**SUPPLEMENTARY INFORMATION**

# Structural basis for protein-RNA recognition in telomerase

Jing Huang[1,2,3], Andrew F. Brown[4], Jian Wu[1], Jing Xue[1], Christopher J. Bley[4], Dustin P. Rand[4], Lijie Wu[1], Rongguang Zhang[1], Julian J.-L. Chen[4], and Ming Lei[1,2,3]

[1]National Center for Protein Science Shanghai, State Key Laboratory of Molecular Biology, Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, Shanghai 200031, China

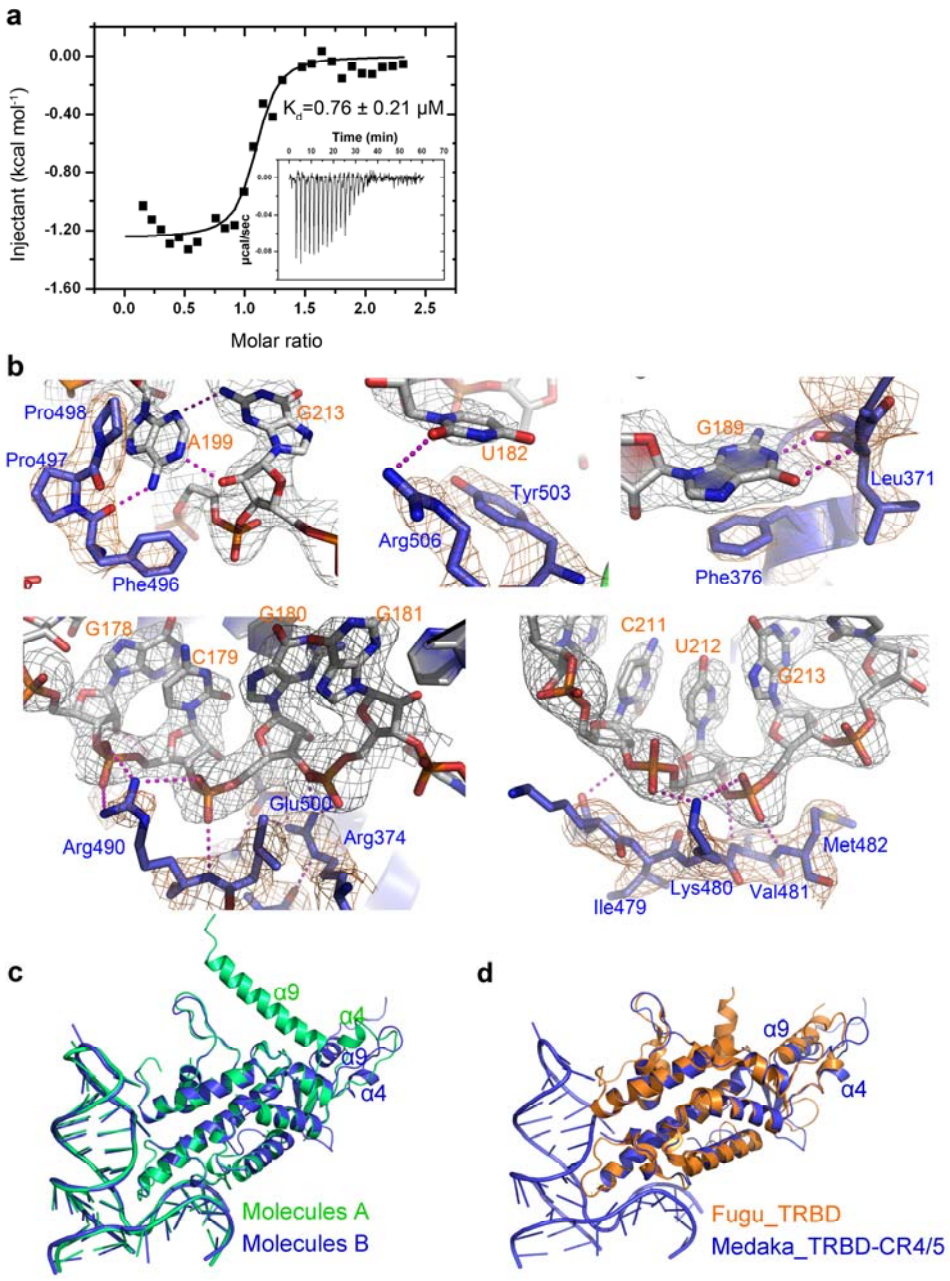
[2]Howard Hughes Medical Institute, University of Michigan Medical School, Ann Arbor, MI 48109, USA

