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1 **Increased basement membrane components in adipose tissue during obesity: links with**
2 **TGFβ and metabolic phenotypes**

3

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24

25 **Abstract**

26 **Context:** Collagen accumulation around adipocytes and vessels (i.e. pericellular fibrosis) is a
27 hallmark of obese adipose tissue associated with altered metabolism.

28 **Objective:** To evaluate components of basement membrane (BM) in adipose tissue, including
29 collagen IV, a major BM component, and its relationships with metabolic parameters and
30 TGF β isoforms.

31 **Design and setting:** We used immuno-techniques and gene expression approaches to detect
32 BM components in subcutaneous and visceral adipose tissue samples. Adipocytes and
33 endothelial cells were isolated from lean and obese adipose tissue. We also focused on the
34 expression of *COL4A1* correlated to metabolic variables in moderate obesity and, in severe
35 obesity before and after bariatric surgery. Using *in vitro* analysis, we explored the impact of
36 TGF β isoforms on the expression of inflammatory and extracellular matrix genes in
37 adipocytes and endothelial cells.

38 **Results:** BM components were detected around adipocytes and endothelial cells, and were
39 increased in obese adipocytes. *COL4A1* expression was positively correlated with insulin-
40 resistance indices in obese subjects, and showed less reduction in severely obese subjects with
41 poorer insulin-resistance outcomes six months after gastric bypass. *COL4A1* expression also
42 correlated with *TGF β 1* and *TGF β 3* gene expressions in subcutaneous adipose tissue.
43 Stimulating isolated adipocytes and endothelial cells *in vitro* with these TGF β isoforms
44 showed an inflammatory and pro-fibrotic phenotype. However, TGF β 1 and TGF β 3 exposure
45 only provoked *COL4A1* over-expression in endothelial cells, and not in adipocytes.

46 **Conclusion:** The disorganization of several BM components, including collagen IV, **could**
47 **contribute** to pathological alterations of obese adipose tissue and cells.

48 **Introduction**

49 Obesity and comorbidities are associated with major alterations of white Adipose Tissue (AT)
50 (1). These alterations include increased interstitial fibrosis defined as an excessive
51 accumulation of extracellular matrix (ECM) components (2). Over the last few years,
52 relationships between fibrotic accumulation in AT and obesity-related metabolic
53 deterioration, such as insulin-resistance and type 2 diabetes, have been described (3). At the
54 level of AT, cell types such as adipocytes, progenitors, endothelial, and immune cells, are all
55 embedded in a three-dimensional fibrillar network of ECM proteins (4,5). In human AT, we
56 have observed increased expression (6) and deposition of ECM proteins, that surround not
57 only adipocytes (i.e. called pericellular fibrosis) but also blood vessels (7). Several studies
58 have also explored specific fibrillar collagens and collagen VI, whose expression in AT is
59 associated with metabolic alterations in humans and rodents, and is regulated by weight loss
60 (5,7,8).

61 A key component of the ECM is the basement membrane (BM), which is found in close
62 proximity to other cell membranes. The BM of endothelial cells (EC) has been well described
63 in many tissues (9), however in AT, these structures have only been demonstrated via
64 morphological studies focusing on adipocytes (10). The BM provides cellular architectural
65 support and also interacts with integrins for signaling (11). This interaction is crucial for
66 correct cell behavior through outside-in signaling. BMs composition and supramolecular
67 organization depend both on tissue type and specific developmental periods or
68 pathophysiological events within those tissues. These modifications are well described, during
69 glomerulogenesis for example, or diabetic nephropathy and retinopathy (12,13). Collagen IV
70 and laminins are two major components, which self-assemble in the extracellular space to
71 form a network which creates BM ultrastructure. Nidogen and perlecan (HSPG2) bridge the
72 laminin and collagen IV network to stabilize and maintain BM integrity (9). The matricellular
73 protein SPARC, expressed in AT (8), also contributes to BM stabilization.

74 Collagen IV, a heterotrimeric glycoprotein, accounts for 50% of the BM and is composed of
75 up to six distinct alpha-chains, $\alpha 1(\text{IV})$ to $\alpha 6(\text{IV})$ (14). The chains interact and assemble with
76 remarkable specificity due to specific recognition of non-collagenous domains (7S and NC1)
77 to form three distinct heterotrimers: $\alpha 1\alpha 1\alpha 2$, $\alpha 3\alpha 4\alpha 5$ and $\alpha 5\alpha 5\alpha 6$ (15). *COL4A1* and *COL4A2*
78 are the most common isoforms and are present in all tissues. Laminins consist of assembled α ,
79 β and γ chains, and five α , three β and three types of γ chains have been identified in
80 vertebrates (9). Depending on the chain combination, at least 15 laminin isoforms have been
81 described (9). Laminin knockout mouse models have highlighted the importance of certain
82 chains in a functional ECM structure (16). For example, $\gamma 1$ null mice are embryonically
83 lethal, producing disorganized extracellular deposits of collagen IV and perlecan (17),
84 showing that this ubiquitous subunit is key for BM assembly. Thus structural anomalies of
85 BM can lead to tissue dysfunction and eventual lethality in some models (18). Among these
86 anomalies, BM thickening is commonly associated with tissue dysfunction, as shown in
87 diabetic nephropathy or retinopathy (12,13). Collagen IV overexpression and oversecretion in
88 the extracellular space is a hallmark of BM thickening (19), and the pro-fibrotic factor,
89 TGF β 1, is an important molecular contributor (20,21).

90 Today, morphological analysis has highlighted the presence of BM around adipocytes in AT
91 (10), but no study has specifically explored BM components in lean and obese AT cells, nor
92 the potential relationships between the major BM component collagen IV and metabolic
93 alterations in obesity. Moreover, whereas previous studies showed that TGF β 1 is expressed in
94 AT and is associated with BMI (22), no study has evaluated the potential relationships
95 between TGF β isoforms, collagen IV, and ECM components in obesity.

