Supplementary Materials

Additional methods

Serum biochemistry

Serum ALT and AST levels in mice, an indicator of hepatocellular injury, were measured by IDEXX Laboratories (Westbrook, ME).

Liver histology

Formalin-fixed, paraffin-embedded liver sections (5μm) were stained with hematoxylin and eosin (HE). The severity of liver IRI was assessed blindly and graded according to the modified Suzuki's criteria on a scale from 0 to 4. No necrosis, congestion/centrilobular ballooning is given a score of 0, while severe congestion >60% lobular necrosis is given a value of 4 (1).

TdT-mediated dUTP nick end labeling (TUNEL) assay

Hepatocellular death in formalin-fixed paraffin-embedded murine liver sections was detected by TUNEL assay using ApopTag Plus Peroxidase in Situ Apoptosis Kit (Millipore, Temecula, CA) following the manufacturer's protocol. Results were scored semi-quantitatively by blindly counting the number of TUNEL+ cells in 10 HPF/section (×400).

Immunohistochemistry

Mouse liver infiltrating neutrophils and macrophages were detected using monoclonal rat anti-Ly6G Ab (BD Biosciences, San Jose, CA) and rat anti-CD68 Ab (AbD Serotec), respectively. Immunostaining signals were visualized with a labeled polymer in the EnVision + system horseradish peroxidase kit (Dako). Positive cells were counted blindly (10 HPF/section ×400).

Cell isolations and in vitro cultures

Femurs and tibias were removed from WT mice, and bone marrow-derived macrophages (BMDM) were generated (2). The siRNA transfection for BMDM was conducted using Lipofectamine reagent (Invitrogen, San Diego, CA) according to the manufacturer's protocol. After 24h, cells were supplemented with LPS (100ng/ml) for 6h. In some experiments, cells were pretreated for 12h with Res (100μM).

Spleens were harvested from WT mice, and splenocytes were suspended to single-cell, treated with Red Cell Lysing Buffer (Sigma-Aldrich), as described (3). For T-cell stimulation, cells were incubated for 24h with ConA (5μg/ml) with or without Res supplement (100μM, 12h).

Western blot analysis

Proteins were extracted from frozen liver tissue samples or cell culture samples. Protein concentration was measured using BCA Protein Assay Kit (Thermo Scientific, Rockford, IL). Equal amount of protein extracts was electrophoresed, blotted, and incubated with primary Ab, secondary HRP-conjugated Ab, and developed. Primary Ab detecting SIRT1, Cox2, iNOS, p-IkBα (Ser32), IkBα, p-Stat1 (Tyr701), cleaved caspase-3, β-actin (Cell Signaling Technology, Danvers, MA) and Tbet (Santa Cruz Biotechnology) were used. To

compare target protein expression in multiple human OLT samples, densitometry quantification was conducted as follows. In a preliminary study, one of the Bx samples expressing all target proteins was chosen and assigned as a “reference” sample. Equal amount of protein lysate from each sample was applied to each well/gel, and the target band intensity was normalized to the reference sample expression in the same gel followed by normalization with β-actin.

Quantitative RT-PCR analysis

Total RNA was extracted from frozen liver tissue samples or cell culture samples using RNAse Mini Kit (Qiagen Inc., Chatsworth, CA). A total of 5.0μg of RNA was reverse-transcribed into cDNA. Quantitative PCR was performed using DNA Engine with Chromo 4 Detector (MJ Research, Waltham, MA) (3). The primers sequences are listed in Supplementary Table 2. The expression of the target gene was normalized to the housekeeping gene HPRT or GAPDH.

Liver lymphocyte isolation and fluorescence-activated cell sorting (FACS)

Liver samples, filtered in PBS using a 70μm nylon filter, were suspended with Percoll (GE Healthcare Bio-Sciences, Uppsala, Sweden) containing heparin to the final concentration of 33%, and centrifuged (600 x g for 20 min). The upper layer and interface was discarded, and the cell pellet was washed with PBS followed by centrifugation (300 x g for 5min). Five ml of ACK lysing buffer (Quality Biological, Inc. Gaithersburg, MD) was used to lyse red blood cells (5min). After washing with PBS, isolated cells were incubated with anti-mouse CD16/32 antibody (clone 93, BioLegend, San Diego, CA) to block the Fc-mediated reactions, followed by staining with anti-CD3-BV785 (clone 17A2, BioLegend). Subsequently, cells were fixed, permeabilized and stained with anti-IFNγ-APC (clone XMG1.1, BioLegend) in PBS containing true-Nuclear Transcription Factor Buffer Set (BioLegend) according to the manufacture’s instruction. Optimal concentrations of antibodies and cells were determined experimentally and appropriate isotope controls were used. FACS analyses were performed using LSRFortessa X-20 SORP (BD Bioscience, San Jose, CA) and results were analyzed using BD FACSDiva software (BD) at the UCLA Jonsson Comprehensive Cancer Center.

