

Ribosome Profiling on GTML5 Mouse Cells under M1071 and Rapamycin Treatment

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1 Motivation

Signaling pathways are responsible for coordinating cell behavior, and when dysregulated they often lead to disease. The PIK3CA-AKT-mTOR pathway is commonly activated in human cancers. It plays an important role in cell cycle regulation, metabolism, and protein synthesis. One of its members, the mechanistic target of rapamycin (mTOR), is an serine/threonine kinase whose over-activation promotes tumor progression. To reverse the effect of increased mTOR activity, mTOR inhibitors, such as rapamycin (first generation) and mTOR kinase inhibitors (second generation), are prescribed as cancer therapy. However, over time, natural selection of tumors results in drug resistance and clinical relapse. To counteract mTOR mutants, Rodrik-Outmezguine et al. recently developed a third-generation mTOR inhibitor called Rapalink-1 (M1071). M1071 has been established by Fan et al. as a potent drug for brain tumors owing to its ability to cross the blood-brain barrier. While the mechanism of action of M1071 is known by design, its downstream effects on biological processes, such as protein translation, are not well understood. The goal of this study is to identify proteins whose biosyntheses are sensitive to M1071 in the context of neural cells. We aim to monitor changes in protein translation using two techniques, ribosome profiling and pulsed SILAC proteomics.

2 Ribosome Profiling Workflow and Data Analysis

1. Ozlem split GTML5 mouse cells into nine cultures. Three were treated with DMSO, three with rapamycin, and three with M1071. Cells were incubated for three hours before harvesting for ribosome footprinting and RNAseq.
2. Arun analyzed the ribosome profiling data with Ribomap. The output STATS files are organized as follows:
 - **refID:** Transcript index in the transcriptome fasta file.
 - **tid:** Transcript header name in the transcriptome fasta file.
 - **rabd:** Ribosome loads, which is the total number of ribosome-protected fragments mapped to the transcript.
 - **tabd:** Relative transcript abundance from RNAseq.
 - **te:** Relative translational efficiency, which is the ratio between **rabd** and **tabd**.
3. Ribomap output was processed and visualized in R.
 - Read STATS files from Ribomap into R.
 - Applied pre-processing to organize transcripts into rows and their descriptors into columns.
 - Merged sample data tables by ENSEMBL transcript IDs to create a master table.
 - Removed uninformative data.
 - Transcripts with **rabd** less than 128 in any sample were omitted.
 - Transcripts with more than one missing value in any condition were omitted.
 - Applied \log_2 -transformation to normalize the data.
 - Subtracted the column median from each value to center columns at \log_2 of 0.
 - Performed Welch's two-sample, two-tailed t-test on replicates to evaluate statistical significance of biological change.
 - Wrote data tables into Excel files.
 - *all_treatment_merged_ribomap_output*: Filtered, transformed, and normalized data containing all three conditions and their corresponding replicates.
 - *ratios_relative_transcript_abundance*: Derived from *all_treatment_merged_ribomap_output* and contains pairwise statistical comparisons of the three conditions by relative transcript abundance.
 - *ratios_ribosome_load*: Derived from *all_treatment_merged_ribomap_output* and contains pairwise statistical comparisons of the three conditions by ribosome load.
 - *ratios_translational_efficiency*: Derived from *all_treatment_merged_ribomap_output* and contains pairwise statistical comparisons of the three conditions by translational efficiency.
 - *Notes on Excel markings in the ratio tables:*
 - Red highlight = *LOG2.RATIO* less than -1 (or greater than 2-fold decrease).

- Green highlight = $LOG2.RATIO$ greater than 1 (or greater than 2-fold increase).
- Bold = $Pvalue$ less than 0.05.

Table 1: Summary statistics on the processed samples

Samples	# transcripts	# rabd ≥ 128	# after merging	# after missing value filtering
DMSO-1	33664	11053	12957	8666
DMSO-2	33378	8168	12957	8666
DMSO-3	32517	9541	12957	8666
M1071-1	34271	11057	12957	8666
M1071-2	34054	9334	12957	8666
M1071-3	34065	9658	12957	8666
Rapamycin-1	32999	10770	12957	8666
Rapamycin-2	34032	10159	12957	8666
Rapamycin-3	33893	10906	12957	8666

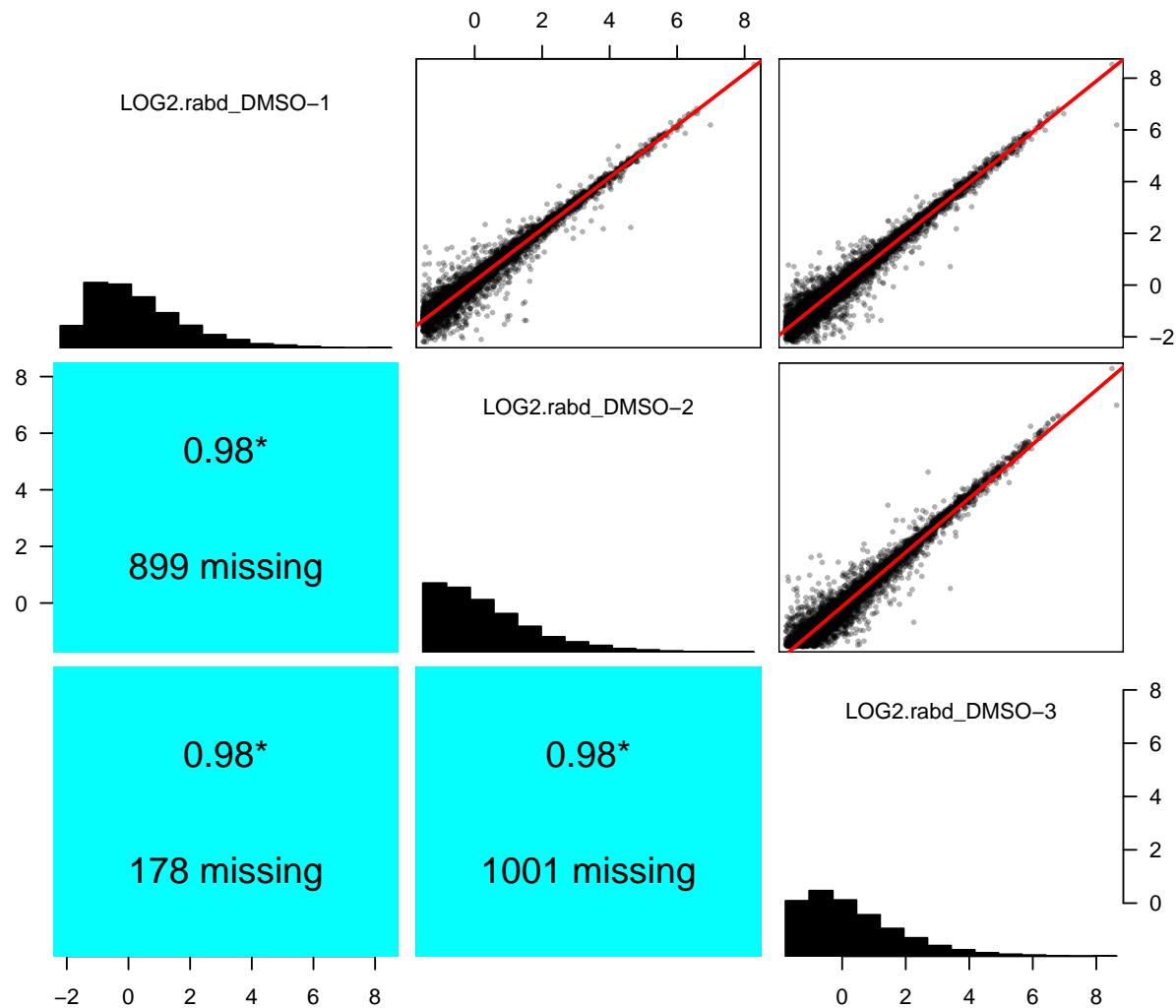
3 About the Figures

- The **correlation figures** show the distribution of each sample along the diagonal. In the upper right corner, the scatter plots depict associations between any two sets of data. The linear regression line is drawn in red. The correlation coefficients are displayed in the lower left corner. Due to the nature of our filter, missing values are allowed and are reported.
- The **volcano plots** display each transcript as a point on the x- and y-axes representing biological and statistical significance, respectively. The statistical test used to call significance is described above. Significantly up- and down-regulated transcripts are colored in green and red, respectively. The dotted horizontal line corresponds to a P-value of 0.05. The dotted vertical lines are drawn arbitrarily to accommodate point labels. It is important to note that the P-values used for plotting are not FDR-controlled. Please see the *ratio* Excel files for P-values corrected by the Benjamini-Hochberg method.
- The **heatmaps** enable comparisons of ribosome load, transcript level, and translational efficiency under individual conditions or ratios between conditions. Each row is a transcript, and its normalized values are coded by color (blue = low, white = medium, red = high). Depending on the figure, either the averages of the replicates were taken or the \log_2 fold changes were calculated.
- All figures are generated from the *ratio* Excel tables.

