

1 **Genome-scale reconstruction of Gcn4/ATF4 networks driving a growth program**

2

3

4 Rajalakshmi Srinivasan<sup>1</sup>, Adhish S. Walvekar<sup>1</sup>, Aswin Seshasayee<sup>2</sup> and Sunil Laxman<sup>1</sup>

5

6 <sup>1</sup> Institute for Stem Cell Science and Regenerative Medicine (inStem)

7 <sup>2</sup> National Centre for Biological Sciences - TIFR

8 GKVK post, Bellary Road

9 Bangalore 560065.

10

11 email: [aswin@ncbs.res.in](mailto:aswin@ncbs.res.in) , [sunil@instem.res.in](mailto:sunil@instem.res.in)

12

13

14 **Abstract:**

15 Growth and starvation are considered opposite ends of a spectrum. To sustain growth, cells must  
16 manage biomolecule supply to balance constructive metabolism with high translation, through  
17 coordinated gene expression programs. Global growth programs couple increased ribosomal  
18 biogenesis with sufficient carbon metabolism, amino acid and nucleotide biosynthesis, and how this  
19 is collectively managed is a fundamental question. Conventionally, the role of the Gcn4/ATF4  
20 transcription factor has been studied only in the context of amino acid starvation. However, high  
21 Gcn4/ATF4 has been observed in contexts of rapid cell proliferation, and the specific role of Gcn4 in  
22 growth contexts are unclear. Here, using a methionine-induced growth program in yeast, we show  
23 that Gcn4/ATF4 is the fulcrum through which metabolic supply dependent sustenance of translation  
24 outputs is maintained. Integrating time-matched transcriptome and CHIP-Seq analysis, we decipher  
25 genome-wide direct and indirect roles for Gcn4 in this growth program. Genes that enable metabolic  
26 precursor biosynthesis indispensably require Gcn4; contrastingly ribosomal genes are partly  
27 repressed by Gcn4. Gcn4 directly binds promoter-regions and transcribes a subset of metabolic  
28 genes, particularly driving lysine and arginine biosynthesis. Gcn4 also globally represses lys/arg  
29 enriched transcripts, which include the translation machinery. The sustained Gcn4 dependent lys/arg  
30 supply is required to maintain sufficient translation capacity, by allowing the synthesis of the  
31 translation machinery itself. Gcn4 thereby enables metabolic-precursor supply to bolster protein  
32 synthesis, and drive a growth program. Thus, we illustrate how growth and starvation outcomes are  
33 both controlled using the same Gcn4 transcriptional outputs, in entirely distinct contexts.

34

35

36

37

## 38 **Introduction**

39

40 Understanding the organizational principles of transcriptional programs that define growth  
41 or starvation is of fundamental importance. In order for cells to sustain growth, and thereby  
42 proliferation, a controlled supply of biosynthetic precursors is essential. These precursors include  
43 amino acids that drive protein translation, nucleotides (to make RNA and DNA), and several co-  
44 factors. Such a balanced cellular economy therefore requires coordinated, genome-wide responses  
45 in order to manage metabolic resources and ensure coordinated growth outputs. Here, the model  
46 eukaryote, *Saccharomyces cerevisiae*, has been instrumental in building our general understanding  
47 of global nutrient-dependent responses, addressing how cells allocate resources, defining  
48 transcriptional and metabolic ‘growth programs’, as well as to uncover general mechanisms of  
49 nutrient-sensing [1–9]. However, much remains unclear about how cells sustain the high  
50 requirement of biosynthetic precursors during growth programs.

51

52 Interestingly, studies from yeast and other systems show that the presence of some  
53 metabolites, even in nutrient-limited conditions, induces cell growth programs, as observed at the  
54 level of transcription, signaling or metabolism. One example is that of acetyl-CoA, which at sufficient  
55 concentrations induces cells to exit quiescence, and activates global gene expression programs  
56 driving proliferation [10–17]. Similarly, methionine (and its metabolite S-adenosyl methionine) turn  
57 on growth programs in cells [18–20]. In mammals, methionine availability correlates with tumor  
58 growth [21,22], and methionine restriction improves cancer therapy, by limiting one-carbon and  
59 nucleotide metabolism [23,24]. In yeast, supplementing methionine inhibits autophagy [25],  
60 activates growth master-regulators [18], and increases cell growth and proliferation [18,26]. At the  
61 level of global transcriptional and metabolic states, methionine triggers a hierarchically organized  
62 growth program, where cells transcriptionally induce ribosomal genes, and key metabolic nodes  
63 including the pentose phosphate pathway, as well as all amino acid, and nucleotide biosynthesis  
64 [20]. These are quintessential hallmarks of a cell growth program [27]. Therefore, using this  
65 controlled growth program, it may be possible to decipher universal regulatory features that  
66 determine a growth state. Further, such a system can be used to address how the metabolic  
67 program couples with the regulation of translation outputs. Unexpectedly, this previous study  
68 suggested that the transcription factor Gcn4 was critical for this growth program [20]. Such a role  
69 played by Gcn4 in a growth program was both unclear and unforeseen. This is because our current  
70 understanding of Gcn4 comes primarily from its role during starvation. Contrastingly, the role of  
71 Gcn4 during high cell growth is largely obscure.

72 Gcn4 (called ATF4 in mammals) is a transcriptional master-regulator, conventionally studied  
73 for its role during starvation and stress [28–31]. During severe amino acid starvation, the translation  
74 of Gcn4 mRNA increases, through the activation of the Gcn2 kinase, and subsequent eIF2-alpha  
75 phosphorylation [28,32,33]. This resultant increase in Gcn4 protein allows it to function as a  
76 transcriptional activator, where it induces transcripts involved in amino acid biosynthesis, thereby  
77 allowing cells to restore amino acid levels and survive starvation [28,31,34,35]. Almost our entire  
78 current knowledge of Gcn4 function comes from studying its roles during amino acid and other  
79 nutrient starvation. Contrastingly, we surprisingly found that in a growth program triggered by  
80 abundant methionine, cells induce Gcn4, in a context of high cell proliferation [20]. Other studies in  
81 several cancers suggest that the mammalian ortholog of Gcn4, called ATF4, is critical to sustain high  
82 growth [36,37]. Since starvation and growth programs are considered to be opposite ends of a  
83 spectrum, we wondered what specific roles does Gcn4 carry out during this growth program?

84 In this study, we find that Gcn4 controls essential components of an anabolic program,  
85 which are coupled with the management of overall translation. During such a growth program, Gcn4  
86 directly transcribes genes required for amino acids and transamination reactions, and indirectly

87 regulates essential ‘nitrogen’ metabolic processes, leading to nucleotide synthesis. We elucidate the  
88 direct and indirect, methionine-dependent roles of Gcn4, and identify separate requirements for this  
89 protein to control the metabolic component of this growth program, as well as manage the  
90 induction of translation-related genes. Thereby, we establish the importance of Gcn4-enabled  
91 biosynthetic precursor supply in appropriately maintaining a high translation capacity. Notably,  
92 comparing this function of Gcn4 during growth programs, to its well-known, conventional roles in  
93 starvation, reveals largely conserved transcriptional outputs of Gcn4 in both scenarios that however  
94 lead to distinct outcomes for the cell (growth vs survival). Through this, we show how a  
95 transcriptional master-regulator, conventionally viewed as a ‘survival-factor’, uses its canonical  
96 outputs to enable a growth program by ensuring specific amino acid synthesis in order to manage  
97 sufficient translation capacity.

98

## 99 **Results**

### 100 ***Methionine induces an universal ‘growth program’***

101

102 Understanding the regulatory logic of transcriptional networks in growth programs is of fundamental  
103 importance. The role of the Gcn4 transcriptional master regulator has been well studied primarily in  
104 the context of severe nutrient starvation, as extensively explained in the subsequent section.  
105 However, several studies of cancers suggest that the mammalian ortholog of Gcn4 (ATF4) is required  
106 for rapid growth [36,37]. Therefore, we first wanted to establish a relevant, universal system where  
107 the role of Gcn4 during a growth program could be rigorously studied. Here, we utilized prior  
108 knowledge suggesting that methionine induces a transcriptional and metabolic growth program.

109

110 The observations showing that methionine switch cells to a growth state come primarily from yeast  
111 cells using lactate as a sole carbon source [18,20,25]. In these lactate-dependent conditions, global  
112 gene expression analysis revealed that providing methionine induces transcripts that represent a  
113 ‘growth signature’ [20]. This includes increased expression of ribosomal transcripts, and induced  
114 expression and metabolic flux through the pentose phosphate pathway, amino acid and nucleotide  
115 biosynthesis [20]. Since current studies are limited to only this lactate carbon source condition, we  
116 first more broadly established that this methionine response is universal, by studying the global  
117 transcriptional response to methionine supplementation in high glucose medium (the most  
118 preferred carbon source for yeast).

119 We performed comprehensive gene-expression analysis comparing transcripts from cells growing in  
120 glucose (MM) or glucose supplemented with methionine (MM+Met), as shown in Supplementary  
121 Figures 1 and 2, Supplementary WS2, Supplementary WS3, and described in the corresponding  
122 supplementary text. These results collectively show that the transcriptional response to methionine  
123 retains all the hallmarks of an anabolic growth program even when glucose is used as a carbon  
124 source. This includes the induction of appropriate metabolic genes (particularly all amino acid  
125 biosynthesis, nucleotide biosynthesis and transamination reaction related genes), as well as  
126 cytoplasmic translation related genes (Supplementary Figure 2, Supplementary WS2, Supplementary  
127 WS3). This transcriptional signature of cells MM+Met overlaps well with earlier studies of cells  
128 growing in lactate as a carbon source (supplemented with methionine) [20]. This induction of the  
129 translation machinery along with amino acid and nucleotide synthesis genes are all classic hallmark  
130 signatures of an anabolic program [27,38,39]. Further, these transcriptional changes also result in an  
131 appropriate metabolic state switch (increased *de novo* amino acid and nucleotide synthesis), as  
132 determined using a quantitative, targeted, stable-isotope pulsed LC/MS/MS based flux approach  
133 (Supplementary Figure 3).

134 In summary, we find that methionine triggers a growth program, with the induction of both  
135 metabolic and ribosomal genes, even in preferred medium with glucose as a carbon source. We  
136 therefore use this system (MM+Met) to address universal principles of cell growth regulation.

137

138 ***Gcn4 is induced by methionine and controls a conserved transcriptional signature in both growth***  
139 ***and starvation programs***

140 Conventionally, Gcn4 (a transcriptional master-regulator), is studied in the context of severe  
141 starvation, as part of the integrated stress response [28,29,31,40,41]. Nearly all existing studies of  
142 Gcn4 use pharmacological inhibitors of amino acid biosynthesis, such as 3-amino triazole (3-AT) or  
143 sulfo meturon (SM) to induce Gcn4, and study its role in starvation responses where cell growth is  
144 minimal [31,34,35,42,43]. Indeed, our current understanding of Gcn4 function comes primarily from  
145 contexts of nutrient-stress and starvation. In contrast, we had earlier observed that supplementing  
146 methionine strongly induces Gcn4 [20], coincident with *increased* cell growth and proliferation. Since  
147 this is distinct from conditions of starvation and low growth, we wanted to understand what the role  
148 of Gcn4 was, during a growth program.

149

150 We therefore used the methionine-induced growth transcriptional program (as described in  
151 Supplementary Figure 2 and the previous section) to address this question. We first asked if Gcn4  
152 protein is induced in methionine-supplemented glucose medium. Indeed, Gcn4 protein levels  
153 substantially increase when methionine is supplemented (MM+Met) (Figure 1A and Supplementary  
154 Figure 4A). This observation reiterates that Gcn4 can be induced by growth signals (methionine)  
155 irrespective of carbon source. We therefore dissected how much of this anabolic program is  
156 mediated by Gcn4. To address this, we compared transcriptomes of wild type and  $\Delta gcn4$  cells in  
157 MM+Met (Supplementary Figure 4B), and found a striking, Gcn4-dependent global response in the  
158 presence of methionine. ~900 genes were differentially expressed in  $\Delta gcn4$ , compared to wild type  
159 cells in MM+Met. Here, 514 genes were upregulated, and 398 genes were downregulated in  $\Delta gcn4$   
160 cells in the presence of methionine (fold change cut-off of  $\geq 2$  fold) (Supplementary Figure 4B &  
161 Supplementary WS1). As a control, in only MM medium (without supplemented methionine), far  
162 fewer genes (~160) showed any differential expression at all in  $\Delta gcn4$  relative to WT (Supplementary  
163 Figure 4B). These data show that Gcn4 has a critical role for the methionine-dependent growth  
164 program in glucose.

165

166 To understand the global consequences of the loss of Gcn4 during this growth program, we used a  
167 GO-based analysis to categorize most altered groups of genes. Upregulated genes in  $\Delta gcn4$  show a  
168 notable enrichment for 'cytoplasmic translation', 'ncRNA processing', 'RNA maturation' and 'RNA  
169 methylation' (Figure 1B). This strikingly revealed that the transcripts associated with protein  
170 translation, which were already induced by methionine, further increase in the absence of Gcn4. i.e.  
171 Gcn4 partially represses cytoplasmic translation even in a growth program. In contrast, genes that  
172 are downregulated in  $\Delta gcn4$  cells are primarily involved in amino acid biosynthetic processes,  
173 nucleotide biosynthetic processes, mitochondrial translation, NADP metabolic processes and  
174 pyruvate metabolism (Figure 1B & Supplementary WS3). Collectively, this reveals that Gcn4 is  
175 essential for the induction of genes involved in these metabolic processes, which is a majority of the  
176 methionine induced anabolic program, but partially represses translation.

177

178 Here, we note a striking observation. In studies of starvation, the induction of Gcn4 represses  
179 ribosomal genes, and induces amino acid biosynthesis genes [35,44,45]. In contrast, in this  
180 methionine-induced growth program, ribosomal and translation related genes are themselves  
181 induced even as Gcn4 is also induced. The loss of Gcn4 further increases ribosomal genes, suggesting

182 that Gcn4 appropriately keeps the extent of ribosomal gene induction in check, while the ribosomal  
183 gene induction occurs through independent regulation. Furthermore, the induction of amino acid  
184 biosynthetic genes remains regardless of starvation or growth programs. This hints that despite  
185 growth and starvation being at opposite ends of a spectrum, the role of Gcn4 in either state might  
186 be conserved. To further address this possibly conserved global role of Gcn4, we compared the  
187 overlap of Gcn4 dependent, induced or repressed genes in this growth program, with existing data  
188 from a conventional starvation program where Gcn4 has high activity. This gene expression data  
189 comes from a conventional mode of inducing Gcn4, via inhibiting amino acid biosynthesis using a  
190 chemical inhibitor of amino acid biosynthesis (sulfometuron or SM) [46]. Notably, we find that  
191 44% of the genes activated by Gcn4 and 56% of the genes repressed by Gcn4 in the  
192 methionine dependent growth program overlap with the genes activated and repressed in  
193 the SM dependent starvation condition (Fisher exact test,  $p < 10^{-10}$ ) (Figure 1C). A GO grouping of  
194 the genes which overlap between the growth and the starvation condition suggests a conserved role  
195 of Gcn4 in inducing amino acid biosynthetic genes and in repressing translation related genes  
196 (Supplementary WS6).

197 Two key points emerge from these analyses. First, the role of Gcn4 appears to be conserved  
198 regardless of whether cells are in a growth or starvation program. This conserved role appears to be  
199 to increase transcripts related to amino acid and nucleotide biosynthesis (all required for  
200 anabolism), while repressing translation related genes. However, during a growth program, there is  
201 already an induction of translation genes (as seen in Figure 1). Therefore, in this context, Gcn4  
202 tempers the extent of induction of translation related genes, while during starvation Gcn4 represses  
203 ribosomal genes below that of non-starved cells.

204

#### 205 ***Gcn4 binds to its target gene promoters related to metabolism during a growth program***

206 Which parts of the transcriptional outputs in this growth program does Gcn4 directly regulate, and  
207 how does this compare to the known, direct roles of Gcn4 during starvation? To address this, we  
208 performed chromatin immunoprecipitation (ChIP)-sequencing of Gcn4 in MM and MM+Met  
209 conditions. Notably, this uniquely integrates directly comparable information from the Gcn4 ChIP-  
210 seq, with a time-matched global transcriptome, *during a growth program*.

