

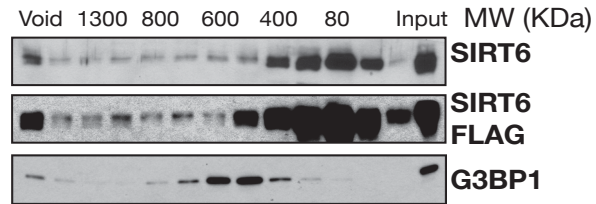
Supplementary Information

Proteomic analysis of the SIRT6 interactome: novel links to genome maintenance and cellular stress signaling

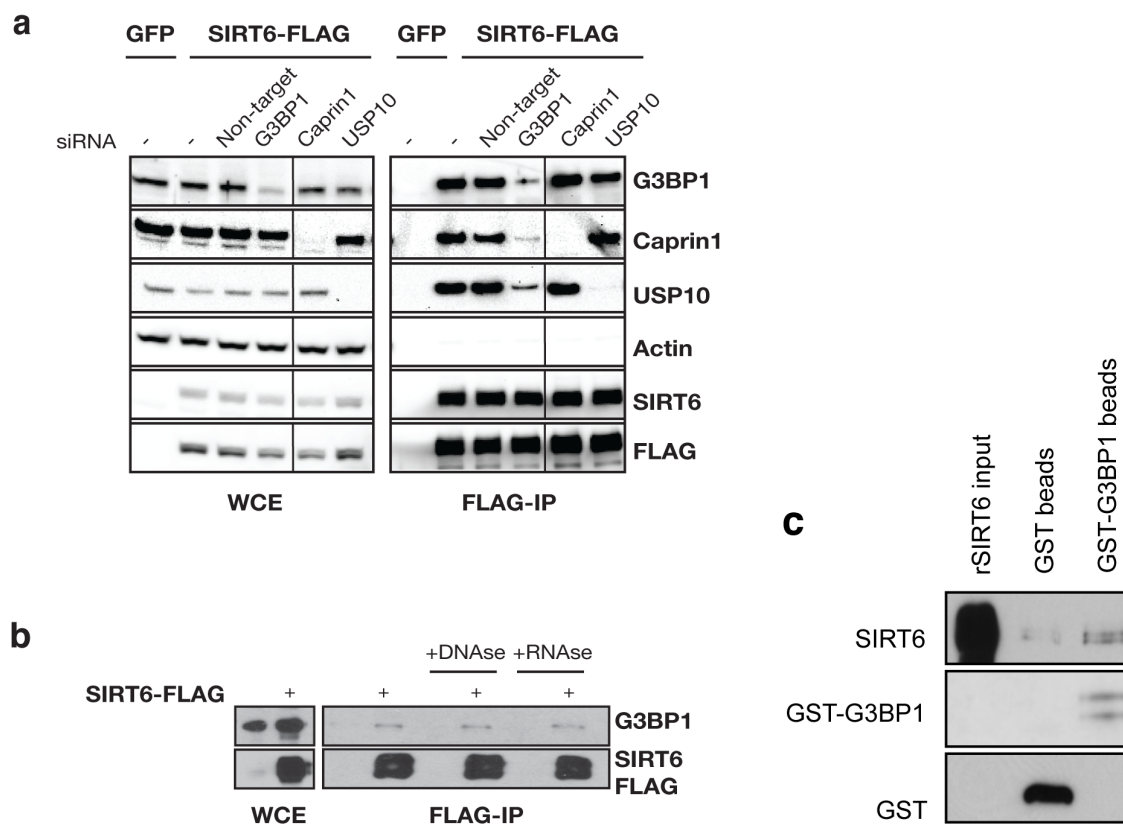
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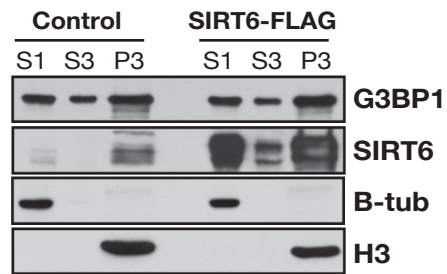
Chua^{1,2,*}



