Supplementary information

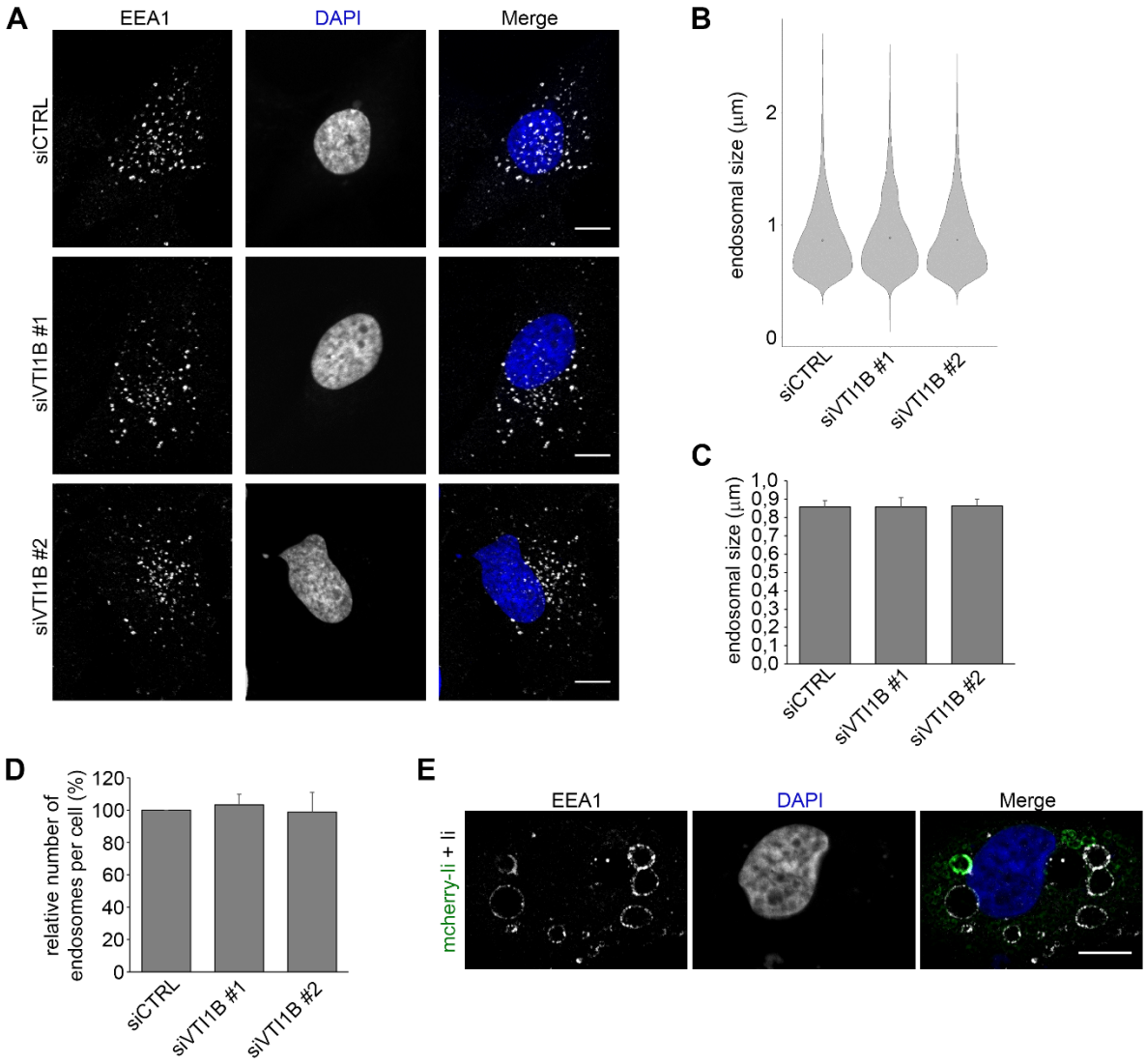

Figure S1

Figure S1. Size of early endosomes is not altered in M1 cells depleted of Vti1b compared to control cells. A) M1 cells (not expressing Ii) have been treated with control siRNA (siCTRL) or siRNAs against VTI1B (siVTI1B #1 or siVTI1B #2). Subsequently, cells have been fixed and stained with α-EEA1 antibody and DAPI. Maximum projections are shown (Scale bars: 10 µm B). Violin plot showing the distribution of the size of endosomes positive for EEA1 in control and VTI1B-depleted cells. Size of at least 2500 endosomes per sample have been analysed. C) Quantification of the average endosomal size in control or VTI1B-depleted cells. Data represent the mean ± s.e.m. of three independent experiments. At least 48 cells per condition have been analysed. D) Quantification of the number of endosomes in control cells and in cells silenced for VTI1B. Data represent the mean ± s.e.m. of three independent experiments. At least 48 cells per condition have been analysed. E) M1 cell transfected with mcherry-Ii and untagged-Ii and stained with α-EEA1 antibody and DAPI is shown. Enlarged EEA1-positive endosomes are shown. Scale bar: 10 µm.

