

1 **Using ribosome profiling to quantify differences in protein expression:**  
2 **a case study in *Saccharomyces cerevisiae* oxidative stress conditions**

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16 **Abstract**

17

18 Cells respond to changes in the environment by modifying the concentration of specific  
19 proteins. Paradoxically, the cellular response is usually examined by measuring variations  
20 in transcript abundance by high throughput RNA sequencing (RNA-Seq), instead of  
21 directly measuring protein concentrations. This happens because RNA-Seq-based  
22 methods provide better quantitative estimates, and more extensive gene coverage, than  
23 proteomics-based ones. However, variations in transcript abundance do not necessarily  
24 reflect changes in the corresponding protein abundance. How can we close this gap? Here  
25 we explore the use of ribosome profiling (Ribo-Seq) to perform differentially gene  
26 expression analysis in a relatively well-characterized system, oxidative stress in baker's  
27 yeast. Ribo-Seq is an RNA sequencing method that specifically targets ribosome-  
28 protected RNA fragments, and thus is expected to provide a more accurate view of  
29 changes at the protein level than classical RNA-Seq. We show that gene quantification  
30 by Ribo-Seq is indeed more highly correlated with protein abundance, as measured from  
31 mass spectrometry data, than quantification by RNA-Seq. The analysis indicates that,  
32 whereas a subset of genes involved in oxidation-reduction processes is detected by both  
33 types of data, the majority of the genes that happen to be significant in the RNA-Seq-  
34 based analysis are not significant in the Ribo-Seq analysis, suggesting that they do not  
35 result in protein level changes. The results illustrate the advantages of Ribo-Seq to make  
36 inferences about changes in protein abundance in comparison with RNA-Seq.

37

## 38 **Introduction**

39

40 In recent years high throughput RNA sequencing (RNA-Seq) has become the method of  
41 choice for comparing gene expression changes of cells grown under different conditions  
42 (Rapaport *et al.*, 2013). The relatively low cost of RNA-Seq, together with the availability  
43 of efficient computational methods to process information from millions of sequencing  
44 reads, has undoubtedly accelerated our understanding of gene regulation. However, a  
45 change in mRNA relative abundance does not always imply a change in the amount of  
46 the encoded protein (Schwanhäusser *et al.*, 2011). Filling this gap in understanding is  
47 essential to discern the functional changes in the cell upon a given stimulus.

48

49 Many studies have shown that mRNA levels only partially explain protein levels in the  
50 cell (de Sousa Abreu *et al.*, 2009; Schwanhäusser *et al.*, 2011; Payne, 2015; Ponnala *et*  
51 *al.*, 2014). In yeast, the correlation between mRNA and protein abundance is typically in  
52 the range 0.6-0.7 (de Sousa Abreu *et al.*, 2009). In addition, the ratio between protein and  
53 mRNA levels may vary across different conditions. For instance, substantial differences  
54 in this ratio have been observed during osmotic stress in yeast (Lee *et al.* 2011) or after  
55 the treatment of human cells with epidermal growth factor (Tebaldi *et al.*, 2012). This  
56 strongly suggests that measuring changes in mRNA levels may often be insufficient to  
57 identify the functional shifts taking place in the cell upon a given stimulus.

58

59 Protein quantification is often performed using whole proteome mass spectrometry-based  
60 methods (Gerber *et al.*, 2003; Edfors *et al.*, 2016). These methods provide a direct  
61 measurement of protein abundance but they also have limitations, especially for the  
62 detection of lowly expressed and/or small proteins (Slavoff *et al.*, 2013). An alternative

63 way to estimate protein levels is the sequencing of ribosome-protected mRNA fragments,  
64 or ribosome profiling (Ribo-Seq) (Ingolia *et al.*, 2009, 2011; Aspden *et al.*, 2014; Ruiz-  
65 Orera *et al.*, 2014). In contrast to RNA-Seq, which measures the total amount of mRNA  
66 in the cell, Ribo-Seq only captures those mRNAs that are being actively translated.  
67 Although Ribo-Seq measures translation, which is an indirect estimate of protein  
68 abundance, it has the advantage over proteomics that virtually any mRNA can be  
69 interrogated. In addition, Ribo-Seq reads can be quantified in the same manner as RNA-  
70 Seq reads. This implies that we can use the same pipelines as for RNA-Seq to identify  
71 differentially expressed genes.

72

73 It has been proposed that alterations in the ratio between the relative number of Ribo-Seq  
74 and RNA-Seq reads mapping to a given locus, known as the translation efficiency (TE),  
75 can be used to identify putative translation activation or repression events (Ingolia, 2016).  
76 Numerous recent studies have used ribosome profiling data has been used to study  
77 translation regulatory mechanisms (Jungfleisch *et al.*, 2017; Yordanova *et al.*, 2018) or  
78 to discover new translated RNA sequences (Michel *et al.* 2012; Aspden *et al.* 2014;  
79 Ingolia *et al.* 2014; Ruiz-Orera *et al.* 2014).

80

81 Here we perform differential gene expression analysis using RNA-Seq and Ribo-Seq data  
82 during oxidative stress in *Saccharomyces cerevisiae*, a condition that is known to trigger  
83 important regulatory changes both at the transcriptional and translational levels (Shenton  
84 *et al.*, 2006; Gerashchenko *et al.*, 2012). We compare the results to proteomics data  
85 obtained from the same samples. The results show that the dynamics of total mRNA and  
86 translated mRNAs are very distinct, and that most changes in the relative amount of  
87 mRNA do not appear to have any consequences at the protein level. The study opens a

88 door for a more generalized use of Ribo-Seq data to measure changes in protein  
89 expression across conditions.

90

## 91 **Results and Discussion**

92

### 93 Quantification of gene expression by Ribo-Seq and RNA-Seq

94

95 We extracted ribosome-protected RNA fragments, as well as total polyadenylated RNAs,  
96 from *Saccharomyces cerevisiae* grown in rich medium (normal) and in H<sub>2</sub>O<sub>2</sub>-induced  
97 oxidative stress conditions (stress). We then sequenced ribosome-protected RNAs (Ribo-  
98 Seq) as well as complete polyA+ mRNAs (RNA-Seq) using a strand-specific protocol.  
99 The Ribo-Seq data corresponded to the translated mRNA fraction (translatome), whereas  
100 the RNA-Seq data corresponded to total mRNAs (transcriptome). For comparison we also  
101 estimated protein concentrations (proteome) in the two conditions by mass spectrometry  
102 (Figure 1).

