Human FCHO1 deficiency reveals role for clathrin-mediated endocytosis in development and function of T cells

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Clathrin-mediated endocytosis (CME) is critical for internalisation of molecules across cell membranes. The FCH domain only 1 (FCHO1) protein is key molecule involved in the early stages of CME formation. The consequences of mutations in FCHO1 in humans were unknown. We identify ten unrelated patients with variable T and B cell lymphopenia, who are homozygous for six distinct mutations in FCHO1. We demonstrate that these mutations either lead to mislocalisation of the protein or prevent its interaction with binding partners. Live-cell imaging of cells expressing mutant variants of FCHO1 provide evidence of impaired formation of clathrin coated pits (CCP). Patient T cells are unresponsive to T cell receptor (TCR) triggering. Internalisation of the TCR receptor is severely perturbed in FCHO1-deficient Jurkat T cells but can be rescued by expression of wild-type FCHO1. Thus, we discovered a previously unrecognised critical role of FCHO1 and CME during T-cell development and function in humans.
ukaryotic cells are characterised by a structural and functional compartmentalisation. Biological membrane systems are critically involved in the establishment and maintenance of the cellular compartments. The highly dynamic modulation of membranes is a key feature for their role in a variety of biological processes, such as endocytosis, secretion, signal transduction or migration. Clathrin, a central molecular scaffold, plays an essential role in the re-organisation of cellular membranes and the transport between compartments. Clathrin-coated vesicles (CCV), first described as “clathrin-coated pits” (CCP) in mosquito oocytes1 and later purified from pig brain2, control internalisation of membrane-associated proteins and protein transport from the trans-Golgi-network3.

Clathrin-dependent endocytosis (CME) is initiated at the plasma membrane by the recruitment of adaptors (hetero-tetrameric AP-2 complex, AP180; clathrin assembly lymphoid myeloid leukaemia, CALM; and anchor proteins (FCH domain only 1 and 2, [FCHO1 and 2]; epidermal growth factor receptor substrate 15, [EPS15]; and intersectin)4-6. These factors are enriched within PIP2 (phosphatidylinositol 4,5-biphosphate)-rich regions of the membrane and trigger the assembly of the clathrin proteins into clusters7. During maturation, the clathrin-enriched membrane patches undergo a local curvature and an invaginated spheric clathrin-coated pit is generated. The connection of this pit to the plasma membrane is detached by dynamin6.

The molecular details of the formation of a new vesicle are still not fully understood. In vitro, the presence of clathrin, an adaptor protein (i.e., AP-1, AP-2) and dynamin are sufficient to form a vesicle9. However, in vivo, the mechanism is much more complex. It has been shown by live-cell total internal reflection fluorescence (TIRF) imaging with single-molecule EGFP sensitivity and high-temporal resolution that the formation of a vesicle can originate from two AP-2 molecules with a clathrin triskelion4. In contrast, another report suggested that FCHO1 and FCHO2 need to be associated with the membrane prior to AP-2 recruitment to initiate the process5. FCHO1/2 interact and show similar kinetics at the membrane as the scaffold proteins EPS15 and intersectin, two factors that are important for clathrin-dependent endocytosis6,10. Upon downregulation of EPS15 expression by small-interfering RNA, FCHO1/2 show a diffuse pattern of distribution at the cell membrane. This observation suggests that EPS15 is critical for the specific agglomeration of FCHO1/2 on the site of the formation of clathrin-coated vesicles5.

Since the early clinical discoveries of monogenic immune disorders by Rolf Kostmann11 and Ogden Bruton12, >400 primary immunodeficiency diseases have been delineated13, many of which have opened unprecedented insights into molecular mechanisms orchestrating differentiation and function of the human immune system.

Here, we describe ten human patients with T-cell deficiency and loss-of-function mutations in FCHO1. Our experiments demonstrate that the absence of functional FCHO1 results in perturbed clathrin-mediated endocytosis in several tissues, as well as dysfunctional internalisation of the TCR. Pharmacological inhibition of CME during in vitro T-cell development results in marked delay of T-cell differentiation. In summary, we identify an essential and non-redundant role for the clathrin adaptor FCHO1 in T-cell differentiation and function, linking CME to the function of the human immune system.

Results

Clinical phenotype and molecular genetics. We collected seven pedigrees with ten patients presenting with features of T-cell immunodeficiency (Fig. 1a and Supplementary Fig. 1). All patients suffered from severe bacterial, viral or fungal infections indicative of a primary immunodeficiency disorder. Three patients (B1, C1, D1) developed B-cell lymphoma prior to allo-genic hematopoietic stem cell transplantation, and three patients (A1, D1, E1) had neurological disease (Table 1). Immunological parameters were ranging from a moderate decrease in peripheral CD4+ T cells (F2 and G1) to severe combined immunodeficiency with virtually absent B- and T-cells (A1, B1, E1). With the exception of G1, all patients had hypogammaglobulinemia (Supplementary Table 1). Thus, the common immunophenotypic denominator for all these patients was CD4+ T-cell deficiency.

In order to identify the underlying genetic defect, we performed whole-exome sequencing (WES) followed by Sanger sequencing of candidate genes on patients and family members (see Online Methods). We have identified six distinct, novel and segregating homozygous mutations in FCHO1 in seven pedigrees (Fig. 1b and Supplementary Figs. 2 and 3; Table 1 and Supplementary Table 2). At the DNA level, the mutation in kindred A results in a nucleotide substitution at the position c.2036 G > C, in kindred B to a nucleotide substitution at the position c.100 G > C, whereas the mutations of the pedigrees C and D (not known to be connected by kinship) are insertions c.2023insG resulting in a frameshift and premature termination of the protein (Fig. 1b, c). The mutations of kindred E (changing the first nucleotide of intron 8 c.489 +1 G > A) and kindred F (contained in the intron 6 splice acceptor site; c.195-2 A > C) affect splicing of pre-mRNA (Fig. 1c and Supplementary Fig. 2). Effects on FCHO1 transcripts in fibroblasts, peripheral blood mononuclear cell (PBMC) and EBV-LCL are shown in Supplementary Figs. 2 and 3. No bands corresponding to wild-type FCHO1 were detected in these analyses. In kindred G, a single-nucleotide substitution at position c.1948C > T results in a predicted premature stop codon at amino acid position 650. All identified patient-associated mutations are summarised in Supplementary Table 2.

FCHO1 consists of three main segments organised into two major domains (Fig. 1c). The N-terminal F-BAR domain (also known as extended FCH), which is responsible for membrane binding, is followed by a structurally less organised and evolutionary poorly conserved segment that binds the adaptor protein AP-2. The C-terminal part (~270 amino acids) forms a µ homology domain (µHD), which directly binds to epidermal growth factor receptor pathway substrate 15 (EPS15) and EPS15-like 1 (EPS15L1) also known as EPS15R5.

