Supporting Information

Canugovi et al. 10.1073/pnas.1204156109

SI Materials and Methods

Behavioral Tests. All tests except rotarod used the Anymaze 4.7 video tracking software system (Stoelting) connected to a digital camera (Panasonic).

Open field. This test measures anxiety and was conducted before any other test. The parameters measured are distance traveled and time spent in the center and peripheral zones during a 15-min period. Anxious animals spend less time in the center zone compared with less anxious animals.

Rotarod. This test was used to analyze the motor function and endurance in mice. The rotarod machine (Med Associates) has nine different fixed speed (1–4) and accelerating speed (5–9) settings. The parameters tested and recorded in each case are number of falls in a 5-min duration and the latency to the first fall. All mice were trained and tested for their motor ability at speed level 5 (2–20 rpm) before middle cerebral artery occlusion/reperfusion (MCAO/R) procedure. After MCAO/R, the rotarod test was repeated at 24-h and 48-h time points.

Novel object recognition. This memory test takes advantage of the natural curiosity of mice. There are two phases, namely, familiarization and novel object presentation. During familiarization, mice are presented with two similar objects in two zones for 30 min followed by a 90-min gap. The mice are then presented with a familiar object and a novel object for 5 min. The number of times the mouse enters the novel object zone and the time it spends near the novel object compared with the familiar object are measured.

Fear conditioning. In this test, an aversive stimulus (an electric shock) is associated with a neutral stimulus (a tone) in a training session of multiple trials. The next day the mice were subjected to a contextual fear session in which they were placed in the conditioning chamber for 5 min without a shock; the percentage of time freezing was the measure of contextual memory. Three hours later, the mice were returned to the chamber but with a different context (cued conditioning).

Water maze. Using methods described previously (1), the mice were trained to find a hidden platform in a pool during an 8-d period and goal latencies were recorded each day. Then probe trials were performed at 1, 2, 3, and 4 d after training and the amount of time the mouse spent in each of the four quadrants during a 1-min period of swimming was determined and used as a measure of memory retention. We used a repeated measures two-way ANOVA followed by Bonferroni's correction to analyze the significance of the data for all behavioral studies.

1. Okun E, et al. (2010) Toll-like receptor 3 inhibits memory retention and constrains adult hippocampal neurogenesis. *Proc Natl Acad Sci USA* 107:15625–15630.

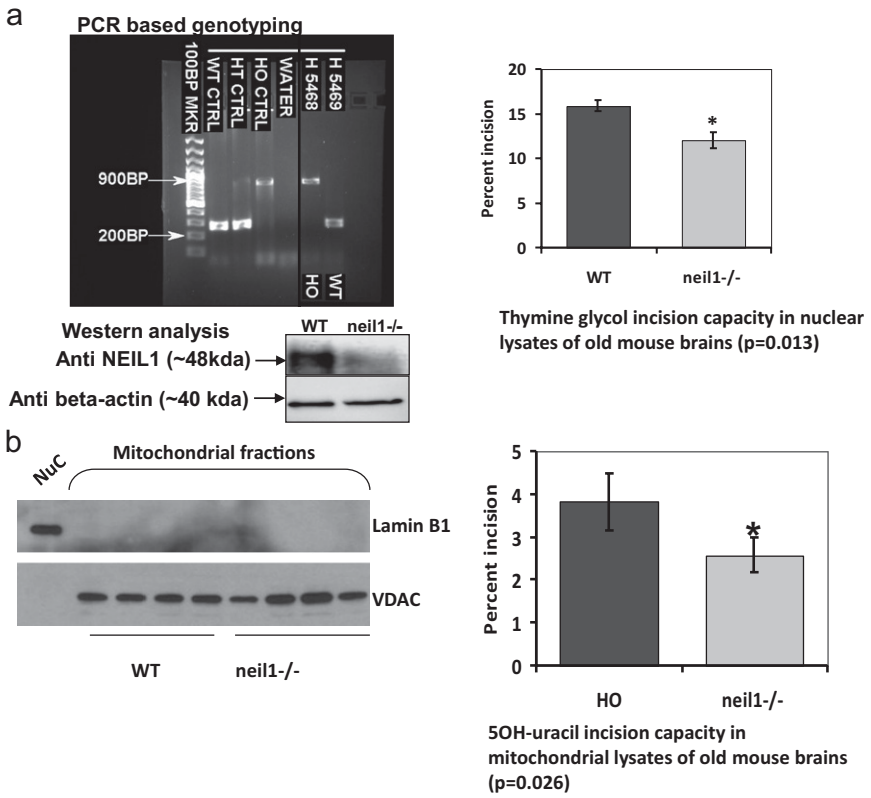

Fig. S1. (*A*) PCR and Western blot analysis confirming the loss of NEIL1 gene and protein in *neil1*$^{-/-}$ (HO) mice (*Left Upper* and *Lower*, respectively). Thymine glycol incision capacity in the nuclear lysates (*Right*) from old WT and *neil1*$^{-/-}$ mice. Data are mean ± SE from six independent samples. A two-tailed Student *t* test was conducted to analyze the significance of the data.(*B*) Mitochondrial fraction lysates purity (*Left*) and incision capacity (*Right*) from old WT and *neil1*$^{-/-}$ mice. Data are mean ± SE from six independent samples. A two-tailed Student *t* test was conducted to analyze the significance of the data.

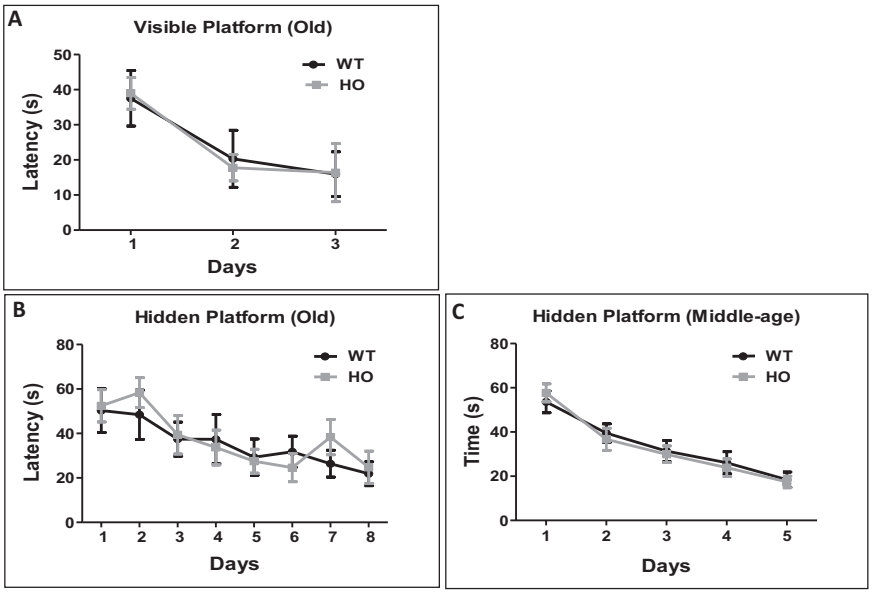

Fig. S2. Water maze training data. (*A*) Visible platform test: Average time spent by each animal before swimming up to the visible platform. (*B*) Hidden platform test: Average time spent by each animal before swimming up to the hidden platform by the old animal cohort (30–33 mo old; $n = 8$ WT and 10 *neil1*$^{-/-}$ mice). (*C*) Middle-aged animal cohort (9–13 mo old; $n = 14$ WT and 17 *neil1*$^{-/-}$ mice). Two-way ANOVA followed by Bonferroni's test was conducted to analyze the significance of the data.