103

104 After quality control of the sequencing reads we obtained 31-36 million reads for Ribo-  
105 Seq and 12-15 million reads for RNA-Seq (Supplementary Table S1). We mapped the  
106 reads to the genome and generated a table of gene counts for each of the samples. After  
107 filtering out non-expressed genes (see Methods), the table contained data for 5,419 *S.*  
108 *cerevisiae* genes. Using mass spectrometry (mass spec) we could quantify the protein  
109 products of 2,200 genes (see Methods), representing about 40% of the genes quantified  
110 by RNA-Seq.

111

112 We normalized the RNA-Seq and Ribo-Seq-based table of counts by calculating counts  
113 per million (CPM) in logarithmic scale, or  $\log_2$ CPM (Supplementary Figure S1). The  
114 gene normalized expression values showed a very high correlation between biological  
115 replicates, with a correlation coefficient large than 0.99 between all pairs of Ribo-Seq or  
116 RNA-Seq replicas (Supplementary Table S2). In contrast, normalized protein abundances  
117 between pairs of proteomics replicates showed correlation coefficients between 0.83 and  
118 0.93 (Supplementary Table S3), indicating that quantification by proteomics is less  
119 reproducible than quantification by RNA-Seq and Ribo-Seq.

120

121 Importantly, the Ribo-Seq data correlated better with the proteomics data than RNA-Seq;  
122 in the first case the correlation was 0.67-0.71 and in the second one 0.46-0.62 (Figure 3).  
123 This supports that notion that Ribo-Seq provides a more accurate view of protein  
124 expression than RNA-Seq (Ingolia *et al.*, 2009).

125

126 We next clustered the RNA-Seq and Ribo-Seq gene expression values using  
127 multidimensional scaling (MDS)(Borg and Groenen, 1997)(Supplementary Figure S2).  
128 Remarkably, the Ribo-Seq measurements for the two conditions (normal and stress) were  
129 more similar to each other than any of them was to the condition-matched RNA-Seq  
130 measurements, and the same thing happened with the RNA-Seq-based measurements.  
131 Thus, the sequencing approach employed is expected to have a strong impact in the results.

132

133 Next, we calculated the fold change (FC) gene expression difference between conditions,  
134 taking the average expression values between replicates of the same experimental  
135 condition. In agreement with the results obtained with MDS, the  $\log_2$ FC distribution  
136 based on the Ribo-Seq data had a lower variance than the  $\log_2$ FC distribution using RNA-

137 Seq data (Figure 4). We considered the possibility that this pattern was due to the number  
138 of Ribo-Seq reads being 2-3 times larger than the number of RNA-Seq reads  
139 (Supplementary Table S1). To test for this, we subsampled the mapped reads so as to  
140 have a similar number of reads in all the RNA-Seq and Ribo-Seq samples (Supplementary  
141 Tables S4 and S5). We again observed a lower  $\log_2FC$  variance for Ribo-Seq than for  
142 RNA-Seq (Supplementary Figure S3), indicating that the observed variance difference  
143 has a biological origin.

144

#### 145 Differential gene expression analysis

146

147 We performed differential gene expression analysis, separately for Ribo-Seq and RNA-  
148 Seq data, using multivariable linear regression with the Limma package (Law *et al.*, 2014).  
149 Limma provides a list of differentially expressed genes with the corresponding adjusted  
150 p-values. We selected genes with an adjusted p-value  $< 0.05$  and a  $\log_2FC$  larger than one  
151 standard deviation; the latter corresponded to a minimum FC of 1.49 for RNA-Seq data  
152 and 1.36 for Ribo-Seq data. We used the standard deviation instead of a fixed value to  
153 accommodate for the differences in the width of the  $\log_2FC$  distributions (Figure 4).

154

155 We obtained 817 up-regulated genes during oxidative stress using RNA-Seq data,  
156 compared to only 92 with Ribo-Seq data. Thus, the vast majority of the genes identified  
157 as up-regulated in stress with RNA-Seq data were not significantly up-regulated when  
158 using the Ribo-Seq data to do the same analysis. The number of down-regulated genes  
159 was 846 and 519 for RNA-Seq and Ribo-Seq, respectively. Overall, only a small fraction  
160 of the differentially expressed genes was common to both approaches (5-10%, see below).

161

162 The induction of oxidative stress by hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) results in an excess of  
163 reactive oxygen species (ROS) in the cell. This is known to activate the expression of  
164 several protein families including thioredoxins, hexokinases, and heat shock proteins  
165 (Morano *et al.*, 2012). The set of up-regulated genes identified by both RNA-Seq and  
166 Ribo-Seq included several members of these families (e.g. HXK2, TDH1, CYC1, HSP10),  
167 consistent with transcriptional activation of genes directly involved in stress response.

168

169 Attempts to use the same pipeline to identify differentially expressed genes using the  
170 proteomics data did not yield significant results. The reproducibility of protein abundance  
171 estimates using mass spec data is not as high as the reproducibility of gene expression  
172 levels in the case of RNA sequencing data, which decreases the power of differential gene  
173 expression analysis using this kind of data (Supplementary Table S3).

174

#### 175 Uncoupling between changes at the transcriptome and translome levels

176

177 The correlation between RNA-Seq and Ribo-Seq gene log<sub>2</sub>FC values was quite low (0.18),  
178 supporting an important disconnect between the two kinds of data (Figure 5). We  
179 quantified the number of genes that showed a significant change in the same direction i.e.  
180 homodirectional changes. There were 38 genes that were up-regulated during stress using  
181 both RNA-Seq and Ribo-Seq data, this is a small number but still more than double the  
182 number expected by chance (15 genes). The number of homodirectional down-regulated  
183 genes was 89, compared to 55 be expected by chance. In summary, while there was a  
184 modest overlap between the stories told by RNA-Seq and Ribo-Seq data (test of  
185 proportions p-value < 1.32x10<sup>-5</sup>), the majority of the differentially expressed genes were  
186 not concordant.



187

188 Dissecting differential regulation by functional class

189

190 To better understand the biological relevance of the above results, we investigated if  
191 certain functional classes were significantly enriched among the sets of differentially  
192 expressed genes. We used DAVID (Huang *et al.*, 2009) to identify significantly over-  
193 represented functional clusters (Figure 4). Only one class, ‘oxidation-reduction process’,  
194 was enriched among genes up-regulated during stress both using RNA-Seq and Ribo-Seq  
195 data. This is consistent with transcriptional activation of this set of genes upon stress,  
196 increasing the signal for both total mRNA and the translated fraction. Three other classes  
197 – ‘translation’, ‘ATPase’ and ‘proteasome’ – showed increased mRNA levels during  
198 stress, but this was not reflected in an increase in the translated fraction. Thus, it is likely  
199 that an important part of these transcripts are stored in a translation inactive form during  
200 stress, for example as P-bodies or stress granules (Zid and O’Shea, 2014; Khong *et al.*,  
201 2017; Luo *et al.*, 2018). In this case, an accumulation of transcripts would be detected by  
202 RNA-Seq but not by Ribo-Seq, as translation of the transcripts is impaired.

