MOLECULAR CHARACTERIZATION OF DISRUPTED IN SCHIZOPHRENIA-1 RISK VARIANT S704C REVEALS THE FORMATION OF ALTERED OLIGOMERIC ASSEMBLY

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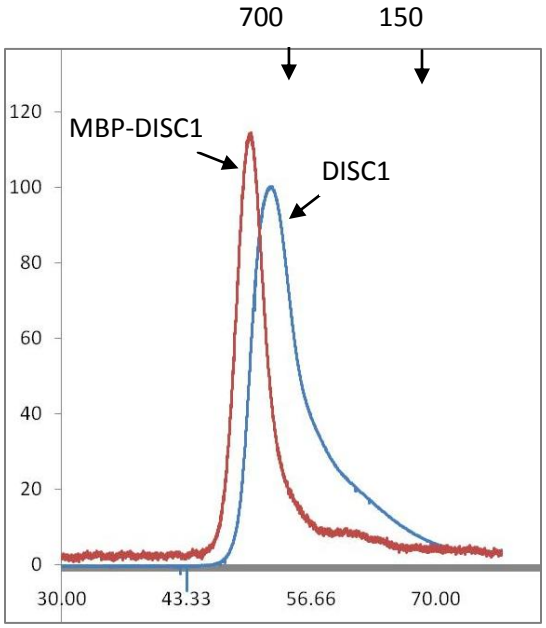
Supporting information:

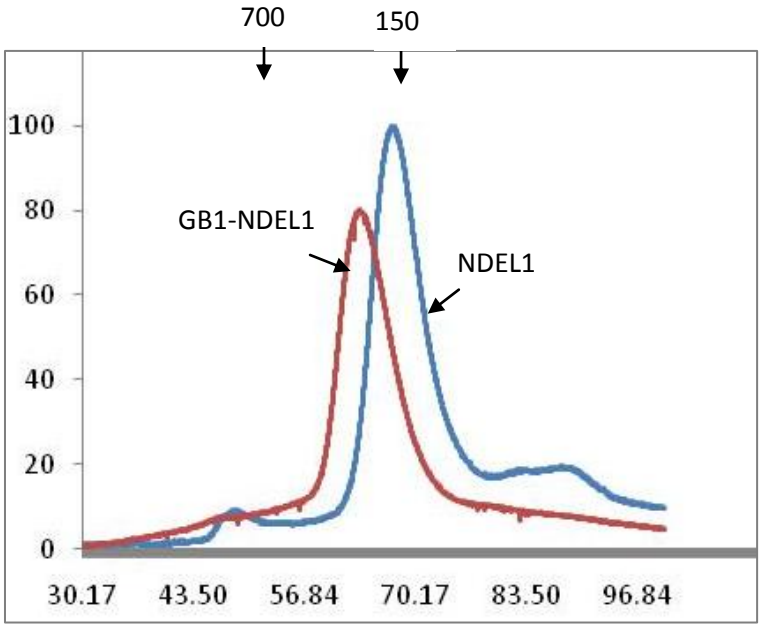
Fig. S1 Fusion tags did not influence oligomerization of (a) DISC1 (b)/NDEL1 as well as (c) NDEL1 binding

a)MBP-DISC1 vs DISC1 oligomerization



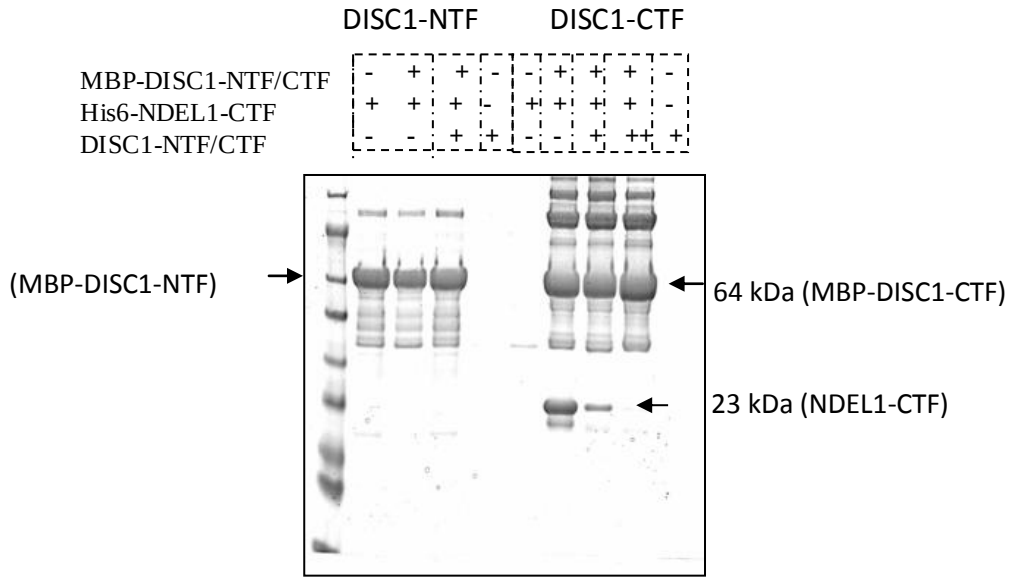
Retention time (min)

