Supplementary Information

Crystal structures of the RNA triphosphatase from *Trypanosoma cruzi* provide insights into how it recognizes the 5' end of the RNA substrate.

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Table S1

Data collections and refinement statistics of the *Trypanosoma cruzi* RNA Triphosphatase, TcCet1(18-243 Δ55-75) , crystals

Ligand(s)	Mn^{2+}, PPPi and I^-	Mn^{2+}	Compound #951	Compound #466
PDB ID	6L7V	6L7W	6L7X	6L7Y
Data Collection				
Beamline	Photon Factory, BL1A	NSRRC, BL15A	Photon Factory, BL1A	Photon Factory, BL1A
Wavelength (Å)	1.9000	1.0000	1.1000	1.1000
Resolution range	33.63 - 2.20 (2.28 - 2.2)	47.9 - 2.60 (2.72 - 2.60)	49.65 - 2.39 (2.48 - 2.39)	49.40 - 2.51 (2.61 - 2.51)
Space group	$P3_221$	$P2_1$	$P3_221$	$P3_221$
Unit cell a,b,c	67.3, 67.3, 76.8	52.4, 73.2, 63.7 β = 114.1	114.7, 114.7, 56.7	115.9,115.9, 56.7
Total reflections	409654	51598	176432	147972
Unique reflections	10613	13646	17288	15320
Multiplicity	38.6	3.8	10.2	9.7
Completeness (%)	100.0 (99.7)	99.6 (98.7)	100.0 (100.0)	99.7 (98.2)
Mean I/sigma(σ)	33.5 (0.7)	9.9 (1.7)	14.9 (1.7)	16.6 (1.8)
Wilson B-factor	39.4	40.1	45.1	57.2
R-merge	0.095 (5.854)	0.121 (0.860)	0.101 (1.843)	0.083 (1.208)
R-meas	0.096 (5.939)	0.142 (1.054)	0.106 (1.934)	0.087 (1.273)
CC1/2	1.000 (0.955)	0.996 (0.709)	0.999 (0.771)	0.999 (0.927)
Phasing				
Number of sites	7	-	-	-
Figure of merit	0.327	-	-	-
Refinement				
R-work	22.6	23.9	20.4	20.1
R-free	26.1	29.4	23.8	24.0
Number of non-hydrogen atoms	1449	2893	1675	1657
macromolecules	1402	2850	1612	1613
ligands	21	2	36	36
solvent	26	41	27	8
Protein residues	180	362	203	203
RMS(bonds)	0.004	0.005	0.004	0.004
RMS(angles)	0.97	0.98	0.95	0.99
Ramachandran favored (%)	95.9	97.7	98.0	97.5
Ramachandran allowed (%)	4.1	2.3	2.0	2.5
Ramachandran outliers (%)	0.0	0.0	0.0	0.0
Rotamer outliers (%)	3.4	1.3	1.2	0.6
Average B-factor	61.2	56.0	67.0	67.6
macromolecules	60.3	56.2	66.8	66.8
ligands	133.5	53.5	98.8	101.7
Mn annd PPPi	97.6	-	-	-
iodine	205.2	-	-	-
solvent	49.4	43.6	56.5	74.8

