Supplemental Material for

STRUCTURAL BASIS OF CARBOHYDRATE RECOGNITION BY CALRETICULIN

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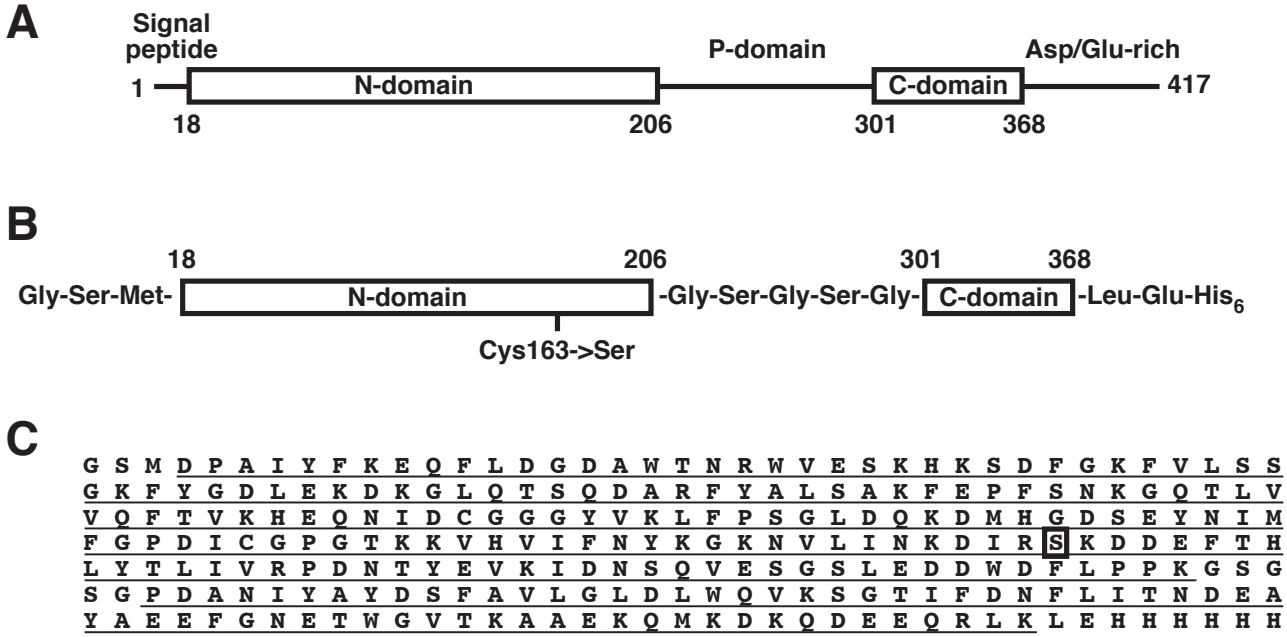
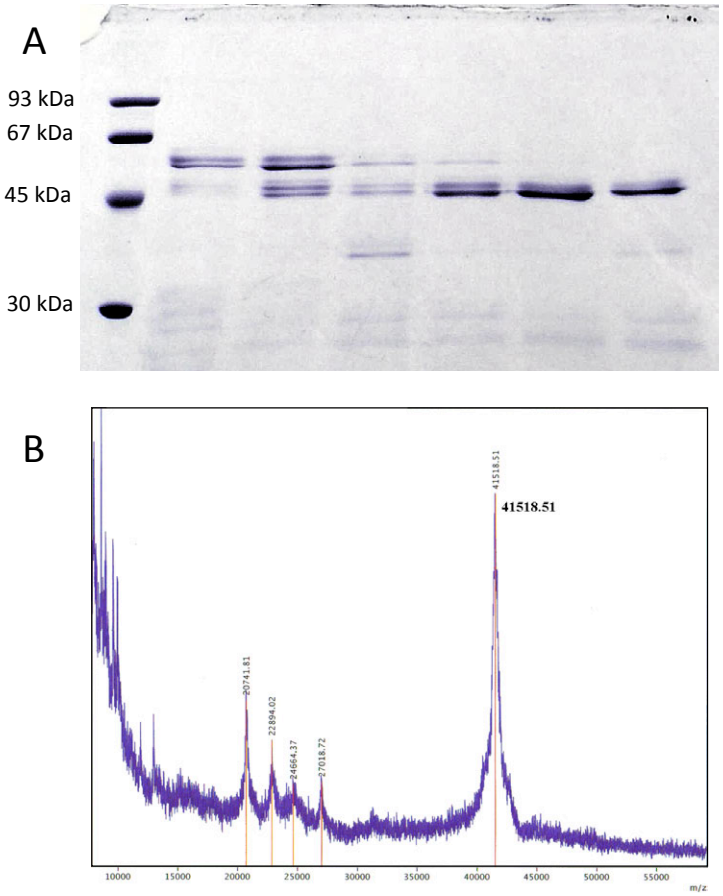
