

Supplemental materials

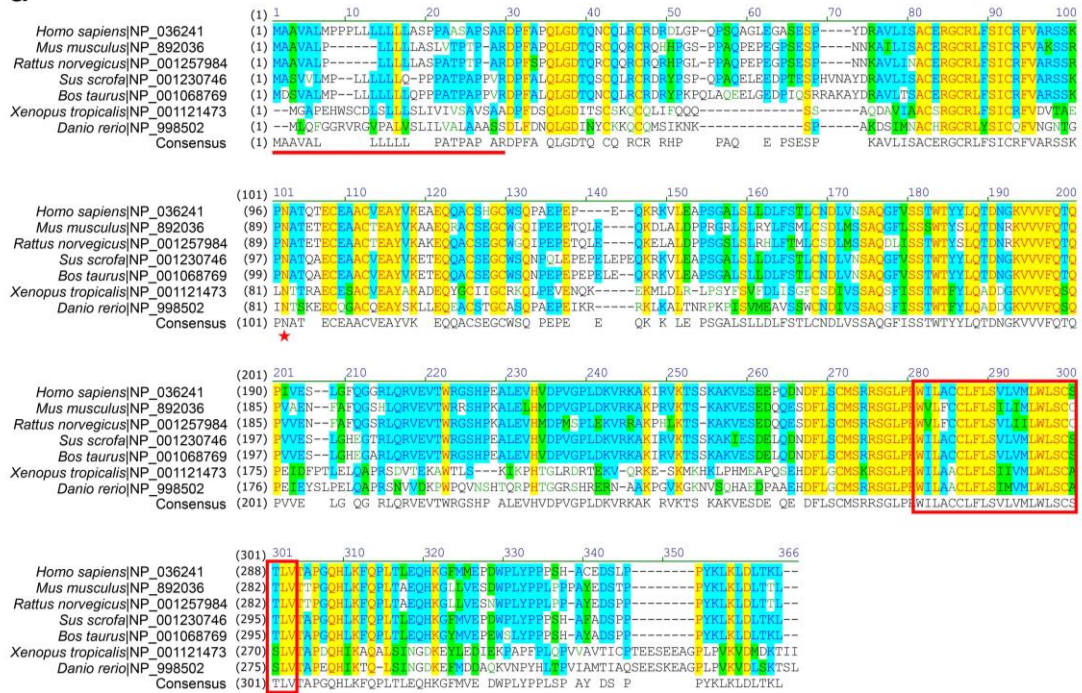
The Neuron-Specific Protein TMEM59L Mediates Oxidative Stress-Induced Cell Death

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b

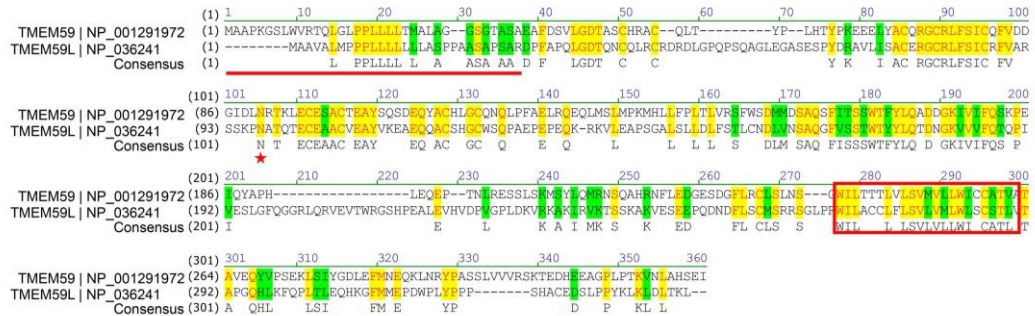


Fig. S1 Sequence alignments of TMEM59L and TMEM59. **a** Protein sequence alignment of TMEM59L among different species. **b** Protein sequence alignment of human TMEM59L and its homolog, TMEM59. The signal peptide region was underlined and the transmembrane domain was boxed. The putative N-glycosylation site was marked with an asterisk. Identical amino acids were on a yellow background. Similar amino acids were on a green background. '-', deletions.

