GPx3 dysregulation impacts adipose tissue insulin receptor expression and sensitivity

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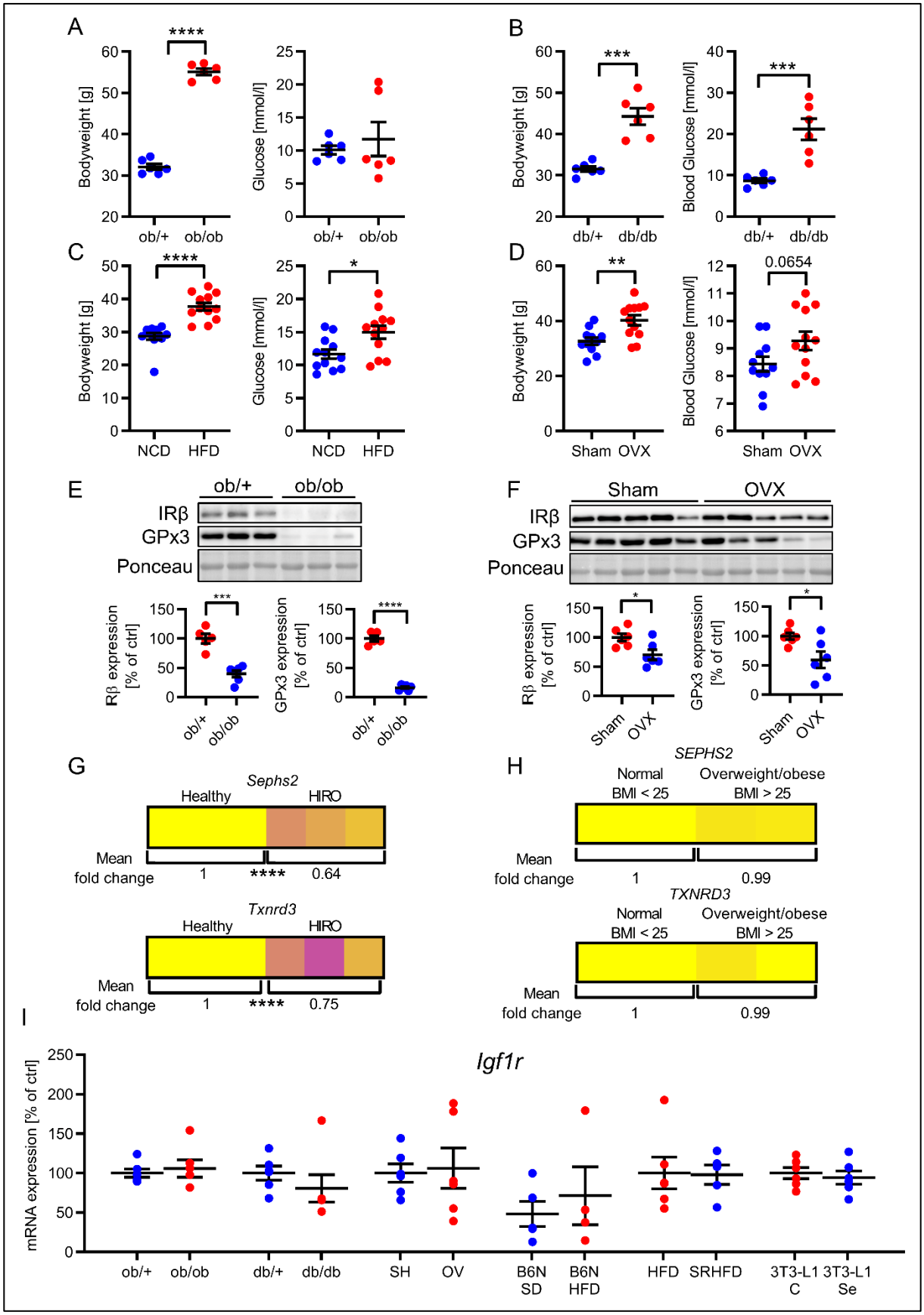
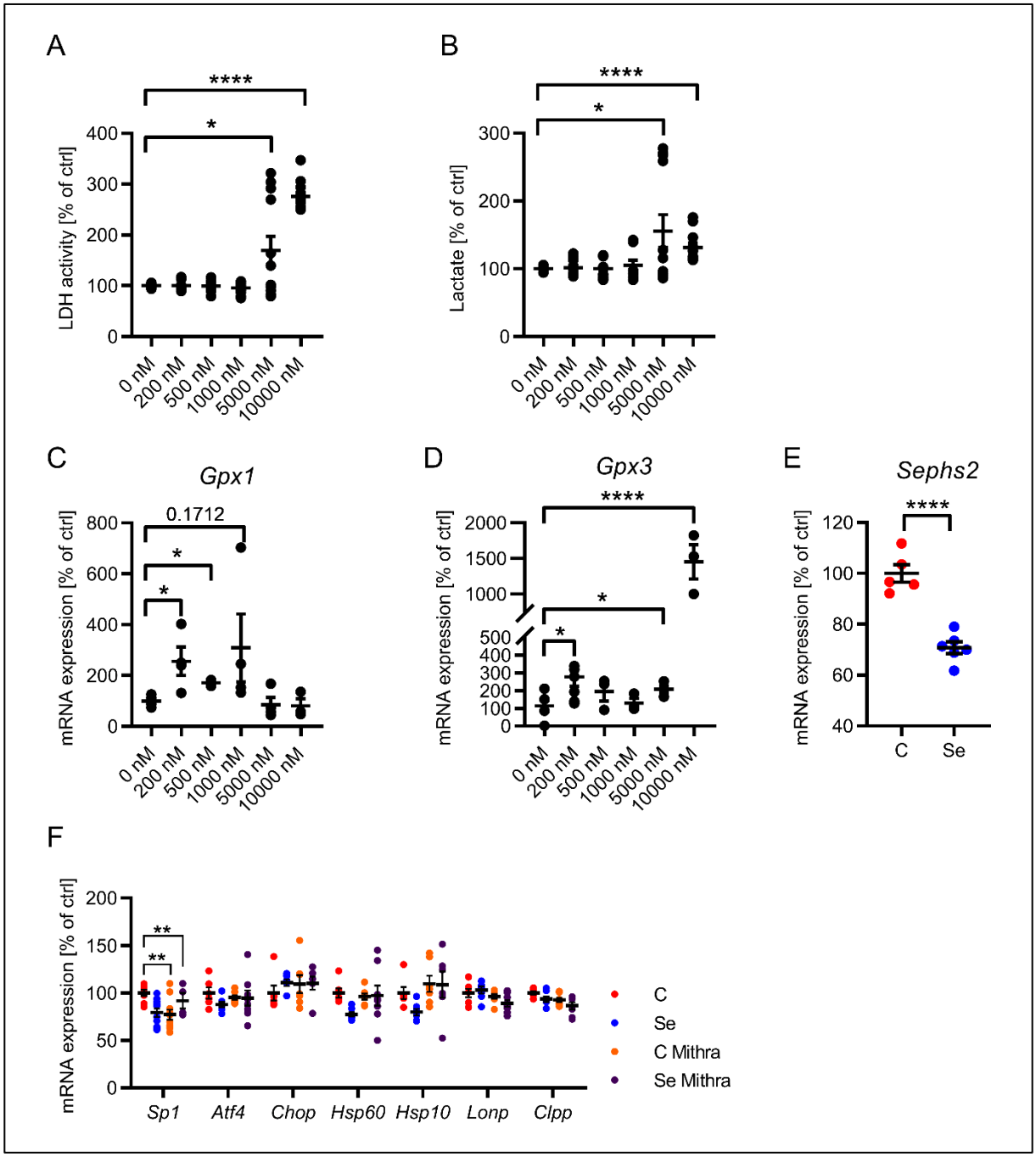
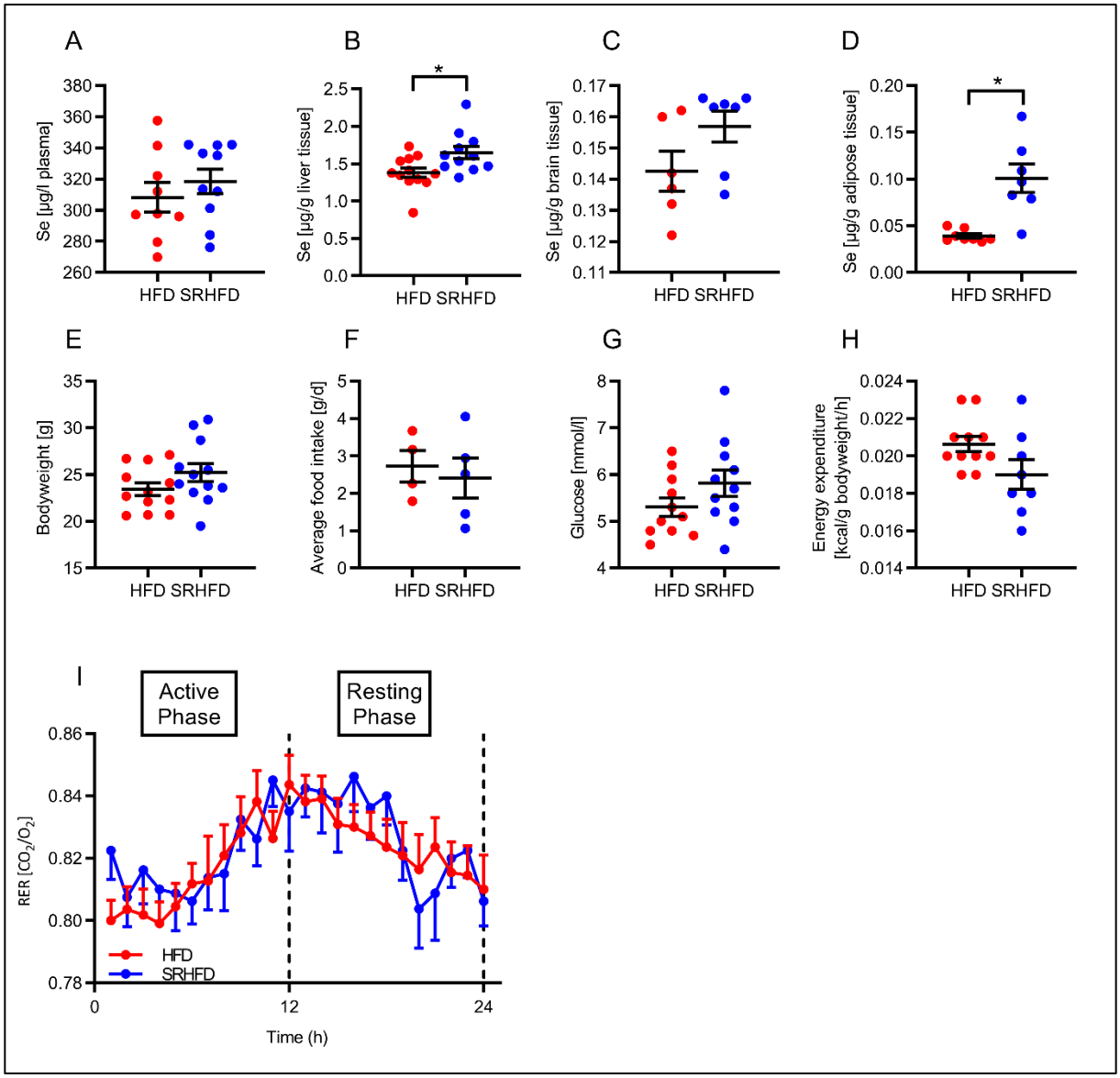
Supplementary Figures and Material


