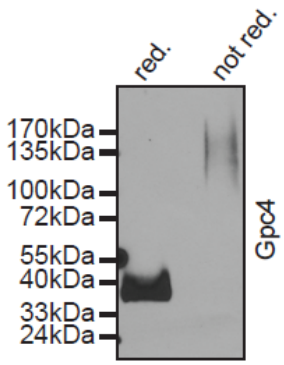
SUPPLEMENTARY DATA

Supplementary Table 1. Shown are clinical parameters for female and male subjects, divided by BMI and body fat distribution used to measure adipose Gpc4 mRNA expression and serum Gpc4 levels. visc. BMI 25-30 and visc. BMI >30 indicates subjects with a CT or MRI ratio between subcutaneous and visceral fat areas >0.4 in the given BMI range.

Female										
Group	BMI <25		BMI 25-30		Visc. BMI 25-30		BMI >30		Visc. BMI >30	
BMI (kg/m²)	23.3	±1.1	27.7	±1.6	27.1	±1.0	36.0	±4.9	37.4	±5.7
WHR	0.7	±0.1	0.9	±0.1	1.0	±0.1	1.0	±0.2	1.2	±0.1
% body fat	21.7	±2.8	30.6	±6.2	26.5	±3.2	40.2	±6.9	36.0	±7.9
FPG (mmol/l)	5.3	±0.4	5.3	±0.6	5.1	±0.6	5.4	±0.4	5.4	±0.3
FPI (pmol/l)	27.5	±12.6	97.0	±65.6	74.3	±16.1	153.0	±95.4	152.9	±78.8
Clamp GIR (µmol/kg/min)	97.4	±10.5	59.1	±25.3	54.3	±24.9	53.9	±24.1	47.6	±33.7
HbA1c (%)	5.3	±0.2	5.4	±0.2	5.6	±0.2	5.5	±0.3	5.5	±0.3
Cholesterol (mmol/l)	5.0	±0.8	4.7	±0.6	5.4	±0.5	4.9	±0.7	5.7	±0.7
HDL-C (mmol/l)	1.5	±0.4	1.3	±0.4	1.6	±0.5	1.4	±0.4	1.6	±0.3
LDL-C (mmol/l)	2.9	±0.9	2.6	±0.5	3.5	±0.5	2.7	±0.6	3.4	±0.5
FFA (mmol/l)	0.3	±0.1	0.4	±0.3	0.6	±0.1	0.6	±0.4	0.9	±0.2
Leptin (ng/ml)	8.7	±4.3	26.8	±10.8	35.6	±15.4	33.0	±11.2	31.5	±7.9
Adiponectin (ng/ml)	9.7	±4.5	8.4	±5.1	3.4	±1.9	7.4	±4.3	5.9	±3.3
Male										
Group	BMI <25		BMI 25-30		Visc. BMI 25-30		BMI >30		Visc. BMI >30	
BMI (kg/m²)	23.9	±0.9	26.8	±1.7	28.0	±1.3	37.1	±5.0	35.9	±5.7
WHR	0.9	±0.1	1.0	±0.1	1.1	±0.1	1.1	±0.1	1.2	±0.1
% body fat	21.3	±2.7	26.6	±6.7	30.3	±3.3	42.5	±8.8	34.5	±5.8
FPG (mmol/l)	5.4	±0.4	5.4	±0.5	5.2	±0.4	5.4	±0.5	5.5	±0.5
FPI (pmol/l)	31.5	±14.9	72.7	±90.6	203.5	±81.4	146.0	±113.9	128.8	±56.3
Clamp GIR (µmol/kg/min)	96.8	±5.5	85.6	±24.1	30.8	±12.9	59.0	±26.2	47.3	±31.1
HbA1c (%)	5.3	±0.2	5.4	±0.3	5.6	±0.2	5.6	±0.2	5.6	±0.3
Cholesterol (mmol/l)	5.1	±0.8	4.8	±0.7	5.6	±0.5	4.8	±0.5	5.5	±1.0
HDL-C (mmol/l)	1.4	±0.4	1.4	±0.3	1.8	±0.4	1.3	±0.3	1.4	±0.2
LDL-C (mmol/l)	2.7	±0.7	2.5	±0.5	3.1	±0.4	2.7	±0.6	3.6	±1.1
FFA (mmol/l)	0.3	±0.2	0.4	±0.2	0.7	±0.3	0.6	±0.4	0.7	±0.4
Leptin (ng/ml)	3.2	±2.9	9.6	±11.8	22.1	±9.7	19.3	±8.3	16.6	±9.9
Adiponectin (ng/ml)	9.7	±2.5	9.2	±6.7	4.3	±3.6	6.1	±2.6	4.3	±2.0

