SUPPLEMENTARY DATA

Supplementary Information for:

Defective natriuretic peptide receptor signaling in skeletal muscle links obesity to type 2 diabetes

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Inventory of Supplemental Information:

1. Supplemental Tables : 5
2. Supplemental Figures : 10

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Supplementary Table 1. Clinical characteristics of the subjects.

	Lean	**Obese**	**IGT/T2D**
Sex (male/female)	6/3	5/4	6/4
Age (yrs)	23.8±0.8	23.7±0.8	46.7±3.4[c,e]
Body weight (kg)	68.3±3.3	87.7±6.3[b]	103.3±4.1[c,d]
BMI (kg/m^2)	22.5±0.5	32.9±0.5[c]	34.3±1.2[c]
Body fat (%)	19.7±2.5	27.9±2.1[a]	30.1±1.4[c]
GDR (mg.min^{-1}.kg^{-1} FFM)	9.4±0.7	7.0±0.4[c]	5.5±0.5[c,d]

Data are Mean ± SEM. BMI: body mass index; GDR: glucose disposal rate; FFM: fat-free mass. [a]$p<0.05$, [b]$p<0.01$, [c]$p<0.001$ versus lean; [d]$p<0.05$, [e]$p<0.01$ versus obese.

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Supplementary Table 2. Correlation between muscle NPRA protein expression and biological variables in humans.

	Human muscle NPRA		
Variables	**r**	**p value**	**p adj. value**
Body weight (kg)	-0.46	0.06	0.06
BMI	-0.46	0.05	0.06
HOMA-IR	-0.48	0.04	0.06
rQUICKI	0.63	0.005	0.025
Fasting Insulin	-0.52	0.03	0.07

BMI: body mass index; HOMA-IR: homeostasis model assessment of insulin resistance; revised QUICKI. r: Spearman correlation coefficients; non adjusted p value; $p_{adj.}$ value: Benjamini-Hochberg false discovery rate considered statistically significant if ≤ 5%.

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Supplementary Table 3. Correlation between log fasting plasma BNP and biological variables in db/+ and db/db mice.

	Mouse Log [BNP]		
Variables	**r**	**p value**	**p adj. value**
Body weight (g)	-0.69	0.0007	0.0008
Fat Mass (%)	-0.79	< 0.0001	0.0002
Fasting glucose	-0.76	< 0.0001	0.0002
Fasting insulin	-0.85	< 0.0001	0.0007
HbA1c	-0.70	0.0006	0.0008
Fructosamines	-0.82	< 0.0001	0.0003
Muscle NPRC	-0.59	0.02	0.02

r: Spearman correlation coefficients; non adjusted p value; $p_{adj.}$ value: Benjamini-Hochberg false discovery rate considered statistically significant if ≤ 5%.

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Supplementary Table 4. Correlation between muscle NPRC protein expression and biological variables in db/+ and db/db mice.

	Mouse muscle NPRC		
Variables	**r**	**p value**	**p adj. value**
Fasting glucose	0.64	0.011	0.014
Fasting insulin	0.61	0.016	0.016
HbA1c	0.65	0.009	0.036
Fructosamines	0.65	0.012	0.024

HOMA-IR: homeostasis model assessment of insulin resistance. r: Spearman correlation coefficients; non adjusted p value; $p_{adj.}$ value: Benjamini-Hochberg false discovery rate considered statistically significant if ≤ 5%.

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Supplementary Table 5. List of mouse primer and probe sequences used for real-time qPCR

Taqman chemistry:

Gene symbol	Taqman Probe ID
UCP1	Mm01244861_m1
TFAM	Mm00447485_m1
GLUT1	Mm00441480_m1
GLUT2	Mm00446229_m1
GLUT4	Mm00436615_m1
CPT1β	Mm00487200_m1
GYS1	Mm00472712_m1
PCK1	Mm00440636_m1
18S	Hs99999901_s1

SYBR chemistry:

Gene symbol	Forward	Reverse
PPARα	AGTTCACGCATGTGAAGGCTG	TGTTCCGGTTCTTCTTCTGAATC
G6P	ACACCGACTACTACAGCAACAG	CCTCGAAAGATAGCAAGAGTAG
PGC1α	CTGTGTCACCACCCAAATCCTTAT	TGTGTCGAGAAAAGGACCTTGA