203

204 Interestingly, there were functions that only appeared when we performed differential  
205 gene expression analysis with the Ribo-Seq data: ‘cell wall’, ‘mitochondrial  
206 intermembrane space’ and ‘catalytic activity’ were enriched among up-regulated genes,  
207 whereas ‘cell cycle’ was enriched among down-regulated genes (Figure 6). As these  
208 classes are not detected by RNA-Seq, they are candidates to be regulated at the  
209 translational level only. An alternative possibility is that the storage of some transcripts  
210 in stress granules distorts the RNA-Seq patterns to such a degree that some truly up-

211 regulated genes become undetectable with RNA-Seq; they would only be detected when  
212 examining actively translated mRNAs with Ribo-Seq.

213

#### 214 Translation inhibition of cell cycle genes

215

216 In order to further identify possible translational regulatory events we compared the  
217 translational efficiency (TE; Ribo-Seq reads divided by RNA-Seq reads) of the different  
218 genes in the two conditions using the program Ribodiff (Zhong *et al.*, 2017). This  
219 approach is based on the assumption that the number of Ribo-Seq reads is proportional to  
220 the amount of translated protein. We detected 470 genes that showed increased TE, and  
221 714 genes that showed decreased TE, in oxidative stress *versus* normal growth conditions  
222 (adjusted p-value < 0.05; see Methods).

223

224 We reasoned that genes whose translation becomes more active during stress should have  
225 increased TE values but also be classified as upregulated when using Ribo-Seq for  
226 differential gene expression analysis. We only found 17 genes fulfilling both conditions  
227 (3.6% of the genes with increased TE), indicating that activation of translation probably  
228 has a relatively small impact in the response to oxidative stress. In the vast majority of  
229 cases the increase in TE could be explained by a decrease in RNA-Seq signal during stress  
230 (Supplementary Table S6).

231

232 By the same token, genes whose translation is repressed during stress are expected to  
233 have decreased TE values but also be classified as down-regulated by Ribo-Seq. We  
234 found 246 such genes (34.4% of the genes with decreased TE), suggesting that this  
235 mechanism may be more prevalent. Among them there were 12 genes from the cell cycle

236 functional category (Supplementary Table S7). The putative translational repression of  
237 these genes did not appear to be mediated by increased translation of upstream ORFs  
238 (Gerashchenko *et al.*, 2012), as we did not detect any increase in the number of Ribo-Seq  
239 reads mapping to 5'UTR regions when compared to coding sequences in stress conditions.

240

## 241 **Concluding remarks**

242

243 The adaptation of organisms to variations in the environmental conditions is associated  
244 with the activation or repression of the expression of particular genes. These changes are  
245 usually studied at the level of complete mRNA molecules using microarrays or next  
246 generation sequencing. However, changes in mRNA concentration do not necessarily  
247 reflect changes in their encoded protein products; rather, uncoupling between total and  
248 polysomal mRNA levels has been observed in many different conditions (Tebaldi *et al.*,  
249 2012; Shenton *et al.*, 2006).

250

251 Ribo-Seq specifically targets ribosome-protected mRNAs, providing a closer view to  
252 protein expression than RNA-Seq, which is for total mRNA sequences. Although Ribo-  
253 Seq data is more labour-intensive than RNA-Seq, the protocols are being simplified and  
254 its use is rapidly growing (Reid *et al.* 2015; Xie *et al.* 2016; Liu *et al.* 2018; Michel *et al.*  
255 2018). Here we have used Ribo-Seq data to perform differential gene expression analysis  
256 during oxidative stress, and compared the results to RNA-Seq and to proteomics data.

257

258 We have shown that gene expression levels inferred from Ribo-Seq data correlate better  
259 with protein abundance than those inferred from RNA-Seq data. Remarkably, many of  
260 the genes that are classified as differentially regulated using RNA-Seq do not show a

261 similar effect when the Ribo-Seq data is analyzed, strongly suggesting that, for these  
262 genes, no significant changes at the protein level take place. The methodological  
263 framework we have developed here can be applied to other conditions and help advance  
264 our understanding of gene regulation.

265

## 266 **Methods**

267

### 268 Biological material

269

270 We grew *S. cerevisiae* (S288C) in 500 ml of rich medium (Tsankov *et al.*, 2010). In order  
271 to induce oxidative stress, 30 minutes before harvesting we added diluted H<sub>2</sub>O<sub>2</sub> to the  
272 medium for a final concentration of 1.5 mM. The cells were harvested in log growth  
273 phase (OD<sub>600</sub> of ~0.25) via vacuum filtration and frozen with liquid nitrogen.

274

### 275 Ribosome profiling

276

277 In order to capture ribosome protected mRNAs, cyclohexamide was added one minute  
278 before the cells were harvested. Cyclohexamide is commonly used as a protein synthesis  
279 inhibitor in order to prevent ribosome run-off and the subsequent loss of ribosome-  
280 transcript complexes. One third of each culture was used for ribosome profiling (Ribo-  
281 Seq); the rest was reserved for RNA-Seq.

282

283 Cells were lysed using the freezer/mill method (SPEX SamplePrep); after preliminary  
284 preparations, lysates were treated with RNaseI (Ambion), and subsequently with  
285 SUPERaseIn (Ambion). Monosomal fractions were collected; SDS was added to stop any

286 possible RNase activity, then samples were flash-frozen with N<sub>2</sub>(l). Digested extracts  
287 were loaded in 7%-47% sucrose gradients. RNA was isolated from monosomal fractions  
288 using the hot acid phenol method. Ribosome-Protected Fragments (RPFs) were selected  
289 by isolating RNA fragments of 28-32 nucleotides (nt) using gel electrophoresis. The  
290 preparation of sequencing libraries for Ribo-Seq and RNA-Seq was based on a previously  
291 described protocol (Ingolia *et al.*, 2012). Pair-end sequencing reads of size 35 nucleotides  
292 (2x35bp) were produced for Ribo-Seq and RNA-Seq on MiSeq and NextSeq platforms,  
293 respectively. The data has been deposited at NCBI Bioproject PRJNA435567  
294 (<https://www.ncbi.nlm.nih.gov/bioproject/435567>).

