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1 **Influenza infection rewires energy metabolism and induces browning**
2 **features in adipose cells and tissues**

3

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24 **Abstract**

25 Like all obligate intracellular pathogens, influenza A virus (IAV) reprograms host cell's
26 glucose and lipid metabolism to promote its own replication. However, the impact of
27 influenza infection on white adipose tissue (WAT), a key tissue in the control of systemic
28 energy homeostasis, has not been yet characterized. Here, we show that influenza infection
29 induces alterations in whole-body glucose metabolism that persist long after the virus has
30 been cleared. We report depot-specific changes in the WAT of IAV-infected mice, notably
31 characterized by the appearance of thermogenic brown-like adipocytes within the
32 subcutaneous fat depot. Importantly, viral RNA- and viral antigen-harboring cells are detected
33 in the WAT of infected mice. Using *in vitro* approaches, we find that IAV infection enhances
34 the expression of brown-adipogenesis-related genes in preadipocytes. Overall, our findings
35 enlighten the role that the white adipose tissue, which lies at the crossroads of nutrition,
36 metabolism and immunity, may have in influenza infection.

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49 **Introduction**

50 Influenza A viruses (IAVs) are important human respiratory pathogens that lead to substantial
51 morbidity and severe disability during annual epidemics¹. Older adults and pediatric
52 populations are particularly at risk for influenza complications. Influenza viruses can also
53 cause recurrent, sporadic and potentially devastating pandemic outbreaks, which create
54 enormous health and economic burdens worldwide. Despite great advances in our
55 understanding of the mechanisms of IAV infection, there is still a major need for more
56 effective virus control modalities; achieving this goal will require a better understanding of
57 the complex virus-host interactions.

58 An IAV infection starts in the respiratory tract, where viruses must attach to, invade and
59 replicate in airway epithelial cells before spreading to neighboring non-immune cells and
60 innate and adaptive immune cells². The antiviral innate immune response has several major
61 roles in host defense: it limits (or at least controls) viral replication during the early phase of
62 infection, and induces the subsequent adaptive immune response that plays a critical role in
63 viral clearance and development of long-lasting immunity during the later stages of infection³.

64 Although influenza infection predominantly results in damage to the respiratory system,
65 various cardiovascular, neurological and gastrointestinal complications have also been
66 reported⁴. Even though the data are subject to debate, it has been suggested that highly
67 pathogenic IAVs can replicate outside the respiratory system - notably in the intestine^{5,6}.

68 The link between influenza infection and metabolic disorders remains elusive. On one hand,
69 data from animal and human studies have identified type 2 diabetes and obesity (both of
70 which are characterized by chronic subclinical inflammation and hyperglycemia⁷) as risk
71 factors for severe influenza outcomes^{8,9}. On the other hand, a few case reports have suggested
72 that influenza infection *per se* may trigger, unmask or aggravate metabolic disorders⁴.

73 At the cellular level, influenza infection dramatically alters host cell's metabolism and thus

74 creates an environment that is propitious for viral replication. Pioneering studies in the 1950s
75 and 1960s observed greater glucose uptake and glycolysis in infected chick embryo cells¹⁰; in
76 contrast, the inhibition of glucose metabolism significantly reduced viral replication¹¹. More
77 recently, Smallwood *et al.* reported elevated glucose metabolism in the lungs of IAV-infected
78 patients and in *in-vitro*-infected primary human bronchial epithelial cells¹². Furthermore,
79 influenza infection increases lipid and cholesterol biosynthesis in the host pulmonary
80 cells^{13,14}; accordingly, the pharmacological inhibition of fatty acid biosynthesis suppresses
81 virus replication¹⁵.

82 White adipose tissue (WAT) is a key metabolic organ. It has a major role in the regulation of
83 whole-body glucose and lipid metabolism¹⁶. In addition to mature, lipid-filled adipocytes,
84 WAT also contains stromal vascular fraction cells (composed of multiple cell types, including
85 adipocyte progenitors (preadipocytes), and innate and adaptive immune cells) whose number
86 and activation status can change as a function of physiological and pathological conditions,
87 such as obesity¹⁷⁻²⁰. As well as being the body's main energy reservoir (triglycerides stored in
88 adipocytes), WAT is acknowledged to be a highly active endocrine tissue that secretes
89 numerous metabolites and hormones¹⁸ - some of which (leptin) have immunoregulatory
90 functions²¹. Unlike other organs, WAT is compartmentalized into discrete depots distributed
91 throughout the body. The subcutaneous adipose tissue (SCAT) and the visceral adipose tissue
92 are the most frequently defined and studied depots. Importantly, these depots differ markedly
93 in their metabolic and endocrine functions, and in their associations with the development of
94 metabolic diseases²².

95 With the exception of the report by Nishimura *et al.* showing that IAV (H5N1) can target
96 WAT *in vivo*²³, the impact of influenza infection on the WAT of lean mice has not yet been
97 investigated. Here, we found that (intranasal) IAV infection (seasonal H3N2 subtype) leads to
98 profound changes in the major fat depots (namely, the inguinal SCAT and the visceral

99 (epididymal) adipose tissue (EWAT)), some of which are common to SCAT and EWAT
100 while others are unique to the SCAT, such as the appearance of thermogenic brown-like/beige
101 adipocytes. Importantly, viral RNA- and viral protein-harboring immune cells and (less
102 frequently) preadipocytes were detected in the WAT's stromal vascular fraction of infected
103 animals. Using *in vitro* approaches, we showed that IAV replication is abortive in
104 preadipocytes, and that infection yields brown-like/beige features in these cells, as evidenced
105 by elevated expression of brown-like/beige specific genes. Transcription analyses further
106 revealed that IAV infection was associated with unique metabolic rewiring in the SCAT of
107 infected mice, and in *in-vitro*-infected preadipocytes. Strikingly, infected mice display
108 alterations in whole-body glucose metabolism that persist long after infection has been
109 resolved.

110 Collectively, our findings may advance our understanding of the ramifications of influenza A
111 infection biology, and may bring novel aspects of metabolic control of the complex virus-host
112 interactions.

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125 **Results**

126 **Influenza alters adipose tissues' functions** C57BL/6 mice were intranasally inoculated with
127 a sublethal dose of H3N2 IAV or with PBS (mock). At 7 days post-infection (7 dpi,
128 corresponding to the peak inflammatory response in the lungs), IAV-infected mice displayed
129 significant body weight loss (Fig. 1a) and reduced inguinal (SCAT) and epididymal (EWAT)
130 fat mass (Fig. 1b). It is noteworthy that the mass of SCAT was positively correlated with the
131 mass of EWAT in both mock-treated and IAV-infected animals (Supplementary Fig. 1); this
132 indicates that influenza infection did not lead to major changes in body fat distribution. As an
133 energy storage organ, adipose tissue stores neutral triglycerides through the lipogenic pathway
134 and releases fatty acids through the lipolytic pathway¹⁶. Lipolysis of triglycerides requires
135 three consecutive steps involving at least three different enzymes: adipose triglyceride lipase
136 (ATGL), hormone-sensitive lipase (HSL), and monoglyceride lipase (MGL)²⁴. Influenza
137 infection resulted in the marked repression of *Atgl* and *Hsl* transcription in SCAT and EWAT.
138 Strikingly, *Mgl* transcription was suppressed in EWAT but enhanced in SCAT. In both fat
139 depots, infection was associated with decreased expression of lipogenic genes, such as those
140 encoding glucose transporter 4 (*Glut4*), malic enzyme 2 (*Me2*) and fatty acid synthase (*Fasn*)
141 (Fig. 1c).

142 Adipose tissue is composed of several cell types that include mature adipocytes,
143 preadipocytes, and a range of innate and adaptive immune cells¹⁷⁻²⁰. As an endocrine organ,
144 WAT secretes numerous cytokines, metabolites and hormones that originate from all these
145 cell types¹⁸. By preserving the physiological *in vivo* cross-talk between cells, explant cultures
146 of WAT allow analyzing the secretory function of the whole tissue²⁵. We therefore used tissue
147 explant cultures to investigate whether influenza infection was associated with changes in
148 SCAT and EWAT's secretory function (Fig. 1d). When compared with controls, the adipose
149 tissues collected from infected mice produced higher levels of the proinflammatory cytokines

150 IL-1 β and IL-6, and of the anti-inflammatory cytokine IL-10. Following IAV infection, the
151 release of leptin - a WAT-derived hormone that regulates feeding behavior, body weight²⁶,
152 and innate and adaptive immune responses²¹ - was enhanced for SCAT and decreased for
153 EWAT.

154 Thus, IAV infection was associated with marked changes in metabolic and inflammatory
155 features of the subcutaneous and visceral adipose tissues. Importantly, infection was
156 associated with depot-specific modifications in WAT's lipid metabolism and leptin secretion.
157

158 **Influenza induces subcutaneous adipose tissue browning** To determine whether SCAT and
159 EWAT undergo qualitative and/or quantitative changes following influenza infection, we
160 performed histologic and histomorphometric analyses at 7 dpi. The EWAT samples from
161 mock-treated and IAV-infected mice had a similar histological appearance: white adipocytes
162 containing large unilocular lipid droplets (a typical WAT histology) (Supplementary Fig. 2a).
163 In contrast, the SCAT samples from mock-treated and IAV-infected mice showed histological
164 differences: numerous pockets of dense, small and multilocular adipocytes - a feature of
165 brown-like adipocytes, also known as beige or brite adipocytes²⁷ - were found interspersed
166 within the SCAT from infected animals (Fig. 2a). As reported in literature²⁸,
167 histomorphometric analyses confirmed that adipocytes are smaller in SCAT than EWAT
168 (Supplementary Fig. 2c). In accordance with the appearance of small brown-like/beige
169 adipocytes, the SCAT from IAV-infected mice showed higher numbers of small adipocytes
170 and reduced numbers of large adipocytes than the SCAT from mock-treated mice (Fig. 2b).
171 The differences in adipocyte size between mock-treated and IAV-infected mice were less
172 pronounced for the EWAT (Supplementary Fig. 2b). Moreover, levels of the mitochondrial
173 uncoupling protein 1 (UCP1, a marker of the brown adipose tissue²⁹) were higher in SCAT
174 protein lysates from infected mice than in those from mock-treated controls. In contrast, no

175 UCP1 was detected in the lungs or EWAT lysates from IAV-infected mice (Fig. 2c, 2d and
176 Supplementary Fig. 2d). In line with increased levels of thermogenic UCP1, the surface
177 temperature generated from the SCAT was higher in IAV-infected mice than in mock-treated
178 mice (Fig. 2e).

179 These results emphasized the depot-specific nature of the effects of influenza infection on fat,
180 with the appearance of thermogenic brown-like/beige adipocytes in the subcutaneous depot
181 being the most notable.

182

183 **Transcriptomics shows fat-depot-specific response to influenza** To gain insights into the
184 molecular basis of the depot-specific effects of influenza infection on fat tissues, we
185 performed transcriptomic analyses on the SCAT and EWAT isolated from mock-treated and
186 IAV-infected mice ($p \leq 0.05$ and fold change (FC) cutoff of ≥ 2). In SCAT, 1,214 genes were
187 found differentially expressed between mock-treated and IAV-infected mice. In EWAT, 660
188 differentially expressed genes (DEGs) were identified. Venn diagrams were used to display
189 comparison of the lists of upregulated or downregulated DEGs (Fig. 3a). Upon infection, 296
190 genes were upregulated in both SCAT and EWAT, 268 genes were upregulated only in
191 SCAT, and 177 genes were upregulated only in EWAT. Regarding infection-associated
192 downregulated genes, 148 genes were commonly downregulated in SCAT and EWAT, 502
193 genes were downregulated only in SCAT, and 39 were downregulated only in EWAT. Then,
194 canonical pathway analyses of these DEG lists were conducted using Ingenuity Pathways
195 Analysis (IPA). The interferon signaling pathway was identified as the top canonical pathway
196 with the highest positive z-score in the list of 296 genes upregulated in SCAT and EWAT,
197 suggesting activation of the pathway in both fat depots during infection (Fig. 3b). For these
198 upregulated genes, IPA predicted potential upstream regulators. The top activated upstream
199 regulators and their targets are listed in Supplementary Table 1. Interestingly, the transcription

200 factors IRF3 and IRF7 are recognized as key regulators of type I IFN gene expression induced
201 by viruses³⁰.

