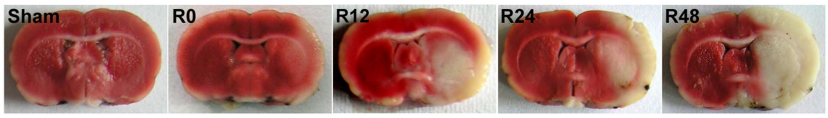
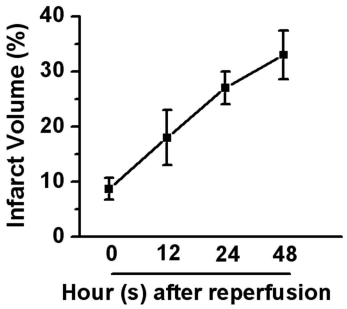
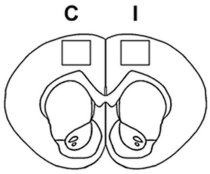
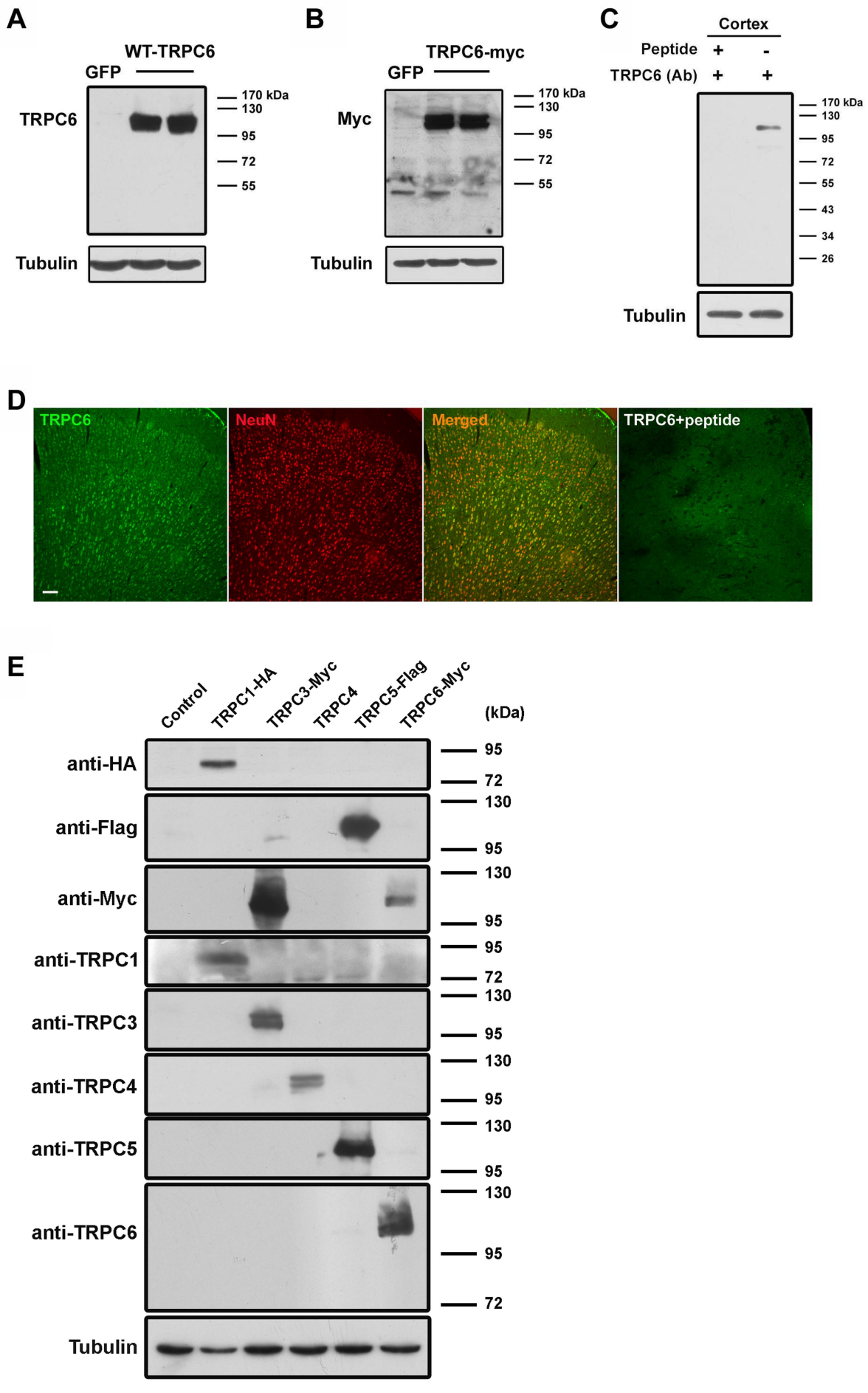




