# NeCaB1 promotes trafficking of GluK5 containing Kainate receptors to the cell surface.

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## Summary

Fast excitatory synaptic transmission is mainly mediated by glutamate receptors in the Central Nervous System (CNS). This family of receptors comprises three different members named after ligand preference: a-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA), *N*-methyl-D-aspartate (NMDA) and kainate. Among these, kainate receptors (KARs) are the less understood from a physiological point of view. An attempt to unveil important aspects of KARs physiology is to elucidate the protein interactome around these receptors. To reach this goal, our lab used a yeast two-hybrid screening to identify possible partners of GluK5 subunits by using its C-terminal domain (CTD) as a bait. During this screening, we identified Neuronal Calcium Binding Protein 1 (NeCaB1) as an interactor of GluK5 CTD.

We further verified the interaction between NeCaB1 and GluK5 by co-inmunoprecipitation in HEK cells expressing both proteins and in pull-down assay. In addition, we found that binding of NeCaB1 to GluK5 CTD is $Ca^{2+}$ dependent in that interaction is disfavored in the presence of $Ca^{2+}$. Bimolecular fluorescence complementation (BiFC) further demonstrated interaction between these two proteins in vivo. This interaction occurs in specific CTD regions that contain endoplasmatic retention signals, likely indicating a role in receptor trafficking.

The increased affinity for glutamate of GluK1/GluK5 heteromeric KARs as compared to homomeric GluK1 receptors served as a readout for detecting GluK1/5 heteromeric receptors at the plasma membrane. Therefore, we found that NeCaB1 promotes the presence of GluK5 containing KARs in the cell surface when internal $Ca^{2+}$ was reduced to a minimum.

Altogether, these data indicate that NeCaB1 binds to CTD of GluK5 subunit containing KARs promoting its trafficking to the cell surface in a low $Ca^{2+}$ environment.


## Figure 1. NeCaB1 interacts with GluK5 C-terminal domain, an interaction which is disfavored by $Ca^{2+}$

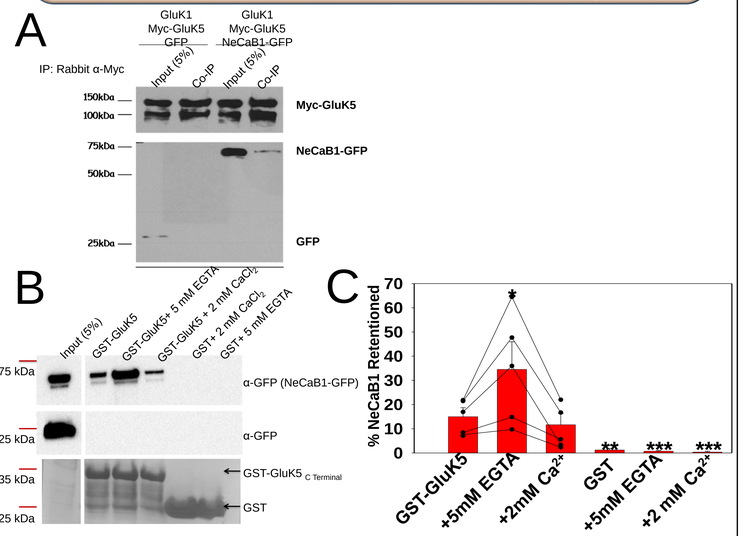


**Figure 1. NeCaB1 protein interacts with the C-terminal of GluK5 subunit containing KARs.** A, validation of NeCaB1 & GluK5 interaction by Co-Inmunoprecipitation. COS cells transfected with GluK1, Myc-GluK5 and GFP or NeCaB1-GFP. B, Pull-down assay where HEK cells extracts expressing NeCaB1-GFP or GFP alone are probed for interaction with the C-terminal domain of GluK5 subunit. NeCaB1-GFP interaction with GluK5 C-terminal is lower in the presence of $Ca^{2+}$. C, Quantification of pull-down assays, where paired experiments are linked with a line. Data are mean + SEM. *p < 0.05, **p < 0.01, ***p < 0.005.

