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Life Sciences Reporting Summary

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Experimental design

1. Sample size

Describe how sample size was determined.

For cell line -related experiments, 5 x 10^6 cells per sample were used for immunoblot analysis, 3 x 10^8 cells per sample were used for mass spec analysis or immunoprecipitation experiments. Cell numbers were decided upon empirically, and each experiments were repeated at least three times. For bone marrow chimeras experiments, four mice per group in two independent experiments were performed. For in vivo bone marrow chimeras studies, sample size was chosen based on previous experience. Relevant data for accurate power calculation was unavailable. A sample size of 4 was sufficient to detect statistically significant differences between the experimental groups.

2. Data exclusions

Describe any data exclusions.

We did not exclude any sample (note - one bone marrow chimera recipient that received virus expressing WT LAT was dead in about ten days after injection).

3. Replication

Describe the measures taken to verify the reproducibility of the experimental findings.

The findings were reliably reproducible. Each set of experiments was repeated at least three times, and different experiments were designed to test the hypothesis from orthogonal perspectives. Regarding CRISPR/Cas9 generated cell lines, multiple clones were tested where possible. We obtained 12 J.LAT (LAT deficient) clones, and randomly chose two different J.LAT clones for reconstitution studies, and similar results were found with each. We also generated proline to alanine point mutation "knock in" clones into the endogenous LAT genomic allele using CRISPR Cas9 technology (data not shown). We obtained four cell clones having knock in mutations as designed, and two other clones had alternative mutations that disrupted the proline rich motif, and all of these cells showed similar phenotype as we observed in reconstitution studies. Even though the data were not shown to keep our report concise, the findings have been reproduced using different experiment designs.

4. Randomization

Describe how samples/organisms/participants were allocated into experimental groups.

Mice were randomized based on the HSC cells expressing WT LAT or mutant LAT.

5. Blinding

Describe whether the investigators were blinded to group allocation during data collection and/or analysis.

When taking down mice, harvesting organs, and acquiring the data, we gave a letter to mouse sample based on the ear tag, which was randomized when the experiment was set up. In calcium and p-ERK experiment, we harvested the thymocytes from bone marrow chimeras that expressed wild type LAT or AIARSA mutant LAT, bar-coded respectively, randomly mixed one WT sample with one AIARSA mutant sample in the same polystyrene tube, and utilized the flow cytometry to examine CD3-crosslinking calcium mobilization. The bar-coded experimental set up allowed us to examine WT and AIARSA samples under the same experimental conditions. Otherwise, no blinding was used. However, Internal negative controls were used to obtain unbiased data (for instance to set gate boundaries in flow cytometric analyses).

Note: all in vivo studies must report how sample size was determined and whether blinding and randomization were used.

6. Statistical parameters

For all figures and tables that use statistical methods, confirm that the following items are present in relevant figure legends (or in the Methods section if additional space is needed).

n/a	Confirmed
☐	☒ The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement (animals, litters, cultures, etc.)
☐	☒ A description of how samples were collected, noting whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒ A statement indicating how many times each experiment was replicated
☐	☒ The statistical test(s) used and whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐ A description of any assumptions or corrections, such as an adjustment for multiple comparisons
☐	☒ Test values indicating whether an effect is present *Provide confidence intervals or give results of significance tests (e.g. P values) as exact values whenever appropriate and with effect sizes noted.*
☐	☒ A clear description of statistics including central tendency (e.g. median, mean) and variation (e.g. standard deviation, interquartile range)
☐	☒ Clearly defined error bars in all relevant figure captions (with explicit mention of central tendency and variation)

See the web collection on statistics for biologists for further resources and guidance.

Software

Policy information about availability of computer code

7. Software

Describe the software used to analyze the data in this study.

BD FACS DIVA
Flojo version 9.9.3 or version 10.1
GraphPad Prism 6
MASCOT version 2.4.1
ProteoWizard software version 3.0.7680
Home-made HTAPP software for mass spectrometry data analysis, and is published as below:
1- Yu, K. & Salomon, A.R. PeptideDepot: flexible relational database for visual analysis of quantitative proteomic data and integration of existing protein information. Proteomics 9, 5350-5358 (2009).
2-Yu, K. & Salomon, A.R. HTAPP: high-throughput autonomous proteomic pipeline. Proteomics 10, 2113-2122 (2010).

