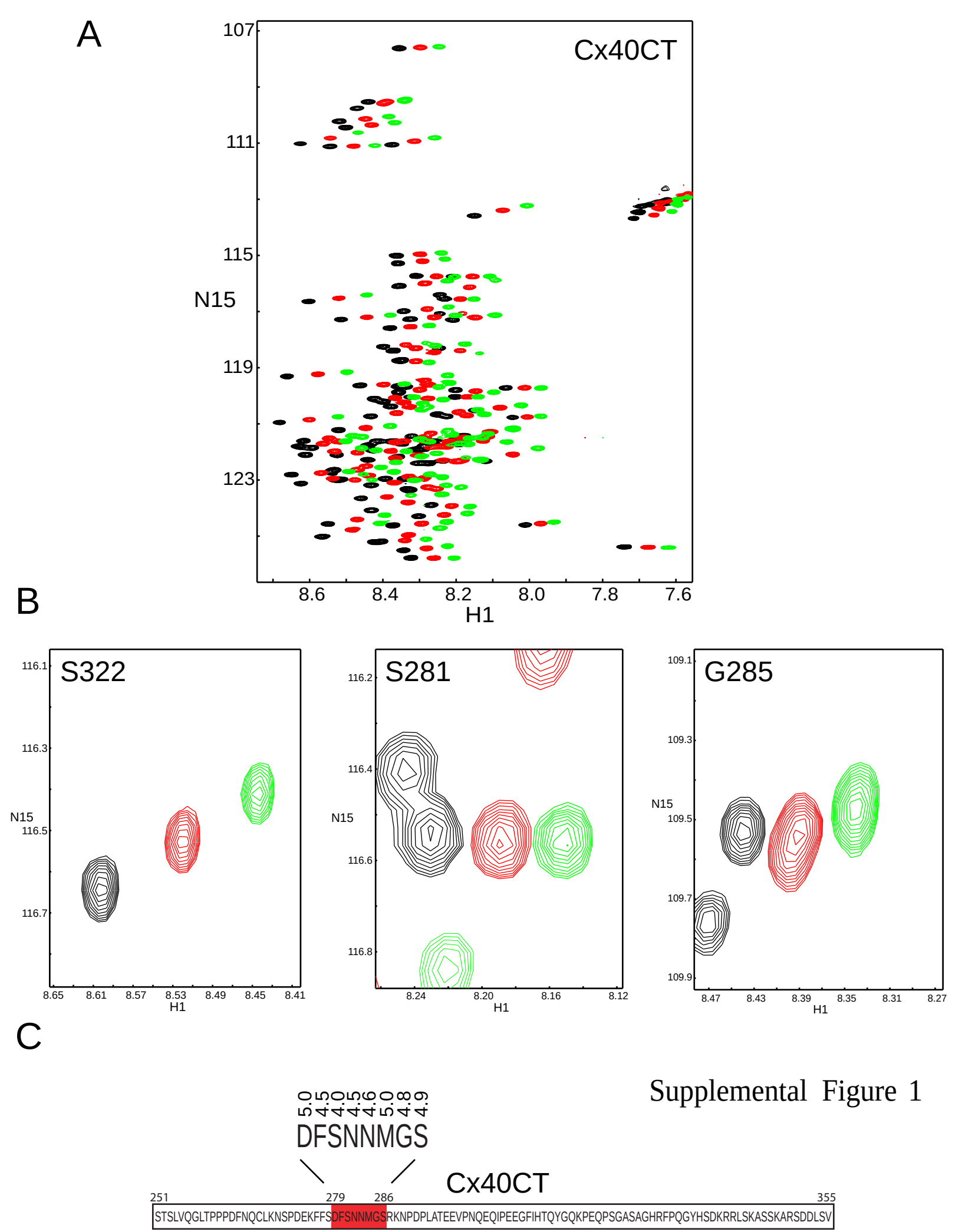
Supplemental Table I. Structural Statistics of the 10 Lowest Energy Cx40CT Structures

