

NeCaB1 modulates trafficking and affinity of GluK5 containing Kainate receptors.

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Fast excitatory synaptic transmission is mainly mediated by glutamate receptors in the Central Nervous System. This family of receptors comprises three different members: AMPA, 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. Hence, our lab used a yeast two-hybrid screening to identify possible partners of GluK5 subunits by using its C-terminal domain (CTD) as bait. Consequently, 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-immunoprecipitation in HEK cells and in pull-down assay. Moreover, 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" and served to narrow down the interacting segment.

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 GluK5 containing KARs in the cell surface when internal Ca^{2+} was reduced to a minimum. In addition, we observed that NeCaB1 increases the density of membrane GluK5 containing receptors at low Ca^{2+} levels. Unexpectedly, NeCaB1 was also found to increase the affinity of GluK5 containing KARs, either in combination with GluK1 or GluK2, in a Ca^{2+} dependent manner.

Altogether, these data demonstrate that NeCaB1 binds to CTD of GluK5 subunit containing KARs promoting their trafficking and increasing their affinity depending on environmental Ca^{2+} , indicating that NeCaB1 could dynamically determine the kind of KARs at synapses according to synaptic activity, constituting a kind of homeostatic plasticity.

NeCaB1 interacts with GluK5 subunit of KARs in a Ca^{2+} dependent manner

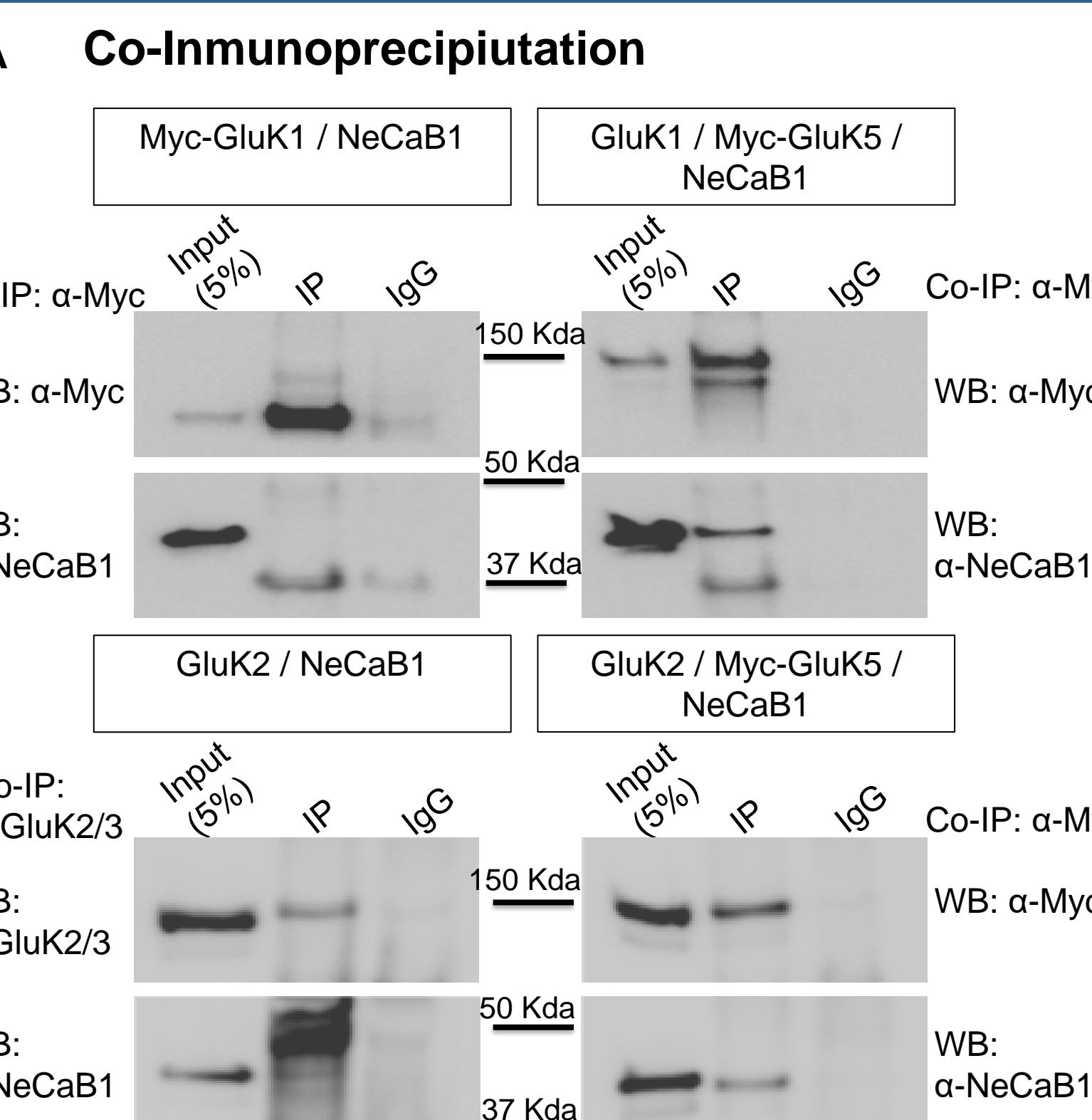


Figure 1. NeCaB1 protein interacts with GluK5 subunit containing KARs. A, validation of NeCaB1 & GluK5 interaction by Co-Immunoprecipitation. HEK cells expressing GluK1 or GluK2 and the heteromeric forms with GluK5 subunit plus NeCaB1 were used to co-immunoprecipitate NeCaB1 protein. Different epitope tag to KARs subunit were used for Co-immunoprecipitation in each case. B, Bimolecular Fluorescence complementation (BiFC) technique diagram. Key illustrations of HEK cells expressing GluK1 and GluK5-VNT or its form without C-terminal constructs is presented where their interaction with NeCaB1-VCT yields yellow fluorescence. For quantification of BiFC index (BiFC fluorescence / (Anti-Myc + Anti-NeCaB1) fluorescence) was taken. In C, 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+} . For quantification of pull-down assays, paired experiments were linked with a line. Data are mean + SEM. *p < 0.05, **p < 0.01, ***p < 0.005.

