# Supplementary online material

# Dimerization of the fungal defense lectin CCL2 is essential for its toxicity against invertebrates

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**Supplementary Tables**

**Supplementary Figures**

**Table S1.** Torsion angles observed in $sLe^x$ bound to CCL2. Phi (Φ) = O5-C1-O'x-C'x and Psi (Ψ) = C1-O'x-C'x-C'(x+1) for a (1-> x) linkage and Φ = O6-C2-O'x-C'x and Ψ = C2-O'x-C'x-C'(x+1) for a (2-> x) linkage

| **Sugar** | **Φ** | **Ψ** |
|---|---|---|
| Fuc | -66.54 | 143.48 |
| Gal | -73.83 | 246.53 |
| Sia | 48.03 | 100 |

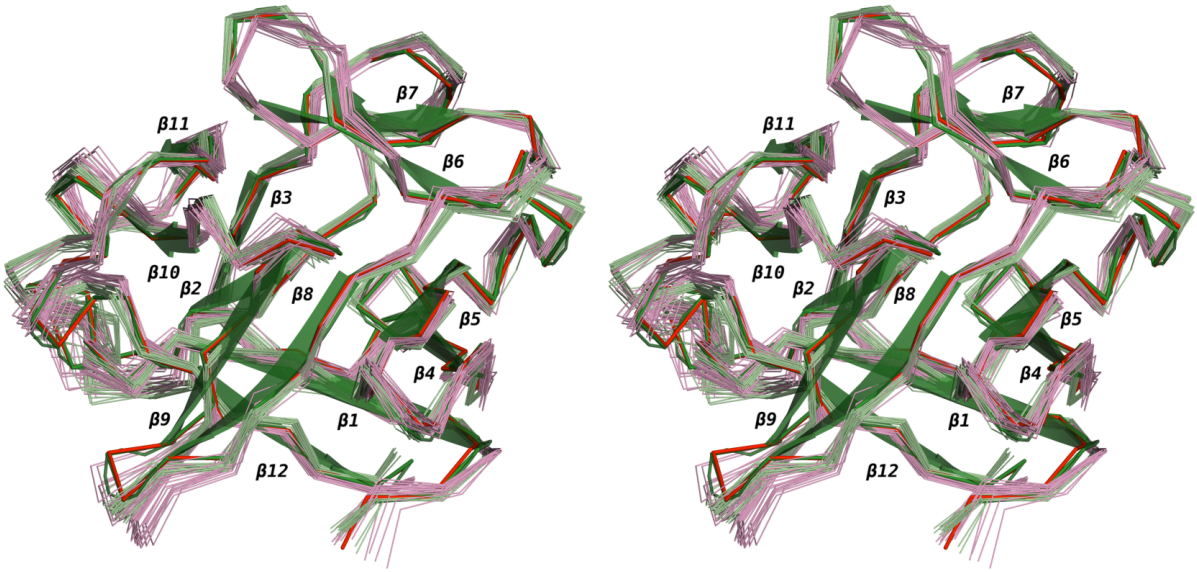


**Figure S1.** ***Superposition of CCL2 overall fold as determined by X-ray and NMR.*** Ribbon representation of the X-ray structures of CCL2 apoprotein (4USP, green), CCL2 complexed with $sLe^X$ (4USO, red) and of the NMR ensemble for CCL2 apoprotein (2LIE, limegreen), and for CCL2 complexed with GlcNAcβ1,4(Fucα1,3)GlcNAcβ1O$(CH_2)_5$COOH (2LIQ, pink). The cartoon representation has been added to visualize the secondary structure elements for 4USP coordinates.