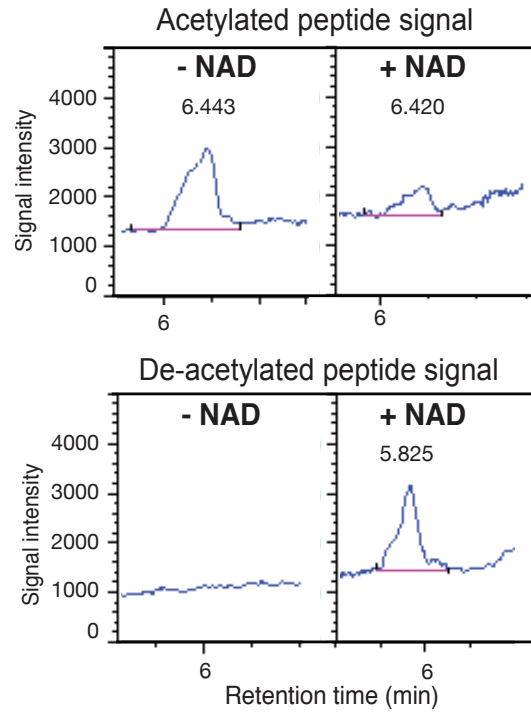
Supplementary figure 1. Elution profiles of endogenous SIRT6, SIRT6-FLAG, and endogenous G3BP1 in gel filtration fractions (Superose 6) of nuclear extracts. Endogenous SIRT6 and SIRT6-FLAG from HeLa S3 nuclear extract show the same elution profile. A subset of SIRT6 co-fractionates with the G3BP1 peak at 600kDa. Alternate (0.5 ml) fractions between void volume and 40 KDa were collected and analyzed. Nuclear extract from HeLa S3 cells were used for detection of endogenous SIRT6, while nuclear extract from HeLa S3 cells stably expressing SIRT6-FLAG were used for detection of SIRT6-FLAG and G3BP1 by western blot.



Supplementary Figure 2. Biochemical characterization of SIRT6-G3BP1 interaction. **(a)** 293T cells were transfected with siRNAs targeting G3BP1, Caprin1, USP10, or control sequence, and after 24 hrs, with FLAG-tagged human SIRT6 or GFP control. 48 hrs after plasmid transfection, FLAG-tagged proteins were immunoprecipitated and analyzed by western blot for G3BP1, Caprin1, USP10, SIRT6 and actin. **(b)** Nuclear extract lysates from HeLa S3 cells stably expressing SIRT6-FLAG or empty vector control were incubated with DNase and RNase, immunoprecipitated and analyzed for the presence of G3BP1 and FLAG by western blot. **(c)** In vitro pull-down assays of recombinant purified SIRT6 by purified GST-SIRT6 or GST control proteins. Lines indicate cropped parts of the same gel, where irrelevant lanes were eliminated.



Supplementary Figure 3. G3BP1 is a chromatin bound protein. HeLa S3 cells stably expressing SIRT6-FLAG or empty vector control were biochemically fractionated as previously described (7). Fractions: S1, cytoplasm; S3, soluble nuclear; P3, chromatin enriched proteins.



Supplementary Figure 4. SIRT6 deacetylates G3BP1(K376Ac) peptide *in vitro*. (a) Recombinant SIRT6 was incubated in presence or absence of NAD⁺ with the G3BP1-(K376Ac) peptide. Retention time of the acetylated and deacetylated G3BP1 peptide are respectively 6.4 and 5.8 min.

Supplementary Table 1. Protein enrichment by Gel Filtration Chromatography (GFC) followed by SIRT6-FLAG pulldown. Comparison of PSMs (peptide-spectrum matches) of proteins identified in SIRT6-FLAG versus Negative Control (NC) pulldown. From 2 independent experiments (1st and 2nd MS analysis), in total, 85 proteins were reproducibly enriched in SIRT6-Flag IPs from the same fractions of Gel Filtration Chromatography (size range: 1300-800 KDa, 800-600 KDa, 600-400 KDa).

Reproducibility of Gel Filtration Chromatography (GFC) samples (1st and 2nd MS analyses)

1st MS analysis

Peptide-spectrum match (PSM)

Experiment A GFC			S6		
NC			S6		
1300_800kDa	800_600kDa	600_400kDa	1300_800kDa	800_600kDa	600_400kDa

