

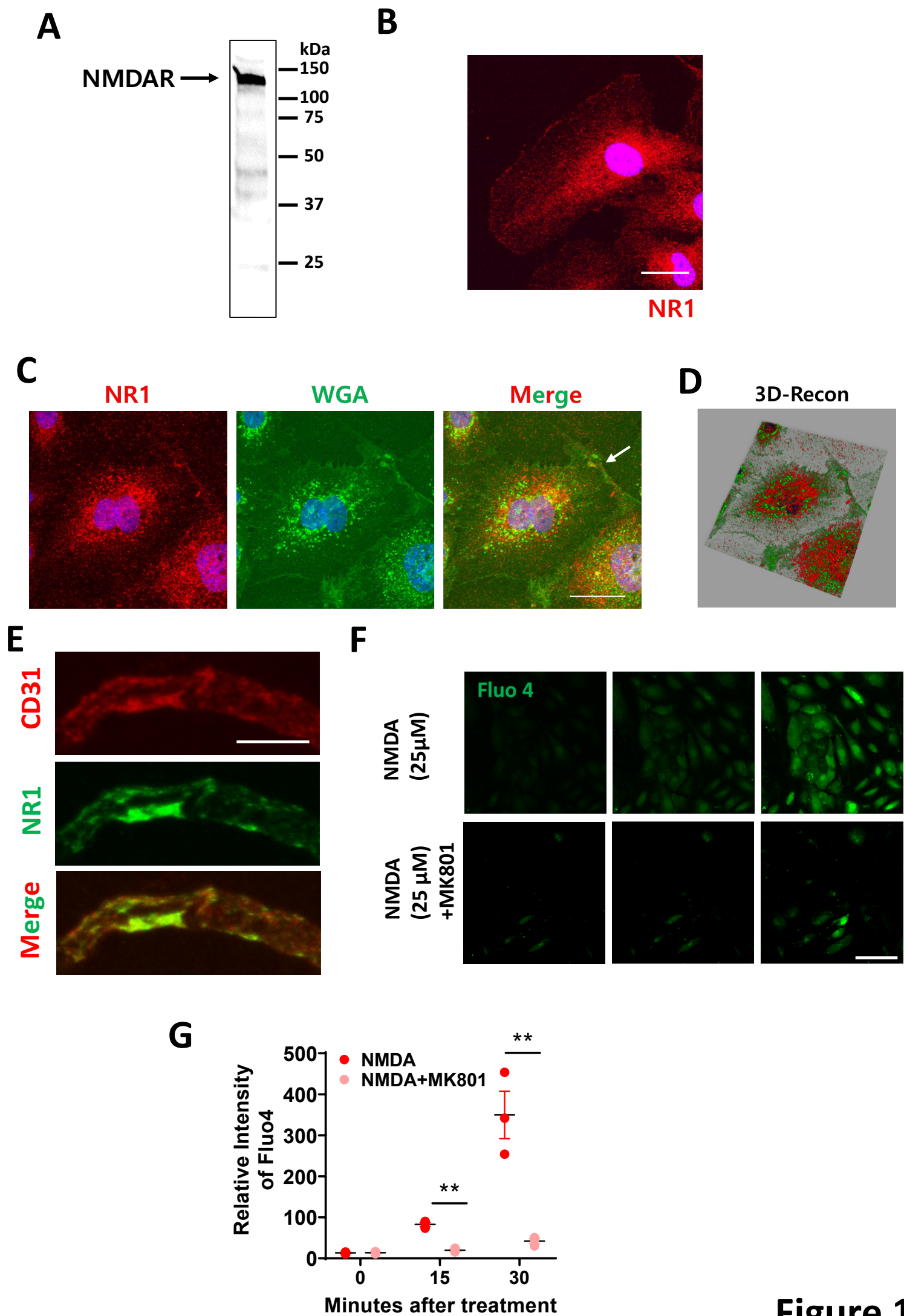


Figure 1

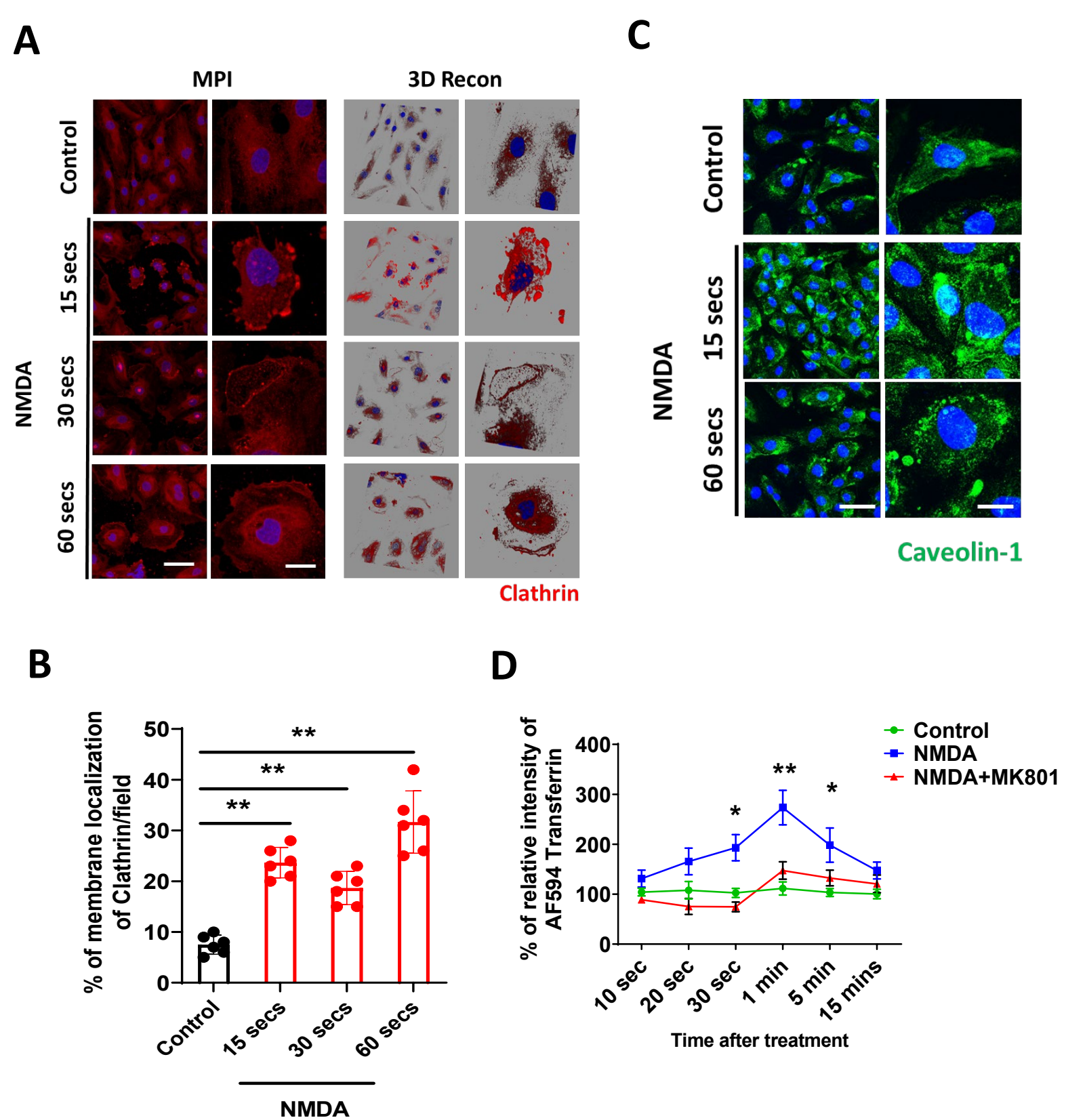


Figure 2

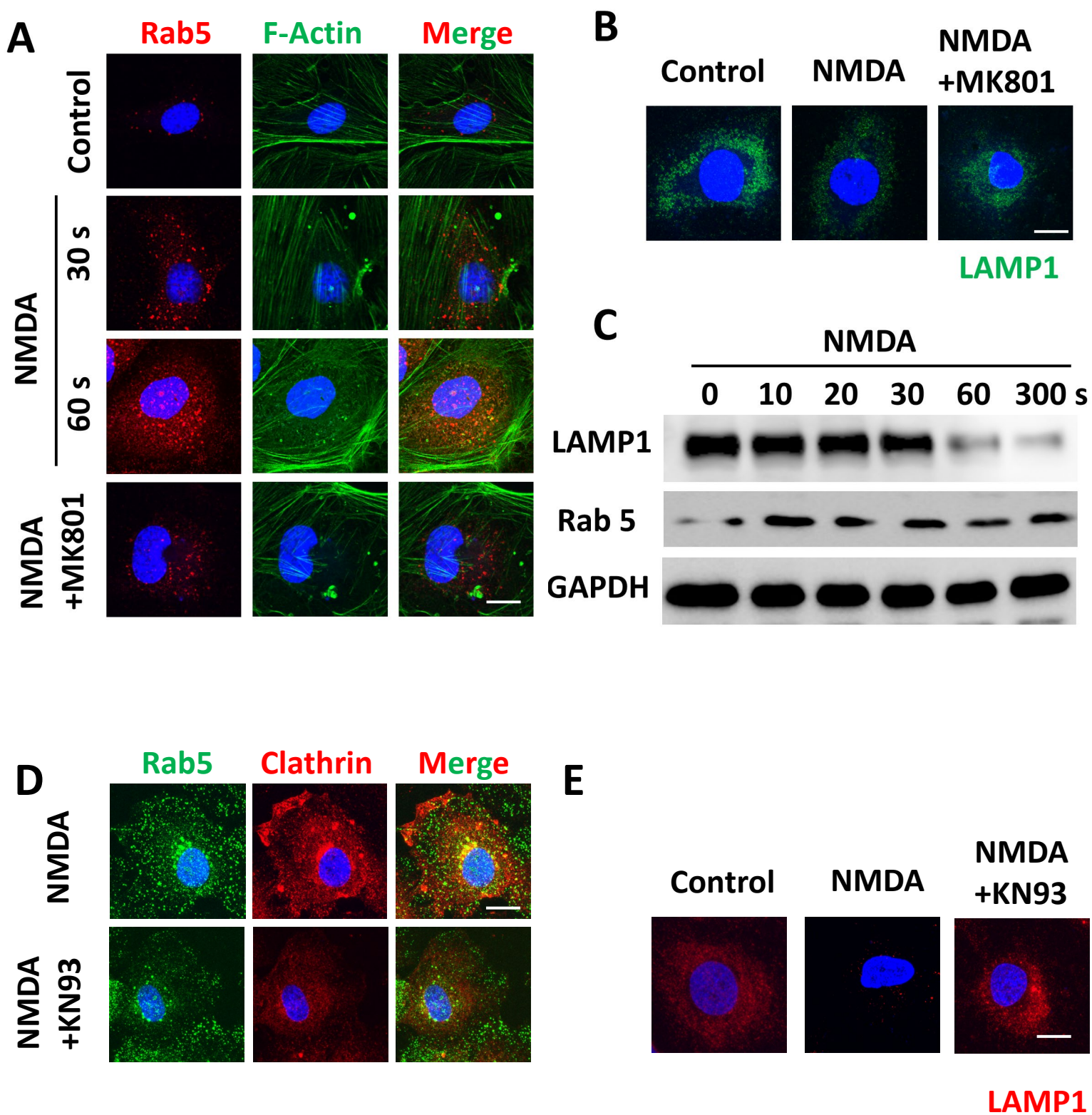
