Supplementary figure legends

Figure 1 Linking of the GluR2 peptide to the carboxyl tail does not change the overall conformation of the PICK1 PDZ-GluR2 peptide complex. The overlay plot of the HSQC spectra of PICK1-3C-GluR2C with or without cleavage of the linker sequence by protease 3C. The insert shows the SDS-PAGE analysis of complete cleavage of the PICK1-3C-GluR2 linkage by protease 3C. Note that after protease 3C cleavage, PICK1 PDZ displayed a slightly smaller molecular weight than the uncleaved complex.

Figure 2 Lipid membrane binding of the peptide ligand-free form PICK1 PDZ . PICK1 PDZ binds to lipid membrane in a peptide ligand independent manner. In this assay, B1 domain of Streptococcal protein G (GB1)-fused form of PICK1 PDZ also robustly binds to lipid membrane in liposome sedimentation assay. As the control, the GB1 domain showed no detectable lipid membrane binding.

Figure 3 Substitution of Cys46 with a Gly does not alter the overall structure of the PICK1 PDZ and GluR2 peptide complex. (A) Overlay plot of the HSQC spectra of ^{15}N-labeled PICK1 PDZ-3C-GluR2 and the C46G mutant of PICK1 PDZ-3C-GluR2. The data show that the majority of the peaks from the two proteins overlap with each other well. (B) Plot as a function of the residue number of combined ^{1}H and ^{15}N chemical shift difference of PICK1 PDZ-3C-GluR2 and the C46G mutant of PICK1 PDZ-3C-GluR2. The combined ^{1}H and ^{15}N chemical shift changes are defined as:

$$\Delta_{ppm} = [(\Delta\delta_{HN})^2 + (\Delta\delta_N \times \alpha_N)^2]^{1/2}$$

Where $\Delta\delta_{HN}$ and $\Delta\delta_N$ represent chemical shift differences of amide proton and nitrogen chemical shifts of each residue of PICK1 PDZ. The scaling factor (α_N) used to normalize the ^{1}H and ^{15}N chemical shifts is 0.17.

Figure 4 Mutation of Cys residues in the CPC-motif does not affect the GluR2 peptide to the PICK1 PDZ domain. Quantitative measurements of the binding affinities of the wild type PICK1 PDZ domain (A) and the CC-GG mutant (B) towards the GluR2 peptide using fluorescence polarization. Fluorescence polarization assay were performed on a PerkinElmer LS-55 fluorimeter equipped with an automated polarizer at 20 ℃. The GluR2 peptide (from GeneScript Corporation) was labeled by fluorescein-5-isothiocynate (FITC, from Molecular Probes) and purified by passing the reaction mixture through a Superdex Peptide HR 10/30 column (from GE healthcare). The labeled peptide (~1 μM) were titrated with increasing amount of binding proteins in 40 mM Hepes buffer containing 100 mM NaCl, 1 mM EDTA and 1 mM DTT (pH 7.4). The dissociation constant, *Kd*, for each titration reaction was derived by fitting the binding curve with the classical one-site binding equation.

