1 **White adipose tissue-infiltrated CD11b+ cells are a source of S100A4, a new potential**
2 **marker of hepatic damage**

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35 **Short title**: S100A4 in adipose tissue CD11b+ cells
36 **Keywords**: S100A4, obesity, inflammation, CD11b+ cells, liver steatosis.
37 **Word count: 3035**.
38 **Funding agencies**
39 DS-I is Investigator of the Miguel Servet Fund from Carlos III National Institute of Health, Spain.
40 RC is a Juan de la Cierva researcher, Ministry of Science and Innovation, Spain. This study was
41 supported by grants CP15/00106 and FIS PI17/01455 from Carlos III National Institute of Health
42 and European Regional Development Fund (ERDF) to DS-I, by the Ministry of Science and
43 Innovation of Spain (SAF2017-83813-C3-1-R to DS and LH, cofunded by the ERDF, and a
44 doctoral fellowship to KI), the CIBEROBN (Grant CB06/03/0001 to DS), the Government of
45 Catalonia (2017SGR278 to DS), and the *Fundació La Marató de TV3* (201627-30 to DS).
46 **Disclosure summary**
47 I certify that neither I nor my co-authors have a conflict of interest as described above that is
48 relevant to the subject matter or materials included in this Work.
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51
52

53 **Abstract**

54 **Context**

55 The endocrine and immunological properties of subcutaneous *vs.* visceral adipose tissue (sWAT
56 and vWAT, respectively) have turned a milestone in the study of metabolic diseases. The cytokine
57 S100A4 has been reported to be elevated in obesity and to have a role in adipose tissue dysfunction.
58 However, the cellular source in adipose tissue and its potential role in hepatic damage in obesity has
59 not been elucidated.

60 **Objective**

61 We aim to study the regulation of *S100A4* in immune cells present in sWAT and vWAT, as well as
62 its potential role as a circulating marker of hepatic inflammation and steatosis.

63 **Design**

64 A cohort of 60 patients with obesity and distinct metabolic status was analyzed. CD11b+ myeloid
65 cells and T cells were isolated from sWAT and vWAT by magnetic-activating cell sorting, and
66 RNA was obtained. *S100A4* gene expression was measured, and correlation analysis with clinical
67 data was performed. Liver biopsies were obtained from 20 patients, and S100A4 circulating levels
68 were measured to check the link with hepatic inflammation and steatosis.

69 **Results**

70 *S100A4* gene expression was strongly upregulated in sWAT- *vs.* vWAT-infiltrated CD11b+ cells,
71 but this modulation was not observed in T resident cells. *S100A4* mRNA levels from sWAT (and
72 not from vWAT) CD11b+ cells positively correlated with glycemia, triglycerides and *TNF-α* gene
73 expression. Finally, circulating S100A4 directly correlated with liver steatosis and hepatic
74 inflammatory markers.

75 **Conclusion**

76 Our data suggest that sWAT-infiltrated CD11b+ cells could be a major source of S100A4 in
77 obesity. Moreover, our correlations identify circulating S100A4 as a potential novel biomarker of
78 hepatic damage and steatosis.

79 **Introduction**

80 Obesity is characterized by a low-grade chronic degree of inflammation associated with
81 altered cytokine secretion and the development of numerous metabolic diseases including type 2
82 diabetes, cardiovascular disease and nonalcoholic fatty liver disease (NAFLD) (1). White adipose
83 tissue (WAT) is considered an endocrine and immunological organ since it is able to recruit
84 immune cells, including T cells and macrophages, and to secrete molecules that participate in a
85 hormonal network communicating several tissues and organs including the liver (2).

86 Macrophage recruitment and secretion of inflammatory cytokines in the WAT have been
87 reported to impact negatively systemic glucose homeostasis (3-6). Unraveling the crosstalk between
88 adipose tissue immune cells and other metabolic organs in the context of obesity is essential to
89 define the pathological bases of related diseases such as insulin resistance and NAFLD.

90 S100A4 is a member of the S100 calcium binding protein family that can modulate cell
91 growth, differentiation, survival, migration, and energy metabolism in different cell types (7, 8). It
92 was identified by Ebradlize and colleagues as a protein associated with tumor metastasis (9), and it
93 binds to cytoskeletal proteins including F-actin (10) and non-muscle myosin heavy chains (11).
94 S100A4 has been reported to be a novel adipocytokine elevated in obesity with a role in sWAT
95 dysfunction and insulin resistance (12). Moreover, it has been shown to increase in serum of
96 adolescent girls with polycystic ovarian syndrome (PCOS), and to correlate with hepato-visceral fat
97 excess in these patients (13). However, the source of S100A4 in adipose tissue and its potential role
98 in hepatic inflammation and steatosis in obesity has not been elucidated.

