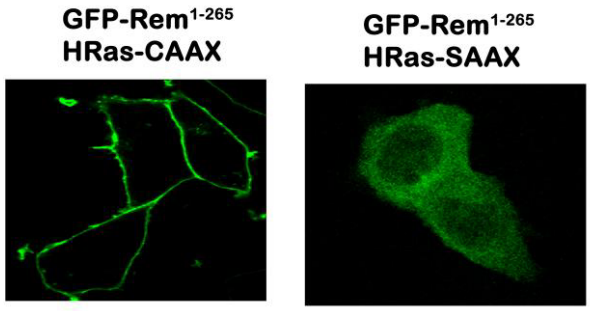
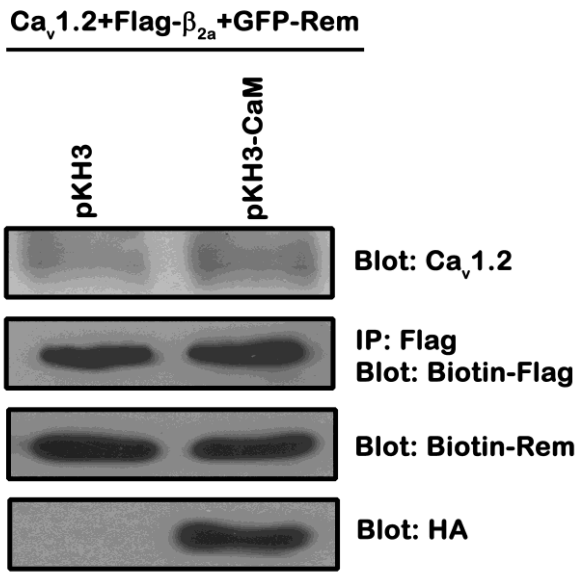
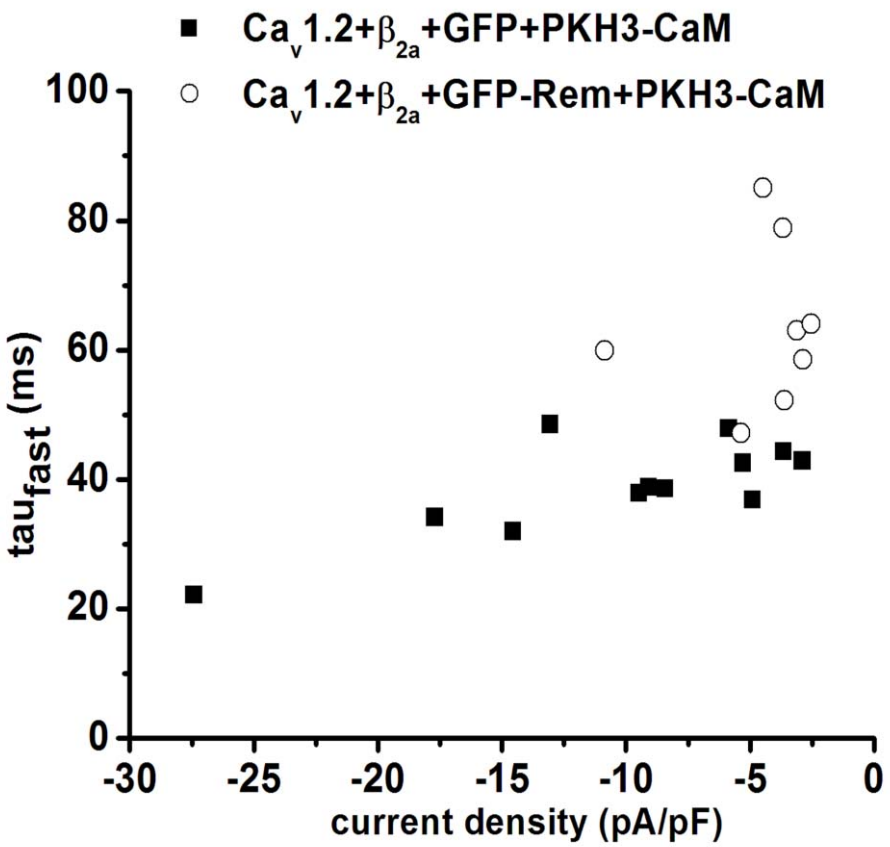