A
Bodyweight [g]
Glucose [mmol/l]
ob/+
ob/ob
B
Bodyweight [g]
Blood Glucose [mmol/l]
db/+
db/db
C
Bodyweight [g]
Glucose [mmol/l]
NCD
HFD
D
Bodyweight [g]
Blood Glucose [mmol/l]
Sham
OVX
0.0654
E
ob/+
ob/ob
IRβ
GPx3
Ponceau
Rβ expression [% of ctrl]
GPx3 expression [% of ctrl]
F
Sham
OVX
G
Sephs2
Healthy
HIRO
Mean fold change
1
0.64
Txnrd3
0.75
H
SEPHS2
Normal BMI < 25
Overweight/obese BMI > 25
0.99
TXNRD3
I
Igf1r
mRNA expression [% of ctrl]
SH
OV
B6N SD
B6N HFD
HFD
SRHFD
3T3-L1 C
3T3-L1 Se

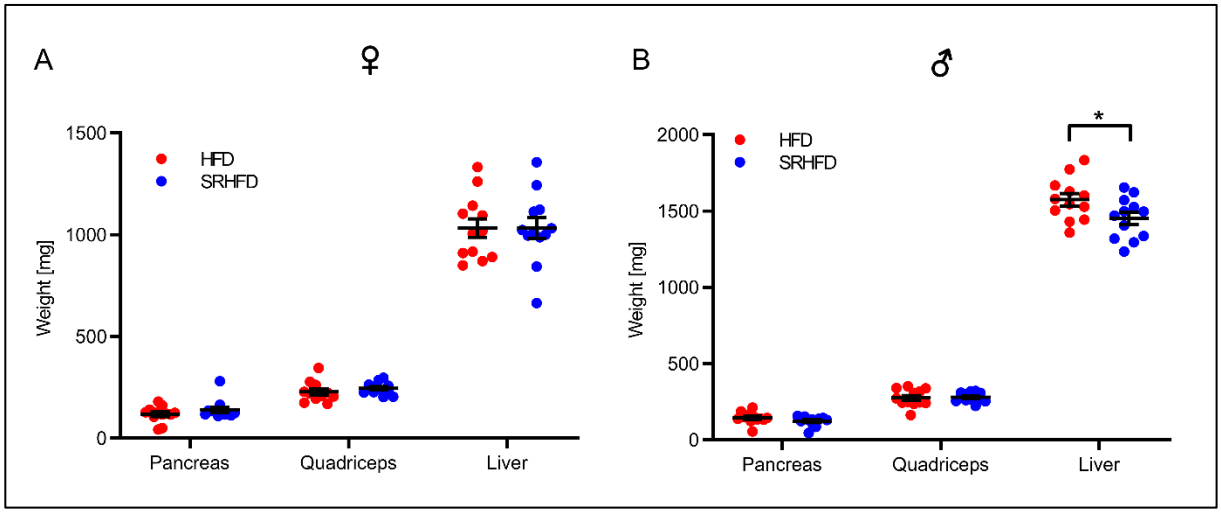
Supplementary figure 1: Metabolic phenotypes of mouse models of obesity and insulin resistance. A: Bodyweight and blood glucose levels of ob/+ and ob/ob mice (n = 6). B: Bodyweight and blood glucose levels of db/+ and db/db mice (n = 6). C: Bodyweight and blood glucose levels of male C57BL/6N mice fed NCD or HFD for 12 weeks (n = 12). D: Bodyweight and blood glucose levels of female sham operated (Sham) and ovariectomized (OVX) mice fed a HFD for 14 weeks (n = 12). E: Representative gWAT IR and GPx3 expression in ob/+ and ob/ob mice (densitometry n = 5-6). F: Representative gWAT IR and GPx3 expression in OVX and Sham mice (densitometry n = 6) G: Mean fold change of *Sephs2* and *Txnrd3* mRNA expression in epigonadal adipose tissue of healthy (NCD, db/+, Sham) and HIRO (HFD, db/db, OVX) mice (n = 6-12). H: Mean fold change of *SEPHS2* and *TXNRD3* mRNA expression in epigonadal adipose tissue of normal weight (BMI < 25, n = 18) and overweight/obese (BMI > 25, n = 37) patients. I: *Igf1r* mRNA expression in gWAT of mouse models of obesity and 3T3-L1 preadipocytes (n = 5-6). *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, ****: $P < 0.0001$ after two-tailed Student's t-test. All data are presented as mean ± SEM.