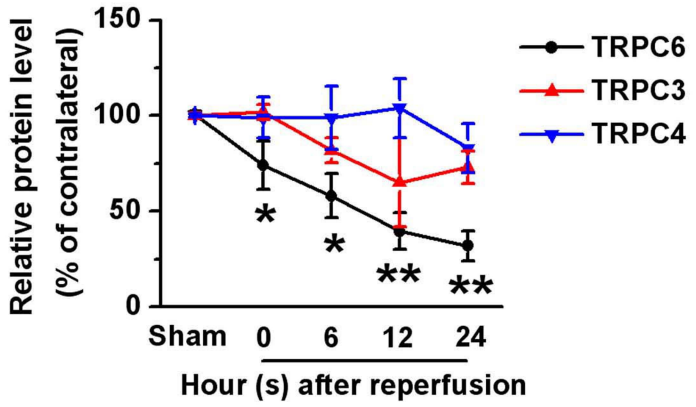


Supplementary Figure 1

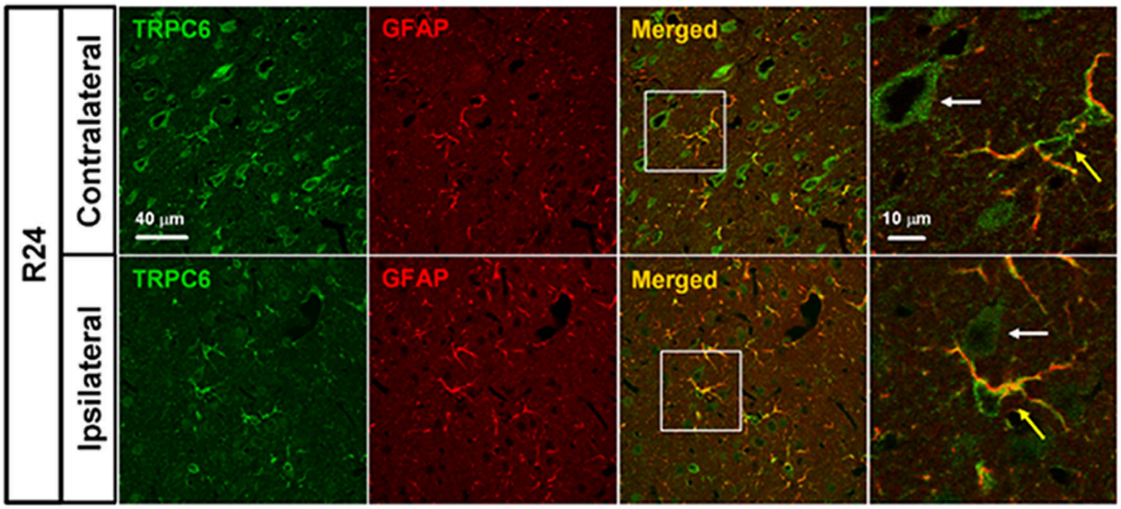


Supplementary Figure 2

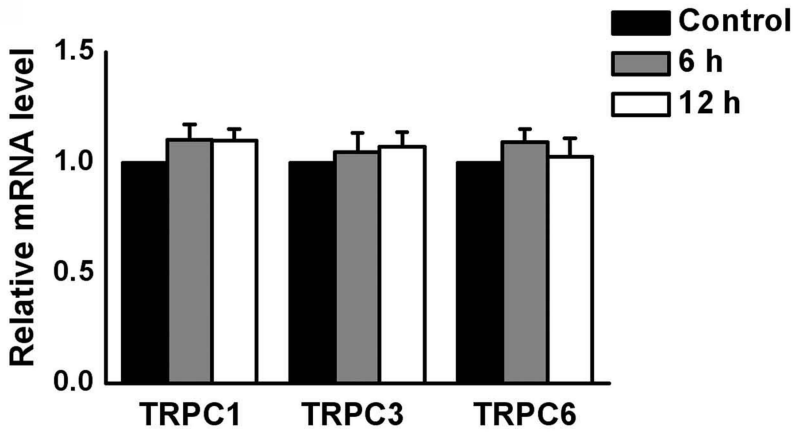
A



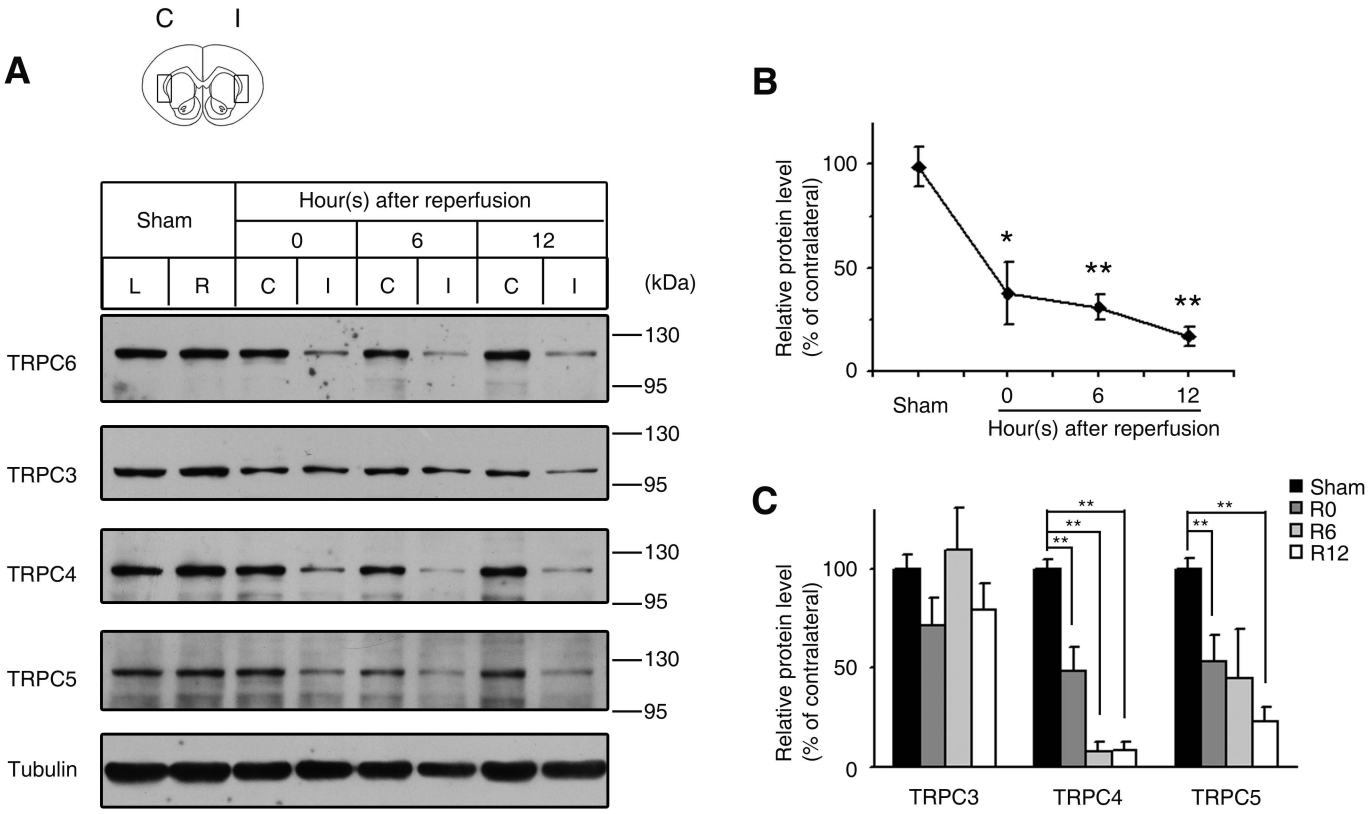
B



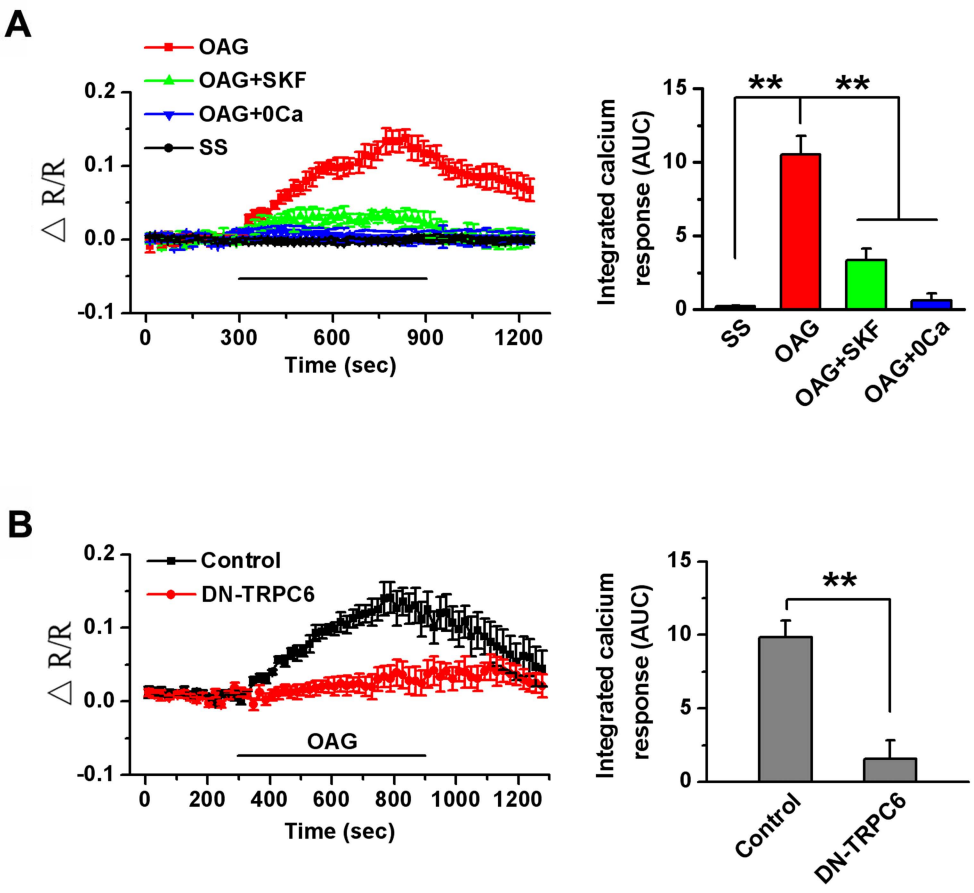
C



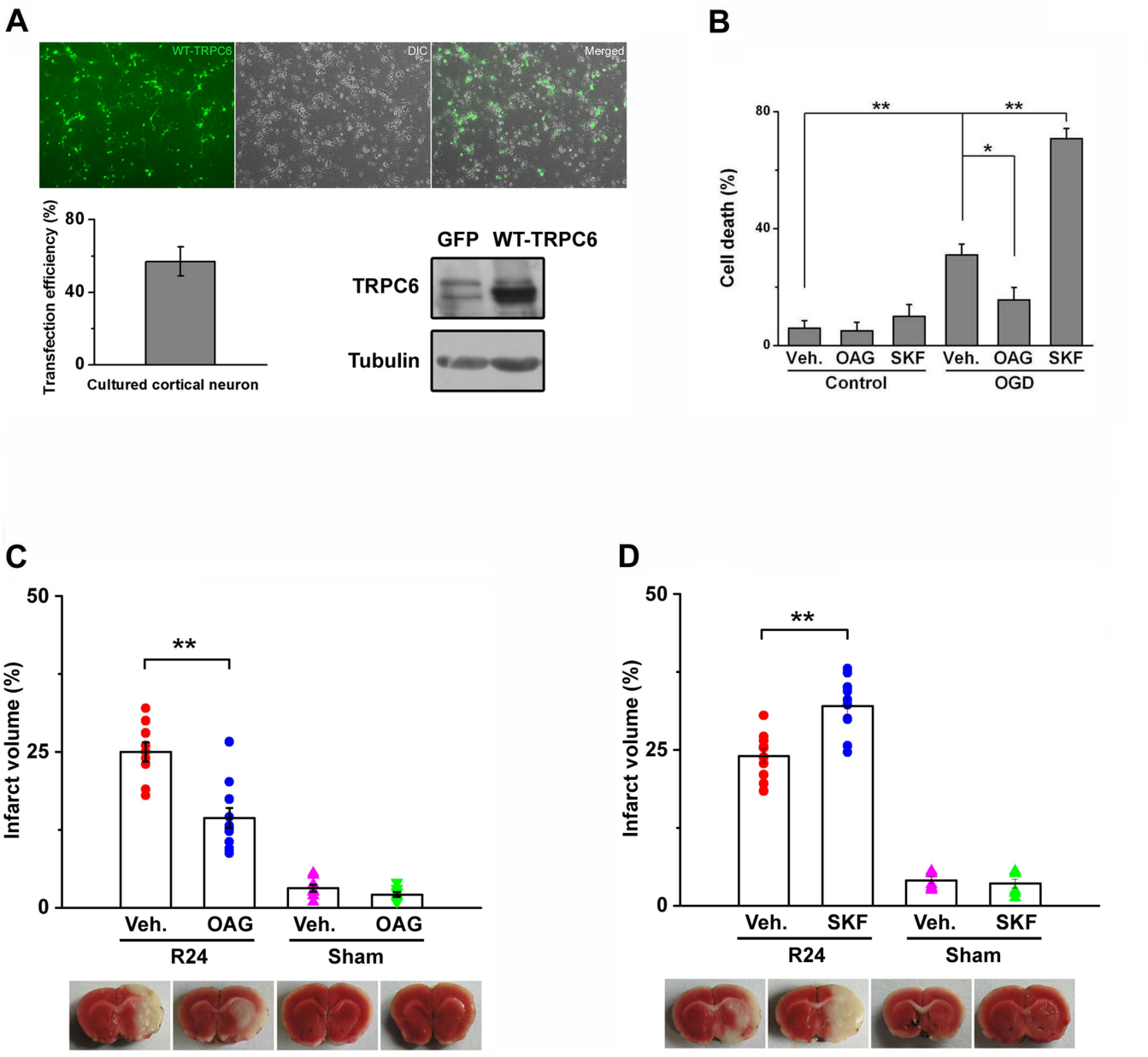
Supplementary Figure 3



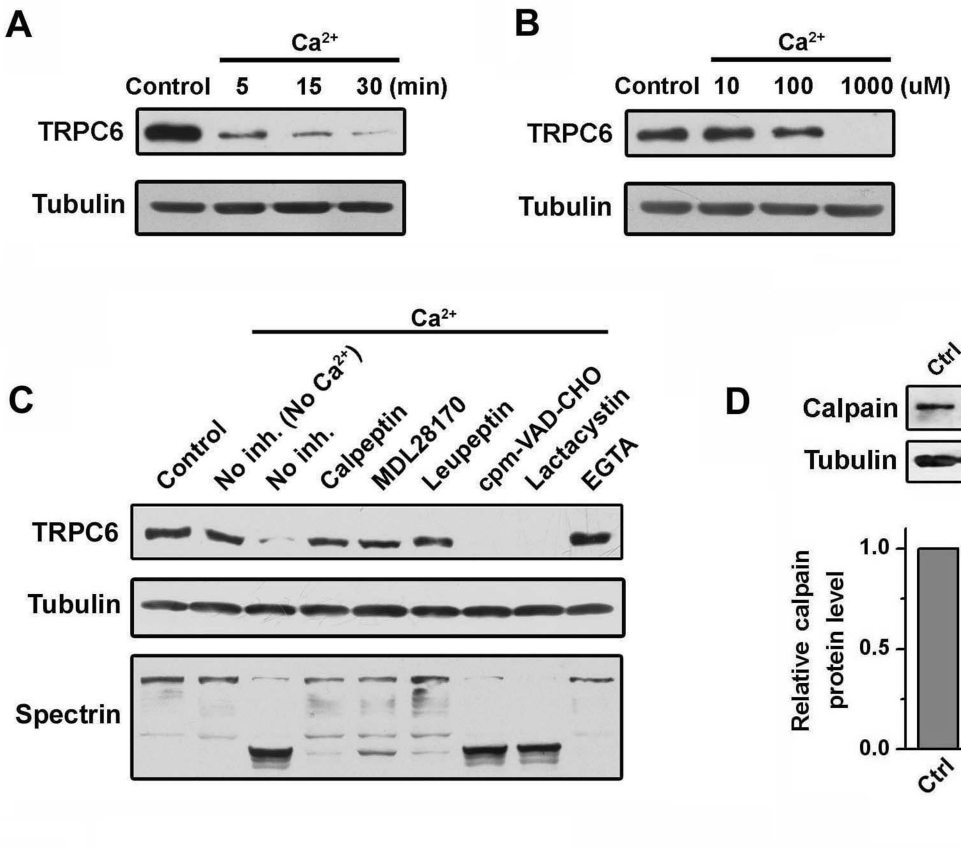
Supplementary Figure 4



Supplementary Figure 5

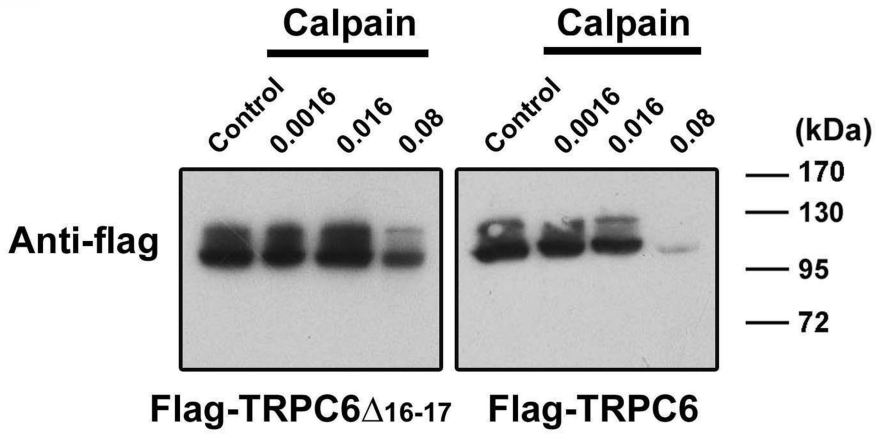


Supplementary Figure 6

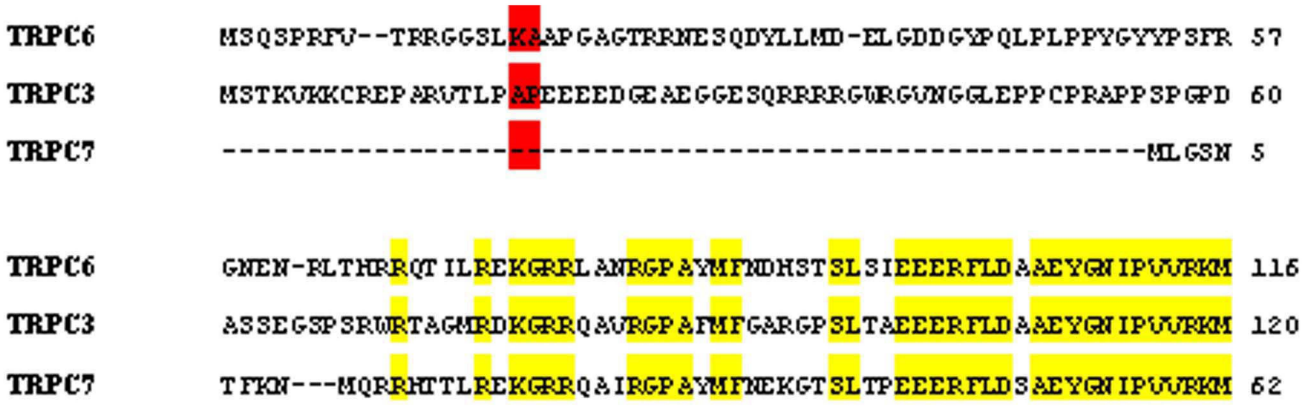


Supplementary Figure 7

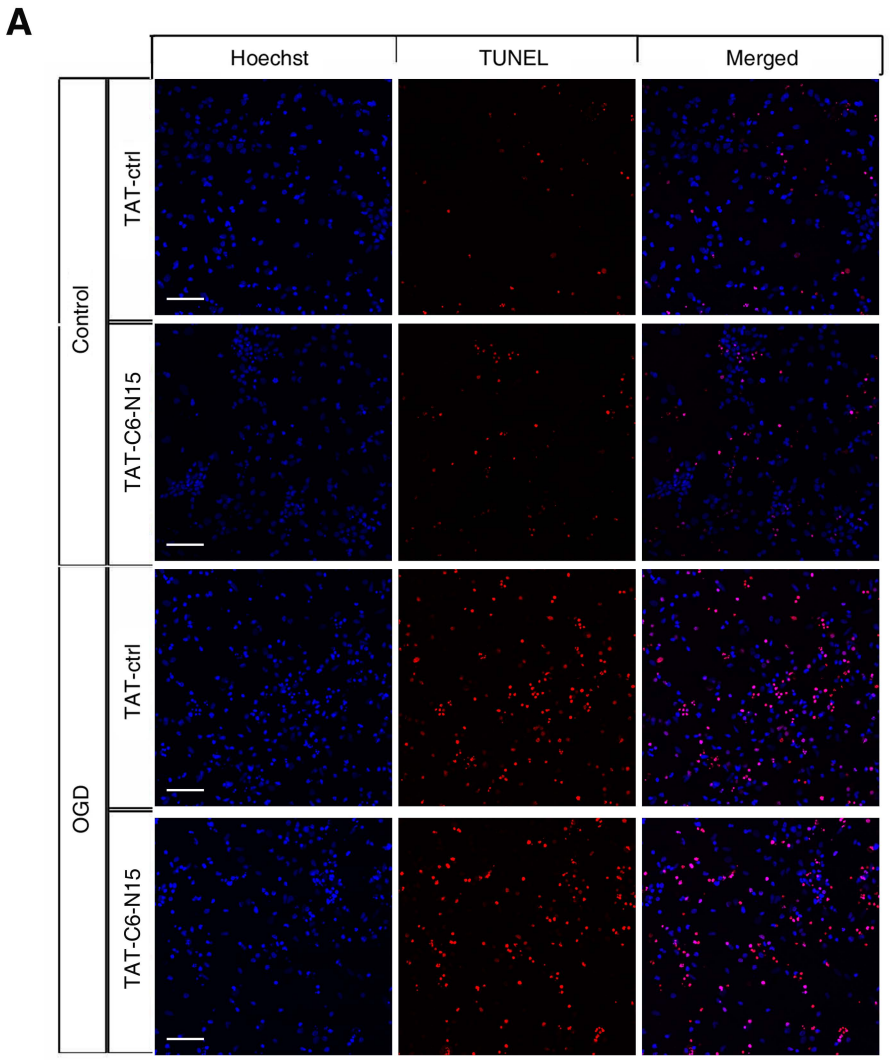
A

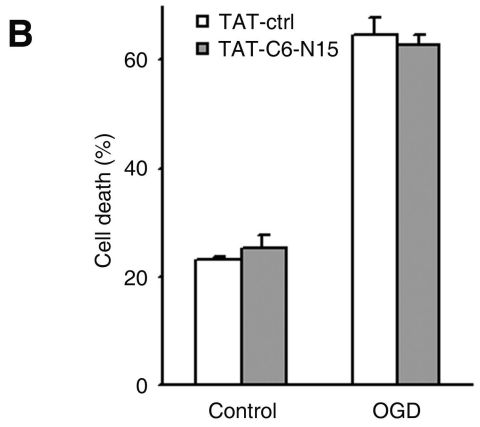


B



Supplementary Figure 8





Supplementary Figure 9

