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## 1           **A metabolic crosstalk between liposarcoma and muscle sustains tumor growth**

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### 13           **Abstract**

14  
15           Dedifferentiated (DD-LPS) and Well-differentiated (WD-LPS) liposarcoma are  
16 characterized by a systematic amplification of the *MDM2* oncogene. We recently demonstrated  
17 that p53-independent metabolic functions of chromatin-bound MDM2 (C-MDM2) are  
18 exacerbated in LPS and mediate an addiction to serine metabolism in order to sustain tumor  
19 growth. Here, we show that metabolic cooperation between LPS and distant muscle, which  
20 raise serine and glycine blood levels, is essential for LPS tumor growth. By releasing IL-6,  
21 tumor influence distant muscle to upregulate their serine synthesis machinery. Blocking IL-6  
22 secretion or treating LPS cells with FDA approved IL-6 inhibitor, decreased serine production  
23 and impaired tumor proliferation. These data reveal IL-6 as a central tumorkine in metabolic  
24 crosstalk between tissues and identifies IL-6 as a plausible treatment for LPS patients.

### 26           **INTRODUCTION**

27           Sarcomas, which represent about 1% of all cancers, are malignant tumors of  
28 mesenchymal origin arising from soft or bone tissues. Among the 100 different histological  
29 subtypes of sarcomas, liposarcoma (LPS) represent the second most frequent subtype after  
30 gastrointestinal stromal tumors (GIST), accounting for 15-20% of all sarcomas<sup>1</sup>. The prognosis

31 of LPS is very heterogeneous and depends on the tumor location, its histological subtype, and  
32 the grade/size of the tumor at diagnosis<sup>2-4</sup>. The risk of recurrence and metastatic dissemination  
33 of advanced LPS varies between 20 to 40% in case of localized tumors, and mainly depends  
34 on the quality of their surgical resection that remains the most efficient therapeutic strategy to  
35 date. LPS are known to be poorly responsive to classical chemotherapies and despite that new  
36 targeted therapies, such as tyrosine kinase inhibitor Pazopanib, have demonstrated efficacy  
37 in patients with several types of sarcomas, they showed no benefit to patients with LPS<sup>5</sup>. The  
38 median overall survival estimated around 15 months<sup>6,7</sup>, and the absence of treatment for  
39 metastatic or unresectable LPS reveal an urge for novel therapeutic strategy for LPS.

40 The most common LPS subtypes, the well differentiated and the dedifferentiated LPS (WD-  
41 LPS and DD-LPS, respectively), are characterized by the systematic amplification of the q13-  
42 15 region of chromosome 12. This region contains the "*murine double-minute 2*" gene (*Mdm2*),  
43 which encodes a well-characterized negative regulator of the p53 tumor suppressor. The  
44 frequency of *Mdm2* amplification is such (almost 100%) that it is currently used for routine  
45 diagnosis to distinguish WD/DD-LPS from other sarcoma subtypes that commonly harbor p53  
46 mutations<sup>8,9</sup>.

47 The *mdm2* gene was first identified as the gene involved in the spontaneous  
48 transformation of an immortalized murine cell line, BALB/c 3T3. *Mdm2* was later labeled as an  
49 oncogene participating in cell transformation. MDM2 oncoprotein is frequently overexpressed  
50 in numerous human cancers<sup>10</sup>, resulting in a loss of the tumor suppressor p53-dependent  
51 activities<sup>11</sup>. Under normal growth conditions, the p53 protein is kept at low levels by MDM2-  
52 mediated polyubiquitylation inducing its degradation by the 26S proteasome. The role of  
53 MDM2 as a major regulator of p53 stability and transcriptional activities has been widely  
54 described by *in vitro* and *in vivo* models<sup>11-13</sup>.

55 Through its E3 ligase activity, MDM2 affects the function of other cellular proteins involved in  
56 cell proliferation, DNA repair, ribosome biosynthesis, and many other processes that can also  
57 contribute to its oncogenic potential<sup>14,15</sup>. However, growing evidences suggest that MDM2 is

58 involved in a complex network of protein interactions that confer MDM2 new functions beyond  
59 its relationship with p53<sup>16–19</sup>. More recently, we started an in-depth analysis of MDM2 functions  
60 independently of p53 and demonstrated a key role in serine metabolism<sup>20</sup>.

61 We have shown that MDM2 is recruited on chromatin (C-MDM2) in a p53 independent  
62 manner and regulates an ATF-3 and ATF-4 dependent transcriptional program. Although we  
63 described that MDM2 is mainly recruited on chromatin under specific stress conditions such  
64 as, oxidative stress. Moreover, performing a screen on a large panel of cancer cell lines, we  
65 identify liposarcoma cell lines as the one which consistently and spontaneously harbor C-  
66 MDM2<sup>20</sup>. The p53-independent metabolic functions of C-MDM2 are exacerbated in LPS and  
67 mediate an addiction to serine metabolism that sustains nucleotide synthesis and tumor  
68 growth<sup>21</sup>. Treatment of LPS cells with Nutlin-3A, a pharmacological inhibitor of the MDM2-p53  
69 interaction, stabilizes p53 but unexpectedly enhances MDM2-mediated control of serine  
70 metabolism by increasing its recruitment to chromatin, likely explaining the poor clinical  
71 efficacy of this MDM2 inhibitors class<sup>22</sup>. In contrast, genetic or pharmacological inhibition of C-  
72 MDM2 by SP141, a distinct MDM2 inhibitor triggering its degradation, and interfering with *de*  
73 *novo* serine synthesis, impaired LPS growth both *in vitro* and in clinically relevant patient-  
74 derived xenograft models. Taken together, our data suggest that targeting MDM2 functions in  
75 serine metabolism represents a potential therapeutic strategy for LPS<sup>21,23</sup>.

76 The impact of cancer cells on their environment, locally and distantly, is known to  
77 promote malignancy and chemoresistance<sup>24</sup>. Understanding the interactions between cancer  
78 cell and surrounding metabolism will be critical for combining metabolism-targeted therapies  
79 with existing chemotherapies. It is known that cancer cells compete with cellular components  
80 of the microenvironment for essential nutrients, such as glucose, amino acids and lipids. For  
81 example, restricting T cell glucose metabolism causes lymphocyte exhaustion<sup>25</sup>, when high  
82 arginine levels promotes enhanced T cell survival and anti-tumor activity<sup>26, 27</sup>. The relationship  
83 between tumors and other organs is not limited to the immune system. Endothelial cells also  
84 undergo factor-induced metabolic reprogramming<sup>28</sup>. Cancer also causes alterations in whole-  
85 body metabolism that may influence how tumor access to essential resources. Acting on

86 nutrient availability through diet composition has been shown to slow cancer progression<sup>29</sup> and  
87 this field is likely to be a productive area of research in the near future.

88 In this study, we demonstrate a new concept in liposarcoma pathogenesis, related to a  
89 potential metabolic “long-distance” cooperation between normal tissues and LPS. Our data  
90 obtained in humanized patient-derived mouse models of LPS (PDX) suggests that LPS  
91 somehow “educate” normal tissues/cells to support their massive demand in serine. While  
92 monitoring LPS growth *in vivo*, we observed an increase of circulating serine and glycine  
93 levels. Interestingly, skeletal muscles of these engrafted animals were found to upregulate a  
94 transcriptional program, involved in *de novo* serine synthesis and described to be regulated by  
95 C-MDM2 in cancer cells<sup>21</sup>. We hypothesized that a metabolic cooperation between LPS cancer  
96 cells and surrounding muscle allows LPS tumors to maintain serine pools and now identify  
97 interleukin-6 (IL-6), as essential for LPS mediated muscle reprogramming. Furthermore,  
98 blocking IL-6 using an antibody, results in exogenous serine starvation in LPS cells and  
99 impaired proliferation *in vitro* and *in vivo*. As such, the exogenous serine provided by  
100 surrounding tissue, such as muscle, is critical for LPS tumor growth and reveals IL-6 as a  
101 plausible target for novel LPS treatments.

## 102 **RESULTS**

### 103 **Muscle reprogramming fuels Liposarcoma serine addiction.**

104 Recently, we classified liposarcoma as serine-dependent tumors that use both, self-  
105 serine production and serine auxotrophy <sup>21</sup>. To further investigate serine metabolism  
106 significance in liposarcoma, we looked at circulating serine and glycine level in nude mice  
107 xenografted with LPS cell lines or humanized patient-derived mouse models (PDX). PDX are  
108 patient-derived tumor xenograft models generated upon transfer of freshly resected human  
109 tumor samples of primary naive LPS into immunodeficient nude mice. Surprisingly, we  
110 observed that serine and glycine levels in the blood stream are significantly higher in mice  
111 harboring LPS tumor relative to control mice. Additionally, in response to MDM2 inhibitor  
112 treatment, which induces a drastic reduction in tumor growth <sup>21</sup>, we were able to rescue serine  
113 and glycine levels at the base line observed pre engraftment, confirming a correlation between

114 circulating levels of serine and glycine and LPS tumorigenesis (Fig. 1A-D). Given that more  
115 than 90% of the serine consumed by tumor cells comes from exogenous sources<sup>21</sup>, we  
116 anticipated that liposarcoma tumors depend on serine and glycine synthesized by surrounding  
117 tissue. To test this hypothesis, we examined the expression of genes encoding limiting  
118 enzymes of the *de novo* serine synthesis pathway (SSP), including *3-phosphoglycerate*  
119 *dehydrogenase (Phgdh)*, *phosphoserine aminotransferase 1 (Psat1)*, and *phosphoserine*  
120 *phosphatase (Psph)* in distant metabolic organs, such as liver and muscles. Interestingly,  
121 these genes were upregulated in skeletal muscles of engrafted animals (Fig. 1E). In contrast,  
122 liver, kidney, brain and adipose tissue did not exhibit such activation of SSP genes (Fig. 1F  
123 and Extended Data Fig. 1A-C). These results indicate that muscle reprogramming, and not  
124 other tissues, sustains liposarcoma need in serine and glycine amino acids. To further  
125 investigate the specificity of muscle-secreted serine on liposarcoma tumorigenesis, control  
126 mice fed with serine/glycine-deprived diet were analyzed. As expected, serine and glycine  
127 levels in blood are lower (Extended Data Fig. 1D) and because liver and kidney are known to  
128 be essential organs involved in serine/glycine homeostasis<sup>30</sup>, SSP genes were activated in  
129 these tissues but not in muscle, brain or adipose tissue (Fig. 1G, H and Extended Data Fig.  
130 1E-G) confirming the specificity of this muscle reprogramming observed in mice harboring  
131 LPS. Of note, in mice with C-MDM2 independent breast cancer PDXs, the *de novo* serine  
132 synthesis transcriptional program regulated by C-MDM2 was not activated (Fig. 1I).  
133 Collectively, these results indicate that liposarcoma tumors potentially induce skeletal muscle  
134 reprogramming to sustain serine levels required for proliferation and survival.

