

Circadian reprogramming of adipose progenitor cells regulates intermittent fasting-mediated adipose tissue remodeling and metabolic improvement

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SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. PWAT of aged DIO mice display pro-inflammatory and reduced adipogenic signatures.

(A) Schematic diagram of young, DIO, aged, and aged DIO mouse model. (B) Body weights of young (n = 6), DIO (n = 6), aged (n = 5), and aged DIO (n = 6) mice. (C) IPGTT of four groups. (D) H&E stained images of the liver. (E) Representative biaxial plots and bar graphs showing macrophage subtypes in young and aged DIO PWAT (n = 3 each). (F) Proportion of eosinophils in PWAT. (G) Proportion of ILC2 in PWAT. Values are presented as means ± SEM. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.005$

Supplementary Figure 2. IF reduces lipid droplet accumulation in the liver of aged DIO and improves insulin sensitivity.

(A) Cumulative food intake over 6 weeks of IF, normalized by per gram of BW. (B) BAT and liver weight after 6 weeks of IF. (C) H&E stained images of BAT and liver. (D) Fasting insulin

level after 24 hours of fasting. (E) HOMA-IR. Calculated by (fasting glucose $\times$ fasting insulin / 22.5). Values are presented as means $\pm$ SEM. * p <0.05, ** p <0.01, *** p <0.005

Supplementary Figure 3. IF does not enhance diet-induced thermogenesis in aged DIO mice.

(A) Hourly oxygen consumption rate during one cycle of IF, normalized by body weight. (B) Oxygen consumption rate on fasting and feeding days. (C) Average oxygen consumption rate during one cycle of IF. (D) Linear regression analysis of oxygen consumption rate with body weight as a covariate during fasting and feeding days. (E) mRNA levels of thermogenic genes in PWAT. (F) mRNA levels of thermogenic genes in BAT. Values are presented as means $\pm$ SEM. * p <0.05, ** p <0.01, *** p <0.005

Supplementary Figure 4. snRNA-sequencing reveals immune and stromal cells that are altered by IF.

(A) Top 3 differentially expressed genes of each cell cluster. (B) Transcript levels of various APC markers in two APC clusters. (C) GO term pathways significantly upregulated and downregulated in macrophage 1 cluster. (D) GO term pathways significantly upregulated and downregulated in macrophage 2 cluster. (E) Body weight during short-term IF. (F) Body composition after 3 weeks of IF. (G) Tissue weight after 3 weeks of IF. (H) Fasting glucose level after 3 weeks of IF. (I) GO term pathways significantly upregulated by short-term IF. Blue arrows indicate thermogenic and circadian pathways. (J) Top 20 differentially regulated genes by short-term IF.

Supplementary Figure 5. Whole-body and APC-specific *Bmal1* knockout mice display altered WAT phenotype.

(A) Hourly oxygen consumption rate of *Bmal1* Ctrl and *Bmal1* KO mice. (B) Oxygen consumption rate during inactive (day) and active (night) phases. (C) Average oxygen consumption rate over a single cycle of IF. (D) Representative macroscopic image of WAT mass of *Bmal1* Ctrl and *Bmal1* KO mice. (E) Histogram showing CD9 expression in PDGFR α ⁺ APCs in PWAT. (F) Transcript level of *Bmal1* in mature adipocytes, PDGFR α ⁺ and PDGFR α ⁻ cells. (n = 3 for each group). (G) Transcript level of *Bmal1* and *Dbp* in in PWAT (n = 5-6 per group). (H) Hourly oxygen consumption rate of APC-*Bmal1* Ctrl and APC-*Bmal1* KO mice. (I) Oxygen consumption rate during day and night. (J) Average oxygen consumption rate. Values are presented as means $\pm$ SEM. * p <0.05, ** p <0.01, *** p <0.005.

Supplementary Figure 6. *Bmal1* KO and APC-*Bmal1*KO mice display diminished metabolic benefits in response to IF.

(A) Adipocyte size of IWAT. (B) Hourly oxygen consumption rate of *Bmal1* Ctrl-AL and *Bmal1* Ctrl-IF mice. (C) Average oxygen consumption rate. (D) Hourly oxygen consumption rate of *Bmal1* KO-AL and *Bmal1* KO-IF mice. (E) Average oxygen consumption rate. (F) Adipocyte size of IWAT. (G) Hourly oxygen consumption rate of APC-*Bmal1* Ctrl-AL and APC-*Bmal1* Ctrl-IF mice. (H) Average oxygen consumption rate. (I) Hourly oxygen consumption rate of APC-*Bmal1* KO-AL and APC-*Bmal1* KO-IF mice. (J) Average oxygen consumption rate. Values are presented as means $\pm$ SEM. * p <0.05, ** p <0.01, *** p <0.005.

