

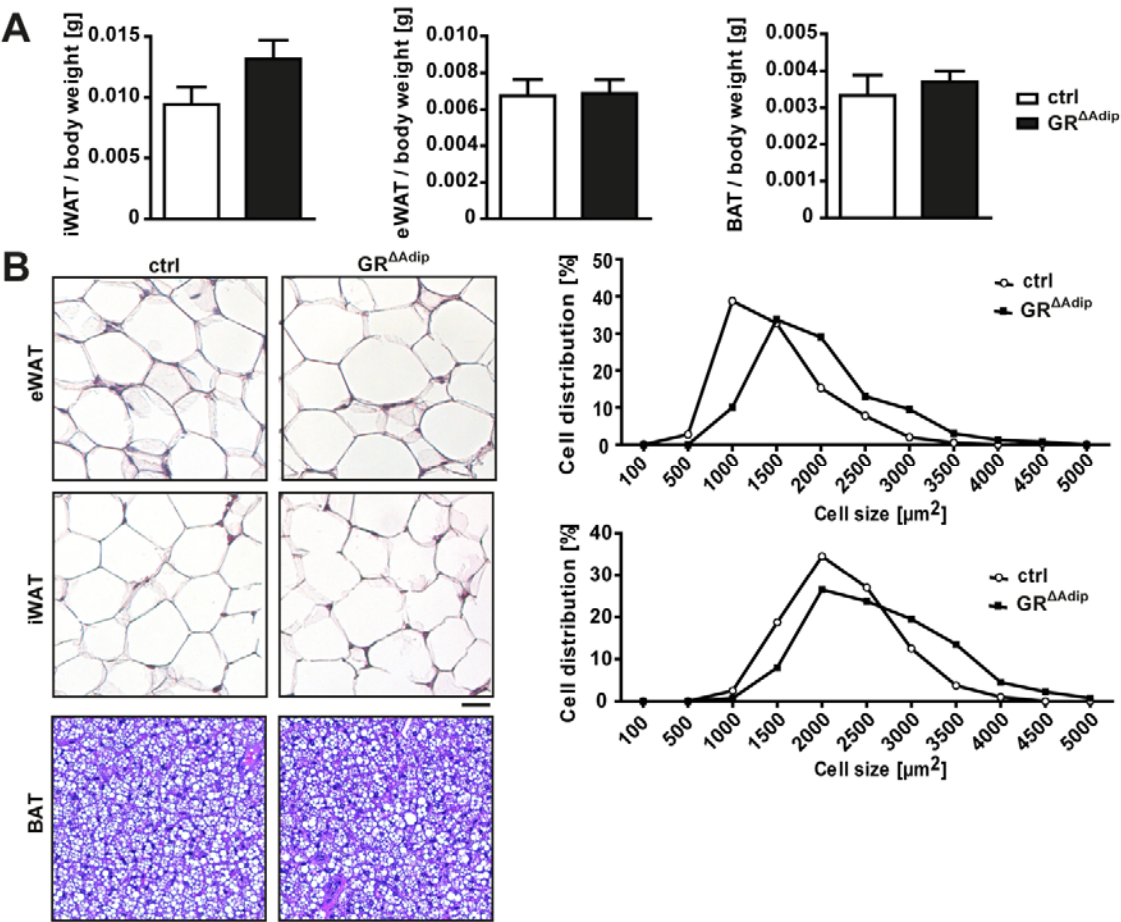
## SUPPLEMENTARY DATA

### **Adipocyte glucocorticoid receptor deficiency attenuates aging- and HFD-induced obesity, and impairs the feeding-fasting transition**

