

Shah et al., Suppl Figure 1

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SIRT1-Δ2/9                                     GGATCCAGATCTGCTAGCATGGAACAAAA
SIRT1-Δ2/9 optimised                             -----
Protein sequence                                -----M E Q K

SIRT1-Δ2/9                                     1 ATGCGCGACGAGGCGG 16
                                           ||.||.|||||||
SIRT1-Δ2/9 optimised ACTCATCTCAGAAGAGGATCTGAATCATACCGGTCATCATCACCATCACCATGAGCTCGCCGATGAGGCGG
Protein sequence     L I S E E D L N H T G H H H H H H E L A D E A
                                           c-Myc epitope                               His epitope
SIRT1-Δ2/9       CCTCGCCCTTCAGCCCGGCGGCTCCCCCTCGGCGGCGGGGCGGACAGGGAGGCGCGCTCGTCCCCCGCC 87
                                           |..|.|.|||||.|||||.|||||.||.|||||..|.|.|||||.||.|||||.|||||.|||||.|||||.|||||.
SIRT1-Δ2/9 optimised CATTGCCCTACAGCCTGGCGGATCTCCAGTGCAGCCGGAGCAGATCGCGAGGCGGCTTCCTCACCCGCA
Protein sequence     A L A L Q P G G S P S A A G A D R E A A S S P A

SIRT1-Δ2/9       GGGGAGCCGCTCCGCAAGAGGCCGCGGAGAGATGGTCCCGGCTCGAGCGGAGCCGGGGGAGCCCGGTTGG 158
                                           |||||.||.|||||.|||||.|||||.|||||.|||||.|||||.|||||.|||||.|||||.|||||.
SIRT1-Δ2/9 optimised GGGGAACCCCTTCGGAAGAGACCGCGCAGAGATGGGCTGGTCTCGAACGGAGCCAGGAGAACCTGGCGG
Protein sequence     G E P L R K R P R R D G P G L E R S P G E P G G

SIRT1-Δ2/9       GGCGGCCCCAGAGCGTGAGGTGCGGCGGCGGCCAGGGGCTGCCCGGTTGCGGCGGCGGCGCTGTGGC 229
                                           .||.||||.||||.||||.||||.||||.||||.||||.||||.||||.||||.||||.||||.||||.
SIRT1-Δ2/9 optimised TGCGAGCTCCAGAGCGTGAAGTACCCGCTGCCGCAAGAGGGTCCCTGGTGGCGCAGCTGCCGCGCTCTGGC
Protein sequence     A A P E R E V P A A A R G C P G A A A A A L W
                                           SEx1
SIRT1-Δ2/9       GGGAGGCGGAGGCAGAGCGCGGCGGCGGAGGAGCAAGAGGCCAGGCGACTCGGCGGCTGGGGAA 300
                                           |.|||||.||.|||||.|||||.||||.||||.||||.||||.||||.||||.||||.||||.||||.
SIRT1-Δ2/9 optimised GAGAGGCCAAGCCGAGGCCGCTGCAGCCGGAGGCGAACAGGAGGCTCAAGCCACAGCTGCAGCCGGGGAA
Protein sequence     R E A E A E A A A A G G E Q E A Q A T A A A G E

SIRT1-Δ2/9       GGAGACAATGGGCCGGGCTGCAGGGCCATCTCGGGAGCCACCGCTGGCCGACAACCTGTACGACGAAGA 371
                                           ||.|||||.||||.||||.||||.||||.||||.||||.||||.||||.||||.||||.||||.
SIRT1-Δ2/9 optimised GGCGACAATGGACCCGGGTTACAGGGCCCAAGCAGGGAACCCCGCTGCGGACAACCTGTATGACGAGGA
Protein sequence     G D N G P G L Q G P S R E P P L A D N L Y D E D