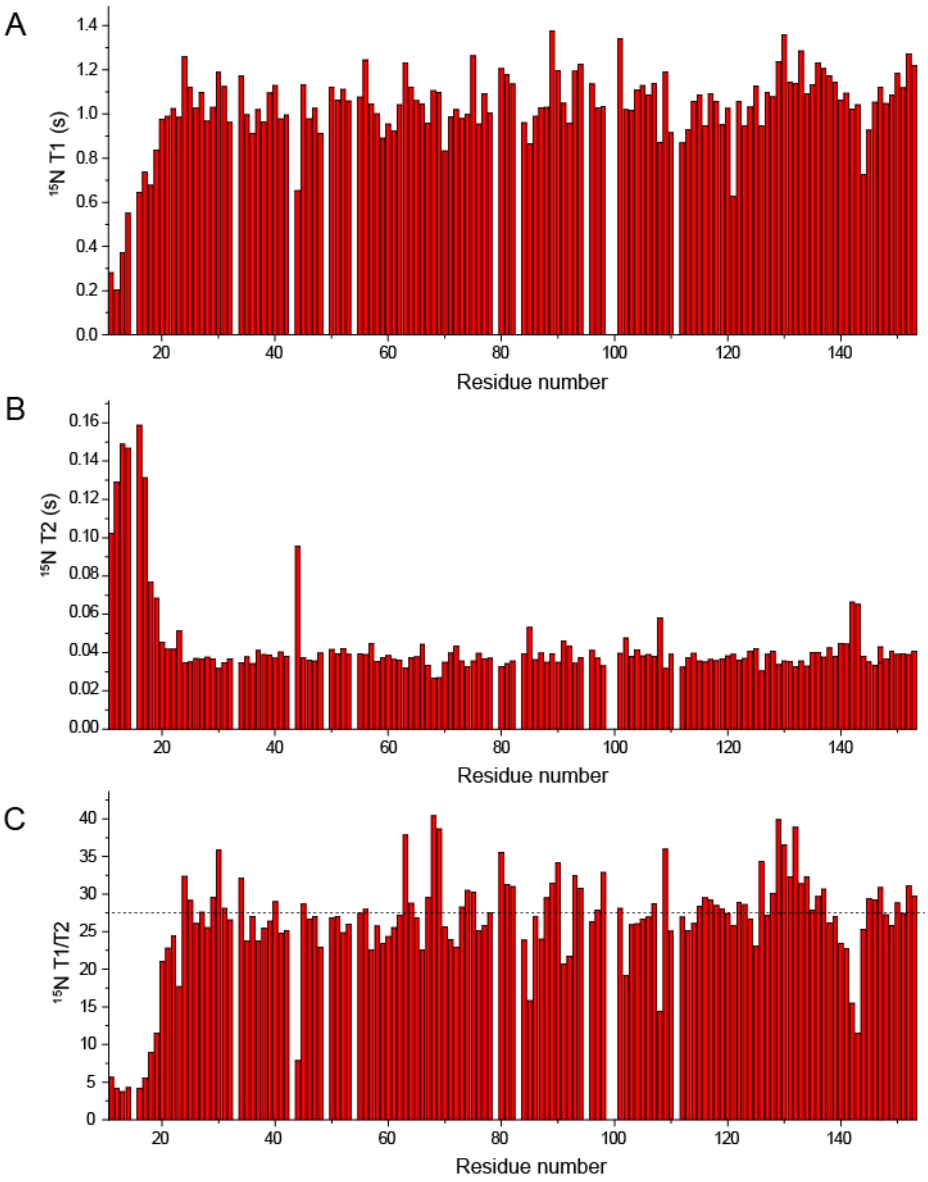


**Figure S2.** ***Backbone $^{15}$N relaxation times of CCL2 ($^{13}$C/$^{15}$N-labeled, 1 mM) measured at a 600 MHz spectrometer at 310 K.*** (A) $^{15}$N longitudinal relaxation times ($T_1$) plotted against residue number. (B) $^{15}$N transverse relaxation times ($T_2$) plotted against residue number. (C) $T_1$ divided by $T_2$ with the average value of 27.4 for the structured region of residue 20-153 indicated as a dotted line.