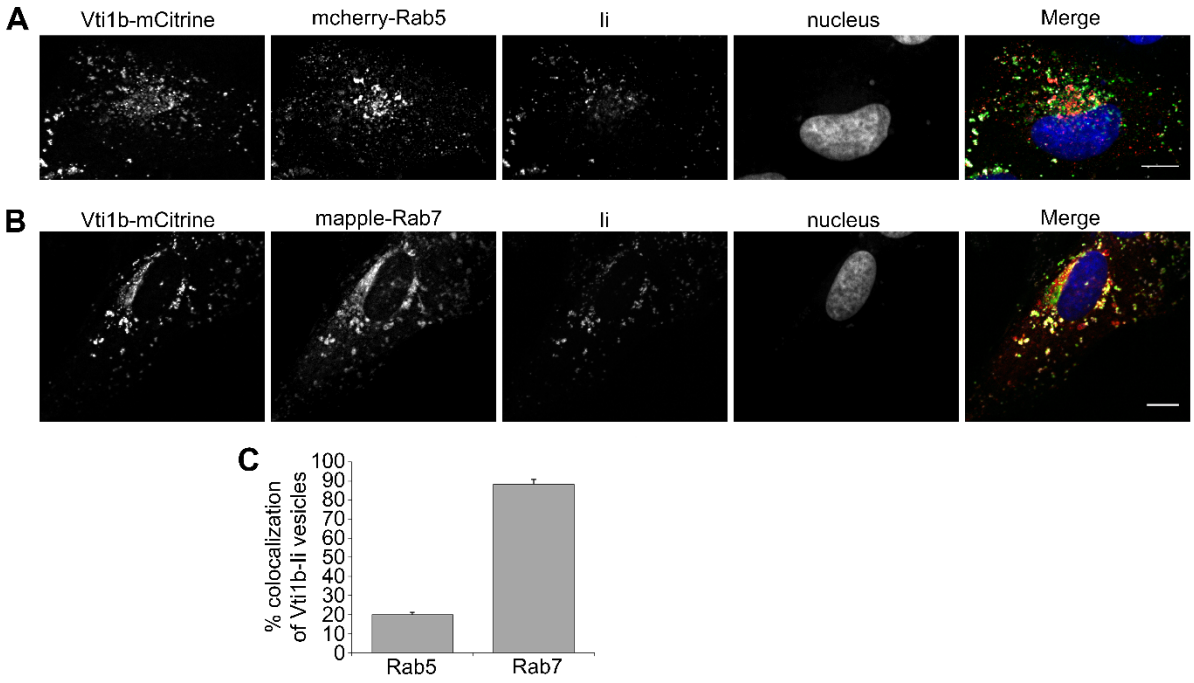

Figure S2

Figure S2. Vti1b and Ii colocalize together on Rab5- or Rab7-positive endosomes. A) MelJuSo control cells have been transfected with Vti1b-mCitrine (green) and mcherry-Rab5 (red) and subsequently fixed and stained for Ii and nuclei. Maximal projections are shown. Scale bar: 10 µm. B) MelJuSo control cells have been transfected with Vti1b-mCitrine (green) and mapple-Rab7 (red) and subsequently fixed and stained for Ii and nuclei. Scale bar: 10 µm. Maximal projections are shown. C) Percentage of colocalization of Vti1b and Ii on Rab5-positive vesicles or Rab7-positive vesicles is indicated. Experiments have been performed in triplicate. Averages ± s.e.m. are indicated. Number of endosomes analysed was 411 per mcherry-Rab5 transfected cells and 767 for mapple-Rab7 transfected cells.

