Isolation of mouse thymocytes and splenic T cells. Thymus and spleen were harvested from 8-10 week old female C57BL/6 mice. Single cell suspensions were prepared by passing the organs through 70 μm sieve. Red blood cells were lysed in the ACK buffer. T cells were isolated from spleen by removing adherent non-T cells in a nylon wool column (reference no. 1 in the Supplement). Thymocytes and splenic T cells were lysed in a modified RIPA buffer (reference no. 12 in the main manuscript).

REFERENCES

1. Eisen SA, Wedner HJ, Parker CW. Isolation of pure human peripheral blood T-lymphocytes using nylon wool columns. *Immunol Commun.* 1972;1(6):571-577.

Primer Set	Amplified Region of cDNA	Primer Direction	Sequence
Set 1	18-944	forward	ATTACAGCCGGCGCAGGCAGCG
		reverse	CCAGGTACCCTTCCTCCCAACA
Set 2	453-1387	forward	TTTGTCCGCTACCAGTTCAC
		reverse	CGGCCTTTGAACAGAAAAGGAAAAAGT

Table S1. Primers used to amplify Unc119 cDNA. Base positions are numbered from 5' to 3' end according to a published human Unc119 mRNA sequence (GeneBank accession # NM_005148.3).

Primer Set	Amplified Exons	Primer Direction	Sequence
Set 1	promoter of the Unc119 gene	forward	GTGTCTGGCGTGTAGTAGGC
		reverse	ATTCCGCAGGCGGCTGTGGTAT
Set 2	exon 1 of the Unc119 gene	forward	ATTCCTGAGCCTGGAACTCAC
		reverse	TATCTGCTGCCGCACGTACCT
Set 3	exon 2 of the Unc119 gene	forward	GCGGAGTTATGTCTCTTCCA
		reverse	GCCTGCAGACTCCTGAGAAT
Set 4	exon 3 of the Unc119 gene	forward	AAGCACAGTGGCGATTGG
		reverse	GCGGAAGTTGTTGACAGG
Set 5	exon 4 of the Unc119 gene	forward	CTGAGCCTGGCTGGCTAGTT
		reverse	TGGTGGCAGACACAGAAGAG
Set 6	exon 5 of the Unc119 gene	forward	TCTGTGTCTGCCACCATTCC
		reverse	CCTCTCCAGCTCCATCATGT

Table S2. Primers used to amplify regions of the Unc119 gene

Primer Set	Amplified Exons	Primer Direction	Sequence
Set 1	exon 2 and 3 of the Lck gene	forward	GCGTGCACACCTCTCCAGTA
		reverse	AGCCTCTCCAGCCTGGTTAG
Set 2	exon 4 and 5 of the Lck gene	forward	GAGAGGCTGAGAGCAGAG
		reverse	TCAGCCGTCGACCTGAGA
Set 3	exon 6 and 7 of the Lck gene	forward	ATACTGAGCGAGCCACACTGA
		reverse	TTACCTGGTTCTGGTCGAAGTC
Set 4	exon 8 of the Lck gene	forward	GCCAGACTCACTGCGTTCTT
		reverse	GGCAGGCCTCGTATTGACAC
Set 5	exon 9 and 10 of the Lck gene	forward	CTTGGAGGTGGTGTCAATACGA
		reverse	TTATGCCACCACACCTGGCTACT
Set 6	exon 11, 12 and 13 of the Lck gene	forward	CTCCTCCATTCTCACTCTTC
		reverse	AGAGGAGATGCAGCTACA
Set 7	exon 14 of the Lck gene	forward	TGCTCACACTGTGCCAGTTA
		reverse	GCCTCCAGACATCCACTCAT

Table S3. Primers used to amplify regions of the Lck gene

Primer/Probe Name	Sequence	Fluorescent Dye
Unc119 forward primer	ACGGCGACGGAGTCC	none
Unc119 reverse primer	AGGCGGCTGTGGTATGG	none
wild type Unc119 allele-specific probe	CCTCGGGCCAGAGC	VIC
mutant Unc119 allele-specific probe	CCCTCGGTCCAGAGC	FAM

Table S4. Primers and probes used in the 5'-nuclease allelic discrimination assay