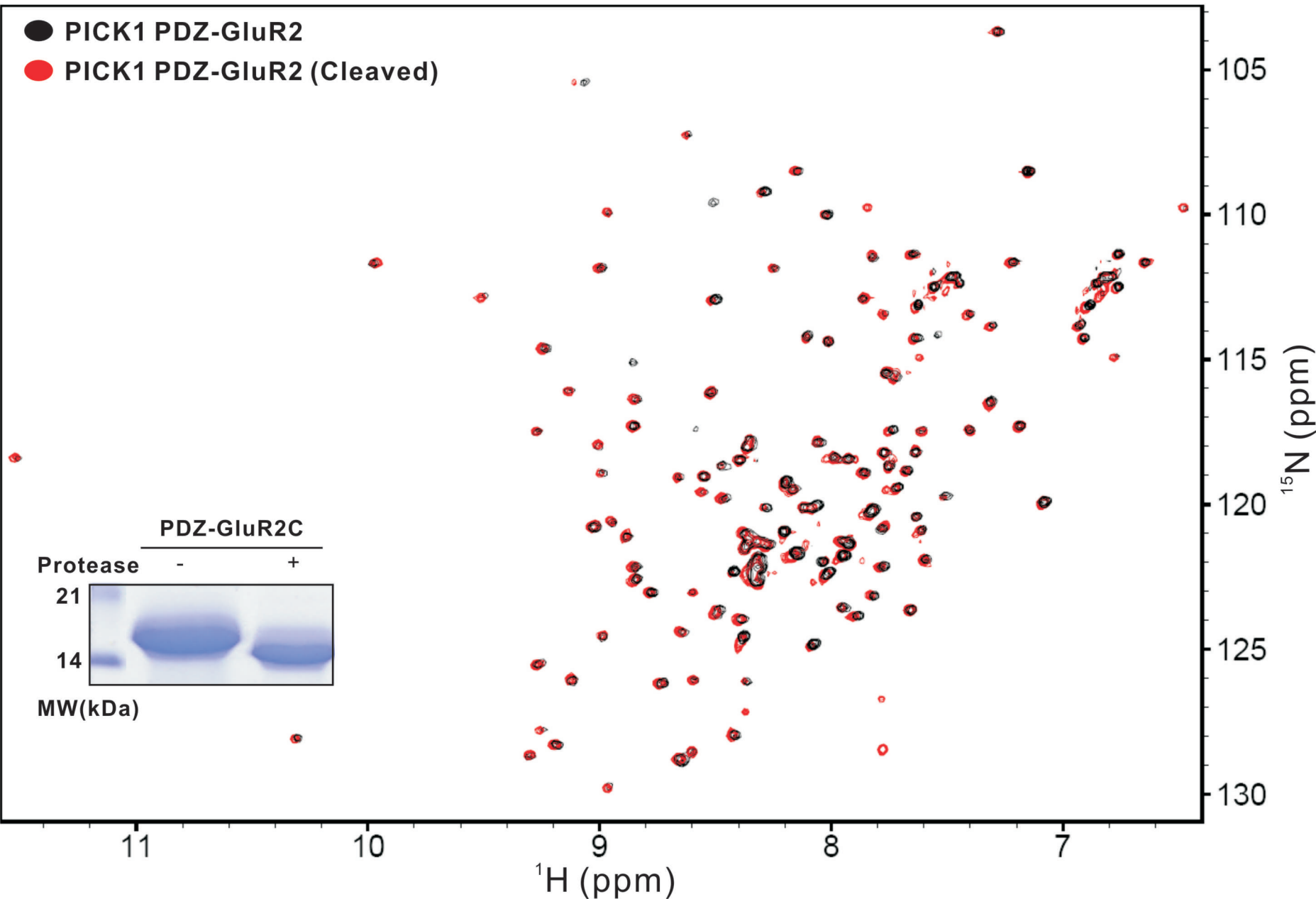
Figure 5: Lipid membrane binding of the full-length PICK1. (A) The PICK1 CC-GG mutant has impaired lipid membrane binding. In this assay, we compared dose-dependent (0-160 μg/ml) lipid membrane bindings of the maltose binding protein (MBP)-fused form of the wild type PICK1 and its CC-GG mutant using liposome sedimentation assay. (B) Quantification of the liposome sedimentation assays of PICK1 shown in panel (B). Values are mean ± SD of three individual experiments.