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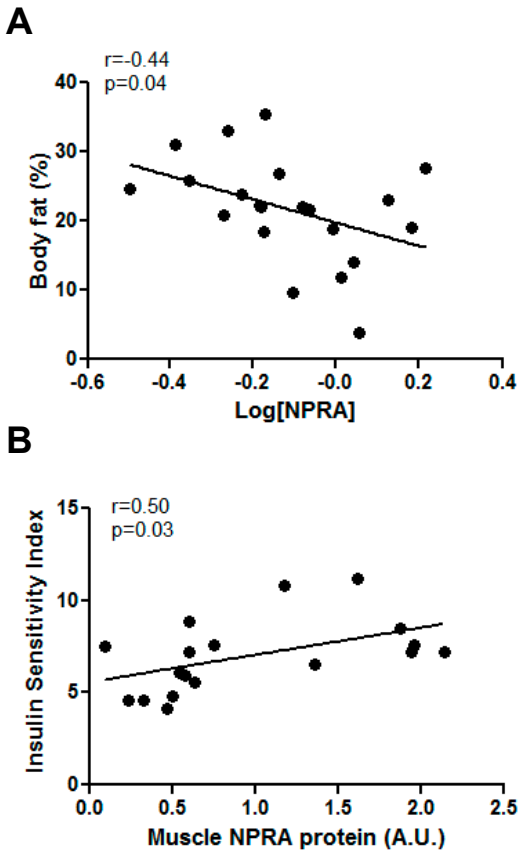
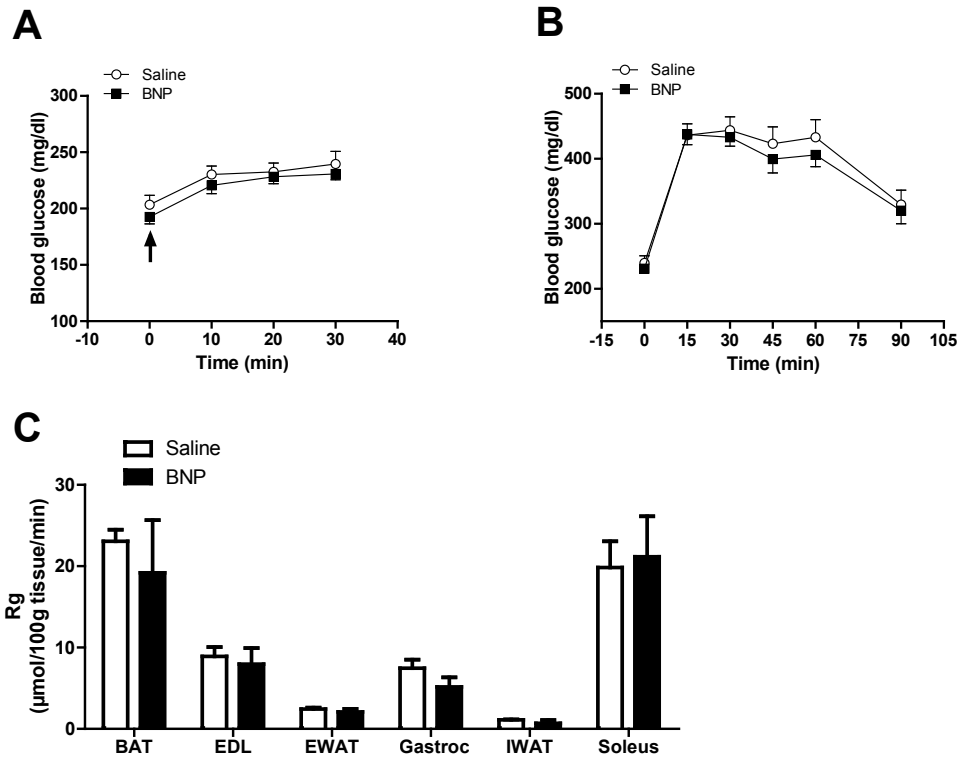
Supplementary Figure S1. Correlation between muscle NPRA protein and clinical variables in humans

Correlation between *vastus lateralis* NPRA protein expression, and (**A**) percent body fat (n=21), and (**B**) the McAuley insulin sensitivity index measured during an oral glucose tolerance test in human subjects with normal glucose tolerance (n=18).



Supplementary Figure S2. Acute effect of BNP injection on insulin sensitivity
Fasted C57BL/6 mice fed standard chow diet were injected intraperitoneously with saline (0.9% NaCl) or with BNP (1 µg/kg) solution (arrow) and fasting blood glucose was measured (**A**) every 10 min in the basal state and (**B**) every 15 min during an i.p. GTT (n=11). (**C**) Tissue-specific glucose uptake was measured during a radiolabeled GTT with [2-^{3}H]deoxyglucose (n=6).



