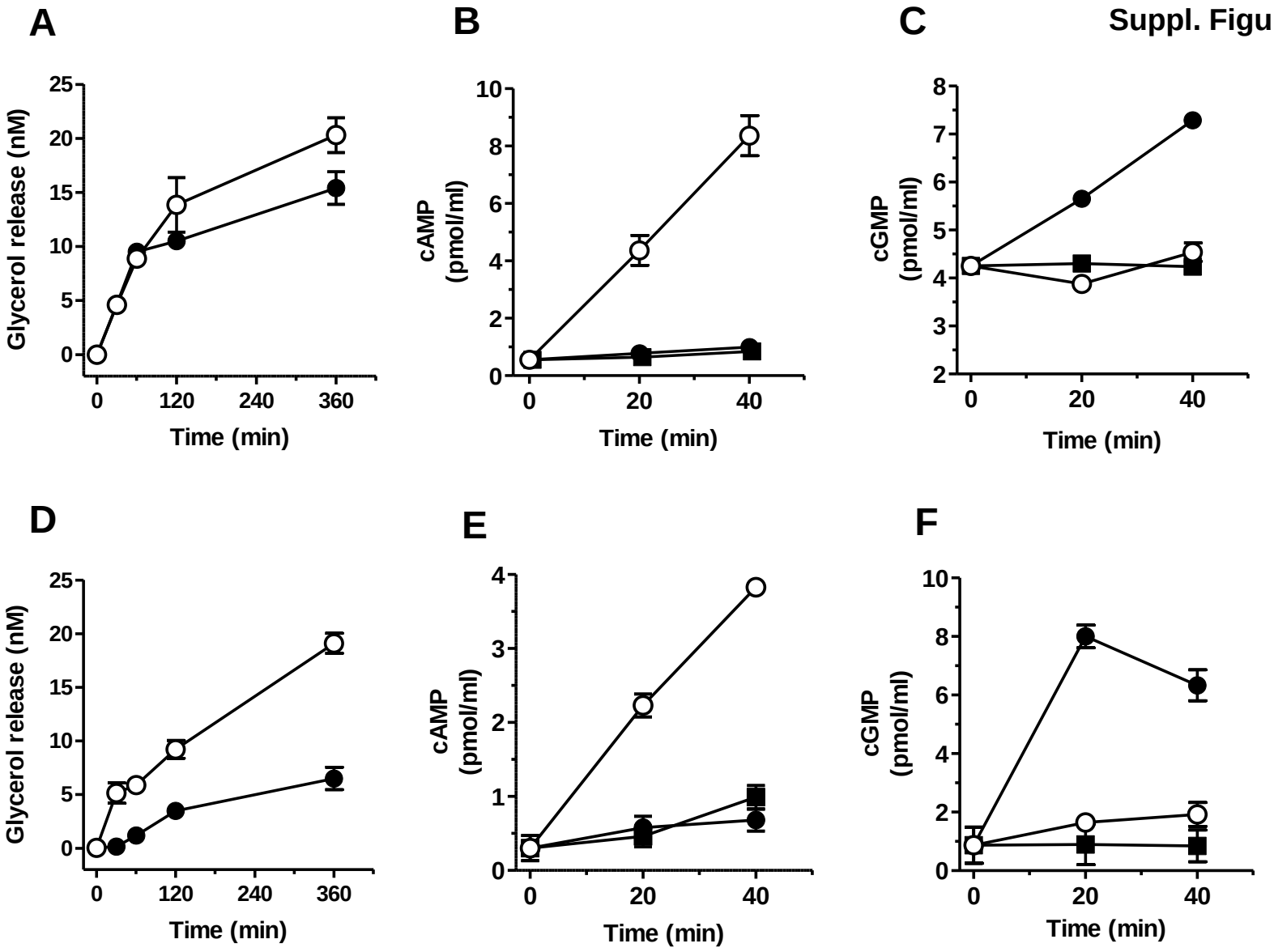
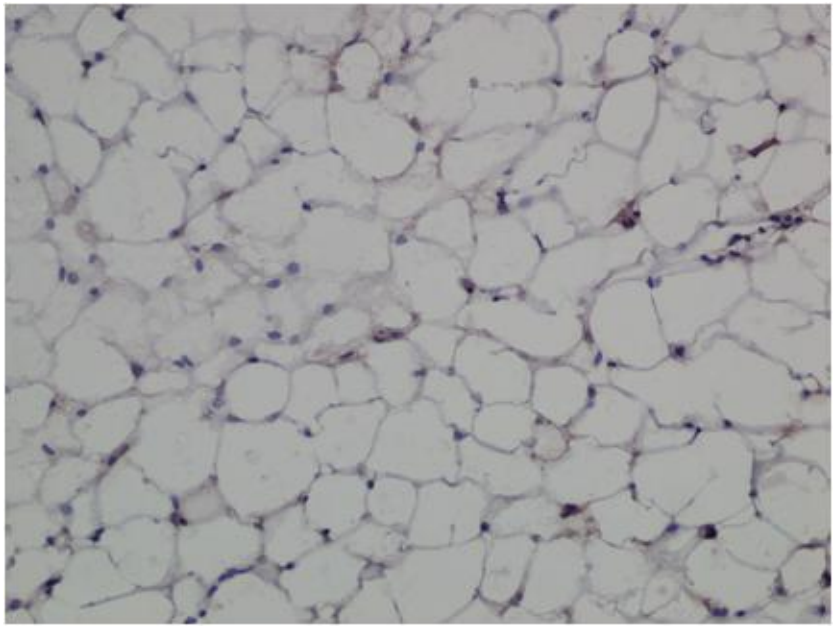


