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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 Authors & Referees and the Editorial Policy Checklist.

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. 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
☐	☒	An indication of 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 statistics 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
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	SerialEM 3.6.0
Data analysis	GraphPad Prism 7.00, Pymol 1.3, PHENIX 1.13-2998, Coot 0.8.9, RELION 2.0, MotionCor2, Gctf 1.06, Gautomatch 0.56, Chimera 1.10.2, CryoSPARC.

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/reviewers upon request. 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

Atomic coordinates for ABCG2-E1S (5D3-Fab variable domain only), and ABCG2-ATP were deposited in the Protein Data Bank under accession codes 6HCO and 6HBU respectively. EM data for the two structures were deposited in the Electron Microscopy Data Bank under accession codes EMD-0196 (ABCG2-E1S) and

EMD-0190 (ABCG2-ATP). Source data for Figures 2e, f, Extended Data Figures 1e, 2b, d, f and 5 are available online. All other data are available from the corresponding author upon reasonable request. A Life Sciences Reporting Summary for this article is available.

Field-specific reporting

Please select 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/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No statistical methods were used to predetermine sample size. For the ATPase assays (Figure 2e, Extended Data Figures 1e, 2b-f and 5) at least three technical replicates (same batch of liposomes or nanodiscs) were completed with four time points recorded for each replicate. For the transport assays (Figure 2f) at least four technical replicates were completed. For all bar graphs (Figures 2e,f and Extended Data Figure 2d,e) the mean rate for each technical replicate has been plotted. Source Data has been provided.
Data exclusions	No data excluded
Replication	All replicates were successful. The experimental conditions described in the Methods and/or referenced were adhered to as strictly as possible.
Randomization	Animals and humans were not used in the study. Randomisation was not applicable as we used predetermined samples, conditions and time points in our assays.
Blinding	Animals and humans were not used in the study. Blinding was not applicable as we used predetermined samples, conditions and time points in our assays.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☒	☐ Human research participants

Methods

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

Antibodies

Antibodies used	The 5D3-antibody (reference 15 in the manuscript) was provided by B.Sorrentino whom we thank in the acknowledgments.
Validation	The 5D3-antibody was generated specifically for ABCG2 and has been biochemically validated (references 13 and 14 in the manuscript).

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	HEK293 EBNA (Thermo Fisher Scientific).
Authentication	No
Mycoplasma contamination	No

Commonly misidentified lines (See ICLAC register)	N/A