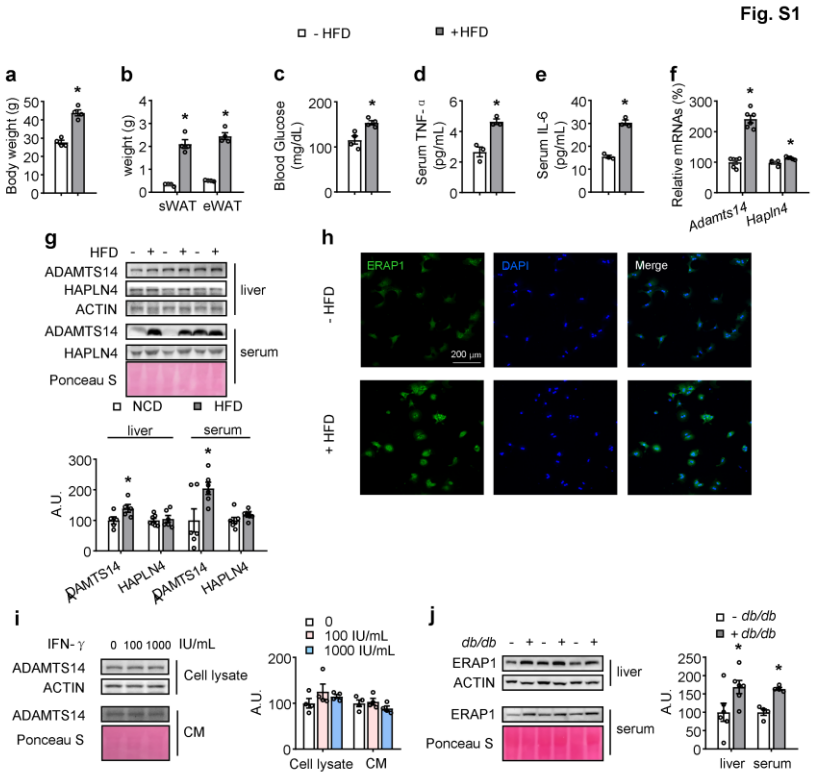
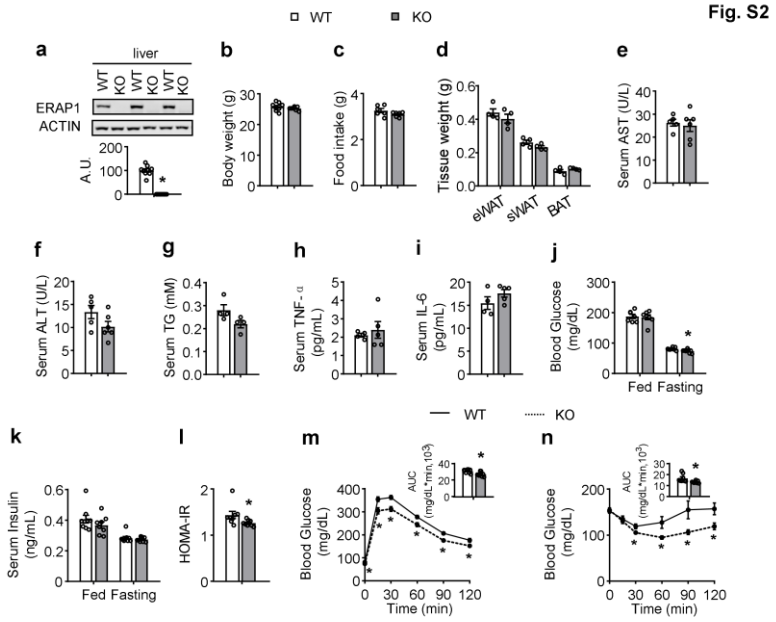
Supplemental Figures



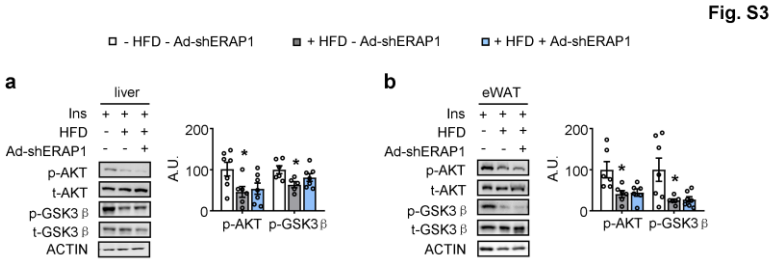
Supplemental Fig. 1. ERAP1 expression is increased in diabetic mice. (**a**) Body weight. (**b**) Epididymal white adipose tissue (eWAT) and subcutaneous white adipose tissue (sWAT) weight. (**c**) Fed blood glucose levels. (**d**) Serum tumor necrosis factor alpha (TNF-α) levels. (**e**) Serum interleukin 6 (IL-6) levels. (**f**) Gene expression of *Adamts14* and *Hapln4* in primary hepatocytes by RT-PCR. (**g, i,** and **j**) ADAMTS14, HAPLN4 or ERAP1 levels in the liver, serum, primary hepatocytes (cell lysate) or culture medium (CM) by western blotting (top or left) and quantified by densitometric analysis (bottom or right); A.U.: arbitrary unit. (**h**) Immunofluorescence staining for ERAP1 (green), DAPI (blue, indicating nucleus) and merge (white) in primary hepatocytes. Studies were conducted using 4-week-old male wild-type (WT) mice fed a