96 Thus, using a combination of immuno-technique microscopy and gene expression approaches
97 in human AT samples, we examined BM composition and its variations in adipocytes from
98 both lean and obese subjects. We also assessed the relationships between collagen IV
99 expression and metabolic phenotypes in obese subjects. Finally using 3D *in vitro* models, we

100 explored the effect of TGF β isoform on the BM, and pro-fibrotic or pro-inflammatory genes
101 in adipocytes and EC from AT.

102

103 **Materials and Methods**

104 The list of antibodies and chemical compounds used in this study are presented in the
105 Supplemental Data.

106

107 **Subjects**

108 First, we obtained **subcutaneous periumbilical** AT (SAT) samples by needle aspiration from
109 an initial cohort of 60 obese subjects (F/M: 40/20, mean age: 51.29 ± 1.18 and BMI: $31.37 \pm$
110 0.44 kg/m^2) as described in (23). These subjects were treatment naïve and had impaired
111 fasting glucose. Subjects were without overt diabetes, and had normal renal, hepatic and
112 thyroid function. We obtained serum glucose and insulin after 12-hours of fasting and
113 calculated insulin resistance indexes from HOMA-IR and HOMA-%S measurements, and
114 beta cell function HOMA-%B using the Homeostatic Model Assessment Insulin Resistance
115 (24).

116 Secondly, 25 severely obese women candidates for bypass surgery (mean age: 48.9 ± 1.8 and
117 BMI: $47 \pm 1.4 \text{ kg/m}^2$) were selected. Among them, 16 subjects were non-diabetic and 9 had
118 type 2 diabetes. The subjects had not been dieting before the surgery and their weight had
119 been stable for at least three months prior to surgery. Subcutaneous periumbilical adipose
120 tissue (SAT) samples were obtained by needle aspiration before the surgical procedure (T0).
121 This group of patients was followed-up six months after the bariatric surgery and we collected
122 **SAT at the periumbilical location using the same procedure (i.e. that needle biopsy (T6)).**
123 Patients were characterized by a series of bioclinical factors just prior to, and six months
124 following surgery, as previously described in (25). **For microscopy analysis, paired SAT**
125 **(periumbilical) and VAT (omental) sample biopsies were obtained from 5 morbidly**

126 **obese subjects (mean age: 38.5 ± 11.6 , BMI: 44.7 ± 7.5 kg/m², F/M: 4/1) during surgery**
127 **by the same surgeon (JLB). A portion of each sample was fixed in 4% formaldehyde for**
128 **immunohistochemistry/immunofluorescence analysis and 0.2% glutaraldehyde-2%**
129 **paraformaldehyde for immuno-electron microscopy analysis.**

130 Thirdly, AT biopsies were also obtained from non-obese women (mean age: 46.5 ± 5.1 and
131 BMI: 23 ± 0.5 kg/m²). These subjects had been admitted for scheduled abdominal surgery.

132 **Subjects were exempt from acute or chronic inflammatory or infectious disease, viral**
133 **infection, cancer and/or known alcohol consumption** and consented to AT sampling from
134 **periumbilical location** as obese subjects.

135 All participants gave written and informed consent. All clinical investigations were performed
136 according to the Declaration of Helsinki and approved by the Hôtel-Dieu hospital ethics
137 committees.

138

139 **Microscopic Analysis of human AT**

140 Immunofluorescence and immunohistochemistry analyses were performed on serial sections
141 of AT from five lean and five obese subjects as described in (26) and (27) respectively.

142 Negative controls were performed by omitting primary antibodies. Immuno-electron
143 microscopy was performed in human SAT as described in supplementary methods.

144

145 **Isolation of adipocytes and endothelial cells from human SAT**

146 SAT biopsies were digested with collagenase then adipocytes and EC were isolated as
147 described in (28) and (29) respectively. For each cells types, one part was kept for gene
148 expression analysis and the other for culture experiments.

149

150

151

152 **3D cultures of isolated adipocytes**

153 Adipocytes were cultured in a 3D-cultured model as described in (26). Culture medium was
154 changed every other day.

155

156 **Gene expression studies**

157 Cultured adipocytes and EC remained untreated or were treated with 5 ng/ml TGF β 1 or
158 TGF β 3 for 48h. mRNA expression was determined by the QuantiGene Plex 2.0 Reagent
159 System (QuantiGene Affymetrix, CA, USA) as described in supplementary methods.

160 Gene expression was quantified by RT-qPCR in adipocytes or EC isolated from human AT.
161 RNA extraction, reverse transcription, and real-time PCR were conducted as described
162 previously (29). Primers are listed in Supplemental Table 1. Values were normalized to *18S*
163 expression.

164

165 **Statistical Analysis**

166 Data were analyzed using GraphPad software (San Diego, CA). Values are expressed as
167 means \pm S.E.M. Differences between **gene expressions** were determined with non-parametric
168 Mann-Whitney tests (obese *vs.* lean subjects) or Wilcoxon tests (SAT *vs.* VAT; prior to *vs.* six
169 months after surgery **and in vitro studies**). **Differences between clinical data (prior to *vs.***
170 **six months after surgery) were determined using paired non-parametric Mann-Whitney**
171 **tests.** Correlations were examined with non-parametric Spearman correlations. Results were
172 considered significant when $p < 0.05$.

173

174

175

176

177

178 **Results**

179 **Basement membrane components in human lean adipocytes and endothelial cells**

180 To investigate the BM in AT, we first performed immunofluorescence microscopy to detect
181 collagen IV protein in SAT from non-obese subjects. This initial morphological exploration
182 revealed collagen IV staining surrounding adipocytes and blood vessels (Figure 1A).
183 Immuno-electron microscopy confirmed that collagen IV staining was located proximate to
184 adipocytes (Figure 1C and **Figure S1A-S1D**) and EC membranes (data not shown). In
185 isolated adipocytes and EC we assessed the expression of genes encoding several BM
186 components, including *COL4A1*, a ubiquitously expressed α chain and *LAMC1*, the most
187 commonly expressed laminin chain. We also evaluated the gene expression level of other BM
188 components such as *NID-1* and *HSPG2*, as well as *SPARC*, a matricellular protein involved in
189 BM stabilization.