Figure S1. ANP and β-agonist increase lipolysis in human adipocytes. Lipolysis and second messenger levels in culture human subcutaneous adipocytes (**A, B, C**) and hMADS adipocytes (**D, E, F**). Cells were treated with Iso (○), or ANP (●) and compared with untreated control cells (■). Glycerol, cAMP and cGMP concentrations were all determined as described in Methods.

Nprc+/+



Nprc-/-

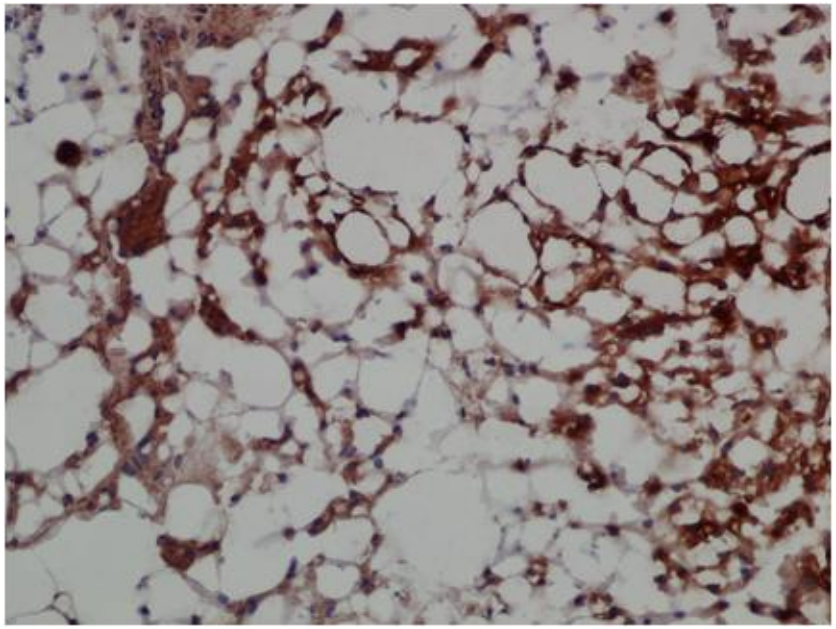


Figure S2. UCP1 immunohistochemical analysis of epididymal WAT. *Nprc+/+* and *Nprc-/-* epididymal WAT were processed to detect UCP1 immunoreactivity.

