

# Proteomic Analysis Identifies Multi-Dimensional Deregulated Signaling Pathways in SCLC Lung Cancer

Klarisa Rikova, Ben Hall, Tyler Levy, Anthony Possemato, Mike Aguiar, Sean Beausoleil, Jian Min Ren, Kimberly Lee Lee, Scott Lonning, Michael Comb Cell Signaling Technology, Inc., Danvers MA 01923

## INTRODUCTION

Lung cancer is the most common type of cancer in the U.S., with 5 yr. survival rates of 18% and 6% for non-small cell lung carcinoma (NSCLC) and small cell lung carcinoma (SCLC) patients, respectively. In spite of success with targeted therapies for 15-20% of patients with NSCLCs, the success is generally short-lived as the tumors become resistant to treatment. In contrast to NSCLC, no successful targeted therapies have been identified in SCLC. Further understanding of the regulatory cell signaling networks for SCLCs, may enable the discovery of new actionable lung cancer drivers. Posttranslational modifications (PTMs), including phosphorylation, acetylation and methylation, act alone and in combination to regulate protein function, cellular behavior and epigenetics. All three types of PTMs are deregulated in various cancers. In this study we developed a highly quantitative MS/MS approach combining TMT labeling with a number of motif and site-specific antibodies to identify deregulated signaling pathways between tumor and normal specimens. PTM-specific antibodies were used to enrich modified peptides followed by 6-plex TMT mass-spec analyses to quantitatively measure over 15,300 differentially phosphorylated, acetylated, and methylated sites on over 4,600 proteins from 17 SCLC patient tissues and 5 para-normals. Understanding cell-signaling networks at the intersection of multiple pathways and protein modification systems in SCLC lung cancers leads to the identification of new druggable disease drivers.

## CONCLUSIONS

- We have developed a unique proteomic approach to survey signaling pathways in cancer, revealing information not available at the chromosomal and transcriptional level.
- This approach was applied to SCLC identifying new deregulated pathways and possible drug targets.

