SUPPLEMENTARY DATA

Procedures for RT-qPCR:

1. *Quantification and integrity measurement of RNA*: Following a primary measurement using a Nanodrop 2000 spectrophotometer (Thermo, Wilmington, DE), the concentrations of RNA samples were adjusted to 200-300 ng/μl using RNase-free H_2O. Subsequently the concentrations of RNA were determined triplicate using a Nanodrop 2000 spectrophotometer. The ratios of 260/280 nm and 260/230 nm in the RNA samples isolated from WAT and liver are 2.03-2.10 and 2.24-2.63, respectively. In addition, the integrity of RNA was confirmed using an Agilent 2100 Bioanalyzer (Palo Alto, CA) with an Agilent RNA 600 Nano Kit. The RNA Integrity Numbers of the RNA samples used in the present study are above 8.2.

2. *RT-qPCR***:** Total RNA (500 ng) was reverse transcribed with MuLV reverse transcriptase and Oligod(T) primers in a 50 μl volume (Table S2). All the reagents for the reverse transcription (RT) were from Applied Biosystems (ABI, Foster City, CA). Resulting RT products were diluted 2 times using RNase-free H_2O and applied in the following qPCR (Table S2). Each target mRNA in all the samples was measured triplicate. In each plate, three wells without template (with the same volume of DNase-free H_2O) were used as negative controls.

3. *Reference gene selection for target gene normalization***:** *18S* has been reported as the most stable endogenous control under various experimental conditions, including adipocytes (1) and adipose tissues from obese and diabetic patients (2). To further confirm *18S* is a proper reference gene in WAT of current study, the expression of *18S*, *β-actin* (forward primer: 5'-GTATGACTCCACTCACGGCAAA-3'; reverse primer: 5'-GGTCTCGCTCCTGGAAGATG-3') and glyceraldehyde-3-phosphate dehydrogenase (*Gapdh,* forward primer: 5'-AGTATGACTCCACTCACGGCAAAT-3'; reverse primer: 5'-GTCTCGCTCCTGGAAGATGGT-3') was determined using the protocol described above. Consistent with previous report (1; 2), *18S* is the most stable endogenous control in WAT among the four experimental groups (*Nrf2*+/+:WT, *Nrf2*-/-:WT, *Nrf2*+/+:*ob/ob*, *Nrf2*-/-:*ob/ob*), with Cq values (mean ± SD) 14.68 ± 0.29, 14.24 ± 0.03, 14.50 ± 0.02 and 14.38 ± 0.16, respectively. There were no significant differences in relative target gene expression when normalized to undiluted *18S* at 12.5 ng compared to diluted *18S* at 12.5 pg, representing a 10 cycle difference in Cq value. In contrast, substantially increased levels of *β-actin* and *Gapdh* (with lower Cq values) were found in WAT of *ob/ob* mice compared to non-*ob/ob* mice. Thus, *18S* was selected as the reference gene for target gene normalization in the present study.

Preparation of tissue lysates of WAT for immunoblotting. WAT (1,000 mg) was homogenized in 500 μl 2× lysis buffer (Cell Signaling; #9803) with protease and phosphatase inhibitors (Sigma; P8340; P0044; P5726) and 1875 μl of a chloroform/methanol (1:2) mixture using a TissueLyser II. Following homogenization, delipidation of tissue lysates was performed by chloroform/methanol extraction (8). Briefly, homogenized tissue was transferred to 10 ml glass tubes and mixed sporadically while kept on ice for 10–15 min. Subsequently the homogenate was diluted with 625 μl of chloroform and 625 μl of water to change the water/chloroform/methanol ratio from 0.8:1:2 to 1.8:2:2 in the final organic solution. Following centrifugation (800*g,* 5 min, 4 °C), protein disk between lipid (lower) phase and aqueous (upper) phase was collected and dissolved in 1× lysis buffer. Resulting protein solution was sonicated for 10 seconds and used for protein quantification and further immunoblot analysis.

SUPPLEMENTARY DATA

Supplementary Table 1. Primer sequences for mouse genotyping

Primer name	Sequence (5'-3')	PCR products (bp)
Nrf2-knockout:		
Nrf2-forward	TGGACGGGACTATTGAAGGCTG	
Nrf2-reverse	CGCCTTTTCAGTAGATGGAGG	733 (WT)
LacZ-reverse	GCGGATTGACCGTAATGGGATAGG	500 (KO)
Nrf2-LoxP:		
Nrf2-LoxP-forward	CACAATGGTATGCCTGCTGT	
Nrf2-LoxP-WT-reverse	TCTGCACCAGAGTTCAAAGG	218 (WT)
Nrf2-LoxP-KI-reverse	AAGAGGGGGTTGGAAAGAGA	174 (KI)
Cre	see The Jackson Laboratory protocol	
Ob	see The Jackson Laboratory protocol	

Supplementary Table 2. Reaction conditions for RT-qPCR

RT				
ABI Reagents	Volume (μl)	RT condition		
RNase-free H2O	9.25	25˚C	10 min	
MgCl2 (25 mM)	11	48˚C	60 min	
GeneAmp 10X PCR Buffer Ⅱ	5	95˚C	5 min	
dNTP (10 mM)	10	4˚C	∞	
Oligd(T)16 (50 μM)	2.5			
Rnase inhibitor (20 U/μl)	1			
MuLV reverse transcriptase (5 U/μl)	1.25			
RNA (50 ng/μl)	10			
Total volume	50			
qPCR	Volume (μl)	PCR condition		Repeats
SYBR	3.5	50˚C	2 min	1
Primer Mix (10 μM)	0.45	95˚C	10 min	1
Dnase-free H2O	1.05	95˚C	0.15 min	40
RT products	2.5	60˚C	1 min	40
Total volume	7.5	4˚C	∞	1

SUPPLEMENTARY DATA

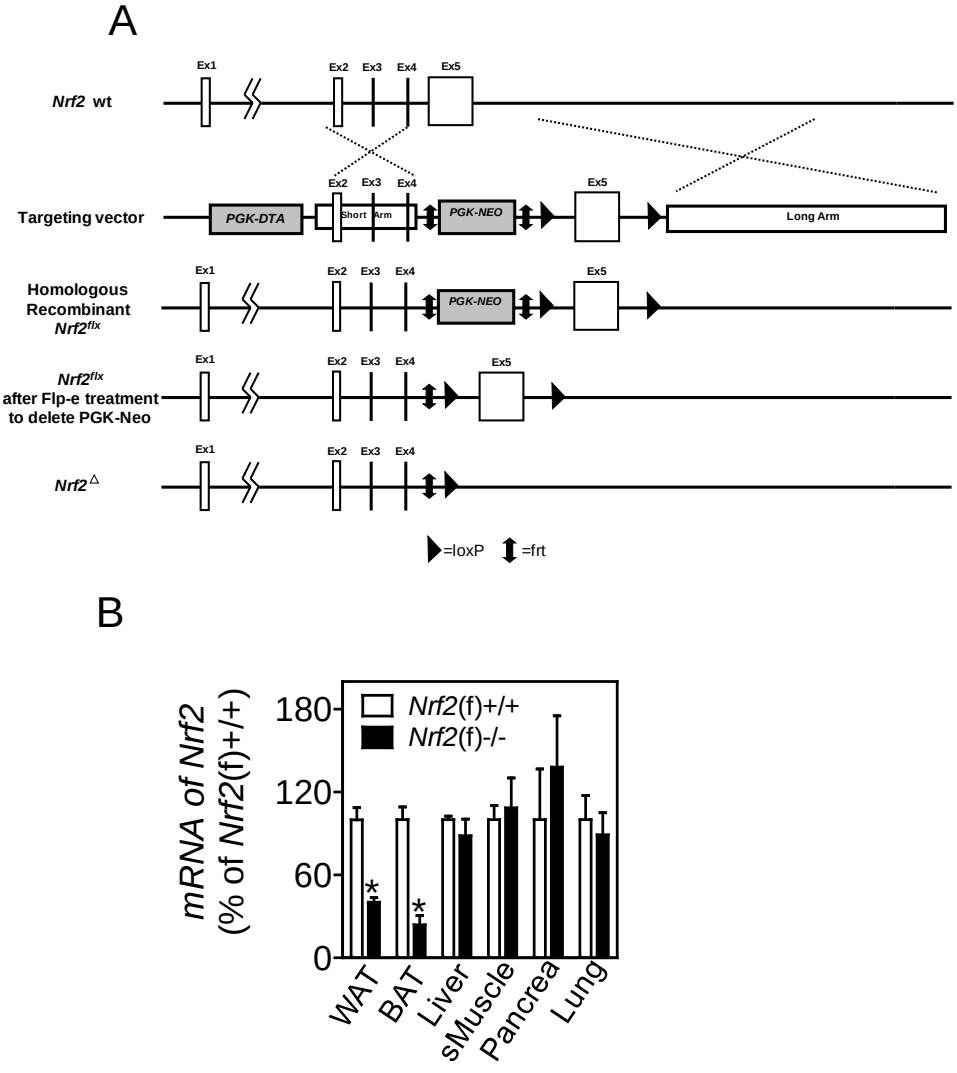
Supplementary Table 3. Primer sequences for RT-qPCR

