Supporting Information

Mikhaylova et al. 10.1073/pnas.0903001106

SI Text

cDNA Constructs. Expression vectors for calneuron-1 and calneuron-2 were pEGFP-C1, pEGFP-N1, pcDNA3.1, pTrcHis2B, pMAL-c2x, and pET-SUMO for calneuron-1 and pEGFP-N1 and pET21a for caldendrin and NCS-1. The GST–PI-4Kβ construct was obtained from T. Balla. mCherry-synaptophysin was a kind gift from A. Fejtova. shRNA constructs against calneuron-1 in pRS-GFP and scrambled controls were generated by Origene (ams Biotechnology). After RNAi construct validation [by immunostaining and Western blot (WB)] the most efficient sequence (5′-GCA-GCC-AAC-CAG-ATC-CTG-CGG-AGC-GGC-AT-3′) and corresponding scrambled control was selected for further experiments.

Antibodies. Antibodies used were: anticalneuron-1 [rabbit, dilutions for immunoblotting (IB) 1:500; immunofluorescence (IF) 1:100] and anticalneuron-2 (rabbit, dilutions for IB 1:500, IF 1:100) generated against full-length proteins (1); mouse polyclonal antibody against calneuron-1 (Abnova; IB: 1:1,000, IF 1:500); calneuron-2/CABP7 MaxPab polyclonal antibody (Abnova; IB: 1:1,000); anticaldendrin raised in guinea pig (IF 1:200) using a GST-Caldendrin fusion protein as antigen. Specificity of these antibodies was proved by various means (blocking experiments with fusion protein, coImmunoprecipitation etc.). Other antibodies used were: mouse monoclonal anti-PI-4Kß and anti-SNAP25 (BD Transduction Laboratories; IB 1:5,000, IF 1:500 and IF 1:100, respectively); rabbit antisyntaxin 6 (Synaptic Systems; IB 1:1,000, IF 1:500); rabbit anti-NCS-1 (Santa Cruz Biotechnology; IB 1:1,000, IF 1:100); monoclonal anti-TGN38 (Novus Biologicals; IF 1:500); rabbit polyclonal anticalreticulin (Upstate Biotechnology; IF 1:200); rabbit monoclonal to GM130 (Abcam; IF 1:500); mouse monoclonal anti-GFP (Babco/Covance; IB 1:5,000), antisynaptophysin (Stressgene; IF 1:200); mouse monoclonal anti-β-COP (IF 1:200) and mouse monoclonal anti-ß-Actin (WB 1:4,000) (Sigma); anti-mouse and anti-guinea pig HRP-linked secondary antibodies (DakoCytomation; IB 1:5,000; anti-rabbit IgG-HRP-linked antibodies (NEB; IB 1:5,000); anti-mouse and anti-rabbit Alexa Fluor 488 and Alexa Fluor 568 (Molecular Probes; IF 1:1,000); and Cy5-conjugated AffiniPure goat anti-mouse and anti-rabbit IgG (Dianova; IF 1:1,000).

Protein Purification and Pull-Down Assay. PI-4Kβ-GST or GST were expressed in BL21 (DE3) bacteria (6 h after induction with 0.5 mM IPTG) and purified from the soluble fraction by glutathione agarose chromatography. Columns with Sepharose-bound GST-PI-4Kβ or GST were extensively washed with Ca^{2+}-free (Chelex100 treated) TBS buffer, and aliquots of these beads were used for pull-down assays. For PI-4Kβ activity assays the enzyme was cleaved from the GST tag with thrombin. Untagged caldendrin, calneuron-1, and NCS-1 were purified after expression with pET21 vectors (for NCS-1 and caldendrin) and pTrcHis2B (for calneuron-1) using hydrophobic columns as described (2). His-SUMO-calneuron-1 was used for competition pull-down assays and the surface plasmon resonance studies. The protein was induced for 4 h at 37 °C and then purified by using ProBond resin (Invitrogen) according to the manufacturer. For the pull-down assay calneuron-1-GFP, calneuron-2-GFP, or GFP heterologously overexpressed in COS-7 cells was extracted with lysis buffer in the presence of 2 mM $CaCl_2$ and 1 mM $MgCl_2$ (Ca^{2+} buffer) or 2 mM EGTA and 1 mM $MgCl_2$ (Ca^{2+}-free buffer). Ten micrograms of GST-PI-4Kβ or GST immobilized on Sepharose beads was washed with the corresponding buffers and incubated overnight at 4 °C with 500 μL of the COS-7 cell extract. After extensive washing with the corresponding buffers protein complexes were eluted with 30 μL of 2×SDS sample buffer and processed for IB. For the competition pull-down 2 μg of GST-PI-4Kβ or 0.5 μg of GST bound to the beads was washed with TBS buffer containing 1 mM $MgCl_2$, 2 mM DTT, and different concentrations of $CaCl_2$: 0 (2 mM EGTA), 0.2, 0.5, and 1 μM. Then equimolar amounts of His-SUMO-calneuron-1 and NCS-1 (0.6 and 0.5 μg) were diluted in corresponding buffers and added to the beads. After incubation for 1 h at the room temperature beads were extensively washed, eluted with 20 μL of 2× SDS sample buffer, and checked by IB with rabbit calneuron-1 or NCS-1 antibody. The same amounts of calneuron-1 or NCS-1 (0.6 and 0.5 μg) were used for the input. The competition experiment was done 5 times and then immunoblots were analyzed. The amounts of NCS-1 bound to GST control at each buffer condition were subtracted from GST-PI-4Kβ pull-down. The conditions with a maximal binding of NCS-1 to GST-PI-4Kβ were taken as 100% and deviations were calculated for each condition.

Subcellular Fractionation, Gel Filtration, and Immunoblotting. Subcellular fractionation was performed as described (3). Microsomal fractions obtained during subcellular fractionation were further extracted with lysis buffer [10 mM Tris·HCl, 150 mM NaCl, 1% Triton X-100, 2 mM DTT, protease inhibitor mixture Complete (Roche), pH 7.5]. Preparations were done at low Ca^{2+} concentrations with 2 mM EDTA or at high Ca^{2+} concentrations with 2 mM Ca^{2+} in the microsomal preparation, which were separated on a gel filtration FPLC column (Amersham Biosciences/GE Healthcare). Fractions 20–80 were precipitated with acetone overnight at −20 °C, washed with ice-cold 70% ethanol, and lyophilized. Dry pellets were solubilized in 50 μL of SDS/PAGE loading buffer and analyzed by IB.