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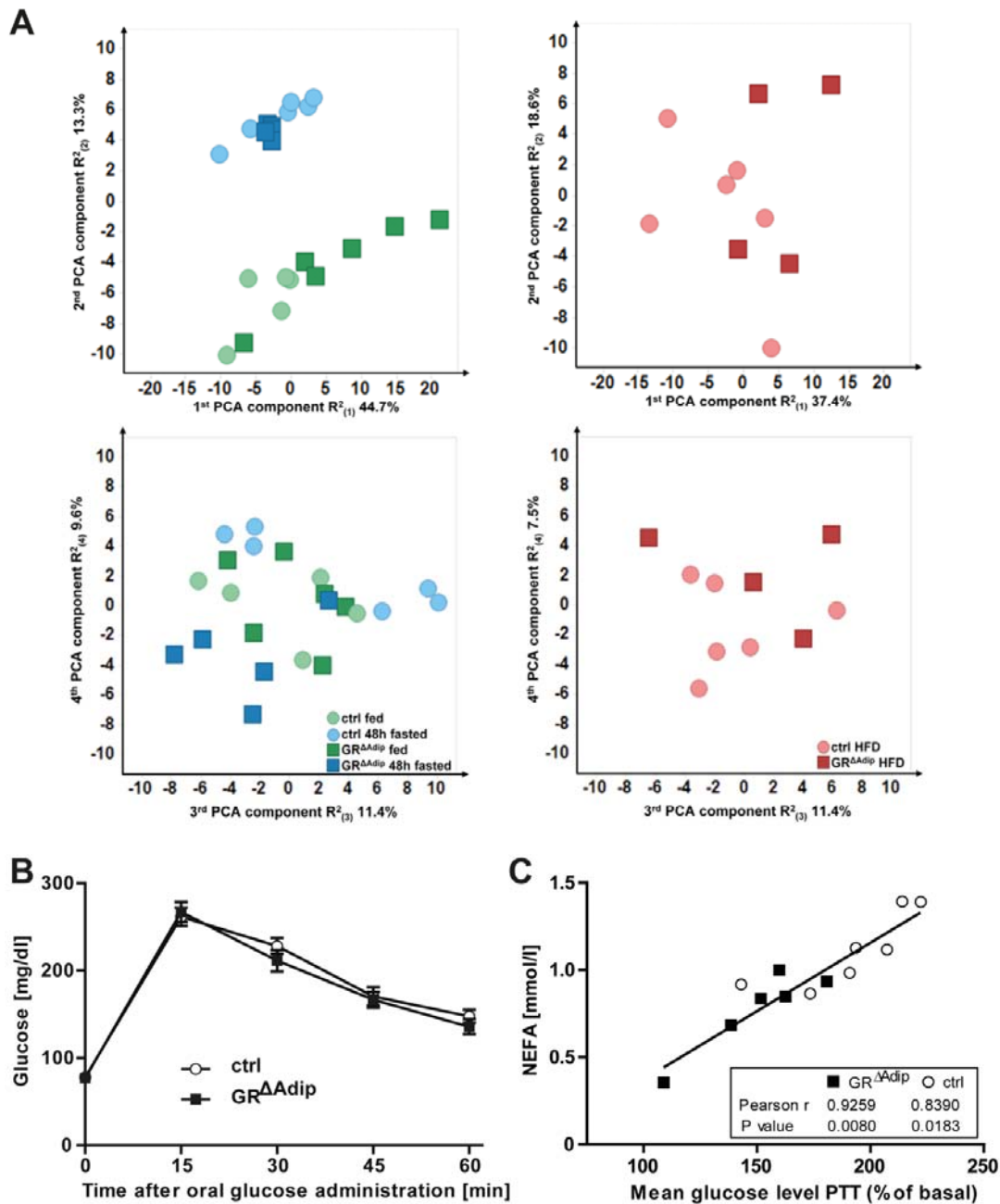
SUPPLEMENTARY DATA

**Supplementary Figure 1. Adipocyte-specific GR deficiency has no impact on adipose tissue size and morphology in 8-week-old mice.** **A** Wet weight of eWAT, iWAT and BAT in relation to body weight (n=4-8/genotype). **B** Representative H&E staining of eWAT, iWAT and BAT from *ad libitum*-fed ctrl and GR<sup>ΔAdip</sup> mice, and quantification of adipocyte cell size of eWAT and iWAT (n=5/genotype). Scale bar indicates 25 μm.



