

## Supplementary information, Table S2

### Cryo-EM data collection, refinement and validation statistics

|   | Inactive GABA <sub>B</sub> receptor | Active GABA <sub>B</sub> receptor    |
|---|-------------------------------------|--------------------------------------|
| <b>Data collection and processing</b>               |                                     |                                      |
| Magnification                                       | 4,9310                              | 4,9310                               |
| Voltage (kV)  | 300                                 | 300                                  |
| Electron exposure (e <sup>-</sup> /Å <sup>2</sup> ) | 62                                  | 62                                   |
| Defocus range (μm)                                  | -0.5 ~ -2.5                         | -0.5 ~ -2.5                          |
| Pixel size (Å)                                      | 1.014                               | 1.014                                |
| Symmetry imposed                                    | C1                                  | C1                                   |
| Initial particle images (no.)                       | 2,969,413                           | 3,075,533                            |
| Final particle images (no.)                         | 374,589                             | 237,606                              |
| Map resolution (Å)                                  |                                     |                                      |
| (Overall/VFT/TM)                                    | 2.9/2.8/3.0                         | 3.0/2.9/3.0                          |
| FSC threshold                                       | 0.143                               | 0.143                                |
| Map resolution range (Å)                            | 2.2-4.0                             | 2.2-4.0                              |
| <b>Refinement</b>                                   |                                     |                                      |
| Initial model used (PDB code)                       | 4MR7                                | inactive GABA <sub>B</sub> structure |
| Model resolution (Å)                                | 2.9                                 | 3.3                                  |
| FSC threshold                                       | 0.5                                 | 0.5                                  |
| Model resolution range (Å)                          | 2.5-4.0                             | 2.5-4.0                              |
| Map sharpening <i>B</i> factor (Å <sup>2</sup> )    |                                     |                                      |
| (Overall/VFT/TM)                                    | 83.6/84.1/67.6                      | 78.5/80.9/190.3                      |
| Model composition                                   |                                     |                                      |
| Non-hydrogen atoms                                  | 10915                               | 10894                                |
| Protein residues                                    | 1355                                | 1348                                 |
| Ligands   | 9                                   | 10                                   |
| <i>B</i> factors (Å <sup>2</sup> )                  |                                     |                                      |
| Protein   | 61.64                               | 66.88                                |
| Ligand  | 63.76                               | 88.08                                |
| R.m.s. deviations                                   |                                     |                                      |
| Bond lengths (Å)                                    | 0.006                               | 0.004                                |
| Bond angles (°)                                     | 0.741                               | 0.684                                |
| Validation  |                                     |                                      |
| MolProbity score                                    | 1.69                                | 1.65                                 |
| Clashscore  | 8.01                                | 7.15                                 |
| Poor rotamers (%)                                   | 0.00                                | 0.00                                 |
| Ramachandran plot                                   |                                     |                                      |
| Favored (%)   | 96.27                               | 96.18                                |
| Allowed (%)   | 3.73                                | 3.82                                 |
| Disallowed (%)                                      | 0.00                                | 0.00                                 |