Abbreviations	Gene name	Forward (5' - 3')	Reverse (5' - 3')	PCR efficiency (%)
18S	18S ribosomal RNA	CGAACGTCTGCCCTATCAACTT	CCGGAATCGAACCCTGATT	101.5
Acacα	Acetyl-CoA carboxylase α	CGGCAGTACCTGCGAGTAGAG	GGGCGAATACACATTTGTCGTA	102.1
Acacβ	Acetyl-CoA carboxylase β	GGGCCCTGGGAGACAAGA	GGGTAAGGTTGGGATTTGCA	103.9
Adfp	Adipose differentiation-related protein	GGTGATGGCAGGCGACAT	CCATCGGACACTTCCTTAAAGG	92.8
Adipoq	Adiponectin	GCTCAGGATGCTACTGTTGCAA	AACGTCATCTTCGGCATGACT	90.7
Adpsn	Adiposin	GCTATCCCAGAATGCCTCGTT	TTCCACTTCTTTGTCCTCGTATTG	92
Akt1	Akt1	TGTCTCGAGAGCGTGTGTTCTC	CAGACACAATCTCCGCACCAT	102.7
Akt2	Akt2	ATGAAGATCCTGCGCAAGGA	GCAGAACCCGGCTCTCTGT	90.25
Mcp1	Monocyte chemotactic protein-1	CTGAAGCCAGCTCTCTCTTCCT	CAGGCCCAGAAGCATGACA	101.2
Cd36	Cluster of differentiation 36	CAGAGTTCGTTATCTAGCCAAGGAA	CATTGGGCTGTACAAAAGACACA	96.9
Cd68	Cluster of differentiation 68	CCCATCCCCACCTGTCTCT	TGATGTAGGTCCTGTTTGAATCCA	94.1
Cebpα	CCAAT-enhancer-binding protein α	CGCAAGAGCCGAGATAAAGC	CGGTCATTGTCACTGGTCAACT	94.7
Cebpβ	CCAAT-enhancer-binding protein β	AAGCTGAGCGACGAGTACAAGA	GTCAGCTCCAGCACCTTGTG	94.2
Cebpδ	CCAAT-enhancer-binding protein δ	GCCGTGCCCACCCTAGA	CGCTTTGTGGTTGCTGTTGA	104.8
F4/80	EGF-like moleculecontaining mucin-like hormone	TCAGCCATGTGGGTAC AGTCA	CACAGCAGGAAGGTGGCTATG	106.9
Fabp4	Fatty acid binding protein 4	GCGTGGAATTCGATGAAATCA	CCCGCCATCTAGGGTTATGA	105
Fas	Fatty acid synthase	CCTGGATAGCATTCCGAACCT	AGCACATCTCGAAGGCTACACA	100.5
Gclc	Glutamate—cysteine ligase catalytic subunit	TGGCCACTATCTGCCCAATT	GTCTGACACGTAGCCTCGGTAA	100.9
Gclm	Glutamate—cysteine ligase regulatory subunit	ACATTGAAGCCCAGGATTGG	CCCCTGCTCTTCACGATGAC	99.3
Glut4	Glucose transporter type 4	CGCACTAGCTGAGCTGAAGGA	AGGAGCTGGAGCAAGGACATT	105.1
Gpat	Glycerol-3-phosphate acyltransferase 1	AGGCTTCTAGGTCCCCTGCTA	CCGCTGAAGTTGTGGACAAA	96.4
Gpx1	Glutathione peroxidase 1	CGCTTTCGTACCATCGACATC	GGGCCGCCTTAGGAGTTG	95.7
Gpx2	Glutathione peroxidase 2	ACCGATCCCAAGCTCATCAT	CAAAGTTCCAGGACACGTCTGA	107.2
Gpx3	Glutathione peroxidase 3	ACAGGAGCCAGGCGAGAA	CCACCTGGTCGAACATACTTGA	99.4
Gpx4	Glutathione peroxidase 4	CCCGATATGCTGAGTGTGGTT	CCTGCCTCCCAAACTGGTT	91.2
Gsr	Glutathione reductase	TTGCGTGAATGTTGGATGTGT	TTCCGAGTGCACTGCTGTGT	94.2
Gss	Glutathione synthetase	TGCGGTGGTGCTACTGATTG	CGGCACGCTGGTCAAATAT	98.6
Ho1	Heme oxygenase 1	CCTCACTGGCAGGAAATCATC	CCTCGTGGAGACGCTTTACATA	97.1
Ikkβ	Inhibitor of nuclear factor kappa-B kinase subunit	AGCTGTCCTTACCCTGCTGAGT	ATGGATGATTCTGTTTTCGTGAAG	100.7
Il6	Interleukin 6	GCCCACCAAGAACGATAGTCA	GAAGGCAACTGGATGGAAGTCT	95.5
IL10	Interleukin 10	GATGCCCCAGGCAGAGAA	CACCCAGGGAATTCAAATGC	104.5
Il1β	Interleukin 1β	GAAACCATGGCACATTCTGTTC	AATAGGTAAGTGGTTGCCCATCA	93.2
Infγ	Interferon γ	TTGGCTTTGCAGCTCTTCCT	TGACTGTGCCGTGGCAGTA	100
Irs1	Insulin receptor substrate 1	CCTCAGTCCCAACCATAACCA	TCCGGCACCCTTGAGTGT	92.8
Nos2	Nitric oxide synthase 2	GCAAACCCAAGGTCTACGTTCA	GAGCACGCTGAGTACCTCATTG	97.88
Nox2	NADPH oxidase gp91phox subunit	CAGGAGTTCCAAGATGCCTG	GATTGGCCTGAGATTCATCC	94.4
Nqo1	NAD(P)H dehydrogenase (quinone 1)	TATCCTTCCGAGTCATCTCTAGCA	TCTGCAGCTTCCAGCTTCTTG	95.4
Nrf2	Nuclear factor E2-related factor 2	A	GCTCGACAATGTTCTCCAGCTT	99.3
Pepck1	Phosphoenolpyruvate carboxykinase 1	CCACAGCTGCTGCAGAACA	GGGTCGCATGGCAAAGG	104.3
Pepck2	Phosphoenolpyruvate carboxykinase 2	GCAAACTCCCCAAGTATAAGAACTG	GCTTTCTACCCGTGCCACAT	95.3
Pgc1α	Peroxisome proliferator-activated receptor γ coac	CCGTAGGCCCAGGTACGA	TGCGGTATTCATCCCTCTTGA	99.3
Atgl	Adipose triglyceride lipase	AGACAGAGCTTTCTCCCAGTGAA	CCCCGTGAAGCCCAACT	106
Pparγ1	Peroxisome proliferator-activated receptor γ1	GGGCTGAGGAGAAGTCACAC	TGGTTCACCGCTTCTTTCA	91
Pparγ2	Peroxisome proliferator-activated receptor γ2	TGCTGTTATGGGTGAAACTCTG	CTGTGTCAACCATGGTAATTTCTT	90
Prx1	Peroxiredoxin 1	GATCCCAAGCGCACCATT	TAATAAAAAGGCCCCTGAAAGAG/	98.2
Retn	Resistin	CACGTACCCACGGGATGAA	GGACAAGGAAGAAAAGGAAAAG(	91
Socs3	Suppressor of cytokine signaling 3	TGGACCCATTCGGGAGTTC	TCTGACCCTTTTGCTCCTTAAAGT	92.1
Sod1	Superoxide dismutase 1	GTGATTGGGATTGCGCAGTA	TGGTTTGAGGGTAGCAGATGAGT	100.1
Sod2	Superoxide dismutase 2	TTAACGCGCAGATCATGCA	GGTGGCGTTGAGATTGTTCA	100
Sod3	Superoxide dismutase 3	CATGCAATCTGCAGGGTACAA	AGAACCAAGCCGGTGATCTG	104
Tcf7l1	transcription factor 7-like 1	CCAGCACACTTGTCCAACAAA	AGCGGGTGCATGTGATGA	96
Tcf7l2	transcription factor 7-like 2	TGCTGCTGGTGGGTGAAAA	CTCGTCGTTAGCGCCTAGGT	95.8
Tnfα	Tumor necrosis factor α	CAGCCGATGGGTTGTACCTT	GGCAGCCTTGTCCCTTGA	91.6
Txn	Thioredoxin	CCGCGGGAGACAAGCTT	GGAATGGAAGAAGGGCTTGATC	108
Txnrd1	Thioredoxin reductase 1	CAATCTGAGCTGCCGAACAA	GGGATCTTTGGAGCCATTCA	107.2
Zfp423	Zinc-finger protein 423	CCGCTGTGTGGTCTGTATGC	ATGTGAAAGGTGCCATGGATCT	106

