**Supplementary Material**

***Patient case and paraclinical findings***

The patient is a 3-year-old boy of Caucasian ancestry born from parents in a non-consanguineous marriage. He was admitted to hospital and was diagnosed with disseminated varicella infection with possible CNS involvement due to altered consciousness. Lumbar puncture performed after transferal to the tertiary center was without pleocytosis (less than 5 x 10^6 cells/L), showed normal glucose and normal protein content (3.0 mmol/L and 0.24g/L respectively) and was PCR negative for herpes simplex virus (HSV) type 1 and 2, CMV, EBV and VZV in the cerebrospinal fluid (CSF), but activated monocytoid cells were identified. He was treated with intravenous acyclovir and broad-spectrum antibiotics. Due to extensive and progressive systemic inflammation together with presence of hemophagocytosis in the bone marrow, HLH (based on the 2004 HLH criteria) was diagnosed and treatment according to the HLH-2004 protocol was initiated. A routine clinical immunological evaluation was performed (supplementary table 2) which revealed that the patient has normal perforin levels, normal NK and T cell degranulation as well as normal NK cell cytotoxicity, thus arguing against a defect in cytotoxic lymphocyte function. The patient had decreased concentrations of NK and B cells in whole blood, which most likely could be ascribed as a secondary feature to HLH [32, 33]. Subsequently, WES was performed using a gene panel comprising 291 PID and HLH associated genes, which did not identify any known disease-causing variants. The patient was considered for bone marrow transplantation, due to the concern that primary HLH could be the case despite absence of gene defects related to fHLH. The boy had not previously demonstrated increased susceptibility or severity to infectious diseases and had been following the Danish routine childhood vaccination program, including live MMR vaccination at age 15 months without major side effects. He made a slow but good recovery, was discharged after one month but did not show full remission and required long-standing maintenance therapy. Finally, HLH-treatment was discontinued after 40 weeks. But acyclovir was continued for prophylaxis.

**Full details of material and methods**

**Patient material**

Whole blood was collected at hospitalization in EDTA stabilized tubes for DNA purification and after recovery in lithium heparin tubes for peripheral blood mononuclear cell (PBMC) isolation. PBMCs were isolated by Ficoll density gradient centrifugation using SepMate PBMC isolation tubes (Stemcell Technologies, #85460) and frozen down in liquid nitrogen. Control PBMCs were purified from healthy donors after obtaining written consent. Genomic DNA was purified using QIAamp DNA Blood Mini Kit (Qiagen, #51104) according to manufacturer’s instructions.

**WES and bioinformatics**

WES was conducted on genomic DNA from the patient using KAPA HTP library preparation and Nimblegen SeqCap EZ MedExome Plus kits and analyzed with Nextseq version2 chemistry [2 × 150 basepairs] (Ilumina). Single nucleotide polymorphisms were called relative to hg19. Variant call files were uploaded to Ingenuity Variant Analysis (IVA) software (Qiagen) and filtered according to rarity (gnomAD frequency <0.1%) and predicted deleteriousness (Combined Annotation Depletion Dependent (CADD) score > 15 and CADD score > Mutation significance cutoff (MSC) score). In addition, variants were filtered based on their biological relevance using gene lists and broad biological filters in IVA related to VZV, HLH, PID and immune response.

**Sanger sequencing of AMFR**

Genomic AMFR DNA from the patient, and the patient’s mother, father, younger sister and younger brother were amplified by PCR using Phusion Hot Start II DNA polymerase (Thermo Fischer Scientific, #F-594S) and the following primers: AMFR forward: 5’AAGCTGCTGCTCCATTATCCG-3’ and AMFR reverse 5’-TACCAGCATCAGAGGTAGACCA-3’. The genotype of amplified AMFR was confirmed using Sanger Sequencing with AMFR forward primer.