99 In the current study, we aimed to identify the role of adipose tissue immune cells in the
100 modulation of S100A4 in obesity. Moreover, we wanted to determine whether circulating S100A4
101 may be a marker for liver inflammation and steatosis in patients with obesity and different hepatic
102 inflammatory and metabolic status as an indicator of these obesity-related comorbidities.

103

104

105 **Materials and Methods**

106 *Subjects*

107 A total of 60 patients undergoing bariatric surgery with severe obesity (41 females and 19
108 males, aged 48 ± 10 years, and BMI 44.1 ± 7.3 kg/m^2) and different metabolic status were included
109 in this study. Anthropometric and clinical parameters are shown in Table 1. More female than male
110 participants could be recruited because the percentage of females undergoing bariatric surgery was
111 strongly higher than males.

112

113 *Adipose tissue, liver samples and serum*

114 Among the total of 60 patients of the cohort, subcutaneous and visceral white adipose tissue
115 of the same individual were obtained from 40 patients and liver biopsies were obtained from the
116 other 20 patients of the cohort. Due to the limited size of the WAT samples, 20 of them were used
117 for T cells isolation and the other 20 for CD11b+ cells isolation. All the tissues were collected
118 during bariatric surgery at the hospital Germans Trias I Pujol, Badalona, Spain. Serum samples
119 were collected after a 12h fasting period.

120

121 *Ethical statement*

122 The Institutional Ethics Committee (Germans Trias I Pujol CEIC), in accordance with the
123 Declaration of Helsinki, approved the study (code PI16-025). All participants gave their written
124 informed consent before collecting clinical data and samples.

125

126 *Isolation of adipose tissue resident CD11b+ cells and T cells from patients with obesity*

127 Immediately after surgical extraction, biopsies were preserved on physiological buffered
128 solution (Dulbeccos's PBS) supplemented with 2% fetal bovine serum (FBS). Samples were cut in
129 small fragments and then enzymatically digested with a solution of 1mg/mL collagenase NB 4 (Cat.

130 No. S1745401 Nordmark Biochemistry) in HBSS Solution, at 37 °C during 60 minutes with
131 agitation every 10 minutes. After enzymatic treatment, samples were centrifuged at 500 g for 5
132 minutes at 4° C, in order to separate stromo-vascular fraction (SVF) (pellet) from released lipids
133 (supernatant). The supernatant was discarded and SVF was resuspended in cold PBS-2%FBS,
134 filtered with 70µm mesh (Miltenyi Biotec, Germany) and centrifuged at 500g for 5 minutes at 4°C.
135 Pellets were washed once again with cold PBS-2%FBS, resuspended in MACS buffer and
136 centrifuged at 300g for 10 minutes. Then, an aliquot was separated to determinate %CD11b$^+$ cells
137 infiltrated in each adipose depot. Total SVF was resuspended in 80 µL of MACS buffer and
138 labeling with 20 µL of CD11b or CD3 antibody-conjugated magnetic microbeads (Miltenyi Biotec,
139 Germany) to isolate either macrophages (plus other CD11+ myeloid cells) or T cells, respectively,
140 and cell suspensions were incubated for 15 minutes at 4°C in the dark. The cells were washed by
141 adding 2 mL of MACS buffer and centrifuged at 300g for 10 minutes. The pellet was finally
142 resuspended in 20 µL of MACS buffer. Positive selection of CD11b+ (for macrophages plus other
143 CD11b+ myeloid cells) or CD3+ (for T cells) was performed using LS magnetic bead column
144 (Miltenyi Biotec, Germany), according to manufacturer´s instructions.
145
146 *Determination of %CD11b+*
147 The percentage of *CD11b+* cells infiltrated in each depot was determined by flow
148 cytometry. For this purpose, SFV aliquots from sWAT and vWAT were labeled for 30 min with 7-
149 AAD and CD11b-PE to quantify the viability and the number of infiltrated CD11b+ cells
150 respectively. Data acquisition was performed in a FACSCanto™ II system (BD Biosciences, San
151 Jose, CA) using the FACSDIVA™ software (BD), and analyzed in FlowJo version X.0.7. (FlowJo
152 LLC, Ashland, OR).
153
154 *S100A4 protein levels in sWAT*