The mutation in kindred A results in an Arg to Pro substitution at the position 679 in the µHD domain. The crystal structure of the Danio rerio µHD domain of Fcho1 has been resolved together with a fragment of EPS15 allowing for direct modelling the effect of the amino acid substitution on this structure (Fig. 1d)14. Although the patient-associated mutations are not directly located at the protein-binding trough, replacing the charged side chain of Arg with a nonpolar and rigid ring of Pro may result in steric alterations in the µHD subdomain A and thus affect interaction with its binding partners (Fig. 1d).

The point mutation in kindred B results in an amino acid substitution in the alpha-helix structure of the F-BAR domain (p. Ala34Pro). The F-BAR domain of FCHO1 has not been crystallised. However, the structure of its functional and structural paralogue FCHO2 has been resolved, which allows for modelling of the patient-associated mutation14. The substitution of Ala with Pro at position 34 is likely to result in a disruption of the alpha-helix structure, which in turn may lead to alterations in the membrane-binding properties of the entire domain (Fig. 1e).

In addition to mutations resulting in amino acid substitutions, we identified three distinct variants resulting in a premature stop...
The two identical mutations in the unrelated families C and D result in a frameshift and premature stop codon at amino acid position 687 (p.Val675GlyTer13, referred to as p.Stop687). Similarly, the mutation associated with kindred G results in a premature stop codon at amino acid position 650 (p.Stop650). In both variants virtually the entire µHD domain is truncated, which presumably alters interaction with the FCHO1-binding partners.

Fig. 1 Homozygous mutations in the FCHO1 gene segregate with patients’ lymphopenia. a Pedigrees of seven unrelated families show ancestral segregation of mutations in the FCHO1 locus. Generations are assigned by Roman numerals from I to III. Index cases are marked with an arrow, small circles and squares denote spontaneous abortions and crossed symbols deceased individuals, mut, mutation; wt, wild-type. b Sanger sequencing chromatograms indicating homozygous mutation c.2036 G > C in index cases of kindred A, c.100 G > C in kindred B and c.2023insG in kindred C and D. Families C and D are not connected by kinship. A–D, index cases; M’, mother; F, father; S, sister; B, brother. Additional kindred analyses are exhibited in Supplementary Figs. 2 and 3. c Schematic representation of FCHO1 protein indicating two main domains and localisation of family-associated mutations. d, e Computed crystal structures with indicated point mutations in µHD domain (d) and F-BAR domain (e).
Table 1 Summary of clinical features of patients carrying mutations in FCHO1.

| Patient | Origin  | Genetic variant          | Consequences of mutation        | Immunological findings                                                                 | Infections                                                                 | Other clinical findings                              | Therapy and outcome                           |
|---------|---------|--------------------------|---------------------------------|----------------------------------------------------------------------------------------|---------------------------------------------------------------------------|-----------------------------------------------------|-----------------------------------------------|
| A1      | Germany | FCHO1 c.2036 G > C       | aa substitution in pHK domain (p.677Arg) | T- and B-cell lymphopenia, hypogammaglobulinemia                                         | Recurrent pneumonia and viral gastroenteritis, Relapsing oro-genital mycoses, Bronchialitis obliterans, Postpneumonic pulmonary fibrosis | Moya-Moya syndrome, Transient left hemiparesis upon cerebral ischemia, Failure to thrive, Microcephaly | Reduced cardiopulmonary performance, stable Moya-Moya 9 years after HLA-matched HSCT |
| B1      | Turkey  | FCHO1 c.100 C > G        | aa substitution in F-BAR domain (p.334Arg) | T- and B-cell lymphopenia, hypogammaglobulinemia                                         | Recurrent pneumonia, Recurrent fungal infections, CMV infection, Recurrent pulmonary infections | Failure to thrive, Liver masses, Xanthogranulomatous pyelonephritis, DLBCL stage I/IV | Deceased as consequence of DLBCL, age 16 years |
| C1      | Turkey  | FCHO1 c.2023insG         | Truncated (p.Stop687)           | CD4⁺ T-cell lymphopenia, hypogammaglobulinemia                                           | Recurrent pneumonia, HSV infection                                       | Failure to thrive, Hepatosplenomegaly, Renal masses, Hemiparesis upon cerebral ischemia | IVIG replacement and antibiotics, awaiting allo-HCT |
| D1      | Turkey  | FCHO1 c.2023insG         | Truncated (p.Stop687)           | CD4⁺ T-cell lymphopenia, hypogammaglobulinemia                                           | Recurrent pneumonia, HSV infection                                       | Failure to thrive, Hepatosplenomegaly, Renal masses, Hemiparesis upon cerebral ischemia | Deceased, age 10 years                          |
| E1      | Palestine| FCHO1 c.489 + 1G > A     | Alternative splicing IVS6 splice donor | CD4⁺ T-cell and B-lymphopenia, hypogammaglobulinemia                                     | Recurrent pneumonia, Chronic diarrehea, CMV infection, Fungal infection, Chronic diarrehea | Recurrent pneumonia, Chronic diarrehea, CMV infection, Fungal infection, Chronic diarrehea | IVIG replacement and antibiotics, awaiting allo-HCT |
| E2      | Palestine| FCHO1 c.489 + 1G > A     | Alternative splicing IVS6 splice donor | CD4⁺ T-cell and B-lymphopenia, hypogammaglobulinemia                                     | Recurrent pneumonia, Chronic diarrehea, CMV infection, Fungal infection, Chronic diarrehea | Recurrent pneumonia, Chronic diarrehea, CMV infection, Fungal infection, Chronic diarrehea | Deceased after cardiac arrest, age 2 years IVIG replacement and antibiotics, awaiting allo-HCT |
| E3      | Palestine| FCHO1 c.489 + 1G > A     | Alternative splicing IVS6 splice donor | CD4⁺ T-cell and B-lymphopenia, hypogammaglobulinemia                                     | Recurrent pneumonia, Chronic diarrehea, CMV infection, Fungal infection, Chronic diarrehea | Recurrent pneumonia, Chronic diarrehea, CMV infection, Fungal infection, Chronic diarrehea | Deceased after cardiac arrest, age 2 years IVIG replacement and antibiotics, awaiting allo-HCT |
| F1      | Saudi Arabia | FCHO1 c.195-2 A > C | Alternative splicing IVS6 splice acceptor | CD4⁺ T-cell and B-lymphopenia, hypogammaglobulinemia                                     | Recurrent pneumonia, Chronic diarrehea, CMV infection, Fungal infection, Chronic diarrehea | Recurrent pneumonia, Chronic diarrehea, CMV infection, Fungal infection, Chronic diarrehea | IVIG replacement and antibiotics, awaiting allo-HCT |
| F2      | Saudi Arabia | FCHO1 c.195-2 A > C | Alternative splicing IVS6 splice acceptor | CD4⁺ T-cell and B-lymphopenia, hypogammaglobulinemia                                     | Recurrent pneumonia, Chronic diarrehea, CMV infection, Fungal infection, Chronic diarrehea | Recurrent pneumonia, Chronic diarrehea, CMV infection, Fungal infection, Chronic diarrehea | IVIG replacement and antibiotics, awaiting allo-HCT |
| G1      | Algeria  | FCHO1 c.1944C > T        | Truncated p.R650X p. Stop650     | CD4⁺ T-cell lymphopenia, Weak response to vaccination                                     | Recurrent bronchopulmonary infections, Candidaemia, CMV infection          | Failure to thrive, Hepatosplenomegaly, Renal masses, Hemiparesis upon cerebral ischemia | IVIG, 12.5 yrs follow up HSCT (MFD) at age 5 years, doing well |

