The RNA-binding protein HuR impairs adipose tissue anabolism in pancreatic cancer cachexia

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ABSTRACT

Background: Cachexia is defined by chronic loss of fat and muscle, is a frequent complication of pancreatic ductal adenocarcinoma (PDAC), and negatively impacts patient outcomes. Nutritional supplementation cannot fully reverse tissue wasting, and the mechanisms underlying this phenotype are unclear. This work aims to define the relative contributions of catabolism and anabolism to adipose wasting in PDAC-bearing mice. Human antigen R (HuR) is an RNA-binding protein recently shown to suppress adipogenesis. We hypothesize that fat wasting results from a loss of adipose anabolism driven by increased HuR activity in adipocytes of PDAC-bearing mice.

Methods: Adult C57BL/6J mice received orthotopic PDAC cell (*Kras*^{G12D}*; p53*$^{R172H/+}$*; Pdx1-cre*) (PDAC) or PBS (sham) injections. Mice exhibiting moderate cachexia (9 days after injection) were fasted for 24h, or fasted 24h and refed 24h before euthanasia. A separate cohort of PDAC mice were treated with an established HuR inhibitor (KH-3, 100 mg/kg) and subjected to the fast/refeed paradigm. We analyzed body mass, gross fat pad mass, and adipose tissue mRNA expression. We quantified lipolytic rate as the normalized quantity of glycerol released from 3T3-L1 adipocytes *in vitro*, and gonadal fat pads (gWAT) *ex vivo*.

Results: 3T3-L1 adipocytes treated with PDAC cell conditioned media (CM) liberated less triglyceride into the culture media than control-treated adipocytes (-28.1%) and had lower expression of lipolysis and lipogenesis genes than control cells. PDAC gWAT cultured *ex vivo* displayed decreased lipolysis compared to sham gWAT (-54.7%). PDAC and sham mice lost equivalent fat mass after a 24h fast, however, PDAC mice could not restore inguinal fat pads (iWAT) (-40.5%) or gWAT (-31.8%) mass after refeeding. RNAseq revealed 572 differentially expressed genes in gWAT from PDAC compared to sham mice. Downregulated genes

(n=126) were associated with adipogenesis (adj p=0.05), and expression of adipogenesis master regulators *Pparg* and *Cebpa* were reduced in gWAT from PDAC mice. Immunohistochemistry revealed increased HuR staining in gWAT (+74.9%) and iWAT (+41.2%) from PDAC mice. Inhibiting HuR binding restored lipogenesis in refed animals with a concomitant increase in iWAT mass (+131.7%) and genes regulating adipogenesis (*Pparγ*, *Cebpa*, *Retn*, *Adipoq*, *Fasn*).

Conclusions: Our work highlights deficient adipose anabolism as a driver of lipid-droplet loss in 3T3-L1 adipocytes treated with PDAC conditioned media and PDAC mice. The small molecule KH-3, which disrupts HuR binding, restored adipose anabolism in PDAC mice. This highlights HuR as a potentially targetable regulatory node for adipose anabolism in cancer cachexia.

Key words: pancreatic ductal adenocarcinoma, cachexia, adipose, adipogenesis, HuR

INTRODUCTION

Cancer-associated cachexia is a wasting condition characterized by systemic inflammation, progressive weight loss, and atrophy of white adipose tissue (WAT) and skeletal muscle [1, S1-2]. In addition to physical deterioration, individuals with cachexia also exhibit fatigue, anorexia, and cognitive decline [1, S1-3], which contribute significantly to reductions in quality of life, ability to tolerate chemotherapy or surgery, and patient mortality [2, 3, S4-6]. Cachexia is estimated to be the direct cause of death in 20-30% of cancer patients [2, S4], and among all malignancies, pancreatic ductal adenocarcinoma (PDAC) is the most highly associated with cachexia, with an estimated 83% of patients suffering from this condition [4, S7-8]. Despite much of cachexia research focusing on improving skeletal muscle mass, a retrospective study of patients with PDAC revealed that fat loss alone is associated with equally poor outcomes as combined muscle and fat mass loss [5]. Additionally, current cachexia clinical trials center around weight maintenance or gain as a primary endpoint. This highlights the importance of understanding the drivers of adipose tissue loss in cachexia.

Cachexia arises when energy catabolism exceeds anabolism, leading to unsustainable levels of fat mobilization and muscle depletion. Multiple factors are known to enhance catabolism, including decreased secretion of anabolic hormones, and altered metabolism of protein, carbohydrate, and lipid substrates [6]. Current work suggests that inflammation drives metabolic abnormalities in cachexia [7, S7]. Pro-inflammatory cytokine activity increases during cancer progression [8, 9] and systemic inflammation can contribute to wasting by inducing hypercatabolism in muscle and adipose tissue [6, 10-12]. Existing literature highlights elevated rates of lipolysis and adipose browning as the primary forces underlying adipose tissue wasting [13, 14]. Browning, in particular, appears to exert a double effect in cachexia by

82 both reducing lipid stores and increasing energy expenditure [14]. However, impaired anabolic
83 processes, like adipogenesis and lipogenesis, also contribute to adipose tissue loss [15].
84 Targeting peroxisome proliferator-activated receptor gamma (PPARG), a transcriptional control
85 point of adipogenesis, with the agonist rosiglitazone was sufficient to improve fat and muscle
86 mass retention in mice with lung cancer [16]. Tumor-derived factors are also capable of
87 impairing adipogenesis in cultured 3T3L-1 adipocytes [17, 18].
88 RNA-binding proteins (RBPs) are essential in governing biogenesis, stabilization,
89 translation, and decay of mRNA transcripts [19, 20]. Several RBPs regulate alternative splicing
90 in adipocytes [21-23] and white adipose tissue browning [24] by inhibiting the translation
91 efficiency of mRNAs such as UCP1 [25]. Until recently, the function of most RBPs in
92 adipocytes was largely unexplored. The RBP human antigen R (HuR), encoded by the
93 embryonic lethal abnormal vision-like 1 (*ELAVL1*) gene, regulates the expression of genes
94 involved in inflammation, stress response, and apoptosis [26]. Although HuR likely exerts
95 complex effects in the context of both PDAC and cachexia, we selected HuR as a target of
96 interest because recent work showed that it is a repressor of adipogenesis in both white and
97 brown adipose tissues [27]. Our goal was to elucidate the relative contributions of enhanced
98 catabolism and impaired anabolism on fat wasting by investigating adipose tissue response to
99 different nutritional contexts and HuR inhibition in cachectic mice.

100 **METHODS**

101 *Cell culture*

102 *KPC PDAC cells*

103 KPC cells expressing pancreas-specific conditional alleles (*Kras*^{G12D}*; p53*$^{R172H/+}$*; Pdx1-*
104 *cre*) [28] were stored in liquid nitrogen until use and then maintained in RMPI 1640

105 supplemented with 10% FBS, 1mM sodium pyruvate, and 50 U/mL penicillin/streptomycin
106 (Gibco, Gaithersburg, MD) at 37C and 5% CO_2. Conditioned media (CM) was collected from
107 confluent KPC cells grown in DMEM, 1% pen/strep (to accommodate later culturing of 3T3-L1
108 cells) after 24 hours incubation, centrifuged at 1,200 g for 10 min, filtered with a 0.2-μm syringe
109 filter, and used immediately or stored at −80°C.

110 *3T3-L1 adipocytes*

111 3T3-L1 adipocytes (ATCC CL-173) were purchased from ATCC and stored in liquid
112 nitrogen until use. Cells were cultured in preadipocyte expansion media (DMEM, 10% bovine
113 calf serum, 1% pen/strep). Then, cells were plated for differentiation at a density of $2x10^5$
114 cells/6-well dish, or 6.7 $x10^3$ cells/96well dish and maintained in preadipocyte expansion media
115 for 2 days after reaching 100% confluency. Media was changed to adipocyte differentiation
116 media (DMEM, 10% FBS, 1% pen/strep, 1 uM dexamethasone, 0.5 mM IBMX, 1 ug/mL bovine
117 insulin) for 2 days before switching to adipocyte maintenance media (DMEM, 10% FBS, 1%
118 pen/strep, 1 ug/mL bovine insulin) for the duration of the experiment. Cells were cultured for up
119 to 6 days after start of maintenance media to reach complete differentiation. For Oil Red O
120 staining, CM was added in place of adipocyte maintenance media and was supplemented with
121 FBS and insulin for 6 days. For lipolysis assay (media glycerol, qPCR, and western blot), cells
122 were completely differentiated and then changed to control media, KPC conditioned media
123 without insulin or FBS. Isoproterenol (10 uM) was added to media as a positive control.
124 Lipolysis end points were collected at 1, 3, and 24 hours after media change.

125 *Oil red O staining*

126 Diluted Oil Red O stock solution was prepared by mixing concentrated Oil Red O (0.5%
127 Oil Red O in 100% isopropyl alcohol) 6:4 with deionized water. This solution was allowed to

128 stand for 10 minutes at 4C and then filtered immediately before use through a 0.22 um filter.
129 3T3-L1 cells were washed with PBS, then incubated in 4% paraformaldehyde for 30 minutes at
130 room temperature, then washed with deionized water twice. Cells were incubated in 60%
131 isopropyl alcohol for 5 minutes, then treated with diluted Oil Red O solution for 15 minutes.
132 Cells were washed 5 times with deionized water, then red signal was quantified at 490 nm in a
133 plate reader (BioTek).

134 *Glycerol quantification*

135 3T3-L1 adipocyte media was collected and run at a 1:2 dilution, and mouse plasma
136 samples were run undiluted for glycerol quantification according to the manufacturer's
137 instructions (Abcam ab65337). Fresh media and KPC CM alone (no 3T3-L1 exposure) were
138 below the detection limit of the assay.

139 *NEFA quantification*

140 Mouse plasma was run undiluted according to the manufacturer's instructions (Wako
141 Diagnostics NEFA-HR series).

142 *Mouse studies*

143 Wildtype C57BL/6J mice (JAX catalog number 000664) were purchased from The
144 Jackson Laboratory (Bar Harbor, ME) and maintained in standard rodent housing at 26°C with
145 12h light/12h dark cycles. Sex is defined in individual figure legends. Animals used for
146 experimentation were 12-15 weeks of age. Mice were individually housed for acclimation for 7
147 days prior to tumor implantation and provided *ad libitum* access to water and food (Rodent diet
148 5001; Purina Mills, St. Louis, MO, USA). Food intake was measured daily. Tumor-bearing
149 animals were euthanized by cardiac puncture under deep isoflurane anesthesia. Studies were
150 conducted in accordance with the U.S. National Institutes of Health Guide for the Care and

151 Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee
152 of Oregon Health & Science University.

153 *Study-specific manipulations*

154 For fasting studies, mice were transferred to clean cages without food for 24 hours prior
155 to euthanasia or refeeding. Pair-feeding was used on the study days indicated in the figure
156 legends by feeding sham mice the average of the food consumed by PDAC mice the day prior.
157 PDAC mice treated with the HuR inhibitor KH-3, were injected intraperitoneally (100 mg/kg) at
158 6-, 8-, and 10-days post-implantation.

159 *Orthotopic PDAC implantation*

160 Wildtype C57BL/6J mice aged 12-15 weeks received orthotopic PDAC tumor injections
161 (*Kras*^{G12D}*; p53*$^{R172H/+}$*; Pdx1-cre*) or sham injections. PDAC mice were injected with $1x10^6$ KPC
162 cells into the tail of the pancreas parenchyma in a volume of 23ul while sham animals were
163 treated with an equal volume of PBS. Animals were euthanized 10-11 days after tumor
164 implantation. N and sex are defined on a per-study basis in the figure legends.

165 *Echo magnetic resonance imaging body composition*

166 Lean mass, fat mass, total body water, and free water were measured using whole-
167 body magnetic resonance imaging (MRI) (EchoMRI, Houston, TX). Measurements were taken
168 pre-implantation (baseline), pre-fasting and at euthanasia in tumor and sham groups to assess
169 body composition.

