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Reporting Summary

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	LAS-4000 Luminescent Image analyzer Ver 2.0 (FujiFilm) was used to acquire western blot images. Multi Gauge V 3.1 was used to export and digitize western blot images.
Data analysis	Results in Fig. 2a is based on the open source tool: Integrated Molecular Pathway Level Analysis (IMPaLA) it is a well-documented and in frequent use. For reference see: Kamburov, A., Cavill, R., Ebbels, T. M., Herwig, R. & Keun, H. C. Integrated pathway-level analysis of transcriptomics and metabolomics data with IMPaLA. Bioinformatics 27, 2917-2918, doi:10.1093/bioinformatics/btr499 (2011). In Figure 4, we have used software FastQC v0.10.1, HISAT2 v2.1.0, FeatureCount v1.5.1, SAMstat v1.5.1, EdgeR R-v3.1.2 to analyze the RNA-seq and proteomics data. We have used GeneSCF v1.1-p1 to perform gene ontology enrichment analysis. For reference see Subhash, S. & Kanduri, C. GeneSCF: a real-time based functional enrichment tool with support for multiple organisms. BMC Bioinformatics 17, 365, doi:10.1186/s12859-016-1250-z (2016). In Extended Data Figure 11, we have used oPOSSUM 3.0 software which is an online prediction tool for transcription factor binding site. For reference see Kwon, A. T., Arenillas, D. J., Worsley Hunt, R. & Wasserman, W. W. oPOSSUM-3: advanced analysis of regulatory motif over-representation across genes or ChIP-Seq datasets. G3 (Bethesda) 2, 987-1002, doi:10.1534/g3.112.003202 (2012). All multicolor flow cytometry data were acquired with a BD LSR Fortessa and analyzed using FlowJo version 10. Excel and GraphPad Prism version 7 was used for statistical analysis and draw figures.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

Transcriptomic data (Fig. 4) has been deposited to the NCBI Gene Expression Omnibus (GEO) with the accession number GSE114258 and can be accessed with the following access link: https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE114258. Proteomic data (Fig. 4) has been included as four tables in Supplementary information with file names: "Proteome_data_Foxk1_KD", Proteome_data_Foxk1_OE", Proteome_data_Foxk2_KD", Proteome_data_Foxk2_OE".

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	In the cell-based experiments we typically work with 3-4 independent experiments with 4 technical replicas each. In our experience and under these circumstances significant changes are reproducible and often biologically meaningful. We would then use a different independent approach to verify the initial findings and gradually by applying more of new experimental approaches build a case. In terms of the in vivo work using mice we have chosen group sizes based on a power analysis indicating that using 6 mice in each group, assuming a normal distribution with a coefficient of variation of 7.5% and comparing the levels with a two-tailed two-sample t-test at global significance level 5% we can detect approximately 25% difference in means with 80% power and a 30% difference in means with 99% power.
Data exclusions	We have not excluded data.
Replication	The number of biological independent replicates and animals are indicated in the figure legends. All attempts at replication were successful.
Randomization	Mice were allocated to groups based on their genotype since the purpose of the experiments were to study any difference relating to genotype.
Blinding	In the phenotypic/experimntal examination of our genetically modified mice cohorts the examiner was blinded to the genotype.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☐	☒ Human research participants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	Antibodies detecting the GLUT-4 (07-1404), GLUT-1 (07-1401), MCT1 (AB3540P), PDH-E1α (pSer293) (AP1062), PDH-E1α (pSer232) (AP1063) and PDH-E1α (pSer300) (AP1064) were from Millipore. Hexokinase II (2867), PKM2 (4053), LDHA (2012), PDP1 (65675), anti-rabbit IgG-HRP (7074) and anti-mouse IgG-HRP (7076) antibodies were from Cell Signaling Technology. PFKM (ab154804), ALDOA (ab169544), PDK1 (ab202468), PDK4 (ab214938), GLUD1 (ab166618), and anti-beta ACTIN-HRP (ab8226) antibodies were from Abcam. Anti-MYOSIN (skeletal, slow type I) (M8421), anti-FLAG (F7425) and anti-FLAG M2-HRP (A8592)

