Supplemental information

Targeting *p21*^{Cip1}-Highly-Expressing Cells in Adipose Tissue Alleviates Insulin Resistance in Obesity

Lichao Wang, Binsheng Wang, Nathan S. Gasek, Yueying Zhou, Rachel L. Cohn, Dominique E. Martin, Wulin Zuo, William F. Flynn, Chun Guo, Evan R. Jellison, Taewan Kim, Larissa G.P. Langhi Prata, Allyson K. Palmer, Ming Li, Christina L. Inman, Lauren S. Barber, Iman M.A. Al-Naggar, Yanjiao Zhou, George A. Kuchel, Alexander Meves, Tamar Tchkonia, James L. Kirkland, Paul Robson, Ming Xu*

*Lead contact

Correspondence should be addressed to: M.X. (mixu@uchc.edu)
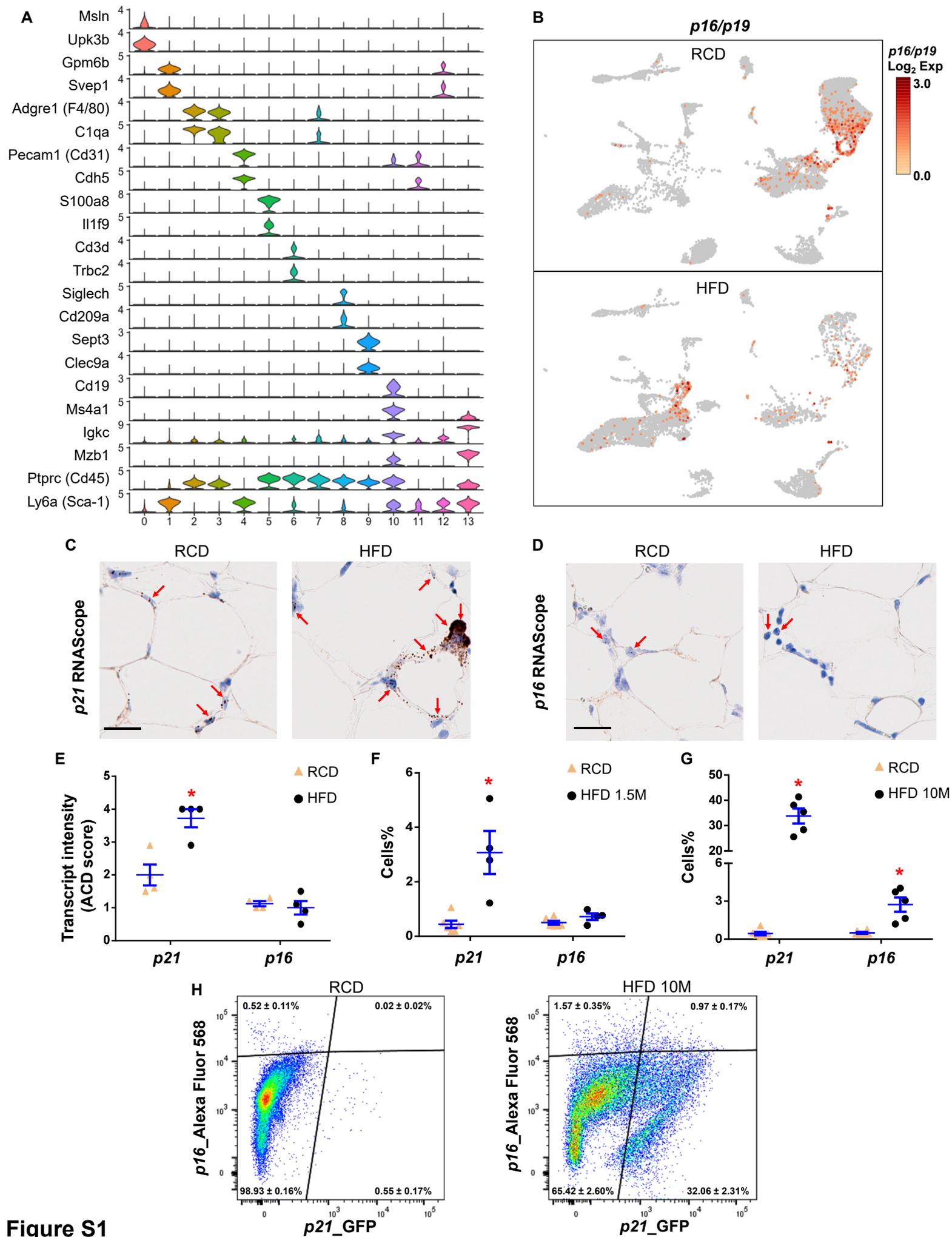
This PDF file includes:
Figure S1 to S7
Table S1

Figure S1

Figure S1. *p21*^{high} and *p16*^{high} cells are two distinct populations. Related to Figure 1.

(A) Violin plots of canonical marker genes in different cell populations. The marker genes were used to define the cell populations (cluster 0-13) in Figure 1A. Mesothelial cell – *Msln*, *Upk3b*; Preadipocyte – *Gpm6b*, *Svep1, Ly6a*; Macrophage – *Adgre1*, *C1qa*, *Siglech*, *Cd209a*; Endothelial cells – *Pecam1*, *Cdh5*; Neutrophil – *S100a8*, *Il1f9*; T cells – *Cd3d*, *Trbc2*; Dendritic cell – *Sept3*, *Clec9a*; B cell – *Cd19*, *Ms4a1*; Plasma cell – *Igkc*, *Mzb1*.

