

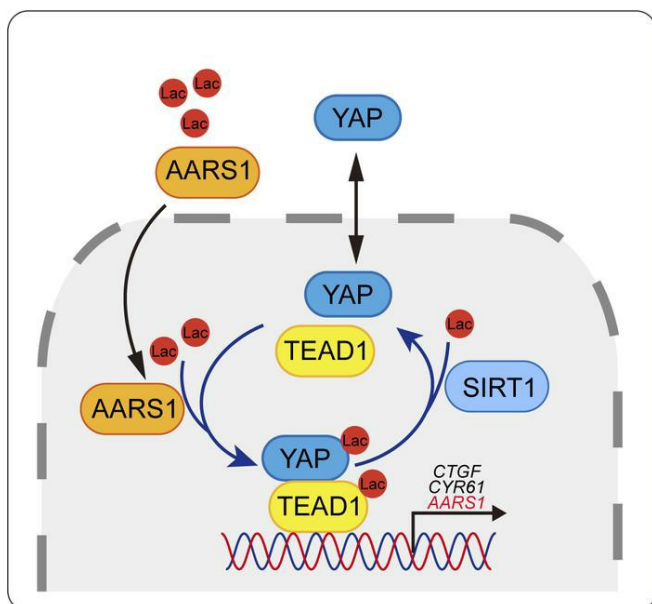
The alanyl-tRNA synthetase AARS1 moonlights as a lactyl-transferase to promote YAP signaling in gastric cancer

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1 **The alanyl-tRNA synthetase AARS1 moonlights as a lactyl-transferase to promote**

2 **YAP signaling in gastric cancer**

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23 **Abstract**

24 Lactylation has been recently identified as a new type of posttranslational modification widely
25 occurring on lysine residues of both histone and non-histone proteins. The acetyl transferase
26 p300 is thought to mediate protein lactylation, yet the cellular concentration of the proposed
27 lactyl-donor, lactyl-coenzyme A is about 1,000 times lower than that of acetyl-CoA, raising the
28 question whether p300 is a genuine lactyl-transferase. Here, we report the Alanyl-tRNA
29 synthetase 1 (AARS1) moonlights as a bona fide lactyl-transferase that directly uses lactate
30 and ATP to catalyze protein lactylation. Among the candidate substrates, we focused on the
31 Hippo pathway that has a well-established role in tumorigenesis. Specifically, AARS1 was
32 found to sense intracellular lactate and translocate into the nucleus to lactylate and activate
33 YAP-TEAD complex; and AARS1 itself was identified as a Hippo target gene that forms a
34 positive feedback loop with YAP-TEAD to promote gastric cancer (GC) cell proliferation.
35 Consistently, the expression of AARS1 was found to be upregulated in GC, and elevated
36 AARS1 expression was found to be associated with poor prognosis for GC patients.
37 Collectively, this work discovered AARS1 with lactyl-transferase activity in vitro and in vivo
38 and revealed how the metabolite lactate is translated into a signal of cell proliferation.

39

40 **Keywords**

41 Protein lactylation, lactyl-transferase, AARS1, Hippo pathway, Gastric cancer

42

43 **Introduction**

44 Lactate is a well-known metabolite found in almost all types of cells, and is highly abundant
45 in proliferating tumor cells due to the Warburg effect (1, 2). Despite of many studies showing
46 that tumor-secreted lactate can enter multiple types of immune cells to shape a
47 microenvironment permissive for tumor growth (3-6), how intracellular lactate is manifested
48 as or translated into signals beneficial for tumor growth remains elusive. Recently, lactylation
49 of lysine residues has been identified as a new type of posttranslational modifications for
50 histones (7, 8), providing a new perspective for non-metabolic functions of lactate. For
51 example, histone lactylation has been found to play essential roles in stem cell pluripotency
52 (9), neural excitation (10), Alzheimer's disease (11), macrophage polarization (12), and tumor
53 development (13). More recently, lactylation has also been found in non-histone proteins (8).
54 For example, an integrative lactylome and proteome analysis of hepatocellular carcinoma
55 identified more than 9,000 lactylated lysines on non-histone proteins (14).

56 Although protein lactylation has increasingly been appreciated as a widespread
57 posttranslational modification especially in tumor cells, the enzyme directly catalyzes this
58 modification, as well as the exact chemical reaction process of catalysis remain debating.
59 Specifically, the acetyltransferase p300 has been proposed to serve as a lactyl-transferase
60 and thus mediate histone lactylation (7), but direct in vitro evidence using purified proteins of
61 p300 and substrate is lacking. More importantly, in that proposed catalysis system, p300 need
62 to use lactyl-coenzyme A (lactyl-CoA) as a lactyl-donor, but the enzymes responsible to
63 produce lactyl-CoA in mammalian cells are still undefined, and the levels of lactyl-CoA in
64 tumor cells are hardly detectable. In fact, the intracellular concentration of lactyl-CoA in

65 general is at least 1,000 times lower than that of acetyl-CoA in mammalian cells (15), which
66 may substantially limit p300's lactyl-transferase activity, if any, in vivo. Therefore, a genuine
67 lactyl-transferase that can directly use lactate as a lactyl-donor to catalyze substrate
68 lactylation in vitro and in vivo is yet to be identified.

69 The evolutionally conserved Hippo signaling pathway plays an essential role in organ
70 size control, tissue homeostasis, and tumorigenesis (16-18). In this pathway, the MST1/2
71 (Hippo)-LATS1/2 kinase cascade controls the subcellular localization and therefore activity of
72 the transcriptional coactivator YAP/TAZ (19, 20). In response to various environmental stimuli,
73 however, the upstream kinase cascade can be inactivated, allowing YAP/TAZ to enter the
74 nucleus and interact with the TEAD family of transcription factors to regulate downstream
75 target gene expression (21, 22). Dysregulation of the Hippo pathway—in particular,
76 hyperactivation of YAP—has been shown to be closely associated with aberrant cell growth
77 and tumorigenesis (23-25). It has been speculated that the Hippo pathway may also directly
78 sense certain metabolic cues (26). However, the specific molecular machineries linking
79 intracellular lactate to Hippo-YAP signaling, if any, are yet to be discovered, especially in
80 tumor cells in which lactate is highly abundant and YAP is hyperactive.

81 In this study, we identified AARS1, which typically functions to catalyze the ligation of L-
82 alanine to tRNA, to be a bona fide lactyl-transferase that can directly use lactate and ATP to
83 catalyze protein lactylation. Moreover, we found that in response to intracellular accumulation
84 of lactate, AARS1 translocated into the nucleus where it directly catalyzed lactylation of YAP
85 at K90 and TEAD1 at K108, thereby activating downstream target gene expression to
86 promote tumor cell proliferation. Furthermore, AARS1 was shown to be a direct target gene

87 of YAP-TEAD1, forming a positive feedback loop to manifest high levels of intracellular lactate
88 as a growth signal. Consistently, we found AARS1 to be upregulated and associated with
89 YAP-TEAD1 lactylation in gastric cancer (GC), and that elevated expression of AARS1 was
90 found to be strongly associated with TNM stages and poor clinical outcomes.

91 **Results**

92 **AARS1 moonlights as a protein lactyl-transferase using lactate as a direct lactyl-donor**

93 To find a lactyl-transferase that can directly use lactate and ATP to catalyze protein lactylation,
94 we reasoned that the candidate enzyme should readily bind both lactate and ATP, as well as
95 a protein substrate. Considering the high similarity between the chemical structure of lactate
96 and that of L-alanine (Figure 1A), we speculated that alanyl-tRNA synthetase 1/2 (AARS1/2)
97 may also act as a lactyl-transferase to catalyze protein lactylation, a scenario reminiscent of
98 AARS1/2 catalyzing the ligation of L-alanine to tRNA. Supporting this idea, molecular docking
99 predicted that lactate could readily bind to the catalytic pocket of AARS1 (Figure 1A). To verify
100 whether AARS1 may directly bind lactate and thus use it as a lactyl-donor, we used purified
101 recombinant protein of AARS1 to examine its interaction with lactate by isothermal titration
102 calorimetry (ITC) assay. Indeed, lactate was found to bind AARS1 with a K_d value of 2.06 μM ,
103 as did the positive control L-alanine ($K_d = 0.45 \mu\text{M}$) (Figure 1B).

104 To further test whether AARS1 is a lactyl-transferase per se, we performed in vitro
105 lactylation experiments using purified recombinant protein of AARS1 (full length or amino acid
106 residues 1-455, corresponding to the catalytic domain) (27) as an enzyme and purified
107 proteins of histone H3 and H4, the most widely studied lactylated proteins as substrates. The
108 results showed that AARS1 was able to directly lactylate histone H3 and H4 in a manner
109 dependent on both lactate and ATP (Figure 1C and Supplemental Figure 1A). The lactylated
110 lysine residues of H3 and H4 in the in vitro lactylation assay were identified via mass
111 spectrometry (Supplemental Figure 1B). Moreover, we used a synthetic H3K18 peptide
112 (APRK¹⁸QLAT) as a substrate in the in vitro lactylation assay. Subsequent mass spectrometry

113 also confirmed that AARS1 was indeed able to directly lactylate the H3 peptide at K18 in the
114 presence of lactate and ATP (Figure 1D). Furthermore, 3D-structural analysis indicated that
115 mutation of amino acid residues (R77A, M100A, W176E, V218D, D239A) lining the catalytic
116 pocket of AARS1 would disrupt its interaction with lactate (Supplemental Figure 1C).
117 Accordingly, we found that 5M mutation of AARS1 totally abolished its lactyl-transferase
118 activity in vitro (Figure 1, C and D), again confirming the necessity of lactate binding for
119 AARS1-mediated protein lactylation and that AARS1 directly uses lactate as a lactyl-donor. In
120 addition, GST pulldown assay also revealed a direct interaction of AARS1 with the substrate
121 histone H3 or H4 (Supplemental Figure 1D).

122 Since aminoacyl-tRNA synthetases catalyze a two-step tRNA aminoacylation reaction,
123 that releasing pyrophosphate (PPi) and forming a reactive acyl adenylate (acyl-AMP)
124 intermediate in the first step-reaction (28), we speculated that AASR1-mediated protein
125 lactylation would also produce PPi. As expected, the amount of PPi released in the in vitro
126 lactylation assay was positively correlated with lactate concentrations used, confirming PPi
127 as a product of the lactylation reaction (Figure 1E). In addition, it was reported that PPi and
128 acyl sulfonyladenosine (acyl-AMS) that mimic the tightly bound acyl-AMP intermediate could
129 inhibit the catalytic activity of aminoacyl-tRNA synthetases (29). Indeed, we found that
130 inclusion of PPi, AMP and the synthetic lactyl-AMS (Supplemental Figure 1E) in our in vitro
131 lactylation system significantly inhibited AARS1-mediated histone lactylation in a dose-
132 dependent manner (Figure 1, C and D and Supplemental Figure 1F). Moreover, as the
133 binding affinity of L-alanine (Kd: 0.45 μ M) to AARS1 is slightly higher but comparable to that
134 of lactate (Kd: 2.06 μ M), inclusion of L-alanine in the reaction system was found to dose-

135 dependently inhibit AARS1-mediated histone H3 lactylation (Supplemental Figure 1F). These
136 data unambiguously demonstrated that AARS1 can directly bind to and transfer lactate to
137 lysine in a similar manner as it catalyzes alanyl-tRNA formation, i.e.: step 1) generate reactive
138 lactyl-AMP and PPI from lactate and ATP; step 2) transfer lactyl-group from lactyl-AMP to
139 lysine residues of substrates (Figure 1F).

140 To compare the AARS1-mediated lactylation with previously reported lactyl-CoA-related
141 lactylation, we employed lactyl-CoA instead of lactate in the in vitro lactylation assay to
142 investigate the potential utilization of lactyl-CoA as a lactyl donor by AARS1. The results
143 showed that AARS1 efficiently catalyzed histone H3 lactylation in the presence of
144 physiological concentrations of ATP and lactate, but it failed to do so in the presence of even
145 100-fold higher concentrations of physiological lactyl-CoA (15) (Supplemental Figure 1G).
146 Intriguingly, we found that high concentration of lactyl-CoA was able to trigger spontaneous
147 protein lactylation in a nonenzymatic manner, a scenario that is not likely exist in vivo because
148 of the extremely low level of cellular lactyl-CoA (Supplemental Figure 1H). We next examined
149 whether AARS1 moonlights as a lactyl-transferase in vivo and found that knockdown of
150 AARS1 significantly decreased histone H3 K18 lactylation levels in human GC cell line
151 HGC27 (Supplemental Figure 1I). Of note, depletion of AARS1 in HGC27 cells substantially
152 and globally decreased protein lactylation levels (Figure 1G), while knockdown of p300 only
153 had a marginal effect (Supplemental Figure 1J). These data suggest that AARS1 catalyzes
154 protein lactylation directly using ATP as energy source and lactate as lactyl-donor.