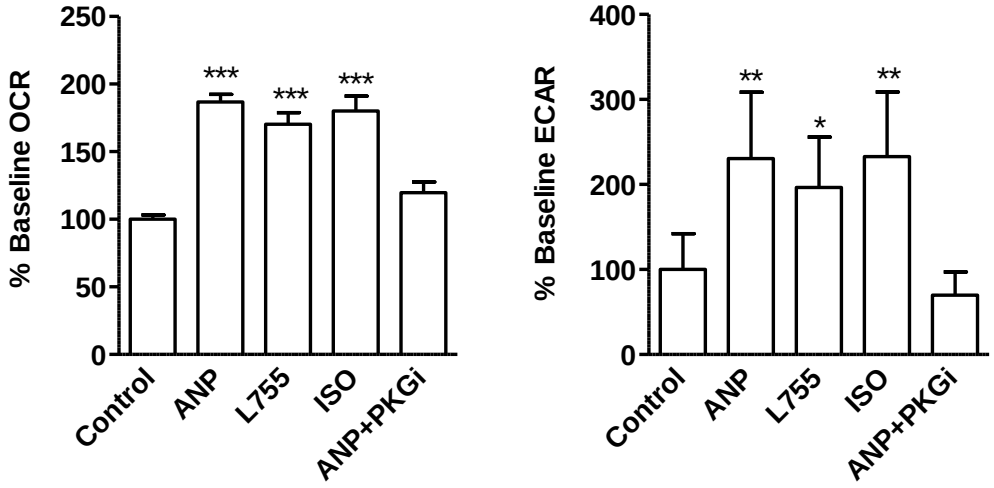


Figure S3. Histograms summarizing the average maximal percent increase of OCR or extracellular acidification rates (ECAR) over their baseline rates. Results are from 6-8 independent experiments. *P< 0.05, **P< 0.01, ***P< 0.001 for treated vs. untreated cells.