170 *Tissue collection and histology*

171 Tissues collected at necropsy were weighed and flash frozen in liquid nitrogen prior to
172 storage at -80C. Tissues for HuR histology were fixed with 4% paraformaldehyde overnight
173 and then transferred to 70% ethanol. Tissues were paraffin-embedded, sectioned, incubated

174 with anti-HuR (1:300 #sc-5261, Santa Cruz) and stained using horseradish peroxidase-
175 conjugated secondary antibody and incubation in 3,3'-diaminobenzidine by the Histopathology
176 Shared Resource Core at OHSU. Whole tissue sections were scanned by the Advanced Light
177 Microscopy Core at OHSU. ZEN Digital Imaging for Light Microscopy (RRID:SCR_013672)
178 was then used to obtain at least five 10x images per tissue. ImageJ software was used to
179 quantify the percent staining for each section (56). All staining was quantified by running color
180 deconvolution on 10x images, applying a standard intensity threshold on the corresponding
181 images, and measuring the percent area covered by the staining.

182 *Ex vivo lipolysis*

183 *Ex vivo* lipolysis assays of adipose explants were performed as previously described
184 [29]. Briefly, gonadal white adipose tissue (gWAT) tissue was collected and cut into
185 approximately 100 mg samples, minced, then incubated in phenol red-free DMEM containing
186 2% fatty acid free bovine serum albumin at 37°C for 1 hour. Media was collected and snap-
187 frozen. The tissue was transferred to a new dish containing media only or media with 10 uM
188 isoproterenol and incubated for 2 hours at 37C. Media was collected and snap-frozen. Media
189 aliquots were thawed and analyzed for glycerol using Sigma Glycerol Assay Kit (MAK117)
190 according to manufacturer's directions.

191 *Quantitative qRT-PCR*

192 Total RNA was extracted from cell pellets or tissues with the E.Z.N.A. Total RNA Kit II
193 (Omega Bio-Tek Inc., Norcross, GA). cDNA was transcribed with the High-Capacity cDNA
194 Reverse Transcription Kit (Applied Biosystems, Waltham, MA). Quantitative real-time
195 polymerase chain reaction (qPCR) was run on the ABI 7300 (Applied Biosystems) using
196 TaqMan Fast Advanced PCR Master Mix (Applied Biosystems) or SYBR Green Master Mix

197 (Applied Biosystems). The relative expression was calculated using the $\Delta\Delta C_t$ method with
198 gene expression relative to beta actin or 18S.
199 *RNA sequencing*
200 RNA sequencing was performed on total RNA isolated as described above. RNA
201 libraries were prepared and sequenced using the Illumina Nova Seq and HiSeq platform
202 according to the Illumina Tru-Seq protocol (Novogene, Sacramento, CA). Detailed analysis
203 methods are included in the supplement.
204 *Western Blot*
205 3T3-L1 protein was collected by scraping cells into lysis buffer followed by brief
206 sonication. Adipose tissue protein was collected using the Minute Total Protein Extraction Kit
207 (Invent Biotechnologies Inc AT-022) and the manufacturer's protocol. We used BCA assay to
208 determine protein concentration. Approximately twenty micrograms of protein was loaded in
209 each lane and run on 4–12% Bis-Tris NuPAGE gel (Invitrogen). Gels were transferred to
210 polyvinylidene difluoride (PVDF) membranes (Millipore) and blocked with 5% BSA for 1 h.
211 Membranes were incubated with primary antibodies overnight at 4°C with gentle agitation.
212 Blots were then washed with Tris-buffered saline with Tween 20 (TBST) and incubated in
213 secondary antibodies for 1 h prior to imaging (LI-COR Odyssey Imaging System). Ladders
214 used were iBright Pre-stained Protein Ladder (Invitrogen)(HuR blot), and Chameleon 700 Pre-
215 stained Protein Ladder (LI-COR)(all other blots). Primary antibodies: Vinculin (Santa Cruz sc-
216 73614), HuR (Santa Cruz sc-5261), ATGL (Cell Signaling 2138), HSL (Cell Signaling 4107),
217 Phospho-HSL (Ser660) (Cell Signaling 45804), and Phospho-HSL (Ser563) (Cell Signaling
218 4139). Secondary antibodies: Mouse IgG and Rabbit IgG Dylight 680 and 800 (Cell Signaling),

219 goat anti-mouse IgG Alexa Fluor Plus 800 (Invitrogen A32730), and goat anti-mouse IgG Alexa
220 Fluor Plus 647 (Invitrogen A32728).

221 *Statistical Analysis*

222 Specific statistical tests and sample size for each study is indicated in the figure
223 legends. Error bars in figures show SEM. Statistical analyses were performed using GraphPad
224 Prism (version 9; GraphPad Software Inc), and graphs were built using GraphPad Prism
225 (GraphPad Software Inc) statistical analysis software or R studio (4.2.3) using ggplot (3.5.1). P
226 values are 2 sided with values less than 0.05 regarded as statistically significant.

227 *Data Availability*

228 Further information and resources, including KPC cells and raw data will be shared
229 upon reasonable request to Aaron J. Grossberg (grossber@ohsu.edu).

230

231 **RESULTS**

232 **Adipocytes suppress lipogenesis in response to PDAC-derived factors**

233 Given a large body of literature suggesting that PDAC cachexia is partly driven by
234 increased lipolysis in the adipose tissue, we tested the effect of PDAC cell conditioned media
235 (KPC CM) on lipolysis *in vitro* [13, 14, 30]. We treated differentiated 3T3-L1 cells with control
236 maintenance media, or with KPC CM for 6 days. At this time, we observed decreased lipid
237 droplet accumulation by brightfield microscopy, which was confirmed with Oil Red O (Figure
238 1A-B). Media glycerol levels, a measure of lipolysis, were not elevated in KPC CM-treated
239 cells, while liberated triglyceride levels were significantly lower in CM-treated cells (Figure 1C,
240 S1A). Correspondingly, protein markers of lipolysis, including activated (phosphorylated)
241 hormone-sensitive lipase (HSL) and adipose triglyceride lipase (ATGL) were not elevated in

242 KPC CM-treated cells (Figure 1D-E, S1B-E, S2) and mRNA levels of lipolysis enzymes *Atgl*
243 and hormone-sensitive lipase (lipase e, *Lipe*) were significantly decreased in KPC CM-treated
244 3T3-L1 cells (Figure 1F). To explain the apparent decrease in lipid accumulation without
245 increased lipolysis, we measured the expression of genes associated with lipogenesis:
246 diacylglycerol O-acyltransferase 1 and 2 (*Dgat1 and Dgat 2),* fatty acid synthase (*Fasn*),
247 lipoprotein lipase (*Lpl*), nuclear receptor subfamily 1 group H member 3 (*Nr1h3*), stearoyl-CoA
248 Desaturase 1 (*Scd1*), solute carrier family 2 member 4 (*Slc2a4*), and sterol regulatory element-
249 binding protein 1c (*Srebp1c*). All of these genes were suppressed after 24 hour KPC CM
250 treatment, indicating that the decreased lipid content in 3T3-L1 cells was due to impaired
251 anabolic activity rather than increased catabolic activity (Figure 1F).

252 **PDAC is associated with decreased fat pad mass *in vivo***

253 We first sought to assess the effects of pancreatic cancer on metabolism by
254 characterizing tissue physiology in a murine orthotopic PDAC model. 12-week-old C57BL/6J
255 mice with PDAC or sham implantations were fed *ad libitum* with or without a 24h fast once
256 mice reached a moderate cachexia burden (9 days after tumor implantation). Body mass
257 maintained stable among all groups prior to fasting (Figure 2A), whereas PDAC mice exhibited
258 a small reduction in food intake (Figure 2B) compared to sham controls. Fasting caused a
259 significant reduction in tumor mass compared to *ad libitum*-fed mice (Figure 2C).EchoMRI
260 body composition analysis of fat mass demonstrated significant decreases in overall adiposity
261 in PDAC animals from baseline to pre-fast due to cachexia progression and reduction in food
262 intake (Figure 2D). As expected, between pre- and post-fast, both PDAC and sham animals
263 lost significant overall adiposity (Figure 2E). Terminal fat pad masses (inguinal, iWAT, and
264 gonadal, gWAT) were significantly decreased in both ad lib and fasted conditions as compared

265 to sham mice (Figure 2F). PDAC mice were also vulnerable to skeletal muscle mass loss as
266 measured by echoMRI and gastrocnemius muscle tissue mass (Figure S3).

267 PDAC impairs lipolysis and adipogenesis

268 Based on our findings *in vitro*, we hypothesized that PDAC mice would not exhibit
269 increased lipolysis, which is characteristic of other cachexia models [13, 30]. To assess
270 products of lipolysis in circulation, we measured plasma glycerol and non-esterified fatty acids
271 (NEFAs). Plasma glycerol levels were significantly lower in fasted PDAC mice than in fasted
272 sham mice (Figure 3A), while there was no significant difference in plasma NEFA levels
273 between fasted groups (Figure 3B). To directly measure the rate of adipose tissue catabolism,
274 we performed an *ex vivo* lipolysis study, using gonadal white adipose tissue (gWAT) collected
275 from sham and PDAC mice. We assessed fat pads both in the presence and absence of the
276 beta-adrenergic agonist isoproterenol (10 uM) to determine baseline and stimulated lipolysis
277 [29]. Baseline and isoproterenol-stimulated glycerol release were significantly decreased in
278 PDAC gWAT (Figure 3C), demonstrating that PDAC suppresses, rather than enhances,
279 lipolytic rate and capacity. To further characterize the molecular biology of adipocytes during
280 PDAC, we assessed mRNA expression of genes regulating lipolysis and browning in adipose
281 tissue. PDAC mice had suppressed protein and mRNA expression of ATGL and suppressed
282 activation (phosphorylation) of HSL in the gWAT (Figure 3D,F, S4). PDAC mice also showed
283 suppressed iWAT and gWAT *Lipe* expression under fasted conditions (Figure 3D-E). We
284 observed suppressed browning-associated gene expression in gWAT, including the genes
285 peroxisome proliferator-activated receptor gamma coactivator 1 alpha (*Pgc1a),* protein domain
286 containing 16 (*Prdm16*), and cell death inducing DFFA like effector a (*Cidea*) in PDAC mice in
287 both *ad libitum* fed and fasted conditions. Uncoupling protein 1 (*Ucp1*), which is also

288 associated with browning, was not significantly increased in gWAT or iWAT from PDAC mice
289 (Figure 3D-E). Therefore, neither enhanced lipolysis nor browning are significant contributors
290 to adipose loss in this model.

291 **PDAC downregulates pathways associated with adipogenesis**

292 To gain further insights into PDAC metabolism from global gene expression analysis,
293 we performed bulk RNA sequencing (RNAseq) on gWAT from sham and PDAC animals. . In
294 gWAT, we identified a total of 572 differentially expressed genes (DEGs) in PDAC versus
295 sham mice, of which 446 were enriched, and 126 were depleted (Figure 4A, S5). Pathway
296 analysis referencing the molecular signatures database (MSigDB) hallmark gene set collection
297 revealed increased expression of gene sets associated with cell cycle control (e.g., E2F
298 targets and G2M checkpoints) and inflammation (e.g. TNFa and JAK/STAT3 signaling) and
299 decreased expression of genes linked to adipogenesis, oxidative phosphorylation, and fatty
300 acid metabolism (Figure 4B) [31]. Because inhibition of adipogenesis could provide an
301 alternative mechanism for adipose wasting, we plotted all significant DEGs in the adipogenesis
302 gene set and observed nearly universal depletion of these transcripts in PDAC mice (Figure
303 4C). We then validated our RNAseq data by performing qPCR on selected adipogenesis-
304 associated DEGs, revealing consistent downregulation of both adipogenesis and lipogenesis
305 genes (Figure 4D). We then repeated qPCR in iWAT to determine whether subcutaneous
306 adipose exhibited the same transcriptomic changes. Transcriptional changes in iWAT were
307 less dramatic than in gWAT and did not reach significance, with the exception of *Lpl*, which
308 was significantly decreased in PDAC tissue (Figure 4E). Together, these results indicate that
309 suppressed transcriptomic programs associated with adipogenesis could account for
310 decreased adipose tissue during cachexia, in the absence of elevated lipolysis.

