Supporting information

The tuberous sclerosis complex subunit TBC1D7 is stabilized by Akt phosphorylation-mediated 14-3-3 binding

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Table S1. Crystallography data and refinement statistics

Figures S1, S2, S3

Table S1. Crystallography data and refinement statistics

	14-3-3 zeta / peptide	TBC1D7
PDB Code	5ULO	3QWL
Data collection		
Space group	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions		
a, *b*, *c* (Å)	69.7, 71.6, 129.9	34.6, 84.0, 87.7
α, β, γ (°)	90.0, 90.0, 90.0	90.0, 90.0, 90.0
Resolution (Å)	50.00–2.14 (2.18–2.14)*	40.00–1.90 (1.97–1.90)
R_{merge} (%)	5.9 (97.9)	6.6 (85.5)
average *I*/average error[a]	53.9 (2.4)	41.3 (2.1)
Completeness (%)	99.5 (100.0)	99.7 (100.0)
Redundancy	8.0 (8.2)	7.0 (7.1)
Refinement		
Resolution (Å)	29.9–2.14	27.15-1.90
No. reflections work/free	35290/1101	19653/1062
R_{work}/R_{free} (%)	22.7/27.0	21.1/25.8
No. atoms		
Protein	3549	2147[c]
Peptide	88	
Water	60	44[c]
B-factors (Å^2)		
Protein	77.0[b]	39.1[c]
Peptide	92.6[b]	
Water	68.9[b]	39.7[c]
RMSD		
Bond lengths (Å)	0.010	0.014
Bond angles (º)	0.98	1.3
Molprobity φ-ψ plot % residues		
favored	98.9	98.5
outliers	0.0	0.0

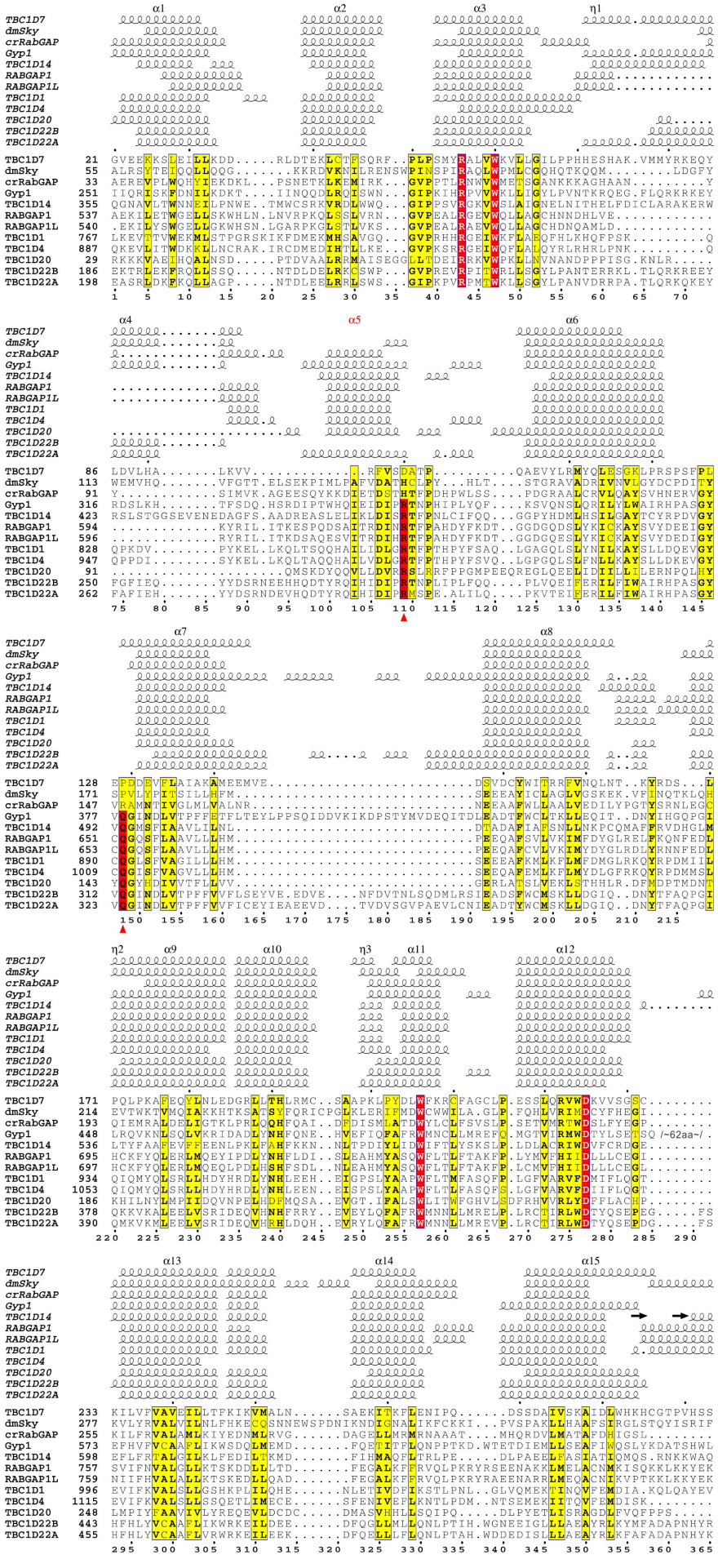
*values in parentheses are for highest resolution shell unless otherwise indicated.