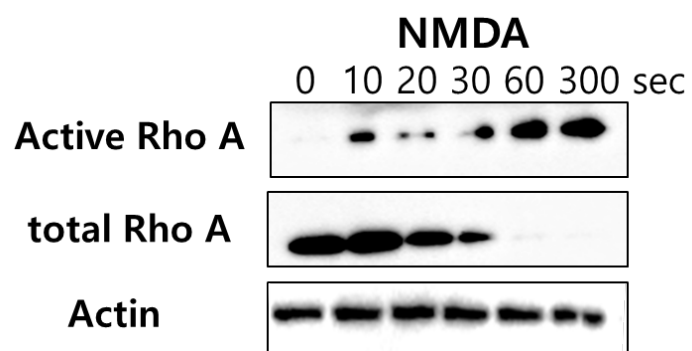
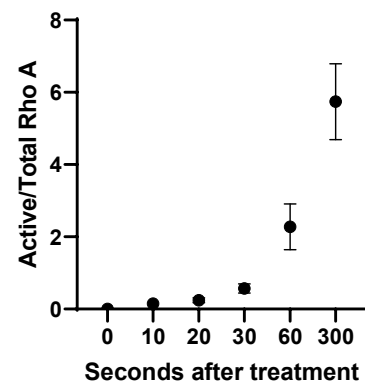
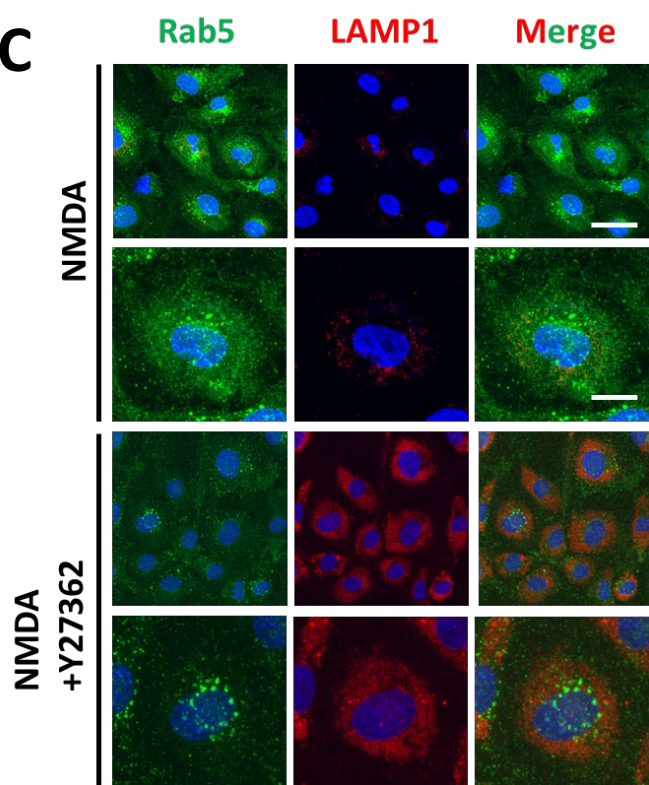
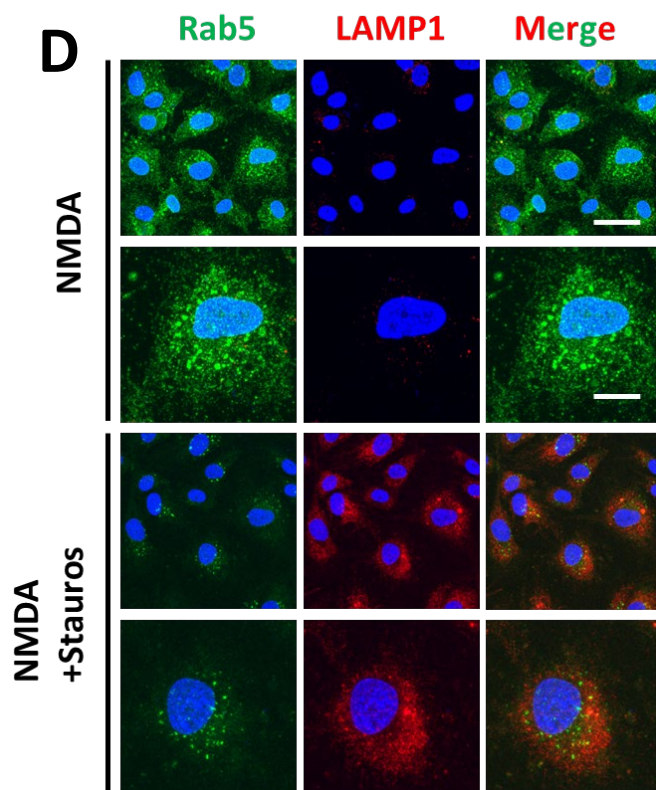