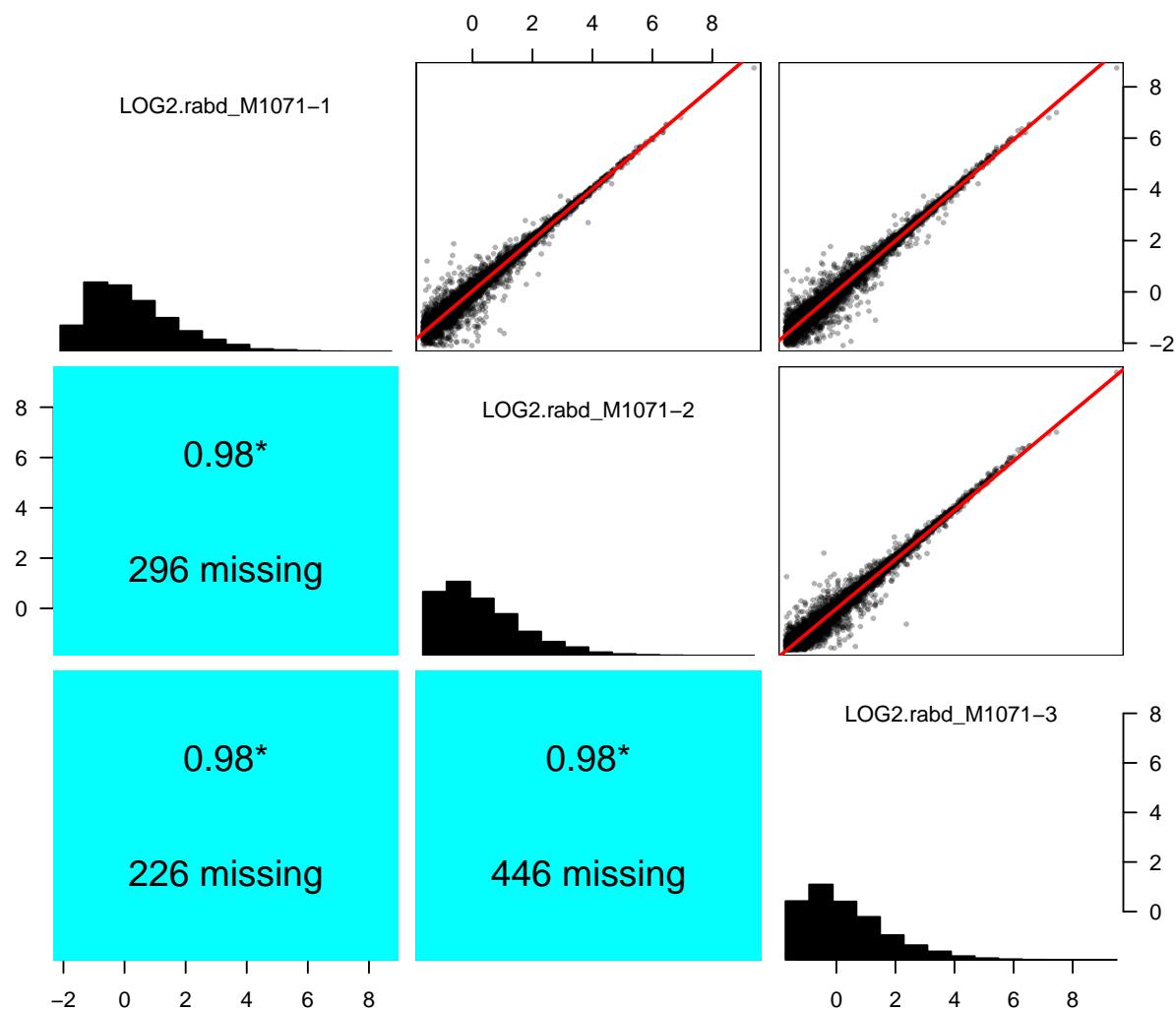
4 Correlation Plots Between Replicates

4.1 Ribomap output: ribosome load

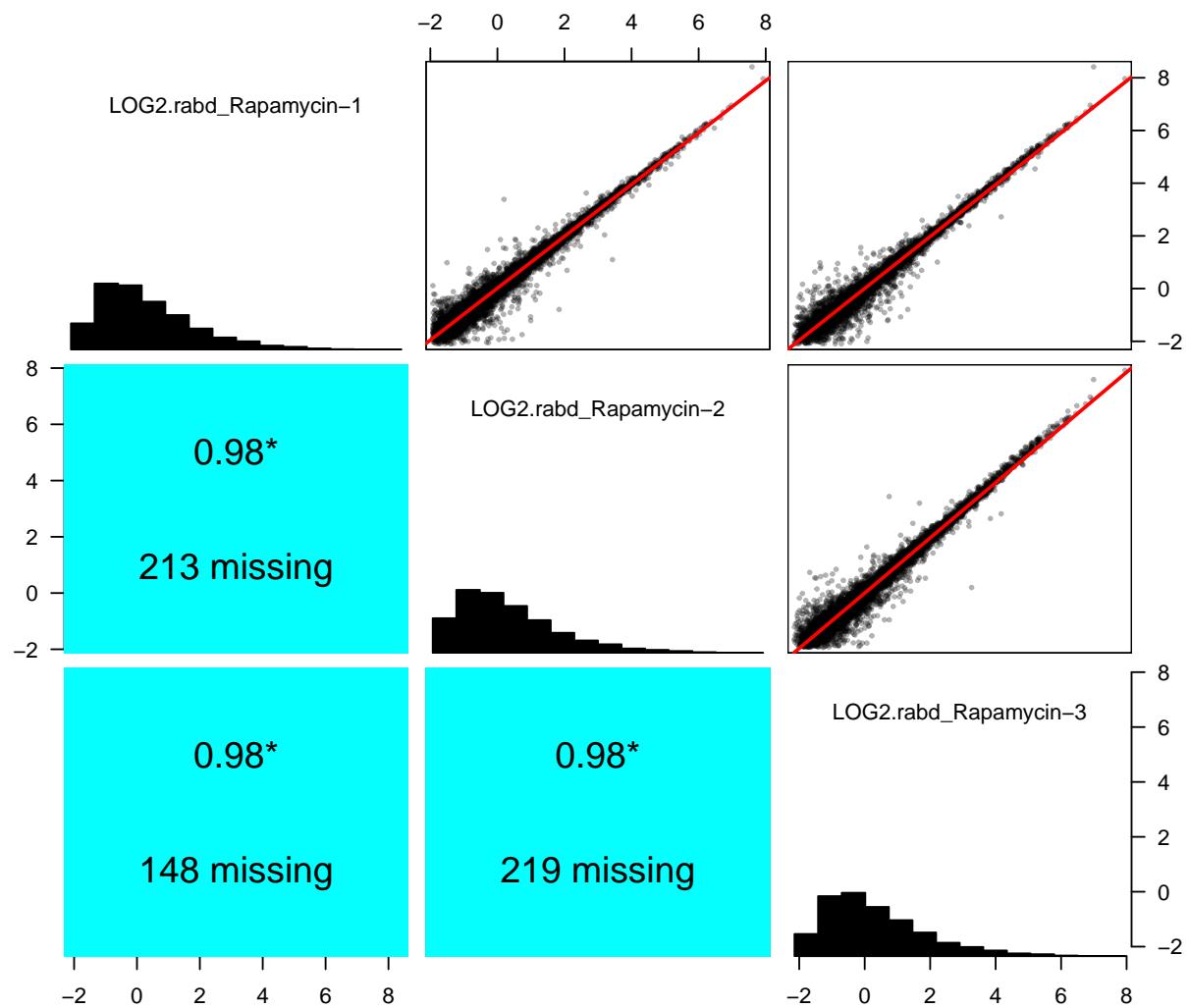
4.1.1 DMSO Treatment



4.1.2 M1071 Treatment

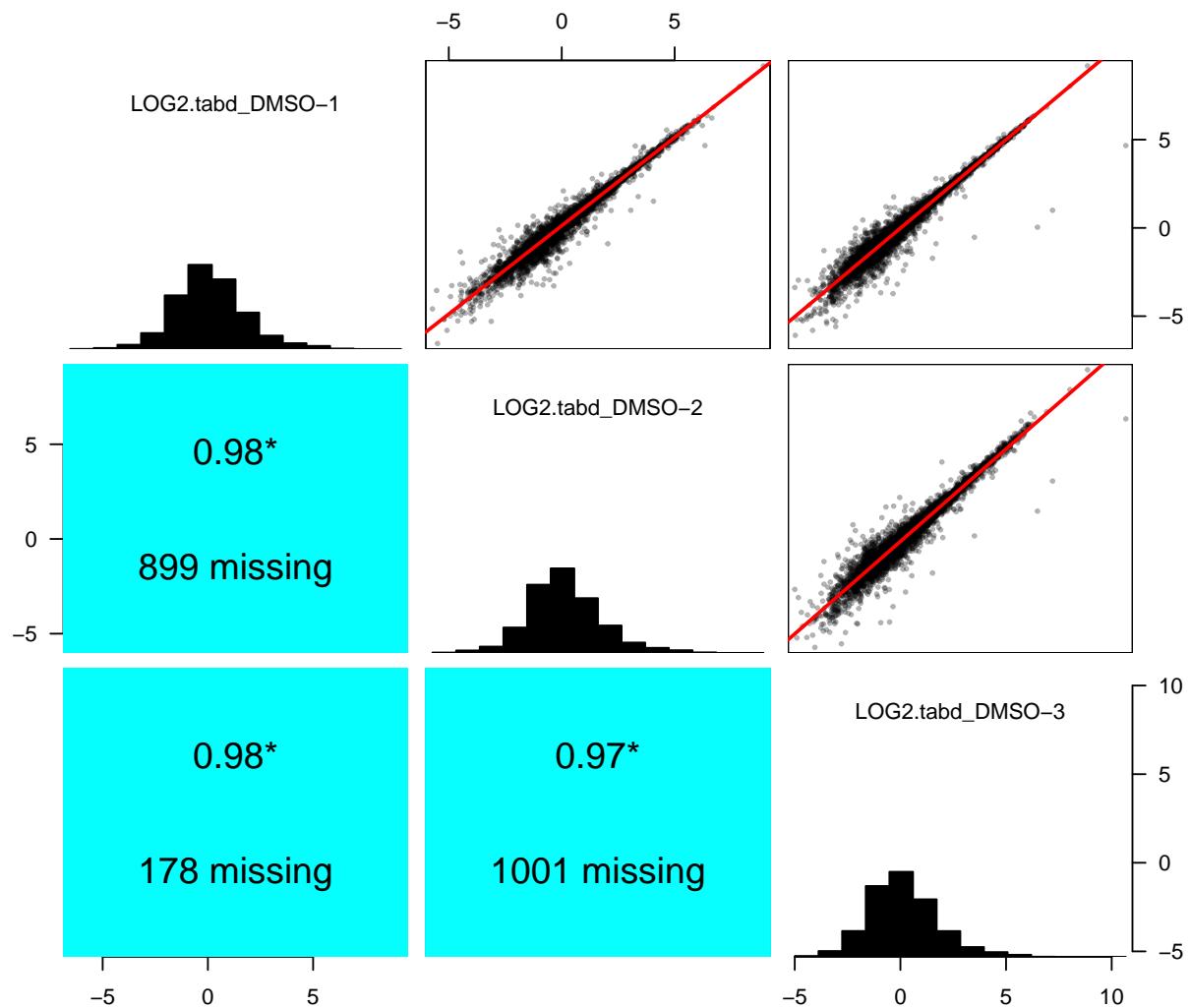


4.1.3 Rapamycin Treatment

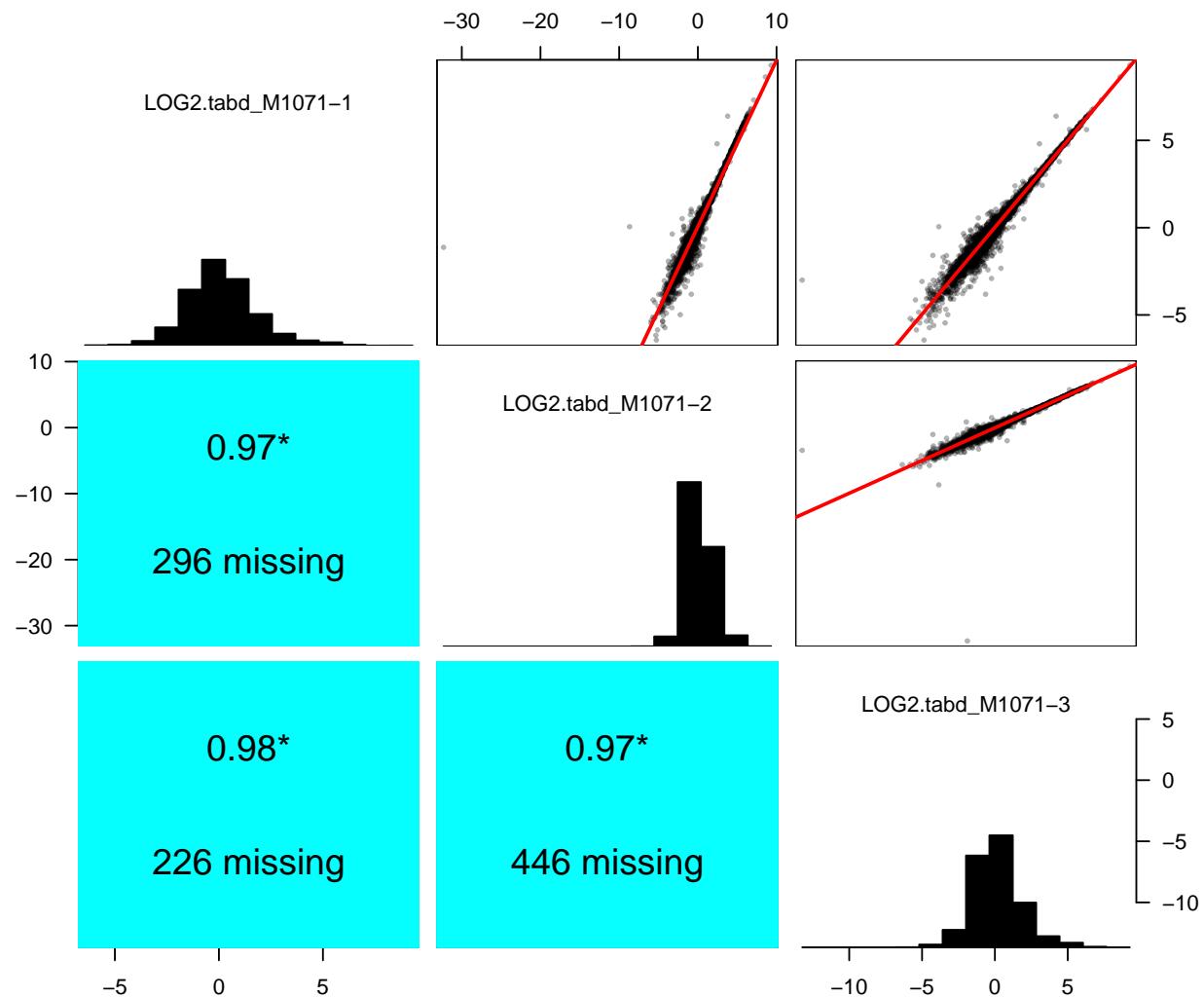


