

1 The MTORC1-AHR pathway sustains translation and autophagy in tumours under 2 tryptophan stress

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57 **Abstract**

58 Tumours face tryptophan (Trp) depletion, but the mechanisms sustaining protein biosynthesis
59 under Trp stress remain unclear. We report that Trp stress increases the levels of the translation
60 repressor EIF4EBP1. Yet, at the same time, EIF4EBP1 is selectively phosphorylated by the
61 metabolic master regulator MTORC1 kinase, preventing EIF4EBP1 from inhibiting translation.
62 MTORC1 activity under Trp stress is unexpected because the absence of amino acids is typically
63 linked with MTORC1 inhibition. EIF4EBP1-sensitive translation in Trp starved cells is sustained
64 by EGFR and RAS signalling to MTORC1. Via this mechanism, Trp stress enhances the synthesis
65 and activity of the aryl hydrocarbon receptor (AHR). This is noteworthy as Trp catabolites are
66 known to activate AHR, and therefore Trp stress was previously considered to inhibit AHR. Trp
67 stress-induced AHR enhances the expression of key regulators of autophagy, which sustains
68 intracellular Trp levels and Trp-charged tRNAs for translation. Hence, Trp stress switches
69 MTORC1 from its established inhibitory function into an enhancer of autophagy, acting through
70 AHR. The clinical potential of this fundamental mechanism is highlighted by the activity of the
71 mTORC1-AHR pathway and an autophagy signature in 20% of glioblastoma patients, opening up
72 new avenues for cancer therapy.

73 **Main**

74 Protein biosynthesis is essential for tumour survival and progression^{1,2} and requires an adequate
75 supply of amino acids. Cancers contain poorly vascularized areas and inefficient tumour blood
76 vessels compromise nutrient delivery. As the least abundant essential amino acid, tryptophan
77 (Trp) will be the first to become limiting upon nutrient restriction. Trp catabolism is often
78 upregulated in cancer including glioblastoma (GB), and activation of the aryl hydrocarbon receptor
79 (AHR) by Trp catabolites promotes tumour progression³⁻⁶. In GB models, Trp levels decline with
80 increasing distance from blood vessels⁷, and GB patients exhibit decreased Trp levels in blood
81 and tumour tissue⁸⁻¹⁰. However, the mechanisms via which tumours sustain protein biosynthesis
82 under Trp stress are poorly understood.

83 Translation in cancer is tightly coupled to the presence of amino acids¹¹. Amino acids and
84 growth factors activate the mechanistic target of rapamycin (MTOR) complex 1 (MTORC1)
85 kinase¹², which enhances translation initiation via phosphorylation of several substrates. These
86 include an activating phosphorylation of S6 kinase (RPS6KB1) at T389^{13,14} and inhibitory
87 phosphorylation of the translation repressive 4E binding protein (EIF4EBP) at multiple sites^{15,16}.
88 Phosphorylated EIF4EBP loses binding to the translation initiation factor 4E (EIF4E), thus
89 enhancing EIF4E association with the translation initiation factor 4G (EIF4G)¹⁷⁻¹⁹. MTORC1
90 repression by amino acid limitation is well documented for arginine, leucine, methionine, glutamine
91 and asparagine²⁰⁻²³. Relatively little is known about how Trp stress signals to MTORC1 and
92 translation. Trp deprivation inhibits phosphorylation of RPS6KB1 at T389²⁴⁻²⁶, which is in line with
93 the idea that MTORC1 activity is low and does not enhance translation. However, we find that
94 translation under Trp stress is enabled by (1) MTORC1-mediated EIF4EBP1 phosphorylation
95 which sustains translation initiation and (2) MTORC1-AHR-driven autophagy providing Trp for
96 tryptophanyl-tRNA charging.

97 **MTORC1 phosphorylates EIF4EBP1 and sustains translation under Trp stress.**

98 Human GB tissues exhibit extensive regions of Trp restriction (**Fig. 1a**), and we wondered whether
99 and how this aggressive tumour sustains protein biosynthesis when Trp is scarce. We compared
100 physiological Trp levels of 78 μ M²⁷ to 24 h of Trp starvation in LN-18 GB cells. Trp stress reduced
101 the amount of charged tryptophanyl-tRNA (**Fig. 1b**), suggesting an impact on translation. We
102 conducted a puromycin incorporation assay (**Fig. 1c,d**) to assess *de novo* protein biosynthesis²⁸.
103 In line with reduced translation, Trp stress decreased puromycin incorporation, but it was even
104 further diminished by the translation elongation inhibitor cycloheximide (CHX)²⁹. Thus, protein
105 biosynthesis under Trp stress continues at a lower level. We analysed the concentration-

106 dependent effects of exogenous Trp and found that decreasing Trp levels reduced
107 phosphorylation of RPS6KB1-T389 (**Fig. 1e,f**) as reported previously^{24,25}, and enhanced the level
108 of the translation repressor EIF4EBP1 (**Fig. 1e,g**). These findings are in line with overall reduced
109 translation. We asked which mechanisms drive protein synthesis when Trp is scarce, and we
110 made the following observations: (1) As the total levels of EIF4EBP1 increased with declining Trp
111 concentrations, also EIF4EBP1 phosphorylation at T37/46 was induced (**Fig. 1e,h**). Normalization
112 of EIF4EBP1 phosphorylation to the EIF4EBP1 total levels showed that as the total levels
113 increased upon Trp stress, phosphorylation of EIF4EBP1 increased such that the ratio between
114 the two was maintained (**Extended Data Fig. 1a & throughout the manuscript**). We
115 corroborated the result in LN-229 GB cells (**Extended Data Fig. 1b-e**), and we reasoned that
116 EIF4EBP1 phosphorylation may sustain translation initiation under Trp stress. (2) As the
117 extracellular Trp concentration dropped from 78 to 7.8 μ M, the intracellular Trp concentration
118 dropped by two orders of magnitude from 267.9 μ M to 3.5 μ M (**Extended Data Fig. 1f-h**). Yet,
119 intracellular Trp declined only marginally with further extracellular Trp reduction (**Extended Data**
120 **Fig. 1f-h**). This suggested that the cells sustained the low intracellular Trp concentration to secure
121 Trp as a building block for translation.

122 We investigated how EIF4EBP1 phosphorylation is regulated and whether it is required for
123 translation under Trp stress. Apart from MTORC1, several other kinases can phosphorylate
124 EIF4EBP1-T37/46³⁰. We inhibited MTORC1 by the ATP-analogue inhibitor AZD8055³¹, which
125 efficiently blocks EIF4EBP1 phosphorylation by MTORC1^{15,16,19}. AZD8055 reduced Trp stress-
126 induced EIF4EBP1 phosphorylation at the MTORC1 substrate sites T37/46 and T70 (**Fig. 1i-k**,
127 **Extended Data Fig. 1i,j**). In further support of MTORC1-mediated phosphorylation of EIF4EBP1
128 under Trp stress, an antibody recognizing non-phosphorylated EIF4EBP1-T46 detected an
129 increased signal upon AZD8055 treatment of Trp-depleted cells (**Fig. 1i,l, Extended Data Fig.**
130 **1k**). An *in vitro* kinase assay confirmed that under Trp stress MTORC1 remained active and
131 phosphorylated EIF4EBP1 (**Fig. 1m,n**). We conclude that MTORC1 phosphorylates EIF4EBP1
132 under Trp stress. EIF4EBP1 phosphorylation inhibits EIF4EBP1 binding to the translation initiation
133 factor EIF4E, thereby enabling EIF4E-EIF4G complex formation and cap-dependent translation
134 initiation³². In line with the puromycin assay (**Fig. 1c,d**), a cap binding assay (**Fig. 1o-q, Extended**
135 **Data Fig. 1l-q**) confirmed that under Trp stress, translation was reduced but remained active: Trp
136 stress enhanced EIF4EBP1-EIF4E binding (**Fig. 1o,p**) while EIF4E-EIF4G association (**Fig. 1o,q**)
137 was preserved at a lower level. Under Trp stress, AZD8055 further enhanced EIF4EBP1-EIF4E
138 binding (**Fig. 1o,p**) and abolished EIF4E-EIF4G complex formation (**Fig. 1o,q**), demonstrating that
139 EIF4EBP1 phosphorylation by MTORC1 is required for cap-dependent translation under Trp

140 stress. The small compound 4EGI-1 is an EIF4EBP1 agonist that enhances EIF4EBP1-EIF4E
141 binding and suppresses EIF4E-EIF4G association, thus inhibiting translation initiation at the
142 cap^{33,34}. Under Trp stress, both 4EGI-1 (**Extended Data Fig. 1r,s**) and AZD8055 (**Fig. 1r-t**,
143 **Extended Data Fig. 1t**) inhibited puromycin incorporation, further supporting that EIF4EBP1
144 phosphorylation by MTORC1 sustains translation under Trp stress. Our data show that MTORC1-
145 mediated phosphorylation prevents the Trp stress-induced increase in total EIF4EBP1 from
146 inhibiting translation, thereby sustaining protein biosynthesis. We conclude that MTORC1 is active
147 under Trp stress, which expands the common view that MTORC1 is inhibited by amino acid
148 deprivation³⁵⁻³⁸ and puts Trp into a unique position in the control of MTORC1.

149

150 **The EGF receptor and RAS signal Trp stress to EIF4EBP1.**

151 Growth factors activate MTORC1 via class I phosphoinositide 3-kinases (PI3Ks)³⁶. The pan class
152 I PI3K inhibitor Pictilisib (GDC-0941)³⁹ inhibited EIF4EBP1 phosphorylation at T37/46 in Trp-
153 restricted cells (**Extended Data Fig. 2a-c**), indicating that PI3K signals to MTORC1 and
154 EIF4EBP1 when Trp is scarce. We went on to investigate which upstream cues mediate Trp stress
155 signalling to EIF4EBP1. PI3K is a key effector of the small GTPase RAS⁴⁰, whose activation has
156 been primarily assigned to growth factor inputs^{41,42}. RAS activation by stress is less established⁴³⁻
157 ⁴⁶, and nutrient stress or Trp restriction have so far not been linked to RAS. In a RAS-GTP pull
158 down assay⁴⁴, Trp restriction enhanced RAS binding to a RAF-RAS-binding domain (GST-RAF1),
159 indicative of enhanced RAS-GTP loading and activity (**Fig. 2a,b**). Knockdown of all RAS isoforms
160 (KRAS/HRAS/NRAS) reduced phosphorylation of EIF4EBP1-T37/46 in Trp-deprived cells (**Fig.**
161 **2c-e, Extended Data Fig. 2d**). The epidermal growth factor (EGF) receptor (EGFR) acts upstream
162 of RAS⁴⁷ and is frequently amplified in GB⁴⁸. Autophosphorylation of the EGFR at Y1068^{49,50} was
163 enhanced in Trp-deprived cells with (**Extended Data Fig. 2e,f**) and without EGF stimulation (**Fig.**
164 **2f,g**). Trp stress enhanced EGFR internalization to perinuclear endosomes (**Fig. 2h,i**), consistent
165 with EGFR activation⁵¹. The pan-ERBB (EGF receptor family) inhibitor Afatinib⁵² as well as the
166 EGFR-specific inhibitor Erlotinib⁵³ reduced Trp stress-induced phosphorylation of EIF4EBP1-
167 T37/46 (**Fig. 2j-l, Extended Data Fig. 2g**), showing that EGFR mediates Trp stress signalling to
168 EIF4EBP1. EGFR activation by Trp stress without exogenous EGF addition (**Fig. 2f,g**) suggested
169 a contribution by an endogenous ligand. Whereas *EGF* mRNA levels were reduced by Trp
170 restriction (**Fig. 2m**), levels of *EREG* (epiregulin) mRNA (**Fig. 2n**) as well as unglycosylated and
171 glycosylated pro-EREG proteins⁵⁴ were enhanced with declining Trp levels (**Fig. 2o-q**). We
172 conclude that EGFR and RAS drive signalling to EIF4EBP1 in Trp-deprived cells. Given the
173 activation of the EGFR-RAS pathway, one would have anticipated both *bona fide* MTORC1

174 substrates EIF4EBP1 and RPS6KB1 to become phosphorylated. Surprisingly, however, Trp
175 stress exerted opposing effects on the two MTORC1 substrates as it enhanced phosphorylation
176 of EIF4EBP1, but reduced phosphorylation of RPS6KB1 (**Fig. 1e,f,h, Extended Data Fig. 1b,c,e**).
177 We found that this divergent regulation was mediated by the MTORC1 suppressor Sestrin2
178 (SESN2)⁵⁵⁻⁶⁰. Trp stress induced SESN2 levels, and SESN2 knockdown selectively enhanced
179 RPS6KB1-T389 phosphorylation (**Fig. 2r-u, Extended Data Fig. 2h**). Thus, SESN2 represses
180 RPS6KB1 phosphorylation but not EIF4EBP1 phosphorylation under Trp stress.

181 **EIF4EBP1-sensitive translation induces AHR expression and activity under Trp stress.**

182 We explored the protein repertoire, which is induced by Trp stress. 364 proteins were increased
183 upon Trp deprivation (**Fig. 3a**). Ribosome profiling showed ribosome pausing at Trp codons (TGG)
184 under Trp stress (**Extended Data Fig. 3a**), potentially leading to accumulation of incomplete
185 polypeptides. However, the proteome data showed that peptide coverage was not altered by Trp
186 stress (**Extended Data Fig. 3b,c**) and extended beyond Trp residues (**Extended Data Fig. 3d**),
187 supporting that Trp stress did not interrupt translation prematurely. We compared the proteomes
188 under Trp stress, generalized amino acid stress in amino acid-free DMEM or HBSS media, or
189 starvation of methionine, another essential amino acid required for translation initiation (**Fig.**
190 **3b,c,d**). Trp stress shared only 29 upregulated proteins with general amino acid starvation
191 (**Extended Data Fig. 3e**), and 31 proteins with methionine stress (**Extended Data Fig. 3f**). 335
192 or 333 upregulated proteins were exclusive to Trp stress, as compared to generalized amino acid
193 or methionine stress, respectively (**Extended Data Fig. 3e,f**). Similar results were found for the
194 downregulated proteins in either condition (**Extended Data Fig. 3g,h**). Neither general amino acid
195 stress nor methionine stress enhanced EIF4EBP1 phosphorylation (**Fig. 3e-i, Extended Data Fig.**
196 **3i**). Thus, Trp stress has a profound impact on the proteome, which differs from generalized amino
197 acid stress and methionine deprivation.

198 Intriguingly, the AHR was specifically induced in the Trp stress proteome (**Fig. 3a**) and AHR
199 levels increased with declining Trp concentrations (**Fig. 3j,k**). We investigated if AHR induction
200 was mediated by EIF4EBP1-sensitive translation. Ribosome profiling showed an increased
201 association of ribosomes with AHR transcripts under Trp stress as well as full ribosome coverage
202 up to the 3' end (**Fig. 3l, Extended Data Fig. 3j**). Also, polysome profiling indicated that the AHR
203 was preferentially translated under Trp stress (**Fig. 3m**). In agreement, the translation elongation
204 inhibitor CHX fully suppressed AHR protein induction by Trp stress (**Extended Data Fig. 3k,l**) and
205 AHR induction upon Trp stress was EGFR- (**Extended Data Fig. 3m-p**), MTOR- (**Fig. 3n,o**,
206 **Extended Data Fig. 3q,r**) and EIF4EBP1-sensitive (**Extended Data Fig. 3s,t**). Trp stress

207 enhanced AHR levels only in cells expressing EIF4EBP1 wildtype but not in cells expressing a
208 non-phosphorylatable EIF4EBP1-T37/46A mutant¹⁵ (**Fig. 3p-r, Extended Data Fig. 3u**).
209 Furthermore, AZD8055 could not reduce AHR levels in cells with non-phosphorylatable
210 EIF4EBP1-T37/46A, showing that MTORC1 controls AHR expression via EIF4EBP1.

211 Notably, Trp stress enhanced not only AHR levels but also AHR activity, as determined by
212 induction of the AHR target gene *CYP1B1* that was suppressed by AHR knockdown (**Fig. 3s**,
213 **Extended Data Fig. 3v**). Also, RNAseq analysis (**Table S2**) revealed that Trp stress enhanced a
214 transcriptional AHR activity signature⁶¹ (**Fig. 3t, Extended Data Fig. 3w-y**). This finding was
215 unexpected as the AHR is typically considered to be activated by Trp metabolites⁶²⁻⁶⁵, but not
216 under Trp restriction when Trp metabolites are low or absent.

217
218 **The MTORC1-AHR pathway enhances autophagy to replenish intracellular Trp levels and**
219 **to sustain tryptophanyl-tRNA charging under Trp stress.**

220 We went on to investigate the functions of the MTORC1-AHR axis under Trp stress. Trp limitation
221 enhanced the enrichment of gene ontology (GO) terms related to macropinocytosis and
222 lysosomes (**Fig. 4a, Table S1**). Macropinocytosis is a non-selective process driven by the EGFR
223 and RAS^{66,67}, leading to uptake of the extracellular fluid-phase and macromolecules⁶⁸. We
224 measured internalization of fluorescently-labeled dextran to stain macropinosomes and found
225 them increased upon Trp depletion (**Fig. 4b,c**). Upon macropinosome maturation, their content is
226 delivered to the lysosomal compartment for degradation⁶⁸. In agreement with the GO term
227 enrichment of lysosomal components in the Trp stress proteome (**Fig. 4a**), the LAMP2 (lysosomal
228 associated membrane protein 2)-positive lysosomal area (**Fig. 4d,e**) and lysosome activity (**Fig.**
229 **4f,g**) were enhanced by Trp stress. Autophagy constitutes a lysosome function, which is critical in
230 conjunction with macropinocytosis to break down macromolecules and fuel cancer metabolism
231 under nutrient limitation⁶⁸⁻⁷⁰. Lipidated LC3 (ubiquitin-like protein microtubule associated protein 1
232 light chain 3 beta, MAP1LC3B), termed LC3-II, decorates autophagosomes and serves as an
233 anchor for autophagy receptor proteins⁷¹. To assess autophagic flux⁷², LC3-II was detected in
234 conjunction with inhibition of lysosome acidification by Bafilomycin A₁ (BafA) (**Fig. 4h-k, Extended**
235 **Data Fig. 4a**). Under Trp replete conditions, BafA only mildly increased LC3-II, indicative of low
236 autophagic flux, and AHR inhibition by stemreginin-1 (SR1)⁷³ did not affect LC3-II levels. MTOR
237 inhibition enhanced LC3-II, consistent with its well-known function as an autophagy suppressor
238 under nutrient replete conditions⁷². Trp stress enhanced autophagic flux, based on LC3-II
239 induction by BafA (**Fig. 4h,i**) and an increased autophagy mRNA signature⁷⁴ (**Fig. 4l, Extended**

240 **Data Fig. 4b-c).** Under Trp stress, MTOR inhibition as well as AHR inhibition reversed the LC3-II
241 level back to that without BafA (**Fig. 4h,i**). Also, the number of foci of the autophagy receptor
242 SQSTM1 (sequestosome 1, p62)⁷², an alternative readout for autophagy, was reduced by MTOR
243 inhibition as well as by AHR inhibition under Trp stress (**Fig. 4m,n**). We conclude that the
244 MTORC1-AHR pathway drives autophagy under Trp stress. This finding is intriguing as MTORC1
245 switches from its canonical, inhibitory role in autophagy to becoming an autophagy activator under
246 Trp stress.

247 Under nutrient replete conditions, MTORC1 inhibits autophagy by repressing the kinase
248 ULK1 (unc-51 like autophagy activating kinase 1) and the TFEB/ TFE3 (transcription factor EB/
249 transcription factor binding to IGHM enhancer 3) transcription factors, two central enhancers of
250 autophagy⁷². These mechanisms cannot provide an explanation for the observed reduction in
251 autophagy by MTOR inhibition under Trp stress: if the MTOR inhibitor were to further decrease
252 ULK1 and TFEB/TFE3 phosphorylation, it would enhance autophagy, contrary to our observation.
253 We therefore reasoned that under Trp stress, the MTORC1-AHR pathway must induce autophagy
254 via another route. Of note, Trp stress enhanced not only LC3 lipidation but also LC3 total levels in
255 an MTOR- and AHR-dependent manner (**Fig. 4k**). In the Trp stress transcriptome, LC3 was
256 among the most upregulated transcripts (**Fig. 4l, Table S2**). In agreement with AHR-driven LC3
257 expression, we identified an AHR binding motif in the LC3 promotor region (**Extended Data Fig.**
258 **4d**). Ribosome profiling showed that also LC3 translation was enhanced under Trp stress
259 (**Extended Data Fig. 4e**). Thus, LC3 is transcribed and translated *de novo* under Trp stress. AHR
260 overexpression (**Fig. 4o-q, Extended Data Fig. 4f,g**) was sufficient to enhance LC3 at mRNA
261 levels (**Fig. 4p**) and LC3 lipidation (**Fig. 4o,q**) demonstrating that the transcription factor AHR
262 induces LC3 expression and autophagy. To investigate a wider array of autophagy regulators
263 under Trp stress (**Fig. 4l**), we quantified a panel of core autophagy regulators by targeted
264 quantitative proteomics upon lysosome blockade by BafA in combination with MTOR or AHR
265 inhibition (**Fig. 4r**). Under Trp replete conditions, most proteins were enhanced by MTOR
266 inhibition, in line with increased autophagic flux. In contrast, under Trp stress several central
267 autophagy receptor, anchor and cargo proteins including GABARAPL1 (GABA type A receptor
268 associated protein like 1) and SQSTM1 (p62) depended on MTOR and AHR (**Fig. 4r**, cluster 1).
269 Taken together, the MTORC1-AHR pathway induces the expression of core components of the
270 autophagy machinery and drives autophagic flux under Trp stress. Via this mechanism, Trp stress
271 triggers a functional switch of MTORC1 in autophagy, making it an autophagy activator. Of note,
272 BafA-mediated inhibition of lysosomal function induced a drop in intracellular Trp (**Fig. 4s**) and
273 increased the proportion of uncharged tryptophanyl-tRNAs (**Fig. 4t**) in Trp-deprived cells. This

274 indicates that lysosome-derived Trp helps cancer cells to overcome Trp limitation by maintaining
275 tryptophanyl-tRNA charging for translation.