References

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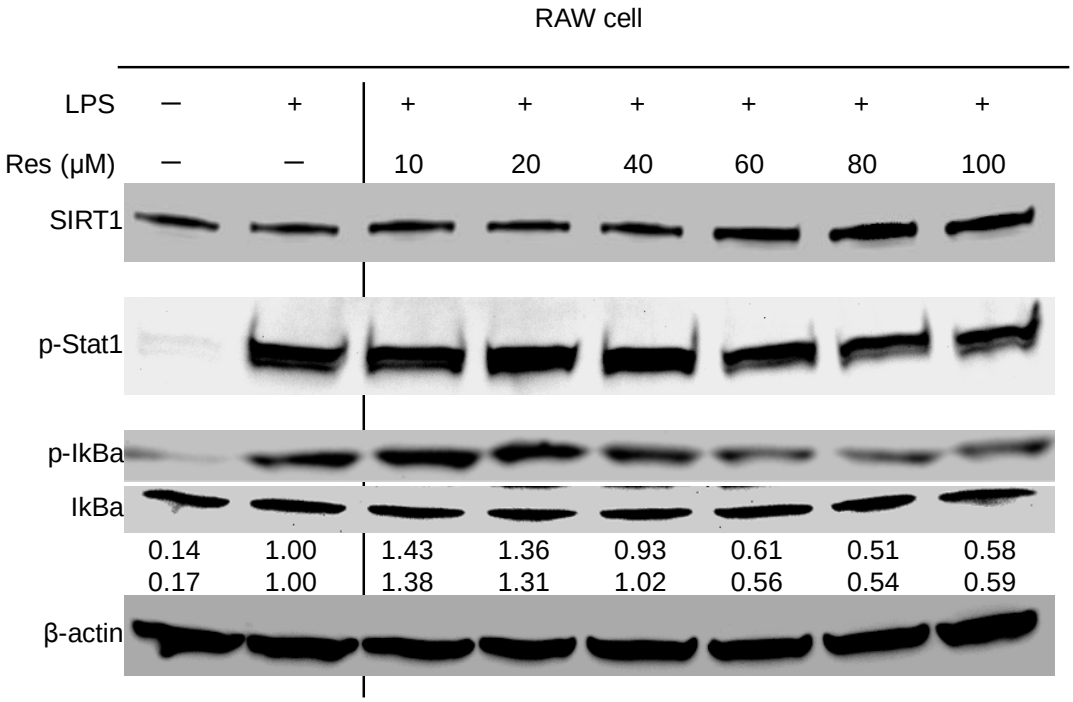
2. Huang J, Shen XD, Yue S, Zhu J, Gao F, Zhai Y, et al. Adoptive transfer of heme oxygenase-1 (HO-1)-modified macrophages rescues the nuclear factor erythroid 2-related factor (Nrf2) antiinflammatory phenotype in liver ischemia/reperfusion injury. Molecular medicine (Cambridge, Mass). 2014;20:448-55.

3. Uchida Y, Ke B, Freitas MC, Yagita H, Akiba H, Busuttil RW, et al. T-cell immunoglobulin mucin-3 determines severity of liver ischemia/reperfusion injury in mice in a TLR4-dependent manner. Gastroenterology. 2010;139(6):2195-206.



Supplementary Fig. 1: SIRT1 downregulates p-Stat1 and p-IkBα in RAW cell cultures

Western blot-assisted detection of SIRT1, p-Stat1, p-IκBα and IκBα in LPS-stimulated RAW cell cultures supplemented with increasing doses of Res (12h). β-actin expression serves as an internal control. The values under the bands represent the relative ratio of normalized intensity compared to that of cells+LPS. Representative of three experiments is shown.

Supplementary Table 1: Donor and recipient demographics.