Supplementary Figure 7. snRNA-seq reveals differential gene expressions in omental fat from insulin-sensitive and insulin-resistant individuals.

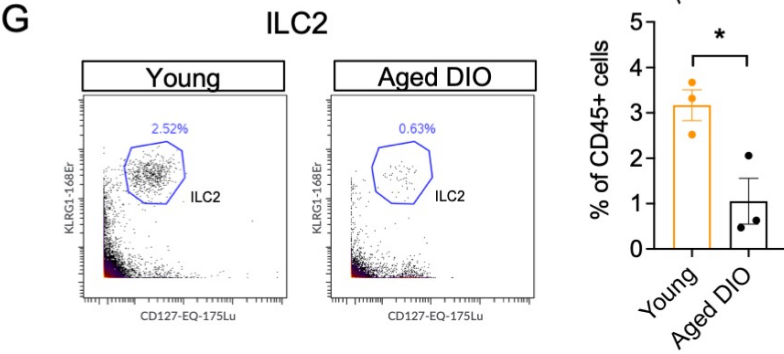
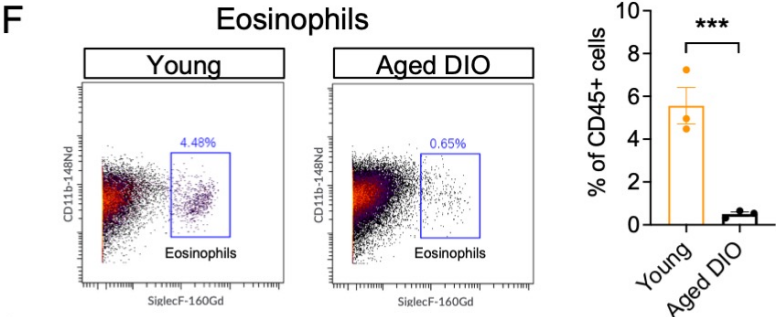
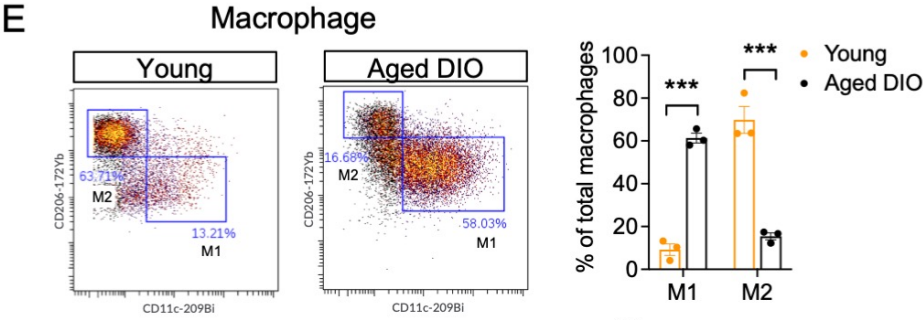
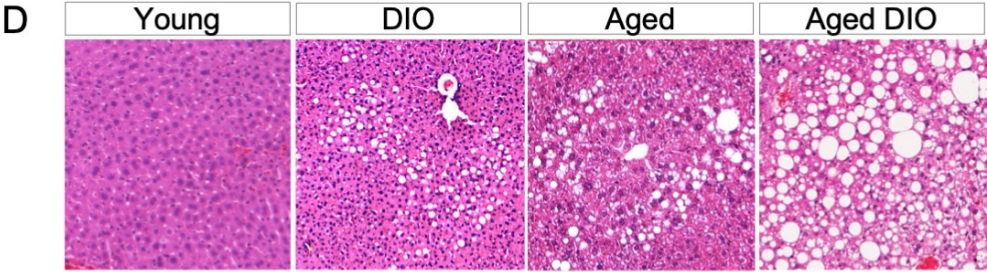
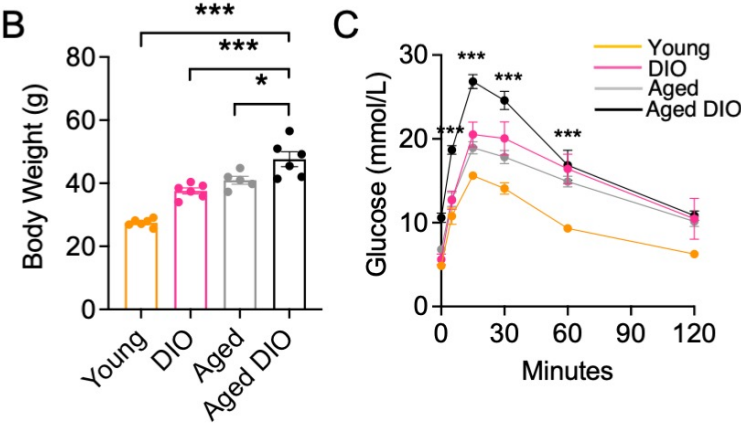
(A) HOMA-IR in insulin-sensitive and insulin-resistant patients. (B) GO terms significantly upregulated and downregulated in adipocytes of insulin-sensitive individuals. (C) Schematic diagram displaying *in vitro* SR9009 treatment on 3T3-L1 cells. (D) Immunofluorescence staining of collagen type VI after treatment of SR9009.