190 *COL4A1* was expressed at similar levels in isolated adipocytes and in EC (Figure 1E). In
191 contrast, *COL4A3* or *COL4A5* transcripts were not detected (data not shown). Expression
192 levels of *LAMC1*, *NID-1*, and *HSPG2*, were similar in lean adipocytes and EC, whereas
193 *SPARC* had increased expression in isolated adipocytes compared to EC ($p=0.018$) (Figure
194 1E). These observations demonstrate that similar to EC, isolated adipocytes express several
195 key BM components, including *COL4A1*.

196

197 **Basement membrane components increase in obese AT**

198 Via immunofluorescent studies, collagen IV staining was more intense around adipocytes and
199 blood vessels in obese compared to lean SAT samples (Figure 1A-B).

200 Immuno-electron microscopy analysis confirmed not only the increased amount of collagen
201 IV close to adipocyte membranes, but also different patterns in obese samples. Collagen IV
202 appeared more disorganized with frequent associated clusters around obese compared to lean
203 adipocytes (Figure 1D). We also found an AT depot-dependent pattern of collagen IV.

204 Collagen IV staining was much more intense in SAT from both lean and obese subjects,
205 compared to VAT from both lean and obese subjects (Figure 1G-J).

206 BM gene expression profiles correlated with histological findings. *SPARC* gene over-
207 expression was observed in obese adipocytes (p=0.021), which also demonstrated increased
208 *COL4A1* (p=0.001), *LAMC1* (p=0.003), *NID-1* (p=0.041) and *HSPG2* (p=0.05) gene
209 expression compared to that of lean adipocytes (Figure 1F). The expression levels of
210 *COL4A1*, *NID-1* and *SPARC* were also significantly higher in adipocytes isolated from obese
211 SAT compared to VAT (*COL4A1*: p=0.005; *NID-1*: p=0.026; *SPARC*: p=0.002) (Figure 1K).
212 *COL4A1* expression was significantly correlated with *LAMC1*, *NID-1*, and *SPARC* genes in
213 both SAT and VAT (Table S2). Both morphological and gene expression explorations
214 demonstrated increased BM components in obese adipocytes, suggesting they could
215 contribute to BM thickening.

216

217 **Collagen IV gene expression in obese SAT is associated with glucose metabolism**

218 In diseases such as diabetic nephropathy, increased collagen IV is induced by hyperglycemia.
219 We thus explored potential links between *COL4A1* expression in SAT (SAT-*COL4A1*) and
220 glucose metabolism from i) moderately obese subjects with increased fasting blood glucose
221 and, ii) severely obese subjects before and after gastric bypass, a condition known to
222 drastically improve glucose metabolism.

223 In 60 obese subjects with impaired fasting glucose but no anti-diabetic treatment (Table S3),
224 we observed significant correlations between SAT-*COL4A1* expression and markers of
225 glucose homeostasis, such as fasting glucose and insulin, markers of insulin resistance
226 (HOMA-IR and HOMA-%S) and of β -cell function, HOMA-%B, as shown in Table 1. These
227 associations remained after adjusting for weight (data not shown).

228 We next investigated the relationship between variations in SAT-*COL4A1* expression and
229 glucose metabolism improvement in 16 non-diabetic subjects with severe obesity before and

230 after gastric bypass (Table S4). Six months after bariatric surgery, SAT-*COL4A1* expression
231 decreased by 29.5% ($p < 0.01$) (Fig. 2A). Individual responses to bariatric surgery intervention
232 were highly variable and treatment dependent, consequently we used a clustering approach to
233 stratify the 16 non-diabetic subjects into two equivalent partitions using the median value for
234 each clinical and biological parameter. For each clinical variable, we were then able to
235 identify those that improved the most (top half) and those with little or no improvement
236 (bottom half). Using these partitions, we found that obese subjects with the greatest
237 improvement in HOMA-IR, the insulin-resistance surrogate, were those in which SAT-
238 *COL4A1* decreased the most following surgery ($p = 0.014$) (Figure 2B). No additional
239 relationships were detected between variations in SAT-*COL4A1* following surgery, and other
240 biological or clinical improvement parameters. We also observed that the other previously
241 described BM components were significantly decreased following surgery (**Figure S2A-**
242 **S2C**). Using the same clustering approach for *COL4A1*, we determined that SAT-*NID1* was
243 also related to HOMA-IR improvement (data not shown).

244

245 **Collagen IV gene expression in obese SAT is associated with TGF β isoforms**

246 TGF β 1, a major pro-fibrotic growth factor correlated with BM thickness in several tissues
247 (30). However this specific aspect has never been explored in obese AT. Moreover, using
248 previous microarray data from our laboratory, we have observed that another TGF β isoform,
249 *TGF β 3*, was also highly expressed in human AT while *TGF β 2* was barely detected (data not
250 shown). We then explored the relationship between both *TGF β 1* and *TGF β 3* and BM
251 components in obese SAT.

252 Similar to *COL4A1*, the expression of both SAT-*TGF β 1* and SAT-*TGF β 3* decreased by 23%
253 and 20% respectively six months after bariatric surgery (Figure 2C and 2D). We found strong
254 positive correlations between SAT-*COL4A1* and SAT-*TGF β 1* expression, as well as between
255 SAT-*COL4A1* and SAT-*TGF β 3*, in the group sampled prior to surgery (Figure 2E and 2F).

