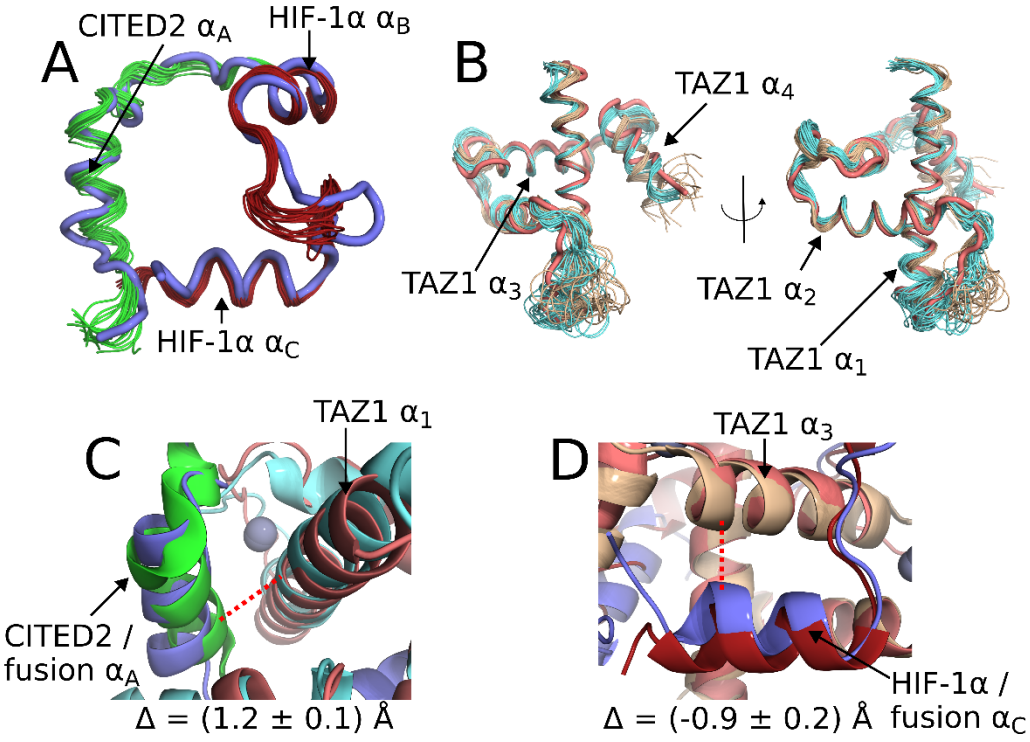

Figure S1. Comparison of the TAZ1:fusion peptide, TAZ1:CITED2, and TAZ1:HIF-1α structures. Related to Figure 2. (A) Comparison of the structures of CITED2 residues 220-246 (green), HIF-1α residues 796-826 (red), and the fusion peptide (slate) in their complexes with TAZ1. For CITED2 (1R8U) (De Guzman et al., 2004) and HIF-1α (1L8C) (Dames et al., 2002), all 20 members of the PDB-deposited NMR ensemble are displayed. The backbones of CITED2 and HIF-1α are represented by ribbons and the backbone of the fusion peptide is represented by a tube. The loop between the α_B and α_C helices in the HIF-1α complex is dynamically disordered in solution but is engaged in multiple lattice contacts in the crystal of the TAZ1:fusion peptide complex. (B) Comparison of the TAZ1 (residues 345-437) component of the TAZ1:CITED2, TAZ1:HIF-1α, and TAZ1:fusion peptide structures. For the TAZ1:CITED2 and TAZ1:HIF-1α structures all 20 members of the PDB-deposited NMR ensemble are displayed and are shown in cyan and tan respectively. The TAZ1 component of the TAZ1:fusion peptide structure is shown in salmon. The TAZ1 backbones of TAZ1:CITED2 and TAZ1:HIF-1α complexes are represented by ribbons and the TAZ1 backbone of the fusion peptide complex is represented by a tube. Zinc atoms are omitted for clarity. (C) Difference in distances between the TAZ1 α_1 (TAZ1 H362) and CITED2 α_A (CITED2 L228 (fusion peptide L18)) helices in the TAZ1:CITED2 and TAZ1:fusion peptide structures. The TAZ1:CITED2 and TAZ1:fusion peptide complexes are colored as described in panels A and B. Zinc atoms are shown as gray spheres. The broken red line connects the approximate positions of the Cα atoms used for distance measurements. The denoted difference in distance is calculated by subtracting the average distance between the CITED2 L228 Cα and the TAZ1 H362 Cα calculated from the 20 members of the NMR ensemble from the distance between the corresponding Cα atoms in the TAZ1:fusion peptide structure. The uncertainty represents the standard deviation of the Cα-Cα distances in the TAZ1:CITED2 NMR ensemble. (D) Difference in distance between the TAZ1 α_3 (TAZ1 K419) and HIF-1α α_C (HIF-1α A821 (fusion peptide A62)) helices in the TAZ1:HIF-1α and TAZ1:fusion peptide structures. TAZ1:HIF-1α and TAZ1:fusion peptide complexes are colored as described in panels A and B. Zinc atoms are shown as gray spheres. The broken red line connects the approximate positions of the Cα atoms used for distance measurements. The denoted difference in distance is calculated by subtracting the average distance between the HIF-1α A821 Cα and the TAZ1 K419 Cα calculated from the 20 members of the NMR ensemble from the distance between the corresponding Cα atoms in the TAZ1:fusion peptide structure. The uncertainty represents the standard deviation of the Cα-Cα distances in the TAZ1:HIF-1α NMR ensemble.