SUPPLEMENTARY TABLE LEGENDS

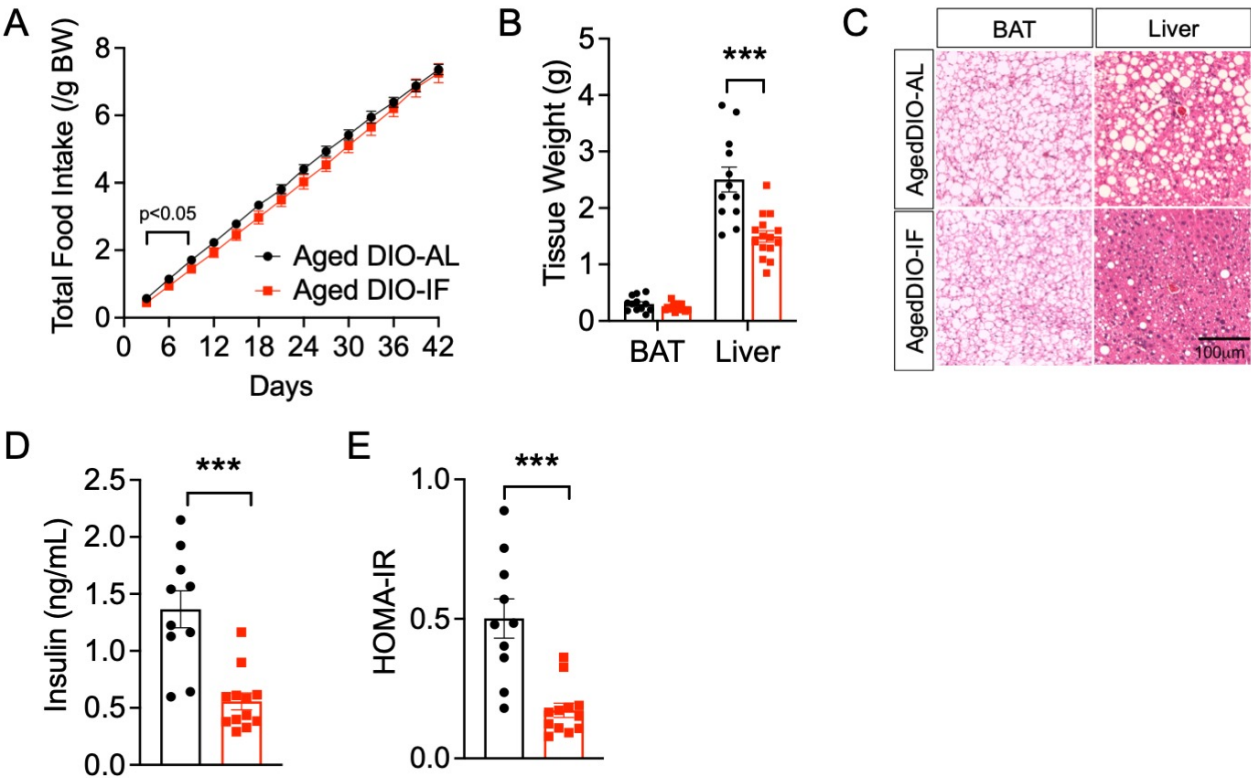
Supplementary Table 1. Characteristics and metabolic parameters of study subjects.

