

## Supplementary information

### Protein expression

All proteins were expressed in *E. coli*, either in BL21DE3-star or in Rosetta strain (see table “Protein constructs used in this study” for details). After transformation by heat shock and selection of recombinant colonies with appropriate antibiotic (see table), bacteria were grown in LB medium + appropriate antibiotic in a shaking incubator at 37 °C until cultures reached an OD<sub>600</sub> ~0.6. Cultures were then cooled to 20 °C, expression was induced by addition of IPTG qsp 0.5 mM final concentration in medium when OD<sub>600</sub> reached ~0.8-1, and bacteria were further grown overnight (except for construct E, which was induced for 3 hours at 37°C).

Bacteria were harvested by centrifugation at 3,000 g at 4 °C for 30 min, LB medium was discarded and pellets were washed in cold PBS, centrifuged again and stored dry at -80 °C after flash-freezing in liquid nitrogen.

### Protein purification

For all proteins, cell pellets were thawed and suspended in 40 mL cold buffer A + Triton-X-100 0.5% v/v + PMSF 1 mM. Suspensions were treated by lysozyme (20 mg for one pellet) at room temperature for 15 min under gentle agitation, and then submitted to sonication at 4 °C (at least 4 cycles of 1 min with pulses of 1 second interspersed with 1 second pause, at 40, 50, 60 and 70% power of a Vibra-Cell 75042 sonicator). Lysates were then supplemented with MgCl<sub>2</sub> qsp 1.5 mM final concentration and treated by Benzonase nuclease (1 µL for one pellet) at room temperature for 15 min. Finally, lysates were centrifuged at 48,000 g at 4 °C for 30 min and resulting supernatants were passed through 0.22 µm filters before application on chromatography column.

The following purification strategy was followed for most protein constructs (specific details for some constructs are provided in next paragraphs): all steps were performed at 4 °C (cold room) or with buffers and sample on ice; filtered supernatant from last step of lysis was immediately applied on a HisTrap HP 5 mL column (GE Healthcare) previously regenerated with Ni<sup>2+</sup> ions and equilibrated in buffer A, loaded column was washed with several volumes of buffer A (until baseline equilibrated) and elution was performed with a linear gradient of 0 to 100% v/v buffer B over 20 column volumes. Fractions containing the protein of interest (as seen by SDS-PAGE) were pooled and set in buffer D (or in PBS for RAP1<sub>[270-399]</sub> construct E) through a HiPrep 26/10 Desalting column (GE Healthcare) to ensure full activity of protease for tag cleavage. Appropriate protease (either thrombin or TEV, see table “Protein constructs used in this study”) was added to the protein solution to a ratio of 2 to 4% w/w and the mix was incubated overnight at 4 °C. Next day, protein solution was adjusted to 300 mM NaCl (400 mM for RAP1 constructs B and C because pI < 5 gives stronger binding to Ni<sup>2+</sup>) and 20 mM imidazole and run through the HisTrap column previously stripped, Ni<sup>2+</sup>-regenerated and equilibrated in buffer C; flow through containing the tag-free protein was collected. Protein solution was finally set in buffer D through the Desalting column, glycerol was added to a final concentration of 5% v/v, and protein was stored as 1 mL aliquots at -80 °C after flash-freezing in liquid nitrogen.

For TRF2, an additional step of affinity chromatography on Heparin HP 5 mL column (GE Healthcare) was performed after the second HisTrap to ensure no DNA from the bacteria remained bound to the protein. For this step, the protein solution was adjusted to 100 mM NaCl before application on the column previously equilibrated in buffer E, and elution was performed with a linear gradient of 0 to 100% v/v buffer F over 20 column volumes. The migration profile of purified TRF2 during gel filtration reveals a first high peak and a

secondary peak as previously observed (Bae & Bauman, 2007, Amiard et al., 2007). SEC-MALS coupled to SDS-PAGE analysis and ITC measurements show that the first peak corresponds to highly pure TRF2 full-length homodimer with an experimental molecular weight of 114.5 kDa (theoretical expected molecular weight = 113.2 kDa, Sup-Figure 1A and 1B), compatible with SAXS analysis.

For RAP1 constructs A and D, an additional step of size exclusion chromatography was performed after the first HisTrap, on Superdex 200 and Superdex 75 columns (GE Healthcare), respectively, equilibrated in buffer G (high salt buffer, to separate potentially interacting contaminants). For ARAGA mutant Circular dichroism spectra in far-UV and temperature denaturation show that these three mutations do not affect the secondary and global structure of RAP1 (Supplementary Figure 7A-7C).

