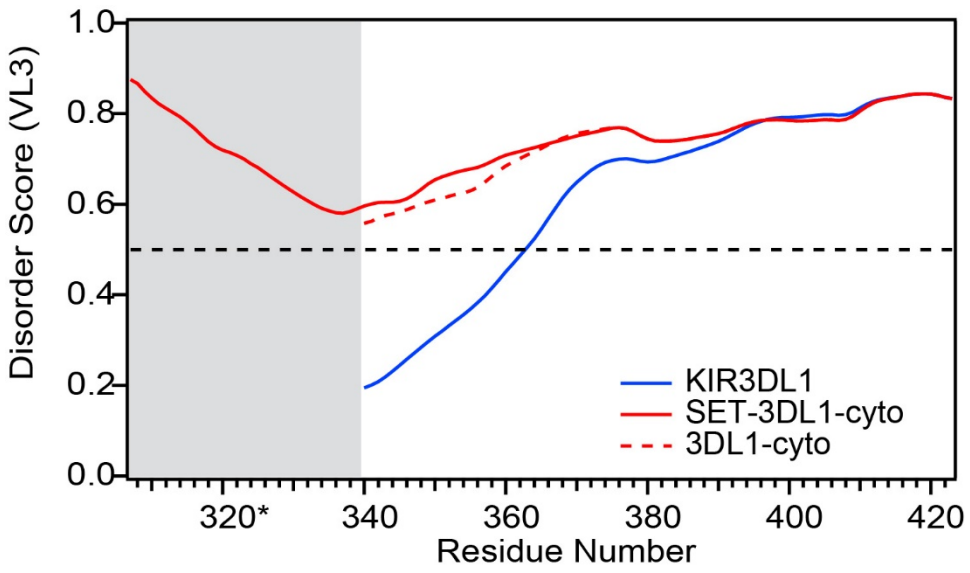

Figure S1. Disorder Prediction. Related to Figure 1. The predictions of disorder were performed using the meta-predictor VL3 (Xue et al., 2010), which uses neural network strategies. The disorder score (> 0.5) is a measure of the certainty that a region of the protein is disordered; a score of 1 indicates 100% certainty. The prediction was performed for intact KIR3DL1 (aa 1-423, blue), SET-3DL1-cyto (solid red), and the 3DL1-cyto only (aa 340-423, dashed red). The SET region is grey shaded. 3DL1-cyto alone, as well as the SET are strongly predicted to be disordered with numeric scores of 0.56 to 0.83. The attachment of the SET at the N-terminus essentially has no impact on the prediction for 3DL1-cyto (residues: 340-423, Fig. 1A). However, the N-terminus of 3DL1-cyto (up to residue A362) is somewhat ordered when predictions were performed for full-length KIR3DL1, suggesting a propensity for non-random structure in the membrane-proximal region.

