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	cryoSPARC v4.3.1
Data analysis	Qiagen's CLC Main Workbench version 23.0.2 PipeBio software suite (PipeBio) Flowlogic software (Miltenyi Biotec) Prism 9.5.0 (Graphpad) Phenix version 1.20.1-4487 Coot version 0.9.6.2-pre Pymol Molecular Graphics System version 1.3 Schrödinger software suite 2022-3

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 model coordinates of the pN162 active state MC4R structure is deposited in the Protein Data Bank under accession number 8QJ2. Reported active and inactive state structures are available via PDB accession codes 7F53 (α-MSH), 7PIU (setmelanotide) and 6W25 (SHU9119), respectively.

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	No human participants involved in this study
Reporting on race, ethnicity, or other socially relevant groupings	No human participants involved in this study
Population characteristics	No human participants involved in this study
Recruitment	No human participants involved in this study
Ethics oversight	No human participants involved in this study

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	No sample-size calculation was performed.
Data exclusions	No data were excluded from the analysis.
Replication	All experiments were minimally performed twice successfully leading to data reproduction, number of repeats explicitly indicated in manuscript.
Randomization	Not relevant as single particle cryo-EM analysis is performed by automatic - software driven - processing.
Blinding	Blinding was not performed as no subjective assessment was required.

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: The source of all antibodies (anti-Flag M2, anti-c-Myc 9E10, or goat anti mouse conjugate) and nanobodies (Nb35 / Cb35 and Nb80 / Cb80) used are indicated in the method section of the manuscript (supplier or article reference are provided).

Validation: Validation of the primary detection antibodies (Flag, c-Myc mAbs are both of mouse origin) was performed by adding positive controls (Flag-tagged receptor or c-Myc tagged nanobodies) in the experiment. Binding of the commercial anti mouse - PE conjugate developed in goat was assessed by applying negative control staining conditions in the flow cytometry experiments (staining of pos control cell line in absence of primary detection mouse antibodies). Nb80/Cb80 and Nb35/Cb35 were validated by Sanger sequencing of the constructs prior to use or for Nb35 / Cb35 by adding positive controls (eg Gs-protein) in the complex purification experiment.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s): The source of all cell lines used (including custom cell lines) is clearly described in the method section of the manuscript.

Authentication: The authentication procedure for each cell line included construct sequencing prior to transient transfections or detection of the tagged receptor via a validated anti-tag commercial monoclonal antibody.

Mycoplasma contamination: In house generated cell lines were not consistenly tested for mycoplasma infections. All custom cell lines provided by contract research organizations were assessed for mycoplasma infections minimally prior to transfer from supplier.

Commonly misidentified lines (See ICLAC register): Cells are not listed in database

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: The laboratory animals belong to the Lama glama species and were at the time of the initiation of the vaccination experiment older than 9 months.

Wild animals: *Provide details on animals observed in or captured in the field; report species and age where possible. Describe how animals were caught and transported and what happened to captive animals after the study (if killed, explain why and describe method; if released, say where and when) OR state that the study did not involve wild animals.*

Reporting on sex: *Indicate if findings apply to only one sex; describe whether sex was considered in study design, methods used for assigning sex. Provide data disaggregated for sex where this information has been collected in the source data as appropriate; provide overall numbers in this Reporting Summary. Please state if this information has not been collected. Report sex-based analyses where performed, justify reasons for lack of sex-based analysis.*

Field-collected samples: *For laboratory work with field-collected samples, describe all relevant parameters such as housing, maintenance, temperature, photoperiod and end-of-experiment protocol OR state that the study did not involve samples collected from the field.*

Ethics oversight: The vaccination experiment was executed in accordance with European and local animal welfare legislation and following approval by a local ethical committee.

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.*

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☐ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Sample preparation and source of cells as described in Method section.
Instrument	MACSQuantX cytometer (Miltenyi Biotec), model 5085
Software	Flowlogic software (Miltenyi Biotec)
Cell population abundance	To detect antibody binding, a minimum of 5000 events following below explained gating strategy (corresponding to intact, Topro negative cells) was recorded.
Gating strategy	Gate 1: SSC x FSC (to detect for morphologically uniform population of single cells) Gate 2: APC - R1 channel (to discriminate intact, Topro-3 negative, from permeable, Topro-3 stained cells) Gate 3: PE - B2 channel (to detect for bound control antibody via a Flag staining or Nanobody via a-c-Myc staining)

☐ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.