Supplementary Table 2. Antibody panel for adipose tissue mass cytometry analysis

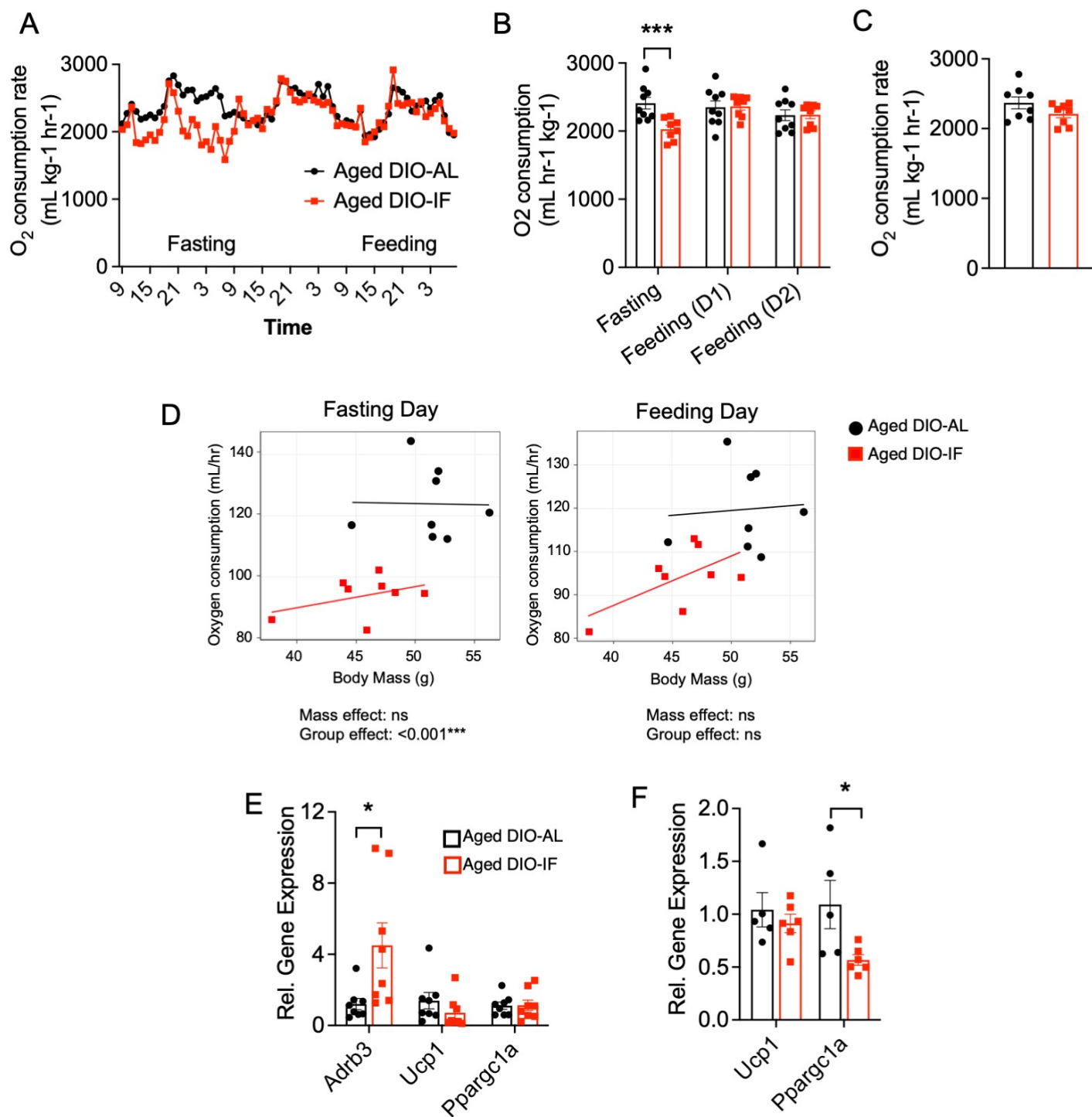
Supplementary Figure 1.