211

212 First, we asked what is the Gcn4 DNA binding activity when induced by methionine. We performed  
213 ChIP of Gcn4 (with a FLAG-epitope incorporated into the C-terminus in the endogenous *GCN4* locus),  
214 using cells grown in MM and MM+Met, with MM essentially acting as a control. We considered  
215 peaks that are represented in both the biological replicates for further analysis, using very well  
216 correlated biological replicates (Supplementary Figure 4C). Here, we identified 320 Gcn4 binding  
217 peaks in the cells grown in MM+Met, whereas, there were no consensus peaks observed in replicate  
218 samples of cells grown in MM (Figure 2A & Supplementary WS4). The enhanced Gcn4 occupancy on  
219 the target gene promoter in MM+Met condition was further validated using ChIP-qPCR analysis  
220 (Supplementary Figure 6). This shows that the GCN4 occupancy on DNA increases in the presence of  
221 methionine.

222

223 Next, we analyzed the Gcn4 binding signals around the transcription and translation start site of the  
224 genes found within 750bp around the identified peaks. Transcription start site data available for cells  
225 growing in rich, glucose medium (the nearest possible condition to that used in this study) was  
226 obtained from the YeastTSS database [47]. The TSS identified using the CAGE method reported in this  
227 database was used for our analysis. Notably, a majority of the Gcn4 binding peaks in MM+Met are  
228 found upstream of these annotated transcription start sites (Figure 2B). A similar analysis with the  
229 translation start sites of the target genes shows higher read coverage upstream of the translation

230 start site (Supplementary Figure 5). We further analyzed the genomic features of the identified  
231 peaks using the HOMER program [48]. Notably, we observed a very apparent enrichment of Gcn4  
232 binding to the promoter region of the targets. 263 out of 320 peaks are found within the promoter  
233 region of target genes (-1kb to +100bp around the TSS), while the remaining peaks bind at intergenic  
234 regions (11), exons (17) or close to transcription termination sites (29) (Figure 2C & Supplementary  
235 WS4). This shows that during a growth program, Gcn4 activity is primarily restricted to binding  
236 promoter sites of target genes.

237

238 We next searched for the enrichment of sequence motifs in the peaks identified in the MM+Met  
239 condition using The MEME-suite [49]. We found that these peaks were enriched for the conserved  
240 Gcn4 binding motifs found previously under amino acid starvation conditions, [35,50,51]. Strikingly,  
241 81% (260 out of 320) of the peaks that we identify have at least one of the variants of the Gcn4  
242 binding motif 'TGANTCA' (Figure 2D), showing that Gcn4 in this context still primarily recognizes its  
243 high-affinity DNA binding motif.

244

245 Finally, how does this compare to studies of Gcn4 activity during starvation, particularly during  
246 severe amino acid biosynthesis inhibition [34,35]? A comprehensive previous study of Gcn4 function  
247 during amino acid starvation indicated substantial Gcn4 binding to regions within ORFs of genes, as  
248 well as to promoter regions [34]. To compare this study from a starvation program with our data  
249 from a growth program, for the non-coding and the ORF peaks regions reported in the previous  
250 study [34], we calculated the Gcn4 binding signal in our Gcn4 ChIP seq data (from the MM+Met  
251 condition). Strikingly, we find that the signal in ORF peaks is significantly lower than the non-coding  
252 peak under MM+Met condition ( $p$ -value  $< 10^{-8}$ ), whereas a similar analysis performed using the Gcn4  
253 ChIP-seq data from [34] show little differences in the signal intensity between ORF and Non-coding  
254 peaks ( $p$ -value of 0.002) (Figure 2E). As a distinct comparison, we used a dataset from a milder  
255 starvation regime [52], where glucose was limited in a chemostat. Here, the occupancy of Gcn4 was  
256 more similar to that observed during our growth program, with a majority of Gcn4 occupancy at  
257 promoter regions of target genes (Figure 2E). These analyses show that during a growth program,  
258 the direct targets of Gcn4 remain highly specific, conserved and restricted to the promoter regions  
259 of genes. The Gcn4 occupancy limited to promoter regions during growth and mild starvation  
260 conditions can be possibly explained by a lower dosage of Gcn4 under these conditions. Under  
261 extreme amino acid starvation conditions, very high Gcn4 levels might result in increased Gcn4  
262 occupancy on the ORFs, in addition to its specific binding to the promoter. Collectively, our data  
263 shows that regardless of the mode of Gcn4 induction, and whether cells are in a growth or starvation  
264 program, it binds specifically to a highly conserved motif.

265

266 Thus, the global role of Gcn4 during either a growth program, or in a starvation response appears  
267 remarkably conserved. However, the cellular outcomes are different, and this can be explained by  
268 two criteria. First, the amounts of Gcn4 protein (as induced by methionine) will be different from the  
269 other conditions tested, as the mode of induction of Gcn4 is entirely different in these studies.  
270 Therefore, since any protein's affinity to its target depends on its dosage in the cell as well as the  
271 presence of other competing factors, there will be differential binding affinity to the targets, as is  
272 well known for most transcription factors [53,54]. Second, the context of Gcn4 induction is entirely  
273 distinct. In this context Gcn4 is supporting an anabolic program, while the cells also have increased  
274 ribosomal genes. Hence, while the function of Gcn4 is the same (primarily to induce amino acid  
275 biosynthesis, and indirectly repress translation), the outcome is entirely different, because in a  
276 growth program the increased production of amino acids and nucleotides might aid the increase in  
277 translational capacity via increased ribosomal biogenesis.

278

## 279 **Direct and Indirect targets of Gcn4 during a growth program**

280 We therefore asked how much of the Gcn4-dependent transcriptional response is directly regulated  
281 by Gcn4, and what its specific targets were? To identify direct targets of Gcn4 in methionine-  
282 dependent gene regulation, we overlaid the transcriptome data ( $\Delta gcn4$  vs *WT* in MM+Met) with the  
283 ChIP-seq data (from MM+Met). Out of the 398 genes that are downregulated in  $\Delta gcn4$ , 133 are  
284 direct targets of Gcn4 (Supplementary WS4). Contrastingly, Gcn4 directly regulates only 24 out of  
285 514 upregulated genes (Supplementary Figures S7A and S7B, and Supplementary WS4). These  
286 results strengthen the role of Gcn4 as a transcriptional activator. GO-based analysis of the genes  
287 directly transcribed by Gcn4 reveals a significant enrichment of amino acid biosynthetic genes.  
288 Notably, the indirectly activated targets are enriched for nucleotide biosynthesis, the pentose  
289 phosphate pathway, and mitochondrial translation (Figure 3A, Supplementary WS3). In addition to  
290 the amino acid biosynthetic genes, Gcn4 directly activates genes involved in other critical functions,  
291 particularly the *Sno1* and *Snz1* genes (pyridoxal synthase), required for transamination reactions that  
292 lead to amino acid synthesis, and *Nde1*- the NADH dehydrogenase (Supplementary Figure 7C). These  
293 genes have Gcn4 binding sites in its promoter [55]. In contrast to the *Snz1* and *Sno1* pair that is  
294 bidirectionally activated by Gcn4, the *Trm1* and *Mdh2* pair of genes are bidirectionally repressed by  
295 Gcn4 (Supplementary Figure 7D). These data show that in the presence of methionine, Gcn4 directly  
296 increases the expression of primarily the amino acid biosynthetic arm, whereas the methionine-  
297 dependent activation of nucleotide biosynthetic genes, pentose phosphate pathway, mitochondrial  
298 translation related genes are indirectly regulated by Gcn4. Collectively, the metabolic component of  
299 the methionine-dependent growth program is directly regulated by Gcn4.

300

301 As discussed, in the presence of methionine Gcn4 directly upregulates the genes of various amino  
302 acid biosynthetic pathways (Figure 3A). In this context, subsets of amino acid biosynthetic genes are  
303 strikingly induced. Notably, every single gene of arginine biosynthetic pathway, and nearly every  
304 gene of lysine, histidine and branched chain amino acid biosynthetic pathways are directly activated  
305 by Gcn4 (Figure 3B and 3C, Supplementary Figure 7E). This suggests that Gcn4 might be critical for  
306 the supply of particularly arginine and lysine, during the methionine mediated anabolic program.

307

308 We estimated the functional contribution of methionine-induced Gcn4 towards individual amino  
309 acid biosynthesis, particularly that of arginine and lysine, using a targeted LC/MS/MS based  
310 approach [56] to measure amino acid synthesis flux, based on stable-isotope incorporation.  
311 Consistent with the transcriptome data, we found a strikingly increased amino acid biosynthesis in  
312 MM+Met, compared to MM, and expectedly; the loss of *GCN4* severely decreased the flux towards  
313 amino acid biosynthesis, including a near-complete loss of arginine and lysine biosynthesis (Figure  
314 3D, Supplementary Figure 8). This reiterates that Gcn4 has a vital role in increasing the amino acid  
315 pools required during a methionine induced growth program, particularly regulating the synthesis of  
316 arginine and lysine.

317

318 How does the role of Gcn4 during this growth program compare to its role during extreme amino  
319 acid starvation? To understand this, we analyzed a publicly available ChIP seq data of Gcn4, where  
320 Gcn4 was induced during severe amino acid starvation (due to SM treatment) [34]. Firstly, we  
321 compared potential Gcn4 targets, which are present 750bp around the Gcn4 peaks, identified in  
322 both the growth and the starvation condition. We found a 47% overlap between the Gcn4 targets  
323 during the methionine induced growth program, and under amino acid starvation [34] (Figure 3E,  
324 and Supplementary WS6) (Fisher's Exact test  $P < 10^{-10}$ ). We also compared the targets identified in  
325 our study with a distinct, simpler starvation regime, where cells were only limited for glucose [52].  
326 About 80% of the targets identified in this study overlap with that of the Gcn4 targets identified in  
327 the glucose limitation study [52] (Figure 3F, and Supplementary WS6) (Fisher's Exact test  $P < 10^{-10}$ ).

328 This indicates that the Gcn4 targets, particularly the activation of amino acid biosynthetic genes, are  
329 conserved irrespective of the growth status of the cell. .

330

331 Finally, during starvation programs, Gcn4 negatively regulates (represses) ribosomal and translation  
332 related genes [31,34,35,44]. In agreement with these ChIP-seq studies in starvation conditions  
333 [34,35], we also find that Gcn4 indirectly represses translation related genes, except for the  
334 following- RPL14B, RPS9A, RPL36B and RRP5, these are directly repressed by Gcn4 under this  
335 condition (Supplementary Figure 7A and 7B). The distinction though is that when methionine is  
336 present, ribosomal genes are induced, but Gcn4 appears to temper the extent of this induction (as  
337 the loss of Gcn4 in this condition further increases ribosomal genes). Therefore, through this  
338 repressive activity, Gcn4 likely enables cells to manage the extent of ribosomal gene induction due  
339 to methionine.

340

341 To summarize, the role of Gcn4 in a methionine-dependent growth program can be broken into two  
342 parts. First, Gcn4 directly induces amino acid biosynthesis genes, as well as transamination reactions.  
343 As part of a feed-forward program, the nucleotide biosynthesis genes and the PPP (which complete  
344 the methionine-mediated anabolic program [20] are indirectly induced. Further, the  
345 ribosomal/translation related genes that are induced by methionine in wild-type cells are further  
346 induced upon the loss of Gcn4 in this condition, suggesting that Gcn4 manages the extent of  
347 ribosomal gene induction due to methionine. Notably, the core function of Gcn4, which is to  
348 increase amino acid (and nucleotide) synthesis, remains unchanged when cells are in a growth state  
349 or dealing with starvation. Importantly, Gcn4 is critical for the high rates of synthesis of arginine,  
350 lysine and histidine. However, the cellular outcome is different, because of this coincident activation  
351 of Gcn4 in conditions where ribosomal biogenesis and translation are high.

352

353 ***Gcn4 globally represses arginine/lysine enriched genes, including the translational machinery.***

354 From our data thus far, it is clear that Gcn4 helps supply cells with several metabolites, particularly  
355 the amino acids arginine and lysine, when methionine triggers a growth program. Given this critical  
356 function of Gcn4 in arginine and lysine biosynthesis and supply, we wondered if there were  
357 correlations of lysine and arginine utilization in genes, and global gene expression programs  
358 controlled by Gcn4. Although the amino acid compositions of proteins are evolutionarily optimized,  
359 our understanding of amino acid supply vs demand remains woefully inadequate [57,58]. As amino  
360 acids are the building blocks of proteins, translation naturally depends on available amino acid pools  
361 in the cell. We therefore asked if there were categories of proteins that were particularly enriched  
362 for arginine and lysine, within the genome, and if this had any correlation with Gcn4 function. For  
363 this, we divided the total number of proteins in the *S. cerevisiae* genome into three bins based on  
364 the percentage of arginine and lysine content of the protein (%R+K). The bin1 comprises of 1491  
365 proteins with the lowest percentage of R+K (bin1; < 10% R+K ), bin2 has 3033 proteins with  
366 moderate %R+K content (bin2; 10-13% R+K), and bin3 comprises of the 1501 proteins, with very high  
367 %R+K ( bin3; >13% R+K) (Figure 4A). We next asked if these bins were enriched for any groups of  
368 functional pathways (based on Gene Ontology). Bin1 and bin2 have very large, disparate groups of  
369 GO terms, with no unique enrichment. However, bin3 was significantly enriched for ribosomal and  
370 translation related genes (Figure 4B). This arginine and lysine distribution in translation related  
371 genes are significantly higher compared to genome wide distributions (Wilcox test, p-value < 10<sup>-10</sup>)  
372 (Figure 4C). Thus, translation related proteins are highly enriched for arginine and lysine amino acids.

373

374 Next, we asked if there is any correlation between the genes regulated by Gcn4 (in MM+Met), and  
375 the percentage of R+K encoded within these encoded proteins. Strikingly, we noticed that a very



376 significant proportion of the genes that are repressed by Gcn4 fall in bin3 (~40%, Fisher's exact test  
377 P-value <  $10^{-10}$ ) (Figure 4D & Supplementary WS5). Therefore, a significant proportion of the genes  
378 induced by methionine, and further induced in  $\Delta gcn4$  are arginine and lysine rich. This suggests the  
379 possibility of a deeper management of overall, methionine-induced anabolism by Gcn4, where the  
380 translation of arginine and lysine enriched proteins will be required for high translation, and this  
381 requires Gcn4-dependent precursors.

382

### 383 ***Gcn4 dependent outputs can sustain high translation capacity during growth***

384 Given this striking observation, we asked if, in a growth program, cells could still sustain the  
385 synthesis of arginine and lysine rich genes if Gcn4 is absent. To evaluate this unambiguously, we  
386 designed inducible, luciferase-based reporters to estimate the translation of a several of R+K  
387 enriched genes, which are induced in cells by methionine (and further increased upon the loss of  
388 Gcn4) (Supplementary WS1). We designed a plasmid, in which the gene of interest (GOI; amplified  
389 from the genomic DNA of *S. cerevisiae*) was cloned in frame with the luciferase coding sequence, in  
390 such a way that the entire fragment (GOI+Luciferase) will be under the control of an inducible  
391 promoter (Supplementary Figure 9). Using this system, measuring luciferase activity after induction  
392 will estimate the specific translation of the specific arginine/lysine enriched gene, in any condition.  
393 This accounts for only newly synthesized protein, and therefore avoids mis-interpretations coming  
394 from already existing protein in the cells before methionine addition. We made reporters for 4 such  
395 candidate genes (RPL32, STM1, NHP2, RPS20) (Supplementary Figure 9).

396

397 First, we contextualized the expression of these lysine and arginine enriched genes (based on  
398 reporter activity) in wild-type cells, under either a growth or a starvation regime where Gcn4  
399 expression is high. The conditions we compared were MM (low Gcn4 expression), addition of  
400 methionine (growth program, strong Gcn4 induction), and the addition of 3-AT (amino acid  
401 starvation condition, high Gcn4). In these conditions, we induced the reporters for Nhp2, Rpl32 and  
402 Rpl20 for 30 min, and compared luciferase activity. Here, the luciferase activity of all three reporters  
403 significantly increase in methionine supplemented conditions, and are decreased in the 3-AT  
404 condition (Figure 5A). This reiterates that the translational outcomes are entirely distinct in a growth  
405 or starvation program, despite high Gcn4 activity in both conditions.

