GlialCAM, a CLC-2 Cl^- Channel Subunit, Activates the Slow Gate of CLC Chloride Channels

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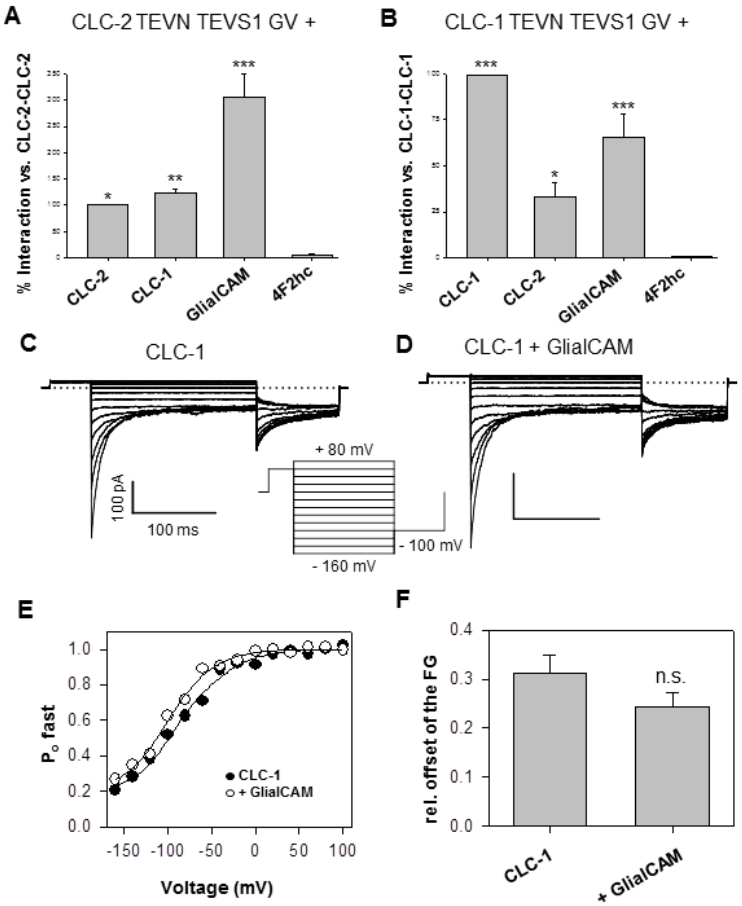
Supplementary Figure 1



Supplementary Figure 1. Interaction of CLC-1 with GlialCAM. (*A*) CLC-2-CLC-1 interaction by split TEV. Supernatants from transfected HeLa cells were measured in a luminometer to detect luciferase activity. % interaction versus homophilic CLC-2 interaction + SEM is presented. **p<0.01 and ***p<0.001 versus the negative interaction CLC-2-4hc. (n=7-12). (*B*) CLC-1 homo-oligomerization and CLC-1-CLC-2 and CLC-1-GlialCAM interactions by split TEV. % interaction versus homophilic CLC-1 interaction + SEM is represented. *p<0.05 and ***p<0.001 versus the negative interaction CLC-1-4F2hc (n=4). Bonferroni's multiple comparison test was used. (*C*, *D*) Representative current traces from inside-out patches of oocytes expressing CLC-1without (*C*) or with (*D*) GlialCAM using the pulse protocol shown in the inset, which provides information of the combined effect of the fast and the slow gate. (*E*, *F*). Gating parameters of the fast gate were unaffected by GlialCAM.

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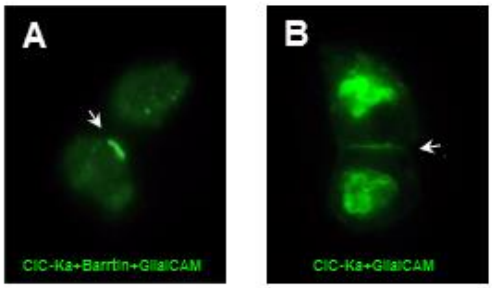
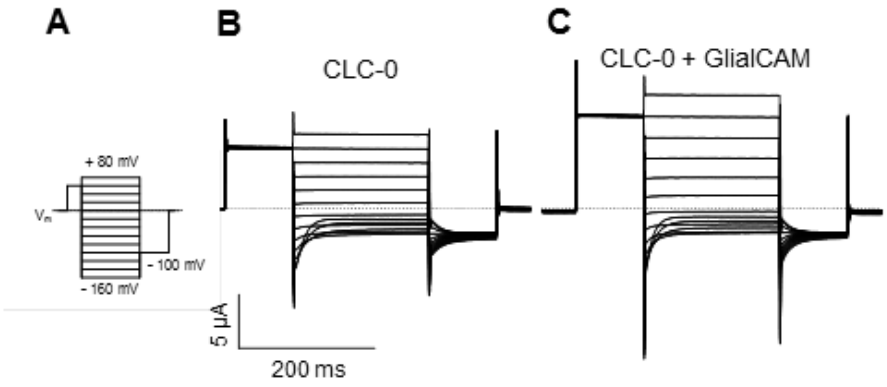
Supplementary Figure 2



Supplementary Figure 2. Co-localization of GlialCAM with CLC-Ka in Hek cells. GlialCAM drives CLC-Ka (tagged with GFP) distribution to cellular contacts when transfected with (*A*) or without (*B*) Barttin.

Supplementary Figure 3



Supplementary Figure 3. The fast gate of CLC-0 is not altered by GlialCAM. (*A*) Pulse protocol used to assay the fast gate. (*B*, *C*) Typical fast gate transients of CLC-0 (*B*) and CLC-0/GlialCAM (*C*) expressing oocytes.

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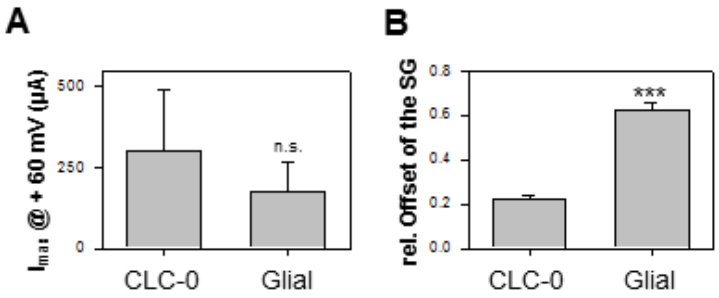
Supplementary Figure 4



Supplementary Figure 4. On-cell macro patch experiments of CLC-0 and GlialCAM co-expressing oocytes. (*A*) Maximal currents at 40 mV as measured using the slow gate protocol shown in Fig. 4 *D*. I_{max} was not significantly different in oocytes co-expressing GlialCAM (n≥25 each). (*B*) Relative offset of the slow gate is significantly increased in CLC-0 / GlialCAM co-expressing oocytes (***p<0.001, Student t test).