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Reporting Summary

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☒	☐	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☒	☐	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☒	☐	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated
☐	☒	Clearly defined error bars *State explicitly what error bars represent (e.g. SD, SE, CI)*

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection	EPU software from FEI was used to collect cryo-EM data. MassLynx software from Waters was used to collect the H/DX-MS data.
Data analysis	Cryo-EM reconstructions were performed with SPHIRE 2017.06.02. Molecular dynamics simulations were performed with NAMD 2.12 and VMD 1.9.3. Molecular figures were created using VMD 1.9.3,Chimera 1.11.2 and MacPymol v1.7.6.4.. Atomic model refinement was performed with Rosetta 2017.08 and 2016.32. Shape complementarity was calculated with SC, included in ccp4 v6.5. Movie frames of the PTC3 and PTC3-D651A dataset were aligned with Motioncorr 1 and Unblur 1.0.2, respectively. Particle picking ofPTC3 dataset was performed with Gautomatch v0.56 Diffraction data were processed and analysed and with XDS, Phaser and the resulting structure was optimized in Coot for further refinement with PHENIX. PLGS 3.0 (Waters) was used to map peptides for the H/DX-MS experiment. H/DX-MS data were analyzed using DynamX 3.0 (Waters).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The densities and atomic coordinates of ABCwt and ABCD651A have been deposited in the EMDB and PDB under the accession numbers EMD-0149, PDB 6H6E and EMD-0150, PDB 6H6F, respectively. The atomic coordinates of the crystal structure of TcdB2/TccC3 without hvr has been deposited in the PDB under the accession number PDB 6H6G. The HDX source data is available under Source Data Figure 4. The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences study design

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

Sample size	BLI experiments: 7 samples were chosen per measurement, determined by the design of the Octet Red 384 instrument enabling 8 parallel measurements with one data point as the negative control. The global fit included all curves with sufficient signal-to-noise level. HDX: H/DX-MS experiments were performed in duplicate, as is standard in the field.
Data exclusions	BLI experiments: All curves were included in the global fit if the signal-to-noise level was sufficient. In the case of empty TcB-TcC, five of seven curves had a sufficient signal-to-noise level to be included in the fits (Extended Data Figure 3). In the case of TcB-D528-534-2Gly-TcC, six of seven curves had a sufficient signal-to-noise level to be included in the fits (Extended Data Figure 5). HDX: No data were excluded.
Replication	BLI experiments: The BLI runs and global fits were replicated at least two times. Replicates confirmed the K(D) values within a factor of two. Gel filtration and negative stain EM (Extended Data Fig 3 und 5): Experiments were replicated at least one time with qualitatively identical results. Intoxication experiments were replicated two times with qualitatively identical results. HDX: All attempts at replication were successful.
Randomization	n/a
Blinding	n/a

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☒	☐ Human research participants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging