SUPPLEMENTARY INFORMATION

Content: Supplementary Tables S1, S2, Supplementary Figures S1, S2, S3

Table S1: The dependence of association constants for TRF2 or $^{\Delta B}$TRF2 binding to telomeric R2 DNA duplex on salt concentration

TRF2 -> DNA-R2

[NaCl] mM	Log [NaCl]	Aver. Value K_d (nM)	K_a ($10^6 M^{-1}$)	Log K_a	Δlog K_a/Δlog[NaCl]	Log K_a^{nel}	Z
50	-1.30	30	33.3	7.3			
70	-1.15	66	15.1	6.9	-3.0	3.7	4.6
100	-1.00	180	5.6	6.4			
140	-0.85	595	1.7	6.0			

$^{\Delta B}$TRF2 -> DNA-R2

[NaCl] mM	Log [NaCl]	Aver. value K_d (nM)	K_a ($10^6 M^{-1}$)	Log K_a	Δlog K_a/Δlog[NaCl]	Log K_a^{nel}	Z
50	-1.30	69	14.5	6.9			
70	-1.15	108	9.3	6.7	-2.2	4.4	3.4
100	-1.00	176	5.7	6.5			
140	-0.85	735	1.4	5.8			

Table S2: Contributions of electrostatic ΔG^{el} and non-electrostatic ΔG^{nel} component to the total free energy for TRF2 binding to DNA duplex R2.

TRF2 -> DNA-R2		**$^{\Delta B}$TRF2 -> DNA-R2**	
ΔG [kJ mol^{-1}]	-42.9	ΔG [kJ mol^{-1}]	-40.8
ΔG^{nel} [kJ mol^{-1}]	-21.3	ΔG^{nel} [kJ mol^{-1}]	-25.0
ΔG^{el} [kJ mol^{-1}]	-21.6	ΔG^{el} [kJ mol^{-1}]	-15.8

The relative standard errors of all presented mean values were less than 3%.

Table S3: SAXS derived parameters for the B-domain free in solution and in complex with DNA duplex R2

Data collection parameters	B-domain	DNA-R2	complex
Wavelength [Å]		1.5418	
q range [$Å^{-1}$]		0.008 – 0.65	
Exposure time [min]		60	
Temperature [C°]		4	
Structural parameters			
Rg [Å] (from Guinier)	16.48	14.89	16.83
Rg [Å] (from P (r))	18.39	16.45	17.64
D max [Å]	77.65	53.18	57.66
Porod volume estimate [$Å^3$]	4310	13520	18530

The relative standard errors of all presented mean values were less than 5%.

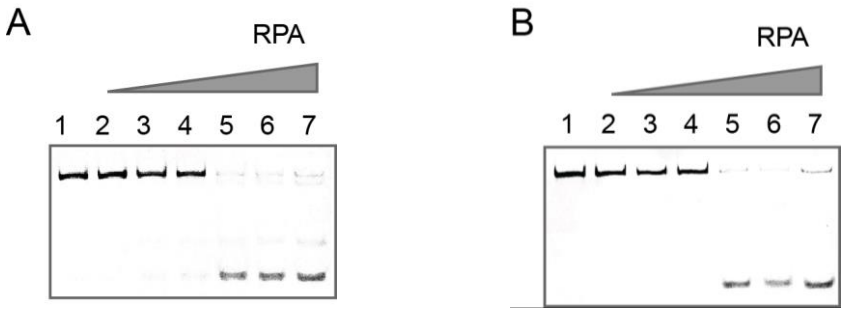

Figure S1. RPA does not unwind D-loops at concentrations used in BLM unwinding experiments. Increasing amounts of RPA were incubated with constant amount of D-loops (3 nM) for 20 min at 37°C. The concentration of RPA in nM was 0, 44, 88, 175, 350, 700, 1400 in lanes 1, 2, 3, 4, 5, 6, and 7, respectively. D-loop R4 containing four telomeric repeats (**A**) or non-telomeric D-loop N (**B**) after incubation with RPA in reaction buffer (25 mM Tris-acetate, pH 7.5, 5 mM CaCl2, 2 mM MgCl2, 5 mM ATP, 5 mM DTT and 100 µg/ml BSA) was separated on a vertical 10% native polyacrylamide gel.