202 IPA identification of pathways populated by genes upregulated during infection only in one
203 type of fat depots showed that the Rho-GTPase family signaling pathways were activated in
204 SCAT, and the T-cell-driven-immune/inflammatory pathways were activated in EWAT (yet
205 with relatively modest *p* values and percentages of overlap) (Supplementary Table 2).

206 From the core set of 148 genes that were downregulated during infection in both SCAT and
207 EWAT, IPA ranked multiple pathways linked to cholesterol biosynthesis as being the most
208 significantly associated with infection (Fig. 3c). Concordantly, the top upstream regulators
209 were predicted to be the transcription factors sterol regulatory element binding proteins
210 (SREBPs, also called SREBFs) and the endoplasmic reticulum (ER) protein SCAP, all of
211 which are master regulators of cholesterol biosynthesis³¹ (Supplementary Table 3).

212 Interestingly, transcriptomic data indicated the inhibition of oxidative phosphorylation
213 (OXPHOS), tricarboxylic acid (TCA) cycle and glycolysis during infection only in SCAT
214 (Fig. 3d). Indeed, within the mitochondrial energy metabolic pathways, numerous genes
215 coding for functional/structural components of the electron transport-linked OXPHOS
216 (predominantly in complex I) as well as TCA cycle genes were found to be downregulated in
217 SCAT (Supplementary Fig. 3). Concomitantly, the sirtuin signaling pathway was activated in
218 this fat depot (Fig. 3d). Sirtuins are a group of NAD⁺-dependent protein-deacetylating stress-
219 responsive enzymes that regulate glucose and lipid metabolism, inflammation and WAT
220 browning, the latter through deacetylation regulation of pathways upstream of UCP1^{32,33}. It is
221 noteworthy that opposite regulation directions of the OXPHOS pathway and the sirtuin
222 signaling pathway have been recently reported³⁴.

223 Overall, these results showed that influenza infection was associated with major
224 transcriptional changes in fat tissues. In both SCAT and EWAT, interferon signaling

225 pathways were activated and cholesterol biosynthesis pathways were repressed. Importantly,
226 infection impacted major metabolic pathways (glycolysis, OXPHOS, TCA cycle) only in
227 SCAT.

228

229 **Influenza durably alters the host's energy metabolism** Adipose tissue is a key metabolic
230 organ in the regulation of whole-body energy homeostasis^{16,18}. Thus, we looked at whether
231 influenza infection impacted on the host systemic metabolism. At 7 dpi, blood glucose levels
232 were lower in IAV-infected mice than in mock-treated controls, whereas insulin levels were
233 higher. Blood levels of resistin - originally described as a WAT-derived hormone modulating
234 insulin resistance in rodents³⁵ - were lower in infected animals than in controls (Fig. 4a).
235 Remarkably, these changes in blood glucose, insulin and resistin levels persisted long after the
236 infection had resolved (up to 20 weeks post-infection) (Fig. 4b). However, these long-lasting
237 metabolic sequelae had no impact on the animals' glucose tolerance, as revealed by an
238 intraperitoneal glucose tolerance test (Supplementary Fig. 4a).

239 Studies in various rodent models have shown that white-to-brown adipose tissue remodeling
240 prevents high-fat-diet (HFD)-induced obesity^{27,36}. Hence, we hypothesized that IAV-infected
241 mice would be protected against obesity. Mock-treated and IAV-infected mice were fed an
242 HFD from 7 dpi (the time when SCAT browning became apparent). As expected, mock-
243 treated mice fed an HFD (mock HFD) gained more weight than mock-treated mice fed a
244 standard diet (mock SD). Although IAV-infected mice fed an HFD (IAV HFD) gained less
245 weight than mock-treated mice fed an HFD (mock HFD), they still gained more weight than
246 when fed a standard diet (IAV SD) (Fig. 4c, and Supplementary Fig. 4b for the corresponding
247 area under the curve (AUC)). Accordingly, IAV-infected mice were not protected against
248 HFD-induced glucose intolerance (Fig. 4d, and Supplementary Fig. 4c for the corresponding
249 area under the curve (AUC)). However, it is noteworthy that HFD-fed IAV-infected animals

250 showed lower levels of blood glucose and resistin than HFD-fed mock-treated mice (Fig. 4e),
251 as was observed at 7 dpi and 20 weeks post-infection under standard diet conditions (see Fig.
252 4a, 4b).

253 These results indicated that influenza infection has a long-lasting impact on whole-body
254 glucose metabolism that is independent of the host's dietary status (it occurred in lean and
255 obese conditions).

256

257 **Viral protein-expressing cells are detected in adipose tissues** Next, we sought to quantify
258 viral RNA in the adipose tissues from IAV-infected mice (*MI* negative strand specific qPCR
259 assay). Albeit at lower levels than in the lungs, viral RNA was detected in SCAT and EWAT
260 (but not in pancreas) at 7 dpi (Fig. 5a). To get some insight into the dynamics of viral RNA
261 accumulation, viral RNA levels were quantified in the lungs, SCAT and EWAT at 2, 4, 7, 14
262 and 28 dpi (Fig. 5b). In the lungs, the viral load rapidly increased from 2 dpi, peaked at 7 dpi,
263 and rapidly declined thereafter. In SCAT and EWAT, we also observed a time-dependency of
264 viral RNA accumulation, which started from 2 dpi, culminated at 7 dpi and decreased
265 afterwards, as noticed in the lungs.

266 WAT's cellularity is highly heterogeneous^{18-20,22}. Mature, lipid-filled adipocytes make up
267 approximately 30% of adipose tissue cells. The remaining stromal vascular fraction (SVF)
268 cells include vascular cells, fibroblasts, innate and adaptive immune cells, and adipocyte
269 precursor cells (preadipocytes). We therefore separated mature adipocytes from SVF cells
270 through enzymatic fractionation of the SCAT and EWAT from mock-treated and IAV-
271 infected mice (Fig. 5c). As shown in Supplementary Fig. 5a, viral RNA was detected in SVF
272 cells isolated from SCAT and EWAT. No viral RNA was detected in adipocytes isolated from
273 either fat depot. Thus, immune cells (identified as CD45⁺ cells) and preadipocytes (identified
274 as CD45⁻ CD31⁻ CD29⁺ CD34⁺ Sca1⁺ cells) from the SVF of the SCAT and EWAT from

275 mock-treated and IAV-infected mice were sorted by fluorescent-activated cell sorting (FACS)
276 (see gating strategy Supplementary Fig. 5b) and stained for the viral protein hemagglutinin
277 (HA) (Fig. 5d). HA-positive immune cells were found in the SCAT of nearly (84%) all
278 infected mice, a frequency that was much lower in the EWAT (17%). Half of infected mice
279 had HA-positive preadipocytes in the SCAT, while none were detected in any of the EWAT
280 samples.

281 Thus, immune cells and (less frequently) preadipocytes bearing viral RNA (M1) and viral
282 protein (HA) were detected in the adipose tissues (mostly in SCAT) of influenza-infected
283 mice. In contrast, no adipocytes bearing viral RNA were found in either fat depot.

284

285 **Influenza induces brown-like adipogenesis in preadipocytes** Preadipocytes share numerous
286 phenotypic and functional properties with macrophages³⁷. IAV replication in macrophages is
287 most often abortive (no release of new infectious virions), yet certain virus strains can
288 replicate productively in these cells^{38,39}. Thus, the detection of HA-positive preadipocytes in
289 the WAT from infected mice (see Fig. 5d) led us to question the IAV-infection ability of
290 murine preadipocytes *in vitro*. We used mouse preadipocyte 3T3-L1 cells, which can
291 differentiate into adipocytes upon induction with a standard hormone cocktail⁴⁰. Hence,
292 undifferentiated 3T3-L1 cells (from now on referred to as preadipocytes) were exposed to
293 graded doses of IAV (at MOI of 0.01, 0.25, 0.5, 1 or 2) and viral RNA levels were evaluated
294 at 6, 24 and 48 hours post-infection (hpi). As shown in Fig. 6a, viral RNA levels rose from 6
295 to 24 hpi at each MOI; this suggested efficient IAV infection and viral genome replication in
296 preadipocytes. It is noteworthy that infection did not affect the viability of preadipocytes, in
297 contrast to highly permissive Madin-Darby Canin Kidney (MDCK) cells (Supplementary Fig.
298 6a).

299 Unlike most RNA viruses, IAVs transcribe and replicate their genome inside the nucleus; this
300 induces major nuclear and nucleolar ultrastructural changes⁴¹. As shown in Fig. 6b, high-
301 resolution transmission electron microscopy revealed the marked accumulation of viral M1-
302 associated rod-like tubular structures in the nucleus, and the complete segregation of nucleolar
303 components in infected preadipocytes (MOI of 5, at 24 hpi), relative to mock-treated cells. In
304 addition, the particles budding from the preadipocytes' plasma membranes appeared to be
305 empty (Fig. 6b). Consistently, no infectious particles were detected in the supernatants of
306 infected preadipocytes while fully infectious virions were released from infected murine lung
307 epithelial cells (MLE-15 cells) (MOI of 0.5, at 6, 24, 48 and 72 hpi) (Supplementary Fig. 6b).
308 We next sought to determine preadipocytes' response to infection (MOI of 1, at 24 hpi) by
309 quantifying the expression of the antiviral innate-immune-related genes *Tlr3* (coding for Toll-
310 like receptor 3), *RigI* (coding for retinoic acid-inducible protein I), *Mda5* (coding for
311 melanoma-differentiation associated protein 5), *Mx1* (coding for Mx1) and *Vip* (coding for
312 viperin) (Fig. 6c). Relative to mock-treated cells, the expression of these genes was enhanced
313 in infected preadipocytes. It is worth mentioning that *in vitro* infection of preadipocytes at
314 much lower MOI (0.01) also led to increased *Tlr3*, *RigI*, *Mda5*, *Mx1* and *Vip* transcript levels
315 (Supplementary Fig. 6c).
316 Importantly, infection enhanced the expression of genes involved in the browning process,
317 such as *Ucp1*, *Pgc1a* (coding for peroxisome proliferator-activated receptor gamma
318 coactivator 1 alpha), *Fgf21* (coding for fibroblast growth factor 21), *Apln* (coding for apelin),
319 *Metnl* (coding for meteorin-like protein) and *Tmem26* (coding for transmembrane protein
320 26)⁴²⁻⁴⁵ in preadipocytes (MOI of 1, at 24, 48 and 72 hpi) (Fig. 6d). Accordingly, adipocytes
321 that had differentiated *in vitro* from IAV-infected preadipocytes had higher *Ucp1* and *Pgc1a*
322 transcript levels than adipocytes that had differentiated from mock-treated preadipocytes
323 (Supplementary Fig. 6d).

