SUPPLEMENTAL MATERIAL

METHODS

Patient cohort and sample collection
Two patients were deceased. F3.1 died at age 40 from pancreatic cancer, and patient F5.1 died at age 59 from metastatic cancer of presumed colorectal source. Patient F2.4 has cerebral palsy and was unable to answer questions.

When feasible, serum, plasma, whole blood RNA, PBMC and fibroblasts were collected. Patients participated in recruiting other patients to the study and identification of outcome measures, and informed consent was obtained under an Institutional Review Board-approved protocol.

Identification of Y254C variant
Exome sequencing of genetically undiagnosed patients through the National Eye Institute clinical protocol (NCT02077894) were reviewed for novel candidate variants in ocular disease genes, including ALPK1, as previously described.(1) Sanger sequencing was used to confirm candidate variants.

CLINICAL PHENOTYPING

Eye exams
Eleven patients with ROSAH syndrome were examined at the National Eye Institute’s (NEI) ophthalmology clinic including visual acuity measurements, anterior segment and dilated fundus exam. Patients who could cooperate with testing completed optical coherence tomography (OCT) (Cirrus HD-OCT; Carl Zeiss Meditec, Dublin, CA), color fundus imaging and fluorescein angiography (Optos ultrawide-field retinal imaging device; Dunfermline, Scotland).(2, 3)

Oral and dental exams
Seven patients with ROSAH syndrome were evaluated in the National Institute of Dental and Craniofacial Research (NIDCR) Dental Clinic using clinical and radiographic examination. Four patients underwent comprehensive oral and craniofacial examinations including unstimulated and stimulated salivary assessments and labial salivary gland (LSG) biopsies. Ultrasounds of the parotid and submandibular glands were performed using a GE Logic ultrasound machine using a 6-12 MHz linear transducer, and results were scored as previously described.(4, 5) Three patients had 3-dimensional (3D) intra oral scans (3shape A/S, Denmark) and cone beam computed tomography (CBCT) performed to further assess dental phenotype. Tooth width was measured on the intraoral scan, and this measurement was used to calibrate the scale in CBCT software. Tooth length was measured in the CBCT software (Planmeca Romexis, Planmeca USA Inc., IL, USA) in the axial orthogonal slices for individual teeth.

FUNCTIONAL STUDIES

Cytokine analysis
Analysis of cytokines, chemokines and other soluble biomarkers was performed on EDTA plasma, sera or cerebrospinal fluid (CSF).

CRP was measured in the clinical laboratories of local health care facilities. Plasma cytokines (tumor necrosis factor (TNF)-alpha, interleukin (IL)-6, interferon (IFN)-beta, IL-10, monocyte
chemoattractant protein-1, IL-1 beta, IFN-gamma, macrophage inflammatory protein-1 alpha, granulocyte-monocyte colony stimulating factor, IL-2 receptor alpha soluble, IFN-alpha, IL-18) were measured through Mayo Clinic Laboratories. Serum and CSF cytokines (TNF-alpha, IL-2, IL-2 receptor soluble, IL-12, IFN-gamma, IL-4, IL-5, IL-10, IL-13, IL-17, IL-1 beta, IL-6, IL-8) were measured through ARUP Laboratories. Additionally, an extended panel of serum and plasma cytokines were tested in-house. Samples were diluted 1:3 in universal assay buffer and analyzed with the ProcartaPlex Cytokine & Chemokine 34-Plex Human Panel 1A according to the manufacturer’s instructions.

**Functional analysis of human neutrophils and monocytes**

Internalization of microbes by neutrophils and monocytes was performed using pHrodo-labeled *Staphylococcus aureus* as previously described.(6)

**Whole blood RNA sequencing for gene expression analysis**

Total RNA was extracted from whole blood collected in PAXgene Blood RNA tubes using the PAXgene Blood RNA Kit (PreAnalytiX) per manufacturer’s instructions. RNA quality was assessed using the Agilent TapeStation and quantified by the Nano Drop-1000 Spectrophotometer.

A total of 1000 ng of RNA (RNA integrity number [RIN] ≥ 7) per sample was used for cDNA library preparation using the NEBNext Ultra II Directional RNA library preparation kit with NEBNext Poly(A) mRNA magnetic isolation module and NEBNext Globin and rRNA Depletion kit (E7765, E7490 and E7755, New England Biolab). Sequencing was performed on an Illumina NovaSeq6000 System in a 2 x 150 bp paired-end mode. Sequenced reads were mapped to the human reference genome (GRCh38) using Hisat2.

BAM files were imported to Partek Flow (www.partek.com) and quantified to hg38. All count data were normalized using DESeq2. Differentially expressed genes (DEGs) were selected as those with a false discovery rate < 0.05, and heat maps showing gene expression patterns were generated using Partek. Ingenuity Pathway Analysis (IPA) software (Qiagen) was used to perform pathway enrichment and network analysis. The RNA-sequencing data are uploaded and available online (Gene Expression Omnibus: GSE198443).

**Cell cultures and plasmids**

293T cells (negative for mycoplasma, originally obtained from the American Type Culture Collection) and skin fibroblast derived from patients with ROSAH syndrome and healthy donors were grown in Dulbecco’s modified Eagle’s medium (Life Technologies) plus 10% fetal bovine serum and 1x antibiotics (Life Technologies).