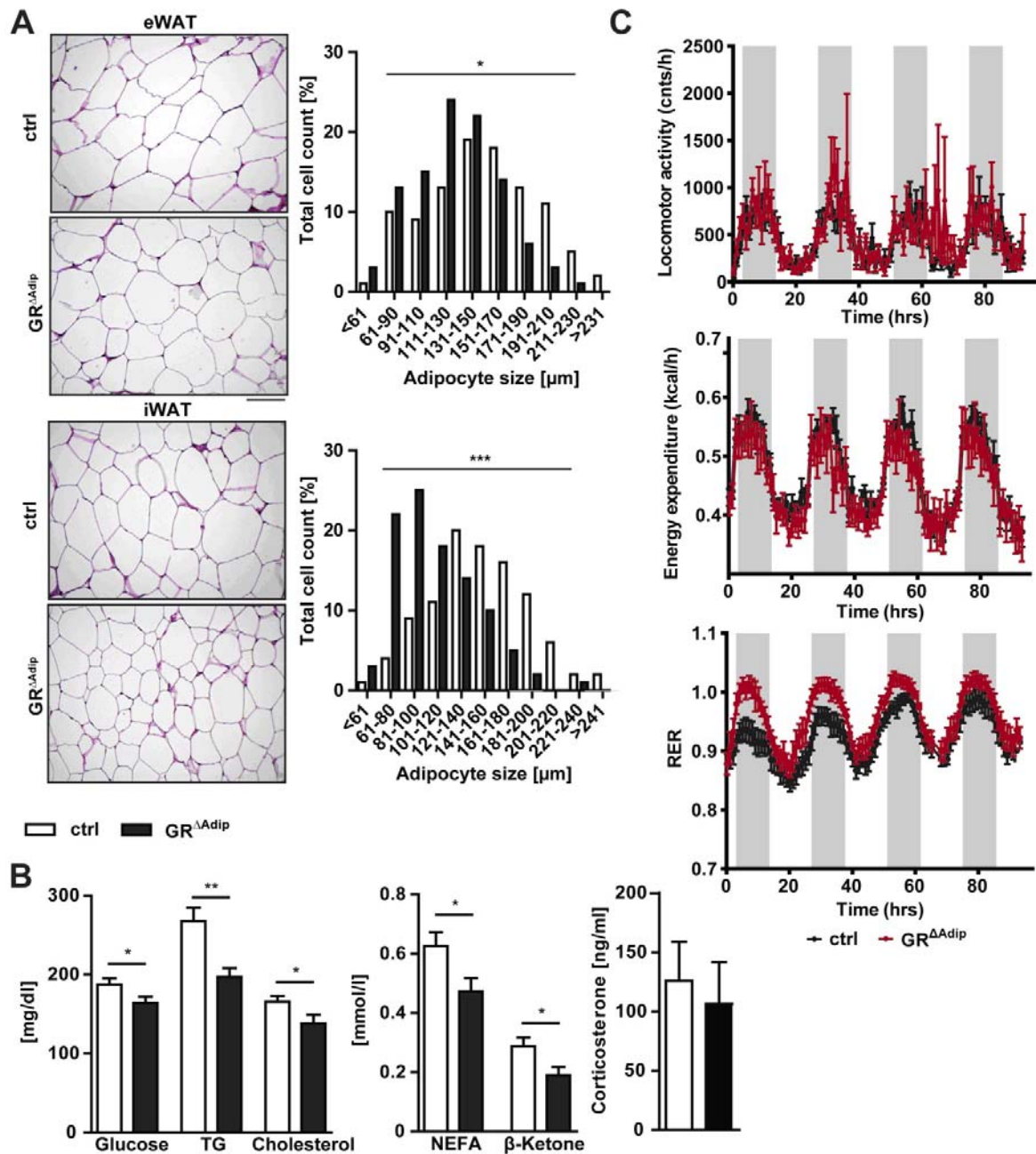
SUPPLEMENTARY DATA

**Supplementary Figure 2. Plasma metabolomics and glucose metabolism in 8-week-old  $GR^{\Delta Adip}$  mice.** **A** Scores plot of the principal component analysis (PCA) with 157 metabolites analyzed by LC-MS. Strong group separation was observed between genotypes in the first two components and a weak group separation between fasted and fed state in the third and fourth component. A tendency for group separation between genotypes under HFD conditions was visible in the first four components. **B** Oral glucose tolerance test. Glucose was administered through oral gavage (2 g/kg body weight) following a 16h fast ( $n=10$ /genotype). **C** Correlation between plasma NEFA and mean of plasma glucose increase during pyruvate tolerance tests in 16h-fasted ctrl and  $GR^{\Delta Adip}$  mice.