324 Although no viral RNA-harboring adipocytes were detected in either fat depots from IAV-
325 infected mice, previous literature reported that adipocytes might be permissive to IAV
326 infection *in vitro*^{46,47}. Thus, differentiated 3T3-L1 preadipocytes (from now on referred to as
327 adipocytes) were infected with IAV, *in vitro*. As shown in Supplementary Fig. 7, infection
328 had no impact on adipocytes' viability (Supplementary Fig. 7a), and resulted in increased
329 levels of viral RNA (Supplementary Fig. 7b), nuclear and nucleolar changes featuring
330 efficient viral genome replication, and budding of empty particles (Supplementary Fig. 7c), as
331 well as increased expression of the antiviral innate-immune-related genes *Tlr3*, *RigI*, *Mda5*,
332 *Mx1* and *Vip* (Supplementary Fig. 7d). As opposed to preadipocytes (see Fig. 6d), IAV
333 infection of adipocytes did not increase the expression of the browning-like/beiging-related
334 genes *Ucp1*, *Pgc1a*, *Fgf21*, *Apln*, *Metrnl* and *Tmem26* (Supplementary Fig. 7e).
335 Importantly, *in vitro* IAV infection of human primary preadipocytes (MOI of 1, 48 hpi)
336 significantly increased the expression of the browning-like/beiging-related genes *Ucp1*,
337 *Prdm16* (coding for PR domain containing 16), *Tmem26* and *Tbx15* (coding for T-Box15). As
338 observed with mouse cells, infection of human adipocytes (differentiated from human primary
339 preadipocytes) did not increase the expression of these genes (with the exception of *Ucp1*)
340 (Supplementary Fig. 7f).
341 These data showed that preadipocytes and adipocytes are permissive to *in vitro* IAV infection;
342 however, virus replication is abortive. Most importantly, *in vitro* infection induced the
343 commitment of preadipocytes to brown-like/beige adipocytes, and not the white-to-beige
344 conversion of mature adipocytes.

345

346 **Influenza induces metabolic reprogramming of preadipocytes** A comparative analysis of
347 the transcriptomes of *in vitro* mock-treated and IAV-infected (MOI of 1, at 24 hpi)
348 preadipocytes (3T3-L1 cells) and adipocytes (differentiated from 3T3-L1 cells) showed that

349 more genes were up- or down-regulated following infection in preadipocytes than in
350 adipocytes (1,472 genes vs. 585 genes, respectively), and that more genes were upregulated
351 than downregulated in both cell types (981 upregulated genes vs. 491 downregulated genes in
352 preadipocytes, and 566 upregulated genes vs. 19 downregulated genes in adipocytes) (Fig.
353 7a). IPA analyses revealed that the 449 genes that were upregulated upon infection both in
354 preadipocytes and adipocytes were related to pathways endowed with interferon signaling,
355 virus sensing and antiviral immune response (Supplementary Table 4).

356 The 117 genes that were only upregulated in adipocytes were related to stress-signaling
357 pathways, yet with rather low *p* values and overlap percentages (Supplementary Table 5).

358 Strikingly, for the 532 genes exclusively upregulated in preadipocytes, the top-scored
359 canonical pathways were the serine-glycine biosynthesis, folate transformations (such as
360 polyglutamylolation), and glycine biosynthesis, pathways (Supplementary Table 6). These
361 metabolic processes are connected by the serine-glycine-one-carbon (SGOC) pathway; an off-
362 shoot of glycolysis⁴⁸. Indeed, the IAV-infection-gene-signature in preadipocytes includes
363 several genes encoding enzymes within the SGOC pathway, such as the cytosolic
364 phosphoglycerate dehydrogenase (PHGDH), phosphoserine phosphatase (PSPH) and serine
365 hydroxymethyltransferase (SHMT) 1, and the mitochondrial SHMT2,
366 methylenetetrahydrofolate dehydrogenase (NADP⁺ dependent) 1 like (MTHFD1L) and
367 MTHFD2 (Fig. 7b). It is worth mentioning that the upregulation of several enzymes involved
368 in SGOC metabolism has been recently reported for IAV-infected macrophages⁴⁹. The
369 upstream regulators predicted to be associated with the genes exclusively upregulated in
370 preadipocytes included activating transcription factor 4 (ATF4), UCP1 and MYC
371 (Supplementary Table 6). ATF4 and MYC form a positive feedback loop for transcriptional
372 activation of the SGOC pathway⁵⁰, and UCP1 was reported to induce *Atf4* expression⁵¹.

373 The SGOC metabolism generates diverse outputs, such as the synthesis of lipids, nucleotides,
374 ATP and proteins⁴⁸, suggesting that the infected preadipocytes' translational machinery might
375 have been activated. Indeed, tRNA charging was also identified as a top-ranked canonical
376 pathway activated in infected preadipocytes (Supplementary Table 6). Concordantly, the
377 mRNA expression levels of genes related to ribosome biogenesis (*Urb2*, coding for unhealthy
378 ribosome biogenesis protein 2, *Wdr75*, coding for WD repeat domain 75, *Zfp593*, coding for
379 zinc finger protein 593, *Mrto4*, coding for MRT4 homolog, ribosome maturation factor and
380 *Nop58*, coding for nucleolar protein 58) were enhanced upon *in vitro* infection in
381 preadipocytes and not in adipocytes (Fig. 7c).

382 Only a small number of genes were downregulated in adipocytes alone (n = 13) or in both
383 preadipocytes and adipocytes (n = 6); no biological functions or pathways could therefore be
384 assigned. In preadipocytes, pathways related to amino acid catabolism and cholesterol
385 biosynthesis were found to be downregulated upon infection, yet with modest *p* values and
386 overlap percentages (Supplementary Table 7).

387 Altogether, transcriptome analyses suggested activation of the interferon-dependent antiviral
388 response in both preadipocytes and adipocytes upon *in vitro* infection, and illuminated
389 intrinsic differences between the two types of cells. Indeed, preadipocytes dealt with infection
390 by acquiring a metabolic program mainly characterized by the transcriptional activation of the
391 SGOC metabolic pathway.

392

393 **Influenza changes mitochondrial bioenergetics of adipose cells** It has been reported that
394 IAV infection decreases host cell's respiration⁵². We above showed that *in vitro* IAV infection
395 of preadipocytes induced their commitment to brown-like/beige adipocytes, increased the
396 expression of genes involved in the SGOC metabolic pathway, and was associated with the
397 elevated expression of genes involved in ribosome biogenesis - all processes known to impact

398 cellular bioenergetics^{53,54}. Thus, we used high-resolution respirometry to record oxygen
399 consumption rates (OCRs) of mock-treated vs. IAV-infected preadipocytes (3T3-L1) and
400 adipocytes (differentiated from 3T3-L1) at 0, 24, 48 and 72 hpi (MOI of 1). The OCRs were
401 measured in the resting respiratory state and after the sequential addition of specific
402 mitochondrial inhibitors or uncouplers. These steps enable us to ascribe OCR values into
403 distinct mitochondrial and cellular processes (proton (H⁺) leakage, maximal respiratory state,
404 and non-mitochondrial residual respiratory state) (Supplementary Fig. 8a). As expected from
405 the literature⁵⁵, all respiratory states were significantly higher in mock-treated adipocytes than
406 in mock-treated preadipocytes (Supplementary Fig. 8b). As shown in Supplementary Fig. 8c,
407 infection impacted bioenergetics differently in preadipocytes and adipocytes. In
408 preadipocytes, IAV infection tended (as expected from the results presented above) to
409 increase the OCRs after 48 hours but this rise was not statistically significant. Conversely, the
410 cellular respiration of adipocytes progressively decreased after IAV infection (as has been
411 described for highly IAV-permissive MDCK cells⁵²) and the difference reached significance
412 at 72 hpi.

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423 **Discussion**

424 Persistent, chronic infections by viruses, parasites or bacteria often lead to metabolic
425 anomalies due to the functionality impairment of metabolic tissues or organs such as the liver,
426 skeletal muscle, pancreas, brain and WAT⁵⁶. Lymphocytic choriomeningitis virus, human and
427 simian immunodeficiency viruses, adenovirus, vaccinia virus, and several species of parasites
428 and bacteria have been reported to target murine and human WAT directly⁵⁷⁻⁶¹. In contrast, it
429 is not clear whether acute infections also result in long-lasting metabolic changes - notably by
430 altering WAT's functions. This is a compelling question, given the frequency and diversity of
431 the acute infections with which modern societies are burdened.

432 Here, we report that a single acute (intranasal) infection with a respiratory pathogen (seasonal
433 influenza A virus subtype H3N2) profoundly modifies WAT's metabolic and immune
434 functions, in mice. Transcriptome analyses of the subcutaneous and visceral fat depots in
435 response to influenza infection (Fig. 8a) revealed the activation of type I interferon signaling
436 pathways associated with the concomitant inhibition of cholesterol biosynthesis pathways
437 both in SCAT and EWAT. The reciprocal negative crosstalk between antiviral type I IFN
438 responses and cholesterol homeostasis is increasingly being recognized⁶². Reconfiguring
439 cholesterol metabolism is now considered as an integral component of the host's response to
440 infection both through heightening antiviral immunity^{63,64}, and creating an unfavorable
441 environment for virus stability and infectivity⁶⁵. Interestingly, the transcriptional response to
442 infection also differed according to the localization of fat depots. Indeed, pathways related to
443 energy metabolism were altered upon infection only in SCAT: the glycolysis, TCA cycle and
444 OXPHOS metabolic pathways were repressed, while the sirtuin signaling pathway was
445 activated. The mammalian sirtuin (SirT) family is categorized as stress-induced-NAD⁺-
446 dependent protein deacetylases that regulate processes such as energy metabolism and
447 inflammation³². Several SirT family members have been reported to promote WAT lipolysis⁶⁶

448 and WAT browning⁶⁷, two processes that are closely linked⁶⁸. In accordance with SirT
449 signaling pathway activation, *Mgl* expression level was increased and brown-like/beige
450 adipocytes were observed in the SCAT from infected mice; this suggested increased
451 lipolysis⁶⁹ and thermogenesis. Indeed, expression of the thermogenic UCP1 protein was
452 increased in SCAT upon infection, and this correlated with elevated surface temperature, as
453 expected from literature⁷⁰.

454 In rodents, SCAT (less frequently EWAT) is readily able to convert to a brown-like state in
455 response to environmental stimuli including chronic cold adaptation, exercise and nutritional
456 challenges; as well as external and internal cues such as pharmacological treatment with β 3-
457 adrenergic receptor agonists and various peptides and hormones⁷¹. Leptin (which secretion is
458 enhanced in the SCAT upon infection) and insulin (which blood level is increased in infected
459 mice) have been reported to promote WAT browning⁷². Influenza infection may also have
460 favored the accumulation of immune cells associated with WAT browning, such as M2
461 macrophages, eosinophils or innate lymphoid type 2 cells⁷³, in the SCAT.

462 Strikingly, we found that immune cells harboring viral proteins (HA) were present in the
463 WAT (mostly SCAT) of influenza-infected mice. Immune cell egress from the lungs has been
464 reported in the context of influenza infection^{74,75}. Thus, immune cells (either infected or
465 harboring remnant viral antigens and RNA) may have entered the bloodstream through the
466 capillaries of damaged alveolar wall of the infected lungs and reached fat depots, participating
467 to the observed changes in WAT's secretory function and to the induction of antiviral-innate-
468 immunity-related pathways. The phenotypic characterization of immune cells that are present
469 in the fat depots of IAV-infected mice is currently under investigation.

470 Alongside immune cells, viral antigen-positive preadipocytes were also found, although less
471 frequently, in the WAT from infected mice. How preadipocytes acquired viral antigens from
472 immune cells that have migrated from infected lungs to WAT, remains to be established.

473 Also, determining whether other WAT's cell types are impacted during influenza infection
474 should be investigated since WAT's functionality result from the concerted interplay between
475 all these cells. Single-cell RNA sequencing combined with flow cytometry or mass cytometry
476 (CyTOF) could help answer this key question⁷⁶.

477 Using *in vitro* approaches (Fig. 8b), we showed that preadipocytes - that share many
478 properties with macrophages³⁷ - are permissive to IAV infection, yet replication was abortive.
479 Importantly, infection induced the browning-like/beiging program in preadipocytes and this
480 was associated with the activation of the folate-dependent SGOC pathway, an offshoot of
481 glycolysis⁴⁸ recently reported to drive effector responses to IAV infection in macrophages⁴⁹.

482

483 The impact of acute infections on the metabolism of the affected hosts has been rather
484 set aside. Here, we reported a systemic response to influenza infection that modulates WAT's
485 metabolic and immune functions. Importantly, infection was also associated with changes of
486 host's metabolism that persist long after infection has been resolved. Altogether, these
487 findings illustrate the complexity of influenza virus-host interactions, in which the adipose
488 tissue may play an important role.