The wild-type (WT) ALPK1 plasmid was generated as previously described and the T237M and Y254C mutants were generated by site-directed mutagenesis and validated by means of Sanger sequencing.(7)

**NF-κB luciferase reporter assay**

Using Lipofectamine 2000 according to the manufacturer’s instructions (Invitrogen), HEK293T cells (seeded at 35,000 cells per well in 96-well plates) we co-transfected with a NanoLuc®
reporter under a NF-κB-responsive element, a firefly expression vector, and an ALPK1 cDNA construct carrying either a WT or mutant sequence. Twenty-four hours after transfection, luciferase activity was measured using the Nano-Glo® Luciferase Assay System (Promega), and the NanoLuc® luciferase activity was normalized against the firefly luciferase activity for control of transfection efficiency and cell number. The reporter activity was then normalized to the result of WT construct.

**Cell stimulation and immunoblotting**

ADP-heptose (Invivogen) was used to stimulate fibroblast and transfected 293T cells (5-10 uM) for indicated times. Whole cell lysates were prepared using ice-cold 1x cell lysis buffer (Cell Signaling) supplemented with complete protease inhibitors. Immunoblotting was conducted using specific antibodies as described previously. ImageJ was used to analyze the immunoblotting images.

Antibodies specific for Phospho-IKKα/β (#2697), IKKα (#11930), IκBα (#4814, #9242), Phospho-IκBα (#2859), Phospho-p38 MAPK (#4511), Phospho-p44/42 MAPK (Erk1/2) (#4370), Phospho-SAPK/JNK (#4668), p44/42 MAPK (Erk1/2) (#4695), SAPK/JNK (#9252), p38 MAPK (#8690), HRP-linked anti-rabbit IgG (#7074), HRP-linked anti-mouse IgG (#7076), Phospho-Stat1 (Tyr701) (#D4A7), Rabbit mAb (#7649); DYKDDDDK Tag (D6W5B) rabbit mAb (#14793) were obtained from Cell Signaling, ALPK1 (PA5-55616) was obtained from Invitrogen and Actin (sc-1615), Anti-OctA-Probe Antibody (sc-166355) were obtained from Santa Cruz.

**In vitro pSTAT1 phosphorylation assay**

PBMCs from ROSAH patients and healthy donors were isolated by Ficoll (Ficoll-Paque PLUS; GE Healthcare) density-gradient centrifugation. STAT1 phosphorylations was assessed by treating PBMCs with IFN-α or IFN-γ (Cell Signaling Technology, Boston, MA) (200ng/ml) at 37°C for 20 minutes. The stimulation was stopped by addition of 1X Lyse/Fix Buffer (BD Biosciences, San Diego, CA). The PBMCs were then washed with PBS and permeabilized in ice-cold Phosflow Perm Buffer III (BD Biosciences, Sand Diego, CA) for 30 minutes in the dark. Cells were washed with FACS buffer (PBS, pH 7.4, 1% BSA, 4 mM EDTA, 0.2% NaN3). Monoclonal antibodies to CD3, CD4, CD8, and CD14 (BD Biosciences, San Diego, CA) were used to identify CD4+ T cells, CD8+ T cells and monocytes respectively. STAT1 phosphorylations were evaluated with 20 mL of Alexa Fluor647 pSTAT1 antibody (BD Biosciences, San Diego, CA). Flow cytometric analysis was performed on a FACS Fortessa (BD Immunocytometry, San Jose, CA) with Diva software. Data were analyzed using FlowJo software (Treestar, Ashland, OR). Median fluorescence intensity (MFI) of the corresponding pSTAT1 were calculated for each cell subset.

**Mice**

All animal studies were performed in accordance with guidelines from Ministry of Health, China or the National Institutes of Health and were approved by the Institutional Animal Care and Use Committee of National Institute of Biological Sciences, Beijing or National Human Genome Research Institute, Bethesda, MD or National Eye Institute, Bethesda, MD, respectively. To generate Alpk1T237M/ T237M mice, gRNA (ACAGGGCATTTCCACATCAC) targeting exon 9 of Alpk1 and donor
atgagagaattccagtctgatttgtttgtttttcctgacagggcatttccATGAGTTTAggcatactggcagacatctttgtttccatgagcaaaaccgattatgaaaa were used. In vitro-transcribed guide RNA, Cas9 mRNA and donor were co-microinjected into C57BL/6N-derived zygotes. The tail genomic DNA of each offspring was amplified with the forward primer 5’-AGCCCAACTTCAAAGTAGCC -3’ and the reverse primer 5’- CATCGAGAGAGGCTGGGATA -3’. Sanger sequencing was performed to analyze the PCR products and identify the founders with T237M mutation. Founders with the same mutation were intercrossed to obtain homozygous Alpk1T237M mice.

Because C57BL/6N mice harbor an rd8 mutation in the Crb1 gene that results in a form of retinal degeneration, the C57BL/6N Alpk1T237M mice were crossed with C57BL/6J mice that lacked the rd8 mutation.(8) Eight Alpk1T237M/T237M Crb1rd8/rd8 mice underwent ophthalmologic evaluation with fundus imaging and histologic evaluation of eyes from sacrificed mice. Three of the mice were imaged and sacrificed at 10 months of age and five of the mice were imaged and sacrificed at 7 months of age.

