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Supplementary Information for

Inherited deficiency of stress granule ZNFX1 in patients with monocytosis and mycobacterial disease

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Supplementary Information Text

Case report

Kindred A. Patient 1 (P1, II.2) was born in 2008 at Sirjan, Iran, to consanguineous parents (**Figure 1A, Supplemental Table 1**). He was vaccinated with BCG at birth. At the age of three months, he developed fever and left axillary lymphadenopathy accompanied by hepatosplenomegaly. An axillary lymph node biopsy culture was positive for *M. bovis*-BCG and histological analysis revealed chronic granulomatous inflammation. Disseminated BCG disease (BCG-osis) was diagnosed. P1 received isoniazid (INH), rifampicin (RIF) and ethambutol (EMB) for one month, but the antibiotics (ATB) prescribed were then replaced by clarithromycin, ofloxacin, amikacin and cycloserine due to hepatotoxicity. P1 also received subcutaneous IFN- γ . After 18 months of treatment, the clinical findings had improved. At the age of four years, P1 had a persistent fever and cough. Chest CT-scan showed diffuse bilateral interstitial lung disease. PCR for cytomegalovirus (CMV) was positive for blood and nasopharynx samples, but negative, at the time for bronchoalveolar lavage (BAL) and urine. Pneumonia with pleural effusion was diagnosed (**Supplemental Figure 1**). P1 was treated with ganciclovir with made a complete recovery. At the age of 10 years, P1 was admitted to hospital for lower left lobar pneumonia. He was treated with antibiotics (ATB) (vancomycin and meropenem), leading to good clinical resolution (**Supplemental Figure 1**). No history of several viral infections was documented, despite seropositivity for multiple viruses, attesting to previous infection (**Supplemental Figure 2**). Nitroblue tetrazolium (NBT) and dihydrorhodamine (DHR) assays gave normal results for neutrophils; immunoglobulin levels were normal (IgG: 1,090; IgM: 53; IgA: 196; IgE: 17 IU/mL); complement C3-C4 and CH50 levels were normal; vaccine-mediated protection against diphtheria and tetanus toxoid was good; no autoantibodies (ANA and anti-ds-DNA) were detected (**Supplemental Table 2**). The sister of P1 (II.1) died at the age of two months, from gastroenteritis and renal failure. The parents are healthy and have not presented any severe infectious disease over the course of their lives.

Kindred B. Patient 2 (P2, II.1) was born in 1998 to a consanguineous family originating from Morocco (first-cousin parents) and living in Belgium (**Figure 1A, Supplemental Table 1**). He did not receive BCG vaccine, in accordance with the routine immunization schedule in Belgium. At the age of 18 days, he was hospitalized in an intensive care unit (ICU) due to failure to thrive, multiple peripheral adenopathies, hepatosplenomegaly, edema, generalized maculopapular cutaneous rash, acute thrombocytopenia and peripheral monocytosis. Cultures of blood, urine and stool for the detection of viruses, bacteria and fungi were negative. A myelogram was performed and revealed significant monocytosis (15%), an absence of megakaryocytes, but no evidence for neoplasia or hemophagocytosis. The peripheral blood karyotype was 46 XY. Vancomycin treatment was indicated. Between the ages of six and 18 months, P2 was hospitalized three times for recurrent episodes of fever, maculopapular or purpuric rash, organomegaly (hepatosplenomegaly and adenomegaly), diarrhea, thrombocytopenia, hepatic cytolysis without documented microbe detection (except one stool sample positive for *Clostridium jejuni*). At the ages of 14 months, 5, 8 and 10 years, P2 was admitted to hospital for focal complex seizures, and a psychodevelopmental delay was also diagnosed. P2 received continuous anti-epileptic therapy. At the age of 12 years, he was hospitalized in the ICU for multiple-organ failure (shock, severe pneumonia with pleural effusion, abscess and maculopapular then purpuric rash). Disseminated *Mycoplasma pneumoniae* infection was suspected. However, P2 has never been hospitalized for a severe documented viral infection (**Supplemental Figure 5, Supplemental Table 2**). During this hospitalization at the age of 12 years, P2 had thrombocytopenia and major peripheral monocytosis ($14 \times 10^3/\text{mm}^3$; 39% of whole blood count); in the bone marrow, 42% of monocytes displayed nuclear destruction, which resolved over a period of three weeks (**Supplemental Figure 1**). At the age of 13 years, P2 was diagnosed with disseminated tuberculosis (TB) (multifocal pneumonia and pleural effusion, meningitis, multiple bone lesions, with spleen and liver involvement). The ferritin level was elevated in blood (2,430 ng/mL), with normal triglyceride and fibrinogen levels. A magnetic resonance imaging (MRI) scan of the brain (**Supplemental Figure 1**) showed an apparent right frontal tuberculoma. Multi-susceptible *Mycobacterium tuberculosis* grew in culture. P2 was treated with five ATB and corticosteroids. After three months of therapy, clinical improvement was observed, and the treatment was replaced by INH, RIF and ciprofloxacin. The clinical