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498 **Methods**

499 **Mice, virus and *in vivo* infection protocol**

500 Male C57BL/6JRj mice (6-week-old; Janvier Labs, Le Genest-Saint-Isle, France) were
501 housed and manipulated in an Animal Biosafety Level-2 facility, in strict accordance with
502 *Institut Pasteur's* guidelines on animal care and use, and in compliance with European animal
503 welfare regulations (European Communities Council Directive of 1986 revised in 2010,
504 2010/63/EU). Protocols were approved by the regional Animal Experimentation Ethics
505 Committee (*Comité d'Ethique en Expérimentation Animale, Hauts-de-France, CEEA 75*) and
506 the French Ministry of Higher Education and Research (*Ministère de l'Enseignement*
507 *Supérieur et de la Recherche*) (authorization numbers: 00357.03 and 00033.02). Mice were
508 fed *ad libitum* with low-fat diet (LFD: 10% kcal from fat) or, when mentioned, with high-fat
509 diet (HFD: 60% kcal from fat) obtained from Research Diets (New Brunswick, NJ, USA).
510 The human-derived, mouse-adapted influenza A/Scotland/20/1974 (H3N2) virus was grown,
511 isolated and titrated in Madin-Darby Canine Kidney (MDCK) cells.

512 Mice were administrated intranasally with a sublethal dose (30 PFU, in 50 µl PBS) of H3N2
513 (IAV groups) or with PBS (mock groups). Body weights were monitored. Intraperitoneal
514 glucose tolerance tests and infrared thermography measurements were performed. Blood,
515 lungs, subcutaneous (inguinal) and visceral (epididymal) white adipose tissue depots (SCAT
516 and EWAT, respectively) were collected and weighed. SCAT and EWAT explants were
517 cultured for 16-24 hours for hormone and cytokine detection in culture supernatants by
518 ELISA. White adipose tissues were processed for further histological, flow cytometry,
519 Western blot or gene expression analyses.

520

521 ***In vivo* assessment of metabolic phenotyping**

522 For determination of intraperitoneal glucose tolerance, mice were fasted for 10-12 hours
523 before being injected (intraperitoneally) with 1g/kg body weight of D-Glucose (Sigma-
524 Aldrich, Saint-Louis, Missouri, USA). Glucose levels were measured by tail-tip bleeding with
525 an automatic glucometer (ACCU-CHEK Performa, Roche, Mannheim, Germany) before
526 injection, and 15, 30, 60, 120 and 180 minutes after glucose administration.

527 For infrared thermography, an infrared camera (FLiR systems) was used to acquire static
528 images of the SCAT region of unrestrained, anesthetized mock-treated and IAV-infected
529 mice, 7 dpi, n = 3 mock-treated animals and n = 4 IAV-infected animals (3 measurements per
530 mouse), as described in Crane *et al.*⁷⁷.

531

532 ***Ex vivo* mouse adipose tissue explant cultures**

533 SCAT and EWAT samples were isolated from mock-treated and IAV-infected mice (7 dpi),
534 and cultured as described in Thalmann *et al.*²⁵. Tissue explants were put into 6-well plates
535 (~50 mg/well) (Sarstedt, Nümbrecht, Germany) and incubated for 24 hours in DMEM without
536 serum and supplemented with 1% penicillin/streptomycin (Life Technologies, CA, USA) in a
537 humidified 37°C, 5% CO₂ incubator. After the incubation period, the medium was aspirated
538 from the floating fat and centrifuge for 5 minutes at 500 g at room temperature to remove any
539 excess lipids or debris. The resultant explant culture supernatants were stored at -80°C until
540 further analysis.

541

542 **Mouse adipose tissue histology and histomorphometry**

543 SCAT and EWAT were harvested from mock-treated and IAV-infected mice, at 7 dpi. After
544 overnight fixation in 4% paraformaldehyde, tissue samples were embedded in paraffin.
545 Multiple sections (5 µm) were de-paraffinized, rehydrated, and stained with hematoxylin and
546 eosin (H&E). For evaluation of adipocyte number and size by morphometry, at least 10 fields

547 per H&E-stained slide were visualized and images were digitally captured using a digital
548 camera (AxioCam HRc, Zeiss, Göttingen, Germany) connected to an optical microscope
549 (Axioplan 2 Imaging, Zeiss). Adipocyte area (in μm^2) was measured using an in-house macro
550 specifically developed for automated image analysis of WAT on the Fiji-ImageJ software
551 (NIH).

552

553 **Cell fractionation of mouse adipose tissue**

554 Individual SCAT and EWAT were carefully excised from mock-treated and IAV-infected
555 mice at 7 dpi. Tissues were thoroughly minced with scissors (1-2 mm^3 pieces) and digested in
556 collagenase I (1 mg/ml, Sigma-Aldrich) for 1 hour at 37°C with gentle shaking by inversion
557 every 20 minutes. Digested tissues were then passed through a 250 μm nylon filter and
558 centrifuged at 150 g for 17 minutes at room temperature (RT). The floating fraction -
559 containing intact, purified mature adipocytes with minimal stromal cell contamination (see
560 Supplementary Fig. 5c) - were carefully recovered with a wide opening plastic transfer pipette
561 and processed for further gene expression analyses. Remaining infranatants were centrifuged
562 at 400 g for 5 minutes, RT. The erythrocytes from the SVF cell-pellets were lysed with cold
563 lysis buffer (3 minutes, on ice) and SVF cells were filtered through a 90 μm and then a 40 μm
564 nylon filter before being washed twice by centrifugation at 400 g for 5 minutes, RT. SVF cells
565 were counted and resuspended in PBS 0.5% BSA for further flow cytometry sorting.

566

567 **Identification and sorting of cells from mouse adipose tissue**

568 SVF cell suspensions were first incubated with an Fc blocking reagent (anti-mouse
569 CD16/CD32, clone 2.4G2, BD Pharmingen, San Jose, CA, USA). Afterward, cells were
570 stained with anti-mouse CD31 (BV421, clone 390), CD45 (BV510, clone 30-F11), CD34
571 (PE, clone HM34), CD29 (AF700, clone HM β 1-1) from BioLegend, and Sca-1 (APC-Cy7,

572 clone D7) from eBioscience, at a dilution of 1:100. Samples were then directly run into the
573 BD Influx™ cell sorter (BD Biosciences) equipped with a 86 µm nozzle and tuned at a
574 pressure of 24.6 psi and a frequency of 48.25 kHz. Sample fluid pressure was adjusted to
575 reach a throughput rate of 10,000 events per second. Immune cells and preadipocytes were
576 selected as CD45⁺ and CD45⁻ CD31⁻ CD34⁺ CD29⁺ Sca-1⁺, respectively. The gating strategy
577 is shown on Supplementary Fig. 5b.

578

579 ***In vitro* differentiation of preadipocytes, and infection procedure**

580 The mouse 3T3-L1 preadipose cell-line (ATCC®, CL-173™) was used. 3T3-L1 cells were
581 cultured in Dulbecco's modified Eagle's medium (DMEM), high glucose, GlutaMAX™
582 (Gibco, ThermoFisher Scientific, Waltham, M.A., USA) supplemented with 10% heat-
583 inactivated fetal bovine serum (FBS) (Gibco) and 1% penicillin/streptomycin in a humidified
584 37°C, 5% CO₂ incubator. The differentiation of 3T3-L1 cells into mature adipocytes was
585 conducted as indicated in⁴⁰: 3T3-L1 preadipose cells were seeded in 6-well plates (Sarstedt) at
586 a density of 4x10⁵ cells per well, in 2 ml of culture medium. Two days after reaching
587 confluence, cells were incubated in the differentiation medium containing 1µg/ml insulin
588 (Sigma-Aldrich, Saint-Louis, Missouri, USA), 0.25µM dexamethasone (Sigma-Aldrich) and
589 0.5mM 3-isobutyl-1-methylxanthine (IBMX) (Sigma-Aldrich) for two days. The medium was
590 then replaced by the maintenance medium that consists of DMEM supplemented with 10%
591 FBS, 1% penicillin/streptomycin and 1µg/ml insulin. The maintenance medium was renewed
592 every two or three days.

593 Human primary preadipocytes were purchased from Lonza (Basel, Switzerland) together with
594 the differentiation kit and the culture medium. Preadipocytes were induced to differentiate
595 into fully mature adipocytes according to manufacturer's instructions.

596 Mouse or human preadipocytes and adipocytes (4×10^5) were infected with IAV at 0.01, 0.25,
597 0.5, 1, 2 or 5 multiplicities of infection (MOIs), as indicated. Mock-treated cells were used as
598 controls. Cell viability was determined using the OranguTM colorimetric assay (Cell Guidance
599 Systems, Cambridge, UK). Supernatants were collected for quantification of released
600 infectious particles by using the plaque-forming assay on MDCK cells. Cells were used for
601 Western blot, transmission electron microscopy, immunostaining, or gene expression
602 analyses.

603

604 **High-resolution respirometry on mouse adipose cells**

605 Oxygen consumption rates (OCRs) of IAV-infected (MOI of 1) and mock-treated
606 preadipocytes and adipocytes (10^6 cells), were determined using high-resolution respirometry
607 (Oxygraph-2k, OROBOROS Instruments, Innsbruck, Austria) at 24, 48 and 72 hours post-
608 infection (hpi). Experiments were performed under controlled temperature (37°C) and stirring
609 (750 rpm).

610 OCRs were measured under baseline conditions (recording resting respiration) and in
611 response to, successively, $2 \mu\text{g/ml}$ oligomycin (ATP synthase inhibitor; recording proton leak
612 respiration, H^+ Leak), pulses of $0.5 \mu\text{M}$ carbonyl cyanide *m*-chlorophenylhydrazone (CCCP)
613 (mitochondrial uncoupler; recording maximal respiration) and $2.5 \mu\text{M}$ antimycin A
614 (mitochondrial chain complex III inhibitor; recording residual respiration). Data acquisition
615 and analyses were performed with DatLab 4 software (OROBOROS Instruments).

616

617 **Western blotting**

618 Tissues (lungs, SCAT and EWAT) were homogenized in lysis buffer supplemented with
619 protease inhibitors (cOmpleteTM Protease Inhibitor Cocktail, Sigma-Aldrich). Whole tissue
620 protein extracts ($40\text{-}50 \mu\text{g}$) were fractionated by 10% SDS-PAGE and transferred onto

621 polyvinylidene difluoride membranes using a transfer apparatus as per the manufacturer's
622 instructions (Life Technologies). Membranes were blocked with 5% Bovine Serum Albumin
623 (BSA) in PBS/0.1% Tween for 1 hour, washed and probed overnight at 4°C with polyclonal
624 antibody against UCP1 (1:500) (Abcam, Cambridge, UK). Membranes were washed and
625 incubated with horseradish peroxidase-conjugated goat anti-rabbit antibody (KPL, Milford,
626 MA, USA) for 2 hours. After three washings, blots were developed with the ECL system
627 (Bio-Rad Laboratories) according to the provided protocol. GAPDH (Santa Cruz
628 Biotechnology, Dallas, Tx, USA) was used as protein loading control.

629

630 **Transmission electron microscopy**

631 *In vitro* mock-treated and IAV-infected (MOI of 5) mouse preadipocytes and adipocytes
632 ($5 \cdot 10^6$) were fixed in 4% glutaraldehyde for 15 minutes at 4°C and post-fixed in cacodylate
633 (0.2 M, pH 7.4)/4% glutaraldehyde (1:1) for 30 minutes at 4°C. Cell pellets were embedded in
634 EPONTM resin (Sigma-Aldrich) that was allowed to polymerize at 60°C for 48 hours.
635 Ultrathin sections were cut, stained with 5% uranyl acetate and 5% citrate and deposited onto
636 gold electron microscopy grids for examination using a JEOL 1011 transmission electron
637 microscope (JEOL Ltd., Tokyo, Japan).