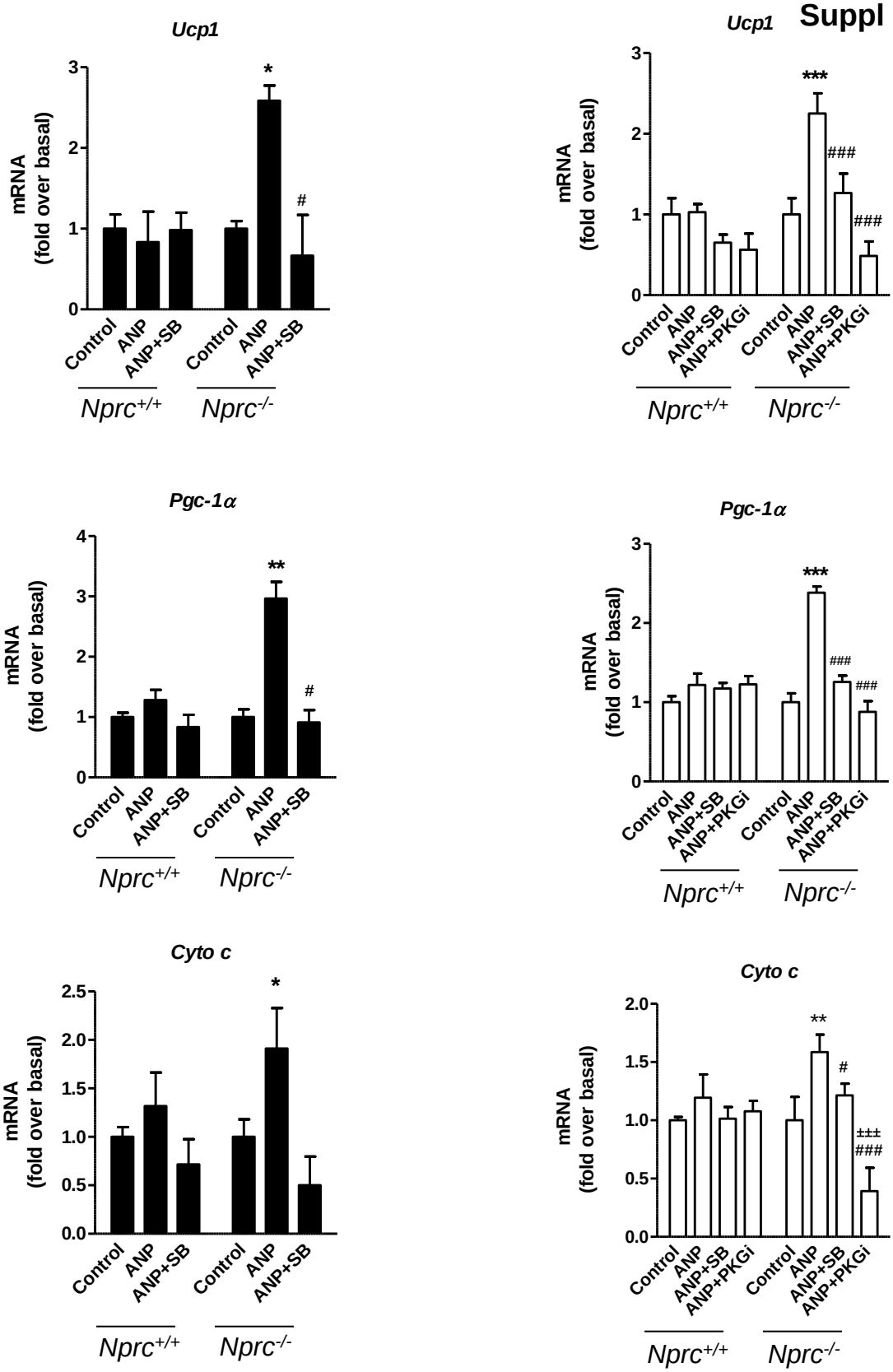


Figure S4. ***Ucp1*, *Pgc-1α*, and *Cyto c* gene expression levels** were evaluated in adipocytes obtained from iBAT (black bars) and epididymal WAT (white bars) of *Nprc*$^{+/+}$ *vs Nprc*$^{-/-}$ mice after treatment or not with ANP and pretreatment or not with PKGi or SB. mRNA levels were measured by quantitative real-time PCR, normalized to 36B4 and expressed relative to untreated control cells as described in Methods. Results are the means ± SD of at least 3 independent experiments.

