Supplemental methods

Patients

Peripheral blood mononuclear cells (PBMCs) were harvested from patients and healthy adult donors after the provision of written consent and in accordance with the tenets of the Declaration of Helsinki. Patients 1 to 5 and Patient 7 were referred to Necker Children's Hospital in Paris, and Patient 6 was from Miami Children's Hospital (now Nicklaus Children's Hospital). Mononuclear cells were isolated by Ficoll-Hypaque density gradient centrifugation (d=1.077 g/ml; Lymphoprep, Axis-Shield, Oslo, Norway).

Transduction of T-cells

A self-inactivating (SIN) pCCLΔU3 LV plasmid containing either an elongation factor 1 alpha (EF1α) promoter and *UNC13D* fused with cyan fluorescent protein (CFP) or CFP alone was used to generate LVs, as previously described [21]. Isolated PBMCs from seven unrelated patients with FHL3 (Supplemental Table 1) were cultured in Pancerin medium (PAN Biotech) supplemented with 5% human AB serum, 100 IU/ml human pro-interleukin-2 (pro-IL2; Novartis) and 1% penicillin-streptomycin. Cells from P1 to P4 were prestimulated with 2.5 µg/ml phytohaemagglutinin (Sigma-Aldrich) and cells from P5-P7 were prestimulated Dynabeads human T-activator CD3/CD28 (Invitrogen) for 48 hours in retronectin-coated plates (CH296, Takara). Concentrated vector was added at the indicated MOI and incubated with the cells overnight at 37°C. The cells were then washed and seeded into culture plates in the presence of pro-IL2 (100 IU/ml). Given that only low mumbers of PHA-activated PBMCs were available for P1 to P4, only the VSVG-LV transduction and the degranulation assay could be performed on these samples. All other comparisons of H/F-LV and VSVG-LV were performed on PBMCs from P5, P6 and P7.

Analysis of Munc 13-4 expression

mRNA expression: RNA was extracted using RNeasy Mini Kit (Qiagen). Reverse transcription was performed using a High Capacity cDNA RT kit (Applied Biosystems). In

qPCR experiments, Munc13-4 and GAPDH (for normalization) were amplified using Hs00397680-m1 and Hs00266705-g1 kits (Life Technologies), respectively.

Protein expression: cells were lysed using RIPA lysis buffer, and cell extracts were separated on a 4-12% bis-tris acrylamide gel (Invitrogen) and transferred onto a PVDF membrane. The membrane was blocked in PBS supplemented with 0.05% Tween 20 and 5% dried milk powder, and then incubated with the primary antibodies: rabbit anti-Munc13-4 (NBP2-19442; Novus Biologicals) and mouse anti-GAPDH (MAB374; Millipore). After staining with horseradish-peroxidase-conjugated secondary antibodies (ThermoFisher Scientific), the immunoblots were developed using Fusion Fx-7 Spectra (Vilber Lourmat).

The degranulation assay

Six days after transduction, activated T-cells were incubated with culture medium alone or culture medium supplemented with 3, 10 or 30 µg/ml of the anti-human CD3 antibody (clone OKT3) in the presence of PE-conjugated anti-CD107a/b (clone H4B4). After a 4-hour incubation at 37°C, cells were stained with the fluorochrome-conjugated antibodies CD3-APC (clone BW264/56) and CD8-FITC (clone BW135/80), and the cell viability dye 7-aminoactinomycin D (7-AAD).

Generation of an EBV-transformed B- lymphoblastoid cell line (B-LCL) and EBV-specific cytotoxic T-cells

Five million PBMCs from P5 (an EBV-seropositive patient) were incubated with 200 µl of concentrated supernatant from the B95-8 EBV-producer cell line for 1 hr at 37°C. Cells were then suspended in complete medium, consisting of RPMI-1640 (Gibco, BRL) supplemented with 10% foetal bovine serum (FBS, Hyclone, Logan, UT), 2 mmol/L L-glutamine (Gibco, BRL), and 1 µg/ml of cyclosporine A (CSA, Sandoz). Cells were fed weekly and expanded for long-term culture.

EBV-specific T-cells (EBV-T-cells) were generated as previously described [22]. Briefly, PBMCs were restimulated with irradiated LCLs at 4000 rads (40:1 ratio). After ten days, cells were restimulated and expanded by weekly restimulation with irradiated LCL at a T-cell:B-LCL ratio of 4:1 in the presence

of human pro-IL 2 (40 U/ml). EBV-T-cells were transduced with H/F-LV expressing Munc13-4/CFP or H/F-LV expressing CFP alone (at an MOI of 5) three days after the third restimulation. Transgene expression was assessed one week after transduction (a FACS analysis of CFP expression).