Surface Plasmon Resonance Analysis. Binding studies for calneuron-1, PI-4Kβ, and myr-NCS-1 were carried out by using the Biacore 2000 instrument and sensor chip CM5 at 25 °C. His-SUMO-calneuron-1 was coupled to the carboxymethylated dextran matrix of a sensor chip cell according to the manufacturer;s instructions. After equilibrating the sensor chip with flow buffer HBS-P [10 mM Hepes (pH 7.4), 150 mM NaCl, 0.005% Surfactant P20] at a flow rate of 5 μL/min, the Dextran matrix was activated with a 7-min pulse of 50 mM *N*-hydroxysuccinimide/200 mM *N*-ethyl-*N*′-(dimethylaminopropyl)- carbodiimide at a flow rate of 5 μL/min. Subsequently, his-SUMO-calneuron-I was immobilized at the surface of the sensor chip cell by injecting a 7-min pulse of ligand solution (20 μg/mL of His-SUMO-calneuron-1 in 10 mM sodium acetate, pH 4.5). Finally, the excess of reactive groups on the chip surface was deactivated with a 7-min pulse of 1 M ethanolamine hydrochloride, pH 8.5, at a flow rate of 5 μL/min. For binding studies including GST-PI-4Kβ (or GST control) and NCS-1 the purified proteins were diluted at the indicated concentrations in the continuous flow buffer HBS-P containing various Ca^{2+} and/or Mg^{2+} concentrations. Each analytic run was performed at 20 μL/min flow rate under the following conditions: 1 min equilibration of the chip with the indicated analysis buffer. Afterward the analyte was injected in a 3-min pulse (association time) followed by a 3-min pulse with analysis buffer alone (dissociation time). Individual runs were finished with the regeneration of the chip

matrix using a 2-min pulse of 50 mM NaOH at a flow rate of 5 μL/min and a terminating 5-min pulse with flow buffer to equilibrate the chip surface again. For the final graph the binding of GST control alone with NCS-1 was subtracted from the corresponding GST-PI-4Kβ values. For estimation of binding of NCS-1 to GST-PI-4Kβ in presence or absence of Mg^{2+} and different Ca^{2+} concentrations NCS-1 was directly coupled to the sensor chip and GST-PI-4Kβ was injected as described above. The molar binding activities were calculated based on the equation from ref. 4.

Steady-State Fluorescence Studies and ITC. Intrinsic fluorescence spectra were recorded on a Hitachi F-4500 fluorescence spectrophotometer. Trp fluorescence was recorded between 300 and 450 nm with the excitation wavelength set at 295 nm. Ca^{2+} and Mg^{2+} binding to calneurons and NCS-1 was evaluated by a Microcal VP-ITC instrument as described (5, 6).

PI-4Kβ Activity Assay. The assay was carried out in a final volume of 120 μL containing 50 mM Tris (pH 7.5), 20 mM $MgCl_2$, 1 mM EGTA, 0.5 mg/mL BSA, 80 μg of substrate (PI) in micelles containing 0.4% Triton X-100 and ATP (mixture of 500 μM cold ATP and 2μCi ATP-P^{32}). Reactions were carried out in Ca^{2+}-free conditions (2 mM EGTA) or in the presence of 2 mM Ca^{2+} at room temperature. Reactions were initiated by adding the ATP mixture. The reaction was terminated after 30 min by adding 3 mL of chloroform/methanol/0.6 M HCl (200:100:0.75 vol/vol%). The labeled phospholipids were extracted with 1.5 mL of chloroform/methanol/0.6 M HCl (3:48:47, vol/vol/vol%). The organic phase was transferred into a scintillation vial. After evaporation, 5 mL of the scintillation fluid containing 1.3 mM POPOP and 22 mM PPO in Toluene were added and radioactivity was measured by liquid scintillation counter. A blank value was subtracted and data are represented as the percentage of basal kinase activity (100%) with or without Ca^{2+}.

PIP Assay. The PIP assay was carried out with a PI(4)P mass strip kit (MoBiTec) in Cos-7 cells. Flasks (75 cm^2) were transfected and after 48 h cells were harvested with cold 1× TBS buffer, homogenized, and centrifuged at 1,000 × *g* for 10 min at 4 °C. The pellet faction was resuspended in 150 μL of TBS and 5 μL was taken for the IB with anti-β-actin (loading control) and antisyntaxin 6 (Golgi marker control). The remaining pellet was used for PI(4)P extraction according to the protocol from the manufacturer. Briefly, the pellet was washed twice with cold 5% TCA/1 mM EDTA buffer by centrifugation and then neutral lipids were extracted by MeOH/$CHCl_3$ (2:1). The obtained pellet was homogenized in $CHCl_3$/MeOH/12 M HCl (40:80:1) for the acetic lipid extraction. The supernatant was collected, then organic and aqueous phases were separated by adding $CHCl_3$ and 0.1 M HCl. The organic phase was transferred into a fresh tube and lyophylized. Dry lipids were reconstituted with 10 μL of $CHCl_3$/MeOH/H_2O (1:2:0,8) and 2 μL was spotted onto a PI(4) strip. Strips were dried, blocked with 3% BSA and incubated with PI(4)P detector solution for 1 h at room temperature. After extensive wash with 1× PBS the strip was incubated for 45 min with secondary detector solution, washed again, and incubated with tertiary detector solution for another 45 min. After the final wash the signals on the membrane were detected by chemiluminescence. Films were scanned, and the optical density of the spots was analyzed by ImageJ.

