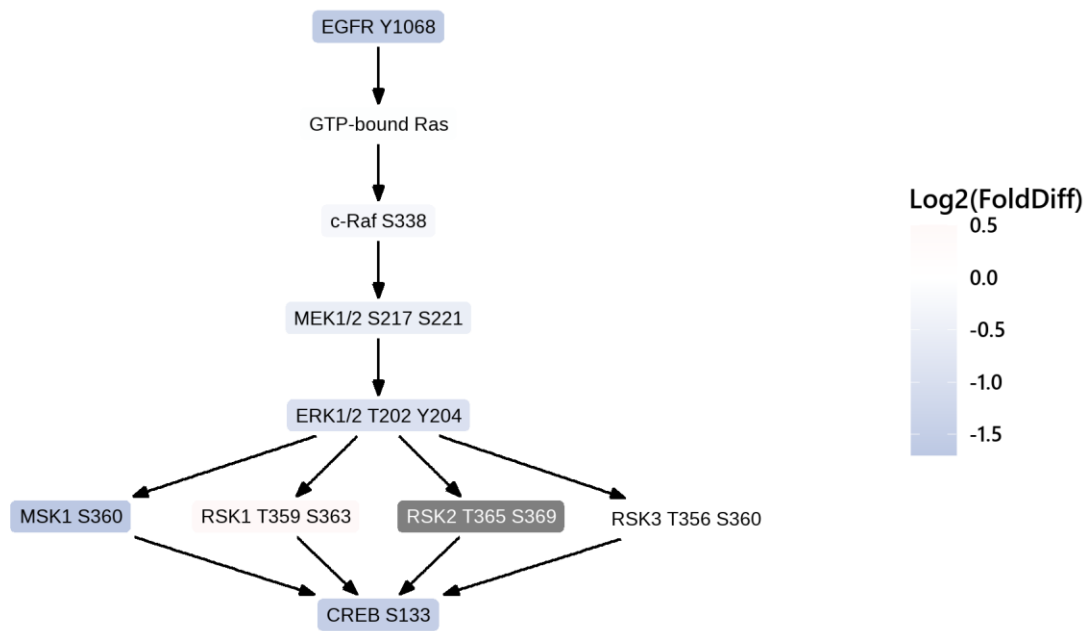


Figure 12: Heatmap PDX Tumor

Pathmaps

For example pathmap interpretations see p. 8.

Drug A 0.1uM vs. Vehicle 0.1uM

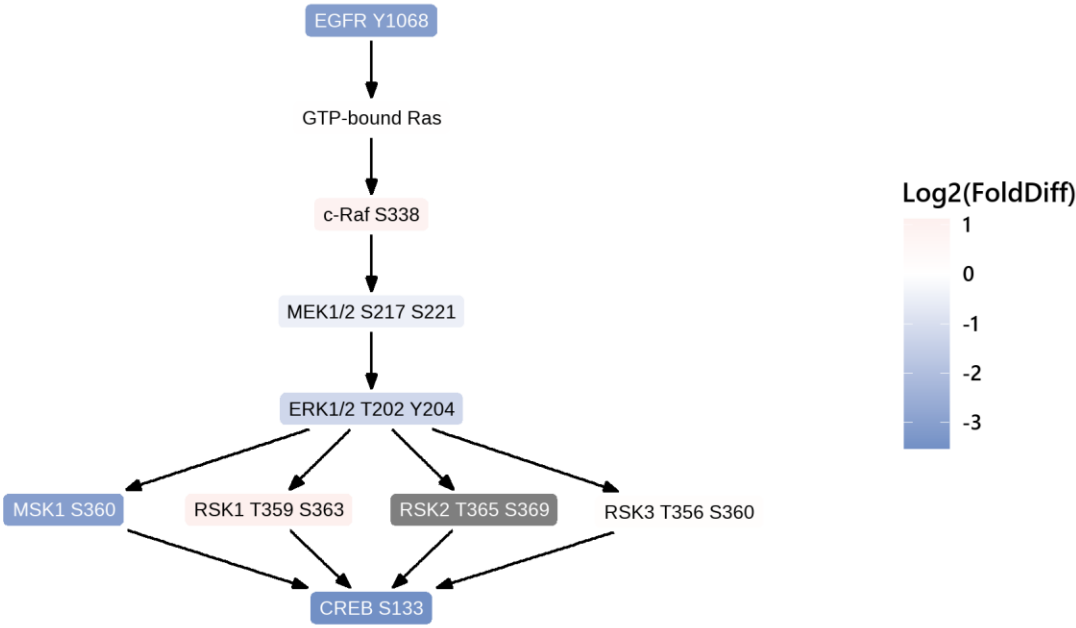


Source: Example Report



Figure 13: Pathmap for Drug A 0.1uM vs. Vehicle 0.1uM

Drug A 1uM vs. Vehicle 1uM

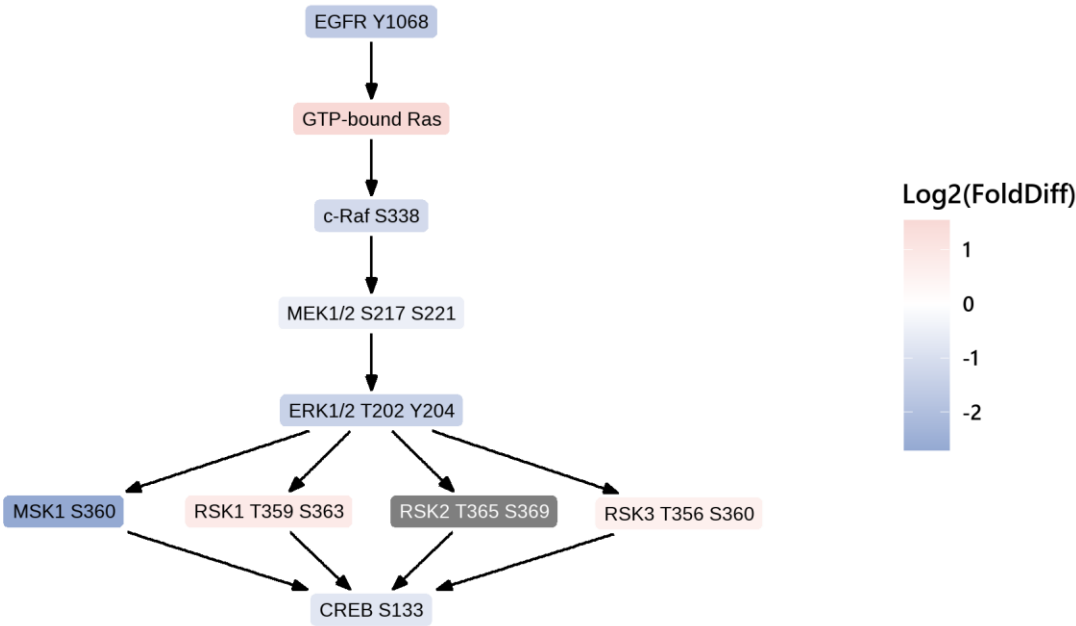


Source: Example Report



Figure 14: Pathmap for Drug A 1uM vs. Vehicle 1uM

Drug A 10uM vs. Vehicle 10uM



Source: Example Report



Figure 15: Pathmap for Drug A 10uM vs. Vehicle 10uM

Correlations

Correlations Sample Size Overview

For an example correlation sample size overview interpretation see p. 10.

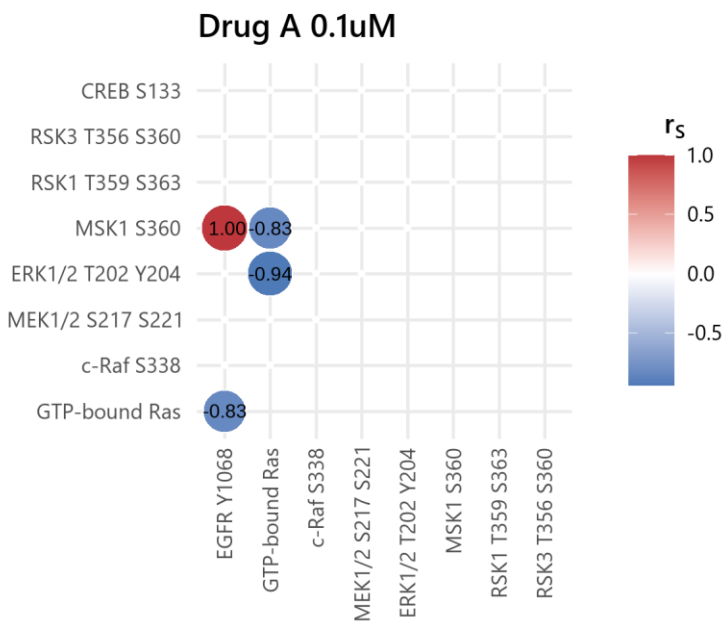
Table 11: Correlations Sample Size

Treatment	N _{minimal}
Drug A 0.1uM	6
Drug A 10uM	2 ^a
Drug A 1uM	5
Vehicle 0.1uM	6
Vehicle 10uM	2 ^a
Vehicle 1uM	5

^aSample size too low

Correlation Analysis per Treatment

For example correlation interpretations see p. 10.



