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NEURON-SPECIFIC SUMO KNOCKDOWN SUPPRESSES GLOBAL GENE EXPRESSION RESPONSE AND WORSENS FUNCTIONAL OUTCOME AFTER TRANSIENT FOREBRAIN ISCHEMIA IN MICE

LIN ZHANG[a,b], **XIAOZHI LIU**[a,b], **HUAXIN SHENG**[a], **SHUAI LIU**[a], **YING LI**[a,c], **JULIA Q. ZHAO**[a], **DAVID S. WARNER**[a], **WULF PASCHEN**[a,*], and **WEI YANG**[a,*]

[a] Multidisciplinary Neuroprotection Laboratories, Department of Anesthesiology, Duke University Medical Center, Durham, NC, USA

[b] Department of Neurosurgery, The Fifth Central Hospital of Tianjin, Tianjin, China

[c] Department of Cardiology, The Fifth Central Hospital of Tianjin, Tianjin, China

Abstract

Small ubiquitin-like modifier (SUMO) conjugation (SUMOylation) plays key roles in neurologic function in health and disease. Neuronal SUMOylation is essential for emotionality and cognition, and this pathway is dramatically activated in post-ischemic neurons, a neuroprotective response to ischemia. It is also known from cell culture studies that SUMOylation modulates gene expression. However, it remains unknown how SUMOylation regulates neuronal gene expression *in vivo*, in the physiologic state and after ischemia, and modulates post-ischemic recovery of neurologic function. To address these important questions, we used a SUMO1-3 knockdown (SUMO-KD) mouse in which a Thy-1 promoter drives expression of 3 distinct microRNAs against SUMO1-3 to silence SUMO expression specifically in neurons. Wild-type and SUMO-KD mice were subjected to transient forebrain ischemia. Microarray analysis was performed in hippocampal CA1 samples, and neurologic function was evaluated. SUMOylation had opposite effects on neuronal gene expression before and after ischemia. In the physiological state, most genes regulated by SUMOylation were up-regulated in SUMO-KD compared to wild-type mice. Brain ischemia/reperfusion significantly modulated the expression levels of more than 400 genes in wild-type mice, with a majority of those genes upregulated. The extent of this post-ischemic transcriptome change was suppressed in SUMO-KD mice. Moreover, SUMO-KD mice exhibited significantly worse functional outcome. This suggests that suppression of global gene expression response in post-ischemic brain due to SUMO knockdown has a negative effect on post-ischemic neurologic function. Together, our data provide a basis for future studies to mechanistically link SUMOylation to neurologic function in health and disease.

[*]Corresponding authors. Address: Department of Anesthesiology, Duke University Medical Center, Box 3094, 152 Sands Building, Research Drive, Durham, NC 27710, USA. wulf.paschen@duke.edu (W. Paschen), wei.yang@duke.edu (W. Yang).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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Keywords

brain ischemia; SUMO; microarray; knockdown; transgenic mice

INTRODUCTION

Small ubiquitin-like modifier (SUMO) conjugation (SUMOylation) is a post-translational protein modification whereby SUMOs are covalently conjugated to lysine residues in target proteins (Flotho and Melchior, 2013). This is an energy-dependent process that is regulated via the action of activating (E1, ATP-dependent), conjugating (E2), and ligating (E3) enzymes. In mammalian cells, three SUMO isoforms have been identified: SUMO1, SUMO2, and SUMO3. SUMO2 and SUMO3 are highly homologous, and are usually referred to as SUMO2/3. SUMO1, however, shares only about 50% homology with SUMO2/3. Notably, while SUMO1 knockout mice and SUMO3 knockout mice do not show any obvious abnormality, SUMO2 knockout is lethal in the embryonic stage (Evdokimov et al., 2008; Wang et al., 2014b).

SUMOylation can modify the activity, stability, and function of target proteins, and thereby modulate almost all major cellular pathways (Flotho and Melchior, 2013). Our knowledge about the significance of SUMO conjugation in cellular homeostasis is primarily based on results from cell culture experiments. In the physiologic state, most SUMO1 is conjugated to target proteins, predominately the Ran GTPase-activating protein-1 (RanGAP1). Thus, under stress conditions, there is only small change in SUMO1 modification profiles. In contrast, most SUMO2/3 is present as free SUMO in the physiologic state. However, under a variety of stress conditions, including heat, metabolic, and oxidative stress, SUMO2/3 conjugation is dramatically activated (Saitoh and Hinchey, 2000; Yang et al., 2012).

Many of the SUMO targets are nuclear proteins involved in gene expression (Golebiowski et al., 2009; Yang et al., 2014). It has been reported that SUMOylation can both activate and suppress transcription. In most cases, however, gene expression is suppressed when the respective transcription factor is SUMO-conjugated (Gill, 2005; Chymkowitch et al., 2015). The mechanisms that link SUMO conjugation to gene expression are still not fully understood. Generally, experimental studies have focused on individual transcription factors to clarify the role of SUMOylation in expression of target genes. Recently, chromatin immunoprecipitation coupled with next-generation sequencing (ChIP-seq) was used to more completely define these mechanisms (Liu et al., 2012; Neyret-Kahn et al., 2013; Seifert et al., 2015). These studies were carried out in cell cultures. A genome-wide analysis of the effects of SUMOylation on gene expression *in vivo* has not yet been performed.

SUMO conjugation plays a pivotal role in neurologic function in the physiologic and pathologic state. For example, SUMOylation is essential for emotionality and cognition, and silencing SUMO1-3 expression specifically in neurons impairs episodic and fear memories (Wang et al., 2014a). However, the pathways that link SUMOylation to memory processes have not been identified. Furthermore, SUMOylation is associated with brain ischemia/stroke and degenerative diseases (Yang et al., 2008b,c; Flotho and Melchior, 2013; Krumova and Weishaupt, 2013). Transient brain ischemia is a severe form of metabolic stress that

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triggers dramatic activation of SUMO2/3 conjugation, and to a lesser extent, SUMO1 conjugation (Yang et al., 2014). It has been proposed that this is a protective response that shields neurons from damage induced by ischemia (Yang et al., 2008a, 2016; Lee and Hallenbeck, 2013). Indeed, results from *in vitro* and *in vivo* studies support this notion. For example, neurons in which SUMO2/3 expression is silenced by lentiviral delivery of SUMO2 and SUMO3 microRNAs (miRNAs), are highly sensitive to transient oxygen/glucose deprivation (OGD, an experimental model that simulates ischemia in cells), whereas transgenic mice overexpressing SUMO conjugating enzyme Ubc9, have higher levels of SUMO1- and SUMO2/3-conjugated proteins and smaller infarcts after stroke (Datwyler et al., 2011; Lee et al., 2011). However, we still do not know the role of SUMO conjugation in post-ischemic neurologic function, which ultimately defines quality of life for patients suffering from ischemic brain damage, and how SUMOylation modulates the genome regulated by transient ischemia. Here, we report our findings from the first experimental study that clarifies the contribution of SUMO conjugation to pre- and post-ischemic gene expression and functional outcome. For this study, we used a recently developed neuron-specific SUMO1-3 knockdown (SUMO-KD) mouse model (Wang et al., 2014a).

EXPERIMENTAL PROCEDURES

Animals

All animal experiments were approved by the Duke University Animal Care and Use Committee. A total of 72 mice were used in this study. SUMO-KD transgenic mice were previously generated in our laboratory (Wang et al., 2014a). In this transgenic mouse model, the transgene contains 3 distinct miRNAs that target SUMO1, 2, and 3, and are expressed under the control of the neuron-specific Thy1 promoter. Green fluorescent protein (GFP) is co-expressed as indicator of transgene expression. SUMOKD mice have been backcrossed with C57Bl/6 mice for more than 10 generations. Male SUMO-KD and wild-type littermates (2–3 months old) were used in this study.

Transient forebrain ischemia

Transient forebrain ischemia was performed as described previously (Yang et al., 2008c). Briefly, anesthesia was induced with 5% isoflurane and maintained with 1.5–1.8% isoflurane during surgery. The rectal temperature was maintained at 37.0 °C ± 0.2 °C by surface heating or cooling. PE-10 tubes were inserted into the right femoral artery and the right internal jugular vein to continuously monitor arterial blood pressure and to withdraw blood, respectively. Forebrain ischemia was induced by a combination of 10-min bilateral common carotid artery occlusion, and blood withdrawal-induced hypotension (mean arterial blood pressure = 30 mmHg). To end the ischemic episode, the carotid arteries were de-occluded and withdrawn blood was re-infused. Sham-operated mice underwent the same procedures except carotid artery occlusion and blood withdrawal. To determine whether SUMO knockdown had any effect on blood flow reduction in our transient forebrain ischemia model, a cohort of mice was subjected to blood flow measurements. Before inducing ischemia, a microprobe (Moor) was affixed to the surface of the right parietal skull to monitor regional cerebral blood flow (rCBF) in the middle cerebral artery territory by Laser Doppler flowmetry (Moor).

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Tissue sample preparation

At the indicated times of reperfusion, mice were sacrificed, and brains were quickly removed. CA1 and cortex samples were excised in a cryostat set at −20 °C. Tissue samples were stored at −80 °C and later used for RNA or protein preparation. For immunohistochemistry analysis, transcardial perfusion was performed using 4% paraformaldehyde. Brains were collected and fixed overnight. The fixed brains were either embedded in paraffin or immersed in 20% sucrose at 4 °C before stored in −80 °C.

Microarray analysis

CA1 subfield tissue samples were harvested from 4 experimental groups: wild-type sham (WS), wild-type ischemia (WI), SUMO-KD sham (TS), and SUMO-KD ischemia (TI). Post-ischemia samples were collected at 3 h reperfusion. For each group, samples were prepared in triplicate. To minimize variation in biological replicates, CA1 subfield samples from two mouse brains were pooled, and used to prepare total RNA for one independent microarray sample. The Affymetrix GeneChip Mouse Genome 430A 2.0 Array, which contains approximately 14,000 well characterized mouse genes, was used. Synthesis of cDNA, labeling of samples, and array processing was performed at the Duke Sequencing and Genomic Technologies facility (Yang et al., 2009). Partek Genomics Suite 6.6 (Partek) was used to identify differentially expressed genes, and to perform principal component analysis (PCA). Robust multi-chip analysis (RMA) normalization was performed on the entire data set. The differentially expressed genes were selected based on a p value <0.05 (as determined by ANOVA), and a fold-change ≥2. The differentially expressed genes were further analyzed using PANTHER (http://www.pantherdb.org/) and DAVID (https://david.ncifcrf.gov/) to identify enriched genes according to gene ontology (GO) classifications and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways.

Quantitative PCR

Quantitative real-time PCR (qPCR) was performed as previously described (Yang et al., 2009). In short, total RNA was prepared from CA1 tissue samples using the TRIzol reagent (Invitrogen). Total RNA (200 ng) was reverse transcribed into cDNA (Invitrogen). qPCR was performed in a Lightcycler 2.0 (Roche). Fold changes were calculated according to the comparative threshold cycle (Ct) method (Schmittgen and Livak, 2008). All primers used in this study are listed in Table 1.

Western blot analysis

Western blotting was conducted as described before (Yang et al., 2014). Briefly, protein samples were run on SDS–PAGE pre-cast gels (Bio-Rad), and were transferred to PVDF membranes. Membranes were blocked with TBST containing 5% milk, and incubated with a primary antibody overnight at 4 °C. After extensive washing, membranes were incubated with a secondary antibody for 1 h at room temperature. Proteins were then visualized using the enhanced chemiluminescence analysis system (GE Healthcare). After exposure, membranes were stripped and re-probed for β-actin as loading control. Image analysis was performed using the ImageJ program (NIH). The following primary antibodies were used: mouse monoclonal anti-SUMO1 (21C7; DSHB Hybridoma), rabbit polyclonal anti-

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SUMO2/3 (Covance), rabbit polyclonal anti-GFP (Invitrogen), and mouse monoclonal anti-β -actin (Sigma).

Immunofluorescence

Immunofluorescence staining was performed on paraffin-embedded brain sections (for SUMO1) or frozen brain sections (for SUMO2/3), as described previously (Yang et al., 2014). In short, after deparaffinization, brain sections (5-μm-thick) were incubated with primary antibodies at 4 °C overnight, followed by appropriate secondary fluorescent antibodies for 1 h at room temperature. The frozen brain sections (25 μm) were immunostained using a free-floating staining method. Confocal images were captured on a Leica SP5 confocal microscope (Leica Microsystems). The following primary antibodies were used: mouse monoclonal anti-SUMO1 (21C7; DSHB Hybridoma), rabbit polyclonal anti-SUMO2/3 (Covance), mouse monoclonal anti-GFP (Millipore), and rabbit polyclonal anti-GFP (Invitrogen).

Neurologic score system

Neurologic deficits were assessed by an observer blinded to the genotype of animals using a 9-point score system (Wellons et al., 2000). The assessment was performed before (Pre) and on day 4 after (Post) forebrain ischemia. After testing was complete, the score for each animal was the sum of the individual scores, with 0 = normal and 9 = severe injury.

Statistical analysis

The Prism 6 software (GraphPad) was used to analyze all data. Statistical analysis was assessed by Mann–Whitney *U* test on all data except the rCBF data. The rCBF data were analyzed by a 2-way analysis of variance (ANOVA) with Bonferroni's post hoc test for multiple comparisons. *P* values ≤0.05 were considered significant.

RESULTS

Effect of ischemia on SUMOylation in SUMO knockdown mice

Detailed characterization of SUMO-KD mice has been described (Wang et al., 2014a). In this mouse model, three miRNAs that target SUMO1-3 are expressed together with GFP as indicator of transgene expression. Expression of designed miRNAs is driven by the Thy-1 promoter to achieve neuron-specific silencing of SUMO1-3. For the current study, we used SUMO-KD line 27 mice in which GFP is widely expressed in layer V of the cerebral cortex, and the hippocampal CA1 subfield (Wang et al., 2014a). Our earlier quantitative analysis revealed that in the hippocampal CA1 subfield, GFP is expressed in about 70% of neurons. To evaluate the change in SUMOylation after ischemia in SUMO-KD mice, we subjected wild-type and SUMO-KD mice to 10-min forebrain ischemia and 1 h reperfusion. Consistent with our previous findings (Yang et al., 2008c, 2014), levels of SUMO2/3-conjugated proteins in wild-type mice increased massively in both the cortex and hippocampus after brain ischemia (Fig. 1A, B). In SUMO-KD mice, however, the increase in post-ischemic SUMOylation was notably less (Fig. 1A, B). Quantitative Western blotting analysis indicated that the induction fold of SUMO2/3 conjugation in SUMO-KD was

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around 50% of that found in wild-type mice (Fig. 1C, D; Mann–Whitney *U* test, $p \leq 0.05$, $n = 3$/group).

We also used immunohistochemistry to evaluate the efficacy of SUMO1-3 miRNAs to silence SUMO expression (Fig. 1E, F). Double fluorescence immunostaining with anti-GFP and anti-SUMO2/3 antibodies revealed that the robust nuclear accumulation of SUMO2/3-conjugated proteins in post-ischemic GFP-negative neurons (Fig. 1E, arrows) was absent in GFP-positive cells (Fig. 1E, arrowheads). As expected, nuclear rim staining was observed for SUMO1. Unlike SUMO2/3, there was no dramatic increase in SUMO1-conjugated proteins nor obvious change in subcellular localization of SUMO1 after ischemia. However, the signal intensity of SUMO1 staining appeared less in GFP-positive neurons (Fig. 1F, arrowheads) than in GFP-negative neurons (Fig. 1F, arrows). Together, our data confirmed that the post-ischemic increase in SUMOylation was effectively suppressed in SUMO-KD mice.

