Angiocrine polyamine production regulates adiposity

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Running title: Endothelial cell-adipocyte metabolic crosstalk

Abstract

Reciprocal interactions between endothelial cells (ECs) and adipocytes are fundamental to maintain white adipose tissue (WAT) homeostasis. This is illustrated by the activation of angiogenesis upon WAT expansion, a process that is impaired in obesity. However, the molecular mechanisms underlying the crosstalk between ECs and adipocytes remain poorly understood. To explore this communication, we promoted enhanced cell-autonomous angiogenesis by deleting *Pten* in the murine endothelium. Endothelial *Pten* loss led to a WAT-selective phenotype, characterized by reduced body weight and adiposity in pathophysiological conditions. This phenotype stemmed from enhanced fatty acid β-oxidation in ECs concomitant with a paracrine lipolytic action on adipocytes, accounting for reduced adiposity. Combined analysis of murine models, isolated ECs and human specimens revealed that EC-induced lipolysis was mediated by mTORC1-dependent production of polyamines. Our results uncover an unprecedented role for angiocrine metabolic signals in tissue homeostasis with important pathological implications.

Introduction

1 Endothelial cells (ECs) line the lumen of the entire vascular system and regulate the dynamic
2 passage of materials and cells. They are located ubiquitously over a uniquely large surface of 4000-
3 7000 m^2 covering the interface between the blood and tissues[1]. This vast contact area permits
4 precise environmental sensing, nutrient transport, and signalling integration from surrounding
5 tissues. Therefore, ECs are regarded as the nutrient gatekeepers of the organism. Despite of this,
6 the role of ECs in the regulation of systemic metabolism and as potential mediators of metabolic
7 disorders remains enigmatic[2,3].

8 Adult ECs are largely quiescent except in some metabolic tissues where vascular expansion
9 is considered the direct response to some tissue adaptations. This is the case for white adipose
10 tissue (WAT) during lipid accumulation[2] or muscle during exercise[4,5] in which adaptations to tissue
11 function are accompanied by vascular growth. ECs mainly expand by angiogenesis, a process in
12 which ECs sprout, branch, connect, and remodel into functional vessel circuits[6,7]. Angiogenesis is
13 guided by several extracellular cues, including growth factors, mechanical forces, flow, and
14 extracellular matrix proteins, that collectively converge on intracellular growth pathways such as
15 phosphatidylinositol 3-kinase (PI3K)/AKT/mTOR and RAS/MAPK/ERK[7,8]. Despite ECs being the first
16 line of nutrient sensing and distribution, the role of nutrients in relation to angiogenesis, and their
17 potential impact in pathophysiology is unclear.

18 PTEN (phosphatase and tensin homolog) is a lipid phosphatase which dephosphorylates
19 membrane phospholipids generated by the class I PI3Ks[9,10], the so called phosphatidylinositol 3,4,5-
20 trisphosphate (PtdIns(3,4,5)P_3, also known as PIP_3). Through the generation of PIP_3, the PI3K/PTEN
21 signalling pathway controls a plethora of cellular functions, including growth, proliferation, migration,
22 metabolism, and vesicular trafficking[8]. ECs are exquisitely sensitive to PI3K fluctuations, with both
23 too much and too little PI3K signalling resulting in defects in vascular development[11,12]. Analysis at
24 single cell resolution has shown that genetic manipulation of the PI3K/PTEN pathway primarily
25 impacts cell proliferation[11,13,14].

26 Here we show that ECs, through the release of specific angiokines, regulate the function of
27 WAT with systemic metabolic implications. Forced activation of the PI3K pathway in ECs results in
28 increased proliferation of the vessel compartment in the adipose tissue and reduced adiposity. The
29 molecular connection between the endothelial function and adipocyte biology is underscored by a
30 metabolite-based angiocrine communication, a process mediated by polyamines. We provide
31 evidence showing that this unprecedented metabolic axis is relevant in pathophysiological
32 conditions, such as obesity.
33

Results

Endothelial *Pten* deletion elicits a lean phenotype

To elucidate endothelium-regulated systemic metabolic processes, we developed a model of enhanced EC-autonomous angiogenesis. Given that EC proliferation is primarily governed by PI3K signalling[11,13,14], we studied the systemic consequences of sustained PI3K activity in the endothelium by *Pten* loss[14]. We bred $Pten^{flox/flox}$ mice[15] with *PdgfbiCreER* transgenic mice[16] (hereafter referred as $Pten^{i\Delta EC}$), which enables the deletion of *Pten* in ECs in a tamoxifen-inducible manner. 4-hydroxytamoxifen (4-OHT) was administered postnatally to mice carrying conditional *Pten* knockout alleles alone or in combination with *PdgfbiCreER*. To confirm *Pten* deletion selectively in the endothelium, we took advantage of the Ribotag model[17]. This strategy allows for the analysis of actively translated mRNAs in a cell-specific manner *in vivo*, when Cre recombinase induces the expression of hemagglutinin A (HA)-tagged ribosomal protein Rpl22. We crossed these mice with *PdgfbiCreER* (hereafter referred as $Ribotag^{iHAEC}$) and demonstrated that only the endothelium was targeted in all tissues analysed (Supplementary Fig. 1a,b). We could confirm that the deletion of *Pten* in the endothelium occurred in all tissues studied in $Pten^{i\Delta EC}$-$Ribotag^{iHAEC}$ mice (Supplementary Fig. 1c). Also, we validated the efficient depletion of *Pten* in isolated ECs from several tissues of $Pten^{i\Delta EC}$ mice (Supplementary Fig. 1d-g).

Next, we evaluated the phenotypic consequences of endothelial-specific *Pten* deletion in adult mice. Endothelial *Pten* loss was associated with a remarkable reduction in body weight and adiposity compared to control littermates, thus resulting in a lean phenotype (Fig. 1a, b). We found a selective reduction in WAT mass and adipocyte area, including both epididymal (eWAT) and inguinal (iWAT) depots (Fig. 1b-h and Supplementary Fig. 2a), a phenotype that was robust by 10 weeks of age. Nuclear magnetic resonance analysis further confirmed the reduction in overall fat mass in adult $Pten^{i\Delta EC}$ mice (Supplementary Fig. 2b,c). Detailed histopathological and molecular characterization did not reveal ectopic lipid deposition in muscle and liver (Fig. 1i), WAT fibrosis (Fig. 1j and Supplementary Fig. 2d,e), altered adipocyte differentiation or cell death (Supplementary Fig.

60 2f-j). Of note, in line with the reduction in adipose tissue mass, the circulating levels of leptin (an
61 adipose-derived adipokine) were markedly reduced in mutant mice (Supplementary Fig. 2k).

62 To explore the impact of loss of endothelial *Pten* on the vasculature, we performed whole-
63 mount or OCT-embedded section staining with isolectin B4 (IB4) or anti-CD31, two well-known
64 markers of ECs, in several tissues. The vast majority of tissues analysed in *Pten*$^{i\Delta EC}$ mice remained
65 phenotypically unaffected at 12 weeks of age despite the deletion of this gene (Supplementary Fig.
66 3a,b). However, we observed vascular hyperplasia in the *Pten*$^{i\Delta EC}$ WAT, based on a progressive
67 increase of vascular content and ECs proliferation, as illustrated by enhanced IB4, ERG (EC-specific
68 nuclear marker) and Ki67 staining (Fig. 2a-h). The vascular phenotype was already apparent by 5-
69 weeks of age (Fig 2a-h), a time point at which adiposity remained largely unaltered (Fig. 1d, g);
70 indicating that vessel growth precedes the reduction in adiposity. The histological data were largely
71 consistent with the molecular evaluation of endothelial markers using quantitative real-time PCR
72 (Supplementary Fig. 3c). Of note, we could not observe VEGF signalling alterations in WAT by
73 means of *VegfA* mRNA abundance, thus suggesting no major role of this growth factor in the
74 enhanced vascularization of *Pten*$^{i\Delta EC}$ mice (Supplementary Fig. 3d). The enhanced vascular content
75 in *Pten*$^{i\Delta EC}$ WAT was not associated with aberrant leakiness (Supplementary Fig. 3e). Due to the
76 regulatory and functional analogies with WAT, we performed a detailed analysis of brown adipose
77 tissue (BAT). *Pten*$^{i\Delta EC}$ BAT showed a mild increase in the vascular content compared to control
78 littermates (Supplementary Fig. 3a,b), although the vascular phenotype was less prominent than in
79 WAT. In line with this, we could not observe alterations in the morphology of brown adipocytes
80 (Supplementary Fig. 3f).