155

156 **AARS1 translocates into the nucleus in response to increased intracellular lactate**

157 We then asked whether and how AARS1, commonly understood as an enzyme residing in
158 the cytoplasm and catalyzing ligation of L-alanine to tRNA, responds to lactate levels in cells.
159 Interestingly, both fractionation and immunofluorescent assays showed that lactate treatment
160 promoted AARS1 shuttling into the nucleus (Figure 1, H and I). Subsequent examination of
161 amino acid sequences of AARS1 from various species revealed an evolutionarily conserved
162 nuclear localization sequence (NLS) motif in its C-terminal region (Figure 1J). We then
163 generated an AARS1 mutant with the NLS deleted (Δ NLS) and examined its subcellular
164 localization. As shown in Figure 1K, wildtype AARS1 was found to be localized in both
165 cytoplasm and nucleus; whereas the Δ NLS mutant was found to be localized only in the
166 cytoplasm. More importantly, addition of lactate significantly promoted the nuclear localization
167 of wildtype AARS1 but not the Δ NLS mutant (Figure 1K).

168 Further, we investigated the mechanism through which lactate promote nuclear
169 translocation of AARS1. Proteins with NLS are usually transported into the nucleus via their
170 interactions with importin- α . In this regard, we performed a lactylation proteomics in HGC27
171 cells, which revealed that several importin- α subunits (KPNA1, 3, 4 and 6) were lactylated
172 (Supplemental Figure 1K). Therefore, we speculated that AARS1 may interact with and
173 directly lactylate these importin(s) in response to accumulation of intracellular lactate. One
174 may expect that intracellular lactate can increase the interaction of AARS1 with importin, thus
175 promoting its nuclear translocation. To test this hypothesis, we examined the interaction of
176 AARS1 with the candidate importins, which revealed KPNA4 as a binding partner of AARS1
177 (Supplemental Figure 1L). Moreover, lactate promoted the interaction of AARS1 with KPNA4,
178 while deletion of the NLS in AARS1 abolished such interaction (Supplemental Figure 1M),

179 results suggesting that KPNA4 binds to the NLS motif of AARS1 to mediate its nuclear
180 translocation.

181

182 **YAP-TEAD are directly lactylated by AARS1 and delactylated by SIRT1**

183 Notably, our lactylation proteomics study in HGC27 cells identified 2,789 unique Klac sites
184 (lactylated lysines) in 1,182 proteins (Figure 2A). Among these proteins were multiple types
185 of histones, which were previously reported to be lactylated (7). Sequence motif analysis
186 showed that the Klac sites preferably locate downstream of serine or arginine residues
187 (Supplemental Figure 2A). A subsequent KEGG analysis indicated multiple cellular pathways
188 including cAMP, insulin and Hippo to be most likely regulated by protein lactylation (Figure
189 2B). To explore the possible role of protein lactylation in driving tumor cell proliferation, we
190 then focused on the Hippo signaling pathway, in which several components or regulatory
191 proteins, such as ACTG1, MOB1A, PPP1CA, YAP and TEAD1, were found to be lactylated
192 (Figure 2A). Of note, YAP and TEAD1 were lactylated at K90 and K108, respectively (Figure
193 2C), and both lactylated sites were found to be highly conserved (Supplemental Figure 2B).

194 We then performed immunoprecipitation assay to validate the mass spectra result.
195 Indeed, immunoblotting using the anti-pan-Klac antibody repeatedly detected strong signals
196 for lactylation of YAP-TEAD (Supplemental Figure 2C). Moreover, lactylation of endogenous
197 YAP and TEAD1 were also observed in HGC27 cells (Figure 2D). Further, to confirm that the
198 K90 and K108 are the primary sites of YAP and TEAD1 lactylation, respectively, we mutated
199 each residue to arginine (R) and examined their lactylation status. Indeed, the YAP-K90R
200 and TEAD1-K108R mutants showed almost no lactylation (Figure 2E). We further examined

201 whether the lactylation of YAP and TEAD1 can respond to lactate levels. To this end, we
202 cultured cells in media with different intracellular lactate concentrations. We found that
203 glucose-deprivation decreased the lactylation levels of YAP-TEAD1, while lactate treatment
204 obviously rescued their lactylation (Supplemental Figure 2, D and E). Similar results were
205 also obtained for endogenous YAP (Figure 2F) and TEAD1 (Figure 2G) in HGC27 cells.

206 To facilitate further study of YAP-K90 lactylation, we generated a rabbit polyclonal
207 antibody recognizing YAP K90 lactylation (hereafter referred as lacYAP^{K90}) using
208 RLRK^{lac}PDSFFKPPC peptide as an antigen. We first applied dot blot assay to test whether
209 this lacYAP^{K90} antibody can recognize lactylated YAP using synthesized peptides
210 corresponding to amino acid residues 87-99 of YAP, and found it can specifically detect the
211 lactylated but not the unmodified peptides (Supplemental Figure 2F). Using this antibody, we
212 then confirmed that lactate treatment increased the lactylation levels of endogenous YAP
213 protein in HGC27 cells (Supplemental Figure 2G) and that YAP lactylation was completely
214 abolished by K90R mutation (Supplemental Figure 2H) and YAP-knockout (Supplemental
215 Figure 2I). Moreover, pretreatment of this homemade antibody with a YAP K90lac peptide
216 totally blocked its signal, i.e., abrogated its ability to recognize lactylated YAP in cells
217 (Supplemental Figure 2J).

218 To investigate whether AARS1 is directly responsible for the lactylation of Hippo pathway
219 components, we first confirmed by co-immunoprecipitation the interaction of AARS1 with
220 YAP-TEAD1 (Figure 2H and Supplemental Figure 2, K and L). Moreover, cellular fractionation
221 assay clearly demonstrated that such interaction mainly occurred in the nucleus
222 (Supplemental Figure 2M). Further in vitro pulldown assays using purified recombinant

223 proteins of AARS1 and YAP-TEAD1 revealed the interaction as a direct one (Figure 2I). We
224 next performed in vitro lactylation experiments using synthetic peptides of TEAD1 K108
225 (RDFHSK¹⁰⁸LKDQTC) and YAP K90 (PMRLRK⁹⁰LPDSFC) as substrates. Mass spectrometry
226 analysis showed that purified AARS1 protein was indeed able to directly lactylate the
227 synthetic TEAD1 K108 and YAP K90 peptides in the presence of lactate and ATP (Figure 2J).
228 Similarly, our in vitro lactylation assay using purified recombinant protein of TEAD1 as a
229 substrate also showed that AARS1 was able to directly lactylate wildtype TEAD1 (Figure 2K),
230 but not its K108R mutant (Supplemental Figure 2N). Also, we found that AARS1 5M mutant
231 failed to lactylate either YAP or TEAD1 (Figure 2J), and that inclusion of PPI or L-alanine
232 significantly inhibited the AARS1-mediated lactylation of YAP-TEAD1 (Figure 2, J and K and
233 Supplemental Figure 2, O and P). Consistently, overexpression of wildtype AARS1 but not its
234 5M mutant in HEK293FT cells promoted lactylation of YAP-TEAD (Figure 2L); while
235 knockdown of AARS1 markedly decreased the lactylation levels of YAP-TEAD (Figure 2M).

236 To probe possible enzymes responsible for the delactylation of YAP, we treated
237 HEK293FT cells with histone deacetylases (HDACs) inhibitor trichostatin A (TSA) or sirtuin
238 inhibitor nicotinamide (NAM). The results showed that NAM treatment significantly increased
239 the lactylation of YAP (Supplemental Figure 2Q). To further identify the specific enzyme
240 responsible for YAP delactylation, we performed a mini-screening in YAP-overexpressing
241 cells co-transfected with individual members of the sirtuin family of deacetylases (SIRT1-7).
242 The results showed that overexpression of SIRT1, but not of other members of this family,
243 substantially reduced the lactylation levels of YAP (Supplemental Figure 2R). Meanwhile, our
244 co-immunoprecipitation assay showed an interaction of SIRT1 with YAP (Supplemental

245 Figure 2S). Moreover, unlike wildtype SIRT1, a catalytically deficient mutant of SIRT1 (H363Y)
246 failed to reduce the lactylation levels of YAP in cells (Supplemental Figure 2T). This was
247 further confirmed by the results of an in vitro delactylation assay showing that purified SIRT1,
248 but not the H363Y mutant, eliminated lactylation of synthetic peptides of both YAP K90lac
249 and TEAD1 K108lac (Supplemental Figure 2U).

250

251 **Lactylation of YAP-TEAD promotes expression of Hippo pathway target genes**

252 To assess the functional consequence of YAP K90 lactylation, we transfected HEK293A cells
253 with wildtype YAP—or with its K90R mutant designed to mimic a lactylation-deficient/resistant
254 state and examined their subcellular localization frequency in glucose-free medium
255 supplemented with or without lactate. Both immunofluorescence (Figure 3A) and cellular
256 fractionation (Figure 3B) assays revealed that lactate strongly promoted nuclear localization
257 of wildtype YAP but not its K90R mutant, suggesting that K90 lactylation is required for lactate-
258 induced YAP activation. Consistently, lactate enhanced the interaction of TEAD1 with
259 wildtype YAP but not its K90R mutant (Figure 3C). Moreover, lactate significantly increased
260 the mRNA levels of *CTGF* and *CYR61* in cells overexpressing wildtype YAP, but a lesser
261 effect was observed in cells overexpressing the YAP K90R mutant (Figure 3D). Also, a
262 luciferase reporter assay showed that lactate promoted wildtype-YAP-induced but not K90R-
263 mutant-induced transactivation of TEADs (Supplemental Figure 3A). Similarly, we also
264 explored the effect of lactylation on TEAD1 by generating a K108R mutant to mimic a
265 lactylation-deficient/resistant state. Chromatin immunoprecipitation (ChIP) assay showed
266 that lactate treatment enhanced the occupancy of wildtype TEAD1, but not its K108R mutant

267 on the promoters of CTGF and CYR61 (Figure 3E). Overall, these data demonstrated that
268 intracellular lactate promotes lactylation levels and transcriptional activity of YAP-TEAD1.
269 Supporting this, RNA-seq analysis indicated that Hippo pathway can indeed respond to
270 treatment with lactate (Figure 3, F and G).

271 Since the lactylation sites of YAP (K90) and TEAD1 (K108) identified in this work were
272 previously found to be ubiquitinated (30, 31), we went on to investigate the interplay between
273 lactylation and ubiquitination of YAP-TEAD1. Given that nuclear localization of YAP is
274 essential for transactivation of TEADs, we first examined the lactylation and ubiquitination
275 levels of YAP and TEAD1 in the nucleus and cytoplasm. Our nucleocytoplasmic fractionation
276 assay showed that most of the lactylated YAP and TEAD1 to be localized in the nucleus,
277 while S127-phosphorylated YAP or ubiquitinated YAP and TEAD1 were mainly distributed in
278 the cytoplasm (Figure 3H and Supplemental Figure 3B). Then we examined a possible effect
279 of lactate on YAP-TEAD1 ubiquitination and found that lactate treatment decreased
280 ubiquitination levels of YAP-TEAD1 in a dose-dependent manner (Supplemental Figure 3C),
281 consistent our findings that increased levels of lactate promote nuclear localization of AARS1
282 and its interaction with YAP-TEAD (Figure 1K and 2H). Moreover, wildtype AARS1 promoted
283 the lactylation of YAP-TEAD1, but the Δ NLS mutant failed to do so (Figure 3I). And depletion
284 of AARS1 significantly promoted ubiquitination of YAP-TEAD1 (Supplemental Figure 3D).
285 Meanwhile, lactate treatment also inhibited the interaction of YAP with XPO1 (Supplemental
286 Figure 3E), a protein previously shown to bind with and facilitate nuclear export of YAP (32),
287 results suggesting that lactylation of YAP impaired its shuttling into the cytoplasm for
288 ubiquitination.

