Supplemental Materials

Molecular Biology of the Cell

Toh et al.

Supplementary Figure Legends

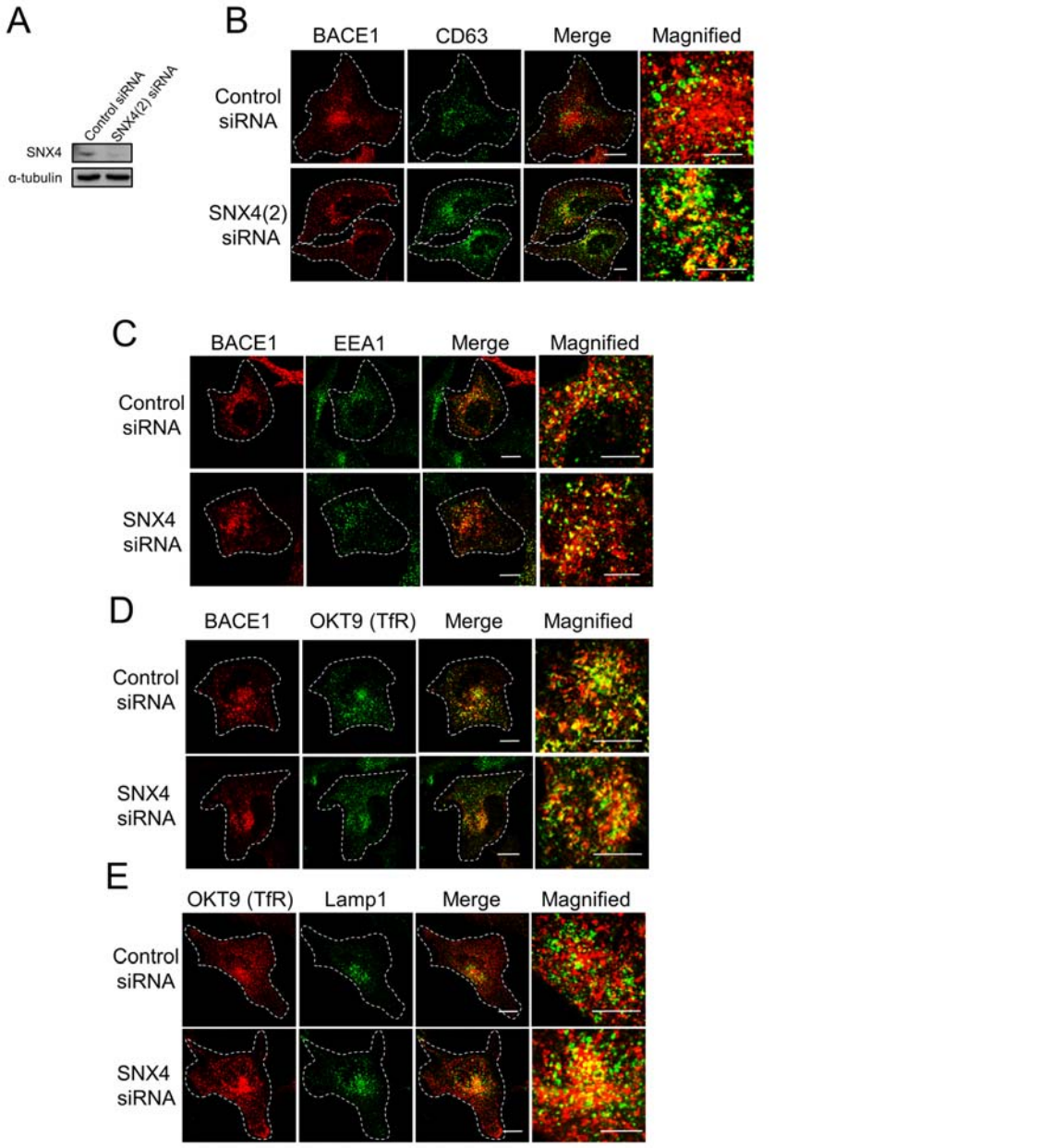

Figure S1: SNX4 depletion increases co-localization of BACE1 with EEA1 and CD63 but has minimal effect on co-localization with the transferrin receptor

HeLa cells transfected with either control siRNA or (A-B) SNX4(2) or (C-E) SNX4 siRNA for 72 h and transfected with (B-D) wtBACE1 constructs for a further 24 h.