For manuscripts utilizing custom algorithms or software that are central to the paper but not yet described in the published literature, software must be made available to editors and reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). *Nature Methods* guidance for providing algorithms and software for publication provides further information on this topic.

Materials and reagents

Policy information about availability of materials

8. Materials availability

Indicate whether there are restrictions on availability of unique materials or if these materials are only available for distribution by a third party.

The LAT deficient mouse strain was a generous kind gift from L. Samelson and C. Sommers (NIH). OVA tetramers were obtained through NIH Tetramer Core Facility. Cell lines were generated in either the Weiss lab or in Stepanek lab. Remaining materials are commercially available as indicated in Materials and methods. Mass spectrometry data have been deposited in the ProteomeXchange Consortium repository via PRIDE (Username: reviewer21222@ebi.ac.uk and password: zI1qcJ3B). The primary data for analysis of all figures and supplementary figures are available upon request.

9. Antibodies

Describe the antibodies used and how they were validated for use in the system under study (i.e. assay and species).

Antibodies for flow cytometry:
CD24-FITC (clone M1/69, BD #561777); TCR-BV421 (clone H57-597, BD #562839); CD69-BV605 (clone FN50, BD # 562989); CD8-BUV737 (clone SK1, BD #564628); CD8-FITC (clone 53-6.7, BD # 53-6.7); CD4- APC (clone RM4-5, BD #553051); CD4-BV395 (clone GK1.5, BD # 563790); CD44-PE-Cy7 (clone IM7, BD # 560569); CD25-BV650 (clone PC61, BD # 564021); CD45.2-APC-Cy7 (clone 104, BD # 560694); CD45.1-PerCP-Cy5.5 (clone A20, BD # 560580); CD5-APC (clone 53-7.3, BD #550035); CD117 (ckit)-PE-Cy7 (clone 2B8, BD #561681); Sca-1-BV421 (clone D7; BD #562729). All the antibodies are from commercial sources and have been validated by the vendors. Validation data are available on the manufacturer's website.

Antibodies for immunoblot analysis:
Lck (clone D88, Cell Signaling Technology, Cat#2984); Lck (clone 1F6, Weiss lab, UCSF); Zap70 (clone 1E7, Weiss lab, UCSF); Zeta-chain (clone 6B10, Weiss lab, UCSF); PLCg1 mixed monoclonal (EMD Millipore, Cat# 05-163); SFK p-Y416 (Cell Signaling Technology, Cat#2751); Phosphotyrosine (4G10, Weiss lab, UCSF); Phosphotyrosine-biotinylated (clone p-Tyr-100, Cell Signaling Technology, #9417); PLCg1 p-Y783 (Invitrogen, Cat#44696G); Erk p-T202/p-Y203 (clone 197G2, Cell Signaling Technology, Cat#4377); LAT p-Y191 (Cell Signaling Technology, Cat#3584); LAT p-Y132 (Abcam, Cat#ab4476); SLP76 p-Y128 (clone J141-668.36.58, BD Biosciences, Cat#558367); Zap70 p-Y493 (Cell Signaling Technology, Cat#2704); Zap70 p-Y319 (Cell Signaling Technology, Cat#2717); Myc-Tag-Biotinylated (clone 71D10, Cell Signaling Technology, Cat#3946); Myc-Tag-HRP (clone 9B11, Cell Signaling Technology, Cat#2040); Myc-Tag (clone 9B11, Cell Signaling Technology, Cat#2276); Tubulin (clone DM1A, Sigma-Aldrich, Cat#T9026); Goat Anti-Rabbit IgG(H+L) Mouse/Human ads-HRP (Southern Biotech, Cat#4050-05); Goat Anti-Mouse IgG(H+L), Human ads-HRP (Southern Biotech, Cat#1031-05); Streptavidin-HRP (Cell Signaling Technology, Cat#3999). All the antibodies are from commercial sources and have been validated by the vendors. Validation data are available on the manufacturer's website.

