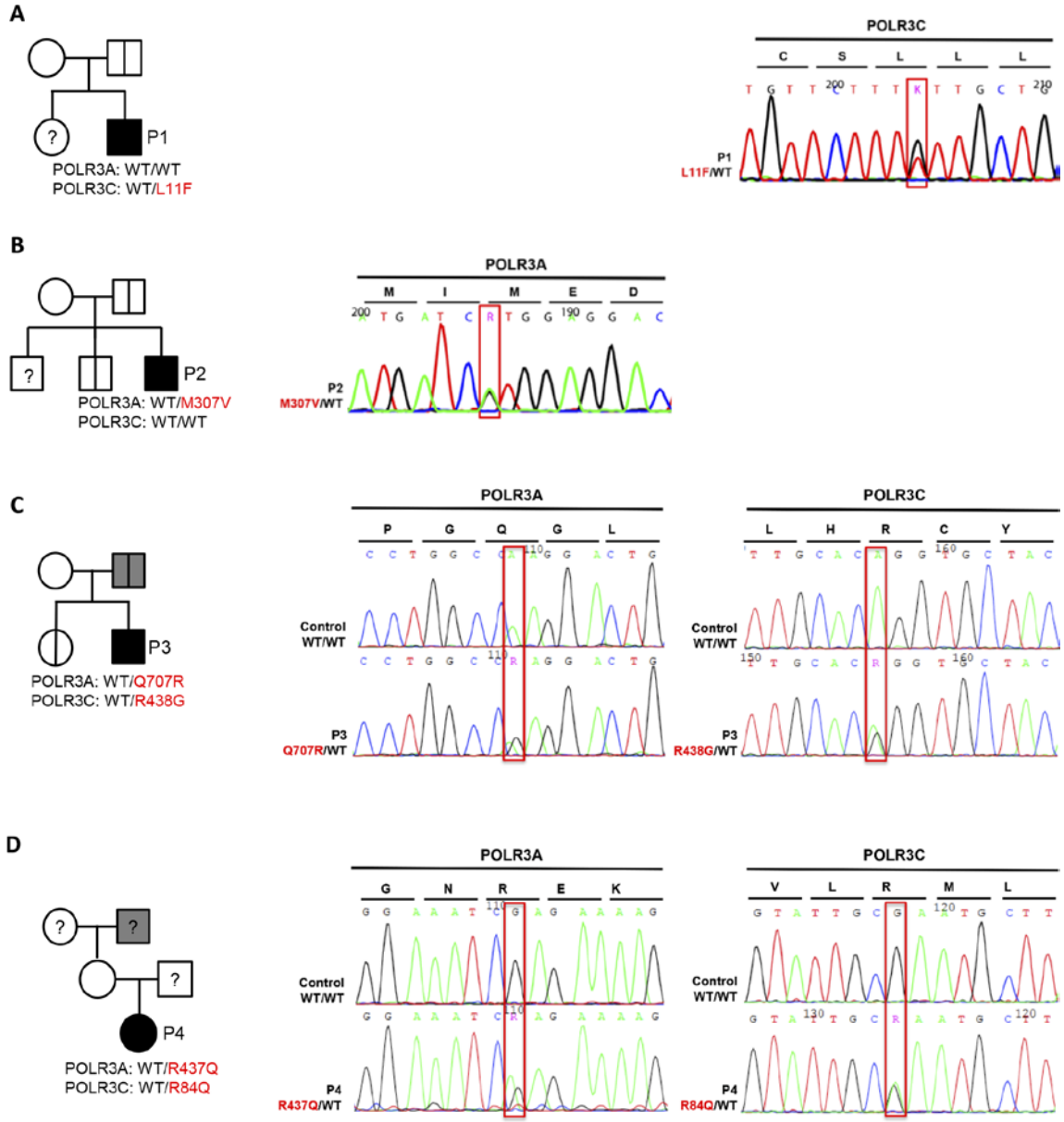
Supplemental material



Supplemental Figure 1. Sanger sequencing of *POLR3A* and *POLR3C*. DNA sequences of the regions in the POLR3A and C genes carrying the mutations.

POLR3A

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                          293                          322
P2                        IKKHRISGAKTQMIYEDWDFLQLQCALYIN
Homo sapiens              IKKHRISGAKTQMIMEDWDFLQLQCALYIN
Macaca mulatta            IKKHRISGAKTQMIMEDWDFLQLQCALYIN
Pan troglodytes           IKKHRISGAKTQMIMEDWDFLQLQCALYIN
Bos taurus                IKKHRISGAKTQMIMEDWDFLQLQCALYIN
Sus scrofa                IKKHRISGAKTQMIMEDWDFLQLQCALYIN
Equus caballus            IKKHRISGAKTQMIMEDWDFLQLQCALYIN
Oryctolagus cuniculus     IKKHRISGAKTQMIMEDWDFLQLQCALYIN
Rattus norvegicus         IKKHRISGAKTQMIMEDWDFLQLQCALYIN
Mus musculus              IKKHRISGAKTQMIMEDWDFLQLQCALYIN
Otolemur garnettii        IKKHRISGAKTQMIMEDWDFLQLQCALYIN
Monodelphis domestica     IKKHRISGAKTQMIMEDWDFLQLQCALYIN
Papio anubis              IKKHRISGAKTQMIMEDWDFLQLQCALYIN
Callithrix jacchus        IKKHRISGAKTQMIMEDWDFLQLQCALYIN
Canis familiaris          IKKHRISGAKTQMIMEDWDFLQLQCALYIN
Loxodonta africana        IKKHRISGAKTQMIMEDWDFLQLQCALYIN
Ovis aries                IKKHRISGAKTQMIMEDWDFLQLQCALYIN
Felis catus               IKKHRISGAKTQMIMEDWDFLQLQCALYIN

                          693                          722
P3                        NRGFSIGIGDVTPGYGLLKAKYELLNAGKY
Homo sapiens              NRGFSIGIGDVTPGQGLLKAKYELLNAGKY
Macaca mulatta            NRGFSIGIGDVTPGQGLLKAKYELLNAGKY
Pan troglodytes           NRGFSIGIGDVTPGQGLLKAKYELLNAGKY
Bos taurus                NRGFSIGIGDVTPGQGLLKAKYELLNAGKY
Sus scrofa                NRGFSIGIGDVTPGQGLLKAKYELLNAGYR
Equus caballus            NRGFSIGIGDVTPGQGLLKAKYELLNAGKY
Oryctolagus cuniculus     NRGFSIGIGDVTPGQGLLKAKYELLNAGKY
Rattus norvegicus         NRGFSIGIGDVTPGQGLLKAKYELLNAGKY
Mus musculus              NRGFSIGIGDVTPGQGLLKAKYELLNAGKY
Otolemur garnettii        NRGFSIGIGDVTPGQGLLTAKYELLNAGKY
Monodelphis domestica     NRGFSIGIGDVTPGQGLLKAKYELLNAGKY
Papio anubis              NRGFSIGIGDVTPGQGLLKAKYELLNAGKY
Callithrix jacchus        NRGFSIGIGDVTPGQGLLKAKYELLNAGKY
Canis familiaris          NRGFSIGIGDVTPGQGLLKAKYELLNAGKY
Loxodonta africana        NRGFSIGIGDVTPGQGLLTAKYELLNAGKY
Ovis aries                NRGFSIGIGDVTPGQGLLKAKHELLNAGKY
Felis catus               NRGFSIGIGDVTPGQGLLKAKYELLNAGKY