4.2 Ribomap output: relative transcript abundance

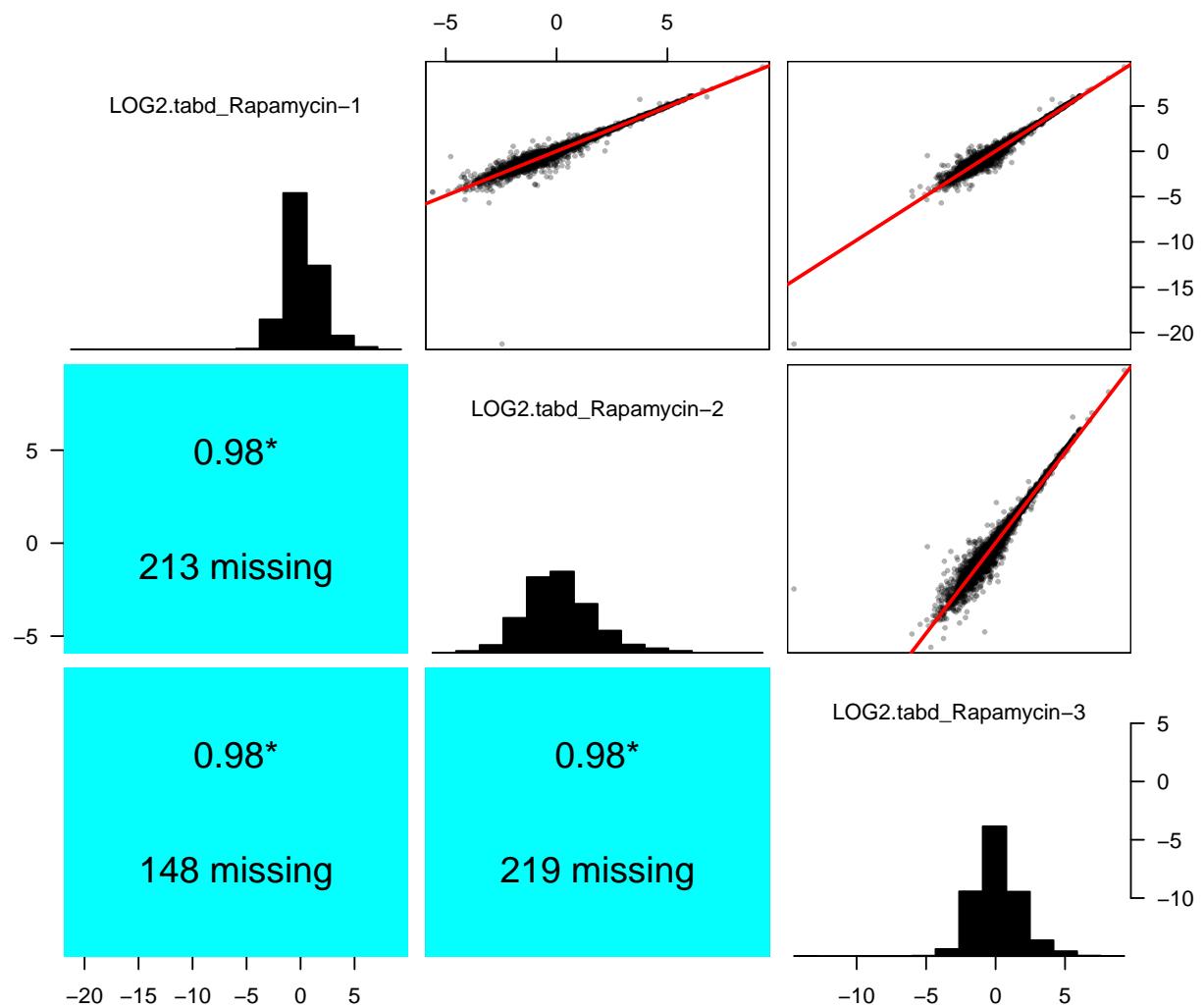
4.2.1 DMSO Treatment



4.2.2 M1071 Treatment

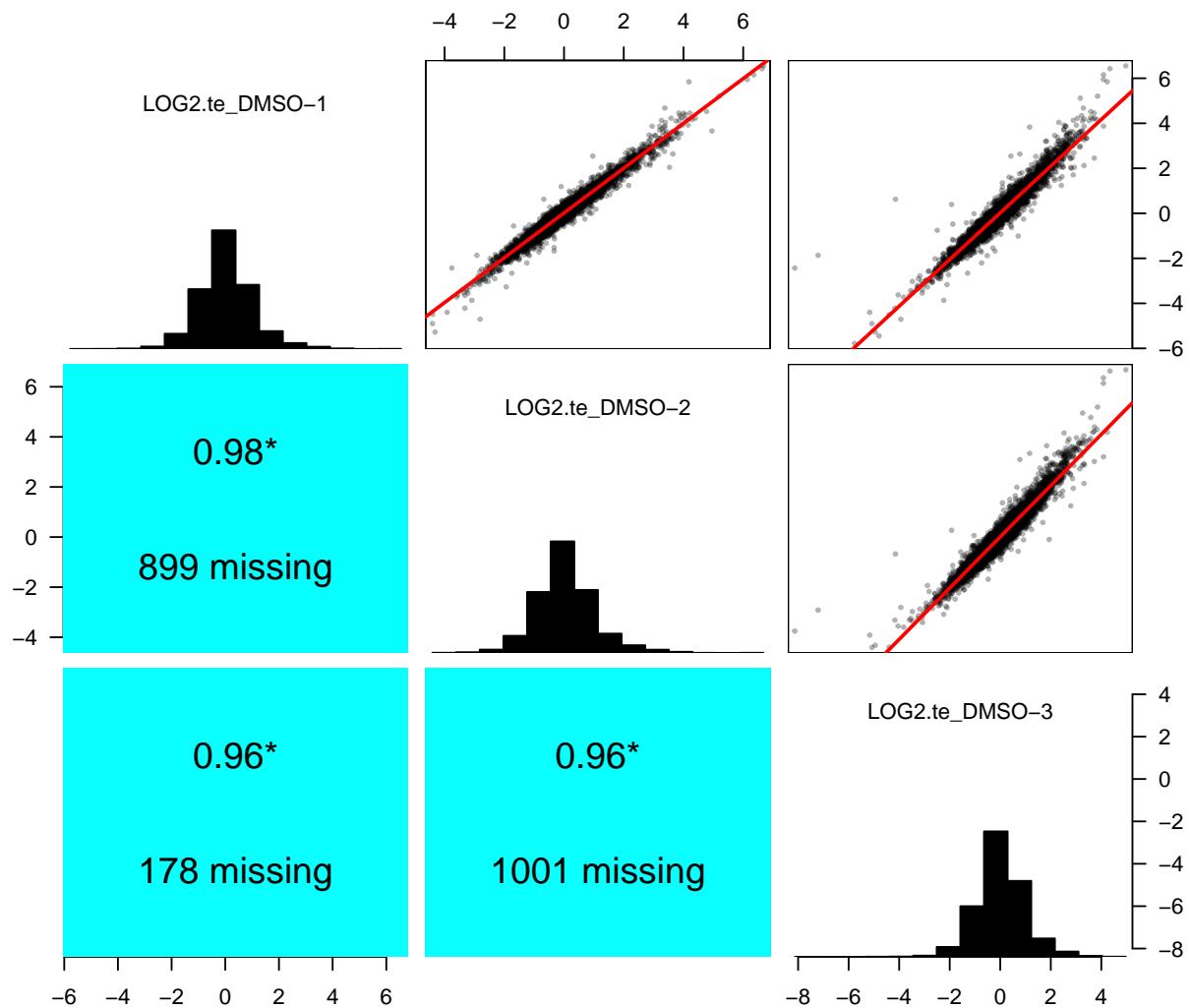


4.2.3 Rapamycin Treatment

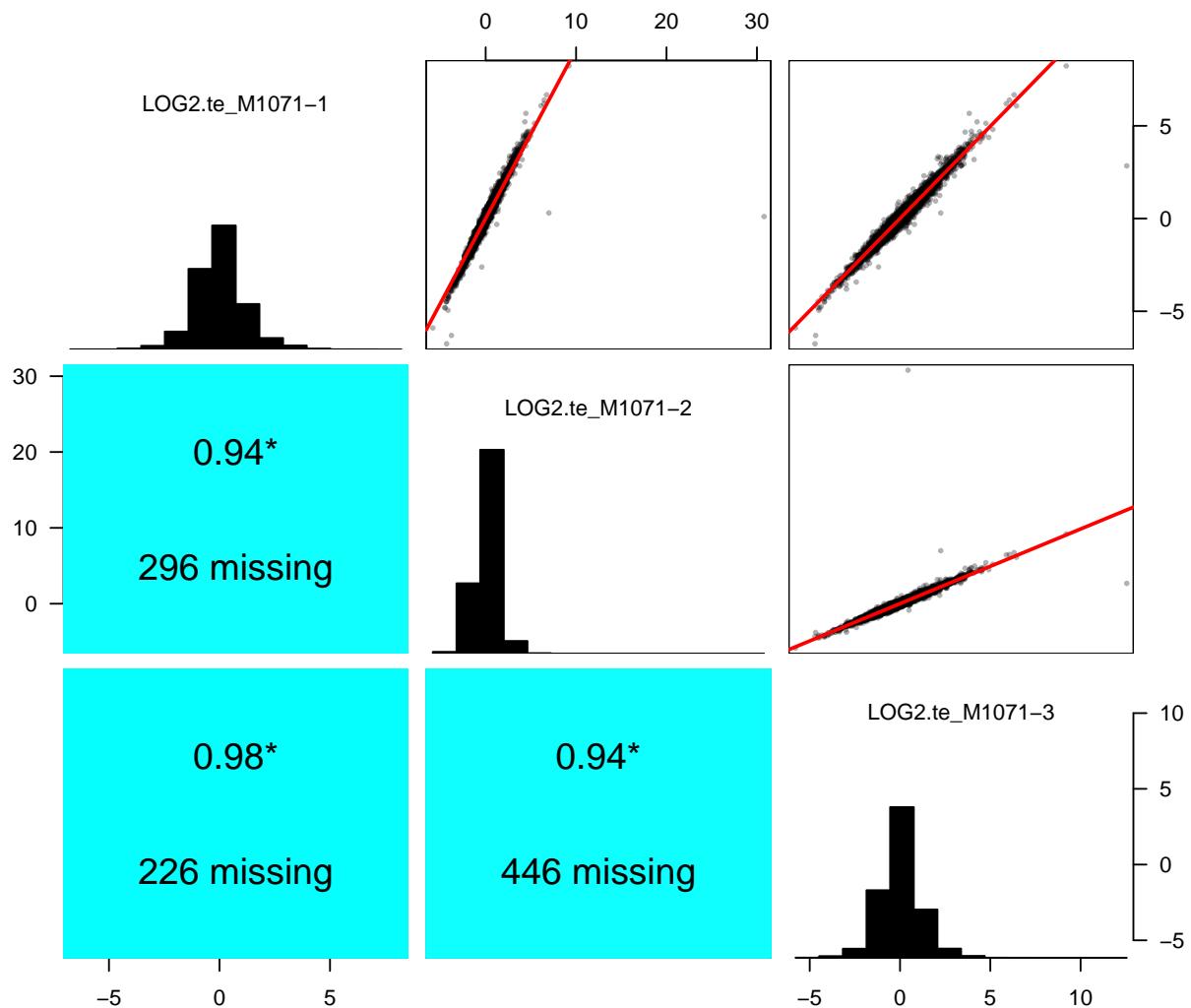


4.3 Ribomap output: translational efficiency

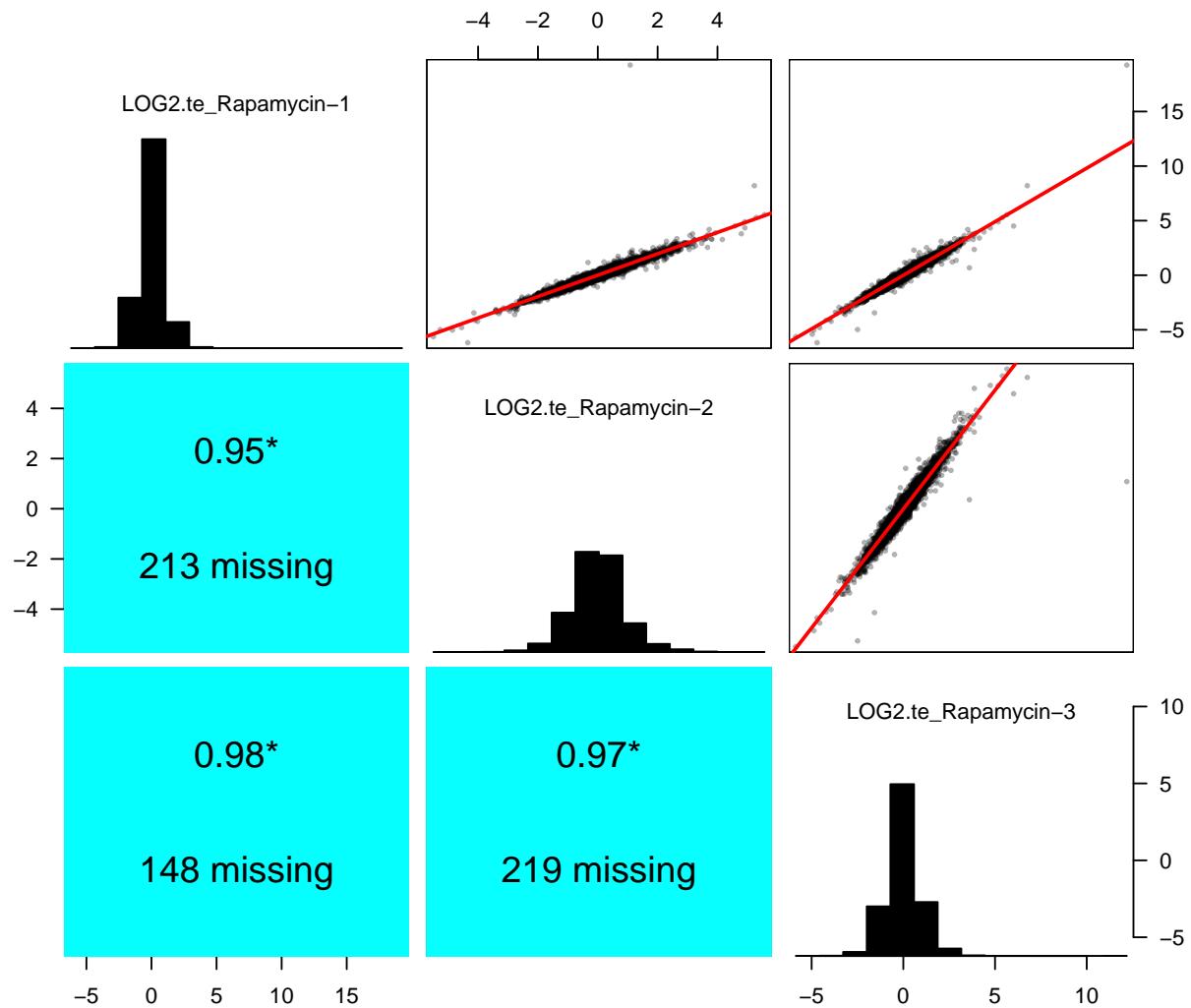
4.3.1 DMSO Treatment