295

#### 296 Processing of the sequencing data

297

298 The RNA-Seq data was filtered using Trimmomatic with default parameters (version  
299 0.36)(Bolger *et al.*, 2014). In the Ribo-Seq data we discarded the second read pair as it  
300 was redundant and of poorer quality than the first read, and then used Cutadapt (Martin,  
301 2011) to eliminate the adapters and to trim five and four nucleotides at 5' and 3' edges,  
302 respectively. Ribosomal RNA was depleted from the Ribo-Seq data *in silico* by removing  
303 all reads which mapped to annotated rRNAs. Ribo-Seq reads shorter than 25 nucleotides  
304 were not used.

305

306 After quality check and read trimming, the reads were aligned against the *S. cerevisiae*  
307 genome (S288C R64-2-1) using Bowtie 2 (Langmead *et al.*, 2009). For annotation we  
308 used a previously generated *S. cerevisiae* transcriptome containing 6,184 annotated  
309 coding sequences plus 1,009 non-annotated assembled transcripts (see Supplementary  
310 data). SAMtools (Li *et al.*, 2009) was used to filter out unmapped reads.

311

312 We counted the number of reads that mapped to each gene with HTSeq-count (Anders *et*  
313 *al.*, 2015). We used the mode ‘intersection strict’ to generate a table of counts from the  
314 data; the procedure removed about 5% of the reads in the case of RNA-Seq, and 8% in  
315 the case of Ribo-Seq. Only genes in which the average read count of the two replicates  
316 was larger than 10 in all conditions (normal and stress, for RNA-Seq and for Ribo-Seq)  
317 were kept. The filtered table of counts contained data for 5,419 genes.

318

319 For subsampling the number of mapped reads we used SAMtools (Li *et al.*, 2009). We  
320 used the function ‘samtools view’ with option ‘-s 0.X’, where X is the percentage of reads  
321 that we wish to keep.

322

### 323 Differential gene expression analysis

324

325 The table of counts was normalized to  $\log_2$  Counts per Million ( $\log_2$ CPM), in order to  
326 account for the different number of total reads in each sample. Before performing  
327 differential gene expression analysis, we normalized the data using Trimmed Mean of M-  
328 values (TMM) as implemented in the package edgeR (Robinson *et al.*, 2010). Finally, we  
329 applied the Limma voom method (Law *et al.*, 2014) to identify differentially expressed  
330 genes, separately for RNA-Seq and Ribo-Seq data (adjusted p-value  $< 0.05$  and  $|\log_2FC| >$   
331  $1 \text{ SD}(\log_2FC)$ ).

332

333 We also performed the same kind of analysis for the proteomics data. We used genes  
334 which had at least 3 unique peptides and could be quantified in all 6 replicates (1,580

335 genes); the procedure did not identify any significantly up or down regulated genes, using  
336 an adjusted p-value < 0.05.

337

### 338 Quantification of protein abundance by mass spectrometry

339

340 For our proteomics experiment, we analysed 3 replicates per condition by LCMSMS  
341 using a 90-min gradient in the Orbitrap Fusion Lumos. These samples were not treated  
342 with cyclohexamide. As a quality control measure, BSA controls were digested in parallel  
343 and ran between each sample to avoid carry-over and assess the instrument performance.  
344 The peptides were searched against SwissProt Yeast database, using the Mascot v2.5.1  
345 search algorithm. The search was performed with the following parameters: peptide mass  
346 tolerance MS1 7 ppm and peptide mass tolerance MS2 0.5 Da; three maximum missed  
347 cleavages; trypsin digestion after K or R except KP or KR; dynamic modifications  
348 oxidation (M) and acetyl (N-term), static modification carbamidomethyl (C). Protein  
349 areas were obtained from the average area of the three most intense unique peptides per  
350 protein group. Considering the data from all 6 samples, we detected proteins from 3,336  
351 genes. We limited our quantitative analysis to a subset of 2,200 proteins which had  
352 proteomics hits for at least 3 unique peptides; this filter eliminates noise arising from  
353 technical challenges of quantifying lowly abundant proteins with LCMSMS.

354

### 355 Analysis of functional clusters

356

357 We identified significantly enriched functional clusters in differentially expressed genes  
358 using DAVID (Huang *et al.*, 2009). The analysis was done separately for over- and under-  
359 expressed genes and for RNA-Seq and Ribo-Seq derived data. Only clusters with

360 enrichment score  $\geq 1.5$  and adjusted p-val  $< 0.05$  were retained. In each cluster we chose  
361 a representative Gene Ontology (GO) term (Ashburner *et al.*, 2000), with the highest  
362 number of genes inside the cluster. Figure 4 integrates the results obtained with the Ribo-  
363 Seq and the RNA-Seq data, the  $\log_{10}$  fold enrichment of the significant GO terms is  
364 plotted.

365

### 366 Analysis of translational efficiency

367

368 We searched for genes with significantly increased or decreased translational efficiency  
369 (TE)(Ingolia *et al.*, 2009) using the RiboDiff program (Zhong *et al.*, 2017). We selected  
370 genes significant at an adjusted p-value  $< 0.05$  and showing  $\log_2(\text{TE}_{\text{stress}}/\text{TE}_{\text{normal}})$  higher  
371 than 0.67 or lower than -0.67 (plus or minus the standard deviation of the distribution).

372

373 We downloaded *S.cerevisiae* 5'UTR sequences from the Yeast Genome Database  
374 ([https://downloads.yeastgenome.org/sequence/S288C\\_reference/SGD\\_all\\_ORFs\\_5prim  
375 e\\_UTRs.fsa](https://downloads.yeastgenome.org/sequence/S288C_reference/SGD_all_ORFs_5prime_UTRs.fsa)). We selected 5'UTR sequences longer than 30 nucleotides, removed  
376 identical sequences and took the longest 5'UTR per gene when several existed. The  
377 resulting annotation file contained the genomic coordinates of the 5'UTRs of 2,424 genes.  
378 We recovered 5'UTR sequences for 5 of the 12 cell cycle-related genes that were  
379 potentially repressed at the translational level (HTL1, SPC19, CDC26, BNS1, DIB1). In  
380 none of these cases the number of Ribo-Seq reads in the 5'UTR divided by the number of  
381 Ribo-Seq reads in the coding sequence increased in oxidative stress with respect to normal  
382 growth conditions.

