

Metabolic Reprogramming in Metastatic Melanoma with Acquired Resistance to Targeted Therapies: Integrative Metabolomic and Proteomic Analysis

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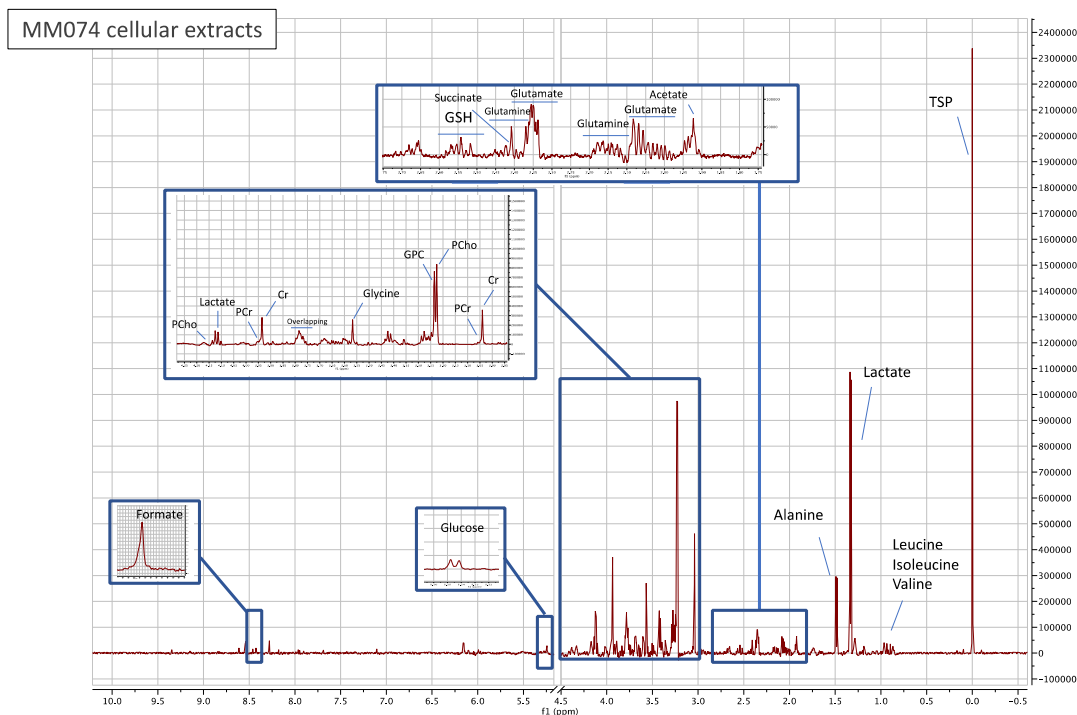


Figure S1. Typical cellular extracts ^1H -NMR spectra of the MM074 cell line.