Prior to any experiment that required thawing of stored aliquots, all proteins were subjected to size exclusion chromatography on Superdex 200 column in order to remove eventual aggregates and to set the appropriate buffer for the experiment. It was of particular importance to avoid the presence of degradation fragments in experiments (TRF2 and RAP1 are prone to degradation due to long unfolded regions).

#### *References:*

Amiard S, Doudeau M, Pinte S, Poulet A, Lenain C, Faivre-Moskalenko C, Angelov D, Hug N, Vindigni A, Bouvet P, Paoletti J, Gilson E, Giraud-Panis MJ (2007) A topological mechanism for TRF2-enhanced strand invasion. *Nat Struct Mol Biol* **14**: 147-154.

Bae NS, Baumann P (2007) A RAP1/TRF2 complex inhibits nonhomologous end-joining at human telomeric DNA ends. *Mol Cell* **26**: 323-334.

#### **Kinetics of RAP1 limited trypsin proteolysis**

RAP1 (construct A) was dialyzed against the following buffer: 20 mM HEPES pH = 7.5, 150 mM NaCl. 45 µg of RAP1 were mixed with trypsin at a ratio of 1/10,000 w/w and incubated at 20 °C. Reaction content at 0, 30 min, 1h, 1h30, 2h, 3h, 4h, 5h, 6h, 7h, 8h, 23h, 24h and 25h was analyzed by SDS-PAGE.

#### **Circular dichroism spectroscopy**

RAP1 WT (construct A), RAP1 ARAGA (construct C) and TRF2 (construct E) were dialyzed against the following buffer: 9 mM Tris pH = 7.5, 300 mM NaF. For each protein, a concentration series of 3, 6 and 15 µM was used for spectra acquisition. Spectra were measured with a Jasco J-815 CD Spectrometer instrument. Each spectrum results from an accumulation of 20 acquisitions, scanning from 250 to 190 nm by steps of 0.1 nm and a bandwidth of 2 nm. Protein spectra were subtracted with buffer spectrum. To be able to compare spectra between the different proteins, they were expressed in molar ellipticity per residue according to the following formula:

$$[\theta] = \frac{\theta}{10 \times c \times l \times n}$$

where  $[\theta]$  is the molar ellipticity per residue ( $\text{deg.cm}^2.\text{dmol}^{-1}.\text{residue}^{-1}$ ),  $\theta$  is the raw CD data from the spectrometer (mdeg),  $c$  is the molar concentration of the protein sample ( $\text{mol.L}^{-1}$ ),  $l$  is the optical path (cm) and  $n$  is the number of residues in the protein. Spectra were also scaled so that their value at 209.5 nm (inflexion point) coincided. This allowed avoiding discrepancies in spectra amplitudes due to uncertainties in protein concentration determination (measured by absorbance at 280 nm with a Nanodrop instrument, which is less sensitive than the CD spectrometer).

Thermal denaturation was followed by measuring the CD signal at 222 nm (alpha helix main signal) while heating the samples from 10 to 95 °C by 1 °C steps. Thermal denaturation data were fitted using SciDAVis software (<http://scidavis.sourceforge.net/>) to determine  $T_m$  values.

### SAXS analysis

We performed Small Angle X-ray Scattering analysis and calculated *ab initio* envelopes that represent the statistical distribution of the three-dimensional conformation of the molecule in solution, including flexible regions (Koch et al., 2003). The data were analyzed using FOXTROT (from SWING beamline) and PRIMUS (Primary Analysis & Manipulations with Small Angle Scattering DATA) from ATSAS 2.1 (Konarev et al., 2006), from which Guinier and normalized Kratky plots (Durand et al., 2010) were generated (Figure 1, Supplementary Figure 5). From the corrected scattering curves, the pair-distribution functions were computed using GNOM (Svergun, 1992), leading to the radius of gyration  $R_g$ , and the maximal distance  $D_{max}$ . The molecular weights of the particles were derived from the extrapolated intensity at the origin  $I_0$  (Figure 4B). In order to calculate reliable *ab initio* averaged envelopes, we generated 100 low-resolution *ab initio* shape envelopes using GASBOR (Svergun, 1999). The best 50 envelopes were selected based on Chi2 factor. After superposition of the envelopes, files were selected based on the double criteria:

- $\chi^2 < \chi_{min} + 1 \text{ ecart.standard}(\chi)$
- $NSD < NSD_{min} + 1 \text{ average deviation}$  ( $NSD$ = Normalized Spatial Discrepancy);

The selected files were averaged with DAMAVER. From this approach, we do not necessary average more envelopes than from the classical approach (8 to 10 envelopes), but the tight selection criteria improved the accuracy of the final envelopes.