276 **The mTORC1-AHR pathway is active in human glioblastomas.**

277 We next interrogated whether the MTORC1-AHR pathway is present in human tumours. We
278 clustered transcriptome data of human GB (The Cancer Genome Atlas, TCGA)⁷⁵ based on the
279 transcripts regulated by Trp stress in the GB cell lines (**Table S2**), yielding seven patient
280 subgroups. Using an AHR activity signature⁶¹ we identified two patient subgroups (1 and 2) with
281 high AHR activity scores (**Fig. 5a**). Patient subgroup 1 (~10% of the GB patients) exhibited low
282 AHR mRNA levels, whereas patient subgroup 2 (~20% of the GB patients) showed high *AHR*
283 mRNA levels (**Fig. 5b**). We reasoned that the latter patient subgroup may have experienced Trp
284 restriction, as Trp stress induces AHR expression. The TCGA reverse phase protein array (RPPA)
285 data revealed that subgroup 2, with high AHR expression, exhibited higher EIF4EBP1-T37/46
286 phosphorylation than subgroup 1 (**Fig. 5c**), whereas RPS6KB1-T389 phosphorylation was similar
287 in both patient groups (**Fig. 5d**). This further supports that GB tumour tissues in subgroup 2 show
288 signs of Trp deprivation, as they featured low RPS6KB1 and high EIF4EBP1 phosphorylation as
289 well as high AHR levels. In agreement, subgroup 2 showed enhanced expression of a
290 transcriptional autophagy regulator signature⁷⁴ (**Fig. 5e**). Thus, an augmented autophagy
291 signature associates with high EIF4EBP1 phosphorylation, high AHR levels, and high AHR
292 activity. Taken together, the data suggest that the MTORC1-AHR pathway is active and drives
293 autophagy in 20% of human GB. The AHR positively regulates various enzymes of the ceramide
294 biosynthetic pathway, including sphingomyelin phosphodiesterases (SMPD,
295 sphingomyelinases)^{76,77} that catalyze the conversion of sphingomyelin to ceramides in
296 lysosomes⁷⁸. Therefore, we investigated ceramides as potential markers for the activity of the
297 MTORC1-AHR pathway, detectable in tumour tissue. In GB cells, Trp stress increased the levels
298 of ceramides, which were suppressed by MTOR and AHR inhibitors (**Fig. 5f, Table S3**). Thus,
299 MTOR and the AHR enhance ceramide levels upon Trp stress, further highlighting the MTORC1-
300 AHR pathway as a driver of lysosomal function. In GB tissues, MALDI-MS imaging revealed that
301 regions of high Trp and high ceramides were mutually exclusive (**Fig. 5g**), suggesting that low Trp
302 and high ceramides – marking the start and endpoints of the MTORC1-AHR pathway – are
303 coupled in human tumours and indicate active MTORC1-AHR signalling.

304 **Discussion**

305 We address the fundamental question of how tumours maintain protein biosynthesis when Trp is
306 scarce (**Fig. 5h**). We report that under Trp stress EIF4EBP1 phosphorylation by MTORC1 sustains
307 translation initiation, and EIF4EBP1-controlled translation induces the AHR. As a result, MTORC1-
308 AHR signalling emerges as a novel pathway under Trp stress, which drives autophagy,
309 replenishing intracellular Trp levels and tryptophanyl-tRNAs for translation. Hence, the MTORC1-
310 AHR pathway enables protein synthesis under Trp stress in a twofold manner, at the level of
311 translation initiation and by providing tryptophanyl-tRNAs. The MTORC1-AHR pathway features
312 several molecular functions that differ from the established picture of MTORC1 or the AHR alone.
313 Trp stress enhances the level of the key translation repressor EIF4EBP1. At the same time,
314 however, MTORC1 remains active and selectively phosphorylates EIF4EBP1, which expands the
315 common view that amino acid limitation inhibits MTORC1^{79,80}. EIF4EBP1-sensitive translation
316 promotes expression of the AHR, known to be activated by Trp via its catabolites⁶²⁻⁶⁵. However,
317 we find that also Trp stress activates the AHR. The MTORC1-AHR pathway induces major
318 controllers of autophagy. Therefore, Trp stress switches MTORC1's role in autophagy from its
319 canonical inhibitory function to becoming an autophagy enhancer, whereby MTORC1 acts via the
320 AHR.

321 Our finding that MTORC1 remains active when the essential amino acid Trp is depleted
322 changes our view on the interplay of amino acids with this key tumour driver. Withdrawal of the
323 single amino acids arginine, leucine, methionine, glutamine, and asparagine represses MTORC1-
324 dependent translation through inhibition of the RRAG GTPases^{20,79} and other lysosomal
325 regulators²¹⁻²³. Trp stress is different in that it selectively enhances phosphorylation of EIF4EBP1,
326 but not RPS6KB1, and the Trp stress proteome profoundly differs from other amino acid stresses.
327 Selective inhibition of RPS6KB1 by the RRAG repressor SESN2^{55,56} has not been described so
328 far^{79,80}, and it requires further investigation whether the lower affinity of RPS6KB1 to
329 MTORC1^{13,19,81} explains RPS6KB1's higher sensitivity to MTORC1 inhibition by SESN2. Long
330 term leucine or arginine starvation has been linked with MTORC1 activation via PI3K and AKT⁸²,
331 however there was no divergence between RPS6KB1 and EIF4EBP1 phosphorylation, and
332 autophagy was down, suggesting that induction of the MTORC1-AHR pathway is Trp-stress-
333 specific. MTORC1 has also been found activated by long term glutamine deprivation^{83,84} but this
334 is mediated by amino acid transporters enhancing the influx of amino acids⁸³, which differs from
335 Trp stress induction of the EGFR-RAS axis upstream of MTORC1. Given that EGFR and RAS
336 also are upstream of the MAPK pathway, which impinges on EIF4E-driven translation in

337 tumours^{1,2,30}, MAPKs may contribute to the MTORC1-mediated Trp stress response upstream of
338 translation.

339 Like MTORC1, translation initiation is generally considered as being inhibited by nutrient
340 starvation and stress⁸⁵. We report that under Trp stress, translation is reduced but remains active.
341 Thus, inhibitory and activating cues balance translation under Trp restriction, allowing for
342 expression of a Trp stress-specific protein repertoire. Trp stress-induced proteome remodelling
343 may have evolved as Trp is the physiologically least abundant amino acid, and a drop in Trp levels
344 is an early indicator of an upcoming starvation for all amino acids. In other words, the Trp stress-
345 sensitive MTORC1-AHR pathway likely serves as a sentinel mechanism that senses an imminent
346 decline in amino acids. By adapting its translation repertoire, the cell can express proteins that are
347 necessary to cope with nutrient starvation while most amino acids are still sufficiently available.
348 The integrated stress response mediated via the GCN2-EIF2A-ATF4 pathway restricts translation
349 initiation under Trp stress^{30,86,87} and it will be intriguing to address its interplay with MTORC1-
350 EIF4EBP1 mediated translation. Incorporation of phenylalanine (Phe) instead of Trp has recently
351 been suggested to sustain translation in Trp-restricted tumours⁸⁸. There was a low overall
352 frequency of such events in our proteome data, and only two peptides harbouring a Trp-Phe
353 exchange increased upon Trp stress (**Table S1**). Thus, mobilization of Trp by autophagy appears
354 sufficient to sustain translation of the Trp-containing proteome, including the AHR.

355 Clinical trials with AHR inhibitors are currently ongoing for cancer immunotherapy⁸⁹. Our
356 findings demonstrate a novel role for AHR in tumour cells, i.e. enabling them to cope with Trp
357 stress by enhanced autophagy. Like the TFEB/TFE transcription factors, which enhance lysosome
358 biogenesis and autophagy⁷², also the AHR enhances autophagy by mediating the expression of
359 a differential autophagy signature that encompasses key components of the autophagy machinery
360 including LC3, GABARAPL1, and SQSTM1 (p62). However, unlike TFEB/TFE3, AHR is activated
361 by MTORC1. This is how MTORC1 switches its role and actively contributes to enhanced
362 autophagy under Trp stress.

363 Trp restriction has been suggested earlier to inhibit the AHR in tumours because Trp
364 metabolites enhance AHR activity⁹⁰. However, our data suggest that this is not a good strategy as
365 Trp stress enhances AHR levels and activity. Rather, reduced Trp levels and enhanced levels of
366 Trp catabolites synergize in boosting AHR activity and its oncogenic outcomes. Indeed, Trp
367 depletion potentiates the effect of Trp catabolites as AHR ligands⁹¹ and promotes Treg
368 differentiation⁹². Our finding that Trp stress activates the AHR highlights the need to not only
369 consider Trp metabolites but also Trp levels to predict AHR activity in tumours. Our data suggest

370 that the MTORC1-AHR pathway is active in 20% of GB patients, attesting to the clinical potential
371 of this fundamental mechanism. These patients may benefit from autophagy suppression by
372 inhibitors of MTORC1 and its upstream cues as well as its novel downstream target AHR^{93,94}. As
373 Trp shortage occurs in many cancers^{6,63}, the MTORC1-AHR pathway may also be active in tumour
374 entities beyond GB. Tumour metabolites attract growing attention as predictive markers, and we
375 determine low Trp and high ceramide levels to be at the start and end points of the MTORC1-AHR
376 pathway. The Trp/ceramide ratio hence warrants clinical testing as a predictive marker for
377 response to drugs targeting the MTORC1-AHR pathway.

378 **Figure 1: MTORC1 phosphorylates EIF4EBP1 and sustains translation under Trp stress.**

379 (a) MALDI mass spectrometry imaging (MALDI-MSI) of Trp distribution in human glioblastoma
380 (GB) sections. Colour scale: purple, low Trp; yellow, high Trp; scale bar: 1 mm. (n = 3).
381 (b) Tryptophanyl-tRNA charging under Trp replete conditions or Trp stress. Relative
382 aminoacylation levels were determined by qRT-PCR using tRNA-specific primers. LN-18 cells.
383 (n = 3).
384 (c) Translation under Trp replete conditions or Trp stress. Puromycin (5 µg/mL, 5 min)
385 incorporation in GB cells, unstimulated or stimulated with epidermal growth factor (EGF, 10 ng/mL,
386 stimulation period as indicated), and treated with the translation elongation inhibitor cycloheximide
387 (CHX) (2 µg/mL, 6.5 h). LN-18 cells. (n = 4).
388 (d) Quantification of puromycin incorporation in (c).
389 (e) Trp stress differentially alters signalling towards translation initiation. Trp concentration row:
390 Cells were cultured in medium with the indicated Trp concentrations for 24 h. LN-18 cells.
391 (n = 3-4).
392 (f) Quantification of RPS6KB1-pT389 (S6K-pT389) in (e). (n = 3).
393 (g) Quantification of EIF4EBP1 (4E-BP1) in (e). (n = 3).
394 (h) Quantification of EIF4EBP1-pT37/46 (4E-BP1-pT37/46) in (e). (n = 4).
395 (i) The MTOR inhibitor AZD8055 (100 nM, 24 h) blocks EIF4EBP1-pT37/46 (4E-BP1-pT37/46)
396 and EIF4EBP1-pT70 (4E-BP1-pT70) and increases unphosphorylated (non-phospho) EIF4EBP1-
397 T46 (4E-BP1-T46) under Trp stress. LN-18 cells. (n = 4).
398 (j) Quantification of EIF4EBP1-pT37/46 (4E-BP1-pT37/46) in (i).
399 (k) Quantification of EIF4EBP1-pT70 (4E-BP1-pT70) in (i).
400 (l) Quantification of (non-phospho) EIF4EBP1-T46 (4E-BP1-T46) in (i).
401 (m) Kinase assay with MTORC1 purified from LN-18 cells under Trp replete conditions or Trp
402 stress. MTOR actively phosphorylates EIF4EBP1-pT37/46 (4E-BP1-pT37/46) upon Trp stress.
403 Substrate: recombinant (rec.) EIF4EBP1 (4E-BP1) (100 ng). Negative controls: No EIF4EBP1 (4E-
404 BP1), mock-IP, MTOR inhibitor AZD8055 (100 nM). Reaction time as indicated. (n = 4).
405 (n) Quantification of EIF4EBP1-pT37/46 (4E-BP1-pT37/46) normalized to MTOR in (m).
406 (o) Cap pull down with m7-GTP beads from LN-18 cells under Trp replete conditions or Trp stress.
407 The MTOR inhibitor AZD8055 (100 nM, 1 h) enhances EIF4EBP1 (4E-BP1)-EIF4E binding and
408 decreases EIF4G-EIF4E binding under Trp stress. (n = 3).
409 (p) Quantification of EIF4EBP1 (4E-BP1) binding to EIF4E in (o).
410 (q) Quantification of EIF4G binding to EIF4E in (o).

411 (r) The MTOR-inhibitor AZD8055 (100 nM, 4 h) inhibits translation upon Trp stress. Puromycin
412 assay. Translation elongation inhibitor cycloheximide (CHX) (2 µg/mL, 4 h), puromycin (5 µg/mL,
413 5 min). LN-18 cells. (n = 3).

414 (s) Quantification of puromycin incorporation in (r).

415 (t) Quantification of EIF4EBP1-pT37/46 (4E-BP1-pT37/46) in (r).

416

417 Cells were cultured in the presence of Trp (+Trp, grey, 78 µM) or absence of Trp (-Trp, blue, 0 µM)
418 for 24 h, if not indicated otherwise. One-way ANOVA followed by a Šídák's multiple comparisons
419 test was applied (d, f, g, h, j, k, l, n, p, q, s, t). For (b) a two-tailed paired Student's t test was
420 performed. Data are presented as mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001. n.s., not
421 significant.

422 **Extended Data Figure 1: Under Trp stress the intracellular Trp concentration is sustained.**

423 (a) Quantification of EIF4EBP1-pT37/46 (4E-BP1-pT37/46) over total EIF4EBP1 (4E-BP1) in
424 Figure 1e.

425 (b) Trp stress differentially alters signalling towards translation initiation also in LN-229 cells. Cells
426 were stimulated with EGF (10 ng/mL) for the indicated time periods. (n = 4).

427 (c) Quantification of EIF4EBP1-pT37/46 (4E-BP1-pT37/46) in (b).

428 (d) Quantification of EIF4EBP1-pT37/46 (4E-BP1-pT37/46) over total EIF4EBP1 (4E-BP1) in (b).

429 (e) Quantification of RPS6KB1-pT389 (S6K-pT389) in (b).

430 (f) Intracellular Trp concentrations in μ M. Cells were cultured in medium with the indicated Trp
431 concentrations for 24 h. LN-18 cells. (n = 4).

432 (g) Intracellular Trp concentrations in molecules per cell. Cells were cultured in medium with the
433 indicated Trp concentrations for 24 h. LN-18 cells. (n = 4).

434 (h) Comparison between extracellular and intracellular Trp concentrations in μ M. Cells were
435 cultured in medium containing 78, 19.5, 7.8 or 0 μ M Trp for 24 h. Cultivation with 7.8 or 0 μ M Trp
436 reduces the intracellular Trp concentration from 267.9 μ M to 3.5-3.4 μ M. LN-18 cells. (n = 4).

437 (i) Quantification of EIF4EBP1-pT37/46 (4E-BP1-pT37/46) over total EIF4EBP1 (4E-BP1) in
438 Figure 1i.

439 (j) Quantification of EIF4EBP1-pT70 (4E-BP1-pT70) over total EIF4EBP1 (4E-BP1) in Figure 1i.

440 (k) Quantification of EIF4EBP1-pT46 (non-phospho) (4E-BP1-pT46) over total EIF4EBP1 (4E-
441 BP1) in Figure 1i.

442 (l) Input for cap pull down in Figure 1o.

443 (m) Quantification of EIF4EBP1-pT37/46 (4E-BP1-pT37/46) in (l).

444 (n) Quantification of EIF4EBP1-pT37/46 (4E-BP1-pT37/46) over total EIF4EBP1 (4E-BP1) in (l).

445 (o) Quantification of RPS6KB1-pT389 (S6K-pT389) in (l).

446 (p) Quantification of EIF4E in (l).

447 (q) Quantification of EIF4G in (l).

448 (r) The EIF4EBP1 (4E-BP1) agonist 4EGI-1 (10 μ M, 24 h) inhibits translation upon Trp stress.

449 Puromycin assay. Translation elongation inhibitor cycloheximide (CHX) (2 μ g/mL, 24 h),
450 puromycin (5 μ g/mL, 5 min). LN-18 cells. (n = 3).

451 (s) Quantification of puromycin incorporation in (r).

452 (t) Quantification of EIF4EBP1-pT37/46 (4E-BP1-pT37/46) over total EIF4EBP1 (4E-BP1) in
453 Figure 1r.

454

455 Cells were cultured in the presence of Trp (+Trp, grey, 78 μ M) or absence of Trp (-Trp, blue, 0 μ M)
456 for 24 h, if not indicated otherwise. One-way ANOVA followed by a Šídák's multiple comparisons

457 test was applied in (a, i, j, k, m, n, o, p, q, s, t), two-way ANOVA followed by a Šídák's multiple
458 comparisons test was applied in (c, d, e). Data are presented as mean \pm SEM. *p < 0.05, **p <
459 0.01, ***p < 0.001. n.s., not significant.

460 **Figure 2: The EGF receptor and RAS signal Trp stress to EIF4EBP1.**

461 (a) Trp stress enhances RAS-GTP binding to RAF1-GST. RAS activity was measured using GST-
462 coupled RAF-RAS-binding domain pull down experiments. Cells treated with EGF (10 ng/mL,
463 30 min). LN-18 cells. (n = 4).

464 (b) Quantification of RAS-GTP in (a).

465 (c) Pan-RAS (KRAS/HRAS/NRAS) knockdown (siRAS) reduces EIF4EBP1-pT37/46 (4E-BP1-
466 pT37/46) induction by Trp stress. Cells were either unstimulated or stimulated with EGF (10
467 ng/mL, 15 min). LN-18 cells. (n = 6).

468 (d) Quantification of KRAS/HRAS/NRAS in (c).

469 (e) Quantification of EIF4EBP1-pT37/46 (4E-BP1-pT37/46) in (c).

470 (f) Trp deprivation gradually enhances EGFR autophosphorylation, similar to the induction of
471 EIF4EBP1-pT37/46 (4E-BP1-pT37/46). Trp concentration row: Cells were cultured in medium with
472 the indicated Trp concentrations for 24 h. Detections of the same samples as in Figure 1e. LN-18
473 cells. (n = 3).

474 (g) Quantification of EGFR-pY1068 in (f).

475 (h) Trp stress enhances EGFR internalization to perinuclear endosomes. Immunofluorescence of
476 EGFR localization. Cells were unstimulated or stimulated with EGF (10 ng/mL, 15 min). EGFR,
477 green; nucleus, blue (DAPI). Scale bar: 10 μ m. LN-18 cells. (n = 4).

478 (i) Quantification of EGFR foci per 100 cells in (h).

479 (j) The pan-ERBB receptor inhibitor Afatinib (10 μ M, 1 h) and the EGFR-specific inhibitor Erlotinib
480 (10 μ M, 1 h) both inhibit Trp restriction-induced EIF4EBP1-pT37/46 (4E-BP1-pT37/46). Cells were
481 stimulated with EGF (10 ng/mL, 30 min). LN-18 cells. (n = 3).

482 (k) Quantification of EGFR-pY1068 in (j).

483 (l) Quantification of EIF4EBP1-pT37/46 (4E-BP1-pT37/46) in (j).

484 (m) Trp stress reduces transcripts of the EGFR ligand EGF. *EGF* mRNA relative to 18S rRNA
485 measured by qRT-PCR. LN-18 cells. (n = 3).

486 (n) Trp stress enhances transcripts of the EGFR ligand Epiregulin (EREG). *EREG* mRNA relative
487 to 18S rRNA measured by qRT-PCR. LN-18 cells. (n = 4).

488 (o) Trp deprivation gradually enhances EREG expression, similar to the induction of EGFR
489 autophosphorylation and EIF4EBP1-pT37/46 (4E-BP1-pT37/46). Trp concentration row: Cells
490 were cultured in medium with the indicated Trp concentrations for 24 h. LN-18 cells. (n = 3).
491 Detections of the same samples as in Figure 1e.

492 (p) Quantification of pro-EREG in (o).

493 (q) Quantification of glycosylated pro-EREG in (o).

494 (r) SESN2 knockdown rescues repressed RPS6KB1-pT389 (S6K-pT389) levels upon Trp stress,
495 but does not affect EIF4EBP1-pT37/46 (4E-BP1-p37/46). LN-229 cells. (n = 4).

496 (s) Quantification of SESN2 in (r).

497 (t) Quantification of RPS6KB1-pT389 (S6K-pT389) in (r).

498 (u) Quantification of EIF4EBP1-pT37/46 (4E-BP1-pT37/46) in (r).

499

500 Cells were cultured in the presence of Trp (+Trp, grey, 78 μ M) or absence of Trp (-Trp, blue, 0 μ M)
501 for 24 h, if not indicated otherwise. One-way ANOVA followed by a Šídák's multiple comparisons
502 test was applied (d, e, g, i, k, l, p, q, s, t, u). For (b,m,n) a two-tailed paired Student's t test was
503 performed. Data are presented as mean \pm SEM. *p < 0.05, **p < 0.01, ***p < 0.001. n.s., not
504 significant.

505 **Extended Data Figure 2: PI3K inhibition blocks Trp stress-induced EIF4EBP1
506 phosphorylation.**

507
508 (a) The class I PI3K inhibitor Pictilisib (GDC0941) (1 μ M, 1 h) blocks Trp stress-induced
509 EIF4EBP1-pT37/46 (4E-BP1-pT37/46). Cells were unstimulated or stimulated with EGF
510 (10 ng/mL, 15 min). LN-18 cells. (n = 4).

511 (b) Quantification of EIF4EBP1-pT37/46 (4E-BP1-pT37/46) in (a).

512 (c) Quantification of EIF4EBP1-pT37/46 (4E-BP1-pT37/46) over EIF4EBP1 (4E-BP1) in (a).

513 (d) Quantification of EIF4EBP1-pT37/46 (4E-BP1-pT37/46) over EIF4EBP1 (4E-BP1) in Figure
514 2c.

515 (e) Trp deprivation enhances autophosphorylation of the EGFR at Y1068. Cells were unstimulated
516 or stimulated with EGF (10 ng/mL, 15 min). LN-18 cells. (n = 3). Detections of the same samples
517 as in (a) (lanes 1 to 4).

518 (f) Quantification of EGFR-pY1068 in (e).

519 (g) Quantification of EIF4EBP1-pT37/46 (4E-BP1-pT37/46) over EIF4EBP1 (4E-BP1) in Figure 2j.

520 (h) Quantification of EIF4EBP1-pT37/46 (4E-BP1-pT37/46) over EIF4EBP1 (4E-BP1) in Figure 2r.

521
522 Cells were cultured in the presence of Trp (+Trp, grey, 78 μ M) or absence of Trp (-Trp, blue, 0 μ M)
523 for 24 h. One-way ANOVA followed by a Šídák's multiple comparisons test was applied (b, c, d, f,
524 g, h). Data are presented as mean \pm SEM. *p < 0.05, **p < 0.01, ***p < 0.001. n.s., not significant.

525 **Figure 3: EIF4EBP1-sensitive translation induces AHR expression and activity under Trp
526 stress.**

527 (a) The Trp stress proteome reveals an increase of the aryl hydrocarbon receptor (AHR). Volcano
528 plot of relative protein abundances. The Trp replete condition (+Trp) was compared to Trp stress
529 (-Trp) for 24 h. The AHR is marked in red, the other colours correspond to the GO terms in Fig.
530 4a. LN-18 cells. (n = 3). Full proteome and list of marked proteins are in Table S1.

531 (b) The proteome upon generalized amino acid stress in DMEM medium shows no increase of the
532 AHR. Volcano plot of relative protein abundances. The amino acid replete condition (+all aa) was
533 compared to withdrawal of all amino acids in DMEM medium (-all aa DMEM) for 24 h. The AHR
534 is marked in red, the other colours correspond to the GO terms in Fig. 4a. LN-18 cells. (n = 4). Full
535 proteome and list of marked proteins are in Table S1.

