

Supplemental Figure 1 Addition of Ca^{2+} ions to permeable PC12 cells that did not undergo priming does not increase the amount of co-precipitated syntaxin. Before cross-linking with 5 mM DTSSP followed by immunoprecipitation (*IP*) with anti Kv2.1 antibody, permeable PC12 cells were incubated in KGlu buffer for 10 min at 30°C in the presence ($+\text{Ca}^{2+}$) or absence ($-\text{Ca}^{2+}$) of 1.6 μM CaCl_2 (1 μM free ionic $[\text{Ca}^{2+}]$) followed by washing with KGlu buffer. Cross-linking and immunoprecipitation were performed as in Fig. 1b except that 1.6 μM CaCl_2 was added throughout these steps to the cells preincubated with Ca^{2+} ($+\text{Ca}^{2+}$). Proteins were separated by SDS–PAGE, blotted, and detected by antibodies as indicated on the left side of the blots. For each reaction we used 1.5×10^6 cracked cells. The experiment shown is one of two similar experiments.

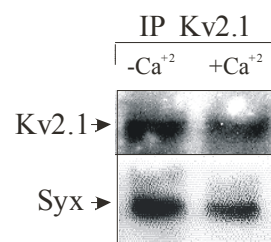


Figure-1 Supplements (Lotan)

Supplemental Figure 2 Kv2.1W365C/Y380T associates with syntaxin 1A and is expressed at the cell surface in oocytes. **(a)** Physical interaction of the Kv2.1W365C/Y380T channel with syntaxin in *Xenopus* oocytes is comparable to that of Kv2.1. Digitized Phosphorimager scans of SDS-PAGE analysis of [³⁵S]Met/Cys-labeled Kv2.1, Kv2.1W365C/Y380T and syntaxin proteins co-precipitated by either Kv2.1 antibody (*IP Kv2.1*) or by syntaxin antibody (*IP Syx*) from 1% Triton X-100 homogenates of oocytes that were injected with Kv2.1 or Kv2.1W365C/Y380T together with syntaxin (0.75 ng/oocyte; +*Syx*), or injected with syntaxin alone (*Syx*). *Arrows* indicate the relevant proteins. Upper scan shows that the amount of syntaxin co-precipitated with the mutant channel (see *IP Kv2.1*) is similar to that co-precipitated with the same amount wild-type channel. Lower scan shows that expression of syntaxin in all groups was similar. **(b)** Immunoprecipitation in manually dissected plasma membranes of oocytes (Michelevski et al., 2003) expressing Kv2.1W365C/Y380T.

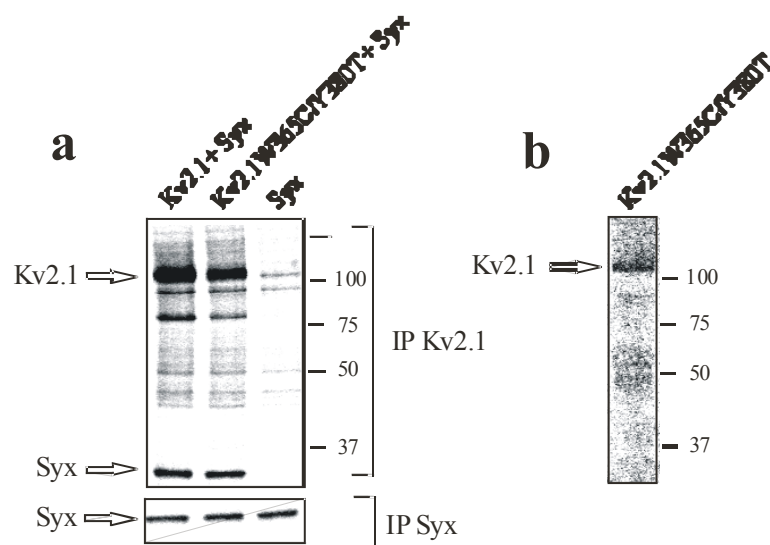


Figure 2 Supplements (Lotan)