Supplemental Materials

Extended Materials and Methods

Tables 1-4: Lists of antibodies and nucleic acid sequences.

Table 1: Antibodies and Applications			
Antibody	**Manufacturer**	**Catalog**	**Use**
Primary:			
β-tubulin	Sigma	T8328	WB
CHIP	Abcam	Ab134064	WB
CHIP	Cell Signaling	2080S	WB
CHIP	Sigma	S1073	WB, IP
FLAG	Sigma	F3165	WB
FLAG	Sigma	F7425	WB
GAPDH	Sigma	G8795	WB
γH2AX	Millipore	05-636	IF
H3	Cell Signaling	9715S	WB
H3K9Ac	Cell Signaling	9671S	WB
H3K9Ac	Sigma	H9286	WB, ChIP
HA-HRP	Roche	3F10	WB
Myc	Santa Cruz	SC-40	WB
Rabbit IgG	Sigma	I5009	ChIP
P27	Abcam	Ab32034	WB
pRPA32 S4/S8	Bethyl	A300-245A	WB
RPA32	Calbiochem	NA19L	WB
SirT6	Cell Signaling	2590S	WB
SirT6	Abcam	Ab62739	WB
SirT6	Sigma	S4322	WB, ChIP
Secondary:			
Goat anti-mouse 488	Molecular Probes	11001	IF
Goat IgG-HRP	Sigma	S5420	WB
Mouse IgG-HRP	Sigma	S9917	WB
Rabbit IgG-HRP	Sigma	S9169	WB
Protein A-HRP	Millipore	18-160	WB

List of antibodies, catalog numbers, and applications. WB = Western blot, IP = immunoprecipitation, IF = immunofluorescence, and ChIP = chromatin immunoprecipitation.

Table 2: siRNA	
siControl	GAGACCCUAUCCGUGAUUAGUA
siCHIP #1	UGCCGCCACUAUCUGUGUAAU
siCHIP #2	AGGCCAAGCACGACAAGUA
siSirT6	AAGAAUGUGCCAAGUGUAAGA

List of siRNA sequences used. A corresponding antisense strand was made and oligos were annealed prior to transfection.

Table 3: ChIP-qPCR Primers		
Gene	**Sense**	**Antisense**
BIRC3	CCACGAGCAATGAAGCAAATGTC	GGGGAACTCCAGCGGTAATAAC
ICAM	ACGTGGTGGATGTCGAGTCTT	ACTAAATATGAGCGGGTGTTTGC
LDHB	TCCGCACGACTGTTACAGAG	CGGATGCTCAGAGCTAAAGG
NFKBIA	GCAGCCCCCTAACCACAGT	CTGGGCGTAGGGATTTGCT
PFKM	TACTGCACAGGCACCGATAA	CCCTCCTGCTCAGGTTATGA
SOD2	AGGTCGGCTTACTTGCAAAGC	CGCCCTTCCAACCCGTAT

List of primer sequences amplifying the promoter regions of the indicated human genes from DNA obtained from ChIP assays. Assays were performed with SYBR green reagent.

Table 4: mRNA primers				
Accession	**Gene**	**Sense**	**Antisense**	**Probe**
Mouse:				
NM_007464.3	Birc3	TCGATGCAGAAGACGAGATG	TTTGTTCTTCCGGATTAGTGC	68
NM_019719.3	Chip	CTCAAGGAGCAGGGAAACC	CACAAGTGGGTTCCGAGTG	93
NM_010493.2	Icam1	CCCACGCTACCTCTGCTC	GATGGATACCTGAGCATCACC	81
NM_008492.2	Ldhb	ACAAGTGGGTATGGCATGTG	ACATCCACCAGGGCAAGTT	106
NM_010907.2	Nfkbia	ACGAGCAAATGGTGAAGGAG	ATGATTGCCAAGTGCAGGA	38
NM_001163487.1	Pfkm	GGACAATCTGCAAGAAAGCAG	ATGATGCTCTTCATGGGTCA	91
NM_181586.3	SirT6	GACCTGATGCTCGCTGATG	GGTACCCAGGGTGACAGACA	18
NM_013671.3	Sod2	GACCCATTGCAAGGAACAA	GTAGTAAGCGTGCTCCCACAC	3
Human:				
NM_001165.4	BIRC3	CTTGTCCTTGCTGGTGCAT	AAGAAGTCGTTTTCCTCCTTTGT	62
NM_005861	CHIP	GTTCGTGGGCCGAAAGTA	GGCCCGGTTGGTGTAATA	49
NM_000201	ICAM1	CCTTCCTCACCGTGTACTGG	AGCGTAGGGTAAGGTTCTTGC	71
NM_002300.6	LDHB	GATGGATTTTGGGGGAACAT	CTGCCACATTCACACCACTC	5
NM_020529	NFKBIA	GTCAAGGAGCTGCAGGAGAT	ATGGCCAAGTGCAGGAAC	38
NM_001166686.1	PFKM	GCCATCAGCCTTTGACAGA	CTCCAAAAGTGCCATCACTG	18
NM_016539.2	SIRT6	AGCTGAGGGACACCATCCTA	ATGTACCCAGCGTGATGGAC	18
NM_000636.2	SOD2	CTGGACAAACCTCAGCCCTA	TGATGGCTTCCAGCAACTC	22
X03205.1	18S	GCAATTATTCCCCATGAACG	GGGACTTAATCAACGCAAGC	48

List of qPCR primer sequences amplifying cDNA for the indicated mouse and human genes with the corresponding probe from the Roche Universal Probe Library indicated. Assays were performed with Taqman reagent.

