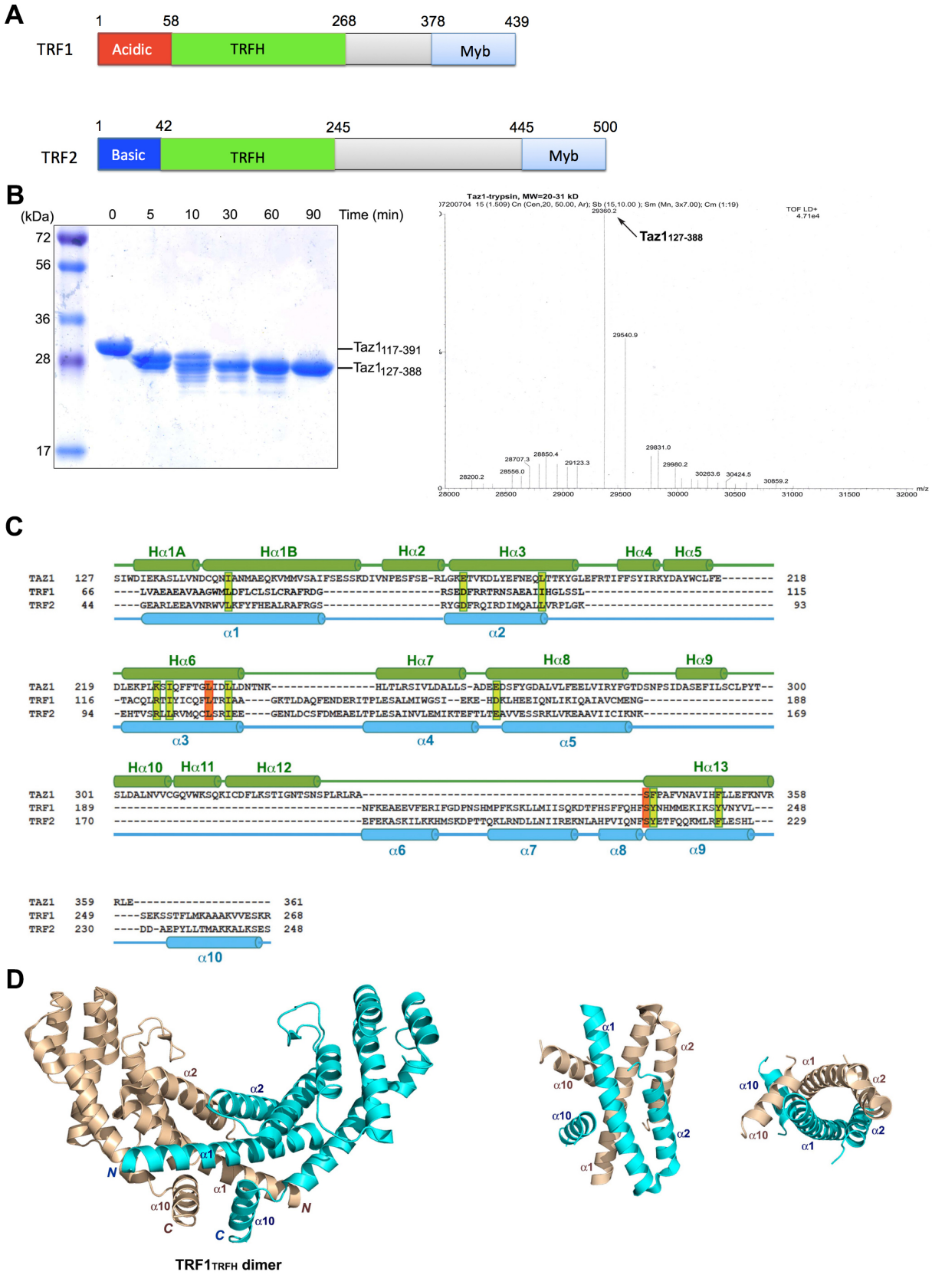
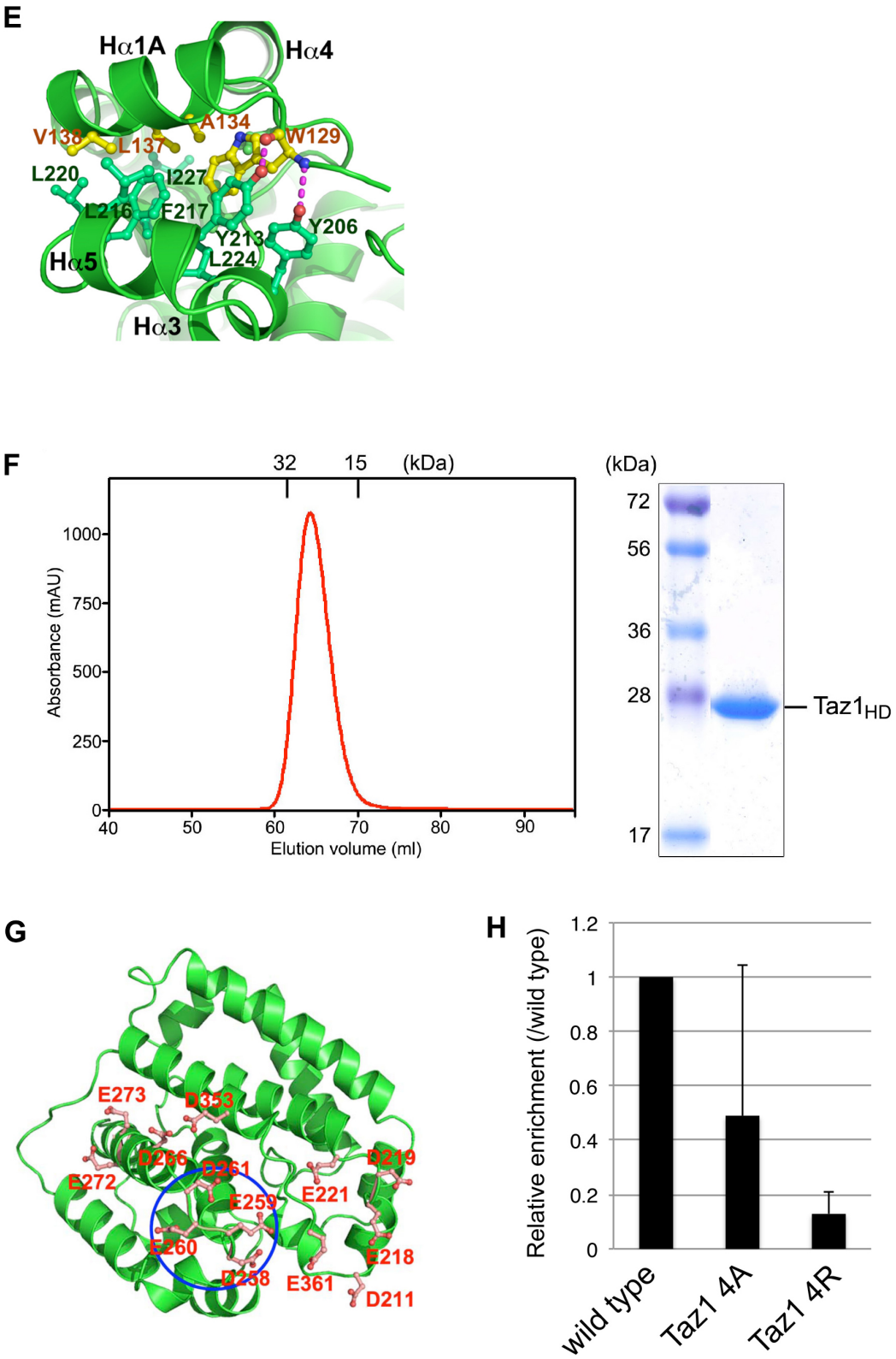