Effects of ischemia on gene expression in SUMO knockdown mice

Our knowledge of the modulating effects of SUMO conjugation on gene expression is mostly based on results from *in vitro* cell culture studies. For this first genome-wide analysis of genes differentially regulated after brain ischemia in wild-type and SUMO-KD mice, we focused on the hippocampal CA1 subfield because CA1 neurons are particularly sensitive to transient ischemia, and SUMO expression is effectively silenced in most CA1 neurons of SUMO-KD mice (Fig. 1). Further, CA1 neurons play a key role in memory processes, and SUMO-KD mice are impaired in episodic and fear memory (Wang et al., 2014a). Since we have shown that the peak level of SUMO conjugated proteins in the brain induced by global forebrain ischemia is at 1 h of reperfusion (Yang et al., 2014), we decided to use brain samples collected at 3 h of reperfusion after ischemia for microarray analysis.

Microarray analysis was performed on samples prepared in triplicate from four groups: WS and WI, and SUMO-KD transgenic sham (TS) and ischemia (TI). The hippocampal CA1 subfield tissues were carefully excised, as indicated in Fig. 2A, and used for RNA preparation and microarray analysis. The raw data from the current study have been deposited in NCBI's Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/geo/), and are accessible through GEO Series accession number GSE80681. An overview of microarray results is depicted in Fig. 2. First, we performed data analysis on the global gene expression profiles of all 12 samples by plotting individual samples in a 3-dimensional space based on principal components analysis (PCA; Fig. 2B). PCA demonstrated a clear separation between sham and ischemia, and between wild-type and SUMO-KD, indicating excellent reproducibility of samples and apparent effects of SUMO knockdown and ischemia on gene expression.

We then defined selection criteria (≥ twofold change and $p < 0.05$) to identify genes differentially regulated in wild-type and SUMO-KD mice subjected to sham or ischemia surgery. The genes identified from different comparisons are listed in the Appendix Table and overviewed in Fig. 2C. To validate microarray data, we selected 11 of these genes, and performed qPCR analysis (Table 2). The same trend of expression change in the respective comparison, ie, up-regulation or down-regulation, was observed for all selected genes,

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although the fold changes of some genes were greater in the qPCR analysis than in the microarray data. Thus, the qPCR results validated our microarray data.

To characterize the effects of genotype and ischemia on gene regulation, we performed pairwise comparison of groups (Appendix Table). The number of differentially regulated genes among all 3 comparisons, and their expression changes after ischemia (up- or down-regulated) are presented in the Venn diagram in Fig. 2C A large number of genes were differentially regulated by ischemia in both wild-type and SUMO-KD mice. In wild-type mice, 419 genes were regulated by ischemia (Fig. 2C; WI vs WS), while 270 genes were regulated by ischemia in SUMO-KD mice (Fig. 2C; TI vs TS). However, a total of 196 genes showed ischemia-induced differential expression uniquely in wild-type mice, more than that in SUMO-KD mice. In sham animals, 64 genes were differently regulated by silencing SUMO expression, and most of these genes were up-regulated (Fig. 2C; TS vs WS). Notably, only one gene showed up in all three pairwise comparisons – the gene coding for the activity-regulated cytoskeleton-associated protein (Arc; also known as Arg3.1). Specifically, Arc was significantly down-regulated in SUMO-KD sham mice. After ischemia, it was up-regulated in both wild-type and SUMO-KD mice.

Microarray data show that the expression levels of SUMO1-3 decreased but with a fold change <2 in SUMO-KD mice compared to wild-type mice, and therefore, according to our pre-defined criteria, none of the three SUMOs were identified as differentially regulated genes (Appendix Table). To verify that expression of all SUMOs was indeed silenced in SUMO-KD mice, we performed qPCR on the hippocampal CA1 samples. The qPCR analysis revealed that SUMO1-3 mRNA levels in SUMO-KD mice were significantly reduced to 51.6% ± 6.7% (SUMO1), 44.3% ± 4.9% (SUMO2), and 53.1% ± 8.4% (SUMO3) compared to wild-type (Fig. 2D; mean ± SEM, Mann–Whitney U test, $p \leq 0.05$, $n = 3$/group). This confirms the high capacity of SUMO miRNAs to silence SUMO expression, considering that SUMOs are expressed in all mammalian cells, and that here, SUMO is knocked down only in neurons of which about 70% express SUMO miRNAs in the hippocampal CA1 subfield. Taken together, the microarray analysis generated a reliable dataset of genes that are differentially regulated by SUMO conjugation in the CA1 subfield after brain ischemia.

Effects of ischemia on processes and pathways in SUMO knockdown mice

We then analyzed our microarray data based on two widely used databases – GO categories and KEGG pathways. GO and KEGG pathway analyses of differentially expressed genes in the CA1 subfield after forebrain ischemia in wild-type and SUMO-KD mice are depicted in Figs. 3 and 4, respectively. Overall, enriched GO terms according to protein class and biological processes, overlapped almost completely between wild-type and SUMO-KD mice. The list of enriched KEGG pathways show some overlaps but also some distinct differences between genotypes. In both datasets, the mitogen-activated protein kinase (MAPK) signaling pathway is particularly enriched, followed by Jak-STAT, p53, and ErbB signaling pathways (Figs. 3 and 4C and C). In these lists, ECM-receptor interaction, endocytosis, and focal adhesion pathways showed up only in SUMO-KD mice after

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ischemia (Fig. 4C), whereas other pathways, such as TGF-beta, Toll-like receptor, and Wnt signaling, were identified only in wild-type mice after ischemia (Fig. 3C).

Gene expression in neurons before and after ischemia in SUMO knockdown mice

Our microarray results suggest that when SUMO expression is silenced in neurons, gene expression is differentially modulated in physiologic vs post-ischemic states (Fig. 2). Fig. 5 shows a visual representation of all genes that were differentially expressed in wild-type and SUMO-KD mice before (Fig. 5A) and after ischemia (Fig. 5B). Notably, of the 64 genes that were regulated by SUMOylation in the physiologic state, 60 were up-regulated, and only four were down-regulated in SUMO-KD mice (Fig. 5A). This observation supports a model whereby in non-stressed neurons SUMO conjugation negatively regulates gene expression *in vivo*. To better visualize the effect of SUMO conjugation on gene expression after ischemia, we compared the fold change of all 223 genes regulated by ischemia in both wild-type and SUMO-KD mice (Fig. 5B). Notably, about 70% of these genes showed a greater fold-change in wild-type compared to SUMO-KD mice (Fig. 5B, fold change ratio below 1), indicating that knockdown of SUMO expression suppresses the post-ischemic activation of gene expression.

Functional recovery after transient forebrain ischemia in SUMO knockdown mice

Results from a variety of *in vitro* and *in vivo* studies suggest that the post-ischemic activation of SUMO conjugation is a neuroprotective stress response. Recovery of neurologic function defines quality of life for patients who have suffered an ischemic attack; however, the effect of SUMO conjugation on functional recovery after ischemia has not yet been studied. To address this important aspect, we first confirmed that neuron-specific SUMO knockdown did not affect blood flow reduction in our transient forebrain ischemia model (Fig. 6A). Then, we used a 9-point scoring system to evaluate neurologic function before and 4 days after 10-min forebrain ischemia in wild-type and SUMO-KD mice. We did not find a significant difference in neurologic scores between wild-type and SUMO-KD mice before ischemia (Fig. 6B). After ischemia, however, impairment of neurologic functions was significantly more pronounced in SUMO-KD compared to wild-type mice (Fig. 6B; Mann–Whitney U test, $p \leq 0.01$; $n = 10$ or 11/group).

DISCUSSION

Recently, SUMO conjugation has attracted attention in neuroscience, because this post-translational protein modification plays key roles in physiologic neurologic functions, including memory processes, and is associated with a variety of diseases of major clinical significance including brain tumor, brain ischemia, and neurodegenerative diseases (Yang et al., 2008b,c, 2013; Flotho and Melchior, 2013; Krumova and Weishaupt, 2013). Therefore, it is important to understand how SUMOylation impacts the physiologic/disease process under investigation. We know that many SUMO targets are nuclear proteins involved in gene expression, but there is little information about how SUMOylation modulates gene expression *in vivo* because prior studies have been conducted predominantly *in vitro*. The tools are now available to modulate SUMO *in vivo* only in cells that are targets of the physiologic/pathologic process under investigation. Recently, we developed a mouse model

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(SUMO-KD) in which expression of all three SUMO iso-forms is silenced specifically in neurons. This new mouse model has allowed us, for the first time, to study the effect of SUMO conjugation on gene expression in neurons *in vivo*, both in the physiologic state and in the diseases associated with SUMO conjugation, and to elucidate the role of SUMOylation in functional recovery of neurons exposed to ischemic stress.

Neurons are extremely sensitive to stress conditions; neurologic function is impaired in many diseases including brain ischemia/stroke, neurodegenerative diseases, and neuropsychiatric conditions. Further, post-ischemic recovery of neuronal function and the capacity of the brain to activate SUMO conjugation when challenged by ischemic stress decline with advanced age (Liu et al., 2016). Therefore, our new mouse model and the results presented here could be of broad interest. It is important to note that the gene expression profiles reported here were evaluated by microarray analysis on the hippocampal CA1 samples. Thus, these findings represent changes that occurred in all cell types present in the sample. However, the effects of SUMOylation on gene expression relate predominantly to neurons because we used a neuron-specific promoter to express SUMO1-3 miRNAs to silence SUMO expression.

For this *in vivo* study on the effects of SUMO conjugation on gene expression, we used brain ischemia as the pathologic state of interest because we have already shown that brain ischemia massively activates SUMO conjugation and nuclear accumulation of SUMO2/3-conjugated proteins (Yang et al., 2008b,c, 2014). Further, it is widely appreciated that the post-ischemic activation of SUMO conjugation is a protective stress response that shields neurons from injury, as evidenced in *in vitro* and *in vivo* studies (Lee et al., 2007, 2009, 2011, 2016; Datwyler et al., 2011). However, the effect of SUMO conjugation on post-ischemic neurologic function has not yet been investigated. The most important findings in the present study can be summarized as follows: (a) in the physiologic state of neurons, expression of most genes regulated by SUMOylation was activated by silencing SUMO expression; (b) in most cases, the effects of ischemia on gene expression were less pronounced in SUMO-KD than in wild-type mice; and (c) silencing SUMO expression worsened neurologic function after brain ischemia. These findings will be discussed in detail below.

Here, we reported a genomic study on hippocampal CA1 subfield tissue samples from mice, to identify genes regulated by ischemia. To date, only a few studies have applied the microarray technology to analyze gene expression modulated by global brain ischemia in samples excised from the CA1 subfield, whole hippocampus, or whole hemisphere (Jin et al., 2001; Kawahara et al., 2004; Yakubov et al., 2004; Feng et al., 2007; Buttner et al., 2009). For a comprehensive discussion see also Schmidt-Kastner, 2015 (Schmidt-Kastner, 2015). All of these studies used rat models, and only 2 studies used microarrays representing more than 10,000 genes (Feng et al., 2007; Buttner et al., 2009). Since these two studies used a post-ischemic time point for analysis similar to our study, we compared our list of genes regulated by ischemia with the lists from these studies. We found a large overlap of genes identified, including Atf3, Jun, Fos, Ptgs2, Gadd45, Hmox1, Hsp70, Hsp27, and also overlap of the highly enriched pathways, based on KEGG analysis, including MAPK, Wnt,

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TGF- β, and Toll-like receptor pathways. These comparisons support the validity of our dataset.

One of the key findings of our study reported here was that silencing SUMO expression in hippocampal CA1 neurons *in vivo* had opposite effects on global gene expression in the physiologic state vs the post-ischemia state. Silencing SUMO expression up-regulated gene expression in non-stressed neurons, but suppressed global gene expression responses induced by transient ischemia. At first glance, the observation that the global gene expression response induced by transient ischemia was suppressed in SUMO-KD mice was an unexpected finding, because until recently, it was generally believed that SUMOylation of most transcription factors negatively regulates their activity. Indeed, studies on a number of individual SUMO targets involved in transcription show that SUMOylation reduces the transcription activity of most of these targets (Chymkowitch et al., 2015). However, comprehensive characterization of transcriptional regulation by SUMOylation on chromatin using ChIP-seq techniques, revealed a more complex role for SUMOylation in gene expression.

Using human fibroblasts for ChIP-seq analysis, SUMOylated proteins were found at promoters of many genes involved in cell growth and proliferation, and inhibition of SUMOylation up-regulated transcription of those genes (Neyret-Kahn et al., 2013). Another study, however, reported that SUMOylation has a positive effect on transcription of genes in stressed cell (Seifert et al., 2015). Notably, using ChIP-seq and RNA-seq techniques, and heat shock as the stress condition, the authors found markedly increased binding of SUMO2-conjugated proteins to active DNA regulatory regions of many pro-survival genes. This observation supports the notion that stress-induced activation of SUMO2/3 conjugation is a protective stress response that shields stressed cells from damage by activating expression of pro-survival genes. Therefore, blocking this pro-survival response in SUMOKD mice may have contributed to the worse functional outcome after transient forebrain ischemia. Together, although our study reported here focused on terminally differentiated neurons *in vivo*, whereas earlier studies performed analyses on dividing cells *in vitro*, the modulating effects of SUMOylation on gene expression were similar, ie, suppressing gene expression in the physiologic state and activating expression of groups of genes when cells are stressed.

Considering the observation that in heat shock-stressed cells, SUMO2 conjugation activates expression of pro-survival genes, we analyzed our dataset to identify pro-survival genes with suppressed ischemia-induced activation in SUMO-KD mice. DAVID analysis identified eight anti-apoptosis genes of which seven were less activated in SUMO-KD mice after ischemia: Bag3, Cited2, Cflar, Bdnf, Hells, Myc, and Cebp β. For example, Bag3, which was identified as an ischemia-regulated gene in an earlier microarray study (Schmidt-Kastner et al., 2002), codes for a protein with anti-apoptosis function through interaction with Hsp70 (Rosati et al., 2011). Activation of the growth arrest and DNA damage 45 (Gadd45) gene family was also suppressed after ischemia in SUMO-KD mice. All 3 members of this gene family – Gadd45a, Gadd45b, and Gadd45g – have been identified as ischemia-regulated genes and they may play a protective role in brain ischemia injury (Chen et al., 1998; Sultan and Sweatt, 2013).

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It is also noteworthy that Arc is one of the few genes that were down-regulated in SUMO-KD mice in the physiologic state. This could be a finding of significant interest, because Arc is critical for embryogenesis, and is a pivotal regulator of synaptic plasticity (Liu et al., 2000; Shepherd and Bear, 2011). Both processes are modulated by SUMOylation. The potential consequences of SUMO-modulated Arc expression in embryogenesis and synaptic plasticity should, therefore, be elucidated in future studies.

In conclusion, we used SUMO knockdown transgenic mice to perform the first *in vivo* analysis of global SUMOylation on the transcriptome in neurons regulated by transient forebrain ischemia. Notably, earlier studies that analyzed the effect of SUMOylation on the transcriptome were performed *in vitro* and exposed cells to a single stress such as heat shock. Further, we provide the first evidence that silencing SUMO expression worsened functional outcome after brain ischemia. Thus, the present findings, together with earlier reports on the role of SUMOylation in brain ischemia, support that SUMOylation is a neuroprotective response, at least in part, through transcriptional regulation of stress response genes.

