

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

2

3

4 Rajalakshmi Srinivasan¹, Adhish S. Walvekar¹, Aswin Seshasayee² and Sunil Laxman¹

5

6 ¹ Institute for Stem Cell Science and Regenerative Medicine (inStem)

7 ² National Centre for Biological Sciences - TIFR

8 GKVK post, Bellary Road

9 Bangalore 560065.

10

11 email: aswin@ncbs.res.in , 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 ≥ 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 < 10⁻¹⁰)
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 β -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 OD₆₀₀ of
512 ~0.2. Once the OD₆₀₀ 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 OD₆₀₀ 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 OD₆₀₀ 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 OD₆₀₀ 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 OD₆₀₀ of 0.2
544 and grown till they reached an OD₆₀₀ 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 OD₆₀₀ of 0.2 and incubated at 30°C until the OD₆₀₀ 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, μ =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 ¹⁵N-label incorporation in amino acids. We used ¹⁵N-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 ¹⁵N labelled ammonium sulfate and incubated for
628 5 minutes or 15 minutes as indicated. After the ¹⁵N 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, C¹³ 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_{RKrich genes} + 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 β -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^r)
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 OD₆₀₀ 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 β -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 μ 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 PPH, 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, Macinskis 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

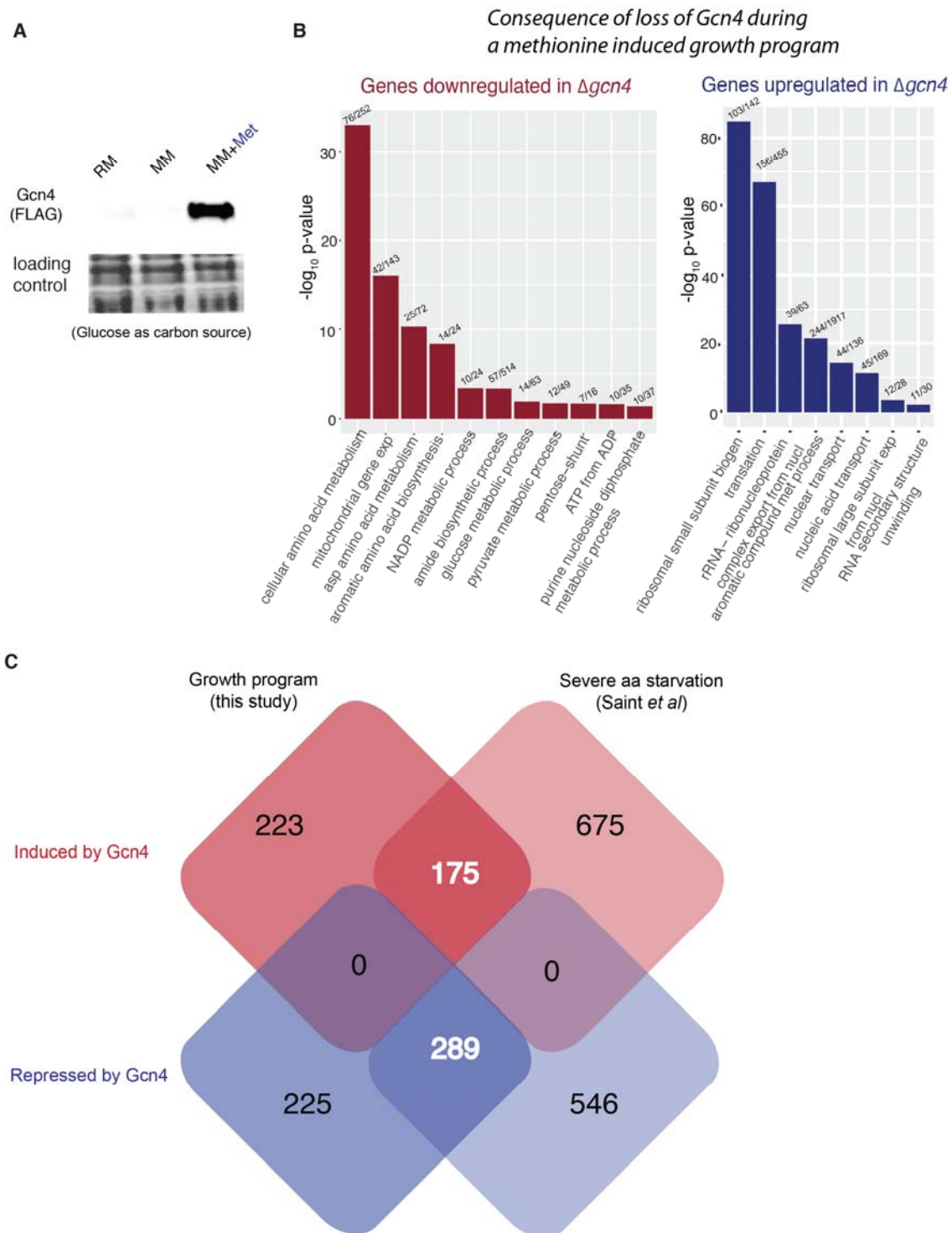
- 957 inducing the SAM-responsive methylation of PP2A. *Cell*. 2013;154: 403–15.
958 doi:10.1016/j.cell.2013.06.041
- 959 19. Lees EK, Banks R, Cook C, Hill S, Morrice N, Grant L, et al. Direct comparison of methionine
960 restriction with leucine restriction on the metabolic health of C57BL/6J mice. *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, Macinskis 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