Supplementary Table 1 Structural statistics for the family of 20 NMR structures of PICK1 PDZ-GluR2 peptide complex[a]

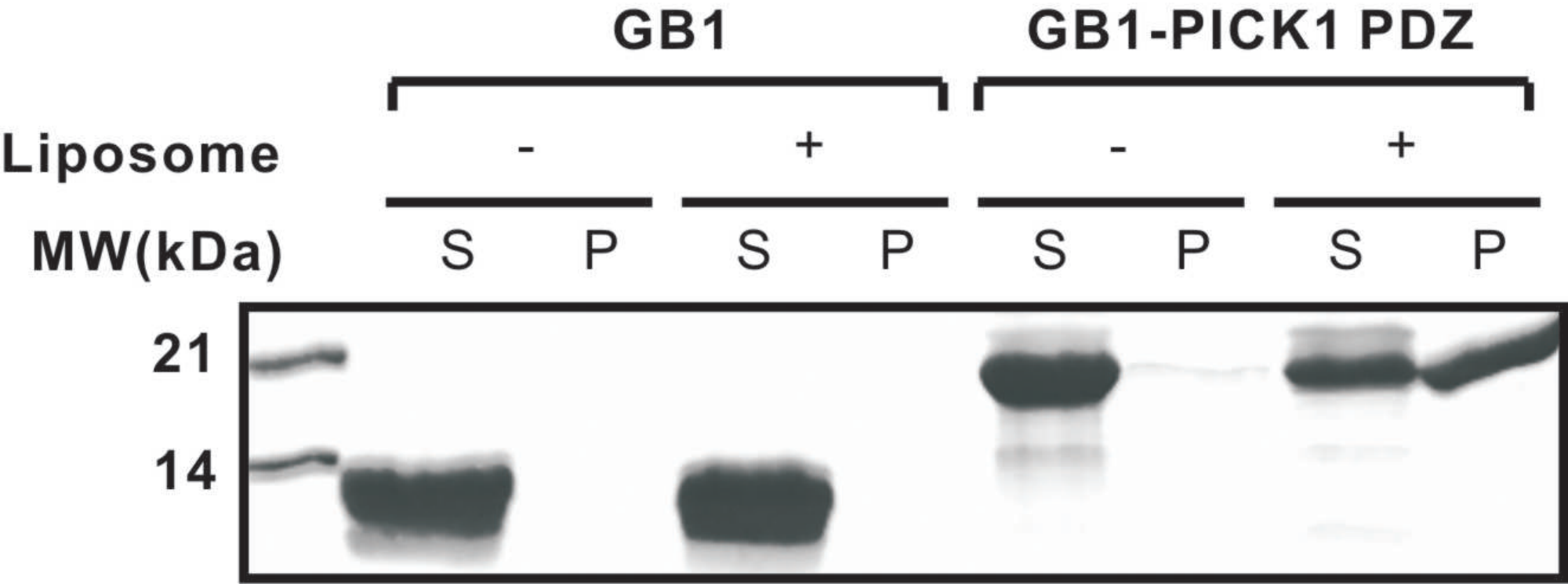
Distance restraints	
Intraresidue*(i-j=0)*	445
Sequential(|*i-j*|=*1*)	481
Medium range(*2* ≤ |*i-j*| ≤ *4*)	316
Long range(|*i-j*| ≥ *5*)	764
Hydrogen bonds	50
Total	2056
Dihedral angle restraints	
Φ	36
Ψ	35
Total	71
Mean r.m.s. deviations from the experimental restraints	
Distance (Å)	0.001±0.000
Dihedral (º)	0.010±0.008
Mean r.m.s. deviations from idealized covalent geometry	
Bond (Å)	0.001±0.000
Angle (º)	0.265±0.002
Improper (º)	0.103±0.003
Mean energies (kcal mol^{-1})[a]	
E_{NOE}[b]	0.20±0.03
E_{cdih}[b]	0.00±0.00
E_{L-J}	-348.91±18.98
Ramachandran plot[c] (Residues 18-104 in PDZ and GluR2[d])	
% residues in the most favorable regions	76.7
additional allowed regions	21.0
generously allowed regions	1.9
Atomic r.m.s. differences (Å)	
Residues 19-40,47-103 in PDZ and GluR2[d]	
Backbone heavy atoms (N, C^{α}, and $C^{'}$)	0.36
Heavy atoms	0.75

