**Supplementary Figure Legends**

**Supplementary Figure 1**: Upper panel: A time-lapse sequence showing the distribution of TfR-GFP (black signal) in a CHO cell transfected with PKD1-WT. Note that vesicles-like structures are distributed throughout the cell cytoplasm, with little accumulation at the Golgi region. Middle panel: A time-lapse sequence showing the distribution and dynamics of TfR-GFP in a CHO cell transfected with PKD1-KD. Note the accumulation of TfR-GFP (black signal) at the Golgi region, and the presence of long tubules that extend up to the cell periphery; note that the tubules are dynamic, elongating and retracting frequently. Lower panels: Another example of the distribution of TfR-GFP (white signal) in a CHO cell with ectopic expression of PKD1-KD. For this experiment, cells were analyzed 12 hours after transfection. Images were taken every 20 sec for periods ranging from 2-10 minutes.

**Supplementary Figure 2**: (A) A time-lapse sequence showing trafficking of TfR-GFP in a hippocampal pyramidal neuron co-transfected with TfR-GFP and GST-PKD1-KD. Note the lack of accumulation of TfR-GFP at the Golgi, and the absence of Golgi-derived tubules; TfR-GFP-containing vesicles distribute throughout the cytoplasm moving in the anterograde and retrograde directions. Images were taken every 5 seconds. For this experiment neurons (8 d.i.v.) were transfected with Lipofectamine 2000 and analyzed by video-microscopy 12-14 hours later. (B) (Upper Panel) Confocal images showing the distribution of TfR-GFP (green) in cells transfected with PKD1-WT or KD and fixed 4, 6, and 8 hours post transfection. No significant differences in the pattern of distribution of TfR-GFP were detected between non-transfected neurons, and cells expressing either PKD1-WT or KD. (Lower Panel) Bars showing quantification of TfR-GFP fluorescence in neurons overexpressing PKD1-WT or KD. 12-bit images (0-4095) were used for this quantification. Measurements were performed

in a 5 $\mu$m area within the cell body and within 10 $\mu$m segments located along inner, middle and distal dendrites.

**Supplementary Figure 3**: The intracellular and surface distribution of HA-mLRP4 in cultured neurons (7-8 d.i.v.). (A-E) Confocal images showing the intracellular distribution of MAP2 and ectopically expressed HA-mLRP4. Note that the HA-mLRP4 localizes to short, branched, MAP2 (+) dendrites. (F) A merge image from D and E showing that HA-mLRP4 vesicular structures do not enter a MAP2 (-) axon-like neurite (arrows). (G-I) Confocal images showing that HA-mLRP4 does not localize with Tau + axons. (J-K) Confocal images showing surface HA-mLRP4 and intracellular MAP2 staining. (L) A merge image from J and K (insert) showing that HA-mLRP4 localizes to the somato-dendritic plasma membrane (arrows).

**Supplementary Figure 4**: Spectral confocal images showing the distribution of TfR-GFP and HA-mLRP4 in a hippocampal neurons (8 d.i.v.) Note that many vesicles display both GFP- and HA-fluorescent signals (green and red arrows).

**Supplementary Figure 5**: Expression of PKD1-KD alters the intracellular distribution of endogenous LRP. (A-D) Confocal images showing the distribution of endogenous LRP and MAP2 in 8 d.i.v. hippocampal pyramidal neurons. Note the colocalization of LRP with the dendritic marker MAP2. (E-G) Confocal images showing the distribution of endogenous LRP and MAP2 in a neuron transfected with PKD1-KD. Note that LRP is also present in the MAP2 (-) processes. (H-J) Confocal images showing the distribution of endogenous LRP and Tau in a neuron transfected with PKD1-KD. Note that LRP is present in the Tau (+) axon.

**Supplementary Figure 6**: (A-C) Confocal images showing the distribution of L1-YFP and HA-mLRP4 in a 7 d.i.v. hippocampal pyramidal neuron. Note that L1-YFP localizes to both

the axon (arrows in A) and dendrites (arrows in B). (D-F) Confocal images showing the distribution of GST-tagged PKD1-KD, L1-YFP, and MAP2 in an 8 d.i.v. hippocampal pyramidal neuron. Note that L1-YFP localizes to both MAP2 (-) (arrows in E) and MAP2 (+) (arrows in F) neurites. (G, H) Confocal images showing the surface distribution of L1 (s-L1) in a neuron co-transfected with L1-YFP and PKD1-KD (not shown). For this experiment, fixed cultures were stained with the L1 mAb before extraction with detergents. Note the absence of L1 surface staining in dendritic processes (short arrows) and its presence along the middle and distal third of the axon (long arrows).

**Supplementary Figure 7**: (A-F) Confocal images showing the distribution of MAP2 and TfR-GFP in neurons co-transfected with PKD1-KD-S916E or PKD1-WT-S916A. Note that after expression of these PKD1 mutants, TfR-GFP localizes to both dendrites (MAP2+ neurites) and axons (MAP2- neurites).

**Supplementary Figure 8**: PKD1-RNAi transfection reduces PKD1 expression in cultured cells. Western blot showing levels of GST-PKD1 obtained from cell extracts of CHO cells co-transfected with the control-sh-GFP plasmid (lane 1) or sh-PKD1-GFP (lane 2). 10 $\mu$g of total cellular protein were loaded in each lane. Western blot showing levels of endogenous PKD1 from cell extracts from control (non-transfected, lane 1), or scrambled-sh-GFP plasmid (lane 2), or sh-PKD1-GFP (lane 3) transfected hippocampal pyramidal neurons. 20 $\mu$g of total cellular protein were loaded in each lane. Densitometric analysis of Western blots from control and RNAi-treated cells. 5 blots were analyzed for each experimental condition. Note the significant decrease in PKD1 protein levels after transfection with the PKD1 RNAi. (B-D) Confocal images showing PKD1 (red) staining in cultured neurons transfected with the sh-PKD1-GFP (green). Note that neurons expressing the RNAi (arrows) show reduced levels of

PKD1 immunofluorescence. (E) Bars showing changes in PKD1 fluorescence intensity after expression of control-GFP, sh-control GFP, and sh-PKD1-GFP. Measurements were performed in the cell body. Note the dramatic decrease in fluorescence intensity. (F-G) Confocal images showing co-expression of sh-PKD1-GFP (green) and HA-mLRP4 (red) in a cultured hippocampal pyramidal neuron (7 d.i.v.). Note the presence of HA labeling in the dendrites (arrowheads) and the axon-like neurite (arrows) of the co-transfected neuron.