Five Alpk1T237M/WT Crb1rd8/WT mice were evaluated by ERG at 9 and 12 months of age. At 12 months of age, the mice were also evaluated for retinal degeneration by fundus imaging and optical coherence tomography (OCT) and Optodrum was used to assess visual acuity, as previously published.(9, 10)

Four Alpk1T237M/WT Crb1WT/WT mice were evaluated by ERG at 6 and 9 months of age. At 9 months of age, the mice were also evaluated for retinal degeneration by fundus imaging and OCT and Optodrum was used to assess visual acuity. Four wild-type control mice were evaluated by ERG at 6 months of age, but one died at 8 months of age so only 3 control mice were evaluated at 9 months of age.

**Statistical analysis**
Continuous variables are presented as means with standard deviations or medians with interquartile ranges and were compared with the use of parametric tests as appropriate.
**SUPPLEMENTAL FIGURES AND TABLES**

Supplemental Table 1: Characteristics of patients with ROSAH syndrome.

| ID   | Current age (years) | Sex  | Nationality | Ophthalmologic diagnosis                                                                 | Previous treatment                                      | Current treatment                           |
|------|---------------------|------|-------------|-----------------------------------------------------------------------------------------|----------------------------------------------------------|--------------------------------------------|
| F1.1 | 23                  | Male | United States | optic nerve elevation, retinal degeneration, uveitis                                     | Colchicine, IVIG                                          | Adalimumab, Methotrexate                   |
| F2.1 | 61                  | Female | United States | optic nerve elevation, retinal degeneration                                                | None                                                     | None                                       |
| F2.2 | 34                  | Female | United States | optic nerve elevation, retinal degeneration, uveitis                                     | Adalimumab                                              | Anakinra, Budesonide slurries Omeprazole   |
| F2.3 | 13                  | Female | United States | optic nerve elevation, uveitis                                                              | Anakinra                                                 | Adalimumab, Tocilizumab                   |
| F2.4 | 8                   | Female | United States | Pale optic discs, retinal scars and pre-retinal peripheral membranes in patient with retinopathy of prematurity status post-laser and vitrectomy* | None                                                     | Levetiracetam, Oxcarbazepine, Clonazepam   |
| F3.1 | Died at 40-pancreatic ca. | Male | United States | optic nerve elevation, retinal degeneration                                                |                                                          |                                            |
| F3.2 | 47                  | Male | United States | optic nerve elevation, uveitis                                                              |                                                          | Colchicine, allopurinol                   |
| F3.3 | 45                  | Male | United States | optic nerve elevation, retinal degeneration, uveitis                                     | Chlorambucil, methotrexate, cyclosporin, mycophenolate mofetil, acetazolamide, oral prednisone | None                                       |
| F3.4 | 14                  | Male | United States | optic nerve elevation, uveitis                                                              | acetazolamide, oral prednisone                           | Adalimumab                                 |
| F3.5 | 7                   | Male | United States | optic nerve elevation                                                                     |                                                          | None                                       |
| F4.1 | 26                  | Male | Italy        | optic nerve elevation, retinal degeneration, uveitis                                     | Colchicine, oral steroids, canakinumab, sarilumab        | Anakinra                                   |
| F5.1 | Died at 59-metastatic ca. of presumed colorectal source | Male | France | **                                                                                       |                                                          |                                            |
| F5.2 | 42                  | Male | France       | optic nerve elevation, uveitis                                                              | Oral steroids                                            | Anakinra                                   |
| F5.3 | 14                  | Male | France       | optic nerve elevation, uveitis, retinal detachment                                         | Anakinra, Adalimumab                                     | Tocilizumab                                |
| Code | Age | Gender | Country | Findings | Treatments |
|------|-----|--------|---------|----------|------------|
| F6.1 62 | Female | Brazil | optic nerve elevation, retinal degeneration, uveitis | Oral prednisone | None |
| F6.2 28 | Female | Brazil | optic nerve elevation, retinal degeneration, uveitis | None | None |
| F7.1 60 | Male | United States | optic nerve elevation, retinal degeneration, uveitis | Oral prednisone | Metformin |
| F8.1 38 | Male | The Netherlands | optic nerve elevation, retinal degeneration | steroids, cyclosporine, octreotide, acetazolamide, mycophenolate mofetil, ocular triamcinolone injections, infliximab, adalimumab | None |
| F9.1 36 | Male | United States | optic nerve elevation, uveitis | None | Lutein |
| F9.2 9 | Female | United States | optic nerve elevation | None | None |
| F9.3 6 | Male | United States | optic nerve elevation | None | None |
| F10.1 42 | Female | England | optic nerve elevation, retinal degeneration | Oral prednisone | None |
| F11.1 51 | Female | Norway | optic nerve elevation | None | None |
| F11.2 27 | Male | Norway | ** | None | None |
| F11.3 25 | Female | Norway | optic nerve elevation, retinal degeneration, uveitis | Oral prednisone | Colchicine |
| F12.1 43 | Female | Japan | optic nerve elevation, retinal degeneration, uveitis, retinal detachment | Adalimumab, oral steroids | Colchicine, NSAIDs |
| F13.1 43 | Female | United States | optic nerve elevation, retinal degeneration, uveitis | methotrexate, mycophenolate mofetil, anakinra (discontinued after 1 week secondary to intolerable site reaction) | Oral prednisone |

*Ocular exam limited by patient’s ability to cooperate.

