Cell Reports, Volume *29*

Supplemental Information

Synaptic Kalirin-7 and Trio Interactomes Reveal a GEF Protein-Dependent Neuroligin-1 Mechanism of Action

Jeremiah D. Paskus, Chen Tian, Erin Fingleton, Christine Shen, Xiaobing Chen, Yan Li, Samuel A. Myers, John D. Badger II, Michael A. Bemben, Bruce E. Herring, and Katherine W. Roche

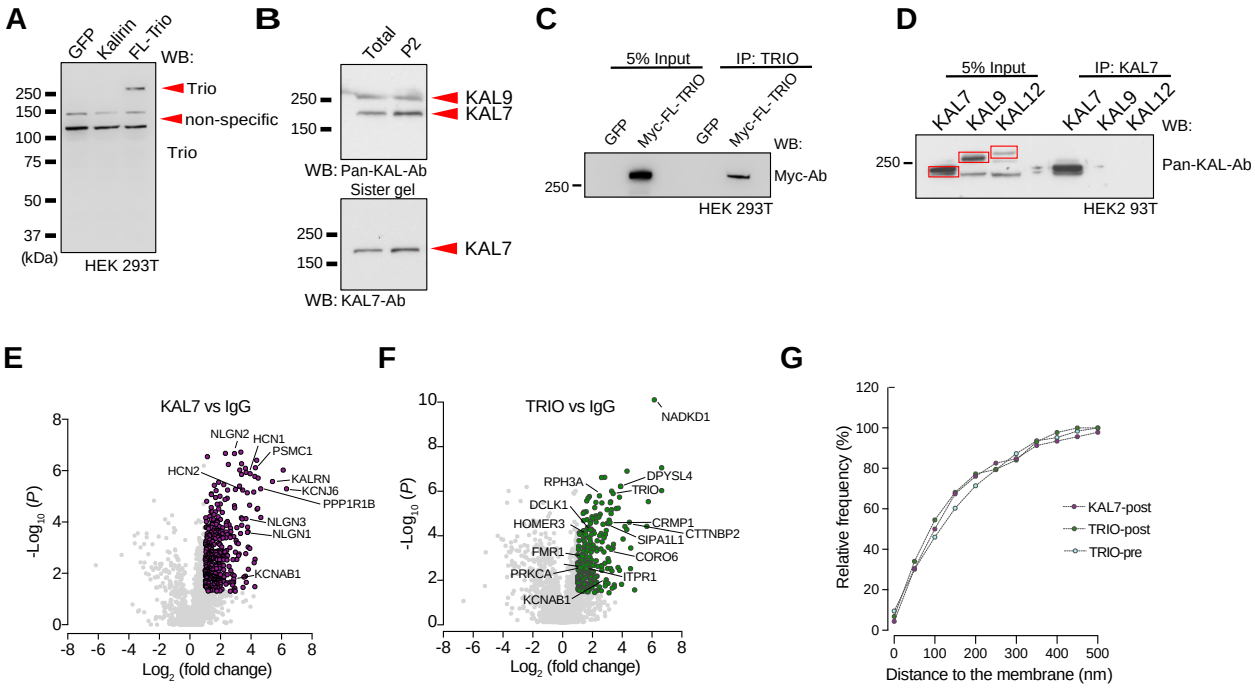

Figure S1. Related to Figure 1 - Kalirin-7 and Trio antibody verification, proteomics, and subcellular localization (A) Immunoblot of lysates from HEK293T cells transfected with GFP, Kalirin-12, or full-length human Trio. Trio antibody generated against an unstructured SH3-linking region (KKLAHKHKKSREV) detects full-length Trio, but not Kalirin-12. Nonspecific bands are detected between 100-150 kDa. (B) Immunoblot analysis of homogenate (total) and P2 brain lysate with pan-Kalirin and Kalirin-7 antibodies, showing Kalirin-7 antibody specificity. (C) Immunoprecipitation of Trio from HEK293T cells expressing Trio. (D) Immunoprecipitation of Kalirin-7 from HEK293T cells expressing Kalirin-7, Kalirin-9, or Kalirin-12, showing enrichment specificity for Kalirin-7. Red boxes in the input lanes delineate Kalirin isoforms from non-specific bands. (E) Volcano plot depicting protein enrichment of Kalirin-7 referenced to IgG. (F) Volcano plot depicting protein enrichment of Trio referenced to IgG. Exact p-values and abundance ratios for E and F are in Supplemental Table 1 and 2. (G) Overlaid accumulative distributions of Trio and Kalirin-7 from EM showing identical distributions ($p>0.99$, Kruskal-Wallis test).

