

Supplementary Figure 1: RAP1 does not bind DNA, does not affect TRF2 binding on linear DNA, and can form a ternary complex with TRF2 and tel H-J.

A: ITC experiments performed at 10°C with RAP1 and double-stranded DNA oligonucleotides in 50 mM Tris pH 7.5, 150 mM NaCl, 10 mM β-ME. B: EMSA performed on 5 nM of a labelled 54 bp linear DNA that contains 4 telomeric repeats (dsT4) using 40 nM of TRF2 dimers and increasing amounts of RAP1. The addition of RAP1 causes a slight change in the aspect of TRF2-containing complexes but no visible changes in affinity. C: EMSA performed on 5 nM of a labelled 106 bp linear DNA containing 16 telomeric repeats (dsT16) using 10 nM of TRF2 dimers and increasing amounts of RAP1. The addition of RAP1 causes both a slight change in the aspect of the TRF2-containing complex and a mild increase in affinity (~10% increase in the amount of complex). D: Drawing of telomeric Holliday Junction (tHJ), a migratable Holliday Junction with 27 bp including 1 telomeric repeat per arm. E: EMSA performed on labelled tHJ (5 nM) using 20 nM of TRF2 dimers and increasing amounts of RAP1. The addition of RAP1 causes a change in the aspect of TRF2-containing complexes but no overt modification of affinity. F: Supershift experiment perfomed using 5 nM of tHJ incubated with 40 nM of TRF2 (dimer) with or without preincubation with 200 nM of RAP1 and post-incubation with 0.2 µg of anti-RPA1 antibody prior to agarose electrophoresis. The shifted DNA complexes obtained with TRF2 and RAP1 are supershifted by the anti-RAP1 antibody showing formation of a ternary TRF2/ RAP1/junction complex.



Supplementary Figure 2: RAP1 totally inhibits TRF2-dependent protection against CCE1 and RusA cleavage of tHJ, and largely inhibits TRF2-dependent protection against Endonuclease I cleavage of tHJ.

A-B: 5 nM of tHJ labelled on either strand 2 (A) or 4 (B) was incubated with 200 nM of TRF2 with and without increasing amount of RAP1 prior to cleavage with either CCE1 A or RusA . Concentrations of RAP1 used were 100, 200, 400, 1000, 1500 nM. In lanes 3, 11 and 18, the junction is incubated with 1500 nM of RAP1. **C-F**: 5 nM of tHJ labelled on either of the 4 strands was incubated with 200 nM of TRF2 with and without increasing amount of RAP1 prior to cleavage with Endonuclease I. Concentrations of RAP1 used were 100, 200, 400, 1000, 1500 nM. In lanes 3 and 10, the junction is incubated with 1500 nM of RAP1.





Supplementary Figure 3: RAP1 does not modify tHJ cleavage in the presence of TRF2^{\Delta B}

5 nM of tHJ labelled on either strand 2 or 4 was incubated with 200 nM of TRF2^{ΔB} with and without increasing amount of RAP1 prior to cleavage with either CCE1 or RusA. Concentrations of RAP1 used were 100, 200, 400, 1000, 1500 nM. In lanes 3, 12 and 20 the junction is incubated with 1500 nM of RAP1.



Supplementary Figure 4: Qualitative analysis of TRF2, RAP1 and RAP1-ARAGA. A: SDS-PAGE of purified TRF2 (1), RAP1 (2), RAP1-ARAGA (3), and acetylated TRF2 (4), RAP1 (5), and RAP1-ARAGA (6) proteins (Coomassie blue stained); MW: Molecular Weights from top to bottom: 250, 150, 100, 70, 50, 40, 30, 20, 15, 10 and 5 kDa. **B-C**: Circular dichroism spectra of TRF2 (**B**) and of RAP1 and RAP1-ARAGA mutant (**C**). **D:** thermal denaturation curve of RAP1 WT (top), of RAP1-ARAGA mutant (bottom). **E:** Limited proteolysis of RAP. The band corresponding to RAP1[1-211] as identified by LC-MS is shown with an arrow. MW: Molecular Weights from top to bottom: 250, 150, 100, 75, 50, 37, 25, 20, 15 and 10 kDa.



