MATERIALS AND METHODS SUPPLEMENTARY FIGURES

Staining of endocytotic vesicles with FM4-64:

Differentiated N2A neuroblastoma cells or CV1 cells were incubated with 15μM FM4-64 (Molecular Probes) for 30 minutes at 37 °C. FM4-64 was observed with a Cy5 filterset.

Staining of lysosomes:

CV1 cells were incubated with 100nM LysoTracker™ Red DND-99 (Molecular Probes) for 30 minutes at 37 °C and observed using rhodamine filter settings.

Immunofluorescence of MAP2 and Tau:

Differentiated N2A neuroblastoma cells were fixed with 3.7% formaldehyde in PBS for 20 minutes at room temperature. Formaldehyde was washed away with PBS and cells were incubated for 15min with 5mM NH_4OH in PBS. After washing, N2A cells were permeabilized with 0.03% Triton X-100 in PBS for 3min at room temperature. Triton X-100 was washed away and cells were blocked for 30 minutes in PBS containing 0.2% fish gelatin. N2A cells were incubated with anti-MAP2 polyclonal antibody (Chemicon – 1/500) or anti-tau monoclonal antibody (Chemicon – 1/100) for 1h at room temperature, washed, and incubated 1 hour with Alexa 647-labeled goat anti-rabbit Ig polyclonal antibody (Ab; 1/500; Molecular Probes) or Alexa 647-labeled goat anti-mouse Ig polyclonal antibody (Ab; 1/500; Molecular Probes), respectively. All samples were viewed with a Fluoview confocal microscope (Olympus) with fully opened confocal aperture to capture the entire cell.

FIGURE LEGENDS TO SUPPLEMENTARY FIGURES

Supplementary figure 1: Nature of processes of differentiated N2A cells.

A, N2A cells were differentiated by serum deprival in the presence of N2-supplement (N2), by addition of 1mM dbcAMP or by incubation with 20μM retinoic acid. After 24h hours of differentiation, the cells were fixed and processed for immunofluorescence. Clearly, processes of N2 and dbcAMP differentiated N2A cells that exhibited the characteristic length reported for axon-like processes in these cells, were negative for the dendrite marker MAP2, while tau, which is reportedly present in both axons and dendrites was clearly visible. On the other hand, processes obtained by differentiation with retinoic acid, which was reported to induce predominantly dendrite-like processes, were positive for MAP2, and tau. Morphologically, the interface between neurite and cell body was more defined in the case of axon-like neurites (arrows). These results strongly indicate that the processes studied here are indeed axon-like.

B, N2A cells were transfected with EGFP, PLCδ-PH, spectrin-PH, or GRP1-PH and differentiated by serum removal. Immunofluorescent detection of MAP2 and tau shows that expression of PH domains did not alter the expression pattern of MAP2 or tau. Thus, the processes remained axon-like.

Supplementary figure 2: PtdIns(4,5)P2-specific PH domains do not influence the distribution of endocytotic vesicles.

N2A cells transfected with EGFP, or PLCδ-PH were stained with the fluorescent lipophylic dye FM4-64 and the distribution of axonal, FM4-64-positive vesicles was