(B) UMAP plots showing $\log_2$ (normalized expression of *p16/p19*) in SVF from lean (left) and obese (right) mice.

(C)-(D) Representative micrographs of *p21* (C) or *p16* (D) RNAScope staining on gVAT from RCD or 2-month HFD fed mice (3-9 images per 4 biological replicates in each group). Red arrows indicate *p21* or *p16* transcripts. Scale bar = 30 µm.

(E) Quantitative analysis of *p21* and *p16* transcripts for gVAT from RCD or HFD fed mice.

(F)-(G) Proportion of *p21*^{high} and *p16*^{high} cells in SVF of gVAT from mice with RCD, 1.5-month HFD (F), and 10-month HFD feeding (G).

(H) Flow cytometry analysis of *p21* and *p16* expression in the SVF of gVAT from RCD and 10-month HFD fed mice.

For E, n = 4 biological replicates with 3-9 technical replicates for both groups. For F-H, n = 6 for RCD, n = 4 for HFD 1.5M, n = 5 for HFD 10M. For F-H, n represents the number of biological replicates with 1 technical replicate. Results are shown as mean ± s.e.m. * $P < 0.05$; two-tailed Welch's t-test.
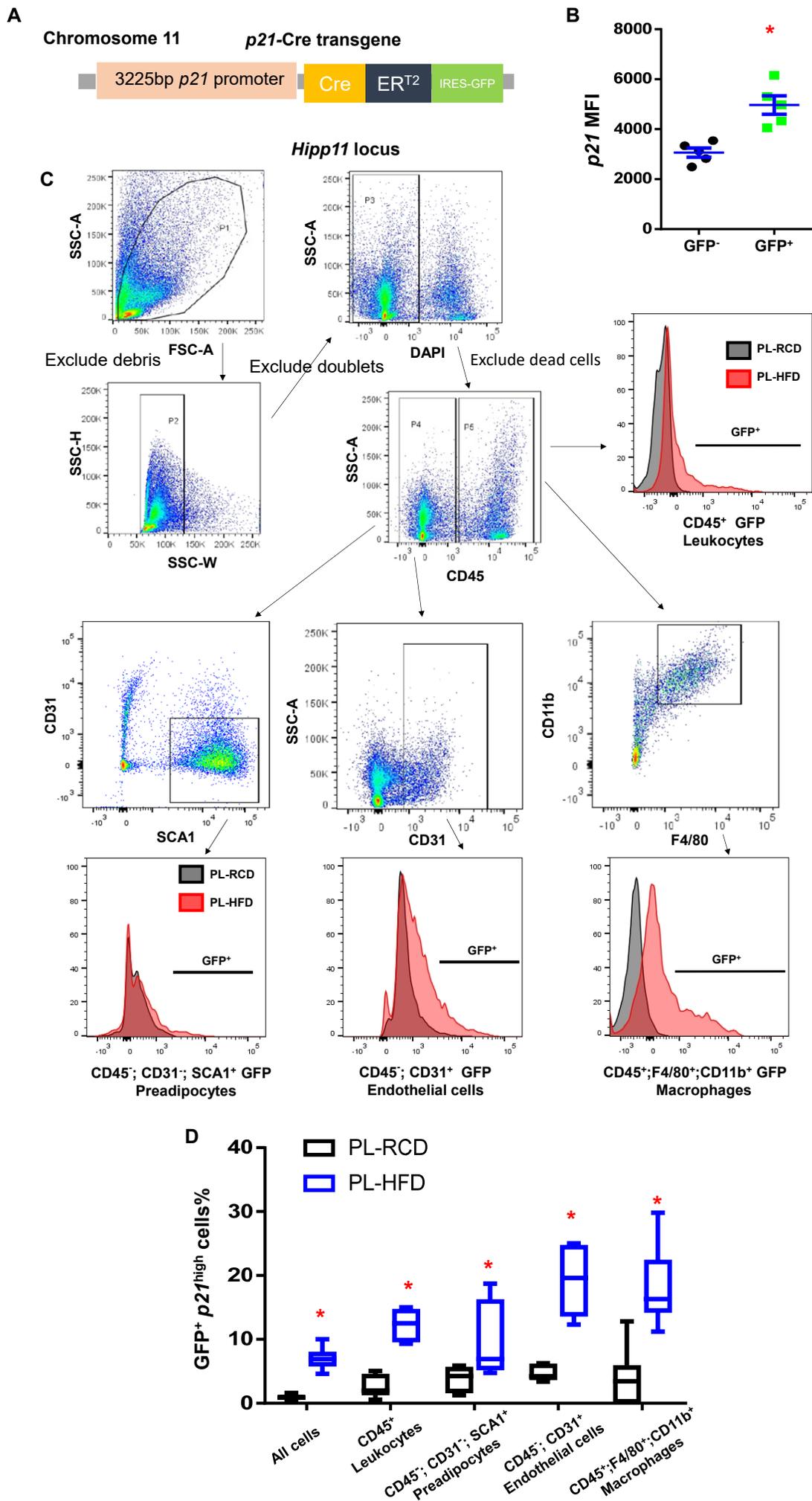
A Chromosome 11 **p21-Cre transgene**