536 (c) The proteome upon generalized amino acid stress in HBSS medium shows no increase of the
537 AHR. Volcano plot of relative protein abundances. The amino acid replete condition (+all aa) was
538 compared to withdrawal of all amino acids in HBSS (-all aa HBSS) for 24 h. The AHR is marked
539 in red, the other colours correspond to the GO terms in Fig. 4a. LN-18 cells. (n = 4). Full proteome
540 and list of marked proteins are in Table S1.

541 (d) The methionine (Met) stress proteome shows no increase of the AHR. Volcano plot of relative
542 protein abundances. The methionine replete condition (+Met) was compared to Met stress (-Met).
543 The AHR is marked in red, the other colours correspond to the GO terms in Fig. 4a. LN-18 cells.
544 (n = 4). Full proteome and list of marked proteins are in Table S1.

545 (e) Hyperphosphorylation of EIF4EBP1 (4E-BP1) at T37/46 is specific to Trp stress. Cells were
546 cultured under amino acid replete conditions in DMEM (control, +Trp), under Trp stress (-Trp), or
547 under withdrawal of all amino acids in HBSS (-all aa HBSS) or DMEM (-all aa DMEM) for 24 h.
548 LN-18 cells. (n = 4).

549 (f) Quantification of EIF4EBP1-pT37/46 (4E-BP1-pT37/46) in (e).

550 (g) Quantification of EIF4EBP1 (4E-BP1) in (e).

551 (h) Hyperphosphorylation of EIF4EBP1 (4E-BP1) is specific to Trp stress. Cells were cultured
552 under methionine replete conditions (control, +Met) or methionine stress (-Met) for 24 h. LN-18
553 cells. (n = 4).

554 (i) Quantification of EIF4EBP1-pT37/46 (4E-BP1-pT37/46) in (h).

555 (j) Trp deprivation gradually enhances AHR expression. Trp concentration row: Cells were cultured
556 in medium with the indicated Trp concentrations for 24 h. LN-229 cells. (n = 5).

557 (k) Quantification of AHR in (j).

558 (l) AHR translation is enhanced upon Trp stress. Ribosome profiling: Ribosome protected
559 fragment (RPF) read density is shown on the AHR transcript in the presence and absence of Trp.

560 Reads per transcript normalized to total number of reads are shown on the y-axis. Bottom panel,
561 short rectangles represent untranslated regions, tall rectangle indicates coding sequence. LN-229
562 cells. (n = 3). Replicates are shown in Extended Data Figure 3j.

563 (m) AHR translation is enhanced upon Trp stress. Polysome profiling: Polysome profiles of AHR
564 upon presence and absence of Trp. Analysis of relative *AHR* mRNA levels per fraction via qRT-
565 PCR. Fraction numbers 1-5 indicate low-molecular weight polysomes, fraction numbers 6-9
566 indicate high-molecular weight polysomes (actively translating fractions, highlighted in light blue).
567 LN-229 cells. (n = 3).

568 On the right: Quantification of the area under the curve (AUC) of *AHR* in fractions 6-9.

569 (n) The MTOR inhibitor AZD8055 (100 nM, 24 h) suppresses AHR induction by Trp stress. LN-
570 229 cells. (n = 5).

571 (o) Quantification of AHR in (n).

572 (p) The MTOR inhibitor AZD8055 (100 nM, 24 h) fails to inhibit AHR levels in cells overexpressing
573 a non-phosphorylatable EIF4EBP1 T37/46A mutant. LN-229 cells. (n = 3).

574 (q) Quantification of AHR in (p).

575 (r) Quantification of EIF4EBP1-pT37/46 (4E-BP1-pT37/46) in (p).

576 (s) Short hairpin-mediated knockdown of the AHR ablates the induction of the AHR target gene
577 *CYP1B1* by Trp stress. mRNA expression normalized to 18S rRNA. qRT-PCR. LN-18 cells.
578 (n = 4).

579 (t) RNAseq analysis reveals an enhanced transcriptional AHR activity signature upon Trp stress.
580 Barcode plots showing the status of AHR activity in cells starved of Trp for 24 h. LN-18 cells.
581 (n = 4). The x-axis represents the values of moderated t-statistic values for all genes in the
582 comparison. The blue and pink coloured segments represent the lower and upper quartiles of all
583 the genes. The vertical barcode lines represent the distribution of the genes. The worm line above
584 the barcode shows the relative density of the AHR-signature genes, which represents the direction
585 of regulation.

586

587 Cells were cultured in the presence of Trp (+Trp, grey, 78 μ M) or absence of Trp (-Trp, blue, 0 μ M)
588 for 24 h, if not indicated otherwise. One-way ANOVA followed by a Šídák's multiple comparisons
589 test was applied (f, g, k, o, q, r, s). For (i) and (m) a two-tailed paired Student's t test was performed.
590 Data are presented as mean \pm SEM. *p < 0.05, **p < 0.01, ***p < 0.001. n.s., not significant.

591 **Extended Data Figure 3: Proteins in the Trp stress proteome are fully translated.**

592 (a) Ribosome profiling: Transcriptome-wide RPF density at Trp codons (TGG) under Trp replete
593 conditions or Trp stress. The peak indicates the accumulation of ribosomes in a window of -/+ 30
594 nucleotides at Trp codons under Trp stress. LN-229 cells. (n = 1).

595 (b-d) Sequence coverage of proteins upregulated in the Trp stress proteome.

596 (b) Density plot: relative distribution of quantified peptides from the N- to the C-terminus of proteins
597 under Trp replete conditions (+Trp, grey) or Trp stress (-Trp, blue). No shift in the distribution of
598 quantified peptides was observed, indicating that there was no increase in premature translation
599 arrest events under Trp stress.

600 (c) Distribution of quantified peptides between the N-terminal and C-terminal half. Proteins
601 upregulated in the stress proteome were analyzed under Trp replete conditions (+Trp) and Trp
602 stress (-Trp).

603 (d) Peptide distribution map for all proteins under Trp replete conditions (upper panel) and Trp
604 stress (lower panel). Relative positions of quantified peptides (green) and Trp residues (magenta)
605 from N- to C-terminus (%) are depicted. Trp stress does not shift the peptide distribution towards
606 the N-terminus, further suggesting that Trp stress does not result in premature translation arrest.

607 (e) The Trp stress proteome exhibits low overlap with generalized amino acid stress in DMEM or
608 HBSS media. Venn-diagram of upregulated proteins in Figures 3a, b and c.

609 (f) The Trp stress proteome exhibits low overlap with methionine stress. Venn-diagram of
610 upregulated proteins in Figures 3a and d.

611 (g) The Trp stress proteome has low overlap with generalized amino acid stress in DMEM or HBSS
612 media. Venn-diagram of downregulated proteins in Figures 3a, b and c.

613 (h) The Trp stress proteome has low overlap with methionine stress. Venn-diagram of
614 downregulated proteins in Figures 3a and d.

615 (i) Quantification of EIF4EBP1 (4E-BP1) in Figure 3h.

616 (j) AHR translation is enhanced upon Trp stress. Two biological replicates of the ribosome profiling
617 data shown in Figure 3l. Ribosome protected fragment (RPF) read density is shown on the AHR
618 transcript in the presence and absence of Trp. Reads per transcript normalized to total number of
619 reads are shown on the y-axis. Bottom panel, short rectangles represent untranslated regions, tall
620 rectangle indicates coding sequence. RPF data show ribosome coverage under Trp stress to be
621 enhanced up to the 3' end. At the Trp codons (depicted as orange lines) the read numbers
622 increase, indicative of ribosome pausing, but this does not lead to premature translation stop. LN-
623 229 cells.

624 (k) Cycloheximide (CHX) (5 µg/mL, 24 h) suppresses AHR induction by Trp stress. LN-229 cells.
625 (n = 4).

626 (l) Quantification of AHR in (k).
627 (m) The pan-ERBB inhibitor Afatinib (10 μ M, 24 h) suppresses AHR induction by Trp stress. LN-
628 229 cells. (n = 4-5).
629 (n) Quantification of AHR in (m).
630 (o) Quantification of EIF4EBP1-pT37/46 (4E-BP1-pT37/46) in (m).
631 (p) Quantification of EIF4EBP1-pT37/46 (4E-BP1-pT37/46) over EIF4EBP1 (4E-BP1) in (m).
632 (q) Quantification of EIF4EBP1-pT37/46 (4E-BP1-pT37/46) in Figure 3n.
633 (r) Quantification of EIF4EBP1-pT37/46 (4E-BP1-pT37/46) over EIF4EBP1 (4E-BP1) in Figure
634 3n.
635 (s) The EIF4EBP1 (4E-BP1) agonist 4EGI-1 (10 μ M, 24 h) suppresses AHR induction by Trp
636 stress. LN-229 cells. (n = 3).
637 (t) Quantification of AHR in (s).
638 (u) Quantification of EIF4EBP1-pT37/46 (4E-BP1-pT37/46) over EIF4EBP1 (4E-BP1) in Figure
639 3p.
640 (v) Short hairpin-mediated knockdown of the AHR in Figure 3s. mRNA expression normalized to
641 18S rRNA. qRT-PCR. LN-18 cells. (n = 4).
642 (w-x) The AHR is active upon Trp stress as determined by the induction of the AHR target gene
643 CYP1B1.
644 (w) CYP1B1 mRNA expression measured relative to 18S rRNA by qRT-PCR, validation of
645 RNASeq data shown in Figure 3t. LN-18 cells. (n = 4).
646 (x) CYP1B1 mRNA expression measured relative to 18S rRNA by qRT-PCR, validation of
647 RNASeq data shown in (y). LN-229 cells. (n = 4).
648 (y) RNAseq analysis reveals an enhanced transcriptional AHR activity signature upon Trp stress.
649 Barcode plots showing the status of AHR activity in cells starved of Trp for 24 h. LN-229 cells.
650 (n = 4). The x-axis represents the values of moderated t-statistic values for all genes in the
651 comparison. The blue and pink coloured segments represent the lower and upper quartiles of all
652 the genes. The vertical barcode lines represent the distribution of the genes. The worm line above
653 the barcode shows the relative density of the AHR-signature genes, which represents the direction
654 of regulation.
655
656 Cells were cultured in the presence of Trp (+Trp, grey, 78 μ M) or absence of Trp (-Trp, blue, 0 μ M)
657 for 24 h, if not indicated otherwise. One-way ANOVA followed by a Šídák's multiple comparisons
658 test was applied (l, n, o, p, q, r, t, u, v), for (i, w, x) a two-tailed paired Student's t test was
659 performed. Data are presented as mean \pm SEM. *p < 0.05, ***p < 0.001. n.s., not significant.

660 **Figure 4: The MTORC1-AHR pathway enhances autophagy to replenish intracellular Trp
661 levels and to sustain tryptophanyl-tRNA charging under Trp stress.**

662 (a) Gene Ontology (GO) terms related to macropinocytosis and lysosomes are enriched in the Trp
663 stress proteome. GO term enrichment analysis of proteins upregulated under Trp stress (-Trp) in
664 Figure 3a. Proteins were considered to be upregulated with $FC \geq 1.5$ and an adjusted p -value < 0.05 . The length of the bar represents the \log_{10} Benjamini-Hochberg corrected p -value. Indicated
665 for each term is the number of associated proteins in the Trp stress proteome; in brackets: total
666 number of associated proteins per term. Proteins that belong to the GO terms are marked in the
667 corresponding colours in the volcano plots in Figure 3a-d and are listed in Table S1.

668 (b) Trp stress increases the area of intracellular macropinosomes. Uptake assay of fluorescently
669 labelled 70 kDa - dextran (dextran, white) in cells stained with DAPI (blue), stimulated with EGF
670 (10 ng/mL, 30 min). Scale bar: 10 μm . LN-18 cells. ($n = 4$).

671 (c) Quantification of the area of macropinosomes per cell in (b).

672 (d) Trp stress increases lysosomal compartment size. Immunofluorescence staining of LAMP2
673 (white) in cells stained with DAPI (blue), stimulated with EGF (10 ng/mL, 15 min). Scale bar:
674 10 μm . LN-18 cells. ($n = 4$).

675 (e) Quantification of the area of LAMP2 staining (lysosomal compartment size) per cell in μm^2 (d).

676 (f) Trp stress increases lysosomal activity. Live cell imaging of Lysotracker Red DND-99
677 (lysotracker, 30 min, white) in cells stained with DAPI (blue). Scale bar: 10 μm . LN-18 cells. ($n = 5$).

678 (g) Quantification of the intensity of lysotracker foci in (f).

679 (h) Trp stress enhances autophagic flux (BafA-induced MAP1LC3B lipidation), which is
680 suppressed by inhibition of MTOR or the AHR. BafA (100 nM, 2 h), MTOR inhibitor AZD8055 (100
681 nM, 24 h), AHR inhibitor SR1 (1 μM , 24 h). LN-18 cells. ($n = 5$).

682 (i) Quantification of lipidated MAP1LC3B-II (LC3-II) in (h).

683 (j) Quantification of EIF4EBP1-pT37/46 (4E-BP1-pT37/46) in (h).

684 (k) Quantification of MAP1LC3B total levels (LC3 I + II) in (h).

685 (l) Volcano plot showing differentially regulated genes in the Trp stress (-Trp) versus the Trp
686 replete condition (+Trp) in RNAseq data of LN-18 cells. Autophagy-related genes are coloured in
687 cyan. AHR and MAP1LC3B (LC3) are shown in red ($n = 4$). Analysis of the same dataset as
688 in Figure 3t. Table S2 lists all the differentially expressed autophagy-related genes.

689 (m) MTOR or AHR inhibition reduces autophagy upon Trp stress. Immunofluorescence staining
690 of p62 foci. BafA (100 nM, 2 h), MTOR inhibitor AZD8055 (100 nM, 24 h), AHR inhibitor SR1
691 (1 μM , 24 h). Scale bar: 20 μm . LN-18 cells. ($n = 4$).

692 (n) Quantification of p62 foci in (m).

693 (o) AHR overexpression (OE) increases AHR and MAP1LC3B (LC3) protein. LN-18 cells. ($n = 4$).

695 (p) AHR overexpression (OE) increases *MAP1LC3B* (*LC3*) mRNA levels. mRNA expression
696 normalized to *18S rRNA*. qRT-PCR. LN-18 cells. (n = 5).
697 (q) Quantification of *MAP1LC3B* (*LC3*) in (o).
698 (r) Heatmap of autophagy regulators measured by targeted quantitative proteomics in cells
699 cultured in the presence or absence of Trp, treated without or with BafA (100 nM, 2 h), and with
700 BafA in combination with the MTOR inhibitor AZD8055 (100 nM, 24 h) or the AHR inhibitor SR1
701 (1 μ M, 24 h). LN-18 cells. Colours represent z-scored averages of the relative abundances n > 4
702 replicates. (n = 5, except for +Trp, +BafA, +SR1 condition with n = 4). Peptides were normalized
703 to their respective heavy-labelled spiked-in peptides. Proteins labelled with an asterisk show a
704 significant difference (p-value < 0.05) between the -Trp BafA condition and the -Trp BafA AZD8055
705 condition.
706 (s) Cells under Trp stress exhibit a further decrease in intracellular Trp when lysosomal function
707 is inhibited. Intracellular Trp concentrations (molecules Trp / cell) in cells treated with and without
708 BafA (100 nM, 2 h). LN-18 cells. (n = 4). The -Trp condition already shown in Extended Data Fig.
709 1g.
710 (t) BafA (100 nM, 2 h) increases the levels of uncharged tryptophanyl-tRNAs upon Trp stress.
711 Relative aminoacylation levels were determined by qRT-PCR using tRNA-specific primers. LN-
712 18 cells. (n = 3). The -Trp condition is also shown in Fig. 1b.
713
714 Cells were cultured in the presence of Trp (+Trp, grey, 78 μ M) or absence of Trp (-Trp, blue, 0 μ M)
715 for 24 h, if not indicated otherwise. One-way ANOVA followed by a Šídák's multiple comparisons
716 test was applied (i, j, k). For (c, e, g, p, q, s, t) a two-tailed paired Student's t test was performed.
717 Data are presented as mean \pm SEM. *p < 0.05, **p < 0.01, ***p < 0.001. n.s., not significant.

718 **Extended Data Figure 4: The MTORC1-AHR pathway enhances autophagy.**

719 (a) Quantification of EIF4EBP1-pT37/46 (4E-BP1-pT37/46) over total EIF4EBP1 (4E-BP1) in
720 Figure 4h.

721 (b) An autophagy mRNA signature is enriched upon Trp stress. Barcode plot showing enrichment
722 of autophagy regulators in RNAseq data of cells starved of Trp for 24 h. LN-18 cells (n = 4).
723 Analysis of the same dataset as in Figure 4l.

724 (c) Interaction network analysis of reported physical interactions between autophagy-related
725 proteins identified in Figure 4l. Clusters generated by k-means clustering were arbitrarily classified
726 based on their functions.

727 (d) AHR binding motif in the *MAP1LC3B* promoter region. The transcription factor target gene
728 database shows an AHR binding motif (V_AHR_01_M00139, chr16: 87421754-87421771)
729 upstream of the *MAP1LC3B* promoter, which is located in a region of H3K27 acetylation
730 enrichment, indicative of active chromatin.

731 (e) *MAP1LC3B* (LC3) translation is enhanced upon Trp stress. Ribosome profiling: Ribosome
732 protected fragment (RPF) read density is shown on the *MAP1LC3B* (LC3) transcript in the
733 presence and absence of Trp. Reads per transcript normalized to total number of reads are shown
734 on the y-axis. LN-229 cells. (n = 3). Bottom panel, short rectangles represent untranslated regions,
735 tall rectangle indicates coding sequence. RPF data show ribosome coverage under Trp stress to
736 be enhanced up to the 3' end.

737 (f) AHR overexpression (OE) increases *AHR* mRNA levels. mRNA expression normalized to 18S
738 rRNA. qRT-PCR. LN-18 cells. (n = 5).

739 (g) Quantification of AHR in Figure 4o.

740

741 Cells were cultured in the presence of Trp (+Trp, grey, 78 µM) or absence of Trp (-Trp, blue, 0 µM)
742 for 24 h, if not indicated otherwise. One-way ANOVA followed by a Šídák's multiple comparisons
743 test was applied in (a). For (f, g) a two-tailed paired Student's t test was performed. Data are
744 presented as mean ± SEM. *p < 0.05, ***p < 0.001. n.s., not significant.

745 **Figure 5: The mTORC1-AHR pathway is active in human glioblastomas.**

746 (a, b) Boxplot representation of the distribution of the (a) AHR activity score and (b) the normalized
747 expression values of AHR in the seven glioblastoma patient subgroups of human GB (The Cancer
748 Genome Atlas, TCGA). The black dotted line represents the mean AHR activity score or
749 normalized AHR expression values across all patient samples. The p-values were determined
750 based on comparing the average score or expression to the corresponding mean of all patient
751 sample groups.

752 (c-e) Boxplot representation of the distribution of TCGA RPPA data of EIF4EBP1-pT37/46 (4E-
753 BP1-pT37/46) (c), P70S6K1-pT389 (S6K-pT389) (d), and the enrichment score of autophagy
754 regulators (e), between groups 1 (high AHR activity and low AHR mRNA expression; grey) and 2
755 (high AHR activity and high AHR mRNA expression; blue).

756 (f) Trp stress enhances the cellular proportion of ceramides in an MTOR- and AHR-dependent
757 manner. Ratio of ceramides to total sphingolipids (SLs). MTOR inhibitor AZD8055 (100 nM, 24 h),
758 AHR inhibitor SR1 (1 μ M, 24 h). LN-18 cells. (n = 7).

759 (g) In human GB, regions of high Trp and high ceramides are mutually exclusive. MALDI-MSI of
760 Trp and ceramide hotspots and their intersections in human GB samples. (n = 4). Scale bar: 1 mm.
761 (h) Schematic representation of the MTORC1-AHR pathway.

762

763 Cells were cultured in the presence of Trp (+Trp, grey, 78 μ M) or absence of Trp (-Trp, blue, 0 μ M)
764 for 24 h. A two-tailed unpaired Student's t test was performed in (f). For bioinformatic analysis,
765 statistic is described in the Method Details section (a-e). Data are presented as mean \pm SEM. *p
766 < 0.05, **p < 0.01, ***p < 0.001. n.s., not significant.

767 **Methods**

768

769 **Cell culture and treatments**

770 Glioblastoma cell lines LN-18 and LN-229 were obtained from ATCC. Cells were cultured in
771 DMEM (Biotech, P04-03600) supplemented with 10% FBS (Gibco, 10270106) and 3 mM L-
772 glutamine (Gibco, 25030-024) or in phenol red-free DMEM (Gibco, 31053-028) supplemented with
773 10% FBS (Gibco, 10270106) and 2 mM L-glutamine (Gibco, 25030-024), 1 mM sodium pyruvate
774 (Gibco, 11360039), 100 U/mL penicillin and 100 µg/mL streptomycin (Gibco, 15140122). All cell
775 lines were maintained at 37 °C and 5% CO₂ and regularly tested for mycoplasma contamination.

776

777 Cell seeding

778 For Ras pull down, 6.5 x 10⁵ LN-18 cells were seeded per 15 cm dish. For cap pull down, 4 x 10⁶
779 LN-18 cells were seeded per 15 cm dish (TPP, 93150), six dishes per condition. For polysome
780 profiling 1.6 x 10⁶ LN-229 cells were seeded per 15 cm dish (TPP, 93150). For the ribosome
781 profiling, 6.5 x 10⁴ LN-229 cells per mL were seeded per 15 cm dish (TPP, 93150). For the
782 proteome analysis 3 x 10⁶ LN-18 cells were seeded per 10 cm dish (TPP, 93100). For tRNA
783 aminoacylation assay, 3 x 10⁶ LN-18 cells were seeded per 10 cm dish (TPP, 93100). For MTOR
784 pathway and simultaneous amino acid - sphingolipid analysis, 1 x 10⁶ LN-18 or LN-229 cells were
785 seeded per 6 cm dish (TPP, 93060). For siRAS experiments, 2.5 x 10⁵ LN-18 cells were seeded
786 per 6 cm dish (TPP, 93060). For analysis of AHR expression and activation, as well as RNA
787 sequencing 4 x 10⁵ LN-229 cells per well were seeded in 6-well plates. For siRNA-mediated
788 knockdown of SESN2 1,6 x 10⁵ cells per 6-well were seeded. For AHR overexpression 3 x 10⁵
789 LN-18 cells per well were seeded in 6-well plates. For EGFR immunofluorescence experiments,
790 1 x 10⁵ LN-18 cells were seeded per well into an 8-well IbiTreat µ-slide (Ibidi, 80826). For all other
791 immunofluorescence experiments, 1 x 10⁵ LN-18 cells were seeded on coverslips (Hecht
792 assistant, 41014). For the uptake assay and lysosome tracking, 5 x 10⁴ LN-18 cells were seeded
793 per well in 8-well IbiTreat µ-slides (Ibidi, 80826).