4.3.2 M1071 Treatment



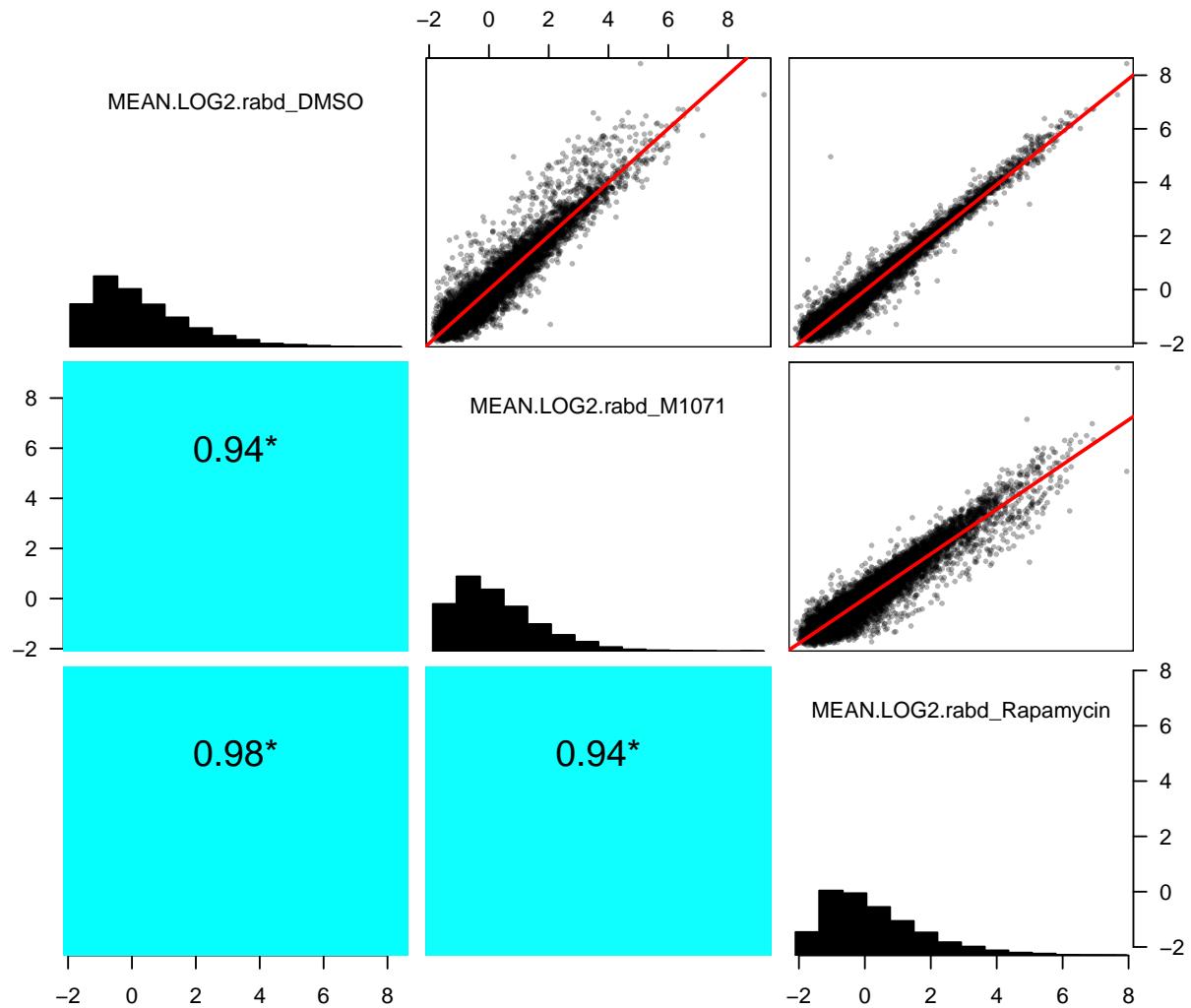
4.3.3 Rapamycin Treatment



5 Correlation Plots Between Treatment Groups

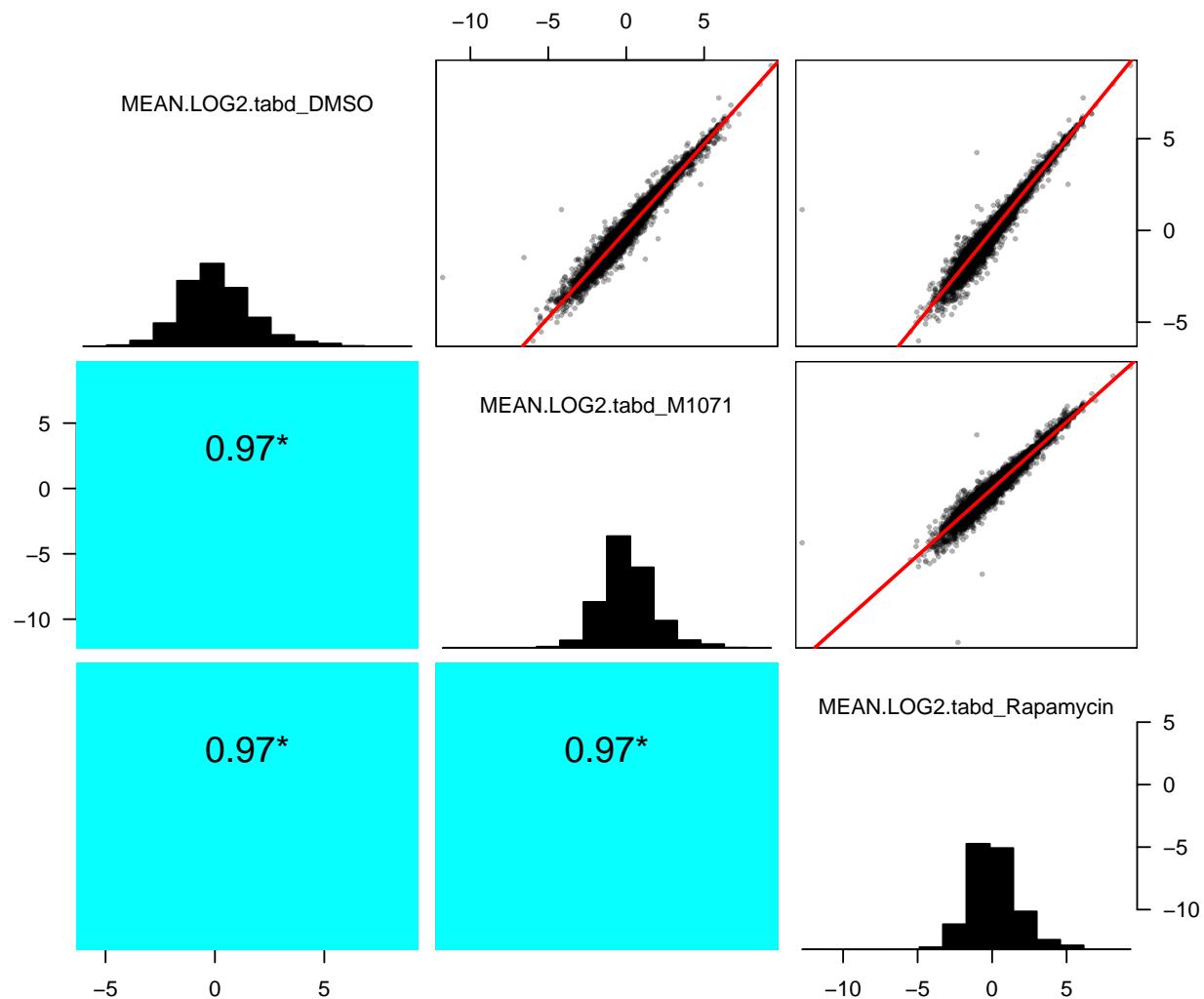
5.1 Ribomap output: ribosome load

5.1.1 DMSO vs M1071 vs Rapamycin



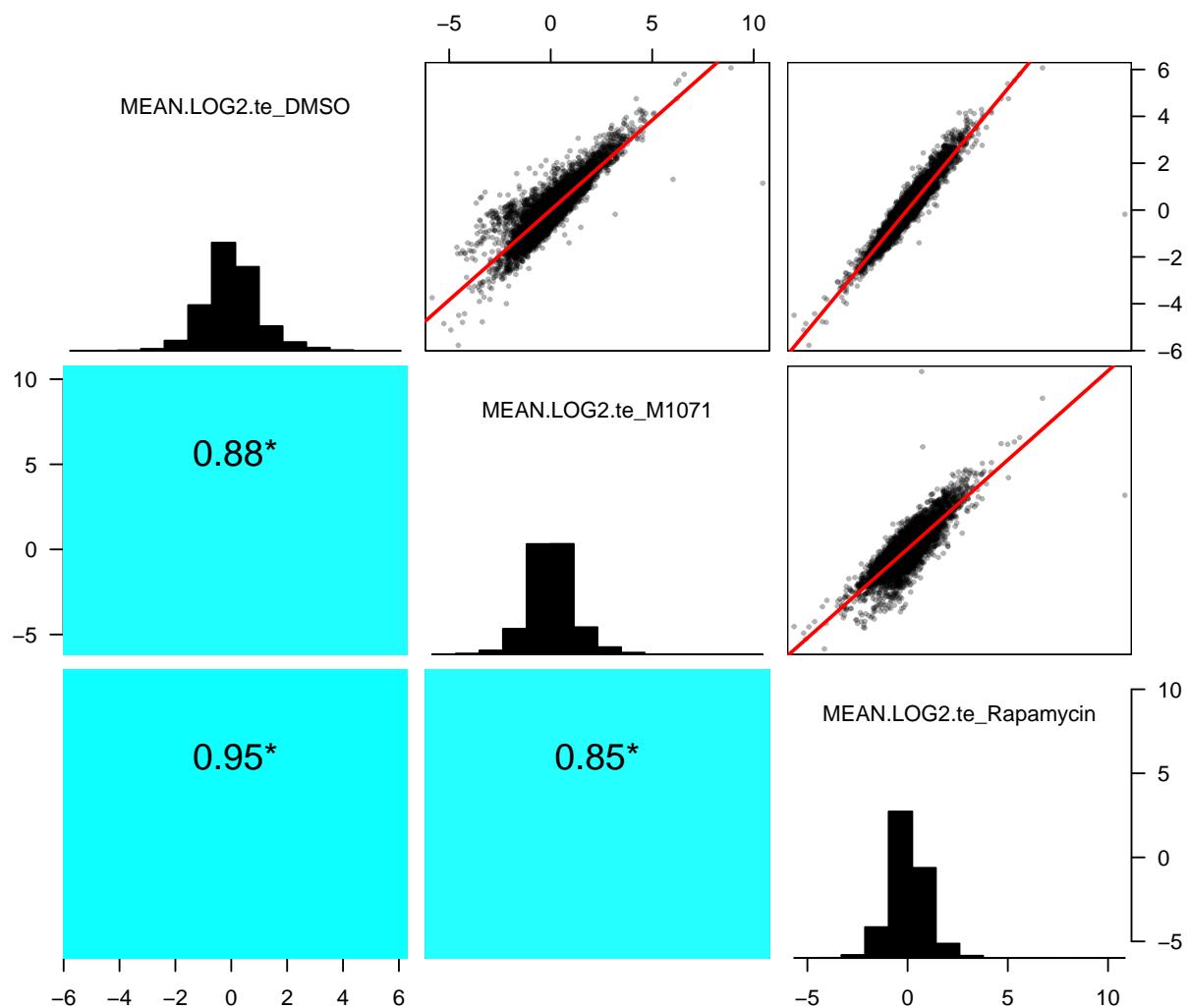
5.2 Ribomap output: relative transcript abundance