289

290 **AARS1 is a direct target gene of YAP-TEAD**

291 To characterize the genome-wide signature genes of YAP-TEAD1 upon lactate stimulation,
292 we performed a ChIP-Seq analysis. Clearly, lactate treatment enhanced the enrichment of
293 YAP-TEAD1 around TSS region (Figure 4A). The ChIP-Seq analysis identified 832 and 923
294 peaks for YAP and TEAD1, respectively, with 412 overlapping peaks. Notably, YAP-TEAD1
295 were enriched on the promoter of AARS1 upon lactate treatment, indicating AARS1 as a
296 downstream target gene of YAP-TEAD1 (Figure 4B). Indeed, a conserved TEAD1-binding
297 motif was found on the promoter of AARS1 (Supplemental Figure 4A). Moreover, a ChIP
298 assay revealed binding of YAP-TEAD1 to the promoter of AARS1 (Figure 4C). These
299 observations were further confirmed by a gel shift assay showing that TEAD1 alone, but not
300 YAP, retarded the DNA probe corresponding to AARS1 promoter and that YAP-TEAD1
301 caused a super-shift of the probe (Figure 4D).

302 To further test whether YAP-TEAD1 regulate the transcription of AARS1 by binding to the
303 predicted TEAD1-binding motif on AARS1 promoter, we constructed luciferase reporter gene
304 vectors containing the wildtype (proAARS1^{WT}) or mutated (proAARS1^{Mu}) TEAD1-binding site
305 (Supplemental Figure 4B). As expected, overexpression of YAP-TEAD1 in HEK293FT cells
306 increased the luciferase reporter gene activity of the wildtype vector in a dose-dependent
307 manner but did not affect the activity of the mutant vector (Figure 4E and Supplemental Figure
308 4C). In addition, both protein and mRNA levels of AARS1 were significantly increased in
309 HGC27 cells upon treatment with lactate, whereas knockout of YAP abolished such effects
310 (Figure 4, F and G). Together, these results indicated that AARS1 is a direct target gene of

311 YAP-TEAD1, and that intracellular lactate drives a positive feedback loop between AARS1
312 and YAP-TEAD1 (Supplemental Figure 4D).

313

314 **AARS1 is upregulated in human GC and associated with poor prognosis**

315 To assess the clinical relevance of AARS1 in GC, we analyzed AARS1 transcription in the
316 GEO database. As expected, the expression of *ATP4B*, a known parietal cell maker in normal
317 gastric epithelium, was lost, while *MKI67*, *YAP1*, *TEAD1*, *CTGF*, and *CYR61* were all
318 significantly upregulated in GC (Figure 5A). Of note, the transcription of *AARS1* but not
319 *AARS2* was much higher in GC tissues than that in healthy tissues (Figure 5A). Moreover,
320 the mRNA levels of *AARS1* were positively correlated with those of *MKI67* and *YAP1*
321 (Supplemental Figure 5A). We then collected 6 human GC samples paired with adjacent
322 normal tissues and confirmed the elevated expression levels of AARS1 in GC (Supplemental
323 Figure 5B). Furthermore, we monitored the expression of AARS1, YAP and TEAD1 during N-
324 methyl-N-nitrosourea (MNU)-induced mouse GC progression and found that expression
325 levels of AARS1 and lactylation levels of YAP-TEAD1 were obviously increased upon MNU
326 treatment (Figure 5B). In addition to the expression of AARS1 and YAP-TEAD1, the levels of
327 lactate were also progressively increased along with the MNU-induced GC progression
328 (Figure 5, C and D). Moreover, not only the expression of YAP and AARS1, but also their
329 nuclear localization was enhanced in MNU-induced tumors than that in normal tissues
330 (Supplemental Figure 5, C and D).

331 Subsequently, we examined the expression of AARS1 by immunohistochemical staining
332 on a human GC tissue array containing 90 GC specimens paired with normal ones.

333 Consistent with the above results, the expressions of AARS1, YAP and TEAD1 were found
334 to be significantly upregulated in GC tissues compared with associated normal tissues
335 (Figure 5, E and F). The expression levels of AARS1 were found to be correlated with those
336 of YAP-TEAD1 in GC tissues (Figure 5G and Supplemental Table 1 and 2). Further Kaplan-
337 Meier survival analysis showed that high expression levels of AARS1, especially in
338 combination with high expression of YAP-TEAD1, strongly predicted a poor prognosis for GC
339 patients of this cohort (Figure 5H). In addition, expression levels of AARS1 were found to be
340 positively correlated with *Helicobacter pylori* infection, tumor size and tumor stages (weakly
341 with lymph node metastasis) (Table 1).

342

343 **AARS1 promotes GC growth dependent on YAP-TEAD lactylation**

344 To investigate whether AARS1 promotes tumor cell growth in a manner dependent on Hippo
345 pathway, we performed an RNA-seq analysis of AARS1-knockdown HGC27 cells (Figure 6A
346 and Supplemental Figure 6A). GSEA analysis showed a negative enrichment of the Hippo
347 pathway signature genes upon AARS1 knockdown (Figure 6B). This result was further
348 validated by a qPCR assay showing that AARS1 knockout dramatically reduced the mRNA
349 expressions of *CTGF* and *CYR61* in the presence of sufficient lactate (normal medium or
350 glucose-free medium with exogenous lactate), but had no effect upon deficiency of lactate
351 (glucose-free medium without exogenous lactate) (Figure 6C). Consistent with these
352 observations, depletion of AARS1 significantly decreased the lactylation levels of
353 endogenous YAP-TEAD1 even in the presence of sufficient lactate (Figure 6D). Also,
354 knockdown of AARS1 abrogated the promoting effect of lactate on the retention of YAP in the

355 nucleus (Figure 6E). These results further confirmed that AARS1 is required for the lactylation
356 of YAP-TEAD1 and therefore lactate-induced expression of Hippo pathway target genes.

357 Next, we assessed the potential regulatory effect of AARS1 on YAP-driven tumor
358 growth. Knockdown of AARS1 in HGC27 cells markedly decreased lactate-induced EdU⁺
359 cell populations (Figure 6F and Supplemental Figure 6B), suppressed cell growth (Figure
360 6G) and the colony formation efficiency (Supplemental Figure 6C). However,
361 overexpression of TEAD1 together with a constitutively active (5A) mutant of YAP rescued
362 the growth of the AARS1-knockdown HGC27 cells (Figure 6H and Supplemental Figure 6,
363 D and E). Conversely, overexpression of AARS1 in wildtype HGC27 cells promoted their
364 growth, while depletion of YAP-TEAD1 abolished such effects (Figure 6I and Supplemental
365 Figure 6, F and G). To further investigate the pathological function of AARS1 in
366 tumorigenesis, we generated subcutaneous and orthotopic mouse GC models and found
367 that knockdown of AARS1 markedly inhibited tumor growth, while enforced expression of
368 YAP-TEAD1 abolished this inhibitory effect (Figure 6J). However, overexpression of the
369 YAP (K90R)-TEAD1 (K108R) mutants only slightly rescued the growth of tumors inhibited
370 by AARS1 knockdown (Figure 6J). Consistently, mice orthotopically injected with HGC27
371 cells stably expressing AARS1 had larger tumors in their stomachs than control group,
372 whereas silencing the expression of YAP-TEAD1 abrogated AARS1-overexpression-
373 induced tumor growth (Figure 6K).

374 To investigate whether the regulatory effect of AARS1 on cell proliferation depends on
375 its canonical function as a tRNA synthetase or its moonlighting function as a lactyl-
376 transferase (i.e. YAP-TEAD1 lactylation), we reintroduced wildtype, ΔNLS and 5M mutant

377 of AARS1 back into AARS1-knockout AGS cells. Immunoblotting showed that AARS1
378 depletion significantly reduced YAP-TEAD1 lactylation levels in cells treated with lactate,
379 while reintroduction of wildtype AARS1, but not Δ NLS and 5M mutant rescued YAP-TEAD1
380 lactylation in AARS1-knockout cells (Supplemental Figure 6H). Subsequently, we used O-
381 propargyl-puromycin (OP-Puro), an analog of puromycin that can incorporate into nascent
382 polypeptide chains within cells, to evaluate the impact of NLS deletion on the tRNA
383 synthetase function of AARS1. The result of flow cytometry analysis showed no significant
384 difference in protein synthesis between AARS1-knockout cells reconstituted with wildtype
385 AARS1 and that reconstituted with Δ NLS mutant AARS1, suggesting that NLS deletion did
386 not affect the canonical function of AARS1 (Supplemental Figure 6I). However, the result
387 of EdU cell proliferation assay showed that only wildtype AARS1, but not the Δ NLS and 5M
388 mutants, rescued the cell proliferation of AARS1-knockout cells (Supplemental Figure 6J).

389 Furthermore, we performed of xenograft GC model to evaluate in vivo the pathological
390 function of AARS1. The results showed that ectopic expression of wildtype AARS1
391 effectively rescued the growth of tumors derived from AARS1-knockout cells, while the
392 Δ NLS mutant failed to do so (Supplemental Figure 6K). In addition, we overexpressed
393 wildtype YAP-TEAD1 and lactylation-deficient mutants of YAP (K90R)-TEAD1 (K108R) in
394 AARS1-overexpressed AGS cells and examined their proliferation. The results of EdU
395 assay showed that wildtype YAP-TEAD1, but not lactylation-deficient mutants, significantly
396 promoted cell proliferation in AARS1-overexpressing cells (Supplemental Figure 6L). Similar
397 results were obtained in a xenograft GC model (Supplemental Figure 6M). Together, these
398 findings indicate that the lactyl-transferase function, instead of the tRNA synthetase

399 function of AARS1 plays an essential role in controlling GC growth.

400

401 **GC-associated R77Q mutation of AARS1 promotes its lactyl-transferase activity**

402 Given the clinical relevance (Figure 5) and the tumor-promoting role (Figure 6) of AARS1, we
403 further analyzed AARS1 mutations in COSMIC and cBioPortal databases and found that
404 R77Q was the most common AARS1 mutation in GC patients (Figure 7A). Since R77 is in
405 the catalytic pocket of AARS1 (Figure 7B), we reasoned that R77Q mutation might affect the
406 substrate binding or enzymatic activity of AARS1. To test this possibility, we first performed a
407 co-immunoprecipitation assay and found no effect of the R77Q mutation on the interaction
408 between AARS1 and YAP-TEAD1 (Supplemental Figure 7). Subsequently, however, our in
409 vitro lactylation assay using TEAD1 as a substrate showed that the R77Q mutation seemingly
410 increased the catalytic efficiency of AARS1 (Figure 7C). Consistently, co-transfection of
411 293FT cells with YAP and wildtype or R77Q-mutated AARS1 showed significantly greater
412 lactylation of YAP when the R77Q mutant was used than when the wildtype AARS1 was used
413 (Figure 7D). Moreover, cell growth and colony formation assay showed significantly greater
414 GC cell growth when the R77Q mutant was overexpressed than when wildtype AARS1 was
415 overexpressed (Figure 7, E and F). In keeping with these observations, the expressions of
416 *CTGF* and *CYR61* were also notably upregulated in the R77Q-mutant-overexpressing cells
417 compared with those in the wildtype AARS1-overexpressing cells (Figure 7G).

418 **Discussion**

419 A newly defined PTM, namely lactylation, has been suggested in recent studies to play
420 important roles in epigenetic regulation of gene expression and to be associated with human
421 diseases such as inflammation, Alzheimer's disease, and cancer (7, 11, 33). In this study, we
422 rediscovered the tRNA synthetase AARS1 to be a moonlighting but bona fide lactyl-
423 transferase that can directly use lactate as a donor of lactyl-group and ATP as an energy
424 source—and on that basis, we revealed a non-canonical function of lactate in tumor cells, i.e.,
425 to transmit a YAP-TEAD1-activating cell-proliferation-promoting signal via lactylation,
426 explaining in a new angle how tumors benefit from the Warburg effect.

427 **AARS1 as a lactyl-transferase and a sensor of intracellular lactate**

428 It has been proposed that p300 may function as a lactyl-transferase to catalyze histone
429 lactylation by using lactyl-CoA as a lactyl-donor (7). However, the enzymes that produce
430 lactyl-CoA from lactate in mammalian cells remains unknown and the levels of lactyl-CoA in
431 tumor cells are extremely low (15). As a major finding of this current work, we unambiguously
432 identified AARS1 as a lactyl-transferase able to catalyze protein lactylation using free lactate
433 and ATP, which are abundant in cells, especially in proliferating tumor cells. Notably, we
434 provided extensive and direct evidence of AARS1's lactyl-transferase activity, in particular, by
435 the in vitro lactylation assay using high-purity recombinant protein of AARS1 as an enzyme,
436 and purified proteins of histones and TEAD1, or synthetic peptides as substrates. Meanwhile,
437 we found that L-alanine can inhibit the lactyl-transferase activity of AARS1 by competing with
438 lactate for binding the same site of the catalytic pocket in AARS1. Therefore, AARS1-
439 mediated protein lactylation may serve as a rapid response mechanism to the dynamic

440 cellular lactate metabolism, which may directly intersect L-alanine abundance and protein
441 synthesis. Interestingly, we accidentally found in vitro that high concentrations of lactyl-CoA
442 may trigger spontaneous protein lactylation, which most likely is an artifact that could not
443 widely occur in vivo due to low levels of cellular lactyl-CoA (34). That said, we could not rule
444 out the possibility that low concentrations of lactyl-CoA in cells, via non-enzymatic lactylation
445 may contribute cumulatively to degenerative processes such as aging.

