

Letter to the Editor

Combined immunodeficiency with severe inflammation and allergy caused by *ARPC1B* deficiency

To the Editor:

One of the major protein complexes in actin polymerization and cellular motility is the Arp2/3 complex, consisting of 7 polypeptides.¹ Two of the subunits are actin-related proteins of the Arp2 and Arp3 subfamilies. The remaining 5 regulatory subunits are referred to as ARPC1 (actin-related protein complex-1), ARPC2, ARPC3, ARPC4, and ARPC5. ARPC1 is present in 2 isoforms in humans, ARPC1A and ARPC1B as a WD40 repeat-containing protein, encoded by different genes, whereas the other ARPC subunits do not contain common sequence motifs. *Arp2/3* gene deletions result in embryonic lethality in the mouse.² The genetic defects in the regulatory proteins for cytoskeletal rearrangements cause different syndromes, mostly dominated by blood and immune phenotypes.³ The activities of nucleation-promoting factors for actin polymerization are mostly regulated by signal-transduction pathways, one of which involves the activation by the Rho-family guanosine triphosphatases CDC42 and RAC2, and the Wiskott-Aldrich Syndrome protein (WASP) family as the intermediary Arp2/3 activator that can control actin assembly downstream of these small guanosine triphosphatases.^{4,5}

Here we describe the first human genetic defect in a component of the Arp2/3 complex itself. The *ARPC1B* mutation results in a combined immunodeficiency with symptoms of immune dysregulation and a mild bleeding tendency, caused by defective actin polymerization in the immune cells, in a 7-year-old male patient born as the first child of consanguineous, healthy Moroccan parents. He has one younger unaffected brother. The first weeks of his postnatal development were uneventful, and his umbilical cord detached spontaneously. At 2 months of age, he was admitted because of gastric bleeding during a febrile period of clinical gastroenteritis. A gastroscopy showed profuse bleeding in the presence of normal coagulation and liver function tests in the presence of a striking leukocytosis (between 25.2 and $55.8 \times 10^6/\text{mL}$), a normochromic anemia and a mild thrombocytopenia. The white blood cell count slowly improved spontaneously during further follow-up, whereas the thrombocytopenia persisted (see Table E1 in this article's Online Repository at www.jacionline.org). The mean platelet volume was repeatedly found to be normal (mean of 8.8 fL , $n = 9$ samples; normal range $7.4\text{--}11.7 \text{ fL}$).

His second admission was due to an auricular infection (perichondritis by *Staphylococcus aureus*), which was treated appropriately, but resulted in scarring of his right pinna (Fig 1, A). At the age of 5 months, he presented again with a clinical picture of fever and purpuric lesions on his legs, arms (Fig 1, B and C), and scrotum, and poor wound healing (data not shown). Skin biopsy showed a clear leukocytoclastic vasculitis with multiple microthrombi in the vascular lesions (Fig 1, D). We measured autoantibodies against nuclear antigens (ANA, ENA), neutrophil cytoplasmic proteins (ANCA-IFT, MPO, and PR3), platelet antigens, and lupus anticoagulants (LAC), and found all tests to be negative (data not shown). He

suffered from 2 similar episodes of vasculitis in the presence of a high number of eosinophils (peaking at $3350 \text{ cells}/\mu\text{L}$ at 25 months of age during an infection) that were treated with corticosteroids for 3 months each. His eosinophil counts remained within the normal range thereafter (Table E1).

At the age of 4 years, he suffered from a period of prolonged bloody diarrhea from which *Salmonella typhimurium* was cultured due to a serious pan-colitis with neutrophil and eosinophil infiltration in the biopsies of his colon (Fig 1, E). Recurrent pneumonias that responded to antibiotics have led to mild bronchiectasis. Apart from infections, he developed serious eczema and showed anaphylaxis after ingestion of nuts. The immunoglobulin spectrum shows increased IgA and IgE (Table E1).

Because of the early leukocytosis and initial bleeding tendency, we excluded leukocyte adhesion defects, including LAD-III.⁶ The most eminent *in vitro* findings consisted of the neutrophil defect in motility and directed movement (chemotaxis) due to an F-actin polymerization defect (Fig 1, F and G); a result supported by confocal analysis (see Fig E1, A, in this article's Online Repository at www.jacionline.org), adhesion was unimpaired (data not shown). Enhanced azurophil granule release on cell activation (see Fig E2 in this article's Online Repository at www.jacionline.org) was noted by the release of proteolytic activity and the upregulation of CD63 as an integral membrane marker for azurophil granules, but normal activation of nicotinamide adenine dinucleotide phosphate oxidase, phagocytosis, and killing of *S aureus* and *Escherichia coli* (Fig E2; data not shown). The initial bleeding tendency seemed to be associated with a very mild platelet dysfunction in a double-colored aggregation assay designed previously to determine platelet function under conditions of reduced platelet counts when standard aggregometry tests fail to be accurate (see Fig E3, A, in this article's Online Repository at www.jacionline.org).⁷ Also, detection of glycoprotein IIb/IIIa integrin activation and upregulation of CD62P and CD63 from the granules were tested and compared with control platelets similarly activated (Fig E3, B). Although spreading of patient platelets was different from control platelets (Fig E1, B) and CD62P and CD63 upregulation was slightly reduced, glycoprotein IIb/IIIa activation seemed intact. Clinically, the bleeding tendency was not apparent anymore after these initial bleeding events during further follow-up in the presence of a mild thrombocytopenia.

Exome sequencing failed to pick up a mutation (because of the default "variant caller" parameter settings in this complex genetic defect), but subsequent proteomics analysis of the platelets and neutrophils indicated a complete lack of ARPC1B (Fig 2, A-D). ARPC1B is an essential hematopoietic component of the Arp2/3 complex for F-actin polymerization. The ARPC1B deficiency was caused by a homozygous complex frameshift mutation in the *ARPC1B* gene (c.491_495TCAAGdelCCTGCC-Cins), as confirmed by additional targeted sequencing approaches with improved, customized "variant caller" parameter settings for the detection of complex mutations (see Fig E4 in this article's Online Repository at www.jacionline.org). The complex nature of the mutation in this family might be the consequence of a double strand break repaired by nonhomologous end joining, or by a microhomology-mediated end-joining