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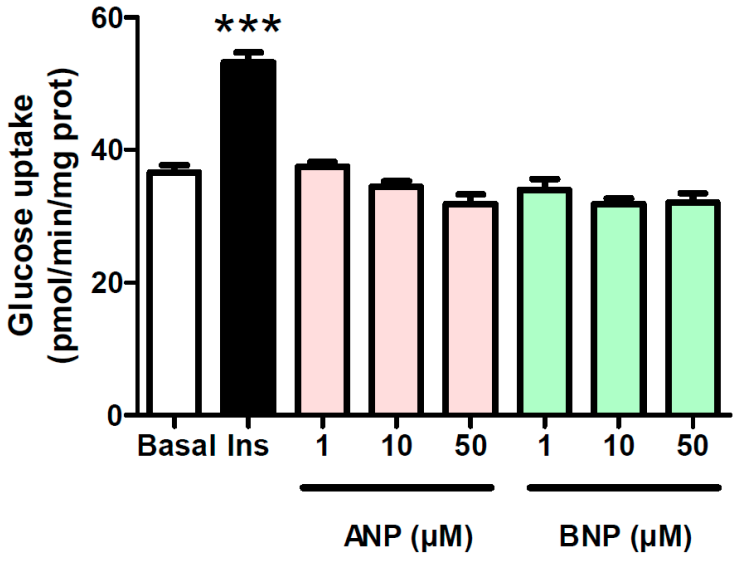
Supplementary Figure S3. Effect of acute NP treatment on glucose uptake in human primary myotubes

Glucose uptake was measured in presence of 1, 10 and 50 µM of ANP or BNP, and 1 µM of insulin in human primary myotubes. *** p<0.001 vs. saline (n=6).



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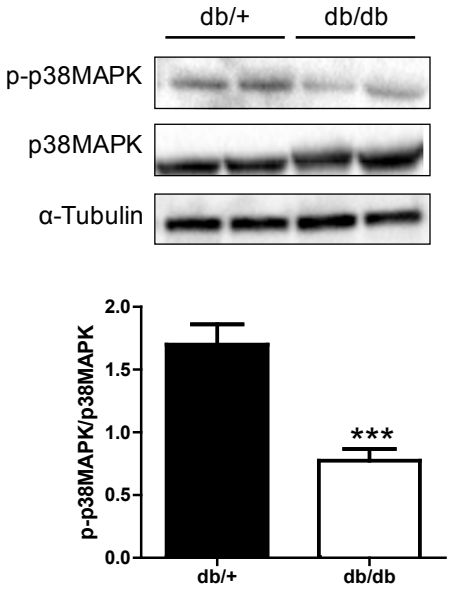
Supplementary Figure S4. Representative blots and quantitative bar graph of p38MAPK phosphorylation relative to total p38MAPK and α-tubulin in *gastrocnemius* muscle of db/+ versus db/db mice. ***p<0.0001 vs. db/+ (n=8).



