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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 uesd
Data analysis	Single nucleus RNA-seq data analysis We used the Seek Soul Tools(Version 1.2.1) pipeline to process the cleaned reads and generated the transcript expression matrix. Firstly, the cell barcodes and UMI sequences were extracted based on the defined pattern about the localization of the barcode, linker and UMI within a read. The barcode was corrected with whitelist. The corrected barcode, together with UMI, were put in the header of their corresponding reads. Secondly, the reads were mapped to the reference genomes using STAR (Version 2.5.1b). Then, the reads with barcode and UMI information were assigned to the transcriptome using featureCounts of package Subread (Version 1.6.4). Finally, similar to the raw_feature_bc_matrix results of Cell Ranger, the raw UMI count matrix according to barcodes and transcripts was generated. A cell-calling algorithm was used to filter the raw UMI count matrix and the cell only filtered_feature_bc_matrix was obtained. The code used to process snRNA-seq data can be found at https://http://seeksoul.seekgene.com/en/v1.2.1. Integration, clustering, visualization, and differential expression analysis were performed using Seurat. To compare cellular profiles with other species, the expression data matrix of liver from Melopsittacus undulatus, Xenopus laevis, Pelodiscus sinensi, Mus musculus and Homo sapiens were collected and re-analyzed using the same criteria. We first generated homologous genes of Melopsittacus undulatus, Xenopus laevis, Mus musculus and Homo sapiens through Orthofinder (Version 2.5.4). We considered one-to-one orthology from all species. In order to compare and analyze liver cell profiles from all species mentioned, we used canonical correlation analysis (CCA) to integrate their data as anchor datasets with dims = 1:40 to batch correction. Based on the shared structure, all batches of data were finally pooled into a single object for downstream analysis. For clustering, 2000 variable genes were used, and mitochondrial genes were removed from the variable gene set before running PCA and calculating clusters using 20 principal components, and a resolution of 0.6. The non-linear dimensional reduction technique was used to visualize and explore these datasets. We used the Wilcoxon rank sum test to find differential expression. We identified differentially

expressed genes by the following criteria: (1) P-value ≤ 0.05; (2) log2(fold change [FC]) ≥ 0.2; and (3) the percentage of cells where the gene is detected in a specific cluster > 10%.The code used to Integration, clustering, visualization, and differential expression analysis can be found at https://satijalab.org/seurat.
Statistical analyses were performed on at least three individual datasets and analyzed using GraphPad Prism software (v.9.5.1). Data are mean ± SEM from at least three independent experiments, performed in triplicates. The one-way ANOVA were used for comparisons for three or more groups. The two-way ANOVA were used for comparisons with multiple factors. The student's t-test was used for comparisons between two groups. For dose–response experiments, data were normalized and analyzed using nonlinear curve fitting for the log (agonist) versus response (three parameters) curves. ANCOVA was performed in R software (version 4.4.0). The R package mainly included "multcomp" (version 1.4-26). The exact value of n (number of biological or experimental replicates) can be found in the figure legends.
Statistical significance was defined as: t-test and ANOVA: ****p < 0.0001; ***p < 0.001; **p < 0.01; *p < 0.05, ANCOVA: p < 0.001; p < 0.01; p < 0.05.
List of all the data analysis software:
software version
ImageJ v1.54g
Graph pad v10.2.0
EnVision Manager 1.14.3049.1193
QuantStudioTM Design & Analysis Software v1.5.2
NIS-Elements BR v5.21
Maga v10.2.6
Image labTM Touch Software v2.4.0.03-DEV MODE
TanonImage v1.00
TSE LabMaster v5.2.4
Zen pro 4.0.30319.42000

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

The dataset about single-nucleus and bulk RNA-sequencing have been deposited in the NCBI database (accession ID: PRJNA1209237; PRJNA1211354). Publicly available datasets and databases used were the following: single nucleus RNA-seq data of Danio rerio: GSE181987, Xenopus laevis: GSM6214275, GSM6214276, Mus musculus:GSE166504, Homo sapiens:GSE158723 (https://www.ncbi.nlm.nih.gov/), and Pelodiscus sinensis:J20080062 (https://ngdc.cncb.ac.cn/)

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	has not been collected
Reporting on race, ethnicity, or other socially relevant groupings	N/A
Population characteristics	N/A
Recruitment	N/A
Ethics oversight	N/A

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

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

Life sciences study design

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

Sample size	n=3-7 individuals/groups
Data exclusions	no data excluded
Replication	Data are mean ± SEM from at least three independent experiments, performed in technical triplicate.
Randomization	Since this study involves multiple animal species, to ensure consistency, we first selected animals of the same species, strain, coat color, fur color, body size, and age/gender. These animals were then randomly assigned to each group.
Blinding	We were blinded to group allocation during data collection and analysis

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
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Clinical data
☒	☐ Dual use research of concern
☒	☐ Plants