3225bp *p21* promoter | Cre | ER^T2 | IRES-GFP

Hipp11 locus

B

C
Exclude debris · Exclude doublets · Exclude dead cells

CD45+ GFP
Leukocytes

CD45−; CD31−; SCA1+ GFP
Preadipocytes

CD45−; CD31+ GFP
Endothelial cells

CD45+;F4/80+;CD11b+ GFP
Macrophages

D

Figure S2

Figure S2. *p21*^{high} (GFP⁺) cells accumulate in adipose tissue with obesity. Related to Figure 2.

(A) Schematic of *p21*-Cre transgene.

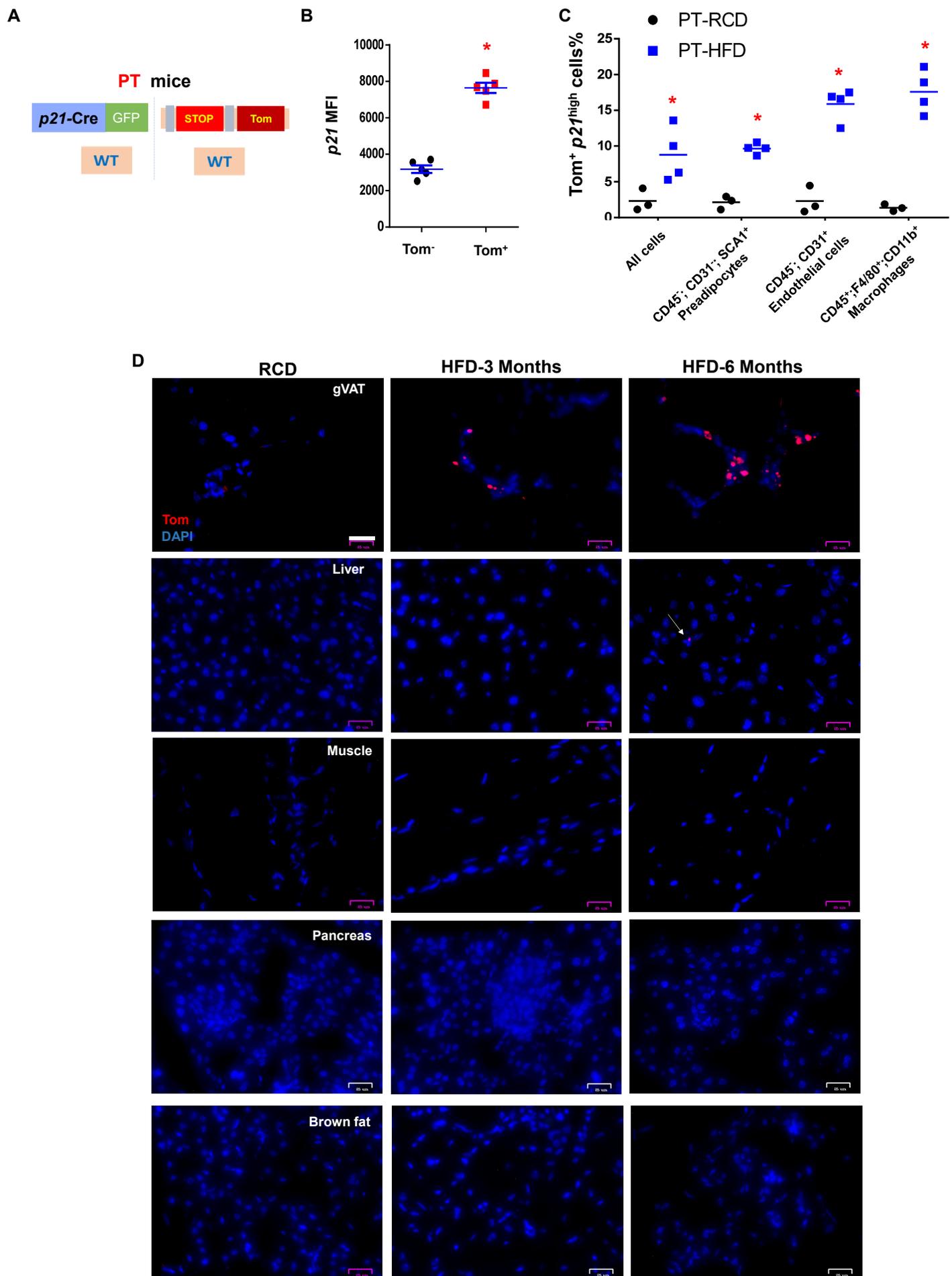(B) Mean fluorescence intensity (MFI) of *p21* staining by flow cytometry in GFP⁺ and GFP⁻ cells.

(C) Gating strategy for analysis of leukocytes, preadipocytes, endothelial cells, and macrophages.

(D) Proportion of GFP⁺ *p21*^{high} cells in all cells, leukocytes, preadipocytes, endothelial cells, and macrophages. Figure S2D was also shown as a part of Figure 2B.

