

SUPPLEMENTARY FIG. S2. Evaluation of Nox1 antibody specificity and expressions of Nox isoforms (Nox2 and Nox4) and regulatory subunits (Noxo1 and Noxa1) in the hippocampus. (A) To verify Nox1 antibody specificity of immunohistochemical analysis, the dopaminergic neurons of the SN tissues of rats injected with 6-OHDA were used as a specific positive control. Nox1 immunostaining (red) was detected in TH-stained neurons (green) that were exposed to 6-OHDA. Nox1 expression in TH⁺ neurons is demonstrated as yellow staining after merging green (TH) and red (Nox1) images. Nox1 antibody specificity also was evaluated by preincubation with a specific blocking peptide for 1 h. The preincubation of the Nox1 antibody with the blocking peptides led to a failure to detect Nox1 (red) in TH-stained dopaminergic neurons in SN exposed to 6-OHDA. (B) Representative photographs of tissue sections stained with Nox2 (red) and NeuN (green) or Nox4 (red) and NeuN (green) antibody from rat hippocampal CA1 taken from sham-operated group (SC, n=4) and 2VO (n=4)-operated rats at 10 weeks after the operation. Nox2 or Nox4 expression in NeuN⁺ neurons is demonstrated as yellow staining after merging green (NeuN) and red (Nox2 or Nox4) images. Expression levels of Nox2 and Nox4 were no different between SC and 2VO groups. (C) Representative photographs of tissue sections stained with Noxo1 (red) and NeuN (green) or Noxa1 (red) and NeuN (green) antibody from rat hippocampal CA1 taken from sham-operated group (SC, n = 4) and 2VO (n = 4)-operated rats at 10 weeks after the operation. Noxal or Noxol expression in NeuN⁺ neurons is demonstrated as yellow staining after merging green (NeuN) and red (Noxa1 or Noxo1) images. Expression levels of Noxa1 and Noxo1 have not been changed by 2VO operation. 6-OHDA, 6-hydroxydopamine; Noxa1, NOX activator 1; Noxo1, NOX organizer 1; SN, substantia nigra. Scale bars = 30 um.