SIRT1-Δ2/9       CGACGACGACGAGGGCGAGGAGGAGGAAGAGCGGCGGCGGCGGCGATTGGGTACCAGGATAAACCCTCTGT 442
                                           |||.|||||.||||.||||.||||.||||.||||.||||.||||.||||.||||.||||.||||.
SIRT1-Δ2/9 optimised CGATGACGATGAGGGCGAGGAAGAGGAGGAGCTGCTGCCGCGCGATTGGCTACAGGACAACCTGCTGT
Protein sequence     D D D E G E E E E A A A A I G Y R D N L L

SIRT1-Δ2/9       TCGTTCTGTGGCAGTAACAGTGATAGTGGGACATGCCAGAGTCCAAGTTTAGAAGAACCATGGAGGATG 513
                                           |||||.||||.||||.||||.||||.||||.||||.||||.||||.||||.||||.||||.
SIRT1-Δ2/9 optimised TCGTGGTGGTGGTGTGACCGTATCGTCCGTCACGCCAGGGTTCAGGCTGAAAGAACCATGGAGGATG
Protein sequence     F V L V A V T V I V G H A R V Q V *

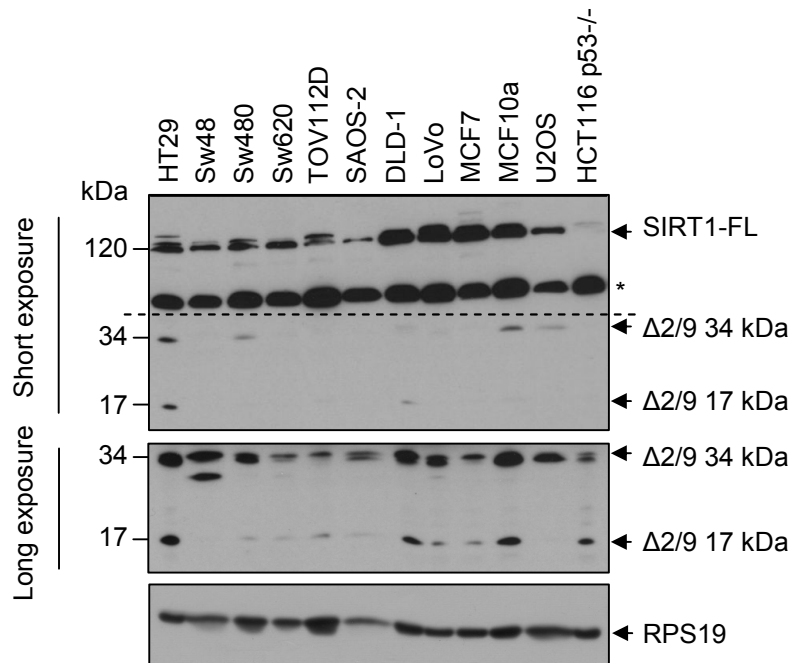
SIRT1-Δ2/9       AAAGTGAATTTGAAGAATCTACAATGGCTTAGAAGATGAGCCTGATGTTCCAGAGAGAGCTGGAGGAGCT 584
                                           |||||.||||.||||.||||.||||.||||.||||.||||.||||.||||.||||.||||.
SIRT1-Δ2/9 optimised AAAGTGAATTTGAAGAATCTACAATGGCTTAGAAGATGAGCCTGATGTTCCAGAGAGAGCTGGAGGAGCT

SIRT1-Δ2/9       GGATTTGGGACTGATGGAGATGATCAAGAGGCAATTAATGAAGCTATATCTGTGAAACAGGAAGTAACAGA 655
                                           |||||.||||.||||.||||.||||.||||.||||.||||.||||.||||.||||.||||.
SIRT1-Δ2/9 optimised GGATTTGGGACTGATGGAGATGATCAAGAGGCAATTAATGAAGCTATATCTGTGAAACAGGAAGTAACAGA

