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Supporting Online Material for

Pyogenic Bacterial Infections in Humans with MyD88 Deficiency

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Supplementary online material

Supplementary note 1: Case reports (see also supplementary tables 9, 10 and 11)

Kindred A

Patient 1, a ten-month-old boy was born to consanguineous parents (first cousins) in France. He was the second child and was born at term, with normal weight. He had received one pentavalent vaccination against *Haemophilus influenzae* Type b, *Diphtheria, Bordetella pertussis*, tetanus and poliovirus, with no adverse event, at the age of three months. The day after his birth, this child underwent surgery for iatrogenic rectal intraperitoneal perforation, and was given intravenous cefotaxime, metronidazol and gentamicin treatment for seven days. At three months of age the patient underwent surgery for bilateral hydrocele. At five months of age, he presented dyspnea and feeding problems due to severe severe *Pseudomonas aeruginosa* pharyngitis. At presentation, he had a temperature of 38°C, a C-reactive protein (CrP) concentration of 19 mg/L, a white blood cell (WBC) count of 5.80 x 10^3/ µl, a polymorphonuclear (PMN) cell count of 1.05 x 10^3/ µl and a thrombocyte count of 535 10^3/ µl. Intravenous treatment (ceftriaxone, metronidazol and amikacin) was adminstered for seven days, followed by treatment with amoxicillin and clavulanic acid. At 8 months, the patient presented strabism and exophthalmus of the left eye due to an abscess in the sphenoidal and ethmoidal sinuses, complicated by diabetes insipidus. The abscess protruded into the cavernous sinus, the sella turcica and both orbits. At initial presentation, the patient had a temperature of 37.7°C, a CrP concentration of 26 mg/L, a WBC count of 7.0 x 10^3/ µl, a PMN count of 1.95 x 10^3/ µl and a thrombocyte count of 769 x 10^3/ µl. Histologic analysis showed the formation of a granuloma containing macrophages, granulocytes, plasmacytoid lymphocytes, multinuclear giant cells and bacteria (*P. aeruginosa*, *Staphylococcus aureus*). The patient was treated with intravenous ceftazidime, vancomycin and amikacin for six

weeks. At 10 months of age, the patient presented meningitis revealed by convulsions, but a paradoxical low temperature of 37.8°C and a low CrP concentration (22 mg/L), a WBC count of 4.7 x 10^3/ µl, a PMN count of 3.62 x 10^3/ µl and a thrombocyte count of 252000/µl. We detected only 30 leukocytes/µl in the cerebrospinal fluid, despite the presence of 7.6 g/l protein, 0.01 mmol/l glucose (blood 6 mmol/l) and the growth of *Streptococcus pneumoniae*. Intravenous treatment with cefotaxime, vancomycin and amikacin was initiated immediately, but the patient died four days later. In addition to severe pyogenic infections, the patient had experienced two hospital-acquired episodes of diarrhea caused by *Adenovirus* and *Rotavirus,* with both infections following a normal course.

Kindred B

Patient 2, a three-year-old Turkish girl, was born at term to nonconsanguineous parents and her birth weight was normal. Her mother is healthy but her father is a chronic carrier of HCV. The patient was fully vaccinated against *Bordetella pertussis*, tetanus, diphtheria, hepatitis B and poliovirus (live viral vaccine) and had also been vaccinated against measles, mumps and rubella without adverse events. She began to develop recurrent skin infections one month after birth. At the age of nine months, she developed an abscess in the left inguinal region, with a CrP concentration of 186 mg/dl, a WBC cell count of 9.6 x 10^3/µl, a PMN count of 2.9 x 10^3/µl and an erythrocyte sedimentation rate (ESR) of 110 mm/h. The abscess was treated surgically and with intravenous antibiotics for 22 days. At the age of one year and eight months, the girl presented convulsions due to meningitis. At presentation, she had a temperature of 38.5°C, a CrP concentration of 153 mg/L, an ESR of 130 mm/h and a WBC count of 7 x 10^3/µl, a PMN count of 4.48 x 10^3/µl, a metamyelocyte count of 1.12 x 10^3/µl and a thrombocyte count of 225 x 10^3/µl. D-dimer concentration had increased to 545 mg/l. In the cerebrospinal fluid, 27.2 mg/dl protein, 4.7 mmol/l glucose (blood 8.6 mmol/l),

1250 leukocytes/µl and *S. pneumoniae* (by Gram staining and culture) were detected. The meningitis was successfully treated with various antibiotics, including ceftriaxone, vancomycin, meropenem, penicillin, rifampicin and chloramphenicol, administered intravenously for more than seven weeks. The patient is now on antibiotic prophylaxis with cotrimoxazole and penicillin and has experienced no infection in more than a year.

Kindred C

Patient 3, a 16-year-old girl, was born to apparently non consanguineous parents originating from the same area of Portugal. One cousin died from meningococcal meningitis. The patient had presented recurrent cutaneous and subcutaneous infections since birth. Despite antibiotic prophylaxis with cotrimoxazole beginning before the age of one year, she suffered seven episodes of meningitis (at the ages of six weeks, 8 months, 14 months, 16 months, 16 months, 3 years and 6 years), due to *S. pneumoniae* on three occasions and non typhoid *Salmonella spp.* on two occasions. The patient is now on antibiotic prophylaxis with cotrimoxazole and penicillin and has suffered no further infection in more than 10 years.

