Supplemental Data

MOLECULAR MECHANISM OF THE NEGATIVE REGULATION OF SMAD1/5 BY CHIP

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Running title: CHIP-mediated repression of Smad1/5

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Supplemental Table S1. Detailed purification procedures for proteins used

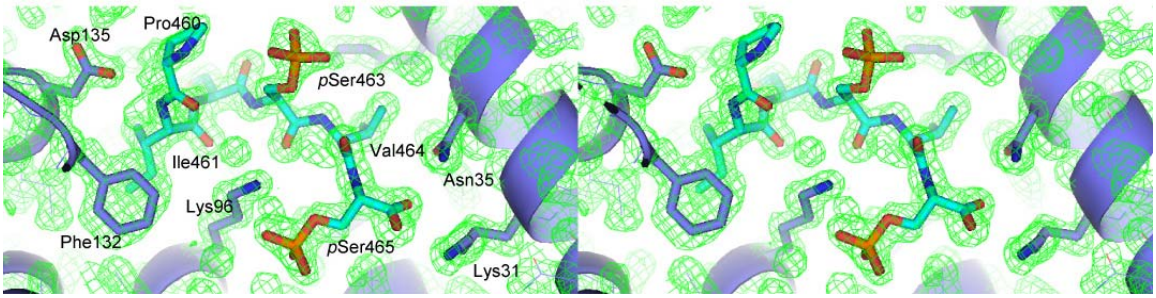
Protein	Vector	Affinity column	Ion exchange	Gel filtration
CHIP	pGEX-4T-2	GST	Source-15Q Source-15S	
CHIP-TPR	pET-Duet-1	Ni-NTA	Source-15S	
CHIP-CC	pGEX-4T-2	GST	Source-15Q	
CHIP-Ubox	pGEX-4T-2	GST		Superdex-200
CHIP-ΔTPR	pGEX-4T-2	GST		Superdex-200
Smad1	pGEX-4T-2	GST	Source-15Q	Superdex-200
Smad1-MH1	pGEX-4T-2	GST		
Smad1-MH1-L	pGEX-4T-2	GST		
Smad1-L-MH2	pGEX-4T-2	GST	Source-15Q	Superdex-200
Smad1-MH2	pGEX-4T-2	GST	Source-15Q	Superdex-200
Smad2-MH1	pGEX-4T-2	GST		
Smad2-L-MH2	pGEX-4T-2	GST	Source-15Q	Superdex-200
Smad2-MH2(DMD)	pET-21b		Sp sepharose Q sepharose	
Smad3-MH1	pGEX-4T-1	GST		
Smad3-L-MH2	pGEX-4T-2	GST	Source-15Q	Superdex-200
Smad4-MH1	pGEX-4T-2	GST		
Smad4-L-MH2	pGEX-4T-2	GST	Source-15Q	Superdex-200
Smad5-MH1	pGEX-4T-2	GST		
Smad5-L-MH2	pGEX-4T-2	GST	Source-15Q	Superdex-200
Hsp70-CTD	pGEX-2T	GST	Source-15Q	
E1	pET-Duet-1	Ni-NTA		
E2(UbcH5a)	pET-Duet-1	Ni-NTA		
Ubiquitin	pET-Duet-1	Ni-NTA	Source-15Q	Superdex-200

GST- or His_6-tag was proteolytically removed from the fusion proteins to prepare nontag proteins. All point mutants used were similarly purified to their corresponding wild-type proteins.

Supplemental Figure S1

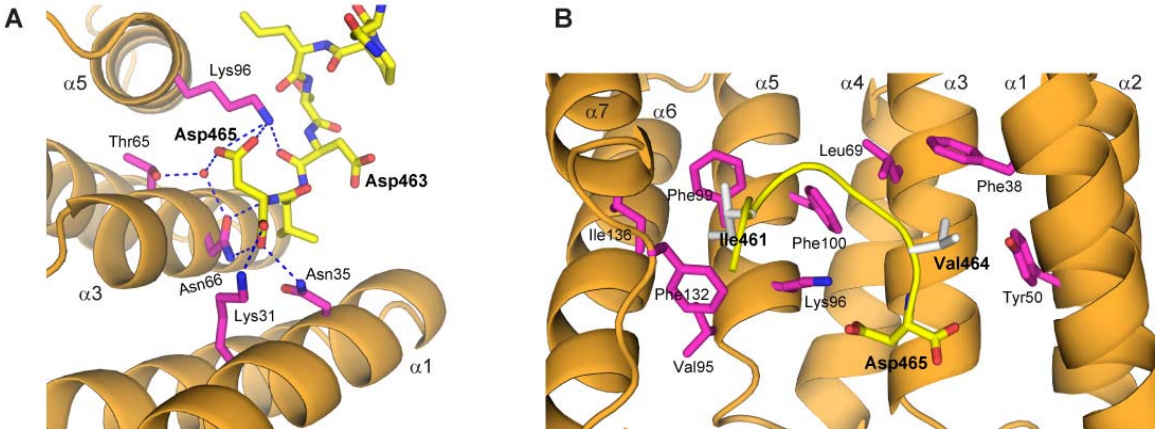
Supplemental Fig. S1 Structure-based sequence alignment of TPR domains that bind to heat shock proteins. The secondary structural elements of CHIP-TPR are shown above the sequences. Residues on CHIP-TPR that are involved in hydrophilic and hydrophobic interactions with Smad1 and Hsp70/90 are indicated by red triangle and green diamond, respectively.