406

407 We now could specifically determine the importance of Gcn4 activity during a growth program  
408 (addition of methionine). First, we compared the extent of transcript expression for these  
409 lysine/arginine enriched transcripts, Nhp2, Rpl32, Rpl20 and Stm1, in wild-type and  $\Delta gcn4$  cells in  
410 the presence of methionine (Figure 5B). The loss of Gcn4 in these conditions further increased  
411 expression of these transcripts, reiterating the role of Gcn4 as a (indirect) repressor of these genes.  
412 We next directly measured the translation of these genes, using the luciferase-based reporters of  
413 these genes. For this, using a similar experimental setup as earlier, we measured luciferase activity in  
414 wild-type and  $\Delta gcn4$  cells after 30 minutes of induction with  $\beta$ -estradiol. Strikingly, all the candidate  
415 reporter genes showed a 3-5 fold reduction in translation in *GCN4* deficient cells, compared to WT  
416 cells (Figure 5C). These data reveal that Gcn4 is critically required to maintain the translation  
417 capacity of the cell, during this growth program. Finally, to determine if this reduced translation  
418 capacity in  $\Delta gcn4$  is due to the reduced supply of arginine and lysine in these conditions, we carried  
419 out a rescue experiment with the addition of only these two amino acids. We supplied both amino  
420 acids (2mM each) to wild-type and  $\Delta gcn4$  cells growing in the presence of methionine, and  
421 measured luciferase activity after induction. Notably, the supply of arginine and lysine substantially  
422 rescued the expression of these reporter proteins, by increasing their translation ~2-3 fold (Figure  
423 5D).

424

425 Collectively, we find that Gcn4 activity is central to sustain a growth program triggered by  
426 methionine. Gcn4 enables sufficient supply of amino acids, particularly arginine and lysine, for the  
427 translation of ribosomal proteins, while also tuning the amount of expression for these transcripts.  
428 This in turn maintains sufficient translational capacity needed by the cell, to sustain the anabolic  
429 program and drive growth. This is in contrast to a conventional starvation program due to amino  
430 acid limitation, where Gcn4 is also high. In such starvation contexts, the amounts of arginine and  
431 lysine enriched transcripts (including translation related transcripts) are low (and repressed by  
432 Gcn4), and the role of Gcn4 is in restoring amino acid levels.

433

434

### 435 **Discussion**

436

437 A central theme emphasized in this study is mechanisms by which cells manage resource allocation,  
438 supply and demand during cell growth. Recent studies in model organisms like yeast and *E. coli* focus  
439 on protein translation, and the need to ‘buffer’ translation capacity during cell growth [8,59]. These  
440 studies alter our perception of how translation is regulated during high cell growth. However, the  
441 process of cell growth requires not just translation reserves (in the form of ribosomes), but also a  
442 constant supply of biosynthetic precursors to meet high demand. This includes: amino acids to  
443 sustain translation as well as drive metabolic functions, nucleotide synthesis (for DNA replication,  
444 transcription and ribosome biogenesis), and sufficient reductive capacity (for reductive  
445 biosynthesis). Even the production of ribosomes is an extremely resource-intensive process [60].  
446 While our understanding of translation-regulation in these contexts is constantly improving, how  
447 metabolic and biosynthetic components are managed, and couple with translation, remain poorly  
448 understood.

449

450 Here, using yeast as a model, we obtain striking, mechanistic insight into how Gcn4 enables cells to  
451 sustain the supply of biosynthetic precursors, during a growth program (induced by methionine). In  
452 this growth program, methionine induces genes involved in ribosomal biogenesis and translation  
453 [20], which is the hallmark of a growth signature [27,60–63]. In addition, cells increase metabolic  
454 processes that sustain anabolism; primarily the pentose phosphate pathway, trans-amination  
455 reactions, an induction in amino acid biosynthesis, and nucleotide synthesis [20]. In particular,  
456 through this study, we now show how the Gcn4 transcription factor functions to critically support  
457 this growth program, by enabling high amino acid supply to maintain a sufficient translation  
458 capacity, as illustrated in a schematic model (Figure 5E).

459

460 Notably, we can now define the roles of Gcn4 during either a growth program, or a more  
461 conventionally studied starvation program. In a growth program (the methionine-induced context),  
462 genes required for ribosome biogenesis and translation that are induced have a nuanced regulation  
463 by Gcn4. Gcn4 represses ribosomal genes (consistent with earlier reports), and in this context  
464 thereby appears to balance or moderate the overall induction of ribosomal genes by methionine.  
465 The methionine-dependent induction of ribosomal genes is likely controlled by directly activating the  
466 TOR pathway [18,64–66], which is a regulator of ribosomal biogenesis. However, despite the high  
467 expression of ribosomal gene mRNAs in  $\Delta gcn4$  cells (in this growth program), and the indication of  
468 an apparent ‘growth signature’ transcriptional profile with high ribosomal transcripts, cells cannot  
469 sustain the required rates of protein synthesis, or maintain the high translation capacity required for  
470 growth. This is because the translation machinery itself is highly enriched for arginine and lysine  
471 amino acids, and so cannot be maintained at sufficient levels without a constant supply of lysine and  
472 arginine. In the presence of methionine, the increased synthesis (and therefore supply) of these two  
473 amino acids depends almost entirely on induced Gcn4. After all, to sustain high growth, and anabolic  
474 programs, cells need to maintain the required high rates of translation, and ribosomal capacity.

475 Thus, through Gcn4, cells can deeply couple translation with metabolism, and manage sufficient  
476 resource allocations to sustain increased anabolism.

477

478 Notably, the specific transcriptional role of Gcn4 in growth or starvation programs remains  
479 conserved. Regardless of context, Gcn4 is required for amino acid biosynthesis (particularly lysine  
480 and arginine biosynthesis), and represses ribosomal genes. However, the different contexts  
481 completely alter the cellular outcomes, since in growth programs ribosomal genes are already high  
482 (and Gcn4 only tempers their expression), while in starvation programs ribosomal genes are low. The  
483 roles of Gcn4 have traditionally only been systematically examined during amino acid starvation or  
484 an ‘integrated stress response’ [29,67]. However, multiple studies now support a role for Gcn4  
485 during contexts of high growth, including recent studies of the mammalian ortholog of Gcn4 (ATF4)  
486 which report high ATF4 activity in several cancers [36,37,68,69]. These studies suggest that ATF4  
487 induction is critical for tumor progression during nutrient limitation, possibly by providing otherwise  
488 limiting metabolites [36,37], without clarity on the specific functions of ATF4 in these conditions.  
489 Separately, observations over decades note that many rapidly proliferating tumors depend on  
490 methionine [70,71], and methionine restriction critically determines tumor progression [23,24]. Here  
491 we directly demonstrate how Gcn4 provides biosynthetic precursor supply to sustain anabolism, in  
492 an otherwise limiting environment. Speculatively, could the ability of methionine to induce  
493 proliferation in cancers rest upon the induction of ATF4, which controls the supply of amino acids  
494 and other biosynthetic precursors?

495

496 Summarizing, here we address Gcn4 function during a growth program triggered by methionine. This  
497 expands the roles of a ‘starvation’ factor, during a contrasting, high anabolism state, showing how  
498 despite conserved function in both contexts, Gcn4 activity can lead to very different outcomes. Our  
499 study provides an illustrative perspective of how cells can manage the supply of important  
500 biosynthetic precursors with overall translation outputs, when a specific growth cues induce high  
501 biosynthetic demands that need to be coordinately sustained, in order to maintain anabolism and  
502 cell growth.

503

504

## 505 **Materials and Methods**

506

### 507 **Strains and growth conditions**

508

509 A fully prototrophic yeast strain *Saccharomyces cerevisiae* strain from a CEN.PK background [72] was  
510 used in all experiments. For all the medium-shift experiments, overnight grown cultures were sub-  
511 cultured in fresh YPD (1% Yeast extract and 2% Peptone, 2% Glucose) medium with an initial OD<sub>600</sub> of  
512 ~0.2. Once the OD<sub>600</sub> of the secondary culture reached 0.8 – 0.9, cells were pelleted down and  
513 washed and shifted to minimal media MM (MM- Yeast Nitrogen Base with glucose as a carbon  
514 source) and MM+Met (MM with 2mM methionine). For the luciferase assay (described later),  
515 overnight grown cultures were prepared by growing the cells in YPD with the antibiotic 1mM  
516 Nourseothricin (NAT). The secondary culture was started with an initial OD<sub>600</sub> of ~0.5 in YPD + NAT  
517 and incubated at 30°C and 250 rpm for 4 hours. After 4 hours of incubation, cells were washed once  
518 in MM, and shifted to MM +Met or MM+Met+arg+lys. 2mM concentration of each amino acid was  
519 used wherever required, unless mentioned otherwise. All the wash steps before shifting to minimal  
520 medium were done by centrifuging the cells at 3500 rpm for 90 seconds.

521

### 522 **Western blot analysis**

523

524 Yeast cells with a 3x-FLAG epitope sequence chromosomally tagged at the carboxy-terminus of Gcn4  
525 (endogenous locus) were used to quantify Gcn4 protein levels using western blotting

526 (Supplementary table 1). Overnight grown cells were sub-cultured into fresh YPD medium, with an  
527 initial OD of 0.2 and grown to an OD<sub>600</sub> of 0.8. Cells were pelleted down at 3500 rpm for 1.5 minutes,  
528 cell pellets were washed once in MM, re-harvested and shifted to MM and MM+ Met after 1 hour of  
529 the shift. ~5 OD<sub>600</sub> of cells were harvested by centrifugation, and proteins were precipitated in 400  
530 µl of 10% trichloro acetic acid (TCA), and extracted by bead beating with glass beads. Lysates were  
531 centrifuged to precipitate all proteins, and total protein pellets were resuspended in 400 µl of SDS-  
532 Glycerol sample buffer. Protein concentrations were quantified using Bicinchoninic assay kit (G-  
533 Biosciences, 786-570) and equal concentrations of proteins were loaded into the 4-12% Bis-tris  
534 polyacrylamide gel (Thermo Fisher, NP0322BOX) and resolved using electrophoresis. Resolved  
535 proteins were transferred to nitrocellulose membranes and detected by standard Western blotting  
536 using monoclonal anti-FLAG M2- mouse primary antibody (Sigma Aldrich, F1804) and HRP labelled  
537 anti-mouse IgG secondary antibody (Cell Signalling technology, 7076S). Blots were developed using  
538 enhanced chemiluminescence reagents (Advansta, K-12045) imaged using Image quant. A different  
539 part of each gel (cut out) was Coomassie stained in order to compare total protein loading amounts.  
540

#### 541 mRNA sequencing and data analysis

542  
543 Overnight grown cells of WT and *Δgcn4* strains were sub-cultured in YPD, with a starting OD<sub>600</sub> of 0.2  
544 and grown till they reached an OD<sub>600</sub> of 0.8-0.9. YPD grown cells were pelleted down at 3500 rpm for  
545 90 seconds and washed once with MM. Washed cells were shifted to MM and MM+Met, and cells  
546 remained in this fresh medium for ~1 hr. The cells were collected an hour after this shift and RNA  
547 was isolated by a hot acid phenol method as described [73]. mRNA libraries were prepared using  
548 TruSeq RNA library preparation kit V2 (Illumina) and quality of the libraries were analyzed using  
549 bioanalyser (Agilent 2100) and libraries were sequenced for 51 cycles using Illumina HiSeq 2500  
550 platform. For every experimental condition, data were obtained from two biological replicates.  
551 Normalized read counts between the biological replicates were well correlated (Figure S1). For each  
552 strain we obtained ~30-35 million uniquely mapped reads. The raw data are available in NCBI-SRA  
553 under the accession PRJNA599001. The transcriptome data were aligned and mapped to the *S.*  
554 *cerevisiae* S288C genome downloaded from the saccharomyces genome database (SGD), using the  
555 Burrows-Wheeler Aligner [74] and the mapped reads with mapping quality of ≥ 20 were used for  
556 further analysis. Number of reads mapped to each gene was calculated and read count matrix was  
557 generated. The EdgeR package was used for normalization and differential gene expression analysis  
558 [75]. Differentially expressed genes with a fold change above 1.5 or 2 fold, with a stringent p-value  
559 cutoff of ≤ 0.0001 were considered for further analysis. Normalized read counts was calculated for  
560 every sample as described earlier [20]. Normalized read counts between the replicates are well  
561 correlated with the Pearson correlation coefficient (R) is more than 0.9 (Figure S1). GO analysis of  
562 the differentially expressed genes were carried out using g:Profiler [76].  
563

#### 564 Chromatin Immunoprecipitation sequencing and data analysis

##### 565 a. Cell growth conditions and sample collection

566 For CHIP sequencing, overnight grown cells were re-inoculated in fresh YPD medium (RM), with the  
567 initial OD<sub>600</sub> of 0.2 and incubated at 30°C until the OD<sub>600</sub> reached 0.8-0.9. Subsequently, 100 mL of  
568 culture was pelleted down, washed and shifted to MM and MM +Met. After 1 hour of the shift, cells  
569 were fixed using 1% formaldehyde, after which the fixing was quenched with 2.5M glycine.

##### 570 b. Spheroplasting of fixed cells

571 Fixed cells were treated with 2-mercapto ethanol, and resuspended in 5 ml of spheroplasting buffer  
572 containing 1M sorbitol, 0.1M sodium citrate, 10mM EDTA, and distilled water, with 4mg/ml of lysing  
573 enzyme from *Trichoderma harzianum* (Sigma L1412-5G). This suspension was incubated at 37°C for 5  
574 hours.

##### 575 c. Lysis and ChIP

576 Spheroplasts were pelleted down at 1000 rpm, washed twice with Buffer 1 (0.25% Triton  
577 X100,10mM EDTA,0.5mM EGTA, 10mM sodium HEPES pH 6.5) and twice with Buffer 2 (200mM NaCl,  
578 1mM EDTA, 0.5mM EGTA, 10mM Sodium HEPES pH 6.5), washed spheroplasts were resuspended in  
579 lysis buffer (50mM sodium HEPES pH 7.4, 1% Triton X, 140mM NaCl,0.1% Sodium deoxy  
580 cholate,10mM EDTA) and lysis and DNA fragmentation were carried out using a bioruptor  
581 (Diagenode, Nextgen) for 30 cycles (30 sec on and off cycles). Lysates were centrifuged to remove  
582 the debris and clear supernatant was used for chromatin immunoprecipitation (ChIP).  
583 Immunoprecipitation was carried out by incubating the lysate with the monoclonal anti-FLAG M2-  
584 mouse primary antibody (Sigma Aldrich, F1804) and protein G Dynabead (Invitrogen, 10004D).  
585 Beads were washed sequentially in low salt, high salt and LiCl buffers, TE buffer and protein-DNA  
586 complex were eluted using elution buffer as reported earlier [77]. Decrosslinking of the immuno-  
587 precipitated proteins were carried out by using a high concentration of NaCl and incubation at 65°C  
588 for 5 hours followed by proteinase-K treatment and DNA purification. Mock samples were also  
589 prepared in parallel, except the antibody treatment. Libraries were prepared for the purified IP DNA  
590 and mock samples (NEBNext Ultra II DNA library preparation kit, Catalog no- E7103L) and sequenced  
591 using Illumina platform HiSeq 2500. Two biological replicates were maintained for all the samples.  
592 The raw data are available in NCBI-SRA under the accession ID PRJNA599001.

593 ChIP sequencing reads were mapped to the *S. cerevisiae* S288C genome downloaded from  
594 SGD. The reads with mapping quality < 20 were discarded, and the remaining reads were used for  
595 further analysis. The number of reads mapped to every 100bp non-overlapping bins were calculated  
596 using 'exomedepth' function of R-package GenomicRanges [78]. Read counts were normalized by  
597 dividing the number of reads falling within each bin by the total number of reads fall within the  
598 range of  $\mu \pm x$ , where,  $\mu$ =mode of the distribution of read counts of each bin,  $x$  = median absolute  
599 deviation of all the bins that has a number of reads that are less than the mean of the distribution.  
600 Subsequently, the regions that have normalized read counts of above 2 were considered for further  
601 analysis. The binding regions which are separated by < 200bp were merged to give a single peak. The  
602 peaks which are conserved in both the replicates with the overlap of at least 50bp were considered  
603 as *bona fide* binding regions of Gcn4. Genes which are encoded around 750 bp on both sides of the  
604 peaks were listed in the Supplementary WS4.

605

#### 606 **Peak feature annotation and motif analysis**

607 Genomic features of the peaks were identified using the annotatePeak.pl function of the HOMER  
608 tool [48]. For motif analysis, nucleotide sequences corresponding to the peak intervals were  
609 extracted from the genome and motif identification was performed using 'meme' function of MEME-  
610 suite [49].

611

#### 612 **Direct and Indirect target analysis**

613 To annotate the genes corresponding to the peaks identified, the open reading frames that are  
614 encoded within 750 bp on both sides of the peak regions were considered as 'possible Gcn4 binding  
615 targets'. Gene sets which are differentially expressed in  $\Delta gcn4$  relative to WT under MM+Met  
616 condition, with a fold change of > 2 (~900 genes) were termed 'Gcn4 regulatory targets'. While  
617 comparing these gene lists, the genes which intersect between these two gene sets are considered  
618 as 'direct Gcn4 binding targets' and the rest of the genes of 'Gcn4 regulatory targets' are 'indirect  
619 targets of Gcn4'.

