SUPPLEMENT MATERIAL

Expanded Materials and Methods

Generation of *PRMT2*$^{+/-}$ and *PRMT2*$^{-/-}$ mice

PRMT2 heterozygous and homozygous null mice were generated as described.[1,2] All animals undergoing experimental procedures were individually genotyped for the *PRMT2* gene by PCR. Except where otherwise mentioned, mice were housed in a temperature, humidity, and light controlled room (14 hr light/10 hr dark cycle) with free access to water and standard chow diet (352 kcal/100 g).

Body weight, heart weight, snout-anus length and food intake measurements

Body weight was measured weekly, beginning at 6 weeks of age. Snout-anus length was measured with a micrometer on 12 week old anaesthetized animals. Food intake was measured daily for 14 days in 12 - 13 week old mice. Heart weight was measured in 10 - 12 week old mice and normalized to femur length.

Blood glucose, serum insulin, triglyceride, and leptin measurements

Whole blood was obtained from the tail veins of fasting or fed state mice (as indicated) at 10 to 11 AM. Blood glucose was assessed by an automatic glucometer (Roche Diagnostic Corp., Indianapolis, IN). Serum was obtained from the hearts of fasting mice at 10 to 11 AM. Serum insulin concentrations were measured by ELISA using rat insulin as a standard (Amersham Biosciences, Piscataway, NJ). Serum triglyceride levels were determined by routine biochemical methods. Serum leptin concentration was measured by ELISA using mouse leptin as a standard (Crystal Chem Inc., Chicago, IL).

High-fat diet studies

Mice were housed 3 to 4 per hanging cage with food and water available ad libitum. The high-fat diet was a modification of the AIN-93G diet with formula added lard in a paste form (Bio-Serv, Frenchtown, NJ) and consisted, as a percentage of caloric content, of 25 % carbohydrate, 21 % protein and 54 % fat. Wild-type and *PRMT2*$^{-/-}$ mice were fed a high-fat diet for 10 weeks. Body weight was measured weekly. For body composition analysis, epidermal, inguinal, subcutaneous and interscapular fat pad masses were dissected and measured at the end of the high-fat feeding. Serum cytokine concentrations were measured using a bead-based suspension micro-array as per the manufacturer's instructions (Assaygate Inc, Ljamsville, MD).

Blood Pressure Analysis

Blood pressure was measured invasively using a micro-tip pressure transducer catheter connected to an electrostatic chart recorder (Millar Instruments, Houston, TX). Animals were anesthetized with 1–3% isoflurane. The right external carotid artery was cannulated and after 5 – 10 min, when hemodynamic stability was observed, baseline blood pressure measurements were recorded.

Histology

Sections (5 μm thick) from formalin fixed paraffin-embedded specimens were stained with hematoxylin and eosin (H & E), and periodic acid-Schiff (PAS), and examined by light microscopy.

Leptin sensitivity study

Nine to 13 week old mice matched for similar body weight at days -4 and 0 were individually caged. Body weight and food intake were measured once daily at 5:30 PM. For the first 7 days, mice were injected i.p. twice daily at 12 PM and 6 PM with PBS to establish baseline measures of weight change and food intake. For the next 6 days,

PBS was replaced with recombinant mouse leptin, 0.1 μg/g body weight (Sigma-Aldrich Inc., St. Louis, MO). Body weight and food intake were measured once daily during these 6 days.

Antibodies

The following antibodies were used: rabbit polyclonal anti-STAT3 (Cell Signaling Technology, Beverly, MA or C-20 from Santa Cruz, CA) and anti-pSTAT3 [pSTAT3 (pY^{705})] (Cell Signaling Technology, Beverly, MA or clone 9E12 from Millipore, Billerica, MA); mouse monoclonal anti-arginine (mono- and di-methyl) antibody (α-metR) (Abcam, Inc., Cambridge, MA, or Novus Biologicals, Littleton, CO); rabbit polyclonal anti-β-actin, rabbit polyclonal and mouse monoclonal anti-Flag antibodies, and anti-Flag M2 Affinity Gel (Sigma-Aldrich Inc.); rabbit polyclonal anti-PRMT2 and mouse monoclonal anti-PRMT2 (Biocarta, San Diego, CA, and A & G Pharmaceutical, Columbia, MD, respectively). Secondary antibodies (Invitrogen, Carlsbad, CA) were used as appropriate.

Plasmids

Mouse PRMT2 cDNA was cloned by reverse transcription PCR using total RNA extracted from mouse cardiac tissues. The following oligonucleotide pairs were used for PCR: 5'- AAGGATCCAGCCCCAGTTATGAGACATGAT -3' and 5'- AAAAGCTTCTTCTTTCACTGAGATGCATGC -3'. pVR1012 was used as a plasmid for subcloning. Mouse PRMT2 motif 1 mutant was generated from the wild-type pVR1012 PRMT2 construct using QuikChange XL Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA).

