Figure S1

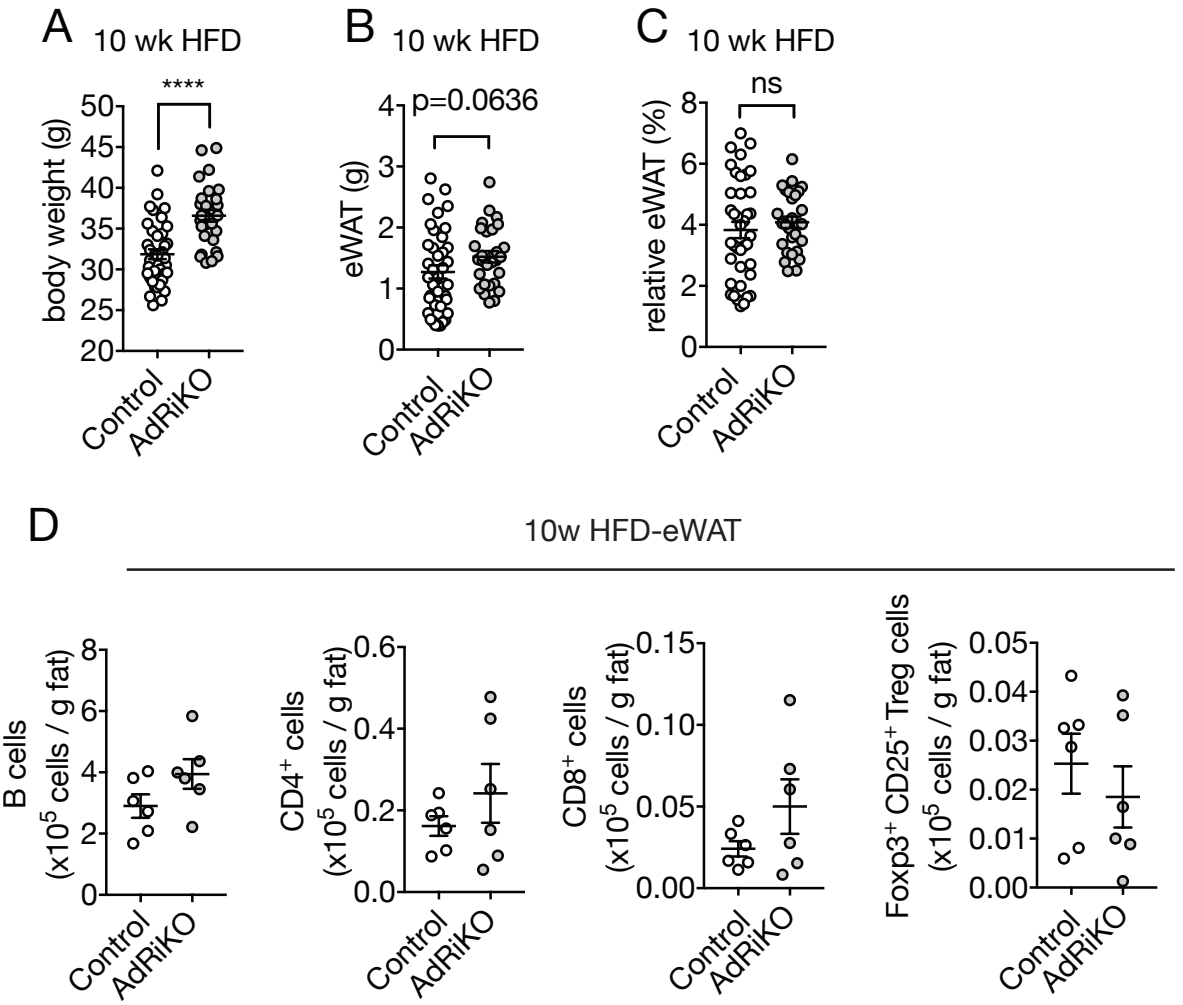

A
10 wk HFD
****
body weight (g)
50
45
40
35
30
25
20
Control
AdRiKO
B
10 wk HFD
p=0.0636
eWAT (g)
4
3
2
1
0
Control
AdRiKO
C
10 wk HFD
ns
relative eWAT (%)
8
6
4
2
0
Control
AdRiKO
D
10w HFD-eWAT
B cells
(x10^5 cells / g fat)
8
6
4
2
0
Control
AdRiKO
CD4+ cells
(x10^5 cells / g fat)
0.6
0.4
0.2
0.0
Control
AdRiKO
CD8+ cells
(x10^5 cells / g fat)
0.15
0.10
0.05
0.00
Control
AdRiKO
Foxp3+ CD25+ Treg cells
(x10^5 cells / g fat)
0.05
0.04
0.03
0.02
0.01
0.00
Control
AdRiKO

Figure S1. Body weight and adiposity of HFD-fed control and AdRiKO mice, and B and T cells in their eWAT.
(**A** and **B)** Body weight (A) and eWAT mass (B) of HFD-fed AdRiKO or control mice. **** $P < 0.0001$, by unpaired Student's *t* test. $n = 39$ (control) and 28 (AdRiKO).
(**C**) Relative eWAT mass was calculated as eWAT mass normalized to body mass. ns (not significant), by unpaired Student's *t* test. $n = 39$ (control) and 28 (AdRiKO).
(**D**) Numbers of B cells ($B220^+$) and $CD4^+$ T cells ($CD3^+$ $CD4^+$), $CD8^+$ T cells ($CD3^+$ $CD8^+$), and Treg ($CD3^+$ $CD4^+$ $FOXP3^+$ $CD25^+$) cells in stromal vascular cells (SVCs) isolated from eWAT of HFD-fed AdRiKO or control mice. $n = 6$. Data are presented as mean ± SEM.

Figure S2

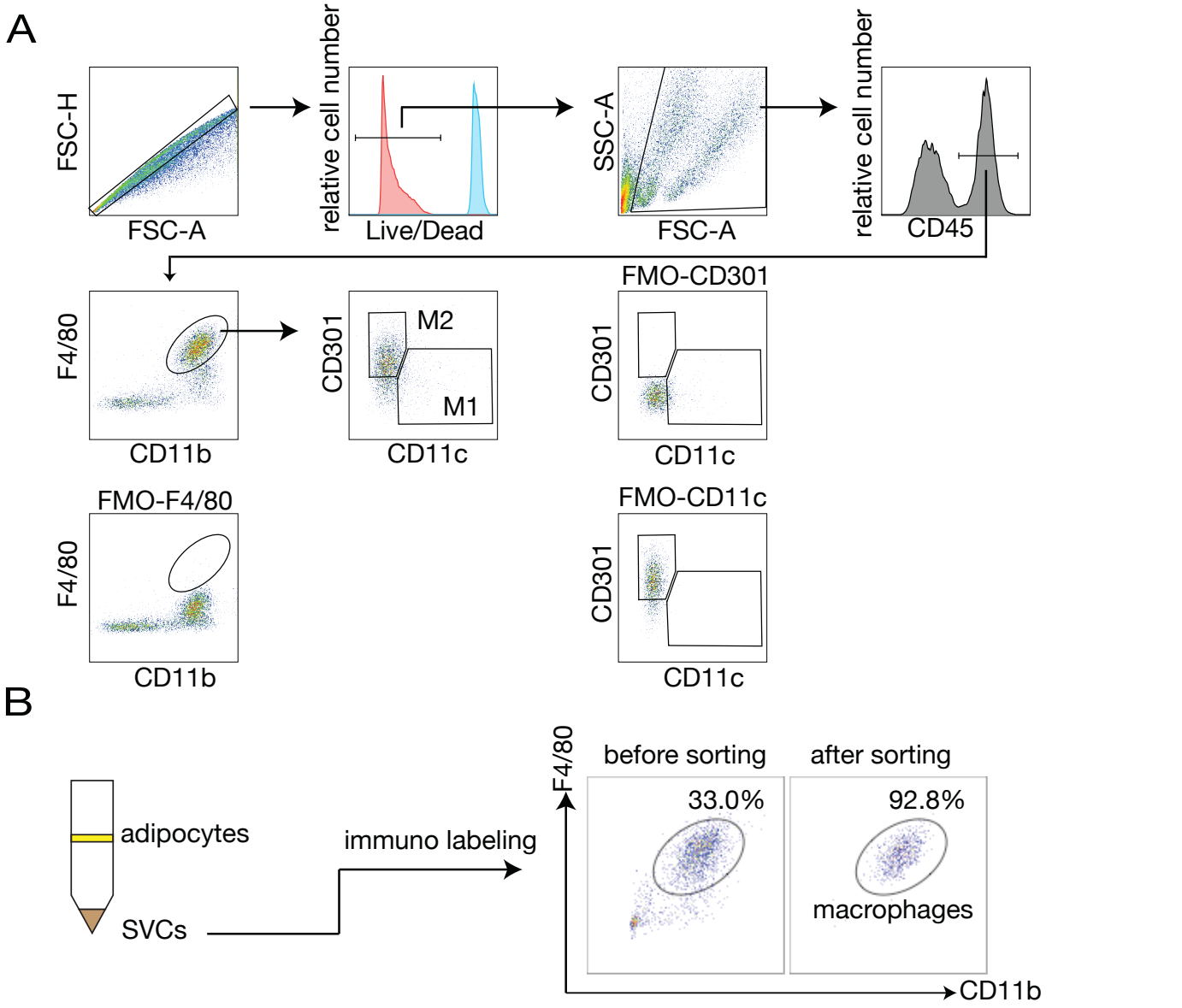

A
FSC-H
FSC-A
relative cell number
Live/Dead
SSC-A
FSC-A
relative cell number
CD45
F4/80
CD11b
CD301
M2
M1
CD11c
FMO-CD301
CD301
CD11c
FMO-F4/80
F4/80
CD11b
FMO-CD11c
CD301
CD11c
B
adipocytes
SVCs
immuno labeling
before sorting
after sorting
F4/80
33.0%
92.8%
macrophages
CD11b
C
Lep mRNA (A.U.)
Cd31 mRNA (A.U.)
Cd45 mRNA (A.U.)
Control
AdRiKO
Adipoq mRNA (A.U.)
F4/80 mRNA (A.U.)
Cd3g mRNA (A.U.)
SVCs adipocytes
SVCs adipocytes
SVCs adipocytes

Figure S2. FACS gating strategy for adipose tissue macrophages and isolation of SVCs and macrophages.
(**A**) FACS gating. Fluorescence minus one (FMO) controls are provided to validate the specificity of the staining.
(**B**) Schematic view for isolation of adipocytes, SVCs, and macrophages. After low speed centrifugation, adipocytes were collected, and macrophages ($CD45^+$ $F4/80^+$ $CD11b^+$) were sorted from SVCs.
(**C**) mRNA levels of *Lep*, *Adipoq*, *F4/80*, *Cd31*, *Cd45*, and *Cd3g* to validate fractionation of adipocytes and SVCs. n = 13-14. Data are presented as mean ± SEM.