**Ocular exam not performed
Supplemental Figure 1: Pedigrees of the 13 families with mutations in ALPK1 leading to ROSAH syndrome.

Pedigrees of 27 patient from 13 families described in this cohort. Clinically affected individuals are shaded, and results are presented for those examined by Sanger sequencing. Variant status is indicated below each individual for whom sequencing data was obtained (T237M indicates allele coding for [p.Thr237Met) and Y254C indicates allele coding for [p.Tyr254Cys]; + indicates normal allele). A diagonal line indicates a deceased individual.

Supplemental Figure 2: Dot plot depicting age of onset for patients with functional visual deficits.
Supplemental Table 2: Post-splenectomy pathology reports for patients F2.2, F5.2, F7.1, F11.2 F11.3 and F12.1.

| ID   | Age (years) | Spleen size at resection             | Splenic pathology                                                                 |
|------|-------------|--------------------------------------|-----------------------------------------------------------------------------------|
| F2.2 | 13          | 1,320 grams; 26 x 15 x 6 cm          | Normal architecture with expanded red pulp and mild histiocytic hyperplasia. Scattered megakaryocytes but no other evidence of extramedullary hematopoiesis (EMH). (IHC stains: CD79a, CD3, CD163, CD68, MPO, CD4, CD8; Congo red negative) |
| F5.2 | 21          | 600 grams; 18 x 10.5 x 4.5 cm        | Hyperplastic aspect of white pulp. Discretely congested red pulp.                  |
| F7.1 | 59          | 1,599 grams; 27 x 17 x 8 cm          | Red pulp congestion                                                              |
| F11.2| 21          | 528 grams, 18 x 10.5 x 5 cm          | Atrophic white pulp. Congestion, some hyperemia and expanded red pulp.            |
| F11.3| 20          | 688 grams, 16 x 6 x 10 cm            | Thickened capsule, thickened walls with sclerosis in walls. Red pulp is expanded, dilated sinusoids and veins. Hemosiderin containing macrophages. Severe congestion. |
| F12.1| 28          | 3100 grams                            | Red pulp is highly congested and white pulp is mildly atrophic                     |

Normal spleen weight for adult male is 28-226 grams and adult female is <230 grams. (11, 12)
Supplemental Figure 3: MRI abnormalities in ROSAH syndrome.

A  White matter changes

Supplemental material
F3.4

A. Bilateral focal hyperintensities (small white arrows) on fluid-attenuated inversion recovery (FLAIR) images in the subinsular regions and right temporal lobe (small white arrows) of patient F7.1. Focal FLAIR hyperintensity in the subcortical white matter of the right temporal lobe of patient F2.3. Focal area of increased FLAIR signal with associated enhancement (red arrows) is seen in the left frontal subcortical white matter in patient F3.4.
B. Focal meningeal enhancement (white arrows) on post-contrast FLAIR imaging for patients F2.2 (left) and F9.1 (right).
C, cont.
C, MRI of the orbits showed papilledema on T2 weighted (small black arrows) and post contrast T1-weighted images (small white arrows) of patient F3.4. Axial post contrast T1 and FLAIR images show thickening/enhancement of the posterior aspects of the globes (thick white arrows) for patient F2.3. Leakage of contrast material into the vitreous humor on delayed post contrast FLAIR images in patients F2.3 and F4.1(red arrows).
Supplemental Figure 4: Oral pathology in ROSAH.

A. Panel of dental panoramic X-rays from ROSAH individuals. Short roots are noted in all except F2.2. Multiple dental restorations present in F2.2 and F5.2, possibly due to enamel defects or caries. Multiple dental implants in F7.1. *marks teeth with taurodontism. + marks decayed or restored teeth.
B. Panoramic X-rays from 14-year-old healthy male for comparison as control.
C. 2D orthogonal slices of maxillary central incisors and mandibular molars in F5.3 (left) and healthy control (right) comparing abnormal teeth roots.
D. Quantification of canine length in four individuals showing the variation in length.
E. Focal lymphocytic sialadenitis (FLS) scores of salivary gland histopathology from healthy volunteers (HV), Sjögren’s Disease (SjD), and patients with ROSAH syndrome.
Supplemental Table 3