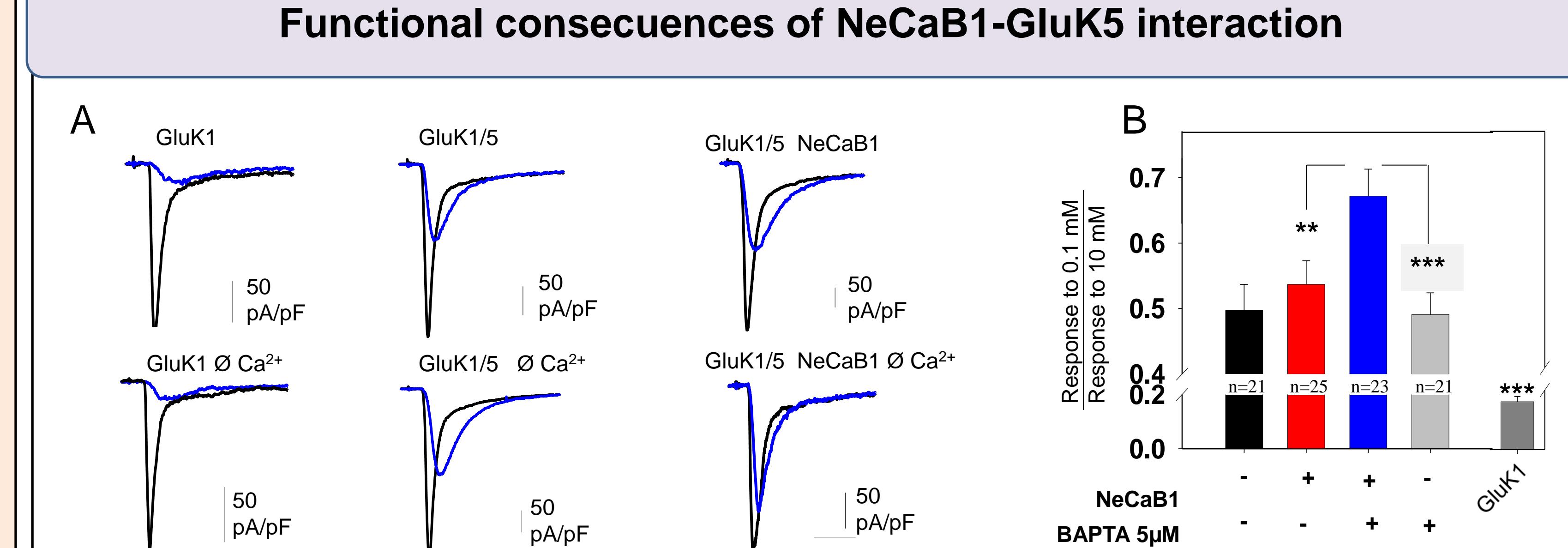
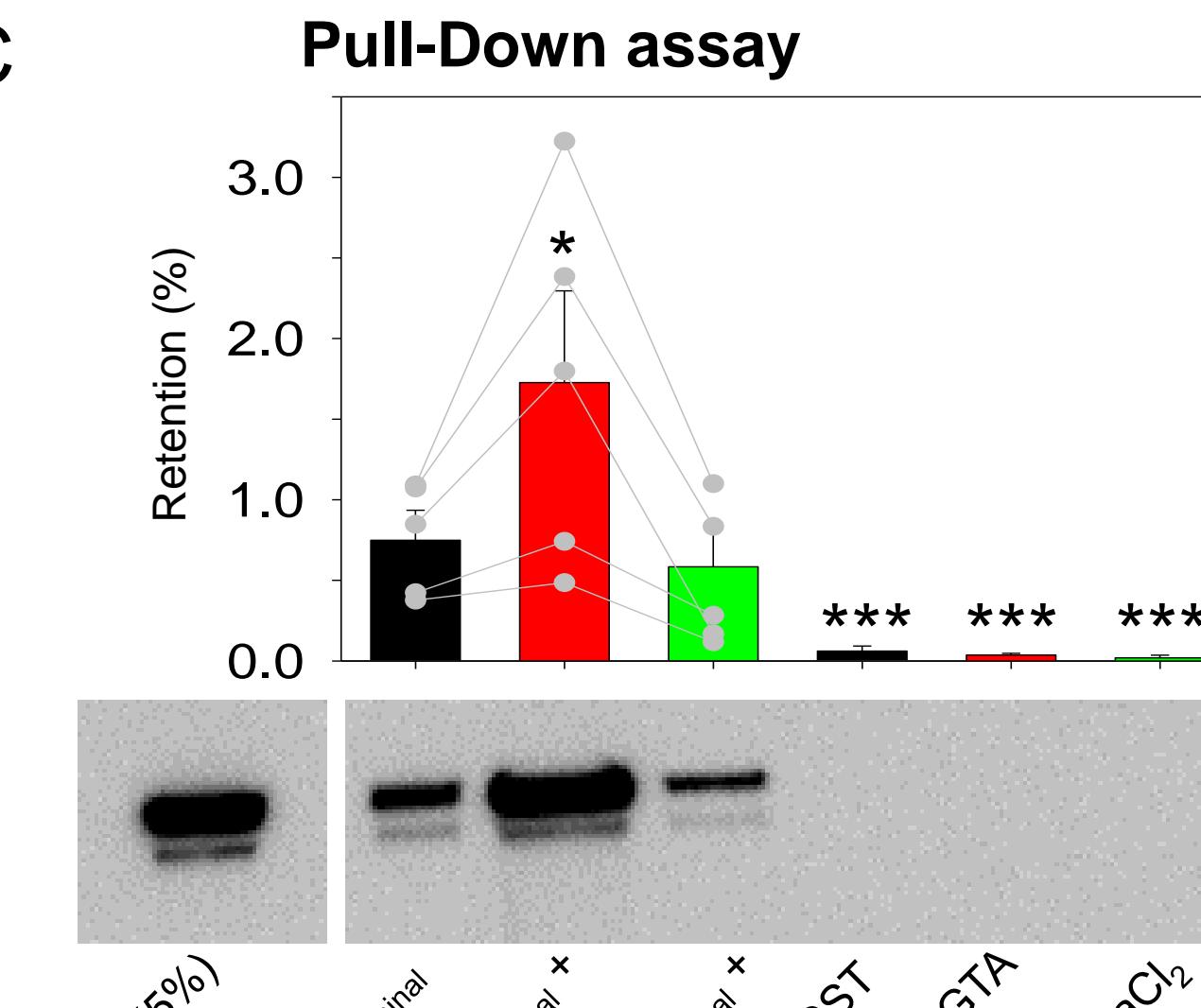


Figure 2. Effect of NeCaB1 on GluK5 containing KARs in normal and low Calcium. In A, responses evoked by 10 mM (black traces) and 0.1 mM (blue traces) of glutamate in HEK cells transfected with different KARs: GluK1, GluK1/5 & GFP and GluK1/5 & NeCaB1-GFP in normal or low Ca^{2+} (BAPTA treated). In B, Ratio of both responses obtained under conditions described in A.