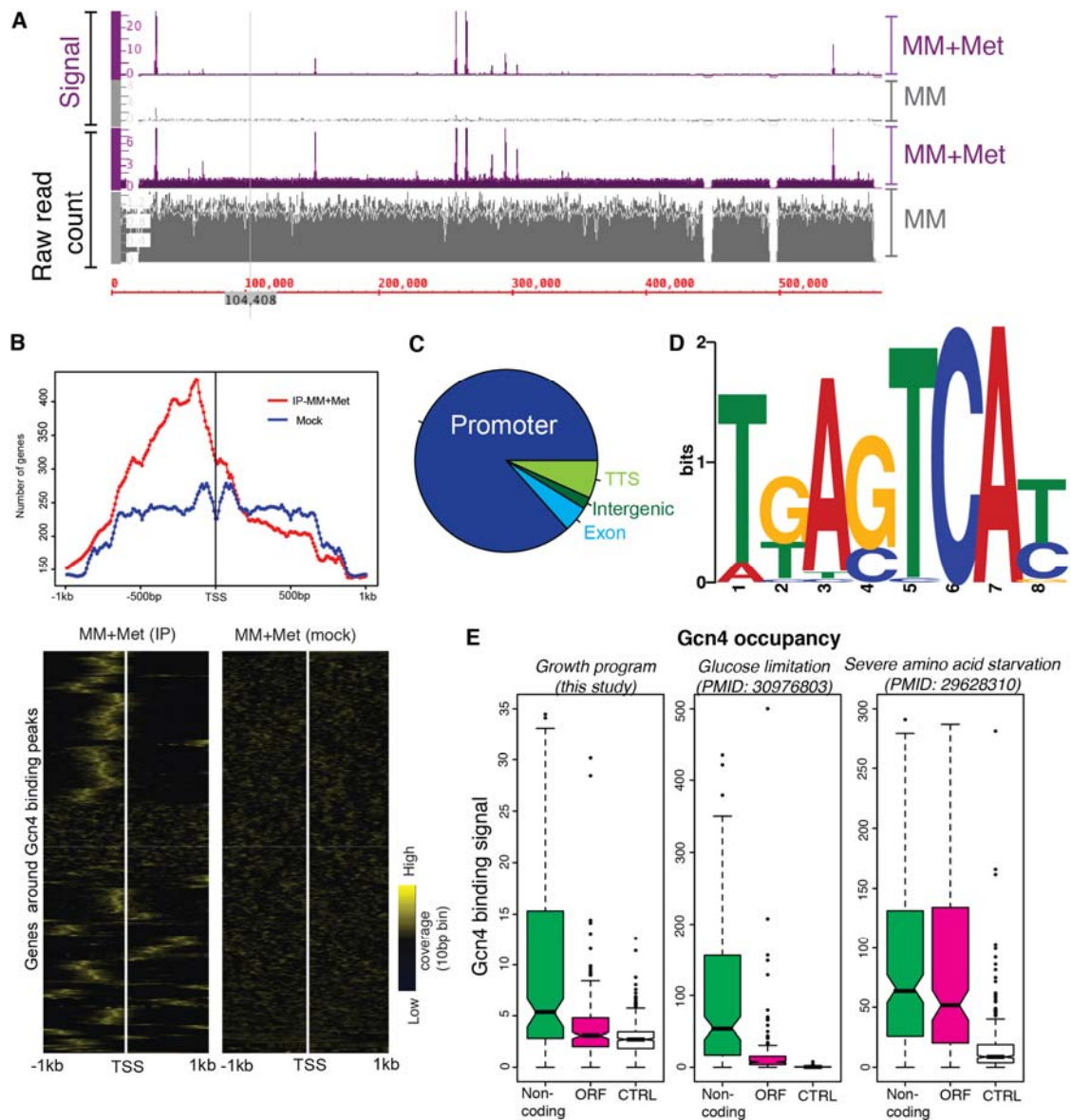


1143

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

1167

1168

1169

1170

1171

1172

1173

1174

1175

1176

1177

1178

1179

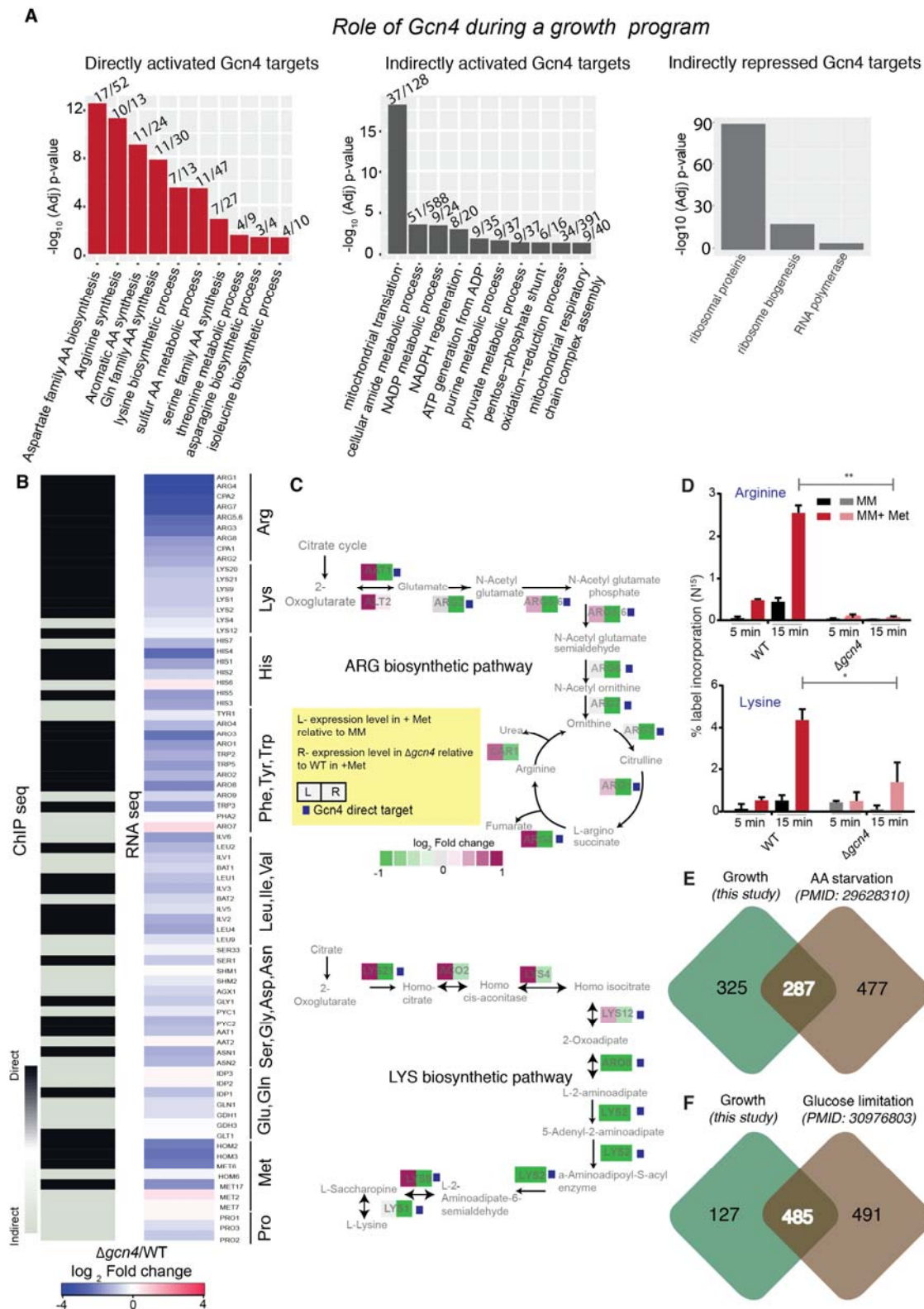
1180

1181

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

- 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.
- (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.
- A pie chart showing the genomic features of the identified peaks annotated using 'annotatepeak' function of Homer tool [48].
- Consensus binding motifs identified in *Gcn4* binding peaks from MM+Met conditions.
- Boxplots, showing the *Gcn4* binding signal corresponding to different genomic

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.