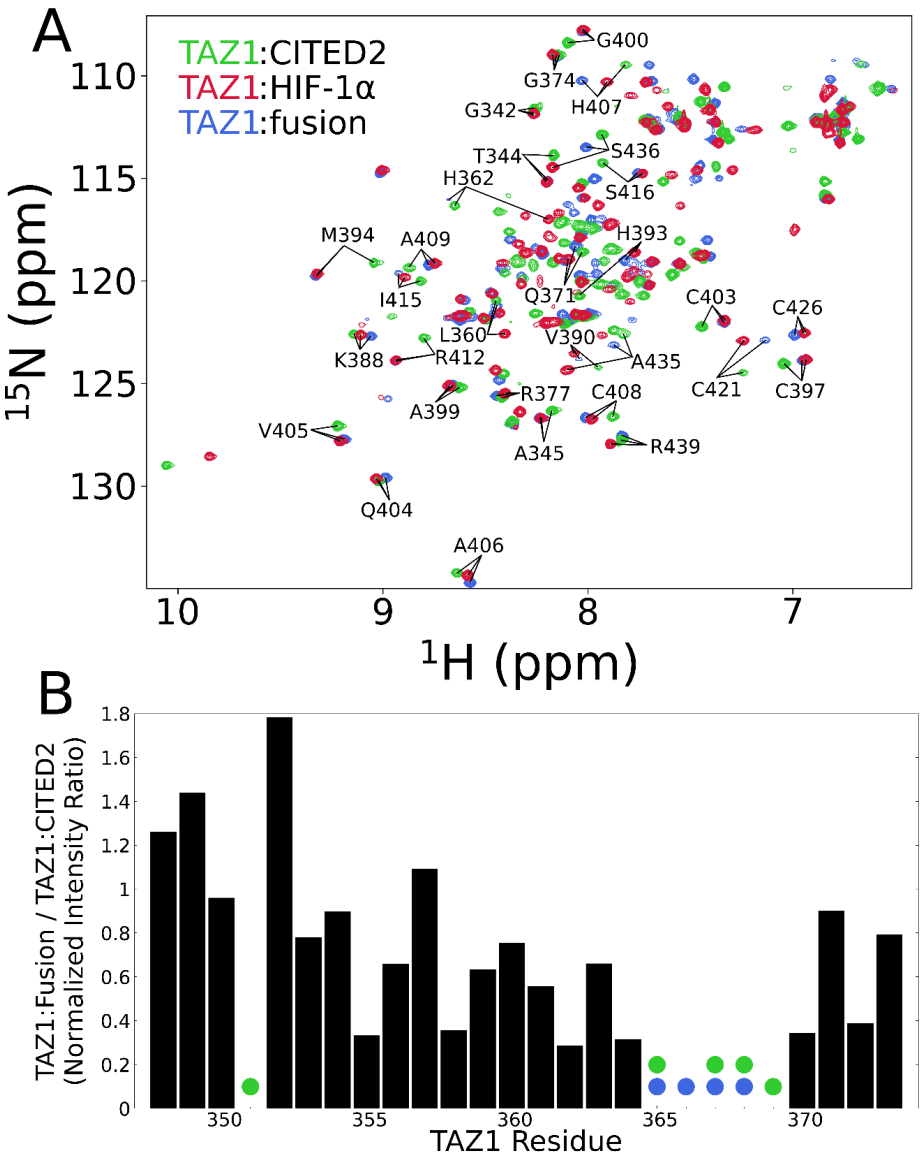

Figure S2. Comparisons of ^{1}H-^{15}N HSQC spectra of ^{15}N-labeled TAZ1 bound to unlabeled CITED2, HIF-1α, or fusion peptide. Related to Figure 3. (A) ^{1}H-^{15}N HSQC spectra (900 MHz ^{1}H frequency) of ^{15}N-labeled TAZ1 bound to unlabeled CITED2 (green), HIF-1α (red), or fusion peptide (blue). Selected backbone amide cross peaks are labeled. (B) Ratios of ^{1}H-^{15}N HSQC cross peak intensities of backbone amides from the TAZ1 α_1 helix in the TAZ1:CITED2 and TAZ1:fusion peptide complexes. Ratios were calculated by first normalizing each cross peak intensity to the C-terminal TAZ1 backbone amide cross peak intensity (R439) from each spectrum. Each normalized TAZ1:fusion intensity was then divided by the corresponding normalized TAZ1:CITED2 intensity. Circles inside the graph denote residues for which backbone amide resonance assignments are missing for TAZ1 in complex with CITED2 (green) or fusion peptide (blue).

