SUPPLEMENTARY DATA

Supplementary Table S1. Major allele frequency (CC) = 0.77. Data are presented as mean ±SD. T2D = Type 2 diabetes; BMI = body mass index

Clinical characteristics of participants Danish family study Danish first degree relatives of patients with T2D	
n (men/women)	30/50
Age (years)	52.5±11.3
BMI (kg m^{-2})	27.8±5.4
Fasting plasma glucose (mmol l^{-1})	5.5±0.4
Fasting plasma insulin (pmol l^{-1})	47.4±38.2
Matsuda ISI	23.9±14.4
ADAMTS9 mRNA muscle	1.2±0.4

SUPPLEMENTARY DATA

Supplementary Table S2. Major allele frequency (CC) = 0.79. Data are presented as mean ±SD. BMI = body mass index; Rd = whole-body glucose disappearance

Clinical characteristics of participants Danish non-diabetic twin cohort	
n (men/women)	98/98
Age (years)	42.8±17.0
BMI (kg m^{-2})	25.0±3.9
Body fat (%)	24.6±8.6
Fasting plasma glucose (mmol l^{-1})	5.4±0.6
Fasting plasma insulin (pmol l^{-1})	35.0±18.8
Rd clamp (mg kg_{FFM}^{-1} min^{-1})	10.9±3.4
Exogenous glucose storage rate clamp (mg kg_{FFM}^{-1} min^{-1})	6.7±3.1
IRTK activity	4.5±1.8
IRS-1 associated PI3K activity	55.0±33.1
Akt308 phosphorylation	35.4±21.8
Akt473 phosphorylation	41.1±22.8
Akt1 activity	32.1±19.6
Akt2 activity	43.5±23.6
GSK3 activity	3.6±0.9
Fractional glycogen synthase activity	32.4±9.5
Complex I - NDUFB6 mRNA basal	0.3±0.1
Complex I - NDUFB6 mRNA insulin	0.4±0.1
Complex I - NDUFV2 mRNA basal	0.9±0.3
Complex I - NDUFV2 mRNA insulin	1.0±0.3
Complex III - UQCRB mRNA basal	0.5±0.2
Complex III - UQCRB mRNA insulin	0.6±0.3
Complex IV - COX7A1 mRNA basal	1.4±0.6
Complex IV - COX7A1 mRNA insulin	1.4±0.6
Complex V - ATP5O mRNA basal	0.2±0.1
Complex V - ATP5O mRNA insulin	0.3±0.1
PPARGC1A mRNA basal	1.2±0.4
PPARGC1A mRNA insulin	1.4±0.5
PPARGC1B mRNA basal	1.2±0.8
PPARGC1B mRNA insulin	2.1±1.3

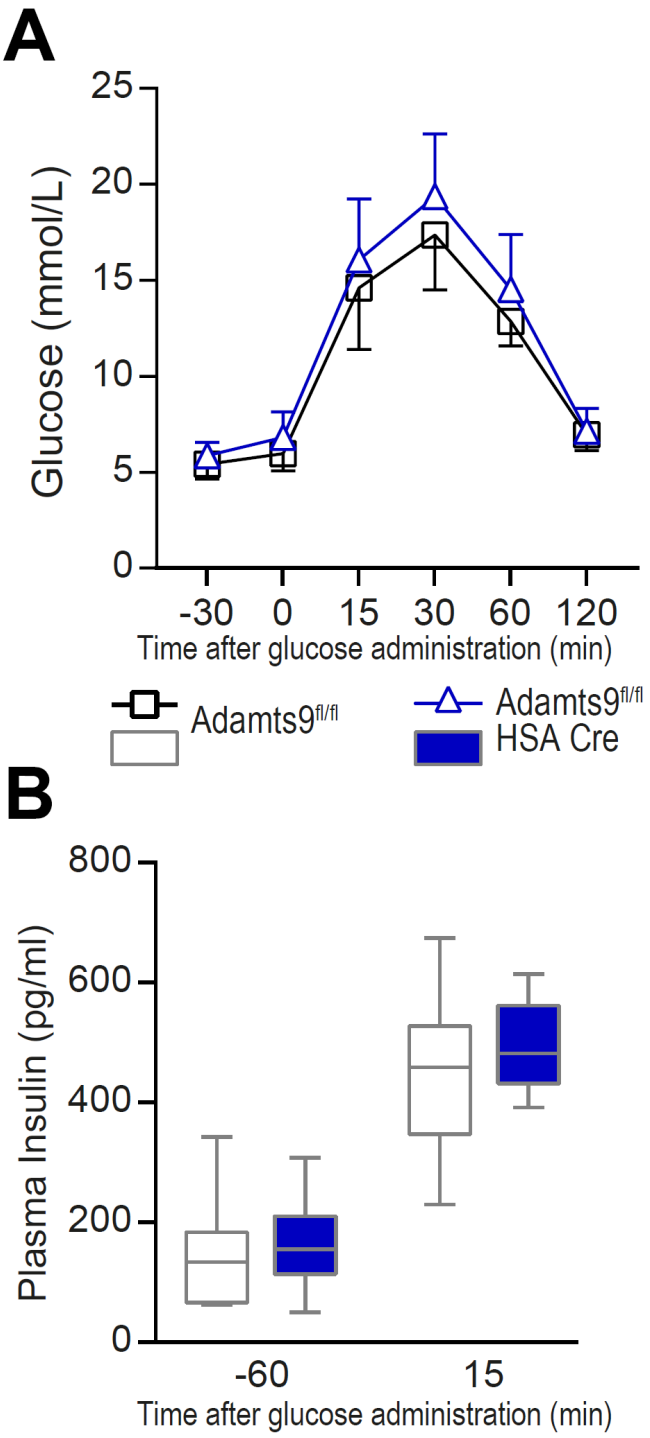
SUPPLEMENTARY DATA

Supplementary Table S3. Associations between the *ADAMTS9* rs4607103 genotype and insulin sensitivity measures as well as mitochondrial measures from the Danish non-diabetic twin cohort. Major allele frequency (CC) = 0.79. Upper part: Enzyme phosphorylations and activities are measured in biopsies taken in the insulin-stimulated state. Phosphorylation of Akt308 was decreased and Rd clamp as well as fractional glycogen synthase activity tended to be decreased in C-risk allele carriers (see Figure 1D-E). Lower part: mRNA expression levels of a number of *complex I-V* subunits and *PGC1α* were measured by TaqMan qPCR in basal and insulin-stimulated biopsies using standard curves. The expression levels were normalized to *PPIA* expression. Associations between *ADAMTS9* rs4607103 genotype and OXPHOS gene expression levels were examined using an additive model adjusting for age, sex, fat percentage as well as twin pair and zygosity status. mRNA expression of *PPARGC1A*, *NDUFB6*, and *UQCRB* was decreased in C-risk allele carriers. Bold indicates P<0.05. Rd = whole-body glucose disappearance.

