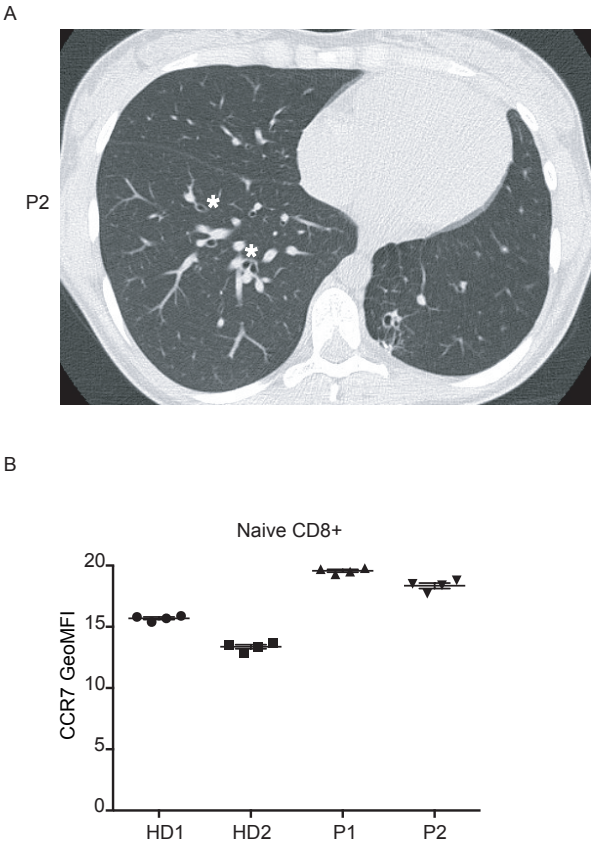
Supplementary Information

Supplementary Table 1. Annotated nonsense mutations in the *ARHGEF1* gene (as of January 2018).

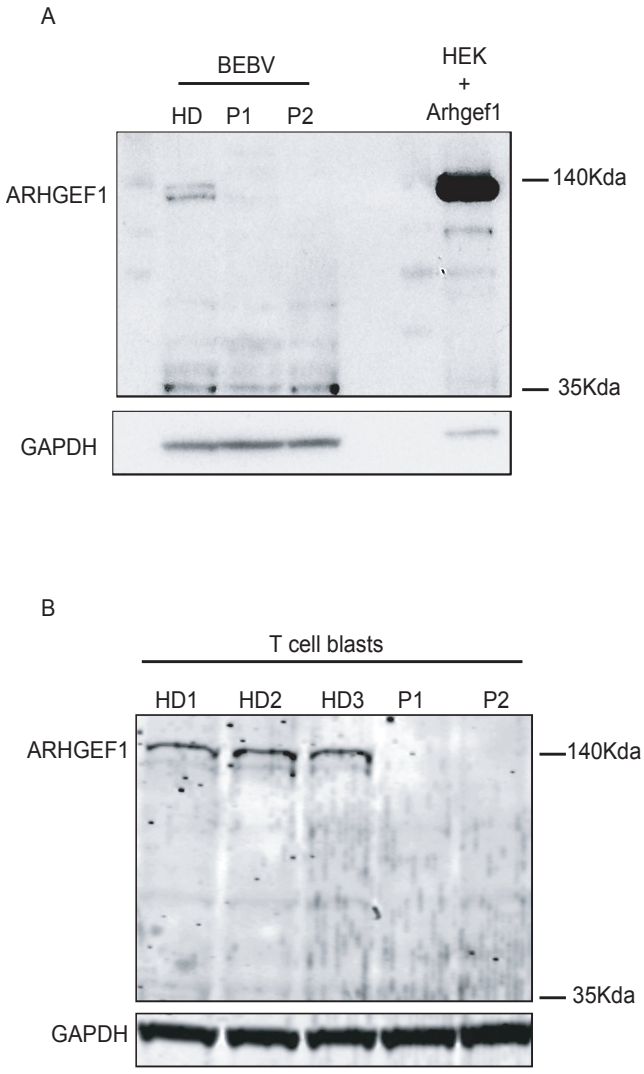
EXON	Type of mutation	Consequence	rs number	Minor allele frequency	Database
2	nonsense	7WX*	782018258	0.00001	ExAC
12	nonsense	R300X			
14	nonsense	E386X	370619761	0.00008	ESP
17	nonsense	Y502X	782346763	0.000008	dbSNP
18	splice-site	E557Kfs34X			
24	nonsense	Q758X	781877132	0.000004	ExAC
24	nonsense	S761X	138482475	0.000004	ExAC
28	nonsense	Q851X	45580632	0.000005	ExAC
28	nonsense	Q883X	782187203	0.000004	ExAC

