Electronic Supplementary Material (ESI) for Molecular BioSystems

Supplementary Material for: Structural insights of SIR2rp3 proteins as promising biotargets to fight against Chagas disease and Leishmaniasis

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The present article describes how three-dimensional models of parasitic NAD^+-dependent deacetylases SIR2rp3 were generated, refined and validated through *in silico* techniques. This supplementary document contains figures and tables that aim at supporting the results reported in the main text. Those data were excluded from the main text for concision and clarity of presentation.

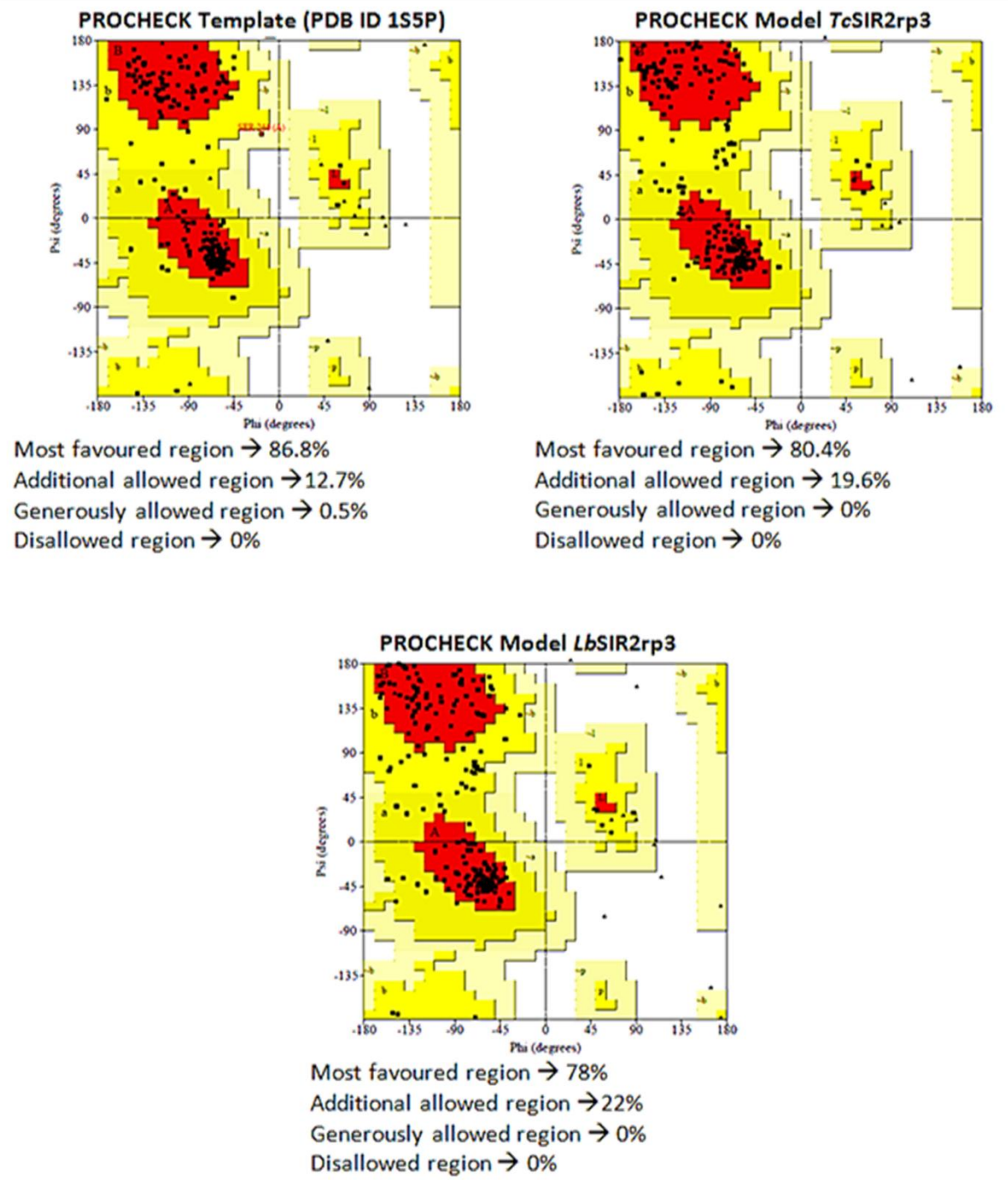

Figure S1. Ramachandran plots of *E. coli* sirtuin (template, PDB code 1S5P), *Tc*SIR2rp3 and *Lb*SIR2rp3 homology models.