620

#### 621 **Metabolic flux analysis using LC/MS/MS**

622 To determine if the rates of biosynthesis of amino acids are altered in MM+Met and Gcn4  
623 dependent manner, we measured <sup>15</sup>N-label incorporation in amino acids. We used <sup>15</sup>N-ammonium  
624 sulfate with all nitrogen atoms labelled. Cells grown in YPD were shifted to fresh minimal medium

625 (with the appropriate carbon source as indicated), containing 0.5 X of unlabelled ammonium sulfate  
626 (0.25%) and MM+Met containing 0.5X of unlabelled ammonium sulfate (0.25%). After 1 hour of shift  
627 to minimal media, cells were pulsed with 0.25 % of <sup>15</sup>N labelled ammonium sulfate and incubated for  
628 5 minutes or 15 minutes as indicated. After the <sup>15</sup>N pulse, metabolites were extracted and label  
629 incorporation into amino acids was analyzed using targeted LC/MS/MS protocols as described earlier  
630 [20,56]. Similarly, C<sup>13</sup> labeled glucose was used to measure rate of biosynthesis of nucleotide.  
631

### 632 **Luciferase based translation reporters for lysine and arginine enriched genes**

633 To measure the translation of specific transcripts that encode arginine and lysine enriched proteins,  
634 the ORF of the following proteins, RPL32, NHP2, STM1, RPS20 were amplified from the genomic DNA  
635 isolated from WT CEN.PK strain of *S. cerevisiae*. The amplified ORFs (without the stop codon) were  
636 ligated to the luciferase cDNA amplified from pGL3 (Supplementary table 2). The resulting fragment  
637 with '*ORF<sub>RKrich genes</sub> + luciferase*' were cloned in a centromeric (CEN.ARS) plasmid pSL207, a modified  
638 version of the plasmid used in the earlier study [79]. Luciferase expression in this construct is under  
639 the control of inducible promoter, which can be induced by  $\beta$ -estradiol [79]. The resulting plasmids  
640 with the following genes RPL32, NHP2, STM1, RPS20 cloned in frame with luciferase and under the  
641 inducible GEV promoter were named pSL218, pSL221, pSL224, pSL234 respectively (Supplementary  
642 Figure 9 and Supplementary table 2). SL217 is a plasmid where only the luciferase cDNA amplified  
643 from pGL3 plasmid was cloned under the inducible promoter, serves as a control. All these plasmids  
644 generated have ampicillin selection (for amplification) and Nourseothricin resistant cassettes (NAT<sup>r</sup>)  
645 for selection in yeast. The generated plasmids were transformed to the WT and  $\Delta gcn4$  strains. To  
646 measure the translation of the genes cloned upstream of luciferase, these strains having plasmid  
647 were grown in YPD for overnight with an antibiotic NAT. Overnight grown cultures were shifted to  
648 fresh YPD+NAT with an initial OD<sub>600</sub> of 0.4, and grown for 4 hours at 30°C. After 4 hrs of incubation in  
649 YPD, cells were washed and shifted to the MM and MM+Met. 75mM of 3-Amino triazole (3-AT)  
650 was used, wherever required. After 1 hour of the shift, cultures were split into two equal parts,  
651 one part of the culture was induced with 200nM  $\beta$ -estradiol (Sigma Aldrich-E8875) and the other half  
652 was left as a mock-induced control. After 30 minutes of induction cells were harvested by  
653 centrifugation at 4°C at 3000 rpm and washed with lysis buffer (1X-PBS containing 1mM PMSF), after  
654 3 washes, were resuspended in 200 $\mu$ l of lysis buffer. Lysed the cells by bead beating at 4° C (1min  
655 ON and 1 min OFF for 10min). After lysis, the protein concentrations were measured by BCA assay  
656 kit. Equal concentrations of protein were used for measuring the luciferase activity. Luciferase  
657 activity was measured using luciferase assay kit (Promega, E1500) and the activity was measured  
658 using a luminometer (Sirius, Titertek Berthold Detection systems). Luciferase activity (measured as  
659 Relative Light Units per Sec (RLU/sec)) were normalized with its respective uninduced control. Similar  
660 experiments were carried out under different media conditions supplemented with different amino  
661 acids, where the conditions are mentioned in the respective sections. The relative difference in  
662 luciferase activities between the strain types and media conditions were used to estimate changes in  
663 the active translation of these proteins.

664

### 665 **Statistical tests**

666 R-Packages and Graph pad prism 7 were used for visualizing data and performing statistical tests.  
667 The respective statistical tests used, and the statistical significance was mentioned wherever  
668 required.

669

### 670 **Acknowledgements**

671 We acknowledge extensive use of the NCBS/inStem/CCAMP next generation sequencing facilities,  
672 and the NCBS/inStem/CCAMP mass spectrometry facilities. RS acknowledges support from SERB  
673 National Postdoctoral Fellowship (PDF/2016/001877), DST, Govt. of India. SL acknowledges support

674 from a DBT-Wellcome trust India Alliance Intermediate Fellowship (IA/I/14/2/501523), and  
675 intramural support for this study. AS acknowledges support from a DBT-Wellcome trust India  
676 Alliance Intermediate Fellowship (IA/I/16/2/502711).

677

678 **Author contributions**

679 RS and SL conceived the study, RS, ASW, ASN and SL designed experiments, RS and AW performed  
680 experiments, RS, ASW, AS and SL analyzed data, RS, AS and SL wrote the manuscript.

681

682

683 **References**

- 684 1. Gresham D, Boer VM, Caudy A, Ziv N, Brandt NJ, Storey JD, et al. System-level analysis of  
685 genes and functions affecting survival during nutrient starvation in *Saccharomyces cerevisiae*.  
686 *Genetics*. 2011; doi:10.1534/genetics.110.120766
- 687 2. Boer VMVM, Crutchfield CACA, Bradley PHPH, Botstein D, Rabinowitz JDJD. Growth-limiting  
688 Intracellular Metabolites in Yeast Growing under Diverse Nutrient Limitations. *Mol Biol Cell*.  
689 2010;21: 198–211. doi:10.1091/mbc.E09-07-0597
- 690 3. Saldanha A, Brauer M, Botstein D. Nutritional Homeostasis in Batch and Steady-State Culture  
691 of Yeast. *Mol Biol Cell*. 2004;15: 4089–104. doi:10.1091/mbc.E04-04-0306
- 692 4. Dikicioglu D, Karabekmez E, Rash B, Pir P, Kirdar B, Oliver SG. How yeast re-programmes its  
693 transcriptional profile in response to different nutrient impulses. *BMC Syst Biol*. *BioMed*  
694 *Central*; 2011;5: 148. doi:10.1186/1752-0509-5-148
- 695 5. Gutteridge A, Pir P, Castrillo JI, Charles PD, Lilley KS, Oliver SG. Nutrient control of eukaryote  
696 cell growth: a systems biology study in yeast. *BMC Biol*. *BioMed Central*; 2010;8: 68.  
697 doi:10.1186/1741-7007-8-68
- 698 6. Brauer MJ, Yuan J, Bennett BD, Lu W, Kimball E, Botstein D, et al. Conservation of the  
699 metabolomic response to starvation across two divergent microbes. *Proc Natl Acad Sci*.  
700 2006;103: 19302–19307.
- 701 7. Gurvich Y, Leshkowitz D, Barkai N. Dual role of starvation signaling in promoting growth and  
702 recovery. *PLoS Biol*. 2017;15: 1–28. doi:10.1371/journal.pbio.2002039
- 703 8. Metzl-Raz E, Kafri M, Yaakov G, Soifer I, Gurvich Y, Barkai N. Principles of cellular resource  
704 allocation revealed by condition-dependent proteome profiling. *Elife*. *eLife Sciences*  
705 *Publications, Ltd*; 2017;6: e28034. doi:10.7554/eLife.28034
- 706 9. Zaman S, Lippman SI, Zhao X, Broach JR. How *Saccharomyces* Responds to Nutrients. *Annu*  
707 *Rev Genet*. 2008;42: 27–81. doi:10.1146/annurev.genet.41.110306.130206
- 708 10. Cai L, Sutter BM, Li B, Tu BP. Acetyl-CoA induces cell growth and proliferation by promoting  
709 the acetylation of histones at growth genes. *Mol Cell*. *Elsevier Inc.*; 2011;42: 426–37.  
710 doi:10.1016/j.molcel.2011.05.004
- 711 11. Cai L, Tu BP. On acetyl-CoA as a gauge of cellular metabolic state. *Cold Spring Harb Perspect*  
712 *Biol*. 2011;76: 195–202.
- 713 12. Kuang Z, Cai L, Zhang X, Ji H, Tu BP, Boeke JD. High-temporal-resolution view of transcription  
714 and chromatin states across distinct metabolic states in budding yeast. *Nat Struct Mol Biol*.  
715 2014/08/31. 2014;21: 854–863. doi:10.1038/nsmb.2881
- 716 13. Shi L, Tu BPBP. Acetyl-CoA induces transcription of the key G1 cyclin CLN3 to promote entry  
717 into the cell division cycle in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci*. 2013;110: 7318–  
718 7323. doi:10.1073/pnas.1302490110
- 719 14. Krishna S, Laxman S. A minimal “push – pull” bistability model explains oscillations between  
720 quiescent and proliferative cell states. *Lew DJ, editor. Mol Biol Cell*. 2018;29: 2243–2258.  
721 doi:10.1091/mbc.E18-01-0017
- 722 15. Wellen KE, Thompson CB. Cellular metabolic stress: considering how cells respond to nutrient  
723 excess. *Mol Cell*. *Elsevier Inc.*; 2010;40: 323–32. doi:10.1016/j.molcel.2010.10.004
- 724 16. Rowicka M, Kudlicki A, Tu BP, Otwinowski Z. High-resolution timing of cell cycle-regulated  
725 gene expression. *Proc Natl Acad Sci U S A*. 2007;104: 16892–7. doi:10.1073/pnas.0706022104
- 726 17. Pedro MB, Madeo F, Pietrocola F, Galluzzi L, Bravo-San Pedro JM, Madeo F, et al. Review  
727 Acetyl Coenzyme A: A Central Metabolite and Second Messenger. *Cell Metab*. *United States*;



- 728 2015;21: 805–821. doi:10.1016/j.cmet.2015.05.014
- 729 18. Sutter BM, Wu X, Laxman S, Tu BP. Methionine inhibits autophagy and promotes growth by  
730 inducing the SAM-responsive methylation of PP2A. *Cell*. 2013;154: 403–15.  
731 doi:10.1016/j.cell.2013.06.041
- 732 19. Lees EK, Banks R, Cook C, Hill S, Morrice N, Grant L, et al. Direct comparison of methionine  
733 restriction with leucine restriction on the metabolic health of C57BL/6J mice. *Sci Rep. Nature*  
734 *Publishing Group UK*; 2017;7: 9977. doi:10.1038/s41598-017-10381-3
- 735 20. Walvekar AS, Srinivasan R, Gupta R, Laxman S. Methionine coordinates a hierarchically  
736 organized anabolic program enabling proliferation. *Mol Biol Cell. American Society for Cell*  
737 *Biology (mboc)*; 2018;29: 3183–3200. doi:10.1091/mbc.E18-08-0515
- 738 21. Halpern BC, Clark BR, Hardy DN, Halpern RM, Smith RA. The effect of replacement of  
739 methionine by homocystine on survival of malignant and normal adult mammalian cells in  
740 culture. *Proc Natl Acad Sci U S A*. 1974;71: 1133–6. doi:10.1073/pnas.71.4.1133
- 741 22. Sugimura T, Birnbaum SM, Winitz M, Greenstein JP. Quantitative nutritional studies with  
742 water-soluble, chemically defined diets. VIII. The forced feeding of diets each lacking in one  
743 essential amino acid. *Arch Biochem Biophys*. 1959;81: 448–455. doi:10.1016/0003-  
744 9861(59)90225-5
- 745 23. Gao X, Sanderson SM, Dai Z, Reid MA, Cooper DE, Lu M, et al. Dietary methionine restriction  
746 targets one carbon metabolism in humans and produces broad therapeutic responses in  
747 cancer. *bioRxiv*. 2019; 627364. doi:10.1101/627364
- 748 24. Gao X, Sanderson SM, Dai Z, Reid MA, Cooper DE, Lu M, et al. Dietary methionine influences  
749 therapy in mouse cancer models and alters human metabolism. *Nature*. 2019/07/31.  
750 *England*; 2019;572: 397–401. doi:10.1038/s41586-019-1437-3
- 751 25. Wu X, Tu BP. Selective regulation of autophagy by the Iml1-Npr2-Npr3 complex in the  
752 absence of nitrogen starvation. *Mol Biol Cell*. 2011;22: 4124–4133. doi:10.1091/mbc.E11-06-  
753 0525
- 754 26. Laxman S, Sutter B, Tu BP. Methionine is a signal of amino acid sufficiency that inhibits  
755 autophagy through the methylation of PP2A. *Autophagy*. 2014;10: 386–387.  
756 doi:10.4161/auto.27485
- 757 27. Brauer MJMJ, Huttenhower C, Airoidi EMEM, Rosenstein R, Matese JCJC, Gresham D, et al.  
758 Coordination of Growth Rate, Cell Cycle, Stress Response, and Metabolic Activity in Yeast.  
759 *Mol Biol Cell*. 2008;19: 352–267. doi:10.1091/mbc.E07-08-0779
- 760 28. Hinnebusch AG. Translational Regulation Of GCN4 and the General Amino Acid Control of  
761 yeast. *Annu Rev Microbiol*. 2005;59: 407–50. doi:10.1146/annurev.micro.59.031805.133833
- 762 29. Mascarenhas C, Edwards-Ingram LC, Zeef L, Shenton D, Ashe MP, Grant CM. Gcn4 is required  
763 for the response to peroxide stress in the yeast *Saccharomyces cerevisiae*. *Mol Biol Cell*.  
764 2008/04/16. *The American Society for Cell Biology*; 2008;19: 2995–3007.  
765 doi:10.1091/mbc.e07-11-1173
- 766 30. Yang R, Wek SA, Wek RC. Glucose limitation induces GCN4 translation by activation of Gcn2  
767 protein kinase. *Mol Cell Biol. American Society for Microbiology*; 2000;20: 2706–2717.  
768 doi:10.1128/mcb.20.8.2706-2717.2000
- 769 31. Natarajan K, Meyer MR, Jackson BM, Slade D, Roberts C, Hinnebusch AG, et al.  
770 Transcriptional profiling shows that Gcn4p is a master regulator of gene expression during  
771 amino acid starvation in yeast. *Mol Cell Biol*. 2001;21: 4347–68.  
772 doi:10.1128/MCB.21.13.4347-4368.2001
- 773 32. Hinnebusch AG, Asano K, Olsen DS, Phan L, Nielsen KH, Valásek L. Study of translational