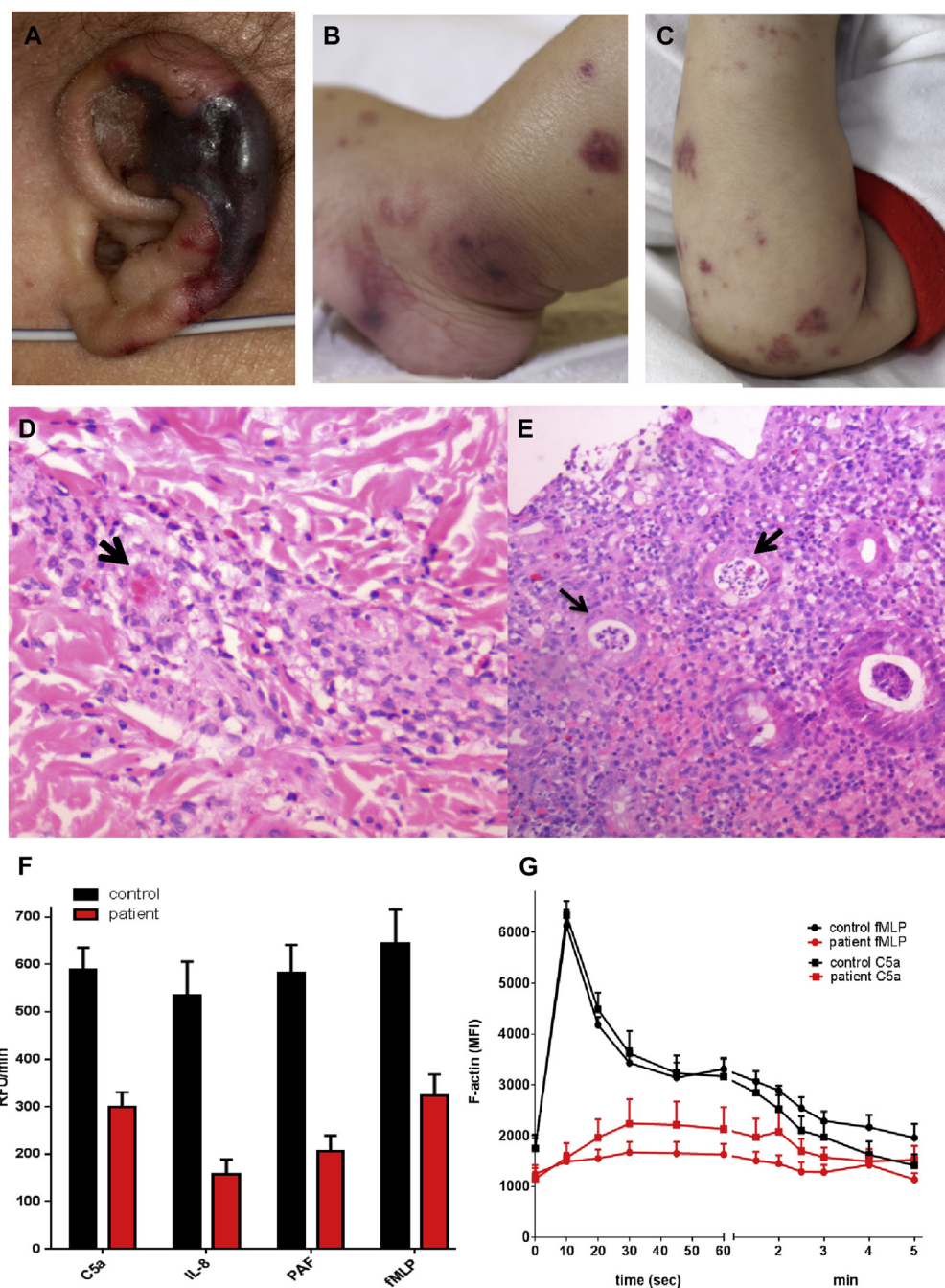


FIG 1. Clinical, histological, and *in vitro* findings in the patient. **A-C**, Remarkable clinical features of the affected index case, with skin manifestations appearing superficially, rather similar to vasculitic purpura-like lesions with poor wound repair under certain conditions. Skin biopsy showing small-vessel vasculitis with leukocytoclasia and thrombosis (**D**, arrow), and colon biopsy with severe active inflammation and crypt abscesses (**E**, arrows) (Hematoxylin and eosin, 200 $\times$). Defective chemotaxis over 3- μ m pore-size filters (**F**), and F-actin polymerization in suspension after stimulation with C5a and fMLP (**G**, mean $\pm$ SEM, $n = 7-9$). fMLP, N-formylmethionine-leucyl-phenylalanine; MFI, mean fluorescence intensity; PAF, platelet-activation factor; RFU, relative fluorescence unit.

mechanism.^{8,9} Proteomics analysis demonstrated the presence of the Arp2/3 complex in normal neutrophils, T cells, and platelets to consist of ARPC1B, ARPC2, ARPC3, ARPC4, ARPC5, and ARPC5L, but there was an absence of ARPC1A. Because ARPC1B was also expressed in tissue cells, the migration defect observed in neutrophils was expected to be present in primary

fibroblasts as well. However, these cells showed normal migratory behavior, and we may conclude that the nonhematopoietic expression of ARPC1A is apparently sufficient to rescue the fibroblasts from a detectable defect (see Fig E5 in this article's Online Repository at www.jacionline.org). Expression of ARPC1A and ARPC1B were variably present in additional nonhematopoietic