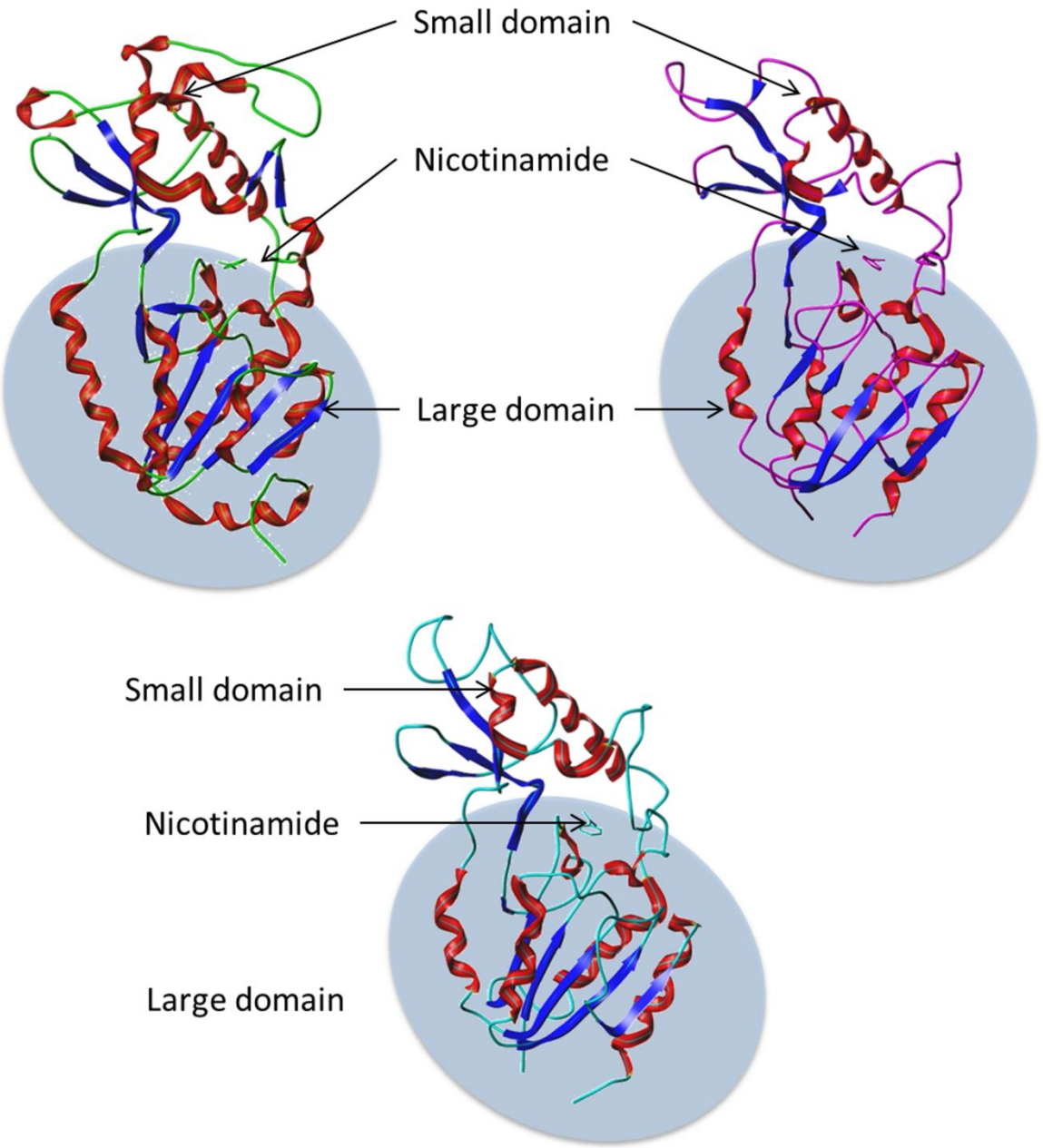

Figure S2. 3D structure of hSIRT5 (green), *Tc*SIR2rp3 (magenta) and *Lb*SIR2rp3 (cyan). Secondary structure elements are highlighted (α helices in red; βsheets in blue). The blue circles enclose the large domain. Small domain and catalytic site are also indicated with black arrows. The crystallographic nicotinamide (PDB code 1YC5) is reported in all proteins, represented as capped sticks.

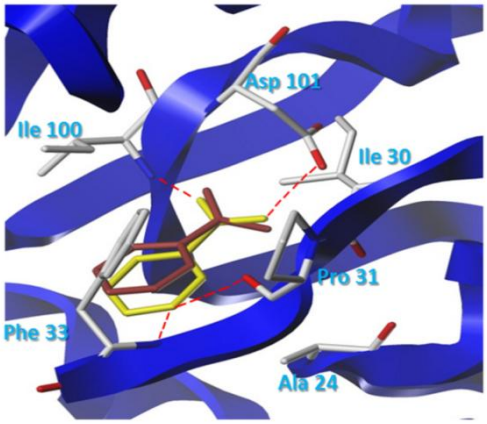

Figure S3. Nicotinamide docked into *T. maritima* (PDB code 1YC5) active pockets. Best-overall poses are represented in brown capped sticks whereas the proteins are represented with blue ribbons. Key amino acids interacting with the ligand are reported in capped sticks, color-coded according to atom types. The crystallographic pose of nicotinamide in *T. maritima* is shown in yellow. Hydrogen bonds are also represented as red dashes.

