Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

**Sample size**
- All of functional experiments were performed in biological triplicates \((n=3)\) in order to allow for the calculation of mean values and the standard error of the mean.

**Data exclusions**
- Cryo-EM micrographs with ice or ethane contamination, empty carbon, and poor CTF fit \((> 5 \text{ Å})\) were excluded manually. Particles belonging to bad classes were discarded and the data processing flowchart were summarized in Supplementary Figures. These criteria were pre-established and the procedure is a common practice in cryo-EM image analysis.

**Replication**
- All attempts at replication were successful according to the detailed protocol described in the methods section. The numbers of replication were described in figure legends.

**Randomization**
- For cryo-EM 3D refinement, all particles were randomly split into two groups. Samples were not allocated into groups for functional experiments, this randomization is not relevant for this study.

**Blinding**
- The investigators were blinded to group allocation during cryo-EM data collection and analysis. Blinding is not relevant for protein structure determination and functional assays because these results are not subjective. Our procedure complies with the common practice in the field.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

### Materials & experimental systems

| n/a | Involved in the study |
|-----|-----------------------|
| ☑   | Antibodies            |
| ☐   | Eukaryotic cell lines |
| ☑   | Palaeontology and archaeology |
| ☑   | Animals and other organisms |
| ☑   | Human research participants |
| ☑   | Clinical data         |
| ☑   | Dual use research of concern |

### Methods

| n/a | Involved in the study |
|-----|-----------------------|
| ☑   | ChIP-seq              |
| ☑   | Flow cytometry        |
| ☑   | MRI-based neuroimaging |

### Antibodies

**Antibodies used**
- Primary antibodies were mouse anti-Strep II tag [Eurbox, cat. no 022140-G3, dilution 1: 5,000 for Western blot], and rabbit anti-HA tag [Cell Signaling Technology, cat. no 3724, dilution 1: 2,000 for Western blot]. The secondary antibodies were goat anti-mouse HRP conjugated IgG [Invitrogen, cat. no 31444, dilution 1: 10,000 for Western blot], and goat anti-rabbit HRP conjugated IgG [Invitrogen, cat. no 31460, dilution 1: 10,000 for Western blot].

**Validation**
- Mouse anti-Strep II tag antibody was validated using Western blot performed in HEK293F cells transfected with strep tagged GFP and strep tagged 5G.1T where bands were only detected in cells with over-expressed strep tagged protein at correct molecular weight.
- Rabbit anti-HA tag was validated by manufacturer [https://www.cellsignal.cn/products/primary-antibodies/ha-tag-c29f4-rabbit-mab/3724?_=16426640009006&nnt=3724&ahead=true]
- Goat anti-mouse HRP conjugated IgG was validated by manufacturer [https://www.thermofisher.cn/cn/zh/antibody/product/Goat-ant-Mouse-IgG-HRP-L-Secondary-Antibody-Polyclona/31444]
- Goat anti-rabbit HRP conjugated IgG was validated by manufacturer [https://www.thermofisher.cn/cn/zh/antibody/product/Goat-ant-Rabbit-IgG-HRP-L-Secondary-Antibody-Polyclona/31460]

### Eukaryotic cell lines

**Policy information about** [cell lines](#)

**Cell line source(s)**
- SF9 and HEK293F cells were from Thermo Fisher Scientific. A0293 cell was from Agilent.

**Authentication**
- None of the cell line used was authenticated.

**Mycoplasma contamination**
- All cell lines were tested negative for mycoplasma contamination.
No commonly misidentified cell lines were used.