A Immunoblotting of cell extracts with (A) goat polyclonal SNX4 antibody and mouse anti-α-tubulin antibody, using a chemiluminescence detection system.

B-D Monolayers were fixed and permeabilized and stained with rabbit polyclonal anti-human BACE1 antibodies (red) and (B) mouse polyclonal antibodies to CD63 (green) or (C) mouse monoclonal antibodies to EEA1 (green) or (D) mouse polyclonal antibodies to TfR (OKT9) (green). Higher magnifications of the merge images are also shown. Bar represents 10 μm.

E Monolayers were fixed and permeabilized and stained with mouse polyclonal antibodies to TfR (OKT9) (red) and rabbit polyclonal antibodies to Lamp1 (green). Higher magnifications of the merge images are also shown. Bar represents 10 μm.

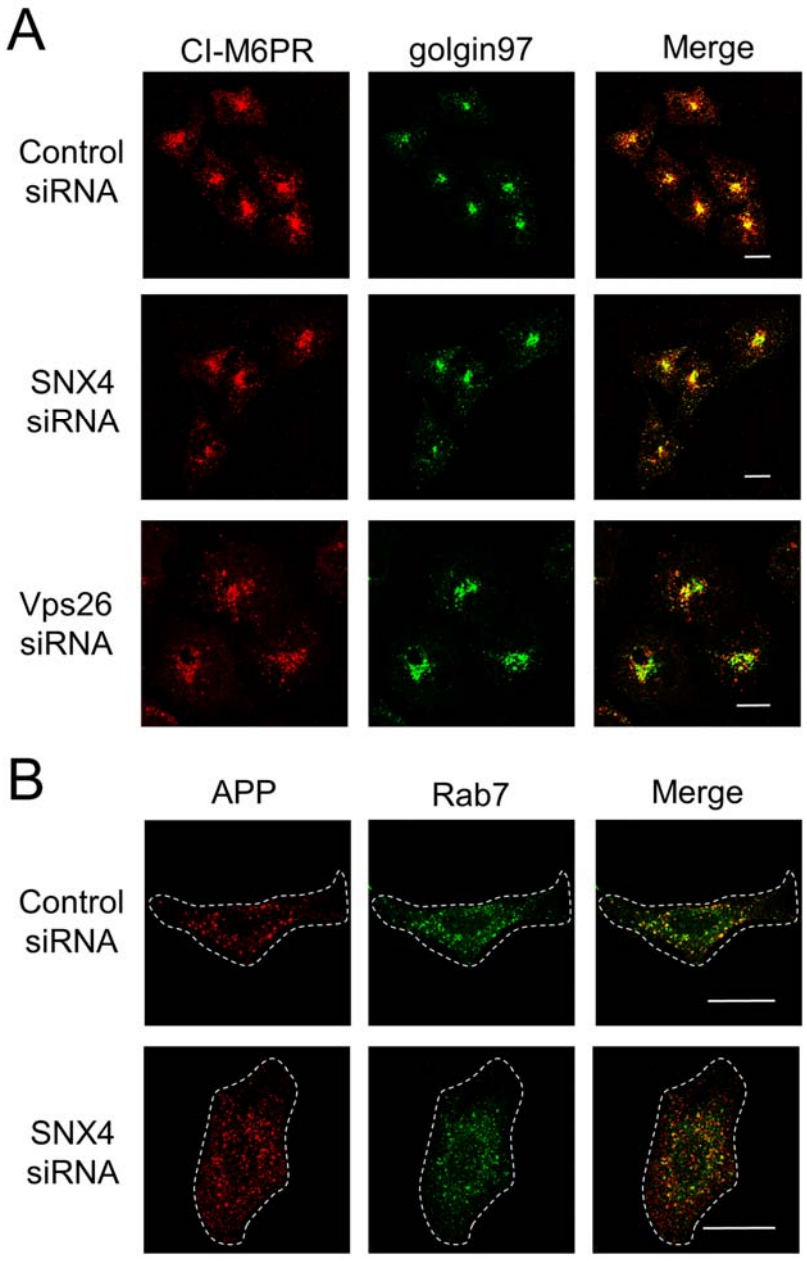

Figure S2: Steady-state distribution of cation-independent mannose-6-phosphate receptor (CI-M6PR) and APP are unaffected by SNX4 depletion

A Confocal images of HeLa cells transfected with either control siRNA or SNX4 siRNA or Vps26 siRNA for 72 h, monolayers fixed and permeabilized and stained with rabbit polyclonal anti-CI-M6PR antibodies and mouse monoclonal antibodies to golgin97. Bars represent 10 μm.