*Highest resolution shells are shown in parenthesis

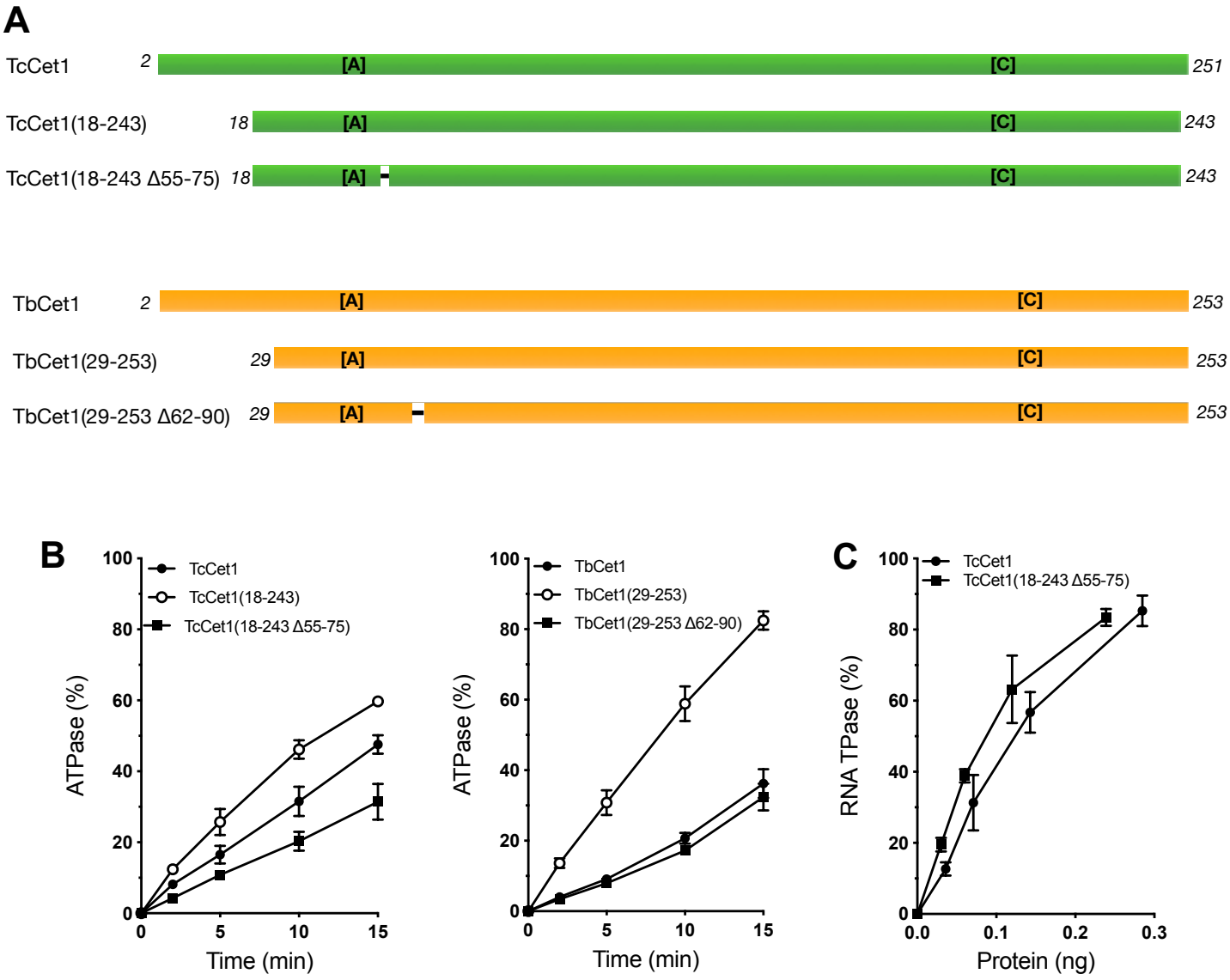


Figure S1. Deletion analysis of Trypanosoma RNA triphosphatase. (A) Schematic diagram of full-length TcCet1, TcCet1(18-243), TcCet1(18-243 Δ55-75), TbCet1, TbCet1(29-253) and TbCet1(29-253 Δ62-90) are shown. Internal deletion is indicated by a dash. Position of conserved motifs, A and C, are indicated. (B) ATPase activity. Reaction mixtures (50 µL) containing 50 mM Tris-HCl (pH 7.5), 2 mM DTT, 2 mM $MnCl_2$, 0.2 mM [γ-^{32}P]ATP, and 5 ng of full-length and truncated proteins were incubated at 30°C. Aliquots were withdrawn at the indicated time and Pi release was plotted as a function of time. (C) RNA triphosphatase activity of TcCet1 and TcCet1(18-243 Δ55-75). Reaction mixtures (10 µL) containing 50 mM Tris-HCl, pH 7.5, 2 mM DTT, 2 mM $MgCl_2$, 1 µM [γ-^{32}P]pppRNA, and indicated amount of full-length TcCet1 and TcCet1(18-243 Δ55-75) were incubated at 30°C for 15 min. Pi release was plotted as a function of input protein. The data shown represent the average of three separate experiments with SE bars.