155 To quantify S100A4 protein levels in adipose tissue, adipocytes and SVF, we performed a
156 specific ELISA kit (Cusabio Biotech Co., LTD, China) of complete adipose tissue and each cell
157 fraction from 4 patients with obesity. First, a piece of tissue was separated to later extract total
158 proteins. This sample was homogenized in PSB 1x using a microtube handheld homogenizer and
159 frozen at -20°C. The rest of tissue was digested with collagenase NB 4 (Cat. No. S1745401
160 Nordmark Biochemistry) for 30 minutes at 37°C at 130 rpm in order to separate adipocytes and
161 SVF. Then, the reaction was stopped with EDTA 0.5M and cell suspension was separated from
162 tissue debris using 250-µm cell strainer (Pierce, IL, USA). Then, we perform a gently centrifugation
163 at 100g for 10 min at room temperature (RT) to isolate adipocytes (upper phase) from the rest of
164 cells. Lower phase was resuspended and passed through 100-µm cell strainer (Thermo Fisher
165 Scientific, MA, USA) to eliminate adipocytes and debris contamination, and centrifugated for 5 min
166 at 500g at RT. Both adipocytes and SVF was washed once, resuspended in PBS 1x and stored at -
167 20°C to further analysis.
168 Protein extraction was performed according to the instructions of ELISA kit. Total protein
169 content of each fraction was assessed using Bradford method, and a dilution of 1:4 was used in
170 S100A4 determination. S100A4 levels of each fraction was normalized using total protein content.
171
172 *Human serological analysis*
173 Glucose, insulin, Hb1Ac levels, lipid profile and other clinical variables were measured in
174 the clinical laboratory at the hospital Germans Trias I Pujol, Badalona, Spain. S100A4 circulating
175 levels were measured using a human protein S100A4 ELISA kit (Cusabio Biotech Co., LTD,
176 China).
177
178 *RNA extraction and RT-PCR Analysis*
179 Tissue samples were homogenized in a TissueLyser LT (QIAGEN, #69980, Hilden,
180 Germany) and total RNA was isolated using TRI Reagent (Sigma, #T9424, St. Louis, MO, USA),

181 following the manufacturer's instructions. cDNA was obtained with the TaqMan RT-PCR Kit
182 (Applied Biosystems, #N808-0234, Foster City, CA, USA) from 1 µg of total tissue RNA. Relative
183 quantification of gene expression was measured by quantitative real-time polymerase chain reaction
184 (qRT-PCR) in the LightCycler 480 Instrument II (Roche, #05015243001, Basel, Switzerland) using
185 SYBR Green (LightCycler 480 SYBR Green I Master, #4887352001, Basel, Switzerland) or
186 TaqMan Probes (LightCycler 480 Probes Master #04707494001, Basel, Switzerland). Primers pair
187 list are provided in supplementary Table S1.
188
189 *Evaluation of hepatic fat accumulation*
190 Two experienced radiologists blinded to participants' clinical characteristics performed all
191 ultrasound examinations. Ultrasonography was performed using Siemens Acuson S2000 (Mountain
192 View, CA, USA) ultrasound equipment and a multifrequency convex transducer (4 MHz). Hepatic
193 steatosis was diagnosed after acquisition of seven standard liver slices: two slices of the left hepatic
194 lobe and two slices of the right hepatic lobe, including the diaphragm and the cortex of the right
195 kidney, one slice of the portal bifurcation and hepatic veins, one slice of the hepatic ileus, and one
196 slice of the gallbladder. The intra- and inter-observer variabilities for the ultrasound diagnosis of
197 hepatic steatosis were within 5%. The presence and grade of NAFLD, classified as mild, moderate
198 or severe, was determined in every patient (15). The sonographic criteria included hepatic
199 parenchymal echogenicity greater than the renal cortex, attenuation of the ultrasound wave, and a
200 poor definition of the diaphragm and intrahepatic vessels.
201
202 *RAW 264.7 macrophage cell culture*
203 RAW 264.7 macrophages (American Type Culture Collection Manassas, Virginia) were maintained
204 in RPMI 1640 Medium (Gibco, Thermo Fisher Scientific, Waltham, Massachusetts) and 10% heat
205 inactivated FBS for 24 hours. The cells were treated with 60 ng/mL lipopolysaccharide (LPS)
206 (Sigma-Aldrich) 12 hours after changing the media to activate the cells to the M1 phenotype,

207 whereas the cells were exposed to 40 ng/mL interleukin-4 (IL4; Pepro Tech EC, Rocky Hill, New
208 Jersey) for 12 hours to induce the M2 phenotype. *S100A4, TNFa* and *ARG1* mRNA levels were
209 measured.

