Electronic Supplementary Material (ESI) for Molecular BioSystems.

SUPPORTING INFORMATION

Title

Selectivity hot-spots of sirtuin catalytic cores

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Table S1. Three-dimensional structures of human sirtuins available from public domain (Protein Data Bank)

Sirtuin	Source	Code	Ligand(s)	Notes
SIRT1	PDB	4I5I	NAD and EX-527 analog	
		4IF6	ADPR	
		4IG9	-	
		4KXQ	ADPR	
SIRT2	PDB	1J8F	-	
		3ZGO	-	
		3ZGV	ADPR	
		4L3O	Cyclic peptide S2iL5	
SIRT3	PDB	3GLT	ADPR bound to AceCS2 peptide	
		3GLS	-	
		3GLR	acetyl-lysine AceCS2 peptide	
		3GLU	AceCS2 peptide	
		4FVT	AceACS peptide and Carba-NAD	
		4HD8	Fluor-de-Lys peptide and piceatannol	
		4FZ3	Acetyl-P53 peptide coupled with MCM	
		4JSR	Inhibitor 11c	
		4JT9	Inhibitor 3	
		4JT8	Inhibitor 28	
		4BN4	ADPR	
		4BN5	SRT1720 and Carba-NAD	
		4BV3	NAD/ADPR and EX-527	
		4BVB	ADPR and EX-527	
		4BVE	Thioalkylimidate formed from thio-acetyl-lysine acs2-peptide	
		4BVF	Thioalkylimidate formed from thio-acetyl-lysine acs2-peptide	
		4BVG	alkylimidate formed from acetyl-lysine acs2-peptide	
		4BVH	2-O-acetyl-ADPR and EX-527	
		4C7B	Bromo-Resveratrol and Fluor-De-Lys peptide	
		4C78	Bromo-Resveratrol and AceCS2 peptide	
SIRT4	SwissModel	Q9Y6E7	-	Based on Sir2 A. Fulgidus (1S7G), identity 31%
SIRT5	PDB	2B4Y	ADPR	
		2NYR	Suramin	
		3RIG	Thioacetyl peptide	
		3RIY	Succinylpeptide and NAD	
		4F4U	Succinylpeptide	
		4F56	H3K9 thiosuccinyl peptide bound with NAD	
		4G1C	Succinylpeptide and Carba-NAD	
		4HDA	Fluor-de-Lys peptide and resveratrol	
SIRT6	PDB	3K35	ADPR	
		3PKI	ADPR	
		3PKJ	2'-N-Acetyl ADPR	
		3ZG6	H3K9 myristoyl peptide and ADPR	
SIRT7	SwissModel	Q9NRC8	-	Based on Sirt6 template (3K35), identity 42%