311 **Adipose tissue anabolism is impaired in orthotopic PDAC mice after refeeding**

312 Based on our results demonstrating downregulation of adipogenic and lipogenic genes
313 in PDAC mice, we next wanted to confirm whether adipose tissue anabolism is functionally
314 impaired in mice implanted with orthotopic PDAC. To do this, wildtype C57BL/6J mice with
315 PDAC or sham implantations were fed *ad libitum* for 9 days, an established timepoint of active
316 cachexia [32], fasted 24h, and then terminated or allowed to re-feed for 24 h. To control for
317 differences in caloric intake, we used a pair-fed refeeding scheme in which sham mice were
318 refed with the average food consumption of the refed PDAC group. Challenging mice with a
319 24h fast depletes adipose mass in both PDAC and sham mice (Figure 2D), enabling us to
320 assess anabolic restoration of fat mass during a 24 h refeeding period. We measured
321 cumulative food intake and daily body mass for the duration of the study (Figure 5A-B). Fasting
322 and refeeding did not impact pancreas tumor mass (Figure S6). There were no differences in
323 iWAT or gWAT mass in fasted PDAC versus sham mice (Figure 5C-D). However, while sham
324 mice regain significant amounts of gWAT and iWAT mass after refeeding, gWAT and iWAT
325 masses in PDAC mice remain equivalent before and after refeeding (Figure 5C-D). Refeeding
326 sham mice caused increased gWAT expression of lipogenic *Fasn* and adipogenic genes *Lep*
327 and *Retn*, while these genes remained suppressed in PDAC gWAT (Figure 5E). Refed sham
328 iWAT showed increased *Fasn,* but not adipogenic genes, while PDAC iWAT had suppressed
329 expression of both adipogenic and lipogenic genes (Figure 5F). These results confirm that
330 downregulation in pathways mediating adipogenesis are indeed accompanied by impaired
331 adipose tissue anabolism in orthotopic PDAC mice after refeeding.

332 **HuR is highly expressed in orthotopic PDAC fat tissue**

333 Following our observation that adipose tissue anabolism is impaired in PDAC animals,
334 we next sought to understand the mechanism of this phenomenon. We used Ingenuity
335 Pathway Analysis upstream regulator analysis to identify potential candidates that could drive
336 impaired anabolism in PDAC adipose tissue. From the DEGs identified in gWAT of PDAC
337 mice, we found that canonical regulators of adipogenesis, such as troglitazone, fenofibrate,
338 PPARA, and PGC1A, were predicted to be inhibited. Inflammatory cytokines associated with
339 PDAC and cachexia, such as MYD88, IL-6, TNFA, IFNG, and IL-1B, were predicted to be
340 activated. Among other targets that were predicted to be activated but were not typically
341 associated with cachexia was human antigen R (HuR, *ELAVL1*), an RNA-binding protein
342 recently established as a suppressor of adipogenesis [27] (Figure 6A). Given the predicted
343 activation of HuR in gWAT and existing literature, we next asked if HuR was more abundant in
344 adipose tissue from PDAC mice. We performed immunohistochemical staining of HuR in
345 formalin-fixed, paraffin-embedded sham and PDAC gWAT, iWAT, pancreas, and muscle
346 tissue. HuR staining was significantly increased in the pancreas, gWAT, and iWAT of PDAC
347 mice versus sham controls (Figure 6B-D, S7-8). We also observed increased HuR protein
348 abundance by western blotting whole gWAT tissue (Figure 6E, S4).

349 **HuR inhibition improves metabolic deficits and restores adipogenesis *in vivo***

350 Since HuR has been established as driving pro-survival pathways in PDAC, we next
351 wanted to determine if preventing HuR binding to target mRNAs could reverse adipose wasting
352 in PDAC cachexia [26]. To do this, we treated PDAC mice with a selective HuR inhibitor, KH-3,
353 which prevents binding of HuR to its target mRNA sequence [33]. All mice received orthotopic
354 PDAC tumor injections and were fed *ad libitum* then fasted 24h with or without a 24h refeed at
355 mid-cachexia (9 days after injection). In each feeding group, mice were treated with the HuR

356 inhibitor KH-3 (100 mg/kg) or vehicle at 6-, 8-, and 10-days post-injection. Cumulative food
357 intake between vehicle and KH-3 groups was not statistically significant prior to fast. KH-3
358 treated PDAC mice gained significantly more weight during refeeding compared to vehicle-
359 treated mice (Figure 7A-B). Refeeding was associated with larger tumors in both vehicle and
360 KH-3 groups, likely due to both log growth and the propensity for mice to become dehydrated
361 during fasting (Figure 7C). KH-3 treatment itself had no effect on tumor growth over this time
362 interval. KH-3 treatment caused a nonsignificant increase in gWAT mass and significantly
363 increased iWAT mass in refed animals (Figure 7D-F). In contrast, vehicle-treated mice did not
364 significantly increase fat pad mass during refeeding. These data indicate that mice treated with
365 KH-3 shows signs of functional anabolism that are lacking in PDAC mice given vehicle
366 treatment. This effect is specific to adipose tissue, as gastrocnemius muscle mass was not
367 changed between fast/refeed, or vehicle/KH-3 treatment (Figure 7F). KH-3 treatment did not
368 restore expression of adipogenic (*Cebpa, Adipoq, Lep*, *Retn*) or lipogenic (*Fasn, Dgat1, Dgat2,*
369 *Nr1h3, Scd1, Srebp1c*) genes (Figure 8B-C, E-F). HuR inhibition also did not impact lipolysis
370 gene expression (*Atgl* and *Lipe*) (Figure 8A, D). Thus, we show a role for HuR in adipose
371 tissue metabolism during PDAC-associated cachexia, whereby inhibiting HuR improved
372 anabolism and adipose retention, although without restoring the expression of genes
373 associated with adipogenesis and lipogenesis.

374 **DISCUSSION**

375 This work evaluates the relative contributions of enhanced catabolism and impaired
376 anabolism on fat wasting by investigating adipose tissue response to different nutritional
377 contexts and HuR inhibition in cachectic mice. We demonstrate that our murine model of
378 PDAC cachexia exhibits a near-complete deficit in adipose tissue anabolism in the context of

379 reduced lipolysis, which corroborates a growing set of literature that challenges the dogma that
380 fat wasting occurs as a result of only enhanced catabolism [16-18]. We further identify HuR as
381 a potential molecular mediator of adipogenesis during pancreatic cancer progression.

382 Our study implicates the RNA-binding protein HuR in the inhibition of adipogenesis and
383 lipogenesis in adipose tissue. HuR can impact the transcriptome through both RNA binding
384 and HuR-dependent splicing [26]. One documented mechanism of HuR-mediated
385 adipogenesis regulation is via HuR binding to adipogenesis upstream regulator *Pparg*, which
386 suppresses *Adipoq* protein expression in a non-transcriptionally dependent manner [34]. In our
387 RNA sequencing, we did not observe increased HuR expression, although others reported
388 increased HuR expression in response to nuclear factor-kappa B (NF-κB) signaling [35].
389 Alternatively, we identified HuR as an upstream regulator based on adipose transcriptomic
390 changes associated with PDAC. In this context, HuR is more likely controlled post-
391 transcriptionally, although further investigation is needed to identify the specific PDAC-
392 associated factors that may activate HuR stability and nuclear translocation [36].

393 Small molecule antagonists of HuR, such as KH-3, are currently in development and
394 have been found to have anti-tumor effects [26, 37]. In our studies, KH-3 ameliorated fat
395 wasting but also induced severe hemolytic anemia in all treated mice (Figure S9). Tumor size
396 was not impacted by KH-3 treatment, likely because of the short period of dosing. However,
397 studies aimed to evaluate tumor growth over 5 weeks documented slower tumor growth and
398 fewer metastases in KH-3-treated mice [38]. KH-3 did not reverse the transcriptional
399 repression of adipogenesis and lipogenesis observed in WAT from PDAC mice. If KH-3
400 restores adipogenesis by blocking HuR binding to RNA, we interpret these data to suggest that
401 HuR acts post-transcriptionally to suppress adipose anabolism, although we cannot rule out

402 non-HuR-mediated mechanisms of action. In our studies, the anti-cachectic effects of KH-3
403 were specific to adipose tissue and did not preserve skeletal muscle mass. Although prior
404 studies link muscle and adipose wasting, these models are all characterized by enhanced
405 lipolysis and browning [30]. As evidenced by the adipose-specific benefits of KH-3 with
406 concurrent muscle wasting, in our model adipose and muscle wasting appear to be driven by
407 independent mechanisms. Continued improvement of HuR antagonists could lead to
408 therapeutics that restore anabolic potential in adipose tissue, providing a novel approach to
409 alleviating one symptom of cachexia, while also providing anti-tumor benefits.

410 In addition to impaired anabolic potential in adipose tissue, our PDAC model also
411 presents suppressed lipolysis. Suppressed lipolysis could contribute to systemic defects in
412 energy utilization in cancer cachexia. Prior work from our group shows that PDAC impairs
413 hepatic lipid oxidation but that this does not result in lipid accumulation or fatty liver [32]. It is
414 possible that impaired lipolysis contributes to global metabolic disruption by limiting circulating
415 lipid substrates. Further interrogation is needed to understand how improved fat retention
416 through HuR inhibition might impact lipid mobilization and systemic metabolism.

417 Our work identifies impaired adipose anabolism and increased HuR signaling as unique
418 mechanisms of adipose wasting in cachexia. Although much of the focus in cachexia research
419 and treatment is on skeletal muscle, multiple recent reports demonstrate that adipose loss in
420 PDAC cachexia is a poor prognostic indicator, independent of muscle loss [5, S9-10]. This
421 further supports existing work describing heterogeneity within cancer cachexia patients [39].
422 Moving forward, accounting for cachexia patient heterogeneity, including adipose wasting and
423 loss of anabolic adipose potential, in clinical trial design may lead to increased therapeutic
424 success. The work presented here provides a strong foundation for continuing to evaluate

425 therapeutics designed to improve adipogenesis and lipogenesis in the context of PDAC
426 cachexia.

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433 HM, BW, PP, GM, AC, AJG. Investigation, PCAW, KP, HM, BW, PP, GM, AC. Writing—
434 Original Draft, PCAW, BLW. Writing – Review and Editing, PCAW, PP, JB, AJG. Visualization,
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453 Declaration of Helsinki and its later amendments. All human subjects provided informed
454 consent and any identifying information of individual patients has been omitted.

455

FIGURE LEGENDS

Figure 1: Adipocytes suppress lipogenesis in response to pancreatic ductal adenocarcinoma-conditioned media. (A) Representative brightfield images of differentiated 3T3-L1 cells treated with control media (top) or KPC CM (bottom) for 8 days. Scale bars represent 70 um. (B) Quantification of Oil Red O staining. N = 4 wells per condition. (C-E) 3T3-L1 cells were fully differentiated and then treated with control, KPC-conditioned, or isoproterenol (10 uM) media for 1 and 3 hours in the absence of FBS and insulin. N = 3 wells per timepoint/condition. (C) Media glycerol levels. Conditioned media and control media did not contain glycerol above background (assay buffer only) levels. (D) Serine 563 phosphorylated HSL protein levels normalized to total HSL protein levels. (E) Serine 660 phosphorylated HSL protein levels normalized to total HSL protein levels. (F) mRNA expression of lipolysis and lipogenesis genes. N= 3 wells per condition, normalized to 18S expression. Panels B and F tested with t-test. Panels C-E tested with two-way ANOVA with Tukey correction for multiple comparisons. $p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, and $^{****}p<0.0001$.

