Supplementary Materials & Methods

Analytical ultracentrifugation experiments:

Sedimentation velocity experiments were performed at 4 °C using a ProteomeLab XL-I analytical ultracentrifuge (Beckman Coulter) equipped with double-UV and Rayleigh interference detection. The samples were used immediately after size-exclusion chromatography in 20 mM Tris/HCl pH8, 150 mM NaCl, 2 mM TCEP and 0.5 mM PMSF. Various protein and protein-DNA complex concentrations (ranging from 6 to 40 µM) were spun at 42,000 rpm using an AN60-Ti rotor and 3 mm or 12 mm thick epon double sector centerpieces. Absorbance and interference profiles were recorded every 4 min. Detection of concentrations as a function of radial position and time was performed by optical density measurements at wavelengths of 280 and 290 nm for proteins and protein-DNA complexes, respectively. Buffer viscosity (η=1.597 cP) and density (ρ=1.00677 g.mL^{-1}) at 4 °C were estimated with Sednterp 1.09 (http://www.rasmb.bbri.org). Partial specific volumes of each construct at 4 °C were estimated based on amino acid sequences using Sednterp 1.09. For Rap1/DNA complexes, partial specific volumes were estimated by weight averaging of the partial specific volume of the amino acid sequences estimated with Sednterp 1.09 and the nucleotide sequences using 0.57 ml/g as value (Table 1). Data were analyzed with Sedfit 12.1 using a continuous size distribution c(S) model (1). The treated data were used to obtain values of sedimentation coefficients at null concentration in our experimental conditions (S_0) (Figure S2), which in turn were processed to get the standard sedimentation coefficients in water at 20 °C ($S_{0,W,20}$) (Table 1).

Overall strategy of building Rap1 and Rap1/DNA molecular architecture:

The size of Rap1, and its high content in unstructured regions restrains the possible approaches to characterize its three-dimensional structure. SAXS provides accurate information about folding and conformation in solution for both rigid and flexible macromolecules. The Fourier transform of the scattering intensity, I(q), yields the P(r)-distribution, a histogram of inter-atomic distances within the macromolecule. From this relationship, one can directly calculate a SAXS profile given a macromolecular structure, although the reverse is not true as the macromolecule atomic details can not be uniquely determined from its experimental SAXS profile alone (2). In order to build a reliable image of Rap1 and of Rap1/DNA architectures, our approach integrates the use of three different constructs of Rap1 that allows a step-by-step structural study, and the combination of SAXS

with ultracentrifugation analysis. *Ab initio* envelopes were calculated in the case of free proteins, but the different specific volumes of protein and DNA prohibit this approach with the complexes. The calculation of the models was performed with an expert version of Bunch, kindly provided by Dr Svergun, that allows adjusting the Rg of the linkers (3). For each data set, 10 to 20 independent runs of Bunch were performed in an iterative manner, using various Rg values of the linkers until agreement between experimental and theoretical scattering data (Table S2). After each Bunch run, the complete coordinates files were re-build using MODELLER (4), allowing to perform energy minimization, and to calculate 5 models for each Bunch output. Theoretical SAXS curves of series of models were calculated with CRYSOL (5) and 4 to 5 models were selected based on the quality of their fits (mean normalized residuals) to experimental data. Cross-validation of the models and final selection were performed based on the agreement between the experimental sedimentation coefficients obtained from ultra-centrifugation analysis and the theoretical sedimentation coefficients calculated with our models using HYDROPRO 7c with a hydrated radius of 3.2 Å for atomic elements (6) (Table 1). In order to further check if the conformation of $Rap1_{[358-827]}$ is maintained in the entire molecule, we also calculated theoretical SAXS curves with CRYSOL using region 358-827 from $Rap1_{[117-827]}$ and Rap1-fl models, and compared them with experimental $Rap1_{[358-827]}$ SAXS curve (Figure S4).