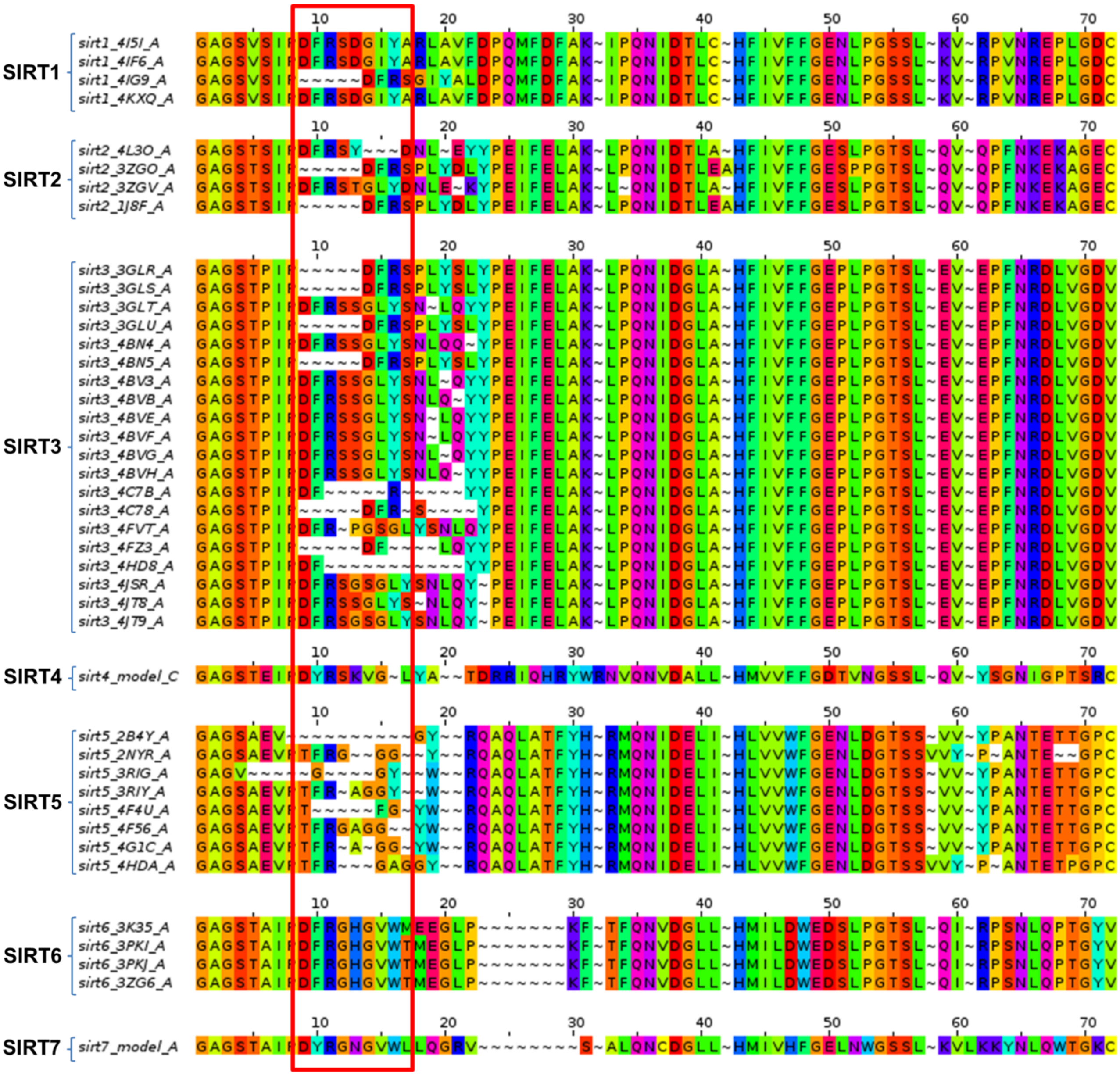
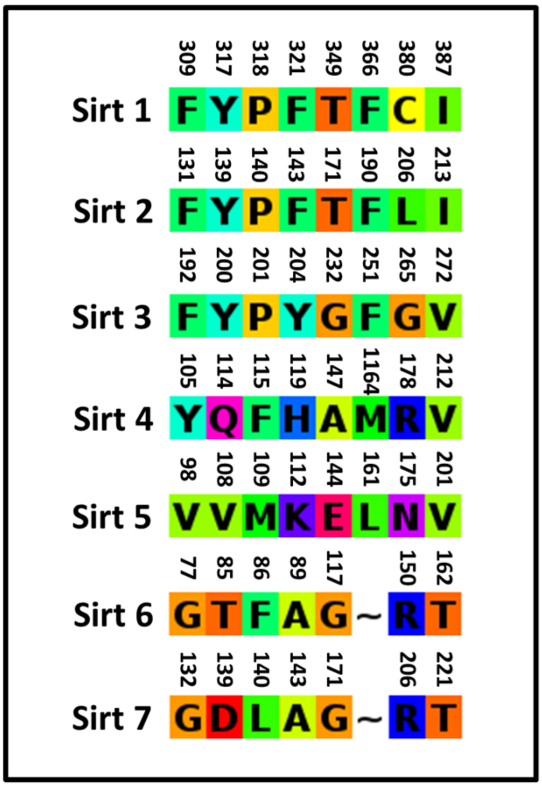


Figure S1. Structural alignment of available three-dimensional structures of human sirtuins. Residues are color-coded using Taylor classification. The red box highlights the area of the cofactor binding loop.