EBV: Epstein-Barr virus, DLBCL: diffuse large B-cell lymphoma, PTLD: post-transplant lymphoproliferative disorder, HLA: human leucocyte antigen, HSCT: haematopoietic stem cell transplantation, MFD: matched family donor, IVIG: intravenous immunoglobulin, a + GvHD: acute and chronic graft versus host disease.

a: Sequence of coding DNA is given from the first nucleotide of the translation start codon.

b: Sequence of protein is given from the first amino acid.
of these amino acids, it is likely that, in case a protein is stably expressed, it disrupts the alpha-helix structure of the F-BAR domain. Finally, the kindred F-associated mutation in the splice acceptor region gives rise to three possible splice variants, each of them resulting in a premature stop codon shortly downstream of exon 6. It prevents correct splicing and by prediction presumably leads to expression of a very short (–7.1 kDa), non-functional form of FCHO1 (Supplementary Fig. 3b–e).

Effects of patient-associated mutations on FCHO1 function. Based on CADD and PolyPhen-2 scores, all patient-associated mutations are deleterious for the FCHO1 protein function (Supplementary Table 2). To test this, we first established a heterologous system, where HEK239T cells were transiently transfected with vectors carrying wild-type (wt) or mutated versions of the FCHO1 complementary DNA (cDNA). None of the herein tested mutations altered protein stability, albeit p.Stop687 resulted in the expression of a shorter protein (Fig. 2a and Supplementary Fig. 4). The encoded proteins were tested for co-immunoprecipitation with their direct interacting partners EPS15 and its homologue EPS15R. Recovery of the EPS15 and EPS15R expression of a shorter protein (Fig. 2a and Supplementary Fig. 3b) was strongly reduced in both mutants affecting the µHD domain (c.R679P and p.Stop687). In contrast, the FCHO1 mutant affecting the F-BAR domain (p.A34P) did not alter the interaction with EPS15 and EPS15R (Fig. 2a).

The function of FCHO1 critically depends not only on its biochemical interaction with partner proteins but also on the spatiotemporal organisation of its interactome. Nucleation of FCHO1–mediated clathrin-coated vesicles (CCV) occurs only at the plasma membrane5, We, therefore, set out to test whether the identified FCHO1 mutations altered the subcellular location of the corresponding protein. We employed SK-MEL-2 cells expressing a RFP-tagged clathrin light chain from one allele of its endogenous locus15. In order to circumvent confounding effects of the endogenously encoded FCHO1 protein, the FCHO1 gene was deleted using CRISPR/Cas9-mediated gene editing. Transient expression of wt FCHO1–GFP fusion protein in knockout (ko) cells showed scattered bright puncta associated with the plasma membrane as previously reported54 (Fig. 2b–d and Supplementary Figs. 5–7). In contrast, expression of the F-BAR mutated (p.A34P) FCHO1 form resulted in the formation of large plasma membrane-dissociated agglomerations (Fig. 2b–d and Supplementary Figs. 5–7).

Further, both pR679P and p.Stop687 µHD mutants failed to form any punctuated structures. These mutants appeared as a diffuse network, mostly dissociated from the plasma membrane (Fig. 2b–d and Supplementary Fig. 5). In accordance with the co-immunoprecipitation results, only wt and p.A34P FCHO1 colocalized with their partners EPS15 and adaptin (Fig. 2b–c, and for quantification Fig. 2e). Importantly, all mutants failed to colocalize with endogenous clathrin (Fig. 2d–e). We chose a model system with physiological expression levels of FCHO1. While this is advantageous in many respects5, it is limited with respect to poor signal-to-noise ratio. To improve signal intensities, we additionally transduced SK-MEL-2 cells to stably over-express RFP-tagged clathrin light chains. As shown in Supplementary Fig 7, these data confirm that only wild-type FCHO1, but none of the mutants, colocalize with clathrin.

Nucleation of CCV is a highly dynamic process. To measure the dynamics of protein–protein interaction between FCHO1 and clathrin, we established live-cell imaging models. Here, we followed wt FCHO1 and both point mutants (p.R679P and p.A34P). As anticipated, wt GFP-FCHO1 initiated the formation of clathrin-coated pits, whereas both mutants failed to do so (Fig. 3a–c and Supplementary Movies 1–3). FCHO1 carrying the µHD-associated p.R679P mutation prevented the formation of productive pits and thus failed to facilitate the formation of CCV. Since the sensitivity of the confocal microscope does not allow to discriminate single molecules, we cannot exclude that small, abortive pits may have been formed. However, we failed to detect any aggregation of FCHO1 carrying this mutation. In striking contrast, the F-BAR-associated mutation p.A34P resulted in the formation of fast moving, large aggregates of GFP-FCHO1 fusion proteins. Moreover, such membrane-dissociated aggregates tended to merge, leading to the formation of cytoplasmic protein intrusions (Supplementary Fig. 5). These pits appeared to be abortive, since they failed to interact with clathrin (Fig. 3a–c).

These results illustrate that the identified mutations in FCHO1 constitute loss-of-function alleles: whereas the mutation in the µHD domain results in loss-of-interaction with its main interacting partners and partial dissociation from the plasma membrane, the mutation in the F-BAR domain alters the subcellular localisation of the FCHO1 protein. Irrespective of their nature, all mutations ultimately result in inefficient nucleation of CCV.

FCHO1 regulates endocytosis of the T-cell receptor. The FCHO1 mutations identified in our patients predominantly result in a severe T-cell defect while other cells of the immune system appear largely unaffected. The key designate of T-cell fate during ontogeny is the quality and strength of the T-cell receptor (TCR) signal16–18. The TCR has no intrinsic catalytic activity. TCR-dependent signal propagation requires a multi-protein complex comprises TCR α- and β-chains non-covalently coupled to immunoreceptor tyrosine-based activation motif (ITAM)-rich CD3 ε, γ, δ and ζ molecules. Internalisation of the CD3:TCR complex depends on the formation of CCP19–24. We hypothesised that FCHO1 may be involved in TCR internalisation during T-cell activation. To test this, we took advantage of the CD4-positive human Jurkat T-cell lymphoma line in which we deleted the endogenous FCHO1 gene using CRISPR/Cas9 gene editing. FCHO1-deficient Jurkat clones were reconstituted with either wt or mutated FCHO1 using retroviral vectors encoding a bicistronic FCHO1 and GFP cDNA separated by an internal ribosomal entry site (IRES). In order to understand the effect of FCHO1 on TCR internalisation and clustering, we analysed TCR distribution upon stimulation using confocal microscopy. After 60 min of TCR triggering by an α-CD3 monoclonal Ab, large intracellular CD3-positive puncta were formed in wt cells, whereas in FCHO1 ko clones CD3 molecules remained in diffuse form (Fig. 4a and Supplementary Fig. 8). Knockout clones reconstituted with wt FCHO1 formed large CD3-positive puncta, essentially indistinguishable from those in wt cells. In contrast, none of the FCHO1 mutants were able to rescue the phenotype and thus nearly all CD3 molecules remained diffused upon TCR-activation (Fig. 4a). We also noted plasma membrane invagination and nucleus segmentation in FCHO1-deficient Jurkat cells. This effect was not observed in SK-MEL-2 cells (Figs. 2b–d and 4a, and Supplementary Fig. 5). It is therefore possible, that FCHO1 deficiency has a more general effect on plasma membrane structure in T cells.