                          423                          452
P4                        QRHTQMK--RFLKYGNQEKMAQELKYGDIVER
Homo sapiens              QRHTQMK--RFLKYGNREKMAQELKYGDIVER
Macaca mulatta            QRHTQMK--RFLKYGNREKMAQELKYGDIVER
Pan troglodytes           QRHTQMK--RFLKYGNREKIAQELKYGDIVER
Bos taurus                QRHTQMK--RFLKYGNREKMAQELKFGDIVER
Sus scrofa                QRHTQMK--RFLKYGNREKMAQELKFGDIVER
Equus caballus            QRHTQMK--RFLKYGNREKMAQELKFGDIVER
Oryctolagus cuniculus     QRHMHMK--RFLKYGNREKMAQELKYGDVVER
Rattus norvegicus         QRHMQMK--RFLKYGNREKMAQELKFGDIVER
Mus musculus              QRHMQMK--RFLKYGNREKMAQELKFGDIVER
Otolemur garnettii        QRHTQMK--RFLKYGNREKMAQELKYGDIVER
Monodelphis domestica     QRHMQMK--RFLKYGNREKMAQELKFGDIVER
Papio anubis              QRHTQMK--RFLKYGNREKMAQELKYGDIVER
Callithrix jacchus        QRHTQMK--RFLKYGNREKMAQELKYGDIVER
Canis familiaris          QRHTQMK--RFLKYGNREKMAQELKYGDIVER
Loxodonta africana        QRHTQMK--RFLKYGNREKMAQELKYGDIVER
Ovis aries                QRHTQMKRLRFLKYGNREKMAQELKFGDIVER
Felis catus               QRHTQMK--RFLKYGNREKMAQELKYGDIVER
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POLR3C

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                          1                            30
P1                        MTQAEIKLCSFLLQEHFGEIVEKIGVHLIR
Homo sapiens              MTQAEIKLCSLLLQEHFGEIVEKIGVHLIR
Macaca mulatta            MTQAEIKLCSLLLQEHFGEIVEKIGVHLIR
Pan troglodytes           MTQAEIKLCSLLLQEHFGEIVEKIGVHLIR
Bos taurus                MTQAEIKLCSLLLQEHFGEIVEKIGVHLIR
Sus scrofa                MTQAEIKLCSLLLQEHFGEIVEKIGVHLIR
Equus caballus            MTQAEIKLCSLLLQEHFGEIVEKIGVHLIR
Oryctolagus cuniculus     MTQAEIKLCSLLLQEHFGEIVEKIGVHLIR
Rattus norvegicus         MTQAEIKLCSLLLQEHFGEIVEKIGVHLIR
Mus musculus              MTQAEIKLCSLLLQEHFGEIVEKIGVHLVR
Otolemur garnettii        MTQAEIKLCSLLLQEHFGEIVEKIGVHLIR
Monodelphis domestica     MTQAEIKLCSLLLQEHFGEIVDKIGTHLIR
Papio anubis              MTQAEIKLCSLLLQEHFGEIVEKIGVHLIR
Callithrix jacchus        MTQAEIKLCSLLLQEHFGEIVEKIGVHLIR
Canis familiaris          MTQAEIKLCSLLLQEHFGEIVEKIGVHLIR
Loxodonta africana        MTQAEIKLCSLLLQEHFGEIVEKIGVHLIR
Ovis aries                MTQAEIKLCSLLLQEHFGEIVEKIGVHLIR
Felis catus               MTQAEIKLCSLLLQEHFGEIVEKIGVHLIR

                          70                           99
P3                        RGVVEYEAQCSRVLDMLRYPRYIYTTKTLY
Homo sapiens              RGVVEYEAQCSRVLRMLRYPRYIYTTKTLY
Macaca mulatta            RGVVEYEAQCSRVLRMLRYPRYIYTTKTLY
Pan troglodytes           RGVVEYEAQCSRVLRMLRYPRYIYTTKTLY
Bos taurus                RGVVEYEAQCNRVLRMLRYPRYIYTAKTLY
Sus scrofa                RGVVEYEAQCSRVLRMLRYPRYIYTAKTLY
Equus caballus            RGVVEYEAQCSRVLRMLRYPRYIYTAKTLY
Oryctolagus cuniculus     RGVVEYEAQCSHMLRMLRYPRYIYTTKTLY
Rattus norvegicus         RGVVEYEAQCSRVLRMLRYPRYIYTTKTLY
Mus musculus              RGVVEYEAQCSRVLRMLRYPRYIYTTKTLY
Otolemur garnettii        RGVVEYEAQCSRVLQMLRYPRYIYTTKTLY
Monodelphis domestica     RGIVEYEAHCSRILRILRYPRYIYTAKTLY
Papio anubis              RGVVEYEAQCSRVLRMLRYPRYIYTTKTLY
Callithrix jacchus        RGVVEYEAQCSRVLRMLRYPRYIYTTKTLY
Canis familiaris          RGVVEYEAQCSRVLRMLRYPRYIYTAKTLY
Loxodonta africana        RGVVEYEAQCSRVLRMLRYPRYIYTSKTLY
Ovis aries                RGVVEYEAQCNRVLRMLRYPRYIYTAKTLY
Felis catus               RGVVEYEAQCSRVLRMLRYPRYIYTAKTLY

                          424                          453
P4                        YTVNILSAARMLLHHCYKSIANLIERRQFE
Homo sapiens              YTVNILSAARMLLHRCYKSIANLIERRQFE
Macaca mulatta            YTVNILSAARMLLHRCYKSIANLIERRQFE
Pan troglodytes           YTVNILSAARMLLHRCYKSIANLIERRQFE
Bos taurus                YTVNILSAARMLLHRCYKSVANLIERRQFE
Sus scrofa                YTVNILSAARMLLHKCYKSVANLIERRQFE
Equus caballus            YTVNILSAARMLLHRCYKSIANLIERRQFE
Oryctolagus cuniculus     YTVNVLSAARMLLHRCYKSIANLIERRQFE
Rattus norvegicus         YTVNVLSAARMLLHRCYKSIANLIERRQFE
Mus musculus              YTVNVLSAARMLLHRCYKSIANLIERRQFE
Otolemur garnettii        YTVNILSAARMLLHRCYKSIANLIERRQFE
Monodelphis domestica     YTVNSLSAARMLLRRCYKSIANLIERRQFE
Papio anubis              YTVNILSAARMLLHRCYKSIANLIERRQFE
Callithrix jacchus        YTVNILSAARMLLHRCYKSIANLIERRQFE
Canis familiaris          YTVNILSAARMLLHRCYKSIANLIERRQFE
Loxodonta africana        YTVNILSAARMLLHRCYKSIANLIERRQFE
Ovis aries                YTVNILSAARMLLHRCYKSVANLIERRQFE
Felis catus               YTVNILSAARMLLHRCYKSIANLIERRQFE
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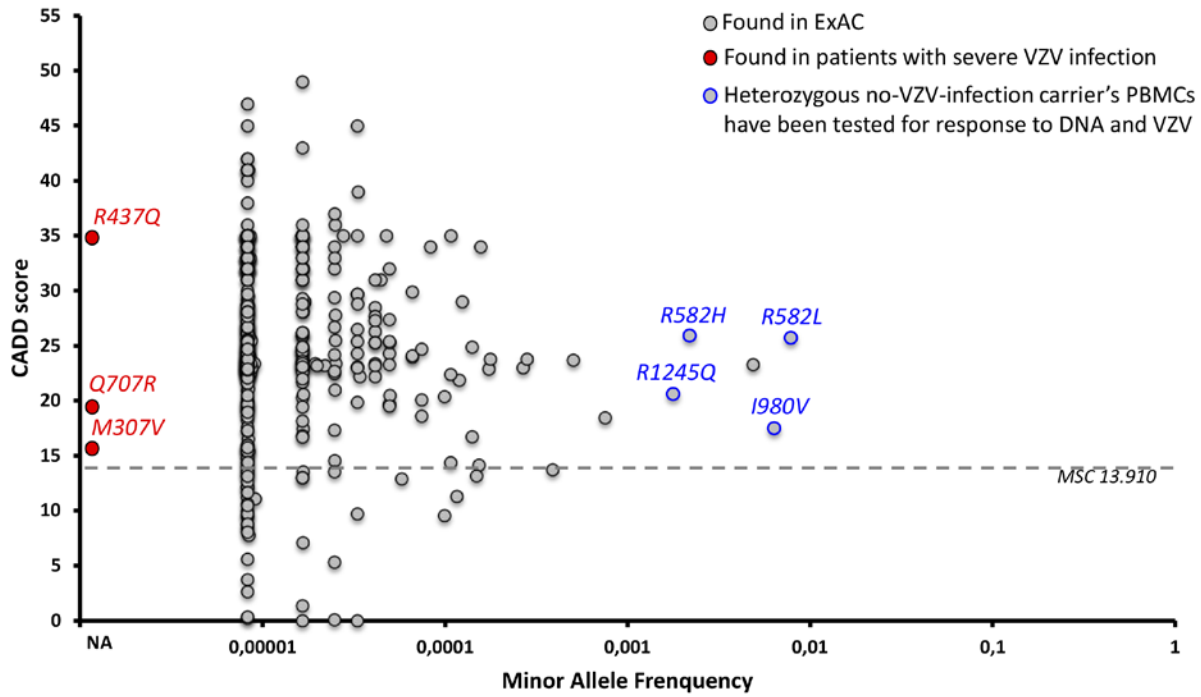
Supplemental Figure 2. Alignments. Alignment across species of the regions surrounding the mutations. Residues marked in red, represent the residue in patients. Residues marked in green represent the residue in the human wildtype allele. Residues marked in yellow are not conserved, but have similar physical properties as the conserved residue at the given position.

