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Life Sciences Reporting Summary

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Experimental design

1. Sample size

   Describe how sample size was determined.

   All experiments were repeated at least four times (in triplicates). With exclusion of supplemental table 5 we are always showing the raw data. Because these data are dependent on temperature, lot number of reagents and passage number of cells, the values might vary from experiment to experiment. Sample size was determinated using a statistical power calculation based on previous experiments analyzing the MC4R signaling properties.

2. Data exclusions

   Describe any data exclusions.

   For most of the experiments we did not exclude any values. If we have excluded values we perform the Grubbs test and removed an indicated outlier or we excluded a value when known that there was a methodical problem.

3. Replication

   Describe the measures taken to verify the reproducibility of the experimental findings.

   All experiments were performed at minimum of four time always in triplicates, for cAMP and NFAT measurement repetitions were performed more often, e.g. for NFAT analysis we performed four independent experiments. We repeated the NFAT and NFAT + AgRP analysis in four independent experiments and again NFAT and NFAT + PTX in four independent experiments.

4. Randomization

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

   not appropriate

5. Blinding

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

   not appropriate

Note: all in vivo studies must report how sample size was determined and 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 *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☐	☒ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ Test values indicating whether an effect is present *Provide confidence intervals or give results of significance tests (e.g. P values) as exact values whenever appropriate and with effect sizes 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 in all relevant figure captions (with explicit mention of central tendency and variation)

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.	We used GraphPad prism 6 for data evaluation.

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 third party.	Setmelanotide (RM-493) and LY2112688 were provided by Rhythm pharmaceuticals. α-MSH was puchased from MERCK (M4135).

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).	No antibody was used.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.	HEK293 and CHO cells were used
b. Describe the method of cell line authentication used.	authentification was performed by SNP analysis
c. Report whether the cell lines were tested for mycoplasma contamination.	all four weeks a mycoplasm test was performed
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.	not appropriate

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 all relevant details on animals and/or animal-derived materials used in the study.	Mice: BKS.Cg-Dock7m +/+ Leprdb/J stock 000642 (male; 8 weeks old; n= 8) and C57BLKS/J stock 000662 (male; 8 weeks old; n = 8) from Jackson Laboratories. Please note that the rodent data have been excluded from the revised manuscript version.

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.

Patients with rare monogenic forms of obesity have been recruited. Two patients with POMC gene mutations and three patients with LEPR gene mutations have been included into this study. All of them had a history of early onset obesity. They were included after giving inormed consent.