446 Most recently, Sun et al. reported that AARS1/2 may potentially function as lactyl-
447 transferases for METTL16 (35), while Mao et al. demonstrated that AARS2 directly catalyze
448 lactylation of mitochondrial proteins PDHA1 and CPT2 (36), implying a general mechanism
449 of catalyzing protein lactylation by AARS1/2. AARS2 has been shown to specifically localize
450 on mitochondria and to not have an NLS motif, while AARS1 was found to have an NLS motif
451 but to normally localize throughout the cytoplasm. Importantly, we found that AARS1 can
452 sense increased levels of intracellular lactate and shuttle into the nucleus, where it interacts
453 with the YAP-TEAD1 complex and lactylates both YAP and TEAD1. Our previous findings
454 suggested that amino acids can enhance the interactions between the corresponding
455 aminoacyl-tRNA synthetases (ARSs) and their substrates to catalyze lysine aminoacylations
456 (37). Similarly, here we found AARS1 interacting with YAP-TEAD1 both in cells and in vitro,
457 and that lactate in the meanwhile can enhance their interactions. Thus, AARS1 appears to
458 be a sensor of intracellular lactate and a general lactyl-transferase.

459 **Function fate of AARS1 as a lactyl-transferase or a tRNA synthetase**

460 An important question elicited by our current work is the cellular signals that control the
461 substrate preference of AARS1 and the mechanism that decides the function fate of AARS1

462 as a lactyl-transferase or a tRNA synthetase. In this regard, note that the intracellular
463 concentration of L-alanine is approximately 0.24 mM in Hela cells (38), and the physiological
464 concentration of lactate ranges from 0.5 to 20 mM (39) and can reach up to 40 mM in tumor
465 tissues (40). Therefore, on one hand, L-alanine can inhibit the lactyl-transferase activity of
466 AARS1 by direct competing with lactate for binding AARS1. On the other hand, increased
467 intracellular lactate might also regulate the tRNA synthetase activity of AARS1 via 1)
468 competing with L-alanine to decrease the alanyl-tRNA synthetase activity; 2) enhancing the
469 expression of AARS1 to increase the activities of both lactyl-transferase and alanyl-tRNA
470 synthetase. We showed that lactate regulation of AARS1 expression plays a more important
471 role in this regulation.

472 Overall, the function fate of AARS1 as a lactyl-transferase or a tRNA synthetase seems
473 to be determined by the intracellular concentrations of lactate and L-alanine. In normal cells
474 primarily relying on oxidative phosphorylation to generate ATP, the intracellular lactate
475 concentration is relatively low, and AARS1 mainly functions as a tRNA synthetase. In
476 proliferating cancer cells addicted to aerobic glycolysis, the intracellular lactate may
477 accumulate to high levels and promote the expression of AARS1, which in turn increases the
478 cellular activity of AARS1 as both lactyl-transferase and a tRNA synthetase. Thus, it is most
479 likely that the function fate of AARS1 may depend on the relative abundance of lactate versus
480 alanine in a specific cellular compartment. In this regard, note that lactate not only increased
481 the expression of AARS1, but also promoted its translocation into the nucleus.

482 **Competitive relationship between lactylation and ubiquitination of YAP-TEAD**

483 Our study identified K90 and K108 as the major lactylation residues of YAP and TEAD1,

484 respectively. Previous studies have reported that YAP K90 and TEAD1 K108 were also sites
485 for ubiquitination (30, 31). Here, we showed that lactylation and ubiquitination of YAP-TEAD1
486 are mutually exclusive and mostly occur in different cellular compartments. AARS1 mainly
487 interacts with and lactylates YAP-TEAD1 in the nucleus in response to increased levels of
488 intracellular lactate, while ubiquitination of YAP-TEAD1 mainly occurs in the cytoplasm. Note
489 that the reciprocal inhibition between lactylation and ubiquitination of YAP-TEAD1 is not
490 merely due to competition of the identical target sites, i.e., lysine residues, but also because
491 of the subcellular localization of YAP-TEAD1. For example, lactylation of YAP inhibited its
492 nuclear export by XPO1 and thus preventing its translocation into the cytoplasm for
493 ubiquitination.

494 **Feedback regulation of AARS1 by YAP-TEAD1**

495 Hyperactivation of YAP has been frequently found in malignant tumors and such
496 hyperactivation has been extensively correlated with tumor growth (16, 18). It has been well
497 established that hyperactivation of YAP promotes tumor cell proliferation. Meanwhile, studies
498 also showed that YAP-TEAD1 can promote glucose uptake and aerobic glycolysis to produce
499 more lactate (41-43). Yet, it was unclear whether and how YAP hyperactivation is coupled to
500 intracellular lactate and global protein lactylation. In this regard, we found AARS1 serving as
501 a direct target gene of YAP-TEAD1. And a lactate treatment was observed to enhance the
502 binding of YAP-TEAD1 onto the promoter region of AARS1, leading to its increased
503 expression. Thus, AARS1 and YAP-TEAD1 were concluded to form a positive feedback loop
504 linking high levels of intracellular lactate with global protein lactylation and accelerated cell
505 proliferation. In addition, previous studies indicated that YAP can be activated by OGT-

506 mediated O-GlcNAcylation to sense cellular glucose levels (26, 44). Since glucose is
507 metabolized to lactate during aerobic glycolysis, this dual sensing mechanism of glucose and
508 lactate by YAP and AARS1, respectively, may further enforce the interpretation of metabolic
509 and nutrient cues into tumor cell proliferation signals.

510 **Clinical implications and therapeutic targeting of AARS1-YAP-TEAD1 axis**

511 Considering the hyperactivation of YAP in GC and other human cancers, tremendous efforts
512 have been focused on developing therapeutic strategies targeting the Hippo-YAP signaling
513 pathway (23, 24, 45). In our current study, AARS1 expression was found to be upregulated
514 in tumor tissues from GC patients and MNU-induced GC mouse models, with this
515 upregulation consistent with our findings of AARS1 serving as a direct target gene of YAP-
516 TEAD1. Moreover, elevated expression levels and gain-of-function mutation of AARS1 and
517 increased lactylation levels of YAP-TEAD1 were found to be closely associated with poor
518 prognosis for GC patients. Here, we found that genetic depletion of AARS1 regressed gastric
519 cancer cell growth. However, as AARS1 plays a fundamental role in tRNA aminoacylation
520 and protein synthesis and its deficiency has been reported to be associated with neurologic
521 disorders and acute liver failure (46, 47), therapeutic targeting of AARS1 to treat GC warrants
522 further investigations.

523 **Physiological function of AARS1-mediated lactylation**

524 We discovered AARS1 as a lactyl-transferase that utilize lactate and ATP to catalyze lysine
525 lactylation on both histones and non-histone proteins, and emphasized its pathological role
526 especially via lactylation of YAP-TEAD1 in a context of tumorigenesis. However,
527 accumulating studies have shown that lactylation can influence various physiological

528 processes as well, and may does so via epigenetic regulation and other mechanisms. For
529 instance, lactate is produced via glycolysis in stimulated M1 macrophages, thus promoting
530 histone lactylation (7). The H3K18lac mark exhibits enrichment on promoter regions of
531 homeostatic genes, thereby activating their expression and facilitating the acquisition of M2-
532 like characteristics to ultimately achieve a biological steady state (7). Moreover, histone
533 lactylation also plays important roles in the process of embryogenesis (48, 49). Lactylation of
534 histones on the promoter regions of genes related to zygotic genome activation (ZGA) seems
535 to facilitate their expression and promote preimplantation embryo development (48). In
536 addition to tumor cells, lactate can be generated through glycolysis and locally accumulated
537 in various types of cells even under physiological state. Thus AARS1 may also play a crucial
538 role in protein lactate in these cells to regulate a variety of biological processes.

539 **Conclusion and limitation**

540 Our study revealed a non-canonical function of AARS1, namely lactyl-transferase activity. In
541 the case of AARS1-mediated lactylation of the Hippo pathway, AARS1 and YAP-TEAD form
542 a positive feedback loop that constitutively pushes forward the conversion of lactate
543 metabolism into tumor cell growth. Meanwhile, our study still has limitations. For example,
544 the clinical relevance of AARS1 to various GC subtypes as well as to other types of malignant
545 tumors remains to be clarified. Moreover, considering the pivotal role of AARS1 in tRNA
546 aminoacylation and protein synthesis, further investigations are warranted to explore the
547 potential of targeting the lactyl-transferase activity of AARS1 for the treatment of GC and
548 other human malignancies. In addition to YAP-TEAD1, other substrates of AARS1 have not
549 yet had their functions fully characterized. Also, the cell type-specific substrate spectrum for

550 AARS1 warrants further investigation. In this regard, due to the accumulation of lactate in the
551 tumor microenvironment, protein lactylation in immune cells and tumor-associated fibroblasts
552 are worthy of attention.

553

554 **Methods**

555 A detailed description of Materials and Methods is provided in the supplemental information.

556 **Sex as a biological variable**

557 Our study examined male and female animals, and similar findings are reported for both
558 sexes.

559 **Study approval**

560 All animal experiments were approved by the Institutional Animal Care and Use Committee
561 of the Institute of Biochemistry and Cell Biology. The approval ID for the use of animals was
562 SIBCB-NAF-14-004-S329-023. The gastric cancer tissue samples used in the study were
563 derived from patients who signed informed consents for the use of the specimen. The studies
564 were performed in accordance with the Declaration of Helsinki and approved by the Hua'shan
565 Hospital Institutional Review Board (HIRB). The human gastric cancer tissue array was
566 purchased from Shanghai Outdo Biotech.

567 **Data availability**

568 RNA-seq and ChIP-seq data reported in this paper are deposited in the Gene Expression
569 Omnibus (GEO) database (<https://www.ncbi.nlm.nih.gov/geo>). The accession numbers are
570 GSE200850, GSE200789 and GSE200790. The human gastric cancer RNA-seq dataset
571 used in **Figure 5A** was obtained from the GEO database with accession number GSE13911.
572 The file containing Supporting data values is provided. All unique/stable reagents generated
573 in this study are available from the Lead Contact with a completed Materials Transfer
574 Agreement.

575

576 **Author contributions**

577 JJ, and HZ performed most of experiments. ZY performed cellular assay. LA performed CHIP-
578 seq. YT and LT performed in vitro biochemical experiments. ML, JY, ZC, DG, FC, WW and
579 YH analyzed and discussed the data. ZZ, SJ, and JJ wrote the manuscript. ZZ, SJ and SZ
580 supervised the project. JJ is listed as the first author in recognition of his significant
581 contribution to the inception of this study.