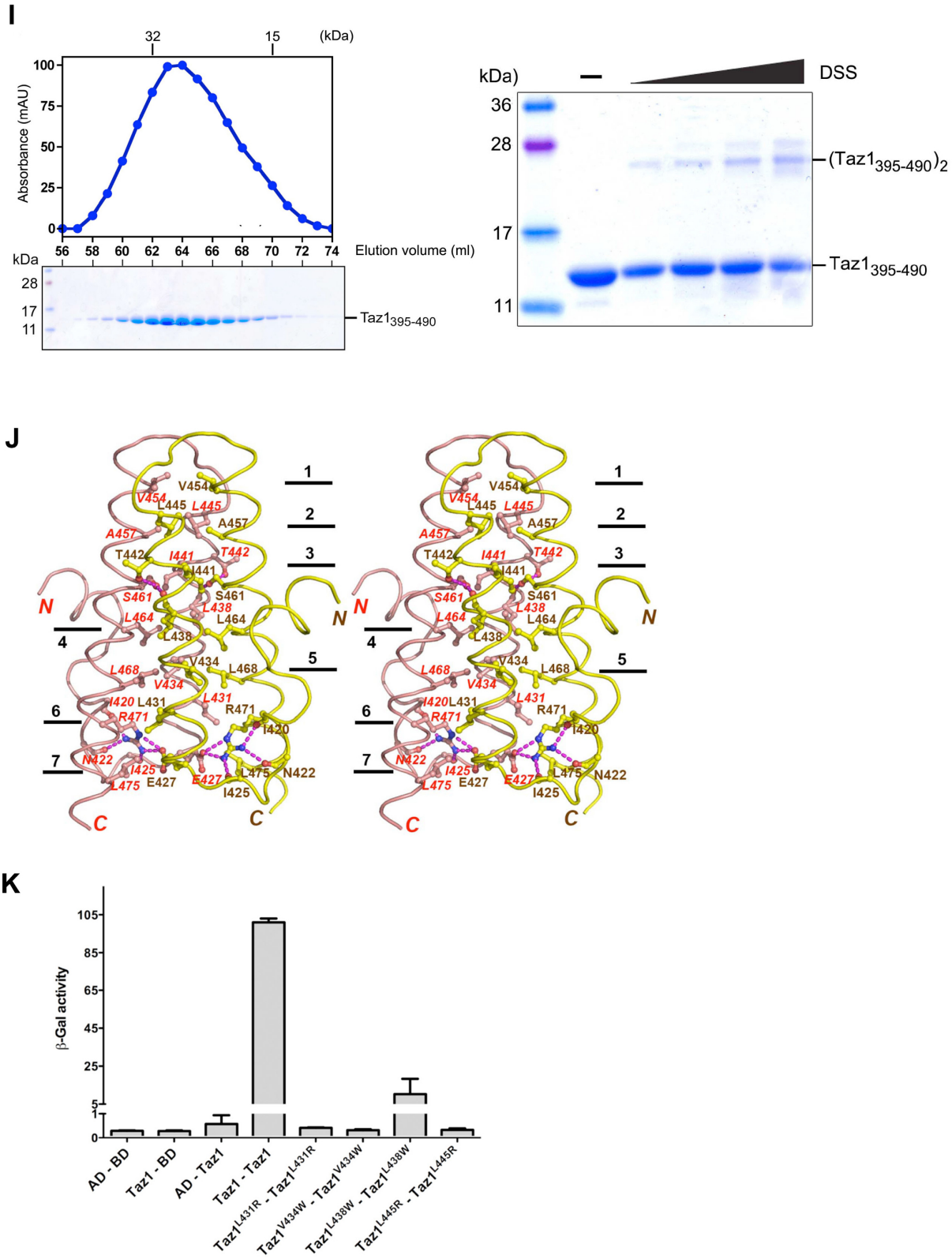

