Supplementary Information

Supplementary Tables

Supplementary Table 1 – Data collection and refinement statistics

|                              | HUWE1<sub>N</sub> native | HUWE1<sub>N</sub> SeMet | HUWE1<sub>N</sub> TaBr |
|------------------------------|---------------------------|-------------------------|------------------------|
| **Data collection**          |                           |                         |                        |
| Space group                  | P1                        | P1                      | P1                     |
| Cell dimensions              |                           |                         |                        |
| a, b, c (Å)                  | 95.5, 96.2, 199.7         | 94.4, 95.3, 199.4       | 94.8, 96.2, 201.3      |
| α, β, γ (°)                  | 92.2, 100.5, 95.3         | 92.7, 100.2, 95.1       | 92.2, 99.6, 94.8       |
| Resolution (Å)               | 196-3.05 (3.39-3.05)      | 98.0-3.42 (3.74-3.42)   | 95.8-4.99 (5.22-4.99)  |
| R<sub>pim</sub>              | 0.059 (0.358)             | 0.057 (0.573)           | 0.064 (0.485)          |
| I / σI                       | 5.6 (1.6)                 | 9.1 (1.5)               | 13.1 (1.7)             |
| Ellipsoidal completeness (%) | 89.4 (68.2)               | 84.9 (5.2)              | 91.6 (92.3)            |
| Redundancy                   | 3.8 (3.8)                 | 10.7 (11.0)             | 13.9 (12.8)            |
| **Refinement**               |                           |                         |                        |
| Resolution (Å)               | 96-3.04                   |                         |                        |
| No. reflections              | 75499 (3733)              |                         |                        |
| R<sub>work</sub> / R<sub>free</sub> (%) | 20.24/23.30             |                         |                        |
| No. atoms                    |                           |                         |                        |
| Protein                      | 34901                     |                         |                        |
| Ligand/ion                   | 0                         |                         |                        |
| Water                        | 0                         |                         |                        |
| B-factors (Å²)               |                           |                         |                        |
| Protein                      | 104.8                     |                         |                        |
| Ligand/ion                   | -                         |                         |                        |
| Water                        | -                         |                         |                        |
| R.m.s. deviations            |                           |                         |                        |
| Bond lengths (Å)             | 0.006                     |                         |                        |
| Bond angles (°)              | 0.85                      |                         |                        |
### Supplementary Table 2 – Cryo-EM data collection, refinement and validation statistics

| Data collection and processing | Class 1 (EMDB-12318) (PDB 7NH1) | Class 2 (EMDB-12319) (PDB 7NH3) |
|------------------------------|----------------------------------|----------------------------------|
| Magnification                | 105,000x                         | 105,000x                         |
| Voltage (kV)                 | 300                              | 300                              |
| Electron exposure (e-/Å²)     | 60.4                             | 60.4                             |
| Defocus range (μm)           | -2 to -3.5                       | -2 to -3.5                       |
| Pixel size (Å)               | 0.86                             | 0.86                             |
| Symmetry imposed             | C1                               | C1                               |
| Initial particle images (no.)| 766368                           | 766368                           |
| Final particle images (no.)  | 56284                            | 57866                            |
| Map resolution (Å)           | 5.29                             | 6.37                             |
| FSC threshold                | 0.143                            | 0.143                            |
| Map resolution range (Å)     | 4.7-8.8                          | 4.7-8.8                          |

### Refinement

| Initial model used (PDB code) | 7BII                             | 7BII                             |
| Model resolution (Å)          | 7.4                              | 8.1                              |
| FSC threshold                 | 0.5                              | 0.5                              |
| Model resolution range (Å)    | -137                             | -178                             |
| Map sharpening B factor (Å²)   |                                   |                                  |
| Model composition             |                                   |                                  |
| Non-hydrogen atoms            | 17417                            | 17417                            |
| Protein residues              | 2138                             | 2138                             |
| Ligands                       | 0                                | 0                                |
| R.m.s. deviations             |                                   |                                  |
| Bond lengths (Å)              | 0.003                            | 0.003                            |
| Bond angles (°)               | 0.639                            | 0.654                            |
| Validation                    |                                   |                                  |
| MolProbity score              | 1.99                             | 2.02                             |
| Clashscore                    | 15.58                            | 16.86                            |
| Poor rotamers (%)             | 0                                | 0                                |
| Ramachandran plot             |                                   |                                  |
| Favored (%)                   | 95.7                             | 95.8                             |
| Allowed (%)                   | 4.23                             | 4.24                             |
| Disallowed (%)                | 0.05                             | 0                                |
Supplementary Figure 1 – CryoEM data processing workflow. (a) Representative summed micrograph from 2738 total used micrographs. (b) 2D classification results showing different views (c) Processing workflow. (d-f) The Euler angle distribution (d), local resolution maps (e), and Fourier shell correlation curves (f) for Class 1 and Class 2.