794

795 Starvation experiments and treatments

796 For Trp starvation experiments, one day after seeding, cells were washed with PBS (Biotech, Cat#
797 P04-36500) and customized Trp-free DMEM (Gibco, ME15175L1) containing 4.5 g/L glucose,
798 supplemented either with 10% dialyzed FBS (Life Technologies, 26400044), 2 mM L-glutamine
799 (Gibco, 25030-024), 1 mM sodium pyruvate (Gibco, 11360039) and 100 U/mL penicillin and
800 100 µg/mL streptomycin (Gibco, 15140122) or supplemented with only 3 mM L-glutamine (Gibco,
801 25030-024) was added to the cells. Trp was dissolved in cell culture grade water (Corning, 25-

802 055-CV) and added fresh at a final concentration of 78 μ M or complete DMEM (Gibco, 31053-
803 028) was used as Trp-containing control medium. For the proteome measurements, the cells were
804 cultured in the presence of 78 μ M (+Trp), or in the presence of less than 0.4 μ M Trp (-Trp). Unless
805 specified otherwise, Trp starvation was performed for 24 h.

806 For the non-Trp stress proteome experiments, all starvations were performed for 24 h. The
807 following media were utilized: -all aa HBSS (Cat# P04-32505, BioTech), -all aa DMEM (Cat# P04-
808 01507, BioTech); Glucose (Cat# A24940-01, Gibco) was added to reach 4.5 g/L, Met control
809 medium (Cat# 21875-034, Gibco) and Met starvation medium (Cat# A14517-01, Gibco).

810 For cell treatments, the following compounds were used: EGF (Peprotech, AF-100-15) was
811 diluted in PBS (SERVA, 47302.03) with 0.1% BSA (Carl Roth, 8076.5) and added directly into the
812 media at a final concentration of 10 ng/mL EGF (immunofluorescence experiments) for the
813 indicated time points. Cycloheximide (Sigma-Aldrich, C4859) was diluted in water and directly
814 added to the media at a final concentration of 2 μ g/mL for the indicated time points or 5 μ g/mL for
815 24 h. Inhibitors were diluted in DMSO (Sigma-Aldrich, D2650) and cells were treated with 10 μ M
816 4EGI-1 (Tocris, 4800), 10 μ M Afatinib (Selleckchem, S1011), 100 nM AZD8055 (MedChem
817 Express, HY-10422), 100 nM Bafilomycin A₁ (MedChem Express, HY-100558), 10 μ M Erlotinib
818 (Selleckchem, S7786), 1 μ M Pictilisib (GDC0941, Axon, 1377), and 1 μ M StemRegnin 1 (SR1,
819 Merck Millipore, 182706) for the indicated time points.

820

821 **Generation of transgenic cell lines**

822 Transient siRNA-mediated knockdown

823 Simultaneous siRNA knockdown of all three RAS isoforms (KRAS/HRAS/NRAS) was induced
824 using 10 nM of each KRAS, HRAS and NRAS (total siRNA 30 nM) ON-TARGETplus human
825 SMARTpool siRNA (Dharmacon, L-005069-00-0005, L-004142-00-0005, L-003919-00-0005) for
826 8 h followed by a medium change. Transfections were preformed using Lipofectamine 3000
827 (Invitrogen, L3000008) according to the manufacturer's protocol 48 h (first transfection) before
828 cells were cultured in Trp-containing or Trp-free medium for 24 h.

829 For siRNA-mediated knockdown of SESN2 ON-TARGETplus human SMARTpool siRNA
830 (Dharmacon, L-019134-02-0005) was used at a final concentration of 40 nM or 60 nM for 8 h
831 followed by a medium change. Transfection was performed with Lipofectamine RNAiMAX
832 (Thermo Fisher Scientific, 13778100) according to the manufacturer's protocol 24 h before cells
833 were cultured in Trp-containing or Trp starvation medium for 24 h.

834 ON-TARGETplus non-targeting pool siRNA (Dharmacon, D-001810-10-05) was used for
835 all knockdowns as a control at the same concentration as indicated. Knockdown efficiency was
836 confirmed by gene and protein expression analysis using qRT-PCR and immunoblot.

837 Stable knockdown of AHR

838 Short hairpin RNA (shRNA) knockdown of AHR in LN-18 cells was performed as previously
839 described in Sadik et al. for U-87 MG cells using the AHR-targeting shERWOOD UltramiR lentiviral
840 shRNA system (transOMIC Technologies, TLHSU1400-196-GVO-TRI) ⁶¹. In brief, cells were
841 incubated with viral culture supernatants containing either the shAHR or shC (control) sequences
842 for 24 h in the presence of 8 µg/mL polybrene (Merck, TR-1003-G) to facilitate viral infection and
843 subsequent integration of the shRNA sequences. Selection of the transduced cells was performed
844 by first cell sorting using a BD FACSaria fusion (BD Biosciences), selecting only cells expressing
845 ZsGreen. Second, transduced cells were selected by adding 1 µg/mL puromycin (AppliChem,
846 A2856) to the culture media. Stable knockdown of AHR was confirmed at the mRNA and protein
847 levels by qRT-PCR and immunoblot analysis.

848

849 AHR overexpression

850 AHR cDNA was amplified by PCR and cloned into the Gateway® pDONR201 vector with primers
851 placed at the end or start positions of the AHR CDS. AttB sites were added to the CDS by a two-
852 step PCR. The first PCR was performed with hybrid primers, consisting of half of the AttB sites
853 and the other half being gene specific. The second PCR was done with primers covering the
854 complete AttB sites.

855 After sequence verification, the AHR CDS was cloned into the following Gateway® (GW)
856 destination vectors (derived from pDEST26 vectors) with a C-terminal protein-tag: pGW-FLAG,
857 pGW-HA and pGW-MYC by Gateway®-specific LR-reaction following the manufacturer's protocol
858 (Invitrogen). In addition, the AHR CDS was generated with STOP-codon in the Gateway-frame
859 and cloned into pGW-FLAG with an N-terminal FLAG-tag by Gateway-specific LR reaction.
860 Plasmids were amplified using DB3.1 and Stabl3 E.coli strains and extraction was performed with
861 the QIAprep Spin Miniprep Kit (Qiagen, 27104) and NucleoBond Xtra Midi kit (MACHEREY-
862 NAGEL, 740410.50).

863 The day after seeding, cells were transiently transfected with Lipofectamine 3000
864 (Invitrogen, L3000008) following the manufacturer's protocol with one of the following plasmids
865 pGW-AHR-HA, pGW-AHR-MYC, pGW-AHR-FLAG or pGW-FLAG-AHR, or the respective empty
866 control plasmids pGW-FLAG, pGW-HA and pGW-MYC. 2.5 µg DNA was used per 6-well plate.
867 Media was changed after 6 h of transfection and cells were harvested after 24h. Overexpression
868 was confirmed on mRNA and protein level by qRT-PCR and immunoblot.

869

870 **EIF4EBP1 Ala phospho mutant expression**

871 The coding sequence of human EIF4EBP1 (NM_004095.4) was amplified from cDNA derived from
872 HepG2 cells using primers containing restriction sites using Q5 polymerase (New England
873 Biolabs, M0491). PCR products were cloned into suitable vectors using restriction digest followed
874 by ligation. pCDNA3.1 (Invitrogen, V86020) with an N-terminal Myc-DKK tag and pETDuet-1
875 (Sigma-Aldrich, 71146) were used for expression in mammalian cells and bacteria, respectively.
876 Mutations T37A T46A were sequentially introduced by PCR using overlapping primers and whole-
877 vector amplification, followed by DpnI (New England Biolabs, R0176S) digestion.

878 Recombinant H6-EIF4EBP1 was produced in BL21 (DE3) E. coli (Novagen, 69450) co-
879 expressing lambda protein phosphatase and purified with the QIAexpress Ni-NTA Protein
880 Purification System (Qiagen, 30210) according to the manufacturers protocol. We quantified the
881 amount of recombinant H6-EIF4EBP1 (100 ng) using immunoblotting. To ensure the
882 concentration was comparable to endogenous levels, we loaded a cell lysate sample alongside
883 our EIF4EBP1 sample. Furthermore, we validated the concentration by loading a commercial
884 EIF4EBP1 sample with a known concentration for comparison.

885 Expression of T37A T46A mutated and *wt* EIF4EBP1 was induced using 0.5 µg of the
886 respective plasmid, created as described above. Transfections were preformed utilizing
887 Lipofectamine 3000 (Invitrogen, L3000008) according to the manufacturer's protocol 48 h before
888 cells were cultured in Trp-containing or Trp-free medium for 24 h.

889

890 **Human glioblastoma samples**

891 Tumour specimens of patients diagnosed with glioblastoma (WHO grade IV, IDH wildtype) were
892 obtained from the Institute of Neuropathology, Heidelberg University Hospital, according to the
893 regulations of the Tissue Bank of the National Center for Tumor Diseases (NCT), Heidelberg
894 University Hospital, under the ethics board approval S-318/2022.

895

896 **MALDI mass spectrometry imaging (MSI)**

897 **MALDI-MSI sample preparation**

898 Frozen glioblastoma samples were cut into 10 µm thick sections with a CM1950 cryostat
899 (Leica Biosystems) and mounted onto ITO coated glass slides (Bruker Daltonics, 8237001) for
900 MALDI MSI. Slides were stored in slide boxes (neoLab, 2-3080), covered with foil, vacuumed
901 (CASO) and stored at -80°C until further processing. Consecutive tissue sections were stained
902 with hematoxylin and eosin (HE) and annotation of tumour tissue regions was performed by a

903 clinically experienced neuropathologist. Immediately before matrix coating, the frozen slides were
904 equilibrated at room temperature (RT) and dried for 10 min in a desiccator (SP Bel-Art).

905 Glioblastoma samples were prepared with the following matrix application protocol: 100 μ L
906 of a 5 mg/mL deuterated tryptophan (D5-Trp) solution (Cayman Chemicals, 34829) in ACN/H₂O
907 (50:50, v/v) (Honeywell, 34967) was added to 25 mg/mL 2,5-dihydroxybenzoic acid (Alfa Aesar,
908 A11459) in ACN/H₂O/TFA (49.4:49.4:0.2, v/v/v) (Merck KGaA, 1.08262.0025) solution and
909 sprayed onto tissue sections with the following parameters: nozzle temperature 75°C, 12 layers,
910 flow rate 0.11 mL/min, velocity 1200, track spacing 2 mm, pattern CC, pressure 10 psi, dry time
911 0s, nozzle height 40 mm.

912

913 **Magnetic resonance MALDI-MSI data acquisition**

914 Data acquisition was performed on a Fourier-transform ion cyclotron resonance (FT-ICR)
915 magnetic resonance mass spectrometer (MRMS; solariX XR 7T, Bruker Daltonics) in two steps
916 (**Table 1**). First, method 1 optimized for detection of Trp was used at 100 μ m step-size. Thereafter,
917 method 2 optimized for detection of ceramides was used on the same tissue section with an XY-
918 offset of 50 μ m at step-size 100 μ m. Peak filtering was set to SNR > 3 and an absolute intensity
919 threshold of 10⁵ a.u.

920

921 **Table 1. MRMS MALDI acquisition methods for glioblastoma samples.**

	Method 1 optimized for Trp	Method 2 optimized for Ceramides
Online calibration	D5-Trp 210.128538	PC (34:1) 760.585082
Size	4M	2M
Mass range	m/z 75-800	m/z 300-800
Transient length	1.47 sec	2.93 sec
Resolving power at m/z 400	200,000	390,000
Laser shots	300	300
Frequency	2000 Hz	2000 Hz
Laser focus	small	small
Q1 Mass	200	500
Funnel RF amplitude	80 V	120 V
Time of flight	0.5 ms	0.5 ms
Q1 isolation	200 \pm 100	off

922

923 **Raw data evaluation and visualization**

924 Centroided data was imported into SCiLS Lab 2023a Pro software (Bruker Daltonics), then
925 exported as .imzml file and uploaded to the Metaspace platform (<https://metaspace2020.eu/>)⁹⁵ for
926 annotation of metabolites. Raw spectra were evaluated in DataAnalysis software (Bruker
927 Daltonics), and the smart formula function was used to generate sum formulas that supported the

928 annotations in METASPACE. Unless stated otherwise, ion images from positive ion mode
929 measurements show protonated adducts ($[M+H]^+$) and ion images from negative ion mode
930 measurements show chloride adducts ($[M+Cl]^-$).

931

932 **Data Processing**

933 Tissue areas were selected using an in-house-built IT tool and saved as regions of interest (ROI)
934 in the raw data file. Data processing was done in R (version 4.2.1). First, centroided data was
935 loaded, data for every ROIs per tissue sample was extracted using an in-house-built R-tool. The
936 data was loaded as a sparse matrix representation using Matrix package, for faster matrix
937 processing. Next, full width at half maximum (FWHM) was calculated as a function of *m/z* per
938 sample, using the *moleculaR* package ⁹⁶. This was followed by peak binning using the
939 MALDIquant package ⁹⁷, and intensity normalization using *moleculaR* package [Root Means
940 Square (RMS) for ceramides-focused datasets and internal standard (IS; D5-tryptophan *m/z*
941 210.12854) for tryptophan-focused datasets]. Finally, peak filtering was performed with a minimum
942 frequency set to 0.01.

943

944 **Calculation of molecular probability maps (MPMs) and collective projection probability maps**
945 **(CPPMs)**

946 Ceramide adduct masses ($[M+H]^+$, $[M+Na]^+$, and $[M+K]^+$) were extracted from the LipidMaps
947 database (www.lipidmaps.org). The data representation was first converted from raw ion
948 intensities into spatial point patterns representations, and then MPMs were calculated per
949 molecule (one ceramide or Trp) of interest (MOI). Subsequently, MPMs for each of the ceramides
950 were standardized and then converted into CPPM representation, as described in Abu Sammour
951 *et al.*, 2021 ⁹⁶. Hotspot areas and contours that indicate significantly increased MOI presence were
952 generated for each tissue sample using the *Spatstat R* package.

953

954 **Calculation of Dice Similarity Coefficient (DSC) values**

955 To calculate overlap between Trp's MPM hotspot contours with each of the ceramides' CPPM
956 hotspot contours, Dice Similarity Coefficient (DSC) was calculated as described in Abu Sammour
957 *et al.*, 2021 ⁹⁶.

958

959 **RNA isolation, cDNA synthesis and qRT-PCR**

960 For RNA isolation, cells were harvested using RTL buffer containing 10 μ L/mL beta-
961 mercaptoethanol (Sigma-Aldrich, M3148) and isolated as recommended in the manufacturer's

962 protocol of the RNeasy Mini Kit (Qiagen, 74106). DNase digest step was performed as
963 recommended in the protocol using the RNase free DNase kit (Qiagen, 79254). RNA
964 concentration and quality were determined by Nanodrop (Thermo Fisher Scientific).
965 Subsequently, cDNA was synthesized using 1 µg RNA and the High Capacity cDNA reverse
966 transcriptase kit (Applied Biosystems, 4368813). Quantitative real-time PCR (qRT-PCR) was
967 performed in a 96-well format using the StepOne Plus Real-Time PCR system (Applied
968 Biosystems) and SYBR Select Master Mix (Thermo Fisher Scientific, 4364346). Expression data
969 was processed using StepOne Software v2.3 (Thermo Fisher Scientific) and analyzed with the $2^{-\Delta\Delta Ct}$
970 method using 18S rRNA as reference gene.

971 For polysome profiling, RNA was isolated using TRI reagent (Zymo, R2050-1-200). In brief,
972 500 µL of each sucrose fraction was mixed with 0.5 µL TRI reagent (Zymo) and 200 µL chloroform
973 (Fisher Scientific, 10452631), mixed and centrifuged at 13,000 x g for 15 min at 4 °C. After
974 centrifugation, 500 µL of the upper phase was transferred in a new tube containing 1 mL
975 isopropanol (VWR, 84881-320) and 2 µL of 15 mg/mL Glycoblue (Invitrogen, AM9515). RNA was
976 precipitated over night at -20 °C followed by centrifugation at 13,000 x g for 15 min at 4°C.
977 Supernatant was discarded and pellet was washed once with 1 mL ice-cold 70% ethanol (Merck,
978 818760.2500) by centrifugation at 13,000 x g for 15 min at 4°C. Supernatant was discarded
979 followed by a second centrifugation step at 4°C to collect the remaining ethanol. Ethanol was
980 discarded and RNA pellet was air dried for 5-15 min at 25 °C. RNA was dissolved in 12 µL
981 nuclease-free water. The whole RNA solution was used for reverse transcription as described
982 above. After cDNA synthesis, a qRT-PCR was performed for AHR as described above. All qRT-
983 PCR primers used in this study are listed in **Table S4**.

984

985 **RNA sequencing**

986 Illumina sequencing libraries were prepared using the TruSeq Stranded mRNA Library Prep Kit
987 (Illumina, 20020595) according to the manufacturer's protocol. Briefly, poly(A)+ RNA was purified
988 from a maximum of 500 ng of total RNA using oligo(dT) beads, fragmented to a median insert
989 length of 155 bp and converted to cDNA. The ds cDNA fragments were then end-repaired,
990 adenylated on the 3' end, adapter ligated and amplified with 15 cycles of PCR. The libraries were
991 quantified using Qubit ds DNA HS Assay kit (Life Technologies-Invitrogen, Q33231) and validated
992 on an Agilent 4200 TapeStation System (Agilent technologies, 5067-5582, 5067-5583). Based on
993 Qubit quantification and sizing analysis multiplexed sequencing libraries were normalized, pooled
994 and sequenced using the NovaSeq 6000 Paired-End 100bp S4 flowcell (Illumina, 20028313) with
995 a final concentration of 300 pM spiked with 1% PhiX control (Illumina, 15051973).

996

997 RNA-seq data processing

998 We used the DKFZ/ODCF workflows for RNAseq v1.3.0-0, Alignment and QC v1.2.73-3
999 (<https://github.com/DKFZ-ODCF>) deployed on the Roddy framework (Roddy v3.5.9; Default-
1000 Plugin v1.2.2-0; Base-Plugin v1.2.1-0; <https://github.com/TheRoddyWMS/>). Paired end FASTQ
1001 reads were aligned using the STAR aligner v2.5.3a⁹⁸ by a 2-pass alignment. The reads were
1002 aligned to a STAR index generated from the 1000 genomes assembly, gencode 19 gene models
1003 (1KGRef_PhiX) and for a sjbdOverhang of 200. The alignment call parameters were --
1004 twopassMode Basic --twopass1readsN -1 --genomeLoad NoSharedMemory --outSAMtype BAM Unsorted
1005 SortedByCoordinate --limitBAMsortRAM 1000000000000 --outBAMsortingThreadN=1 --outSAMstrandField
1006 intronMotif --outSAMunmapped Within KeepPairs --outFilterMultimapNmax 1 --outFilterMismatchNmax 5
1007 --outFilterMismatchNoverLmax 0.3 --chimSegmentMin 15 --chimScoreMin 1 --chimScoreJunctionNonGTAG
1008 0 --chimJunctionOverhangMin 15 --chimSegmentReadGapMax 3 --alignSJstitchMismatchNmax 5 -1 5 5 --
1009 alignIntronMax 1100000 --alignMatesGapMax 1100000 --alignSJDBoverhangMin 3 --alignIntronMin 20.
1010 Duplicate marking of the resultant main alignment files, as well as generating BAM indices was
1011 done with sambamba v0.6.5⁹⁹. Quality control analysis was performed using the samtools flagstat
1012 command (samtools v1.6), and the rnaseqc tool¹⁰⁰.

1013 Featurecounts from the subread package v1.6.5 was used to perform gene specific read
1014 counting over exon features based on the gencode 19 gene models¹⁰¹. Strand unspecific counting
1015 was used. Both reads of a paired fragment were used for counting and the quality threshold was
1016 set to 255.

1017

1018 Gene expression analysis and gene set testing

1019 The raw RNA-seq counts were imported into R and saved as DGELists¹⁰². Genes with low counts
1020 across all samples were filtered using the function filterByExpr followed by trimmed mean of M
1021 values (TMM) normalization¹⁰² and variance modeling using voom¹⁰³. Batch effects were
1022 determined on the principal component analysis (PCA) projections and were corrected by a linear
1023 regression model. Differential gene expression was performed using the limma RNA-seq
1024 pipeline¹⁰³. Differentially regulated genes were considered significant at a p-value of less than or
1025 equal to 0.05. We retrieved the gene sets of the AHR-signature⁶¹ and autophagy regulators⁷⁴ for
1026 gene set testing. Comparing the state of activity of any gene set was performed by a non-
1027 competitive gene set test using ROAST¹⁰⁴. Multiple testing correction was performed by applying
1028 the Benjamini-Hochberg procedure.

1029

1030 **TCGA glioblastoma expression data**

1031 Data download

1032 We downloaded and manually curated the metadata entries of 614 submitted glioblastoma patient
1033 samples. We excluded 42 entries that were either, duplicates, referring to normal tissue, control
1034 analytes, resected from the wrong site or of recurrent tumours. We selected the patient data
1035 generated on the two channel Agilent 244K Custom Gene Expression array because it was used
1036 for all remaining 572 samples. The Cy3 channel was hybridized with the Stratagene Universal
1037 RNA Reference and the Cy5 channel was hybridized with the sample. We used the unique
1038 identifiers to download the raw microarray data using GDC-client v1.1.0.
1039 (<https://portal.gdc.cancer.gov/legacy-archive/search/f>)

1040

1041 Data annotation

1042 Two different versions of the custom array were used, G4502A-07-1 and G4502A-07-2. Both
1043 arrays had ~87% of common probes, which were later used to merge the patient data from both
1044 versions together. The array design files (ADF) and FASTA files were downloaded from
1045 <https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga/using-tcga/technology>). We created a new annotation file by aligning the 60 k-mer probes to the non-
1046 redundant nucleotide database (<https://ftp.ncbi.nlm.nih.gov/blast/db/FASTA/nt.gz>; reference build
1047 hg38; downloaded on 21.07.2016) by using BLAST+ v2.2.30 and the following call parameters
1048 blastn -query unique.probes -task blastn -db nt -out resultblastn.txt -evalue 0.0001 -outfmt "6 std sgi nident
1049 staxids sscinames sstitle scomnames sstrand qcovhsp" -num_threads 14. The blast result was annotated
1050 using mygene v1.8 and additional gene information was added using the NCBI gene-info file
1051 (ftp://ftp.ncbi.nlm.nih.gov/gene/DATA/gene_info.gz; downloaded on 15.08.2016). The annotation
1052 file was filtered by removing all hits without the human taxid (9606), and with less than 60 bp
1053 matching, a mismatch > 0, and without an "NM_" RefSeq accession prefix.
1054

1055

1056 Data processing

1057 We used the Sample and Data Relationship Format files (SDRF) to group the microarray data
1058 according to the chip version used. For each group the raw files were imported using the
1059 read.maimages function from the limma package. The probes of the raw matrix were background
1060 corrected using the "normexp" method with a setoff value of 50, followed by within array
1061 normalization using the LOESS-smoothing algorithm. Only probes that were successfully
1062 annotated as described above were retained. For every gene, probes were summarized into a
1063 single value (gene-set). If a gene was represented by more than three probes, we calculated the

1064 median absolute deviation (mad), and if a probe had a value outside the closed interval [-1.5, 1.5],
1065 it was counted as an outlier and was filtered out. The remaining probes were averaged to represent
1066 the single gene value. All genes with 3 probes or less were averaged, and in the case of genes
1067 reported as a single probe, the single probe value was used. The resulting normalized matrix was
1068 saved into an MA-list object also including the curated meta- and clinical data. Finally, samples
1069 were filtered out if they had a reported IDH mutation, any missing clinical data, or patient age was
1070 below 30 years. The final MA-list comprised of 406 patients (GBM406).