Source: Example Report



Figure 16: Correlogram for Drug A 0.1uM

Table 12: Correlations for Drug A 0.1uM

Analyte 1	Analyte 2	Pearson's r ^a	p _P	Spearman's rho	p _S
EGFR Y1068	MSK1 S360	0.96	< 0.01	1.00	< 0.01
EGFR Y1068	GTP-bound Ras	-0.90	0.04	-0.83	0.04
GTP-bound Ras	MSK1 S360	-0.89	0.04	-0.83	0.04
GTP-bound Ras	ERK1/2 T202 Y204	-0.83	< 0.01	-0.94	< 0.01
c-Raf S338	RSK3 T356 S360	0.79	0.47	0.37	0.47
EGFR Y1068	ERK1/2 T202 Y204	0.77	0.11	0.71	0.11
ERK1/2 T202 Y204	MSK1 S360	0.71	0.11	0.71	0.11
MEK1/2 S217 S221	CREB S133	0.60	0.27	0.54	0.27
MSK1 S360	CREB S133	0.59	0.21	0.60	0.21
ERK1/2 T202 Y204	CREB S133	0.58	0.27	0.54	0.27
EGFR Y1068	RSK1 T359 S363	-0.58	0.87	0.09	0.87
c-Raf S338	ERK1/2 T202 Y204	-0.56	0.07	-0.77	0.07
EGFR Y1068	CREB S133	0.55	0.21	0.60	0.21
RSK1 T359 S363	RSK3 T356 S360	0.53	0.27	0.54	0.27
RSK3 T356 S360	CREB S133	0.49	0.21	0.60	0.21
ERK1/2 T202 Y204	RSK1 T359 S363	-0.49	0.62	-0.26	0.62
MEK1/2 S217 S221	MSK1 S360	0.46	0.21	0.60	0.21
GTP-bound Ras	c-Raf S338	0.40	0.11	0.71	0.11
EGFR Y1068	MEK1/2 S217 S221	0.39	0.21	0.60	0.21
GTP-bound Ras	RSK1 T359 S363	0.37	0.87	0.09	0.87
MEK1/2 S217 S221	ERK1/2 T202 Y204	0.34	0.27	0.54	0.27
MSK1 S360	RSK1 T359 S363	-0.33	0.87	0.09	0.87
GTP-bound Ras	CREB S133	-0.31	0.40	-0.43	0.40
GTP-bound Ras	RSK3 T356 S360	0.31	0.54	0.31	0.54
GTP-bound Ras	MEK1/2 S217 S221	-0.31	0.40	-0.43	0.40
ERK1/2 T202 Y204	RSK3 T356 S360	-0.30	0.62	-0.26	0.62
RSK1 T359 S363	CREB S133	-0.24	0.96	0.03	0.96
c-Raf S338	CREB S133	0.22	0.87	-0.09	0.87
c-Raf S338	RSK1 T359 S363	0.20	0.96	0.03	0.96
MEK1/2 S217 S221	RSK3 T356 S360	0.17	0.96	-0.03	0.96
MEK1/2 S217 S221	RSK1 T359 S363	-0.14	0.70	-0.20	0.70
MSK1 S360	RSK3 T356 S360	0.10	0.96	-0.03	0.96
EGFR Y1068	RSK3 T356 S360	-0.10	0.96	-0.03	0.96
c-Raf S338	MEK1/2 S217 S221	0.07	0.70	-0.20	0.70
EGFR Y1068	c-Raf S338	-0.06	0.62	-0.26	0.62
c-Raf S338	MSK1 S360	0.04	0.62	-0.26	0.62

^aSorted by absolute value of Pearsons' r

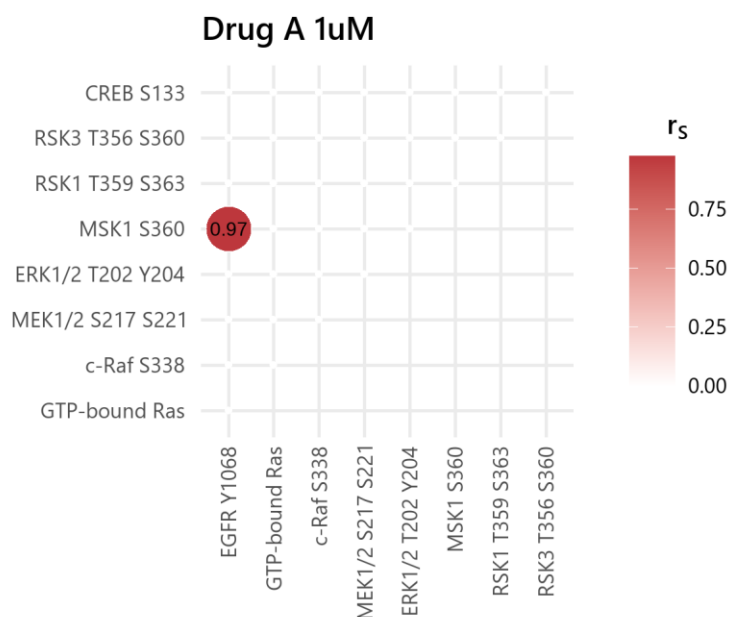


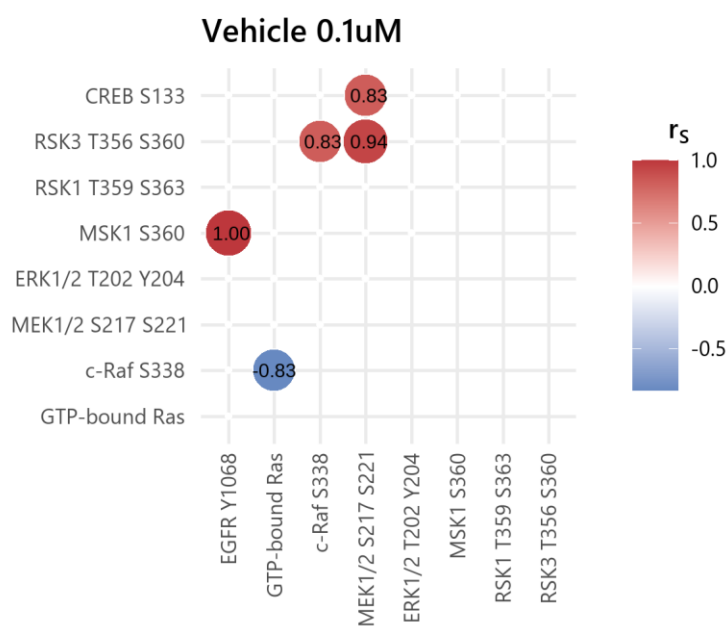
Figure 17: Correlogram for Drug A 1uM

Table 13: Correlations for Drug A 1uM

Analyte 1	Analyte 2	Pearson's r^a	p_P	Spearman's rho	p_s
EGFR Y1068	MSK1 S360	0.89	< 0.01	0.97	< 0.01
c-Raf S338	CREB S133	-0.84	0.05	-0.87	0.05
RSK1 T359 S363	CREB S133	-0.80	0.22	-0.67	0.22
ERK1/2 T202 Y204	RSK1 T359 S363	-0.75	0.28	-0.60	0.28
GTP-bound Ras	CREB S133	0.69	0.05	0.87	0.05
MEK1/2 S217 S221	RSK3 T356 S360	-0.67	0.19	-0.70	0.19
GTP-bound Ras	ERK1/2 T202 Y204	0.65	0.62	0.30	0.62
GTP-bound Ras	RSK3 T356 S360	0.63	0.19	0.70	0.19
MSK1 S360	RSK1 T359 S363	0.63	0.62	0.30	0.62
EGFR Y1068	c-Raf S338	-0.61	0.17	-0.72	0.17
GTP-bound Ras	RSK1 T359 S363	-0.58	0.28	-0.60	0.28
MEK1/2 S217 S221	ERK1/2 T202 Y204	0.56	0.19	0.70	0.19
RSK3 T356 S360	CREB S133	0.55	0.17	0.72	0.17
RSK1 T359 S363	RSK3 T356 S360	-0.54	0.28	-0.60	0.28
ERK1/2 T202 Y204	CREB S133	0.49	0.74	0.21	0.74
EGFR Y1068	RSK1 T359 S363	0.45	0.55	0.36	0.55
EGFR Y1068	ERK1/2 T202 Y204	-0.45	0.49	-0.41	0.49
c-Raf S338	RSK1 T359 S363	0.40	0.62	0.30	0.62
ERK1/2 T202 Y204	MSK1 S360	-0.37	0.39	-0.50	0.39
MSK1 S360	RSK3 T356 S360	-0.36	0.87	0.10	0.87