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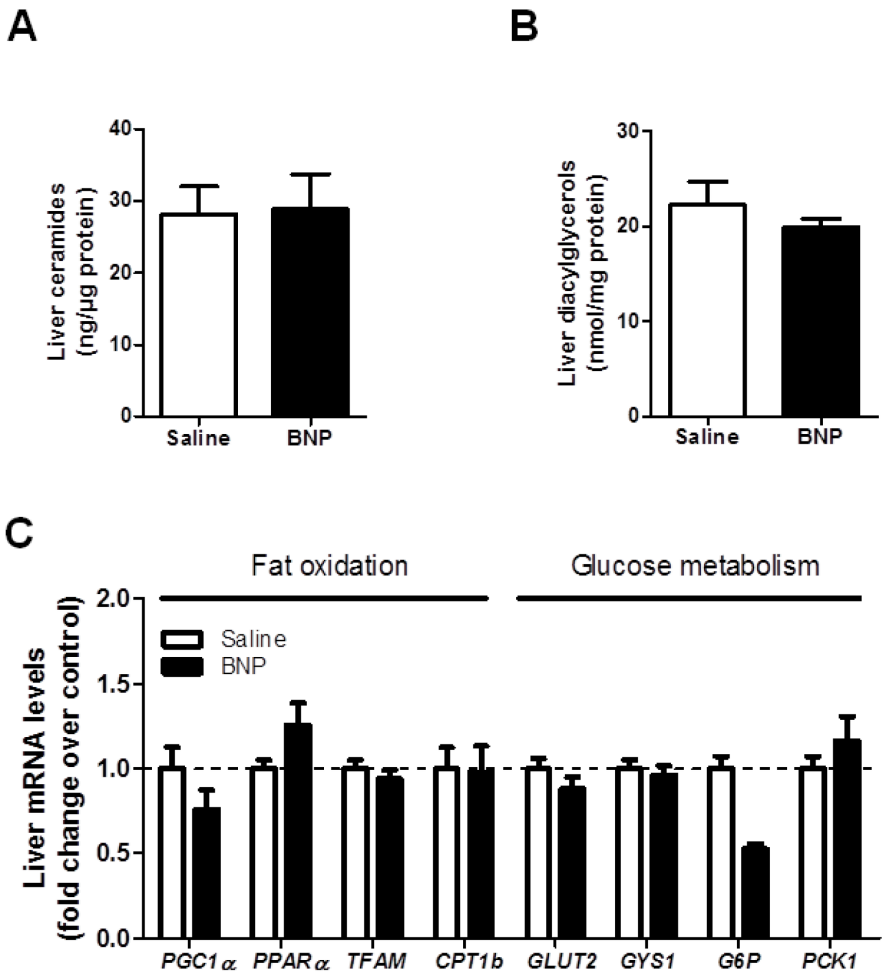
Supplementary Figure S5. Chronic BNP treatment does not change lipid levels and gene expression in liver of db/db mice

(**A**) Total ceramides, (**B**) total diacylglycerols levels, and (**C**) mRNA levels of genes involved in fat oxidation and glucose metabolism in liver of saline- and BNP-treated db/db mice.



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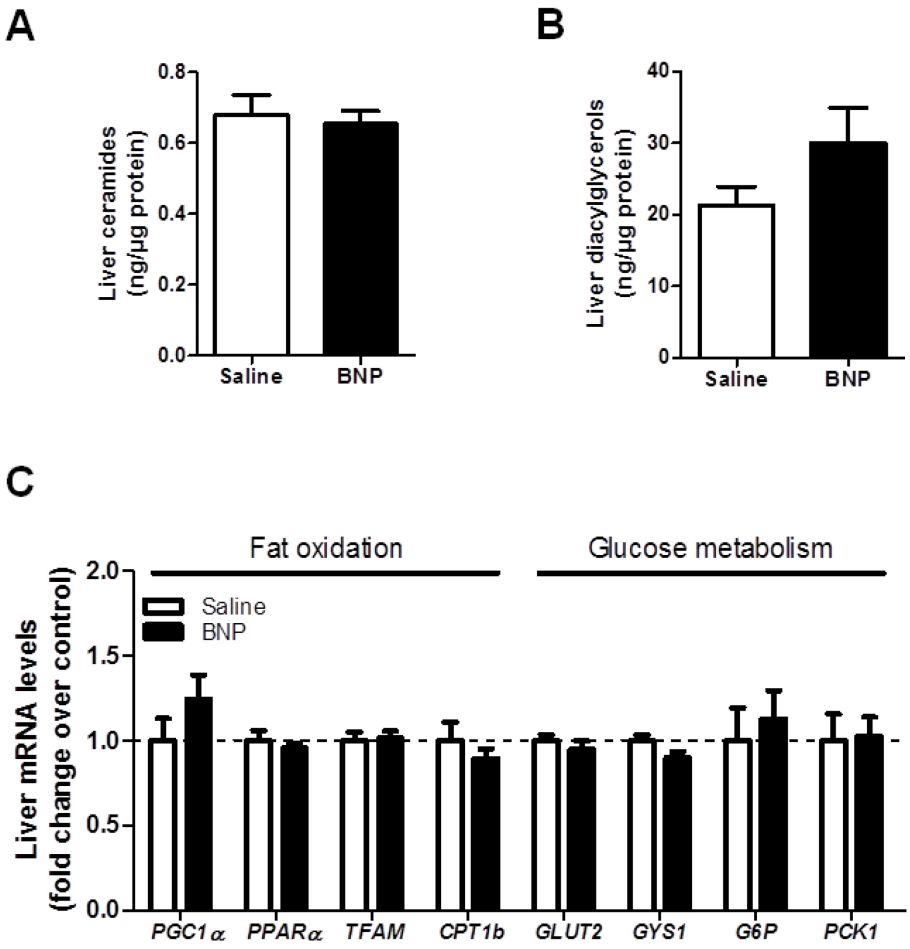
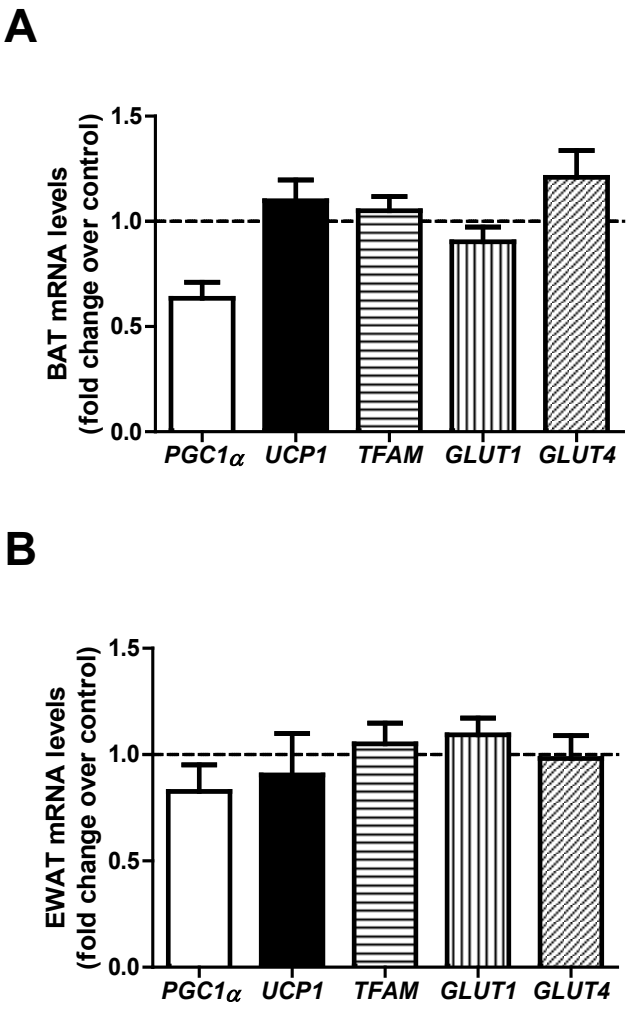
Supplementary Figure S6. Chronic BNP treatment does not change lipid levels and gene expression in liver of HFD-fed mice