1071

1072 Feature selection for identifying glioblastoma subgroups

1073 We performed a feature selection step to identify glioblastoma patient subgroups showing high
1074 AHR expression and activity, while also reflecting the starvation phenotype observed in the LN-
1075 18 and LN-229 RNA-seq experiments. First, we compiled all differentially expressed genes from
1076 the topTables that had an average expression greater than or equal to 1 log2 counts per million,
1077 a log2 fold change of 0.58 or higher for upregulated genes and -0.58 or lower for downregulated
1078 genes, and adjusted p-value of at least 0.05. The genes fulfilling this criterion in those experiments
1079 were 2812 (starvation-features). Next, we estimated immune infiltration scores for the GBM406
1080 patient dataset using the MCP-counter package v1.2.0¹⁰⁵. Principle component analysis using the
1081 FactoMineR package v2.6 was performed with MCP-scores. The starvation-features were
1082 correlated using Spearman correlation with the Eigenvalues of each of the first five principal
1083 components. Only 1628 genes were left after filtering all other genes that didn't have a correlation
1084 coefficient greater than or equal to 0.3 or less than or equal to -0.3, and a p-value of at least 0.05,
1085 with at least one of the first five principal components.

1086

1087 Defining glioblastoma subgroups

1088 We applied a graph-based approach to identify glioblastoma subgroups. The subset of the
1089 expression matrix comprising the 1628 genes was used for identifying glioblastoma subgroups.
1090 First, we created a nearest neighbour graph using the cccd package v1.6. We used the correlation
1091 between the genes as a measure of distance, set the k-nearest neighbours to 10, and selected
1092 the kd-tree algorithm for the graph embedding¹⁰⁶⁻¹⁰⁸. We used the Louvain algorithm¹⁰⁹ for
1093 community detection, which defined the seven GB subgroups.

1094

1095 Generating enrichment scores

1096 Single sample enrichment scores for the AHR signature⁶¹ and autophagy regulators⁷⁴ were
1097 generated using the GSVA package¹¹⁰. In brief, this method accounts for biases resulting from the
1098 difference in GC content across genes. Using a Gaussian kernel, the expression values were

1099 scaled by estimating the non-parametric kernel of its cumulative density function, which was used
1100 for estimating a rank distribution. Kolmogorov Smirnov like random walk statistic was used to
1101 calculate a normalized enrichment score based on the absolute difference of the magnitude of the
1102 positive and negative random walk deviations.

1103

1104 **TCGA RPPA data**

1105 We downloaded level-4 normalized reverse phase protein arrays (RPPA) of TCGA glioblastoma
1106 patients from The Cancer Proteome Atlas (TCPA) (<http://tcpaportal.org/tcpa>). The data was
1107 filtered to include 169 samples, which were in common between both the RPPA and the GBM406
1108 dataset.

1109

1110 **Interaction network analysis**

1111 Volcano plot representation of LN-18 RNA-seq data was performed using tidyverse package in
1112 Rstudio. A cut-off ($|Log2FC| > 0.5$ and adjusted p-value < 0.05) was applied to count significantly
1113 altered genes. Autophagy-related genes that were upregulated upon Trp restriction were extracted
1114 from Bordi et al⁷⁴. The identified autophagy-related genes were subjected to interaction network
1115 analysis using STRING database¹¹¹. Physical interaction networks were generated and subjected
1116 to k-means clustering (cluster n = 5) to obtain subnetworks, which were further classified based
1117 on the functions of the proteins⁷⁴.

1118

1119 **Promoter binding analysis**

1120 The AHR binding motif in the *MAP1LC3B* promotor region was retrieved from the Transcription
1121 Factor Target Gene Database (<http://tfbsdb.systemsbiology.net/>)¹¹². Information on chromatin state
1122 and regulatory elements were derived from the UCSC browser, including ENCODE histone H3 lysine 27
1123 acetylation (ENCODE histone modification tracks), DNase hypersensitivity cluster information
1124 (Integrated Regulation from ENCODE, V3), chromatin segmentation states (Broad ChromHMM)
1125 and regulatory element interactions based on GeneHancer (GeneHancer Regulatory Elements
1126 and Gene Interactions, V2¹¹³). The reference genome used was hg19. Binding motifs for AHR
1127 (*MAP1LC3B* promoter) were visualized in conjunction with chromatin state and interaction data
1128 from the UCSC browser.

1129

1130 **Protein isolation and immunoblot**

1131 For protein harvest, cells were washed once with ice-cold PBS (Gibco, 14190169) and lysed with
1132 radio immunoprecipitation assay (RIPA) buffer containing 1% IGEPAL CA-630 (Sigma-Aldrich,
1133 I8896), 0.1% SDS (Carl Roth, 8029.3), and 0.5% sodium deoxycholate (AppliChem, A1531) in
1134 PBS supplemented with Phosphatase Inhibitor Cocktail 2 and Cocktail 3 (Sigma-Aldrich, P5726,
1135 P0044) and Complete Protease Inhibitor Cocktail (Roche, 11836145001) and centrifuged for 10
1136 min at 13,000 g and 4°C. Protein concentration was determined using Protein Assay Dye Reagent
1137 Concentrate (Bio-Rad, 5000006), and absorbance was measured at 595 nm using a
1138 spectrophotometer (GE Healthcare). All samples within one experiment were adjusted to the
1139 lowest absorbance value. Cell lysates were mixed with 5x Laemmli buffer containing 10% glycerol
1140 (Sigma-Aldrich, 15523), 1% beta-mercaptoethanol (Sigma-Aldrich, M3148), 1.7% SDS (Carl Roth,
1141 8029.3), 62.5 mM TRIS base (Sigma-Aldrich, T1503) [pH 6.8], and bromophenol blue (Sigma-
1142 Aldrich, B5525), and boiled for 5 min at 95°C. Separation of proteins was performed with SDS
1143 polyacrylamide gel electrophoresis (PAGE) using gels with a concentration of 8%, 10%, 14% or
1144 15% acrylamide (Carl Roth, 3029.1) in a Mini-PROTEAN Tetra Vertical Electrophoresis Cell
1145 system (Bio-Rad, 1658029FC) with running buffer containing 0.2 M glycine (Sigma-Aldrich,
1146 33226), 25 mM TRIS base (Sigma-Aldrich, T1503), and 0.1% SDS (Carl Roth, 8029.3) at 80 - 150
1147 V. Proteins were blotted onto a PVDF membrane (Merck Millipore, IPVH00010) or nitrocellulose
1148 membrane (Sigma-Aldrich, GE10600001) at 45 V for 2 h using the Mini-PROTEAN Tetra Vertical
1149 Electrophoresis Cell System (Bio-Rad, 1658029FC) and the blotting buffer containing 0.1 M
1150 glycine (Sigma-Aldrich, 33226), 50 mM TRIS base (Sigma-Aldrich, T1503), 0.01% SDS (Carl
1151 Roth, 8029.3) [pH 8.3], and 10% methanol (Merck, 1.06009.2511). Membranes were blocked for
1152 1 h at RT in 5% BSA (Carl Roth, 8076.5) in Tris-buffered saline tween (TBST) buffer (0.15 M NaCl
1153 (Sigma-Aldrich, S7653), 60 mM TRIS base (Sigma-Aldrich, T1503), 3 mM KCl (Sigma-Aldrich,
1154 P405), and 0.1% Tween-20 (Sigma-Aldrich, P9416), [pH 7.4]). Primary antibodies were diluted as
1155 recommended by the manufacturer in 5% BSA in TBST and incubated overnight at 4°C. On the
1156 next day, membranes were washed three times for 10 min in TBST buffer and subsequently
1157 incubated for 2 h with the respective horseradish peroxidase (HRP)-coupled secondary antibody
1158 dissolved in 5% BSA in TBST buffer. After another three 10 min wash steps in TBST buffer,
1159 proteins were detected using ECL Western Blotting Substrate (Thermo Fisher Scientific, 32106,
1160 Amersham, RPN2235), or SuperSignal West FEMTO (Thermo Fisher Scientific, 34096) under a
1161 ChemiDoc XRS+ camera system (Bio-Rad, 1708265) or a Fusion Fx camera (Vilber). Images
1162 taken with the ChemiDoc XRS+ were quantified with the Image Lab software (Bio-Rad, v6.0.1).
1163 Images taken with the Fusion FX camera were quantified with the ImageQuant TL 1D (v8.2.0).
1164 Normalization was performed as described¹². In brief, the images were first normalized by the pixel

1165 values of a single lane to the average value of all lanes in a blot for each antibody. Subsequently,
1166 the internally normalized proteins were normalized to the loading control glycerinaldehyd-3-
1167 phosphat-dehydrogenase (GAPDH), tubulin (TUBA1B), or vinculin (VCL), as indicated.
1168 Upon separation by SDS-PAGE, EIF4EBP1 runs in three discernible bands (alpha, beta, gamma),
1169 all of which can be phosphorylated at T37/46 by MTORC1^{114,115}. We therefore quantified across
1170 all EIF4EBP1-pT37/46 signals.

1171

1172 **Puromycin Assay**

1173 Protein synthesis was measured by puromycin assay. Therefore, 5 µg/mL puromycin (Sigma-
1174 Aldrich, P8833) was added directly to the media 5 min prior lysis. Puromycin incorporation was
1175 detected by immunoblot analysis, as described above. The entire lane was used for quantification
1176 of the puromycin blots.

1177

1178 **Cap pull down**

1179 The cells were washed two times in ice-cold PBS and harvested in 1 mL per 15 cm plate of cap
1180 pull down lysis buffer (40 mM HEPES, 120 mM NaCl, [pH 7.5], 0.3% CHAPS) supplemented with
1181 Phosphatase Inhibitor Cocktail 2 and Cocktail 3 (Sigma-Aldrich, P5726 and P0044) and Complete
1182 Protease Inhibitor Cocktail (Roche, 11836145001). The lysed cells of every condition were pooled
1183 together (six dishes per condition were seeded). Afterwards, the samples were end over end
1184 rotated at 4°C for 20 min and then centrifuged for 3 min at 600 g and 4°C. The protein
1185 concentrations were measured in the supernatants using Protein Assay Dye Reagent Concentrate
1186 and all samples were adjusted to the lowest value. For the input analysis, 160 µL per condition
1187 were mixed with 40 µL of 5x Laemmli buffer [10% glycerol (Sigma-Aldrich, 15523), 1% beta-
1188 mercaptoethanol (Sigma-Aldrich, M3148), 1.7% SDS (Carl Roth, 8029.3), 62.5 mM TRIS base
1189 (Sigma-Aldrich, T1503) [pH 6.8], and bromophenol blue (Sigma-Aldrich, B5525)], incubated at
1190 95°C for 5 min, vortexed, centrifuged and stored at -20°C. Cap pull down beads (Immobilized γ-
1191 Aminophenyl-m7GTP, Jena Bioscience, AC-155) and mock beads (Jena Bioscience, AC-001)
1192 were washed twice with lysis buffer. The samples were respectively split into halves. 17.5 µL of
1193 cap pull down beads / lysate were added to one half of a sample, 17.5 µL of mock beads / lysate
1194 were added to the other half of a sample. The sample-beads mixtures were end over end rotated
1195 at 4°C for two hours and then centrifuged at 500 g for 1 min. The supernatants were removed and
1196 the pellets resuspended in 500 µL of lysis buffer. After transferring the resuspended pellets into
1197 new tubes, they were washed three times with lysis buffer by inverting the tubes six times and
1198 centrifugation at 500 g for 1 min. After the last washing step, the buffer was carefully removed and

1199 the pellets were dissolved in 65 μ L of 1x Laemmli buffer. The samples were gently vortexed and
1200 incubated for 10 min at 95°C before freezing them at -20°C.

1201

1202 **Ras pull down assay**

1203 For Ras pull down, cells were collected in MLB buffer (RAS Activation Assay Kit, Merck Millipore,
1204 17-218) after 30 min of 10 ng/mL EGF (Preprotech, AF-100-15) stimulation. Protein concentration
1205 was determined using BCA assay (Thermo Fisher Scientific, 23227) and pellets were frozen in -
1206 80°C. The RAS GTP pull-down assay was performed as described in Heberle et al, 2019⁴⁴. In
1207 short, 500 μ L protein extracts (800 μ g - 1 mg, adjusted depending on the lowest concentration in
1208 each replicate) were incubated for 45 min at 4°C with 10 μ L agarose beads using a RAS-GTP
1209 pull-down assay kit (RAS Activation Assay Kit, Merck Millipore, 17-218). Supernatant was
1210 recovered after centrifugation, mixed with 40 μ L of 1x Laemmli buffer, incubated at 95°C for 5 min,
1211 centrifuged and stored at -20°C. For immunoblot analysis of RAS-GTP levels 20 μ L of protein
1212 extract was separated by gel electrophoresis, blotted and incubated overnight in 5% skim milk
1213 (GERBU Biotechnik, 70166) in TBST at 4°C with an anti-RAS antibody (Millipore, 05-516). As a
1214 loading control, glutathione-S transferase (GST; CST, 2622) was tested in 5% skim milk in TBST
1215 for 2 h at RT. Immunoblots were quantified using ImageJ v.153k. Single lane chemiluminescence
1216 values were normalized to the average value of all lanes in a blot for each antibody, and
1217 subsequently normalized to the internal loading control GST.

1218

1219 **Kinase assay**

1220 The kinase assays were developed based on previous work^{116,117}. Media exchange was performed
1221 once 10 cm dish (TPP, 93100) was confluent. After treatment, the cells were washed three times
1222 in ice-cold PBS and then harvested in 600 μ L per plate CHAPS-based IP lysis buffer [40 mM
1223 HEPES (Gibco, 15630056), 120 mM NaCl (Sigma-Aldrich, S7653), [pH 7.5] and 0.3% CHAPS
1224 (Merck, 331717-45-4)] supplemented with 500 nM Benzamidine (Benzamidine, B6506-5G) and
1225 20 μ g/mL Heparin (Sigma-Aldrich, H3149-25KU). The lysate was incubated under gentle agitation
1226 for 20 min at 4°C, centrifuged for 3 min at 600 g at 4°C, the pellet was discarded and the
1227 supernatant was transferred to fresh tubes. In case of multiple samples, the protein concentration
1228 was measured using Protein Assay Dye Reagent Concentrate (Bio-Rad, 5000006) and all
1229 samples were adjusted to the lowest value. The lysates were pre-incubated with 10 μ L pre-
1230 washed Protein G covered Dynabeads (Life Technologies, 10009D) per mL of lysate for 30 min
1231 at 4°C under gentle agitation. The pre-cleaned lysates were subdivided, and anti-RPTOR
1232 antibodies (Helmholtz Center Munich, 20D12) or isotype control IgG antibodies (Helmholtz Center
1233 Munich, 7H8) were added using 7.5 μ g antibody per mL of pre-cleaned lysate. Isotype control IgG

1234 antibodies (mock antibodies) were used in the same concentration as the protein-specific
1235 antibodies. After 30 min at 4°C under gentle agitation, 37.5 µL pre-washed Protein G covered
1236 Dynabeads / mL lysate were added, and the incubation was continued for 90 min at 4°C under
1237 gentle agitation. Next, beads were washed with CHAPS lysis buffer three times shortly and three
1238 times for 10 min at 4°C under gentle agitation.

1239 Following the last wash step with CHAPS lysis buffer, the beads were subdivided and excess
1240 liquid was removed. The kinase assays were performed in a final volume of 30 µL, containing
1241 kinase assay buffer (final concentration: 40 mM HEPES, 120 mM NaCl, [pH 7.5] and 0.3%
1242 CHAPS, 4 mM MnCl₂ (Merck, 1059270100), 10 mM DTT (Sigma-Aldrich, D0631), supplemented
1243 with 1x Protease inhibitor cocktail without EDTA and 2 µg/mL Heparin), 100 ng recombinant H6-
1244 EIF4EBP1 (cloned and purified as described above), 500 nM AZD8055 (MedChem Express, HY-
1245 50706) and 0.133 mM ATP (Merck, 74804-12-9).

1246 First, 24.5 µL kinase assay buffer was added to each condition, before 0.5 µL of dried
1247 AZD8055, dissolved in kinase assay buffer, or 0.5 µL of kinase assay buffer was added and pre-
1248 incubated for 15 min at 4°C before initiation of the kinase reaction. The kinase reactions were
1249 started by adding 1 µL of recombinant H6-EIF4EBP1, or kinase assay buffer, and addition of 4 µL
1250 1 mM ATP. The reactions were incubated at 30 °C for the indicated time points, and stopped by
1251 the addition of 30 µL 2x Laemmli buffer. Samples were heated for 5 min at 95°C and separated
1252 by SDS-PAGE. The MTORC1-mediated phosphorylation of EIF4EBP1-pT37/46 and MTOR levels
1253 were run on gradient gels and detected by immunoblotting with specific antibodies. Signals were
1254 quantified using ImageQuant (v8.2.0) and are shown as the EIF4EBP1-pT37/46/MTOR ratio.

1255
1256 **Immunofluorescence**

1257 For EGFR immunofluorescence experiments, cells were washed with ice-cold PBS (Gibco,
1258 14190169), fixed in 100% methanol (VWR, 85681-320) for 10 min at RT and permeabilized with
1259 0.3% Triton X-100 (Sigma-Aldrich, T8787) in TBS [0.15 M NaCl (Sigma-Aldrich, S7653), 60 mM
1260 TRIS base (Sigma-Aldrich, T1503), and 3 mM KCl (Sigma-Aldrich, P405) [pH 7.4]] for 10 min at
1261 37°C. Prior to immunofluorescence, blocking was performed in TBS + 1% BSA (Carl Roth, 8076.5)
1262 for 2 h and incubated with anti-EGFR (CST, 4267) and anti-LAMP2 (DSHB, H4B4) antibodies for
1263 3 h at RT. After three wash steps in TBST [0.15 M NaCl (Sigma-Aldrich, S7653), 60 mM TRIS
1264 base (Sigma-Aldrich, T1503), 3 mM KCl (Sigma-Aldrich, P405), and 0.1% Tween-20 (Sigma-
1265 Aldrich, P9416), [pH 7.4]], anti-rabbit Alexa-488 (A-11008, Invitrogen) and anti-mouse Alexa-647
1266 antibodies (A-32728, Invitrogen) were added for 2 h at RT in the dark. Finally, nuclei were
1267 counterstained with 5 µg/mL DAPI (BD Biosciences, 564907) in TBS for 1 min. Microscopy was
1268 performed using a CQ1 Confocal Quantitative Image Cytometer (Yokogawa Electric). For nuclear,

1269 EGFR focus and counting, binary masks were generated from intensity-thresholded images. For
1270 LAMP2 compartment size measurement, images were thresholded using an IJ_Isodata algorithm.
1271 The total measured area was normalized to nuclear count per image to determine the mean
1272 LAMP2 compartment size per cell. Image analysis was performed using ImageJ v.153k.

1273 For p62 autophagy immunofluorescence experiments, the cells were washed twice with
1274 ice cold PBS (Gibco, 14190169) and fixed with 4% PFA (AppliChem, A3813) in PBS for 20 min.
1275 Afterwards, the cells were washed three times with PBS before permeabilizing them with 0.1%
1276 Triton X-100 (Sigma-Aldrich, T8787) in PBS for 60 seconds. After another three washing steps
1277 with PBS, blocking solution (5% FBS and 0.05% Tween20 in PBS) was added to the cells for 60
1278 min. The p62 primary antibody (Progen, GP62-C) was diluted in blocking solution, and the cells
1279 incubated in the diluted primary antibody overnight at 4°C in a humid chamber. The next day, the
1280 cells were washed three times short and two times for 10 min with PBS. The Alexa Fluor 568
1281 labelled secondary antibody (Invitrogen, A-11075) and Hoechst 33342 (Invitrogen, H3570) were
1282 diluted in blocking solution, and added to the cells for 60 min at RT in a dark humid chamber. The
1283 cells were washed three times with PBS and then twice with deionized water, before mounting the
1284 coverslips with Mowiol® 4-88 with DABCO (1,4-diazabicyclo[2.2.2]octane) and 10% n-propyl-
1285 gallate (NPG). For image collection, the Axiocam 702 mono camera was utilized, acquiring seven
1286 stacks (0.5 µM thickness) per image. The exposure time was adjusted to the condition with the
1287 strongest signal and kept constant throughout all conditions. For quantifying the p62 foci per cell,
1288 CellProfiler 4.2.1 was used.

1289

1290 **Macropinocytosis Assay**

1291 For the uptake assay, 20 µL of medium with 70 kDa-Dextran Oregon Green (dextran) (Invitrogen,
1292 D7173) with a final concentration of 0.1 mg/mL and 10 ng/mL EGF were added for 30 min. Next,
1293 cells were washed twice with ice-cold PBS and fixed with 4% formaldehyde (AppliChem, A3813)
1294 in PBS for 20 min at RT. Fixed cells were washed with PBS and incubated with 10 mg/mL DAPI
1295 (Serva Electrophoresis, 18860) in PBS for 10 min. Finally, cells were washed again with PBS and
1296 imaged using an AxioObserver.Z1, equipped with an LSM780 ConfoCor 3 microscope with a 63x
1297 / 1.4 Oil DIC M27 Plan-Apochromat objective and ZEN 2012 (Zeiss, black edition, v8.1.0,484)
1298 software. Nuclear staining using DAPI was imaged with an UV diode (405 nm) and the dextran
1299 detection (488 nm) was performed using an argon multiline (458/488/514 nm). Detector gain and
1300 detector offset were adjusted once and never changed for an entire dataset. Raw images (CZI
1301 files) were subjected for further analyses in Fiji.

1302 Dextran fluorescence was analyzed with Fiji version 1.52p using a background subtraction
1303 of 3, a Gaussian Blur filter of 1, threshold adjustment from 3500-max, a prominence of 10, and the

1304 'Analyze Particles' function with a particle size from 5-infinity. The number ('count') of
1305 macropinosomes was then divided by the number of respective cells displayed in the DAPI
1306 channel in the analysed microscopy picture. The number of macropinosomes per cell were
1307 compared between at least 5 independent fields of view from 4 independent datasets. For
1308 presentation in figures, ZEN 3.0 (Zeiss, blue edition) was used, and representative regions of
1309 interest for each condition were exported as TIFF with no compression using the ZEN 'Best Fit'
1310 option. Dextran green fluorescence was pseudo-coloured white. Finally, brightness or contrast
1311 were adjusted for better visibility.

