

Supplementary figure legends

Figure 1 Characterization of ZO-1 PDZ2. (A) ^1H - ^{15}N HSQC spectrum of the wild-type ZO-1 PDZ2 showing that a subset of peaks with very broad line-widths. (B) The R193A mutant of ZO-1 PDZ2 exhibit an excellent ^1H - ^{15}N HSQC spectrum, and the overall spectrum of the mutant is highly similar to that of the wild-type ZO-1 PDZ2. (C) Sedimentation equilibrium analysis of ZO-1 PDZ2 at three different concentrations showing that the protein can be nicely fitted to a single specie model with MW of ~ 21.8 kD. The result indicates that ZO-1 PDZ2 adopts a stable dimer in solution. (D) Superposition plot of the HSQC spectra of ^{15}N labeled ZO-1 PDZ2 alone and the protein mixed with equal amount of unlabeled ZO-2 PDZ2 and incubated at 30°C for 1 week. No chemical shift change of ZO-1 PDZ2 could be detected, indicating that there was no detectable hetero-PDZ dimer formation in the mixture.

Figure 2 Molecular details mediating the domain swapped ZO-1 PDZ2 dimer formation. (A) Amino acid sequence alignment analysis showing that DLG5 PDZ2 shares the sequence features found in PDZ2 of ZO-1, ZO-2 and ZO-3 (the βB and βC regions in particular). For comparison, amino acid sequences of two known monomeric PDZ domains (PICK1 PDZ and PSD95 PDZ3) are included in the alignment. In this drawing, the conserved hydrophobic residues are shown in orange, negatively charged residues in magenta, positively charged residues in blue and the rest of the highly conserved residues in green. The βB and βC regions are boxed in red. (B) Combined stick model and ribbon representation showing the details of inter-PDZ interactions in the ZO-1 PDZ2 dimer. These inter-domain interactions are concentrated in three regions, namely the inter-

domain $\beta A/\beta F$ -, $\beta BC/\beta BC$ - pairings, and the inter-PDZ salt bridges involving Lys209 and Glu238. The two PDZ molecules in the dimer are drawn in marine and orange, respectively. (C) The Coomassie Blue-stained SDS-PAGE gel showing DLG5 PDZ2 before and after lysine-specific cross-linking with DSG. The figure demonstrates that DLG5 PDZ2 forms dimer in solution.

Figure 3 The GGGA-insertion mutant and the wild type ZO-1 PDZ2 adopt the highly similar secondary structures. The figure plots the combined C_α/C_β secondary chemical shifts as a function of amino acid residues. The secondary chemical shift, Δppm , of each residue is defined as:

