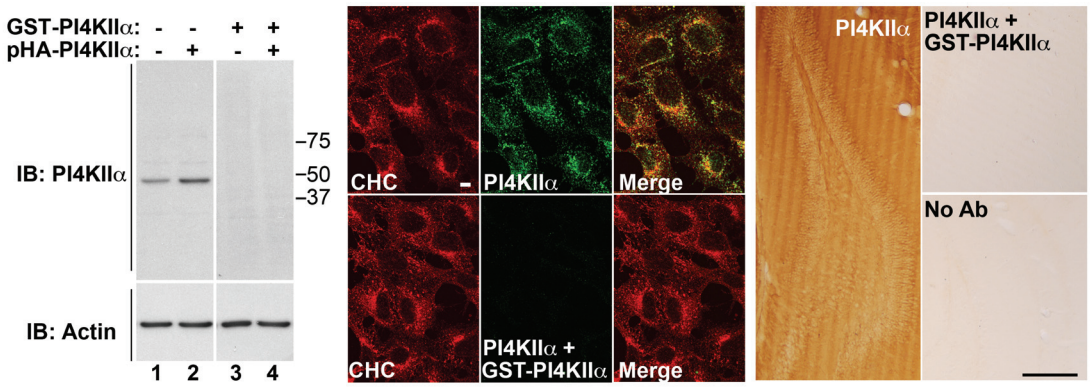
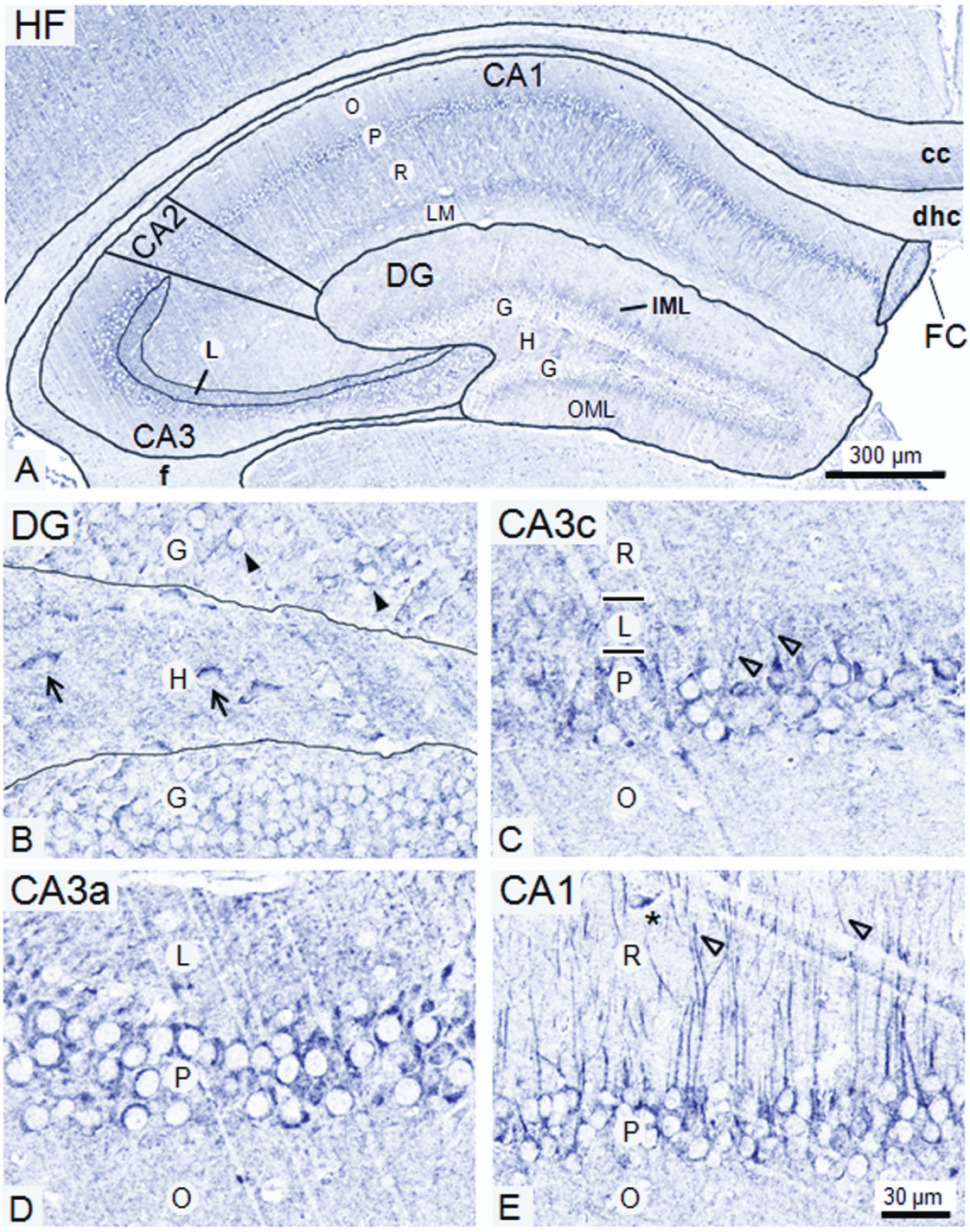