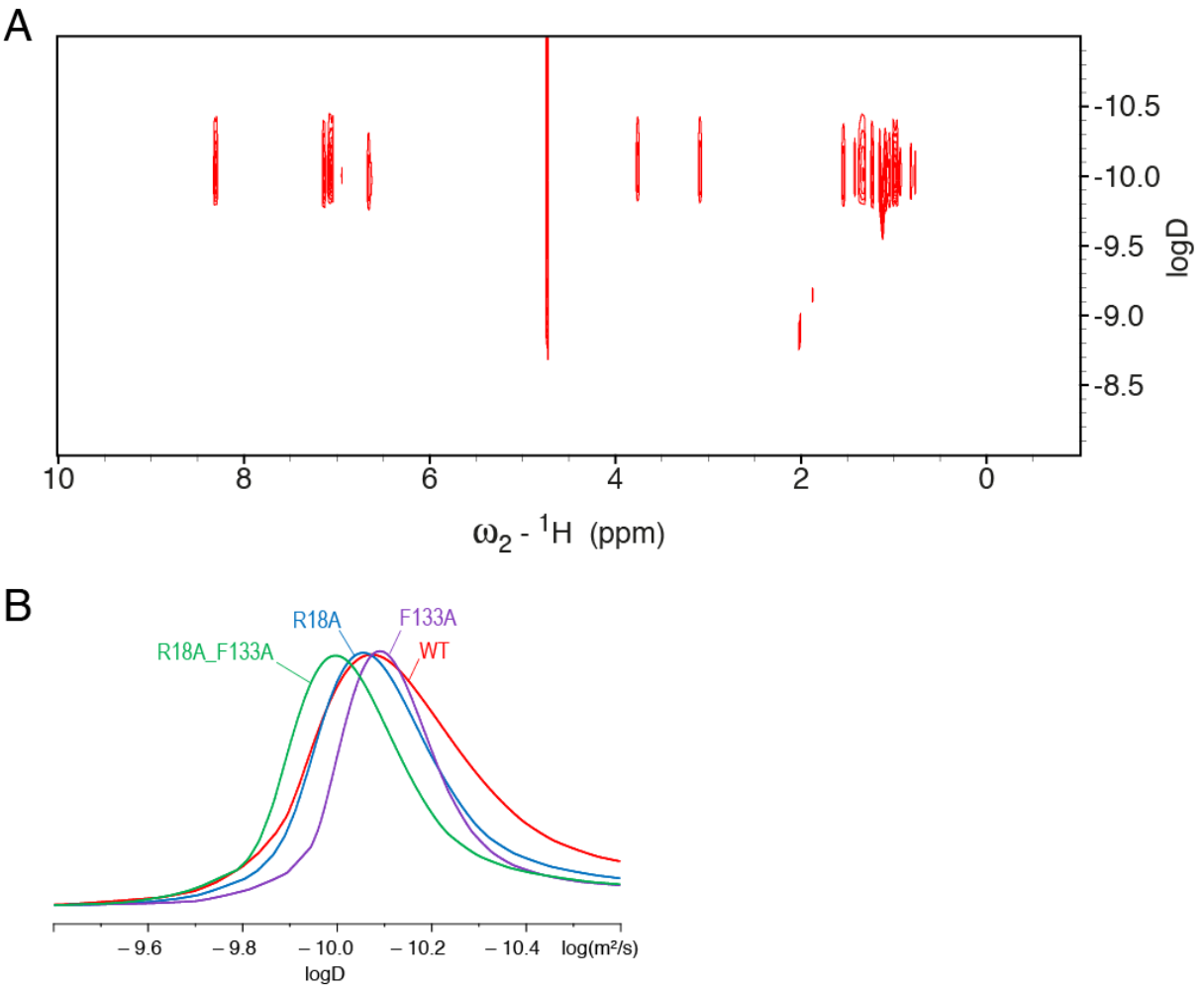


**Figure S3.** ***Diffusion-ordered SpectroscopY (DOSY).*** (A) 2D DOSY spectrum of CCL2 (R18A_F133A) in aqueous buffer at a concentration of 0.3 mM recorded at 298 K and 600 MHz using 128 scan, 32 increments, a gradient duration of δ=1.2ms and an echo delay of Δ=400ms. The standard Bruker stebpgp1s19 pulse sequence, with stimulated echo, bipolar gradient pulses, one spoil gradient and 3-9-19 water suppression, was run with a linear gradient (53.5 G $cm^{-1}$) stepped between 2 and 95%. (B) Projections of logD of CCL2 WT, CCL2(R18A), CCL2(F133A) and CCL2(R18A_F133A) recorded with comparable parameters and concentrations. The region of methyl groups (1.04–0.5 ppm) was used for the projection.