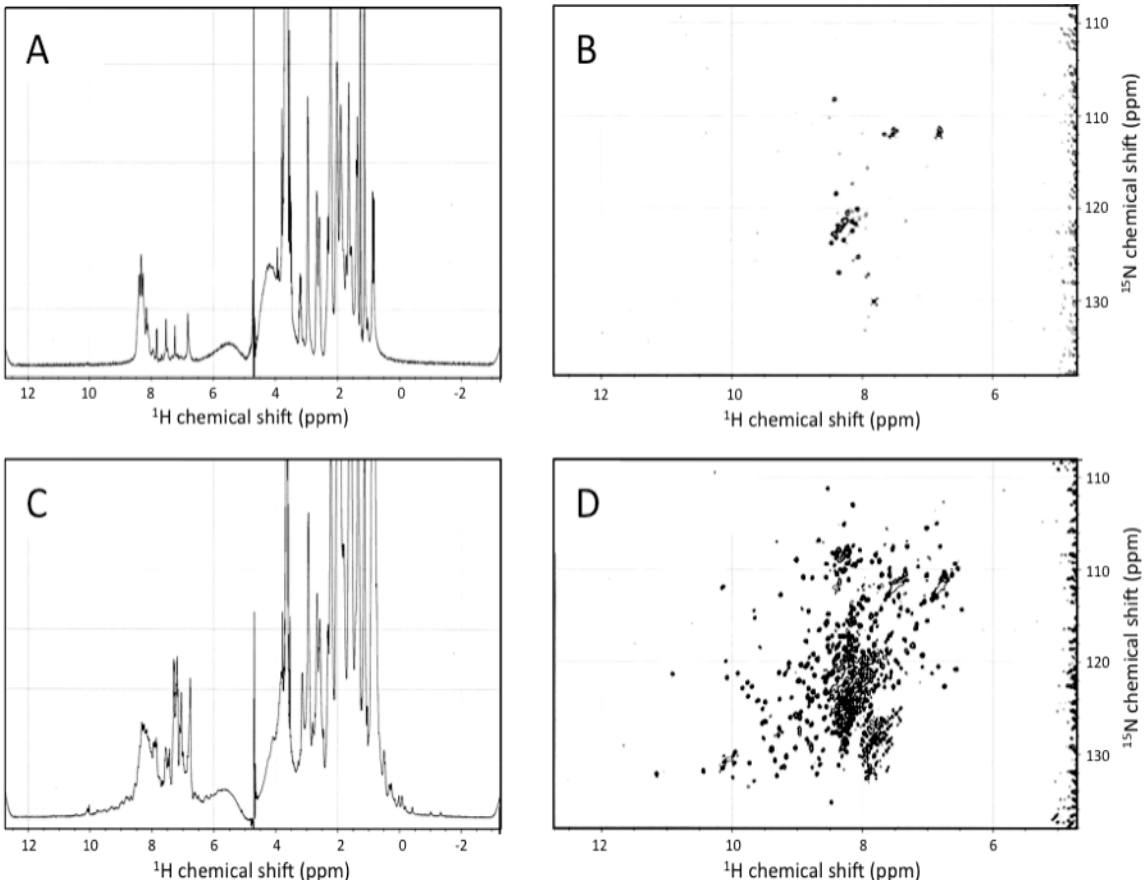


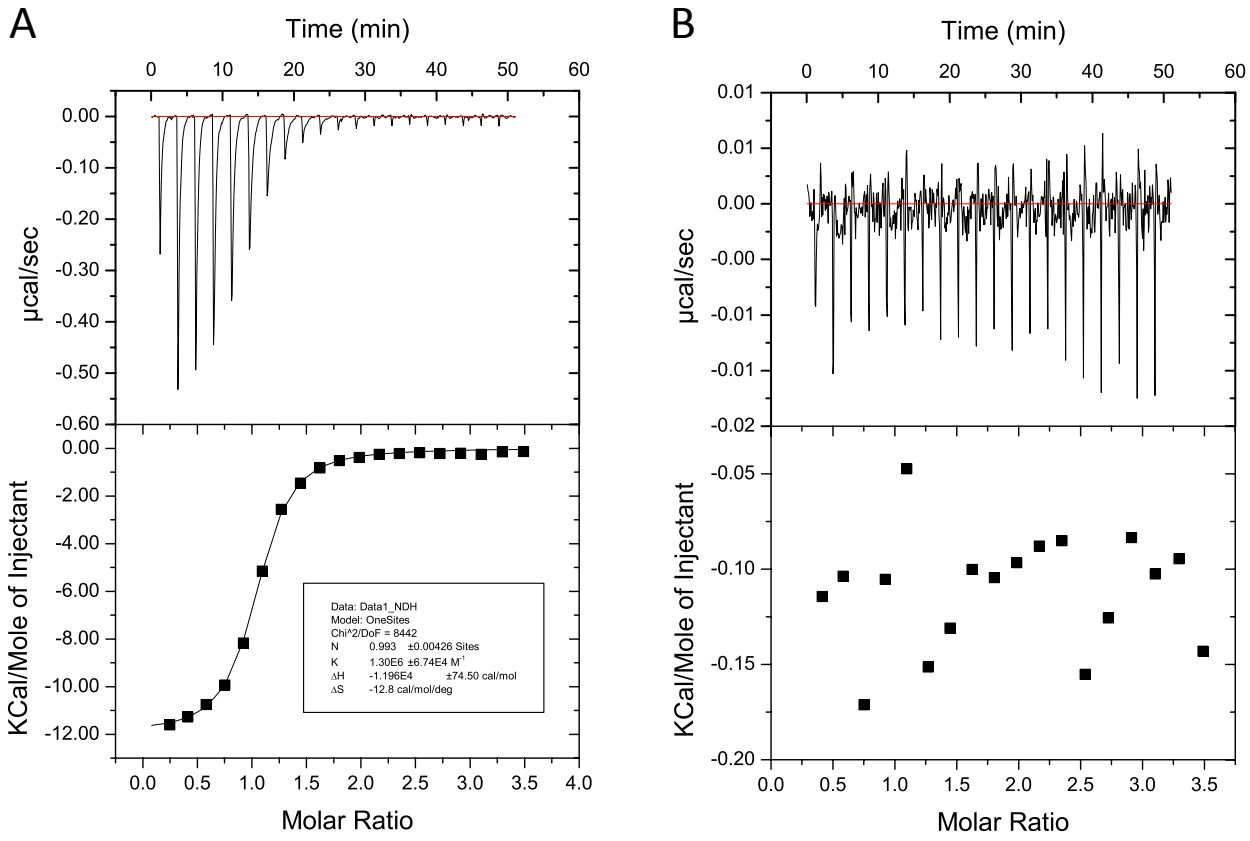
Suppl. Figure 1. Calreticulin lectin domain. (A) Schematic drawing of mouse calreticulin showing the signal peptide, two discontinuous segments of the lectin domain (N-and C-domains), P-domain and acidic C-terminus. (B) Schematic drawing and (C) amino acid sequence of the fragment crystallized. The N-, C- terminal extensions, C163S mutation, and five amino acid linker are indicated.