control diet (– HFD) or high-fat diet (+ HFD) for 16 weeks in (a–h). For (i), primary hepatocytes isolated from 10-week-old male WT mice were incubated with indicated concentration of interferon-gamma (IFN-γ) for 48 h. Studies were conducted using 10-week-old male WT (– *db/db*) or *db/db* (+ *db/db*) mice in (j). Data are expressed as the mean ± SEM (n = 4–6 per group, as indicated), with individual datapoints. * $p < 0.05$ for the effect of any group vs. control group.



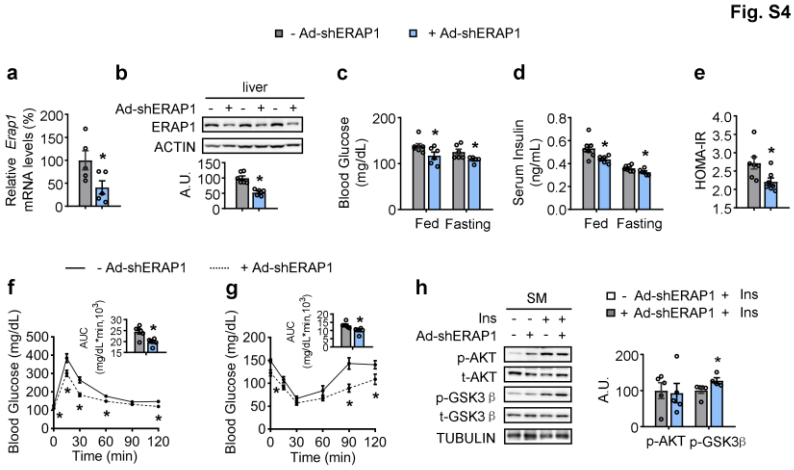
Supplemental Fig. 2. Global knockout of *Erap1* improves systemic insulin sensitivity. (**a**) ERAP1 levels in the liver by western blotting (top) and quantified by densitometric analysis (bottom); A.U.: arbitrary unit. (**b**) Body weight. (**c**) Food intake. (**d**) Tissue weight (eWAT: epididymal adipose tissue; sWAT: subcutaneous adipose tissue; BAT: brown adipose tissue). (**e**) Serum aspartate aminotransferase (AST) levels. (**f**) Serum alanine aminotransferase (ALT) levels. (**g**) Serum triglyceride (TG) levels.

(**h**) Serum tumor necrosis factor alpha (TNF-α) levels. (**i**) Serum interleukin 6 (IL-6) levels. (**j**) Fed and fasting blood glucose levels. (**k**) Fed and fasting serum insulin levels. (**l**) Homeostatic model assessment of insulin resistance (HOMA-IR) index. (**m**) Glucose tolerance tests; AUC: area under the curve. (**n**) Insulin tolerance tests. Studies were conducted using 10-week-old wild-type (WT) or global *Erap1* knockout (KO) mice. Data are expressed as the mean ± SEM (n = 3–10 mice per group, as indicated), with individual datapoints. * $p < 0.05$ for the effect of KO vs. WT.



