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1 **Genetic deficiency of Indoleamine 2, 3-dioxygenase promotes gut microbiota-mediated metabolic**
2 **health**

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40 **Abstract**

41 **The association between altered gut microbiota, intestinal permeability, inflammation and**
42 **cardiometabolic diseases is becoming increasingly clear but remains poorly understood^{1,2}.**
43 **Indoleamine 2, 3-dioxygenase (IDO) is an enzyme induced in many types of immune cells**
44 **including macrophages in response to inflammatory stimuli, and catalyses the degradation of**
45 **tryptophan (Trp) along the kynurenine (Kyn) pathway. IDO activity is better known for its**
46 **suppression of effector T-cell immunity and its activation of regulatory T cells^{3,4}. However, high**
47 **IDO activity predicts worse cardiovascular outcome⁵⁻⁹ and may promote atherosclerosis and**
48 **vascular inflammation⁶, suggesting a more complex role in chronic inflammatory settings. IDO**
49 **activity is also increased in obesity¹⁰⁻¹³. Yet, the role of IDO in metabolic disease is still**
50 **unexplored. Here we show that obesity is associated with an increase of intestinal IDO activity,**
51 **which shifts Trp metabolism from indole derivative and interleukin (IL)-22 production towards**
52 **Kyn production. IDO deletion or inhibition improves insulin sensitivity, preserves gut mucosal**
53 **barrier, decreases endotoxaemia and chronic inflammation, and regulates lipid metabolism in**
54 **liver and adipose tissues. These beneficial effects are due to rewiring of Trp metabolism towards**
55 **a microbiota-dependent production of IL-22 and are abrogated after treatment with a**
56 **neutralizing anti-IL-22 antibody. In summary, we identify an unexpected function of IDO in the**
57 **fine tuning of intestinal Trp metabolism with major consequences on microbiota-dependent**
58 **control of metabolic disease, which suggests IDO as a potential therapeutic target.**

59

60 To address the role of IDO in obesity, we used high fat diet (HFD) to promote metabolic disease in
61 wild-type (WT) and *Ido1*^{-/-} mice. HFD -fed WT mice compared to those on a normal chow diet (NCD)
62 induced *Ido1* mRNA in epididymal (epi) and inguinal (ing) white adipose tissue (WAT) as well as in
63 soleus muscle (**Supplementary Fig. 1a**), whereas no *Ido1* mRNA was detected in liver (data not
64 shown). Of note, IFN- γ , known as a potent inducer of IDO¹⁴ was also higher in the HFD-fed mice
65 compared to those on a NCD (**Supplementary Fig. 1b**). Accordingly, we found that HFD-feeding
66 resulted in significantly greater IDO activity (as assessed by measurement of Kyn/Trp ratio) in
67 plasma, epiWAT, brown adipose tissue (AT) and the soleus muscle, in comparison to WT mice on a
68 NCD (**Fig. 1a**).

69 *Ido1*^{-/-} mice fed a NCD showed no major differences in body mass, adiposity and insulin sensitivity
70 compared to WT on the same diet (**Supplementary Fig. 2**). Interestingly, when put on a HFD, *Ido1*^{-/-}
71 mice still had a similar weight curve as WT or *Ido1*^{-/-} mice fed a NCD (**Fig. 1b**) and had a lower fat
72 mass as evaluated by magnetic resonance imaging (MRI), as compared to HFD-fed WT mice (**Fig.**
73 **1c**), without any change in lean mass (**Supplementary Fig. 3a**). In particular, the weights of epiWAT,
74 inguinal (ing) WAT and retroperitoneal (ret) WAT were lower in HFD-fed *Ido1*^{-/-} mice compared to
75 HFD-fed WT mice (**Fig. 1d**). Consistent with lower adiposity and plasma leptin levels¹⁵ (**Fig. 1e**),
76 HFD-fed *Ido1*^{-/-} mice compared to WT on the same diet also had lower liver weights (**Fig. 1f**), and
77 their livers were characterized by less lipid accumulation (**Fig. 1g**), and lower macrophage infiltration
78 (**Supplementary Fig. 3b**), indicating a protection from liver steatosis.

79 Obesity is known to contribute to the development of adipose tissue inflammation leading to insulin
80 resistance^{16,17}. Examination of ingWAT and epiWAT revealed less macrophage infiltration (**Fig. 1h**),
81 and higher content of CD11b+F4/80+CD206+ M2-like macrophages in epiWAT (**Fig. 1i**), with no
82 change of CD11b+F4/80+CD11c+ M1-like (data not shown), in HFD-fed *Ido1*^{-/-} mice compared to
83 WT on the same diet. Similarly, epiWAT explants from HFD-fed *Ido1*^{-/-} mice produced higher levels
84 of protective adiponectin¹⁸ (**Fig. 1j**) compared to explants from WT mice on the same diet, whereas
85 ingWAT produced higher type 2 immune cytokines IL-10, IL-4 and IL-5 (ref. 17) (**Supplementary**
86 **Fig. 3c**), indicating a lower inflammatory status in adipose tissues of HFD-fed *Ido1*^{-/-} mice compared
87 to HFD-fed WT. Consistent with lower fat mass, insulin concentrations were lesser in fasting HFD-fed

88 *Ido1*^{-/-} mice compared to WT on the same diet and during oral glucose tolerance test (OGTT)
89 (**Supplementary Fig. 3d**). HFD-fed *Ido1*^{-/-} mice also showed improved insulin tolerance test (ITT)
90 (**Fig. 1k**), lower AUC insulin/AUC glucose (**Fig. 1l**) and better insulin signalling (P-AKT) in the
91 soleus muscle (**Fig. 1m**), but not in liver, ingWAT and epiWAT (**Supplementary Fig. 3e**), compared
92 to HFD-fed WT mice. These results indicated that HFD-fed *Ido1*^{-/-} mice were protected from obesity
93 and related metabolic complications, including liver steatosis and insulin resistance.

94 To explain the weight differences between HFD-fed *Ido1*^{-/-} mice and their controls, we performed
95 detailed metabolic analyses. Covariate analysis of relationship between body weight and energy
96 expenditure revealed a significant difference between HFD-fed WT and HFD-fed *Ido1*^{-/-} mice pointing
97 towards a higher metabolic efficiency in absence of IDO without any change in food intake and total
98 energy excretion (**Supplementary Fig. 3f-h**), and any difference in spontaneous locomotor activity or
99 preferential substrate use (data not shown). To determine which tissues contributed to the higher
100 energy expenditure, we used positron emission tomography-computed tomography (PET-CT). ¹⁸F
101 fluorodeoxyglucose (FDG) uptake was higher in the muscle of HFD-fed *Ido1*^{-/-} mice compared to
102 HFD-fed WT, without any observed differences in brain, brown AT and heart (**Fig. 1n**). This is in
103 agreement with higher membrane glucose transporter type 4 (GLUT4) expression, mitochondrial
104 marker staining, and greater adenosine triphosphate (ATP) production in soleus muscle of HFD-fed
105 *Ido1*^{-/-} mice compared to HFD-fed WT mice (**Supplementary Fig. 4**), suggesting a higher muscular
106 metabolic rate.

107 Then, we sought to inhibit IDO activity using L-1Methyl Tryptophan (1MT) in drinking water. We
108 found no differences in body weight in 1MT- treated WT mice compared to untreated mice fed with a
109 HFD (**Supplementary Fig. 5a**) that may due to several factors such as the duration, extent and
110 sustainability of IDO inhibition. However, HFD-fed WT mice treated with 1MT showed lower plasma
111 IDO activity (as measured by the Kyn/Trp ratio), a higher production of adiponectin by epiWAT
112 explants, improved insulin tolerance, and a lower insulin-resistance index (HOMA-IR), compared to
113 untreated WT mice (**Supplementary Fig. 5b-e**). We observed similar results in genetically obese
114 leptin-deficient (*ob/ob*) mice treated with 1MT compared to untreated *ob/ob* mice (**Supplementary**
115 **Fig. 5f-g**), indicating that the inhibition of IDO activity improved insulin resistance in obesity.

116 IDO is expressed by both myeloid and non-myeloid compartments^{4,19}. To distinguish between the
117 roles of IDO in those compartments, we generated chimeric mice. Reconstitution of WT mice with
118 bone marrow from *Ido1*^{-/-} mice compared to bone marrow from WT mice only slightly affected plasma
119 IDO activity (*i.e.*, the Kyn/Trp ratio) (**Fig. 2a**), mouse body weight, WAT weights and insulin
120 sensitivity (**Fig. 2b-d**). Moreover, mice deleted for IDO in macrophages (*Ido1*^{flox/flox} LysM-cre), the
121 main cells that express IDO in the myeloid compartment¹⁴, showed similar weight curves and insulin
122 sensitivity, compared to HFD-fed *Ido1*^{flox/flox} control mice, indicating that IDO in myeloid
123 compartment is dispensable for obesity and insulin-resistance (**Supplementary Fig. 6**). Interestingly,
124 mice deficient for IDO in non-myeloid cells had a marked lower plasma IDO activity (Kyn/Trp) (**Fig.**
125 **2a**), gained less body weight on HFD and had lower ingWAT, epiWAT, retWAT and liver weights
126 (**Fig. 2b-c**), as well as improved insulin tolerance and glucose homeostasis (**Fig. 2d-e**), compared to
127 HFD-fed WT mice transplanted with WT bone marrow. The results strongly support the importance of
128 IDO expressed in non-myeloid compartment in the induction of metabolic disease.

129 Increased gut-derived lipopolysaccharide (LPS) translocation and intestinal dysbiosis were observed in
130 obesity²⁰. Since IDO is expressed in the gastrointestinal tract¹⁹, we analyzed intestinal IDO activity
131 during HFD feeding. HFD feeding resulted in a markedly greater IDO activity (as measured by
132 changes in the Kyn/Trp ratio) in both the small intestine and colon (**Fig. 2f**). We therefore
133 hypothesised that intestinal IDO activity may hijack local Trp metabolism and shift it away from use
134 by the gut microbiota.

135 To address the importance of the microbiota, we depleted the gut microbiota in HFD- fed WT and
136 *Ido1*^{-/-} mice using a broad spectrum antibiotic cocktail supplemented in drinking water. In agreement
137 with a previous study²¹, depletion of the microbiota protected the mice against HFD-induced weight
138 gain (**Fig. 2g**). Moreover, antibiotic treatment abrogated the differences of body weight previously
139 seen between HFD-fed WT and HFD-fed *Ido1*^{-/-} mice (**Fig. 2g**). To test whether the gut microbiota is
140 involved in the phenotype, WT and *Ido1*^{-/-} mice were co-housed after weaning (mix) and compared to
141 mice housed in cages separated by genotype. As shown in **Fig. 2h**, the weight of co-housed animals
142 (whether WT or *Ido1*^{-/-}) was similar to those of *Ido1*^{-/-} mice housed in separate cages, indicating a
143 dominant protective effect against weight gain of microbiota from *Ido1*^{-/-} mice. Moreover, antibiotic
144 treatment and co-housing abrogated the genotype-related differences in HOMA-IR (**Fig. 2i**).

145 We then sought to explore whether microbiota transfer might suffice to recapitulate the phenotype
146 observed in HFD-fed *Ido1*^{-/-} mice. We thus forced-fed WT mice with feces collected from *ob/ob* mice
147 treated or not with 1MT. We used *ob/ob* mice because they are already obese and they showed
148 improved insulin sensitivity but no difference in body weight in response to 1MT treatment (data not
149 shown), in association with a significant lower Kyn/Trp ratio in the feces (**Fig. 2j**). As shown in **Fig.**
150 **2k-n**, repetitive gavage of WT mice with feces from 1MT-treated *ob/ob* mice led to a lower increase
151 of total body, WAT and liver weights, to a higher content of M2-like macrophages in epiWAT, and a
152 lower HOMA-IR, compared to WT mice transferred with feces from control *ob/ob* mice, indicating
153 protective effects of microbiota collected from mice treated with IDO inhibitor.

154 We next explored the bacterial fecal composition of the microbiota by use of 16S rDNA sequencing.
155 Principal component analysis (PCA) on the basis of genus composition revealed major differences
156 between WT and *Ido1*^{-/-} mice fed with HFD (**Fig. 3a**) and between *ob/ob* mice treated or not with 1MT
157 (**Supplementary Fig. 7a**). No differences regarding bacterial biodiversity were observed between WT
158 and *Ido1*^{-/-} mice fed with HFD, and between *ob/ob* mice treated or not with 1MT (**Supplementary Fig.**
159 **7b**). At the phylum level, important differences were observed between WT and *Ido1*^{-/-} mice fed with
160 either a NCD or a HFD (**Fig. 3b**). In particular, we found that the HFD led to higher *Firmicutes* to
161 *Bacteroidetes* ratio in WT mice, as previously reported²², whereas HFD-fed *Ido1*^{-/-} mice showed a
162 reduction of this ratio, compared to NCD-fed *Ido1*^{-/-} mice (**Fig. 3b**). At the family level, significantly
163 greater proportions of *Ruminococcaeae* and lower proportions of *Rikenellaceae* were observed in
164 HFD-fed WT mice compared to NCD-fed WT mice (**Fig. 3c**), in agreement with previous reports^{23,24}.
165 Whereas in HFD-fed *Ido1*^{-/-} mice compared to NCD-fed *Ido1*^{-/-} mice, the decrease of *Firmicutes* was
166 mainly due to a lower proportion of *Clostridiales*, in particular *Lachnospiraceae* (**Fig. 3c** and
167 **Supplementary Fig. 7c**). The decrease of *Lachnospiraceae* was also observed on 1MT-treated *ob/ob*
168 mice compared to untreated mice (**Supplementary Fig. 7d**). Moreover, a positive correlation was
169 observed between the proportion of *Clostridiales lachnospiraceae* in feces and LPS levels in plasma
170 (**Supplementary Fig. 7e**), suggesting a beneficial impact of the decrease of a selective bacterial
171 species on inflammation in HFD-fed *Ido1*^{-/-} and 1MT-treated *ob/ob* mice. These results were
172 confirmed, using the linear discriminant analysis (LDA) effect size (LEFSE) pipeline comparing
173 HFD-fed WT and HFD-fed *Ido1*^{-/-} mice (**Supplementary Fig. 7f**). Overall, these data demonstrate that
174 IDO has an important role in shaping gut microbiota, which is required to control body weight and
175 insulin-resistance.

176 We next examined whether Kyn or derived metabolites played a direct role in obesity, as previously
177 suggested²⁵. In particular, administration of kynurenic acid (Kna), a metabolite downstream of Kyn, to
178 WT mice has been shown to activate G protein-coupled receptor (GPR) 35 and rises energy
179 expenditure²⁶. To this end, we supplemented *Ido1*^{-/-} mice with Kyn or Kna added in drinking water.
180 Kyn supplementation in *Ido1*^{-/-} mice did not change body weight, WAT weights or insulin sensitivity
181 despite a higher plasma Kyn levels (**Supplementary Fig. 8**). Moreover, Kna supplementation in *Ido1*
182 ^{-/-} mice did not alter body weight (data not shown). Our results indicate that the absence of Kyn or
183 derived metabolites in *Ido1*^{-/-} mice does not explain the observed protection against metabolic disease.

184 Trp is either metabolized by IDO to produce Kyn or by gut bacteria into indole derivatives, such as
185 indole-3-acetic acid (IAA) that activates aryl hydrocarbon receptor (AHR)²⁷ (**Supplementary Fig.**
186 **9a**). We hypothesised that in obesity the increase of IDO activity shifts Trp metabolism from
187 generation of indole derivatives towards Kyn production. To test this, we examined intestinal content
188 of IAA, Trp and Kyn in NCD or HFD-fed WT or *Ido1*^{-/-} mice, *ob/ob* mice treated or not with IMT,
189 and in WT mice that received feces from IMT-treated or untreated *ob/ob* mice. As shown in **Fig. 3d**,
190 HFD in WT mice led to lower intestinal content of IAA, whereas it markedly induced Kyn levels in
191 the gastrointestinal tract, indicating that HFD-induced obesity causes a major shift of Trp metabolism
192 towards Kyn production. Consistently, in the case of a low level of intestinal Kyn as in HFD-fed *Ido1*^{-/-}
193 mice (**Fig. 3d**) and in IMT-treated *ob/ob* mice (**Supplementary Fig. 9b**), a substantially higher IAA
194 intestinal content was observed, as compared with HFD-fed WT mice (**Fig. 3d**) and control *ob/ob*
195 mice (**Supplementary Fig. 9c**), without major changes of intestinal Trp levels (**Supplementary Fig.**
196 **9d-e**). Moreover, a higher intestinal IAA was observed in WT mice that received feces from IMT-
197 treated mice compared to non-treated mice (**Supplementary Fig. 9f**), indicating the importance of
198 IDO-dependent changes of microbiota in IAA production. Using an AHR reporter system, we found
199 that small intestines contents of HFD-fed *Ido1*^{-/-} mice activated AHR more than those recovered from
200 HFD-fed WT mice (**Supplementary Fig. 9g**). This data supports the importance of IDO in controlling
201 Kyn and IAA-activating AHR balance. Moreover, AHR activation or IAA supplementation in WT
202 mice fed a HFD reduced insulin resistance and epiWAT inflammation (**Supplementary Fig. 10a-f**),
203 without significant changes in body weight (data not shown).