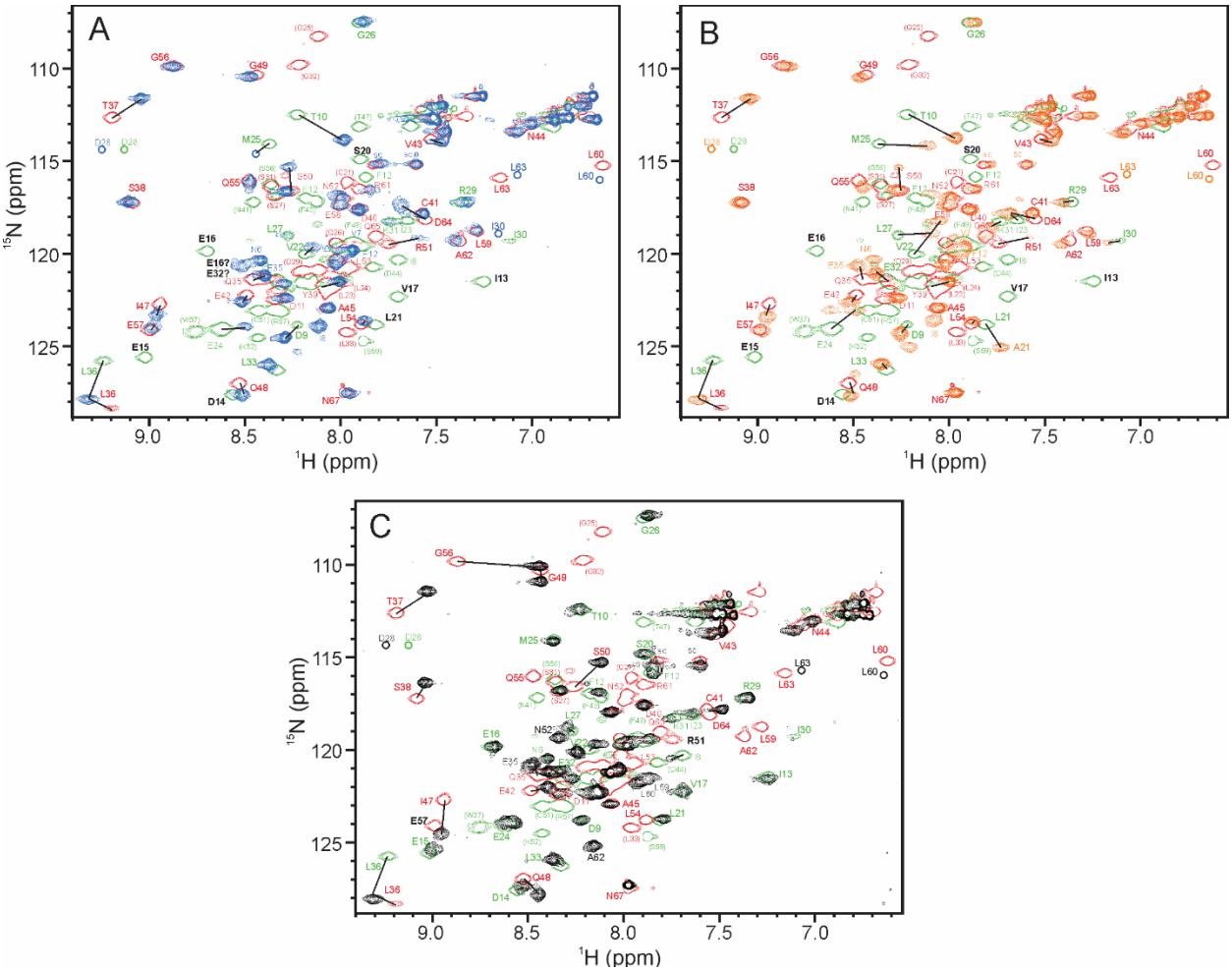

Figure S3. Comparisons of ^{1}H-^{15}N HSQC spectra of ^{15}N-labeled CITED2, HIF-1α, and fusion peptides bound to unlabeled TAZ1. Related to Figure 5. In each panel, the ^{1}H-^{15}N spectrum of CITED2 in complex with unlabeled TAZ1 is shown in green, and that of HIF-1α is shown in red. (A) The CITED2 and HIF-1α spectra superimposed on the ^{1}H-^{15}N spectrum of the fusion peptide in complex with unlabeled TAZ1 (blue). (B) The CITED2 and HIF-1α spectra superimposed on the ^{1}H-^{15}N spectrum of the L21A mutant fusion peptide in complex with unlabeled TAZ1 (orange). (C) The CITED2 and HIF-1α spectra superimposed on the ^{1}H-^{15}N spectrum of the L63A mutant fusion peptide in complex with unlabeled TAZ1 (black). Cross peaks from the CITED2 and HIF-1α complexes are labeled in green and red, respectively, and connected with the corresponding cross peaks from the fusion peptide complexes by black lines. Cross peaks from the N-terminus of the HIF-1α complex and the C-terminus of the CITED2 complex have no corresponding cross peak in the fusion peptide complex spectrum and are labeled in smaller font in parentheses. Cross peaks that appear in the CITED2 complex spectrum but are absent from the spectrum of the fusion peptides are labeled in bold font, black. The positions of very weak cross peaks that are observed at very low contour levels are shown as labeled circles.