Establishment of the NSG xenograft mouse model

The xenograft NOD/SCID/IL2rγ^{null} (NSG) mouse model and bioluminescence imaging were implemented as described elsewhere [14]. Briefly, NSG mice were inoculated with 5×10^6 B-LCLs engineered to express firefly luciferase subcutaneously on the nape of the neck on day 0. Seven days later, mice received 5×10^6 transduced autologous EBV-T-cells intravenously (7 days after transduction of EBV-T-cells). Mice also received a daily intraperitoneal injection of human pro-IL2 (2500 U) for 8 days, starting on the day before the injection of EBV-T-cells. Tumour growth was evaluated using the IVIS imaging system monitoring the expression of luciferase by the tumour mass (Xenogen; Caliper life Sciences, Hopkinton, MA). For flow cytometry analysis, tumour samples were first minced through a 70-μm pore size nylon mesh. Single cells were collected, stained with an anti-human CD3 antibody (BD Bioscience) and measured with an LSR II FACS system (BD Biosciences). Samples were analysed with Flow Jo software ((version 10.0.7; Treestar, Ashland, OR) by comparing human T cells with BFP-expressing lymphoma B cells. Dead cells (stained with 7-AAD) were excluded.

Flow cytometry analysis

Cells were processed on a MACSQuant flow cytometer (Miltenyi Biotec), and the results were analyzed with FlowJo software (Treestar). Monoclonal antibodies against CD3-APC (BW264/56), CD8-Vioblue (BW135/80), CD45RA-APCVIO770 (T6D11), CD62L-PE (DREG-56), CD95-PECY7 (DX2) and 7-AAD were used for cell surface staining. All antibodies used in flow cytometry analysis were purchased from BD Biosciences and Miltenyi Biotec.

Supplemental figure legends

Supplemental Figure 1. Lentiviral constructs and T-cell infiltration into a B-LCL lymphoma tumour. (A) A schematic representation of the lentiviral constructs used in this study, containing CFP alone or *UNC13D* cDNA fused with CFP (B) Upper panel: immunohistochemistry of human T-cell infiltration (CD3 staining) on a tumour section from xenograft NSG mice 30 days after EBV-T-cell infusion. The image evidences high levels of human $CD3^+$ T-cell infiltration into the B-cell lymphoma in mice treated with Munc13-4/CFP-transduced EBV-T-cells (relative to mice receiving control CFP EBV-T-cells). A representative image is shown. Scale bar: 100 μm; lower panel: flow cytometry analysis of tumour digests from NSG mice. Tumor cells expressing luciferase were also stained with an anti-human CD3 monoclonal antibody.

Supplemental Figure 2. T_{SCM} targeting and persistence during the *ex vivo* transduction protocol. (A) Plots of the $CD8^+$ T-cell population from two representative FHL3 patients (of the three patient samples tested: P5 to P7), showing the proportions (in %) of different T-cell subpopulations; T_N: naïve precursors, T_{CM}: T central memory, T_{EM}: T effector memory, T_{EMRA}: T effector memory $45RA^+$. (B) The mean number of Munc13-4/CFP^+ cells as a percentage of T_{SCM} ($CD45RA^+$, $CD62L^+$, and $CD95^+$) cells six days after the transduction of activated T-cells. $n=3$; $*p<0.05$ in an unpaired t-test (C) PBMCs from FHL3 patients were stimulated *in vitro* with anti-human T-cell activator CD3/CD28 Dynabeads + pro-IL2 (100 U/ml) for 48 hours and then cultured for 6 days with pro-IL2 (100 U/ml). The graph presents the proportions of $CD8^+$ T-cell subpopulations prior to stimulation and then 48 hours and 8 days after stimulation. d8 (H/F): cells transduced with H/F-LV (MOI: 5) on day 8 of culture (6 days post-transduction). d8 (VSVG): cells transduced with VSVG-LV (MOI: 100) on day 8 of culture (6 days post-transduction).

Supplemental Table and Figures

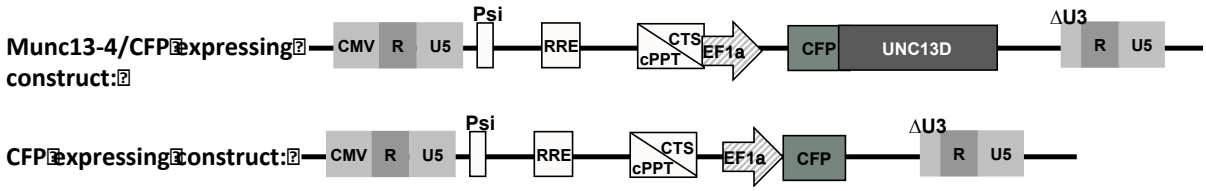
Supplementary Table 1. Data on the patients' mutations and initial laboratory results