210

211 *Statistical analysis*

212 Data are presented as mean ± SD or SEM (clinical parameters or gene expression,
213 respectively) unless otherwise stated. Normality of datasets was tested using the Shapiro-Wilk test.
214 Potential associations were analysed with standard least-squares linear regression. Two-tailed t test,
215 Mann-Whitney's U test for non-normal distributed data in two-group comparisons, or one-way
216 parametric or nonparametric ANOVA with Tukey's post-hoc test for comparisons of more than two
217 groups were used to determine statistical significance. Statistical analyses were conducted in JMP®
218 v14.1 (SAS Institute Inc., Cary, NC, USA), SPSS 23 (IBM, Chicago, IL, USA) and GraphPad
219 Prism (California, CA, USA). $P<0.05$ was set as the significance threshold.

220

221

222 **Results**

223 *S100A4 is upregulated in sWAT vs. vWAT CD11b+ cells but not in T cells, and its protein levels are*
224 *higher in SVF from patients with obesity compared to adipocyte fraction*

225 S100A4 has been reported to be elevated in sWAT and vWAT from patients with obesity
226 compared to lean individuals (12,13). In this context, we aimed to check the role of resident
227 CD11b+ cells and T cells in the regulation of *S100A4* in sWAT *vs.* vWAT in a cohort of patients
228 with severe obesity. *S100A4* was significantly overexpressed in sWAT-infiltrated CD11b+ cells
229 compared to those cells obtained from vWAT of the same patient (Figure 1A). Conversely,
230 although WAT-infiltrated T cells also expressed *S100A4*, its mRNA levels were similar in both
231 subcutaneous and visceral depots (Figure 1B). Interestingly, S100A4 mRNA levels were higher in
232 CD11b+ cells compared to T cells only in sWAT, but not in vWAT (Supplementary Figure S1A-B),
233 and no differences were found between the relative abundance of CD11b+ cells in sWAT *vs.* vWAT
234 in our samples (Supplementary Figure S1C). Finally, S100A4 protein levels were higher in the SVF
235 of sWAT from patients with obesity compared to adipocyte fraction (Supplementary Figure S2).
236

237 *S100A4 gene expression in adipose tissue CD11b+ cells directly correlates with circulating glucose*
238 *and triglycerides and TNF-α expression*

239 Since *S100A4* was not differentially regulated in T cells present in sWAT *vs.* vWAT, and
240 CD11b+ cells presented higher mRNA levels compared to T cells in sWAT, we focused on
241 CD11b+ cells to study potential associations with clinical data. The expression of S100A4 in
242 isolated sWAT CD11b+ cells positively correlated with circulating glucose and triglyceride levels
243 as well as with their TNF-α mRNA expression (Figure 2). No correlations were found between
244 S100A4 gene expression in vWAT CD11b+ cells and clinical parameters or mRNA levels, neither
245 didS100A4 gene expression correlate with BMI, age, sex and other clinical parameters
246 (Supplementary Table S2).

247 *S100A4 gene expression in adipose tissue CD11b+ cells directly correlates with markers of*
248 *proliferation*

249 The mRNA levels of some markers of inflammation, proliferation and quiescence were
250 checked in our CD11b+ cells. A strong correlation of *S100A4* expression with that of proliferation
251 markers (*MKI67, CD81, MIF*, and *CSF1*) was observed (Figure 3). No correlation was found
252 between S100A4 and inflammatory markers (IFNgamma, IL1beta, or CD86), and a moderate
253 association with markers of quiescence (GADD45G, ITGB1, GLS2) was detected (Supplementary
254 Figure S3).