1312

1313 **Lysotracker**

1314 For lysosome tracking, 20 min before live cell imaging, cells were washed with PBS and 10 nM
1315 LysoTracker™ Red DND-99 (lysotracker) (Invitrogen, L7528) and 10 mg/mL DAPI (Serva
1316 Electrophoresis, 18860) in PBS were added. Living cells were imaged as above with an
1317 AxioObserver.Z1, equipped with an LSM780 ConfoCor 3 microscope with a 63x / 1.4 Oil DIC M27
1318 Plan-Apochromat objective and ZEN 2012 (black edition, v8.1,0,484) software. Nuclear staining
1319 using DAPI was imaged with an UV diode (405 nm) and lysotracker with a 561 nm laser. Detector
1320 gain and detector offset were adjusted once and never changed for an entire dataset. Raw images
1321 (CZI files) were subjected for further analyses in Fiji. Lysotracker was analyzed with Fiji version
1322 1.52p using a background subtraction of 3, a Gaussian Blur filter of 1, threshold adjustment from
1323 3500-max, a prominence of 10, and the 'Analyze Particles' function with a particle size from 15-
1324 infinity. The raw integrated density (RawIntDen) value was then divided by the number of
1325 respective cells displayed in the DAPI channel in the analysed microscopy picture. The intensity
1326 of lysotracker foci per cell was then compared across at least 3 independent fields of view from 5
1327 independent datasets.

1328

1329 **Simultaneous proteo-metabolome liquid-liquid extraction and measurement**

1330 Proteome extraction from cells was done by a simultaneous proteo-metabolome liquid-liquid
1331 extraction ¹¹⁸. The cells were washed three times with PBS and cell metabolism was quenched by
1332 addition of 500 µL ice-cold methanol (Fisher Chemical, 10653963) and 500 µL MS-grade water
1333 (Millipore, Direct Water Purification System). Lysates were scraped and transferred to tubes
1334 followed by the addition of 500 µL chloroform. After agitation in a cell shaker at 4°C for 20 min and
1335 500 rpm, phase separation was performed by centrifugation at 4°C for 5 min at 16,100 g.
1336 Subsequently, after removing the liquid polar and non-polar phases, the solid interphases
1337 containing the proteomes were washed with methanol. Finally, interphases were dried, covered
1338 with 50 µL methanol, and stored at -80°C until further processing.

1339 Protein extraction from interphases

1340 To extract proteins, 60 μ L of 8 M urea (Sigma-Aldrich, 51456) in 100 mM ammonium bicarbonate
1341 (Sigma-Aldrich, 09830-500G), pH 8.2 were added to the interphases followed by 240 μ L of 100
1342 mM NH_4HCO_3 , pH 8.2. To bring proteins into solution, samples were sonicated with a tip sonicator
1343 (Thermo Fisher Scientific, 10588013). Protein concentration was determined using a microplate
1344 BCA protein assay kit (Thermo-Fisher-Scientific, 23227) following the manufacturer's instructions.
1345 For protein determination, samples were diluted 1:50 in MilliQ water. A BSA standard was used
1346 to calibrate the assay across the concentration range of 0 - 200 $\mu\text{g/mL}$. The absorbance was
1347 measured at 580 nm using a plate reader (BMG Labtech, PHERAstar FSX). Extracts from samples
1348 that had been cultured with 0.4, 0.2 and 0 μM Trp were pooled.

1349

1350 Digestion and desalting

1351 100 μg of dissolved protein was transferred into a new vial and filled to a final volume of 100 μL
1352 with the extraction buffer. Samples were incubated with 1 M DTT (Sigma-Aldrich, D0631) in 0.1 M
1353 triethylammonium bicarbonate (TEAB) (Sigma-Aldrich, 15715-58-9) to a final concentration of 10
1354 mM DTT on a shaker for 30 min at 55°C and 800 rpm. Afterwards, alkylation was performed by
1355 0.5 M iodoacetamide (IAA) (Sigma-Aldrich, I1149). IAA was added to a final concentration of 20
1356 mM and incubated in the dark for 30 min. To quench the remaining IAA, DTT (1 M DTT in 0.1 M
1357 TEAB) was added. Digestion of the proteins was performed by the addition of trypsin (Gibco,
1358 15400054) in a trypsin:protein ratio of 1:20. After overnight digestion at 37°C, the reaction was
1359 stopped by adding 100% formic acid (FA) (Fisher Scientific, 10596814) to achieve a final
1360 concentration of 1% FA in each sample. Afterwards, peptide samples were desalting using Oasis
1361 HLB 1 cc Vac Cartridge (Waters, 186000383). For this, the cartridges were first activated with 1
1362 mL of 100% methanol, followed by 1 mL of 95% ACN (Fisher Scientific, 10616653), 0.1% FA.
1363 Next, equilibration was performed by adding twice 1 mL of 0.1% FA. Peptide samples were slowly
1364 loaded onto the cartridge in 1 mL 0.1% FA. After washing twice with 1 mL 0.1% FA, samples were
1365 eluted from the cartridge with 1 mL 70% ACN, 0.1% FA. Samples were dried in a SpeedVac
1366 (Eppendorf, Concentrator 5301) and dried peptides were stored at -80°C until further processing.

1367

1368 LC-MS/MS analysis

1369 For LC-MS/MS analysis of the Trp stress proteome, the dried tryptic peptides were dissolved in
1370 20 μL 0.1% FA. The samples were injected on a nano-ultra pressure liquid chromatography
1371 system (Dionex UltiMate 3000 RSLCnano pro flow, Thermo Fisher Scientific) coupled via an
1372 electrospray ionization (ESI) source to an orbitrap hybrid mass spectrometer (QExactive, Thermo
1373 Scientific). The samples were loaded (5 $\mu\text{L}/\text{min}$) on a trapping column (nanoE MZ Sym C18, 5

1374 μ m, 180 μ m x 20 mm, Waters; buffer A: 0.1% FA in HPLC-H₂O; buffer B: 100% ACN, 0.1% FA)
1375 with 100% buffer A. After sample loading, the trapping column was washed for 5 min with 100%
1376 buffer A (5 μ L/min) and the peptides were eluted (300 nL/min) onto the separation column (nanoE
1377 MZ PST CSH, 130 A, C18, 1.7 μ m, 75 μ m x 250 mm, Waters) and separated with a gradient of
1378 2–30% B in 60 min. The spray was generated from a steel emitter (Fisher Scientific) at a capillary
1379 voltage of 1850 V. MS/MS measurements were carried out in data-dependent acquisition mode
1380 (DDA) using a normalized HCD collision energy of 25% and a loop count of 15. MS scan was
1381 performed over an m/z range from 400-1200, with a resolution of 70,000 at m/z 200 (maximum
1382 injection time = 240 ms, AGC target = 1e6). MS/MS spectra were recorded over a m/z range of
1383 100-2000 m/z with a resolution of 17,500 at m/z 200 (maximum injection time = 50 ms, maximum
1384 AGC target = 1e5, intensity threshold: 5e3), a quadrupole isolation width of 2 Da and an exclusion
1385 time of 20 seconds.

1386 For LC-MS/MS analysis of the -all aa DMEM, -all aa HBSS and Met stress proteomes, the
1387 dried tryptic peptides were dissolved in 20 μ L 0.1% FA. Samples were injected on a nano-ultra
1388 pressure liquid chromatography system (Vanquish Neo UHPLC System, Thermo Fisher Scientific)
1389 coupled via an electrospray ionization (ESI) source to an Orbitrap Eclipse (Thermo Scientific). The
1390 samples were loaded (60 μ L/min) on a trapping column (nanoE MZ Sym C18, 5 μ m, 180 μ m x 20
1391 mm, Waters; buffer A: 0.1% FA in HPLC-H₂O; buffer B: 80% ACN, 0.1% FA) with 100% buffer A.
1392 After sample loading, the trapping column was washed and the peptides were eluted (300 nL/min)
1393 onto the separation column (nanoE MZ PST CSH, 130 A, C18 1.7 μ , 75 μ m x 250 mm, Waters)
1394 and separated with a gradient of 1-40% B in 90 min. The spray was generated from a steel emitter
1395 (Fisher Scientific) at a capillary voltage of 1850 V. MS/MS measurements were carried out in data-
1396 dependent acquisition mode (DDA) using a normalized HCD collision energy of 30% and a cycle
1397 time of 3s. The MS scan was performed over an m/z range from 375-1500, with a resolution of
1398 240,000 at m/z 200 (maximum injection time= 50 ms, AGC target= 4e5). MS/MS spectra were
1399 recorded over a m/z range of 135-2000 m/z (maximum injection time= 35 ms, maximum AGC
1400 target= 1e5, intensity threshold: 5e3), a quadrupole isolation width of 0.8 Da and an exclusion time
1401 of 60 seconds in the ion trap.

1402
1403 LC-MS/MS data processing
1404 LC-MS/MS raw files were analysed with ProteomeDiscoverer 2.4 (Thermo Fisher Scientific). For
1405 peptide and protein identification, the LC-MS/MS were searched with SequesHT against a human
1406 database (SwissProt, 20,369 entries) and a contaminant database (116 entries). The following
1407 parameters were used for the data-base search: mass tolerance MS1: 10 ppm, mass tolerance
1408 MS2: 0.02 Da for MS/MS analysis in the orbitrap and 0.5 for MS/MS analysis in the ion trap, fixed

1409 modification: carbamidomethylation (cysteine), variable modification: Oxidation (Methionine),
1410 variable modification at protein N-terminus: Acetylation, Methionine loss, Methionine loss +
1411 Acetylation. Trp-Phe exchanges were included as a variable modification for analysis of the Trp
1412 stress proteome. Percolator were used for FDR calculation. For feature detection, Minora Feature
1413 Detection was used with default settings. For label-free quantification, the Precursor Ions
1414 Quantifier was used with the following parameters: Peptides to use: unique peptides, Precursor
1415 Abundance Based On: Area, Minimum Replicate Features: 100% for the Trp stress proteome and
1416 75% for generalized amino acid stress (HBSS, DMEM) and methionine stress proteomes,
1417 Normalization Mode: Total Peptide Amount, Protein Abundance Calculation: Summed
1418 Abundances, Top N: 3, Hypothesis testing: t-test (Background Based). Adjusted p-values were
1419 calculated using Benjamini–Hochberg correction. Venn diagrams show proteins up- or
1420 downregulated in different conditions. Proteins are defined as regulated if they have a fold change
1421 of at least 1.5 with an adjusted p-value of lower than 0.05.

1422 GO enrichment was performed with g:profiler¹¹⁹. Resulting p-values were corrected with the
1423 Benjamini-Hochberg method. Visualization of results was done using the ggplot2 package in R¹²⁰.

1424 To assess if protein synthesis was disrupted at tryptophan positions leading to shorter
1425 proteins, the protein coverage by mass spectrometry was analyzed. For this, proteins that were
1426 upregulated in low tryptophan conditions were considered. A density plot was created showing
1427 the distribution of peptides of proteins upregulated under Trp starvation. Additionally, a heatmap
1428 was created showing the peptide coverage for all regulated proteins. Peptides were considered
1429 as „quantified“ if they were assigned by Proteome Discoverer with at least confidence „High“ in
1430 any of the low tryptophan samples.

1431
1432 Extraction of intracellular Trp and quantification by mixed mode reversed phase-anion exchange
1433 UPLC-MS/MS

1434 The cells were treated as described in the Simultaneous proteo-metabolome liquid-liquid
1435 extraction paragraph. A fully ¹³C, ¹⁵N labelled amino acid standard (Cambridge Isotope
1436 Laboratories, MSK-CAA-1) was spiked into samples at the first step of the extraction. Dried polar
1437 phases obtained from simultaneous extraction were dissolved in 100 µL of water containing 5 mM
1438 ammonium formate (NH₄FA) (Sigma-Aldrich, 70221-100G-F) and 0.15% FA (Fisher Scientific,
1439 10596814). 1 µL of each sample was injected. Analytes were separated at 40°C on an Atlantis
1440 Premier BEH C18 AX column (1.7 µm, 2.1 x 150 mm, Waters, 186009361) using an Acquity
1441 Premier UPLC system (Waters).

1442 A gradient was run at a flowrate of 0.3 mL/min with mobile phase A (5 mM NH₄FA and 0.15% FA
1443 in water) and mobile phase B (10 mM NH₄FA and 0.15% FA 80% ACN) as follows: 5% B to 15%

1444 B in 2 min, 15% B to 70% B in 1.5 min, 70% B to 95% B in 0.5 min followed by 1 min of elution at
1445 95% B and re-equilibration of the column to initial conditions over 2 min. Trp was detected using
1446 a Xevo-TQ XS Mass spectrometer (Waters) equipped with an electrospray ionization source
1447 running in positive mode. The transition from 205.1 -> 146.2 for endogenous Trp and 218.1 ->
1448 156.1 were used for quantification. The cone voltage was set to 14 V and the collision energy was
1449 set to 18 V. Raw files were analysed in TargetLynx (Waters, V4.2 SCN1012). Resulting peak
1450 areas of endogenous and ¹³C, ¹⁵N tryptophan were further analysed in R and resulting tryptophan
1451 concentrations were normalised to cell numbers.

1452
1453 For the determination of the intracellular Trp concentration in μ M, the average cell size (11.4 μ m)
1454 was determined as the size per cell via the CytoSMART Exact cell counter (Axion BioSystems)
1455 with a lower size gate of 8 μ m. Assuming a sphere, the cell volume was estimated to be 775.7
1456 μ m³.

1457
1458 Extraction of sphingolipids
1459 For the measurement of sphingolipids in LN-18 cells, the cells were washed with 5 mL PBS (4°C)
1460 and trypsinized with 1 mL 0.25% Trypsin-EDTA per dish (Gibco, 25200-056) for 5 min at 37°C and
1461 5% CO₂. After culture medium (4 mL) has been added, cells were pelleted (500 g, 5 min, 4°C),
1462 washed twice with ice-cold PBS (1.0 mL and 0.5 mL, 4°C), centrifuged (3000 g, 5 min, 4°C), frozen
1463 in liquid nitrogen, and stored at -80°C.

1464 Sphingolipids were extracted from LN-18 cell pellets by successive addition of PBS pH 7.4,
1465 methanol, chloroform, and saline to a final ratio of 14:34:35:17^{121,122}. The organic phase was
1466 evaporated to dryness using an Eppendorf Concentrator Plus System (Eppendorf, 5305000509;
1467 high vapor pressure application mode), and the remaining lipid film was dissolved in methanol,
1468 centrifuged twice at 21,100 \times g, 4°C for 5 min, and subjected to UPLC-MS/MS analysis. Internal
1469 standards used (Sigma-Aldrich): D-erythro-sphingosine-d7, N-heptadecanoyl-D-erythro-
1470 sphingosine, D-glucosyl- β -1,1'-N-heptadecanoyl-D-erythrosphingosine, N-lauroyl-ceramide-1-
1471 phosphate, N-heptadecanoyl-D-erythro-sphingosylphosphorylcholine, and D-erythro-
1472 sphingosine-d7-1-phosphate.

1473
1474 Analysis of sphingolipids by reversed phase UPLC-MS/MS
1475 Chromatographic separation of sphingosines (Sph), (dihydro)ceramides ([dh]Cer), hexosyl-
1476 ceramides (HexCer), ceramide-1-phosphates (C1P), and (dihydro)sphingomyelines ([dh]SM) was
1477 carried out at 45°C on an Acquity UPLC BEH C8 column (130Å, 1.7 μ m, 2.1 \times 100 mm, Waters,
1478 186002878) using an ExionLC AD UHPLC system (Sciex). The gradient of mobile phase A

1479 (water/ACN, 90/10, 2 mM ammonium acetate) and mobile phase B (ACN/water, 95/5, 2 mM
1480 ammonium acetate) was ramped at a flow rate of 0.75 mL/min from 75% to 85% B within 5 min
1481 and to 100% B within another 2 min, followed by 13 min of isocratic elution.

1482 Sphingolipids were analyzed in the positive ion mode by scheduled multiple reaction
1483 monitoring (MRM) using a QTRAP 6500⁺ Mass Spectrometer (Sciex), which was equipped with
1484 an electrospray ionization source. Transitions from $[M+H]^+$ to $[M+H-H_2O]^+$ (Sph, dhCer), m/z =
1485 184.1 ([dh]SM), and m/z = 264.4 (Cer, HexCer, C1P) were selected for quantitation. The curtain
1486 gas was set to 40 psi, the collision gas to medium, the ion spray voltage to 5000 V, the heated
1487 capillary temperature to 500°C, and the sheath and auxiliary gas pressure to 40 psi. The
1488 declustering potential was adjusted to 30 V (Sph, [dh]Cer, C1P) or 40 V (HexCer, [dh]SM), the
1489 entrance potential to 5 V (HexCer) or 10 V (Sph, [dh]Cer, C1P, [dh]SM), the collision energy to 20
1490 eV (Sph), 30 eV ([dh]SM), 40 eV ([dh]Cer, C1P), or 50 eV (HexCer), and the collision cell exit
1491 potential to 5 V (C1P), 10 V ([dh]SM), 20 V ([dh]Cer, HexCer), or 25 V (Sph).

1492 In variation to the procedure described above, sphingosine-1-phosphate (S1P) was
1493 separated on an Acquity UPLC CSH C18 column (130Å, 1.7 µm, 2.1 × 50 mm, Waters,
1494 186005296) at 55°C. The LC system was operated at a flow rate of 0.55 mL/min using water/ACN
1495 (80/20) with 0.1% formic acid as mobile phase A and isopropanol/ACN (80/20) with 0.1% formic
1496 acid as mobile phase B. Initial conditions (60% B) were kept for 3 min, linearly increased to 70%
1497 B within 2 min and further to 100% B within 0.4 min, followed by isocratic elution for 1.6 min.

1498 For the analysis of S1P in the positive ion mode ($[M+H]^+$) by MRM, $[M+H-H_3PO_4-H_2O]^+$
1499 (m/z = 264.2) was detected as fragment ion. The curtain gas was set to 40 psi, the collision gas
1500 to low, the ion spray voltage to 4500 V, the heated capillary temperature to 500°C, the sheath gas
1501 pressure to 60 psi, the auxiliary gas pressure to 30 psi, the declustering potential to 40 V, the
1502 entrance potential to 10 V, the collision energy to 20 eV, and the collision cell exit potential to 20
1503 V. Relative proportions of total ceramides (calculated as sum of ceramide species analyzed) are
1504 given as percentage of the sum of all sphingolipids determined in the corresponding sample
1505 (= 100%). Mass spectra were acquired and processed using Analyst 1.7.1 or 1.7.2 (Sciex) and
1506 Analyst 1.6.3 (Sciex), respectively.

1507
1508 Targeted Proteomics samples preparation
1509 Samples were extracted via the simultaneous proteo-metabolome liquid-liquid extraction as
1510 described above. The dried interphases were solubilized in 50 µL 1% sodium deoxycholate in
1511 50 mM ammonium bicarbonate buffer pH 8.5. Samples were heated at 95°C for 5 min and
1512 sonicated in bath sonicator for 10 min. Protein concentration was measured by BCA assay
1513 (Thermo Fischer Scientific) and 30 µg proteins per samples were spiked with synthetic heavy-

1514 labelled peptides (**Table 2**). Synthetic peptides were C-terminally labeled with heavy lysine (¹³C₆,
1515 ¹⁵N₂) or arginine (¹³C₆, ¹⁵N₄) residues and spiked to reach an amount of approx. 12 or 120 fmol on
1516 column depending on the previously measured peptide signals. Samples were reduced with 1 mM
1517 DTT for 30 min at RT and alkylated for 30 min with 5.5 mM IAA for 30 min in the dark. Trypsin
1518 (Promega) was added to a 1/100 trypsin to protein ratio and the samples were incubated overnight
1519 at 37°C. Samples were acidified to 2% TFA and vortexed to precipitate the deoxycholate. Samples
1520 were spun and the supernatant was desalted on reverse-phase S cartridges (Agilent) using an
1521 Agilent Bravo automated liquid handling platform (Agilent). Samples were lyophilized overnight
1522 and resuspended in 0.1% formic acid in water. Peptide concentrations were measured on a
1523 NanoDrop (Thermo Fischer Scientific) and adjusted to 100 ng/μL.

1524

1525 **Table 2. Synthetic heavy-labelled peptides**

Protein Gene	Peptide	Precursor
OPTN	ADLLGIVSELQLK	703.9209++ (heavy)
OPTN	TSDSDQQAYLVQR	760.8644++ (heavy)
GABARAPL1	EDHPFEYR	551.7450++ (heavy)
NBR1	VSFDLNTIQIK	643.3657++ (heavy)
NBR1	IHLWNSIHGLQSPK	546.6382+++ (heavy)
NBR1	GALSVAASAYK	523.2920++ (heavy)
SQSTM1	VAALFPALR	484.3018++ (heavy)
SQSTM1	LTPVSPESSSTEEK	749.8718++ (heavy)
SQSTM1	NYDIGAACLDIQYSK	840.4220++ (heavy)
CALCOCO2	DYWETELLQLK	723.3737++ (heavy)
CALCOCO2	ENDHLFLSLTEQR	537.9382+++ (heavy)
STBD1	EHVPSGQFPDTEAPATSETSNSR	818.7063+++ (heavy)
BNIP3L	ILLDAQHESGQSSSR	546.6088+++ (heavy)
BNIP3	ILLDAQHESGR	416.8905+++ (heavy)
CCPG1	GELQQLSGSQLHGK	745.3961++ (heavy)
CCPG1	FFLNGVFIHDQK	736.8924++ (heavy)
NCOA4	DLELAIGGVLR	583.3444++ (heavy)
TAX1BP1	AHQLEEDIVSVTHK	538.6173+++ (heavy)
TAX1BP1	LSDQSANNNNVFTK	780.3806++ (heavy)
WDFY3	SEGVVPSPVSLVPEEK	830.9478++ (heavy)
FKBP8	ADFVLAANSYDLAIK	809.9320++ (heavy)
FKBP8	VLAQQGEYSEAIPILR	898.9927++ (heavy)
GABARAPL2	VSGSQIVDIDK	584.8186++ (heavy)
GABARAPL2	IQLPSEK	411.7442++ (heavy)
PNPLA2	VSDGENVIISHFNSK	551.9489+++ (heavy)
PNPLA2	YVDGGISDNLPLYELK	902.4664++ (heavy)
PHB2	LLLGAGAVAYGVR	635.3813++ (heavy)
PHB2	IGGVQQDTILAEGLHFR	622.0032+++ (heavy)
PHB2	IVQAEGEAAAK	612.3215++ (heavy)

1526 Targeted Proteomics measurements

1527 LC-MS/MS measurements were performed on a Q-Exactive HF-X mass spectrometer coupled to
1528 an EasyLC 1200 nanoflow-HPLC (all Thermo Scientific). 500 ng peptides were separated on a
1529 fused silica HPLC-column tip (I.D. 75 μ m, New Objective, self-packed with Acquity CSH C18-AQ,
1530 1.7 μ m (Waters) to a length of 20 cm) using a gradient of A (0.1% formic acid in water) and B
1531 (0.1% formic acid in 80% acetonitrile in water): samples were loaded with 0% B with a flow rate of
1532 600 nL/min; peptides were separated by 4%–30% B within 85 min with a flow rate of 250 nL/min.
1533 Spray voltage was set to 2.3 kV and the ion-transfer tube temperature to 250°C; no sheath and
1534 auxiliary gas were used. Mass spectrometer was operated in PRM mode with a resolution of
1535 60,000, maximum injection time of 118 ms, AGC target value of 1×10^6 and an isolation window
1536 of 1.5 m/z and normalized collision energy of 27. Targeted precursors were acquired in a
1537 scheduled manner in 3 min time windows. Loop count was set at 30 and full MS scans were
1538 acquired at a resolution of 30,000 with an AGC target value of 1×10^6 and a maximal injection time
1539 of 54 ms in a range of 400 to 1200 m/z. The raw MS files were analyzed using Skyline^{123,124}.
1540 Precursors were manually filtered for interferences. A minimum of 3 fragment ions were
1541 considered for quantification. Quantification values were normalized on heavy peptides and all
1542 quantities were extracted from skyline.

