

Supplemental Figure 1. Changes in activation kinetics of the chimeric and truncation mutant Eag channels. (A) Comparison of activation kinetics of Eag, mEag and the two chimeric channels. Activation time course was determined by measuring time to half maximal current amplitude $(t_{1/2})$ at +60 mV in extracellular solutions containing 2 mM Mg²⁺. Values are shown as mean ± SEM. Data shown are measured from transfected CHO cells cultivated at 26°C. For mEag and the two chimeric channels that conduct K⁺ currents at 37°C, their $t_{1/2}$ values are not significantly different from that at 26°C. (B) Compared with the respective full length channels, CT-truncated mutant channels exhibited slower activation kinetics when they expressed ionic currents at 26°C.



Supplemental Figure 2. Non-conducting mutant Eag channels are targeted to the plasma membrane. Cell surface proteins were biotinylated using a membrane-impermeable reagent and isolated with streptavidin beads. Channel immunoreactivity in the streptavidin precipitates (*first panel*) and in the lysates (*third panel*) was detected with an anti-Eag antibody which recognizes a region of the Eag carboxyl terminal domain. GAPDH was probed either as a loading control (*fourth panel*), or to verify that the biotinylation reagent did not label intracellular proteins, (*second panel*). Images shown are representative data from transfected tsA 201 cells cultivated at 26°C. Similar results were obtained from cells maintained at 37°C. Note that expression levels of different mutant channel proteins varied because they were expressed by three different vectors.