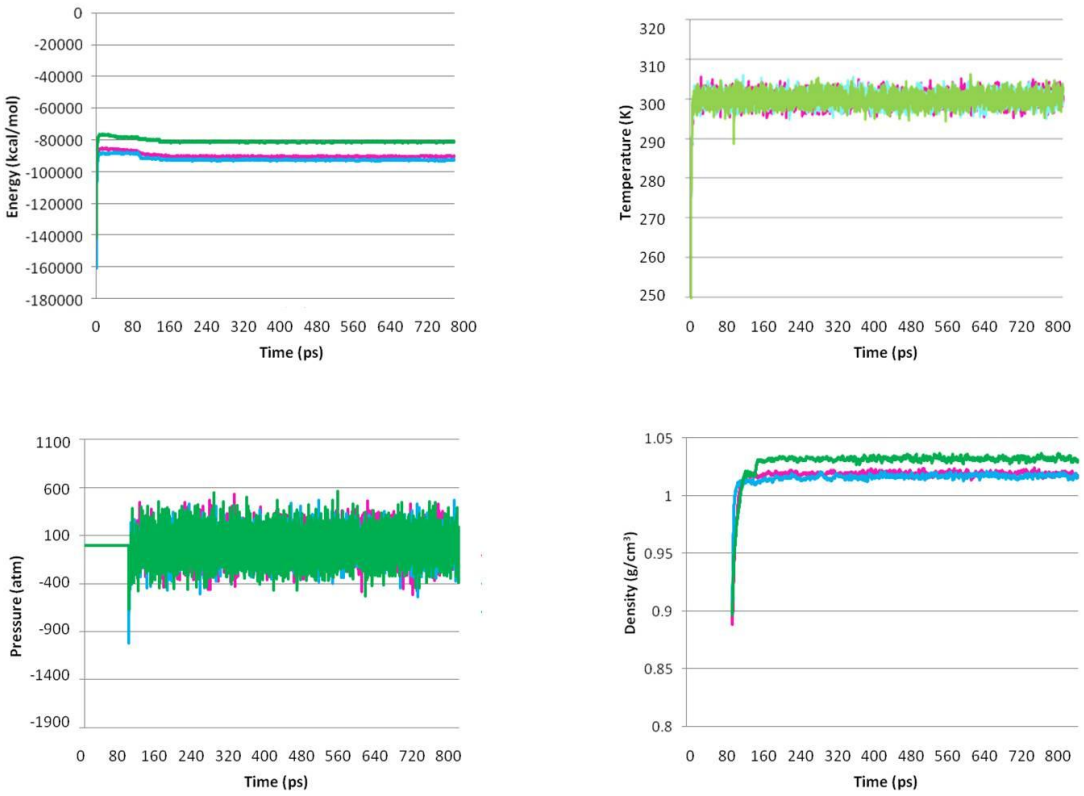

Figure S4. Plots against time for the heating and equilibration phases of the total energies (A), temperature (B), pressure (C) and density (D) in nicotinamide-protein complex simulations. hSIRT5 (green), *Tc*SIR2rp3 (magenta), *Lb*SIR2rp3 (cyan).