NeCaB1 increases membrane delivery of GluK5 containing KARs

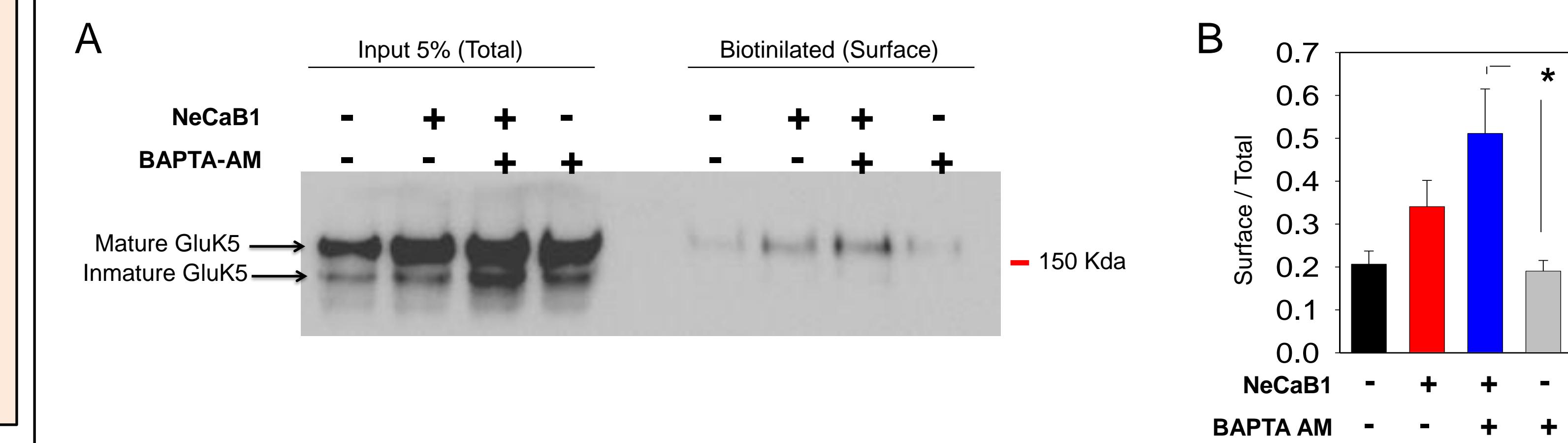


Figure 3. NeCaB1 increases GluK5 containing KARs at the cellular surface in low Calcium. A, biotinylation experiment of HEK cells transfected with GluK1/5 and GFP or NeCaB1-GFP in a normal or low Ca^{2+} . Input (5%) represents 5% of total protein and biotynilated proteins are the fraction in the plasma membrane. The Western-Blot membrane was blotted against myc antibody. In B, quantification of panel A.