Analyte 1	Analyte 2	Pearson's r ^a	p _P	Spearman's rho	p _S
GTP-bound Ras	c-Raf S338	-0.34	0.28	-0.60	0.28
EGFR Y1068	RSK3 T356 S360	-0.34	0.87	-0.10	0.87
c-Raf S338	MSK1 S360	-0.30	0.10	-0.80	0.10
c-Raf S338	RSK3 T356 S360	-0.27	0.39	-0.50	0.39
MEK1/2 S217 S221	RSK1 T359 S363	-0.25	0.87	-0.10	0.87
ERK1/2 T202 Y204	RSK3 T356 S360	0.13	0.75	-0.20	0.75
EGFR Y1068	CREB S133	0.13	0.64	0.29	0.64
GTP-bound Ras	MEK1/2 S217 S221	-0.12	0.62	-0.30	0.62
EGFR Y1068	GTP-bound Ras	-0.10	0.93	-0.05	0.93
MSK1 S360	CREB S133	-0.09	0.49	0.41	0.49
MEK1/2 S217 S221	CREB S133	0.09	0.80	-0.15	0.80
MEK1/2 S217 S221	MSK1 S360	-0.07	0.87	-0.10	0.87
c-Raf S338	MEK1/2 S217 S221	-0.04	0.87	0.10	0.87
GTP-bound Ras	MSK1 S360	0.04	0.87	0.10	0.87
c-Raf S338	ERK1/2 T202 Y204	-0.03	0.87	0.10	0.87
EGFR Y1068	MEK1/2 S217 S221	0.02	0.87	0.10	0.87

^aSorted by absolute value of Pearson's r



Source: Example Report



Figure 18: Correlogram for Vehicle 0.1uM

Table 14: Correlations for Vehicle 0.1uM

Analyte 1	Analyte 2	Pearson's r ^a	p _P	Spearman's rho	p _S
MEK1/2 S217 S221	RSK3 T356 S360	0.98	< 0.01	0.94	< 0.01

Analyte 1	Analyte 2	Pearson's r ^a	p _P	Spearman's rho	p _S
EGFR Y1068	MSK1 S360	0.95	< 0.01	1.00	< 0.01
GTP-bound Ras	c-Raf S338	-0.88	0.04	-0.83	0.04
MEK1/2 S217 S221	CREB S133	0.80	0.04	0.83	0.04
c-Raf S338	MEK1/2 S217 S221	0.76	0.07	0.77	0.07
RSK1 T359 S363	CREB S133	-0.76	0.27	-0.54	0.27
RSK3 T356 S360	CREB S133	0.75	0.07	0.77	0.07
c-Raf S338	RSK3 T356 S360	0.74	0.04	0.83	0.04
GTP-bound Ras	MEK1/2 S217 S221	-0.74	0.27	-0.54	0.27
GTP-bound Ras	MSK1 S360	0.65	0.21	0.60	0.21
GTP-bound Ras	RSK3 T356 S360	-0.64	0.21	-0.60	0.21
GTP-bound Ras	CREB S133	-0.59	0.27	-0.54	0.27
RSK1 T359 S363	RSK3 T356 S360	-0.58	0.40	-0.43	0.40
c-Raf S338	ERK1/2 T202 Y204	0.53	0.54	0.31	0.54
MEK1/2 S217 S221	RSK1 T359 S363	-0.53	0.21	-0.60	0.21
EGFR Y1068	CREB S133	-0.50	0.21	-0.60	0.21
c-Raf S338	CREB S133	0.48	0.33	0.49	0.33
MSK1 S360	CREB S133	-0.45	0.21	-0.60	0.21
EGFR Y1068	GTP-bound Ras	0.44	0.21	0.60	0.21
c-Raf S338	MSK1 S360	-0.44	0.70	-0.20	0.70
MEK1/2 S217 S221	MSK1 S360	-0.43	0.62	-0.26	0.62
EGFR Y1068	ERK1/2 T202 Y204	0.41	0.70	0.20	0.70
EGFR Y1068	MEK1/2 S217 S221	-0.38	0.62	-0.26	0.62
GTP-bound Ras	ERK1/2 T202 Y204	-0.33	0.54	-0.31	0.54
MSK1 S360	RSK3 T356 S360	-0.30	0.54	-0.31	0.54
EGFR Y1068	RSK3 T356 S360	-0.27	0.54	-0.31	0.54
ERK1/2 T202 Y204	RSK1 T359 S363	0.25	0.62	0.26	0.62
EGFR Y1068	c-Raf S338	-0.23	0.70	-0.20	0.70
ERK1/2 T202 Y204	CREB S133	-0.18	0.62	-0.26	0.62
ERK1/2 T202 Y204	MSK1 S360	0.18	0.70	0.20	0.70
MSK1 S360	RSK1 T359 S363	-0.18	0.54	-0.31	0.54
c-Raf S338	RSK1 T359 S363	-0.09	0.79	-0.14	0.79
MEK1/2 S217 S221	ERK1/2 T202 Y204	-0.08	0.70	-0.20	0.70
ERK1/2 T202 Y204	RSK3 T356 S360	-0.06	0.96	0.03	0.96
GTP-bound Ras	RSK1 T359 S363	0.02	0.79	-0.14	0.79
EGFR Y1068	RSK1 T359 S363	-0.02	0.54	-0.31	0.54

^aSorted by absolute value of Pearson's r

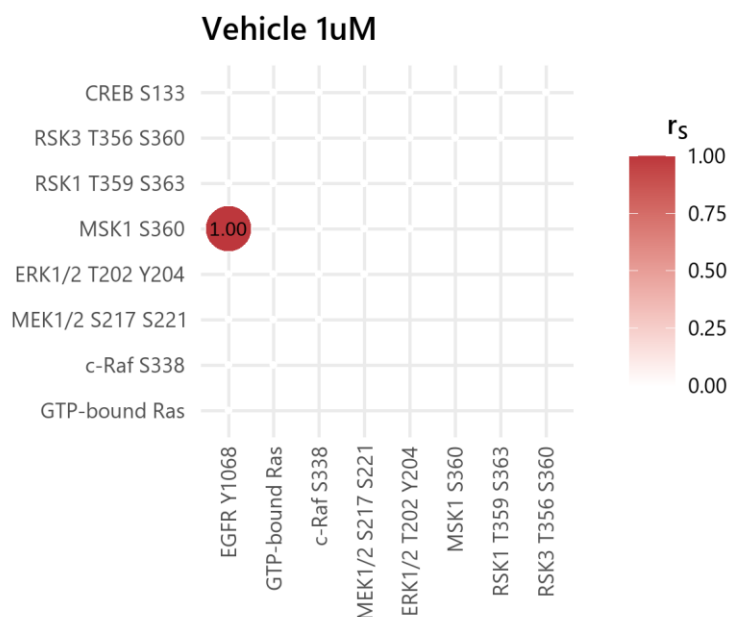


Figure 19: Correlogram for Vehicle 1uM

Table 15: Correlations for Vehicle 1uM

Analyte 1	Analyte 2	Pearson's r^a	p_P	Spearman's rho	p_S
ERK1/2 T202 Y204	MSK1 S360	-0.97	0.10	-0.80	0.10
MSK1 S360	CREB S133	0.92	0.19	0.70	0.19
EGFR Y1068	MSK1 S360	0.86	< 0.01	1.00	< 0.01
GTP-bound Ras	RSK1 T359 S363	-0.85	0.19	-0.70	0.19
ERK1/2 T202 Y204	CREB S133	-0.84	0.62	-0.30	0.62
EGFR Y1068	ERK1/2 T202 Y204	-0.78	0.10	-0.80	0.10
EGFR Y1068	CREB S133	0.76	0.19	0.70	0.19
RSK1 T359 S363	CREB S133	0.65	0.19	0.70	0.19
EGFR Y1068	RSK3 T356 S360	0.62	0.28	0.60	0.28
c-Raf S338	RSK3 T356 S360	-0.58	0.10	-0.80	0.10
GTP-bound Ras	MEK1/2 S217 S221	-0.56	0.19	-0.70	0.19
c-Raf S338	MEK1/2 S217 S221	-0.55	0.50	-0.40	0.50
ERK1/2 T202 Y204	RSK3 T356 S360	-0.55	0.28	-0.60	0.28
MSK1 S360	RSK3 T356 S360	0.46	0.28	0.60	0.28
RSK1 T359 S363	RSK3 T356 S360	-0.43	0.39	-0.50	0.39
EGFR Y1068	MEK1/2 S217 S221	-0.40	0.62	-0.30	0.62
GTP-bound Ras	c-Raf S338	0.39	1.00	0.00	1.00
MSK1 S360	RSK1 T359 S363	0.37	0.62	0.30	0.62
MEK1/2 S217 S221	CREB S133	-0.37	0.62	-0.30	0.62
EGFR Y1068	c-Raf S338	-0.33	0.28	-0.60	0.28

Analyte 1	Analyte 2	Pearson's r ^a	p _P	Spearman's rho	p _S
ERK1/2 T202 Y204	RSK1 T359 S363	-0.32	0.62	-0.30	0.62
GTP-bound Ras	CREB S133	-0.29	0.62	-0.30	0.62
GTP-bound Ras	RSK3 T356 S360	0.28	0.28	0.60	0.28
MEK1/2 S217 S221	MSK1 S360	-0.27	0.62	-0.30	0.62
MEK1/2 S217 S221	RSK3 T356 S360	0.25	0.87	-0.10	0.87
EGFR Y1068	GTP-bound Ras	0.15	0.75	0.20	0.75
c-Raf S338	ERK1/2 T202 Y204	0.15	0.28	0.60	0.28
GTP-bound Ras	ERK1/2 T202 Y204	0.13	0.75	-0.20	0.75
RSK3 T356 S360	CREB S133	0.12	0.87	-0.10	0.87
EGFR Y1068	RSK1 T359 S363	0.11	0.62	0.30	0.62
c-Raf S338	MSK1 S360	-0.10	0.28	-0.60	0.28
c-Raf S338	RSK1 T359 S363	-0.08	0.87	0.10	0.87
GTP-bound Ras	MSK1 S360	-0.08	0.75	0.20	0.75
MEK1/2 S217 S221	ERK1/2 T202 Y204	0.07	1.00	0.00	1.00
MEK1/2 S217 S221	RSK1 T359 S363	0.07	0.75	0.20	0.75
c-Raf S338	CREB S133	-0.03	0.87	-0.10	0.87

^aSorted by absolute value of Pearson's r

PDX Model Data - Stroma

PDX Stroma: Data Overview

For an example data overview interpretation see p. 4.