Note: The specificity of primers has been validated using melting curve analysis. The PCR efficiency was determined using mRNA of WAT, liver, 3T3-L1 cells, RAW 264.7 cells and/or MIN6 cells and calculated from equation [Efficiency (%) = $100 \times (10^{(-1/\text{slope})}-1)$]. In addition, most of the primer sets have been tested and confirmed in mouse cells with specific gene knockdown, overexpression and/or exposure to chemical activators (3-7).

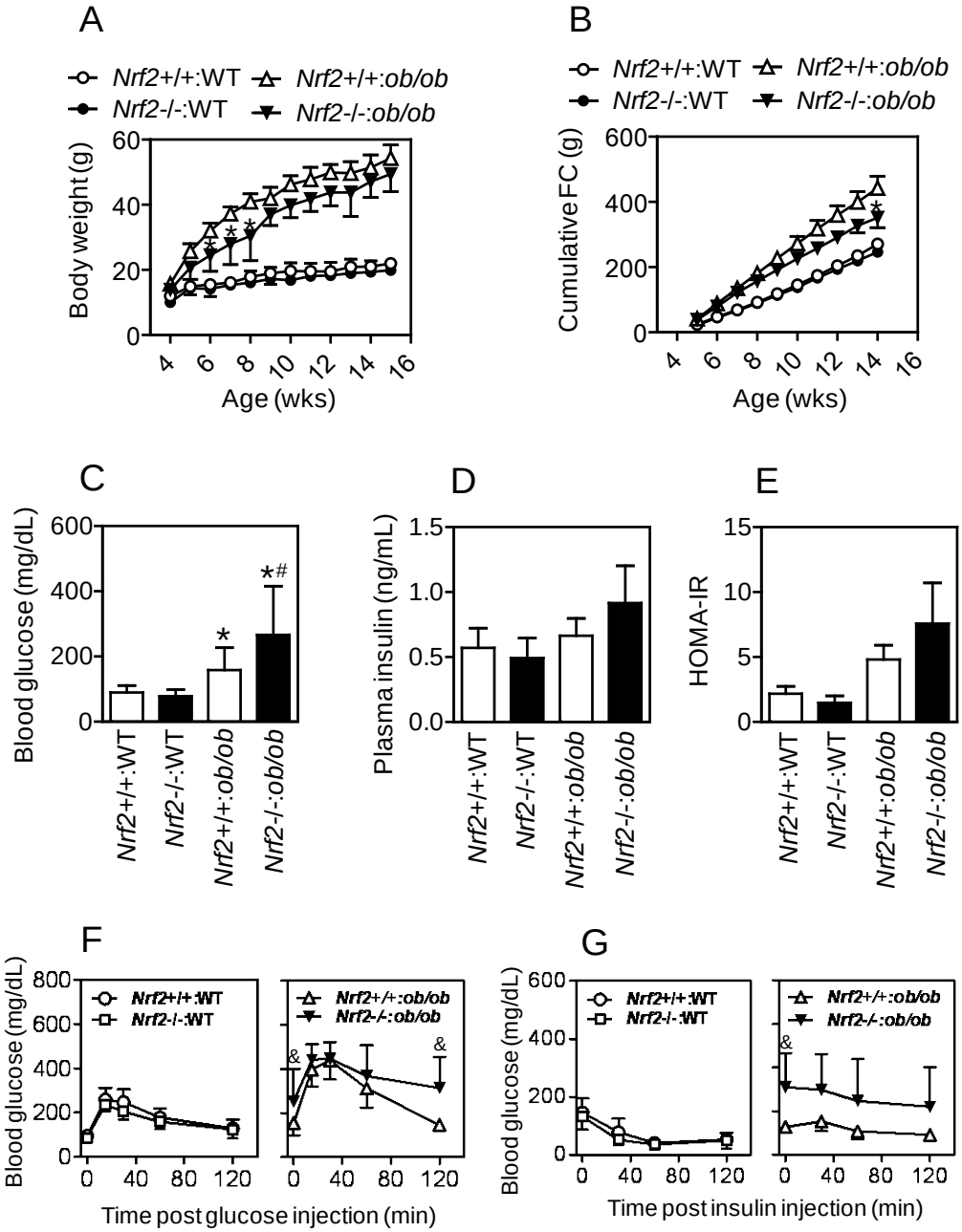
SUPPLEMENTARY DATA

Supplementary Figure 1. (*A*) Generation of a conditional allele, via a sequence replacement strategy to knock-out the *Nrf2* gene. The construct contains loxP sites that flank exon 5, a 2.6 kb 5' short arm of homology (containing exons 2, 3 & 4), a 9.8 kb 3' long arm of homology, a Diphtheria Toxin A (DTA) cassette, and a Neomycin (Neo) cassette flanked by frt sites for selective deletion. The Neo element allows for positive selection in ES cells, while the DTA element permits negative selection in ES cells. After homologous recombination of the conditional knock-out construct, the PGK-Neo is excised via Flp-e electroporation. The *Nrf2* gene has normal expression until Cre-mediated deletion of exon 5. This recombination creates a drastic premature stop, which renders the *Nrf2* gene inactive. (*B*) Gene expression of *Nrf2* in adipose tissue-specific *Nrf2*-knockout mice (*Nrf2(f)-/-)*. Deletion of the floxed *Nrf2* in adipose tissue was achieved by crossing homozygous $Nrf2^{LoxP/LoxP}$ with B6.Cg-Tg(Fabp4-cre)1Rev/J heterozygous mice (Cre-positive). *Nrf2* expression was determined by real-time RT-PCR. *Nrf2*(f)+/+, $Nrf2^{LoxP/LoxP}$ and Cre-negative; *Nrf2*(f)-/-, $Nrf2^{LoxP/LoxP}$ and Cre-positive. n = 4-5 males (age = 14-15 weeks). *$p < 0.05$ vs. *Nrf2*(f)+/+.