The mouse STAT3 pcDNA3 plasmid was a kind gift from Dr. J. E. Darnel (Rockefeller University, New York, NY). The plasmid encoding GST fusion proteins of STAT3 was generated by PCR from a mouse STAT3 pcDNA template using the following oligonucleotide pair: 5'- GGCGAATTCACTGCAGCAGGATGGCTCAGTG -3'

and 5'- GCTGTCGACTTGTGGTTGGCCTGGCCCCCTTG -3'. The resulting PCR product was cloned into the EcoRI and SalI sites of pGEX6P-3 (Amersham Biosciences). The same region with the $Arg^{31} \rightarrow Ala^{31}$ mutant was generated from the pGEX6P-3 wild-type STAT3 construct using the above kit.

Bacterial expression and purification of wild-type and mutated STAT3

Glutathione *S*-transferase (GST)-STAT3 fusion proteins were prepared according to the manufacturer's protocol (Amersham Biosciences) with the following modifications. An overnight culture of Top10 One Shot Competent cells containing the pGEX6P3 wild-type or mutated STAT3 plasmid grown in Luria broth supplemented with carbenicillin (100 µg/ml) was diluted 1:15 into 400 ml of the same medium and grown until OD^{650} 0.5 at 22 °C followed by another culture until $OD^{650} > 2.0$ in the presence of 0.1 mM isopropyl-β-D-thiogalactopyranoside (Sigma-Aldrich Inc.). Cells were then pelleted, resuspended in 20 ml of ice cold PBS containing 1 % Triton-X 100, and sonicated. Wild-type and mutated GST-STAT3 were bound to glutathione sepharose 4B (Amersham Biosciences), and after extensive washing, eluted in elution buffer (50 mM Tris-HCl, pH 9.6, 10 mM glutathione, reduced form). Fusion proteins were dialyzed by PBS using Slide-A-Lyzer 10K Dialysis Cassettes (Pierce, Rockford, IL) and stored at -80 °C.

Cell culture and transient transfection

VSMCs were prepared from the thoracic aortas of 12 week old male wild-type and *PRMT2*$^{-/-}$ mice by explant methods.[3] MEFs were prepared from 13.5 day old wild-type and *PRMT2*$^{-/-}$ embryos. VSMCs and MEFs were cultured in Dulbecco's modified Eagle's medium (DMEM; GIBCO, Grand Island, NY) containing 10 % fetal calf serum (FCS) at 37 °C in a humidified atmosphere of 95 % air with 5 % CO_2. The integrity of PRMT2 expression in the established VSMCs and MEFs lines was confirmed by Western blot and immunofluorescence analysis (data not shown). Quiescent VSMCs

(3rd - 6th passages) were used in the described experiments following 48 hr serum starvation, and were treated with sodium orthovanadate (o-vanadate, Na_3VO_4, Sigma-Aldrich Inc.) as indicated. HEK293 cells were also grown in DMEM plus 10 % FCS and were transiently transfected with FuGENE6 transfection reagent (Roche Diagnostic Corp.) according to the manufacture's protocol. For each transfection, 2 µg of mouse PRMT2-related and 8 µg of mouse STAT3-related expression constructs were used. After 24 to 48 hr, cells were used for the various experiments as indicated.

In situ hybridization

Brains were removed immediately after decapitation and snap-frozen in 2-methylbutane (Sigma-Aldrich Inc.) on a bed of crushed dry ice for 6 sec. Coronal sections were cut at a thickness of 16 µm on a cryostat at -20 °C and thaw-mounted on RNase-free slides (KD Medical Inc., Columbia, MD). Labeled riboprope was generated by an in vitro transcription reaction using linerization of the cDNA template and 2 µCi of [^{35}S]UTP (Amersham Biosciences), and unincorporated label was separated using Autoseq (G-50) column (Amersham Biosciences). After pretreatment with 0.25 % acetic anhydride, sections were hybridized with sense and antisense riboprobes corresponding to nucleotides 295 - 798 of mouse PRMT2 cDNA or to the nucleotides 883 - 1398 of mouse STAT3 cDNA in the presence of hybridization buffer (20 mM Tris-HCl, pH7.5, 50 % Formamide, 300 mM NaCl, 1.0 mM EDTA, 1x Denhard's solution, 10 % dextrane sulfate, 150 mM DTT, 0.2 % SDS) at 54 °C. After incubation for 18 hr, sections were washed in 0.1x SSC (15 mM NaCl, 1 mM NaH_2PO_4, and 0.1 mM EDTA) containing 2 mM DTT at 22 °C, dried, and autoradiographed with film for 5 days at room temperature. Results were quantified by densitometry using Photoshop version 6.0 (Adobe Systems, San Jose, CA) to determine the mean pixel deviation of the PVH and ARC hypothalamic nuclei from background. The same densitometry assessment methodology was also used to quantify other results as presented in Figures 5b, 6b and 6e.