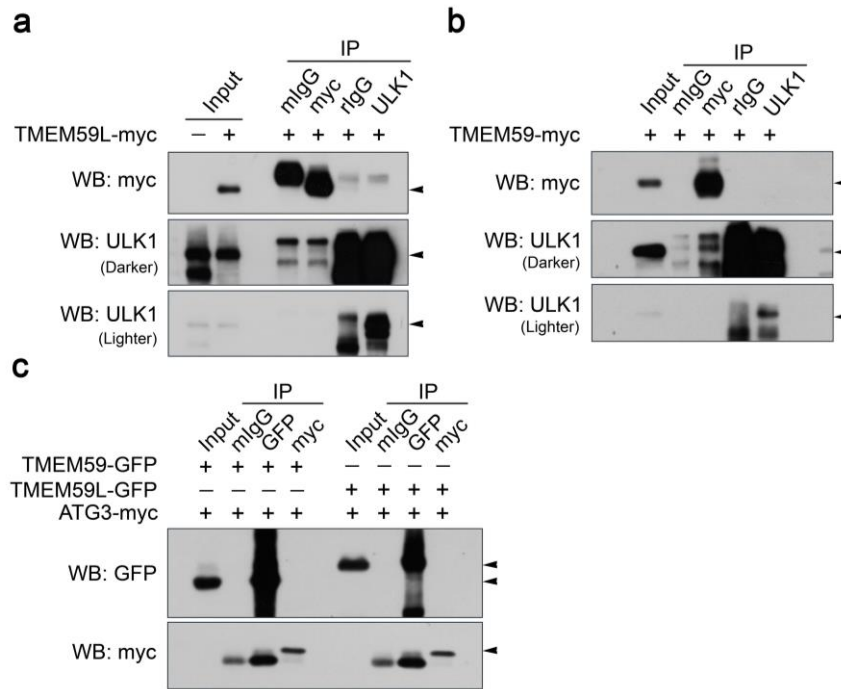


Fig. S2 a, b Neither TMEM59L nor TMEM59 interact with ULK1 and ATG3. HEK293T cells were transfected with (a) TMEM59L-myc or (b) TMEM59-myc. Equal amounts of cell lysates were subjected to immunoprecipitation (IP) with mouse normal IgG (mIgG), mouse antibody against myc, rabbit normal IgG (rIgG), or rabbit antibody against ULK1, followed by Western blot (WB) analysis. c TMEM59-GFP or TMEM59L-GFP were co-transfected with myc-tagged ATG3 into HEK293T cells. Equal amounts of cell lysates were subjected to IP with mIgG, mouse antibody against GFP, or mouse antibody against HA, followed by WB analysis. Five percent of cell lysates were used as input.

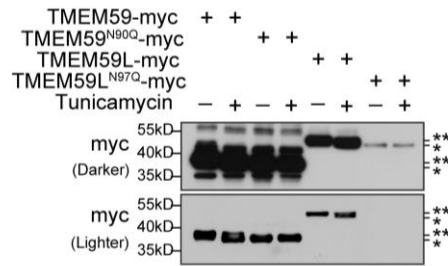


Fig. S3 TMEM59L and TMEM59 are glycosylated proteins. HEK293T cells were transfected with TMEM59-myc, TMEM59L-myc, TMEM59^{N90Q}-myc, and TMEM59L^{N97Q}-myc for 24 hr, and then treated with 10 μ g/ml tunicamycin for additional 12 hr. Cell lysates were subjected to Western blot for myc. “*” indicated the non-glycosylated form. “***” indicated the glycosylated form.

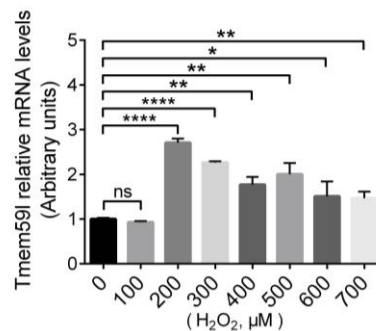


Fig. S4 The mRNA level of *Tmem59l* is significantly increased upon hydrogen peroxide insult. Primary cortical neurons were treated with H₂O₂ at indicated concentrations for 5 hr at DIV10. Total RNAs were extracted, reverse transcribed, and then subjected to qRT-PCR. The mRNA levels of *Tmem59l* were normalized to those of β -actin and compared to that of control (set as one arbitrary unit). n=3, ns, not significant, * p <0.05, ** p <0.01, **** p <0.0001.