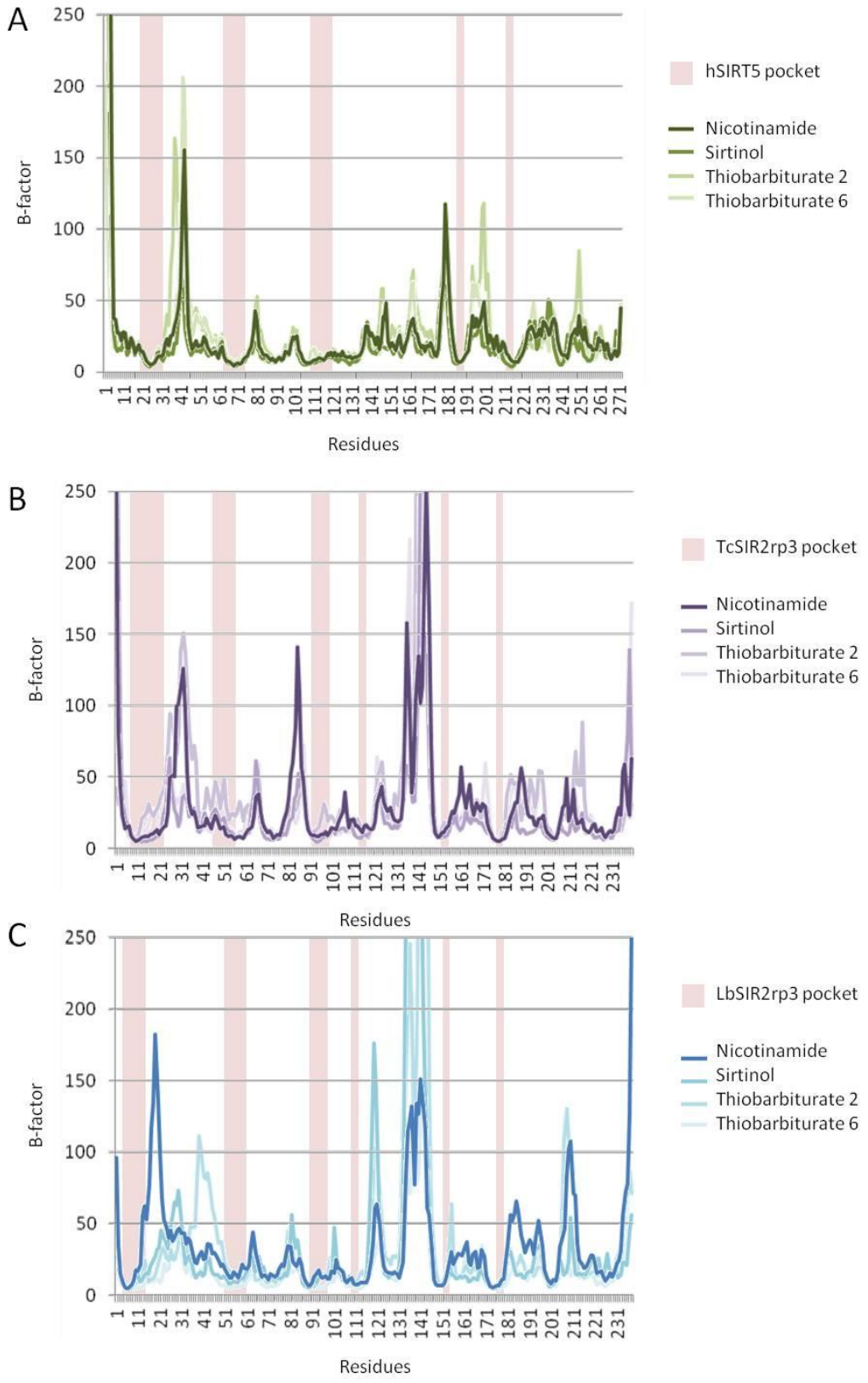

Figure S5. Theoretical B-factors (backbone atoms) of hSIRT5 (A), *Tc*SIR2rp3 (B), *Lb*SIR2rp3 (C) in complex with Nicotinamide, Sirtinol, Thiobarbiturate compound 2 and 6. B-factor values associated to binding site residues are shaded in light pink.