A
TRF1
1
58
268
378
439
Acidic
TRFH
Myb
TRF2
1
42
245
445
500
Basic
TRFH
Myb
B
(kDa)
0
5
10
30
60
90
Time (min)
72
56
36
28
17
Taz1117-391
Taz1127-388
Taz1-trypsin, MW=20-31 kD
Taz1127-388
29540.9
29831.0
28850.4
28707.3
29123.3
28556.0
29980.2
28200.2
30263.6
30424.5
30859.2
m/z
C
Hα1A
Hα1B
Hα2
Hα3
Hα4
Hα5
TAZ1 127 SIWDIEKASLLVNDCQNIANMAEQKVMMVSAIFSESSKDIVNPESFSE-RLGKETVKDLYEFNEQLTTKYGLEFRTIFFSYIRKYDAYWCLFE-------- 218
TRF1 66 ----LVAEAEAVAAGWMLDFLCLSLCRAFRDG-----------------RSEDFRRTRNSAEAIIHGLSSL---------------------------- 115
TRF2 44 ----GEARLEEAVNRWVLKFYFHEALRAFRGS-----------------RYGDFRQIRDIMQALLVRPLGK---------------------------- 93
α1
α2
Hα6
Hα7
Hα8
Hα9
TAZ1 219 DLEKPLKSIQFFTGLIDLLDNTNK----------------HLTLRSIVLDALLS-ADEEDSFYGDALVLFEELVIRYFGTDSNPSIDASEFILSCLPYT-- 300
TRF1 116 -TACQLRTIYICQFLTRIAA----GKTLDAQFENDERITPLESALMIWGSI--EKE-HDKLHEEIQNLIKIQAIAVCMENG---------------------- 188
TRF2 94 -EHTVSKLLRVMQCLSRIEE----GENLDCSFDMEAELTPLESAINVLEMIKTEFTLTEAVVESSRKLVKEAAVIICIKNK---------------------- 169
α3
α4
α5
Hα10
Hα11
Hα12
Hα13
TAZ1 301 SLDALNVVCGQVWKSQRICDFLKSTIGNTSNSPLRLRA--SFPAFVNAVIHFLLEFKNVR 358
TRF1 189 ------------------------------------NFKEAEEVFERIFGDPNSHMPFKSKLLMIISQKDTFHSFFQHFSYNHMMEKIKSYVNYVL--- 248
TRF2 170 ------------------------------------EFEKASKILKKHMSKDPTTQKLRNDLLNIIREKNLAHFVIQNFSYETFQQRMLFFLESHL--- 229
α6
α7
α8
α9
TAZ1 359 RLE-------------------- 361
TRF1 249 ----SEKSSTFLMKAAAKVVESKR 268
TRF2 230 ----DD-AEPYLLTMAKKALKSES 248
α10
D
α2
α2
α1
N
α1
α10
C
N
C
α10
TRF1TRFH dimer
α1
α2
α10
α10
α2
α1
α1
α2
α10
α2
α10
α1


