

## 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   |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> The exact sample size ( $n$ ) for each experimental group/condition, given as a discrete number and unit of measurement  |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly  |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> The statistical test(s) used AND whether they are one- or two-sided<br><i>Only common tests should be described solely by name; describe more complex techniques in the Methods section.</i>   |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> A description of all covariates tested   |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons  |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> 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) |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For null hypothesis testing, the test statistic (e.g. $F$ , $t$ , $r$ ) with confidence intervals, effect sizes, degrees of freedom and $P$ value noted<br><i>Give <math>P</math> values as exact values whenever suitable.</i>                            |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings   |
| <input type="checkbox"/>            | <input type="checkbox"/> For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes   |
| <input checked="" type="checkbox"/> | <input type="checkbox"/> 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 Zoo system (X-ray), SerialEM (cryoEM)

Data analysis KAMO, XDS, ccp4, PHENIX, Relion 3.2, MotionCorr2.1, CTFFIND, Coot0.9.2, PYMOL, Chimera, ChimeraX, GraphPad PRISM 4, CLUSTASLW,

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](#)

Atomic coordinates and a cryo EM density map reported in this study have been deposited in the Protein Data Bank (<http://www.rcsb.org>) and the Electron Microscopy Data Bank (<https://ebi.ac.uk/pdbe/emdb/>) as follows;

7EFL : Crystal structure of the gastric proton pump K791S mutant in Rb+-bound (BYK)E2BeF state

7EFM: Crystal structure of the gastric proton pump K791S/E820D/Y340N mutant in Rb+-bound (BYK)E2BeF state

7EFN: Crystal structure of the gastric proton pump K791S/E820D/Y340N/E936V mutant in Rb+-bound (BYK)E2BeF state

7ET1: Cryo-EM structure of the gastric proton pump K791S/E820D/Y340N/E936V/Y799W mutant in K+-occluded (K+)E2-AIF state

EMDB-31294 : Cryo-EM structure of the gastric proton pump K791S/E820D/Y340N/E936V/Y799W mutant in K+-occluded (K+)E2-AIF state

## 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	No statistical method was used to determine sample size.
Data exclusions	No data was excluded
Replication	Biochemical assays, including protein expression, thermal stability and ATPase activity were examined at least three times independently, and their average or representative ones were shown in the manuscript
Randomization	n/a. Animals or human research participants were not involved in this study. Thus sample were not randomized for the experiments.
Blinding	n/a. Animals or human research participants were not involved in this study. No blinding used in this study.

## 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
<input type="checkbox"/>	<input checked="" type="checkbox"/> Antibodies
<input type="checkbox"/>	<input checked="" type="checkbox"/> Eukaryotic cell lines
<input checked="" type="checkbox"/>	<input type="checkbox"/> Palaeontology and archaeology
<input checked="" type="checkbox"/>	<input type="checkbox"/> Animals and other organisms
<input type="checkbox"/>	<input type="checkbox"/> Human research participants
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data
<input type="checkbox"/>	<input type="checkbox"/> Dual use research of concern

### Methods

n/a	Involved in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> ChIP-seq
<input checked="" type="checkbox"/>	<input type="checkbox"/> Flow cytometry
<input checked="" type="checkbox"/>	<input type="checkbox"/> MRI-based neuroimaging

## Antibodies

Antibodies used	anti-FLAG M2 affinity resin
Validation	<a href="https://www.sigmaaldrich.com/catalog/CertOfAnalysisPage.do?symbol=A2220&amp;LotNo=SLBT8835&amp;brandTest=SIGMA&amp;returnUrl=%2Fproduct%2FSIGMA%2FA2220">https://www.sigmaaldrich.com/catalog/CertOfAnalysisPage.do?symbol=A2220&amp;LotNo=SLBT8835&amp;brandTest=SIGMA&amp;returnUrl=%2Fproduct%2FSIGMA%2FA2220</a>

## Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)	HEK293S GnT1- (ATCC CRL-3022)
Authentication	No further authentication was performed for cell lines.
Mycoplasma contamination	Not tested for mycoplasma contamination.
Commonly misidentified lines (See <a href="#">ICLAC</a> register)	No commonly misidentified cell lines were used.

## Human research participants

Policy information about [studies involving human research participants](#)

### Population characteristics

*Describe the covariate-relevant population characteristics of the human research participants (e.g. age, gender, genotypic information, past and current diagnosis and treatment categories). If you filled out the behavioural & social sciences study design questions and have nothing to add here, write "See above."*

### Recruitment

*Describe how participants were recruited. Outline any potential self-selection bias or other biases that may be present and how these are likely to impact results.*

### Ethics oversight

*Identify the organization(s) that approved the study protocol.*

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

## Dual use research of concern

Policy information about [dual use research of concern](#)

### Hazards

Could the accidental, deliberate or reckless misuse of agents or technologies generated in the work, or the application of information presented in the manuscript, pose a threat to:

- | No                       | Yes                      |                            |
|--------------------------|--------------------------|----------------------------|
| <input type="checkbox"/> | <input type="checkbox"/> | Public health              |
| <input type="checkbox"/> | <input type="checkbox"/> | National security          |
| <input type="checkbox"/> | <input type="checkbox"/> | Crops and/or livestock     |
| <input type="checkbox"/> | <input type="checkbox"/> | Ecosystems                 |
| <input type="checkbox"/> | <input type="checkbox"/> | Any other significant area |

### Experiments of concern

Does the work involve any of these experiments of concern:

- | No                       | Yes                      |   |
|--------------------------|--------------------------|---|
| <input type="checkbox"/> | <input type="checkbox"/> | Demonstrate how to render a vaccine ineffective                             |
| <input type="checkbox"/> | <input type="checkbox"/> | Confer resistance to therapeutically useful antibiotics or antiviral agents |
| <input type="checkbox"/> | <input type="checkbox"/> | Enhance the virulence of a pathogen or render a nonpathogen virulent        |
| <input type="checkbox"/> | <input type="checkbox"/> | Increase transmissibility of a pathogen                                     |
| <input type="checkbox"/> | <input type="checkbox"/> | Alter the host range of a pathogen  |
| <input type="checkbox"/> | <input type="checkbox"/> | Enable evasion of diagnostic/detection modalities                           |
| <input type="checkbox"/> | <input type="checkbox"/> | Enable the weaponization of a biological agent or toxin                     |
| <input type="checkbox"/> | <input type="checkbox"/> | Any other potentially harmful combination of experiments and agents         |