582

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589

590 **Declaration of Interests**

591 The authors have declared that no conflict of interest exists.

592

593 **References**

- 594 1. Vander Heiden MG, Cantley LC, and Thompson CB. Understanding the Warburg effect: the metabolic
595 requirements of cell proliferation. *Science*. 2009;324(5930):1029-33.
- 596 2. Warburg O. On the origin of cancer cells. *Science*. 1956;123(3191):309-14.
- 597 3. Haas R, Smith J, Rocher-Ros V, Nadkarni S, Montero-Melendez T, D'Acquisto F, et al. Lactate Regulates
598 Metabolic and Pro-inflammatory Circuits in Control of T Cell Migration and Effector Functions. *PLoS Biol*.
599 2015;13(7):e1002202.
- 600 4. Watson MJ, Vignali PDA, Mullett SJ, Overacre-Delgoffe AE, Peralta RM, Grebinoski S, et al. Metabolic
601 support of tumour-infiltrating regulatory T cells by lactic acid. *Nature*. 2021;591(7851):645-51.
- 602 5. Kumagai S, Koyama S, Itahashi K, Tanegashima T, Lin YT, Togashi Y, et al. Lactic acid promotes PD-1
603 expression in regulatory T cells in highly glycolytic tumor microenvironments. *Cancer Cell*. 2022;40(2):201-
604 18 e9.
- 605 6. Colegio OR, Chu NQ, Szabo AL, Chu T, Rhebergen AM, Jairam V, et al. Functional polarization of tumour-
606 associated macrophages by tumour-derived lactic acid. *Nature*. 2014;513(7519):559-63.
- 607 7. Zhang D, Tang Z, Huang H, Zhou G, Cui C, Weng Y, et al. Metabolic regulation of gene expression by
608 histone lactylation. *Nature*. 2019;574(7779):575-80.
- 609 8. Wan N, Wang N, Yu S, Zhang H, Tang S, Wang D, et al. Cyclic immonium ion of lactyllysine reveals
610 widespread lactylation in the human proteome. *Nat Methods*. 2022;19(7):854-64.
- 611 9. Li L, Chen K, Wang T, Wu Y, Xing G, Chen M, et al. Glis1 facilitates induction of pluripotency via an
612 epigenome-metabolome-epigenome signalling cascade. *Nat Metab*. 2020;2(9):882-92.
- 613 10. Hagihara H, Shoji H, Otabi H, Toyoda A, Katoh K, Namihira M, et al. Protein lactylation induced by neural
614 excitation. *Cell Rep*. 2021;37(2):109820.
- 615 11. Pan RY, He L, Zhang J, Liu X, Liao Y, Gao J, et al. Positive feedback regulation of microglial glucose
616 metabolism by histone H4 lysine 12 lactylation in Alzheimer's disease. *Cell Metab*. 2022;34(4):634-48 e6.
- 617 12. Irizarry-Caro RA, McDaniel MM, Overcast GR, Jain VG, Troutman TD, and Pasare C. TLR signaling adapter
618 BCAP regulates inflammatory to reparatory macrophage transition by promoting histone lactylation. *Proc
619 Natl Acad Sci U S A*. 2020;117(48):30628-38.
- 620 13. Sun L, Zhang H, and Gao P. Metabolic reprogramming and epigenetic modifications on the path to cancer.
621 *Protein Cell*. 2022;13(12):877-919.
- 622 14. Yang Z, Yan C, Ma J, Peng P, Ren X, Cai S, et al. Lactylome analysis suggests lactylation-dependent
623 mechanisms of metabolic adaptation in hepatocellular carcinoma. *Nat Metab*. 2023.
- 624 15. Varner EL, Trefely S, Bartee D, von Krusenstiern E, Izzo L, Bekeova C, et al. Quantification of lactoyl-CoA
625 (lactyl-CoA) by liquid chromatography mass spectrometry in mammalian cells and tissues. *Open Biol*.
626 2020;10(9):200187.
- 627 16. Dey A, Varelas X, and Guan KL. Targeting the Hippo pathway in cancer, fibrosis, wound healing and
628 regenerative medicine. *Nat Rev Drug Discov*. 2020;19(7):480-94.
- 629 17. Pan D. The hippo signaling pathway in development and cancer. *Dev Cell*. 2010;19(4):491-505.
- 630 18. Harvey KF, Zhang X, and Thomas DM. The Hippo pathway and human cancer. *Nat Rev Cancer*.
631 2013;13(4):246-57.
- 632 19. Zhou D, Conrad C, Xia F, Park JS, Payer B, Yin Y, et al. Mst1 and Mst2 maintain hepatocyte quiescence and
633 suppress hepatocellular carcinoma development through inactivation of the Yap1 oncogene. *Cancer Cell*.
634 2009;16(5):425-38.
- 635 20. Zhao B, Li L, Tumaneng K, Wang CY, and Guan KL. A coordinated phosphorylation by Lats and CK1

- 636 regulates YAP stability through SCF(beta-TRCP). *Genes Dev.* 2010;24(1):72-85.
- 637 21. Dong J, Feldmann G, Huang J, Wu S, Zhang N, Comerford SA, et al. Elucidation of a universal size-control
638 mechanism in Drosophila and mammals. *Cell.* 2007;130(6):1120-33.
- 639 22. Wu S, Liu Y, Zheng Y, Dong J, and Pan D. The TEAD/TEF family protein Scalloped mediates transcriptional
640 output of the Hippo growth-regulatory pathway. *Dev Cell.* 2008;14(3):388-98.
- 641 23. Jiao S, Wang H, Shi Z, Dong A, Zhang W, Song X, et al. A peptide mimicking VGLL4 function acts as a YAP
642 antagonist therapy against gastric cancer. *Cancer Cell.* 2014;25(2):166-80.
- 643 24. Tang Y, Fang G, Guo F, Zhang H, Chen X, An L, et al. Selective Inhibition of STRN3-Containing PP2A
644 Phosphatase Restores Hippo Tumor-Suppressor Activity in Gastric Cancer. *Cancer Cell.* 2020;38(1):115-28
645 e9.
- 646 25. An L, Nie P, Chen M, Tang Y, Zhang H, Guan J, et al. MST4 kinase suppresses gastric tumorigenesis by
647 limiting YAP activation via a non-canonical pathway. *J Exp Med.* 2020;217(6).
- 648 26. Peng C, Zhu Y, Zhang W, Liao Q, Chen Y, Zhao X, et al. Regulation of the Hippo-YAP Pathway by Glucose
649 Sensor O-GlcNAcylation. *Mol Cell.* 2017;68(3):591-604 e5.
- 650 27. Song Y, Zhou H, Vo MN, Shi Y, Nawaz MH, Vargas-Rodriguez O, et al. Double mimicry evades tRNA
651 synthetase editing by toxic vegetable-sourced non-proteinogenic amino acid. *Nat Commun.*
652 2017;8(1):2281.
- 653 28. Kwon NH, Fox PL, and Kim S. Aminoacyl-tRNA synthetases as therapeutic targets. *Nat Rev Drug Discov.*
654 2019;18(8):629-50.
- 655 29. Lux MC, Standke LC, and Tan DS. Targeting adenylate-forming enzymes with designed sulfonyl-adenosine
656 inhibitors. *J Antibiot (Tokyo).* 2019;72(6):325-49.
- 657 30. Zhou X, Li Y, Wang W, Wang S, Hou J, Zhang A, et al. Regulation of Hippo/YAP signaling and Esophageal
658 Squamous Carcinoma progression by an E3 ubiquitin ligase PARK2. *Theranostics.* 2020;10(21):9443-57.
- 659 31. Akimov V, Barrio-Hernandez I, Hansen SVF, Hallenborg P, Pedersen AK, Bekker-Jensen DB, et al. UbiSite
660 approach for comprehensive mapping of lysine and N-terminal ubiquitination sites. *Nat Struct Mol Biol.*
661 2018;25(7):631-40.
- 662 32. Fang L, Teng H, Wang Y, Liao G, Weng L, Li Y, et al. SET1A-Mediated Mono-Methylation at K342 Regulates
663 YAP Activation by Blocking Its Nuclear Export and Promotes Tumorigenesis. *Cancer Cell.* 2018;34(1):103-
664 18 e9.
- 665 33. Yu J, Chai P, Xie M, Ge S, Ruan J, Fan X, et al. Histone lactylation drives oncogenesis by facilitating m(6)A
666 reader protein YTHDF2 expression in ocular melanoma. *Genome Biol.* 2021;22(1):85.
- 667 34. James AM, Smith CL, Smith AC, Robinson AJ, Hoogewijs K, and Murphy MP. The Causes and Consequences
668 of Nonenzymatic Protein Acylation. *Trends Biochem Sci.* 2018;43(11):921-32.
- 669 35. Sun L, Zhang Y, Yang B, Sun S, Zhang P, Luo Z, et al. Lactylation of METTL16 promotes cuproptosis via
670 m(6)A-modification on FDX1 mRNA in gastric cancer. *Nat Commun.* 2023;14(1):6523.
- 671 36. Mao Y, Zhang J, Zhou Q, He X, Zheng Z, Wei Y, et al. Hypoxia induces mitochondrial protein lactylation to
672 limit oxidative phosphorylation. *Cell Res.* 2024;34(1):13-30.
- 673 37. He XD, Gong W, Zhang JN, Nie J, Yao CF, Guo FS, et al. Sensing and Transmitting Intracellular Amino Acid
674 Signals through Reversible Lysine Aminoacylations. *Cell Metab.* 2018;27(1):151-66 e6.
- 675 38. Yoshida T, Nakajima H, Takahashi S, Kakizuka A, and Imamura H. OLIVE: A Genetically Encoded Fluorescent
676 Biosensor for Quantitative Imaging of Branched-Chain Amino Acid Levels inside Single Living Cells. *ACS*
677 *Sens.* 2019;4(12):3333-42.
- 678 39. Brooks GA. Lactate as a fulcrum of metabolism. *Redox Biol.* 2020;35:101454.
- 679 40. Walenta S, Wetterling M, Lehrke M, Schwickert G, Sundfor K, Rofstad EK, et al. High lactate levels predict

680 likelihood of metastases, tumor recurrence, and restricted patient survival in human cervical cancers.
681 *Cancer Res.* 2000;60(4):916-21.

682 41. Kuo CC, Ling HH, Chiang MC, Chung CH, Lee WY, Chu CY, et al. Metastatic Colorectal Cancer Rewrites
683 Metabolic Program Through a Glut3-YAP-dependent Signaling Circuit. *Theranostics.* 2019;9(9):2526-40.

684 42. Cox AG, Tsomides A, Yimlamai D, Hwang KL, Miesfeld J, Galli GG, et al. Yap regulates glucose utilization
685 and sustains nucleotide synthesis to enable organ growth. *EMBO J.* 2018;37(22).

686 43. Zheng X, Han H, Liu GP, Ma YX, Pan RL, Sang LJ, et al. LncRNA wires up Hippo and Hedgehog signaling to
687 reprogramme glucose metabolism. *EMBO J.* 2017;36(22):3325-35.

688 44. Zhang X, Qiao Y, Wu Q, Chen Y, Zou S, Liu X, et al. The essential role of YAP O-GlcNAcylation in high-
689 glucose-stimulated liver tumorigenesis. *Nat Commun.* 2017;8:15280.

690 45. Calses PC, Crawford JJ, Lill JR, and Dey A. Hippo Pathway in Cancer: Aberrant Regulation and Therapeutic
691 Opportunities. *Trends Cancer.* 2019;5(5):297-307.

692 46. Marten LM, Brinkert F, Smith DEC, Prokisch H, Hempel M, and Santer R. Recurrent acute liver failure in
693 alanyl-tRNA synthetase-1 (AARS1) deficiency. *Mol Genet Metab Rep.* 2020;25:100681.

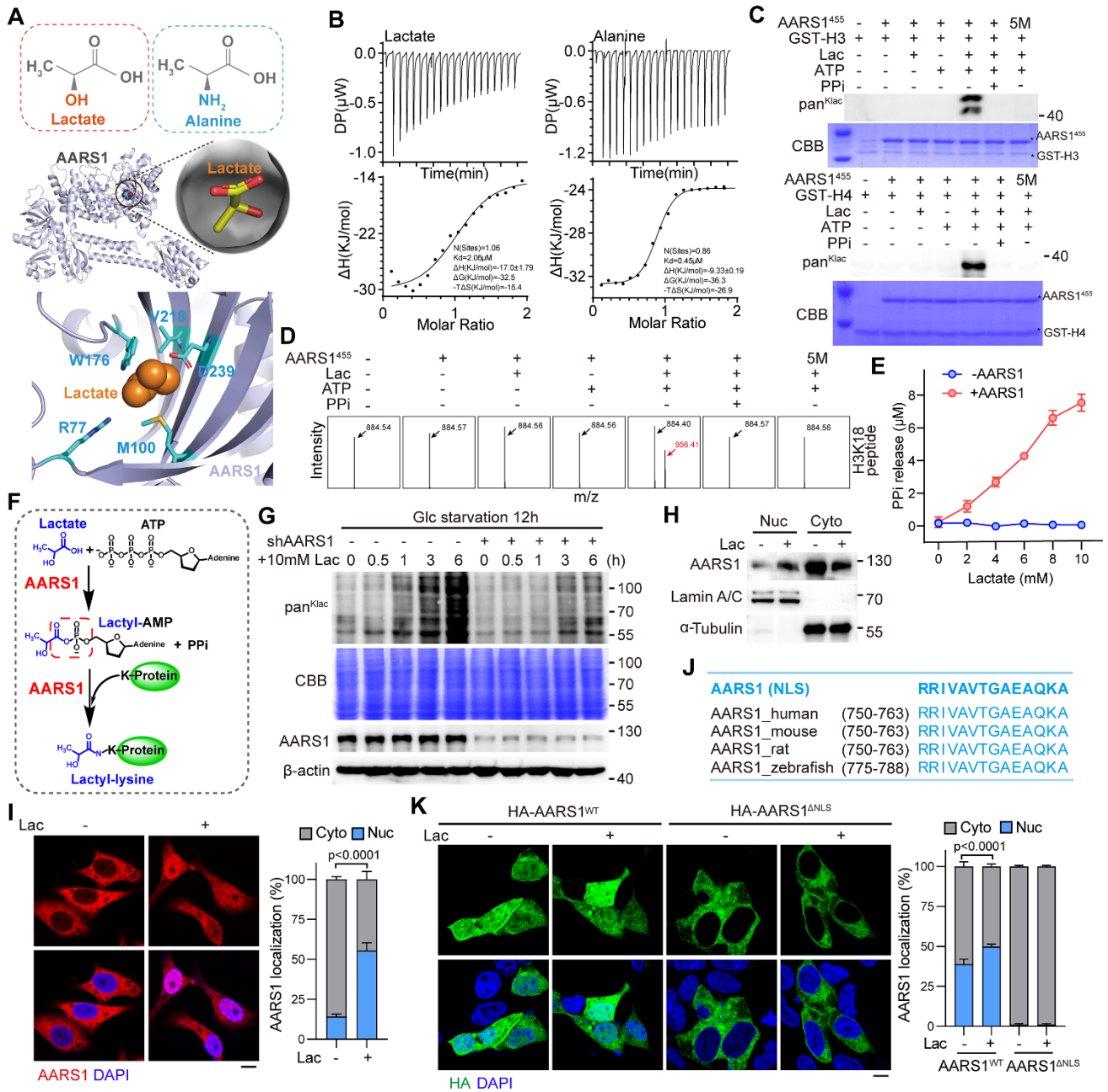
694 47. Nakayama T, Wu J, Galvin-Parton P, Weiss J, Andriola MR, Hill RS, et al. Deficient activity of alanyl-tRNA
695 synthetase underlies an autosomal recessive syndrome of progressive microcephaly, hypomyelination, and
696 epileptic encephalopathy. *Hum Mutat.* 2017;38(10):1348-54.