manifestations resolved well, but neutrophilia, monocytosis, high C-reactive protein (CRP) and abnormal erythrocyte sedimentation rate (ERS) persisted, even three years after the diagnosis of disseminated TB. Bone marrow aspiration showed a normal frequency (3%) and morphology of monocytes. P2 had three sisters and three brothers. Two sisters (II.2, born in 1999 and II.3 born in 2002) and one brother (II.6, born in 2005) are healthy. One sister (II.4, born in 2003) died at of the age of five years from “atypical hemolytic uremic syndrome (HUS)” with a decrease in factor H concentration and activity. One brother (**P3**, II.7, born in 2011) has presented recurrent episodes of thrombocytopenia and a high degree of monocytosis, but without severe infectious diseases. Another brother (**P4**, II.6, born in 2010) died at the age of 14 months from disseminated TB with severe meningoencephalitis. His cerebral T1, T2 and FLAIR weighted MRI scans showed an altered parenchymal signal in the supratentorial and mesencephalic regions, and in the gray matter nuclei, suggesting diffuse edema associated with a diagnosis of encephalomalacia with multiple arachnoid cysts. None of the siblings received BCG vaccine, in accordance with the routine immunization schedule in Belgium. Finally, the parents are alive and healthy with no history of severe infections.

Supplementary Figures

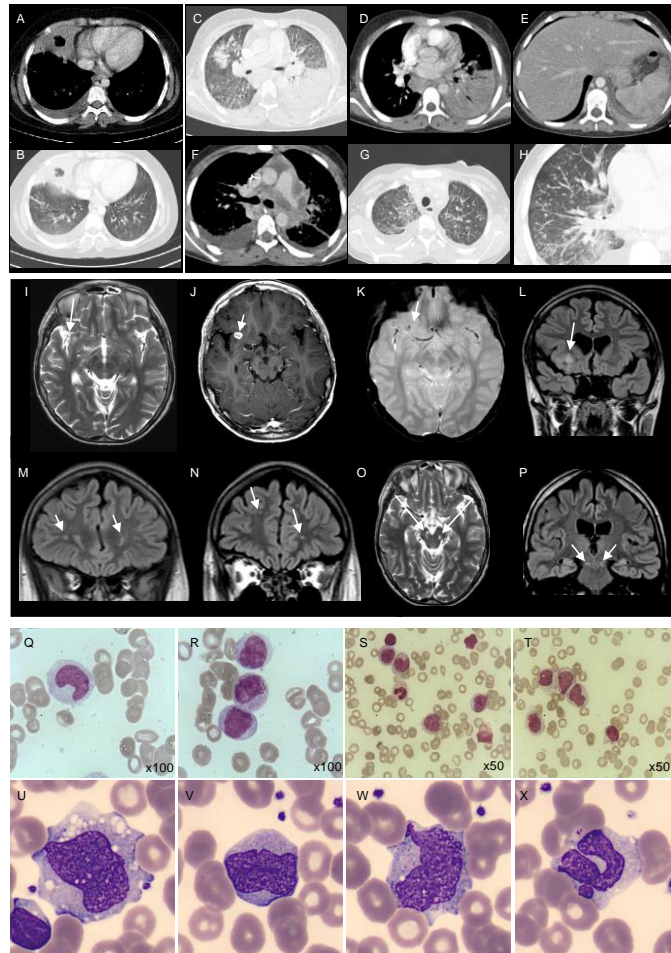


Fig. S1. Images of pulmonary, cerebral and hematological manifestations in ZNF1-deficient patients. **A and B.** CT-scan lung and mediastinal windows for P1 at the age of 10 years, showing a right lung abscess associated with right pleural effusion. **C-D.** CT scan lung and mediastinal windows for P2 at the age of 12 years, showing necrotizing pneumonia of the left inferior lobe, **(C)** a rounded peribronchial consolidation in the right upper lobe and patchy areas of ground-glass opacities. In the mediastinal window **(D)**: enlarged bilateral hypodense lymph nodes. **E.** Homogeneous hepatomegaly and large necrotic lesions of the spleen in P2. **F.** CT scan performed at the age of 13 years: the mediastinal window shows diffuse necrotic quasicystic lymph nodes and bilateral pleural effusions. **G.** Lung CT-scan of P2 showing a large area of ground-glass opacity, associated with smooth thickened interlobular septa, which are not usually described in mycobacterial infections. **H.** Detail of the lung parenchyma on maximal intensity projection reconstruction, revealing diffuse, randomly distributed (miliary pattern) micronodular disease. **I-P.** Brain MRI scan for P2 at the age of 13 years, showing a right frontal lesion with T2 and FLAIR hyperintensity **(I and L)** and contrast enhancement after gadolinium staining (T1 weighted sequences, **(J)**) with hypointensity reflecting calcification processing (T2* weighted sequences, **(K)**). Brain MRI showed mild cortical atrophy **(I, L)**. Bilateral brainstem and supratentorial hyperintensities highlighted probable sequelae of previous tuberculosis disease (T2 and FLAIR weighted sequences). **Q-T.** Bone marrow aspiration for P1 performed at the age of 11 years, showing an increase in the proportion of monocytes (42%) without cytological aberrations (optical magnification x100 or x50). **U-X.** Blood smear from P1 at the age of 11 years.