B Confocal images of HeLa cells stably expressing APP transfected with either control siRNA or SNX4 siRNA for 72 h, monolayers fixed and permeabilized and stained with mouse monoclonal APP antibodies (red) and rabbit polyclonal antibodies to Rab7 (green). Bars represent 10 μm.

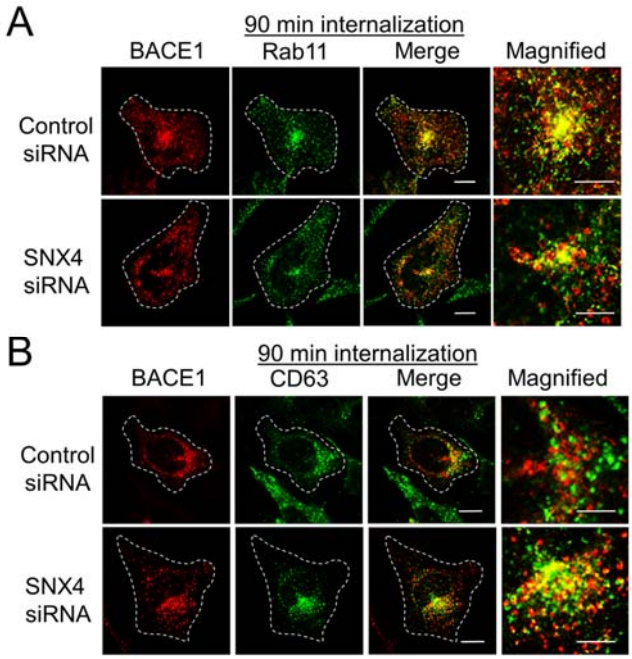

Figure S3: Reduced trafficking of BACE1 to the recycling endosomes in SNX4-depleted cells

Confocal images of HeLa cells transfected with either control siRNA or SNX4 siRNA for 72 h and then transfected with wtBACE1 for a further 24 h. Transfected cells were incubated with anti-BACE1 antibodies on ice for 30 min and the monolayers were shifted to 37ºC for 90 min before fixation and permeabilization. Cells were stained for the internalized BACE1-antibody complexes with Alexa-conjugated secondary antibodies and either (A) mouse monoclonal antibodies to Rab11 or (B) mouse polyclonal antibodies to CD63. Higher magnifications of the merge images are also shown. Bars represent 10 μm.