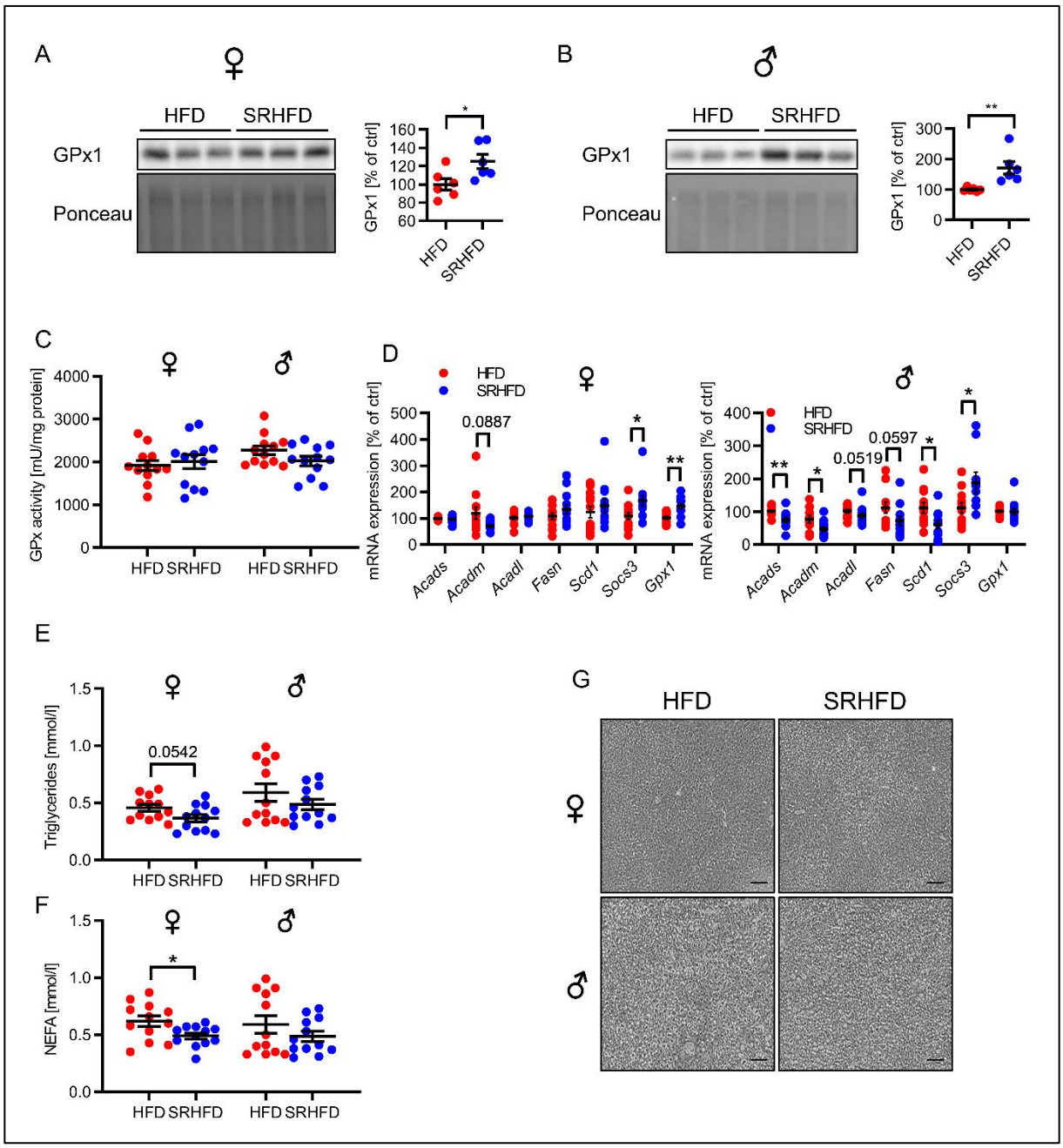
Supplementary figure 2: Dose-dependent effects of selenite treatment on 3T3-L1 preadipocytes. C = control, Se = 200 nM selenite treatment. A: Lactate dehydrogenase (LDH) activity in media of 3T3-L1 preadipocytes after 96h treatment with indicated selenite concentrations (n = 12). B: Lactate concentration of media of 3T3-L1 preadipocytes after 96h treatment with indicated selenite concentrations (n = 12). C: *Gpx1* mRNA expression of 3T3-L1 preadipocytes after 96h treatment with indicated selenite concentrations (n = 4). D: *Gpx3* mRNA expression of 3T3-L1 preadipocytes after 96h treatment with indicated selenite concentrations (n = 4-6). E: *Sephs2* mRNA expression of 3T3-L1 preadipocytes after 96h treatment with or without 200 nM selenite (n = 6). F: mRNA expression levels of *Sp1* and members of the integrated stress response after selenite and mithramycin (Mithra) treatment (n = 8). *: $P < 0.05$, ****: $P < 0.0001$ after two-tailed Student's t-test. All data are presented as mean ± SEM.