Table 16: Data Overview

Analyte	Groups	N	Missing	Mean	SD	Spread	Density
c-Raf S338	6	26	0	10,292.73	5,883.86		
CREB S133	6	26	0	7,054.26	2,107.19		
EGFR Y1068	6	26	0	18,558.93	12,949.48		
ERK1/2 T202 Y204	6	26	0	27,081.22	13,976.22		
MEK1/2 S217 S221	6	26	0	8,171.41	1,856.33		
MSK1 S360	6	26	0	73,763.45	36,702.54		
RSK1 T359 S363	6	26	0	1,513.47	924.66		
GTP-bound Ras	6	26	0	12,880.54	5,781.42		
RSK3 T356 S360	6	26	0	5,075.02	3,339.98		

Mean Comparison Statistics

For an example mean comparison interpretation see p. 4.

Table 17: Mean Comparison Statistics

Var ₁	Var ₂	N ₁	N ₂	log2FD	Mean ₁ ^a	SD ₁ ^a	Mean ₂ ^a	SD ₂ ^a	p	p _{adj}
EGFR Y1068										
Drug A 0.1uM	Vehicle 0.1uM	6	6	-1.62	9.31	6.48	28.58	3.97	< 0.01	< 0.01
Drug A 1uM	Vehicle 1uM	5	5	-2.65	5.37	3.48	33.75	3.45	< 0.01	< 0.01
Drug A 10uM	Vehicle 10uM	2	2	-3.37	2.63	3.72	27.17	0.08	N/A ^b	N/A ^b
GTP-bound Ras										
Drug A 0.1uM	Vehicle 0.1uM	6	6	-0.19	11.02	4.95	12.61	3.50	0.82	1.00
Drug A 1uM	Vehicle 1uM	5	5	0.26	15.18	4.65	12.71	7.49	1.00	1.00
Drug A 10uM	Vehicle 10uM	2	2	0.26	14.65	13.85	12.19	9.72	N/A ^b	N/A ^b
c-Raf S338										
Drug A 0.1uM	Vehicle 0.1uM	6	6	-0.23	10.37	7.41	12.15	5.29	0.59	1.00
Drug A 1uM	Vehicle 1uM	5	5	-0.07	10.69	7.08	11.25	3.45	0.84	1.00
Drug A 10uM	Vehicle 10uM	2	2	0.95	7.50	8.66	3.88	1.90	N/A ^b	N/A ^b

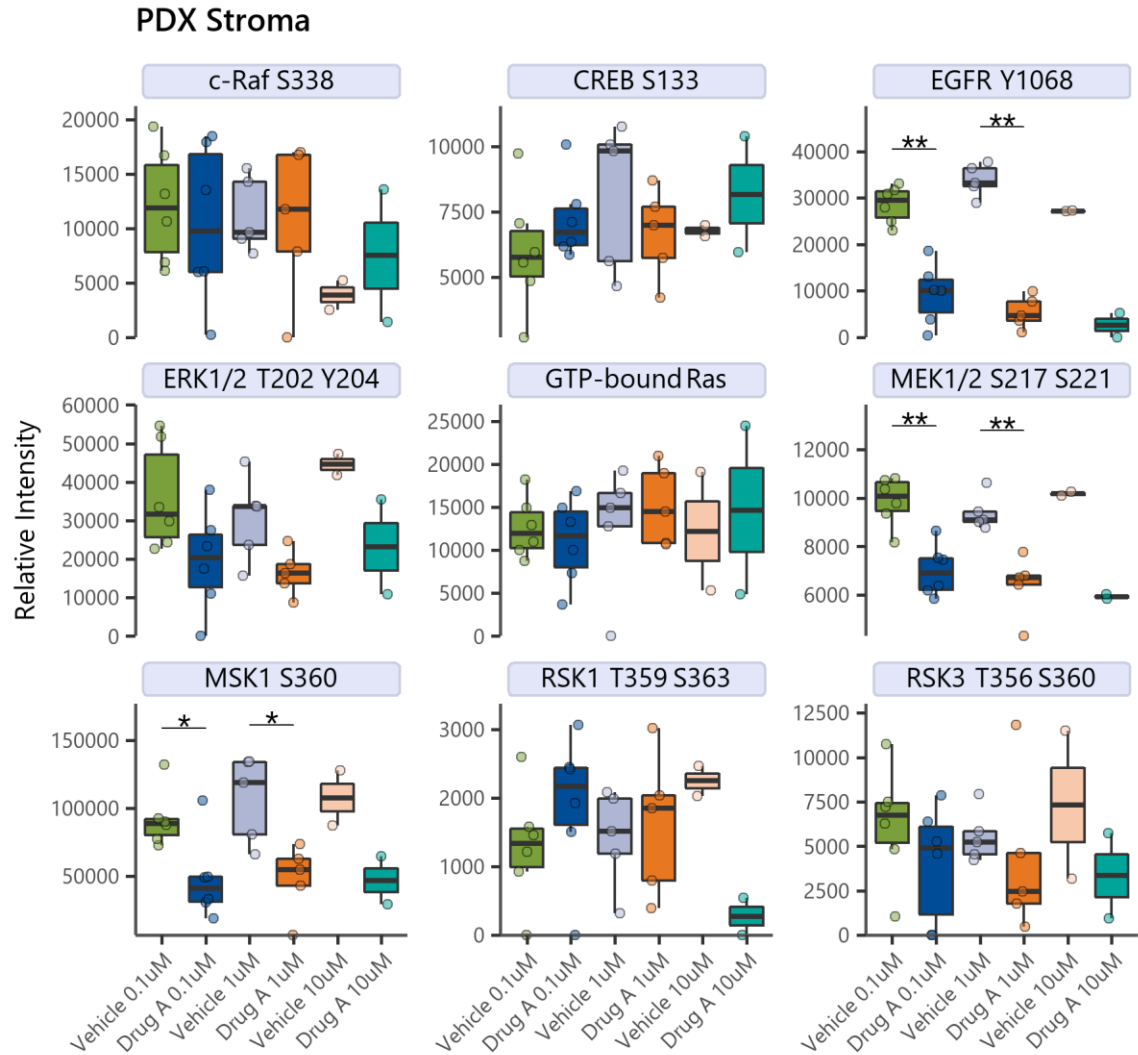
Var ₁	Var ₂	N ₁	N ₂	log2FD	Mean ₁ ^a	SD ₁ ^a	Mean ₂ ^a	SD ₂ ^a	p	p _{adj}
MEK1/2 S217 S221										
Drug A 0.1uM	Vehicle 0.1uM	6	6	-0.50	7.00	1.06	9.87	1.01	< 0.01	< 0.01
Drug A 1uM	Vehicle 1uM	5	5	-0.55	6.40	1.28	9.40	0.73	< 0.01	< 0.01
Drug A 10uM	Vehicle 10uM	2	2	-0.78	5.93	0.13	10.17	0.11	N/A ^b	N/A ^b
ERK1/2 T202 Y204										
Drug A 0.1uM	Vehicle 0.1uM	6	6	-0.88	19.56	13.21	36.10	13.80	0.09	0.19
Drug A 1uM	Vehicle 1uM	5	5	-0.89	16.45	5.93	30.47	11.23	0.10	0.19
Drug A 10uM	Vehicle 10uM	2	2	-0.95	23.16	17.45	44.59	3.92	N/A ^b	N/A ^b
MSK1 S360										
Drug A 0.1uM	Vehicle 0.1uM	6	6	-0.95	47.61	30.76	91.91	21.09	0.04	0.04
Drug A 1uM	Vehicle 1uM	5	5	-1.16	47.85	25.82	106.63	31.69	0.02	0.03
Drug A 10uM	Vehicle 10uM	2	2	-1.20	46.65	24.85	107.51	28.86	N/A ^b	N/A ^b
RSK1 T359 S363										
Drug A 0.1uM	Vehicle 0.1uM	6	6	0.55	1.89	1.07	1.29	0.85	0.26	0.52
Drug A 1uM	Vehicle 1uM	5	5	0.19	1.62	1.04	1.42	0.71	0.84	0.84
Drug A 10uM	Vehicle 10uM	2	2	-3.04	0.27	0.39	2.25	0.31	N/A ^b	N/A ^b
RSK3 T356 S360										
Drug A 0.1uM	Vehicle 0.1uM	6	6	-0.64	4.02	3.30	6.27	3.22	0.30	0.59
Drug A 1uM	Vehicle 1uM	5	5	-0.39	4.23	4.51	5.56	1.46	0.31	0.59
Drug A 10uM	Vehicle 10uM	2	2	-1.13	3.34	3.39	7.33	5.90	N/A ^b	N/A ^b
CREB S133										
Drug A 0.1uM	Vehicle 0.1uM	6	6	0.28	7.23	1.57	5.97	2.35	0.18	0.36
Drug A 1uM	Vehicle 1uM	5	5	-0.30	6.67	1.75	8.19	2.83	0.42	0.42
Drug A 10uM	Vehicle 10uM	2	2	0.27	8.18	3.13	6.78	0.28	N/A ^b	N/A ^b

^ax * 1000

^bSample size too low

Boxplots

For example boxplot interpretations see p. 6.



