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Supplemental Information

JNK Phosphorylates SIRT6 to Stimulate DNA Double-Strand Break Repair in Response to Oxidative Stress by Recruiting PARP1 to DNA Breaks

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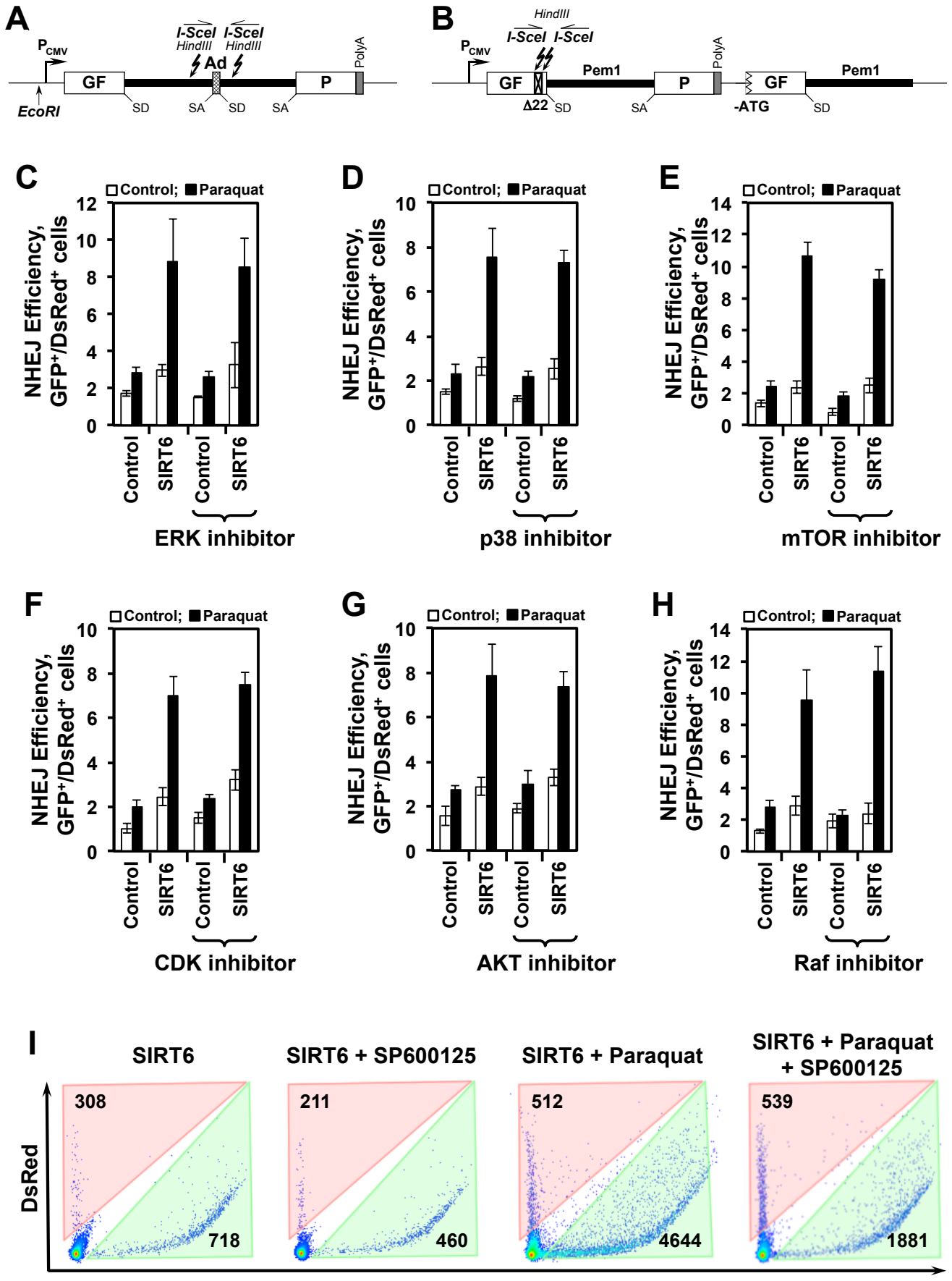