For B, $n = 5$ for both groups. Results are shown as mean ± s.e.m. For D, $n = 7$ for both groups. For B and D, *n* represents the number of biological replicates with 1 technical replicate. Results were shown as box-and-whisker plots, where a box extends from the 25th to 75th percentile with the median shown as a line in the middle, and whiskers indicate the smallest and largest values. * $P < 0.05$; two-tailed Welch's t-test.

A

PT mice

p21-Cre GFP | STOP Tom

WT | WT

B

p21 MFI

Tom⁻   Tom⁺

C

Tom⁺ p21^high cells%

● PT-RCD
■ PT-HFD

All cells | CD45⁻; CD31⁻; SCA1⁺ Preadipocytes | CD45⁻; CD31⁺ Endothelial cells | CD45⁺;F4/80⁺;CD11b⁺ Macrophages

D

RCD | HFD-3 Months | HFD-6 Months

gVAT

Tom
DAPI

Liver

Muscle

Pancreas

Brown fat

Figure S3

Figure S3. *p21*^{high} (Tom⁺) cells accumulate in adipose tissue with obesity. Related to Figure 2.

(A) PT mice have one copy of *p21*-Cre transgene and one copy of floxed knock-in tdTomato.

(B) MFI of *p21* staining by flow cytometry in Tom$^+$ and Tom$^-$ cells.

(C) Proportion of Tom$^+$ *p21*^{high} cells in all cells, preadipocytes, endothelial cells, and macrophages.

(D) Representative micrographs of 5 tissues in PT mice (3 images per 3 biological replicates in each group). Red: tdTomato, Blue: DAPI. Scale bar = 25 μm.

For B, $n = 5$ for both groups. For C, $n = 3$ for PT-RCD, $n = 4$ for PT-HFD. For B and C, n represents the number of biological replicates with 1 technical replicate. Results are shown as mean ± s.e.m. * $P < 0.05$; two-tailed Welch's t-test.
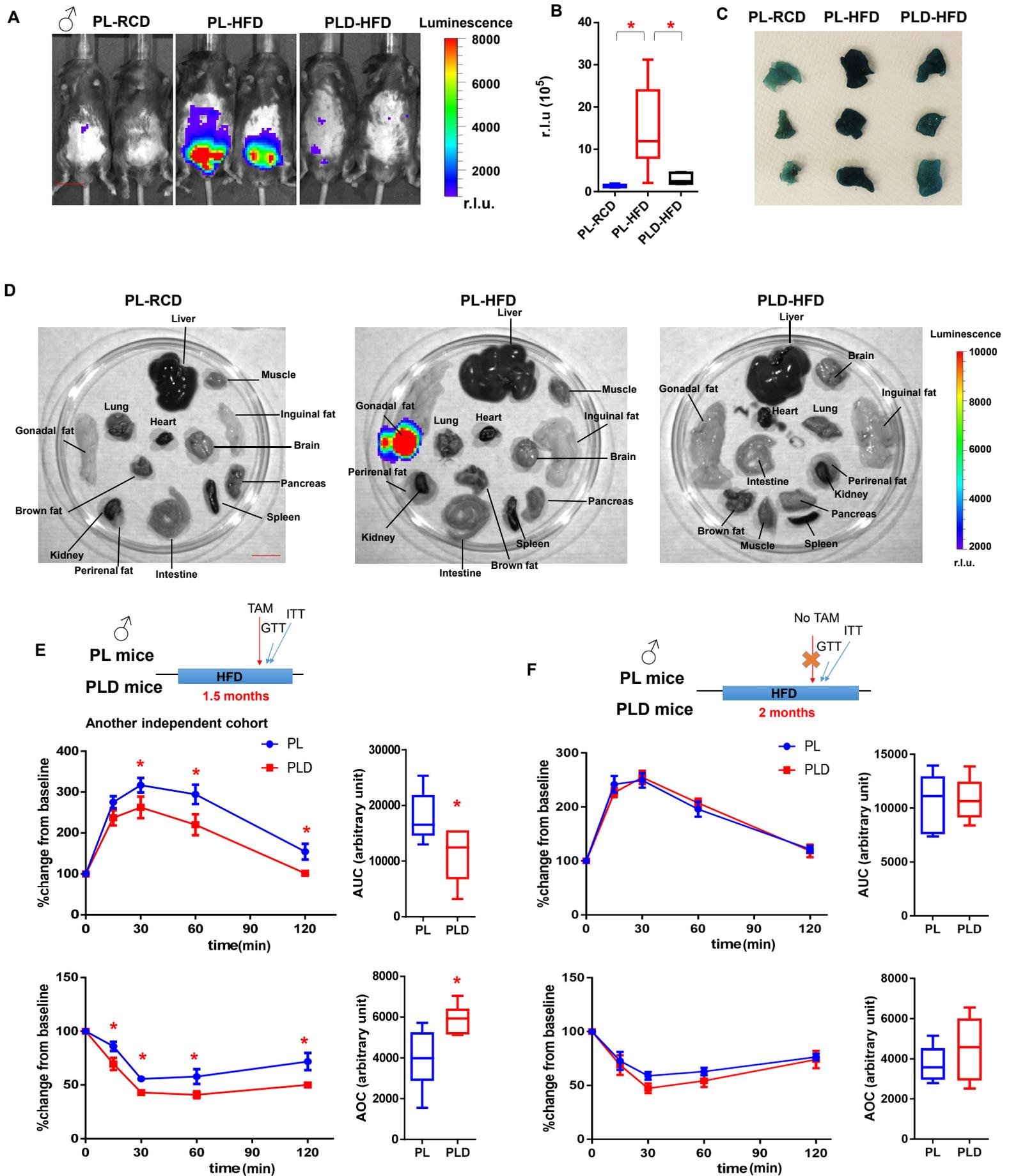
A ♂ PL-RCD PL-HFD PLD-HFD Luminescence
8000
6000
4000
2000
r.l.u.