5.2.1 DMSO vs M1071 vs Rapamycin



5.3 Ribomap output: translational efficiency

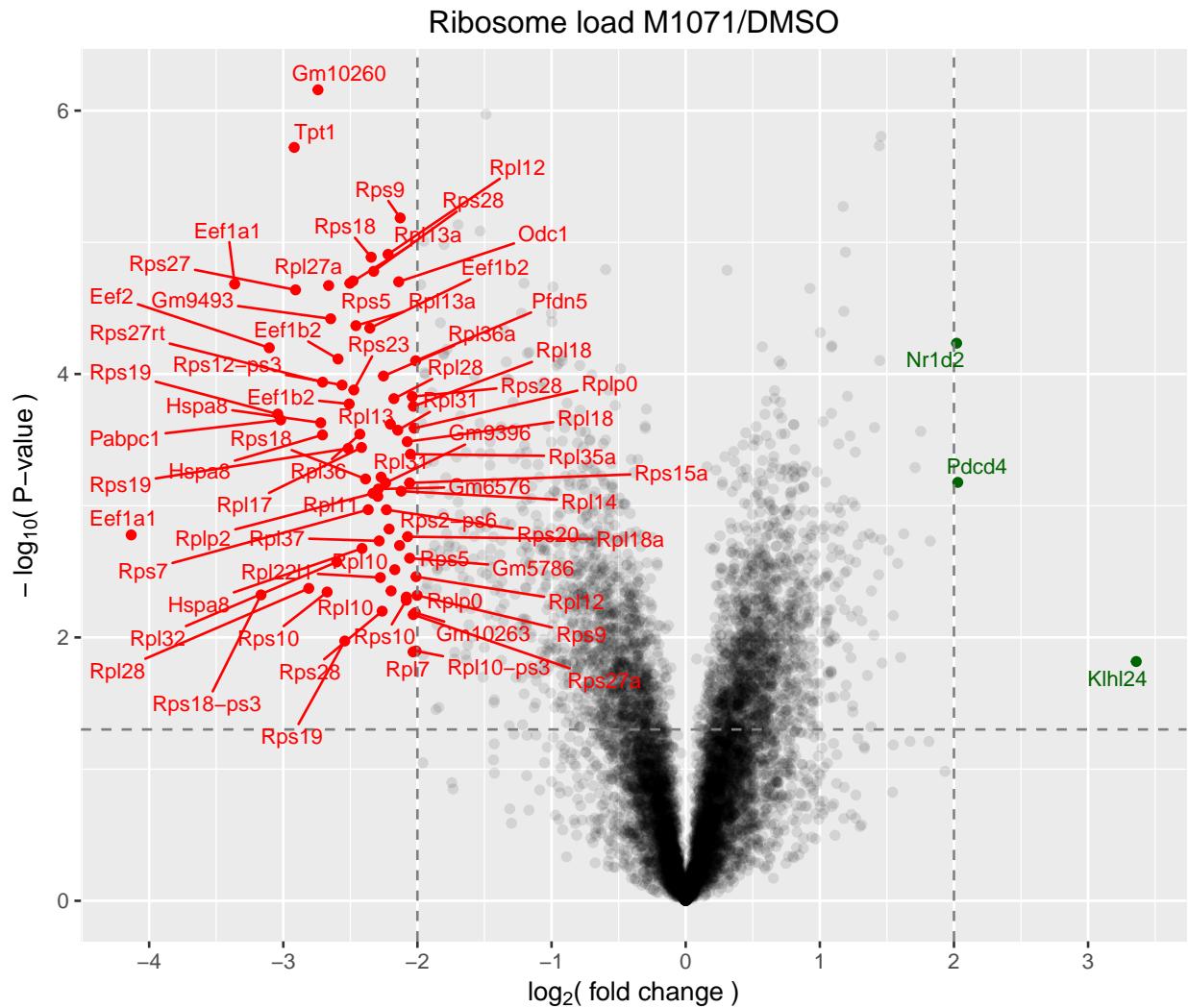
5.3.1 DMSO vs M1071 vs Rapamycin



6 Volcano Plots Between Treatment Groups

6.1 Ribomap output: ribosome load

6.1.1 M1071 vs DMSO



6.1.2 Rapamycin vs DMSO

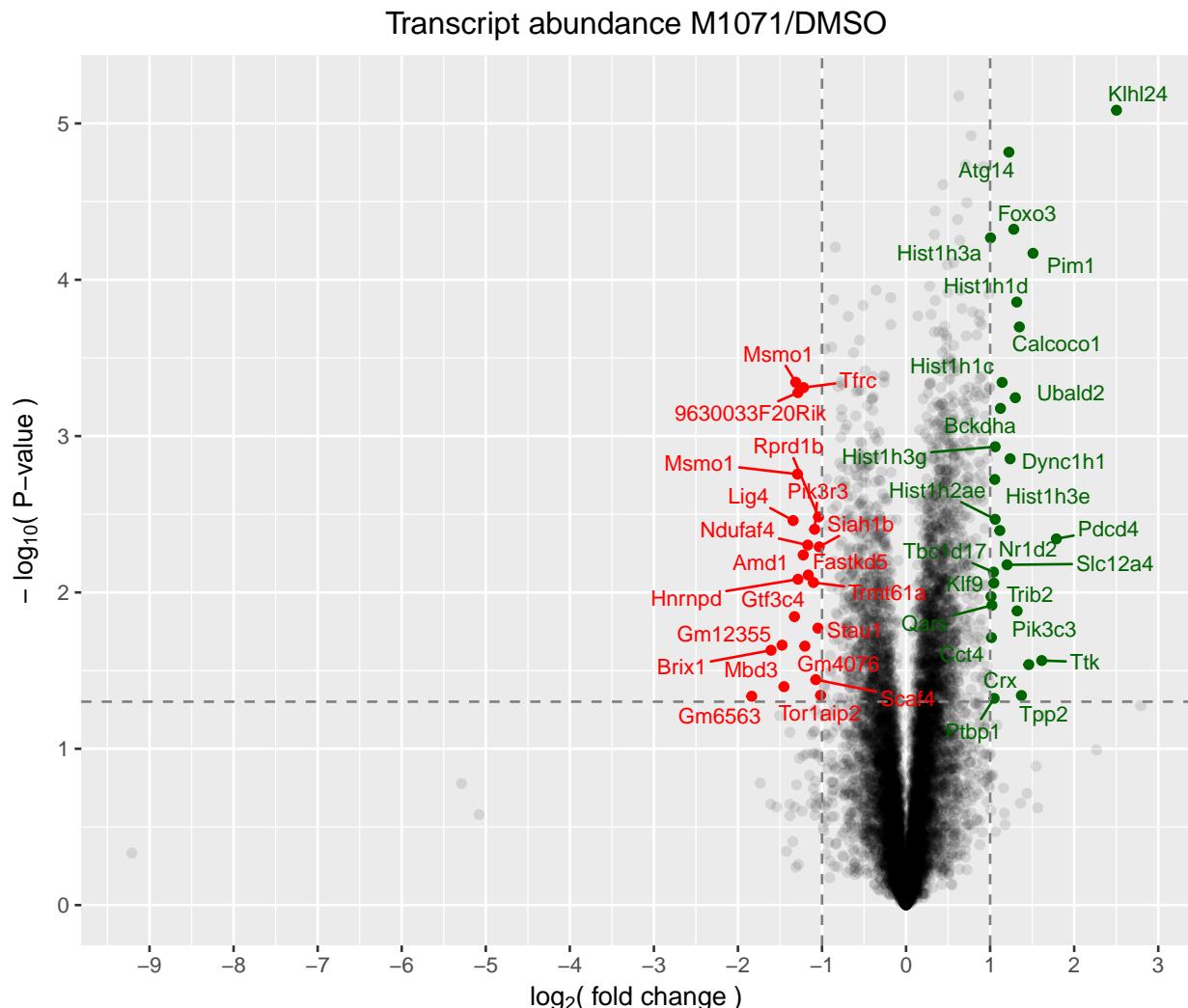