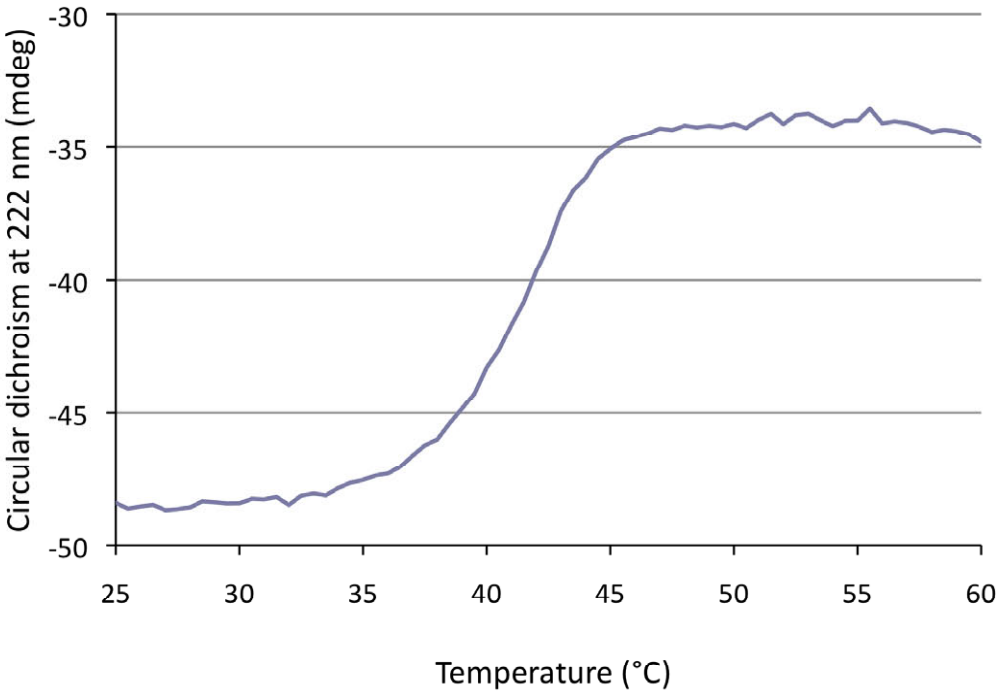
Suppl. Figure 2. Limited trypsin proteolysis of mouse calreticulin (CRT residues 18-416 with an N-terminal his-tag MGSSHHHHHHSSGRENLYFQGHM and a C163S mutation). (A) SDS-PAGE. Lanes from left to right are molecular weight markers and digests with trypsin at 1/500 for 3 min, 15 min, 30 min, 1 h, 2 h, and 3 h. CRT migrates anomalously at higher molecular weight than expected. (B) MALDI-TOF mass spectrum of the main CRT tryptic fragments. The major product was at 41518 m/z, which is close to the theoretical mass for a fragment starting after the arginine in the tag and ending at K368.



