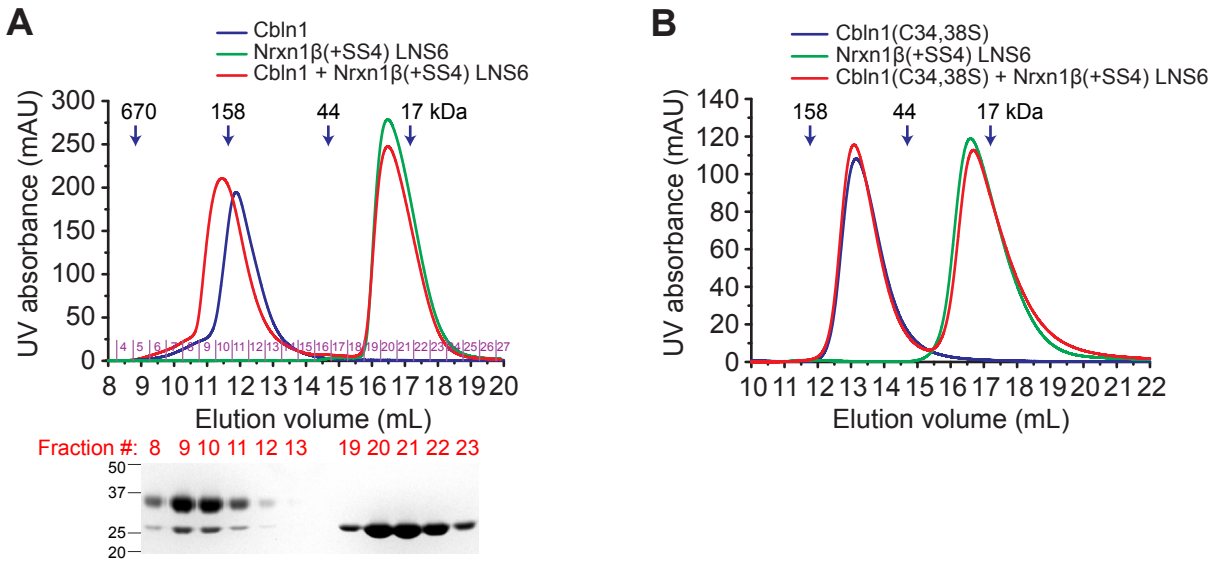

Figure S1. Related to Figure 1. The LNS6 domain of Nrxn1 is sufficient for binding to Cbln1, and the disulfide-forming cysteines in the CRN domain of Cbln1 are necessary for Nrxn1 binding.

A. Cbln1 binds the LNS6 domain of Nrxn1β(+SS4) only. The LNS6 only construct of Nrxn1β includes residues H82 to P292. Fractions from the Cbln1 + Nrxn1β(+SS4) LNS6 chromatogram (red) are run on a non-reducing, denaturing 12% polyacrylamide gel.