	Secondary CD4 lymphopenic patients								Healthy subjects
	P4	P5	P6	P7	P8	P9	P10	P11	
Total lymphocytes/µl	969	672	1397	1044	1377	1432	710	980	1000-4800
CD3+ lymphocytes/µl	640	373	780	493	947	785	381	504	678-2504
CD4+ lymphocytes/µl	298	202	328	299	306	295	222	295	414-1679
CD8+ lymphocytes/µl	206	170	462	149	477	463	160	205	162-1038
CD4/CD8 ratio	2.02	1.8	0.7	2.01	0.64	0.63	1.3	1.4	1-3.60
CD19+ lymphocytes/µl	114	149	87	375	233	401	169	268	96-515
CD16/56+ NK cells/µl	224	139	524	166	149	325	149	210	45-523

Table S5. Lymphocyte subsets in the blood of patients with secondary CD4 lymphopenia

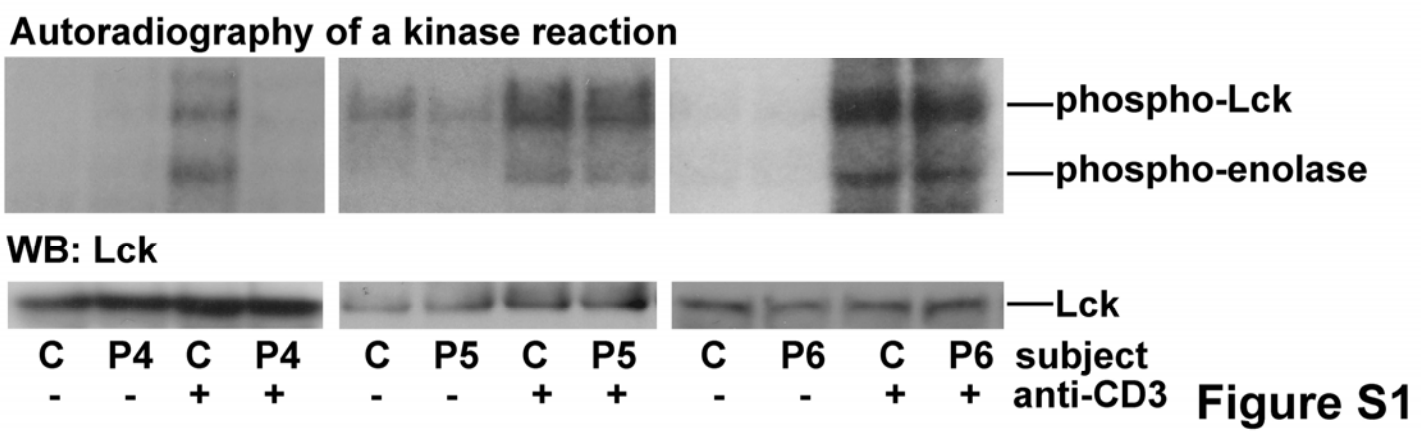

Figure S1. Lck activity in CD4 T cells from patients with secondary CD4 lymphopenia. Peripheral blood CD4 T cells from healthy control subjects (C), patients with CD4 lymphopenia in the course of mycobacterial infection (P4), SLE (P5), and CVID (P6) were incubated with (+) or without (-) a plate-bound anti-CD3 antibody for 5 min and lysed. The enzymatic activity of Lck in lysates was examined as in Figure 1. Each radiography image and a corresponding blot is representative of 3 experiments.

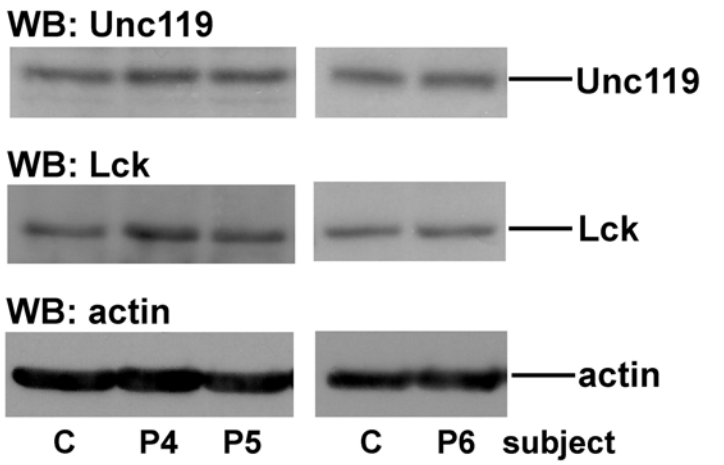

Figure S2

Figure S2. Expression of Unc119 and Lck proteins in patients with secondary CD4 lymphopenia. CD4 T cells from subjects as in Figure S1 were lysed and blotted with anti-Unc119, anti-Lck and anti-actin antibodies. Each blot represents one of 3 independent experiments.