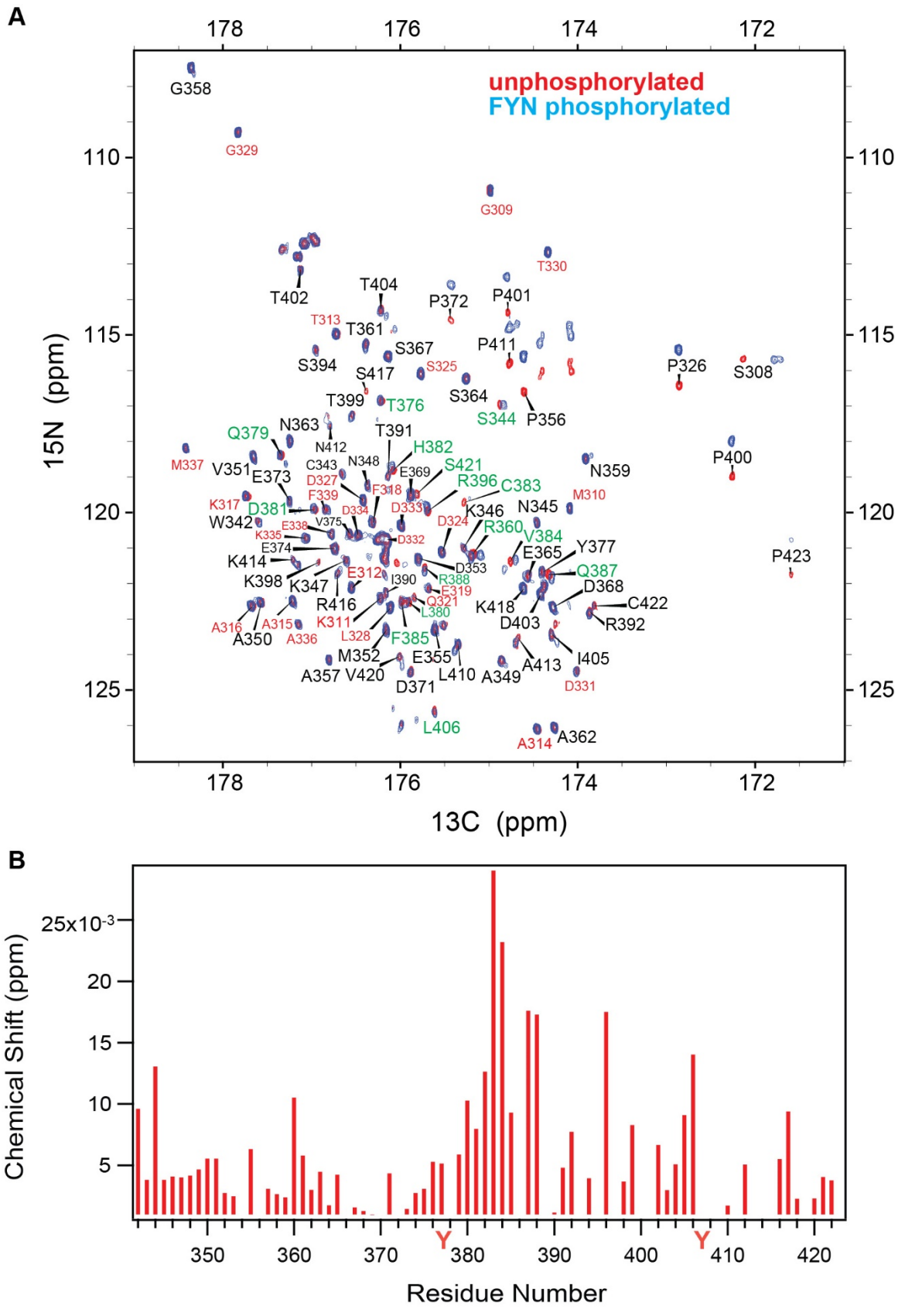
A
unphosphorylated
FYN phosphorylated
15N (ppm)
13C (ppm)
B
Chemical Shift (ppm)
Residue Number

Figure S2. FYN-mediated tyrosine phosphorylation of SET-3DL1-cyto. Related to Figure 6. (A) Superimposed plot of CON spectra of unphosphorylated (red) and FYN phosphorylated 3DL1-cyto (blue) 40 hours after adding FYN (10 μL of 7.8 μM, ~800 units of FYN in ~ 500 μL 1 mM of 3DL1-cyto). The positions of proline residues are evidently folded inside the spectrum due to the spectral width used in the nitrogen dimension. (B) Combined chemical shift differences for the peaks in CON spectra (A) versus residue number. The tyrosines (Y) are marked in red to denote the positions of ITIMs. Peaks are colored based on segments.