Figure 2: Pancreatic ductal adenocarcinoma is associated with decreased fat pad mass *in vivo*. (A-E)Wildtype C57BL/6J mice with PDAC or sham implantations were fed *ad libitum* or fasted 16 h at mid-cachexia (9 days after injection). Animals were euthanized 10 or 11 days after tumor implantation following a 16 h fast. N=5 sham, 6 PDAC male mice per feeding condition. (A) Daily body mass. Statistically tested with 3-way ANOVA $p<0.0001$ for time, time x fast; $p=0.0073$ for fast; $p=0.0097$ for time x tumor status; $p=0.0167$ for tumor status. (B) Cumulative food intake. Statistically tested with 3-way ANOVA $p<0.0001$ for time, time x fast; $p=0.0035$ for time x tumor status. (C) Terminal pancreas/tumor mass. (D-E) Body composition changes in total adiposity were characterized from baseline (pre-tumor implantation) to pre-fast between sham and PDAC mice (C), and from pre- to post-fast (D). (F) Terminal gWAT and iWAT mass following 24 h fast 14 days after injection. N=5 male mice per *ad lib* group, 5 male mice sham fast, 3 male mice PDAC fast. 2x2 analyses were statistically tested with two-way ANOVA or mixed effects model with Tukey multiple comparisons. $^{*}p<0.05$, $^{**}p<0.01$, and $^{****}p<0.0001$.

Figure 3: Pancreatic ductal adenocarcinoma impairs lipolysis and adipogenesis *in vivo*. (A) Measurement of lipolysis (glycerol release) in explants derived from gWAT collected 10 days after tumor implantation. Lipolysis was induced by the β-adrenergic agonist isoproterenol (10 μM). N=6 male mice per group. Statistically tested with two-way ANOVA with uncorrected Fisher's LSD test to compare groups. (D) mRNA levels of lipolysis and browning genes in gWAT collected 10 days post-implantation from 24 h-fasted and *ad libitum*-fed mice, normalized to 18S expression. N=5 male mice sham/fasted; 3 male, 3 female mice sham/ad lib; 6 male mice PDAC/fasted; 3 male, 2 female mice PDAC/ad lib. (E) mRNA expression of lipolysis and browning genes in iWAT collected 11 days post-implantation from *ad libitum*-fed mice, normalized to beta actin expression. N=4 male mice per group. (F) Western blot analysis of PDAC and Sham gWAT protein for HSL (83 kDa) and ATGL (55 kDa) (left) and Serine 563- and Serine 660-phosphorylated HSL protein levels normalized to total HSL protein levels (right). N=5 male mice per group. Protein normalized to vinculin (124 kDa). 2x2 data were statistically tested with two-way ANOVA with Tukey multiple comparisons comparing

tumor experimental groups to sham controls. Pairwise comparisons were made with unpaired t-test. *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001.

Figure 4: PDAC downregulates pathways associated with adipogenesis. RNAseq on gWAT from *ad libitum*-fed PDAC and sham mice collected 14 days post tumor implantation. N=2 female, 3 male PDAC; 3 female, 2 male sham. (A) Volcano plot of differentially expressed genes in gWAT. Blue dots represent significantly downregulated genes (log_2 fold change < -1 and adj p<0.05). while red dots represent significantly upregulated genes (log_2 fold change >1 and adj p<0.05). Grey dots are insignificant. Labels denote significant differentially expressed genes from the Hallmark Adipogenesis pathway. (B) Broad Institute GSEA showing significantly enriched pathways in PDAC gWAT relative to sham gWAT (pathway p<0.05). Blue is decreased expression, red is increased expression. (C) Heat map of Hallmark Adipogenesis pathways constituents in PDAC and Sham gWAT. Scale represents row-normalized raw counts. (D) qPCR validation of adipogenesis and lipogenesis genes in in gWAT collected 10 days post-implantation from 24 h-fasted and *ad libitum*-fed mice. N=5 male mice sham/fasted; 3 male, 3 female mice sham/ad lib; 6 male mice PDAC/fasted; 3 male, 2 female mice PDAC/ad lib. Statistically tested with two-way ANOVA with Tukey multiple comparisons comparing tumor experimental groups to sham controls. (E) qPCR validation of adipogenesis and lipogenesis genes in iWAT from ad lib fed mice collected 11 days post implantation. N=4 male mice per group. Statistically tested with unpaired t-test. *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001.

Figure 5: Adipose tissue anabolism is impaired in orthotopic PDAC mice after refeeding. Wildtype C57BL/6J mice with PDAC were fed *ad libitum* for 9 days then fasted 24 h with or without a 24 h refeeding period. Sham mice were pair-fed daily to the average food intake of the PDAC group from 2 days post-implantation until study end. Animals were euthanized 10 or 11 days after tumor implantation following the 24 h fast (10 d) or 24 h refeed (11 d). N=3 male and 3 female mice per group. (A) Cumulative food intake. Statistically tested with 3-way ANOVA. P<0.0001 for time, p=0.0344 for time x tumor status. (B) Daily body mass. Statistically tested with 3-way ANOVA. P<0.0001 for time, p=0.002 for time x tumor status. iWAT (C) and gWAT (D) mass normalized to initial body mass. mRNA expression of anabolic genes in gWAT (E) and iWAT (F), normalized to beta actin expression. Data represented in C-F were statistically tested with two-way ANOVA with Tukey multiple comparisons comparing PDAC groups to sham controls. *p<0.05, and ****p<0.0001.

Figure 6: HuR is highly expressed in PDAC orthotopic pancreas and fat tissue. (A) Selected upstream regulator predictions from Ingenuity Pathway Analysis of differentially expressed genes in gWAT. (B-D) Representative images and quantification of immunohistochemical detection of HuR in tissue from PDAC and sham mice. (B) Pancreas N=4 male and 4 female mice per group. (C) gWAT N=4 male and 4 female mice per group. (D) iWAT N=3 female, 2 male sham and 2 female, 3 male PDAC. (E) Western blot analysis of PDAC and Sham gWAT protein for HuR. N=5 male mice per group. HuR protein (36 kDa) normalized to vinculin protein (124 kDa). Scale bars represent 100 um. Statistically tested with t-test. *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001.

544
545 Figure 7: HuR inhibition prevents adipose tissue loss *in vivo*. Wildtype C57BL/6J mice with
546 PDAC were fed *ad libitum* for 9 days then fasted 24 h with or without a 24 h refeed. In each
547 feeding paradigm, groups were treated with either vehicle or the HuR antagonist, KH-3, then
548 euthanized 10 or 11 days after tumor implantation following the 24 h fast or 24 h refeed.
549 Vehicle-treated mice were pair-fed to the KH-3 treated group's average food intake from 8
550 days post-implantation until study end. N=7 male mice per group. (A) Cumulative food intake,
551 statistically tested as an unpaired t-test of vehicle vs KH-3 treatment at day 9 (prior to fast) p
552 =0.1121. (B) Change in body weight after refeeding, statistically tested with unpaired t-test. (C)
553 Terminal pancreas mass. (D) Terminal gWAT mass. (E) Terminal iWAT mass. (F) Terminal
554 gastrocnemius mass. 2x2 analyses were statistically tested with two-way ANOVA with Tukey
555 multiple comparisons*p<0.05, and **p<0.01.
556
557 Figure 8: HuR inhibition partially restores adipogenesis and lipogenesis *in vivo*. mRNA levels
558 of lipolysis (A), adipogenesis (B), and lipogenesis (C) genes in gWAT, normalized to beta actin
559 expression. mRNA levels of lipolysis (D), adipogenesis (E), and lipogenesis (F) genes in iWAT,
560 normalized to beta actin expression. Data were statistically tested with two-way ANOVA with
561 Tukey multiple comparisons correction. N=7 male mice per group. *p<0.05, **p<0.01,
562 ***p<0.001, and ****p<0.0001.
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566 **REFERENCES**

567 1. Baracos VE, Martin L, Korc M, Guttridge DC, Fearon KCH. Cancer-associated
568 cachexia. Nat Rev Dis Primers. 2018;4:17105. doi:10.1038/nrdp.2017.105
569 2. von Haehling S, Anker MS, Anker SD. Prevalence and clinical impact of cachexia in
570 chronic illness in Europe, USA, and Japan: facts and numbers update 2016. J Cachexia
571 Sarcopenia Muscle. 2016;7:507-9. doi:10.1002/jcsm.12167
572 3. Fearon K, Strasser F, Anker SD, Bosaeus I, Bruera E, Fainsinger RL, et al. Definition
573 and classification of cancer cachexia: an international consensus. Lancet Oncol. 2011;12:489-
574 95. doi:10.1016/S1470-2045(10)70218-7
575 4. Fearon K, Arends J, Baracos V. Understanding the mechanisms and treatment options
576 in cancer cachexia. Nat Rev Clin Oncol. 2013;10:90-9. doi:10.1038/nrclinonc.2012.209
577 5. Kays JK, Shahda S, Stanley M, Bell TM, O'Neill BH, Kohli MD, et al. Three cachexia
578 phenotypes and the impact of fat-only loss on survival in FOLFIRINOX therapy for pancreatic
579 cancer. Journal of cachexia, sarcopenia and muscle. 2018;9:673-84.
580 6. Mantovani G, Maccio A, Lai P, Massa E, Ghiani M, Santona MC. Cytokine activity in
581 cancer-related anorexia/cachexia: role of megestrol acetate and medroxyprogesterone
582 acetate. Semin Oncol. 1998;25:45-52.
583 7. Straub RH, Cutolo M, Buttgereit F, Pongratz G. Energy regulation and neuroendocrine-
584 immune control in chronic inflammatory diseases. J Intern Med. 2010;267:543-60.
585 doi:10.1111/j.1365-2796.2010.02218.x
586 8. Argiles JM, Busquets S, Toledo M, Lopez-Soriano FJ. The role of cytokines in cancer
587 cachexia. Curr Opin Support Palliat Care. 2009;3:263-8. doi:10.1097/SPC.0b013e3283311d09

588 9. MacDonald N, Easson AM, Mazurak VC, Dunn GP, Baracos VE. Understanding and
589 managing cancer cachexia. J Am Coll Surg. 2003;197:143-61. doi:10.1016/S1072-
590 7515(03)00382-X
591 10. Carson JL, Hernandez M, Jaspers I, Mills K, Brighton L, Zhou H, et al. Interleukin-13
592 stimulates production of nitric oxide in cultured human nasal epithelium. In Vitro Cell Dev Biol
593 Anim. 2018;54:200-4. doi:10.1007/s11626-018-0233-y
594 11. Haslett PA. Anticytokine approaches to the treatment of anorexia and cachexia. Semin
595 Oncol. 1998;25:53-7.
596 12. Moldawer LL, Copeland EM, 3rd. Proinflammatory cytokines, nutritional support, and
597 the cachexia syndrome: interactions and therapeutic options. Cancer. 1997;79:1828-39.
598 13. Das SK, Eder S, Schauer S, Diwoky C, Temmel H, Guertl B, et al. Adipose triglyceride
599 lipase contributes to cancer-associated cachexia. Science. 2011;333:233-8.
600 14. Petruzzelli M, Schweiger M, Schreiber R, Campos-Olivas R, Tsoli M, Allen J, et al. A
601 switch from white to brown fat increases energy expenditure in cancer-associated cachexia.
602 Cell metabolism. 2014;20:433-47.
603 15. Taylor J, Uhl L, Moll I, Hasan SS, Wiedmann L, Morgenstern J, et al. Endothelial Notch1
604 signaling in white adipose tissue promotes cancer cachexia. Nature Cancer. 2023;4:1544-60.
605 16. Langer HT, Ramsamooj S, Dantas E, Murthy A, Ahmed M, Ahmed T, et al. Restoring
606 adiponectin via rosiglitazone ameliorates tissue wasting in mice with lung cancer. Acta
607 Physiologica. 2024;e14167.
608 17. Jang HJ, Kim HY, Lyu JH, Muthamil S, Shin UC, Kim HS, et al. Bee (Apis mellifera L.
609 1758) wax restores adipogenesis and lipid accumulation of 3T3-L1 cells in cancer-associated
610 cachexia condition. Food Science & Nutrition. 2024;