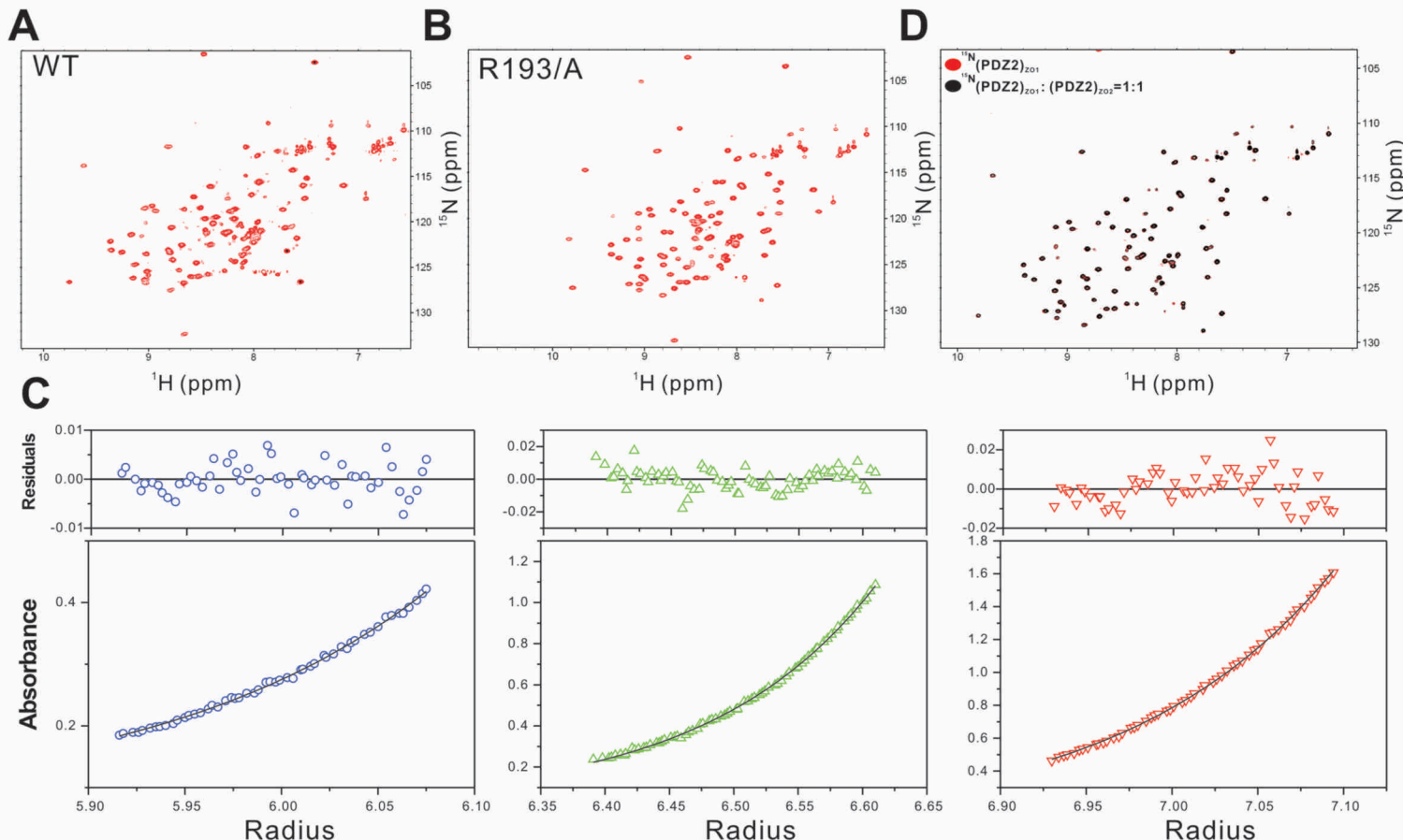
$$\Delta\text{ppm} = ({}^{13}\text{C}_{\alpha,\text{exp}} - {}^{13}\text{C}_{\alpha,\text{rc}}) - ({}^{13}\text{C}_{\beta,\text{exp}} - {}^{13}\text{C}_{\beta,\text{rc}})$$

where ${}^{13}\text{C}_{\alpha,\text{exp}}$ and ${}^{13}\text{C}_{\beta,\text{exp}}$ are experimental chemical shifts of ${}^{13}\text{C}_\alpha$ and ${}^{13}\text{C}_\beta$, respectively, and ${}^{13}\text{C}_{\alpha,\text{rc}}$ and ${}^{13}\text{C}_{\beta,\text{rc}}$ are random coil chemical shifts for each residue. Each data point was smoothed by averaging the secondary chemical shifts of one residue immediately before and after a given amino acid. The regions corresponding to the α -helix and β -strands of the GGGA-insertion mutant derived from the secondary chemical shifts are indicated and colored in black. For comparison, the secondary structure of the wild type ZO-1 PDZ2 swapped dimer derived from the crystal structure is also included at the top of the figure.