- 774 control of eukaryotic gene expression using yeast. *Ann N Y Acad Sci. United States*;  
775 2004;1038: 60–74. doi:10.1196/annals.1315.012
- 776 33. Hinnebusch AG. Translational control of GCN4: an in vivo barometer of initiation-factor  
777 activity. *Trends Biochem Sci. England*; 1994;19: 409–414. doi:10.1016/0968-0004(94)90089-2
- 778 34. Rawal Y, Chereji R V, Valabhoju V, Qiu H, Ocampo J, Clark DJ, et al. Gcn4 Binding in Coding  
779 Regions Can Activate Internal and Canonical 5' Promoters in Yeast. *Mol Cell. 2018/04/05.*  
780 2018;70: 297–311.e4. doi:10.1016/j.molcel.2018.03.007
- 781 35. Mittal N, Guimaraes JC, Gross T, Schmidt A, Vina-Vilaseca A, Nedialkova DD, et al. The Gcn4  
782 transcription factor reduces protein synthesis capacity and extends yeast lifespan. *Nat*  
783 *Commun. Nature Publishing Group UK*; 2017;8: 457. doi:10.1038/s41467-017-00539-y
- 784 36. Ye J, Kumanova M, Hart LS, Sloane K, Zhang H, De Panis DN, et al. The GCN2-ATF4 pathway is  
785 critical for tumour cell survival and proliferation in response to nutrient deprivation. *EMBO J.*  
786 2010;29: 2082–96. doi:10.1038/emboj.2010.81
- 787 37. Tameire F, Verginadis II, Leli NM, Polte C, Conn CS, Ojha R, et al. ATF4 couples MYC-  
788 dependent translational activity to bioenergetic demands during tumour progression. *Nat*  
789 *Cell Biol. 2019/07/01. Springer US*; 2019;21: 889–899. doi:10.1038/s41556-019-0347-9
- 790 38. Xiao L, Grove A. Coordination of Ribosomal Protein and Ribosomal RNA Gene Expression in  
791 Response to TOR Signaling. *Curr Genomics. Bentham Science Publishers Ltd*; 2009;10: 198–  
792 205. doi:10.2174/138920209788185261
- 793 39. Airoidi EM, Huttenhower C, Gresham D, Lu C, Caudy AA, Dunham MJ, et al. Predicting cellular  
794 growth from gene expression signatures. *PLoS Comput Biol. 2009/01/02. Public Library of*  
795 *Science*; 2009;5: e1000257–e1000257. doi:10.1371/journal.pcbi.1000257
- 796 40. Hinnebusch AG, Natarajan K. Gcn4p, a master regulator of gene expression, is controlled at  
797 multiple levels by diverse signals of starvation and stress. *Eukaryot Cell. American Society for*  
798 *Microbiology*; 2002;1: 22–32. doi:10.1128/ec.01.1.22-32.2002
- 799 41. Pakos-Zebrucka K, Koryga I, Mnich K, Ljujic M, Samali A, Gorman AM. The integrated stress  
800 response. *EMBO Rep. 2016/09/14. John Wiley and Sons Inc.*; 2016;17: 1374–1395.  
801 doi:10.15252/embr.201642195
- 802 42. Akhter A, Rosonina E. Chromatin Association of Gcn4 Is Limited by Post-translational  
803 Modifications Triggered by its DNA-Binding in *Saccharomyces cerevisiae*. *Genetics.*  
804 2016/10/21. *Genetics Society of America*; 2016;204: 1433–1445.  
805 doi:10.1534/genetics.116.194134
- 806 43. Albrecht G, Mösch HU, Hoffmann B, Reusser U, Braus GH. Monitoring the Gcn4 protein-  
807 mediated response in the yeast *Saccharomyces cerevisiae*. *J Biol Chem. United States*;  
808 1998;273: 12696–12702. doi:10.1074/jbc.273.21.12696
- 809 44. Joo YJ, Kim J, Kang U, Yu M, Kim J. Gcn4p-mediated transcriptional repression of ribosomal  
810 protein genes under amino-acid starvation. *EMBO J. Nature Publishing Group*; 2010;30: 859–  
811 872. doi:10.1038/emboj.2010.332
- 812 45. Bose T, Lee KK, Lu S, Xu B, Harris B, Slaughter B, et al. Cohesin proteins promote ribosomal  
813 RNA production and protein translation in yeast and human cells. *PLoS Genet. 2012/06/14.*  
814 *Public Library of Science*; 2012;8: e1002749–e1002749. doi:10.1371/journal.pgen.1002749
- 815 46. Saint M, Sawhney S, Sinha I, Singh RP, Dahiya R, Thakur A, et al. The TAF9 C-terminal  
816 conserved region domain is required for SAGA and TFIID promoter occupancy to promote  
817 transcriptional activation. *Mol Cell Biol. 2014/02/18. American Society for Microbiology*;  
818 2014;34: 1547–1563. doi:10.1128/MCB.01060-13
- 819 47. McMillan J, Lu Z, Rodriguez JS, Ahn T-H, Lin Z. YeastTSS: an integrative web database of yeast

- 820 transcription start sites. Database (Oxford). Oxford University Press; 2019;2019: baz048.  
821 doi:10.1093/database/baz048
- 822 48. Heinz S, Benner C, Spann N, Bertolino E, Lin YC, Laslo P, et al. Simple combinations of lineage-  
823 determining transcription factors prime cis-regulatory elements required for macrophage and  
824 B cell identities. *Mol Cell*. 2010;38: 576–589. doi:10.1016/j.molcel.2010.05.004
- 825 49. Bailey TL, Boden M, Buske FA, Frith M, Grant CE, Clementi L, et al. MEME SUITE: tools for  
826 motif discovery and searching. *Nucleic Acids Res*. 2009/05/20. Oxford University Press;  
827 2009;37: W202–W208. doi:10.1093/nar/gkp335
- 828 50. Arndt K, Fink GR. GCN4 protein, a positive transcription factor in yeast, binds general control  
829 promoters at all 5' TGACTC 3' sequences. *Proc Natl Acad Sci U S A*. 1986;83: 8516–8520.  
830 doi:10.1073/pnas.83.22.8516
- 831 51. Oakley MG, Dervan PB. Structural motif of the GCN4 DNA binding domain characterized by  
832 affinity cleaving. *Science*. United States; 1990;248: 847–850. doi:10.1126/science.2111578
- 833 52. Holland P, Bergenholm D, Börlin CS, Liu G, Nielsen J. Predictive models of eukaryotic  
834 transcriptional regulation reveals changes in transcription factor roles and promoter usage  
835 between metabolic conditions. *Nucleic Acids Res*. Oxford University Press; 2019;47: 4986–  
836 5000. doi:10.1093/nar/gkz253
- 837 53. Aow JSZ, Xue X, Run J-Q, Lim GFS, Goh WS, Clarke ND. Differential binding of the related  
838 transcription factors Pho4 and Cbf1 can tune the sensitivity of promoters to different levels of  
839 an induction signal. *Nucleic Acids Res*. 2013/04/04. Oxford University Press; 2013;41: 4877–  
840 4887. doi:10.1093/nar/gkt210
- 841 54. Kribelbauer JF, Rastogi C, Bussemaker HJ, Mann RS. Low-Affinity Binding Sites and the  
842 Transcription Factor Specificity Paradox in Eukaryotes. *Annu Rev Cell Dev Biol*. 2019/07/05.  
843 2019;35: 357–379. doi:10.1146/annurev-cellbio-100617-062719
- 844 55. Nishizawa M, Komai T, Morohashi N, Shimizu M, Toh-e A. Transcriptional repression by the  
845 Pho4 transcription factor controls the timing of SNZ1 expression. *Eukaryot Cell*. 2008/04/11.  
846 American Society for Microbiology (ASM); 2008;7: 949–957. doi:10.1128/EC.00366-07
- 847 56. Walvekar A, Rashida Z, Maddali H, Laxman S. A versatile LC-MS/MS approach for  
848 comprehensive, quantitative analysis of central metabolic pathways. *Wellcome open Res*.  
849 F1000 Research Limited; 2018;3: 122. doi:10.12688/wellcomeopenres.14832.1
- 850 57. Hofmeyr JHS, Cornish-Bowden A. Regulating the cellular economy of supply and demand.  
851 *FEBS Lett*. 2000;476: 47–51. doi:10.1016/S0014-5793(00)01668-9
- 852 58. Hofmeyr J-HSHS. The harmony of the cell: the regulatory design of cellular processes.  
853 Wolkenhauer O, Wellstead P, Cho K-H, editors. *Essays Biochem*. 2008;45: 57–66.  
854 doi:10.1042/bse0450057
- 855 59. Hui S, Silverman JM, Chen SS, Erickson DW, Basan M, Wang J, et al. Quantitative proteomic  
856 analysis reveals a simple strategy of global resource allocation in bacteria. *Mol Syst Biol*.  
857 BlackWell Publishing Ltd; 2015;11: 784. doi:10.15252/msb.20145697
- 858 60. Warner JR, Vilardell J, Sohn JH. Economics of ribosome biosynthesis. *Cold Spring Harb Symp*  
859 *Quant Biol*. 2001;66: 567–74. doi:10.1101/sqb.2001.66.567
- 860 61. Bosdriesz E, Molenaar D, Teusink B, Bruggeman FJ. How fast-growing bacteria robustly tune  
861 their ribosome concentration to approximate growth-rate maximization. *FEBS J*. 2015/03/26.  
862 John Wiley & Sons, Ltd; 2015;282: 2029–2044. doi:10.1111/febs.13258
- 863 62. Klumpp S, Scott M, Pedersen S, Hwa T. Molecular crowding limits translation and cell growth.  
864 *Proc Natl Acad Sci U S A*. 2013/09/30. National Academy of Sciences; 2013;110: 16754–  
865 16759. doi:10.1073/pnas.1310377110

- 866 63. Tu BP, Tu BP, Kudlicki A, Rowicka M, Mcknight SL. Logic of the Yeast Metabolic Cycle of  
867 Cellular Processes. *Science*. 2005; doi:10.1126/science.1120499
- 868 64. Martin DE, Soulard A, Hall MN. TOR regulates ribosomal protein gene expression via PKA and  
869 the Forkhead transcription factor *FHL1*. *Cell*. 2004;119: 969–79.  
870 doi:10.1016/j.cell.2004.11.047
- 871 65. Mayer C, Grummt I. Ribosome biogenesis and cell growth: mTOR coordinates transcription by  
872 all three classes of nuclear RNA polymerases. *Oncogene*. England; 2006;25: 6384–6391.  
873 doi:10.1038/sj.onc.1209883
- 874 66. Gu X, Orozco JM, Saxton RA, Condon KJ, Liu GY, Krawczyk PA, et al. SAMTOR is an S -  
875 adenosylmethionine sensor for the mTORC1 pathway. *Science* (80- ). 2017;818: 813–818.
- 876 67. Dey S, Baird TD, Zhou D, Palam LR, Spandau DF, Wek RC. Both transcriptional regulation and  
877 translational control of ATF4 are central to the integrated stress response. *J Biol Chem*.  
878 2010/08/23. American Society for Biochemistry and Molecular Biology; 2010;285: 33165–  
879 33174. doi:10.1074/jbc.M110.167213
- 880 68. Singleton DC, Harris AL. Targeting the ATF4 pathway in cancer therapy. *Expert Opin Ther*  
881 *Targets*. 2012/09/26. England; 2012;16: 1189–1202. doi:10.1517/14728222.2012.728207
- 882 69. Pällmann N, Livgård M, Tesikova M, Zeynep Nenseth H, Akkus E, Sikkeland J, et al. Regulation  
883 of the unfolded protein response through ATF4 and FAM129A in prostate cancer. *Oncogene*.  
884 2019/07/16. England; 2019;38: 6301–6318. doi:10.1038/s41388-019-0879-2
- 885 70. Breillout F, Antoine E, Poupon MF. Methionine dependency of malignant tumors: a possible  
886 approach for therapy. *J Natl Cancer Inst*. 1990;82: 1628–32. doi:10.1093/jnci/82.20.1628
- 887 71. Poirson-Bichat F, Gonçalves RA, Miccoli L, Dutrillaux B, Poupon MF. Methionine depletion  
888 enhances the antitumoral efficacy of cytotoxic agents in drug-resistant human tumor  
889 xenografts. *Clin Cancer Res*. 2000;6: 643–53.
- 890 72. van Dijken JP, Bauer, Brambilla, Duboc, Francois, Gancedo, et al. An interlaboratory  
891 comparison of physiological and genetic properties of four *Saccharomyces cerevisiae* strains.  
892 *Enzyme Microb Technol*. 2000;26: 706–714. doi:10.1016/S0141-0229(00)00162-9
- 893 73. Collart MA, Oliviero S. Preparation of Yeast RNA. *Current Protocols in Molecular Biology*.  
894 Hoboken, NJ, USA: John Wiley & Sons, Inc.; 2001. doi:10.1002/0471142727.mb1312s23
- 895 74. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform.  
896 *Bioinformatics*. 2009;25: 1754–1760. doi:10.1093/bioinformatics/btp324
- 897 75. Robinson MD, McCarthy DJ, Smyth GK. edgeR: a Bioconductor package for differential  
898 expression analysis of digital gene expression data. *Bioinformatics*. 2010;26: 139–40.  
899 doi:10.1093/bioinformatics/btp616
- 900 76. Raudvere U, Kolberg L, Kuzmin I, Arak T, Adler P, Peterson H, et al. g:Profiler: a web server for  
901 functional enrichment analysis and conversions of gene lists (2019 update). *Nucleic Acids Res*.  
902 Oxford University Press; 2019;47: W191–W198. doi:10.1093/nar/gkz369
- 903 77. Lelandais G, Blugeon C, Merhej J. ChIPseq in Yeast Species: From Chromatin  
904 Immunoprecipitation to High-Throughput Sequencing and Bioinformatics Data Analyses.  
905 *Methods Mol Biol*. United States; 2016;1361: 185–202. doi:10.1007/978-1-4939-3079-1\_11
- 906 78. Lawrence M, Huber W, Pagès H, Aboyoun P, Carlson M, Gentleman R, et al. Software for  
907 computing and annotating genomic ranges. *PLoS Comput Biol*. 2013/08/08. Public Library of  
908 Science; 2013;9: e1003118–e1003118. doi:10.1371/journal.pcbi.1003118
- 909 79. McIsaac RS, Silverman SJ, McClean MN, Gibney PA, Macinskas J, Hickman MJ, et al. Fast-  
910 acting and nearly gratuitous induction of gene expression and protein depletion in  
911 *Saccharomyces cerevisiae*. *Mol Biol Cell*. 2011/09/30. The American Society for Cell Biology;

- 912 2011;22: 4447–4459. doi:10.1091/mbc.E11-05-0466
- 913
- 914 2. Boer VMVM, Crutchfield CACA, Bradley PHPH, Botstein D, Rabinowitz JDJD. Growth-limiting  
915 Intracellular Metabolites in Yeast Growing under Diverse Nutrient Limitations. *Mol Biol Cell*.  
916 2010;21: 198–211. doi:10.1091/mbc.E09-07-0597
- 917 3. Saldanha A, Brauer M, Botstein D. Nutritional Homeostasis in Batch and Steady-State Culture  
918 of Yeast. *Mol Biol Cell*. 2004;15: 4089–104. doi:10.1091/mbc.E04-04-0306
- 919 4. Dikicioglu D, Karabekmez E, Rash B, Pir P, Kirdar B, Oliver SG. How yeast re-programmes its  
920 transcriptional profile in response to different nutrient impulses. *BMC Syst Biol*. *BioMed*  
921 *Central*; 2011;5: 148. doi:10.1186/1752-0509-5-148
- 922 5. Gutteridge A, Pir P, Castrillo JI, Charles PD, Lilley KS, Oliver SG. Nutrient control of eukaryote  
923 cell growth: a systems biology study in yeast. *BMC Biol*. *BioMed Central*; 2010;8: 68.  
924 doi:10.1186/1741-7007-8-68
- 925 6. Brauer MJ, Yuan J, Bennett BD, Lu W, Kimball E, Botstein D, et al. Conservation of the  
926 metabolomic response to starvation across two divergent microbes. *Proc Natl Acad Sci*.  
927 2006;103: 19302–19307.
- 928 7. Gurvich Y, Leshkowitz D, Barkai N. Dual role of starvation signaling in promoting growth and  
929 recovery. *PLoS Biol*. 2017;15: 1–28. doi:10.1371/journal.pbio.2002039
- 930 8. Metzl-Raz E, Kafri M, Yaakov G, Soifer I, Gurvich Y, Barkai N. Principles of cellular resource  
931 allocation revealed by condition-dependent proteome profiling. *Elife*. *eLife Sciences*  
932 *Publications, Ltd*; 2017;6: e28034. doi:10.7554/eLife.28034
- 933 9. Zaman S, Lippman SI, Zhao X, Broach JR. How *Saccharomyces* Responds to Nutrients. *Annu*  
934 *Rev Genet*. 2008;42: 27–81. doi:10.1146/annurev.genet.41.110306.130206
- 935 10. Cai L, Sutter BM, Li B, Tu BP. Acetyl-CoA induces cell growth and proliferation by promoting  
936 the acetylation of histones at growth genes. *Mol Cell*. Elsevier Inc.; 2011;42: 426–37.  
937 doi:10.1016/j.molcel.2011.05.004
- 938 11. Cai L, Tu BP. On acetyl-CoA as a gauge of cellular metabolic state. *Cold Spring Harb Perspect*  
939 *Biol*. 2011;76: 195–202.
- 940 12. Kuang Z, Cai L, Zhang X, Ji H, Tu BP, Boeke JD. High-temporal-resolution view of transcription  
941 and chromatin states across distinct metabolic states in budding yeast. *Nat Struct Mol Biol*.  
942 2014/08/31. 2014;21: 854–863. doi:10.1038/nsmb.2881
- 943 13. Shi L, Tu BPBP. Acetyl-CoA induces transcription of the key G1 cyclin CLN3 to promote entry  
944 into the cell division cycle in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci*. 2013;110: 7318–  
945 7323. doi:10.1073/pnas.1302490110
- 946 14. Krishna S, Laxman S. A minimal “push – pull” bistability model explains oscillations between  
947 quiescent and proliferative cell states. Lew DJ, editor. *Mol Biol Cell*. 2018;29: 2243–2258.  
948 doi:10.1091/mbc.E18-01-0017
- 949 15. Wellen KE, Thompson CB. Cellular metabolic stress: considering how cells respond to nutrient  
950 excess. *Mol Cell*. Elsevier Inc.; 2010;40: 323–32. doi:10.1016/j.molcel.2010.10.004
- 951 16. Rowicka M, Kudlicki A, Tu BP, Otwinowski Z. High-resolution timing of cell cycle-regulated  
952 gene expression. *Proc Natl Acad Sci U S A*. 2007;104: 16892–7. doi:10.1073/pnas.0706022104
- 953 17. Pedro MB, Madeo F, Pietrocola F, Galluzzi L, Bravo-San Pedro JM, Madeo F, et al. Review  
954 Acetyl Coenzyme A: A Central Metabolite and Second Messenger. *Cell Metab*. United States;  
955 2015;21: 805–821. doi:10.1016/j.cmet.2015.05.014
- 956 18. Sutter BM, Wu X, Laxman S, Tu BP. Methionine inhibits autophagy and promotes growth by