Figure 3

A**B****C****D****Figure 4**

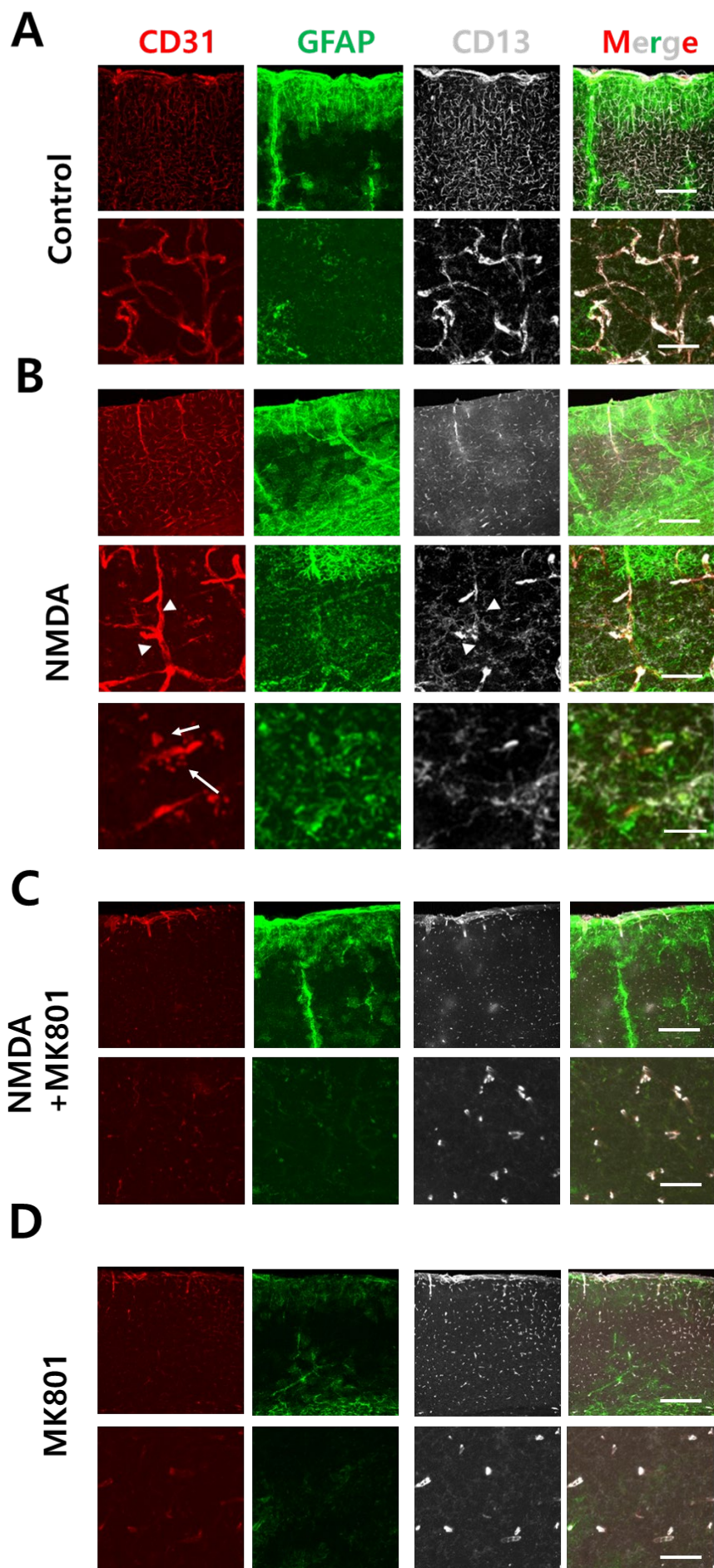
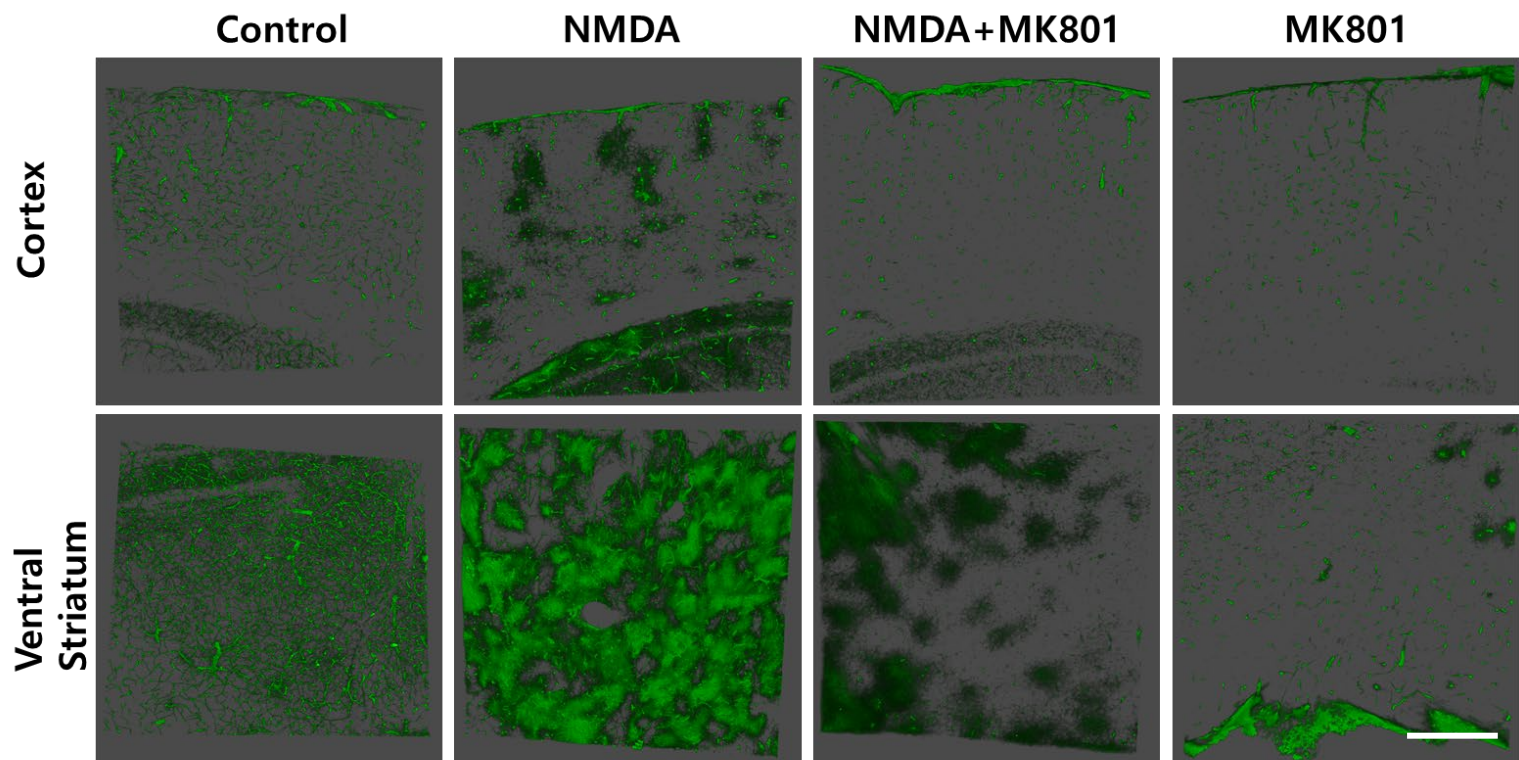
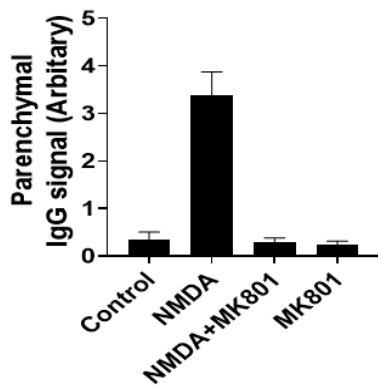
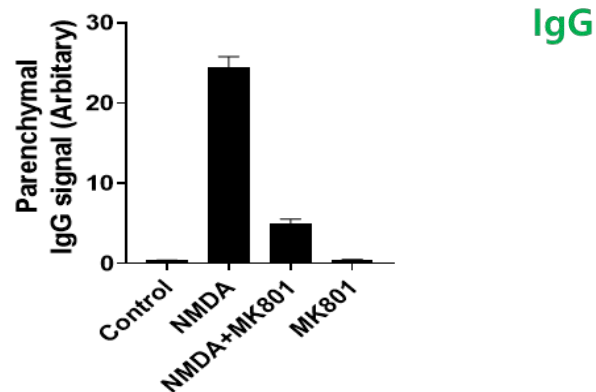
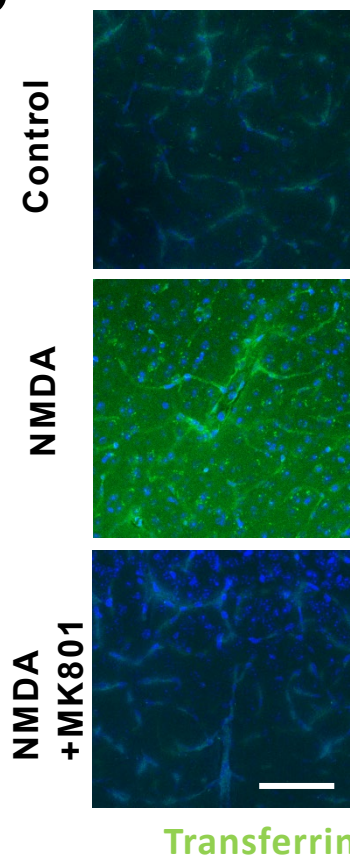
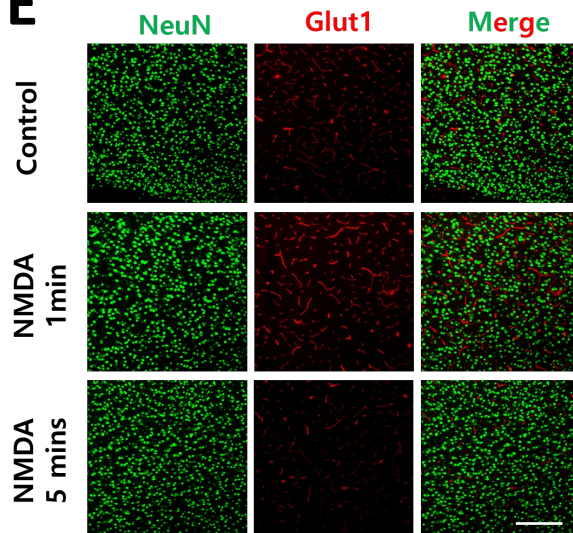
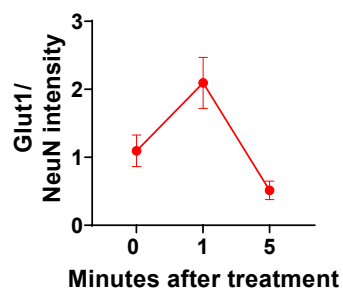
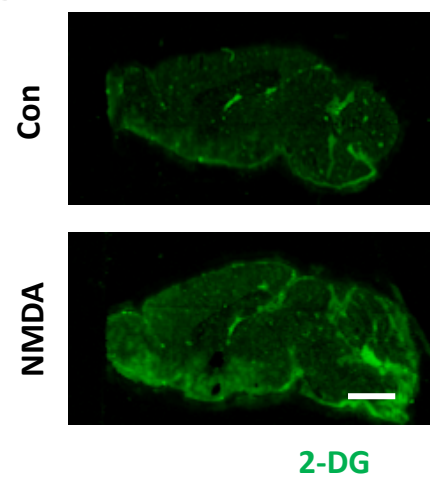
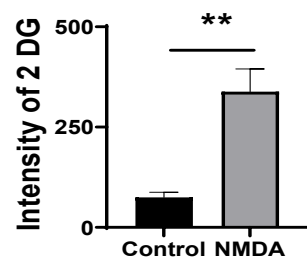