B. Cbln1(C34,38S) double mutant does not bind Nrxn1β(+SS4) LNS6.

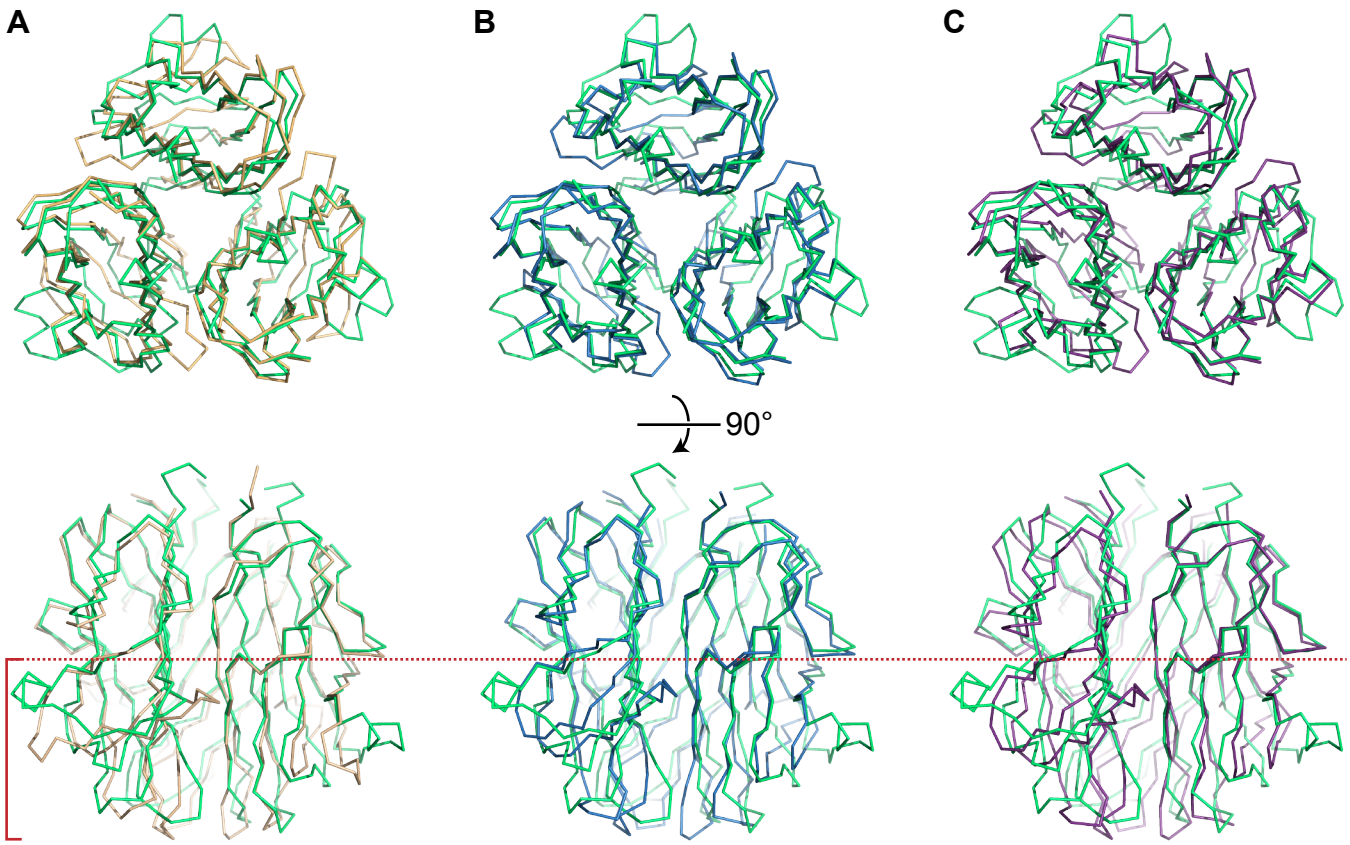

Figure S2. Related ro Figure 3. Structure of the cleaved Cbln1 and its comparison to other C1q domain proteins.
Comparison of trimers of Cerebellin C1q domains (green) with those of **(A)** human Caprin-2 (PDB ID: 4OUL), **(B)** mouse C1QL-1 (PDB ID: 4D7Y), and **(C)** mouse C1QL-3 D207A (Ca^{2+}-binding mutant; PDB ID: 4QQP). The bottom half of the C1q domain, highlighted by a red bracket, of Cerebellin varies significantly from the other C1q-domain proteins.