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APPENDIX A

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WI vs WS (194 genes)							
Probeset ID	**Entrez Gene**	**Gene Symbol**	**Gene Title**	**RefSeq Transcript ID**	**WI vs WS**	**TI vs TS**	**TS vs WS**
1449827_at	11595	Acan	aggrecan	NM_007424	7.3	2.2	−1.1
1422053_at	16323	Inhba	inhibin beta-A	NM_008380	5.4	1.2	−1.4
1421009_at	58185	Rsad2	radical S-adenosyl methionine domain containing 2	NM_021384	5.3	2.6	−1.0
1419220_at	22437	Xirp1	xin actin-binding repeat containing 1	NM_001081339 /// NM_011724	5.2	1.1	−1.1
1437279_x_at	20969	Sdc1	syndecan 1	NM_011519	5.2	2.0	1.3
1418930_at	15945	Cxcl10	chemokine (C-X-C motif) ligand 10	NM_021274	5.1	2.3	−1.1
1419247_at	19735	Rgs2	regulator of G-protein signaling 2	NM_009061	4.1	1.9	−1.1
1423310_at	21983	Tpbg	trophoblast glycoprotein	NM_001164792 /// NM_011627	3.8	1.8	1.9
1424229_at	226419	Dyrk3	dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 3	NM_145508	3.8	1.5	1.1
1418392_a_at	55932	Gbp3	guanylate binding protein 3	NM_018734	3.8	1.7	1.2
1435137_s_at	319269	1200015M12Rik /// A130040M12Rik	RIKEN cDNA 1200015M12 gene /// RIKEN cDNA A130040M12 gene	NR_002860	3.7	2.8	−1.2
1416579_a_at	17075	Epcam	epithelial cell adhesion molecule	NM_008532	3.6	1.8	−1.0
1418203_at	58801	Pmaip1	phorbol-12-myristate-13-acetate-induced protein 1	NM_021451	3.6	1.8	1.7
1421134_at	11839	Areg	amphiregulin	NM_009704	3.5	1.4	−1.0
1428781_at	73712	Dmkn	dermokine	NM_001166173 /// NM_001166174 /// NM_028618 /// NM_172899	3.5	1.3	−1.2
1455271_at	620695	Gm13889	predicted gene 13889	NM_001145034	3.3	2.0	1.4
1418240_at	14469	Gbp2	guanylate binding protein 2	NM_010260	3.3	1.7	−1.2
1417487_at	14283	Fosl1	fos-like antigen 1	NM_010235	3.2	1.9	1.1
1424711_at	83921	Tmem2	transmembrane protein 2	NM_001033759 /// NM_031997	3.2	1.6	1.2
1450698_at	13537	Dusp2	dual specificity phosphatase 2	NM_010090	3.2	1.7	1.0
1418538_at	105785	Kdelr3	KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 3	NM_134090	3.1	1.7	−1.1
1420591_at	80910	Gpr84	G protein-coupled receptor 84	NM_030720	3.1	2.0	−1.1
1419766_at	17691	Sik1	salt inducible kinase 1	NM_010831	3.1	1.9	1.2
1453238_s_at	319269	A130040M12Rik	RIKEN cDNA A130040M12 gene	NR_002860	3.0	2.5	−1.2

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WI vs WS (194 genes)							
Probeset ID	**Entrez Gene**	**Gene Symbol**	**Gene Title**	**RefSeq Transcript ID**	**WI vs WS**	**TI vs TS**	**TS vs WS**
1449824_at	96875	Prg4	proteoglycan 4 (megakaryocyte stimulating factor, articular superficial zone protein)	NM_001110146 /// NM_021400	3.0	2.1	−1.5
1449484_at	20856	Stc2	stanniocalcin 2	NM_011491	2.9	2.3	−1.3
1423233_at	12609	Cebpd	CCAAT/enhancer binding protein (C/EBP), delta	NM_007679	2.9	2.0	1.4
1424130_a_at	19285	Ptrf	polymerase I and transcript release factor	NM_008986	2.9	1.4	−1.2
1427359_at	338523	Jhdm1d	jumonji C domain-containing histone demethylase 1 homolog D (S. cerevisiae)	NM_001033430	2.8	1.7	−1.1
1449025_at	15959	Ifit3	interferon-induced protein with tetratricopeptide repeats 3	NM_010501	2.8	1.5	1.2
1449031_at	12705	Cited1	Cbp/p300-interacting transactivator with Glu/Asp-rich carboxy-terminal domain 1	NM_001276466 /// NM_001276473 /// NM_001276474 /// NM_007709	2.8	1.8	1.3
1450783_at	15957	Ifit1	interferon-induced protein with tetratricopeptide repeats 1	NM_008331	2.8	2.1	−1.2
1417051_at	18530	Pcdh8	protocadherin 8	NM_001042726 /// NM_021543	2.8	1.5	1.0
1424594_at	74480	Samd4	sterile alpha moif domain containing 4	NM_001037221 /// NM_001163433 /// NM_028966	2.7	1.9	−1.1
1424289_at	209212	Osgin2	oxidative stress induced growth inhibitor family member 2	NM_145950	2.7	1.8	1.1
1423703_at	235036	Ppan	peter pan homolog (Drosophila)	NM_145610	2.7	2.0	1.2
1423389_at	17131	Smad7	SMAD family member 7	NM_001042660 /// NM_008543	2.7	1.8	−1.2
1430978_at	75617	Rps25	ribosomal protein S25	NM_024266	2.6	1.6	1.0
1428374_at	93683	Glce	glucuronyl C5-epimerase	NM_033320	2.6	1.6	1.3
1418135_at	17355	Aff1	AF4/FMR2 family, member 1	NM_001080798 /// NM_133919	2.6	1.5	1.6
1448961_at	18828	Plscr2	phospholipid scramblase 2	NM_001195084 /// NM_008880	2.6	2.0	1.2
1435655_at	269261	Rpl12	ribosomal protein L12	NM_009076	2.6	1.8	1.2
1440831_at	12013	Bach1	BTB and CNC homology 1	NM_007520	2.6	1.9	1.1
1452869_at	66921	Prpf38b	PRP38 pre-mRNA processing factor 38 (yeast) domain containing B	NM_025845	2.6	2.0	−1.0
1448480_at	66164	Nip7	nuclear import 7 homolog (S. cerevisiae)	NM_001164472 /// NM_025391 /// NR_028367	2.6	1.7	1.2
1418666_at	19288	Ptx3	pentraxin related gene	NM_008987	2.6	1.9	−1.2
1424932_at	13649	Egfr	epidermal growth factor receptor	NM_007912 /// NM_207655	2.6	1.9	1.3

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WI vs WS (194 genes)							
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1415996_at	56338	Txnip	thioredoxin interacting protein	NM_001009935 /// NM_023719	2.6	2.3	1.1
1421336_at	19130	Prox1	prospero-related homeobox 1	NM_008937	2.6	1.2	1.4
1416762_at	20194	S100a10	S100 calcium binding protein A10 (calpactin)	NM_009112	2.5	1.9	1.2
1426648_at	17164	Mapkapk2	MAP kinase-activated protein kinase 2	NM_008551	2.5	1.6	1.0
1416123_at	12444	Ccnd2	cyclin D2	NM_009829	2.5	1.8	1.2
1429783_at	56376	Pdlim5	PDZ and LIM domain 5	NM_001190852 /// NM_001190853 /// NM_001190854 /// NM_001190855 /// NM_001190856 ///NM	2.5	1.9	1.4
1426871_at	70611	Fbxo33	F-box protein 33	NM_001033156	2.5	1.9	−1.1
1418492_at	23893	Grem2	gremlin 2 homolog, cysteine knot superfamily (Xenopus laevis)	NM_011825	2.5	1.7	1.1
1450986_at	55989	Nop58	NOP58 ribonucleoprotein	NM_018868	2.5	2.0	1.1
1425037_at	224014	Fgd4	FYVE, RhoGEF and PH domain containing 4	NM_139232 /// NM_139233 /// NM_139234	2.5	1.7	1.3
1418648_at	112407	Egln3	EGL nine homolog 3 (C. elegans)	NM_028133	2.5	1.5	−1.3
1424371_at	228769	Psmf1	proteasome (prosome, macropain) inhibitor subunit 1	NM_144889 /// NM_212446	2.5	1.7	1.4
1451714_a_at	26397	Map2k3	mitogen-activated protein kinase kinase 3	NM_008928	2.5	1.9	1.1
1460335_at	80289	Lysmd3	LysM, putative peptidoglycan-binding, domain containing 3	NM_030257	2.5	1.8	1.1
1424001_at	67949	Mki67ip	Mki67 (FHA domain) interacting nucleolar phosphoprotein	NM_026472	2.4	1.6	1.0
1420376_a_at	15078	Gm10257 /// Gm12657 /// Gm6749/// H3f3a /// H3f3b /// H3f3c /// LOC101056659	predicted gene 10257 /// predicted gene 12657 /// predicted pseudogene 6749 /// H3 hist	NM_001081019 /// NM_008210 /// NM_008211 /// XM_003084942 /// XM_003945939 /// XM_00394	2.4	1.8	1.2
1421375_a_at	20200	S100a6	S100 calcium binding protein A6 (calcyclin)	NM_011313	2.4	1.8	1.2
1452192_at	234344	Naf1	nuclear assembly factor 1 homolog (S. cerevisiae)	NM_001163564	2.4	1.3	1.1
1434380_at	229900	Gbp7	guanylate binding protein 7	NM_001083312 /// NM_145545	2.4	1.5	1.2
1422706_at	65112	Pmepa1	prostate transmembrane protein, androgen induced 1	NM_022995	2.4	1.7	1.0
1425503_at	14538	Gcnt2	glucosaminyl (N-acetyl) transferase 2, I-branching enzyme	NM_008105 /// NM_023887 /// NM_133219	2.4	1.8	1.4

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WI vs WS (194 genes)							
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1420380_at	20296	Ccl2	chemokine (C-C motif) ligand 2	NM_011333	2.4	1.8	1.1
1417639_at	30805	Slc22a4	solute carrier family 22 (organic cation transporter), member 4	NM_019687	2.4	1.6	1.3
1419169_at	50772	Mapk6	mitogen-activated protein kinase 6	NM_015806 /// NM_027418	2.4	1.9	−1.1
1424355_a_at	20467	Sin3b	transcriptional regulator, SIN3B (yeast)	NM_001113248 /// NM_009188	2.4	1.8	1.1
1450718_at	23921	Sh2b2	SH2B adaptor protein 2	NM_018825	2.4	1.9	1.4
1460695_a_at	72061	2010111I01Rik	RIKEN cDNA 2010111I01 gene	NM_028079	2.4	1.7	1.2
1434128_a_at	232976	Zfp574	zinc finger protein 574	NM_001168506 /// NM_175477	2.3	1.7	1.0
1417508_at	30945	Rnf19a	ring finger protein 19A	NM_013923	2.3	1.3	1.1
1437843_s_at	71844	Nupl1	nucleoporin like 1	NM_170591	2.3	1.5	1.5
1421365_at	14313	Fst	follistatin	NM_008046	2.3	1.6	1.1
1449317_at	12633	Cflar	CASP8 and FADD-like apoptosis regulator	NM_009805 /// NM_207653	2.3	1.7	1.1
1426351_at	15510	Hspd1 /// LOC101056370	heat shock protein 1 (chaperonin) /// 60 kDa heat shock protein, mitochondrial-like	NM_010477 /// XM_003946023	2.3	1.9	−1.0
1424107_at	228421	Kif18a	kinesin family member 18A	NM_139303	2.3	1.5	1.1
1418825_at	15944	Irgm1	immunity-related GTPase family M member 1	NM_008326	2.3	1.1	1.8
1451680_at	76650	Srxn1	sulfiredoxin 1 homolog (S. cerevisiae)	NM_029688	2.3	1.4	−1.0
1433502_s_at	104662	Tsr1	TSR1 20S rRNA accumulation	NM_177325	2.3	1.7	1.2
1438761_a_at	18263	Odc1	ornithine decarboxylase, structural 1	NM_013614	2.3	1.5	1.2
1431422_a_at	56405	Dusp14	dual specificity phosphatase 14	NM_019819	2.3	1.5	1.3
1425671_at	26556	Homer1	homer homolog 1 (Drosophila)	NM_011982 /// NM_147176 /// NM_152134	2.3	1.7	−1.1
1452358_at	24004	Rai2	retinoic acid induced 2	NM_001103367 /// NM_198409	2.3	1.1	1.1
1422473_at	18578	Pde4b	phosphodiesterase 4B, cAMP specific	NM_001177980 /// NM_001177981 /// NM_001177982 /// NM_001177983 /// NM_019840	2.3	1.7	1.4
1426798_a_at	108954	Ppp1r15b	protein phosphatase 1, regulatory (inhibitor) subunit 15b	NM_133819	2.3	1.9	1.0
1418834_at	117197	Bloc1s4	biogenesis of organelles complex-1, subunit 4, cappuccino	NM_133724	2.3	1.9	1.1

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WI vs WS (194 genes)							
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1449007_at	12228	Btg3 /// Gm7334	B cell translocation gene 3 /// B-cell translocation gene 3 pseudogene	NM_009770 /// NR_002700	2.3	1.7	1.3
1419765_at	71745	Cul2	cullin 2	NM_029402	2.3	1.8	1.4
1425565_at	19712	Rest	RE1-silencing transcription factor	NM_011263			
1424270_at	13175	Dclk1	doublecortin-like kinase 1	NM_001111051 /// NM_001111052 /// NM_001111053 /// NM_001195538 /// NM_001195539 /// NM	2.2	1.4	1.0
1438992_x_at	11911	Atf4	activating transcription factor 4	NM_009716	2.2	1.9	−1.2
1427321_s_at	13052	Cxadr	coxsackie virus and adenovirus receptor	NM_001025192 /// NM_001276263 /// NM_009988	2.2	1.7	1.3
1436684_a_at	67045	Riok2	RIO kinase 2 (yeast)	NM_025934	2.2	1.5	1.5
1439154_at	269966	Nup98	nucleoporin 98	NM_022979	2.2	1.8	1.1
1435561_at	13875	Erf	Ets2 repressor factor	NM_010155	2.2	1.7	1.1
1449227_at	12642	Ch25h	cholesterol 25-hydroxylase	NM_009890	2.2	1.9	1.1
1448133_at	97112	Nmd3	NMD3 homolog (S. cerevisiae)	NM_133787	2.2	1.6	1.2
1416106_at	100087	Kti12	KTI12 homolog, chromatin associated (S. cerevisiae)	NM_029571	2.2	1.6	1.1
1415806_at	18791	Plat	plasminogen activator, tissue	NM_008872	2.2	1.8	1.2
1417719_at	60406	Sap30	sin3 associated polypeptide	NM_021788	2.2	1.9	1.1
1421267_a_at	17684	Cited2	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2	NM_010828	2.2	1.4	1.6
1450297_at	16193	Il6	interleukin 6	NM_031168	2.2	1.8	1.0
1434436_at	75746	Morc4	microrchidia 4	NM_001193309 /// NM_029413	2.2	1.8	1.1
1419702_at	21339	Taf1a	TATA box binding protein (Tbp)-associated factor, RNA polymerase I, A	NM_001277957 /// NM_001277958 /// NM_001277959 /// NM_021466	2.2	1.5	−1.1
1421855_at	14190	Fgl2	fibrinogen-like protein 2	NM_008013	2.2	1.3	1.2
1452997_at	381598	2610005L07Rik	cadherin 11 pseudogene	NM_001024708 /// NM_001033456 /// NR_028428	2.2	1.5	1.5
1460351_at	20195	Gm12854 /// Gm5068 /// S100a11	predicted gene 12854 /// predicted gene 5068 /// S100 calcium binding protein A11 (calg	NM_016740 /// XM_003085822 /// XM_003086388 /// XM_204772	2.2	1.8	−1.1