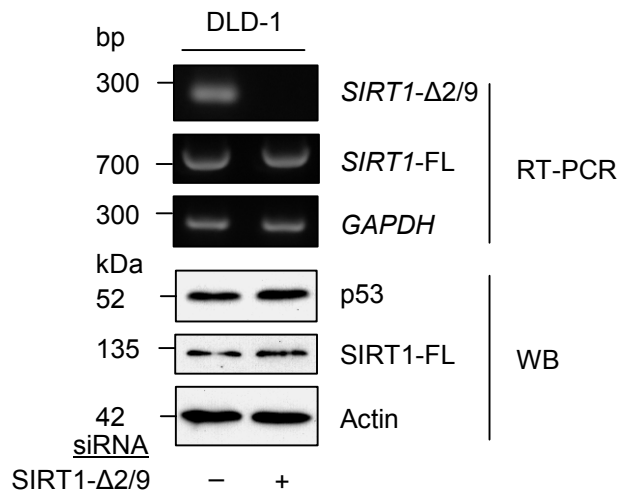
SIRT1-Δ2/9       CATGAACTATCCATCAAACAATCATAG 683
                                           |||||.||||.||||.||||.||||.||||.||||.||||.||||.||||.||||.
SIRT1-Δ2/9 optimised CATGAACTATCCATCAAACAATCATAGAAGCTTTCGTCGACGATATCagcacagtggggccgctcgag
                                           ←
                                           10R primer
                                           tctagaggcccggttcgaacaaaaactcatctcagaaggatctgaatatgcataccgggtcatcatc
                                           accatcaccattgagtttaaacccgctgatcagcctcgactgtgccttcta
                                           ←
                                           BGH-Rvs

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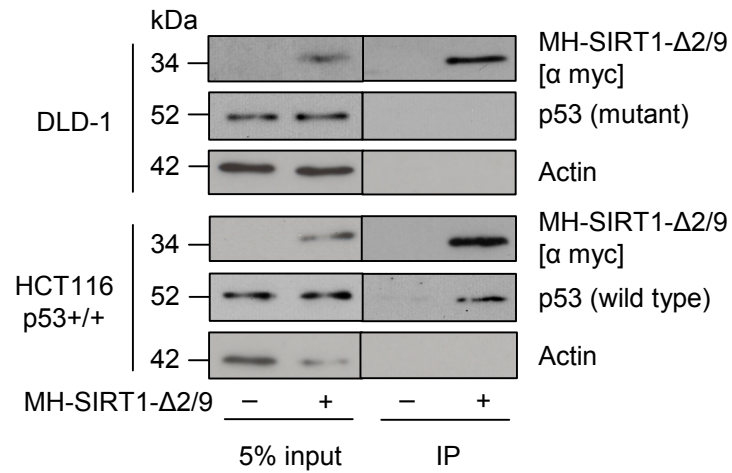
**Supplemental Figure 1. Alignment of *SIRT1*- $\Delta$ 2/9 wild type and codon-optimised sequences in pcDNA3.1 expression construct.** *SIRT1*- $\Delta$ 2/9 sequence including part of its 3'-UTR (full 3'-UTR of *SIRT1*- $\Delta$ 2/9 is not known) together with sequences for c-Myc and His epitope tags (underlined) was codon-optimised, chemically synthesized and cloned in pcDNA3.1 vector. The codon-optimised sequence is compared with the original wild type nucleotide sequence of *SIRT1*- $\Delta$ 2/9, vertical bars and dots indicate identity and silent changes introduced for codon-optimisation respectively. ATG start and TAG stop codons are shown bold. Boxed, yellow and red labeled sequences indicate exon 2, 2-9F primer and novel C-terminal amino acids respectively. Arrows represent location of other primers as indicated. Primer pairs Sex1/BGH-Rvs and 2-9F/10R amplify exogenous- and endogenous-specific *SIRT1*- $\Delta$ 2/9 transcripts respectively. Small lettered nucleotides are pcDNA3.1 vector sequence.