204 We then explored the role of the 2 cytokines related to indole metabolites²⁷, IL-17 and IL-22, in our
205 findings. In agreement with previous reports showing that HFD diminished IL-17 and IL-22 (ref. 28,
206 29), we found lower levels of these cytokines in Peyer's patches (PP) of HFD-fed WT compared to
207 NCD-fed WT mice (**Fig. 3e**). Moreover, in agreement with higher IAA²⁷, we observed more IL-17 and
208 IL-22 in HFD-fed *Ido1*^{-/-} mice compared to HFD-fed WT (**Fig. 3e**) as well as more IL-22 in IMT-
209 treated WT mice compared to non-treated mice (**Supplementary Fig. 10g**). Furthermore, a higher
210 intestinal IL-22 level was observed in WT mice that received feces from IMT-treated *ob/ob* mice
211 compared to non-treated *ob/ob* mice (**Supplementary Fig. 10h**). Intestinal IAA levels were positively
212 correlated with intestinal IL-22 levels and negatively with HOMA-IR (**Supplementary Fig. 10i-j**).
213 We further found an increase of IL-22-target genes such as antimicrobial proteins³⁰, regenerating islet-
214 derived (*Reg3g*, *Reg3b*) mRNA (**Fig. 3f**) in intestines of HFD-fed *Ido1*^{-/-} compared to HFD-fed WT
215 mice. Short-chain fatty acids (SCFAs), mainly acetate, propionate and butyrate, are the end products
216 of fermentation of dietary fibres by the anaerobic intestinal microbiota, and have been shown to exert
217 multiple beneficial effects². Interestingly, a higher fecal level of SCFAs was observed in HFD-fed
218 *Ido1*^{-/-} compared to WT mice fed with the same diet (**Fig. 3g**) supporting a restoration of the intestinal
219 ecosystem. Moreover, we observed lower expression of inflammation-associated genes (differentially
220 expressed between HFD-fed WT and *Ido1*^{-/-} mice) in intestines of HFD-fed *Ido1*^{-/-} compared to HFD-
221 fed WT mice, using NanoString technology (**Fig. 3h**). As previously published²⁰, we found that HFD
222 led to a higher plasma LPS (**Fig. 3i**). However, HFD-fed *Ido1*^{-/-} mice showed lower plasma LPS in
223 comparison to HFD-fed WT mice (**Fig. 3i**), which was also the case in IMT-treated WT and *ob/ob*
224 mice compared to untreated controls (data not shown). Altogether these results provide a strong
225 evidence for a protective role of IDO deletion in preserving intestinal immune barrier during obesity.
226 IL-22 was shown to exert essential roles in eliciting antimicrobial immunity and maintaining mucosal
227 barrier integrity within the intestine^{31,32}. Given the observed higher levels of IL-22 in HFD-fed *Ido1*^{-/-}
228 mice compared to HFD-fed WT mice, we injected mouse anti-IL-22 neutralizing antibody or control
229 IgG1 to WT and *Ido1*^{-/-} mice during HFD period. Neutralization of IL-22 in HFD-fed *Ido1*^{-/-} mice
230 compared to HFD-fed WT mice abrogated the protective effects of IDO deletion on obesity, insulin
231 sensitivity and intestinal permeability (**Fig. 3j-o** and **Supplementary Fig. 11**).

232 As rodents may differ from humans regarding the regulation of IDO activity³³, we then explored the
233 relevance of our data in the human setting of obesity (**Supplementary Table**). In line with the

234 dysfunction of gut barrier function in obesity, we detected a higher circulating endotoxin level in
235 subjects with obesity in comparison with non-obese individuals (**Fig. 4a**). Moreover, plasma Kyn level
236 was higher in subjects with obesity or with type 2 diabetes compared to controls (**Fig. 4b**). We then
237 analysed for the first time the levels of fecal Trp, Kyn and IAA in the context of human obesity. In
238 agreement with our mouse data, we observed a shift of Trp metabolism towards more Kyn and less
239 IAA in feces of subjects with obesity or diabetes compared to non-obese subjects (**Fig. 4c**). We found
240 no correlation between plasma and feces levels of Kyn/Trp ratio ($r = 0.04$, $P = 0.75$), suggesting a
241 specific micro-environmental regulation of IDO in intestine of subjects with obesity. We then
242 examined correlations between feces or plasma Kyn levels and metabolic and clinical parameters in
243 subjects with obesity. We found positive correlations between plasma Kyn and body weight ($r = 0.37$,
244 $P = 0.007$), waist-circumference ($r = 0.34$, $P = 0.01$), fat mass ($r = 0.38$, $P = 0.01$), plasma LPS ($r =$
245 0.31 , $P = 0.02$), aspartate aminotransferase AST ($r = 0.34$, $P = 0.02$), area under the curve during an
246 oral glucose tolerance test ($r = 0.37$, $P = 0.008$), but negative correlations with HDL-cholesterol ($r = -$
247 0.31 , $P = 0.03$) and glucose rate during euglycemic hyperinsulinemic clamp ($r = -0.40$, $P = 0.006$). We
248 observed an inverse correlation between feces Kyn levels and HDL-cholesterol ($r = -0.35$, $P = 0.01$),
249 but detected positive correlations between feces Kyn levels and plasma triglycerides ($r = 0.38$, $P =$
250 0.007), and triglycerides/HDL-cholesterol ratio ($r = 0.42$, $P = 0.002$), the latter being a surrogate
251 marker for cardiometabolic risk³⁴. These data indicate that high levels of Kyn in plasma and feces are
252 associated with a deleterious metabolic profile in the setting of obesity.

253

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267 **Author Contribution statement**

268 L.L. was involved in experimental design, conducted most experiments and analyzed data. N.V.
269 provided technical and conceptual helps on obesity experiments and discussed results. Y.H., M.C.,
270 S.M., B.S. helped in some experiments. R.G.P.D., designed, performed, analyzed and interpreted the
271 indirect calorimetry exploration. F.A. helped with experiments and performed immunohistological
272 staining. M.S., C.M. and S.J. provided technical help for microbiota analysis. T.V. and B.T. performed
273 and discussed PET analysis. B.E. helped with in vivo studies. J-M.L., J.D. and J.C. measured all
274 biochemical parameters in mouse and human samples. S.L. provided funding and contributed to
275 calorimetry data analysis and interpretation. M.C., J-M. M-N., M.F., JM.F-R. and R.B. provided
276 human material and clinical data. A.T. and Z.M. discussed results and edited the manuscript. H.S.
277 performed and interpreted gut microbiota analysis, provided some of human samples and discussed
278 results. S.T. designed the study, analyzed and interpreted the data, and wrote the manuscript.

279
280 **Competing financial interests**

281 No conflict of interest

282

283

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360

361

362

363 **Figure Legends**

364 **Figure 1** *Ido1*^{-/-} mice are protected from obesity, inflammation, liver steatosis and insulin resistance.
365 (a) IDO activity (Kyn/Trp ratio) in plasma, ingWAT, epiWAT, brown AT, liver and soleus from WT
366 mice fed with either a HFD (*n* = 5) or a NCD (*n* = 5) or HFD-fed *Ido1*^{-/-} mice after 20 weeks. HFD-fed
367 *Ido1*^{-/-} mice values are displayed only as controls. (b) body mass of WT and *Ido1*^{-/-} mice fed with either
368 HFD (*n* = 10 per group) or NCD (*n* = 5 per group) during 20 weeks. This result was confirmed in a
369 total of 4 independent experiments. (c-f) Body fat % (c), weights in grams of epiWAT, ingWAT and
370 retWAT (d), plasma leptin (e) and liver weight (f) of WT and *Ido1*^{-/-} mice fed with HFD during 20
371 weeks. (g) Representative pictures (left) and quantification (right) of lipid areas in liver cross-sections
372 from HFD-fed WT and *Ido1*^{-/-} mice (*n* = 9 per group). Scale bars, 100 μ m. (h) Representative images
373 (left) and quantifications (right) of F4/80 staining in ingWAT and epiWAT (*n* = 5 per group). Scale
374 bars, 100 μ m. F4/80 staining (in red) was normalized on Plin1 staining (in green). (i-l) quantification
375 of M2-like macrophages (F4/80+CD11b+CD206+) in epiWAT (*n* = 5 per group) (i), adiponectin
376 production by epiWAT explants (*n* = 10 per group) (j), insulin tolerance test (ITT) adjusted on body
377 weight (confirmed in a total of 3 independent experiments) (k), ratio of area under curve (AUC) of
378 insulin to AUC glucose during oral glucose tolerance test (OGTT) in WT and *Ido1*^{-/-} mice fed with
379 HFD during 20 weeks (*n* = 10 per group) (l). (m) Insulin signaling (pAKT-S473) in soleus after 15
380 min of 5 U/kg insulin injection. Cropped blot images are shown, the full scans are available in
381 **Supplementary Fig. 12**. The result is representative of two independent experiments. (n)
382 Representative FDG-PET images (left) and quantification (right) of tissue FDG uptake in WT and
383 *Ido1*^{-/-} mice fed with HFD during 14 weeks (*n* = 5 per group). SUV, standardized uptake values. White
384 arrows show the FDG uptake in muscle. Data are expressed as mean \pm sem. Mann-Whitney test (a-l)
385 or two-tailed unpaired Student's *t*-test (n) was used for statistical analysis. **P* < 0.05, ***P* < 0.001,
386 ****P* < 0.0001.

387 **Figure 2** IDO activity controls gut microbiota-dependent regulation of obesity and its complications.
388 (a-e) IDO activity (Kyn/Trp ratio) in plasma (*n* = 5 per group) (a), % of weight gain (b), weights in
389 grams of ingWAT, epiWAT, retWAT and liver (c), insulin test tolerance (ITT) (d), oral glucose
390 tolerance test (OGTT) (e) in WT mice irradiated and transplanted with either WT or *Ido1*^{-/-} bone
391 marrow (*Ido1*^{-/-}-> WT (*n* = 10) and WT -> WT (*n* = 10) groups) or *Ido1*^{-/-} mice irradiated and
392 transplanted with WT bone marrow (WT -> *Ido1*^{-/-} (*n* = 10)) after 20 weeks of HFD. (f) IDO activity
393 (*i.e.* Kyn/Trp) in small intestines and colons of WT mice fed with either a NCD (*n* = 5) or a HFD (*n* =
394 4) and HFD-fed *Ido1*^{-/-} mice (*n* = 4). (g-i) weight curves (non-mixed and non-treated HFD-fed WT and
395 *Ido1*^{-/-} mice weight curves are displayed only as controls), (g and h) HOMA-IR index normalized to
396 body weight of WT and *Ido1*^{-/-} mice either on antibiotic treatment (Ab) (*n* = 10 per group) or WT and
397 *Ido1*^{-/-} mice mixed in the same cages from 4 weeks of age (mix) (*n* = 8 per group) or WT and *Ido1*^{-/-}
398 mice untreated and separated in different cages (*n* = 10 per group) (i). (j-n) gavage of WT mice with
399 feces from 1MT-treated or not treated *ob/ob* mice (*n* = 10 per group). Ratio of Kyn/Trp in feces of
400 1MT-treated or not treated *ob/ob* mice (*n* = 4 per group) (j), body mass (k), weights in grams of
401 ingWAT, epiWAT, retWAT and liver (l), representative cytometry (left) and quantification (right) of
402 M2-like macrophages (F4/80+CD11b+CD206+) in epiWAT (*n* = 5 per group) (m) and HOMA-IR in
403 WT mice that received feces from 1MT-treated or untreated *ob/ob* mice (*n* = 10 per group). n.s., non-
404 significant. Data are expressed as mean \pm sem. Mann-Whitney test was used for statistical analysis (a-
405 n). **P* < 0.05, ***P* < 0.001, ****P* < 0.0001.

406 **Figure 3** IDO deficiency preserves the intestinal barrier through IL-22 in the setting of obesity. (a)
407 PCA plot based on bacterial 16S rDNA gene sequence abundance in fecal content of WT and *Ido1*^{-/-}
408 mice fed with either a NCD or a HFD. Axes correspond to principal components 1 (x-axis), 2 (y-axis)
409 and 3 (z-axis). (b, c) bacterial-taxon-based analysis at the phylum level (b) and at family level (c) in
410 the fecal microbiota. (d) IAA (left) and Kyn (right) levels in small intestines and colons of WT fed
411 with either a NCD (*n* = 5) or a HFD (*n* = 4) and HFD-fed *Ido1*^{-/-} mice (*n* = 4). (e) IL-17 (left) and IL-

412 22 (right) contents in Peyer's patches (PP) of WT and *Ido1*^{-/-} mice fed with either a NCD (*n* = 4 per
413 group) or a HFD (*n* = 3 per group). The result was confirmed in two independent experiments. **(f)**
414 *Reg3b* (left) and *3g* (right) mRNA in intestines of HFD-fed WT (*n* = 3) and *Ido1*^{-/-} mice (*n* = 4). **(g)**
415 SCFA contents in the fecal microbiota from HFD-fed WT (*n* = 10) and *Ido1*^{-/-} mice (*n* = 9). The result
416 is a pool of 2 independent experiments. **(h)** The heat map generated using the hierarchical clustering
417 shows expression of genes differentially expressed in intestines of HFD-fed WT (*n* = 3) and *Ido1*^{-/-}
418 mice (*n* = 4). **(i)** Plasma LPS in WT and *Ido1*^{-/-} mice fed with either NCD or HFD (*n* = 5 per group)
419 after 20 weeks. The result was confirmed in two independent experiments. **(j-n)** % of weight gain after
420 12 weeks of HFD **(j)**, OGTT **(k)**, ITT **(l)**, HOMA-IR **(m)**, liver steatosis **(n)** and plasma LPS **(o)** in
421 WT and *Ido1*^{-/-} mice injected with mouse anti-IL-22 neutralising antibody, 3 times per week during 12
422 weeks of HFD. Scale Bars 100μM. Data are expressed as mean ± sem. Mann-Whitney test **(d, g, i** and
423 **m)** or two-tailed unpaired Student's *t*-test **(e** and **f)** was used for statistical analysis. **P* < 0.05, ***P* <
424 0.001, ****P* < 0.0001.

425 **Figure 4** A shift of Trp metabolism towards more Kyn and less IAA in the context of human obesity
426 and type 2 diabetes. **(a)** plasma LPS, **(b)** plasma Trp (left) and Kyn (right) in subjects with obesity (*n* =
427 49) or type 2 diabetes (*n* = 43) and non-obese individuals (*n* = 20). **(c)** Trp (left), Kyn (middle) and
428 Kyn/Trp (right) in feces from subjects with obesity (*n* = 49) or type 2 diabetes (*n* = 43) and non-obese
429 subjects (*n* = 34). **(d)** IAA contents in feces from subjects with obesity (*n* = 49) or type 2 diabetes (*n* =
430 43) and non-obese subjects (*n* = 34). Mann-Whitney test **(a)** or Kruskal-Wallis **(b-d)** was used for
431 statistical analysis. Data are expressed as mean ± sem. **P* < 0.05, ****P* < 0.0001.

432

433 **Online Methods**

434 **Mice**

435 Male C57Bl/6 *Ido1*^{-/-} mice were bought from the Jackson Laboratory (Jax) and bred in our facility. At
436 weaning, mice were separated according to the genotype. Male *ob/ob* mice were bought from Janvier
437 Laboratory at 4 weeks of age. To generate *Ido1*^{fllox/fllox} LysM-Cre, *Ido1*^{fllox/fllox} mice were crossed to
438 LysM-Cre mice. Mice were fed with either a normal chow diet (NCD) (A03, SAFE, France) or
439 subjected to diet-induced obesity containing 60% FAT (E15742-347, SSNIFF, Germany). High fat
440 diet (HFD) was started at 7 weeks of age and continued for 20 weeks or less with ad libitum access to
441 water and food. For chimerism experiment, we subjected 10 weeks old C57Bl/6 WT and C57Bl/6
442 *Ido1*^{-/-} mice to medullar aplasia by 9.5 gray lethal total body irradiation. We repopulated the mice with
443 an intravenous injection of bone marrow cells isolated from femurs and tibias of male C57Bl/6 WT
444 and C57Bl/6 *Ido1*^{-/-} mice. After 4 weeks of recovery, mice were fed a HFD for 20 weeks. In some
445 experiments, IDO inhibitor (L-1methyl tryptophan, 1MT) (Sigma Aldrich) or IAA (indole acetic acid;
446 Sigma Aldrich) was used at 2mg/ml diluted in drinking water. The 6-formylindolo(3,2-b)carbazole
447 (Ficz; Sigma Aldrich) was resuspended in dimethyl sulfoxide (DMSO; Sigma Aldrich) and
448 administered intraperitoneally (1 ug/mouse, 1-3 times per week) during 15 weeks of HFD. In another
449 experiment, kynurenine (2 mg/ml diluted in drinking water) supplementation to *Ido1*^{-/-} mice was
450 performed during 15 weeks of HFD. We also subjected some mice to antibiotic treatment as described
451 before³⁵. All mice used in these experiments were bred and housed in a specific pathogen-free barrier
452 facility. Animal experiments were performed according to the European directive (2010/63/UE) and to
453 the institutional guidelines approved by the local ethics committee of the French authorities, the
454 'Comité d'Ethique en Experimentation Animale' (CEEA) under the following number 17-018.