FRAP and Life Imaging Experiments. FRAP experiments were carried out by using a confocal laser scanning microscope (Leica M IRE2) equipped with a krypton-argon-ion laser (488/568/647 nm) and an acousto-optic-tunable filter for selection and intensity adaptation of laser lines. DIV 5–7cortical neurons 24 h after transfection with mCherry-synaptophysin and GFP or calneuron-1-GFP and 72 h after for the calneuron-1 shRNA were transferred into the recording chamber with ACSF (125 mM NaCl, 2.5 mM KCl, 2 mM $MgSO_4$, 2 mM Ca^{2+}, 10 mM glucose, 30 mM Hepes, pH 7.3) at 37 °C. A 488-nm laser line was used to monitor the GFP channel and a 568-nm line was used for both imaging of mCherry and photobleaching. A 63× oil objective and 2× zoom were used. Pictures were taken every 5 min as a *z*-stack (300-nm *z*-step) with resolution 524 × 524 pixels. The first 5 images were recorded before the bleaching to establish a baseline and the stabilizing of the coverslip in the recording chamber. Then, the first 15- to 20-μm segment of the longest neurite (axon) was bleached with the maximal laser power for 5–8 min (12× zoom) because of the high photo-stability of mCherry, followed by 1 h of postbleach recordings at the same conditions as for the baseline. After the bleaching, 100 μM ATP was added for 3 min to increase the intracellular Ca^{2+} and stimulate the Golgi trafficking in the case of calneuron-1-GFP or GFP transfection. For the BAPTA experiments cells were preincubated with 10 μM of BAPTA-AM for 1 h at 37 °C in serum free medium. Images were analyzed with ImageJ (National Institutes of Health). *Z*-stacks were processed as maximal projections, and a 10-μm initial segment of the bleached neurite was selected as the region of interest (ROI). In each experiment, the ROI was applied to the all time points and the mean gray value (represented as 255 different gray values) was calculated. Data were normalized on the photo-bleaching during the image acquisition by introducing the "bleaching coefficient" (unrelated area with the background fluorescence was monitored during the experiment after the neurite was specifically bleached, initial fluorescence intensity was taken as 1 and each change of the intensity was calculated as the proportion). For the evaluation of percentage of FRAP the initial fluorescence was taken as 100% and data were graphically plotted. The differences between fluorescence intensity after bleaching at time point 0 min and time point 60 min were taken as percentage of recovery.

For the line analysis of vesicle trafficking the same groups as described above were studied. For the BAPTA-AM experiments cells were preincubated for 1 h at 37 °C. Coverslips were transferred to an experimental chamber on the stage of an upright microscope (Zeiss Axioscope FS). Cells were continuously superfused with Hepes-buffered ACSF at 35 °C. mCherry fluorescence was excited at 575 nm. Excitation light was directed onto the specimen via a 60× objective and a 580-nm dichroic. Emission was filtered with a 590-nm long-pass filter. Images were acquired every 30 s (exposure times 200–500 ms) via a water-immersion objective (LUMPLFL 60× W; Olympus) and a cooled CCD camera (Pentamax; Princeton Instruments). Data acquisition was performed with Metafluor software (Universal Imaging). For off-line analysis and processing of fluorescence images ImageJ software was used.

Analysis of Golgi Complexes and PTVs in Primary Neurons. Transfected primary cortical neurons were fixed at DIV5 and stained with TGN markers (syntaxin-6, TGN38), the endosomal/Golgi marker β-Cop, or the *cis*-Golgi marker GM130 in combination with PI-4Kβ or different presynaptic markers (SNAP25, Piccolo, synaptophysin). For the colocalization study images were scanned with a confocal microscope (see above) as 300-nm *z*-stacks (63× oil objective) and calculated as maximum intensity projections with ImageJ software (see above). The size of the Golgi complex was analyzed 24 h after transfection with calneuron-1-GFP and GFP-calneuron-2, NCS-1-GFP, or GFP constructs and 72 h after introduction of the calneuron-1 shRNA. Images were scanned as 126-nm *z*-stacks (63× oil objective and 4× confocal zoom) and analyzed with Imaris image analysis software (version 6.2, Bitplane). The syntaxin-6 channel was reconstructed as 3D with the same parameters for all groups and then volume, total surface area, and the number of TGN clusters

were measured. For the analysis of PTV number MAP2 was used as a dendritic marker and Piccolo was used as a marker of PTVs. The longest MAP2-negative neurite was identified as the axon. For quantitative analysis 50-μm stretches were taken 30 μm away from the cell body to exclude the axon initial segment, scanned as *z*-stack (63× oil objective, 2× zoom) and then transferred to the maximal projection as described above. The GFP cannel was adjusted to saturation level and inverted to a binary image, which was then used to calculate the total area of the axonal segment. Subsequently, the Piccolo immunofluorescence was also converted to a binary image with the same threshold for all groups. Only the area overlapping with the GFP image was analyzed. Data are presented as the ratio between the area covered by PTVs (Piccolo positive) and the total area of the axon (GFP positive). GFP-transfected neurons were taken as control (set to 100%) and percentage deviations were calculated. For analysis of PTV size and intensity of fluorescence for Piccolo on PTVs, the mask created from the binary Piccolo image was applied to the original unmodified Piccolo channel. The size and fluorescence intensity of ROI was measured.