reference

Gene symbol

reference	Gene symbol	1300_800kDa	800_600kDa	600_400kDa	S6 1300_800kDa	S6 800_600kDa	S6 600_400kDa
Q8N6T7	SIRT6	7		3	397	452	713
P07355	ANXA2	10	2	52	25	40	96
P61978	HNRNPK		3	38	26	36	75
P13639	EEF2			2		5	22
Q86UV5	USP48			2			14
Q96HC4	PDLIM5						13
P68104	EEF1A1	13	13	57	70	91	135
P11142	HSPA8	2	3	11	61	60	50
P49005	POLD2					18	34
P04406	GAPDH	5	2	3	4	18	6
P54652	HSPA2			3	13	15	14
P34931	HSPA1L	2	2	8	12	15	17
Q9BR76	CORO1B			3			14
P06748	NPM1			3	12	23	29
Q71U36	TUBA1A	3	1	3	74	63	57
Q13885	TUBB2A			3	56	55	64
P43246	MSH2			10	2	23	53
P06702	S100A9	2	2		4	33	
Q16531	DDB1		1	3	10	45	33
Q15054	POLD3					5	12
Q13283	G3BP1				12	23	5
P43243	MATR3		3	3	33	63	37
P26641	EEF1G	21	15	7	75	29	20
P52701	MSH6			2	8	33	44
Q14444	CAPRN1				16	11	
O14744	PRMT5	13	68	27	97	221	67
P02545	LMNA	20	2	1	122	41	5
P12814	ACTN1		8		26	122	6
P15924	DSP	53	4	9	12	21	1
P53396	ACLY			16	2	82	96

P78347	GTF2I	6	10		77	40	5
Q93009	USP7		4	27	9	49	41
O00267	SUPT5H					17	40
Q5VTR2	RNF20				34	8	
P12268	IMPDH2			2		7	34
Q14839	CHD4	3			33	2	
Q8N163	KIAA1967				30		
Q13263	TRIM28				29	7	
O75150	RNF40				28	9	
P05109	S100A8	3	4	2	5	20	4
P50990	CCT8				7	12	
Q14694	USP10				1	10	
O95239	KIF4A				12	22	
O75717	WDHD1				21	3	
Q8WX93	PALLD			3		3	18
Q15021	NCAPD2				17	3	
P47756	CAPZB				17	10	
Q7Z5K2	WAPAL				16	14	
Q15029	EFTUD2				16	3	
Q14258	TRIM25					15	11
P62333	PSMC6				14		
P17987	TCP1				4	13	
P52907	CAPZA1				12	6	
Q15020	SART3				12		
P12035	KRT3	5	1			7	
Q4G0J3	LARP7				10		
P40222	TXLNA				9	1	
P48643	CCT5					7	
Q9BTA9	WAC				6		
O60506	SYNCRIP				6	10	5
P31943	HNRNPH1	3	10	13	18	28	22
P78527	PRKDC	10			456	140	14
P21333	FLNA	33	3		258	39	5
O75369	FLNB	22			244	9	
Q09028	RBBP4	5	2	3	22	4	
Q92769	HDAC2	4			20	5	2
O94776	MTA2	2			30	5	1
Q14683	SMC1A				56	4	
O95347	SMC2				41	3	
Q9NTJ3	SMC4				48	7	

Q9UQE7	SMC3	46	2	
Q29RF7	PDS5A	9	9	2
Q9Y265	RUVBL1	43	34	7
Q9Y230	RUVBL2	64	38	16
O75643	SNRNP200	31	8	2
Q13428	TCOF1	6	5	
Q16576	RBBP7	31	8	2
O95071	UBR5	10		
O60216	RAD21	11	1	
P30260	CDC27	17		
Q8WXI9	GATAD2B	16		
Q9UJX3	ANAPC7	29	7	1
Q13547	HDAC1	25	8	
Q6P2Q9	PRPF8	26	2	
Q92878	RAD50	14		
Q9BZK7	TBL1XR1	13		
Q9H1A4	ANAPC1	20		

2nd MS analysis

Peptide-spectrum match (PSM)

Experiment B GFC					
NC			S6		
1300_800kDa	800_600kDa	600_400kDa	1300_800kDa	800_600kDa	600_400kDa

reference	Gene symbol							
Q8N6T7	SIRT6	6			324	259	363	
P07355	ANXA2	10	3	6	21	27	26	
P61978	HNRNPK				18	11	30	
P13639	EEF2					2	23	
Q86UV5	USP48						10	
Q96HC4	PDLIM5						6	
P68104	EEF1A1	7	6		40	37	36	
P11142	HSPA8				15	12	22	
P49005	POLD2					2	37	
P04406	GAPDH	6		3	1	15	12	
P54652	HSPA2				5	8	8	
P34931	HSPA1L				8	3	3	
Q9BR76	CORO1B						10	
P06748	NPM1				8	18	20	
Q71U36	TUBA1A				3	10	4	
Q13885	TUBB2A				5	4	6	