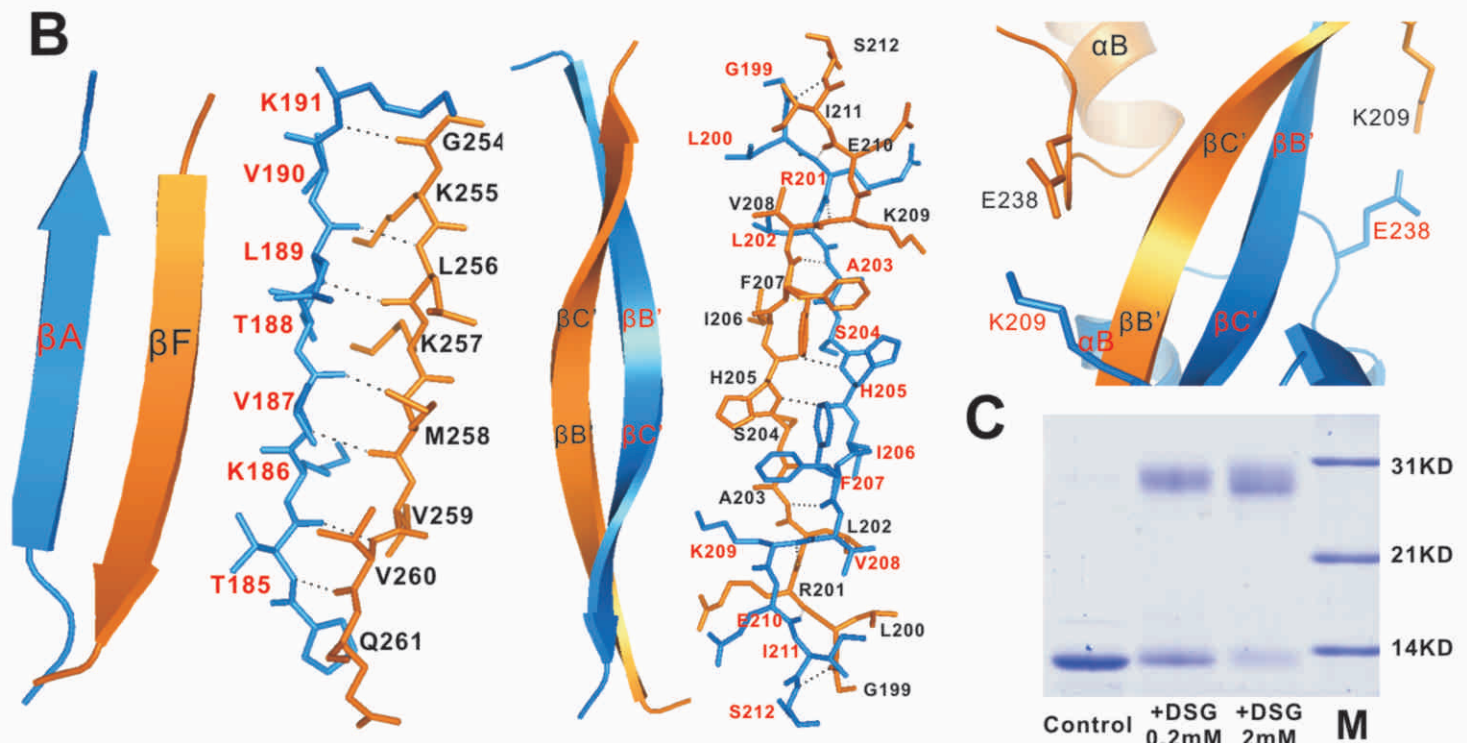
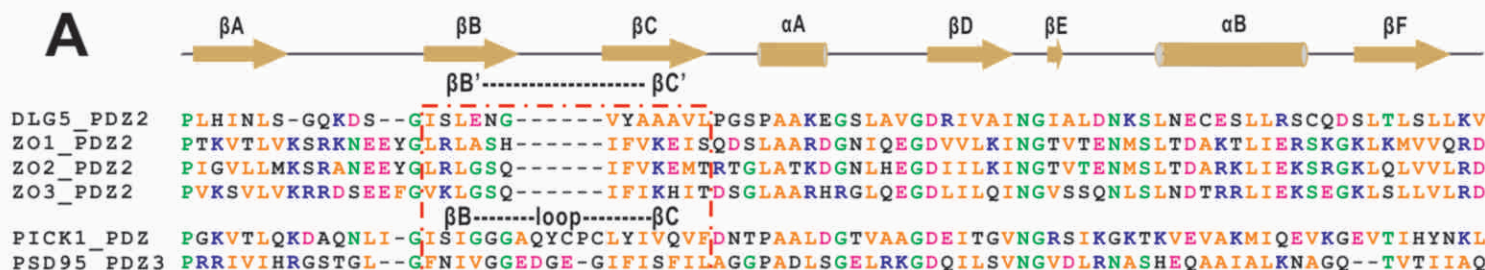
Figure 4 ZO-2 PDZ2 displays very weak binding to the Cx43 peptide. (A) Structure-based sequence comparison of human ZO-1 PDZ2 and ZO-2 PDZ2. The residues of ZO-1 PDZ2 that are directly involved in the binding to the Cx43 peptide are boxed in red and

the residue in the α B5 position is also highlighted. (B) Comparison of the conformation of the α B/ β B-groove of ZO-1 PDZ2 (green, using the structure of the ZO-1PDZ2/Cx43 peptide complex in this work) with that of ZO-2 PDZ2 (purple, using the ligand free NMR structure³³). The two structures are superimposed with each other using their respective α B helices. The side chains of Arg in the α B5 position of ZO-2 PDZ2, Lys in the α B5 position of ZO-1 PDZ2 and Leu(-2) of the Cx43 peptide are shown in stick model. (C) Comparison of the binding affinities of the wild-type ZO-2 PDZ2, the wild-type ZO-1 PDZ2 and the K246R ZO-1 PDZ2 mutant towards the CX43 peptide measured by fluorescence spectroscopy.