Figure 5

A**B****C****D****E****F****G****H****Figure 6**

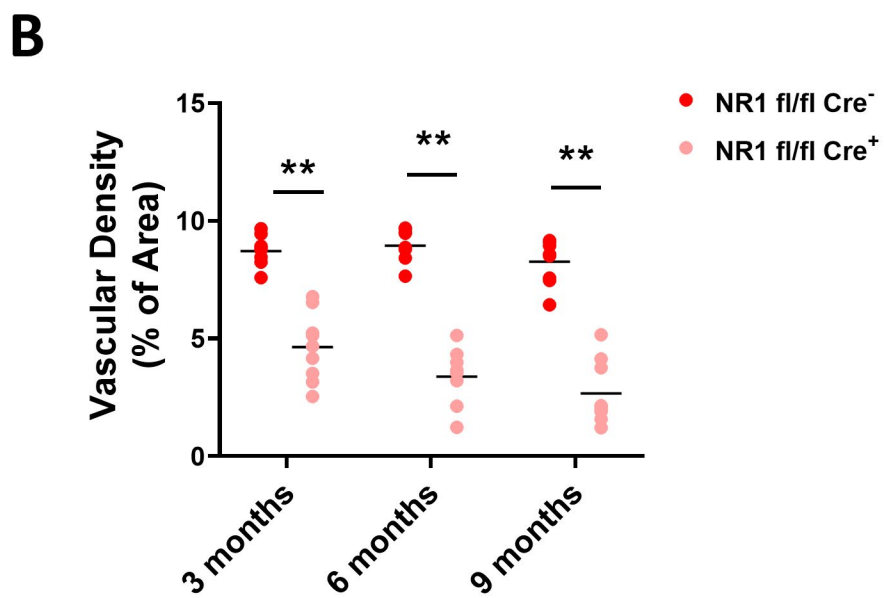
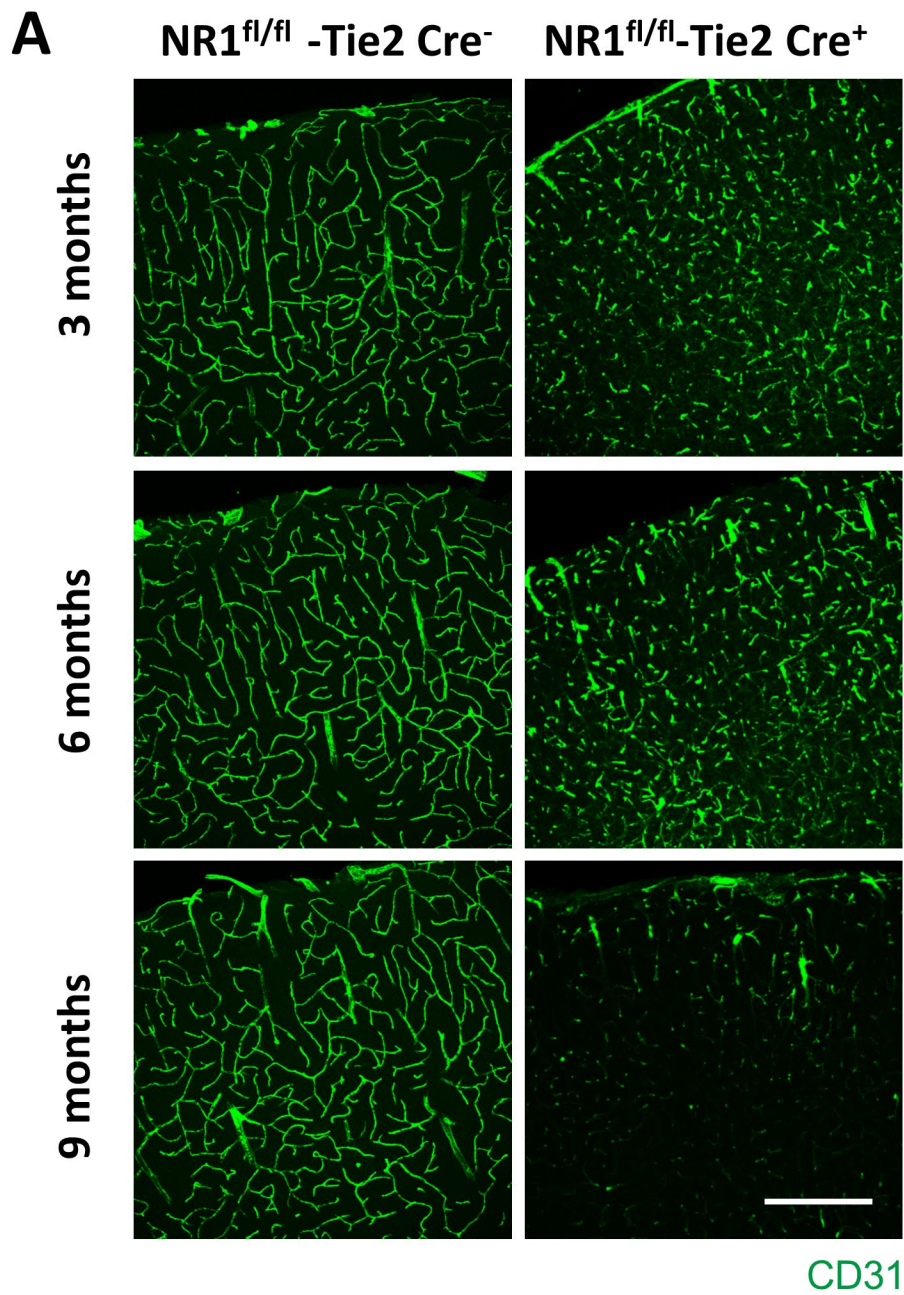
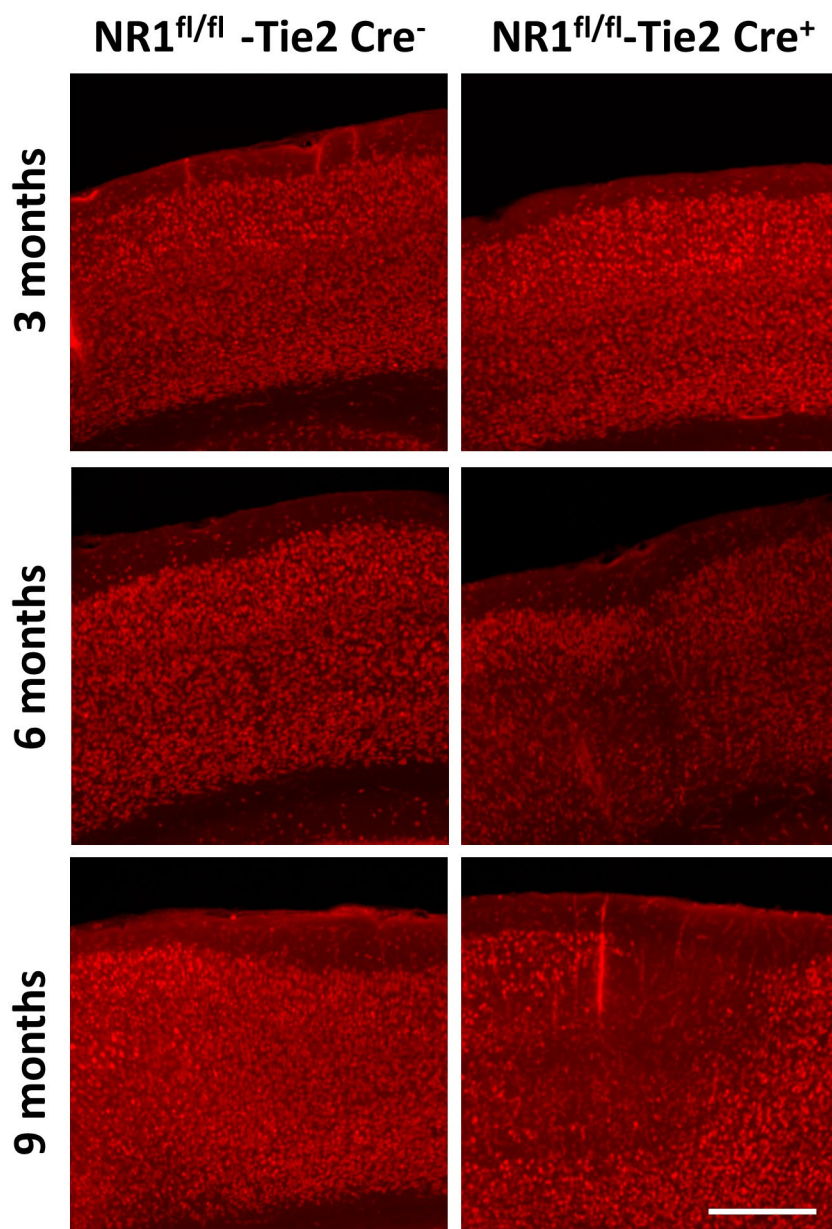