Source: Example Report

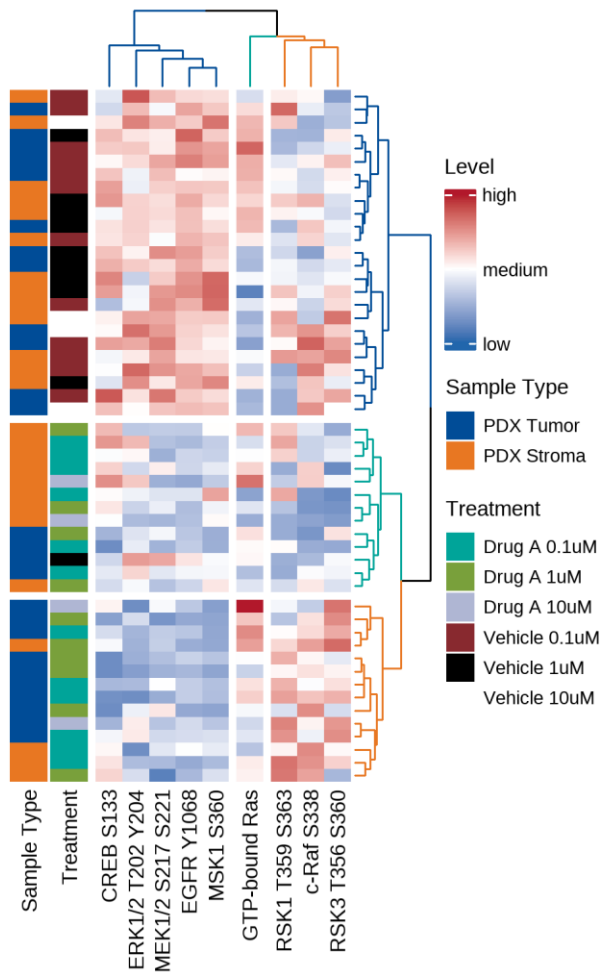


Figure 20: Boxplots by Analyte

Heatmap

For an example heatmap interpretation see p. 7.

PDX Tumor/Stroma Heatmap



Source: Example Report

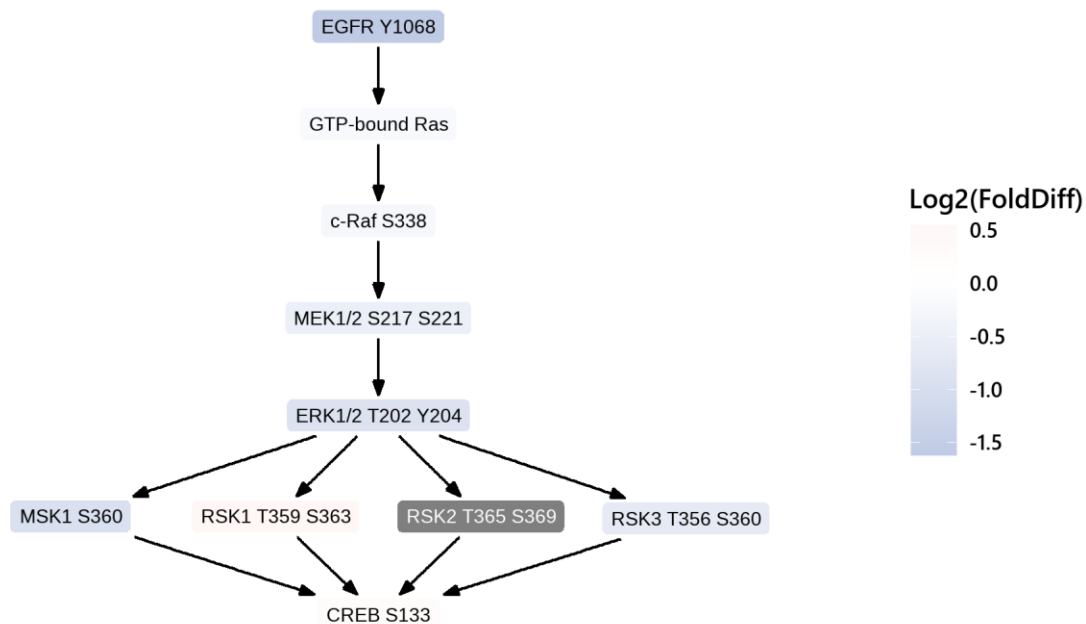


Figure 21: Heatmap PDX Stroma

Pathmaps

For example pathmap interpretations see p. 8.

Drug A 0.1uM vs. Vehicle 0.1uM

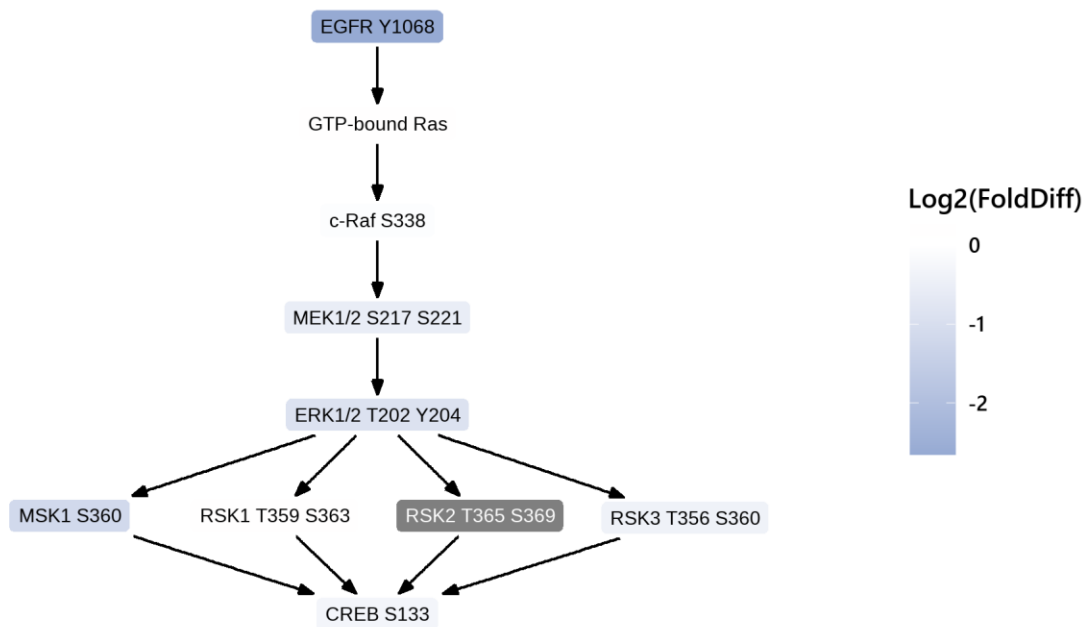


Source: Example Report



Figure 22: Pathmap for Drug A 0.1uM vs. Vehicle 0.1uM

Drug A 1uM vs. Vehicle 1uM

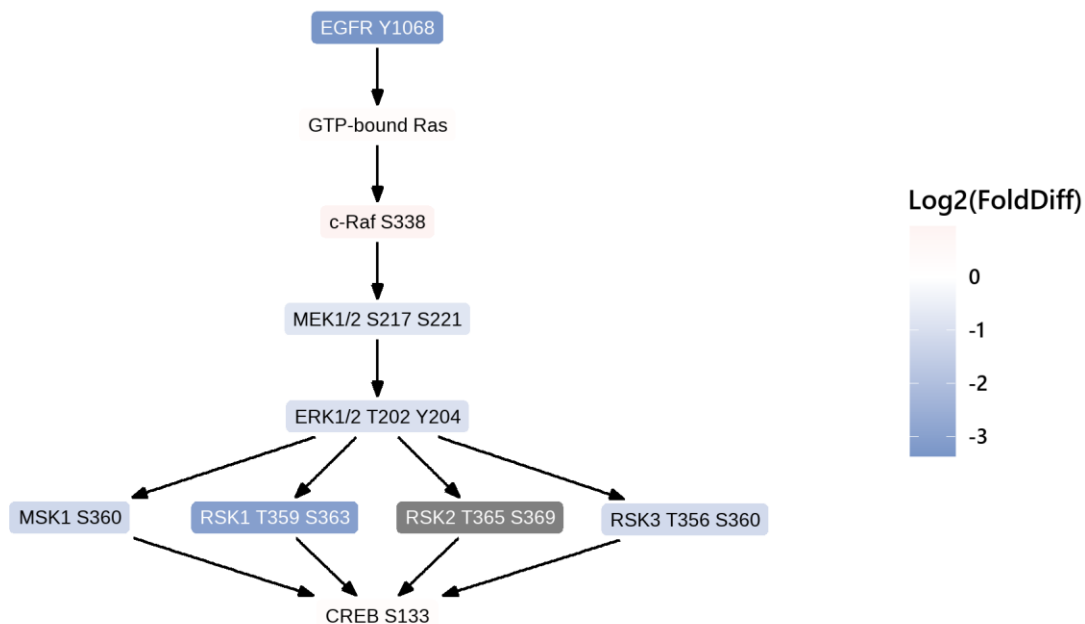


Source: Example Report



Figure 23: Pathmap for Drug A 1uM vs. Vehicle 1uM

Drug A 10uM vs. Vehicle 10uM



Source: Example Report



Figure 24: Pathmap for Drug A 10uM vs. Vehicle 10uM

Correlations

Correlations Sample Size Overview

For an example correlation sample size overview interpretation see p. 10.