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WI vs WS (194 genes)							
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1448328_at	24055	Sh3bp2	SH3-domain binding protein 2	NM_001136088 /// NM_001145858 /// NM_001145859 /// NM_011893	2.2	2.0	1.3
1420502_at	20229	Sat1	spermidine/spermine N1-acetyl transferase 1	NM_009121	2.2	1.9	−1.0
1459902_at	212772	Arl14ep	ADP-ribosylation factor-like 14 effector protein	NM_001025102 /// NM_173750	2.2	1.8	1.0
1451533_at	217887	BC022687	cDNA sequence BC022687	NM_145450	2.2	1.5	1.5
1451969_s_at	235587	Parp3	poly (ADP-ribose) polymerase family, member 3	NM_145619	2.2	2.0	−1.1
1418804_at	84112	Sucnr1	succinate receptor 1	NM_032400	2.2	1.2	1.0
1448678_at	73225	Fam118a	family with sequence similarity 118, member A	NM_133750 /// NM_177067	2.2	1.9	1.1
1423169_at	24074	Taf7	TAF7 RNA polymerase II, TATA box binding protein (TBP)-associated factor	NM_175770	2.1	1.6	1.6
1455105_at	19248	Ptpn12	protein tyrosine phosphatase, non-receptor type 12	NM_011203	2.1	1.5	−1.0
1452416_at	16194	Il6ra	interleukin 6 receptor, alpha	NM_010559	2.1	1.9	1.2
1424211_at	70556	Slc25a33	solute carrier family 25, member 33	NM_027460 /// XM_003945705	2.1	1.4	1.3
1451780_at	17060	Blnk	B cell linker	NM_008528	2.1	1.6	−1.1
1460428_at	68420	Ankrd13a	ankyrin repeat domain 13a	NM_026718	2.1	1.8	1.0
1452045_at	226442	Zfp281	zinc finger protein 281	NM_001160251 /// NM_177643	2.1	1.9	−1.0
1436893_a_at	57438	41705	membrane-associated ring finger (C3HC4) 7	NM_020575	2.1	1.6	1.3
1422554_at	66647	Ndnl2	necdin-like 2	NM_023239	2.1	1.8	−1.0
1460657_at	22409	Wnt10a	wingless related MMTV integration site 10a	NM_009518	2.1	1.3	1.1
1418711_at	18590	Pdgfa	platelet derived growth factor, alpha	NM_008808	2.1	1.7	−1.0
1454064_a_at	56515	Rnf138	ring finger protein 138	NM_019706 /// NM_207623	2.1	1.5	1.2
1448749_at	56193	Plek	pleckstrin	NM_019549	2.1	1.9	1.1
1451069_at	223775	Pim3	proviral integration site 3	NM_145478	2.1	1.9	−1.1
1426858_at	16324	Inhbb	inhibin beta-B	NM_008381	2.1	1.6	1.6
1416359_at	170625	Snx18	sorting nexin 18	NM_130796	2.1	2.0	−1.1
1437111_at	244871	Zc3h12c	zinc finger CCCH type containing 12C	NM_001162921	2.1	1.8	−1.1
1426609_at	72662	Dis3	DIS3 mitotic control homolog (S. cerevisiae)	NM_028315	2.1	1.8	1.1
1450685_at	59046	Arpp19	cAMP-regulated phosphoprotein 19	NM_001142655 /// NM_021548	2.1	1.5	−1.2

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1416081_at	17125	Smad1	SMAD family member 1	NM_008539	2.1	1.9	1.1
1425321_a_at	94040	Clmn	calmin	NM_001040682 /// NM_053155	2.1	1.9	1.1
1418918_at	16006	Igfbp1	insulin-like growth factor binding protein 1	NM_008341	2.1	1.5	1.1
1423904_a_at	52118	Pvr	poliovirus receptor	NM_027514	2.1	1.5	1.2
1448802_at	27275	Nufip1	nuclear fragile X mental retardation protein interacting protein 1	NM_013745	2.1	1.7	1.1
1434425_at	99681	Tchh	trichohyalin	NM_001163098	2.1	1.3	1.4
1428114_at	108052	Slc14a1	solute carrier family 14 (urea transporter), member 1	NM_001171010 /// NM_001171011 /// NM_028122	2.1	1.3	1.7
1437696_at	381066	Zfp948	zinc finger protein 948	NM_001002008	2.1	1.4	1.1
1428942_at	17750	Mt2	metallothionein 2	NM_008630	2.1	1.8	1.1
1429040_at	547150	6820431F20Rik	cadherin 11 pseudogene	NR_030708	2.1	1.3	1.8
1418323_at	14155	Fem1b	feminization 1 homolog b (C. elegans)	NM_010193	2.1	1.9	1.0
1438511_a_at	66214	Rgcc	regulator of cell cycle	NM_025427	2.0	1.3	1.1
1449188_at	59090	Midn	midnolin	NM_021565	2.0	1.7	−1.1
1453100_at	13000	Csnk2a2	casein kinase 2, alpha prime polypeptide	NM_009974	2.0	1.7	1.1
1419749_at	13434	Trdmt1	tRNA aspartic acid methyltransferase 1	NM_010067	2.0	1.7	1.3
1436871_at	225027	Srsf7	serine/arginine rich splicing factor 7 -	NM_001195485 /// NM_001195486 /// NM_001195487 /// NM_146083 /// NR_036615	2.0	1.7	1.3
1437396_at	208647	Creb3l2	cAMP responsive element binding protein 3-like 2	NM_178661	2.0	1.7	1.0
1460179_at	15502	Dnaja1	DnaJ (Hsp40) homolog, subfamily A, member 1	NM_001164671 /// NM_001164672 /// NM_008298	2.0	1.8	−1.1
1416751_a_at	53975	Ddx20	DEAD (Asp-Glu-Ala-Asp) box polypeptide 20	NM_017397	2.0	1.4	1.1
1415940_at	100494	Zfand2a	zinc finger, AN1-type domain 2A	NM_001159908 /// NM_133349	2.0	1.9	1.1
1420930_s_at	54366	Ctnnal1	catenin (cadherin associated protein), alpha-like 1	NM_018761	2.0	1.7	1.1
1431057_a_at	76453	Prss23	protease, serine, 23	NM_029614	2.0	1.3	1.1
1417426_at	19073	Srgn	serglycin	NM_011157	2.0	1.7	−1.1
1449858_at	12524	Cd86	CD86 antigen	NM_019388	2.0	1.7	−1.0
1448413_at	71952	2410016O06Rik	RIKEN cDNA 2410016O06 gene	NM_023633	2.0	1.5	1.0

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WI vs WS (194 genes)							
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1420664_s_at	19124	Procr	protein C receptor, endothelial	NM_011171	2.0	1.7	−1.0
1419940_at	109260	C030018P15Rik	RIKEN cDNA C030018P15 gene	---	2.0	1.9	−1.1
1437630_at	224092	Lsg1	large subunit GTPase 1 homolog (S. cerevisiae)	NM_178069	2.0	1.4	1.2
1437320_s_at	22590	Xpa	xeroderma pigmentosum, complementation group A	NM_011728	−2.0	−1.9	−1.0
1439453_x_at	68209	Rnaseh2c	ribonuclease H2, subunit C	NM_026616	−2.0	−1.8	−1.1
1416053_at	16979	Lrrn1	leucine rich repeat protein 1, neuronal	NM_008516	−2.0	−1.9	1.0
1429177_x_at	20671	Sox17	SRY-box containing gene 17	NM_011441	−2.0	−1.9	1.1
1419332_at	54156	Egfl6	EGF-like-domain, multiple 6	NM_019397	−2.0	−1.4	1.0
1426544_a_at	67120	Ttc14	tetratricopeptide repeat domain 14	NM_025978 /// NM_027619	−2.0	−1.8	1.2
1450803_at	18205	Ntf3	neurotrophin 3	NM_001164034 /// NM_001164035 /// NM_008742	−2.1	−1.6	−1.3
1428483_a_at	66578	Mis18a	MIS18 kinetochore protein homolog A (S. pombe)	NM_025642	−2.1	−1.6	−1.0
1455092_at	22680	Zfp207	zinc finger protein 207	NM_001130169 /// NM_001130170 /// NM_001130171 /// NM_011751 /// NR_045038	−2.1	−1.8	1.1
1448530_at	66355	Gmpr	guanosine monophosphate reductase	NM_025508	−2.1	−1.4	−1.2
1455831_at	233908	Fus	fused in sarcoma	NM_139149	−2.1	−1.7	−1.1
1425111_at	66673	Sorcs3	sortilin-related VPS10 domain containing receptor 3	NM_025696	−2.1	−1.3	−1.4
1451901_at	20585	Hltf	helicase-like transcription factor	NM_009210 /// NM_144959	−2.1	−1.7	1.0
1418266_at	11686	Alox12b	arachidonate 12-lipoxygenase, 12R type	NM_009659	−2.1	−1.4	1.5
1423084_at	26878	B3galt2	UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase, polypeptide 2	NM_020025	−2.1	−1.7	1.1
1438710_at	15550	Htr1a	5-hydroxytryptamine (serotonin) receptor 1A	NM_008308	−2.1	−1.5	−1.2
1425175_at	227580	C1ql3	C1q-like 3	NM_153155	−2.1	−1.7	1.0
1421818_at	12053	Bcl6	B cell leukemia/lymphoma 6	NM_009744	−2.1	−1.7	−1.3
1438034_at	66302	Rmdn1	regulator of microtubule dynamics 1	NM_025476	−2.2	−1.9	1.1
1451344_at	231633	Tmem119	transmembrane protein 119	NM_146162	−2.2	−1.9	−1.0
1425344_at	67608	Narf	nuclear prelamin A recognition factor	NM_026272	−2.2	−1.6	−1.1

WI vs WS (194 genes)							
Probeset ID	**Entrez Gene**	**Gene Symbol**	**Gene Title**	**RefSeq Transcript ID**	**WI vs WS**	**TI vs TS**	**TS vs WS**
1417574_at	20315	Cxcl12	chemokine (C-X-C motif) ligand 12	NM_001012477 /// NM_013655 /// NM_021704	−2.2	−1.8	−1.2
1428052_a_at	68310	Zmym1	zinc finger, MYM domain containing 1	NM_026670	−2.2	−1.9	1.1
1427017_at	212712	Satb2	special AT-rich sequence binding protein 2	NM_139146	−2.3	1.2	−1.4
1438465_at	414758	5830428H23Rik	RIKEN cDNA 5830428H23 gene	NM_001001737 /// XR_105997	−2.4	−1.8	−1.2
1426552_a_at	14025	Bcl11a	B cell CLL/lymphoma 11A (zinc finger protein)	NM_001159289 /// NM_001159290 /// NM_001242934 /// NM_016707	−2.4	−1.7	−1.2
1424303_at	211896	Depdc7	DEP domain containing 7	NM_144804	−2.5	−1.2	−2.0
1427975_at	75668	Rasl10a	RAS-like, family 10, member A	NM_145216	−2.9	−2.0	−1.1

TI vs TS (44 genes)							
Probeset ID	**Entrez Gene**	**Gene Symbol**	**Gene Title**	**ReSeq Transcript ID**	**WI vs WS**	**TI vs TS**	**TS vs WS**
1454770_at	12426	Cckbr	cholecystokinin B receptor	NM_007627	1.48	2.71	−1.54
1450700_at	260409	Cdc42ep3	CDC42 effector protein (Rho GTPase binding) 3	NM_026514	1.58	2.48	1.10
1450843_a_at	12406	Serpinh1	serine (or cysteine) peptidase inhibitor, clade H, member 1	NM_001111043 /// NM_001111044 /// NM_009825	1.85	2.43	−1.38
1423614_at	100604	Lrrc8c	leucine rich repeat containing 8 family, member C	NM_133897	1.68	2.43	−1.25
1416897_at	80285	Parp9	poly (ADP-ribose) polymerase family, member 9	NM_030253	1.89	2.40	−1.03
1417812_a_at	16780	Lamb3	laminin, beta 3	NM_001277928 /// NM_008484	1.96	2.40	1.17
1419654_at	21887	Tle3	transducin-like enhancer of split 3, homolog of Drosophila E(spl)	NM_001083927 /// NM_001083928 /// NM_009389	1.94	2.37	−1.07
1417332_at	19725	Rfx2	regulatory factor X, 2 (influences HLA class II expression)	NM_009056 /// NM_027787	1.67	2.37	−1.25
1420589_at	15118	Has3	hyaluronan synthase 3	NM_008217	1.44	2.33	−1.80
1417240_at	22793	Zyx	zyxin	NM_011777	1.96	2.29	−1.05
1449161_at	13615	Edn2	endothelin 2	NM_007902	1.79	2.29	−1.27
1448228_at	16948	Lox	lysyl oxidase	NM_010728	1.10	2.27	1.59
1451794_at	319880	Tmcc3	transmembrane and coiled coil domains 3	NM_001168684 /// NM_172051 /// NM_177026	2.00	2.27	−1.04
1451751_at	73284	Ddit4l	DNA-damage-inducible transcript 4-like	NM_030143	1.59	2.26	−1.34
1418025_at	20893	Bhlhe40	basic helix-loop-helix family, member e40	NM_011498	1.86	2.24	−1.25

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TI vs TS (44 genes)							
Probeset ID	**Entrez Gene**	**Gene Symbol**	**Gene Title**	**ReSeq Transcript ID**	**WI vs WS**	**TI vs TS**	**TS vs WS**
1416287_at	19736	Rgs4	regulator of G-protein signaling 4	NM_009062	1.92	2.23	−1.30
1415874_at	24063	Spry1	sprouty homolog 1 (Drosophila)	NM_011896	1.42	2.21	−1.25
1451873_a_at	17129	Smad5	SMAD family member 5	NM_001164041 /// NM_001164042 /// NM_008541	1.73	2.19	−1.09
1425420_s_at	50523	Lats2	large tumor suppressor 2	NM_015771 /// NM_153382	1.97	2.17	1.04
1450350_a_at	81703	Jdp2	Jun dimerization protein 2	NM_001205052 /// NM_001205053 /// NM_030887	1.63	2.14	−1.58
1431734_a_at	67035	Dnajb4	DnaJ (Hsp40) homolog, subfamily B, member 4	NM_025926 /// NM_027287	1.95	2.14	−1.01
1448860_at	140743	Rem2	rad and gem related GTP binding protein 2	NM_080726	1.46	2.13	−1.57
1450229_at	26896	Med14	mediator complex subunit 14	NM_001048208 /// NM_012005	1.83	2.12	−1.03
1417884_at	104681	Slc16a6	solute carrier family 16 (monocarboxylic acid transporters), member 6	NM_001029842 /// NM_134038	1.35	2.12	−1.18
1453678_at	17190	Mbd1	methyl-CpG binding domain protein 1	NM_013594	1.82	2.11	1.02
1417379_at	29875	Iqgap1	IQ motif containing GTPase activating protein 1	NM_016721	1.90	2.09	−1.02
1416564_at	20680	Sox7	SRY-box containing gene 7	NM_011446	1.51	2.09	−1.30
1416360_at	170625	Snx18	sorting nexin 18	NM_130796	1.98	2.08	−1.12
1434901_at	381990	Zbtb2	zinc finger and BTB domain containing 2	NM_001033466	1.75	2.08	−1.33
1449545_at	14172	Fgf18	fibroblast growth factor 18	NM_008005 /// NR_102395	1.77	2.08	1.14
1453392_at	69863	Ttc39b	tetratricopeptide repeat domain 39B	NM_025782 /// NM_027238	1.58	2.05	−1.22
1450734_at	89867	Sec16b	SEC16 homolog B (S. cerevisiae)	NM_001159986 /// NM_033354 /// NR_027641	1.33	2.02	−1.20
1451021_a_at	12224	Klf5	Kruppel-like factor 5	NM_009769	1.48	2.02	−1.14
1418255_s_at	20807	Srf	serum response factor	NM_020493	1.94	2.01	−1.38
1425400_a_at	56222	Cited4	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 4	NM_019563	1.87	2.01	1.73
1439079_a_at	59079	Erbb2ip	Erbb2 interacting protein	NM_001005868 /// NM_021563	1.58	2.00	−1.03
1449482_at	382522	Hist3h2ba /// Hist3h2bb-ps	histone cluster 3, H2ba /// histone cluster 3, H2bb, pseudogene	NM_030082 /// NM_206882	0.51	−2.03	1.29
1416174_at	26450	Rbbp9	retinoblastoma binding protein 9	NM_015754	0.54	−2.04	1.17
1451832_at	75458	Cklf	chemokine-like factor	NM_001037840 /// NM_001037841 /// NM_029295 /// NM_029313	0.60	−2.18	1.54
1418788_at	21687	Tek	endothelial-specific receptor tyrosine kinase	NM_013690	0.52	−2.26	1.01