A



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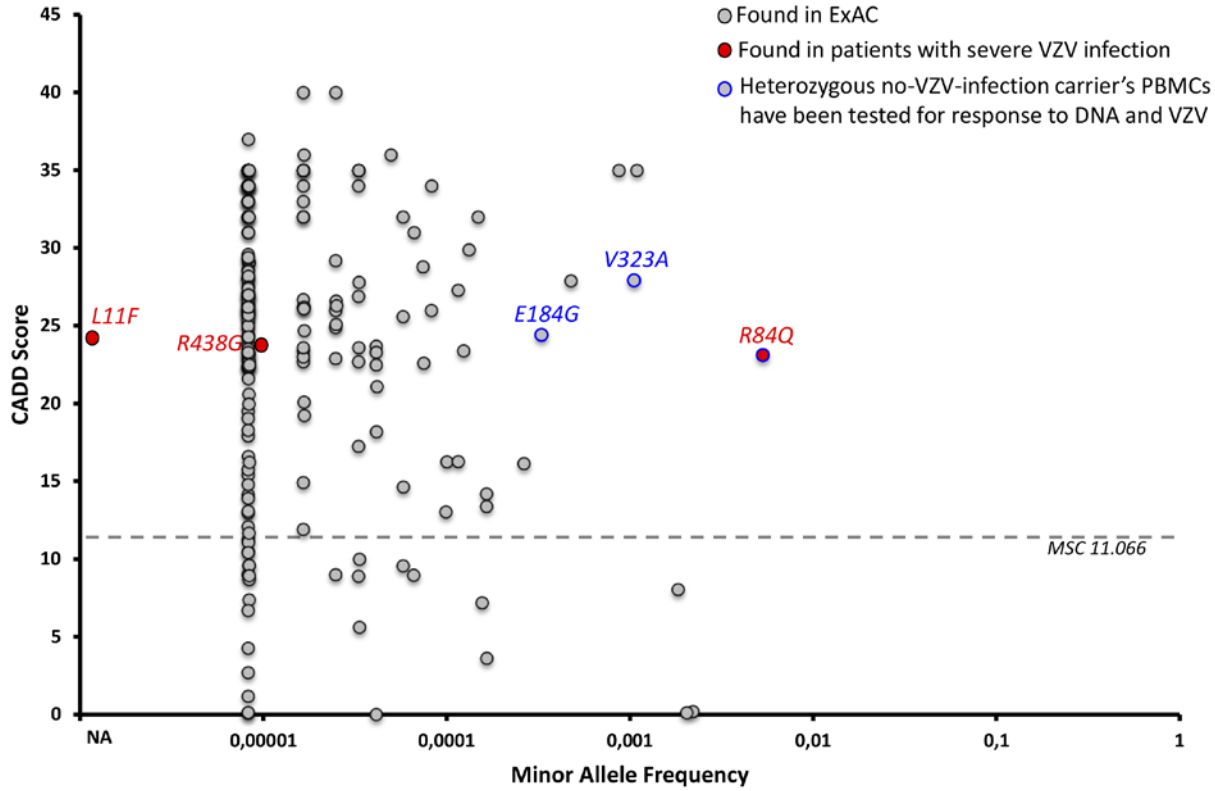
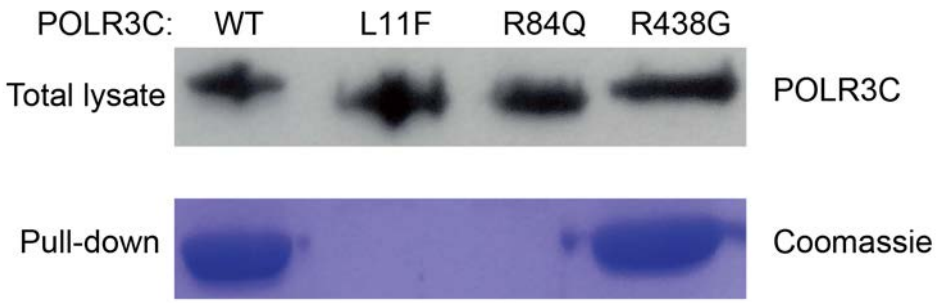
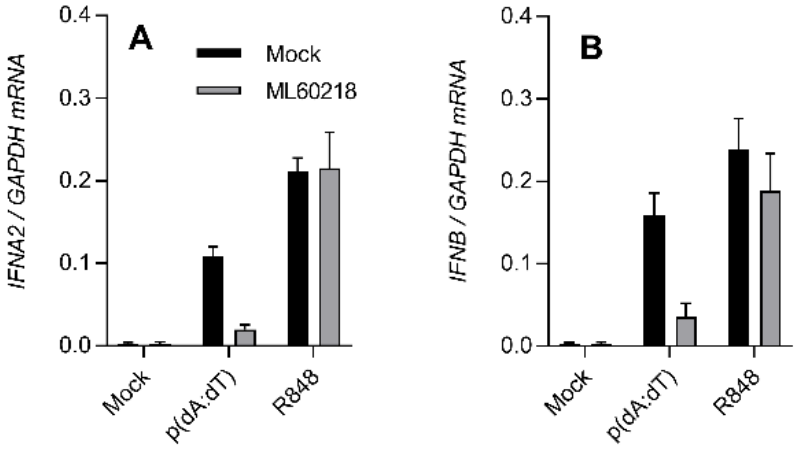


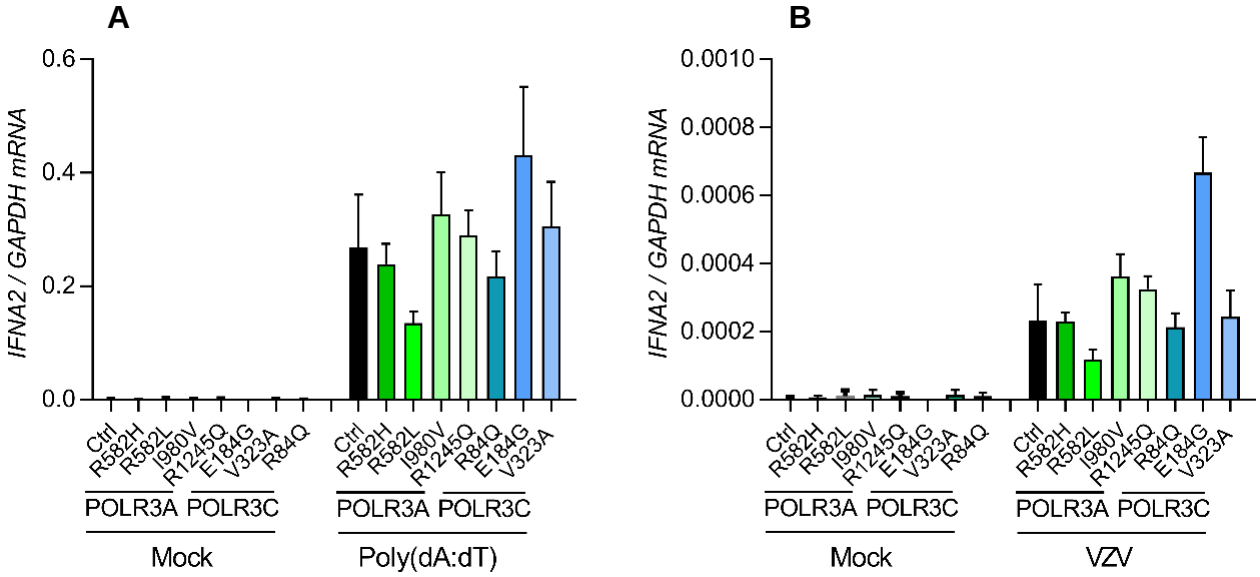
Supplemental Figure 3. Analysis of CADD score versus allele frequency variations in ExAC and VZV patients. (**A**) *POLR3A*, (**B**) *POLR3C*. MSC, Mutation Significance Cutoffs.