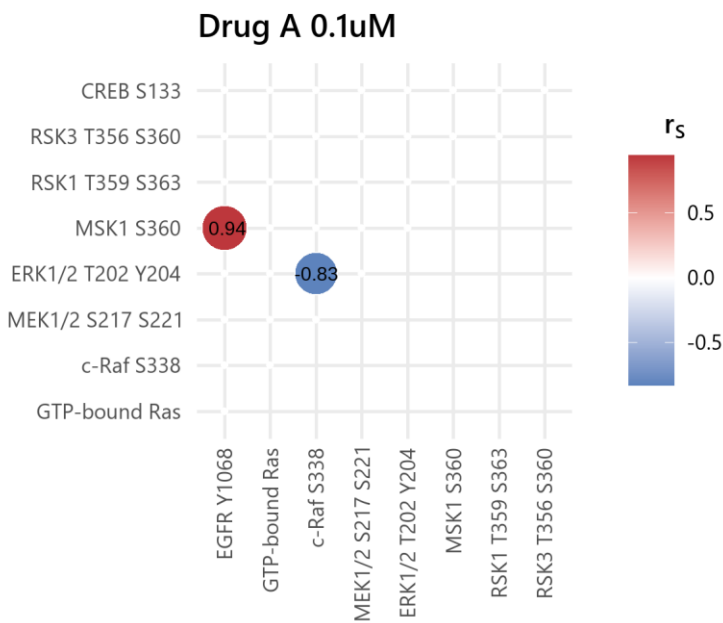
Table 18: Correlations Sample Size

Treatment	N _{minimal}
Drug A 0.1uM	6
Drug A 10uM	2 ^a
Drug A 1uM	5
Vehicle 0.1uM	6
Vehicle 10uM	2 ^a
Vehicle 1uM	5

^aSample size too low

Correlation Analysis per Treatment

For example correlation interpretations see p. 10.



Source: Example Report



Figure 25: Correlogram for Drug A 0.1uM

Table 19: Correlations for Drug A 0.1uM

Analyte 1	Analyte 2	Pearson's r ^a	p _P	Spearman's rho	p _S
MEK1/2 S217 S221	RSK1 T359 S363	-0.78	0.16	-0.66	0.16
c-Raf S338	ERK1/2 T202 Y204	-0.78	0.04	-0.83	0.04
MSK1 S360	RSK3 T356 S360	-0.74	0.26	-0.55	0.26
GTP-bound Ras	MSK1 S360	-0.73	0.21	-0.60	0.21
MEK1/2 S217 S221	RSK3 T356 S360	-0.69	0.32	-0.49	0.32
EGFR Y1068	MSK1 S360	0.58	< 0.01	0.94	< 0.01
c-Raf S338	MSK1 S360	-0.57	0.70	-0.20	0.70
c-Raf S338	RSK3 T356 S360	0.56	0.12	0.70	0.12
RSK1 T359 S363	RSK3 T356 S360	0.54	0.42	0.41	0.42
ERK1/2 T202 Y204	CREB S133	0.53	0.87	0.09	0.87
EGFR Y1068	CREB S133	-0.53	0.47	-0.37	0.47
EGFR Y1068	MEK1/2 S217 S221	0.53	0.16	0.66	0.16
EGFR Y1068	GTP-bound Ras	-0.52	0.27	-0.54	0.27
MEK1/2 S217 S221	MSK1 S360	0.49	0.21	0.60	0.21
EGFR Y1068	RSK1 T359 S363	-0.48	0.11	-0.71	0.11
GTP-bound Ras	c-Raf S338	0.48	0.47	0.37	0.47
EGFR Y1068	ERK1/2 T202 Y204	-0.44	0.47	-0.37	0.47
MEK1/2 S217 S221	ERK1/2 T202 Y204	-0.37	0.40	-0.43	0.40
MSK1 S360	CREB S133	-0.36	0.40	-0.43	0.40
GTP-bound Ras	RSK1 T359 S363	-0.36	0.79	-0.14	0.79
EGFR Y1068	RSK3 T356 S360	-0.31	0.50	-0.35	0.50
ERK1/2 T202 Y204	RSK3 T356 S360	-0.30	0.35	-0.46	0.35
c-Raf S338	RSK1 T359 S363	-0.21	0.62	-0.26	0.62
GTP-bound Ras	CREB S133	0.20	0.47	0.37	0.47
ERK1/2 T202 Y204	RSK1 T359 S363	0.20	0.62	0.26	0.62
GTP-bound Ras	RSK3 T356 S360	0.18	0.83	0.12	0.83
c-Raf S338	MEK1/2 S217 S221	0.17	0.70	0.20	0.70
MSK1 S360	RSK1 T359 S363	-0.10	0.21	-0.60	0.21
c-Raf S338	CREB S133	-0.04	0.87	0.09	0.87
GTP-bound Ras	MEK1/2 S217 S221	-0.03	0.96	-0.03	0.96
EGFR Y1068	c-Raf S338	-0.03	0.87	0.09	0.87
RSK3 T356 S360	CREB S133	-0.03	0.83	-0.12	0.83
RSK1 T359 S363	CREB S133	0.01	0.79	0.14	0.79
MEK1/2 S217 S221	CREB S133	-0.01	0.47	0.37	0.47
GTP-bound Ras	ERK1/2 T202 Y204	-0.00	0.87	-0.09	0.87
ERK1/2 T202 Y204	MSK1 S360	-0.00	0.70	-0.20	0.70

