Supporting Information

Zhang et al. 10.1073/pnas.1104391108

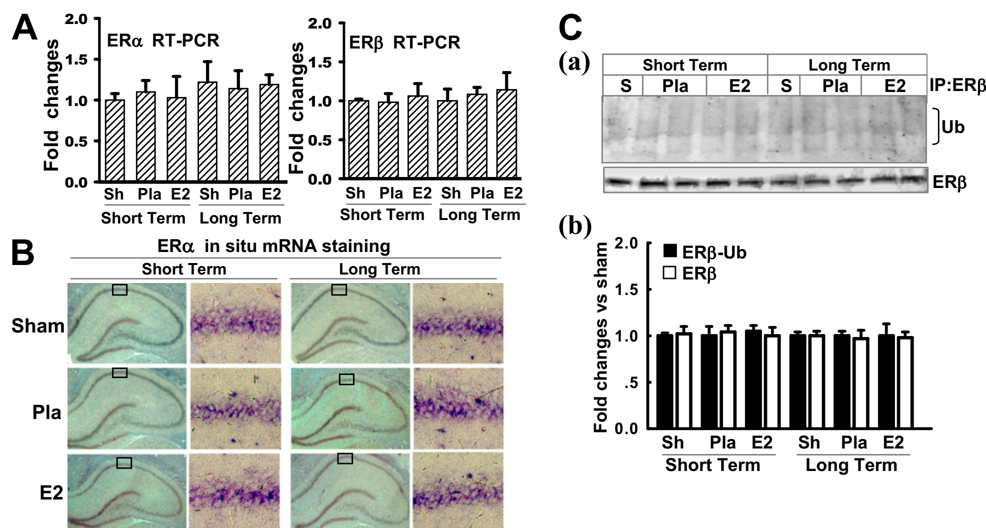

Fig. S1. ERβ ubiquitination was not significantly changed following 10-wk E2 deprivation; ERα and ERβ mRNA were not significantly changed following LTED. (*A*) Quantitative analysis of ERα and ERβ mRNA expression by RT-PCR from the hippocampal CA1 region of the brain from sham and 3-h ischemic reperfusion animals in the short-term and LTED (10 wk later) groups. ERα and ERβ mRNA expression was not significantly changed following LTED. Data were shown as mean ± SE from independent animals (n = 4–5) and expressed as fold vs. sham. Sh, sham. (*B*) ERα mRNA expression was further confirmed by ERα in situ hybridization. Representative photographs are from four to six (n = 4–6) sham and 3-h ischemic reperfusion animals, showing that ERα mRNA detected in the cytoplasm was not apparently changed. (*C*, *a*) Hippocampal CA1 protein samples at 3 h of reperfusion from the short-term and 10-wk later group animals were immunoprecipitated with anti-ERβ antibody and separately blotted with antiubiquitin (Ub) antibody or anti-ERβ antibody. UB, ubiquitin. (*C*, *b*) Data were expressed as fold vs. sham from four to five rats per group. No significant differences were found between the groups.

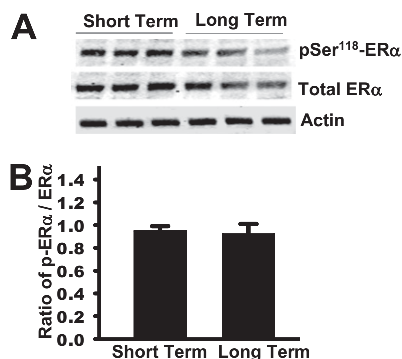

Fig. S2. No significant changes in pSER118-ERα levels following LTED. (*A*) At 3 h after cerebral ischemia reperfusion, Western blotting analyses of pSER118-ERα, total ERα levels, and β-actin were performed with hippocampal CA1 protein from short-term E2-deprived and LTED animals. (*B*) Phospho-ERα levels were corrected with total ERα protein, and values were expressed as ratio changes between the two groups. No significant changes were observed between short-term E2-deprived and LTED animals (n = 4–5 per group).

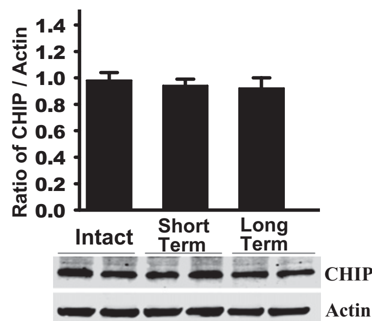

Fig. S3. CHIP protein expression was not significantly changed following ovariectomy. Hippocampal CA1 protein from intact animals and from short-term and long-term ovariectomized rats was subjected to Western blot analyses with anti-CHIP or antiactin antibody. Data shown are mean ± SE from independent animals (n = 5–6), and CHIP levels were corrected with β-actin. Total CHIP protein was not significantly changed in any group.