Associations for *ADAMTS9* rs4607103 with insulin sensitivity and related molecular measures in skeletal muscle Danish non-diabetic twin cohort		
Response variable	**Effect size** (95% CI)	**P-value**
Rd clamp (mg kg_{FFM}^{-1} min^{-1})	-0.64 (-0.10;1.38)	**0.08**
Exogenous glucose storage rate clamp (mg kg_{FFM}^{-1} min^{-1})	-0.38 (-0.40;1.16)	0.3
IRTK activity	0.068 (-0.18;0.043)	0.2
IRS-1 associated PI3K activity	0.078 (-0.27;0.11)	0.4
Akt308 phosphorylation	-0.19 (0.011;0.36)	**0.04**
Akt473 phosphorylation	-0.038 (-0.10;0.18)	0.6
Akt1 activity	0.14 (-0.34;0.057)	0.2
Akt2 activity	-0.0079 (-0.15;0.17)	0.9
GSK3 activity	-0.17 (-0.046;0.39)	0.2
Fractional glycogen synthase activity	-2.48 (-0.14;5.10)	0.06
Complex I - NDUFB6 mRNA basal	-0.032 (0.0029;0.061)	**0.03**
Complex I - NDUFB6 mRNA insulin	-0.028 (-0.0068;0.063)	0.1
Complex I - NDUFV2 mRNA basal	-0.075 (-0.0035;0.15)	0.06
Complex I - NDUFV2 mRNA insulin	-0.047 (-0.049;0.14)	0.3
Complex III - UQCRB mRNA basal	-0.049 (-0.0067;0.10)	0.08
Complex III - UQCRB mRNA insulin	-0.10 (0.023;0.18)	**0.01**
Complex IV - COX7A1 mRNA basal	-0.075 (-0.072;0.22)	0.3
Complex IV - COX7A1 mRNA insulin	-0.071 (-0.066;0.21)	0.3
Complex V - ATP5O mRNA basal	-0.018 (-0.0016;0.038)	0.07
Complex V - ATP5O mRNA insulin	-0.013 (-0.011;0.037)	0.3
PPARGC1A mRNA basal	-0.11 (-0.020;0.24)	0.09
PPARGC1A mRNA insulin	-0.17 (0.026;0.32)	**0.02**
PPARGC1B mRNA basal	-0.14 (-0.059;0.34)	0.2
PPARGC1B mRNA insulin	-0.16 (-0.21;0.52)	0.4

SUPPLEMENTARY DATA

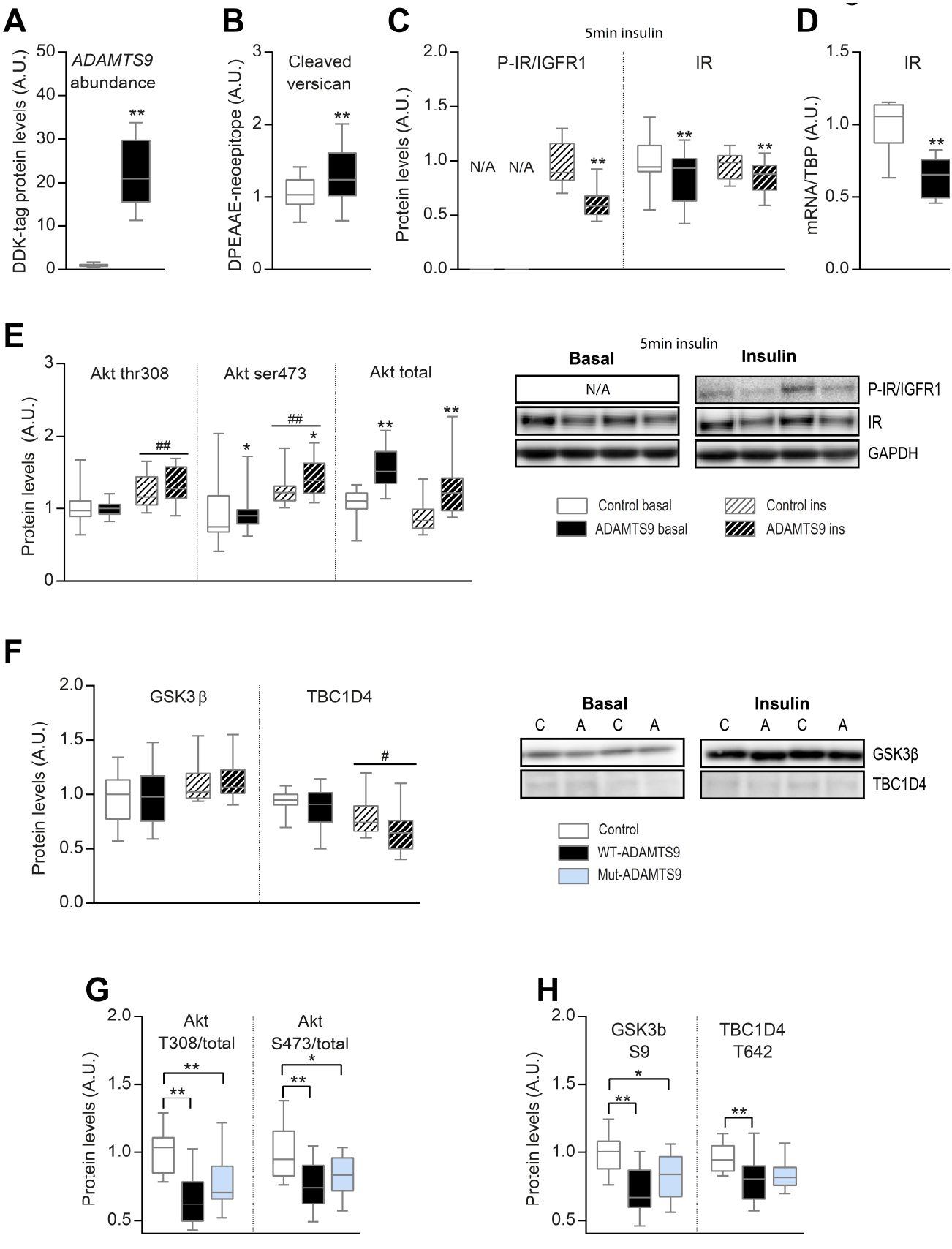
Supplementary Figure S1. Muscle-specific KO of *Adamts9* does not alter glucose metabolism or insulin secretion on chow diet. (A+B) *Adamts9*$^{fl/fl}$ (littermate controls) and *Adamts9*$^{fl/fl}$ HSA Cre (muscle-specific *Adamts9* KO) mice on chow diet at 8 weeks of age (n = 12 for *Adamts9*$^{fl/fl}$; n = 10 for *Adamts9*$^{fl/fl}$ HSA Cre). (A) Oral glucose tolerance test (OGTT) and (B) insulin secretion during OGTT.



SUPPLEMENTARY DATA

Supplementary Figure S2. ADAMTS9 overexpression in mouse skeletal muscle decreases insulin signaling dependent on catalytic activity. (A-C+E-G) Mice overexpressing ADAMTS9 or control plasmid in TA muscles were stimulated with either saline (basal) or 0.5U/kg insulin for (C) 5min or (A+B+E+F) 25min (ins). The TA muscles were isolated and used for western blot analysis against: (A) ADAMTS9-DDK (n = 24 for each group; results replicated twice and then pooled; saline and insulin pooled). (B) Cleaved versican (DPEAAE-neoepitope) (n = 18 for each group; results replicated twice and then pooled; saline and insulin pooled) (C) P-IR/IGFR1 and IR (n = 15-16 for each group) (E) Akt (n = 12 for each group; results replicated twice and then pooled) (F) GSK3β and TBC1D4 (n = 11-12 for each group, results replicated twice and then pooled). (D) TA muscles from mice overexpressing ADAMTS9 or control plasmid were isolated and used for mRNA expression level analysis (n = 8 in each group). Statistical significance was determined using (A+B+D) paired Student's t-test or (C+E+F) paired 2-way ANOVA with Sidak's test for multiple comparisons. */** indicates genotype effect ($p<0.05/0.01$), whereas #/## indicates main effect of insulin vs. basal treatment ($p<0.05/0.01$). (G+HI) Mouse TA muscles overexpressing WT-ADAMTS9, mut-ADAMTS9 or control plasmid were isolated and used for western blot analysis (n = 13 for WT-ADAMTS9 and control groups, n = 14 for mut-ADAMTS9; results replicated twice and then pooled). Statistical significance was determined using 1-way ANOVA with Tukey's test for multiple comparisons. */** indicates genotype effect (WT-ADAMTS9 vs. mut-ADAMTS9 vs. control) where specified ($p<0.05/0.01$).