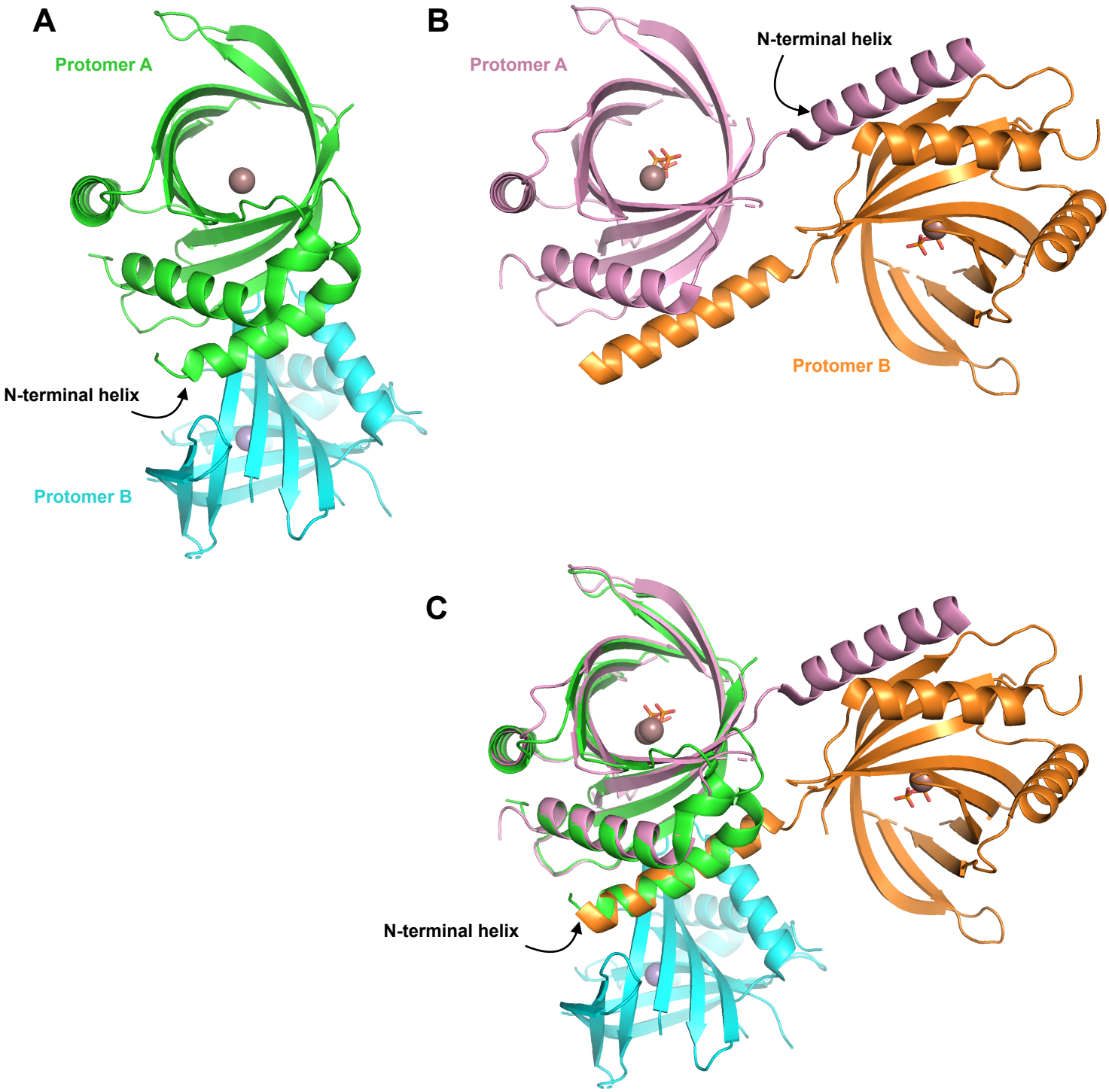
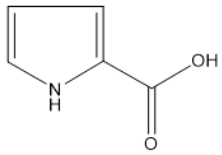
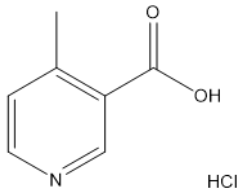


Figure S2. Crystal structures of TcCet1(18-243 Δ55-75). (A) Mn-bound structure. Ordered amino acids in protomer A/B include 18–50, 79-165, 178-218, 220-242/ 20-51, 79-114, 116-164, 180-215, 219-242. (B) Mn•PPPi-bound structure. The ordered amino acids include 18–51, 77-112, 117-164, 179–217, 220–242. (C) Superimposition of Mn-bound and Mn•PPPi-bound structure of TcCet1(18-243 Δ55-75). Note that position of N-terminal helix (amino acid residues 19-36) in the protomer A of the Mn-bound form (green) and protomer B of the Mn•PPPi bound form (orange) overlap each other.