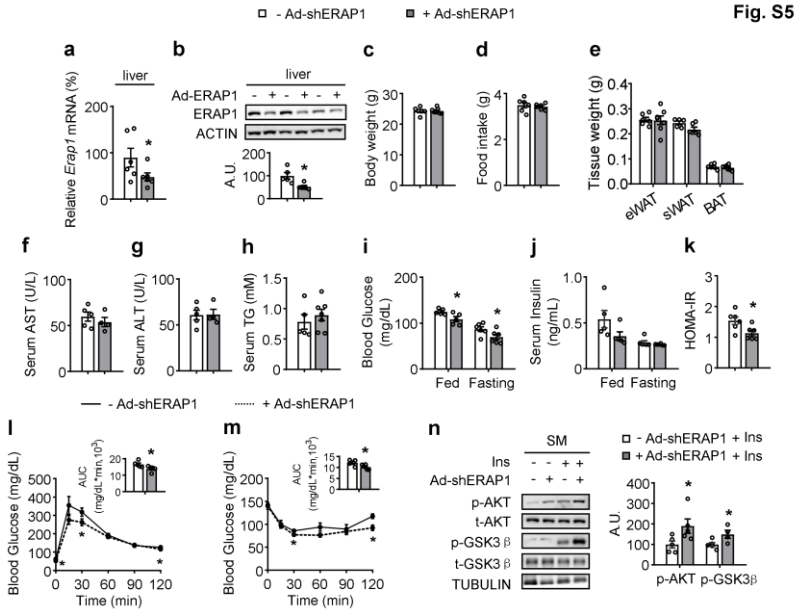
Supplemental Fig. 3. Liver-specific knockdown of *Erap1* does not affect white adipose tissue (eWAT) or liver insulin sensitivity in high-fat diet (HFD) mice. (**a** and **b**) p-AKT, t-AKT, p-GSK3β, and t-GSK3β levels in the liver or eWAT by western blotting after insulin (+ Ins) stimulation (left) and quantified by densitometric analysis of the relative abundance of phosphorylated protein normalized to their total protein levels (right). Studies were conducted using 4-week-old male wild-type mice fed a control diet (− HFD) or high-fat diet (+ HFD) for 16 weeks and then injected with negative control adenovirus (− Ad-shERAP1) or adenovirus expressing small-hairpin RNA specific for mouse *Erap1* (+ Ad-shERAP1). Data are expressed as the mean ±

SEM (n = 6–7 mice per group, as indicated), with individual datapoints. * $p < 0.05$ for the effect of + HFD vs. − HFD under − Ad-shERAP1 conditions; $^{\#}$ $p < 0.05$ for the effect of + Ad-shERAP1 vs. − Ad-shERAP1 under + HFD conditions.



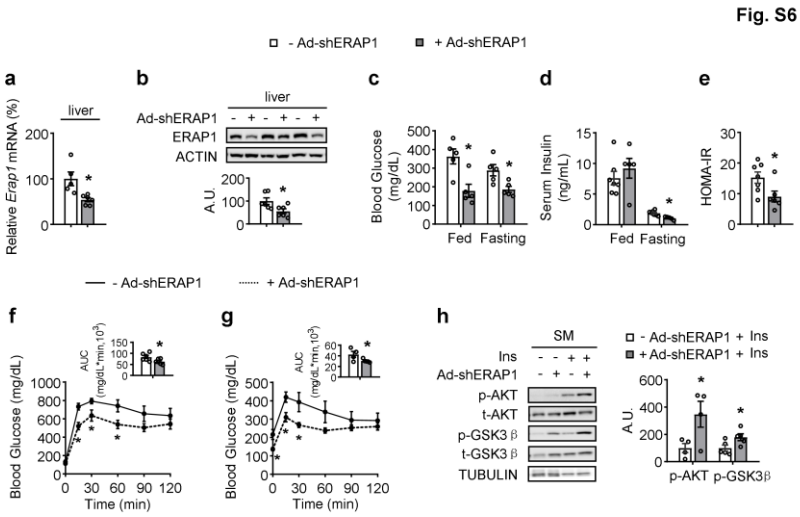
Supplemental Figure 4. Liver-specific knockdown of *Erap1* ameliorates insulin resistance in high-fat diet (HFD)-fed female mice. (**a**) Gene expression of *Erap1* in the liver by RT-PCR. (**b**) ERAP1 levels in the liver by western blotting (top) and quantified by densitometric analysis (bottom); A.U.: arbitrary unit. (**c**) Fed and fasting blood glucose levels. (**d**) Fed and fasting serum insulin levels. (**e**) Homeostatic model assessment of insulin resistance (HOMA-IR) index. (**f**) Glucose tolerance tests; AUC: area under the curve. (**g**) Insulin tolerance tests. (**h**) p-AKT, t-AKT, p-GSK3β, and t-GSK3β levels in the skeletal muscle (SM) by western blotting after insulin (+ Ins) stimulation (left) and quantified by densitometric analysis of the relative abundance of phosphorylated protein normalized to their total protein levels (right). Studies were conducted using 4-week-old female wild-type mice fed HFD for 9 weeks and then

injected with negative control adenovirus (− Ad-shERAP1) or adenovirus expressing small-hairpin RNA specific for mouse *Erap1* (+ Ad-shERAP1). Data are expressed as the mean ± SEM (n = 5–6 mice per group, as indicated), with individual datapoints. * $p < 0.05$ for the effect of + Ad-shERAP1 vs. − Ad-shERAP1.