455 **In vivo Studies**

456 For oral glucose tolerance test (OGTT), mice were fasted overnight prior to an oral administration of
457 1-5 g/kg glucose. Blood was sampled from the tail vein at 0, 5, 15, 30, 60, 90 and 120 min in order to
458 assay glucose concentration (OneTouch Ultra glucometer, LifeScan Europe). At 0, 15, 30, 60 min tail
459 vein blood was collected, plasma samples were stored at -20°C until they were analyzed for insulin
460 concentration (Crystal Chem Inc., Downers Grove, USA). Insulin tolerance test (ITT) was performed
461 in mice food deprived for 5 h prior to an intraperitoneal injection of 1 U/kg insulin. Blood was sampled
462 from the tail vein at 0, 5, 15, 30, 60 and 90 min in order to assay glucose concentration. For insulin
463 signaling assays C57Bl/6 WT and C57Bl/6 *Ido1*^{-/-} mice were fasted overnight and then treated by
464 intraperitoneal injection with 5 U/kg insulin (15 min). Tissue samples were examined by immunoblot
465 analysis by probing with antibodies to phospho-AKT, AKT (Cell Signaling). Experiments with fecal
466 gavage were done with fresh stool samples from either *ob/ob* control mice or *ob/ob* mice
467 supplemented with 1MT during 6 weeks until 19 weeks. Briefly, stool were suspended in water and
468 sieved through a 70 µm cell strainer (BD). These fecal suspensions were inoculated to C57Bl/6 WT
469 mice via oral gavage with 400 µL of fecal suspension 4-5 times per week during 15 weeks of HFD.
470 For anti-IL-22 neutralizing antibody treatment, WT and *Ido1*^{-/-} mice were injected intraperitoneally
471 three times per week with mouse anti-IL22 neutralizing antibody (50 µg/mouse) (Genentech, South
472 San Francisco, CA, USA) or an equivalent amount of isotype control (IgG1) (Genentech) for a period
473 of 12 weeks of HFD.

474 **Analysis of metabolic parameters**

475 Blood glucose level was measured using a glucometer (OneTouch Ultra, LifeScan Europe). Plasma
476 insulin (Crystal Chem Inc., Downers Grove, USA) and plasma leptin was determined by ELISA
477 (R&D Systems). HOMA-IR in mice was calculated using the equation ((fasting glucose concentration
478 x fasting insulin concentration)/405) as previously described³⁶. Areas under the curve (AUCs) for
479 glucose and insulin were calculated for both C57Bl/6 and C57Bl/6 *Ido1*^{-/-} mice during the 2h OGTT
480 using the trapezoid method³⁷. LPS in plasma was measured with a colorimetric diagnostic kit (Pierce,
481 USA).

482 Measurement of short chain fatty acids (SCFA) was performed as described previously³⁸ with slight
483 modifications. A stock solution of SCFA metabolites (Sigma Aldrich, France) was prepared and
484 serially diluted to get 10 calibration solutions. A working solution of internal standards was prepared
485 in 0.15 M NaOH to get the following final concentrations: 75 mmol/L of D₃-acetate, 3.8 mmol/L of
486 D₅-propionate, 2.5 mmol/L of ¹³C-butyrate, 0.5 mmol/L of D₉-valerate (Sigma Aldrich). Stool samples
487 were weighed (~50 mg), dissolved in 200 μL of sodium hydroxide solution at 0.15 M (NaOH, Sigma
488 Aldrich). Twenty microliters of the internal standard solution were added to stool samples and
489 calibration solutions. Each sample was then acidified with 5 μL of hydrochloric acid 37% (Sigma
490 Aldrich, France) and then extracted with 1.7 mL of diethyl ether (Biosolve, France). Samples were
491 stirred gently for 1 hour and then centrifuged 2 min (5000 rpm, 4°C). The organic layers were
492 transferred into 1.5 ml glass vials and SCFAs were derivatized with 20 μL of *tert*-butyldimethylsilyl
493 imidazole (Sigma Aldrich, France). Samples were incubated 30 min at 60°C before analysis. Samples
494 were finally analyzed by GC-MS (model 7890A-5975C, Agilent Technologies, France) using a 30 m ×
495 0.25 mm × 0.25 μm capillary column (HP1-MS, Agilent Technologies, France). The temperature
496 program started at 50 °C for 1 min, ramped to 90°C at 5°C/min, then up to 300 °C at 70°C/min.
497 Selected ion monitoring (SIM) mode was used to measure SCFA concentrations with ions at m/z 117
498 (acetate), 120 (D₃-acetate), 131 (propionate), 136 (D₅-propionate), 145 (butyrate and isobutyrate), 146
499 (¹³C-butyrate), 159 (valerate), 168 (D₉- valerate).

500 Immunohistochemical analyses

501 Liver sections (7 μm) were used for Oil Red O coloration to visualize lipid accumulation and to
502 perform CD68 staining. Briefly, sections were fixed 5 min in PFA prior to staining. Macrophages were
503 detected using anti-CD68 antibody (AbD Serotec MCA1975) overnight at 4°C. Anti-Rat peroxidase
504 polymer (Histofine) and IHC chromogen substrate (Thermo scientific) were used for revelation.
505 Immediately after collection, adipose tissue samples were drop-fixed in formaldehyde solution for 24
506 hours, then put through an automated carousel processor for dehydration, clearing and paraffin
507 embedding (Leica). Briefly, tissue sections were dewaxed through three consecutive incubations (5
508 min) in xylene and then dehydrated by three consecutive incubations (3 min) in 96–100% ethanol,
509 followed by 2 washes (2 min) in de-ionized water. Sections were then incubated for 15 min at room
510 temperature in 3% (v/v) hydrogen peroxide, followed by two washes (2 min) in de-ionized water.
511 Photo-bleaching with 5 min under UV lamp and Oil Red O emersion for 2 min were applied to quench
512 auto-fluorescence. Sections were then blocked for 30 min with Tris-buffered saline (TBS) solution and
513 3% bovine serum albumin (w/v). Primary antibodies anti-F4/80 (1:50, Cl:A3-1, MCA497, AbD
514 Serotec), anti-Plin1 (1:100, D1D8, 08/2016 Cell Signalling Technologies) diluted in TBS + 1% (w/v)
515 BSA were applied for 2 h at room temperature, slides were then washed in TBS + 0.1% (v/v) Tween20
516 three times for 5 min, and the secondary antibodies anti-Rat TRITC-conjugated secondary antibody
517 (A18870, ThermoFisher); anti-Rabbit FITC-conjugated secondary antibody (F2765, Life
518 Technologies) were applied for 1 h at room temperature in the dark. Slides were then washed in TBS +
519 0.1% (v/v) Tween20 three times for 5 min, slides were mounted with VectaShield hardset mounting
520 media with DAPI, slides were cured overnight at 4°C before imaging. Images were acquired on an
521 Axiovert 200M microscope using appropriate filters. Quantitative expression of immunostainings was
522 performed using positive pixels algorithm (Indica Labs) on digital slides (Zeiss). F4/80 staining is
523 expressed as a ratio of Plin1 staining. Quantification method of random fields is an automated
524 observer-independent process based on publicly accessible algorithms. Each biological replicate
525 represented one slide, which was mounted with at least 3 tissue sections, representing 3 technical
526 replicates, the mean of which was presented as the result per biological replicate.

527 Frozen soleus muscle sections were stained according to standard immunofluorescence protocols.
528 Briefly, transversal sections were brought to room temperature and fixed with 10% formalin for 10
529 minutes, followed by permeabilisation through two 5 minute washes with Tris buffered saline (TBS)
530 solution containing 0.5 % (v/v) Tween 20. Sections were then blocked with TBS solution containing 3
531 % bovine serum albumin. Slides were incubated at 4°C in a humidified chamber overnight with anti-

532 AIF at a 1:50 dilution in TBS containing 1% BSA (Sigma SAB3501107) or anti-GLUT4 (Cell
533 Signaling 11/2008) antibodies. Following incubation slides were washed three times for 10 minutes
534 with TBS and incubated in a humidified chamber for 2 hours at room temperature with the following
535 secondary antibodies: AlexaFlour 647 goat-anti-rabbit at 1:200 in TBS containing 1% BSA (Life
536 technologies A21244) and rabbit-anti-mouse at 1:200 in TBS containing 1% BSA (abcam ab150127).
537 Slides were washed three times for 5 minutes with TBS. Slides were then mounted with Vectashield
538 Hard-set mounting media containing DAPI. Whole slide scanning was carried out on a Zeiss Axioscan
539 scanner and Zen software. Image quantification was carried out using Visiopharm software applied to
540 whole tissue scans in an observer independent manner following definition of membrane and cytosolic
541 regions. Unless otherwise stated, standard reagents were sourced from Sigma. Quantification was
542 carried out through positive pixel counts of morphologically defined membrane and cytoplasmic
543 regions with the use of Visiopharm software.

544 **Adipose cell isolation and flow cytometry analyses**

545 The stromal vascular fraction (SVF) containing mononuclear cells and preadipocytes was extracted
546 from adipose tissue. Adipose tissue from mice was digested using 10 mL digestion solution (7 mL
547 Hank's Solution, 3 mL 7.5% BSA and 20 mg collagenase type II, Sigma). The digestion was
548 performed at 37°C using a shaker at 100 rpm for 20 min. After digestion, the adipocyte fraction
549 (floating) was isolated and the solution containing the SVF was centrifuged at 1500 rpm at 4°C for 5
550 min. The SVF pellet was resuspended in 1 mL fluorescence-activated cell sorter (FACS) buffer. After
551 15 min incubation with Fc Block (2.4G2, BD Biosciences), SVF cells were stained with appropriate
552 antibodies conjugated to fluorochromes or isotype controls for 30 min at 4°C in the dark: CD45 (30-
553 F11), F4/80 (BM8), CD11b (M1/70), CMHII (M5/114.15.2) from eBiosciences, CD11c (HL3) from
554 BD Biosciences and CD206 (C068C2) from Biolegend. Samples were acquired using an Fortessa
555 cytometer (Becton Dickinson) and analyzed with FlowJo (TreeStar) software programs.

556 **Adipose tissue culture**

557 Mouse adipose tissue biopsies (0.1g) were minced and incubated in 1mL of endothelial cell basal
558 medium (PromoCell) containing 1% bovine serum albumin, penicillin (100 U/mL) and streptomycin
559 (100 U/mL). Adipose tissue-conditioned medium (ATCM) were recovered after 24h and stored at -
560 80°C until analysis.

561 **Cytokine quantification**

562 Cytokine concentrations from ATCM were analyzed using ELISA kits. Adiponectin, IL-17, IL-5
563 ELISA kits were from R&D Systems. IFN- γ , IL-6, IL-4 and IL-10 ELISA kits were bought from BD.
564 IL-22 ELISA kit was from eBiosciences. IL-17 and IL-22 were measured in PPs (Peyer's patches) or
565 intestine extracts. Briefly, PPs or intestines were lysed in detergent buffer (RIPA) containing protease
566 inhibitor (Roche). After centrifugation 13000 g – 10 min at 4°C, protein quantification was performed
567 on supernatants and then supernatants were stored at -20° until ELISA assay. IFN γ levels in plasma
568 and WAT were determined using the murine IFN- γ ELISA kit from Diaclone SAS (Besançon, France)
569 and according to the manufacturer's instructions.

570 **Quantitative Real time PCR**

571 Macrophages and intestines were lysed in detergent buffer RLT and then subjected to RNA extraction
572 and reverse transcription (Qiagen). Then, quantitative real-time PCR was performed on an ABI
573 PRISM 7700 (Applied Biosystems) in triplicates. Cycle threshold for Gapdh (primers: Gapdh-R, 5'-
574 CGTCCCGTAGACAAAATGGTGAA-3'; Gapdh-L, 5'-GCCGTGAGTGGAGTCATACTGGAACA-
575 3') was used to normalize gene expression. Primers for *Ido1* are: *Ido1*-R, 5'-
576 ATATATGCGGAGAACGTGGAAAAAC-3', *Ido1*-L, 5'-CAATCAAAGCAATCCCCACTGTATC-
577 3'. *Reg3g*-R 5'-TTCCTGTCTCCATGATCAAAA-3' and *Reg3g*-L 5'-

578 CATCCACCTCTGTTGGGTTCA-3'; and *Reg3b*-R 5'-ATGCTGCTCTCCTGCCTGATG-3' and
579 *Reg3b*-L 5'-CTAATGCGTGCGGAGGGTATATTC-3. PCR conditions were 10 min at 95°C ; 35
580 cycles of 95°C for 15 s, 60°C for 20 s and 72°C for 20 s and a final extension at 72°C for 20 s.

581 **Western blot analysis**

582 Tissue samples were lysed in detergent buffer (RIPA, Thermo Scientific) containing protease and
583 phosphatase inhibitor mixture (Roche) and sodium orthovanadate. Proteins were separated on 4-12%
584 NuPage Tris-Bis gels using NuPage MES-SDS running buffer (Invitrogen) and then were transferred
585 onto nitrocellulose membranes. The filters were probed with antibodies directed against phospho-AKT
586 (Cell Signaling #4060P), AKT (Cell Signaling #9272S).

587 **Indirect calorimetric measurements**

588 Calorimetry exploration was performed at the “Functional and Physiological Exploration” facility
589 (Université Paris-Diderot) as previously described³⁹. Briefly, body composition was assessed using an
590 Echo Medical systems EchoMRI 100 (Whole Body Composition Analyser, EchoMRI, Houston,
591 USA). Energy expenditure, oxygen consumption and carbon dioxide production, respiratory exchange
592 ratio, food intake and homecage activity were obtained using calorimetric chambers (Labmaster, TSE
593 Systems GmbH, Bad Homburg, Germany). Activity was recorded using infrared light beam-based
594 locomotion monitoring system. Mice were individually housed, fed with a HFD (SSNIFF, Germany)
595 and acclimated to the chambers and drinking and food holders for 48 hr before experimental
596 measurements. Calorimetric data represent mean of at least 96 hr measurement.
597 Extensive analysis of energy expenditure (kcal/hr) and VO₂ consumption (mL/hr) was performed
598 using a regression based approach, analysis of covariance (ANCOVA, Minitab 16, Paris France), as
599 previously described⁴⁰, to take account of the mass effects with body weight, lean mass and fat free
600 mass as covariance. Whole body mass and lean body mass were found to be dependent variables ($P >$
601 0.05 from Analysis of covariance). Total energy excretion in feces was estimated by bomb calorimetry
602 (Phenomin facility).

603

604 **Intestinal content DNA extraction**

605 Fecal genomic DNA was extracted from the weighted stool samples using a method that was
606 previously described²⁷, which is based on the European MetaHIT DNA extraction method.
607

608 **16s rRNA gene sequencing**

609 16s rDNA gene sequencing of fecal DNA samples was performed as previously described²⁷. Briefly,
610 the V3-V4 region (16S (sense) 5'-TACGGRAGGCAGCAG-3' and (antisense) 5'-
611 CTACCNNGGTATCTAAT-3') was amplified and sequencing was done using an Illumina MiSeq
612 platform (GenoScreen, Lille, France). Raw paired-end reads were subjected to the following process:
613 (1) quality-filtering using the PRINSEQ-lite PERL script³⁸ by truncating the bases from the 3' end
614 that did not exhibit a quality < 30 based on the Phred algorithm; (2) paired-end read assembly using
615 FLASH (fast length adjustment of short reads to improve genome assemblies)⁴¹ with a minimum
616 overlap of 30 bases and a 97% overlap identity; and (3) searching and removing both forward and
617 reverse primer sequences using CutAdapt, with no mismatches allowed in the primers sequences.
618 Assembled sequences for which perfect forward and reverse primers were not found were eliminated.
619 Sequencing data were analyzed using the quantitative insights into microbial ecology (QIIME 1.9.1)
620 software package. The sequences were assigned to OTUs using the UCLUST algorithm⁴² with a 97%
621 threshold of pairwise identity and classified taxonomically using the Greengenes reference database⁴³.
622 Rarefaction was performed (8,000 sequences per sample) and used to compare abundance of OTUs
623 across samples. Biodiversity indexes were used to assess alpha diversity and α and β diversities were
624 estimated using phylogenetic diversity and unweighted UniFrac. Principal component analyses (PCA)
625 of The Bray Curtis distance with each sample colored according to phenotype were built and used to
626 assess the variation between experimental groups. The LDA effect size algorithm was used to identify
627 taxa that are specific to experimental group⁴⁴.

628 **Luciferase assay**

629 The H1L1.1c2 cell line containing a stably integrated dioxin response elements (DRE)-driven firefly
630 luciferase reporter plasmid pGudLuc1.1 has been described previously²⁷. The cells were seeded in 96-
631 well plates at 7.5×10^4 cells/well in 100 μ l of complete Dulbecco's modified Eagle's medium
632 (DMEM) (with 10% heat-inactivated FCS, 50 IU/ml penicillin, and 50 μ g/ml streptomycin; Sigma-
633 Aldrich) and cultured (37 °C, 10% CO₂) for 24 h before treatment. This cell line tested negative for
634 mycoplasma contamination and was used in this study to determine AHR activity of small intestine
635 content. To assess agonistic activity, the cells were treated with small intestine content suspensions
636 diluted at 1:10 in complete DMEM. Controls consisted of cells treated with DMEM as the negative
637 control, or FICZ (Sigma) diluted in DMEM as the positive control. After 24 h of incubation, wells
638 were washed with 100 μ l PBS, and 50 μ l Promega lysis buffer was added to each well. The plates
639 were shaken for 30 min to lyse the cells. After adding 100 μ l of luciferase reagent (Promega),
640 luciferase activity was measured using a luminometer. The results were normalized based on the
641 negative luciferase activity of the control.