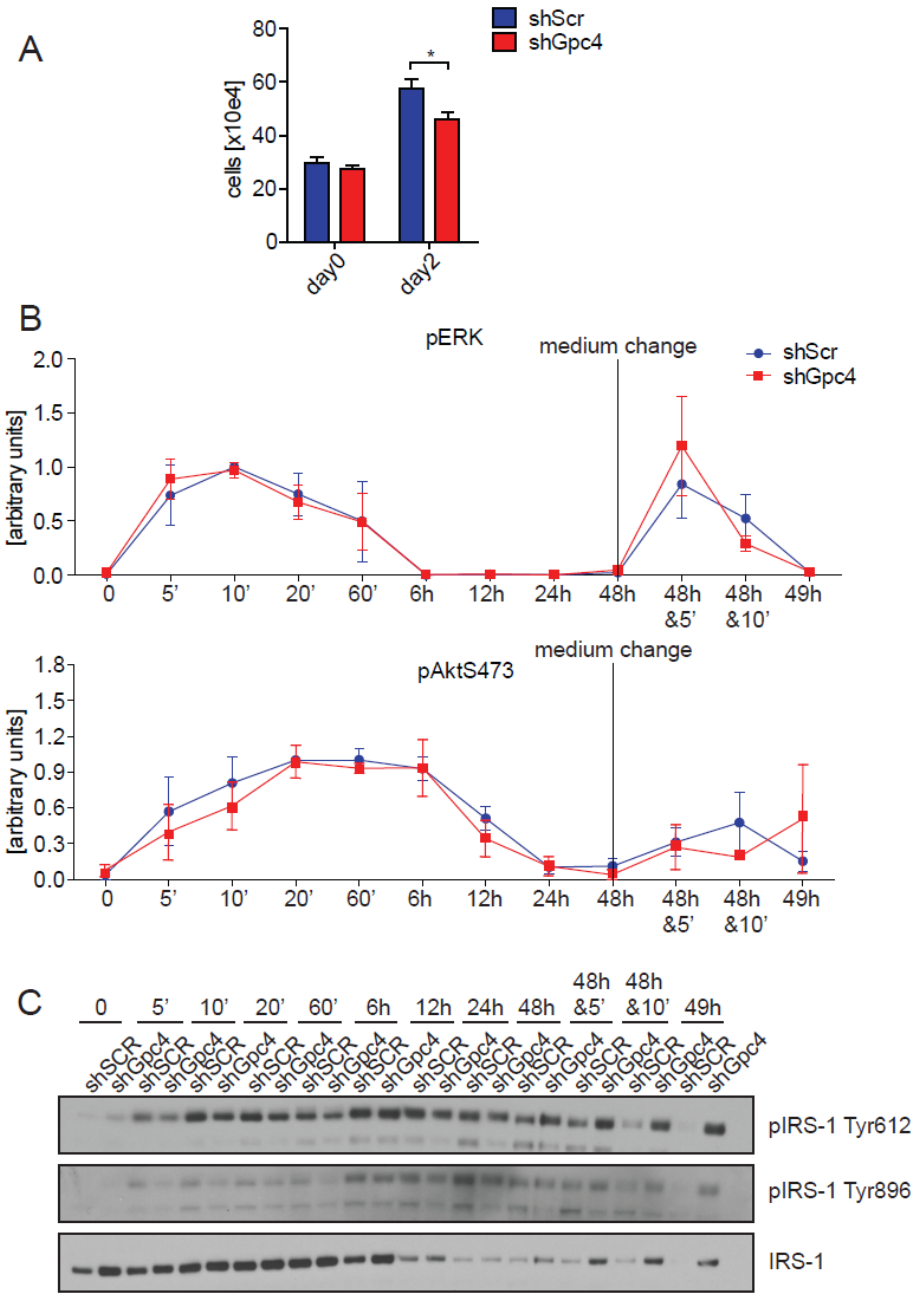
SUPPLEMENTARY DATA

Supplementary Figure 1. Western blot for Gpc4 from purified Gpc4 under reduced (red.) or not reduced (not. red.) conditions.



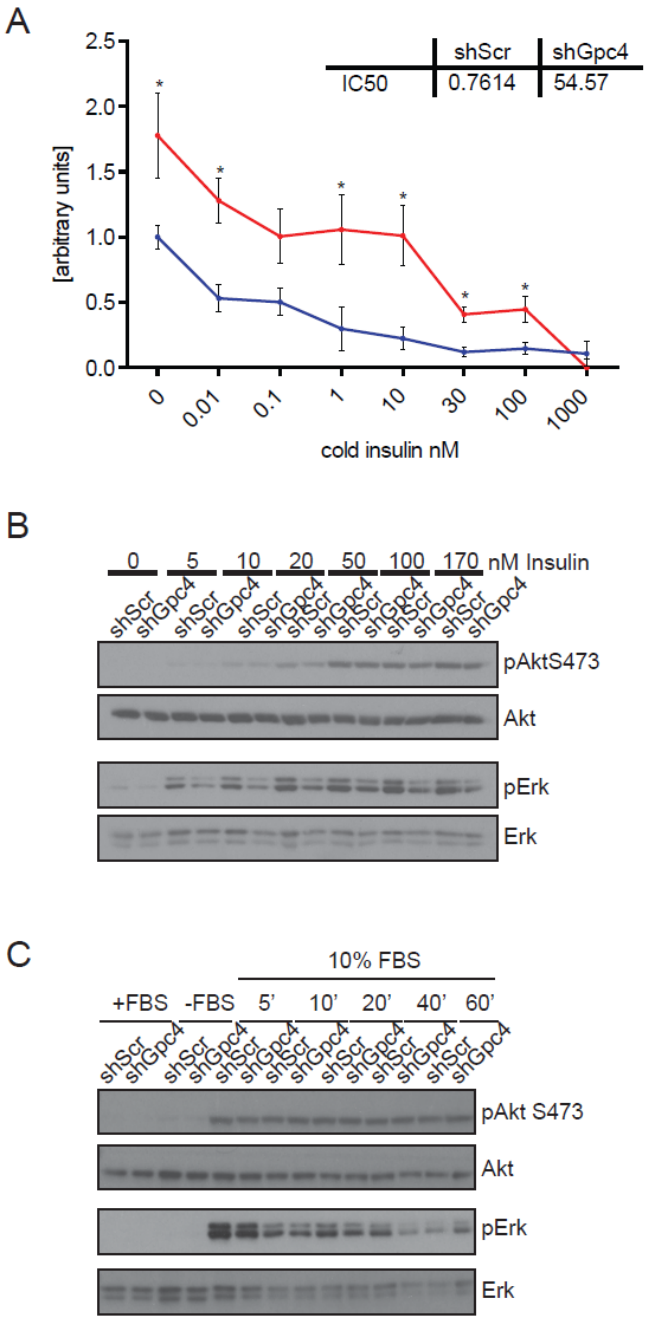
Supplementary Figure 2. (A) Cell number of control and shGpc4 3T3-L1 at day 0 and day 2 of differentiation (n=3). (B) Quantification of Western blots for ERK and AktS473 phosphorylation during the first 49 hours of differentiation. Phospho- signals were normalized to total ERK and Akt, respectively. Induction medium was changed after 48 hours to growth medium containing 10% FBS and 170 nM insulin (n=3). (C) Western Blot for pIRS-1Y612 and pY896 and total IRS-1. Differentiation was induced at time point 0. Induction medium was changed to growth medium after 48 hours.