^aSorted by absolute value of Pearson's r

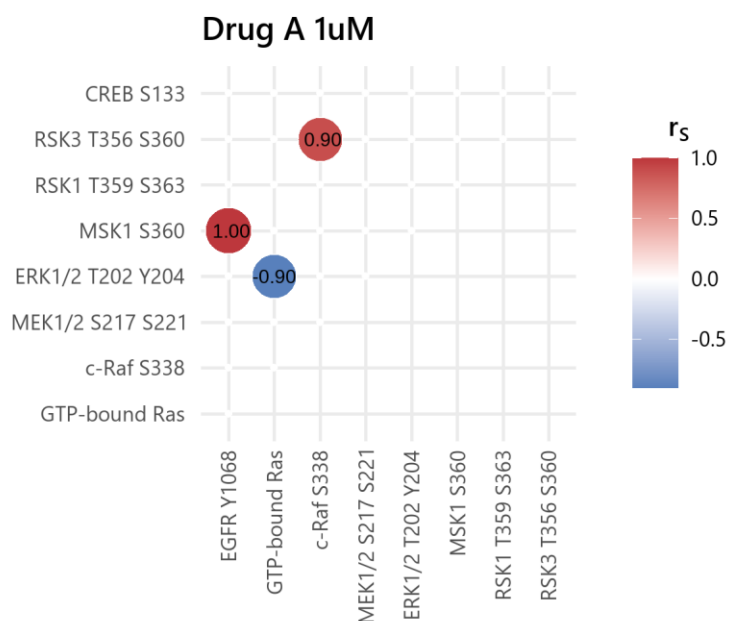


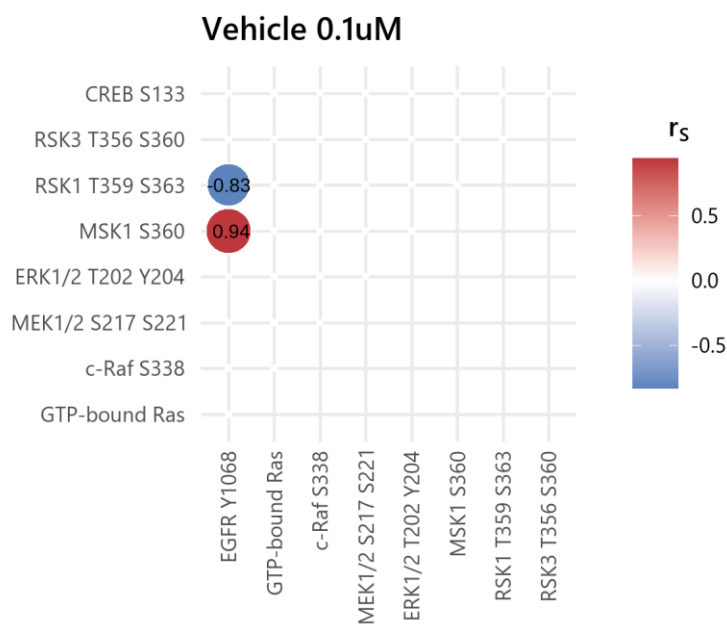
Figure 26: Correlogram for Drug A 1uM

Table 20: Correlations for Drug A 1uM

Analyte 1	Analyte 2	Pearson's r^a	p_P	Spearman's rho	p_S
EGFR Y1068	MSK1 S360	0.93	< 0.01	1.00	< 0.01
GTP-bound Ras	ERK1/2 T202 Y204	-0.84	0.04	-0.90	0.04
MEK1/2 S217 S221	RSK1 T359 S363	-0.82	0.19	-0.70	0.19
ERK1/2 T202 Y204	MSK1 S360	0.79	0.28	0.60	0.28
MSK1 S360	RSK3 T356 S360	-0.77	0.62	-0.30	0.62
EGFR Y1068	ERK1/2 T202 Y204	0.74	0.28	0.60	0.28
c-Raf S338	RSK1 T359 S363	0.71	0.39	0.50	0.39
c-Raf S338	MEK1/2 S217 S221	-0.71	0.50	-0.40	0.50
GTP-bound Ras	MSK1 S360	-0.67	0.19	-0.70	0.19
c-Raf S338	RSK3 T356 S360	0.66	0.04	0.90	0.04
GTP-bound Ras	RSK3 T356 S360	0.60	0.62	0.30	0.62
GTP-bound Ras	RSK1 T359 S363	0.56	0.39	0.50	0.39
c-Raf S338	MSK1 S360	-0.52	0.28	-0.60	0.28
RSK1 T359 S363	CREB S133	0.51	0.28	0.60	0.28
RSK3 T356 S360	CREB S133	-0.51	0.28	-0.60	0.28
EGFR Y1068	GTP-bound Ras	-0.50	0.19	-0.70	0.19
EGFR Y1068	RSK3 T356 S360	-0.50	0.62	-0.30	0.62
ERK1/2 T202 Y204	RSK3 T356 S360	-0.49	0.87	-0.10	0.87
GTP-bound Ras	c-Raf S338	0.48	0.39	0.50	0.39
ERK1/2 T202 Y204	CREB S133	-0.40	0.62	-0.30	0.62

Analyte 1	Analyte 2	Pearson's r ^a	p _P	Spearman's rho	p _S
EGFR Y1068	RSK1 T359 S363	-0.38	0.62	-0.30	0.62
MSK1 S360	RSK1 T359 S363	-0.37	0.62	-0.30	0.62
GTP-bound Ras	CREB S133	0.35	0.50	0.40	0.50
EGFR Y1068	c-Raf S338	-0.33	0.28	-0.60	0.28
MEK1/2 S217 S221	ERK1/2 T202 Y204	-0.28	0.28	-0.60	0.28
ERK1/2 T202 Y204	RSK1 T359 S363	-0.26	0.87	-0.10	0.87
c-Raf S338	CREB S133	-0.23	0.62	-0.30	0.62
EGFR Y1068	CREB S133	-0.19	0.87	-0.10	0.87
MEK1/2 S217 S221	CREB S133	-0.17	0.87	-0.10	0.87
RSK1 T359 S363	RSK3 T356 S360	0.13	0.75	0.20	0.75
c-Raf S338	ERK1/2 T202 Y204	-0.11	0.75	-0.20	0.75
EGFR Y1068	MEK1/2 S217 S221	0.11	0.75	-0.20	0.75
MEK1/2 S217 S221	MSK1 S360	0.06	0.75	-0.20	0.75
GTP-bound Ras	MEK1/2 S217 S221	-0.05	0.75	0.20	0.75
MEK1/2 S217 S221	RSK3 T356 S360	0.04	0.62	-0.30	0.62
MSK1 S360	CREB S133	0.01	0.87	-0.10	0.87

^aSorted by absolute value of Pearson's r



Source: Example Report



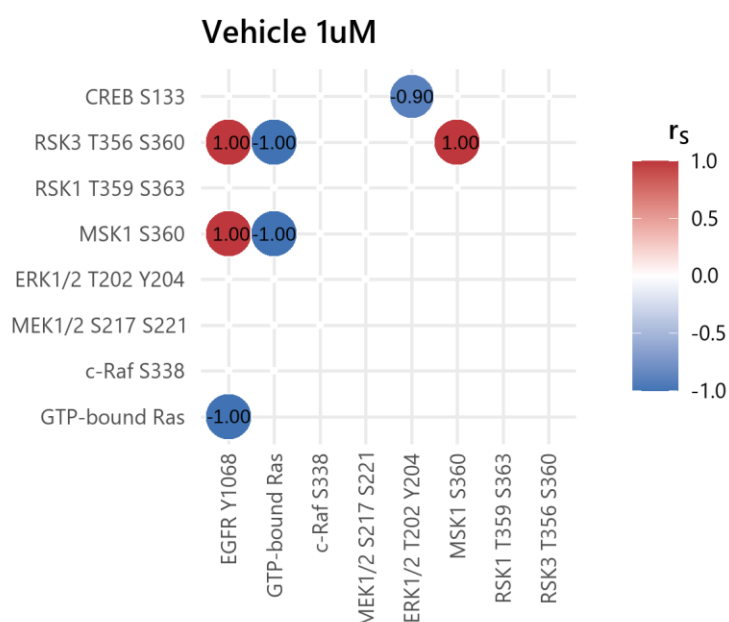
Figure 27: Correlogram for Vehicle 0.1uM

Table 21: Correlations for Vehicle 0.1uM

Analyte 1	Analyte 2	Pearson's r ^a	p _P	Spearman's rho	p _S
EGFR Y1068	RSK1 T359 S363	-0.85	0.04	-0.83	0.04

Analyte 1	Analyte 2	Pearson's r ^a	p _P	Spearman's rho	p _S
GTP-bound Ras	CREB S133	0.78	0.21	0.60	0.21
GTP-bound Ras	ERK1/2 T202 Y204	-0.71	0.11	-0.71	0.11
EGFR Y1068	MSK1 S360	0.68	< 0.01	0.94	< 0.01
GTP-bound Ras	MEK1/2 S217 S221	-0.65	0.16	-0.66	0.16
MEK1/2 S217 S221	CREB S133	-0.59	0.11	-0.71	0.11
ERK1/2 T202 Y204	RSK3 T356 S360	-0.54	0.33	-0.49	0.33
c-Raf S338	ERK1/2 T202 Y204	0.53	0.27	0.54	0.27
c-Raf S338	MSK1 S360	-0.51	0.62	-0.26	0.62
MSK1 S360	CREB S133	-0.51	0.79	-0.14	0.79
MEK1/2 S217 S221	RSK3 T356 S360	0.43	0.16	0.66	0.16
GTP-bound Ras	c-Raf S338	-0.43	0.47	-0.37	0.47
ERK1/2 T202 Y204	RSK1 T359 S363	-0.39	0.87	-0.09	0.87
ERK1/2 T202 Y204	MSK1 S360	-0.38	0.79	-0.14	0.79
MEK1/2 S217 S221	MSK1 S360	0.34	0.40	0.43	0.40
c-Raf S338	RSK3 T356 S360	0.31	0.79	0.14	0.79
RSK1 T359 S363	RSK3 T356 S360	0.28	0.62	0.26	0.62
MSK1 S360	RSK1 T359 S363	-0.25	0.16	-0.66	0.16
c-Raf S338	RSK1 T359 S363	-0.21	0.62	-0.26	0.62
RSK1 T359 S363	CREB S133	-0.21	0.27	-0.54	0.27
ERK1/2 T202 Y204	CREB S133	-0.21	0.62	-0.26	0.62
GTP-bound Ras	RSK1 T359 S363	0.20	0.87	0.09	0.87
GTP-bound Ras	MSK1 S360	-0.17	0.62	-0.26	0.62
EGFR Y1068	GTP-bound Ras	-0.17	0.47	-0.37	0.47
c-Raf S338	MEK1/2 S217 S221	0.15	0.96	-0.03	0.96
MEK1/2 S217 S221	ERK1/2 T202 Y204	0.13	0.96	-0.03	0.96
MSK1 S360	RSK3 T356 S360	0.12	0.87	0.09	0.87
GTP-bound Ras	RSK3 T356 S360	0.10	0.96	-0.03	0.96
MEK1/2 S217 S221	RSK1 T359 S363	0.07	0.79	0.14	0.79
EGFR Y1068	CREB S133	-0.07	0.96	0.03	0.96
RSK3 T356 S360	CREB S133	-0.04	0.62	-0.26	0.62
EGFR Y1068	MEK1/2 S217 S221	0.03	0.47	0.37	0.47
EGFR Y1068	RSK3 T356 S360	-0.02	0.96	0.03	0.96
EGFR Y1068	c-Raf S338	-0.01	0.96	0.03	0.96
EGFR Y1068	ERK1/2 T202 Y204	0.01	0.96	0.03	0.96
c-Raf S338	CREB S133	0.01	0.62	0.26	0.62