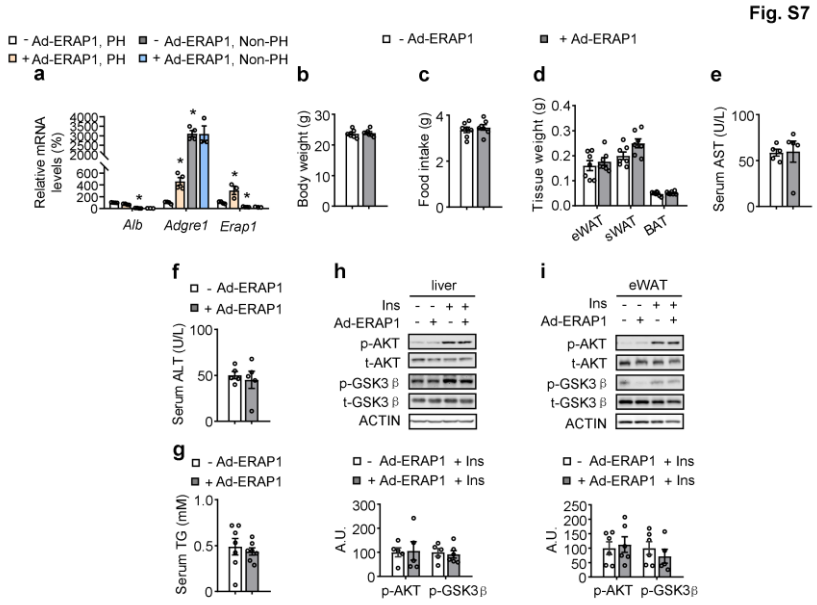
Supplemental Fig. 5. Liver-specific knockdown of *Erap1* increases skeletal muscle (SM) insulin sensitivity in wild-type mice. (**a**) Gene expression of *Erap1* in the liver by RT-PCR. (**b**) ERAP1 levels in the liver by western blotting (top) and quantified by densitometric analysis (bottom); A.U.: arbitrary unit. (**c**) Body weight. (**d**) Food intake. (**e**) Tissue weight (eWAT: epididymal adipose tissue; sWAT: subcutaneous adipose tissue; BAT: brown adipose tissue). (**f**) Serum aspartate aminotransferase (AST) levels. (**g**) Serum alanine aminotransferase (ALT) levels. (**h**) Serum triglyceride (TG) levels. (**i**) Fed and fasting blood glucose levels. (**j**) Fed and fasting serum insulin levels. (**k**)

Homeostatic model assessment of insulin resistance (HOMA-IR) index. (**l**) Glucose tolerance tests; AUC: area under the curve. (**m**) Insulin tolerance tests (0.5 U/kg). (**n**) p-AKT, t-AKT, p-GSK3β, and t-GSK3β levels in the SM by western blotting before (− Ins) or after insulin (+ Ins) stimulation (left) and quantified by densitometric analysis of the relative abundance of phosphorylated protein normalized to their total protein levels (right). Studies were conducted using 10-week-old male wild-type mice injected with negative control adenovirus (− Ad-shERAP1) or adenovirus expressing small-hairpin RNA specific for mouse *Erap1* (+ Ad-shERAP1). Data are expressed as the mean ± SEM (n = 6–7 mice per group, as indicated), with individual datapoints. * $p < 0.05$ for the effect of + Ad-shERAP1 vs. − Ad-shERAP1.



Supplemental Fig. 6. Liver-specific knockdown of *Erap1* ameliorates skeletal muscle (SM) insulin sensitivity in *db/db* mice. (**a**) Gene expression of *Erap1* in the liver by RT-PCR. (**b**) ERAP1 levels in the liver by western blotting (top) and quantified by densitometric analysis (bottom); A.U.: arbitrary unit. (**c**) Fed and fasting blood

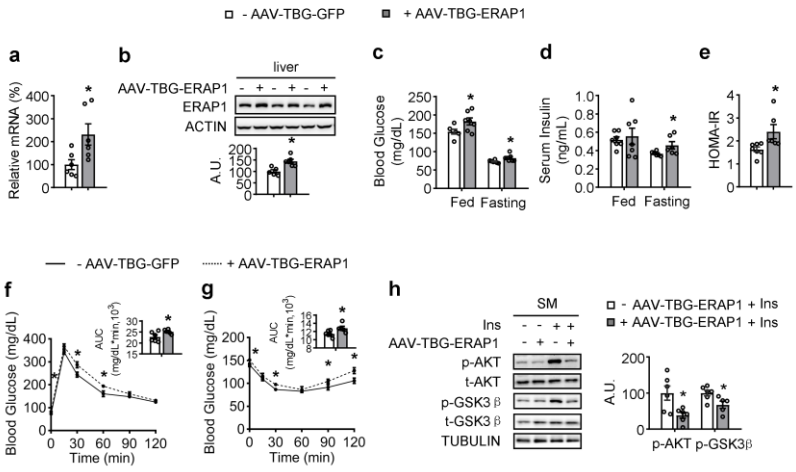
glucose levels. (**d**) Fed and fasting serum insulin levels. (**e**) Homeostatic model assessment of insulin resistance (HOMA-IR) index. (**f**) Glucose tolerance tests; AUC: area under the curve. (**g**) Insulin tolerance tests. (**h**) p-AKT, t-AKT, p-GSK3β, and t-GSK3β levels in the SM by western blotting before (− Ins) or after (+ Ins) insulin stimulation (left) and quantified by densitometric analysis of the relative abundance of phosphorylated protein normalized to their total protein levels (right). Studies were conducted using 10-week-old male *db/db* mice injected with negative control adenovirus (− Ad-shERAP1) or adenovirus expressing small-hairpin RNA specific for mouse *Erap1* (+ Ad-shERAP1). Data are expressed as the mean ± SEM (n = 6–7 mice per group, as indicated), with individual datapoints. * $p < 0.05$ for the effect of + Ad-shERAP1 vs. − Ad-shERAP1.