SUPPLEMENTARY DATA



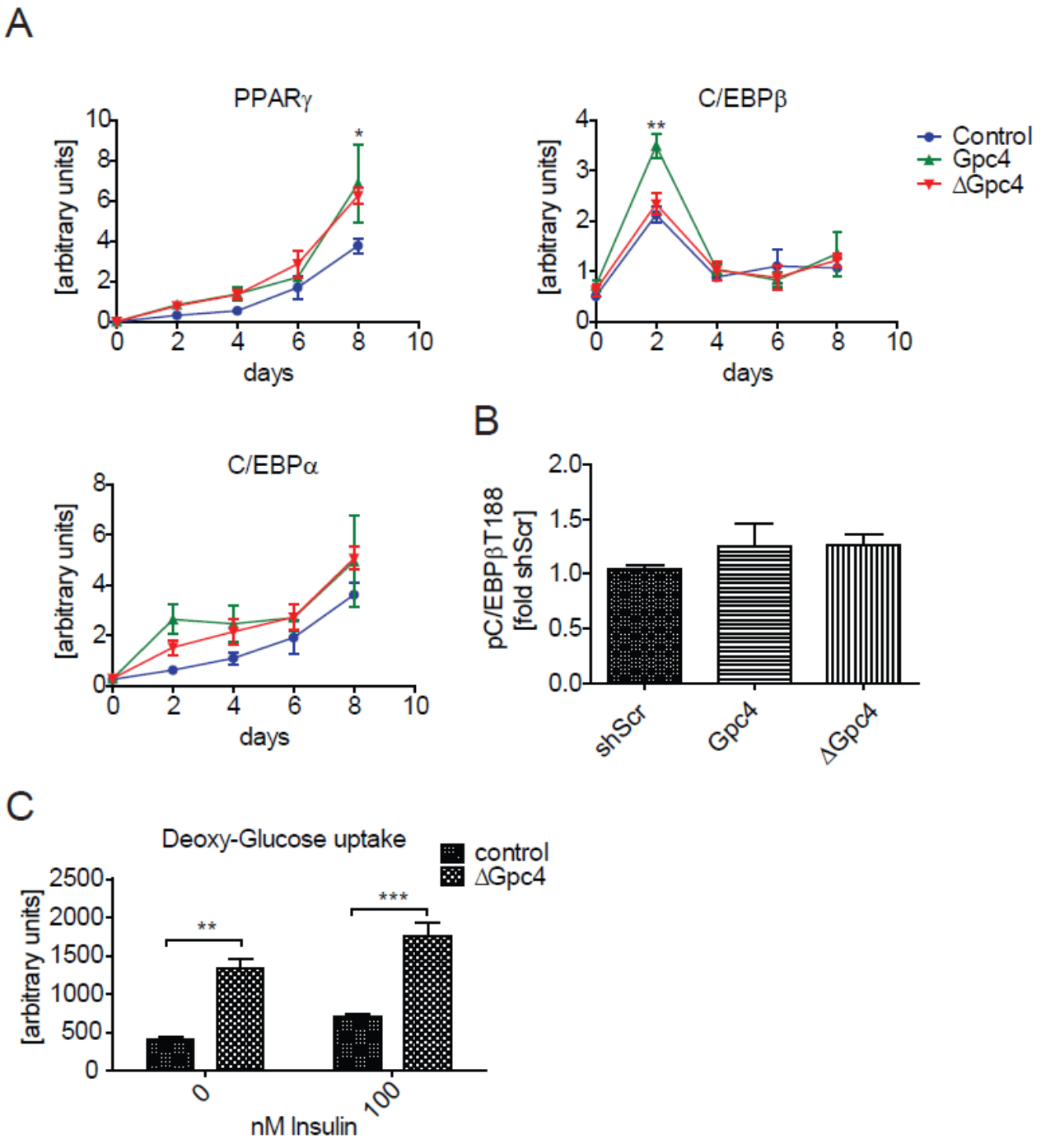
SUPPLEMENTARY DATA

Supplementary Figure 3. (A) Insulin binding to confluent shScr and shGpc4 preadipocytes. 125I-insulin was competed with increasing concentrations of unlabeled insulin. Values were background subtracted and normalized to protein concentration. (n=6). (B) Western Blot for pAktS473, pErk and the respective unphosphorylated proteins of shScr and shGpc4 cells stimulated with the indicated concentrations of insulin for 20 minutes. (C) Western Blot for pAktS473, pErk and the respective unphosphorylated proteins of shScr and shGpc4 cells stimulated with 10% FBS after 3 hours serum withdrawal.