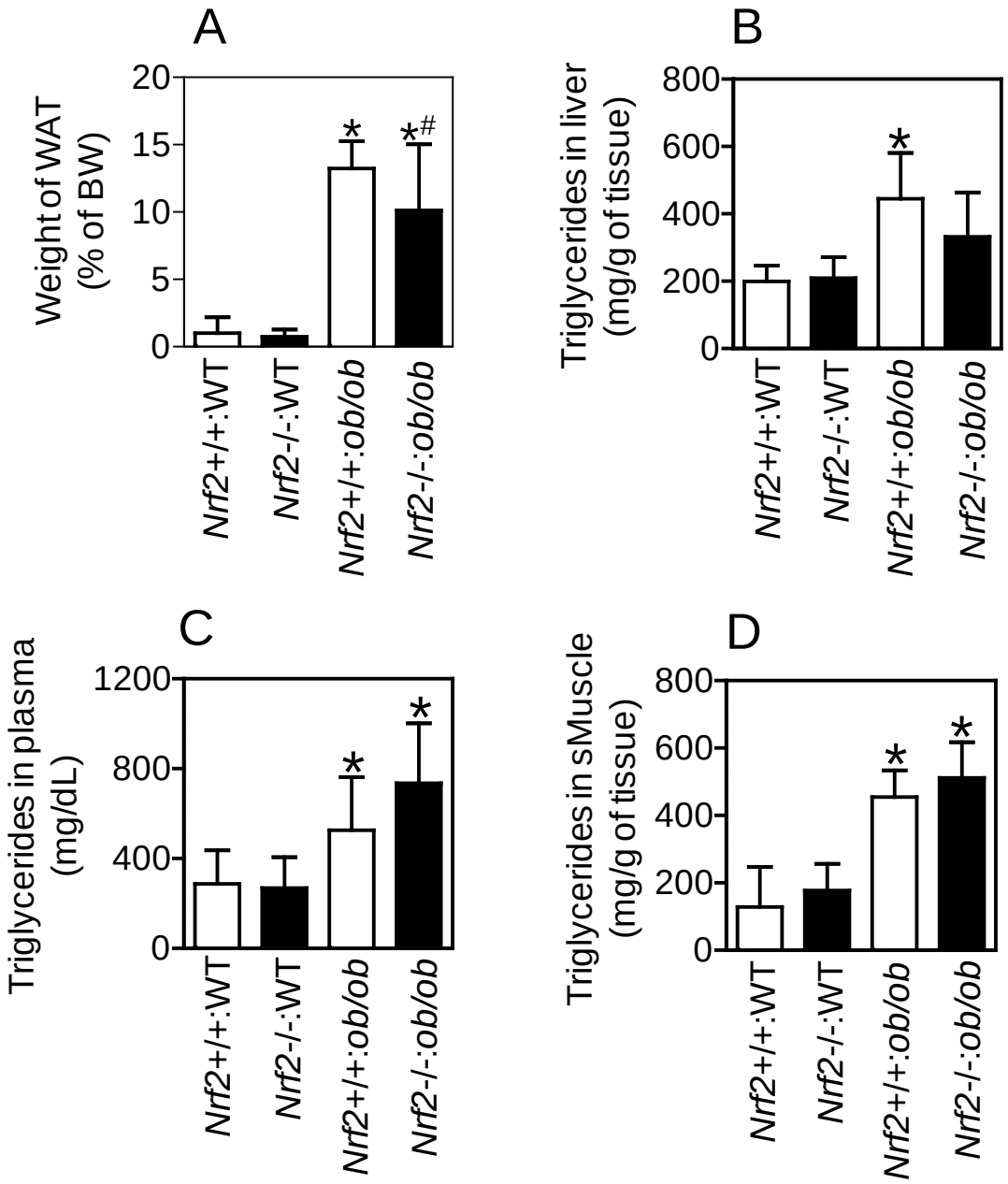
SUPPLEMENTARY DATA

Supplementary Figure 2. *Ob/ob* female mice with global *Nrf2* deletion exhibit reduced body weight, aggravated insulin resistance and hyperglycemia. ***A***: Body weight analysis of mice maintained on a chow diet. n = 6-10. **p* < 0.05 vs. *Nrf2*+/+:*ob*/*ob* mice at the same age. ***B***: Cumulative food consumption (FC). n = 5. **p* < 0.05 vs. *Nrf2*+/+:*ob*/*ob* mice at the same age. ***C***: Fasting blood glucose. n = 16-30. **p* < 0.05 vs. non-*ob*/*ob* mice with the same *Nrf2* genotype; $^{\#}p$ < 0.05 vs. *Nrf2*+/+:*ob*/*ob* mice. ***D***: Fasting plasma insulin. n = 3-8. ***E***: Homeostatic model assessment for insulin resistance (HOMA-IR). n = 3-8. ***F***: Intraperitoneal glucose tolerance test. Mice were challenged with 0.5 mg of glucose/g body weight. n = 10-11. $^{\&}p$ < 0.05 vs. *Nrf2*+/+:*ob*/*ob* mice with the same treatment. ***G***: Intraperitoneal insulin tolerance test. Mice were challenged with insulin at 0.75 and 4 U/g of BW in non-*ob/ob* and *ob/ob* mice, respectively. n = 9-11.



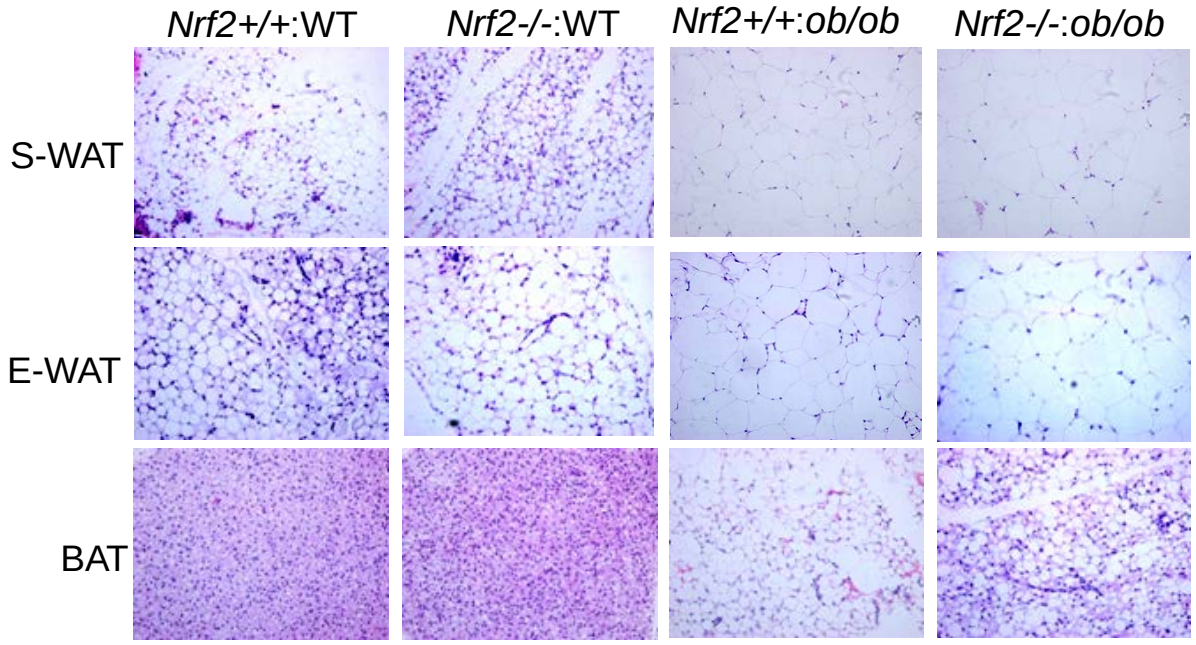
SUPPLEMENTARY DATA

Supplementary Figure 3. *Nrf2*-/-:*ob/ob* female mice show reduced WAT mass and mild hepatic steatosis but trended increased plasma triglycerides. ***A***: Weight of WAT. Retroperitoneal and gonadal depots were measured. n = 11-15. Animal age is 8-15 wks. ***B***: Levels of triglycerides in liver. n = 7. ***C***: Levels of triglycerides in plasma. n = 6. ***D***: Levels of triglycerides in skeletal muscle. n = 5-6. Values in *A-D* are mean ± SD. **p* < 0.05 vs. non-*ob*/*ob* mice with the same *Nrf2* genotype; $^{\#}p$ < 0.05 vs. *Nrf2*+/+:*ob*/*ob* mice.