383

### 384 Supplementary data



385

386 Supplementary data files have been uploaded to Figshare and can be accessed at  
387 <http://dx.doi.org/10.6084/m9.figshare.5809812>. This includes the transcriptome genomic  
388 coordinates, the filtered table of counts and the list of differentially expressed genes  
389 obtained using RNA-Seq and Ribo-Seq. The RNA-Seq and Ribo-Seq original sequencing  
390 data is available from <https://www.ncbi.nlm.nih.gov/bioproject/435567> (NCBI  
391 Bioproject PRJNA435567). Processed data, including annotation files and differentially  
392 regulated genes can be found at <http://dx.doi.org/10.6084/m9.figshare.5809812>.

393

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395

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407

### 408 **Figure legends**

409

410 **Figure 1. Experimental design.** Baker's yeast (*S. cerevisiae*) was grown in rich medium  
411 or oxidative stress conditions. The cultures were used to extract total RNA, ribosome-  
412 protected RNA fragments and proteins.

413

414 **Figure 2. Representative gene expression correlations between RNA sequencing**  
415 **samples. A.** RNA-Seq normal replicate 1 *versus* Ribo-Seq normal replicate 1. **B.** RNA-  
416 Seq stress replicate 1 *versus* Ribo-Seq stress replicate 1. **C.** RNA-Seq normal replicate 1  
417 *versus* RNA-Seq normal replicate 2. **D.** Ribo-Seq normal replicate 1 *versus* Ribo-Seq  
418 normal replicate 2. Expression units are CPM in logarithm scale; R: Spearman correlation  
419 value. N: normal growth conditions (two replicates N1 and N2); S: stress conditions (two  
420 replicates S1 and S2).

421

422 **Figure 3. Proteomics shows a stronger correlation with Ribo-Seq than with RNA-**  
423 **Seq data. A.** RNA-Seq *versus* proteomics, normal growth conditions. **B.** RNA-Seq *versus*  
424 proteomics, oxidative stress. **C.** Ribo-Seq *versus* proteomics, normal growth conditions.  
425 **D.** Ribo-Seq *versus* proteomics, oxidative stress. CPM: counts per million for RNA-Seq  
426 and RNA-Seq data (represented in logarithmic scale, average between replicates).  $\log_2$   
427 normalized area: relative abundance for proteomics data (average between replicates). R:  
428 Spearman correlation value. Plot and correlations represent 2200 genes for which  $\geq 3$   
429 unique peptides were detected by LCMSMS.

430

431 **Figure 4. Distribution of gene expression fold change (FC) differences in logarithmic**  
432 **scale.** FC was calculated as the ratio between the number of reads in oxidative stress and  
433 normal conditions. We took the average number of reads per gene among the replicates.  
434 The standard deviation of  $\log_2$ FC was 0.44 for Ribo-Seq (RP) and 0.57 for RNA-Seq  
435 (RNA).

436

437 **Figure 5. Correlation between gene expression fold changes with RNA-Seq and**  
438 **Ribo-Seq data.** Fold change (FC) gene expression values are shown in logarithmic scale.  
439 The X axis corresponds to the RNA-Seq data, or transcriptome, the Y axis to the Ribo-  
440 Seq data, or translome. The number of down-regulated and up-regulated genes is  
441 indicated. Coloured dots correspond to differentially expressed genes. In the legend

442 homodirectional means up-regulated, or down-regulated, at the transcriptome and  
443 translome levels; opposite\_change is up-regulated at one level and down-regulated at  
444 the other one.

445

446 **Figure 6. Significant gene functional classes among differentially expressed genes.**

447 Shown is a 2-D plot of the enrichment score values, in logarithmic scale, provided by the  
448 software DAVID for differentially expressed genes using RNA-Seq (transcriptome) or  
449 Ribo-Seq (translatome) data. Significant enrichment scores are associated with a p-val <  
450 0.05. Functional classes associated with positive values are significantly enriched among  
451 up-regulated genes, and functional classes with negative values are significantly enriched  
452 among down-regulated genes. Non-significant enrichment scores are given a value of 0  
453 in the plot.

454

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456

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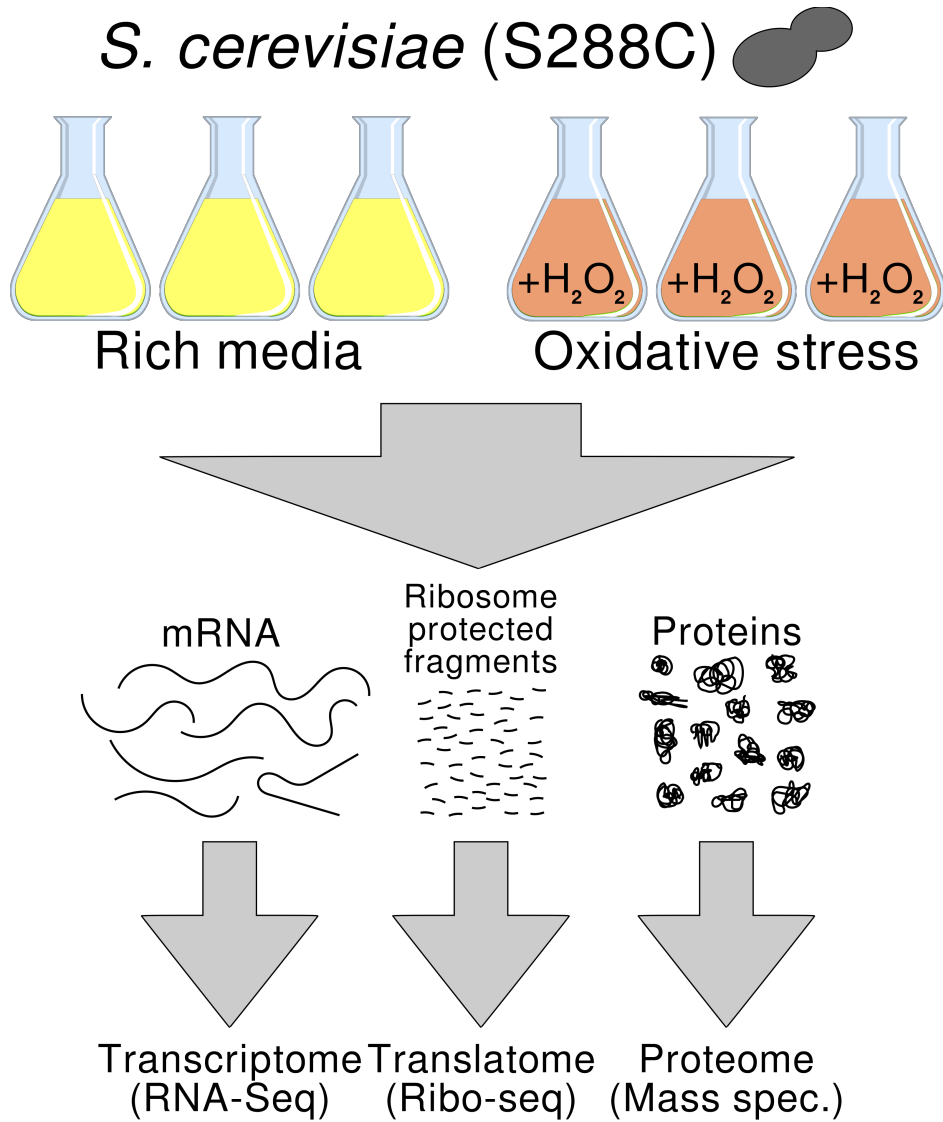
544 **Figures**