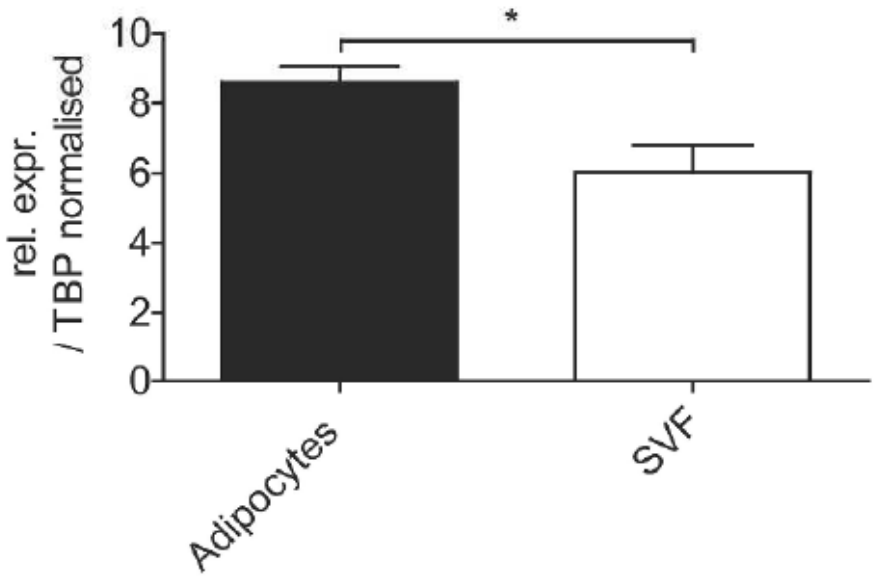
SUPPLEMENTARY DATA

Supplementary Figure 4. (A) Realtime PCR for Pparγ, C/EBPα and C/EBPβ during an eight day time-course of differentiation of control Gpc4 and ΔGpc4 overexpressing cells. * indicates significantly higher expression in ΔGpc4 and Gpc4 vs. control cells (n=5). (B) Quantification of phospho-C/EBPβThr188 normalized to total C/EBPβ of control Gpc4 and ΔGpc4 overexpressing cells 24h after induction (n=3). ^{14}C-Deoxy-glucose uptake was measured in serum starved 3T3-L1 control or ΔGpc4 overexpressing adipocytes exposed for 45 minutes to 0 or 100nM insulin (n=3).