1543

1544 **tRNA aminoacylation assay**

1545 For the tRNA aminoacylation assay, cells were collected and suspended in a solution containing
1546 0.3 M sodium acetate/acetic acid (NaOAc/HOAc) at pH 4.5. Subsequently, total RNA extraction
1547 was carried out using acetate-saturated phenol/CHCl₃ at pH 4.5 (Thermo Fisher Scientific,
1548 AM9720). The isolated RNA was then resuspended in 10 mM NaOAc/HOAc at pH 4.5. The
1549 samples were divided into two portions: one-half (2 μ g) underwent oxidation with 50 mM NaIO₄ in
1550 100 mM NaOAc/HOAc at pH 4.5 for 15 min, while the other half (2 μ g) was incubated in 50 mM
1551 NaCl in 100 mM NaOAc/HOAc at pH 4.5 for 15 min. Glucose (100 mM) was used to quench the
1552 reactions for 5 min at RT, followed by purification in G50 columns (Cytiva, 27533001) and
1553 precipitation with ethanol. The tRNAs were deacylated in 50 mM Tris-HCl at pH 9 for 30 min at
1554 37°C. After precipitation, the RNA was ligated to the 3' adaptor tRNA using T4 RNA ligase 2 (NEB,
1555 M0351L) for 2 h at 37°C. Reverse transcription was performed with the SuperScript IV synthesis
1556 kit (Thermo Fisher Scientific, 18091050). Relative aminoacylation levels were determined by qRT–
1557 PCR using tRNA-specific primers.

1558

1559

1560 **Isolation of polysome-associated mRNA**

1561 For polysome profiling, cells were resuspended in lysis buffer (20 mM Tris-HCl, pH 7.8, 100 mM
1562 KCl, 10 mM MgCl₂, 1% Triton X-100, 2 mM DTT, 100 µg/mL cycloheximide, 1X complete protease
1563 inhibitor). The cytosolic fraction was obtained through centrifugation at 1300 g for 10 min. This
1564 extract was then carefully layered onto a 7% to 47% linear sucrose gradient and subjected to
1565 centrifugation in a SW41Ti rotor (Beckman Coulter) at 36,000 rpm for 2 h at 4°C. Nine fractions
1566 were collected from the resulting gradients, and RNA isolation from each fraction was isolated
1567 using TRI reagent (Zymo, R2050-1-200) according to manufacturer's protocol as described briefly
1568 above. Reverse transcription and qRT-PCR for *AHR* were performed as mentioned above. Using
1569 the cycle threshold (CT) values, the percent (%) distribution for the mRNAs across the gradients
1570 were calculated using the ΔCT method.

1571

1572 **Ribosome Profiling**

1573 For the ribosome profiling, cells were washed with ice-cold PBS supplemented with 100 µg/mL
1574 CHX (Sigma Aldrich, C7698) and RP-Lysis buffer (20 mM Tris-HCl pH 7.5 (Thermo Fisher
1575 Scientific, 15567-027), 10 mM MgCl₂ (Sigma-Aldrich, M2393), 100 mM KCl (Sigma-Aldrich, P405),
1576 1% Triton-X 100 (Sigma-Aldrich, T8787), 2 mM DTT (Sigma-Aldrich, D0631), 100 µg/mL CHX, 1x
1577 EDTA-free Complete Protease Inhibitor Cocktail (Sigma-Aldrich, 11873580001)) was added. After
1578 lysis, all samples were centrifuged at 6400 rpm, 4°C for 5 min. The supernatant was taken and
1579 digested with 1 U/µL RNaseI (Thermo Fisher Scientific, AM2295) for 45 min at RT under rotation.
1580 Digested lysates were run through 7% - 47% sucrose gradients using a Beckman Coulter
1581 ultracentrifuge and SW41 Ti rotor (Beckman Coulter) with 36,000 rpm at 4°C for 2 h. Monosome
1582 fractions were obtained and digested with 1% SDS (Sigma-Aldrich, 05030) and 0.113 µg/µL
1583 Proteinase K (Roche, 3115828001) for 45 min at 45°C. Resulting footprint RNA was extracted
1584 following a standard Phenol-Chloroform extraction (Zymo Research, R2050-1-200) and size-
1585 selected using a 10% denaturing PAGE gel.

1586 RP library construction in brief: Footprint RNA was dephosphorylated using 5 U of T4 PNK
1587 (New England Biolabs, M0201S). Subsequently, preadenylated UMI-linkers were ligated to the
1588 RNA 3'end using 100 U T4 RNA Ligase 2, truncated K227Q (New England Biolabs, M0351L).
1589 Residual linker was eliminated by 25 U 5'Deadenylase and 15 U RecJf for 60 min at 30°C.
1590 Ribosomal RNA was subtracted using a biotinylated rRNA oligo pool in 1x SSC buffer (3 M NaCl,
1591 300 mM trisodium citrate, pH 7), which were pull down using MyOne Streptavidin C1 DynaBeads
1592 (Thermo Fisher Scientific, 65001). Resulting RNA footprints were reverse transcribed using the
1593 SuperScript III First-Strand Synthesis System (Thermo Fisher Scientific, 2232161). cDNA was
1594 size-selected using a 8% denaturing PAGE gel. cDNA was circularized by using the CircLigaseII

1595 Kit (Lucigen, CL9021K). The samples were subjected to PCR to introduce Illumina i7 indexes,
1596 followed by size-selection on an 8% non-denaturing PAGE gel. Resulting sample concentrations
1597 were measured with Qubit 3.0 (Thermo Fisher Scientific) using Qubit DNA HS kit (New England
1598 Biolabs, M0494S). The final RP libraries were single-end sequenced with a NextSeq2000 P2
1599 system (Illumina). All samples were sequenced at the DKFZ Sequencing Open Lab, associated
1600 with the DKFZ Genomics & Proteomics Core Facility.

1601

1602 RiboSeq data processing

1603 The FASTQ raw data was provided by the DKFZ Genomics & Proteomics Core Facility. In brief,
1604 sample adapters were trimmed using cutadapt (v3.4)¹²⁵ and demultiplexed with barcode_splitter
1605 from FASTX-toolkit (v0.0.6)¹²⁶. Fragments smaller than 30 nt were dropped. UMIs extraction was
1606 performed using umi_tools (v1.1.1)¹²⁷. By BLAST-Like Alignment Tool (BLAT) (v36x2), rRNA
1607 reads were filtered and discarded¹²⁸. The rRNA index for RNA18S5, RNA28S5 and RNA5-8S5
1608 was constructed manually from NCBI RefSeq annotation. Remaining reads were aligned with
1609 Spliced Transcripts Alignment using STAR (v2.5.3a)⁹⁸ to the GRCh37/hg19 reference with the
1610 following call parameters --outSAMtype BAM Unsorted --readFilesCommand zcat --quantMode
1611 TranscriptomeSAM GeneCounts --outSAMmapqUnique 0. Genome browser bigwig tracks were
1612 obtained using samtools (v1.15.1) and bedtools (v2.24.0). RPF 5' density was calculated as
1613 previously described in Loayza-Puch et al.¹²⁹.

1614

1615 **Statistical analysis**

1616 GraphPad Prism (v9.4.1 or v8.4.3) was used for statistical analysis and statistical presentation
1617 unless otherwise specified. In case two conditions were compared, a paired or unpaired two-tailed
1618 Student's t-test was performed. If more than two conditions were compared, a one-way ANOVA
1619 followed by a Šídák's multiple comparisons test was applied. Immunoblot time courses with more
1620 than two conditions were compared using a two-way ANOVA followed by a Šídák's multiple
1621 comparisons test. For each experiment the number of replicates and the statistical test applied
1622 are indicated in the figure legend. Data are presented as mean \pm SEM. *p < 0.05, **p < 0.01, ***p
1623 < 0.001, n.s.: not significant.

1624 For bioinformatic analysis, unless otherwise stated, all pairwise comparisons were
1625 performed using Kruskal-Wallis and Wilcoxon sum rank tests, and all reported p-values were
1626 adjusted using the Benjamini-Hochberg procedure. All analyses were run in R, versions 3.3 and
1627 4.2.2, (<https://cran.r-project.org/>) and Bioconductor version 3.3 and 3.15
1628 (<https://bioconductor.org/>). All representations were generated using ggplot2, ggpurr, gridExtra
1629 and RcolorBrewer.

1630 **Generation of schematic representations**

1631 The schematic representations in the graphical abstract were generated using Biorender.org.

1632

1633 **Contact for reagent and resource sharing**

1634 Further information and requests for resources and reagents should be directed to and will be
1635 fulfilled by the lead contact, Kathrin Thedieck (Kathrin.Thedieck@uibk.ac.at).

1636

1637 **Materials availability**

1638 All unique materials and reagents generated as part of this study are available from the lead
1639 contact with a completed Material Transfer Agreement.

1640

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1684 Views & opinions are those of the authors.

1685

1686 **Author contributions**

1687 PH, LH, MTP and PRN designed and performed experiments, analysed data, and contributed to
1688 manuscript writing. MS conducted and analysed experimental data. AS designed, performed and
1689 wrote bioinformatics methods and analyses for cancer transcriptome and RPPA data. UR
1690 designed and conducted kinase assays. AH designed experiments and contributed to scientific
1691 discussions. SS and LR designed and performed experiments and analysed data. TB developed
1692 MALDI-MS Imaging methods, performed MSI experiments and wrote the corresponding methods.
1693 AL and JD conducted targeted proteomics of autophagy regulators. ASE developed, performed
1694 and analysed intracellular Trp measurements and analysed proteome data. BB, LFSP, MR, DS,
1695 VP, TK, MH, JRP, ILK, TB, SO, LET, FH, AR, DS, IK, CB, MRP and LEE performed and supported
1696 experiments and analyses. ST performed analysis of regulatory regions. AvP and YZ supported

1697 proteome sample preparation and data analysis. SK measured the Trp stress proteome,
1698 supervised by HS. FL-P and AK performed ribosome profiling. LW established and LW and AG
1699 performed LC-MS lipid analyses and analysed lipid MS data. TK and VIK performed autophagy
1700 gene analysis, and supported autophagy data analysis. JZ supported data analysis. SM performed
1701 analysis of MALDI-MS imaging. PS, FS performed tissue collection and pathological assessment.
1702 AK supervised lipid measurements by LC-MS. CH supervised MALDI-MS Imaging. MK designed
1703 and supervised proteome and intracellular Trp measurement and analysis. CS designed and
1704 supervised EGFR and RAS experiments and LAMP stainings. BVdE analysed data and
1705 contributed to scientific discussions. CO and KT conceived and supervised the study, designed
1706 and analysed experiments, and wrote the manuscript. All the authors read, revised, and approved
1707 the manuscript.

1708

1709 **Declaration of interests**

1710 AS, ST and CO are founders and AS and CO are managing directors of cAHRmeleon Bioscience
1711 GmbH. VIK is a Scientific Advisor for Longaeus Technologies. Authors of this manuscript have
1712 patents on AHR inhibitors in cancer (WO2013034685, CO); A method to multiplex tryptophan and
1713 its metabolites (WO2017072368, CO); A transcriptional signature to determine AHR activity
1714 (WO2020201825, AS, ST, CO); Interleukin-4-induced gene 1 (IL4I1) as a biomarker
1715 (WO2020208190, AS, ST, LFSP, MTP, CO) Interleukin-4-induced gene 1 (IL4I1) and its
1716 metabolites as biomarkers for cancer (WO2021116357, AS, ST, LFSP, CO); a targeted
1717 proteomics method to monitor autophagy (EP23182541, AL, JD).

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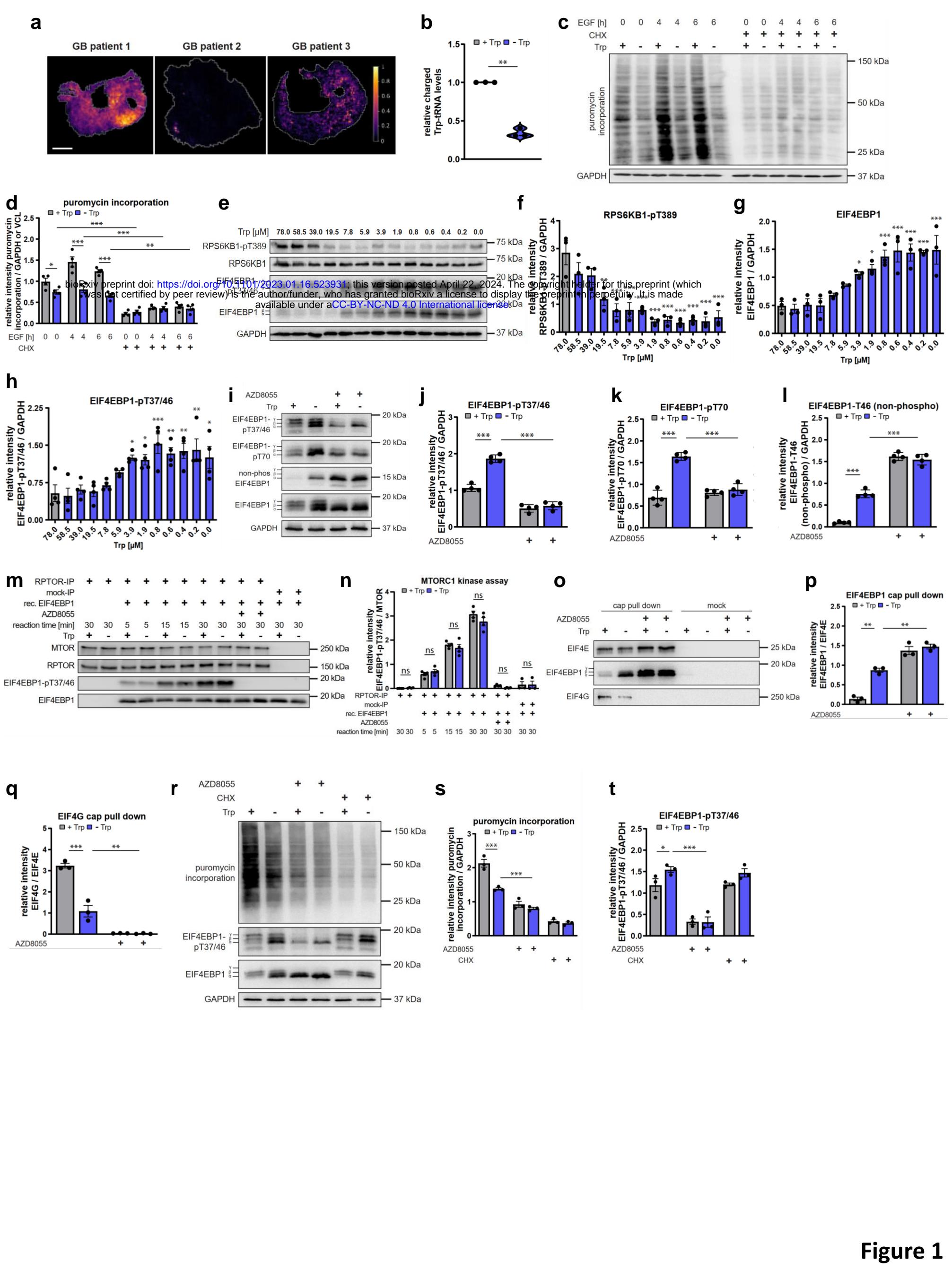
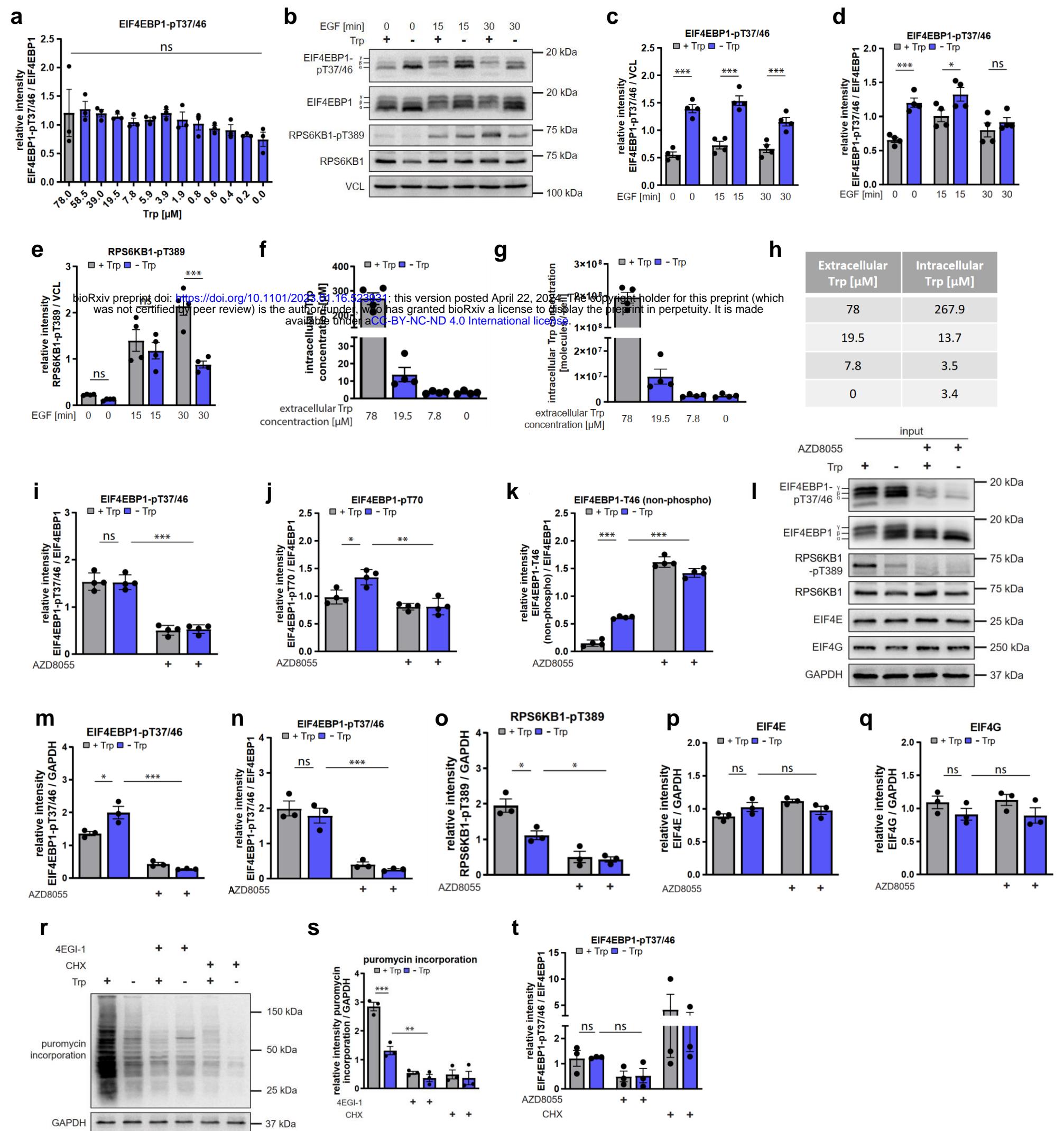