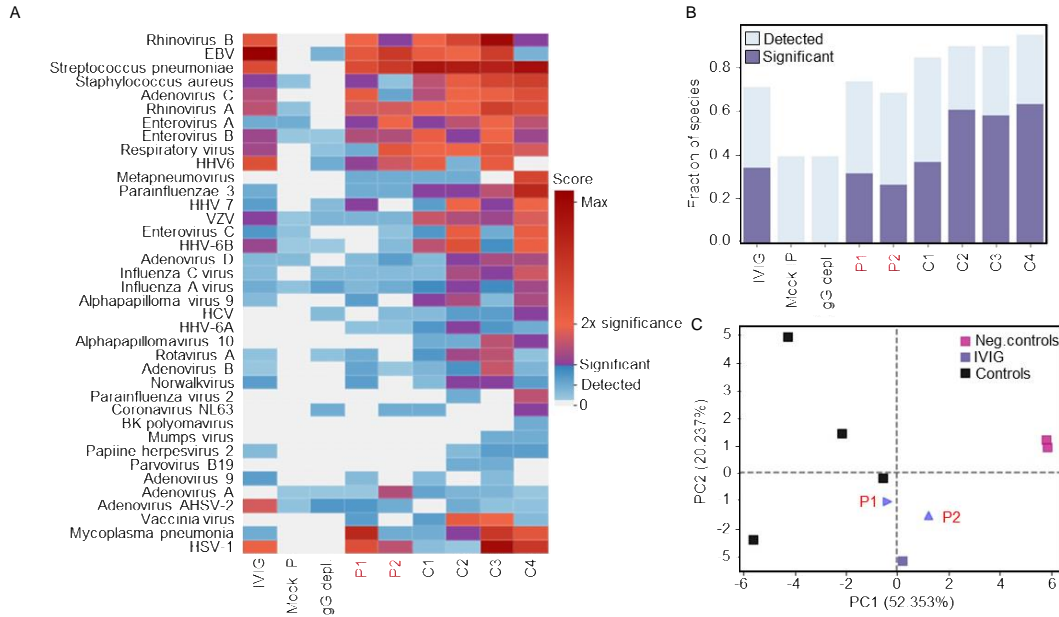


Fig. S2: Exposure to microbes in the ZNFX1-deficient patients. **A.** Heatmap plot representing previous microbial exposure in ZNFX1-deficient patients (P1 and P2), evaluated by phage immunoprecipitation–sequencing (PhIP-Seq) with comparison to unrelated matched controls (C1-C4), pooled plasma used for intravenous immunoglobulin (IVIG) therapy, IgG-depleted serum (IgG_Depleted) and a mock IP sample. IVIG was used as a positive control and IgG-depleted serum and the mockIP sample were used as negative controls. Antimicrobial antibody species-specific score values equivalent to the count of significantly enriched peptides. The color indicates the scores, with scores above the threshold shown in shades of violet to red. Max, maximum. **B.** Fraction of species shown in A for which a given sample was seropositive. **C.** Principal component analysis of the species-specific score values shown in A.