Patient	Mutation	Munc13-4 expression (Western blot)	CD107 surface expression by CTLs*
1	c.2093(-1)G>A (splice site) c.2346_49delGGAG (p.Arg782fsX12)	Absent	Lower than in controls
2	c.1822_1833del (del V608_A611)	Absent	Lower than in controls
3	c.2296C>T (p.Q766X) c.2477_80del (p.L826fsX20)	Absent	Lower than in controls
4	c.1389(+1)G>A (splice site) c.753(+1)G>T (splice site)	Absent	Lower than in controls
5	c.1822_1833del (del V608_A611)	Absent	Lower than in controls
6	c.154(-1)G>C (splice site) 253kb inversion	Absent	Lower than in controls
7	c.916C>T (p.Q306X) c.1193C>T (p.S398L)	Very low	Lower than in controls

*CTL: cytotoxic T lymphocyte activity, determined as the level of CD107 surface expression by fluorescence-activated cell sorting after 4 hours of stimulation with anti-CD3 antibody.

Supplemental Figure 1.

A.



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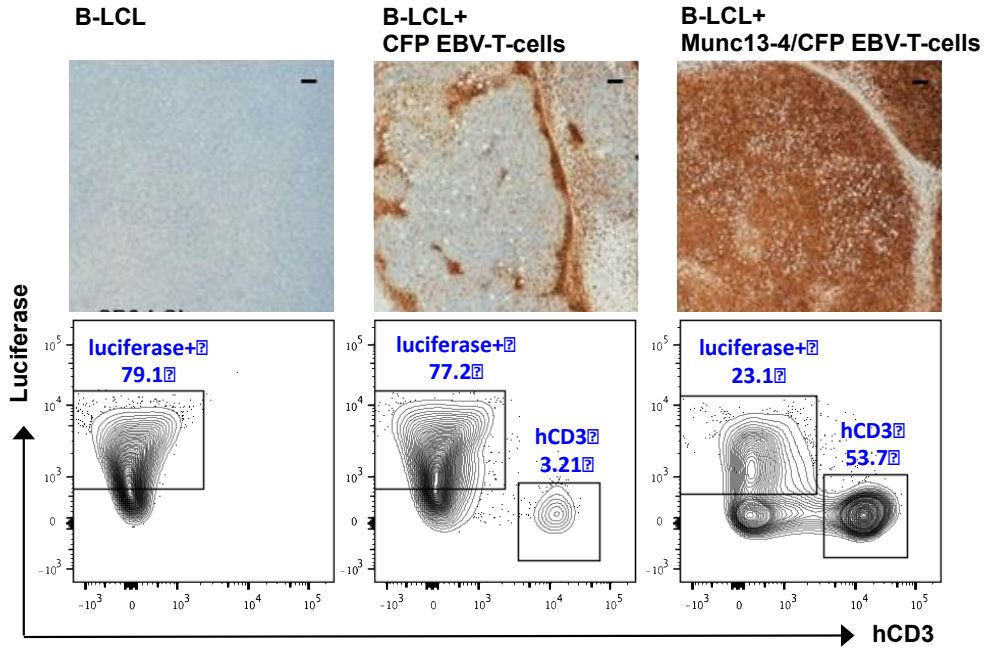
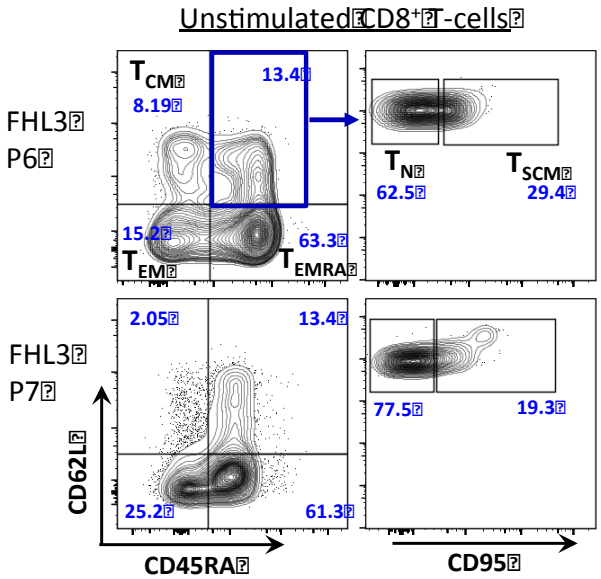


Supplemental Figure 2.

A.



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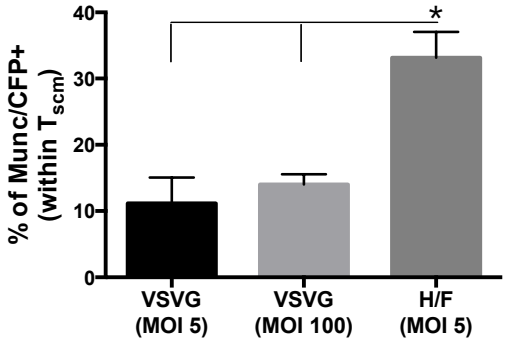

C.