Patient 4, the nine-year-old brother of patient 3, was born at term and had a normal birth weight. At two months of age he presented severe cervical adenitis, inguinal abscess and bacteremia with *S. aureus*, *P. aeruginosa* and *Proteus spp*. At presentation, he had a temperature of 38.2°C, a CrP concentration of 0.16 mg/l, a WBC count of 11.2 x 10^3/µl, a PMN count of 5.7 x 10^3/µl and a thrombocyte count of 571 x 10^3/µl. At the age of four months, he presented meningitis due to β-hemolytic group B *Streptococcus*. At presentation, he had a temperature of 39°C, a CrP concentration of 2.18 mg/l, a WBC count of 7 x 10^3/µl, a PMN count of 3.9 x 10^3/µl and a thrombocyte count of 329 x 10^3/µl. At the age of 15 months, the patient suffered meningitis due to *S. pneumoniae*, with neurological sequelae. At presentation, he had a temperature below 38°C, a CrP concentration of 8.8 mg/l, a WBC

count of 10.4 x 10^3/µl, a PMN count of 6.4 x 10^3/µl and a thrombocyte count of 402 x 10^3/µl. At the age of two years he suffered from lobar pneumonia. At the age of three years he presented gastroenteritis caused by *S. enteritidis,* leading to sacroiliitis caused by the same pathogen. On admission, he had a CrP concentration of 0.22 mg/l, an ESR of 78 mm/h and a WBC count of 7.4 x 10^3/µl, a PMN count of 2.9 x 10^3/µl and a thrombocyte count of 393 x 10^3/µl. At the age of five years, he suffered from left hip arthritis caused by *S. aureus,* with a CrP concentration of 0.05 mg/l, an erythrocyte sedimentation rate of 19 mm/h and a WBC count of 4.9 x 10^3/µl, a PMN count of 1.76 x 10^3/µl and a thrombocyte count of 423 x 10^3/µl. At the age of six years, he suffered from meningitis due to *S. pneumoniae*. At presentation, he had no fever, and presented no signs of meningitis other than vomiting and headache. His CrP concentration was 0.9 mg/l, and he had a WBC count of 12.3 x 10^3/µl, a PMN count of 10 x 10^3/µl and a thrombocyte count of 280 x 10^3/µl. At the age of seven years, he suffered from arthritis of the left ankle due to *S. aureus*. On admission, he had a CrP concentration of 1.98 mg/l, a WBC count of 10.9 x 10^3/µl, and a thrombocyte count of 266 x 10^3/µl. He is now on antibiotic prophylaxis with cotrimoxazole and penicillin and has experienced no further infection over a period of more than three years.

Kindred D

Patient 5 was born to healthy non consanguineous gypsy parents living in Spain. She suffered from several submandibular abscesses at the ages of 5 and 8 months, without fever. One of these abscesses was caused by *S. aureus*. On presentation, at the age of 8 months, she had a CrP concentration below 3 mg/l, a WBC count of 11.3 x 10^3/µl and a PMN count of 1.8 x 10^3/µl. She died at the age of 11 months from pneumococcal meningitis.

Patient 6, the 3 and half year-old brother of patient 5 presented at three months of age with cervical adenitis caused by *S. aureus*. At presentation, he had a CrP concentration of 1

mg/l, a procalcitonin (PCT) concentration below 0.5 ng/ml, a WBC count of 15.6 x 10^3/µl and a PMN count of 4 x 10^3/µl. At the ages of two and three years, he presented retroauricular adenitis caused by *S. pneumococcus.* During the second of these episodes, he developed no temperature, a CrP concentration of 1.4 mg/l, a PCT of 0.02 ng/ml, a WBC count of 8.4 x 10^3/µl, and a thrombocyte count of 391 x 10^3/µl. At 39 months of age, the patient presented submaxillary adenitis without local inflammatory signs, without positive culture and responding well to antibiotic treatment. On admission, he had a CrP concentration of 3.8 mg/l, a PCT of 0.03 ng/ml, a WBC count of 8.5 x 10^3/µl, a PMN count of 2.9 x 10^3/µl and a thrombocyte count of 373 x 10^3/µl. At 42 months of age he presented with cellulitis and lymphadenitis with severe local inflammatory signs in the left groin and thigh. He also had paronychia in the big toe of the left foot, with positive cultures of *S. aureus* and *Citrobacter sp*. The outcome on antibiotic treatment was good. On admission, he had a CrP concentration of 8.8 mg/l, a PCT of 0.07 ng/ml, a WBC count of 12.2 x 10^3/µl, and PMN count of 5.2 x 10^3/µl.

Patient 7, the brother of patients 5 and 6 died from undocumented sepsis at the age of 1 month.