### 135 **Liposarcoma cells take advantage of surrounding muscle cells for growth**

136 To understand mechanisms by which liposarcoma cancer cells reprogram muscle cells, mRNA  
137 levels of SSP enzymes were assessed in co-cultures of human liposarcoma cells IB115 or  
138 IB111 and murine myoblast C2C12. It should be noted that direct interaction between human  
139 and murine cells allowed us to discriminate genes expression from the two population of cells  
140 present in the same dish using specific primers. Interestingly, we demonstrated that C2C12

141 co-cultured with LPS cells exhibit an increase of SSP genes expression compared to C2C12  
142 cultured alone or co-cultured with other cancer type, such as breast cancer (MCF7) and  
143 melanoma (A375) cell lines (Fig. 2A and Extended Data Fig. 2A-C). Moreover, because we  
144 observed a distant muscle reprogramming in mice, we suggest that liposarcoma tumors could  
145 produce factors invoking muscle metabolism rewiring. To decipher these mechanisms, we  
146 collected culture media from LPS cells; conditioned media (CM), and test the impact of CM on  
147 C2C12 SSP genes. CM from IB115 and IB111 were added to C2C12 cells for 48h and mRNA  
148 extraction was used to evaluate SSP genes response. Interestingly, CM from LPS cancer cell  
149 lines increased *Psat1*, *Phgdh* and *Psph* mRNA expression in C2C12 indicating that a factor  
150 released specifically by liposarcoma cells induces serine metabolism reprogramming in muscle  
151 cells (Fig. 2B, C). Similar results were obtained using myotubes and luciferase technology  
152 (Extended Data Fig. 2D, E). We also confirmed these results using transformed human  
153 myoblast (Myo-E7) (Extended Data Fig. 2F). To guarantee the exclusiveness of the special  
154 crosstalk between liposarcoma and muscle cells, liposarcoma cells were co-cultured with  
155 murine liver cells, BMEL, and we observed that SSP genes were not induced (Fig. 2D)  
156 Previous report from our lab demonstrated that ATF4 dependent C-MDM2 regulates the  
157 transcription of serine metabolism genes in LPS<sup>21</sup>. To assess the role of Mdm2 in our model,  
158 we targeted Mdm2 in C2C12 cells (C2C12 sh*Mdm2*). Whereas IB115 induced the 3 SSP genes  
159 in C2C12 sh*Scr*, IB115 failed to induce the same transcriptional program in sh*Mdm2*  
160 expressing C2C12 (Fig. 2E, F and Extended Data Fig. 2G, H). Depleting Mdm2 co-transcription  
161 factor, Atf4 by shRNA in C2C12 also lead to similar results (Fig. 2G, H and Extended Data Fig.  
162 2I, J) confirming the importance of Mdm2-Atf4 complex in the activation of the SSP  
163 transcriptional program LPS-mediated in C2C12. To confirm that increase serine *de novo*  
164 biosynthesis in the myoblast is associated with intercellular serine releasing, we assessed  
165 serine levels in C2C12 media. As expected, serine levels were higher in C2C12 media  
166 cocultured with IB115 compared to IB115 alone (Fig. 2I).

167           Given that liposarcoma induces muscle reprogramming, we anticipated that they  
168 depend on muscle serine production for growth. To test this hypothesis, LPS were cultured in  
169 media without serine and glycine (-Ser/Gly) and in the presence or not of C2C12 cells. We  
170 observed proliferation defect in the absence of serine and glycine relative to cells grown in full  
171 media (Complete). Interestingly, co-culturing LPS cells with C2C12 in -Ser/Gly, fully rescued  
172 LPS cells proliferation (Fig. 2J). Similar results were obtained using human myoblast  
173 (Extended Data Fig. 2K). In contrast, co-culturing LPS cells with C2C12 lacking enzymes  
174 involved in the SSP (C2C12 sh*Psat1*, sh*Phgdh*) failed to rescue IB115 proliferation (Fig. 2K  
175 and Extended Data Fig. 2L, M). Collectively, these results indicate that LPS cells require  
176 muscle cells to reach serine requirement and sustain proliferation in a MDM2 dependent  
177 manner.

### 178 **Liposarcoma-released IL-6 regulates muscle reprogramming**

179 We hypothesized that soluble factors might be released by LPS cells to initiate serine  
180 metabolism in distant muscle. To test the extent to which secreted factors from the liposarcoma  
181 cells promote muscle reprogramming, we collected LPS cells media. The analysis of this  
182 culture supernatant using a cytokines array shows that LPS cells release significant number  
183 of different cytokines including interleukin-6 (IL-6) (Fig. 3A). Interestingly, previous reports  
184 linked ATF4 with IL-6 expression in macrophages<sup>31,32</sup>. Furthermore, MDM2 has been involved  
185 in a IL-6-mediated degradation of p53<sup>33</sup>. For those reasons, we decided to concentrate our  
186 effort on IL-6. We then examined *IL-6* gene expression in liposarcoma cell lines relative to  
187 other cancer cell lines and observed higher *IL-6* mRNA levels in LPS cell lines (Fig. 3B). IL-6  
188 protein levels were also overrepresented in supernatant of liposarcoma cell lines analyzed by  
189 ELISA (Fig. 3C). In addition, to confirm the efficiency of IB115-released IL-6, we treated IL-6-  
190 dependent myeloma cell line XG-6 with IB115 CM. CM from IB115 was able to increase XG-6  
191 proliferation while CM from MCF7 not (Fig. 3D and Extended Fig. 3A). To further investigate  
192 by which mechanism IL-6 is released from LPS tumors, we performed CHIP experiments and  
193 observed ATF4 and MDM2 binding on *IL-6* promoter (Fig. 3E), suggesting that ATF4/MDM2



194 complex regulates *IL-6* transcription. Moreover, we confirmed IL-6 regulation through ATF4  
195 and MDM2 in LPS cells using shRNA technology. As expected, *IL-6* mRNA in LPS cells and  
196 excreted IL-6 protein from LPS cells were lowered upon shRNA-*ATF4* and –*MDM2* treatment  
197 (Fig. 3F, G and Extended Fig. 3B, C).

198 To determine the uncharacterized role of IL-6 on muscle reprogramming, we analyzed  
199 the level of SSP genes in C2C12 murine myoblast cultured in the presence of recombinant IL-  
200 6. Interestingly, 50 pg/ml of IL-6 were sufficient to activate serine synthesis genes expression  
201 in C2C12 (Fig. 3H) We also assess the effect of targeting the IL-6 pathway operating between  
202 LPS cells and distant muscle. Serine genes activation was not observed in C2C12 cells when  
203 cocultured with IB115 sh/*IL-6* (Fig. 3I and Extended Data Fig. 3D, E). Similar results were  
204 observed with C2C12 sh/*IL-6R* cocultured with IB115 (Fig. 3J and Extended Data Fig. 3F, G).  
205 Collectively, these results indicate that liposarcoma cells-released IL-6 promotes serine  
206 metabolism reprogramming on muscle cells.

207

208 **Targeting the IL-6/STAT3 pathway impairs serine biosynthesis activation in**  
209 **reprogrammed muscle cells**

210 Being a cytokine with numerous functions, IL-6 affects metabolism of many organs, such as  
211 white adipose tissue, liver, skeletal muscle, or pancreas<sup>34</sup>. The main approach for inhibition of  
212 IL-6-mediated signaling is currently the use of antibodies. LPS serine-deprived cell lines  
213 treated with three different IL-6 antibodies (BE8, Siltuximab, hBE8) exhibited proliferation  
214 defects reflecting the absence of IL6-mediated *de novo* serine induction in C2C12 cells (Fig.  
215 4A, B and Extended Data Fig. 4A-D). Similar results were observed with bazedoxifene (BZA),  
216 drug-targeting GP130 (IL-6R $\alpha$ ) (Fig. 4C and Extended Data Fig. 4E). Mechanistically, to  
217 activate its classic signaling pathway, IL-6 binds to its membrane-bound receptor (IL-6R $\alpha$ )  
218 inducing the JAK (Janus Kinase) activation cascade, which then serve as docking sites for  
219 proteins initiation such as, PI3K/AKT, MAPK, Ras or STAT3 signaling pathways<sup>35</sup>. Our work  
220 and other have shown a link between ATF4/MDM2<sup>20</sup> and ATF4/STAT3<sup>36</sup> suggesting that  
221 JAK/STAT3 signaling pathway could play a prominent role in mediating effects of IL-6 on  
222 muscle reprogramming. We hypothesized that our observed IL-6-dependent proliferation  
223 phenotype was at least partially mediated through STAT3 signaling axis in C2C12. STAT3  
224 phosphorylation was induced in C2C12 myoblasts stimulated by IB115 CM and correlate with  
225 accumulation of the 3 SSP enzymes (Fig.4D, E). Moreover, STAT3 inhibitors also led to serine  
226 metabolism transcriptional program and LPS proliferation defects (Fig.4F, G and Extended  
227 Data Fig. 4F). Therefore, how STAT3 is connected to serine biosynthesis will required more  
228 investigation in the future. Taken together, our data suggest that LPS-released IL-6 appears  
229 to be critical to sustain STAT3 signaling and control muscle reprogramming. Blocking LPS-  
230 muscle crosstalk through IL-6 inhibitors and/or serine restriction could offer a potential  
231 combination therapy for patients with liposarcoma.

232

233 ***IL-6 as a novel target in liposarcoma***

234 After the discovery of IL-6/STAT3/Serine axis and its role in liposarcoma carcinogenesis, we  
235 proposed that immunotherapy using IL-6 antibody could be useful to reduce liposarcoma tumor  
236 proliferation on mice and becoming the mainstay for future liposarcoma diagnosis and  
237 treatment. To evaluate the clinical relevance of our findings, we first measured circulating IL-6  
238 levels in mice harboring LPS-PDX relative to normal mice. Elisa analysis revealed a higher IL-  
239 6 blood concentration in the presence of liposarcoma (Fig. 5A). Moreover, we compared *IL-6*  
240 mRNA expression in human tumor samples from different type of cancers. As expected,  
241 liposarcoma samples exhibit elevated *IL-6* mRNA abundance compared to other cancer types  
242 (Fig. 5B). Collectively, these results suggest that IL-6 screening could be potentially used to  
243 detect liposarcoma before any clinical signs appear.

244 As well as guiding diagnostic, IL-6 could be exploited as a good target for treating liposarcoma.  
245 To assess the *in vivo* relevance of IL-6 pathway blockade, we combined Siltuximab, anti-IL-6  
246 antibody, and doxorubicin, first-line treatment for primary LPS. PDX-LPS mice generated from  
247 patient samples were treated with Siltuximab (10 mg/kg twice a week) or doxorubicin (2 mg/kg,  
248 twice a week), and tumor growth was monitored. Anti-IL-6 antibody treatment decreased tumor  
249 growth, whereas doxorubicin had no statistically significant effect (Fig. 5C and Extended Fig.  
250 5A). In contrast to doxorubicin, a 3-week treatment with Siltuximab did not result in obvious  
251 side effects in mice (data not shown). As validated by RT Q-PCR, *de novo* serine synthesis  
252 genes expression was decreased specifically in muscle tissue after Siltuximab treatment  
253 (Extended Fig. 5B, C).

254 Moreover, to evaluate the therapeutic use of our findings, suggesting that food-provided serine  
255 and IL-6-mediated blood serine elevation can promote liposarcoma tumor growth, we injected  
256 nude mice with LPS PDX and provided either a serine/glycine-free or normal diet. Then, mice  
257 were treated with anti-IL-6 leading to LPS tumor growth defects (Fig. 5D and Extended Fig.  
258 5D). Interestingly, these data support the notion that serine/glycine metabolism initiated in  
259 muscle by LPS tumors is a driving event to sustain LPS tumor growth.

260 Collaboration between clinicians of the 'Institut du Cancer de Montpellier' (ICM), allowed us to  
261 generate a clinical-biobank (BCB) regrouping a collection of, fresh tumor and blood samples  
262 from LPS and other sarcoma patients. Using this biobank, we conducted a preliminary study  
263 on a small cohort and noticed that LPS patients exhibited higher serine blood levels before  
264 surgery and decreased drastically after surgery (Fig. 5E). Of note, serine levels did not move  
265 in other sarcoma subtypes (Fig. 5F). Two patients showed early recurrence and surprisingly,  
266 when looking at their blood serine concentration profile, we noticed that serine levels did not  
267 drop as low as levels observed in other patients (Fig. 5E). It seems essential to continue this  
268 study on a larger number of patients, to do so, clinical trial is ongoing on LPS patients to  
269 measure serine levels before and after surgery with a follow up of 2 years to anticipate potential  
270 recurrence.

271 Collectively, our findings reveal that liposarcoma cells reprogram distant muscle to  
272 meet their serine requirement and maintain proliferation through a STAT3/ATF4/C-MDM2 axis  
273 (Fig. 5F). Finally, serine levels could be use as early detection marker in liposarcoma and to  
274 predict recurrence after surgery. IL-6 may also be a new therapeutic strategy that, either singly  
275 or in combination, could benefit patients with liposarcoma.

