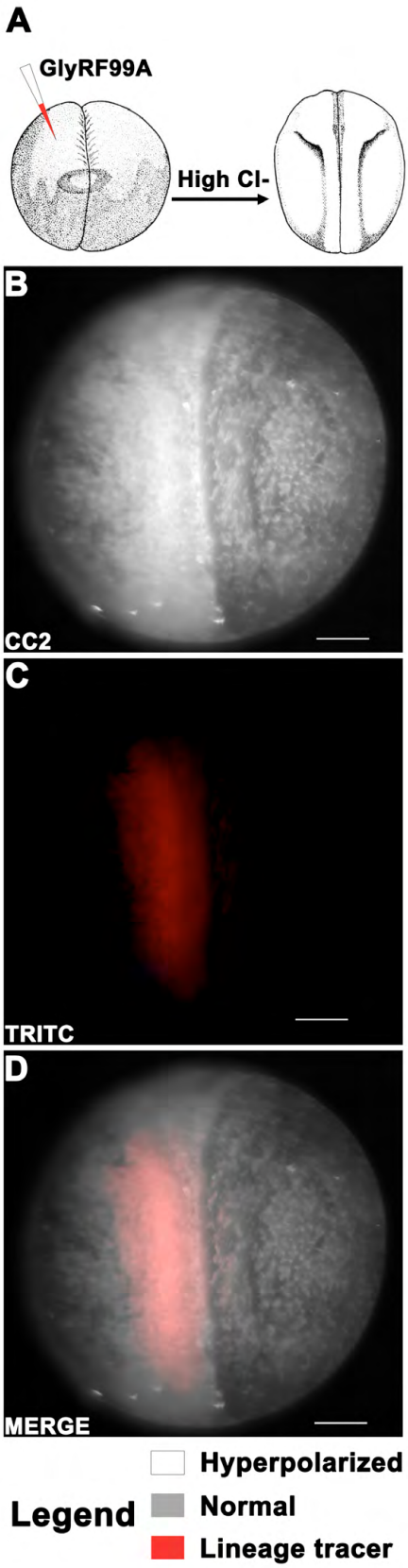

Fig. S1. V_{mem} changes quantified via electrophysiology. Fertilized 1-cell stage embryos were divided into five treatment categories: uninjected controls in 0.1x MMR, uninjected controls in high Cl^-, GlyRF99A injected embryos in high Cl^-, Xrel3 injected embryos, and Xrel3 and GlyRF99A co-injected embryos in high Cl^-. Standard electrophysiology was used to measure V_{mem} from embryos in each category: uninjected control group in 0.1x MMR averaged −22.6 mV (*n*=5), uninjected controls in high Cl^- averaged −25.6 mV (*n*=5), and GlyRF99A injected embryos, when compared to the two control groups, showed a significantly hyperpolarized V_{mem} (−42.06 mV; *n*=5). Consistent with data obtained via voltage-sensitive dye imaging, Xrel3-injected embryos are significantly depolarized (average of −11.4 mV; *n*=5) compared to controls, while co injecting the GlyRF99A channel together with Xrel3 significantly counteracts the depolarization (average of −24.7 mV; *n*=12). (One-way ANOVA with Bonferroni post hoc analysis, ***P<0.001).