638

639 **Immunofluorescence using confocal microscopy**

640 Immune cells and preadipocytes sorted from the SCAT and EWAT of mock-treated and IAV-
641 infected mice (7 dpi) were fixed in 4% paraformaldehyde for 15 minutes at room temperature
642 (RT), carefully washed with PBS/0.1% Tween-20, and permeabilized with 0.1% Triton X-
643 100. After a blocking step with PBS/1% BSA (1 hour, RT), cells were overnight (4°C)
644 labelled with the anti-H3N2 hemagglutinin (HA) polyclonal antibody (ThermoFisher
645 Scientific, Waltham, MA, USA) before being incubated (1 hour, RT) with the secondary

646 antibody (goat anti-rabbit Alexa Fluor[®] 488-labeled, Invitrogen), at concentration
647 recommended by the suppliers. Nuclei were then stained with DAPI (ThermoFisher
648 Scientific) and coverslips and slides were assembled using a mounting medium (ProLong[®]
649 Gold Antifade Mountant, ThermoFisher Scientific). Preparations were observed on a confocal
650 laser microscope (Zeiss LSM 880, Oberkochen, Germany).

651

652 **Gene expression quantification by real-time RT-PCR**

653 Total RNAs were extracted from snap-frozen tissues (SCAT, EWAT and lungs), from the
654 stromal vascular and adipocyte fractions isolated from adipose tissues, or from *in vitro*
655 cultured murine or human preadipocytes and adipocytes, according to the protocol supplied
656 with the RNeasy Lipid Tissue mini-kit (Qiagen, Hilden, Germany).

657 For RT-PCR amplification of all genes except the gene encoding the viral protein M1, RT
658 was performed with the High-capacity RNA-to-cDNA kit (Applied Biosystems, Foster City,
659 CA, USA) with oligo(dT)₁₆ primers and PCR was performed with the Power SYBR Green
660 PCR Master Mix (Applied Biosystems) using the QuantStudio[™] 12K Flex Real-Time PCR
661 System (Applied Biosystems). Reactions were run in duplicate and the geometric mean of the
662 housekeeping gene *Eef2* transcript levels was used as internal control to normalize the
663 variability in expression levels. Expression data were analyzed using the 2^{-ΔΔCt} method.

664 For quantification of viral RNA, a negative strand specific RT-qPCR assay for IAV RNA
665 encoding the *M1* gene (segment 7) was performed. Total RNAs were treated with RNase
666 OUT (Invitrogen) and reverse-transcribed with SuperScript[®] II Reverse Transcriptase
667 (Invitrogen) using primer specific for *M1* (5'-TCT AAC CGA GGT CGA AAC GTA-3').
668 PCR was performed with TaqMan Universal PCR Master Mix (Applied Biosystems),
669 detection primer pairs for *M1* (sense: 5'-AAG ACC AAT CCT GTC ACC TCT GA-3'; anti-
670 sense: 5'-CAA AGC GTC TAC GCT GCA GTC C-3') and *M1* specific TaqMan probe

671 ((FAM)5'-TTT GTG TTC ACG CTC ACC GTG CC-3'(TAMRA)), using the QuantStudio™
672 12K Flex Real-Time PCR System (Applied Biosystems).

673

674 **Whole-genome transcriptomics of adipose cells and tissues**

675 To prevent DNA contamination, RNAs extracted from adipose cells and tissues were treated
676 with DNase. The isolated RNAs were then dissolved in RNase-free water, after which the
677 concentration of total RNAs was measured and the quality was assessed for further
678 microarray expression analyses. The transcriptional profilings of mock-treated vs. IAV-
679 infected adipose cells and tissues were performed using Agilent's SurePrint G3 Mouse Gene
680 Expression v2 8x60K Microarray kits (Agilent Technologies, Santa Clara, CA, USA),
681 according to manufacturer's instructions.

682 The microarray analysis of *in vitro* mock-treated and IAV-infected mouse preadipocytes and
683 adipocytes (MOI of 1, 24 hpi) was performed at the *Centre de Ressources Biologiques pour la*
684 *Génomique des Animaux Domestiques et d'Intérêt Economique* (CRB GADIE), INRA Jouy-
685 en-Josas Research Center, France. Separate microarrays were run for each experimental
686 sample (one sample per culture condition, four independent experiments). A single-color
687 design was used to provide two types of comparisons: [mock-treated preadipocytes vs. IAV-
688 infected preadipocytes] and [mock-treated mature adipocytes vs. IAV-infected mature
689 adipocytes]. Identification of differentially expressed genes and functional investigation were
690 done using GeneSpring software (Version 14.8, Agilent Technologies). Differentially
691 expressed genes were identified using a moderated T-test. A Benjamini-Hochberg False
692 Discover Rate (FDR) was then used as multiple testing correction method and a corrected *p*-
693 value cutoff of 1% was applied. A fold-change >2 cutoff was then added to select for the
694 differentially expressed genes between the mock and infected conditions.

695 The microarray analysis of adipose tissues (SCAT and EWAT) of mock-treated and IAV-
696 infected mice (30 PFU, 7 dpi) was performed at the *Institut Pasteur*'s platform of
697 Transcriptomics and Applied Genomics. Separate microarrays were run for each experimental
698 sample (2 experimental groups, 4 mice per group, 2 tissues per mouse). After hybridization,
699 slides were scanned on a SureScan Microarray scanner (Agilent Technologies) and further
700 processed using Feature extraction v10.7.3.1 software. The resulting text files were uploaded
701 into language R v3.5.1 for LIMMA (Linear Model for Microarray Data) differential
702 expression analysis. A within-array normalization was performed using LOWESS (locally
703 weighed linear regression) to correct for dye and spatial effects. Moderated T-statistic with
704 empirical Bayes shrinkage of the standard errors was then used to determine significantly
705 modulated genes. Statistics were corrected for multiple testing using a FDR approach.
706 For functional analyses, Gene Ontology categories (www.geneontology.org) and Ingenuity
707 Pathway Analyses (IPA, www.qiagen.com/ingenuity, Ingenuity Systems, Inc. Redwood City,
708 CA, USA) were performed on the lists of commonly or exclusively differentially up- or
709 down- regulated genes.

710

711 **ELISA**

712 Insulin levels were quantified using an Ultrasensitive Mouse Insulin ELISA (Merckodia,
713 Uppsala, Sweden). Leptin and resistin levels were measured using: Mouse/Rat Leptin and
714 Mouse Resistin Quantikine ELISA kits (R&D Systems, Minneapolis, MN, USA). IL-1 β and
715 IL-6 dosages were carried out with ELISA Ready-SET-Go kits (eBioscience/Affymetrix,
716 ThermoFisher Scientific). IL-10 levels were determined using BD OptEIA™ kits (BD
717 Biosciences, Franklin Lakes, NJ, USA).

718

719 **Statistics and reproducibility**

720 Differences between experimental groups were determined using unpaired two-tailed
721 student's *t* test or analysis of variance (ANOVA) as appropriate and were considered
722 significant when *p*-values were lower than 0.05. For repeated measures (body weight
723 evolution, IP-GTT tests), two-way ANOVA analyses with Bonferroni post-test were
724 performed. For respirometry analyses, a one-way ANOVA followed by Tukey post-hoc test
725 was carried out when comparing four groups or more. Statistics were performed using
726 GraphPad Prism 6 software.

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745 **Data Availability**

746 Minimum information about Microarray Experiment (MIAME) on *in vitro* mock-treated vs.
747 IAV-infected preadipocytes, and *in vitro* mock-treated vs. IAV-infected adipocytes, was
748 deposited in ArrayExpress database (<https://www.ebi.ac.uk/arrayexpress/>) under the platform
749 ID E-MTAB-6646.

750 Minimum information about Microarray Experiment (MIAME) on the subcutaneous
751 (inguinal) adipose tissue (SCAT) of mock-treated vs. IAV-infected mice, and the visceral
752 (epididymal) adipose tissue (EWAT) of mock-treated vs. IAV-infected mice, was deposited in
753 ArrayExpress database (<https://www.ebi.ac.uk/arrayexpress/>) under the platform ID E-
754 MTAB-6646.

755 All data are available from the corresponding author upon reasonable request.

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770 **Author contributions**

771 A. A., S. L., J. B., A. P., A. M. -L., M. B., L. D., D. S., T. J., O. M. -C., L. S. and V. S.
772 performed the experiments. A. A., M. R. -C., S. L., A. M. -L., M. B., D. H., C. F., O. M. -C.,
773 D. D., R. L. G., F. T. and I. W. planned the experiments and/or analyzed the data, I. W., A.
774 A., and F. T. wrote the manuscript, I. W. oversaw the project. All authors provided critical
775 feedback and approved the content and the submission of the paper.

776

777 **Competing interests statement**

778 The authors declare no financial and non-financial competing interests.

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819 **References**

- 820 1. Ahmed, R., Oldstone, M. B. & Palese, P. Protective immunity and susceptibility to
821 infectious diseases: lessons from the 1918 influenza pandemic. *Nat. Immunol.* **8**, 1188-
822 1193 (2007).
- 823 2. Taubenberger, J. K. & Morens, D. M. The pathology of influenza virus infections. *Annu.*
824 *Rev. Pathol.* **3**, 499-522 (2008).
- 825 3. Iwasaki, A. & Pillai, P. S. Innate immunity to influenza virus infection. *Nat. Rev. Immunol.*
826 **14**, 315-328 (2014).
- 827 4. Sellers, S. A., Hagan, R. S., Hayden, F. G. & Fischer, W. A. The hidden burden of
828 influenza: A review of the extra-pulmonary complications of influenza infection. *Influenza*
829 *Other Respir. Viruses.* **11**, 372-393 (2017).
- 830 5. Openshaw, P. J. Crossing barriers: infections of the lung and the gut. *Mucosal Immunol.* **2**,
831 100-102 (2009).
- 832 6. Shu, Y., Li, C. K., Li, Z., Gao, R., Liang, Q., Zhang, Y., Dong, L., Zhou, J., Dong, J.,
833 Wang, D., Wen, L., Wang, M., Bai, T., Li, D., Dong, X., Yu, H., Yang, W., Wang, Y., Feng,
834 Z, McMichael, A. J. & Xu, X. N. Avian influenza A (H5N1) viruses can directly infect and
835 replicate in human gut tissues. *J. Infect. Dis.* **201**, 1173-1177 (2010).
- 836 7. Das, A. & Mukhopadhyay, S. The evil axis of obesity, inflammation and type-2 diabetes.
837 *Endocr. Metab. Immune Disord. Drug Targets.* **11**, 23-31 (2011).
- 838 8. Milner, J. J. & Beck, M. A. Obesity and influenza infection severity. *Future Virol.* **9**, 223-
839 225 (2014).
- 840 9. Hulme, K. D., Gallo, L. A. & Short, K. R. Influenza virus and glycemic variability in
841 diabetes: A killer combination? *Front. Microbiol.* **8**, 861-868 (2017).
- 842 10. Klemperer, H. Glucose breakdown in chick embryo cells infected with influenza virus.
843 *Virology.* **13**, 68-77 (1961).