81 To validate the contribution of endothelial-specific *Pten* deletion on vessel density and
82 adiposity, we carried out two complementary approaches. First, we ruled out developmental effects
83 associated with early activation of Cre by studying the consequences of *Pten* deletion in adult mice.
84 Administration of tamoxifen at 8 weeks of age in *Pten*$^{i\Delta EC}$ mice recapitulated the phenotype in
85 vascularity and adiposity (Supplementary Fig. 4a-h). We also took advantage of a second inducible

86 Cre delivery system targeted to the endothelium, under the control of the *VE-Cadherin* promoter[18];
87 the so called *Cdh5-CreER^{T2}*. 4-OHT administration in postnatal mice resulted in a similar phenotype
88 to that of the *PdgfbiCreER* model, albeit milder (Supplementary Fig. 4i-p). Together, these results
89 reveal an adipose tissue-specific contribution of endothelial *Pten* that enables the control of
90 adiposity.
91

92 **Endothelial *Pten*-loss protects from obesity**

93 Excessive adiposity is a hallmark of obesity and is associated with suboptimal WAT
94 vascularization[2,3,19-21]. Thus, we interrogated whether obesity-associated reduced vascularization in
95 experimental systems and human specimens could be correlated with alterations in PTEN levels.
96 The elevated adiposity elicited by high-fat diet (HFD) feeding in mice was associated with reduced
97 vascularization and higher expression of *Pten* in the adipose endothelium (Supplementary Fig. 5a-
98 c). We confirmed that reduced vascularization and elevated expression of *PTEN* is also observed in
99 surgical specimens of visceral adipose tissue (VAT) from obese individuals (Supplementary Fig. 5d-
100 f, refer to Material and methods for details on the human cohort).

101 Given that induction of angiogenesis in WAT reportedly ameliorates obesity-associated
102 metabolic complications[22-26], we predicted that our mutant mouse model would be protected from
103 obesity. First, we challenged control and *Pten$^{i\Delta EC}$* mice with HFD. Loss of *Pten* in this context led to
104 reduced body weight, WAT mass and adipocyte area (Fig. 3a-d), with no significant changes in other
105 tissues (Supplementary Fig. 5g). Similar to mice fed with chow diet, *Pten$^{i\Delta EC}$* mice showed higher
106 vascularization in WAT upon exposure to HFD (Fig. 3e,f). Moreover, *Pten* deletion in ECs resulted
107 in improved glucose metabolism in mice fed with HFD for 10 weeks (Fig. 3g and Supplementary Fig.
108 5h-j). In line with previous observations, we did not detect overt alterations in WAT fibrosis in *Pten$^{i\Delta EC}$*
109 mice under HFD (Supplementary Fig. 5k,l). Second, we evaluated the consequences of endothelial-
110 specific deletion of *Pten* in established obesity (Fig. 3h). Administration of tamoxifen in mice fed with
111 HFD for 12 weeks resulted in a robust correction of body weight, reduced adiposity, increased WAT

112 vascularity, and improved glucose tolerance in the absence of WAT fibrosis (Fig. 3h-n, and
113 Supplementary Fig. 5m-q). Together, these data reveal that endothelial PTEN is instrumental for the
114 control of adiposity in pathophysiological conditions.

115

116 **Endothelial *Pten*-loss leads to enhanced WAT metabolic rate**

117 To address how loss of *Pten* in the endothelium resulted in reduced adiposity, we monitored
118 factors involved in nutritional uptake and energy consumption in the mouse model. Intriguingly, the
119 phenotype of *Pten*$^{i\Delta EC}$ mice was not due to differences in food intake, intestinal malabsorption,
120 locomotor activity, BAT thermogenesis, or Ucp1-dependent or independent WAT browning (Fig. 4a-
121 h). However, we did detect a significant increase in energy expenditure in mutant mice, consistent
122 with reduced adiposity (Fig. 4i). In line with this, the plasma triglyceride concentration was reduced
123 in *Pten*$^{i\Delta EC}$ mice compared to controls (Fig. 4j). To associate the energy expenditure phenotype with
124 the reduction in adiposity, we sought to assess metabolic activity at tissue resolution. We evaluated
125 mitochondrial function *ex vivo* by high-resolution respirometry in various freshly isolated tissues.
126 Only WAT, the tissue exhibiting the most prominent phenotype in *Pten*$^{i\Delta EC}$ mice, presented an
127 increase in mitochondrial respiration (Fig. 4k-n). In summary, our results reveal a causal association
128 between EC proliferation, increased local and systemic energy expenditure and reduced adiposity.

129

130 ***Pten*-deficient EC proliferation relies on fatty acid metabolism**

131 The increased local energy consumption in WAT suggested that this phenotype could be
132 due, at least in part, to enhanced metabolic activity by the mutant endothelium. Thus, we interrogated
133 whether the unique access to lipids in the adipose tissue could favour the activation of lipid catabolic
134 transcriptional programs in *Pten*$^{i\Delta EC}$ WAT endothelium to promote enhanced vascularization
135 selectively in this tissue. First, we evaluated endothelial expression of genes involved in lipid
136 catabolism *in vivo* (using *Pten*$^{i\Delta EC}$-*Ribotag*^{iHAEC} mice, Fig. 5a) and in isolated adipose ECs *(*Fig. 5b).
137 Upregulated genes in *Pten*$^{i\Delta EC}$-*Ribotag*^{iHAEC} WAT endothelium included fatty acid transporters, genes

138 involved in fatty acid β oxidation (FAO), and central regulators of cellular energy metabolic pathways.
139 In line with our previous results, the activation of this program was unique to WAT endothelium, with
140 no major differences in the endothelium of BAT, muscle, and liver (Supplementary Fig. 6a-c). The
141 lipid catabolic reprogramming was associated with enhanced fatty acid oxidation (FAO; Fig. 5c).
142 Consistently, we observed increased mitochondrial respiration when lipids were supplemented in
143 the media (Fig. 5d,e), whereas no changes in glucose uptake (Supplementary Fig. 6d) or in glucose-
144 induced oxygen consumption were observed (Fig. 5f,g). We speculated that the vascular phenotype
145 in WAT was the result of increased local fatty acid availability. Indeed, cultured *Pten*$^{i\Delta EC}$ ECs derived
146 from lung, a tissue that did not manifest a phenotype *in vivo* (Supplementary Fig. 3c), exhibited
147 biological and metabolic features similar to adipose ECs when lipids were supplied in the media
148 (Supplementary Fig. 6e-i). To further investigate this, we evaluated the impact of etomoxir (a
149 pharmacological inhibitor of FAO) on cell proliferation[27]. Etomoxir hampered the proliferative burst
150 elicited by *Pten* loss in adipose ECs *in vitro* and *in vivo* (Fig. 5h-j), without overt macroscopic or
151 weight alterations in any tissue analysed (Supplementary Fig. 6j). Next, we undertook a genetic
152 approach to selectively interfere with lipid catabolism in ECs *in vivo*. Our gene expression data
153 showed a prominent increase in genes related to lipid transport, fatty-acid β-oxidation and oxidative
154 phosphorylation, which are transcriptionally regulated by PGC1 family factors (Fig. 5a,b). Also, we
155 identified a mild upregulation of *Ppargc1b*, the transcript that encodes for Pgc1β (Fig. 5a,b). Hence,
156 we took advantage of *Pgc1β* conditional knockout mice[28], which we bred into the endothelial-specific
157 *Pten* deficient mice. *Pgc1β* deletion in the context of endothelial *Pten* loss partially rescued the
158 elevation in adipose vascular density (Supplementary Fig. 6k,l). Together, these data indicate that
159 *Pten* deletion makes ECs prone to proliferate in the presence of fatty acids, which represents a
160 microenvironmental prerequisite for these cells.
161
162 **Polyamines are pro-lipolytic angiocrine metabolic mediators**