255

256 *S100A4 is not regulated in M1 or M2 macrophages in vitro*

257 M1 and M2 polarization was induced in RAW 264.7 macrophages. Then, pro-inflammatory
258 markers characteristic of M1 and anti-inflammatory genes involved in M2 phenotype were
259 measured. As expected, a strong induction of TNFa in M1 and ARG1 in M2 was found in our cells
260 (Supplementary Figure S4A-B). Finally, the mRNA levels of S100A4 were measured, and similar
261 expression was observed in the M1 and M2 polarization states (Supplementary Figure S4C).

262

263 *Circulating S100A4 correlates with the hepatic steatosis degree and GPT transaminase levels*

264 Since *S100A4* gene expression in sWAT CD11b+ cells correlated with triglyceride levels,
265 and previous studies suggested that circulating S100A4 could be an indicator of hepatic fat
266 accumulation in adolescent individuals with PCOS (14), we aimed to analyze the link between
267 S100A4 circulating levels and the hepatic steatosis and inflammatory status of patients with obesity.
268 To address this, liver biopsies were obtained from 20 patients of our cohort, and expression of genes
269 involved in inflammation and glucose and lipid homeostasis was measured alongside the liver
270 steatosis degree and S100A4 circulating levels. We observed that S100A4 circulating levels directly
271 correlated with liver steatosis index and GPT transaminase, and inversely with *ARG1* gene

272 expression (Table 2). No correlations were found between circulating S100A4 and BMI, age, sex or
273 expression levels of other genes involved in inflammation, ER stress, glucose or lipid metabolism
274 (Table 2).

275 Moreover, to decipher whether the circulating levels of S100A4 could reflect not only the
276 adipose tissue contribution, but also that of other organs including liver, we compared the mRNA
277 levels of S100A4 in adipose tissue *vs.* liver in human samples from patients with obesity. Higher
278 levels of S100A4 mRNA were detected in adipose tissue compared to liver (Supplementary Figure
279 S5).

280

281 **Discussion**

282 Increased immune cell infiltration and proinflammatory cytokine release in adipose tissue is
283 a key event for the establishment of a low-grade, chronic inflammatory status that can lead to type 2
284 diabetes mellitus. We report for the first time that *S100A4* gene expression is specifically
285 upregulated in sWAT-infiltrated CD11b+ cells compared to those present in vWAT from patients
286 with obesity. This fat depot-dependent modulation was not observed in other adipose tissue immune
287 cells populations such as T cells, in which S100A4 mRNA was similarly expressed when infiltrated
288 in sWAT or vWAT.

289 S100A4 levels have been reported to be upregulated in whole-tissue WAT from patients
290 with obesity compared to lean controls (14, 15). Adipose tissue immune cells were the second
291 fraction after adipocyte precursors that express the highest levels of S100A4 in sWAT from healthy
292 lean patients (14), but the specific role of immune cell types, i.e. myeloid-derived CD11b+ cells or
293 T cells, in sWAT *vs.* vWAT was unknown. In line with previous observations at the transcript level
294 (14), S100A4 protein levels were higher in the SVF of sWAT from patients with obesity compared
295 to the adipocyte fraction (Figure 1A), which is indicative that such increase comes from alterations
296 in cells within the SVF and not from mature adipocytes. In this context, we aimed to check the role

297 of resident CD11b+ cells and T cells in the regulation of *S100A4* in sWAT *vs.* vWAT in a cohort of
298 patients with severe obesity. Our data show that sWAT-infiltrated CD11b+ cells in conditions of
299 obesity overexpress S100A4 compared to those located in vWAT, and suggest that CD11b+ cells
300 might be the main immune cell population regulating systemic S100A4 levels in WAT. Moreover,
301 our results show a positive correlation between S100A4 mRNA expression in sWAT CD11b+ cells
302 and glycemia, triglyceride levels and *TNF-α* gene expression. These results are in agreement with a
303 study describing similar direct correlations between the whole sWAT *S100A4* expression and
304 metabolic parameters in patients with obesity (14), and suggest that S100A4 produced by CD11b+
305 cells infiltrated in sWAT of patients with obesity is accountable for the alterations reported in
306 conditions of adipose tissue dysfunction.