Figure S1. Related to Figure 1

Figure S1, related to Figure 1

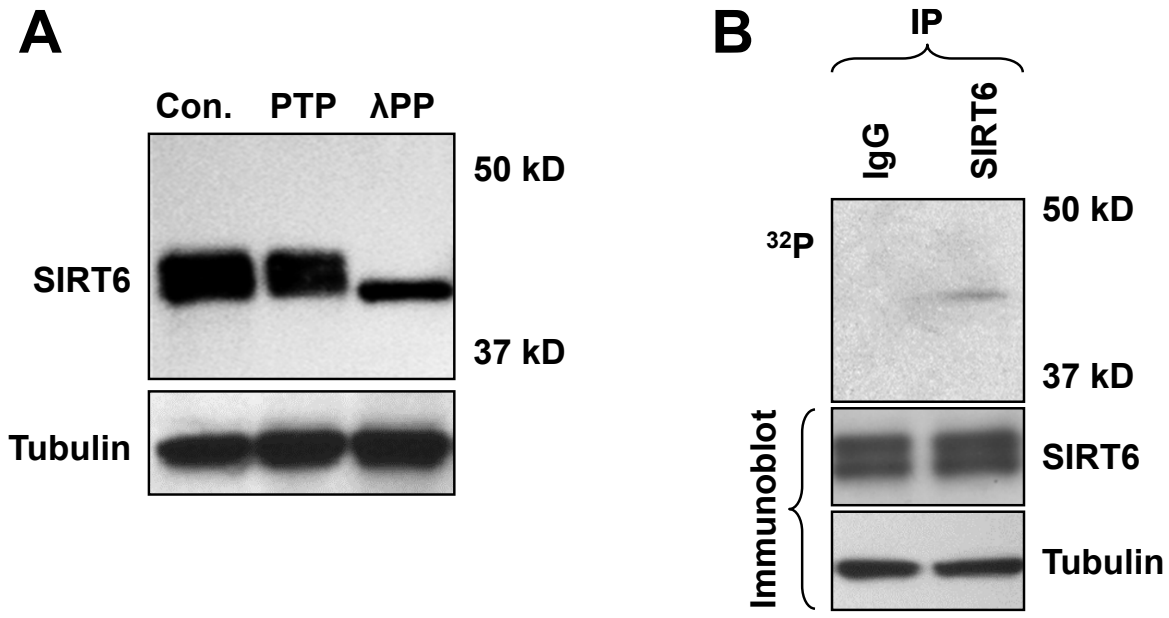
Construct integrated into HCA2-hTERT cells for detecting NHEJ and HR

(A) NHEJ reporter cassette. The construct consists of a GFP gene containing an intron from the rat Pem1 gene, interrupted by an adenoviral exon (Ad). This exon is flanked by inverted I-SceI recognition sites for induction of DSBs. In the starting construct, the GFP gene is inactive. Induction of a DSB by expression of I-SceI followed by a successful NHEJ event reconstitutes the functional GFP gene. SD, splice donor; SA, splice acceptor; shaded squares, polyadenylation sites.

(B) HR reporter cassette. The reporter cassette consists of two mutated copies of GFP-Pem1. In the first copy of GFP-Pem1, the first GFP exon contains a deletion of 22 nucleotides and an insertion of two I-SceI cleavage sites in an inverted orientation. The 22 nucleotide deletion ensures that GFP cannot be reconstituted by an NHEJ event. The second copy of GFP-Pem1 lacks the ATG start codon and the second exon of GFP. Upon induction of DSBs by I-SceI, gene conversion events reconstitute an active GFP gene.

(C-H) Other kinase inhibitors do not affect the ability of SIRT6 to stimulate DSB repair under oxidative stress. (C) ERK signaling is not required for SIRT6 to stimulate NHEJ in response to stress. HCA2-hTERT-NHEJ cells were treated as described in Figure 1 in the presence or absence of ERK inhibitors. **(D)** p38 signaling is not required for SIRT6 to stimulates NHEJ in response to stress. HCA2-hTERT-NHEJ cells were treated as described in Figure 1 in the presence or absence of p38 inhibitors. **(E)** mTOR signaling is not required for SIRT6 to stimulates NHEJ in response to stress. HCA2-hTERT-NHEJ cells were treated as described in Figure 1 in the presence or absence of mTOR inhibitors. **(F)** CDK signaling is not required for SIRT6 to stimulates NHEJ in response to stress. HCA2-hTERT-NHEJ cells were treated as described in Figure 1 in the presence or absence of CDK inhibitors. **(G)** AKT signaling is not required for SIRT6 to stimulates NHEJ in response to stress. HCA2-hTERT-NHEJ cells were treated as described in Figure 1 in the presence or absence of AKT inhibitors. **(H)** Raf signaling is not required for SIRT6 to stimulates NHEJ in response to stress. HCA2-hTERT-NHEJ cells were treated as described in Figure 1 in the presence or absence of Raf inhibitors.

(I) Representative FACS traces for the experiment shown in Figure 1A.