To assess TCR internalisation in a quantitative manner, we next measured the intracellular accumulation of CD3:TCR complexes upon anti-CD3-mediated TCR triggering over time using flow cytometry. Consistent with our confocal microscopy studies, we noted that FCHO1 ko cells accumulated approximately two-fold less CD3:TCR complexes when compared to wt cells (Fig. 4b). Finally, as the TCR cross-linking and its subsequent internalisation are essential for quality and strength of the triggered signal, we assessed whether FCHO1 directly

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modulated TCR responsiveness. To this end, Jurkat cells sufficient or deficient for FCHO1 were stimulated with an α-CD3 antibody and assessed for release of intracellular Ca^{2+}. When compared to controls, FCHO1-deficient cells released less Ca^{2+} upon CD3:TCR triggering (Fig. 4c). Furthermore, only reconstitution with wt but not mutated forms of FCHO1 restored normal levels of Ca^{2+}, directly demonstrating that physiological TCR signalling depends on FCHO1.

During CME adaptor proteins recognise cargoes at the cell surface and direct them to the clathrin pits. FCHO1 binds its
cargo through its µHD domain. It has been proposed that Syt1, the yeast FCHO1 homologue, recognises Dxy motifs on its cargo. We hypothesised that human FCHO1 may also recognise its cargo via Dxy motifs, in particular since CD3e and CD3y have Dxy motifs in their cytoplasmic domain (Supplementary Fig. 9a). While we could confirm interaction of FCHO1 and EPS15 in Jurkat cells stably transduced with N’- or C’-flag FCHO1 fusion proteins, we could not observe any direct interaction with either of the CD3 molecules (Supplementary Fig. 9c–f). Thus, although CD3 molecules possess the putative sorting motifs recognised by FCHO1, we could not provide experimental evidence for direct FCHO1-CD3 interaction.

In sum, we provide evidence that FCHO1 plays a role in TCR-dependent T-cell activation. It affects TCR clustering upon receptor triggering and modulates its internalisation. Finally, FCHO1 deficiency results in impaired mobilisation of Ca2+, directly linking the FCHO1 to TCR-associated signalling.

FCHO1 deficiency does not affect entry of VSV-G pseudotyped HIV-1. Infection of cells with vesicular stomatitis virus (VSV) is strongly dependent on CME. CME of VSV particles is initiated after interaction of the viral glycoprotein (VSV-G) with the cellular LDL (low-density lipoprotein) receptor. Entry of VSV or VSV-G pseudotyped lentiviruses into the cytoplasm occurs after endosomal acidification, which induces a conformational change of VSV-G followed by fusion of viral and cellular membranes.

To evaluate whether the absence of FCHO1 in Jurkat T cells has an impact on CME-dependent virus infection, Jurkat wt and
FCHO1 ko clones were challenged with HIV-1 particles, that are devoid of their own envelope glycoprotein but had been pseudotyped with VSV-G. The interaction of T cells with VSV-G HIV-1 was monitored using two established readouts, i.e., intracellular staining for Env (BlaM-Vpr) (Fig. 5a and Supplementary Fig. 10a) or VSV-G HIV-1 pseudotypes can be quantified by intracellular staining for HIV-1 p24 antigen.

Jurkat wt and FCHO1 ko clones were challenged with different multiplicities of infection of either VSV-G HIV-1 ΔEnv (BlaM-Vpr) (Fig. 5a and Supplementary Fig. 10a) or VSV-G HIV-1 ΔEnv (Fig. 5b and Supplementary Fig. 10b). FCHO1-deficient Jurkat T clones displayed a susceptibility to VSV-G pseudotyped HIV-1,
whereas patient T cells failed to proliferate (Fig. 6b). Similarly, T cells of healthy siblings responded vigorously to the stimulation assessed after 3 and 5 days. At both time points, CD4 and CD8 α C1 (Fig. 6a). Although largely reduced in number when compared to her heterozygous siblings, peripheral blood T cells were abundant enough to perform a functional assay. To this end, FCHO1-deficient clones from either virion fusion (e) or infection (f). Patient fibroblast or healthy donor fibroblast were subjected to the transferrin uptake assay. Cells were exposed to fluorescently labelled transferrin for the indicated time at 37 °C and subsequently analysed by FACS. Data are pooled of four independent assays; error bars represent standard deviation. a–e Statistical analysis was performed using two-way ANOVA (p-values for the effect of the genotype). Source data are provided as a Source Data file.

FCHO1 deficiency does not alter global clathrin-mediated endocytosis. To further assess whether FCHO1 deficiency has a global impact on CME, we tested transferrin receptor internalisation, a well-recognised model of a clathrin-dependent process33. To this end, we incubated patient and healthy donor fibroblasts with fluorescently labelled transferrin in the cold and, following a temperature shift, its internalisation was monitored over time. Both wild-type and FCHO1-deficient primary cells were able to internalise transferrin through the TIR comparably (Fig. 5c), suggesting that FCHO1 deficiency does not affect general CME endocytosis, but selectively CME endocytosis of certain molecules.

Inhibition of CME arrests development of murine T cells in vitro. Our data highlighted a mechanistic link between impaired CME and ensuing T-cell deficiency. To further validate this notion, we employed OP9-DL1 and OP9 co-culture systems allowing us to study in vitro T-cell differentiation, as well as differentiation of B cells and myeloid cells34. Purified thymus-derived, T-cell-committed double-negative (DN) three progenitors were co-cultured with OP9 DL1 cells for 10 days until ~50% of them co-expressed CD4 and CD8 co-receptors (double-positive, DP). At day 5 of culture, we exposed the cells to chlorpromazine, a known inhibitor of CME 35 (Fig. 7a). In a concentration-dependent manner, we observed inhibition of T-cell development. Whereas >50% of cells reached the DP stage in the absence of CME inhibition, only 30–35% of chlorpromazine-treated showed progression to the DP stage. Even though minusculue surface expression levels of pre-TCR at this stage of thymocyte development prevented any direct measurements of TCR internalisation, it is known that thymocyte differentiation from DN3 to DP strongly depends on the quality of pre-TCR signal36,37.