[a]None of the structures exhibits distance violations greater than 0.3 Å or dihedral angle violations greater than 4˚.
[b]The final values of the square-well NOE and dihedral angle potentials were calculated with force constants of 50 kcal $mol^{-1}Å^{-1}$ and 200 kcal $mol^{-1}rad^{-1}$.
[c]The PROCHECK-NMR program (Laskowski et al,1996) was used to assess the overall quality of the structures.
[d]GluR2 refers to the last 5 amino acid residues(ESVKI) of the GluR2 subunit of the AMPA receptors.

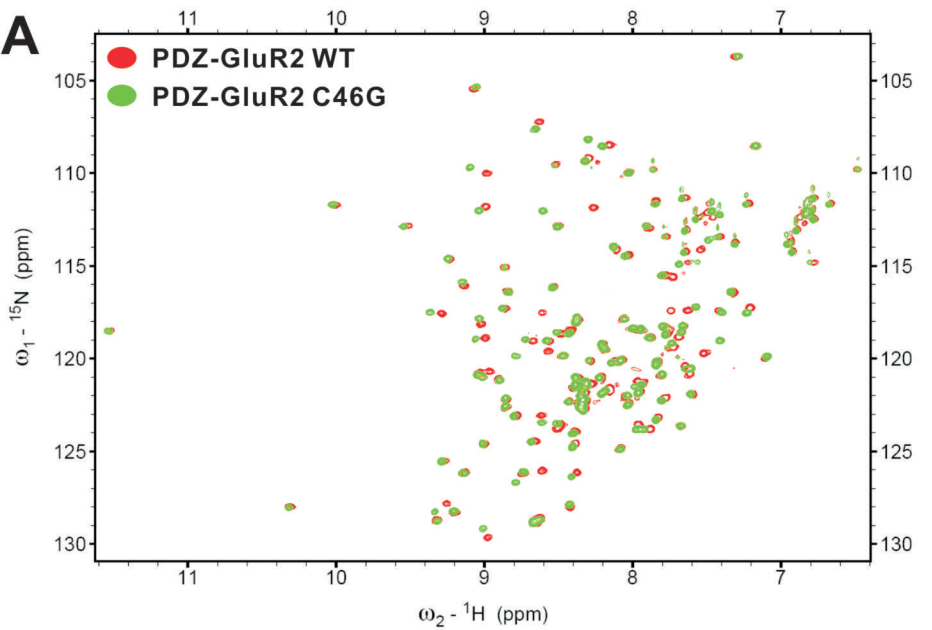
Supplementary Figure 1

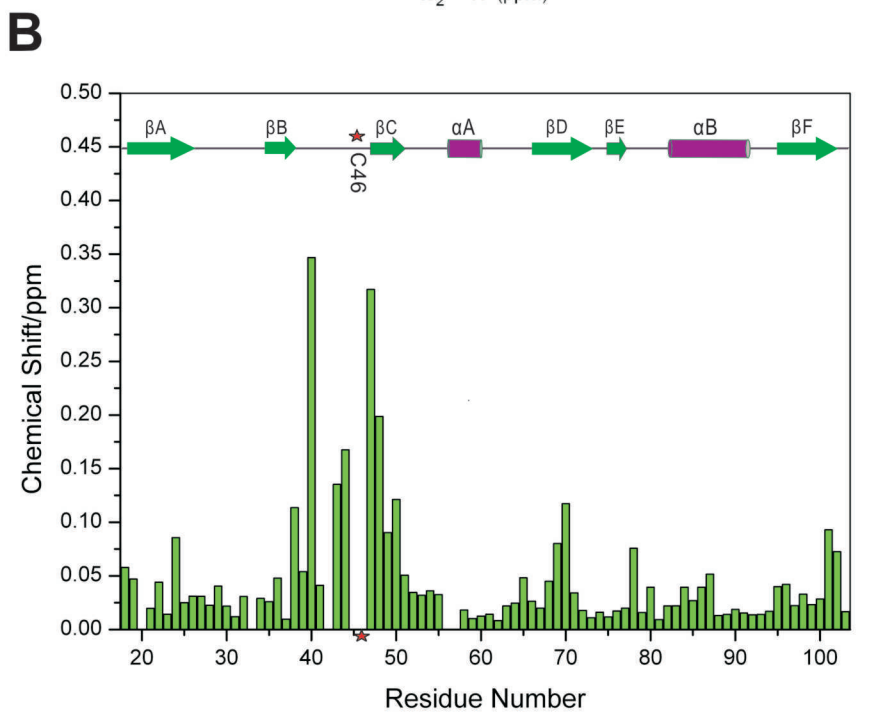


Supplementary Figure 2

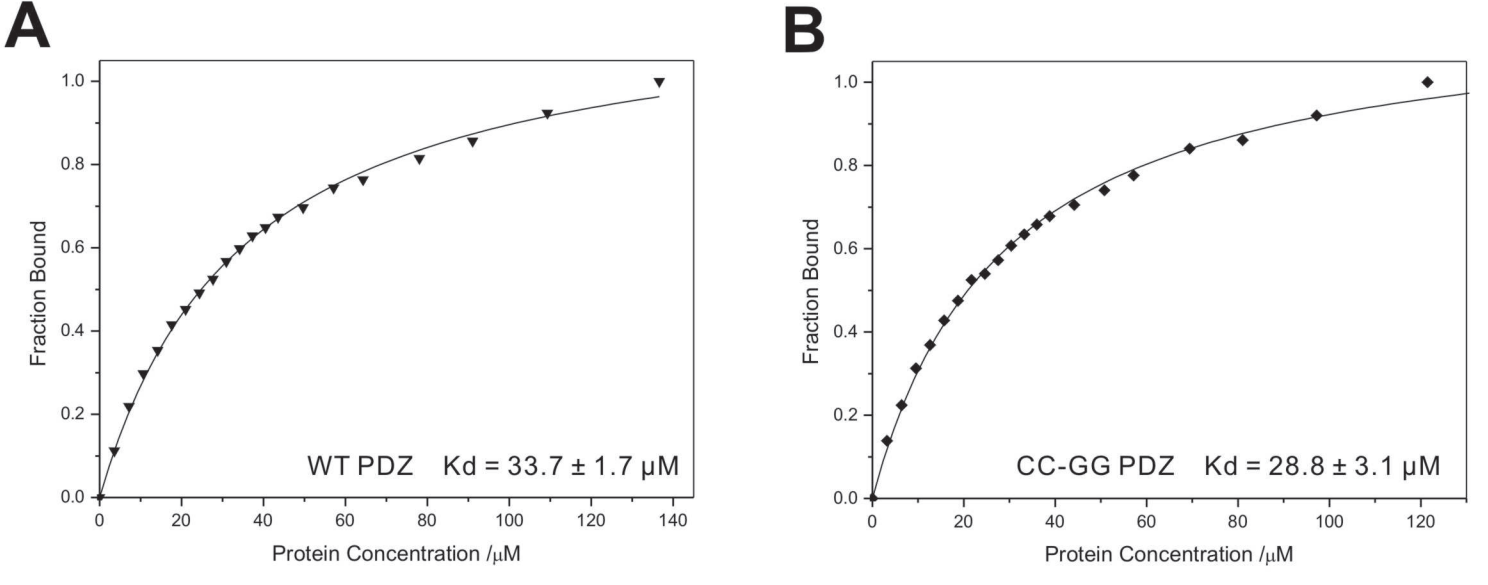


Supplementary Figure 3





Supplementary Figure 4



Supplementary Figure 5