| IkBα–UB–UBE2D crosslink (C211 C107A C111D) Substrate Receptor | UBE2D2 2x2way XL | UBE2D3 3way XL | UBE2D3 3way XL | UBE2D3 3way XL | UBE2D2 β-TRCP1 175-C | UBE2D2 β-TRCP1 175-C | UBE2D2 β-TRCP1 175-C | UBE2D2 β-TRCP1 175-C | UBE2D2 β-TRCP1 175-C | none | none |
|---------------------------------------------------------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|-------|
| RBX1                                                          | WT             | WT             | N98R           | N98R           | N98R           | N98R           | WT             | WT             | WT             | yes             | no    |
| NEDD8                                                         | yes            | yes            | yes            | yes            | yes            | yes            | no             | yes            | yes            | yes             | yes   |
| SKP1                                                          | EMD-           | EMD-           | EMD-           | EMD-           | EMD-           | EMD-           | EMD-           | EMD-           | EMD-           | 10585           | 10582          |

**Data collection and processing**

| Microscope       | Krios         | Arctica       | Arctica       | Arctica       | Krios         | Glacios       | Glacios       |
|------------------|---------------|---------------|---------------|---------------|---------------|---------------|---------------|
| Magnification    | 105,000       | 92,000        | 73,000        | 73,000        | 130,000       | 36,000        | 22,000        |
| Voltage (kV)     | 300           | 200           | 200           | 200           | 300           | 200           | 200           |
| Electron exposure (e-/Å²) | 56            | 61.3          | 60.8          | 70            | 70.2          | 60            | 59            |
| Defocus range (µm) | -1.2 ~ -3.6   | -1.5 ~ -3.5   | -1.5 ~ -3.5   | -1.5 ~ -3.5   | -1.2 ~ -3.3   | -1.2 ~ -3.3   | -1.2 ~ -3.3   |
| Pixel size (Å)   | 1.34          | 1.612         | 1.997         | 1.997         | 1.06          | 1.181         | 1.885         |
| Symmetry imposed | C2            | C1            | C1            | C1            | C1            | C1            | C1            |
| Initial particle images (no.) | 2,575,161    | 464,344       | 601,121       | 459,011       | 1,661,870     | 2,051,804     | 1,666,293     |
| Final particle images (no.) | 33,738       | 47,246        | 107,311       | 40,835        | 106,257       | 262,116       | 349,803       |
| Map resolution (Å) | 9.3           | 8.6           | 9.4           | 8.4           | 3.72          | 4.64          | 6.7           |
| FSC threshold    | (0.143)       | (0.143)       | (0.143)       | (0.143)       | (0.143)       | (0.143)       | (0.143)       |
| Map resolution range (Å) | -             | -             | -             | -             | 3.46 ~ 6.0    | -             | -             |

**Refinement**

| Initial model used (PDB code) | 1LDJ 6M90 4P5O 4V3L |
|-------------------------------|----------------------|
| Model resolution (Å) | 3.7 |
| FSC threshold | (0.143) |

| Map sharpening B factor (Å²) | -578.9 | -1159 | -1272 | -983.5 | -94.2 | -199 | -338 |
|-------------------------------|--------|-------|-------|--------|-------|------|------|

**Model composition**

- Non-hydrogen atoms: 13028
- Protein residues: 1616
- Ligands: 3(ZN)

**B factors (Å²)**

- Protein: 88.6
- Ligand: 203.8

**R.m.s. deviations**

- Bond lengths (Å): 0.006
- Bond angles (°): 0.850

**Validation**

- MolProbity score: 2.23
- Clashscore: 13.09
- Poor rotamers (%): 0.07

**Ramachandran plot**

- Favored (%): 91
- Allowed (%): 9
- Disallowed (%): 0