276

## 277 **DISCUSSION**

278 WD- and DD-LPS, the most frequent LPS subtypes, are poorly responsive to classical  
279 chemotherapies, and there is currently no cure available for metastatic or unresectable  
280 LPS. First line treatment for LPS is surgery and high doses of doxorubicin could be  
281 administrated, but it provides limited clinical benefit and commonly results in severe  
282 side effects (7). We recently demonstrated that in contrast to other types of sarcoma,  
283 all LPS display constitutive recruitment of C-MDM2 and are highly dependent on  
284 exogenous serine<sup>21</sup>. In mammals, serine is considered a nonessential amino acid, the  
285 majority of which is synthesized *de novo* from glycolysis. Interestingly, many tumors

286 such as triple-negative breast cancer and melanoma, up-regulate PHGDH expression  
287 leading to SSP flux increase and tumor growth *in vivo*. However, many tumors such as  
288 liposarcoma and lung cancer depend on the availability of extracellular serine  
289 suggesting that serine needs vary among cancer types. Nevertheless, liposarcoma  
290 cells consume large amounts of extracellular serine regardless of SSP activity and  
291 could affect exogenous serine availability. Counterintuitively, we observed that PDX  
292 mice with liposarcoma exhibit high level of circulating serine in blood compared to  
293 control mice fed with the same diet. We show here that surrounding muscles provide  
294 extracellular serine to sustain liposarcoma proliferation. Generating LPS-PDXs mice,  
295 which increased serum serine levels, demonstrate that *de novo* serine synthesis  
296 machinery was activated in these mice muscles. This was surprising, due to the  
297 distance between muscle and tumor, but muscle has already been described as a  
298 signaling organ releasing cytokines and metabolites during exercise. Our data reveal  
299 that conditioned media from LPS proliferating cells was sufficient to increase serine  
300 synthesis genes in muscle murine cell lines. The underlying signaling events linking  
301 LPS proliferation and muscle serine metabolism are likely to be complex but previous  
302 studies revealed that tumorkines are intimately involved in the initiation and  
303 progression of cancer, and circulating levels of many tumorkines are elevated in  
304 diverse cancer types. We found that IL-6 expression and concentration were elevated  
305 in LPS tumors compared to other cancer types tested. Using functional *in vitro* and *in*  
306 *vivo* studies, we demonstrate that LPS cells establish a metabolic cooperation with  
307 normal surrounding muscles to sustain serine requirements via IL-6 release. We  
308 observed that, using IL-6 pathways, tumor had the amazing power to reprogram distant  
309 muscle and understanding tumor biology beyond their own metabolic regulation seems  
310 to be essential to develop new therapeutic strategies. The molecular basis of increased

311 IL-6 expression is mediated through MDM2/ATF4 chromatin function involved in serine  
312 homeostasis in LPS cells. As for MDM2, we provide genetic and pharmacological  
313 evidence that targeting IL-6-associated metabolic functions represents an efficient  
314 alternative strategy for LPS. IL-6 is a cytokine that plays roles in immunity, tissue  
315 regeneration, and metabolism. Production of IL-6 contributes to the defense during  
316 infection, but dysregulation of IL-6 signaling is also involved in disease pathology. The  
317 biological role of IL-6 has been implicated in various autoimmune disease such as  
318 rheumatoid arthritis and some other acute and chronic inflammation<sup>37</sup>. In addition to its  
319 critical role in several disease, IL-6 has very recently been reported key in the  
320 pathogenesis of multicentric Castleman disease (MCD)<sup>38</sup>. These features and our data  
321 make IL-6 and IL6-R $\alpha$  attractive therapeutic targets, leading to the development of IL-  
322 6 pathway inhibitors including siltuximab, which has been granted full approval by the  
323 food and drug administration (FDA). In addition to our *in vitro* and *in vivo* experiments  
324 using siltuximab, further studies will be required to assess the efficacy and clinical utility  
325 of repurposing Siltuximab for liposarcoma therapy. Finally, our work highlights clearly  
326 the intriguing and emerging metabolic field of tumor - organ communication and may  
327 also suggest new combination therapies.

328

329

330

331 **Methods**

332 All material and methods used are described in the Extended data.

333

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343

344 **Author contributions**

345 G.M, R.R and L.K.L., designed the studies, interpreted the data, and wrote the  
346 manuscript; G.M., J.P.R., B.R.M., A.A., S.J. and M.Y.C. performed *in vitro* the  
347 experiments; L.G, G.M, L.K.L contributed to the *in vivo* experiments. N.F. provided  
348 medical expertise.

349 **Competing interests:** L.K.L., N.F., and G.M. are inventors on patent application n° 21  
350 306099.9 submitted by ICM that covers “methods for the treatment of cancer”. All other  
351 authors declare that they have no competing interests.

352 **Data and materials availability:** All the data used for this study are present in the  
353 paper or the Supplementary Materials.

354 Correspondence and requests for materials should be addressed to Laetitia K Linares.

355

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449 **EXPERIMENTAL MODEL AND SUBJECT DETAILS**

450 **Reagents.** Recombinant Human IL-6 protein, Bazedoxifène, C188-9, Stattic, Phosphatase  
451 inhibitor cocktail 2, all unlabeled amino acids, formic acid (98% LC-MS grade), acetonitrile  
452 (>99.9% LC-MS grade), and methanol (>99.9% LC-MS grade) were purchased from Sigma-  
453 Aldrich. Protease inhibitor complete EDTA-free was purchased from Roche. The MDM2  
454 inhibitor SP141 was synthesized based on the published structure<sup>1</sup>. Siltuximab (SYLVANT®)  
455 and Tocilizumab were purchased at the “ICM” pharmacy. Elsilimomab (BE8) et mAB 1339  
456 (humanized all-6) were purchased by Evitria.

457 **Cell culture.** Unless otherwise stated, cell culture reagents were purchased from Gibco  
458 (Invitrogen). Cells were kept at 37 °C in a humidified 5% CO<sub>2</sub> incubator and maintained in  
459 DMEM Glutamax supplemented with 10% FBS (Hyclone, Thermo Scientific) and 1%  
460 Penicilline/Streptavidin. For Serine and Glycine starvation proliferation experiments, cells were  
461 cultured in MEM lacking amino acids supplemented with 1% Dialysed serum (Sigma-Merck,  
462 F0392), 1% Penicillin/Streptavidin, alanine (430µM), asparagine (50 µM), aspartic acid (20  
463 µM), glutamic acid (80 µM), proline (200 µM) with (7AA medium) or without (5AA medium)  
464 serine (150 µM) and Glycine (300 µM).

465 **Construct and viral transduction.** The following constructs were used for knockdown  
466 experiments:

467 For human protein: pLKO.1\_Puro shMDM2 (Sigma-Aldrich mission clone #3377),  
468 pLKO.1\_Puro shATF4 (Sigma-Aldrich mission clone #13577), pLKO.1\_Puro shIL-6 (Sigma-  
469 Aldrich mission clones #59205, #59206, #59207).

470 For Murine protein: pLKO.1\_Puro shMDM2 (Sigma-Aldrich mission clones #302276),  
471 pLKO.1\_Puro shATF4 (Sigma-Aldrich mission clones #301646, #301731, #71724),  
472 pLKO.1\_Puro shPHGDH (Sigma-Aldrich mission clones #41624, #41627, #41625),  
473 pLKO.1\_Puro shPSAT1 (Sigma-Aldrich mission clones #120417, #120420, #120421),  
474 pLKO.1\_Puro shPSPH (Sigma-Aldrich mission clones #81493, #81494, #81497),  
475 pLKO.1\_Puro shIL6-R $\alpha$  (Sigma-Aldrich mission clones #375089, #68293, #305257).

476 Lentivirus was prepared by co-transfection of 293T cells with shRNA of interest along with  
477 packaging plasmids pMD2.G (AddGene, cat. 12259), psPAX2 (AddGene, cat. 12260) and  
478 Lipofectamine 2000 transfection reagent (Invitrogen, cat. 15338). Lentivirus-containing media  
479 was collected from plates at 48h post-transfection, filtered using a 0.45µm filter, and stored  
480 at -80°C. For viral transduction, cells were incubated with lentivirus-containing medium and  
481 8 µg/mL polybrene for 6h. Cells were allowed to recover for another 48h before selection with  
482 puromycin. All experiments were performed with cells that survived puromycin selection and  
483 displayed knockdown of MDM2, ATF4, PHGDH, PSAT1, IL6 and IL6Rα as assayed by  
484 western blot.

485 C2C12 cells were transfected using Lipofectamine 2000 to express Serine synthesis reporter  
486 gene : *Phgdh* (C2C12 Luc-*Phgdh*) or *Psat1* (C2C12 Luc-*Psat1*). Transduced myoblasts cells  
487 were selected with Hygromycine (2 µg/ml, Invitrogen) for 72 h.

488 **Serine and Glycine starvation Assay.** C2C12 cells were conditioned to produce serine by  
489 seeding them at 8K/insert (Starsted, #833930040), 5h later inserts were transferred to a 6 well-  
490 plate containing 80K of IBGFP, and incubated for 72h. IB115-GFP were seeded in DMEM  
491 10%FBS at 20K/well in 6 wells plate (Falcon #353046) after 5 hours plates and insert were  
492 washed 2 times with PBS and 5AA or 7AA medium were added to each well. LPS Cells  
493 proliferation was monitored in real time for 3 to 6 days using the Incucyte S3 Live-Cell Analysis  
494 system, whole-well module. The confluence value of each well was automatically monitored  
495 by the Incucyte system for 3-6 days and expressed as a value representing relative confluence  
496 area. Normalized Confluence was calculated by dividing the Confluence for each time point by  
497 the original Confluence. Effects of serine and Glycine deprivation on cell proliferation were  
498 confirmed by manual counting after trypan blue exclusion performed at the end of the  
499 experiments.

500 **Co-culture assay.** C2C12 and LPS-cells (IB111, IB115), were seeded in 6-well plate with a  
501 ratio 1:2: 90K C2C12, 180K LPS-cells, in 2ml DMEM medium, for 48h. Similarly, cells (ratio  
502 1:2) were cultivated separately using an insert. These experiments were also carried out using

503 the shRNA-mediated depleted cells or with addition of drugs. Cells in culture alone or co-  
504 culture with LPS-cells were treated or not with a blocking anti-IL-6 antibody (Elsilimomab  
505 (BE8), mAB 1339 (humanized BE8), Siltuximab, 10 µg/ml), an anti-IL-6 receptor (Tocilizumab  
506 (20 µg/ml)) or inhibitor (Bazedoxifène, 100nM), or STAT3 inhibitors such as C188-9 and Stattic  
507 (10nM).

508 **Conditioned media (CM) assay.** Myoblasts were seeded at 100K/well in a 6-well plate. About  
509 30h after, medium was changed and 100-200µl of LPS-cells conditioned media (quantity  
510 corresponding to an IL-6 concentration finale of 150-300 pg/ml) or Fresh media was added to  
511 each well. Cells were collected after 16 hours. IL6 recombinant experiments were done using  
512 same experimental setting but by adding 50pg/ml of Human IL-6 recombinant proteins instead  
513 of CM on C2C12 cells. All myoblast metabolic reprogramming assay data were normalized to  
514 the corresponding control samples (mean +/-SD; n=3 independent experiments) and statistical  
515 significance was evaluated using non-parametric Mann Whitney U tests.

516 **RNA extraction and quantitative PCR.** Total mRNAs were prepared using TriZol Reagent  
517 (Invitrogen). cDNAs were synthesized from 1µg of total RNA using SuperScript III Reverse  
518 Transcriptase (Invitrogen). Real-time quantitative PCRs were performed on a LightCycler 480  
519 SW 1.5 apparatus (Roche) using the Platinum Taq DNA polymerase (Invitrogen) and a SYBR  
520 Green mix containing 3 mM MgCl<sub>2</sub> and 30 µM of each dNTP using the following amplification  
521 procedure: 45 cycles of 95°C for 4 s, 60°C for 10 s, and 72°C for 15 s. The relative mRNA copy  
522 number was calculated using Ct value and normalized to at 2 or 3 housekeeping genes.  
523 Sequence of primers used for qPCR are listed in table S1.