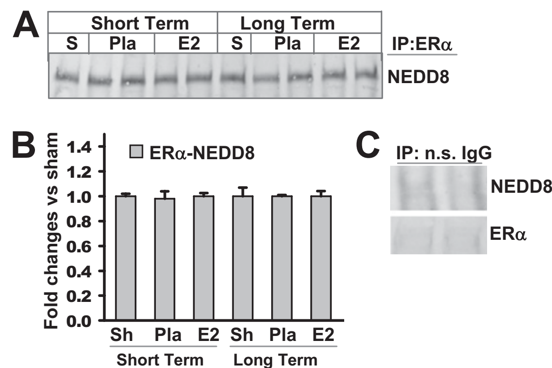

Fig. S4. No significant changes in the interactions between ERα and NEDD8 proteins following LTED. (*A* and *B*) Hippocampal CA1 protein samples from sham and short-term and long-term Pla/E2-replaced rats at 3 h of reperfusion were immunoprecipitated with anti-ERα antibody and blotted with anti-NEDD8 antibody. No significant changes in ERα-NEDD8 binding were observed between groups (n = 4–5 per group). S, sham; Sh, sham. (*C*) In the control experiments, hippocampal CA1 homogenate samples were subjected to IP with nonspecific IgG and the immunocomplexes were probed for the presence of NEDD8 and ERα. No apparent bands were observed in the precipitates. n.s., nonspecific.

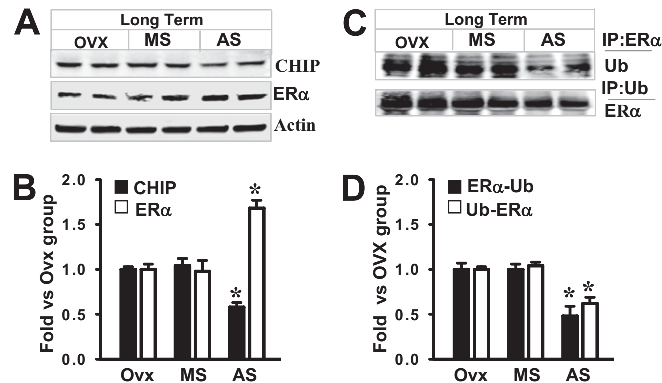

Fig. S5. Evidence that CHIP mediates the ubiquitination and degradation of ERα following LTED. (*A* and *B*) Western blot analysis demonstrates a robust reduction in CHIP expression in the hippocampal CA1 region after treatment with CHIP antisense oligodeoxynucleotides compared with missense oligodeoxynucleotide controls or ovariectomy controls in the LTED animals. Note that ERα protein attenuation was reversed in the rats treated with CHIP antisense oligodeoxynucleotides compared with control groups. AS, antisense; MS, missense; OVX, ovariectomy. (*C* and *D*) Effects of CHIP antisense oligodeoxynucleotides on ERα ubiquitination. The high levels of ERα ubiquitination following LTED were significantly attenuated following CHIP knockdown. Ub, ubiquitination. All data are expressed as mean ± SE (n = 4–5 in each group). *P < 0.05 vs. MS group.

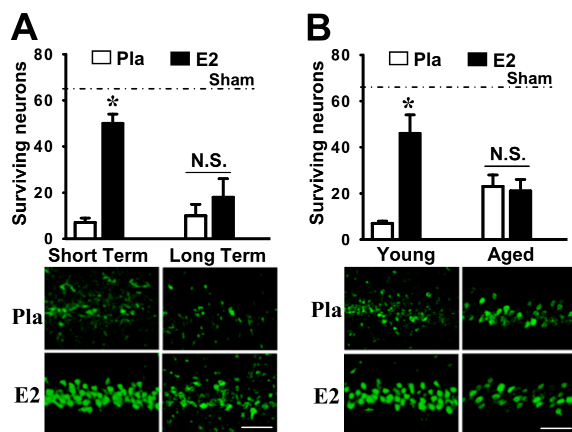

Fig. S6. No neuroprotective effects of higher dose E2 (0.050 mg, 21-d release) in LTED and aged animals following global ischemia. (*A*) Short-term E2-deprived or LTED young SD rats were treated with E2 or Pla; 7 d after ischemic reperfusion, typical NeuN staining in the hippocampal CA1 region was analyzed. Note that E2 treatment in the short term (but not in LTED animals) resulted in significant neuroprotection, as evidenced by an increased number of surviving neurons in the medial CA1 region. N.S., no significant difference. (*B*) Typical NeuN staining in the hippocampal CA1 region treated with E2 or Pla in young F344 rats following 7 d of reperfusion and in aged F344 rats following 3 d of reperfusion. E2 loses its ability to protect CA1 neurons against ischemic damage in the aged rats. All the data (mean ± SE) were represented as the numbers of surviving neurons per 250-μm length of medial CA1 (n = 5–8 in each group). (Magnification: 40×; scale bar: 50 μm.) *$P < 0.05$ vs. Pla group.