163 Whereas FAO was necessary to sustain adipose EC proliferation, adiposity remained
164 reduced both upon pharmacological and genetic blockade of fatty acid catabolism (Fig. 5k, and
165 Supplementary Fig. 6m). These data suggest that reduced adiposity is not solely the consequence
166 of the proliferative burst of *Pten*$^{i\Delta EC}$ adipose endothelium. We hypothesized that angiocrine signals
167 induced by *Pten* deletion were instructing a lipolytic response in adipocytes that would explain the
168 lack of rescue in adiposity by inhibition of FAO. To explore this, we measured basal and stimulated
169 fatty acid secretion into the media. Explants from *Pten*$^{i\Delta EC}$ WAT exhibited higher release of free fatty
170 acids (FFA) than control counterparts (Supplementary Fig. 7a, Fig. 6a). This greater effect was
171 confirmed in primary adipocyte cultures upon exposure to conditioned media derived from control or
172 *Pten*$^{i\Delta EC}$ ECs (Fig. 6b). In line with this, fasted *Pten*$^{i\Delta EC}$ mice exhibited elevated circulating free fatty
173 acids (FFA) (Fig. 6c). Collectively, these data demonstrate that endothelial-specific *Pten* deletion
174 leads to elevated lipid mobilization from the adipose tissue. Stimulation of the beta-adrenergic
175 receptors (betaADR) activates lipolysis in WAT[29-31]. Thus, we tested the involvement of these
176 membrane proteins in the lipolytic phenotype observed in *Pten*$^{i\Delta EC}$ mice. We treated mutant animals
177 with the pan-betaADR inhibitor propranolol for 4 days and then measured lipolysis in WAT explants.
178 The results showed that propranolol reduced lipolysis in the explants, thus demonstrating that
179 betaADR receptor activity is relevant for endothelial *Pten* loss-induced lipolysis in WAT (Fig. 6d).

180 The lipolytic phenotype observed with conditioned media from *Pten*$^{i\Delta EC}$ ECs suggests that an
181 angiocrine signal may be responsible for the metabolic effects described above. Angiocrine signals
182 control a variety of pathophysiological processes and are emerging as key mediators of EC function
183 and tissue homeostasis[32-34]. These molecular cues could be polypeptides, metabolites, or
184 extracellular vesicles[32-36]. To discern the identity of angiocrine signals in our model, we filtered
185 conditioned media to separate the metabolite fraction from that containing proteins and vesicles
186 (Supplementary Fig. 7b). Interestingly, only the *Pten*$^{i\Delta EC}$ metabolite-rich conditioned media fraction
187 retained lipolytic activity (Fig. 6e,f). These data indicate that the angiocrine signal which stimulates
188 lipolysis in *Pten*$^{i\Delta EC}$ WAT is a metabolite.

189 Next, we investigated in further detail the metabolic nature of the lipolytic angiocrine signal.
190 To this end, we searched for metabolic pathways fulfilling three criteria: i) to be previously linked to
191 adiposity, ii) to exert paracrine biological effects, and iii) to respond to PI3K activity. Polyamines
192 satisfy these criteria, as they can function in a paracrine manner[37-39], reduce adiposity in the context
193 of obesity[40-44], and it was previously reported that PI3K activation enhances polyamine synthesis to
194 sustain oncogenicity[45,46]. Thus, we hypothesized that polyamines may act as WAT angiocrine
195 lipolytic signals. Polyamines are polycationic metabolites synthesized from methionine and
196 ornithine[38]. ^{13}C-methionine tracing revealed a remarkable increase in metabolites related to
197 polyamine biosynthesis in isolated *Pten*-deficient ECs, including ^{13}C-labelled spermidine and
198 spermine (Fig. 6g, Supplementary Fig. 7c). In line with these results, label-free metabolomics
199 confirmed the elevation in polyamine levels in $Pten^{i\Delta EC}$ isolated EC cultures and in $Pten^{i\Delta EC}$ WAT
200 (Fig. 6h,i and Supplementary Fig 7d, e). Moreover, elevated polyamine levels were observed in the
201 supernatant of $Pten^{i\Delta EC}$ EC, in agreement with their secreted nature (Fig. 6j).
202
203 **Polyamines activate betaADRs to promote lipolysis**
204 We next ascertained the role of polyamines in regulating WAT biology. Supplementation of
205 ^{13}C-Spermidine in adipocyte cultures showed that this polyamine was efficiently taken up (Fig. 7a).
206 As shown above, increased lipolysis in $Pten^{i\Delta EC}$ WAT is dependent on betaADR activation.
207 Interestingly, polyamines reportedly promote betaADR activity[47]. In turn, we tested whether
208 polyamines promoted the release of adipose FFA in a betaADR-dependent fashion.
209 Supplementation of polyamines to WAT explants increased lipolysis, an effect that was prevented
210 by propranolol (Fig. 7b, Supplementary Fig. 8a). Also, we showed that polyamines stimulated
211 lipolysis in freshly isolated primary adipocyte cultures in a betaADR receptor dependent manner (Fig.
212 7c, Supplementary Fig. 8b). BetaADR receptors signal through PKA activation and cAMP
213 production[29], and we could confirm that spermidine increased the production of intracellular cAMP
214 in adipocytes[47] (Fig. 7d, Supplementary Fig. 8c). The capacity of propranolol to block the action of

215 spermidine on betaADR in WAT explants suggests that there is sufficient natural agonist in the assay
216 (tissue-intrinsic sympathetic innervation) to sustain a basal betaADR activity. Indeed, we detected
217 the presence of these agonists by LC/MS in the adipose tissue explants employed in our assays
218 (Supplementary Fig. 8d). Overall, our data suggest that polyamines are angiocrine mediators of
219 lipolysis through the regulation of betaADR activity in adipocytes.

220 To demonstrate that angiocrine polyamines are key regulators of adiposity, we evaluated the
221 effect of polyamine supplementation *in vivo*. The administration of spermidine to diet-induced obese
222 mice for 6 weeks tempered the obesogenic phenotype, selectively reduced WAT mass and
223 adipocyte size, and improved glucose tolerance (Fig. 7e-j and Supplementary Fig. 8e-g). Of note,
224 polyamine supplementation in HFD significantly reduced food intake in mice (Supplementary Fig.
225 8h). To rule out that the effect of polyamines was solely due to reduced food intake, we performed
226 pair feeding studies in HFD-fed mice. After 10 weeks on HFD, vehicle-treated animals were heavier
227 than the ones supplemented with spermidine, but when the control group was pair-fed with
228 spermidine-treated mice, the differences in body weight were partially maintained (Supplementary
229 Fig. 8i). We also examined whether obesity states are associated with reduced WAT polyamine
230 levels. Remarkably, WAT from both mice subjected to HFD and obese human individuals exhibited
231 reduced polyamine content (Fig. 7k,l, Supplementary Fig. 8j).