Protein preparation, immunoprecipitation and immunoblotting

STAT3 IP was performed as described previously.[4] Cells were washed with PBS and lysed in Nonidet P-40 (NP-40) 0.5 % buffer [50 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1.5 mM $MgCl_2$, 1 mM EDTA, 1.0 % Triton-X, 0.5 % NP-40, 10 % glycerol, 50 mM NaF, 7.5 mM $Na_4P_2O_7$, 1 mM Na_3VO_4, 1 mM dithiotreitol, 1x protease inhibitor cocktail (Roche Diagnostic Corp.)] or RIPA buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 1 % NP-40, 0.5 % Na-deoxycholate, 0.1 % SDS, 1x protease inhibitor cocktail). Lysates were then incubated to solubilize for 30 min at 4 °C, followed by centrifugation at 15,000 rpm for 10 min at 4 °C. Supernatants were then rocked with either polyclonal anti-STAT3 or monoclonal anti-Flag antibody for 14 hr at 4 °C, and then protein G agarose for 3 hr at 4 °C. Beads were washed 3 times in lysis buffer without protease inhibitors, solubilized in 15 µl 3x SDS-polyacrylamide gel electrophoresis (PAGE) buffer (187.5 mM Tris-HCl, 6 % SDS, 30 % glycerol, 150 mM dithiothreitol, 0.3 % bromophenol blue, pH 6.8) and subjected to immunoblotting. For IP experiments described in Figure 5, overnight fasting wild-type and *PRMT2*$^{-/-}$ mice were treated with or without mouse leptin (1.67 µg/g body weight) and tissue harvest performed after 90 min. Tissue extracts were prepared by dissection and homogenization in lysis buffer (75 mM KCl, 2.5 mM MgCl2, 20 mM HEPES, 0.1% NP40, 1 mM DTT, 0.5 mM PMSF, 1 ug/ml leupeptin, 1 ug/ml pepstatin) using a Polytron homogenizer (Brinkman Instruments, Westbury, NY). Extracts were immunoprecipitated with either mouse IgG or α-metR antibody, then subjected to immunoblotting with a rabbit polyclonal anti-STAT3 antibody.

For western blot, cell extracts were prepared in lysis buffer (25 mM HEPES, pH 7.9, 1 % NP-40, 137 mM NaCl, 1x protease inhibitor cocktail). Samples were centrifuged at 2,000 x g for 5 min at 4 °C, the resulting supernatants were centrifuged at 14,000 x g for 20 min at 4 °C, and the protein content of the supernatants were resolved to 3x SDS-PAGE buffer.

Immunoblotting was performed as described previously.[4] After samples were boiled for 5 min at 95 °C and centrifuged for 1 min at 4 °C, aliquots of supernatant were subjected to 7.5 %, 10 %, 15 % or 4 - 15 % SDS-PAGE. Proteins in the gel were transferred to a nitrocellulose membrane by electroblotting. Membranes were treated with specific primary antibodies as indicated, followed by appropriate secondary antibodies conjugated with HRP. Immunoreactive proteins were detected using the ECL plus system (Amersham Biosciences).

In vitro methylation assay

293 cells were transiently transfected with expression vectors encoding Flag-tagged wild-type or mutant mouse PRMT2. After incubation for 48 hr cells were lysed with 500 µl of RIPA buffer, and 1.8 mg of lysate was incubated with 40 µl of Anti-Flag M2 Affinity Gel (Sigma-Aldrich Inc.) for 3 hr at 4 °C. Immune complexes were washed 3 times with lysis buffer without proteinase inhibitors and subjected to the in vitro methylation assay. MEFs were grown to 80 % confluence on a 10 cm plate. Cells were washed and scraped off the plate into 500 µl of PBS (pH 7.4), and were lysed by sonication. After centrifugation at 15,000 rpm for 10 min at 4 °C, the supernatants were used as another applicable enzyme source. In vitro methylation reactions were performed by adding the immune complexes or cell lysates to 1 µg of GST, GST-STAT3 or GST-STAT3 $Arg^{31} \rightarrow Ala^{31}$ and 2 µCi of the methyl donor S-adenosyl-l [methyl-^{3}H]methionine ([^{3}H]-Ado-Met) (Amersham Biosciences) in a final volume of 35 µl. Reactions were incubated for 1 hr at 4 °C and terminated by addition of 3x SDS-loading buffer. Samples were subjected to SDS-PAGE in 4 - 15 % Tris-HCl gradient gel (Bio-Rad Laboratories, Inc., Hercules, CA), transferred to a polyvinylidene difluoride membrane, sprayed with En^{3}hance (PerkinElmer Life and Analytical Sciences, Boston, MA), and exposed to Kodak BioMax MS film (Eastman Kodak Company) with Transcreen LE Intensifying Screen (Eastman Kodak Company) for 7

days at -80 °C. After autography, membranes were washed twice with buffer and stained with Coomassie brilliant blue for 5 min to detect GST protein amounts.