307 In our study, we report for the first time a direct correlation of mRNA levels of *S100A4* and
308 markers of proliferation in our CD11b+ cells. In obesity, immune cell infiltration to adipose tissue
309 is increased, but macrophages can proliferate and activate to a proinflammatory state, or undergo
310 apoptosis (16). In this regard, our results suggest that S100A4 could be a novel potential marker of
311 proliferation of CD11b+ cells, such as macrophages, in obesity. In addition, in accordance with our
312 human data showing a lack of correlation between *S100A4* mRNA levels and pro-inflamatory or
313 anti-inflammatory markers in CD11b+ cells, our *in vitro* results in murine RAW 264.7 macrophages
314 revealedsimilar *S100A4* expression levels in the M1 and M2 polarization statuses. These results
315 suggest that S100A4 is not specifically modulated by pro or anti-inflammatory macrophages.
316 Therefore, these data could indicate that S100A4 could have a role in the proliferation of
317 macrophages, a very important process in adipose tissue biology in obesity, but not in their
318 activation. Further studies are necessary to decipher whether S100A4 is involved in the WAT
319 proliferation signaling and the crosstalk with other metabolic organs.

320 Moreover, a positive correlation between the mRNA levels of S100A4 in sWAT CD11b+
321 cells and circulating triglycerides was observed. It is known that, when WAT is not able to further
322 store the excess of triglycerides, these lipids are ectopically incorporated in other organs that are not

323 designed to accumulate fat in healthy conditions, such as the muscle or the liver (17, 18). According
324 to that, it is possible that S100A4 expressed mainly by sWAT macrophages could be an indicator of
325 lipotoxicity, and its detection in circulation might be a potential marker of fat accumulation in other
326 metabolic organs such as the liver. In addition, we observed an inverse correlation between S100A4
327 circulating levels and hepatic Arginase 1 (*ARG1*) gene expression. This goes in line with previous
328 studies in which we reported that S100A4 could be a potential circulating marker of hepato-visceral
329 fat excess in adolescent girls with PCOS (13). Moreover, an association between the decrease of
330 hepato-visceral fat after pharmacological treatment of PCOS and the reduction of S100A4
331 circulating levels was found. In agreement with this observation, here we observed a positive
332 correlation between circulating levels of S100A4 and liver fat accumulation and glutamate-pyruvate
333 transaminase (GPT) in patients with severe obesity. These results suggest that the adipokine
334 S100A4 could also be an indicator of fat accumulation and hepatic damage in patients with
335 obesity. Finally, the negative correlation between circulating S100A4 and hepatic *ARG1* mRNA
336 may indicate its involvement in hepatic inflammation. To provide a better insight on the
337 contribution of different endocrine tissues in the circulating levels of S100A4, we compared the
338 *S100A4* mRNA levels in the adipose tissue *vs.* the liver. Since S100A4 expression levels in the liver
339 were dramatically lower to those in the WAT, these results suggest that the liver is not a main
340 source of this protein in humans with obesity.

341 We are aware that, because of the difficulty to obtain human liver samples, the main
342 limitation of our present study is the relatively low number of patients with liver biopsy and the lack
343 of lean controls due to the difficulty to obtain liver biopsies in healthy lean individuals. However,
344 the strength of our study is the clinical characterization of the patients including liver images and
345 serum samples and the different range of metabolic status of the cohort that allowed us to perform a
346 pilot study of potential biomarkers of obesity-related metabolic complications. Another limitation is
347 the lack of an identification of specific acute or chronic inflammatory reactions in obesity. Data
348 from different laboratories demonstrate that a first transient, acute inflammatory status in adipose

tissue contributes to a healthy and physiological adaptation to store an excess of fat (19, 20). In this context molecules secreted by immune cells, including S100A4, may act mediating this phenomenon in order to balance early alterations of the metabolic status before eventually becoming chronically dysregulated, but further research is needed in this regard.

In conclusion, our data show for the first time that CD11b+ cells infiltrated in sWAT in obesity overexpress S100A4 compared to CD11b+ cells recruited in vWAT in conditions of obesity. Moreover, S100A4 positively correlates with parameters indicating glucose and lipid homeostasis dysfunction, and could be a novel potential circulating marker of liver alterations in patients with obesity. Further studies with a larger cohort of patients remain to be performed at this point to figure out the potential of interventions addressed to S100A4 as a therapeutic strategy in metabolic diseases related to obesity, including liver steatosis.

Acknowledgements

Samples from patients included in this study were provided by the IGTP HUGTP Biobank integrated in the Spanish National Biobanks Network of *Instituto de Salud Carlos III* (PT13/0010/0009) and they were processed following standard operating procedures with the appropriate approval of the Ethical and Scientific Committees.