**American College of Rheumatology-European League Against Rheumatism (ACR-EULAR) Classification Criteria for Sjögren’s Disease**

| Classification criteria (positive range) | Points for positive score | Patient 5.2 (points) | Patient 5.3 (points) | Patient 2.2 (points) | Patient 7.1 (points) |
|-----------------------------------------|---------------------------|----------------------|----------------------|----------------------|----------------------|
| Focus score (≥ 1 foci/mm²)              | 3                         | 1 (3)                | N/A*                 | 1 (3)                | 2 (3)                |
| Anti-SSA (Ro) (positive)                | 3                         | Negative (0)         | Negative (0)         | Negative (0)         | Negative (0)         |
| Unstimulated whole saliva (≤ 1.5 ml/15 min) | 1                         | 3.4218 (0)           | 1.9443 (0)           | 0.7644 (1)           | 4.4442 (0)           |
| Ocular staining score (≥ 5)             | 1                         | 5 (1)                | 3 (0)                | N/A                  | 4 (0)                |
| Schirmer test (≤ 5 mm/5 min)            | 1                         | 11 (0)               | 18 (0)               | N/A                  | 5 (1)                |
| Total score (≥ 4 meet the criteria for SjD) |                           | 4                    | 0                    | 4                    | 4                    |

*biopsy not performed on Patient 5.3, a minor.*
Supplemental Figure 5: Inflammation and cytopenias in ROSAH syndrome.

A). Cell counts and serum CRP levels for patient F4.1 demonstrate that his cytopenia frequently coincide with CRP elevations. Cell counts are plotted on level axis and shown in black and serum CRP levels are plotted on right axis and shown in red. Blue boxes outline time patient was taking canakinumab. Green line denotes administration of single dose of sarilumab that was suspended after development of neutropenia.
B. Post-splenectomy, cytopenias appear to resolve but immune activation continues. B) Pre- and post-splenectomy blood counts for patient F7.1. The timing of splenectomy is indicated by the vertical red dotted line.
Supplemental Figure 6:

**Lymphocyte phenotyping**

Immunophenotyping of blood cells in patients with ROSAH syndrome (n=12) is depicted. Normal reference ranges are indicated by the gray shaded areas. The indicated reference ranges do not apply to all plotted values. Three pediatric patients are plotted and indicated by the symbols as followed: “X” Indicates value from 8-year-old (Patient F2.4). “+” Indicates values from patient between 11-18 years old (Patients F2.3 and F5.3). For pediatric patients, the following reference ranges can be used: For 6-10 years old - Total lymphocyte count 1500-7000/uL, total T 700-4200/uL, Helper T 300-2000/uL, Cytotoxic T 300-1800/uL, Total B 200-1600/uL, NK 90-900/uL. For 11-18 years old - Total lymphocyte count 1000-5200/uL, total T 800-3500/uL, Helper T 400-2100/uL, Cytotoxic T 200-1200/uL, Total B 200-600/uL, NK 70-1200/uL. Also, patient F8.1 had an NK cell count of 90/uL and the normal reference range for NK cell count from the laboratory performing the test was reported to be 90-600/uL.
Supplemental Figure 7: **ALPK1 transcription in immune cells and pHrodo dye-based detection of phagocytosis.**

**A.** ALPK1 expression in human immune cells. (13)

**B-C.** Ingestion of *Staphylococcus aureus* labelled with pH-sensitive dye by polymorphonuclear leukocytes (PMNs) (b) and monocytes (c) was assessed by flow cytometry. MFI: mean fluorescence intensity. HV: healthy volunteer.
Supplemental Figure 8: Serum immunoglobulins

Serum immunoglobulins measured in 16 patients with ROSAH syndrome. As compared to age-adjusted normal values, patient F1.1 and F2.4 had low IgG and patients F1.1, F2.4, F4.1, F5.2 and F11.2 had low IgM (14). Gray shaded areas represent the indicated normal reference range for individuals >10 years of age. *denotes pediatric patient. Normal reference range for patient between age 6-8 years is IgG: 633-1280 mg/dL, IgA: 45-236 mg/dL, IgM: 52-242 mg/dL.

Supplemental Table 4: Autoantibodies in ROSAH syndrome cohort. Most patients with ROSAH syndrome lacked high-titer autoantibodies.

| Patient ID | ANA          | RF            | anti-CCP | anti-ds DNA | ENA screen (anti-RNP, SM, SSA, SSB, Jo-1, Scl-70) | Anti-SS-A (Ro) | Anti-SS-B (La) |
|------------|--------------|---------------|----------|-------------|-------------------------------------------------|----------------|----------------|
| F1.1       | negative     | negative      | negative | negative    | negative                                        |                |                |
| F2.1       | negative     | negative      | negative | negative    | negative                                        |                |                |
| F2.2       | 1:320 Homogeneous | negative   | negative | negative    | negative                                        |                |                |
| F2.3       | 1.4 EU (positive) | negative   | negative | negative    | negative                                        |                |                |
| F2.4       | negative     | negative      | negative | negative    | negative                                        |                |                |
| F3.3       | Non-reactive x 3 | negative   | negative | negative    | negative                                        |                |                |
| F3.4       | Non-reactive x 3 | negative   | negative | negative    | negative                                        |                |                |
| F4.1       | <0.1         | negative      | negative | negative    | negative                                        |                |                |
| F5.2       | <1:80        | negative      | negative | negative    | negative                                        |                |                |
| F5.3       | 1:320 Homogeneous | negative   | negative | negative    | negative                                        |                |                |
| F6.2       | negative     | negative      | negative | negative    | negative                                        |                |                |
| F7.1       | negative     | negative      | negative | negative    | negative                                        | negative       | negative       |
| F8.1       | negative     | negative      | negative | negative    | negative                                        | negative       | negative       |
| F9.1       | negative     | negative      | negative | negative    | negative                                        |                |                |
| 11.1       | negative     | negative      | negative | negative    | negative                                        |                |                |
| 11.2       | negative     | negative      | negative |            |                                                 |                |                |
| 11.3       | negative     | negative      | negative |            |                                                 |                |                |
| 12.1       | <40 (negative) | negative     | negative | negative    | negative                                        |                | negative       |
| F13.1      | 0.2 (negative) | negative     | negative |            |                                                 |                |                |
Supplemental Figure 9: **Inflammatory signature in patients with ROSAH syndrome.**