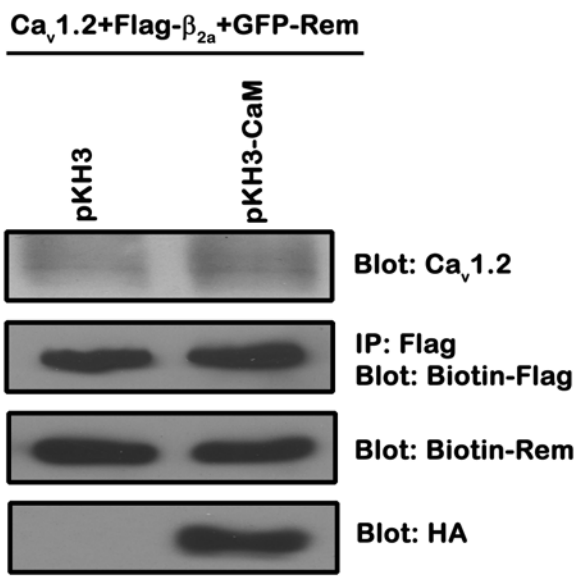

**Supplemental Figure S1**
**Localization of green fluorescent protein (GFP)-Rem-(1-265)-HCAAX and GFP-Rem-(1-265)-HSAAX.**
TsA201 cells were transfected with plasmids expressing GFP-Rem-(1-265)-HCAAX or GFP-Rem-(1-265)-HSAAX. 48 h post transfection cells were examined by confocal microscopy. Note that Rem-(1-265)-HCAAX is enriched at the cell periphery, which is consistent with plasma membrane localization. However, Rem-(1-265)-HSAAX displays predominant cytosolic localization.




**Supplemental Figure S2**
**Overexpression of CaM does not alter expression of $Ca^{2+}$ channel subunits or GFP-Rem.**
TsA201 cells were transiently transfected with $Ca_v1.2$, Flag-$\beta_{2a}$, GFP-Rem, and pKH3-CaM or pKH3 as control. 48 hrs after the transfection, cell lysates were immunoblotted with the appropriate antibodies to monitor recombinant protein expression.




**Supplemental Figure S3**
**Relation between the fast component of the time constant of inactivation ($tau_{fast}$) and peak current density.**
$Tau_{fast}$ was derived as described under "Experimental Procedures" and plotted against the peak current density measured at +20 mV.

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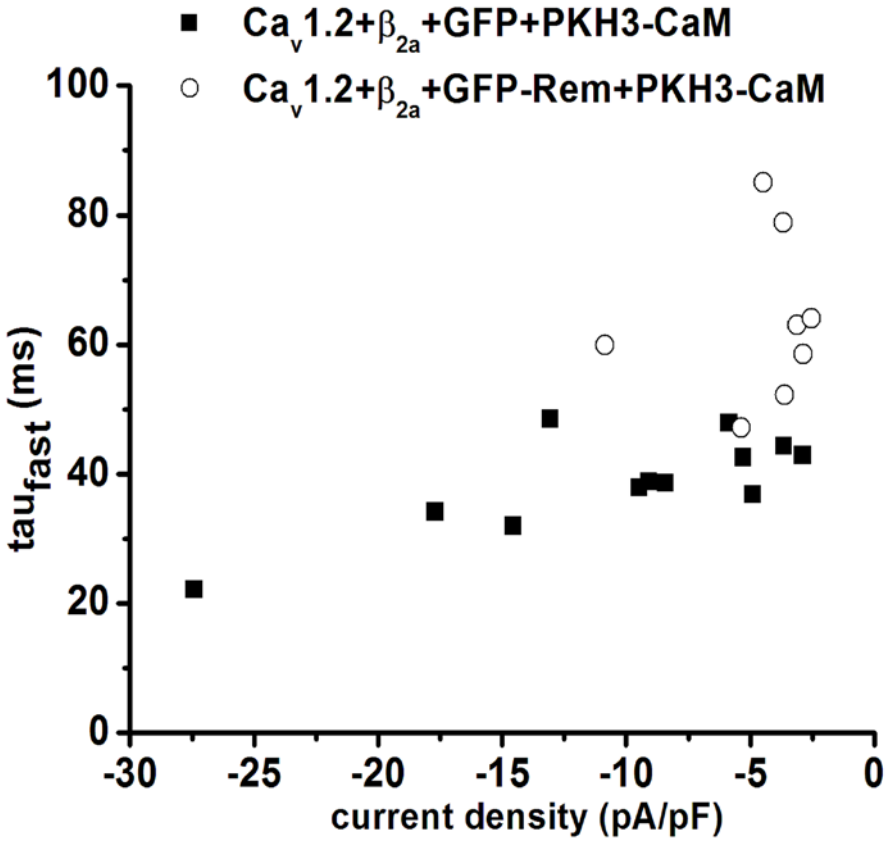