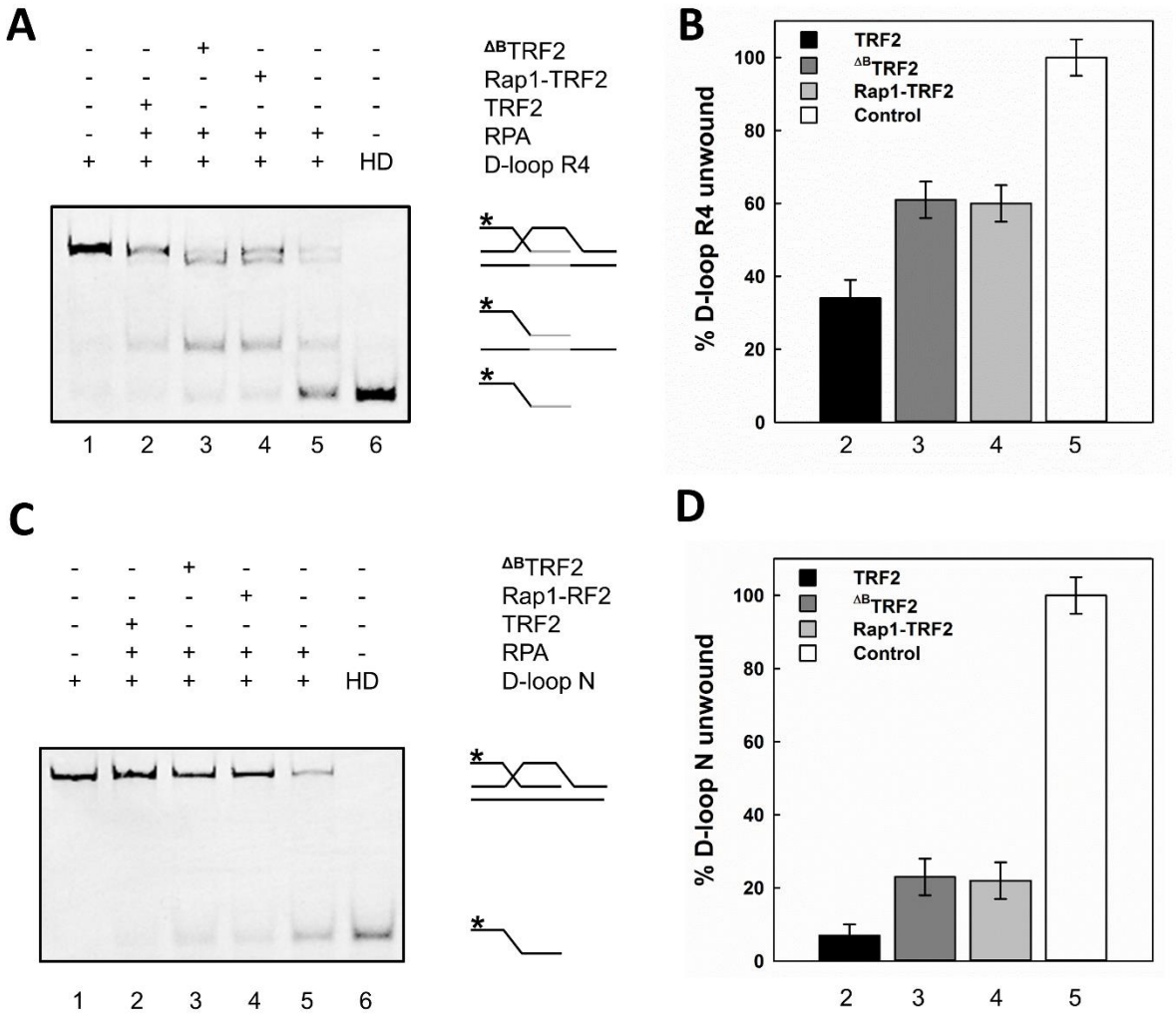

Figure S2. The B-domain of TRF2 reduces dsDNA unwinding by RPA. (**A**) D-loop R4 containing four telomeric repeats or (**C**) non-telomeric D-loop N was unwound by RPA. Reaction mixtures (10 µl) contained D-loop (3 nM) labeled by fluorescein and incubated with RPA (1500 nM; lane 5) and either TRF2, $^{\Delta B}$TRF2 or Rap1-TRF2 complex (750 nM; lanes 2, 3, 4). D-loop only (lane 1); HD heat denatured D-loop (lane 6). An asterisk denotes the fluorescently labeled invasion strand of the D-loop. Telomeric repeats are shown in gray. Products of D-loop unwinding assay were analyzed on a vertical 10% native polyacrylamide gel. (**B**) The bar graph exhibits the percentage of D-loop R4 unwinding of selected reactions from part A. The lane 5 was assumed as 100 % unwinding which corresponds to the open column denominated as Control. (**D**) The bar graph demonstrates the percentage of D-loop N unwinding of selected reactions from part C. The error bars represent the mean and standard deviation from three independent experiments.