Figure S3

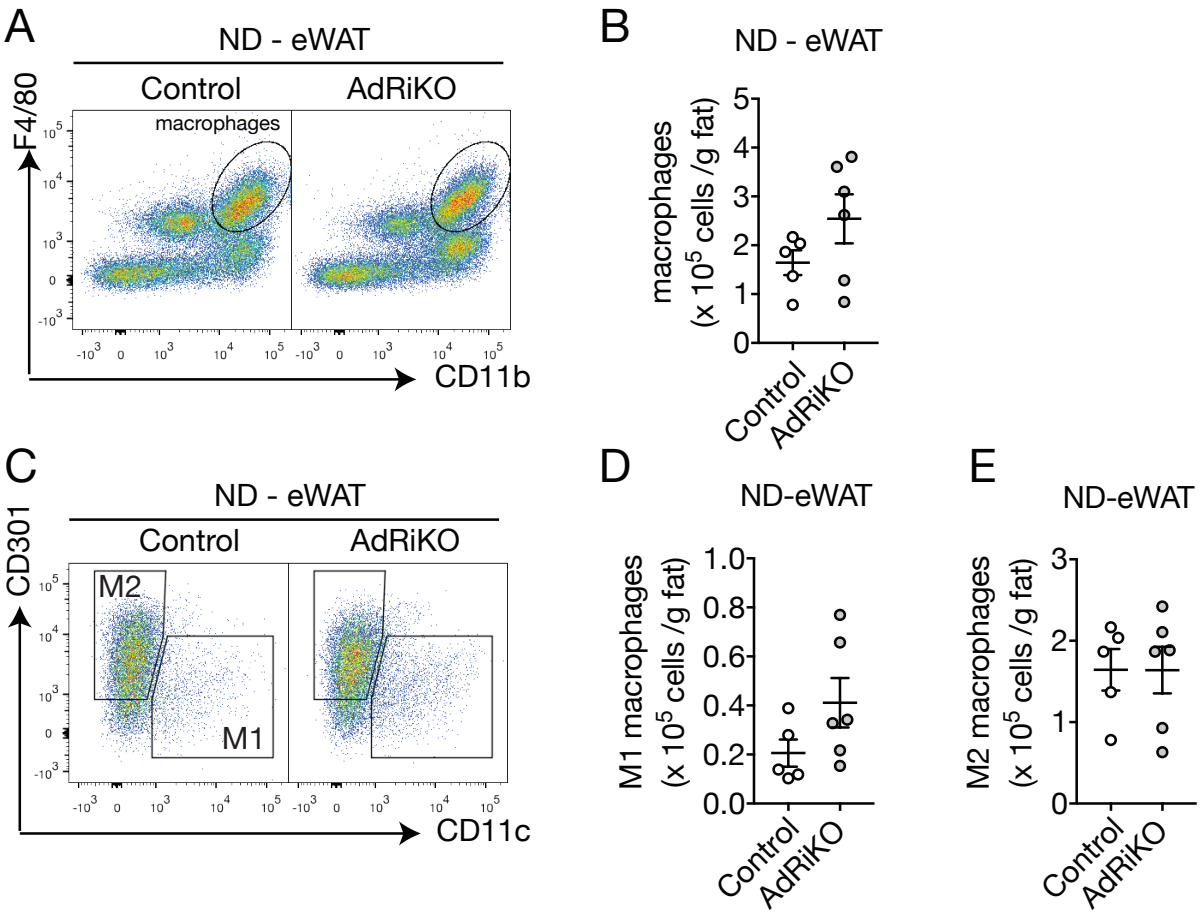

A
ND - eWAT
Control
AdRiKO
macrophages
F4/80
CD11b
B
ND - eWAT
macrophages
(x 10^5 cells /g fat)
Control
AdRiKO
C
ND - eWAT
Control
AdRiKO
M2
M1
CD301
CD11c
D
ND-eWAT
M1 macrophages
(x 10^5 cells /g fat)
Control
AdRiKO
E
ND-eWAT
M2 macrophages
(x 10^5 cells /g fat)
Control
AdRiKO

Figure S3. AdRiKO eWAT does not accumulate macrophages in a ND-fed condition.
(**A** and **B**) Numbers of macrophages ($CD45^+$ $F4/80^+$ $CD11b^+$) in stromal vascular cells (SVCs) isolated from eWAT of ND-fed AdRiKO or control mice. Representative FACS profiles are shown in **A**, and quantification is shown in **B**. n = 5-6.
(**C-E**) Numbers of M1 macrophages ($CD45^+$ $F4/80^+$ $CD11b^+$ $CD11c^+$) and M2 macrophages ($CD45^+$ $F4/80^+$ $CD11b^+$ $CD301^+$) in SVCs from eWAT of AdRiKO or control mice fed with ND. Representative FACS profiles are shown in **C**, and quantification is shown in **D** and **E**. n = 5-6. Data are presented as mean ± SEM.

Figure S4

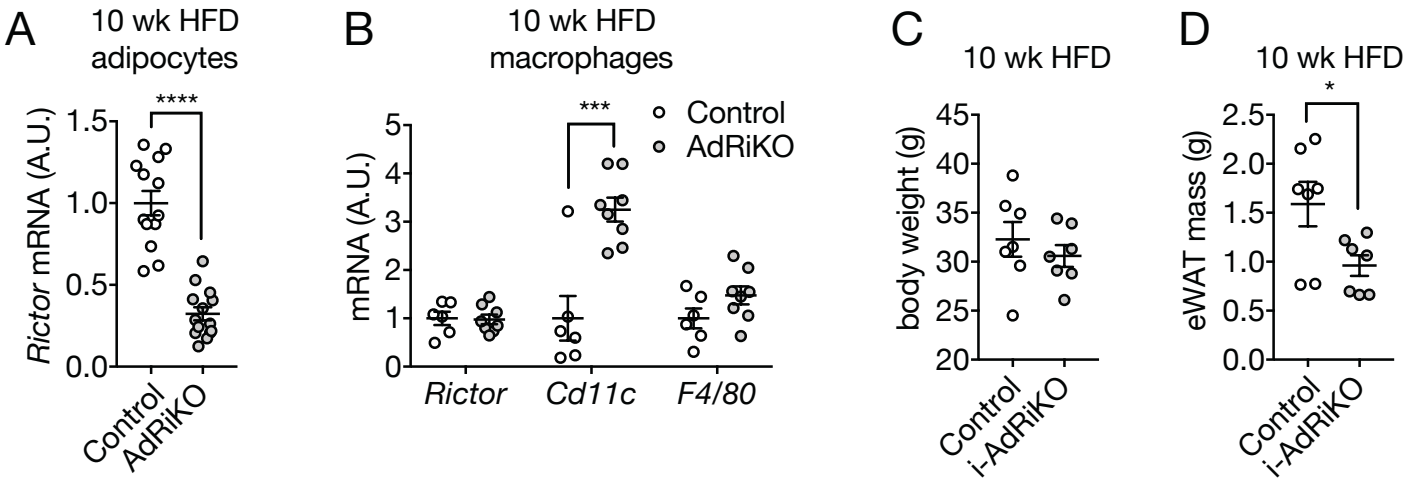

A
10 wk HFD
adipocytes
Rictor mRNA (A.U.)
****
Control
AdRiKO
B
10 wk HFD
macrophages
mRNA (A.U.)
***
Control
AdRiKO
Rictor
Cd11c
F4/80
C
10 wk HFD
body weight (g)
Control
i-AdRiKO
D
10 wk HFD
eWAT mass (g)
*
Control
i-AdRiKO

Figure S4. Macrophage accumulation in AdRiKO eWAT is not due to confounding effects of ectopic knockout of *Rictor* in macrophages.
(**A**) *Rictor* mRNA level in adipocytes isolated from HFD-fed AdRiKO or control mice. . **** $P < 0.0001$, by unpaired Student's *t* test. $n = 13\text{-}14$.
(**B**) *Rictor*, *Cd11c*, and *F4/80* mRNA levels in macrophages isolated from HFD-fed AdRiKO or control mice. *** $P < 0.001$, by unpaired Student's *t* test. $n = 6\text{-}8$.
(**C** and **D**) Body weight (**C**) and eWAT mass (**D**) of HFD-fed i-AdRiKO or control mice. * $P < 0.05$, unpaired Student's *t* test. $n = 7$. Data are presented as mean ± SEM