## Figure 2. NECAB1 and GluK5 interact *"In Vivo"*

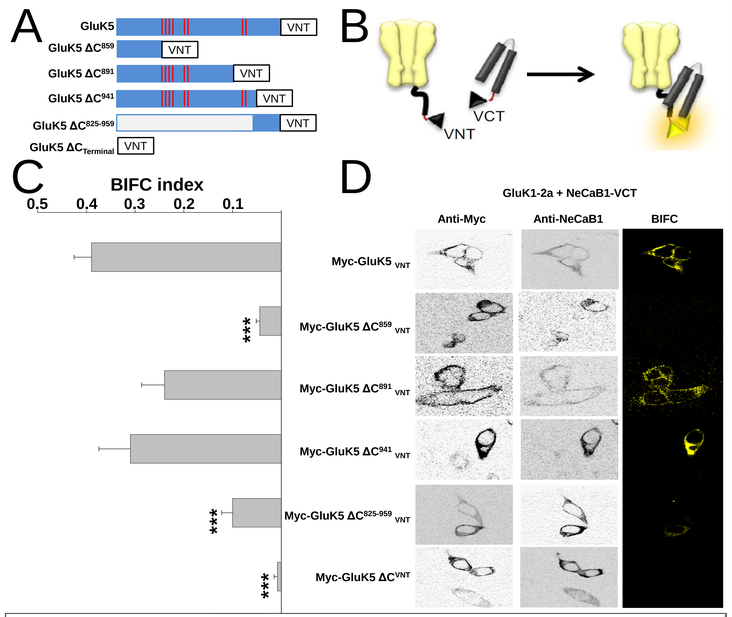


**Figure 2. *"In vivo"* interaction between NeCaB1 and GluK5.** A, schematic structure of different deletion constructs of the C-terminal domain of GluK5 subunit fused to Venus N-terminal (VNT) domain. In B, Bimolecular Fluorescence complementation (BiFC) technique diagram. C, quantification of BiFC index (BiFC fluorescence / (Anti-Myc + Anti-NeCaB1) fluorescence). In D, key illustrations of HEK cells expressing GluK1 and different GluK5 constructs where their interaction with NeCaB1-VCT yields yellow fluorescence. Data are mean + SEM. ***p < 0.005.

## Figure 3. NeCaB1 increases GluK5 expression at the membrane in a $Ca^{2+}$ dependent manner

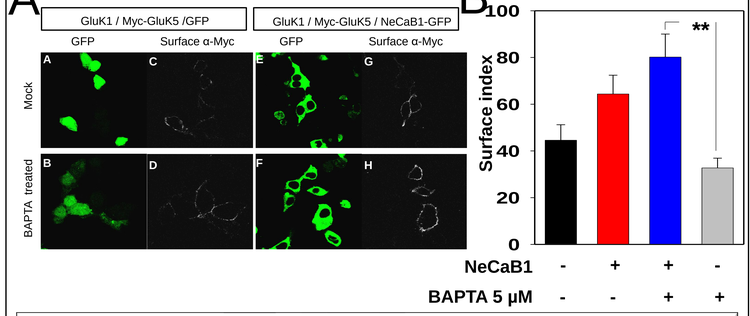


**Figure 3. Increased GluK5 expression at the plasma membrane by NeCaB1 action.** In A, HEK cells expressing GluK1/5 KARs were tested for GluK5 membrane expression upon coexpression of GFP or NeCaB1-GFP under normal or low $Ca^{2+}$ (BAPTA treated). In B, quantification of surface expression of GluK5 subunit KARs. Data are mean + SEM. p **< 0.01.

