nature portfolio

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Reporting Summary

Nature Portfolio 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 Portfolio policies, see our Editorial Policies 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	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on 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 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)
☐	☒	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

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	No software was used.
Data analysis	GraphPad Prism 8.0 and G*Power (open-source)

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 and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio 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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

All data is available in the manuscript or the supplementary materials.

Field-specific reporting

Life sciences study design

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

Sample size	Group sizes were estimated using open-source G*Power software based on the literature, and adequate power was ensured to detect differences.
Data exclusions	No data were excluded.
Replication	The experiments that do not involve animals were independently replicated at least twice and repeated at least three times within each of the experimental runs.
Randomization	In all the animal studies, animals were randomized.
Blinding	Blinding was performed in animal studies and immunoassays.

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
[ ]	[x] Antibodies
[ ]	[x] Eukaryotic cell lines
[x]	[ ] Palaeontology and archaeology
[ ]	[x] Animals and other organisms
[x]	[ ] Human research participants
[x]	[ ] Clinical data
[x]	[ ] Dual use research of concern

Methods

n/a	Involved in the study
[x]	[ ] ChIP-seq
[x]	[ ] Flow cytometry
[x]	[ ] MRI-based neuroimaging

Antibodies

Antibodies used	Anti-OVA IgG (Abcam #17293), mouse anti-exendin IgG (Abcam #23407), anti-exendin (NBP1-05179H; Novus Biologicals; neutralizing antibody assay only),mouse anti-PEG IgG (Abcam #195350), R-Phycoerythrin-conjugated goat anti-mouse IgG (Jackson Immunoresearch; 115-115-164), biotinylated goat anti-mouse IgM (Jackson Immunoresearch; 115-065-075).
Validation	We tested if the control antibodies (anti-exendin IgG, anti-OVA IgG, anti-PEG IgG, anti-mouse IgG, and anti-mouse IgM) showed any cross-reactivity to the drug-coupled beads by incubating singleplex and multiplexed beads with a single type or multiple types of antibodies. Cross-reactivity of a bead set to a control antibody was calculated as the percent MFI signal of a true positive bead set and was less than 1% for all drug-coupled beads at 1 µg ml-1 antibody concentration. This result indicated that the control reagents were of high specificity.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	HEK293/CRE-Luc/GLP1R
Authentication	The cell line was authenticated via short tandem repeat (STR) analysis by the American Type Culture Collection (ATCC).
Mycoplasma contamination	No mycoplasma contamination was found.
Commonly misidentified lines (See ICLAC register)	Intracellular cAMP concentrations were quantified by treating Human Embryonic Kidney 293 cells recombinantly expressing GLP1R and luciferase fused cAMP (HEK293/CRE-Luc/GLP1R) with exendin variants. This recombinant cell line is one of the most commonly used cell lines for genetic modification to express endogenous receptor of a drug candidate.

Animals and other organisms

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

Laboratory animals	In vivo studies were conducted by employing six-week-old male C57BL/6J (Jackson Laboratories; stock no. 000664) or B6.BKS(D)-LepRdb/J mice (db/db; Jackson Laboratories; stock no: 000697).

Wild animals	No wild animals were used.
Field-collected samples	No field-collected samples were used.
Ethics oversight	In vivo studies were conducted under protocols approved by Duke Institutional Animal Care and Use Committee (IACUC).

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