Conformational Restraints		
NOE distance restraints		
Total		1123
Intra-residue (|i-j|=0)		535
Sequential (|i-j|=1)		334
Medium range (2≤|i-j|<5)		254
Long range (|i-j|≥5)		0
Unambiguous restraints		1123
Ambiguous restraints		0
Backbone hydrogen bonds		0
Residual Violations		Average Number per residue
Distance restraints > 0.3 Å		0
Distance restraints > 0.5 Å		0
RMSD[a] from Standard Geometry		
Bond lengths (Å)		0.0065 ± 0.0001
Bond angles (degrees)		0.82 ± 0.01
Impropers (degrees)		0.76 ± 0.03
Energies		
NOE		27.4 kcal/mol ± 6.3
Van der Waals		-654.6 kcal/mol ± 14.4
Electrostatic		-3589.7 kcal/mol ± 56.6
Ramachandran Maps		
Residues in most favored regions		81.0%
Residues in additional allowed regions		7.4%
Average RMSD[a]	Backbone (Å)	All non-hydrogens (Å)
Cx40CT domain (251-355)	18.75 ± 3.59	18.46 ± 3.44

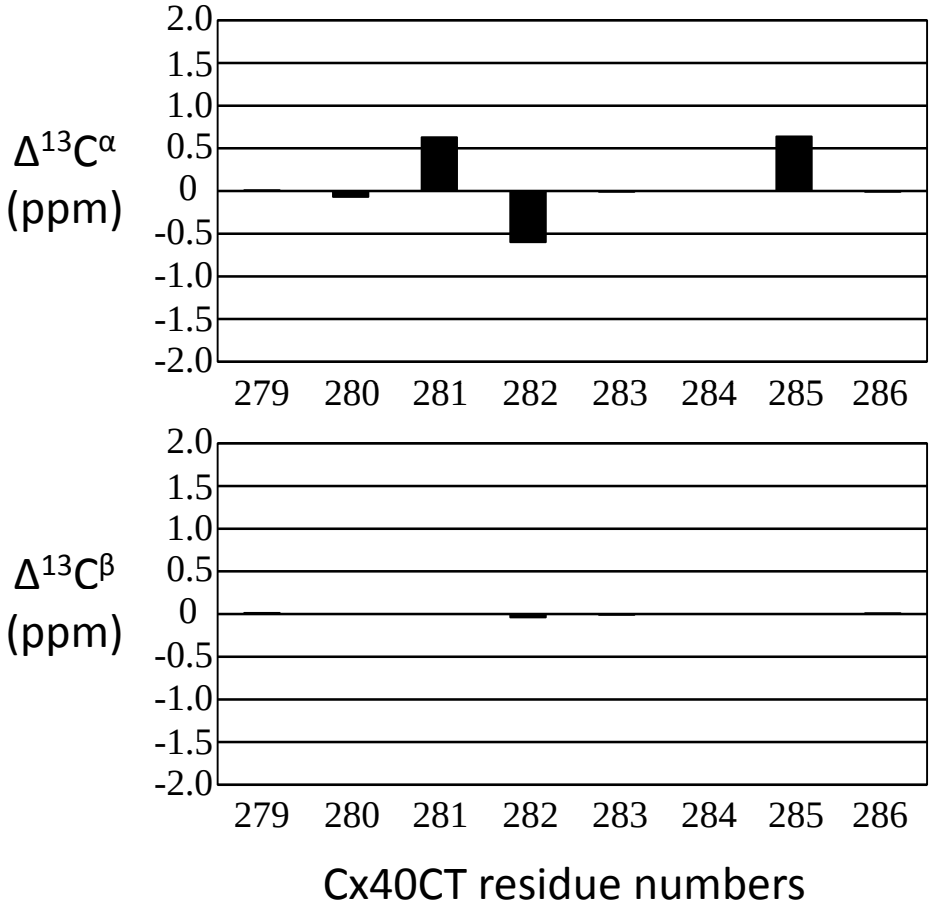
[a]RMSD, root mean square deviation.