611 18. Tien S-C, Chang C-C, Huang C-H, Peng H-Y, Chang Y-T, Chang M-C, et al. Exosomal
612 miRNA 16-5p/29a-3p from pancreatic cancer induce adipose atrophy by inhibiting
613 adipogenesis and promoting lipolysis. Iscience. 2024;27:
614 19. Cook KB, Kazan H, Zuberi K, Morris Q, Hughes TR. RBPDB: a database of RNA-
615 binding specificities. Nucleic Acids Res. 2011;39:D301-8. doi:10.1093/nar/gkq1069
616 20. Gerstberger S, Hafner M, Tuschl T. A census of human RNA-binding proteins. Nat Rev
617 Genet. 2014;15:829-45. doi:10.1038/nrg3813
618 21. Pihlajamaki J, Lerin C, Itkonen P, Boes T, Floss T, Schroeder J, et al. Expression of the
619 splicing factor gene SFRS10 is reduced in human obesity and contributes to enhanced
620 lipogenesis. Cell Metab. 2011;14:208-18. doi:10.1016/j.cmet.2011.06.007
621 22. Brosch M, von Schonfels W, Ahrens M, Nothnagel M, Krawczak M, Laudes M, et al.
622 SFRS10--a splicing factor gene reduced in human obesity? Cell Metab. 2012;15:265-6; author
623 reply 7-9. doi:10.1016/j.cmet.2012.02.002
624 23. Huot ME, Vogel G, Zabarauskas A, Ngo CT, Coulombe-Huntington J, Majewski J, et al.
625 The Sam68 STAR RNA-binding protein regulates mTOR alternative splicing during
626 adipogenesis. Mol Cell. 2012;46:187-99. doi:10.1016/j.molcel.2012.02.007
627 24. Chou CF, Lin YY, Wang HK, Zhu X, Giovarelli M, Briata P, et al. KSRP ablation
628 enhances brown fat gene program in white adipose tissue through reduced miR-150
629 expression. Diabetes. 2014;63:2949-61. doi:10.2337/db13-1901
630 25. Dai N, Zhao L, Wrighting D, Kramer D, Majithia A, Wang Y, et al. IGF2BP2/IMP2-
631 Deficient mice resist obesity through enhanced translation of Ucp1 mRNA and Other mRNAs
632 encoding mitochondrial proteins. Cell Metab. 2015;21:609-21. doi:10.1016/j.cmet.2015.03.006

633 26. Finan JM, Sutton TL, Dixon DA, Brody JR. Targeting the RNA-binding protein HuR in
634 cancer. Cancer Research. 2023;83:3507-16.
635 27. Siang DTC, Lim YC, Kyaw AMM, Win KN, Chia SY, Degirmenci U, et al. The RNA-
636 binding protein HuR is a negative regulator in adipogenesis. Nat Commun. 2020;11:213.
637 doi:10.1038/s41467-019-14001-8
638 28. Michaelis KA, Zhu X, Burfeind KG, Krasnow SM, Levasseur PR, Morgan TK, et al.
639 Establishment and characterization of a novel murine model of pancreatic cancer cachexia. J
640 Cachexia Sarcopenia Muscle. 2017;8:824-38. doi:10.1002/jcsm.12225
641 29. Schweiger M, Eichmann TO, Taschler U, Zimmermann R, Zechner R, Lass A.
642 Measurement of lipolysis. In: Elsevier; 2014. pp. 171-93.
643 30. Rupert JE, Narasimhan A, Jengelley DH, Jiang Y, Liu J, Au E, et al. Tumor-derived IL-6
644 and trans-signaling among tumor, fat, and muscle mediate pancreatic cancer cachexia.
645 Journal of Experimental Medicine. 2021;218:e20190450.
646 31. Liberzon A, Birger C, Thorvaldsdóttir H, Ghandi M, Mesirov JP, Tamayo P. The
647 molecular signatures database hallmark gene set collection. Cell systems. 2015;1:417-25.
648 32. Arneson-Wissink PC, Mendez H, Pelz K, Dickie J, Bartlett AQ, Worley BL, et al. Hepatic
649 signal transducer and activator of transcription-3 signalling drives early-stage pancreatic
650 cancer cachexia via suppressed ketogenesis. J Cachexia Sarcopenia Muscle. 2024;15:975-88.
651 doi:10.1002/jcsm.13466
652 33. Wu X, Gardashova G, Lan L, Han S, Zhong C, Marquez RT, et al. Targeting the
653 interaction between RNA-binding protein HuR and FOXQ1 suppresses breast cancer invasion
654 and metastasis. Commun Biol. 2020;3:193. doi:10.1038/s42003-020-0933-1

655 34. Hwang JS, Lee WJ, Hur J, Lee HG, Kim E, Lee GH, et al. Rosiglitazone-dependent
656 dissociation of HuR from PPAR-γ regulates adiponectin expression at the posttranscriptional
657 level. The FASEB Journal. 2019;33:7707-20. doi:10.1096/fj.201802643r
658 35. Kang MJ, Ryu BK, Lee MG, Han J, Lee JH, Ha TK, et al. NF-κB Activates
659 Transcription of the RNA-Binding Factor HuR, via PI3K-AKT Signaling, to Promote Gastric
660 Tumorigenesis. Gastroenterology. 2008;135:2030-42.e3. doi:10.1053/j.gastro.2008.08.009
661 36. Grammatikakis I, Abdelmohsen K, Gorospe M. Posttranslational control of HuR
662 function. Wiley Interdisciplinary Reviews: RNA. 2017;8:e1372.
663 37. Huang Z, Liu S, Tang A, Wu X, Aube J, Xu L, et al. Targeting RNA-binding protein HuR
664 to inhibit the progression of renal tubular fibrosis. Journal of Translational Medicine.
665 2023;21:428.
666 38. Dong R, Chen P, Polireddy K, Wu X, Wang T, Ramesh R, et al. An RNA-Binding
667 Protein, Hu-antigen R, in Pancreatic Cancer Epithelial to Mesenchymal Transition, Metastasis,
668 and Cancer Stem Cells. Mol Cancer Ther. 2020;19:2267-77. doi:10.1158/1535-7163.Mct-19-
669 0822
670 39. Fearon Kenneth CH, Glass David J, Guttridge Denis C. Cancer Cachexia: Mediators,
671 Signaling, and Metabolic Pathways. Cell Metabolism. 2012;16:153-66.
672 doi:10.1016/j.cmet.2012.06.011
673 40. von Haehling S, Coats AJ, Anker SD. Ethical guidelines for publishing in the Journal of
674 Cachexia, Sarcopenia and Muscle: update 2021. Wiley Online Library; 2021. pp. 2259-61.