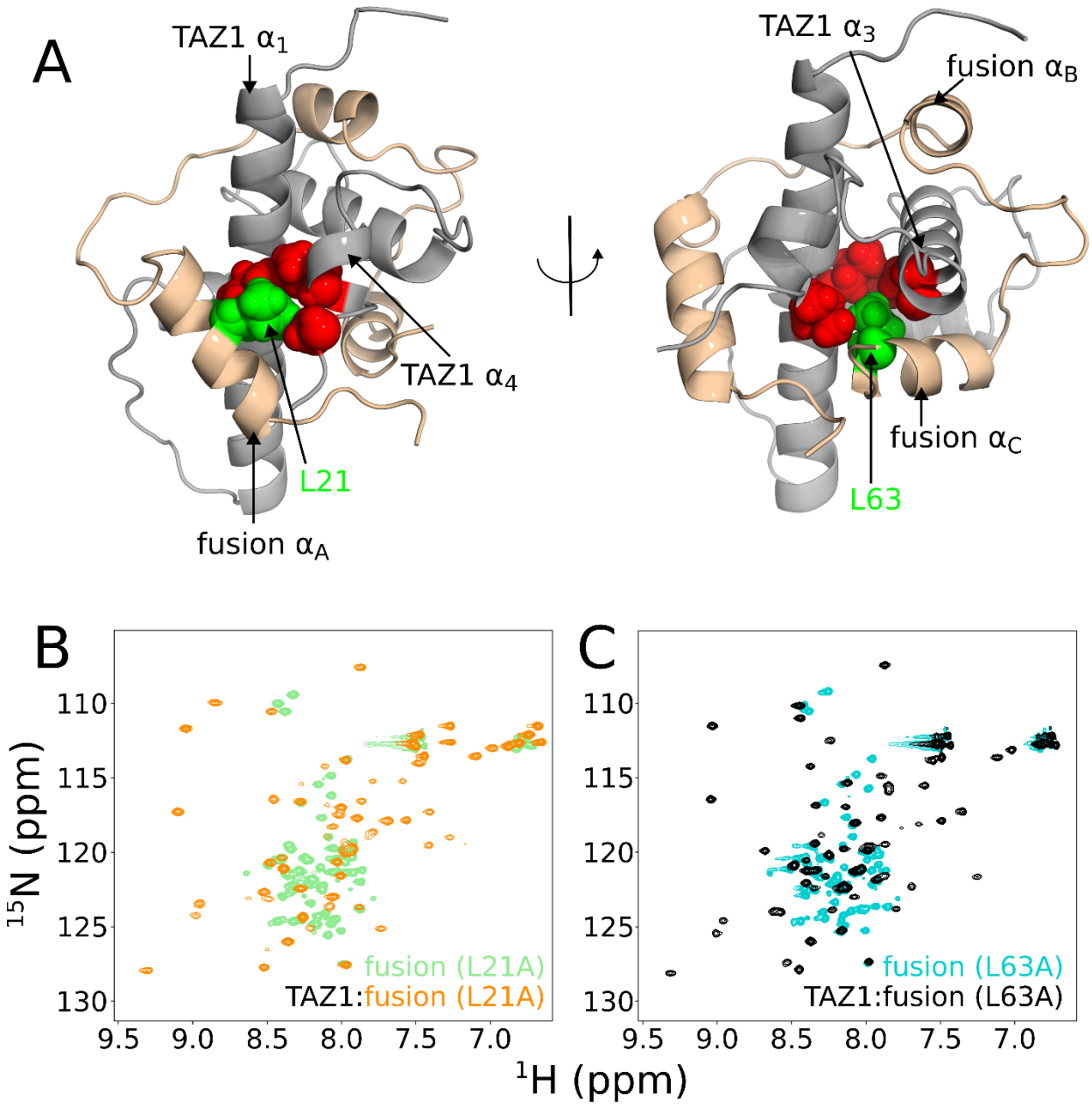

Figure S4. Mutant fusion peptides. Related to Figure 6. (A) Left panel: interactions between L21 (green spheres) and TAZ1 V358, L432, and A435 (red spheres). Right panel: interactions between L63 (green spheres) and TAZ1 L360, L361, and I415 (red spheres). TAZ1 is colored gray and the fusion peptide is colored tan. Zn atoms are omitted for clarity. (B) Overlay of the amide region of ^{1}H-^{15}N HSQC spectra (500 MHz ^{1}H frequency) of ^{15}N-labeled L21A fusion peptide free in solution (green) or bound to TAZ1 (orange). (C) Overlay of the amide region of ^{1}H-^{15}N HSQC spectra (900 MHz ^{1}H frequency) of ^{15}N-labeled L63A fusion peptide free in solution (cyan) or bound to TAZ1 (black).