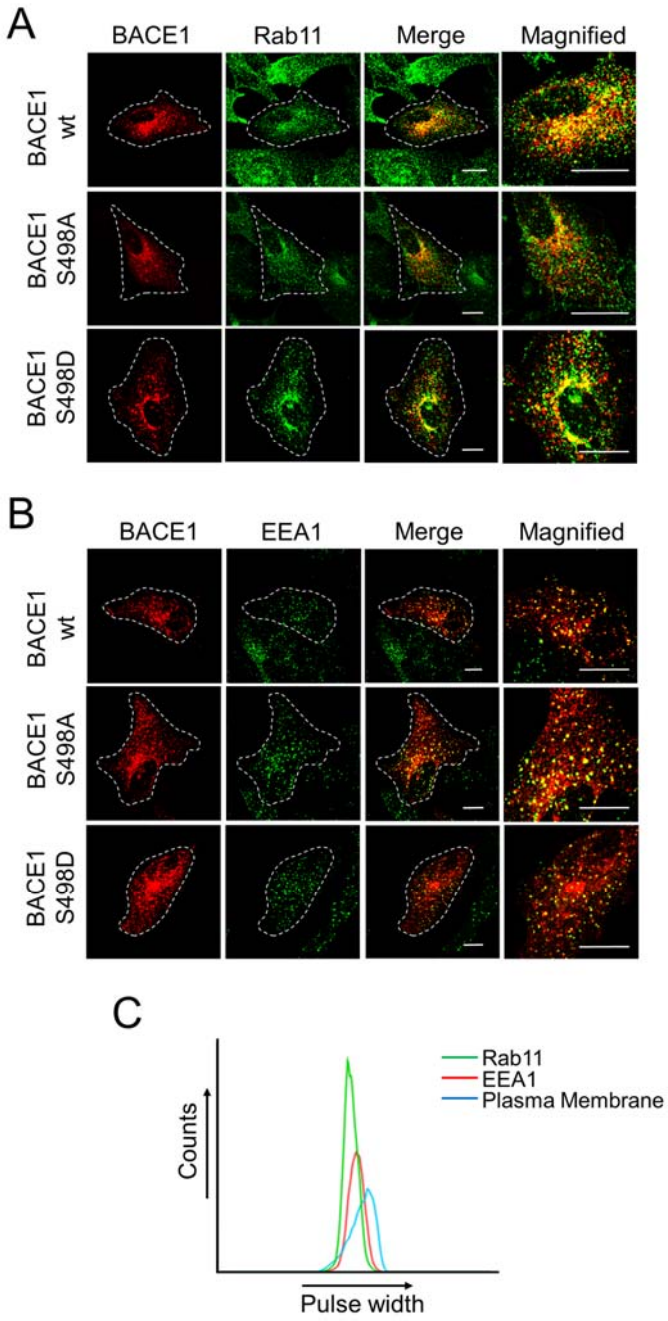

Figure S4: Steady-state distribution of BACE1 phospho-mutants in SK-N-SH cells and Pulse-Width analysis (PulSA) by flow cytometry

A, B Confocal images of SK-N-SH cells transfected with either wtBACE1 or BACE1 phospho-mutants constructs for 24 h, fixed and permeabilized and stained with rabbit polyclonal anti-human BACE1 antibodies and (A) mouse monoclonal antibodies to Rab11 or (B) mouse monoclonal antibodies to EEA1. Higher magnifications of the merge images are also shown. Bars represent 10 μm.

C PulSA: HeLa monolayers were harvested by incubation with 5 mM EDTA/PBS and cells in suspension fixed and stained with either mouse anti-Rab11, mouse anti-EEA1 antibodies or surface stained with OKT9 (TfR) antibodies. Stained cells were analysed by flow cytometry and 10,000 events were collected per sample. Histograms of pulse width are shown. A rightward shift in the histogram represents an increased pulse width while a leftward shift represents a decreased pulse width. Histograms were overlaid using Flowjo.

