



**Supplementary fig. 3. Rat mutant and chimeric receptors. Relative calcium permeability.** (A, B, C, E and G) Representative I-V curves obtained by application of voltage ramps (-120 to +50 mV, 2 s) at the plateau of the response to 100  $\mu$ M ACh in oocytes superfused with N-methyl glucamine-based solutions containing different  $\text{Ca}^{2+}$  concentrations (0.5 and 5 mM) for oocytes expressing rat  $\alpha 9\alpha 10$  mutant or chimeric receptors. Insets: magnification near the  $E_{rev}$ . (A) Extracellular vestibule mutant receptors: rat  $\alpha 9D110N\alpha 10$  (left) and rat  $\alpha 9S127F\alpha 10$  (right). (B) Rat  $\alpha 9A-4'D\alpha 10$  mutant receptor. (C) TM2 domain mutant receptors: rat  $\alpha 9A24'P\alpha 10$  (left) and rat  $\alpha 9I7'V/M11'L\alpha 10$  (right). (E) Rat  $\alpha 9(IC-chick \alpha 9)\alpha 10$  chimeric receptor. (G) Rat  $\alpha 9D110N/S127F/A-4'D\alpha 10$  triple mutant receptor. (D and F) Plots of  $E_{rev}$  values as a function of extracellular  $\text{Ca}^{2+}$  concentration for rat  $\alpha 9\alpha 10$  mutant or chimeric receptors.  $E_{rev}$  values for rat  $\alpha 9\alpha 10$  (red) and chicken  $\alpha 9\alpha 10$  (blue) are shown for comparison. Values are mean  $\pm$  S.E.M. of 5-11 experiments per group. Solid lines are fit to the GHK equation (see Methods). (D) TM2 domain mutant receptors: rat  $\alpha 9A24'P\alpha 10$  and rat  $\alpha 9I7'V/M11'L\alpha 10$ . (F). Rat  $\alpha 9(IC-chick \alpha 9)\alpha 10$  chimeric receptor.