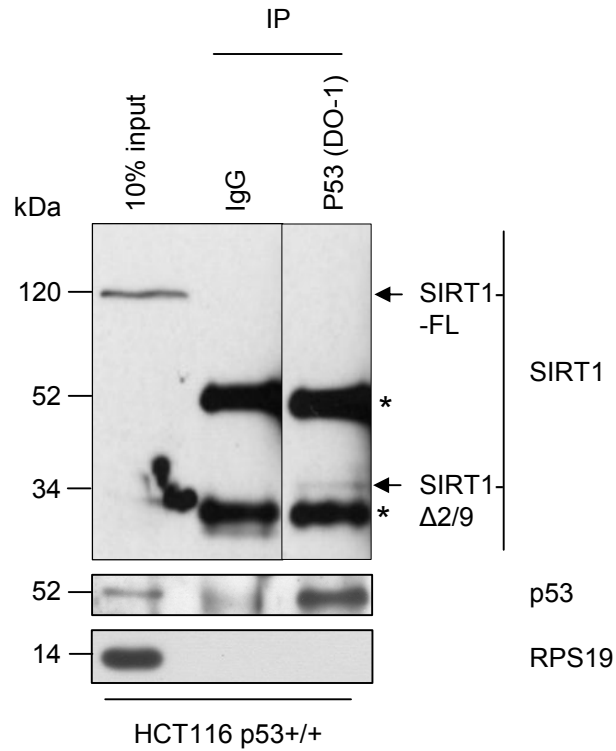
**Supplemental Figure 2. Expression of SIRT1-FL and SIRT1- $\Delta$ 2/9 in a range of human cell lines.** Total cell lysates from indicated cell lines were immunoblotted for SIRT1 protein using anti-SIRT1 antibodies (N-terminus; Epitomics); similar but weaker results were obtained with an independent SIRT1 N-terminal antibody (Millipore). Loading control was the ribosomal protein RPS19. SIRT-FL was readily detectable in short exposures (upper panel) whilst longer exposure was required to detect SIRT1- $\Delta$ 2/9 in most of the cell lines (lower panel). Dotted line in short exposure panel delineates area shown in the panel below (long exposure). Both 17kDa and 34kDa forms of SIRT1- $\Delta$ 2/9 are evident (see Results section). \* = a strong, non-specific band cross reactive with the Epitomics antibody.



**Supplemental Figure 2. Mutant p53 protein levels are not affected by SIRT1-Δ2/9 depletion.** Protein levels of mutant p53 (S241F), SIRT1-FL and actin in DLD-1 cell line following depletion of *SIRT1-Δ2/9* by siRNA (lower panel) and mRNA levels of *SIRT1-Δ2/9*, *SIRT1-FL* and *GAPDH* (upper panel).



**Supplemental Figure 3. SIRT1-Δ2/9 does not interact with mutant p53.** MH-SIRT1-Δ2/9 was expressed in HCT116 p53+/+ and DLD-1 cells, immunoprecipitated with anti-His antibody and probed for SIRT1-Δ2/9 and p53.



**Supplemental Figure 5. Complexing between endogenous SIRT1-Δ2/9 and p53**

**proteins.** Lysates of HCT116 cells cultured under normal conditions (i.e. in the absence of applied stress) were immunoprecipitated using anti-p53 monoclonal antibody DO-1, followed by immunoblotting for SIRT1 (N-terminus, Epitomics), p53 (FL-393) or RPS19 as indicated. SIRT1-FL and SIRT1-Δ2/9 proteins are indicated by arrows; \* = heavy and light IgG chains of DO-1 antibody. On long exposures the IgG light chain signal masks the co-immunoprecipitated SIRT1-Δ2/9 protein band (not shown). Note that SIRT1-FL was undetectable in p53 pull-downs even in long exposures of the immunoblot (see text, Results section for further discussion of this observation).