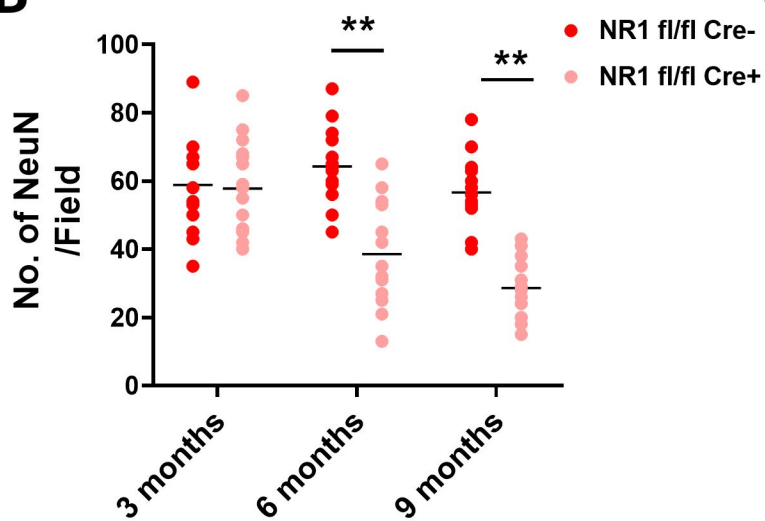
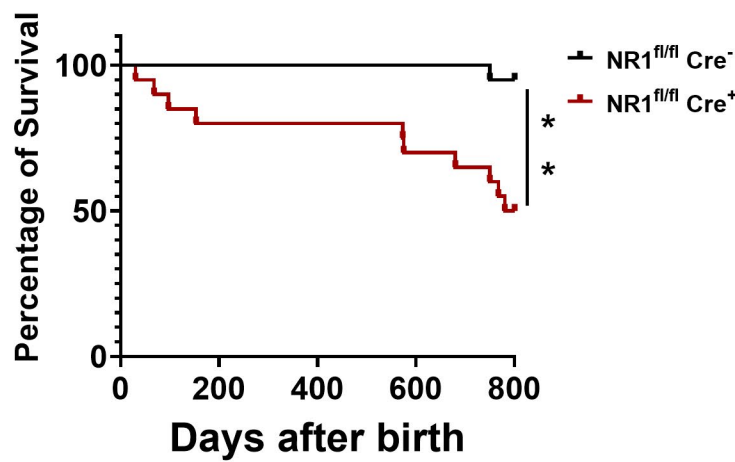
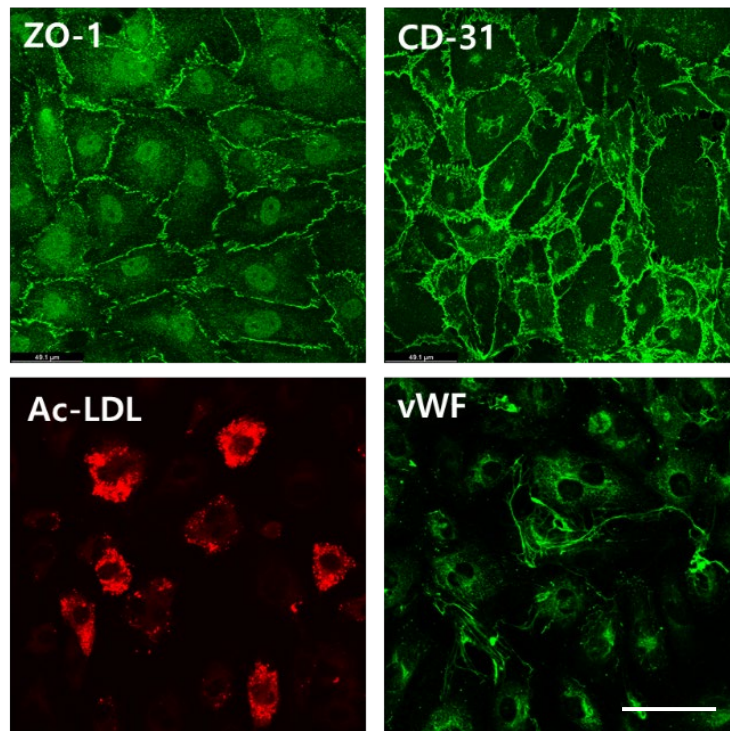
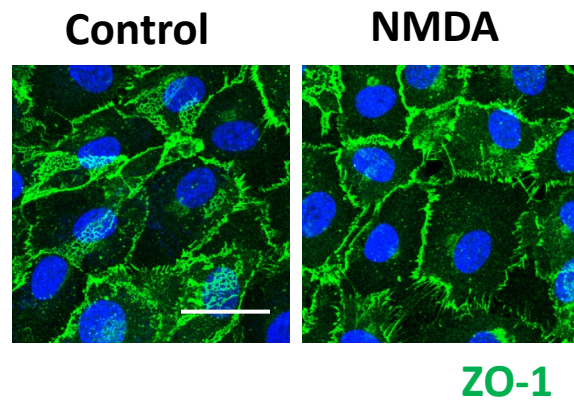