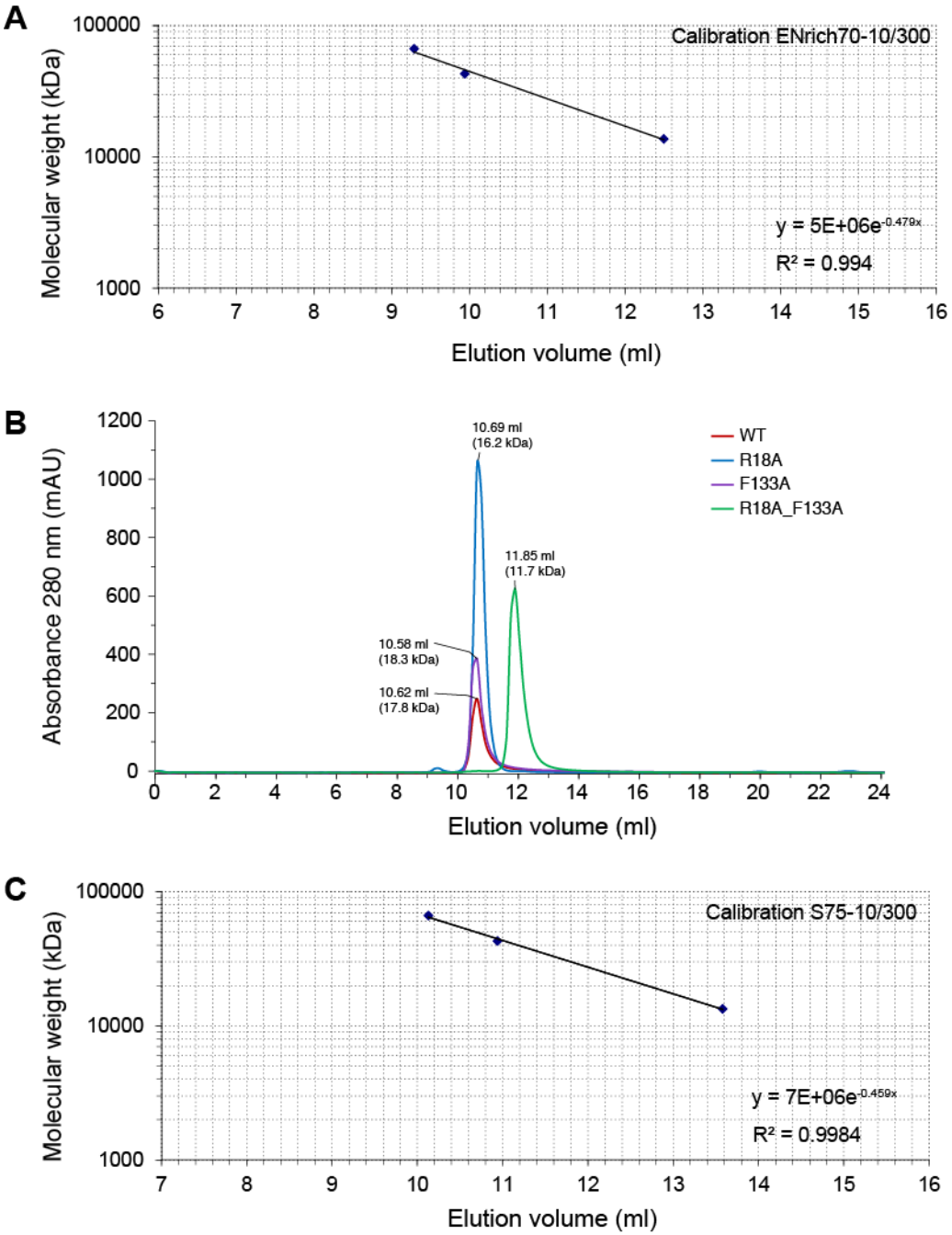


**Figure S4.** ***Size exclusion chromatography of CCL2 variants.*** (A) Calibration curve of the ENrich SEC 70 10 x 300 mm column was done with the following standards: bovine serum albumin 66.5 kDa (9.29 ml), ovalbumin 43 kDa (9.94 ml) and RNase A 13.7 kDa (12.5 ml). (B) SEC Chromatograms for CCL2 wild-type, CCL2(R18A), CCL2(F133A) and CCL2(R18A_F133A) proteins on a Superdex 75 10 x 300 mm column (C) Calibration curve of the Superdex 75 10 x 300 column with the same standards as above. The elution volumes were 10.13 ml (BSA), 10.94 (Ova) and 13.58 ml (RNase A). All experiments were done using 50 mM $KH_2PO_4/K_2HPO_4$ pH 5.7 and 150 mM NaCl as buffer.

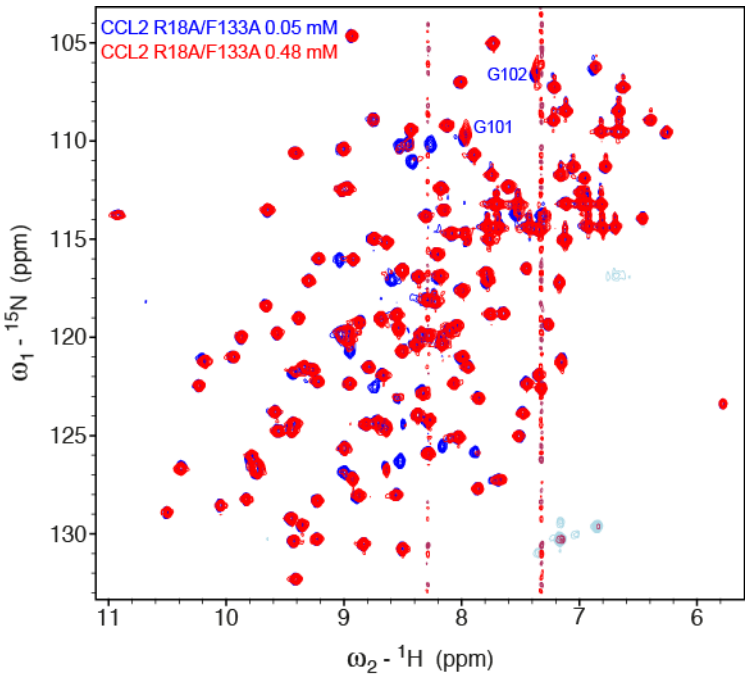


**Figure S5.** ***The R18A/F133A double mutant shows concentration dependent chemical shift deviations.*** The $^{15}$N-$^{1}$H HSQC spectrum at a concentration of 50 μM (blue) shows the expected number of sharp signals whereas an identical spectrum recorded at 480 μM (red) indicates line broadening (signals of G101 and G102 at the dimer interface) and the disappearance of some signals from the dimer interface. The latter signals (blue contours without red contours in front) showed large chemical shift changes in the R18A F133A double mutant compared to WT indicating their involvement in the dimer interface.