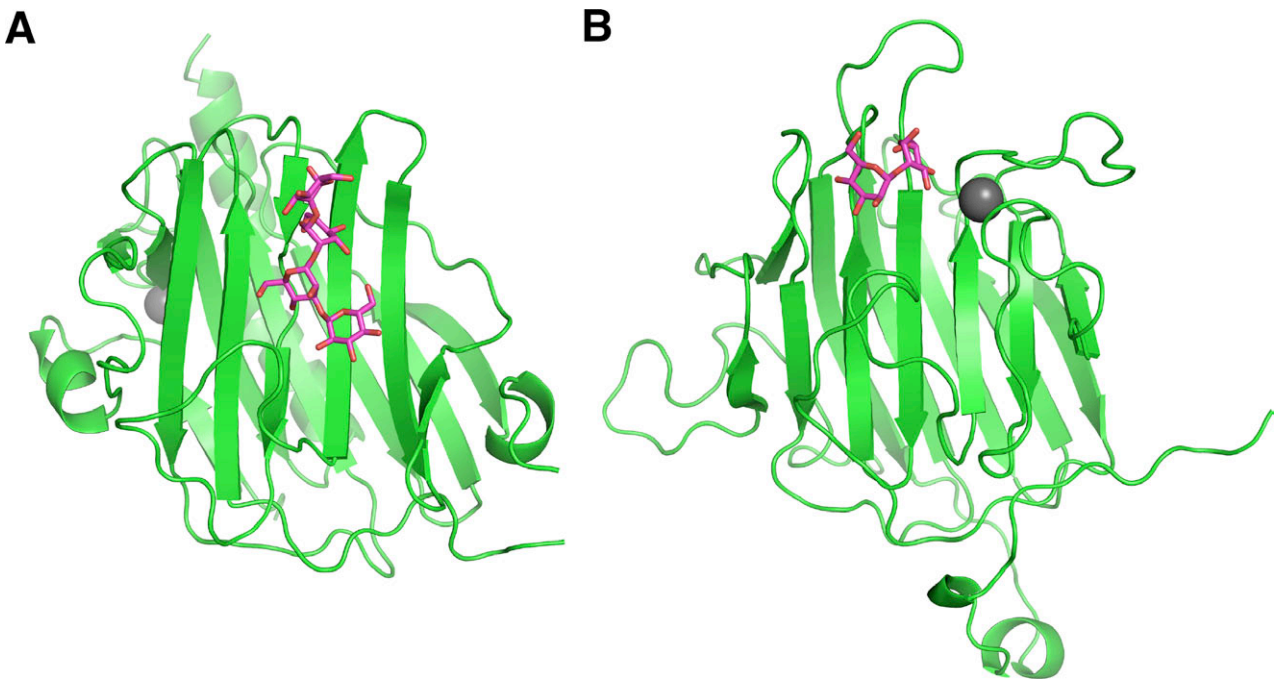
Suppl. Figure 3. NMR spectroscopy of C163S CRT (residues 18-206 and 301-416 with a GSGSG linker and N-terminal his-tag) before (A, B) and following tryptic digestion and partial purification (C, D). Left hand panels (A, C) are one-dimensional proton spectra. Right had panels (B, D) are two-dimensional ^{1}H-^{15}N correlation spectra. Proteolysis was with 0.04 mg/ml trypsin for 3h, followed by size-exclusion chromatography on a Superdex 75 column (GE Healthcare).



Suppl. Figure 4. Calorimetric titration of the C163S CRT lectin domain with Glc_1Man_3 in the (A) absence and (B) presence of the reducing agent 10 mM TCEP (tris(2-carboxyethyl)phosphine). In each panel, the top graph shows the heat released following injections of Glc_1Man_3 into a cell containing CRT lectin domain. The bottom graph shows the integrated heat after correction for the heat of dilution and normalization of the amount of Glc_1Man_3 injected (squares). The curve represents the best fit to a model involving a single site. No binding could be detected in the presence of TCEP.



Suppl. Figure 5. Circular dichroism of the calreticulin lectin domain as a function of temperature. The C163S CRT lectin domain (0.4 mg/ml) in 20 mM HEPES, 150 mM NaCl, 1 mM $CaCl_2$, pH 7.5 was heated at 1 °C minute and the dichroism measured at 222 nm in a 2 mm cuvette. The melting temperature is 42 ± 1 °C.



Suppl. Figure 6. CRT and VIP36 use different structural elements to bind oligosaccharides. (A) CRT uses the surface of the concave β-sheet to bind carbohydrates (magenta, stick representation). A calcium ion (grey ball) is positioned on the opposite side of the protein and does not participate in sugar binding. (B) VIP36 utilizes loops off the concave β-sheet to engage oligomannose and calcium is required for efficient protein-carbohydrate interactions.