Immunofluorescence: Cells were fixed in 4% paraformaldehyde for 10 min, then incubated in permeabilization buffer (PBS, 0.5% Triton X-100, 1% BSA) for 10 min. Primary and secondary antibodies were prepared at 1:500 and 1:800 dilutions, respectively, in blocking buffer (PBS, 0.05% Triton X-100, 1% BSA). Coverslips were mounted using Fluoro-Gel II with DAPI (Electron Microscopy Services). Cells were visualized using a Zeiss LSM 710 spectral confocal

microscope with a 63X oil immersion lens and Zeiss ZEN software package. γH2AX foci larger than 10 pixels were quantified using ImageJ.

SirT6 Half-Life – shControl or shCHIP 293 cells stably expressing FLAG-SirT6 (shControl+FLAG-SirT6 or shCHIP+FLAG-SirT6), or shControl or shCHIP 293 cells transfected for 24 h with FLAG-K170R SirT6, were split into 4 6-cm plates per cell type. After PBS wash, cells were incubated in depletion media (methionine/cysteine-free DMEM (Gibco), 10% dialyzed FBS) for 30 min, then incubated for 30 min in depletion media containing 0.2 mCi ^{35}S methionine/cysteine (EasyTag Express Protein Labeling Mix, Perkin Elmer) per plate. Cells were rinsed three times with PBS, then incubated in chase media (DMEM, 10% FBS, 5 mM methionine, 5 mM cysteine). The cells used for 72-h timepoint samples were transferred into larger plates to prevent overconfluence after 48 h. At the indicated time points, cells were harvested on ice and pellets were stored at -80°C. Pellets were resuspended in lysis buffer, immunoprecipitations were performed as described in the manuscript, and bound protein was eluted using FLAG peptide. Lysates were separated on a NuPage 4-12% Bis-Tris gel, which was incubated in gel fixing solution (20% methanol, 10% acetic acid, 3% glycerol) for 15 min, dried on Whatman paper, and exposed in a dark cassette for 48 h on Blue Devil Autoradiography film (Genesee Scientific) prior to developing. For steady-state SirT6 protein level measurements, cells were incubated with actinomycin (1 μg/mL), chloroquine (5 μg/mL), cycloheximide (50 μg/mL), MG132 (2 μg/mL), or ammonium chloride (1 mg/mL) for 16 h prior to harvesting.

Chromatin immunoprecipitation: Following crosslinking with 1% formaldehyde, cells were sonicated with a Misonix XL2010 sonicator. Soluble nuclear material from approximately 2

million cells was used per immunoprecipitation. ChIP was performed using Magna ChIP Protein A+G Magnetic Beads (Millipore) and the indicated antibodies in Table 1. Eluted DNA was isolated using the PCR Purification Kit (Qiagen). Real time PCR was performed on DNA samples using Sso Advanced SYBR Green Supermix (Bio-Rad) and validated primers amplifying the promoter regions of the indicated genes (Integrated DNA Technologies, see Table 3) on a Roche LightCycler 480 real-time PCR instrument. Results were expressed relative to 1% input control and normalized to shControl samples.

ChIP-seq: Chromatin immunoprecipitation was performed as described, except the procedure was scaled up to accommodate approximately 60 million cells per immunoprecipitation. Library preparation was performed using NEB enzymes. End repair was performed with T4 DNA polymerase, T4 PNK, and Klenow polymerase for 30 min at 20°C. Adeosine bases were added using Klenow 3′-5′ exonuclease for 30 min at 37°C. Paired-end adapters (Illumina) were ligated using Quick DNA ligase for 25 min at 20°C. DNA was purified using Agencourt AMPure XP beads. DNA was amplified in a PCR using Phusion polymerase (Finnzymes), and 200-400 bp chromatin fragments were gel-purified in a low-melt 2% agarose gel (Bio-Rad). Paired-end sequencing was performed on a Genome Analyzer II (Illumina) at the High Throughput Sequencing Facility at UNC-Chapel Hill. Data was processed using the Illumina pipeline, and raw data was aligned to the genome using Bowtie (http://bowtie-bio.sourceforge.net/index.shtml). SICER was used for peak calling, with a window size of 200 bp. A list of exons, introns, and 5′/3′ regions within 20K bp of genes from the UCSC Known Gene data set was generated, and peaks were assigned to features overlapping the peak area. Peaks with a *p* value greater than 0.05 were excluded from analysis. Annotated peak information

78 was analyzed using Ingenuity Pathway Analysis (www.ingenuity.com) and GATHER

79 (gather.genome.duke.edu).