were from Sigma-Aldrich. Antibody detecting CYCLOPHILIN B (VPA00583) was from Bio-Rad. Vector® M.O.M. ™ Immunodetection Kit (PK-2200) was from Vector Laboratories. PDH Rodent Immunocapture Kit (ab133988) for PDH complex IP was purchased from Abcam.
The following antibodies were used for FACS analysis: CD3 – PerCP (clone UCHT1; Cat# 300428) and FLAG – PE (Clone L5; Cat# 637310) from biolegend and CD4 - APC-H7 (Clone RPA-T4; Cat# 560158), CD8 - BV510 (Clone RPA-TA8; Cat# 563256) and Ki-67 – BV786 (Clone B56; Cat# 563756) from BD Bioscience. In addition, fixable viability stain 700 (FVS700; BD Bioscience; Cat# 564997) was used to exclude dead cells.

Validation

All the antibodies were validated by the manufactures. Foxk1 and Foxk2 antibodies were also identified by shRNA in cell experiments. Antibody for T cell analysis has been titrated for validation and determination of optimal volume prior to being used in these analyses.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

293T, 3T3-L1, C2C12, L6 cells and cancer cells including SH-SY5Y and FaDu were purchased from ATCC.
Human skeletal muscle myoblasts were purchased from Lonza (Cat No: CC-2580).
Primary human white adipocytes were perchased from Promo Cell(C-12730).
Human peripheral blood CD3+ cells were purified from buffy coats (Oslo University Hospital Blood Bank) by density-gradient centrifugation with Lymphoprep (Axis-Shiel) followed by the RosetteSep™ Human T cell enrichment protocol as described.

Authentication

Cells were directly bought from ATCC, Lonza and Promo Cell company. No other authentication was performed, than that we noted expected morphology and general behavior of cells as we cultured them.

Mycoplasma contamination

Cell lines were screened for mycoplasma and they were negative for mycoplasma.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified lines were used.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals

Mice: C57BL/6 mice used for breeding, in starvation and exercise experiments were obtained from Charles River Laboratories (Germany). EIIa-cre (Stock# 003724) and Mck-cre (Stock #006475) were obtained from The Jackson Laboratory, Bar Harbor, ME. OB/OB mice were obtained from The Jackson Laboratory, Bar Harbor, Maine.
We used male mice which were two months old at the beginning of each experiment.

Wild animals

No wild animals were involved in this study.

Field-collected samples

No field collected samples were involved in this study.

Human research participants

Policy information about studies involving human research participants

Population characteristics

Primary cells from humans were used and all appropriate and required permission and ethical considerations have been undertaken as out-lined in Method section under "Human primary adipocytes". All subjects complied with ethical regulations.

Recruitment

Subjects undergoing reconstructive surgical procedures at Sahlgrenska University hospital were recruited as donors of primary cells.

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation

As described in the materials and methods, but in short, CD3 positive cells were obtained from buffy coats from the University Hospital blood bank and isolated by density-gradient centrifugation with Lymphoprep (Axis-Shiel) and RosetteSep™ Human T cell enrichment cocktail.
The CD3 positive cells were activated with CD3/CD28 antibodies for 48 hours. The cells were then transduced with retrovirus

	carrying FLAG-Foxk1 and FLAG-Foxk2 overexpression vectors for 48 hours. After additional incubation for 72 hours the cells were stained with CD3 – PerCP (UCHT1) and FLAG – PE (L5) from biolegend and CD4 - APC-H7 (RPA-T4), CD8 - BV510 (RPA-TA8) and Ki-67 – BV786 (B56) from BD Bioscience. In addition, fixable viability stain 700 (FVS700; BD Bioscience) was used to exclude dead cells.
Instrument	All multicolor flow cytometry data were acquired with a BD LSR Fortessa.
Software	All multicolor flow cytometry data were collected with DIVA software version 8 and further analyzed using FlowJo version 10.
Cell population abundance	There was no FACS sorting before analysis, but CD3 positive cells were isolated from buffy coats using RosetteSep™ Human T cell enrichment cocktail.
Gating strategy	The gating strategy is described in supplemental figure 2. After the exclusion of dead cells, doublets and CD3 negative cells, CD4 and CD8 positive populations were gated. The subsequent FLAG gate was set based on cells transduced with an empty vector. The Ki-67 gate was set on the observable positive population of FLAG negative cells and copied into the FLAG positive population.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.