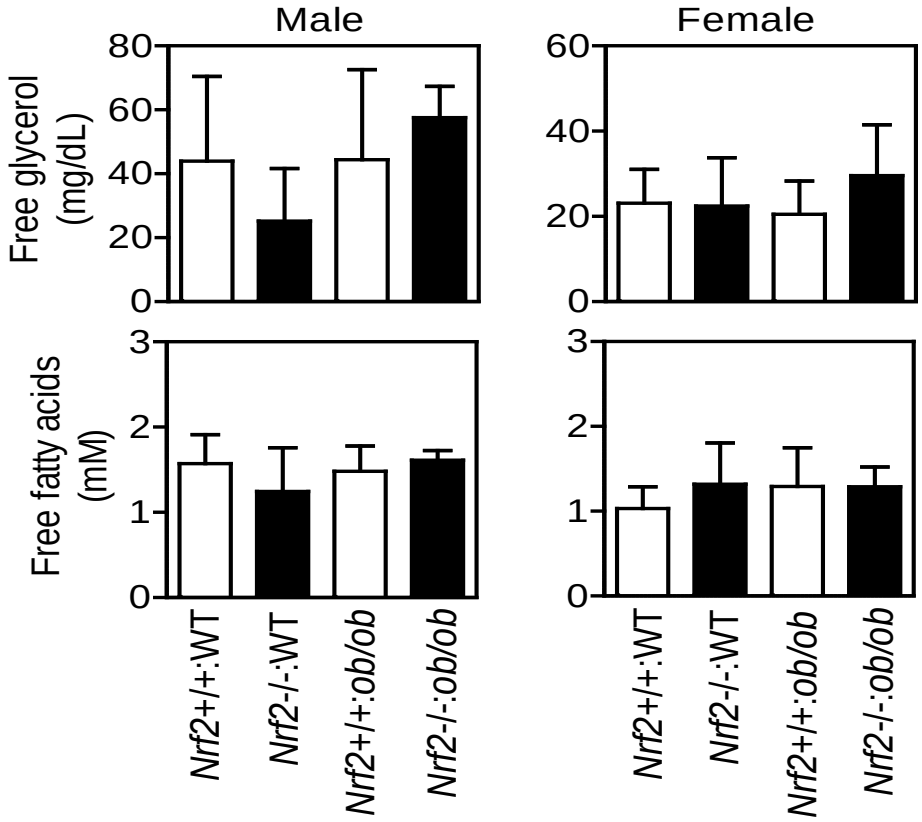


SUPPLEMENTARY DATA

Supplementary Figure 4. Representative images of H&E stained adipose tissues (20×). S-WAT, subcutaneous WAT; E-WAT, epididymal WAT. BAT, brown adipose tissue. Animal age is 12-15 wks.

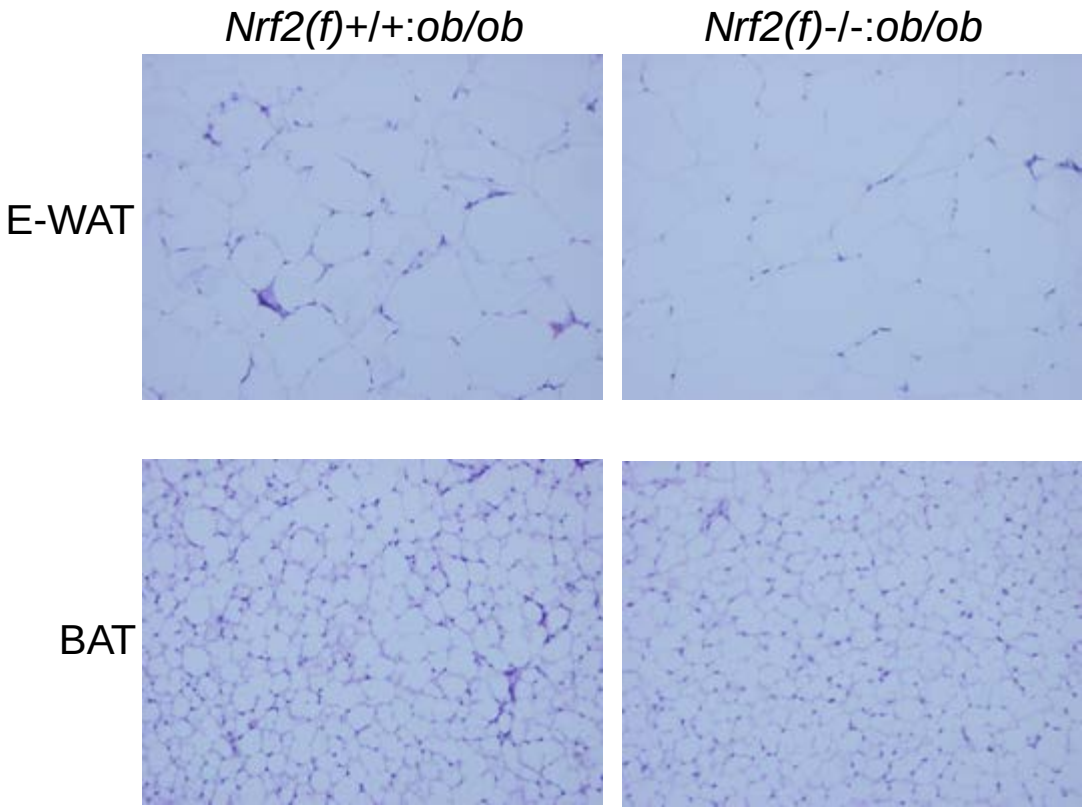


Supplementary Figure 5. Plasma levels of free glycerol and free fatty acids in mice. Values are mean ± SD. n = 6 male or female mice



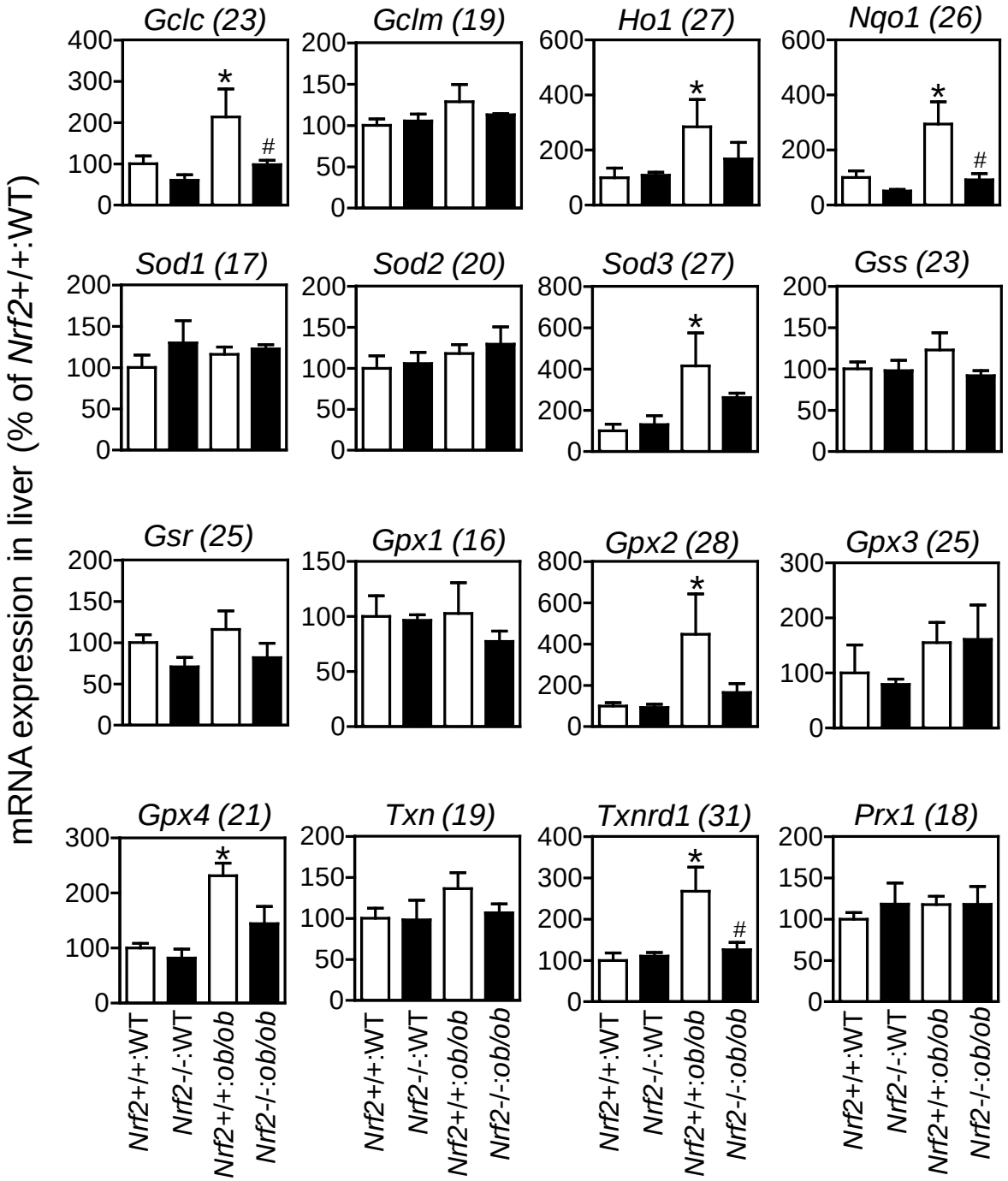
SUPPLEMENTARY DATA

Supplementary Figure 6. Representative images of H&E stained adipose tissues (20×) in male mice. E-WAT, epididymal WAT; BAT, brown adipose tissue. Animal age is 8-12 wks.