642 **HPLC quantifications**

643 Thawed stools from mice were extracted as previously described⁴⁵. L-tryptophan (Trp) and L-
644 kynurenine (Kyn) were measured via HPLC using a coulometric electrode array (ESA Coultronics,
645 ESA Laboratories, Chelsford, MA, USA)⁴⁶. Quantifications were performed by referencing calibration
646 curves obtained with internal standards. Other compounds (IAA) were quantified via liquid
647 chromatography coupled to mass spectrometry (LC-MS) by using a Waters ACQUITY
648 ultraperformance liquid chromatography (UPLC) system equipped with a binary solvent delivery
649 manager and sample manager (Waters Corporation, Milford, MA, USA) and that was coupled to a
650 tandem quadrupole-time-of-flight (Q-TOF) mass spectrometer equipped with an electrospray interface
651 (Waters Corporation). Compounds were identified by comparing with the accurate mass and the
652 retention time of reference standards in our in-house library, and the accurate masses of the
653 compounds were obtained from web-based resources, such as the Human Metabolome Database
654 (<http://www.hmdb.ca>) and the METLIN database (<http://metlin.scripps.edu>).

655 **ATP measurements**

656 The firefly luciferase bioluminometric assay was used to measure the muscular ATP content as
657 previously described⁴⁷.

658 **2-deoxy-2-[¹⁸F]fluoro-D-glucose ([¹⁸F]FDG) PET-CT Imaging**

659 Mice were anesthetized with isoflurane (IsoVet 100%; Centravet, France) in 100% O₂ (4% isoflurane
660 for induction; 1-2% for maintenance). Mice were weighted, and placed on a heated plate (Minerve,
661 France). A customized catheter with a 26 G needle (Fischer Scientific, France) connected to a 5-cm
662 polyethylene tubing (Tygon Microbore Tubing, 0.010" x 0.030"OD; Fisher Scientific, France) was
663 installed in the lateral tail vein of the mice. 10 MBq of 2-deoxy-2-[¹⁸F]fluoro-D-glucose ([¹⁸F]FDG;
664 Gluscan, Advanced Applied Applications, France) in 200 μ l of saline solution were injected in the
665 mice. The mice were then put back in their cages and left awake for 30 min. Mice were anesthetized
666 again and installed in the imaging bed of the PET/CT scan (nanoScan, Mediso, Hungary). CT scans
667 were performed first using the following parameters: mode semi-circular, tension of 39kV, 720
668 projections full scan, 300 ms per projection, binning 1:4. List-mode PET data were collected between
669 45 and 60 min post injection of [¹⁸F]FDG, binned using a 5-ns time window, a 400- to 600-keV energy
670 window, and a 1:5 coincidence mode. *In vivo* PET acquisitions were reconstructed using the Tera-
671 Tomo reconstruction engine (3D-OSEM based manufactured customized algorithm) with expectation
672 maximization iterations, scatter and attenuation corrections. Images were analyzed using the software
673 PMOD (PMOD Technologies LLC). Standardized Volume of Interest (VOI) was drawn in each organ

674 of interest and Standardized Uptake Values (SUV) were calculated by dividing the mean tissue
675 radioactivity concentration by the injected dose and body weight.

676 **NanoString.**

677 NanoString analysis was performed and analyzed according to the manufacturer's recommendations.

678 **Human population**

679 Feces from healthy non obese individuals were recruited in the Gastroenterology Department of the
680 Saint Antoine Hospital (Paris, France) and provided informed consent. Plasmas were from healthy non
681 obese individuals ($n = 20$) from EFS, HEGP hospital (Paris, France).

682 49 women with morbid obesity were followed at the Endocrinology Service of the Hospital
683 Universitari de Girona Dr Josep Trueta (Girona, Spain, $n = 25$) and at the Center for Atherosclerosis of
684 Policlinico Tor Vergata University of Rome (Rome, Italy, $n = 24$). Pre-established inclusion criteria:
685 all subjects were of Caucasian origin; the subjects reported a stable body weight 3 months preceding
686 the study, were free of any infections 1 month before and had no systemic disease. Pre-established
687 exclusion criteria: subjects with liver disease, specifically HCV infection and tumor disease, and
688 subjects with thyroid dysfunction were excluded by biochemical workup. All subjects gave written
689 informed consent, validated and approved by the ethical committee of the Hospital Universitari Dr
690 Josep Trueta (Comitè d'Ètica d'Investigació Clínica) and Policlinico Tor 388 Vergata University of
691 Rome (Comitato Etico Indipendente).

692

693 **Subjects with type 2 diabetes**

694

695 We recruited subjects with treatment-naïve type 2 diabetes (T2D), as recently reported⁴⁸. Inclusion
696 criteria were: (i) aged between 18 and 65 years; (ii) T2D diagnosis in the previous 6 months, as
697 defined by the American Diabetes Association Criteria; (iii) absence of systemic and metabolic disease
698 other than T2D, and absence of infection within the previous month; (iv) absence of diet or medication
699 that might interfere with glucose homeostasis, such as glucocorticoids or antibiotics in the previous 3
700 months; and (v) HbA1c lower than 9%. Exclusion criteria were: (i) clinically significant major
701 systemic disease, including malignancy; (ii) clinical evidence of hemoglobinopathies or anemia; (iii)
702 history of drug or alcohol abuse, defined as > 80 g/d in men and > 40 g/d in women; (iv) acute major
703 cardiovascular event in the previous 6 months; (v) acute illnesses or current evidence of acute or
704 chronic inflammatory or infective disease; and (vi) mental illness rendering the participants unable to
705 understand the nature, scope, and possible consequences of the study.

706 All individuals gave written informed consent. The experimental protocol was approved by the Ethics
707 Committee and the Committee for Clinical Investigation of the Hospital Universitari Dr. Josep Trueta
708 (Girona, Spain). We certify that all applicable institutional regulations concerning the ethical use of
709 information and samples from human volunteers were followed during this research. Complete clinical
710 trial registration is deposited in the EU clinical trials register (EudraCT number 2010-022394-34). We
711 report here the findings in the baseline samples from these subjects before entering the trial.

712

713 **Analytical determinations (human)**

714

715 Total plasma cholesterol was measured by an enzymatic, colorimetric method through the cholesterol
716 esterase/cholesterol oxidase/peroxidase reaction (Cobas CHOL2). HDL cholesterol was quantified by
717 a homogeneous enzymatic colorimetric assay through the cholesterol esterase/cholesterol
718 oxidase/peroxidase reaction (Cobas HDLC3). Total plasma triglycerides were measured by an
719 enzymatic, colorimetric method with glycerol phosphate oxidase and peroxidase (Cobas TRIGL).
720 Serum aspartate transaminase (AST) was measured by colorimetry using automated tests (Roche
721 Diagnostics GmbH, Mannheim, Germany). Intra- and inter-assay coefficients of variation were $< 4\%$.

722

723 **Euglycemic hyperinsulinemic clamp (human)**

724 Insulin action was determined by the euglycemic hyperinsulinemic clamp. After an overnight fast, two
725 catheters were inserted into an antecubital vein, one for each arm, used to administer constant
726 infusions of glucose and insulin, and to obtain arterialized venous blood samples. A 2-h euglycemic
727 hyperinsulinemic clamp was initiated by a two step primed infusion of insulin (80 mU/m²/min for 5
728 min, 60 mU/m²/min for 5 min) immediately followed by a continuous infusion of insulin at a rate of
729 40 mU/m²/min (regular insulin; Actrapid, Novo Nordisk, NJ). Glucose infusion began at minute 4 at
730 an initial perfusion rate of 2 mg/kg/min, then was adjusted to maintain plasma glucose concentration at
731 4.9–5.5 mmol/L. Blood samples were collected every 5 min for determination of plasma glucose and
732 insulin. Insulin sensitivity was assessed as the mean glucose infusion rate during the last 40 min. In the
733 stationary equilibrium, the amount of glucose administered (M) equals the glucose taken by the body
734 tissues and is a measure of overall insulin sensitivity.

735

736 **Statistical analysis.**

737 Values are expressed as means ± s.e.m. The differences between groups were assessed using non-
738 parametric Mann-Whitney test or an unpaired Student's *t*-test (two-sided). Values were considered
739 significant at *P* < 0.05. For human data, given the sample size, non-parametric Mann-Whitney test was
740 used to assess the differences between 2 groups or Kruskal-Wallis for multiple comparisons.
741 Spearman's rank correlation coefficient (*r*) was used to analyze the correlations between Kyn and
742 metabolic and clinical parameters in subjects with obesity. Differences corresponding to *P* < 0.05 were
743 considered significant. Statistical analysis was performed with GraphPad Prism (San Diego, CA,
744 USA).

745 **Life Sciences Reporting Summary.**

746 Further information on experimental design is available in the Life Sciences Reporting Summary.

747 **Data availability**

748 The data for the findings of this study are available from the corresponding author upon reasonable
749 request. European Nucleotide Archive: the sequencing data are deposited under accession number
750 PRJEB25438.