ExAC: Exome Aggregation Consortium; ESP: Exome Sequencing Project; dbSNP: Short Genetic Variations Database; * only present in NM_199002.1, the longest annotated ARHGEF1 transcript; variations investigated in the present study are shown in blue type.



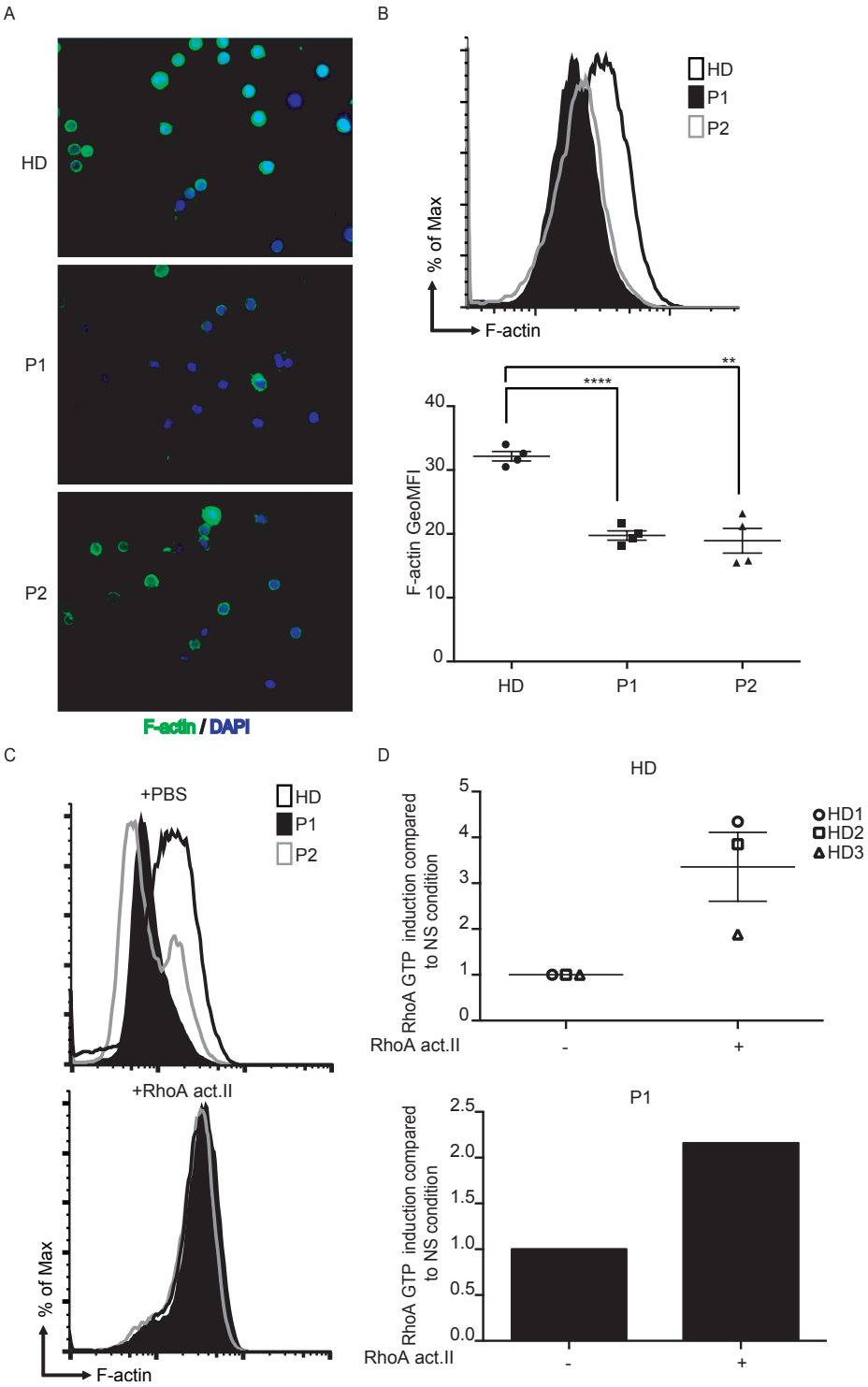
Supplementary Information Figure 1. Patients with ARHGEF1 deficiency display bronchiectasis and elevated CCR7 expression in the naïve CD8+ compartment.