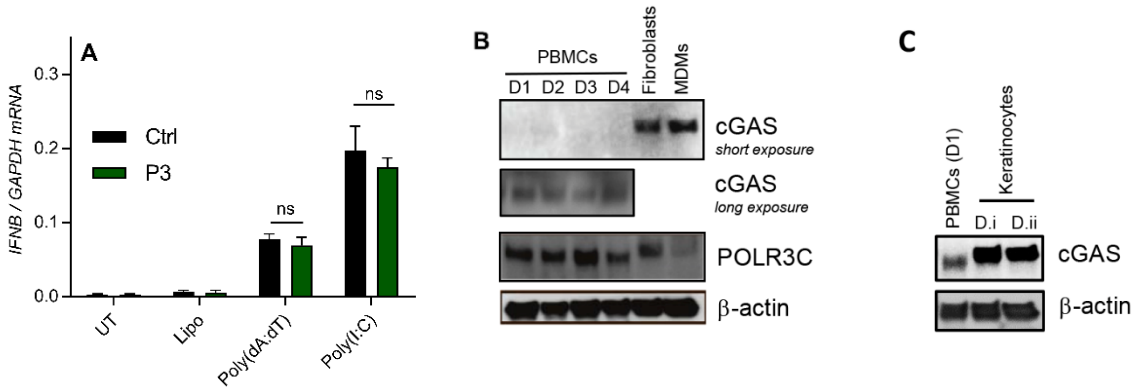
Supplemental Figure 4. Expression of WT and patient POLR3C in bacteria. Bacteria were transduced with plasmids encoding WT POLR3C and the 3 mutant forms from P1, P3, and P4 with a His-tag at the N-terminus. POLR3C expression was induced by addition of 1 mM IPTG treatment. Total lysates were analyzed for expression of POLR3C by Western blot, thus demonstrating that all mutants were expressed (top panel). Lysates were collected and evaluated for protein retention by the His-tag Nickel affinity resin.



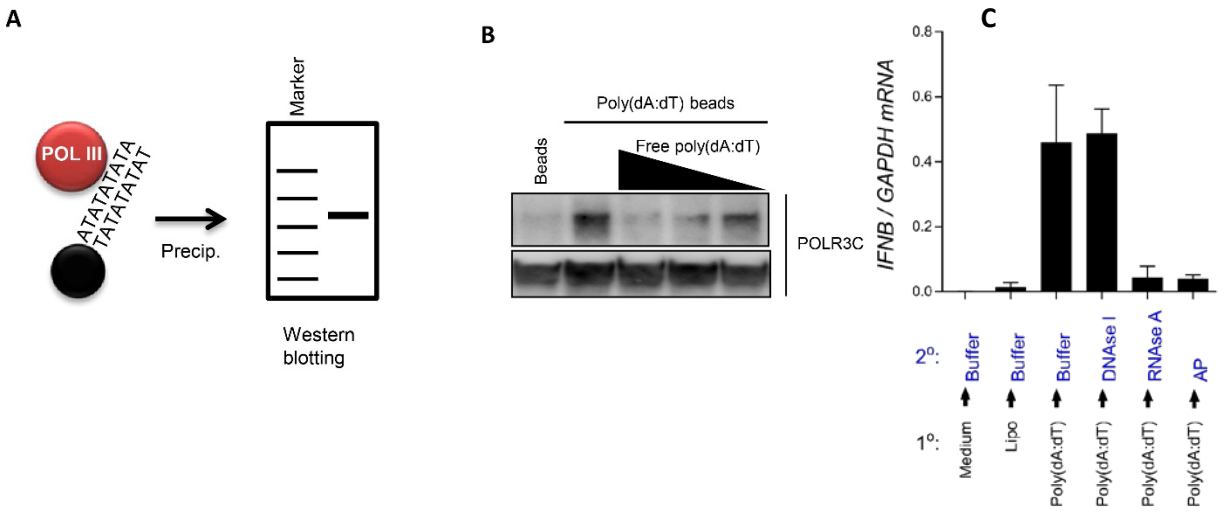
Supplemental Figure 5. POL III-dependent stimulation of IFN expression by poly(dA:dT) in PBMCs. PBMCs from a healthy donor were treated with the Pol III inhibitor ML-60218 (10 µM) for 16 h prior to transfection of poly(dA:dT) (2 µg/mL) or treatment with R848 (1 µg/mL). Total RNA was harvested 6 h after stimulation and levels of (**A**) *IFNA2* and (**B**) *IFNB* mRNA were determined by RT-qPCR as shown. Data are shown as means +/- st.dev.



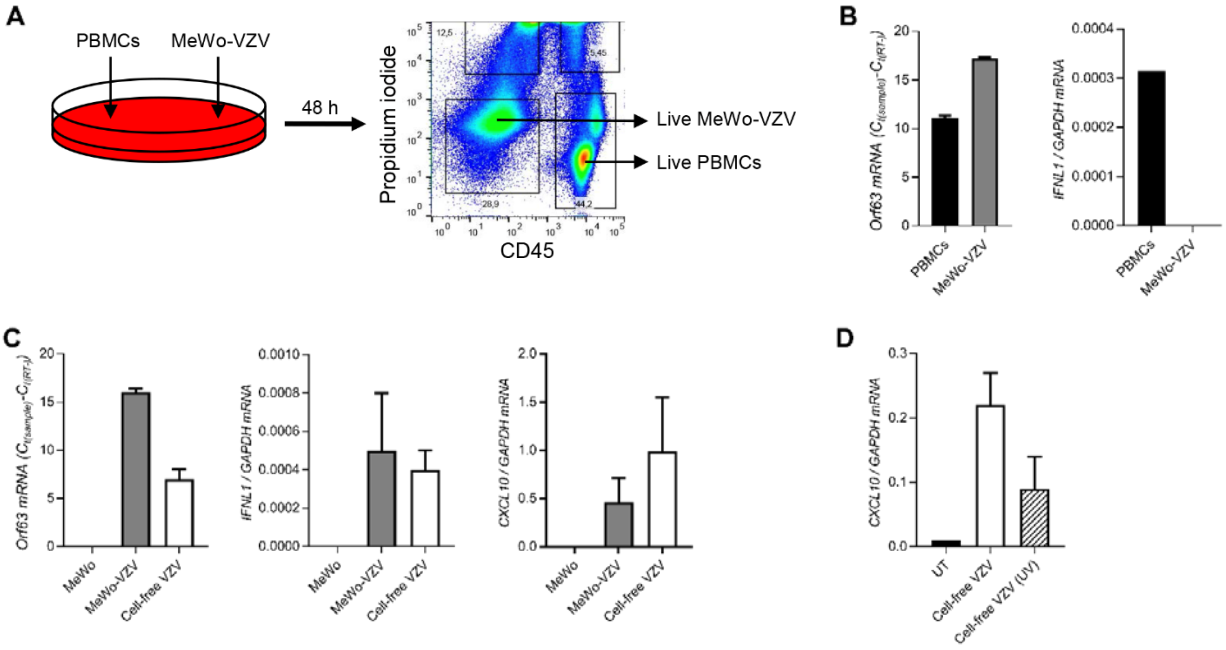
Supplemental Figure 6. Expression of *IFNA2* by PBMCs from donors with other POLR3 mutations. PBMCs from donors with other POLR3 mutations than the ones found in the patients were (**A**) transfected with poly(dA:dT) (2 µg/mL), or (**B**) VZV-infected MeWo cells. Total RNA was harvested 6h after poly(dA:dT) stimulation and 48 h after initiation of co-culture with VZV-MeWo cells. Levels of *IFNA2* mRNA were determined by RT-qPCR as indicated. The levels of cytokine mRNAs were normalized to *GAPDH*, and data are presented as means +/- st.dev. Ctrl, Control. Average of 4 sex- and age-matched controls.