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TI vs TS (44 genes)							
Probeset ID	**Entrez Gene**	**Gene Symbol**	**Gene Title**	**ReSeq Transcript ID**	**WI vs WS**	**TI vs TS**	**TS vs WS**
1454858_x_at	70152	Mettl7a1	methyltransferase like 7A1	NM_027334	0.62	−2.42	1.64
1437461_s_at	67225	Rnpc3	RNA-binding region (RNP1, RRM) containing 3	NM_001038696 /// NM_026043	0.50	−2.47	1.10
1420048_at	97455	C78859	expressed sequence C78859	---	0.61	−2.56	1.63
1456010_x_at	15208	Hes5	hairy and enhancer of split 5 (Drosophila)	NM_010419	0.54	−2.63	1.74

TS vs WS (58 genes)							
Probeset ID	**Entrez Gene**	**Gene Symbol**	**Gene Title**	**RefSeq Transcript ID**	**WI vs WS**	**TI vs TS**	**TS vs WS**
1456515_s_at	277353	Tcfl5	transcription factor-like 5 (basic helix-loop-helix)	NM_178254	1.59	−1.28	38.59
1433579_at	238257	Tmem30b	transmembrane protein 30B	NM_178715	1.03	−1.11	13.88
1417797_a_at	69073	1810019J16Rik	RIKEN cDNA 1810019J16 gene	NM_001083916 /// NM_133707	−1.02	−1.55	13.43
1422825_at	27220	Cartpt	CART prepropeptide	NM_001081493 /// NM_013732	−1.13	−1.15	10.15
1424525_at	225642	Grp	gastrin releasing peptide	NM_175012	1.27	−1.21	9.75
1416121_at	16948	Lox	lysyl oxidase	NM_010728	1.65	1.25	6.91
1418304_at	170677	Cdhr1	cadherin-related family member 1	NM_130878	1.28	−1.48	6.55
1427509_at	545192	Baiap3	BAI1-associated protein 3	NM_001163270	1.45	−1.53	5.96
1418941_at	93893	Pcdhb22	protocadherin beta 22	NM_053147	1.09	−1.63	5.93
1420422_at	93892	Pcdhb21	protocadherin beta 21	NM_053146	−1.28	−1.81	5.15
1417680_at	16493	Kcna5	potassium voltage-gated channel, shaker-related subfamily, member 5	NM_145983	1.33	−1.09	4.62
1417430_at	12585	Cdr2	cerebellar degeneration-related 2	NM_007672	1.56	−1.04	4.32
1422530_at	19132	Prph	peripherin	NM_001163588 /// NM_001163589 /// NM_013639	1.07	−1.36	4.05
1417920_at	93835	Amn	amnionless	NM_033603	1.74	1.32	3.90
1416627_at	20732	Spint1	serine protease inhibitor, Kunitz type 1	NM_016907	1.34	−1.53	3.58
1421396_at	18548	Pcsk1	proprotein convertase subtlisin/kexin type 1	NM_013628	1.06	−1.60	3.55
1418301_at	54139	Irf6	interferon regulatory factor 6	NM_016851 /// NM_178083	−1.65	−1.13	3.48
1449283_a_at	29857	Mapk12	mitogen-activated protein kinase 12	NM_013871	1.04	−1.18	3.33
1452270_s_at	65969	Cubn	cubilin (intrinsic factor-cobalamin receptor)	NM_001081084	1.92	−1.32	3.20
1449491_at	105844	Card10	caspase recruitment domain family, member 10	NM_130859	1.05	−1.27	3.19
1422586_at	13599	Ecel1	endothelin converting enzyme-like 1	NM_001277925 /// NM_021306	−1.16	−1.25	3.05

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TS vs WS (58 genes)							
Probeset ID	**Entrez Gene**	**Gene Symbol**	**Gene Title**	**RefSeq Transcript ID**	**WI vs WS**	**TI vs TS**	**TS vs WS**
1421129_a_at	53313	Atp2a3	ATPase, Ca++ transporting, ubiquitous	NM_001163336 /// NM_001163337 /// NM_016745	−1.01	−1.64	2.97
1449583_at	93891	Pcdhb20	protocadherin beta 20	NM_053145	−1.57	−1.96	2.89
1425288_at	231004	Samd11	sterile alpha motif domain containing 11	NM_001110516 /// NM_173736	1.29	1.10	2.86
1419021_at	109904	Mcf2	mcf.2 transforming sequence	NM_133197	−1.38	−1.33	2.86
1418488_s_at	72388	Ripk4	receptor-interacting serine-threonine kinase 4	NM_023663	1.25	1.41	2.82
1434674_at	17101	Lyst	lysosomal trafficking regulator	NM_010748	−1.05	−1.35	2.68
1418373_at	56012	Pgam2	phosphoglycerate mutase 2	NM_018870	−1.39	−1.71	2.67
1426271_at	226026	Smc5	structural maintenance of chromosomes 5	NM_001252684 /// NM_001252685 /// NM_153808	1.03	−1.30	2.67
1423770_at	217353	Tmc6	transmembrane channel-like gene family 6	NM_145439 /// NM_181321	1.23	−1.11	2.56
1424581_at	217154	Stac2	SH3 and cysteine rich domain 2	NM_146028	1.06	−1.08	2.50
1425050_at	66307	Isoc1	isochorismatase domain containing 1	NM_025478	−1.06	−1.60	2.48
1435477_s_at	14130	Fcgr2b	Fc receptor, IgG, low affinity IIb	NM_001077189 /// NM_010187	1.67	−1.12	2.48
1417933_at	16012	Igfbp6	insulin-like growth factor binding protein 6	NM_008344	−1.31	−1.27	2.43
1437226_x_at	17357	Marcksl1	MARCKS-like 1	NM_010807	1.37	−1.16	2.39
1419517_at	72978	Cnih3	cornichon homolog 3 (Drosophila)	NM_001160211 /// NM_001160212 /// NM_028408	1.23	−1.10	2.38
1424767_at	104010	Cdh22	cadherin 22	NM_174988	1.57	−1.08	2.36
1427115_at	17883	Myh3	myosin, heavy polypeptide 3, skeletal muscle, embryonic	NM_001099635	1.05	−1.04	2.31
1416125_at	14229	Fkbp5	FK506 binding protein 5	NM_010220	1.10	−1.35	2.29
1460366_at	225898	Eml3	echinoderm microtubule associated protein like 3	NM_144872	−1.07	−1.23	2.28
1422552_at	67874	Rprm	reprimo, TP53 dependent G2 arrest mediator candidate	NM_023396	1.50	−1.44	2.23
1424451_at	235674	Acaa1b	acetyl-Coenzyme A acyltransferase 1B	NM_146230	1.08	1.10	2.21
1422659_at	108058	Camk2d	calcium/calmodulin-dependent protein kinase II, delta	NM_001025438 /// NM_001025439 /// NM_023813	1.53	−1.30	2.20
1419470_at	14696	Gnb4	guanine nucleotide binding protein (G protein), beta 4	NM_013531	1.20	−1.03	2.18
1448807_at	99296	Hrh3	histamine receptor H3	NM_133849 /// NR_102309	1.00	−1.18	2.17
1449839_at	12367	Casp3	caspase 3	NM_009810	1.93	1.51	2.16
1421505_at	27217	Mixl1	Mix1 homeobox-like 1 (Xenopus laevis)	NM_013729	1.03	−1.54	2.15

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TS vs WS (58 genes)							
Probeset ID	**Entrez Gene**	**Gene Symbol**	**Gene Title**	**RefSeq Transcript ID**	**WI vs WS**	**TI vs TS**	**TS vs WS**
1423551_at	12554	Cdh13	cadherin 13	NM_019707	−1.31	−1.22	2.13
1449837_at	66712	Spesp1	sperm equatorial segment protein 1	NM_025721	−1.14	−1.72	2.10
1419606_a_at	21955	Tnnt1	troponin T1, skeletal, slow	NM_001277903 /// NM_001277904 /// NM_011618	−1.02	−1.47	2.07
1438673_at	218756	Slc4a7	solute carrier family 4, sodium bicarbonate cotransporter, member 7	NM_001033270	1.61	1.01	2.07
1424902_at	72324	Plxdc1	plexin domain containing 1	NM_001163608 /// NM_028199	−1.31	−1.14	2.06
1448635_at	14211	Smc2	structural maintenance of chromosomes 2	NM_008017	−1.45	−1.57	2.05
1426936_at	192885 /// 215866 /// 629242 /// 641366	BC005512 /// F630007L15Rik /// Gm6958 /// LOC215866	cDNA sequence BC005512 /// RIKEN cDNA F630007L15 gene /// predicted gene 6958 /// uncha	XM_001479180 /// XM_001480210 /// XM_003688870 /// XM_003689442 /// XM_989008 /// XR_14	1.65	1.11	2.04
1450998_at	65020	Zfp110	zinc finger protein 110	NM_022981	1.64	1.08	2.04
1448823_at	20315	Cxcl12	chemokine (C-X-C motif) ligand 12	NM_001012477 /// NM_013655 /// NM_021704	−1.41	−1.33	−2.76
1418476_at	12931	Crlf1	cytokine receptor-like factor 1	NM_018827	−1.12	−1.05	−2.77
1427351_s_at	16019	Ighm	immunoglobulin heavy constant mu	---	1.08	1.42	−3.06

Overlap (222 genes)

Probeset ID	Entrez Gene	Gene Symbol	Gene Title	RefSeq Transcript ID	WI vs WS	TI vs TS	TS vs WS
1450716_at	11504	Adamts1	A disintegrin-like and metallopeptidase (reprolysin type) with thrombospondin type 1 mo	NM_009621	4.0	5.0	1.0
1419706_a_at	83397	Akap12	A kinase (PRKA) anchor protein (gravin) 12	NM_031185	3.5	3.8	−1.0
1449363_at	11910	Atf3	Activating transcription factor 3	NM_007498	46.7	33.1	−1.2
1432007_s_at	11772	Ap2a2	Adaptor-related protein complex 2, alpha 2 subunit	NM_007459	2.3	2.2	1.2
1418823_at	11845	Arf6	ADP-ribosylation factor 6	NM_007481	2.5	2.0	1.0
1418250_at	80981	Arl4d	ADP-ribosylation factor-like 4D	NM_025404	4.5	2.6	1.1
1423420_at	11554	Adrb1	Adrenergic receptor, beta 1	NM_007419	3.1	2.3	1.0
1416077_at	11535	Adm	Adrenomedullin	NM_009627	2.2	3.0	−1.1
1417130_s_at	57875	Angptl4	Angiopoietin-like 4	NM_020581	4.2	2.7	1.4
1453287_at	67434	Ankrd33b	Ankyrin repeat domain 33B	NM_001164441 /// NM_026153/// NM_027496	2.7	2.2	−1.1

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Overlap (222 genes)

Probeset ID	Entrez Gene	Gene Symbol	Gene Title	RefSeq Transcript ID	WI vs WS	TI vs TS	TS vs WS
1419091_a_at	12306	Anxa2	Annexin A2	NM_007585	2.8	2.5	−1.1
1424481_s_at	494468	Armcx5	Armadillo repeat containing, X-linked 5	NM_001009575	2.5	2.1	1.2
1451340_at	214855	Arid5a	AT rich interactive domain 5A (MRF1-like)	NM_001172205 /// NM_001172206 /// NM_145996 /// NR_033310	3.3	4.1	−1.3
1420973_at	71371	Arid5b	AT rich interactive domain 5B (MRF1-like)	NM_023598	2.7	2.2	1.1
1419004_s_at	12044/// 12045/// 12047	Bcl2a1a /// Bcl2a1b /// Bcl2a1d	B cell leukemia/lymphoma 2 related protein A1a /// B cell leukemia/lymphoma 2 related p	NM_007534 /// NM_007536 /// NM_009742	3.1	2.6	−1.1
1416250_at	12227	Btg2	B cell translocation gene 2, anti-proliferative	NM_007570	4.2	3.2	1.2
1422452_at	29810	Bag3	BCL2-associated athanogene 3	NM_013863	5.5	3.0	1.2
1423753_at	68010	Bambi	BMP and activin membrane-bound inhibitor	NM_026505	2.1	2.2	1.5
1437419_at	140780	Bmp2k	BMP2 inducible kinase	NM_080708	3.7	2.2	1.4
1422169_a_at	12064	Bdnf	Brain derived neurotrophic factor	NM_001048139 /// NM_001048141 /// NM_001048142 /// NM_007540	2.3	2.3	−1.4
1433956_at	12562	Cdh5	Cadherin 5	NM_009868	2.6	2.5	−1.1
1437270_a_at	56708	Clcf1	Cardiotrophin-like cytokine factor 1	NM_019952	2.4	2.1	1.0
1427844_a_at	12608	Cebpb	CCAAT/enhancer binding protein (C/EBP), beta	NM_009883	2.9	2.2	−1.1
1417268_at	12475	Cd14	CD14 antigen	NM_009841	5.1	4.2	−1.1
1416034_at	12484	Cd24a	CD24a antigen	NM_009846	2.8	2.1	2.1
1423760_at	12505	Cd44	CD44 antigen	NM_001039150 /// NM_001039151 /// NM_001177785 /// NM_001177786 /// NM_001177787 /// NM	6.2	4.4	−1.0
1419589_at	17064	Cd93	CD93 antigen	NM_010740	3.0	3.0	−1.2
1450842_a_at	12615	Cenpa	Centromere protein A	NM_007681	6.2	5.2	−1.2
1427205_x_at	76380	Cep112	Centrosomal protein 112	NM_029586 /// NM_029606 /// NM_145688	2.0	2.1	1.0
1451382_at	69065	Chac1	ChaC, cation transport regulator 1	NM_026929	7.9	5.9	1.0
1419561_at	20302	Ccl3	Chemokine (C-C motif) ligand 3	NM_011337	4.5	4.6	−1.3
1419209_at	14825	Cxcl1	Chemokine (C-X-C motif) ligand 1	NM_008176	9.4	8.4	1.0
1424143_a_at	67177	Cdt1	Chromatin licensing and DNA replication factor 1	NM_026014	2.1	2.1	1.1
1431166_at	12648	Chd1	Chromodomain helicase DNA binding protein 1	NM_007690	2.2	2.2	1.0
1424409_at	71908	Cldn23	Claudin 23	NM_027998	2.2	2.1	−1.0
1452414_s_at	108673	Ccdc86	Coiled-coil domain containing 86	NM_023731	4.8	3.5	1.2
1452035_at	12826	Col4a1	Collagen, type IV, alpha 1	NM_009931	2.8	2.2	1.3