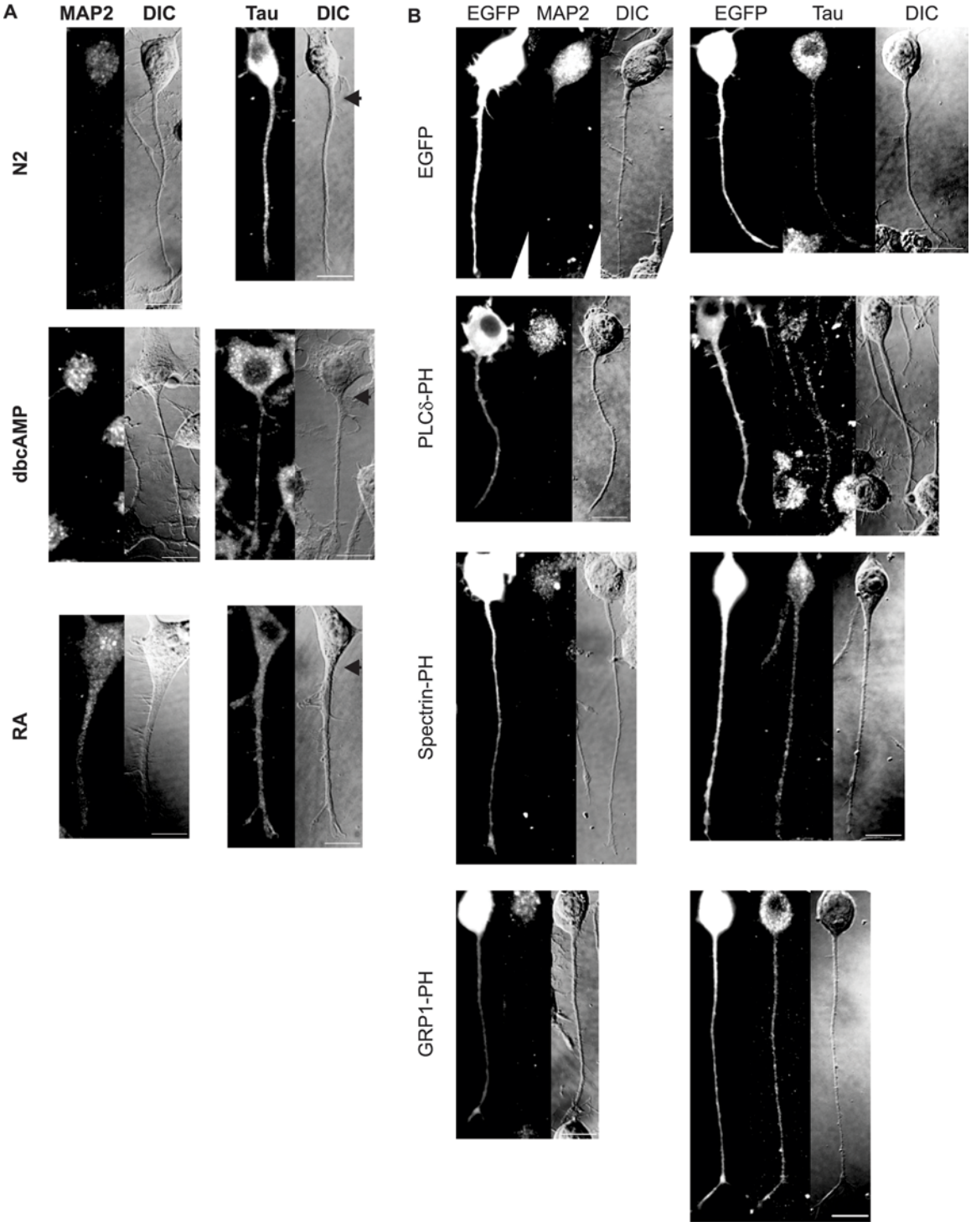
determined. A, Representative fluorescence images showing the distribution of axonal endosomes and the corresponding fluorescence profile are presented – Scale bar = 20μm. B, The axon was divided in 3 equal parts ranging from the cell body (CB), over the middle of the axon (AX) to the distal part at the growth cone (GC) and the number of vesicles in each part was determined. Data shown is the mean and SEM of 9 and 12 cells, respectively. Statistical analysis (t-test) revealed that there is no significant difference between the number of vesicles found in the three axonal subdivisions (CB, AX, and GC – $p>0.05$).

Supplementary figure 3: PH domains do not influence the distribution of mitochondria, endocytotic vesicles, and lysosomes in CV1 fibroblasts.

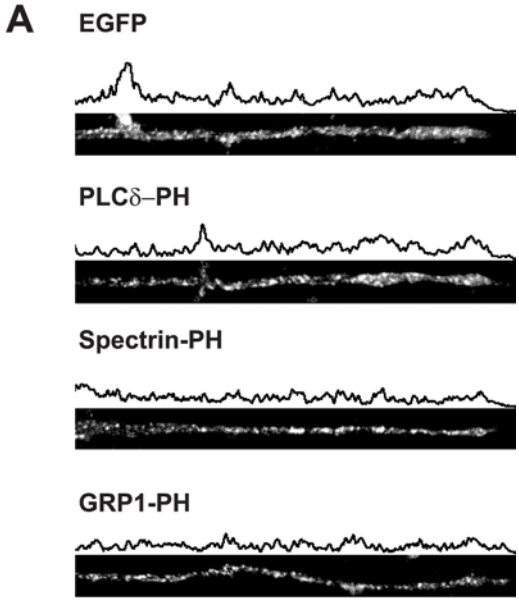
Mitochondria, endocytotic vesicles, or lysosomes were visualized in CV1 cells transfected with EGFP, PLCδ-PH, or spectrin-PH by co-transfection with DsRed1-mito, or staining with FM4-64 or LysoTracker, respectively.

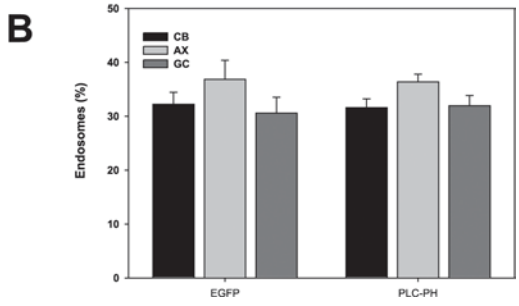
Clearly, PH domains did not affect the distribution of mitochondria (A), lysosomes (B), or endocytotic vesicles (C).

Suplementary figure 1



Supplementary Figure 2





supplementary figure 3