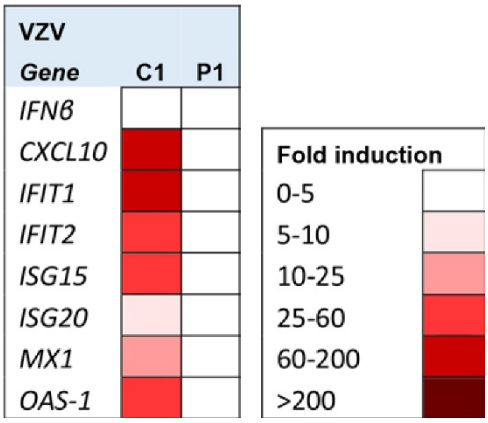
Supplemental Figure 7. Normal response to DNA in fibroblasts from P3. Fibroblasts from P3 and a healthy donor were transfected with (**A**) poly(dA:dT) or poly(I:C) (both 2 µg/mL). Total RNA was harvested 6 h later and levels of *IFNB* mRNA were determined by RT-qPCR, normalized to *GAPDH*, and presented as means +/- st.dev. ns, p>0.05. (**B**) Total cell lysates from PBMCs (4 donors), skin fibroblasts, and monocyte-derived macrophages were immunoblotted for the levels of cGAS, POLR3C, and β-actin. (**C**) Total cell lysates from PBMCs (1 donor), and keratinocytes (2 donors) were immunoblotted for the levels of cGAS and β-actin.



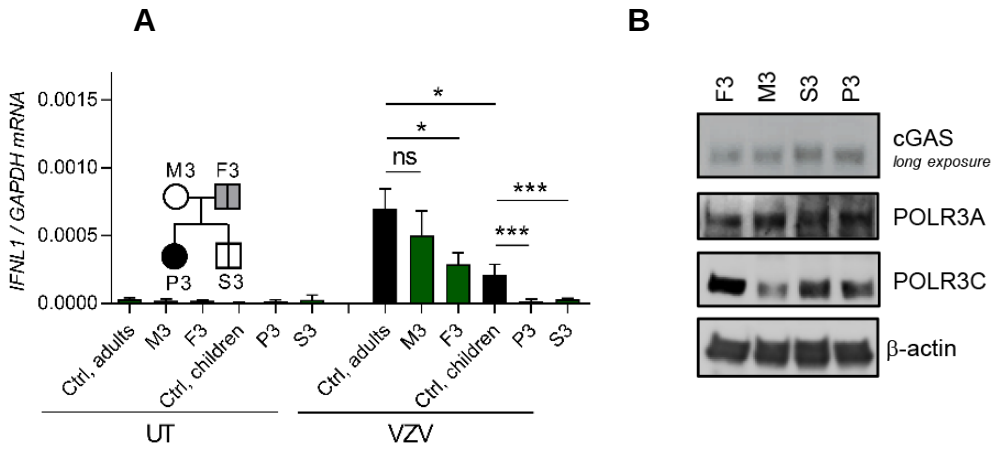
Supplemental Figure 8. Poly(dA:dT) interacts with POL III and leads to generation of immunostimulatory 5'-triphosphorylated RNA. (**A**) Illustration of the principle steps in precipitation and identification of poly(dA.dT)-interacting proteins. (**B**) Poly(dA:dT) beads incubated with cytoplasmic extracts of PBMCs from a healthy donor in the presence and absence of increasing levels of free poly(dA:dT) were precipitated and immunoblotted with anti-POLR3C. (**C**) PBMCs from one healthy donor were treated as indicated under 1º. Total RNA was isolated 14 h later, and the RNA was treated as specified under 2º before transfection into HEK293 cells. Total RNA was harvested 6 h later and levels of *IFNB* mRNA were determined by RT-qPCR and normalized to *GAPDH*. Data are presented as means +/- st.dev.



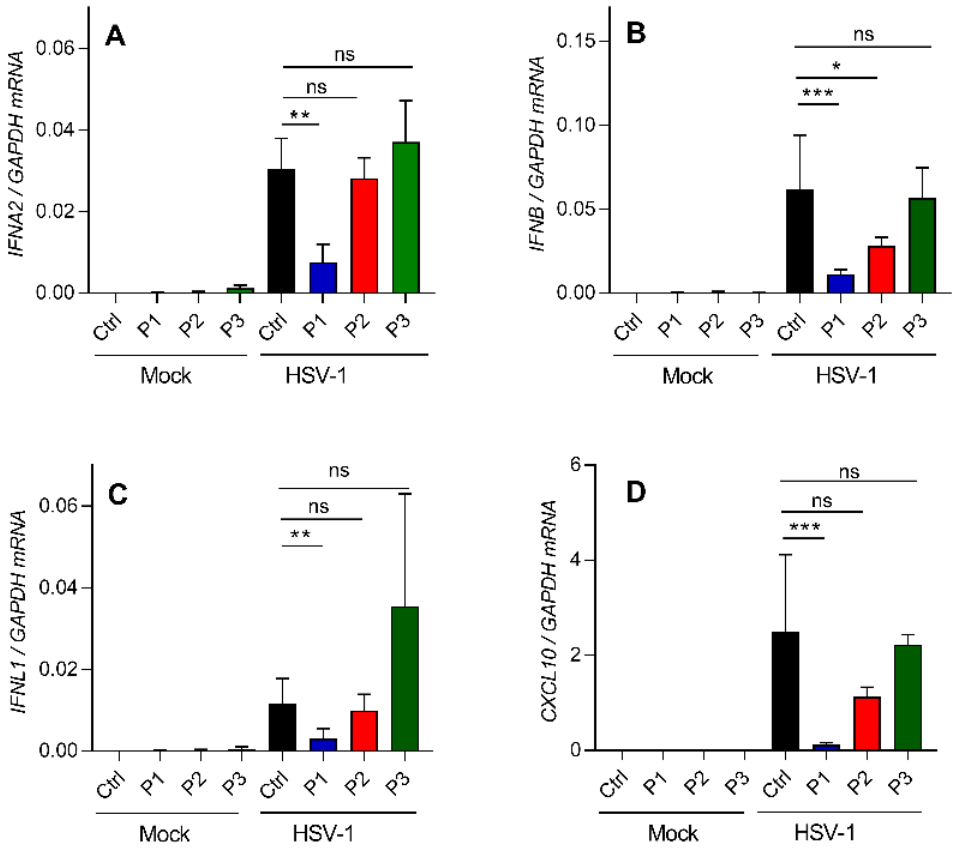
Supplemental Figure 9. Characterization of the PBMC-MeWo coculture system. (**A, B**) PBMCs from a healthy donor were co-cultured with MeWo cells (VZV-infected or non-infected) in a 1:1 ratio. The cells two cell populations were separated 48 h later by sorting for CD45 expression, and levels of *Orf63* and *IFNL1* mRNA were determined by RT-qPCR. (**C**) PBMCs were co-cultured with (i) uninfected MeWo cells, (ii) VZV-infected MeWo cells in a 1:1 ratio, or (iii) sonicated and cleared lysates from infected MeWo cells (cell-free VZV). (**D**) In a different experimental set-up PBMCs were co-cultured with cell-free VZV +/- UV-treatment prior to addition to the PBMCs. Total RNA was harvested 12 h after initiation of co-culture/infection, and levels of Orf63, *IFNL1, and CXCL10* mRNA was determined by RT-qPCR. Data are presented as means +/- st.dev.