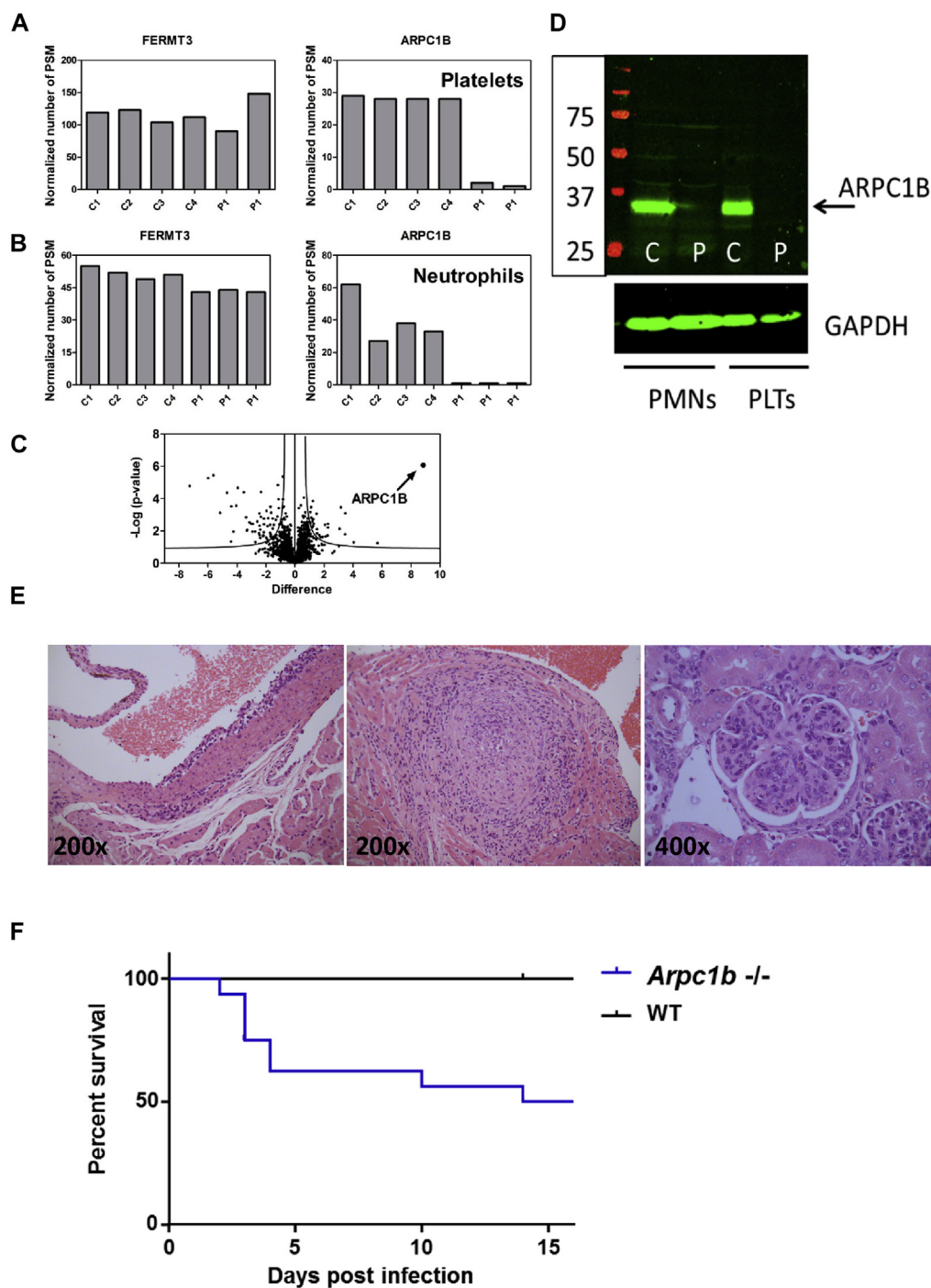


FIG 2. ARPC1B deficiency and *Arpc1b*-knock-out mouse model. **A-C**, Mass spectrometry analysis showing the significant difference in protein levels between the neutrophils of healthy controls and the patient ($n = 3$; Volcano plot in **C**); ARPC1A is absent in proteomics analysis in control platelets and neutrophils (data not shown). **D**, Absence of ARPC1B was confirmed by Western blotting with anti-ARPC1B antibody of PMN and platelet lysates. Anti-glyceraldehyde-3-phosphate dehydrogenase antibody was used as loading control ($n = 3$). WASP was present by Western blotting (data not shown). **E**, Vasculitis and renal glomerulonephritis in *Arpc1b*^{-/-} mice with aortic vasculitis (200 $\times$; left panel), cardiac subendocardial vasculitis (200 $\times$; middle panel), and proliferative glomerulonephritis (400 $\times$; right panel). **F**, Kaplan-Meier curve for survival following infection of *Arpc1b*^{-/-} and wild-type (WT) mice with *S typhimurium* M525. The results shown are pooled from 2 independent experiments of 8 mice each (4 males, 4 females; 16 mice total). In both experiments half of the *Arpc1b*^{-/-} animals reach the severity limit (see Methods). PSM, peptide spectral matches.

cell lines (HeLa, SKBR3, and HEK273), indicating redundancy of ARPC1 proteins, whereas only ARPC1B protein was detected in hematopoietic cell lines (Daudi, Ramos, Jurkat, NB4, U937), which is similar to the various findings with fibroblasts versus blood cells tested (data not shown). Together, these novel data on ARPC1 protein expression may well explain why this ARPC1B defect manifests primarily as a hematological and immunological disease.

When *Arpc1b*^{-/-} mice (*Arpc1b*^{tm1a(EUCOMM)Wtsi}) were generated and compared with the patient (see Table E2 in this article's Online Repository at www.jacionline.org) as part of a phenotyping screen, no overt changes in whole blood cell counts were found. However, plasma total IgE levels were increased at 16 weeks (0 ± 0 ng/mL in wild-type mice vs 220.6 ± 266.9 ng/mL in *Arpc1b*^{-/-} mice). In 75% of the *Arpc1b*-deficient mice, mild inflammation of the blood vessels was observed, targeting the aorta and/or the mesenteric and pancreatic arteries (Fig 2, E). On being challenged with the attenuated *S typhimurium* M525 and unlike their wild-type equivalents, *Arpc1b*^{-/-} mice showed signs of moderate salmonellosis by day 3 postinfection, with 8 of 18 mice having to be sacrificed by day 5 (Fig 2, F). In contrast, all wild-type mice survived the challenge.

The fact that ARPC1B is the only ARPC1 isoform in hematopoietic cells, whereas both ARPC1A and ARPC1B are present in tissue cells, suggests a differential use of Arp2/3 activities in tissue and blood cells. Severe food allergies, eczema, and autoimmunity are also observed in WASP- and WIP-deficient patients causing Wiskott-Aldrich(-like) syndrome,^{3,5,10} but this was genetically excluded in our patient. These immunodeficiencies tend to have a more severe bleeding tendency and more pronounced thrombocytopenia.

We have identified a novel combined immunodeficiency with features of recurrent infections, allergic reactions, vasculitis, and mild bleeding tendency. The biology as well as the supportive evidence from the mouse model suggests a direct causal relationship between the *ARPC1B* mutation and the clinical manifestations.

We are very grateful to the patient, his family, and healthy donors. We also thank Dr Ester van Leeuwen for immunological screening and Hans Janssen for his electron microscopic evaluations of blood cells, Simon Forman for IgE measurements, and Dr Hibret Adissu for histopathology.

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Disclosure of potential conflict of interest: The authors declare that they have no relevant conflicts of interest.

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<http://dx.doi.org/10.1016/j.jaci.2016.09.061>

METHODS

Isolation of blood cells

Heparinized venous blood was collected from healthy donors and the patient after informed consent had been obtained. The study was approved by the Academic Medical Center Institutional Medical Ethics Committee in accordance with the 1964 Declaration of Helsinki.

Human neutrophils were isolated with a Percoll solution with a density of 1.076 g/mL. Erythrocytes were lysed with isotonic $\text{NH}_4\text{Cl}/\text{KHCO}_3$, washed twice in PBS and resuspended in HEPES buffer for further functional testing.^{E1-E3}

PBMCs were collected from the surface of the Percoll solution, washed twice and resuspended in PBS containing 0.5% (wt/vol) BSA for immunophenotyping or for further functional studies in Iscove modified Dulbecco medium supplemented with 10% (vol/vol) fetal calf serum and antibiotics.^{E4,E5} Platelet-rich plasma and washed platelets were prepared as previously described.^{E6} Isolated platelets were washed twice with sequestrene buffer followed by centrifuging for 5 minutes at 2310g. Platelets were resuspended in HEPES medium supplemented with 0.02 U/mL apyrase. Studies were performed within 6 hours after blood collection.

NADPH-oxidase activity

Nicotinamide adenine dinucleotide phosphate (NADPH)-oxidase activity was assessed as the release of hydrogen peroxide determined with an Amplex Red kit (Molecular Probes, Eugene, Ore). Neutrophils ($0.25 \times 10^6/\text{mL}$) were stimulated with 1 mg/mL unopsonized zymosan (MP Biomedicals, Solon, Ohio), serum-treated zymosan (previously described^{E4}), phorbol 12-myristate 13-acetate (PMA; 100 ng/mL) or platelet-activation factor (PAF)/N-formylmethionine-leucyl-phenylalanine (fMLP) (both 1 $\mu\text{mol/L}$) in the presence of Amplex Red (0.5 $\mu\text{mol/L}$) and horseradish peroxidase (1 U/mL). Fluorescence was measured at 30-second intervals for 30 minutes with an Infinite F200-pro plate reader (Tecan, Männedorf, Switzerland) at an excitation wavelength of 535 nm and an emission wavelength of 595 nm. Maximal slope of H_2O_2 release was assessed per 2-minute interval.

Neutrophil chemotaxis and adhesion

Neutrophils ($5 \times 10^6/\text{mL}$) were incubated with calcein-AM (1 $\mu\text{mol/L}$ final concentration; Molecular Probes) for 30 minutes at 37°C, washed twice, and resuspended in HEPES buffer at a concentration of $2 \times 10^6/\text{mL}$. Adhesion was determined in an uncoated 96-well MaxiSorp plate (Nunc, Wiesbaden, Germany). Calcein-labeled cells ($2 \times 10^5/\text{well}$, final volume 100 μL) were stimulated with one of the following: granulocyte-colony stimulating factor (G-CSF) (20 ng/mL), 10 mmol/L dithiothreitol (DTT); Sigma Aldrich, St Louis, Mo), 20 $\mu\text{g/mL}$ Pam3Cys (EMC Microcollections, Tübingen, Germany), 20 ng/mL bacterial Toll-like receptor-4 ligand LPS (isolated from *E coli* strain 055:B5; Sigma Aldrich) in the presence of 50 ng/mL LPS-binding protein (R&D Systems, Minneapolis, Minn), 1 $\mu\text{mol/L}$ PAF (Sigma Aldrich), 10 nmol/L C5a (Sigma Aldrich), 1 $\mu\text{mol/L}$ fMLP, C5a (10^{-8} mol/L), TNF- α (10 ng/mL), or 100 ng/mL PMA. Plates were incubated for 30 minutes at 37°C and washed 3 times with PBS. Adherent cells were lysed in 0.5% (wt/vol) Triton X-100 in H_2O for 5 minutes at room temperature. Fluorescence was measured with a Tecan Infinite F200-pro plate reader at an excitation wavelength of 485 nm and an emission wavelength of 535 nm. Adhesion was determined as percentage of total input of calcein-labeled cells.

