

## Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see [Authors & Referees](#) and the [Editorial Policy Checklist](#).

### Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- |                                     |                                                                                                                                                                                                                                                                                                |
|-------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| n/a                                 | Confirmed                                                                                                                                                                                                                                                                                      |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> The exact sample size ( $n$ ) for each experimental group/condition, given as a discrete number and unit of measurement                                                                                                                                    |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly                                                                                                                                    |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> The statistical test(s) used AND whether they are one- or two-sided<br><i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>                                                               |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> A description of all covariates tested                                                                                                                                                                                                                                |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons                                                                                                                                                   |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> A full description of the statistical parameters 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) |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> For null hypothesis testing, the test statistic (e.g. $F$ , $t$ , $r$ ) with confidence intervals, effect sizes, degrees of freedom and $P$ value noted<br><i>Give <math>P</math> values as exact values whenever suitable.</i>                            |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings                                                                                                                                                                      |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes                                                                                                                                                |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Estimates of effect sizes (e.g. Cohen's $d$ , Pearson's $r$ ), indicating how they were calculated                                                                                                                                                                    |

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

### Software and code

Policy information about [availability of computer code](#)

|                 |                                                                                                                                                               |
|-----------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Data collection | Xcalibur (v. 4.1.31.9, Thermofisher), Amsheram Typhoon (1.0.0.7, GE Healthcare), iQ (v 2.9.1, Andor)                                                          |
| Data analysis   | Microsoft Excel for Office 365 MSO, R (v 3.6.2), ImageJ (v 1.51, NIH), Tracefinder (v 4.1, Thermofisher) and Progenesis (2.3.6275.47961, nonlinear dynamics). |

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. 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

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## Field-specific reporting

Please select the one below that is 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/documents/nr-reporting-summary-flat.pdf](https://www.nature.com/documents/nr-reporting-summary-flat.pdf)

## Life sciences study design

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

|                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                            |
|-----------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Sample size     | Statistical tests were not used to predefine sample size. For insulin clamp and related experiments, sample sizes of 8-10 were chosen to demonstrate moderate differences in commonly measured metabolic parameters, and because these are typical sample sizes reported for such experiments in the literature. For the in vivo LbNOX/EtOH experiments and subsequent metabolomics experiments, sample of n=8 were targeted as these numbers were sufficient in hepatocyte experiments to demonstrate differences in secreted aHB levels. |
| Data exclusions | For in vivo mouse experiments involving adenovirus-mediated expression of LbNOX or control, mice were not included in experiments which failed due to technical reasons. A single sample was excluded from figure S3H and from Figure 6B (urate measurements) as they were outliers as determined by Grubbs statistical tests. These criteria were not predefined before performing the experiment.                                                                                                                                        |
| Replication     | All attempts at replication were successful. All data points represent either experiments performed on an individual mouse (for in vivo data), hepatocytes isolated from an individual mouse (for data points in each experimental group in primary hepatocyte data), or an experiment performed on a separate day (for data in each experimental group in cell line experiments).                                                                                                                                                         |
| Randomization   | For in vivo experiments involving LbNOX or luciferase injections, cagemates were randomly assigned to a particular experimental condition. Mass spec runs involved randomization of sample order.                                                                                                                                                                                                                                                                                                                                          |
| Blinding        | Blinding was not performed for experiments described in this paper.                                                                                                                                                                                                                                                                                                                                                                                                                                                                        |

## Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

### Materials & experimental systems

| n/a                                 | Involved in the study                                           |
|-------------------------------------|-----------------------------------------------------------------|
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Antibodies                  |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Eukaryotic cell lines       |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Palaeontology                          |
| <input type="checkbox"/>            | <input checked="" type="checkbox"/> Animals and other organisms |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Human research participants            |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Clinical data                          |

### Methods

| n/a                                 | Involved in the study                           |
|-------------------------------------|-------------------------------------------------|
| <input checked="" type="checkbox"/> | <input type="checkbox"/> ChIP-seq               |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> Flow cytometry         |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> MRI-based neuroimaging |

## Antibodies

|                 |                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                                         |
|-----------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Antibodies used | <ol style="list-style-type: none"> <li>1. GAPDH (Cell Signaling Technology, Catalog #2118, Clone 14C10, Lot 1, 1:1000 Dilution)</li> <li>2. Flag (Cell Signaling Technology, Catalog #2368, Polyclonal, Lot 12, 1:1000 Dilution)</li> <li>3. Pan AKT (Cell Signaling Technology, Catalog #4691, Clone C67E7, Lot 20, 1:1000 Dilution)</li> <li>4. Phospho-AKT2 Ser474 (Cell Signaling Technology, Catalog #8599, Clone D3H2, Lot 2, 1:1000 Dilution)</li> <li>5. Phospho-AKT Thr308 (Cell Signaling Technology, Catalog #13038, Clone D25E6, Lot 5, 1:1000 Dilution)</li> <li>6. Cyclophilin B (Abcam, Catalog #ab178397, Clone EPR12703(b), LotGR317882-10, 1:500 Dilution)</li> <li>7. GCKR (Cell Signaling Technology, Catalog #14328, Clone D1W9P, Lot 1, 1:1000 Dilution)</li> <li>8. TPI aka TIM (Santa Cruz Biotechnology, Catalog #sc-166785, Clone H-11, Lot#B2117, Dilution 1:200)</li> </ol> |
| Validation      | <p>GCKR and Flag antibodies were validated in this paper through detection of adenoviral overexpression of targets of the appropriate molecular weight. pan-AKT was validated by the supplier through detection of recombinant AKT1, AKT2, and AKT3. phospho-AKT2 ser474 was validated by the supplier in genetic knockout of endogenous AKT2 in mouse embryonic fibroblasts. Phospho-AKT Thr308 was not validated by the supplier in a genetic knockout, but has been shown to detect AKT in the context of hPDGF exposure in human cell lines. Cyclophilin B was validated by the supplier in a genetic knockout of endogenous protein in a human cell line. GAPDH and TPI were not validated by the supplier or in this paper.</p>                                                                                                                                                                   |

## Eukaryotic cell lines

Policy information about [cell lines](#)

|                                                                      |                                                                                                                                                                                                            |
|----------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Cell line source(s)                                                  | Hela cells were obtained from ATCC, and then modified as previously described (see Titov et al. Science 2016 DOI: 10.1126/science.aad401). 293A for adenovirus amplification were also obtained from ATCC. |
| Authentication                                                       | The cell lines used in this paper from ATCC were not specifically re-authenticated.                                                                                                                        |
| Mycoplasma contamination                                             | All cell lines were tested for mycoplasma contamination monthly which was negative.                                                                                                                        |
| Commonly misidentified lines<br>(See <a href="#">ICLAC</a> register) | No commonly misidentified lines were used in this paper.                                                                                                                                                   |

## Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

|                         |                                                                                         |
|-------------------------|-----------------------------------------------------------------------------------------|
| Laboratory animals      | All animals used were male C57BL6/J mice purchased from Jackson labs, aged 12-16 weeks. |
| Wild animals            | This study did not involve wild animals.                                                |
| Field-collected samples | This study did not involve field-collected samples.                                     |
| Ethics oversight        | All animal protocols were approved by the MGH IACUC                                     |

Note that full information on the approval of the study protocol must also be provided in the manuscript.