524 **Immunoblotting.** Protein extracts were subjected to SDS-PAGE and immunoblotted with the  
525 following primary antibodies: Mouse monoclonal: TBP (Santa Cruz, sc-56795), β-tubulin  
526 (Sigma-Aldrich, T6199), MDM2 (clones 4B11, and 2A10, Cell signaling and Millipore), STAT3  
527 (Cell Signaling, 9139). Rabbit polyclonal: p-STAT3 (Cell signaling, 9145T), PHGDH (Cell  
528 Signaling, 13428), PSAT1 (Sigma, SAB2108040), PSPH (Santa Cruz, sc-365183), HSP90α

529 (Cell Signalling, 8165), TBP (Cell Signalling, 8515). The proteins of interest were then detected  
530 either by incubation with horseradish peroxidase-conjugated anti-mouse and anti-rabbit IgG  
531 (Cell Signaling) secondary antibodies and revealed using the Pierce ECL Western Blotting  
532 Substrate or the SuperSignal West Femto Maximum Sensitivity Substrate (Thermo Fisher  
533 Scientific), or 488-, 680- fluorescent Antibodies (Li-Cor). Quantification of immunoblots was  
534 performed by densitometric analysis of the corresponding bands using ImageStudio and  
535 ImageJ software.

536 **IL-6 concentration Assay.** IL-6 concentration in the supernatant of the different cancer cell  
537 lines, and mice Serum was measured by an immunoassay (*Murine or Human IL-6 Standard*  
538 *TMB ELISA Development Kit* Catalog Number: 900-T50) following the manufacturer's  
539 instructions. Cells supernatant were collected after 48h of culture, and centrifuge at 1 200 rpm.  
540 Mice serum was prepared by collecting 500 $\mu$ L to 1ml of blood from mice upon sacrifice in  
541 anticoagulant (Heparin 10 $\mu$ l/tube, 5000UI/ml). Mice blood was then centrifuged at 800g for  
542 20min without the brakes, serum was then transferred to a vial, and store frozen prior analysis.

543 **XG-6 viability assay.** The IL-6 dependent cell line XG-6 was cultured at a concentration of  
544  $1,5 \times 10^5$  cells/ml in RPMI medium in presence of recombinant IL-6 (control, 2ng/ml) or  
545 supernatant from two different cell lines (MCF7 breast cancer cells or IB115 liposarcoma cells)  
546 for 72hours. The quantity of XG-6 cells (AU) was measured by manual counting after trypan  
547 blue exclusion and CellTiter-Glo® Luminescent Cell Viability Assay (Promega, # G7570)

548 **ChIP experiment assay.** Cells were collected and processed as previously described by  
549 Riscal et al. (2016). The Immunoprecipitation was performed using an anti-MDM2 (Santa  
550 Cruz, sc-813), anti-ATF4 (Cell signaling, 11815S) or a polyclonal rabbit IgG (Cell Signaling,  
551 2729S) that serve as a control. Samples were than analyzed by qPCR using primers specific  
552 to IL-6 and TRIB3 (table S2).

553 **HPLC.** Mice and patients serum samples was prepared directly upon collection as follow:  
554 Their blood were collected in anticoagulant (Heparin, EDTA coated collection tubes), then

555 centrifuged at 800g for 20min, without the brakes. 30% acid salicylic was added to the samples  
556 and centrifuged again, supernatant was transferred to a vial, and store frozen prior analysis.  
557 The measurement was performed using an Agilent HPLC and data were analyzed using R  
558 studio.

559 ***In vivo* Experiments.** Mice liposarcoma PDX models were established in collaboration with  
560 the surgical and pathology departments of the “Institut du Cancer de Montpellier” (ICM) by  
561 inserting a human tumor fragment of approximately 40 mm<sup>3</sup> subcutaneously on 8-week-old  
562 Nude mice (Charles River). Volumetric measurements of xenografted tumors were performed  
563 every 3 days by the same person using a manual caliper (volume = length × width<sup>2</sup>/2). All  
564 animals were euthanized when the first animal reached the ethical endpoint (volume = 1000  
565 cm<sup>3</sup> or ulceration). During our experiments mice were either fed with control diet (called Amino  
566 Acid diet; TD 99366 Harlan ENVIGO) or a test diet (Harlan Envigo, TD 130775: diet lacking  
567 serine and glycine) during 4 weeks. The diets had equal caloric value (3.9 kCal/g), an equal  
568 amount of total amino acids (179.6 g/kg) and are in a form of kibbles for mice. Total food intake  
569 was controlled to be identical in all experimental groups. Mice were housed in a pathogen-free  
570 barrier facility in accordance with the regional ethics committee for animal warfare (n°CEEA-  
571 LR-12067). Anti-IL-6 antibody (BE8 and Siltuximab), SP141 and doxorubicin were  
572 administered by IP injection at the dose of 10mg/kg twice a week for 3 weeks, 40mg/kg weekly  
573 and 2mg/kg weekly respectively.

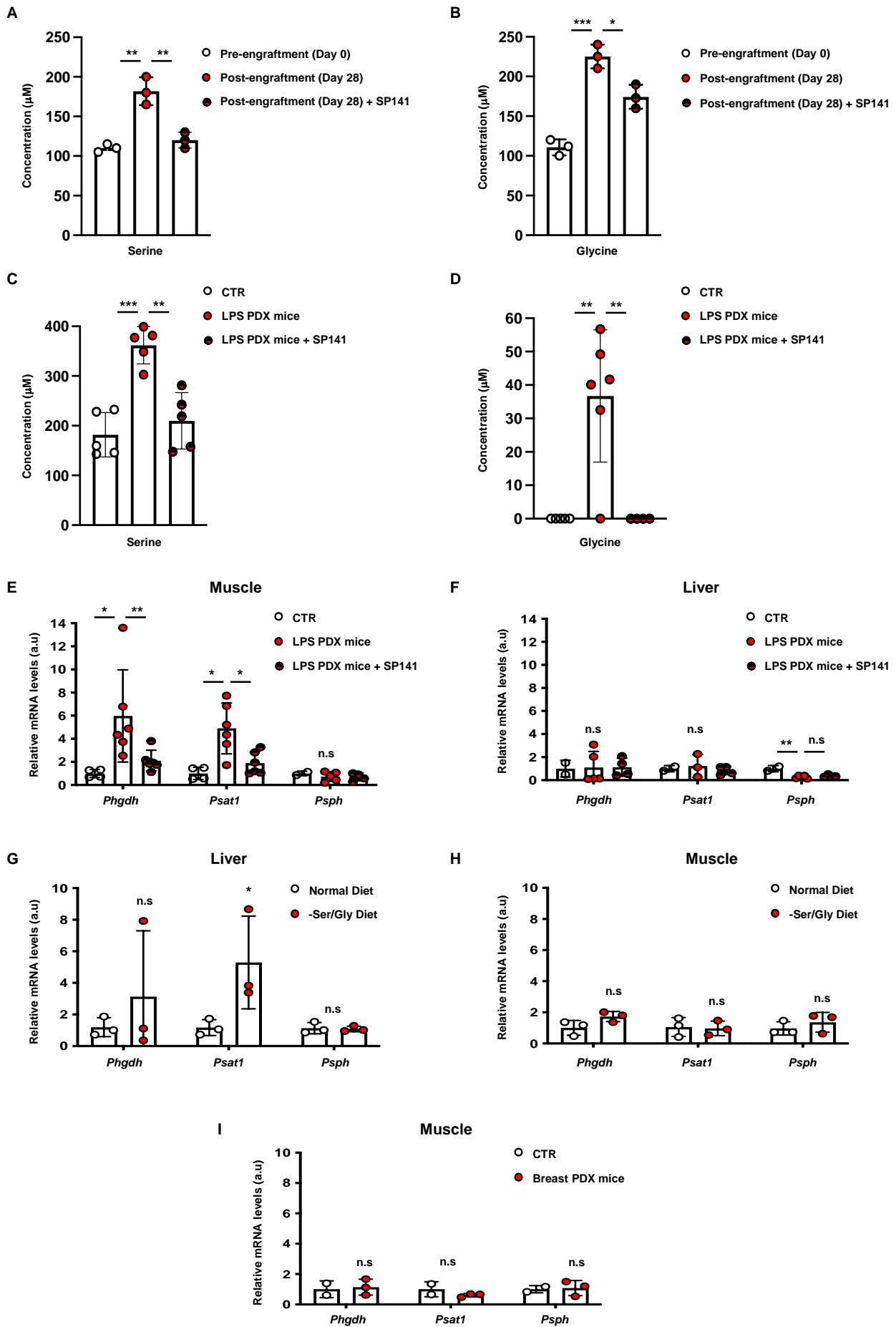
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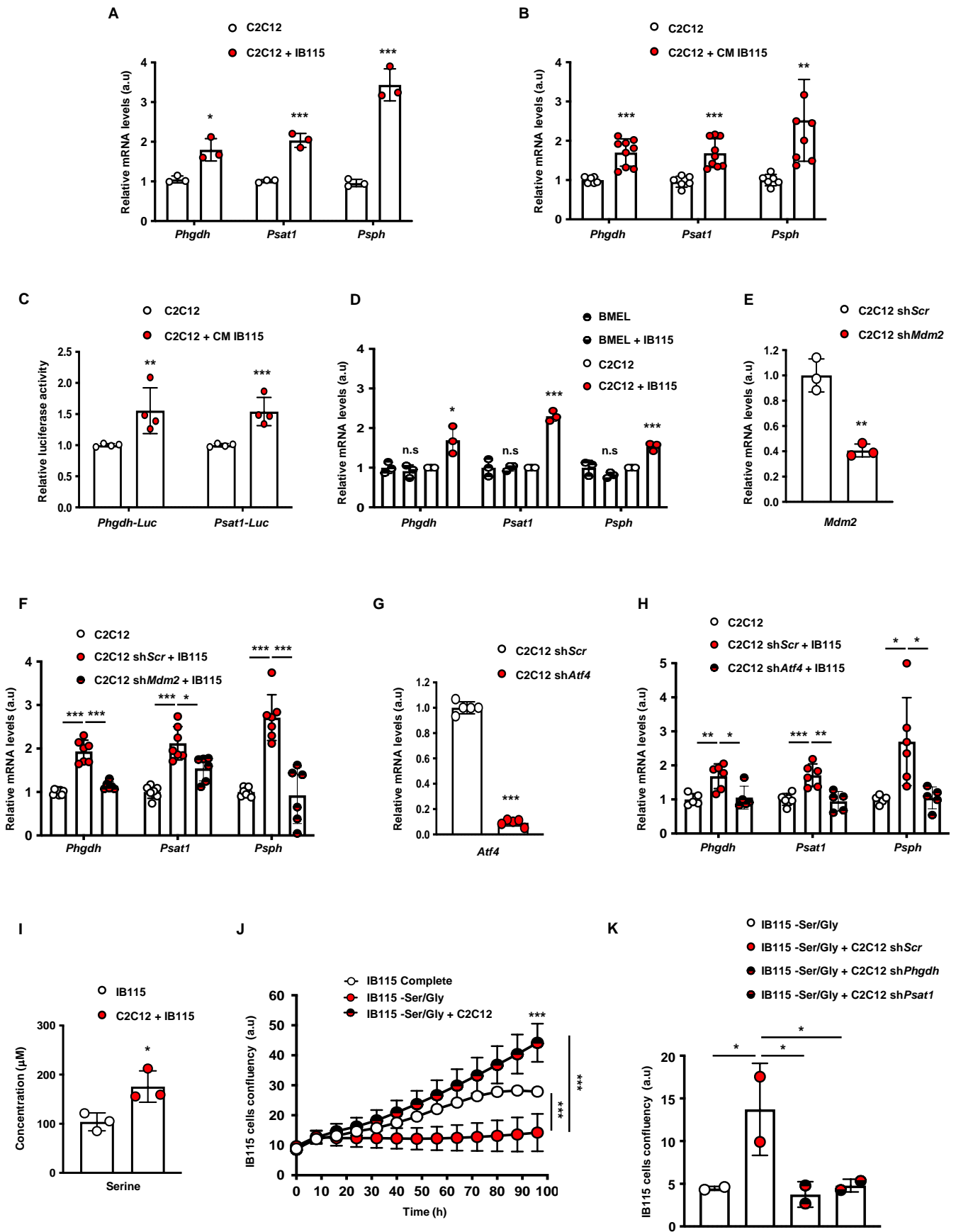
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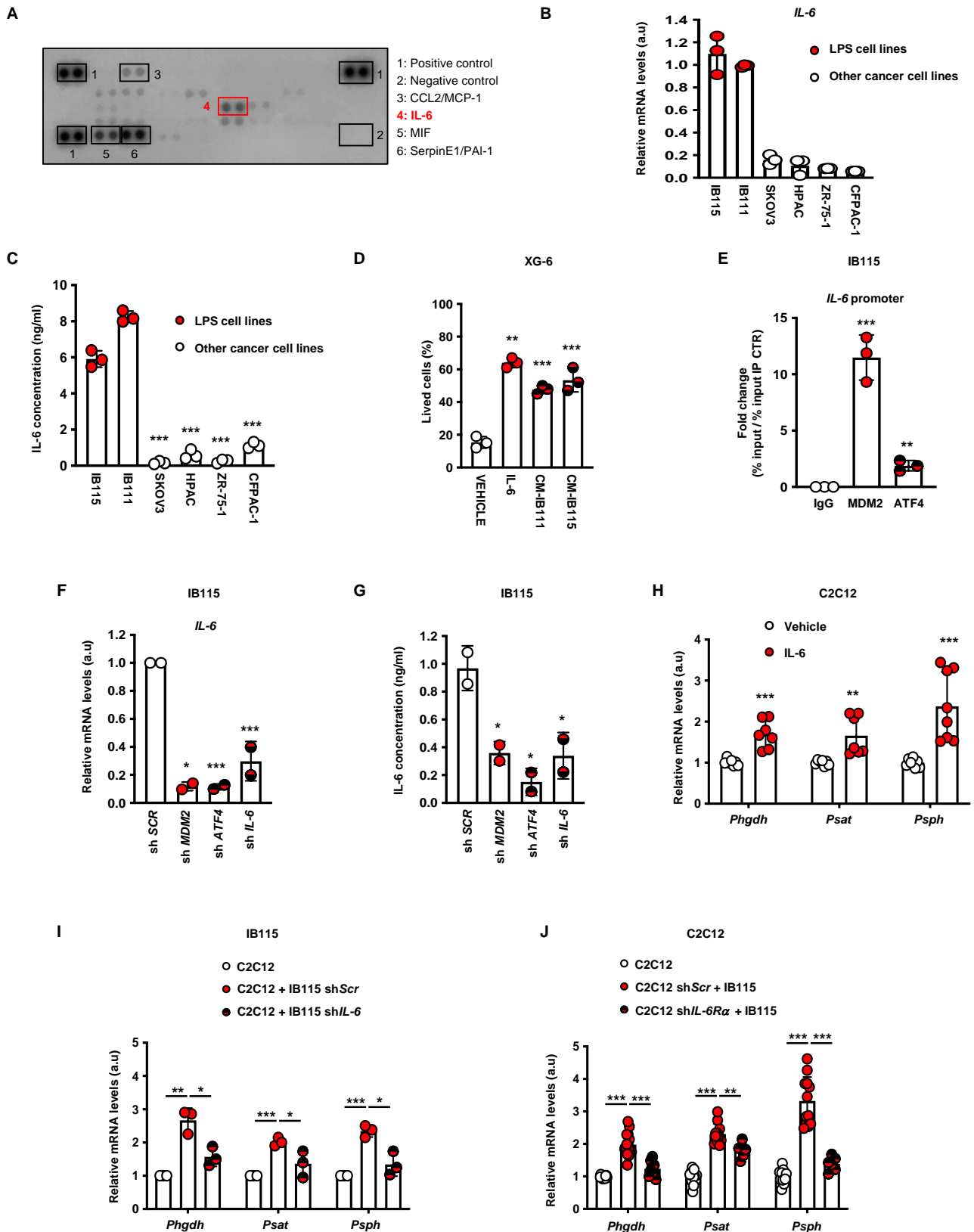