Figure S5

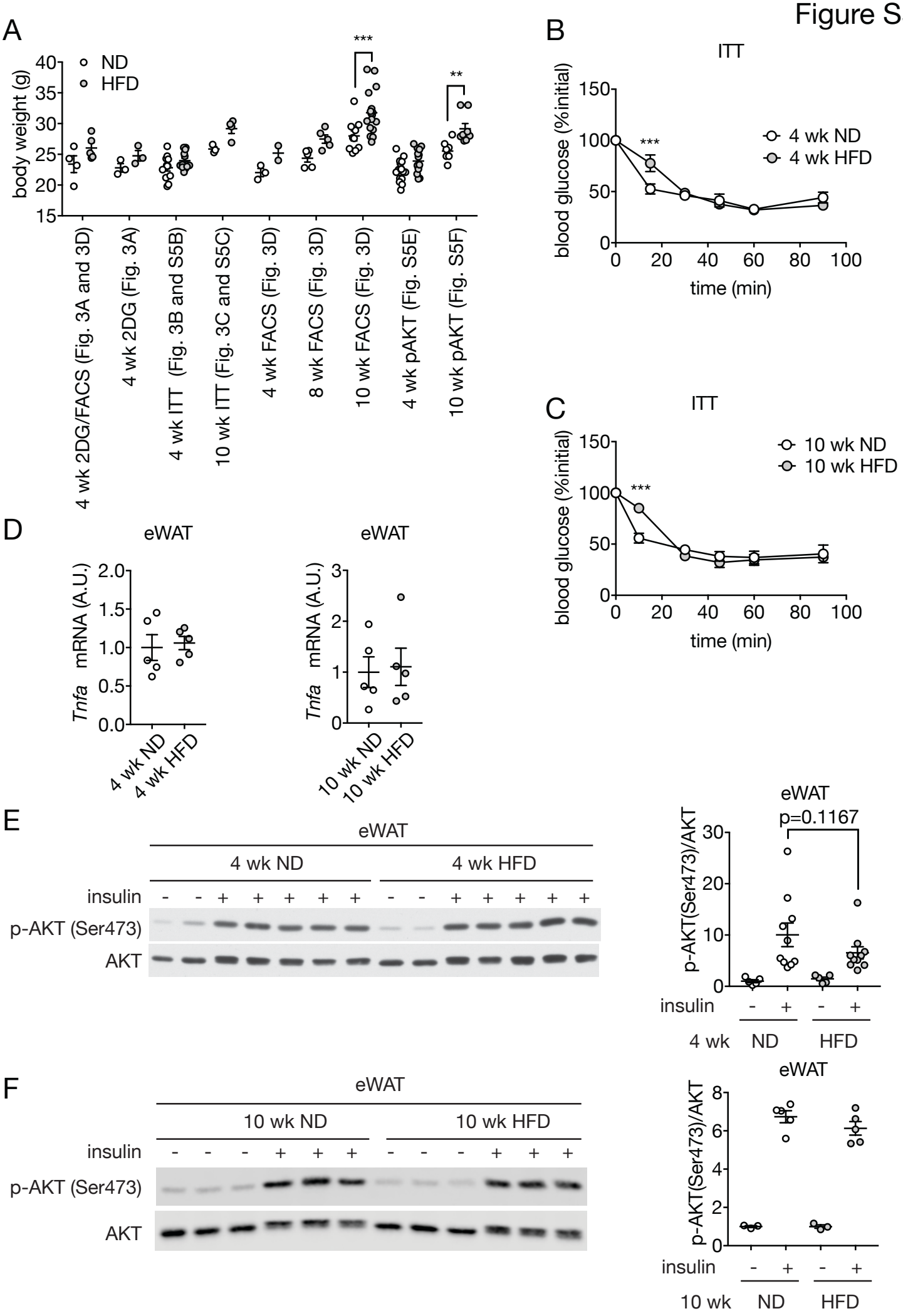

A
ND
HFD
body weight (g)
***
**
4 wk 2DG/FACS (Fig. 3A and 3D)
4 wk 2DG (Fig. 3A)
4 wk ITT (Fig. 3B and S5B)
10 wk ITT (Fig. 3C and S5C)
4 wk FACS (Fig. 3D)
8 wk FACS (Fig. 3D)
10 wk FACS (Fig. 3D)
4 wk pAKT (Fig. S5E)
10 wk pAKT (Fig. S5F)
B
ITT
blood glucose (%initial)
4 wk ND
4 wk HFD
time (min)
C
ITT
10 wk ND
10 wk HFD
D
eWAT
Tnfa mRNA (A.U.)
4 wk ND
4 wk HFD
10 wk ND
10 wk HFD
E
eWAT
4 wk ND
4 wk HFD
insulin
p-AKT (Ser473)
AKT
p-AKT(Ser473)/AKT
p=0.1167
4 wk ND HFD
F
eWAT
10 wk ND
10 wk HFD
10 wk ND HFD

Figure S5. Related to Figure 3.
(**A**) Body weight of WT mice used for a longitudinal study. *** $P < 0.001$, by multiple Student's *t* test.
(**B**) ITT for WT mice fed a ND or HFD for 4 weeks. Blood glucose levels were normalized to initial glucose levels. Two-way ANOVA, *** $P < 0.001$, by 2-way ANOVA. $n = 15$ (ND) and 17 (HFD).
(**C**) ITT for WT mice fed a ND or HFD for 10 weeks. Blood glucose levels were normalized to the initial glucose levels. *** $P < 0.001$, by 2-way ANOVA. $n = 3$ (ND) and 4 (HFD).
(**D**) *Tnfa* mRNA level in eWAT from mice fed a ND or HFD for 4 or 10 weeks. not significant, by unpaired Student's *t* test. $n = 5$.
(**E** and **F**) Immunoblots of eWAT from WT mice fed with ND and HFD for 4 weeks (**E**) or 10 weeks (**F**). Mice were fasted for 5 hours, and then treated with PBS or insulin. p-AKT (Ser473) signals were normalized to AKT. 1-way ANOVA. $n = 5$-10 (4 weeks) and $n = 3$-5 (10 weeks). Data are presented as mean ± SEM.

Figure S6

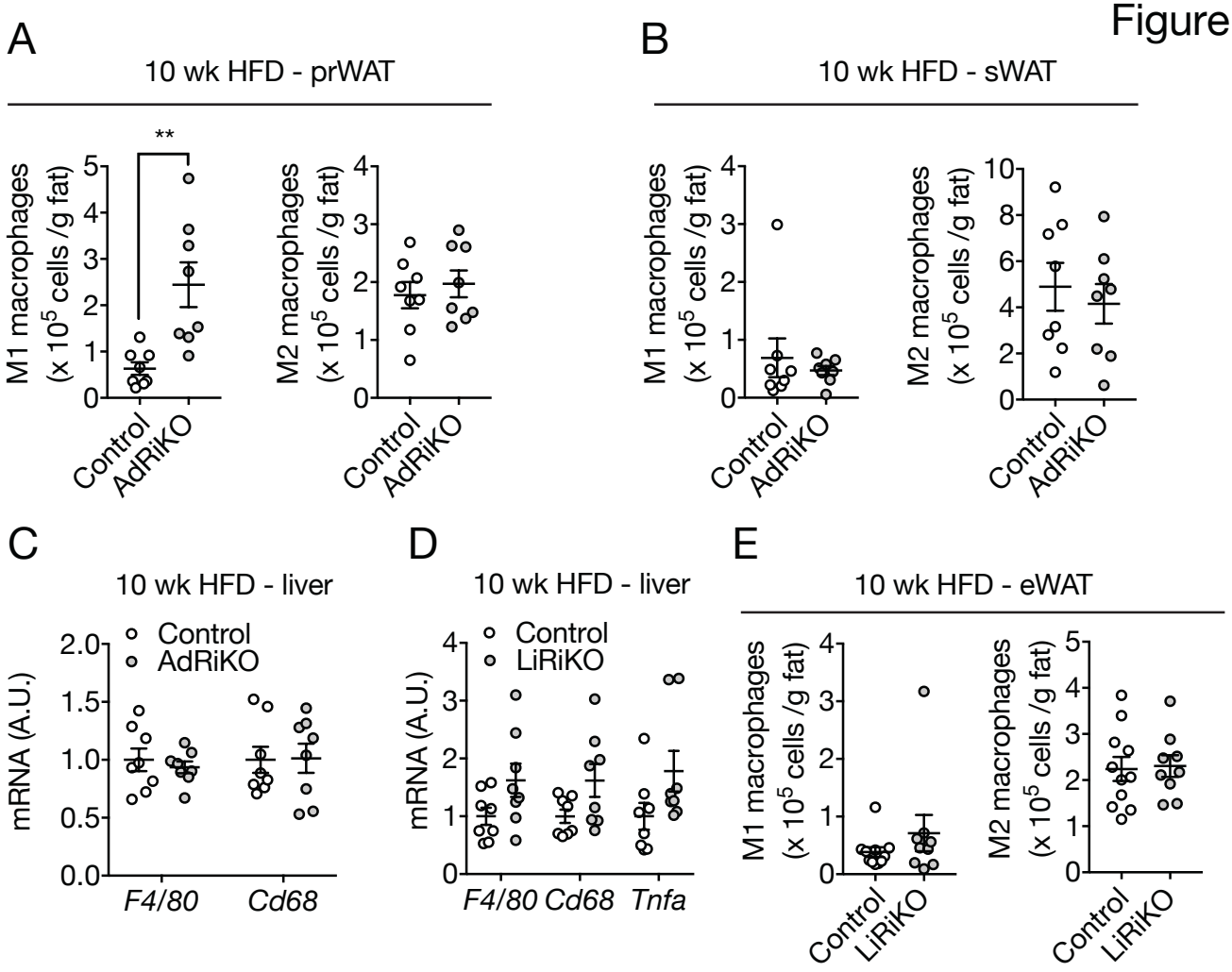

A
10 wk HFD - prWAT
M1 macrophages (x 10^5 cells /g fat)
M2 macrophages (x 10^5 cells /g fat)
**
Control
AdRiKO
B
10 wk HFD - sWAT
C
10 wk HFD - liver
mRNA (A.U.)
Control
AdRiKO
F4/80
Cd68
D
10 wk HFD - liver
Control
LiRiKO
F4/80
Cd68
Tnfa
E
10 wk HFD - eWAT
Control
LiRiKO