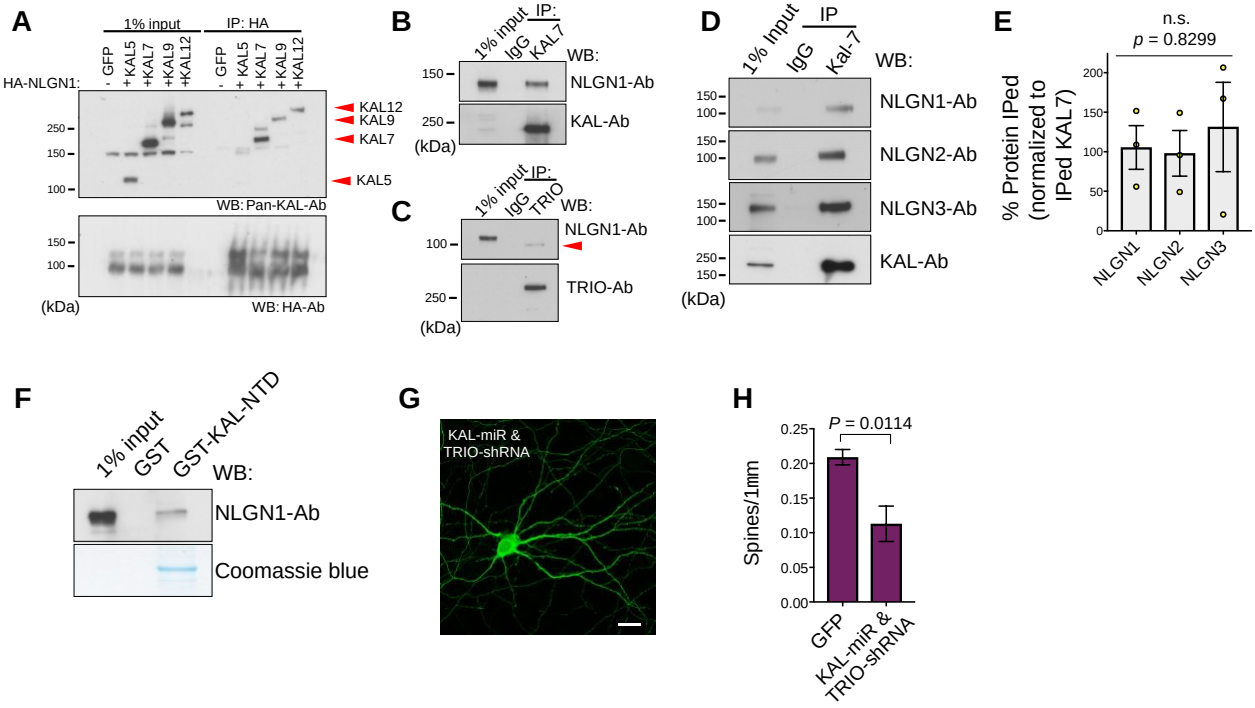

Figure S2. Related to Figure 2 and 3 – Kalirin-7 associates with NLGN1-3. (A) Immunoblot analysis showing co-immunoprecipitation of HA-NLGN1 with Kalirin-5, Kalirin-7, Kalirin-9, and Kalirin-12 in HEK293T cells. Kalirin-5 does not interact with NLGN1, however, Kalirin-7, -9, and -12 do. (B) Immunoblot analysis showing co-immunoprecipitation of endogenous Kalirin-7 with NLGN1 in rat cortical neurons. (C) Immunoblot analysis showing co-immunoprecipitation of endogenous Trio with NLGN1 in rat cortical neurons. Red arrow indicates non-specific band. (D) Immunoblot analysis showing co-immunoprecipitation of endogenous Kalirin-7 with NLGN1-3 from the synaptic fraction of adult rat brain. (E) Quantification of D. No significant difference in interaction strength between NLGN isoforms (one-way ANOVA, p=0.8299). (F) Pull-down from synaptic fraction brain lysate using GST-sec14p fusion protein (Kalirin-NTD) as bait. (G) Image of cultured hippocampal neurons transfected at DIV 5 with KAL-miR & TRIO-shRNA. Cells were stained for GFP and HA and imaged at DIV 12-14. Scale bar is 20 μm. GFP image is found in Figure 3A. (H) Quantification of G is for all observations (Mann-Whitney U test).