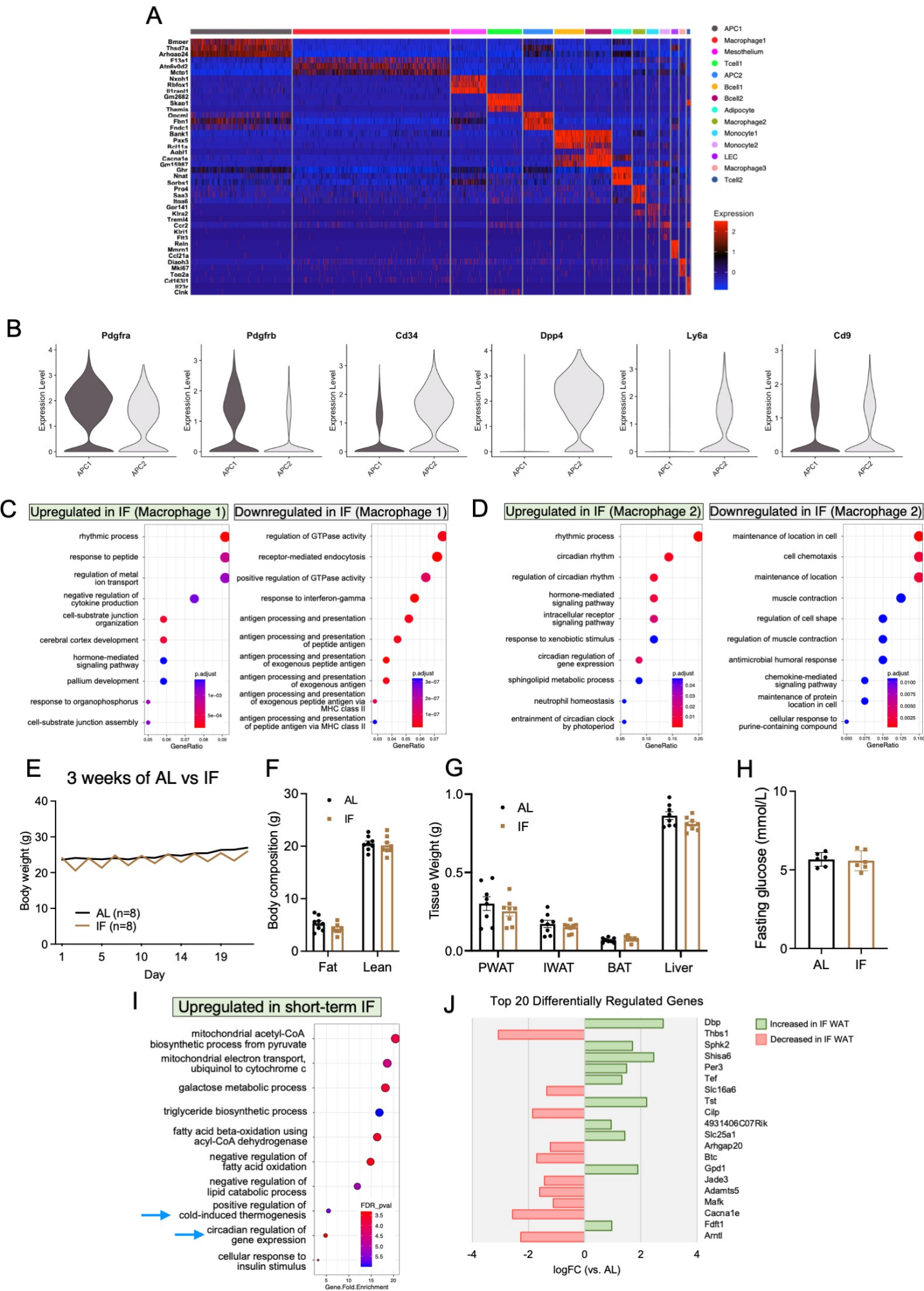
Supplementary Figure 2.