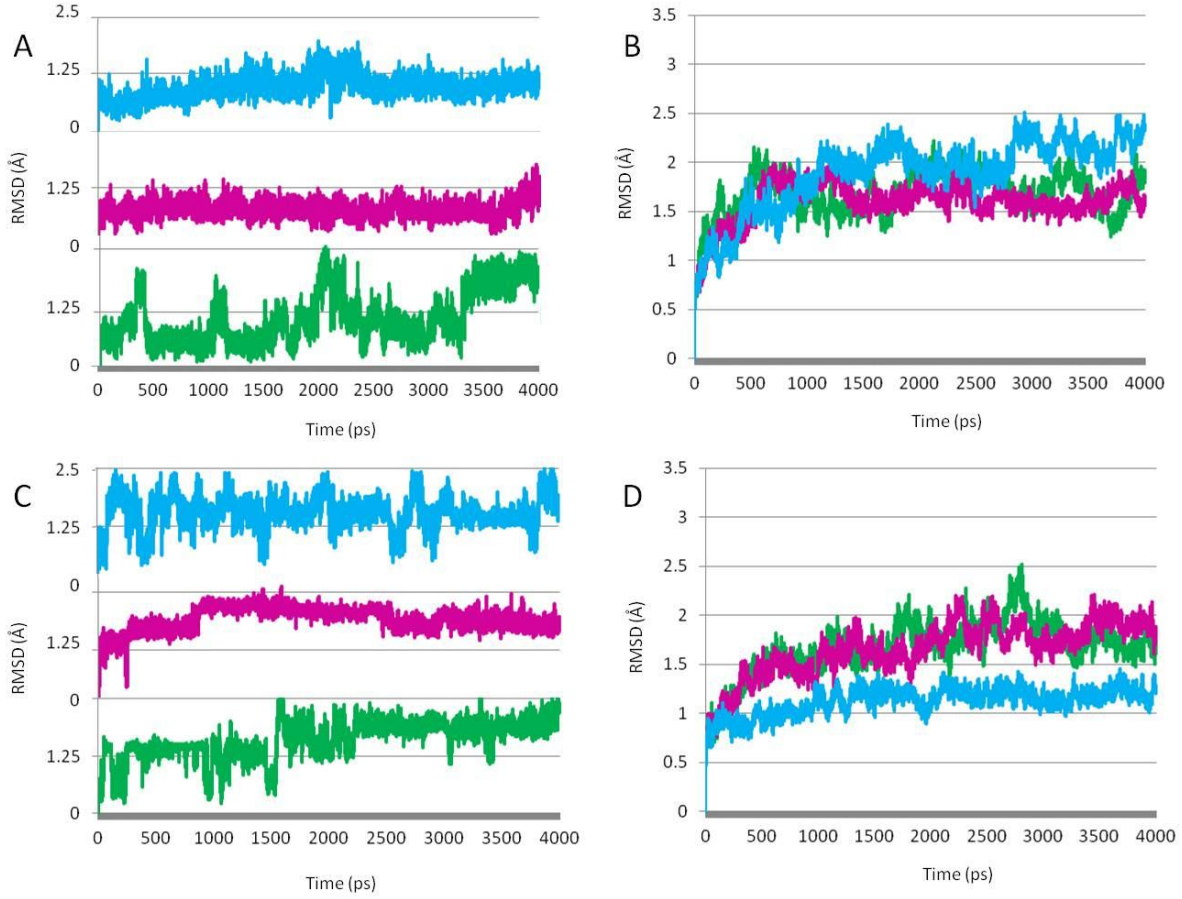

Figure S6. RMSD plots against time calculated for the atoms belonging to: Sirtinol (A); Thiobarbiturate compound 6 (B); backbone atoms of the proteins in complex with Sirtinol (C) and with Thiobarbiturate compound 6 (D). hSIRT5, *Tc*SIR2rp3 and *Lb*SIR2rp3 are colored in green, magenta and cyan, respectively.

Figure S7 Multiple sequence alignment between TcSIR2rp3, LbSIR2rp3, and *Tb*SIR2rp3. Amino acids in a 10 Å sphere radius around the ligand are shaded in red whereas the nicotinamide recognition sequence is shaded in green. Identity between amino acids is marked with an asterisk.

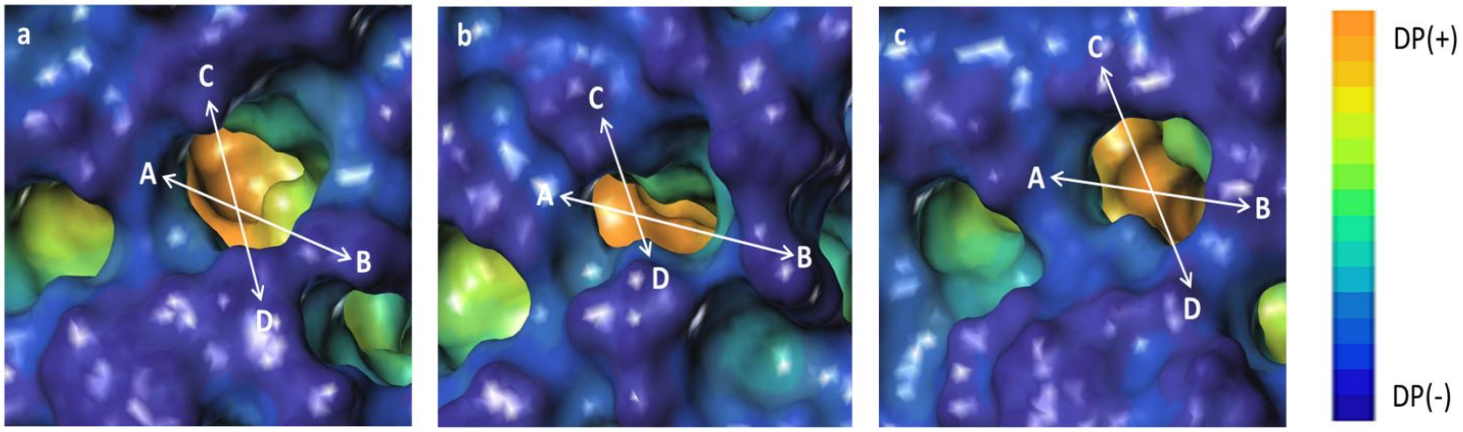

Figure S8. Cavity depth properties calculated on hSIRT5 (a), *Tc*SIR2rp3 (b) and *Lb*SIR2rp3 (c) solvent accessible surfaces. Surfaces are colored from blue (low depth, outside of the protein, DP(-)) to orange (high depth, cavities deep inside the protein, DP(+)). The white arrows delimit the channel rim of each protein, characterized by four regions named A, B, C and D.