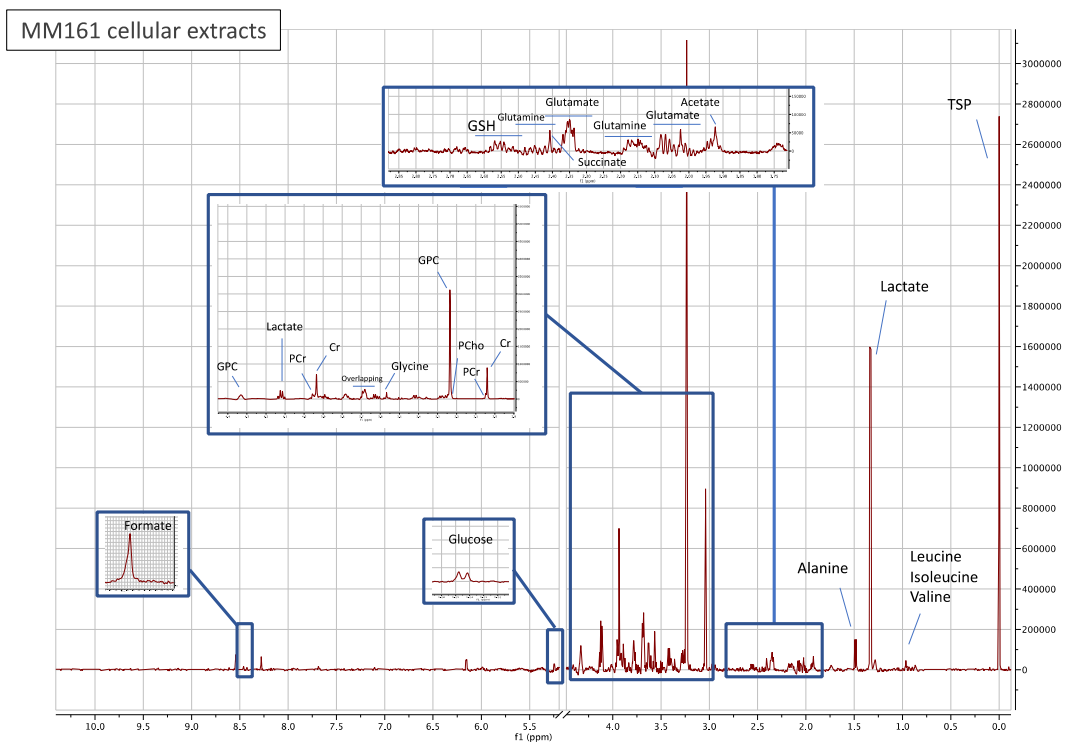


Figure S2. Typical cellular extracts ^1H -NMR spectra of the MM161 cell line.

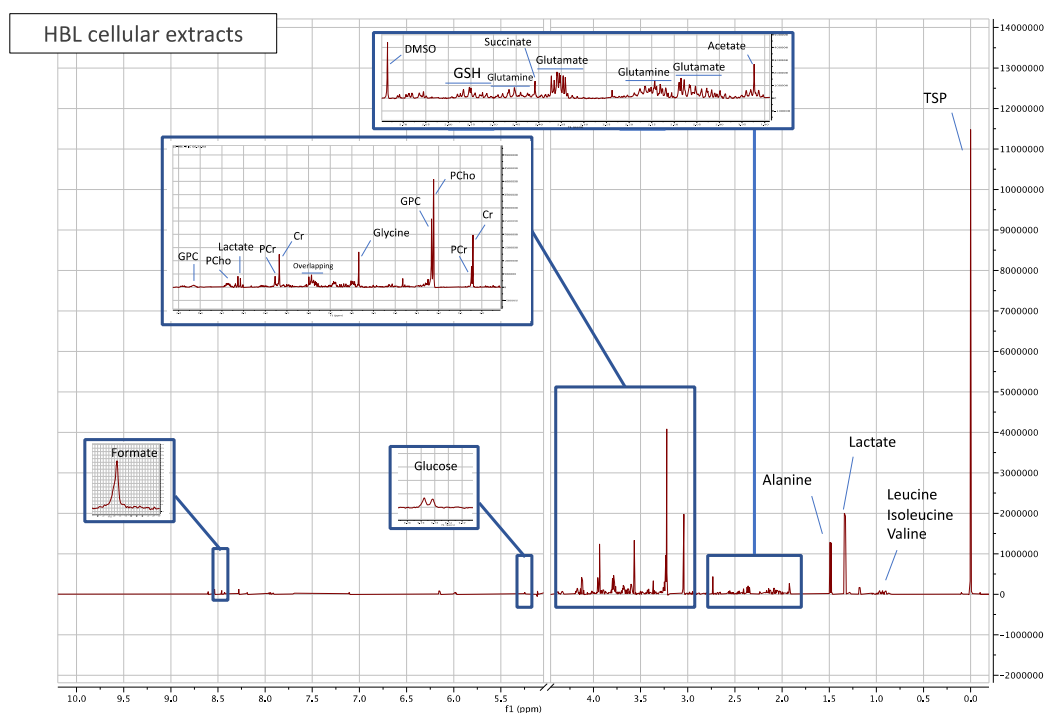


Figure S3. Typical cellular extracts ^1H -NMR spectra of the HBL cell line.

Table S1. Metabonomic signatures of the MM161 cells in various experimental conditions.**(A) Intracellular metabonomics signatures**