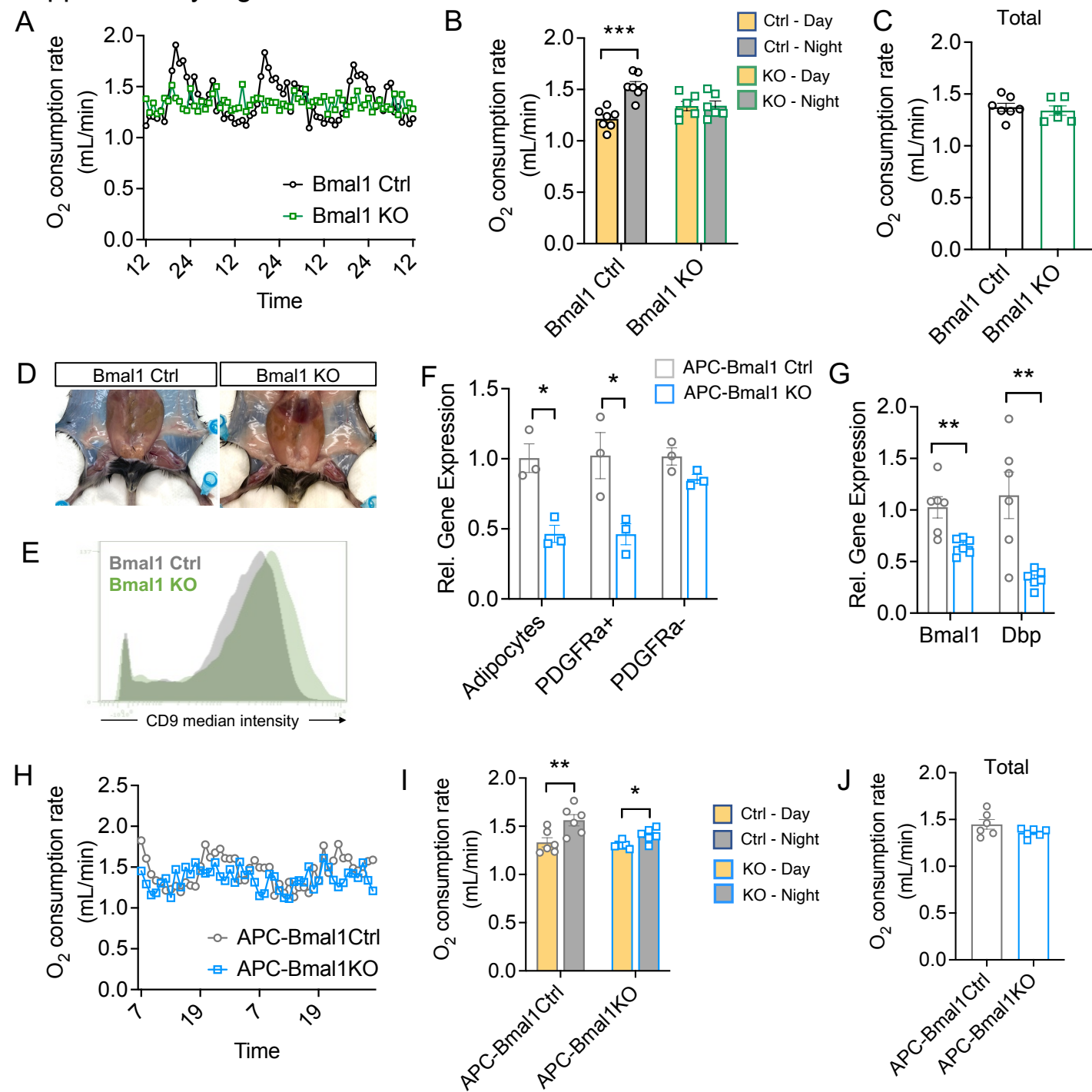
Supplementary Figure 3.



