Supplementary Figure 1. **Normal IF and internalisation assays of cytoplasmic domain-deletion KIAA0319 proteins.** HEK293T cells were transiently transfected with constructs encoding mycHIS-tagged deletion proteins Kd20-21 (A-C), Kd20-21a (D-F), and Kd21b (G-I) (lacking residues 984-1072, 984-1023 and 1031-1072, respectively). KIAA0319 proteins were detected with polyclonal R2 antiserum and monoclonal anti-myc 9E10 antibody. (a) Cells were processed for normal IF; all the three deletion proteins reach the plasma membrane, and can also be detected inside the cell, although only Kd21b is found in large vesicles. (b) R2 antiserum internalisation assays show that Kd20-21 (B) and Kd20-21a (E) are not internalised once they reach the plasma membrane, while Kd21b (H) is found in internal large vesicles as the wild type (see Fig. 1). Panels C, F and I from Sup. Fig. 1b are shown in Fig. 1 (M, N, O, respectively). Scale bar: 10μm.

Supplementary Figure 2. Internalisation of KIAA0319 is not dependent upon antibody-mediated cross-linking. *A*, SDS-PAGE in reducing conditions showing the purification of Fab fragments of the specific anti-KIAA0319 antibodies. Affinity-purified anti-peptide A (residues 471-485 of human KIAA0319 protein) obtained from R2 antiserum (27) (lane 1) were processed with the Pierce Fab Micro Preparation Kit according to the manufacturer's instructions. Detection of the Fc fragments (lane 2) confirms digestion of the IgG molecules with papain. The resulting Fab fragments (lanes 3 and 4) were purified from the Fc fragments and the remaining un-digested IgG molecules (lane 5) through a protein A column. *B*, internalisation assays in HEK293T cells expressing the mycHIS-tagged KIAA0319 protein were performed as described in Fig. 1 with either untreated (A-F) or the Fab fragments of (G-L) affinity-purified anti-KIAA0319 antibodies. In both cases the results are the same than those obtained with R2 antiserum (see Figs. 1 and 2), indicating that the internalisation of the KIAA0319 protein is not triggered by the binding of the antibodies. 10μm bar is shown in panel C; same magnification was used in all panels. BSA, bovine serum albumin; Hc, Lc, Fc, and Fab, indicate the heavy chain, light chain, Fc fragments, and Fab fragments, respectively, of the IgG molecules.

Supplementary Figure 3. KIAA0319 undergoes clathrin-mediated endocytosis. HeLa cells were treated with transfection reagent alone (mock), with 100nM scrambled siRNA, or with 100nM siRNA directed against either μ2 or CLTC. After siRNA treatment, cells were transfected with a full length KIAA0319 construct containing a V5 tag. KIAA0319 protein at the plasma membrane was labelled with anti-KIAA0319 R2 antiserum and after a period of internalisation cells were fixed and processed to detect internalised R2 (B, E, H, K). Cells silenced for CLTC can be identified by the absence of clathrin staining (J). In mock transfected and scrambled control cells, R2-labelled KIAA0319 is observed intracellularly, where it co-localises with clathrin (A-F). In cells depleted for CLTC, R2-labelled KIAA0319 is observed only on the cell surface (K). Clathrin intracellular distribution is not modified by AP-2 siRNA (G). Arrowhead in panel L indicates a non-depleted cell which serves as an internal negative control. Scale bar:10μm.

Supplementary Figure 4. KIAA0319 is internalised in the neuronal cell line KELLY and Tyrosine 995 is needed for this endocytosis. Using lipofectamin 2000 (Invitrogen), Kelly cells were transfected with constructs expressing either wild type (KAv) (A-C) or mutant (KAv-Y995A) (D-F) V5-tagged full-length KIAA0319 protein. Proteins were detected by immunofluorescence with R2 antiserum (A, D) or by R2 antiserum internalisation assays (B, E). KAv is observed on the cell surface and in internal structures, while KAv-Y995A is found only at the cell surface and R2 antibodies are not internalised. Internalisation of transferrin-594 is unaffected by the expression of either construct demonstrating clathrin-dependent endocytosis occurs normally in both cases (C, F). Scale bar: 10 μm.