Supplementary Figure 5: SAXS analysis of Rap1_[266-399] (yellow curves), RAP1 (red curves), TRF2 (blue curves), RAP1_[266-399]/TRF2 (violet curves), and RAP1/TRF2 (green curves), and characterization of TRF2 dimer.

A: SAXS curves with Intensity at the origin in the insert; **B**: SEC-MALS and SDS-PAGE analysis of TRF2; **C**: ITC analysis of TRF2 dilution; **D**: distance distribution function p(r); **E**: Normalized Kratky plots. **F-G**: *Ab initio* averaged envelopes RAP1-RCT (**F**) and TRF2/RAP1-RCT (**G**) with superimposed globular domains as references



Supplementary Figure 6: Superposition of isolated RAP1 or RAP1-RCT envelopes with envelopes of their complexes with TRF2.

A: Superposition of two RAP1-RCT envelopes (red and magenta) with TRF2/ RAP1-RCT envelope (grey) based on the known interaction between RAP1-RCT and TRF2-RBM; **B:** Superposition of TRF2 and TRF2/RAP1 *ab initio* envelopes with TRF2 Dmax in blue and TRF2/RAP1 Dmax in green; **C-D**: Superposition of two RAP1 envelopes (blue and magenta) with TRF2/RAP1 envelope (yellow), based on (B) the known interaction between RAP1-RCT and TRF2-RBM or (C) the optimal occupancy of the upper part of TRF2/RAP1 envelope by the N-terminal region of RAP1

Condition	Janoušková et al (2015)	Our study		
[TRF2-monomer]	5 μM (cell)	13 μM (cell)		
[RAP1]	44μM (injector)	130μM (injector)		
Injections	10 μL	10µL		
Temp.	25°C	10°C		
Buffer	[Na2PO4] = 20 mM pH = 7.0 [NaCl] = 50 mM	[Tris-HCl] = 50mM pH = 7.5 [NaCl] = 150 mM [2-mercaptoethanol] = 10 mM		



Supplementary Figure 7: ITC experiments. (A) Comparison of experimental condition of ITC measurement of the interaction between TRF2 and RAP1. (B-F) ITC characterisation of the interaction between TRFH and Apollo-YLP peptide ⁴⁹⁴ATEFRGLALKYLLTPVNF⁵¹¹ (B), TRFH and RAP1-YLP peptide ⁸⁹ERNERLELEAYRLGPASA¹⁰⁶ (C), the pre-formed TRF2/RAP1 complex with Apollo-YLP peptide (D), TRF2 and Apollo-YLP peptide (E), Apollo-YLP/TRF2 preformed complex and RAP1 (F).

Α



Supplementary Figure 8: Comparison of MS spectra for TRF2 free vs in complex.

A: summary of footprinting results in TRF2 (K58 to K267). Lysine residues protected (blue) or not affected (grey) in TRF2/RAP1 and TRF2/ARAGA complex, or protected only in TRF2/RAP1 complex (green). Region protected in TRF2/RAP1 and TRF2/ ARAGA complex, but precise lysine undetermined (light blue). Lysine residues not detected, or with signal too weak to be interpreted (white). **B-F**: MS peaks of interest, with corresponding fragment indicated above each peak. Lysine residues are color-coded according to **A**, acetylated lysines are underlined, and number of acetyl groups in each fragment is indicated. Red and orange curves: TRF2 alone; dark blue and blue curves: TRF2 in complex with RAP1 (with ARAGA in **D**, right panel).