232

233 **AMD1 inhibition hampers *Pten*-loss elicited reduction of adiposity**

234 We have previously identified a molecular link between the PI3K pathway and polyamine
235 biosynthesis in prostate cancer[46]. Activation of mTOR complex 1 (mTORC1) as a consequence of
236 *Pten* deletion results in elevated AMD1 protein levels and the elevation of decarboxylated dcSAM,
237 thus promoting the synthesis of polyamines[46]. As shown in Supplementary Fig. 7d, e, dcSAM was
238 significantly increased in cultured ECs and WAT from $Pten^{i\Delta EC}$ mice. In agreement, we confirmed
239 that Amd1 protein levels, but not mRNA gene expression, were higher in *Pten* null ECs (Fig. 8a and

240 Supplementary Fig. 8k), an effect that was counteracted by the inhibition of mTORC1 with rapamycin
241 (Supplementary Fig. 8l,m).

242 To address the relevance of Amd1 activity in the phenotype elicited upon endothelial *Pten*
243 loss, and in the absence of a conditional *Amd1* knockout mouse model, we took advantage of a
244 selective inhibitor of this enzyme, SAM486A. We confirmed that the conditioned media from *Pten*-
245 deficient ECs pre-treated with SAM486A was unable to increase lipolysis in WAT explants (Fig.
246 8c,d). In addition, explants from $Pten^{i\Delta EC}$ mice treated with SAM486A exhibited lower lipolytic rates
247 (Fig. 8e), and $Pten^{i\Delta EC}$ mice treated with SAM486A demonstrated greater body weight (Fig. 8f) and
248 adiposity than vehicle-treated counterparts (fig. 8g-k). Together, these data uncover polyamines as
249 angiocrine metabolic regulators of lipolysis and adiposity under the control of endothelial PTEN.

250

251 **Discussion**

252 ECs have been long studied as structural components of blood vessels with a bystander role in
253 systemic metabolic homeostasis. Our data, together with others[2], challenge this view and show that
254 manipulation of EC biology influences systemic metabolism with pathobiological implications. We
255 provide evidence that EC function modulates adiposity through angiocrine production of polyamines
256 acting directly on adipocytes in a paracrine fashion (Fig. 8I). Remarkably, we demonstrate that ECs
257 exploit the metabolic angiocrine communication mode to stimulate the release of FFA from
258 adipocytes to sustain cell proliferation and promote vascular growth. By doing so, ECs protect WAT
259 from pathological expansion in the context of obesity.

260 Angiocrine is a term used to define endothelial-derived paracrine signals which mediate
261 parenchymal function and regenerative functions in an organ-specific manner[32-34,48]. Yet, little is
262 known about the intercellular endothelial-to-adipocyte communication. We uncover for the first time
263 that ECs communicate with adipocytes through metabolites, the so-called polyamines. Other
264 examples of angiocrine-related organ regulation include liver and lung regeneration, neuronal stem
265 cell function, tumour angiogenesis and heart development[32,49-54]. However, angiocrine signals in
266 those contexts are of a protein nature. Recent evidence has recognised that metabolites produced
267 and secreted by ECs also function as angiocrine signals[35,55]. This is the case for muscle and retinal
268 ECs which secrete lactate, a product of glycolysis[35,55]. However, this is not surprising given that ECs
269 are largely glycolytic cells[56]. An interesting observation from these studies is that lactate does not
270 directly interact with parenchymal cells, but it engages macrophages which act as mediators of tissue
271 regeneration and angiogenesis[35,55]. In contrast, we identify that endothelial-derived polyamines,
272 upon secretion, directly stimulate lipolysis in adjacent adipocytes; thus, demonstrating that
273 angiocrine metabolic signalling directly regulate parenchyma function.

274 A remarkable result from our study is that loss of *Pten* in the endothelium result in a WAT-
275 restricted phenotype. This is consistent with the observation that ECs specialize in each type of
276 organ to fulfil tissue-specific tasks by cues that are essential for organ function[33]. Given that *Pten*

null cells primarily use lipids to proliferate, and that WAT serves as a reservoir of lipids, our results suggest that the environmental milieu educates EC behaviour. This would also explain why in BAT, a tissue with a mild accumulation of lipids, *Pten* null ECs exhibit a moderate increase in proliferation. While at present it is not clear how organotypic differences between EC emerge, our data call for considering the uniqueness of each environmental milieu as a key determinant of this EC specialization. We propose that understanding which signals from each organ microenvironment regulate EC unique properties could offer tissue-specific vascular therapies not only to repair malfunctional or degenerative tissues, but also to improve their homesotatic function.

The exhaustive pathophysiological analyses of the *Pten*$^{i\Delta EC}$ mice included here have allowed us to identify a causal relationship between reduced body weight and adiposity with enhanced local metabolic rate in WAT and increased usage of FFA by *Pten*$^{i\Delta EC}$ ECs. While we cannot rule out that the adipocytes contribute to the enhanced basal metabolic rate in *Pten*$^{i\Delta EC}$ mice, our data support the concept that adipose *Pten*$^{i\Delta EC}$ ECs actively contribute to energy consumption by lipid oxidation. This is consistent with the observation that ECs can use lipids to sustain a proliferative phenotype[26,57,58]. We believe that the observation that loss of endothelial *Pten* in BAT also results in a mildly enhanced vascularisation relates to the lipid availability in this tissue. This, in fact, further supports the conclusion that *Pten* deletion makes ECs prone to proliferate in the presence of fatty acids, which represents a microenvironmental prerequisite for these cells.

We have not been able to confirm a role for improved BAT function or WAT browning in the phenotype of *Pten*$^{i\Delta EC}$ mice. This is surprising given that VEGF-related enhanced angiogenesis in iWAT leads to improved systemic metabolic health by stimulating browning in this tissue[22-24]. Although the reason for this difference is not clear, collectively these studies reinforce the notion that enhanced vascularity improves WAT function. This fits with recent studies showing that age-dependent organ decline is associated with reduced vessel density[59,60], and that VEGF signalling prevents age-associated capillary loss, improves organ perfusion and function, and extends life span[60].

303 Polyamines are small polycations that are considered instrumental in proliferative cells[38].
304 Indeed, we show that polyamines are produced in the proliferative adipose endothelium, and
305 extrapolate evidence acquired in cancer studies to the endothelial field [46]. We demonstrate that the
306 connection between the PI3K/mTORC1 pathway and AMD1 activity is operative in ECs. Importantly,
307 beyond the role of these metabolites in cell proliferation, we show that they function as paracrine
308 regulators of adipocyte biology. An interesting follow-up question would be to identify whether
309 polyamines are angiocrine mediators in any EC that undergoes proliferation, or if they selectively
310 function as angiocrine mediators of adipose ECs. A critical finding from our study is that EC-derived
311 secreted polyamines regulate the activity of bADR, a concept for which there was solely isolated
312 evidence to date[47,61,62]. These previous studies demonstrated that polyamines stimulate bADR
313 signalling in the absence of natural ligand, and this triggered a physiologically relevant reponse[47].
314 We also observed that polyamines activate the release of FFA in the absence of natural ligand in
315 primary adipocytes. However, it seems rather unlikely that polyamines alone sufficiently contribute
316 to lipolysis *in vivo*, as shown by the poor lipolytic effect compared to natural ligands. Instead, we
317 propose that polyamines function as fine tuners of canonical bADR signals. This would be also
318 supported by the observation that in adipose tissue explants, we could detect sufficient endogenous
319 norepinephrine and epinephrine levels to stimulate basal lipolysis, an effect that was further
320 enhanced when polyamines were supplemented in the media. Of note, our data agree with previous
321 observations showing that exogenous administration of spermidine regulates lipid metabolism and
322 in turn ameliorates the WAT pathophysiological response to HFD[63]. This provides further support
323 that polyamines, and in particular spermidine, may open new therapeutic avenues to treat obesity.
324 Taken together, we uncover an unappreciated mode of communication between ECs and
325 adipocytes with implications for systemic metabolism. Our data provide evidence that this mode of
326 communication is disrupted in obesity, thus, opening exciting new research avenues to comprehend
327 and treat this disease. Also, we propose that understanding the extent of the contribution of

328 polyamines to adrenergic signalling-driven cellular responses may be important in many other

329 biological contexts.