Figure S6. Loss of insulin-mTORC2 signaling in adipocytes causes accumulation of M1 macrophages in visceral WAT.
(**A**) Numbers of M1 macrophages ($CD45^+$ $F4/80^+$ $CD11b^+$ $CD11c^+$) and M2 macrophages ($CD45^+$ $F4/80^+$ $CD11b^+$ $CD301^+$) in SVCs isolated from peri-renal WAT (prWAT) of AdRiKO or control mice fed a HFD for 10 weeks. ** $P < 0.01$, by unpaired Student's *t* test. $n = 8$.
(**B**) Numbers of M1 macrophages ($CD45^+$ $F4/80^+$ $CD11b^+$ $CD11c^+$) and M2 macrophages ($CD45^+$ $F4/80^+$ $CD11b^+$ $CD301^+$) in SVCs isolated from subcutaneous WAT (sWAT) of AdRiKO or control mice fed a HFD for 10 weeks. $n = 8$.
(**C**) mRNA levels of *F4/80* and *Cd68* in liver of AdRiKO or control mice fed a HFD for 10 weeks. $n = 8$.
(**D**) mRNA levels of *F4/80*, *Cd68*, and *Tnfa* in liver of liver-specific *Rictor*-knockout (LiRiKO) or control mice fed a HFD for 10 weeks. $n = 8$.
(**E**) Numbers of M1 macrophages ($CD45^+$ $F4/80^+$ $CD11b^+$ $CD11c^+$) and M2 macrophages ($CD45^+$ $F4/80^+$ $CD11b^+$ $CD301^+$) in SVCs isolated from eWAT of LiRiKO or control mice fed a HFD for 10 weeks. $n = 9$-11. Data are presented as mean ± SEM.

Figure S7

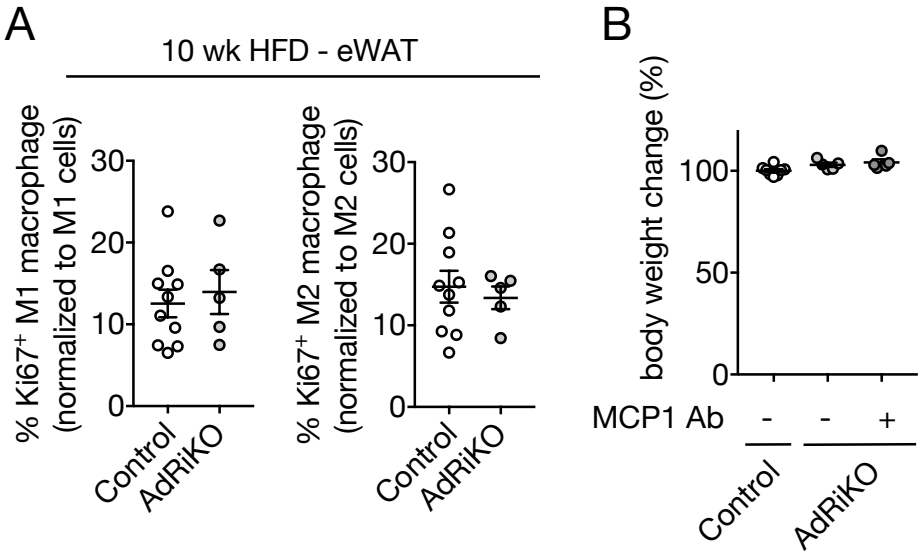

A
10 wk HFD - eWAT
% Ki67+ M1 macrophage (normalized to M1 cells)
30
20
10
0
Control
AdRiKO
% Ki67+ M2 macrophage (normalized to M2 cells)
30
20
10
0
Control
AdRiKO
B
body weight change (%)
100
50
0
MCP1 Ab
-
-
+
Control
AdRiKO

Figure S7. Related to Figure 4. MCP1 is responsible for M1 macrophage accumulation in eWAT of AdRiKO mice fed a HFD.
(**A**) Percentage of $Ki67^+$ M1 macrophages ($CD45^+$ $F4/80^+$ $CD11b^+$ $CD11c^+$ $Ki67^+$) and M2 macrophages ($CD45^+$ $F4/80^+$ $CD11b^+$ $CD301^+$ $Ki67^+$) in respective M1 and M2 macrophages from eWAT of AdRiKO or control mice fed a HFD for 10 weeks. n= 5-10.
(**B**) Body weight change before and after an MCP1 neutralizing antibody or control antibody treatment. n = 5-8.

Figure S8

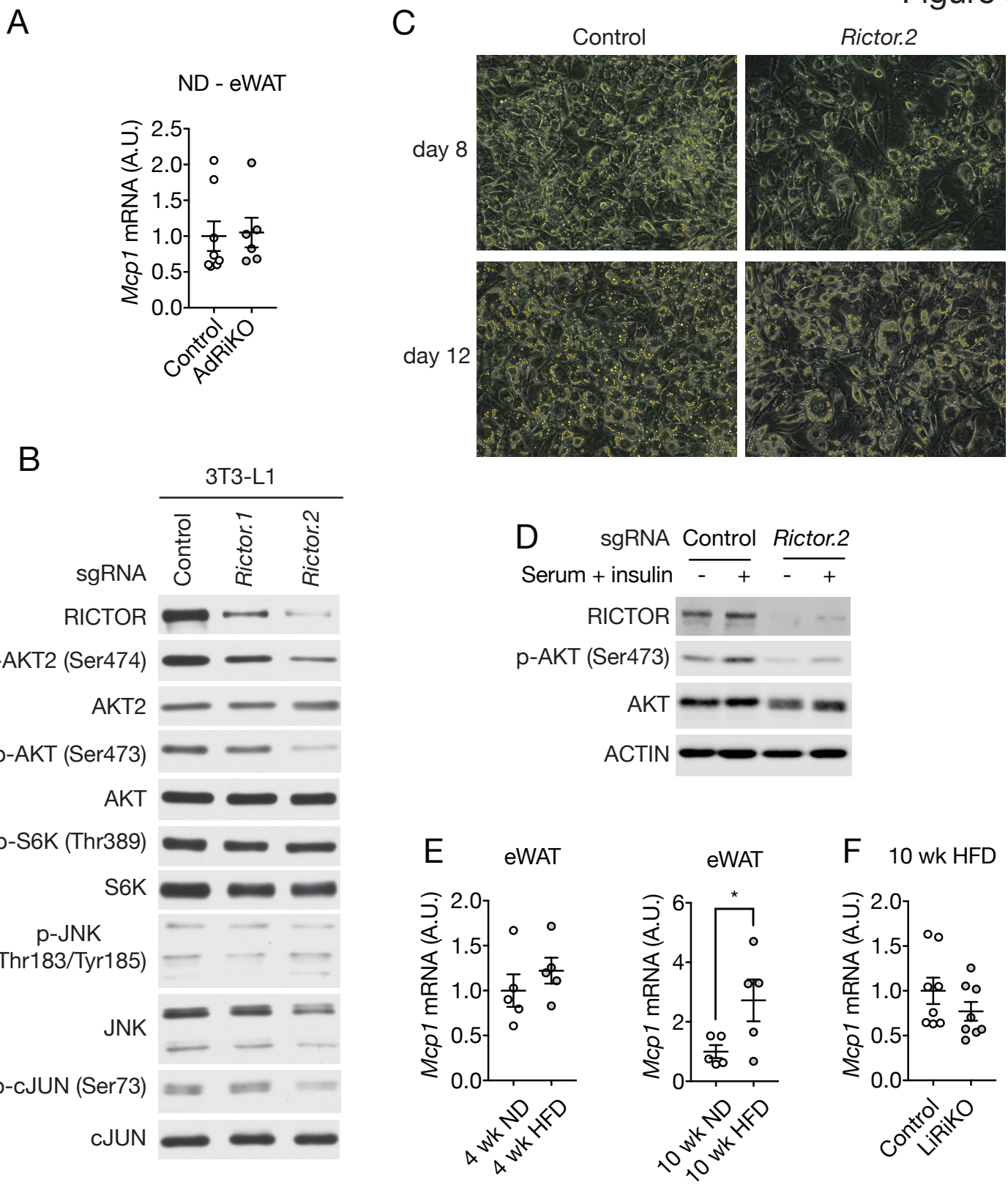

A
ND - eWAT
Mcp1 mRNA (A.U.)
2.5
2.0
1.5
1.0
0.5
0.0
Control
AdRiKO
B
3T3-L1
sgRNA
Control
Rictor.1
Rictor.2
RICTOR
p-AKT2 (Ser474)
AKT2
p-AKT (Ser473)
AKT
p-S6K (Thr389)
S6K
p-JNK (Thr183/Tyr185)
JNK
p-cJUN (Ser73)
cJUN
C
Control
Rictor.2
day 8
day 12
D
sgRNA
Control
Rictor.2
Serum + insulin
- + - +
RICTOR
p-AKT (Ser473)
AKT
ACTIN
E
eWAT
Mcp1 mRNA (A.U.)
2.0
1.5
1.0
0.5
0.0
4 wk ND
4 wk HFD
eWAT
*
6
4
2
0
10 wk ND
10 wk HFD
F
10 wk HFD
Control
LiRiKO

Figure S8. Loss of insulin-mTORC2 signaling leads to *Mcp1* transcription.
(**A**) *Mcp1* mRNA level in eWAT from AdRiKO or control mice fed a ND. n = 6-8
(**B**) Immunoblots of *Rictor* knockout or control 3T3-L1 adipocytes for the indicated proteins. $N = 3$.
(**C**) Bright field images of *Rictor* knockout or control 3T3-L1 adipocytes.
(**D**) Immunoblots of *Rictor* knockout or control 3T3-L1 adipocytes treated with or without serum and insulin. $N = 3$.
(**E**) *Tnfa* mRNA level in eWAT from WT mice fed a ND or HFD for 4 or 10 weeks. * $P < 0.05$, by unpaired Student's t test. $n = 5$.
(**F**) *Mcp1* mRNA level in liver from LiRiKO or control mice fed a HFD for 10 weeks. Not significant by unpaired Student's t test. $n = 8$. Data are presented as mean ± SEM.

