**Supplementary File 2. Summary of cryo-EM data for the Pgp mutants.**

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| **Pgp mutants** | **Structures** | **Resolution** | **Conformation** | **Bound cyclopeptides** | **Bound nucleotides** |
| Pgp335 (L335C/TM6) | IF335-2lig | 3.8 Å | Inward-facing | 2 ligands (1 covalent and 1 non-covalent) | Not present |
| OF335-apo | 2.9 Å | Outward-facing | No ligand | ATP |
| OF335-nolig | 2.6 Å | Outward-facing | No ligand | ATP |
| OF335-1lig | 2.6 Å | Outward-facing | 1 ligand (covalent) | ATP |
| OF335-2lig | 3.1 Å | Outward-facing | 2 ligands (1 covalent and 1 non-covalent) | ATP |
| Pgp978 (V978C/TM12) | IF978-2lig | 4.7 Å | Inward-facing | 2 ligands (1 covalent and 1 non-covalent | Not present |
| OF978-1lig | 3.0 Å | Outward-facing | 1 ligand (covalent) | ATP |
| Pgp971 (L971C/TM12) | IF971-1lig | 4.3 Å | Inward-facing | 1 ligand (non-covalent) | Not present |
| OF971-1lig | 3.0 Å | Inward-facing | 1 ligand (covalent) | ADP/vanadate |