Experimental Procedure

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
AKT Phospho (Thr308)	Cell Signaling	4056
AKT Phospho (Ser373) XP	Cell Signaling	4060
AKT	Cell Signaling	9272
Erk 1/2 (p44/42 MAPK) Phospho(Thr202/Tyr204) XP	Cell Signaling	9101
Erk 1/2 (p44/42 MAPK)	Cell Signaling	9102
IRS1	Millipore	06248
IRS2	Millipore	MAB515
PI3K p85	Cell Signaling	4257S
PI3K p85α	Abcam	Ab133595
PI3K p55γ	Abcam	Ab238509
Tubulin α/β	Cell Signaling	2148S
PI3Kα	Cell Signaling	4249
PI3Kβ	Cell Signaling	3011
PI3Kδ	Millipore	04-401
PI3Kγ	a gift of Prof. Matthias Wymann University of Basel	(Russian)
Chemicals, Peptides, Recombinant Proteins and Inhibitors		
Insulin	Humalog Lilly	VL7510
Glucose	Acros	410955000
BSA	Fisher Scientific	BP9702
ECL-anti rabbit IgG HRP	GE Healthcare	NA934V
ECL-anti mouse IgG HRP	GE Healthcare	LNA931V
Collagenase P	Roche	11213865001
GSK2636771 (Inhibitor PI3Kβ)	SelleckChem	S8002
TGX-221 (Inhibitor PI3Kβ)	MedChem Express	HY-10114
IPI549 (Inhibitor PI3Kγ)	MedChem Express(SelleckChem)	HY-100716
PIK294 (Inhibitor PI3Kδ)	MedChem Express	HY-10303
Buparlisib (BKM120, panPI3K inhibitor)	MedChem Express	HY-70063
Taselisib (GDC-0032, panPI3K inhibitor)	MedChem Express	HY-13898
Critical Commercial Assays		
Insulin ELISA Kit	Crystal Chem	90080
C-peptide Elisa Kit	Crystal Chem	90050
FFA (NEFA-HR set)	Fuji Film	436-91995, 434-91795 91096 (standard)
Leptin Elisa Kit	R&D	MOB00B
Adipsin Elisa Kit	R&D	DY5430-05
FABP4 Elisa Kit	BioVendor	RD191036200R
Asprosin Elisa Kit	Abbexa	abx585287
Immobilon Western Chemiluminescence HRP substrate	Millipore	WBKLS0500
Hematoxylin Solution (Mayer's, Modified)	Abcam	AB220365
Eosin	Sigma	HT110116
Experimental Models: Organisms/Strains		

WT	C57BL/6 background	Janvier Labs
PI3Kα^{Flox}	Mixed background	Provided by Victoria Rotter Sopasakis
PI3Kα^{AdQ}	Mixed background	Provided by Victoria Rotter Sopasakis
Software and Algorithms		
Image Lab software (version 5.2.1)	Bio-Rad	http://www.bio-rad.com/en-us/product/image-lab-software
Graph Pad Prism 7.0	Graph Pad Software	N/A
AxioVision Software	Imaging Sofware	Carl Zeiss Microscopy
Deposited Data		
	Immunoblots images	

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for reagents may be directed to the Lead Contact, Giovanni Solinas (giovanni.solinas@wlab.gu.se)

Mice and In Vivo Studies

All mouse studies were approved by the Ethics Committee on Animal Care Use in Gothenburg, Sweden. Only male mice were used in this study. Mice were maintained at the EBM specific pathogen free facility of the University of Gothenburg under a 12-hour light /12-hour dark cycles and at a room temperature of 22°C.

PI3Kα LoxP floxed mice (PI3Kα$^{F/F}$) in 129/SvJ and C57BL/6J mixed background were previously described (Molinaro et al. 2019). PI3Kα^{AdQ} mice and littermate control PI3Kα$^{F/F}$ mice were obtained by crossing Adipoq-Cre mice in pure C57BL/6J background (Jackson labs) with the PI3Kα$^{F/F}$ mice from above. Wild type (WT) C57BL/6J mice were obtained from Janvier Labs.