[3]Department of Biological Chemistry, University of Michigan Medical School, Ann Arbor, MI 48109, USA

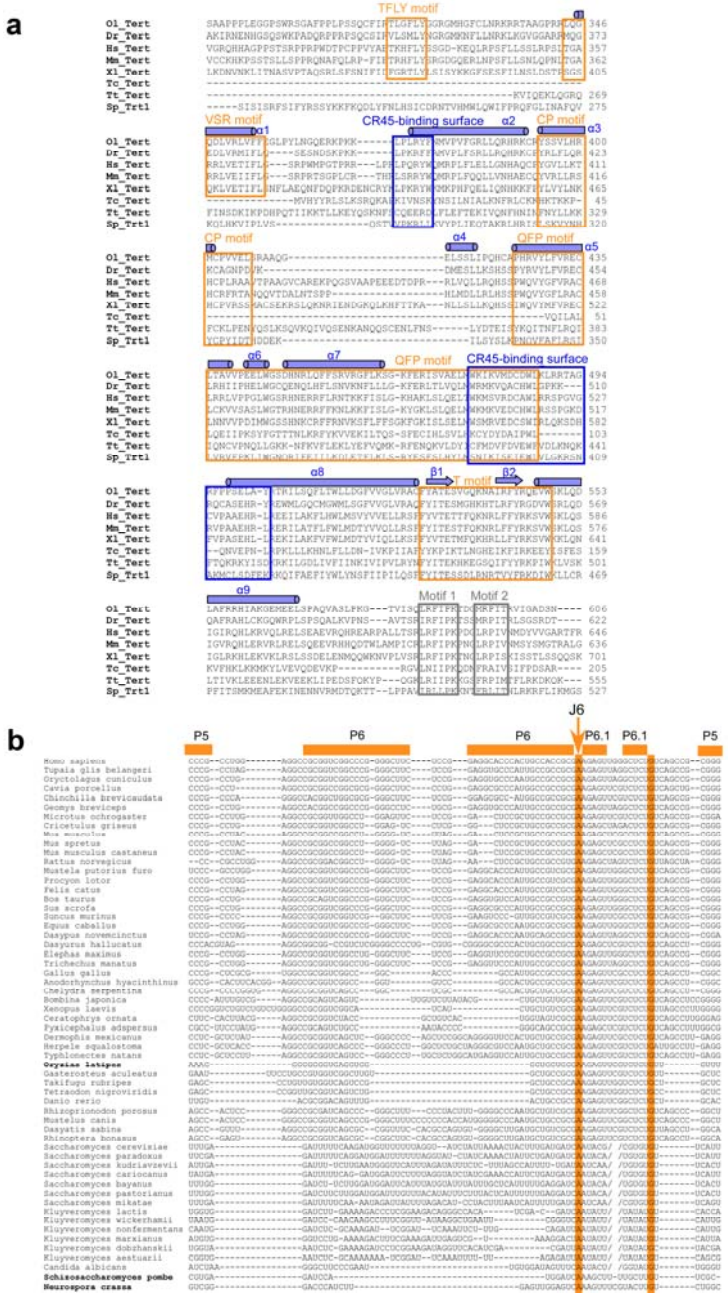
[4]Department of Chemistry and Biochemistry, Arizona State University, Tempe, AZ 85287, USA

Correspondence should be addressed to M.L. (leim@sibcb.ac.cn) or J.J.-L.C. (jlchen@asu.edu).

