Identification and validation of *NAT2* as an insulin sensitivity gene

Supplementary appendix

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GWAS study populations and phenotyping, detailed descriptions

RISC (Relationship between Insulin Sensitivity and Cardiovascular disease)

The RISC study is a prospective study of 1500 healthy men and women of European ancestry, aged between 30-60 years, from 20 centers in 13 European countries. Subjects underwent a hyperinsulinemic-euglycemic clamp (1) and those with diabetes, hypertension or dyslipidemia were excluded, yielding a total of 1046 subjects appropriate for inclusion of which 1004 remained after excluding non-Caucasians and 4SD outliers of insulin sensitivity. Insulin sensitivity was measured by hyperinsulinaemic-euglycemic clamp as previously described (1). Exogenous insulin was administered as a primed-continuous intravenous infusion at a rate of 240 pmol $min^{-1}m^{-2}$ for 120 min, simultaneously with a variable 20% (wt/vol) glucose infusion. This was adjusted every 5 to10 min to maintain plasma glucose concentration within 0.8 mmol/l of the target glucose concentration (4.5–5.5 mmol/l). Insulin sensitivity was assessed as the mean glucose infusion rate over the last 40 min of the clamp, corrected for the lean body mass (M value; micromol/kg bodywt/min). To ensure consistency across study centres, the clamp procedure was standardized.

ULSAM (Uppsala Longitudinal Study of Adult Men)

ULSAM is an ongoing, population based study of all men, born 1920-1924, in Uppsala, Sweden. All 50- year-old men living in Uppsala in 1970-1974 were invited to participate. Of the 2841 eligible men, 2322 (82%) participated (2-5). Major health outcomes are being tracked by linking to Swedish national registries. Participants were examined, had blood drawn for basic metabolic profiles and other measures and underwent diagnostic tests. The EC was performed between 1991-1995. DNA has been extracted and is stored at the SNP technology platform, Uppsala (6,

7). Insulin sensitivity was determined using the hyperinsulinaemic-euglycemic insulin clamp technique, according to DeFronzo *et al.* (1979) (8), but with a higher insulin infusion rate [56 vs.40 mU min^{-1} $(m^2)^{-1}$] to achieve nearly complete suppression of hepatic glucose output (9). Glucose disposal rate, representing insulin sensitivity, was calculated as the amount of glucose taken up during the last 60 minutes of the clamp procedure and is presented in mg/kg of body weight per minute.

EUGENE2 (European network on Functional Genomics of type 2 diabetes)

EUGENE2 is a European Union-funded study involving eight countries focusing on the causes and consequences of T2D. One goal is to identify new candidate genes for T2D and IR. All participants in EUGENE2 were healthy non-diabetic offspring of parents with T2D. For inclusion, one of the parents had to have T2D and the other parent normal OGTT. Eight hundred and forty-six non-diabetic offspring were included and 617 of these subjects underwent EC using well-established protocols (8, 10, 11), and 591 were available for the genetic analysis. Participants underwent 75-g oral glucose tolerance test (OGTT) and intravenous glucose tolerance tests (IVGTT). A bolus of glucose (300 mg/kg in a 50% solution) was given into the antecubital vein within 30 s for the IVGTT. At 60 min after the glucose bolus a hyperinsulinaemic-euglycemic clamp was initiated (insulin infusion: 240 pmol m−2 min−1 for 120 min) to evaluate insulin sensitivity (8). Glucose was clamped at 5.0 mmol/l for the next 120 min by infusion of 20% glucose at various rates according to glucose measurements performed at 5 min intervals. The glucose disposal during the clamp was expressed as the amount of glucose infused per kilogram body weight per minute during the last 60 min of the clamp examination (micromol/kgbodywt/min).

Stanford Insulin Suppression Test (IST) cohort

This cohort includes a subset of all subjects participating in various clinical research studies at Stanford University Medical center that required an insulin suppression test (IST) since 2002 (12-14). Participants in these studies are volunteers from the surrounding Stanford communities and were all free of major chronic medical conditions at the time of the IST, including T2D, cardiovascular disease (CVD), hypertension, liver or kidney disease. Subjects were excluded from participation if they reported being on medications known to influence insulin sensitivity including corticosteroids, metformin, sulfonylureas or thiazolidinediones. For the current study, 270 Caucasian subjects were included. Insulin sensitivity was measured by the modified insulin suppression test (IST)(15). The steady state plasma glucose (SSPG) value from the IST is highly inversely correlated to M-value ($r < -0.9$, $P<0.001$) (16-18).

Replication genotyping cohorts, detailed descriptions

GUARDIAN (Genetics UndeRlying DIAbetes in HispaNics)

Seven cohorts are included in the GWAS phase of the GUARDIAN study: five family-based studies (IRAS Family, BetaGene, MACAD, HTN-IR, NIDDM-Athero) and two non-family based studies (IRAS, TRIPOD) (19). All cohorts are of self-reported Hispanic ancestry (majority of Mexican origin). Persons with self-reported and laboratory confirmed diabetes are not included. Insulin sensitivity was measured by euglycemic clamp in MACAD, HTN-IR, and NIDDM-Athero under an identical protocol. During the hyperinsulinemic-euglycemic clamp (8), a priming dose of human insulin (Novolin, Clayton, NC) was given and followed by infusion for 120 minutes at a constant rate (60 mU m^{-2} min^{-1}) to establish hyperinsulinemia. Blood was

sampled every 5 minutes, and the rate of 20% dextrose co-infused was adjusted to maintain plasma glucose concentrations at 95 to 100 mg/dL. The glucose infusion rate (M value) over the last 30 minutes of steady-state insulin and glucose concentrations reflects glucose uptake by all tissues of the body (primarily insulin-mediated glucose uptake in muscle) and is therefore directly correlated with tissue insulin sensitivity (8). Additional descriptions of the three cohorts from GUARDIAN are below:

The Hypertension-Insulin Resistance Family Study (HTN-IR) was designed as a family study to examine the genetic basis of hypertension and insulin resistance (20). Family members of probands with documented hypertension were recruited in the Los Angeles area. GUARDIAN includes 708 of these individuals from 156 families (of which 624 were available for the genetic study here). Insulin sensitivity was obtained by euglycemic clamp. Other phenotypes include OGTT, carotid intima-media thickness by B-mode ultrasonography, and salt sensitivity.

The Mexican-American Coronary Artery Disease (MACAD) Study was designed as a family study to examine the genetic basis of coronary artery disease and insulin resistance (21). Family members of probands with documented coronary artery disease were recruited from the Los Angeles area. GUARDIAN includes 772 of these individuals from 208 families (of which 737 were available for the current study). Insulin sensitivity was obtained by euglycemic clamp. Other phenotypes include OGTT, carotid intima-media thickness by B-mode ultrasonography, total body fat by DXA scan, and post-heparin lipase activity assessment.

The **NIDDM-Atherosclerosis Study** was designed as a family study to examine the genetic basis of subclinical atherosclerosis and diabetes (22). Family members of probands with T2D were recruited in the Los Angeles area. GUARDIAN includes 188 of these individuals from 93 families (of which 179 were available for the current study). Insulin sensitivity was obtained by euglycemic clamp. Other phenotypes include OGTT and carotid intima-media thickness by B-mode ultrasonography.

The GUARDIAN cohort was genotyped using the Illumina Human Omni Express platform.

Minnesota cohorts

Three separate studies of Minnesota children and adolescents were part of the effort, described separately below (23-25). Genotyping data was obtained from 930 subjects from Minnesota recruited into three separate studies of school children and adolescents aimed at the study of insulin resistance and related traits such as obesity and hypertension. Many of the subjects in these studies underwent EC testing either at the time of enrollment or at a later time point For the current study, we excluded data on subjects < 18 years of age because these measurements often fluctuate in adolescence. Characteristics of subjects who had EC are summarized in Supplementary Table 1.

The **Insulin Resistance Study** cohort included subjects of European ancestry (n = 570) from a study of Minnesota schoolchildren (n=195), their siblings (n=152) and parents (n=223). These children participated in a longitudinal study of the role of insulin resistance and adiposity in the development of cardiovascular risk and type 2 diabetes, and were randomly selected from a

sampling frame of 12,043 11-14 year old Minneapolis school children (representing 93% of all students in those grades) who underwent blood pressure screening in 1995. The children had repeated exams and ECs as part of the study (up to four total), and the most recent available EC was used in analysis. Some siblings and parents of these children were also examined and had ECs.

The Prevention of High Blood Pressure in Children Study (PHBPC) study is a cohort (n = 265) selected from blood pressure screening of 10,4231st-3rd grade children (99% of all children enrolled in those grades) in the Minneapolis Public Schools in 1977-78. Approximately 30 years later (2007-12), original PHBPC participants along with their eligible children were recontacted and invited to participate in a follow-up examination that included a EC.

The Sodium-Potassium Blood Pressure Trial in Children (NaKS) cohort (n=121) was originally recruited to participate in, a clinical trial designed to evaluate modification of dietary sodium and potassium intake in a healthy, free living population of children and adolescents. Screening was conducted in 19,452 students in grades 5-8 in Minneapolis and St. Paul between January 1986 and May 1987. At the conclusion of four prerandomization visits, 243 children agreed to be randomized to one of four trial groups: low-sodium diet; potassium supplement administration; placebo-treated control group; and a no-treatment control group (to test the effect of acclimatization on blood pressure measurements). A EC was conducted as part of a post-trial follow-up exam in young adulthood when participants were between 23-32 years of age.

<u>Scandinavian cohorts</u>

Genotyping data was also obtained from 329 subjects from Scandinavia had undergone ECs as part of two separate studies related to insulin resistance and diabetes: 1) Botnia, 2) Malmö-sib (26-28). Altogether genotype data was available from 278 individuals from the Botnia study (29) and 51 and individuals from the Malmö-sib study. In this analysis, diabetic individuals were excluded.

The Stanford Asian and Pacific Program for Hypertension and IR (SAPPHIRe) Cohort

SAPPHIRe was part of the NHLBI Family Blood Pressure Program (FBPP). The primary aim of this study was identifying genes contributing to the risk of hypertension and IR. SAPPHIRe has a sibpair study design and includes a total of 1588 East Asian subjects recruited mostly from Taiwan and Hawaii. Exclusion criteria included diabetes, BMI > 35 and severe renal or liver disease. A smaller subset of 491 sibs from 202 families successfully underwent an IST (30, 31).