AUC values	MM161 ^(a)	MM161 PIMA 72 h ^(b)	MM161-R PIMA ^(c)
Lactate	17.30 ± 3.36 * ^(b)	10.54 ± 1.52 * ^(a) *** ^(c)	25.96 ± 1.66 *** ^(b)
Alanine	1.19 ± 0.36	1.35 ± 0.20 * ^(c)	0.66 ± 0.13 * ^(b)
Acetate	0.79 ± 0.47	0.39 ± 0.18	0.59 ± 0.18
Glutamine	0.19 ± 0.14 * ^(b)	0.46 ± 0.14 * ^(a)	0.27 ± 0.11
Glutamate	1.82 ± 0.22	1.51 ± 0.30	1.52 ± 0.27
GSH	0.82 ± 0.23	0.72 ± 0.18 * ^(c)	1.30 ± 0.35 * ^(b)
Succinate	0.55 ± 0.11	0.40 ± 0.12 ** ^(c)	0.96 ± 0.12 ** ^(b)
Aspartate	0.12 ± 0.23	0.11 ± 0.10 * ^(c)	0.01 ± 0.01 * ^(b)
Creatine	5.86 ± 1.36 * ^(b)	7.68 ± 0.31 * ^(a) ** ^(c)	3.80 ± 0.53 ** ^(b)
Phosphocreatine	0.83 ± 0.25	1.22 ± 0.31	0.88 ± 0.09
Phosphocholine	0.72 ± 1.46 * ^(c)	1.55 ± 1.42	3.83 ± 1.96 * ^(a)
Glycerophosphocholine (GPC)	23.57 ± 4.15 * ^(b)	16.20 ± 3.04 * ^(a)	17.58 ± 2.31
Glucose	2.78 ± 0.51	2.40 ± 0.78	1.57 ± 0.90
Glycine	1.52 ± 0.31 * ^(b)	0.96 ± 0.44 * ^(a)	1.20 ± 0.30
Formate	0.09 ± 0.02	0.12 ± 0.04	0.13 ± 0.02
Heatmap values	MM161 ^(a)	MM161 PIMA 72 h ^(b)	MM161-R PIMA ^(c)
Lactate	0.67	0.41	1.0
Alanine	0.88	1.0	0.49
Acetate	1.0	0.49	0.75
Glutamine	0.41	1.0	0.59
Glutamate	1.0	0.83	0.83
GSH	0.63	0.55	1.0
Succinate	0.57	0.42	1.0
Aspartate	1.0	0.92	0.08
Creatine	0.76	1.0	0.49
Phosphocreatine	0.68	1.0	0.72
Phosphocholine	0.19	0.40	1.0
Glycerophosphocholine (GPC)	1.0	0.69	0.75
Glucose	1.0	0.86	0.56
Glycine	1.0	0.63	0.79
Formate	0.69	0.92	1.0

(B) Extracellular metabonomics signatures

AUC values	MM161 ^(a)	MM161 PIMA 72 h ^(b)	MM161-R PIMA ^(c)
Glucose	3.18 ± 0.06 * ^(b)	3.98 ± 0.45 * ^(a) * ^(c)	3.21 ± 0.27 * ^(b)
Lactate	91.30 ± 10.29 *** ^(b)	42.32 ± 13.35 *** ^(a)	67.31 ± 5.71
Alanine	1.47 ± 0.18	1.31 ± 0.20	1.25 ± 0.11
Glutamine	0.82 ± 0.29	0.63 ± 0.08 ** ^(c)	1.23 ± 0.09 ** ^(b)
Heatmap values	MM161 ^(a)	MM161 PIMA 72 h ^(b)	MM161-R PIMA ^(c)
Glucose	0.80	1.0	0.81
Lactate	1.0	0.46	0.74
Alanine	1.0	0.89	0.85
Glutamine	0.67	0.51	1.0

Area under the curve (AUC) of resonances from selected metabolites within intra- **(A)** or extracellular **(B)** ¹H-NMR spectra, presented as mean ± standard deviation. Heatmap values were calculated using univariate data conversion, dividing each metabolite AUC values by the highest one. Letters are used to tag groups (a: sensitive cells, b: sensitive cells exposed for 72 h to pimasertib, c: resistant cells exposed to pimasertib) and highlight the statistical significance resulting from the multiple Kruskal-Wallis test comparison of the groups as follows: * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$.

Table S2. Metabonomics signatures of the MM074 cells in various experimental conditions.

