

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 (<i>n</i>) 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 <i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>
☒	☒ 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. <i>F</i> , <i>t</i> , <i>r</i>) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
☒	☒ 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 <i>d</i> , Pearson's <i>r</i>), 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	Cryo-EM data were collected with the EPU 2.9 software. MD simulations were performed using GROMACS 2021.4.
Data analysis	Cryo-EM data were analyzed with CryoSPARC (v.3.3.1 and 4.2.1), Phenix 1.20.1, ChimeraX 1.6.1, Coot 0.9.8.7, GraphPad Prism 9.0.2, LigPlot+ v.2.2, Clustal Omega(https://www.ebi.ac.uk/jdispatcher/msa/clustalo). Phylogenetic analysis was performed using Muscle (codon-based) implemented in MEGAX, ModelTest-NG (x.y.z), and RAXML-NG (v.1.0.0). Phylogenetic visualization was performed using R (v.4.3.2) packages ggtree 3.10.0, tidyverse 2.0.0, seqinr 4.2-36, ape 5.7-1, phangorn 2.11.1, ggimage 0.3.3, and rsvg 2.6.0.

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 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 cryo-EM density map and coordinate have been deposited in the Electron Microscopy Data Bank (EMDB) and the Protein Data Bank (PDB) under accession number EMD-38240[<https://www.ebi.ac.uk/pdbe/entry/emdb/EMD-38240>], 8XCD[<https://doi.org/10.2210/pdb8XCD/pdb>], respectively.

The GenBank accession numbers of sequences used in the phylogenetic analysis are as follows: NM_003049.4, XM_003824101.4, XM_510035.6, XM_002824890.3, XM_030812638.1, KT382282, KT326156, KT382286, XM_007987122.2, XM_011726495.2, XM_012053949.1, XM_003901980.5, XM_012000900.1, XM_010359747.2, XM_003924480.3, KR153327.1, XM_032245781.1, KT382285, KT382284, XM_001500563.4, XM_537494.7, XM_026008484.1, XM_044232291.1, XM_004739020.3, XM_003987782.5, XM_042943638.1, XM_019451401.2, XM_007095141.3, XM_012438199.2, XM_008052390.1, XM_006091030.3, XM_033108900.1, XM_005686021.2, XM_027971954.2, NM_001046339.1, XM_006186424.3, XM_001927695.5, XM_026385176.1, XM_005322843.4, KT184810, JQ608471, NM_001177561.1, XM_015490579.1, and NM_017047.2. The sequences were downloaded from the NCBI database (<https://www.ncbi.nlm.nih.gov/nucleotide/>).

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	N/A
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](https://www.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	Sample sizes were not predetermined for this study. The size of Cryo-EM data were determined by available time of microscope and the single particle density on grid. For cell-based assay, at least three independent samples were used to ascertain the reproducibility and consistency of experiments.
Data exclusions	For phylogenetic analysis, if the downloaded sequences contained bases upstream of the start codon or downstream of the stop codon, those bases were excluded.
Replication	Cell-based experiments and purification of protein were repeated at least three times in independent experiments. Experimental findings were reproduced reliably. Structural analysis was not repeated because the analysis does not change by replications and does not need to be verified in replication study. A Maximum Likelihood phylogenetic tree was reconstructed using RAxML-NG v. 1.0.0 with 1,000 bootstrap resamplings.
Randomization	Cryo-EM data are randomly divided as two sets (half maps) and used for resolution determination. Randomization is not required to other experiments because there are no unknown covariates.
Blinding	No blinding was needed for this study because experiments performed in this study do not require subject assessment of the data.

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	In house antibody Fab fragment against macaque NTCP (YN69083Fab) for cryoEM analysis, anti-c-Myc (9E10) (mouse monoclonal antibody, sc-40, Santa Cruz Biotechnology) for immunofluorescence (used at 500-fold dilution with blockage solution:KAC, UKB80) and immunoblot analysis (used at 2,000-fold dilution with solution I:TOYOBO, NKB-201), anti-beta actin (mouse monoclonal antibody, A5441, Sigma-Aldrich) for immunoblot analysis (used at 10,000-fold dilution with solution I:TOYOBO, NKB-201).
Validation	Binding of YN69083Fab against macaque NTCP was shown by size-exclusion chromatography.

Eukaryotic cell lines

Policy information about [cell lines and Sex and Gender in Research](#)

Cell line source(s)	Sf9 (Thermo Fisher Scientific, A35243), HepG2 (ATCC, HB-8065; kindly gifted from Dr. Tatsuo Miyamura at National Institute of Infectious Diseases), HepG2-hNTCP-C4 (HepG2 cells overexpressing human NTCP; established in National Institute of Infectious Diseases [Iwamoto et al. Biochem Biophys Res Commun 443: 808-13, 2014])
Authentication	These cells were authenticated by growth properties and cell morphologies according to instruction provided by Thermo Fisher Scientific and ATCC.
Mycoplasma contamination	Mycoplasma contamination was routinely tested every two months for HepG2 and HepG2-hNTCP-C4 cells, and was not tested for Sf9 cells.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

Plants

Seed stocks	N/A
Novel plant genotypes	N/A
Authentication	N/A