Supplemental Fig. 7. Liver-specific *Erap1* overexpression does not affect epididymal white adipose tissue (eWAT) or liver insulin sensitivity. (**a**) Gene

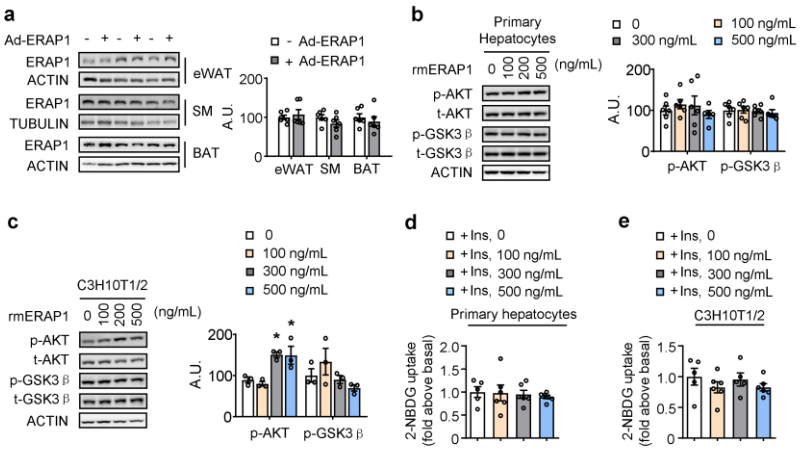
expression of *Albumin* (*Alb*), adhesion G protein-coupled receptor E1 (*Adgre1*), or *Erap1* in primary hepatocytes (PH) or non-hepatocytes (non-PH) by RT-PCR. (**b**) Body weight. (**c**) Food intake. (**d**) Tissue weight (sWAT: subcutaneous adipose tissue; BAT: brown adipose tissue). (**e**) Serum aspartate aminotransferase (AST) levels. (**f**) Serum alanine aminotransferase (ALT) levels. (**g**) Serum triglyceride (TG) levels. (**h** and **i**) p-AKT, t-AKT, p-GSK3β, and t-GSK3β levels in the liver or eWAT by western blotting before (− Ins) or after (+ Ins) insulin stimulation (left) and quantified by densitometric analysis of the relative abundance of phosphorylated protein normalized to their total protein levels (right). Studies were conducted using 10-week-old male wild-type mice injected with adenovirus expressing green fluorescent protein (− Ad-ERAP1) or *Erap1* (+ Ad-ERAP1). Data are expressed as the mean ± SEM (n =3–6 mice per group, as indicated), with individual datapoints. * $p < 0.05$ for the effect of any group vs. control group.