**Supplemental Table 1. Primers used in this study.**

Primers	Sequence
1F	5'-ataaccttctgttcgttct-3'
2-9F	5'-ataaccttctgttcgttct-3'
8Rvs	5'-aagaggtgtgggtggcaactctg-3'
10R	5'-ctatgattgttgatggatagttc-3'
<i>APAF-1-F</i>	5'-tgcgctgctctgccctct-3'
<i>APAF-1-R</i>	5'-ccatgggtagcagctcctct-3'
BGH-Rvs	5'-tagaaggcacagtcgagg-3'
<i>CUGBP1-F</i>	5'-gataggagccaaaaccgcc-3'
<i>CUGBP1-R</i>	5'-tcacaaatgcacaacctcgg-3'
<i>CUGBP2-F</i>	5'-gtgaaaagtccaacgctgtg-3'
<i>CUGBP2-R</i>	5'-ccaggtggcagtggtgagc-3'
<i>GAPDH-F</i>	5'-cggagtcaacggatttggtcg-3'
<i>GAPDH-R</i>	5'-agcctctccatggtggtgaa-3'
<i>HO-1-F</i>	5'-ccagtgccaccaagtcaag-3'
<i>HO-1-R</i>	5'-cagctctgcaactcctcaa -3'
<i>HO-1pF</i>	5'-gtcaacgcctgcctcctctc-3'
<i>HO-1pR</i>	5'-tcgggttgccggacgctccat-3'
<i>IGFBP3-iF</i>	5'-agagatgtaacggggacctaga-3'
<i>IGFBP3-iR</i>	5'-accagtaccgtcctcaatgct-3'
<i>IGFBP3-F</i>	5'-gacagaatatggcctcctgccg-3'
<i>IGFBP3-R</i>	5'-ttggaagggcgacactgct-3'
<i>IGFBP3-pF</i>	5'-cgggcacacctggttcttg-3'
<i>IGFBP3-pR</i>	5'-cttcgccctgagcagccg-3'
<i>MMP-1-F</i>	5'-ttgatgtaccctagctacacctca-3'
<i>MMP-1-R</i>	5'-aaaggttagcttactgtcacatgcttt-3'
<i>TP53E5F</i>	5'-cccctgccctcaacaagatgt-3'
<i>TP53E8R</i>	5'-ctgaagggtgaaatattctcc-3'
<i>TP53-up1</i>	5'-atggaggagccgcagtcagat-3'
<i>TP53-dn1</i>	5'-tcagtctgagtcaggcccttc-3'
SEx1	5'-ccagagcgtgaggtgcc-3'
SEx4	5'-gggatggtatttatgctcgc-3'
<i>TBP-F</i>	5'-caggagccaagagtgaagaaca-3'
<i>TBP-R</i>	5'-agctggaaaaccaacttctgt-3'
<i>PLAT-F</i>	5'-atgcccattcagaagagg-3'
<i>PLAT-R</i>	5'-gacaggcactgagtgccact-3'

**Supplemental Table 2. RT-PCR annealing and cycling conditions.**

Primer sets for gene	Annealing temperature/ no. of cycles
Standard RT-PCR	
<i>CUGBP1</i>	58°C/35
<i>GAPDH</i>	58°C/30
<i>TP53</i>	58°C/35
<i>SIRT1-Δ2/9</i>	53°C/44
<i>SIRT1-FL</i>	58°C/34
qRT-PCR	
<i>APAF-1</i>	60°C/40
<i>CUGBP2</i>	58°C/35
<i>HO-1</i>	60°C/40
<i>HO-1</i> promoter	60°C/45
<i>IGFBP3</i>	60°C/40
<i>IGFBP3</i> intron	60°C/45
<i>IGFBP3</i> promoter	60°C/45
<i>MMP-1</i>	60°C/40
<i>TP53</i>	58°C/33
<i>TBP</i>	58°C/35
<i>PLAT</i>	60°C/35