- 844 11. Kohio, H. P. & Adamson, A. L. Glycolytic control of vacuolar-type ATPase activity: A
845 mechanism to regulate influenza viral infection. *Virology*. **444**, 301-309 (2013).
- 846 12. Smallwood, H. S., Duan, S., Morfouace, M., Rezinciuc, S., Shulkin, B. L., Shelat, A.,
847 Zink, E. E., Milasta, S., Bajracharya, R., Oluwaseun, A. J., Roussel, M. F., Green, D. R.,
848 Pasa-Tolic, L. & Thomas, P. G. Targeting metabolic reprogramming by influenza infection
849 for therapeutic intervention. *Cell Rep*. **19**, 1640-1653 (2017).
- 850 13. Lin, S., Liu, N., Yang, Z., Song, W., Wang, P., Chen, H., Lucio, M., Schmitt-Kopplin, P.,
851 Chen, G. & Cai, Z. GC/MS-based metabolomics reveals fatty acid biosynthesis and
852 cholesterol metabolism in cell lines infected with influenza A virus. *Talanta*. **83**, 262-268
853 (2010).
- 854 14. Tisoncik-Go, J., Gasper, D. J., Kyle, J. E., Einfeld, A. J., Selinger, C., Hatta, M.,
855 Morrison, J., Korth, M. J., Zink, E. M., Kim, Y. M., Schepmoes, A. A., Nicora, C. D.,
856 Purvine, S. O., Weitz, K. K., Peng, X., Green, R. R., Tilton, S. C., Webb-Robertson, B. J.,
857 Waters, K. M., Metz, T. O., Smith, R. D., Kawaoka, Y., Suresh, M., Josset, L. & Katze, M. G.
858 Integrated Omics analysis of pathogenic host responses during pandemic H1N1 influenza
859 virus infection: The crucial role of lipid metabolism. *Cell Host Microbe*. **19**, 254-266 (2016).
- 860 15. Munger, J., Bennett, B. D., Parikh, A., Feng, X. J., McArdle, J., Rabitz, H. A., Shenk, T.
861 & Rabinowitz, J. D. Systems-level metabolic flux profiling identifies fatty acid synthesis as a
862 target for antiviral therapy. *Nat. Biotechnol*. **26**, 1179-1186 (2008).
- 863 16. Luo, L. & Liu, M. Adipose tissue in control of metabolism. *J. Endocrinol*. **231**, R77-R99
864 (2016).
- 865 17. Pellegrinelli, V., Carobbio, S. & Vidal-Puig, A. Adipose tissue plasticity: how fat depots
866 respond differently to pathophysiological cues. *Diabetologia*. **59**, 1075-1088 (2016).
- 867 18. Ayari, A. & Wolowczuk, I. Physiology and pathophysiology of adipose tissue-derived
868 cytokine networks. In: *Cytokine effector functions in tissues*. Elsevier Ed (2017).

- 869 19. Ferrante, A.W. The immune cells in adipose tissue. *Diabetes Obes. Metab.* **15**, 34-38
870 (2013).
- 871 20. Mathis, D. Immunological goings-on in visceral adipose tissue. *Cell Metab.* **17**, 851-859
872 (2013).
- 873 21. La Cava, A. & Matarese, G. The weight of leptin in immunity. *Nat. Rev. Immunol.* **4**, 371-
874 379 (2004).
- 875 22. Lee, M. J., Wu, Y., & Fried, S. K. Adipose tissue heterogeneity: implication of depot
876 differences in adipose tissue for obesity complications. *Mol. Aspects Med.* **34**, 1-11 (2013).
- 877 23. Nishimura, H., Itamura, S., Iwasaki, T., Kurata, T. & Tashiro, M. Characterization of
878 human influenza A (H5N1) virus infection in mice: neuro-, pneumo-, and adipotropic
879 infection. *J. Gen. Virol.* **81**, 2503-2510 (2000).
- 880 24. Zechner, R., Zimmermann, R., Eichmann, T. O., Kolhwein, S. D., Haemmerle, G., Lass,
881 A., & Madeo, F. Fat signals. Lipases and lipolysis in lipid metabolism and signaling. *Cell*
882 *Metab.* **15**, 279-291 (2012).
- 883 25. Thalmann, S., Juge-Aubry, C. E. & Meier, C. A. Explant cultures of white adipose tissue.
884 *Methods Mol. Biol.* **456**, 195-199 (2008).
- 885 26. Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L. & Friedman, J. M. Positional
886 cloning of the mouse obese gene and its human homologue. *Nature.* **372**, 425-432 (1994).
- 887 27. Harms, M. & Seale, P. Brown and beige fat: development, function and therapeutic
888 potential. *Nat. Med.* **19**, 1252-1263 (2013).
- 889 28. Parlee, S. D., Lentz, S. I., Mori, H. & MacDougald O. A. Quantifying size and number of
890 adipocytes in adipose tissue. *Methods Enzymol.* **537**, 93-122 (2014).
- 891 29. Klaus, S., Casteilla, L., Bouillaud, F. & Riquier, D. The uncoupling protein UCP: a
892 membraneous mitochondrial ion carrier exclusively expressed in brown adipose tissue. *Int. J*
893 *Biochem.* **23**, 791-801 (1991).

- 894 30. Honda, K., Takaoka, A. & Taniguchi, T. Type I interferon gene induction by the
895 interferon regulatory factor family of transcription factors. *Immunity*. **25**, 349-360 (2006).
- 896 31. Brown, M. S. & Goldstein, J. L. The SREBP pathway: Regulation of cholesterol
897 metabolism by proteolysis of a membrane-bound transcription factor. *Cell*. **89**, 331-340
898 (1997).
- 899 32. Chang, H. C. & Guarente, L. SIRT1 and other sirtuins in metabolism. *Trends Endocrinol.*
900 *Metab.* **25**, 138-145 (2014).
- 901 33. Sebaa, R., Johnson, J., Pileggi, C., Norgren, M., Xuan, J., Sai, Y., Tong, Q., Krystkowiak,
902 I., Bondy-Chorney, E., Davey, N. E., Krogan, N., Downey, M. & Harper, M. H. SIRT3
903 controls brown fat thermogenesis by deacetylation regulation of pathways upstream of UCP1.
904 *Mol. Metab.* **25**, 35-49 (2019).
- 905 34. Chu, Q., Ding, Y., Cai, W., Liu, L., Zhang, H., & Song, J. Marek's disease virus infection
906 induced mitochondria changes in chickens. *Int. J. Mol. Sci.* **20**, 3150-3166 (2019).
- 907 35. Steppan, C. M., Bailey, S. T., Bhat, S., Brown, E. J., Banerjee, R. R., Wright, C. M., Patel,
908 H. R., Ahima, R. S. & Lazar, M. A. The hormone resistin links obesity to diabetes. *Nature*.
909 **409**, 307-312 (2001).
- 910 36. Vegiopoulos, A., Müller-Decker, K., Strzoda, D., Schmitt, I., Chichelnitskiy, E., Ostertag,
911 A., Berriel Diaz, M., Rozman, J., Hrabec de Angelis, M., Nüsing, R. M., Meyer, C. W., Wahli,
912 W., Klingenspor, M. & Herzig, S. Cyclooxygenase-2 controls energy homeostasis in mice by
913 de novo recruitment of brown adipocytes. *Science*. **28**, 1158-1161 (2010).
- 914 37. Charrière, G., Cousin, B., Arnaud, E., André, M., Bacou, F., Pénicaud, L. & Casteilla, L.
915 Preadipocyte conversion to macrophage. Evidence of plasticity. *J. Biol. Chem.* **278**, 9850-
916 9855 (2003).
- 917 38. Cline, T. D., Beck, D. & Bianchini, E. Influenza virus replication in macrophages:
918 balancing protection and pathogenesis. *J. Gen. Virol.* **98**, 2401-2412 (2017).

919 39. Cline, T. D., Karlsson, E. A., Seufzer, B. J. & Schultz-Cherry, S. The hemagglutinin
920 protein of highly pathogenic H5N1 influenza viruses overcomes an early block in the
921 replication cycle to promote productive replication in macrophages. *J. Virol.* **87**, 1411-1419
922 (2013).

923 40. Green, H. & Meuth, M. An established pre-adipose cell line and its differentiation in
924 culture. *Cell.* **3**, 127-133 (1974).

925 41. Terrier, O., Carron, C., Cartet, G., Traversier, A., Julien, T., Valette, M., Lina, B., Moules,
926 V. & Rosa-Calatrava, M. Ultrastructural fingerprints of avian influenza A (H7N9) virus in
927 infected human lung cells. *Virology.* **456-457**, 39-42 (2014).

928 42. Garcia, R. A., Roemmich, J. N. & Claycombe, K. J. Evaluation of markers of beige
929 adipocytes in white adipose tissue of the mouse. *Nutr. Metab. (Lond).* **13**, 24-38 (2016).

930 43. Fisher, F. M., Kleiner, S., Douris, N., Fox, E. C., Mepani, R. J., Verdeguer, F., Wu, J.,
931 Kharitononkov, A., Flier, J. S., Maratos-Flier, E. & Spiegelman, B. M. FGF21 regulates PGC-
932 1 α and browning of white adipose tissues in adaptive thermogenesis. *Genes Dev.* **26**, 271-281
933 (2012).

934 44. Wu, J., Boström, P., Sparks, L. M., Ye, L., Choi, J. H., Giang, A. H., Khandekar, M.,
935 Virtanen, K. A., Nuutila, P., Schaart, G., Huang, K., Tu, H., van Marken Lichtenbelt, W. D.,
936 Hoeks, J., Enerbäck, S., Schrauwen, P. & Spiegelman, B. M. Beige adipocytes are a distinct
937 type of thermogenic fat cell in mouse and human. *Cell.* **150**, 366-376 (2012).

938 45. Rao, R. R., Long, J. Z., White, J. P., Svensson, K. J., Lou, J., Lokurkar, I., Jedrychowski,
939 M. P., Ruas, J. L., Wrann, C. D., Lo, J. C., Camera, D. M., Lachey, J., Gygi, S., Seehra, J.,
940 Hawley, J. A. & Spiegelman, B. M. Meteorin-like is a hormone that regulates immune-
941 adipose interactions to increase beige fat thermogenesis. *Cell.* **157**, 1279-1291 (2014).

942 46. Harmon, A. W. & Beck, M. A. Influenza infection disrupts glucose uptake in 3T3-L1
943 adipocytes. *FASEB J.* **20**, A163-A163 (2006).

944 47. Bouwman, J. J. M., Visseren, F. L. J., Bouter, K. P. & Diepersloot, R. J. A. Infection-
945 induced inflammatory response of adipocytes *in vitro*. *Int. J. Obes. (Lond.)*. **32**, 892-901
946 (2008).

947 48. Locasale, J.W. Serine, glycine and one-carbon units: cancer metabolism in full circle. *Nat.*
948 *Rev. Cancer*. **13**, 572-583 (2013).

949 49. Woods, P., Nigdelioglu, R., Meliton, A. Y., Sun, K.A., Witt, L., Wu, D., Hamanaka, R. B.
950 & Mutlu, G. M. Serine glycine one-carbon metabolism drives effector responses to influenza
951 infection in macrophages. *Am. J. Respir. Crit. Care Med.* **197**, A3860-A3860 (2018).

952 50. Xia, Y., Ye, B., Ding, J., Yu, Y., Alptekin, A., Thangaraju, M., Prasad, P. D., Ding, Z. C.,
953 Park, E. J., Choi, J. H., Gao, B., Fiehn, O., Yan, C., Dong, Z., Zha, Y. & Ding, H. F.
954 Metabolic reprogramming by MYCN confers dependence on the serine-glycine-one-carbon
955 biosynthetic pathway. *Cancer Res.* **79**, 3837-3850 (2019).

956 51. Keipert, S., Ost, M., Johann, K., Imber, F., Jastroch, M., van Schothorst, E. M., Keijer, J.
957 & Klaus, S. Skeletal muscle mitochondrial uncoupling drives endocrine cross-talk through
958 the induction of FGF21 as a myokine. *Am. J. Physiol. Endocrinol. Metab.* **306**, E469-E482
959 (2014).

960 52. Derakhshan, M., Willcocks, M. M., Salako, M. A., Kass, G. E. & Carter, M. J. Human
961 herpesvirus 1 protein US3 induces an inhibition of mitochondrial electron transport. *J. Gen.*
962 *Viol.* **87**, 2155-2159 (2006).

963 53. de Andrade, P. B. M., Bolsoni-Lopes, A. & Alonso-Vale, M. I. C. Mitochondrial actions
964 for fat browning and energy expenditure in white adipose tissue. *J. Obes. Overweig.* **2**, 201-
965 216 (2016).

966 54. Peña, C., Hurt, E. & Panse, V. G. Eukaryotic ribosome assembly, transport and quality
967 control. *Nat. Struct. Mol. Biol.* **24**, 689-699 (2017).