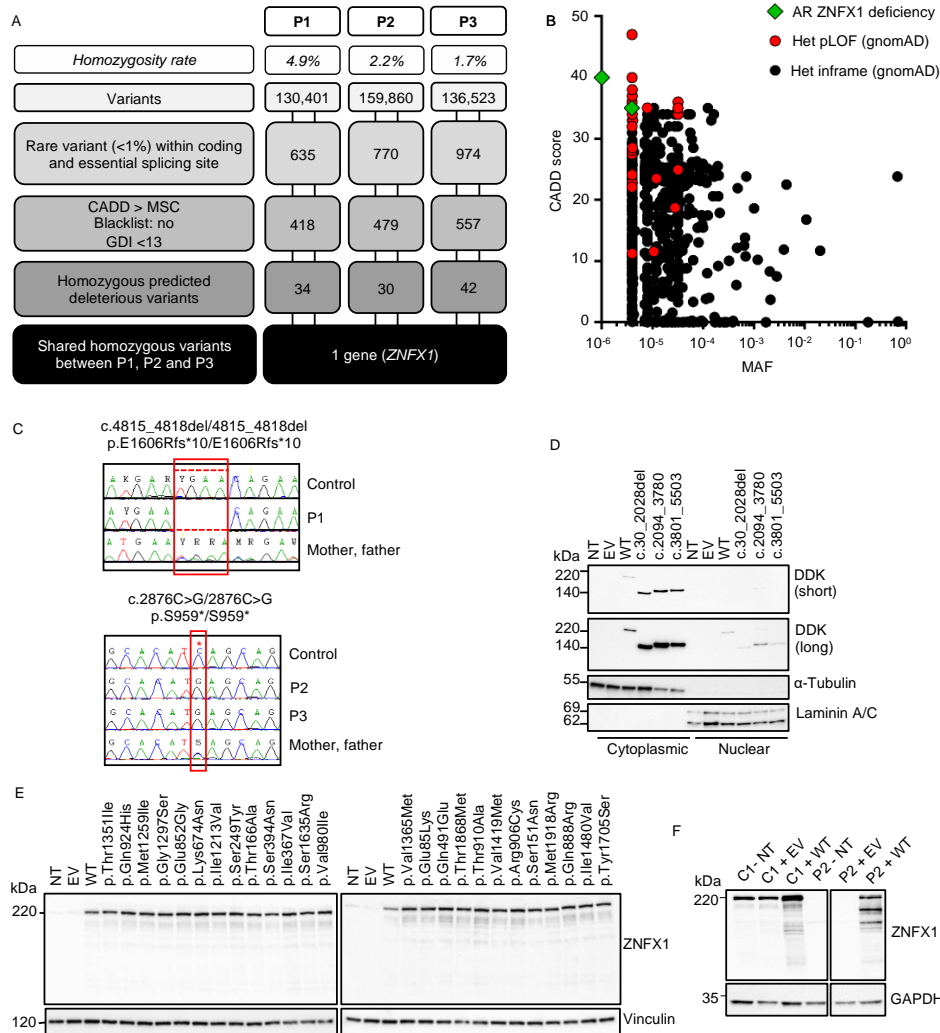


Fig. S3: Genetics of *ZNF1* deficiency: **A.** Analyses of whole-exome sequencing (WES) data identified *ZNF1* as the only gene carrying a homozygous coding mutation common to the three kindreds. **B.** The minor allele frequency (MAF) and combined annotation-dependent depletion (CADD) score of the homozygous *ZNF1* variants found in P1-P3 (red symbols) or in all heterozygous gnomAD v.2.1.1 and Bravo/TOP2 variants (black symbols). The dotted line corresponds to the mutation significance cutoff (MSC) with its 99% confidence interval. The MSC for *ZNF1* is 3.3. **C.** Sanger sequencing electropherogram confirming the homozygous *ZNF1* variant in P1, P2, and P3 and heterozygous in parents. **D.** Cytoplasmic and nuclear protein fractions extracted from HEK293T cells transiently transfected with an empty plasmid (EV), wild-type (WT)-*ZNF1*, or constructs with deletions of the ARM (c.30_2028del), helicase (c.2094_3780del) or ZNF (c.3801_5503del) domain, with an antibody directed against the C-ter DDK tag (short and long exposure). An antibody against tubulin or laminin A/C was used as a cytoplasmic and nuclear loading control, respectively. **E.** Levels of protein for the homozygous *ZNF1* variants found in public databases (gnomAD and BRAVO/TOPmed). Western blots were performed with total protein extracts from HEK293T cells either left NT or transfected with EV, WT or *ZNF1* variants. *ZNF1* was detected with a monoclonal antibody directed against the N-terminus of *ZNF1*. An antibody against vinculin was used as a loading control. **F.** Western blot of total protein extracts from the SV40 fibroblasts of a healthy control (C1) and a *ZNF1*-deficient patient (P2) either left non-transfected (NT), or transfected with the EV or *ZNF1*-wild type (WT) cDNA. A monoclonal antibody against the N-terminus of *ZNF1* was used. An anti-GAPDH antibody was used as a loading control. The results shown are representative of two independent experiments.