- 957 inducing the SAM-responsive methylation of PP2A. *Cell*. 2013;154: 403–15.  
958 doi:10.1016/j.cell.2013.06.041
- 959 19. Lees EK, Banks R, Cook C, Hill S, Morrice N, Grant L, et al. Direct comparison of methionine  
960 restriction with leucine restriction on the metabolic health of C57BL/6J mice. *Sci Rep. Nature*  
961 *Publishing Group UK*; 2017;7: 9977. doi:10.1038/s41598-017-10381-3
- 962 20. Walvekar AS, Srinivasan R, Gupta R, Laxman S. Methionine coordinates a hierarchically  
963 organized anabolic program enabling proliferation. *Mol Biol Cell. American Society for Cell*  
964 *Biology (mboc)*; 2018;29: 3183–3200. doi:10.1091/mbc.E18-08-0515
- 965 21. Halpern BC, Clark BR, Hardy DN, Halpern RM, Smith RA. The effect of replacement of  
966 methionine by homocystine on survival of malignant and normal adult mammalian cells in  
967 culture. *Proc Natl Acad Sci U S A*. 1974;71: 1133–6. doi:10.1073/pnas.71.4.1133
- 968 22. Sugimura T, Birnbaum SM, Winitz M, Greenstein JP. Quantitative nutritional studies with  
969 water-soluble, chemically defined diets. VIII. The forced feeding of diets each lacking in one  
970 essential amino acid. *Arch Biochem Biophys*. 1959;81: 448–455. doi:10.1016/0003-  
971 9861(59)90225-5
- 972 23. Gao X, Sanderson SM, Dai Z, Reid MA, Cooper DE, Lu M, et al. Dietary methionine restriction  
973 targets one carbon metabolism in humans and produces broad therapeutic responses in  
974 cancer. *bioRxiv*. 2019; 627364. doi:10.1101/627364
- 975 24. Gao X, Sanderson SM, Dai Z, Reid MA, Cooper DE, Lu M, et al. Dietary methionine influences  
976 therapy in mouse cancer models and alters human metabolism. *Nature*. 2019/07/31.  
977 *England*; 2019;572: 397–401. doi:10.1038/s41586-019-1437-3
- 978 25. Wu X, Tu BP. Selective regulation of autophagy by the Iml1-Npr2-Npr3 complex in the  
979 absence of nitrogen starvation. *Mol Biol Cell*. 2011;22: 4124–4133. doi:10.1091/mbc.E11-06-  
980 0525
- 981 26. Laxman S, Sutter B, Tu BP. Methionine is a signal of amino acid sufficiency that inhibits  
982 autophagy through the methylation of PP2A. *Autophagy*. 2014;10: 386–387.  
983 doi:10.4161/auto.27485
- 984 27. Brauer MJMJ, Huttenhower C, Airoidi EMEM, Rosenstein R, Matese JJC, Gresham D, et al.  
985 Coordination of Growth Rate, Cell Cycle, Stress Response, and Metabolic Activity in Yeast.  
986 *Mol Biol Cell*. 2008;19: 352–267. doi:10.1091/mbc.E07-08-0779
- 987 28. Hinnebusch AG. Translational Regulation Of GCN4 and the General Amino Acid Control of  
988 yeast. *Annu Rev Microbiol*. 2005;59: 407–50. doi:10.1146/annurev.micro.59.031805.133833
- 989 29. Mascarenhas C, Edwards-Ingram LC, Zeef L, Shenton D, Ashe MP, Grant CM. Gcn4 is required  
990 for the response to peroxide stress in the yeast *Saccharomyces cerevisiae*. *Mol Biol Cell*.  
991 2008/04/16. *The American Society for Cell Biology*; 2008;19: 2995–3007.  
992 doi:10.1091/mbc.e07-11-1173
- 993 30. Yang R, Wek SA, Wek RC. Glucose limitation induces GCN4 translation by activation of Gcn2  
994 protein kinase. *Mol Cell Biol. American Society for Microbiology*; 2000;20: 2706–2717.  
995 doi:10.1128/mcb.20.8.2706-2717.2000
- 996 31. Natarajan K, Meyer MR, Jackson BM, Slade D, Roberts C, Hinnebusch AG, et al.  
997 Transcriptional profiling shows that Gcn4p is a master regulator of gene expression during  
998 amino acid starvation in yeast. *Mol Cell Biol*. 2001;21: 4347–68.  
999 doi:10.1128/MCB.21.13.4347-4368.2001
- 1000 32. Hinnebusch AG, Asano K, Olsen DS, Phan L, Nielsen KH, Valásek L. Study of translational  
1001 control of eukaryotic gene expression using yeast. *Ann N Y Acad Sci. United States*;  
1002 2004;1038: 60–74. doi:10.1196/annals.1315.012

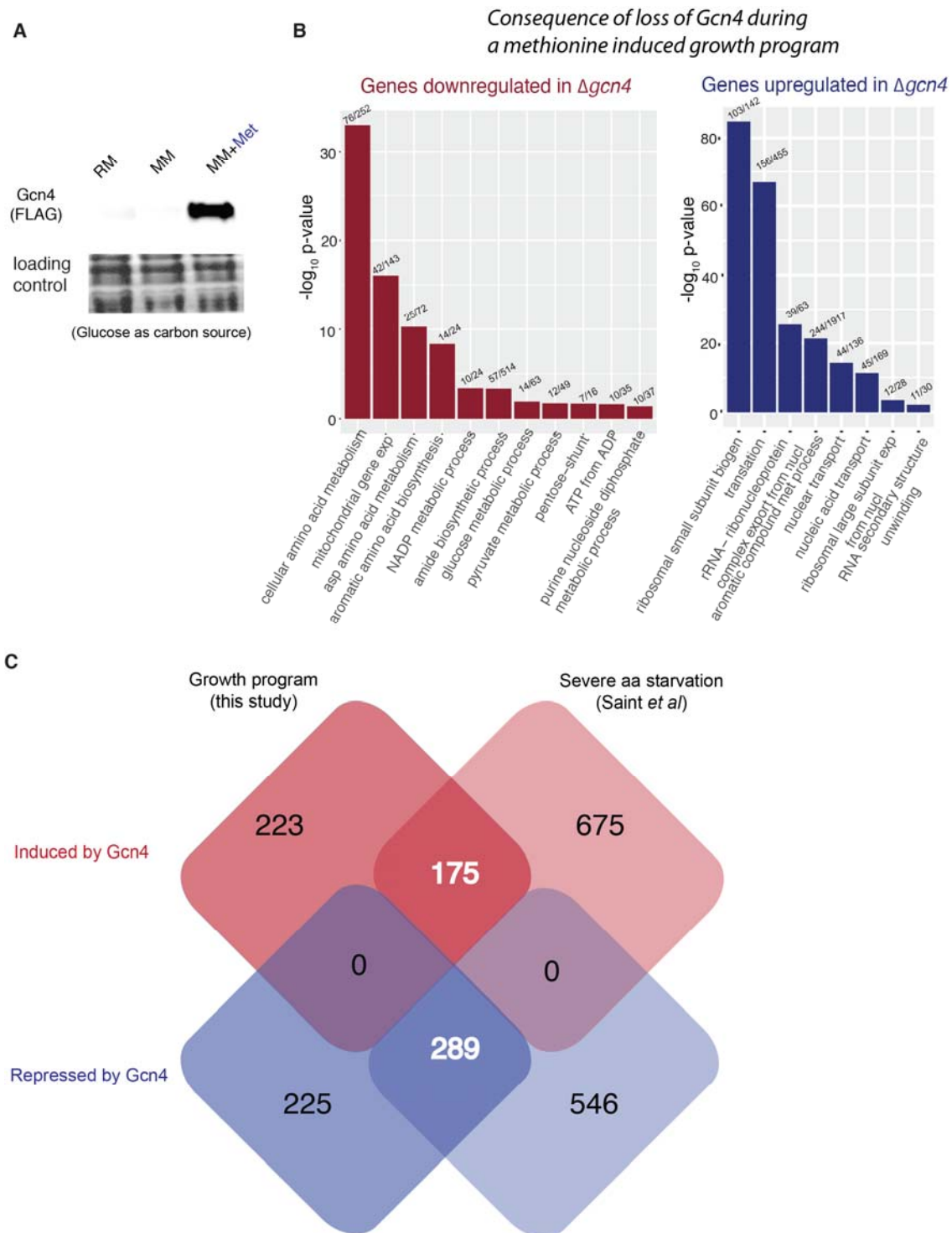
- 1003 33. Hinnebusch AG. Translational control of GCN4: an in vivo barometer of initiation-factor  
1004 activity. *Trends Biochem Sci. England*; 1994;19: 409–414. doi:10.1016/0968-0004(94)90089-2
- 1005 34. Rawal Y, Chereji R V, Valabhoju V, Qiu H, Ocampo J, Clark DJ, et al. Gcn4 Binding in Coding  
1006 Regions Can Activate Internal and Canonical 5' Promoters in Yeast. *Mol Cell*. 2018/04/05.  
1007 2018;70: 297-311.e4. doi:10.1016/j.molcel.2018.03.007
- 1008 35. Mittal N, Guimaraes JC, Gross T, Schmidt A, Vina-Vilaseca A, Nedialkova DD, et al. The Gcn4  
1009 transcription factor reduces protein synthesis capacity and extends yeast lifespan. *Nat*  
1010 *Commun. Nature Publishing Group UK*; 2017;8: 457. doi:10.1038/s41467-017-00539-y
- 1011 36. Ye J, Kumanova M, Hart LS, Sloane K, Zhang H, De Panis DN, et al. The GCN2-ATF4 pathway is  
1012 critical for tumour cell survival and proliferation in response to nutrient deprivation. *EMBO J*.  
1013 2010;29: 2082–96. doi:10.1038/emboj.2010.81
- 1014 37. Tameire F, Verginadis II, Leli NM, Polte C, Conn CS, Ojha R, et al. ATF4 couples MYC-  
1015 dependent translational activity to bioenergetic demands during tumour progression. *Nat*  
1016 *Cell Biol*. 2019/07/01. Springer US; 2019;21: 889–899. doi:10.1038/s41556-019-0347-9
- 1017 38. Xiao L, Grove A. Coordination of Ribosomal Protein and Ribosomal RNA Gene Expression in  
1018 Response to TOR Signaling. *Curr Genomics*. Bentham Science Publishers Ltd; 2009;10: 198–  
1019 205. doi:10.2174/138920209788185261
- 1020 39. Airoidi EM, Huttenhower C, Gresham D, Lu C, Caudy AA, Dunham MJ, et al. Predicting cellular  
1021 growth from gene expression signatures. *PLoS Comput Biol*. 2009/01/02. Public Library of  
1022 Science; 2009;5: e1000257–e1000257. doi:10.1371/journal.pcbi.1000257
- 1023 40. Hinnebusch AG, Natarajan K. Gcn4p, a master regulator of gene expression, is controlled at  
1024 multiple levels by diverse signals of starvation and stress. *Eukaryot Cell. American Society for*  
1025 *Microbiology*; 2002;1: 22–32. doi:10.1128/ec.01.1.22-32.2002
- 1026 41. Pakos-Zebrucka K, Koryga I, Mnich K, Ljujic M, Samali A, Gorman AM. The integrated stress  
1027 response. *EMBO Rep*. 2016/09/14. John Wiley and Sons Inc.; 2016;17: 1374–1395.  
1028 doi:10.15252/embr.201642195
- 1029 42. Akhter A, Rosonina E. Chromatin Association of Gcn4 Is Limited by Post-translational  
1030 Modifications Triggered by its DNA-Binding in *Saccharomyces cerevisiae*. *Genetics*.  
1031 2016/10/21. Genetics Society of America; 2016;204: 1433–1445.  
1032 doi:10.1534/genetics.116.194134
- 1033 43. Albrecht G, Mösch HU, Hoffmann B, Reusser U, Braus GH. Monitoring the Gcn4 protein-  
1034 mediated response in the yeast *Saccharomyces cerevisiae*. *J Biol Chem. United States*;  
1035 1998;273: 12696–12702. doi:10.1074/jbc.273.21.12696
- 1036 44. Joo YJ, Kim J, Kang U, Yu M, Kim J. Gcn4p-mediated transcriptional repression of ribosomal  
1037 protein genes under amino-acid starvation. *EMBO J. Nature Publishing Group*; 2010;30: 859–  
1038 872. doi:10.1038/emboj.2010.332
- 1039 45. Bose T, Lee KK, Lu S, Xu B, Harris B, Slaughter B, et al. Cohesin proteins promote ribosomal  
1040 RNA production and protein translation in yeast and human cells. *PLoS Genet*. 2012/06/14.  
1041 Public Library of Science; 2012;8: e1002749–e1002749. doi:10.1371/journal.pgen.1002749
- 1042 46. Saint M, Sawhney S, Sinha I, Singh RP, Dahiya R, Thakur A, et al. The TAF9 C-terminal  
1043 conserved region domain is required for SAGA and TFIID promoter occupancy to promote  
1044 transcriptional activation. *Mol Cell Biol*. 2014/02/18. American Society for Microbiology;  
1045 2014;34: 1547–1563. doi:10.1128/MCB.01060-13
- 1046 47. McMillan J, Lu Z, Rodriguez JS, Ahn T-H, Lin Z. YeastTSS: an integrative web database of yeast  
1047 transcription start sites. *Database (Oxford)*. Oxford University Press; 2019;2019: baz048.  
1048 doi:10.1093/database/baz048