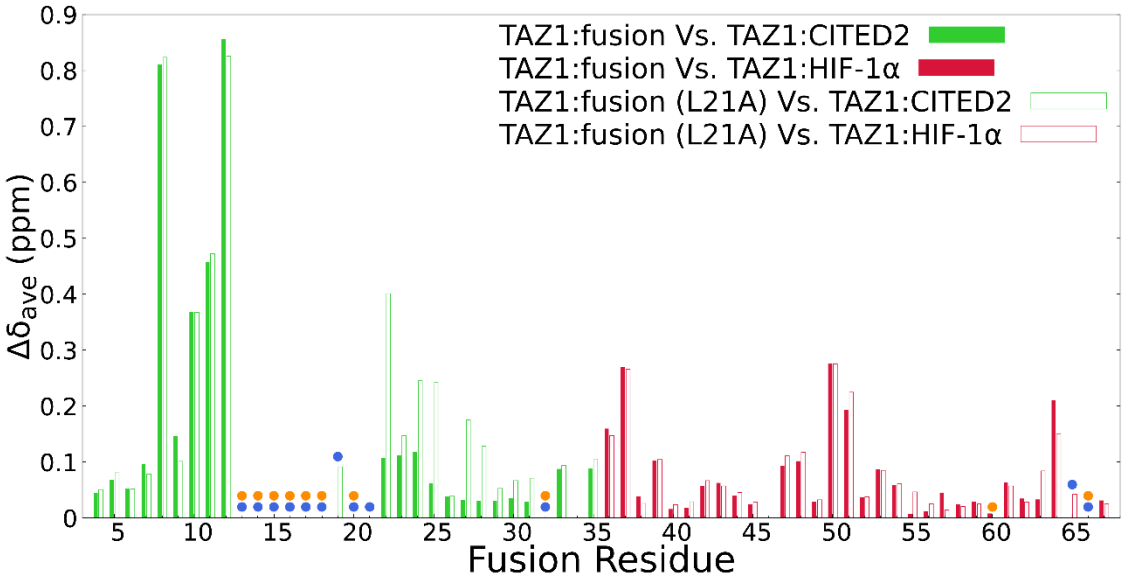

Figure S5. Weighted average $^{1}H,^{15}N$ chemical shift differences between ^{15}N-labeled original or L21A fusion peptide in complex with unlabeled TAZ1 complexes and ^{15}N-labeled CITED2 or HIF-1α in complex with unlabeled TAZ1. Related to Figure 6, 7. Comparisons with CITED2 (residues 222-245) are shown in green and those with HIF-1α (residues 795-826) are shown in red. Comparisons with the original fusion peptide are shown as solid bars, and those with the L21A fusion peptide are shown as empty bars. Circles denote missing backbone amide resonance assignments of the original fusion peptide in complex with unlabeled TAZ1 (blue) or the L21A fusion peptide in complex with unlabeled TAZ1 (orange).