Donor	low SIRT1 (n=11)	high SIRT1 (n=10)	p value
Age (years)	39.6±18.0	35.5±15.7	p=0.48
Gender (M/F)	5 / 6	7 / 3	p=0.38
Weight (kg)	79.6±11.9	80.9±16.4	p=1.00
BMI (kg/m^2)	28.1±4.8	27.2±5.8	p=0.48
Cold Ischemic Time (min)	494±95	426±164	p=0.09
Donation status	All of the donors were donation after brain death		

Recipient	low SIRT1 (n=11)	high SIRT1 (n=10)	p value
Age (years)	57.6±6.1	47.7±10.8	p=0.13
Gender (M/F)	8 / 3	6 / 4	p=0.65
Weight (kg)	89.2±23.7	84.5±34.4	p=0.62
BMI (kg/m^2)	29.2±6.1	29.3±8.6	p=1.00
Race			p=0.09
Asian	1	1	
Black/African American	0	0	
Caucasian/White	6	3	
Hispanic/Latino(a)	3	6	
other	0	1	
Disease ethiology			p=0.31
Alchol	4	1	
HCV	2	5	
NASH	1	2	
other	4	2	
HCC (with/without)	4 / 7	1 / 9	p=0.31
ABO			p=1.00
identical	10	9	
compatible	1	1	
MELD score	33.5±7.1	34.8±5.7	p=0.51
Pre-transplant ALT (IU/L)	62.0±43.1	54.2±35.4	p=0.80
Pre-transplant AST (IU/L)	88.8±60.9	116.4±89.2	p=0.48
Pre-transplant T-Bil (mg/dl)	16.4±13.1	16.6±16.3	p=0.83
Intraoperative blood loss (L)	6.6±4.3	5.1±5.0	p=0.44

Twenty-one post-transplant human liver biopsy samples were divided based on the relative SIRT1 expression levels (low - n=11 or high - n=10). Correlations between donor and recipient demographic parameters and post-transplant SIRT1 levels were analyzed using Fisher's exact test for categorical variables and Mann–Whitney U test for continuous values. Values are expressed as means ± SD.

Supplementary Table 2: Primer sequences used for Real-Time Quantitative PCR

Gene	Forward	Reverse
mouse SIRT1	5'-ATCGGCTACCGAGACAAC-3'	5'-GTCACTAGAGCTGGCGTGT-3'
mouse IL1β	5'-TGTAATGAAAGACGGCACACC-3'	5'-TCTTCTTTGGGTATTGCTTGG-3'
mouse MCP1	5'-CATCCACGTGTTGGCTCA-3'	5'-GATCATCTTGCTGGTGAATGAGT-3'
mouse TNFα	5'-GCCTCTTCTCATTCCTGCTTGT-3'	5'-GATGATCTGAGTGTGAGGGTCTG-3'
mouse CXCL10	5'-GCTGCCGTCATTTTCTGC-3'	5'-TCTCACTGGCCCGTCATC-3'
mouse CCL5	5'-CAGCAGCAAGTGCTCCAATCTT-3'	5'-TTCTTGAACCCACTTCTTCTCTGG-3'
mouse IFNγ	5'-TCTGGAGGAACTGGCAAAAG-3'	5'-TTCAAGACTTCAAAGAGTCTGAGG-3'
mouse IL2	5'-GCTGTTGATGGACCTACAGGA-3'	5'-ATCCTGGGGAGTTTCAGGTT-3'
mouse HPRT	5'-TCAACGGGGGACATAAAAGT-3'	5'-TGCATTGTTTTACCAGTGTCAA-3'
human IL1β	5'-CTGTCCTGCGTGTTGAAAGA-3'	5'-TTGGGTAATTTTTGGGATCTACA-3'
human MCP1	5'-TTCTGTGCCTGCTGCTCAT-3'	5'-GGGGCATTGATTGCATCT-3'
human TNFα	5'-CAGCCTCTTCTCCTTCCTGA-3'	5'-GCCAGAGGGCTGATTAGAGA-3'
human CXCL10	5'-CCAGAATCGAAGGCCATCAA-3'	5'-CATTTCCTTGCTAACTGCTTTCAG-3'
human IFNγ	5'-CTAATTATTCGGTAACTGACTTGA-3'	5'-ACAGTTCAGCCATCACTTGGA-3'
human GAPDH	5'-AGCCACATCGCTCAGACAC-3'	5'-GCCCAATACGACCAAATCC-3'