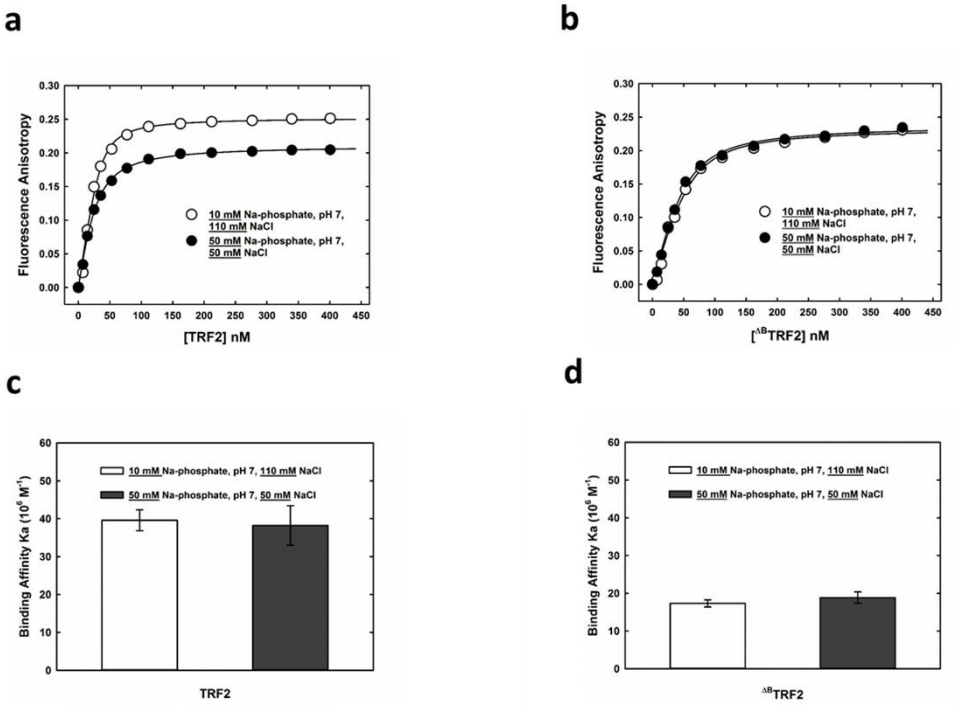

Figure S3 TRF2 variants show the same binding affinity for telomeric DNA in buffers with different concentrations of sodium phosphate and NaCl. (a) Binding isotherms of full length TRF2 binding to telomeric DNA duplex R2 measured by fluorescence anisotropy in 10 mM sodium phosphate, pH 7 containing 110 mM NaCl (opened circles) or 50 mM sodium phosphate, pH 7 containing 50 mM NaCl (closed circles). (b) Binding isotherms of $^{\Delta B}$TRF2 binding to telomeric DNA duplex R2 measured by fluorescence anisotropy in 10 mM sodium phosphate, pH 7 containing 110 mM NaCl (opened circles) or 50 mM sodium phosphate, pH 7 containing 50 mM NaCl (closed circles). **(c, d)** The quantification of binding affinity constants of TRF2 or $^{\Delta B}$TRF2 for telomeric DNA R2 in 10 mM sodium phosphate, pH 7 containing 110 mM NaCl (white bars) or 50 mM sodium phosphate, pH 7 containing 50 mM NaCl (dark grey bars). The comparison of binding affinity revealed that TRF2/$^{\Delta B}$TRF2 binding to telomeric DNA in 10 mM sodium phosphate, pH 7 with 110 mM NaCl is comparable with binding affinity in 50 mM sodium phosphate, pH 7 with 50 mM NaCl. Oligonucleotides in the cuvette (7.5 nM) were labelled by Alexa Fluor® 488. Measurement conditions were the same as described in the Materials and methods.

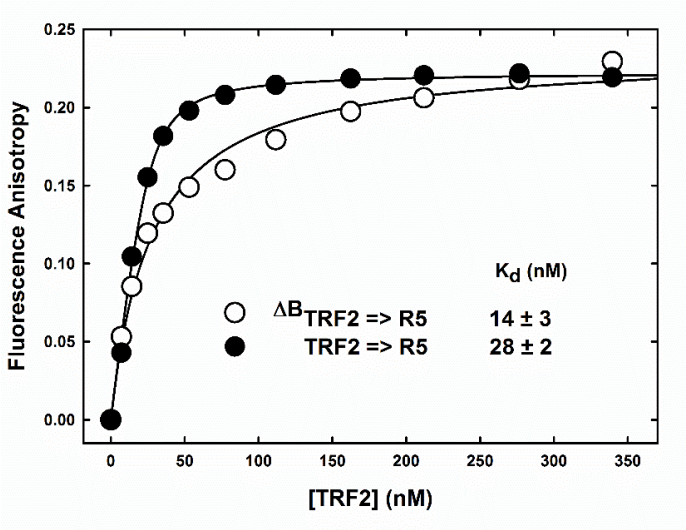

Figure S4. The B-domain doubles TRF2 binding affinity to telomeric DNA R5 with five telomeric repeats. TRF2 or $^{\Delta B}$TRF2 (5 μM) was titrated to Alexa Fluor 488 labeled DNA duplex (7.5 nM) in 50 mM sodium phosphate, pH 7.0, 50 mM NaCl at 25°C. The binding was monitored by fluorescence anisotropy change (excitation 490 nm, emission 520 nm). Binding isotherms of full length TRF2 (closed circles) and truncated $^{\Delta B}$TRF2 (open circles) binding to telomeric DNA duplex R5 are shown together with the corresponding K_d values and standard deviation error of three independent measurements.