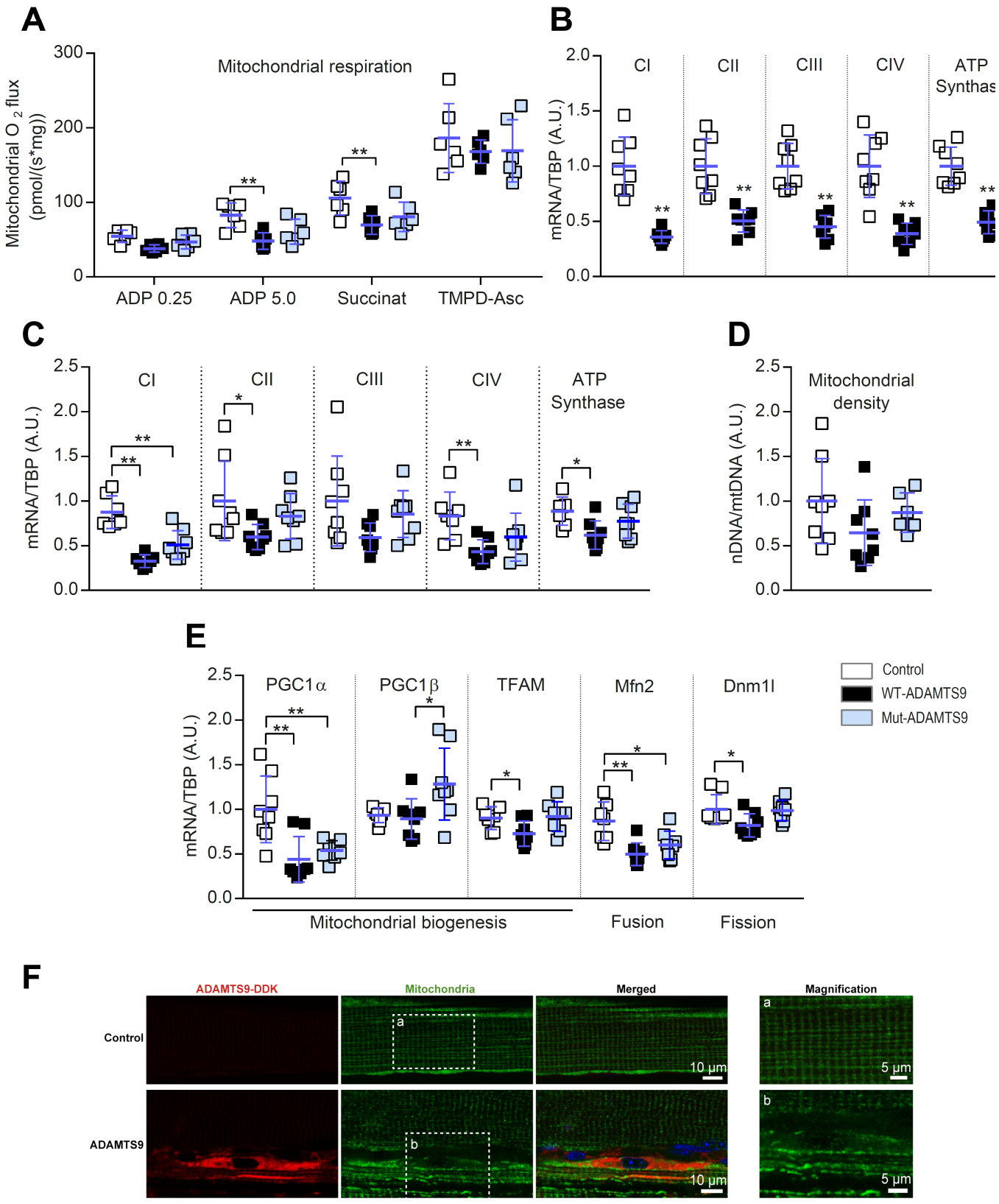
SUPPLEMENTARY DATA



SUPPLEMENTARY DATA

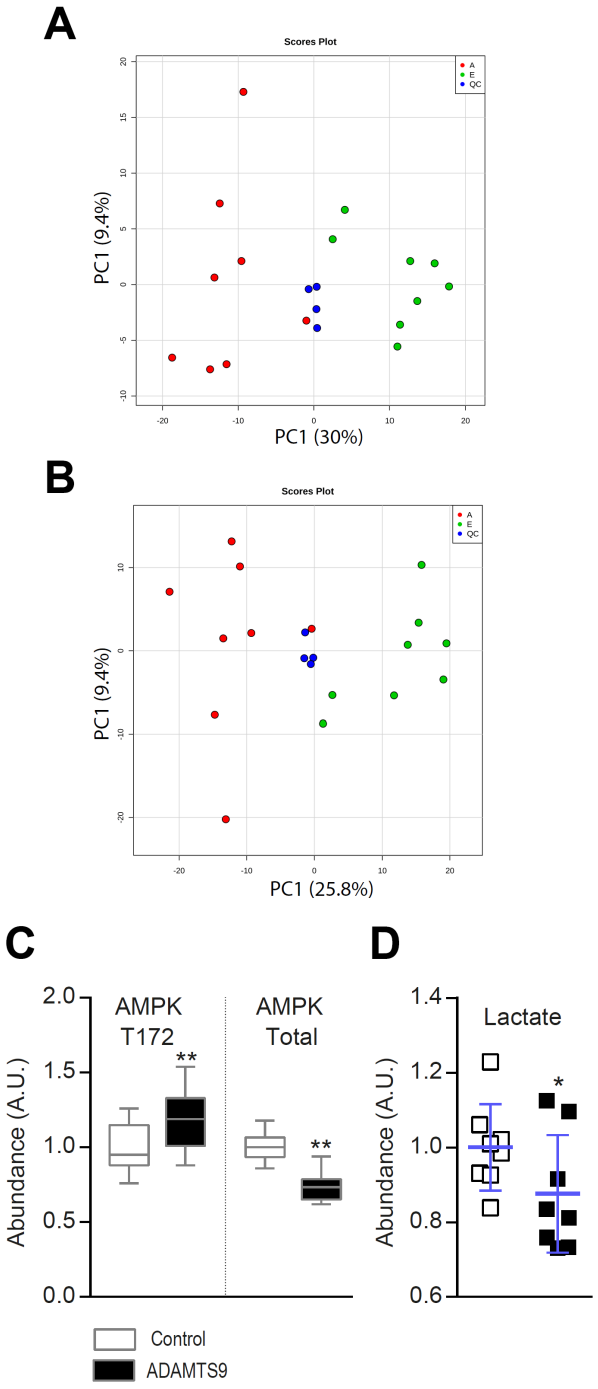
Supplementary Figure S3. ADAMTS9 induces mitochondrial changes in mouse skeletal muscles dependent on catalytic activity. (A+C-E) Mouse TA muscles overexpressing WT-ADAMTS9, mut-ADAMTS9 or control plasmid were used for: (A) Mitochondrial respiration (four replicates for each TA muscle; n = 6 for each group). (C+E) mRNA expression levels measured by qPCR normalized to *TBP* (n = 8 for each group). (D) Mitochondrial density determined by mitochondrial encoded DNA normalized to nuclear encoded DNA in TA muscles (n = 8 for WT-ADAMTS9 and control groups, n = 6 for mut-ADAMTS9). Statistical significance was determined using (A) 2-way ANOVA with repeated measurements and Tukey's test for multiple comparisons or (C-E) 1-way ANOVA with Tukey's test for multiple comparisons. Scatter plots are presented as mean values ±SD. */** indicates genotype effect (WT-ADAMTS9 vs. mut-ADAMTS9 vs. control) where specified ($p<0.05/0.01$). (B+F) Mouse TA muscles overexpressing ADAMTS9 or control plasmid were isolated and used for (B) mRNA expression levels of mitochondrial *complex I-V* (*CI-CV*) measured by qPCR normalized to *TBP* (n = 8 for each group) and (F) Immunohistochemistry with representative pictures from n = 2. Doubled stained for DDK-tag (ADAMTS9) (red) and mitochondrial COXIV (green). Nuclei were visualized using Hoechst stain (blue). Statistical significance was determined by paired Student's t-test. */** indicates effect of ADAMTS9 overexpression vs. control plasmid ($p<0.05/0.01$).