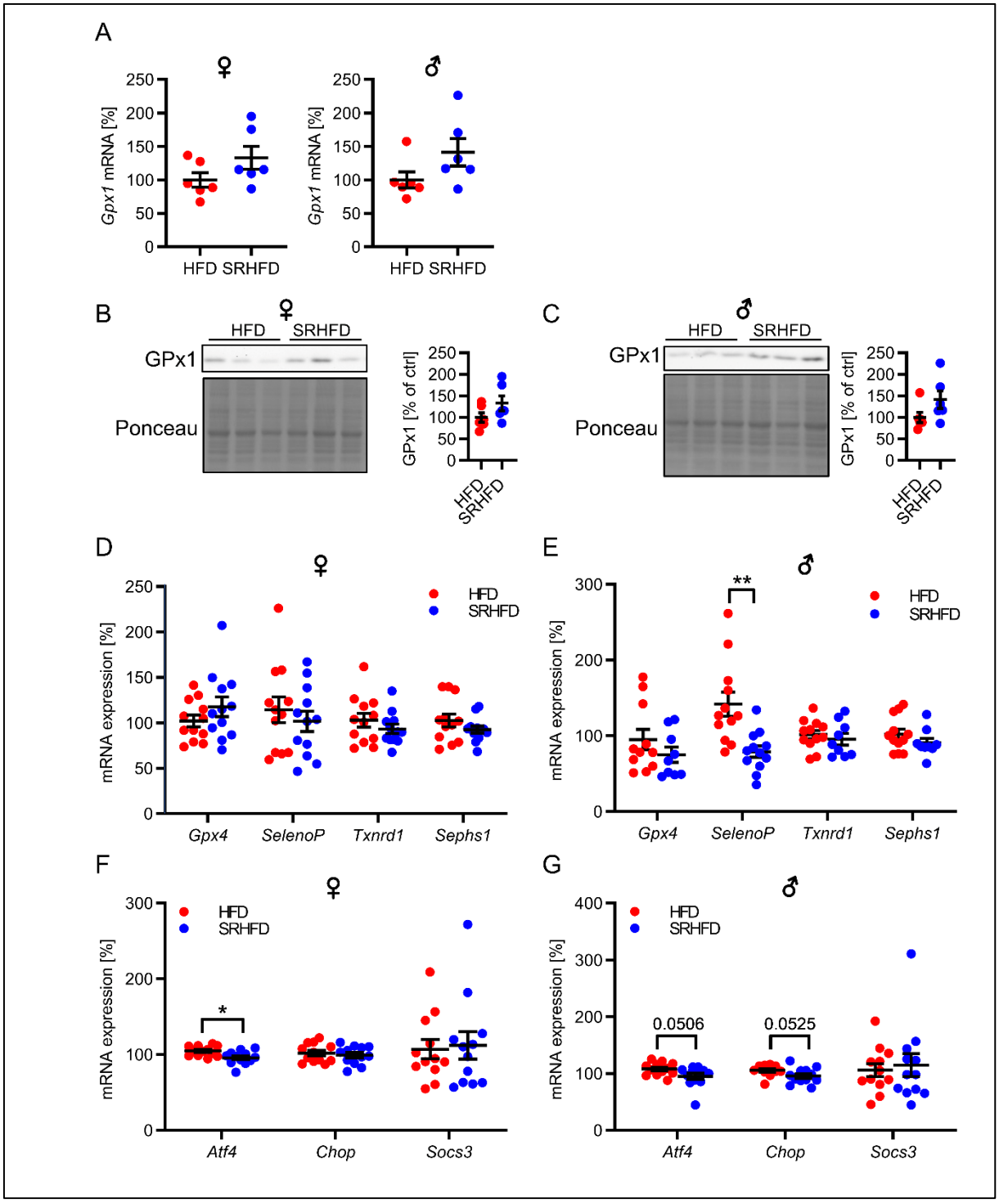
Supplementary figure 3: Unaltered energy homeostasis in female mice fed a selenium-rich HFD. A, B, C, D: Selenium content in plasma (A), liver (B), brain (C), and adipose tissue (D) of female mice after 12 weeks of either HFD or SRHFD (n = 8-12). E: Bodyweight of mice after 12 weeks of either HFD or SRHFD (n = 8-12). F: Average food intake of female mice fed either HFD or SRHFD (n = 6). G: Fasting blood glucose of female mice fed either HFD or SRHFD (n = 8-12). H: Energy expenditure of female mice fed either HFD or SRHFD (n = 8-12). I: Respiratory exchange ratio of mice fed either HFD or SRHFD (n = 8-12). *: $P < 0.05$ after two-tailed Student's t-test. All data are presented as mean ± SEM.



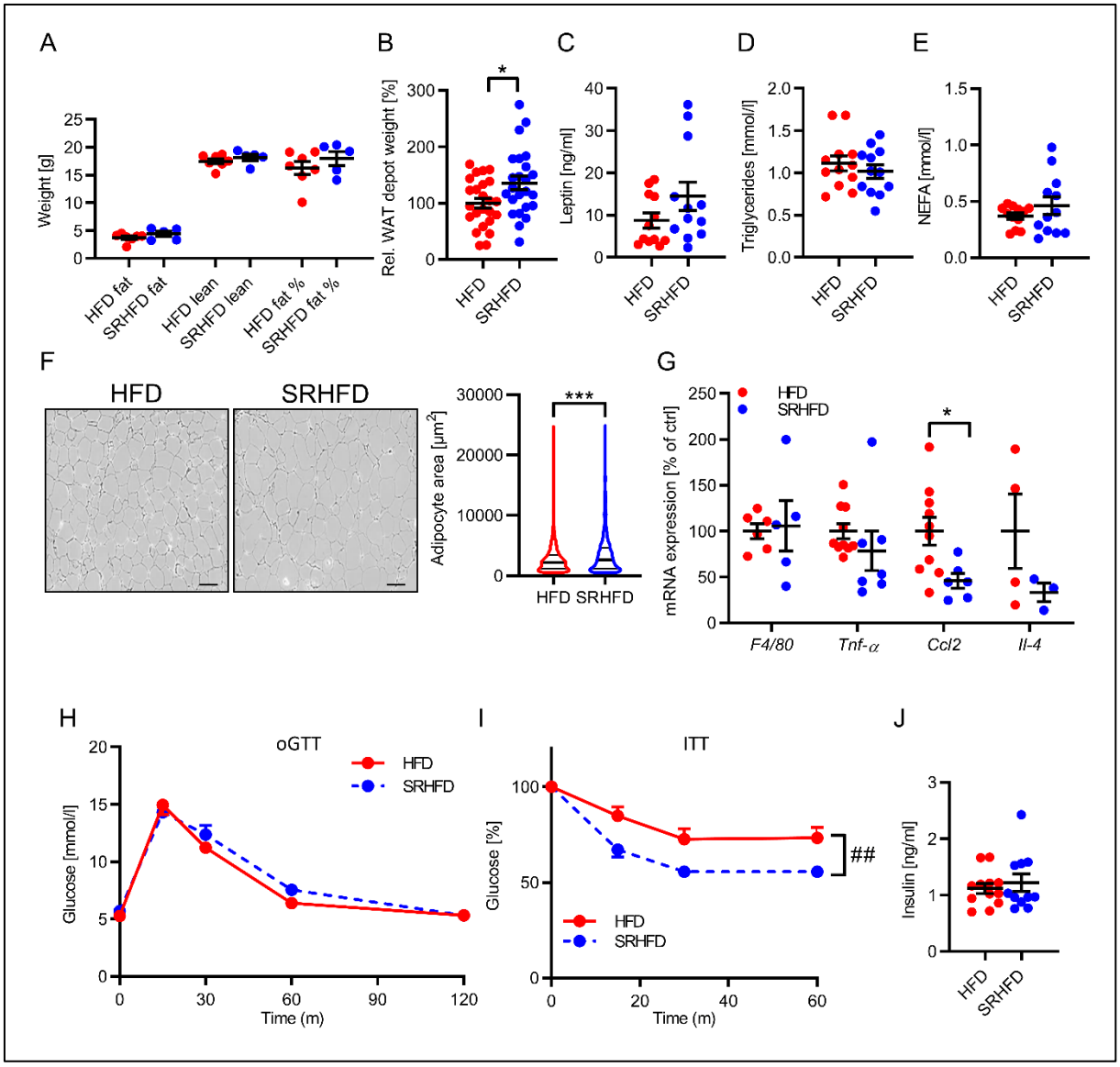
Supplementary Figure 4: Organ weights of C57BL/6N mice after 12 weeks selenite supplementation. A, B: Weight of pancreas, quadriceps and liver of female (A) and male (B) mice after 12 weeks of either HFD or SRHFD (n = 12). *: $P < 0.05$ after two-tailed Student's t-test. All data are presented as mean ± SEM.