GWAS genotyping, quality control and imputation of individual studies

RISC

Samples were genotyped on the Affymetrix 6.0 microarray platform. We used standard QC criteria, including genotyping call rate >95%, Hardy–Weinberg equilibrium P-values (HWE-*P)*>0.0001 and MAF > 1%, which left 747,423 single nucleotide polymorphisms (SNPs) for analysis. Using these SNPs we excluded samples that had sex-mismatches, were related with a PI_hat > 0.2 or were non-European individuals based on EIGENSTRAT analysis (32). We then used MACH (33) to first phase the haplotypes and then ran MiniMac (34)to impute the genotypes on the 1000 Genomes Project data (Interim 20101123 phase 1) against all-population reference panel (monomorphic and singleton sites excluded). After imputation, the exclusion

criteria for quality control were: imputation quality r^2 <0.3 and minor allele count (MAC)<5. After imputation and quality filters, a total of 8,207,865 SNPs were used for association analysis.

ULSAM

Genotyping was performed using the Illumina Omni2.5M and Illumina Metabochip at the SNP&SEQ Technology Platform in Uppsala (www.genotyping.se). Sample exclusion criteria included: 1) genotype call rate <95%; 2) heterozygosity >3 SD; 3) gender discordance; 4) duplicated samples; 5) identity-by-descent match; and 6) ethnic outliers. SNP exclusion criteria of genotyped data before imputation included: 1) monomorphic SNPs; 2) Hardy-Weinberg equilibrium (HWE) p-value<$1x10^{-6}$; 3) genotype call rate<0.99 (SNPs with MAF<5%) or <0.95 (SNPs with MAF≥5%); 4) MAF<1%. Missing genotypes were imputed on the 1000 Genomes Project data (Interim 20101123 phase 1) against all-population reference panel in IMPUTE. After imputation, the exclusion criteria for quality control were: imputation quality <0.4 and minor allele count (MAC) <5.

EUGENE2

Samples were genotyped on the Illumina 550K platform in the Helsinki Genome Centre. In total, 561,301 SNPs were called. SNPs were filtered on genotyping call rate >95%, HWE-*P*>0.0001 and MAF > 1%, then imputed on the 1000 Genomes Project data (Interim 20101123 phase 1) against all-population reference panel. After imputation, the exclusion criteria for quality control were: imputation quality r^2 <0.3 and minor allele count (MAC) <5.

Stanford IST

Samples from the Stanford IST cohort were genotyped on the Affymetrix 6.0 microarray platform. In total, 909,508 SNPs were called. SNPs were filtered on genotyping call rate >90%, HWE-*P*>$1x10^{-6}$ and MAF > 1%, then imputed on the 1000 Genomes Project data (Interim 20101123 phase 1) against all-population reference panel. Individuals showing non-European ancestry and sex-mismatches were removed. After imputation, the exclusion criteria for quality control were: imputation quality r^2<0.3 and minor allele count (MAC) <5.

Study-specific statistical analyses

We carried out GWAS separately within each cohort using MACH2QTL or SNPTEST based on an additive genetic model (33, 35, 36). EUGENE2 was a family-based study: there were 610 pairs of related individuals in EUGENE2 (by the cut-off P(IBD=2)+0.5*P(IBD=1)>0.2 in PLINK(37)).We accounted for the relatedness using the genome-wide efficient mixed-model association (GEMMA)(38) software.

In silico lookup and de novo replication genotyping

The SNPs carried forward were: rs9877159 (chr3: near *GMNC,* GWAS P = 5.6×10^{-6}); rs1208 and rs1801280 (chr8: spanning *NAT2,* with GWAS P =9.8×10^{-7} and P = 3.7×10^{-6}); rs117421960 (chr8: near *TMEM64* and *NECAB1*, GWAS P = 3.6×10^{-6}); rs1775921 (near *BAMBI,* GWAS P value 4.3×10^{-6}). If the GWAS SNP was not available we chose to look at the best proxy. For instance, rs1208 was not directly genotyped so rs7832071 was used as a proxy (r^2 = 0.97).

NAT2 predicted acetylator phenotype analysis

The analysis was tested in RISC, Stanford and EUGENE2 (the subgroup of unrelated individuals)(total N=1753). The 6-SNPs used to predict acetylation phenotype are: rs1208 (803A>G), rs1041983 (282C>T), rs1799929 (481C>T), rs1801280 (341T>C), rs1799930 (590G>A) and rs1799931 (857G>A).

3T3-L1 cell culture

3T3-L1 cells were cultured in growth medium, consisting of Dulbecco's Modified Eagle's Medium (DMEM) (Invitrogen; Carlsbad, CA) supplemented with 10% FBS, 100 U/ml penicillin and 100 ug/ml streptomycin. Differentiation of 2-day postconfluent 3T3-L1 cells was initiated with 0.5 mM 3-isobutyl-1-methylxanthine, 1μM dexamethasone, and 1.25 μM insulin in DMEM supplemented with 10% FBS. After 48 h, the culture medium was replaced with DMEM supplemented with 10% FBS and 1 μg/mL insulin for an additional 48 h, and the cells were then fed every other day with DMEM containing 10% FBS. The degree of differentiation was assayed by Oil-Red O stain. For experiments, overnight-serum-starved, differentiated adipocytes were used.

RNA extraction, reverse transcription and real-time PCR

Total RNA from cells and mouse tissues were isolated using an RNeasy minikit (Qiagen) according to the manufacturer's protocol. Two microgram of total RNA was reverse transcribed using the high capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA, USA). Quantitative real-time PCR was performed on ViiA™ 7 Light Cycler (Applied Biosystems) using the Power SYBR® Green PCR Master Mix (Applied Biosystems). The gene expression values were normalized to cyclophilin A as a housekeeping gene. The data were

analyzed by the public domain program Relative Expression Software Tool – REST. Values are presented as mean ± SEM

Lipolysis in 3T3-L1 cells

Differentiated 3T3-L1 adipocytes were incubated in DMEM with 1.5% fatty acid-free BSA and exposed to insulin and/or isoproterenol (an adrenergic agonist known to stimulate lipolysis) for 1 h. Free fatty acid (FFA) levels present in the medium were determined with a colorimetric kit (non-esterified fatty acid kit; Wako) and normalized to cell density (39).

Glucose uptake in 3T3-L1 cells

Insulin-stimulated glucose uptake was measured as previously described using the fluorescently labeled glucose analog 2-deoxy-2-[(7-nitro-2,1,3-benzoxadizol-4-yl) amino]-D-glucose (2-NBDG) (Cayman Chemical) in fully differentiated 3T3-L1 cells . Confluent 3T3-L1 cells were starved overnight in DMEM with no glucose or fetal calf serum (FCS). Cells were incubated with insulin (1, 10, 100 nM) or vehicle for 15 minutes at 37^{o}C in 100uL DMEM containing 150 ug/ml 2-NBGD but no glucose or FCS. The known glucose transport inhibitors apigenin (1:1000 dilution) and cytocholasin B were used as negative controls (40).

Adipogenesis of 3T3-L1 preadipocytes

3T3-L1 preadipocytes were plated onto 24-well tissue culture plates maintained in regular growth DMEM (10% FBS) until they reached 70% confluence for the first transfection (Day 0). For knockdown experiments, 3T3-L1 adipocytes were transfected with 20nM synthetic predesigned siRNA targeting *Nat1* or non-silencing siRNA (scr siRNA). Six hours post-

transfection, the medium containing transfection reagent was changed to differentiation medium (regular growth DMEM containing 0.5mM IBMX, 1uM dexamethasone, 1.25uM Insulin). Two days later (Day 2) the medium was changed to growth DMEM medium containing 1.25uM insulin for two days. The second transfection of 3T3-L1 cells was accomplished as described above on day 4. Six hours after transfection, the 3T3-L1 cells were kept in regular growth DMEM medium that was changed every two days until last day when they were fully mature adipocytes.

Nat1 targeted mice

The ES cell clone was originally generated by Regeneron Pharmaceuticals, Inc. Methods used to generate the Velocigene targeted alleles have been published previously (41).

Glucose tolerance test (GTT):

For GTT the mice (10 - 12 week old) were injected i.p. after overnight fasting with 2 g glucose/kg body weight. Blood was collected at 0, 15, 30, 60 and 120 min after injection from the tail vein of conscious animals and blood glucose was measured using a glucometer (TRUEbalance, Nipro Diagnostics, Inc.) (42).

Insulin tolerance test (ITT):

ITTs were performed by injecting 1.0 U per kg body weight human insulin i.p. into mice (10 - 12 week old) after 6 h fasting, followed by blood collection at 0, 30, 60 and 120 min after injection. Blood glucose values were determined using a glucose monitor (TRUEbalance, Nipro Diagnostics, Inc.)(42).