## Profiling of PTM Spaces with TMT labeling

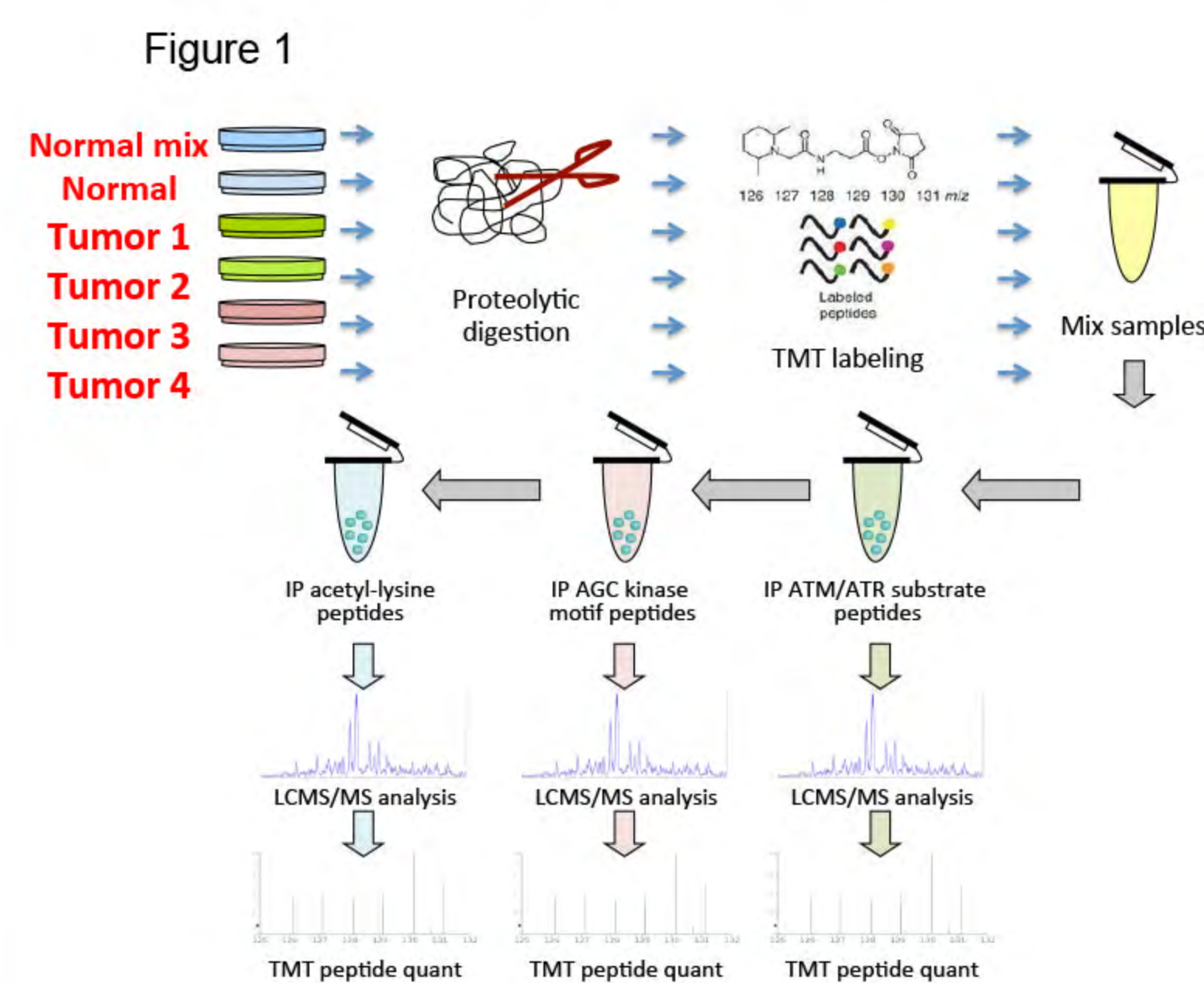


Figure 1: 2mg of peptides from trypsin digested tissue were labelled with TMT reagent and combined into 20 six-plexes. The mix of normals was a concatenation of the normal samples. Following a test mix to ensure a 1:1:1:1:1 mix across all channels, samples were appropriately combined and serially fractionated through immunoprecipitation with antibodies specific for phosphotyrosine, ATM/ATR substrate motif, AGC/CAMK/STE family kinase motif, acetyl-lysine, and methyl-arginine. For all phospho-spaces, an additional Immobilized Metal Affinity Chromatography (IMAC) step was used to further enrich samples. The peptides from each immunoprecipitation were analyzed by LCMS/MS using a Q-Exactive\*, with quantification of the TMT labels enabled by CID fragmentation. Data processing was performed using CORE. (Protein Sieve, Protein Assembler, Protein Quant.). In the analysis of our data, we defined upregulated protein meets the criteria of having >5 fold change based on 2 sigma cutoff (95% confidence).

Table 1 Sequential IP Reagents

Ab Target	Clone #	Motif
pY-1000	D1G10/D2D1	Phospho Tyr
AGC/PSD	D3E5/D8D9/D4E2/D8B11	AGC/CAMK/STE kinase activation loop
Akt substrate	100B7	RXX(s/t)
AMPK	D72H3/D78C9	(s/t)Q
ATM/ATR substrate	D23/D69	(s/t)Q
ATM/ATR substrate	D14/D86	(s/t)Q
Ac-K	D10G3/11D/16E	Acetyl lysine
K-Me	D4P3J, D3Z9J, D8R1C	K-Me
R-Me	Me-R4-100	R-Me
Epigenetic Regulator Mix	-	-
Ubiquitin Library	D4A7A10	kGG