## Figure 4. NeCaB1 increases both the the fraction of GluK5 containing KARs at the membrane and their affinity for glutamate under low $Ca^{2+}$

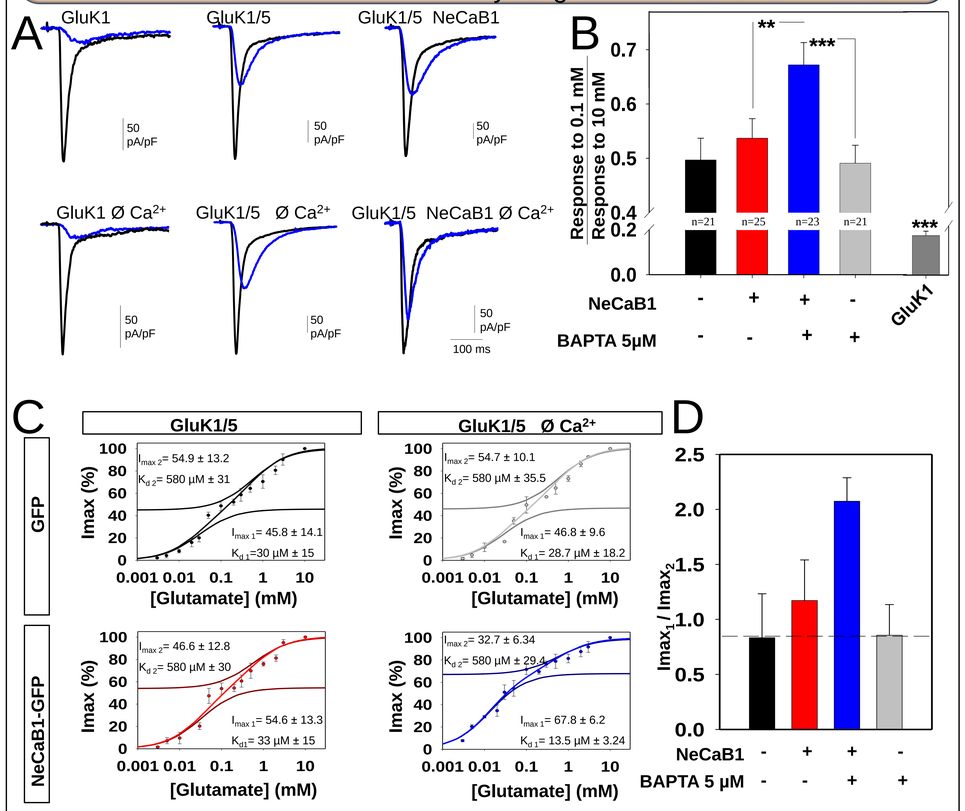


**Figure 4. NeCaB1 increases GluK5 containing KARs glutamate affinity in low Calcium environment.** In A, traces evoked by 10 mM (black traces) and 0.1 mM (blue traces) of glutamate in HEK cells transfected with different KARs: GluK1, GluK1/5 & GFP and GluK1/5 & NeCaB1-GFP in normal or low $Ca^{2+}$ environment (BAPTA treated). In B, quantification of panel A. C, dose-response curves for GluK1/5 KARs in cells coexpressing GFP or NeCaB1-GFP in normal and low $Ca^{2+}$ environment. Data were fitted to the sum of two logistic equation [$F_{(X)} = I_{max}*X/(K_d+X)$]. In D, the ratio between the asymptotic values of both equations. This indicates the prevalence of a higher affinity population in GluK1/5 & NeCaB1-GFP under low $Ca^{2+}$. Data are mean + SEM. **p < 0.01, ***p < 0.005.

## Concluding remarks

- NeCaB1 interacts with GluK5 subunit of Kainate Receptors.
- Interaction between NeCaB1 and GluK5 is disfavored by $Ca^{2+}$.
- NeCaB1 promotes GluK5 containing Kainate receptors to the cell surface and increases their affinity