A) Serum CRP as well as plasma and serum cytokines and chemokine levels were trended in 3 untreated patients (Patient F2.4, F5.2, F7.1), one patient with continued fever and CRP elevations despite anti-IL-1 therapy (patient F4.1) and one patient on 5 mg of prednisone daily (patient F13.1). CRP (mg/dL) is shown in yellow and plotted on the left y axis. Cytokines and chemokines are graphed as multiples of the upper limit of normal (ULN). ULN determined by reference laboratory performing the assay.
B) Additional plasma cytokines and chemokines measure in patients F1.1, F2.2, F2.3, F2.4 and F7.1. Gray shaded areas represent the mean plus or minus 2 standard deviation from 114 healthy controls.

C) Additional serum cytokines and chemokines measure in patients F2.1, F2.2, F2.3, F2.4, F3.3 and F3.4. Patients with ROSAH syndrome are plotted on left with blue circles. Health controls are plotted on right with black squares (n=3).
Supplemental Table 5:

|                                      | Reference range | F1.1 | F2.4 |
|--------------------------------------|-----------------|------|------|
| Interferon-gamma Positive CD4        | 8-24%           | 21   | 7 (L) |
| Interferon-gamma Positive CD8        | 20-48%          | 23   | 16 (L) |
| Interferon-gamma Positive NK         | 48-80%          | 56   | 75   |
| Interferon-gamma Positive NKT        | 18-64%          | 40   | Insufficient NKT cells in sample |
| TNF-alpha Positive CD4               | 39-67%          | 66   | 49   |
| TNF-alpha Positive CD8               | 17-61%          | 28   | 24   |
| TNF-alpha Positive NK                | 29-61%          | 39   | 55   |
| TNF-alpha Positive NKT               | 23-73%          | 49   | Insufficient NKT cells in sample |
| IL-4 Positive CD4                    | 0-3.7%          | 0.8  | 0.6  |
| IL-4 Positive CD8                    | 0-3%            | 0.5  | 0.0  |
| IL-4 Positive NK                     | <1.6%           | 0.4  | 0.0  |
| IL-4 Positive NKT                    | 0-3.1%          | 0.4  | Insufficient NKT cells in sample |

Stimulated ROSAH leukocytes did not display increased intracellular cytokines (n=2). Whole blood cells from patient F1.1 and F2.4 were incubated with Phorbol 12-Myristate 13 Acetate and Ionomycin for 4 hours at 37°C to stimulate cytokine production. Activation was carried out in the presence of Brefeldin A, to inhibit intracellular transport, causing all cytokines produced during the activation to be retained inside the cell. The activated cells were stained with surface monoclonal antibodies for phenotyping, fixed, permeabilized and stained with antibodies to the cytokines IFN-γ, TNF-α and IL-4. The cells were then analyzed by flow cytometry. Percent positive results for IFN-γ, TNF-α and IL-4 were reported for CD4, CD8, NK and NKT cells. At the time of cell collection, patient F1.1 was on adalimumab and patient F2.4 was not receiving any anti-cytokine treatment. (L) indicates value below reference range.
Supplemental Table 6: **Selected studies of CSF obtained by lumbar puncture from patients with ROSAH syndrome.**

|                              | F2.2 (33 yo) | F3.4 (10 yo) | F10.1 (33 yo) |
|------------------------------|--------------|--------------|--------------|
| **Results [normal reference]** |              |              |              |
| Opening pressure (cm H2O)    | 17 [6-25, adult] | 26.6 [12-28, pediatric] | 22 [6-25, adult] |
| WBC (cells/mm³)              | 3 [0-5]      | 2 [0-7]      | 4 (75% lymph, 23% mono, 2% unclassified; Large mononuclear cells with basophilic cytoplasm and fine chromatin) |
| Protein (mg/dL)              | 39 [23-38]   | 23 [25-45]   | 60 [5-55]    |
| CSF neopterin (nmol/L)       | 71 [8-28]    | 65 [7-40]    | Not measured |
| **Current treatment**        | TNF-inhibitor | None         | None         |
| **Cytology**                 |              |              | Numerous medium-sized cells with round to folded and indented nuclei, prominent nucleoli and moderately abundant basophilic cytoplasm, some with cytoplasmic vacuoles and granules, consistent with activated monocytes and lymphocytes. The overall findings are consistent with a reactive process. |
Supplemental Table 7: **Paired serum and CSF cytokines for patient F2.2.**