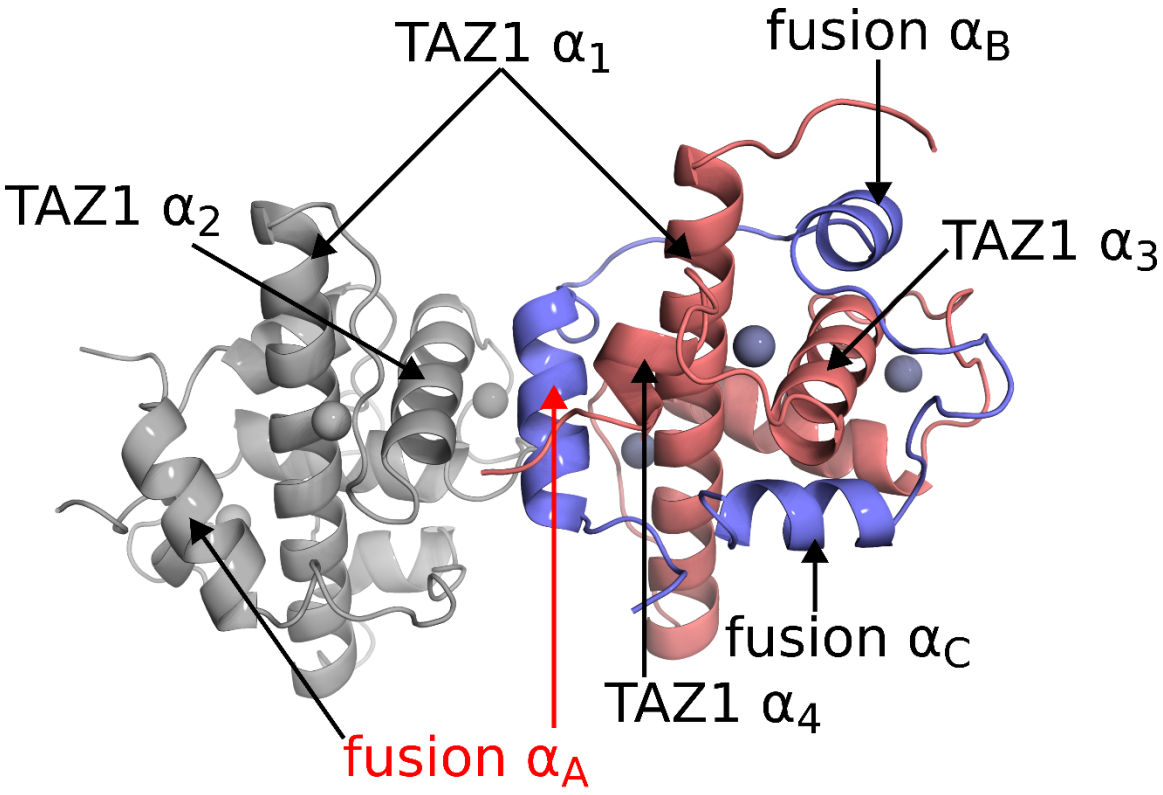

Figure S6. Stabilization of the TAZ1-fusion peptide α_A interaction through intermolecular crystal contacts. Related to Figure 2. TAZ1 is shown in salmon, the fusion peptide is shown in slate, a neighboring molecule of the crystal lattice is shown in gray. Zinc atoms are