b)GB1- NDEL1 vs NDEL1 oligomerization



Retention time (min)

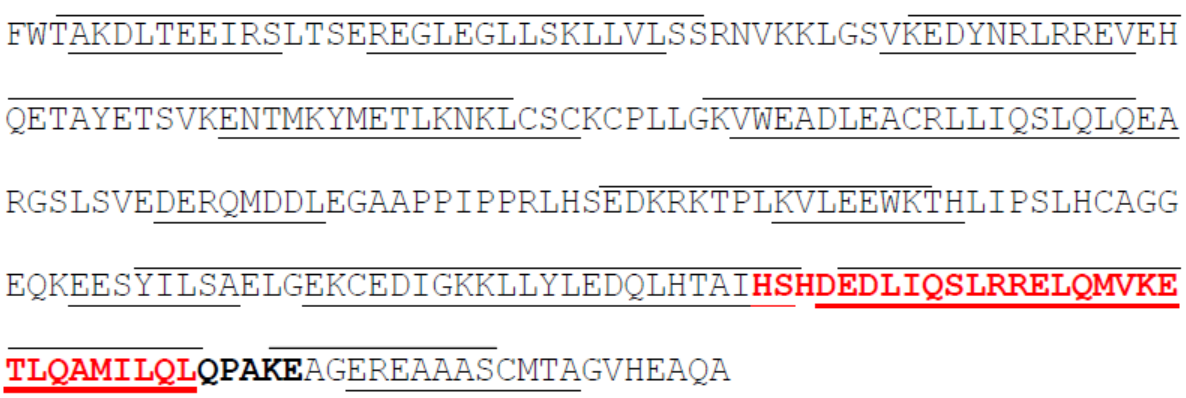
c) Pull-down assay confirm no influence of fusion tag on the complex formation



Size exclusion chromatography profile of a) MBP-DISC1 (Red) and DISC1(Blue) and b) GB1-NDEL1(Red) and NDEL1(Blue). The addition of MBP/GB1 tag did not influence the oligomerization of DISC1/NDEL1, as predominant oligomeric DISC1/NDEL1 peak remain intact. C. In order to show that the fusion protein has no influence on binding to NDEL1, we used two DISC1 constructs (supplementary table S1), MBP-DISC1 N-terminus (a.a 72-290) and MBP-DISC1C-terminus (a.a 680-832), in which the later contains the