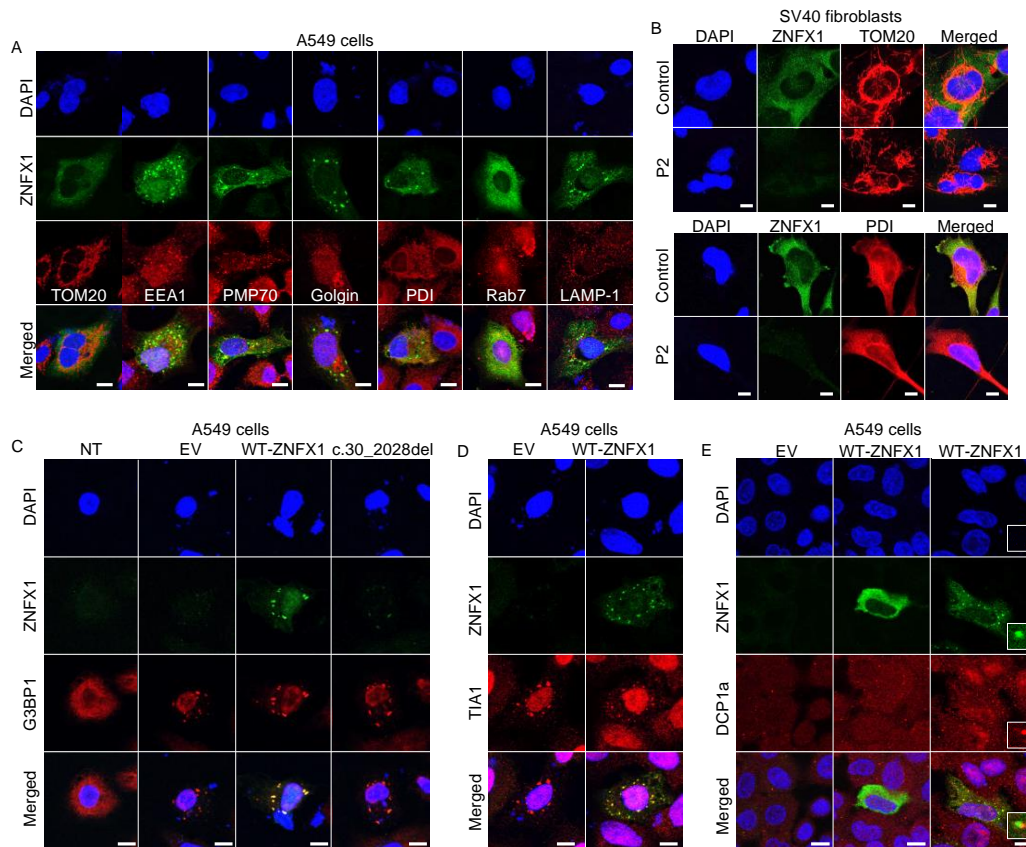


Fig. S4: Localization of ZNFX1 in A549 cell lines and SV40 fibroblasts. **A.** Confocal microscopy of A549 cell lines transiently transfected with the WT-ZNFX1 and stained with antibody recognizing mitochondria (TOM20), early endosomes (anti-EEA1), late endosomes (anti-RAB7), Golgi apparatus (Golgin), peroxisomes (anti-PMP70), lysosomes (anti-LAMP-1) and an antibody directed against the N-terminus of ZNFX1. **B.** Confocal microscopy of SV40 fibroblasts stimulated 24h with 25 μ g/mL of poly(I:C) from a healthy control and a ZNFX1-deficient patient (P2) stained with antibodies recognizing mitochondria (TOM20) or endoplasmic reticulum (PDI). **C.** Colocalisation of ZNFX1 and G3BP1 in A549 cells transiently transfected with an empty vector (EV), the WT-ZNFX1 or with a plasmid with an inframe deletion (c.30_2028del), coding for a mutant lacking the ARM domain recognized by the N-terminal ZNFX1 antibody. **D.** Colocalisation of ZNFX1 and TIA1 in A549 cells transiently transfected with an empty vector (EV) or the WT-ZNFX1. **E.** Colocalisation of ZNFX1 and DCP1a in A549 cells transiently transfected with an empty vector (EV) or the WT-ZNFX1.