Methods

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

Antibodies

Antibodies used

GCGR, General gene, UPA61074
beta Actin Ab (HRP), Abways, AB2001
HRP Anti-DDDDK tag, Abcam, ab49763
Goat Anti-Mouse IgG (HRP), Abways, AB0102
Goat Anti-Rabbit IgG (HRP), Abways, AB0101
HRP Anti-DDDDK tag, Sigma, A8592
anti-insulin, Cell Signaling Technnology, 23413S
anti-glucagon, Cell Signaling Technnology, 8233S
Alexa Fluor 488 conjugated Anti-rabbit, Cell Signaling Technnology, 4412S
V5-tag, Cell Signaling Technnology, 13202S

Validation

GCGR constructs from different species with an N-terminal FLAG tag were cloned into pcDNA3.1 (fusion expression) and transiently transfected into HEK293T cells. Western blot (WB) analysis was performed using anti-FLAG and anti-GCGR antibodies to detect protein expression levels, and band size comparison was used to validate the antibody efficacy.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	HEK293 (human fetal kidney), HEK293T (human fetal kidney) cells and AML12 (alpha mouse liver 12) were all obtained from National Collection of Authenticated Cell Cultures (Chinese Academy of Sciences, Shanghai, China). LMH cells (chicken hepatocellular carcinoma epithelial) were obtained from American type culture collection (ATCC, No. CRL-2117)
Authentication	HEK293, HEK293T, AML12, and LMH cells were authenticated by short tandem repeat (STR) testing.
Mycoplasma contamination	Cell lines were tested negatively for mycoplasma by qPCR in the lab

Commonly misidentified lines (See ICLAC register)	no commonly misidentified cell lines used in the study

Animals and other research organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research, and Sex and Gender in Research

Laboratory animals

Common name	Current scientific name	Age	Gender
Placental mammals			
Rhesus monkey	Macaca mulatta	4years	Male
Mouse(C57BL/6JGpt)	Mus musculus	6-8weeks	Male
Norway rat	Rattus norvegicus	3month	Male
Cattle	Bos taurus	8month	Male
Goat	Capra hircus	8month	Male
Non-placental mammals			
Sugar glider	Petaurus breviceps	4month	Male
Birds			
Common ostrich	Struthio camelus	2years	Male
Chinese yellow-feathered broiler	Gallus gallus	60-90day	Male/Female
Chicks	Gallus gallus	30day	Male
Chicken	Gallus gallus	1 month -3 years	Male
Mallard	Anas platyrhyncha	15-17month	Male
Pigeon	Columba livia	140days	Male
Budgerigar	Melopsittacus undulatus	180days	Male
Vinaceous Rosefinch	Carpodacus vinaceus	1-2years	Male
White-rumped munia	Lonchura striata	3month	Male
Common canary	Serinus canaria	8month	Male
Zebra finch	Taeniopygia guttata	5-6month	Male
Ferruginous flycatcher	Muscicapa ferruginea	8month	Male
Green-backed Tit	Parus monticolus	180days	Male
House sparrow	Passer domesticus	1year	Male
Large-billed leaf warbler	Phylloscopus magnirostris	7month	Male
Red-flanked bluetail	Tarsiger cyanurus	8month	Male
Reptiles			
Green anole	Anolis carolinensis	12-14month	Male
Western painted turtle	Chrysemys picta bellii	4years	Male
Leopard gecko	Eublepharis macularius	1.1years	Male
Peking gecko	Gekko swinhonis	3years	Male
Papenfuss rock agama	Laudakia papenfussi	2years	Male
Kashmir rock agama	Laudakia tuberculata	2years	Male
Theobald's toad-headed agama	Phrynocephalus theobaldi	2years	Male
Chinese blue-tailed skink	Plestiodon chinensis	3years	Male
Anan's rock agama	Laudakia sacra	2years	Male
Central bearded dragon	Pogona vitticeps	1year	Male
Painted turtle	Chrysemys picta picta	3years	Male
Siamese crocodile	Crocodylus siamensis	10years	Male
Beauty rat snake	Elaphe taeniura	18month	Male
Beauty snake	Orthriophis taeniurus	24month	Male
Taiwan stink snake	Elaphe carinata	4years	Male
Mongolia racerunner	Eremias argus	2years	Male
Amphibians			
African clawed frog	Xenopus laevis	1years	Male
Dark-spotted frog	Pelophylax nigromaculatus	2years	Male
Fish			
Zebrafish	Danio rerio	3-5month	Male
Channel catfish	Ictalurus punctatus	2years	Male

Wild animals	No wild animal was used in this study
Reporting on sex	males
Field-collected samples	o field collected samples were used in the study.
Ethics oversight	All animal care and experimental procedures complied with the Animal Management Regulations of the Ministry of Health, China (Document No. 55, 2001) and were approved by the Science and Technology Department of Jiangsu Province [IACUC-20231210].

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

Plants

Seed stocks

Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.

Novel plant genotypes

Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.

Authentication

Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.