751

752 **References**

753

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- 788

Figure 1

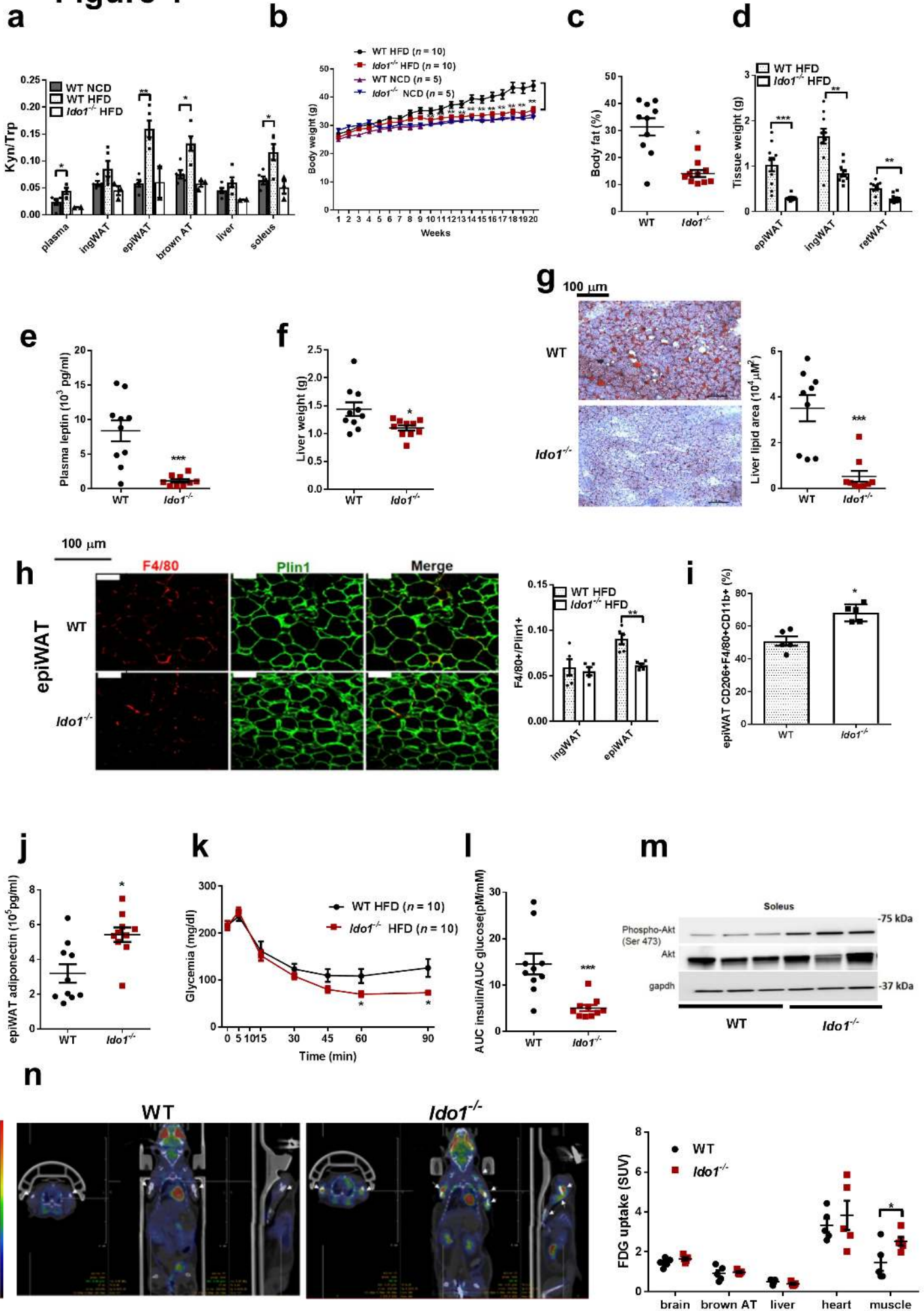


Figure 2

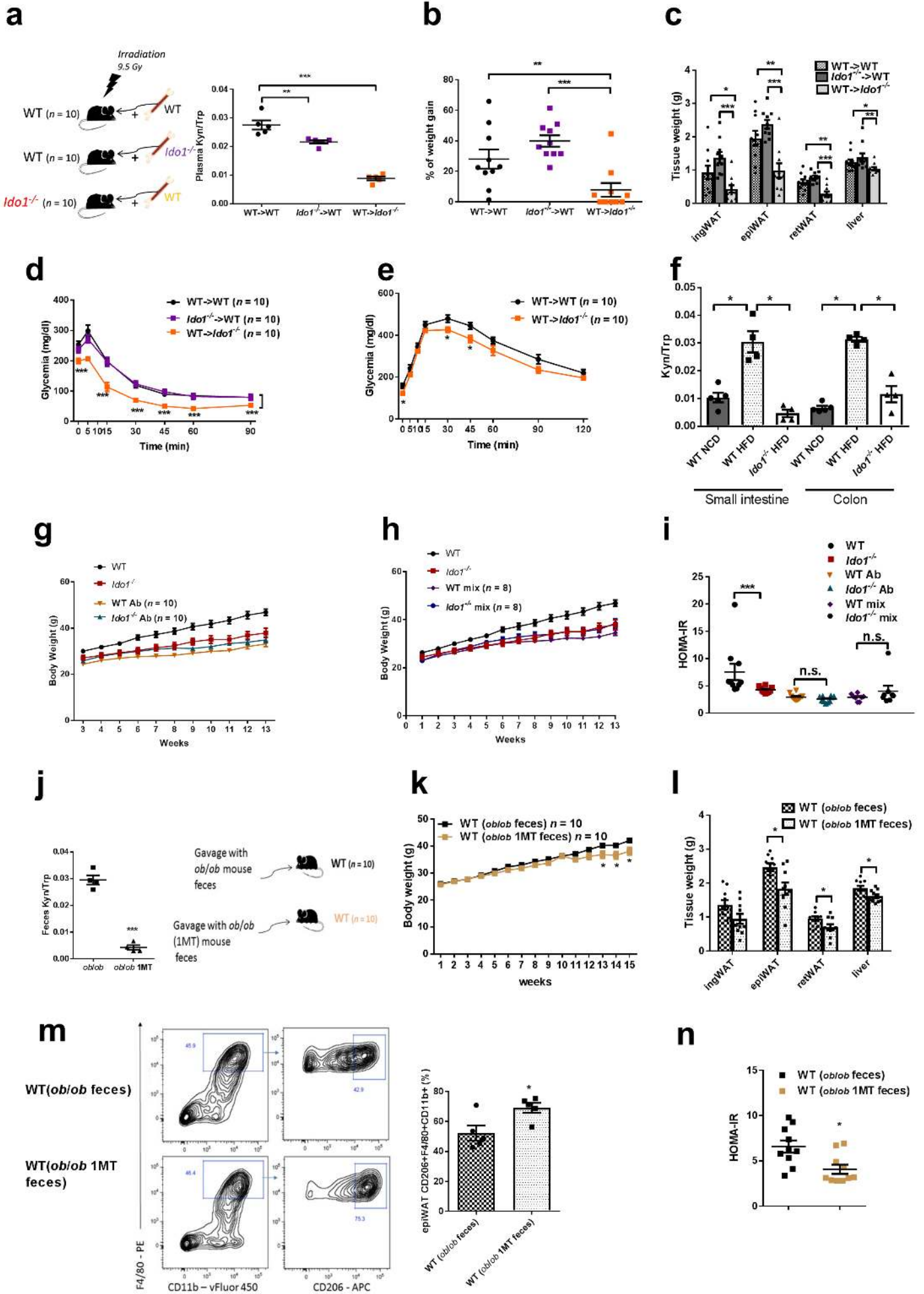


Figure 3

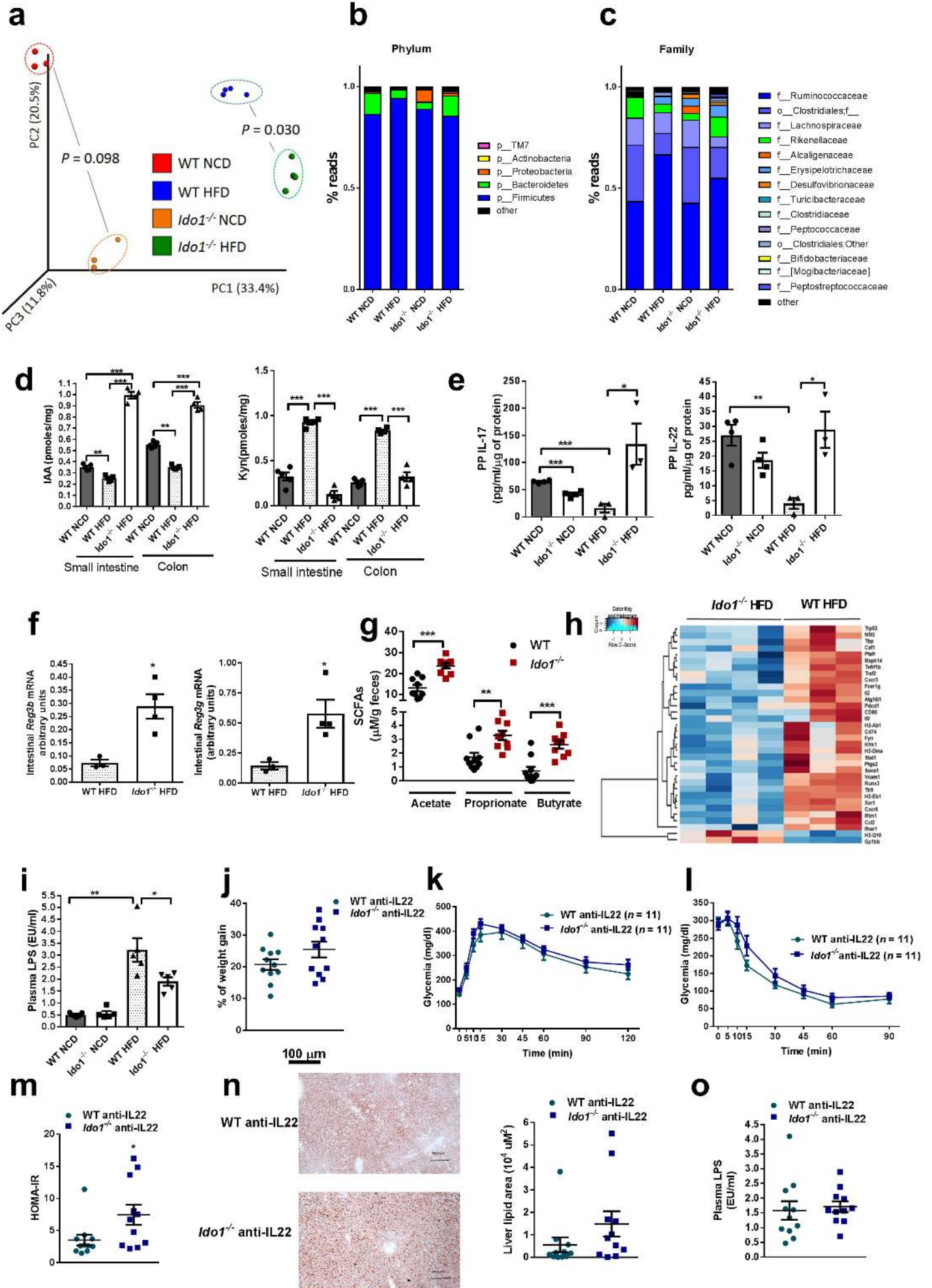
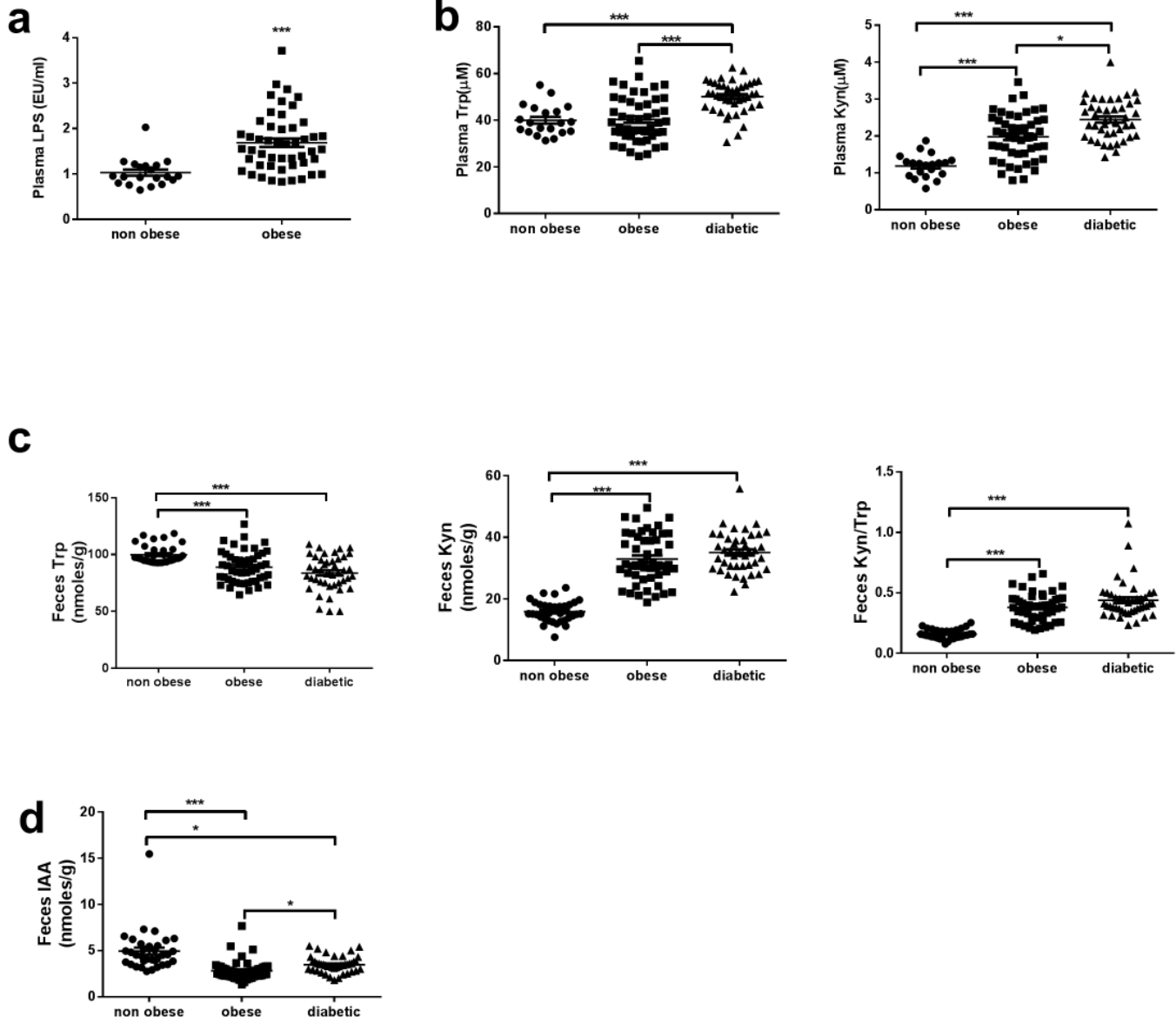


Figure 4



Supplementary Table *Clinical characteristics of the subjects*

	Obese	Diabetic	Controls 1 (feces)	Controls 2 (plasma)
Number	49	43	34	20
Sex Ratio (f/m)	39/10	24/19	20/14	15/5
Age (years)	43.29±1.53	52.28±1.38	35.08±2.26	37.35±3.36
Weight (kg)	128.60±3.98	96.70±3.64	64.79±1.74	69.05±3.20
BMI (kg/m²)	47.29±1.19	36.42±1.22	22.65±0.43	23.80±0.79
Waist (cm)	135.2±3.78	112.42±2.5		
Fat mass (%)	57.99±2.02	36.91±3.42		
AST (U/L)	24.59±1.68	41.47±6.5		
AUC^{glucose} OGTT (mmol/L/120 min)	36.46±3.43			
GDR (mg/kg/min)	3.09±0.34			
Total cholesterol (mmol/l)	187.8±4.08	203.56±5.22		
HDL-cholesterol (mmol/l)	45.75±1.58	45.77±1.70		
Triglycerides (mmol/l)	125.30±6.62	143.53±13.40		
TG/HDL-cholesterol	3.04±0.24	3.59±0.44		
LPS (EU/ml)	1.68±0.09			1.03±0.06
Plasma Trp (μM)	40.35±1.39	50.09±1.05		39.99±1.45
Plasma Kyn (μM)	1.97±0.08	2.44±0.08		1.18±0.07
Feces Trp (nmol/g)	88.83±1.98	83.74±2.32	99.98±1.34	
Feces Kyn (nmol/g)	32.97±1.16	35.09±0.98	15.88±0.60	
Feces IAA (nmol/g)	2.83±0.15	3.47±0.13	4.94±0.37	

Values are mean±sem

BMI, body mass index; AUC^{glucose} OGTT: area under the curve of glucose during an oral glucose tolerance test; GDR (glucose disposal rate) glucose infusion during euglycemic hyperinsulinemic clamp, AST aspartate aminotransferase, TG triglycerides, Trp tryptophan, Kyn kynurenine, LPS lipopolysaccharide, IAA Indole-3-acetic acid.

Supplemental Figure Legends

Supplementary Fig. 1 *Ido1* mRNA is induced by the high fat diet (HFD). (a) *Ido1* mRNA in epiWAT, ingWAT and soleus muscle in WT and *Ido1*^{-/-} mice fed with either NCD or HFD during 20 weeks (*n* = 5 per group). (b) IFN- γ levels in epiWAT and plasma of WT mice on either NCD or HFD during 20 weeks (*n* = 5 per group). Data are expressed as mean \pm sem. ***P* < 0.001, ****P* < 0.0001.

Supplementary Fig. 2 IDO deficiency has no major effects in basal conditions. (a-d) Weights of epiWAT, ingWAT, retWAT and liver (a), OGTT(b), ITT (c), and HOMA-IR (d), in WT and *Ido1*^{-/-} mice fed with NCD during 20 weeks (*n* = 5 per group). Data are expressed as mean \pm sem. **P* < 0.05.

Supplementary Fig. 3 *Ido1*^{-/-} mice fed with HFD are protected against obesity and its complications. (a-c) Lean mass measured by Echo-MRI (a), representative pictures (left) and quantification of CD68 (right) staining surface in livers of WT and *Ido1*^{-/-} mice fed with HFD during 20 weeks (*n* = 9 per group)(b), cytokine production by ingWAT explants from WT and *Ido1*^{-/-} mice fed with HFD (*n* = 5 per group) (c). Scale bars 100 μ M. (d) Plasma insulin concentrations during OGTT in WT and *Ido1*^{-/-} mice fed with either NCD (*n* = 5 per group) or HFD (*n* = 10 per group) during 20 weeks. (e) Insulin signaling (pAKT-S473) in liver, ingWAT and epiWAT after 15 min of 5 U/kg insulin injection in WT and *Ido1*^{-/-} mice fed with HFD. (f) Representative Scatter plots from the analysis of covariance showing energy expenditure (kcal/hr) plotted against body weight of HFD-fed WT (*n* = 11) and HFD-fed *Ido1*^{-/-} mice (*n* = 12) with a significant genotype effect (*P* < 0.05) and a significant body weight effect (*P* < 0.01). (g) Food intake normalized on body weight (g/kg body weight/hr; *n* = 5-6 per group) expressed as mean of 4 daylight and 4 nights. (h) Energy excretion measured by calorimetric bomb in feces of WT and *Ido1*^{-/-} mice fed with HFD (*n* = 4 per group). Data are expressed as mean \pm sem. **P* < 0.05, ***P* < 0.001, ****P* < 0.0001.

Supplementary Fig. 4 *Ido1*^{-/-} mice fed with HFD have a high muscular metabolism. (a, b) Representative pictures and quantifications of staining for respectively glucose transporter 4 (GLUT4) (a), and for apoptosis inducing factor (AIF) as a mitochondrial marker (b), on transverse sections of soleus muscle from *Ido1*^{-/-} mice (*n* = 6) and WT mice fed with HFD (*n* = 5). Nuclei were counterstained with DAPI. (c) ATP concentrations in soleus muscle of WT (*n* = 5) and *Ido1*^{-/-} mice (*n* = 3) fed with HFD. Data are expressed as mean \pm sem. **P* < 0.05, ***P* < 0.001.

Supplementary Fig. 5 IDO inhibition protects against insulin resistance in obesity. (a-e) Weight curves of HFD-fed WT mice treated or not with 1MT diluted in drinking water (2mg/ml) during 19 weeks (*n* = 10 per group) (a), plasma IDO activity (Kyn/Trp) (b), production of adiponectin by epiWAT explants (*n* = 5 per group) (c), ITT (d) and HOMA-IR (e), in HFD-fed WT mice treated or not with 1MT (*n* = 7 per group). (f, g) ITT and OGTT in ob/ob mice fed with NCD and treated or not with 1MT during 14 weeks (*n* = 10 per group). Data are expressed as mean \pm sem. **P* < 0.05, ***P* < 0.001, ****P* < 0.0001.

Supplementary Fig. 6 IDO expressed in macrophages has no effects in obesity. (a) *Ido1* mRNA in peritoneal macrophages (pMac) isolated from *Ido1*^{flox/flox} mice and *Ido1*^{flox/flox} LysM-cre mice after 20 weeks of HFD. (b) Weight curves, (c) weights of epiWAT, ingWAT and retWAT, (d, e) ITT and HOMA-IR in *Ido1*^{flox/flox} mice or *Ido1*^{flox/flox} LysM-cre mice (*n* = 5 per group) after 20 weeks of HFD. Data are expressed as mean \pm sem. ****P* < 0.0001.

Supplementary Fig. 7 IDO inhibition and invalidation effects on gut microbiota. (a) PCA plot based on bacterial 16S rDNA gene sequence abundance in fecal content of ob/ob mice treated or not with 1MT. Axes correspond to principal components 1(x-axis), 2 (y-axis) and 3 (z-axis). (b) Bacterial diversity on the basis of shannon in the fecal samples from WT and *Ido1*^{-/-} mice fed with either NCD or HFD and ob/ob mice treated or not with 1MT. (c) Bacterial taxons differentially expressed in feces from WT or *Ido1*^{-/-} mice fed with either NCD or HFD. (d) Bacterial-taxon-based analysis at the phylum level in feces of ob/ob mice treated or not with 1MT. (e) A correlation between presence of

Clostridiales Lachnospiraceae in feces and plasma LPS. (f) Bacterial taxa differentially enriched in HFD-fed WT and *Ido1*^{-/-} mice (generated using LeFSE analysis). Data are expressed as mean ± sem. ***P* < 0.001.

Supplementary Fig. 8 Kynurenine has no effects in metabolic syndrome. (a, b) Plasma Kyn (a) and Trp (b) in *Ido1*^{-/-} mice supplemented or not with Kyn diluted in drinking water (2 mg/ml) during 15 weeks of HFD (*n* = 10 per group). (c-d) weight curves (c), and weights of ingWAT, epiWAT, retWAT and liver (d). (e, f) ITT and HOMA-IR in WT, *Ido1*^{-/-} and Kyn-supplemented *Ido1*^{-/-} mice. Data of HOMA-IR are presented as box-and-whisker plots, with the midline representing the median and the whiskers representing maximum and minimum values. Data are expressed as mean ± sem. ****P* < 0.0001.

Supplementary Fig. 9 Trp and Trp-derived metabolites in gastrointestinal tract. (a) Schematic representation of the use of Trp in gastrointestinal tract. (b-c) Kyn and IAA levels in small intestines and colons of *ob/ob* mice treated or not with 1MT (*n* = 4 per group). (d) Trp levels in small intestines and colons from WT mice fed with either NCD or HFD and HFD-fed *Ido1*^{-/-} mice (*n* = 4-5 per group). (e) Trp levels in small intestines and colons of *ob/ob* mice treated or not with 1MT during 15 weeks (*n* = 4 per group). (f) IAA levels in small intestines of WT mice which received feces from either 1MT-treated or not treated *ob/ob* mice (*n* = 10 per group). (g) Quantification of AHR activity in fecal samples recovered from WT and *Ido1*^{-/-} small intestines (*n* = 5 per group) after 7 weeks of HFD. Data are expressed as mean ± sem. **P* < 0.05, ****P* < 0.0001.