## Deregulated Tumor Signaling: Multiple Dimensions

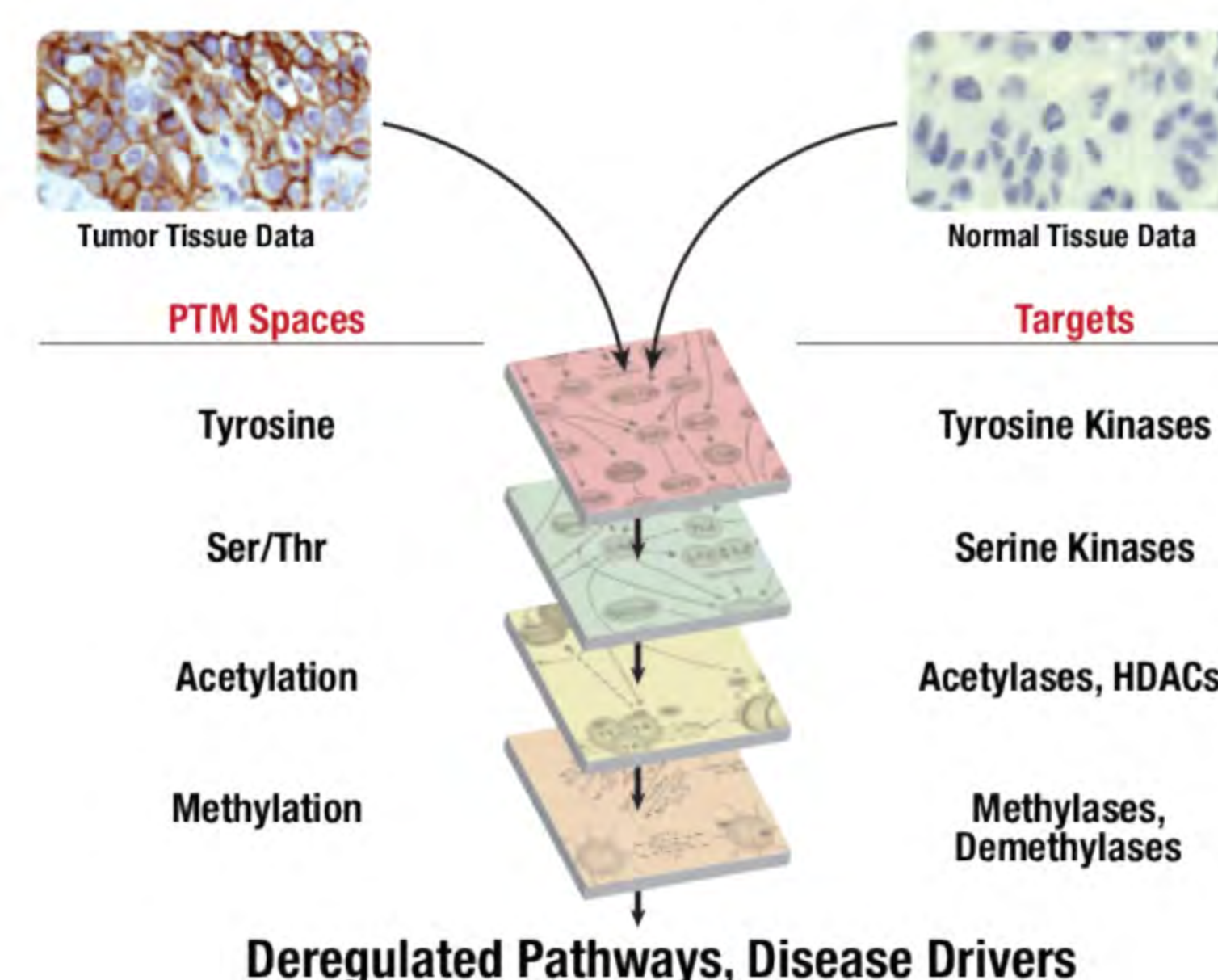


Figure 3. Unsupervised Clustering of Kinases with Tyrosine Phosphorylation