256 These associations remained at six months following the weight loss induced by bariatric
257 surgery (data not shown). We also found positive associations between variations in SAT-
258 *COL4A1* expression and variations in both SAT-*TGFβ1* (Figure 2G) and SAT-*TGFβ3* (Figure
259 2H) between baseline and six months post-surgery. Of note, the other previously explored
260 BM components were also positively correlated to SAT-*TGFβ1* or SAT-*TGFβ3* expression.
261 To a lesser extent, we also found significant positive associations between the variations in
262 *LAMC1*, *HSPG2*, and *SPARC* expression and those of *TGFβ1* and *TGFβ3* (**Figure S2D**).
263 These associations suggest potential relationships between pro-fibrotic TGFβ isoforms and
264 the modulation of BM components, particularly *COL4A1*.

265

266 **TGFβ1 and TGFβ3 effects on human adipocytes and endothelial cells**

267 Since SAT-*TGFβ1* and SAT-*TGFβ3* were associated with upregulated SAT-*COL4A1*
268 expression (and other BM components), we hypothesized that these pro-fibrotic factors could
269 induce the synthesis of BM components in AT cells. TGFβ1 is indeed known to induce
270 *COL4A1* over-expression in epithelial cells (31). We used a previously described 3D model to
271 test the effects of these TGFβ isoforms on the genetic expression of two BM components
272 (*COL4A1*, *LAMC1*) and a series of ECM remodeling markers (*COL3A1*, *COL6A3*, *SPARC*,
273 and *LOX*, as well as *CTGF*, *TGFβ1*, *PAI-1*, and *α-SMA*) and inflammation markers (*CCL2*,
274 *IL-6*), in isolated human lean adipocytes and EC.

275 Firstly, we observed that TGFβ1 treatment induced significant over-expression of *PAI-1*,
276 *TGFβ1*, *CTGF* and *IL6* in both 3D cultured adipocytes (Figure 3A) and EC (Figure 3C).
277 TGFβ1 treatment also increased *SPARC* and *LOX* expression in both types of isolated cells.

278 In contrast, the gene expression of BM components (*COL4A1*, *LAMC1*) and other collagens
279 (*COL3A1*) in isolated adipocytes was not significantly changed by TGFβ1 stimulation (Figure
280 3B). *COL4A1* and *COL6A3* expression were however significantly induced by TGFβ1 in EC

281 (Figure 3D). As observed for TGF β 1, TGF β 3 treatment also provoked a significant up-
282 regulation of *PAI-1*, *TGF β 1*, *α -SMA* and *IL6* in both 3D cultured adipocytes (Figure 4A) and
283 EC (Figure 4C). *CTGF* expression was also strongly induced under these conditions (Figure
284 4C).

285 Importantly, TGF β 3 treatment only induced expression of *COL4A1*, *COL6A3*, and *LOX* in EC
286 (Figure 4D).

287

288 **Discussion**

289 Here we have provided novel insights regarding the expression of BM components in
290 adipocytes and EC, and their modifications in AT during human obesity. We have shown that
291 adipocytes from obese AT samples exhibit augmentations in several BM components.
292 Amongst them *COL4A1* expression positively associates with markers of **insulin resistance**,
293 and with two TGF β isoforms in obesity. *In vitro* data on human cells indicated that while
294 TGF β 1 and TGF β 3 can stimulate remodeling and the up-regulation of pro-fibrotic and
295 inflammatory genes in both adipocytes and EC, they can only induce *COL4A1* expression in
296 EC, and not in isolated human adipocytes.

297 The BM of EC has been characterized in many tissues, but rarely in human AT, and has never
298 been compared to isolated adipocytes. We observed that EC and adipocytes express common
299 BM components such as a collagen IV protomer (α 1 α 1 α 2), a laminin heterotrimer (containing
300 the γ 1 chain), nidogen and perlecan. While also detected in both of these cell types, transcripts
301 of the matricellular protein SPARC were more highly expressed in adipocytes. This
302 glycoprotein exhibits diverse functions, such as modulating the interaction of cells with the
303 ECM (both the BM and the interstitial matrix) and BM permeability.

304 By focusing our study on a few major BM components, we can theorize that while adipocytes
305 and EC share some common structural components, organizational differences could be

306 highlighted by variable *SPARC* gene expression, which as others modulates BM architecture
307 to adapt to cell functions.

308 Obesity induces significant BM component modifications in both EC and adipocytes, as
309 illustrated by increased deposition of collagen IV protein around these cells, and increased
310 BM gene expression in obese compared to lean adipocytes. The increase in BM components
311 was also greater in SAT compared to VAT. Our team recently highlighted the importance of
312 collagen accumulation located not only around adipocytes but also EC (i.e. called pericellular
313 fibrosis) in obese AT (7). Here it is tempting to consider that increases in BM components
314 around these cells, such as collagen IV, contribute to peri-adipocyte and peri-vascular
315 fibrosis. Additional detailed imagery analysis of human AT would be necessary to provide
316 further insights regarding the structural organization of pericellular fibrosis.

317 In many tissues such as kidney or retina, BM remodeling is related to insulin resistance and a
318 hyperglycemic environment. We have previously shown that pericellular fibrosis in AT is
319 associated with deterioration of metabolic parameters, and also with diabetes in severe obesity
320 (3,32). BM components were not explored in this pathological context, instead focus was
321 made on collagen VI, a type of collagen, which forms the interface between BM and thick
322 bundles of collagen I. The expression of collagen VI in AT has been correlated to glucose
323 metabolism impairment in human populations (33). Here, we have shown that the expression
324 of collagen IV, a major component of adipose BM, also associates with several variables
325 related to glucose homeostasis and insulin-resistance in obese individuals with impaired
326 fasting glucose and naïve of treatment. While this quantitative association was not observed at
327 baseline in more severe forms of obesity, *COL4A1* expression in AT significantly decreased
328 six months after bariatric surgery, and was associated with improved metabolic condition.
329 This observation correlates with a previous study from our team demonstrating that the gene
330 expression of several types of collagen were down-regulated in AT one year after gastric
331 bypass (32). In the current study, subjects with the greatest HOMA-IR improvement had the

332 lowest *COL4A1* expression six months after the surgery. These data are only correlative, but
333 they do help predict the impact of hyperglycemic/hyperinsulinemic tissue environments on
334 BM component synthesis in AT cells.