SUPPLEMENTARY DATA



SUPPLEMENTARY DATA

Supplementary Figure S4. Metabolome analysis. (A+B) Principal component analysis of (A) negative mode and (B) positive MS mode. Red = TA muscles overexpressing ADAMTS9, green = TA muscles with control plasmid, blue = quality control samples. Mouse TA muscles overexpressing ADAMTS9 or control plasmid were isolated and used for (C) western blot analyses (n = 12 for each group; results replicated twice and then pooled) or (D) metabolite analysis of lactic acid (n = 8 for each group). (C+D) Statistical significance was determined by paired Student's t-test. Scatter plots are presented as mean values ±SD. */** indicates effect of ADAMTS9 overexpression vs. control plasmid ($p<0.05/0.01$).



SUPPLEMENTARY DATA

Supplemental Material description

Human studies

Study populations

The meta-analysis was performed in non-diabetic individuals from six study groups using intravenously stimulated estimates of insulin sensitivity. For four studies "ULSAM", "EUGENE2", "German study" and "Danish non-diabetic twins", we included the M- or Rd-value from an euglycaemic-hyperinsulinaemic clamp. For two studies ("Young healthy individuals" and "Danish family study"), we included the insulin sensitivity index obtained by the Bergmans minimal model (1) from a frequently sampled intravenous glucose tolerance test. See below for further details.

The "Uppsala Longitudinal Study of Adult Men" ("ULSAM") cohort (http://www.pubcare.uu.se/ULSAM/) is a health investigation focusing on identifying metabolic risk factors for cardiovascular disease. All 50-year-old men living in Uppsala, Sweden, in 1970-1974 were invited to participate. Of these, 2,322 (82%) participated in the investigation. The cohort was reinvestigated 20 years later (1990-1995). Of the 1,681 available 71-year-old men invited to the follow-up investigation, 1,221 (73%) attended. At the 71-years investigation a hyperinsulinemic-euglycemic clamp was carried out. In the current study we investigated 963 non-diabetic participants from the 71-years investigation where hyperinsulinemic-euglycemic clamp was carried out as reported (2).
Further studies of quantitative metabolic traits were performed in 377 individuals from a population-based sample of "Danish young healthy individuals" recruited at the Research Centre for Prevention and Health (3). Each of the participants underwent an intravenous glucose tolerance test (IVGTT) after a 12 h overnight fast. Baseline values of serum insulin, serum C-peptide, and plasma glucose were taken prior to injection of glucose intravenously over a period of 60 sec (0.3 grams/kg body weight of 50% glucose). At 20 min a bolus of 3 mg tolbutamide/kg body weight (Rastinon, Hoechst, Germany) was injected during 5 s to elicit a secondary pancreatic β-cell response. Venous blood was sampled at 2, 4, 8, 19, 22, 30, 40, 50, 70, 90, and 180 min for measurements of plasma glucose, serum insulin and serum C-peptide (3). The insulin sensitivity index was calculated using the Bergman minimal model MINMOD computer programme (1).

The "Danish non-diabetic twin" cohort comprises 98 monozygotic and dizygotic twin pairs, which were recruited in the years 1997–1999 as previously described in detail (4, 5). The participants were recruited as two age groups, (28.0±2.3 and 61.5±3.1 years), but pooled in the present analyses adjusting for age group. None of the participants were pre-diagnosed with diabetes; however, an oral glucose tolerance test revealed that 24 had impaired glucose tolerance and four had type 2 diabetes according to World Health Organization criteria. The participants were examined by a 2-hour hyperinsulinemic-euglycemic clamp (40 mU/m2/min) using a ^{3}H-tritiated glucose infusion in order to determine rates of peripheral glucose disposal (Rd).

Three other study samples, for which association results of *ADAMTS9* rs4607103 have already been published, were included in the meta-analysis of estimates of insulin sensitivity. First, a European collaboration of offspring of first degree relatives of T2D individuals from five countries was included (6). A hyperinsulinemic-euglycemic clamp was performed in 596 individuals ("EUGENE2") and an IVGTT in the Danish subset of "EUGENE2" ("Danish family study") consisting of 66 families including 253 participants (6). Second, a "German study", in which a subgroup of participants (n=330), were examined by a hyperinsulinemic-euglycermic clamp, was included in the meta-analysis (7). These study samples have been described in detail previously (6, 7).

For mRNA expression analysis, 38 of 66 families from the "Danish family" study involved in the Danish part of the "EUGENE2" collaboration were investigated in a follow-up study 11 years after

baseline. Here 93 participants were examined by anthropometric measurements and a standard 75 g OGTT. Furthermore, a muscle biopsy was obtained in 80 non-diabetic participants at a second day of investigation (8). Muscle tissue was obtained from the vastus lateralis muscle under local anesthesia using a Bergström needle. This study was used to investigate skeletal muscle *ADAMTS9* mRNA expression in relation to ADAMTS9 rs4607103 genotype, and the present analysis evaluated the 80 first-degree offspring without known diabetes of a parent with type 2 diabetes with available *ADAMTS9* rs4607103 genotype and muscle *ADAMTS9* mRNA data. Peripheral insulin sensitivity was estimated by the OGTT-based Matsuda insulin sensitivity index (ISI) calculated as reported (9). The "Danish non-diabetic twin" population was used to investigate the insulin signaling cascade and OXPHOS and GLUT4 gene expression in relation to ADAMTS9 rs4607103 genotype.

In all study samples, diabetes and states of pre-diabetes were diagnosed according to World Health Organization 1999 criteria (10). All study protocols were approved by the local Ethics Committees and conducted in accordance with the principles of the Helsinki Declaration II.

Biochemical measurements

In the "ULSAM" cohort plasma concentrations of specific insulin was analyzed by the Access Immunoassay System (SanofiPasteur Diagnostics). Other measurements in the "ULSAM" sample were analyzed as described (2). In the cohorts of "Danish young healthy individuals" and "Danish family study", plasma glucose concentration was measured by an automated glucose oxidase method (Granu-test: Merck, Damstadt, Germany) and concentration of insulin (excluding des(31, 32)- and intact proinsulin) in serum was measured by ELISA applying the Dako insulin kit with overnight incubation (code NO. K6219; Dako Diagnostics Ltd., Ely, United Kingdom) (3). In the "Danish non-diabetic twin" cohort, plasma glucose concentrations were analyzed by the glucose dehydrogenase oxidation method. Plasma insulin concentrations were measured using a two-site, two-step time-resolved immunofluoremetric assay (Delfia, Turku, Finland) (4).

Genotyping

Genotyping of rs4607103 in the "ULSAM" cohort was performed using the Illumina GoldenGate assay with succes rate of 98.9% and an error rate of 0% in 15 duplicate samples. Genotyping in the "Danish young healty individuals", the "Danish family" study and the "Danish non-diabetic twin" cohort was performed by the KASPar SNP Genotyping system (KBiosciences, Hoddesdon, UK) with a success rate of 96.9% and error rate <1% in 1,090 duplicate samples genotyped in the same batch. Genotype distributions were in Hardy-Weinberg equilibrium in all study subgroups (p>0.05). Genotyping in other study samples included in the meta-analyses have been previously reported (6, 7).