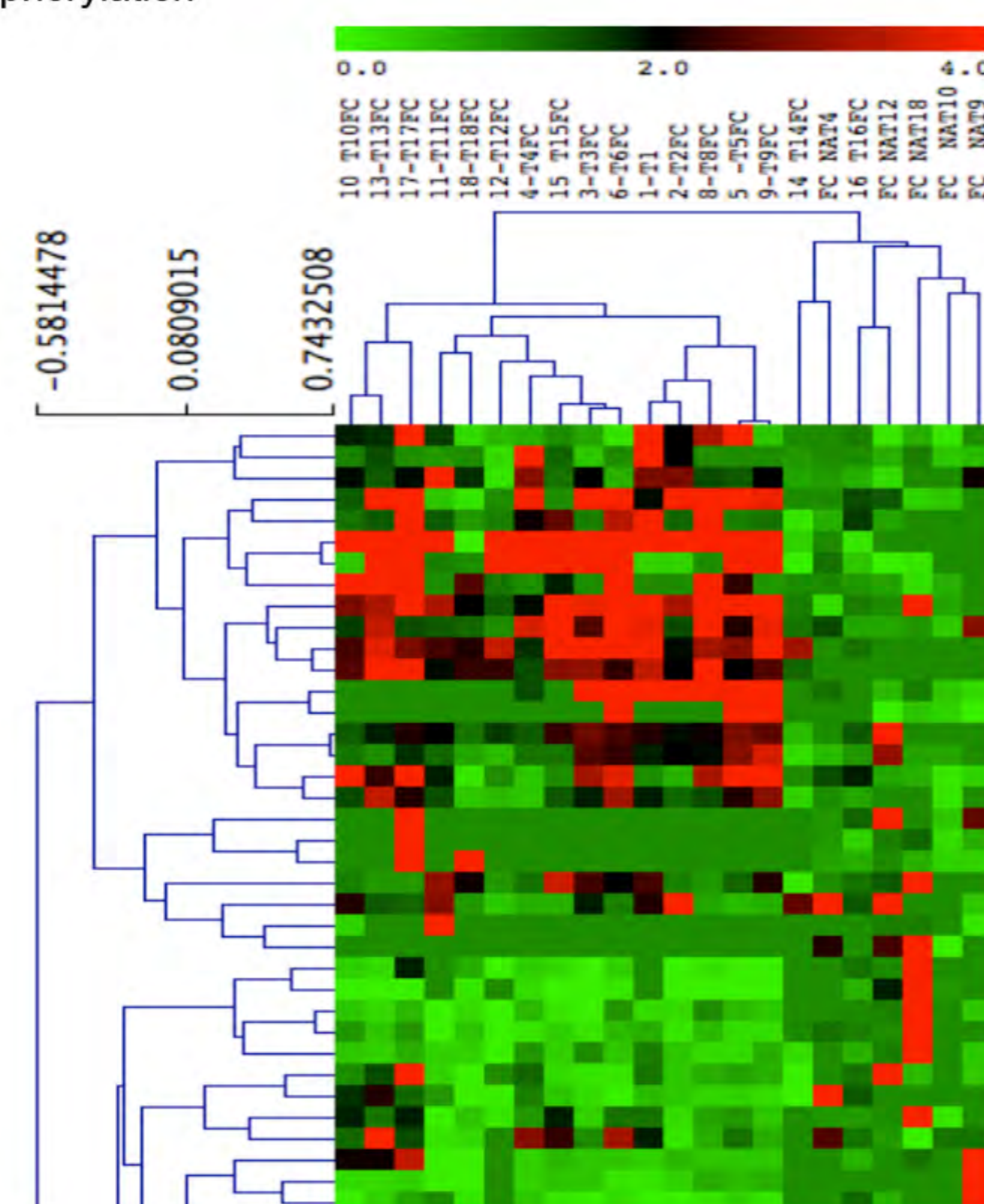


Figure 4 and 5. Unsupervised Clustering of Enzymes with Ser/Thr Phosphorylation and Me/Ac