**Supplemental Figure S3**
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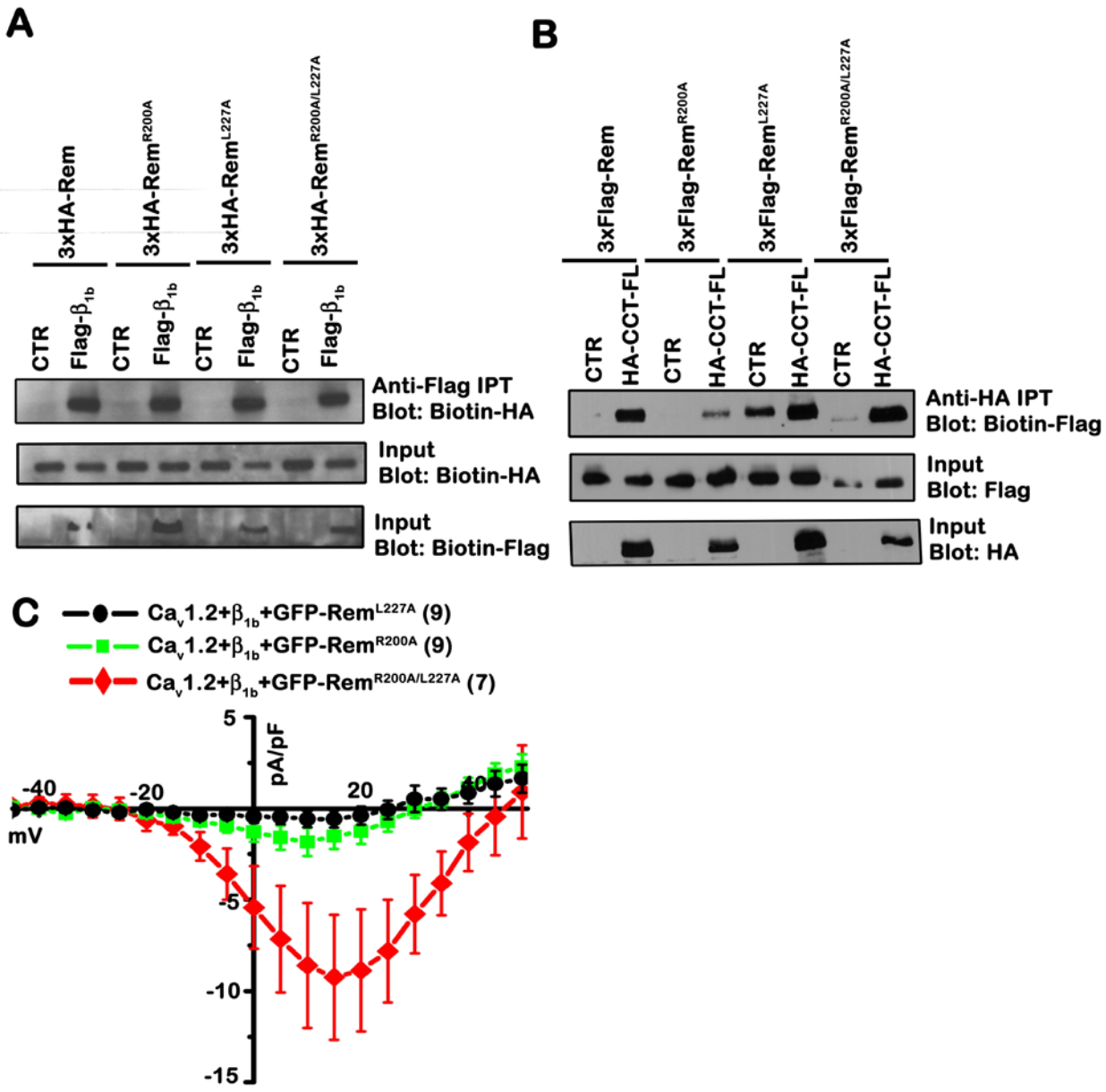
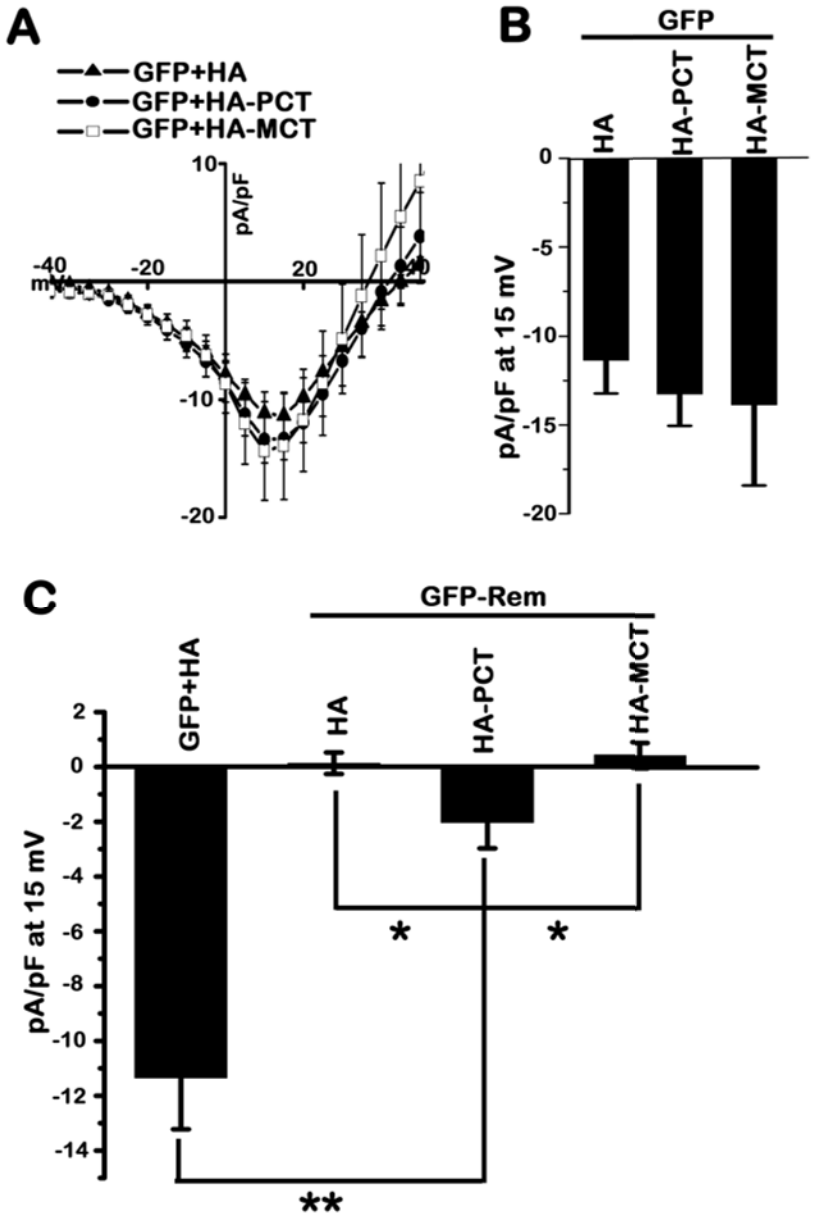