330

Materials and methods

Reagents. All reagents (chemical, primers, and antibodies) used in this work are listed in Supplementary Tables 1,2, and 3, including commercial references and experimental dosage.

Mice. Mice were maintained under specific pathogen-free conditions and kept in individually ventilated cages. Experiments were conducted in accordance with the guidelines and laws of the Catalan Departament d'Agricultura, Ramaderia i Pesca (Catalunya, Spain), following protocols approved by the local Ethics Committees of Institut d'Investigació Biomèdica de Bellvitge (IDIBELL)-CEEA. Mice were fed ad libitum either with chow diet or high fat diet (HFD, 45% fat enriched diet). When HFD was required, mice were switch from chow diet to HFD at 6 weeks of age. When stated, mice were fasted for 6h or 16h. $Pten^{flox}$ mice[15] were crossed into the transgenic mice expressing the tamoxifen-inducible recombinase CreER under the control of the endothelial specific *Pdgfb* promoter (*Pdgfb-iCreER*) transgenic mice[16] to obtain $Pten^{i\Delta EC}$ mice and controls or into the control of the endothelial specific Cdh5 promoter ($Cdh5\text{-}CreER^{T2}$)[18]. For isolation of endothelial cell-specific actively translating mRNA, $Ribotag^{flox}$ mice ($Rpl22^{tm1.1Psam}$)[17] were crossed with *Pdgfb-iCre-ER* ($Ribotag^{iHAEC}$) and $Pten^{i\Delta EC}$ ($Pten^{i\Delta EC}$ - $Ribotag^{iHAEC}$). Cre activity and gene deletion were induced by intraperitoneal (I.P) injection of 4-OH tamoxifen (4-OHT) at 25mg (2.5 μl of a 10 mg ml^{-1} solution in absolute ethanol) in pups at postnatal day (P)2 and P3 and tissues were collected at 5 and 12 weeks of age. In adult mice, gene deletion was induced by I.P. injections of tamoxifen (75 mg kg^{-1}; resuspended in peanut oil) at 8 or 18 weeks of age for three alternate days. Both Cre positive and negative mice were treated with 4-OHT or tamoxifen. For combined endothelial-cell-specific loss-of-function of *Pten* and $Pgc1\beta$, we crossed $Pten^{flox}$ mice[15] with $Pgc1\beta^{flox}$ mice[28] and *PdgfbiCreER*[16] mice. For pharmacological rescue studies, control and $Pten^{i\Delta EC}$ mice were treated with vehicle (saline), etomoxir (25 mg kg^{-1}) or SAM486A (5 mg kg^{-1}) from 5 to 10 weeks of age. For explants, mice were treated 4 days with vehicle (saline), SAM486A (5 mg kg^{-1}), spermidine (20 mg kg^{-1}) or propranolol (20 mg kg^{-1}). Etomoxir and SAM486A *in vivo* experiments were planned by choosing a dose, route of administration and drug regimen previously published by us [27,46]. For spermidine treatment, wild-

357 type C57Bl6 mice (purchased from Charles River) were fed with HFD and were treated with vehicle
358 or spermidine (20 mg kg^{-1}) from 6 to 12 weeks of age daily excluding weekends. Of note, the
359 spermidine supplementation strategy 20 mg kg^{-1} is within physiological ranges of spermidine
360 supplementation[43,64]. All *in vivo* experiments of our study, including chow and high-fat diet conditions,
361 were performed without altering the normal presence of dietary polyamines. Before starting *in vivo*
362 experiments, mice were weighted and homogenously distributed in experimental groups. All other
363 criteria were not considered and as such, randomized. Those mice showing distress features not
364 compatible with our ethic protocols were sacrificed.

365 ***Human Cohort.*** We studied omental VAT from morbid obese patients (BMI>35) undergoing bariatric
366 surgery and non-obese subjects (BMI<30) undergoing abdominal surgery at the Hospital Clinic of
367 Barcelona. For the study of polyamine levels, 12 obese female patients (BMI of 42.9±4.9) and 6 non-
368 obese females (BMI of 26.7±1.5) age-matched (≥ 53) were included. For vascular analysis and *Pten*
369 mRNA expression analysis, morbidly obese (BMI of 42.84±4,43, 3 females and 7 males, age of
370 52±13,24 years) and non-obese individuals (BMI of 26.86±2.8, 1 female and 5 males, age of
371 60.5±5.31 years) were included. The Hospital Clinical of Barcelona Ethical Committee approved the
372 studies, and all study participants provided informed consent to donate tissue samples.

373 ***RiboTag.*** Enrichment of the active translating RNA was achieved by immunoprecipitation (IP) of
374 ribosomes via HA. Briefly, snap frozen tissues (50-100mg) were homogenized in 500 µL of polysome
375 buffer [65] with a pestle motor and centrifuged at 10000 g for 10 min at 4ºC. An aliquot of 25 µL of the
376 supernatant was kept as input; the rest was incubated with 4 µL of anti-HA antibody for 2 h at 4ºC in
377 gentle rotation. After that, 200 µL of Pierce™ Protein A/G Magnetic Beads were added and incubated
378 for 2 h at 4ºC in gentle rotation. Beads were washed 3 times in high salt buffer[65] and then
379 resuspended in 350 µL of RLT buffer (from RNeasy Micro).

380 ***RNA extraction, cDNA synthesis and qPCR.*** RNA was isolated from tissues, ECs lysates, and
381 Ribotag IPs using RNeasy Plus Mini and RNeasy Micro Kit, respectively, following manufacturer's
382 instructions. To obtain cDNA, reverse transcription was performed from 500ng of RNA by using High-

383 Capacity cDNA Reverse Transcription Kit (Applied Biosystems). For quantitative PCR, a LightCycler
384 480 System (Roche) was used with LightCycler 480 SYBR Green I Master kit (Roche) or a
385 QuantStudio™ 5 System (Applied Biosystems) with TaqMan™ Universal PCR Master Mix (Applied
386 Biosystems). Specific primers detailed on Supplementary Table 2. *mL32* or *Hprt1* were used as
387 housekeeping genes.

388 ***Vascular density analysis.*** To study the vasculature of mouse and human WAT, we used whole
389 mount immunostaining. Briefly, approximately 3 mm^3 cubes of tissue were cut from each sample and
390 permeabilized for 1 h with PBS + 1% Triton-x 100. Afterwards, tissues were blocked with blocking
391 buffer (1x PBS + 0,3% Triton-x 100 + 5% goat serum) for 2 h at RT. Primary and secondary
392 antibodies (references are listed in Supplementary Table 3) diluted in blocking buffer were incubated
393 overnight at room temperature, with over day washings with PBS + 0,3% Triton-x 100 after both
394 incubations. Mouse WAT pads were stained with Alexa-conjugated isolectin B4 (IB4). Human WAT
395 pads were stained with anti-CD31 antibody. To study the vasculature of BAT, muscle, liver, heart
396 and brain, we used cryosection immunostaining. Freshly isolated tissues were fixed overnight in 4%
397 paraformaldehyde; washed in PBS; dehydrated in 30% sucrose overnight and embedded in OCT.
398 BAT (14 μm), muscle, heart, brain (10 μm) and liver (5 μm) cryosections were cut using the Cryostat
399 (Leica Microsystems). BAT sections were blocked with 1xPBS 0,3% Triton-x 100 + 5% goat serum
400 for 1 h at RT. Afterwards, sections were incubated overnight at 4°C with Alexa-conjugated IB4.
401 Muscle, liver, heart and brain sections were incubated in Tris-EDTA solution (0.1 M EDTA + 0.001
402 M Tris-base + 0.05% Tween 20) in a steamer for 30min. After cooling down, sections were blocked
403 with 1x PBS + 5% donkey serum + 0.4% Triton-x 100 for 1 h at room temperature. Afterwards,
404 sections were incubated overnight at 4°C with anti-CD31 antibody and with secondary antibody 1 h
405 at RT (listed in Supplementary Table 3) in blocking solution. Sections were washed in 1xPBS 3 times
406 and mounted with Immuno-mount.