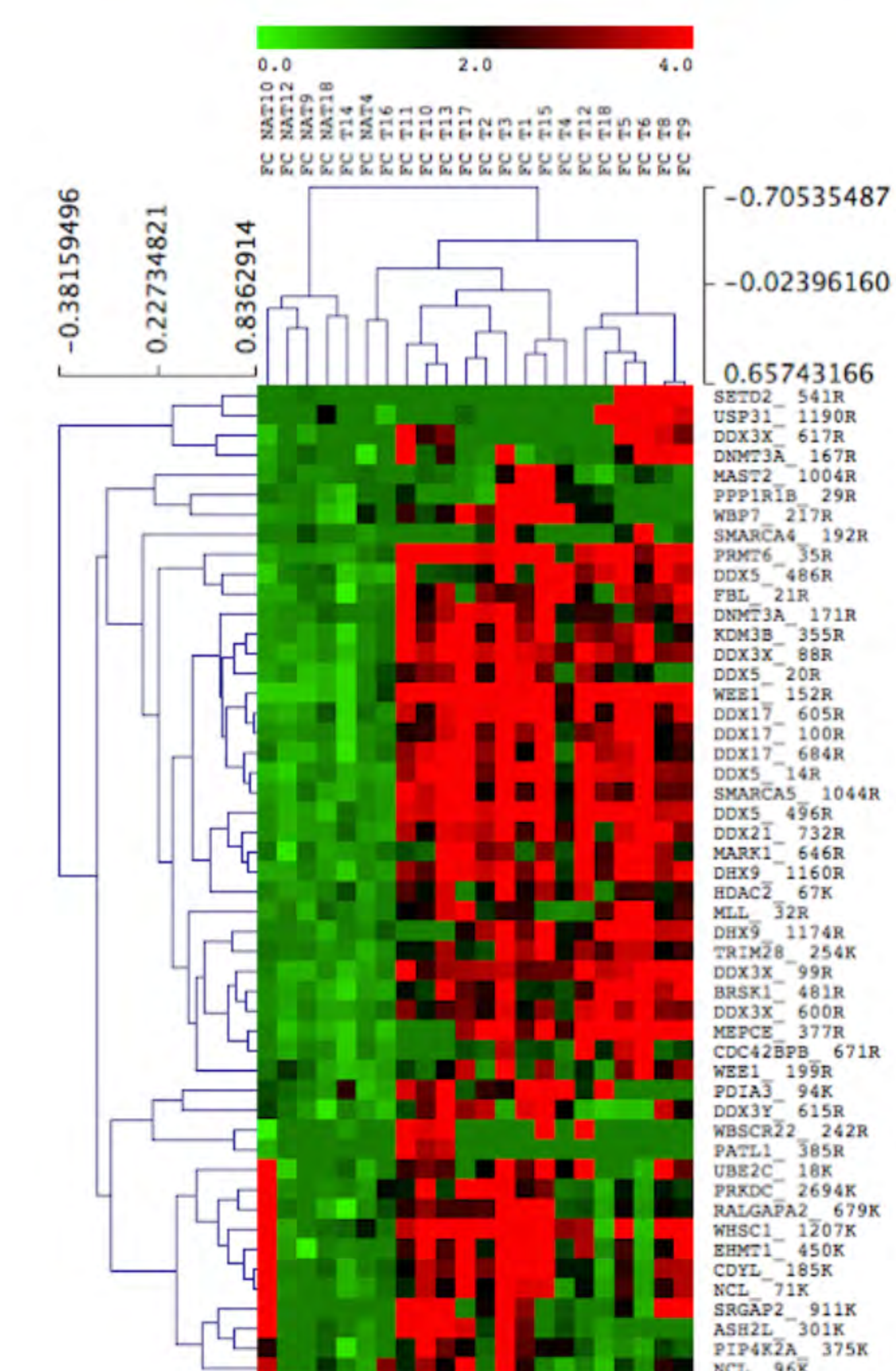