[a] SCALEPACK reports "Average I" and "Average error"

[b] GJ Kleywegt: Moleman software

[c] PHENIX.PDBTOOLS [Adams PD, Afonine PV, Bunkóczi G, Chen VB, Davis IW, Echols N, Headd JJ, Hung LW, Kapral GJ, Grosse-Kunstleve RW, McCoy AJ, Moriarty NW, Oeffner R, Read RJ, Richardson DC, Richardson JS, Terwilliger TC, Zwart PH (2010) PHENIX: a comprehensive Python-based system for macromolecular structure solution. *Acta Crystallogr D Biol Crystallogr.* **66**(Pt 2): 213-221.]

Figure S1. Structure-based alignment of TBC domains of all known TBC structures. The same pdb codes as those in Fig. 1C were used for the alignment. The Gyp1 has an insertion of 57 unstructured residues between a.a. 510 and 568 that are invisible in the crystal structure (PDB:2G77). This insertion does not exist in other TBC domains and was removed from the alignment. The numbering of the helices was based on that of Gyp1. Helix 5 is missing in TBC1D7 and is colored red.



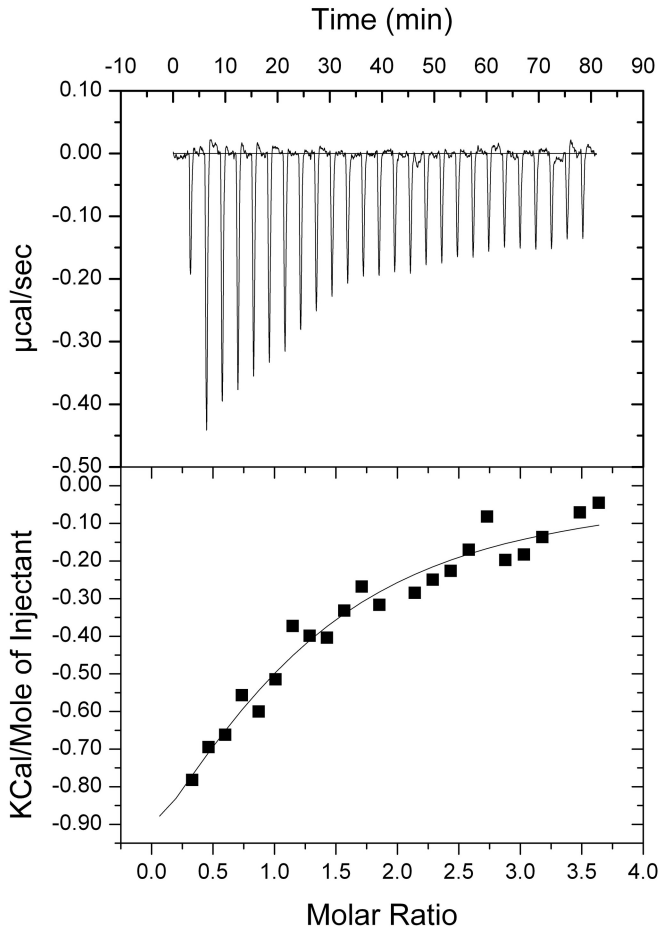


Figure S2. Isothermal titration calorimetry measurement of 14-3-3 zeta and TBC1D7 phosphopeptide binding. 12-mer TBC1D7 phosphopeptide at 1 mM in a syringe was titrated into 52 µM 14-3-3 protein in a sample cell. One-site binding fitting gives a dissociation constant KD = 58.5 µM