407 To analyse vessel area and proliferation, images were taken with a Leica SP5 laser-scanning
408 confocal microscope or Nikon 80i microscope using 20X and 40X objectives. Confocal images are

409 maximal intensity z-stack projections and images were processed using Volocity, Fiji and Adobe
410 Photoshop CS5. Vessel density was quantified by measuring IB4 positive or CD31 positive area,
411 using the ImageJ software; data were expressed as percentage of total area. EC proliferation was
412 quantified by counting the number of Ki67/ERG double positive nuclei. Endothelial branch points
413 were quantified in 4 images per each sample (taken with 40X oil immersion objective) and
414 represented as average.

415 ***Histology analysis.*** WAT depots, liver and muscle were fixed and embedded in paraffin following a
416 standard procedure. 5 µm thick paraffin sections were stained with hematoxylin/eosin and mounted
417 with DPX mounting media. Adipocyte area was calculated with the Adiposoft tool of ImageJ software.
418 Fibrosis was evaluated by Trichrome Stain Kit following manufacturer's instructions. Cell death was
419 evaluated by immunostaining of cleaved caspase3 in paraffin sections following standard protocol.
420 Mouse prostate cancer specimen was used as positive control (data not shown). Briefly, antigen
421 retrieval was done with citrate buffer and permeabilization with PBS + 0.2% Tween20, primary
422 antibody was incubated over night at 4°C and secondary 2 h at RT (listed in Supplementary Table
423 3). Samples were mounted with Shandon Immu-Mount. Images were taken with a Nikon 80i
424 microscope using the 20X objective.

425 ***Glucose tolerance test (GTT) and Insulin tolerance test (ITT) and HOMA-IR index.*** GTT and ITT
426 were performed in 6h fasted mice. Both compounds were I.P. injected, glucose (1.5 g kg^{-1}) and
427 insulin (0.75 UI kg^{-1}), and blood glucose was monitored for 90-120min. HOMA-IR index was
428 calculated applying the following formula: (fasting glucose (mg dL^{-1}) x fasting insulin (mU L^{-1}))/ 405.
429 Insulin was measured with Ultra-Sensitive Mouse Insulin ELISA Kit following manufacturer's
430 instructions.

431 ***Daily food intake.*** Mice were single housed, acclimatized for 1 week prior to study and a weighted
432 amount of food was provided. Food intake was measured for 5 consecutive days at 8 and 12 weeks
433 of age. Peer feeding was done to determine the extent to which the body weight-reducing effect of
434 spermidine treatment was the consequence of changes in food intake. All mice were individualized

435 during the pair-feeding protocol. The average food intake of the spermidine-treated group was daily
436 measured between 9.00AM and 10.00AM. Subsequently, the pair-fed group was offered the same
437 amount of food eaten by spermidine-treated mice on the previous day. This protocol lasted for 2
438 weeks.

439 ***Body composition, Indirect calorimetry and locomotor activity.*** Body weight and discrete
440 adipose tissue pad mass were measured using a precision scale. Whole body composition was
441 measured using nuclear magnetic resonance imaging (Whole Body Composition Analyzer;
442 EchoMRI, Houston, TX)***.*** Indirect calorimetry and locomotor activity was assessed using a TSE
443 LabMaster modular research platform (TSE Systems) as previously described[66,67]. Briefly, mice were
444 acclimatized for 24h into test chambers and monitored for additional 48h. O_2 consumption and CO_2
445 production were measured every 45 min during 48 h, to indirectly determine Energy Expenditure
446 (EE). Locomotor activity was determined using a multidimensional infrared light beam system with
447 the parameters defined by the LabMaster software. These analyses were performed at 7-week-of-
448 age, before body weight differences between control and *Pten*$^{i\Delta EC}$ mice were apparent.

449 ***Thermographic imaging.*** Heat production was visualized using a high-resolution infrared camera
450 (FLIR PM280; FLIR Systems), as previously described[68]. Infrared thermography images were taken
451 from the upper half of the body to specifically visualize heat production from the BAT. Mice were
452 shaved in the interscapular area before imaging to minimize interference. Images were analysed,
453 within a fixed rectangular area, using the Flir Tools program.

454 ***Western blot.*** Primary ECs were lysed in 50 mM Tris HCl pH 7.4, 5 mM EDTA, 150 mM NaCl and
455 1 % Triton X-100 supplemented with 2 mg ml^{-1} aprotinin, 1 mM sodium fluoride, 1 mM pepstatin, 1
456 ng ml^{-1} leupeptin, 1 mM phenylmethysulfonylfluoridem, 10 g ml^{-1} Na-p-tosyl-L-lysine chloro-methyl
457 ketone hydrochloride, 1 mM sodium orthovanadate, 1 µM okadaic acid and 1 mM DTT. Adipose
458 tissue was manually homogenized with a pestle in 400-500µl of lysis buffer (described above). Cell
459 lysis, SDS-PAGE and immunoblot were performed as previously described[14]. Antibodies used are

460 listed in Supplementary Table 3. Quantification of band intensities by densitometry was carried out
461 using the ImageJ software. Unncropped blots are found in Supplementary Fig. 9.

462 ***Mitochondrial respiration.*** Mitochondrial respiration was assessed in freshly isolated eWAT,
463 iWAT, muscle, and liver by high-resolution respirometry in an Oroboros Oxygraph-2k system
464 (Oroboros Instruments, Innsbruck, Austria) as previously reported[69]. In brief, tissues were first
465 mechanically and chemically permeabilized in respiratory medium[69] in the presence of digitonin (8
466 µM) or saponin (50 µg mL^{-1}). LEAK respiration was measured by the addition of NADH-linked
467 substrates (complex I linked) Malate (2 mM) and Pyruvate (5 mM) in the absence of ADP. OXPHOS
468 state was measured by adding ADP+$MgCl_2$ [5 mM] and cytochrome C (10 µM), followed by the
469 subsequent addition of Glutamate [10 mM] (NADH-linked pathway) and Succinate [10 mM]
470 (convergent electron flow through both, NADH- and succinate-linked pathways). FCCP [0.5 µM] was
471 titrated to evaluate the maximal capacity of the Electron Transfer System (ETS CI+CII). Finally,
472 rotenone [0.5 µM] was used to inhibit CI and measure ETS fuelled by succinate-linked pathway.
473 Oxygen flux values were expressed relative to tissue wet weight per second (pmol O_2 mg^{-1} s^{-1}).
474 Finally, residual oxygen consumption (ROX) was determined by the inhibition of complex III adding
475 Antimycin A (2.5 µM) and this value was subtracted from O2 flux as a baseline for all respiratory
476 states.

477 ***Faces collection and analysis.*** Faces were collected from individually housed mice cages for four
478 days, stored at -20ºC and desiccated at 60ºC before processing. Energy content in faces was
479 measured using a calorimetric bomb in the Laboratorio de Nutrición Animal SERIDA (Villaviciosa,
480 Spain). For triglycerides measurement, frozen faeces samples were pulverized under liquid nitrogen,
481 and 100 mg portions were digested in 3 M KOH for 1 h at 70ºC, followed by overnight incubation at
482 RT. Samples were diluted to a final concentration of 100 mg tissue in 500 µL Tris–HCl 50 mM before
483 using Triglycerides-LQ Kit under manufacturer’s instructions.