To exclude the general cytotoxic effect of chlorpromazine we co-cultured bone marrow-derived lineage-Sca-1+CD117+ (LSK) cells, the most versatile progenitors, which in similar conditions develop to nearly all hematopoietic lineages (albeit with different kinetics), and assessed how chlorpromazine affects development of various lymphoid and non-lymphoid cells. To this end, LSK co-cultured on OP9-DL1 cells gave rise to all pre-TCR independent thymocyte populations in an indistinguishable manner.
Fig. 6 Patient-associated mutation in FCHO1 gene alters development and activation of T lymphocytes. a Density plots of blood leucocytes (CD45+) of the index case (upper row) homozygous for a mutation in the FCHO1 locus and her siblings carrying the heterozygous mutation (middle and bottom rows). T helper cells were defined as CD45+CD3+SSC+CD3+CD4+ and cytotoxic T cells were CD45+CD3−SSC+CD3+CD8+. Numbers adjacent to the gates indicate percentages. b Histograms of CFSE-labelled T lymphocytes stimulated with anti-CD3 and anti-CD28 Ab for 3 (red line) or 5 (blue line) days. Unstimulated controls are depicted in grey. c Cytokine production by lymphocytes of patient and family members after anti-CD3 and anti-CD28 stimulation for the indicated periods of time. IL-2, IFN-γ, TNF-α and IL-4 were determined using cytometric bead assays. a–c Data are representative of two independent experiments, except data of day 3 shown in c, which was assessed once. Source data are provided as a Source Data file.

Discussion

Here, we identify autosomal recessive FCHO1 deficiency as a human genetic defect associated with combined immunodeficiency (CID). We show that six different mutations in the FCHO1 gene, either point mutations resulting in amino acid substitutions, premature stop codons or affecting pre-mRNA splicing are deleterious for FCHO1 function. T-cell deficiency predisposes affected patients to severe and persistent viral and fungal infections. Hypogammaglobulinemia is seen in all patients, except G1. It remains unclear whether B-cell defects are intrinsic or strictly dependent on defective T cells.

FCHO1 deficiency predisposes not only to infections but also to lymphoma13. Several patients died secondary to malignancies before definitive therapy in form of an allogeneic hematopoietic stem cell transplant could be done. Thus, FCHO1 deficiency warrants rapid genetic diagnosis and provision of access to definitive cure.

Our studies have provided insights into the structural and functional biology of FCHO1. In a structure-guided prediction, Ma and colleagues14 concluded that a minimum of two aa substitutions in FCHO1 (at positions K877E + R879A), located directly in the peptide-binding groove, are required to abolish interaction of the domain with its main interacting partner EPS15. Here, we show that a single substitution at R679P, spatially distant from the groove, is sufficient to severely alter this interaction. Although localised opposite of the groove, replacement of the charged side chain of Arg with a nonpolar and rigid ring of Pro is sufficient to alter the domain function. Three mutations resulting in a premature stop codon at the beginning of µHD domain further strengthen their significance for FCHO1 function. Alterations in the F-BAR domain also prove to be deleterious, yet in a different mode. Substitution of Ala at position 34 with Pro, a known alpha-helix breaker, leads to dissociation of the FCHO1 homodimers from the plasma membrane and hence abolishes the function. Although not directly tested, it is plausible that alternative splicing resulting in loss of 51 aa located within the F-BAR domain disrupts the protein structure. From a clinical point of view, the severity of the immunodeficiency, however, cannot directly be correlated to the type of the FCHO1 mutation.

Given the central role of FCHO1 for the formation of clathrin-coated pits and endocytosis it is surprising that FCHO1 deficiency results in T-cell immunodeficiency rather than more global defects of development. In contrast, Caenorhabditis elegans deficient for FCHO show body malformation and uncoordinated locomotion38. FCHO interacts with AP-2 and induces a conformational change, leading to AP-2 activation. Mutations in either FCHO or AP-2 result in a strikingly similar phenotype, indicating that the severe defects seen in FCHO-deficient worms are caused by aberrant activation of AP-2. In vertebrates, however, the interplay between AP-2 and FCHO is more complex. Morpholino-mediated knockdown of Fcho1 in Danio rerio causes dorsoventral patterning defects and severe malformation at an early developmental stage, whereas transcriptional silencing of Fcho2 is associated with notochord and somite malformations38. In zebrafish, AP-2 deficiency results in a more severe, broader and earlier developmental phenotype than combined Fcho1/2 deficiency, suggesting that AP-2 function is, at least in part, Fcho1/2-independent39. The idea that the functional relevance of FCHO has changed during evolution is supported by the observation that a) two FCHO paralogs have emerged40 and b) FCHO may act as receptor-specific adaptors (e.g., BMP-mediated signalling in zebrafish) rather than universally manner irrespective of presence or absence of chlorpromazine (Fig. 7b). Similarly, presence or absence of chlorpromazine in LSK and OP9 co-culture did not interfere with development of neither B220+CD19+ B-cell committed progenitors nor CD11b+Gr-1+ neutrophilic granulocytes (Fig. 7c, d).

Thus, chlorpromazine, a chemical inhibitor of CME, shows rather selective effects on the DN3-DP transition of thymocyte differentiation, known to be particularly vulnerable to disturbances of pre-TCR-signalling strength36,37. Taken together, these data indirectly support our concept that T-cell differentiation in the FCHO1 homodimers from the plasma membrane and hence regulates the clathrin-mediated endocytosis of their main interacting partner EPS15. Here, we show that a single substitution at R679P, located

R879A), located
**Fig. 7 Chlorpromazine-induced inhibition of CME arrests development of thymocytes.** a Thymi-derived double-negative three progenitors (DN3: CD4⁻CD8⁻CD44⁺CD25⁻) were sorted and cultured on OP9-DL1 stroma cells in the presence of Flt-3L, SCF and IL-7. At day 5 of co-culture cells were left untreated (ctrl) or treated with chlorpromazine (15 μM) to partially inhibit CME (chlorpromazine IC₅₀ for CME was established at 17.4 μM). Subsequently, development of double-positive (DP: CD4⁺CD8⁺) progenitors was assessed by FACS (left) and quantified (right). b Bone marrow-derived LSK progenitors (lineage⁻Sca-1⁻CD117⁺) were sorted and cultured on OP9-DL1 stroma cells in the presence of Flt-3L, SCF and IL-7. Analogous to a, at day 8 of culture cells were left untreated (ctrl) or treated with 15 μM chlorpromazine. Effect of partial CME inhibition on early-thymocyte progenitor development was assessed by FACS. Double-negative (DN) cells were defined as follows: DN1-CD4⁺CD25⁻; DN2-CD4⁺CD25⁺ and DN3-CD44⁺CD25⁻. c, d Bone marrow-derived LSK progenitors (lineage⁻Sca-1⁻CD117⁺) were sorted and cultured on OP9 stroma cells in the presence of Flt-3L, SCF and IL-7. At day 8 of culture cells were left untreated (ctrl) or treated with chlorpromazine (15 μM) to partially inhibit CME. Subsequently, development of c B-cell committed progenitors defined as B220⁺CD19⁺ and d granulocytes (CD11b⁺Gr-1⁻) was assessed by FACS (left) and quantified (right). a–d Density FACS plots and charts are representative of two independent experiments where one to four wells were measured at each indicated time point. Number adjacent or within the gates indicate frequency. Each dot on the chart represents one well. Whiskers indicate the range (min to max). Arrows indicate time points when chlorpromazine was added. Statistical analysis was performed using two-way ANOVA (p-values for effect of chlorpromazine). Source data are provided as a Source Data file.

important initiators of clathrin-mediated endocytosis. This notion is also supported by the lack of functional consequences of FCHO1 deficiency observed on both VSV-G-mediated virus entry and TIR endocytosis in T cells. Both processes strictly rely on CME, but are apparently not affected in FCHO1-deficient cells. We conclude that FCHO1 deficiency does not generally affect cellular processes that require CME, underscoring a selective role of FCHO1.