C

GPS 2.1 Kinase Predictions for SIRT6 S10

(Peptide: VNYAAGL**S**PYADKGK)

Rank	Kinase	Score	Cutoff
1	JNK1	15.111	3.444
2	p38 γ	9.333	6
3	MEK6	9	3.333
4	p38 δ	8.667	5.667
5	CDK5	8.474	3.895

D

Diluted from 1mg/ml	Antibody Concentration (ng/ml)	Anti-SIRT6 S10-P Specific antibody Rabbit #5159		Rabbit #5160	
		SIRT6-P	SIRT6	SIRT6-P	SIRT6
1:1,000	1000	2.3	0.387	2.339	0.676
1:2,000	500	2.241	0.230	2.293	0.424
1:4,000	250	2.224	0.167	2.289	0.271
1:8,000	125	2.208	0.113	2.273	0.179
1:16,000	62.5	2.005	0.078	2.032	0.110
1:32,000	31.25	1.934	0.073	1.948	0.085
1:64,000	15.62	1.698	0.066	1.645	0.074
1:128,000	7.81	1.149	0.063	1.020	0.070
1:256,000	3.90	1.030	0.059	0.866	0.065
1:512,000	1.95	0.595	0.055	0.470	0.061
Blank	Blank	0.057	0.060	0.057	0.060
Blank	Blank	0.057	0.060	0.057	0.060
Titer	Titer	>1:512,000	1:4000	1:512,000	1:8000

Figure S2. Related to Figure 2

Figure S2, related to Figure 2

SIRT6 is a phospho-protein in HCA2-hTERT cells

(A) HCA2-hTERT protein extracts were treated with either a protein tyrosine phosphatase (PTP) or a lambda phosphatase (λPP) and then separated by SDS-PAGE. Immunoblotting, probing with SIRT6 antibodies, revealed that treatment with λPP, but not PTP, ablates the upper band of the SIRT6 doublet.

(B) HCA2-hTERT cells were incubated overnight with ^{32}P-orthophosphoric acid. Immunoprecipitation with SIRT6 antibodies revealed that SIRT6 specifically incorporated the radiolabel.

(C) SIRT6 is a predicted JNK substrate. Group based prediction system (GPS 2.1 Kinase site prediction software) software ranks JNK1 as the most highly predicted kinase for phosphorylating SIRT6 at reside S10 compared to all other serine/threonine kinases.

(D) Specificity of SIRT6 S10 phospho-specific antibodies. Two custom rabbit polyclonal antibodies (Rb5159 and Rb5160) were generated by immunizing rabbits with YAAGL**pS**PYADKGKC peptide. Specificity of each anti-SIRT6-pS10 purified antibody (from each rabbit) was assayed by ELISA by comparing the binding of serial dilution of each antibody against pS10-peptide or the non-phosphorylated equivalent peptide.

