

## 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](#).

Please do not complete any field with "not applicable" or n/a. Refer to the help text for what text to use if an item is not relevant to your study.

[For final submission](#): please carefully check your responses for accuracy; you will not be able to make changes later.

### 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 type="checkbox"/>	<input checked="" type="checkbox"/> The exact sample size ( $n$ ) for each experimental group/condition, given as a discrete number and unit of measurement
<input type="checkbox"/>	<input checked="" 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 <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 <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 checked="" 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 Thermo-Scientific EPU 2.10.0

Data analysis WARP v1.0.8, cryoSPARC v2.5.0, Phenix v1.19, COOT 0.8.9.2, Chimera 1.15.0, ChimeraX-1.1.1

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](#)

Cryo-EM maps have been deposited in the Electron Microscopy Data Bank under accession numbers EMD-13062 (DNA-PKcs in complex with NU7441), EMD-13064 (DNA-PKcs in complex with ATPγS), EMD-13067 (DNA-PKcs in complex with wortmannin), EMD-13068 (DNA-PKcs in complex with AZD7648), EMD-13069 (DNA-PKcs in complex with M3814) and EMD-13443 (DNA-PK in complex with ATPγS). Model coordinates have been deposited in the Protein Data Bank under accession numbers 7OTM (DNA-PKcs in complex with NU7441), 7OTP (DNA-PKcs in complex with ATPγS), 7OTV (DNA-PKcs in complex with wortmannin), 7OTW (DNA-PKcs in complex with AZD7648) and 7OTY (DNA-PKcs in complex with M3814). The model referenced for model building (6ZFP) is available in the Protein Data Bank. The Hotspots API is available from <https://github.com/prcurran/hotspots> under the MIT license, dependent upon the commercial CSD Python API.

## 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

## Life sciences study design

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

Sample size	There were no statistical methods used to predetermine sample size. Each cryo-EM dataset was collected independently. The final micrograph and particle numbers are comparable to recent cryo-EM studies in the field for the final reconstruction.
Data exclusions	During the cryo-EM data analysis, bad particles and contamination were discarded through iterative 3D classifications. This procedure is standard in the single particle analysis cryo-EM field.
Replication	The attempts at replication of sample preparation and DNA-PKcs/ligand complex reconstruction for cryo-EM investigation and EMSA were successful.
Randomization	Randomization was not relevant to our study since no clinical trials or drug treatment assays were performed.
Blinding	Blinding was not relevant to our study since no clinical trials or drug treatment assays were performed.

## 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	Involvement in the study
<input checked="" type="checkbox"/>	<input type="checkbox"/> Antibodies
<input checked="" type="checkbox"/>	<input 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 checked="" type="checkbox"/>	<input type="checkbox"/> Human research participants
<input checked="" type="checkbox"/>	<input type="checkbox"/> Clinical data
<input checked="" type="checkbox"/>	<input type="checkbox"/> Dual use research of concern

### Methods

n/a	Involvement 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