shown as gray spheres. Helices of TAZ1 and the fusion peptide are labeled.

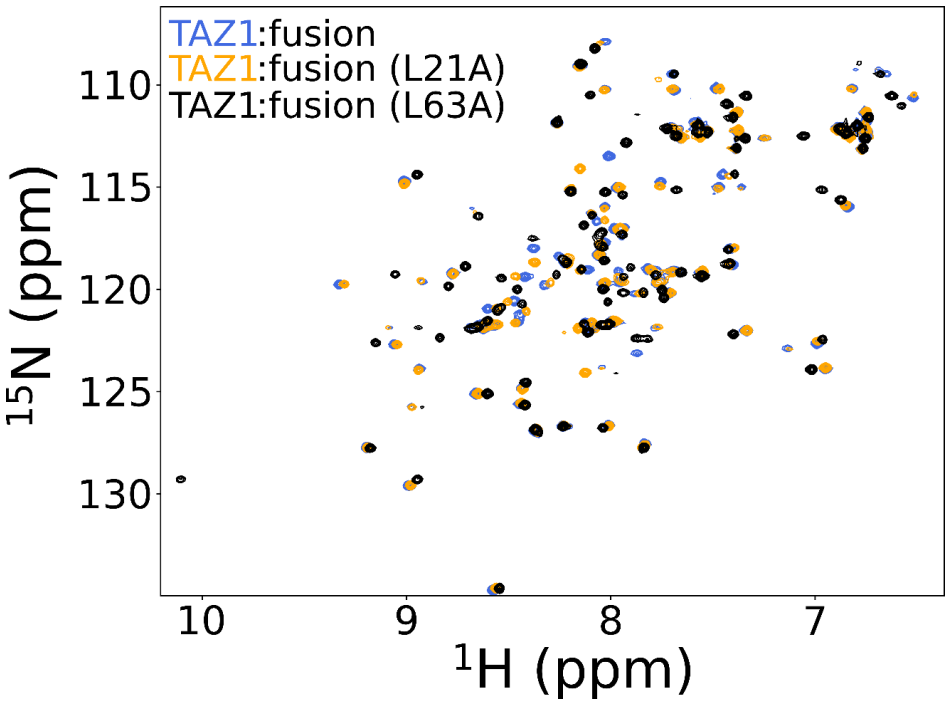

Figure S7. ^{1}H-^{15}N HSQC spectra (900 MHz ^{1}H frequency) of ^{15}N-labeled TAZ1 in complex with unlabeled original (blue), L21A (orange), or L63A (black) fusion peptide. Related to Figure 7.