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure 1. The rat model of focal cerebral ischemia.
Representative images of TTC-stained brain sections of sham (Sham) or ischemic rats after 0, 12, 24, 48 h reperfusion (R0, R12, R24 and R48). Quantitative analysis of the infarct volume was shown on the lower panel. Data were mean ± s.e.m of 3 rats per time point. The rectangles in the diagram shown in the right were the area from where the brain samples were taken for immunoblots and immunostaining analyses. Contralateral (**C**) or ipsilateral (**I**) cortex.

Supplementary Figure 2. Characterization of the TRPC6 antibody.
(**A**) Immunoblots for TRPC6 in HEK293 cells transfected with GFP or WT-TRPC6 constructs using the antibody against TRPC6.
(**B**) Immunoblots for TRPC6 in HEK293 cells transfected with GFP or TRPC6-*myc* constructs using the antibody against *myc* epitope.
(**C**) Immunoblots for TRPC6 in rat brain lysates using the antibody against TRPC6 with or without pre-incubation with the antigenic peptide.
(**D**) Representative images of the rat brain section double-stained with antibodies against TRPC6 and NeuN or in the presence of the antigenic peptide for TRPC6. Scar bar, 50 μm.
(**E**) Immunoblots of the extracts from HEK293 cells transfected with TRPC1-*HA*, TRPC3-*Myc*, TRPC4, TRPC5-*Flag* or TRPC6-*Myc* constructs. The antibody for TRPC6 specifically recognized overexpressed TRPC6 proteins, but not overexpressed TRPC1, 3, 4 or 5 proteins. TRPC6 antibodies