^aSorted by absolute value of Pearson's r



Source: Example Report



Figure 28: Correlogram for Vehicle 1uM

Table 22: Correlations for Vehicle 1uM

Analyte 1	Analyte 2	Pearson's r^a	p_P	Spearman's rho	p_s
GTP-bound Ras	RSK3 T356 S360	-0.99	< 0.01	-1.00	< 0.01
GTP-bound Ras	MEK1/2 S217 S221	-0.93	0.28	-0.60	0.28
EGFR Y1068	MSK1 S360	0.93	< 0.01	1.00	< 0.01
MEK1/2 S217 S221	RSK3 T356 S360	0.92	0.28	0.60	0.28
EGFR Y1068	RSK3 T356 S360	0.89	< 0.01	1.00	< 0.01
EGFR Y1068	GTP-bound Ras	-0.85	< 0.01	-1.00	< 0.01
ERK1/2 T202 Y204	CREB S133	-0.82	0.04	-0.90	0.04
MSK1 S360	RSK3 T356 S360	0.81	< 0.01	1.00	< 0.01
c-Raf S338	ERK1/2 T202 Y204	0.78	0.19	0.70	0.19
GTP-bound Ras	MSK1 S360	-0.73	< 0.01	-1.00	< 0.01
EGFR Y1068	MEK1/2 S217 S221	0.72	0.28	0.60	0.28
RSK1 T359 S363	CREB S133	0.64	0.62	0.30	0.62
EGFR Y1068	CREB S133	0.64	0.62	0.30	0.62
MEK1/2 S217 S221	ERK1/2 T202 Y204	-0.62	0.10	-0.80	0.10
EGFR Y1068	ERK1/2 T202 Y204	-0.60	0.28	-0.60	0.28
MEK1/2 S217 S221	RSK1 T359 S363	0.59	0.28	0.60	0.28
c-Raf S338	MEK1/2 S217 S221	-0.59	0.10	-0.80	0.10
MEK1/2 S217 S221	MSK1 S360	0.55	0.28	0.60	0.28
MEK1/2 S217 S221	CREB S133	0.53	0.39	0.50	0.39
ERK1/2 T202 Y204	RSK3 T356 S360	-0.50	0.28	-0.60	0.28

Analyte 1	Analyte 2	Pearson's r ^a	p _P	Spearman's rho	p _S
ERK1/2 T202 Y204	RSK1 T359 S363	-0.47	0.50	-0.40	0.50
c-Raf S338	RSK3 T356 S360	-0.46	0.28	-0.60	0.28
GTP-bound Ras	ERK1/2 T202 Y204	0.45	0.28	0.60	0.28
GTP-bound Ras	CREB S133	-0.45	0.62	-0.30	0.62
RSK3 T356 S360	CREB S133	0.44	0.62	0.30	0.62
ERK1/2 T202 Y204	MSK1 S360	-0.44	0.28	-0.60	0.28
GTP-bound Ras	c-Raf S338	0.39	0.28	0.60	0.28
c-Raf S338	MSK1 S360	-0.38	0.28	-0.60	0.28
GTP-bound Ras	RSK1 T359 S363	-0.37	0.75	-0.20	0.75
EGFR Y1068	c-Raf S338	-0.36	0.28	-0.60	0.28
MSK1 S360	CREB S133	0.36	0.62	0.30	0.62
c-Raf S338	CREB S133	-0.29	0.50	-0.40	0.50
RSK1 T359 S363	RSK3 T356 S360	0.28	0.75	0.20	0.75
MSK1 S360	RSK1 T359 S363	-0.22	0.75	0.20	0.75
c-Raf S338	RSK1 T359 S363	-0.17	1.00	0.00	1.00
EGFR Y1068	RSK1 T359 S363	0.14	0.75	0.20	0.75

^aSorted by absolute value of Pearson's r

Materials

Analyte Information

Table 23: Analyte/Antibody Information

Name		Epitope	Vendor	CatNo
CREB S133	CREB S133		Cell Signaling Technology	9198
EGFR Y1068	EGFR Y1068		Cell Signaling Technology	3777
ERK1/2 T202 Y204	ERK1 T202 Y204, ERK2 T185 T187		Cell Signaling Technology	4370
MEK1/2 S217 S221	MEK1 S217 S221, MEK2 S222 S226		Invitrogen	MA5-15016
RSK1 T359 S363	RSK1 T359 S363		Cell Signaling Technology	9344
GTP-bound Ras	H-Ras (GTP)		Cell Signaling Technology	8821
RSK3 T356 S360	RSK3 T356 S360, RSK1 T359 S363, RSK2 T365 S369		Cell Signaling Technology	9348
c-Raf S338	c-Raf S338		Cell Signaling Technology	9427
MSK1 S360	MSK1 S360		Abcam	ab81294

Sample Information

Table 24: Sample Information

Sample Type	Treatment	N
Cell Line A	Drug A 0.1uM	8
Cell Line A	Drug A 10uM	2
Cell Line A	Drug A 1uM	5
Cell Line A	Vehicle 0.1uM	9
Cell Line A	Vehicle 10uM	9
Cell Line A	Vehicle 1uM	9
PDX Stroma	Drug A 0.1uM	6
PDX Stroma	Drug A 10uM	2
PDX Stroma	Drug A 1uM	5
PDX Stroma	Vehicle 0.1uM	6
PDX Stroma	Vehicle 10uM	2
PDX Stroma	Vehicle 1uM	5
PDX Tumor	Drug A 0.1uM	6
PDX Tumor	Drug A 10uM	2
PDX Tumor	Drug A 1uM	5
PDX Tumor	Vehicle 0.1uM	6

Sample Type	Treatment	N
PDX Tumor	Vehicle 10uM	2
PDX Tumor	Vehicle 1uM	5

Methods

Western Blot Confirmation of Antibody Specificity

Before a primary antibody can be used in the RPPA process, antibody specificity must be established first in-house via Western Blot. To establish specificity, a panel of cell line lysates are separated first by SDS-PAGE, transferred to a blotting membrane, and then probed with the primary antibody of interest.

An antibody is considered validated for use on the RPPA if they meet the following criterion: a predominant band (80% of the signal intensity) must be observed at the protein's expected molecular weight. After validation, the primary antibody is tested at multiple dilutions on RPPAs that have calibrators and controls printed on them to: 1) optimize the dilution factor; 2) ensure low background signal, and 3) confirm a suitable linear dynamic range of the antibody. Repeat testing of this entire process occurs until an antibody is selected that meet these criteria for assay performance.

Sample Preparation

Cell Lysates

Samples were mixed 1:1 with Theralink's lysis buffer (45% 2X Tris-Glycine SDS, 45% T-PER Reagent, 10% TCEP) and boiled for 8 minutes.

Reverse Phase Protein Microarray Construction

Lysates were printed onto nitrocellulose backed slides in technical triplicates. Additionally, analyte control, analyte calibrators (JTY7: Jurkat + TPA mixed with Y79 at varying ratios), bovine serum albumin (BSA) standards, and matrix blanks were printed alongside the samples in triplicate. Nitrocellulose slides were stored at -20°C prior to antibody staining.

Reverse Phase Protein Microarray Staining

Prior to staining, nitrocellulose slides were first treated with ReBlot (Millipore), washed twice in DPBS (Gibco), and placed into a blocking reagent (I-Block; Applied Biosystems). Nitrocellulose slides were stained using the DAKO GenPoint™ kit (DAKO) using a DAKO Autostainer Plus according to the Theralink Technologies® RPPA staining protocol. Slides are incubated with primary antibodies for 30 min at room temperature and each staining run also has a single negative control slide (only antibody diluent; no primary antibody). Slides are then incubated with a secondary antibody. Protein detection is amplified via horseradish peroxidase mediated biotinyl tyramide deposition and visualized using a fluorescent probe (LiCoR). For total protein determination, a nitrocellulose slide is treated with 1% sodium hydroxide, incubated in a destain solution (7% acetic acid; 30% methanol), and a 0.2% Fast green solution is applied.

Reverse Phase Protein Array Visualization and Data Analysis

Images of stained RPPA and Fast green stained slides are captured on an InnoScan 710-AL (Innopsys). Individual spots are detected using the InnoScan software program, Mapix, and spots with non-standard morphology and/or staining are flagged and removed from analysis.

For each analyte, the median pixel value for each individual spot (designated in blue, below) is calculated and the local background pixel value (designated in yellow, below) is subtracted to generate a raw median intensity value (See Raw Data Tab in the excel file). Similarly, a negative slide is scanned at same scanner parameters as each analyte slide, and the raw median intensity value from the negative slide is calculated.

Since all samples on the array are printed with technical replicates, the average median intensity value is calculated for each sample on the array for each analyte as well as the negative control slide. For each sample, the average median intensity value of the negative control slide is subtracted from the average median intensity value for each analyte to generate a negative subtracted median intensity value (See Negative Subtracted Data Tab).

For fast green stained slides, the average median intensity values for individual spots are calculated as described above. Fast green is a dye-based stain and, therefore, the raw median intensity values for each spot are used in the total protein calculations and for the total protein normalization procedures. Total protein standard curves are generated by printing serial two-fold dilutions of bovine serum albumin (BSA) from 1000 $\mu\text{g/mL}$ to 7.8125 $\mu\text{g/mL}$ in the same sample buffer as the project samples. The linear regression of the BSA curve is used to convert the raw median intensity values of all the samples on the array to a protein concentration (mg/mL).

If a sample's technical replicates have a coefficient of variation (CV) greater than 30%, the outlying technical replicate is removed and the CV is recalculated. If the CV is less than 30%, the negative subtracted mean value is reported; if the CV is still greater than 30% after removing the outlier, the data is reported as not available "NA".

For every analyte, the median intensity value of a sample is then divided by that sample's protein concentration (in mg/mL) to create a final total protein normalized intensity value. This total protein normalization data corrects for the amount of protein deposited on the array. Only total protein normalized data should be used for later downstream analyses.