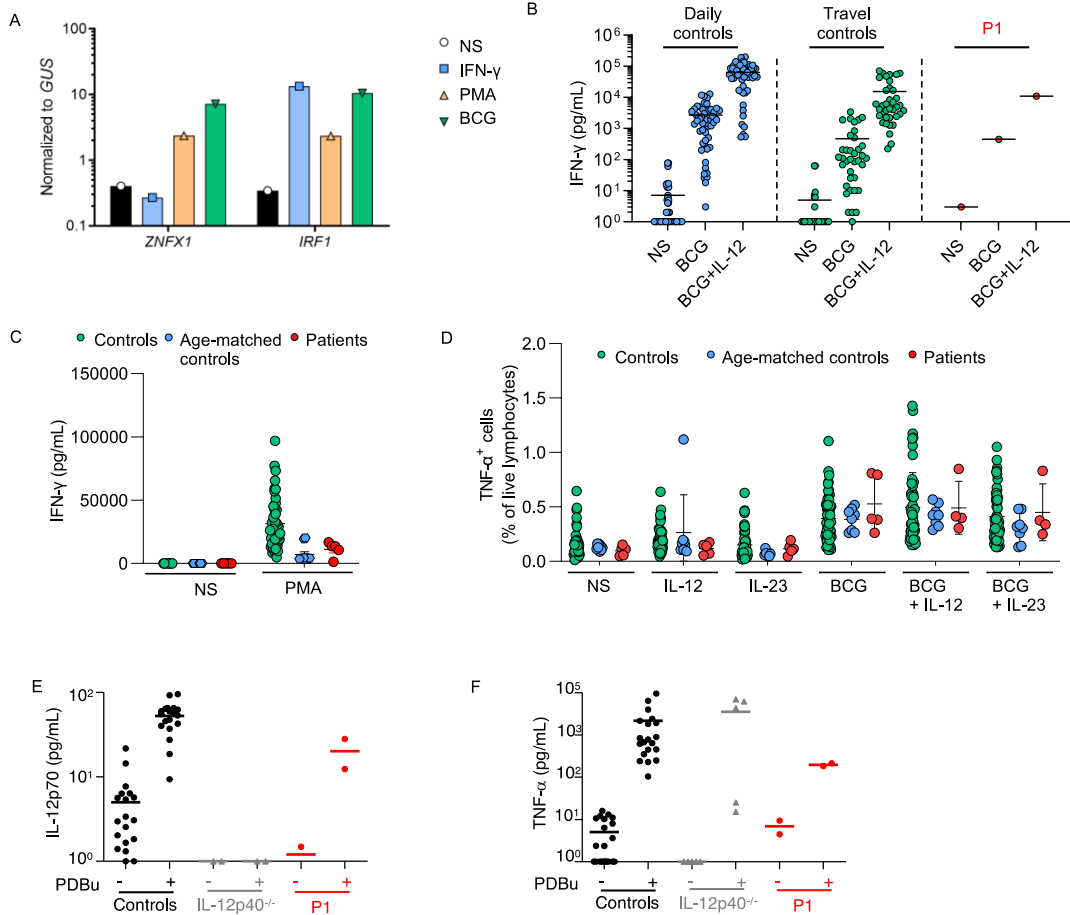


Fig. S5: Induction of *ZNFX1* expression in THP1 cells and cytokine secretion in the cells of *ZNFX1*-deficient patients. **A.** *ZNFX1* transcription, assessed by RT-qPCR in THP1 cells with and without stimulation with IFN- γ , PMA, or BCG for 36 h. *IRF1* transcription was also evaluated in the same samples. Expression is normalized against *GUS*, by the $2^{-\Delta\Delta C_t}$ method. **B.** Secretion of IFN- γ in whole blood from daily controls ($n=55$), travel controls ($n=37$), or P1, with and without (NS) stimulation with BCG alone or BCG + IL-12, as assessed by ELISA. **C.** Secretion of IFN- γ , assessed by ELISA on the supernatant of PBMC after the PMA-ionomycin stimulation of cells from healthy controls (green), aged-matched controls (blue), P1, P2 and P3 (red). Values are expressed as means \pm SEM. **D.** TNF- α level in the supernants of PBMCs after the stimulation with IL-12, IL-23, BCG, BCG+IL-12 or BCG+IL-23, as assessed by multiplex ELISA on cells from healthy controls (green), aged-matched controls (blue) and patients with AR *ZNFX1* deficiency (red). **E.** Production of IL-12p70 and **F.** TNF- α by EBV-B cells from controls, patients with AR complete IL-12p40 complete deficiency (IL-12p40 $^{-/-}$) and P1, after stimulation with 10^{-7} M PDBu for 24 hours. ELISA was used to determine the levels of these cytokines.

Table S1. Genetic, demographic, and clinical information for patients with AR ZNF1 deficiency