| Cytokine          | CSF Result (pg/mL) | CSF Reference range | Serum Result | Serum Reference range |
|-------------------|-------------------|---------------------|--------------|----------------------|
| CRP               | 7.3               | 0-4.99 mg/L         |              |                      |
| TNF-α             | <1.7              | <=1.7               | 7            | <=7.2                |
| IL-2              | <2.1              | <=2.1               | <2.1         | <=2.1                |
| IL-2 receptor α, soluble | **36.3** | <=26.8               | 627.6        | 175.3-858.2         |
| IL-12             | <1.9              | <=1.9               | <1.9         | <=1.9                |
| IFN-γ             | <4.2              | <=4.2               | <4.2         | <=4.2                |
| IL-4              | <2.2              | <=5.2               | <2.2         | <=2.2                |
| IL-5              | <2.1              | <=2.1               | <2.1         | <=2.1                |
| IL-10             | 5.7               | <=12.7              | <2.8         | <=2.8                |
| IL-13             | **38.3**          | <=7.3               | <1.7         | <=2.3                |
| IL-17             | <1.4              | <=4.6               | <1.4         | <=1.4                |
| IL-1β             | <6.5              | <=6.5               | <6.5         | <=6.7                |
| IL-6              | 7.1               | <=7.5               | <2           | <=2.0                |
| IL-8              | **186.3**         | 4.6-283.5           | <3.0         | <=3.0                |
Supplemental Figure 10: **NF-κB signature.**

Heatmap showing differentially expressed NF-κB response genes (GO:0007249) in whole blood of pre-treatment (n=4) patients with ROSAH syndrome and healthy volunteers (n=3). Up-regulated genes are shown in red, and down-regulated genes in blue. For patients F1.1 and F3.3, samples were collected on days for which sera and plasma cytokines were also significantly elevated, consistent with disease flare.
Supplemental Figure 11: Mice harboring ROSAH-associated mutations in Alpk1 demonstrated serum cytokine and chemokine elevations similar to those seen in patients with ROSAH syndrome, but splenomegaly, visual deficits, and retinal degeneration were not observed.

A  Mouse Serum Cytokines and Chemokines

|               | 2 months of age | 3 months of age |
|---------------|-----------------|-----------------|
|               | Male            | Female          | Male            | Female          |
| CXCL1 (GRO-α) | ![CXCL1](image) | ![CXCL1](image) | ![CXCL1](image) | ![CXCL1](image) |
| CXCL10 (IP-10)| ![CXCL10](image)| ![CXCL10](image)| ![CXCL10](image)| ![CXCL10](image) |
| CCL2 (MCP-1)  | ![CCL2](image)  | ![CCL2](image)  | ![CCL2](image)  | ![CCL2](image)  |
| IL-6          | ![IL-6](image)  | ![IL-6](image)  | ![IL-6](image)  | ![IL-6](image)  |
| TNF-α         | ![TNF-α](image) | ![TNF-α](image) | ![TNF-α](image) | ![TNF-α](image) |
| IL-13         | ![IL-13](image) | ![IL-13](image) | ![IL-13](image) | ![IL-13](image) |
Activation of NF-κB by ADP-Heptose involves ALPK1 and is reported to be TIFA (TRAF-interacting protein with forkhead-associated domain) dependent (7).

A. Cytokine profiling by multiplex immunoassay was performed on serum from wild-type (WT) C57BL/6 mice, Alpk1^{T237M/T237M} mice and Alpk1^{T237M/T237M}/TIFA^{−/−} mice at 2 and 3 months of age. Cytokine concentrations in the serum shown as mean ± s.e.m. As compared to WT and Alpk1^{T237M/T237M}/TIFA^{−/−} mice, Alpk1^{T237M/T237M} mice showed elevation of inflammatory biomarkers including CXCL1, CXCL10 and CCL2, suggesting that this inflammation is secondary to activation of the ALPK1-TIFA axis.

B. Spleen and body weight

B. Spleen and body weights were determined for mice of 6 weeks, 12 weeks, and 16 weeks of age.
C. Visual acuity in $Alpk1^{T237M/WT}$ mice by OptoDrum at 9 months of age.
D. Visual function by ERG at 9 months of age

D. Visual function in $Alpk^T237M/WT$ mice by ERG at 9 months of age.
E ALPK1 - WT
Fundus Imaging  OCT

| Image | Description |
|-------|-------------|
| JY0572 | Alpk1<sup>WT/WT</sup> Crb1<sup>WT/WT</sup>, 9 months of age |
| JY0573 | Alpk1<sup>WT/WT</sup> Crb1<sup>WT/WT</sup>, 9 months of age |
| JY0582 | Alpk1<sup>WT/WT</sup> Crb1<sup>WT/WT</sup>, 9 months of age |

ERG

| Amplitude (µV) | Dark | Light |
|---------------|------|-------|
| a             |      |       |
| b             |      |       |

OptoDrum

| Visual acuity (logMAR) | Dark | Light |
|------------------------|------|-------|
|                         |      |       |