B
r.l.u (10⁵)
40
30
20
10
*   *
PL-RCD PL-HFD PLD-HFD

C PL-RCD PL-HFD PLD-HFD

D

PL-RCD
Liver
Muscle
Lung
Heart
Inguinal fat
Gonadal fat
Brain
Pancreas
Brown fat
Spleen
Kidney
Perirenal fat
Intestine

PL-HFD
Liver
Muscle
Gonadal fat
Lung
Heart
Inguinal fat
Brain
Perirenal fat
Pancreas
Kidney
Spleen
Brown fat
Intestine

PLD-HFD
Liver
Gonadal fat
Brain
Heart
Lung
Inguinal fat
Intestine
Perirenal fat
Kidney
Brown fat
Pancreas
Muscle
Spleen

Luminescence
10000
8000
6000
4000
2000
r.l.u.

E ♂ PL mice / PLD mice
TAM / GTT / ITT
HFD
1.5 months

Another independent cohort
%change from baseline
400
300
200
100
*   *   *   *
PL (blue), PLD (red)
0 30 60 90 120
time(min)

AUC (arbitrary unit)
30000
20000
10000
*
PL PLD

%change from baseline
150
100
50
*   *   *   *
0 30 60 90 120
time(min)

AOC (arbitrary unit)
8000
6000
4000
2000
*
PL PLD

F ♂ PL mice / PLD mice
No TAM / GTT / ITT
HFD
2 months

%change from baseline
300
200
100
PL (blue), PLD (red)
0 30 60 90 120
time(min)

AUC (arbitrary unit)
15000
10000
5000
PL PLD

%change from baseline
150
100
50
0 30 60 90 120
time (min)

AOC (arbitrary unit)
8000
6000
4000
2000
PL PLD

Figure S4

Figure S4. Clearance of *p21*^{high} cells in gVAT alleviates metabolic dysfunction induced by HFD. Related to Figure 2.

(A) Representative images of LUC activity in male PL-RCD ($n = 6$), PL-HFD ($n = 8$), and PLD-HFD ($n = 8$) mice (1 image per 6-8 biological replicates). r.l.u., relative luciferase units. Scale bar = 15 mm.

 (B) Quantification of LUC activity. Part of Figure S4B was also shown in Figure 4C.

(C) Representative images of SA-β-gal staining for gVAT (1 image per 6 biological replicates in each group).

(D) Representative images of LUC activity in various tissues. One image per 6 (RCD) or 8 (HFD) biological replicates. Scale bar = 10 mm.

(E) Experimental design, GTT curve (mean ± s.e.m.), AUC, ITT curve (mean ± s.e.m.), and AOC in another independent cohort of PL and PLD mice on a HFD.

(F) Experimental design, GTT curve (mean ± s.e.m.), AUC, ITT curve (mean ± s.e.m.), and AOC in PL and PLD mice on a HFD without TAM administration.