Figure 7

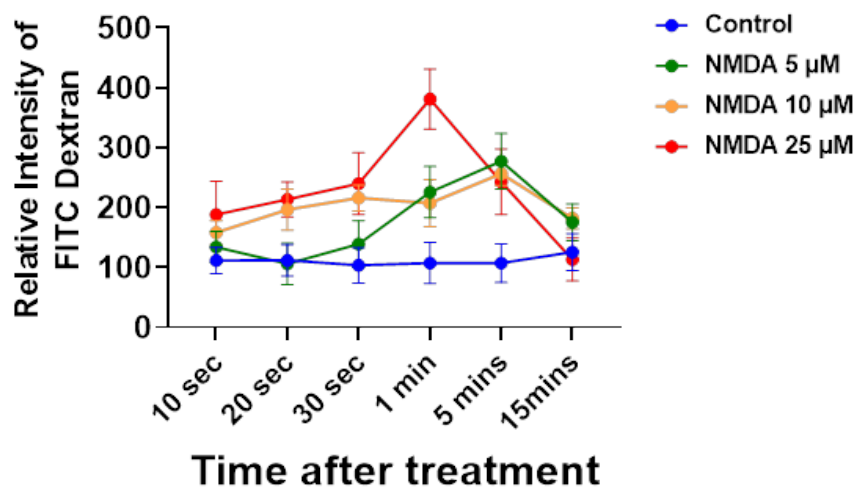
A**B****C****Figure 8**



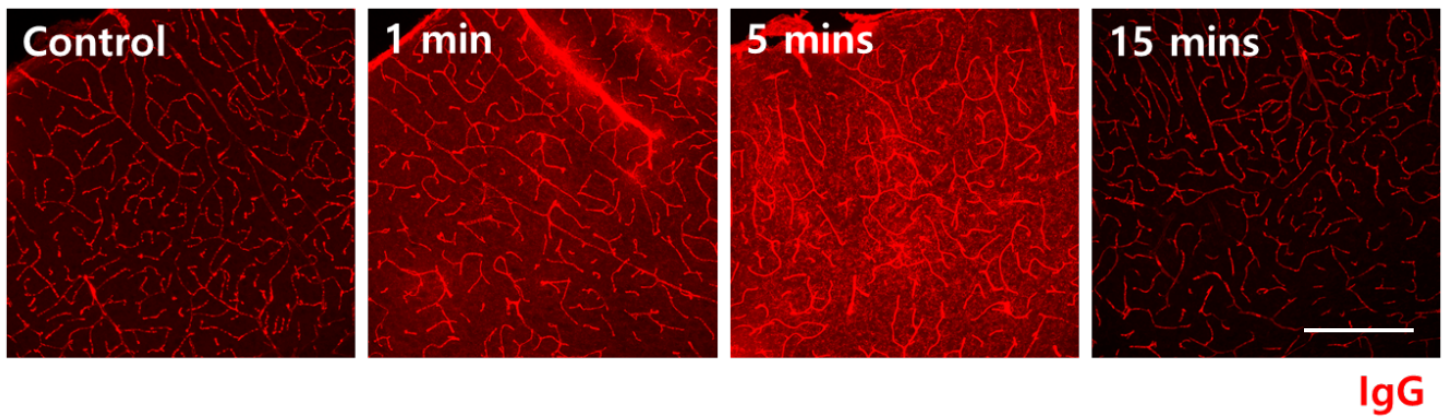
Supplementary Figure 1. Characterization of the primary brain endothelial cell used in the study. Expression of tight junction protein, zona occludens (ZO1), adhesion molecule (CD31), accumulation of acetylated low density lipoprotein (Ac-LDL), and expression of von Willebrand Factor (vWF) showing the characteristics of the brain endothelial cells were confirmed. Scale bar indicates 75 μm



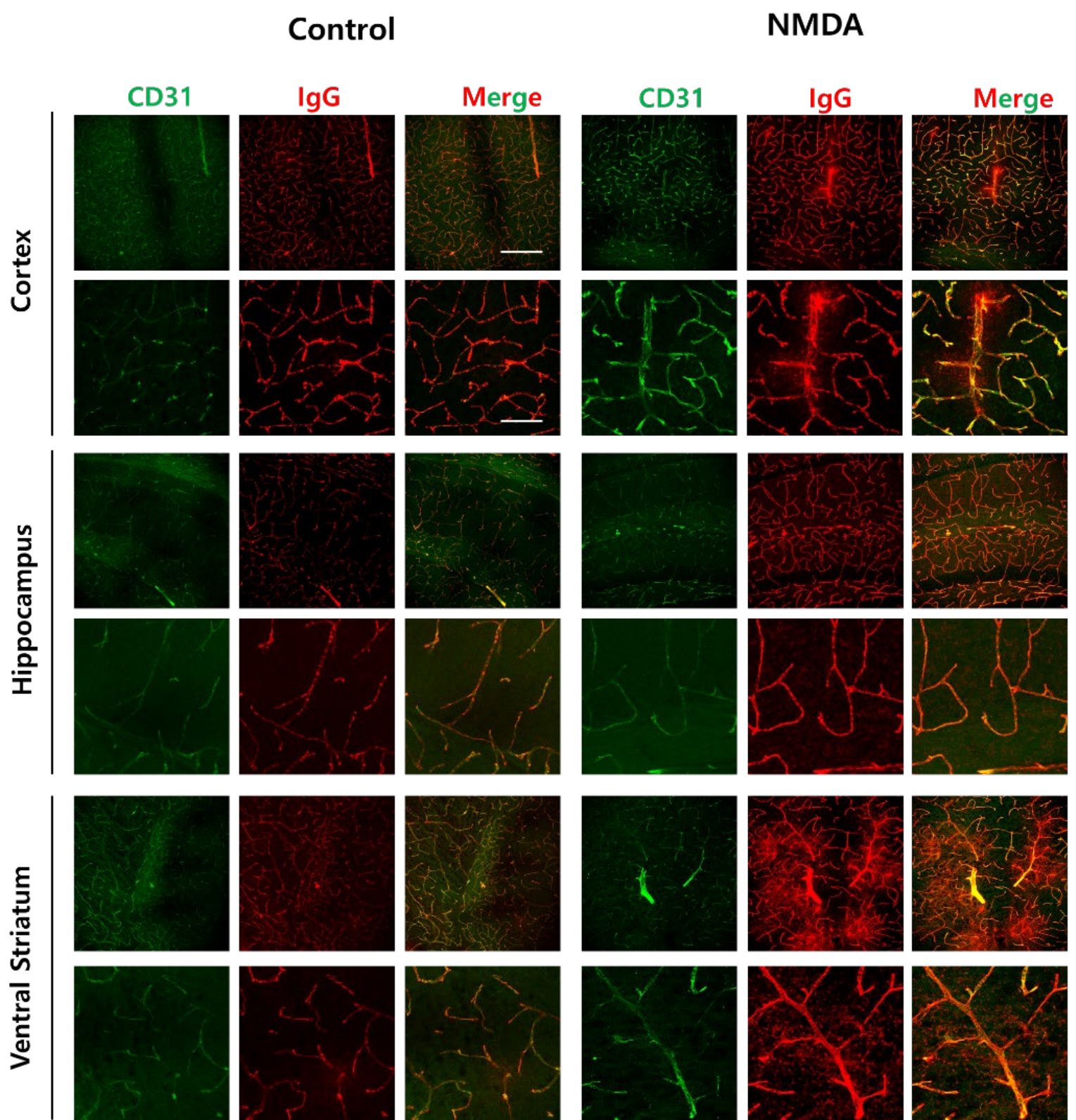
Supplementary Figure 2. Effect of NMDA receptor activation on the integrity of the brain endothelial cell Monolayer. Primary brain endothelial cell monolayer were activated with 25 μ M NMDA for 1 minute and stained with ZO-1 (Green) to visualize tight junction. Scale bar indicates 50 μ m.