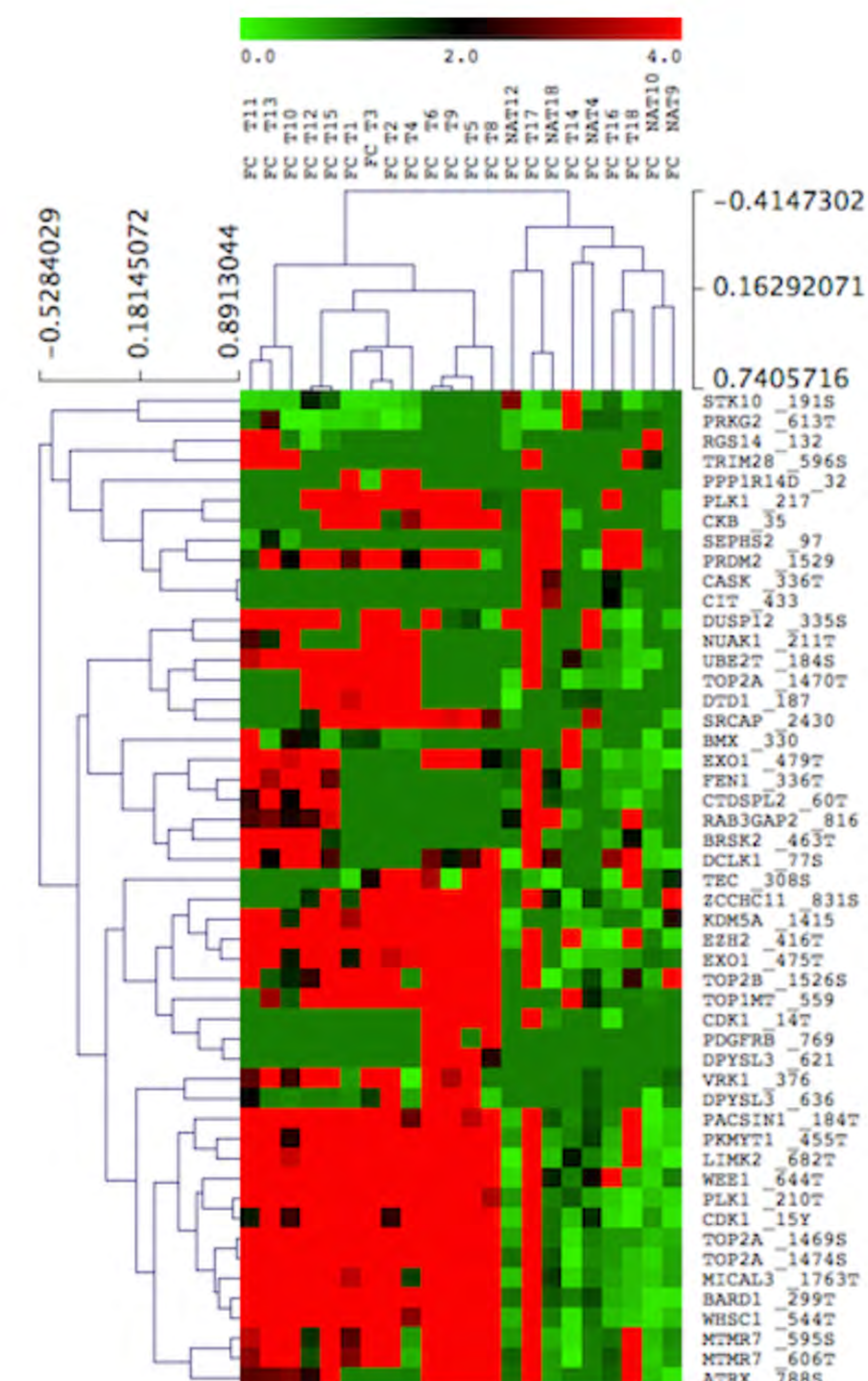