This concept is further corroborated by a series of other studies focusing on central elements orchestrating clathrin-mediated endocytosis. Defects in prime molecules such as clathrin, epsin, AP-2 and dynamin, result in embryonic lethality in model organisms reviewed in ref. 5. Mutations in proteins that are deemed to be less pivotal for the clathrin-mediated endocytosis have been linked to a variety of diseases such as cancer, neuro-psychiatric disorders, metabolic syndromes, but not to inherited defects of the immune system.9,35 Of note, a large number of FCHO1 and FCHO2 interacting partners have been identified, yet none of them have been linked to the adaptive immune system.27,35,39,41,42

The quality of signal transmitted by pre-T-cell receptor (pre-TCR) and TCR is essential for T-cell development and homeostasis. During T-cell ontogeny, there are three stages in which such signal is critically required for selection, differentiation and commitment of developing thymocytes, respectively (reviewed in refs. 43,44).
The precise localisation of the TCR that is continuously internalised and recycled back to the plasma membrane is of critical importance for the quality of signal transduction. The TCR can be internalised via clathrin-dependent\(^{16-21,43}\) or clathrin-independent mechanisms\(^{46}\). This process may be ligand-dependent or ligand-independent\(^{16,20,22,44,47,48}\). Even though previous studies have linked TCR internalisation to a di-leucine sorting motif present in the CD3\(\gamma\) subunit of the TCR/CD3 complex\(^{36,90}\), we could not provide definitive evidence that FCHO1 directly binds to the CD3\(\gamma\) sorting motifs. Nevertheless, we have shown that FCHO1 deficiency results in impaired TCR internalisation. Our data thus suggest that (a) we were not in the position to prove FCHO1/CD3 interaction using crude biochemical methods or (b) that FCHO1 interacts with other membrane-bound molecules to indirectly induce TCR internalisation.

In theory, FCHO1-dependent TCR internalisation can serve several purposes: (1) activation signals could be amplified via the scaffolding on early endosomes, (2) activation signals could be attenuated via lysosomal degradation of the TCR or (3) recirculation of the TCR by internalisation can activate recycling endosomes that modulate TCR-dependent activation signals upon encountering antigen presenting cells. Integrating signal strengths relies on spatiotemporal organisation of the TCR and associated signalling molecules. Although the quality of the signal provided by TCR is essential for the outcome of thymopoiesis, our studies in FCHO1 deficiency do not provide definitive answers on detailed mechanisms of FCHO1 in T-cell differentiation. Indirectly, the link between CME and TCR-signalling is highlighted by our in vitro T-cell differentiation studies. In the presence of chlorpromazine, a chemical inhibitor of CME, we observed a rather specific effect of differentiation blockade at the DN3-DP transition. This step is known to be highly dependent on TCR-signalling strength\(^{36,37}\).

While our study was under review, Calzoni et al. reported biallelic mutations in FCHO1 in four families\(^{51}\). The phenotypes of these patients resembled the phenotype of our patients. Based on experiments in activated T-cell blasts, the authors concluded that in the absence of FCHO1, CME of the transferrin receptor is affected\(^{51}\). In contrast, our data support the concept that FCHO1 does not control internalization of the transferrin receptor and mediates more subtle effects on CME.

In sum, our studies unravel a previously unrecognised role of FCHO1 in orchestrating the T-cell development and function. Our discovery also exemplifies how systematic studies of patients with inherited disorders help to uncover genes and pathways that were previously not associated with the function of the immune system. FCHO1 deficiency thus highlights a critical role of clathrin-mediated endocytosis for the development and function of human T cells.

**Methods**

**Patients.** Patients were referred to the clinical and scientific team of Professor Christoph Klein for further investigations. Informed consent/assent for the genetic and immunological studies, as well as their publication, was obtained from all legal representatives and patients. Genetic and functional studies on biosamples from patients and their relatives were performed under the framework of a scientific project entitled "Genetic characterisation of congenital bone marrow failure and immunodeficiency syndromes". This study was approved in 2011 by the ethics committee at LMU (438-11) and includes permission to publish the results.

**Whole-exome sequencing and variant filtering.** For family A we performed whole-exome sequencing (WES) for patient and father using Agilent V4 + UTR library preparation and a SOLID sequencing platform. WES for the patient from family B, the patient from family C, as well as for patient, mother and siblings (from family D) was performed after Agilent library preparation (V5 + UTR or V6 + UTR (SY265)) using an IlluminaNextSeq 5000 platform. BWA (Version 0.7.15) was used to align short reads to the human reference genome Grch37.p13. Variants were called and recalibrated according to the best practice pipeline by GATK (version 3.6). The final variants were then annotated with VEP release 85. A custom in-house database was used to filter variants to be rare (not reported in gnomAD or ExAC), as well as severe effects according to the Ensembl guidelines. Effects of filtered variants on protein were predicted with SIFT\(^{52}\) and PolyPhen-2\(^{53}\). The remaining variants were compiled and filtered for rare homozygous and low frequency heterozygous mutations following a patient- and inheritance-filtering approach. Whole-exome sequencing of genomic DNA of kindred F patients was conducted using Illumina sequencing platforms. Bioinformatics analysis for detection of rare sequence variants following Mendelian inheritance patterns were performed as described previously\(^{54}\).

**Sanger sequencing.** FCHO1 Sanger sequencing was performed to confirm WES-detected variants and their segregation with the clinical phenotype across the family members. Genomic DNA was PCR-amplified using OneTag Polymerase (NEB), specific primers are provided in Supplementary Table 3. Amplicons were sequenced either in-house or by using the commercial service of Eurofins Genomics.

**Structural analysis of FCHO1 mutants.** Crystal structures of FCHO1 domain were modelled using PyMol software with the maturizer wizard\(^{35}\). Mu homology domain of Danio rerio with bound Epis15 peptide (SIP2; https://doi.org/10.2210/pdb9hS5/pdb) and human FCHO2 F-Bar domain (pdb2Y00; https://doi.org/10.2210/pdb2Y00/pdb) were chosen as template for structure modelling. To calculate effect of point mutations on the structures, rotamer configurations of the highest probability were chosen.