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442 **Figure Legends**

443 **Figure 1. *S100A4* gene expression in adipose tissue resident CD11b+ cells and T cells.** Total
444 RNA was extracted from sWAT and vWAT immune cells from patients that underwent bariatric
445 surgery. The mRNA expression of *S100A4* was quantified using RT-qPCR. (A) *S100A4* gene
446 expression in adipose tissue resident CD11b+ cells. (B) *S100A4* gene expression in adipose tissue
447 resident T cells. Lines indicate the mean values for each group. Each dot represents a single
448 individual.

449

450 **Figure 2. Correlation between *S100A4* gene expression of adipose tissue resident CD11b+ cells**
451 **and clinical variables from patients.** Plots showing the linear correlation between *S100A4* gene
452 expression of sWAT resident CD11b+ cells with (A) glucose circulating levels, (B) triglycerides
453 circulating levels, and (C) *TNF*α gene expression.

454

455 **Figure 3. Correlation between *S100A4* gene expression of human adipose tissue resident**
456 **CD11b+ cells and markers of proliferation.** Plots showing the linear correlation between *S100A4*
457 gene expression of sWAT resident CD11b+ cells with (A) *MKI67* gene expression, (B) *CD81* gene
458 expression (C) *MIF* gene expression, and (D) *CSF1* gene expression.

459

460 **Supplementary Figure S1. mRNA expression levels of *S100A4* in T cells and CD11b+ cells**
461 **isolated from sWAT and vWAT, and percentage of CD11b+ cells isolated from both tissues.**
462 *S100A4* mRNA expression levels in T cells vs. CD11b+ cells present in sWAT (A) and vWAT (B).
463 C) % CD11b+ cells that were isolated from sWAT and vWAT from the above described cohort of
464 patients with obesity. Lines indicate the mean values for each group. Each dot represents a single
465 individual. ns, $P > 0.05$. *, $P \leq 0.05$. **, $P \leq 0.01$. ***, $P \leq 0.001$. ****, $P \leq 0.0001$.

466

467 **Supplementary Figure S2. S100A4 protein levels in stromo-vascular fraction (SVF) and**

468 **adipocytes.** The protein levels were obtained from the SVF, adipocytes and the whole adipose
469 tissue, and measured by ELISA. S100A4 protein levels in SVF compared to adipocytes. ns, P >
470 0.05. *, P ≤ 0.05. **, P ≤ 0.01. ***, P ≤ 0.001. ****, P ≤ 0.0001.
471
472 **Supplementary Figure S3. Correlation between *S100A4* gene expression of human adipose**
473 **tissue resident CD11b+ cells and markers of inflammation and quiescence.** Plots showing the
474 linear correlation between *S100A4* gene expression of sWAT resident CD11b+ cells with (A)
475 *IFNgamma* gene expression, (B) *IL-1β* gene expression, (C) *CD86* gene expression, (D) *GADD45G*
476 gene expression, (E) *ITGB1* gene expression, (F) *GLS2* gene expression.
477
478 **Supplementary Figure S4. *S100A4* gene expression is not modulated in M1 and M2 *in vitro***
479 **macrophages.** Total RNA was extracted from RAW 264.7 macrophages cell line pretreated with
480 60-ng/mL LPS or 40-ng/mL IL4 respectively for 12 hours and the controls. The mRNA expression
481 of *S100A4* was quantified using RT-qPCR. (A) *TNFα* gene expression in control, M1 and M2
482 macrophages, (B) *ARG1* gene expression in control, M1 and M2 macrophages, and (C) *S100A4*
483 gene expression in control, M1 and M2 macrophages.
484
485 **Supplementary Figure S5. *S100A4* gene expression in adipose tissue vs liver from patients**
486 **with obesity.** Total RNA was extracted from vWAT and liver from patients that underwent
487 bariatric surgery. The mRNA expression of *S100A4* was quantified using RT-qPCR.
488
489 **Table 1.** Anthropometric parameters, biochemical and lipid profile of our cohort of 60 patients with
490 obesity. Data are shown as mean (minimal value – maximal value).
491
492 **Table 2.** Correlations between circulating S100A4 and clinical parameters and genes involved in
493 inflammation, glucose and lipid metabolism.

494

495 **Supplementary Table S1.** Primers pairs for qRT-PCR detection and relative quantification of

496 genes in humans.

497

498 **Supplementary Table S2.** Correlations between the *S100A4* relative gene level of sWAT and

499 vWAT CD11b+ cells and clinical parameters.

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