VSMC immune-staining

VSMCs were plated on 4-chamber glass slides (Lab-Tek: Nunc Inc., Naperville, IL). After 48 hr serum-starvation, quiescent cells were treated with or without mouse leptin for the indicated times. Stimulation was terminated by removal of medium and cells were washed 3 times with ice-cold PBS followed by fixation in 4 % paraformaldehyde for 15 min at room temperature. After washing with ice-cold PBS, slides were immersed in PBS containing 0.2 % Triton X-100 for 5 min at room temperature, and then unspecific binding was blocked by treatment with PBS containing 3 % bovine serum albumin (BSA) for 1 hr at room temperature. Samples were then incubated with anti-pSTAT3 antibody in Tris-buffered saline containing 1 % BSA overnight at 4 °C. Next, slides were washed 4 times with ice-cold PBS and incubated with FITC conjugated anti-rabbit IgG secondary antibody (Invitrogen) for 1 hr at room temperature. Slides were then washed with ice-cold PBS and mounted with Vectashield mounting medium with diamidnophenolindole (DAPI) (Vector Laboratories Inc., Burlingame, CA) for nuclear staining. Slides were visualized using a fluorescence microscope (Nikon Eclipse E800, Nikon, Tokyo, Japan) and images captured by digital camera (Retiga 1300, QImaging , Burnaby, Canada).

Immunohistochemistry and immunofluorescence staining

After overnight fasting wild-type and *PRMT2*$^{-/-}$ mice were treated with or without mouse leptin (1.67 μg/g body weight) and euthanized after 90 min. Brains, liver, skeletal muscle and heart were immediately removed and frozen in 2-methylbutane on a bed of crushed dry ice for 6 sec. Coronal sections were cut at a thickness of 16 μm on a cryostat at -20 °C and thaw-mounted on slides (KD Medical Inc.). Sections were fixed with acetone for 10 min at 4 °C and then incubated in methanol for 30 min at -20

°C. After treatment with 0.3 % H_2O_2 in methanol for 30 min at room temperature to block endogenous peroxidase, sections were blocked in Tris-buffered saline (TBS)-Ca (100 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM $CaCl_2$) containing 10 % normal goat serum and 3 % BSA, and then incubated with a rabbit anti-pSTAT3 (pY^{705}) diluted 1:100 in blocking solution (see above) overnight at 4 °C. Next, sections were washed 3 times in TBS-Ca, incubated with a biotinylated secondary goat antibody (Invitrogen) in TBS-Ca containing 1 % BSA for 1 hr at room temperature and then treated with ABC solution (Vector laboratories Inc.) for 1 hr. The signal was then developed by DAB solution (Vector laboratories) giving a brown precipitate, dehydrated, and mounted using Permount (Sigma-Aldrich Inc.). Images were acquired as described above. Immunofluorescence staining was performed on fresh frozen sections from liver, skeletal muscle and heart with primary antibody against pSTAT3 (pY^{705}), then secondary antibody anti-mouse Alexa Fluor 555 (Invitrogen). Mounting medium containing DAPI (H-1200; Vector Laboratories) was then applied. Images were acquired using a Zeiss LSM 510 UV laser scanning confocal microscope system (Carl Zeiss GmbH).

Quantitative real-time PCR

Mice 8 - 12 weeks of age, permitted free access to chow and water, were injected for 3 days with either PBS or recombinant mouse leptin i.p. (Sigma-Aldrich Inc.) at a dose of 0.5 μg/g body weight twice daily (9:00 AM and 7:00 PM). Six hr after final leptin treatment, hypothalamic tissue was isolated and snap-frozen. Total hypothalamic RNA was isolated using Trizol (Invitrogen) according to the manufacturer's instructions. Total RNA was treated with ribonuclease-free deoxyribonuclease (DNase) for 30 min using a commercially available kit (Invitrogen) to eliminate genomic DNA. RNA samples were reverse transcribed with TaqMan Reverse Transcription Reagents (Applied Biosystems, Foster City, CA) and the cDNA products then subjected to automated fluorescent qRT-PCR using an ABI PRISM 7700 Sequence Detection