**Cell lines.** The SK-MEL-2 cell line, engineered using zinc finger nucleases (ZNF) genome editing to stably express GFP under the endogenous human clathrin light chain A locus (CLTA-RFP), was kindly provided by David G. Drubin, University of Berkeley\(^{57}\). The cell line with only one GFP-tagged CLTA locus was used to produce mRFP tagged CLTA. The expression of the Clathrin light chain tagged with mRFP was kindly provided by Klemens Rottner from Technical University in Braunschweig\(^{56}\). It was cloned to lentiviral pRRL vector for production of virus particles. The correctness of the sequences was routinely monitored by Sanger sequencing.

**Plasmid DNA cloning.** Full-length cDNA of the human FCHO1 isoform b (IMAGE 5757146, cloned into the pEGFP-C3 vector with modified MCS (EcorRI/ Sall) was kindly provided by Emmanuel Boucot (UCD London) and Harvey McMahon (MRG-LMB, Cambridge, UK). Patient mutations were introduced by site-directed PCR mutagenesis using Q5 site-directed mutagenesis kit (NEB) according to manufacturer’s instruction with specific primers designed with NEBase Changer (NEB). WT and mutated FCHO1 cDNAs were cloned as either GFP fusion proteins or as IRES-containing bicistronic lentiviral pRRL vectors with mRFP as a reporter gene, respectively. Full-length cDNA of human clathrin light chain tagged with mRFP was kindly provided by Klemens Rottner from Technical University in Braunschweig. It was cloned to lentiviral pRRL vector for production of virus particles. The correctness of the sequences was routinely monitored by Sanger sequencing.

**CRISPR/Cas9 genome editing.** The genomic locus of FCHO1 (transcript ENST00000599202.1) was designated for gene disruption by introducing double-strand breaks in exon 7 (5′-GGACCCTTCGCTGCAGCGC AGG-3′) and intron 7 (5′-GTGAGCCTGATGAACCCTGGGTGTG-3′) and introduced by site-directed PCR mutagenesis using Q5 site-directed mutagenesis kit (NEB). The correctness of the sequences was monitored by Sanger sequencing. As endogenous expression of FCHO1 is too low for faithful assessment by western blot, deletion of FCHO1 was confirmed by PCR. The following primer pair was used for validation: (5′-GTGACCCTGCAGCGCAGCCCGTAGTGGTGCTG-3′, 5′-TGACATGTGGGTGGACAGATCAGAC-3′). Cells carrying homozygous mutations are expected to have a long deletion, spanning between exon 7 and intron 7. A minimum of five clones carrying homozygous mutations resulting in frameshift were used for subsequent experiments (Supplementary Fig. 12). Unmodified clones were used as a FCHO1-positive control. To ensure deletion specificity, the ten most probable off-target sites were tested using direct Sanger sequencing, showing no signs of unspecific cuts.

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Transient transfection. FCHO1 ko SK-MEL-2 cells and FCHO1 ko Jurkat cells were transfected using calcium phosphate transfection kit (Sigma, USA) and 0.1–1 µg of the various lentiviral plasmids carrying either wt or mutant FCHO1 cDNAs. Cells were typically incubated 24 h to express the constructs before imaging.

Lentiviral vector production and cell transduction. For production of vesicular stomatitis virus G glycoprotein (VSV-G)-pseudotyped lentiviral particles HEK293T cells were transfected using calcium phosphate transfection kit (Sigma, USA) and 5 µg respective lentiviral vector, 12 µg pcDNA3.GFp.αCITE (which expresses herpes simplex virus 1 (HSV)-1-αCITE), 5 µg pHSV-Rev (which expresses VSV-G) in the presence of 25 µM chloroquine (Sigma, USA). Eight hours after transfection, medium was exchanged and supernatant containing lentiviral particles was collected after 24, 48 and 72 h post transfection. Viral titre were determined on 3T3 cells.

FCHO1 ko SK-MEL-2 cells and FCHO1 ko Jurkat cells were transduced with lentiviral particles through centrifugation at 900 × g for 4 h at 32 °C in the presence of polybrene (8 µg/ml) (Sigma, USA).

Production of HIV-1 stocks. The HIV-1NL4-3ΔEnv plasmid was obtained from Nathaniel Landau (Alexandria Centre for Life Science, NYU, USA), the HIV-1NL4-3ΔEnv plasmid was a kind gift of Oliver T. Fackler (Universitätsklinikum Heidelberg, Germany) and the BlaM-Vpr plasmid was a gift from Thomas J. Hope (Northwestern University, Chicago, USA). HIV-1NL4-3ΔEnv and HIV-1NL4-3ΔEnv (BlaM-Vpr), both VSV-G pseudotyped, or HIV-1NL4-3ΔEnv (BlaM-Vpr) stocks were produced by PEI co-transfection of HEK293T cells. Forty-eight hours later, supernatants were collected and filtered through a 0.45 µm Stericup (Millipore). After sucrose cushion (25% in 1x phosphate-buffered saline (PBS) purification, medium was exchanged directly before start of the experiment. During the experiment dishes were placed into a temperature-controlled chamber on the microscope stage with 95% air, 5% CO2 and 100% humidity. Live-cell imaging data were acquired using a fully motorised inverted confocal microscope (Zeiss LSM880) using either 40x/1.4 NA or 63x/1.4 NA objectives) under control of the Definite Focus for Axio Observer Z1 (Zeiss). Sixteen-bits digital images were obtained with GaAsP high-sensitivity detectors, confocal module and 488 and 561 nm laser lines and a dual (525/50; 605/70) BP filters. Cell regions were selected to allow for 500–950 ms-lasting intervals. Time dependence of the fluorescent intensity of FCHO1 and endogenous clathrin were assessed on movies from three independent experiments, using Zen Blue software (Zeiss). Up to three 25 µm2 regions per cells was quantified. Both GFP and RFP channels were normalised to the background and initial fluorescence was set to 1.

Immunoprecipitation and western blot. To test for protein–protein interactions, FCHO1-deficient cell lines were used. SK-MEL-2 cells overexpressing different variants of GFP-FCHO1 fusion proteins were starved in serum-free medium for 1 h at 37 °C. Western blot bands were cut out and subunits were resolved using 5 µg PMG-D (which entirely includes VSV-G) in the presence of 25 µM chloroquine (Sigma, USA). Eight hours after transfection, medium was exchanged and supernatant containing lentiviral particles was collected after 24, 48 and 72 h post transfection. Viral titre were determined on 3T3 cells.