For B, $n = 6$ for PL-RCD, $n = 8$ for PL-HFD, and $n = 8$ for PLD-HFD. For E, $n = 6$ for both groups. For F, $n = 5$ for both groups. For B, E and F, n represents the number of biological replicates with 1 technical replicate. Results were shown as box-and-whisker plots, where a box extends from the 25th to 75th percentile with the median shown as a line in the middle, and whiskers indicate the smallest and largest values. * $P < 0.05$ vs PL-HFD by one-way ANOVA (B), by two-way ANOVA (GTT and ITT curves), by two-tailed Welch's t-test (AUC and AOC).
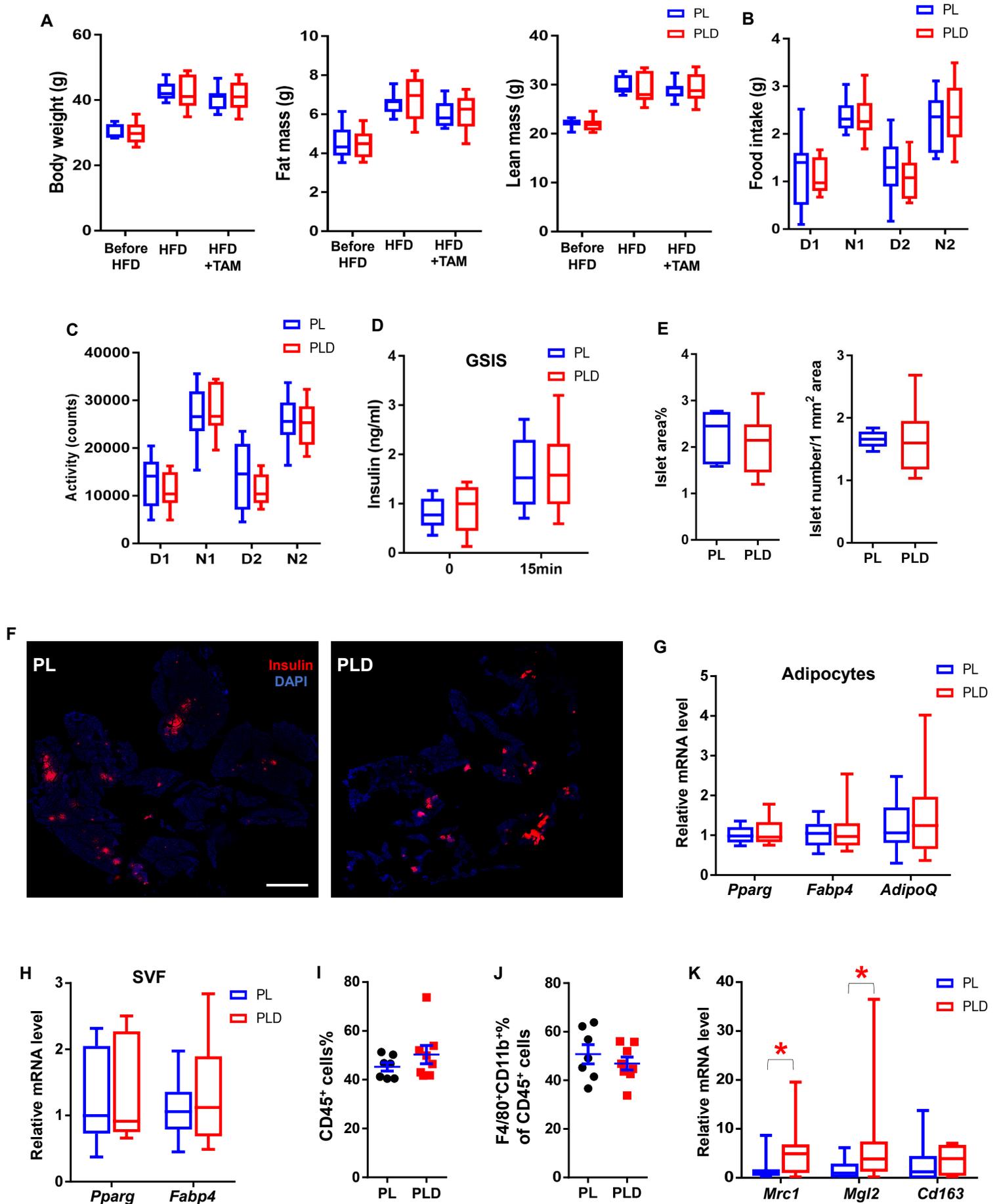
Figure S5

Figure S5. Clearance of *p21*^{high} cells does not substantially affect food intake, activity, or pancreatic β-cell function. Related to Figure 2.

(A) Body composition in PL and PLD mice before HFD feeding, 2 months after HFD feeding but before TAM treatment, and 5 days after TAM treatment.

(B-C) Food intake (B) and activity (C) during daytime (D) and night (N) for 2 days in HFD-fed PL and PLD mice.

(D) Plasma insulin level at baseline and 15 minutes after glucose injection in HFD-fed PL and PLD mice.

(E) Islet area (insulin staining positive area) over total pancreatic area and islet number in HFD-fed PL and PLD mice.

(F) Representative images of insulin staining of pancreatic tissues from HFD-fed PL and PLD mice (1 image per 6 biological replicates in each group). Red: insulin, Blue: DAPI. Scale bar = 1 mm.

(G-K) PL and PLD mice were fed with HFD for 2 months, and then treated with TAM. (G) Relative mRNA abundance of adipogenesis genes in adipocytes and (H) in SVF. (I) Proportion of CD45$^+$ cells. (J) Proportion of macrophages (F4/80$^+$CD11b$^+$ of CD45$^+$ cells). (K) Relative mRNA expression for 3 key M2 macrophage markers in SVF.

For A-D, n = 8 for both groups. For E, n = 6 for both groups. For G, H and K, n = 11 for both groups. For I and J, n = 7 for PL, and n = 8 for PLD. For A-E, G-K, n represents the number of biological replicates with 1 technical replicate. Results were shown as box-and-whisker plots,

where a box extends from the 25th to 75th percentile with the median shown as a line in the middle, and whiskers indicate the smallest and largest values. * $P < 0.05$; two-tailed Welch's t-test.