System (Applied Biosystems). All TaqMan probes were labeled with a fluorescein reporter at the 5'-end and a tetramethylrhodamine quencher at the 3'-end. The following oligonucleotides were synthesized and used as primers and probes for qRT-PCR: POMC, sense 5'-CTGCTTCAGACCTCCATAGATGTG-3', antisense 5'-CAGCGAGAGGTCGAGTTTGC -3', probe 5'-6FAM-CAACCTGCTGGCTTGCATCCGG-TAMRA -3'; NPY, sense 5'-TCAGACCTCTTAATGAAGGAAAGCA-3', antisense 5'-GAGAACAAGTTTCATTTCCCATCA-3', probe 5'-6FAM-CCAGAACAAGGCTTGAAGACCCTTCCAT-TAMRA-3'. PRMT1-8 isoforms were quantified from hypothalamus, liver, skeletal muscle and heart RNA isolated from 8 - 10 week old males receiving normal chow diet using 20x TaqMan Gene Expression Assays (Applied Biosystems). References for the primers and probes for the PRMT isoforms are: PRMT1 Mm00480133_m1, PRMT2 Mm00459994_m1, PRMT3 Mm00659701_m1, PRMT4 Mm00491417_m1, PRMT5 Mm00550472_m1, PRMT6 Mm00619134_m1, PRMT7 Mm01250624_m1, PRMT8 Mm01182915_m1. TaqMan Rodent GAPDH Control Reagents (Applied Biosystems) were used as control primers and probe for each template. Each predicted qRT-PCR product spanned an intron/exon junction. Reactions were incubated for 2 min at 50 °C, followed by 10 min at 95 °C, and then 40 cycles of 15 sec at 95 °C and 1 min at 60 °C. Each qRT-PCR reaction was performed in triplicate in a final volume of 20 µl and assessed by the comparative Ct ($\Delta\Delta$Ct) method (Applied Biosystems, ABI Prism 7700 Users Bulletin #2).

Online Tables

Online Table I. Metabolic phenotypes of *PRMT2*$^{+/+}$*, PRMT2*$^{+/-}$*, and PRMT2*$^{-/-}$ mice

	Male			**Female**	
Genotype	$^{+/+}$	$^{+/-}$	$^{-/-}$	$^{+/+}$	$^{-/-}$
Body weight at 12 weeks (g)	32.4 ± 0.9	30.8 ± 0.7**	27.8 ± 0.7**	23.0 ± 0.8	22.8 ± 0.7
Body weight at 30 weeks (g)	45.2 ± 1.3	42.7 ± 0.7*	39.8 ± 0.4**	29.8 ± 1.2	28.2 ± 0.9*
Snout-anus length (cm)	10.6 ± 0.1	10.3 ± 0.1*	10.3 ± 0.1*	10.0 ± 0.1	9.7 ± 0.1**
Food intake (g per 14 days)	62.3 ± 4.4	(NR)	50.6 ± 2.8*	52.6 ± 1.9	42.8 ± 1.2**
Fasting glucose (mg/dl)	106.0 ± 3.3	97.1 ± 2.5	91.1 ± 3.9*	106.7 ± 2.0	92.8 ± 3.7*
Fed-state glucose (mg/dl)	156.4 ± 3.9	144.9 ± 4.5*	141.7 ± 4.3*	130.6 ± 3.5	127.7 ± 3.0
Insulin (ng/ml)	27.8 ± 9.5	9.4 ± 1.1	8.9 ± 0.8	18.7 ± 4.1	6.9 ± 0.7*
Triglyceride (mg/dl)	82.7 ± 9.3	55.9 ± 11.5	58.9 ± 7.1	37.6 ± 5.7	20.1 ± 2.2*
Leptin (ng/ml)	6.7 ± 1.5	3.6 ± 1.0	2.7 ± 0.6*	3.9 ± 0.9	1.2 ± 0.3*

Male and female PRMT2 wild-type ($^{+/+}$), heterozygote ($^{+/-}$), and null ($^{-/-}$) mice were fed a standard chow diet. Snout-anus length and food intake were measured at 12 - 13 weeks of age. Blood glucose levels at fasting and fed states, and serum insulin, triglyceride, and leptin concentrations at fasting states were measured at 8 - 12 weeks of age. Values represent mean ± SEM. n = 8 for all data, except n = 12 for body weight for male wild-type and *PRMT2*$^{-/-}$ mice. *P < 0.05 vs. wild-type. **P < 0.01 vs. wild-type. NR = not recorded.

Online Table II. Blood pressure and normalized heart weight in wild-type and *PRMT2*$^{-/-}$ mice

	Wild-type	*PRMT2*$^{-/-}$
Mean blood pressure (mmHg)	70.2 ± 1.2	76.3 ± 9.7 (*n.s.*)
Normalized heart weight (g/mm)	0.01082 ± 0.0003	0.0116 ± 0.0004 (*n.s.*)

Blood pressure and heart weight were measured in wild-type (+/+) and *PRMT2*$^{-/-}$ (-/-) male mice. Heart weight was normalized to femoral length (mm). Values represent mean ± SEM. n = 3 for mean blood pressure, n = 12 for heart weight.