NMR assignment of $Rap1_{[675-827]}$ frequencies:

NMR spectra were acquired at 25 °C on Bruker Avance 600 and 700 MHz spectrometers (CEA-Saclay, France) equipped with triple resonance cryoprobes. Different samples of $Rap1_{[675-827]}$ (uniformly ^{15}N- or ^{15}N, ^{13}C- labeled and at 0.15-0.35 mM concentrations) were prepared in 25 mM Tris-HCl buffer (91 % H_2O/9 % D_2O), pH 7.5, 150 mM NaCl, 10 mM β-mercaptoethanol, 0.5 mM PMSF. The NMR data were processed using Topspin (Bruker Biospin, Germany) and the spectra were analyzed using Sparky (T.D. Goddard and D.G. Kneller, University of California, San Francisco). Backbone chemical shift assignments were obtained from the analysis of a combination of several 3D NMR experiments (HNCA, HNCO, HN(CA)CO, HN(CO)CA, HNCACB, CBCA(CO)NH). The assignments were facilitated by using the MARS software (7), and enabled to assign 98.6% of the backbone signals (Figure S6 A). The prolines and the Asn 749 and Phe 822 weren't assigned. Prediction of secondary structure elements from chemical shifts using TALOS (8) showed that α-helices and β-sheets observed in solution are similar to those identified in the crystal structure (PDB code: 3CZ6).

Titration of Rif2 and Sir3$_{[450-487]}$ onto Rap1$_{[675-827]}$:

Titrations of ^{15}N Rap1$_{[675-827]}$ with Rif2, and Sir3$_{[450-487]}$ were followed by recording series of 2D ^{1}H-^{15}N HSQC spectra of ^{15}N Rap1$_{[675-827]}$ in the presence of increasing ratios of partners. In the case of titration with Sir3$_{[450-487]}$, the initial concentration of ^{15}N Rap1$_{[675-827]}$ was 168 µM, and the Rap1$_{[675-827]}$: Sir3$_{[450-487]}$ ratios varied from 0.13 to 3.4. Titration with Rif2 full length was performed using ^{15}N Rap1$_{[675-827]}$ and Rif2 (at initial concentrations of 125.2 µM and 218.5 µM respectively), and a final Rap1$_{[675-827]}$:Rif2 ratio of 0.2. In this latter case, a Rap1$_{[675-827]}$:Rif2 ratio of 1 leads to no detectable signals (dilution effect and/or too broad peaks). Assigned peaks affected upon titration were reported on the crystal structure. Mapping the differences in chemical shift and peak intensity allowed the identification of the surfaces involved in the interaction with Rif2 and Sir3$_{[450-487]}$ (Figure S6 B-D).

Titration of N-terminal fragments onto Rap1$_{[675-827]}$:

We built three different constructs corresponding to the N-terminal end of Rap1: Rap1$_{[1-224]}$ (N-terminal extremity *plus* the BRCT domain), Rap1$_{[117-224]}$ (BRCT domain), and Rap1$_{[117-352]}$ (BRCT *plus* the linker between BRCT and DBD). Titrations of ^{15}N Rap1$_{[675-827]}$ with Rap1$_{[1-224]}$, Rap1$_{[117-224]}$, Rap1$_{[117-352]}$, Rif2, and Sir3$_{[450-487]}$ were followed by recording series of 2D ^{1}H-^{15}N HSQC spectra of ^{15}N Rap1$_{[675-827]}$ in the presence of increasing ratios of partners. Titrations with Rap1$_{[1-224]}$, Rap1$_{[117-224]}$, and Rap1$_{[117-352]}$, were performed using ^{15}N Rap1$_{[675-827]}$:"N-terminal end construct" ratios from 1:0 to 1:1. NMR titration of ^{15}N labeled Rap1$_{[675-827]}$ with each of these N-terminal constructs did not reveal any chemical shift perturbation, suggesting an absence of interaction between the N- and C-terminal regions of Rap1.

ITC experiments:

ITC titrations were performed on a VP-ITC calorimeter (GE Healthcare). Prior to the measurements, all the solutions were degassed under vacuum. All partners were prepared in the same buffer containing 50 mM Tris, pH 7.5, 150 mM NaCl, 10 mM β-mercaptoethanol. A 10 µM Rap1$_{[1-224]}$ solution in the calorimeter cell was titrated by a 95 µM Rap1$_{[675-827]}$ solution in the syringe, using automatic injections of 8 µL, with 240 s intervals between each injection. Titration of Rap1$_{[1-224]}$ by Rap1$_{[675-827]}$ (performed at 30°C) showed no interaction. Titrations of Rap1$_{[1-827]}$, Rap1[Y592A-K597A] and Rap1Δ[591-597] (4 µM) by a 21 bp telomeric DNA fragment (40 µM) were realised at 10 °C, using 10 µL automatic injections. Thermodynamic parameters Δ*H* (enthalpy change), *n* (stoichiometry), and *K*a (association constant) were obtained by non-linear least-squares fitting of the experimental data of the

Origin software provided with the instrument. The free energy of binding (ΔG) and entropy (ΔS) were calculated from classical equations.