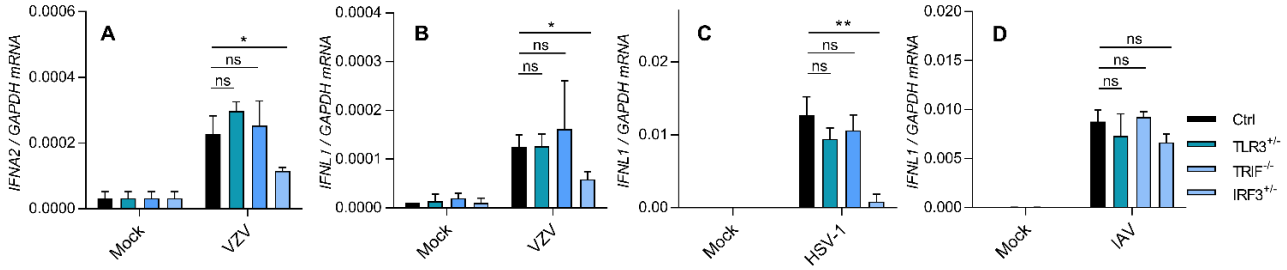
Supplemental Figure 10. Reduced ISG expression in P1 PBMCs after VZV infection. PBMCs from P1 and an age- and gender-matched healthy control were treated with VZV-infected MeWo cells for 48 h. Total RNA was harvested and levels of IFNβ and selected ISGs were determined by Fluidigm. The data were normalized to β-actin levels (which were comparable between the two groups) and are shown as heat-maps, with each color point representing means of 3 biological replicates.



Supplemental Figure 11. Characterization of *IFNλ1* production in response to VZV infection by PBMCs from the family of P3. (**A**) PBMCs from P3, family members, and age-matched healthy controls were treated with VZV-infected MeWo cells. Total RNA was harvested 48 h after initiation of co-culture with VZV-MeWo cells and levels of *IFNL1* mRNA were determined by RT-qPCR. The levels of the mRNA of interest was normalized to *GAPDH*, and data are presented as means +/- st.dev. Ctrl, Control. Average of 7 and 12 age-matched adult controls in the adult and children groups, respectively. ns, $p>0.05$; *, $0.01<p<0.05$; **, $0.01<p<0.001$; ***, $0.001<p<0.0001$. (**B**) Expression levels of cGAS, POLR3A, POLR3C, and β-actin in PBMCs lysates from the family of P3.



Supplemental Figure 12. Expression of IFNs and ISGs by PBMCs from P1-P3 after HSV-1 infection. PBMCs from P1, P2, P3 and healthy controls were infected with HSV-1 (MOI 3). Total RNA was harvested 6 h after infection and levels of (**A**) *IFNA2*, (**B**) *IFNB*, (**C**) *IFNL1*, and (**D**) *CXCL10* mRNA were determined by RT-qPCR as indicated. The levels of cytokine mRNAs were normalized to *GAPDH*, and data are presented as means +/- st.dev. Ctrl, Control. Average of 4 sex- and age-matched controls. ns, $p>0.05$; *, $0.01<p<0.05$; **, $0.01<p<0.001$; ***, $0.001<p<0.0001$.



Supplemental Figure 13. PBMCs from patients with mutations in TLR3 pathway genes have normal response to VZV infection. PBMCs from healthy controls and patients with the indicated mutations in genes of the TLR3 pathway were (**A, B**) treated with VZV-infected MeWo cells, (**C**) infected with HSV-1 (MOI 3), or (**D**) IAV (MOI 8). Total RNA was harvested 48 h after initiation of co-culture with VZV-MeWo cells and 6 h after HSV and IAV infections. Levels of *IFNA2* and *IFNL1* mRNA were determined by RT-qPCR as indicated. The levels of the mRNA of interest were normalized to *GAPDH*, and data are presented as means +/- st.dev. Ctrl, Control. Average of 4 age-matched controls. ns, $p>0.05$; *, $0.01<p<0.05$; **, $0.01<p<0.001$; ***, $0.001<p<0.0001$.

Supplemental Table 1. Genes involved in IFN production and function

Gene symbol	Gene name	HGNC ID	Chromosome Location
ADAR1	Adenosine deaminase	103	1q21.3
AIM2	Absent in melanoma 2	357	1q22
AMFR	Autocrine Motility Factor Receptor	463	16q21
APOBEC3G	apolipoprotein B mRNA editing enzyme catalytic subunit 3G	17357	22q13.1-q13.2
ATF2	Activating transcription factor 2	784	2q32
ATM	Ataxia telangiectasia mutated	795	11q22-q23
BST2	Bone marrow stromal cell antigen 2	1119	19p13.1
BTK	Bruton agammaglobulinemia tyrosine kinase	1133	Xq21.33-q22
CASP1	Caspase 1	1499	11q23
CASP10	Caspase 10	1500	2q33-q34
CASP8	Caspase 8	1509	2q33-q34
CD74	CD74	1697	5q32
CHUK	Conserved helix-loop-helix ubiquitous kinase / Nuclear Factor NF-Kappa-B Inhibitor Kinase Alpha	1974	10q24-q25
CIITA	Class II, major histocompatibility complex, transactivator	7067	16p13
CXCL9	C-X-C motif chemokine ligand 9	4283	4q21
CXCL10	C-X-C motif chemokine ligand 10	3627	4q21
CXCL11	C-X-C motif chemokine ligand 11	6373	4q21.2
DDX41	DEAD (Asp-Glu-Ala-Asp) box polypeptide 41	18674	5q35.3
DDX58	DEAD (Asp-Glu-Ala-Asp) box polypeptide 58 (RIG-I)	23586	9p12
DDX60	DEAD (Asp-Glu-Ala-Asp) box polypeptide 58	25942	4q32.3
DHX9	DEAH-box helicase 9	2750	1q25
DHX36	DEAH-box helicase 36	14410	3q25.2
DHX58	DEAH-box helicase 58 (LGP2)	29517	17q21.2
DOCK2	Dedicator of cytokinesis 2	1794	5q35.1
DOCK8	Dedicator of cytokinesis 8	19191	9p24.3
GBP1	Guanylate binding protein 1	4182	1p22.2
GBP2	Guanylate binding protein 2	4183	1p22.2
GATA2	GATA binding protein 2	4171	3q21
HPSE	Heparanase	5164	4q21.3
IFI6	Interferon alpha inducible protein 6	4054	1p35
IFI16	Interferon, gamma-inducible protein 16	5395	1q22
IFIH1	Interferon induced with helicase C domain 1 (MDA5)	18873	2q24.2
IFIT1	Interferon induced protein with tetratricopeptide repeats 1	5407	10q23.31
IFIT2	Interferon induced protein with tetratricopeptide repeats 2	5409	10q23.31
IFIT3	Interferon induced protein with tetratricopeptide repeats 3	5411	10q24