For insulin-tolerance test (ITT) mice were fasted for 4 hours and injected intraperitoneally with 1 I.U. of insulin per Kg of body weight. Blood was collected from the tail at the indicated post-injection times and blood glucose concentrations were measured using a glucometer (Contour Next, Ascensia). For glucose tolerance test (GTT) mice were fasted for either 4 hours (GTT in Figure S2) or overnight for glucose-mediate suppression of lipolysis (Figure 2), and injected intraperitoneally with either 1g or 2 g of glucose per Kg of body weight respectively. Blood was collected from the tail at the indicated time-points and used to measure blood glucose and prepare serum for measurements of hormones and metabolites.

For in vivo injection of PI3K-selective inhibitors, the indicated dose of compound was injected intraperitoneally into eight to twelve-months-old PI3KaAdQ mice and PI3KaF/F littermate controls or C57BL/6 mice. The injectable solutions of the PI3K inhibitors were freshly

prepared as following: PI3K inhibitor was first dissolved in pure DMSO, and to this master liquid were added one by one and in this order PEG300, Tween 80, and water. The solution was mixed after each addition. Final concentration for the single components was as following: 5% DMSO, 30% PEG, 1% Tween-80. Control ‘‘vehicle solution’’ was 5% DMSO, 30% PEG, 1% Tween-80, and water. For solution including nicotinic acid (NA), the NA powder was directly dissolved in the final mix at the desired concentration, the acidity was adjusted at a value of pH 7 by adding NaOH 1M to the solution.
Blood glucose was measured at the indicated time points after injection and blood was collected for further measurements of serum insulin, serum FFA, serum leptin and plasma levels of C-peptide, BCAA, BCKA, free and acyl-carnitines.
At sacrifice mice were anesthetized with Isofluorane (Baxter KDG 9623) and euthanized by CO_2 and bleeding.

Insulin, C-peptide, Leptin and adipokine measurements

Insulin, C-peptide, levels in serum and plasma were measured using Elisa kit from Crystal Chem. Serum leptin was measured by ELISA kit from R&D. Plasma Adipsin (Mouse Complement Factor D) was measured by quantitative sandwich enzyme immunoassay using kits obtained from R&D Systems and adipocyte FABP4 abundance in plasma was measured using an ELISA kit from BioVendor. All ELISA assays were performed according to the instruction of the manufacturer.

Hematoxylin and eosin (H&E) staining

Samples of adipose tissue pads and liver were obtained from 13-week-old mice. The samples were fixed in 10% formalin, embedded in paraffin, cut into 5 μm sections, and stained with hematoxylin (Abcam) and eosin (Sigma). H&E stained sections were imaged at 20X magnification with an AxioImager.M1 microscope (Zeiss, Germany).

Adipocyte size distribution

Images of two fields for six mice were analyzed in Fiji using the open source plugin adiposoft. Cell surface area was calculated in microns and classified based on area μm^2.

Immunoblot Analysis

Protein samples were resolved by SDS-PAGE electrophoresis and then transferred to a PVDF membrane. The membrane blocking procedure was carried out at room temperature in phosphate saline buffer (PBS) with 3% bovine serum albumin (BSA), and 0.3% tween solution (Cell Metabolism 30, 1400–1409.e1–e5, June 4, 2019 e4). The primary antibody was incubated in cold room overnight in PBS with 0.3% tween and 3% BSA; the membranes

were then washed three times with a PBS 0.3% tween solution and incubated with a horseradish peroxidase conjugated secondary antibody (GE Healthcare) in PBS 0.3% tween 3% BSA for one hour at room temperature. After three washes in a PBS 0.3% tween solution and incubation with detection reagent (Millipore), signal acquisition was carried out using the Biorad ChemiDoc apparatus.