Gel filtration analysis of N- and C-terminal moieties of Rap1 interaction:

We explored by gel filtration the possible interactions between the N-terminal region of Rap1 and a large C-terminal region 358-827 (comprising the DBD and the RCT domain) (Figure 1A). Purified $Rap1_{[1-224]}$, $Rap1_{[117-352]}$ and $Rap1_{[358-827]}$, or equimolar mixes of $Rap1_{[1-224]}$ and $Rap1_{[358-827]}$ or $Rap1_{[117-352]}$ and $Rap1_{[358-827]}$, were loaded at 35 µM on a 4.6 mm Agilent Bio SEC-3 size exclusion chromatography column in a buffer containing 25 mM Tris/HCl pH 7.5, 150 mM NaCl and 10 mM β-mercaptoethanol. Comparable fractions were collected for each run, and analyzed by SDS-PAGE. Analysis of the elution volumes of $Rap1_{[1-224]}$/$Rap1_{[358-827]}$ and $Rap1_{[117-352]}$/$Rap1_{[358-827]}$ showed no difference between the Rap1 fragments alone or together with their Rap1 partners, in agreement with an absence of interaction (Figure S8).

EMSA experiments:

Mixes containing either 8 µM of 21-mere telomeric DNA duplex, or 8 µM of 21-mere telomeric DNA duplex and 4 µM of Rap1 WT, $Rap1_{[Y592A-K597A]}$ or $Rap1\Delta_{[591-597]}$, were incubated 15 min at 4°C in a buffer containing 25 mM Tris/HCl pH8, 150 mM NaCl, 2.5 mM β-mercaptoethanol, 0.2 mM PMSF and 12 % glycerol. Samples were then loaded on a 1.5×200×200 mm 8 % Acrylamide/bisacrylamide 29/1, 0.5 X TBE, followed by a migration of 2 hours at 300 V and 4°C. DNA was revealed using BET staining.

Supplementary information:

NMR mapping Orientation of C-terminal domain within Rap1/DNA complex is compatible with its interaction with functional partners Rif2 and Sir3:

In order to check if the orientation of RCT in the Rap1/DNA complex is compatible with its interaction with functional partners in solution, we performed NMR titrations of ^{15}N-labeled $Rap1_{[675-827]}$ with a $Sir3_{[450-487]}$ interacting peptide designed on the basis of previous double-hybrid experiments (11) and Rif2 full length (Figure S6). In order to analyze these titrations, we assigned the NMR spectra of $Rap1_{[675-827]}$ (Figure S6 A). Then we identified residues whose peaks were affected upon titration with $Sir3_{[450-487]}$ and Rif2. Our mapping analysis leads to surfaces of 4354 Å^2 and 3016 Å^2 for $Sir3_{[450-487]}$ and Rif2 respectively, for a total Rap1 surface of 8473 Å^2 (Figure S6). Both surfaces include a large cavity consistent with that described by Lei's group in the case of Sir3 (10), and with biological data from Wohlberger's

group for both Sir3 and Rif2 (9) (Figure S6). Comparison of the surfaces involved in $Sir3_{[450-487]}$ and Rif2 interaction reveals a large overlap corresponding to 1967 $Å^2$ (Figure S7A). This provides structural elements that confirm previous observation of mutually exclusive Rif and Sir binding to RCT (9, 12). In addition, Sir3 and Rif2 interacting surfaces on Rap1 RCT are accessible in the average structure of Rap1 full length in complex with DNA (Figure S7B).

Supplementary references:

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Table S1: Summary of oligo-nucleotides, and protein constructs used during the study.