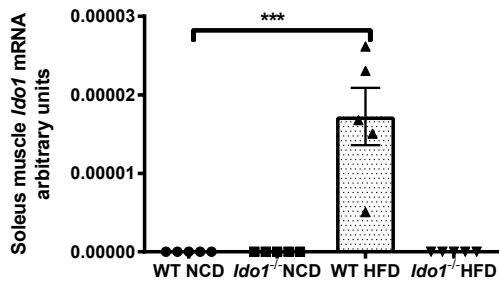
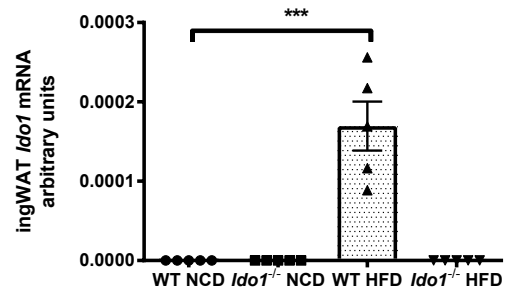
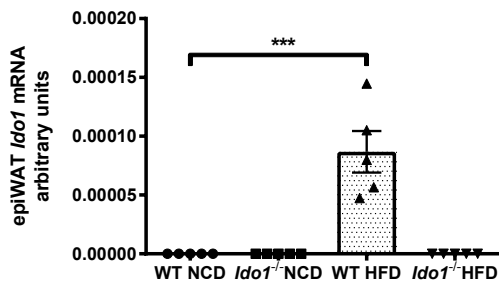
Supplementary Fig. 10 IAA decreases insulin resistance and adipose inflammation. (a-b) HOMA-IR (a), representative cytometry (left) and quantification (right) of M2-like macrophages (F4/80+CD11b+CD206+ in epiWAT) (*n* = 5 per group) (b), from WT mice treated with either the vehicle (DMSO) (*n* = 8) or FICZ (6-formylindolo[3,2-b]carbazole) (*n* = 7) during 15 weeks of HFD. (c-f) Feces IAA levels (c), intestinal IL-22 concentrations (d), ITT (e), and representative cytometry and quantification of CD45+ cells and M2-like macrophages (F4/80+CD11b+CD206+ in epiWAT) (*n* = 5 per group) (f), in WT mice supplemented or not with IAA (in drinking water) and put on HFD during 11 weeks (*n* = 9-10 per group). (g) IL-12 levels in PP of WT mice treated or not with 1MT during 7 weeks of HFD (*n* = 5 per group). (h) IL-22 levels in small intestines from WT mice which received feces from 1MT-treated or untreated *ob/ob* mice after 15 weeks of HFD. (i-j) Correlations between intestinal IL-22 levels and intestinal IAA (i), or HOMA-IR (j), in WT which received feces from 1MT-treated or untreated *ob/ob* mice. Data are expressed as mean ± sem. **P* ≤ 0.05, ***P* < 0.001, ****P* < 0.0001.

Supplementary Fig. 11 IL22 neutralisation abrogates protective effects of IDO deficiency against obesity and its complications. (a-b) Body fat % (*n* = 6 per group) (a), weights of epiWAT, ingWAT and retWAT from WT and *Ido1*^{-/-} mice fed with HFD during 12 weeks and treated with mouse neutralizing anti-IL22 antibody (b) (*n* = 11 per group). (c) Representative cytometry (left) and quantification (right) of M2-like macrophages (F4/80+CD11b+CD206+ in epiWAT) (*n* = 5 per group) from WT and *Ido1*^{-/-} mice fed with HFD during 12 weeks and treated with neutralizing anti-IL22 antibody. (d-h) % of body fat (d), weights of epiWAT, ingWAT and retWAT (e), ITT (f), OGTT (g) and plasma LPS (h) in WT (*n* = 5) and *Ido1*^{-/-} (*n* = 4) mice treated with control IgG1 three times per week during 12 weeks of HFD. (d, e and h) Data are presented as box-and-whisker plots, with the midline representing the median and the whiskers representing maximum and minimum values. Data are expressed as mean ± sem. **P* < 0.05, ***P* < 0.001

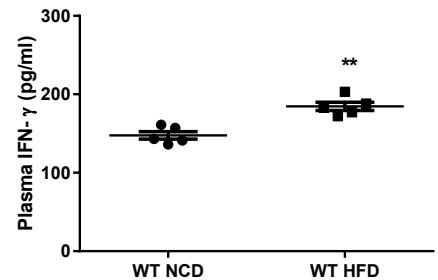
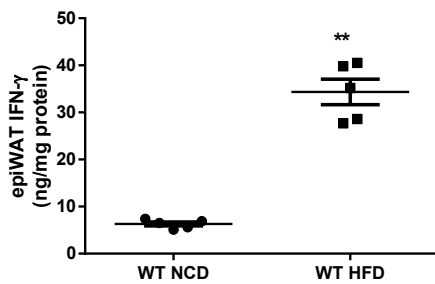
Supplementary Fig. 12 Full blot scans of P-AKT, AKT and GAPDH. The blots shown in Fig. 1m are within squares.

Supplementary Fig 1

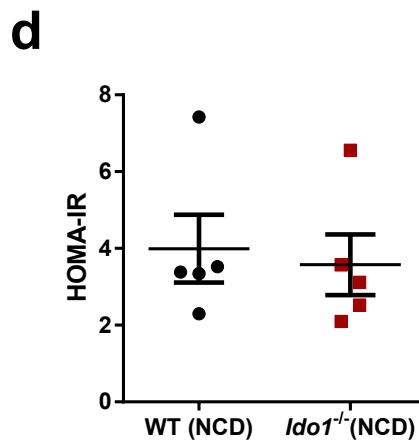
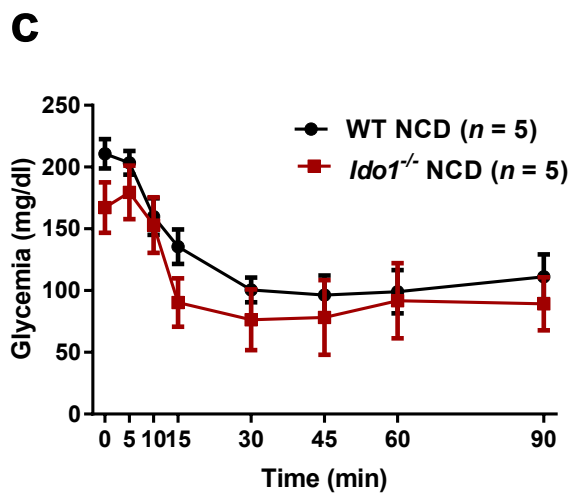
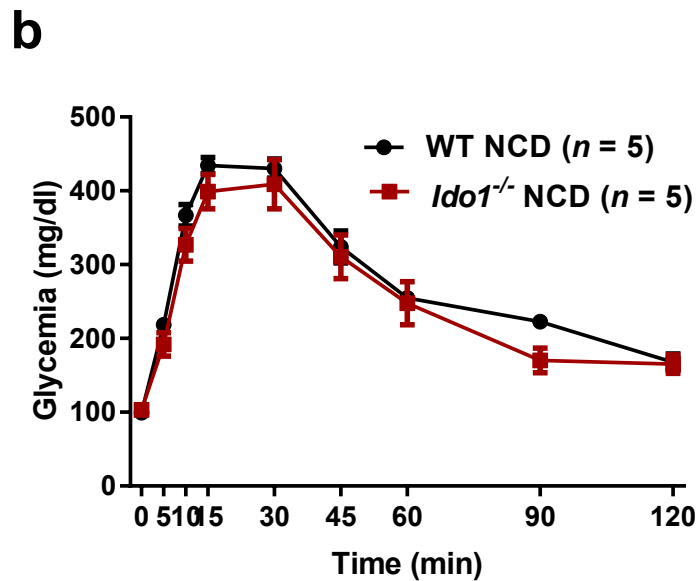
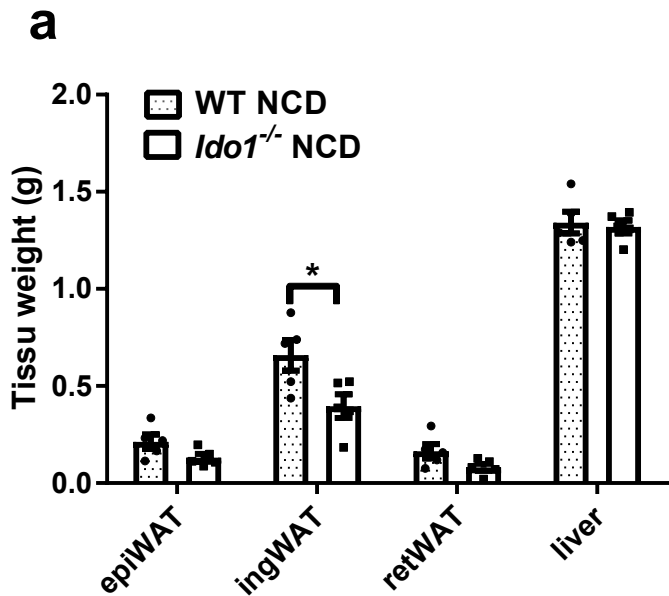
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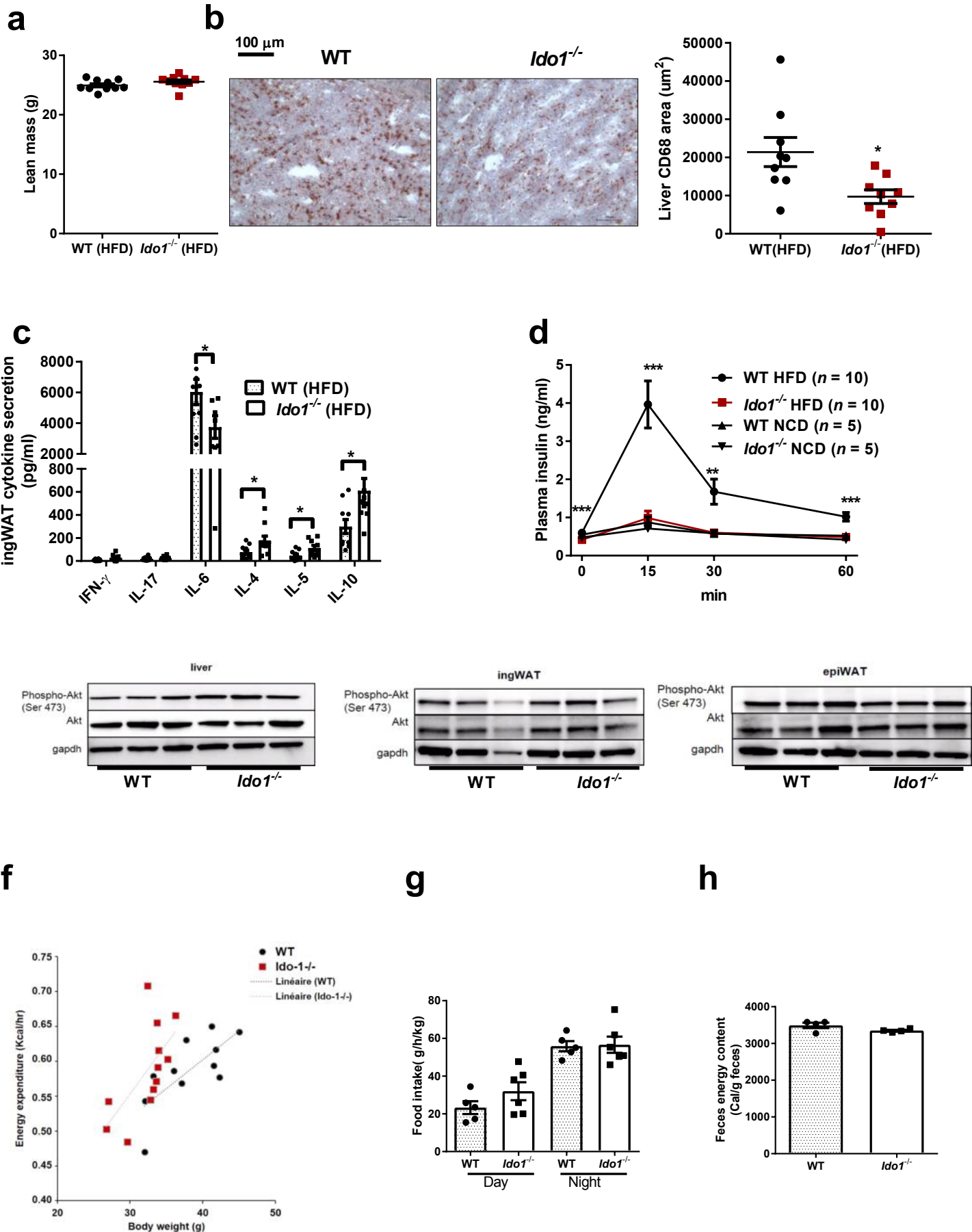
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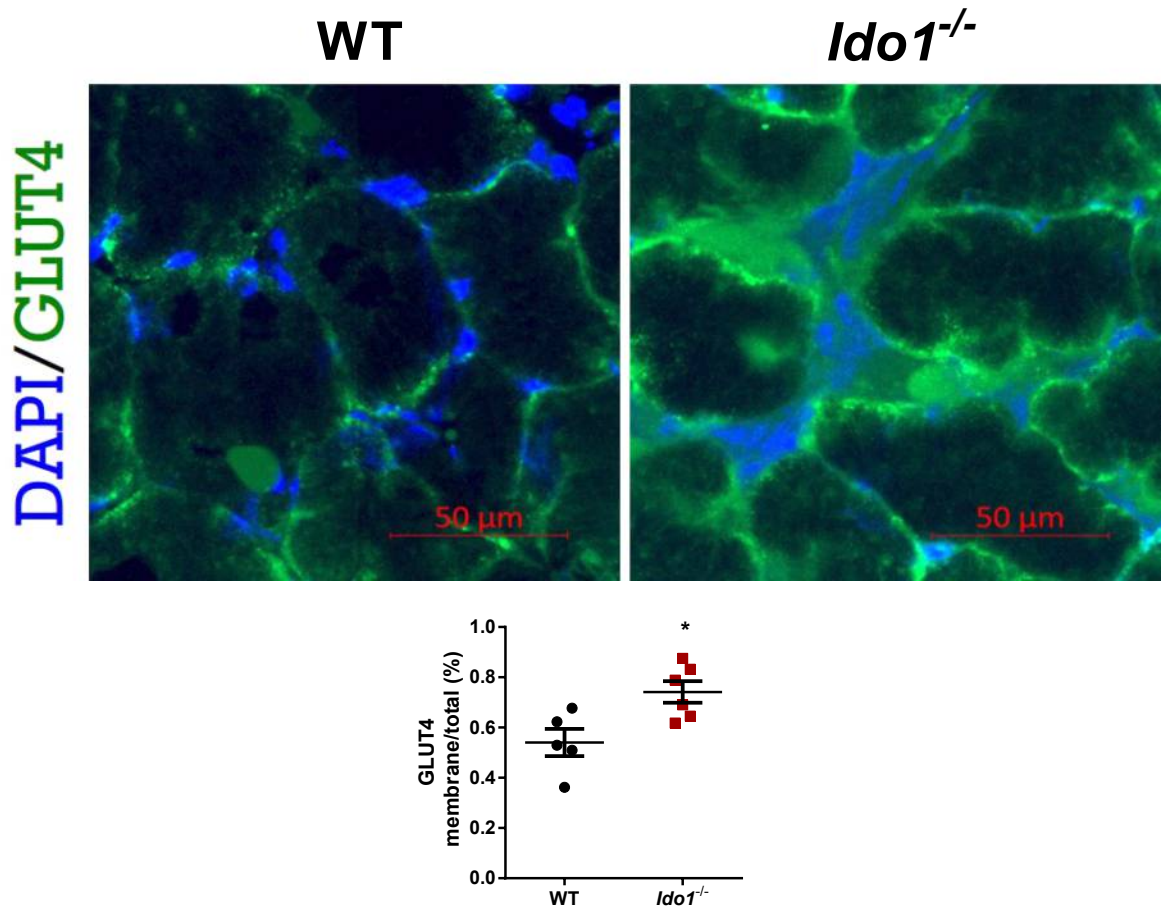
Supplementary Fig 2



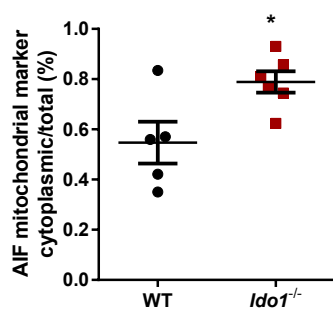
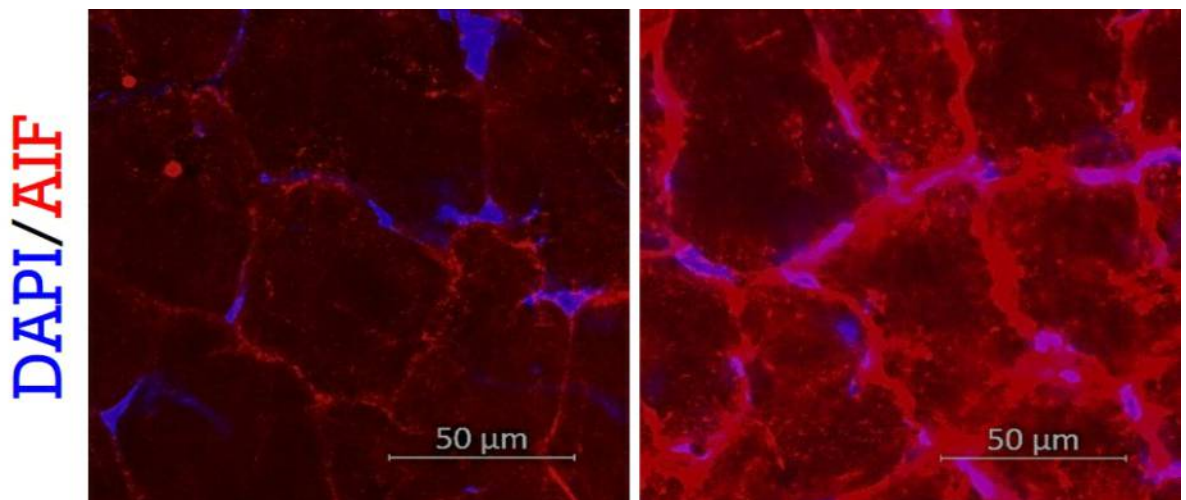
Supplementary Fig 3