335 Deeper insights into molecular interactions are also needed. As such, TGF β isoforms are
336 relevant candidates as in addition to their pro-fibrotic role, TGF β 1 can induce collagen IV
337 expression and BM thickening in other tissues, such as kidney and retina. This link may also
338 exist in AT. Our association studies indeed showed strong positive correlations between
339 *COL4A1* and *TGF β 1* expression in the SAT of severely obese subjects, both prior to, and six
340 months post-bariatric surgery. Similar associations were found for the TGF β 3 isoform, also
341 highly expressed in AT. These observations prompted us to examine whether both TGF β
342 isoforms could induce the expression of *COL4A1* and other remodeling genes in human
343 isolated adipocytes and EC, using a previously described *in vitro* 3D model (26). Both TGF β 1
344 and TGF β 3 induced the expression of several pro-fibrotic and inflammatory genes in
345 adipocytes and EC, but *COL4A1* expression was only significantly induced in EC.

346 This raises important questions about whether other pro-fibrotic stimuli can induce adipocyte
347 BM remodeling and change ECM composition in obese AT. In our 3D model, we tested other
348 known collagen IV-inducers, such as activin A, but neither induced BM component
349 expression in isolated adipocytes (data not shown).

350 Recently, one study demonstrated that another TGF β superfamily member, BMP4, induces
351 *COL4A1* and *COL4A2* expression in isolated glomeruli from mouse kidneys (34). While a
352 recent study highlighted that BMP4 has an anti-inflammatory effect in adipocytes, its action
353 on collagen IV expression in adipocytes should also be explored (35).

354 **While obesity is characterized by deregulated production of adipokines, it remains also**
355 **to understand whether the two main adipokines, leptin and adiponectin, could also**
356 **contribute to fibrosis development in AT. Indeed in liver, leptin exerts pro-fibrotic**
357 **impact while adiponectin is anti-fibrotic (36). In a recent in vitro study performed on**

358 **HUVEC, leptin treatment induced collagen IV over-secretion while when adding**
359 **adiponectin, collagen IV secretion returns to control secretion (37). Based on these**
360 **findings, it would be interesting to assess further the effects of these adipokines on the**
361 **secretion of collagen IV in endothelial cells and adipocytes. Overall further studies are**
362 **needed to understand how the production of basement membrane components by**
363 **adipocytes is regulated.**

364 Treating isolated adipocytes and EC with TGF β isoforms induced *LOX* expression, an
365 enzyme that plays a key role in catalyzing covalent collagen crosslinks in the extracellular
366 environment, and regulates ECM mechanical properties. Both adipocytes and EC may thus
367 contribute to AT fibrosis via the thickening of their own BM due to pro-fibrotic stimuli. We
368 have also shown that *LOX* expression is induced in obese AT (28) and is decreased after
369 weight loss (32) suggesting modulation of collagen cross-linking with subsequent effects
370 impacts on cell constraints. **Furthermore, AT tensile strength has been shown to be a**
371 **feature that promotes co-morbidities development (38) suggesting a potential role of BM**
372 **component in this phenomenon.**

373 Our laboratory indeed demonstrated that adipocytes could also be considered as mechano-
374 sensitive cells subject to bilateral mechanical forces during obesity (28). On one hand, lipid
375 droplet growth exerts physical stress from inside the cell, and on the other, AT fibrosis
376 characterized by fibrillar cross-linked collagen can create constraints from the outside in.
377 Interestingly, collagen IV knockout studies in *C. elegans* and mice have demonstrated that
378 this BM component is crucial in enabling cells to respond to mechanical constraints by
379 transmitting signals between cells and interstitial matrix (39,40). It is thus tempting to
380 suggest that collagen IV accumulation around obese adipocytes could be governed by
381 mechanical stresses, either induced by lipid droplet enlargement and/or fibrosis accumulation.
382 Further studies are needed to investigate this aspect in more depth.

383 In conclusion we have described major BM component modification in adipocytes and EC
384 during obesity. Collagen IV is a major BM component associated with *in vivo* metabolic
385 alterations in human obesity, thus future molecular studies are needed to determine both its
386 regulation and contribution to altered adipocyte biology.

387

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397

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531 **Figure legends**

532 **Figure 1: BM components in lean and obese human adipose tissue and isolated adipocyte**
533 **and endothelial cells.**

534 (A-D) Collagen IV immunofluorescence in lean (**n=5**) (A) and obese (**n=5**) human
535 subcutaneous adipose tissue collected by surgical biopsy (B) Collagen IV immuno-electron
536 microscopy in lean (C) and obese (D) human subcutaneous adipose tissue. Representative
537 samples are shown from a set of five explored samples. (E) *COL4A1*, *LAMC1*, *NID-1*, *HSPG2*
538 (perlecan), and *SPARC* expression was quantified by real-time PCR and normalized to *18S* in
539 adipocytes (n=12; white bar) and compared to endothelial cells (n=5; grey bar) from lean
540 subcutaneous adipose tissue. (F)- *COL4A1*, *LAMC1*, *NID-1*, *HSPG2* (perlecan), and *SPARC*
541 expression was quantified using real-time PCR and normalized to *18S* in lean (n=12; white
542 bar) or obese (n=12; black bar) adipocytes. (G-J) Collagen IV immunohistochemistry in either
543 lean (**n=5**) human subcutaneous, (G) or visceral adipose tissue (I); and in obese (**n=5**) human
544 subcutaneous (H) or visceral adipose tissue (J). Representative samples are shown after the
545 histological analysis of 5 subjects for each condition. (K) *COL4A1*, *LAMC1*, *NID-1*, *HSPG2*
546 (perlecan), and *SPARC* expression was quantified by real-time PCR and normalized to *18S* in
547 adipocytes isolated from either obese subcutaneous (n=16; black bars) or visceral adipose
548 tissue (n=16; hatched bars). **For RT-qPCR, values are obtained from the average of the**
549 **reference sample Ct.** Ad: adipocytes; EC: endothelial cells; SAT: subcutaneous adipose
550 tissue; VAT: visceral adipose tissue; arrow: collagen IV. Data are expressed as mean \pm SEM.
551 Significant differences between groups are indicated *** p<0.001; **p<0.01; *p <0.05 Mann-
552 Whitney's test or Wilcoxon's test for subcutaneous versus visceral adipose tissue comparison.