E
Hα1A
Hα4
V138
A134
W129
L137
L220
I227
L216
E217
Y206
Y213
L224
Hα5
Hα3
F
32
15
(kDa)
1000
750
500
250
0
Absorbance (mAU)
40
50
60
70
80
90
Elution volume (ml)
(kDa)
72
56
36
28
17
Taz1HD
G
E273
D353
D256
D261
D219
E272
E259
E221
E260
E218
D258
E361
D211
H
Relative enrichment (/wild type)
1.2
1
0.8
0.6
0.4
0.2
0
wild type
Taz1 4A
Taz1 4R


I
32
15
(kDa)
Absorbance (mAU)
100
75
50
25
0
56 58 60 62 64 66 68 70 72 74
Elution volume (ml)
kDa
28
17
11
Taz1395-490
kDa)
DSS
36
28
17
11
(Taz1395-490)2
Taz1395-490
J
V454
L445
A457
T442
I441
S461
L438
L464
V434
L468
I420
L431
R471
N422
I425
L475
E427
1
2
3
4
5
6
7
N
C
K
β-Gal activity
105
85
65
45
25
5
1
0
AD - BD
Taz1 - BD
AD - Taz1
Taz1 - Taz1
Taz1L431R - Taz1L431R
Taz1V434W - Taz1V434W
Taz1L438W - Taz1L438W
Taz1L445R - Taz1L445R

Supplementary information, Figure S1 (A) Organization of the human TRF1 and TRF2 polypeptide chains. In TRF1, the N-terminal acidic region is colored in red, the C-terminal Myb domain in light blue, and the TRFH domain in green. In TRF2, the N-terminal basic region is colored in blue, the C-terminal Myb domain in light blue, and the TRFH domain in green. **(B)** Identification of the stable $Taz1_{127\text{-}388}$ fragment. SDS-PAGE time course of limited protease (trypsin) cleavage of $Taz1_{117\text{-}391}$. Lanes in minutes of time of the reaction are labeled. MALDI-TOF mass spectrum result of the trypsin digestion products. **(C)** Structure-based sequence alignment of $Taz1_{HD}$ against the TRFH domains of TRF1 and TRF2. Secondary structure assignments from the $Taz1_{HD}$ and TRFH crystal structures are shown as colored cylinders (α helices) above ($Taz1_{HD}$) and under (TRFH) the aligned sequences. **(D)** The dimerization interface of human $TRF1_{TRFH}$ dimer. Left panel: overview of crystal structure of human $TRF1_{TRFH}$ dimer in ribbon diagram. The two monomers are colored in cyan and salmon, respectively. Right panel: two orthogonal views of the $TRF1_{TRFH}$ dimerization interface in ribbon diagrams. The extended N-terminal portion of helix α1 interacts with helix α10 from the other molecule in the dimer. **(E)** Crystal structure of the helical domain of Taz1 ($Taz1_{HD}$). The bent helix Ha1A fits into a hydrophobic groove formed by helices Hα3, Hα4, and Hα5. Residues in helices Hα1A and Hα5 are shown in stick model and colored in yellow and green, respectively. **(F)** $Taz1_{HD}$ is a monomer in solution. Gel filtration chromatography profile (Hiload Superdex 75) of $Taz1_{HD}$. Elution positions of the 15 and 32 kDa protein markers are indicated. SDS-PAGE of $Taz1_{HD}$ corresponding to the peak fraction in the gel filtration profile. **(G)** An acidic patch on $Taz1_{HD}$ surface is required for telomere length regulation. $Taz1_{HD}$ is in ribbon representation and in the same orientation as in