Figure 6. Kegg: Pathways Activated in SCLC

KEGG Pathway	Category	Gene	Count	Score	Significance
S04106	Metabolic pathways	18	0.4	4.849	4.2E-7
S04107	Metabolic pathways	17	0.3	7.769	3.4E-7
S04108	Metabolic pathways	21	0.4	6.776	5.6E-7
S04109	Metabolic pathways	21	0.4	6.776	5.6E-7
S04110	Metabolic pathways	19	0.4	6.607	1.3E-6
S04111	Metabolic pathways	14	0.3	1.154	2.0E-5
S04112	Metabolic pathways	14	0.3	1.154	2.0E-5
S04113	Metabolic pathways	11	0.2	3.265	4.4E-4
S04114	Metabolic pathways	11	0.2	3.265	4.4E-4
S04115	Metabolic pathways	11	0.2	3.265	4.4E-4
S04116	Metabolic pathways	11	0.2	3.265	4.4E-4
S04117	Metabolic pathways	11	0.2	3.265	4.4E-4
S04118	Metabolic pathways	11	0.2	3.265	4.4E-4
S04119	Metabolic pathways	11	0.2	3.265	4.4E-4
S04120	Metabolic pathways	11	0.2	3.265	4.4E-4
S04121	Metabolic pathways	11	0.2	3.265	4.4E-4
S04122	Metabolic pathways	11	0.2	3.265	4.4E-4
S04123	Metabolic pathways	11	0.2	3.265	4.4E-4
S04124	Metabolic pathways	11	0.2	3.265	4.4E-4
S04125	Metabolic pathways	11	0.2	3.265	4.4E-4
S04126	Metabolic pathways	11	0.2	3.265	4.4E-4
S04127	Metabolic pathways	11	0.2	3.265	4.4E-4
S04128	Metabolic pathways	11	0.2	3.265	4.4E-4
S04129	Metabolic pathways	11	0.2	3.265	4.4E-4
S04130	Metabolic pathways	11	0.2	3.265	4.4E-4
S04131	Metabolic pathways	11	0.2	3.265	4.4E-4
S04132	Metabolic pathways	11	0.2	3.265	4.4E-4
S04133	Metabolic pathways	11	0.2	3.265	4.4E-4
S04134	Metabolic pathways	11	0.2	3.265	4.4E-4
S04135	Metabolic pathways	11	0.2	3.265	4.4E-4
S04136	Metabolic pathways	11	0.2	3.265	4.4E-4
S04137	Metabolic pathways	11	0.2	3.265	4.4E-4
S04138	Metabolic pathways	11	0.2	3.265	4.4E-4
S04139	Metabolic pathways	11	0.2	3.265	4.4E-4
S04140	Metabolic pathways	11	0.2	3.265	4.4E-4
S04141	Metabolic pathways	11	0.2	3.265	4.4E-4
S04142	Metabolic pathways	11	0.2	3.265	4.4E-4
S04143	Metabolic pathways	11	0.2	3.265	4.4E-4
S04144	Metabolic pathways	11	0.2	3.265	4.4E-4
S04145	Metabolic pathways	11	0.2	3.265	4.4E-4
S04146	Metabolic pathways	11	0.2	3.265	4.4E-4
S04147	Metabolic pathways	11	0.2	3.265	4.4E-4
S04148	Metabolic pathways	11	0.2	3.265	4.4E-4
S04149	Metabolic pathways	11	0.2	3.265	4.4E-4
S04150	Metabolic pathways	11	0.2	3.265	4.4E-4
S04151	Metabolic pathways	11	0.2	3.265	4.4E-4
S04152	Metabolic pathways	11	0.2	3.265	4.4E-4
S04153	Metabolic pathways	11	0.2	3.265	4.4E-4
S04154	Metabolic pathways	11	0.2	3.265	4.4E-4
S04155	Metabolic pathways	11	0.2	3.265	4.4E-4
S04156	Metabolic pathways	11	0.2	3.265	4.4E-4
S04157	Metabolic pathways	11	0.2	3.265	4.4E-4
S04158	Metabolic pathways	11	0.2	3.265	4.4E-4
S04159	Metabolic pathways	11	0.2	3.265	4.4E-4
S04160	Metabolic pathways	11	0.2	3.265	4.4E-4
S04161	Metabolic pathways	11	0.2	3.265	4.4E-4
S04162	Metabolic pathways	11	0.2	3.265	4.4E-4
S04163	Metabolic pathways	11	0.2	3.265	4.4E-4
S04164	Metabolic pathways	11	0.2	3.265	4.4E-4
S04165	Metabolic pathways	11	0.2	3.265	4.4E-4
S04166	Metabolic pathways	11	0.2	3.265	4.4E-4
S04167	Metabolic pathways	11	0.2	3.265	4.4E-4
S04168	Metabolic pathways	11	0.2	3.265	4.4E-4
S04169	Metabolic pathways	11	0.2	3.265	4.4E-4
S04170	Metabolic pathways	11	0.2	3.265	4.4E-4
S04171	Metabolic pathways	11	0.2	3.265	4.4E-4
S04172	Metabolic pathways	11	0.2	3.265	4.4E-4
S04173	Metabolic pathways	11	0.2	3.265	4.4E-4
S04174	Metabolic pathways	11	0.2	3.265	4.4E-4
S04175	Metabolic pathways	11	0.2	3.265	4.4E-4
S04176	Metabolic pathways	11	0.2	3.265	4.4E-4
S04177	Metabolic pathways	11	0.2	3.265	4.4E-4
S04178	Metabolic pathways	11	0.2	3.265	4.4E-4
S04179	Metabolic pathways	11	0.2	3.265	4.4E-4
S04180	Metabolic pathways	11	0.2	3.265	4.4E-4
S04181	Metabolic pathways	11	0.2	3.265	4.4E-4
S04182	Metabolic pathways	11	0.2	3.265	4.4E-4
S04183	Metabolic pathways	11	0.2	3.265	4.4E-4
S04184	Metabolic pathways	11	0.2	3.265	4.4E-4
S04185	Metabolic pathways	11	0.2	3.265	4.4E-4
S04186	Metabolic pathways	11	0.2	3.265	4.4E-4
S04187	Metabolic pathways	11	0.2	3.265	4.4E-4
S04188	Metabolic pathways	11	0.2	3.265	4.4E-4
S04189	Metabolic pathways	11	0.2	3.265	4.4E-4
S04190	Metabolic pathways	11	0.2	3.265	4.4E-4
S04191	Metabolic pathways	11	0.2	3.265	4.4E-4
S04192	Metabolic pathways	11	0.2	3.265	4.4E-4
S04193	Metabolic pathways	11	0.2	3.265	4.4E-4
S04194	Metabolic pathways	11	0.2	3.265	4.4E-4
S04195	Metabolic pathways	11	0.2	3.265	4.4E-4
S04196	Metabolic pathways	11	0.2	3.265	4.4E-4
S04197	Metabolic pathways	11	0.2	3.265	4.4E-4
S04198	Metabolic pathways	11	0.2	3.265	4.4E-4
S04199	Metabolic pathways	11	0.2	3.265	4.4E-4
S04200	Metabolic pathways	11	0.2	3.265	4.4E-4