- 1049 48. Heinz S, Benner C, Spann N, Bertolino E, Lin YC, Laslo P, et al. Simple combinations of lineage-  
1050 determining transcription factors prime cis-regulatory elements required for macrophage and  
1051 B cell identities. *Mol Cell*. 2010;38: 576–589. doi:10.1016/j.molcel.2010.05.004
- 1052 49. Bailey TL, Boden M, Buske FA, Frith M, Grant CE, Clementi L, et al. MEME SUITE: tools for  
1053 motif discovery and searching. *Nucleic Acids Res*. 2009/05/20. Oxford University Press;  
1054 2009;37: W202–W208. doi:10.1093/nar/gkp335
- 1055 50. Arndt K, Fink GR. GCN4 protein, a positive transcription factor in yeast, binds general control  
1056 promoters at all 5' TGACTC 3' sequences. *Proc Natl Acad Sci U S A*. 1986;83: 8516–8520.  
1057 doi:10.1073/pnas.83.22.8516
- 1058 51. Oakley MG, Dervan PB. Structural motif of the GCN4 DNA binding domain characterized by  
1059 affinity cleaving. *Science*. United States; 1990;248: 847–850. doi:10.1126/science.2111578
- 1060 52. Holland P, Bergenholm D, Börlin CS, Liu G, Nielsen J. Predictive models of eukaryotic  
1061 transcriptional regulation reveals changes in transcription factor roles and promoter usage  
1062 between metabolic conditions. *Nucleic Acids Res*. Oxford University Press; 2019;47: 4986–  
1063 5000. doi:10.1093/nar/gkz253
- 1064 53. Aow JSZ, Xue X, Run J-Q, Lim GFS, Goh WS, Clarke ND. Differential binding of the related  
1065 transcription factors Pho4 and Cbf1 can tune the sensitivity of promoters to different levels of  
1066 an induction signal. *Nucleic Acids Res*. 2013/04/04. Oxford University Press; 2013;41: 4877–  
1067 4887. doi:10.1093/nar/gkt210
- 1068 54. Kribelbauer JF, Rastogi C, Bussemaker HJ, Mann RS. Low-Affinity Binding Sites and the  
1069 Transcription Factor Specificity Paradox in Eukaryotes. *Annu Rev Cell Dev Biol*. 2019/07/05.  
1070 2019;35: 357–379. doi:10.1146/annurev-cellbio-100617-062719
- 1071 55. Nishizawa M, Komai T, Morohashi N, Shimizu M, Toh-e A. Transcriptional repression by the  
1072 Pho4 transcription factor controls the timing of SNZ1 expression. *Eukaryot Cell*. 2008/04/11.  
1073 American Society for Microbiology (ASM); 2008;7: 949–957. doi:10.1128/EC.00366-07
- 1074 56. Walvekar A, Rashida Z, Maddali H, Laxman S. A versatile LC-MS/MS approach for  
1075 comprehensive, quantitative analysis of central metabolic pathways. *Wellcome open Res*.  
1076 F1000 Research Limited; 2018;3: 122. doi:10.12688/wellcomeopenres.14832.1
- 1077 57. Hofmeyr JHS, Cornish-Bowden A. Regulating the cellular economy of supply and demand.  
1078 *FEBS Lett*. 2000;476: 47–51. doi:10.1016/S0014-5793(00)01668-9
- 1079 58. Hofmeyr J-HSHS. The harmony of the cell: the regulatory design of cellular processes.  
1080 Wolkenhauer O, Wellstead P, Cho K-H, editors. *Essays Biochem*. 2008;45: 57–66.  
1081 doi:10.1042/bse0450057
- 1082 59. Hui S, Silverman JM, Chen SS, Erickson DW, Basan M, Wang J, et al. Quantitative proteomic  
1083 analysis reveals a simple strategy of global resource allocation in bacteria. *Mol Syst Biol*.  
1084 BlackWell Publishing Ltd; 2015;11: 784. doi:10.15252/msb.20145697
- 1085 60. Warner JR, Vilardell J, Sohn JH. Economics of ribosome biosynthesis. *Cold Spring Harb Symp*  
1086 *Quant Biol*. 2001;66: 567–74. doi:10.1101/sqb.2001.66.567
- 1087 61. Bosdriesz E, Molenaar D, Teusink B, Bruggeman FJ. How fast-growing bacteria robustly tune  
1088 their ribosome concentration to approximate growth-rate maximization. *FEBS J*. 2015/03/26.  
1089 John Wiley & Sons, Ltd; 2015;282: 2029–2044. doi:10.1111/febs.13258
- 1090 62. Klumpp S, Scott M, Pedersen S, Hwa T. Molecular crowding limits translation and cell growth.  
1091 *Proc Natl Acad Sci U S A*. 2013/09/30. National Academy of Sciences; 2013;110: 16754–  
1092 16759. doi:10.1073/pnas.1310377110
- 1093 63. Tu BP, Tu BP, Kudlicki A, Rowicka M, Mcknight SL. Logic of the Yeast Metabolic Cycle of  
1094 Cellular Processes. *Science*. 2005; doi:10.1126/science.1120499



- 1095 64. Martin DE, Soulard A, Hall MN. TOR regulates ribosomal protein gene expression via PKA and  
1096 the Forkhead transcription factor *FHL1*. *Cell*. 2004;119: 969–79.  
1097 doi:10.1016/j.cell.2004.11.047
- 1098 65. Mayer C, Grummt I. Ribosome biogenesis and cell growth: mTOR coordinates transcription by  
1099 all three classes of nuclear RNA polymerases. *Oncogene*. England; 2006;25: 6384–6391.  
1100 doi:10.1038/sj.onc.1209883
- 1101 66. Gu X, Orozco JM, Saxton RA, Condon KJ, Liu GY, Krawczyk PA, et al. SAMTOR is an S -  
1102 adenosylmethionine sensor for the mTORC1 pathway. *Science* (80- ). 2017;818: 813–818.
- 1103 67. Dey S, Baird TD, Zhou D, Palam LR, Spandau DF, Wek RC. Both transcriptional regulation and  
1104 translational control of ATF4 are central to the integrated stress response. *J Biol Chem*.  
1105 2010/08/23. American Society for Biochemistry and Molecular Biology; 2010;285: 33165–  
1106 33174. doi:10.1074/jbc.M110.167213
- 1107 68. Singleton DC, Harris AL. Targeting the ATF4 pathway in cancer therapy. *Expert Opin Ther*  
1108 *Targets*. 2012/09/26. England; 2012;16: 1189–1202. doi:10.1517/14728222.2012.728207
- 1109 69. Pällmann N, Livgård M, Tesikova M, Zeynep Nenseth H, Akkus E, Sikkeland J, et al. Regulation  
1110 of the unfolded protein response through ATF4 and FAM129A in prostate cancer. *Oncogene*.  
1111 2019/07/16. England; 2019;38: 6301–6318. doi:10.1038/s41388-019-0879-2
- 1112 70. Breillout F, Antoine E, Poupon MF. Methionine dependency of malignant tumors: a possible  
1113 approach for therapy. *J Natl Cancer Inst*. 1990;82: 1628–32. doi:10.1093/jnci/82.20.1628
- 1114 71. Poirson-Bichat F, Gonçalves RA, Miccoli L, Dutrillaux B, Poupon MF. Methionine depletion  
1115 enhances the antitumoral efficacy of cytotoxic agents in drug-resistant human tumor  
1116 xenografts. *Clin Cancer Res*. 2000;6: 643–53.
- 1117 72. van Dijken JP, Bauer, Brambilla, Duboc, Francois, Gancedo, et al. An interlaboratory  
1118 comparison of physiological and genetic properties of four *Saccharomyces cerevisiae* strains.  
1119 *Enzyme Microb Technol*. 2000;26: 706–714. doi:10.1016/S0141-0229(00)00162-9
- 1120 73. Collart MA, Oliviero S. Preparation of Yeast RNA. *Current Protocols in Molecular Biology*.  
1121 Hoboken, NJ, USA: John Wiley & Sons, Inc.; 2001. doi:10.1002/0471142727.mb1312s23
- 1122 74. Li H, Durbin R. Fast and accurate short read alignment with Burrows-Wheeler transform.  
1123 *Bioinformatics*. 2009;25: 1754–1760. doi:10.1093/bioinformatics/btp324
- 1124 75. Robinson MD, McCarthy DJ, Smyth GK. edgeR: a Bioconductor package for differential  
1125 expression analysis of digital gene expression data. *Bioinformatics*. 2010;26: 139–40.  
1126 doi:10.1093/bioinformatics/btp616
- 1127 76. Raudvere U, Kolberg L, Kuzmin I, Arak T, Adler P, Peterson H, et al. g:Profiler: a web server for  
1128 functional enrichment analysis and conversions of gene lists (2019 update). *Nucleic Acids Res*.  
1129 Oxford University Press; 2019;47: W191–W198. doi:10.1093/nar/gkz369
- 1130 77. Lelandais G, Blugeon C, Merhej J. ChIPseq in Yeast Species: From Chromatin  
1131 Immunoprecipitation to High-Throughput Sequencing and Bioinformatics Data Analyses.  
1132 *Methods Mol Biol*. United States; 2016;1361: 185–202. doi:10.1007/978-1-4939-3079-1\_11
- 1133 78. Lawrence M, Huber W, Pagès H, Aboyoun P, Carlson M, Gentleman R, et al. Software for  
1134 computing and annotating genomic ranges. *PLoS Comput Biol*. 2013/08/08. Public Library of  
1135 Science; 2013;9: e1003118–e1003118. doi:10.1371/journal.pcbi.1003118
- 1136 79. McIsaac RS, Silverman SJ, McClean MN, Gibney PA, Macinskas J, Hickman MJ, et al. Fast-  
1137 acting and nearly gratuitous induction of gene expression and protein depletion in  
1138 *Saccharomyces cerevisiae*. *Mol Biol Cell*. 2011/09/30. The American Society for Cell Biology;  
1139 2011;22: 4447–4459. doi:10.1091/mbc.E11-05-0466
- 1140

1141 **Figures and Figure Legends:**  
 1142

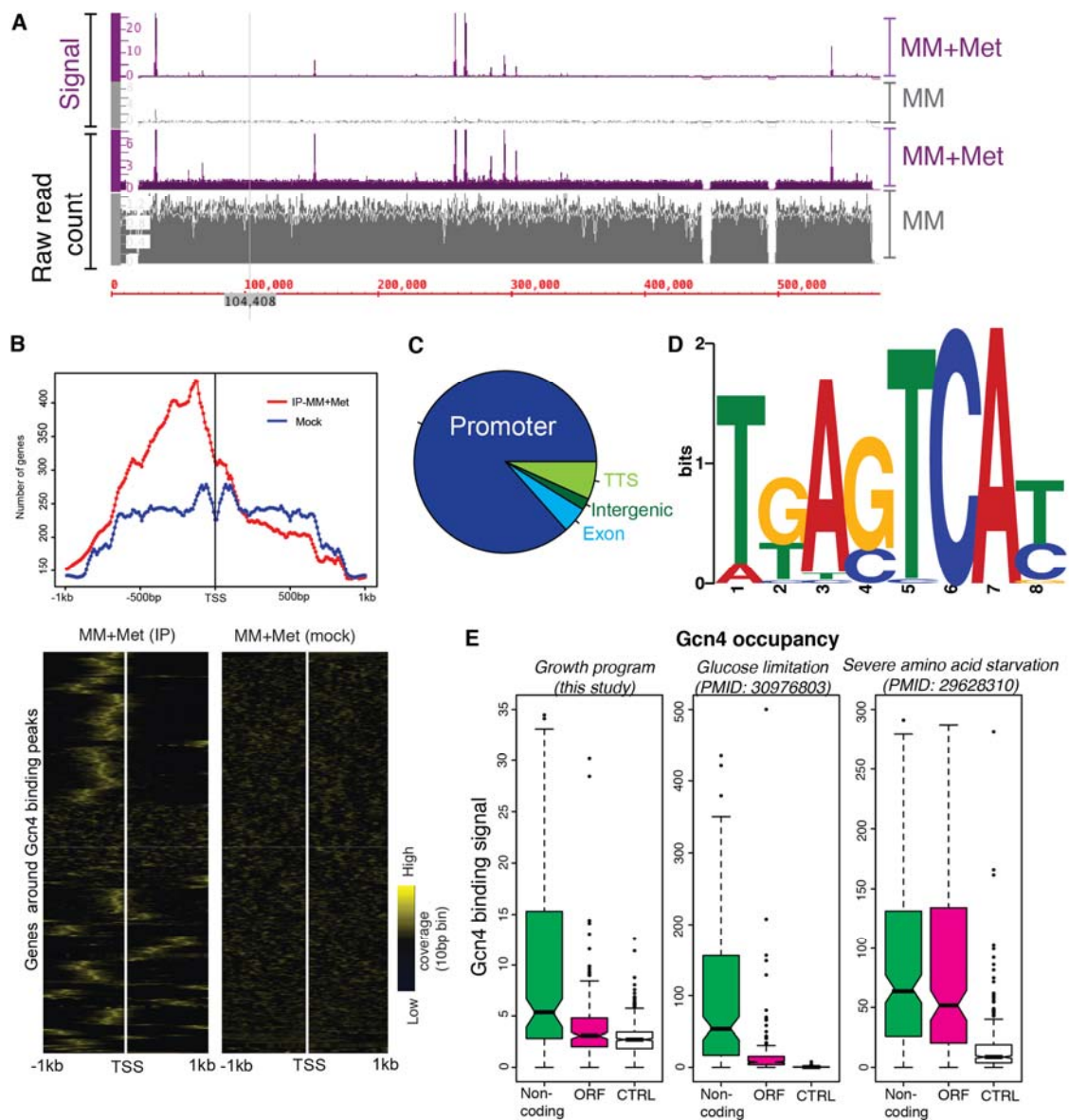


1143

1144 **Figure 1: *Gcn4* binds to its target gene promoters related to metabolism during a growth program.**

1145 A. *Gcn4* is strongly induced by methionine addition. A representative western blot shows high  
 1146 *Gcn4* protein levels in MM+Met (*Gcn4* tagged with the FLAG epitope at the endogenous locus).  
 1147 RM - rich medium, MM - minimal medium without amino acids and with glucose as a carbon

1148 source, and MM+Met - minimal medium without amino acids and with glucose as a carbon  
1149 source supplemented with 2mM methionine. Also see Supplementary Figure 4A.  
1150 B. GO based analysis and grouping of transcripts down regulated in *Δgcn4*. All the terms shown  
1151 here are significantly enriched terms with the corrected p-value < 0.05 (hypergeometric test,  
1152 Bonferroni Correction). GO based analysis and grouping of the transcripts up-regulated in  
1153 *Δgcn4*. All the terms shown here are significantly enriched terms with the corrected p-value <  
1154 0.05 (hypergeometric test, Bonferroni Correction). Also see Supplementary WS3 and  
1155 Supplementary Figure 4B for gene expression volcano plots.  
1156 C. The Venn diagram shows the number of differentially expressed genes that overlap,  
1157 from data obtained from distinct cell growth conditions where Gcn4 levels are high.  
1158 The boxes on the left are data from this study (methionine induced growth program),  
1159 while the boxes on the right use data from a severe amino acid starvation condition  
1160 (sulfo meturon addition) [46]. Also see Supplementary WS6.  
1161  
1162  
1163  
1164



1165

1166

**Figure 2: *Gcn4* binds to its target gene promoters related to metabolism during a growth program**

1167

A. Genomic tracts showing *Gcn4* binding to DNA regions in MM and MM+Met. Raw read counts and signal around the binding region of *Gcn4* are shown.

1168

B. (Top) Density plots showing that most target genes have *Gcn4* binding peaks upstream of the Transcription Start Site (TSS) in the ChIP samples (red), whereas no such enrichment of genes is observed in mock samples (blue). (Bottom) A heat map showing read coverage for *Gcn4* binding, including 1kb upstream and downstream of predicted/known transcription start sites (TSS) of target genes. All the genes that fall in the vicinity of 750bp around the identified *Gcn4* binding peaks are considered to be target genes. The heat map on the left shows read coverage in IP samples, and on the right shows coverage in mock-IP (control) under in MM+Met condition. Also see Supplementary Figure 5 which shows read coverage for *Gcn4*, in the context of the translation start site (ATG) of each gene.

1169

1170

1171

1172

1173

1174

1175

1176

1177

C. A pie chart showing the genomic features of the identified peaks annotated using 'annotatepeak' function of Homer tool [48].

1178

1179

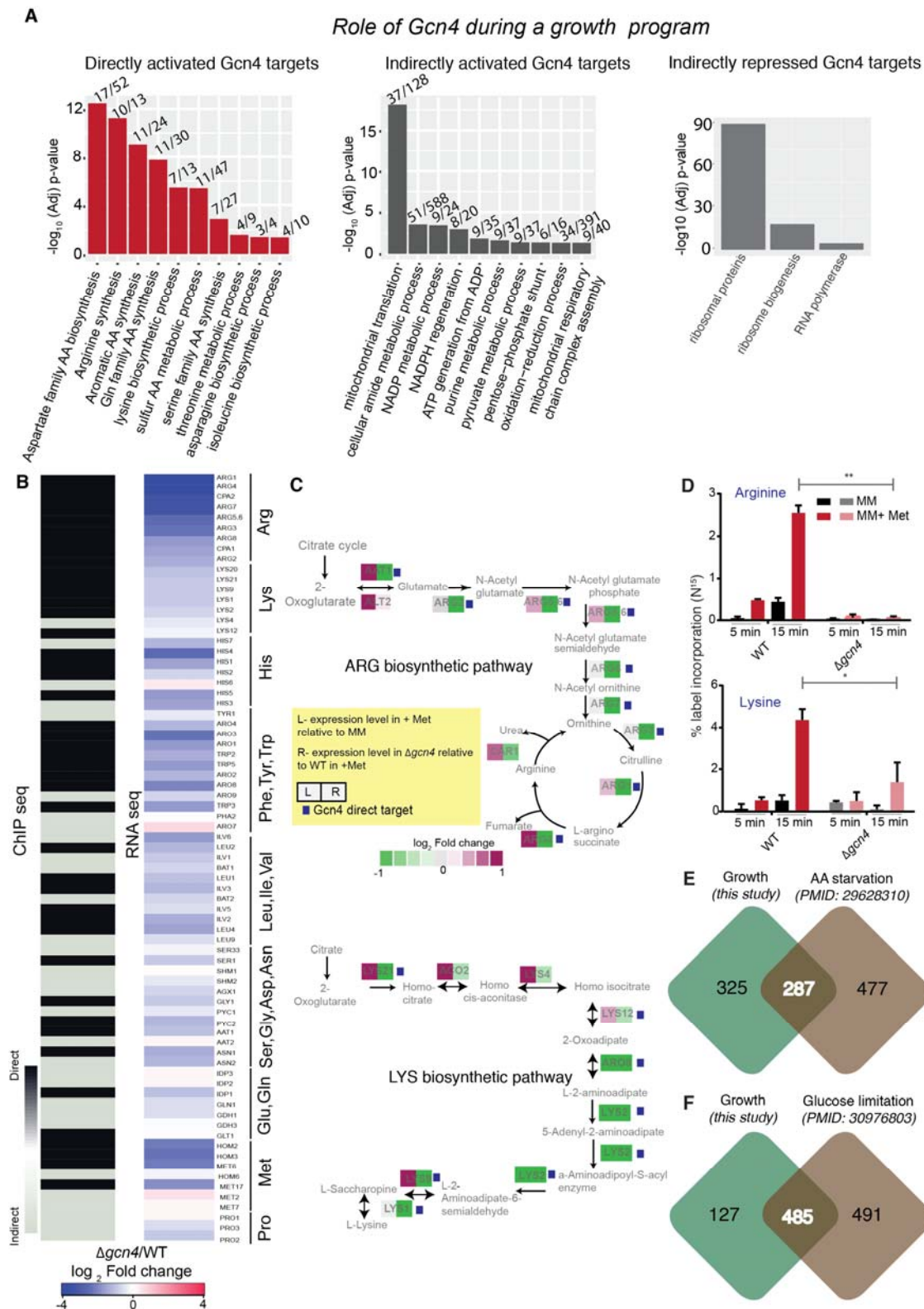
D. Consensus binding motifs identified in *Gcn4* binding peaks from MM+Met conditions.