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556 **Figure 2: Decreased expression of collagen IV, TGFβ1, and TGFβ3 genes in**
557 **subcutaneous adipose tissue following weight-loss is associated with improved glucose**
558 **metabolism parameters.**

559 (A). *COL4A1*, (C) *TGFβ1*, and (D) *TGFβ3* gene expression was quantified by real-time PCR
560 and normalized to *18S* in human obese subcutaneous adipose tissue before (black bars) and
561 after weight loss (hatched bars). T0: Before weight loss; T6: six months after weight loss;
562 SAT: subcutaneous adipose tissue. (B). Average *COL4A1* expression in subcutaneous adipose
563 tissue after patient samples were clustered depending on HOMA-IR improvement six months
564 after surgery. Data are expressed as mean ± SEM. Statistically significant differences between
565 groups are indicated ** p<0.01; *p <0.05. n=25. Correlation analysis between subcutaneous
566 adipose tissue *COL4A1* gene expression and *TGFβ1* before (E) and six months after surgery
567 (G); and *TGFβ3* before (F) and six months after surgery (H). Wilcoxon's tests were used in
568 (A), (B), (C), and (D), and Pearson's correlations were used in (E), (F), (G), and (H). n=25
569

570 **Figure 3: TGFβ1 effects on ECM and inflammatory genes in 3D adipocytes and**
571 **endothelial cells isolated from lean human subcutaneous adipose tissue.**

572 3D adipocytes and endothelial cells were treated for 48h with 5 ng/mL recombinant TGFβ1.
573 (A-B) Inflammatory genes were quantified by Quantiplex Assays in either non-treated (white
574 bars) or treated (black bars) 3D adipocytes (A); and non-treated (light grey bars) or treated
575 (dark grey bars) endothelial cells (B). (C-D) ECM remodeling genes were quantified by
576 Quantiplex Assays in either non-treated (white bars) or treated (black bars) 3D adipocytes
577 (C); and non-treated (light grey bars) or treated (dark grey bars) endothelial cells (D). Ad:
578 adipocyte (n=12); EC: endothelial cells (n=8). Statistically significant differences between
579 groups are indicated *** p<0.001; ** p<0.01; *p <0.05 Wilcoxon's test.

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581 **Figure 4: TGFβ3 effects on ECM and inflammatory genes in 3D adipocytes and**
582 **endothelial cells isolated from lean human subcutaneous adipose tissue.**