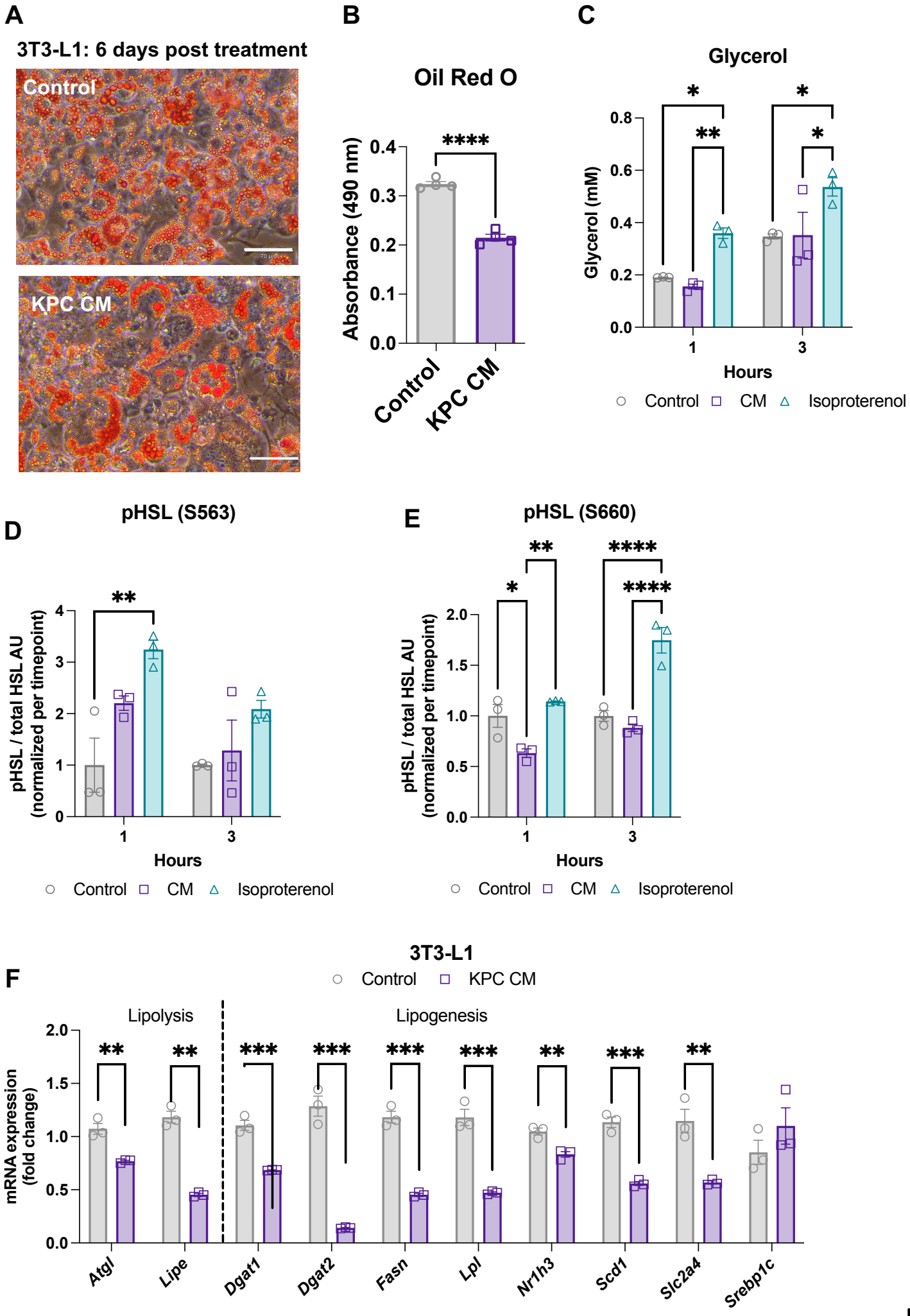
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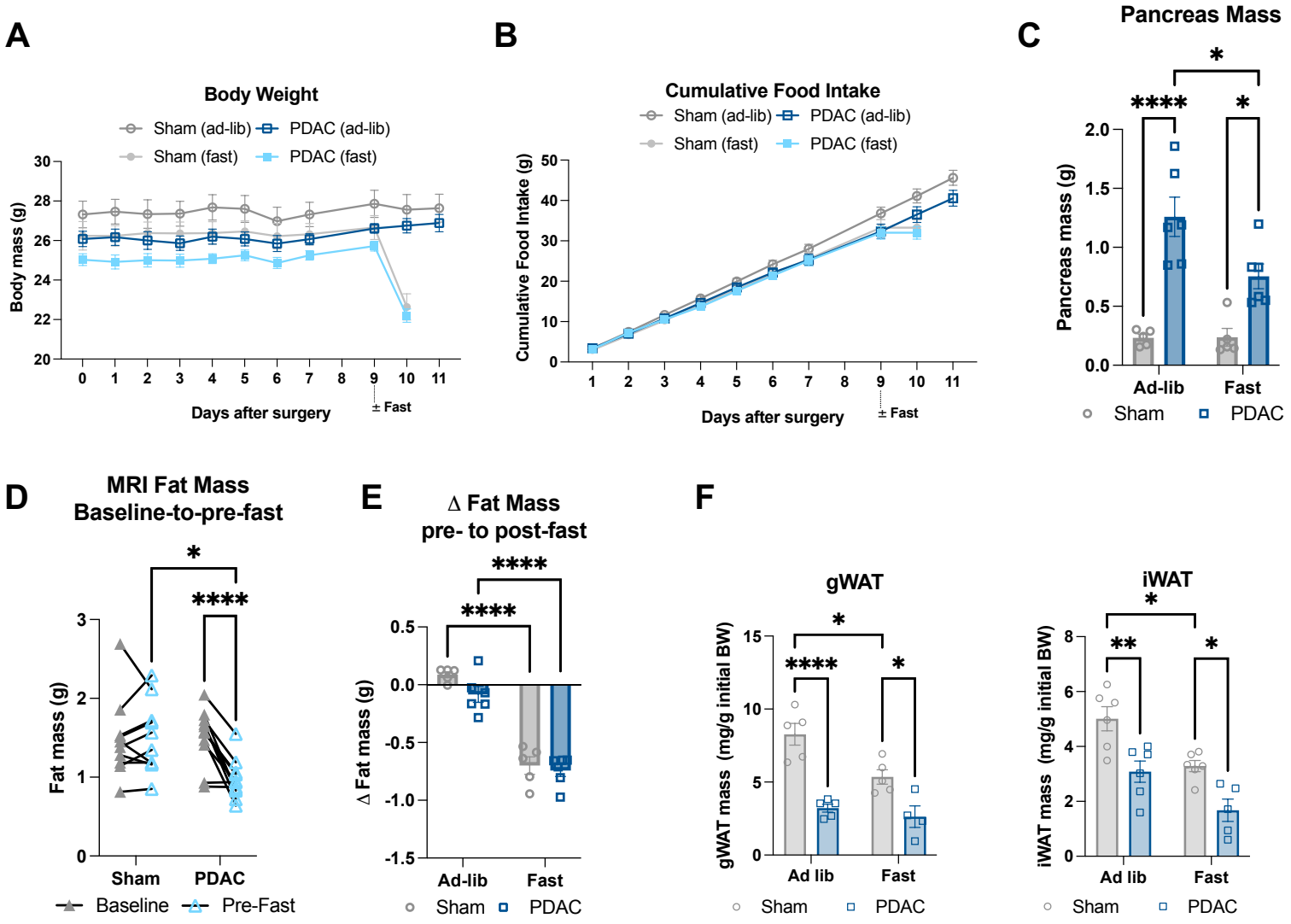
Figure 1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Pre-Fast
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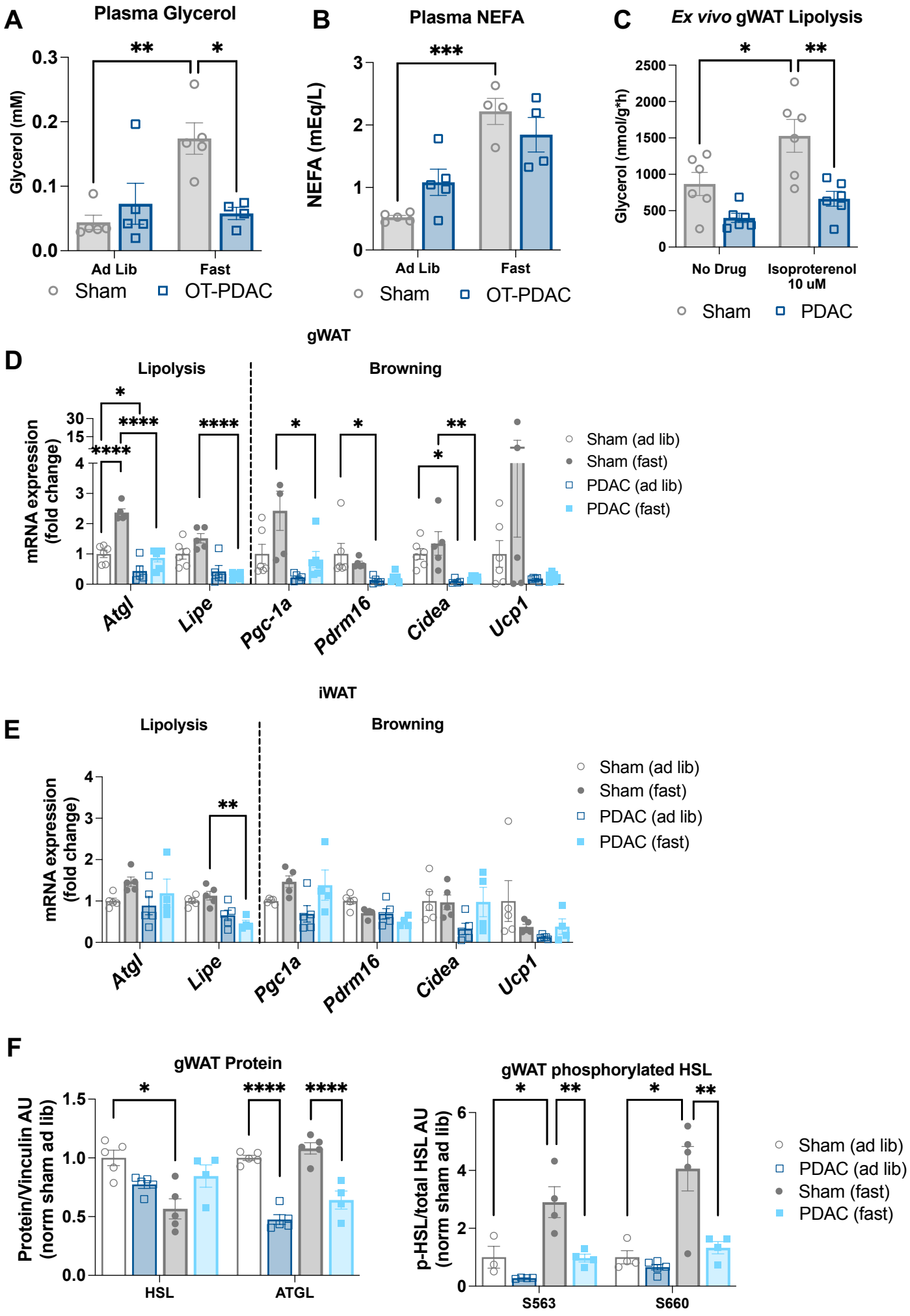
Figure 2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Ad Lib
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C
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Pdrm16
Cidea
Ucp1
Sham (ad lib)
Sham (fast)
PDAC (ad lib)
PDAC (fast)
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Browning
mRNA expression (fold change)
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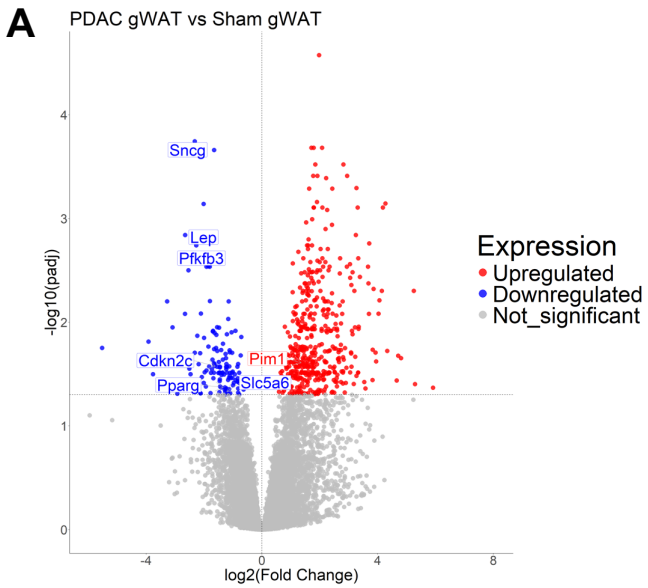
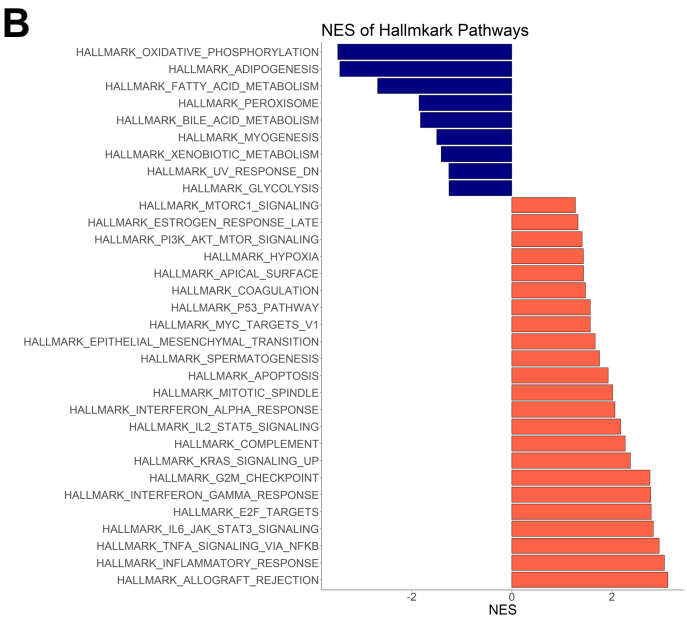
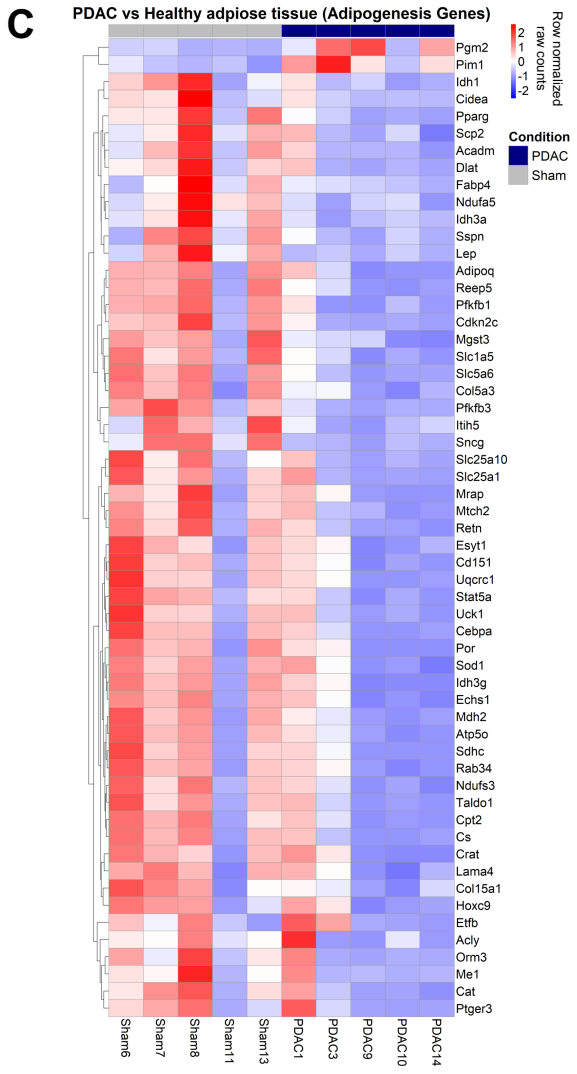
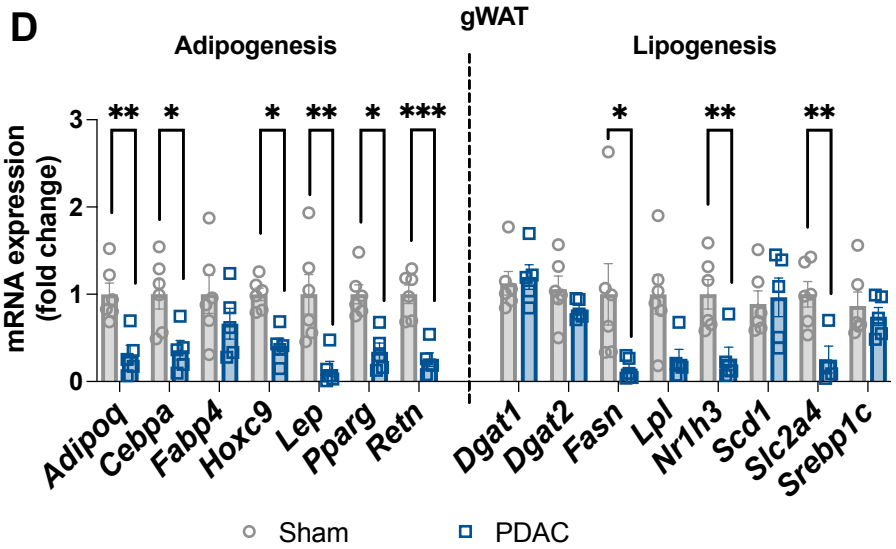
3
2
1
0
**
Atgl
Lipe
Pgc1a
Pdrm16
Cidea
Ucp1
Sham (ad lib)
Sham (fast)
PDAC (ad lib)
PDAC (fast)
F
gWAT Protein
Protein/Vinculin AU (norm sham ad lib)
1.5
1.0
0.5
0.0
*
****
****
HSL
ATGL
gWAT phosphorylated HSL
p-HSL/total HSL AU (norm sham ad lib)
6
4
2
0
*
**
*
**
S563
S660
Sham (ad lib)
PDAC (ad lib)
Sham (fast)
PDAC (fast)