**S Fig.1. Specificity of the PI4KIIα antibody**

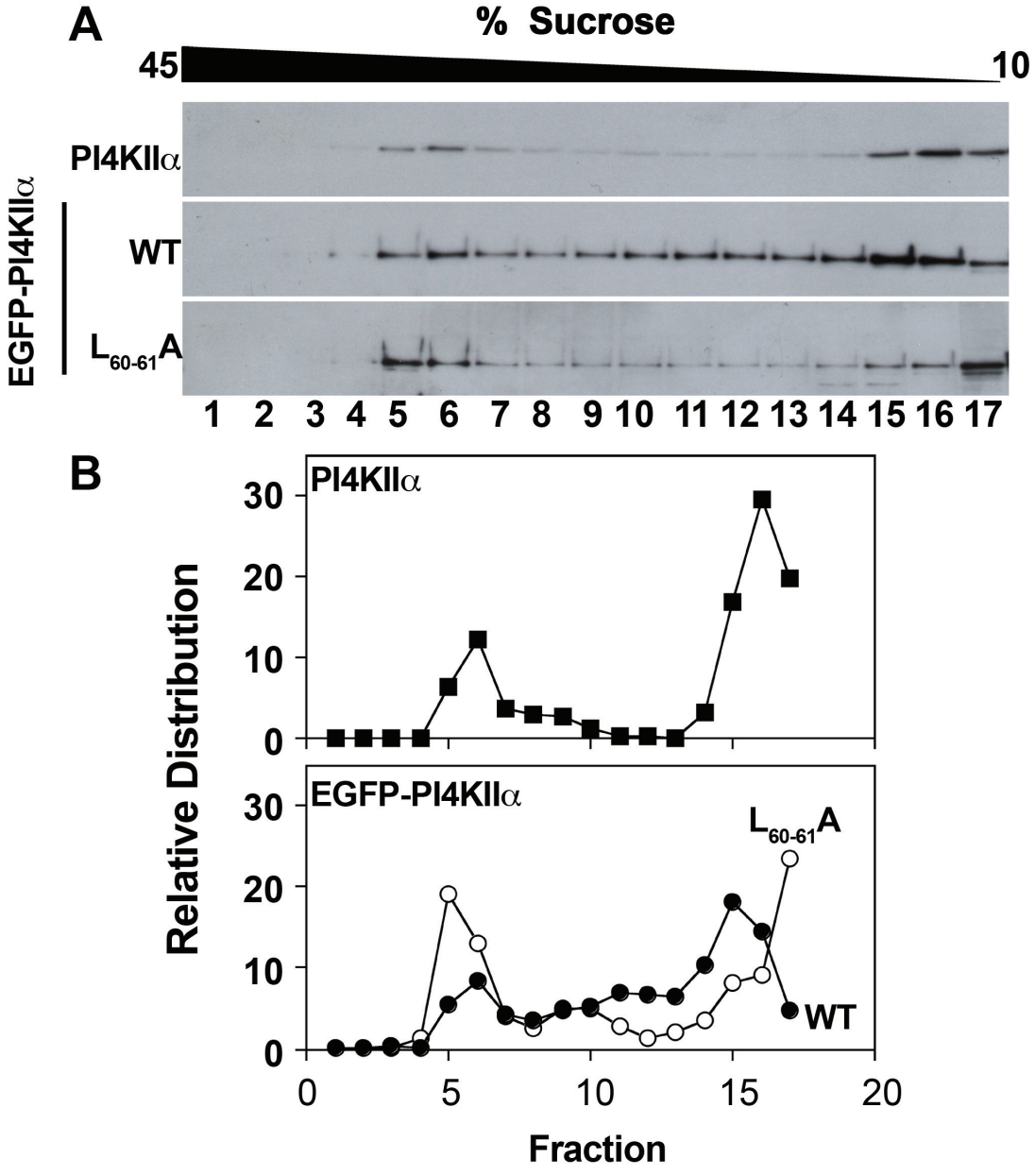
A. Lysates from wild type PC12 and PC12 cells stably transfected with PI4KIIα-HA were probed with either anti-PI4KIIα or with the antibody plus GST-PI4KIIα fusion protein to outcompete antibody binding. Beta actin levels were used as controls. B. Immunofluorescence labeling in HEK293 cells with anti-PI4KIIα (green) and anti-clathrin heavy chain X22 mAb (red). The bottom panel also used a GST-PI4KIIα fusion protein during the primary antibody incubation for outcompetition. Bars equal 10 μm. C. Light microscopy images of PI4KIIα distribution in mouse dentate detected with peroxidase-anti-peroxidase reactions in wild type dentate gyrus. Reactions were performed in the presence of the PI4KIIα antibody (C1). Background was determined either by omitting primary PI4KIIα antibody (C2) or by out competition with full length GST-PI4KIIα recombinant protein (C3). Scale bars are 250 μm.