	Sirt 1	Sirt 2	Sirt 3	Sirt 4	Sirt 5	Sirt 6
Sirt 2	V266/T89 Q294/E116 R446/Q267					
Sirt 3	Q294/E177 C482/V366 R446/E325 V266/T150	C324/V366 Q267/E325				
Sirt 4	V266/T67 I279/G80 F273/Y74 E467/G288 R466/I287 D481/R303 F312/R104 Q294/R88 P293/R87 I316/N109 F297/Q91 R446/Y266 ~/R108	L103/G80 F96/Y74 E288/G288 K287/I287 E323/R303 L134/R104 E116/R88 P115/R87 ~/R108 L138/N109 F119/Q91 Q267/Y266	L164/G80 F157/Y74 D346/G288 R345/I287 V366/C304 D365/R303 L195/R104 E177/R88 P176/R87 ~/R108 L199/N109 F180/Q91 E325/Y266			
Sirt 5	I279/G75 R466/I287 D481/P292 I316/R105 R446/Y255	L103/G75 K287/T276 E323/P292 T89/A63 E116/Q83 ~/M109 L138/R105 Q267/Y255	L164/G75 R345/T276 V366/C293 D365/P292 T150/A63 E177/Q83 ~/M109 L199/R105 E325/Y255	Y74/F70 G288/E277 I287/T276 R303/P292 T67/A63 R88/Q83 ~/M109 N109/R105 R104/F101 R87/A82 R108/~ A86/Q91		
Sirt 6	E467/Q242 R466/L241 C482/V258 D481/Y257 V266/T57 F312/~ Q294/~ P293/~ ~/F82 ~/F86 I316/~ F297/~	E288/Q242 K287/L241 C324/V258 E323/Y257 L134/~ E116/~ P115/~ ~/F82 ~/F86 L138/~ F119/~ Q267/R220	D346/Q242 R345/L241 D365/Y257 L195/~ E177/~ P176/~ ~/F82 ~/F86 L199/~ F180/~ E325/R220	G288/Q242 R303/Y257 C304/V258 Y74/F64 G80/V70 R104/~ R88/~ R87/~ N109/~ Q91/~ R108/~ ~/F82 ~/F86 Y266/R220	E277/Q242 P292/Y257 C293/V258 G75/V70 A63/T57 F101/~ Q83/~ A82/~ R105/~ A86/~ ~/F82 M109/F86 Y255/R220	
Sirt 7	I347/C169 F273/Y119 E467/Q299 R466/L298 D481/K314 V266/T112 F312/~ ~/S136 ~/L140 I316/~ F297/~ ~/L274 Q294/~ P293/~	I169/C169 F96/Y119 E288/Q299 K287/L298 E323/K314 L134/~ E116/~ P115/~ ~/S136 ~/L140 L138/~ F119/~ ~/L274 Q267/K275	I230/C169 F157/Y119 D346/Q299 R345/L298 D365/K314 V366/C315 L195/~ P176/~ E177/~ L199/~ F180/~ ~/S136 ~/L140 ~/L274 I230/C169 E325/K275	V145/C169 G80/V125 G288/Q299 I287/L298 R303/K314 R104/~ R87/~ R88/~ N109/~ Q91/~ ~/S136 ~/L140 ~/L274 V145/C169 Y266/K275	I142/C169 V70/Y119 E277/Q299 T276/L298 P292/K314 G75/V125 A63/T112 F101/~ F82/~ Q83/~ R105/~ A86/~ ~/S136 ~/L274 Y255/K275	V115/C169 F64/Y119 Y257/K314 V258/C315 F82/S136 ~/L274

Figure S2. Selectivity hot-spots table. Each cell reports the aminoacidic differences between a couple of sirtuins that are supposed to be important for selectivity. For each difference, the first residue refers to the sirtuin in the row and the second refers to the sirtuin in the column.



	309	317	318	321	349	366	380	387
Sirt 1	F	Y	P	F	T	F	C	I
	131	139	140	143	171	190	206	213
Sirt 2	F	Y	P	F	T	F	L	I
	192	200	201	204	232	251	265	272
Sirt 3	F	Y	P	Y	G	F	G	V
	105	114	115	119	147	1164	178	212
Sirt 4	Y	Q	F	H	A	M	R	V
	98	108	109	112	144	161	175	201
Sirt 5	V	V	M	K	E	L	N	V
	77	85	86	89	117		150	162
Sirt 6	G	T	F	A	G	~	R	T
	132	139	140	143	171		206	221
Sirt 7	G	D	L	A	G	~	R	T

Figure S3. Structural alignment of selectivity pocket induced by SirReal2 ligand binding. Derived from structural alignment published by Rumpf et al. (Nat Commun. 2015 Feb 12;6:6263). The alignments were generated using residues in contact with ligand, excluded those already reported in Figure 4 in the main text. The residues were color-coded using Taylor classification and numbered following gene sequences taken from the Uniprot database.