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1419483_at	12267	C3ar1	Complement component 3a receptor 1	NM_009779	3.0	2.5	−1.1
1416953_at	14219	Ctgf	Connective tissue growth factor	NM_010217	3.5	3.7	−1.2
1434618_at	233490	Crebzf	CREB/ATF bZIP transcription factor	NM_145151 /// NR_073436 /// NR_073437 /// NR_073438	−2.8	−2.5	−1.1
1433733_a_at	12952	Cry1	Cryptochrome 1 (photolyase-like)	NM_007771	2.7	2.4	−1.0
1423622_a_at	56706	Ccnl1	Cyclin L1	NM_001025442 /// NM_001025443 /// NM_019937	2.3	2.1	−1.1
1421679_a_at	12575	Cdkn1a	Cyclin-dependent kinase inhibitor 1A (P21)	NM_001111099 /// NM_007669	4.1	3.0	1.2
1438133_a_at	16007	Cyr61	Cysteine rich protein 61	NM_010516	14.5	21.3	1.1
1422533_at	13121	Cyp51	Cytochrome P450, family 51	NM_020010	2.8	2.1	1.1
1455372_at	208922	Cpeb3	Cytoplasmic polyadenylation element binding protein 3	NM_198300	2.6	2.0	−1.0
1449931_at	67579	Cpeb4	Cytoplasmic polyadenylation element binding protein 4	NM_026252	2.8	2.1	1.2
1416814_at	21841	Tia1	Cytotoxic granule-associated RNA binding protein 1	NM_001164078 /// NM_001164079 /// NM_011585	−2.2	−2.1	1.1
1448471_a_at	13024	Ctla2a	Cytotoxic T lymphocyte-associated protein 2 alpha	NM_001145799 /// NM_007796	4.4	3.6	−1.2
1452352_at	13025	Ctla2b	Cytotoxic T lymphocyte-associated protein 2 beta	NM_001145801 /// NM_007797	2.6	2.5	−1.1
1417937_at	59036	Dact1	Dapper homolog 1, antagonist of beta-catenin (xenopus)	NM_001190466 /// NM_021532	2.5	3.2	1.2
1426081_a_at	13371	Dio2	Deiodinase, iodothyronine, type II	NM_010050	3.0	2.8	1.1
1417516_at	13198	Ddit3	DNA-damage inducible transcript 3	NM_007837	2.4	2.1	1.0
1416756_at	81489	Dnajb1	DnaJ (Hsp40) homolog, subfamily B, member 1	NM_018808	5.5	5.7	−1.4
1448830_at	19252	Dusp1	Dual specificity phosphatase 1	NM_013642	2.7	4.0	−1.2
1418401_a_at	70686	Dusp16	Dual specificity phosphatase 16	NM_001048054 /// NM_130447/// NM_181320	3.3	3.1	−1.0
1415834_at	67603	Dusp6	Dual specificity phosphatase 6	NM_026268	2.1	3.0	−1.7
1417065_at	13653	Egr1	Early growth response 1	NM_007913	2.6	4.3	−2.0
1427683_at	13654	Egr2	Early growth response 2	NM_010118	17.4	22.0	−2.3
1449977_at	13656	Egr4	Early growth response 4	NM_020596	2.3	2.2	−1.4
1416529_at	13730	Emp1	Epithelial membrane protein 1	NM_010128	7.4	5.7	1.1
1416129_at	74155	Errfi1	ERBB receptor feedback inhibitor 1	NM_133753	3.0	2.0	−1.0
1418294_at	54357	Epb4.1l4b	Erythrocyte protein band 4.1-like 4b	NM_019427	2.5	2.6	1.0
1418635_at	27049	Etv3	ets variant gene 3	NM_001083318 /// NM_012051	4.3	2.3	1.0
1441023_at	67204	Eif2s2	Eukaryotic translation initiation factor 2, subunit 2 (beta)	NM_026030	2.7	2.1	−1.0

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1417562_at	13685	Eif4ebp1	Eukaryotic translation initiation factor 4E binding protein 1	NM_007918	3.8	2.9	1.2
1438427_at	67544	Fam120b	Family with sequence similarity 120, member B	NM_024203 /// NR_033586	3.4	2.4	1.3
1423100_at	14281	Fos	FBJ osteosarcoma oncogene	NM_010234	11.4	20.2	−1.8
1422134_at	14282	Fosb	FBJ osteosarcoma oncogene B	NM_008036	8.9	6.6	−1.1
1451264_at	319710	Frmd6	FERM domain containing 6	NM_028127	4.3	3.2	−1.2
1418534_at	57265	Fzd2	frizzled homolog 2 (Drosophila)	NM_020510	−2.5	−2.3	1.1
1450135_at	14365	Fzd3	frizzled homolog 3 (Drosophila)	NM_021458	3.2	2.7	1.0
1419301_at	14366	Fzd4	frizzled homolog 4 (Drosophila)	NM_008055	2.9	3.0	−1.0
1419322_at	13998	Fgd6	FYVE, RhoGEF and PH domain containing 6	NM_053072	2.7	2.5	1.1
1422542_at	23890	Gpr34	G protein-coupled receptor 34	NM_011823	−2.5	−2.7	1.1
1460275_at	14748	Gpr3	G-protein coupled receptor 3	NM_008154	2.5	2.0	1.0
1420394_s_at	14727 /// 14728	Gp49a /// Lilrb4	Glycoprotein 49 A /// leukocyte immunoglobulin-like receptor, subfamily B, member 4	NM_008147 /// NM_013532	4.1	2.9	1.0
1424825_a_at	14663	Glycam1	Glycosylation dependent cell adhesion molecule 1	NM_008134	2.5	2.0	−1.0
1449519_at	13197	Gadd45a	Growth arrest and DNA-damage-inducible 45 alpha	NM_007836	4.4	3.7	−1.2
1449773_s_at	17873	Gadd45b	Growth arrest and DNA-damage-inducible 45 beta	NM_008655	11.7	6.5	1.0
1453851_a_at	23882	Gadd45g	Growth arrest and DNA-damage-inducible 45 gamma	NM_011817	10.8	6.5	1.1
1426063_a_at	14579	Gem	GTP binding protein (gene overexpressed in skeletal muscle)	NM_010276	8.3	10.1	1.0
1423143_at	69237	Gtpbp4	GTP binding protein 4	NM_027000	2.9	2.2	1.1
1420499_at	14528	Gch1	GTP cyclohydrolase 1	NM_008102	2.3	2.0	−1.4
1430295_at	14674	Gna13	Guanine nucleotide binding protein, alpha 13	NM_010303	2.0	2.1	1.1
1423566_a_at	15505	Hsph1	Heat shock 105kDa/110kDa protein 1	NM_013559	3.8	3.2	−1.2
1425964_x_at	15507	Hspb1	Heat shock protein 1	NM_013560	10.7	15.2	−1.2
1422579_at	15528	Hspe1	Heat shock protein 1 (chaperonin 10)	NM_008303	2.6	2.1	1.0
1452388_at	193740	Hspa1a	Heat shock protein 1A	NM_010479	13.9	17.6	−1.4
1427126_at	15511	Hspa1b	Heat shock protein 1B	NM_010478	12.9	17.6	−1.5
1449872_at	56534	Hspb3	Heat shock protein 3	NM_019960	2.7	2.2	−1.0
1431182_at	15481	Hspa8	Heat shock protein 8	NM_031165	4.4	4.6	−1.4
1425378_at	235439	Herc1	Hect (homologous to the E6-AP (UBE3A) carboxyl terminus) domain and RCC1 (CHC1)-like do	NM_145617	3.9	3.2	1.3
1417541_at	15201	Hells	Helicase, lymphoid specific	NM_008234	2.7	2.4	1.3

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1448239_at	15368	Hmox1	Heme oxygenase (decycling) 1	NM_010442	7.1	5.3	1.3
1418350_at	15200	Hbegf	Heparin-binding EGF-like growth factor	NM_010415	5.5	3.2	1.4
1452534_a_at	97165	Hmgb2	High mobility group box 2	NM_008252	3.2	3.4	1.0
1435866_s_at	319162	Hist3h2a	Histone cluster 3, H2a	NM_178218	−2.0	−2.6	1.3
1419905_s_at	15446	Hpgd	Hydroxyprostaglandin dehydrogenase 15 (NAD)	NM_008278	−2.7	−2.2	1.3
1416442_at	15936	Ier2	Immediate early response 2	NM_010499	7.4	6.6	−1.0
1417612_at	15939	Ier5	Immediate early response 5	NM_010500	3.8	2.8	−1.4
1437103_at	319765	Igf2bp2	Insulin-like growth factor 2 mRNA binding protein 2	NM_183029	3.3	2.0	−1.1
1424067_at	15894	Icam1	Intercellular adhesion molecule 1	NM_010493	2.2	2.3	−1.3
1416067_at	15982	Ifrd1	Interferon-related developmental regulator 1	NM_013562	3.7	2.4	1.1
1426597_s_at	212632	Iffo2	Intermediate filament family orphan 2	NM_001205173 /// NM_183148	2.5	2.0	1.1
1417409_at	16476	Jun	Jun oncogene	NM_010591	5.8	4.2	1.4
1449117_at	16478	Jund	Jun proto-oncogene related gene d	NM_010592	2.1	2.1	1.1
1415899_at	16477	Junb	Jun-B oncogene	NM_008416	3.2	4.1	−1.7
1425270_at	16561	Kif1b	kinesin family member 1B	NM_008441 /// NM_207682	2.5	2.6	−1.2
1416029_at	21847	Klf10	Kruppel-like factor 10	NM_013692	3.1	2.6	−1.4
1417394_at	16600	Klf4	Kruppel-like factor 4 (gut)	NM_010637	2.4	5.9	1.0
1418280_at	23849	Klf6	Kruppel-like factor 6	NM_011803	4.7	5.5	1.1
1439479_at	226413	Lct	Lactase	NM_001081078	−2.5	−2.2	−1.0
1421654_a_at	16905	Lmna	Lamin A	NM_001002011 /// NM_001111102 /// NM_019390	5.1	4.0	1.2
1426808_at	16854	Lgals3	Lectin, galactose binding, soluble 3	NM_001145953 /// NM_010705	4.7	3.9	−1.1
1433842_at	16978	Lrrfip1	Leucine rich repeat (in FLII) interacting protein 1	NM_001111311 /// NM_001111312 /// NM_008515	6.6	5.2	1.0
1422725_at	17152	Mak	Male germ cell-associated kinase	NM_001145802 /// NM_001145803 /// NM_008547	3.3	4.1	−1.0
1429170_a_at	17764	Mtf1	Metal response element binding transcription factor 1	NM_008636	4.5	4.8	1.1
1451612_at	17748	Mt1	Metallothionein 1	NM_013602	2.8	3.0	1.0
1419254_at	17768	Mthfd2	Methylenetetrahydrofolate dehydrogenase (NAD+ dependent), methenyltetrahydrofolate cycl	NM_008638	3.8	2.8	1.0
1434364_at	53859	Map3k14	Mitogen-activated protein kinase kinase kinase 14	NM_016896	2.4	2.2	1.0

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1424942_a_at	17869	Myc	Myelocytomatosis oncogene	NM_001177352 /// NM_001177353 /// NM_001177354 /// NM_010849	4.0	3.8	1.1
1448503_at	17210	Mcl1	Myeloid cell leukemia sequence 1	NM_008562	6.1	4.8	1.1
1418589_a_at	17349	Mlf1	Myeloid leukemia factor 1	NM_001039543 /// NM_010801	3.4	3.1	−1.1
1422818_at	18003	Nedd9	Neural precursor cell expressed, developmentally down-regulated gene 9	NM_001111324 /// NM_017464	3.6	3.8	1.0
1420720_at	53324	Nptx2	Neuronal pentraxin 2	NM_016789	7.2	3.0	1.0
1417930_at	17937	Nab2	Ngfi-A binding protein 2	NM_001122895 /// NM_008668	2.9	2.3	−1.2
1417483_at	80859	Nfkbiz	Nuclear factor of kappa light polypeptide gene enhancer in B cells inhibitor, zeta	NM_001159394 /// NM_001159395 /// NM_030612	6.4	4.6	1.4
1418932_at	18030	Nfil3	Nuclear factor, interleukin 3, regulated	NM_017373	5.9	7.1	−1.4
1428083_at	66961	Neat1	Nuclear paraspeckle assembly transcript 1 (non-protein coding)	NR_003513	2.2	2.5	−1.2
1416505_at	15370	Nr4a1	Nuclear receptor subfamily 4, group A, member 1	NM_010444	2.1	3.6	−2.4
1436780_at	108155	Ogt	O-linked N-acetylglucosamine (GlcNAc) transferase (UDP-N-acetylglucosamine:polypeptide-	NM_139144	−2.3	−2.2	1.2
1418674_at	18414	Osmr	Oncostatin M receptor	NM_011019	7.2	4.1	1.3
1423174_a_at	58220	Pard6b	par-6 (partitioning defective 6) homolog beta (C. elegans)	NM_021409	3.4	2.6	−1.1
1422324_a_at	19227	Pthlh	Parathyroid hormone-like peptide	NM_008970	3.5	4.0	−1.1
1417372_a_at	67245	Peli1	Pellino 1	NM_023324	2.2	2.2	1.2
1419006_s_at	93834	Peli2	Pellino 2	NM_033602	2.1	2.1	−1.0
1449851_at	18626	Per1	Period circadian clock 1	NM_001159367 /// NM_011065	2.5	2.3	1.1
1417602_at	18627	Per2	Period circadian clock 2	NM_011066	2.5	2.8	−1.6
1420715_a_at	19016	Pparg	Peroxisome proliferator activated receptor gamma	NM_001127330 /// NM_011146	3.4	2.1	1.0
1421811_at	21825	Thbs1	Thrombospondin 1	NM_011580/// NM_013753	13.7	8.0	1.3
1421917_at	18595	Pdgfra	Platelet derived growth factor receptor, alpha polypeptide	NM_001083316 /// NM_011058	−2.0	−2.0	1.1
1418835_at	21664	Phlda1	Pleckstrin homology-like domain, family A, member 1	NM_009344	3.4	3.4	−1.2
1434496_at	12795	Plk3	Polo-like kinase 3	NM_013807	2.2	2.4	−1.0
1429456_a_at	26939	Polr3e	Polymerase (RNA) III (DNA directed) polypeptide E	NM_001164096 /// NM_025298	2.5	2.4	1.4
1424874_a_at	19205	Ptbp1	Polypyrimidine tract binding protein 1	NM_001077363 /// NM_008956	2.6	2.0	1.1
1425341_at	16527	Kcnk3	Potassium channel, subfamily K, member 3	NM_010608	3.1	2.1	−1.2
1431213_a_at	67527 /// 100041932	Gm3579 /// LOC67527	Predicted gene 3579 /// murine leukemia retrovirus	XM_001477842	2.5	2.1	−1.2
1433668_at	108767	Pnrc1	Proline-rich nuclear receptor coactivator 1	NM_001033225	−2.1	−2.1	−1.0