Chemotaxis was measured with 3- μm pore-size Fluoroblock inserts (Corning Inc, Corning, NY), in a Falcon 24-well plate. Calcein-labeled PMNs ($2 \times 10^6/\text{mL}$, 0.3 mL) were pipetted in the insert (upper compartment), and placed in the lower compartment containing 0.8 mL of C5a (10^{-8} mol/L), IL-8 (10^{-8} mol/L), or PAF (10^{-7} mol/L). Fluorescence was measured underneath the filter every 2.5 minutes for 45 minutes with a Tecan Infinite F200-pro plate reader at an excitation wavelength of 485 nm and an emission wavelength of 535 nm. Alternatively, TaxiScan chemotaxis was assessed with an EZ-TAXIScan (ECI Inc, Tokyo, Japan) using a 5- μm chip (260 μm length) at 37°C. Chemotaxis was induced by 2 μL of C5a (stock concentration 10^{-7} mol/L) or 2 μL of fMLP (stock concentration 10^{-7} mol/L). Serial images were taken every 30 seconds for 60 minutes per experiment.

F-actin measurement

Neutrophils ($2 \times 10^6/\text{mL}$) were stimulated with fMLP (1 $\mu\text{mol/L}$) or C5a (10^{-8} mol/L) for the indicated times. Reaction was stopped by adding 2×10^5 cells to ice-cold PBS with 0.5% (wt/vol) paraformaldehyde (PFA), 20 mmol/L NaF and 1% (wt/vol) BSA for 10 minutes. Thereafter, cells were washed and incubated for 30 minutes at 4°C with Alexa Fluor 488 phalloidin (Invitrogen, Carlsbad, Calif), 1:1000 in IntraPrep Permeabilization Reagent (Beckman Coulter, Indianapolis, Ind), washed twice, and analyzed on an FACSCanto-II flow cytometer equipped with FACSDiva software (BD, San Jose, Calif). Data are expressed as mean fluorescence intensity (MFI).

Neutrophil degranulation assay

Neutrophils ($2 \times 10^6/\text{mL}$) were preincubated with the (priming) agents PAF (1 $\mu\text{mol/L}$) or cytochalasin B (5 $\mu\text{g/mL}$) for 5 minutes and were subsequently stimulated with fMLP (1 $\mu\text{mol/L}$) for 10 minutes. Thereafter, cells were put on ice, washed with HEPES buffer once, and subsequently stained with an antibody against neutrophil granule markers: CD63-APC (IgG1, clone 9A152; USBiological, Salem, Mass). The cells were analyzed on a FACSCanto-II flow cytometer with FACSDiva software. Data are expressed as MFI.

Protease release measurement with DQ-green-BSA

Protease release after degranulation was measured with DQ-green BSA (Molecular Probes), which becomes fluorescent on cleavage by proteases. Neutrophils ($2.5 \times 10^6/\text{mL}$) were preincubated with PAF (1 $\mu\text{mol/L}$) or cytochalasin B (5 $\mu\text{g/mL}$) for 5 minutes at 37°C in the presence of DQ-BSA (10 $\mu\text{g/mL}$) and were then stimulated with fMLP (1 $\mu\text{mol/L}$). Also, a 100% content value with Triton X-100 0.5% (wt/vol) was determined. Fluorescence was measured with an Infinite F200-pro plate reader at an excitation wavelength of 485 nm and an emission wavelength of 535 nm. Data are expressed as relative fluorescence units/minute.

Phagocytosis assay

Fluorescein isothiocyanate (FITC)-labeled zymosan was opsonized with 10% (vol/vol) human pool serum for 10 minutes at 37°C (serum treated zymosan), and then added in a concentration of 1 mg/mL to neutrophils at a final concentration of 5×10^6 PMN/mL. At the indicated time points, the cellular uptake was instantly stopped by adding 2×10^5 cells to ice-cold PBS with 0.5% (wt/vol) PFA, 20 mmol/L NaF, and 1% (vol/vol) BSA. The PMNs were analyzed on a FACSCanto-II flow cytometer equipped with FACSDiva software. The “phagocytosis index” was defined as the percentage of FITC-positive PMNs multiplied by the MFI.

Bacterial killing

Granulocyte bactericidal activity was determined with *E coli*, strain ML-35, and *S. aureus*, strain 502A. Bacterial survival was measured by assaying bacterial colony formation as previously described.^{E7}

Platelet activation and aggregation

Platelet aggregation tests employing flow cytometry were performed as previously described.^{E6,E8} Briefly, platelets were washed with sequestrene buffer. Carboxyfluorescein diacetate succinimidyl ester (Molecular Probes) and PKH26-labeled platelets were mixed 1:1 and preincubated with agonists: 20 $\mu\text{mol/L}$ thrombin-related activation peptide-6 (TRAP6; Bachem, Bubendorf, Switzerland); 100 ng/mL PMA (Sigma Aldrich). Samples were taken in time and were fixed in 0.5% (wt/vol) formaldehyde, PBS.

For antigen upregulation of CD62P and CD63 (FITC-labeled; Sanquin Reagents, Amsterdam, The Netherlands) platelets were stimulated 5 minutes with 100 ng/mL PMA, 10 $\mu\text{g/mL}$ collagen, or 20 $\mu\text{g/mL}$ TRAP6. Total expression of the $\beta 3$ integrin (glycoprotein IIb/IIIa) was measured with C17-FITC (Sanquin Reagents), whereas the high-affinity conformation of integrin $\beta 3$ with first procaspase activating compound (PAC-1)-FITC (BD

Biosciences, San Jose, Calif). The extent of activated integrin was determined relative to total integrin expression, that is, after background correction with the isotype control and normalizing ratios of unstimulated platelets to 1.^{E8}

Both double-colored events and antigen expression were assessed with a LSR II flow cytometer equipped with High Throughput Sampler (HTS) and data were analyzed by FACSDiva version 6.1 software (both from BD).

Confocal conditions

For confocal microscopy, a total of 250,000 neutrophils were seeded on 12-mm coverslips (Menzel-Gläser, Thermo Fisher Scientific, Braunschweig, Germany) and stimulated either with fMLP (0.1 μ mol/L; Sigma-Aldrich) or C5a (0.1 μ mol/L; Sigma-Aldrich). Cells were then fixed in 3.7% paraformaldehyde for 30 minutes at room temperature and permeabilized with 0.1% Triton in PBS. In case of washed platelets, an equal number was seeded and activated by collagen (10 μ g/mL; Sigma-Aldrich) or TRAP (20 μ mol/L; Bachem). After fixation and permeabilization, cells were stained with Phalloidin Texas Red (Invitrogen), Hoechst (Sigma-Aldrich), anti- α -tubulin (Sigma-Aldrich) followed by secondary antibody goat antimouse IgG Alexa Fluor 488 (Thermo Fisher Scientific) for neutrophils, or Phalloidin Alexa 488 for platelets. Coverslips were analyzed on a Zeiss LSM 510 confocal microscope (Jena, Germany). Images were deconvoluted with Huygens deconvolution software.

Fibroblast culture and scratch assay

Fibroblasts were cultured in 10% (vol/vol) FCS in Dulbecco modified Eagle medium (DMEM) and passaged twice a week. For the migration assays, 6.0×10^4 fibroblasts were seeded in a 24-well plate and grown to confluence. Cells from passages 4 to 6 were used. Cells were starved in DMEM 20 hours prior to scratching. The cells were scratched with a yellow pipette tip and washed with PBS. Cells were stimulated with either 10% (vol/vol) FCS in DMEM or 10 ng/mL platelet-derived growth factor-BB (PDGF-BB) (Peprotech, Oak Park, Calif). The wound area was captured every 20 minutes for 45 hours at 37°C and 5% CO₂ on a Zeiss observer Z1 microscope with a 10 $\times$ objective. Healing was quantified manually by measuring wound size in ImageJ (National Institutes of Health, Bethesda, Md).