545

546 **Figure 1**

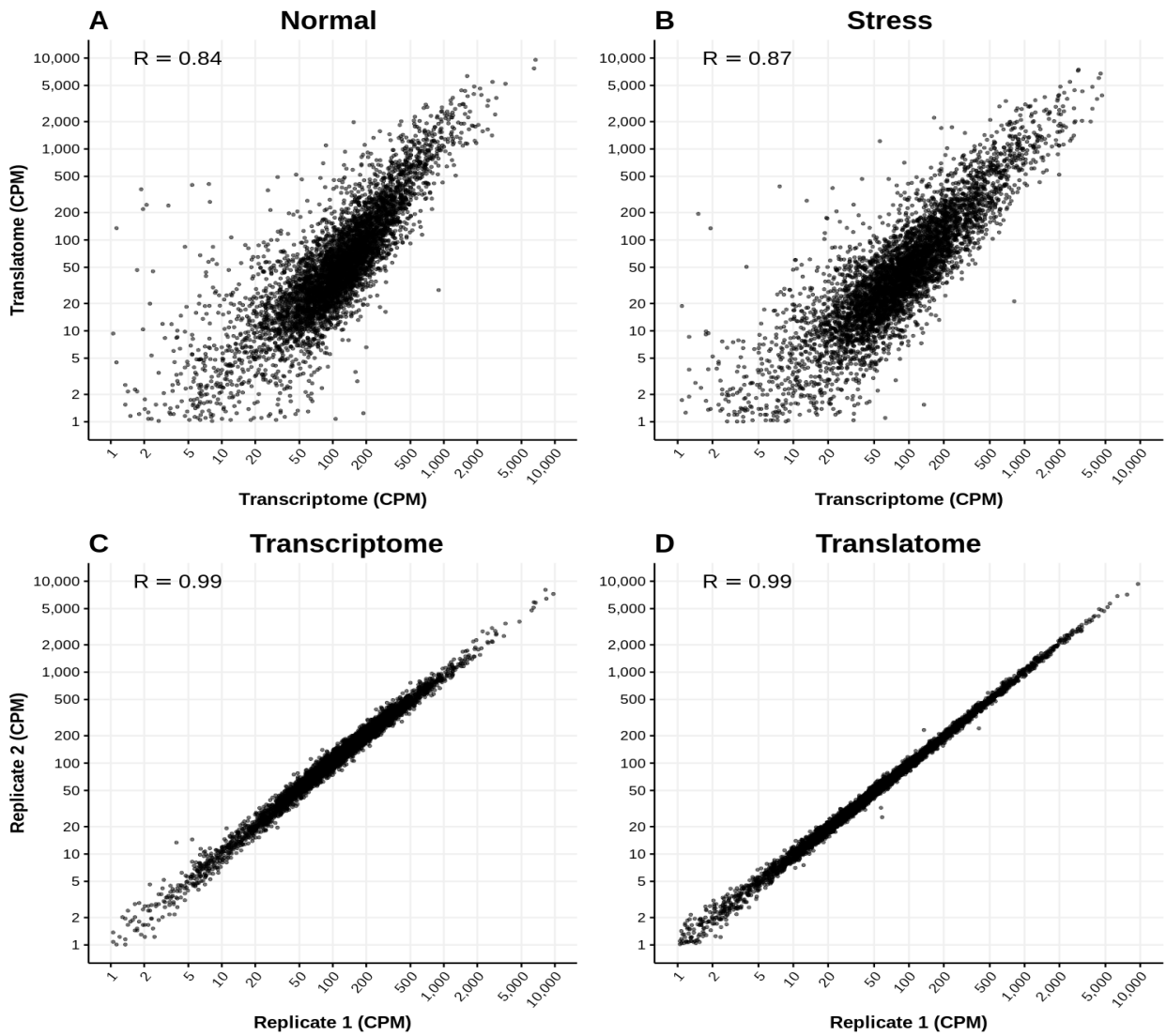
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549 **Figure 2**