(A) Intracellular metabonomics signatures

AUC values	MM074 ^(a)	MM074 DABRA 24 h (b)	MM074 DABRA 72 h (c)	MM074-R DABRA (d)
Lactate	19.27 ± 2.25 * (b)	12.92 ± 3.10 * (a)	15.48 ± 1.14	17.34 ± 2.31
Alanine	4.13 ± 0.47 * (c)	2.09 ± 0.17 ** (d)	1.33 ± 0.28 * (a) *** (d)	7.40 ± 0.27 ** (b) *** (c)
Acetate	0.48 ± 0.64	0.91 ± 0.39	1.23 ± 0.69	1.18 ± 1.03
Glutamine	0.02 ± 0.04	0.28 ± 0.09 * (d)	0.34 ± 0.24 * (d)	0.01 ± 0.02 * (b) * (c)
Glutamate	2.85 ± 0.36 * (b) ** (c)	1.46 ± 0.17 * (a) * (d)	0.45 ± 0.24 ** (a) ** (d)	2.78 ± 0.36 * (b) ** (c)
GSH	0.75 ± 0.10	0.95 ± 0.19 ** (c)	0.22 ± 0.13 ** (b)	0.70 ± 0.17
Succinate	0.66 ± 0.08 * (b)	0.39 ± 0.10 * (a) *** (c)	1.03 ± 0.28 *** (b)	0.64 ± 0.07
Aspartate	0.02 ± 0.03 * (b)	0.10 ± 0.04 * (a) * (d)	0.31 ± 0.55	0.20 ± 0.53 * (b)
Creatine	4.17 ± 0.49 * (c)	4.94 ± 0.28 *** (c) * (d)	1.13 ± 0.24 * (a) *** (b)	3.40 ± 0.25 * (b)
Phosphocreatine	0.33 ± 0.15	0.62 ± 0.07 * (d)	0.36 ± 0.10	0.27 ± 0.28 * (b)
Phosphocholine	9.19 ± 1.52	7.63 ± 0.86 * (d)	2.47 ± 1.91 *** (d)	12.78 ± 0.91 * (b) *** (c)
GPC	10.49 ± 0.74 * (b) ** (c)	6.52 ± 0.83 * (a) * (d)	2.45 ± 1.04 ** (a) ** (d)	10.34 ± 0.86 * (b) ** (c)
Glucose	1.14 ± 0.45 * (c)	1.96 ± 0.51	4.17 ± 0.65 * (a) ** (d)	0.99 ± 0.58 ** (c)
Glycine	2.81 ± 0.48	1.60 ± 0.50 ** (d)	1.54 ± 0.25 ** (d)	5.68 ± 0.60 ** (b) ** (c)
Formate	0.19 ± 0.14 * (c)	0.14 ± 0.04 * (c)	0.30 ± 0.04 * (a) * (b)	0.15 ± 0.02
Heatmap values	MM074 ^(a)	MM074 DABRA 24 h (b)	MM074 DABRA 72 h (c)	MM074-R DABRA (d)
Lactate	1.0	0.67	0.80	0.90
Alanine	0.56	0.28	0.18	1.0
Acetate	0.39	0.74	1.0	0.96
Glutamine	0.06	0.82	1.0	0.03
Glutamate	1.0	0.51	0.16	0.98
GSH	0.79	1.0	0.23	0.74
Succinate	0.64	0.38	1.0	0.62
Aspartate	0.06	0.32	1.0	0.65
Creatine	0.84	1.0	0.23	0.69
Phosphocreatine	0.53	1.0	0.58	0.44
Phosphocholine	0.72	0.6	0.19	1.0
GPC	1.0	0.62	0.23	0.99
Glucose	0.27	0.47	1.0	0.24
Glycine	0.49	0.28	0.27	1.0
Formate	0.63	0.47	1.0	0.50

(B) Extracellular metabonomics signatures

AUC values	MM074 ^(a)	MM074 DABRA 24 h (b)	MM074 DABRA 72 h (c)	MM074-R DABRA (d)
Glucose	3.81 ± 0.43	4.81 ± 0.21 * (d)	4.67 ± 0.22 * (d)	3.44 ± 0.31 * (b) * (c)
Lactate	68.38 ± 11.97 * (c)	36.27 ± 14.77	26.46 ± 12.96 * (a) ** (d)	69.16 ± 16.47 ** (c)
Alanine	2.41 ± 0.34 * (c)	1.46 ± 0.37	1.38 ± 0.19 * (a) * (d)	2.33 ± 0.50 * (c)
Glutamine	0.10 ± 0.09	0.39 ± 0.12	0.62 ± 0.12	0.24 ± 0.37
Heatmap values	MM074 ^(a)	MM074 DABRA 24 h (b)	MM074 DABRA 72 h (c)	MM074-R DABRA (d)
Glucose	0.79	1.0	0.97	0.72
Lactate	0.99	0.52	0.38	1.0
Alanine	1.0	0.61	0.57	0.97
Glutamine	0.16	0.63	1.0	0.39

Area under the curve (AUC) of resonances from selected metabolites within intra- (A) or extracellular (B) ¹H-NMR spectra, presented as mean ± standard deviation. Heatmap values were calculated using univariate data conversion, dividing each metabolite AUC values by the highest one. Letters are used to tag groups (a: sensitive cells, b: sensitive cells exposed for 24h to dabrafenib, c: sensitive cells exposed for 72h to dabrafenib, d: resistant cells exposed to dabrafenib) and highlight the statistical significance resulting from the multiple Kruskal-Wallis test comparison of the groups as follows: * $p < 0.05$ ** $p < 0.01$ *** $p < 0.001$.

