Auraptene Enhances Junction Assembly in Cerebrovascular Endothelial Cells by Promoting Resilience to Mitochondrial Stress through Activation of Antioxidant Enzymes and mtUPR

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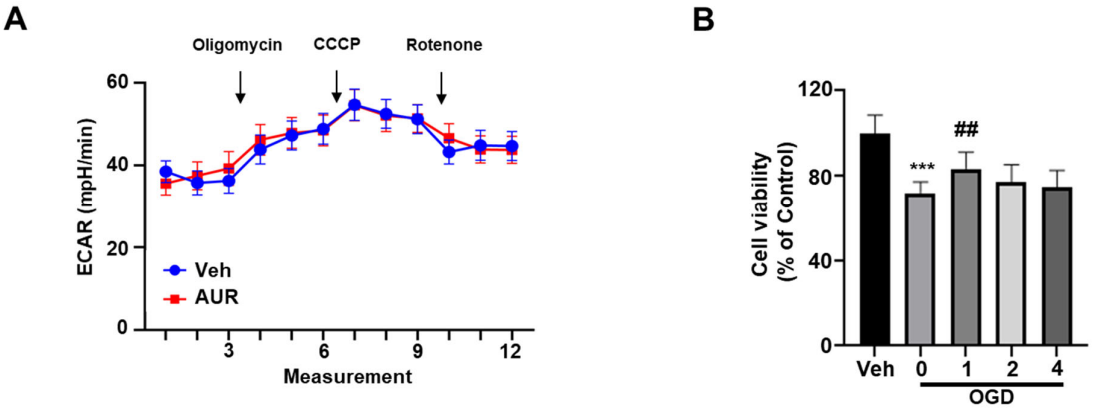
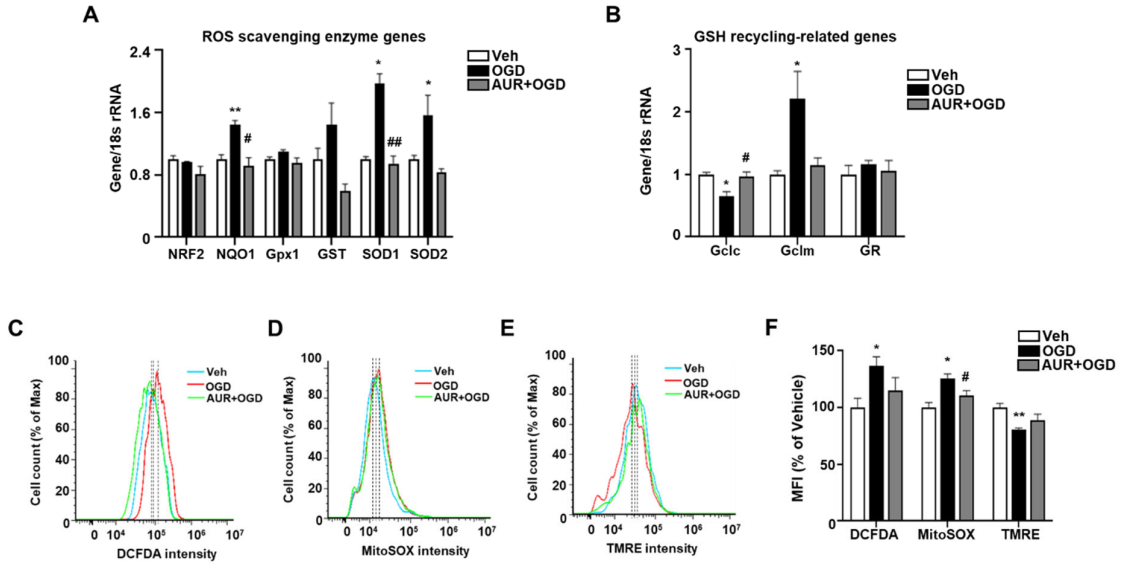


Supplementary Figure 1.

(A) extracellular acidification rate (ECAR) was measured in bEnd.3 cells treated with vehicle or 1 μM AUR for 24h.

(B) bEnd.3 cells (5 x 10^3 cells per well) seeded in 96-well plates were incubated in OGD condition for 3h after incubated in media containing 0, 1, 2, 4 μM of AUR for 18h. Vehicle group was

incubated in normal condition for 24h. Cell viability was measured by SRB assay (*** $p < 0.001$ compared to Veh, # $p < 0.05$ compared to OGD).



Supplementary Figure 2.

(A, B) mRNA expressions for ROS scavenging antioxidant enzymes and GSH recycling-related genes were analyzed using qPCR with bEnd.3 cells incubated in OGD 3h after treatment of vehicle or 1 μM AUR.

(C, D) bEnd.3 cells were incubated in OGD 3h after treatment of vehicle or 1 μM AUR. The cells were stained with 5 μM CM-H_2DCFDA or 5 μM MitoSOX™ or 100 nM TMRE and analyzed by flow cytometry. Total ROS was determined by DCFDA-stained cells(C), mitochondrial ROS was determined by MitoSOX™ – stained cells (D) and mitochondrial membrane potential was determined by TMRE-stained cells (E). (F) Median fluorescence intensity (MFI) values are analyzed by FlowJo program. Data are presented as mean and ± SEM of three independent experiments (* $p < 0.05$, ** $p < 0.01$ compared to Veh, # $p < 0.05$ compared to OGD).

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