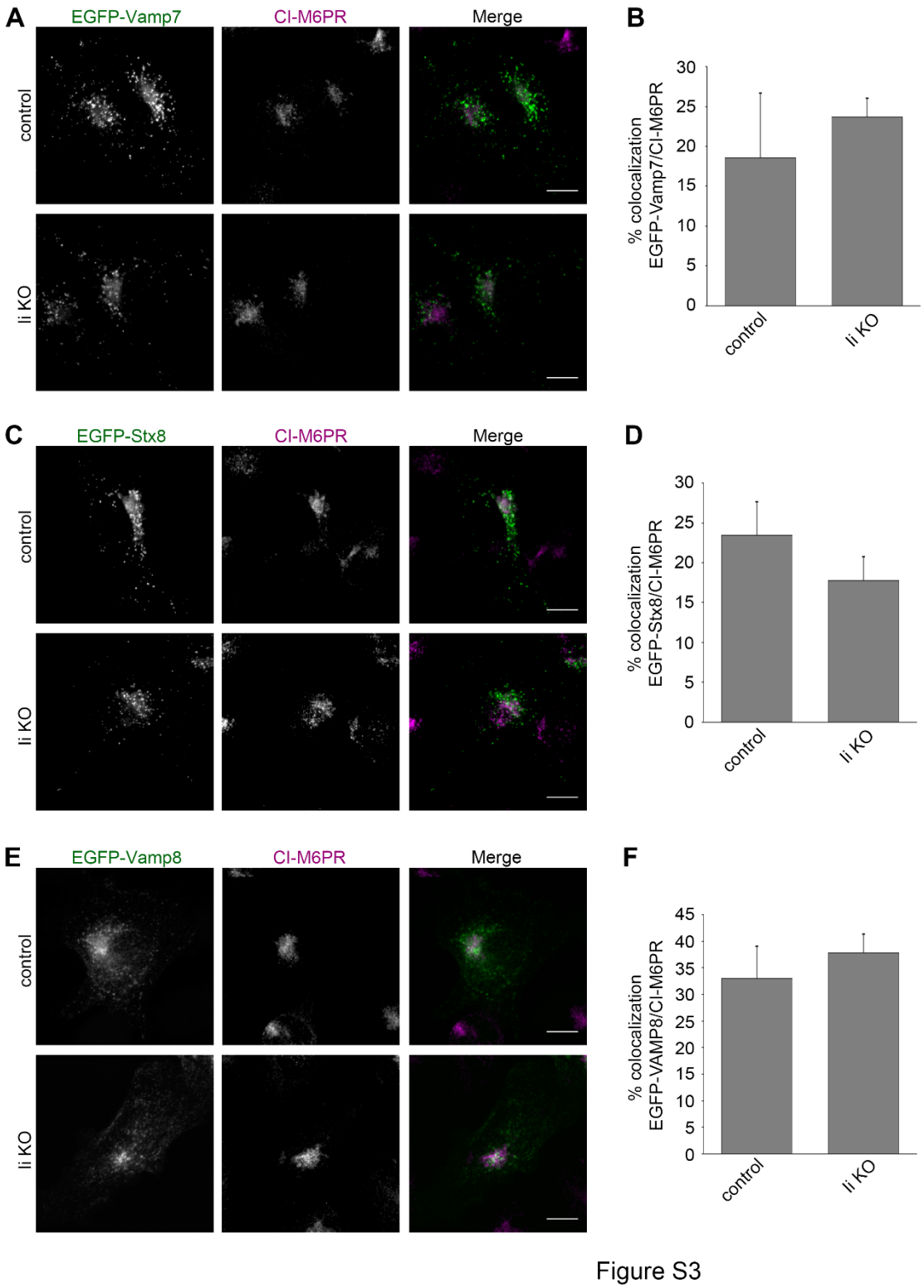

Figure S3

Figure S3. Localization of Vamp7, Stx8 and Vamp8 is not altered in MelJuSo Ii KO cells. A) MelJuSo control and Ii KO cells have been transfected with EGFP-Vamp7 and subsequently stained after fixation with an anti-CI-M6PR antibody. Representative images (maximal projections) of EGFP-Vamp7 (green) and CI-M6PR (magenta) and merge are shown. Scale bars: 10 µm. B) Quantification of the percentage of colocalization between

EGFP-Vamp7 and CI-M6PR in control and Ii KO cells is shown. C) MelJuSo control and Ii KO cells have been transfected with EGFP-Stx8 and subsequently stained after fixation with an anti-CI-M6PR antibody. Representative images (maximal projections) of EGFP-Stx8 (green) and CI-M6PR (magenta) and merge are shown. Scale bars: 10 µm. D) Quantification of the percentage of colocalization between EGFP-Stx8 and CI-M6PR in control and Ii KO cells is shown. E) MelJuSo control and Ii KO cells have been transfected with EGFP-Vamp8 and subsequently stained after fixation with an anti-CI-M6PR antibody. Representative images (maximal projections) of EGFP-Vamp8 (green) and CI-M6PR (magenta) and merge are shown. Scale bars: 10 µm. D) Quantification of the percentage of colocalization between EGFP-Vamp8 and CI-M6PR in control and Ii KO cells is shown.