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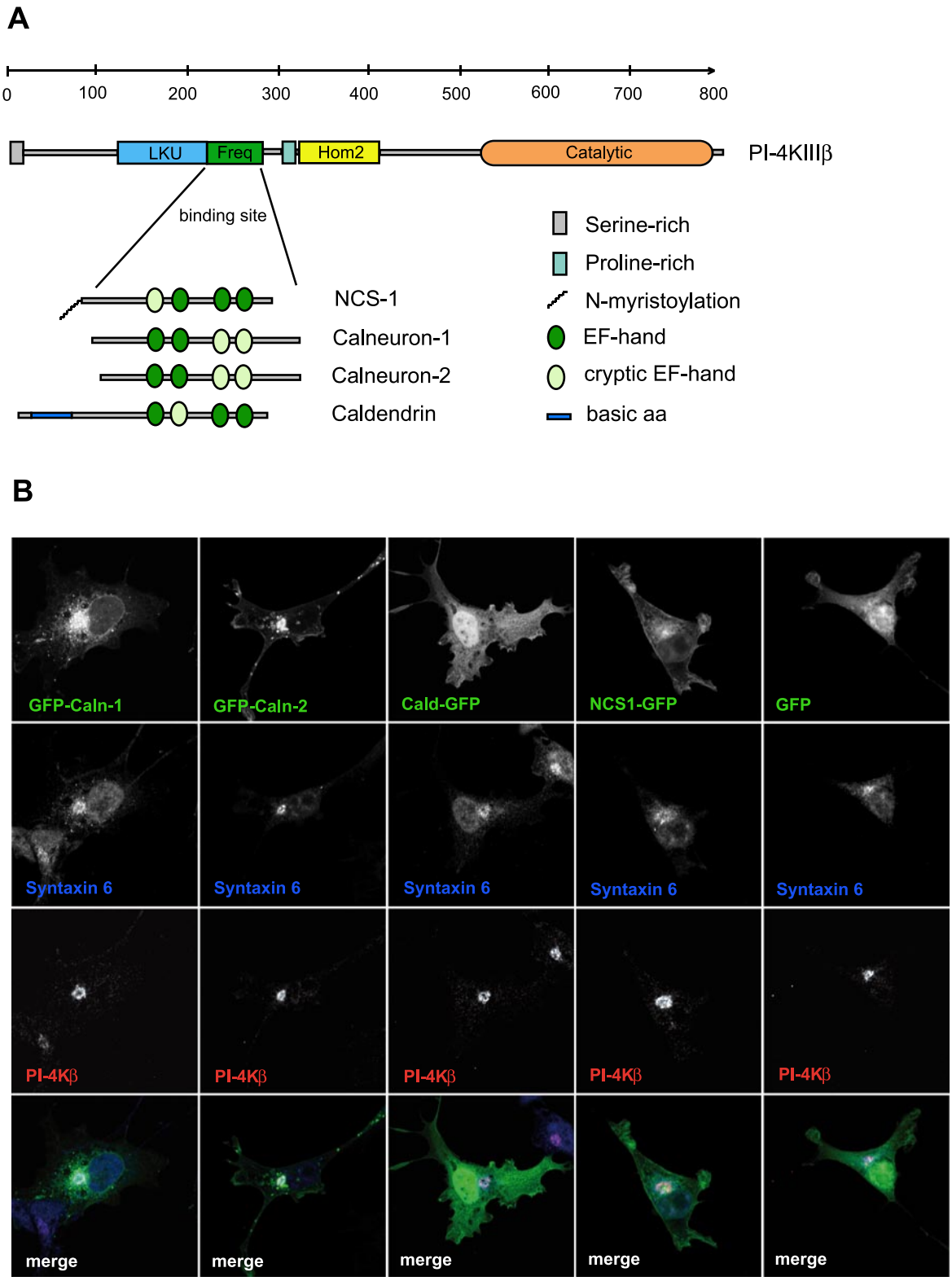

Fig. S1. (*A*) Primary structure of caldendrin, calneurons, NCS-1, and PI-4Kβ. Shown is a schematic representation as predicted from their cDNA sequences. The main sequence features including EF-hand structures, N-myristoylation, the lipid kinase unique (LKU), catalytic domain, and the Frequenin/NCS-1 binding site (Freq) in PI-4Kβ are depicted. (*B*) Overexpression of calneuron-1, calneuron-2, caldendrin, NCS-1 GFP-fusion constructs, and a GFP-control in COS-7 cells. Syntaxin-6 (blue) immunofluorescence staining was used as a TGN marker. Note that calneurons as compared witho NCS-1 and caldendrin are particularly abundant at the Golgi. Importantly, they show a very good colocalization with PI-4Kβ (red) on confocal laser scans.