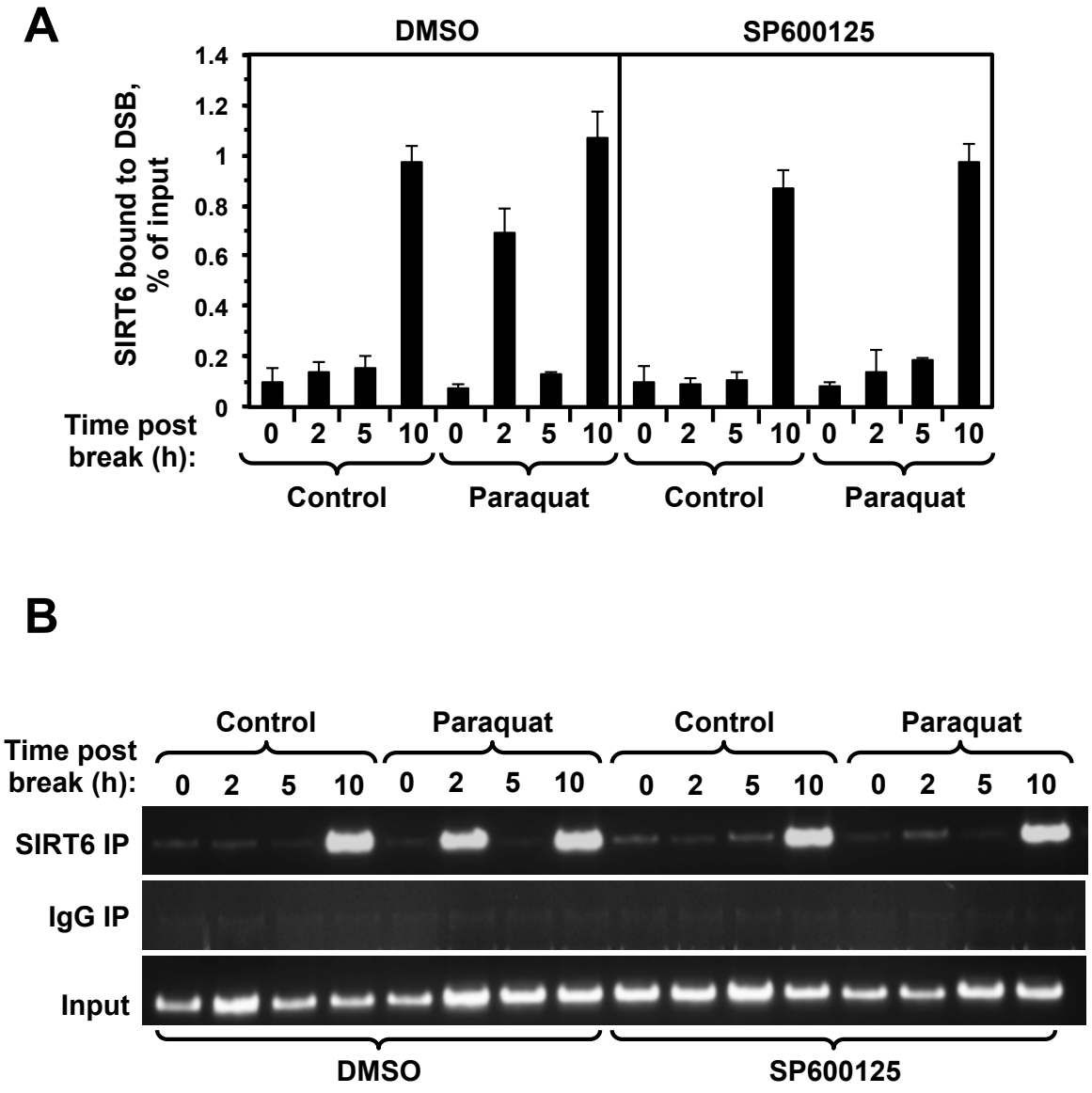


Figure S3. Related to Figure 3

Figure S3, related to Figure 3

ChIP analysis of SIRT6 recruitment to site-specific DSB

(A) ChIP analysis showing kinetics of SIRT6 recruitment to sequences flanking I-SceI-induced DSBs after transfection in the presence or absence of a JNK inhibitor. HCA2-hTERT-NHEJ cells were pretreated with 1 mM paraquat for 16 hours prior to induction of DSB. Two waves of SIRT6 recruitment are observed in these cells: an early wave (2 h) coinciding with maximum expression of I-SceI (Mao et al., 2008), and a late wave (10 h). When these cells were pretreated with a JNK inhibitor, SIRT6 failed to mobilize to DSB sites at the early time point. Asterisks indicate values significantly different from corresponding zero time points ($P < 0.05$). Error bars indicate SD; $n = 3$.

(B) Semi-quantitative representation of ChIP experiments. DNA precipitated by ChIP was amplified with primers proximal to the I-SceI induced break site by PCR. Gel is a representative image of amplification products. IgG, immunoglobulin G.

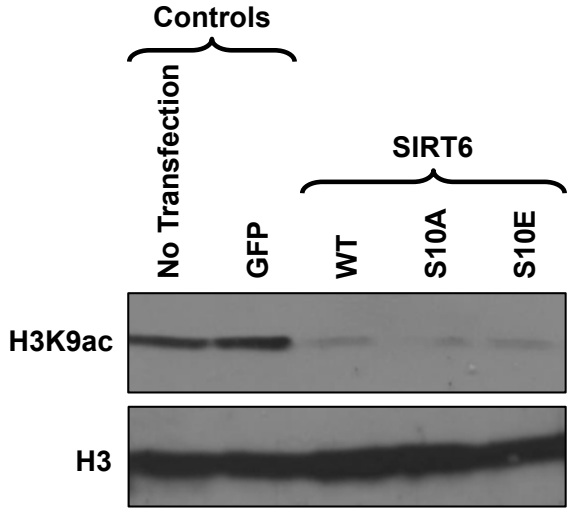


Figure S4. Related to Figure 4

Figure S4, related to Figure 4

SIRT6 S10 phosphorylation does not affect SIRT6 deacetylation activity

Human diploid fibroblasts were transfected with WT SIRT6, S10A or S10E mutants. Western blot shows the level of acetylated H3K9 24 hours after transfection.