NeCaB1 increases affinity of GluK1/5 KARs

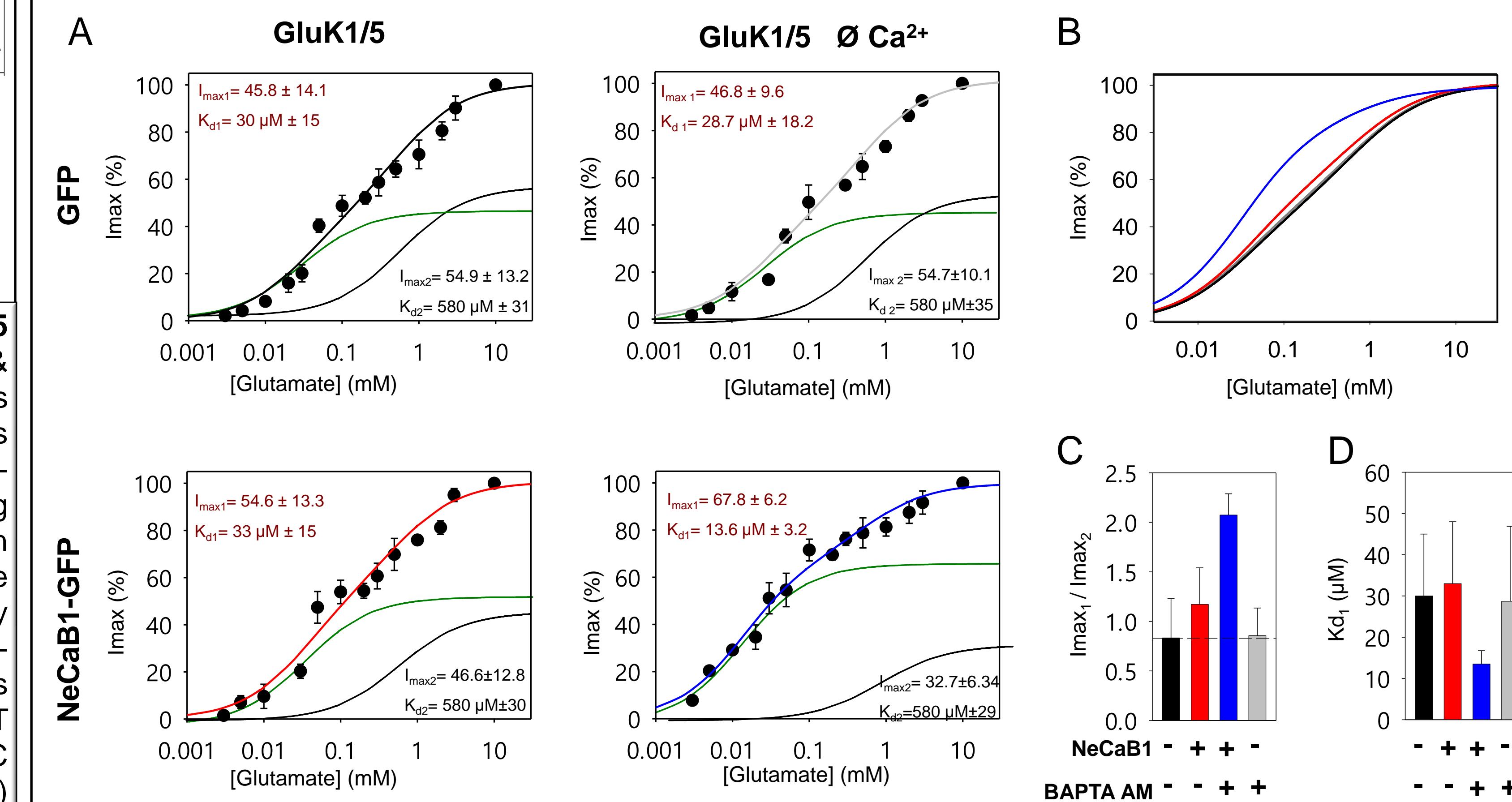


Figure 4. NeCaB1 increases the affinity of GluK5 containing KARs under low Ca^{2+} conditions. A, dose-response curves for GluK1/5 KARs in cells coexpressing GFP or NeCaB1-GFP in normal and low Ca^{2+} . Data were fitted to the sum of two logistic equations [$F(x) = I_{\max} \cdot X / (K_d + X)$]. B, superposition of resulting fits for each condition. C, the ratio between the asymptotic values (I_{\max}) for both fitted equations are presented. This indicates the prevalence of a higher affinity population in GluK1/5 & NeCaB1-GFP under low Ca^{2+} . D, comparison of the K_d values for each condition. Data are mean + SEM. **p < 0.01, ***p < 0.005.