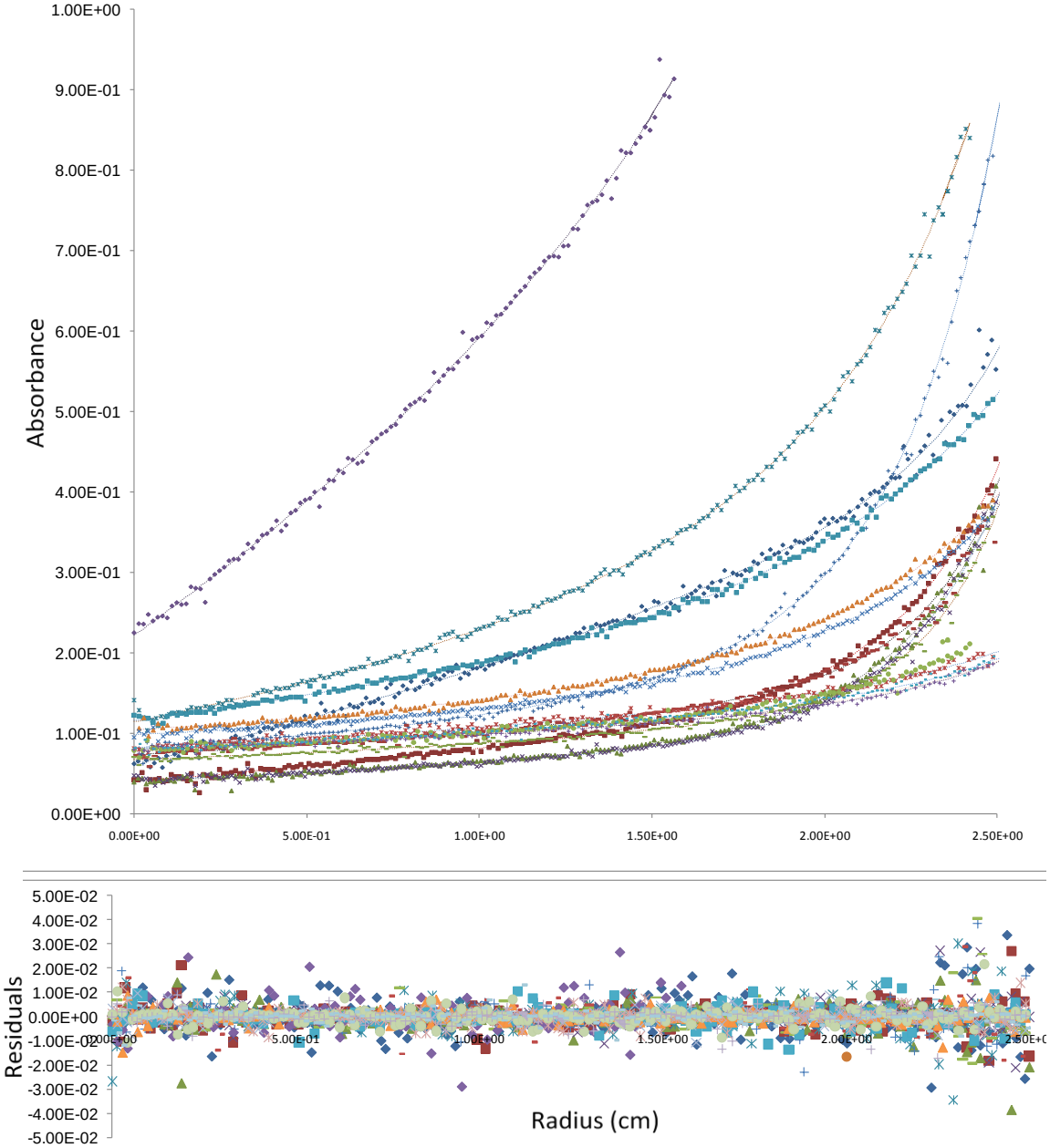
NDEL1 binding site. Lysates of these expressed proteins together with NDEL1-CTF were co-incubated in amylose beads . After 3 hours, the beads were washed and the eluted proteins were subjected to SDS-PAGE analysis. His_6 -DISC1 C-terminus or N-terminus were used to compete for the binding. The C-termimal domain that encompasses NDEL1 binding site could pull-down the NDEL1 binding domain, while N-terminal DISC1 could not, clearly shows that the MBP has no influence in binding.

