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	MicroCal VP-ITC Analysis Add On Software Package 7.20 for ORIGIN 7.0 Maestro (Schrödinger release 2020 3) BIOVIA Discovery Studio (Version 4.0) BIOVIA Draw (Version 22.1)
Data analysis	Phoenix NLME (Version 8.4) GraphPad Prism (Version 10.2.1) Microsoft Excel for Microsoft 365 (Version 2406 Build 16.0.17726.20078)

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

Source files of the 3D structural models of NNC2215 shown in Figure 1b are available at https://doi.org/10.6084/m9.figshare.26526460. All other data that support the findings of this study are available within this paper, its Supplementary information and the Source data that are provided with this paper. Any additional information is available upon request.

Human research participants

Policy information about studies involving human research participants and Sex and Gender in Research.

Reporting on sex and gender	No data from humans are included.
Population characteristics	No data from humans are included.
Recruitment	No data from humans are included.
Ethics oversight	No data from humans are included.

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 calculations were performed. For the in vitro and ex vivo studies, the sample size was at least three biological replicates to be able to calculate a variability measure in addition to the mean. For the in vivo studies in rats and pigs, three to eight animals were included per group as typical for these types of studies.
Data exclusions	No data were excluded.
Replication	The native MS study included three technical replicates. The binding studies included three independent experiments and each independent experiment included two or three technical replicates. The cell based and in vivo studies included at least three biological replicates. Specific n values are provided in each table/figure legend. All attempts at replication were successful and all experimental data were reproducible between independent experiments.
Randomization	Randomization is not applicable for the included types of studies. For the in vivo studies in rats, animals were stratified into the required number of groups based on body weight.
Blinding	Blinding for experimental group allocation is not applicable for these types of studies. Firstly, to minimize the risk of making unintentional human errors during the experiments, the study protocols were kept as simple as possible, which included working with non-blinded study designs. Secondly, it is unlikely in these types of studies that the results can be affected by knowing the allocation to experimental groups.

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

Methods

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

Antibodies

Antibodies used

Anti-hIR 83.7 (1212-0000-0228; Novo Nordisk; batch 4C), 25 ng per binding reaction
Anti-IGF-1R 24-31 (1212-0000-0131; Novo Nordisk; batch 4B), 25 ng per binding reaction
Quantification of NNC2215: Sandwich assay with combination of antibodies mAb HUI-018 (1212-0000-0087; Novo Nordisk; batch 27B; working concentration: 33.3 µg/mL acceptor beads conjugated with antibody) and biotinylated pAb GP a-HI 4080-E (Novo Nordisk; working concentration: 15 nM = 283x dilution).
Quantification of insulin degludec: Combination of antibodies mAb NN454-1F31 (1212-0000-0096; Novo Nordisk; batch 17B; working concentration: 66.7 µg/mL acceptor beads conjugated with antibody) and biotinylated mAb S1 (1212-0000-0107; Novo Nordisk; batch 4B; working concentration: 3.27 nM).
Quantification of human insulin: Combination of antibodies mAb HUI-018 (1212-0000-0087; Novo Nordisk; batch 27B; working concentration: 33.3 µg/mL acceptor beads conjugated with antibody) and biotinylated mAb OXI-005 (1212-0000-0415; Novo Nordisk; batch 3B; working concentration: 8 nM).
Quantification of pig C-peptide: Combination of antibodies: mAb M-grC-pe-1F34A1 (1212-0000-0583; Novo Nordisk; batch 1B; working concentration: 33.3 µg/mL acceptor beads conjugated with antibody) and biotinylated mAb 4F16A6 (1212-000-0581; Novo Nordisk; batch 2B; working concentration: 12 nM).

Validation

Validation of the specificity of anti-hIR 83.7 for the insulin receptor and anti-IGF-1R 24-31 for the IGF-1R has previously be done by Soos, M. A. et al. (1986) Biochem. J. 235, 199-208 and Soos, M. A. et al. (1992) J. Biol. Chem 267, 12955-12963. The specificity of the batches expressed in-house were qualified by immunoprecipitation and Western blot analysis.
The antibodies for quantification of insulin, insulin analogues and C-peptide were produced at Novo Nordisk. Their identity and quality have been checked by using Nanodrop, SEC-HPLC, LC-MS and SDS Page. The combination of antibodies used in the assays were qualified for purpose by covering the following issues: 1) selectivity (cross reactivity of rat insulin in human insulin assay is <1% and cross reaction of pig insulin in C-peptide assay is <0.01%), 2) sensitivity and range, 3) inter- and intra-assay variability, 4) dilution linearity and 5) interference.

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)

Chinese Hamster Ovary cells (ATTC, Rockville, MD, USA) were transfected with cDNA encoding human insulin receptor (CHO-hIR) as described in Hansen et al. (1996) Biochem J. 315, 271-279.

Authentication

The CHO-hIR cells expressing the cloned human insulin receptor were not authenticated, but grew as expected with the appropriate selection pressure. The morphology was as expected, and it was confirmed that the cells expressed the insulin receptor.

Mycoplasma contamination

Negative for mycoplasma contamination.

Commonly misidentified lines
(See ICLAC register)

No commonly misidentified cell lines were 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

L-glucose rat model: Male Sprague-Dawley rats (11 weeks of age)
Hypoglycaemia study in pigs: Female Landrace-Yorkshire-Duroc pigs (approximately 18 weeks of age)
S.c. and i.v. pharmacokinetic study of NNC2215 in pigs: Female Landrace-Yorkshire-Duroc pigs (approximately 22 weeks of age)
Glucose challenge study in diabetic rats: Male Sprague-Dawley rats (approximately 9-10 weeks of age)

Wild animals

No wild animals were used

Reporting on sex

There is generally no need for sex segregation with insulin pharmacology

Field-collected samples

No field collected samples

Ethics oversight	Danish national Animal Experiments Inspectorate

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