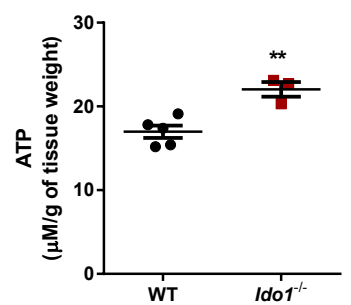
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b

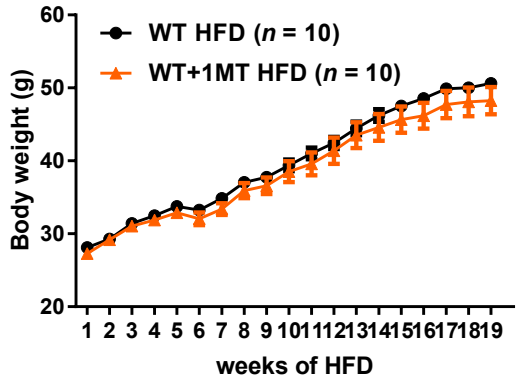


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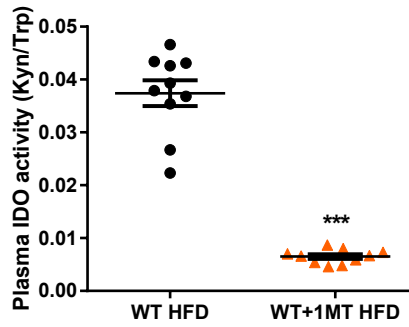


Supplementary Fig 5

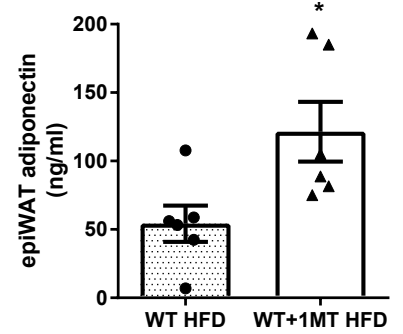
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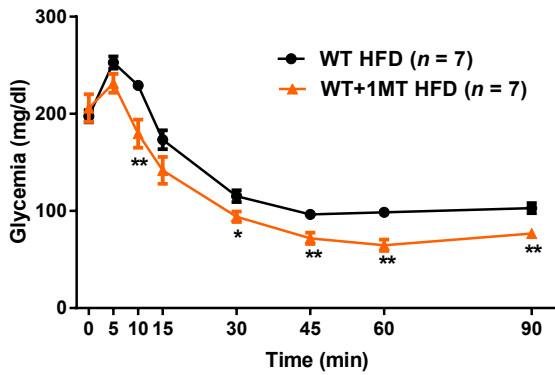
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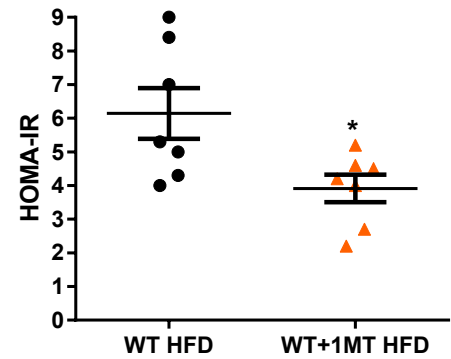
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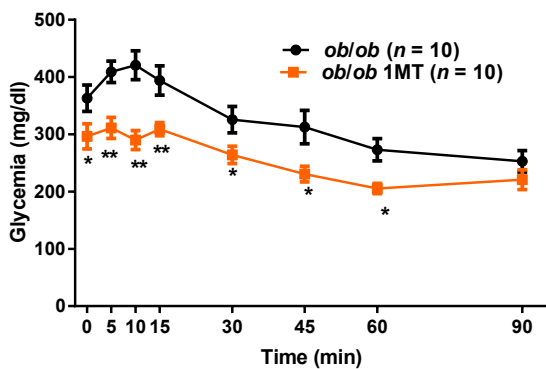
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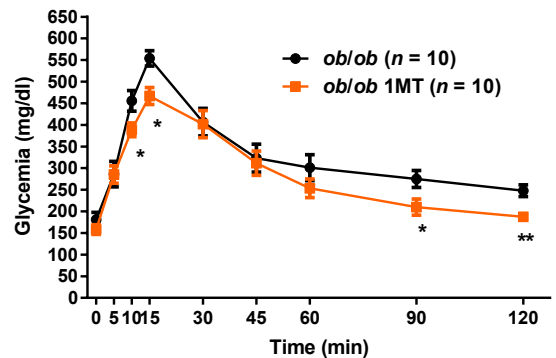
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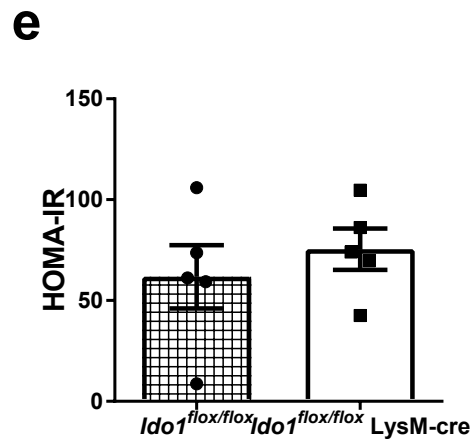
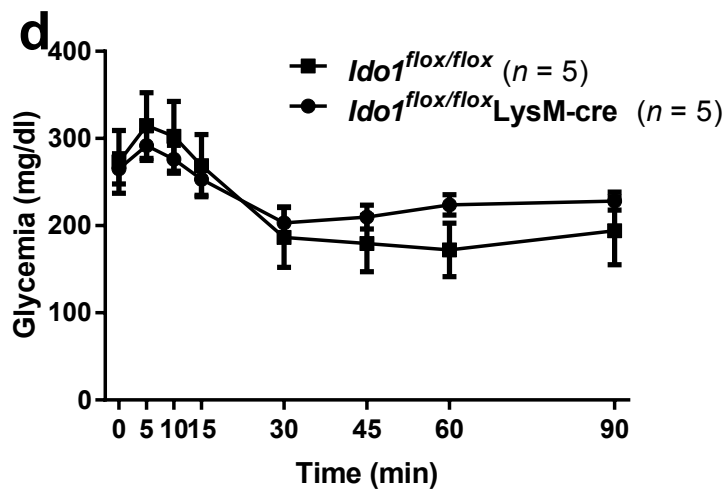
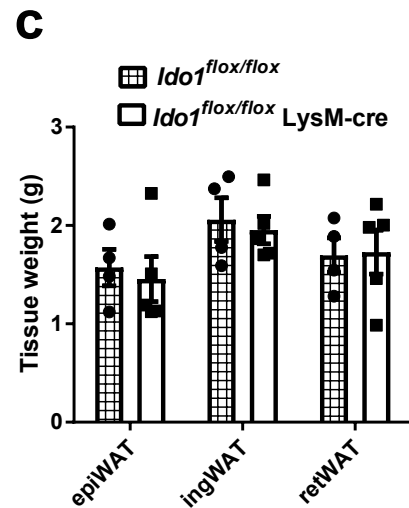
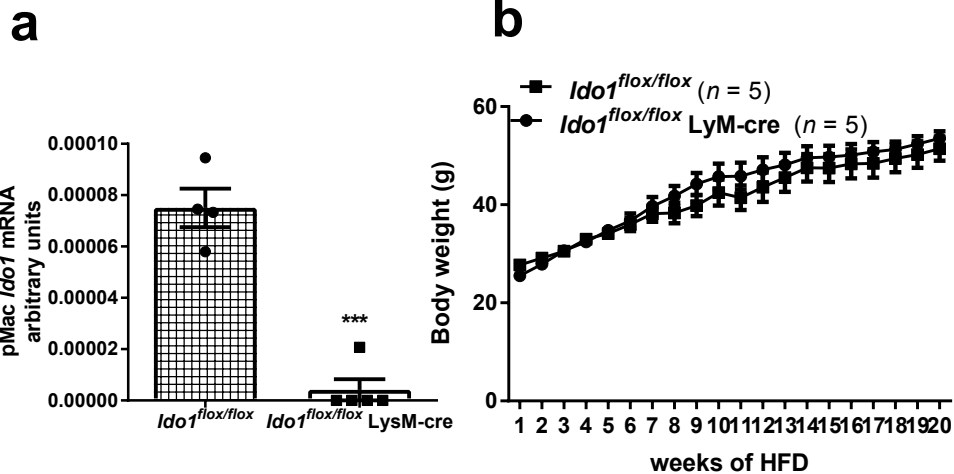
f



g

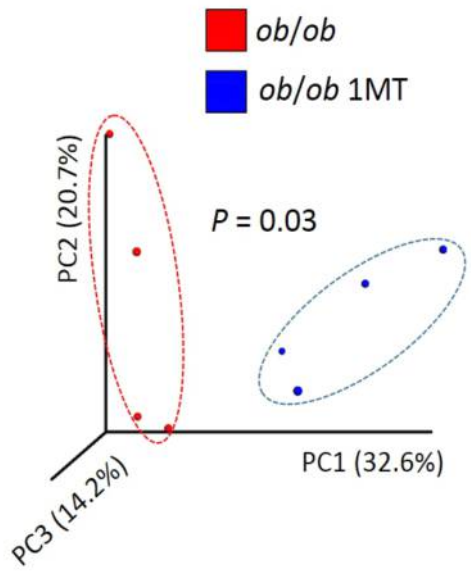


Supplementary Fig 6

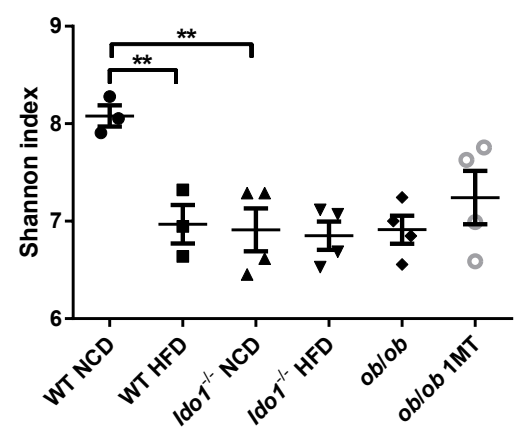


Supplementary Fig 7

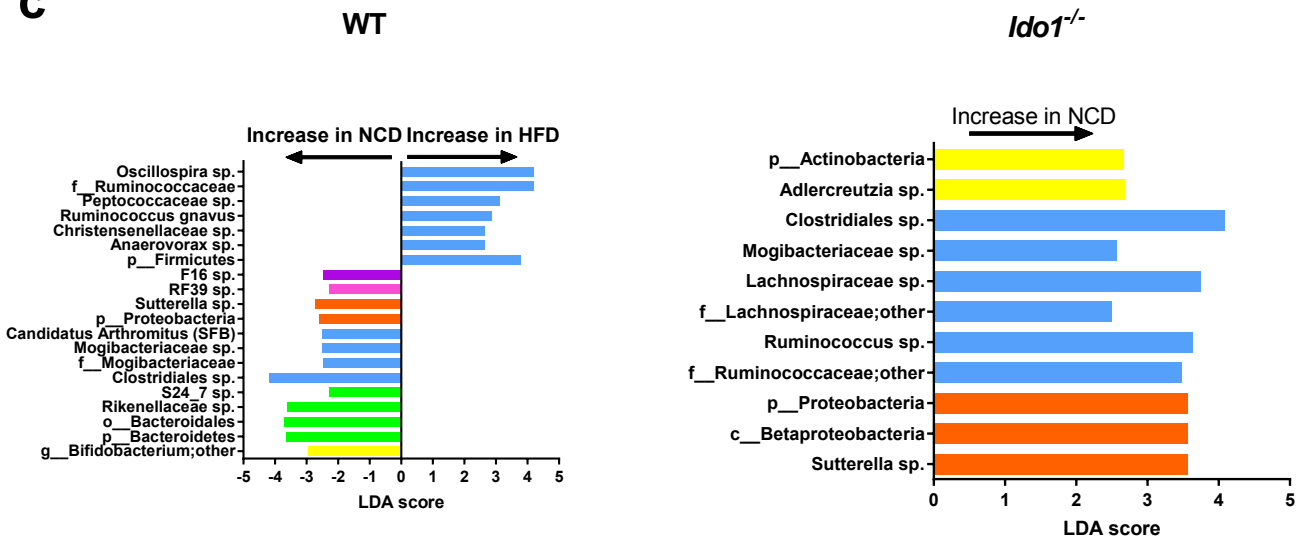
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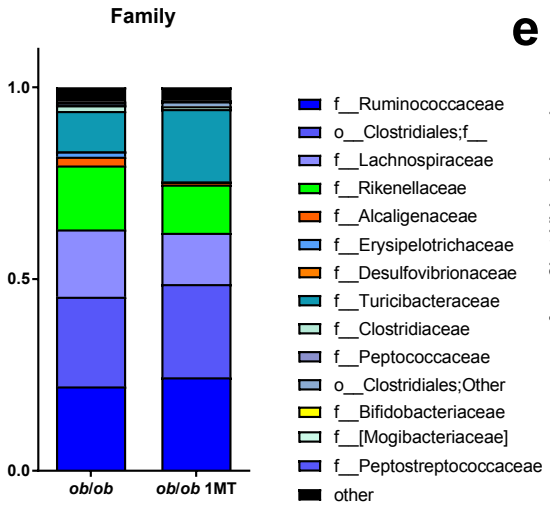
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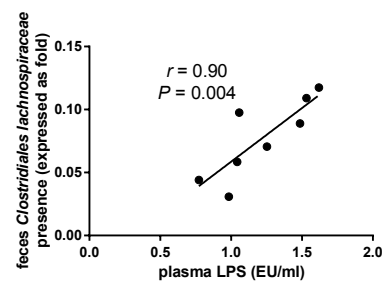
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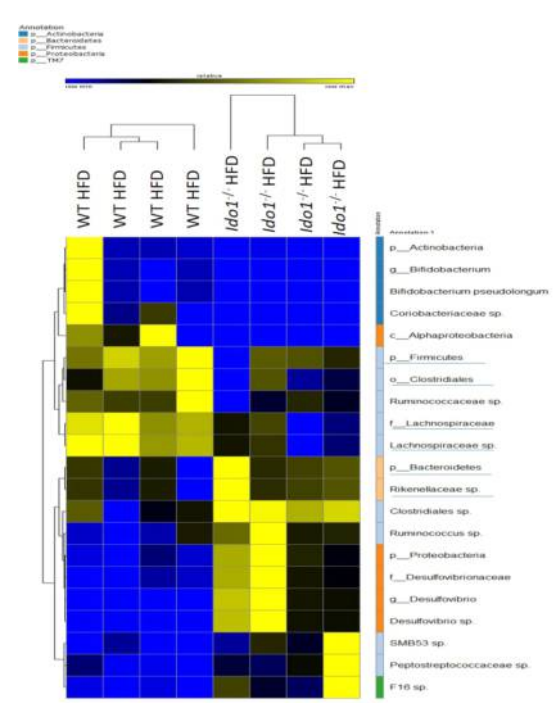
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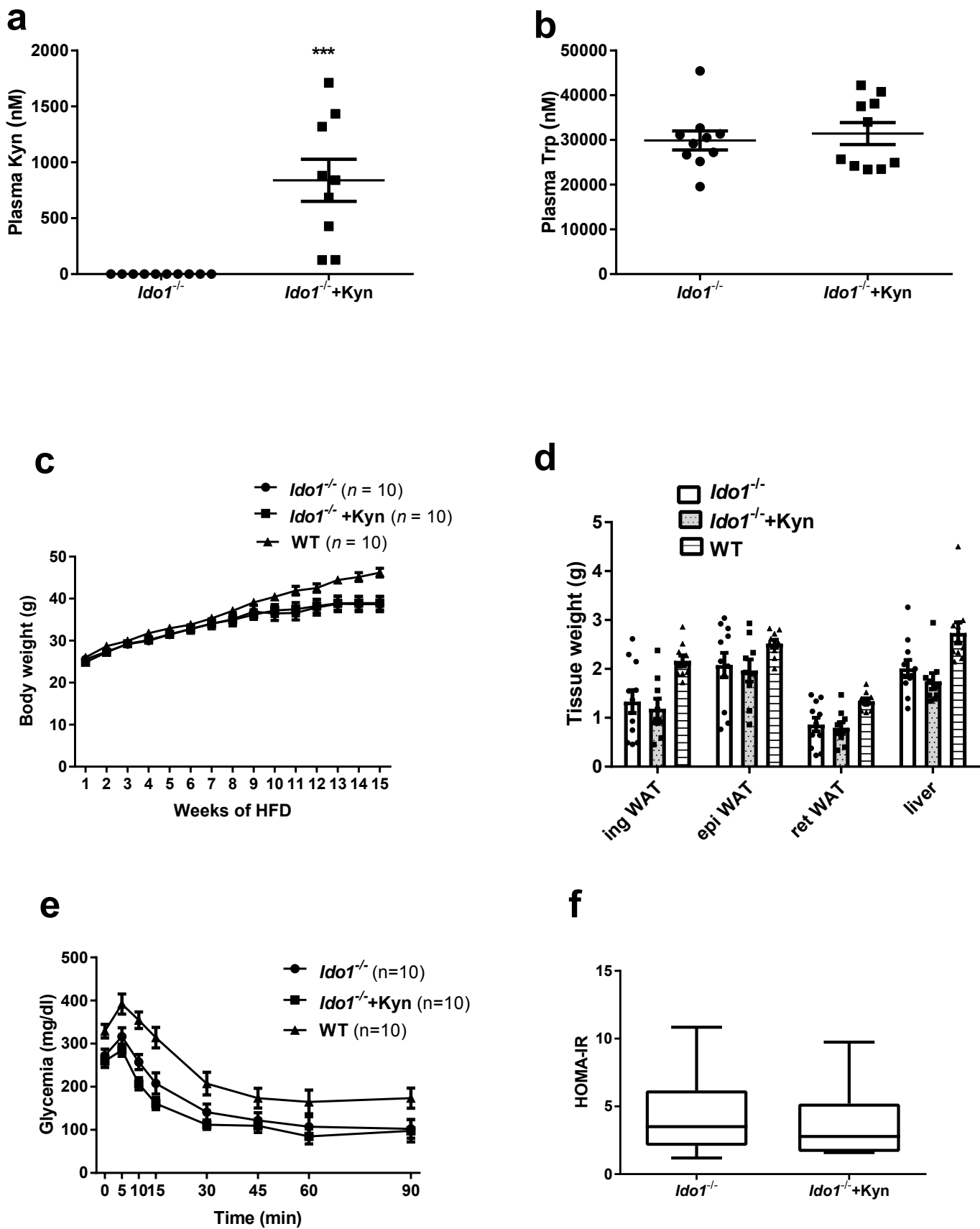
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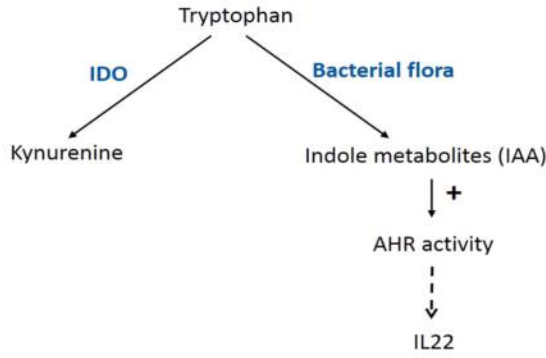
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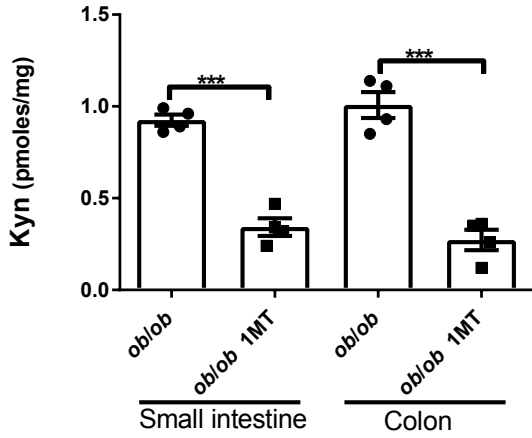
Supplementary Fig 8