(**A**) Total ceramides, (**B**) total diacylglycerols levels, and (**C**) mRNA levels of genes involved in fat oxidation and glucose metabolism in liver of saline- and BNP-treated HFD-fed mice.



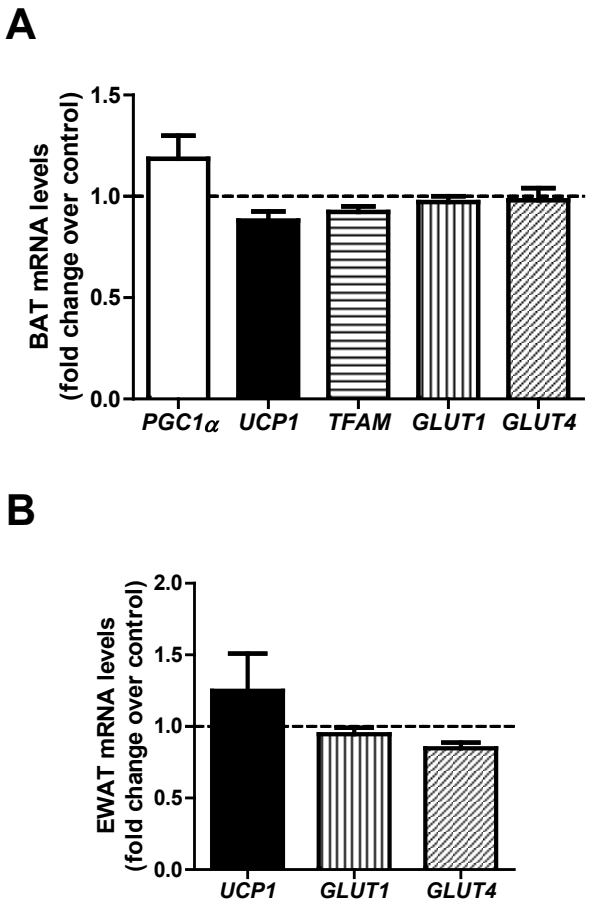
Supplementary Figure S7. Expression of thermogenic and brown/beige gene markers in adipose tissues of db/db mice

PGC1α, UCP1, TFAM, GLUT1 and *GLUT4* mRNA levels in (**A**) BAT and (**B**) EWAT of db/db mice treated for 4 weeks with BNP (n=8-10).