697 48. Li J, Hou W, Zhao Q, Han W, Cui H, Xiao S, et al. Lactate regulates major zygotic genome activation by
698 H3K18 lactylation in mammals. *National Science Review.* 2023.

699 49. Yang Q, Liu J, Wang Y, Zhao W, Wang W, Cui J, et al. A proteomic atlas of ligand-receptor interactions at
700 the ovine maternal-fetal interface reveals the role of histone lactylation in uterine remodeling. *J Biol Chem.*
701 2022;298(1):101456.

702

Figure Legends



704
705 **Figure 1. AARS1 is a lactyl-transferase sensitive to intracellular lactate levels**

706 **(A)** Comparison of the chemical formula of lactate and L-alanine (top). A predicted overall structural view
707 of lactate with AARS1 (middle). Detailed interactions between lactate (orange) and amino acid residues in
708 the catalytic core of AARS1 (cyan) (bottom). **(B)** ITC analysis of the interaction between lactate (left) or L-
709 alanine (right) and AARS1. **(C)** Immunoblotting with pan-Klac antibody to detect AARS1⁴⁵⁵-induced

710 lactylation of GST-H3 and GST-H4 in vitro. Coomassie brilliant blue (CBB) staining showing the purified
711 AARS1⁴⁵⁵, GST-H3 and GST-H4 used in in vitro lactylation assay. Asterisks represent the AARS1⁴⁵⁵, GST-
712 H3 and H4 proteins. Lac, lactate. **(D)** Mass spectrometry to determine the lactylation of the synthetic
713 H3K18 peptide catalyzed by AARS1⁴⁵⁵ and its catalytic-dead mutant 5M in vitro. 5M, R77A, M100A,
714 W176E, V218D, D239A. Lac, lactate. **(E)** PPi production in in vitro lactylation assay in the absence and
715 presence of AARS1 ($n = 3$). Data are presented as mean \pm SD. **(F)** Schematic illustration showing the
716 proposed catalytic mechanism of AARS1-induced protein lysine lactylation. **(G)** Immunoblotting with pan-
717 Klac antibody to detect global protein lactylation levels in glucose-deprived AARS1-knockdown HGC27
718 cells stimulated with 10mM lactate for indicated times. Lac, lactate. **(H)** Nucleocytoplasmic distribution of
719 AARS1 in lactate-treated HGC27 cells. Lac, lactate. **(I)** Immunofluorescence staining of AARS1 in lactate-
720 treated HGC27 cells (left). Scale bar = 5 μ m. Statistical analysis of AARS1 cellular distribution (right) ($n =$
721 10). Data are presented as mean \pm SD. Lac, lactate. **(J)** Alignment of nuclear localization signal (NLS)
722 sequences of AARS1 in the indicated species. **(K)** Immunofluorescence staining of HA-AARS1 in lactate-
723 treated HEK293A cells after transfection with HA-tagged AARS1 or its NLS-deletion (Δ NLS) mutant (left).
724 Scale bar = 5 μ m. Statistical analysis of HA-AARS1 cellular localization (right) ($n = 10$). Data are presented
725 as mean \pm SD. Lac, lactate. Unpaired Student's t test (**I** and **K**).

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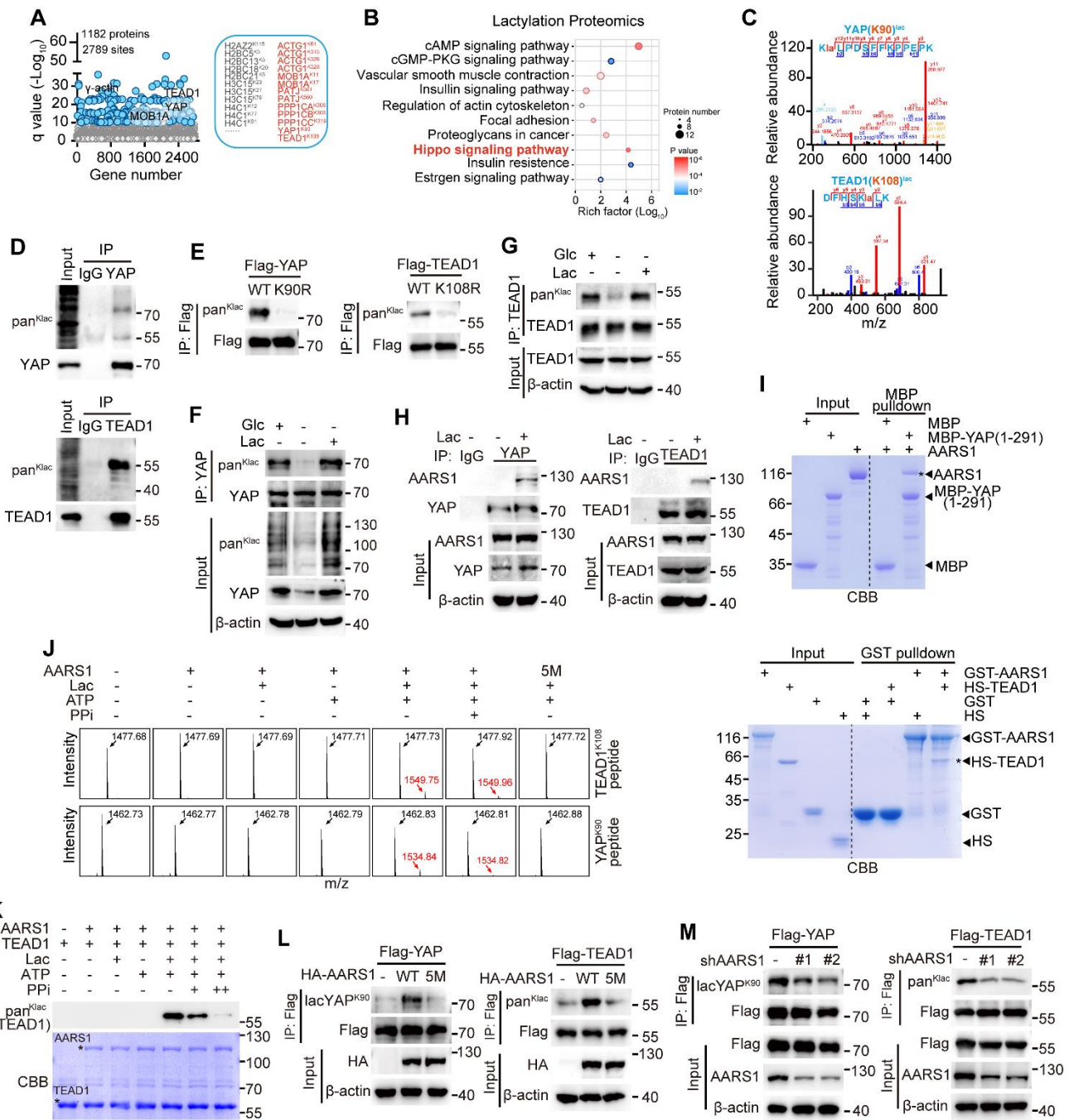


Figure 2. AARS1 directly interacts with and lactylates YAP-TEAD

(A) Lysine lactylome in lactate-treated HGC27 cells. A total of 1,182 lactylated proteins and 2,789 lactylated sites with q value (-Log₁₀) > 10 were identified. Histone sites modified through lactylation are shown (gray). Lactylation on Hippo pathway-associated components are shown (red). (B) KEGG pathway analysis of lactylated proteins identified using lactylation proteomics in HGC27 cells cultured in glucose-free medium supplemented with 25 mM lactate. (C) Mass spectra of lactylated sites of YAP (K90) and TEAD1 (K108).

734 **(D)** Immunoblotting showing the lactylation of endogenous YAP and TEAD1 proteins using pan-Klac
735 antibody. **(E)** Lactylation levels of exogenous YAP and TEAD1 in cells transfected with the indicated
736 plasmids. **(F and G)** Lactylation levels of endogenous YAP (F) and TEAD1 (G) in lactate-treated HGC27
737 cells. Glc, glucose. Lac, lactate. **(H)** Co-immunoprecipitation analysis of the endogenous interaction
738 between YAP (left) or TEAD1 (right) and AARS1 in lactate-treated HGC27 cells. Lac, lactate. **(I)** Pulldown
739 assay showing the direct interaction between AARS1 and YAP/TEAD1. MBP-pulldown assay to detect the
740 interaction between AARS1 and MBP-YAP (1-291) (top). GST-pulldown assay to detect the interaction
741 between GST-AARS1 and His-sumo-TEAD1 (HS-TEAD1) (bottom). Asterisks represent the indicated
742 proteins. **(J)** Mass spectrometry to determine the lactylation of the synthetic YAP K90 and TEAD1 K108
743 peptides catalyzed by AARS1 and its catalytic-dead mutant 5M in vitro. Lac, lactate. **(K)** Immunoblotting
744 with pan-Klac antibody to detect AARS1-induced lactylation of TEAD1 in vitro. Coomassie brilliant blue
745 (CBB) staining showing the purified AARS1 and TEAD1 used in in vitro lactylation assay. Asterisks
746 represent the AARS1 and TEAD1 proteins. The final concentrations of PPI in the reaction mixture were 2
747 mM (+) and 10 mM (++), respectively. Lac, lactate. **(L)** Lactylation levels of YAP (left) and TEAD1 (right) in
748 AARS1- or its 5M mutant-overexpressing cells. **(M)** Lactylation levels of YAP (left) and TEAD1 (right) in
749 AARS1-knockdown HEK293FT cells.

