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☐ Initial submission ☒ Revised version ☐ Final submission

Life Sciences Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form is intended for publication with all accepted life science papers and provides structure for consistency and transparency in reporting. Every life science submission will use this form; some list items might not apply to an individual manuscript, but all fields must be completed for clarity.

For further information on the points included in this form, see Reporting Life Sciences Research. For further information on Nature Research policies, including our data availability policy, see Authors & Referees and the Editorial Policy Checklist.

Experimental design

1. Sample size

Describe how sample size was determined.

The sample sizes (indicated throughout) were chosen to equal to three or greater for in vitro studies and five or greater (e.g. mice per group) for in vivo experiments.

2. Data exclusions

Describe any data exclusions.

Presented data, and statistical treatment, include the entirety of the planned experiments; no data were considered outliers by testing or arbitrarily excluded.

3. Replication

Describe whether the experimental findings were reliably reproduced.

Key findings/conclusions regarding peripheral insulin resistance in the LDKO model of hepatic insulin resistance, as well as regulation of follistatin by hepatic FoxO1 were reproduced/corroborated by contrasting the results of like assays in diverse genetic contexts, including:
1) control (floxed Irs1/2) versus LDKO mice,
2) LDKO versus LTKO (LDKO with hepatic FoxO1 knockout) mice,
3) LTKO + adenoviral FoxO1 restoration,
4) acutely generated LDKO mice (flox mice plus viral Cre) to examine developmental dependency

Key findings/conclusions concerning the role of follistatin in vivo were reproduced/corroborated by:
1) Overexpression of Fst in wild-type (high fat-fed) mice
2) Knockdown of hepatic follistatin expression in LDKO mice: two separate methods were used for knockdown (shFst and sgFst/CRISPR) with similar results. Additionally, the results of two sgFst sequences are shown.
3) Knockdown of hepatic follistatin in mice maintained on high fat diet
4) Viral reconstitution of follistatin expression in hepatocytes of LTKO mice
5) Fst expression was checked/confirmed repeatedly at both hepatic message (mRNA) and secreted protein (serum) levels in the above models plus/minus the experimental manipulations.

Gene expression (increased or decreased) implied by transcriptomic approach (Affymetrix arrays) was confirmed by qPCR of follistatin and other mRNAs. In vivo overerexpression (screening) of putative hepatokines was also confirmed by qPCR.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

Mice were randomly divided into control and treatment groups. All included human data are from treated individuals (bariatric surgery).

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

Data collection for screening of putative hepatokines (Fig. 2) in WT mice was performed in blind fashion and unblinded for treatment (hepatokine). Microarray assays (Fig S8a-b) were performed by service for fee that was blind to genotype. Note: all human data herein come from subjects in the same (surgery) treatment group.

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ A statement indicating how many times each experiment was replicated
☐	☒ The statistical test(s) used and whether they are one- or two-sided (note: only common tests should be described solely by name; more complex techniques should be described in the Methods section)
☐	☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ The test results (e.g. *P* values) given as exact values whenever possible and with confidence intervals noted
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.	Alpha Innotech FluorChem 5500 was used to quantified the blots. GraphPad Prism 7 was used to perform analysis.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a for-profit company.	No unique materials as described under the policy information were used.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

Phospho-Akt (Ser473) Cell Signaling Technology CST#4058
Phospho-Akt (Thr308) Cell Signaling Technology CST #4056
Akt Cell Signaling Technology CST #9272
Phospho-HSL (Ser660) Cell Signaling Technology CST #4126
HSL Cell Signaling Technology CST #4107
FoxO1 Cell Signaling Technology CST #2880
β-Actin Cell Signaling Technology CST #4967
Phospho-Smad2 (Ser465/467) Cell Signaling Technology CST #3101
Smad2 (D43B4) Cell Signaling Technology CST #5339
GAPDH (D16H11) Cell Signaling Technology CST #5174
Lamin A/C Cell Signaling Technology CST #2032
Actinin Santa Cruz Biotechnology SC-15335
Irs1 mouse polyclonal Produced in our laboratory
Irs2 mouse polyclonal Produced in our laboratory
Srebp1 Abcam #3259
Prolong Antifade Gold with DAPI Life Technologies #1652731
Fst primary antibodies for immunostaining Abcam #203131
Fst primary antibodies for cell culture R&D Systems #AF669
Goat anti-rabbit secondary antibody Molecular Probes #A11012

Validation: Irs1 and Irs2 antibodies were validated using liver samples from Irs1/2-flox and Irs1/2-LDKO mice. Fst antibody was validated by correlation with viral overexpression of Fst in mouse liver. All others were used according to instruction of the products.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

3T3-L1 adipocytes (gift of Dr. Evan Rosen's lab)
HEK293 ATCC CRL-1573

b. Describe the method of cell line authentication used.

3T3-L1 adipocyte and HEK293 cells were authenticated by STR Profiling.

c. Report whether the cell lines were tested for mycoplasma contamination.

Both cell lines were tested negative for mycoplasma contamination

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

No commonly misidentified cell lines were used

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide details on animals and/or animal-derived materials used in the study.

C57BL6/J mice The Jackson Laboratory #000664
Ob/Ob mice The Jackson Laboratory #000632
B6;FVB-Tg(Adipoq-cre)1Evdr/J The Jackson Laboratory #010803
Irs1-flox Generated in our laboratory
Irs2-flox Generated in our laboratory
FoxO1-flox From Ronald DePinho's lab
B6.129S2-Il6tm1Kopf/J The Jackson Laboratory #002650

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

Human serum samples: Human serum used in this study was obtained as a part of a larger clinical study approved by both the Boston Children's Hospital IRB (IRB-P00021478; PI: Nicholas Stylopoulos) and the University of Pittsburgh Medical Center IRB (MOD15090464-02 / PRO15090464; PI: Anita Courcoulas). This is an ongoing, prospective, single-center study. Patients underwent RYGBS by the Minimally Invasive Bariatric and General Surgery (MIBGS) group of the University of Pittsburgh (UPMC). Blood was collected by venipuncture at a research visit prior to surgery (baseline) and 6 months after surgery. All bariatric surgeries were performed on a clinical basis and were not performed on a research basis. 15 of 18 patients, or 83.33% of participants, were female. Average baseline BMI was 45.29 ±7.29 kg/m2 and did not differ between diabetic and non-diabetic patients. Patients taking Metformin or statins were asked to temporarily discontinue these medications starting on the night prior to surgery. Measurements were collected including weight, % body fat using the Tanita Scale, and the height using a stadiometer. Diabetes was defined as either a documented fasting blood glucose level >126 mg/dl or an HbA1c of 6.5% or higher, or treatment with an anti-diabetic medication. Average HbA1c in non-diabetic patients was 5.55±0.36% and, in diabetic patients, 8.35±1.26%. Detailed information provided in Table S2.