Fig. S8

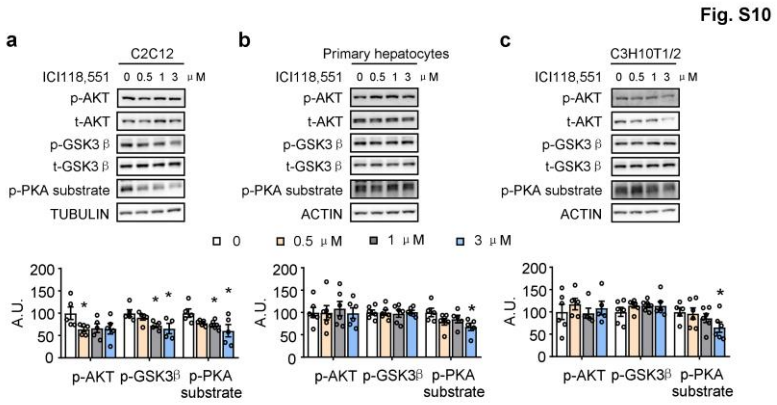


Supplemental Figure 8. Adeno-associated virus mediated overexpression of ERAP1 in the liver impairs skeletal muscle (SM) insulin sensitivity. (a) Gene expression of *Erap1* in the liver by RT-PCR. (b) ERAP1 levels in the liver by western blotting (top) and quantified by densitometric analysis (bottom); A.U.: arbitrary unit. (c) Fed and fasting blood glucose levels. (d) Fed and fasting serum insulin levels. (e) Homeostatic model assessment of insulin resistance (HOMA-IR) index. (f) Glucose tolerance tests; AUC: area under the curve. (g) Insulin tolerance tests. (h) p-AKT, t-AKT, p-GSK3β, and t-GSK3β levels in the SM by western blotting before (− Ins) or after (+ Ins) insulin stimulation in the SM (left) and quantified by densitometric analysis of the relative abundance of phosphorylated protein normalized to their total protein levels (right). Studies were conducted using 10-week-old male wild-type mice injected with AAV8-TBG-GFP (− AAV-TBG-ERAP1) or AAV8-TBG-ERAP1 (+ AAV-TBG-ERAP1). Data are expressed as the mean ± SEM (n = 5–7 mice per group, as indicated), with individual datapoints. * $p < 0.05$ for the effect of + AAV-TBG-ERAP1 vs. − AAV-TBG-ERAP1.

Fig. S9

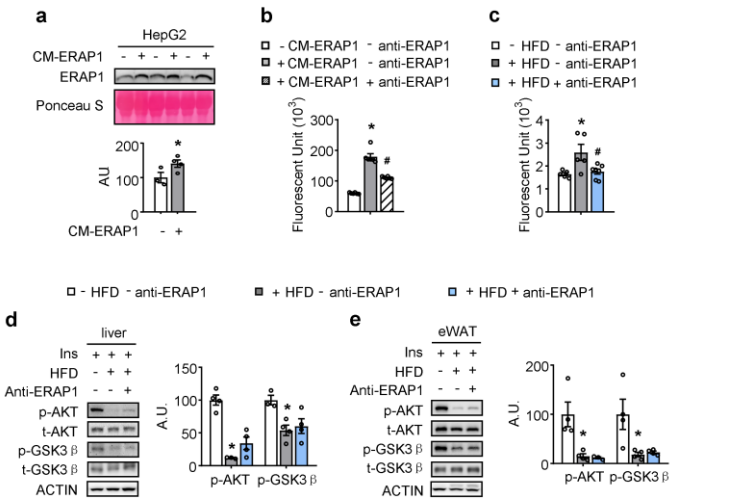


Supplemental Fig. 9. ERAP1 does not affect insulin sensitivity in hepatocytes or adipocytes. (**a**) ERAP1 levels in epididymal white adipose tissue (eWAT), skeletal muscle (SM), or brown adipose tissue (BAT) by western blotting (left) and quantified by densitometric analysis (right); A.U.: arbitrary unit. (**b** and **c**) p-AKT, t-AKT, p-GSK3β, and t-GSK3β levels in primary hepatocytes or C3H10T1/2 adipocytes by western blotting after insulin stimulation (left) and quantified by densitometric analysis of the relative abundance of phosphorylated protein normalized to their total protein levels (right). (**d** and **e**) Insulin stimulated 2-NBDG uptake in primary hepatocytes or C3H10T1/2 adipocytes. Studies for (a) were conducted using 10-week-old male wild-type mice injected with adenovirus expressing green fluorescent protein (−Ad-ERAP1) or *Erap1* (+ Ad-ERAP1). Cells were incubated with indicated concertation of recombinant mouse ERAP1 (rmERAP1) (b−e) for 20 min, with 100 nM insulin (+ Ins) stimulation for 20 min at the same time. Data are expressed as the mean ± SEM (n = 3–6 per group, as indicated), with individual datapoints. * $p < 0.05$ for the effect of any group vs. control group.



Supplemental Fig. 10. Inhibition of β2 adrenergic receptor (ADRB2) specifically inhibits skeletal muscle insulin signaling *in vitro*. (a−c) p-AKT, t-AKT, p-GSK3β, t-GSK3β, ADRB2, and p-PKA substrate levels by western blotting after insulin stimulation (top) and quantified by densitometric analysis (bottom); A.U.: arbitrary unit. *C2C12* myotubes (a), primary hepatocytes (b), or *C3H10T1/2* adipocytes (c) were incubated with indicated concentration of ICI118,551 for 6 h, followed by 100 nM insulin stimulation for 20 min. Data are expressed as the mean ± SEM (n = 4–6 per group, as indicated), with individual datapoints. * $p < 0.05$ for the effect of any group vs. control group.