**Reviewer Figure R1.**

**Analysis of putative β-null Rem mutants.** ***A***, TsA201 cells were transiently co-transfected with plasmids expressing HA-tagged Rem, $Rem^{R200A}$, $Rem^{L227A}$, or $Rem^{R200A/L227A}$, and either empty pCMVT7/F2 or Flag-$\beta_{1b}$. Co-immunoprecipitation was performed with Flag antibody and interaction with Rem proteins examined by immunoblotting with biotinylated anti-HA antibody. Total cell lysates were analyzed by immunoblotting with biotin-HA to detect Rem or by immunoblotting with biotin-Flag to detect $\beta_{1b}$. Results are representative of two independent experiments. ***B***, TsA201 cells were transiently co-transfected with plasmids expressing 3xFlag-tagged Rem, $Rem^{R200A}$, $Rem^{L227A}$, or $Rem^{R200A/L227A}$ and either empty pCDNA3.1+3xHAa (vector control) or HA-tagged CCT-FL. Co-immunoprecipitation was performed with anti-HA antibody and interaction with Rem examined by immunoblotting with biotinylated FLAG antibody. Total cell lysates were analyzed by immunoblotting with anti-HA to detect CCT or by immunoblotting with anti-Flag to detect Rem or Rem mutants. Results are representative of two independent experiments. ***C,*** TsA201 cells were transfected with plasmids expressing $Ca_v1.2$, $\beta_{1b}$ and either GFP-$Rem^{R200A}$, GFP-$Rem^{L227A}$, or GFP-$Rem^{R200A/L227A}$. Current was examined using the whole-cell patch clamp configuration in the presence of 30 mM $Ba^{2+}$.




**Reviewer Figure R2**

**Co-expression of PCT partially recovers L-type current from Rem-mediated regulation in HIT-T15 cells.**

***A***, Current-voltage relationships were determined for HIT-T15 cells co-transfected with GFP+HA (*filled triangles*, n=9), GFP+HA-PCT (*filled circles*, n=6) or GFP+HA-MCT (*open squares*, n=7) in the presence of 30 mM $Ba^{2+}$. ***B***, Current density at 15mV is displayed for experiments in panel A. There is no significant difference between the treatments. ***C***, Current density at 15 mV from HIT-T15 cells co-transfected with GFP+HA (n=9), GFP-Rem+HA (n=9), GFP-Rem+HA-PCT (n=13), or GFP-Rem+HA-MCT (n=15). The error bars represent the standard error of the mean. Analysis of the results by student's t test revealed a significant difference between treatments denoted by single ($P<0.05$) or double ($P<0.00005$) asterisks.