A) Thoracic computed tomography scans of P1's lungs at the age of 20, showing mild bronchiectasis (white stars) and a small left lower lobe (due to previous lobectomy). **B)** Dot plot of a representative experiment showing the expression of CCR7 in the naïve CD8+ T cell population in blood samples from healthy donors (HD1, circle; HD2, square) and patients (P1, up-pointing triangle; P2 down pointing triangle). Each blood sample was stained and analyzed four times. The experiment was performed twice.



Supplementary Information Figure 2. Effect of *ARHGEF1* splice-site mutation and the absence of ARHGEF1 truncated proteins in patients' cells

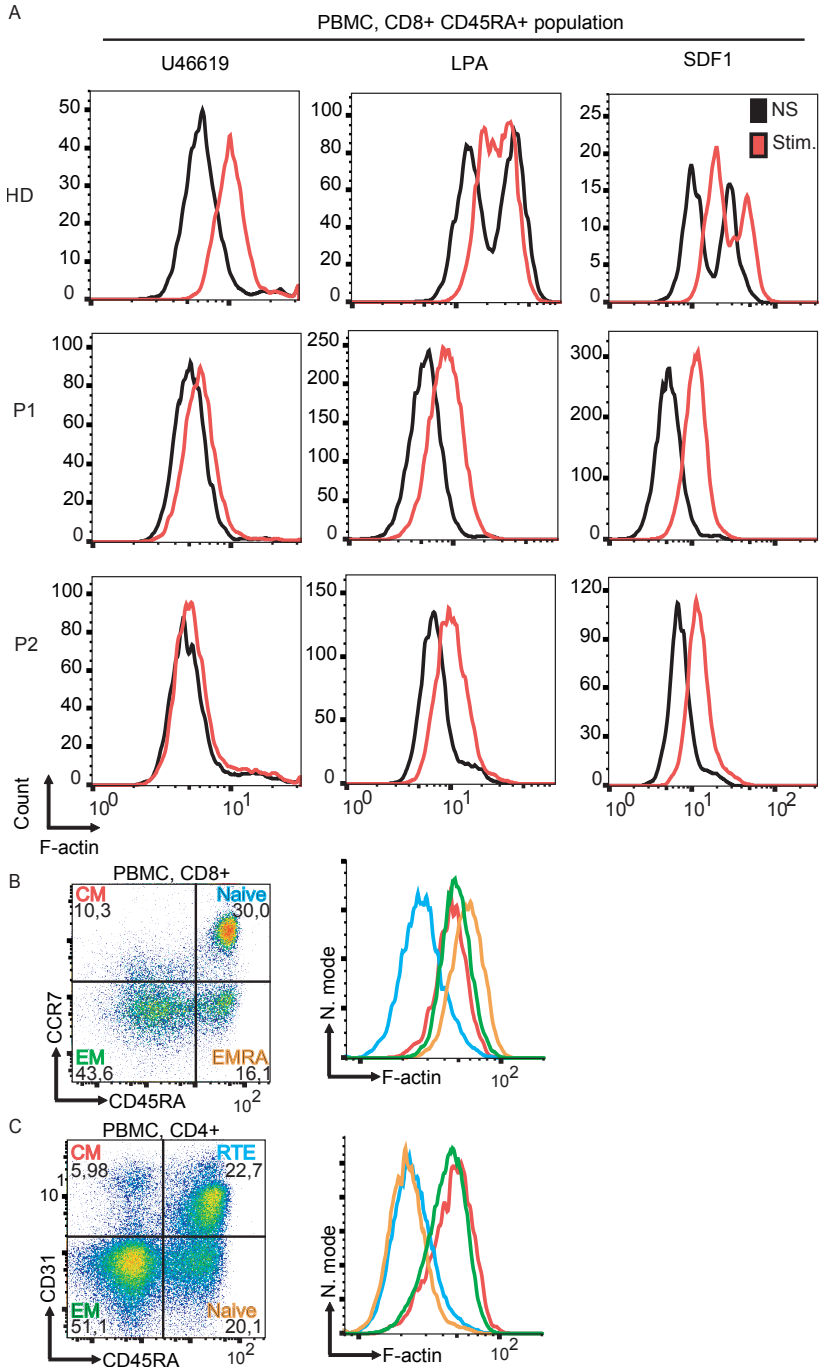
A and B) Western blots of ARHGEF1 expression in (A) B-EBV cell lines and (B) T cell blasts derived from patients (P1 and P2) and healthy donors (HD, n=3). Lysates of HEK 293 cells over-expressing ARHGEF1' protein were used to confirm the specificity of the antibody raised against the N-terminal part of ARHGEF1. GAPDH was included as loading control.