Figure 1E. **(H)** Levels of telomeric enrichment in Taz1-GFP immunoprecipitates (IPs) derived from a synchronously growing cells analyzed by ChIP followed by Southern analysis with the telomere-specific probe. Relative enrichments at telomeres compared with the wild type were normalized by the values at rDNA (n = 3). **(I)** Identification of the Taz1 dimerization domain. Left panel: gel filtration chromatography profile (Hiload Superdex 75) of the $Taz1_{395\text{-}490}$ fragment. Elution positions of the 15 and 32 kDa protein markers are indicated. SDS-PAGE of the $Taz1_{395\text{-}490}$ fragment corresponding to the peak fraction in the gel filtration profile. Due to the fact that $Taz1_{395\text{-}490}$ does not have UV_{280} absorbance, the gel filtration chromatography profile was drawn based on the band intensity in the SDS-PAGE. Right panel: SDS-PAGE of the $Taz1_{395\text{-}490}$ dimer that was cross-linked by DSS cross-linking reagent (Disuccinimidyl suberate, Thermo Scientific). **(J)** Stereo view of the $Taz1_{DD}$ dimer. The dimeric interface involves six layers of hydrophobic and one layer of hydrophilic interactions between the two subunits. Residues important for the interaction are shown in stick models and hydrogen-bonding interactions are denoted by magenta dashed-lines. **(K)** Effects of four mutations on dimer formation of $Taz1_{DD}$ in yeast two-hybrid assays. Dimeric interaction between LexA-$Taz1_{DD}$ and GAD-$Taz1_{DD}$ was determined by measuring the β-galactosidase activity produced by the reporter gene. Data are averages of three independent β-galactosidase measurements normalized to the wild-type dimeric interaction, arbitrarily set to 100.

Supplementary information, Table S1A Data collection and refinement statistics

	$Taz1_H$ (Native)	**$Taz1_H$ (Se-Peak)**	**$Taz1_H$ (Se-Edge)**	**$Taz1_H$ (Se-Remote)**
Data collection				
Space group	*C*222	*C*222	*C*222	*C*222
Cell dimensions				
a, b, c (Å)	68.0, 160.1, 50.1	67.9, 160.0, 50.0	68.0, 160.3, 49.9	68.0, 160.4, 49.9
α, β, γ (°)	90.0, 90.0, 90.0	90.0, 90.0, 90.0	90.0, 90.0, 90.0	90.0, 90.0, 90.0
Resolution (Å)	2.3	2.7	2.7	2.7
R_{merge}*	0.043 (0.373)	0.083 (0.510)	0.091 (0.525)	0.088 (0.497)
I / σ*	57.9 (3.6)	50.1 (2.4)	51.2 (2.2)	51.3 (2.1)
Completeness (%)*	96.6 (80.3)	96.7 (94.2)	95.3 (94.5)	95.2 (95.0)
Redundancy*	6.7 (5.4)	13.8 (11.2)	12.8 (10.4)	13.1 (10.9)
Refinement				
Resolution (Å)	50.0-2.3			
No. of reflections	12,289			
R_{work} / R_{free} (%)	19.4/23.3			
B-factors				
Protein	79.4			
Ion	52.2			
Water	81.4			
R.m.s. deviations				
Bond lengths (Å)	0.008			
Bond angles (°)	1.166			
Ramanchandran plot				
Favored region	96.6%			
Allowed region	100.0%			
Outlier region	0.0%			