Fig: S2 DISC1 Secondary structure prediction and NDEL1 binding site



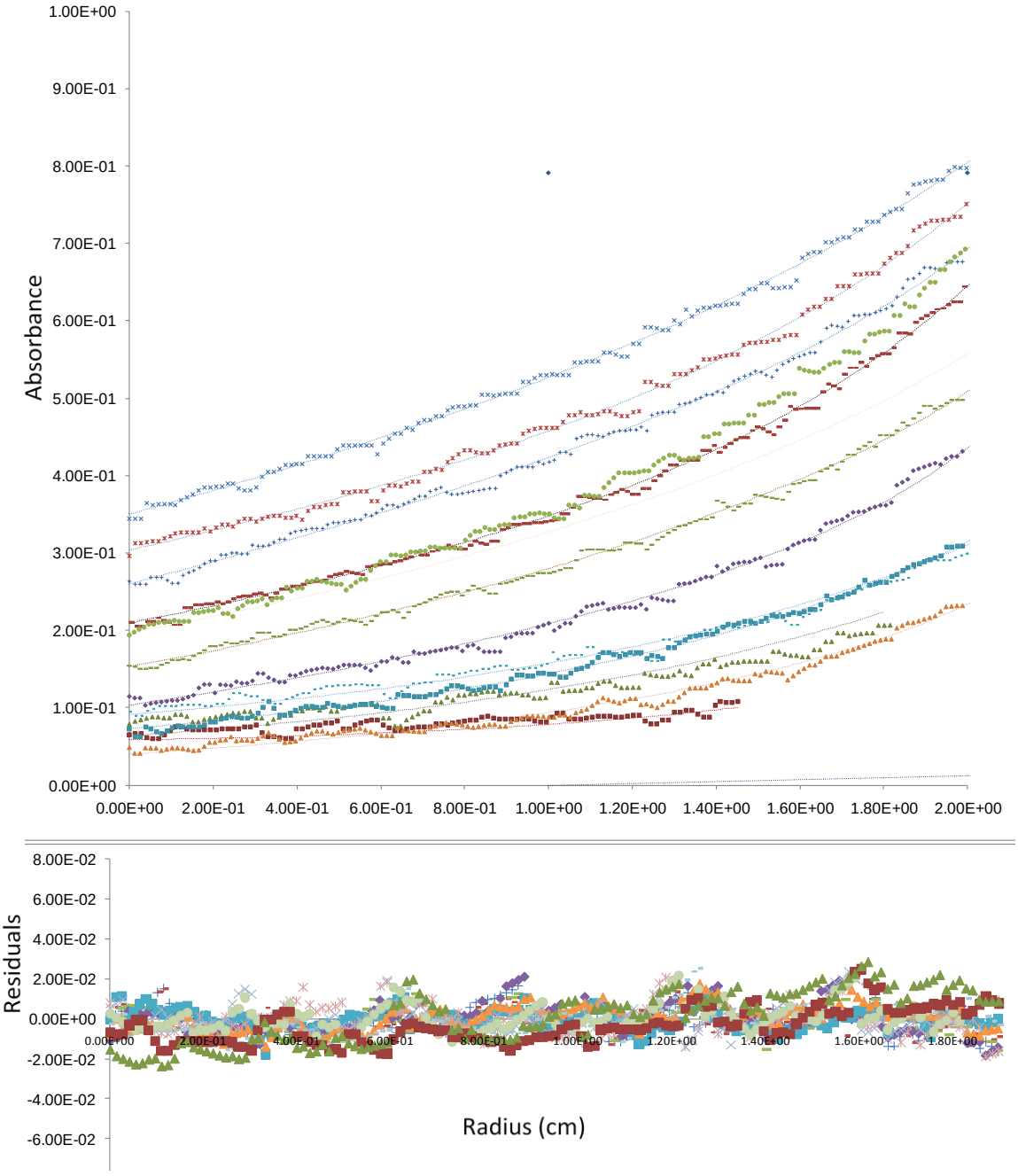
Secondary structure prediction of DISC1 (DISC1-CTF shown here): Two independent prediction softwares were used: HNN(http://npsa-pbil.ibcp.fr/NPSA/npsa_hnn.html) and Psipred (http://bioinf.cs.ucl.ac.uk/psipred/). Predicted Helices are underlined (from HNN prediction) or represented by a line above the sequence (prediction from Psipred). Consistently both algorithms predict the NDEL1 binding site (marked in Red) is helical in nature.

Fig. S3 Equilibrium Analytical Ultracentrifugation experiment on DISC1 at various concentrations and speeds



Equilibrium analytical ultracentrifugation (AUC) experiments on DISC1. The experimental data are represented in closed circles and model fitting are indicated in dotted line. The residues are plotted at the bottom panel. Colors represent fitting same model but independent data with different combination of centrifugal speeds as well as concentration.

Fig. S4 Equilibrium Analytical Ultracentrifugation experiment on NDEL1 at various concentrations and speeds



Equilibrium analytical ultracentrifugation (AUC) experiments on NDEL1. The experimental data are represented in closed circles and model fitting are indicated in dotted line. The residues are plotted at the bottom panel. Colors represent fitting same model but independent data with different combination of centrifugal speeds as well as concentrations.

Fig. S5a. SDS-PAGE analysis of the co-eluted DISC1/NDEL1 complex.

