## Experimental design

1. **Sample size**
   - Describe how sample size was determined.
   - Standard practices for crystallographic, biochemical and cellbiological experiments were followed.

2. **Data exclusions**
   - Describe any data exclusions.
   - Data exclusion in crystallographic datasets (outlier reflection rejection) was carried out automatically as implemented in the program aimless using pre-established criteria.

3. **Replication**
   - Describe whether the experimental findings were reliably reproduced.
   - All experimental findings were confirmed in at least two, usually three to five independent experiments. See the figure captions for more details.

4. **Randomization**
   - Describe how samples/organisms/participants were allocated into experimental groups.
   - n/a

5. **Blinding**
   - Describe whether the investigators were blinded to group allocation during data collection and/or analysis.
   - n/a

Note: all studies involving animals and/or human research participants must disclose whether blinding and randomization were used.

6. **Statistical parameters**
   - For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

<table>
<thead>
<tr>
<th>Item</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>The exact sample size ((n)) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)</td>
<td>Confirmed</td>
</tr>
<tr>
<td>A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly</td>
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</tbody>
</table>
7. Software

Describe the software used to analyze the data in this study.

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). Nature Methods guidance for providing algorithms and software for publication provides further information on this topic.

8. Materials availability

Materials will be available from the authors upon request. Lys6-specific affimers will be available from Avacta (Wetherby, UK).

9. Antibodies

See the methods section for more details. CISD1 (16006-1-AP, proteintech), GAPDH (AM4300, Ambion), MIRO1 (HPA010687, Atlas Antibodies), Mitofusin 2 (ab56889, abcam), Parkin (ab77924, abcam), TOM20 (FL-145, sc-11414, Santa Cruz Biotechnology), Tubulin (clone DM1A, T6199, Sigma Aldrich), USP30 (HPA016952, Atlas Antibodies), VDAC1 (75-204, Neumob), USP30 (HPA016952, Atlas Antibodies), ubiquitin (Ubi-1, NB300-130, Novus Biologicals), pSer65 ubiquitin (ABS1513-I, Millipore), anti-rabbit or anti-mouse IgG-HRP (NA934V or NXA931, GE Healthcare). All antibodies were validated by the supplier for human samples, and were checked in the lab by Western Blotting on cell lysate and by comparing to the manufacturer’s results.

10. Eukaryotic cell lines

Inducible HeLa Flp-In T-REx cells expressing wild-type Parkin were a gift from W. Harper, Harvard University.

The cell line has not been authenticated. Cells displayed homogeneous, clear HeLa-like morphology and displayed Parkin expression upon dox treatment as expected.

The cell line was tested negative for mycoplasma contamination (Lonza MycoAlert™ Assay)

No commonly misidentified cells were used

11. Description of research animals

n/a

12. Description of human research participants

n/a