Bordicchia et al.
Suppl Fig 5

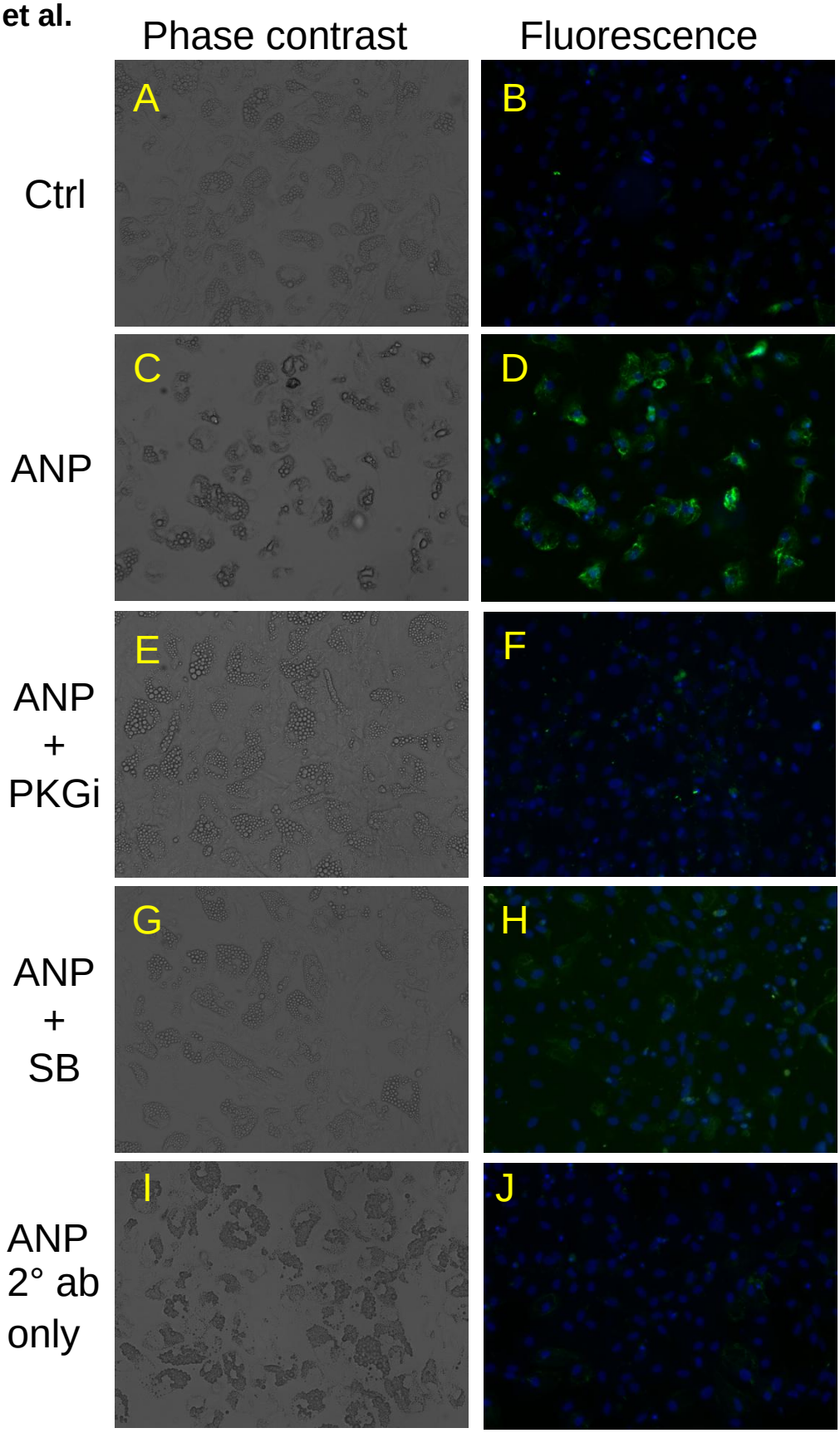


Figure S5. Images of hMADS immunostained for UCP1 and visualized under phase-contrast or fluorescence microscopy. Cells were differentiated on the slide and either untreated (**A**, **B**), or treated with ANP (**C**, **D**) alone or after a 30 min pre-treatment period with PKGi (**E**, **F**), or SB (**G**, **H**). Specificity of UCP1 immunostaining was verified by absence of fluorescence with secondary antibody alone (**I**, **J**). In all slides nuclei are shown by the blue DAPI staining.