Table S3. Metabonomics signatures of the HBL cells in various experimental conditions.

(A) Intracellular metabonomics signatures

AUC values	HBL ^(a)	HBL DASA 72 h ^(b)	HBL-R DASA ^(c)
Lactate	9.00 ± 1.76 ** (b) * (c)	6.67 ± 0.49 ** (a)	6.92 ± 0.88 * (a)
Alanine	2.32 ± 0.19 *** (b)	4.02 ± 0.42 *** (a) * (c)	3.00 ± 0.18 * (b)
Acetate	0.52 ± 0.09 * (b)	0.32 ± 0.16 * (a)	0.45 ± 0.09
Glutamine	1.70 ± 0.12 ** (b) ** (c)	0.90 ± 0.15 ** (a)	0.84 ± 0.06 ** (a)
Glutamate	1.87 ± 0.19	1.86 ± 0.24	1.91 ± 0.16
GSH	1.11 ± 0.14	0.99 ± 0.26	1.05 ± 0.12
Succinate	0.24 ± 0.06	0.27 ± 0.05	0.23 ± 0.03
Aspartate	0.13 ± 0.02 * (c)	0.12 ± 0.02 * (c)	0.07 ± 0.02 * (a) * (b)
Creatine	3.72 ± 0.51	5.25 ± 1.74 * (c)	3.47 ± 0.48 * (b)
Phosphocreatine	1.57 ± 0.38 * (b)	2.63 ± 0.95 * (a)	1.76 ± 0.31
Phosphocholine	12.65 ± 1.24 * (b)	10.04 ± 1.65 * (a)	11.18 ± 1.12
GPC	5.97 ± 0.60 *** (b)	9.05 ± 1.04 *** (a)	7.09 ± 0.53
Glucose	1.83 ± 0.26	1.82 ± 0.36	1.86 ± 0.24
Glycine	0.09 ± 0.02 * (b) *** (c)	0.21 ± 0.05 * (a) * (c)	2.10 ± 0.22 *** (a) * (b)
Formate	0.12 ± 0.02	0.09 ± 0.02	0.11 ± 0.02
Heatmap values	HBL ^(a)	HBL DASA 72 h ^(b)	HBL-R DASA ^(c)
Lactate	1.0	0.74	0.77
Alanine	0.58	1.0	0.75
Acetate	1.0	0.62	0.87
Glutamine	1.0	0.53	0.49
Glutamate	0.98	0.97	1.0
GSH	1.0	0.89	0.95
Succinate	0.88	1.0	0.85
Aspartate	1.0	0.92	0.54
Creatine	0.71	1.0	0.66
Phosphocreatine	0.6	1.0	0.67
Phosphocholine	1.0	0.79	0.88
GPC	0.66	1.0	0.78
Glucose	0.98	0.98	1.0
Glycine	0.04	0.10	1.0
Formate	1.0	0.75	0.92

(B) Extracellular metabonomics signatures

AUC values	HBL ^(a)	HBL DASA 72 h ^(b)	HBL-R DASA ^(c)
Glucose	4.67 ± 0.11 *** (c)	4.44 ± 0.20 * (c)	3.90 ± 0.23 *** (a) * (b)
Lactate	50.82 ± 6.56 *** (b)	33.88 ± 2.93 *** (a)	41.54 ± 3.23
Alanine	1.95 ± 0.23	1.82 ± 0.18	1.84 ± 0.24
Glutamine	1.15 ± 0.16 * (b) *** (c)	0.56 ± 0.07 * (a)	0.43 ± 0.06 *** (a)
Heatmap values	HBL ^(a)	HBL DASA 72 h ^(b)	HBL-R DASA ^(c)
Glucose	1.0	0.95	0.84
Lactate	1.0	0.67	0.82
Alanine	1.0	0.93	0.94
Glutamine	1.0	0.49	0.37

Area under the curve (AUC) of resonances from selected metabolites within intra- (**A**) or extracellular (**B**) ¹H-NMR spectra, presented as mean ± standard deviation. Heatmap values were calculated using univariate data conversion, dividing each metabolite AUC values by the highest one. Letters are used to tag groups (a: sensitive cells, b: sensitive cells exposed for 72h to dasatinib, c: resistant cells exposed to dasatinib) and highlight the statistical significance resulting from the multiple Kruskal-Wallis test comparison of the groups as follows: * $p < 0.05$ - ** $p < 0.01$ - *** $p < 0.001$.