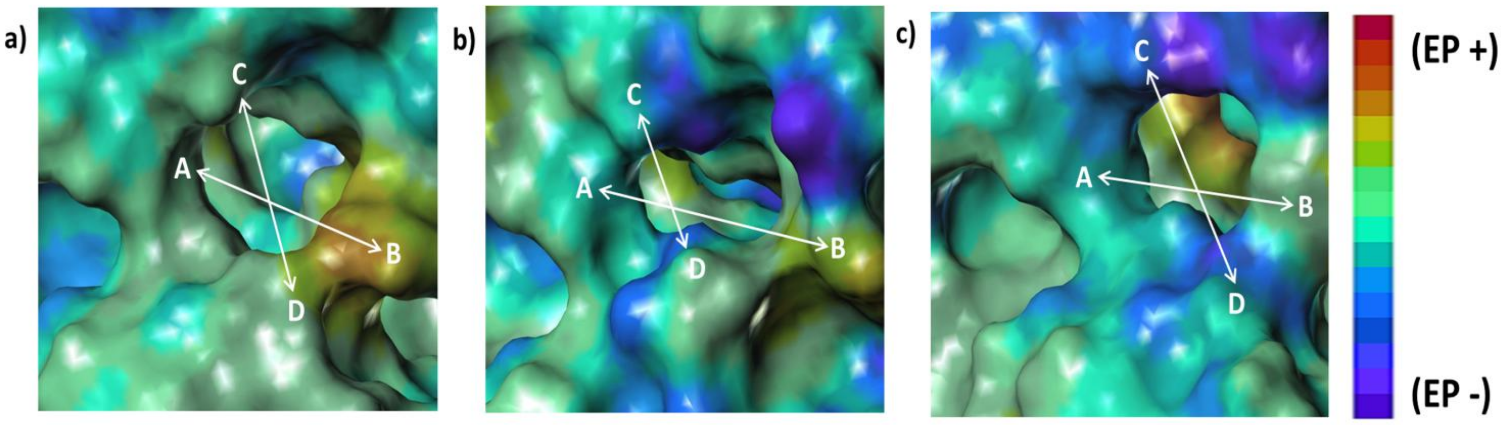

Figure S9. Electropotential calculated on hSIRT5 (a), *Tc*SIR2rp3 (b) and *Lb*SIR2rp3 (c) solvent accessible surfaces. Surfaces are colored from blue (negative region of the protein) to red (Positive region of the protein). The white arrows delimit the channel rim of each protein, characterized by four regions named A, B, C and D.