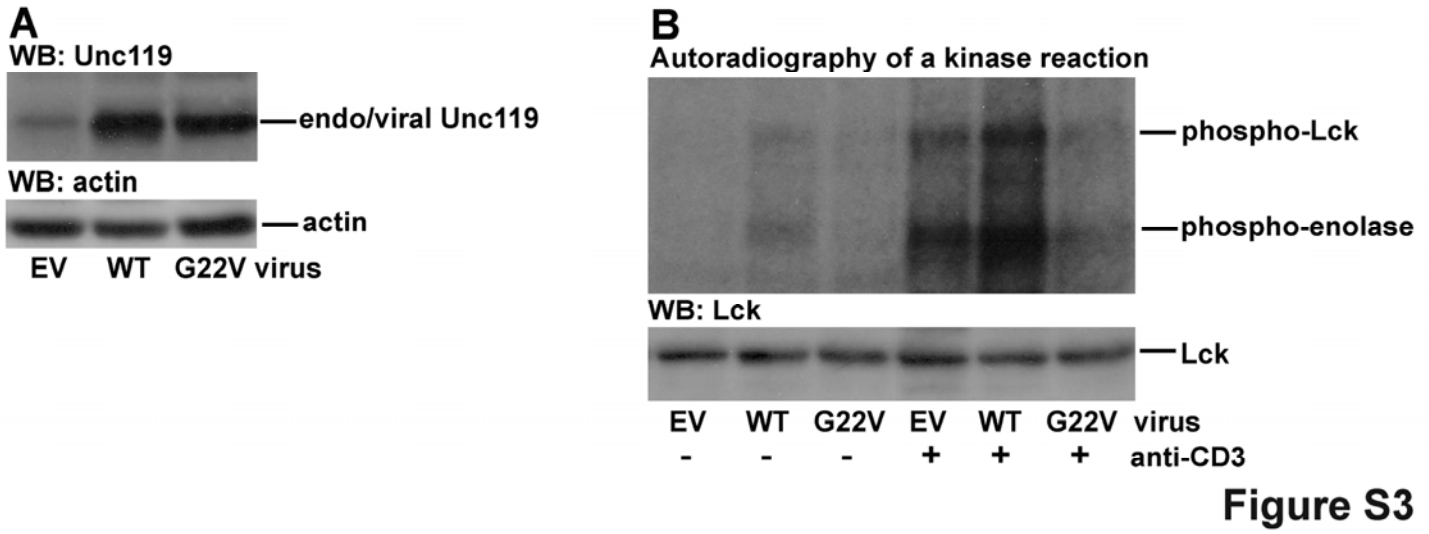

Figure S3. Effect of Unc119 G22V on activation of Lck. (A) Jurkat cells were infected separately with an empty retrovirus (EV), and retroviruses for untagged wild type Unc119 (WT) or untagged mutant Unc119 (G22V). The GFP-negative retroviral vector pMSCVneo was used for cloning[13]. Jurkat clones were isolated by limiting dilution. The lysates from the clones were blotted as in Figure 3A. endo, endogenous (B) Jurkat clones were analyzed for Lck activity as in Figure 1. Figures S3A and S3B are representative of three different experiments.