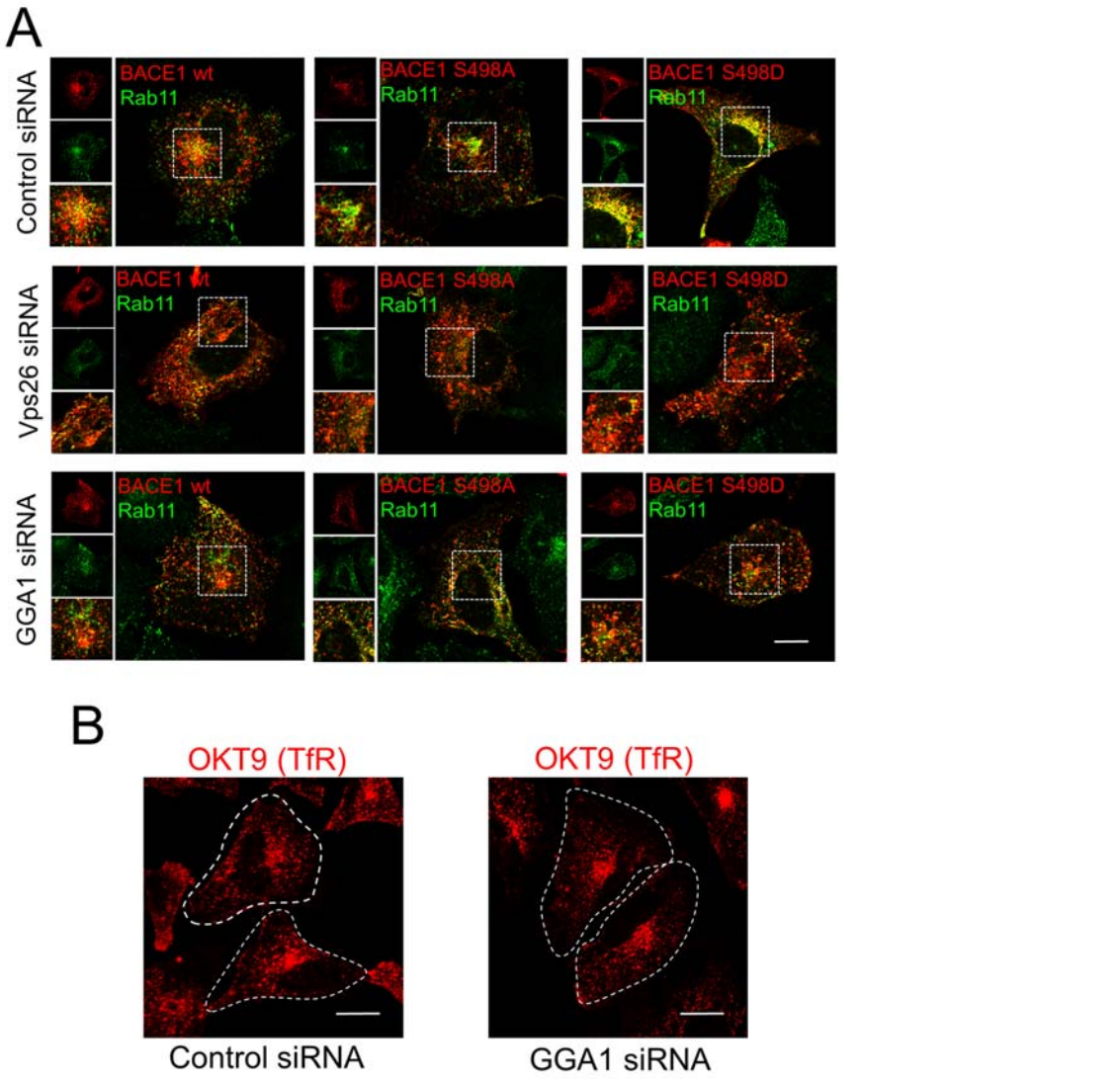

Figure S5: Steady-state distribution of BACE1 phospho-mutants and transferrin receptor following GGA1 depletion

A Confocal images of HeLa cells transfected with either control siRNA, Vps26 or GGA1 siRNA for 72 h and transfected with either wtBACE1 or BACE1 phospho-mutants constructs for a further 24 h. Monolayers were fixed and permeabilized and stained with rabbit polyclonal anti-human BACE1 antibodies (red) and mouse polyclonal antibodies to Rab11 (green). Higher magnifications of the merge images are also shown. Bar represents 10 µm.

B Confocal images of HeLa cells transfected with either control siRNA or GGA1 siRNA for 72 h. Monolayers were fixed, permeabilized and stained with mouse monoclonal antibodies to transferrin receptor (TfR). Bars represent 10 µm.

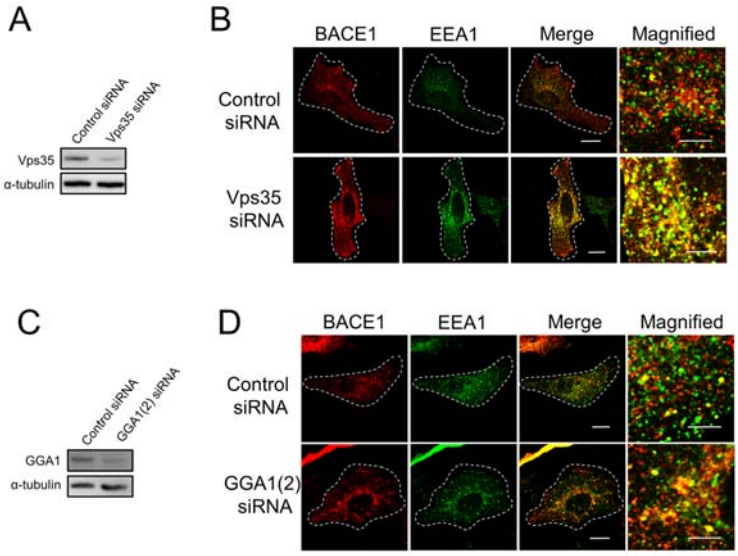

Figure S6: Vps35 and GGA1 depletion increases co-localization of BACE1 with EEA1

A-D HeLa cells transfected with either control siRNA, Vps35 or GGA1(2) siRNA for 72 h and transfected with wtBACE1 constructs for a further 24 h.

A, C Immunoblotting of cell extracts with (A) mouse monoclonal Vps35 antibody or (C) rabbit monoclonal GGA1 antibody and mouse anti-α-tubulin antibody, using a chemiluminescence detection system.

B, D Monolayers were fixed and permeabilized and stained with rabbit polyclonal anti-human BACE1 antibodies (red) and mouse monoclonal antibodies to EEA1 (green). Higher magnifications of the merge images are also shown. Bar represents 10 µm.