Supplementary Table 1: Demographic and clinical characteristics of replication cohorts

Traits	GUARDIAN MACAD (n=743)	GUARDIAN HTN-IR (n=680)	GUARDIAN NIDDM- Athero (n=178)	Insulin Resistance Study (n=570)	PHBPC (n=265)	NaKs (n=121)	Botnia (n=399)	Malmo-sib (n=77)	SAPPHIRe (n=489)†
Female (%)	57%	59%	57%	52%	46%	50%	44%	50.6%	55%
Age (yrs)*	34 (18, 64)	37 (18, 87)	32 (18, 69)	32.0 (18, 64)	38 (18, 42)	27.0 (23, 32)	50.5 (18, 84)	49.2 (18, 73)	48.3 (26, 72)
Fasting Insulin (pmol/L)	96.3 (18, 513)	93.2 (18, 630)	88.8 (25.2, 327)	61.4 (6, 1243)	55.9 (6.9, 590)	76.0 (13.9, 476)	41.7 [7.0-419.5]	52.1 (0.83-152.1)	46.8 (2.4, 233.4)
Fasting Glucose (mmol/L)	5.1 (3.3, 6.9)	5.3 (3.4, 6.9)	4.9 (2.4, 6.8)	4.9 (3.5, 6.9)	4.9 (3.2, 6.9)	5.3 (0.3, 6.8)	6.3 (3.8, 20.2)	6.7 (4.3, 21.2)	5.1 (2.8, 18.1)
BMI (kg/m^2)	28.9 (16.9, 48.6)	28.7 (17.3, 52.8)	28.6 (16.3, 60.1)	26.9 (16.5, 59.8)	28.1 (16.3, 55.2)	28.5 (18.6, 48.5)	26.8 (16.9, 52.6)	25.7 (18.1, 41.9)	25.3 (18.3, 39.2)
Insulin Sensitivity †	33.1 (1.3, 91.3)	31.6 (4.4, 83.2)	31.0 (5.5, 72.4)	43.7 (8.5, 99.4)	45.0 (5.0, 91.6)	40.7 (2.9, 108.9)	35.6 (5.6-76.7)	38.9 (8.9-82.8)	175.8 (40.9, 371.8)
Systolic BP (mmHg)	114.1 (71, 167)	122.6 (85, 201)	117.3 (87, 157)	112.7 (88, 168)	114.0 (83, 160)	115.1 (89, 159)	129.6 (95, 195)	126.6 (105, 180)	128.3 (81, 235)
Total Cholesterol (mmol/L)	4.7 (3.4, 6.9)	4.6 (2.1, 8.3)	4.5 (2.8, 7.6)	4.5 (2.6, 7.6)	4.7 (2.9, 7.5)	4.3 (0.1, 4.6)	5.6 (3.0, 12.5)	5.3 (3.2, 8.1)	4.9 (2.2, 9.2)
LDL (mmol/L)	2.8 (3.0, 5.2)	2.7 (0.8, 5.7)	2.7 (0.7, 5.2)	2.7 (0.8, 5.9)	2.8 (1.1, 4.7)	2.5 (.01, 4.6)	3.6 (1.3, 10.7)	3.4 (1.2, 5.9)	3.2 (0.2, 7.6)
HDL (mmol/L)	1.2 (0.6, 2.7)	1.3 (0.6, 2.7)	1.2 (0.3, 2.2)	1.2 (0.5, 2.5)	1.3 (0.6, 2.6)	1.1 (.02, 2.3)	1.3 (0.8, 2.4)	1.2 (0.5, 7.2)	1.1 (0.4, 2.5)
TG (mmol/L)	1.5 (0.3, 11.0)	1.3 (0.3, 5.5)	1.4 (0.3, 13.3)	1.3 (0.2, 10.4)	1.5 (0.4, 12.5)	1.4 (.01, 6.9)	1.4 (0.4, 6.0)	1.5 (0.5, 7.2)	1.5 (0.3, 9.5)
Smokers (%) (at time of 'clamp')	0.2	NA	NA	22%	24%	27%	NA	NA	18%

* Age is mean (range), † In all studies except Stanford and SAPPHIRe, the insulin sensitivity was measured by hyperinsulinaemic-euglycemic clamp (M-value (micromol/kgbodywt/min)). The M-value has a positive correlation with insulin sensitivity (i.e. an individual with a high M-value has high insulin sensitivity). In the Stanford and SAPPHIRe studies, insulin sensitivity was measured by steady-state plasma glucose (SSPG) method (mg/dl). The SSPG value is highly inversely correlated to M-value (r ~ -0.9)(17, 18). **Conversion factors:** Total Cholesterol, LDL and HDL: 1 mmol/l = 38.6 mg/dl, TGs: 1 mmol/l = 88.5 mg/dl, Glucose: 1mmol/l = 18.0 mg/dl,

Supplementary table 2: Association statistics for rs1208

Age and sex adjusted including all cohorts				
group	Weight	Effect	Std error	P
RISC	1004	-0.12	0.04	0.007
ULSAM	899	-0.10	0.05	0.044
EUGENE2	591	-0.04	0.06	0.546
Stanford	270	-0.10	0.09	0.270
BOTNIA	278	0.01	0.08	0.885
MALMO-SIB	51	0.17	0.19	0.373
MACAD	743	0.00	0.06	0.995
HTN-IR	680	-0.08	0.06	0.170
NIDDM-Athero	178	0.03	0.11	0.764
Insulin Resistance Study	564	-0.04	0.06	0.570
PHPBC	251	-0.14	0.09	0.110
NaKs	115	0.14	0.13	0.310
Meta Analysis	5624	-0.056	0.019	0.002
Age and sex adjusted excluding NaKs				
Meta Analysis	5509	-0.064	0.02	0.001
Age, sex and BMI adjusted including all cohorts				
group	Weight	Effect	Std error	P
RISC	1004	-0.13	0.04	0.003
ULSAM	899	-0.14	0.05	0.004
EUGENE2	591	-0.12	0.06	0.045
Stanford	270	-0.16	0.09	0.095
BOTNIA	278	-0.07	0.08	0.426
MALMO-SIB	51	0.16	0.18	0.397
MACAD	743	-0.01	0.05	0.783
HTN-IR	680	-0.10	0.05	0.036
NIDDM-Athero	178	-0.03	0.09	0.752
Insulin Resistance Study	564	-0.04	0.06	0.570
PHPBC	251	-0.14	0.09	0.110
NaKs	115	0.29	0.13	0.030
Meta Analysis	5624	-0.09	0.02	2.83E-06
Age, sex and BMI adjusted excluding NaKs				
Meta Analysis	5509	-0.094	0.018877551	6.38E-07

Supplementary Table 3: The loci influencing glycemic traits from Scott *et al*. (2012) and their associations to clamp-measured insulin sensitivity in our study

SNP	chr	position (Hg19)	gene	trait-raising allele /other allele	trait *P*	IS effect Direction*	IS *P*	IS (bmi) effect Direction*	IS (bmi) *P*
Fasting insulin									
rs2972143	2	227,116,365	*IRS1*	G/A	3.2×10^{-8}	-	0.46	-	0.05
rs731839	19	33,899,065	*PEPD*	G/A	1.7×10^{-8}	+	0.04	+	0.12
rs7903146	10	114,758,349	*TCF7L2*	C/T	6.1×10^{-11}	-	0.27	-	0.23
rs2820436	1	219,640,680	*LYPLAL1*	C/A	4.4×10^{-9}	-	0.17	-	0.23
rs10195252	2	165,513,091	*GRB14**	T/C	4.9×10^{-10}	+	0.98	-	0.28
rs1421085	16	53,800,954	*FTO*	C/T	1.9×10^{-15}	-	0.01	-	0.37
rs4865796	5	53,272,664	*ARL15*	A/G	2.1×10^{-8}	-	0.91	-	0.40
rs983309	8	9,177,732	*PPP1R3B**	T/G	3.8×10^{-14}	+	0.33	+	0.52
rs2745353	6	127,452,935	*RSPO3*	T/C	5.5×10^{-9}	-	0.24	-	0.50
rs9884482	4	106,081,636	*TET2*	C/T	1.4×10^{-11}	-	0.81	-	0.64
rs1167800	7	75,176,196	*HIP1*	A/G	2.6×10^{-9}	-	0.77	+	0.91
rs1530559	2	135,755,629	*YSK4*	A/G	3.4×10^{-8}	-	0.73	-	0.92
Fasting insulin adjusted for BMI									
rs3822072	4	89,741,269	*FAM13A*	A/G	1.8×10^{-8}	-	0.04	-	0.02
rs2943645	2	227,099,180	*IRS1*	T/C	2.3×10^{-19}	-	0.54	-	0.06
rs6912327	6	34,764,922	*UHRF1BP1*	T/C	2.3×10^{-8}	+	0.07	+	0.09
rs731839	19	33,899,065	*PEPD*	G/A	5.1×10^{-12}	+	0.04	+	0.12
rs459193	5	55,806,751	*ANKRD55-MAP3K1*	G/A	1.12×10^{-10}	-	0.18	-	0.16
rs6822892	4	157,734,675	*PDGFC*	A/G	2.6×10^{-10}	-	0.03	-	0.18
rs17036328	3	12,390,484	*PPARG*	T/C	3.6×10^{-12}	-	0.68	-	0.21
rs2126259	8	9,185,146	*PPP1R3B*	T/C	3.3×10^{-13}	+	0.27	+	0.26
rs10195252	2	165,513,091	*GRB14**	T/C	1.3×10^{-16}				
rs4846565	1	219,722,104	*LYPLAL1*	G/A	1.8×10^{-9}	-	0.47	-	0.35
rs4865796	5	53,272,664	*ARL15*	A/G	2.2×10^{-12}	-	0.91	-	0.40
rs974801	4	106,071,064	*TET2*	G/A	3.3×10^{-11}	-	0.97	-	0.78
Fasting glucose									
rs11715915	3	49,455,330	*AMT*	C/T	4.9×10^{-8}	-	0.01	-	4.24×10^{-3}
rs10747083	12	133,041,618	*P2RX2*	A/G	7.6×10^{-9}	-	0.01	-	0.03

