Supporting Information

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SI Materials and Methods

Cell Lines, Antibodies, and Plasmids. HEK293 cells were maintained in DMEM (Invitrogen Life Technologies) and Jurkat cells were cultured with RPMI1640. The media was supplemented with 10% FBS, 100 U/mL penicillin, 200 μg/mL streptomycin, and 0.25 μg/mL amphotericin B. Polyclonal antibodies against early responsive gene (EGR) 2, EGR3, and β-Actin were obtained from Santa Cruz Biotechnology. Anti-CD3 and anti-CD28 were from eBioscience. The anti-Flag antibody was from Sigma. Antibodies against forkhead O family transcription factor 3a (FoxO3a), phospho-FoxO3a, phosphor-Akt, and total Akt are purchased from Cell Signaling Technology. pCMV and Flag-Sirt1 expression plasmids were used as previously reported (1). Mouse Sirt1 promoter DNA was amplified by PCR using forward primers accactgagctatagcaacact (2 kb), acctagggcctctgcaggagc (1 kb), and ACCGCGGCGATCCCGGACGA (250 bp), together with a common reverse primer CTTCCAACTGCCTCTCTGGC. DNA fragments were subcloned into pGL4-luciferase plasmid (Promega).

Mice. C57/BL6 and OT-II TCR transgenic mice were purchased from the Jackson Laboratory. All mice used in this study were maintained and used at the Northwestern University mouse facility under pathogen-free conditions according to institutional guidelines and animal study proposals approved by the Institutional Animal Care and Use Committees.

Transfection, Immunoprecipitation, and Western Blotting. Transient transfection was performed by using Lipofectamine 2000 (Invitrogen Life Technologies), according to the manufacturer's instructions, with 60-mm dishes and 2–3 μg of total DNA per transfection. Two days after transfection, cells were lysed in 1× Nonidet P-40 lysis buffer (1% Nonidet P-40, 20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 5 mM EDTA), and freshly added protease inhibitor mixture. The cell lysates were mixed with antibodies (1 μg) for 2 h, followed by the addition of 30 μL of fast-flow protein G-Sepharose beads (GE Healthcare Bioscience) for an additional 2 h at 4 °C. Immunoprecipitates were washed four times with Nonidet P-40 lysis buffer and boiled in 20 μL of 2× Laemmle buffer. Samples were subjected to 8% or 10% SDS-PAGE analysis and electrotransferred onto polyvinylidene difluoride membranes (Millipore). Membranes were probed with the indicated primary antibodies, followed by horseradish peroxidase-conjugated secondary antibodies. Membranes were then washed and visualized with an enhanced chemiluminescence detection system (Amersham Pharmacia Biotech). When necessary membranes were stripped by incubation in stripping buffer (BioRad), washed, and then reprobed with other antibodies as indicated.

Chromatin Immunoprecipitation. Briefly, naïve, activated, and anergic $CD4^+$ T cells were stimulated with anti-CD3 plus anti-CD28 or with PI3K inhibitors LY294002 (EMD Biosciences) or recombinant IL-2 (eBioScience), as indicated. Cells were cross-linked with 1% formaldehyde, lysed with SDS lysis buffer, and sonicated for 15 min. Five percent of the cell lysate was used to determine the total amount of target DNA in the input. Remaining cell lysates were diluted in ChIP dilution buffer. Immunoprecipitation was performed with 4 μg of polyclonal anti-FoxO3a or anti-EGR2 antibodies at 4 °C overnight. Immune complexes were then mixed with Salmon sperm DNA/protein agarose-50% slurry 4 °C for 1 h. Immunoprecipitates were then washed sequentially with low-salt buffer, high-salt buffer, LiCl wash buffer, and TE buffer. DNA-protein complexes were eluted with elution buffer and cross-linking was reversed. Genomic DNA was extracted using phenol/chloroform and ethanol precipitated DNA was resuspended in Tris-EDTA buffer. PCR was performed with specific primers for mouse *sirt1* promoters: 5′-GTGTTGTGGTCCGGCCCGC-3′ and 5′-CTCCGCTCGACGCGCGGCA CT-3′.

Real-Time RT-PCR Analysis of *sirt1* Gene Transcription. Total RNA was extracted using TRIzol reagent (Invitrogen Corporation). cDNA was synthesized using oligo-dT primers and SuperScript polymerase (Invitrogen) following the manufacturer's recommendations. The levels of *sirt1* cDNA were analyzed by real-time PCR using SyBRGreen reagent (Invitrogen). The primers for *sirt1* are 5′-gttctgactggagctggggtttctg-3′ and 5′-tgatttgtctgatggatagtttac-3′; for *egr2* are 5′-gaacggagtggcgggagatgg-3′ and 5′-gcggattaagggtggzagtcac-3′. Amplification of *β-actin* cDNA was used as an inter control. The primers were 5′-tgggccgctctaggcaccaa-3′ and 5′-ctcttgatgtcacgcacgatttc-3′.

Luciferase Assay. Experiments were performed as previously described (2). Briefly, HEK293 cells in 12-well plates were transfected with pRL-TK (Promega) and each of the Sirt1-luc plasmids, along with EGR2, EGR3, or FoxO3a expression plasmids, or both as indicated. Transfected cells were lysed 2 d after transfection. The luciferase activities in the cell lysates were analyzed using a Dual Luciferase Reporter assay kit (Promega). Luciferase activity was measured using a luminometer (Turner BioSystems) and expressed in relative light units.

1. Kong S, et al. (2011) The type III histone deacetylase Sirt1 protein suppresses p300-mediated histone H3 lysine 56 acetylation at Bclaf1 promoter to inhibit T cell activation. *J Biol Chem* 286:16967–16975.

2. Chen A, et al. (2009) The HECT-type E3 ubiquitin ligase AIP2 inhibits activation-induced T-cell death by catalyzing EGR2 ubiquitination. *Mol Cell Biol* 29:5348–5356.