### References:

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- Svergun DI (1992) Determination of the regularization parameter in indirect-transform methods using perceptual criteria. *J Appl Crystallogr* **25**: 495-503.
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### Setup of protein footprinting protocol

Protocol was adapted from Delphine Benarroch-Popivker method (personal communication) as well as methods reported in the following references to our specific study of protein / protein interactions. Therefore, we had to take into account the number of lysines present in TRF2 and RAP1 and use an excess of one protein to ensure the other one is engaged in complexes at 100%. TRF2 (construct F), RAP1 (construct B) and RAP1-ARAGA (construct C) were set in buffer H by size exclusion chromatography (Superdex 200 10/300 GL, GE Healthcare).

The following mixtures were then prepared to a final volume of 20  $\mu$ L:

- free proteins: TRF2 at 20  $\mu$ M (monomers) +/- sulfo-NHS-acetate at 4.075 mM (same absolute concentration as in the condition where we look at TRF2 engaged in the complex) ; RAP1 or ARAGA at 20  $\mu$ M +/- sulfo-NHS-acetate at 7.85 mM (same absolute concentration as in the condition where we look at RAP1 or ARAGA engaged in the complex)

- complexes: TRF2 at 20  $\mu$ M (monomers) + RAP1 or ARAGA at 30  $\mu$ M (1.5-fold molar excess versus TRF2) + sulfo-NHS-acetate at 4.075 mM (2.5-fold molar excess versus total number of lysines in this condition) ; RAP1 or ARAGA at 20  $\mu$ M + TRF2 at 30  $\mu$ M (monomers, 1.5-fold molar excess versus RAP1 or ARAGA) + sulfo-NHS-acetate at 7.85 mM (2.5-fold molar excess versus total number of lysines in this condition).

Before addition of sulfo-NHS-acetate, all preparations were incubated 10 min at 20 °C to allow complex formation. Excess of one protein was necessary to ensure that the other one is engaged in complexes at 100%. Sulfo-NHS-acetate was dissolved in water shortly before use, as advised by the manufacturer (Thermo Scientific). Control experiments were also performed for free proteins by adding water instead of sulfo-NHS-acetate. Reactions were incubated 30 min at 20 °C, and finally quenched by addition of 2  $\mu$ L of 1M Tris buffer pH = 7.5 to consume the remaining excess of free sulfo-NHS-acetate. Samples were then analyzed by SDS-PAGE, and excised bands were analyzed by mass spectrometry PMF (peptide mass fingerprinting) after protease digestion.

#### *References:*

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#### **Mass spectrometry analysis of footprinting products**

Protein bands excised from SDS-PAGE gels were washed twice with 500  $\mu$ L ammonium bicarbonate 100 mM pH = 8, washed again twice with 500  $\mu$ L ammonium bicarbonate 50 mM pH = 8 / acetonitrile 50%, partially dried by lyophilization, and rehydrated by 20  $\mu$ L ammonium bicarbonate 100 mM pH = 8.

All bands were digested by trypsin at 10 ng/ $\mu$ L in ammonium bicarbonate 100 mM pH = 8, incubated 2h at 50 °C and under agitation. RAP1 and ARAGA replicate bands were also digested by V8 protease at 10 ng/ $\mu$ L in ammonium bicarbonate 100 mM pH = 8, incubated overnight at 30 °C and under agitation. Proteolysis reactions were stopped by addition of TFA at 1% v/v final concentration.

0.5  $\mu$ L of each sample were directly spotted on MALDI plates with 0.5  $\mu$ L 4-HCCA matrix at 10 mg/mL in acetonitrile 50% / water 49.9% / TFA 0.1%. For MS/MS acquisitions, samples were concentrated by MicroZipTip C18 before spotting.