Patient	P1	P2	P3	P4
Kindred (code)	A (II-2)	B (II-1)	B (II-7)	B(II.6)
Genetic characteristics				
Mutation	c.4815_4818del/ c.4815_4818del	c.2876C>G/ c.2876C>G	c.2876C>G/ c.2876C>G	E?
Predicted cDNA	p.E1606Rfs*10/ p.E1606Rfs*10	p.S959*/ p.S959*	p.S959*/ p.S959*	E?
CADD score	35	40	40	-
Demographic data				
Country of origin	Iran	Morocco	Morocco	Morocco
Sex (male/female)	Male	Male	Male	Male
BCG vaccine	At birth	No	No	No
Onset of clinical manifestations	2.5 months	16 days	NR	NR
Age (follow-up)	13 years (alive)	21 years (alive)	9 years (alive)	Died
Infectious manifestations	BCG-osis, CMV viremia, oral candidiasis, pneumonia	Disseminated TB, <i>Campylobacter jejuni</i> gastroenteritis	None	Disseminated TB (meningoencephalitis)
Inflammatory features				
Organomegalies	Hepatomegaly	Hepatomegaly, splenomegaly	NR	Hepatomegaly, splenomegaly
Recurrent inflammatory episodes	Recurrent fever	Recurrent fever, dyspnea, skin rash, hepatitis	No	NR
Laboratory parameters during acute flare-up	High acute-phase reactant	High acute-phase reactant	-	NR
Other clinical manifestations				
Pulmonary disease	Interstitial pulmonary disease	Interstitial pulmonary disease	No	NR
Neurological disease	No	Seizures, neuro-developmental delay	No	NR
Hematological findings				
Peripheral leukocytosis	Neutrophilia Monocytosis	Neutrophilia Lymphocytosis Monocytosis	Monocytosis	NR
Peripheral cytopenia	No	Recurrent thrombocytopenia	Recurrent thrombocytopenia	NR