Kindred E

Patient 8, a seven-year-old boy, was born to healthy consanguineous (first-cousin) gypsy parents living in Spain. At 4 months of age, he presented an acute urinary tract infection caused by *Klebsiella pneumoniae* related to a left pelvic dilation, with favourable outcome on amoxicillin and clavulanic acid. During the episode he has no fever, a CrP concentration under 3 mg/L, a WBC count of 16.8 x 10^3/µl and PMN count of 3.3 x 10^3/µl. At 9 months of age, he presented with acute osteomyelitis of astragalus caused by *Group C Streptococcus beta-haemolyticus* (positive in blood culture). Osteomyelitis was successfully

treated with ceftriaxone. At the presentation, he had fever, a CrP concentration under 3 mg/L, a WBC count of 13.5 x 10^3/µl, a PMN count of 4.82 x 10^3/µl and a thrombocyte count of 691 x 10^3/µl. At 16 months of age, he had one episode of gastroenteritis caused by *Campylobacter jejuni*. In the course of this infection (10 days later) he developed a submaxilar adenitis without bacterial documentation with good outcome on amoxicilin-clavulanic. At the presentation, he had a CrP concentration under 3 mg/L, a ESR 57 mm/h, a WBC count of 6.58 x 10^3/µl and a PMN count of 1.6 x 10^3/µl and a thrombocyte count of 599 x 10^3/µl.

Patient 9, the four-year-old sister of patient 8, presented at 27 months of age with septic arthritis of the right knee caused by *S. pneumoniae.* This condition responded well to antibiotic treatment. During this episode, she had a slight fever (37.4°C), an ESR of 49 mm/h, a WBC count of 1.99 x 10^3/µl and a PMN count of 0.38 x 10^3/µl. At 30 months of age, she presented with septic arthritis of the right ankle caused by *S. pneumoniae,* which responded well to antibiotic treatment. During this episode, she developed a slight fever (37.4°C), her ESR and CrP concentration were normal and she had a WBC count of 11.8 x 10^3/µl and a PMN count of 8.9 x 10^3/µl. At 50 months of age, she presented mediastinal and mesenteric lymphadenitis caused by *S. aureus*. During this episode, she developed a slight fever (38°C), an ESR of 61 mm/h, a CrP concentration of 69 mg/l, a WBC count of 6.6 x 10^3/µl and a PMN count of 3.6 x 10^3/µl.

Lymphocyte subsets were evaluated in the patients and found to be within normal limits (see **Supplementary tables 9 and 10**).

Supplementary note 2: Details of transcriptional signatures

At 2 hours, 100 to 173 genes were regulated by IL-1β in control cells, 2 were regulated in IRAK-4-deficient cells, and 27 were regulated in MyD88-deficient cells (**Supplementary Table 4**). The few transcripts showing a change in MyD88-deficient cells were not regulated by IL-1β in control cells. IL-8 and CXCL1, which were moderately induced in IRAK-4-deficient cells, did not reach the levels observed in unstimulated control cells. Similar results were obtained at 8 hours, with 382 to 412 genes regulated by IL-1β in control cells, 1 regulated in IRAK-4-deficient cells, and 24 regulated in MyD88-deficient cells. By contrast, the number of transcripts regulated in IRAK4- and MyD88-deficient cells in response to TNF-α or poly(I:C) fell within the normal range, and gene signatures were also consistent with those of control cells (**Figure 3B**, **Supplementary Table 4**). For a list of transcripts regulated in at least two of the three control fibroblast lines stimulated for 2 hours with IL-1β, TNF-α, or poly(I:C) (275 transcripts), see **Supplementary Table 5**. For a list of transcripts regulated in at least two of the three control fibroblast lines stimulated for 8 hours with IL-1β, TNF-α, or poly(I:C) (1451 transcripts), see **Supplementary Table 6.** For a list of transcripts regulated by IL-1β at 2 h in MyD88 -/-, IRAK4 -/- and control cells, see **Supplementary Table 7.** For a list of transcripts regulated by IL-1β at 8 h in MyD88 -/-, IRAK4 -/- and control cells, see **Supplementary Table 8.**

Supplementary note 3: Materials and Methods

Subjects and kindreds. Our study was conducted in accordance with the Helsinki Declaration, with informed consent obtained from each patient or the patient's family. The study was approved by the local ethics committee, the CCPPRB of Necker-Enfants Malades Hospital, Paris, France. Patient 1 was recruited in a study on genetic susceptibility to IPD in France, conducted by CP. Patients 2 to 9 were recruited in a study on genetic susceptibility to

pyogenic infections, conducted by JLC. IRAK-4-deficient patients were recruited in both studies.

