Table E1. Immunologic workup over time. (For Online Repository)

	Normal	Abnormal
Initial workup during admission for CMV (3 mo)	Ferritin HIV PCR Recent thymic emigrants	*Lymphocyte subsets:* low CD8+ (174), low B cells (365), low NK cells (15) IgM (7), IgG (53), IgA (<7)
Extended workup (3 mo – 25 mo)	MHC I & MHC II, Zap 70 Mitogen stimulation (PHA & PWM) Immunoglobulins normalized at 5 mo B and T cells normalized at 9 mo	NK cell function low x 3 samples by 12 mo *Whole exome sequencing: ZBTB24* mutation c.1492_1493del, p.Q498Vfs, homozygous (returned at age 25 mo)
Prior to second presentation (32 mo)	Recent thymic emigrants Tetanus and diphtheria titers IgM (33), IgG (541), IgA (76)	*B-cell panel:* decreased CD27+ IgM-/IgD- (2, 0.3%), decreased CD19+/CD5+ (286, 36%) *Lymphocyte subsets:* low NK cells (93) *Pneumococcal titers:* 0/14 protective
Admission for EBV-driven lymphoproliferative disease (34 mo)	Mitogen stimulation (borderline-low response to PHA, but low viability of sample) IgM (36), IgA (49)	*Lymphocyte subsets:* low CD4+ (671), low CD8+ (548), low B cells, low NK cells (48) *Pneumococcal titers:* 0/14 protective after PPSV23 (administered at 32 mo) Low tetanus and diphtheria titers IgG (362)

Units: Immunoglobulins (mg/dL), cell frequencies (cells/mm^3)

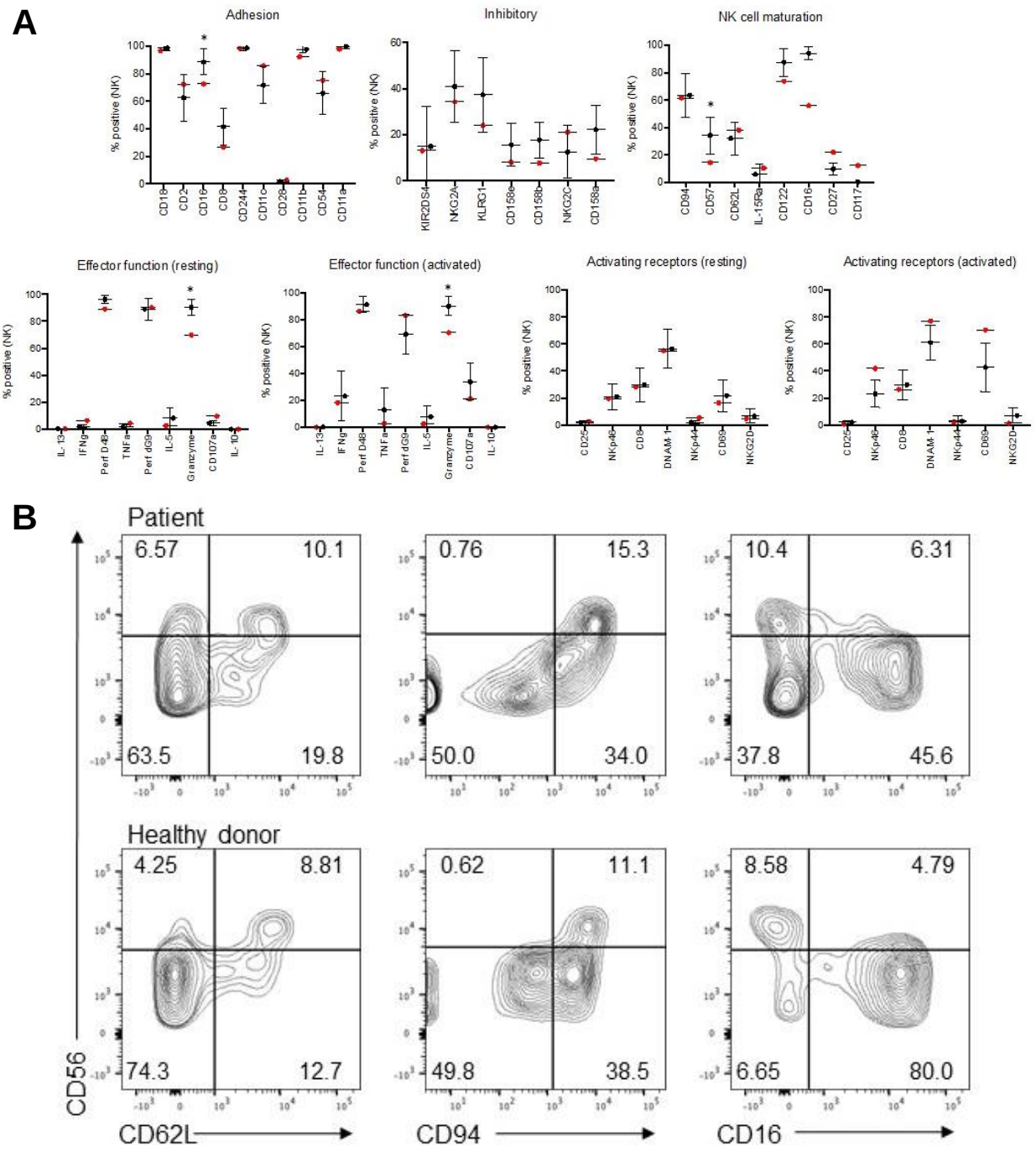

Figure E1. High-resolution phenotyping of patient NK cells. (For Online Repository) PBMC from the patient were analyzed using the flow cytometry panels described in Methods. A) Frequency of NK cells positive for the parameter of interest were calculated for the patient and are shown (red symbol) with the average ± SD of 45 pediatric healthy donors (black symbols). Markers associated with NK cell maturation that are out of normal ranges are marked with an asterisk. B) Representative plots showing the expression of markers associated with NK cell maturation and their expression on patient (top) and healthy donor NK cells (bottom).