Molecule	Method	Sequence or construct
DNA	Crystallography	5′-ACCT**GGTGT**GTG**GGTGT**TGTGT**GGTGT**TCAC -3′ 3′-GA**CCACA**CAC**CCACA**ACACA**CCACA**AGTGTG -5′
	AUC	5′-TCCT**GGTGT**GTG**GGTGT**GCG - 3′ 3′-GA**CCACA**CAC**CCACA**CGCAG - 5′
	SAXS	5′-TCCT**GGTGT**GTG**GGTGT**GCG - 3′ 3′-GA**CCACA**CAC**CCACA**CGCAG - 5′
	ITC and EMSA	5′-TCCT**GGTGT**GTG**GGTGT**GCGG - 3′ 3′-CCAGGA**CCACA**CAC**CCACA**CG - 5′
Rap1	Crystallography	$Rap1_{[358-827]}$
	NMR	$Rap1_{[1-224]}$, $Rap1_{[117-224]}$, $Rap1_{[117-352]}$, and $Rap1_{[675-827]}$
	SAXS	$Rap1_{[1-827]}$, $Rap1_{[117-827]}$, $Rap1_{[358-827]}$
	AUC	$Rap1_{[1-827]}$, $Rap1_{[117-827]}$, $Rap1_{[358-827]}$
	ITC	$Rap1_{[1-827]}$, $Rap1_{[117-827]}$, $Rap1_{[358-827]}$, $Rap1_{[Y592A-K597A]}$, $Rap1_{\Delta[591-597]}$
	EMSA	$Rap1_{[1-827]}$, $Rap1_{[Y592A-K597A]}$, $Rap1_{\Delta[591-597]}$
Rif2	NMR	1-395
$Sir3_{[450-487]}$	NMR	NENPTPEKGNAKMIDFATLSKLKKKYQIILDRFAPDNQ

Table S2: Summary of the Rg linkers tested during the iterative approach used with BUNCH to calculate our models:

	Rg linkers tested (Å)			Rg linker best model (Å)		
Construct	L0: 1-117	L1: 215-357	L3: 601-680	L0	L1	L2
Rap1[358-827]			10-20			10
Rap1[117-827]		30-60	15-50		50	30
Rap1-fl	10-30	20-50	15-50	30	50	20
Rap1[358-827]/DNA			15-35			25
Rap1[117-827]/DNA		15-65	13-50		60	23
Rap1-fl	10-30	30-60	23-50	20	60	23

Table S3: Thermodynamic parameters for Rap1 wild type, and of mutants $Rap1_{[Y592A-K597A]}$ and $Rap1_{\Delta[591-597]}$ binding to DNA as measured by ITC.

	Ka (±error) (M^{-1})	ΔG (kcal/mol)	ΔH (±error) (kcal/mol)	TΔS (kcal/mol)	n
Rap1-fl	2.5 10^8 (0.44)	10.8	6.8 (0.04)	17.6	1.04
$Rap1_{[Y592A, K597A]}$	1.5 10^8 (0.36)	10.5	6.8 (0.07)	17.3	0.95
$Rap1_{\Delta[591-597]}$	6.5 10^7 (0.58)	10.0	18.6 (0.1)	28.6	0.92

Supplementary figures legends:

Figure S1: SAXS analysis:

A-B: SAXS curves of $Rap1_{[358-827]}$, $Rap1_{[117-827]}$, and Rap1 full length alone (A), or in complex with DNA (B). C: Guinier plots. D: Normalized Kratky plots. D: Pair distribution function. A-D color code: $Rap1_{[358-827]}$ light blue, $Rap1_{[117-827]}$ light green, Rap1 full length orange, $Rap1_{[358-827]}$/DNA blue, $Rap1_{[117-827]}$/DNA green, and Rap1 full length/DNA red. E: *Ab initio* envelops of free proteins calculated with DAMMIN with $Rap1_{[358-827]}$ in light blue, $Rap1_{[117-827]}$ in light green, Rap1 full length in orange, and superposition of the three envelops.

Figure S2: Analytical ultracentrifugation sedimentation:

Normalized sedimentation coefficient (S_0) distribution profiles based on sedimentation velocity experiments performed at 4 °C. Sedimentation coefficients are expressed in Svedbergs, where 1 S = 10^{-13} s.

Figure S3: SAXS analysis of intermediate construct $Rap1_{[117-827]}$:

A-B: Cross-validated models with fit and residual of $Rap1_{[117-827]}$ in A and $Rap1_{[117-827]}$/DNA in B.

C-D: Superposition of $Rap1_{[117-827]}$ and Rap1-fl free or in complex with DNA. The color code of the domains is the same than in Figure 1 A.

Figure S4: $Rap1_{[358-827]}$ conformational adjustment:

A-B: Shape conservation of region 358-827 in Rap1 and Rap1/DNA. A: Superposition of region 358-827 from $Rap1_{[117-827]}$ (green) and Rap1-fl (orange) with $Rap1_{[358-827]}$ model (blue), with fits between theoretical and experimental curve. B: Superposition of region 358-827/DNA from $Rap1_{[117-827]}$/DNA (dark green) and Rap1-fl/DNA (red) with $Rap1_{[358-827]}$ model (dark blue), with fits between theoretical and experimental curve.