MS data were acquired on a 4800 MALDI-TOF/TOF instrument (ABSciex, Foster City, USA). Spectra were analyzed with Mascot (<http://www.matrixscience.com>), against non-redundant NCBI protein database, to determine sequence coverage and identify TRF2 and RAP1 fragments containing lysine residues, which were subsequently confirmed by MS/MS sequencing. Peaks of interest were also identified by manual comparison of spectra acquired on free proteins treated or not treated by sulfo-NHS-acetate.

### Acetylation profiles analysis

Using mMass software, MS spectra were normalized relative to the highest peak (peak at  $m/z$  = 1064 for RAP1 and ARAGA digested by trypsin; peak at  $m/z$  = 1483 for RAP1 and ARAGA digested by V8 endopeptidase Glu-C; peak at  $m/z$  = 909 for TRF2 digested by trypsin). This relative scale allowed comparison of spectra obtained from different samples, which had otherwise different amplitudes on their absolute scales. For each fragment containing lysine residues and confirmed experimentally by MS/MS, we compared peak heights for the free protein versus the protein bound to its partner. Results are shown in Supplementary Figures 8 (TRF2) and 9 (RAP1). Identical results were obtained with RAP1 and ARAGA, except when a spectrum is displayed for ARAGA (Supplementary Figures 8 D and 9 G). Taken together, the modifications in acetylation profiles highlight regions in TRFH, TRF2-RBM, TRF2-Myb, RAP1-RCT, RAP1-Myb and RAP1-BRCT.

*Reference:* Strohal M, Kavan D, Novák P, Volný M, Havlíček V (2010) mMass 3: A Cross-Platform Software Environment for Precise Analysis of Mass Spectrometric Data. *Anal. Chem.* **82**: 4648–4651

### TRF2

As expected, the known interaction between RAP1-RCT and TRF2-RBM is associated to protection of Lys293 from TRF2-RBM. In addition, Lys327, Lys329 and Lys333 from TRF2, and Lys383 and Lys384 from RAP1-RCT are affected by TRF2/RAP1 interactions, which extends the RBM region of TRF2 by about 20 residues beyond the one described in the crystal structure (Supplementary Figure 10, PDB entry 3K6G, 24). TRFH domain contains 17 accessible lysines located mainly on the external helices of the dimer or on the inner side. We observe acetylation for 6 of them and interestingly the acetylation profile is modified upon RAP1 interaction for 4 of them. Lys58, Lys93, located close to **YRLGP** interaction site, and the more external Lys183 and Lys190, are protected by RAP1. Interestingly, Lys58 and Lys93 are protected by RAP1-ARAGA but not the more external Lys183 and Lys190, suggesting that the interaction of the external region is driven by the **YRLGP** sequence. Finally, Lys202 and Lys220, located on the opposite face of the monomer, are always acetylated, which implies that this region does not belong to the interaction path.

Strong decrease in peak height for peaks corresponding to acetylated peptides 55-66 and 81-99 show that K58 and K93 are protected in TRF2/RAP1 complex (Supplementary Figure 8, B and C). Peak corresponding to di-acetylated peptide 181-192 decreases for TRF2 in complex with RAP1 (Supplementary Figure 8 D, left panel) but not in complex with ARAGA (Supplementary Figure 8 D, right panel), showing that K184 and K190 are only protected in TRF2/RAP1 complex and not in TRF2/ARAGA complex. No variation of peak height for peaks corresponding to peptides 201-220 and 203-220 show that these lysines are not affected by the presence of RAP1 nor ARAGA (Supplementary Figure 8 E). Strong decrease in peak height for peak corresponding to 4-acetylated peptide 242-274 shows that this region is

protected in TRF2/RAP1 or TRF2/ARAGA complex. MS/MS analysis revealed that this peak results from a mix of peptides 242-274 acetylated on different lysine residues, and the decrease in peak height indicates that one of the lysines is protected in the complex but we cannot say precisely which one. This is consistent with this region being the beginning of a flexible linker, immediately in C-ter with respect to TRFH domain. Variations of peak height for peaks corresponding to peptides 290-307 (acetylated), 294-307, 294-311 (acetylated), 312-327, 312-329 (acetylated) and 312-332 (di-acetylated) were all correlated due to the fact that trypsin is unable to cleave after an acetylated lysine, giving a complex cleavage pattern that depends on acetylation state. The data presented in Supplementary Figure 8 H can be explained by:

- A protection of K293, explaining the decrease seen in top left panel
- K307 becoming more accessible (thus more frequently acetylated), explaining the decrease seen in top middle and left panels and the increase seen in top right panel
- K311 (lysine marked by a star) becoming more accessible (thus more frequently acetylated), explaining the decrease seen in bottom left panel instead of the expected increase due to K327 protection
- A protection of K327, explaining the decrease seen in bottom middle and right panel
- A protection of K329, explaining the decrease seen in bottom right panel.