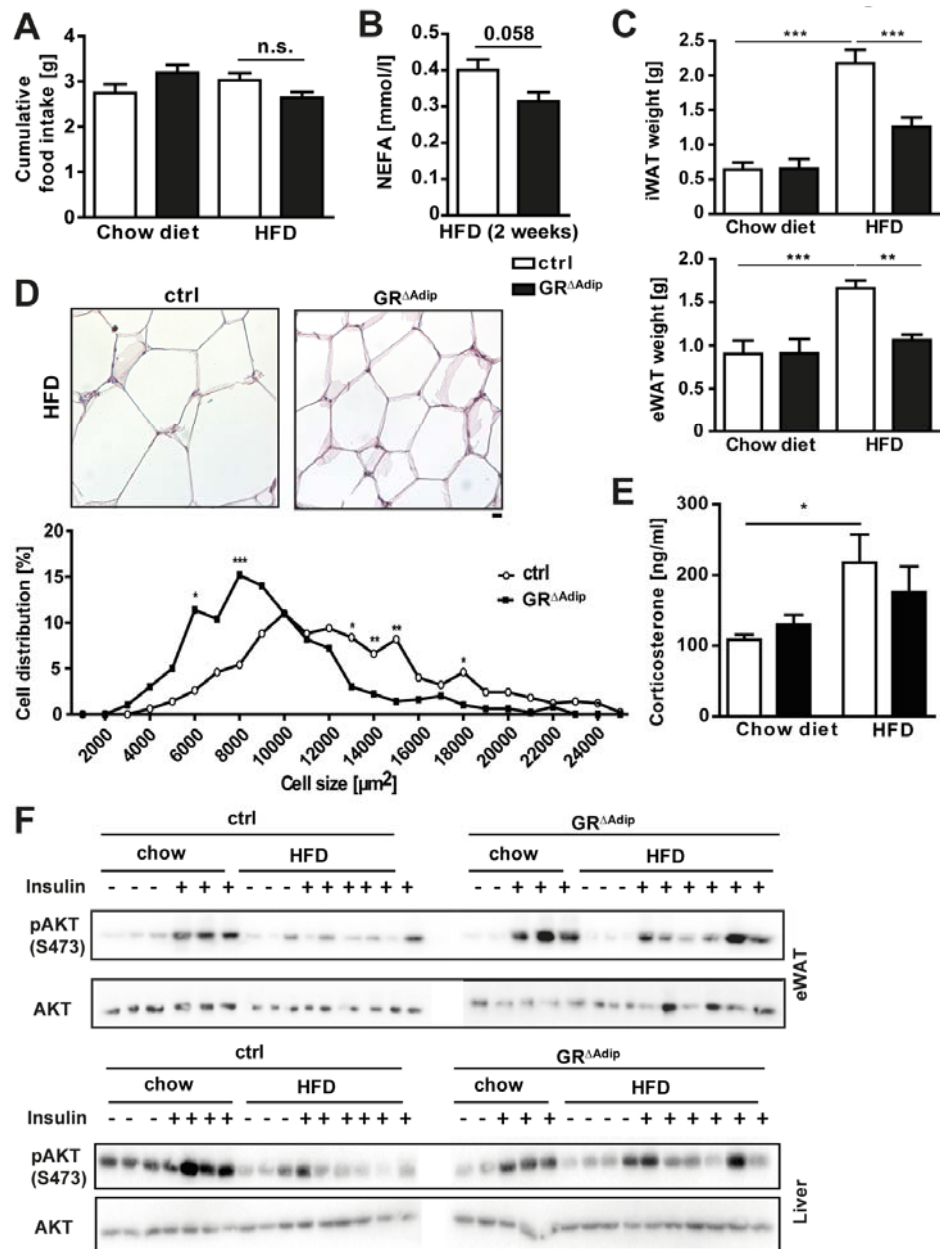
SUPPLEMENTARY DATA

**Supplementary Figure 3. Aging-associated metabolic phenotype 52-week-old  $GR^{\Delta Adip}$  mice.** **A** Representative H&E staining of eWAT and iWAT, and quantification of adipocyte sizes ( $n \geq 5$ /genotype). Scale bar indicates 100  $\mu m$ . **B** Plasma TG, cholesterol, NEFA, blood  $\beta$ -ketone level and plasma corticosterone level. Plasma metabolites were determined by colorimetric assays ( $n \geq 8$ /genotype); corticosterone was determined by ELISA ( $n \geq 7$ /genotype). **C** Patterns of locomotor activity, energy expenditure and respiratory exchange ratios (RER) during light and dark cycles (93.4h;  $n \geq 6$ /genotype). Data are shown as the mean  $\pm$  SEM. \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .



