

SUPPLEMENTAL MATERIAL

FIGURES

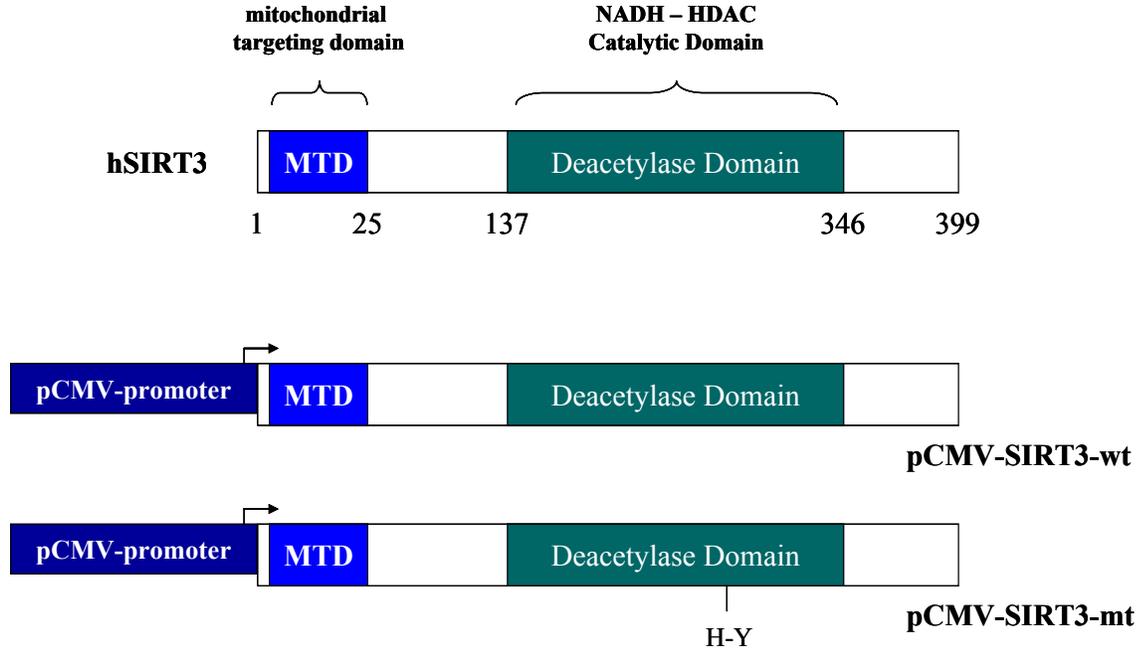


Figure S1. *SIRT3* wild type and deacetylation mutant CMV based expression vectors. The expression vector pcDNA4 was obtained from Promega, Inc. The wild type and deacetylation mutant, where amino acids 248 has been changed from a histidine to tyrosine to express an acetylation null protein, *SIRT3* genes were cloned downstream of the CMV promoter of pcDNA4 to create pCMV-SRIT3-wt and pCMV-SIRT3-mt (Shi et al., 2005) and were a kind gift from Toren Finkel (National Institutes of Health).

