Supplementary figure 1 : Expression levels of endocytic markers in LCLs from euploids and individuals with DS.

Quantitative western blot analysis of LCL extracts from controls (n=10) and DS (n=10)probed with anti-EEA1 and anti-Rab5 antibodies (t-test, $p=0.43$ for EEA1 and $p=0.1$ for Rab5).

Supplementary figure 2: non-perfused Tg(SYNJ1) show enlarged endosomes in prefrontal cortex neurons.

(A) Quantitative western blot analysis of non perfused brain extracts from Tg(SYNJ1) (n=4) and WT mice (n=4) probed with anti-Rab5 antibody. (t-test $p=0.64$).(B) Mean number of EEA1-positive early endosomes in neurons from Tg(SYNJ1) mice as compared to WT mice. (C) Mean endosome size of neurons from Tg(SYNJ1) mice as compared to WT mice. (D) Endosome size distribution into small-, medium- and large-sized endosomes, based on K-means clusters. (E). Representation of the WT (green circles) and Tg(SYNJ1) mice (red circles) in the plane defined by the percentage of large and small endosomes in their neurons. *** $p<0.0001$.

Supplementary Figure 3: Fibroblasts from individuals with DS exhibit enlarged endosomes as compared to fibrablats from euploids.

(A) Representative confocal images of EEA1-positive early endosomes (red) from euploid and DS fibroblasts. (B) Mean number of EEA-1 positive early endosomes from euploid and DS fibroblasts (C) Mean endosome size from euploid and DS fibroblasts transfected. (D) Endosome size distribution into small-, medium- and large-sized endosomes, based on K-means clusters. *** $p<0.0001$.

Supplementary Figure 4: Reduction of SYNJ1 expression levels by *SYNJ1*-shRNA in HEK293 cells overexpressing Flag-SYNJ1-145 or 170 isoforms.

(A) Western blots of cell homogenates showing Flag expression levels in HEK293 cells transfected with Flag-SYNJ1-145 (neuronal) or 170 (ubiquitous) isoforms plasmid constructs with or without random-shRNA or SYNJ1-shRNA plasmid constructs containing GFP 72 hours after transfection. Quantitative western blot analysis of cell homogenates probed with anti-Flag antibody shows a strong decrease of Flag expression levels in the presence of *SYNJ1*-shRNA GFP construct. Note that random-shRNA GFP construct did not change the expression of Flag. (B) Immunofluorescence showing Flag and GFP staining in HEK293 cells 72 hours after transfection. Flag labelling revealed with anti-Flag antibody (in red) shows a strong decrease of intensity with Synj1-shRNA. Note that random-shRNA did not alter Flag staining intensity. * $p < 0.05$