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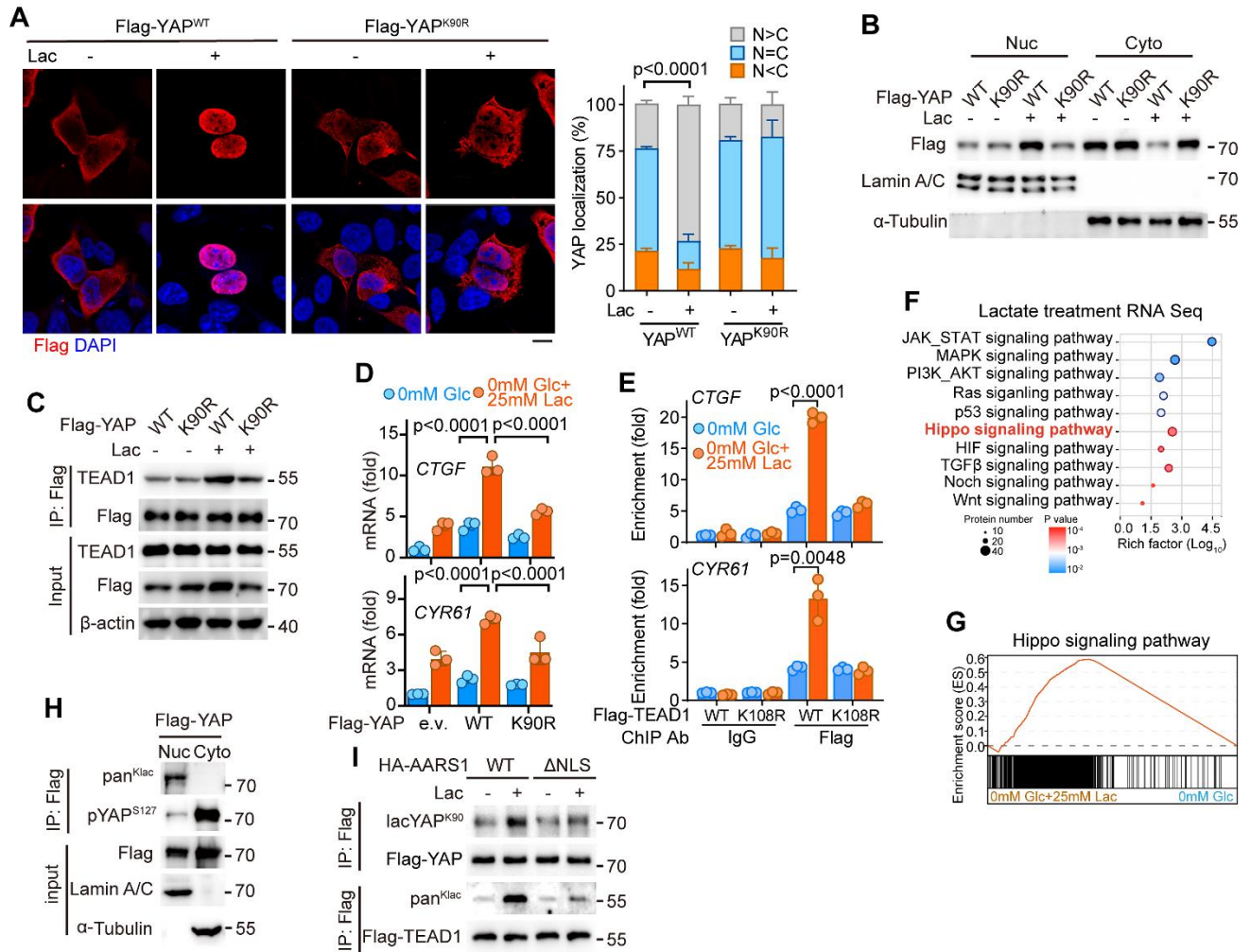


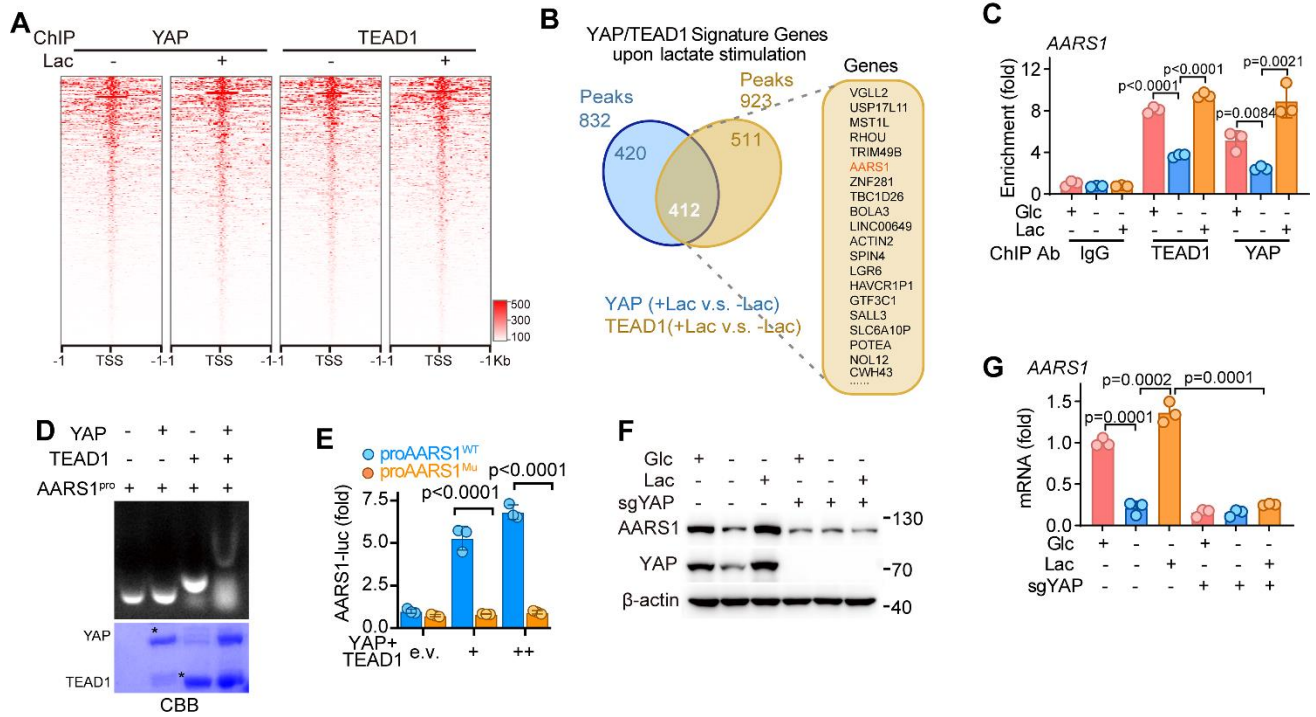
Figure 3. Lactylation promotes nuclear localization and stabilization of YAP-TEAD

(A) Immunofluorescence analysis using anti-Flag antibody showing nuclear translocation of YAP in HEK293A cells transfected with Flag-tagged YAP or its K90R mutant following lactate treatment (left). The signal intensity of Flag-YAP was quantified using ImageJ software (right) ($n = 3$). N, nuclear localization. C, cytosolic localization. Lac, lactate. Data are presented as mean \pm SD. Scale bar = 5 μ m. (B) Nucleocytoplasmic distribution of heterologously expressed YAP or its K90R mutant in lactate-treated cells. Nuc, nuclear localization. Cyto, cytosolic localization. Lac, lactate. (C) Co-immunoprecipitation analysis showing the interaction of YAP or its K90R mutant with TEAD1 in lactate-treated cells. Lac, lactate. (D) Real-time qPCR showing the mRNA levels of *CTGF* and *CYR61* in HEK293A cells overexpressing YAP or its K90R mutant following lactate treatment ($n = 3$). Data are presented as mean \pm SD. Glc, glucose. Lac,

762 lactate. **(E)** ChIP-qPCR analysis for the enrichment of TEAD1 or its K108R mutant on the indicated genes'
763 promoter in lactate-treated HEK293FT cells ($n = 3$). Data are presented as mean \pm SD. Glc, glucose. Lac,
764 lactate. **(F)** KEGG analysis of the differentially expressed genes in the glucose-deprived HGC27 cells with
765 or without 25 mM lactate. **(G)** GSEA of the Hippo pathway signature in the glucose-deprived HGC27 cells
766 with or without 25 mM lactate. **(H)** Nucleocytoplasmic distribution of lactylation and phosphorylation of YAP
767 in YAP-overexpressing HEK293FT cells. **(I)** Lactylation of exogenous YAP and TEAD1 in lactate-treated
768 HEK293A cells transfected with AARS1 or its NLS-deletion (Δ NLS) mutant. Lac, lactate. Unpaired
769 Student's *t* test (**A**, **D**, and **E**).

770

771



772

773 **Figure 4. AARS1 and YAP-TEAD form a positive feedback loop**

774 **(A)** ChIP-Seq analysis heatmap representing the distribution of YAP- or TEAD1-binding relative to the
 775 gene transcription start site (TSS) in cells cultured in glucose-medium treated with or without lactate. Lac,
 776 lactate. **(B)** Venn diagram illustrating the overlap of YAP- and TEAD1-enriched genes upon lactate
 777 stimulation. The top 20 genes are shown. **(C)** ChIP-qPCR showing the enrichment of YAP and TEAD1 on
 778 AARS1 promoter in lactate-treated HGC27 cells ($n = 3$). Glc, glucose. Lac, lactate. Data are presented as
 779 mean \pm SD. **(D)** Gel shift analysis showing the binding of YAP and TEAD1 to the synthetic DNA probe
 780 containing TEAD1 binding site on AARS1 promoter (top). CBB staining of purified recombinant YAP (MBP-
 781 YAP 1-291) and TEAD1 proteins used in gel shift assay (bottom). **(E)** Luciferase activity of wildtype (WT)
 782 or mutant (Mu) AARS1 promoter vectors in YAP and TEAD1-overexpressing HEK293FT cells ($n = 3$). Data
 783 are presented as mean \pm SD. **(F)** Immunoblotting of AARS1 protein levels in YAP-knockout AGS cells upon
 784 lactate treatment. Glc, glucose. Lac, lactate. **(G)** AARS1 mRNA levels in YAP-knockout cells upon lactate
 785 stimulation ($n = 3$). Data are presented as mean \pm SD. Unpaired Student's t test (**C**, **E**, and **G**).

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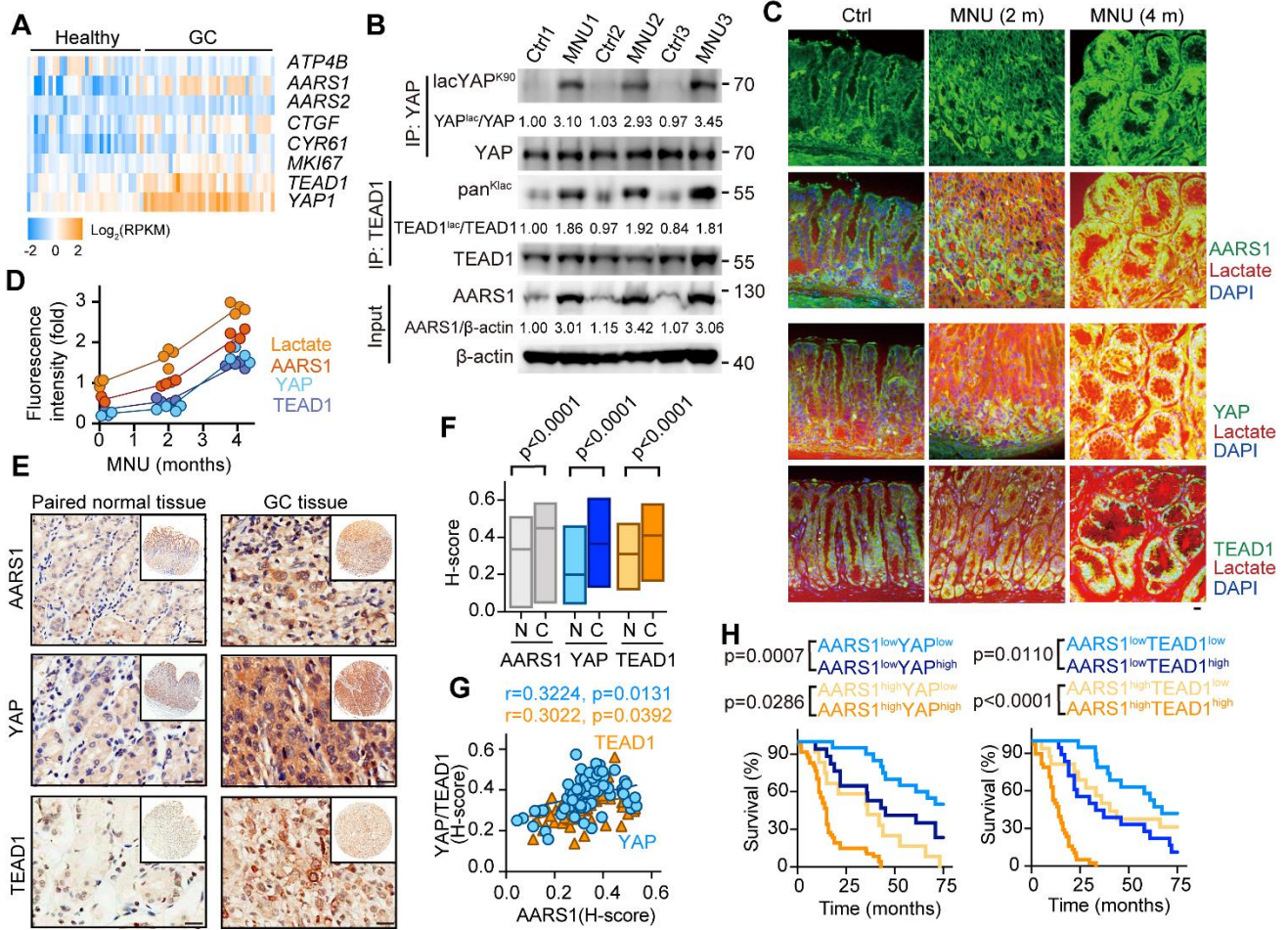