Mass spectrometry analysis of neutrophils and platelets

All chemicals were obtained from Life Technologies (Carlsbad, Calif) unless stated otherwise. To isolate platelets, platelet-rich plasma was collected via centrifugation of whole blood for 20 minutes at 120g. Platelets were spun down by centrifugation for 10 minutes at 2000g, washed and resuspended in a buffer comprising 36 mmol/L citric acid, 103 mmol/L NaCl, 5 mmol/L KCl, 5 mmol/L EDTA, 5.6 mmol/L D-glucose, and 10% (vol/vol) ACD-A (BD, Plymouth, UK) at pH 6.5. About 100×10^6 platelets in 25 μ L wash buffer were lysed in 8 mol/L urea in 100 mmol/L Tris-HCl (pH 8). Disulfide bonds were reduced with 10 mmol/L DTT for 60 minutes at 20°C, alkylated with 55 mmol/L iodoacetamide for 45 minutes at 20°C, and samples were digested overnight at 20°C with MS-grade trypsin (Promega Benelux, Leiden, The Netherlands). Peptides were desalted and concentrated with Empore-C18 StageTips prepared in-house and eluted with 0.5% (vol/vol) acetic acid, 80% (vol/vol) acetonitrile as described. Sample volume was reduced by SpeedVac and supplemented with 2% (wt/vol) acetonitrile, 0.1% (wt/vol) trifluoroacetic acid to a final volume of 5 μ L. Neutrophils were lysed in 4% (wt/vol) SDS, 100 mmol/L DTT, 100 mmol/L Tris-HCl pH 7.5 supplemented with HALT protease and phosphatase inhibitor cocktail (Thermo Fisher Scientific) and processed into tryptic peptides with the filter aided sample preparation method.^{E9} Peptides derived from neutrophils were desalted as described for the platelets. The tryptic peptides derived from platelet or neutrophil proteins (3 μ L sample) were separated by nanoscale C18 reverse chromatography coupled on line to an Orbitrap Fusion Tribrid mass spectrometer (Thermo Fisher Scientific) via a nanoelectrospray ion source (Nanospray Flex Ion Source, Thermo Fisher Scientific) as described.^{E10} All mass spectrometry data were acquired with Xcalibur software (Thermo Fisher Scientific).

Mass spectrometry data analysis

The raw mass spectrometry files were processed with Proteome Discoverer 1.4.1.14 (Thermo Fisher Scientific). The SEQUEST-HT search algorithm and a protein database with *Homo sapiens* proteins from human Uniprot database^{E11} (downloaded February 2015) were employed to identify the peptides and the number of peptide spectral matches (PSMs) for the individual proteins. The peptide precursor mass tolerance was set to 10 parts per million, and fragment ion mass tolerance was set to 0.6 Da. Carbamidomethylation on cysteine residues was used as fixed modification, and oxidation of methionine was used as a dynamic modification. The percolator node in proteome discoverer was utilized for peptide validation (false discovery rate < 1% based on peptide *q* value). To compare samples, the obtained number of PSMs for a protein was corrected for the difference in the total number of PSMs for all identified proteins in the individual samples. The correction factor for a sample was calculated by dividing the total number of PSMs of healthy control C1 by the total number of PSMs of the sample. Mass spectrometry raw files obtained from the neutrophil samples of healthy individuals and the patient were also processed with the MaxQuant 1.5.2.8 computational platform as described.^{E10,E12} Label-free quantification based on precursor intensity was used to evaluate the difference in protein level between control and patient samples. Quantitative significance was assessed with an adapted permutation-based false discovery rate *t* test from Perseus 1.5.1.6 software.

PAGE and Western blot analysis

Samples were separated by SDS-PAGE and transferred onto a nitrocellulose membrane. Individual proteins were detected with antibodies against ArpC1b (goat polyclonal antibodies, Thermo Fisher Scientific, Rockford, Ill) and against human Glyceraldehyde-3-Phosphate Dehydrogenase (mouse monoclonal antibody; Merck Millipore, Darmstadt, Germany). Secondary antibodies were either donkey-anti-goat-IgG IRDye 800CW, goat-anti-mouse-IgG IRDye 800CW, or goat-anti-rabbit-IgG IRDye 680CW (LI-COR Biosciences, Lincoln, Neb). Quantification of bound antibodies was performed on an Odyssey Infrared Imaging system (LI-COR Biosciences).

PCR

Exon 5 plus flanking intron sequences of the *ARPC1B* gene was amplified from genomic DNA. We used the Rapid Cycler technology (BioFire Diagnostics, Salt Lake City, Utah) with 50 cycles of 5 seconds at 95°C, 30 seconds at 65°C, and 60 seconds at 72°C, in 15 μ L of PCR buffer containing 2 U of Taq polymerase (Promega Benelux), 2 U of TaqStart antibody (Takara, Mountain View, Calif), 50 ng of each primer, 200 μ mol/L for each of the deoxynucleotide triphosphate, 50 mmol/L KCl, 1.5 mmol/L MgCl₂, 0.1% (vol/vol) Triton X-100, 10 mmol/L Tris, pH 9.0 at 25°C, in 10- μ L glass capillaries (BioFire Diagnostics). The *ARPC1B* exon 5 primer sequences are: fw-primer: 5'-ctgtctgcctgaccaccgtcc-3', reverse primer: 5'-ctatgatgtcagcagtcagttg-3'.

Ion Torrent PGM sequencing

An Ampliseq custom panel (Thermo Fisher Scientific, Waltham, Mass) was used to sequence the coding regions of the following genes: *ACTR2*, *ACTR3*, *ARPC1A*, *ARPC1B*, *ARPC2*, *ARPC3*, *ARPC4*, *ARPC5*, *ARPC5L*, *MKLI*, *WASP*, *XIAP*. Library preparation and sequencing was performed according to manufacturer protocols on a PGM system (Thermo Fisher Scientific). Ion Reporter software (Thermo Fisher Scientific) with customized variant caller parameter settings to enable the calling of complex mutations was used to analyze the sequence data.

Sanger sequencing

PCR products from genomic DNA were sequenced on an automated fluorescent sequencer (ABI 3130XL; Thermo Fisher Scientific) with the use of Big Dye Terminator (version 1.1) chemistry (Thermo Fisher Scientific). Primers used for sequencing were the same as those used for the PCR. For

cDNA numbering, the A of the ATG translation initiation codon was taken as 1. This codon is codon 1. NM_005720 was used as reference sequence.

Mouse methods

C57BL/6N ES cells were used to generate a knock-out first allele, as described.^{E13} The ES cell clone ID was EPD0423_1_B04. Terminal blood was collected at 16 weeks of age by puncture of the retro-orbital sinus under anesthesia, into K2 EDTA-coated pediatric tubes (Kabe Labortechnik, Numbrecht, Germany) for hematology and into heparinized pediatric tubes (Kabe Labortechnik) for plasma preparation and peripheral blood leukocyte analysis. Plasma was analyzed with an Olympus AU400 analyzer (Beckman Coulter, Buckinghamshire, United Kingdom) and complete blood counts were generated with a Scil Vetabc system (Montpellier, France). Flow cytometric analysis of peripheral blood leukocytes was performed on heparinized blood collected from 16-week-old mice. After lysis of red cells, washing with PBS with 0.5% (wt/vol) BSA, and blocking with BD Fc-Block for 10 minutes on ice, fluorescently conjugated titrated antibodies were added, and the mixture was incubated for 20 minutes on ice. After staining, propidium iodide (Sigma-Aldrich) solution was added to a final concentration of 2.5 mg/mL and incubated for a further 5 minutes on ice. After washing 3 times, cells were resuspended in fluorescence-activated cell sorting buffer and analyzed on a BD-LSRII. Data were analyzed with FlowJo software (version 9.5, TreeStar Inc; Ashland, Ore).