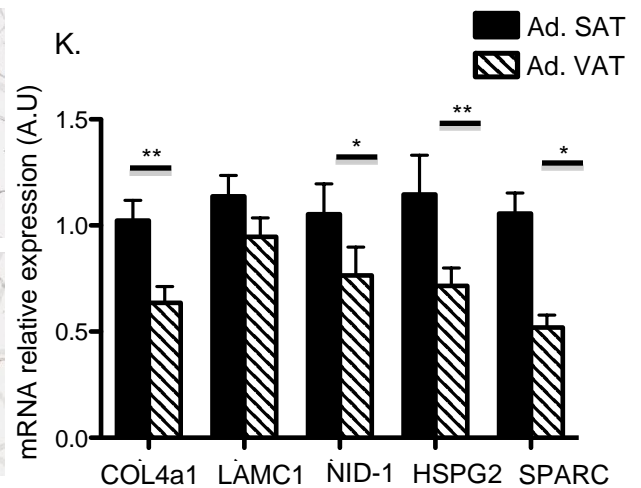
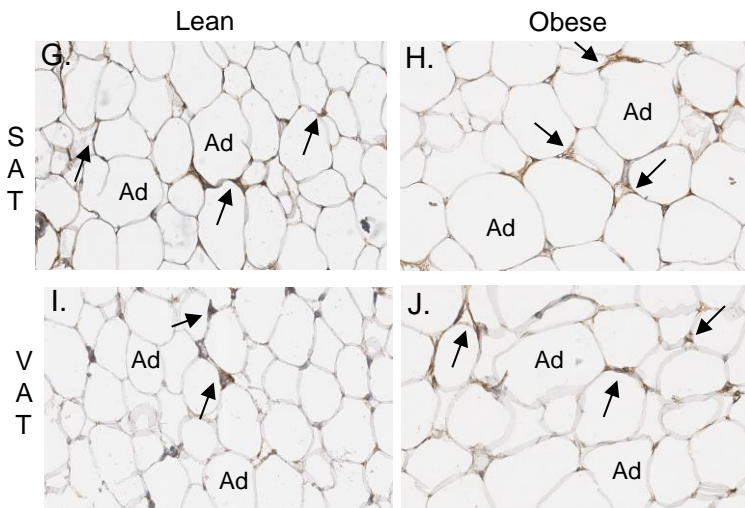
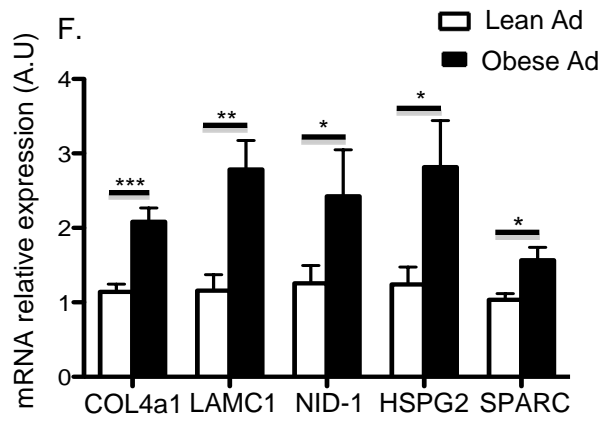
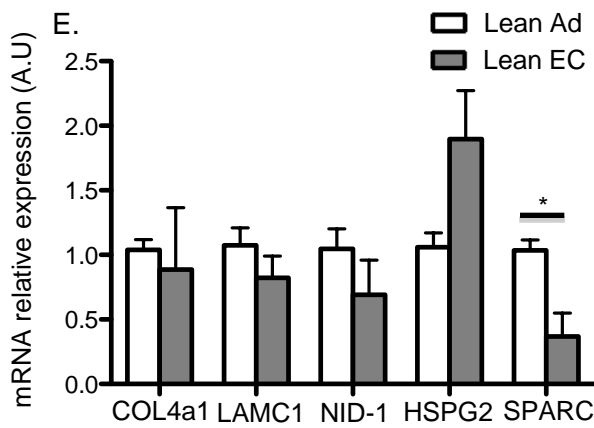
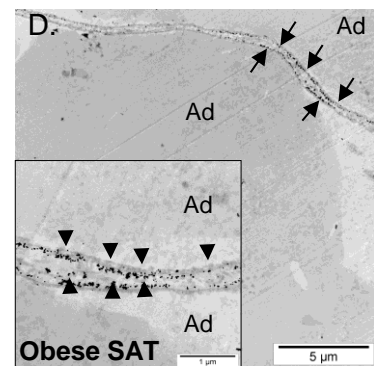
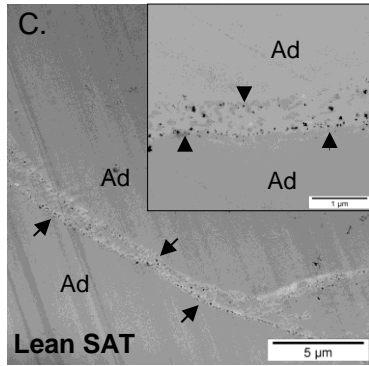
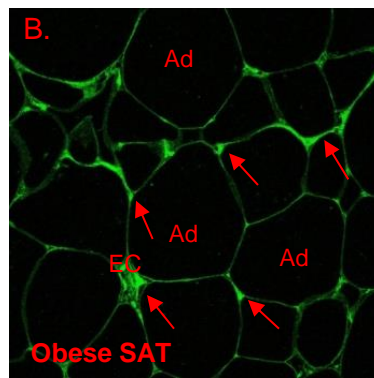
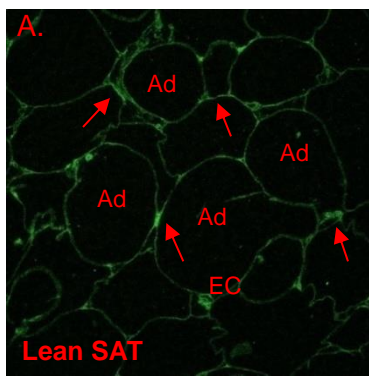
583 3D adipocytes and endothelial cells were treated for 48h with 5 ng/mL recombinant TGFβ3.
584 A-B Inflammatory genes were quantified by Quantiplex Assays in either non-treated (white
585 bars) or treated (black bars) 3D adipocytes (A); and non-treated (light grey bars) or treated
586 (dark grey bars) endothelial cells (B). C-D ECM remodeling genes were quantified by
587 Quantiplex Assays in either non-treated (white bars) or treated (black bars) 3D adipocytes
588 (C); and non-treated (light grey bars) or treated (dark grey bars) endothelial cells (D). Ad:
589 adipocyte (n=9); EC: endothelial cells (n=6). Statistically significant differences between
590 groups are indicated *** p<0.001; ** p<0.01; *p <0.05 Wilcoxon's test.