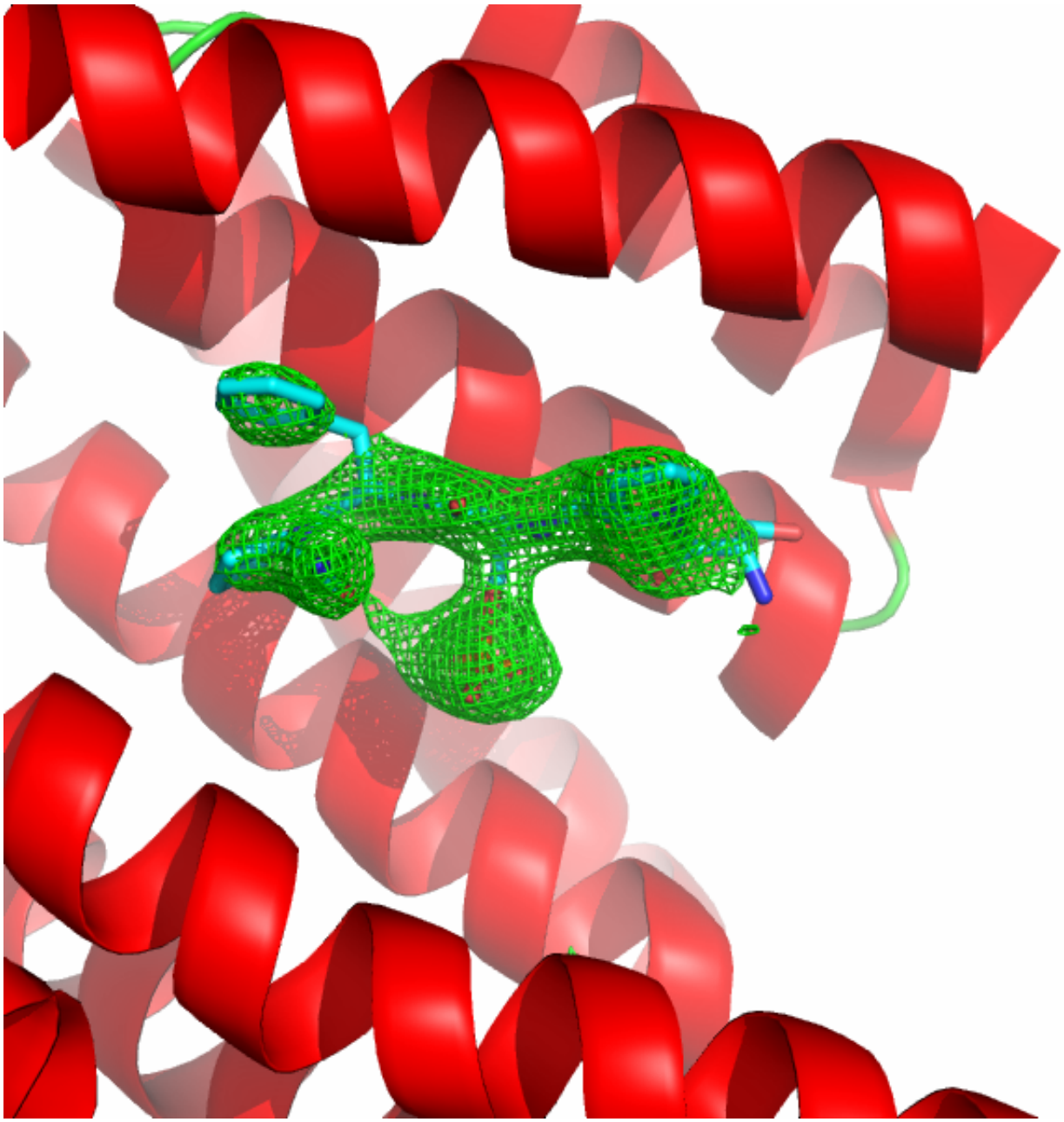


Figure S3. Omit map of the TBC1D7 peptide in the complex structure. The omit Fo-Fc electron density map for the peptide was contoured at 4 σ.