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1424208_at	19219	Ptger4	Prostaglandin E receptor 4 (subtype EP4)	NM_001136079 /// NM_008965	4.6	2.6	1.4
1417262_at	19225	Ptgs2	Prostaglandin-endoperoxide synthase 2	NM_011198	8.5	3.3	−1.3
1448325_at	17872	Ppp1r15a	Protein phosphatase 1, regulatory (inhibitor) subunit 15A	NM_008654	4.2	4.7	1.0
1429715_at	71978	Ppp2r2a	Protein phosphatase 2 (formerly 2A), regulatory subunit B (PR 52), alpha isoform	NM_001205188 /// NM_028032	2.8	2.1	1.1
1449249_at	54216	Pcdh7	Protocadherin 7	NM_001122758 /// NM_018764	3.0	2.7	−1.2
1435458_at	18712	Pim1	Proviral integration site 1	NM_008842	5.6	2.5	−1.2
1431724_a_at	70839	P2ry12	Purinergic receptor P2Y, G-protein coupled 12	NM_027571	−2.8	−2.4	1.1
1427931_s_at	216134	Pdxk	Pyridoxal (pyridoxine, vitamin B6) kinase	NM_172134	2.4	2.0	1.1
1417273_at	27273	Pdk4	Pyruvate dehydrogenase kinase, isoenzyme 4	NM_013743	3.0	2.2	1.1
1426452_a_at	75985	Rab30	RAB30, member RAS oncogene family	NM_029494	2.3	2.1	−1.1
1448414_at	19355	Rad1	RAD1 homolog (S. pombe)	NM_011232	−2.1	−2.1	1.0
1418892_at	80837	Rhoj	ras homolog gene family, member J	NM_023275	3.4	2.2	1.3
1423854_a_at	68939	Rasl11b	RAS-like, family 11, member B	NM_026878	2.0	2.6	−1.6
1422562_at	56437	Rrad	Ras-related associated with diabetes	NM_019662	14.6	4.5	1.8
1426037_a_at	19734	Rgs16	regulator of G-protein signaling 16	NM_011267	2.3	2.0	1.3
1452359_at	100532	Rell1	RELT-like 1	NM_145923	2.4	2.2	1.1
1420710_at	19696	Rel	Reticuloendotheliosis oncogene	NM_009044	3.4	2.6	−1.1
1416700_at	74194	Rnd3	Rho family GTPase 3	NM_028810	3.2	3.3	−1.0
1438502_x_at	20068	Rps17	Ribosomal protein S17	NM_009092	3.1	2.8	−1.1
1427299_at	110651	Rps6ka3	Ribosomal protein S6 kinase polypeptide 3	NM_148945	2.3	2.0	−1.1
1427932_s_at	71719 /// 71739 /// 319269	1200003I10Rik /// 1200015M12Rik /// A130040M12Rik	RIKEN cDNA 1200003I10 gene /// RIKEN cDNA 1200015M12 gene /// RIKEN cDNA A130040M12 gen	NR_002860	3.7	3.8	−1.2
1451415_at	69068	1810011O10Rik	RIKEN cDNA 1810011O10 gene	NM_026931	2.3	2.5	1.3
1428552_at	66520	2610001J05Rik	RIKEN cDNA 2610001J05 gene	NM_183258 /// NR_024619	−2.1	−2.2	1.1
1416355_at	19655	Rbmx	RNA binding motif protein, X chromosome	NM_001166623 /// NM_011252 /// NR_029425	−2.1	−2.1	−1.2
1424704_at	12393	Runx2	Runt related transcription factor 2	NM_001145920 /// NM_001146038 /// NM_001271627 /// NM_001271630 /// NM_001271631 /// NM	2.6	2.3	−1.1
1419149_at	18787	Serpine1	Serine (or cysteine) peptidase inhibitor, clade E, member 1	NM_008871	3.2	3.0	−1.1
1429650_at	74178	Stk40	Serine/threonine kinase 40	NM_001145827 /// NM_028800	3.4	2.4	1.0

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1417406_at	55942	Sertad1	SERTA domain containing 1	NM_018820	2.9	2.4	−1.2
1425139_at	230784	Sesn2	Sestrin 2	NM_144907	2.7	2.3	1.0
1448170_at	20439	Siah2	Seven in absentia 2	NM_009174	2.6	2.6	−1.1
1433674_a_at	83673	Snhg1	Small nucleolar RNA host gene (non-protein coding) 1	NR_002896	4.6	3.6	1.2
1428529_at	72655	Snhg5	Small nucleolar RNA host gene 5	NR_040721	2.6	2.2	1.1
1428776_at	75750	Slc10a6	Solute carrier family 10 (sodium/bile acid cotransporter family), member 6	NM_029415	5.4	5.4	−1.2
1420697_at	65221	Slc15a3	Solute carrier family 15, member 3	NM_023044	2.6	2.6	−1.2
1438824_at	20515	Slc20a1	Solute carrier family 20, member 1	NM_001159593 /// NM_015747	2.3	2.7	1.1
1422786_at	22782	Slc30a1	Solute carrier family 30 (zinc transporter), member 1	NM_009579	2.6	2.4	1.0
1420405_at	28250		Solute carrier organic anion transporter family, member 1a4	NM_030687	−2.6	−2.3	−1.1
1422256_at	20606	Sstr2	Somatostatin receptor 2	NM_001042606 /// NM_009217	3.4	2.8	−1.1
1451596_a_at	20698	Sphk1	Sphingosine kinase 1	NM_001172472 /// NM_001172473 /// NM_001172475 /// NM_011451 /// NM_025367	2.5	2.7	−1.3
1420150_at	74646	Spsb1	splA/ryanodine receptor domain and SOCS box containing 1	NM_029035	2.6	2.4	−1.2
1436584_at	24064	Spry2	Sprouty homolog 2 (Drosophila)	NM_011897	3.5	2.4	−1.2
1449109_at	216233	Socs2	Suppressor of cytokine signaling 2	NM_001168655 /// NM_001168656 /// NM_001168657 /// NM_007706	2.1	2.3	−1.1
1456212_x_at	12702	Socs3	Suppressor of cytokine signaling 3	NM_007707	11.6	13.1	−1.3
1434089_at	104027	Synpo	Synaptopodin	NM_001109975 /// NM_177340	2.3	2.5	−1.1
1430271_x_at	75316	Taf1d	TATA box binding protein (Tbp)-associated factor, RNA polymerase I, D	NM_026541 /// NM_027261 /// NM_029248 /// NR_028401	2.1	2.3	1.1
1452161_at	99929	Tiparp	TCDD-inducible poly(ADP-ribose) polymerase	NM_178892	4.8	2.8	1.2
1416342_at	21923	Tnc	Tenascin C	NM_011607	5.8	4.3	1.2
1424012_at	78802	Ttc30a1	Tetratricopeptide repeat domain 30A1	NM_030188	−2.4	−2.1	1.0
1418547_at	21789	Tfpi2	Tissue factor pathway inhibitor 2	NM_009364	2.6	2.0	−1.1
1454018_at	24086	Tlk2	Tousled-like kinase 2 (Arabidopsis)	NM_001112705 /// NM_011903	2.6	2.5	1.2
1436854_at	22064	Trpc2	Transient receptor potential cation channel, subfamily C, member 2	NM_001109897 /// NM_011644	−2.2	−2.4	1.1
1423289_a_at	66282	Tma16	Translation machinery associated 16 homolog (S. cerevisiae)	NM_025465	2.5	2.2	1.1
1424880_at	211770	Trib1	Tribbles homolog 1 (Drosophila)	NM_144549	3.9	3.8	−1.3
1430576_at	22019	Tpp2	Tripeptidyl peptidase II	NM_009418	2.5	2.4	1.2

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Overlap (222 genes)

Probeset ID	Entrez Gene	Gene Symbol	Gene Title	RefSeq Transcript ID	WI vs WS	TI vs TS	TS vs WS
1416431_at	67951	Tubb6	Tubulin, beta 6 class V	NM_026473	5.9	6.4	−1.0
1418572_x_at	27279	Tnfrsf12a	Tumor necrosis factor receptor superfamily, member 12a	NM_001161746 /// NM_013749	7.0	6.7	−1.2
1456094_at	72344	Usp36	Ubiquitin specific peptidase 36	NM_001033528	3.1	2.4	−1.1
1452385_at	99526	Usp53	Ubiquitin specific peptidase 53	NM_133857	3.1	2.3	1.1
1436882_at	66177	Ubl5	Ubiquitin-like 5	NM_025401	2.1	2.1	1.2
1454842_a_at	97884	B3galnt2	UDP-GalNAc:betaGlcNAcbeta 1,3-galactosaminyltransferase, polypeptide 2	NM_178640	−2.9	−3.2	1.2
1420994_at	108105	B3gnt5	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 5	NM_001159407 /// NM_001159408 /// NM_054052	2.5	2.8	−1.2
1434606_at	13867	Erbb3	v-erb-b2 erythroblastic leukemia viral oncogene homolog 3 (avian)	NM_010153	−3.5	−2.3	−1.4
1418936_at	17133	Maff	v-maf musculoaponeurotic fibrosarcoma oncogene family, protein F (avian)	NM_010755	6.0	5.6	−1.1
1455812_x_at	246154	Vasn	vasorin	NM_139307	2.3	2.1	1.1
1456292_a_at	22352	Vim	Vimentin	NM_011701	2.5	2.1	−1.1
1434744_at	230734	Yrdc	yrdC domain containing (E.coli)	NM_153566	2.1	2.1	1.0
1422570_at	22632	Yy1	YY1 transcription factor	NM_009537	2.2	2.1	1.0
1425305_at	114565	Zbtb21	Zinc finger and BTB domain containing 21	NM_001081684 /// NM_001081685 /// NM_175428	2.4	2.1	1.0
1452519_a_at	22695	Zfp36	Zinc finger protein 36	NM_011756	4.1	5.2	−1.1
1424974_at	232854	Zfp418	Zinc finger protein 418	NM_146179	−2.3	−2.3	−1.1
1449946_a_at	68040	Zfp593	Zinc finger protein 593	NM_024215	2.5	2.4	−1.1
1452623_at	268670	Zfp759	Zinc finger protein 759	NM_172392	−2.1	−2.4	1.3
1427539_a_at	52696	Zwint	ZW10 interactor	NM_025635	4.9	4.1	1.2

Overlap (1 genes)

RefSeq transcript ID	WI vs WS	TI vs TS	TS vs WS
NM_001276684 /// NM_018790	6.15	8.25	−2.46

Overlap (2 genes)

Gene symbol	Gene title	RefSeq transcript ID	WI vs WS	TI vs TS	TS vs WS
Sox11	SRY-box containing gene 11	NM_009234	3.51	−1.37	3.32

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Overlap (2 genes)

Gene symbol	Gene title	RefSeq transcript ID	WI vs WS	TI vs TS	TS vs WS
Upp1	uridine phosphorylase 1	NM_001159401 /// NM_001159402 /// NM_009477	2.14	1.04	2.14

Overlap (3 genes)

Probeset ID	Entrez gene	Gene symbol	gene title	RefSeq transcript ID	WI vs WS	TI vs TS	TS vs WS
1418160_at	22652	Mkrn3	makorin, ring finger protein, 3	NM_011746	−1.47	−2.05	5.40
1418475_at	20277	Scnn1b	sodium channel, nonvoltage-gated 1 beta	NM_001272023 /// NM_011325 /// NR_073548	−1.20	−2.61	3.66
1419663_at	18295	Ogn	osteoglycin	NM_008760	−1.30	−2.81	5.70

Abbreviations

ANOVA	analysis of variance
Arc	activity-regulated cytoskeleton-associated protein
GFP	green fluorescent protein
GO	gene ontology

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KEGG	Kyoto Encyclopedia of Genes and Genomes
MAPK	mitogen-activated protein kinase
miRNA	microRNA
OGD	oxygen/glucose deprivation
PCA	principal component analysis
qPCR	quantitative real-time PCR
RanGAP1	Ran GTPase-activating protein-1
rCBF	regional cerebral blood flow
SUMO	small ubiquitin-like modifier
SUMO-KD	SUMO1-3 knockdown
WI	wild-type ischemia
WS	wild-type sham

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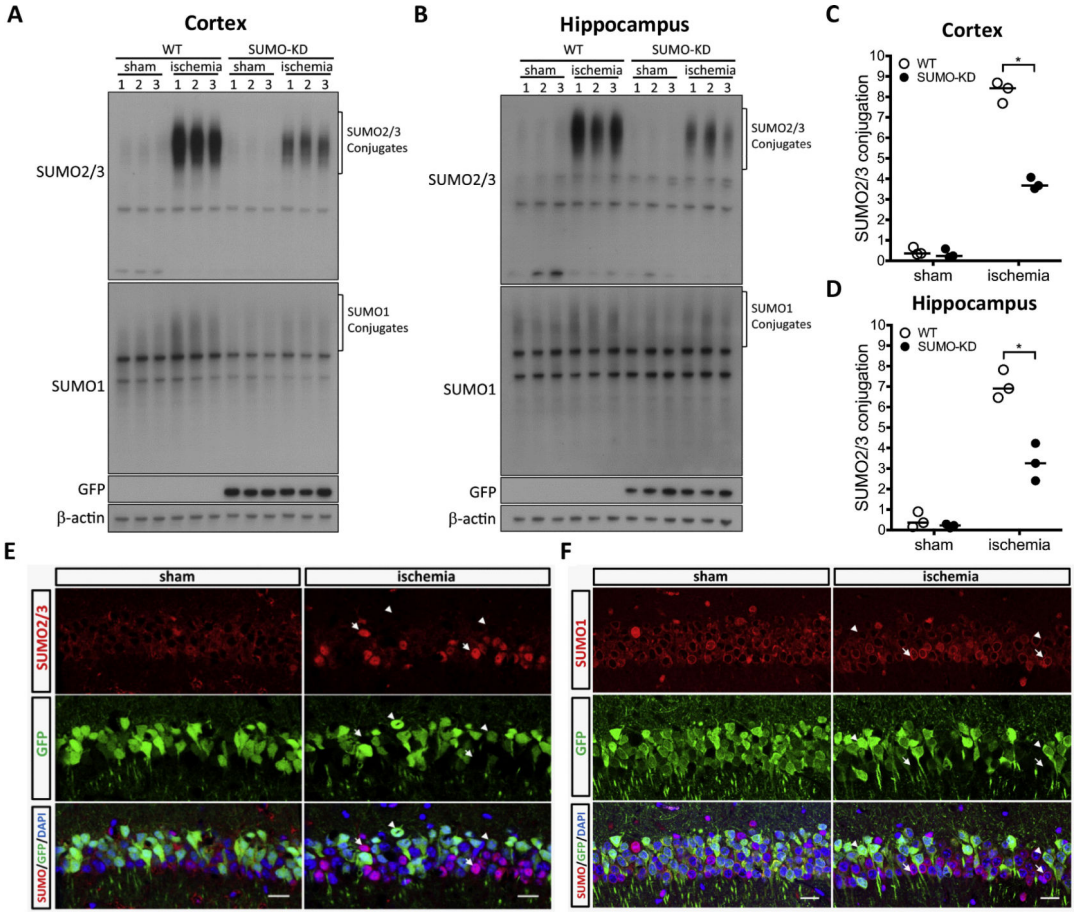

Fig. 1.