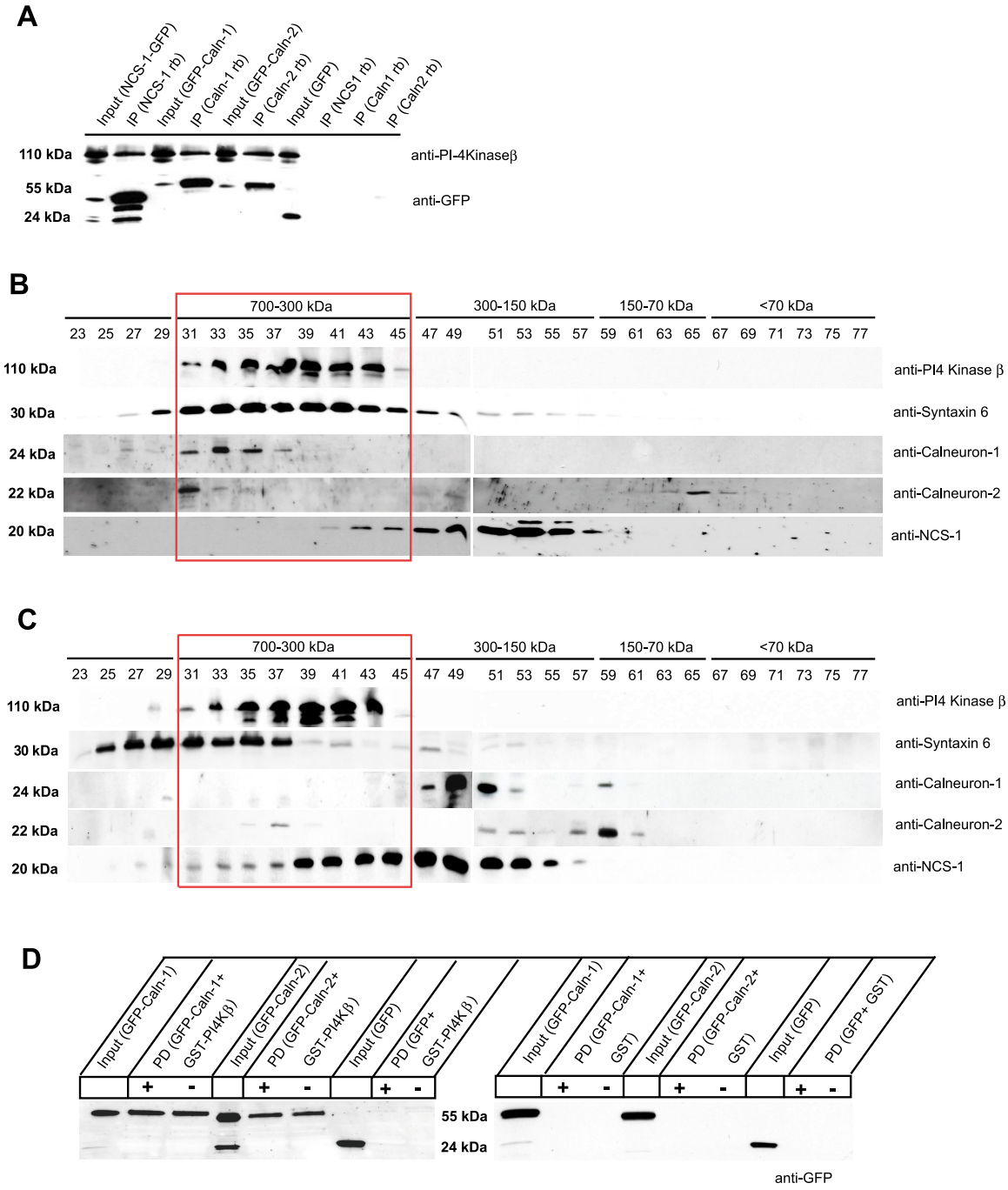

Fig. S2. (*A*) CoImmunoprecipitation of GFP-tagged NCS-1, calneuron-1 and calneuron-2 with PI-4Kß after heterologous expression in COS-7 cells. PI-4Kß coprecipitates in 1 complex with all 3 fusion proteins of interest but not with GFP alone. Staining with a GFP antibody demonstrates the amount of immunoprecipitated protein. IP: immunoprecipitate. (*B*) Gel filtration of proteins from a microsomal preparation under low Ca^{2+} conditions. Every second fraction is loaded on the gel. Fractions covering the range from ≈900 to ≈20 kDa were collected from EDTA-treated extracted extracts, loaded on SDS/PAGE, and analyzed by Western blotting. The red box highlights the PI-4Kß containing fractions. (*C*) Gel filtration of proteins from a microsomal preparation under high Ca^{2+} conditions (2 mM Ca^{2+} and 1 mM Mg^{2+}). The red box highlights the PI-4Kβ-containing fractions. (*D*) (*Left*) A pull-down assay with GST-PI4-Kβ coupled to the matrix and GFP-tagged calneuron-1 and calneuron-2 demonstrates a Ca^{2+}-independent interaction of these proteins. + indicates the presence of Ca^{2+} (2 mM Ca^{2+} and 1 mM Mg^{2+}) and − indicates Ca^{2+}-free conditions (2 mM EGTA and 1 mM Mg^{2+}). The blots were developed with a GFP-antibody. In the lane containing the calneuron-2 GFP some breakdown product is visible. (*Right*) No specific pull-down was visible in GFP and GST controls.

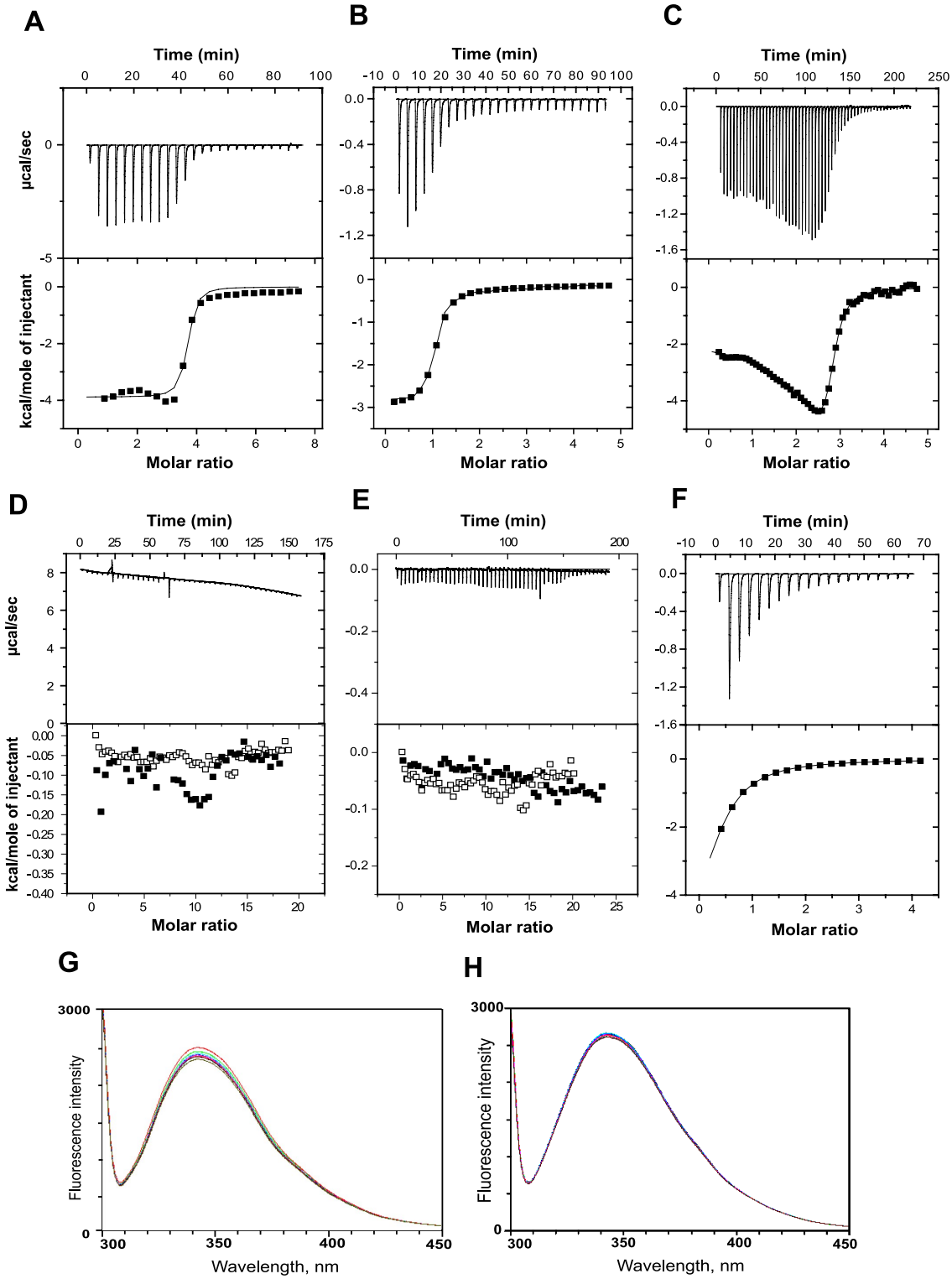

Fig. S3. (*A-C*) ITC measurements of Ca^{2+}- and Mg^{2+}-binding affinities of calneuron-1, calneuron-2, and NCS-1. The ligand concentrations for the titration were 5 mM Ca^{2+} and 10 mM Mg^{2+}. Depicted are isotherms of Ca^{2+}-binding to untagged calneuron-1 (*A*), MBP-tagged calneuron-2 (*B*), and untagged Mg^{2+}-bound myristolated NCS-1 (*C*). (*D–F*) Isotherms of Mg^{2+} binding to untagged calneuron-1 (*D*), MBP-tagged calneuron-2 (*E*), and untagged myristoylated NCS-1 (*F*). (*G* and *H*) Tryptophan fluorescence spectroscopy of untagged calneuron-1 in the presence of Ca^{2+} (*G*) or Mg^{2+} (*H*). Note that Ca^{2+} but not Mg^{2+} changes the conformation of the protein.