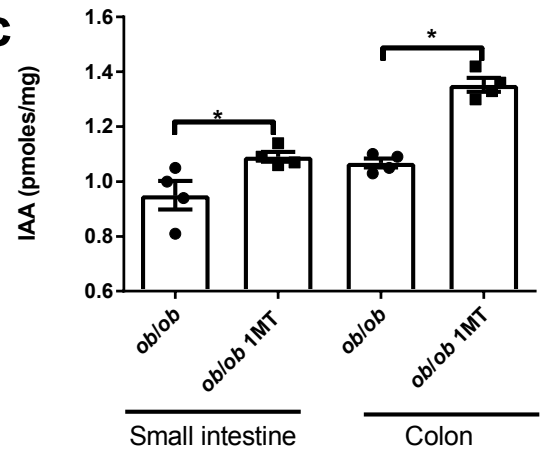
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b



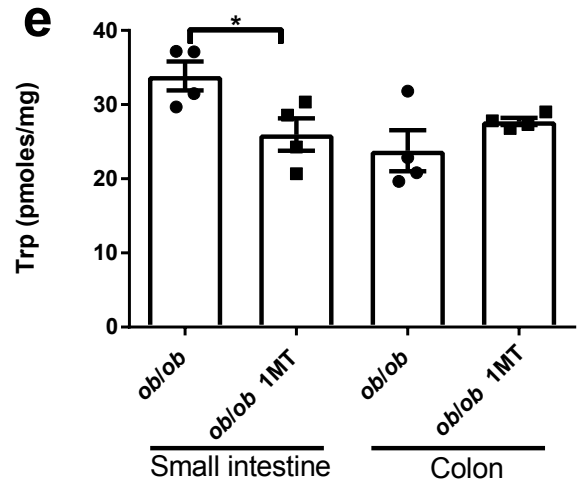
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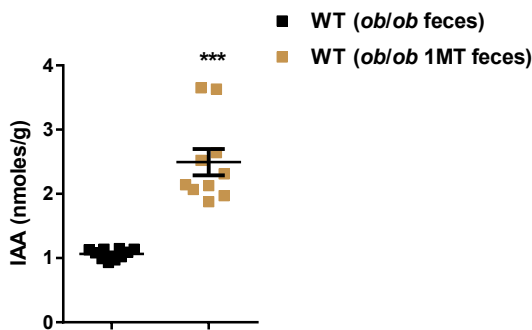
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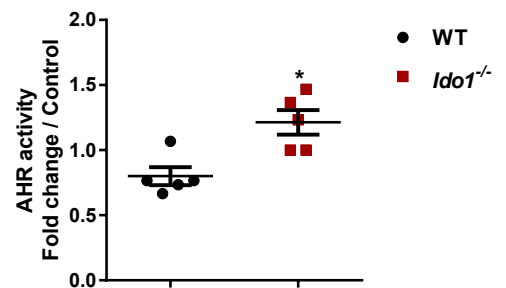
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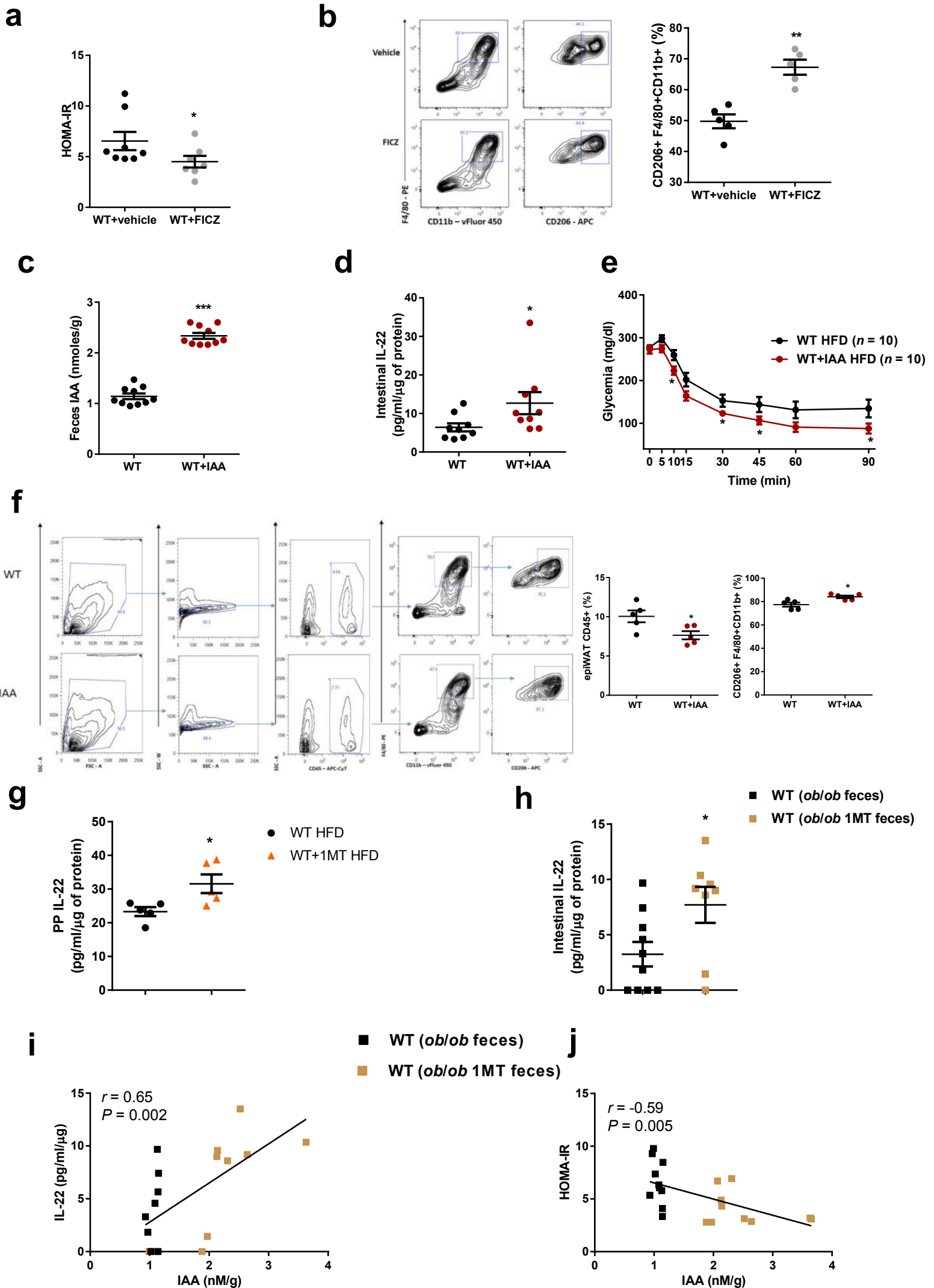
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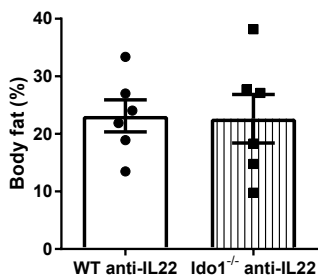


Supplementary Fig. 10

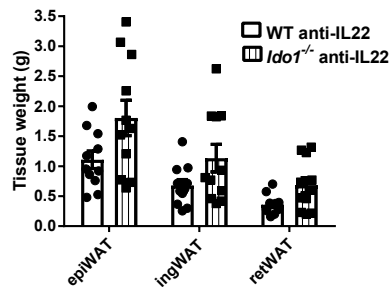


Supplementary Fig 11

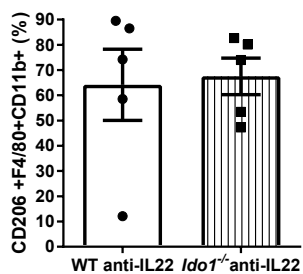
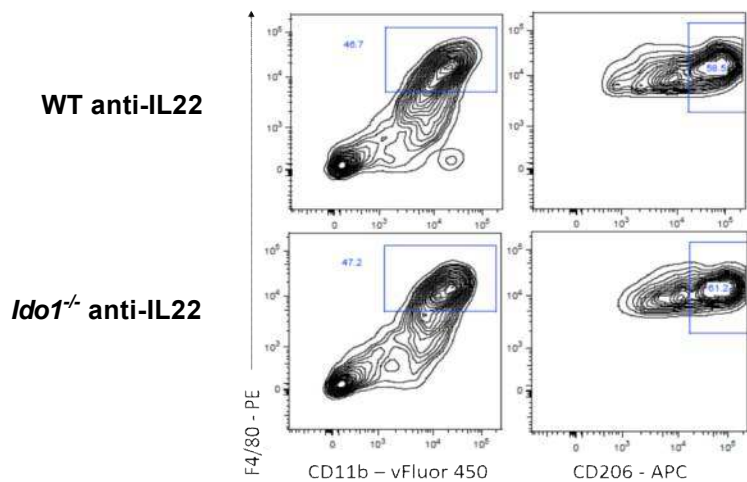
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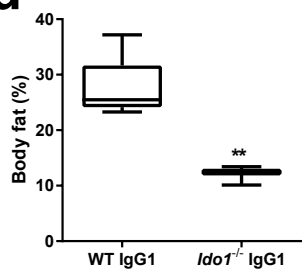
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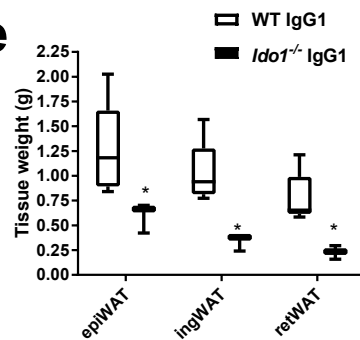
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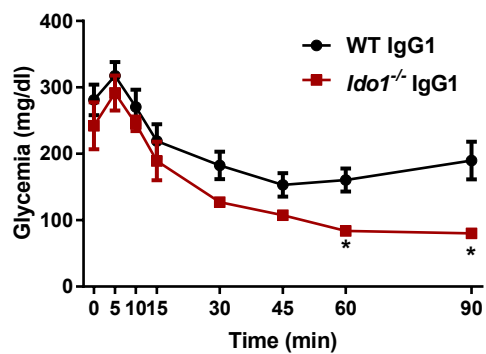
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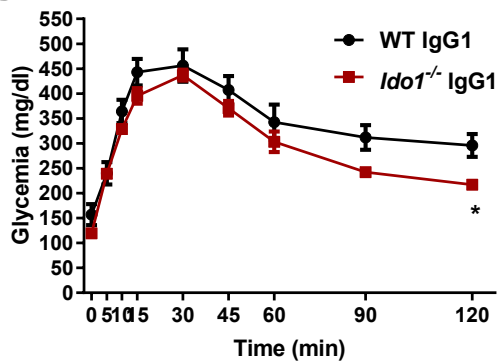
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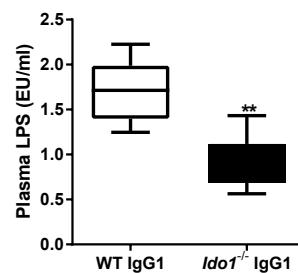
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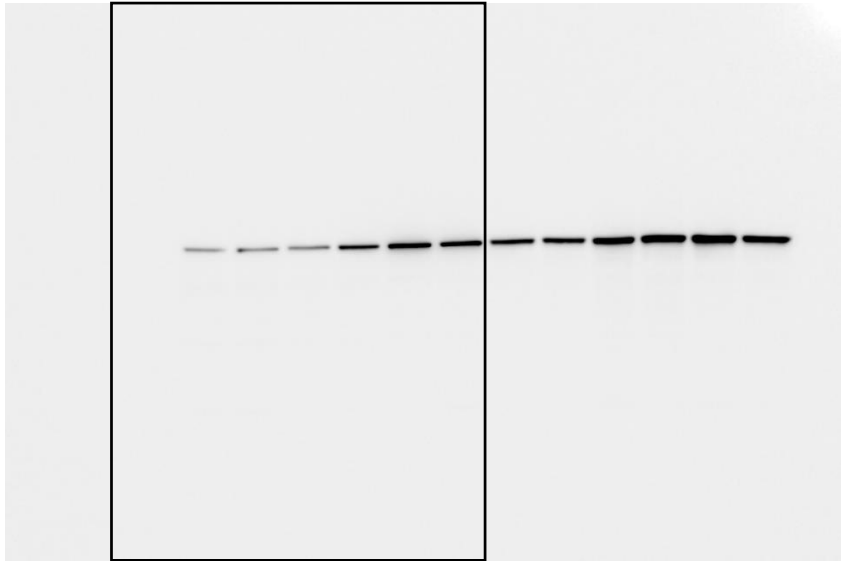


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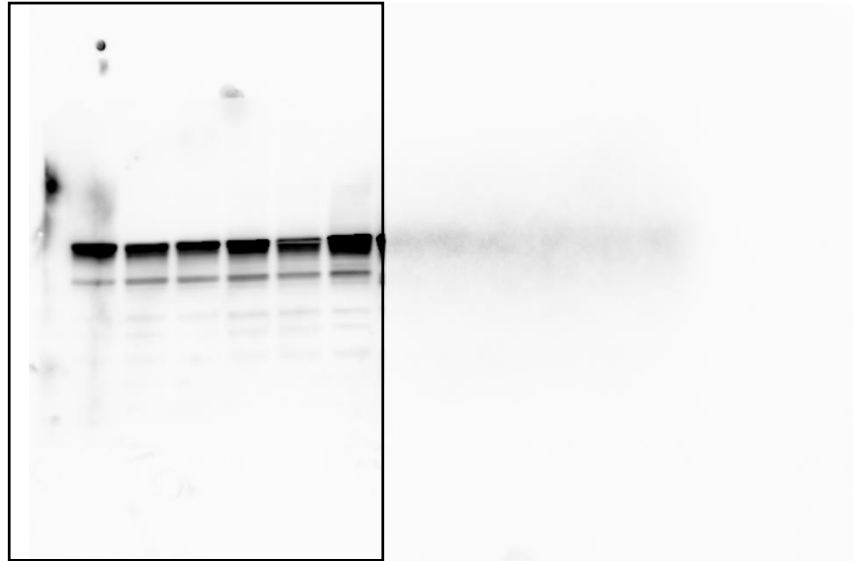


Supplementary Fig. 12

P-AKT



AKT



GAPDH