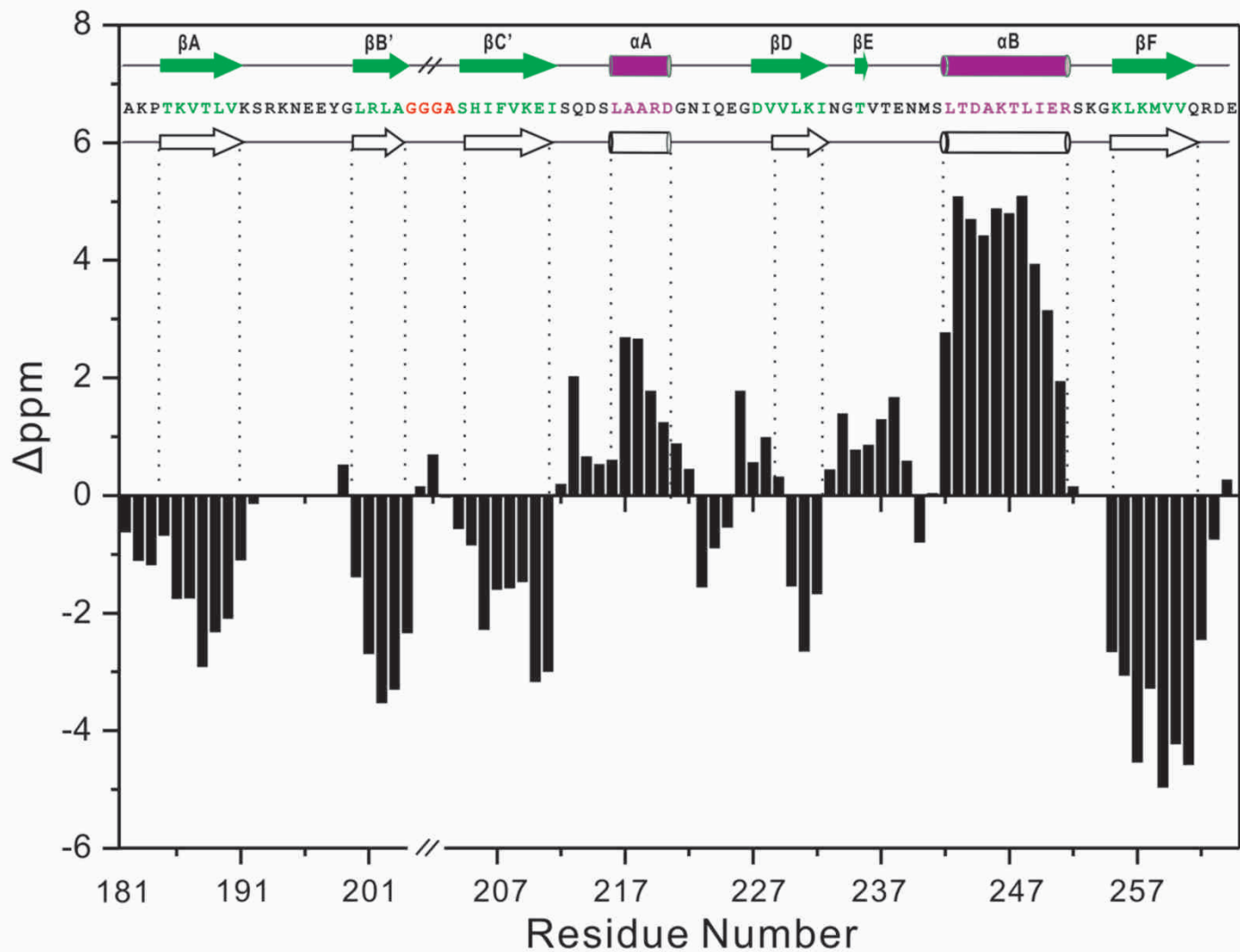
Supplementary Fig 1



Supplementary Fig2



Supplementary Fig3



Supplementary Fig4