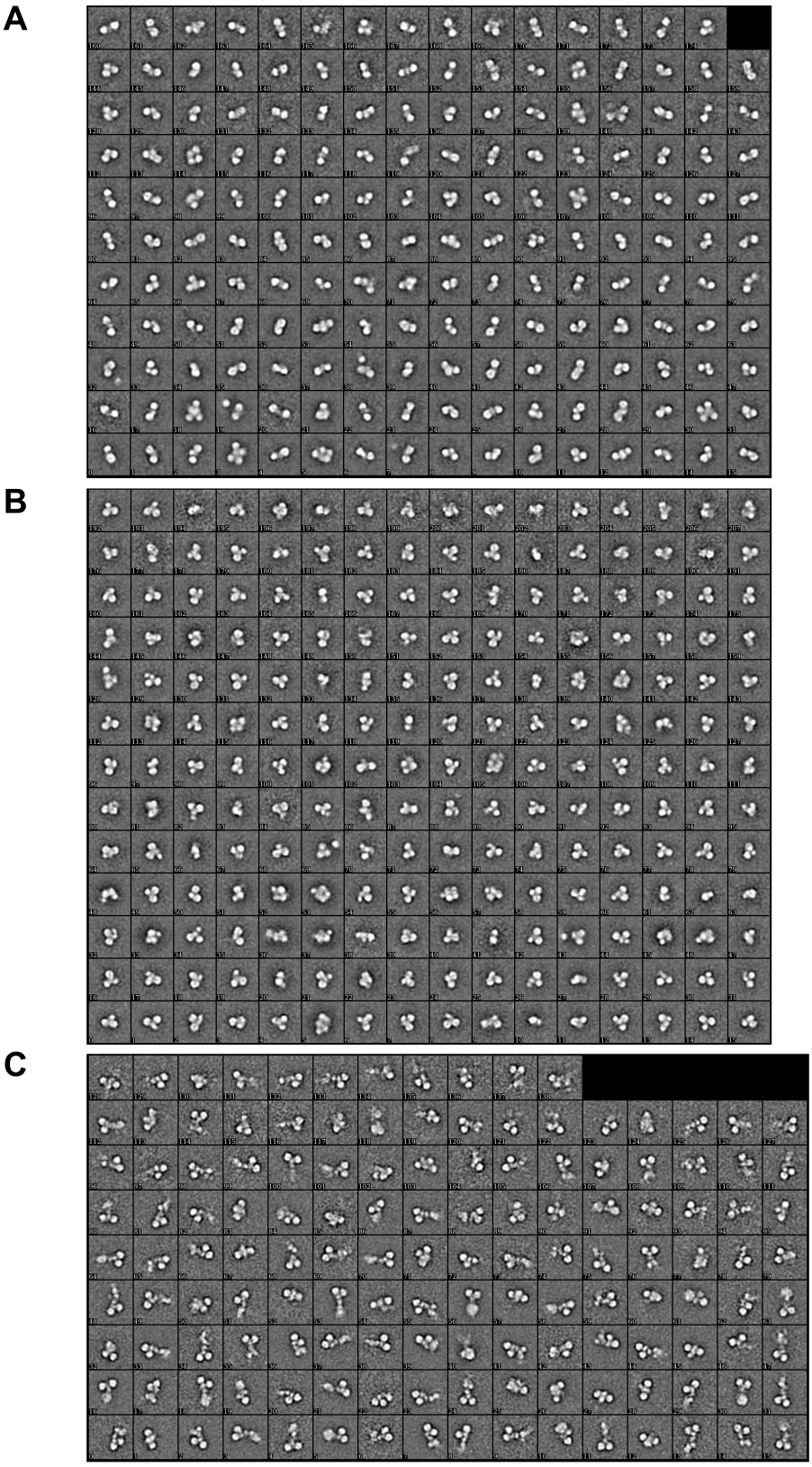

Figure S3. Related to Figure 4. 2D EM class averages of negative-stained (A) rat Cbln1 (full-length), (B) rat Cbln1 + Nrxn1β(+SS4) LNS6 (residues His82 to Asn292), and (C) rat Cbln1 + Nrxn1α(+SS4) LNS2 to LNS6 (residues Glu296 to Pro1330).