# SUPPLEMENTARY DATA

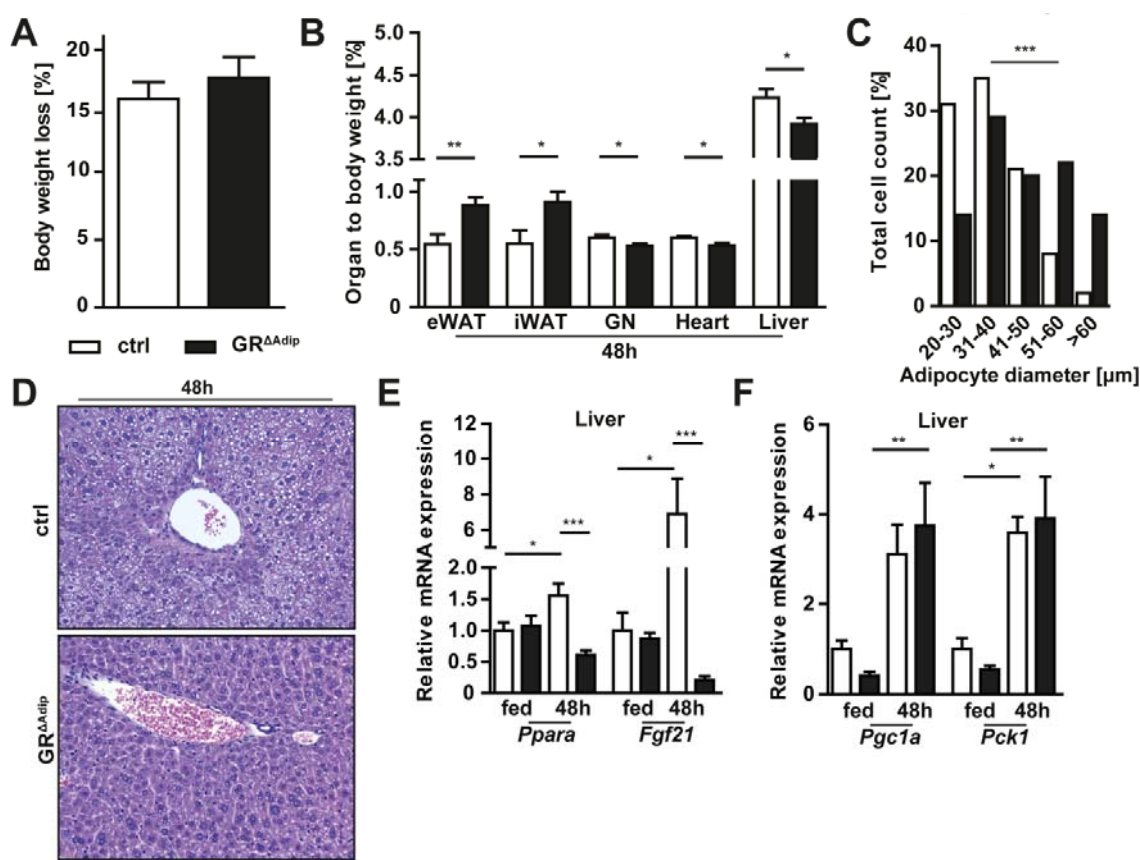
**Supplementary Figure 4. Reduced weight gain of HFD-fed  $GR^{\Delta Adip}$  mice is associated with diminished adipocyte hypertrophy and improved insulin signaling.** **A** Measurement of cumulative food intake over a time period of 3 days from ctrl and  $GR^{\Delta Adip}$  mice that either received a chow or a high fat diet (HFD) ( $n=5-10$ /genotype). **B** Plasma NEFA levels of ctrl and  $GR^{\Delta Adip}$  mice after 2 weeks of HFD feeding. Parameters were determined by colorimetric assays ( $n \geq 5$ /genotype). **C** Wet weight of iWAT and eWAT after chow and HFD of the indicated genotypes ( $n \geq 5$ /genotype). **D** Representative H&E staining of iWAT and quantification of adipocyte cell sizes in iWAT from ctrl and  $GR^{\Delta Adip}$  mice after 20 weeks of HFD ( $n=5$ /genotype). Scale bar indicates 25  $\mu m$ . **E** Plasma corticosterone levels of chow- and HFD-fed mice as determined by ELISA ( $n \geq 5$ /genotype). **F** Western blot analysis of insulin-stimulated phosphorylation of AKT in eWAT and liver (1 U/kg body weight) of chow- or HFD-fed ctrl and  $GR^{\Delta Adip}$  mice. Data are shown as the mean  $\pm$  SEM; \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ .





## SUPPLEMENTARY DATA

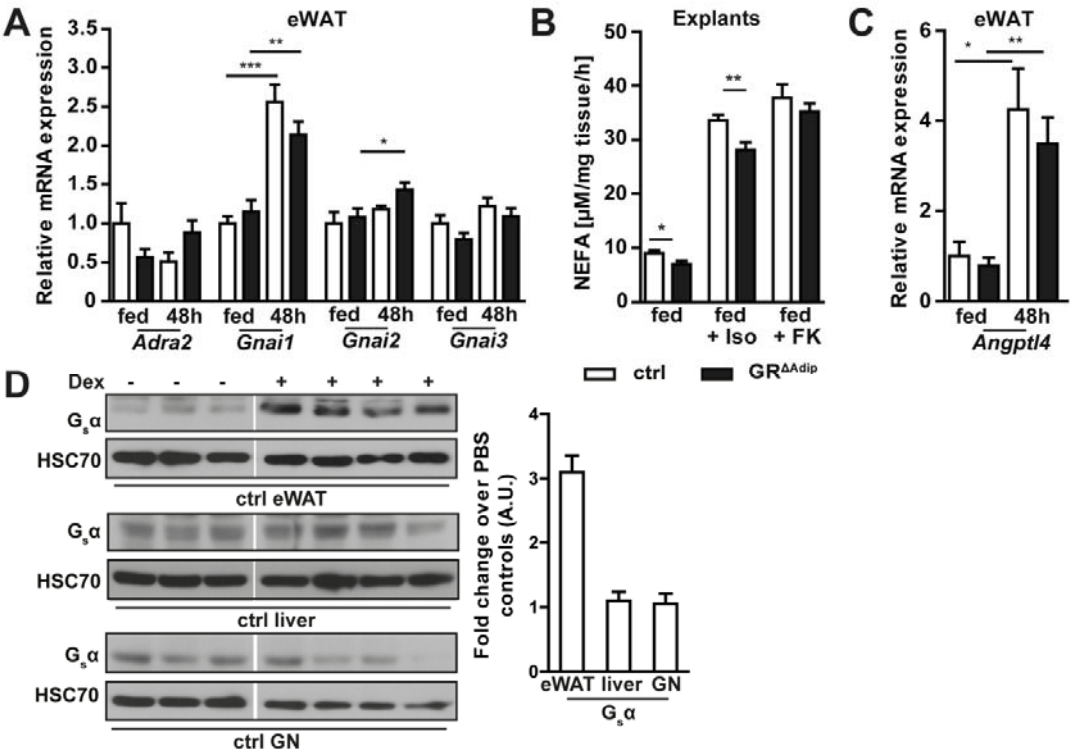
**Supplementary Figure 5. Adipocyte-specific GR-deficiency reduces fasting-induced lipolysis in white adipose tissues and impairs energy metabolism.** **A** Percent body weight loss of 48h-fasted  $GR^{\Delta Adip}$  and ctrl mice ( $n \geq 8$ /genotype). **B** Wet weight of eWAT, iWAT, gastrocnemius muscle (GN), heart and liver in relation to body weight (48h-fasted;  $n \geq 6$ /genotype). **C** Quantification of adipocyte cell size of eWAT of 48h-fasted mice ( $n \geq 4$ /genotype). **D** Representative H&E staining of livers of 48h-fasted  $GR^{\Delta Adip}$  and ctrl mice. Scale bar indicates 100  $\mu m$ . **E** Relative mRNA expression of *Ppara* and *Fgf21* as determined by qRT-PCR in livers of *ad libitum*-fed and 48h-fasted mice. Ct values were normalized to *Gapdh* ( $n \geq 5$ /genotype). **F** Relative mRNA expression of *Pgc1a* and *Pck1* as determined by qRT-PCR in livers of *ad libitum*-fed and 48h-fasted mice. Ct values were normalized to *Gapdh* ( $n \geq 5$ /genotype). Data are shown as the mean  $\pm$  SEM; \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ . *Ppara*: Peroxisome proliferator-activated receptor alpha; *Fgf21*: Fibroblast growth factor 21; *Pgc1a*: Peroxisome proliferator-activated receptor gamma coactivator 1-alpha; *Pck1*: Phosphoenolpyruvate carboxykinase; *Gapdh*: Glyceraldehyde 3-phosphate dehydrogenase.



SUPPLEMENTARY DATA

**Supplementary Figure 6. Adipocyte-specific GR-deficiency reduces fasting-induced lipolysis in white adipose tissue.**

**A** Relative mRNA expression of *Adra2* and *Gnai1-3* as determined by qRT-PCR in eWAT of ad libitum-fed and 48h-fasted mice. Ct values were normalized to *Gapdh* ( $n \geq 8$ /genotype). **B** NEFA release from eWAT explants of *ad libitum*-fed mice in absence or presence of indicated agonists (10  $\mu$ M;  $n \geq 2$  mice/genotype/treatment;  $n = 10$  explants/genotype). **C** Relative mRNA expression of *Angptl4* as determined by qRT-PCR in eWAT of *ad libitum*-fed and 48h-fasted mice. Ct values were normalized to *Gapdh* ( $n \geq 8$ /genotype). **D** Western blot of  $G_s\alpha$  in eWAT, liver and gastrocnemius muscle (GN) of dexamethasone-treated control mice (10 days; 5 mg/kg i.p.). HSC70 served as loading control. Quantification of  $G_s\alpha$  levels ( $n \geq 3$ /genotype). Protein bands were quantified by densitometry; total protein expression was corrected for the respective loading control. Data are shown as the mean  $\pm$  SEM; \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ . *Adra2*: Alpha-2-adrenergic receptor; *Gnai*: Inhibitory G-protein  $\alpha$ -subunit isoform; *Angptl4*: Angiopoietin-like 4; *Gapdh*: Glyceraldehyde 3-phosphate dehydrogenase; Iso: Isoproterenol; FK: Forskolin.



SUPPLEMENTARY DATA

**Supplementary Table 1. qRT-PCR primer sequences for specific amplification of cDNA.**

Gene symbol	Sequence (5' - 3')
<i>Cebpb</i>	ATCGACTTCAGCCCCTACCT
	TAGTCGTCGGCGAAGAGG
<i>Pparg</i>	GAAAGACAACGGACAAATCACC
	GGGGGTGATATGTTTGAAGTTG
<i>Fabp4</i>	GGATGGAAAGTCGACCACAA
	TGGAAGTCACGCCTTTCATA
<i>Ucp1</i>	GACGTCCCCTGCCATTTAC
	CGCAGAAAAGAAGCCACAA
<i>Adipoq</i>	GGAGAGAAAGGAGATGCAGGT
	CTTTCCTGCCAGGGGTTT
<i>Leptin</i>	CAGGGAGGAAAATGTGCTGGAG
	CCGACTGCGTGTGTGAAATGT
<i>Nr3c1</i>	GGCCGCTCAGTGTTTTCTAA
	GCAGAGTTTGGGAGGTGGT
<i>Fasn</i>	GCTGCTGTTGGAAGTCAGC
	AGTGTTTCGTTCTCGGAGTG
<i>Gpat1</i>	GGAAGGTGCTGCTATTCCTG
	TGGGATACTGGGGTTGAAAA
<i>Cd36</i>	TTGTACCTATACTGTGGCTAATGAGA
	CTTGTGTTTTGAACATTTCTGCTT
<i>Slc27a4</i>	CTTGCCTGAGCTGCACAA
	GCGGGTCTTTCACAACAGT
<i>Actb</i>	GCACCAGGGTGTGATGGTG
	CCAGATCTTCTCCATGTCGTCC
<i>Pnpla2</i>	GTTGAAGGAGGGATGCAGAG
	GCCACTCACATCTACGGAGC
<i>Lipe</i>	GGAGAGAGTCTGCAGGAACG
	CCTGCAAGAGTATGTCACGC
<i>Abhd5</i>	GATGTGGGACACCAGGTAGG
	CGGTGATGAAAGCGATGG
<i>Ppara</i>	ATCGCGTACGGCAATGGCTTTA
	GCCTCGGAGGTCCCTGAACAG
<i>Fgf21</i>	GTCCTCCAGCAGCAGTTCTC
	CCTGGGTGTCAAAGCCTCTA
<i>Angptl4</i>	GGAAAAGTCCACTGTGCCTC
	TTTCCAAGATGACCCAGCTC
<i>Pck1</i>	CGTTTTCTGGGTTGATAGCC
	CCTAGTGCCTGTGGGAAGAC
<i>Pgc1a</i>	TGAGGACCGCTAGCAAGTTT
	TGAAGTGGTGTAGCGACCAA
<i>Adrb2</i>	TGACTAGATCAGCACACGCC
	GCCATCCTCATGTCGGTTAT
<i>Adrb3</i>	TCGAGCATAGACGAAGAGCA
	ACAGGAATGCCACTCCAATC