Compound # 202
Tm = 37.0°C



Compound # 212
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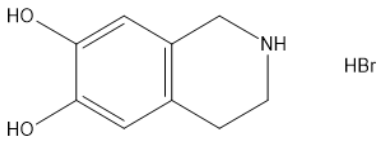

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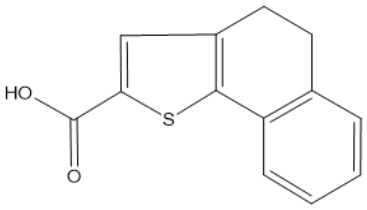

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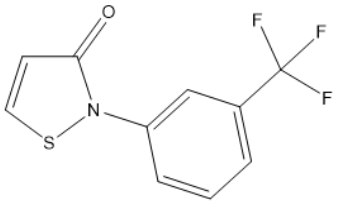

Compound # 375
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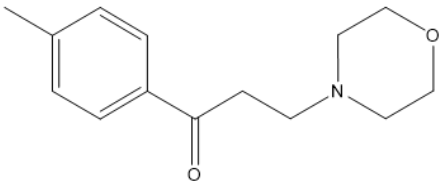
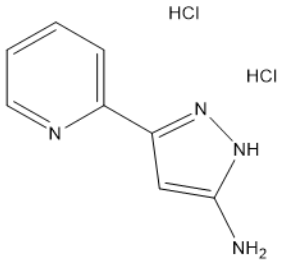
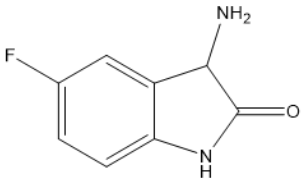


Compound # 378
Tm = 36.9°C



Compound # 521
Tm = 35.8°C



Compound # 524
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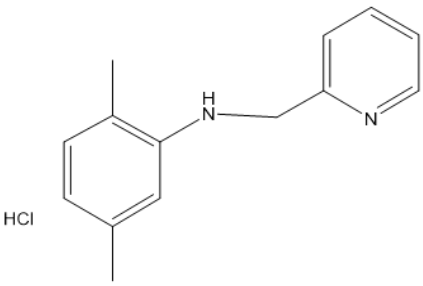
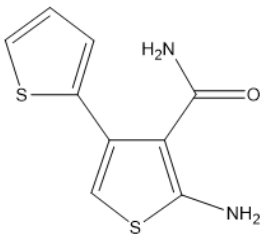


Compound # 809
Tm = 33.0°C



Compound # 818
Tm = 36.2°C

Figure S3. Small molecules that destabilize TcCet1. Melting temperature (Tm) of TcCet1(18-243 Δ55-75) in the presence of 2 mM compounds were determined by thermal shift assay (Figure 3A). Structures of ten compounds that reduce the Tm to below 37°C are shown.