from the three vendors were examined while the western blot results using the antibody from Alomone Labs were shown here. Unless stated, TRPC6 antibodies from Millipore or Alomone Labs were used for Supplementary Fig. 2D, 3B or 4, 6 ,7, respectively.

Supplementary Figure 3. TRPC6 in the glia was not downregulated after focal cerebral ischemia.
(**A**) Quantitative analysis of the protein levels of TRPC6, 3 and 4 at indicated time points after cerebral ischemia. Data were mean ± s.e.m of 3-5 rats per time point. * $p < 0.05$, ** $p < 0.01$ versus sham.
(**B**) Representative images of the contralateral and ipsilateral cortex after 24 h reperfusion (R24) double-stained with antibodies against TRPC6 and GFAP. The right panel was the enlarged images taken from the indicated squares. Yellow arrow indicated GFAP-positive glial cells. White arrow indicated adjacent neurons.
(**C**) Real-time RT-PCR analysis of the mRNA levels of TRPC1, 3 and 6 in neurons after OGD at indicated time point.

Supplementary Figure 4: TRPC proteins were downregulated in infarct core regions
(**A**) Immunoblots of the extracts from the contralateral (**C**) or ipsilateral (**I**) infarct core region (Sham; left (L) or right (R) hemisphere as shown by the rectangles in the diagram.) using the indicated antibodies. Tubulin served as a loading control.
(**B**) Quantification of the normalized TRPC6 protein levels. (n = 5-6 rats per time point) ∗ $p < 0.05$, ∗∗ $p < 0.01$ versus sham.
(**C**) Quantification of the normalized TRPC protein levels. (n = 5-6 rats per time point) ∗ $p < 0.05$,

** $p < 0.01$ versus sham.

Supplementary Figure 5. Effect of OAG on $[Ca^{2+}]_i$ in cortical neurons.

(**A**) The $[Ca^{2+}]_i$ elevation determined by the ratio F340/F380 was depicted by ΔR/R and normalized to the ratio of baseline. The horizontal line indicated the duration of application of indicated drugs. For control, SS (HPSS buffer, see **Methods**) was applied. For Ca^{2+}-free condition; SS + 2 mM EGTA. OAG (100 μM), SKF; SKF96365 (10 μM). Right panel: quantitative analysis of AUC (area under the curve) in each condition. Data were mean ± s.e.m of 15-25 cells per group. ** $p < 0.01$ versus SS or OAG.