F ALPK1 - HET
Fundus Imaging  OCT

| Image | Description |
|-------|-------------|
| JY0574 | Alpk1<sup>12237M/WT</sup> Crb1<sup>WT/WT</sup>, 9 months of age |
| JY0575 | Alpk1<sup>12237M/WT</sup> Crb1<sup>WT/WT</sup>, 9 months of age |
| JY0576 | Alpk1<sup>12237M/WT</sup> Crb1<sup>WT/WT</sup>, 9 months of age |

ERG

| Amplitude (µV) | Dark | Light |
|---------------|------|-------|
| a             |      |       |
| b             |      |       |

OptoDrum

| Visual acuity (logMAR) | Dark | Light |
|------------------------|------|-------|
|                         |      |       |
E. Most recent results for fundus imaging, OCT, ERG and OptoDrum evaluations. Age and genotypes as listed.
Supplemental Table 8: List of the 99 differentially expressed inflammatory response genes (GO: 0006954) in whole blood of pre-treatment (n=4) and post-treatment with adalimumab (n=2) patients with ROSAH syndrome.

| Gene  | Gene  | Gene  | Gene  |
|-------|-------|-------|-------|
| C3    | LRRK2 | KLRG1 | OGG1  |
| C3AR1 | PPARG | TNFRSF1B | ALOX5AP |
| ICAM1 | LACC1 | ITGB2 | NFAM1 |
| PRKCD | FFAR3 | CSAR1 | STAT3 |
| IL23R | PIK3CG | HIF1A | CD14 |
| PTX3  | SNAP23 | LYN   | PTGIR |
| MYD88 | NLRC4 | IL1RL1 | FPR1 |
| TNFAIP3 | CXCL8 | NLRC3 | IL1R1 |
| MAPK14 | GBA   | GGT1  | NLRX1 |
| LOXL3 | RELB  | IL6R  | SLAMF1 |
| FGR   | BCL6  | DAB2IP | NDST1 |
| IL17RA | CD36  | CEBPA | NFE2L2 |
| HCK   | ADAM8 | FPR2  | GGT5  |
| CYBB  | ALOX5 | PELO  | F11R  |
| CXCR6 | IL10RB | NLRP12 | PTAFR |
| PRCP  | STAP1 | TLR1  | ORM1  |
| NCF1  | NFKB1 | EXT1  | NOTCH2 |
| VAMP8 | SERPINA1 | ADGRE2 | TLR4 |
| SIRPA | IL1B  | PGLYRP1 | STAT5B |
| CCRL2 | PLCG2 | ZC3H12A | MAPKAPK2 |
| NFKBIZ | PIK3AP1 | TLR2  | RAC1  |
| GRN   | PARP4 | PLA2G7 | UNC13D |
| SYK   | AREL1 | TNIP1  | THEMIS2 |
| TICAM2 | SIGLEC10 | JAK2  | AOAH  |
| PTGES | CD44  | ADORA2A |       |
Members of the Undiagnosed Diseases Network

Maria T. Acosta
Margaret Adam
David R. Adams
Justin Alvey
Laura Amendola
Ashley Andrews
Euan A. Ashley
Mahshid S. Azamian
Carlos A. Bacino
Guney Bademci
Ashok Balasubramanyam
Dustin Baldridge
Jim Bale
Michael Bamshad
Deborah Barbouth
Pinar Bayrak-Toydemir
Anita Beck
Alan H. Beggs
Edward Behrens
Gill Bejerano
Jimmy Bennet
Beverly Berg-Rood
Jonathan A. Bernstein
Gerard T. Berry
Anna Bican
Stephanie Bivona
Elizabeth Blue
John Bohnsack
Devon Bonner
Lorenzo Botto
Brenna Boyd
Lauren C. Briere
Elly Brokamp
Gabrielle Brown
Elizabeth A. Burke
Lindsay C. Burrage
Manish J. Butte
Peter Byers
William E. Byrd
John Carey
Olveen Carrasquillo
Thomas Cassini
Ta Chen Peter Chang
Sirisak Chanprasert
Hsiao-Tuan Chao
Gary D. Clark
Terra R. Coakley
Laurel A. Cobban
Joy D. Cogan
Matthew Coggins
F. Sessions Cole
Heather A. Colley
Cynthia M. Cooper
Heidi Cope
William J. Craigen
Andrew B. Crouse
Michael Cunningham
Precilla D'Souza
Hongzheng Dai
Surendra Dasari
Joie Davis
Jyoti G. Dayal
Matthew Deardorff
Esteban C. Dell'Angelica
Katrina Dipple
Daniel Doherty
Naghmeh Dorrani
Arjenia L. Doss
Emilie D. Douine
Laura Duncan
Dawn Earl
David J. Eckstein
Lisa T. Emrick
Christine M. Eng
Cecilia Esteves
Marni Falk
Liliana Fernandez
Elizabeth L. Fieg
Paul G. Fisher
Brent L. Fogel
Irman Forghani
William A. Gahl
Ian Glass
Bernadette Gochuico
Rena A. Godfrey
Katie Golden-Grant
Madison P. Goldrich
Alana Grajewski
Ima Gutierrez
Don Hadley
Sihoun Hahn
Rizwan Hamid
Kelly Hassey
Nichole Hayes
Frances High
Anne Hing
Fuki M. Hisama
Ingrid A. Holm
Jason Hom
Martha Horike-Pyne
Alden Huang
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