484 ***Primary ECs.*** Mouse iWAT depots, BAT, muscle, and lungs were digested with 4 U mL^{-1} of dispase
485 II and 0.5 mg mL^{-1} of collagenase A. Selection and culture conditions for adipose-derived and lung-

486 derived endothelial cells were as previously reported[14]. Cells were seeded on a 12-well plate and
487 were coated with gelatin (0.5%) in DMEM/F12 supplemented with 20% fetal bovine serum (FBS)
488 and endothelial cell growth factors (PromoCell) and 1% penicillin/streptomycin. After the first
489 passage, the cells were re-purified with VE-cadherin antibody coated magnetic beads. Cells were
490 cultured until passage 5. To assess cell proliferation 1 $\times 10^4$ primary endothelial cells were cultured
491 in DMEM/F12 with 1% FBS, 2 mg mL^{-1} of AlbuMAX and half dose of EC growth factors in the
492 presence or absence of etomoxir. Cell growth was measured by staining with crystal violet (0.1% in
493 20% methanol) at day 2 and 3 and normalized by day 0 values. For conditioned media collection, 8x
494 10^5 cells were cultured in 1 ml of DMEM 1 g L^{-1} glucose + 0,5% FFA free BSA. After 4 h, media was
495 collected, centrifuged for 5 min at 1200 rpm, filtered with a 0.22 filter and kept at 4ºC (up to 2 days).
496 When indicated, cells were pre-treated 24h with vehicle (H_2O) or SAM486A (0.5 µM) in growing
497 media. For media fractioning, 2.5 mL of media was loaded into Amicon Ultra-4 filters (membrane
498 PLBC Ultracel-3, 3 kDa) and centrifuged at 3800g, 4ºC for 1h. The flow-through (metabolite fraction)
499 was collected and stored at 4 ºC. The retained protein and vesicle fraction (150 µL), was washed
500 with 2 mL of DMEM 1g L^{-1} glucose, centrifuged again, diluted in 2ml of DMEM 1g L^{-1} glucose and
501 stored at 4 ºC. For pharmacological studies, primary adipose endothelial cells were cultured for 24
502 h with vehicle (DMSO) or mTOR inhibitor (rapamycin, 1 µm).
503 ***Primary adipocytes.*** The stromal vascular fraction (SVF) was isolated from iWAT depots of 6-week-
504 old male mice. iWAT was digested in Hanks' Balanced Salt solution with 4 U mL^{-1} of dispase II and
505 0.5 mg mL^{-1} of collagenase during 40 min; digested tissue was passed through a cell strainer (mesh
506 size 100 µm) and digestion was stopped by adding one volume of DMEM + 10% FBS. Cell
507 suspension was centrifuged for 15 min at 1250 rpm, and resuspended in DMEM + 10% FBS, 1%
508 penicillin/streptomycin (DMEM complete). Cells were plated in 12-well plates (4 wells per mouse).
509 Differentiation was induced 48 h after cells reached confluence by adding the induction media:
510 DMEM complete with insulin (100 nM), dexamethasone (1 µM), 3-Isobutyl-1-methylxanthine (IBMX)
511 (0,5 mM) and rosiglitazone (1 µM). After 48h, induction media was replaced by DMEM complete +

512 insulin (100 nM). Experiments were performed at day 8 of differentiation. Before performing any
513 experiments, each well was carefully checked at the microscope to discard wells that were not
514 properly differentiated. The capability of differentiation from control and Pten$^{i\Delta EC}$ adipose tissue was
515 assessed in SVF isolated from 5 biological replicates per genotype.

516 ***In vitro free fatty acid β-oxidation (FAO).*** *In vitro* FAO was measured as previously described[70].
517 Briefly, after 4 h incubation of cells with [^{3}H] palmitate, medium was collected to analyse the released
518 ^3H_2O formed during cellular oxidation, normalized to protein content.

519 ***Seahorse.*** Control and *Pten$^{i\Delta EC}$* primary endothelial cells were seeded in customized Seahorse 24-
520 well plates. 1h before the assay cells were maintained in XF Assay Medium Modified DMEM
521 (Seahorse Bioscience) supplemented with 5 mM glucose in a non-CO_2 incubator and just prior the
522 assay 10 µl of AlbuMAX were added (to a final concentration of 2mg mL^{-1}). Oxygen consumption
523 rate (OCR) was measured using the Seahorse XFe24 analyser (Agilent) following the manufacturer's
524 protocols. The Mito Stress test was used to assay the mitochondrial respiration rate under basal
525 condition, in presence of 3 µM oligomycin to block ATP synthase, 1 µM of the mitochondrial
526 uncoupler carbonyl cyanide-4-(trifluoromethoxy) phenyl-hydrazine (FCCP) to measure maximal
527 respiratory capacity; and the respiratory chain inhibitors 1.5 µM antimycin A and 3 µM rotenone. The
528 protocol and injection strategies of the Mito Stress Assay were followed. OCR was calculated by
529 plotting the O_2 tension of media as a function of time (pmol min^{-1}), and data were normalized by the
530 protein concentration measured in each individual well. Calculations were performed using the
531 Agilent Seahorse Wave Desktop software.

532 ***Glucose uptake.*** Primary adipose endothelial cells (15 x 10^4) were seeded 12 h prior the
533 experiment. Cells were then incubated in 900 µL of KRH buffer (20 mM HEPES, 136 mM NaCl, 4.7
534 mM KCl, 1.25 mM $MgSO_4$, 1.25 mM $CaCl_2$) for 15 min at 37ºC and 100 µL of 10x START solution (1
535 mM 2-deoxyglucose, 5 µCi mL^{-1} [^{3}H]- 2-deoxyglucose) were added afterwards. Following 10 min of
536 incubation at 37ºC, cells were washed and 1 mL of 0.03% SDS was added. Counts per minute (cpm)
537 were determined and normalized by mg of protein.

538 ***Lipolysis assay in primary adipocytes***. Lipolysis was evaluated by measuring FFA release in
539 differentiated adipocytes. Briefly, differentiated adipocytes were washed with 1x PBS and stimulated
540 by: (a) conditioned media from control and *Pten*$^{i\Delta EC}$ endothelial cells or (b) DMEM 1g L^{-1} glucose +
541 0,5% FFA free BSA +/- 1 μM spermidine. DMEM 1g L^{-1} glucose + 0,5% FFA free BSA + 1 μM
542 CL316243 (betaADR agonist) was included in all assays as positive control. When stated, cells were
543 pre-treated with 100 μM propranolol in DMEM complete + insulin (100 nM) during 2h prior the
544 experiment. FFA release was measured at minute 0 and minute 180, and the difference between
545 time points was calculated. FFA were measured using FFA assay HR-NEFA kit following
546 manufacturer's instructions.
547 ***cAMP production in primary adipocytes.*** cAMP was measured in primary adipocytes cell lysate
548 after 15 min incubation with DMEM 1g L^{-1} glucose + 0,5% FFA free BSA +/- 1μM spermidine. Time
549 0 was taken 20 min after switching media to exclude alteration in cAMP due to the different
550 composition of assay media compared to culture media. 10 μM Forskolin was used as positive
551 control. cAMP was measured using Cyclic AMP colorimetric Assay, following manufacturer's
552 instructions.
553 ***Plasma leptin, triglycerides (TG) and FFA measurement.*** Blood was collected in EDTA
554 microtubes from the tail vein of mice before and after 16 h of starvation. Plasma was obtained after
555 20 min of centrifugation at 3600 rpm at 4ºC. Leptin was measured with Mouse Leptin ELISA Kit
556 following manufacturer's instructions after 6 h starvation. TGs were measured by a chemistry panel
557 following standard protocols. FFA were measured using FFA assay HR-NEFA kit following
558 manufacturer's instructions.
559 ***Ex vivo eWAT explants.*** Fresh eWAT depots were collected in ice-cold DMEM 1g L^{-1} glucose +
560 0,5% FFA free BSA, cut in small pieces and explants of ~25 mg of tissue and were placed in 24-well
561 plates (one piece per well) with 500 μl of DMEM 1g L^{-1} glucose + 0,5% FFA free BSA. Baseline
562 media samples were obtained after 1 minute of incubating the tissue at 37ºC with gentle shaking
563 (150 rpm). Then, spermidine (50μM) or equal volume of DMEM 1g L^{-1} glucose + 0,5% FFA free BSA