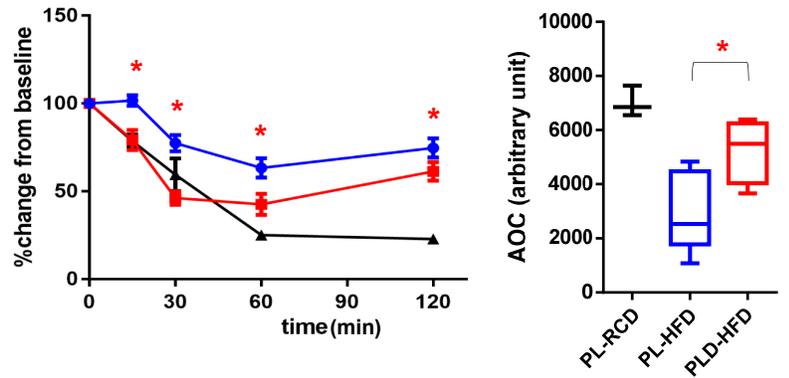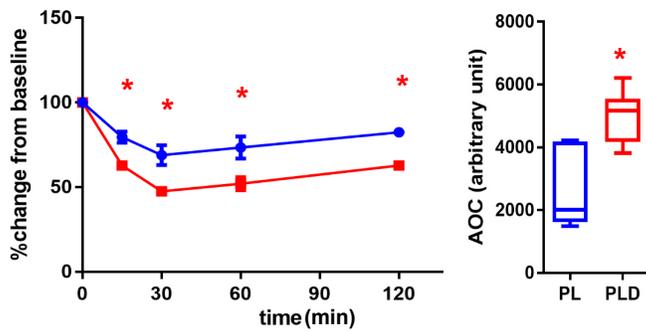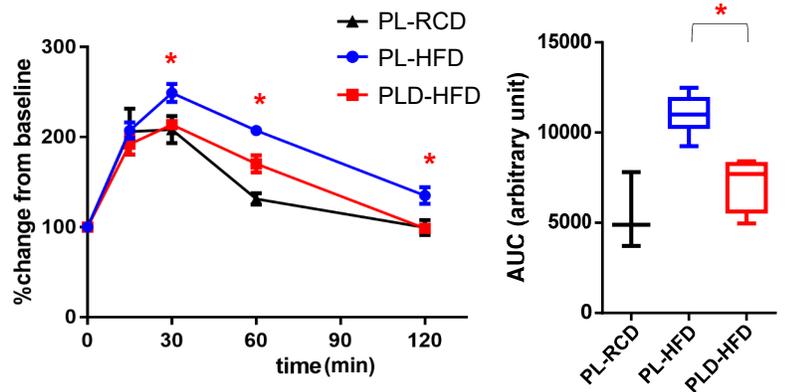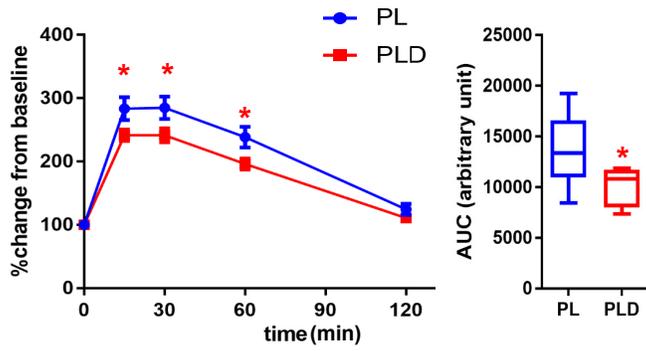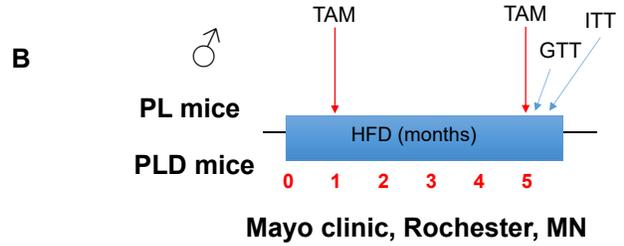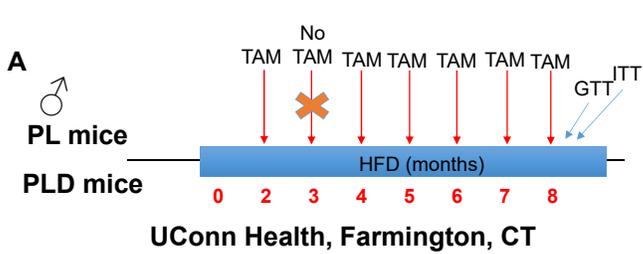Figure S6

Figure S6. Intermittent clearance of *p21*^high cells has long-term protective benefit for metabolic function with obesity. Related to Figure 3.

(A) Experimental design, GTT curve (mean ± s.e.m.), AUC, ITT curve (mean ± s.e.m.), and AOC in PL and PLD mice after 8 months of HFD feeding. One mouse died in each group during the 8 months.

(B) Experimental design, GTT curve (mean ± s.e.m.), AUC, ITT curve (mean ± s.e.m.), and AOC in RCD-fed PL, HFD-fed PL and HFD-fed PLD mice. Notably, these experiments were performed at Mayo Clinic while the rest of the experiments in this study were performed at UConn Health.