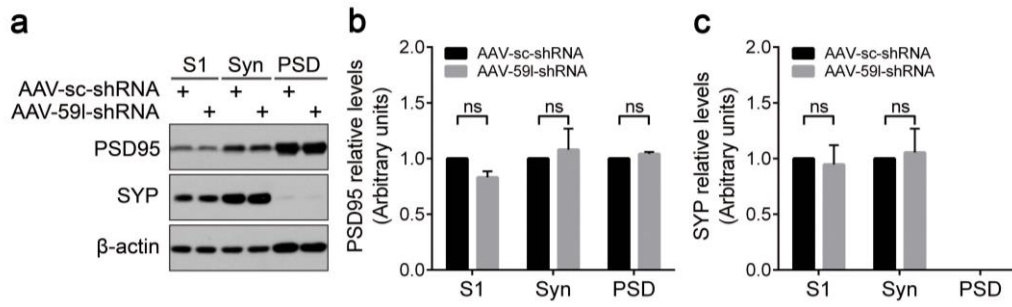


Fig. S5 Downregulation of TMEM59L does not affect synaptic components. **a** Western blot analyses of PSD95 and Synaptophysin (SYP) in postnuclear (S1), synaptosomal (Syn), and post-synaptic density (PSD) fractions isolated from hippocampus of mice injected with AAV-sc-shRNA or AAV-59l-shRNA. **b** PSD95 and **(c)** SYP protein levels in different fractions were quantified by densitometry for comparison. n=3, ns, not significant.

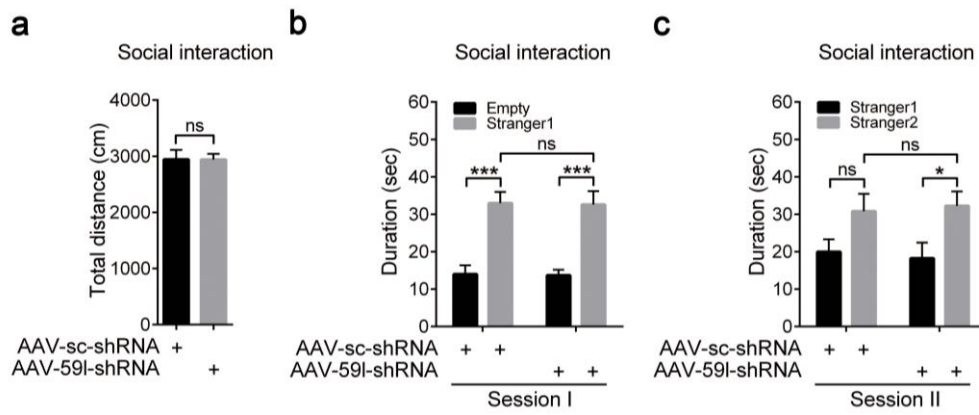


Fig. S6 Downregulation of TMEM59L does not affect mouse social interaction ability.

a-c Mice injected with AAV-sc-shRNA (n=16) and AAV-59l-shRNA (n=16) were subjected to social interaction test to determine **(a)** total distance (cm), **(b)** percentage duration (session I), and **(c)** percentage duration (session II). ns, not significant, * $p < 0.05$, *** $p < 0.001$.

Table S1 Primers used for quantitative real-time PCR

Gene	Primer sequences
<i>β-actin</i>	Forward: AGCCATGTACGTAGCCATCCA Reverse: TCTCCGGAGTCCATCACAATG
<i>Tmem59l</i>	Forward: TGGCTGTCGGCTTTTCTCC Reverse: GGCAGAACTCATCAGGTCGCT
<i>Tmem59</i>	Forward: CCAGTTTGTGGATGATGGGCTT Reverse: GCATTCTTGGCATCAGGGACA
<i>Caspase-3</i>	Forward: GGCTTGCCAGAAGATACCGGTG Reverse: GCATAAATTCTAGCTTGTGCGCGTA