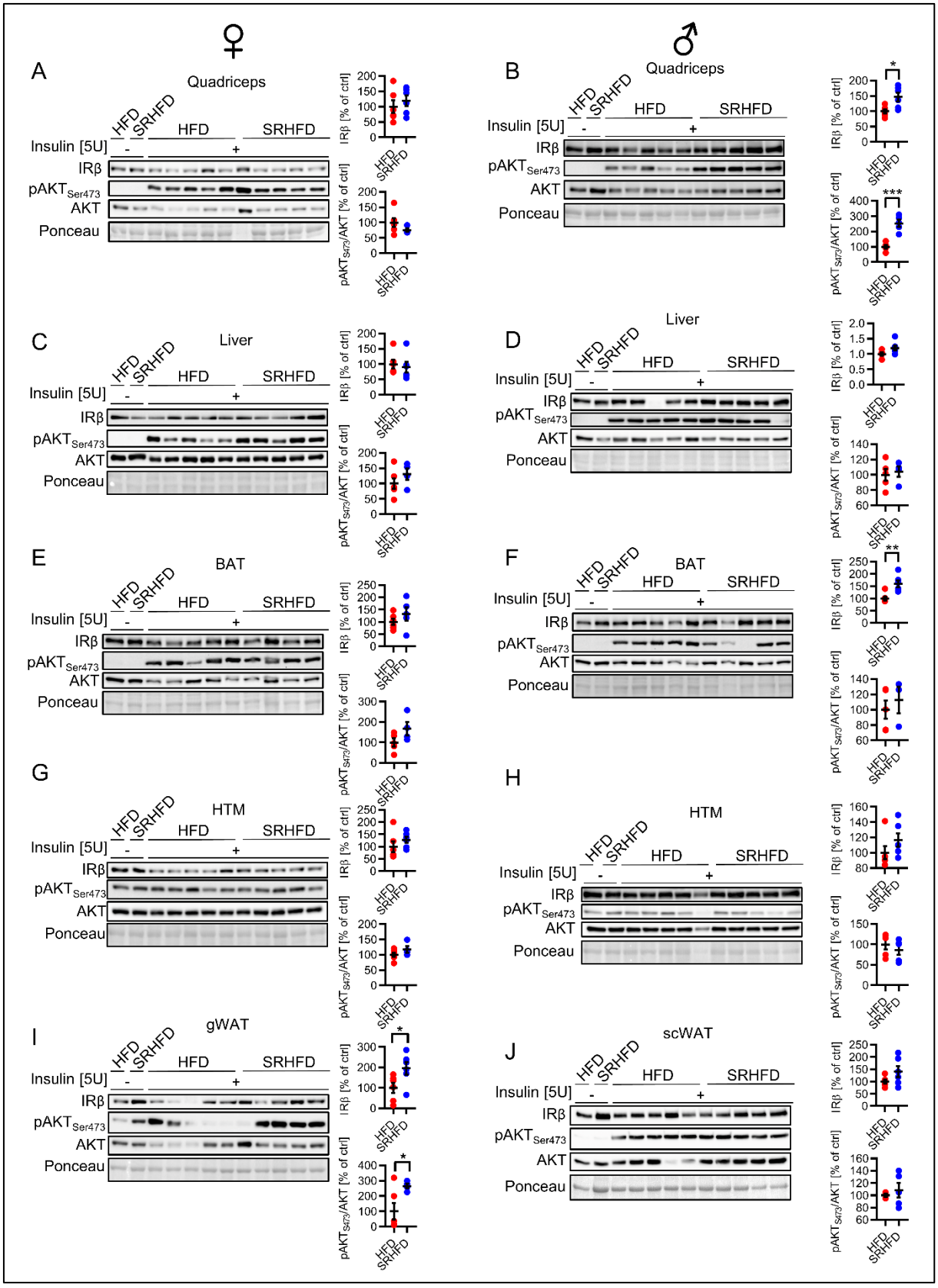
Supplementary Figure 5: Selenite supplementation affects liver metabolism. A, B: Representative protein expression and densitometric analysis of GPx1 in liver tissue of female (A) and male (B) mice after 12 weeks of either HFD or SRHFD (n = 6). C: Total GPx activity in liver tissue of male and female mice after 12 weeks of either HFD or SRHFD (n = 12). D: mRNA expression levels of members of fatty acid metabolism pathways and stress markers in liver tissue of female and male mice after 12 weeks of either HFD or SRHFD (n = 12). E: Plasma triglyceride content of female and male mice after 12 weeks of either HFD or SRHFD (n = 12). F: Non-esterified fatty acid (NEFA) levels of female and male mice after 12 weeks of either HFD or SRHFD (n = 12). G: Representative hematoxylin and eosin stain of liver tissue of mice after 12 weeks of either HFD or SRHFD, scale bars 100 µM. *: $P < 0.05$. **: $P < 0.01$ after two-tailed Student's t-test. All data are presented as mean ± SEM.



Supplementary Figure 6: No major differences in the hypothalamus of SRHFD fed mice. A: *Gpx1* mRNA expression in the hypothalamus after 12 weeks of either HFD or SRHFD (n = 6). B, C: Representative protein expression and densitometric analysis of GPx1 in the hypothalamus of female (B) and male (C) mice after 12 weeks of either HFD or SRHFD (n = 6). D, E: mRNA expression of different selenoproteins in the hypothalamus of mice after 12 weeks of either HFD or SRHFD (n = 12). F, G: mRNA expression of stress markers in the hypothalamus of mice after 12 weeks of either HFD or SRHFD (n = 12). *: P < 0.05, **: P < 0.01 after two-tailed Student's t-test. All data are presented as mean ± SEM.