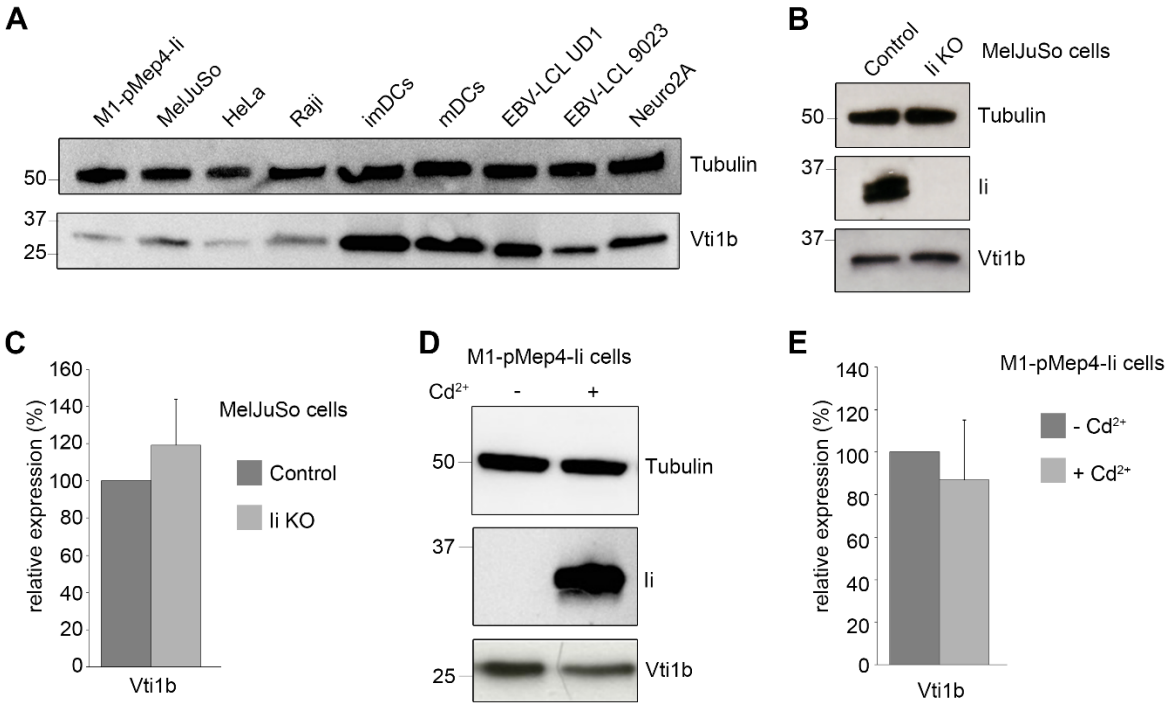

Figure S4

Fig. S4. Expression levels of candidate proteins in different cell types. A) Lysates of several cell types as indicated in the figure have been subjected to western blot analysis using anti-Vti1b and anti-tubulin antibodies. B) MelJuSo control and Ii KO cells have been lysed and relative samples have been subjected to western blot analysis. Antibodies against Vti1b, Ii and tubulin have been used. C) Quantification of Vti1b abundance in MelJuSo control and Ii KO cells. Data represent the mean ± s.e.m. of three independent experiments. D) Control M1-pMep4-Ii wt cells not expressing Ii ($-Cd^{2+}$) or after treatment with 7µM $CdCl_2$ overnight to induce the expression of Ii ($+Cd^{2+}$) have been lysed. Lysates were subjected to western blot analysis using anti-Vti1b, anti-Ii and anti-tubulin antibodies. E) Quantification of Vti1b abundance in M1-pMep4-Ii wt cells expressing ($+Cd^{2+}$) or not ($-Cd^{2+}$) Ii is shown. Data represent the mean ± s.e.m. of three independent experiments.

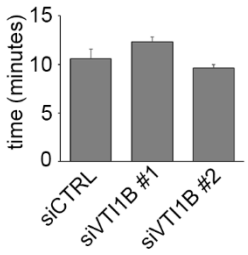

Figure S5

Fig. S5. Vti1b depletion does not affect endosomal maturation in absence of Ii. Quantification of the time until the Rab5-Rab7 switch is shown. Mean ± s.e.m. for at least three independent experiments. 65 endosomes were analysed for control siRNA, 30 for Vti1b siRNA #1, and 53 for Vti1b siRNA #2 cells) from several cells. The measurements of each individual endosome depend on its visibility in time and space and, therefore, are not precisely accurate.