6.1.3 Rapamycin vs M1071

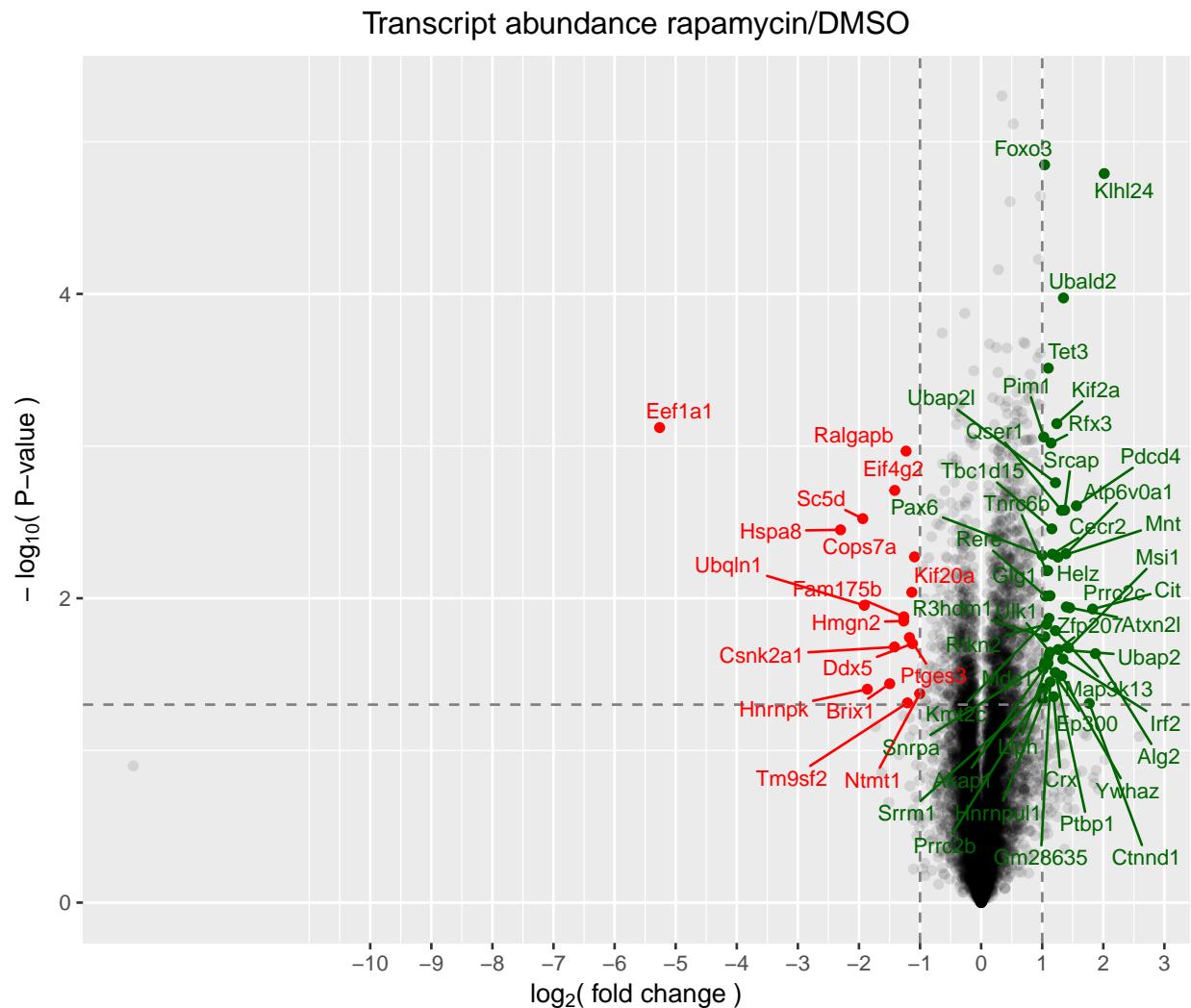


6.2 Ribomap output: relative transcript abundance

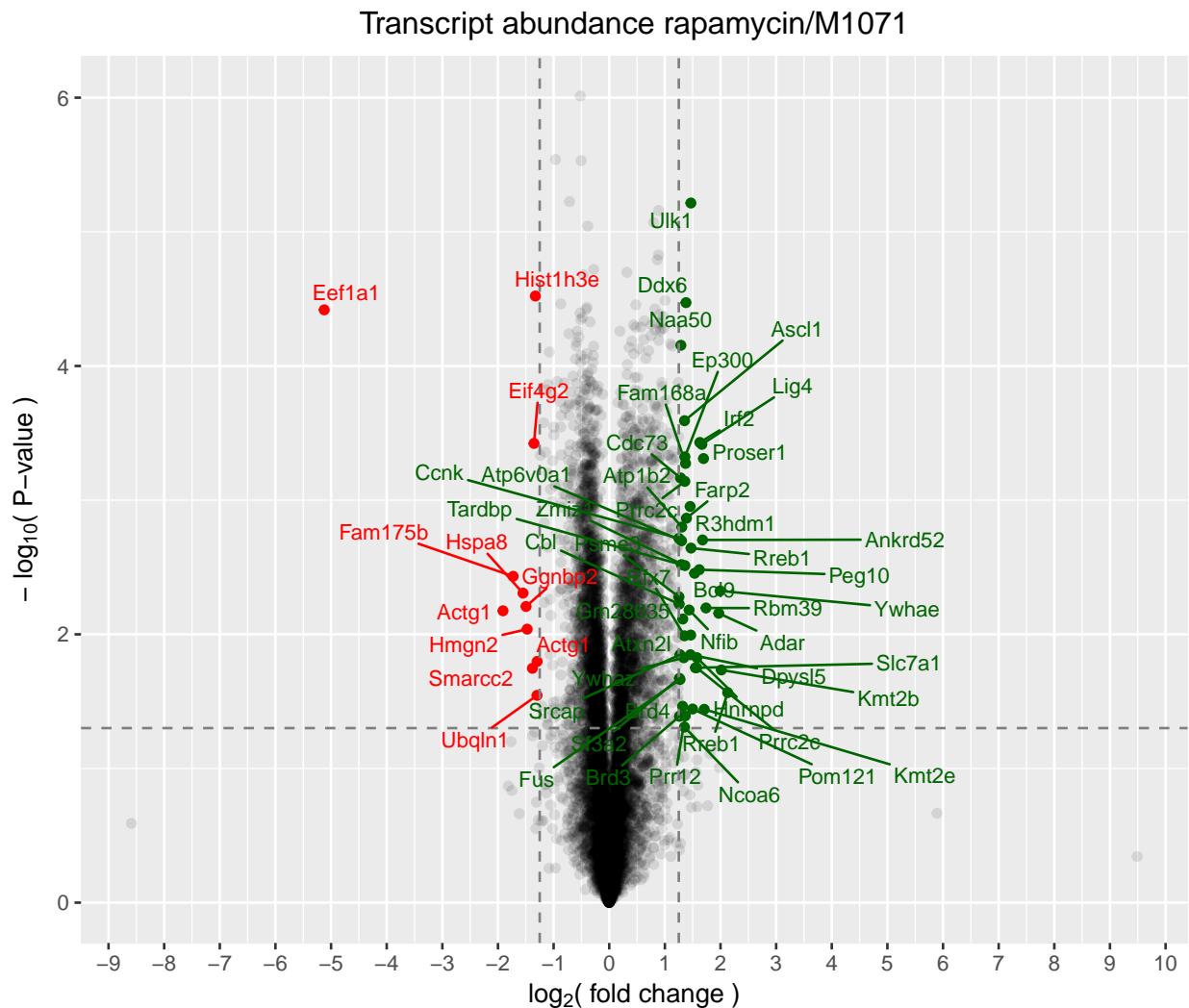
6.2.1 M1071 vs DMSO



6.2.2 Rapamycin vs DMSO

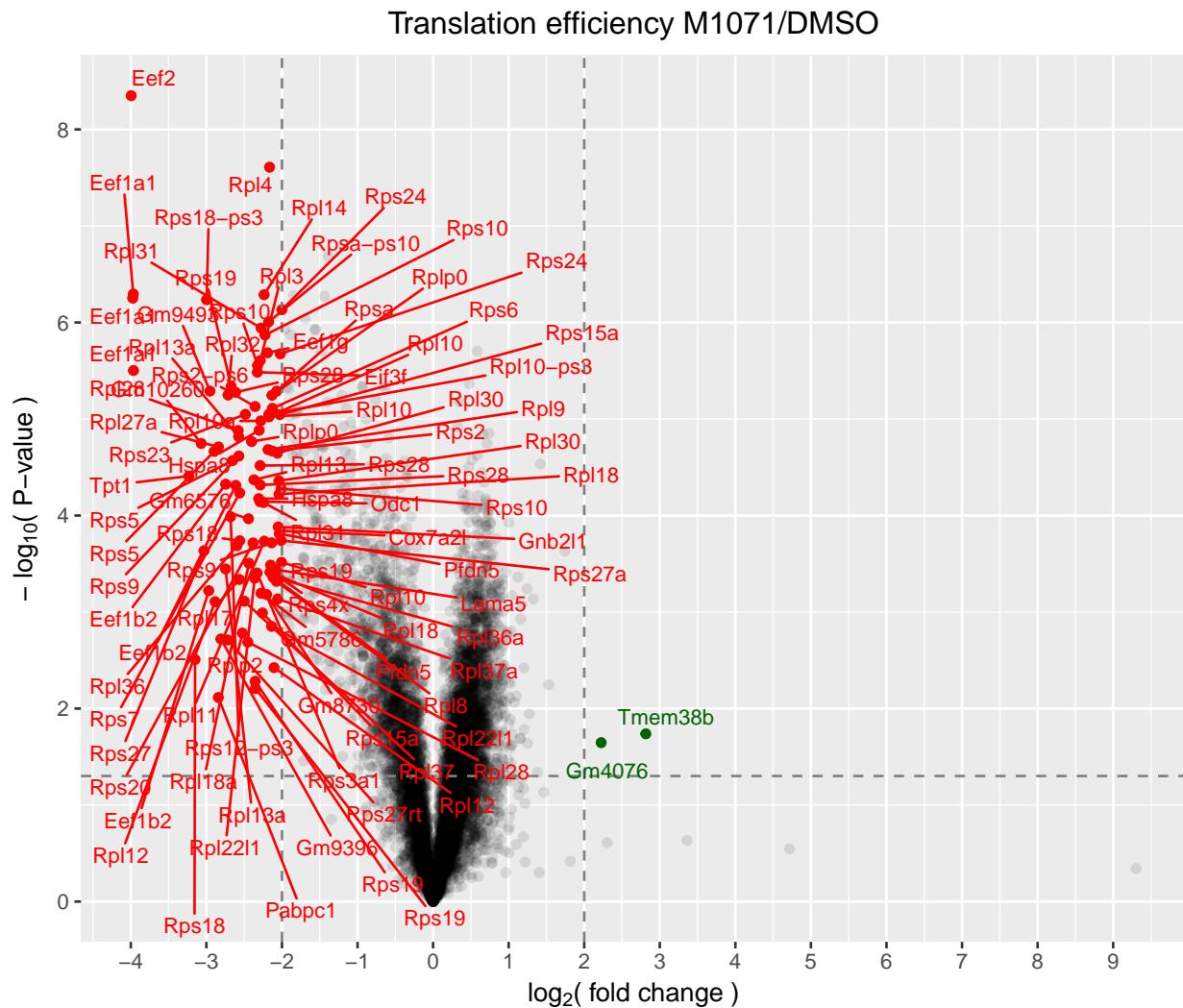


6.2.3 Rapamycin vs M1071