Gene expression analysis

Total RNA was extracted from the muscle biopsies using TRI reagent (Sigma-Aldrich, St. Louis, MO, USA). cDNA was synthesized from RNA by use of QuantiTect® Reverse Transcription kit (Qiagen, Valencia, CA, USA) (8). Quantitative real-time PCR was performed with the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA) using TaqMan gene-specific primers (Applied Biosystems). Each sample was measured in duplicate and the standard curve approach was used for quantification. Human pooled RNA samples originating from several tissues were obtained commercially (ClonTech Laboratories Inc., Mountain View, CA) as a panel to examine the *ADAMTS9* (Hs00172025_m1) expression differences among tissues. The expression levels were normalized to the endogenous control *PPIA* (4326316E). In the Danish family study, TaqMan and the same probes for *ADAMTS9* assay was used (Hs00172025_m1) (Applied Biosystems). Each sample was measured in duplicate and the standard curve approach was used for quantification. Transcript transcript quantity was

normalized to the relative amount of total cDNA content of the sample as measured in triplicate with the Quant-iT OliGreen ssDNA Assay Kit (Invitrogen, Carlsbad, CA, USA) as described previously (11). In the Danish non-diabetic twin cohort, the expression levels of mitochondrial complexes and peroxisome proliferator activating factors were measured in basal and insulin-stimulated biopsies using using the standard curve method and TaqMan assays for *NDUFB6* (Hs00159583_m1), *UQCRB* (Hs00559884_m1), *COX7A1* (Hs00156989_m1), *ATP5O* (Hs00426889_m1), PGC-1β (Hs00370186_m1), and peroxisome proliferator-activated receptor gamma, coactivator 1 alpha (PGC-1α) (Hs00173304_m1) as described previously (12–16). The expression levels were normalized to *PPIA* (4326316E) expression.

Human tissue mRNA samples pooled from donors or suddenly deceased individuals of Caucasian or Asian ancestry were obtained commercially; Human Aorta Poly A+ RNA (#636153); Human Blood, Peripheral Leukocytes Poly A+ RNA (#636170); Human Brain Poly A+ RNA (#636102); Human Colon Poly A+ RNA (#636146); Human Small Intestine Poly A+ RNA (#636125); Human Adipose Tissue Poly A+ RNA (#636162); Human Kidney Poly A+ RNA (#636118); Human Liver Poly A+ RNA (#636101); Human Pancreas Poly A+ RNA (#636119); Human Skeletal Muscle Poly A+ RNA (#636120); Human Placenta Poly A+ RNA (#636103). The expression levels were normalized to the endogenous control *PPIA* (4326316E).
In the "Danish non-diabetic twin" cohort, the basal and insulin-stimulated biopsies activities of IRS-1-associated PI3K (IRS-1-PI3K), Akt1 and Akt2, GSK3 β and glycogen synthase were measured as described previously (17, 18).

Statistical analysis
We performed meta-analysis of all available data in relation to intravenously estimated insulin sensitivity following intravenous glucose stimulation in relation to *ADAMTS9* rs4607103 genotype assuming an additive genetic model. Four studies with hyperinsulinemic-euglycemic clamp data reported the M-value ("ULSAM", "EUGENE2" and "German study") or Rd ("Danish non-diabetic twin cohort") while insulin sensitivity index obtained by Bergmans minimal model from a frequently sampled IVGTT data was included for the two studies ("Danish young healthy individuals" and "Danish family" study). In each cohort, the measure of insulin sensitivity was logarithmically transformed (base 10) prior to analysis and regression analyses were adjusted for age and sex. In "ULSAM", "Danish young healthy individuals" and "German study" analyses were made by linear models while "EUGENE2", the "Danish family" study and the "Danish non-diabetic twin" cohort were analyzed by a linear mixed model including family ("Danish family study"), family and center ("EUGENE2") or twin pair and zygosity ("Danish non-diabetic twin cohort") as random factors. All data were standardized by conversion to Z-scores to enable combination of results from different methods. Z-score standardized effect size estimates and standard errors (SE) were meta-analyzed by RGui version 3.1.1 applying the "meta" package. The fixed effects method (weight of studies estimated using inverse variance) was applied. The rs4607103 variant was included applying an additive model and male sex was base. In the "Danish family" study, analysis of *ADAMTS9* mRNA levels in skeletal muscle was performed in 80 Danes from 38 families by mixed linear model including family as a random factor. In the "Danish non-diabetic twin" cohort insulin signaling protein and OXPHOS gene expression data were evaluated by a mixed adjusting for age, sex and body fat percentage. Zygosity and pair number were included as random factors. Mixed model analysis was performed using Statistical Package for Social Science (SPSS Inc., Chicago, IL, USA) version 14 or in SAS (version 9.4, SAS Institute, Cary, USA, and all other analyses were performed by RGui version 2.10.0 (http://www.r-project.org). A *p*-value below 0.05 was considered significant.

SUPPLEMENTARY DATA

Mouse studies

Mouse models

In vivo gene electrotransfer model: 9-week-old male C57BL/6JBomTac mice were used for *in vivo* gene electrotransfer after allowing a 1-week acclimatization period. The mice were group-housed in an enriched, temperature-controlled environment with a 12 h light:12 h dark cycle and *ad libitum* regular chow (#1310, Altromin) and water. Plasmid DNA was extracted (#12381, Qiagen) and diluted to a final concentration on 2 μg/μL in isotonic saline. Electroporated TA muscles were isolated seven days after the gene electro-transfer following 4 h of fasting and 25 min stimulation with either control (gelofusine (B. Braun, Denmark)) or insulin (0.5 U/kg, actrapid, Novo Nordisk). The mice were euthanized by cervical dislocation and the TA muscles were dissected and snap-frozen in liquid nitrogen.

Adamts9$^{fl/fl}$ *HSA-Cre model*: *Adamts9*$^{fl/fl}$ mice with loxP sites inserted in intron 4 and 8 (19) were crossed with mice carrying a Cre recombinase encoding gene under the muscle-specific human α-skeletal actin (HSA) promoter (20). The mice were group-housed until 12 weeks of age and were then single housed until termination. The mice were housed on a 12 h light; 12 h dark cycle in an enriched, temperature-controlled environment and had free access to water and chow diet (until 15 weeks of age) or a high fat-high sucrose diet (#D12451, Open Source Diets) (after 15 weeks of age). At 8 weeks of age while still on chow diet, an oral glucose tolerance test (OGTT), including plasma insulin measurements, was carried out. Before HFHS and every second week during HFHS feeding, fat and lean mass of the mice were determined by magnetic resonance imaging (MRI) scanner (EchoMRI^{TM}, Echo Medical Systems, Houston, Texas). Fasting blood glucose and plasma insulin were measured following 4 h of fast. Blood glucose was then measured using a glucometer (Bayer-Contour; Bayer) and approx. 70 μl blood was drawn for plasma insulin measurements (#K152BZC; Meso Scale Discovery).

Successful deletion of exons 5–8 in *Adamts9*$^{fl/fl}$ HSA KO mice was detected by RT-PCR of cDNA from skeletal muscles. Primers used were located around the floxed region and therefore detected a band on 704 bp if the region was intact and a band on 357 bp if the region was deleted. Forward primer in exon 4: 5'AGAATGCGAAAACGGAGAAA3'. Reverse primer in exon 9: 5' ATCCAGGGGTTGGTGTAGAA3'.

Adamts9$^{lacZ/+}$ mice: *Adamts9* targeted mice with an intragenic LacZ gene were described previously (21). Muscles were obtained between 4-6 months of age.