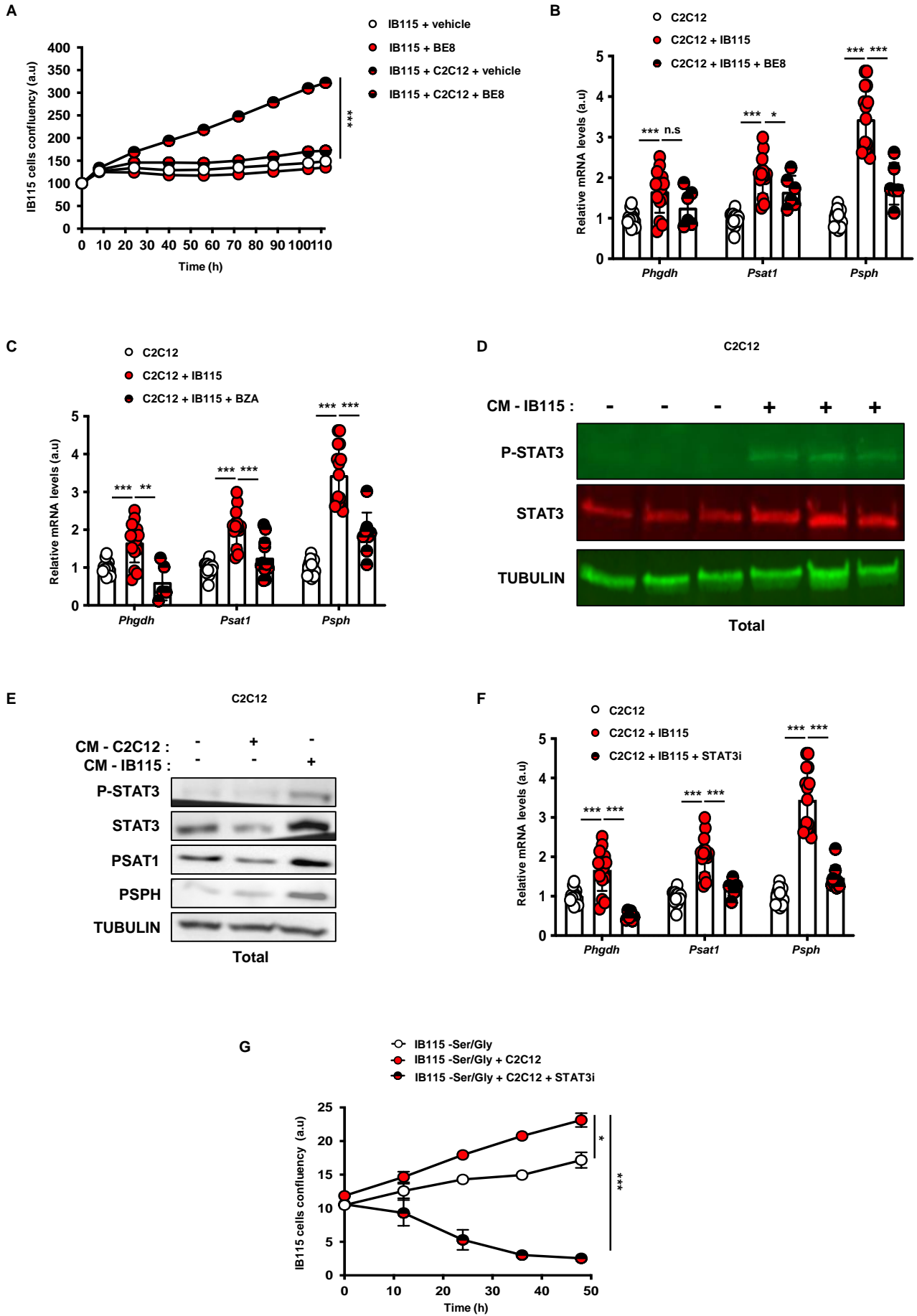
**A**, Serine and **B**, Glycine levels ( $\mu\text{M}$ ) measured by HPLC, in nude mice before and 28 days post-engraftment of liposarcoma cells (IB115). Mice were treated daily with placebo or SP141 (40mg/ml). **C**, Serine and **D**, Glycine levels ( $\mu\text{M}$ ) measured by HPLC. Control mice were compared to liposarcoma PDX Mice (LPS-PDX). Mice were treated daily with placebo or SP141 (40mg/ml). **E**, Real-time qPCR analysis performed on LPS-PDX and control mice muscle, evaluating expression of serine synthesis pathway genes: *Phgdh*, *Psat1* and *Psph*. Mice were treated daily with placebo or SP141 (40mg/ml). **F**, Real-time qPCR analysis performed on LPS-PDX and control mice liver, evaluating expression of serine synthesis pathway genes: *Phgdh*, *Psat1* and *Psph*. Mice were treated daily with placebo or SP141 (40mg/ml). **G**, Real-time qPCR analysis performed on control mice liver, evaluating expression of serine synthesis pathway genes: *Phgdh*, *Psat1* and *Psph*. Mice were fed with normal or -Ser/Gly diet. **H**, Real-time qPCR analysis performed on control mice muscle, evaluating expression of serine synthesis pathway genes: *Phgdh*, *Psat1* and *Psph*. Mice were fed with normal or -Ser/Gly diet. **I**, Real-time qPCR analysis performed on Breast-PDX and control mice muscle, evaluating expression of serine synthesis pathway genes: *Phgdh*, *Psat1* and *Psph*. (All experiments were performed in at least triplicates and statistical analysis was applied with \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , n.s.=non-significant).