Supplemental Figure S2



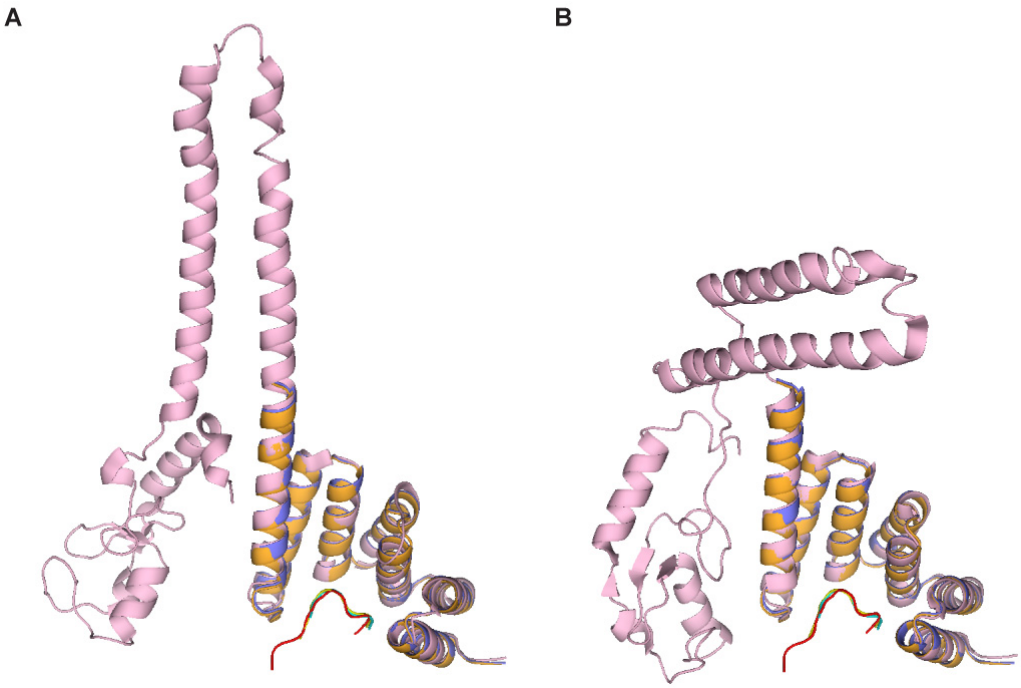
Supplemental Fig. S2 Stereo image of the $2F_o–F_c$ map (green mesh) around the phosphor-Smad1 peptide (cyan sticks). CHIP-TPR is colored in slate, and residues surrounding Smad1 peptide are shown in sticks.

Supplemental Figure S3



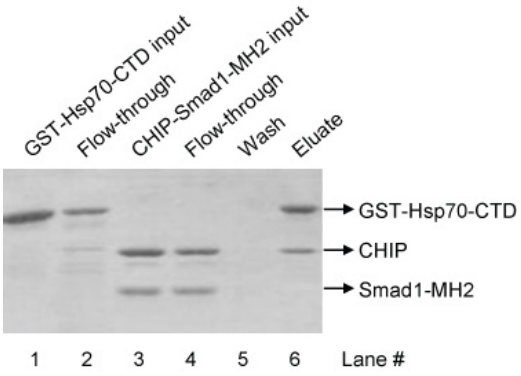
Supplemental Fig. S3 Hydrophilic and hydrophobic contacts between CHIP-TPR and the pseudophosphorylated Smad1(DVD) peptide. The two views were oriented same as the ones in Fig. **3C** and **3D**, respectively. (**A**) Hydrogen bond networks at the interface of CHIP-TPR and Smad1(DVD) peptide. CHIP-TPR is colored in orange and the critical residues are highlighted in magentas sticks. The Smad1 peptide is shown as yellow sticks. (**B**) van der Waals contacts between CHIP-TPR and pseudophosphorylated Smad1 peptide. The Smad1 peptide is shown as yellow ribbon and the hydrophobic residues are highlighted in grey sticks.