1180

E. Boxplots, showing the *Gcn4* binding signal corresponding to different genomic

1181

1182 features, under distinct growth scenarios. For Gcn4 binding in non-coding and open  
1183 reading frame (ORF) regions (as reported in a previous study) [34], we compared the  
1184 Gcn4 binding signal in the Gcn4 ChIP sequencing data from cells in MM+Met  
1185 (current study), or under different starvation conditions (severe AA-starvation [34], or  
1186 in glucose limitation [52]). Notably, in either MM+Met or during glucose limitation  
1187 the Gcn4 binding signal in ORF peaks is significantly lower than the non-coding  
1188 region peaks ( $p < 10^{-8}$ ). Contrarily, under severe amino acid starvation [34], the Gcn4  
1189 binding signal found in ORF and non-coding regions are very similar.  
1190  
1191



1192

1193

1194

1195

**Figure 3: Direct and Indirect targets of Gcn4 during a growth program**

A. Role of Gcn4 during a growth program (methionine addition): Bar plots shows enriched GO term and the corresponding  $-\log_{10}(\text{p-value})$  for the genes which are directly or indirectly

1196 activated, or directly/indirectly repressed by Gcn4 when methionine is supplemented (growth  
1197 program). Also see Supplementary Figure 7B.

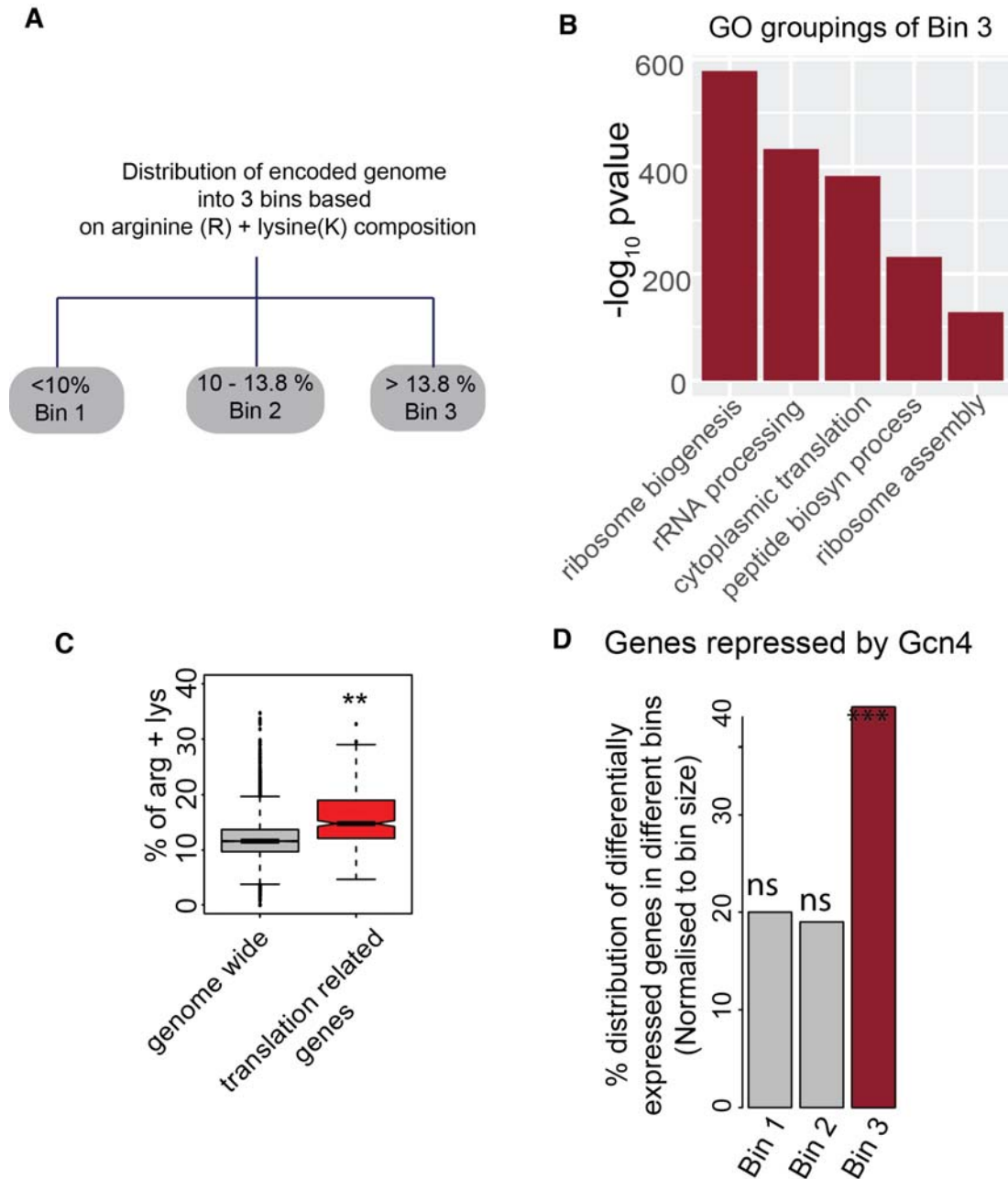
1198 B. Comparing direct targets of Gcn4 regulon, and gene expression profiles of WT and  $\Delta gcn4$  cells  
1199 (Gcn4 dependence) in a growth program. The heat map on the left shows whether the  
1200 indicated gene (involved in amino acid biosynthesis) is directly or indirectly regulated by Gcn4  
1201 based on ChIP-Seq data from cells in MM+Met medium. The black color indicates a direct  
1202 target of Gcn4 and grey indicates an indirect target (Gcn4 does not bind the promoter of this  
1203 gene). The heatmap on the right shows the gene expression fold changes in  $\Delta gcn4$  relative to  
1204 WT cells, grown in MM+Met medium.

1205 C. (Top panel) Representative pathway maps of the arginine biosynthetic pathway. This map  
1206 shows the fold change in gene expression due to methionine (MM+Met compared to MM) in  
1207 WT cells (**left box**), the change in gene expression due to loss of Gcn4 (WT compared to  $\Delta gcn4$ )  
1208 in the presence of methionine (MM+Met) (**right box**). Genes that are direct targets of Gcn4 are  
1209 also indicated with a small purple box next to the gene name.  
1210 (Lower panel) A representative pathway map of the lysine biosynthetic pathway, represented  
1211 similar to that of arginine biosynthesis.

1212 D. Increased arginine and lysine biosynthesis in a methionine dependent growth program depend  
1213 entirely on Gcn4. Data from quantitative LC/MS/MS based metabolic flux analysis experiments,  
1214 using  $N^{15}$  ammonium sulfate labeling to estimate new amino acid synthesis in a methionine and  
1215 Gcn4 dependent manner, are shown. The comparisons are between WT and  $\Delta gcn4$  cells  
1216 treated identically in MM+Met medium. The data are presented for arginine, lysine. Also see  
1217 Figure Supplementary Figure 8. \* $p < 0.05$ , \*\* $p < 0.01$  (t-test).

1218 E. Overlap between potential Gcn4 binding targets identified by ChIP-seq in a growth program  
1219 (this study), vs the targets identified under a severe amino acid starvation response [34].

1220 F. Overlap between potential Gcn4 binding targets identified by ChIP-seq in a growth program  
1221 (this study), vs the targets identified during moderate starvation induced by glucose limitation  
1222 [52].  
1223  
1224  
1225



1226

1227

1228

**Figure 4: Gcn4 globally represses arginine/lysine enriched genes, including the translational machinery.**

1229

A. “Binning” of the yeast proteome into three equal parts, based on the percentage of arginine and lysine in these proteins. The percentages of arginine and lysine (together) in these bins are indicated.

1230

1231

1232

B. GO based analysis reveals that bin3, which has the high percentage of arginine and lysine, is significantly enriched for ribosomal and translation related genes. The graph plots the most enriched GO term against  $-\log_{10}(P\text{ value})$ .

1233

1234

1235

C. Boxplot, comparing the arginine and lysine composition of the entire proteome (excluding translation related genes), and the translation related genes. The translation related genes have a significantly higher than genome wide composition of arginine and lysine.

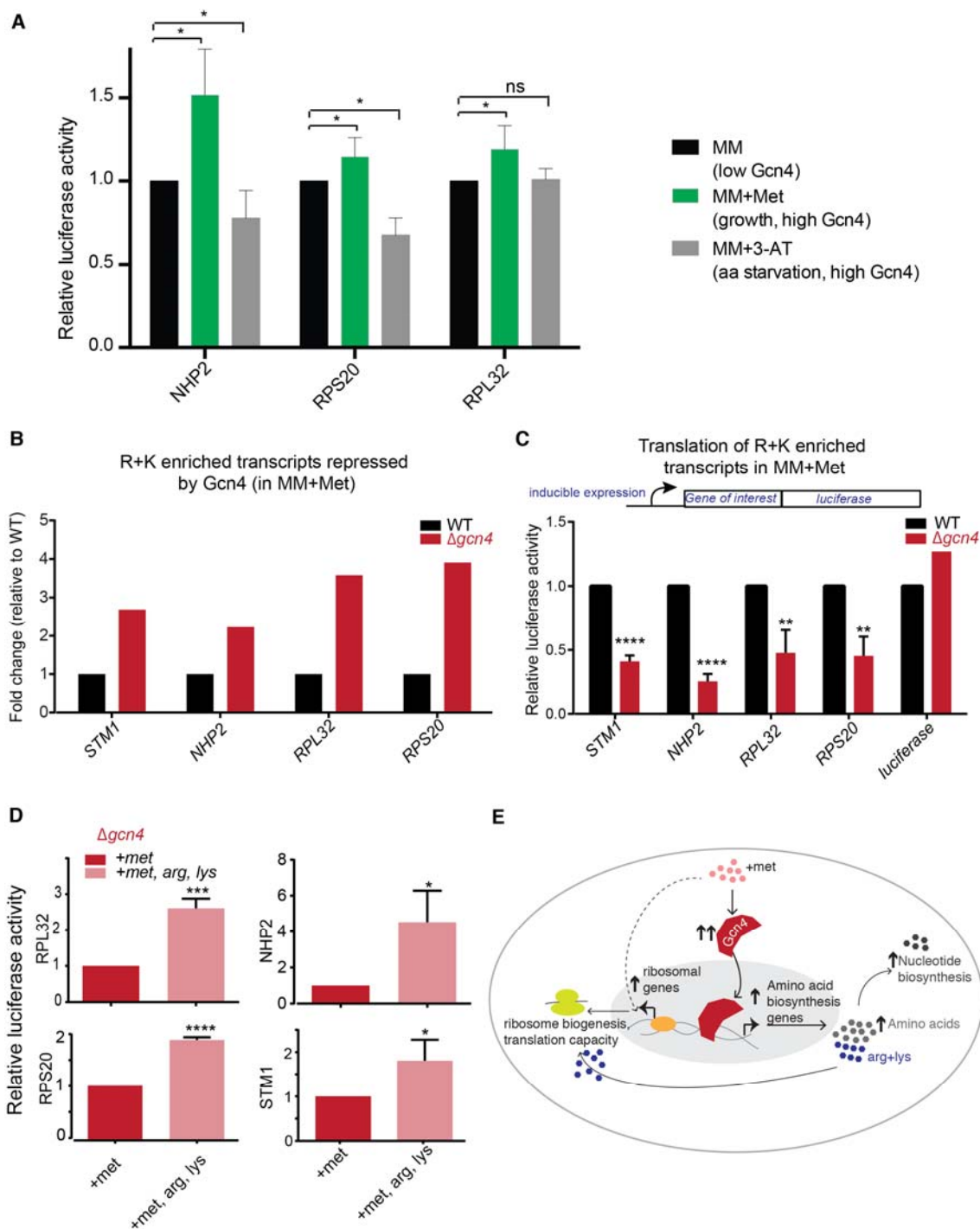
1236

1237



1238 D. Barplots, indicating in which bin (as shown in Figure 4A) the genes repressed by Gcn4 (i.e.  
1239 induced in  $\Delta gcn4$ ) fall under. A significant majority of the genes repressed by Gcn4 are  
1240 enriched for arginine and lysine rich (Fisher exact test,  $p < 10e-10$ ).

1241



1242

1243

1244

1245

1246

1247

1248

1249

1250

**Figure 5: Gcn4 dependent outputs can sustain high translation capacity during growth**

- A. Translation of arg/lys genes increase during a growth program (methionine addition), and decrease during amino acid starvation (3-AT addition), both of which are conditions where Gcn4 is induced.
- B. Arg/lys enriched genes are induced in  $\Delta gcn4$  cells in methionine supplemented medium. Barplots comparing relative transcript amounts for selected, highly induced, arginine and lysine enriched genes, between WT and  $\Delta gcn4$  cells. Data shown are taken from the RNA seq data.

- 1251 C. Arg/lys enriched gene transcripts cannot be translated in *Δgcn4* cells in methionine  
1252 supplemented medium. Barplots, comparing the relative amount of proteins translated (in a  
1253 methionine-dependent manner), for the genes shown in Figure 5B. These selected genes  
1254 were cloned in frame with luciferase in an inducible system, to create a translation-reporter,  
1255 and translation of these were induced in WT or *Δgcn4* cells in methionine supplemented  
1256 medium (MM+Met ). Data shown are mean+/- SD from ≥ 3 biological replicates.  
1257 \*p<0.05, \*\*p<0.01, \*\*\*<0.0001 (t-test).  
1258 D. Supplementing arginine and lysine restores translational capacity in *Δgcn4* cells. Barplots,  
1259 comparing the relative amount of proteins translated (in a methionine-dependent manner),  
1260 for the genes shown in Figure 5C, in methionine-supplemented medium (MM+Met) or in  
1261 MM+Met+Arg+lys . Data shown are mean+/- SD from ≥ 3 biological replicates.  
1262 \*p<0.05, \*\*\*p<0.001, \*\*\*\*<0.0001 (t-test).  
1263 E. A mechanistic model illustrating how high Gcn4 enables a methionine dependent anabolic  
1264 response, by supplying amino acids, and maintaining translation capacity.  
1265  
1266  
1267  
1268

1269 **Supporting Information and Legends**

1270

1271 The Supporting Information is provided as the following files:

1272

1273 1) Supplementary results, Supplementary Figures 1-9, and Supplementary Tables 1 and 2

1274 (single pdf file)

1275 2) Supplementary Worksheet 1 (.xlsx format), of differentially expressed genes in the

1276 indicated conditions.

1277 3) Supplementary Worksheet 2 (.xlsx format), list of anabolic and translation related genes

1278 induced in the indicated conditions.

1279 4) Supplementary Worksheet 3 (.xlsx format), of GO categories of genes up/down regulated

1280 in the indicated conditions.

1281 5) Supplementary Worksheet 4 (.xlsx format), of Gcn4 targets based on ChIP-seq analysis.

1282 6) Supplementary Worksheet 5 (.xlsx format), of genes repressed by Gcn4 that are in bin 3

1283 (from Figure 4).

1284 7) Supplementary Worksheet 6 (.xlsx format), of genes induced by Gcn4 and repressed by

1285 Gcn4, and overlap of Gcn4 targets from starvation studies.

1286