**RNA isolation qRT-PCR**

Total RNA was purified using Nucleospin 96 RNA core kit (Macherey-Nagel, #740466.4) and reverse transcribed into cDNA using Iscript™ gDNA Clear cDNA Synthesis Kit (Bio-Rad, #1725035). This was followed by measurement of GAPDH housekeeping gene levels and viral ORF gene induction using SYBRGreen Brilliant III Ultra-Fast SYBR® Green QPCR Master Mix (Agilent, #600882), and the following primers: GAPDH forward: 5’-TCTTTTGCGTCG-3’, GAPDH reverse 5’-ACCAGGCGCCCA-3’, ORF63 forward: 5’-GCGCCGGCATGA-3’, ORF63 reverse: 5’-GACACGAGCCAA-3’; ORF40 forward: 5’-ACTTGGTAACCG3-‘ ORF40 reverse: 5’-CGGGCTACATCA-3’, ORF9 forward: 5’-GGGAGCAGGCGC-3’ and ORF9 reverse: 5’-TTTGGTGCAGTG-3’.

**STING immunoprecipitation**

HEK293T cells were seeded at a density of 2.5 \*106 cells in 6 cm petri dish and following overnight incubation, transfected with 1 ug of the following plasmids: pcDNA3/FLAG-STING, pRK5/HA-K27-Ubiquitin and pcDNA3/Myc-AMFR WT or pcDNA3/Myc-AMFR R594C. Controls transfected only with FLAG-STING, HA-K27-Ubiquitin and pcDNA3-Empty vector (No AMFR control), or HA-K27-Ubiquitin, AMFR WT and FLAG-Empty vector (No STING control) were included. Cells were lysed 24 hrs post transfection in Pierce IP lysis buffer (Thermo Fischer Scientific, #8788) supplemented with 1x protease inhibitor cocktail (Roche, #05.892.970.001) and 1x PhosSTOP cocktail (Roche, #04.906.837.001) for 1 hr at 4°C with rotation and centrifuged for 10 min at 1400 x g at 4°C. Lysates were then incubated with FLAG M2 Dynabeads (Sigma, # M8823) for 1.5 hrs at room temperature, washed 3 times in TBS with 0.05% Tween20 and phosphatase inhibitors (TBS-T+) and incubated with 3XFLAG peptide (Sigma Aldrich) for 15 min at room temperature to elute STING. Then, eluents were denatured by boiling for 5 min at 95°C in the presence of 1% sodium dodecyl sulphate (SDS) after which samples were diluted in lysis buffer with protease-, phosphatase-, and deubiquinitylating enzyme inhibitiors (Merck, #662141) and incubated overnight at 4°C with FLAG M2 Dynabeads. Finally, samples were washed three times in TBS-T and FLAG-STING eluted by boiling the samples in Laemmeli concentrate Sample buffer (Sigma, #S3401-10VL) at 95 °C for 10 min. Lastly, samples were immunoblotted for expression of FLAG-STING, Myc-AMFR and HA-Ubiquitin.

**Western blotting**

For PMBC stimulations, cells were washed twice with PBS and lysed in RIPA buffer (Thermo Fischer Scientific, #89901) supplemented with Halt Protease and Phosphatase Inhibitor Cocktail (Thermo Fischer Scientific, #78440) and Benzonase Nuclease (Sigma Aldrich, #E1014-25KU). Protein concentrations were measured using Pierce BCA protein Assay Kit (Thermo Scientific, #23227) and cell lysates were denatured at 95C for 5 min with 50 mM DTT (Sigma Aldrich, #43816-10ML) and 4xLaemmli buffer (Bio-rad, #1610747). Samples were subjected to SDS gel electrophoresis and transferred to a PVDF-membrane using the Transfer-Blot Turbo systems. The membrane was blocked in 5% skimmed milk in PBS-T or TBS-T for 1 hour, followed by incubation overnight at 4C with primary antibodies against: STING (Cell Signaling, #13647S, 1:1000), phospho-STING (Cell Signaling, #19781S, 1:1000), TBK1 (Cell signaling, #3013S 1:1000), phospho-TBK1 (Cell Signaling, #5483S, 1:1000), IRF3 (Cell signaling, #11904S, 1:1000), phospho-IRF3 (Cell Signaling, #4947S, 1:1000), ISG15 (Cell signaling, #2758S, 1:1000), Vinculin (Cell signaling, #13901S, 1:2000), AMFR (Proteintech, #16674-1-ap) FLAG-Tag (Cell signaling, #17793S, 1:500), Myc-Tag (Cell Signaling, #2278S, 1:1000) or HA-Tag (Cell Signaling, #3724S, 1:1000). Primary antibodies were visualized using secoundary horseradish-peroxidase-coupled anti-rabbit or anti-mouse antibodies (Jackson ImmunoResearch, #715-036-150, #711-035-152) at 1:10.000 on ChemiDoc gel imaging system (Bio-rad).