rs9368222	6	20,686,996	*CDKAL1*	A/C	1×10^{-9}	+	0.02	+	0.05
rs2302593	19	46,196,634	*GIPR*	C/G	9.3×10^{-10}	+	0.67	+	0.14
rs6113722	20	22,557,099	*FOXA2*	G/A	2.5×10^{-11}	-	0.72	-	0.24
rs11619319	13	28,487,599	*PDX1*	G/A	1.3×10^{-15}	-	0.54	-	0.28
rs11603334	11	72,432,985	*ARAP1*	G/A	1.1×10^{-11}	+	0.88	-	0.38
rs10811661	9	22,134,094	*CDKN2B*	T/C	5.6×10^{-18}	+	0.57	+	0.39
rs7651090	3	185,513,392	*IGF2BP2*	G/A	1.75×10^{-8}	-	0.40	+	0.44
rs983309	8	9,177,732	*PPP1R3B**	T/G	6.3×10^{-15}	+	0.33	+	0.52
rs6072275	20	39,743,905	*TOP1*	A/G	1.7×10^{-8}	-	0.94	-	0.58
rs6943153	7	50,791,579	*GRB10*	T/C	1.6×10^{-12}	+	0.47	+	0.71
rs3829109	9	139,256,766	*DNLZ*	G/A	1.1×10^{-10}	+	0.56	-	0.89
rs4869272	5	95,539,448	*PCSK1**	T/C	1×10^{-15}	+	0.68	+	0.75
rs16913693	9	111,680,359	*IKBKAP*	T/G	3.5×10^{-11}	-	0.95	+	0.99
rs3783347	14	100,839,261	*WARS*	G/T	1.3×10^{-10}	+	0.83	-	0.99
rs576674	13	33,554,302	*KL*	G/A	2.3×10^{-8}	+	0.51	+	0.94
Fasting glucose adjusted for BMI									
rs17762454	6	7,213,200	*RREB1*	T/C	9.6×10^{-9}	+	0.18	+	0.03
rs2657879	12	56,865,338	*GLS2*	G/A	3.9×10^{-8}	+	0.44	+	0.64
rs7708285	5	76,425,867	*ZBED3*	G/A	1.2×10^{-8}	-	0.73	+	0.67
2hr glucose									
rs6975024	7	44,231,886	*GCK*	C/T	5.2×10^{-11}	+	0.07	+	0.04
rs11782386	8	9,201,787	*PPP1R3B**	C/T	2.2×10^{-9}	-	0.02	-	0.07
rs1019503	5	96,254,817	*ERAP2*	A/G	8.9×10^{-9}	-	0.15	-	0.48
2hr glucose adjusted for BMI									
rs7651090	3	185,513,392	*IGF2BP2*	G/A	4.5×10^{-8}	-	0.40	+	0.44

*The effect directions on clamp-measured insulin sensitivity were reported on the glycemic trait-raising alleles as reported in Scott et al (2012) (43). Scott et al (2012) reported 53 glycemic loci in total, only 48 passed quality control for imputation data in our study.

Supplementary Table 4: Association of previously known glycemic trait loci with insulin sensitivity

SNP	Chr	T2D effect allele	Risk allele frequency	Combined_OR (95% CI)	Combined_*p*-value	Locus	Insulin sensitivity effect	stder	insulin sensitivity p_value	direction
rs10923931	1	T	[0.09-0.14]	1.08 (1.04-1.12)	1.3E-05	*NOTCH2*	0.0225	0.0493	0.649	-+--
rs2075423	1	G	[0.55-0.69]	1.07 (1.05-1.10)	8.1E-09	*PROX1*	0.0226	0.028	0.4199	+---
rs340874	1	C	[0.50-0.59]	1.07 (1.04-1.09)	1.1E-07		0.0095	0.0266	0.72	+---
rs780094	2	C	[0.56-0.66]	1.06 (1.04-1.09)	5.4E-07	*GCKR*	-0.0086	0.0282	0.7588	++--
rs10203174	2	C	[0.88-0.94]	1.14 (1.10-1.19)	9.5E-12	*THADA*	-0.0682	0.0461	0.1394	+-++
rs11899863	2	C	[0.89-0.95]	1.15 (1.10-1.20)	9.5E-11		-0.103	0.0498	0.03858	+-++
rs243088	2	T	[0.45-0.48]	1.07 (1.04-1.09)	1.8E-08	*BCL11A*	-0.0152	0.0267	0.5683	++--
rs243021	2	A	[0.44-0.49]	1.09 (1.05-1.13)	5.3E-06		0.0089	0.0266	0.7365	-+++
rs243019	2	C	[0.44-0.49]	1.07 (1.04-1.09)	2.2E-08		0.0089	0.0266	0.7383	+---
rs7569522	2	A	[0.43-0.47]	1.05 (1.03-1.07)	4.1E-05	*RBMS1*	-0.0059	0.0274	0.8296	+--+
rs7593730	2	C	[0.76-0.82]	1.11 (1.06-1.15)	1.5E-06		-0.0078	0.0331	0.8128	+-+-
rs4410242	2	G	[0.77-0.82]	1.04 (1.01-1.07)	1.4E-02		-0.0085	0.0332	0.7977	+-+-
rs13389219	2	C	[0.52-0.65]	1.07 (1.05-1.10)	1.0E-08	*GRB14*	-0.0218	0.0282	0.4398	-+++
rs3923113	2	A	[0.57-0.69]	1.07 (1.05-1.10)	3.3E-08		-0.0381	0.0287	0.1842	+---
rs2943640	2	C	[0.61-0.66]	1.10 (1.07-1.12)	2.7E-14	*IRS1*	-0.0489	0.0275	0.07472	+++-
rs7578326	2	A	[0.62-0.68]	1.08 (1.06-1.11)	3.8E-10		-0.0659	0.0284	0.02018	---+
rs1801282	3	C	[0.82-0.90]	1.13 (1.09-1.17)	1.1E-12	*PPARG*	-0.0584	0.0408	0.1521	--+-
rs13081389	3	A	[0.90-0.95]	1.12 (1.07-1.18)	8.2E-07		-0.0629	0.0538	0.2421	--++
rs1496653	3	A	[0.66-0.83]	1.09 (1.06-1.12)	3.6E-09	*UBE2E2*	0.0042	0.0329	0.8977	++--
rs7612463	3	C	[0.82-0.89]	1.10 (1.04-1.16)	9.8E-04		0.0576	0.041	0.1597	---+
rs12497268	3	G	[0.75-0.85]	1.03 (1.01-1.07)	2.1E-02	*PSMD6*	-0.0429	0.034	0.2062	+--+
rs831571	3	C	[0.80-0.82]	1.03 (0.99-1.08)	1.8E-01		-0.0459	0.0343	0.1815	++++
rs13059603	3	A	[0.72-0.77]	1.00 (0.97-1.03)	8.7E-01		-0.0544	0.0313	0.08282	--+-
rs6795735	3	C	[0.54-0.65]	1.08 (1.06-1.11)	7.4E-11	*ADAMTS9*	0.0254	0.0271	0.3501	--+-
rs11717195	3	T	[0.74-0.82]	1.11 (1.08-1.14)	6.5E-14	*ADCY5*	-0.0301	0.0327	0.3583	--++
rs11708067	3	A	[0.74-0.83]	1.11 (1.08-1.14)	7.2E-14		-0.0416	0.033	0.2079	--++
rs4402960	3	T	[0.27-0.33]	1.13 (1.10-1.16)	2.4E-23	*IGF2BP2*	0.023	0.0296	0.4375	+-+-
rs1470579	3	C	[0.28-0.33]	1.12 (1.08-1.16)	7.5E-11		0.0165	0.0295	0.575	-+-+
rs6769511	3	C	[0.28-0.33]	1.13 (1.10-1.16)	2.0E-21		0.0167	0.0295	0.5718	-+-+
rs17301514	3	A	[0.09-0.15]	1.05 (1.01-1.09)	1.4E-02	*ST64GAL1*	0.0171	0.0413	0.6795	++--

rs16861329	3	C	[0.84-0.89]	1.03 (0.93-1.10)	4.1E-01		0.0527	0.0413	0.2016	--++
rs6819243	4	T	[0.95-0.99]	1.07 (1.01-1.14)	3.0E-02	*MAEA*	0.1138	0.0758	0.1333	+++-
rs6815464	4	-	-	-	-		0.0967	0.0767	0.2077	-++-
rs4458523	4	G	[0.56-0.63]	1.10 (1.07-1.12)	2.0E-15	*WFS1*	0.0107	0.0282	0.7041	--+-
rs1801214	4	T	[0.54-0.64]	1.10 (1.08-1.13)	3.3E-15		0.0093	0.0284	0.7444	-+-+
rs459193	5	G	[0.68-0.76]	1.08 (1.05-1.11)	6.0E-09	*ANKRD55*	-0.0439	0.0311	0.1579	++-+
rs6878122	5	G	[0.08-0.32]	1.10 (1.07-1.13)	5.0E-11	*ZBED3*	0.0136	0.0324	0.6746	-++-
rs4457053	5	G	[0.10-0.33]	1.09 (1.06-1.12)	1.8E-10		0.0118	0.0328	0.7182	-++-
rs7756992	6	G	[0.23-0.34]	1.17 (1.14-1.20)	7.0E-35	*CDKAL1*	0.0519	0.0294	0.07682	----
rs10440833	6	A	[0.23-0.34]	1.22 (1.17-1.27)	3.6E-22		0.0592	0.0297	0.04621	++++
rs9368222	6	A	[0.23-0.34]	1.17 (1.14-1.20)	7.0E-34		0.0574	0.0297	0.05322	++++
rs4299828	6	A	[0.78-0.83]	1.04 (1.01-1.07)	1.1E-02	*ZFAND3*	0.0449	0.0328	0.1713	++-+
rs9470794	6	T	[0.87-0.94]	1.01 (0.94-1.08)	8.1E-01		0.0347	0.0482	0.471	-+-+
rs3734621	6	C	[0.02-0.04]	1.07 (1.00-1.15)	6.6E-02	*KCNK16*	-0.0414	0.0807	0.608	++-+
rs1535500	6	-	-	-	-		-0.0085	0.0267	0.7507	-+-+
rs17168486	7	T	[0.12-0.22]	1.11 (1.07-1.14)	5.9E-11	*DGKB*	-0.0414	0.0358	0.2477	----
rs6960043	7	C	[0.51-0.57]	1.06 (1.04-1.09)	3.4E-07		-0.0291	0.0272	0.2857	++--
rs2191349	7	T	[0.44-0.50]	1.05 (1.03-1.08)	3.0E-05		-0.032	0.0275	0.245	--++
rs849135	7	G	[0.50-0.53]	1.11 (1.08-1.13)	3.1E-17	*JAZF1*	-0.0072	0.0273	0.7915	-+++
rs849134	7	A	[0.47-0.50]	1.12 (1.08-1.16)	3.2E-10		-0.0045	0.0273	0.8688	+---
rs10278336	7	A	[0.50-0.72]	1.07 (1.04-1.10)	6.4E-06	*GCK*	0.0376	0.0295	0.2018	-++-
rs4607517	7	A	[0.10-0.19]	1.08 (1.04-1.11)	1.0E-05		0.0749	0.0378	0.04783	++++
rs17867832	7	T	[0.89-0.91]	1.09 (1.03-1.15)	1.9E-03	*GCC1*	0.0377	0.0533	0.4789	-++-
rs6467136	7	A	[0.42-0.49]	1.01 (0.97-1.05)	5.5E-01		-0.0282	0.027	0.296	+---
rs13233731	7	G	[0.50-0.56]	1.05 (1.02-1.07)	2.3E-04	*KLF14*	-0.0134	0.0273	0.6245	++-+
rs972283	7	G	[0.50-0.56]	1.04 (1.02-1.07)	6.0E-04		-0.0178	0.0278	0.5219	++-+
rs516946	8	C	[0.72-0.81]	1.09 (1.06-1.12)	2.5E-10	*ANK1*	0.0219	0.0318	0.4914	-++-
rs7845219	8	T	[0.50-0.55]	1.06 (1.03-1.08)	4.6E-06	*TP53INP1*	0.0202	0.0269	0.4523	+-++
rs896854	8	T	[0.46-0.50]	1.05 (1.03-1.08)	2.1E-05		-0.025	0.032	0.4348	--??
rs3802177	8	G	[0.60-0.91]	1.14 (1.11-1.17)	1.3E-21	*SLC30A8*	-0.0433	0.0295	0.1423	++++
rs10758593	9	A	[0.40-0.46]	1.06 (1.04-1.09)	2.6E-07	*GLIS3*	0.0122	0.0273	0.6538	0-+-
rs7041847	9	A	[0.50-0.53]	1.04 (1.02-1.07)	7.2E-04		-0.015	0.027	0.5791	--+-
rs16927668	9	T	[0.19-0.26]	1.04 (1.01-1.07)	2.8E-03	*PTPRD*	0.0623	0.0324	0.05439	++++