Ex vivo studies

Western blot analysis

The TA muscles were then homogenized in lysis buffer (pH 7.4, 10% glycerol, 1% IGEPAL, 150 mM NaCl, 50 mM Hepes, 20 mM β-glycerophosphate, 10 mM NaF, 1 mM EDTA, 1 mM EGTA, 1 mM sodium butyrate, 2 mM sodium orthovanadate and protease inhibitors (#S8820, Sigma)). The homogenate was centrifuged at 16,000 g for 20 min and the supernatant was used for western blot analyses. Protein concentrations were determined with a BCA kit (#23225, Pierce) and equal amount of proteins were diluted in NuPage LDS sample buffer (#NP0007, Life Technologies) containing 50mM DTT and heated to 92°C for 5min. 20 μg protein pr. lane were separated on 3-7% Tris acetate or 10% Bis-tris gels (#WG160 or #WG120, Life Technologies) and electroblotted to PDVF membranes (#88518, Thermo Scientific). The membranes were blocked for 30 min (#37543, Thermo Scientific)

before incubating membrane with primary antibody (see table below) over night at 4°C. The membranes were washed in PBS + 0.1% tween-20 and incubated 1 h at RT with a horseradish peroxidase-conjugated (HRP) secondary antibody (#NA934V, GE healthcare). After 3 washes the membranes were incubated 5min with chemiluminescent HRP substrate luminol (#WBKLS0500, Millipore) and visualized using the FlourChem E Imaging System (ProteinSimple, San Jose, CA). The bands were quantified using Alphaview software (ProteinSimple, San Jose, CA). Membranes were stripped using Restore Western Blot Stripping Buffer (#21059, Thermo Scientific).

Antibody information			
Primary antibody	**Dilution**	**Catalog number**	**Manufacturer**
Akt T308	1:1000	9275	Cell Signaling
Akt S473	1:1000	9271	Cell Signaling
Akt	1:1000	9272	Cell Signaling
AMPKα T172	1:1000	2531	Cell Signaling
AMPKα (α1+α2)	1:1000	2532	Cell Signaling
β1 integrin*	1:1000	MA2910	Thermo Scientific
Caveolin	1:10.000	610060	BD Trans
Complex I (NDUFB8)	1:2000	459210	Invitrogen
Complex II (SDHA)	1:4000	459200	Invitrogen
Complex III (UQCRC1)	1:1000	459140	Invitrogen
Complex IV (COX4)	1:10000	459600	Invitrogen
ATP Synthase (ATP5O)	1:1000	sc-74786	Santa Cruz
DDK-tag	1:2000	TA50011	Origene
FAK Y397	1:1000	3283	Cell Signaling
GSK-3β S9	1:1000	9336	Cell Signaling
GAPDH	1:1000	3683	Cell Signaling
ILK	1:1000	3862	Cell Signaling
Insulin receptor β	1:1000	sc-711	Santa Cruz
IGF-I Receptor β (Tyr1135/1136)/IRβ (Tyr1150/1151)	1:1000	3024	Cell Signaling
IRS1 Y612	1:1000	44-816G	Invitrogen
Myc-tag	1:200	sc40	Santa Cruz
PINCH	1:5000	ab76112	Abcam
PGC1α	1:1000	ab54481	Abcam
PDH S293	1:1000	ABS204	Millipore
PDH	1:1000	3205	Cell signaling
TBC1D4 T642	1:4000	8881	Cell Signaling
Versican (DPEAAE)	1:2000	PA1-1748A	Thermo Scientific

Primary antibodies used for western blot analysis. *Analyzed under non-reducing conditions.

SUPPLEMENTARY DATA

Quantitative PCR (qPCR) for mouse samples

RNA was isolated from TA muscles using RNeasy mini kit (#74804, Qiagen) with Dnase digestion according to instructions from manufacturer. cDNA was produced using SuperScript III (#18080044, Invitrogen) and DNA was purified from TA muscles using DNA miniprep kit (#C1N70, Sigma) according to instructions from manufacturer. qPCR analysis was performed on LightCycler480 (Roche Applied Science, Penzberg, Germany) using PrecisionPLUS Mastermix (#PrecisionPLUS-SY, Primerdesign) and specific primers (see table below). All runs consisted of a preincubation step at 95°C for 2 min followed by 45 cycles of: 95°C, 15 sec; 60°C, 45 sec. The expression levels were normalized to the average expression of the housekeeping genes *TATA-box binding protein* (*TBP*) for RNA samples or average of nuclear DNA (nDNA) for DNA samples, using the ΔΔCt method (22). Control plasmid was set to 1 and all data was normalized to the corresponding control plasmid.

qPCR primer sequences		
Target gene (mouse)	**Forward primer**	**Reverse primer**
PGC-1α	5'CCC TGC CAT TGT TAA GAC C 3'	5'TGC TGC TGT TCC TGT TTT C 3'
PGC-1β	5'CGC TCC AGG AGA CTG AAT CCA G 3'	5'CTT GAC TAC TGT CTG TGA GGC 3'
TFAM*	5'CCG AAG TGT TTT TCC AGC AT 3'	5'GGC TGC AAT TTT CCT AAC CA 3'
Mfn2*	5'CCA GCT TCC TTG AAG ACA CC 3'	5'CTG ATA CCC CTG ACC TTG GA 3'
Dnm1l*	5'CGG TTC CCT AAA CTT CAC GA 3'	5'ACG CTA GCT CAA TTG CCA CT 3'
Acad-m*	5'GCC CAG AGA GCT CTA GAC GA 3'	5'GCG AGC AGA AAT GAA ACT CC 3'
Acad-l*	5'ACT TGG GAA GAG CAA GCG TA 3'	5'TCG CAA TAT AGG GCA TGA CA 3'
Acad-vl*	5'TTC GAC TGT TTG TGG CTC TG 3'	5'GAT TCC TGT CCT CCG TCT CA 3'
Desmin	5'GAG CGC AGA ATC GAA TCC CT 3'	5'CCT GCT GTT CCT GAA GCT GG 3'
MYH2	5'CGG AGC CCA AGG AAT CCT TT 3'	5'TTC TTT CAC GGT CAG GGT CG 3'
nDNA (RIP140)	5'TCC CCG ACA CGA AAA AGA AAG 3'	5'ACA TCC ATT CAA AAG CCC AGG 3'
nDNA (UCP1)	5'GAG GCA GTC AAG AGC AGC TT 3'	5'GCC CAA TAC ACA AGC CCT AA 3'
nDNA (PPARγ)	5'TTT GGA ATT CTC ACA AAA CTT CA 3'	5'TTT TCT ACT GCT GGG GAT GG 3'
mtDNA (CO2)	5'ATT TAG TCG GCC TGG GAT G 3'	5'ACC GAG TCG TTC TGC CAA TA 3'
mtDNA (CytB)	5'ATT CCT TCA TGT CGG AC GAG 3'	5'CTG TGG CTA TGA CTG CGA AC 3'
Complex I (Nd1)	5'GGA TCC GAG CAT CTT ATC CA 3'	5'GGT GGT ACT CCC GCT GTA AA 3'
Complex II (Sdha)	5'AAC ACT GGA GGA AGC ACA CC 3'	5'GCA CAG TCA GCC TCA TTC AA 3'
Complex III (Cyc1)	5'GAG CTT TAC CCC CTG ACC TC 3'	5'GTA GCC AGT GAG CAG GGA AA 3'
Complex IV (Cox7a1)	5'AAA ACC GTG TGG CAG AGA AG 3'	5'CAG CGT CAT GGT CAG TCT GT 3'
ATP Synthase (Atp5g1)	5'TTG GCA CAG TGT TTG GTA GC 3'	5'CAA ACC CCA GAA TGG CAT AG 3'
Insulin receptor	5'TCC TGA AGG AGC TGG AGG AGT 3'	5'CTT TCG GGA TGG CCT GG 3'
MCT1	5'AAT GAT CGC TGG TGG TTG TC 3'	5'AAG TTG AAA GCA AGC CCA AG 3'
MCT4	5'CTT TGC GTC CCT GGG AAT 3'	5'TGG AAG TTG AGA GCC AGA CC 3'
qPCR primer sequences		
Target gene (mouse)	**Forward primer**	**Reverse primer**
TBP	5'TCA AAC CCA GAA TTG TTC TCC 3'	5'GGT AGA TGT TTT CAA ATG CTT CA 3'

Primer sequences used for qPCR. *Unpublished primer sequences from Elahu Gosney Sustarsic and Zach Gerhart-Hines.