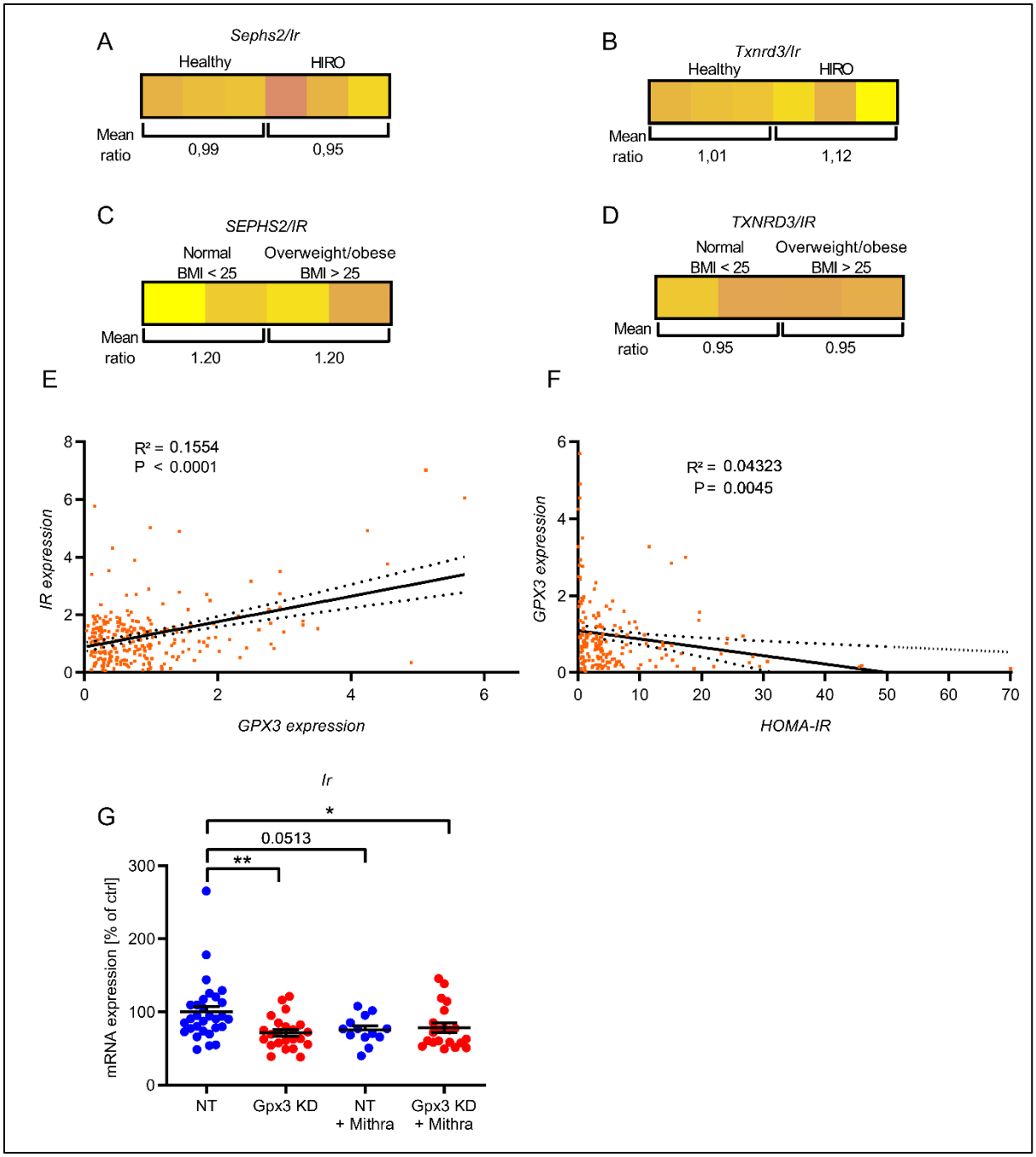


Supplementary Figure 7: Characterization of female phenotype. A: NMR (Nuclear Magnetic Resonance) measurement of mice after 9 weeks of either HFD or SRHFD (n = 7). B: Combined white adipose tissue (WAT) weight relative to bodyweight after 12 weeks of either HFD or SRHFD (n = 24). C, D, E: Leptin (C), Triglyceride (D) and non-esterified fatty acids (NEFA, E) levels of female mice after 12 weeks of either HFD or SRHFD (n = 12). F: Representative hematoxylin and eosin stain and area analysis of epigonadal white adipose tissue of mice after 12 weeks of either HFD or SRHFD, scale bars 100 µM (n = 6). G: mRNA expression levels of inflammatory markers in epigonadal white adipose tissue of mice after 12 weeks of either HFD or SRHFD. H: Blood glucose levels during an oral glucose tolerance test of mice fed either HFD or SRHFD (n = 12). I: Blood glucose levels during an intraperitoneal insulin tolerance test of mice fed either HFD or SRHFD (n = 15-17). J: Plasma Insulin levels of mice after 9 weeks of either HFD or SRHFD (n = 12). *: $P < 0.05$, ***: $P < 0.001$ after two-tailed Student's t-test. ##: $P < 0.01$ after two-way ANOVA. All data are presented as mean ± SEM. All data are presented as mean ± SEM.

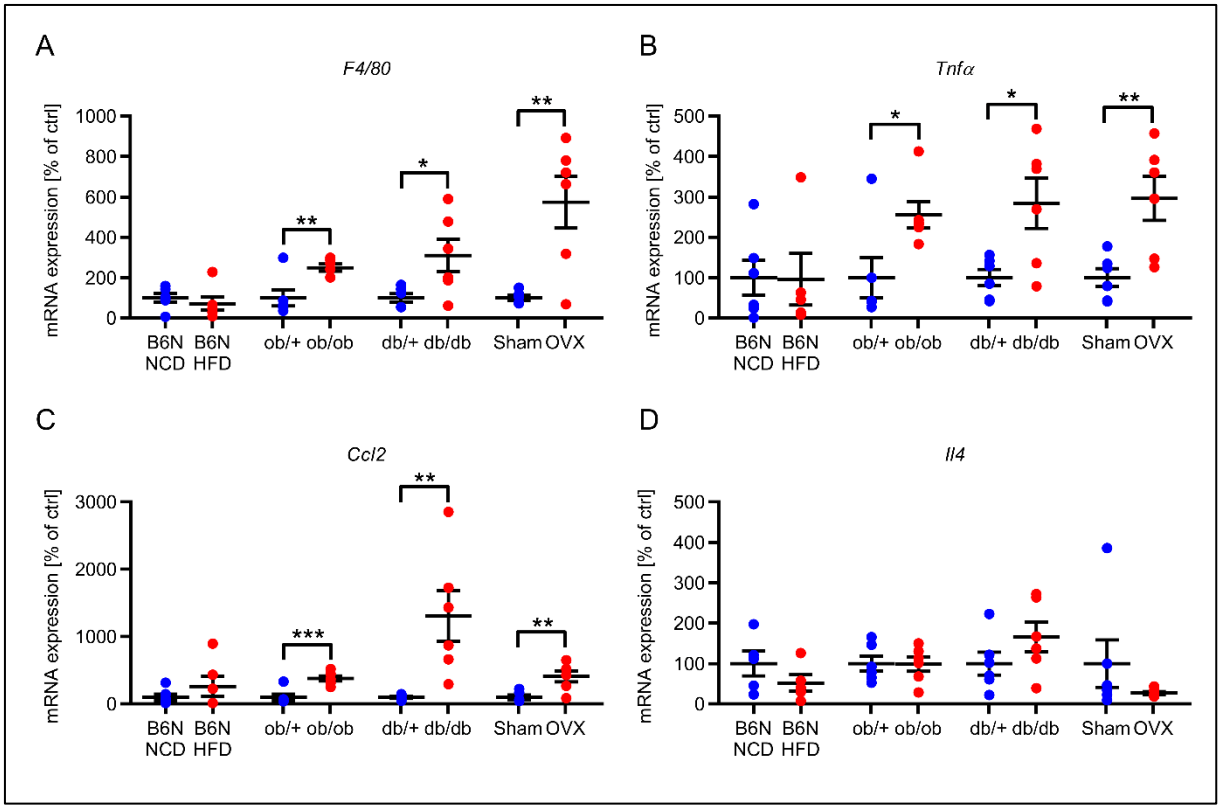


Supplementary Figure 8: Selenite supplementation increases insulin signaling. A-I: Protein expression and densitometric analysis of members of the insulin signaling pathway in the quadriceps (A, B), liver (C, D), brown adipose tissue (BAT, E, F), hypothalamus (HTM, G, H), gWAT (I), and scWAT (J)