*Highest resolution shell is shown in parenthesis

Supplementary information, Table S1B Data collection and refinement statistics

	Taz1$_D$ -Native	**Taz1$_D$ -NaI**
Data collection		
Space group	$P4_32_12$	$P4_32_12$
Cell dimensions		
a, b, c (Å)	45.1, 45.1, 78.7	45.2, 45.2, 79.3
α, β, γ (°)	90.0, 90.0, 90.0	90.0, 90.0, 90.0
Resolution (Å)	1.5	1.3
R_{merge}*	0.046 (0.271)	0.086 (0.355)
I / σ*	38.6 (2.1)	54.7 (2.6)
Completeness (%)*	97.1 (85.9)	95.3 (64.7)
Redundancy*	6.5 (4.0)	11.6 (4.1)
Refinement		
Resolution (Å)	20.0-1.5	
No. of reflections	13,237	
R_{work} / R_{free} (%)	15.9/20.1	
B-factors		
Protein	23.4	
Water	39.6	
R.m.s. deviations		
Bond lengths (Å)	0.006	
Bond angles (°)	0.796	
Ramanchandran plot		
Favored region	100.0%	
Allowed region	100.0%	
Outlier region	0.0%	

*Highest resolution shell is shown in parenthesis

Supplementary information, Materials and Methods

Protein expression and purification

The helical domain (HD) of Taz1 (residues 128 – 388) and dimerization domain (DD) of Taz1 (residues 395 – 490) were cloned into a modified pET28b vector with a SUMO protein fused at the amino-terminus after the His_6 tag [1, 2]. $Taz1_{HD}$ was expressed in *E. coli.* BL21(DE3). After induction for 16 hours with 0.1 mM IPTG at 25 °C, the cells were harvested by centrifugation and the pellets were resuspended in lysis buffer (50 mM Tris-HCl pH 8.0, 50 mM NaH_2PO_4, 400 mM NaCl, 3 mM imidazole, 10% glycerol, 1mM PMSF, 0.1 mg/ml lysozyme, 2mM 2-mercaptoethanol, and home-made protease inhibitor cocktail). The cells were then lysed by sonication and the cell debris was removed by ultracentrifugation. The supernatant was mixed with Ni-NTA agarose beads (Qiagen) and rocked for 6 hours at 4 °C before elution with 250 mM imidazole. The ULP1 protease was added to remove the His_6-SUMO tag. Finally $Taz1_{HD}$ was further purified by passage through Mono-Q ion-exchange column and by gel-filtration chromatography on Hiload Superdex 75 equilibrated with 25 mM Tris-HCl pH 8.0, 150 mM NaCl and 5 mM dithiothreitol (DTT). The purified $Taz1_{HD}$ protein was concentrated to 25 mg/ml and stored at -80 °C. The Seleno-Met substituted $Taz1_{HD}$ protein was similarly purified.

The protein of dimerization domain of Taz1 was expressed in *E. coli.* and purified following the same procedures as described above.

Electrophoretic mobility shift assay (EMSA)

The protein of $Taz1_{408\text{-}663}$ in binding buffer (25 mM Tris-HCl pH 8.0, 150 mM NaCl, and 5 mM DTT) was mixed with 1 μM ^{32}P-labeled telomeric dsDNAs (32mer) in a total volume of 20 μL. The reaction mixtures were incubated at 4 °C for 30 min. Then

the mixtures were directly loaded onto a 4−20% nondenaturing polyacrylamide gel. Electrophoresis was carried out in TBE buffer at 150 V for 85 min at 4 °C. The gels were dried, and radiolabeled dsDNA was visualized using a PhosphorImager. (32mer dsDNA was annealed by the following ssDNA with its complementary strand: 5' - GATCTCAGCT GGTTACA GGTTACA GGTTACA G- 3') The signal of the radiolabeled dsDNA was quantified by using ImageQuant (GE Healthcare Life Sciences). The dissociation constant was determined by using the software of GraphPad Prism.

Crystallization, data collection and structure determination

$Taz1_{HD}$ was crystallized by hanging-drop-vapor-diffusion at 4 °C. The precipitant/well solution contained 100 mM Tris-HCl pH7.0, 18% PEG 3350, 300mM KNO_3, 10 mM $Co(NH_4)_6Cl_3$ and 10 mM DTT. Crystals were gradually transferred to a harvesting solution containing 100 mM Tris-HCl pH7.0, 25% PEG 3350, 300 mM KNO_3, 10 mM $Co(NH_4)_6Cl_3$ and 25% glycerol before flash-frozen in liquid nitrogen for storage and data collection under cryogenic conditions (100 K). Se-Met-MAD datasets with the resolution of 2.7 Å were collected at beam line 21ID-D at APS and processed using HKL2000 [3]. Crystals belong to space group *C*222 and contain one $Taz1_{HD}$ per asymmetric unit. Two selenium sites were located and refined, and MAD phases calculated using SHARP [4]. A model was automatically built into the modified experimental electron density using ARP/WARP [5]; the model was then further refined in CNS [6] with manual rebuilding using program O [7].