Figure 1



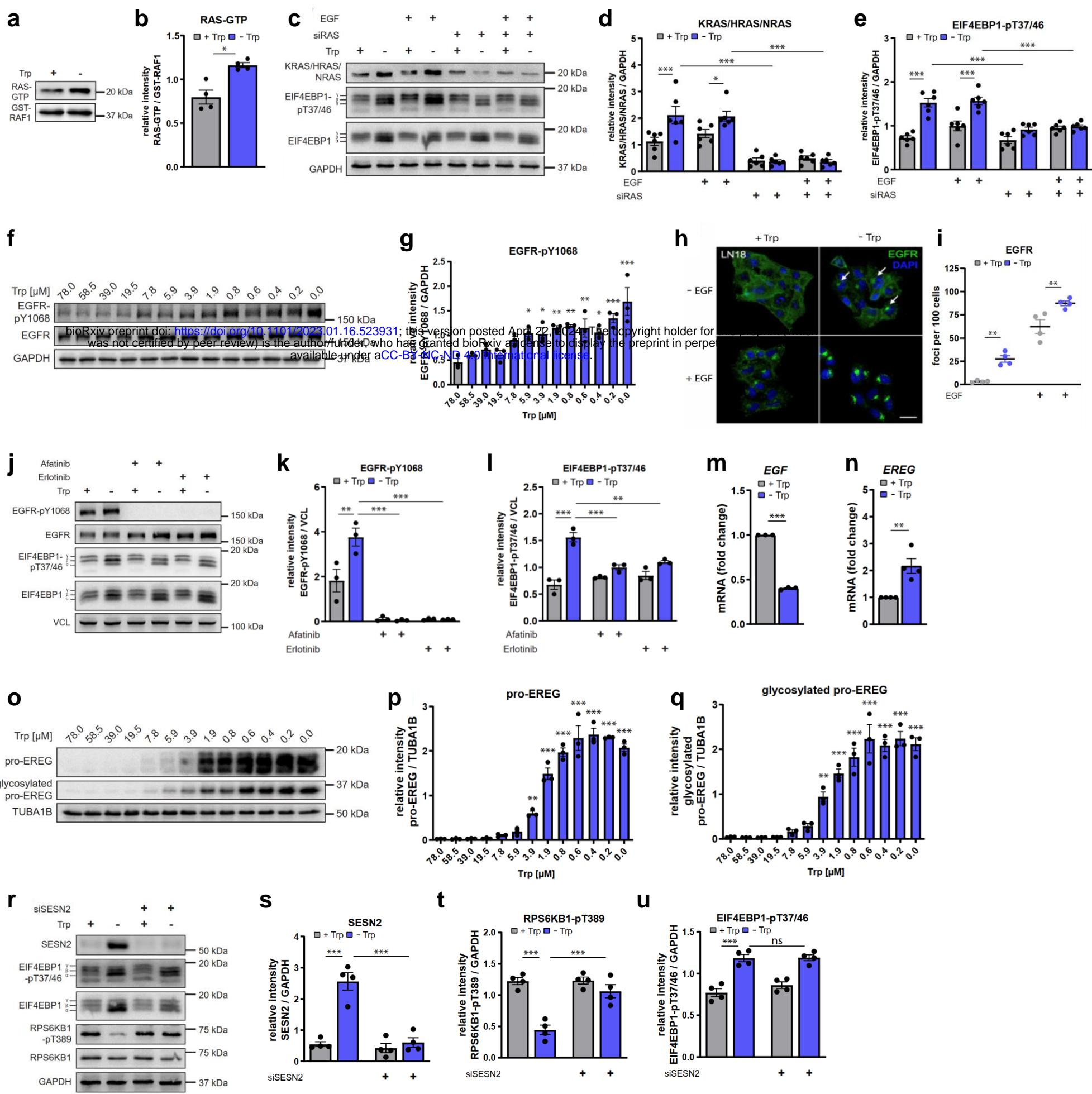
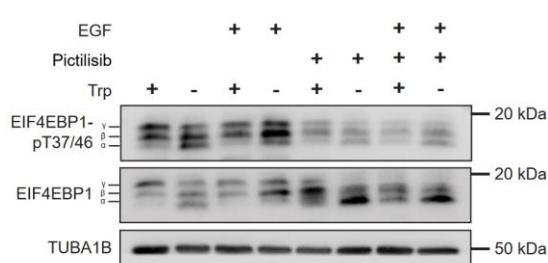
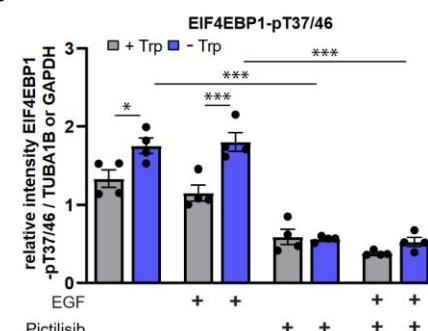
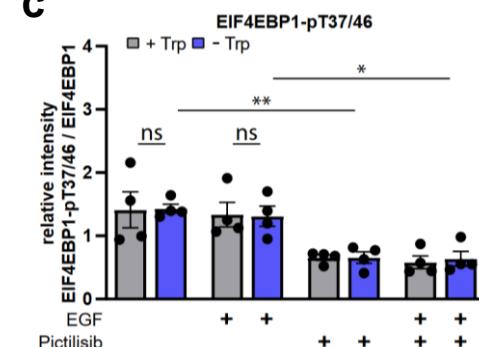
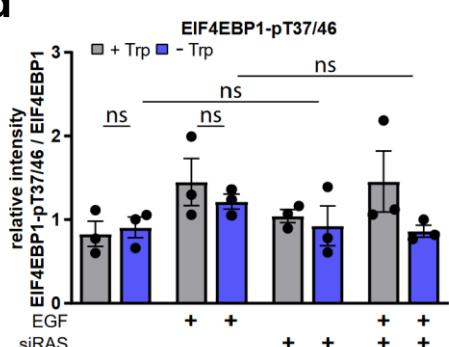
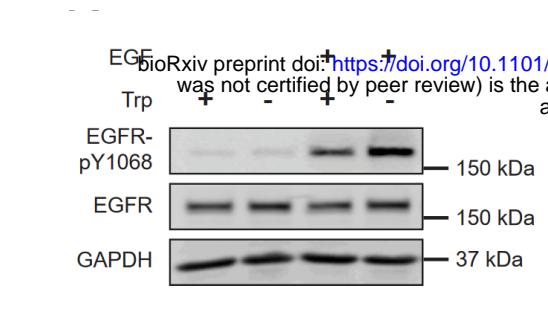
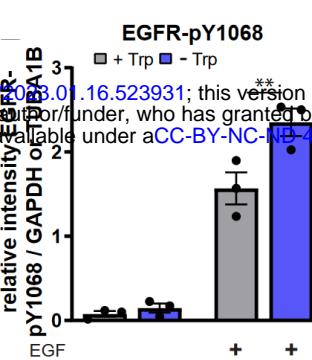
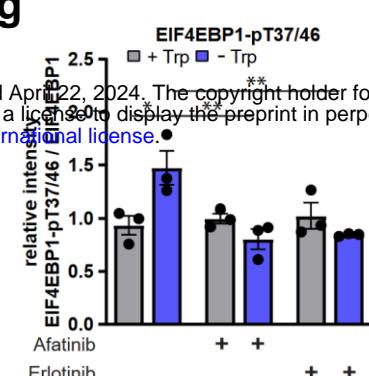
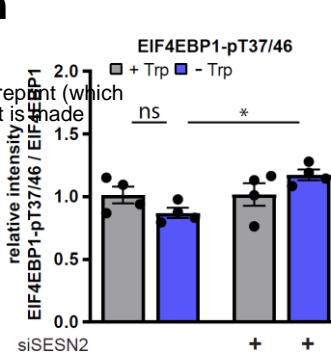
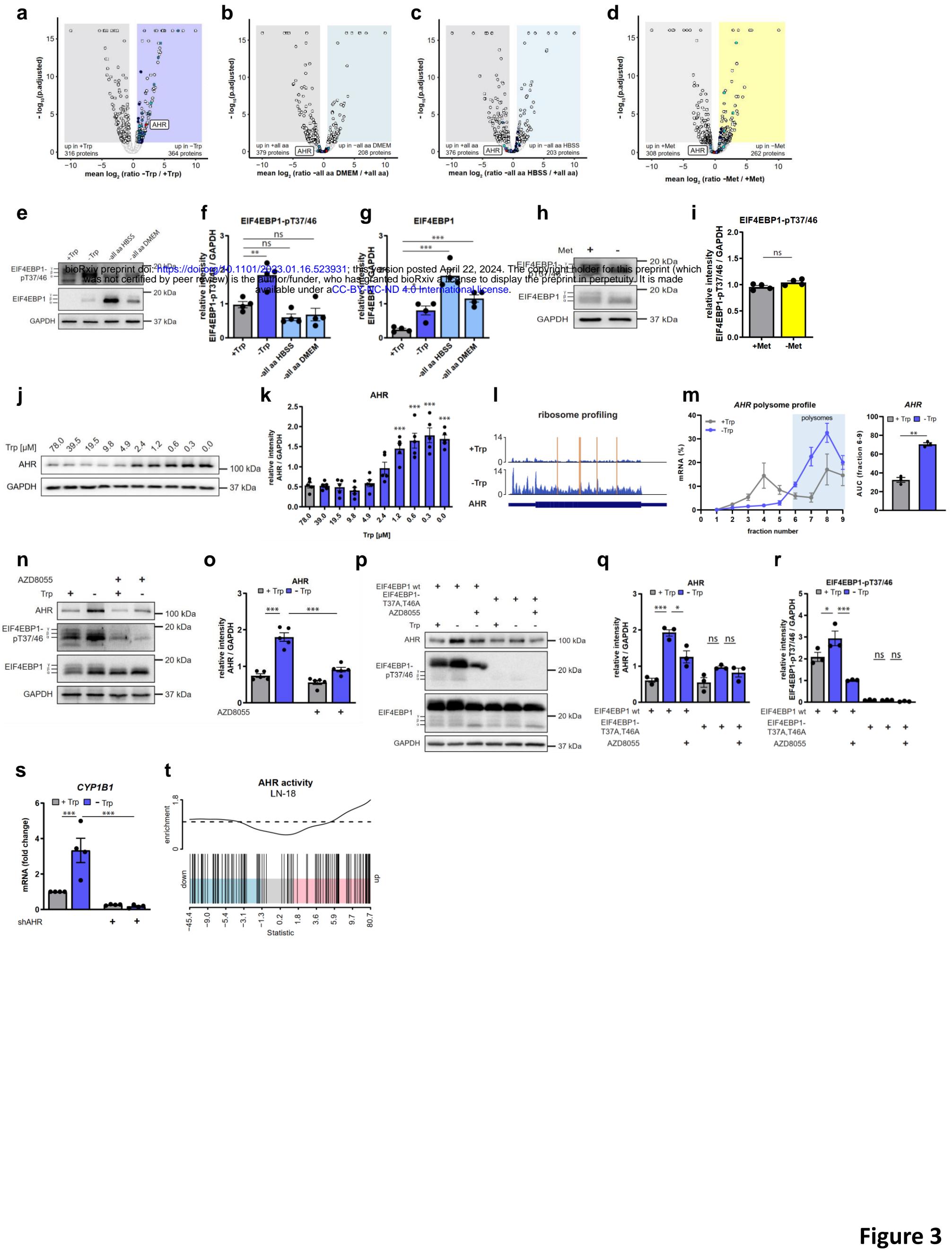
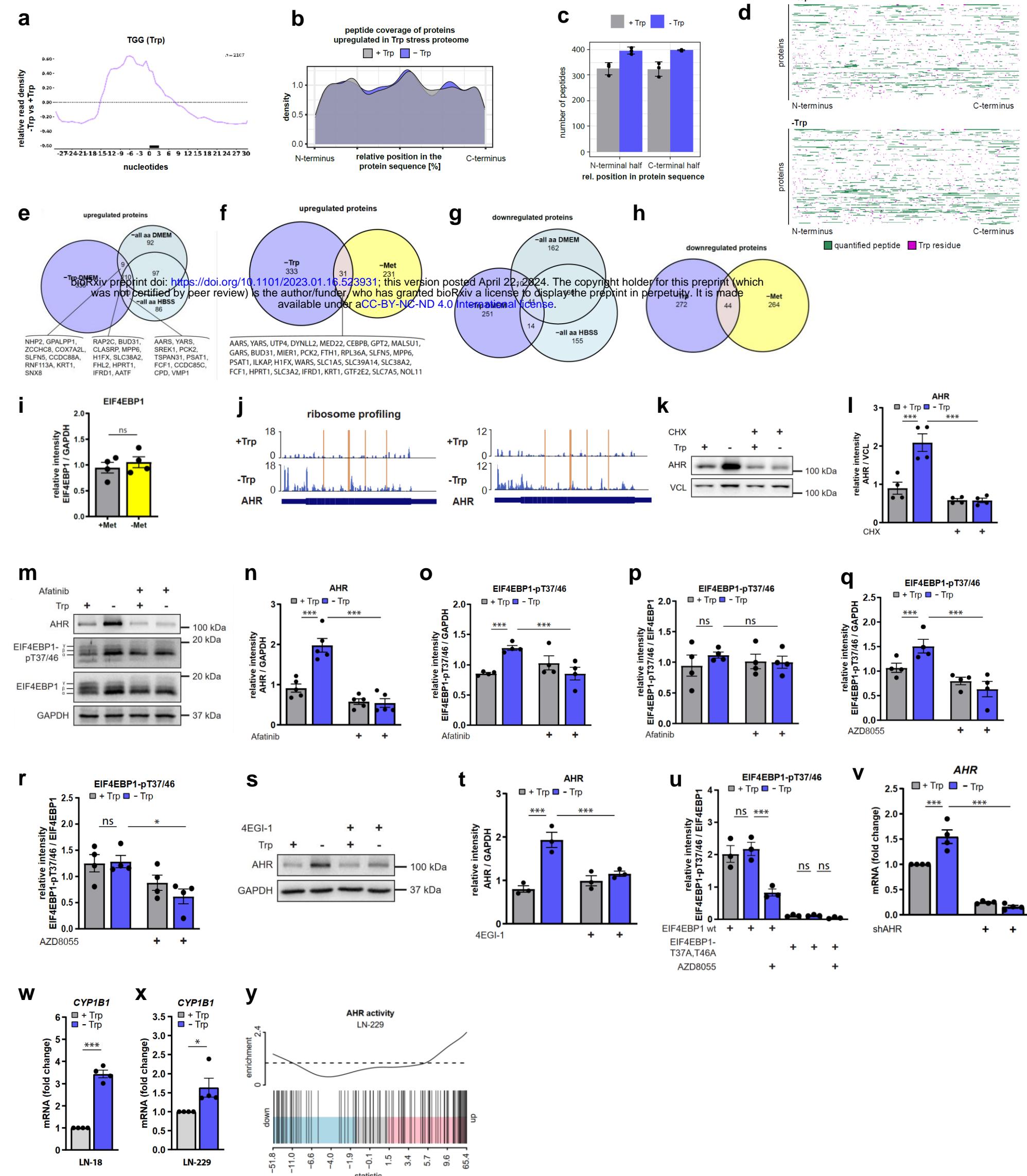
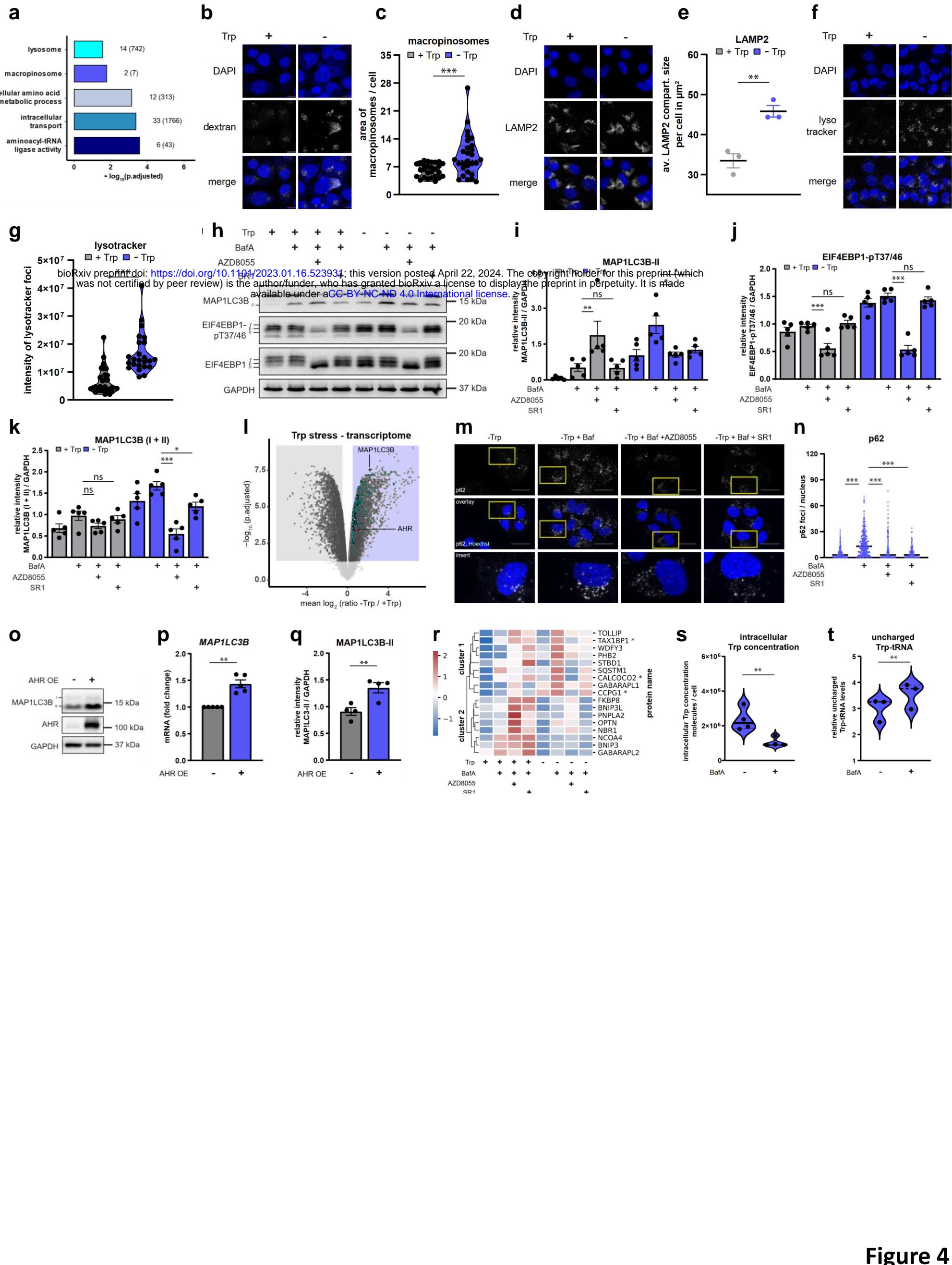


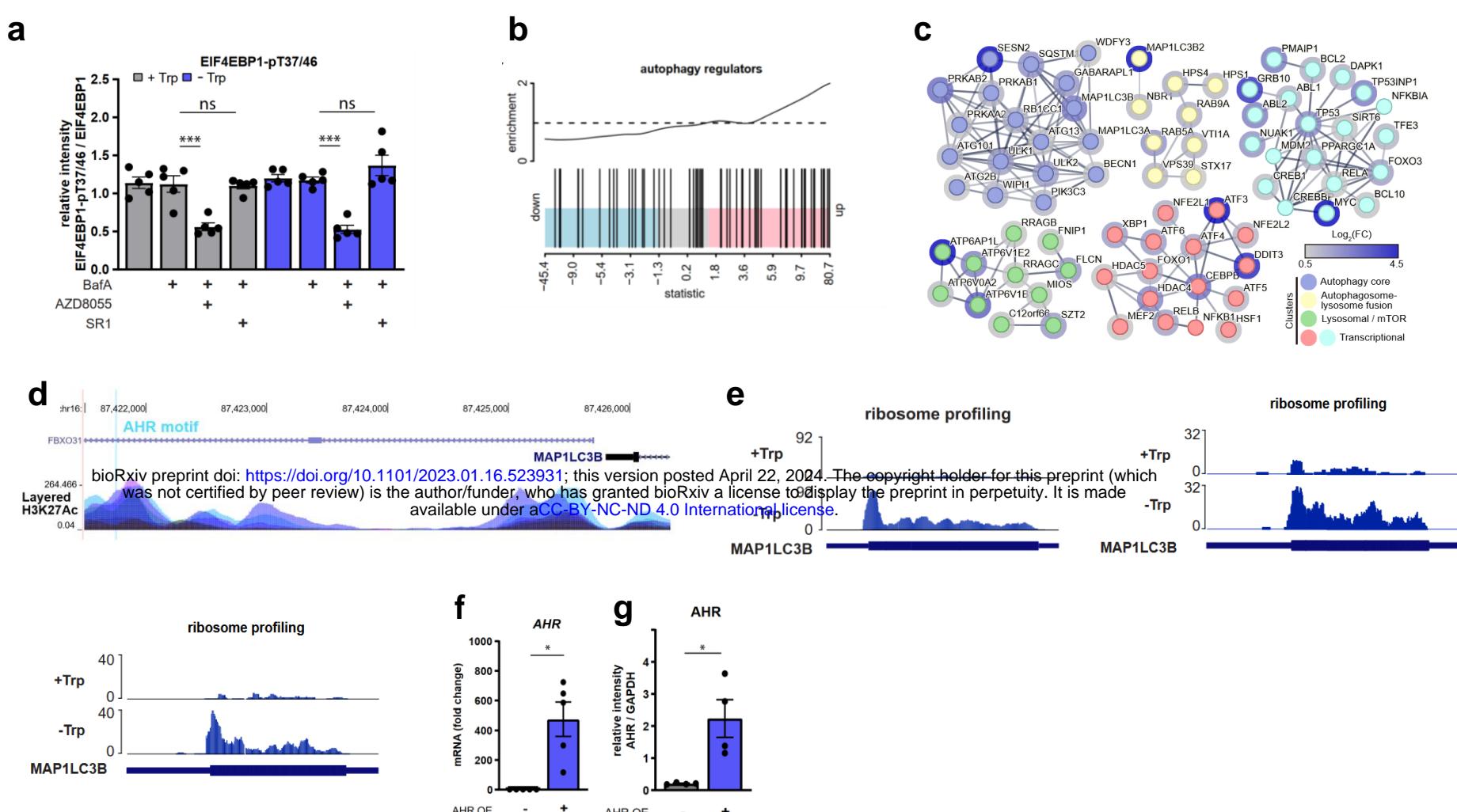
Figure 2

a**b****c****d****e****f****g****h**









Extended Data Figure 4

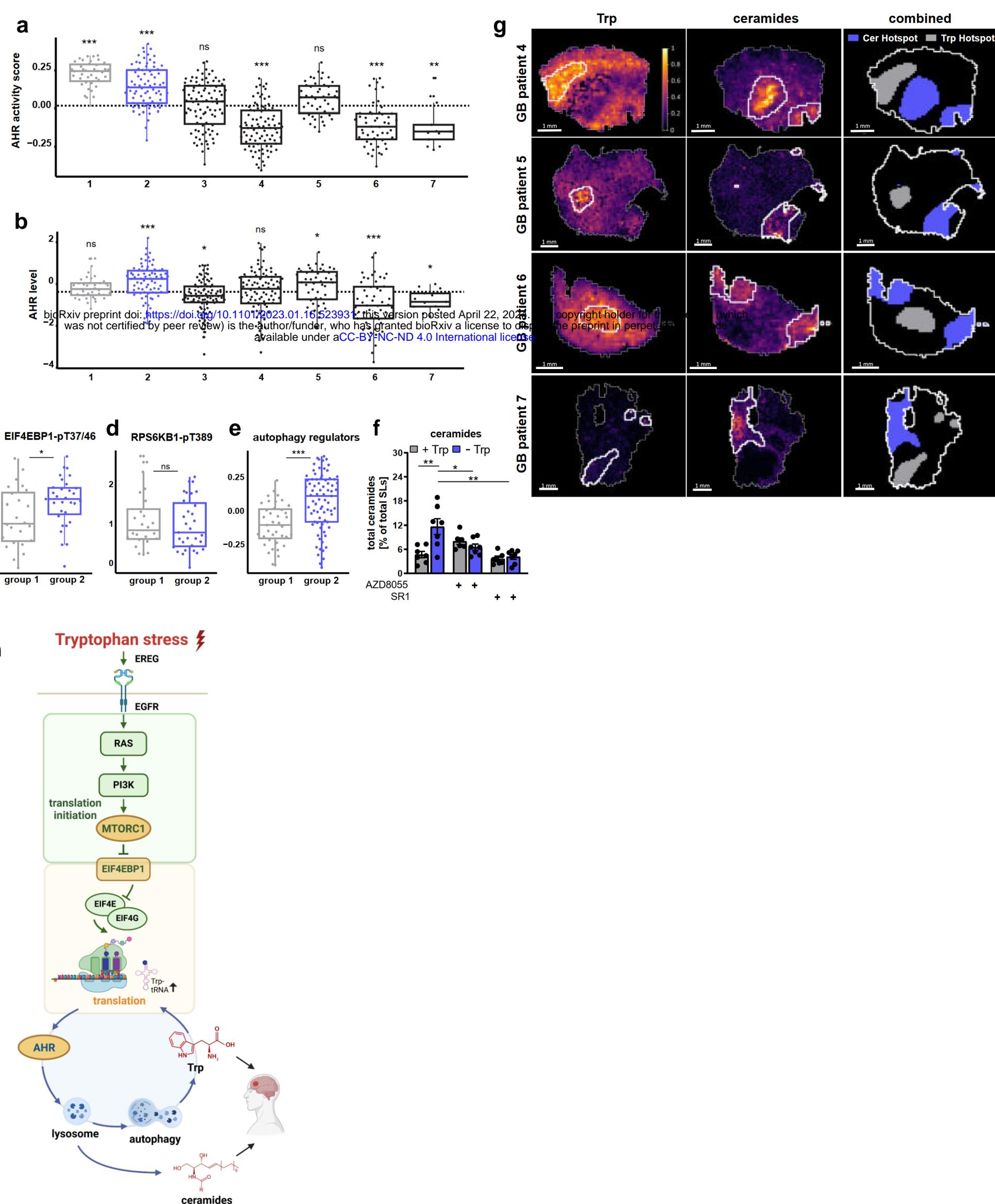


Figure 5