564 was added. CL316243 was used as a positive control in all assays. Plates were incubated at 37ºC
565 while shaking (120 rpm) and aliquots of media (15 µL) were taken at 30, 60, 90 and 120 min.
566 Alternatively, explants were incubated with 500 µL of conditioned media and aliquots of media were
567 collected at baseline and 90min. Samples were stored at -20ºC until processed. FFA concentration
568 was quantified using FFA assay HR-NEFA kit following manufacturer's instructions. When stated
569 explants were preincubated 10 min with 100 µM propranolol at 37ºC before adding spermidine.
570 When indicated *Pten*$^{i\Delta EC}$ mice were pre-treated with either vehicle (saline), SAM468A (5 mg kg^{-1}),
571 spermidine (20 mg kg^{-1}) or propranolol (20 mg kg^{-1}) *in vivo* for 4 days prior the experiment.
572 ***Targeted metabolomics.*** For *in vitro* metabolomic analysis, growing endothelial cells were washed
573 with PBS and snap-frozen in liquid nitrogen. For metabolomic flux analysis endothelial cells were
574 incubated 2 and 4 h with DMEM high glucose, no glutamine, no methionine, no cystine plus [U-
575 13C5]l-methionine 30 µg mL^{-1}, washed with PBS and snap-frozen in liquid nitrogen. For in vivo
576 metabolomic flux analysis, [U-13C5]l-methionine was injected through the tail vein at 100 mg kg^{-1};
577 mice were sacrificed 20 h after injection and adipose tissue was collected and snap-frozen in liquid
578 nitrogen. For targeted metabolomic of conditioned media, endothelial cells were incubated 4 h in
579 DMEM 1 g L^{-1} glucose + 0,5% FFA free BSA, media was collected and kept at -80°C; protein content
580 was used to normalize data (the experiment was performed with technical triplicates of 6 biological
581 replicates for each genotype). To measure spermidine uptake, primary adipocytes were incubated
582 with $^{13}C_4$ spermidine in DMEM 1g L^{-1} glucose + 0,5% FFA free BSA, washed with PBS and snap-
583 frozen in liquid nitrogen; samples were taken at t0, 15, 30 and 60min. For ex vivo metabolomic
584 analysis adipose tissue was collected and immediately snap-frozen in liquid nitrogen. Levels of
585 dcSAM and spermidine in WAT and adipose-derived primary endothelial cells were analysed by
586 UPLC–MS, as previously described[46]. Briefly, extraction and homogenization were done in
587 methanol/acetic acid (80/20% v/v). Speed-vacuum-dried metabolites were solubilized in 100 µL of a
588 mixture of water/acetonitrile (40/60% v/v) and injected onto the UPLC–MS system (Acquity and

589 SYNAPT G2, Waters). The extracted ion traces were obtained for dcSAM, spermine and spermidine.
590 Corrected signals were normalized to protein content or mg of tissue.
591 ***Epinephrine and norepinephrine measure in WAT.*** WAT was homogenized in 500 µL of ice-cold
592 extraction liquid with a tissue homogenizer (FastPrep) in a single 40 seconds cycle at 6000 rpm. The
593 extraction liquid consisted of a mixture of ice-cold methanol/water (50/50 %v/v) with 10 mM acetic
594 acid. Subsequently 400 µL of the homogenate plus 400uL of chloroform was transferred to a new
595 aliquot and shaken at 1400 rpm for 60 minutes at 4 °C. Next, the aliquots were centrifuged for 30' at
596 13000 rpm at 4 °C. The organic phase was separated from the aqueous phase. From the aqueous
597 phase 250µL was transferred to a fresh aliquot and placed at -80 °C for 20'. The chilled supernatants
598 were evaporated with a speedvac in approximately 2h. The resulting pellets were resuspended in
599 150 µL water/acetonitrile (MeCN) /formic acid (40/60/0.1 v/v/%). Samples were measured with a
600 UPLC system (Acquity, Waters Inc., Manchester, UK) coupled to a Time-of-Flight mass spectrometer
601 (ToF MS, SYNAPT G2, Waters Inc.). A 2.1 x 100 mm, 1.7 µm BEH amide column (Waters Inc.),
602 thermostated at 40 °C, was used to separate the analytes before entering the MS. Mobile phase
603 solvent A (aqueous phase) consisted of 99.5% water, 0.5% FA and 5 mM ammonium formate while
604 solvent B (organic phase) consisted of 10% water, 90% MeCN, 0.5% FA and 5 mM ammonium
605 formate. To obtain a good separation of the analytes the following gradient was used: from 25% A
606 to 95% A in 1.5 minutes in curved gradient (#7, as defined by Waters), constant at 95% A for 1.5
607 minutes, back to 25% A in 0.2 minutes. The flow rate was 0.250 mL/min and the injection volume
608 was 4 µL. All samples were injected randomly and in duplicate. After every 7 injections QC sample
609 was injected. The MS was operated in positive electrospray ionization mode in full scan (50 Da to
610 1200 Da). The cone voltage was 25 V and capillary voltage was 250 V. Source temperature was set
611 to 120 °C and capillary temperature to 450 °C. The flow of the cone and desolvation gas (both
612 nitrogen) were set to 5 L h^{-1} and 600 L h^{-1}, respectively. A 2 ng mL^{-1} leucine-enkephalin solution in
613 water/acetonitrile/formic acid (49.9/50/0.1 %v/v/v) was infused at 10 µL min^{-1} and used for a lock
614 mass which was measured each 36 seconds for 0.5 seconds. Spectral peaks were automatically

corrected for deviations in the lock mass. For both metabolites signals of in-source fragments were used for quantification. Extracted ion traces were obtained for the epinephrine fragment (m/z = 166.0859) and for norepinephrine fragment (m/z = 152.0731), in a 20 mDa window and subsequently smoothed and integrated with TargetLynx software (Waters, Manchester, UK). These calculated raw signals were adjusted for by median fold-change (MFC) adjustment. This is a robust adjustment factor for global variations in signal due to e.g., difference in tissue amounts, signal drift or evaporation. The MFC is based on the total amount of detected mass spectrometric features (unique retention time/mass pairs). The calculations and performance of the MFC adjustment factors are described in the following publications.

Statistics. Data was analysed using GraphPad Prism (Graphpad Software Inc.). Data in graphs are expressed as means ± SEM and obtained from at least two independent experiments. Sample size and experimental replicates were indicated in figure legends. Mice and samples received a correlative number independent of their genotype, ensuring the blindness of data collection and analysis. Although no statistical methods were used to predetermine sample size, we aimed to have experimental groups of at least 7 mice for all *in vivo* experiments. This group sampling was based on our expertise (minimum number of mice to provide robust and reliable data) and was feasible to obtain within our animal resources. Unpaired Mann Whitney's t-test was used for comparing two unmatched groups, one-tail t-test was used for hypothesis driven analysis, one-way ANOVA for three or more unmatched groups and two-way ANOVA for two or more matched groups. ***$p<0.001$, **$p<0.01$, *$p<0.05$.

Data Availability

The data that support the findings of this study are available from the corresponding author upon request.

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681 **Author's contribution**

682 E.M., M.C., A.C and M.G. conceived the project; E.M., P.V., L.G., A. Z-L., A. M-R., J.LL., D.B., L.G.,
683 I.C., S.Z., P.G-P. O.O-C., L.M., A.M-G., S.D.C., N. M-M, P.C., L. V-J., I.G-G., and S. F-R.,
684 performed experiments and analyzed data with the supervision of J.V., D.S., L.H., R.B., P. G-R,
685 R.N., P.C., M.C., A.C. and M.G.; E.M., P.V., L.G., S.D.C., A.C. and M.G. wrote the manuscript and
686 designed the figures. L.H., D.B., R.N., P. G-R., M.C., A.C. and M.G. provided funding.
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688 **Disclosure**

689 M.G. has a research agreement with Merck and Venthera. None of those have a relationship with
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