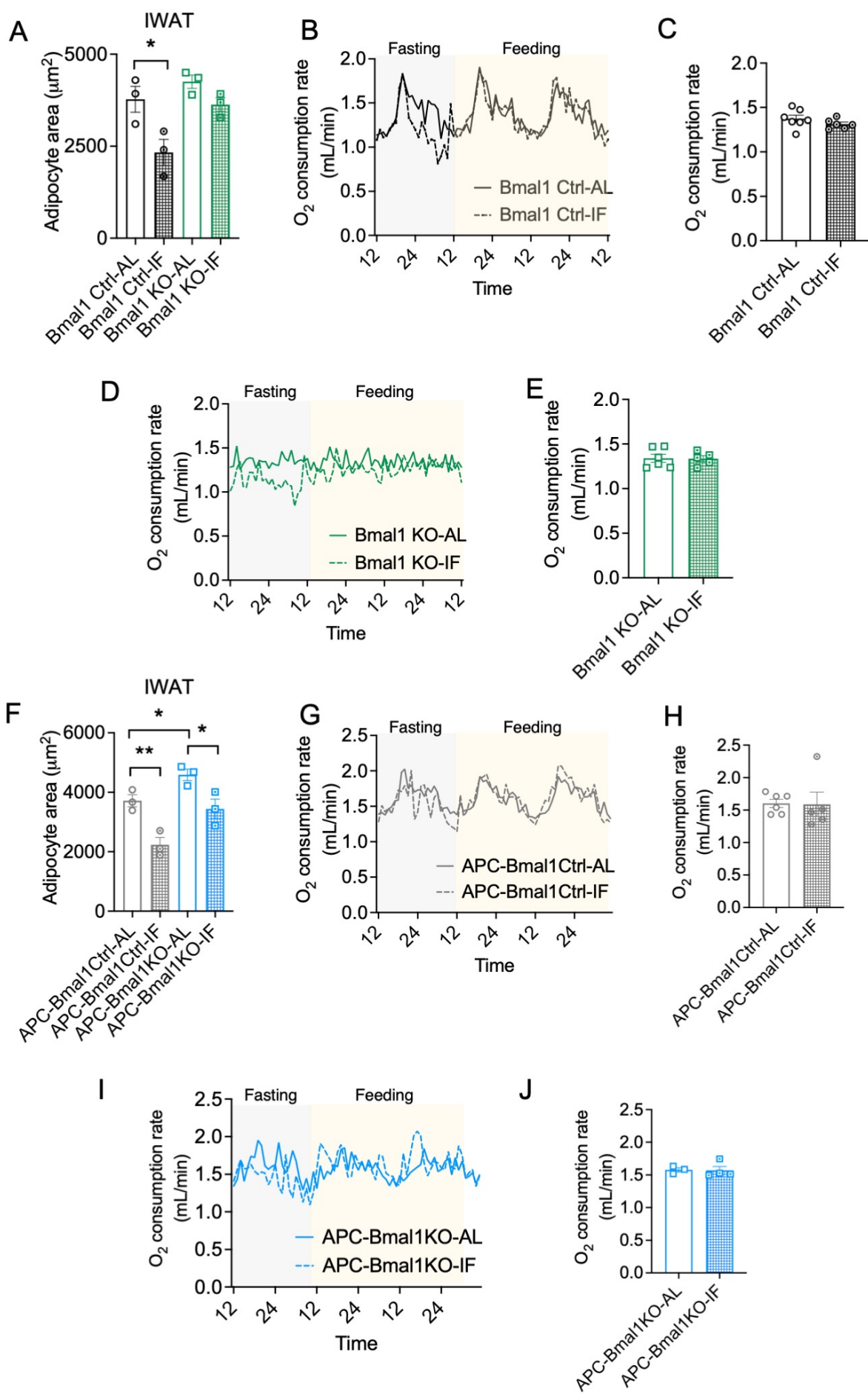
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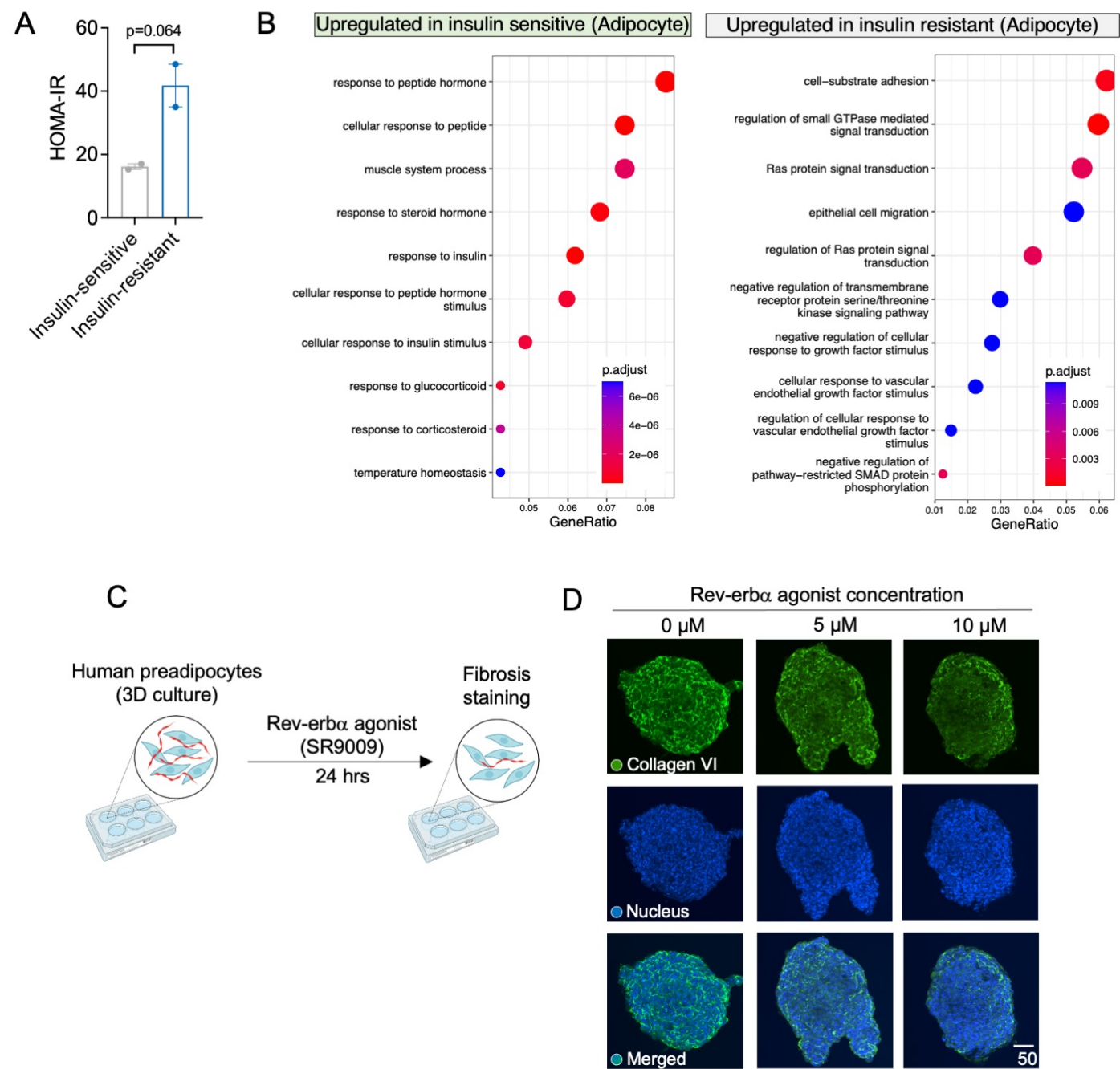
Supplementary Figure 5.