Background-matched wild-type and *Arpc1b*^{-/-} mice 6 to 7 weeks of age were infected intravenously with a sublethal dose of *S typhimurium* M525, as part of the Sanger mouse primary infection screen.^{E14} Mice were weighed daily and culled if they lost 20% body weight or became moribund. All mice were monitored daily, using the humane end points of >20% bodyweight loss or inability to feed and water itself in accordance with UK Home Office license guidelines and license PPL80/2596.

Statistics

Graphs were drawn and statistical analysis was performed with GraphPad Prism version 5.00 for Windows (GraphPad Software, San Diego, Calif). Data were evaluated by Student *t* test. The criterion for significance was *P* < .05 for all comparisons.

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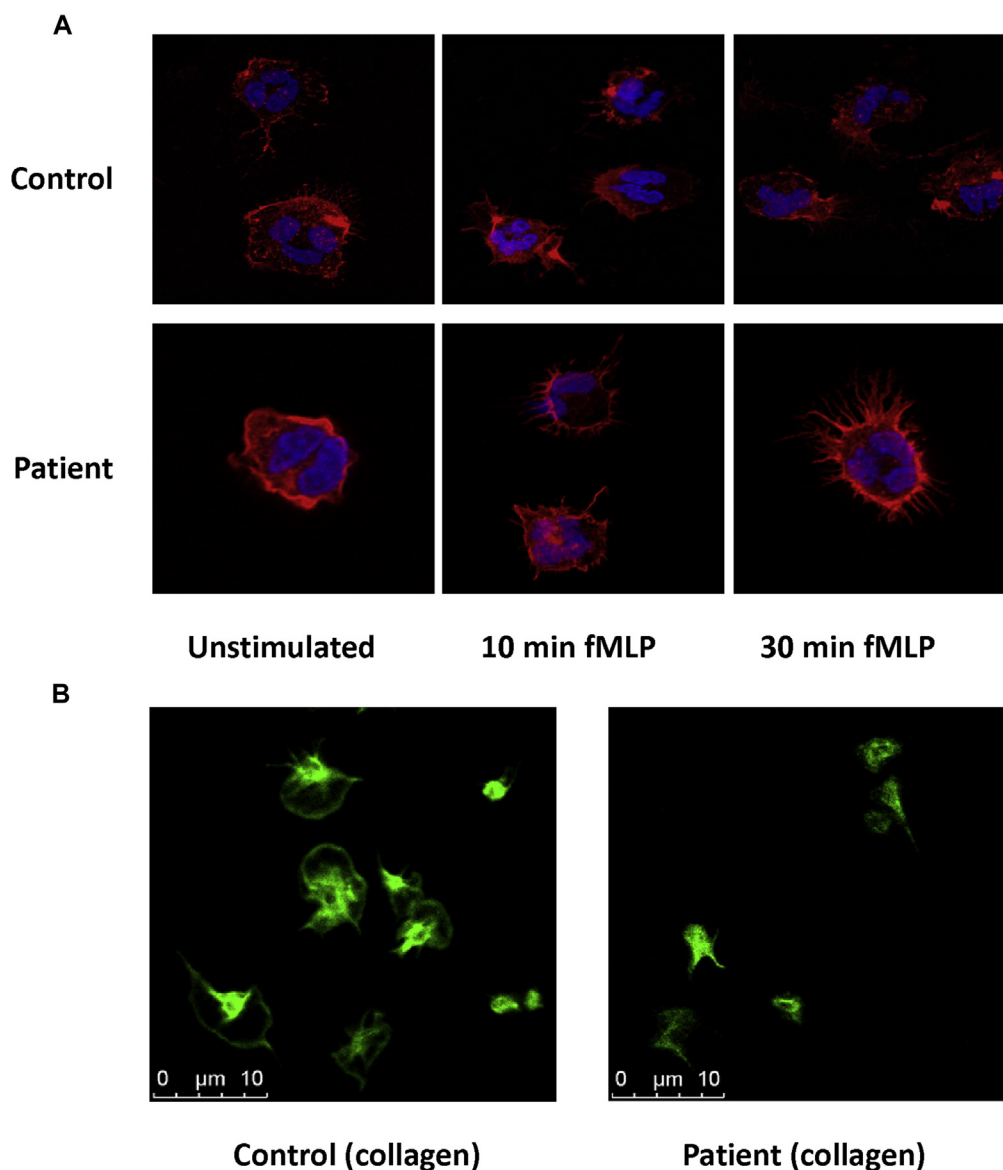


FIG E1. Actin localization on neutrophil activation. **A**, Polymerized F-actin was stained in control and patient PMNs. Polymerized actin staining on glass cover slide (*red* = actin, *blue* = nucleus). Control cells were activated by fMLP (*upper panel*) and compared with patient cells (*lower panel*) at the indicated time points. On activation with fMLP, the normal lamellipodia are not observed with the patient cells and instead the formation of filopodia are the major effect induced in the patient cells, which is in line with expected role of a dysfunctional Arp2/3 complex, resulting in lack of actin polymerization by branching. **B**, Polymerized F-actin was stained in control and patient platelets activated by collagen and showed defective spreading behavior similar to what was observed with neutrophils. These are representative for 3 independent experiments (see the [Methods](#) in the Online Repository).