Figure S9

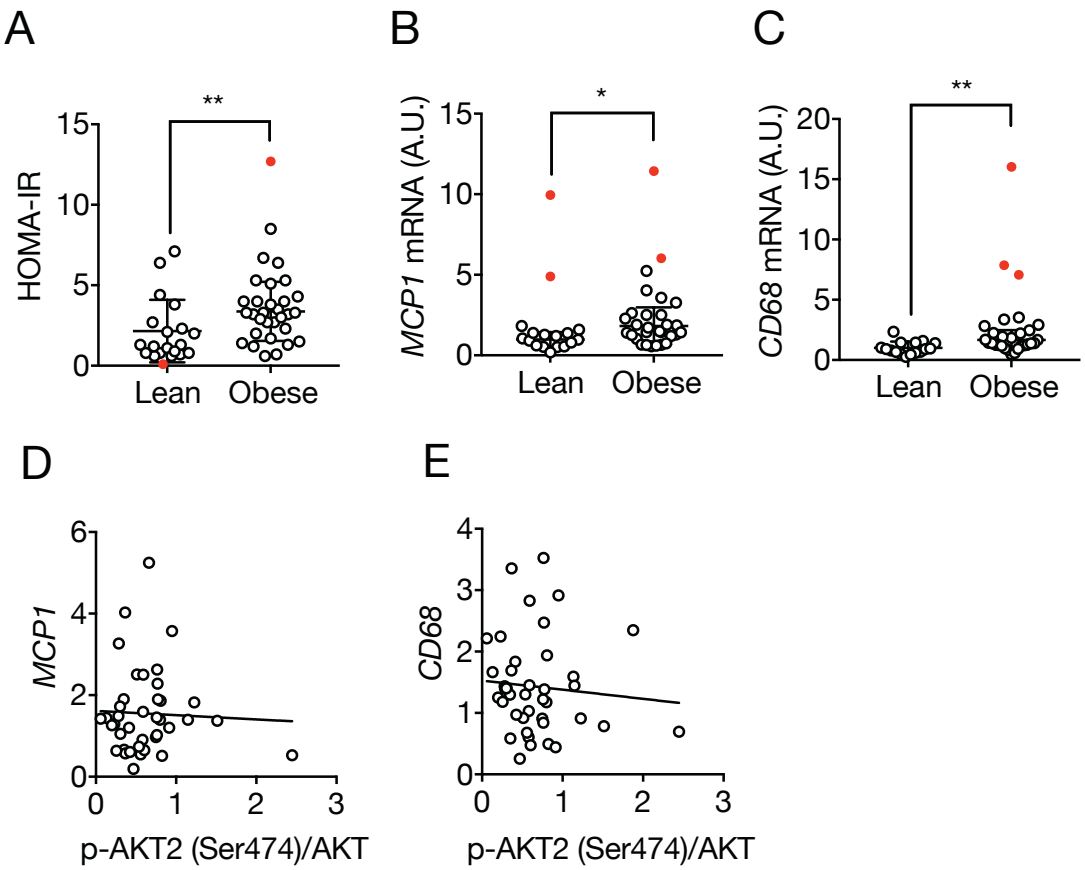

A
B
C
HOMA-IR
MCP1 mRNA (A.U.)
CD68 mRNA (A.U.)
**
*
**
Lean
Obese
D
E
MCP1
CD68
p-AKT2 (Ser474)/AKT
p-AKT2 (Ser474)/AKT

Figure S9. Related to figure 6.
(**A**) HOMA-IR including outliers.
Same plots as in Figure 6B but including outlier values as shown in red dots. Outliers were identified by ROUT outlier test (Q=1%). ** $P < 0.01$, by Mann-Whitney test.
(**B**) *MCP1* mRNA levels including outliers in human oWAT.
Same plots as in Figure 6F but including outlier values as shown in red dots. Outliers were identified by ROUT outlier test (Q=1%). * $P < 0.05$, by Mann-Whitney test.
(**C**) *CD68* mRNA levels including outliers in human oWAT.
Same plots as in Figure 6H but including outlier values as shown in red dots. Outliers were identified by ROUT outlier test (Q=1%). ** $P < 0.01$, by Mann-Whitney test.
(**D** and **E**) p-AKT2 (Ser474)/AKT does not correlate with *MCP1* mRNA (D) and *CD68* (E). Pearson's correlation analysis.

Figure S10

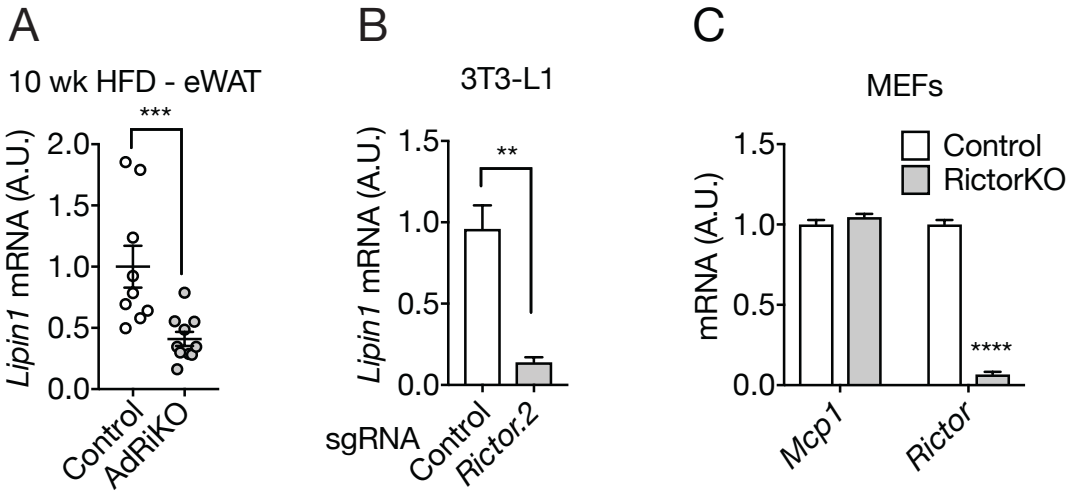
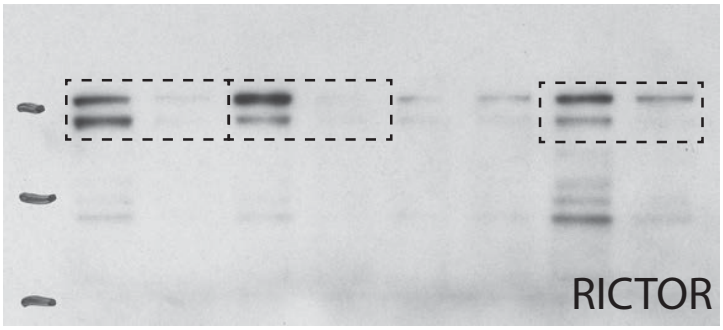
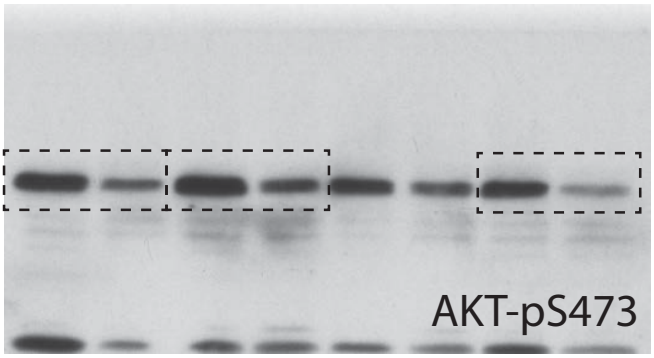
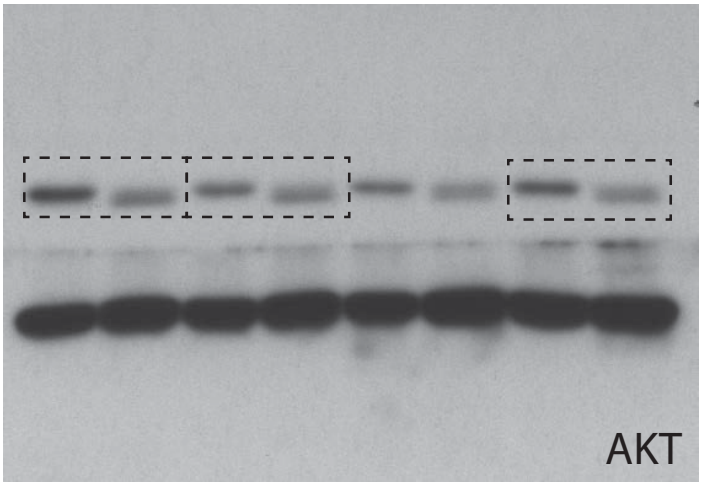

A
10 wk HFD - eWAT
***
Lipin1 mRNA (A.U.)
2.0
1.5
1.0
0.5
0.0
Control
AdRiKO
B
3T3-L1
**
Lipin1 mRNA (A.U.)
1.5
1.0
0.5
0.0
sgRNA
Control
Rictor.2
C
MEFs
Control
RictorKO
mRNA (A.U.)
1.5
1.0
0.5
0.0
****
Mcp1
Rictor