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Mitochondrial respiration

Mitochondrial respiration was assessed *in situ* in saponin-permeabilized muscle fibers using an Oxygraph-2k (Oroboros, Innsbruck, Austria). Respiration measurements in the Oxygraph-2K champers were performed at 37 °C (physiological temperature) after hyperoxygenation to avoid oxygen limitation. All measurements were made in four replicates performed simultaneously. The applied protocol for muscle fibers preparation and measuring mitochondrial is a slightly modified version of a detailed description given elsewhere (23). Complex I respiration was measured by addition of the NADH-generating substrates glutamate (10 mM), malate (5 mM), and pyruvate (15 mM) followed by addition of ADP at a non-saturating concentration (0.25 mM) and a saturating concentration (5 mM) respectively. Intactness of the outer mitochondrial membrane was established by observing no stimulation of respiration following cytochrome c (5 mM) addition. Complex II-linked respiration was evaluated by addition of the $FADH_2$-generating substrate succinate (20 mM). Since Complex I was not inhibited at this step, simultaneous respiration at Complex I and II was assessed with succinate. Next, Complex III was inhibited using 1 mM antimycin A to enable subsequent evaluation of Complex IV-linked respiration alone. Complex IV respiration was measured by addition of the artificial substrate for Complex IV, N,N,N',N'-tetramethyl-p-phenylenediamine (TMPD, 0.5 mM) as well as ascorbate (2 mM) to keep TMPD in a reduced state. Each step in the substrate-inhibitor titration protocol was performed after stable respiration rate was observed. All chemicals were obtained from Sigma-Aldrich (St Louis, MO, USA) or Fluka Chemie (Buchs, Switzerland). Oxygen fluxes were expressed as ρmol per second per mg wet weight tissue (ρmol O2/(s·mg)) and were acquired using the DatLab software (version 5.1.1.91, Oroboros Instruments, Innsbruck, Austria). Before determining the oxygen flux upon addition of TMPD+ascorbate, a correction for auto-oxidation of TMPD and ascorbate was applied. 3 sample measurements were excluded from the statistical analysis, due to a >15% increase in the oxygen consumption after addition of cytochrome c.

Immunohistochemistry

Muscle fibers were prepared and stained for mitochondrial networks as previously described (24). To enable visualization of mitochondrial networks in muscle fibers overexpressing ADAMTS9, muscle fibers were double-stained for mitochondria (complex IV) and ADAMTS9. ADAMTS9 expressed from the pCMV6-Entry vector or pcDNA3.1/Myc-His A vector was stained using a mouse IgG antibody against the DDK-tag (1:700, #TA50011, Origene) or a sheep IgG against the Myc-tag (1:250, #PA3-981, Thermo Scientific), respectively. Secondary antibodies used were anti-rabbit conjugated to Alexa Fluor 488 (1:500, #A21206, Invitrogen) for COXIV visualization and donkey anti-mouse conjugated to Alexa Fluor 568 (1:500, #A10037, Invitrogen) or donkey anti-sheep conjugated to Alexa Fluor 568 (1:500, #A21099, Invitrogen) for ADAMTS9 visualization. Confocal images were acquired with a Zeiss LSM700 confocal microscope (Carl Zeiss Microscopy, München, Germany) through a 63x/1.40 Oil DIC Plan-Apochromat objective. Alexa Fluor 488 was excited with a 488 nm laser, Alexa Fluor 568 with a 555 nm laser, and Hoechst 33342 (nuclear stain) with a 405 nm laser. Detector gain and laser power were set to obtain the minimum of empty or saturated pixels and to minimize the degree of background staining, and all images were acquired with the same settings. Imaging was performed in 3-5 muscle fibers per TA muscle, selectively chosen according to their overexpression of ADAMTS9.

Whole mount LacZ staining of *Adamts9*$^{lacZ/+}$ quadriceps was followed by paraffin embedding. The 5 µm sections were rehydrated and digested with 5 µg/ml proteinase K (Invitrogen, Carlsbad, CA) for 3 min at room temperature, then incubated with 5% normal goat serum for 30 min and with anti-laminin rabbit polyclonal antibody (dilution: 1/400; catalog number L9393, Sigma-Aldrich, St-Louis, MO) followed by incubation with a goat anti-rabbit secondary antibody coupled to alkaline phosphatase and staining was obtained using the SIGMAFAST Fast Red TR/Naphtol AS-MX Tablets according to the manufacturer

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instructions.

Lipid quantification - DAG analysis by thin-layer chromatography

The DAG isomers sn-1,2 and sn-1,3 content was measured on 1 mg freeze-dried and dissected tibialis anterior muscle by Thin-layer chromatography. The lipids were extracted in Chloroform-Methanol (2:1) using the method of Folch (25) and dissolved in chloroform as previously described (26). The DAG isomers were separated on silica-gel coated glass plates using chloroform–methanol–acetic acid (98:2:0.5) for sn-1,2-DAG and sn-1,3-DAG. Butylated hydroxytoluene (50 mg/l) was added to the mobile phases as described previously (27). The lipids were developed by dipping the silica glass plates in a 10 % copper sulfate pentahydrate and 8% phosphoric acid solution and heated to 120°C for 15 min. The lipids were visualized on a Typhoon FLA 7000 IP fluorescent scanner and analyzed according to weight using ImageQuant TL (GE Healthcare Life Sciences, Little Chalfont, United Kingdom).

Metabolomics analysis

Chemicals and reagents: All chemicals and reagents used were of liquid chromatography-mass spectrometry (LC-MS) grade. D5-tryptophan, methanol, water, acetonitrile, 2-propanol, formic acid, ammonium hydroxide were purchased from Sigma Aldrich (Denmark) and hexakis(2,2-difluoroethoxy)phosphazene from Apollo Scientific (UK). *Metabolites extraction:* Whole TA muscle sample was pulverized under liquid nitrogen and 24±2 mg underwent metabolite extraction. Samples were randomized for processing and blanks (empty microcentrifuge tubes) were included in the preparation. 20 µl of 0.2 mg/ml D5-tryptophan was added as an internal standard to each sample and blanks. 500 µl of cold 50% methanol solution was added and muscles underwent homogenization in the autolyser (TissueLyser II, Qiagen, USA) for 1 min at frequency 25/sec. Lysed tissues were shortly vortexed and shaken for 20 min at 1°C and 1400 rpm (Thermomixer Comfort, Eppendorf, Germany). Following the centrifugation at 0°C, 4000 rpm for 10min (Centrifuge 5424R, Eppendorf, Germany) supernatant was collected into pre-chilled microcentrifuge tube. 500 µl 80% cold methanol solution was added to the remaining pellet, shortly vortexed and shaken for 20 min using the same settings as previously described. After the second centrifugation, the supernatant was collected and combined with the previous extract. The same procedure was repeated using 500 µl 100% methanol and the remaining pellet was disregarded. Combined extracts were dried using speed vacuum (EZ-2 Personal Evaporator, GeneVac, USA) without heating (~3 h). Extracts were re-suspended in 100 µl of 5% acetonitrile with 0.1% formic acid cold solution and shaken at 1°C for 20 min at 1400 rpm. After centrifugation at 12,000 g at 0°C for 10 min, supernatant was collected in new pre-chilled microcentrtifuge tubes and after short vortexing 5 µl of each sample was collected to one pre-chilled microcentrifuge tube, creating a Quality Control sample (QC). Finally, remaining extracts were stored at -80°C until LC-MS analysis. *LC-MS metabolic profiling:* Muscle extracts, QC sample and blanks were defrosted on ice, vortexed and 10 µl of the sample was dissolved in in 90 µl of cold 5% acetonitrile with 0.1% formic acid solution in a pre-chilled LC-MS vial (Verex Vial, µVial i3 Qsert, Phenomenex) with a screw-cap (Verex Cert+ MSQ Cap, Phenomenex). Leftover samples were stored at -80°C. Metabolic profiling was conducted using LC-MS system: UHPLC Dionex Ultimate 3000 (Thermo Scientific, Germany) coupled to a QToF Impact II mass spectrometer (Bruker Daltonics, Germany) operating in electrospray ionization. Samples were analyzed in positive and negative mode. Chromatographic separation was performed using Luna Polar C18 column (1.6 µm, 2.1x100 mm, Phenomenex, USA) with EVO C18 guard column (sub-2µm, 2.1mm, Phenomenex, USA) kept at 40°C. Solvent A and B were 0.1% formic acid in acetonitrile and 0.1% formic acid with 5mM ammonium hydroxide in LC-MS grade water, respectively. A flow rate of 0.3 ml/min was applied with a gradient elution profile: 95% B 0-1 min, 95%-5% B 1.0-10.0 min, 5% B 10.0-12.0 min, 5-95% B 12.0-12.5 min, 95% B 12.5-14.5 min (equilibration step). 5 µl of the extracts was injected in positive mode and 10 µl in the negative. Line and profile MS spectra were acquired in the mass range 50-1000 mass to charge ratio (m/z) at 2.00 Hz spectra rate using the source settings for