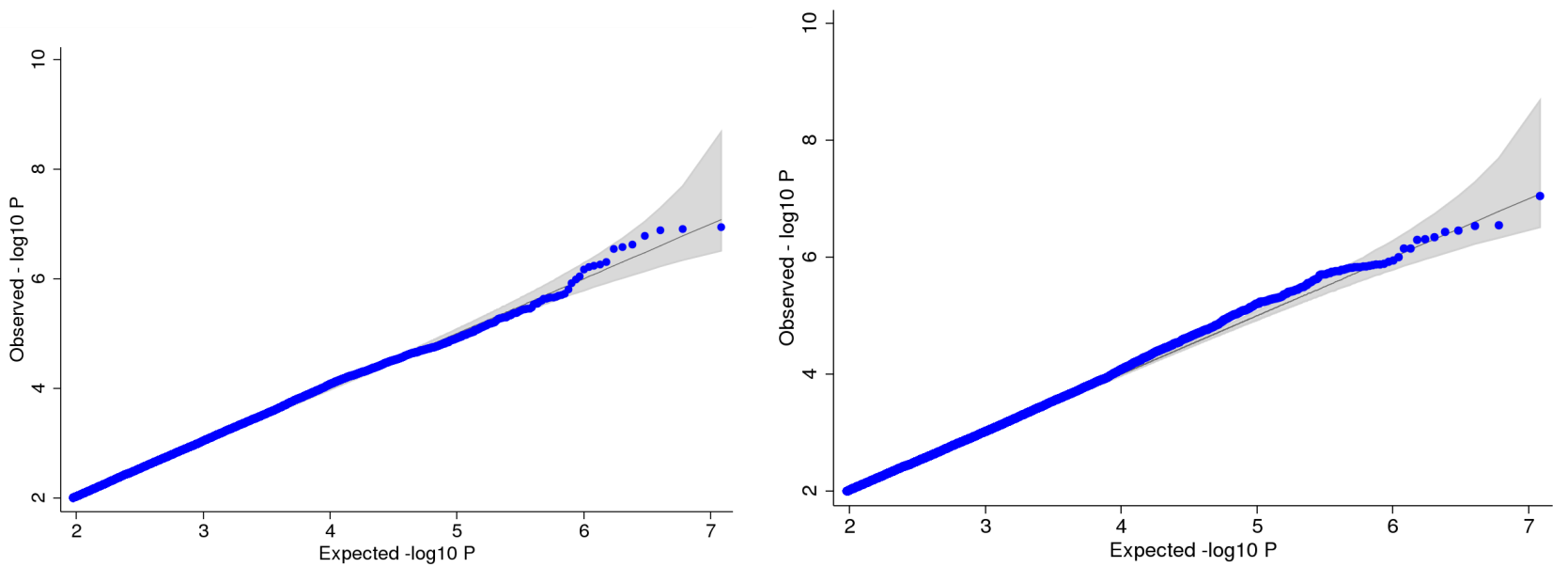
rs17584499	9	T	[0.05-0.24]	1.00 (0.95-1.06)	9.4E-01		0.0195	0.0366	0.5936	-++-
rs10811661	9	T	[0.81-0.84]	1.18 (1.15-1.22)	3.7E-27	*CDKN2A/B*	0.0313	0.0361	0.386	+++-
rs944801	9	C	[0.54-0.62]	1.08 (1.05-1.10)	2.4E-09		-0.042	0.0274	0.1258	----
rs10965250	9	G	[0.82-0.85]	1.19 (1.15-1.23)	1.8E-25		0.0296	0.0359	0.41	-+-+
rs17791513	9	A	[0.87-0.96]	1.12 (1.07-1.17)	2.8E-07	*TLE4*	0.0142	0.0549	0.7962	++--
rs13292136	9	C	[0.86-0.94]	1.19 (1.11-1.27)	8.5E-07		-0.0044	0.0534	0.934	--++
rs2796441	9	G	[0.59-0.91]	1.07 (1.05-1.10)	5.4E-09	*TLE1*	0.0161	0.0276	0.5602	--++
rs11257655	10	T	[0.20-0.25]	1.07 (1.04-1.10)	2.1E-06	*CDC123/CAMK1D*	0.0032	0.0335	0.9229	-+++
rs12779790	10	G	[0.17-0.23]	1.08 (1.03-1.13)	1.2E-03		0.0184	0.035	0.5979	--+-
rs12242953	10	G	[0.91-0.95]	1.07 (1.02-1.12)	3.9E-03	*VPS26A*	-0.0541	0.0567	0.3398	++-+
rs1802295	10	T	[0.27-0.36]	1.00 (0.98-1.03)	8.0E-01		0.0112	0.0294	0.7044	-+-+
rs12571751	10	A	[0.52-0.56]	1.08 (1.05-1.10)	1.0E-10	*ZMIZ1*	0.0105	0.0273	0.7019	+-++
rs1111875	10	C	[0.53-0.63]	1.11 (1.09-1.14)	2.0E-19	*HHEX/IDE*	-0.0117	0.0276	0.6711	+---
rs5015480	10	C	[0.53-0.62]	1.15 (1.11-1.19)	2.2E-16		-0.0106	0.0276	0.701	+---
rs7903146	10	T	[0.18-0.38]	1.39 (1.35-1.42)	1.2E-139	*TCF7L2*	0.0373	0.0309	0.227	-++-
rs2334499	11	T	[0.37-0.45]	1.04 (1.02-1.06)	1.2E-03	*DUSP8*	0.0209	0.0317	0.5091	++?-
rs163184	11	G	[0.37-0.49]	1.09 (1.06-1.11)	1.2E-11	*KCNQ1*	0.0416	0.0286	0.1457	+---
rs231361	11	A	[0.19-0.30]	1.09 (1.06-1.12)	1.2E-09		-0.0501	0.0306	0.1015	--+-
rs231362	11	G	[0.44-0.50]	1.08 (1.05-1.11)	1.7E-09		-0.0497	0.0272	0.06774	++-+
rs5215	11	C	[0.35-0.46]	1.07 (1.05-1.10)	8.5E-10	*KCNJ11*	-0.0406	0.0277	0.1431	+++-
rs1552224	11	A	[0.76-0.87]	1.11 (1.07-1.14)	1.8E-10	*ARAP1 (CENTD2)*	-0.0294	0.0362	0.4172	----
rs10830963	11	G	[0.22-0.34]	1.10 (1.07-1.13)	5.3E-13	*MTNR1B*	-0.0038	0.0318	0.9042	-++-
rs1387153	11	T	[0.21-0.32]	1.09 (1.06-1.12)	1.6E-11		0.0189	0.0325	0.5612	+--+
rs11063069	12	G	[0.19-0.27]	1.08 (1.05-1.11)	3.3E-07	*CCND2*	-0.0183	0.0344	0.5955	+-+-
rs10842994	12	C	[0.75-0.92]	1.10 (1.06-1.13)	6.1E-10	*KLHDC5*	0.0493	0.0351	0.1603	--++
rs2261181	12	T	[0.06-0.13]	1.13 (1.08-1.17)	1.2E-09	*HMGA2*	-0.1194	0.0492	0.01511	----
rs1531343	12	C	[0.07-0.13]	1.15 (1.09-1.22)	4.9E-07		-0.1159	0.0486	0.01711	----
rs2612035	12	G	[0.06-0.12]	1.12 (1.08-1.17)	3.0E-09		-0.1188	0.0487	0.01471	++++
rs7955901	12	C	[0.42-0.49]	1.07 (1.05-1.10)	6.5E-09	*TSPAN8/LGR5*	-0.0248	0.0275	0.3668	+-++
rs4760790	12	A	[0.21-0.30]	1.10 (1.05-1.14)	8.0E-06		0.0254	0.0307	0.4074	-+++
rs4760915	12	T	[0.21-0.30]	1.06 (1.03-1.09)	1.1E-05		0.0265	0.0307	0.3878	-+++
rs12427353	12	G	[0.72-0.83]	1.08 (1.05-1.12)	6.5E-08	*HNF1A*	0.0158	0.0339	0.6412	+---