Activation by TLR agonists and cytokines. Cytokine production by PBMC (P1) was assessed in RPMI 1640 supplemented with 1% FCS, incubated for 48 h without stimulation, and then with LPS, R-848 or phorbol-12-myristate-acetate (PMA) plus ionomycin at the following concentrations: LPS (*Salmonella minnesota* Re595 LPS, Sigma, L-9764) 10 ng/ml; resiquimod (R-848) (3M, provided specially for this study) 3 μg/ml; PMA/ionomycin (Sigma®, P-8139/ Calbiochem®, 407952) 10^{-6} M/ 10^{-5} M. Cytokine production by whole blood cells (P2-4) was assessed in whole blood mixed with an equal volume of RPMI 1640, incubated for 48 h without stimulation, and then incubated with IL-1β, LPS or phorbol-12-myristate-acetate (PMA) plus ionomycin at the following concentrations: IL-1β (R&D Systems Europe) 10 ng/ml, LPS 1 ng/ml, PMA/ionomycin (Sigma®, P-8139/ Calbiochem®, 407952) 10^{-7} M/ 10^{-5} M. TNF-α production by EBV-transformed B cells was assessed in RPMI supplemented with 10% FCS incubated for 24 h without stimulation, and then with R-848 (3 μg/ml) or PMA/ionomycin ($5x10^{-8}$ M / $5x10^{-6}$ M). Cytokine and interferon production by fibroblasts was assessed in DMEM supplemented with 10% FCS incubated for 48 h without stimulation, and then with TNF-α, with IL-1β, with poly(I:C) (Amersham, cat # 27-4729-01) or with PMA/ionomycin at the following concentrations: TNF-α (R&D Systems Europe) 20 ng/ml, IL-1β (R&D Systems Europe) 10 ng/ml, poly(I:C) 25 μg/ ml, PMA/ionomycin $5x10^{-8}$ M/ $5x10^{-6}$ M.

Cytokine measurement. Levels of TNF-α, IL-6, IL-8, IFN-β and IFN-λ secretion were determined with an ELISA kit (*5, 6*). We also used a fluorescence-based assay capable of detecting 25 cytokines (Human Cytokine Twenty-Five-Plex Antibody Bead kit, LHC0009, Biosource, CA, USA). For genome-wide transcription analysis, whole blood was activated either as described above (poly (I:C) and R-848), or with the following agonists at the

following final concentrations: PAM_3CSK_4 (100 ng/ml), PAM_2CSK_4 (100 ng/ml), LPS (10 ng/ ml), flagellin (100 ng/ ml), 3M13 (3 μg/ ml), 3M2 (3 μg/ ml), D19 (3 μg/ ml), CPG-C (3 μg/ ml), IL-18 (50 ng/ ml), IL-33 (50 ng/ ml), PMA/ionomycin (10^{-7} M/ 10^{-5} M)), for the simultaneous determination of multiple cytokines. Fluorescence was measured with a Luminex 100 IS™ System (Luminex Corporation, TX, USA). The assay and analysis were carried out according to the manufacturer's instructions, as previously described (*7*).

Analysis of selectin (CD62L) shedding from granulocytes. Granulocytes were isolated by Ficoll density gradient centrifugation, activated with TLR agonists, stained with anti-CD62L-FITC (BD) antibody and analyzed by flow cytometry, as previously described (*15*).

Sequencing analysis. Genomic DNA was isolated by phenol/chloroform extraction. RNA was isolated with Trizol (BibcoBRL Life Technologies, Invitrogen SARL). Genomic DNA and cDNAs for *IRAK4* and *MyD88* were amplified, sequenced, and analyzed on an ABI Prism 3700 apparatus (BigDye Terminator sequencing kit, Applied Biosystems).

Western blotting and electrophoretic mobility shift assay (EMSA). Cell extracts were prepared from SV40-transformed fibroblasts left unstimulated or stimulated with TNF-α (20 ng/ml) or IL-1β (10 ng/ml). MyD88 expression, IRAK-1 degradation and the phosphorylation of MAP-kinases were monitored by western blotting and staining the membranes with the corresponding antibodies (anti-human MyD88, CSA-510, Stressgen; anti-human IRAK-1, H-273, sc-7883, anti-human phospho-p38, #9211, Cell Signaling; anti-human phospho-SAPK/JNK, #9251, Cell Signaling). EMSA was performed by incubating 7.5 μg of nuclear extract with a ^{32}P-labeled double-stranded NF-κB-specific oligonucleotide κB-probe (5'GATCATGGGGAATCCCCA-3' and 5'-GATCTGGGGATTCCCCAT-3'), as previously described (*3*).

Plasmids and site-directed mutagenesis. The full-length human *MYD88* cDNA was inserted into the pcDNA3.2-V5 vector (Invitrogen # 12489019). We generated various *MYD88* mutations, using the Quick-Change site-directed mutagenesis kit (Stratagene) in accordance with the manufacturer's protocol. The primers used to generate mutations are available upon request.

Transfection of SV40-transformed fibroblasts for the complementation of patients' fibroblasts with plasmids carrying wild-type *MYD88* and of HEK293 cells with plasmids carrying wild-type and mutant *MYD88* genes was achieved with Lipofectamine (Invitrogen # 18324-012). Fibroblasts were incubated with a complex containing Lipofectamine reagent+ and plasmids. This complex was then removed and fibroblasts were incubated for 18 h in DMEM supplemented with 10% FCS before activation with TNF-α (20 ng/ml) and IL-1β (10 ng/ml). IL-8 production was assayed by ELISA.