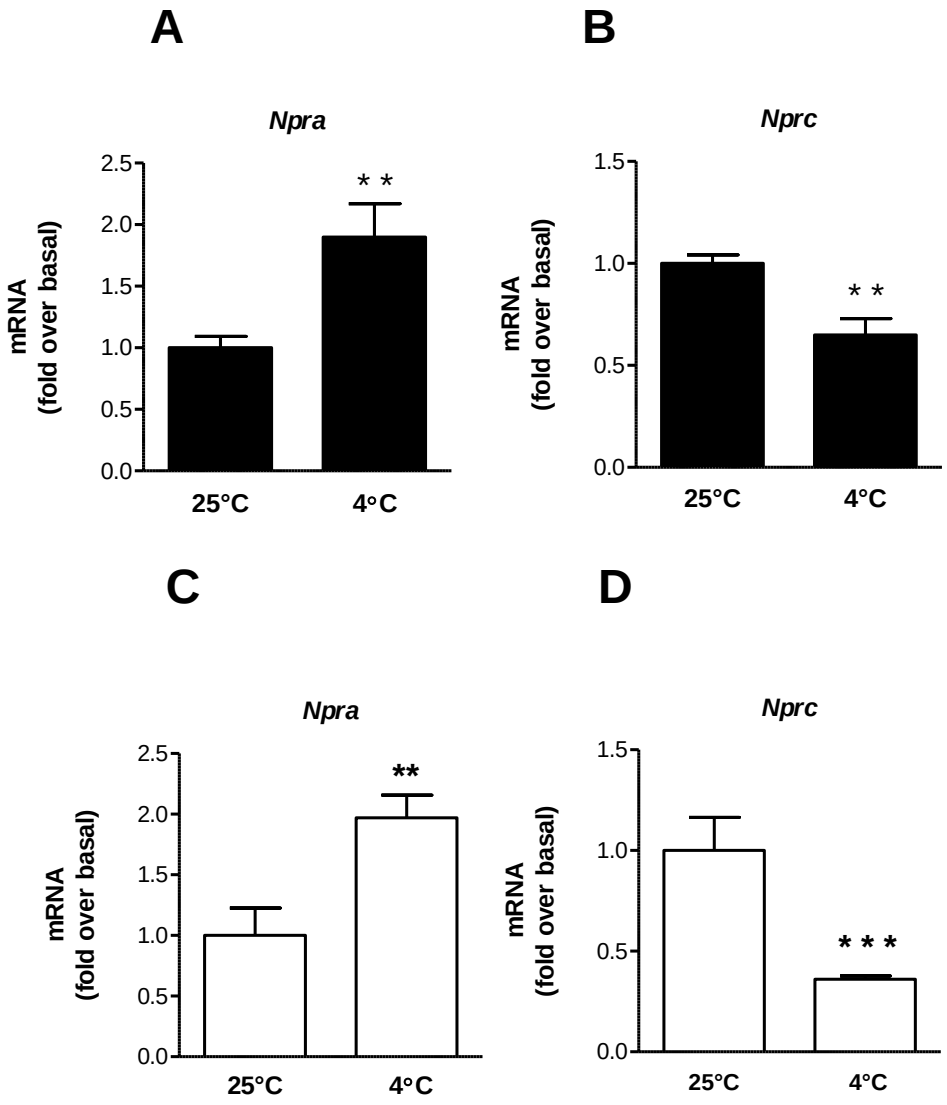


Figure S6. Gene expression analysis of natriuretic peptide receptors in C57BL6 after 6 hrs of cold exposure (4°C; n=9) compared to control mice maintained at room temperature (25°C; n=8). **A** and **B)** *Npra* and *Nprc* mRNA levels in BAT, respectively; **C** and **D)** *Npra* and *Nprc* mRNA levels in epididymal WAT, respectively.