SUMOylation after transient forebrain ischemia in SUMO knockdown (SUMO-KD) mice. Wild-type (WT) and SUMO-KD mice were subjected to sham surgery or 10 10-min forebrain ischemia and 1 h reperfusion. Ischemia-induced changes in SUMOylation, and its subcellular localization were evaluated by Western blotting (A–D) and immunohistochemistry (E, F). (A–D) Global SUMOylation in cortex (A, C) and hippocampus (B, D). Global SUMOylation induced by ischemia/reperfusion was significantly less in SUMO-KD mice. The high-molecular-weight regions, marked by brackets, were used for quantification of SUMO2/3 conjugation. Intensities of SUMO2/3 conjugates were measured by image analysis, and normalized to β-actin. Horizontal bar = median values; $*^{p} \leq 0.05$. (E, F) Immunohistochemistry analysis of SUMO2/3 (E) and SUMO1 (F) in brains of SUMO-KD mice subjected to sham or ischemia surgery. After ischemia, strong nuclear SUMO2/3 staining was observed in GFP-negative hippocampus neurons (arrows), but was absent in SUMO knockdown neurons (GFP GFP-positive; arrowheads). Nuclear rim staining by SUMO1 appeared more intense in GFP GFP-negative neurons (arrows) compared to GFP GFP-positive cells (arrowheads). Scale bar = 20 μm.

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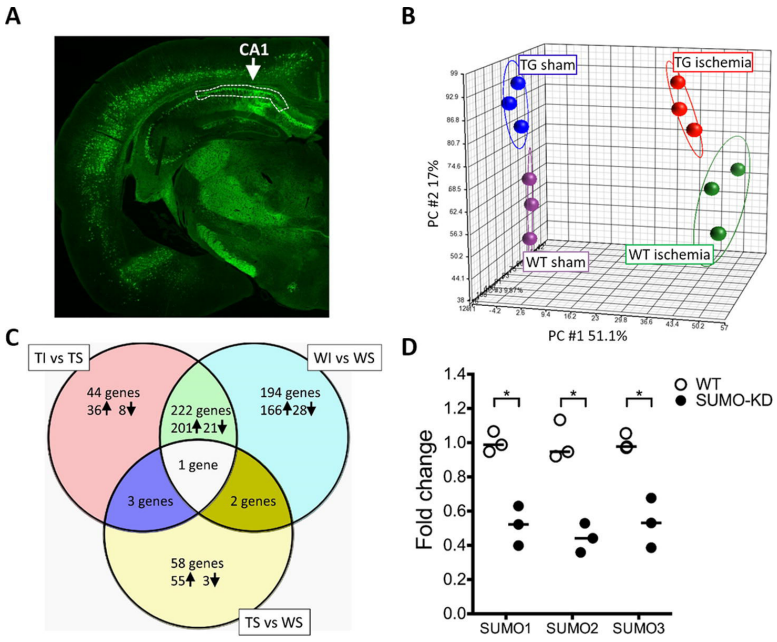

Fig. 2.

Overview of microarray data. Data analysis was performed on the global gene expression profiles of 12 samples from four groups: wild-type (WT) sham (WS), and ischemia (WI); and SUMO-KD (TG) sham (TS), and ischemia (TI). (A) The sampling regions. The region of hippocampal CA1 subfield that was dissected out and used for microarray analysis and qPCR is marked with white dot lines in a representative brain slice of SUMO-KD mice with GFP fluorescence. (B) Principal component analysis (PCA). The individual samples were plotted in a 3-dimensional space based on three principal components. Four groups of samples are clustered according to the genotype and surgery. (C) Venn diagram. The numbers of differentially regulated genes that were identified by pairwise comparisons of groups, with a cut-off of ≥ 2-twofold increase (↑) or decrease (↓) in gene expression are shown. (D) Verification of SUMO1-3 knockdown in SUMO-KD mice. The RNA samples from the sham group that were used for the microarray study, were analyzed to determine the levels of SUMO1-3 mRNA levels in WT and SUMO-KD mice. All individual data were normalized to β-actin. To calculate fold change, the mean values of WT mouse samples were set to 1.0. Horizontal bar = median values; *$p \leq 0.05$.

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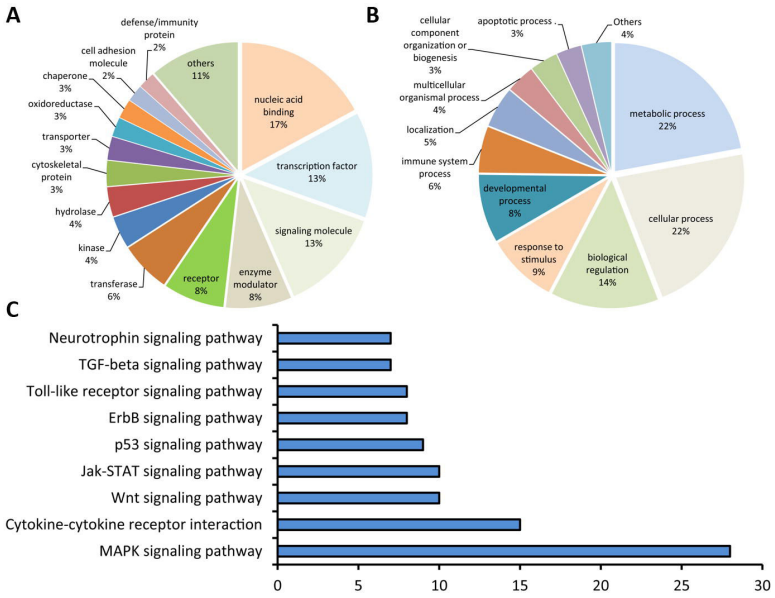

Fig. 3.

GO classification and KEGG pathway analysis of differentially expressed genes in the CA1 samples after forebrain ischemia in wild-type mice. (A) Enriched GO terms according to protein class. (B) Enriched GO terms according to biological processes. (C) Enriched KEGG pathways.

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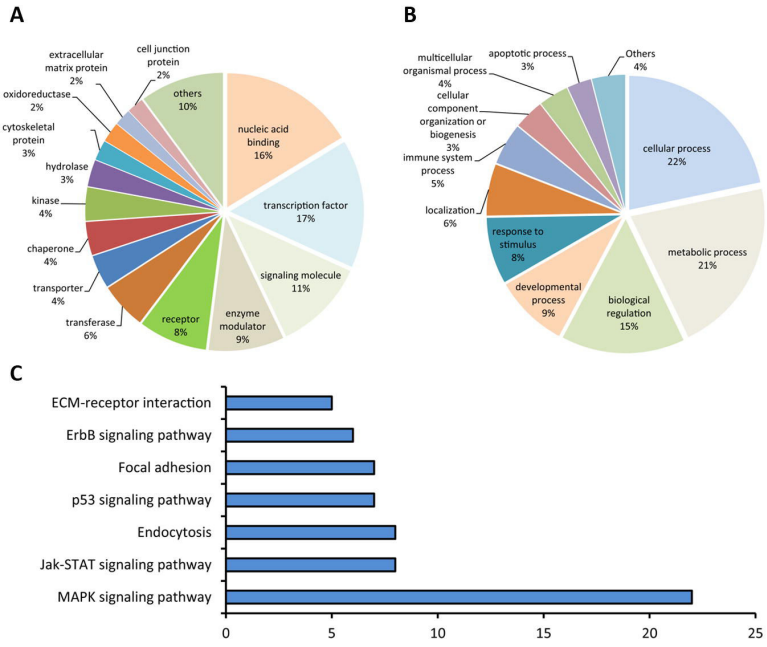

Fig. 4.

GO classification and KEGG pathway analysis of differentially expressed genes in the CA1 samples after forebrain ischemia in SUMO-KD mice. (A) Enriched GO terms according to protein class. (B) Enriched GO terms according to biological processes. (C) Enriched KEGG pathways.

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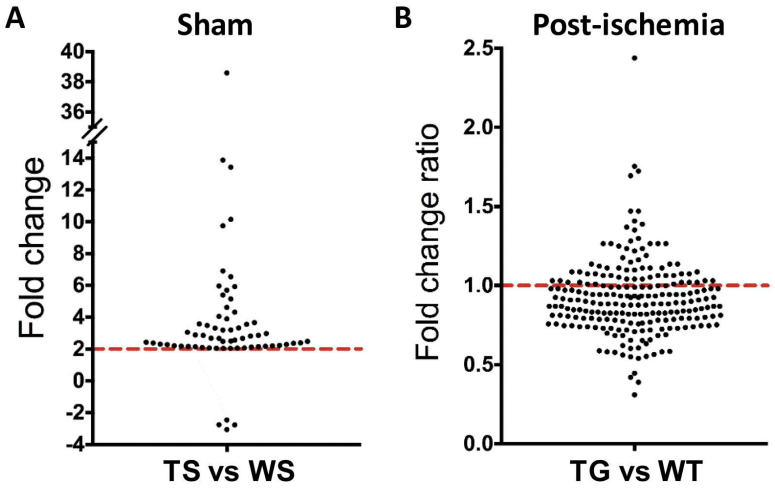

Fig. 5.

Effect of SUMO knockdown on differentially expressed genes in the CA1 subfield before and after ischemia. (A) Fold change of genes differentially regulated in the SUMO-KD sham group (TS) compared to the wild-type sham group (WS). (B) The ratio of fold change of the genes differentially regulated by ischemia in both SUMO-KD (TG) and wild-type (WT) mice. The ratio was calculated by dividing an ischemia-induced fold change of a gene between TG and WT mice. If the ratio of a gene is < 1, the fold change of this gene regulated by ischemia is greater in WT mice.

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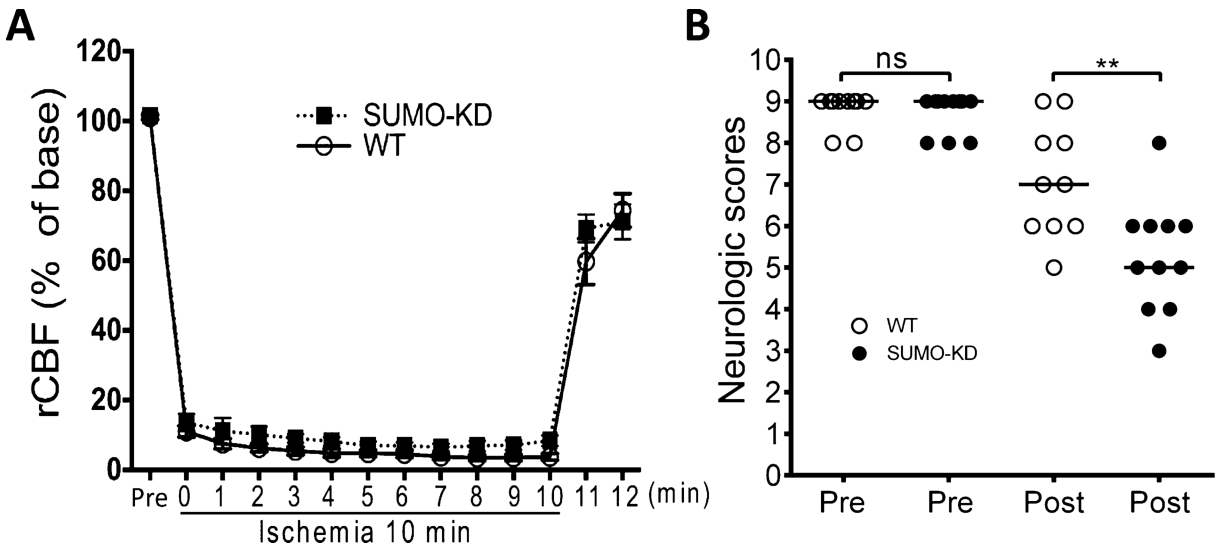

Fig. 6.

Functional outcome after transient forebrain ischemia. (A) Effect of SUMO knockdown on rCBF in our transient forebrain ischemia model. Wild-type (WT; $n = 5$) and SUMO-KD ($n = 5$) mice were subjected to 10 10-min forebrain ischemia. Data are presented as means ± SEM. Laser-Doppler measurements revealed a similar change in rCBF between WT and SUMO-KD mice during ischemia and reperfusion. (B) Wild-type (WT; $n = 10$) and SUMO-KD ($n = 11$) mice were subjected to 10 10-min forebrain ischemia. Neurologic function was evaluated 1 day before (Pre) and 4 days after (Post) forebrain ischemia. Horizontal bar = median values. ns, not significant; $^{**}p \leq 0.01$.

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Table 1

List of primers

Gene	Primer sequences (5′->3′)
Sumo1	Forward: CAGGAGGCAAAACCTTCAAC
	Reverse: CTCCATTCCCAGTTCTTTCG
Sumo2	Forward: ACGAGAAACCCAAGGAAGGA
	Reverse: CTCCATTTCCAACTGTGCAG
Sumo3	Forward: AGAAGCCCAAGGAGGGTGT
	Reverse: CCTCGGGAGGCTGATCCT
Atf3	Forward: CCAGGTCTCTGCCTCAGAAG
	Reverse: CCTTCAGCTCAGCATTCACA
Hspb1	Forward: GCCTCTTCGATCAAGCTTTC
	Reverse: CCTCAGGGGATAGGGAAGAG
Fos	Forward: ATGGGCTCTCCTGTCAACAC
	Reverse: GCAGCCATCTTATTCCGTTC
Arc	Forward: AGCAGCAGACCTGACATCCT
	Reverse: GTGATGCCCTTTCCAGACAT
Rgs2	Forward: GAGGAGAAGCGGGAGAAAAT
	Reverse: GAGGACAGTTTTTGGGGTGA
Tcfl5	Forward: GGAGGACCGCTTCAACAGTA
	Reverse: GGCAGTCCAATATCCTGGTG
Cxcl12	Forward: GCGCTCTGCATCAGTGAC
	Reverse: TAATTTCGGGTCAATGCACA
Socs2	Forward: CGCGAGCTCAGTCAAACAG
	Reverse: GCAGAGTGGGTGCTGATGTA
Ogt	Forward: GCGTTTTCCAGCAGTAGGAG
	Reverse: CCAGACTTTGCCACGAATTT
Slco1a4	Forward: AATGCCAAAGAGGAGAAGCA
	Reverse: TGGGAAATTGTCACAGGTCA
Rasl10a	Forward: TCATCCGCCAATTCTTGTTT
	Reverse: TTGTTGCCTACCACCAGGAT
β-actin	Forward: TAGGCACCAGGGTGTGATG
	Reverse: GGGGTGTTGAAGGTCTCAAA

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Table 2

Verification of microarray data by qPCR analysis

Entrez gene	Gene symbol	WI vs WS		TI vs TS		TS vs WS	
		qPCR	Microarray	qPCR	Microarray	qPCR	Microarray
11910	Atf3	123.93	46.7	103.25	33.1	−1.46	−1.2
15507	Hspb1	42.71	10.7	3.83	15.2	2.28	−1.2
14281	Fos	39.58	11.4	64.74	20.2	−1.79	−1.8
11838	Arc	11.00	6.15	12.38	8.25	−2.73	−2.45
19735	Rgs2	3.86	4.1	1.43	1.9	−1.08	−1.1
277353	Tcfl5	3.36	1.56	0.83	−1.27	261.38	38.58
20315	Cxcl12	−1.82	−1.4	−1.96	−1.32	−1.91	−2.45
216233	Socs2	−1.36	1.95	1.17	2.18	−2.40	−1.1
108155	Ogt	−2.21	−2.3	−1.89	−2.2	−0.96	1.2
28250	Slco1a4	−5.15	−2.6	−2.13	−2.3	−1.04	−1.1
75668	Rasl10a	−6.39	−2.9	−3.26	−2.0	−1.13	−1.1