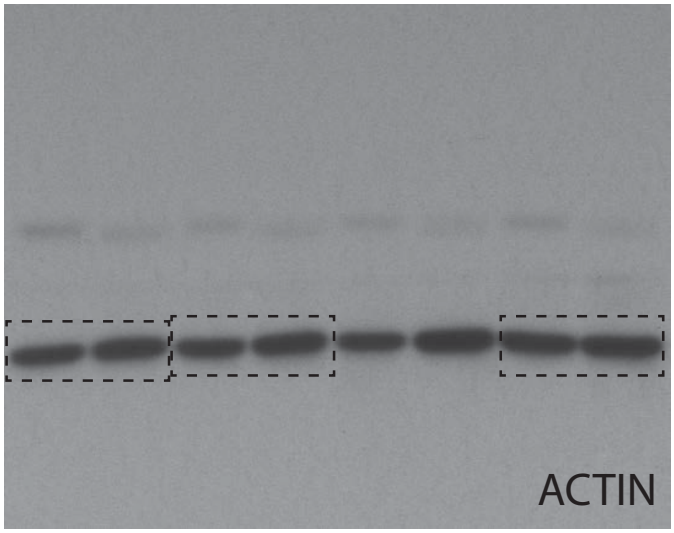
Figure S10. *Lipin1* mRNA levels were downregulated in AdRiKO eWAT and *Rictor* knockout 3T3-L1 adipocytes.
(**A**) *Lipin1* mRNA levels in eWAT from AdRiKO or control mice fed a HFD for 10 weeks. *** $P < 0.001$, by unpaired Student's *t* test. $n = 9$.
(**B**) *Lipin1* mRNA levels in *Rictor* knockout or control 3T3-L1 adipocytes. ** $P < 0.01$, by unpaired Student's *t* test. $N = 3$.
(**C**) *Mcp1* mRNA levels in *Rictor* knockout or control mouse embryonic fibroblasts (MEFs). **** $P < 0.0001$, by unpaired Student's *t* test. $N = 3$.