**Supplementary Results**

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| **Gene** | **Transcript ID** | **Transcript Variant; Protein Variant** | **Translationalimpact** | **CADD; MSC scores** | **SIFT Function Prediction** | **gnomAD frequency (%)** | **dsSNP ID** |
| ***ECM1*** | NM\_004425.4;  | c.135\_136insGC; p.P46fs\*133 | frameshift | 24.800; 0.001 |  NA |  - |  - |
| ***ITGB8*** | NM\_002214.3 | c.1198T>G; p.Y400D | missense | 15.610; 3.313 | Tolerated | 0.002 | 778043359 |
| ***EGR2*** | NM\_000399.5 | c.644C>T; c.494C>T; p.T215M | missense | 21.100; 1.828 | Tolerated | 0.063 | 139147487 |
| ***NECTIN1*** | NM\_002855.5 | c.1331\_1333dupAGG; p.E444dup | in-frame | 16.670; NA |  NA |  - | 753988307 |
| ***BRIP1*** | NM\_032043.2 | c.139C>G; p.P47A | missense | 25.200; 0.001 | Damaging | 0.025 | 28903098 |
| ***PRKCA*** | NM\_002737.3 | c.556A>G; p.M186V | missense | 24.500; 3.313 |  NA | 0.010 | 200732611 |
| ***MYO5B*** | NM\_001080467.3 | c.5449A>G; p.M1817V | missense | 18.940; 0.017 | Activating | 0.006 | 200172993 |
| ***RNASEH2A*** | NM\_006397.2 | c.518C>T; p.P173L | missense | 24.400; 10.51 | Damaging | 0.003 | 369355807 |
| ***PIK3R2*** | NM\_005027.4 | c.1938C>T; p.I646I | synonymous | 19.070; 23.5 |  NA | 0.068 | 146987351 |
| ***LRBA*** | NM\_006726.4 | c.1903\_1905delGGT; p.G635del | in-frame |  NA; 23.5 |  NA |  - |  - |

**Supplementary Table 1. Additional genetic variants identified in the patient by whole exome sequencing and not ascribed a disease-causing potential.** CADD, Combined Annotation Dependent Depletion; MSC, Mutation significance cutoff; SIFT, Sorting Intolerant From Tolerant; gnomAD, The genome aggregation database; NA, Information not available; -, variant not reported in gnomAD.

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|  | Patient value | Normal value |
| Total lymphocytes | 0.3\*109 cells/L | 1.10-9.90\*109 cells/L |
| CD3- CD56+ (NK) cells |  0.01\*109 cells/L | 0.05-0.85\*109 cells/L |
| CD3+ (T) cells | 1.26\*109 cells/L | 1.95-6.70\*109 cells/L |
| CD3+ CD4+ (T) cells | 0.79\*109 cells/L | 0.7-2.5\*109 cells/L |
| CD3+ CD8+ (T) cells | 0.44\*109 cells/L | 0.2-1.20\*109 cells/L |
| CD19+ (B) cells | 0.07\*109 cells/L | 1.10-9.90\*109 cells/L |
| NK cell degranulation (CD107a)a | 24% | 19%b |
| T cell degranulation (CD107a)c | 77% |  >5%  |
| NK cell-mediated cytotoxicityd | 31 lytic units | >10 lytic units |
| IgG | 6.5 g/L | 4.1-12.0 g/L |
| IgM | 0.25 g/L | 0.30-1.84 g/L |
| IgA | 0.48 g/L | 0.09-1.37 g/L |

**Supplementary Table 2. Results from clinical Immunological investigations in the patient during hospital admission.**

aNK cell (CD3- CD16+ CD56+) degranulation was measured as the proportion of CD107a on the surface of NK cells following stimulation with K562 lymphoblast cell line.

bMeasured in an age-matched healthy control.

cT cell (CD3+) degranulation after stimulation with anti-CD3 antibodies.

dNK cell (CD3- CD56+) mediated cytotoxicity on the K562 lymphoblast cell line.