Supplementary Figure 8 I shows that one of the three lysines K333, K353 or K355 is protected in the complex, explaining the decrease seen for peaks corresponding to di- and tri-acetylated peptide 333-356 (middle and right panels, respectively) while peak corresponding to mono-acetylated peptide 333-356 does not vary between free and complexed TRF2 (left panel). It has been shown that TIN2 and RAP1 can interact together with TRF2. Since TIN2 interaction site includes K353 and K355, we concluded that the protected lysine is K333, which is also consistent with the proximal protected K327 and K329. Peaks corresponding to di-acetylated peptide 447-459 and mono-acetylated peptide 448-459 do not vary between free and complexed TRF2 (Supplementary Figure 8 J), showing that K447, K449 and K459 are identically exposed either in free TRF2 or in the complex. Decrease in peak height for peaks corresponding to di-acetylated peptide 460-482 and mono-acetylated peptide 465-482 (Supplementary Figure 8 K) show that K464 and K475 are protected in the complex.

## RAP1

Slight increase in peak height for peak corresponding to acetylated peptide 24-40 (Supplementary Figure 9 B) shows that K39 is more accessible in the complex. This is observed only with RAP1-WT, not with ARAGA. Increase in peak height for peak corresponding to peptide 101-129, and no significant variation in peak height for peak corresponding to acetylated peptide 101-129 (Supplementary Figure 9 C) show that K114 is protected in the complex. K149, K165 and K181 remain equally accessible in the complex as in free RAP1, as shown by no significant variation of peak height for peaks corresponding to peptide 134-149 and acetylated peptide 134-153 (Supplementary Figure 9 D), acetylated peptide 154-169 (Supplementary Figure 9 E), and peptide 170-183 and its acetylated form (Supplementary Figure 9 F). K186 is protected in the complex with RAP1-WT only (not with ARAGA), as shown by a decrease in peak height for peak corresponding to acetylated peptide 183-192 (Supplementary Figure 9 G). Slight increase in peak height for peak corresponding to acetylated peptide 190-208 (Supplementary Figure 9 H) shows that K194 is more accessible in the complex, only with RAP1-WT. K208, K210, K212 and K228 remain equally accessible in the complex as in free RAP1, as shown by no significant variation of peak height for peaks corresponding to mono- and di-acetylated peptide 195-211 (Supplementary Figure 9 J) and

non-acetylated, mono-acetylated and di-acetylated forms of peptide 212-229 (Supplementary Figure 9 K). Strong increase in peak height for peak corresponding to acetylated peptide 324-344 (Supplementary Figure 9 L) shows that K324 is more accessible in the complex than in free RAP1. K383 is protected in the complex, as shown by an increase in peak height for peak corresponding to peptide 380-395 and a correlated decrease in peak height for peak corresponding to an acetylated form of this peptide (Supplementary Figure 9 M). K384 is also protected, as shown by the same variations for peaks corresponding to non-acetylated and acetylated peptide 384-392 (Supplementary Figure 9 N).

### **RAP1 / TRF2-Myb interaction assay**

Footprinting experiments showed that TRF2 K464 and K475 are protected in the complex (Supplementary Figure 10, A and B), which was not expected because these lysines are located in the Myb DNA-binding domain, a region not expected to interact with RAP1. We therefore tested the possibility of a contact between RAP1 and TRF2-Myb by size exclusion chromatography. The chromatogram displays two peaks at same elution volumes as the peaks obtained with both proteins run separately through the column (Supplementary Figure 11), and SDS-PAGE analysis of the contents of those peaks confirms that the two proteins do not co-elute. Protein concentration in the output from the column was estimated to be around 25-30  $\mu$ M for RAP1 and around 55-60  $\mu$ M for TRF2-Myb. We can therefore conclude that RAP1 does not interact with TRF2-Myb with a detectable affinity, and that the protection detected in footprinting experiments could be due to conformational adjustment of TRF2 or intra-TRF2 (potentially inter-monomer) contacts.