Analysis of plasma branched chain amino acids and branched chain keto acids

25µl plasma and standard was precipitated with 250µl methanol containing 1µM of deuterated internal standards (Valine-$^{13}C_5$,^{15}N, Leucine d_3, Isoleucine d_{10}, keto-leucine d_7 and keto-isolucine d_8). After 10 minutes of vortex at 1400 rpm the samples were centrifuged at 4000g for 10 minutes. 25µl µl was removed and evaporated under a stream of nitrogen gas. The samples were reconstituted in 125µl water and injected on the UPLC-MS/MS system. Separation was performed on a Waters Acquity BEH C8 column (2.1 x 100mm 1.7µm) with a gradient consisting of water and acetonitrile, both with 0.1% formic acid. The detection was made on a Waters Xevo TQ-XS triple quadrupole mass spectrometer in MRM mode using intra-run polarity switching where the detection of amino acids were made in positive mode between 0-3 minutes. The polarity was then switched into negative mode for the detection of the keto acids between 3-10 minutes. The standard curve containing all six amino- and keto acids was prepared in 20% methanol and treated the same way as the samples.

Analysis of plasma acylcarnitines and glutamine

25µl of plasma was precipitated with 250µl of methanol containing 1µM of deuterated glutamine. After 10 minutes of vortex at 1400 rpm the samples were centrifuged at 4000g for 10 minutes. 25µl µl was removed and evaporated under a stream of nitrogen gas. The samples were reconstituted in 125µl of an acylcarnitine internal standard mix (NSK-B reference mix, Cambridge Isotope Laboratories) prepared in acetonitril:methanol [3:1]. The samples were separated in HILIC mode on a Waters BEH Amide column (2.1 x 100 1.7µm) using 5mM ammonium formate and 0.06% formic acid in 95% acetonitrile as mobile phase A and 10 mM amonium formate and 0.12% formic acid in 100% Milli-Q water as mobile phase B. The detection was made on a Waters Xevo TQ triple quadrupole mass spectrometer in positive MRM mode. Quantification of acylcarnitines was made using a one point calibration against the deuterated analogue. For the acylcarnitines without a deuterated analogue, the quantification was made against the deuterated acylcarnitine closest in retention time. Glutamine was quantified using an external standard curve.

Glucose Stimulated insulin secretion (GSIS)

Primary mouse islet isolation was performed as previously described (Hugill A, Shimomura K, Cox RD. Islet Insulin Secretion Measurements in the Mouse. Curr Protoc Mouse Biol. 2016 Sep 1;6(3):256-271. doi: 10.1002/cpmo.14. PMID: 27584553). After isolation and overnight incubation at 37°C with humidified 5% CO_2 air in RPMI supplemented with 1% P/S and 10% FBS, islets were transferred into a 24-well plate. 5 islets were hand-picked and transferred into each well in presence of 200ul low glucose buffer (Krebs Ringer Buffer KRB supplemented with 0.2% BSA and 2.8mM glucose) and pre-incubated for 1 hour at 37°C. Islets underwent successive incubations for 20 minutes at 37°C with 200µL of fresh KRB 0.2% BSA containing low glucose (2.8mM), followed by high glucose (16.7mM). At the end of each incubation islets were transferred into a new well and supernatant was collected for analysis of insulin levels. At the end of the stimulation protocol, cells were lysed in 70µL acid ethanol (75% absolute ethanol, 23.5% water, 1.5% HCl) and stored at 4°C overnight. Samples were dried with a SpeedVac and resuspended in 30µL ultrapure water. DNA was quantified by Nanodrop (ThermoFisher, USA) and insulin was quantified using Elisa kit (Crystal chem) and normalized to DNA content.

QUANTIFICATION AND STATISTICAL ANALYSIS

Data are expressed as means, and error bars indicate standard errors.
The exact number of mice for a specific experiment is indicated in the figure legends.
For in vivo mouse studies weight matched littermate mice were used.
Concerning sample size determination, in the time course after injection a minimum of 5 mice was used; for all other in vivo studies a minimum of 3 biological replicates was analyzed when parametric statistical tests is used, whereas a minimum of 4 biological replicates was analyzed when non-parametric statistical tests were used. In analysis where two different categorical variables are considered (GTT, ITT, mice weight gain, and time-course analyses), repeated measures (RM) two-way ANOVA was used.
All statistical analysis was performed with GraphPad Prism software.