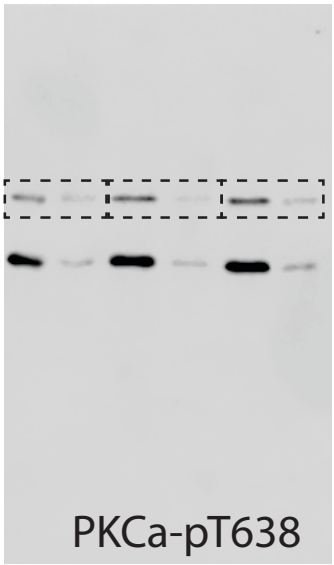
Unedited blots for Figure 1B

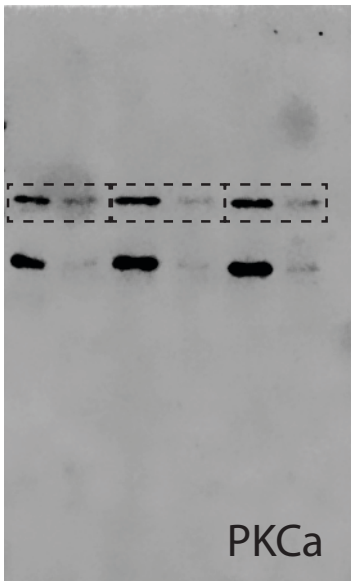


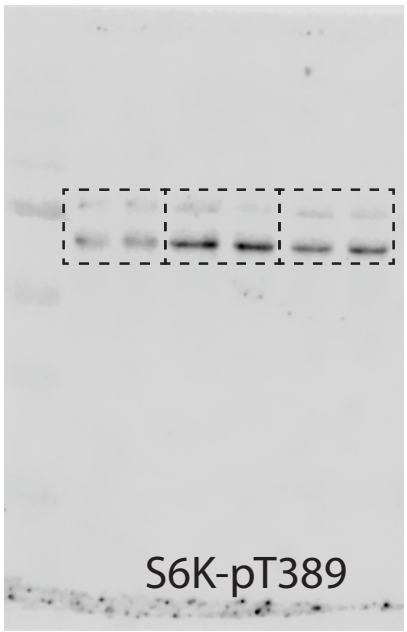




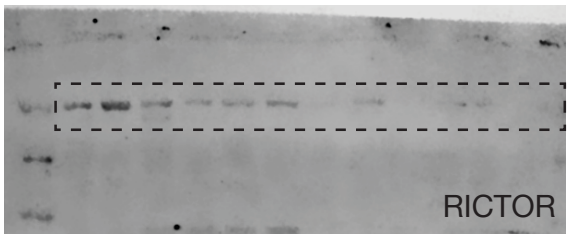


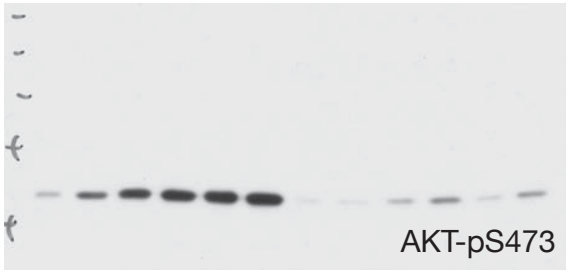


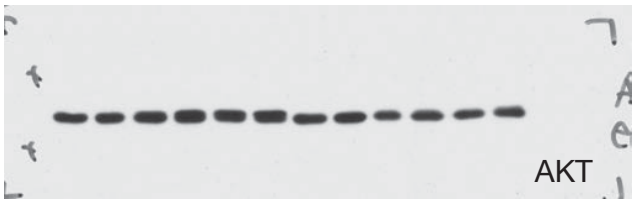




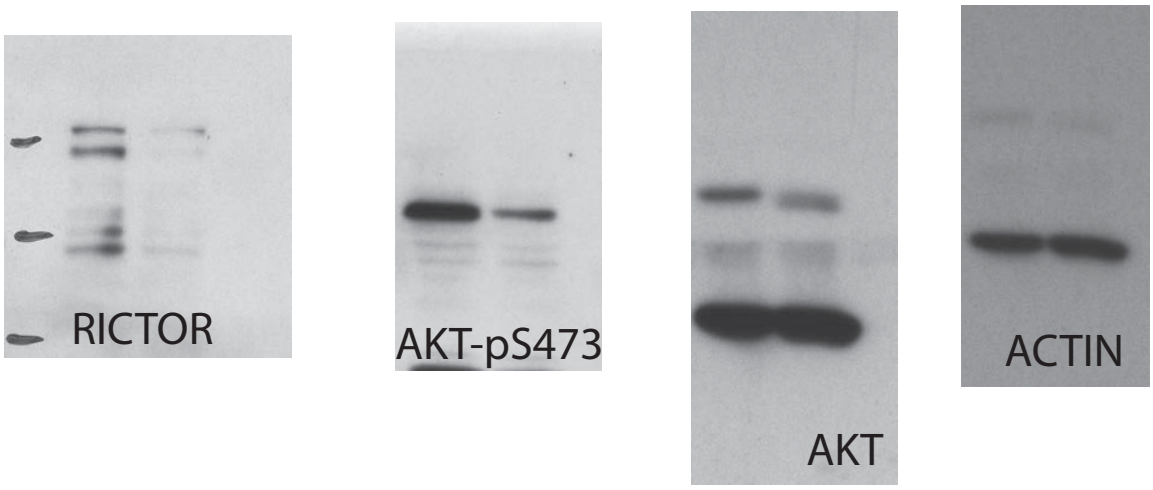
Unedited blots for Figure 2J

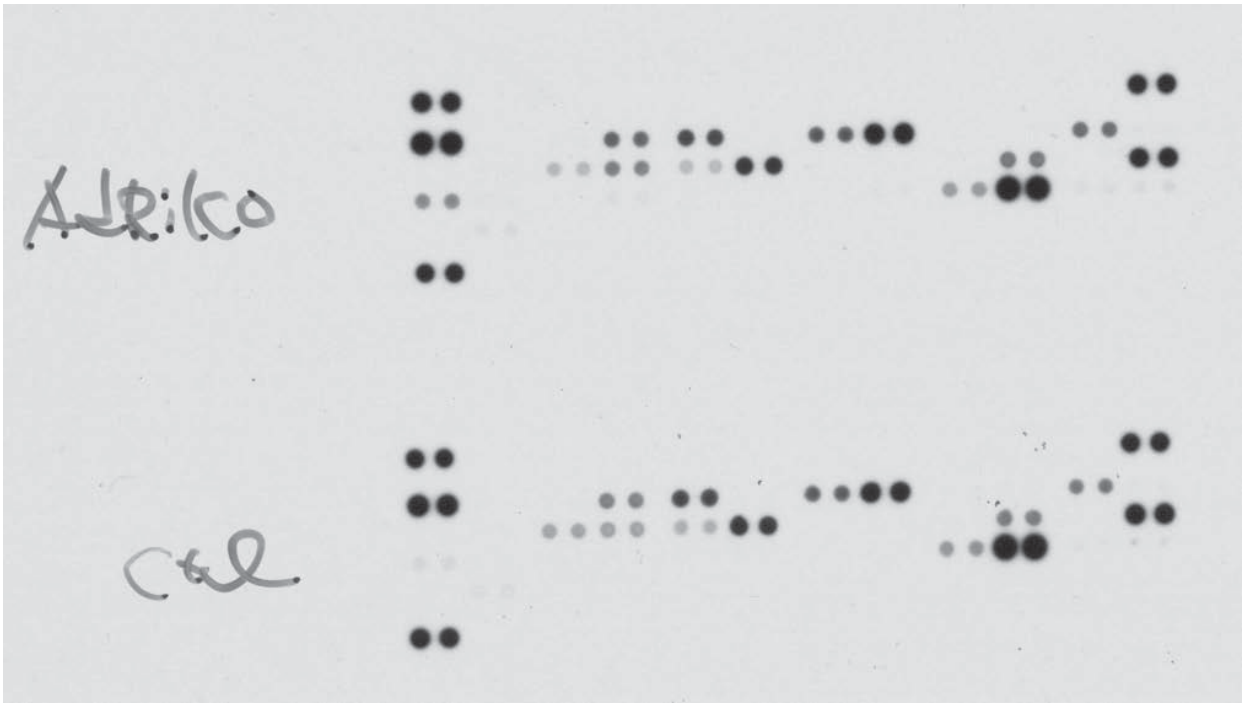




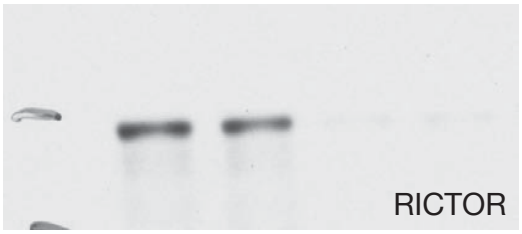


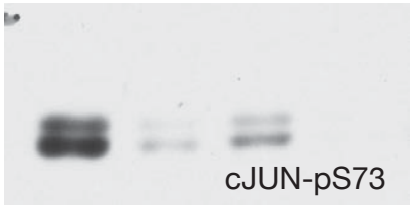
Unedited blots for Figure 4A

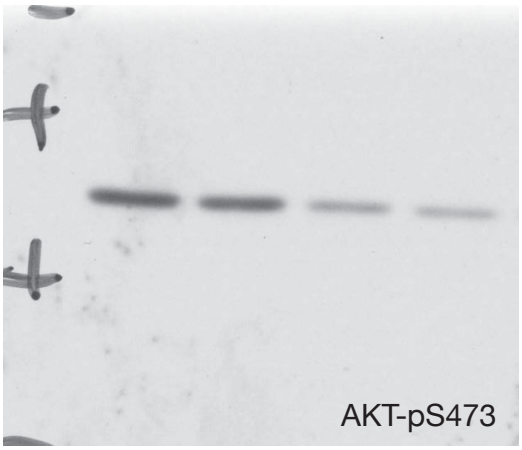


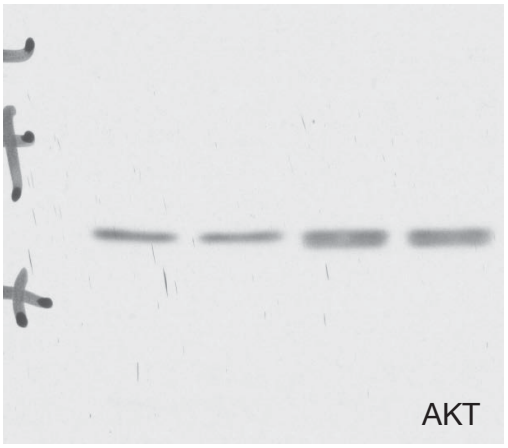


Unedited blots for Figure 5J

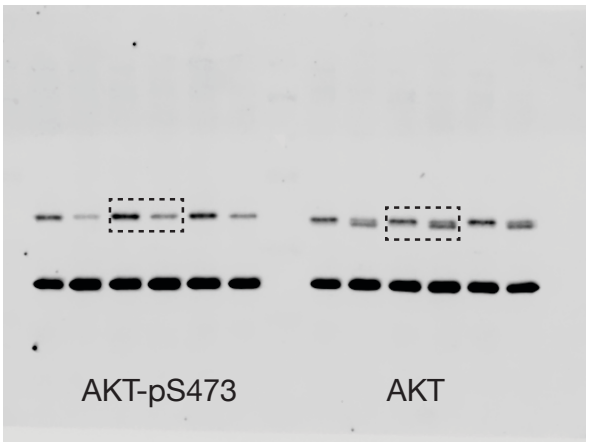


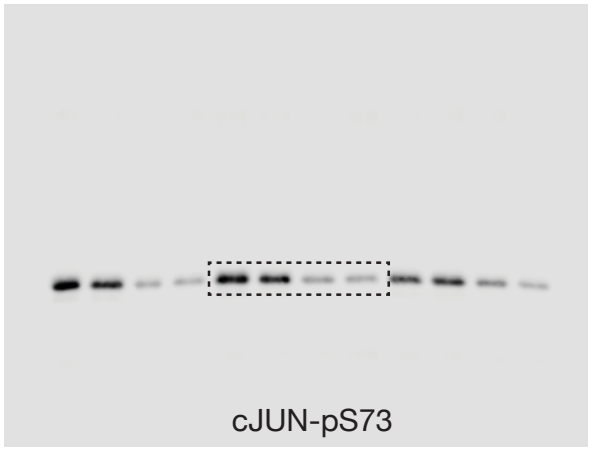


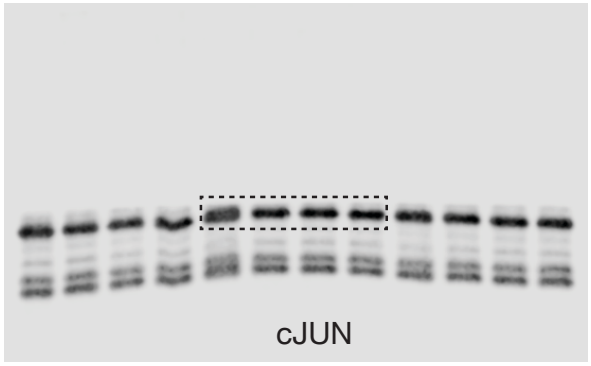


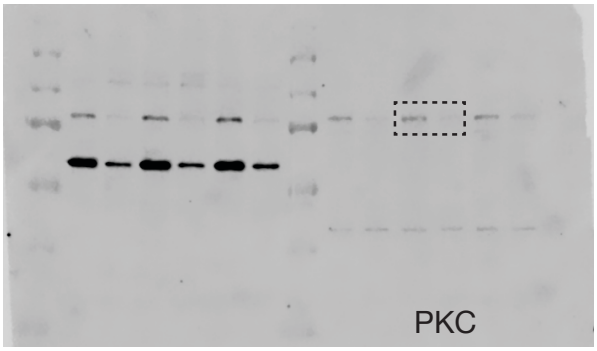


Unedited blots for Figure 5K