### **Preparation of nuclear extracts**

Nuclear extracts were prepared following the protocol described in (Abmayr *et al*, 2001), with the following minor modifications : all buffers contained 2-mercaptoethanol at a concentration of 5 mM instead of DTT; PMSF concentration in all buffers was 1 mM instead of 0.2 mM; conductivity of the extracts was not measured but final dialysis was performed against 100 volumes of dialysis buffer (molecular weight cut-off 6-8 kDa) during 1h30; final centrifugation was done at 16,100 g instead of 25,000 g. Quantification by Bradford assay showed that in our hands this protocol yielded nuclear extracts containing from 3 to 5 mg/mL total proteins.

## Supplementary data

**Supplementary Table 1: Protein constructs used in this study**

Protein construct		Expression vector	Tag (all at N-ter)	Cleavage site	<i>E. coli</i> strain	Antibiotic
A	RAP1 [1-399]	pET-16b	DsRed - 9-His	TEV	Rosetta	Amp 100 µg/mL Cm 35 µg/mL
B	RAP1 [1-399]	pET-16b	9-His	TEV	Rosetta	Amp 100 µg/mL Cm 35 µg/mL
C	RAP1 [1-399] ARAGA	pET-16b	9-His	TEV	Rosetta pLysS	Amp 100 µg/mL Cm 35 µg/mL
D	RAP1 [270-399] RCT	pET-14b	6-His	Thrombin	BL21DE3-star	Amp 50 µg/mL
E	RAP1[1-208]	pET-16b	9-His	TEV	Rosetta pLysS	Amp 100 µg/mL Cm 35 µg/mL
F	TRF2 [3-500]	pTrcHisB	6-His	TEV	BL21DE3-star	Amp 50 µg/mL
G	TRF2 [1-500]	pETM-13 <sup>a</sup>	8-His	TEV	BL21DE3-star	Kan 30 µg/mL
H	TRF2 [43-245] TRFH	pETM-13 <sup>a</sup>	8-His	TEV	BL21DE3-star	Kan 30 µg/mL
I	TRF2 [400-500] Myb	pETM-13 <sup>a</sup>	8-His	TEV	BL21DE3-star	Kan 30 µg/mL

Amp: ampicillin; Cm: chloramphenicol; Kan: kanamycin; a: from Genscript

**Supplementary Table 2: Oligonucleotides used in this study.**

Oligonucleotide	Number of TTAGGG repeats	Sequence	Ends	Used in
T2-S1-S2	2	5'-CTAGGGTTAGGGTTAG-3'	Blunt	Supp. Fig. 1
T2-S5-S8-biotin	2	biotin-5'-TACTAGGGTTAGGGTTAGCA-3'	1-base 5'-overhang	Supp. Fig. 12

### Buffers used for protein purification

All buffers are freshly prepared and filtered through 0.22 µm diameter pores.

Buffer A: HisTrap loading buffer

- Na<sub>2</sub>HPO<sub>4</sub>/NaH<sub>2</sub>PO<sub>4</sub> pH = 8, 20 mM
- NaCl 500 mM
- Imidazole 60 mM in case of 8- or 9-His tag, 20 mM in case of 6-His tag
- 2-mercaptoethanol 10 mM

Buffer B: HisTrap elution buffer

- Na<sub>2</sub>HPO<sub>4</sub>/NaH<sub>2</sub>PO<sub>4</sub> pH = 8, 20 mM
- NaCl 500 mM (1 M for RAP1 constructs B and C because pI < 5 gives stronger binding to Ni<sup>2+</sup>)
- Imidazole 1 M
- 2-mercaptoethanol 10 mM



Buffer C: “Reverse HisTrap” loading buffer

- Tris-HCl pH = 7.5, 20 mM
- NaCl 300 mM (400 for RAP1 constructs B and C because pI < 5 gives stronger binding to Ni<sup>2+</sup>)
- Imidazole 20 mM
- 2-mercaptoethanol 10 mM

Buffer D: Desalting buffer

- Tris-HCl pH = 7.5, 20 mM
- NaCl 150 mM
- 2-mercaptoethanol 5 mM

Buffer E: Heparin low salt buffer

- Na<sub>2</sub>HPO<sub>4</sub>/NaH<sub>2</sub>PO<sub>4</sub> pH = 7.5, 10 mM
- NaCl 50 mM
- 2-mercaptoethanol 5 mM