Supplementary Information Figure 3. Cultured T cell blasts derived from the patients recapitulate the F-actin defects observed *ex vivo*

A) Representative image of polymerized actin (F-actin) staining in T cell blasts derived from P1, P2 and a healthy donor (HD). DAPI staining highlights the nuclei

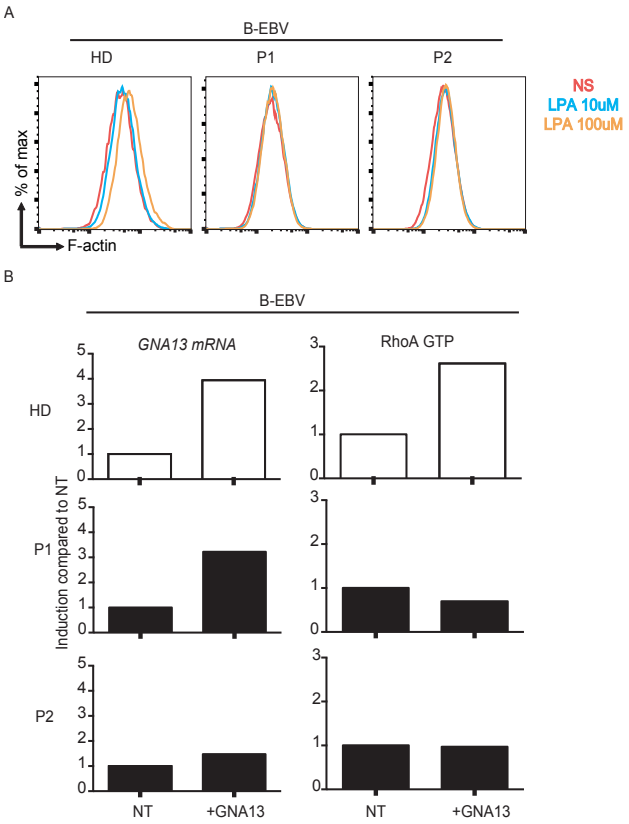
(magnification: x25). The experiment was performed twice. **B)** Representative FACS analysis of F-actin content in T cell blasts derived from patients and HDs. Dot plot of the geometric mean fluorescence intensity of F-actin (as determined by FACS) of a representative experiment in which T cell blasts from each culture (HD, circle; P1, square; P2, triangle) were stained and analyzed four times. **:$p<0.01$ and ****: $p<0.0001$ and in a 2-tailed Welch t-test. The experiment was performed 4 times. **C)** FACS plot showing the effect of the RhoA activator II (RhoA act., 32 µg/ml for 1hour) on actin polymerization in T cell blasts from patients and HD. The experiment was performed twice with T cell blasts from the same culture from P2 and the HD. D) ELISA measurement of active RhoA (RhoA GTP) after stimulation with RhoA activator II as in C. HD (n=3), P1 (n=1).