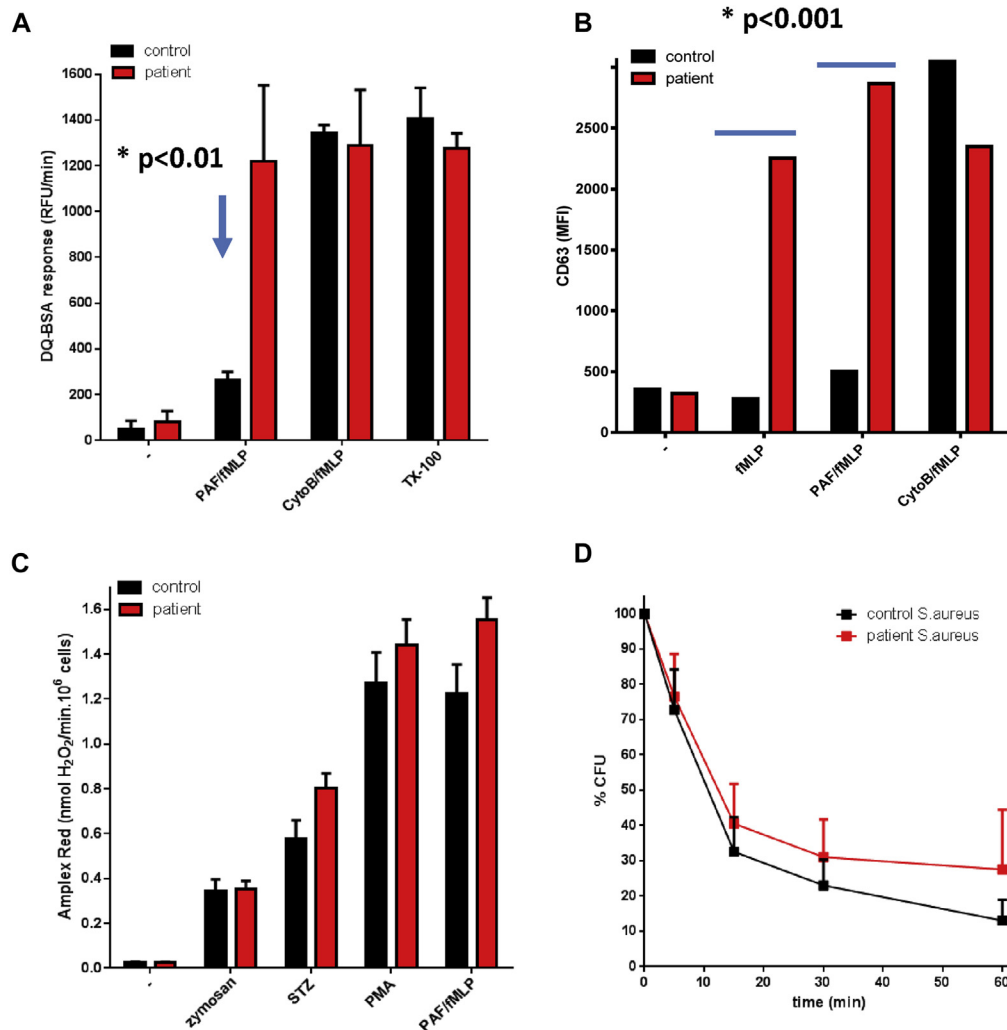


FIG E2. Neutrophil function tests. **A**, Protease release of control and patient PMNs after stimulation with fMLP, PAF/fMLP, cytochalasin B (CytoB)/fMLP, and TX-100 (maximal slope in relative fluorescence units [RFU]/min; mean $\pm$ SEM, $n = 3$). **B**, CD63 expression on control and patient PMNs after stimulation with fMLP, PAF/fMLP, and CytoB/fMLP (mean of 2 independent experiments). **C**, Nicotinamide adenine dinucleotide phosphate-oxidase activity was quantified as H₂O₂ release after stimulation with zymosan, serum-treated zymosan (STZ), PMA, or PAF/fMLP (nmol/L H₂O₂/min/10⁶ PMNs; mean $\pm$ SEM, $n = 4$). **(D)** Killing of *S. aureus* by control and patient PMNs. Killing was quantified by determining colony-forming units after incubation with PMNs at different time points, $t = 0$ was defined as 100% (mean $\pm$ SEM, $n = 4$). See the [Methods](#) in the Online Repository.

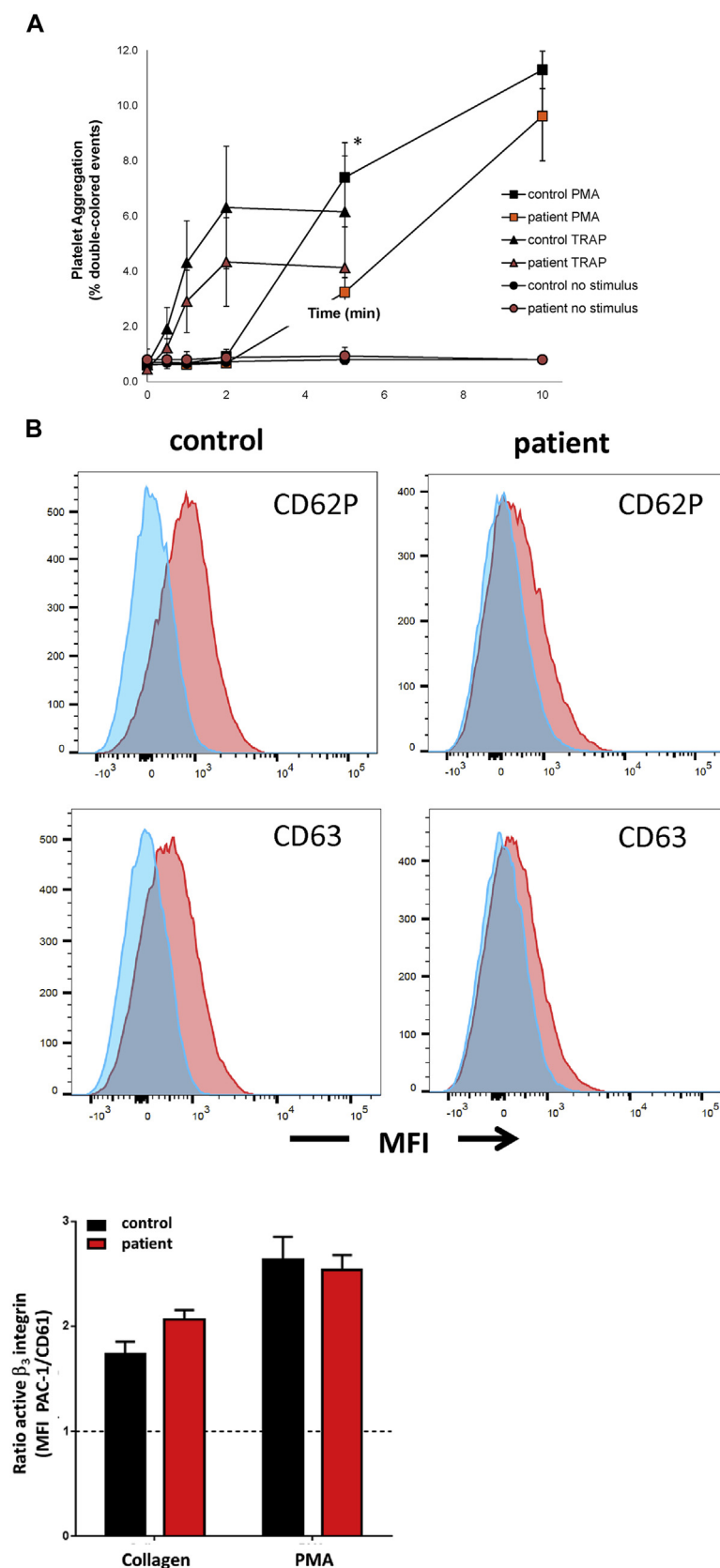


FIG E3. Mild defect in patient platelet aggregation and activation to multiple ligands. **A**, Platelet aggregation was determined as double-colored events (see the [Methods](#) in the Online Repository). PMA (100 ng/mL), TRAP (20 μ mol/L) and background levels without stimulation are shown (mean $\pm$ SEM, $n = 4$; $*P < .05$). **B**, Platelet expression of CD62P, CD63, and PAC-1 compared with CD61 upregulation (expressed as a ratio) on activation by TRAP (CD62P and CD63, platelet-rich plasma), or by collagen and PMA (PAC-1–CD61 ratio, washed platelets) (mean $\pm$ SEM; $n = 3$).

40 bp

98,987,620 bp

98,987,630 bp

AMPL1 159893536

Position: chr7, 98987625-98987630
 Reference: TCAG*
 Type: INDEL
 Alternate: [CCTGCCCC]

T G T G A C T T C A A G T G T C G

C D F K C

The figure displays four Sanger sequencing chromatograms, each showing a DNA sequence trace with peaks for Adenine (A), Cytosine (C), Guanine (G), and Thymine (T). The sequences are aligned horizontally, with positions marked at the top and bottom of each trace. The top trace is labeled '1 fw-Control' and shows a sequence from position 1117 to 1320. The second trace is labeled '2 fw-Patient' and shows a sequence from position 1117 to 1352, with a pink shaded region indicating a deletion between positions 1117 and 1125. The third trace is labeled '3 fw-Mother' and shows a sequence from position 1124 to 1360. The bottom trace is labeled '4 fw-Father' and shows a sequence from position 1125 to 1360. The Patient's sequence (2) is missing the region between positions 1117 and 1125, which is present in the Control, Mother, and Father sequences.