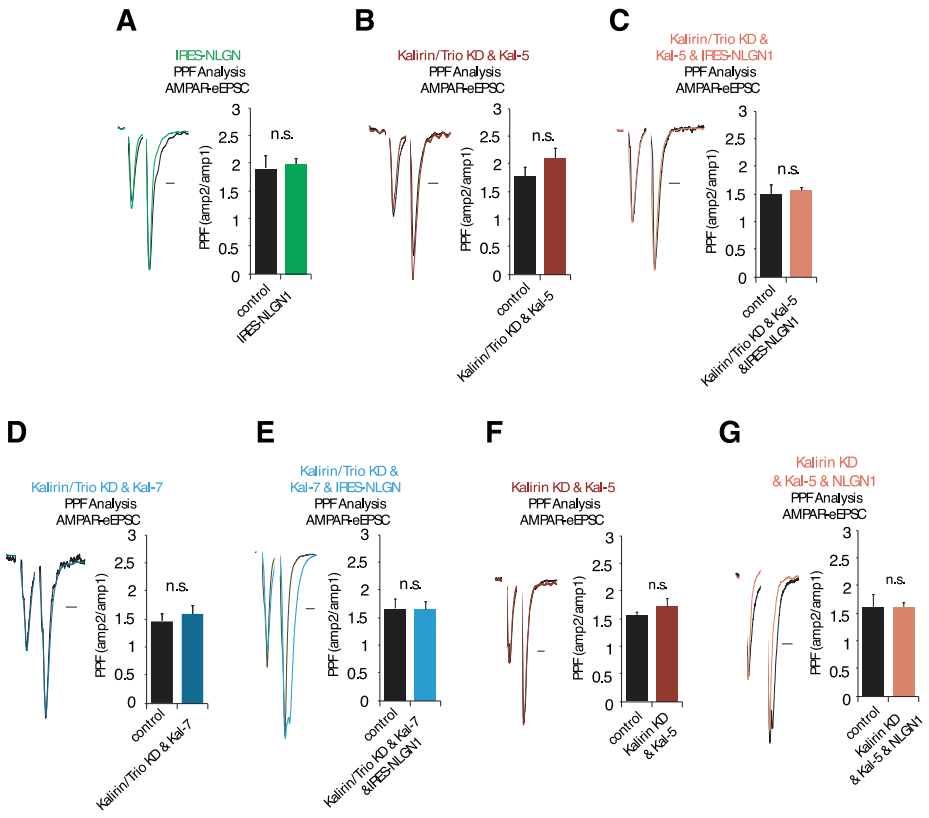

Figure S3. Related to Figure 4 - Postsynaptic manipulation of Kalirin, Trio and Neuroligin-1 function does not affect presynaptic release probability. Paired-pulse facilitation (PPF) second EPSC over first EPSC for consecutive stimuli separated by 40 ms. Example traces are normalized at first EPSC for A-E, No significant change in PPR was observed following (A) NLGN1 overexpression (n=5; p>0.05), (B) Kalirin/Trio KD & Kal-5 (n=6; p>0.05), (C) Kalirin/Trio KD & Kal-5 & NLGN1 (n=5; p>0.05), (D) Kalirin/Trio KD & Kal-7 (n=6; p>0.05), (E) Kalirin/Trio KD & Kal-7 & NLGN1 (n=6; p>0.05), (F) Kalirin KD & Kal-5 (n=5; p>0.05) or (G) Kalirin KD & Kal-5 & NLGN1 (n=5; p>0.05).