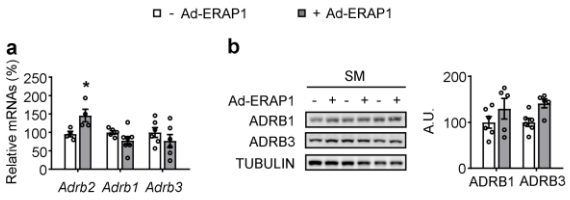
Fig. S11



Supplemental Fig. 11. ERAP1 neutralizing antibodies have no effect on epididymal white adipose tissue (eWAT) or liver insulin sensitivity. (**a**) ERAP1 levels in conditioned medium by western blotting (top) and quantified by densitometric analysis (bottom); A.U.: arbitrary unit. (**b and c**) Leucine aminopeptidase activity. (**d** and **e**) p-AKT, t-AKT, p-GSK3β, and t-GSK3β levels in the liver or eWAT after insulin (+ Ins) stimulation (left) and quantified by densitometric analysis of the relative abundance of phosphorylated protein normalized to their total protein levels (right). *HepG2* cells were infected with adenovirus expressing green fluorescent protein (− Ad-ERAP1) or *Erap1* (+ Ad-ERAP1). − Ad-ERAP1 infected conditioned medium (− CM-ERAP1) or + Ad-ERAP1 infected high-ERAP1 conditioned medium (+ CM-ERAP1) were collected 48 h later in (a). For (b), − CM-ERAP1 or + CM-ERAP1 incubated with 12 μg/mL anti-IgG antibodies (− anti-ERAP1) or anti-ERAP1 antibodies (+ anti-ERAP1) were detected for leucine aminopeptidase activity. For (c−e), 4-week-old male wild-type mice were fed a control diet (− HFD) or high-fat diet (+ HFD) for 16 weeks and then intraperitoneally injected with 1 mg/kg − anti-ERAP1

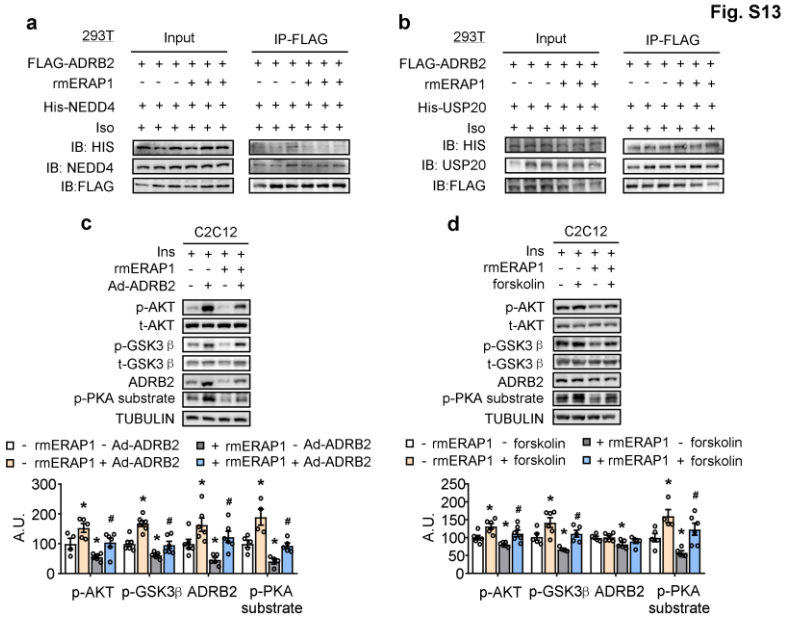
or + anti-ERAP1. Data are expressed as the mean ± SEM (n = 3–4 per group, as indicated), with individual datapoints. * $p < 0.05$ for the effect of any group vs. control group; # $p < 0.05$ for the effect of + anti-ERAP1 vs. – anti-ERAP1 under + CM-ERAP1 conditions in (b).

Fig. S12



Supplemental Fig. 12. ERAP1 does not affect β1 adrenergic receptor (ADRB1) and β3 adrenergic receptor (ADRB3) levels in skeletal muscle (SM). (**a**) Gene expression of *Adrb2*, *Adrb1*, and *Adrb3* levels in the SM. (**b**) ADRB1 and ADRB3 levels in the SM by western blotting (left) and quantified by densitometric analysis (right); A.U.: arbitrary unit. Studies were conducted using 10-week-old male wild-type mice injected with adenovirus expressing green fluorescent protein (− Ad-ERAP1) or *Erap1* (+ Ad-ERAP1). Data are expressed as the mean ± SEM (n = 4–6 mice per group, as indicated), with individual datapoints. * $p < 0.05$ for the effect of + Ad-ERAP1 vs. − Ad-ERAP1.