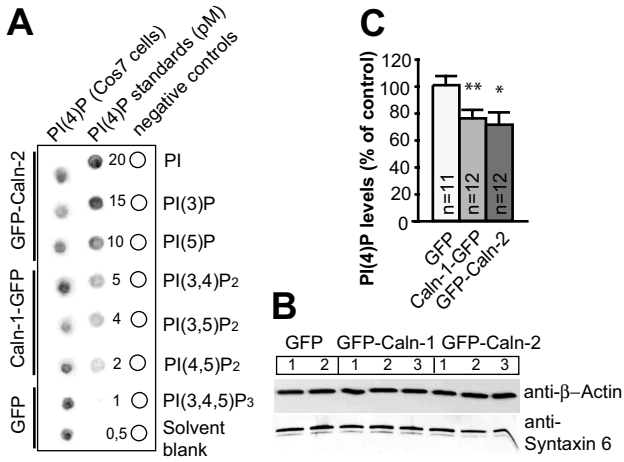

Fig. S4. Overexpression of calneuron-1-GFP and calneuron-2-GFP reduces PIP production in transfected Cos7 cells. (*A*) Representative dot blots showing a reduction of PIP levels as evidenced by a protein-lipid overlay assay. (*B*) The presence of Golgi membranes was checked by immunoblotting of Syntaxin-6 and was in used in combination with β-actin immunoblots as loading controls to normalize the loading for the dot blot assay. (*C*) Quantification of PIP levels. The amount of PIP in GFP-transfected cells was taken as 100%, and the deviation for calneuron-GFP-transfected cells was calculated. Error bars represent the SEM. **, $P < 0.01$; *, $P < 0.05$.