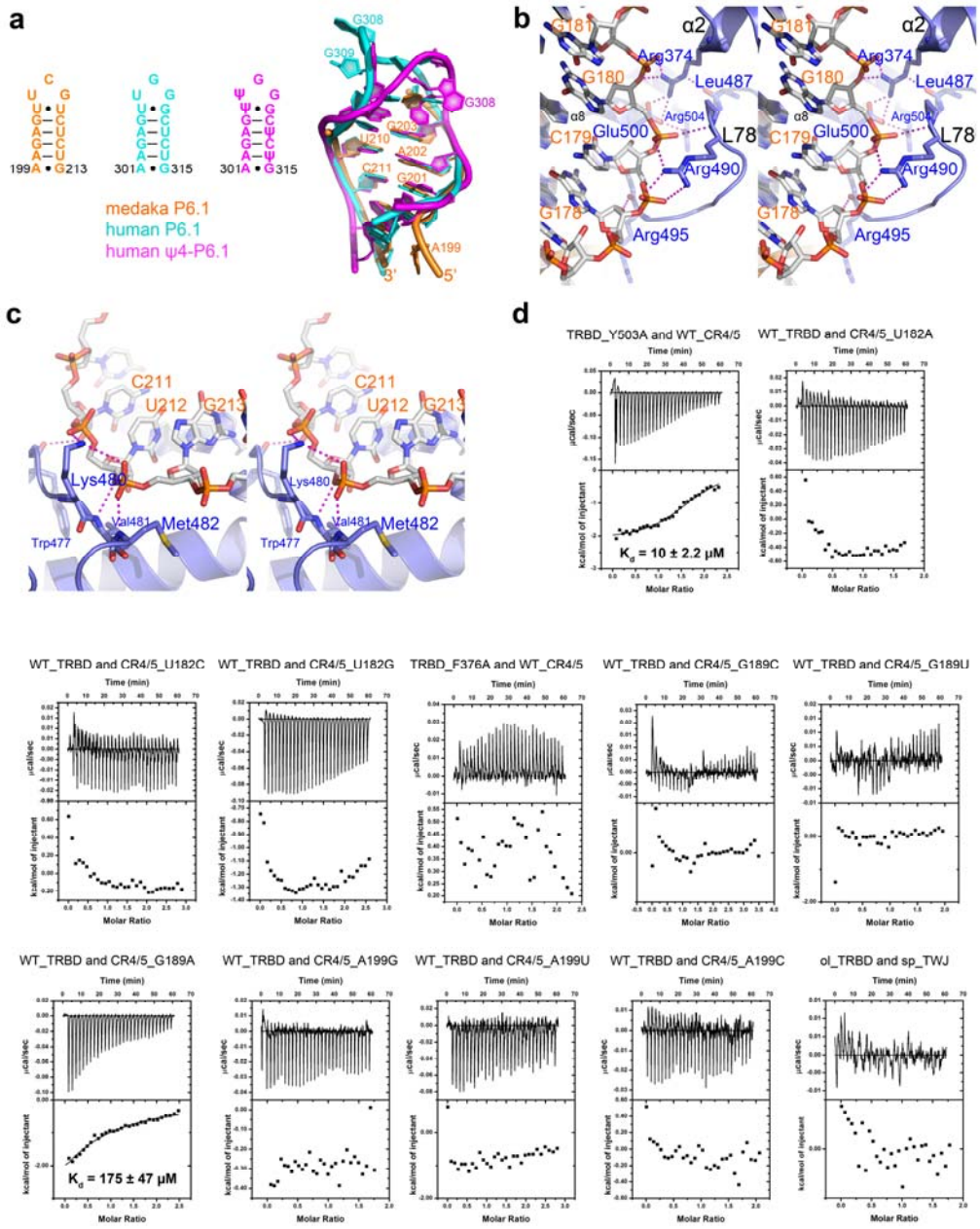
Nature Structural and Molecular Biology: doi:10.1038/nsmb.2819