968 55. Wilson-Fritch, L., Burkart, A., Bell, G., Mendelson, K., Leszyk, J., Nicoloso, S., Czech,
969 M. & Corvera, S. Mitochondrial biogenesis and remodeling during adipogenesis and in
970 response to the insulin sensitizer rosiglitazone. *Mol. Cell. Biol.* **23**, 1085-1094 (2003).

971 56. Hegde, V. & Dhurandhar, N. V. Microbes and obesity--interrelationship between
972 infection, adipose tissue and the immune system. *Clin. Microbiol. Infect.* **19**, 314-320 (2013).

973 57. Misumi, I., Starmer, J., Uchimura, T., Beck, M. A., Magnuson, T. & Whitmire, J. K.
974 Obesity expands a distinct population of T cells in adipose tissue and increases vulnerability
975 to infection. *Cell Rep.* **27**, 514-524 (2019).

976 58. Damouche, A., Lazure, T., Avettand-Fènoël, V., Huot, N., Dejuq-Rainsford, N., Satie, A.
977 P., Mélard, A., David, L., Gommel, C., Ghosn, J., Noel, N., Pourcher, G, Martinez, V.,
978 Benoist, S., Béréziat, V., Cosma, A., Favier, B., Vaslin, B., Rouzioux, C., Capeau, J., Müller-
979 Trutwin, M., Dereuddre-Bosquet, N., Le Grand, R., Lambotte, O. & Bourgeois, C. Adipose
980 tissue is a neglected viral reservoir and an inflammatory site during chronic HIV and SIV
981 infection. *PLoS Pathog.* **11**, e1005153 (2015).

982 59. Ponterio, E., Cangemi, R., Mariani, S., Casella, G., De Cesare, A., Trovato, F. M.,
983 Garozzo, A. & Gnessi, L. Adenovirus 36 DNA in human adipose tissue. *Int. J. Obes. (Lond.)*.
984 **39**, 1761-1764 (2015).

985 60. Franke-Fayard, B., Fonager, J., Braks, A., Khan, S. M. & Janse, C. J. Sequestration and
986 tissue accumulation of human malaria parasites: Can we learn anything from rodent models of
987 malaria? *PLoS Pathog.* **6**, e1001032 (2010).

988 61. Beigier-Bompadre, M., Montagna, G. N., Külh, A. A., Lozza, L., Weiner, J., Kupz, A.,
989 Vogelzang, A., Mollenkopf, H. J., Löwe, D., Banderhann, S., Dorhoi, A., Brinkmann, V.,
990 Matuschewski, K. & Kaufmann, S. H. E. *Mycobacterium tuberculosis* infection modulates
991 adipose tissue biology. *PLoS Pathog.* **13**, e1006676 (2017).

992 62. Robertson, K. A. & Ghazal, P. Interferon control of the sterol metabolic network:
993 bidirectional molecular circuitry-mediating host protection. *Front. Immunol.* **7**, 634 (2016).

994 63. Ghazal, P. Host defense against viral infection involves interferon mediated down-
995 regulation of sterol biosynthesis. *PLoS Pathog.* **9**, e1000598 (2011).

996 64. York, A. G., Williams, K. J., Argus, J. P., Zhou, Q. D., Brar, G., Vergnes, L., Gray, E. E.,
997 Zhen, A., Wu, N. C., Yamada, D. H., Cunningham, C. R., Tarling, E. J., Wilks, M. Q.,
998 Casero, D., Gray, D. H., Yu, A. K., Wang, E. S., Brooks, D. G., Sun, R., Kitchen, S. G., Wu,
999 T. T., Reue, K., Stetson, D. B. & Bensinger, S. J. Limiting cholesterol biosynthetic flux
1000 spontaneously engages type I IFN signaling. *Cell.* **163**, 1716-1729 (2015).

1001 65. Bajimaya, S., Frankl, T., Hayashi, T. & Takimoto, T. Cholesterol is required for stability
1002 and infectivity of influenza A and respiratory syncytial viruses. *Virology.* **510**, 234-241
1003 (2017).

1004 66. Ye, X., Li, M., Hou, T., Gao, T., Zhu, W. G. & Yang, Y. Sirtuins in glucose and lipid
1005 metabolism. *Oncotarget.* **8**, 1845-1859 (2017).

1006 67. Qiang, L., Wang, L., Kon, N., Zhao, W., Lee, S., Zhang, Y., Rosenbaum, M., Zhao, Y.,
1007 Gu, W., Farmer, S. R. & Accili, D. Brown remodeling of white adipose tissue by SirT1-
1008 dependent deacetylation of Ppar γ . *Cell.* **150**, 620-632 (2012).

1009 68. Haemmerle, G., Lass, A., Zimmermann, R., Gorkiewicz, G., Meyer, C., Rozman, J.,
1010 Heldmaier, G., Maier, R., Theussl, C., Eder, S., Kratky, D., Wagner, E. F., Klingenspor, M.,
1011 Hoefler, G. & Zechner, R. Defective lipolysis and altered energy metabolism in mice lacking
1012 adipose triglyceride lipase. *Science.* **312**, 734-737 (2006).

1013 69. Taschler, U., Radner, F. P., Heier, C., Schreiber, R., Schweiger, M., Schoiswohl, G.,
1014 Preiss-Landl, K., Jaeger, D., Reiter, B., Koefeler, H. C., Wojciechowski, J., Theussl, C.,
1015 Penninger, J. M., Lass, A., Haemmerle, G., Zechner, R. & Zimmermann, R. Monoglyceride

1016 lipase deficiency in mice impairs lipolysis and attenuates diet-induced insulin resistance. *J.*
1017 *Biol. Chem.* **286**, 17467-17477 (2011).

1018 70. Brenmoehl, J., Ohde, D., Albrecht, E., Walz, C., Tuchscherer, A. & Hoeflich, A.
1019 Browning of subcutaneous fat and higher surface temperature in response to phenotype
1020 selection for advanced endurance exercise performance in male DUhTP mice. *J. Comp.*
1021 *Physiol. B.* **187**, 361-373 (2017).

1022 71. Kaisanlathi, A. & Glumoff, T. Browning of white fat: agents and implications for beige
1023 adipose tissue to type 2 diabetes. *J. Physiol. Biochem.* **75**, 1-10 (2019).

1024 72. Dodd, G. T., Descherf, S., Loh, K., Simonds, S. E., Wiede, F., Balland, E., Merry, T. L.,
1025 Münzberg, H., Zhang, Z. Y., Kahn, B. B., Neel, B. G., Bence, K. K., Andrews, Z. B., Colley,
1026 M. A. & Tiganis, T. Leptin and insulin act on POMC neurons to promote the browning of
1027 white fat. *Cell.* **160**, 88-104 (2015).

1028 73. Villarroya, F., Cereijo, R., Villarroya, J., Gavaldà-Navarro, A., & Giralt, M. Toward an
1029 understanding of how immune cells control brown and beige adipobiology. *Cell Metab.* **27**,
1030 954-961 (2018).

1031 74. Jennrich, S., Lee, M. H., Lynn, R. C., Dewberry, K. & Debes, G. F. Tissue exit: a novel
1032 control point in the accumulation of antigen-specific CD8 T cells in the Influenza A Virus-
1033 infected lung. *J. Virol.* **86**, 3436-3445 (2012).

1034 75. Legge, K. L. & Braciale, T. J. Accelerated migration of respiratory dendritic cells to the
1035 regional lymph nodes is limited to the early phase of pulmonary infection. *Immunity.* **18**, 265-
1036 277 (2003).

1037 76. Steuerman, Y., Cohen, M., Peshes-Yaloz, N., Valadarsky, L., Cohn, O., David, A.,
1038 Frishberg, A., Mayo, L., Bacharach, E., Amit, I. & Gat-Vicks, I. Dissection of influenza
1039 infection in vivo by single-cell RNA sequencing. *Cell Syst.* **6**, 679-691 (2018).

1040 77. Crane, J. D., Mottillo, E. P., Francombe, T. H., Morrison, K. M. & Steinberg, G. R. A
1041 standardized infrared imaging technique that specifically detects UCP1-mediated
1042 thermogenesis *in vivo*. *Mol. Metab.* **3**, 490-494 (2014).

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1065 **Figure titles and legends**

1066 **Fig. 1** Influenza alters adipose tissues' functions. **a** Body weight loss (% from initial body
1067 weight (BW)) of mock-treated and IAV-infected mice, at day 7 post-infection (7 dpi), n = 33
1068 mock-treated animals and n = 46 IAV-infected animals. Individuals values, as well as means
1069 \pm SD are shown. **** $p < 0.0001$. **b** Adipose tissue (AT) mass (g/g BW) of mock-treated and
1070 IAV-infected mice at 7 dpi, n = 25 mock-treated animals and n = 28 IAV-infected animals.
1071 * $p < 0.05$, ** $p < 0.01$. **c** Real-time quantitative PCR (RT-qPCR) analysis of the expression of
1072 lipolytic genes (*Atgl*, *Hsl*, *Mgl*) and lipogenic genes (*Glut4*, *Me2*, *Fasn*) in SCAT and EWAT
1073 from mock-treated and IAV-infected mice at 7 dpi. For lipolytic genes: n = 5 mock-treated
1074 animals and n = 8 IAV-infected animals. For lipogenic genes: n = 5 mock-treated animals and
1075 n=18 IAV-infected animals. RT-qPCR data were normalized to *Eef2* housekeeping gene
1076 expression and expressed relative to the expression obtained in the samples from mock-
1077 treated mice. * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$, ns = not significant. **d** Determination of IL-1 β ,
1078 IL-10, IL-6 and leptin levels (by ELISA) in the supernatants of SCAT and EWAT explants
1079 from mock-treated and IAV-infected mice at 7 dpi. For IL-1 β and leptin: n = 7 mock-treated
1080 animals and n = 13 IAV-infected animals. For IL-10 and IL-6: n = 14 mock-treated animals
1081 and n = 20 IAV-infected animals. Data were normalized to the mass of the explants (mg).
1082 * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.
1083 Differences between mock-treated and IAV-infected groups (**a** to **d**) were considered
1084 significant when $p < 0.05$.

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1086 **Fig. 2** Influenza induces subcutaneous adipose tissue browning. **a** Representative images of
1087 hematoxylin/eosin staining of SCAT sections from mock-treated and IAV-infected mice at 7
1088 dpi, n = 7 mock-treated animals and n = 7 IAV-infected animals. Brown-like/beige adipocytes
1089 are arrowheaded. **b** Frequency distributions (%) of adipocyte sizes (areas, μm^2) in the SCAT

1090 from mock-treated and IAV-infected mice, 7 dpi, n = 6 mock-treated animals and n = 6 IAV-
1091 infected animals. Individual values, as well as means \pm SD are shown. * $p < 0.05$, ** $p < 0.01$. **c**
1092 Western blot of UCP1 in lanes loaded with EWAT, lung and SCAT protein lysates from
1093 mock-treated and IAV-infected mice at 7 dpi. GAPDH was used as an internal loading
1094 control. The figure is representative of four independent blots covering a total of: SCAT from
1095 n = 5 mock-treated animals (SCAT mock), SCAT from n = 35 IAV-infected animals (SCAT
1096 IAV), EWAT from n = 5 IAV-infected animals (EWAT IAV) and lungs from n = 5 IAV-
1097 infected animals (Lung IAV). Of note, the uncropped version of the blot is shown on
1098 Supplementary Fig. 2d. **d** Quantification of normalized densitometric UCP1/GAPDH ratios
1099 (arbitrary units) of all Western blots. ** $p < 0.01$, **** $p < 0.0001$. **e** Infrared thermography
1100 measurement ($^{\circ}\text{C}$) of SCAT surface temperature at 7 dpi, n = 3 mock-treated animals and n =
1101 4 IAV-infected animals. Individual values, as well as means \pm SD are shown. ** $p < 0.01$.
1102 Representative infrared images of the SCAT area from mock-treated and IAV-infected mice
1103 are shown.
1104 Differences between mock-treated and IAV-infected groups (**b**, **d**, **e**), and between SCAT
1105 IAV and EWAT IAV (**d**) were considered significant when $p < 0.05$.