Online Table III. Pro-inflammatory cytokine levels in wild-type and *PRMT2*$^{-/-}$ mice

	IL-1α	IL-2	IL-4	IL-5	IL-6	IL-17	TNF-α	IFN-γ
Wild-type / normal chow	1051 ± 557	8.8 ± 4.6	1.3 ± 1.3	7.5 ± 3.8	26.8 ± 15.5	9.8 ± 2.7	3.2 ± 3.2	3.2 ± 3.2
Wild-type / high-fat diet	1452 ± 734	1.2 ± 0.6	0.0 ± 0.0	4.2 ± 2.3	18.5 ± 10.7	6.3 ± 2.3	1.7 ± 1.7	1.6 ± 1.6
PRMT2 $^{-/-}$ / normal chow	747 ± 210	14.4 ± 14.4	7.9 ± 3.3	14.0 ± 3.7	37.7 ± 21.7	18.8 ± 4.7	11.9 ± 9	13.3 ± 13.3
PRMT2 $^{-/-}$ / high-fat diet	1422 ± 598	10.8 ± 3.2*	9.8 ± 2.2	4.9 ± 2.2	16.5 ± 9.5	5.9 ± 3.3*	0.5 ± 0.5	6.0 ± 10.8

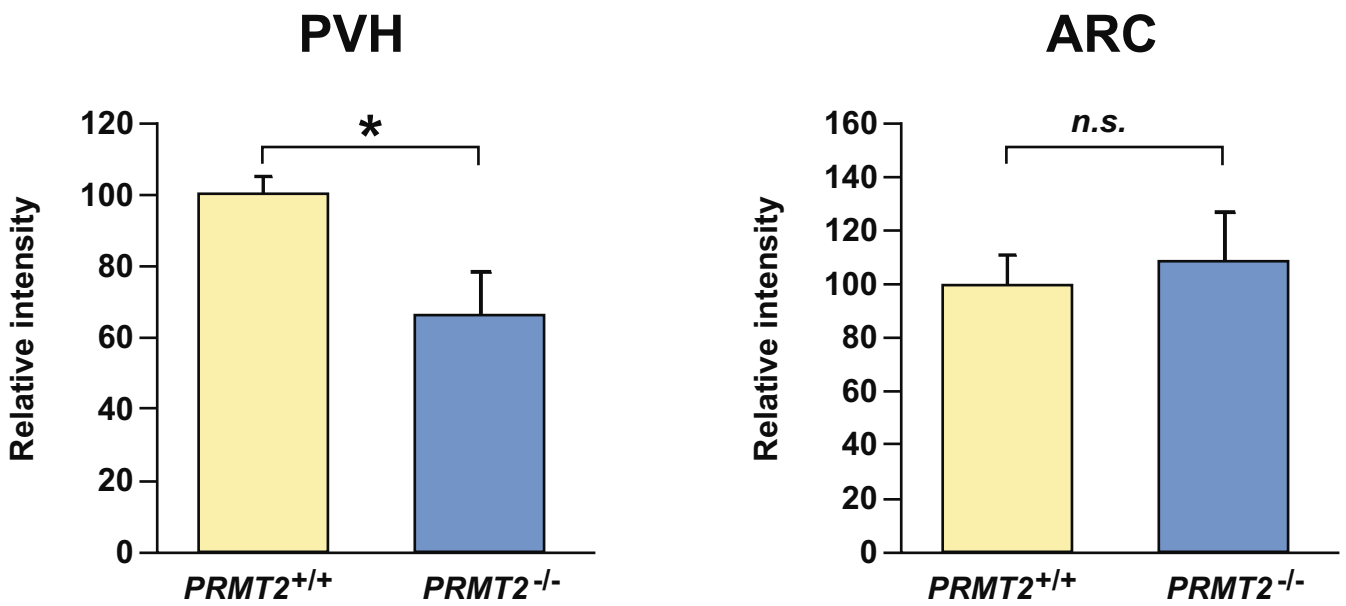
Male PRMT2 wild-type and null (*PRMT2*$^{-/-}$) were fed a standard normal chow diet or high-fat diet for 10 weeks starting at 8 weeks of age. Serum cytokine levels were measured (pg/ml). Values represent mean ± SEM. n = 3, *$P < 0.05$. IL-2: High-fat diet, wild-type vs. *PRMT2*$^{-/-}$. IL-17: *PRMT2*$^{-/-}$ normal chow vs. high-fat diet.