Table S4. Median centered Log2 data of the relative protein levels between the HBL, MM074 and MM161 resistant and sensitive counterparts.

Protein name	Sample description					
	HBL	HBL-R	MM074	MM074-R	MM161	MM161-R
DUSP4	-1.903073954	-0.535891047	-0.281792955	0.955274349	0.658281949	0.281792955
HIF1A	-0.574084212	-0.094590894	0.232850745	1.506291346	0.094590894	-0.305689909
MAPK1,3	-1.029546585	-0.99650041	-0.126599225	1.057120703	0.126599225	0.947461899
PLCG1	0.152585769	0.097659941	-1.476234731	-0.137862469	-0.097659941	0.702727645
Tau	-0.93692358	-0.053104712	-1.052763994	0.053104712	0.267849423	0.278592465
ATRAX	-0.25039738	-0.006105834	0.452982029	-0.611029244	0.006105834	0.705620881
Caveolin 1	0.157388396	0.269891226	1.647437576	-0.157388396	-0.169231058	-0.199839293
EPHA2	0.093669683	-0.560703649	2.627886222	0.38645617	-0.212129776	-0.093669683
EPHA2	-0.190491324	-0.302815025	1.787832647	0.375551256	-0.144898341	0.144898341
Rho GTPase activating protein 45	-1.843519301	-1.2423631	0.768232202	-0.768232202	1.38918177	2.171147282
PARG	-0.404217551	-0.080766764	1.242504695	-0.228860887	0.080766764	0.126884143
MLKL	-0.104545873	-0.193535146	0.104545873	-1.171105769	1.91805893	1.93223713
STAT5A	-0.371694476	-0.241862147	0.241862147	-0.797745855	1.181642243	0.796311949
BCL2L1	-0.629146848	0.426960563	-0.744560383	-0.131129775	0.541157293	0.131129775
DNMT1	-0.242796493	0.790347808	-0.721359808	-0.405990615	0.242796493	0.311450513
FOXO1	-0.726127382	0.408681713	-0.726989284	-0.408681713	0.685997615	0.81260914
MSH6	-0.528605186	0.756253597	-0.752770362	-0.321893606	0.321893606	0.657301197
RB1	-0.609487278	0.749124156	-1.009323131	-0.675610405	0.614782846	0.609487278
AKT 1/2/3	2.476047665	0.280506987	0.090616579	-0.291949794	-0.090616579	-0.178265815
AKT 1/2/3	1.213853733	-0.001435672	-0.084215398	0.009653353	-0.185219727	0.001435672
GYS1	0.820206376	-0.228717023	0.25539653	0.228717023	-0.329946576	-0.447764884
p21	1.374127741	-0.91393387	1.343531022	0.91393387	-2.520938357	-2.644286415
RPS6KA1	-0.112089914	-1.912268939	0.564518527	0.175202995	-1.087055761	0.112089914
SLC1A5	0.800748501	-0.428186856	0.062005418	-0.062005418	0.2772064	-0.362238978
SRC	0.362008929	-3.050474284	0.189322812	-0.227050453	0.655644903	-0.189322812
Rps6	-0.112089914	-1.912268939	0.564518527	0.175202995	-1.087055761	0.112089914
Rps6	0.504791002	-0.9750599	0.623395095	0.245045029	-1.781288812	-0.245045029
ZAP70	2.4889699	-0.00801409	0.120294259	-0.060367097	0.00801409	-0.016139345
CDC6	-0.104391808	0.138162769	-0.72861176	-0.110228026	0.104391808	1.109268053
ER-a	-0.35330408	-0.016668567	-0.267597011	0.016668567	0.393382995	2.962696234
Histone H3	-0.104545932	0.152519008	-0.097293528	0.097293528	-0.139922002	2.169434008
MAP2K1,2	-0.201238483	-0.285805012	0.475508867	0.013175291	-0.013175291	1.001260984
HK2	-0.005088175	0.005088175	-0.419560549	0.502940827	0.80444892	-0.518766725
c-kit	1.791504654	2.26639649	-0.535834229	-0.478565734	0.478565734	-2.789795411
COX1	0.640978672	0.503342035	-0.550204093	-0.503342035	0.856353098	-0.71268049