Microarray analysis. *Data acquisition:* Total RNA was isolated from fibroblasts and whole blood cells were obtained from patients with primary immunodeficiencies or control subjects and stimulated with IL-1β, TNF-α or poly(I:C), or left unstimulated for 2 or 8 hours (RNeasy kit, Qiagen, Cat# 74104). RNA integrity was assessed on an Agilent 2100 Bioanalyzer (Agilent, Palo Alto, CA). Biotinylated cRNA targets were prepared from 200 ng of total RNA, using the Illumina TotalPrep RNA Amplification Kit (Ambion, Cat# IL1791). We then incubated 1500 ng of cRNA for 16 hours with Sentrix Human-6 V2 BeadChip arrays (49,295 probes, Illumina, Cat# 11223189). Beadchip arrays were then washed, stained and scanned on an Illumina BeadStation 500 according to the manufacturer's instructions.

Data preprocessing: Following background subtraction, the raw signal values extracted with Illumina Beadstudio version 2 softwares were scaled to the mean intensity of the dataset. Minimum intensity was set to 10. Only the probes called present in at least one

sample ($p<0.01$) were retained for downstream analysis (n=18519 at 2 hours, n= 16929 at 8 hours).

Data analysis: Transcripts differentially regulated upon stimulation were defined based on a minimum 1.5-fold change (up- or downregulation) and a minimum absolute crude intensity difference of 200 with respect to the equivalent unstimulated sample. A gene expression analysis program, GeneSpring, version 7.3.1 (Agilent), was used for hierarchical clustering and the generation of heat maps. Functional networks were resolved with Ingenuity Pathway Analysis software (Ingenuity® Systems, www.ingenuity.com).

Data availability: Raw data for the 78 microarray analyses performed here are available from the public repository of the NCBI (GEO: www.ncbi.nlm.nih.gov/geo/). Networks were resolved with commercially available software and a knowledge base constructed from published data identifying relevant functional links between transcripts differentially regulated by each stimulus in control fibroblasts.

Supplementary Note 4: Supplementary Tables

Supplementary Table 1. *In vivo* mortality in MyD88-deficient and wild-type mice in response to pathogens in experimental conditions

Pathogen group	*Strain*	*In vivo* mortality
Bacteria, Gram-positive	*Staphylococcus aureus*	enhanced (1) / unchanged (2)
	Streptococcus agalacticae	enhanced / diminished (3) *
	Streptococcus pneumoniae	enhanced (4, 5)
	Listeria monocytogenes	enhanced (6)
	Mycobacterium avium	enhanced (7)
	Mycobacterium bovis	unchanged (8)
	Mycobacterium tuberculosis	enhanced (9, 10) / unchanged (11)
Bacteria, Gram-negative	*Anaplasma phagocytophilum*	unchanged (12)
	Borrelia hermsii	enhanced (13)
	Chlamydia pneumoniae	enhanced (14)
	Citrobacter rodentium	enhanced (15, 16)
	Francisella novicida	enhanced (17)
	Francisella tularenis	enhanced (18)
	Neisseria meningitidis	diminished (19)
	Pseudomonas aeruginosa	enhanced (2, 20) / unchanged (21)
Viruses	*Coxsackievirus*	diminished (22)
	Herpes simplex virus 1	enhanced (23)
	Herpes simplex virus 2	enhanced (24)
	Lymphocytic choriomeningits virus	enhanced (25)
	Murine cytomegalovirus	enhanced (26, 27)
	Reovirus	unchanged (28)
	Vesicular stomatitis virus	enhanced (29, 30)
Parasites	*Cryptosporidium parvum*	enhanced (31)
	Plasmodium berghei	diminished (32)
	Plasmodium chabaudi	unchanged (33)
	Toxoplasma gondii	enhanced (34-39)
	Trypanosoma brucei	enhanced (40)
	Trypanosoma cruzii	enhanced (41-43)
Fungi	*Aspergillus*	enhanced (44)
	Candida albicans	enhanced (44, 45)
	Cryptococcus neoformans	enhanced (46, 47)
Nematodes	*Trichuris muris*	diminished (48)

* enhanced in low-dose model/ diminished in high-dose model

+ enhanced after short period of follow-up/ unchanged after long period of follow-up of infected mice

Supplementary Table 2. *In vivo* pathogen growth in MyD88-deficient and wild-type mice under experimental conditions