Figure 5. AARS1 is upregulated in gastric cancer and associated with bad clinical outcomes

(A) Heatmap showing the transcription of the indicated genes in healthy gastric tissues and GC tissues from the GEO database (GSE13911). (B) Immunoblotting of AARS1 levels and YAP-TEAD1 lactylation levels in mouse normal (Ctrl) and MNU-induced GC tissues. Relative YAP-TEAD1 lactylation and AARS1 levels calculated by gray value analysis are shown. (C) Immunofluorescence images of lactate, AARS1, YAP, and TEAD1 in the gastric tissues of the MNU-induced GC model at the indicated times. Scale bar = 10 μm . (D) Fluorescence intensity of lactate, AARS1, YAP, and TEAD1 in the murine GC model from panel E ($n=4$). Data are presented as mean \pm SD. (E) Immunohistochemical staining of AARS1, YAP, and TEAD1 in GC tissues and paired healthy tissues. Scale bar = 50 μm . (F) Histoscore (H-score) of AARS1, YAP, and TEAD1 in GC tissues (C) and paired healthy tissues (N) by a semi-quantitative assessment. (G) Correlation between the H score for YAP or TEAD1 and that for AARS1 in GC tissues. (H) Kaplan-Meier

799 survival curve of GC patients with AARS1/YAP (left) or AARS1/TEAD1 (right) at high or low levels from
800 tissue microarray. Unpaired Student's *t* test (**F**). Spearman rank correlation (**G**). Logrank test (**H**).
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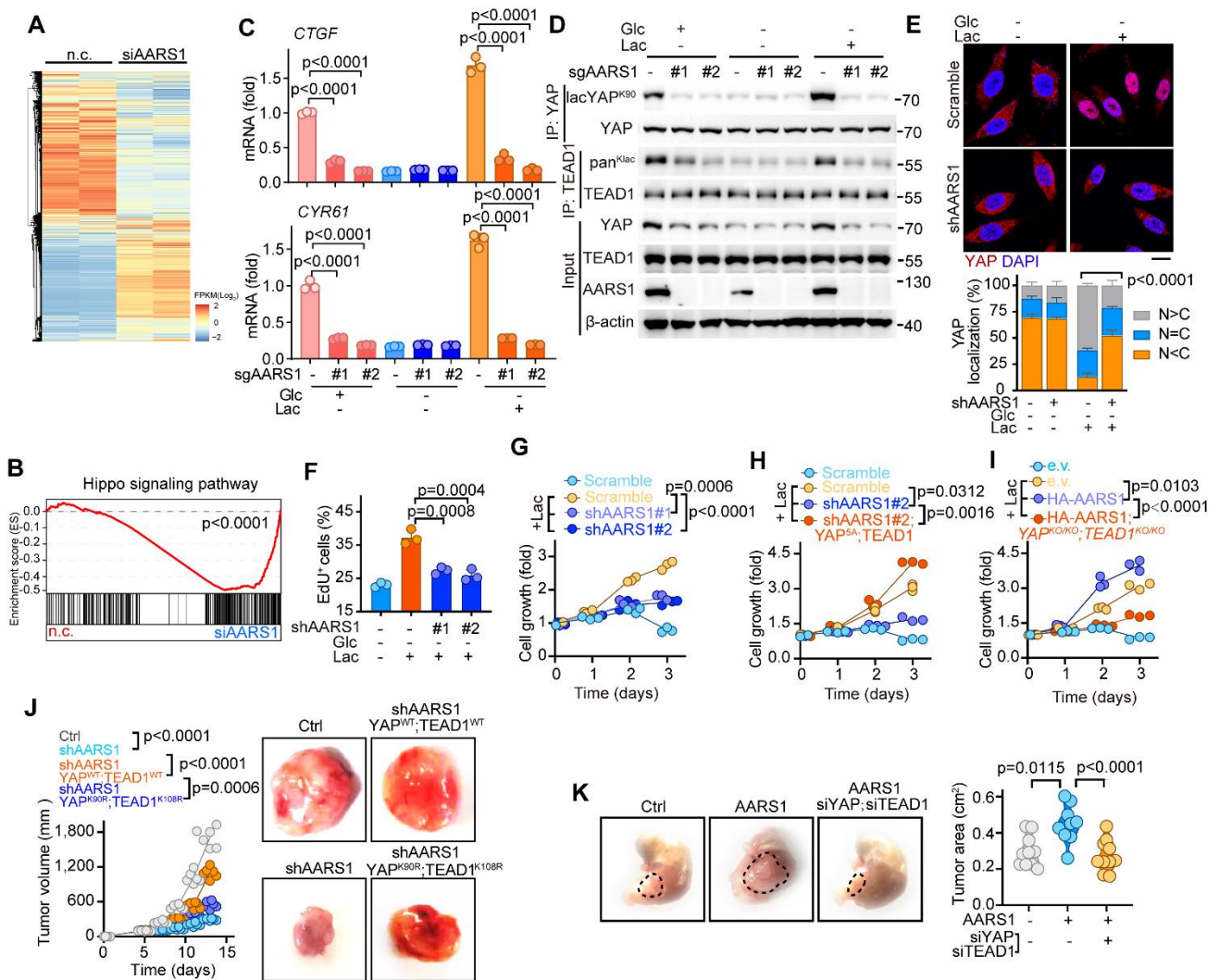
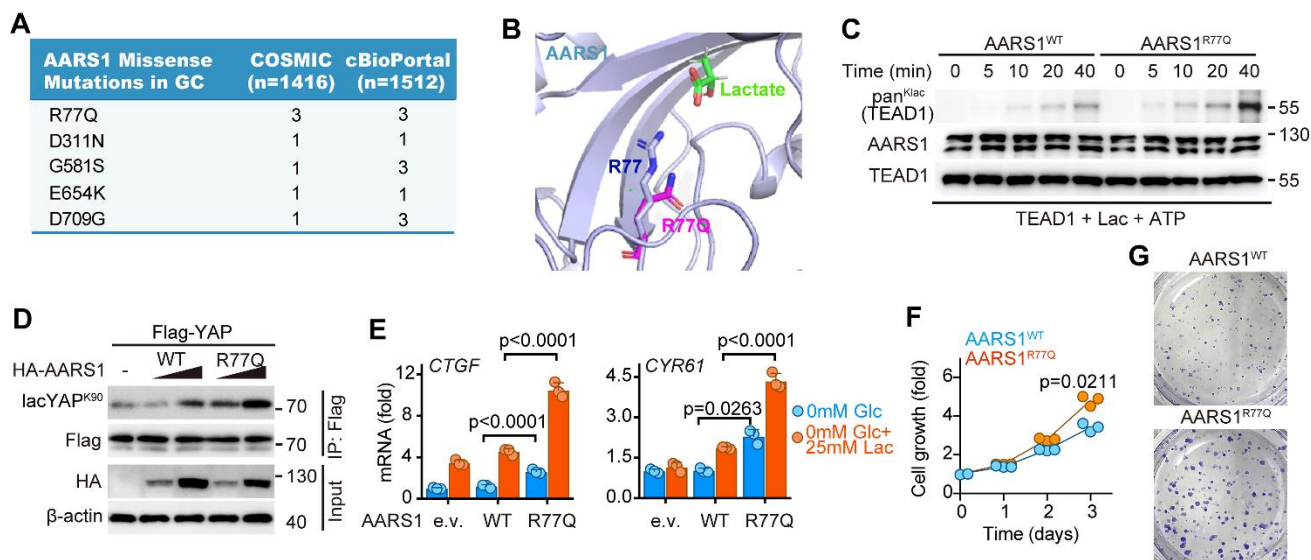


Figure 6. AARS1 promotes gastric cancer overgrowth via YAP-TEAD lactylation

(A) Heatmap of differentially expressed genes in AARS1-knockdown cells upon lactate treatment. RNA-sequencing (RNA-seq) was performed to evaluate the transcriptomics of AARS1 siRNAs-transfected HGC27 cells cultured in glucose-free medium with lactate for 12h. (B) Gene set enrichment analysis (GSEA) of Hippo signature genes in lactate-treated HGC27 cells. Normalized enrichment score (NES) and FDR are shown. (C) mRNA levels of *CTGF* and *CYR61* in AARS1-knockout cells upon lactate treatment ($n = 3$). Glc, glucose. Lac, lactate. Data are presented as mean \pm SD. (D) Lactylation levels of YAP and TEAD1 in AARS1-knockout cells upon lactate treatment. (E) Immunofluorescence images of YAP localization in scramble control and AARS1-knockdown cells upon lactate treatment for 12h (top).

812 Quantification of YAP signal intensity (bottom) ($n = 3$). N, nuclear localization. C, cytosolic localization. Data
813 are presented as mean \pm SD. Scale bar = 5 μm . **(F)** Percentage of EdU+ cells in AARS1-knockdown cells
814 treated with lactate for 12 h ($n = 3$). Data are presented as mean \pm SD. **(G)** Cell growth curves of scramble
815 control and AARS1-knockdown cells upon lactate treatment ($n = 3$). Data are presented as mean \pm SD. **(H)**
816 Rescue assay showing the cell growth of AARS1-knockdown cells after enforced expression of YAP^{5A} and
817 TEAD1 upon lactate treatment ($n = 3$). YAP^{5A}, S61A, S109A, S127A, S164A, S397A. Data are presented
818 as mean \pm SD. **(I)** Cell growth curves of HA-AARS1-overexpressing cells after YAP and TEAD1-depletion
819 upon lactate treatment ($n = 3$). Data are presented as mean \pm SD. **(J)** Xenograft murine GC model after
820 subcutaneous injection with HGC27 cells transfected with indicated plasmids. Tumor growth curves (left)
821 and representative tumor images (right) were shown ($n = 6$). **(K)** Orthotopic murine GC model after injection
822 with HGC27 cells transfected with indicated plasmids. Representative images (left) were shown. Tumor
823 area (right) were measured ($n = 10$). Unpaired Student's *t* test (**C**, **E**, **F**, and **K**). One-way ANOVA (**G-J**).
824



825

826 **Figure 7. R77Q mutation promotes AARS1 activity and GC cell growth**

827 **(A)** AARS1 mutations in GC patients from COSMIC and cBioPortal databases. **(B)** Structural view of R77,

828 R77Q and lactate in the catalytic core of AARS1. **(C)** In vitro lactylation assay to assess the catalytic

829 efficiencies of WT AARS1 and R77Q mutant AARS1. **(D)** Immunoblot analysis of YAP lactylation in

830 HEK293FT cells co-transfected with Flag-YAP and WT or R77Q mutant AARS1. **(E)** Real-time qPCR

831 analysis of *CTGF* and *CYR61* mRNA levels in HEK293FT cells co-transfected with Flag-YAP and WT

832 AARS1 or R77Q mutant AARS1 treated with or without lactate ($n = 3$). Data are presented as mean \pm SD.

833 **(F and G)** Cell growth curves (F) and colony formation assay (G) of AARS-WT- and R77Q-mutant-

834 overexpressing AGS cells ($n = 3$). Data are presented as mean \pm SD. Unpaired Student's *t* test (E). One-

835 way ANOVA (F).

836

Table 1. AARS1 Expression Correlates with Poor Prognosis of GC Patients in the Tissue Array

Groups	AARS1(IOD)		n	p value (Fisher's test)
	Increased	Non- increased		
Age (years)				
< 60	12	13	25	0.1365
>= 60	47	18	65	
Gender				
Male	43	17	60	0.1025
Female	16	14	30	
<i>Helicobacter pylori</i>				
Positive	40	12	52	0.0129*
Negative	19	19	38	
Lauren				
Intestinal	41	15	56	0.0675
Non-intestinal	18	16	34	
Differentiation				
Low	19	14	33	0.2554
Moderate or High	40	17	57	
Lymphatic invasion				
Ly0-1	14	13	27	0.0922
Ly2-3	45	18	63	
Tumor Size				
pT1 + pT2 (<= 5 cm)	3	10	13	0.0010*
pT3 + pT4 (> 5 cm)	56	21	77	
Lymph node metastasis				
N0 + N1	19	17	36	0.0442*
N2 + N3	40	14	54	
Distant metastasis				
M0	54	31	85	0.1600
M1	5	0	5	
Tumor stage				
Stage I + Stage II	19	19	38	0.0129*
Stage III + Stage IV	40	12	52	
Total	59	31	90	

838 A Fisher's exact test was used to test the association between two categorical variables. *Represents
839 statistical significance at $p < 0.05$.