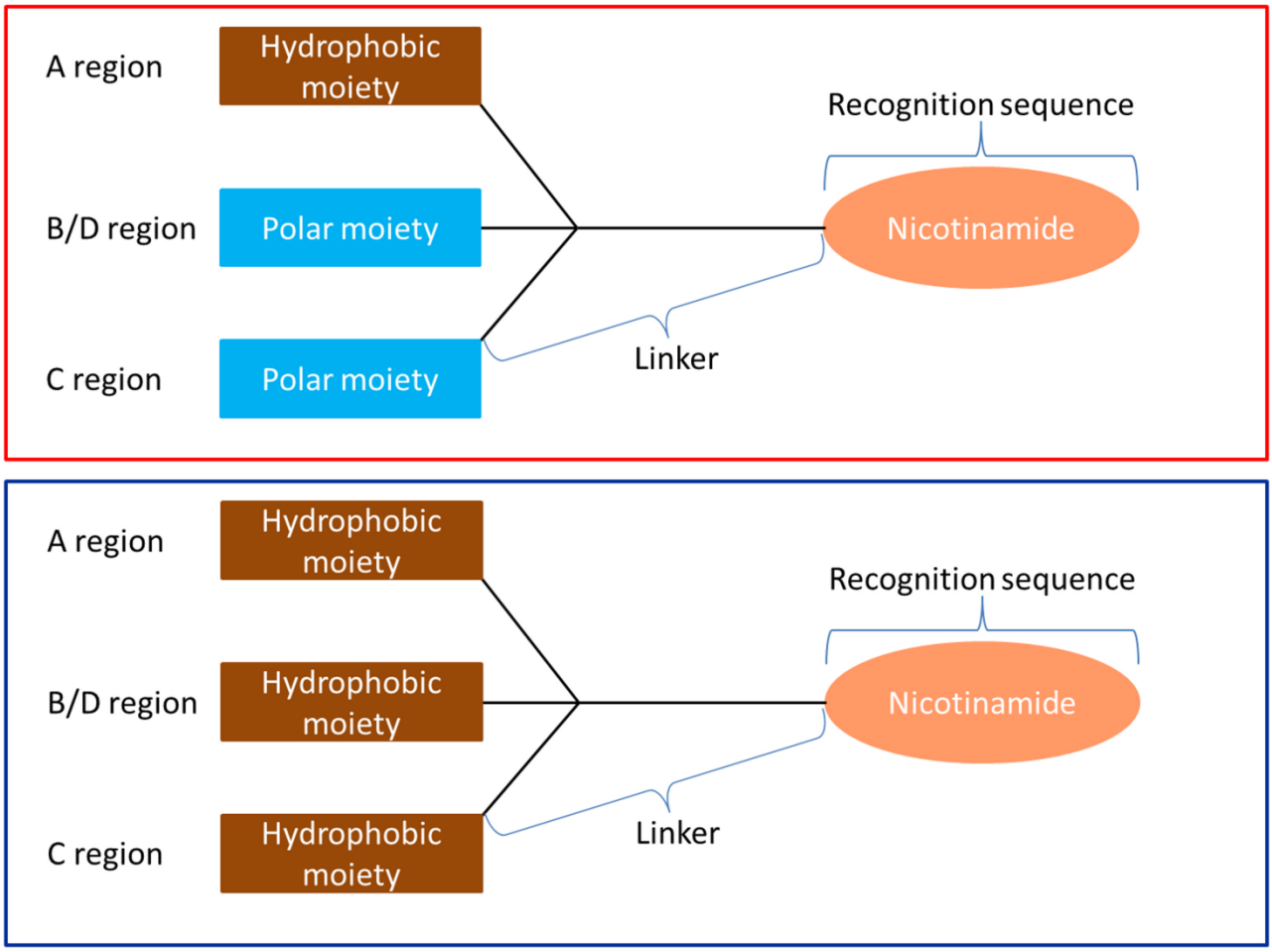

Fig. S10 Qualitative scheme for the design of new selective inhibitors against parasitic SIR2rp3 (red box). Scheme for human SIRT5 is reported in the blue box.

Table S1. Residues from hSIRT5, *Tc*SIR2rp3, *Lb*SIR2rp3 involved in hydrogen bonds and Van der Waals interactions with the docked inhibitors.

Nicotinamide	**hSIRT5**	***Tc*SIR2rp3**	***Lb*SIR2rp3**
Hydrogen bonds	Ile112 Asp113	Val96 Asp97	Val96 Asp95
Van der Waals interactions	Phe40 Ile112 Ala29	Phe24 Val96 Ala13	Phe21 Val94 Ala10
Sirtinol	**hSIRT5**	***Tc*SIR2rp3**	***Lb*SIR2rp3**
Van der Waals interactions	Ile112 Ala29 Phe40 Gly30 Gly219	Val96 Ala13 Phe24 Gly14 Gly180	Val94 Ala10 Phe21 Gly11 Gly178
Thiobarbiturate 2	**hSIRT5**	***Tc*SIR2rp3**	***Lb*SIR2rp3**
Hydrogen bonds	Arg75 Tyr72	Arg59 Tyr56	Arg56 Tyr53
Van der Waals interactions	Phe193 His128	Phe156 His112	Phe154 His110
Thiobarbiturate 6	**hSIRT5**	***Tc*SIR2rp3**	***Lb*SIR2rp3**
Hydrogen bonds	Arg75 Tyr72	Arg59 Tyr56	Arg56 Tyr53
Van der Waals interactions	Phe193 His128	Phe156 His112	Phe154 His110

Table S2. Details on the systems submitted to molecular dynamics MD simulations.

	Solvated system (number of atoms)	42235	39794	35402
		hSIRT5	***Tc*Sir2rp3**	***Lb*SIR2rp3**
	Residues Number	273	241	239
	Added ions	3 Cl-	4 Na+	4 Na+
Nicotinamide - protein complex	**Waters (number of residues)**	12838	11907	9839
	Solvated system (number of atoms)	42666	39476	33212
Sirtinol - protein complex	**Waters (number of residues)**	12970	11979	9768
	Solvated system (number of atoms)	43094	39729	33036
Thiobarbiturate 2 - protein complex	**Waters (number of residues)**	12851	11958	10648
	Solvated system (number of atoms)	42729	39652	35662
Thiobarbiturate 6 - protein complex	**Waters (number of residues)**	12683	12002	10558

Table S3. RMSD values (Å) and standard deviations (in brackets), calculated along 4ns MD simulations.

	hSIRT5	***Tc*SIR2rp3**	***Lb*SIR2rp3**
Nicotinamide (N)	0.2 (0.04)	0.2 (0.03)	0.2 (0.04)
Backbone (N complex)	1.7 (0.3)	1.6 (0.3)	1.6 (0.3)
Sirtinol (S)	1.1 (0.5)	0.8 (0.2)	1.0 (0.2)
Backbone (S complex)	1.7 (0.3)	1.6 (0.3)	1.7 (0.3)
Thiobarbiturate 2 (T2)	0.7 (0.2)	1.0 (0.2)	1.0 (0.2)
Backbone (T2 complex)	2.0 (0.5)	2.0 (0.5)	1.9 (0.4)
Thiobarbiturate 6 (T6)	1.6 (0.4)	1.9 (0.3)	1.6 (0.4)
Backbone (T6 complex)	1.7 (0.3)	1.7 (0.3)	1.1 (0.1)

Table S4. Identity and similarity between the template (PDB code 1S5P), hSIRT5, the modelled *Tc*SIR2rp3, *Lb*SIR2rp3 and *Tb*SIR2rp3 sequences and the parasitic sequences encoded by the SIR2rp1 genes.