rs7957197	12	T	[0.73-0.83]	1.08 (1.05-1.11)	3.3E-07	*(TCF1)*	0.0155	0.0337	0.6455	+---
rs1359790	13	G	[0.69-0.77]	1.08 (1.05-1.10)	1.4E-08	*SPRY2*	0.0129	0.0301	0.6688	+--+
rs4502156	15	T	[0.51-0.61]	1.06 (1.03-1.08)	2.3E-06	*C2CD4A*	0.0339	0.0275	0.2177	++++
rs7163757	15	C	[0.50-0.61]	1.06 (1.02-1.10)	1.3E-03		0.0518	0.0273	0.05746	----
rs7177055	15	A	[0.68-0.73]	1.08 (1.05-1.10)	4.6E-09	*HMG20A*	-0.0169	0.0293	0.5633	--+-
rs7178572	15	G	[0.66-0.71]	1.07 (1.05-1.10)	2.2E-08		-0.0191	0.0291	0.5121	+--+
rs11634397	15	G	[0.61-0.68]	1.05 (1.02-1.07)	1.4E-04	*ZFAND6*	0.0585	0.0281	0.03754	---+
rs2007084	15	G	[0.88-0.93]	1.02 (0.98-1.07)	3.6E-01	*AP3S2*	-0.0333	0.0572	0.5601	--++
rs2028299	15	C	[0.24-0.30]	1.04 (1.00-1.09)	4.4E-02		-0.0244	0.0306	0.4256	++-+
rs12899811	15	G	[0.29-0.34]	1.08 (1.05-1.10)	6.3E-09	*PRC1*	0.0018	0.0291	0.9502	+--+
rs8042680	15	A	[0.28-0.35]	1.07 (1.04-1.09)	1.9E-07		0.0126	0.0288	0.6612	-++-
rs9936385	16	C	[0.36-0.41]	1.13 (1.10-1.16)	2.6E-23	*FTO*	-0.0235	0.0272	0.3879	++--
rs11642841	16	A	[0.39-0.44]	1.12 (1.09-1.14)	1.1E-19		-0.0557	0.029	0.0551	---+
rs7202877	16	T	[0.86-0.92]	1.12 (1.07-1.16)	3.5E-08	*BCAR1*	0.0072	0.0457	0.875	+-+-
rs2447090	17	A	[0.57-0.67]	1.04 (1.01-1.06)	3.8E-03	*SRR*	-0.0416	0.0288	0.1483	---+
rs391300	17	T	[0.34-0.40]	1.01 (0.99-1.04)	3.9E-01		-0.0342	0.0298	0.2511	+---
rs11651052	17	A	-	1.10 (1.07-1.14)	2.0E-11	*HNF1B (TCF2)*	0.0155	0.0277	0.5759	++++
rs4430796	17	G	[0.50-0.66]	1.13 (1.07-1.19)	2.4E-06		0.0087	0.0277	0.7541	-+--
rs11651755	17	C	-	1.10 (1.07-1.13)	1.8E-10		0.0103	0.0275	0.7075	----
rs12970134	18	A	[0.20-0.30]	1.08 (1.05-1.11)	1.2E-08	*MC4R*	0.0325	0.0311	0.2959	++++
rs11873305	18	A	[0.03-0.05]	1.18 (1.11-1.26)	3.8E-07		0.0006	0.0685	0.9932	-+--
rs10401969	19	C	[0.03-0.09]	1.13 (1.09-1.18)	7.0E-09	*CILP2*	-0.0808	0.0562	0.1502	++??
rs8182584	19	T	[0.34-0.42]	1.04 (1.01-1.07)	2.2E-03	*PEPD*	0.039	0.0281	0.1646	+++-
rs3786897	19	A	[0.55-0.61]	1.03 (1.00-1.05)	3.7E-02		0.0005	0.027	0.9848	+-++
rs8108269	19	G	[0.21-0.33]	1.07 (1.04-1.10)	4.4E-07	*GIPR*	0.0423	0.0297	0.1544	----
rs4812829	20	A	[0.13-0.19]	1.06 (1.03-1.09)	1.5E-04	*HNF4A*	-0.0617	0.0353	0.08053	----

Supplementary Table 5: Primer sequences

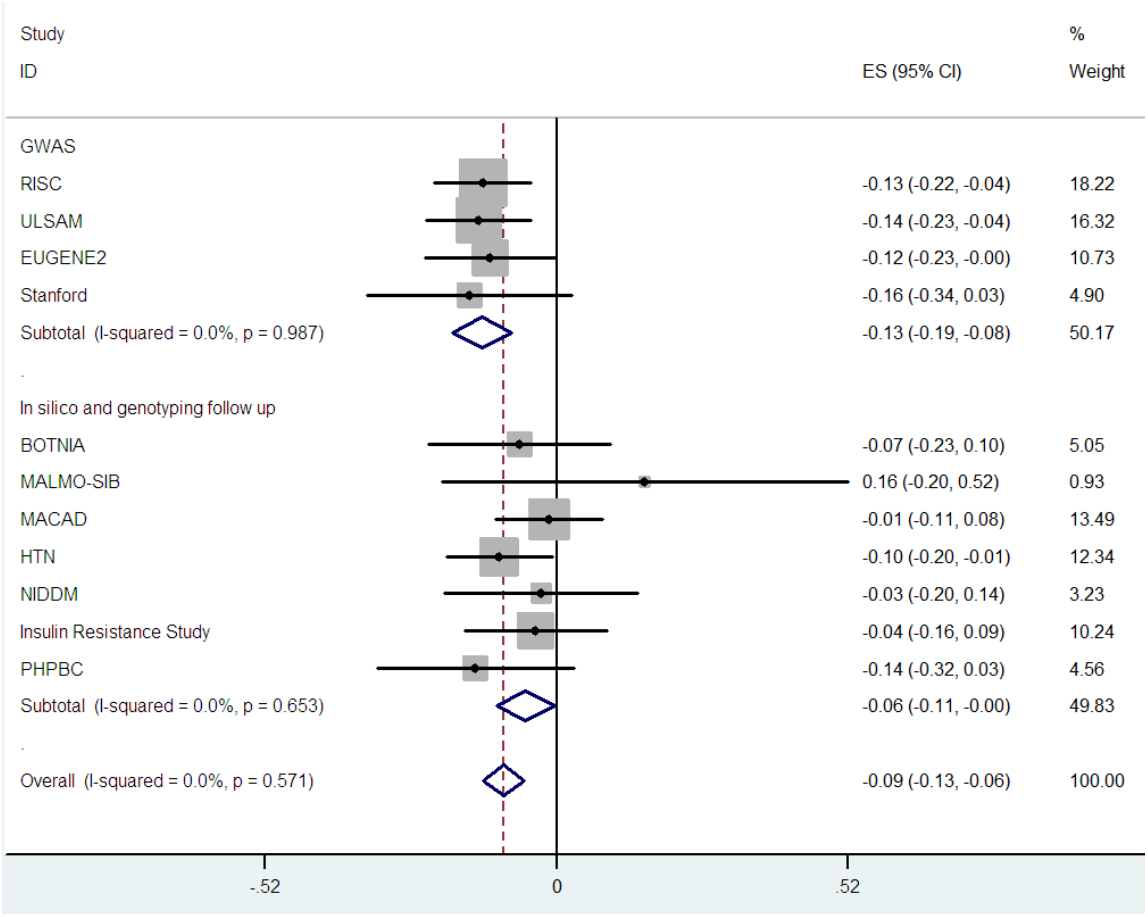
	FWD	REV
mNat1	AGATGCGAGCAGTTCCTTTTG	CCTGTACTAGAAGGTGGACCATT
mNat2	TCTTGAGCCCCGAACTATTGA	GCCAACCAAACAATGAACTCCT
Srebf1	CTCAGCAGCCACCATCTAGCCT	GCTGATGCCTGCAGTCTTCACG
Pparg	CCATTCTGGCCCACCAAC	AATGCGAGTGGTCTTCCATCA
Cyclophilin	TTCCAGGATTCATGTGCCAG	CCATCCAGCCATTCAGTCTT
Cebpa	GCGGGCAAAGCCAAGAA	GCGTTCCCGCCGTACC
Adiponectin	GATGGCACTCCTGGAGAGAA	TCTCCAGGCTCTCCTTTCCT
Leptin	GAGACCCCTGTGTCGGTTC	CTGCGTGTGTGAAATGTCATTG

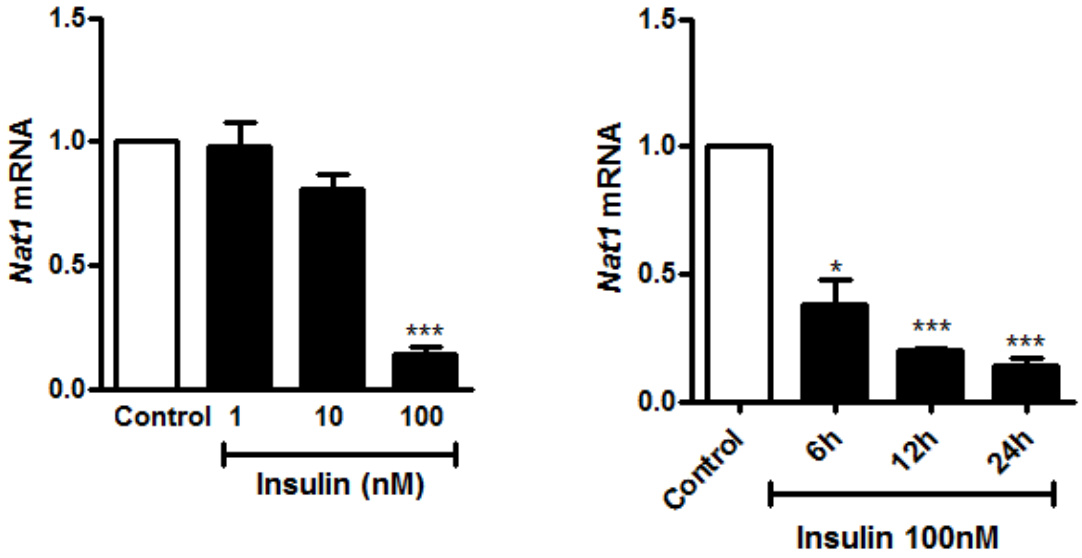
Supplementary Figure 1: The quantile-quantile (QQ) plots of insulin sensitivity in the meta-analysed GWAS