NK cell phenotyping

Comprehensive NK cell phenotyping was performed to better understand the altered NK cell subsets observed in the patient. When the NK cell population was considered as a whole, the frequency of expression of markers associated with NK cell maturation, including CD16, CD57 and granzyme B, were decreased when compared to the mean of 45 healthy pediatric donors (Supp Fig 1A). In addition, the expression of killer immunoglobulin-like receptors was at the low end of the normal range. Conversely, those receptors associated with an immature phenotype, such as CD117 and CD62L, had increased expression or expression at the high end of the normal range. Despite these changes, there were no other significant abnormalities detected in the expression of activating, inhibitory, or adhesion molecules on patient NK cells. While granzyme B, and to a certain extent perforin expression was low in the patient, IFNγ was produced in response to activation at a level consistent with healthy controls (Supp Fig 1A). Taken together, these data suggest that the alteration in receptors identified by FACS analyses is a result of over-representation of a bona fide $CD56^{bright}$ subset. This was confirmed by further analyses that identified the appropriate distribution of maturation markers on $CD56^{bright}$ and $CD56^{dim}$ subsets respectively (Supp Fig 1B).

METHODS

Human study approval

All studies were performed in accordance with the Declaration of Helsinki with the written and informed consent of all participants under the guidance of the Institutional Review Boards of Baylor College of Medicine and Texas Children's Hospital. The patient's family gave their consent for their daughter's case to be formally described.

Whole exome sequencing and analysis

Whole exome sequencing was performed on genomic DNA as previously described[E1]. Bioinformatic analyses were performed as previously described using filters of an allelic frequency of less than 0.0005 in the BCMG, ESP5400, 1000 Genomes and ExAC databases. Variants were subsequently confirmed by Sanger sequencing using primers constructed by Primer3[E2].

Cell isolation and cell lines

Primary peripheral blood mononuclear cells were isolated by Ficoll-Paque density centrifugation. K562 erythroleukemic target cells were maintained in complete RPMI media with 10% FCS.

^{51}Cr cytotoxicity assay

^{51}Cr assays were performed as previously described.[E3] Briefly, 10^4 K562 target cells were labeled with 100 μCi of 51Cr, washed, and subsequently incubated with PBMC from patient or healthy donor in the presence or absence of 1000 U/ml IL-2 (Roche). After 4 hours, plates were centrifuged and supernatant was transferred to a LUMA plate and dried overnight. Emission was read with a TopCount NXT and % specific lysis was calculated as follows: (sample – average

spontaneous release) / (average total release – average spontaneous release) x 100.

Flow cytometry

NK cell phenotyping panels were performed as described in Mahapatra et al.[E4] For panels which did not require stimulation, cells were immunostained with antibodies diluted in PBS-2% FBS for 20-25 minutes. For panels assessing effector function and activation, cells were stimulated with phorbol 12-myristate 13-acetate (10ng/ml, Sigma-Aldrich) and ionomycin (1 μg/ml, Sigma-Aldrich) for 4 hours at 37° C and unstimulated control cells were incubated in parallel. Brefeldin A (10 μg/ml, Sigma-Aldrich) and anti-CD107a were added at the beginning of incubation for both stimulated and unstimulated cells for detection of intracellular cytokines. Cells were then permeabilized with BD Cytofix/Cytoperm (BD Biosciences), followed by antibody staining for 45-60 minutes in BD Fix/Perm buffer. Activated cells were stained for surface markers for 20-25 minutes following the 4-hour incubation. Data were acquired on a modified LSR Fortessa (BD Biosciences) with the capacity to detect 18 fluorescent parameters and exported to FlowJo 10.5.3 (TreeStar) for analysis. Frequency of cells positive for each parameter were compared to the average and standard deviation of 45 healthy donor pediatric samples ages 5-20 years.[E4] Data showing comparisons was visualized with Prism 6.0 (GraphPad Software).

SUPPLEMENTAL REFERENCES

E1. Lupski JR, Gonzaga-Jauregui C, Yang Y, Bainbridge MN, Jhangiani S, Buhay CJ, et al. Exome sequencing resolves apparent incidental findings and reveals further complexity of SH3TC2 variant alleles causing Charcot-Marie-Tooth neuropathy. Genome Med. 2013 Jun 27;5(6):57.

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E3. Orange JS, Ramesh N, Remold-O'Donnell E, Sasahara Y, Koopman L, Byrne M, et al. Wiskott-Aldrich syndrome protein is required for NK cell cytotoxicity and colocalizes with actin to NK cell-activating immunologic synapses. Proc Natl Acad Sci U S A. 2002 Aug 20;99(17):11351-6.

E4. Mahapatra S, Mace EM, Minard CG, Forbes LR, Vargas-Hernandez A, Duryea TK, et al. High-resolution phenotyping identifies NK cell subsets that distinguish healthy children from adults. PLoS One. 2017 Aug 2;12(8):e0181134.