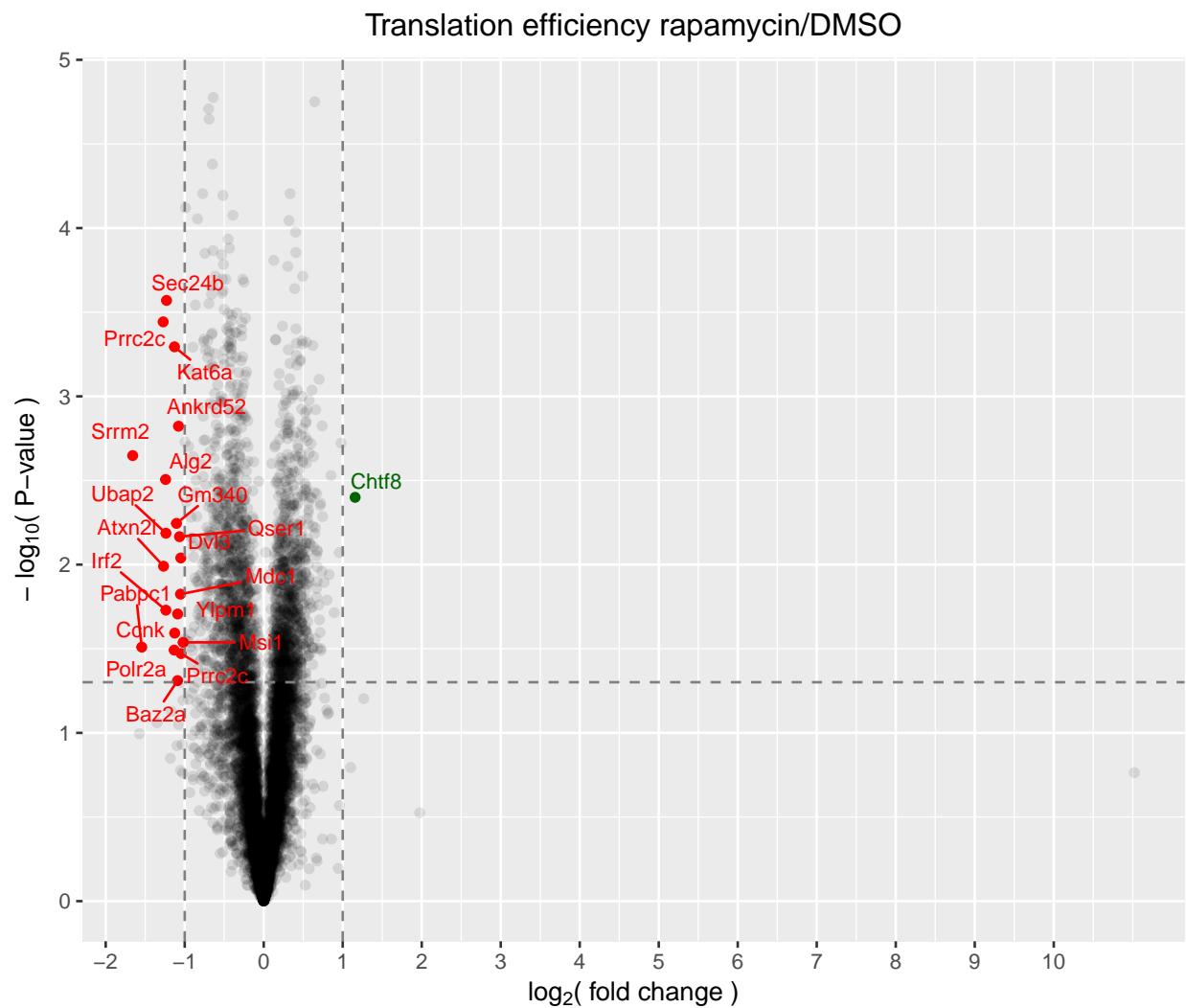


6.3 Ribomap output: translational efficiency

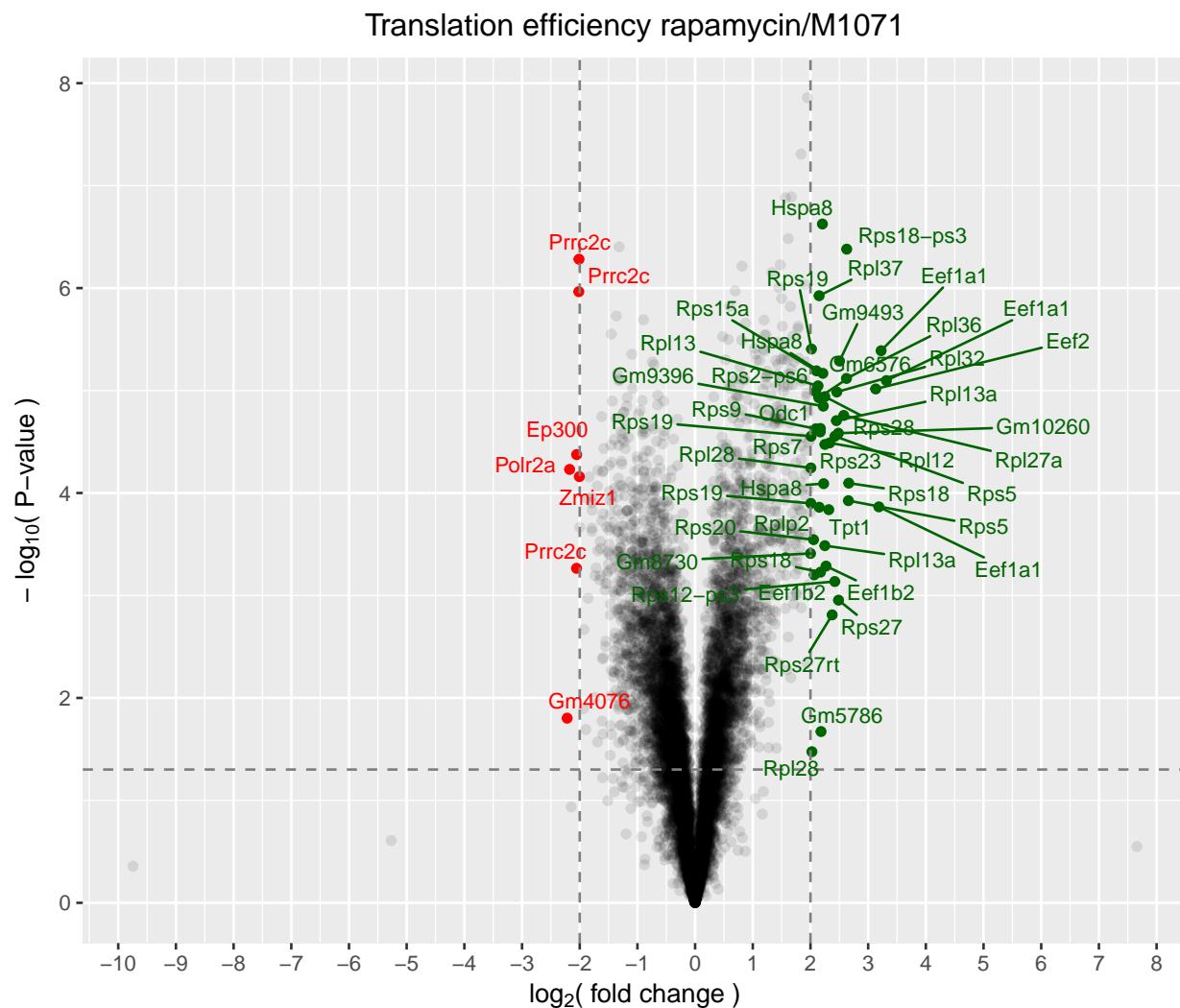
6.3.1 M1071 vs DMSO



6.3.2 Rapamycin vs DMSO



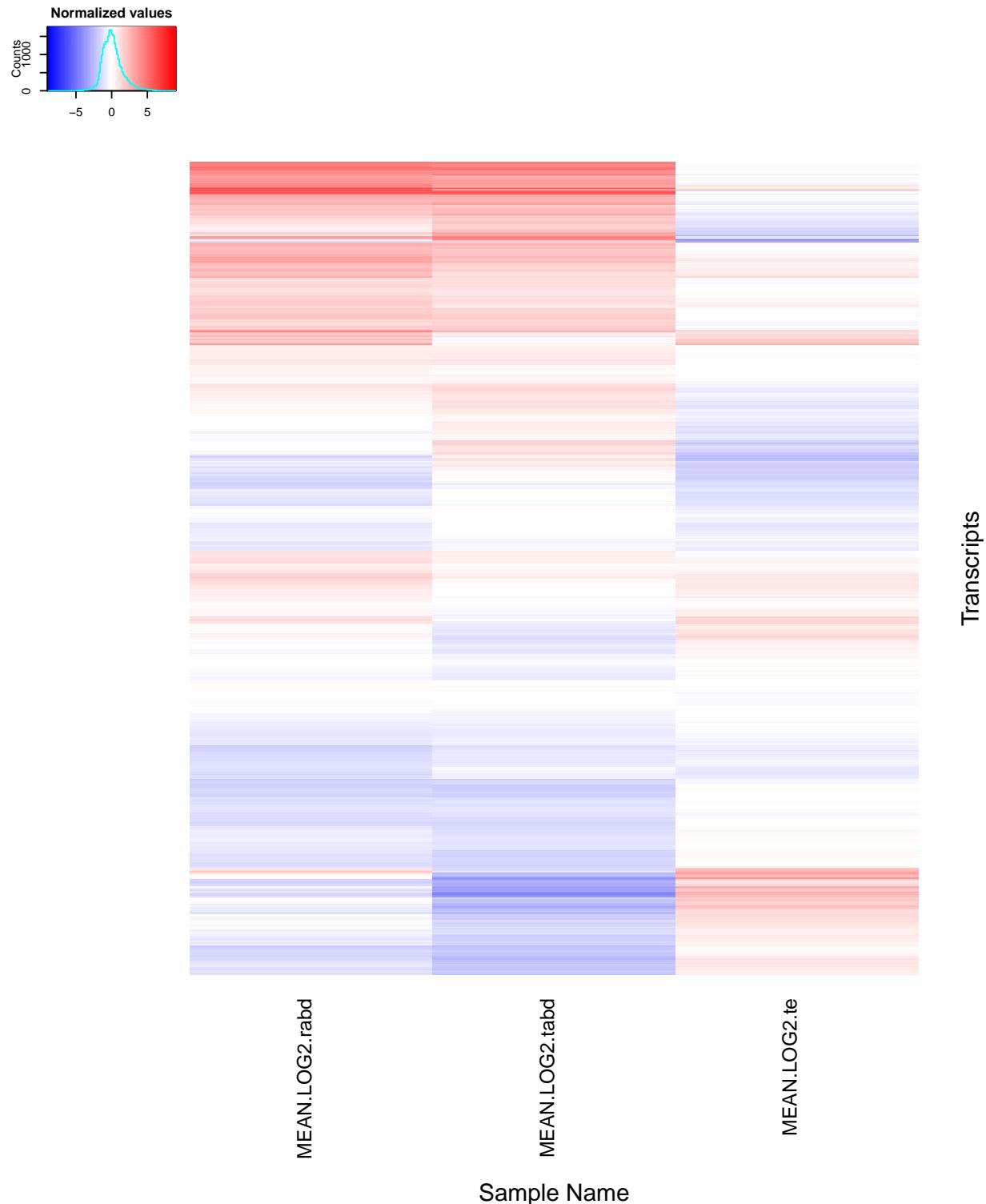
6.3.3 Rapamycin vs M1071



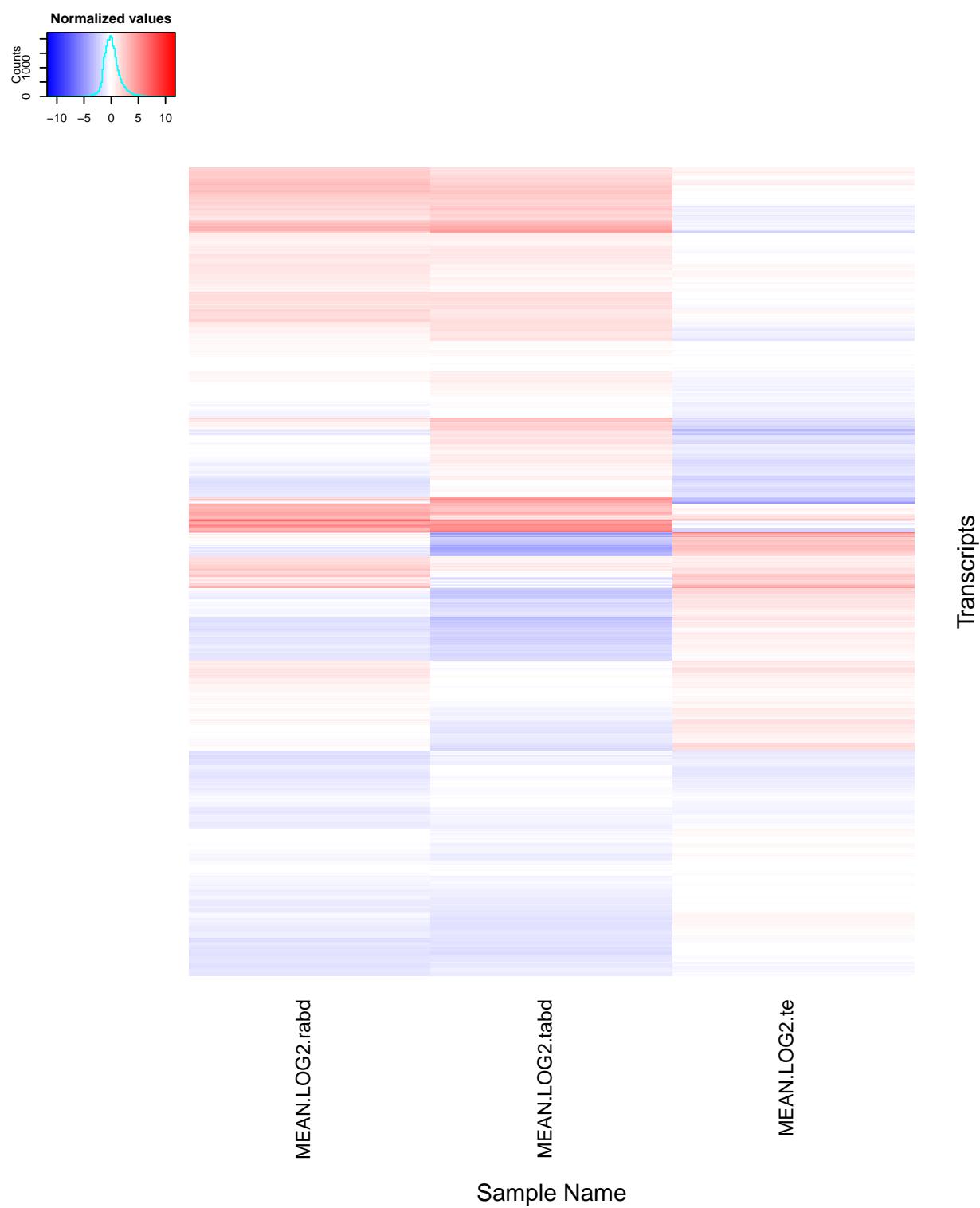
7 Heatmaps

7.1 Ribomap Output Comparisons