Pathogen group	Strain	*In vivo* pathogen growth
Bacteria, Gram-positive	*Staphylococcus aureus*	enhanced (*1, 49*) / unchanged (*2*)
	Streptococcus agalacticae	enhanced / unchanged (*3*) *
	Streptococcus pneumoniae	enhanced (*5*)
	Listeria monocytogenes	enhanced (*6, 50-52*)
	Mycobacterium avium	enhanced (*7*)
	Mycobacterium bovis	enhanced (*8*)
	Mycobacterium tuberculosis	enhanced (*9, 10, 53*)
Bacteria, Gram-negative	*Borrelia burgdorferi*	enhanced (*54-56*)
	Borrelia hermsii	enhanced (*13*)
	Brucella abortus	enhanced (*57, 58*)
	Campylobacter jejunii	enhanced (*59*)
	Chlamydia pneumoniae	enhanced (*14*)
	Citrobacter rodentium	enhanced (*15, 16*)
	Francisella tularenis	enhanced (*18*)
	Haemophilus influenzae	enhanced (*60*)
	Legionella pneumoniae	enhanced (*61, 62*)
	Neiisseria meningitidis	enhanced (*19*)
	Pseudomonas aeruginosa	enhanced (*2, 20, 63*) / unchanged (*21*) +
Viruses	*Coxsackievirus*	diminished (*22*)
	Herpes simplex virus1	enhanced (*23*) / unchanged (*64*)
	Herpes simplex virus 2	enhanced (*24*)
	Influenza A virus	enhanced (*65*)
	Lymphocytic choriomeningitis virus	enhanced (*25, 66*)
	Murine cytomegalovirus	enhanced (*26, 27, 67, 68*)
	Reovirus	unchanged (*28*)
	Respiratory syncytial virus	enhanced (*69*)
	Vesicular stomatitis virus	enhanced (*29, 30)*
Parasites	*Cryptosporidium parvum*	enhanced (*31*)
	Leishmania major	enhanced (*70, 71*)
	Plasmodium berghei	unchanged (*32*)
	Plasmodium chabaudi	unchanged (*33*)
	Toxoplasma gondii	enhanced (*34-36, 72*)
	Trypanosoma brucei	enhanced (*40*)
	Trypanosoma cruzii	enhanced (*41, 43*)
Fungi	*Aspergillus*	enhanced (*44*)
	Candida albicans	enhanced (*44, 45, 73*)
	Cryptococcus neoformans	enhanced (*46, 47*)
	Fusobacterium oxysporum	enhanced *(80)*

* enhanced in low-dose model/ unchanged in high-dose model

+ in an identical model, enhanced after a short period of follow-up/ unchanged after a long period of follow-up of infected mice (*21, 63*)

Supplementary Table 3. *In vivo* morbidity of MyD88-deficient and wild-type mice in response to pathogens, under experimental conditions

Pathogen group	Strain	*In vivo* morbidity
Bacteria, Gram-positive	*Staphylococcus aureus*	enhanced (*49*) / unchanged (*2*)
	Streptococcus pneumoniae	enhanced (*74*) / diminished (*75*)
	Listeria monocytogenes	enhanced (*50, 51*)
	Mycobacterium avium	enhanced (*7*)
	Mycobacterium bovis	enhanced (*8*)
	Mycobacterium tuberculosis	enhanced (*9-11, 53*)
Bacteria, Gram-negative	*Borrelia burgdorferi*	enhanced (*56*) / unchanged (*54, 55*)
	Chlamydia pneumoniae	enhanced (*14*)
	Citrobacter rodentium	enhanced (*15, 16*)
	Legionella pneumoniae	enhanced (*61*)
	Pseudomonas aeruginosa	enhanced (*2, 20*) / unchanged (*21*)
Viruses	*Coxsackievirus*	diminished (*22*)
	Herpes simplex virus 1	enhanced (*23*)
	Herpes simplex virus 2	enhanced (*24*)
	Respiratory syncytial virus	enhanced (*76*)
Parasites	*Cryptosporidium parvum*	enhanced (*31*)
	Leishmania major	enhanced (*77*)
	Plasmodium berghei	unchanged (*78*) / diminished (*32, 79*)
	Plasmodium chabaudi	unchanged (*33*)
Fungi	*Cryptococcus neoformans*	unchanged (*46*)
	Fusobacterium oxysporum	enhanced *(80)*

Supplementary Table 4. Number of transcripts regulated in fibroblast cultures stimulated for 2 hours or 8 hours with IL-1β, TNF-α, or poly(I:C).

	IL-1β		TNF-α		poly(I:C)	
	Up	**Down**	**Up**	**Down**	**Up**	**Down**
			2 hours			
C (2 of 3)	103	40	107	32	127	41
C1	141	70	138	25	121	59
C2	100	46	284	215	156	65
C3	173	44	129	55	182	65
IRAK4 -/-	2	3	125	83	118	166
MYD88 -/-	27	13	179	49	224	128
UNC93B-/- 1	175	146	216	216	131	257
UNC93B-/- 2	309	56	241	34	31	87
STAT1 -/-	134	27	133	40	194	84
NEMO -/-	25	1	5	1	15	10
			8 hours			
C (2 of 3)	349	229	262	68	594	526
C1	385	243	298	84	573	547
C2	412	514	249	85	693	946
C3	382	282	304	138	650	515
IRAK4 -/-	1	1	283	113	510	458
MYD88 -/-	24	2	340	178	804	795
UNC93B-/- 1	382	235	336	149	53	7
UNC93B-/- 2	328	328	253	155	23	3
STAT1 -/-	213	87	210	93	294	149
NEMO -/-	13	46	29	75	29	46