For A, $n = 7$ for both groups. For B, $n = 3$ for PL-RCD, $n = 7$ for PL-HFD, $n = 4$ for PLD-HFD. *n* represents the number of biological replicates with 1 technical replicate. Results were shown as box-and-whisker plots, where a box extends from the 25th to 75th percentile with the median shown as a line in the middle, and whiskers indicate the smallest and largest values. * $P < 0.05$ vs PL-HFD by two-tailed Welch's t-test (AUC and AOC in (A)), by one-way ANOVA (AUC and AOC in (B)), by two-way ANOVA (GTT and ITT curves).
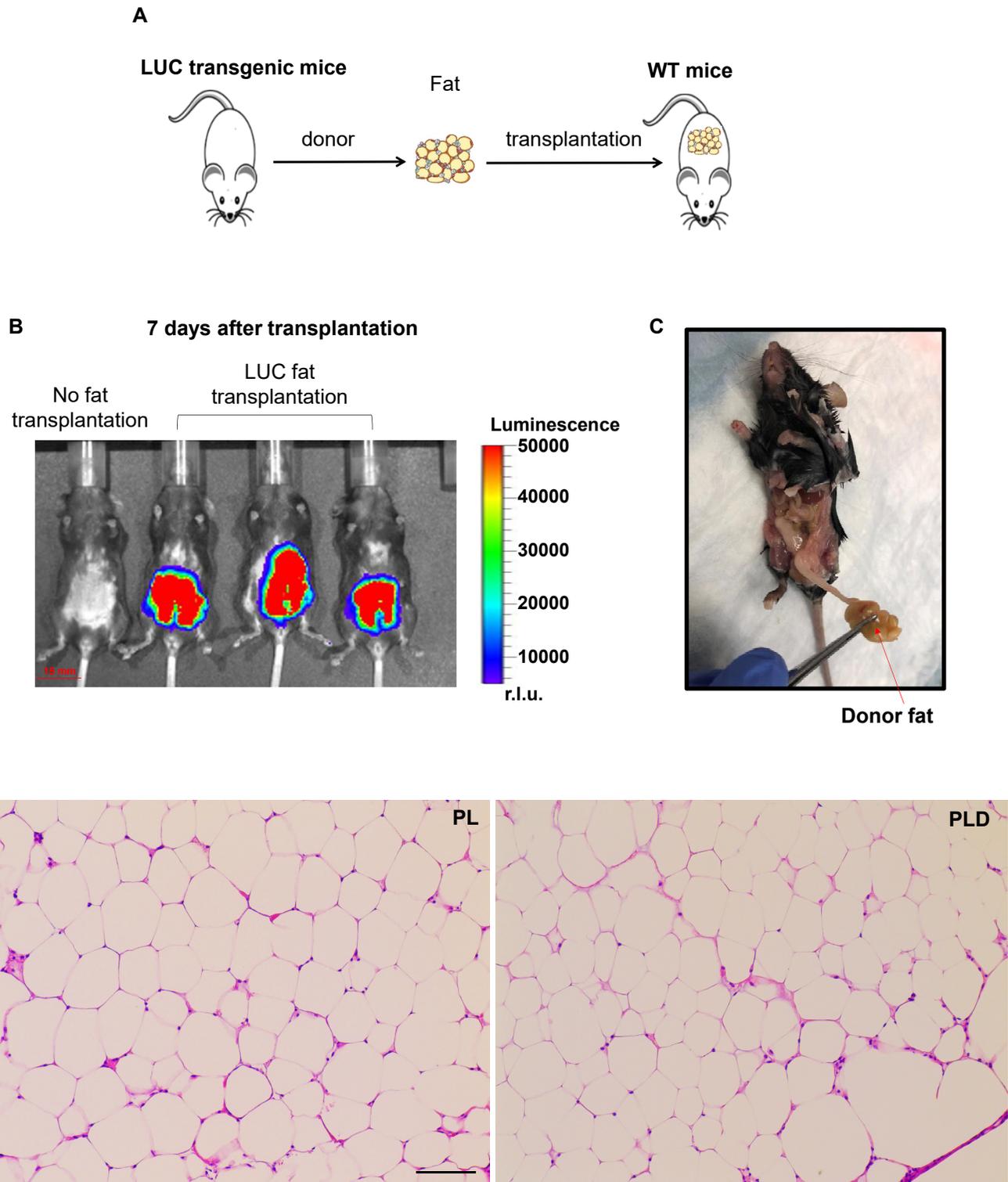
A

LUC transgenic mice → Fat (donor) → transplantation → WT mice

B

7 days after transplantation

No fat transplantation

LUC fat transplantation

Luminescence

50000

40000

30000

20000

10000

r.l.u.

C

Donor fat

D

PL

PLD

Figure S7

Figure S7. VAT remains viable and biologically active in the host after transplantation. Related to Figure 6.

(A) Experimental design.

(B) Representative images of LUC activity in WT mice transplanted with LUC VAT or PBS (6 biological replicates with 1 technical replicate in each group).

(C) Representative photograph of donor VAT 2 months after transplantation (3 biological replicates with 1 technical replicate in each group). Donor VAT remained connected to the host adipose tissue.

(D) Representative H&E-stained micrographs of donor PL and PLD VAT 2 months after transplantation (3 images per 3 biological replicates in each group). Scale bar = 100 μm.

Table S1. Gender, age, racial and BMI information of the study participants, related to STAR methods.

Donor ID	Gender	Age	Racial	BMI
Donor patient OM-1	Female	28	Caucasian	37.08
Donor patient OM-2	Female	49	Caucasian	52.5
Donor patient OM-3	Female	68	Caucasian	41.73
Donor patient OM-4	Male	62	Caucasian	42.38