10. Eukaryotic cell lines

a. State the source of each eukaryotic cell line used.

Jurkat cell lines and related variants were generated in Weiss lab or in Stepanek lab. HEK293 cells were obtained from UCSF Cell Culture Facility. The Weiss Lab has deposited the original parental Jurkat E6-1 in the ATCC.

b. Describe the method of cell line authentication used.

Identity of Jurkat is routinely validated using an anti-TCR Vbeta mAb (C305) generated by Dr. Weiss.

c. Report whether the cell lines were tested for mycoplasma contamination.

The parental Jurkat line has been tested for mycoplasma in past years. But derivative lines used here were not.

d. If any of the cell lines used are listed in the database of commonly misidentified cell lines maintained by ICLAC, provide a scientific rationale for their use.

The Jurkat cell line was not among the misidentified cell lines published from the most recent version 8.0 of the database. HEK293 cell line was not listed in ICLAC database.

Animals and human research participants

Policy information about studies involving animals; when reporting animal research, follow the ARRIVE guidelines

11. Description of research animals

Provide all relevant details on animals and/or animal-derived materials used in the study.

The LAT deficient mouse strain was from L. Samelson and C. Sommers (NIH) are on C57BL/6 background . BoyJ (CD45.1) mice were obtained from Jackson (B6.SJL-Ptprca Pepcb/BoyJ). Both male and female mice were used. 6-8 weeks old of LAT deficient mice were used for experiments. 8 weeks old of BoyJ were used for experiments. Genotypes of LAT deficient mouse strains were confirmed using PCR analysis.

Policy information about studies involving human research participants

12. Description of human research participants

Describe the covariate-relevant population characteristics of the human research participants.

This study did not involve human research participants.

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Flow Cytometry Reporting Summary

Form fields will expand as needed. Please do not leave fields blank.

Data presentation

For all flow cytometry data, confirm that:

☐ 1. The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ 2. The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ 3. All plots are contour plots with outliers or pseudocolor plots.

☒ 4. A numerical value for number of cells or percentage (with statistics) is provided.

Methodological details

5. Describe the sample preparation.	Thymus, spleens, and lymph node single cell suspensions were prepared by gently tweezing samples in cold PBS buffer containing 0.5% FBS and 0.2% EDTA.
6. Identify the instrument used for data collection.	BD LSR Fortessa (from Weiss lab; 5 lasers: blue laser, red laser, violet laser, yellow-green laser, and UV laser.) The mouse bone marrow HSC cells were sorted on BD FACSAria II Cell Sorter (from Parnassus Flow Cytometry Core at UCSF, machine #Booboo)
7. Describe the software used to collect and analyze the flow cytometry data.	Data Collection: BD FACS DIVA Data Analysis: Flojo version 9.9.3 or version 10.1
8. Describe the abundance of the relevant cell populations within post-sort fractions.	The c-kit+Sca1+ HSC population was about 7-10% among pre-sort populations, and after cell sort, was about 98-99% among post-sort populations.
9. Describe the gating strategy used.	Doublets were excluded using forward light-scatter gating followed by gating on lymphocytes based on FSC-SSC. CD45.2+ B6 T cells were gated, and subject to mCherry+ gates (as in Fig 3a). CD45.2+ mCherry+ populations were used to examine the each T cell populations as described in Fig 3, 4 and Supplementary Fig 3. The gating of DN, DP, CD4, CD8 followed convention consensus based on the cell surface marker staining (Fig 3), as well as peripheral naive or memory, CD4 or CD8 T cells (Supplementary Fig 3). In Fig.4a,b, the whole DP T cell population was gated to examine the calcium responses or ERK phosphorylation (we did not specifically gate on pre-selection DP T cells.). In Fig 4c, mature CD8SP cells were gated on CD24-TCR+CD4-CD8+ to avoid ISP populations.

Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information. ☐