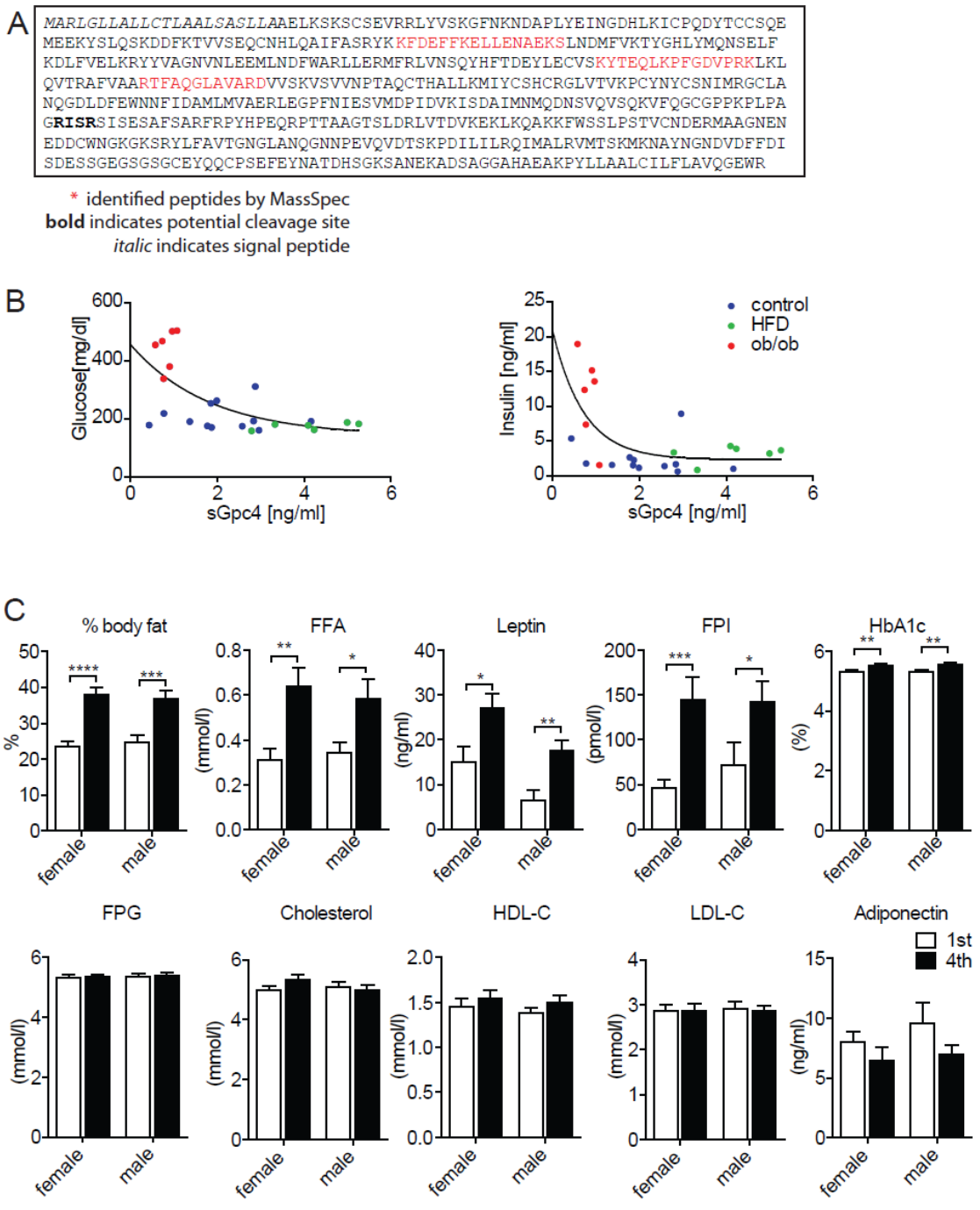
SUPPLEMENTARY DATA

Supplementary Figure 5. qPCR for Gpc4 from freshly isolated perigonadal adipocytes and the corresponding SVF. Gpc4 expression was normalized to TBP (n=3).