$Taz1_{DD}$ was crystallized by sitting-drop-vapor-diffusion at 4 °C. The precipitant/well solution contained 2.3 M $(NH_4)_2SO_4$, 100 mM Bicine pH 8.5 and 10 mM DTT. Crystals were gradually transferred to a harvesting solution containing 2.3

M $(NH_4)_2SO_4$, 100 mM Bicine pH 8.5 and 5 M Sodium Formate. Final concentration of 125 mM NaI was added into harvesting solution 1 minute before the flash-frozen in liquid nitrogen for storage and data collection under cryogenic conditions (100 K). Crystals belong to space group $P4_32_12$ with one $Taz1_{DD}$ monomer per asymmetric unit. Datasets with the resolution of 1.5 Å were collected at beam line 21ID-D at APS and processed using HKL2000 [3]. Native-/I- datasets were combined and scaled in ccp4i and then were input into SHARP [4]. The initial SIR map was significantly improved by solvent flattening. A model was automatically built into the modified experimental electron density using ARP/WARP [5]; the model was then further refined using simulated-annealing and positional refinement in CNS [6] with manual rebuilding using program O [7].

Yeast two-hybrid assay

The yeast two-hybrid assays were performed using L40 strains harboring pBTM116 and pACT2 (Clonetech) fusion plasmids. The colonies containing both plasmids were selected on –Leu –Trp plates. The β-galactosidase activities were measured according to Clontech MATCHMAKER library protocol.

Yeast Strains

A region of *taz1*$^+$ open reading frame (from bases pair 315 to 501) has been replaced by *ura4*$^+$ gene to generate a *taz1::ura* (Tomita K., unpublished data). Vectors *pACT2-taz1-*$L_{431}R$ */ taz1-*$V_{434}W$ */ taz1-*$L_{438}W$ */ taz1-*$L_{445}R$ as well as vectors *pET-SUMO-Taz1* $I_{379}R$ */ Taz1-*$L_{380}R$ */ Taz1-*$L_{383}R$ */ Taz1-*$L_{387}R$ were digested with BamHI / XhoI, generating a taz1 full length fragment containing a set of point mutation in Taz1 dimerization domain (*taz1-*$L_{431}R$ */ taz1-*$V_{434}W$ */ taz1-*$L_{438}W$ */ taz1-*$L_{445}R$) and Taz1-

Rap1 interaction domain (*taz1* $I_{379}R$ / *taz1*-$L_{380}R$ / *taz1*-$L_{383}R$ / *taz1*-$L_{387}R$). *taz1::ura* strain was transformed with the BamHI / XhoI fragments from *pACT2* and *pET-SUMO* and plated on replica-plated twice on EMM + FOA to select for the loss of *ura4*+ gene. Transformants were screened by PCR using two sets of primers. Primers 59/60 hybridize outside *taz1*+ ORF; primers 146/60 hybridize inside and outside *taz1*+ ORF respectively.

Measurement of telomere length

Telomere length was measured by Southern hybridization according to the procedure described by Cooper J.P. *et al* 1997 [8]. Briefly, genomic *S.pombe* DNA was prepared by glass beads lysis method, digested with EcoRI, resolved on 1% agarose gel, transferred to Hybond membrane (Amersham) and hybridized at 65°C to ^{32}P random primed telomeric probe (SacI-PstI fragment from pIRT2-TELO vector).

Pulse-field gel electrophoresis for fission yeast

PFGE for detection of telomere end fusion was carried out as described [9]. Cells were grown in EMM with nitrogen, and then incubated in EMM without nitrogen for 24 hrs to arrest cells in G1 phase. Genomic DNAs were digested by Not1. Telomere repeats (~300 bp) were used as the probe.

ChIP

Cell fixation and IP were performed as described [10]. For dot blot analysis, signal intensities were quantified with Imagequant software and those of IP DNA normalized to input genomic DNA. Each sample was measured in triplicate and values expressed as IP/WCE.

Reference

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