Supplementary Information Figure 4. F-actin polymerization in CD8+CD45RA+ T cells after stimulation with lysophospholipids

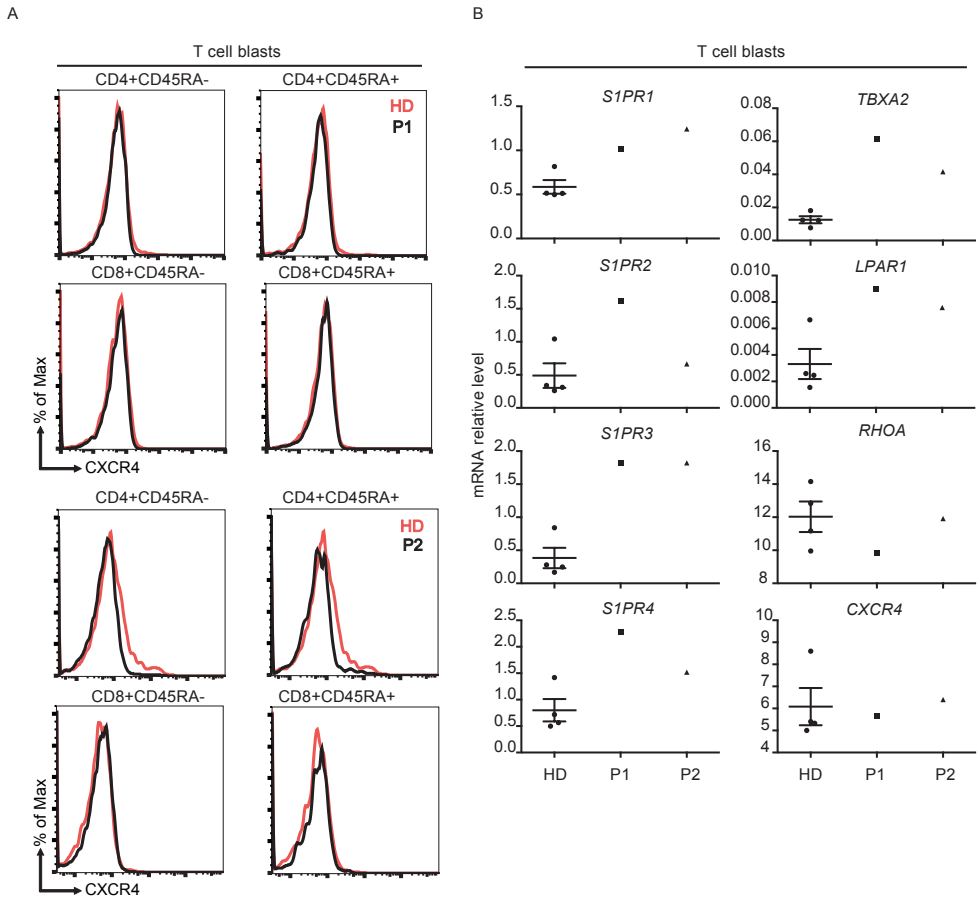
A) Representative FACS analyses showing the induction of actin polymerization in the CD8+CD45RA+ T cell compartment of PBMCs from P1, P2 and a healthy donor (HD) treated with various lysophospholipids. The experiment was performed twice. Cells were stimulated with S1P (10 µM for 1 min), the thromboxane analogue U46619 (1 µM

for 1 min) and LPA (7.7 μM for 15 mins). Stimulation with SDF1 (3ug/ml for 1 min) was assessed as a lysophospholipid-independent means of inducing actin polymerization. **B and C)** Representative FACS analysis of the level of polymerized actin (F-actin) in the different populations of CD8 (B) and CD4 (C) T cells from an HD. RTE: recent thymic emigrant cells; CM: central memory; EM: effector memory; EMRA: terminally differentiated effector memory cells. The experiment presented in **B** and **C** was performed 3 times.



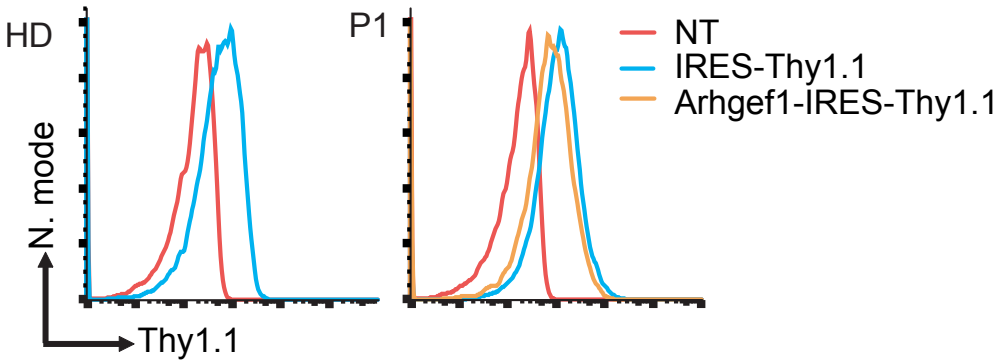
Supplementary Information Figure 5. B-EBV cells from patients exhibit impaired lysophospholipid activation of actin polymerization and Ga13-mediated RhoA activation