C-D: Comparison of domains orientation in region 358-827 C: Superposition on double-Myb moiety of region 358-827 from $Rap1_{[358-827]}$ (blue), $Rap1_{[117-827]}$ (green) and Rap1-fl (orange). D: Superposition on double-Myb/DNA moiety of region 358-827/DNA from $Rap1_{[358-827]}$/DNA (dark blue), $Rap1_{[117-827]}$/DNA (dark green) and Rap1-fl/DNA (red).

Figure S5: Crystal structure of $Rap1_{DBD}$/DNA.

A: Cartoon representation of fold-back region 565-571 with 1 sigma level 2FoFc electron density map in blue. Residues involved in interaction network that folds-back the wrapping loop toward DNA are represented in sticks. B: stick representation of fragment 575-583

(magenta) with DNA C-rich strand in white, DNA G-rich strand in yellow, and 1 sigma level 2FoFc electron density map in light blue.

Figure S6: NMR titration

A: 2D ^{1}H-^{15}N HSQC spectrum of $Rap1_{[675-827]}$ at 298K. Assignments of resonances are indicated using one-letter codes for amino acids.

B: At a 2 $Rap1_{[675-827]}$:$Sir3_{[675-827]}$ ratio, residues corresponding to peaks decreasing by more than 40 % in intensity (slow exchange) are colored in red, those corresponding to peaks shifting by more than 1.5σ (σ being the chemical shift standard deviation; fast exchange) are colored in yellow and those corresponding to both decreasing and shifting peaks (intermediate exchange) are colored in red. The arrows highlight residues for which mutation affects Sir3 interaction in Feeser *et al*., 2008.

C: Comparison of NMR surface mapping of $Rap1_{[675-827]}$ with $Sir3_{[450-487]}$ and crystal structure of $Rap1_{[677-824]}$ in complex with $Sir3_{[461-480]}$ (Chen et al., 2011, PDB entry 3OWT). Residues which NMR backbone chemical shifts were affected by $Sir3_{[450-487]}$ are colored in red, those located to less than 5 Å to $Sir3_{[461-480]}$ in green, those which NMR backbone chemical shifts were affected by $Sir3_{[450-487]}$ and located to less than 5 Å to $Sir3_{[461-480]}$ in salmon, and those located to less than 5 Å to $Sir3_{[461-480]}$ but unassigned by NMR in blue. $Sir3_{[461-480]}$ is represented in green sticks, and green arrows highlights each end of the peptide.

D: At a 5 $Rap1_{[675-827]}$:Rif2 ratio, residues corresponding to peaks decreasing by more than 65% in intensity (slow exchange with strong effect) are colored in blue and those decreasing by more than 50% (slow exchange with weak effect) are colored in cyan. The arrows highlight residues for which mutation affects Rif2 interaction in Feeser *et al*., 2008.

Figure S7: **Interaction of Rap1 C-terminal domain with functional partners.**

A: Mapping the NMR interaction surfaces of ^{15}N-labeled $Rap1_{[675-827]}$ common for Sir3 peptide and full length Rif2 protein. $Rap1_{[675-827]}$ residues whose NMR backbone chemical shifts were affected by the addition of $Sir3_{[450-487]}$ are colored in red, those affected by the addition of Rif2 are colored in cyan and those affected both by the addition of $Sir3_{[450-487]}$ or Rif2 protein are colored in yellow. Upper side: surface mode representation; lower side: cartoon mode representation. B: Average structure of Rap1/DNA showing common NMR interaction surface of $Rap1_{[675-827]}$ from titration with $Sir3_{[450-487]}$ or Rif2 in yellow. Color code of the domains is the same than in A.

Figure S8: **Interaction analysis between $Rap1_{[1-224]}$ or $Rap1_{[117-352]}$ and $Rap1_{[358-827]}$.**

A: Size exclusion chromatography (SEC) profiles of $Rap1_{[1-224]}$, $Rap1_{[117-352]}$ or $Rap1_{[358-827]}$ alone and of $Rap1_{[1-224]}$ / $Rap1_{[358-827]}$ and $Rap1_{[117-352]}$ / $Rap1_{[358-827]}$ mixtures.

B: SDS-PAGE analysis of comparable fractions for each run of SEC.