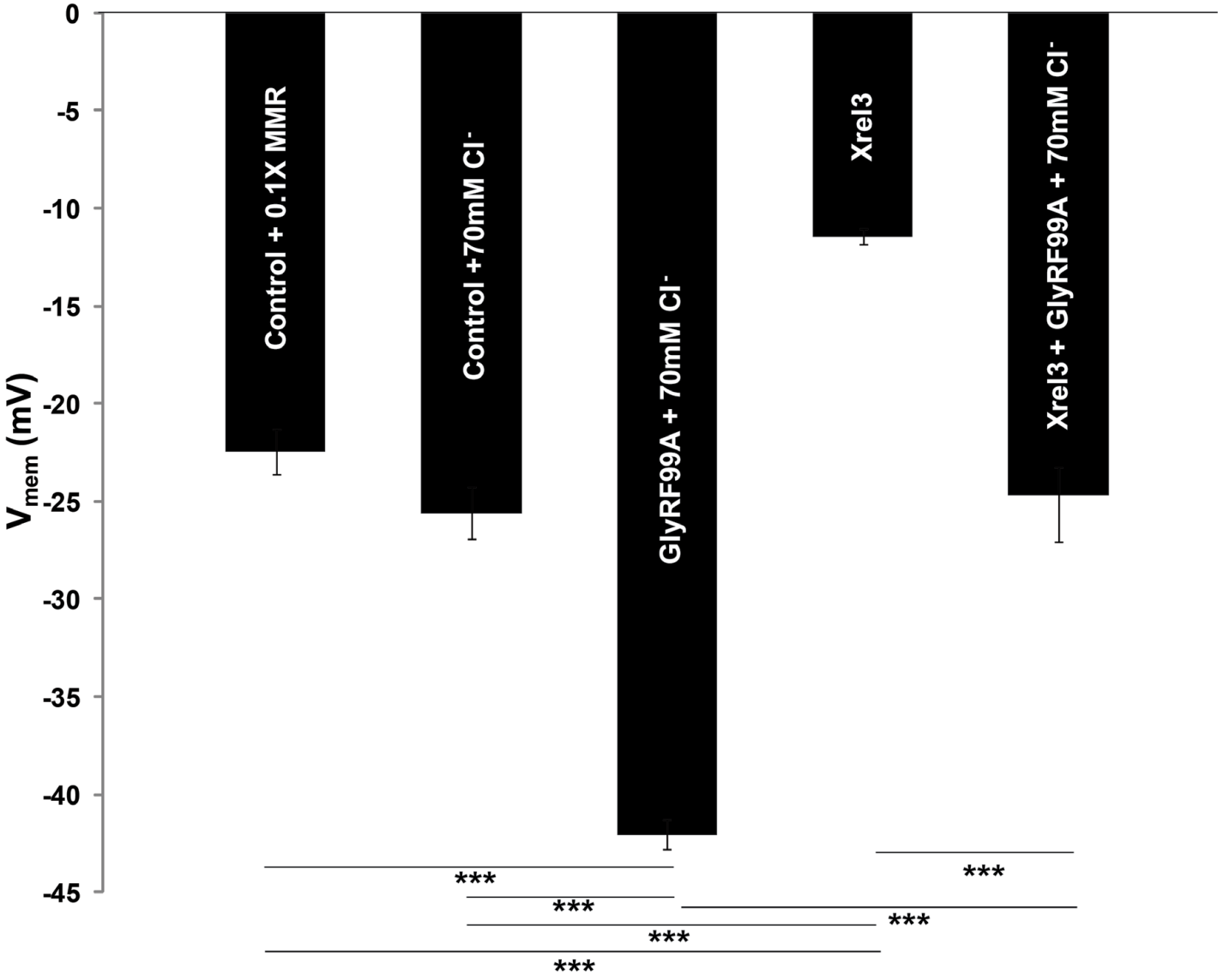

Fig. S2. **Hyperpolarization is detected by the voltage sensitive dye CC2-DMPE.** (A) Schematic of forcing hyperpolarization by a constitutively open Cl^- channel: embryos were co-injected with GlyRF99A mRNA and dextran-conjugated Alexa Fluor 555 (lineage dye) into 1 cell of a 2-cell embryo, allowing the uninjected side to serve as an internal control. Embryos were raised to stage 18 in a high (70 mM) Cl^- medium. (B) At stage 18, embryos were soaked in 5 μM CC2 dye in 0.1x MMR, and imaged using a CC2 filter set (EX 405/20; BS 425; EM 460/50). The injected left half of the embryo was highly fluorescent, indicating relative hyperpolarization compared to the right half of the embryo. (C) Imaging of the same embryo using a TRITC filter set confirmed the presence of injected mRNA mix, evidenced by the red fluorescent signal of the lineage tracer. (D) Overlay of CC2 and TRITC images showing the co-localization of hyperpolarization signal and hyperpolarizing channel mRNA.