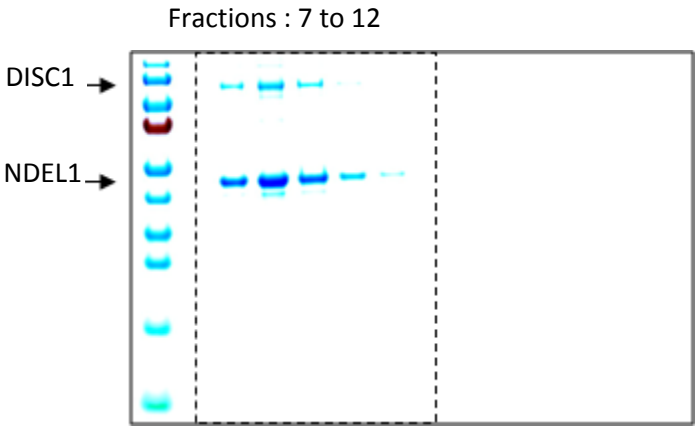
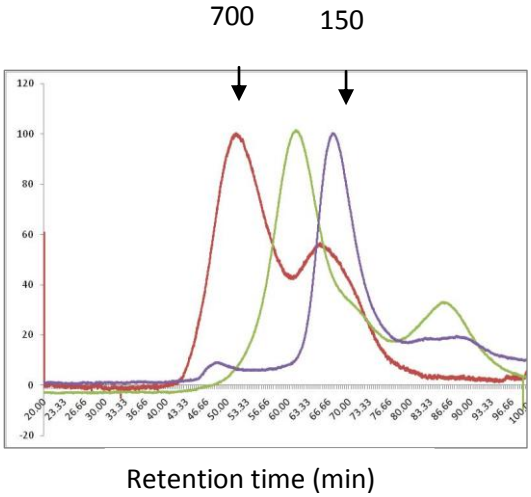
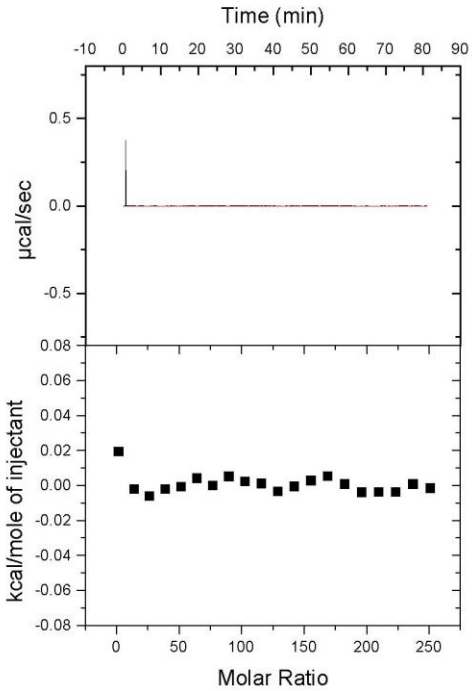


Fig. S5b. DISC1/NDEL1 interaction is mediated through oligomers of DISC1(320-854) and NDEL1.