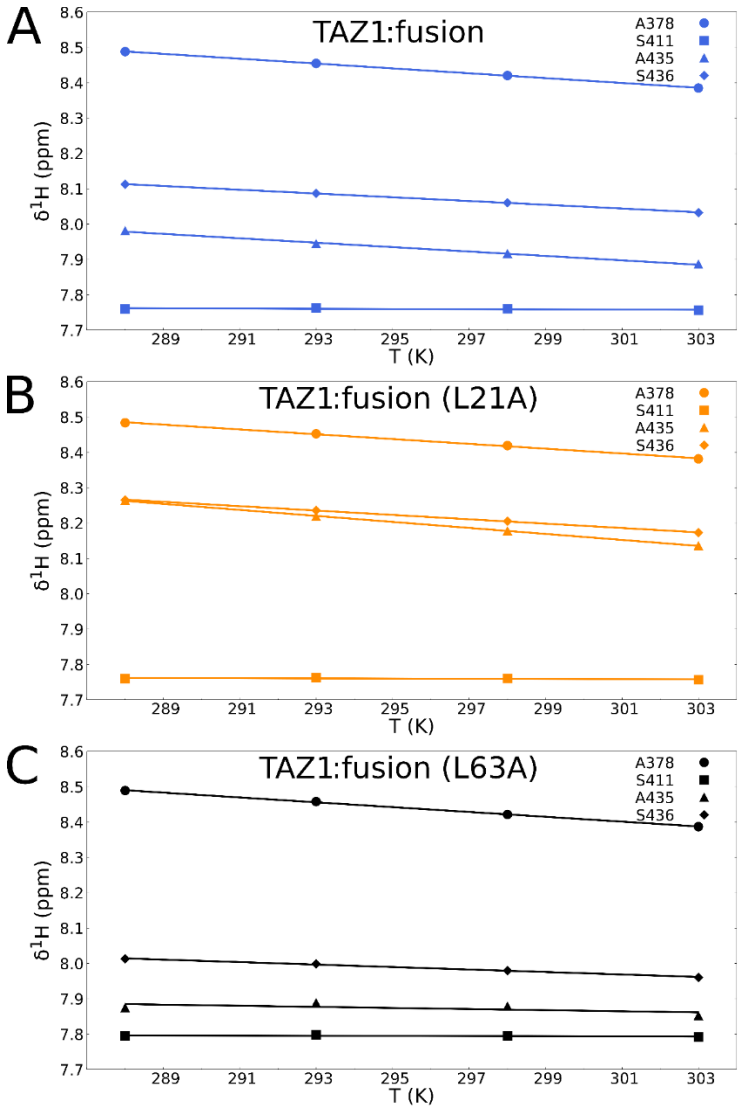

Figure S8. Temperature dependence of backbone amide ^{1}H chemical shifts of selected residues from TAZ1 in complex with the original, L21A, or L63A fusion peptides. Related to Figure 7. (A) ^{1}H chemical shifts of TAZ1 A378 (circles), S411 (squares), A435 (triangles), S436 (diamonds) from the TAZ1:fusion peptide complex. (B) ^{1}H chemical shifts of TAZ1 A378 (circles), S411 (squares), A435 (triangles), S436 (diamonds) from the TAZ1:fusion peptide (L21A) complex. (C) ^{1}H chemical shifts of TAZ1 A378 (circles), S411 (squares), A435 (triangles), S436 (diamonds) from the TAZ1:fusion peptide (L63A) complex. Lines in each graph represent linear fits of the data.