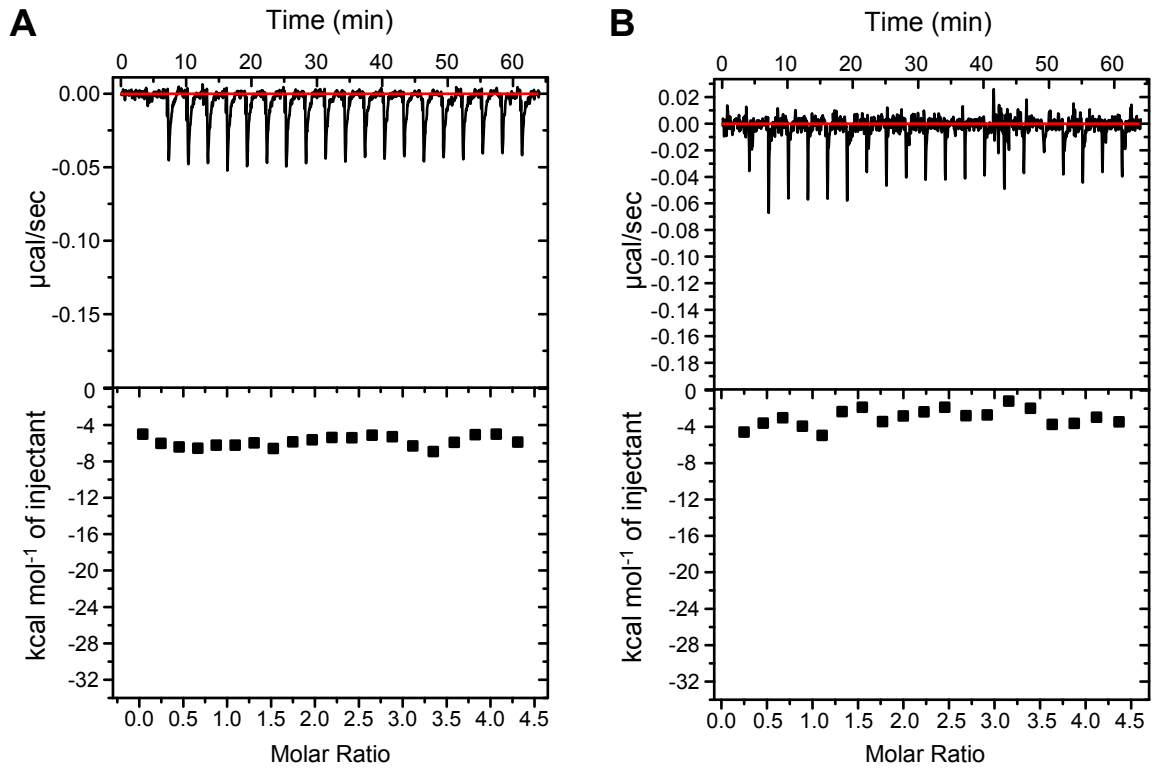

Figure S4. Related to Figure 5. Isothermal titration calorimetry experiments for binding of Cbln1 to tetramerized ATD of GluD2 (GluD2 ATD-4Z) in the absence of Calcium (A), and in the presence of 3 mM $CaCl_2$ (B). The molar ratio reported is the ratio of Cbln hexamers to GluD2 tetramers. The expected ratio is 2 (two hexamers to one tetramer).

Supplemental Figure Legends

Figure S1. Related to Figure 1. The LNS6 domain of Nrxn1 is sufficient for binding to Cbln1, and the disulfide-forming cysteines in the CRN domain of Cbln1 are necessary for Nrxn1 binding.

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Supplemental Experimental Procedures

Multi-angle light scattering (MALS) analysis

For MALS, ASTRA software version 5.3.4 was used (Wyatt Technology Corp). Line broadening effects were accounted by in the Cerebellin-1 alone curves, but could not be effectively applied in the Cbln1–Nrxn1β measurements. *dn*/*dc* values used in the analysis were calculated by mass-weighted averages of protein (0.185) and glycan (0.142) *dn*/*dc* values. For this analysis, we assumed two N-linked glycans on rat Cbln1 (N_{23}ETE and N_{79}RTM) and one on Neurexin-1 (N_{1246}ATL in α numbering), where each insect N-glycan group added 1.3 kDa of mass.

