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☐ Initial submission ☒ Revised version ☐ Final submission

Life Sciences Reporting Summary

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Experimental design

1. Sample size

Describe how sample size was determined.

Our sample sizes were chosen based on 1) number of fish or samples available for use, 2) ethical guidelines of our IACUC protocol and 3) constraints of the experimental design (i.e. time it takes to collect and process samples simultaneously). Below is a summary of the sample size considerations for each figure.

Figure1: b, c Sample size was determined based on the number of fish available and the minimum amount to determine average blood glucose level for each population. d, e, We chose the maximum sample size that would allow us to perform the experiment over the specified time course accounting for the time it takes to inject the fish and collect blood. f,g We chose the maximum sample size that would allow us to perform a western blot on all of the lysates simultaneously to control for technical differences in the western blot between replicates.

Figure 2. d, We chose to use greater than 2,500 live cells per treatment to quantify insulin binding based on the time it takes to run the flow cytometry assay. This number allowed us to reliably reproduce highly significant results.

Figure 3. a, The sampling frequency was determined based on the limited number of samples that were available to collect in the wild. b, We used six individuals to measure weight in parental surface and Tinaja cavefish as there is less variation in weight within each population as they are relatively genetically homogenous. c, d Differently, there is more genetic variation in the F2 population therefore we chose a higher sample size to measure F2 weight. Our sample size was based on the number of F2s available that could be housed separately to control for differences in food consumption. Our F2 sample size revealed a significant effect of genotype on weight and weight gain. f, Sample size was chosen based on the number of fish available and the time constraints of the experimental set-up (ie amount of time it takes to process each fillet while maintaining the same treatment length in PBS versus insulin for each fillet). g,h sample size was chosen based on the number of fish available at a developmental stage prior to maturation.

Figure 4. a, The images represent the limited number of fish that have been kept in captivity for greater than 14 years. d, We used the greatest number of samples that would allow us to run the assay simultaneously for all populations.

2. Data exclusions

Describe any data exclusions.

Figure 1. c, We measured fasting blood glucose levels in surface and Tinaja at multiple time points over 21 days of fasting; however, we chose to include the data only from 1 day and 21 days as we found those days to be significantly different between the populations and representative of the differences in surface and Tinaja blood glucose regulation. g, we only present the quantification of data from skeletal muscle treated with the highest concentration of insulin. We show representative blots of lystates from untreated and low-concentration treatments in figure f and felt it was not nessesary to include the quantification of those values in figure g.

Figure 2. d, We excluded the data from dead cells for this analysis as described in the figure legend.

Figure 3. b-d, As described in the text, female body weight was excluded from our analysis of body weight as we found that the female gonad had a large contribution to total body weight in females and changes depending on gravidity (Extended Data Fig. 6).

Extended data Figure 3. We took additional insulin measurements from the fish used in this experiment after the fish were housed individual for several weeks. We never observed a significant difference in insulin levels so only include the initial insulin measurement.

Extended data Figure 4c. We excluded the data for the 30-minute time point as we found that there was not a difference in blood glucose levels comparing 15 and 30 minutes after insulin injection in surface fish (Extended data Figure 4b).

3. Replication

Describe whether the experimental findings were reliably reproduced.

Figure1: a, The reported difference in blood glucose levels between surface and cave populations was reproducible (Fig. 1b,c (1day), e (pbs), (and data not shown) in multiple replications. c,d,e Each of these experiments was replicated at least once and the results were reproducible. f, g. We were able to reproduce the results shown in the western blot two times. In other replicates the results were not reproduced, but this was due to technical difficulty with the western blot (ie overloading the gel, inefficient transfer of protein to membrane, or high background).

Fig2. d, The insulin binding experiment was performed two times and the results were highly reproducible as shown in the Figure 2, d.

Figure 3. b-d, Weight measurements were taken multiple times on the same fish and the results were reproducible. f, Three biological replicates were used per genotype and condition in this experiment. The pAKT/AKT ELISA assay was carried out one time for each biological replicate due to the limitations of tissue size.

Figure 4. d, Four biological replicates were used to determine the AGE level for each population. The AGE assay was carried out one time for each biological replicate and we observed a significant difference between the surface and Molino values.

4. Randomization

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

It was not possible to randomize the samples due to the obvious morphological differences between the surface and cave populations. Groups were chosen based on population or genotype.

5. Blinding

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

For most measurements, it was not possible to be blinded due to the obvious morphological differences between the surface and cave populations. However, the genotypes of the F2 surface/cave hybrids as well as the zebrafish were unknown to the researcher who collected the weight data. The zebrafish scale data was also quantified without knowing the zebrafish genotype.

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.	All analysis was done using R version 3.3.1 (2016-06-21).

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.	All unique materials used in this study are readily available. Glucose, arginine, insulin (Sigma), blood glucose meter with test strips (Freestyle lite), pAKT/AKT Elisa kit (Abcam), Glucagon radioimmunoassay (Millipore), OxiSelect™ Advanced Glycation End Product Competitive ELISA Kit (Cell Biolabs) and antibodies (see below) are readily available from standard commercial sources as indicated in the manuscript. Cell lines and fish are readily available from the authors.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	We used AKT (9272S) and phospho-AKT (4060S) antibodies from Cell Signaling Technologies. We detected a single band with expected molecular weight when these antibodies were used to probe cell lysates from A. mexicanus skeletal muscle. We used anti-glucagon (Abcam ab36215), and anti-insulin (Dako A0564) antibodies to determine the number of insulin- and glucagon-expressing cells in juvenile A. mexicanus and to quantify glucagon and insulin levels in the serum. Using the antibodies, we detected a signal in the anatomical location of the fish pancreas and when the serum was probed using the antibodies we detected a single band with expected molecular weight.

10. Eukaryotic cell lines

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

The FLP-In-293 cell line was originally purchased from Invitrogen/ThermoFisher (cat#R75007), then cell banked internally at the Stowers Institute.

b. Describe the method of cell line authentication used.

The cell line was sent to *Promega/ATCC for authenticated by Short Tandem Repeat (STR) Profiling. 17 simple sequence repeats (SSR) were used. The test was performed in July 2014.

*Promega and ATCC collaborated as a joint effort in July 2014.

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

The cell line gets tested every quarter. The last two tests were performed on 01/12/17 and 05/09/17, and were both negative. The Universal Mycoplasma Detection Kit (ATCC® 30-1012K™) was used.

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.

n/a

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.

Cave (Pachon, Tinaja, and Molino) and surface populations of Astyanax mexicanus were used in this study. Age and sex of the fish used are indicated in the methods or figure legends for each experiment. The animals used in the study were raised in the lab and are from fish bred in the laboratory with the exception of the wild-caught populations that were sampled for genotype analysis (Figure 3a) and >14-year-old fish that were wild caught and then maintained in the laboratory (Figure 4a). For the zebrafish (Danio rerio) experiments we used the AB line. Age and sex of the fish used are indicated in the methods or main text for each experiment.

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.

This study did not involve human research participants.