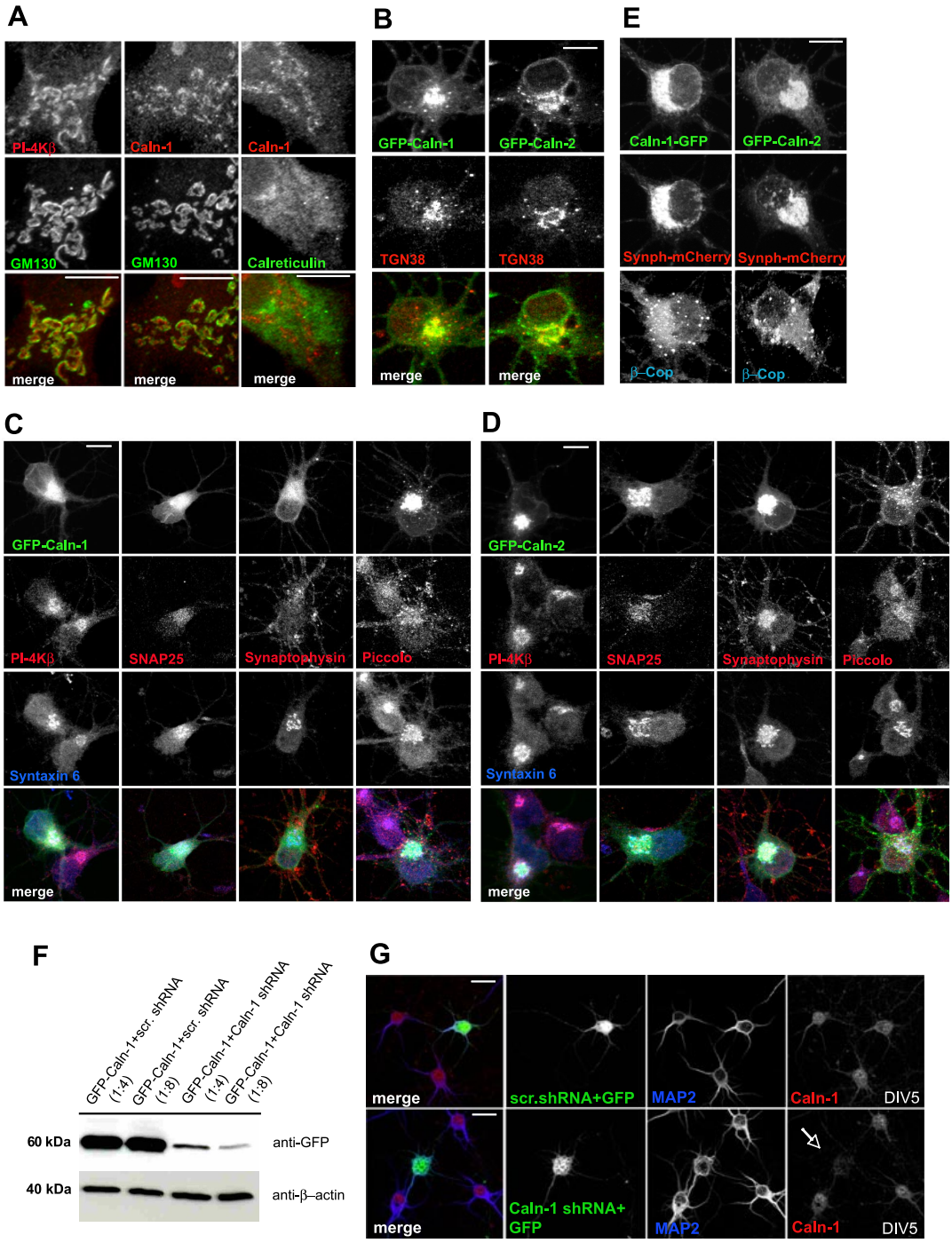

Fig. S5. Endogenous and overexpressed calneurons colocalize with Golgi markers and proteins known to be part of Golgi-derived vesicles and PTVs. (*A*) Cortical neurons (DIV21) costained with anti-PI-4Kβ or calneuron-1 antibody and GM130, a *cis*-Golgi marker. Note that all 3 proteins are restricted to the same structure but show very little overlap, indicating that their localization is restricted to different Golgi subcompartments. Calreticulin, a marker for the endoplasmic reticulum, showed very little overlap with calneuron-1 staining. (*B*) GFP-calneuron-1 and calneuron-2 colocalize with the TGN markers, TGN38 and syntaxin-6. (Scale bar: 10 μm.) (*C* and *D*) GFP-calneuron-1- and calneuron-2-transfected neurons costained with PI-4Kβ, SNAP25, synaptophysin, and Piccolo. Note the accumulation of vesicular proteins in the Golgi after transfection with calneurons. (Scale bar: 20 μm.) (*E*) mcherry-Synaptophysin and calneuron-1-GFP or GFP-calneuron-2 cotransfected primary cortical neurons (DIV5) stained with another endosomal/TGN marker (β-Cop) show very limited colocalization of all 3 proteins at the Golgi. Note the strong colocalization of GFP-tagged calneurons and mcherry-Synaptophysin at the Golgi. (Scale bar: 10 μm.) (*F*) Cos7 cells double-transfected with shRNA for calneuron-1 or the scrambled construct and calneuron-1 in pEGFP-C1 vector in a ratio of 4:1 or 8:1. Seventy-two hours later cells were harvested and protein levels were checked by Western blot with GFP and actin antibodies (the GFP antibody does not recognize tGFP from the pRS-GFP plasmid carrying the shRNA sequence). Strong inhibition of calneuron-1 expression can be observed with a 8:1 ratio of constructs. (*G*) Cortical neurons transfected on DIV2 and fixed 72 h later were stained with a calneuron-1 rabbit antibody. Arrow indicates the transfected neuron with significant reduction of calneuron-1 immunoreactivity.

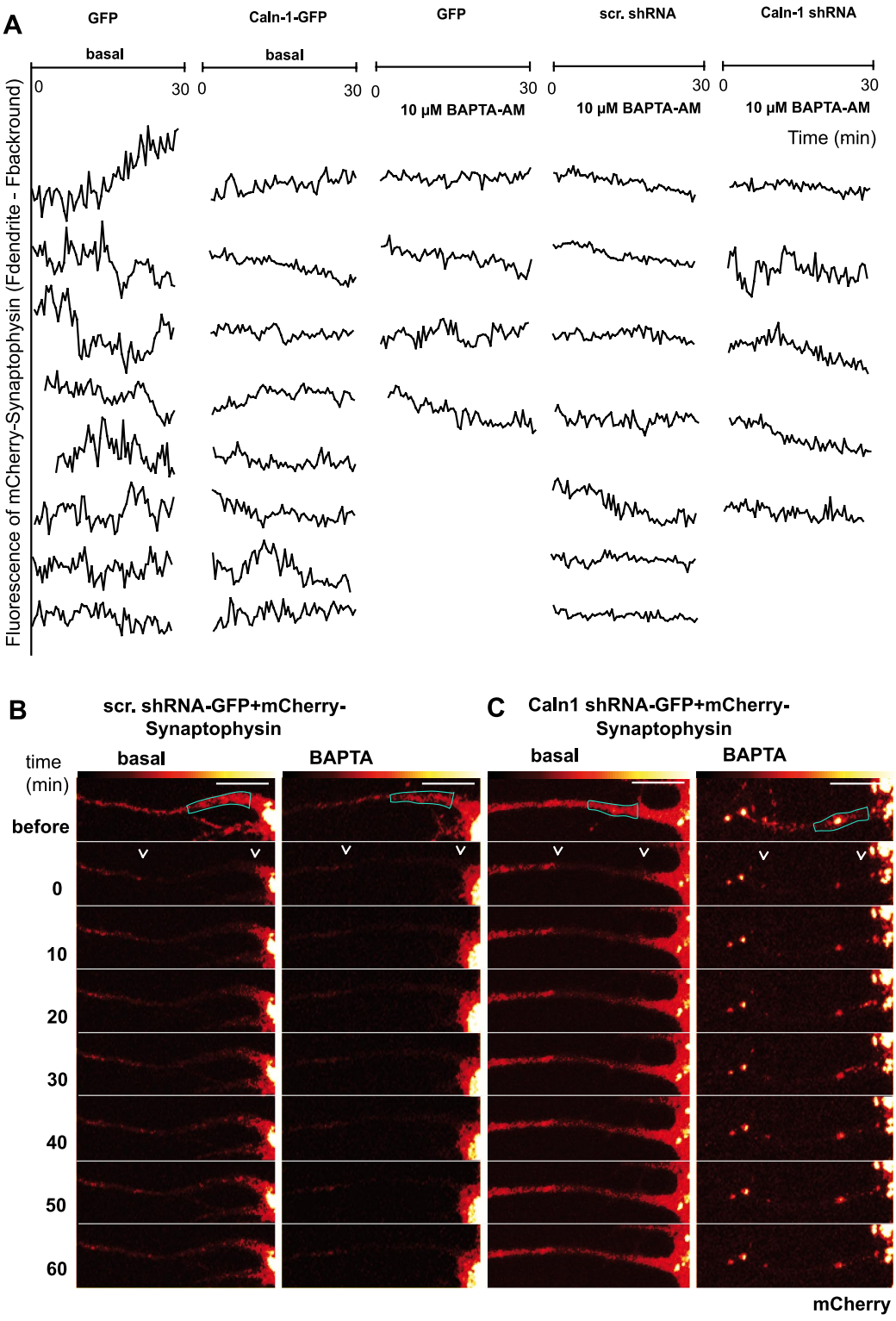

Fig. S6. (*A*) Line analysis for the life imaging of vesicle trafficking via the proximal part of its major neurite of DIV 5–7 cortical neurons cotranfected with mcherry-synaptophysin and calneuron-1-GFP shows a decreased amplitude of mCherry fluorescence compared with GFP controls. Traces of each individual experiment are shown with the same scale. (*B* and *C*) FRAP experiments for the neurons cotransfected with calneuron-1 shRNA (or scrambled shRNA) and mcherry-synaptophysin. Preincubation with BAPTA-AM strongly reduced mcherry fluorescence recovery, indicating the requirement of free intracellular Ca^{2+} for Golgi trafficking. shRNA knock of calneuron-1 was able to remove the BAPTA-AM-induced inhibition of Golgi trafficking.

