



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  $\pm$  SE (n=3-4).