Expanded View Figures

A

WT

Arhgap45

5'UT-Ex1 Ex3 Ex4 Ex6 Ex7 Ex8 Ex9 Ex10 Ex22 Ex23-3'UT

5'UT-Ex1 Ex3 Ex4 Ex6 Ex7 Ex8 Ex9 Ex10 Ex22 Ex23-3'UT

B

WT

ARHGAP45

ARHGAP45

ARHGAP45

ARHGAP45

WT

ARHGAP45

ARHGAP45

Anti-ARHGAP45

Anti-β-ACTIN

T cells

B cells

C

Arhgap45

FRT

LoxP

LacZ

Neo

5'UT-Ex1 Ex2

5'UT-Ex1 Ex2

5'UT-Ex1 Ex2

5'UT-Ex1 Ex2

Ex3 Ex4 Ex5 Ex6 Ex7 Ex8 Ex9 Ex10

Ex3 Ex4 Ex5 Ex6 Ex7 Ex8 Ex9 Ex10

Ex3-Ex10

Ex3-Ex10

Ex3-Ex10

Ex3-Ex10

5'UT-Ex1 Ex2

5'UT-Ex1 Ex2

5'UT-Ex1 Ex2

5'UT-Ex1 Ex2

Ex11 Ex22 Ex23-3'UT

Ex11 Ex22 Ex23-3'UT

Ex11 Ex22 Ex23-3'UT

Ex11 Ex22 Ex23-3'UT

Arhgap45

Δ

Flp

CD4-Cre

Arhgap45

Δ

Δ

Figure EV1.
Figure EV1. Generation of mutant mouse lacking ARHGAP45, or expressing a loxP-flanked Arhgap45 allele.

A Schematic representation of the WT Arghap45 and Arghap45<sup>−/−</sup> alleles. Exons 1–23 are shown and numbered and the 5' and 3' UTR shown as gray box. The deletion engineered in the Arghap45<sup>−/−</sup> allele encompasses exon 4 (http://www.ensembl.org/Mus_musculus/Transcript/Summary?db=core;g=ENSMUSG00000035697;r=10:80016653-80031472;t=ENSMUST00000099501).

B Immunoblot analysis of equal amounts of total lysates of thymocytes (left panel) and of B and T cells (right panel) purified from WT, Arghap45<sup>−/−</sup>, and Arghap45<sup>ΔT/ΔT</sup> mice probed with anti-ARHGAP45 and anti-β-Actin (loading control). Molecular weights are shown on the left. Results are representative of two experiments.

C Schematic representation of the Arhgap<sup>45<sup>tm1a</sup></sup>, Arhgap<sup>45<sup>fl</sup></sup>, and Arhgap<sup>45<sup>ΔT</sup></sup> alleles. See “Generation of mice with a loxP-flanked Arhgap45 allele and of mice conditionally deprived of ARHGAP45 in T cells” in Materials and Methods. Exons 1–23 are shown and numbered and the 5' and 3' UTR shown as gray boxes.

Source data are available online for this figure.

Figure EV2.
Figure EV2. Numbers of B and T cells in the specified organs of WT mice and of mice deficient in ARHGAP45 (Arhgap45<sup>−/−</sup>), or expressing a loxP-flanked Arhgap45 allele prior to (Arhgap45<sup>fl/fl</sup>) or after (Arhgap45<sup>ΔT/ΔT</sup>) crossing with CD4-Cre transgenic mice.

A Cellularity of thymus from the specified mice.
B Numbers of T cells found in the blood of the specified mice.
C Numbers of B cells found in the blood of the specified mice.
D Numbers of T cells found in the LNs of the specified mice.
E Numbers of B cells found in the LNs of the specified mice.
F Numbers of T cells found in the spleen of the specified mice.
G Numbers of B cells found in the spleen of the specified mice.

Data information: Each dot corresponds to a mouse and the mean and SD are indicated. Data are representative of three independent experiments involving each a total of 8–16 mice. A one-way Anova test was used to compare each mouse model against WT mouse controls. The resulting probability is indicated above each model. ns, non-significant, **P ≤ 0.002, ****P ≤ 0.0001.

Figure EV3. Naive T cells do not polarize and migrate on ICAM-1 coated surface in absence of added chemokine.

A Analysis of migration patterns of WT and Arhgap45<sup>−/−</sup> naive T cells on 2D surface coated with ICAM-1. Each track represents the migratory path of individual WT and Arhgap45<sup>−/−</sup> naive T cells recorded over 466 s in a single field of view. Trajectories were plotted to a common starting point, and > 200 T cells were recorded per plot using time-lapse microscopy at 10× magnification.
B Average cell speed of WT cell (black) and Arhgap45<sup>−/−</sup> naive T cells (red). Three independent experiments were performed involving more than 200 cells and each dot corresponds to the mean of the speed in one given experiment. The speed of 10 μm/min correspond to Brownian motion, which means that cells are not motile (two-tailed Student’s t-test).
Figure EV4. Analysis of the projected adhesion area of WT and ARHGP45^−/− naive T cells on 2D surface coated with ICAM-1 and CCL21.

A–F Processing of images from RICM microscopy to infer projected adhesion area. The projected area of cells is extracted from bright field images (A), which are binarized (B) to extract the contour in red (C). The area of adhesion fingerprint is assessed from RICM images (D) that are inverted and binarized (E) to extract the area of the contact zone in green (F). To illustrate image processing, the final image of the migration sequence shown in Fig 6C has been used. Scale bar: 10 µm.

G, H Histograms of instant projected adherent area for WT (G) and ARHGP45^−/− (H) naive T cells on 2D surface coated with ICAM-1 and CCL21.
Figure EVS. Analysis of hematopoietic progenitors in the BM of WT and Arhgap45<sup>−/−</sup> mice.

A Gating strategy for the specified BM hematopoietic cell progenitors as described in Cordeiro Gomes et al (2016). HSC: hematopoietic stem cells, MPP: multipotent progenitors, CLP: common lymphoid progenitors, CMP: common myeloid progenitors, GMP: granulocyte and monocyte progenitors, and MEP: megakaryocyte and erythroid progenitors.

B Numbers of HSC, MPP and CLP per femur of WT and Arhgap45<sup>−/−</sup> mice.

C Numbers of CMP, GMP and MEP per femur of WT and Arhgap45<sup>−/−</sup> mice.

Data information: Each dot corresponds to a mouse and the mean and SD are indicated. Data are representative of three independent experiments. *P ≤ 0.01, ***P ≤ 0.001, unpaired Student’s t-test.