Figure 3


A
PDAC gWAT vs Sham gWAT
Sncg
Lep
Pfkfb3
Cdkn2c
Pparg
Pim1
Slc5a6
Expression
Upregulated
Downregulated
Not_significant
-log10(padj)
log2(Fold Change)


B
NES of Hallmkark Pathways
HALLMARK_OXIDATIVE_PHOSPHORYLATION
HALLMARK_ADIPOGENESIS
HALLMARK_FATTY_ACID_METABOLISM
HALLMARK_PEROXISOME
HALLMARK_BILE_ACID_METABOLISM
HALLMARK_MYOGENESIS
HALLMARK_XENOBIOTIC_METABOLISM
HALLMARK_UV_RESPONSE_DN
HALLMARK_GLYCOLYSIS
HALLMARK_MTORC1_SIGNALING
HALLMARK_ESTROGEN_RESPONSE_LATE
HALLMARK_PI3K_AKT_MTOR_SIGNALING
HALLMARK_HYPOXIA
HALLMARK_APICAL_SURFACE
HALLMARK_COAGULATION
HALLMARK_P53_PATHWAY
HALLMARK_MYC_TARGETS_V1
HALLMARK_EPITHELIAL_MESENCHYMAL_TRANSITION
HALLMARK_SPERMATOGENESIS
HALLMARK_APOPTOSIS
HALLMARK_MITOTIC_SPINDLE
HALLMARK_INTERFERON_ALPHA_RESPONSE
HALLMARK_IL2_STAT5_SIGNALING
HALLMARK_COMPLEMENT
HALLMARK_KRAS_SIGNALING_UP
HALLMARK_G2M_CHECKPOINT
HALLMARK_INTERFERON_GAMMA_RESPONSE
HALLMARK_E2F_TARGETS
HALLMARK_IL6_JAK_STAT3_SIGNALING
HALLMARK_TNFA_SIGNALING_VIA_NFKB
HALLMARK_INFLAMMATORY_RESPONSE
HALLMARK_ALLOGRAFT_REJECTION
NES


C
PDAC vs Healthy adpiose tissue (Adipogenesis Genes)
Row normalized raw counts
Condition
PDAC
Sham
Sham6
Sham7
Sham8
Sham11
Sham13
PDAC1
PDAC3
PDAC9
PDAC10
PDAC14


D
gWAT
Adipogenesis
Lipogenesis
mRNA expression (fold change)
Adipoq
Cebpa
Fabp4
Hoxc9
Lep
Pparg
Retn
Dgat1
Dgat2
Fasn
Lpl
Nr1h3
Scd1
Slc2a4
Srebp1c
Sham
PDAC

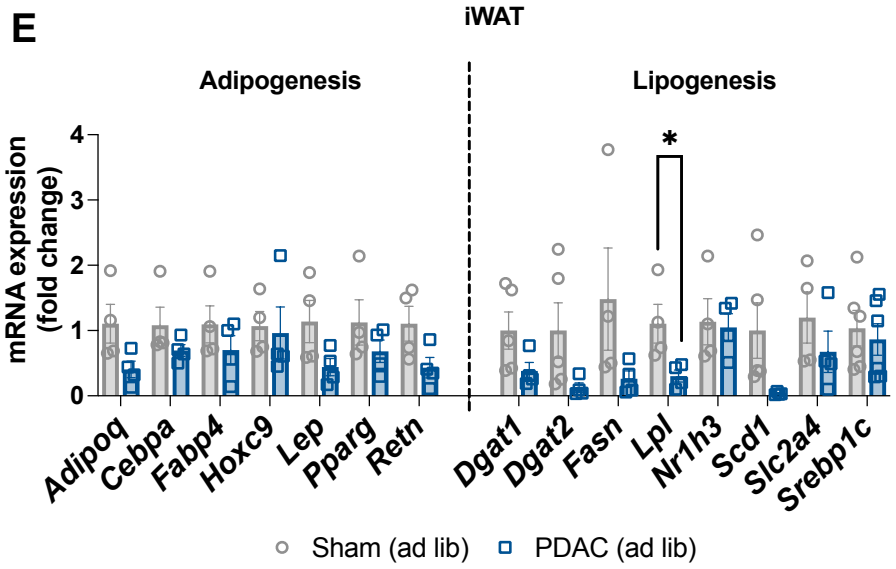

E
iWAT
Adipogenesis
Lipogenesis
mRNA expression (fold change)
Adipoq
Cebpa
Fabp4
Hoxc9
Lep
Pparg
Retn
Dgat1
Dgat2
Fasn
Lpl
Nr1h3
Scd1
Slc2a4
Srebp1c
Sham (ad lib)
PDAC (ad lib)

Figure 4

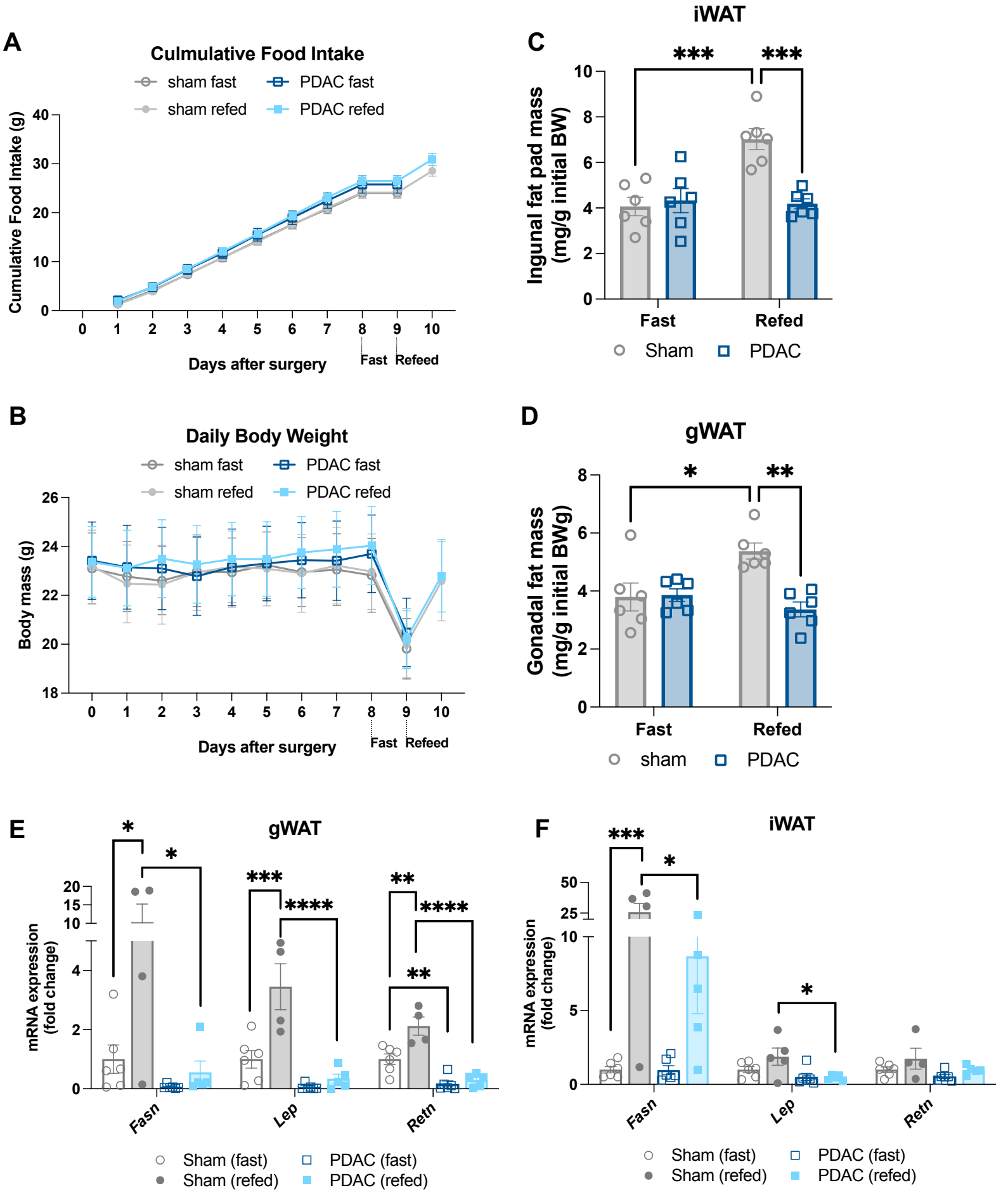

A
Culmulative Food Intake
sham fast
PDAC fast
sham refed
PDAC refed
Cumulative Food Intake (g)
Days after surgery
Fast
Refeed
B
Daily Body Weight
sham fast
PDAC fast
sham refed
PDAC refed
Body mass (g)
Days after surgery
Fast
Refeed
C
iWAT
Ingunal fat pad mass (mg/g initial BW)
Fast
Refed
Sham
PDAC
D
gWAT
Gonadal fat mass (mg/g initial BWg)
Fast
Refed
sham
PDAC
E
gWAT
mRNA expression (fold change)
Fasn
Lep
Retn
Sham (fast)
Sham (refed)
PDAC (fast)
PDAC (refed)
F
iWAT
mRNA expression (fold change)
Fasn
Lep
Retn
Sham (fast)
Sham (refed)
PDAC (fast)
PDAC (refed)