Crystallization of Cerebellin-1

All proteins were secreted with C-terminal hexahistidine tags using baculovirus-infected High Five cells in serum-free Insect XPRESS medium (Lonza). For crystallization, rat Cerebellin-1 was purified over Ni^{2+}-NTA Agarose resin, followed by size-exclusion chromatography using a Superdex 200 Increase 10/300 column. Purified Cbln1 was mixed with Neurexin-1β, and the complex was purified over the same size-exclusion column. For crystallization, we used a Mosquito crystallization robot (TTP Lab Biotech) and a sitting-drop vapor diffusion strategy with 100 nl of protein + 100 nl of crystallization condition. We could grow similar crystals from both Cbln1 alone and Cbln1–Nrxn1β samples, after they were treated with Carboxypeptidase A and B to remove the hexahistidine tags, indicating that the crystals contained hexahistidine-tag-(partially) removed Cbln1 only. The best crystal diffracting to 1.8 Å resolution was grown from a 22 mg/ml Cbln1–Nrxn1β LNS6 (+SS4) complex using 0.1 M Tris, pH 7.5, 3 M Sodium formate at 21°C. This crystal was cryo-protected in 0.083 M Tris, pH 8.0, 1.0 M Potassium/sodium tartrate, 25% Glycerol.

The structure of Cerebellin-1 C1q trimer

Here, we report a 1.8-Å structure of the trimeric C1q domain of Cerebellin-1. The structure shows a canonical C1q architecture of mostly β-strand in nature, where 51% of residues are assigned as extended and only 7% as helical by DSSP over 137 residues observed in our crystal structure. In the Protein Databank, Cerebellin is most similar to the zebrafish and human Caprin-2 structures, which are 39-40% identical to rat Cbln1 over 132 residues aligned, and mammalian C1QL-1 to -3, at 37% identity over 134 residues (Kakegawa et al., 2015; Miao et al., 2014; Ressl et al., 2015). Structural alignments of Cbln1 to Caprin-2 and C1QL-1 demonstrate shared topology of secondary structural elements (Figures S2A-C).

The C1q like domain of Caprin-2 and C1QLs are known to bind Calcium ions at their trimeric interface (Kakegawa et al., 2015; Miao et al., 2014; Ressl et al., 2015). Neither the sample buffer not the crystallization condition for Cerebellin included any calcium, and the electron density maps do not support the presence of calcium or a calcium coordination sphere by Cerebellin residues. Overall, Cbln1 did not show any signs of a dissociation of trimers, and was still able to bind Neurexin with high affinity in the absence of Calcium ions (Figures 1, 2 and 4).

X-ray crystallography for GluD2 ectodomain

GluD2 ATD+LBD was crystallized using protein at 10.4 mg/ml with 0.1 M Sodium cacodylate, pH 6.6, 1.3 M $NH_4H_2PO_4$. Crystals were cryo-protected in 0.1 M Sodium citrate, pH 5.6, 1 M ammonium sulfate, 30% Glycerol. 399 images out of the 600 collected were deemed to be undamaged by radiation, based on scaling B-factors reported by *HKL2000* (Otwinowski and Minor, 1997). Data scaled with acceptable statistics in $P6_{2/4}22$; however, tests indicated the presence of twinning. Therefore, data was re-processed in all subgroups despite no improvements to redundancy-corrected scaling *R*-merge statistics, indicating that twinning was likely to be near-perfect. Molecular replacement in *PHASER* (McCoy et al., 2007) revealed the correct space group to be $P3_221$. Molecular replacement searches were done with three copies each of a GluD2 ATD monomer (PDB: 5KC8) and a GluD2 LBD monomer (PDB: 2V3T). NCS-averaged maps of GluD2 electron density were created in *Coot* (Emsley et al., 2010).

Supplemental References

All Supplemental References are also cited in the main article.