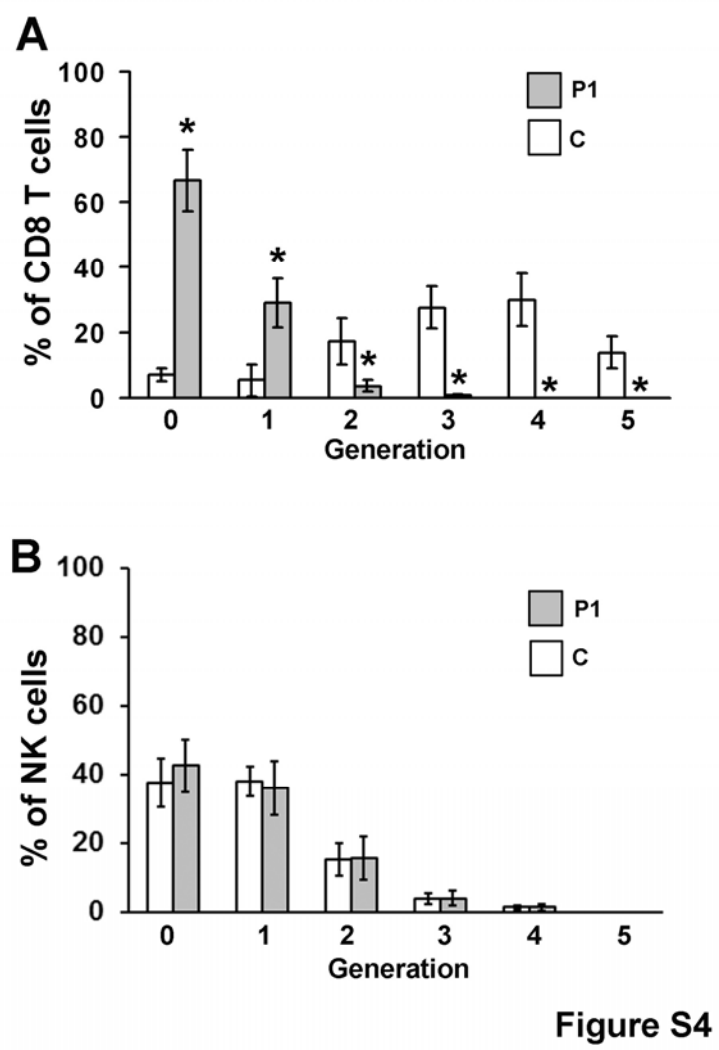

Figure S4. Proliferation of CD8 T cells and NK cells of the patient P1. (A) Peripheral blood mononuclear cells from the patient P1 and healthy controls (C) were labeled with CFSE and stimulated with plastic-bound anti-CD3 and anti-CD28 antibodies for 72h. Cells were then stained with a PE-labeled anti-CD8 antibody. The progressive dilution of the CFSE dye in daughter CD8 T cell generations ("0" denotes undivided cells, "1" denotes cells after first division etc) was analyzed by flow cytometry. The graph shows percentage (mean ± SD) of CD8 T cells in each generation. N=4 experiments; *, $P<0.05$ (Mann-Whitney test, pairwise comparison). (B) CFSE-labeled peripheral blood mononuclear cells from the patient P1 and healthy control subjects were stimulated with IL-15 for 5 days and, then, stained with PE-labeled anti-CD3 and APC-labeled anti-CD56 antibodies. The dilution of the CFSE dye in the $CD3^{negative}CD56^{positive}$ cell (NK cell) population was studied by flow cytometry as in S4A. N=3 experiments.

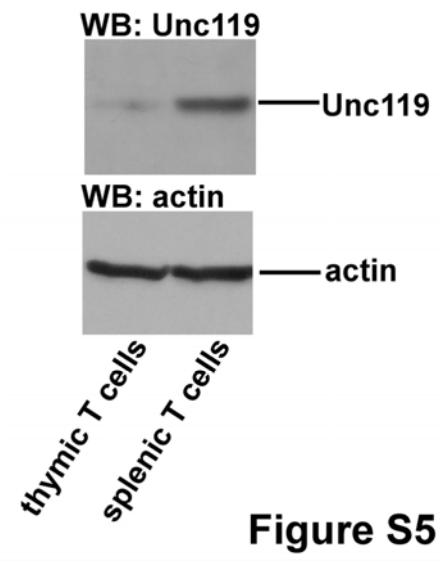

Figure S5. Expression of Unc119 in thymocytes and splenic T cells. Thymocytes and splenic T cells from C57BL/6 mice were lysed and blotted with anti-Unc119 and anti-actin antibodies (N=3).