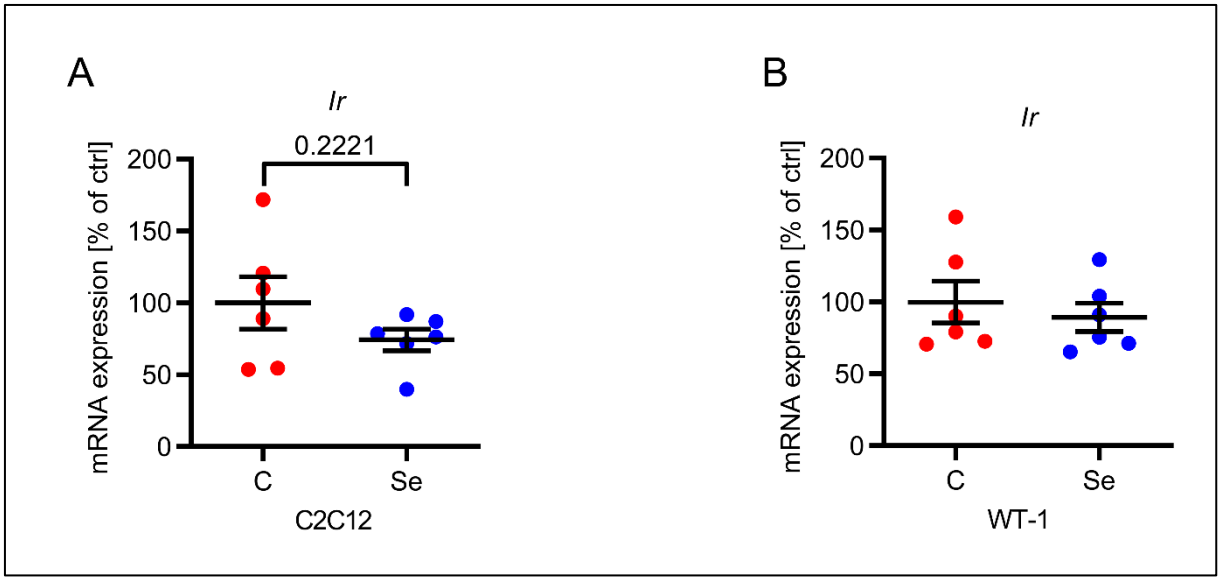
after bolus injection of 5 U insulin into the *vena cava* in female and male mice fed either HFD or SRHFD for 12 weeks. *: $P < 0.05$. **: $P < 0.01$ ***: $P < 0.001$ after two-tailed Student's t-test. All data are presented as mean ± SEM.



Supplementary Figure 9: Dysregulation of *Gpx3*, *Sephs2* and *Txnrd3* in murine and human adipose tissue. C = control, Se = 200 nM selenite treatment. A-D: Mean *Sephs2/Ir* and *Txnrd3/Ir* ratios in gWAT of healthy and LTIR mice (A, B, n = 12), and in normal weight (BMI < 25, n = 18) and overweight/obese (BMI > 25, n = 37) human patients (C, D). E: Correlation between *IR* and *GPX3* in human viscWAT (n = 302). F: Correlation between HOMA-IR and *GPX3* expression in human viscWAT (n = 215). G: *Ir* mRNA expression in siRNA mediated KD of *Gpx3* in 3T3-L1 cells after selenite and mithramycin (Mithra) treatment (NT = non-target). Correlation calculated using Pearson correlation coefficients, linear regression analysis was used for best fit curve showing 95 % CI in dashed lines. *: P < 0.05. **: P < 0.01 after two-tailed Student's t-test. All data are presented as mean ± SEM.



Supplementary Figure 10: Regulation of inflammatory markers in gWAT of mouse models of obesity. A-D: mRNA expression of *F4/80* (A), *Tnfα* (B), *Ccl2* (C), and *Il4* (D) (n = 6, % of ctrl refers to each respective healthy model, *i.e* NCD, ob/+, db/+, and Sham). *: P < 0.05. **: P < 0.01 after two-tailed Student's t-test. All data are presented as mean ± SEM.



Supplementary Figure 11: *In vitro* selenite treatment of Myoblasts (C2C12) and brown preadipocytes (WT1) does not regulate *Ir* expression. C = control, Se = 200 nM selenite treatment. A: *Ir* mRNA expression in C2C12 myoblasts after selenite treatment (n = 6). B: *Ir* mRNA expression in WT-1 brown preadipocytes after selenite treatment (n = 6). All data are presented as mean ± SEM.

Supplementary table 1: Analysis of trace element composition of selenium-controlled mouse diets

Element	Se ng/g	Mg mg/g	Ca mg/g	Mn µg/g	Fe µg/g	Cu µg/g	Zn ng/g	Cd ng/g	Mo ng/g	As ng/g
HFD	230.07	1.22	7.25	29.76	51.36	9.12	68.77	23.22	64.58	25.86
	6.70	0.00	0.07	2.71	0.30	1.58	0.83	0.83	1.56	0.39
SRHFD	676.67	1.20	7.16	24.95	46.95	9.00	68.53	22.65	50.77	26.25
	18.71	0.01	0.10	0.01	0.01	1.60	0.49	0.86	1.08	1.08

Concentrations and standard deviations of Selenium (Se), magnesium (Mg), calcium (Ca), iron (Fe), copper (Cu), zinc (Zn), cadmium (Cd), molybdenum (Mo) and arsenic (As) in HFD and SRHFD rodent diets measured via ICP-MSMS.

Supplementary table 2: Anthropometric and clinical characteristics of the study

Parameter	Entire cohort	Normal weight	Overweight/obese
N (women/men)	302 (205/97)	28 (15/13)	274 (190/83)
Age (years)	49 ± 15	68 ± 11	47.1 ± 13.5*
BMI	44.1 ± 12.8	22.7 ± 1.9	46.3 ± 11.3*
HOMA-IR (N)	5.6 ± 8.6 (215)	0.6 ± 0.8	6.2 ± 8.9*

Clinical parameters of 302 individuals and normal or overweight/obese subgroups. Data are means ± SD. *P value < 0.05 for differences between normal weight and overweight/obese subgroups.

Supplementary table 3: Real-time quantitative PCR primer pairs

Target gene	Forward sequence	Reverse sequence
Tbp (TATA-binding-protein	CTGGAATTGTACCGCAGCTT	ATGATGACTGCAGCAAATCG
Acadl (Acyl-CoA dehydrogenase, long chain)	GGTGGAAAACGGAATGAAAGG	GGCAATCGGACATCTTCAAAG
Acadm (Acyl-CoA dehydrogenase, medium chain)	TGTTAATCGGTGAAGGAGCAG	CTATCCAGGGCATACTTCGTG
Acads (Acyl-CoA dehydrogenase, small chain)	CCTGGGATGGGCTTCAAAATAG	GGTTCTCGGCATACTTCACAG
Fasn (Fatty acid synthase)	GAGGACACTCAAGTGGCTGA	GTGAGGTTGCTGTCGTCTGT
Atf4 (Activating transcription factor 4)	CCTGAACAGCGAAGTGTTGG	TGGAGAACCCATGAGGTTTCAA
Chop (C/EBP homologous protein)	CTGCCTTTCACCTTGGAGAC	CGTTTCCTGGGGATGAGATA
Scd1 (Stearoyl-CoA desaturase-1)	CTGACCTGAAAGCCGAGAAG	AGAAGGTGCTAACGAACAGG
Socs3 (Suppressor of cytokine signaling 3)	CCTATGAGAAAGTGACCCAGC	TTTGTGCTTGTGCCATGTG
Pppary (Peroxisome proliferator-activated receptor gamma)	CCCAGAGCATGGTGCCTTCGC	AGTTGGTGGGCCAGAATGGCA
Ap2 (Adipocyte protein 2)	AAGGTGAAGAGCATCATAACCCT	TCACGCCTTTCATAACACATTCC
Glut4 (Glucose transporter type 4)	CATTCCCTGGTTCATTGTGG	GAAGACGTAAGGACCCATAGC
Ir (Insulin receptor)	AATGGCAACATCACACACTACC	AATGGCAACATCACACACTACC
Gpx1 (Glutathione peroxidase 1)	ACAGTCCACCGTGTATGCCTTC	CTCTTCATTCTTGCCATTCTCCTG
Gpx2 (Glutathione peroxidase 2)	GTGCTGATTGAGAATGTGGC	AGGATGCTCGTTCTGCCCA
Gpx3 (Glutathione	CCATTTGGCTTGGTCATTCTGGG	CACCTGGTCGAACATACTTGAGAC