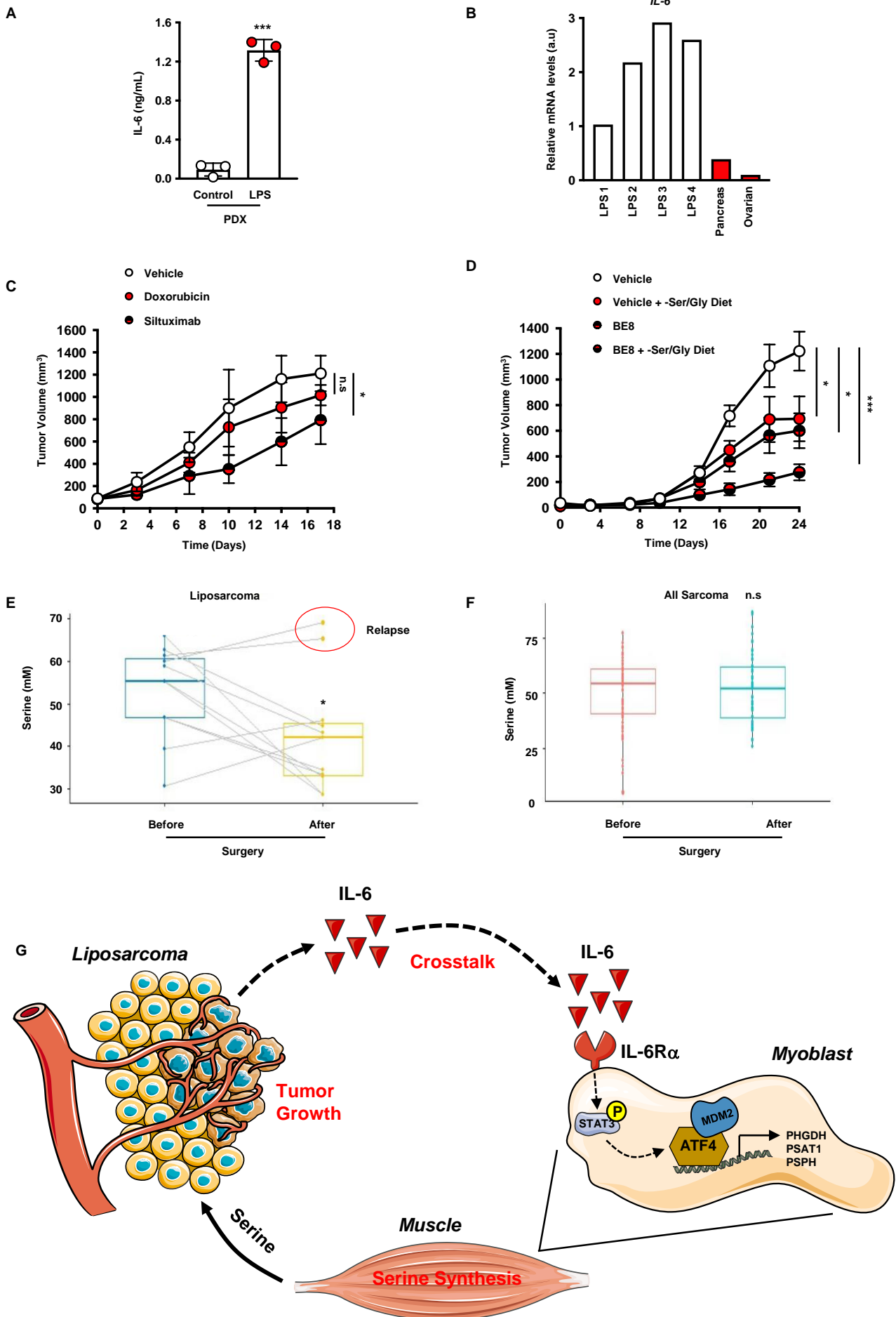
**A**, Real-time qPCR analysis performed on C2C12 cocultured with IB115 cells (ratio 1:2), evaluating expression of serine synthesis pathway genes: *Phgdh*, *Psat1* and *Psph*. **B**, Real-time qPCR analysis performed on C2C12 cells incubated 16h with IB115 conditioned media, evaluating relative expression of serine synthesis pathway genes: *Phgdh*, *Psat1* and *Psph*. **C**, Luciferase assay performed on C2C12 cells incubated 16h with IB115 conditioned media, evaluating relative luciferase activity of *Phgdh* and *Psat1* reporter. **D**, Real-time qPCR analysis performed on C2C12 or BMEL murine cells cocultured with IB115 cells (ratio 1:2), evaluating expression of serine synthesis pathway genes: *Phgdh*, *Psat1* and *Psph*. **E**, Real-time qPCR analysis of *Mdm2* mRNA level in C2C12 cells after shScr or shMdm2 lentiviral infection, puromycin selection (48h, 2 $\mu$ g/mL). **F**, Real-time qPCR analysis performed on C2C12 cells after shScr or shMdm2 cocultured with IB115 cells (ratio 1:2), evaluating expression of serine synthesis pathway genes: *Phgdh*, *Psat1* and *Psph*. **G**, Real-time qPCR analysis of *Atf4* mRNA level in C2C12 cells after shScr or shAtf4 lentiviral infection, puromycin selection (48h, 2 $\mu$ g/mL). **H**, Real-time qPCR analysis performed on C2C12 cells after shScr or shAtf4 cocultured with IB115 cells (ratio 1:2), evaluating expression of serine synthesis pathway genes: *Phgdh*, *Psat1* and *Psph*. **I**, Serine levels ( $\mu$ M) measured by HPLC in IB115 alone or cocultured with C2C12 cells (ratio 1:2). **J**, Proliferation assay performed on IB115 cells grown in media supplemented with or without Serine and Glycine and cocultured with C2C12 cells. **K**, End point of proliferation assay performed on IB115 cells grown in media without Serine and Glycine and cocultured with C2C12 cells after shScr, shPhgdh or shPsat1 cells. (All experiments were performed in at least triplicates and statistical analysis was applied with \*= $P < 0.05$ , \*\*= $P < 0.01$ , \*\*\*= $P < 0.001$ , n.s.=non-significant).



**A**, Proteome Profiler Human Cytokine Array. **B**, Real-time qPCR analysis of *IL-6* mRNA level of different cancer cell lines, SKOV3, HPAC, ZR-75.1, CFPAC-1, including LPS cell lines, IB115 and IB111. **C**, IL6 content (ng/ml), measured by Elisa, of different cancer cell lines, SKOV3, HPAC, ZR-75.1, CFPAC-1, including LPS cell lines, IB115 and IB111. **D**, End point of proliferation assay performed on XG-6 cells grown in media supplemented with vehicle, recombinant IL-6, IB115 or IB111 conditioned media. **E**, Chromatin immunoprecipitation-qPCR experiment performed in IB115 cells. Results are represented as the relative ratio between the mean value of immunoprecipitated chromatin (calculated as a percentage of the input) with the indicated antibodies. **F**, Real-time qPCR analysis of *IL-6* mRNA level in IB115 cells after shScr, shMDM2, shATF4 or shIL-6 lentiviral infection, puromycin selection (48h, 2 $\mu$ g/mL). **G**, IL6 content (ng/ml), measured by Elisa, of IB115 cells after shScr, shMDM2, shATF4 or shIL-6 lentiviral infection, puromycin selection (48h, 2 $\mu$ g/mL). **H**, Real-time qPCR analysis performed on C2C12 cells grown in media supplemented with vehicle or recombinant IL6, evaluating expression of serine synthesis pathway genes: *Phgdh*, *Psat1* and *Psph*. **I**, Real-time qPCR analysis performed on IB115 cells after shScr or shIL-6 cocultured with C2C12 cells (ratio 1:2), evaluating expression of serine synthesis pathway genes: *Phgdh*, *Psat1* and *Psph*. **J**, Real-time qPCR analysis performed on C2C12 cells after shScr or shIL-6Ra cocultured with IB115 cells (ratio 1:2), evaluating expression of serine synthesis pathway genes: *Phgdh*, *Psat1* and *Psph*. (All experiments were performed in at least triplicates and statistical analysis was applied with \* $P$ <0.05, \*\* $P$ <0.01, \*\*\* $P$ <0.001, n.s.=non-significant).

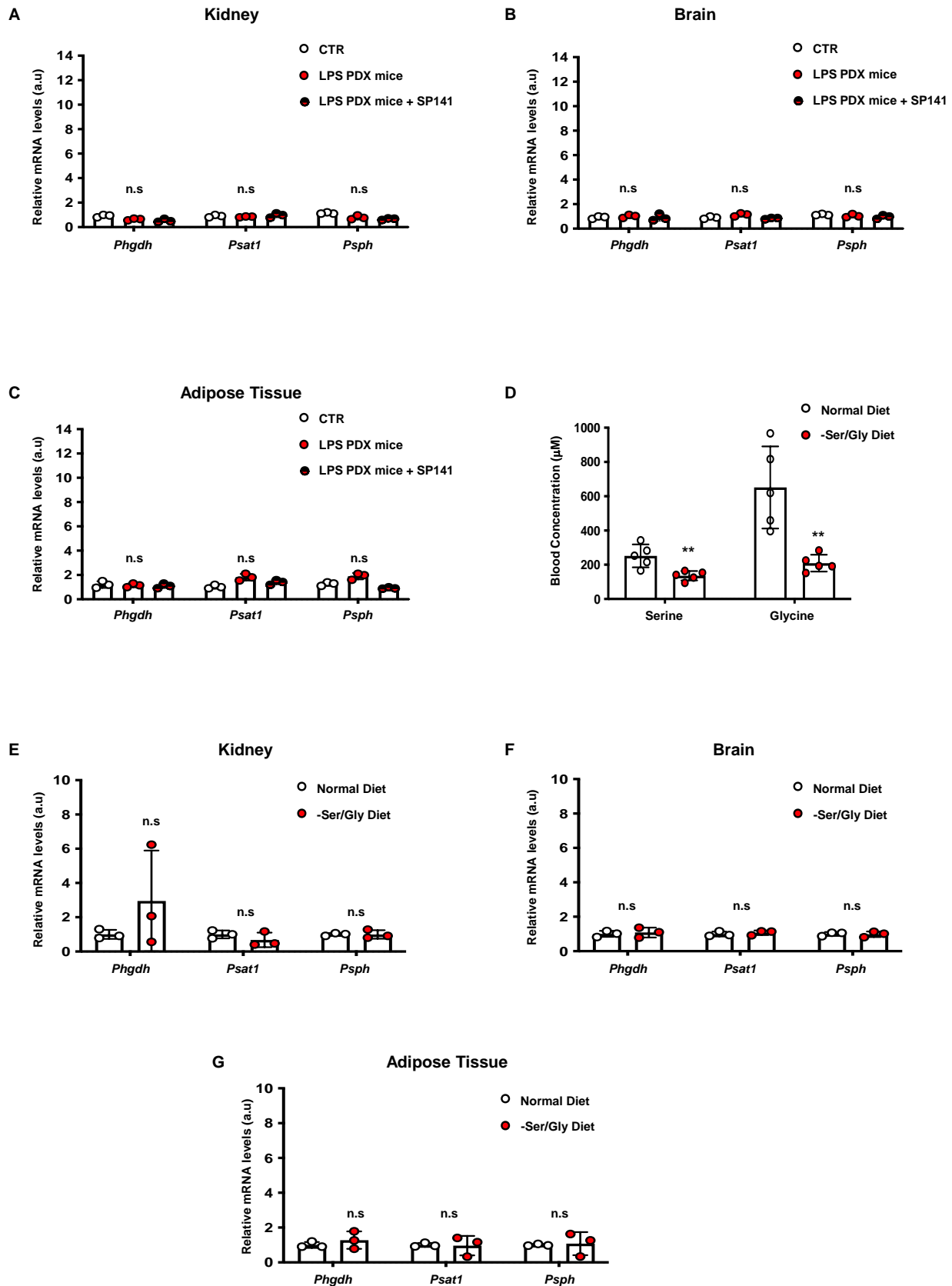


**A**, Proliferation assay performed on IB115 cells alone or cocultured with C2C12 cells grown in media without Serine and Glycine and supplemented with vehicle or anti IL-6 (BE8). **B**, Real-time qPCR analysis performed on C2C12 cells alone or cocultured with IB115 cells grown in media supplemented with vehicle or anti IL-6 (BE8), evaluating expression of serine synthesis pathway genes: *Phgdh*, *Psat1* and *PspH*. **C**, Real-time qPCR analysis performed on C2C12 cells alone or cocultured with IB115 cells grown in media supplemented with vehicle or GP130 inhibitor (BZA), evaluating expression of serine synthesis pathway genes: *Phgdh*, *Psat1* and *PspH*. **D**, STAT3 and P-STAT3 protein expression assessed by fluo-immunoblots in C2C12 cells incubated 16h with IB115 conditioned media. TUBULIN was used as the loading control. **E**, STAT3, P-STAT3, PSAT1 and PSPH protein expression assessed by immunoblots in C2C12 cells incubated 16h with IB115 or C2C12 conditioned media. TUBULIN was used as the loading control. **F**, Real-time qPCR analysis performed on C2C12 cells alone or cocultured with IB115 cells (ratio 1:2) grown in media supplemented with vehicle or STAT3 inhibitor, evaluating expression of serine synthesis pathway genes: *Phgdh*, *Psat1* and *PspH*. **G**, Proliferation assay performed on IB115 cells alone or cocultured with C2C12 cells grown in media without Serine and Glycine and supplemented with vehicle or STAT3i. (All experiments were performed in at least triplicates and statistical analysis was applied with \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ , n.s.=non-significant).