Live-cell imaging. For live-cell imaging experiments stably transduced SK-MEL-2 cell lines were used. Cells were seeded on μ-dish 35 mm, high Glass Bottom (IBIDI, glass coverslip no.1.5 H, selected quality, 170 µm) in 24-well tissue culture plates and cultured in complete DMEM/F12 medium for 12 h before imaging. Samples were analysed using confocal fluorescent microscopy using the Zeiss LSM880 and Zeiss LSM880 inverted microscopes. Images were collected using 63x/1.4 NA or 40x/1.4 NA oil objectives (Zeiss). Four solid-state 5 and 10 mW laser (405, 488, 561, 640 nm) were used as light source; scanner frequency was 400 Hz; line-averaging 2. All images were obtained with GaAsP high-sensitivity detectors. Pearson correlation or co-localisation coefficient was assessed on 16-bits raw digital files on Zen Blue software (Zeiss). Representative cells from two to three independent experiments were chosen for analysis. Two to four 25 µm2 square regions were selected and minimum ten cells per condition were analysed. To avoid bias during analysis, only one fluorescent channel was active while selecting regions.

Fusion of VSV-G-pseudotyped HIV-1 ΔEnv to Jurkat T cells. The virion fusion assay was performed in principle as reported employing HIV-1 particles carrying the HIV-1 Vpr protein fused to β-lactamase (BlaM-Vpr). In brief, after fusion of virus particles to target cells, the incorporated BlaM-Vpr protein is released into the cytoplasm and is able to cleave the CCF2 dye. This leads to a shift of the dye's excitation and emission wavelengths 10–15 nm, which can be detected and quantified by flow cytometry. Jurkat T cells were plated at a density of 2 × 105 cells per well (96-well conical plate, Corning, New York, USA). Where indicated, the HIV-1 fusion inhibitor T20 (50 µM, enfuvirtide, Roche, Rotkreuz, Switzerland) was added 1 h prior to virus challenge. Serial dilutions of VSV-G-HIV-1NL4-3ΔEnv (BlaM-Vpr) were added in PBS. 4 h following challenge with 1:2000 diluted virus (VSV-G HIV-1NL4-3ΔEnv (BlaM-Vpr) or HIV-1NL4-3ΔEnv (BlaM-Vpr)), Jurkat T cells were washed and incubated with the CCF2 dye as previously reported. The following day, cells were fixed for 90 min with 4% PFA and analysed by flow cytometry.

Infection of Jurkat T cells with VSV-G-HIV-1ΔEnv. Jurkat T cells were plated at a density of 2 × 105 cells per well. T20 (50 µM), the V-ATPase inhibitor bafilomycin A1 (100 nM, Sigma Aldrich, St. Louis, USA) or PBS were added 1 h prior to virus challenge. Cells were challenged with VSV-G-HIV-1NL4-3ΔEnv and 4 h later, 200 µl of fresh medium were added to each well of the 96-well plate. 24 h post infection, the supernatant was monitored for p24 and/or HIV infection was measured using an intracellular p24 staining (anti-p24 antibody, clone KC57-FITC, Beckmann Coulter, Brea, USA) in principle as reported.

Transferrin uptake. Fibroblasts from healthy donor (HD) and patient (kindred E) were detached using PBS containing 10 mM EDTA and serum-starved for 30 min in DMEM at 37 °C. Cells and transferrin conjugated to AlexaFluor 633 (ThermoFischer Scientific) were washed in glucose buffer (PBS supplemented with 20 mM Glucose and 1% BSA) and cooled down on ice. 25 µg/ml AlexaFluor 633-conjugated transferrin were added to cells and incubated on ice for 10 min. Cells were then transferred to 37 °C for indicated periods of time. Upon indicated time, surface-bound transferrin was stained by acid wash (PBS supplemented with 0.1 M glycine and 150 mM NaCl at pH 3) and uptake of fluorescent transferrin was determined by flow cytometry. Data are pooled from four independent experiments. Error bars indicate mean ± SD. Statistical analysis using two-way ANOVA followed by Sidak’s multiple comparisons test revealed no significant difference in transferrin uptake between HD and patient fibroblasts.
Flow cytometry. Blood samples were washed with PBS and stained with the following antibodies for 20 min at RT: α-CD45 PerCp-Cy7 (BD), α-CD1 @ APC (BD) and α-CD6 PE-Cy5 (BD), α-CD3 PE- H7 (eBioscience), α-CD19 ETC (BD), α-CD4 PE-APC (RPA-T8), α-CD4 PE-Cy7 (A161A1) all from Biolegend. All antibodies used are summarised in Supplementary Table 4. Red blood cells were lysed using 1 x BD FACs Lysing Solution (BD Biosciences) according to the manufacturer’s instructions. The samples were acquired using an LSRFortessa (BD Bioscience) cytometer. Data were analysed using FlowJo Software (Treestar) and TNF. Gating strategy was according to instructions. The positive and negative control samples were used to calibrate the gates. Data were analyzed using GraphPad Prism software version 6.5. Pearson correlation and colocalization coefficient on selected cell fragments were assessed on raw files using Zen Blue software (Zeiss). Cell regions or entire cells are referred to as n unless indicated otherwise. No method of randomization was used, and no samples were excluded from analysis. To avoid bias during analysis, cell regions were selected based on the signal from only one fluorescent channel. No statistical methods were used to determine sample size for analyses. Two-way analysis of variance (ANOVA) or ANOVA analysis followed by Sidak’s multiple comparison test were used to assess differences between groups. p-values < 0.05 were considered to be statistically significant.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability
The source data underlying Figs. 2e, 3b, 3c, 4b, 5a-c, 6, 7a-d, Supplementary Figs. 1–5, 10, 11 are provided as a Source Data file. All other data are available from the corresponding authors upon reasonable request. The identified FCHO1 mutations have been submitted to the ClinVar database with accession numbers SCV001146883, SCV001146884, SCV001146885, SCV001146886, SCV001146887 and SCV001146888.

According to current regulatory frameworks, exome sequencing data cannot be made publicly available. For any further questions, the corresponding authors will share additional data in accordance with regulatory guidelines.

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**Author contributions**

M.L. and N.Z. initiated the project, designed the studies, performed experiments and analysed the data (pedigrees A-D); L.F. performed analyses of the data (pedigrees A-D); U.P. designed and performed experiments, analysed the data (pedigree F); M.S. performed HIV infection experiments and analysed the data; Y.L. designed and performed CRISPR/Cas9 knockout experiments; Y.F. performed IP experiments (pedigree A-D); J.P. and S.H. performed bioinformatical analysis of WES data (pedigree A-D); L.S. performed genotyping and WB experiments, analysed the data (pedigrees A-D); E.Ü provided patient material, clinical data (pedigree C); Y.F. performed Sanger sequencing and in silico prediction analyses of mutations (pedigrees A-D); K.-W.S. provided clinical data (pedigree A); O.T.K designed and analysed HIV infection experiments; E.U. provided patient material, clinical data (pedigree C); M.K. provided patient material, clinical data (pedigree B, D); A.L. provided patient material, clinical data and genetic analysis (pedigree E); M.H. J.R. and K.S. designed the studies, provided patient material, clinical data and genetic analysis (pedigree F and G); C.K. performed family analyses, RNA and protein detection (Pedigree F). C.K. initiated the project, designed the studies and provided clinical and laboratory resources; (pedigrees A-E); M.L. and N.Z. and C.K. wrote the manuscript.

**Competing interests**

The authors declare no competing interests.

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