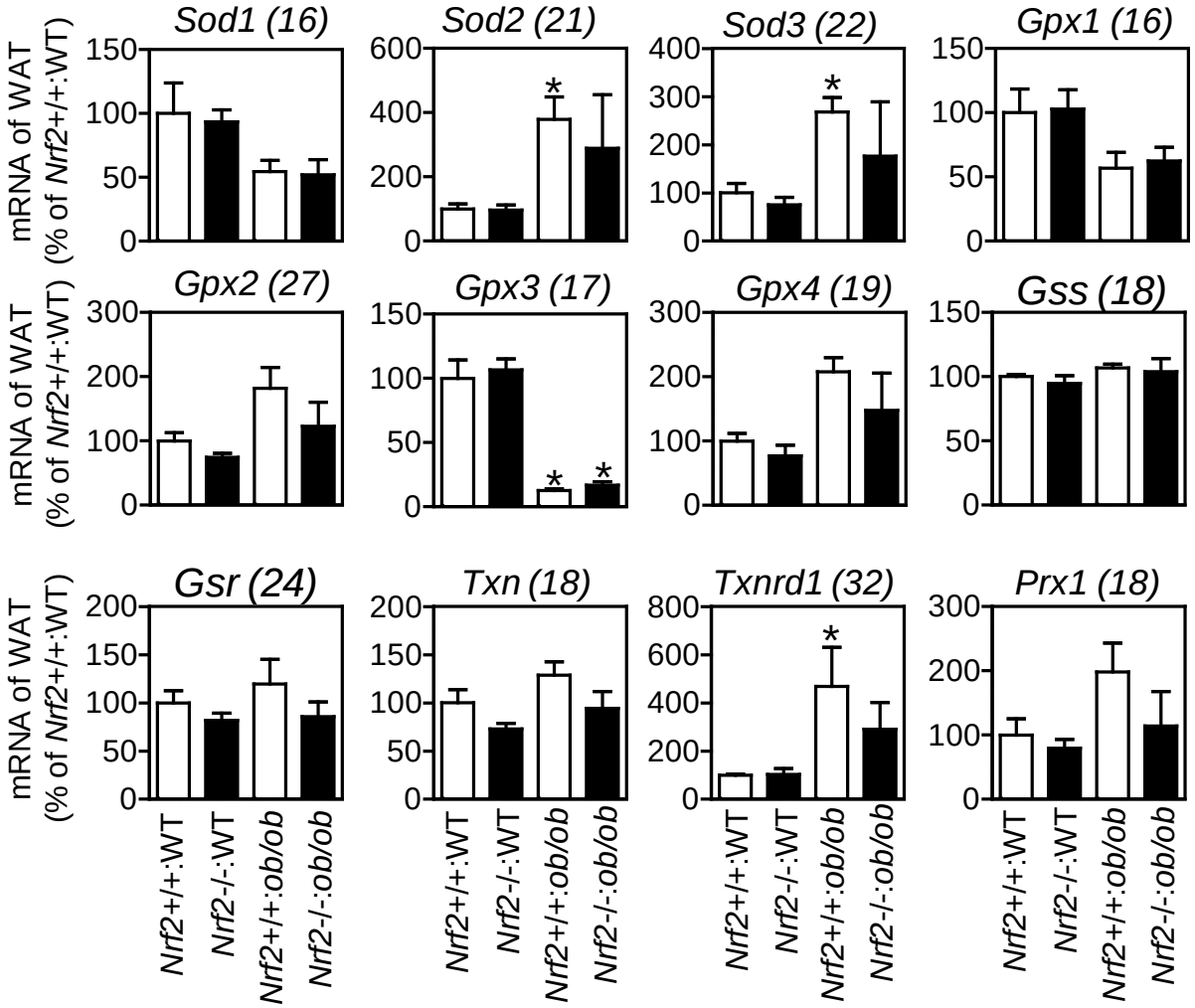
SUPPLEMENTARY DATA

Supplementary Figure 7. mRNA expression of antioxidant enzymes in liver. n = 3-6 males. Animal age = 8-10 wks. Values are mean ± SD. *$p < 0.05$ vs. *Nrf2*+/+:WT. #$p < 0.05$ vs. *Nrf2*+/+:*ob/ob* mice. The number in brackets following each gene name is the Cq value of that gene in *Nrf2*+/+:WT. The average Cq value of reference gene 18S is 14.



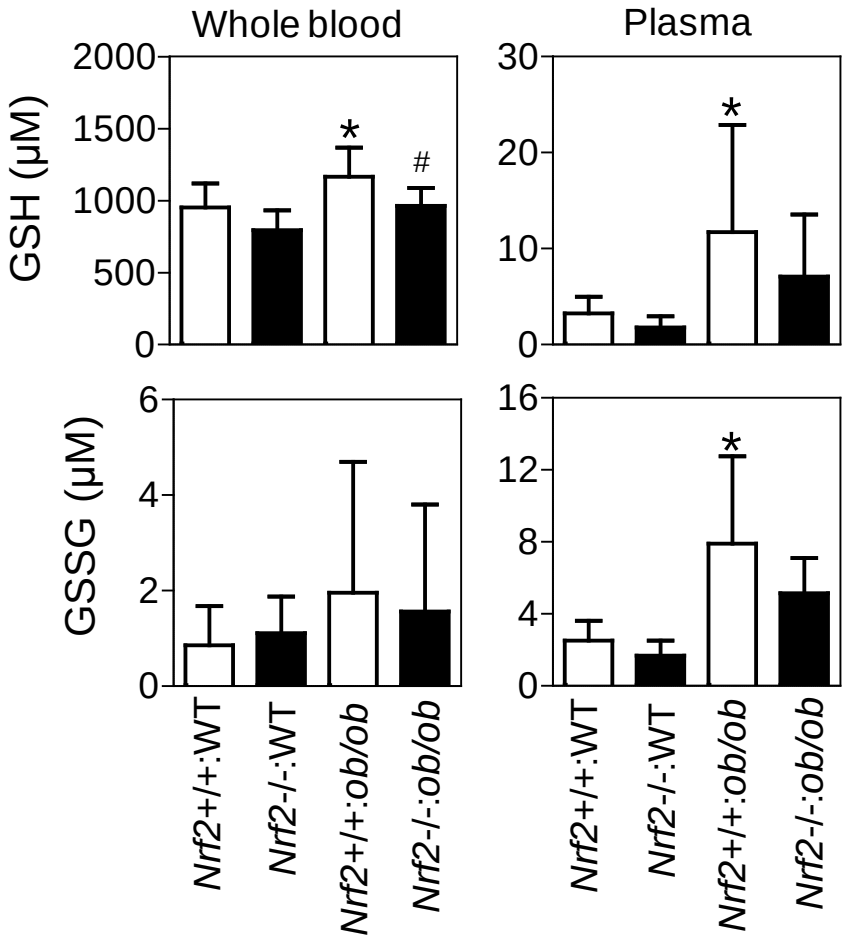
SUPPLEMENTARY DATA

Supplementary Figure 8. Expression of antioxidant genes in epididymal WAT. n = 3-6 males. Animal age is 8-10 wks. Values are mean ± SEM. *$p < 0.05$ vs. *Nrf2*+/+:WT. The number in brackets following each gene name is the Cq value of that gene in *Nrf2*+/+:WT. The average Cq value of reference gene 18S is 14.