IFITM1	Interferon induced transmembrane protein 1	5412	11p15.5
IFITM2	Interferon induced transmembrane protein 2	5413	11p15.5
IFITM3	Interferon induced transmembrane protein 3	5414	11p15.5
IFNA1	Interferon alpha1	3439	9p22
IFNA2	Interferon alpha2	3440	9p22
IFNA4	Interferon alpha4	3441	9p22
IFNA5	Interferon alpha5	3442	9p22
IFNA6	Interferon alpha6	3443	9p22
IFNA7	Interferon alpha7	3444	9p22
IFNA8	Interferon alpha8	3445	9p22
IFNA10	Interferon alpha10	3446	9p22
IFNA13	Interferon alpha13	3447	9p22
IFNA14	Interferon alpha14	3448	9p22
IFNA16	Interferon alpha16	3449	9p22
IFNA17	Interferon alpha17	3451	9p22
IFNA21	Interferon alpha21	3452	9p22
IFNAR1	Interferon alpha and beta receptor subunit 1	3454	21q22.11
IFNAR2	Interferon alpha and beta receptor subunit 1	3455	21q22.11
IFNB1	Interferon, beta1	3456	9p21
IFNG	Interferon gamma	5438	12q14
IFNGR1	Interferon gamma receptor 1	5439	6q23-q24
IFNGR2	Interferon gamma receptor 2 (interferon gamma transducer 1)	5440	21q22.1
IFNL1	Interferon lambda 1	18363	19q13.13
IFNL2	Interferon lambda 2	18364	19q13.13
IFNL3	Interferon lambda 3	18365	19q13.13
IFNL4	Interferon lambda 4	44480	19q13.12
IFNLR1	Interferon lambda receptor 1	18584	1p36.11
IKBKB	Inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase beta	5960	8p11.2
IKBKE	Inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase epsilon	14552	1q31
IKBKG	Inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase gamma	5961	Xq28
IL10RB	interleukin 10 receptor subunit beta	5965	21q22.11
IL1RN	Interleukin 1 receptor antagonist	6000	2q14.2
IRAK1	Interleukin-1 receptor-associated kinase 1	3654	Xq28
IRAK2	Interleukin-1 receptor-associated kinase 2	3656	3p25.3
IRAK4	Interleukin-1 receptor-associated kinase 4	17967	12q12
IRF1	Interferon regulatory factor 1	6116	5q31.1
IRF3	Interferon regulatory factor 3	6118	19q13.3-q13.4
IRF5	Interferon regulatory factor 5	6210	7q32
IRF7	Interferon regulatory factor 7	6122	11p15.5
IRF8	Interferon regulatory factor 8	5358	16q24.1

IRF9	Interferon regulatory factor 9	6131	14q11.2
ISG15	Interferon stimulated gene 15, ubiquitin-like modifier	4053	1p36.33
ISG20	Interferon stimulated gene 20, exonuclease	6130	15q26
JAK1	Janus kinase 1	6190	1p32.3-p31.3
JAK2	Janus kinase 2	3717	9p24
JAK3	Janus kinase 3	6193	19p13-p12
MB21D1	Mab-21 domain containing 1 (cGAS)	21367	6q13
MAP3K14	Mitogen-activated protein kinase kinase kinase 14	6853	17q21
MAVS	Mitochondrial antiviral signaling protein	29233	20p13
MRE11A	MRE11 meiotic recombination 11 homolog A (S. cerevisiae)	7230	11q21
MYD88	Myeloid differentiation primary response 88	7562	3p22
MX1	MX dynamin like GTPase 1	7532	21q22.3
MX2	MX dynamin like GTPase 2	7533	21q22.3
NAMPT	Nicotinamide phosphoribosyltransferase	30092	7q22.3
NFKB2	Nuclear factor of kappa light polypeptide gene enhancer in B-cells 2 (p49/p100)	7795	10q24
NFKBIA	Nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, alpha	7797	14q13
NHEJ1	Nonhomologous end-joining factor 1	25737	2q35
NLRP12	NLR family, pyrin domain containing 12	22938	19q13.42
NLRP2	NLR family, pyrin domain containing 2	22948	19q13.42
NLRP3	NLR family, pyrin domain containing 3	16400	1q44
NOD2	Nucleotide-binding oligomerization domain containing 2	5331	16q12
OAS1	2'-5'-oligoadenylate synthetase 1	8086	12q24.2
OAS2	2'-5'-oligoadenylate synthetase 2	8087	12q24.2
OAS3	2'-5'-oligoadenylate synthetase 3	8088	12q24.2
OASL	2'-5'-oligoadenylate synthetase like	8090	12q24.2
PGM3	Phosphoglucomutase 3	8907	6q14.1-q15
PACT	Protein activator of PKR	9438	2q31.2
PKR	Protein kinase activated by double-stranded RNA	2p22-p21	2p22-p21
POLR3A	RNA polymerase III subunit A	30074	10q22.3
POLR3B	RNA polymerase III subunit B	30348	12q23.3
POLR3C	RNA polymerase III subunit C	30076	1q21
POLR3D	RNA polymerase III subunit D	1080	8q21
POLR3E	RNA polymerase III subunit E	30347	16p12.2
POLR3F	RNA polymerase III subunit F	15763	20p11.23
POLR3G	RNA polymerase III subunit G	30075	5q14.3
POLR3H	RNA polymerase III subunit H	30349	22q13.2
POLR3GL	RNA polymerase III subunit G like	28466	1q21.1
POLR3K	RNA polymerase III subunit K	14121	16p13.3
PRKDC	Protein kinase, DNA-activated, catalytic polypeptide	9413	8q11
PYCARD	PYD and CARD domain containing	16608	16p11.2
RELA	RELA proto-oncogene, NF-kB subunit	9955	11q13
RHBDF2	Rhomboid 5 homolog 2 (iRhom2)	20788	17q25.1

RIPK1	Receptor (TNFRSF)-interacting serine-threonine kinase 1	10019	6p25.2
RIPK3	Receptor-interacting serine-threonine kinase 3	10021	14q12
RNaseH2A	ribonuclease H2 subunit A	18518	19p13.2
RNaseH2B	ribonuclease H2 subunit B	25671	13q14.3
RNaseH2C	ribonuclease H2 subunit C	24116	11q13.1
RNASEL	Ribonuclease L	6041	1q25
RNF135	Ring Finger protein 135	21158	17q11.2
RTP4	receptor transporter protein 4	23992	3q27.3
SAMHD1	SAM and HD domain containing deoxynucleoside triphosphate triphosphohydrolase 1	15925	20pter-q12
STAT1	Signal transducer and activator of transcription 1, 91kDa	11362	2q32.2-q32.3
STAT2	Signal transducer and activator of transcription 2	11363	12q13.3
STAT3	Signal transducer and activator of transcription 3 (acute-phase response factor)	11364	17q21
STAT4	Signal transducer and activator of transcription 4	11365	2q32.2-q32.3
STAT5A	Signal transducer and activator of transcription 5A	11366	17q11.2
STAT5B	signal transducer and activator of transcription 5B	11367	17q11.2
TANK	TRAF family member-associated NFKB activator	11562	2q24-q31
TBK1	TANK-binding kinase 1	11584	12q14.2
TICAM1	Toll-like receptor adaptor molecule 1 (TRIF)	18348	19p13.3
TICAM2	Toll-like receptor adaptor molecule 2 (TRIF)	21354	5q23.1
TIRAP	Toll-interleukin 1 receptor (TIR) domain containing adaptor protein	17192	11q24.2
TLR2	Toll-like receptor 2	11848	4q32
TLR3	Toll-like receptor 3	11849	4q35
TLR4	Toll-like receptor 4	11850	9q33.1
TLR7	Toll-like receptor 7	15631	Xp22.3
TLR8	Toll-like receptor 8	15632	Xp22
TLR9	Toll-like receptor 9	15633	3p21.3
TMEM173	Transmembrane protein 173 (STING)	27962	5q31.2
TRAF3	TNF receptor-associated factor 3	12033	14q32.32
TRAF6	TNF receptor-associated factor 6, E3 ubiquitin protein ligase	12036	11p12
TRIM5	Tripartite motif containing 5	16276	11p15
TRIM19	Tripartite motif containing 19	9113	15q22
TRIM21	Tripartite motif containing 21	11312	11p15.5
TRIM22	Tripartite motif containing 22	16367	11p15
TRIM25	Tripartite motif containing 25	12932	17q23.2
TRIM32	Tripartite motif containing 32	16380	9q33.1
TRIM56	Tripartite motif containing 56	19028	7q22.1

TREX1	Three prime repair exonuclease 1	12269	3p21.31
TYK2	Tyrosine kinase 2	12440	19p13.2
UNC93B1	Unc-93 homolog B1 (C. elegans)	13481	11q13.2
VPS45	Vacuolar protein sorting 45 homolog (S. cerevisiae)	14579	1q21.2
XIAP	X-linked inhibitor of apoptosis	592	Xq25
XRCC5	X-ray repair cross complementing 5	12833	2q35
XRCC6	X-ray repair cross complementing 6	4055	22q13.2
ZBP1	Z-DNA binding protein 1	16176	20q13.31
ZDHHC1	Zinc Finger DHHC-Type Containing 1	17916	16q22.1