Buffer F: Heparin high salt buffer

- Na<sub>2</sub>HPO<sub>4</sub>/NaH<sub>2</sub>PO<sub>4</sub> pH = 7.5, 10 mM
- NaCl 2 M
- 2-mercaptoethanol 5 mM

Buffer G: Gel filtration high salt buffer

- Tris-HCl pH = 7.5, 20 mM
- NaCl 1 M
- 2-mercaptoethanol 5 mM

Buffer H: Protein footprinting buffer

- Hepes-NaOH pH = 7.5,
- 20 mM NaCl 150 mM
- 2-mercaptoethanol 5 mM

**Supplementary Table 2: ITC experiments**

Cell	Syringe	Buffer	Temperature	Vol inj.
TRF2 (13 μM)	RAP1 (130 μM)	50 mM Tris pH 7.5, 150 mM NaCl, 10 mM βMe	283 K	10 μL
TRF2 (10 μM)	RAP1 <sub>[270-399]</sub> (91 μM)	50 mM Tris pH 7.5, 150 mM NaCl, 10 mM βMe	283 K	10 μL
TRF2 (13 μM)	RAP1-ARAGA (130 μM)	50 mM Tris pH 7.5, 150 mM NaCl, 10 mM βMe	283 K	10 μL
TRF2 (13 μM)	Apollo-YLP (130 μM)	50 mM Tris pH 7.5, 150 mM NaCl, 10 mM βMe	283 K	10 μL
TRFH (25 μM)	RAP1-YLP (250 μM)	25 mM Tris pH 7.5, 100 mM NaCl, 10 mM βMe	283K 293K 279 K	10 μL
TRF2/RAP1 (13 μM/13μM)	Apollo-YLP (130 μM)	50 mM Tris pH 7.5, 150 mM NaCl, 10 mM βMe	283 K	10 μL
Apollo-YLP (25 μM)	TRFH (250 μM)	25 mM Tris pH 7.5, 100 mM NaCl, 10 mM βMe	293 K	10 μL
TRF2 (13 μM)	Apollo-YLP (130 μM)	50 mM Tris pH 7.5, 150 mM NaCl, 10 mM βMe	283 K	10 μL
TRF2 (27 μM)	TIN2-YLP (270 μM)	50 mM Tris pH 7.5, 150 mM NaCl, 10 mM βMe	283 K	1.5 μL (ITC200)

TRF2/RAP1 (27 $\mu$ M/27 $\mu$ M)	TIN2-YLP (270 $\mu$ M)	50 mM Tris pH 7.5, 150 mM NaCl, 10 mM $\beta$ Me	283 K	1.5 $\mu$ L (ITC200)
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$\beta$ Me = 2-mercaptoethanol

#### Sequences used in conservation analysis:

##### TRF2:

1. Q15554 TERF2\_HUMAN
2. Q9PU53 TERF2\_CHICK
3. Q1WM12 Q1WM12\_XENLA
4. B8QB44 B8QB44\_XENTR
5. F6V5V7 F6V5V7\_CALJA
6. G1TE57 G1TE57\_RABIT
7. F1MMS4 F1MMS4\_BOVIN
8. D3ZJF7 D3ZJF7\_RAT
9. O35144 TERF2\_MOUSE
10. I2CTL7 I2CTL7\_MACMU
11. F7FBA4 F7FBA4\_MONDO
12. F1PBX6 F1PBX6\_CANFA
13. H0ZCM5 H0ZCM5\_TAEGU
14. Q8JGS4 Q8JGS4\_DANRE

##### RAP1:

1. Q9NYB0 TE2IP\_HUMAN
2. Q7T0L4 TE2IP\_CHICK
3. Q71M44 TE2IP\_XENLA
4. B8QB46 TE2IP\_XENTR
5. F7IE94 F7IE94\_CALJA
6. G1T9Q1 G1T9Q1\_RABIT
7. Q0VCT3 TE2IP\_BOVIN
8. Q5EAN7 TE2IP\_RAT
9. Q91VL8 TE2IP\_MOUSE
10. H9FW08 H9FW08\_MACMU
11. F7DF74 F7DF74\_MONDO
12. E2RSQ7 E2RSQ7\_CANFA
13. H0ZDI5 H0ZDI5\_TAEGU
14. Q6NYJ3 TE2IP\_DANRE