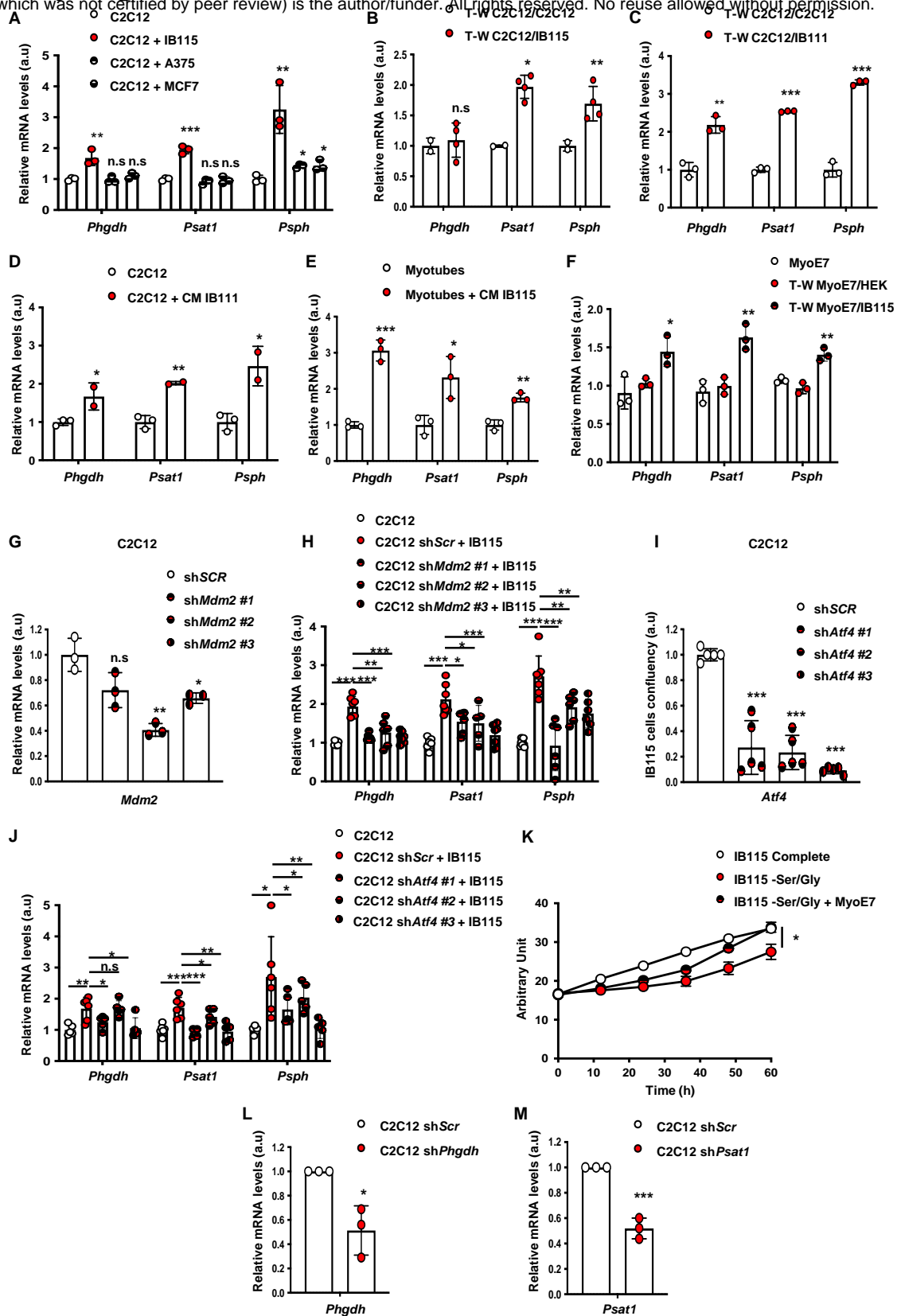


**A**, IL6 content (ng/ml), measured by Elisa. Control mice were compared to liposarcoma PDX Mice (LPS-PDX). **B**, Real-time qPCR analysis of *IL-6* mRNA level in frozen patient samples from liposarcoma, pancreas and ovarian cancer. **C**, Tumor growth curves from patient liposarcoma tumor subcutaneously implanted in nude mice treated or not with Siltuximab (10 mg/kg) or doxorubicine (2mg/kg) by IP weekly after tumor volume reached approximately 150mm<sup>3</sup>. Tumor volume was assessed at the indicated timepoints using caliper measurements (n=6 mice per group). **D**, Tumor growth curves from patient liposarcoma tumor subcutaneously implanted in nude mice, fed a normal or a no Serine/Glycine diet and treated or not with anti-IL-6, BE8 (10 mg/kg) by IP weekly after tumor volume reached approximately 150mm<sup>3</sup>. Tumor volume was assessed at the indicated timepoints using caliper measurements (n=5 mice per group). **E** and **F**, Quantification of serum Serine levels (mM) from sarcoma patients before and 30 days after surgery, using liquid chromatography-high resolution mass spectrometry (LC/HRMS). **G**, Schematic representing crosstalk between liposarcoma tumors and surrounding muscles initiated through IL-6/STAT3 pathway activation. (All experiments were performed in at least triplicates and statistical analysis was applied with \*= $P < 0.05$ , \*\*= $P < 0.01$ , \*\*\*= $P < 0.001$ , n.s.=non-significant).

# Supplementary Figure 1

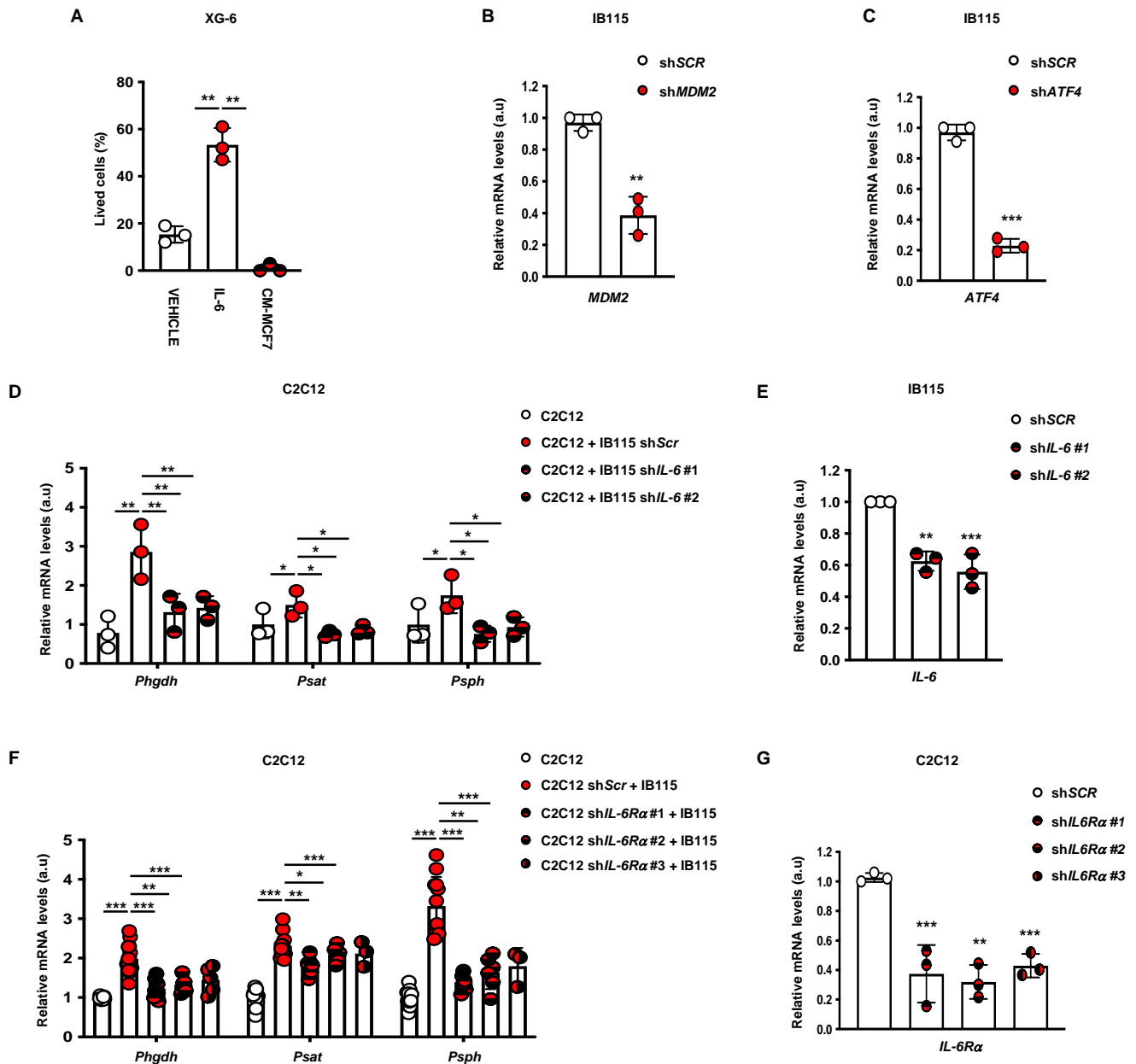


**Supplementary Figure 1:** **A**, Real-time qPCR analysis performed on LPS-PDX and control mice kidney, evaluating expression of serine synthesis pathway genes: *Phgdh*, *Psat1* and *Psph*. Mice were treated daily with placebo or SP141 (40mg/ml). **B**, Real-time qPCR analysis performed on LPS-PDX and control mice brain, evaluating expression of serine synthesis pathway genes: *Phgdh*, *Psat1* and *Psph*. Mice were treated daily with placebo or SP141 (40mg/ml). **C**, Real-time qPCR analysis performed on LPS-PDX and control mice adipose tissue, evaluating expression of serine synthesis pathway genes: *Phgdh*, *Psat1* and *Psph*. Mice were treated daily with placebo or SP141 (40mg/ml). **D**, Serum Serine and Glycine levels ( $\mu\text{M}$ ) measured by HPLC. Mice were fed with normal or -Ser/Gly diet. **E**, Real-time qPCR analysis performed on control mice kidney, evaluating expression of serine synthesis pathway genes: *Phgdh*, *Psat1* and *Psph*. Mice were fed with normal or -Ser/Gly diet. **F**, Real-time qPCR analysis performed on control mice brain, evaluating expression of serine synthesis pathway genes: *Phgdh*, *Psat1* and *Psph*. Mice were fed with normal or -Ser/Gly diet. **G**, Real-time qPCR analysis performed on control mice adipose tissue, evaluating expression of serine synthesis pathway genes: *Phgdh*, *Psat1* and *Psph*. Mice were fed with normal or -Ser/Gly diet. (All experiments were performed in at least triplicates and statistical analysis was applied with  $*=P<0.05$ ,  $**=P<0.01$ ,  $***=<0.001$ , n.s=non-significant).



**Supplementary Figure 2:** **A**, Real-time qPCR analysis performed on C2C12 cocultured with IB115, A375 and MCF7 cells (ratio 1:2), evaluating expression of serine synthesis pathway genes: *Phgdh*, *Psat1* and *PspH*. **B**, Real-time qPCR analysis performed on C2C12 cocultured in transwell for 48h with IB115 cells, evaluating expression of serine synthesis pathway genes: *Phgdh*, *Psat1* and *PspH*. **C**, Real-time qPCR analysis performed on C2C12 cocultured in transwell for 48h with IB111 cells, evaluating expression of serine synthesis pathway genes: *Phgdh*, *Psat1* and *PspH*. **D**, Real-time qPCR analysis performed on C2C12 differentiated in myotubes incubated 16h with IB115 conditioned media, evaluating expression of serine synthesis pathway genes: *Phgdh*, *Psat1* and *PspH*. **E**, Luciferase assay performed on C2C12 cells incubated 16h with IB115 conditioned media, evaluating relative luciferase activity of *Phgdh* and *Psat1* reporter. **F**, Real-time qPCR analysis performed on human myoblasts MyoE7 cocultured in transwell for 48h with IB115 or HEK cells, evaluating expression of serine synthesis pathway genes: *Phgdh*, *Psat1* and *PspH*. **G**, Real-time qPCR analysis of *Mdm2* mRNA level in C2C12 cells after shScr or 3 different sh*Mdm2* lentiviral infection, puromycin selection (48h, 2μg/mL). **H**, Real-time qPCR analysis performed on C2C12 cells after shScr or 3 different sh*Mdm2* cocultured with IB115 cells (ratio 1:2), evaluating expression of serine synthesis pathway genes: *Phgdh*, *Psat1* and *PspH*. **I**, Real-time qPCR analysis of *Atf4* mRNA level in C2C12 cells after shScr or 3 different sh*Atf4* lentiviral infection, puromycin selection (48h, 2mg/mL). **J**, Real-time qPCR analysis performed on C2C12 cells after shScr or 3 different sh*Atf4* cocultured with IB115 cells (ratio 1:2), evaluating expression of serine synthesis pathway genes: *Phgdh*, *Psat1* and *PspH*. **K**, Proliferation assay performed on IB115 cells grown in media supplemented with or without Serine and Glycine and cocultured with human myoblasts MyoE7. **L**, Real-time qPCR analysis of *Phgdh* mRNA level in C2C12 cells after shScr or sh*Phgdh* lentiviral infection, puromycin selection (48h, 2mg/mL). **M**, Real-time qPCR analysis of *Psat1* mRNA level in C2C12 cells after shScr or sh*Psat1* lentiviral infection, puromycin selection (48h, 2mg/mL). (All experiments were performed in at least triplicates and statistical analysis was applied with \*= $P < 0.05$ , \*\*= $P < 0.01$ , \*\*\*= $P < 0.001$ , n.s.=non-significant).