Supplementary Figure S8. Expression of thermogenic and brown/beige gene markers in adipose tissues of HFD-fed mice

(**A**) *PGC1a, UCP1, TFAM, GLUT1* and *GLUT4* mRNA levels in BAT and (**B**) *UCP1, GLUT1* and *GLUT4* gene expression in EWAT of HFD mice treated for 4 weeks with BNP (n=8-10).



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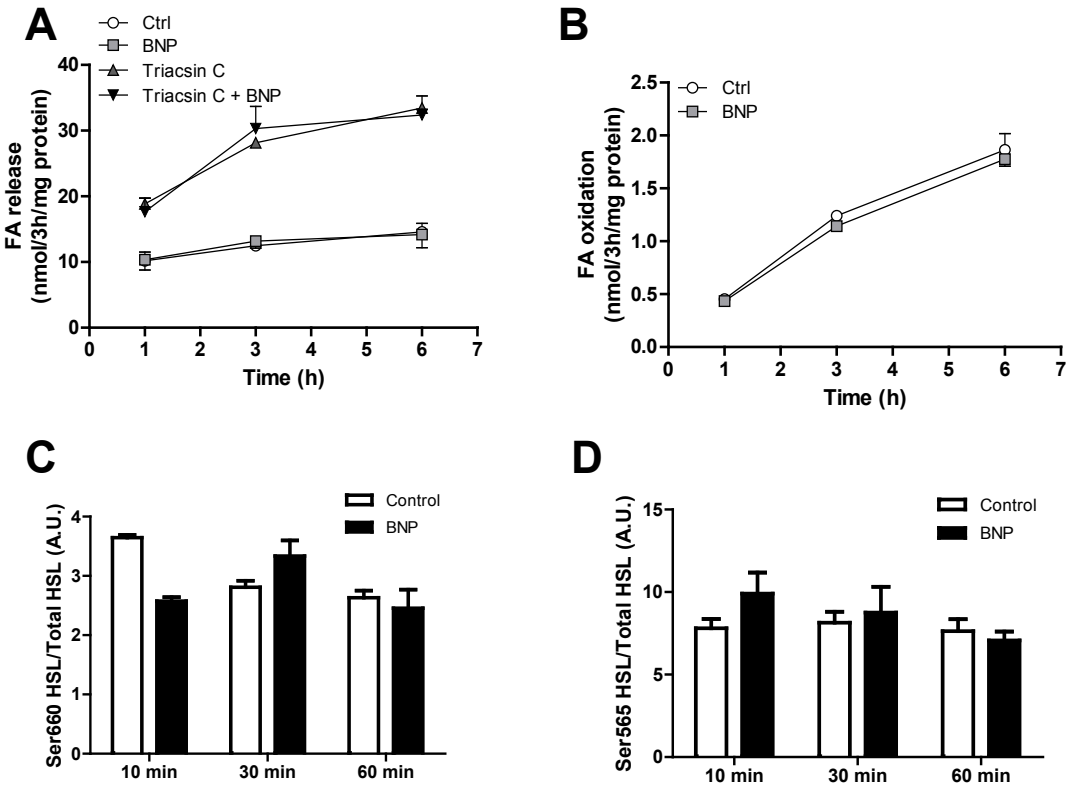
Supplementary Figure S9. Effect of acute BNP treatment on lipolysis in human primary myotubes

Time-course of (**A**) fatty acid (FA) release and (**B**) FA oxidation from endogenous pre-labeled TAG pools in response to 1, 3 or 6 hours BNP treatment in the presence or absence of triacsin C to block FA recycling into TAG pools. (**C**) HSL Ser660 and (**D**) HSL Ser565 phosphorylation were measured after 10, 30 and 60 min acute stimulation with 100 nM of BNP in human primary myotubes (n=3-5).



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Supplementary Figure S10. Effect of chronic NP treatment on basal ceramides content in human primary myotubes.

Ceramide species content in human primary myotubes in basal condition (BSA) in control myotubes and in response to 3-days treatment with 100 nM of ANP or BNP (n=4).