FIG E4. Identification of ARPC1B c.191_195 TCAAGdelCCTGCCins mutation. **A**, Screenshot from the Integrative Genome Viewer (Broad Institute, Cambridge, Mass) shows the exon 5 sequence reads from the *ARPC1B* gene of the patient obtained with the Ion Torrent PGM system. All reads show the c.191_195 TCAAGdelCCTGCCins mutation. **B**, Electrophorograms are shown from the Sanger sequencing results obtained with the exon-5 forward primer. The *pink shading* indicates the start of the homozygous c.191_195 TCAAGdelCCTGCCins mutation in the genomic DNA of the patient in exon 5 of *ARPC1* gene (*second row*). The mother and father of the patient (*third and fourth rows*) show a heterozygous pattern. The healthy control (*upper row*) shows the wild-type sequence.

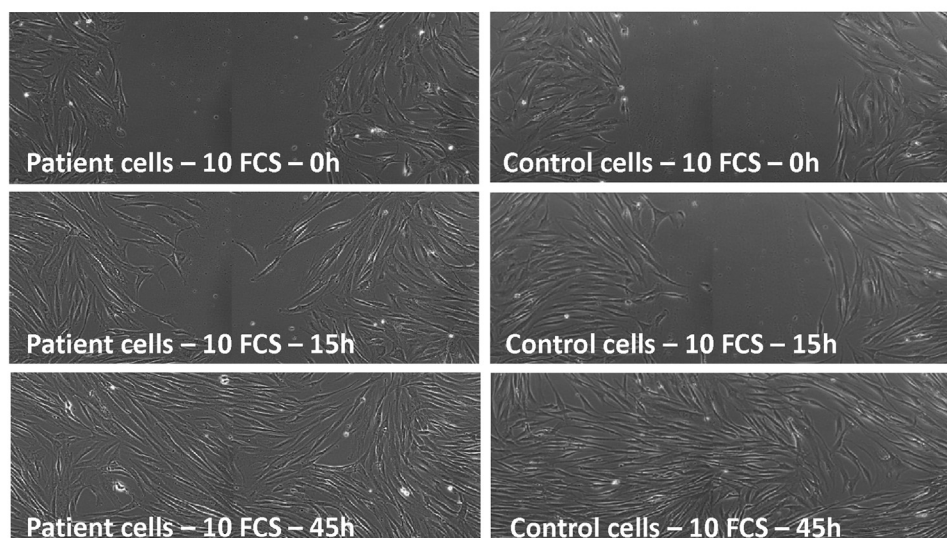
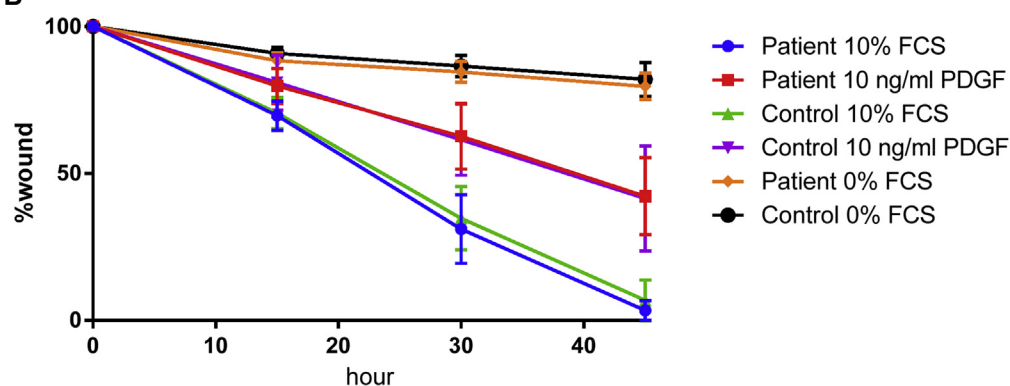
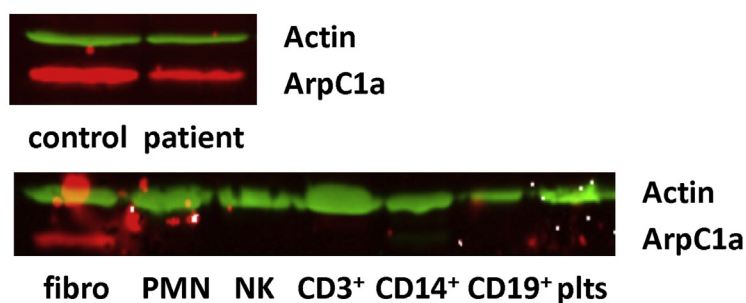
A**B****C**

FIG E5. Fibroblast migration and ARPC1A/ARPC1B expression. Comparison of fibroblast migration in a scratch assay showed no difference between patient and control cells in response to PDGF and FCS. **A**, Still images of patient and control cells after stimulation with 10% (vol/vol) FCS. **B**, Quantification of the wound area after 0, 15, 30, and 45 hours after stimulation (mean $\pm$ SD of 12 different areas). The wound area at $t = 0$ was defined as 100%. **C**, Western blot of patient and control fibroblast lysates (*upper panel*) and control fibroblasts and blood cell lysates (*lower panel*) with anti-ARPC1A antibody and actin as a loading control.

TABLE E1. Hematology and immunology parameters of the *ARPC1B*-deficient patient

Laboratory results	First patient admission	Year 1	Year 2	Year 5	Normal ranges
Hematology					
Hemoglobin (mmol/L)	4.2	6.8	6.7	7.0	6.0-9.0
Platelets (10 ⁶ /mL)	58	86	127	116	150-350
WBCs (10 ⁶ /mL)	36.2	24.5	12.2	10.8	4-16
Neutrophils (10 ⁶ /mL)	22.0	16.1	7.3	7.1	1.5-9.0
Eosinophils (10 ⁶ /mL)	2.6	0.3	0.6	0.5	<0.8
Monocytes (10 ⁶ /mL)	3.2	1.6	0.9	0.9	0.2-1.0
Lymphocytes (total, 10 ⁶ /mL)	8.8	4.4	3.1	2.7	1.5-6.0
Immunology, humoral*					
IgG, range (g/L)	8.0	13.2	8.9	9.0	<10.3
IgA, range (g/L)	3.5	3.8	4.6	5.2	<1.7
IgM, range (g/L)	0.25	0.39	0.32	0.52	<0.75
IgE, range (kU/L)	3,000	12,430	7,730	7,590	<100

WBCs, White blood cells.

*Data were taken from admission up to the first 5 years of follow-up.

TABLE E2. Morphological and mechanistic abnormalities comparing the *ARPC1B*-deficient patient and *Arpc1b*^{-/-} mice

Symptoms	Patient	Knock-out mouse
Growth	Small for age (−2.0 SD)	Males at lower end of normal range (using 95% CI)
Dysmorphic features	No	No
Severe organ involvement	No	No
Heart	No (anatomical or functional)	No
Lung	Bronchiectasis	No
Liver	Hepatomegaly (mild)*	No
Kidney	No	Proliferative glomerulopathy (variable)
GI tract	Feeding difficulties (anaphylaxis)†	No
Skeletal	No abnormalities (Dexa, x-ray)	No gross abnormalities observed
Neurological	Normal development	No gross abnormalities
Susceptibility to infection	Yes	Yes (<i>Salmonella</i>)
Vasculitis	Yes (infection related)	Yes (specific pathogen free) targeting aorta and/or mesenteric and pancreatic arteries
Allergy	Yes (skin, lung, food allergy)	High IgE in naive mice
Bleeding tendency	Yes (mild)	Not tested
Malignancy	None thus far	None thus far

GI, Gastrointestinal.

*No lymphadenopathy or splenomegaly.

†He has hypoallergic nutrition because of food refusal for a very long period and severe anaphylaxis.