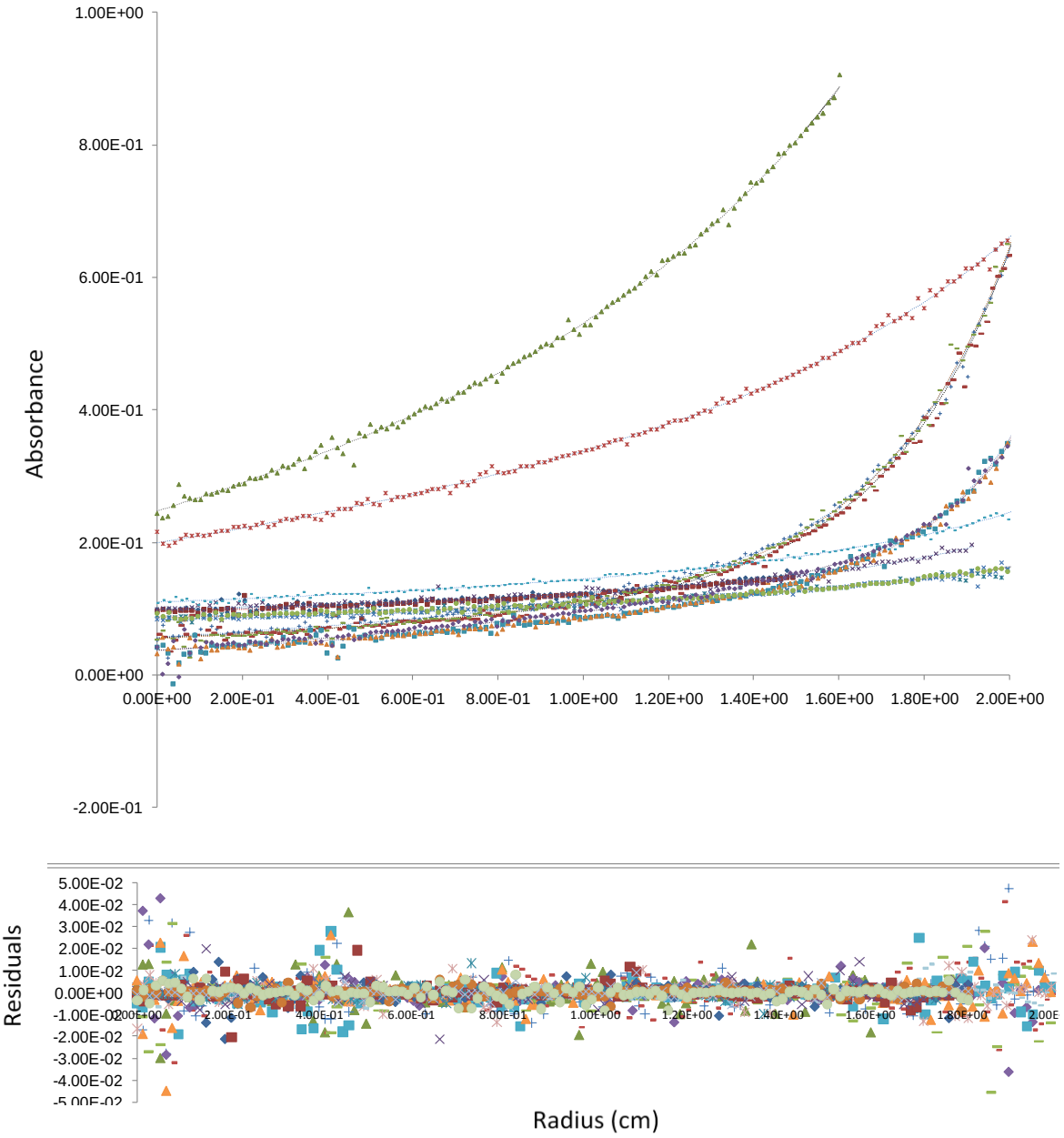
a) SDS-PAGE analysis of the co-elution of DISC1-NDEL1 by size exclusion chromatography confirms the stable complex formation of DISC1-NDEL1. b)Size exclusion chromatography profile of a) DISC1(320-854)/NDEL1 complex (Red) and DISC1(320-854) (Green) and b) NDEL1(Purple). NDEL1 interaction with oligomers of DISC1 (320-854) is evident as the complex migrates within the characterization limit of superdex-200 size exclusion column.

Fig. S6 Control Isothermal titration calorimetry experiment



Performing isothermal titration calorimetry experiment on binding buffer upon titrating with NDEL1 confirms the heat of dilution is insignificant.

Fig. S7 Equilibrium Analytical Ultracentrifugation experiment on MBP-DISC1-S704C at various concentrations and speeds



Equilibrium analytical ultracentrifugation (AUC) experiments on MBP-DISC1-S704C. The experimental data are represented in dots and model fitting are indicated in dotted line. The residues are plotted at the bottom panel. Colors represent fitting same model but independent data with different combination of centrifugal speeds as well as concentration.

Supplementary Table –S1 List of expression constructs used in this study

Proteins used	M.Wt (kDa)	Name of the construct	Tag used	Vector backbone	Restriction site
MBP-DISC1 (WT)	135	Full length DISC1	MBP fusion	pMal-C4E	SacI/HindIII
DISC1 (WT)	93				
MBP-DISC1-S704C	135	Full length DISC1-S704C	MBP fusion	pMal-C4E	SacI/HindIII
DISC1-S704C	93				
GB1-NDEL1	51	Full length NDEL1	N-GB1 fusion	PET-24b	SalI/NotI
NDEL1	43				
DISC1-CTF	19	DISC1(680-832)	N-His7	PET-24b	EcorI/NotI
DISC1-CTF	28	DISC1(680-832)	N-GB1 fusion	PET-24b	EcorI/NotI
DISC1-CTF	64	DISC1(680-832)	MBP fusion	pMal-C2	SacI/BamH1
DISC1-CTF(680-804)	24	DISC1(680-804)	N-His7	PET-24b	EcorI/NotI
DISC1-NTF	67	DISC1(72-290)	MBP fusion	pMal-C2	EcorI/NotI
DISC1(320-834)	59	DISC1(320-834)	MBP fusion	pMal-C4E	SacI/HindIII
NDEL1-CTF	23	NDEL1(122-314)	N-His7	PET-24b	EcorI/NotI