positive mode: absolute threshold 50 cts per 1000 sum, End Plate Offset 500V, Capillary 4500 V, Nebulizer 2.0 Bar, Dry Gas 10.0 l/min, Dry Temperature 220°C. In negative mode Capillary voltage was set to 3000. The other parameters, as follow, were identical for both modes, with Transfer: Funnel 1RF 150.0 Vpp, Funnel 2FR 200.0 Vpp, isCID Energy 0.0 eV, Hexapole RF 50.0 Vpp; Quadrupole: Ion Energy 4.0 eV, Low Mass 100.0 m/z; Collision Cell: Collision Energy 7.0 eV, Transfer Time 65.0 µs, Collision RF 650.0 Vpp, Pre Pulse Storage 5.0 µs. MS spectra were divided into 3 segments: pre-analysis 0-0.1min, calibration 0.1-0.5 min, analysis 0.5-14.5 min. External and internal calibration was based on sodium formate clusters in 2-propanol with Zoom of 1.0% and HPC mode. Additionally, lock-mass calibration based on hexakis(2,2-difluoroethoxy) phosphazene in 2-propanol (0.1 mg/ml) throughout the whole scan was applied. Targeted MS/MS analysis was performed at the same LC-MS settings as the MS scans with additional collision energy set to 20 and scan width 1.0 m/z for both negative and positive mode. Samples were maintained at 4°C throughout the analysis. QC samples and blanks were injected after each 5th sample. *LC-MS data analysis:* Raw data from the positive and negative mode were automatically calibrated according to the sodium-clusters and lock-mass shifts throughout the analysis, using the Compass Data Analysis 4.3 (Bruker Daltonics, Germany). Files were converted to NetCDF format through the Bruker software and metabolic features were extracted using R-based(28), XCMS (29), following CAMERA (30) analysis in search for isotops and adducts. Data were normalized according to the internal standard abundance and samples weight. Statistical analysis was performed using online analytical tools within MetaboAnalyst 3.0 (31). CAMERA-generated buckets were log-transformed and Pareto-scaled. Non-informative variables were removed based on their standard deviation. Student's T-test with False Discovery Rate (FDR) analysis was used to select significantly different metabolic features between ADAMTS9 overexpressing muscles and control. Targeted MS/MS analysis was performed on the significantly different metabolic features (FDR<0.1, P<0.05) and matched with metabolites in Human Metabolome Database (32), Metlin Database (33) and/or Lipid Maps database (34) according to the mass to charge ratio, MS/MS profile and evaluated according to their structure in relation to the retention time in the current study. However, with the LC-MS technique used we were not able to distinguish between carbon-6-sugars and positioning of the phosphorylation.

In vivo studies

OGTT

Mice were fasted overnight and experiments were performed the next morning. Blood glucose was measured from a tail vein puncture before and 0, 15, 30, 60 and 120 min after glucose administration (1.5 g/kg; Sigma-Aldrich) using a glucometer (Bayer-Contour; Bayer). Approximately 70 µl blood was also drawn from the retroorbial vein before and 15 min after glucose administration. The blood was centrifuged at 8000 rpm in 10 min at 4°C and plasma was isolated for insulin measurements. Plasma insulin was determined in duplicates using the mouse/rat insulin kit (#K152BZC; Meso Scale Discovery).

Hyperinsulinemic euglycemic clamp

Basal and insulin-stimulated glucose metabolism: Postabsorptive (i.e., overnight fasted 22:00 to 09:00), body weight–matched male mice were anesthetized with 6.25 mg/kg acepromazine (Plegicil, Dechra, Denmark), 6.25 mg/kg midazolam (Roche, Denmark), and 0.31 mg/kg fentanyl (Janssen-Cilag, Denmark). Tissue-specific glucose uptake was determined in hyperinsulinemic-euglycemic state. In the basal period the *Adamts9$^{fl/fl}$* or *Adamts9$^{fl/fl}$* HSA-Cre mice were infused with [^{3}H]glucose (0.3 µCi/kg/min; Amersham) for 60 min to attain steady state levels of [^{3}H]glucose in plasma, and to determine endogenous glucose production. In the hyperinsulinemic euglycemic clamp period, insulin (Humulin; Eli Lilly) was administered intravenously by a primed dose (4.1 mU), and by continuous (5

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mU/kg/min (35 g)) infusion to attain steady-state insulin levels, together with [^{3}H]glucose (0.3 μCi/kg/min) for 90 min. This infusion rate of insulin was chosen based on previous dose-response studies of hyperinsulinemia, aimed at a 5-fold increase in insulin levels that both inhibited endogenous glucose production and stimulated glucose uptake (35). A variable intravenous infusion of a 12.5% glucose solution was used to maintain euglycemia as determined at 5-min intervals via tail bleeding (<3 μL, Contour NEXT, Bayer Group, Germany). For assessment of basal and insulin-mediated glucose uptake in individual tissues, 2-deoxy-d-[^{14}C]glucose (2-[^{14}C]DG) (Amersham) was administered as a bolus (1 μCi) 30 min before the end of the experiment. In the last 20 min of the experiment, blood samples were taken at intervals of 10 min. Subsequently, the mice were terminated, and organs were quickly harvested and snap-frozen in liquid nitrogen. *Plasma analysis:* Blood samples were taken from the tail tip or toes into Pasteur pipettes and transferred to chilled heparin. The tubes were placed on ice and centrifuged at 4°C at 3000 g for 10 min. Plasma insulin levels were measured using an insulin ELISA kit (Crystal Chem, Downers Grove, IL). Total plasma [^{14}C]glucose and [^{3}H]glucose were determined in supernatant of 7.5 μl plasma after protein precipitation using 20% trichloroacetic acid and evaporation to eliminate tritiated water. *Tissue analysis*: For determination of tissue 2-[^{14}C]DG uptake, homogenates of gastrocnemius skeletal muscle were boiled, and the supernatants were subjected to an ion-exchange column to separate 2-[^{14}C]DG-6-phosphate (which is trapped within the organ and not further metabolized) from 2-[^{14}C]DG. *Calculations*: Turnover rates of glucose (micromoles per minute per kilogram) were calculated for the basal state and for the hyperinsulinemic-euglycemic state as the rate of tracer infusion (disintegrations per minute per minute) divided by plasma-specific activities of [^{3}H]glucose (disintegrations per minute per micromole). The ratio was corrected for body weight. EGP was calculated as the difference between the tracer-derived rate of glucose appearance and the GIR. Tissue-specific glucose uptake in muscle was calculated from tissue 2-[^{14}C]DG content, corrected for plasma-specific activity, and expressed as micromoles per gram of tissue.

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