Supplemental Table 2. Enrichment of rare POLR3A, POLR3C variations in severe acute VZV infection cohort

	VZV cohort (21 individuals)	Control cohort (3724 individuals)	VZV vs Control *p-value*
POLR3A	3	54	0.0020
POLR3C	2	14	0.0046
POLR3A or POLR3C	4	68	0.00036
POLR3A and POLR3C*	2	4	0.0012

Notes:

1. Ethnic origin of the whole-exome/genome-sequenced individuals, from the VZV cohort or from Control cohort, has been assessed by principal component analysis (PCA) using their exome data. The VZV cohort contains 14 Caucasians, 4 North Africans, 1 African, and 2 Americans. The Control cohort was constructed by including individuals from the Human Genetics of Infectious Diseases (HGID) lab no-viral infection cohorts and from the 1000Genome database [(1)].
2. The 'Control cohort' consists of 1220 individuals from the no-viral infection cohorts of the HGID lab, and 2504 individuals from the 1000Genome project. Total number of individuals in the cohort of severe acute VZV infection or the Control cohort is indicated under the name of each cohort.
3. Under a POLR3A or POLR3C monogenic model, rare alleles with a MAF<0.0001 in ExAC, and with a CADD score higher than predicted Mutational Significance Cut-off of 95% confidence (13.910 for *POLR3A*, 11.066 for *POLR3C*) were considered in the test. Under a POLR3A and POLR3C digenic model*, rare alleles with a MAF<0.01 in ExAC, and with a CADD score higher than predicted Mutational Significance Cut-off of 95% confidence were considered in the test.
4. Number of individuals from VZV cohort or Control cohort who harbor rare *POLR3A* or *POLR3C* variations is listed in the table.
5. Ethnic-adjusted *POLR3A* and/or *POLR3C* mutation enrichment assessment was performed using logistic regression adjusting on the three first components of the PCA.

Reference:

Genomes Project, C., Auton, A., Brooks, L.D., Durbin, R.M., Garrison, E.P., Kang, H.M., Korbel, J.O., Marchini, J.L., McCarthy, S., McVean, G.A., et al. 2015. A global reference for human genetic variation. *Nature* 526:68-74.

Supplemental Table 3. Clinical data

Patient # (sex, age)	Clinical presentation	Paraclinical findings	MR/EEG findings	Diagnosis and treatment
1 (M, 3 years)	- Fever - Convulsions, day 6 - Loss of consciousness - Hypertonia - Anisocoria - Meningism	- VZV PCR (CSF[1]), +[2] - WBC[3], 50 cells/µl - Glucose and protein, N[4]	- Brain CAT[7], N - MRI (4 mo), N - EEG, slowed	Encephalitis Acyclovir 330 mg x3 i.v. for 21 days Day 14: methylprednisolone pulse therapy for 3 days
2 (M, 5 years)	- Ataxia and nystagmus during chickenpox - Chickenpox twice	- VZV PCR (CSF), ÷[5]	MRI: Arnold-Chiari type I with tonsillar herniation	Cerebellitis
3 (M, 5 years)	- diffuse necrotic varicella - severe pneumonia	VZV PCR (TSM)[6], +		Pneumonitis ECMO support needed
4 (F, 11 years)	- bitemporofrontal acute headache - varicella at 6 years old	- VZV PCR (CSF), +		Encephalitis

[1] CSF, *Cerebrospinal fluid*
[2] +, *positive;* ÷, *negative*
[3] WBC, White blood cells
[4] N, Normal
[5] It has been reported that only a minority of patients with VZV cerebellitis harbor detectable levels of viral DNA in the CSF (1). Therefore, since the onset and resolution of the clinical symptoms of cerebellitis coincided with the chickenpox, it was considered highly likely that VZV was the cause of the CNS symptoms.
[6] Tracheal suction material
[7] CAT, Computed Axial Tomography; MRI, *Magnetic resonance imaging*; EEG, Electroencephalography

1. Rack,A.L. *et al*. Neurologic varicella complications before routine immunization in Germany. *Pediatr. Neurol.* **42**, 40-48 (2010).

Supplemental Table 4. Immunophenotyping of available patient blood cells

	P1	P2	VZV patient (no POL III mutation)	Normal range
Hematology				
Neutrophils	3.96 x 10^9 cells/L	6.43 x 10^9 cells/L	2.57 x 10^9 cells/L	1.8-8.0 x 10^9 cells/L
Lymphocytes	2.86 x 10^9 cells/L	2.72 x 10^9 cells/L	3.05 x 10^9 cells/L	1.2-6.5 x 10^9 cells/L
Monocytes	0.96 x 10^9 cells/L	0.73 x 10^9 cells/L	0.64 x 10^9 cells/L	0.1-0.8 x 10^9 cells/L
Eosinophils	0.13 x 10^9 cells/L	0.19 x 10^9 cells/L	0.75 x 10^9 cells/L	0-0.5 x 10^9 cells/L
Basophils	0.03 x 10^9 cells/L	0.03 x 10^9 cells/L	0.02 x 10^9 cells/L	0.02-0.1x10^9 cells/L
Flow cytometry				
T cells				
CD3+	1945 cells /µL	1706 cells /µL	1937 cells /µL	700-4200 cells /µL
CD3+CD4+	826 cells /µL	1100 cells /µL	1075 cells /µL	300-2000 cells /µL
CD3+CD8+	889 cells /µL	491 cells /µL	692 cells /µL	300-1800 cells /µL
CD4+/CD8+	0.93	2.24	1.55	0.9 – 2.6
NK cells				
CD16+CD56+CD3÷	191 cells /µL	593 cells /µL	485 cells /µL	90-900 cells /µL
B cells				
CD19+	167 cells /µL	462 cells /µL	492 cells /µL	200-1600 cells /µL
Kappa	53.7% of CD19+	NA	66.9% of CD19+	
Lambda	46.3% of CD19+	NA	33.2% of CD19+	
Kappa/Lambda	1.16	NA	2.02	0.26 - 1.65
CD10	0.5% of CD19+	NA	0.9% of CD19+	
Plasma cells				
CD38++	0.2%	NA	0.1%	
Granulocytes	55.4%	65.1%	65.1%	35-80%

NA, information no available

Supplemental Table 5. Structural characteristics of the POL III mutations

Patient, mutation	Localization	Comments
P1, POLR3C L11F	Relatively buried in the core of the protein	May alter the local or general folding of POLR3C
P2, POLR3A M307V	At the interface between POLR3A and POLR3C	May perturb the interaction with POLR3C and its partners, POLR3F and POLR3G
P3, POLR3A Q707R	Exposed to the solvent	May perturb the interaction with associated factors
P3, POLR3C R438G	At the interface with POLR3F	May alter the POLR3C-POLR3F interaction
P4, POLR3A R437Q	Exposed to the solvent	May perturb the interaction with associated factors
P4, POLR3C R84Q	Buried in the core of the protein	May alter folding of POLR3C

Supplemental Table 6. Summary of phenotypes in PBMCs

		Poly(dA:dT)				VZV			
Patient	**Genotype**	*IFNA2*	*IFNB*	*IFNL1*	*CXCL10*	*IFNA2*	*IFNL1*	*CXCL10*	*Orf63*
P1	POLR3C L11F	R[1]	R	R, resc[2]	R	N	R, resc	R[3]	E, resc
P2	POLR3A M307V	R	R	R	R	N	N	N	N
P3	POLR3A Q707R, POLR3C R438Q	R	R	R, resc	R	R	R, resc	R	E, resc
P4	POLR3A R437Q POLR3C R84Q	R	R	R	R	R	R	R	E

[1] R, reduced compared to controls; N, normal; E, elevated compared to controls
[2] resc, rescue of phenotype by transduction with wildtype allele
[3] VZV-infected PBMCs from P3 displayed reduced expression of a panel of IFN-stimulated genes compared to controls