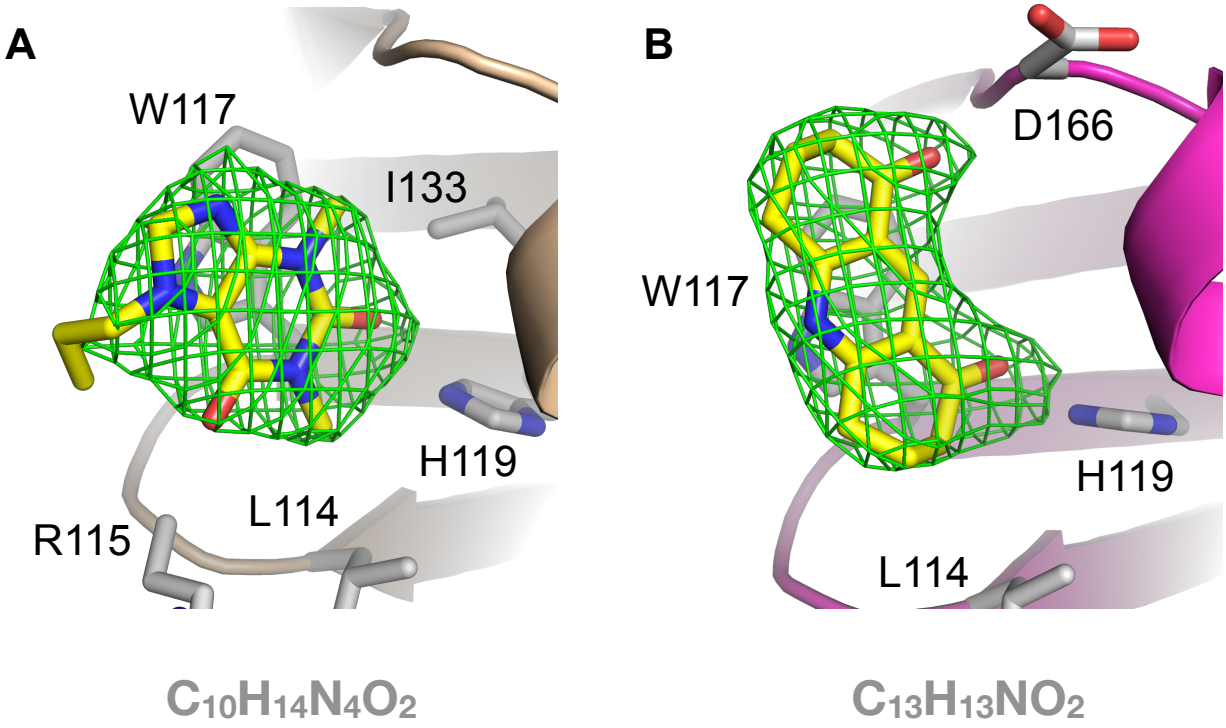


Figure S4. Electron-density of ligands bound to TcCet1(18-243 Δ55-75). Omit (Fo-Fc) map of (A) $C_{10}H_{14}N_4O_2$; compound #951 and (B) $C_{13}H_{13}NO_2$; compound #466. Omit maps are shown as green mesh, contoured at 3 σ. Maps are carved at 2.0 Å around the ligands. Proteins are shown in cartoon representations and ligands as sticks colored according to the atom type (nitrogen in blue, oxygen in red and carbon in yellow).

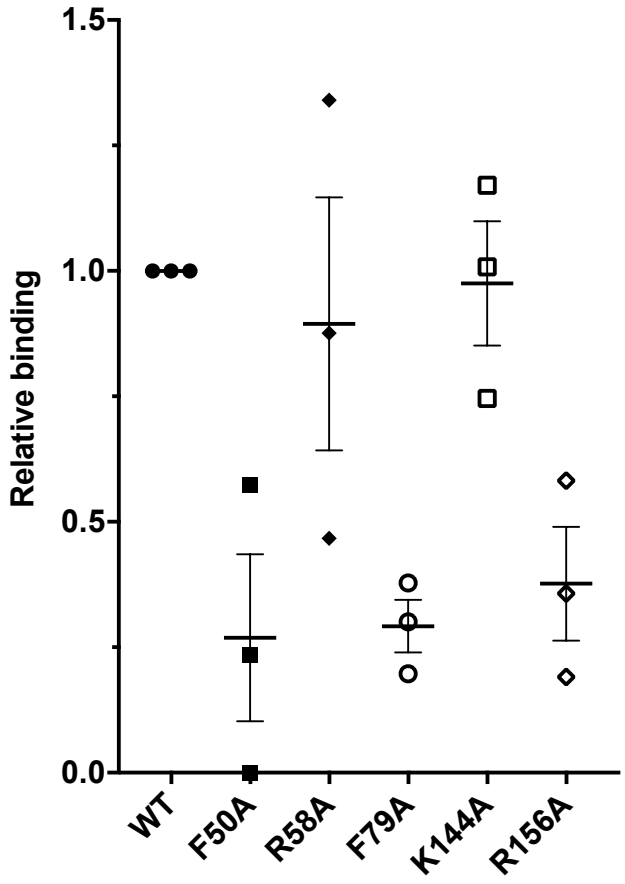


Figure S5. Filter binding Assay. Nucleic acid binding of wild type and mutant TcCet1 proteins were analyzed by filter binding assay. One pmol of 5' [^{32}P]-labeled 17-mer oligonucleotide was incubated with 10 pmol of protein in a 20 µL buffer containing 50 mM Tris-HCl (pH 7.5), 2 mM DTT and 50 mM NaCl for 15 min at 23°C. The mixture was then spotted onto MF-Millipore membrane filter (Merck), and washed twice with the buffer. The dried membrane was exposed to a PhosphorImager plate, scanned by BAS-2000, and quantitated by Image Gauge software. The amount of labeled oligonucleotide bound by the WT was defined as an arbitrary unit of 1.0, against which all other values were normalized. The average of three independent experiments and standard error of the mean are plotted.