

Supplementary Figure Legends

Supplementary Figure 1. All anti-pannexin antibodies are specific. To rule-out any possible crosstalk between anti-pannexin antibodies used in the immunoprecipitation studies, cells expressing Panx1 or Panx2 were immunoprecipitated with the anti-Panx3 antibody (A, C) or the anti-Panx2 antibody (B) and immunolabeled for Panx1 (A, B) or Panx2 (C). No cross-reactivity of any of the antibodies was detected.

Supplementary Figure 2. Cell lysates from 293T cells expressing Panx2 together with Panx1 or Panx3 were immunoprecipitated with anti-Panx2 antibodies and immunoblotted with anti- Panx1, Panx3 or Panx2 antibodies. These studies revealed the interaction of Panx2 with the Gly0 and Gly1 species of Panx1 and confirm the lack of interaction between Panx2 and Panx3. The same blot was probed sequentially with anti-Panx3 and Panx2 after Panx1 without stripping. (A). Cell lysates from 293T cells co-expressing Panx1 or Panx1^{N254Q} together with Panx3 or Panx3-GFP were immunoprecipitated with anti-Panx1 antibodies and immunoblotted with anti- Panx3 or -Panx1 antibodies. These studies revealed that Panx1 and Panx1^{N254Q} interacted with Panx3-GFP (B). An aliquot of cell lysates were immunoblotted with anti-pannexin antibodies to assess pannexin levels. Line indicates higher exposure on the right panel of the same blot.

Supplementary Figure 3. Immunolocalization of Panx1, Panx3 and mutants in 293T cells. Panx1 and Panx3 exhibited a similar pattern of cell surface and intracellular localization. Panx3-GFP can be rescued to the cell surface upon co-expression with either Panx3 or Panx1, while co-transfection of Panx3-GFP and Panx1^{N254Q} results in a marked co-localization in intracellular compartments. Bars: 20 μ m