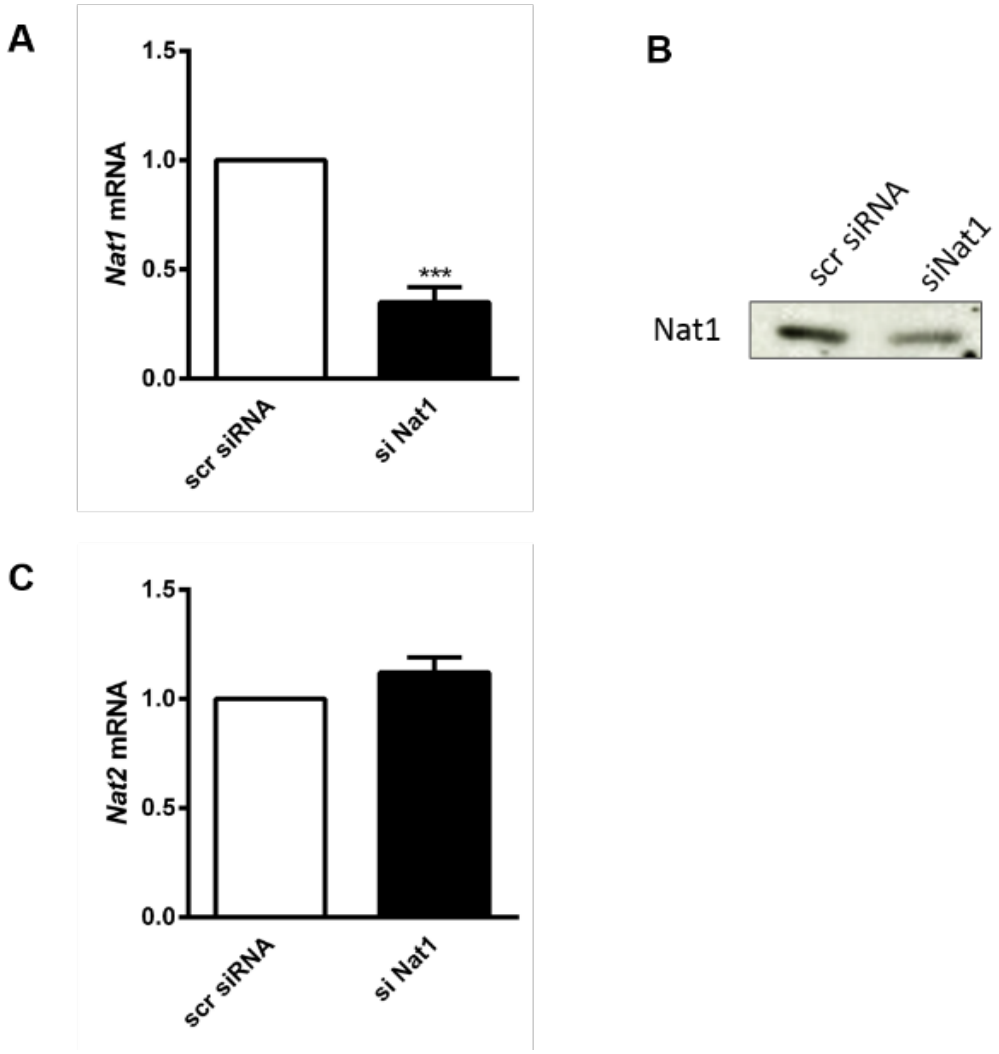
Left panel: GWAS results without adjustment for BMI; **Right panel**: GWAS results with adjustment for BMI
The x-axis represents the expected –log10 *P-values*. The y-axis represents the observed –log10 *P-values*. The observed *P-value* for each SNP are sorted and plotted against the expected *P-value* in a theoretical χ^2-distribution.

Supplementary Figure 2: Forest plot excluding small cohort (NaKs) with significant heterogeneity for rs1208 (effect allele "A", frequency 0.57) in analyses adjusted for age, g and BMI.

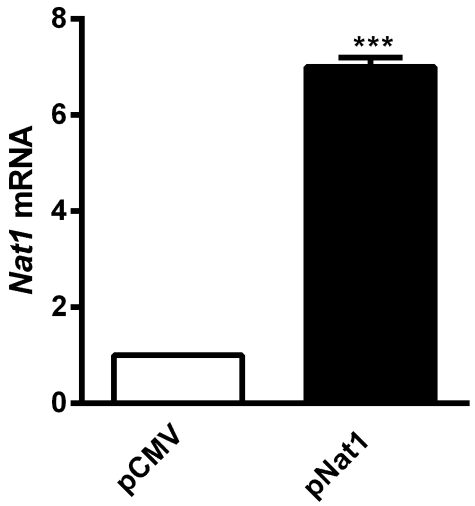




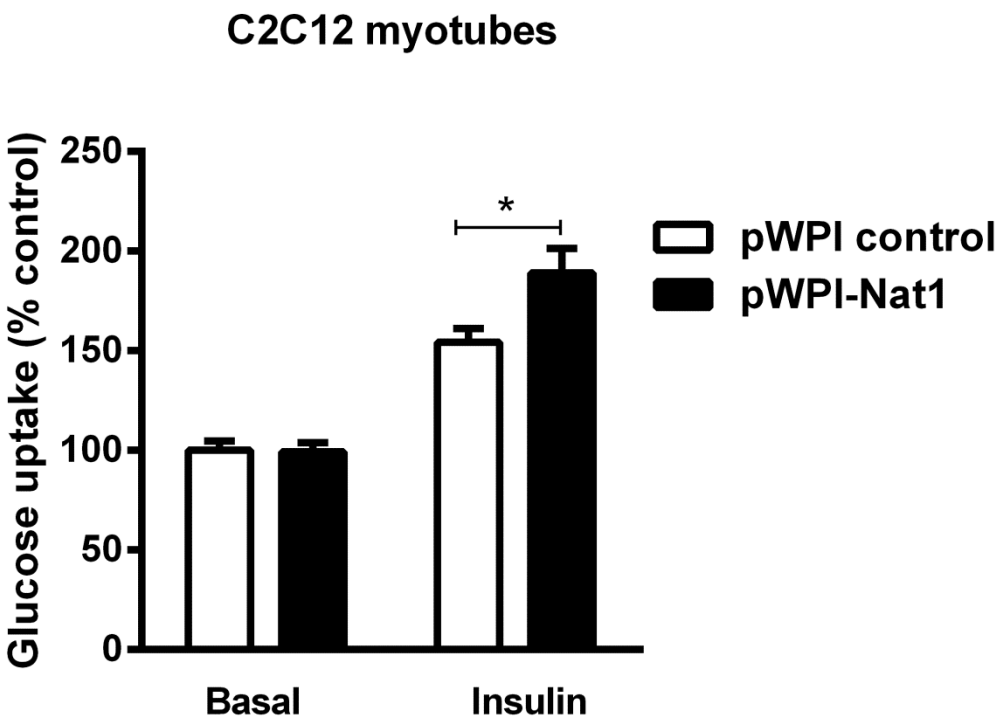
Supplementary Figure 3: In 3T3-L1 adipocytes, stimulation with insulin decreases *Nat1* expression levels.



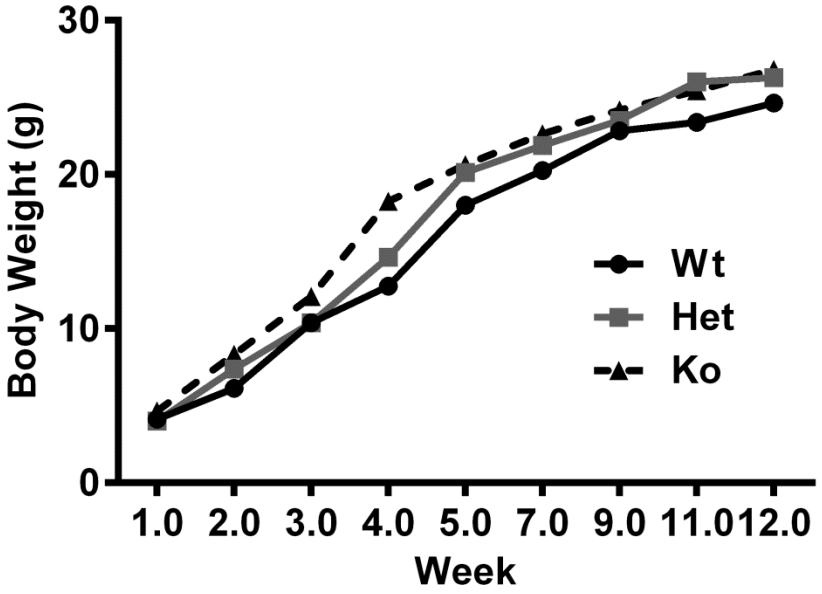
Supplementary Figure 4: *Nat1* knockdown in 3T3-L1 adipocytes. Differentiated 3T3-L1 adipocytes were transfected with scrambled siRNA (scr siRNA) or with siRNA against *Nat1* (si Nat1). (A) mRNA levels of *Nat1* were analyzed by real-time quantitative PCR, normalized to cyclophilin and expressed relative to scrambled controls. Results represent the mean ± SEM of three independent experiments (*** $p \leq 0.001$). (B) Western blotting analysis of *Nat1* expression in whole cell lysates from 3T3-L1 adipocytes transfected with scr siRNA or with si Nat1. (C) mRNA levels of *Nat2* were analyzed by real-time quantitative PCR and expressed relative to scrambled controls.



Supplementary Figure 5: *Nat1* over expression in 3T3-L1 adipocytes. Differentiated 3T3-L1 adipocytes were transfected with empty vector (pCMV control) or with expression plasmid for *Nat1* (p *Nat1*). mRNA levels of *Nat1* were analyzed by real-time quantitative PCR, normalized to cyclophilin and expressed relative to controls. Results represent the mean ± SEM of three independent experiments (*** $p \leq 0.001$).