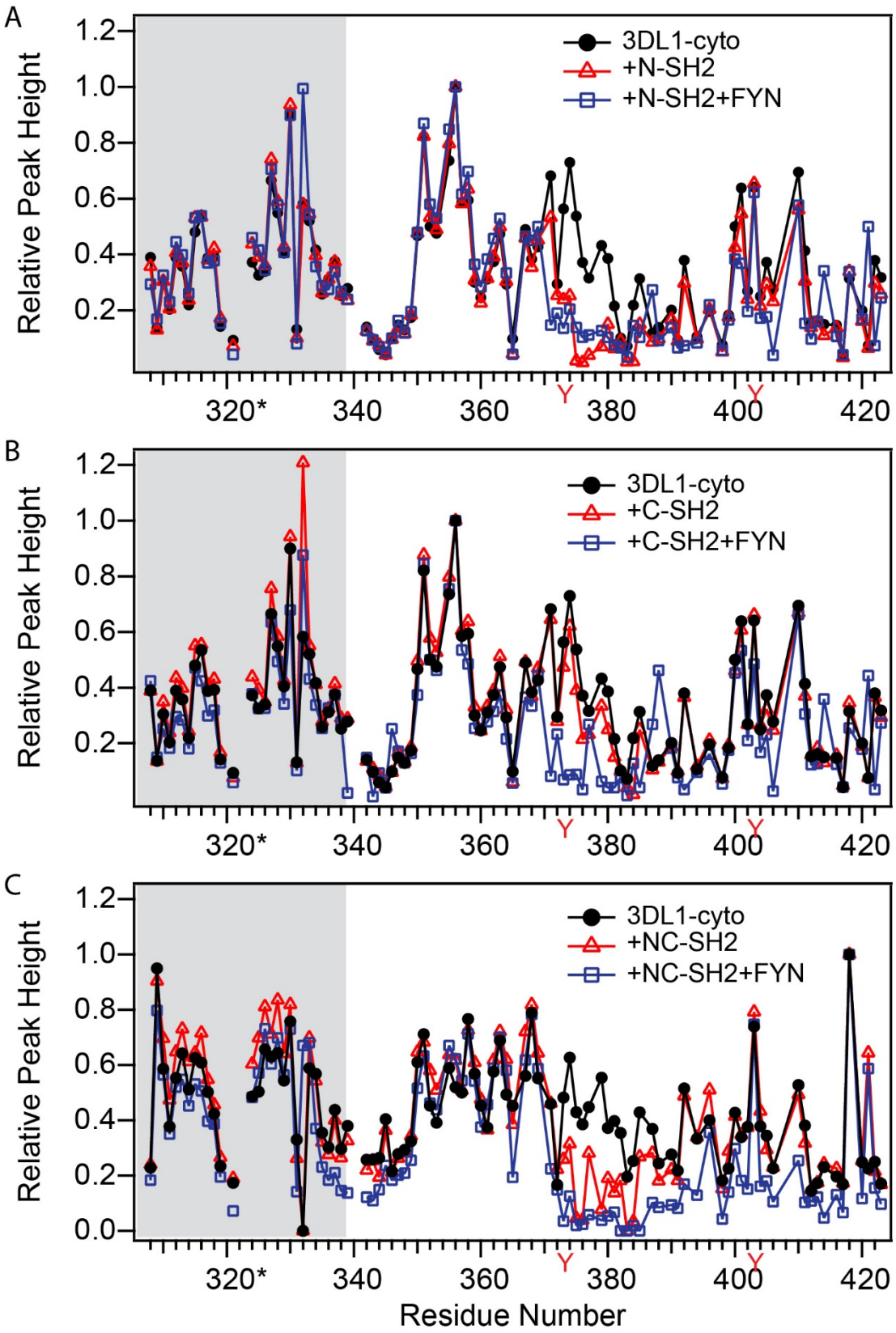
A
Relative Peak Height
1.2
1.0
0.8
0.6
0.4
0.2
3DL1-cyto
+N-SH2
+N-SH2+FYN
320*
340
360
380
400
420
Y
Y
B
Relative Peak Height
1.2
1.0
0.8
0.6
0.4
0.2
3DL1-cyto
+C-SH2
+C-SH2+FYN
320*
340
360
380
400
420
Y
Y
C
Relative Peak Height
1.2
1.0
0.8
0.6
0.4
0.2
0.0
3DL1-cyto
+NC-SH2
+NC-SH2+FYN
320*
340
360
380
400
420
Y
Y
Residue Number

Figure S3. Relative NMR peak intensities in the spectra of 3DL1-cyto without or with SHP-2 SH2 domains either in the absence or presence of FYN. Related to Figure 7. The residues in the SET are shaded. (A) The effects of the binding to SHP-2 N-SH2 on 3DL1-cyto peak intensity in the CON spectra plotted as relative peak height versus residue number for 1 mM [^{13}C, ^{15}N] 3DL1-cyto alone (●); after addition of SHP-2 N-SH2 into 1 mM [^{13}C, ^{15}N] 3DL1-cyto at molar ratio of 0.2:1 (Δ); and subsequent to FYN phosphorylating tyrosine residues in 3DL1-cyto in the presence of SHP-2 N-SH2 (□). (B) The effects of the binding to SHP-2 C-SH2 on 3DL1-cyto peak intensity plotted with relative peak height in the CON spectra versus residue number for 1 mM [^{13}C, ^{15}N] 3DL1-cyto alone (●); with SHP-2 C-SH2 at molar ratio of 1:0.2 (Δ); and subsequent to addition of FYN and SHP-2 N-SH2 (□). (C) The effects of the binding to SHP-2 NC-SH2 on 3DL1-cyto peak intensity plotted with relative peak height in the (HCA)CON spectra versus residue number for 1 mM [^{13}C, ^{15}N] 3DL1-cyto alone (●); with SHP-2 NC-SH2 at molar ratio of 1:0.1 (Δ); and subsequent to addition of FYN in the presence of SHP-2 NC-SH2 (□). Positions of the ITIM tyrosines are marked with Y on the bottom axis and SET is shaded in grey.