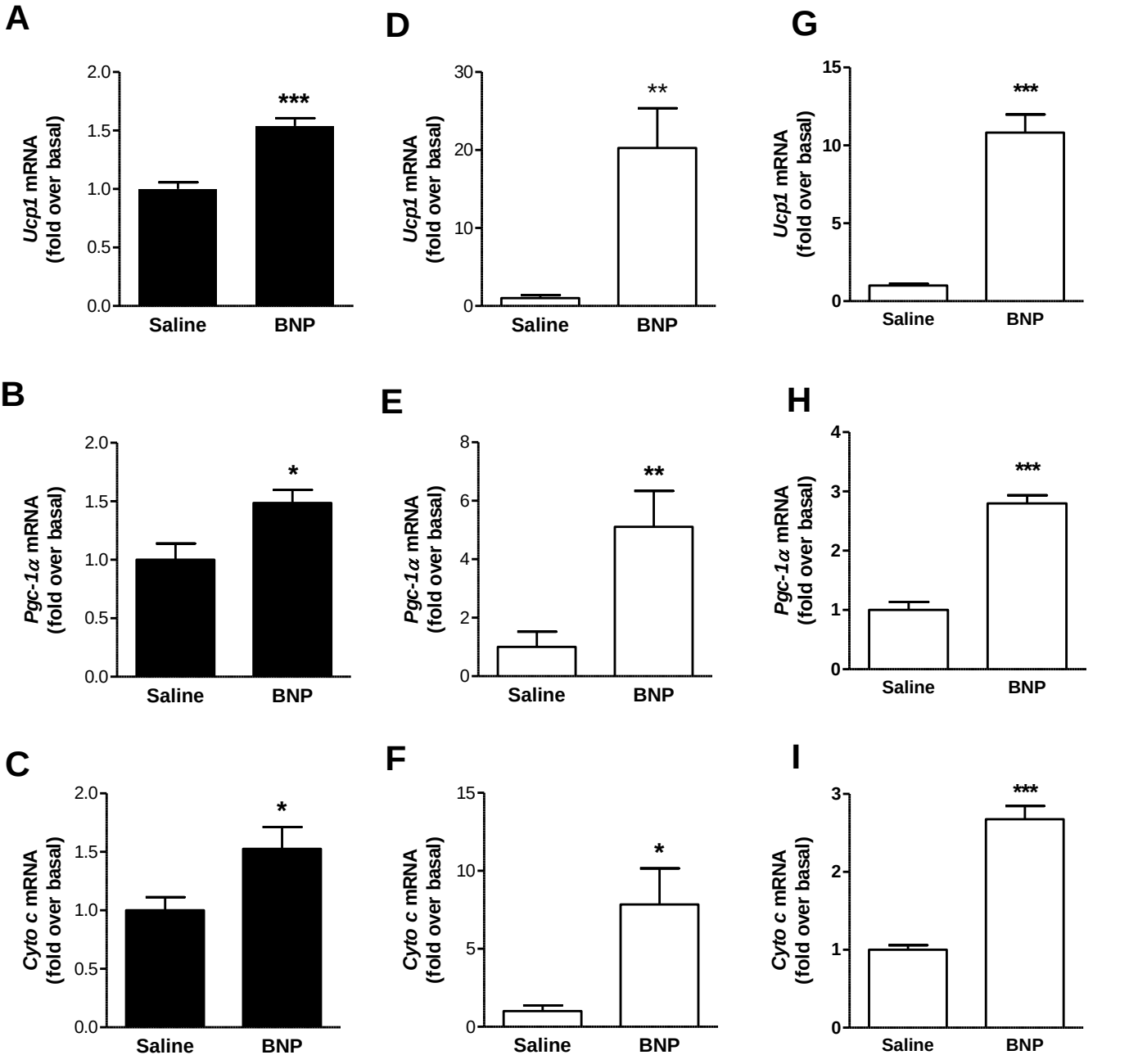


Figure S7. Adipose tissues from C57BL/6 mice treated with BNP (2ng/kg/hr) express higher levels of brown adipocyte marker genes. Gene expression analysis of *Ucp1*, *Pgc-1α* and *Cyto c* in iBAT (panel **A** to **C**, respectively), inguinal WAT (panel **D** to **F**, respectively) *and epididymal WAT (panel **G** to **I**, respectively)* from C57BL/6 mice treated with saline or BNP by Mini-pump. * P < 0.05 vs. Saline ** P <0.01 vs. Saline.

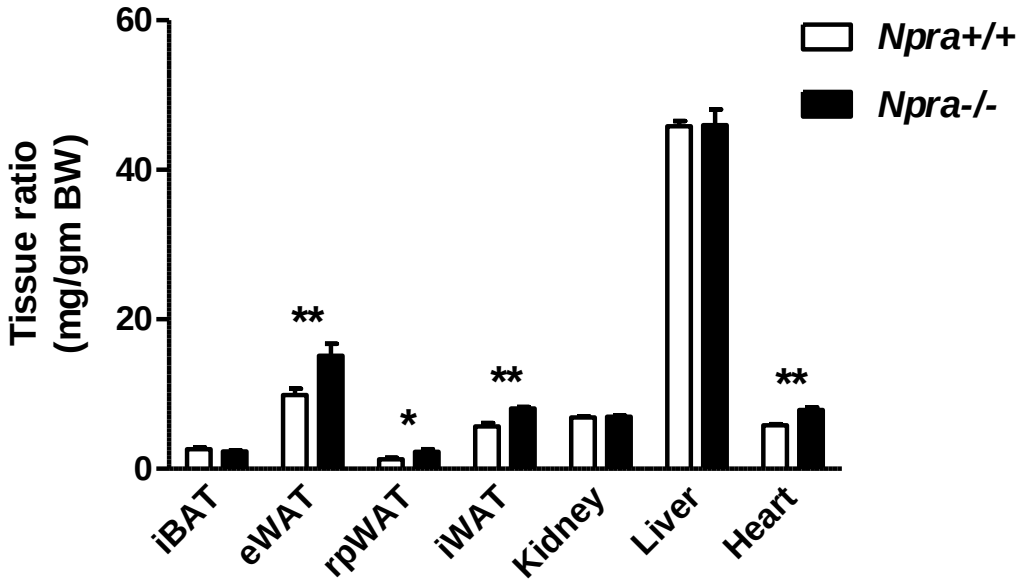


Figure S8. Ratio between weight of isolated tissues and total body weight of *Npra*$^{+/+}$ vs *Npra*$^{-/-}$ mice.