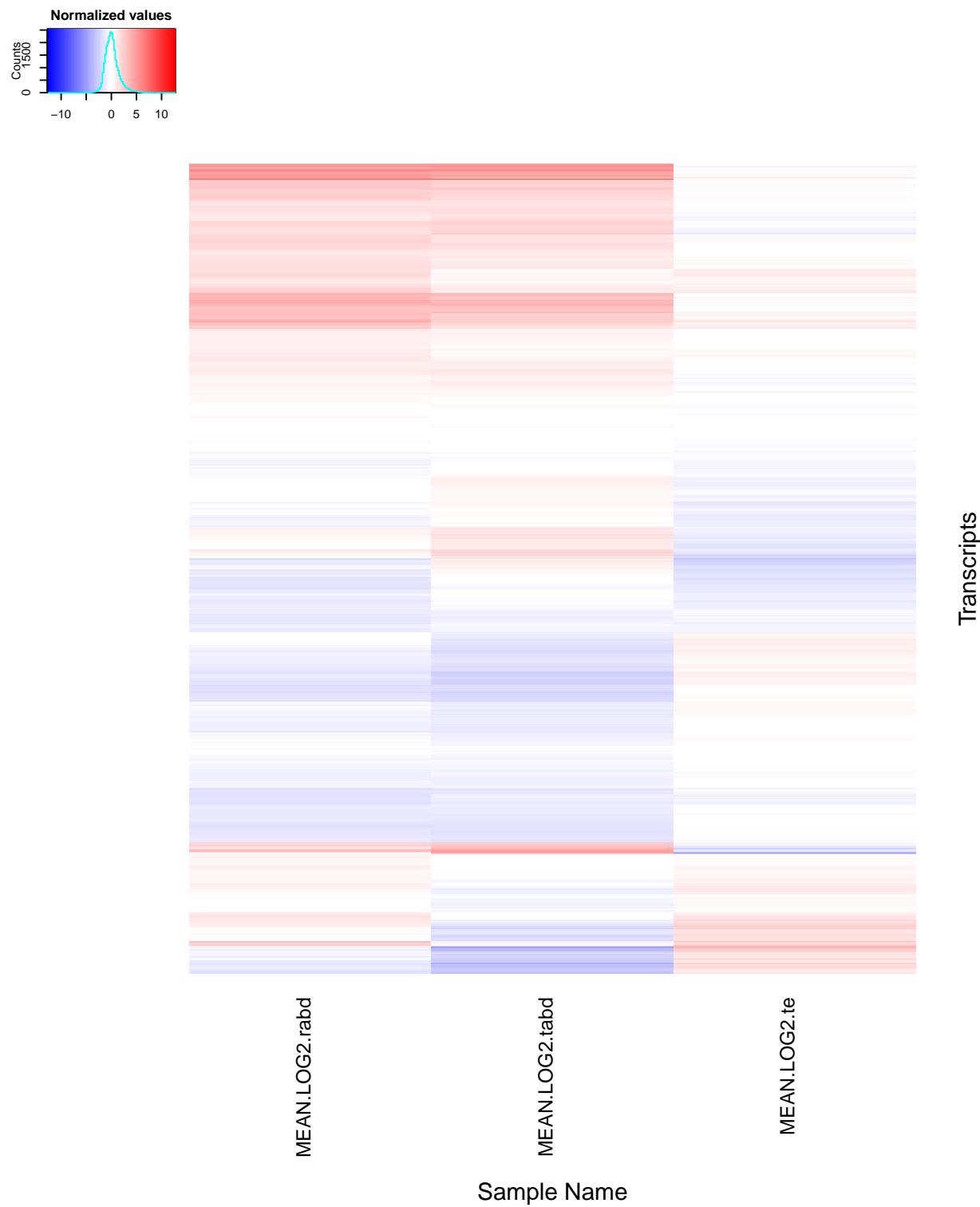
7.1.1 DMSO Treatment



7.1.2 M1071 Treatment

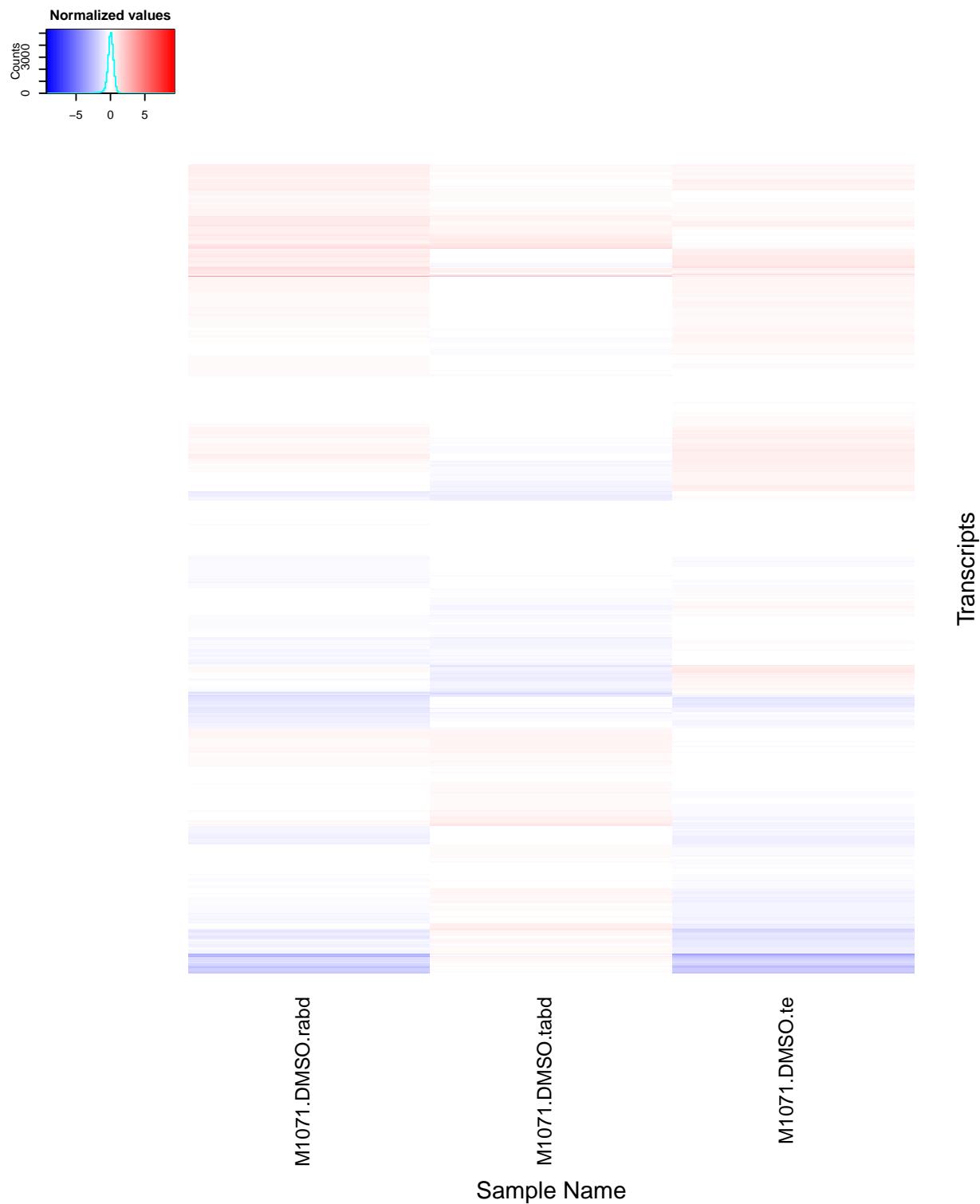


7.1.3 Rapamycin Treatment

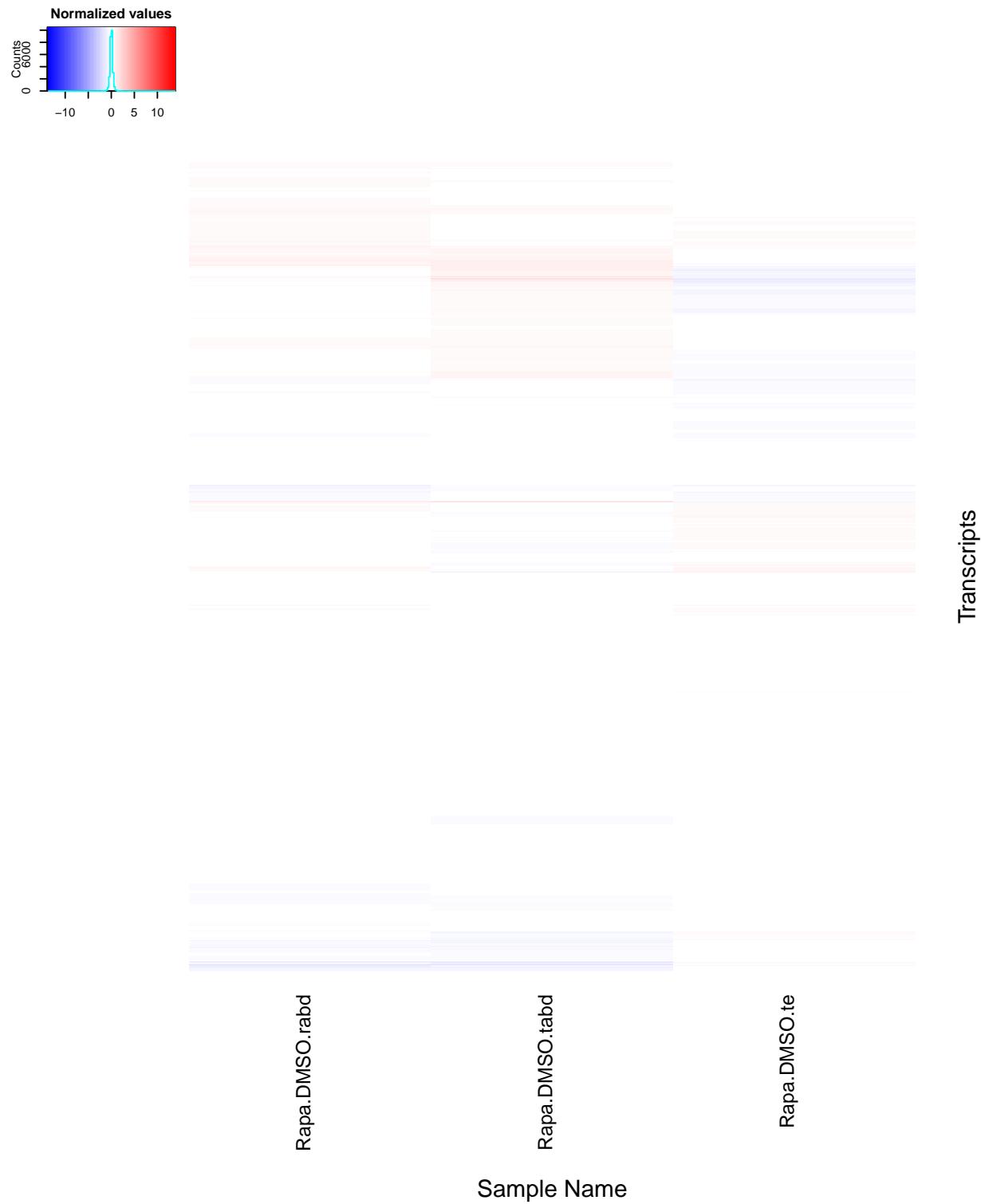


7.2 Treatment Comparisons

7.2.1 M1071 vs DMSO



7.2.2 Rapamycin vs DMSO



7.2.3 Rapamycin vs M1071