**Suppl. Fig. 2**

**S Fig. 2. PI4KIIα distribution in the hippocampal formation of adult C57Bl6/J mice.**
**PI4KIIα distribution in the hippocampal formation (HF) of adult C57Bl6/J mice.**
A. Low magnification view of the HF (hippocampal fields CA1-3 plus dentate gyrus, DG) showing the laminar pattern of immunoreactivity visualized with a blue reaction

product due to nickel sulfate amplification. Note that PI4KIIα is concentrated in two major presynaptic fields: (1) the inner molecular layer (IML) of the DG and (2) the stratum lucidum (L) of CA3B-E. Higher magnification views showing cellular localization of immunoreactivity, which was non-nuclear and neuronal (not glial) in HF gray matter. B. PI4KIIα is evident in cell bodies, but not dendrites, of granule (arrow heads) and large hilar (arrows) neurons in the DG. The levels are low in the case of granule cells and only low to moderate in hilar cells. C-D. PI4KIIα is seen at higher levels in pyramidal cells of CA3 (as in CA1 and 2), where it extends into their apical dendrites (open arrowhead). Stratum lucidum is filled with diffuse immunoreactivity probably derived from DG granule cells. CA3c and CA3a are the medial and lateral segments of CA3, respectively. E. Spread of PI4KIIα far into distal portions of pyramidal cell apical dendrites (open arrowhead) was clearest in CA1, probably because only in CA1 are such dendrites parallel to the coronal plane. Distal tips of labeled dendrites in CA1 and CA2 bend to form the immunoreactive band seen in A between stratum radiatum (R) and stratum lacunosum-moleculare (L-M). A subset of unidentified interneurons expressed PI4KIIα in stratum oriens (O) and stratum radiatum, an example of which is marked by an asterisk in E. cc = corpus callosum, dhc = dorsal hippocampal commissure, f = fimbria, FC = fasciola cinereum, and OML = outer molecular layer of DG. The scale bar in E also applies to B-D.




**Suppl. Fig. 3**

**S Fig.3. The PI4KIIαL60-61A does not fractionate to microvesicles**
A. PC12 cell microvesicles either non-transfected or expressing wild type or PI4KIIαL60-61A tagged with EGFP were isolated on a 10-45% sucrose gradient. Fractions were resolved by SDS-PAGE and immunoblotted for endogenous PI4KIIα or GFP. B. Relative distribution of PI4KIIα and PI4KIIαL60-61A protein. Synaptic-like microvesicles sediment at the top of the gradient (fractions 15-17) whereas donor endosomes migrate in fractions 5-6.