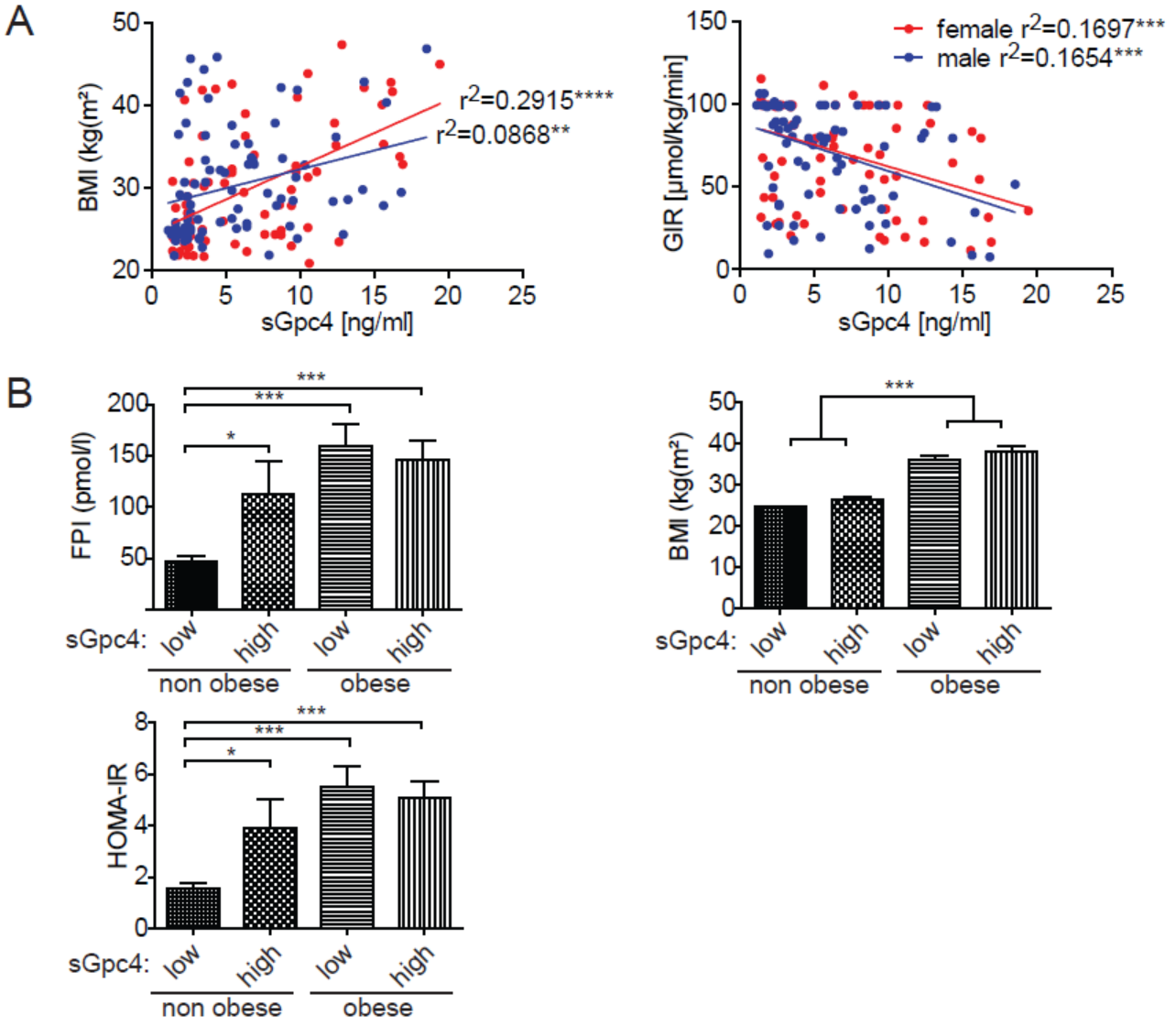
SUPPLEMENTARY DATA

Supplementary Figure 6. (A) Murine Gpc4 protein sequence. Peptides identified by mass spectrometry are highlighted in red. (B) Correlation between serum Gpc4 and glucose and insulin levels in control, HFD fed (8 weeks) and ob/ob mice. (C) Comparison of clinical parameters from the lowest and highest quartile of serum Gpc4 levels of 160 patients shown in Figure 5D (n=40 per quartile).



SUPPLEMENTARY DATA

Supplementary Figure 7. (A) Correlation of serum Gpc4 with BMI and GIR in non-diabetic females (n=77) and males (n=83). (B) Comparison of HOMA-IR and BMI from non-obese (BMI<30) and obese (BMI>30) subjects divided into groups with low serum Gpc4 levels (≤5 ng/ml) and high serum Gpc4 levels (≥ 9 ng/ml).



SUPPLEMENTARY DATA

Supplementary Figure 8. Real Time PCR for the GPI lipases Gpld1 and Notum in liver, subcutaneous (SCF) and perigonadal fat (PGF) of control (ob/+) and ob/ob mice. Expression values were normalized to TBP (n=6).