TABLE S1

Effect of ANP on glycerol release from cultured wild-type and *Nprc*$^{-/-}$ adipocytes

	Brown adipocytes		***White adipocytes***	
Treatment	*Nprc*$^{+/+}$	*Nprc*$^{-/-}$	*Nprc*$^{+/+}$	*Nprc*$^{-/-}$
	x sd	x sd	x sd	x sd
Ctrl	61.30 ± 9.45 (3)	64.00 ± 4.86 (3)	65.66 ± 11.71 (3)	53.64 ± 4.39 (3)
ANP	71.20 ± 4.12 (3)	90.04 ± 7.75 (3)**	68.16 ± 7.47 (3)	72.95 ± 0.84 (3)**

Cultured brown and white adipocytes from *Nprc*$^{+/+}$ and *Nprc*$^{-/-}$ were treated with ANP (100 nM), and aliquots of culture medium were collected and assayed for glycerol release. Concentrations were determined from a standard curve. **P < 0.01 vs. untreated cells.

Table S2

Human primers

UCP1	FWD primer RV primer	5'- ACGACACGGTCCAGGAGTTC -3' 5'- CTGAGCCCGCAGTGTGG -3'
CYTO C	FWD primer RV primer	5'- TGGCCCCTCCCATCTACAC -3' 5'- ATCCTTGGCTATCTGGGACATG -3'
PGC-1α	FWD primer RV primer	5'- CACCAAACCCACAGAGAACA -3' 5'- GGGTCATTTGGTGACTCTGG -3'
PRDM16	FWD primer RV primer	5'- CAGCACGGTGAAGCCATTC -3' 5'- GCGTGCATCCGCTTGTG -3'
hCPT1B	FWD primer RV primer	5'- CTGGAAGAAACGCCTGATCC -3' 5'- GCATCTCTGGATGCAACTGA -3'
hMFN1	FWD primer RV primer	5'- CTGTTGCCGGGTGATAGTTG -3' 5'- AGGAGTCAGGGCCAGAGC -3'
hMFN2	FWD primer RV primer	5'- TCCCTGCTAGGAGTTGCTGTAC -3' 5'- CACCTCAGCCCATGTGTCTCTT -3'
hCOX10	FWD primer RV primer	5'- CACACTCTCTCCTCACGCCTC -3' 5'- TTCTTTCAAGATACCAGACAGAGC -3'
SIRT3	FWD primer RV primer	5'- CTTGTGCAGCGGGAAACT -3' 5'- TCCTATGTTACCATTTATTGTGTGC -3'
GAPDH	FWD primer RV primer	5'- TGGTCTCCTCTGACTTCAAC -3' 5'- GTGAGGGTCTCTCTCTTCCT -3'

Mouse primers

Ucp1	FWD primer RV primer	5'- TAAGCCGGCTGAGATCTTGT -3' 5'- GGCCTCTACGACTCAGTCCA -3'
Pgc-1α	FWD primer RV primer	5'- CGGAAATCATATCCAACCAG -3' 5'- TGAGAACCGCTAGCAAGTTTG -3'
Cyto c	FWD primer RV primer	5'- TGGCCCCTCCCATCTACAC -3' 5'- ATCCTTGGCTATCTGGGACATG -3'
Npra	FWD primer RV primer	5'- CGAAGACAAGTGCATCCTGAG -3' 5'- TGGAGACACAGTCAACACAGC -3'
Nprc	FWD primer RV primer	5'- AGCTGGCTACAGCAAGAAGG -3' 5'- CGGCGATACCTTCAAATGTC -3'
36B4	FWD primer RV primer	5'- GATGCCCAGGGAAGACAG -3' 5'- ACAATGAAGCATTTTGGATAATCA -3'

Primers for quantification of mtDNA

NADH dehydrogenase Subunit 1 (mitochondrial)	FWD primer RV primer	5'- CCCTAAAACCCGCCACATCT -3' 5'- GAGCGATGGTGAGAGCTAAGGT -3'
Lipoprotein Lipase (genomic)	FWD primer RV primer	5'- CGAGTCGTCTTTCTCCTGATGAT -3' 5'- TTCTGGATTCCAATGCTTCGA -3'

Primers for ChIP assay

UCP1 enhancer	FWD primer RV primer	5'- GTGCAGCGATTTCTGATTGA -3' 5'- AGGGTCACAGGAGAAGCTGA -3'
UCP1 exon 2	FWD primer RV primer	5'- ACGACACGGTCCAGGAGTTC -3' 5'- GTTTGGGGAAGGGAGAGTTC -3'

Suppl Table 1. Primer sequences used for gene expression analysis, chromatin immune-precipitation (ChIP) assay and quantification of mitochondria DNA content (mtDNA).