Figure 5

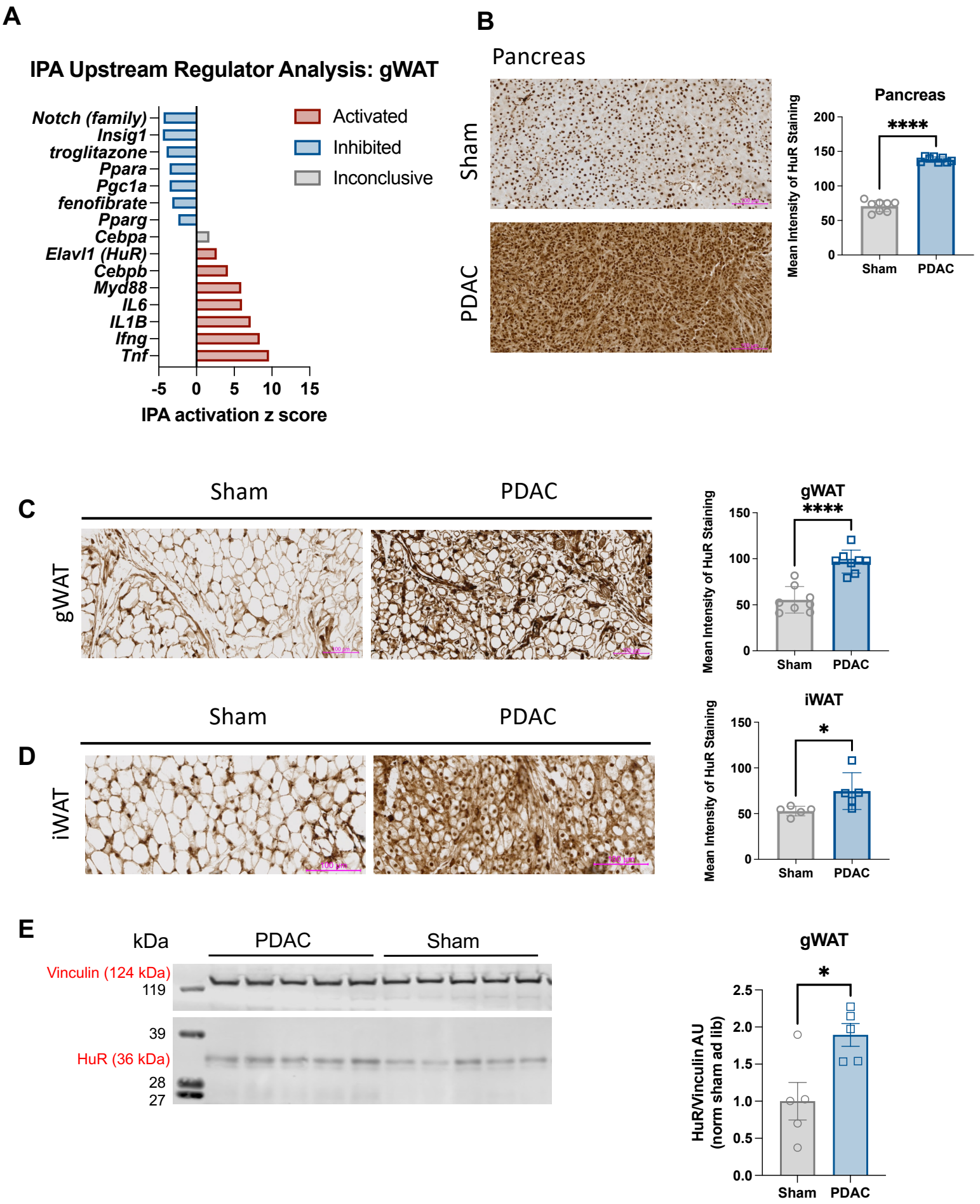

A
IPA Upstream Regulator Analysis: gWAT
Notch (family)
Insig1
troglitazone
Ppara
Pgc1a
fenofibrate
Pparg
Cebpa
Elavl1 (HuR)
Cebpb
Myd88
IL6
IL1B
Ifng
Tnf
Activated
Inhibited
Inconclusive
-5
0
5
10
15
IPA activation z score
B
Pancreas
Sham
PDAC
Pancreas
Mean Intensity of HuR Staining
****
Sham
PDAC
C
Sham
PDAC
gWAT
gWAT
Mean Intensity of HuR Staining
****
Sham
PDAC
D
Sham
PDAC
iWAT
iWAT
Mean Intensity of HuR Staining
*
Sham
PDAC
E
kDa
PDAC
Sham
Vinculin (124 kDa)
119
39
HuR (36 kDa)
28
27
gWAT
*
HuR/Vinculin AU
(norm sham ad lib)
Sham
PDAC

Figure 6

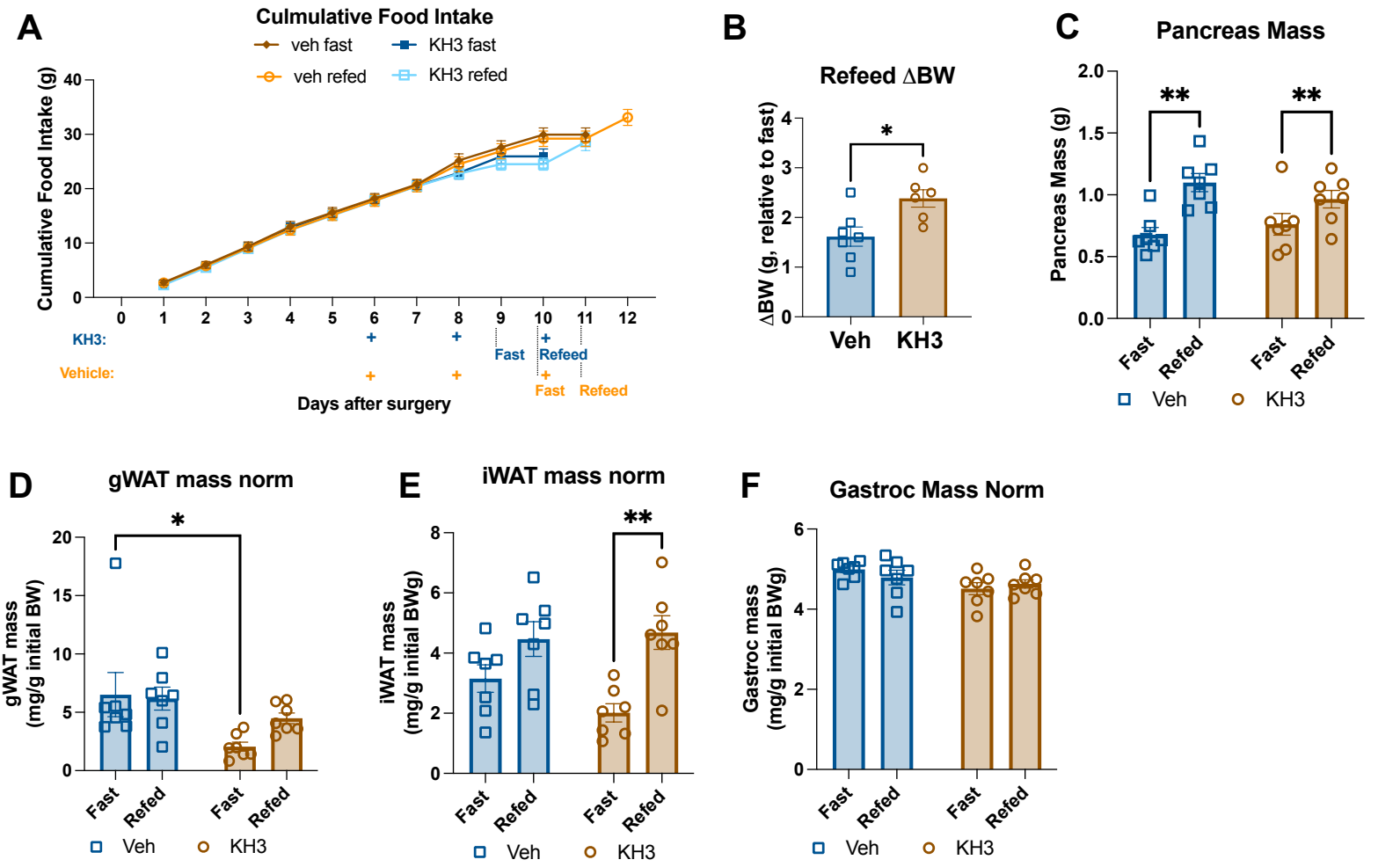

A
Culmulative Food Intake
veh fast
KH3 fast
veh refed
KH3 refed
Cumulative Food Intake (g)
KH3:
Vehicle:
Fast
Refeed
Days after surgery
B
Refeed ΔBW
ΔBW (g, relative to fast)
Veh
KH3
C
Pancreas Mass
Pancreas Mass (g)
Fast
Refed
Veh
KH3
D
gWAT mass norm
gWAT mass (mg/g initial BW)
E
iWAT mass norm
iWAT mass (mg/g initial BWg)
F
Gastroc Mass Norm
Gastroc mass (mg/g initial BWg)

Figure 7

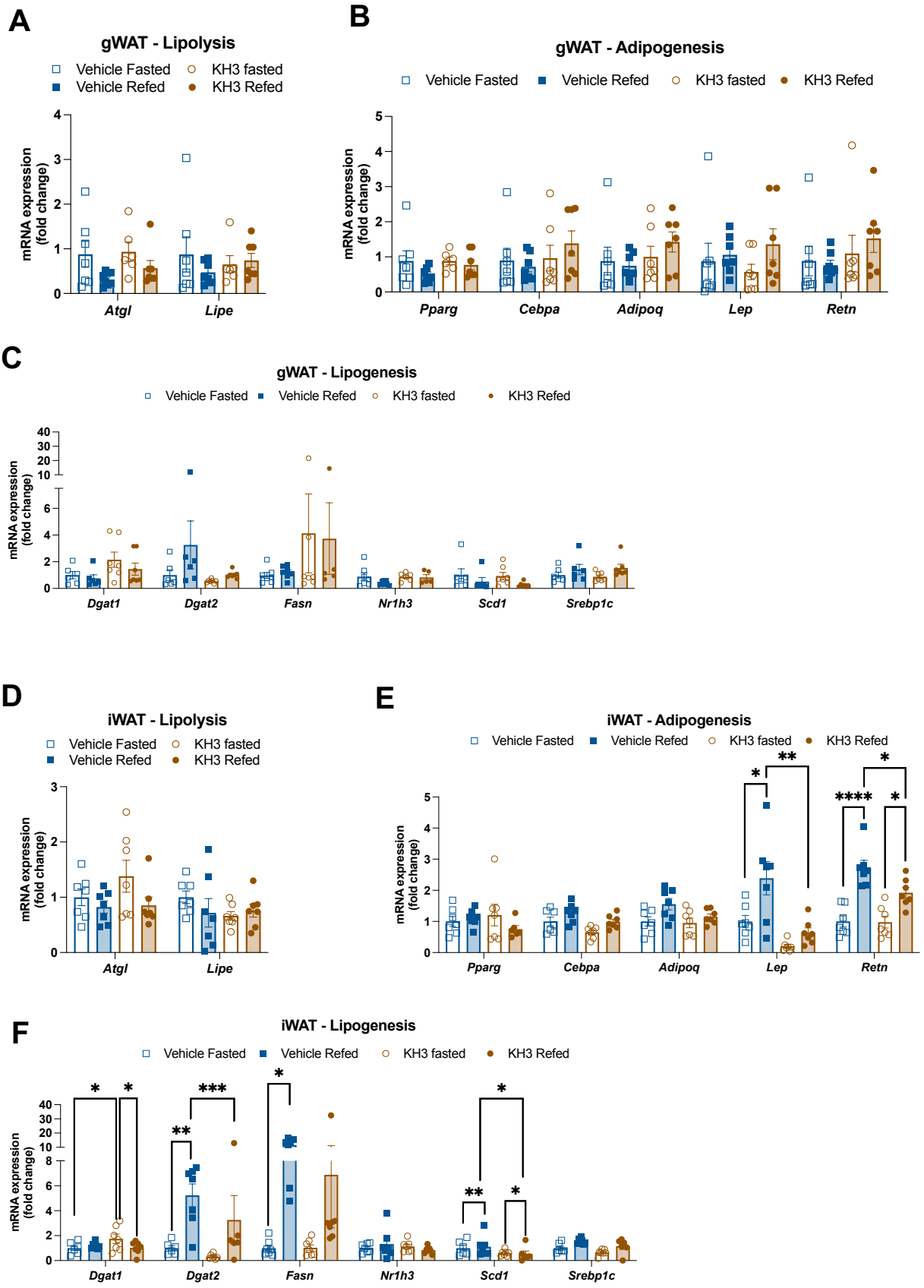

A
gWAT - Lipolysis
Vehicle Fasted
KH3 fasted
Vehicle Refed
KH3 Refed
mRNA expression (fold change)
Atgl
Lipe
B
gWAT - Adipogenesis
Vehicle Fasted
Vehicle Refed
KH3 fasted
KH3 Refed
mRNA expression (fold change)
Pparg
Cebpa
Adipoq
Lep
Retn
C
gWAT - Lipogenesis
Vehicle Fasted
Vehicle Refed
KH3 fasted
KH3 Refed
mRNA expression (fold change)
Dgat1
Dgat2
Fasn
Nr1h3
Scd1
Srebp1c
D
iWAT - Lipolysis
Vehicle Fasted
KH3 fasted
Vehicle Refed
KH3 Refed
mRNA expression (fold change)
Atgl
Lipe
E
iWAT - Adipogenesis
Vehicle Fasted
Vehicle Refed
KH3 fasted
KH3 Refed
mRNA expression (fold change)
Pparg
Cebpa
Adipoq
Lep
Retn
F
iWAT - Lipogenesis
Vehicle Fasted
Vehicle Refed
KH3 fasted
KH3 Refed
mRNA expression (fold change)
Dgat1
Dgat2
Fasn
Nr1h3
Scd1
Srebp1c

Figure 8