NeCaB1 increases trafficking and affinity of GluK2/5 heteromeric KARs

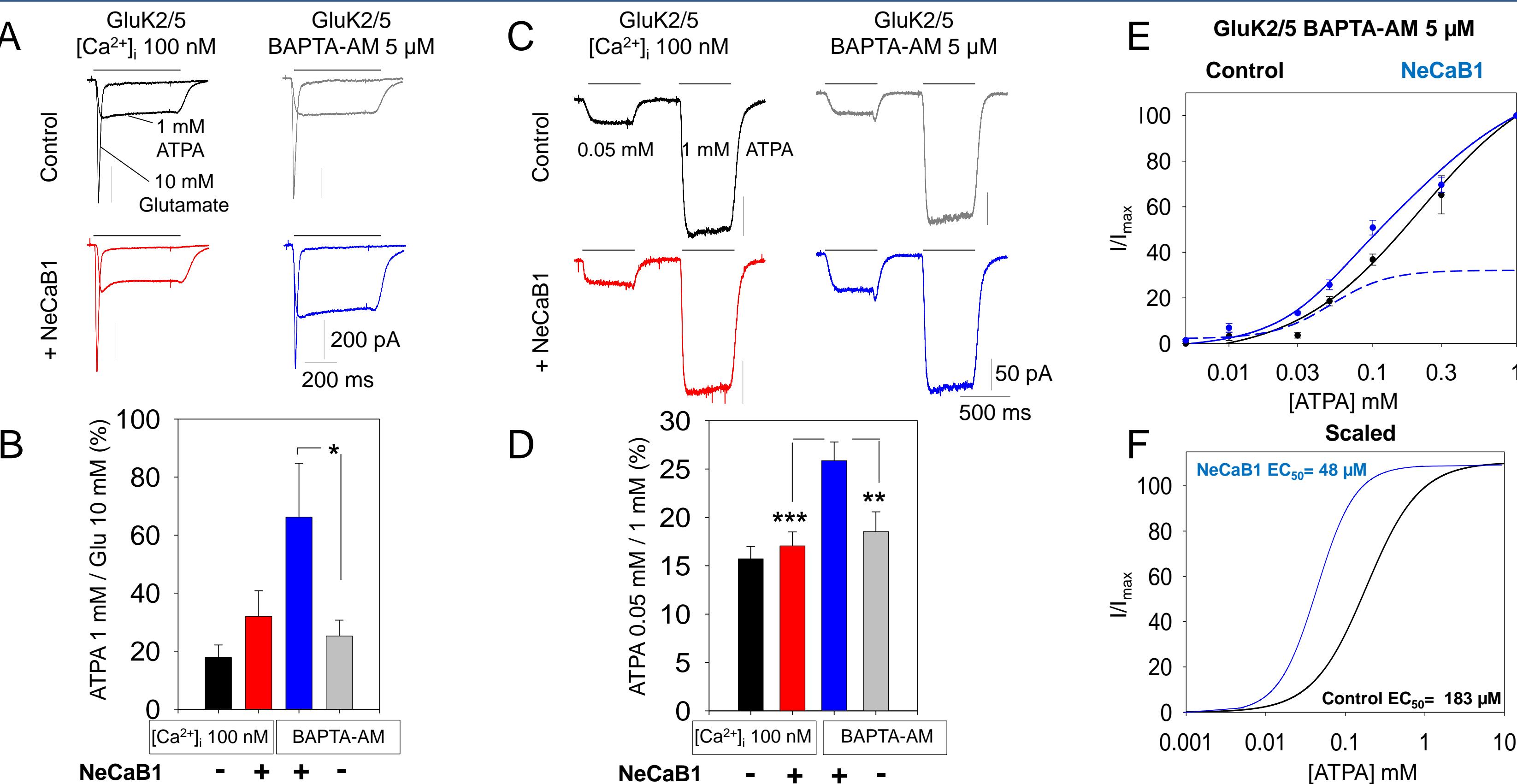


Figure 5. NeCaB1 increases number and affinity of GluK2/5 KARs at low Ca^{2+} concentration. A, responses from HEK cells expressing GluK2/5 and GFP or NeCaB1-GFP were evoked by either glutamate 10 mM or ATPA 1mM to exclusively measure heteromeric receptors, in normal or low Ca^{2+} . B, quantification of panel A. C, responses from HEK cells transfected like in panel A evoked by different concentrations of ATPA to measure relative affinity between 0.05 mM and 1 mM, in normal or low Ca^{2+} environment. D, quantification of experiments shown in panel C. E, a dose-response curve is presented for cells expressing GluK2/5 plus GFP or NeCaB1-GFP in low Ca^{2+} . NeCaB1 curve (blue line) was better fitted by adding a second logistic equation (dashed blue) to control fitted curve (black line). F, control fitted curve and the second fitted curve once scaled. Data are mean + SEM. *p < 0.05, **p < 0.01 and ***p < 0.005.