peroxidase 3)		
Gpx4 (Glutathione peroxidase 4)	TCTGTGTAAATGGGGACGATGC	TCTCTATCACCTGGGGCTCCTC
Gpx6 (Glutathione peroxidase 1)	GCACATCCTCTTTGTCAACG	CTTTCCAGGTTCTTGCTTTCC
Txnrd1 (Thioredoxin reductase 1)	ATGAGAATGCTTACGGGAGGT	GGAACCGCTCTGCTGAATAGAT
Txnrd2 (Thioredoxin reductase 2)	GATCCGGTGGCCTAGCTTG	TCGGGGAGAAGGTTCCACAT
Txnrd3 (Thioredoxin reductase 3)	CGACAACGAACGTGTGGTGG	AGTAGCTGCTTCGTGAGCCC
Dio1 (Iodothyronine deiodinase 1)	AGAGACTCGTAGATGACTTTGCC	GCCGGATGTCCACGTTGTT
Dio2 (Iodothyronine deiodinase 2)	TTTGATGTGTCAGGAGTCGGG	CCAACATTCCCTACCCCAAGA
Dio3 (Iodothyronine deiodinase 3)	CACGGCCTTCATGCTCTGG	CGGTTGTCGTCTGATACGCA
Sephs1 (Selenophosphate synthetase 1)	TGAACTGAAAGGCACAGGCTGC	CGCAAGTATCCATCCCAATGC
Sephs2 (Selenophosphate synthetase 2)	ACCGACTTCTTTTACCCCTTGG	TCACCTTCTCTCGTTCCTTTTCAC
Sep15 (15 kDa selenoprotein)	GTTTCAAGCGGCGTCTGCTC	TGCTTCTTCCTGACAGCACCC
SelenoH (Selenoprotein H)	CCTTATTCCACCAACGCGCCA	GCGTCAGCTCGTACAATGCTC
SelenoK (Selenoprotein K)	ATGGAAGAGGGCCACCAGGA	TTACCTTCCTCATCCACCAGCC
SelenoI (Selenoprotein I)	ACTGGTTACTGCTTCCTCTCCTC	CTGCTTCACCACTTGTACGCC
SelenoM (Selenoprotein M)	CGGATTGGAACCGTCTTCGAG	CACCTCCTTTAGGCGATTCAAC
SelenoO (Selenoprotein O)	TGACACTGAGTTCCAAAGGCAC	GTTAGTGAAGTCAGCACCAGTCAG
SelenoP (Selenoprotein P)	CCTTGGTTTGCCTTACTCCTTCC	TTTGTTGTGGTGTTTGTGGTGG
SelenoS (Selenoprotein S)	CAGAAGATTGAAATGTGGGACAGC	CCTTTGGGGATGACAGATGAAGTAG

SelenoT (Selenoprotein T)	CTTTAAATGATGTGCCAGTGTGGT	GGTAGGGCTATGATCGATGATGTG
SelenoV (Selenoprotein V)	CCCAACAGAATCTTGATCCGTG	TTCAAACTCCCCTGTAACCTG
SelenoW (Selenoprotein W)	GCCGTTCGAGTCGTGTATTGT	CACTTCAAAGAACCCGGTGAC
SelenoX (Selenoprotein X)	ACTTCGAGCCAGGTGTCTACG	GGCACTTGGTCACACTGTCTG
Pgc1α (Peroxisome proliferator-activated receptor gamma coactivator 1-alpha)	AGCCGTGACCACTGACAACGAG	GCTGCATGGTTCTGAGTGCTAAG
Tfam (Mitochondrial transcription factor A)	CACCCAGATGCAAAACTTTCAG	CTGCTCTTTATACTTGCTCACAG
Cox2 (Cytochrome c oxidase subunit II)	CCTGGTGAACTACGACTGCT	GAATAACCCTGGTCGGTTTG
Cox3 (Cytochrome c oxidase subunit III)	GCAGGATTCTTCTGAGCGTTCT	GTCAGCAGCCTCCTAGATCATGT
mrRNA (mitochondrial ribosomal RNA)	AGCCCATTTCTTCCCATTTC	CGATAAACCCCGCTCTACCT
Nd1 (NADH-ubiquinone oxidoreductase chain 1)	GGATCCGAGCATCTTATCCA	GGTGGTACTCCCGCTGTAAA
Nd6 (NADH-ubiquinone oxidoreductase chain 2)	ATTAAACAACCAACAAACCCAC	TTTGGTTGGTTGTCTTGGGTT
Hsp60 (Heat-shock protein 60)	AGTGTTCAGTCCATTGTCCC	TGACTGCCACAACCTGAAG
Hsp10 (Heat-shock protein 60)	CTGCCGAAACTGTAACCAAAG	TCTCCAACTTTCACACTGACAG
Lonp Lon protease homolog, mitochondrial)	CTTCCGTTTCAGTGTTGGTG	GGGTTCTCTGTCTTGGTCTTC
Clpp (Endopeptidase Clp)	ATATACTCGAGGCTGTTGCG	CCACCTGGGCTGTTGATATAC

F4/80 (EGF-like module-containing mucin-like hormone receptor-like 1)	GAATCTTGGCCAAGAAGAGAC	GAATTCTCCTTGTATATCATCAGC
Tnfα (Tumor necrosis factor alpha)	CTTCTGTCTACTGAACTTCGGG	CAGGCTTGTCACTCGAATTTTG
Ccl2 (chemokine (C-C motif) ligand 2)	GTCCCTGTCATGCTTCTGG	GCTCTCCAGCCTACTCATTG
Il-4 (Interleukin 4)	ACAGGAGAAGGGACGCCAT	GAAGCCCTACAGACGAGCTCA
Igf1r (Insulin-like growth factor receptor)	TGCTGTCTATGTCAAGGCTG	AGAGGAAGAGTTTGATGCTGAG
Transcription factor *Sp1*	CTCCAGACCATTAACCTCAGTG	ACCAGATCCATGAAGACCAAG

Supplementary table 4: Primary antibodies for Western Blotting

Antigen	Company	Cat.-Nº.
AKT	Cell Signaling Technology, Inc	9272
$pAKT_{Ser473}$	Cell Signaling Technology, Inc	9271
GPx1	Abcam plc.	ab22604
GPx3	Thermo Fisher Scientific, Inc.	PA5-18677
IRβ	Cell Signaling Technology, Inc	3025
DNP	Merck KGaA	D9656

Supplementary table 5: Secondary Antibodies for Western Blotting

Species	Company	Cat.-Nº.
Rabbit	Cell Signaling Technology, Inc	7074S
Mouse	Cell Signaling Technology, Inc	7076S
Goat	Jackson Immuno Research, Inc.	305-035-006

Supplementary table 6: Various compounds

Compound	Company	Cat.-No.
Mithramycin A	Merck KGaA	M6891
Cytochalasin B	Merck KGaA	C6762
^{14}C-deoxyglucose	Perkin Elmer Inc.	NEC720A250UC
Saponin	Merck KGaA	47036
Insulin	Merck KGaA	I9278
Oil-Red-O	Merck KGaA	O0625