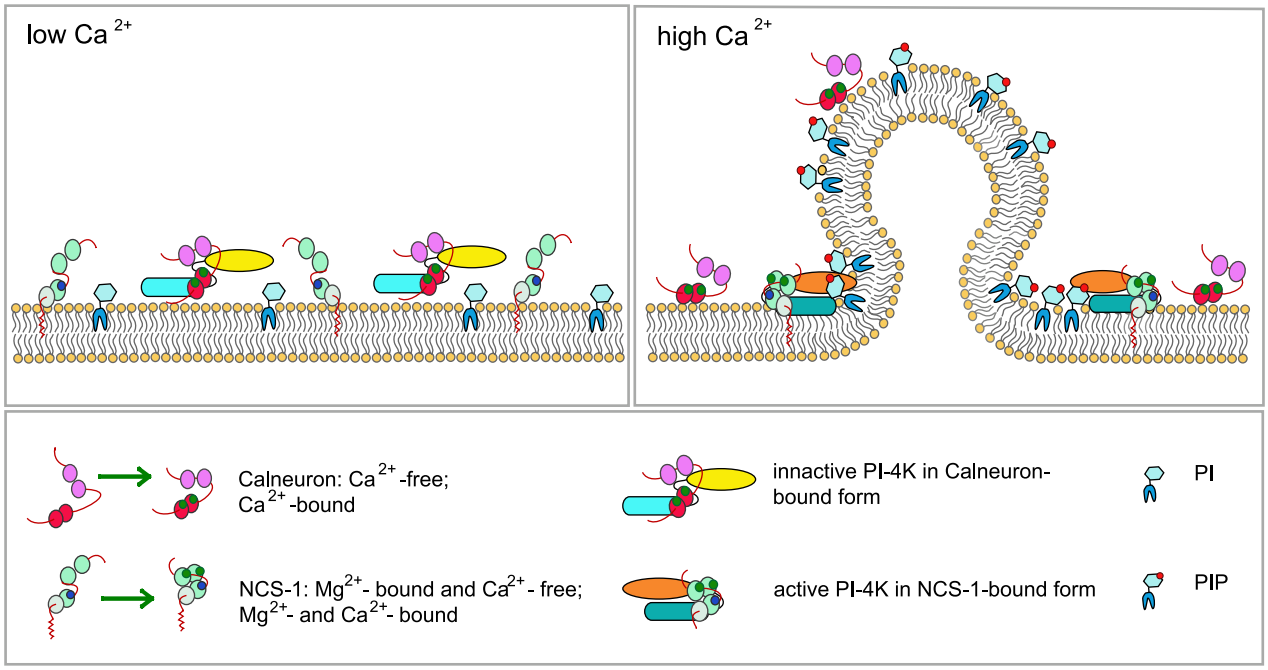

Fig. S7. Model of a Ca^{2+}-dependent switch between inhibition and activation of PI-4Kβ at Golgi membranes. (*Left*) At low Ca^{2+} concentrations PI4-Kβ will be predominantly in the calneuron-bound form. (*Right*) PI4-Kß activity will be low and Golgi trafficking will be slowed down until Ca^{2+} concentrations will rise. Under these conditions NCS-1 will be in a Ca^{2+}-bound form and calneurons are released from the PI4-Kβ complex. NCS-1 will stimulate the activity of PI4-Kβ and induce Golgi trafficking via a 3- to 4-fold increase in PIP and PIP2 production.

Table S1. Molar binding activity of myr-NCS-1 surfaces for PI-4Kß-GST under different Ca^{2+}/Mg^{2+} conditions

Myr-NCS-1 immobilized, ng/mm^2	PI-4Kß-GST, μM	Ca^{2+} concentration, μM	Mg^{2+} concentration, μM	Signal, RU	Molar binding activity
5.6	1	0,2	0	30	$9.9E10^{-4}$
5.6	1	0,2	200	23	$7.9E10^{-4}$
5.8	1	0,4	0	30	$9.4E10^{-4}$
5.6	1	0,4	200	22	$7.2E10^{-4}$

Table S2. Molar binding activity of his-SUMO-Calneuron-1 surfaces for PI-4Kß-GST under different Ca^{2+}/Mg^{2+} conditions

His-SUMO-Calneuron-1 immobilized, ng/mm^2	PI-4Kß-GST, μM	Ca^{2+} concentration, μM	Mg^{2+} concentration, μM	Signal, RU	Molar binding activity
7.6	5	0,2	0	74	$3.9E10^{-3}$
7.7	5	0,2	200	80	$4E10^{-3}$
7.8	5	0,4	0	62	$3.6E10^{-3}$
7.7	5	0,4	200	75	$3.9E10^{-3}$

Table S3. Thermodynamic and Ca^{2+} and Mg^{2+} binding parameter obtained for Calneuron-1 and Calneuron-2 calculated by ITC

Proteins	Model	Association constant K_A, M^{-1}	Dissociation constant K_D, μM	ΔH, kcal/mol	$T\Delta S$, kcal/mol	$\Delta G°$, kcal/mol
Calneuron 1 Mg^{2+} binding	Does not bind	-	-	-	-	-
Calneuron 1 Ca^{2+} binding	1 set of sites	5.38×10^6	0.18	-3.75 ± 0.058	5.57	−9.32
Calneuron2* Mg^{2+} binding	Does not bind	-	-	-	-	-
Calneuron 2* Ca^{2+} binding	1 set of sites (sequential binding sites)	4.34×10^6	0.23	-3.43 ± 0.368	5.51	−8.94
Myr-NCS-1 Mg^{2+} binding	1 set of site	3.97×10^4	25.18	-6.84 ± 0.52	−0.463	−6.37
Mg^{2+} bound Myr-NCS-1 Ca^{2+} binding	2 set of sites	1.96×10^6	0.89	-1.61 ± 0.13	7.12	−8.73
		6.43×10^5		-42.32 ± 13.8	−34.23	−8.09

Data on myristoylated NCS-1 are also included .
* MBP-tagged protein was used.