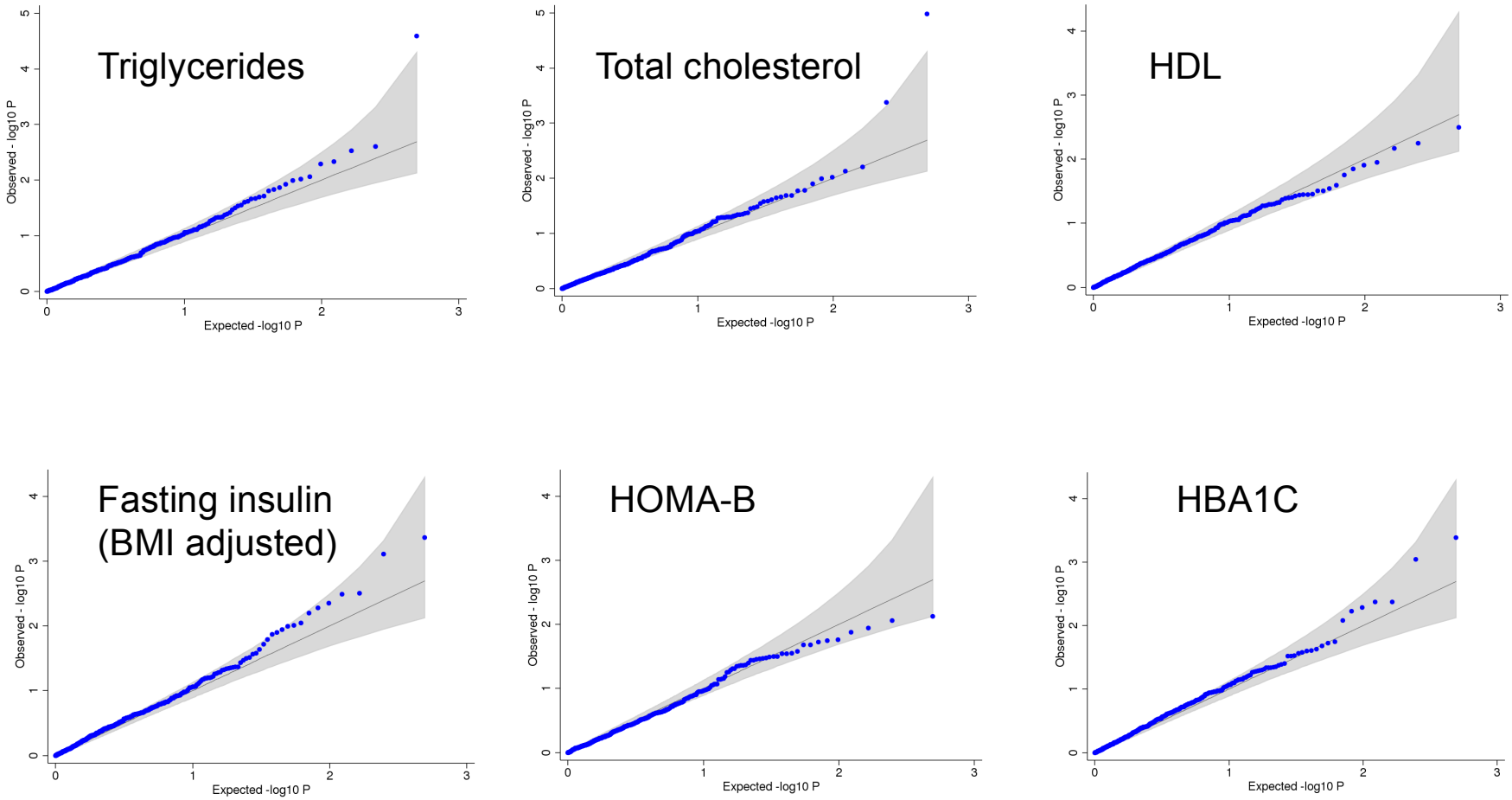


Supplementary Figure 6: *Nat1 increased glucose uptake in C2C12 myotubes*. C2C12 cells transduced with lenti pWPI (control) and pWPI-Nat1 were differentiated into myotubes. Cells were serum and glucose starved for overnight and the assay was done in the presence or absence of insulin. Radiolabeled glucose in lysates was measured in a high-flashpoint scintillation cocktail using a liquid scintillation counter. Results represent mean ± SEM from two separate experiments with 3-4 wells per condition (**$p \leq 0.01$).

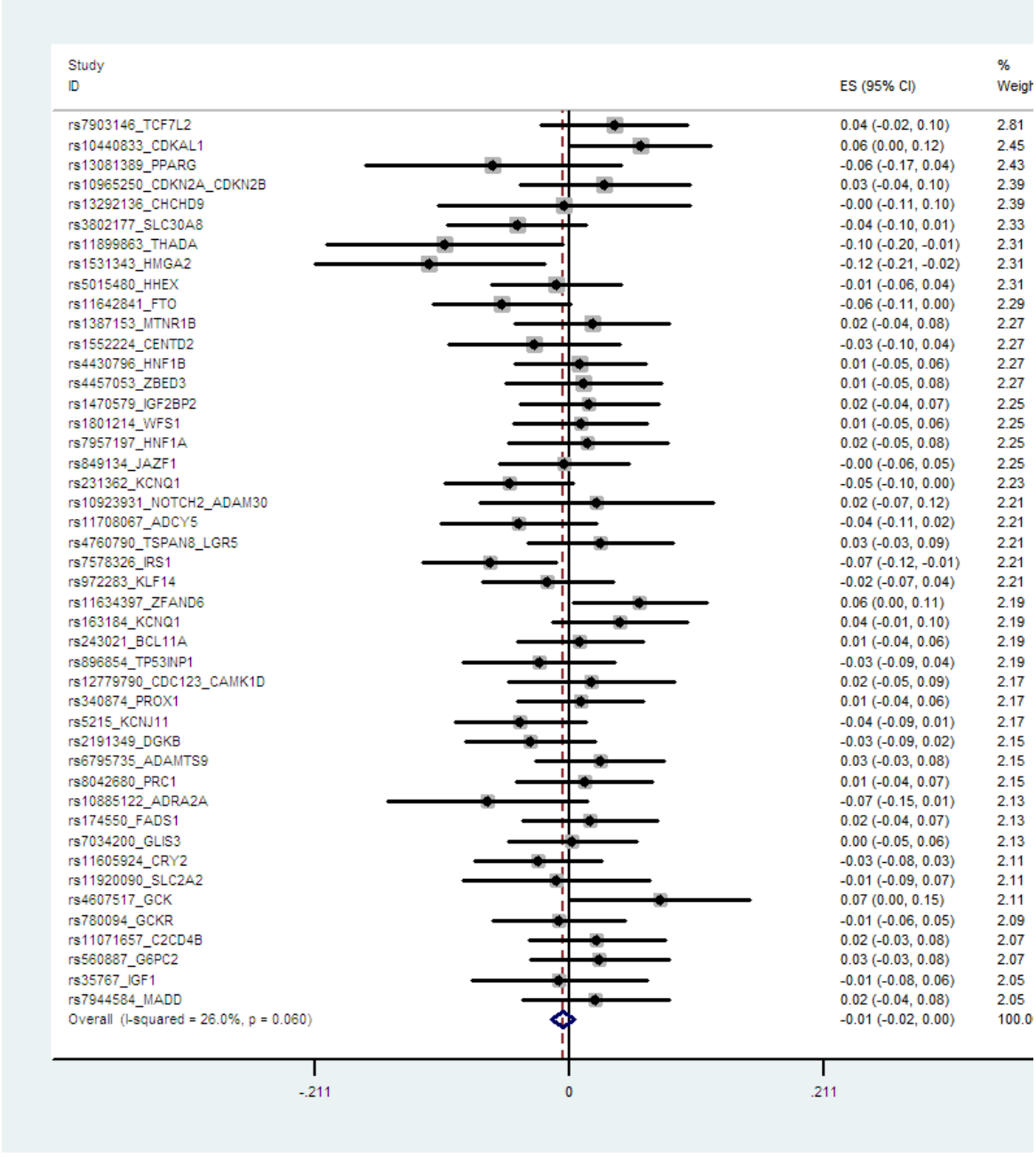


Supplementary Figure 7: Body weight was monitored weekly for 12 weeks in male mice (*n* = 10 per genotype).

Supplementary Figure 8: No enrichment of insulin sensitivity associated SNPs identified in the GWAS (age, gender, BMI adjusted analyses) with glycemic or lipid traits. Includes insulin sensitivity SNPs with P values < 10-3 (n=185 SNPs).



Supplementary Figure 9: Association of T2D SNPs with insulin sensitivity



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Joshua. W. Knowles contributed to the original conception, design, and worked to organize collaboration, GWAS implementation, supervised the basic science experiments and did primary work on writing the manuscript.

Weijia Xie contributed to discussion, performed data analyses and helped to write the manuscript.

Zhongyang Zhang contributed to data analysis and helped to write the manuscript.

Themistocles L. Assimes contributed to the original design of the GENESIS GWAS, the approach to association analysis, and manuscript writing.

Jussi Paananen contributed to data generation and analysis.

Ola Hansson provided samples and assisted with genotyping and data analysis.

James Pankow contributed samples and assisted with analysis.

Indumathi Chennamsetty contributed to basic science experiments in particular in vitro and in vivo characterization of *NAT2*.

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Ivan Carcamo-Orive contributed to basic science experiments

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Fahim Abbasi recruited subjects and assisted with data analysis.

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Pek Lum assisted with administration with early phases of the project.

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Alan Sinaiko contributed samples and assisted with analysis.

Gerald Reaven contributed samples and helped with overall study design and discussion.

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Agnes Hsiung contributed samples and to study design, discussion.

Leif Groop contributed samples and contributed to the discussion.

Heather J. Cordell contributed to the original design and analysis approach.

Markku Laakso contributed to study design, discussion, manuscript preparation.

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Erik Ingelsson contributed samples as well as to design, data generation, supervised data analyses and manuscript preparation.

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Thomas Quertermous co-conceived of the GENESIS project and organized the investigators, contributed to analyses and manuscript writing.

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Merck: Other collaborating investigators for this mansuscript included: **Christine Suver** and **Daniel M. Kemp** (both while at Merck Research Labs, 33 Ave. Louis Pasteur, Boston, MA 02115, USA).

The consortium provided assisted with genotyping, data analyses, coordination of results and manuscript approval. Danielle Greenawalt and Cliona Molony were authorized to act on behalf of the group for this manuscript.

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Investigators in **bold** played a more direct role in the project

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