Supplemental Materials and Methods

Preparation of Synaptosomal and PSD Fractions

Synaptosomal and PSD fractions were prepared as previously described [1]. Briefly, mouse brains were dissected and homogenized in cold sucrose buffer (0.32 M sucrose and 25 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), pH 7.4). The homogenates were centrifuged at 300 g for 5 min to separate the supernatant (S1) from the nuclei and large debris fraction. The S1 fraction was centrifuged at 10,000 g for 12 min to separate the supernatant (S2, light membrane and cytosolic fraction) and the pellet (P2, crude synaptosomal fraction). The P2 fraction was resuspended in cold HBS buffer (25 mM HEPES, pH7.4, and 150 mM NaCl) to get the synaptosomal fraction. The PSD fraction was prepared by solubilizing the synaptosomal fraction in 1% Triton HBS buffer and centrifuging at 10,000 g for 20 min. Solubilized pellet were re-dissolved in 3% SDS.

Behavioral Tests

Open Field Test. The open field box is 40cm (L) × 40cm (W) × 40cm (H). Each mouse was placed in the box for 10 min. During the experiments, the open field was video recorded. Total travel distance and time spent in the center areas of the maze were computed by a Smart 3.0 video tracking system (Panlab, Harvard Apparatus).

Elevated Plus Maze Test. The elevated plus maze (30cm (L) × 6cm (W) × 15cm (H)) was used to analyze anxiety. Mice were placed onto the center of the maze and allowed free access to all four arms for 5 min. Total travel distance and time spent on the open arms were calculated.

Forced Swim Test [2]. The forced swim test was used for evaluation of the depressive-like behavior. The tanks (15cm (D) × 30cm (H)) were filled with tap water set at 22 °C. Mice were placed in the water and their escape-related mobility behavior was measured for 5 min.

Social Interaction Test [3]. The social interaction apparatus is a rectangular, three chambered plexiglas box, with each chamber measuring 20cm (L) × 42cm (W) × 22cm (H). Dividing walls are clear with small semicircular openings (3.5cm radius) allowing access into each chamber. The middle chamber is empty and each of the two outer chambers contains a small, round wire cage. Before test, the subject mouse was placed at the center of the middle chamber for habituating for 5 min. For social affiliation aspect test (session I), one control mouse (stranger 1) was placed inside a wire cage and then the walls between the three chambers were removed to allow the subject mouse to explore for 10 min. For the social novelty/preference test (session II), a second control mouse (stranger 2) was placed inside the other wire cage and then the subject mouse was allowed to explore for additional 10 min. The duration of contact between the subject mouse and the empty wire cage vs the cage housing stranger 1 (in session I), or between the subject mouse and the cage housing stranger 1 vs the cage housing stranger 2 (in session II) were recorded.

Y-Maze Test. The Y-maze was used for evaluation of spontaneous alternation for spatial working memory in mice. The Y-shaped maze was constructed with three symmetrical white solid plastic arms at a 120-degree angle (30cm (L) × 6cm (W) × 15cm (H)). Each session began with placement of the mouse in the center of the maze. The mouse was allowed to freely explore the three arms for 5 min. Arm entry was defined as all four limbs within the arm. A triad was defined as a set of three arm entries, when each entry was to a different arm of the maze. Total travel distance, the

number of arm entries and the number of triads were recorded. Spontaneous alternations were defined as consecutive triplets of different arm choices.

Morris Water Maze Test [4,5]. Spatial reference memory testing was performed in a circular tank (90cm (D) × 35cm (H)) filled with tap water set at 22 °C. The walls surrounding the tank contained bright and contrasting shapes that served as reference cues. The tank contained a fixed platform in a target quadrant. On the acquisition trials, the platform was submerged and the mice were placed into the maze at one of four points randomly. Mice were allowed to search for a platform for 60 sec, and if they did not find a platform, they were gently guided to it. Four trials a day were conducted with at least 1 hr intertrial interval. The escape latency, an indication of learning progress, was scored for each trial. On day 8, the platform was removed and a probe test was conducted. The time spent in each of the four quadrants and the number of target (platform) area crossings, were recorded.

Reference

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