A) Representative FACS analysis of actin polymerization in B-EBV cell derived from patients and a healthy donor after stimulation (15mins) with the indicated dose of LPA. NS, non stimulated. The experiment was performed twice. **B)** *GNA13* mRNA level and the corresponding activity of RhoA measured by ELISA after lentiviral transduction of full length GNA13 construct in a healthy donor and patients derived B-EBV cells. NT, not transduced cells. The experiment was performed once.



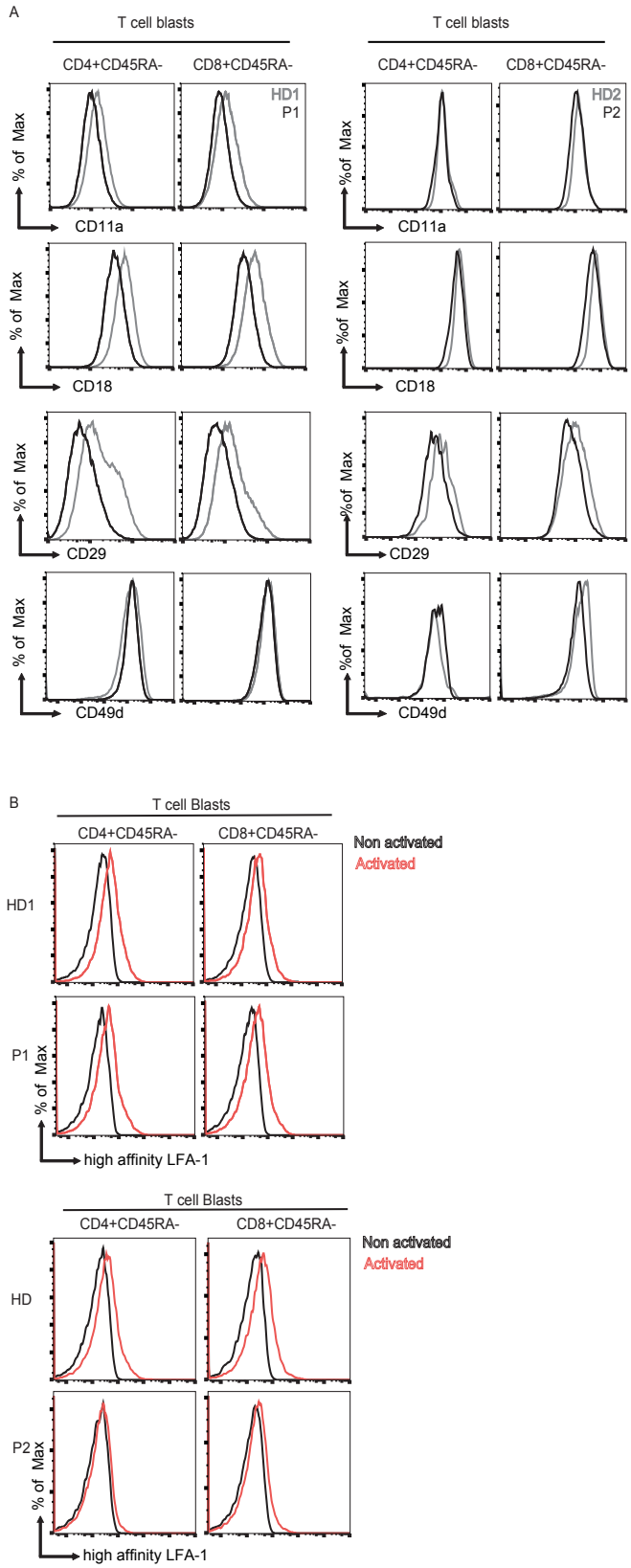
Supplementary Information Figure 6. CXCR4 and lysophospholipds receptors expressions are maintained in patients cells with ARHGEF1 deficiency

A) FACS analysis of two independent experiments showing surface expression of CXCR4 in the naïve (CD4+CD45RA+) and memory (CD4+CD45RA-) CD4 T cells compartments in patients (P1 and P2) and healthy donors T cell blasts cultures. The experiment was performed 3 times (HD, n=3; P1, n=1 and P2, n=2). **B)** mRNA expression analysis of the indicated lysophospholipid receptors in healthy donor (HD, n=4) and patients (P1, n=1 and P2, n=1) T cell blast cultures.