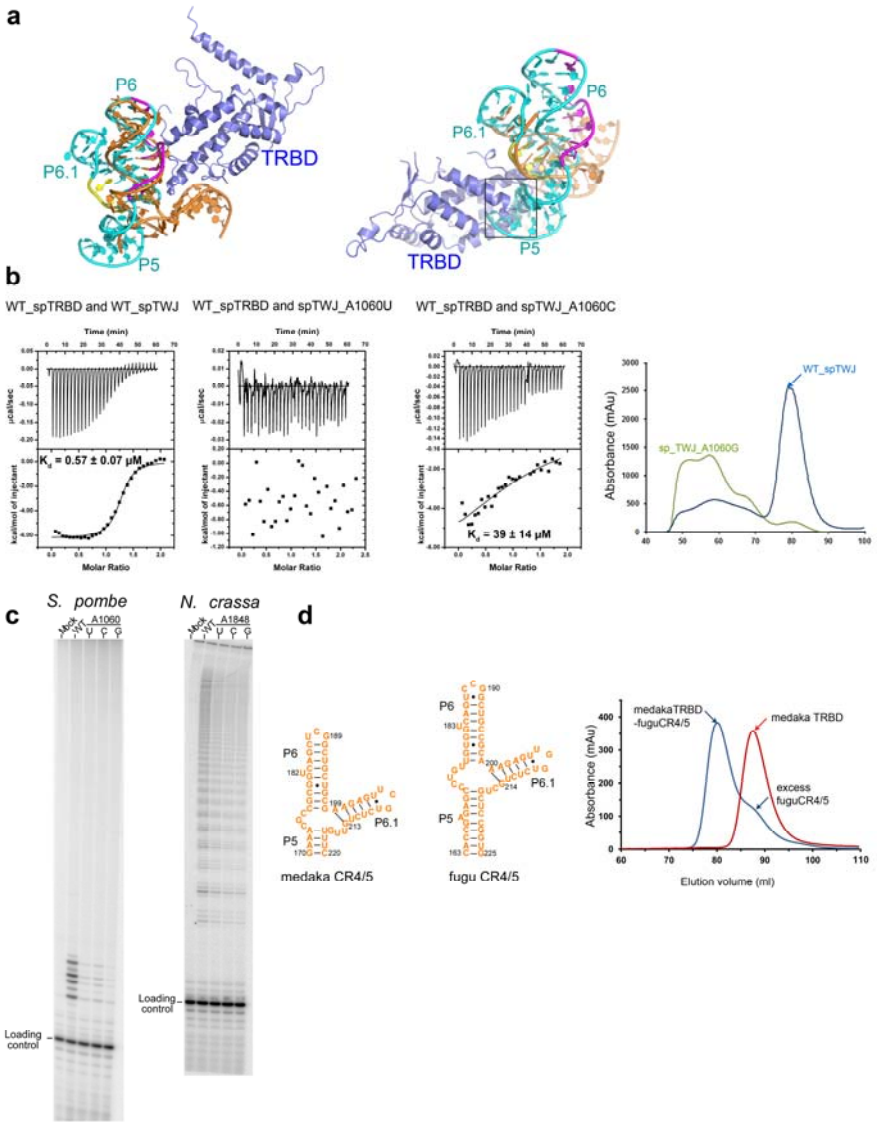

**Supplementary Figure 1. Structure of the TRBD-CR4/5 complex. (a)** ITC measurement of the interaction between medaka TRBD (residues 318-579) and CR4/5 (nt 170-220). **(b)** Electron density map of the TRBD-CR4/5 interface. $2F_o$-$F_c$ map of the CR4/5 nucleotides (orange) and the TRBD residues (blue) at the TRBD-CR4/5 interface are contoured at 1.5 σ, related to Fig. 3c,d,f and Supplementary Fig. 3b,c. **(c)** Superposition of the two sets of TRBD-CR4/5 molecules in the asymmetric unit. The helices α4 and α9 in the molecules A have an altered orientation due to crystal packing. **(d)** Structural superposition of the *Oryzias latipes* (medaka) TRBD-CR4/5 complex (blue) with the *Takifugu rubripes* (fugu) TRBD domain (orange, PDB accession code 4LMO[1]).