(**B**) Effect of DN-TRPC6 on $[Ca^{2+}]_i$ elevation induced by OAG. The neurons transfected with DN-TRPC6 or control vectors were stimulated with OAG (100 μM) and $[Ca^{2+}]_i$ elevation was determined. The horizontal line indicated the duration of OAG application. Right panel: quantitative analysis of AUC in each group. Data were mean ± s.e.m of 20 cells per group. ** $p <$ 0.01 versus control.

Supplementary Figure 6. Increasing TRPC6 amount protected neurons from OGD-induced damage.

(**A**) Representative images of cultured rat cortical neurons transfected with WT-TRPC6 and quantitative analysis of the transfection efficiency. Immunoblots of the lysates isolated from the neurons transfected with GFP or WT-TRPC6 using the TRPC6 antibody.

(**B**) Effect of vehicle (Veh), OAG (100 μM) or SKF96365 (10 μM) on OGD-induced cell death assayed by PI staining. Data were mean ± s.e.m of three independent experiments. * $p < 0.05$, ** $p < 0.01$ versus Veh.

(**C, D**) TTC-stained brain sections showed the infarct volumes (bar graph) or area (image) in the rat brains 24 h after reperfusion (R24) from following groups: (**C**) R24 or sham: vehicle (Veh, DMSO/5 μl) or OAG (OAG, 100 mM/5 μl). Data were mean ± s.e.m of 8-11 rats per group. ** $p < 0.01$ versus Veh. (**D**) R24 or sham: vehicle (Veh, saline/5 μl) or SKF96365 (SKF, 20 mM/5 μl). Data were mean ± s.e.m of 8-10 rats per group. ** $p < 0.01$ versus Veh.

Supplementary Figure 7. TRPC6 protein was proteolyzed by a Ca^{2+}-dependent protease.

(**A**) Time-dependent decrease in TRPC6 protein levels in the rat brain lysates incubated with Ca^{2+} (1 mM) at 37°C assayed by immunoblotting.

(**B**) Dose-dependent decrease in TRPC6 protein levels in the brain lysates incubated with indicated concentrations of Ca^{2+} for 30 min at 37°C .

(**C**) Western blot analysis of the rat brain lysates incubated with Ca^{2+} in the presence or absence (No inh.) of the indicated agents using the antibodies against TRPC6 or α-spectrin. Calpeptin; 20 μM, Leupeptin; 100 μM, MDL28170; 60 μM, cpm-VAD-CHO; 20 μM, Lactacystin; 10 μM, and EGTA; 5 mM.

(**D**) Immunoblots for calpain in the neurons (ctrl) transfected with nonsense-siRNA (NS), or two calpain siRNA (CAPN i_1 and CAPN i_2). Lower panel, quantification of calpain levels. (n = 3) ** $p < 0.01$ versus NS.

(**E**) Immunoblots for spectrin in the cortical extracts from ischemic rats. Sham; left (L) or right (R) hemisphere. Contralateral (C) or ipsilateral (I) cortex.

(**F**) Western blot analysis of the cell lysates of cortical neurons subjected to OGD in the presence

of vehicle (Veh) or MK801 (10 μM) using TRPC6 antibody.
(**G**) Immunoblots for NR1 in the neurons (ctrl) transfected with nonsense-siRNA (NS) or two NR1 siRNA (NR1 i_1 and NR1 i_2). Lower panel, quantification of NR1 levels. (n = 3) ** $p < 0.01$ versus NS.

Supplementary Figure 8. Two-amino-acid deletion surrounding the cleavage site in TRPC6 (TRPC6$\Delta_{16\text{-}17}$) reduced the calpain-proteolysis of TRPC6.
(**A**) Immunoblots of the lysates of HEK293 cells transfected with Flag-tagged TRPC6 or Flag-tagged TRPC6$\Delta_{16\text{-}17}$ (both K^{16} and A^{17} were deleted) and incubated with μ-calpain at the indicated concentrations using the indicated antibodies.
(**B**) Sequence alignments for TRPC3, 6 and 7. Red: the calpain cleavage site in TRPC6 was unique. Yellow: the sequence similarity among these proteins.

Supplementary Figure 9. The N-terminal fragment of TRPC6 was not neurotoxic
(**A**) Representative images of cortical neurons incubated with 5μM TAT-ctrl or TAT-C6-N15 2 hours before OGD and stained with TUNEL labeling 24 hours after OGD. Hoechst labeled the nucleus. Scale bar, 50 μm.
(**B**) Quantification of OGD-induced cell death (at 24 h) in the presence of TAT-ctrl or TAT-C6-N15.