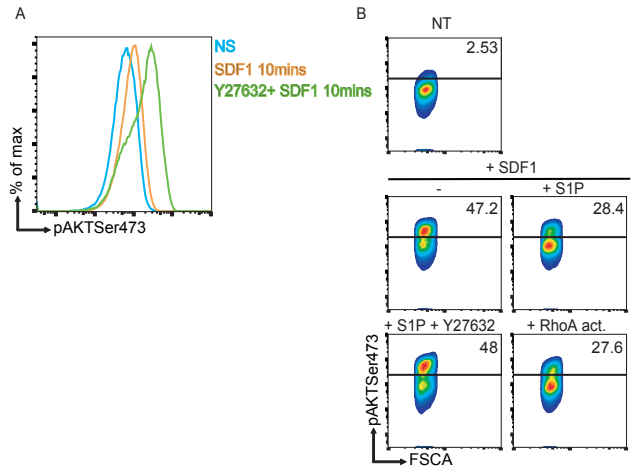
Supplementary Information Figure 7. Evaluation of ARHGEF1 retroviral transduction efficiency

FACS plots showing the efficiency of retroviral transduction for either a Thy1.1 or Thy1.1 plus ARHGEF1 construction, followed by the expression of the Thy1.1 marker in T cell blasts from a healthy donor and from P1 48 hours post-transduction. NT, not transduced cells. The experiment was performed 4 times.



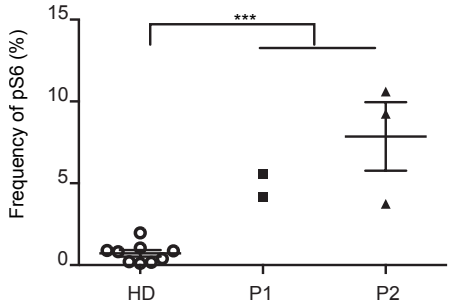
Supplementary Information Figure 8. Integrin surface expression in patients cells

A) FACS analysis of two independent experiments showing the expression of LFA-1 and VLA-4 components in patients and healthy donor T cell blasts. **B)** FACS plots of two independent experiments showing high affinity LFA-1 surface expression in patients and healthy donors T cell blasts in non activating (EDTA 10mM) or activating (MgCL2, 1mM and SDF1, 100ng/ml) conditions. The experiment presented in **A** and **B** was performed twice for P1 and once for P2.



Supplementary Information Figure 9. RhoA-ROCK activation regulates AKT pathway in human T cell blasts cultures

A) FACS plot showing the level of AKTSer473 after T cell blasts stimulation with SDF1 (6ug/ml for 10 mins) alone or after a pre-treatment with the Y27632 (0.6 mg/ml, a 1-hour pre-treatment). **B)** FACS plots showing the requirement for a functional RhoA-ROCK pathway in the control of AKT phosphorylation (Ser473) in T cell blasts from a healthy donor (HD). Cells were stimulated with SDF1 (6ug/ml for 10 mins) and S1P (20 µM for 10 mins) in the presence of the ROCK inhibitor Y27632 (0.6 mg/ml, a 1-hour pre-treatment) or the RhoA activator II (RhoA act., 16 µg/ml, a 3-hour pre-treatment). Experiments presented in **A** and **B** were performed twice.



Supplementary Information Figure 10. Patients' B lymphocytes display elevated levels of pS6 phosphorylation

Dot plot showing the frequency of B cells (CD19+) with a high level of S6 Serine 235/236 phosphorylation (pS6) in fresh blood samples from patients and healthy donors (HDs) less than 4 h after sampling. ***: $p<0.001$ in a Mann-Whitney test. The results correspond to independent blood samples for HDs (circles; n=9), P1 (squares; n=2) and P2 (triangles; n=3).