Fig. S13



Supplemental Fig. 13. ERAP1 does not affect the interaction between neural precursor cell expressed, developmentally downregulated 4 (NEDD4) or ubiquitin specific peptidase 20 (USP20) with β2 adrenergic receptor (ADRB2). (**a**) The effect of ERAP1 on the interaction between ADRB2 and NEDD4; (**b**) The effect of ERAP1 on the interaction between ADRB2 and USP20. (**c and d**) p-AKT, t-AKT, p-GSK3β, t-GSK3β, ADRB2, and p-PKA substrate levels in *C2C12* myotubes (left) and quantified by densitometric analysis (right); A.U.: arbitrary unit. Studies for (a and b) were conducted using *293T* cells transfected with indicated plasmids for 24 h and treated with (+ rmERAP1) or without (− rmERAP1) 500 ng/mL recombinant mouse ERAP1 (rmERAP1) for 2 h and stimulated with 10 μM isoprenaline (Iso) for 30 min. Then, cells were immunoprecipitated (IP) with anti-FLAG antibodies, followed by immunoblotting (IB) with indicated antibodies in (a and b). Studies for (c) were conducted using *C2C12* myotubes infected with adenovirus expressing control green fluorescent protein (− Ad-ADRB2), or *Adrb2* (+ Ad-ADRB2) for 48 h and then

stimulated with or without 500 ng/mL rmERAP1 for 2h. Studies for (d) were conducted using *C2C12* myotubes treated with (+ forskolin) or without (− forskolin) 100 μM forskolin for 24 h and stimulated with or without 500 ng/mL rmERAP1 for 20 min, with 100 nM insulin (+ Ins) stimulation for 20 min at the same time. Data are expressed as the mean ± SEM (n = 5–6 per group, as indicated), with individual datapoints. * $p < 0.05$ for the effect of any group vs. control group. # $p < 0.05$ for the effect of + Ad-ADRB2 vs. – Ad-ADRB2, or + forskolin vs. − forskolin under + rmERAP1 conditions.

Supplemental Table 1. Antibodies for western blotting.

Antibodies	Company	Cat No.	Dilution
Phospho-AKT (Ser 473)	Cell Signaling Technology	9271S	1:1000
AKT	Cell Signaling Technology	9272S	1:1000
Phospho-GSK3β (Ser 9)	Cell Signaling Technology	9336S	1:1000
GSK3β	Cell Signaling Technology	9315S	1:1000
Phospho-PKA substrates	Cell Signaling Technology	9621S	1:1000
ERAP1	Abcam (for WB)	ab124669	1:1000
ERAP1	Santa Cruz Biotechnology (for IF)	sc-271823	1:50
ADRB2	Proteintech (for WB)	13096-1-AP	1:1000
ADRB2	Norvus (for IP)	NBP2-67187	1:200
USP33	Proteintech	20445-1-AP	1:1000
USP20	Proteintech	17491-1-AP	1:1000
NEDD4	Proteintech	21698-1-AP	1:1000
HAPLN4	Proteintech	21228-1-AP	1:1000
β-actin	Proteintech	66009-1-Ig	1:5000
α-tubulin	Sigma–Aldrich	T6199	1:5000

HA	Sigma–Aldrich	H9658-.2ML	1:500
FLAG	Sigma–Aldrich	F3165	1:500
ADRB1	Sigma–Aldrich	SAB2100064-50UG	1:1000
ADRB3	Signalway Antibody	40617-1	1:1000
MYC	Santa Cruz Biotechnology	sc-40	1:500
ADAMTS14	Invitrogen	PA5-103578	1:1000

Supplemental Table 2. RT-PCR primers.

Genes	Forward primer (5′–3′)	Reverse primer (5′–3′)
Erap1	GGCGGCTGTGATGAAGGTAA	TCCCCAGAAAGTCAGAGTGC
Gapdh	*TGTGTCCGTCGTGGATCTGA*	*CCTGCTTCACCACCTTCTTGAT*
Actin	CAACGAGCGGTTCCGATG	GCCACAGGATTCCATACCCA
Adamts14	TTTCGGGAACTCTTCCGACAA	CCAATCCATCACAGTTGCTGA
Hapln4	CCCTTTGGCTTTCGCCGAT	CCCCAGGTCCGTCATTCTG
Adrb1	GCATTGAGACCCTGTGTGTCA	AGCAAACTCTGGTAGCGAAAGG
Adrb2	CCACCCACAAGAAAGCTATCG	GGCCTGGTTCGTGAAGAAGTC
Adrb3	ACGCCGAGACTACAGACCATA	CTGGTGGCATTACGAGGA
Albumin	*AGAAGACACCCTGATTACTCT*	*TCGAGAAGCAGGTGTCCTTGT*
Adgre1	*TTTCCTCGCCTGCTTCTTC*	*CCCCGTCTCTGTATTCAACC*

Supplemental data 1. NCD vs HFD differentially expressed genes and proteins.