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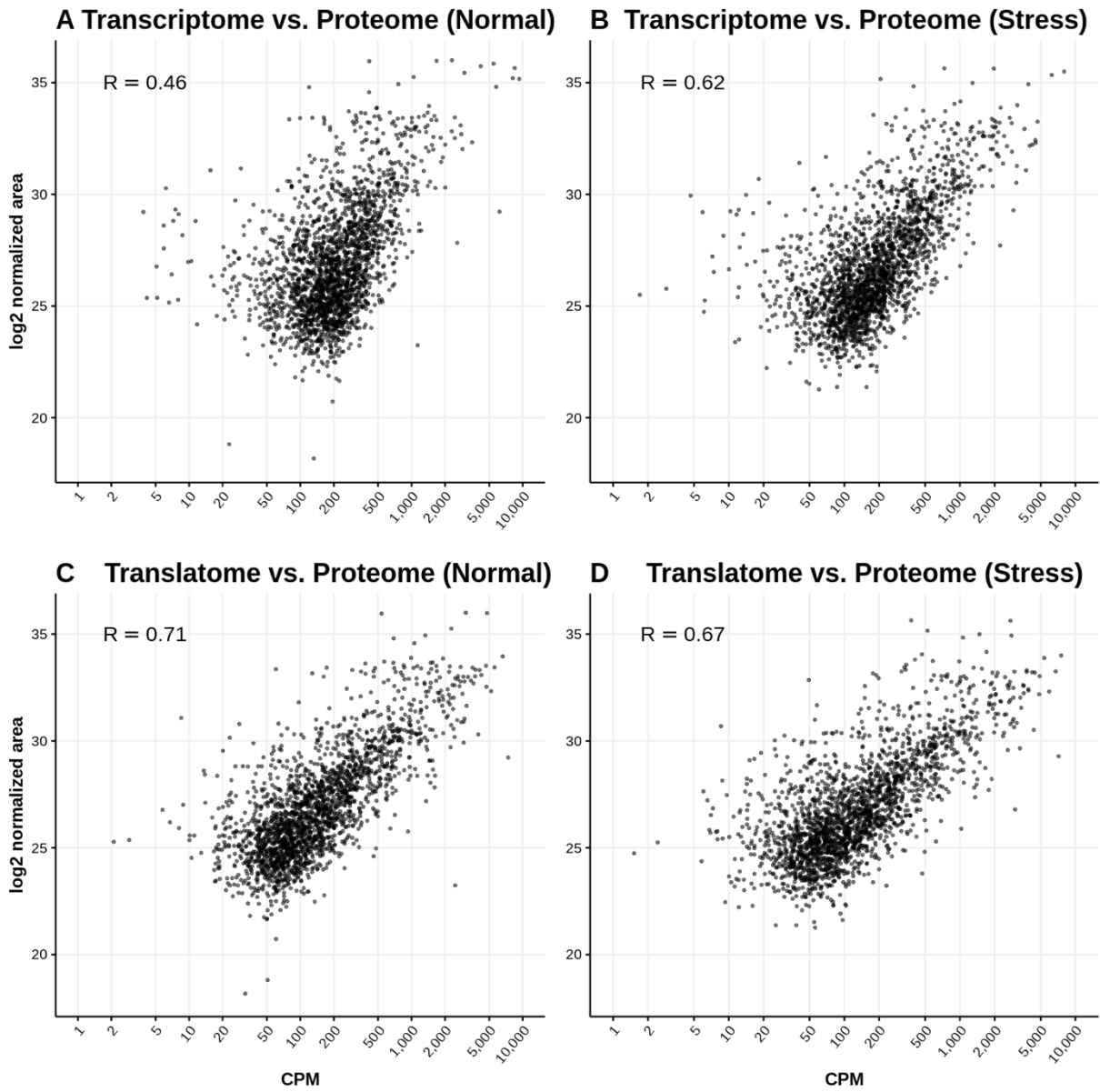
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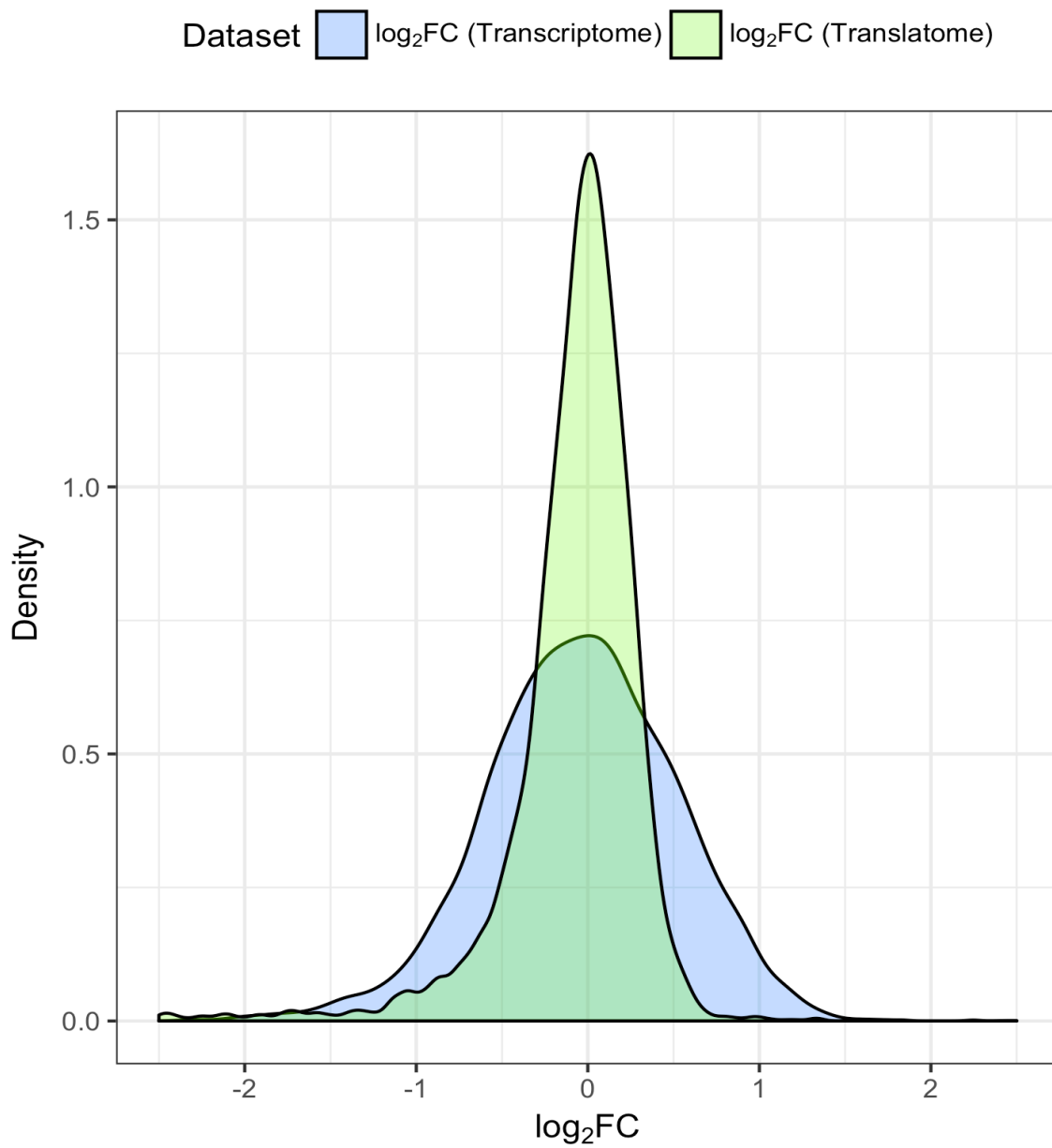
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556 **Figure 4**