Supplementary Figure 8 (continued)

G: summary of footprinting results in TRF2 (K289 to K495). Lysine residues protected (blue), more accessible (red) or not affected (grey) in TRF2/RAP1 and TRF2/ARAGA complex. Region protected in TRF2/RAP1 and TRF2/ARAGA complex, but precise lysine undetermined (light blue). Lysine residues not detected, or with signal too weak to be interpreted (white). **H-K**: MS peaks of interest, with corresponding fragment indicated above each peak. Lysine residues are color-coded according to **G**, acetylated lysines are underlined, and number of acetyl groups in each fragment is indicated. Red and orange curves: TRF2 alone; dark blue and blue curves: TRF2 in complex with RAP1.



Supplementary Figure 9: Comparison of MS spectra for RAP1 free or in complex.

A: summary of footprinting results in RAP1 (K9 to K194). Lysine residues protected (blue) or not affected (grey) in TRF2/RAP1 and TRF2/ARAGA complex. Lysine residues protected (green) or more accessible (orange) only in TRF2/RAP1 complex. Lysine residues not detected, or with signal too weak to be interpreted (white).

B-H: MS peaks of interest, with corresponding fragment indicated above each peak. Lysine residues are color-coded according to **A**, acetylated lysines are underlined, and number of acetyl groups in each fragment is indicated. Red and orange curves: RAP1 alone; dark blue and blue curves: RAP1 in complex with TRF2 (ARAGA instead of RAP1 in **G**, right panel).



Supplementary Figure 9 (continued)

I: summary of footprinting results in RAP1 (K208 to K399). Lysine residues protected (blue), more accessible (red) or not affected (grey) in TRF2/RAP1 and TRF2/ARAGA complex. Lysine residues not detected, or with signal too weak to be interpreted (white).

m/z

J-N: MS peaks of interest, with corresponding fragment indicated above each peak. Lysine residues are color-coded according to **I**, acetylated lysines are underlined, and number of acetyl groups in each fragment is indicated. Red and orange curves: RAP1 alone; dark blue and blue curves: RAP1 in complex with TRF2.



Supplementary Figure 10 :

Acetylation profile of lysine residues in TRF2, RAP1 or RAP1-ARAGA alone, compared to TRF2/RAP1 or TRF2/RAP1-ARAGA shown on the RAP1-RCT / TRF2-RBM crystal structure (PDB entry code 3K6G). Lysine residues are represented in small sphere and coloured differently if they are protected (blue) or more accessible (red) in both TRF2/RAP1 and TRF2/RAP1-ARAGA complex. The inserted table shows the lysine number in the sequence, highlighted in blue in RAP1 globular domain, in yellow in TRF2 RBM, and in grey in TIN2 interacting region (Chen et al., 2008). The acetylation profile follows the same colour code as in the structure representation.



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TRF2 lysines	446	447	449	459	464	475	488	495
acetylation profile								



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Supplementary Figure 11: RAP1 does not interact with TRF2 Myb domain.

A: TRF2 K464 and K475 (blue) on the structure of TRF2 Myb domain (yellow, drawn from coordinates in PDB 1W0U).

B: summary of footprinting results in TRF2 Myb domain. Lysines underlined in grey: partially acetylated or not acetylated, and not affected by RAP1 nor ARAGA binding. Lysines underlined in blue: protected when TRF2 is bound to RAP1 or ARAGA. Lysines underlined in white: not detected, or signal to weak to allow interpretation.

C: size exclusion chromatograms of RAP1 alone (blue curve), TRF2 Myb alone (green curve) and a mix of RAP1 and TRF2 Myb at a 1:2 molar ratio, 90 and 180 μ M respectively (red curve).

D: SDS-PAGE analysis (Coomassie blue stained) of the contents of the two peaks from the red chromatogram in C.



Supplementary Figure 12: Streptavidin-DNA pulldown chromatography elution profile.

HeLa nuclear extracts were loaded on streptavidin/(T2AG3)2-DNA column preequilibrated with TRF2 (**A**), RAP1/TRF2 (**B**), RAP1-ARAGA/TRF2 (**C**), or nothing (**D**) with two experiments for each case. The region corresponding to the elution appears in the green box.