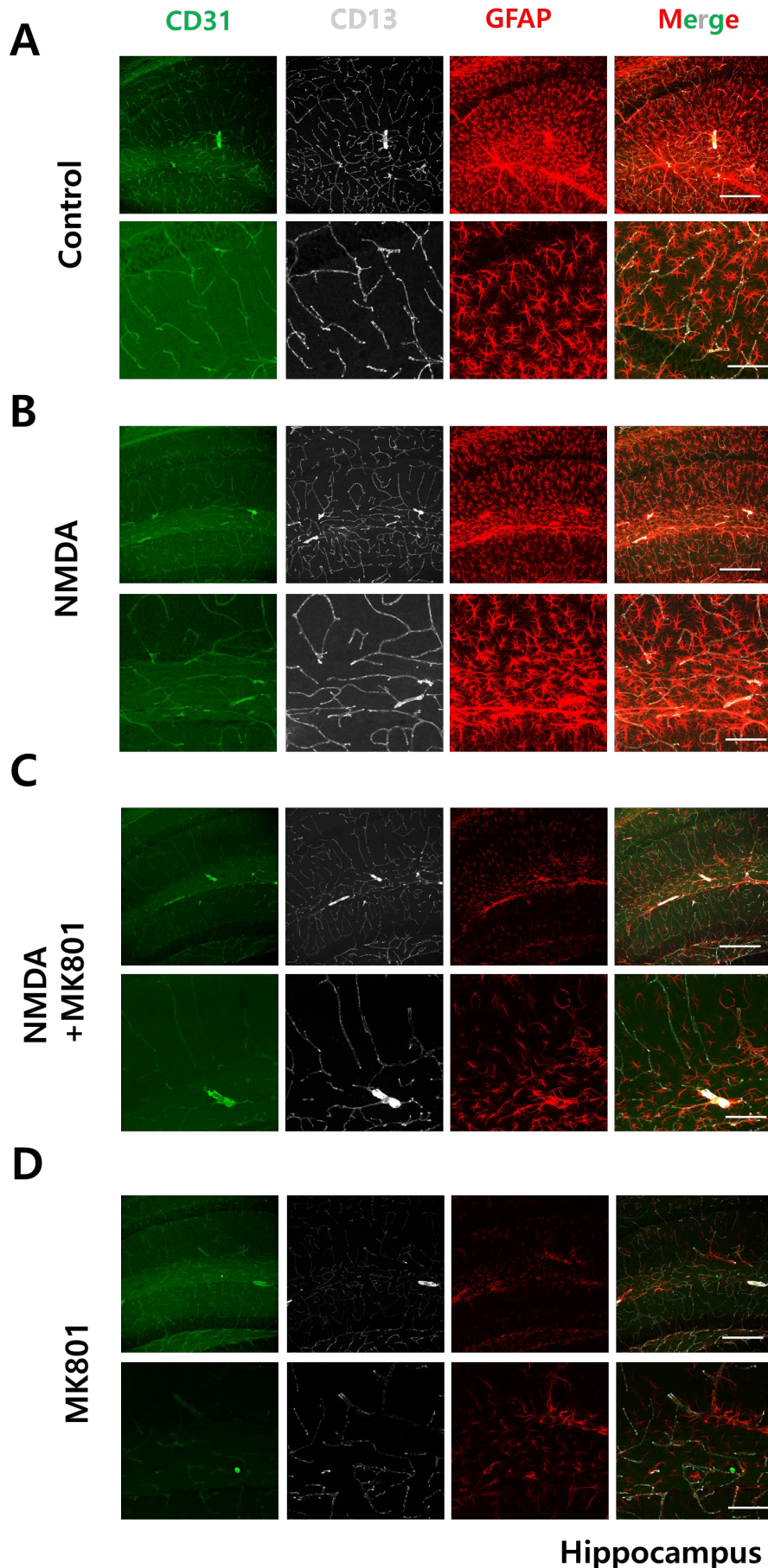
Supplementary Figure 3. Time dependent effect of NMDA receptor activation on the permeability to FITC-Dextran. HBMVEC cells cultured on the porous membrane were activated with different concentration of NMDA (5, 10, and 25 μ M) for upto 15 minutes and concentration of FITC-Dextran at the bottom chambers were quantified and depicted as a graph (n=4).



Supplementary Figure 4. Time dependent changes of the BBB permeability to IgG upon NMDA activation. Activation of the NMDA receptor induces increased permeability to the immunoglobulin G from peripheral system in a time dependent manner. C57/BL6 mouse was injected retro-orbitally with PBS, NMDA for 1,5, and 15 minutes. Brain sections were stained with IgG (Red) to visualize immunoglobulin G. Scale bar indicates 200 μm .

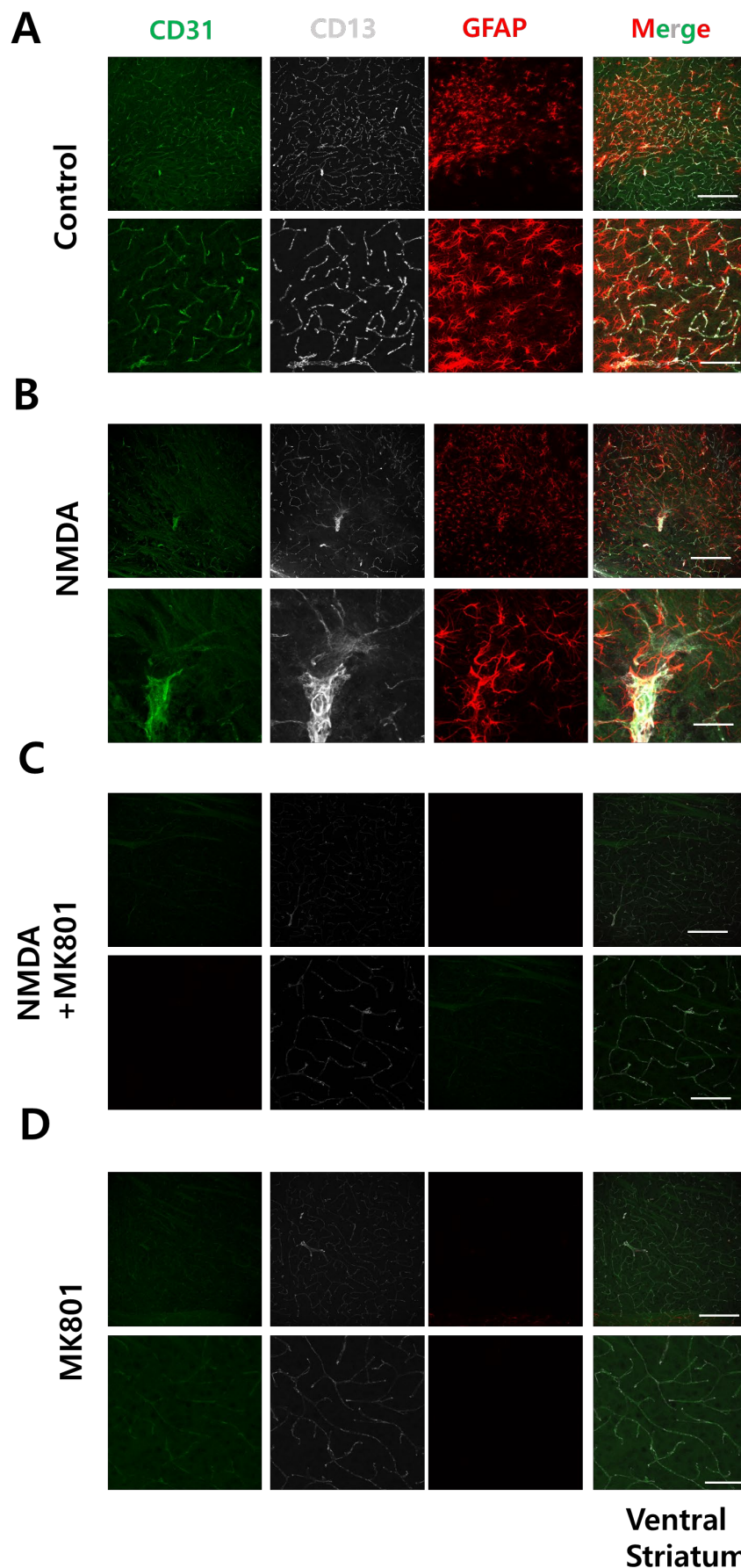


Supplementary Figure 5. Activation of NMDA receptor induces permeability changes of the BBB in a heterogeneous manner. Activation of the NMDA receptor induces increased permeability to the immunoglobulin G from peripheral system. C57/BL6 mouse was injected retro-orbitally with PBS, NMDA for 1 minute. Brain sections were stained with CD31 (Green) and IgG (Red) to visualize endothelial cells and immunoglobulin G, respectively. Images were taken from Cortex, Hippocampus, and Ventral Striatum region. Scale bar indicates 200 and 75 μm at the top and bottom panel.