BGC, bacillus Calmette-Guérin; CADD, combined annotation-dependent depletion; E?, unknown genotype; CMV, cytomegalovirus; NR, not reported; TB, tuberculosis

Table S2. Immunological parameters of patients with autosomal recessive complete ZNF1 deficiency

Patient	P1	P2		P3	
Mutation	p.Glu1606Argfs*10/ Glu1606Argfs*10	p.Ser959*/ Ser959*		p.Ser959*/ Ser959*	
Age at testing	10 years	13 years		6 months	
			Normal range		Normal range
Lymphocytes					
T cells					
CD3+	7669	5,743	1,500-2.600/ μ L	2563	1,500-6,800/ μ L
CD4+	1,872	3,521	400-1,200/ μ L	2,034	1,400-4,300/ μ L
CD8+	5,178	1,845	330-920/ μ L	493	500-1,700/ μ L
B cells					
CD19+	722	128	193-628/ μ L	558	523-2,149/ μ L
NK cells					
CD16+CD56+	368	633	70-480/ μ L	33	160-950/ μ L
Platelets	278	1061	150-440 $\times 10^9$ /L	4	150-440 $\times 10^9$ /L
Neutrophils	11.0	10.6	1.5-8.0 $\times 10^9$ /L	2.5	1.5-5.0 $\times 10^9$ /L
Monocytes	2.5	2.3	0.1-0.85 $\times 10^9$ /L	4.8	0.2-1.2 $\times 10^9$ /L
Eosinophils	0.4	0	<0.81 $\times 10^9$ /L	0.1	<0.81 $\times 10^9$ /L
Basophils	ND	0	<0.21 $\times 10^9$ /L	0	<0.21 $\times 10^9$ /L
Immunoglobulins					
IgG	10.4	31.3	7.1-15.3 g/L	5.50	2.95-7.72 g/L
IgA	1.96	4.14	0.63-3.04 g/L	1.00	0.11-0.76 g/L
IgM	0.53	1.26	0.5-1.87 g/L	0.97	0.33-1.25 g/L
IgE	ND	41	<200 kIU/L	ND	<15 kIU/L
Autoantibodies	Negative	ND	-	ND	-
Anti-ds-DNA	Negative	ND	-	ND	-
Antigen T-cell proliferation	ND	Normal after candidin and tetanus toxoid	-	ND	-
Reactive oxygen species (ROS) by neutrophils in response to PMA	100%	100%	>70%	ND	
Mitogen T-cell proliferation	ND	Low after anti-CD3 antibody or PHA	-	ND	-

Post-vaccinal antibodies	Presence of anti-tetanus and anti-diphtheria antibodies	Presence of anti-pneumococcal and anti-tetanus antibodies; no anti-diphtheria antibodies (<0.1 IU/mL)	-	ND	-
IgG HIV	negative		negative	negative	
IgG CMV	Positive	ND	-	ND	-
IgG EBV	Positive	ND	-	ND	-
IgG VZV	Negative	ND	-	ND	-
IgG HSV-1	Positive	ND	-	ND	-

CMV, cytomegalovirus; EBV, Epstein-Barr virus; HSV-1, herpes simplex virus type 1; ND, not done; PHA, phytohemagglutinin; VZV, varicella zoster virus