The SNARE protein Vti1b is recruited to the sites of BCR activation but redundant for antigen internalisation, processing and presentation

Running title: Vti1b in B cell activation

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Keywords: Adaptive immune system, B cells, intracellular traffic, BCR, signalling, SNARE, Vti1b

Supplementary Information
Supplementary Figure 1. (A) A20 D1.3 B cells were transfected with human GFP-Vti1b (green) to test the specificity of the Vti1b antibodies (magenta; Proteintech). Scale bar: 5 µm. (B) Non-transfected Raji D1.3 (human) or A20 D1.3 (mouse) B cells were stained with anti-Vti1b antibodies (Proteintech) to detect the endogenous protein. Scale bar: 5 µm. (C) A20 D1.3 cells were transfected with GFP or GFP-Vti1b and analysed by flow cytometry. The percentage of transfected cells (GFP+) is shown. (D) Untransfected or transfected A20 D1.3 were stained with anti-IgM (anti-BCR) or anti-MHCII antibodies and analysed by flow
cytometry. The geometric mean is shown. Every dot represents one measurement. (E) Live imaging of GFP-Vti1b transfected A20 D1.3s to better visualise the plasma membrane structures. Cells were transfected, seeded on MatTek dishes and imaged using a spinning disk confocal microscope without PFA fixation. Left: plane. Right: 3D stack reconstruction. (F) A20 D1.3 B cells were transfected with human GFP-Vti1b (green) and stained with WGA-AF633 (magenta) to check the plasma membrane localisation. Scale bar: 5 µm. (G) GFP-Vti1b transfected cells were analysed using Fiji ImageJ. The MFI (total, membrane and intracellular; see schematics) was obtained and the total intensity was plotted against the membrane accumulation (ratio membrane/intracellular) to analyse the potential correlation between overexpression (total MFI) and membrane localisation of Vti1b. Every dot represents one cell.

Supplementary Figure 2. Supplement to Figure 3E-G. A20 D1.3 cells transfected with GFP-Vti1b (green) were seeded on anti-IgM coated glass for 30 min and stained with anti-Rab6, Rab7 or Rab11 (magenta) and phalloidin (actin; yellow). Scale bar: 5 µm.
Supplementary Figure 3. (A) Surface levels of anti-IgM BCR in non-stimulated primary cells isolated from the spleen of Vti1b HEZ or KO mice. (B) Cell Trace Violet (CTV) labelled OT-II T cells were incubated with WT, HEZ or Vti1b KO B cells activated with anti-IgM or anti-IgM:OVA (2 different ratios) coated beads for 3 days. After 3 days, T cell proliferation in response to OVA peptide presentation is measured by flow cytometry.

Supplementary Movie 1. A20 D1.3 cells transfected with GFP-Vti1b were pre-labelled with anti-IgM-FITC-647 antibodies (“antigen”) on ice and seeded on poly-L-lysine coated MatTek dishes. After 10-15 minutes incubation at 37 °C to allow activation and internalisation of the antigen, a quencher probe was added and cells were imaged using a spinning disk confocal microscope (EVOLVE camera). Magenta: Vti1b-GFP. Cyan: internalised antigen. Merge. One plane was acquired every 5 seconds. Scale bar: 5 µm.

Supplementary Table 1. List of antibodies used in the study.

| Antibody               | Company                   | Clone/Number | Dilution | Technique |
|------------------------|---------------------------|--------------|----------|-----------|
| Anti-Rab5              | Cell Signalling Technologies (CST) | C8B1         | 1:150    | IF        |
| Anti-Rab7              | CST                       | D95F2        | 1:100    | IF        |
| Anti-Rab11             | CST                       | D4F5         | 1:200    | IF        |
| Anti-Rab6              | CST                       | D37C7        | 1:200    | IF        |
| Anti-PCMI-647          | Santa Cruz                | G-6          | 1:200    | IF        |
| Anti-Vti1b             | Proteintech               | 14495-1      | 1:100    | IF        |
| Donkey anti-Rabbit IgG (H+L) AF555 | Thermo                   | A-31572      | 1:500    | IF        |
| Anti-pCD19             | Cell Signalling Technologies (CST) | 3571         | 1:1000   | WB        |
| Anti-pSyk              | CST                       | 2701         | 1:1000   | WB        |
| Anti-pAKT              | CST                       | 4058         | 1:1000   | WB        |
| Anti-pERK1/2           | CST                       | 9101         | 1:1000   | WB        |
| Anti-Vti1b             | Produced in-house (Antonin et al., 2000a) | 1:1000      | WB        |
| Anti-GAPDH             | Proteintech               | 60004-1-Ig   | 1:10000  | WB        |
| Anti-tubulin           | Proteintech               | 66240-1-Ig   | 1:10000  | WB        |