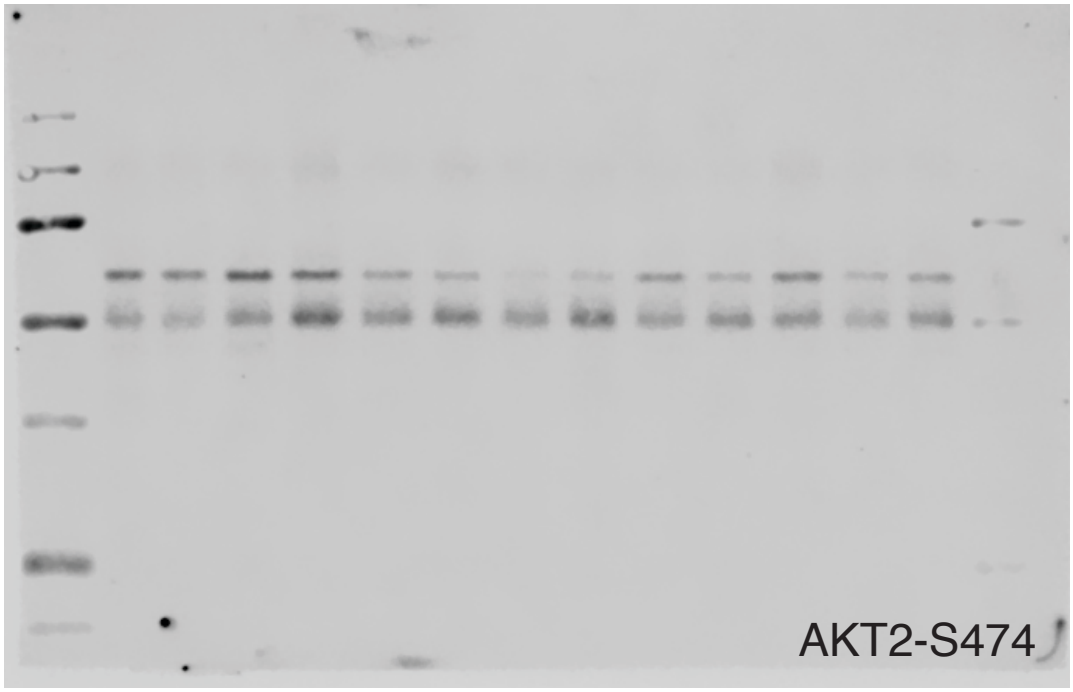


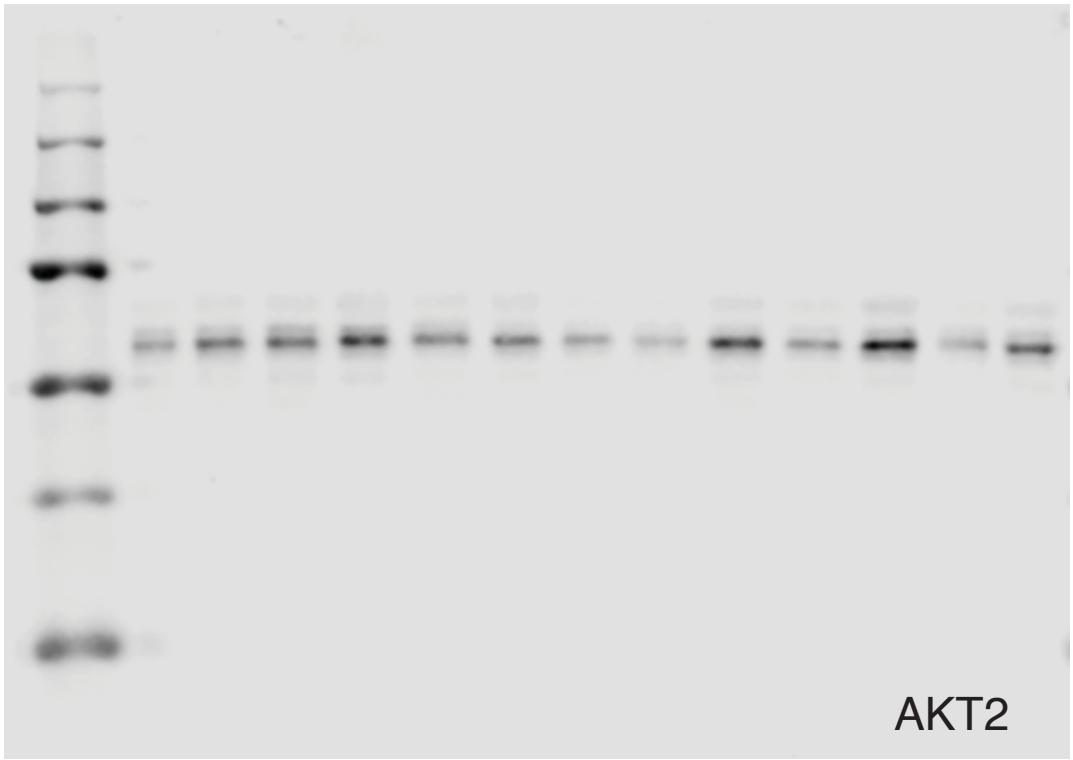




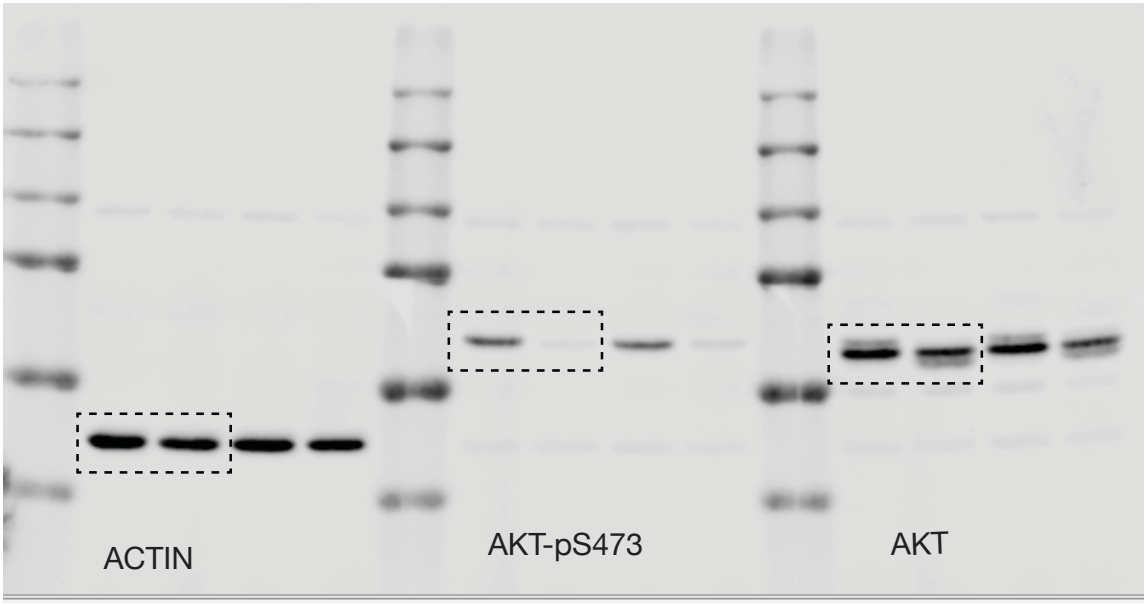


Unedited blots for Figure 6C



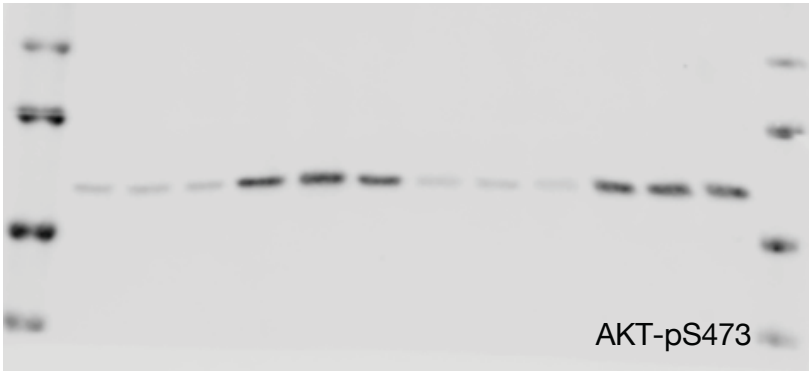


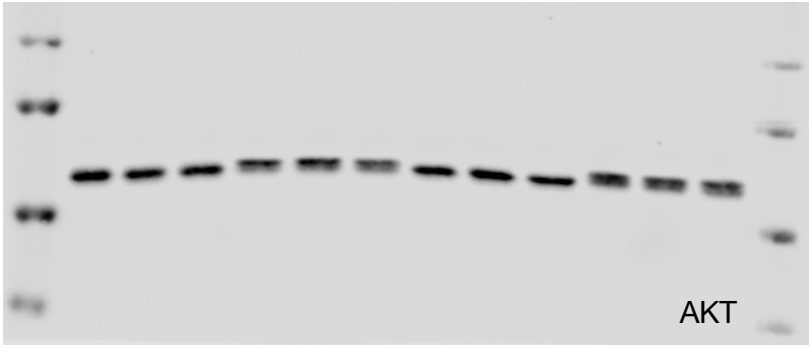
Unedited blots for Figure 6N



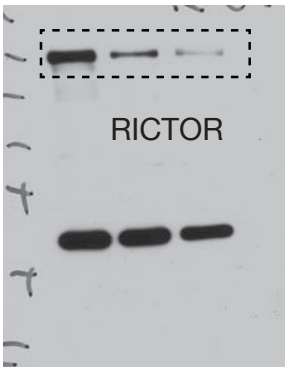
Unedited blots for Figure S5D

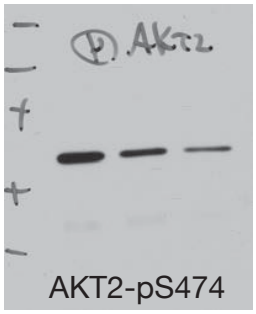
Unedited blots for Figure S5E

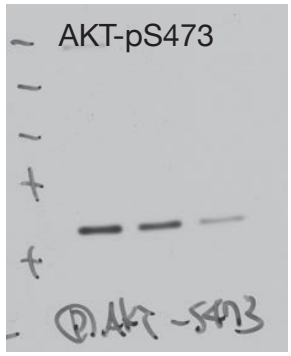


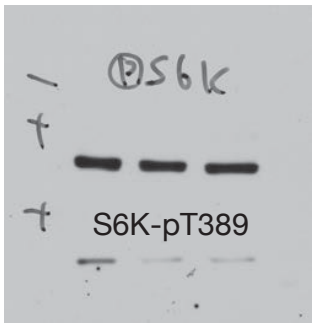


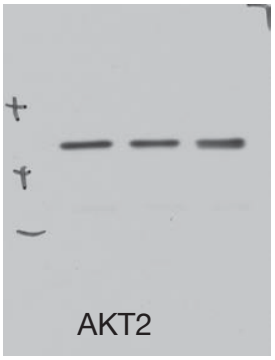
Unedited blots for Figure S8B

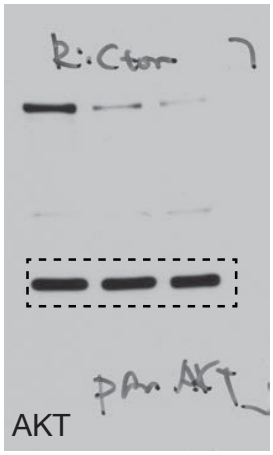


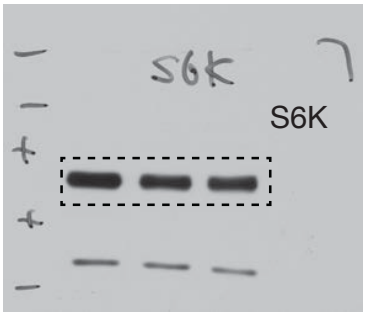


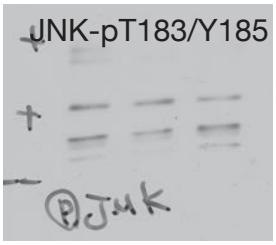


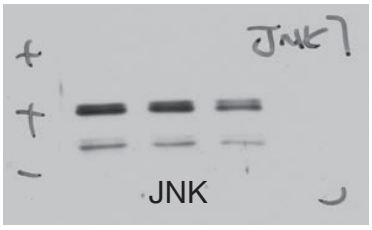


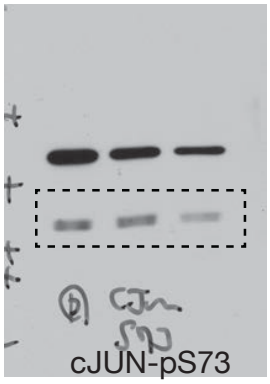


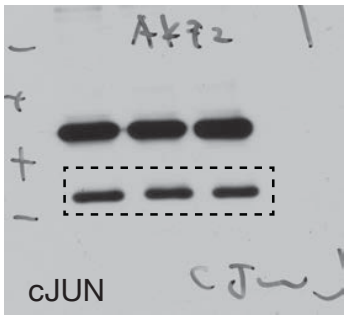












Unedited blots for Figure D