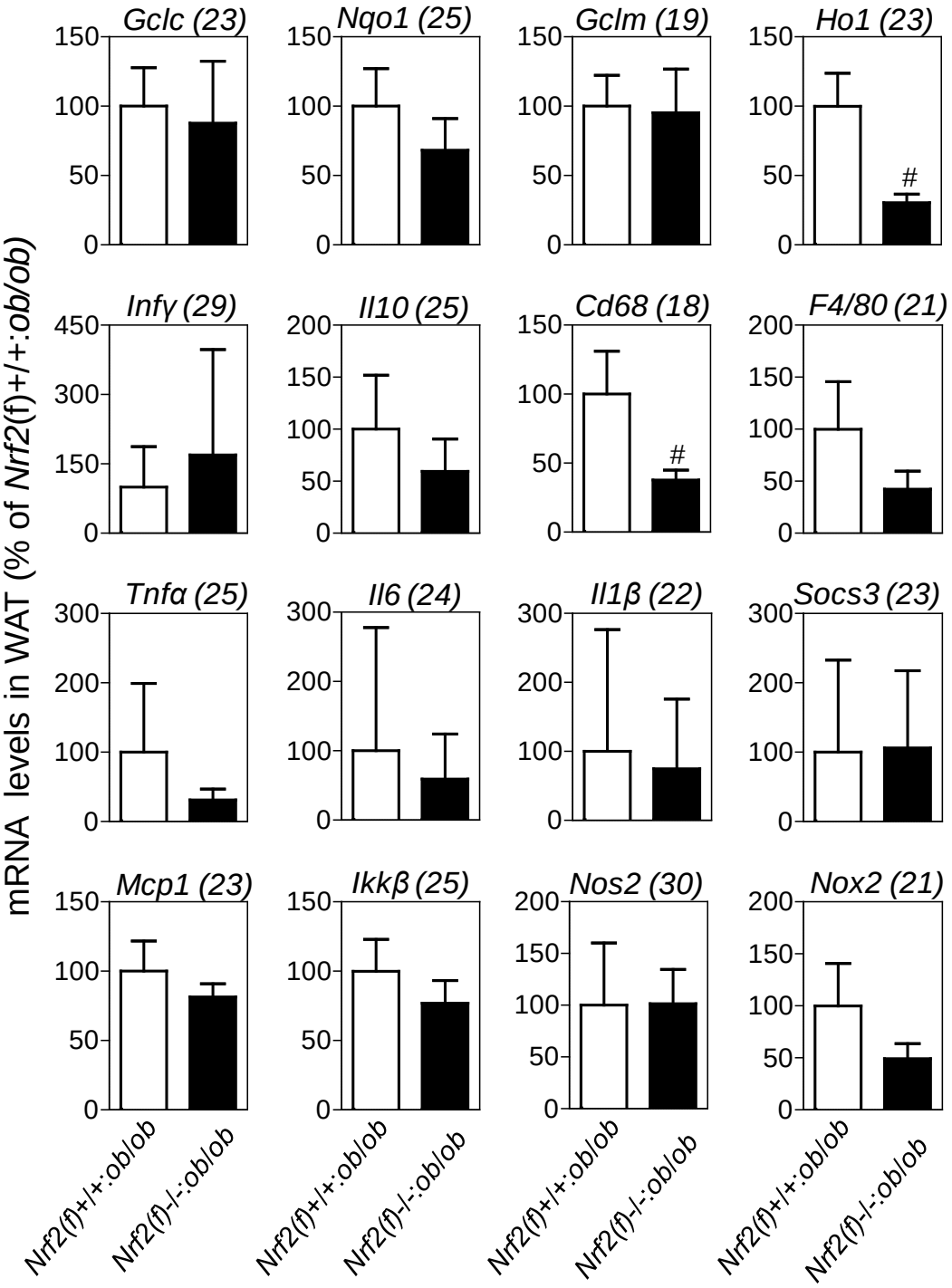
SUPPLEMENTARY DATA

Supplementary Figure 9. GSH and GSSG levels in whole blood and plasma in female mice. n = 8-18. Animal age = 8-15 wks. Values are mean ± SD. *$p < 0.05$ vs. *Nrf2*+/+:WT; #$p < 0.05$ vs. *Nrf2*+/+:*ob/ob* mice.



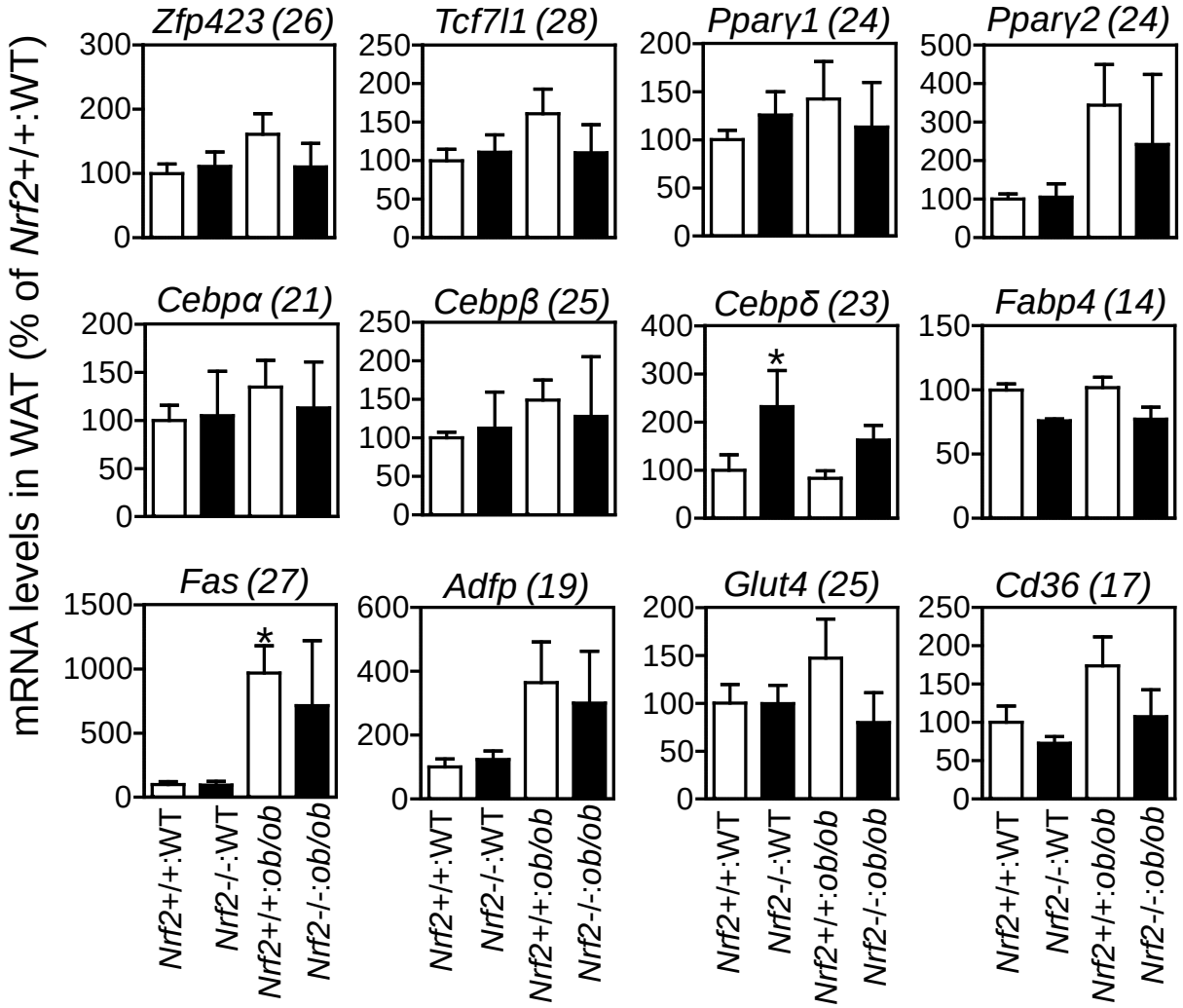
SUPPLEMENTARY DATA

Supplementary Figure 10. mRNA expression of antioxidant and inflammatory response genes in epididymal WAT of *Nrf2*(f)-/-:*ob/ob* mice. n = 3-4 males. Animal age is 8-10 wks. Values are mean ± SD. *$p < 0.05$ vs. *Nrf2*(f)+/+:*ob/ob*. The number in brackets following each gene name is the Cq value of that gene in *Nrf2*(f)+/+:WT. The average Cq value of reference gene 18S is 14.