NeCaB1 dynamically changes KARs affinity in a rapid manner

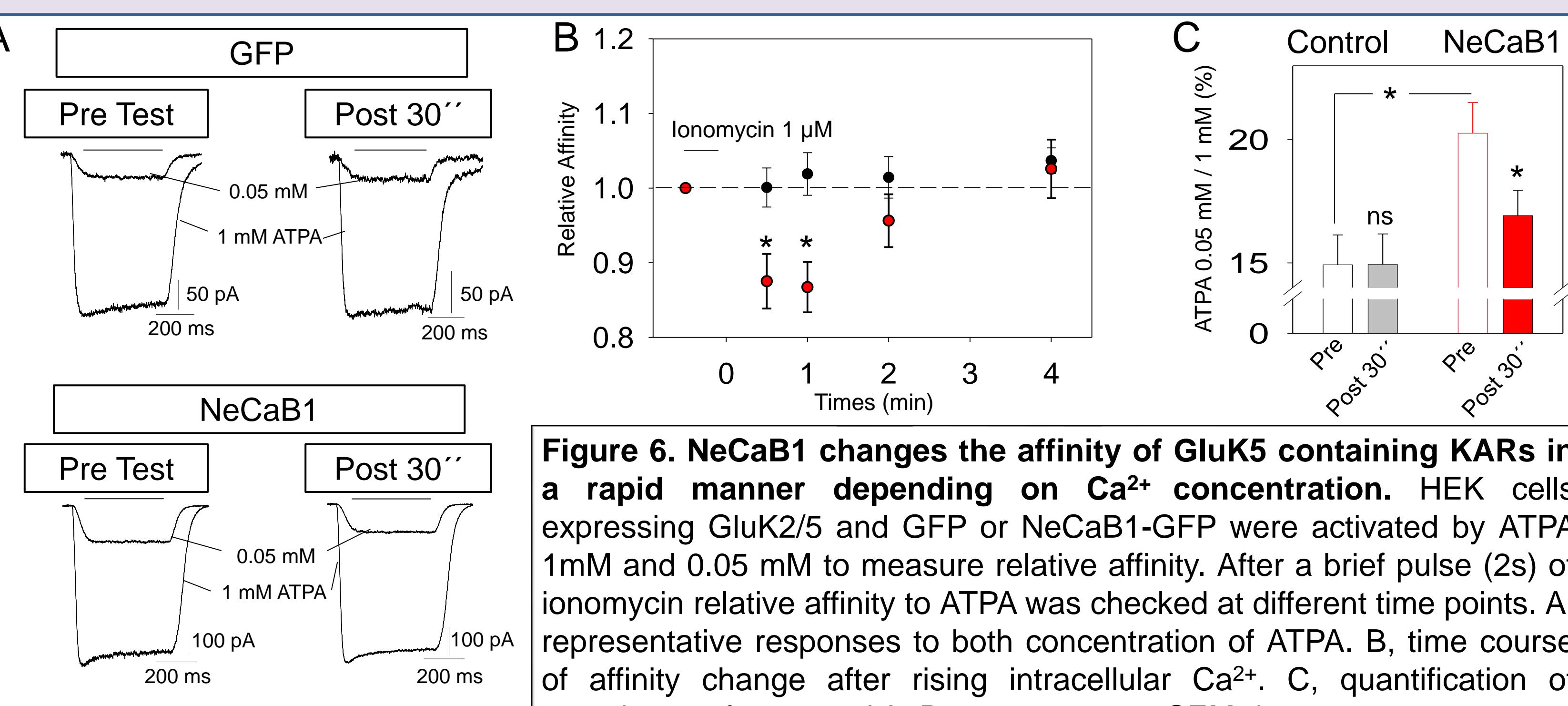


Figure 6. NeCaB1 changes the affinity of GluK5 containing KARs in a rapid manner depending on Ca^{2+} concentration. HEK cells expressing GluK2/5 and GFP or NeCaB1-GFP were activated by ATPA 1mM and 0.05 mM to measure relative affinity. After a brief pulse (2s) of ionomycin relative affinity to ATPA was checked at different time points. A, representative responses to both concentration of ATPA. B, time course of affinity change after rising intracellular Ca^{2+} . C, quantification of experiments from panel A. Data are mean + SEM. *p < 0.05.

