SUPPLEMENTARY RESEARCH DESIGN AND METHODS

Muscle protein expression. Approximately 50 mg of biopsy specimen was used for muscle homogenate and lysate preparation. The tissue was pulverized and homogenized by means of a homogenizer (Polytron PT 3100, Kinematica, Swiss) in a buffer (1:20, weight:vol) containing 50 mM HEPES (pH 7.5), 150 mM NaCl, 20 mM Na-pyrophosphate, 20 mM β-glycerophosphate, 10 mM NaF, 2 mM Na-ortovanadate, 2 mM EDTA, 1% Nonidet P-40, 10% glycerol, 2 mM PMSF, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 10 µg/ml leupeptin, 10 µg/ml aprotinin, and 3 mM Benzamindine. All chemicals were obtained from Sigma-Aldrich, MO, USA. Homogenates were rotated end over end for 1 hour at 4 ^oC and then cleared by centrifugation for 1 hour at 17,500 g at 4 ^oC. The supernatant (lysate) was harvested and divided into several aliquots, frozen in liquid nitrogen and stored at −80 ^oC for later analysis. The protein content was measured using the bicinchoninic acid assay as recommended by the manufacturer (Pierce, Rockford, IL). p85α and p110β contents were determined by SDS-PAGE and Western blotting using a Criterion Gel System (BioRad, Hercules, CA). The proteins were separated using 26 wells 7.5% Bis-Tris gels (BioRad), and transferred to Immobilon Transfer Membranes (Millipore, Billerica, MA). The membranes were incubated in primary and secondary antibodies as recommended by the manufacturers. *p85α*: Anti-p85α, 06-497 (Upstate Biotechnology Inc., MA), HRP-linked anti-rabbit, P0448 (DAKO, Glostrup, Denmark). *p110β*: Anti-p110β, Y384 (Abcam, Cambridge, UK), HRP-linked anti-rabbit, 7074 (Cell Signaling, Danvers, MA). Protein quantities of p85α and p110β were expressed in arbitrary units relative to a human skeletal muscle standard. Analysis of assay linearity between signal and amount of protein loaded was verified for each of the antibodies used.

SUPPLEMENTARY TABLES

Supplementary table 1. Characteristics of twin subjects stratified according to *PIK3R1* rs3730089 genotypes.

***PIK3R1* rs3730089**	**GG**	**GA**	**AA**	P_{add}
Genotype frequency	75.3%	24.2%	0.5%	.
n (male/female)	146 (70/76)	47 (26/21)	1 (1/1)	.
Age (years)	42.9±17.0	42.7±17.0	63	.
BMI (kg/m^2)	25.1±3.9	24.6±3.9	29.0	0.4
Fat %	25.3±8.7	22.6±8.4	24.8	0.2
Fasting insulin (pmol/l)	34.9±17.6	35.4±22.6	26.0	0.9
30 min insulin (pmol/l)	325.4±177.3	329.4±177.2	269.0	0.3
120 min insulin (pmol/l)	179.6±137.1	164.5±132.6	194.0	0.6
Fasting glucose (mmol/l)	5.4±0.5	5.3±0.9	5.2	0.1
30 min glucose (mmol/l)	8.5±1.7	8.6±1.8	9.2	0.4
120 min glucose (mmol/l)	6.4±1.5	6.8±2.1	7.1	0.07
R_d clamp ($mg \cdot kg_{FFM}^{-1} \cdot min^{-1}$)	10.9±3.5	10.9±3.2	6.1	0.4
HGP clamp ($mg \cdot kg_{FFM}^{-1} \cdot min^{-1}$)	1.6±0.5	1.4±0.7	1.6	0.2
HGP basal ($mg \cdot kg_{FFM}^{-1} \cdot min^{-1}$)	3.1±0.5	3.0±0.4	2.9	0.2
Hepatic IR index	108.5±56.1	103.7±65.1	74.1	0.8
D_i	$1.8±1.2 \cdot 10^{-7}$	$1.8±1.0 \cdot 10^{-7}$	$0.3 \cdot 10^{-7}$	0.9

Association of *PIK3R1* rs3730089 with quantitative traits is shown in 194 Danish twins. Data are presented as mean±SD. All P_{add} values are adjusted for sex, age, and twin pair and zygosity status. P_{add} values for plasma levels of glucose and insulin, and for metabolic rates and indices are additionally adjusted for body fat percentage. D_i: Disposition index, FFM: Fat-free body mass, HGP: Hepatic glucose production, IR: insulin resistance, R_d: Glucose disposal rate.

Supplementary table 2. Characteristics of Inter99 subjects stratified according to *PIK3R1* rs3730089 genotypes.

***PIK3R1* rs3730089**	**GG**	**GA**	**AA**	P_{add}
Genotype frequency	70.1%	27.3%	2.6%	.
n (male/female)	4039 (2017/2022)	1572 (764/808)	152 (83/69)	.
Age (years)	46.1±7.9	46.2±7.9	46.5±7.6	.
BMI (kg/m^2)	26.3±4.6	26.1±4.5	26.1±4.2	0.1
Fasting insulin (pmol/l)	42.5±28.3	40.4±25.8	41.7±34.4	0.046
30 min insulin (pmol/l)	294.0±189.5	282.1±168.4	290.0±187.5	0.2
120 min insulin (pmol/l)	218.6±212.1	211.5±205.9	234.4±267.1	0.9
Fasting glucose (mmol/l)	5.5±0.8	5.5±0.9	5.6±0.8	0.8
30 min glucose (mmol/l)	8.7±1.9	8.6±1.9	8.7±1.9	0.3
120 min glucose (mmol/l)	6.2±2.1	6.2±2.2	6.3±2.4	0.2
HOMA-IR	10.7±8.1	10.2±7.6	10.7±10.6	0.04
BIGTT-SI	9.1±4.1	9.4±4.1	9.3±4.4	0.04
BIGTT-AIR	1852.7±1124.6	1822.9±962.6	1865.3±1247.6	0.7
D_i	15236.5±7195.3	15743.6±7338.4	15081.0±6992.5	0.1

Association of *PIK3R1* rs3730089 with quantitative traits in 5,763 Danish subjects. Data are presented as mean±SD. All P_{add} values are adjusted for sex and age. P_{add} values for plasma levels of glucose and insulin, HOMA-IR, BIGTT-SI, and BIGTT-AIR are additionally adjusted for BMI. D_i: Disposition index, HOMA-IR: Homeostasis model assessment for insulin resistance, BIGTT-SI: Insulin sensitivity index, BIGTT-AIR: Acute insulin response index.

Supplementary table 3. p85α and p110β muscle proteins in twins stratified according to *PIK3R1* rs3730089 genotypes.

***PIK3R1* rs3730089**	**GG**	**GA**	**AA**	P_{add}
Basal p85α (AU)	717.0±238.8	690.8±145.2	953.5	0.4
Basal p110β (AU)	485.5±18.5	468.2±139.7	435.8	0.3
Basal p85α:p110β (AU)	1.62±0.76	1.55±0.37	2.19	0.6
Insulin p85α (AU)	708.5±236.2	733.0±145.2	786.7	0.7
Insulin p110β (AU)	499.7±187.2	501.1±196.7	357.7	0.6
Insulin p85α:p110β (AU)	1.60±0.78	1.64±0.73	2.20	0.2

Skeletal muscle protein quantities are shown in arbitrary units (AU) for 184 Danish twins. Data are presented as mean±SD. P_{add} values are adjusted for sex, age, body fat percentage, and twin pair and zygosity status.