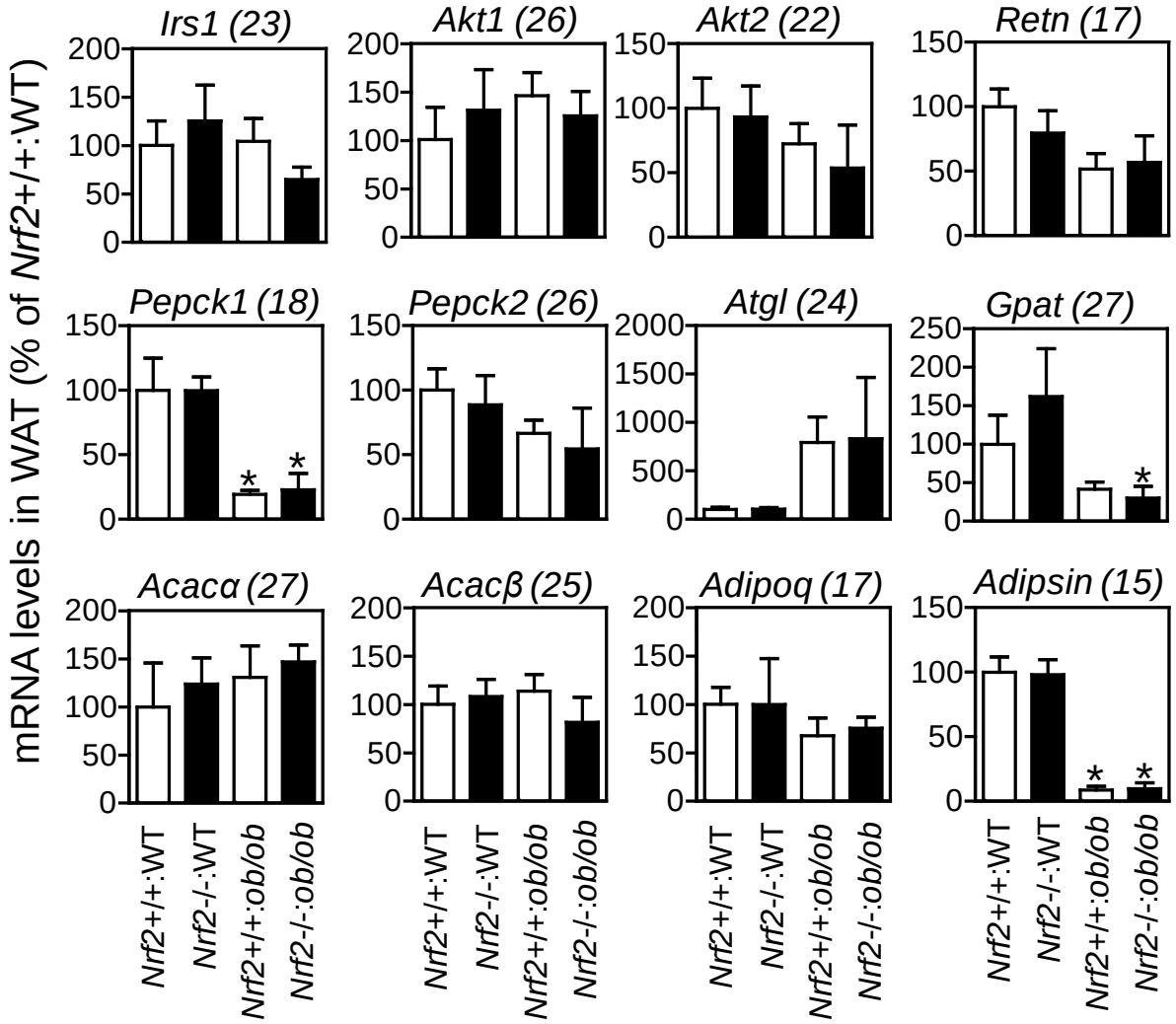
SUPPLEMENTARY DATA

Supplementary Figure 11. Adipogenic gene expression in epididymal WAT. n = 3-6 males. Animal age is 8-10 wks. Values are mean ± SD. *, $p < 0.05$ vs. *Nrf2*+/+:WT; $^{\#}p < 0.05$ vs. *Nrf2*+/+:*ob/ob* mice. The number in brackets following each gene name is the Cq value of that gene in *Nrf2*+/+:WT. The average Cq value of reference gene 18S is 14.



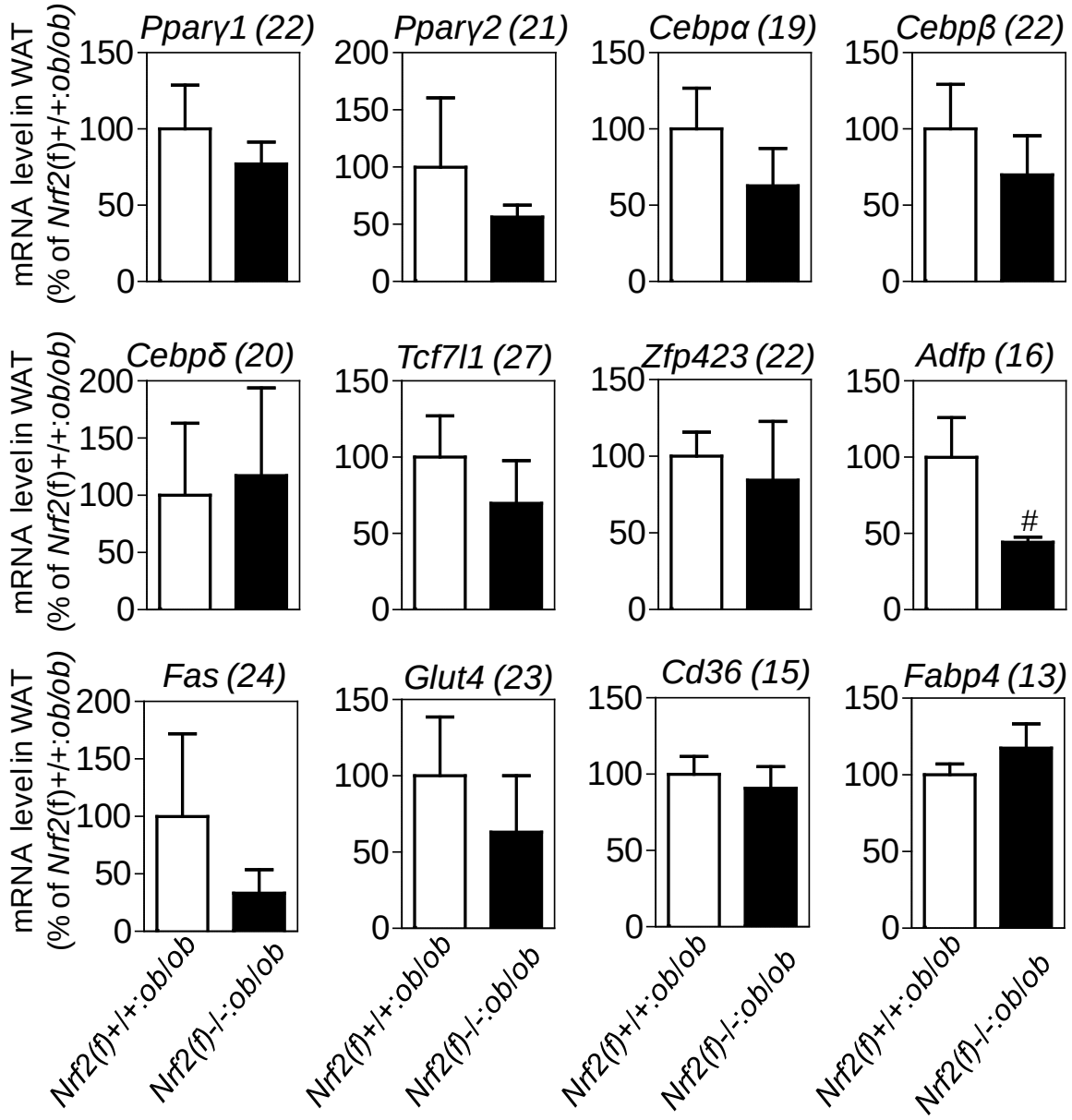
SUPPLEMENTARY DATA

Supplementary Figure 12. mRNA expression profile in epididymal WAT. n = 3-6 males. Animal age is 8-10 wks. Values are mean ± SD. *, $p < 0.05$ vs. *Nrf2*+/+:WT. The number in brackets following each gene name is the Cq value of that gene in *Nrf2*+/+:WT. The average Cq value of reference gene 18S is 14.



SUPPLEMENTARY DATA

Supplementary Figure 13. mRNA expression of adipogenic genes in epididymal WAT. n = 3-4 males. Animal age is 8-10 wks. Values are mean ± SD. # $p < 0.05$ vs. *Nrf2(f)*+/+:*ob/ob*. The number in brackets following each gene name is the Cq value of that gene in *Nrf2*(f)+/+:WT. The average Cq value of reference gene 18S is 14



SUPPLEMENTARY DATA

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