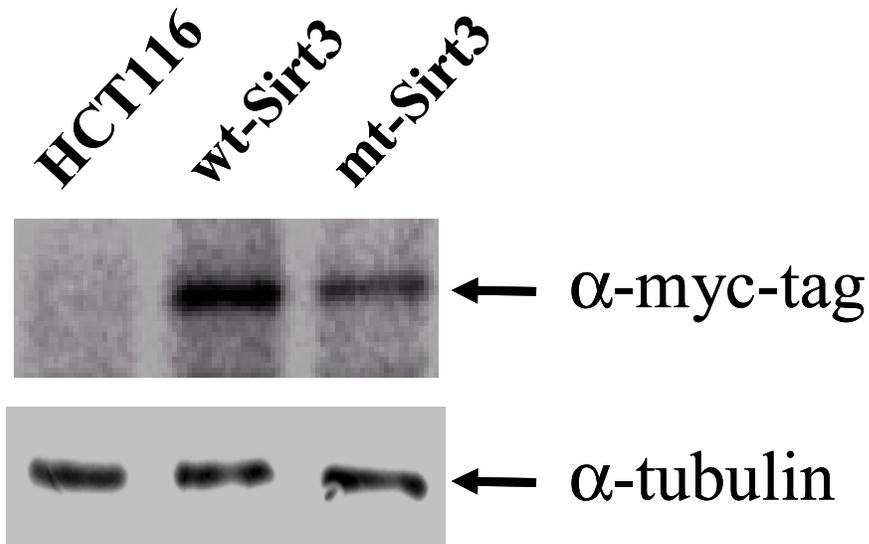


Figure S2. SIRT3 myc-tagged protein levels in the HCT116, wt-SIRT3, and mt-SIRT3 selected perminant cell lines. wt-SIRT3, and mt-SIRT3 cells were harvested and immunoreactivity to myc-SRIT3-tagged protein levels were determined. 20ug of cellular protein was isolated and separated by SDS-PAGE, transferred onto nitrocellulose, and processed for immunoblotting with myc antibody (Santa Cruz, Inc) or an anti-tubulin antibody . Equal protein loading was determined using a Bradford protein assay

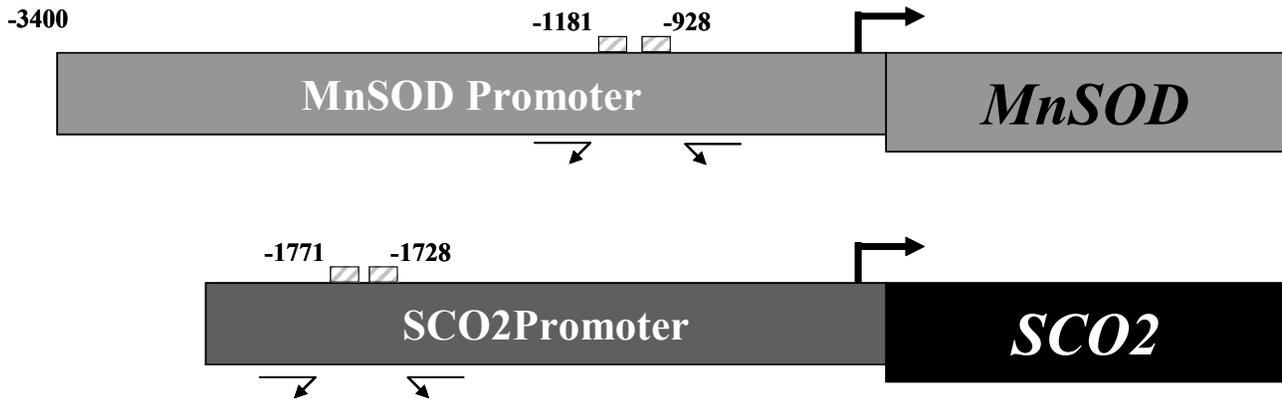


Figure S3. *MnSOD* and *SCO2* contain FOXO3a DNA-binding sites in the upstream regulatory promoter regions. Promoter region analyses of the *MnSOD* (upper panel) and *SCO2* (lower panel) genes. The 5'-upstream regulatory regions were analyzed for potential FOXO3a cis-acting DNA binding / enhancer sites using software available at Entrez Genome and the Genetics Computer Group. The two potential FOXO3a for each promoter is shown as a diagonally filled rectangle at positions -1181 and -928 for *MnSOD* and -1771 and -1728 for *SCO2*, respectively.

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ChIP Primers -

MnSOD:

Fwd 5' - TCTGACGTCTGTAAACAAGCCCAG

Rev 5' - TTCTTTCCTGCGCTGTCTTGTAGC

SCO2

Fwd 5' - GCAACGTCTGTAAACAAGCGTCA

Rev 5' - CTCATTCCTGCGCTGTTTCGTCAA

p3x-FOXO3a-tk-Luc -

cAGGCTGGGCGGCGGgagctcacgcgtCCGCGAAGAAACgctagcctcgagCCTCCTGGCT
TTa and gatct AAACGCCAGGAGG ctcgag gctagcGTTTCTTCGCGG acgc-gtgagtc
CCGCCGCCAGCCT ggtac.