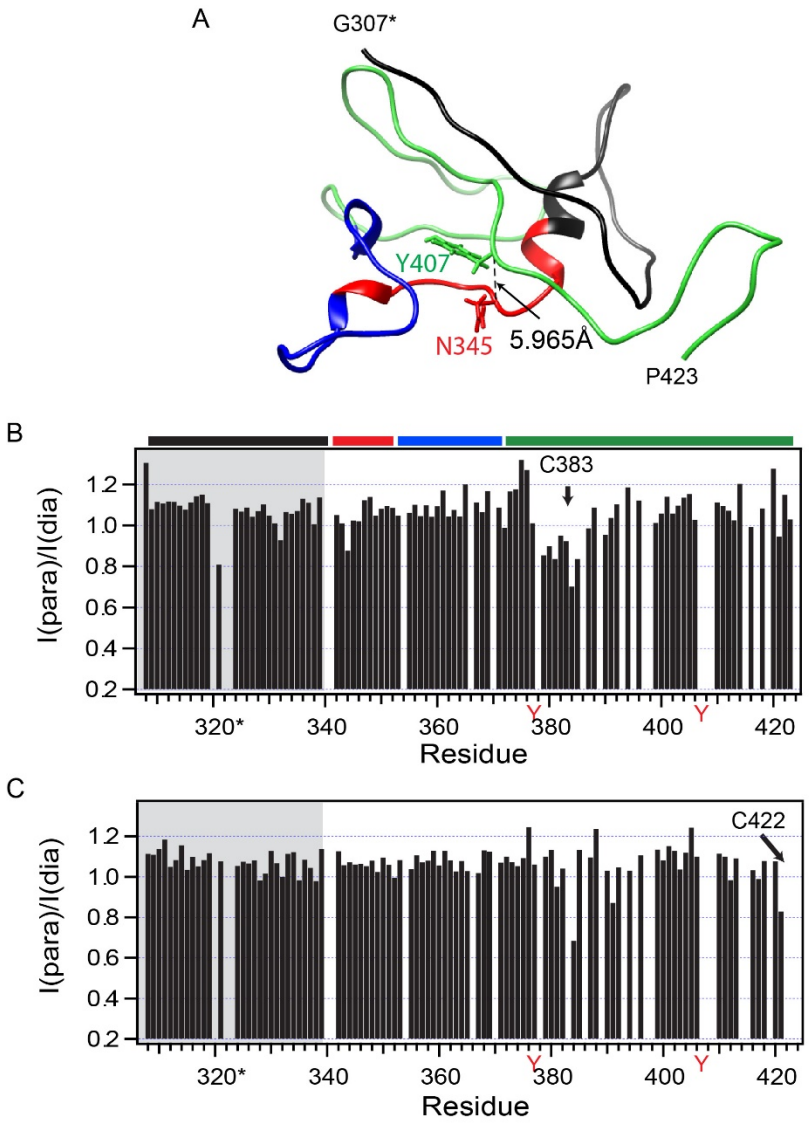

Figure S4. Intramolecular interactions Related to Figure 7. (A) Ribbon Diagram of the best-ranked structure of CS-Rosetta. Segments I, II, and III are in red, blue, and green, respectively. SET is in black. Segments I and III approach each other in this model. The distance between Cαs of N345 and Y407 is shown. (B) and (C) Paramagnetic enhancement to nuclear spin relaxation for SET-3DL1-cyto. The histograms show the experimental intensity ratios (Ipara/Idia) for each residue with an adequately resolved crosspeak in the ^{13}CO-^{15}N CON spectrum of (B) C342A/383Cp/C422A, (C) 342A/C383A/422Cp. An arrow indicates the location of the spin label. The spectra were recorded for 1 mM SET-3DL1-cyto recorded at 37 °C in 20 mM HEPES (pH 7.4), 20 mM NaCl, 3 mM EDTA. The diamagnetic controls were recorded by introducing 10 mM DTT into the NMR samples. The residues in SET are marked by the black bar at the top in B and C.