		Identity (%)	**Similarity (%)**
1S5P	***Tc*SIR2rp3**	59	72
	***Lb*SIR2rp3**	57	73
hSIRT5	***Tc*SIR2rp3**	35	50
	***Lb*SIR2rp3**	36	53
***Tc*SIR2rp3**	**1S5P**	27	45
	hSIRT5	32	45
	***Tc*SIR2rp3**	28	43
***Lb*SIR2rp1**	**1S5P**	26	37
	hSIRT5	29	40
	***Lb*SIR2rp3**	27	37
***Tb*SIR2rp3**	**hSIRT5**	40	51
	***Tc*SIR2rp3**	64	78
	***Lb*SIR2rp3**	58	74

Table S5. Summary of the residues characterizing the A-C pockets of hSIRT5, *Tc*SIR2rp3, *Lb*SIR2rp3 and *Tb*SIR2rp3. Residues potentially interacting with nicotinamide, also used in MD analyses, are reported.

	hSIRT5	***Tc*SIR2rp3**	***Lb*SIR2rp3**	***Tb*SIR2rp3**
Pocket A	Gly60 Arg71 Gly249 Ala272 Glu273 Phe274 Cys293 Gly294	Gly15 Arg26 Gly181 Leu204 Glu205 Leu206 Ala225 Ser226	Gly15 Arg25 Gly181 Leu204 Glu205 Leu206 Ala225 Ser226	Gly15 Arg26 Gly181 Leu204 Glu205 Leu206 Ala225 Ser226
Pocket B	His158 Gln140	His113 Gln95	His 113 Gln95	His113 Gln95
Pocket C	Gly58 Ala59 Gly60 Val61 Ser62 Gly66 Val67 Pro68 Thr69 Phe70 His118 Thr139 Gln140 Asn141 Ile142 Asp143 Leu145	Gly13 Ala14 Gly15 Ile16 Ser17 Gly21 Leu22 Ser23 Thr24 Phe25 His75 Thr94 Gln95 Asn96 Val97 Asp98 Leu100	Gly12 Ala13 Gly14 Ile15 Ser16 Gly20 Ile21 Pro22 Thr23 Phe24 His74 Thr94 Gln95 Asn96 Val97 Asp98 Leu100	Gly13 Ala14 Gly15 Ile16 Ser17 Gly21 Ile22 Ser23 Thr24 Phe25 His75 Thr94 Gln95 Asn96 Val97 Asp98 Leu100
Amino acid residues 6 Å around nicotinamide	Gly58 Ala59 Ser62 Ala63 Val67 Pro68 Thr69 Phe70 Arg105 Gln140 Asn141 Ile142 Asp143 Glu144 Leu145 Leu161	Gly13 Ala14 Ser17 Ala18 Leu22 Ser23 Thr24 Phe25 Arg60 Gln95 Asn96 Val97 Asp98 Asn99 Leu100 Leu116	Gly12 Ala13 Ser16 Ala17 Ile21 Pro22 Thr23 Phe24 Arg59 Gln95 Asn96 Val97 Asp98 Asp99 Leu100 Leu116	Gly13 Ala14 Ser17 Ala18 Ile22 Ser23 Thr24 Phe25 Arg60 Gln95 Asn96 Val97 Asp98 Asp99 Leu100 Leu116

Table S6. Definition of the A-D areas characterizing the catalytic rims of hSIRT5, *Tc*SIR2rp3 and *Lb*SIR2rp3.

	hSIRT5	***Tc*SIR2rp3**	***Lb*SIR2rp3**
Area A **Area B**	Cζ Phe223 Cζ Arg71	Cζ Phe157 Cζ Arg26	Cζ Phe157 Cζ Arg25
Distance Area A - Area B (Å)	10.7	11.7	10.7
Area C **Area D**	Cγ Gln83 Cγ2 Val253	Cγ Glu38 Oδ1 Asn185	Cγ Glu37 Oδ1 Asp185
Distance Area C – Area D (Å)	8.5	6.0	8.9

Table S7. Amino acids belonging to the four regions (A-D) of the rim channel of hSIRT5, *Tc*SIR2rp3 and *Lb*SIR2rp3 proteins. Punctual differences are highlighted in bold.

	hSIRT5	***Tc*SIR2rp3**	***Lb*SIR2rp3**
A	His158 Val220 Val221 Phe223 **Leu227** Val254 Pro256	His113 Ile154 Val155 Phe157 Pro161 Val186 Pro188	His113 Ile154 Val155 Phe157 Pro161 Val186 Pro188
B	Thr69 Arg71 **Gly72** Ser251 Glu277	Thr24 Arg26 Asp27 Ser183 **Asp209**	Thr23 Arg25 Asp26 Ser183 Gln209
C	**Gly72** **Ala73** **Gln81** **Gln38**	Asp27 **Lys28** Arg36 Glu38	Asp26 **Gly27** Arg36 Glu38
D	Ser251 **Val253**	Ser183 Asn185	Ser183 Asn185