Supplementary Table 5. List of transcripts regulated in at least 2 of the 3 control fibroblast lines stimulated for 2 hours with IL-1β, TNF-α, or poly(I:C) (275 transcripts).
(available online)

Supplementary Table 6. List of transcripts regulated in at least 2 of the 3 control fibroblast lines stimulated for 8 hours with IL-1β, TNF-α, or poly(I:C) (1451 transcripts).
(available online)

Supplementary Table 7. List of transcripts regulated by IL-1β, at 2 h, in MyD88 -/-, IRAK4 -/- and control cells.
(available online)

Supplementary Table 8. List of transcripts regulated by IL-1β, at 8 h, in MyD88 -/-, IRAK4 -/- and control cells.
(available online)

Supplementary Table 9. Blood leukocyte subsets in MyD88-deficient patients

Patients (age)	**P1 10 mo**	**P2 2 y**	**Normal values 1-2y**	**P7 3 y**	**P9 3 y**	**Normal values 2-4y**	**P4 5 y**	**P8 7 y**	**Normal values 5-8 y**
Lymphocytes ($10^9/\mu L$)	ND	2.4	(3.6-9)	Normal	3.32	(2.3-5.7)	ND	2.43	(2.3-5.7)
T cells %									
CD3	ND	64	(53-75)	Normal	64	(56-76)	77	47	(56-76)
CD4	ND	39	(32–51)	Normal	39	(28–47)	43	25	(28–47)
CD8	ND	27	(14-30)	Normal	25	(16-35)	27	22	(16-35)
CD45RA/CD4	ND	ND	(64-93)	ND	ND		46		
CD45RO/CD4	ND	ND		ND	ND		59		
NK cells % CD16 CD56	ND	20	(4-31)	ND	11.5	(4-31)	4	26	(4-31)
B cells % CD19	27	18	(6-35)	ND	17.5	(6-35)	18	17	(6-35)

Total lymphocyte counts, percentages of T cells, NK cells and B cells. Age-specific normal values are shown in brackets (normal values).

Supplementary Table 10. T-cell proliferation, Ig levels and humoral responses to recall antigens and to glycans in MyD88-deficient patients.

Patients	**P2**	**Normal values**	**P6**	**P9**	**Normal values**	**P8**	**P4**	**Normal values**	**P3**	**Normal values**
(age)	**2 y**	**1-2 y**	**3 y**	**3 y**	**4 y**	**7 y**	**8 y**	**5-8 y**	**15 y**	**14 y-adult**
Proliferation (x10^{3}cpm)										
CD3	46	(> 30)	ND	ND	(> 30)	ND	56	(> 30)	44	(> 30)
PHA	175.5	(> 50)	ND	50	(> 50)	ND	113	(> 50)	98.5	(> 50)
PPD	4.2	(> 10)	ND	ND	(> 10)	ND	10.3	(> 10)	38.5	(> 10)
Candidin	1.0	(> 10)	ND	ND	(> 10)	ND	15.2	(> 10)	17	(> 10)
Tetanus	26.0	(>10)	ND	ND	(>10)	ND	21	(>10)	49	(>10)
Serum Ig (g/l)										
IgG	12.4	(5-10)	Normal	29.2	(4.35-11.2)	12.5	8.31	(7-12)	12,5	(9-14.8)
IgG1	10.3	(3)	Normal	20.4	(4)	8.0	6.61	(4)		(>4)
IgG2	0.148	(0.3)	Normal	5.19	(0.4)	3.2	0.96	(0.5)		(>0.6)
IgG3	0.59	(0.12)	Normal	1.7	(0.16)	0.47	0.24	(0.17)		(>0.17)
IgG4	0.44		Normal	3.51		3.7	0.1	-		
IgA	0.5	(0.2-0.8)	Normal	1.6	(0.16-1.18)	1.32	1.21	(0.7-1.6)	1.36	(1.1-2.6)
IgM	1.59	(0.5-1.1)	Normal	1.24	(0.45-1.59)	0.29	0.9	(0.5-1.2)	0.69	(0.88-1.8)
IgE (U/ml)	24.5	(<30)	Increased	ND	(<40)	ND	199	(<80)	10.4	(<150)
Specific antibodies										
Anti-tetanus		(>0.1 IU/ml)	Normal	0.9	(>0.1 IU/ml)		1.3	(>0.1 IU/ml)		(>0.1 IU/ml)
S. pneumoniae	17.96	(>1.4 mg/l)	Normal	488	(>400 UA/ml)		2.74	(>0.8mg/dl)	3.85	(8.7 mg/l)
Allohemagglutinin	1/4						1/16		1/16	

Proliferative responses to OKT3 (50 ng/ml) ("CD3"), the mitogen PHA and various antigens (PPD, candidin, tetanus), serum immunoglobulin levels and titers for specific antibodies. Age-specific normal values are shown in brackets. Ig: immunoglobulin.