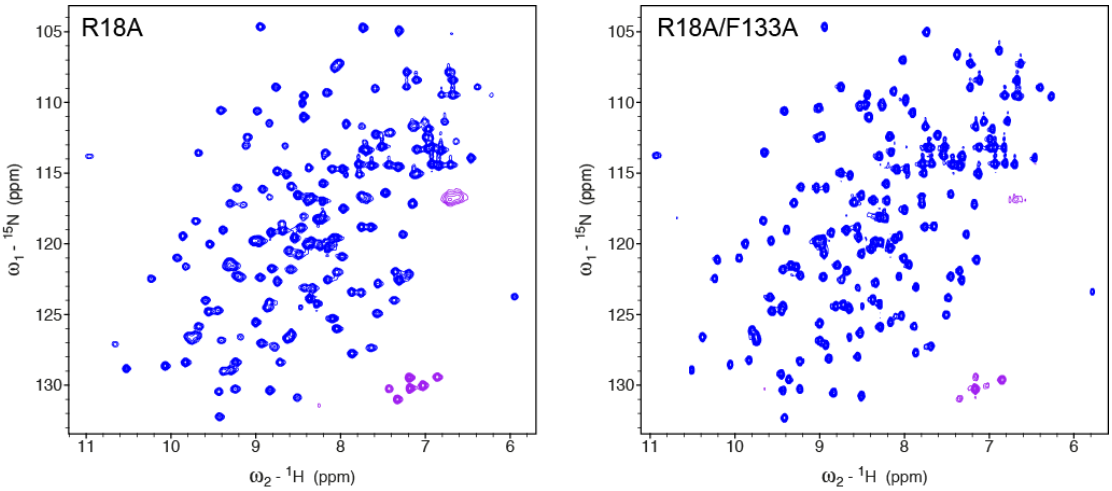


**Figure S6.** ***All point mutant proteins are folded.*** $^{15}$N-$^{1}$H HSQC spectra of $^{15}$N-labeled proteins show a nicely dispersed signal distribution which is typical for a β-sheet protein and comparable to WT.