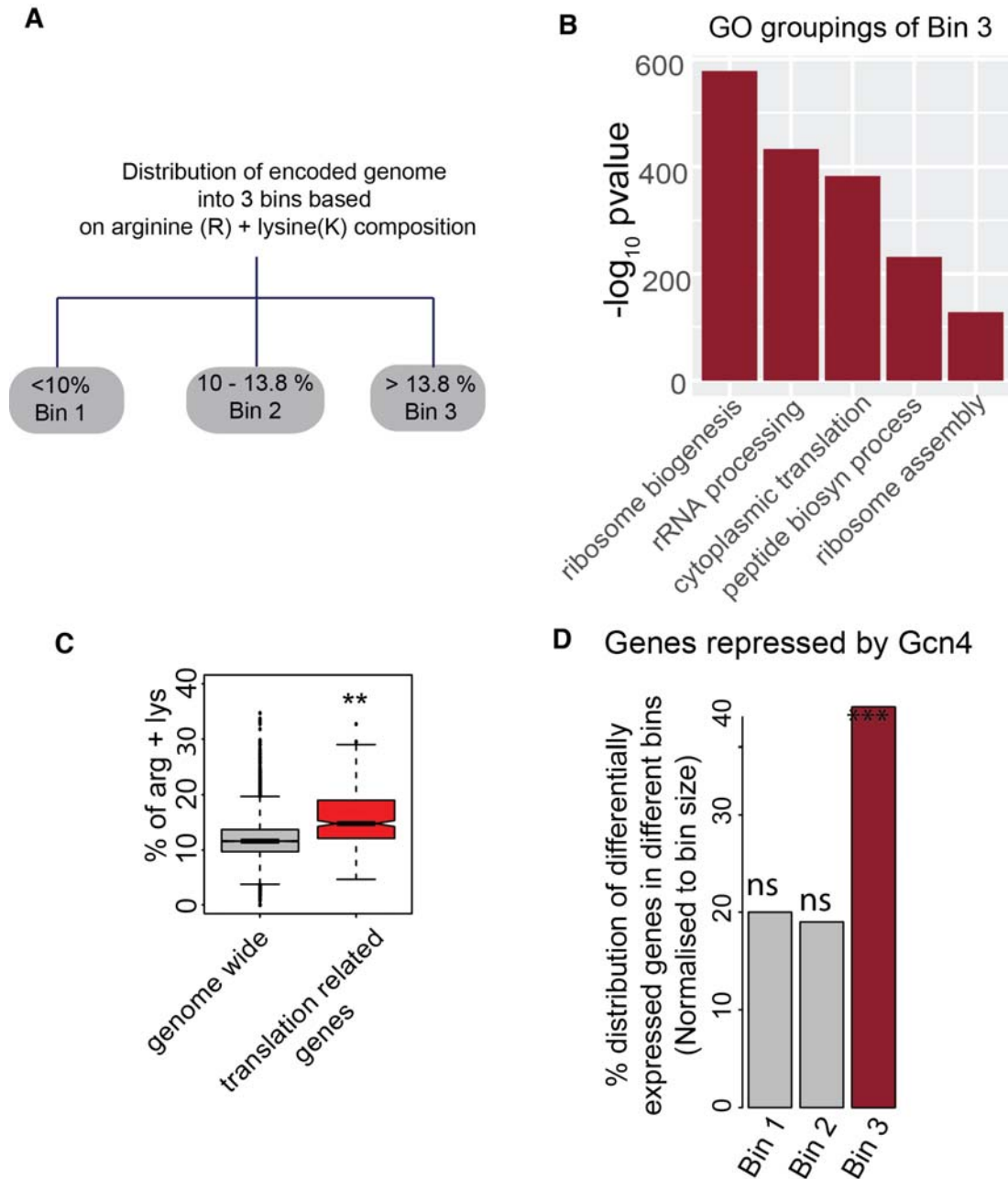
1198 B. Comparing direct targets of Gcn4 regulon, and gene expression profiles of WT and $\Delta gcn4$ cells
1199 (Gcn4 dependence) in a growth program. The heat map on the left shows whether the
1200 indicated gene (involved in amino acid biosynthesis) is directly or indirectly regulated by Gcn4
1201 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