Online Figure Legends

Online Figure I. Quantification of STAT3 mRNA in the PVH and ARC nuclei in wild-type and *PRMT2*$^{-/-}$ mice. The relative levels of STAT3 mRNA in PVH and ARC nuclei were determined by densitometry using the in-situ hybridization results as presented in Figure 3b and normalized to non treated wild-type mice. Data represent the mean ± SEM of 3 mice per group, **P* < 0.05.

Online Figure II. Tyrosine phosphorylated STAT3 was detected in liver sections from wild-type and *PRMT2*$^{-/-}$ mice at baseline and at 90 min after peripheral leptin treatment (1.67 µg/g body weight). Immunohistochemistry was performed using anti-pSTAT3 (pY^{705}) antibody (red) and nuclei were counterstained with DAPI.

Online Figure III. PRMT family mRNA levels in wild type and *PRMT2*$^{-/-}$ mice.
a) Hypothalamic, **b)** heart, **c)** liver, and **d)** skeletal muscle mRNA levels of PRMT 1 – 8 from untreated wild-type and *PRMT2*$^{-/-}$ mice were measured by qRT-PCR. Data are expressed as fold detection compared to wild-type and represent the mean ± SEM of 3 mice per group, **P* < 0.05, ***P* < 0.01, ****P* < 0.005.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100
80
60
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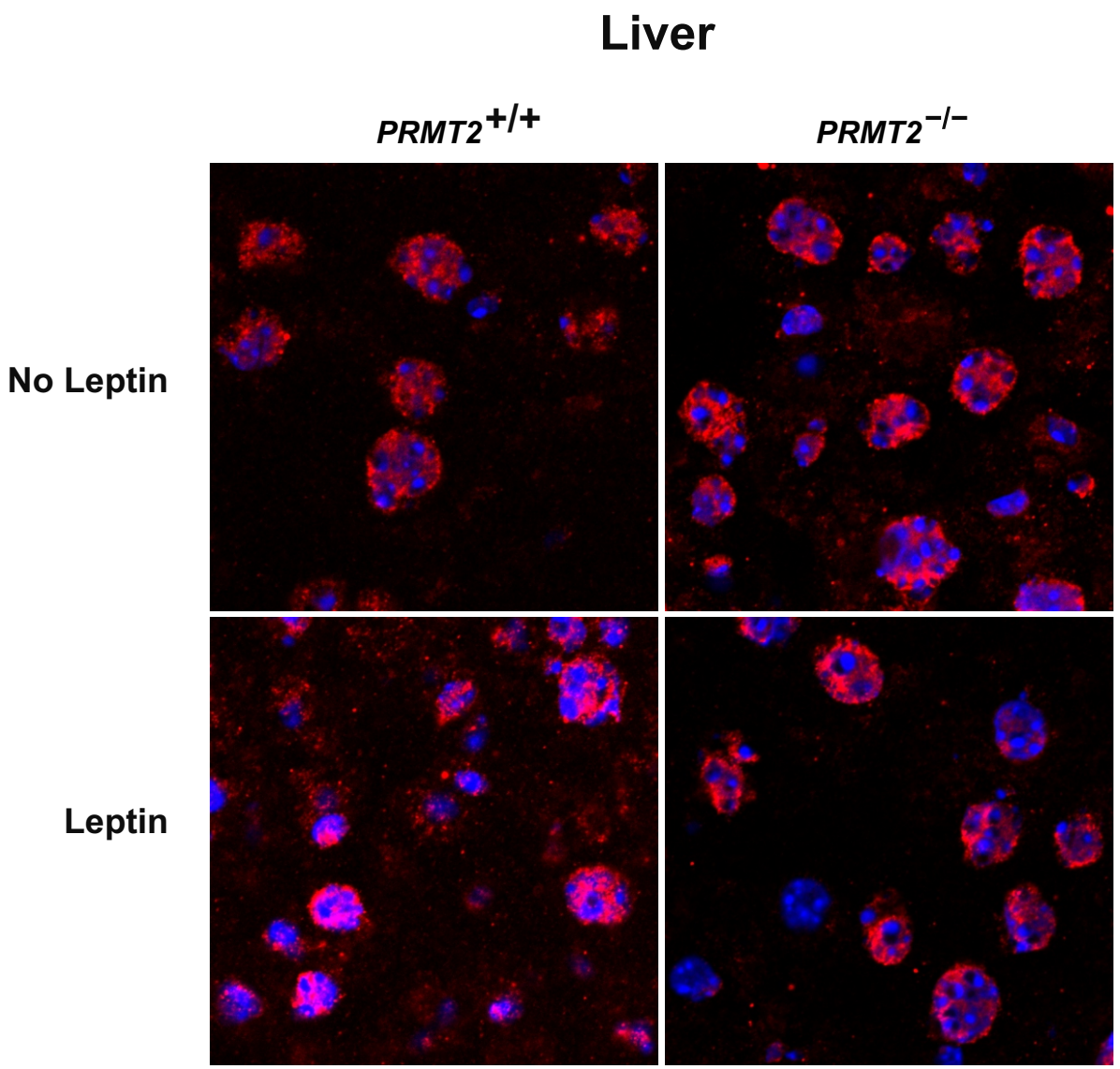
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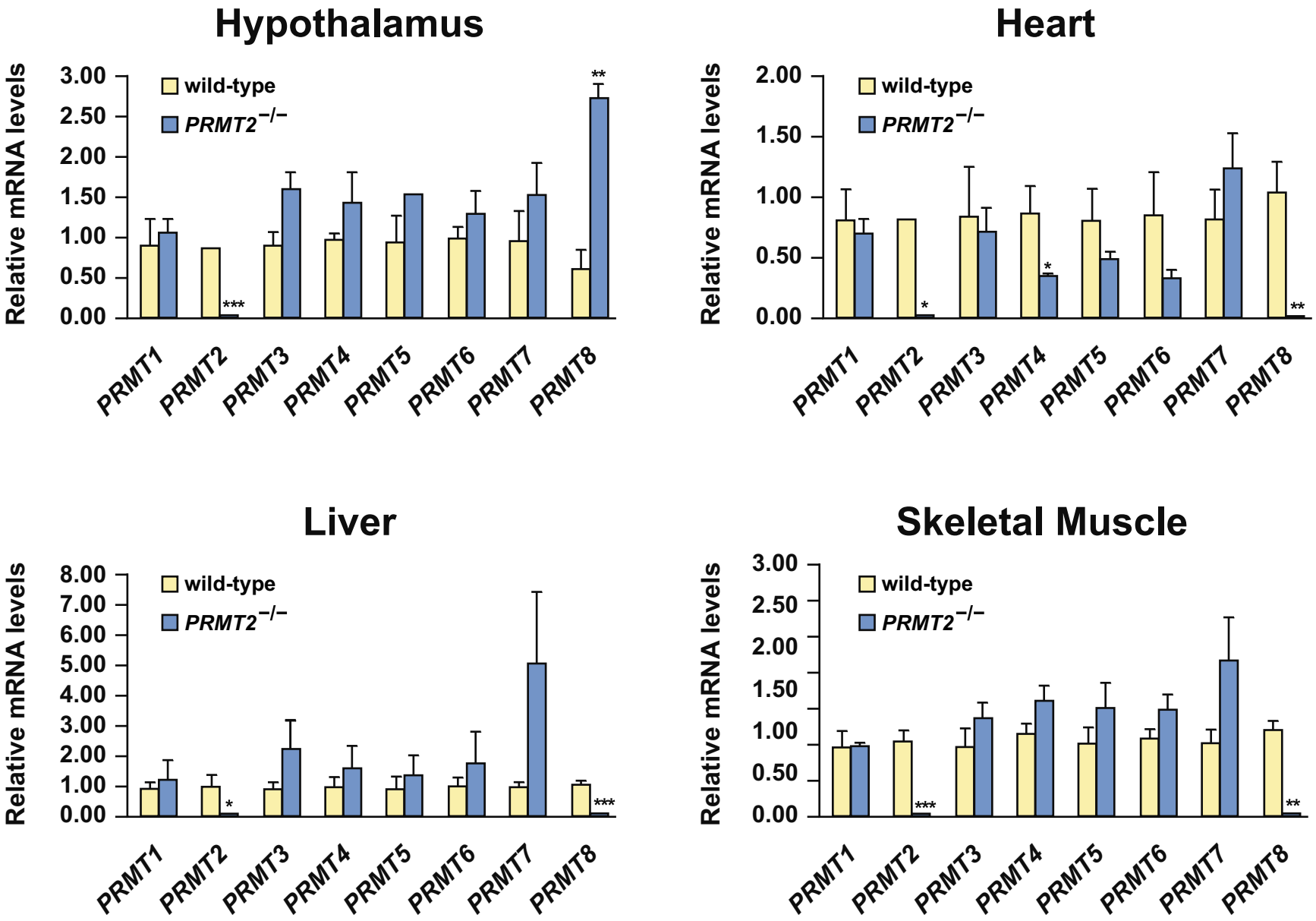
PRMT2+/+
PRMT2−/−
No Leptin
Leptin


Hypothalamus
Heart
Liver
Skeletal Muscle
Relative mRNA levels
wild-type
PRMT2−/−
PRMT1
PRMT2
PRMT3
PRMT4
PRMT5
PRMT6
PRMT7
PRMT8

References

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