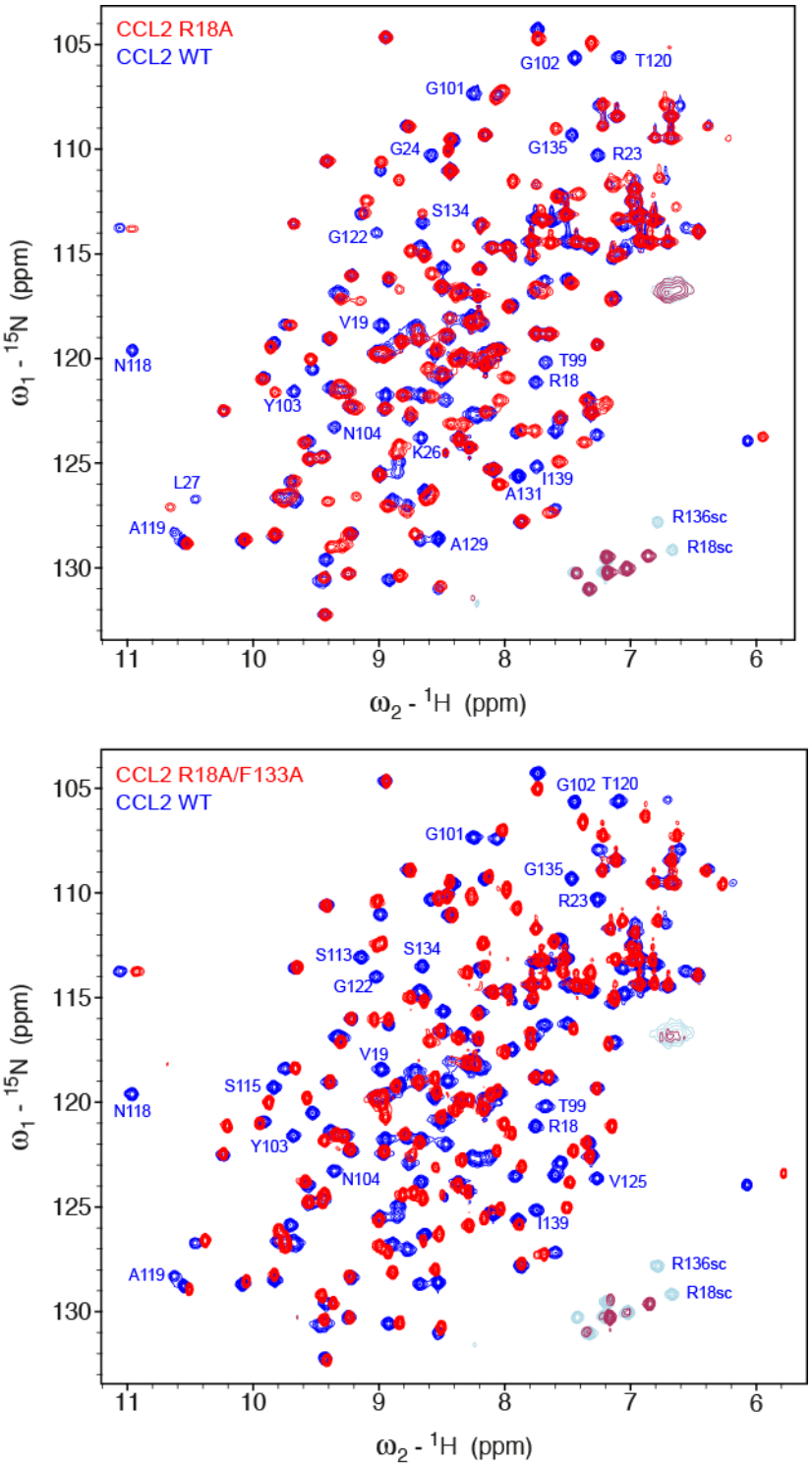


**Figure S7.** ***Protein regions affected by point mutations.*** $^{15}$N-$^{1}$H HSQC spectra of $^{15}$N-labeled mutant proteins (red) in comparison to WT (blue). Most prominent residues influenced by the point mutations are labeled at the peak positions of the WT. Signals from side chains are indicated by 'sc'.

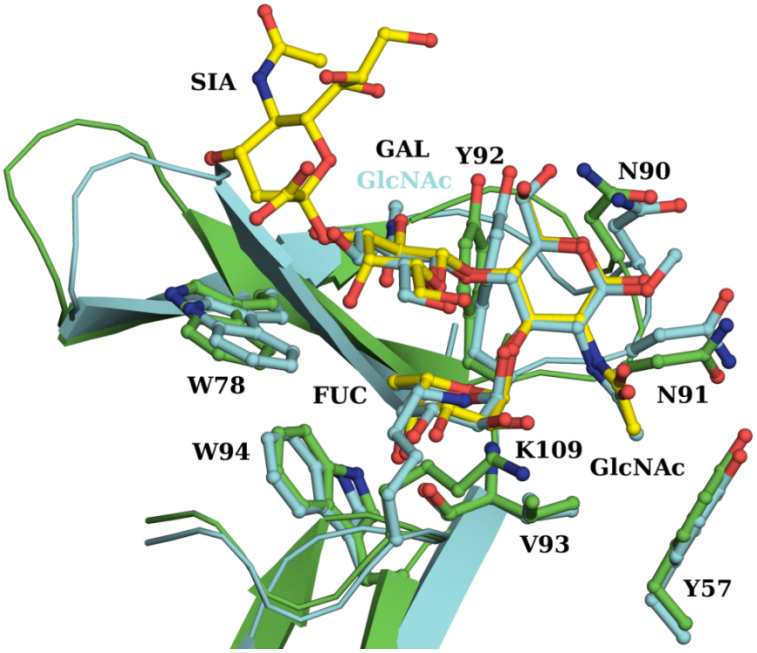


**Figure S8.** ***Superposition of the carbohydrate-binding site of CCL2 for sLe$^x$ and GlcNAcβ1,4(Fucα1,3)GlcNAcβ1O(CH$_2$)$_5$COOH.*** The sLe$^X$ complex (4USO) is represented in green/yellow and the GlcNAcβ1,4(Fucα1,3)GlcNAcβ1O(CH$_2$)$_5$COOH complex (2LIQ) in cyan.