Supplemental Figure S4



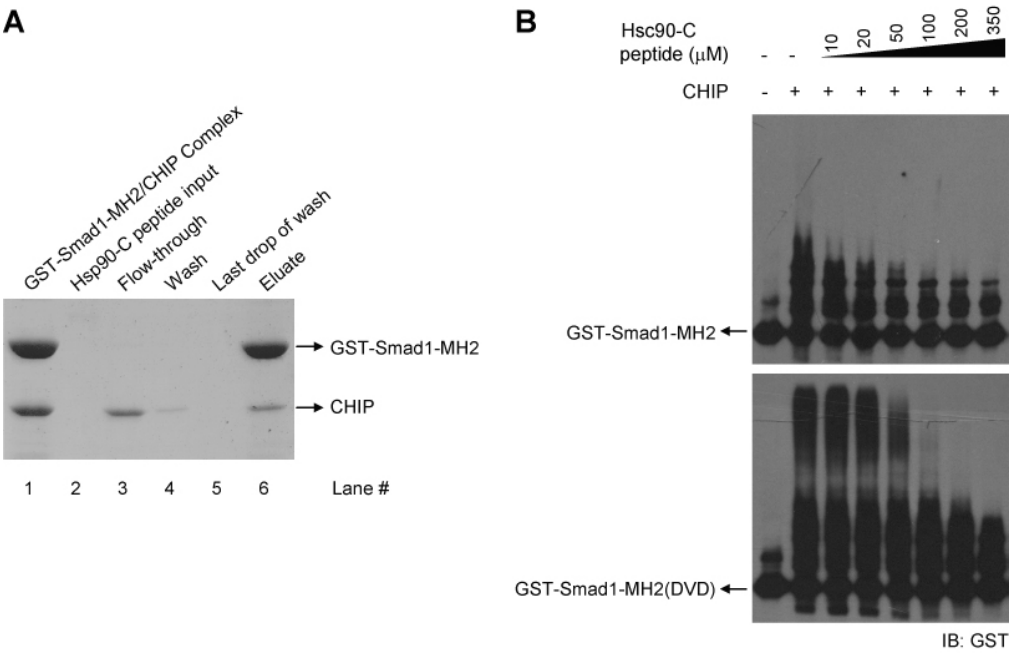
Supplemental Fig. S4 Superposition of the CHIP-Smad1 structures with the counterparts in the dimeric structure of full-length CHIP in complex with Hsp90-C peptide (PDB ID: 2C2L). The two asymmetric full-length CHIP molecules are colored in pink and the bound Hsp90-C peptides in red. The CHIP-Smad1 peptide structures are colored the same as Fig. **3A** and **3B**.

Supplemental Figure S5



Supplemental Fig. S5 Disruption of CHIP-Smad1 complex by Hsp70-CTD. Recombinant GST-Hsp70-CTD was first bound to 0.2 ml Glutathione Sepharose 4B resin. The resin was washed five times with 1.0 ml buffer to remove excess unbound Hsp70 or other contaminants. Then, non-tagged CHIP-Smad1 complex was allowed to flow through the resin. After extensive washing, the bound proteins were eluted with 5 mM reduced glutathione, and all fractions were visualized by SDS-PAGE with Coomassie Blue staining.

Supplemental Figure S6



Supplemental Fig. S6 Hsp90-C peptide disrupts CHIP-Smad1 interaction and inhibits CHIP-mediated Smad1 ubiquitination. (**A**) Hsp90-C peptide competing with Smad1 for CHIP binding. The complex of GST-Smad1 and CHIP was loaded to the resin, followed by the addition of the Hsp90-C peptide. The column was then washed extensively and eluated with reduced glutathione. Samples were visualized by SDS-PAGE with Coomassie Blue staining. (**B**) Hsp90-C peptide antagonizing CHIP-mediated Smad1 ubiquitination. *In vitro* ubiquitination assays were performed in the absence or presence of increasing amount of Hsp90-C peptide.