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1107 **Fig. 3** Transcriptomics shows fat-depot-specific response to influenza. SCAT and EWAT
1108 from mock-treated and IAV-infected mice were treated for transcriptomic analysis at 7 dpi, n
1109 = 4 mock-treated animals and n = 4 IAV-infected animals. **a** Venn diagrams summarizing the
1110 number of distinct and overlapping differentially expressed genes (DEGs) in the SCAT and
1111 EWAT from IAV-infected mice vs. the SCAT and EWAT from mock-treated mice.
1112 Upregulated (UR) and downregulated (DR) DEGs numbers are shown. **b** The top five
1113 canonical pathways identified by Ingenuity Pathway Analysis (IPA) for the genes upregulated
1114 in SCAT and EWAT upon infection (IFN = interferon, IRF = interferon regulatory factor, RA

1115 = retinoic acid). The corresponding upstream regulators and their target molecules are shown
1116 in Supplementary Table 1. **c** The top five canonical pathways identified by IPA for the genes
1117 downregulated in SCAT and EWAT upon infection (SP = superpathway). The corresponding
1118 upstream regulators and their target molecules are shown in Supplementary Table 3. **d** The
1119 top five canonical pathways identified by IPA for the genes downregulated upon infection
1120 exclusively in SCAT (TCA cycle = tricarboxylic acid cycle). For **b** to **d**, pathways were
1121 sorted by *p*-values and absolute *z*-scores. Intensities of red indicate the higher or lower value
1122 of positive *z*-scores (activated pathways), intensities of blue indicate the higher or lower value
1123 of negative *z*-scores (inhibited pathways), grey indicates pathways having no activity pattern
1124 available since no *z*-score could be calculated. The spotted line indicates IPA's default
1125 threshold.

1126

1127 **Fig. 4** Influenza durably alters the host's energy metabolism. **a** Blood levels of glucose (10-12
1128 hours fasting, mg/dl), insulin (10-12 hours fasting, $\mu\text{g/l}$) and resistin (fed state, ng/ml) in
1129 mock-treated and IAV-infected mice at 7 dpi. For glucose: $n = 20$ mock-treated animals and n
1130 $= 30$ IAV-infected animals. For insulin and resistin: $n = 15$ mock-treated animals and $n = 15$
1131 IAV-infected animals. Individuals values, as well as means \pm SD are shown. ** $p < 0.01$,
1132 **** $p < 0.0001$. **b** Blood levels of glucose, insulin and resistin in mock-treated and IAV-infected
1133 mice at week 20 post-infection, $n = 8$ mock-treated animals and $n = 6$ IAV-infected animals.
1134 Individuals values, as well as means \pm SD are shown. * $p < 0.05$, ** $p < 0.01$. **c** Body weight
1135 change (% initial body weight) of mock-treated and IAV-infected mice fed with standard diet
1136 (SD) or with high-fat diet (HFD) from 7 dpi (arrow), $n = 5$ mock-treated SD-fed animals
1137 (mock SD), $n = 10$ IAV-infected SD-fed animals (IAV SD), $n = 10$ mock-treated HFD-fed
1138 animals (mock HFD) and $n = 15$ IAV-infected HFD-fed animals (IAV HFD). Data are
1139 expressed as mean \pm SD. **** $p < 0.0001$. Corresponding area under the curve (AUC) is shown

1140 in Supplementary Fig. 4b. **d** Intraperitoneal glucose tolerance test (IP-GTT) was performed in
1141 10-12 hours-fasted mice, 18 weeks post-infection. Blood samples for glucose level
1142 determination were taken from the tail vein before glucose administration (i.p. injection, 1
1143 g/kg BW) and after 15, 30, 60, 120 and 180 minutes, n = 5 mock-treated SD-fed animals, n =
1144 10 IAV-infected SD-fed animals, n = 9 mock-treated HFD-fed animals and n = 12 IAV-
1145 infected HFD-fed animals. Data are expressed as mean \pm SD. **** p <0.0001. Corresponding
1146 area under the curve (AUC) is shown in Supplementary Fig. 4c. **e** Blood levels of glucose,
1147 insulin and resistin (10-12 hours fasting) at sacrifice, n = 6 mock-treated HFD-fed animals
1148 and n = 11 IAV-infected HFD-fed animals. The dotted lines indicate the values obtained for
1149 mock-treated SD-fed animals (n = 4). Individuals values, as well as means \pm SD are shown.
1150 * p <0.05, ns = not significant.
1151 Differences between mock-treated and IAV-infected groups were considered significant when
1152 p <0.05.

1153

1154 **Fig. 5** Viral protein-expressing cells are detected in adipose tissues. **a** Viral RNA levels
1155 (expressed as cycle threshold (Ct) values) in the lungs, SCAT, EWAT and pancreas at 7 dpi.
1156 Lungs and pancreas from n = 10 IAV-infected animals, and SCAT and EWAT from n = 25
1157 IAV-infected animals. The dotted line indicates pancreas' mean Ct value. Individuals values,
1158 as well as means \pm SD are shown. **** p <0.0001. **b** Viral RNA levels (expressed as Ct values)
1159 in the lungs, SCAT and EWAT from mock-treated and IAV-infected mice, at 0, 2, 4, 7, 14
1160 and 28 dpi, n = 5 mock-treated animals and n = 8 IAV-infected animals per time point. The
1161 dotted line indicates day 0 of infection. Data are expressed as mean \pm SD. * p <0.05, ** p <0.01.
1162 **c** Fractionation procedure used to separate stromal vascular fraction (SVF) cells from mature
1163 adipocytes, of the adipose tissues. Immune cells (characterized as CD45⁺ cells) and
1164 preadipocytes (characterized as CD45⁻ CD31⁻ CD29⁺ CD34⁺ Sca1⁺ cells) from the SVF were

1165 then sorted by flow cytometry (see gating strategy Supplementary Fig. 5b). **d** A selection of
1166 sorted immune cells and preadipocytes expressing the viral protein hemagglutinin (green)
1167 observed by immunofluorescence and confocal microscopy. The nuclear counterstain DAPI
1168 was used (blue). All images were taken at x63 magnification, n = 3 mock-treated animals and
1169 n = 6 IAV-infected animals.

1170 Differences between pancreas and lungs, pancreas and SCAT and pancreas and EWAT (**a**)
1171 and between day 0 and days 2, 4, 7, 14 or 28 (**b**) were considered significant when $p < 0.05$.

1172

1173 **Fig. 6** Influenza induces brown-like adipogenesis in preadipocytes. **a** Viral RNA levels
1174 (expressed as Ct values) in IAV-infected preadipocytes (3T3-L1 cells) (MOI of 0.01, 0.25,
1175 0.5, 1 or 2) at 6, 24 and 48 hours post-infection (hpi), n = 4 biologically independent samples
1176 per condition. The dotted line indicates the 6 hpi/0.01 MOI mean Ct value. **** $p < 0.0001$. **b**

1177 Representative transmission electron microscopy sections of mock-treated and IAV-infected
1178 preadipocytes (MOI of 5, 24 hpi), n = 3 biologically independent samples per condition.

1179 Classical nuclear (yellow arrowheads) and nucleolar (yellow dashed frame) fingerprints
1180 associated with IAV infection are shown. A detailed view of empty viral particles at budding
1181 regions is presented. N = nucleus. **c** Relative mRNA expression (RT-qPCR) of the antiviral

1182 innate-immune-related genes *Tlr3*, *RigI*, *Mda5*, *Mx1* and *Vip* in mock-treated and IAV-
1183 infected preadipocytes (MOI of 1, 24 hpi). For *Tlr3* and *Vip*: n = 5 biologically independent
1184 samples per condition. For *RigI*, *Mda5* and *Mx1*: n = 7 biologically independent mock-treated
1185 preadipocyte samples and n = 18 biologically independent IAV-infected preadipocyte

1186 samples. ** $p < 0.01$, **** $p < 0.0001$, ns = not significant. **d** Relative mRNA expression of the

1187 genes involved in the browning-like/beiging adipogenesis process *Ucp1*, *Pgc1a*, *Fgf21*, *Apln*,

1188 *Metrn1* and *Tmem26* in mock-treated and IAV-infected preadipocytes at 24, 48 and 72 hpi

1189 (MOI of 1), n = 6 biologically independent samples per condition. Of note, for *Tmem26*

1190 expression, the 24 hpi time was not done. Data are expressed as mean \pm SD. * p <0.05,
1191 ** p <0.01, *** p <0.001, ns = not significant.

1192 Differences between 6 hpi and 24 hpi and between 6 hpi and 48 hpi (**a**), and between mock-
1193 treated and IAV-infected groups (**c, d**) were considered significant when p <0.05.

1194

1195 **Fig. 7** Influenza induces metabolic reprogramming of preadipocytes. Murine preadipocytes
1196 (3T3-L1 cells) and adipocytes (differentiated from 3T3-L1 cells) were infected at a MOI of 1,
1197 and samples were prepared for transcriptomic analysis at 24 hpi, $n = 4$ biologically
1198 independent samples per condition. **a** Venn diagrams showing the numbers and intersections
1199 of the differentially expressed genes (DEGs), either upregulated or downregulated, in IAV-
1200 infected vs. mock-treated preadipocytes and adipocytes in the whole-genome expression
1201 profiling microarray data set. Top canonical pathways and related upstream regulators are
1202 listed in Supplementary Table 4 (genes commonly upregulated upon infection in
1203 preadipocytes and adipocytes), Supplementary Table 5 (genes only upregulated upon
1204 infection in adipocytes), Supplementary Table 6 (genes only upregulated upon infection in
1205 preadipocytes), and Supplementary Table 7 (genes only downregulated upon infection in
1206 preadipocytes). **b** Scheme of the serine-glycine-one-carbon (SGOC) metabolic pathway.
1207 Genes coding for cytosolic and mitochondrial SGOC enzymes (in red) that were upregulated
1208 in preadipocytes after IAV infection are framed. **c** Relative mRNA expression (RT-qPCR) of
1209 genes related to ribosome biogenesis (*Urb2*, *Wdr75*, *Zfp593*, *Mrto4* and *Nop58*) in mock-
1210 treated and IAV-infected preadipocytes and adipocytes (MOI of 1, 24 hpi), $n = 12$
1211 biologically independent samples per condition. Data are expressed as mean \pm SD. * p <0.05,
1212 *** p <0.001, **** p <0.0001.

1213 Differences between mock-treated and IAV-infected groups (**c**) were considered significant
1214 when p <0.05.

1215

1216 **Fig. 8** Metabolic reprogramming associated with influenza infection in subcutaneous adipose
1217 tissue (*in vivo*) and in preadipocytes (*in vitro*). **a** Transcriptome analysis of the subcutaneous
1218 (SCAT) and the visceral (EWAT) adipose tissues from mock-treated and IAV-infected mice
1219 revealed activation of the STAT1-dependent-IFN-mediated signaling pathway and the
1220 concomitant inhibition of the cholesterol biosynthesis pathways in both SCAT and EWAT.
1221 IAV infection was associated with rewired energy metabolism only in the SCAT, as reflected
1222 by activation of the sirtuin signaling pathway and inhibition of glycolysis, OXPHOS, and
1223 TCA cycle. This was associated with a white-to-brown phenotypic change of the SCAT. **b** *In*
1224 *vitro* IAV infection of preadipocytes and adipocytes led to activation of the IFN signaling
1225 pathways in both cell types. In preadipocytes only, IAV infection rewired cell metabolism
1226 through activation of the SGOC metabolic pathway. This was associated with the induction of
1227 a brown-like/beige genetic program in preadipocytes.
1228 OXPHOS = oxidative phosphorylation, ETC = electron transport chain, SGOC metabolism =
1229 serine-glycine-one-carbon metabolism. Activated and inhibited pathways are indicated in red
1230 and blue, respectively. Top upstream regulators are indicated in brackets.