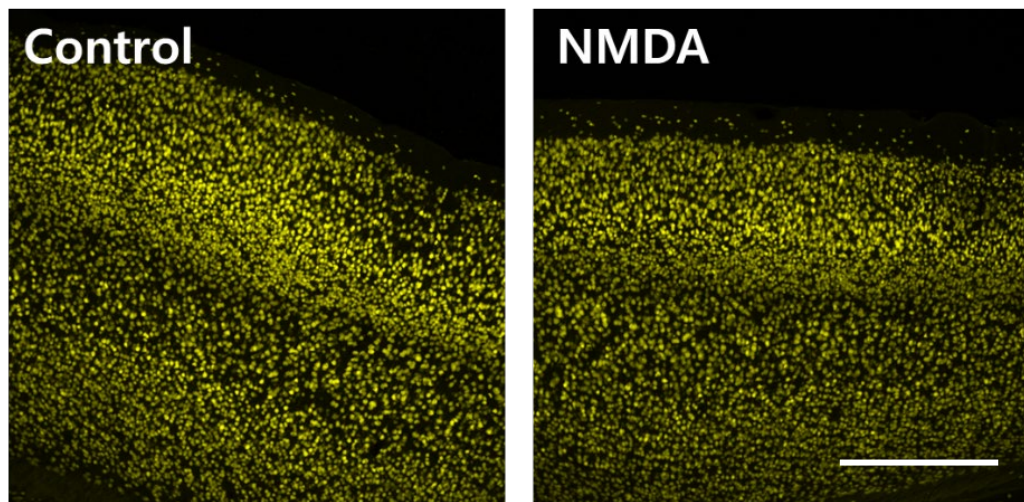


Hippocampus

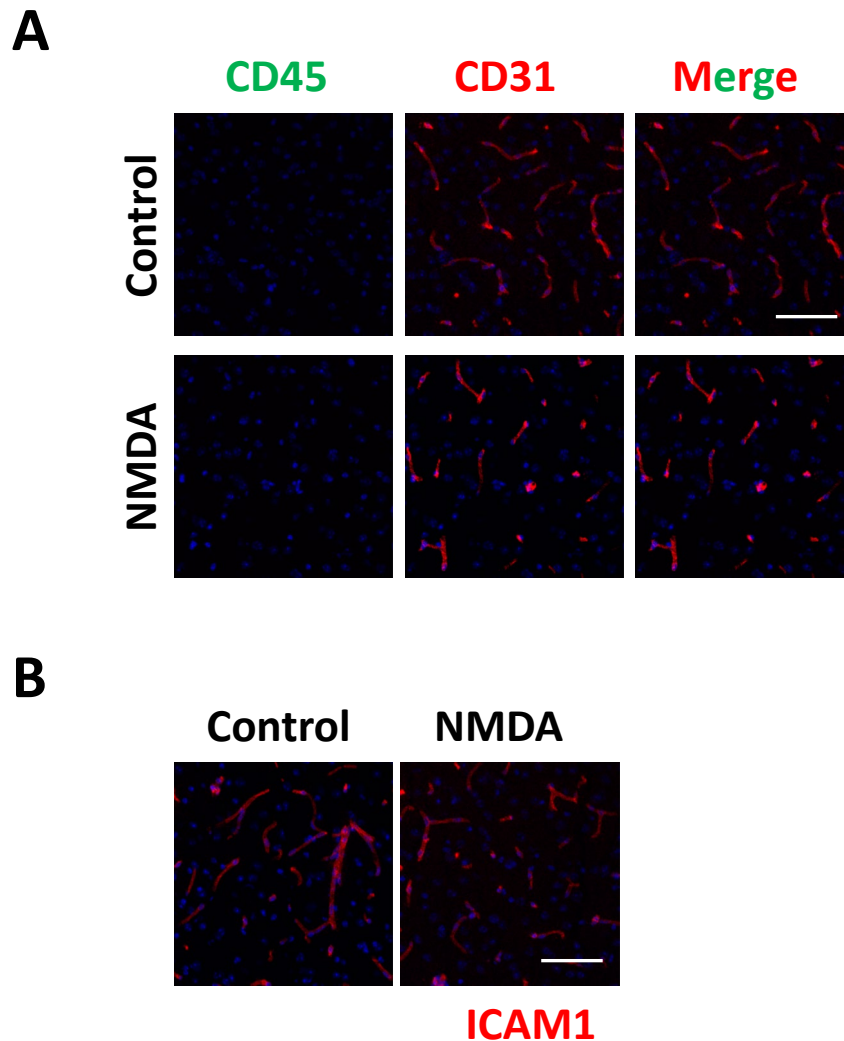
Supplementary Figure 6. Activation of NMDA receptor and its effect on markers for the BBB at the capillary level in the mouse hippocampus. (A-D) C57/BL6 mouse was injected retro-orbitally with PBS (A), NMDA (0.5 mg/kg) (B), NMDA with MK801 (0.5 mg/kg each) (C), and MK801 (0.5 mg/kg) (D) for 1 minute and mice were sacrificed and fixed with 4 % PFA overnight. Sections were stained with different markers for the blood brain barrier (BBB), CD31 (Green, 1:500), CD13 (Gray, 1:500), and GFAP (Red,1:500), a marker for endothelial cells, pericyte, and astrocyte, respectively. Magnified images of each treatment are displayed at the bottom panel. Scale bar indicates 200 μ m and 75 μ m for upper and bottom panel, respectively.



Supplementary Figure 7. Activation of NMDA receptor and its effect on markers for the BBB at the capillary level in the mouse ventral striatum. (A-D) C57/BL6 mouse was injected retro-orbitally with PBS (A), NMDA (0.5 mg/kg) (B), NMDA with MK801 (0.5 mg/kg each) (C), and MK801 (0.5 mg/kg) (D) for 1 minute and mice were sacrificed and fixed with 4 % PFA overnight. Sections were stained with different markers for the blood brain barrier (BBB), CD31 (Green, 1:500), CD13 (Gray, 1:500), and GFAP (Red, 1:500), a marker for endothelial cells, pericyte, and astrocyte, respectively. Magnified images of each treatment are displayed at the bottom panel. Scale bar indicates 200 μm and 75 μm for upper and bottom panel, respectively.

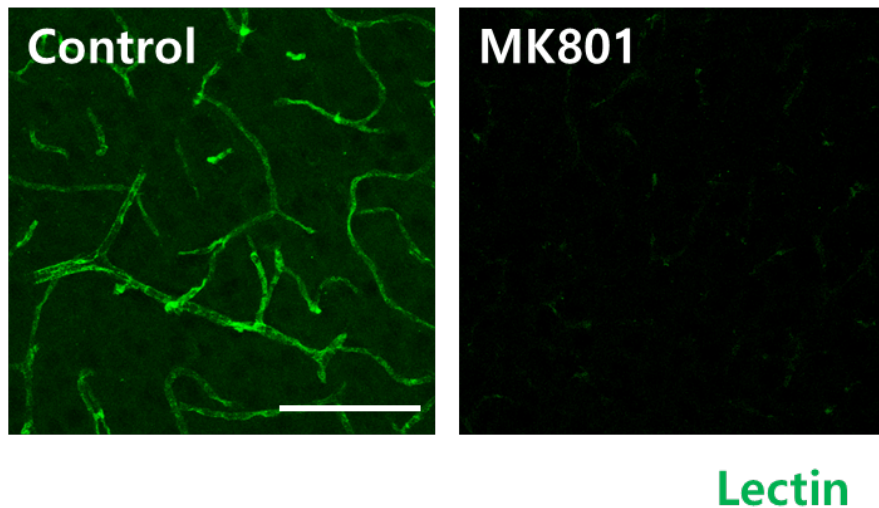


Supplementary Figure 7. Activation of NMDA receptor and absence of its toxic effect on neuronal cells. C57/BL6 mouse was injected retro-orbitally with PBS (A), NMDA (0.5 mg/kg) for 1 minute and mice were sacrificed and fixed with 4 % PFA overnight. Sections were stained with NeuN (yellow), a marker for neuronal cells. Scale bar indicates 500 μ m.

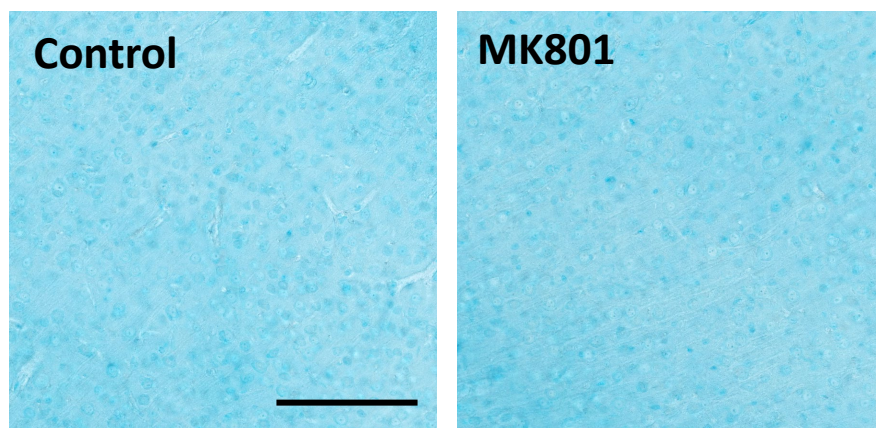


Supplementary Figure 8. Activation of NMDA receptor and absence of its inflammatory responses in the brain. C57/BL6 mouse was injected retro-orbitally with PBS or NMDA (0.5 mg/kg) for 1 minute. (A) Fresh frozen brain sections were stained with anti-CD45 (Green) and anti-CD31 (Red), marker of peripheral immune cells and brain endothelial cells, respectively. Scale bar indicates 75 μ m. (B) Fresh frozen sections were stained with anti-intercellular adhesion molecule 1 (ICAM1, red) to test the increased level of adhesion molecule. Scale bar indicates 75 μ m.

A



B



Supplementary Figure 8. Activation of NMDA receptor reduces lectin and glyocalyx in the brain endothelial cells. C57/BL6 mouse was injected retro-orbitally with PBS or NMDA (0.5 mg/kg) for 1 minute. (A) PFA fixed brain sections were stained with AF488-conjugated wheat germ agglutinin (Green) to stain lectin. Scale bar indicates 75 μ m. (B) PFA fixed brain section was stained with alcian blue to visualize glyocalyx. Arrow indicates the presence of brain vasculature stained with alcian blue. Scale bar indicates 75 μ m.