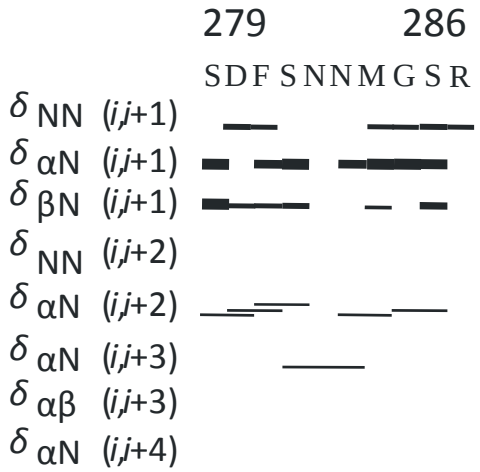
Supplemental Figure 1

Supplemental Figure 2

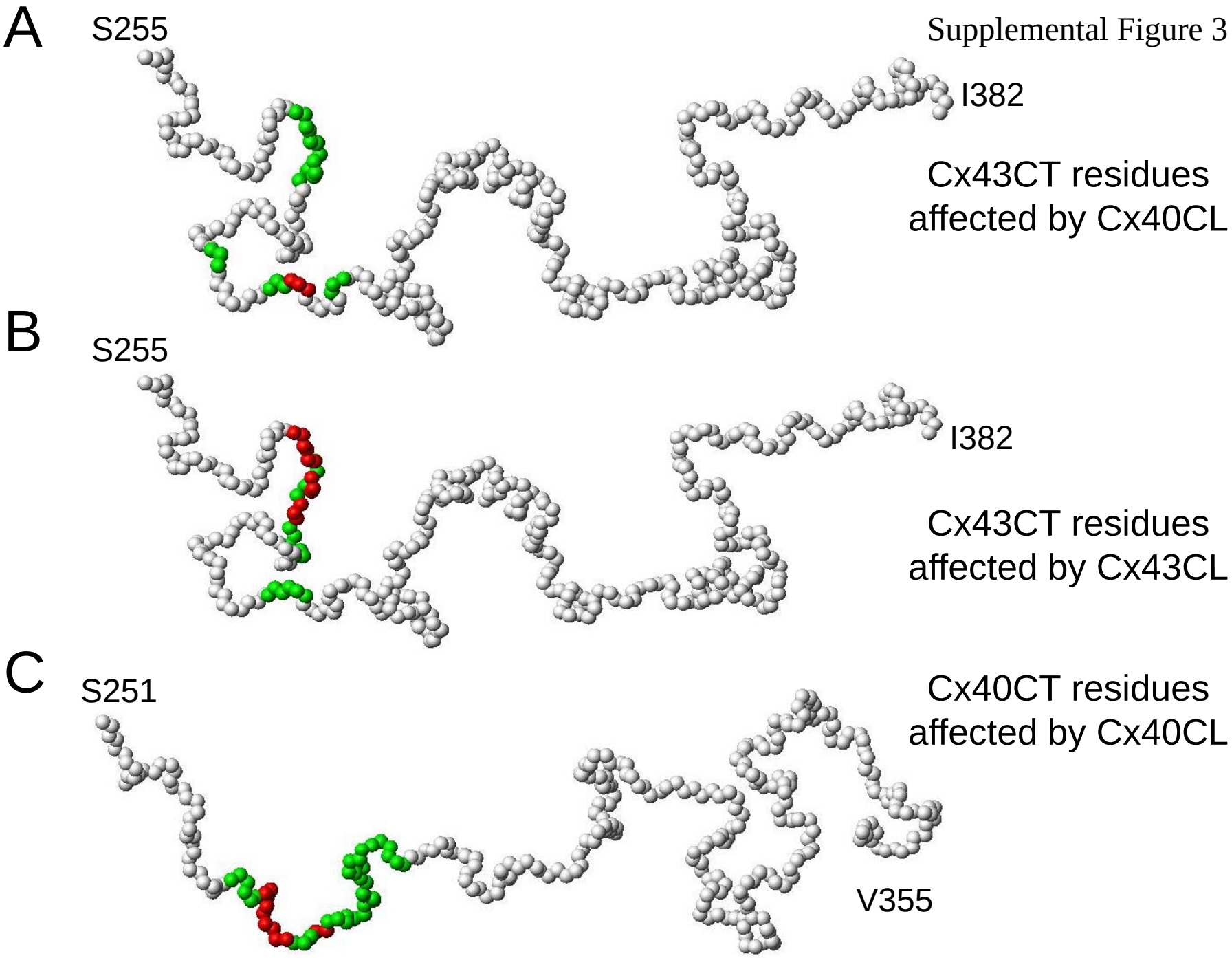
A



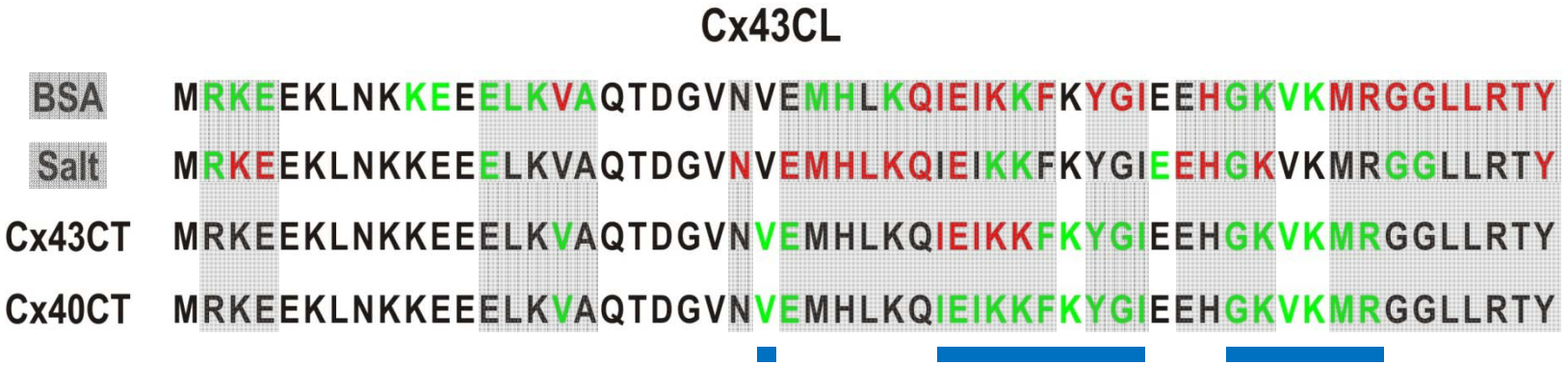
B



Supplemental Figure 3



Supplemental Figure 4



Supplemental Figure 1. NMR temperature coefficient experiment. A) ^{15}N-HSQC spectra of the Cx40CT were collected at 7°C, 12°C, 17°C, 22°C, 27°C, and 32°C. Displayed are the spectra from 7°C (black), 17°C (red), and 27°C (green). Peaks that shifted less than 5.0 ppb/K were considered likely to be hydrogen-bonded. B) Blow-up of a residue that shifted greater than 5.0 ppb/K (S322) and two residues that shifted less than 5.0 ppb/K (S281 and G285). C) Displayed in red are all the Cx40CT residues that shifted ≤5.0 ppb/K (D279-S286). Calculated temperature coefficient values are provided for residues D279-G285. All other residues had values > 5.0 ppb/K.

Supplemental Figure 2. Summary of the CA and CB chemical shift index and NOE pattern for Cx40CT residues D279-S286. A) Analysis of the chemical shift deviations from random coil for $^{13}C^{\alpha}$ and $^{13}C^{\beta}$. B) Backbone NOEs used in the structural analysis.

Supplemental Figure 3. Summary of the Cx43CT and Cx40CT residues affected by a CL domain. 3D model of the Cx43CT illustrating the areas affected by the (A) Cx40CL and (B) Cx43CL domains. C) 3D model of the Cx40CT illustrating the areas affected by the Cx40CL domain. Red areas were affected greater than the green areas (grey areas were unaffected).

Supplemental Figure 4. Summary of the Cx43CL residues affected by bovine serum albumin (BSA) and salt concentration. Displayed is the amino acid sequence for the Cx43CL domain. On the left side of each sequence is the ligand titrated into the CL domain and the affected residues have been high-lighted (red residues were affected greater than the green residues; black residues were unaffected). Grey shaded regions indicate the CL residues that are affected by BSA and salt. After analyzing the Cx43CL residues affected by BSA and salt, underlined in blue are the CL residues affected by the presence of the indicated CT ligand.