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Reporting Summary

Nature Research 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 Research 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	SerialEM-3.6.11
Data analysis	MotionCor2-1.1.0, GCTF-1.0.6, Gautomatch-0.56, RELION 3.1, cryoSPARC-2.14.2, PHENIX-1.18rc1-3777, Coot-0.8.6, UCSF Chimera-1.12, Pymol-1.7.0.5, Microsoft Excel-2013, GraphPad Prism 6, Chimera X-1.1, SigmaPlot-12.0, phyre2 server, Rosetta Web Server, RaptorX Contact Prediction server, EM builder-1.0

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 Research 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 list of figures that have associated raw data
- A description of any restrictions on data availability

Data supporting the findings of this manuscript are available from the corresponding author upon reasonable request. The cryo-EM map of DUOX1-DUOXA1 in the high-calcium and low-calcium states have been deposited in the EMDB under ID code EMD-30556 [https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-30556] and EMD-30555 [https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-30555]. The atomic coordinate of DUOX1-DUOXA1 in the high-calcium and low-calcium states have been deposited in the PDB under ID code 7D3F [https://doi.org/10.2210/pdb7D3F/pdb] and 7D3E [https://doi.org/10.2210/pdb7D3E/pdb], respectively. The following PDB entries used in this study were downloaded from Protein Data Bank: 6ERC [https://doi.org/10.2210/pdb6ERC /pdb], 5O0T [https://doi.org/10.2210/pdb5O0T /pdb], 4IL1 [https://doi.org/10.2210/pdb4IL1/pdb], 5O0X [https://doi.org/10.2210/pdb5O0X /pdb], 1QFZ [https://doi.org/10.2210/pdb1QFZ /pdb], 6OV2

[https://doi.org/10.2210/pdb6OV2/pdb], 3A1F [https://doi.org/10.2210/pdb3A1F /pdb], and 3KZ1 [https://doi.org/10.2210/pdb3KZ1/pdb]. The cryo-EM map of mouse DUOX1-DUOXA1 used in this study was downloaded from the EMDB under ID code EMD-21964 [https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-21964]. Source Data underlying Fig. 1b and c, Supplementary Fig. 1d and f are provided as a source data file.

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 predetermination of sample size was perform. Sufficient cryo-EM data were collected to achieve adequate map resolution for model building. All of functional experiments were repeated at least 3 times and the sample size is determined based on the reproducibility of the experiments.
Data exclusions	Cryo-EM micrographs with ice or ethane contamination, empty carbon, and poor CTF fit (> 5 Å) were excluded manually. Particles belonging to bad classes were discarded and the data processing flowchart were summarized in Supplementary Figures 2 and 4. These criteria were pre-established and the procedure is a common practise in cryo-EM image analysis.
Replication	All attempts at replication were successful. All attempts at replication were successful according to the detailed protocol described in the methods section. The numbers of replication were described in the figure legends.
Randomization	For cryo-EM 3D refinement, all particles were randomly split into two groups. Randomization was not applicable to other experiments because no covariates were involved.
Blinding	The investigators were blinded to group allocation during cryo-EM data collection and analysis. No blinding was performed in other experiments because there is no comparison inside each experimental group.

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
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

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

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	FreeStyle 293F and Sf9 were from Thermo Fisher Scientific
Authentication	None of the cell line used was authenticated.
Mycoplasma contamination	All cell lines were tested negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.