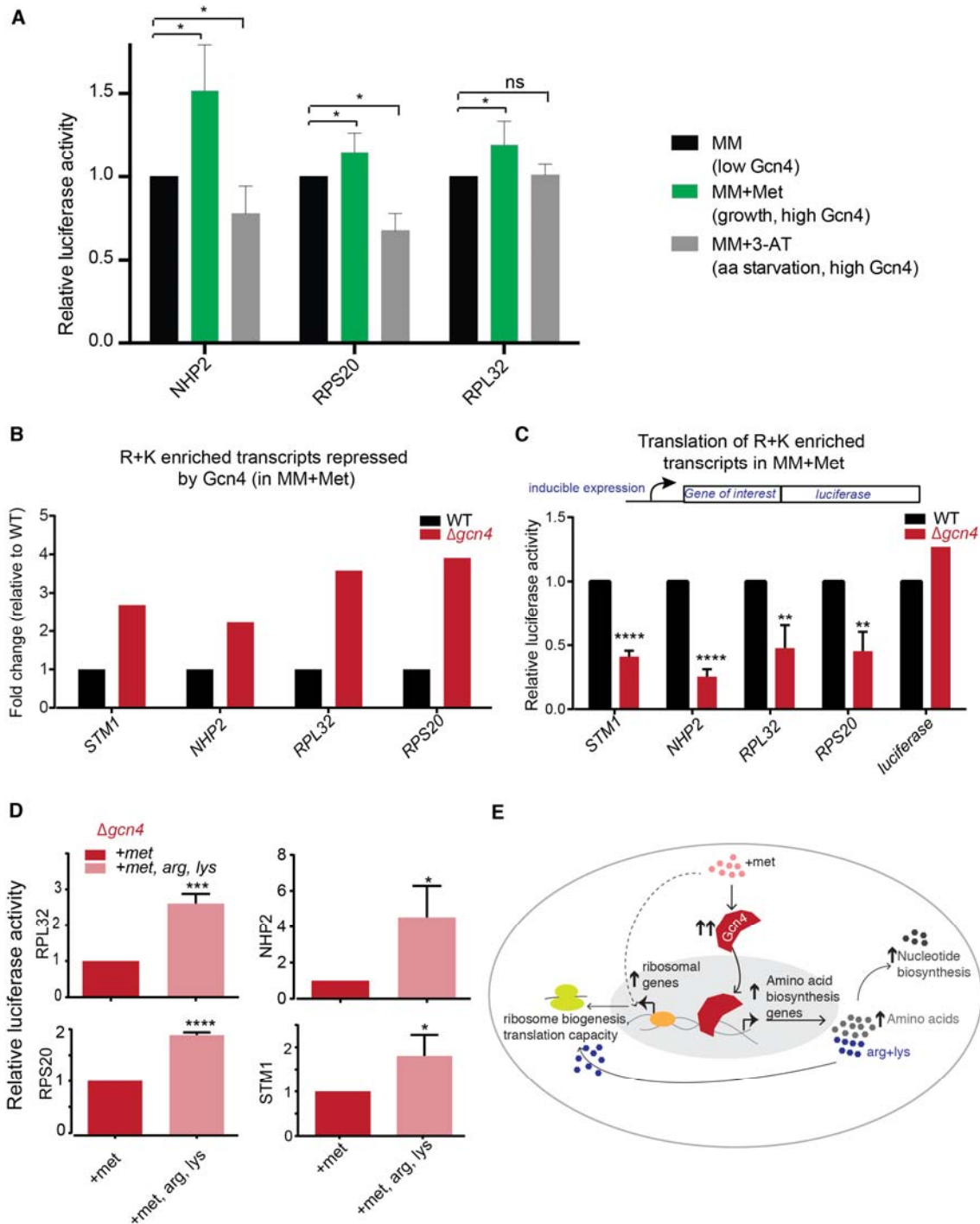
C. Boxplot, comparing the arginine and lysine composition of the entire proteome (excluding translation related genes), and the translation related genes. The translation related genes have a significantly higher than genome wide composition of arginine and lysine.

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