Supplementary Fig. 1.

Design of preconditioning and injury exposure paradigms. Most cell types typically exhibit minor variations from day to day in their sensitivity to preconditioning and injury. To overcome this problem, preconditioning was induced by 3-4 concentrations of tamoxifen or H_2O_2, one or two of which often fell in the lethal range. After an overnight recovery, the cultures were re-exposed to higher concentrations of tamoxifen or H_2O_2 and their survival determined. These concentrations were slightly higher and intended to kill the cultures that were not preconditioned. In parallel, duplicate control and preconditioned cultures were analyzed for Cx43 levels by western blotting and/or immunocytochemistry, hemichannel activity by dye uptake, ATP release, or whole cell recordings. The viability data selected for the figures represents the preconditioned group that had the best survival compared to non-preconditioned cultures exposed to the same severity of subsequent injury. The bar histograms are representative examples of an experiment comparing the protective effect of preconditioning in C6-Cx43+ cells (A) and C6-Cx43- cells (B) exposed in parallel. Data represent average ± SD (n=3-4).

Supplementary Fig. 2 MCA occlusion is associated with a comparable fall in cerebral perfusion in all groups studied. (A) Histogram mapping the relative decrease in perfusion during 45 min MCA occlusion and recovery at 10 min after reperfusion (55 min after initiation of MCA occlusion) detected by laser doppler flowcytometry. (B) No significant differences in blood flow were noted among the groups studied. Data represent average ± SEM (n=5-8). One limitation of the laser Doppler analysis is that only relative changes in

blood flow are measured in the ischemic core. It is possible that accumulation of adenosine in WT mice increased blood flow in the penumbral areas relative to Cx43 null mice and thereby resulted in smaller infarcts.

Supplementary Fig. 3 Uptake of PI in C6-Cx43 cells following preconditioning requires exposure to a Ca^{2+}-free solution and does not reflect loss of viability. (A) Representative example of PI uptake in control C6-Cx43+ cells prior to exposure to a low Ca^{2+} solution. (B) Same culture after exposure to a low Ca^{2+} solution, and (C) uptake of the viability indicator calcein/am (2 μM) in the same field as B. (D) Uptake of PI in a C6-Cx43+ culture exposed to a preconditioning stimulation prior to exposure to a low Ca^{2+} solution. (E) Uptake of PI after exposure to a low Ca^{2+} solution. A marked increase in PI uptake is noted. (F) Calcein/am (2 μM) in the same field shows that the cells are viable and able to retain calcien.