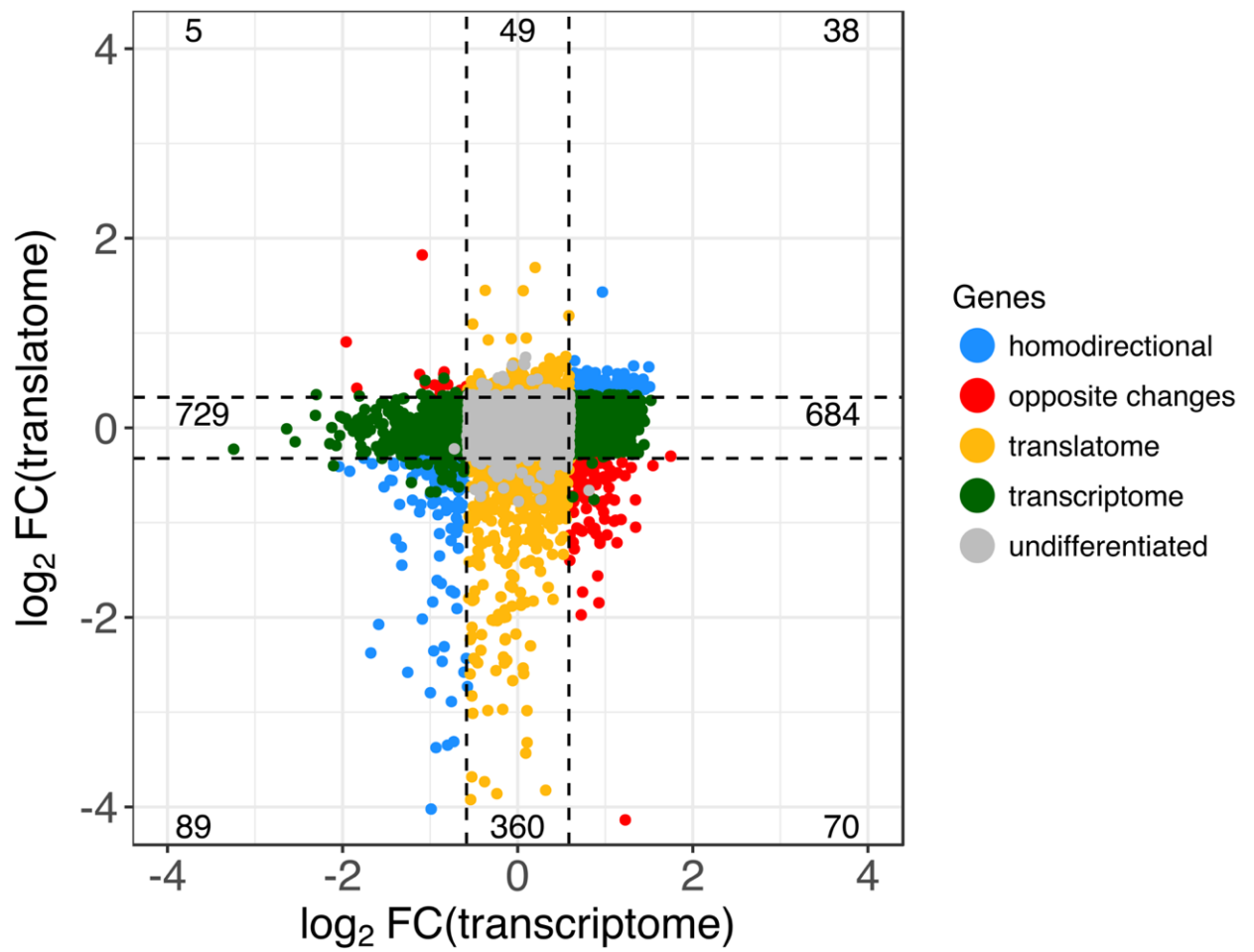
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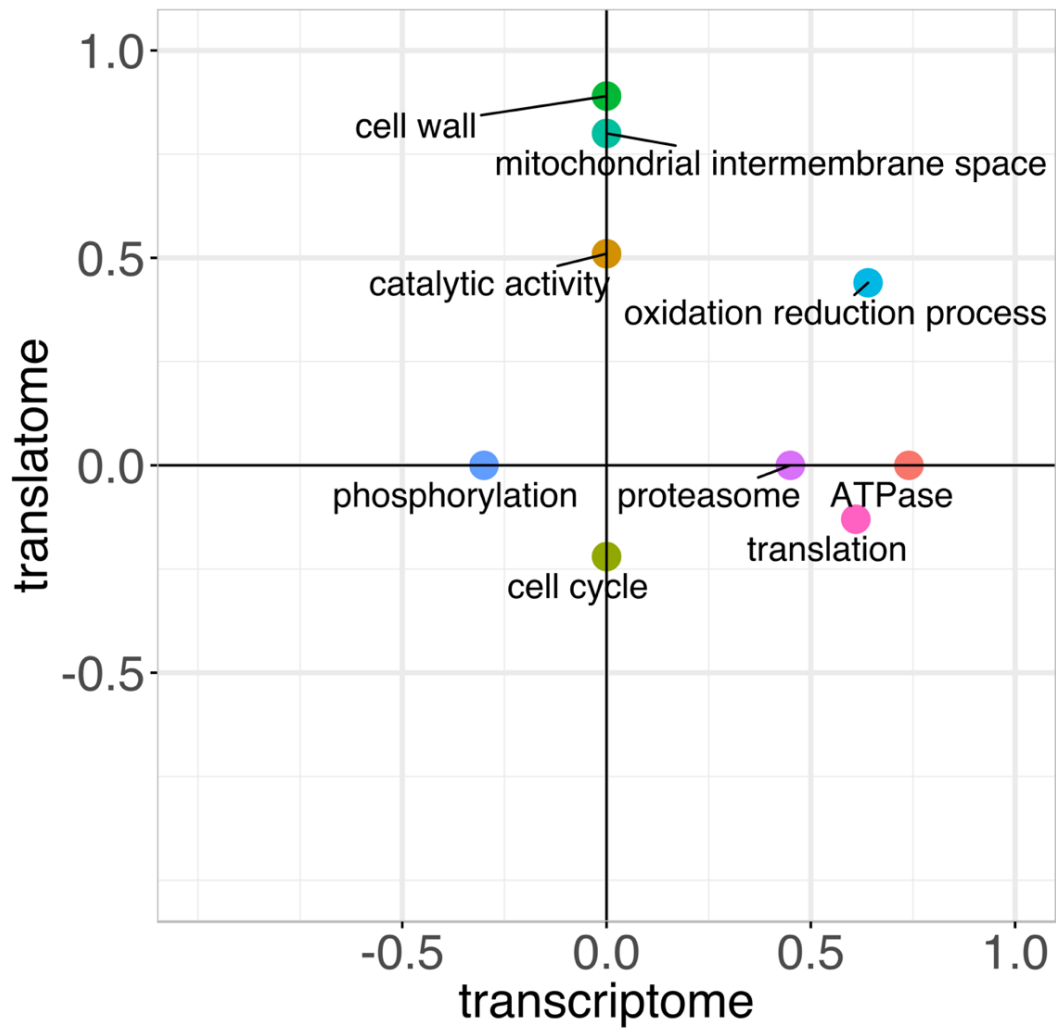
559 **Figure 5**

560



561

562 **Figure 6**  
563



564