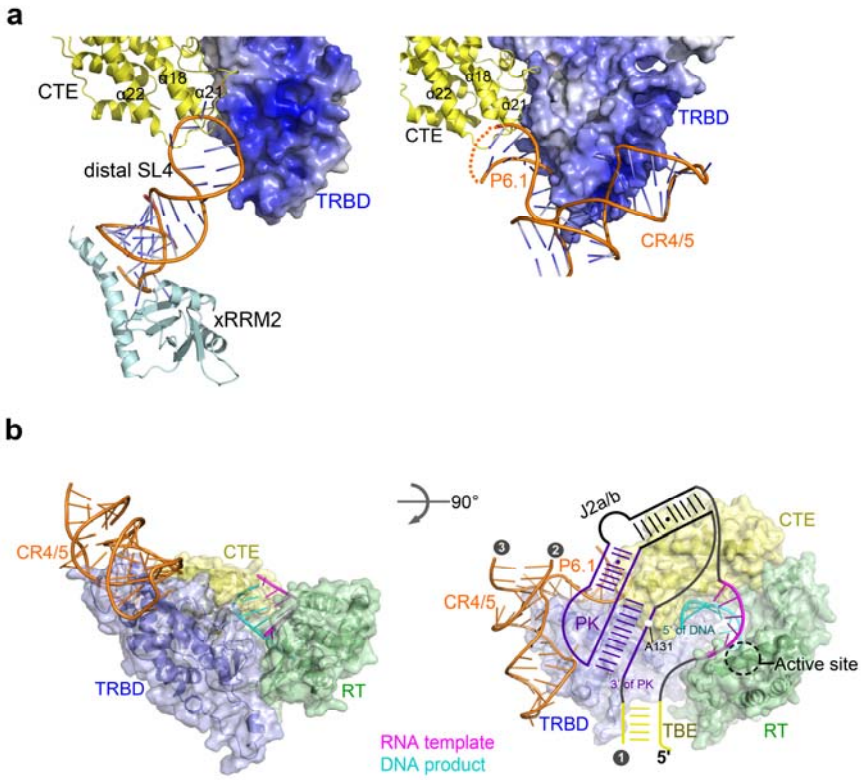

**Supplementary Figure 2. Sequence alignments of the TRBD domain of TERT and the CR4/5 (TWJ) domain of TR. (a)** Primary sequence alignment of the TRBD domain of TERT. Conserved motifs of TRBD are boxed. Secondary structure assignments are shown as cylinders (α helices) and arrows (β strands) and are colored blue. Ol_Tert, *Oryzias latipes*; Dr_Tert, *Danio rerio*; Hs_Tert, *Homo sapiens*; Mm_Tert, *Mus musculus*; Xl_Tert, *Xenopus laevis*; Tc_Tert, *Tribolium castaneum*; Tt_Tert, *Tetrahymena thermophila*; Sp_Tert, *Schizosaccharomyces pombe*. **(b)** Sequence alignment of the vertebrate CR4/5 domains and the fungi TWJ domains, based on predicted secondary structures. Conserved RNA secondary structures are denoted. The conserved nucleotide A at the junction J6/J6.1 as well as its interacting nucleotide G are highlighted in orange.




**Supplementary Figure 3. Structural and mutational analyses of the TRBD-CR4/5 interaction. (a)** Structural superposition of the crystal structure of medaka P6.1 (orange) with the NMR structures of human P6.1 (cyan, PDB accession code 1OQ0[2]) and pseudouridylated human P6.1 (magenta, PDB accession code 2KYE[3]). **(b)** Stereo view of the stem P6-mediated TRBD-CR4/5 interactions. The intermolecular hydrogen bonds are shown as dashed magenta lines. **(c)** Stereo view of the stem P6.1-mediated TRBD-CR4/5 interactions. **(d)** ITC measurements of the interactions between wild-type and mutant TRBD and CR4/5, related to Table 2. ol_TRBD, *Oryzias latipes* TRBD; sp_TWJ, *Schizosaccharomyces pombe* three-way junction.




**Supplementary Figure 4. Analyses of the TRBD-CR4/5 and TRBD-TWJ interactions. (a)** Superposition of the TRBD-CR4/5 complex structure with the NMR structure of the medaka CR4/5 based on stem P6 (left panel) and stem P6.1 (right panel), respectively. TRBD is colored blue and the CR4/5 free and in the complex is colored cyan and orange, respectively. In the structure of the free CR4/5, the TRBD-binding nucleotides on stem P6 and stem P6.1 are colored magenta and yellow, respectively. The black box denotes the spatial collision happens between TRBD and stem P5 in the structural superposition based on stem P6.1. **(b)** ITC measurements of the interactions between wild-type and the A1060 mutants of *S. pombe* TWJ and TRBD, related to Table 3. The TWJ_A1060G mutant RNA was not correctly folded, as shown in its gel filtration profile compared with that of the wild-type TWJ RNA. **(c)** Telomerase primer-extension assays of wild type and mutant *S. pombe* and *N. crassa* telomerases, related to Fig. 4c. **(d)** Fugu CR4/5 can bind to medaka TRBD efficiently, as shown in the HiLoad 200 gel filtration profile.




**Supplementary Figure 5. Implication of the TRBD-CR4/5 structure on the architecture of vertebrate telomerase. (a)** Comparison of the structural organizations between the distal SL4 region of ciliate telomerase RNA (left panel) and the stem P6.1 of vertebrate CR4/5 (right panel). The organization of SL4 in *Tetrahymena* telomerase is adapted from Fig. 2g in Ref. 27 of the main text. In both structures, TRBD is shown as electrostatic potential surface. The medaka CR4/5-TRBD structure is oriented in the same direction as that of the *Tetrahymena* TRBD. **(b)** A proposed architecture of vertebrate telomerase based on the CR4/5-TRBD crystal structure, shown in two perpendicular views. The color theme and the figure denotation are the same as that of Fig. 1a in the main text.

## SUPPLEMENTARY REFERENCES


1. Harkisheimer, M., Mason, M., Shuvaeva, E. & Skordalakes, E. A Motif in the Vertebrate Telomerase N-Terminal Linker of TERT Contributes to RNA Binding and Telomerase Activity and Processivity. *Structure* **21**, 1870-8 (2013).

2. Leeper, T., Leulliot, N. & Varani, G. The solution structure of an essential stem-loop of human telomerase RNA. *Nucleic Acids Res* **31**, 2614-21 (2003).

3. Kim, N.K., Theimer, C.A., Mitchell, J.R., Collins, K. & Feigon, J. Effect of pseudouridylation on the structure and activity of the catalytically essential P6.1 hairpin in human telomerase RNA. *Nucleic Acids Res* **38**, 6746-56 (2010).


Nature Structural and Molecular Biology: doi:10.1038/nsmb.2819