Supplementary Figure 6.



Supplementary Figure 7.



Supplementary Table 1. Characteristics and metabolic parameters of study subjects.

	Age	Sex	Weight (kg)	Height (m)	BMI	Waist Circumference (cm)	Blood Pressure	Fasting Glucose (mmol/L)	Fasting Insulin	HOMA- IR	Triglyceride
S021 (Insulin-sensitive)	63	F	173.5	1.637	64.7	162	137/87	4.8	80	2.82	1.48
S049 (Insulin-sensitive)	47	F	206.7	1.7	71.4	137	130/77	5.3	65	2.53	1.7
S050 (Insulin-resistant)	40	F	172	1.65	63.2	141	142/88	5.1	214	8.01	0.76
S058 (Insulin-resistant)	57	F	134.6	152.6	57.8	137.2	n/a	6.2	127	5.78	1.1

Supplementary Table 2. Antibody panel for adipose tissue mass cytometry analysis.

Antibody	Clone	Vendor	Concentration
89Y-CD45	30-F11	Fluidigm	1:300
115In-Ly6C	HK1.4	Biolegend	1:100
142Nd-CD11c	N418	Biolegend	1:300
143Nd-SiglecF	E50-2440	BD	1:1000
144Nd-MHC2	M5/114.15.2	Biolegend	1:500
146Nd-PDGFR β	APB5	Biolegend	1:100
148Nd-CD11b	M1/70	Biolegend	1:800
149Sm-CD19	1D3	BD	1:500
150Nd-CD24	M1/69 (Maxpar ready)	Biolegend	1:500
151Eu-CD31	390 (Maxpar ready)	Biolegend	1:1000
152Sm-DPP4	H194-112	Biolegend	1:800
154Sm-ICAM1	YN1/1.7.4	Biolegend	1:1000
156Gd-PDGFR α	APA5	ThermoFisher	1:800
159Tb-CD64	X54-5/7.1	Biolegend	1:300
160Gd-CD142	AF3178	R&D	1:300
161Dy-CD25	3C7	Biolegend	1:100
163Dy-CD8b	H35-17.2	ThermoFisher	1:2500
164Dy-Sca1	E13-161.7	Biolegend	1:1000

165Ho-CD4	RM4-5 (Maxpar ready)	Biolegend	1:300
166Er-CD9	EM-04	Novus	1:500
167Er-Ly6G	1A8	Biolegend	1:500
168Er-KLRG1	2F1	BD	1:1000
169Tm-TCR β	H57-597	Biolegend	1:500
170Er-NK1.1	PK136	Biolegend	1:100
171Yb-CD29	HM β 1-1	Biolegend	1:1000
172Yb-CD206	C068C2	Biolegend	1:800
173Yb-CD34	RAM34	ThermoFisher	1:100
174Yb-CD3	145-2C11 (Maxpar ready)	Biolegend	1:200
175Lu-CD127	A7R34 (Maxpar ready)	Biolegend	1:300
176Yb-B220	RA3-6B2	Biolegend	1:500