SUPPLEMENTARY DATA

Supplementary Materials and Methods

Genotyping of Animals

Genotyping was performed by PCR of tail clip DNA. Primers TTCACATTGCATGTGTGTGG and TAGCCTGCAGTGTGGTG amplify a 423-bp fragment from the normal sirt1 allele whereas a 526-bp fragment from the null allele is amplified from the first primer. \(^1\)

Induction of permanent cerebral ischemia

All experiments have been performed and quantified in a randomized manner by investigators blinded to treatment groups for the prevention of bias. Mice were anesthetized with 3% isoflurane (in 70% N\(_2\)O, 30% O\(_2\)) for induction and with 1.5% isoflurane for maintenance. Rectal temperature was maintained at 36.5°C with a heating pad. Common carotid (CCA) and middle cerebral artery (MCA) were exposed and occluded permanently by ligation as previously described\(^2\) with some modifications. Due to the anatomical differences between Sirt1\(^{-/-}\) and WT mice (the trunk of the MCA was not easily accessible for ligation in Sirt1\(^{-/-}\) animals), MCA was occluded at its rostral branch in both WT and knockout mice in the set of experiments aimed to study the effect of the genetic deletion of Sirt1 on infarct volume. In contrast, in the set of experiments to ascertain the effect of the pharmacological modulation of SIRT1 on infarct volume, the artery was occluded in its trunk. Finally, in a third set of experiments, aimed to the effect of pharmacological treatments and Sirt1 deletion on the levels of acetylated SIRT1 substrates, the ligature was performed at the branch of the MCA to compare all the experimental groups.
Complete interruption of the blood flow was confirmed under an operating microscope. Sham-operated animals were subjected to anesthesia and the surgical procedure but the occlusion of the arteries was omitted. Following surgery, subjects were returned to their cages and allowed free access to water and food. The survival rate of the animals until the end of the experiment was 90%.

**Determination of physiological parameters**

Mice were placed on a homeothermic blanket (Harvard Apparatus) throughout the duration of the measurement to prevent anesthesia-induced hypothermia. A polyethylene catheter was inserted into the right femoral artery. All parameters were measured 10 minutes before surgery and 30 minutes after ischemia. Mean arterial pressure was recorded through a Lab-Trax-4/24T (World Precision Instruments). Physiological parameters were tested using iStat System blood analyzer (Ven-Bios ES).

**Neurological deficit evaluation**

Two days after surgical procedure, neurological deficit was assessed by two independent researchers blinded to experimental conditions. Neurological assessment was performed by using a modified neurological severity score (mNSS).\(^2\) mNSS is graded on a scale of 0 to 16, with a higher score indicating more severe sensory-motor deficits, the test is sensitive to unilateral cortical injury because it reflects multiple asymmetries, including postural, sensory, and forelimb and hind limb use asymmetries.
**Determination of infarct size**

Two days after permanent MCAO (pMCAO), animals were killed by cervical dislocation to assess infarct outcome. Brain was removed and cut into 1mm thick coronal slices and stained with 2,3,5-triphenyltetrazolium chloride (1% TTC in 0.1M phosphate buffer). Infarct size was determined as follows: infarct volumes were measured by sampling stained sections with a digital camera (Nikon Coolpix 990, Nikon Corporation, Tokyo, Japan), and the image of each section was analyzed using ImageJ 1.44i (NIH, Bethesda, MD, USA). The digitalized image was displayed on a video monitor. With the observer masked to the experimental conditions, the areas of the infarcted tissue (InfArea), the whole ipsilesional hemisphere (IpsArea) and the whole contralesional hemisphere (ContrArea) are delineated for each slice. Then, infarct volume, expressed as % of the hemisphere that is infarcted (%IH), is calculated using the formula:

\[
\%IH = \frac{\text{InfVol}}{\text{ContrVol}} \times 100
\]

where

\[
\text{InfVol} (\text{Infarcted Tissue Volume}) = \sum \frac{\text{InfArea}}{\text{SwellingIndex}}
\]

\[
\text{ContrVol} (\text{Contralesional Hemisphere Volume}) = \sum \text{ContrArea}
\]

and

\[
\text{SwellingIndex} = \frac{\text{IpsArea}}{\text{ContrArea}}.
\]

**Protein determination by western blot**

Protein concentration was determined in tissue homogenates with the Bradford protein assay. Equal amounts of total protein (35 µg) were resolved by SDS-PAGE and transferred to nitrocellulose membranes. Immunodetection was performed by standard procedures. The membranes were blocked with 5% nonfat milk in TBS-T (0.05% Tween 20 in TBS) and probed with specific primary
antibodies against Sirt1 (Cell Signaling; 1:500 dilution), acetyl-histone H3 (Millipore; 1:500), acetyl-histone H4 (Millipore; 1:500), p53 (acetyl K381) (Abcam; 1:1000), NFκB (acetyl K310) (Abcam; 1:1000) and mouse anti-β-actin (Sigma; 1:10000) was included to ensure equal protein loading. Specific signals were quantified using densitometry analysis software (ImageJ).

**Immunofluorescence and confocal microscopy**

Free-floating coronal brain slices (30 µm) were processed as described previously. In brief, brain sections were blocked with 5% goat serum and incubated with mouse anti-neuronal nuclei (NeuN) and anti-glial fibrillary acidic protein (GFAP), and rabbit anti-Sirt1 (Santa Cruz) 1:100 at 4ºC overnight followed by the appropriate rabbit secondary antibody Alexa 488 (Invitrogen A-11008), anti-mouse Cy3 (Jackson Immuno Research; 715-165-151) 2 h at room temperature. All immunofluorescence images were obtained in a blinded manner from seven correlative slices of each brain. Image acquisition was performed with a laser-scanning confocal imaging system (Zeiss LSM710) and image analysis was performed with the ZEN 2009 software (Zeiss). All co-localization images shown were confirmed by orthogonal projection of z-stack files.

**Supplementary References**