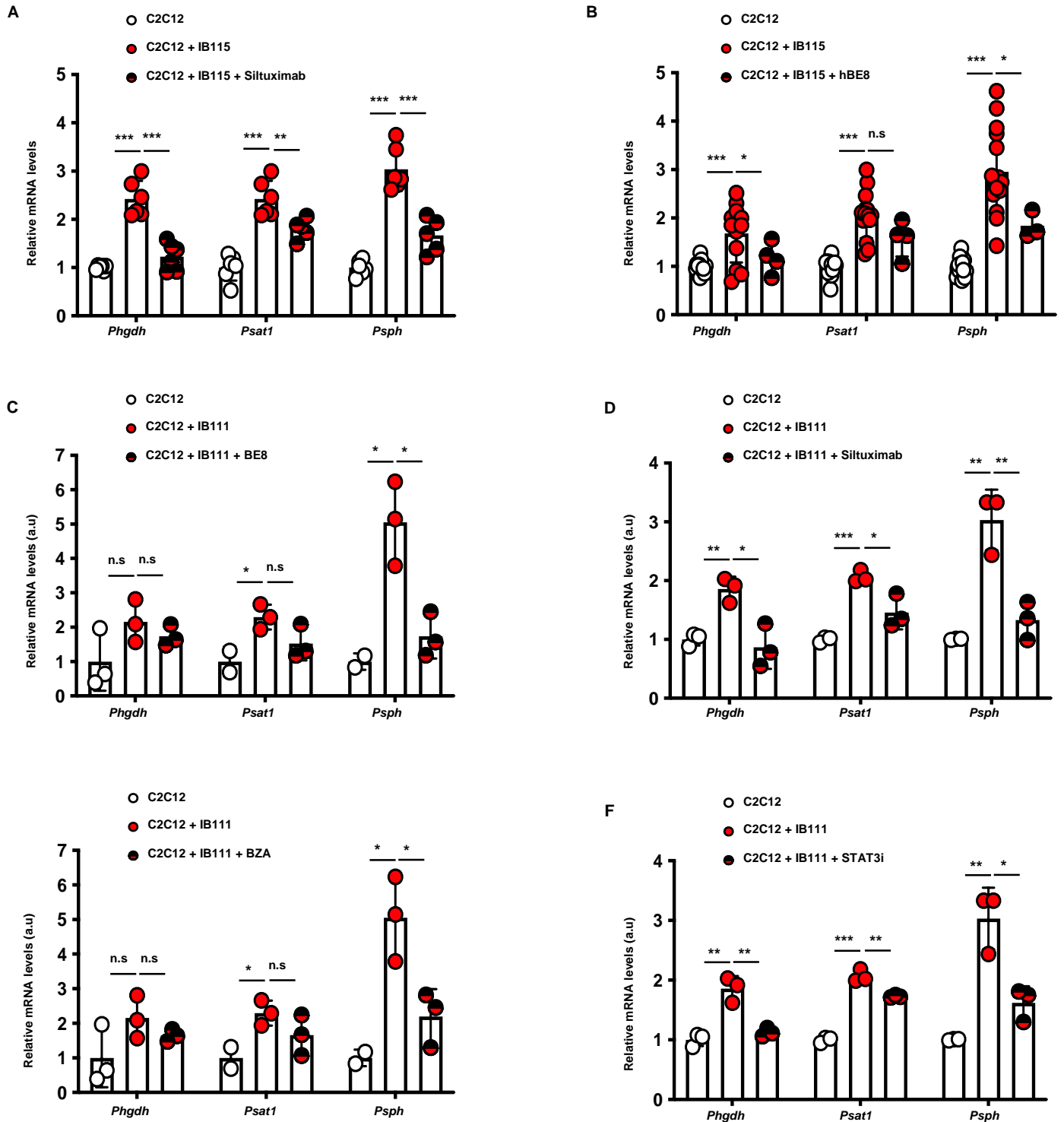
# Supplementary Figure 3



**Supplementary Figure 3:** **A**, End point of proliferation assay performed on XG-6 cells grown in media supplemented with vehicle, recombinant IL-6 or MCF7 conditioned media. **B**, Real-time qPCR analysis of *MDM2* mRNA level in IB115 cells after shSCR or shMDM2 lentiviral infection, puromycin selection (48h, 2 $\mu$ g/mL). **C**, Real-time qPCR analysis of *ATF4* mRNA level in IB115 cells after shSCR or shATF4 lentiviral infection, puromycin selection (48h, 2 $\mu$ g/mL). **D**, Real-time qPCR analysis performed on C2C12 cells cocultured with IB115 cells after shSCR or 2 different shIL-6 (ratio 1:2), evaluating expression of serine synthesis pathway genes: *Phgdh*, *Psat1* and *Psph*. **E**, Real-time qPCR analysis of *IL-6* mRNA level in IB115 cells after shScr or 2 different shIL-6 lentiviral infection, puromycin selection (48h, 2 $\mu$ g/mL). **F**, Real-time qPCR analysis performed on C2C12 cells after shSCR or 3 different shIL-6R $\alpha$  cocultured with IB115 cells (ratio 1:2), evaluating expression of serine synthesis pathway genes: *Phgdh*, *Psat1* and *Psph*. **G**, Real-time qPCR analysis of *IL-6R $\alpha$*  mRNA level in C2C12 cells after shScr or 3 different shIL-6R $\alpha$  lentiviral infection, puromycin selection (48h, 2 $\mu$ g/mL). (All experiments were performed in at least triplicates and statistical analysis was applied with \*= $P$ <0.05, \*\*= $P$ <0.01, \*\*\*= $P$ <0.001, n.s.=non-significant).

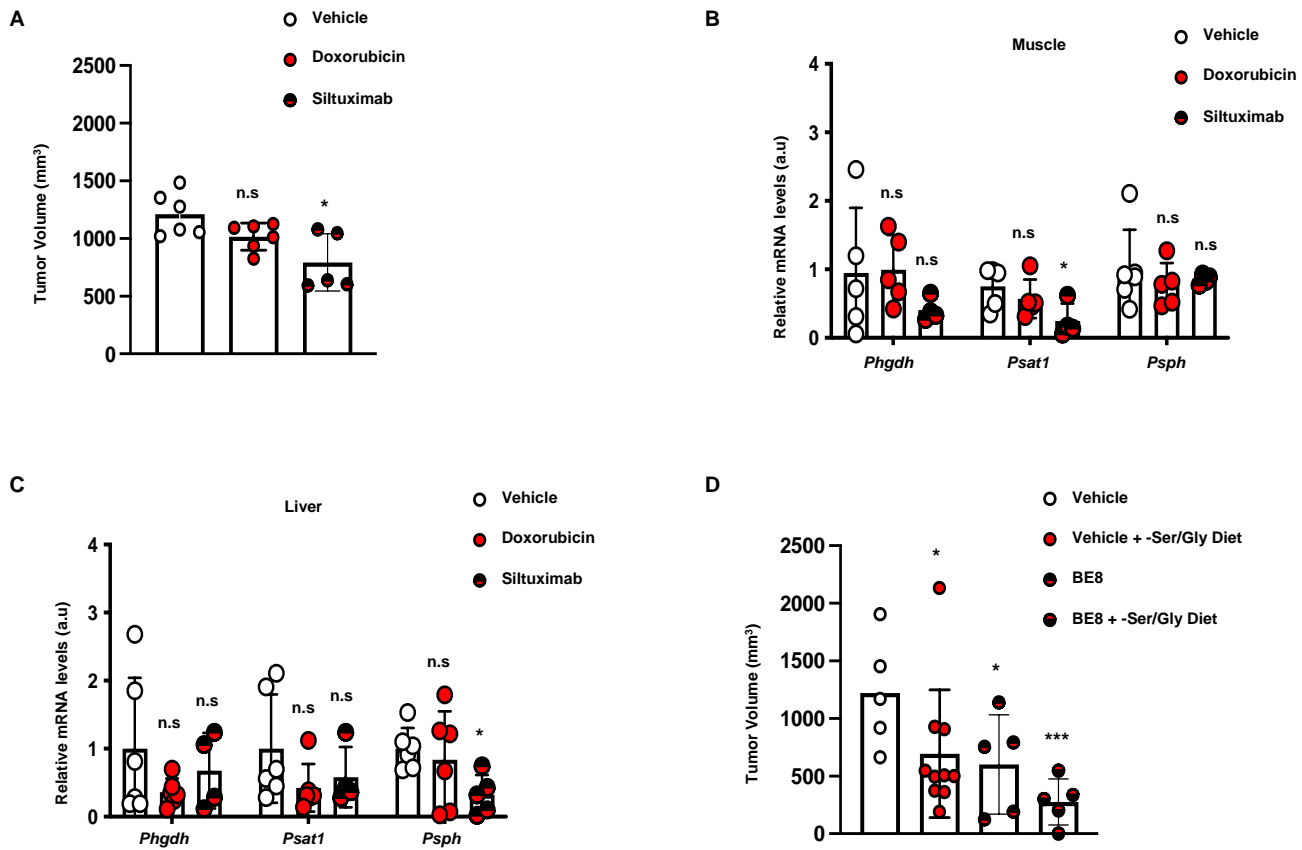


# Supplementary Figure 4



**Supplementary Figure 4:** **A**, Real-time qPCR analysis performed on C2C12 cells alone or cocultured with IB115 cells grown in media supplemented with vehicle or siltuximab, evaluating expression of serine synthesis pathway genes: *Phgdh*, *Psat1* and *Psph*. **B**, Real-time qPCR analysis performed on C2C12 cells alone or cocultured with IB115 cells grown in media supplemented with vehicle or anti IL-6 (hBE8), evaluating expression of serine synthesis pathway genes: *Phgdh*, *Psat1* and *Psph*. **C**, Real-time qPCR analysis performed on C2C12 cells alone or cocultured with IB111 cells grown in media supplemented with vehicle or anti IL-6 (BE8), evaluating expression of serine synthesis pathway genes: *Phgdh*, *Psat1* and *Psph*. **D**, Real-time qPCR analysis performed on C2C12 cells alone or cocultured with IB111 cells grown in media supplemented with vehicle or siltuximab, evaluating expression of serine synthesis pathway genes: *Phgdh*, *Psat1* and *Psph*. **E**, Real-time qPCR analysis performed on C2C12 cells alone or cocultured with IB111 cells grown in media supplemented with vehicle or GP130 inhibitor (BZA), evaluating expression of serine synthesis pathway genes: *Phgdh*, *Psat1* and *Psph*. **F**, Real-time qPCR analysis performed on C2C12 cells alone or cocultured with IB111 cells grown in media supplemented with vehicle or STAT3 inhibitor, evaluating expression of serine synthesis pathway genes: *Phgdh*, *Psat1* and *Psph*. (All experiments were performed in at least triplicates and statistical analysis was applied with \*= $P < 0.05$ , \*\*= $P < 0.01$ , \*\*\*= $P < 0.001$ , n.s.=non-significant).

# Supplementary Figure 5



**Supplementary Figure 5:** **A**, Tumor weight from patient liposarcoma tumor subcutaneously implanted in nude mice treated or not with Siltuximab (10 mg/kg) or doxorubicine (2mg/kg), 17 days after implantation. **B**, Real-time qPCR analysis performed on mice muscle, evaluating expression of serine synthesis pathway genes: *Phgdh*, *Psat1* and *Psph*. Mice were treated or not with Siltuximab (10 mg/kg) or doxorubicine (2mg/kg) by IP weekly **C**, Real-time qPCR analysis performed on mice liver, evaluating expression of serine synthesis pathway genes: *Phgdh*, *Psat1* and *Psph*. Mice were treated or not with Siltuximab (10 mg/kg) or doxorubicine (2mg/kg) by IP weekly **D**, Tumor weight from patient liposarcoma tumor subcutaneously implanted in nude mice, fed a normal or a no Serine/Glycine diet and treated or not with anti IL-6, BE8 (10 mg/kg), 24 days after implantation. (All experiments were performed in at least triplicates and statistical analysis was applied with  $*=P<0.05$ ,  $**=P<0.01$ ,  $***=<0.001$ , n.s=non-significant).