# SUPPLEMENTARY DATA

<i>Adra2</i>	CAGCGCCCTTCTTCTCTATG
	CAGGCCATCGAGTACAACCT
<i>Gnas</i>	TCTGTGGGAGGATGAGGGAG
	TGGTCACTTGGCACGTAAGTC
<i>Gnai1</i>	CAAGATGATCGACCGCAACC
	ACCTCCCCATGGCTCTAATG
<i>Gnai2</i>	GGTGTTGCTGTAGACCACGG
	GGGAGGTGAAGTTGCTTCTG
<i>Gnai3</i>	TCGTCCTCTGAATAGCCGTC
	GAGAAAGCGGCCAAAGAAGT
<i>Gapdh</i>	TTGAGGTCAATGAAGGGGTC
	TCGTCCCGTAGACAAAATGG
<i>Acaca</i>	GAAGCCACAGTGAAATCTCG
	GATGGTTTGGCCTTTCACAT
<i>Gpat3</i>	GTGCTGGGTGTCCTAGTGC
	AAGCTGATCCCAATGAAAGC
<i>Dgat2</i>	GGCGCTACTTCCGAGACTAC
	TGGTCAGCAGGTTGTGTGTC
<i>Slc27a1</i>	CGGCGTTCTGTGTGTACG
	CCGAACACGAATCAGAACAG

# SUPPLEMENTARY DATA

## Supplementary Table 2. Relative gene expression of adipogenic and adipocyte-specific markers.

Relative mRNA expression of *Cebpb*, *Pparg*, *Fabp4*, *Adiponectin*, *Leptin* and *Ucp1* as determined by qRT-PCR in iWAT, eWAT and BAT of ctrl and GR<sup>ΔAdip</sup> mice. Ct values were normalized to Actb (n≥8/genotype). Data are shown as the mean ±SEM. Cebpb: CCAAT/enhancer binding protein beta; Pparg: peroxisome proliferator-activated receptor gamma; Fabp4: fatty acid binding protein 4; Ucp1: uncoupling protein 1; Actb: β-Actin.

Gene	Tissue	ctrl	GR <sup>ΔAdip</sup>
<i>Cebpb</i>	iWAT	1.000 ± 0.1384	0.7921 ± 0.05627
	eWAT	0.6018 ± 0.1329	0.6798 ± 0.09286
	BAT	2.847 ± 0.6499	2.523 ± 0.3279
<i>Pparg</i>	iWAT	1.000 ± 0.1810	0.9196 ± 0.09747
	eWAT	0.8784 ± 0.1901	1.007 ± 0.1297
	BAT	2.958 ± 0.6028	2.961 ± 0.1977
<i>Fabp4</i>	iWAT	1.000 ± 0.1541	0.8510 ± 0.09064
	eWAT	0.3600 ± 0.07333	0.3523 ± 0.03811
	BAT	0.5616 ± 0.1018	0.4811 ± 0.03504
<i>Adiponectin</i>	iWAT	1.0 ± 0.1102	0.8592 ± 0.07639
	eWAT	0.4778 ± 0.09838	0.4613 ± 0.04948
	BAT	0.5216 ± 0.05834	0.4826 ± 0.02067
<i>Leptin</i>	iWAT	1.000 ± 0.03984	0.9988 ± 0.03957
	eWAT	0.9005 ± 0.02766	0.8336 ± 0.02457
	BAT	0.6903 ± 0.03423	0.7355 ± 0.01381
<i>Ucp1</i>	iWAT	0.003017 ± 0.002504	0.01176 ± 0.01135
	eWAT	0.0001496 ± 0.00006723	0.02537 ± 0.02471
	BAT	1.000 ± 0.1248	1.156 ± 0.09576