P43246	MSH2				4	10	19
P06702	S100A9	17	3	5	9	49	13
Q16531	DDB1				8	10	7
Q15054	POLD3						22
Q13283	G3BP1				16	40	30
P43243	MATR3				16	54	94
P26641	EEF1G	2	1		45	13	7
P52701	MSH6				4	5	22
Q14444	CAPRIN1				15	28	5
O14744	PRMT5	4	9		32	46	17
P02545	LMNA	4			108	18	1
P12814	ACTN1				3	17	
P15924	DSP	109	44	39	51	112	63
P53396	ACLY					18	26
P78347	GTF2I				28	17	
Q93009	USP7				6	11	23
O00267	SUPT5H						12
Q5VTR2	RNF20				38	6	
P12268	IMPDH2					2	7
Q14839	CHD4				9		
Q8N163	KIAA1967				30		
Q13263	TRIM28				16	7	
O75150	RNF40				20		
P05109	S100A8	17	3	4	5	25	13
P50990	CCT8				6	25	2
Q14694	USP10				3	24	7
O95239	KIF4A				9	10	
O75717	WDHD1				9	2	
Q8WX93	PALLD						9
Q15021	NCAPD2				17		
P47756	CAPZB				6		
Q7Z5K2	WAPAL				12		
Q15029	EFTUD2				11		
Q14258	TRIM25				4	15	5
P62333	PSMC6				7		
P17987	TCP1				1	12	
P52907	CAPZA1				9	2	
Q15020	SART3				10		
P12035	KRT3	7			5	10	3
Q4G0J3	LARP7				9		

P40222	TXLNA		7		
P48643	CCT5		1	7	1
Q9BTA9	WAC		7		
O60506	SYNCRIP		6	4	1
P31943	HNRNPH1		12	5	15
P78527	PRKDC		81	70	80
P21333	FLNA	48	161	8	
O75369	FLNB		125		
Q09028	RBBP4		8	2	
Q92769	HDAC2		7		
O94776	MTA2		26		
Q14683	SMC1A		57	9	
O95347	SMC2		60	5	
Q9NTJ3	SMC4		58	6	
Q9UQE7	SMC3		53	3	
Q29RF7	PDS5A		37	2	
Q9Y265	RUVBL1		21	7	4
Q9Y230	RUVBL2		13	5	2
O75643	SNRNP200		17		
Q13428	TCOF1		13		
Q16576	RBBP7		13		
O95071	UBR5		12		
O60216	RAD21		11		
P30260	CDC27		11		
Q8WXI9	GATAD2B		11		
Q9UJX3	ANAPC7		11		
Q13547	HDAC1		10		
Q6P2Q9	PRPF8		8		
Q92878	RAD50		7		
Q9BZK7	TBL1XR1		6		
Q9H1A4	ANAPC1		6		

Supplementary Table 2. SIRT6-FLAG interacting proteins in the three size ranges (1300-800 KDa, 800-600 KDa, 600-400 KDa) of Gel Filtration Chromatography. Gene Ontology (GO) was performed using DAVID, categories Panther_BP_all. The human genome was used as background.

List of SIRT6 Interactors enriched in specific fractions after Gel Filtration Chromatography.

1300-800KDa	800-600KDa	600-400KDa
HNRNPK	HNRNPK	EEF1A1
EEF1A1	EEF1A1	HSPA8
HSPA8	HSPA8	NPM1
HSPA1L	NPM1	DDB1
NPM1	DDB1	MATR3
DDB1	G3BP1	PRMT5
G3BP1	MATR3	PRKDC
MATR3	CAPRIN1	HSPA2
EEF1G	PRMT5	MSH2
CAPRIN1	LMNA	ACLY
PRMT5	GTF2I	TUBB2A
LMNA	USP7	MSH6
GTF2I	RNF20	POLD2
USP7	TRIM28	SUPT5H
RNF20	CCT8	IMPDH2
CHD4	KIF4A	EEF2
KIAA1967	PRKDC	USP48
TRIM28	FLNA	PDLIM5
RNF40	SMC4	CORO1B
CCT8	RUVBL1	POLD3
KIF4A	HSPA2	PALLD
WDHD1	TUBA1A	
NCAPD2	ACTN1	
CAPZB	ANXA2	
WAPAL	GAPDH	
EFTUD2	MSH2	
PSMC6	S100A9	
CAPZA1	DSP	
SART3	ACLY	
LARP7	S100A8	
TXLNA	USP10	
WAC	TRIM25	
SYNCRIP	TCP1	
HNRNPH1	KRT3	
PRKDC	CCT5	
FLNA		
FLNB		
RBBP4		
HDAC2		
MTA2		
SMC1A		
SMC2		
SMC4		
SMC3		
PDS5A		
RUVBL1		
RUVBL2		

List of SIRT6 Interactors enriched in specific fractions after Gel Filtration Chromatography.

SNRNP200
TCOF1
RBBP7
UBR5
RAD21
CDC27
GATAD2B
ANAPC7
HDAC1
PRPF8
RAD50
TBL1XR1
ANAPC1