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Figure 1



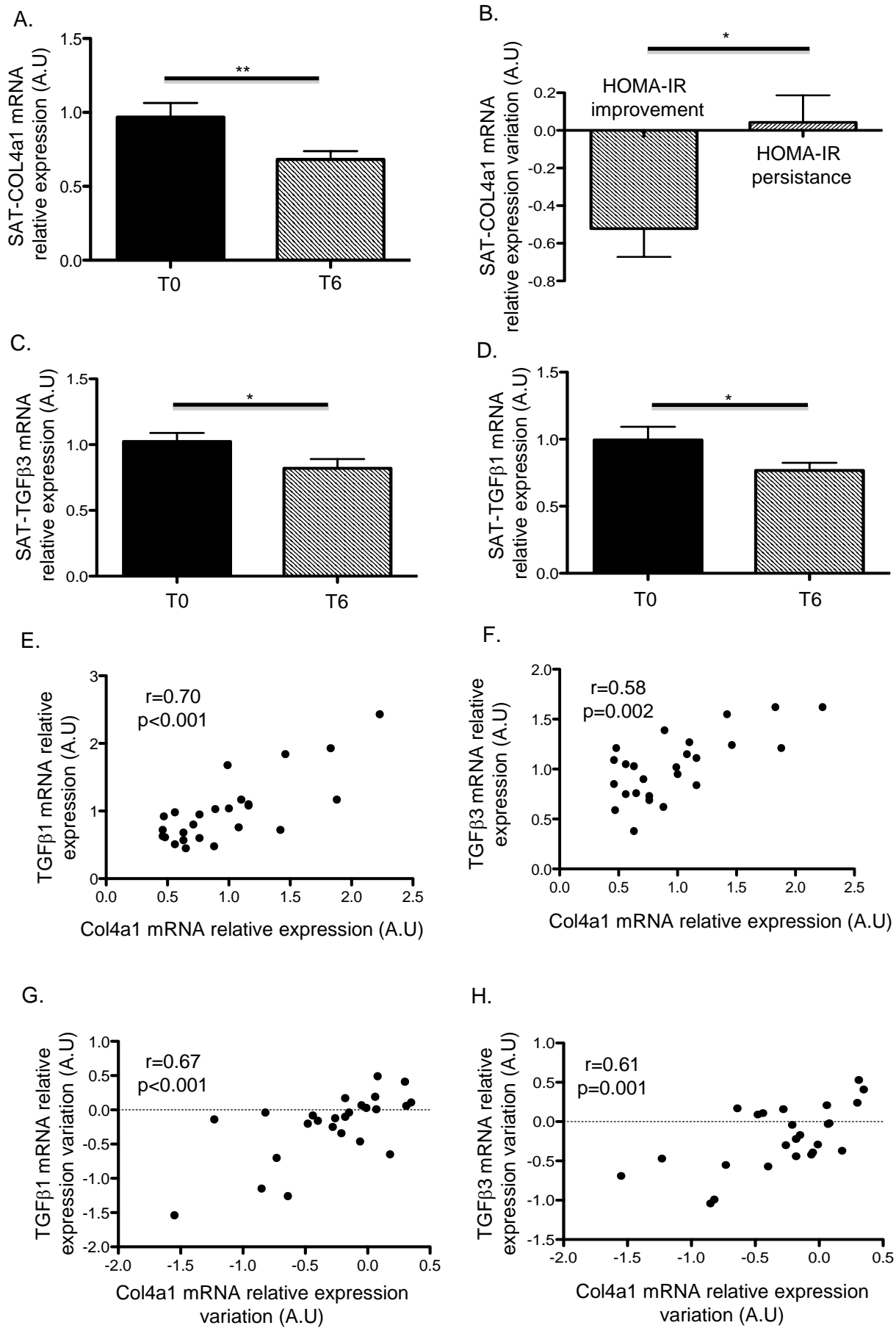


Figure 3

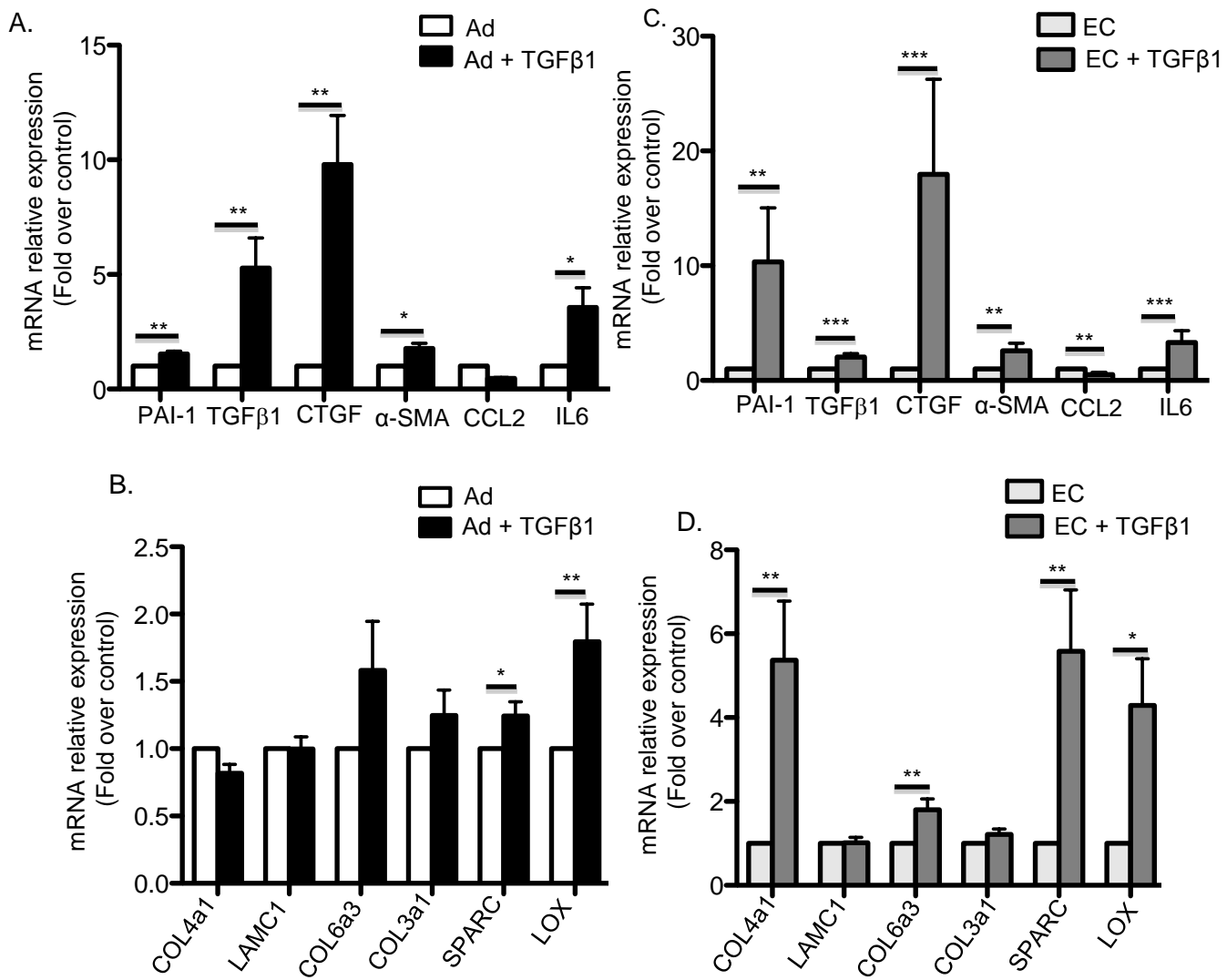


Figure 4