Supplementary Table 11. Humoral responses to viruses and *Toxoplasma gondii* in MyD88-deficient patients

Patients (age) **Serological test results**	**P1 (11 mo)**	**P2 (2 y)**	**P7 (3 y)**	**P9 (3 y)**	**P4 (8 y)**	**P3 (15 y)**
Herpes Simplex Virus 1	negative	negative	not done	not done	positive	positive
Herpes Simplex Virus 2	not done	not done	not done	not done	positive	negative
Varicella Zoster Virus	not done	negative	not done	not done	positive	positive
Cytomegalovirus	negative	IgG positive	not done	not done	IgG positive	IgG positive
Epstein Barr Virus	negative	negative	not done	IgG anti VCA positive	IgG anti VCA positive IgG anti EBNA positive	IgG anti VCA positive IgG anti EBNA positive
Rubella	negative	IgG positive	IgG positive	not done	IgG positive	IgG positive
Measles	negative	IgG positive	IgG positive	not done	negative	IgG positive
Mumps	negative	not done	IgG positive	not done	negative	IgG positive
Rotaviruses	positive	not done	not done	not done	not done	not done
Adenoviruses	positive	not done	not done	not done	not done	not done
Toxoplasma	negative	not done	not done	not done	positive	negative

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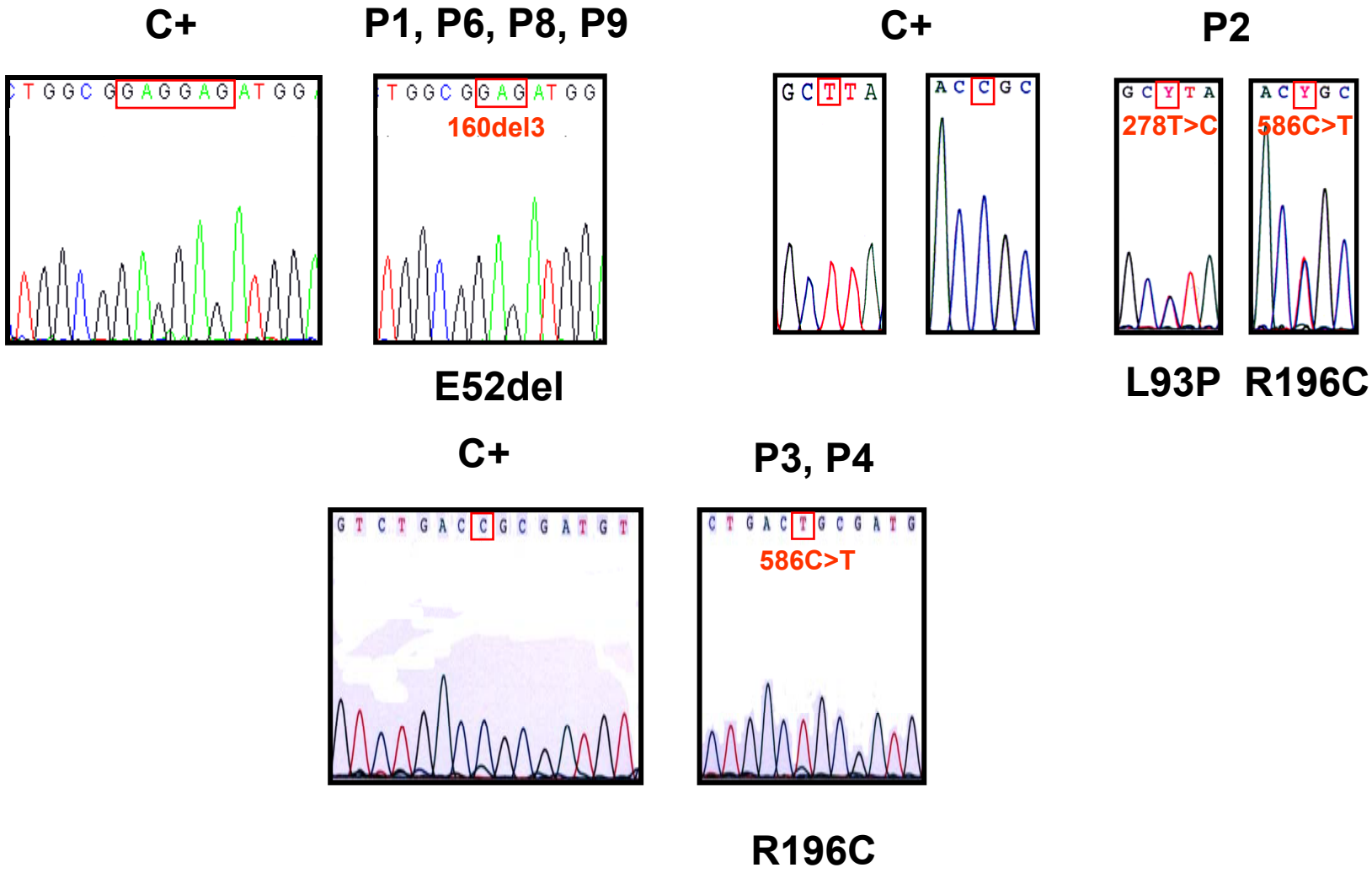
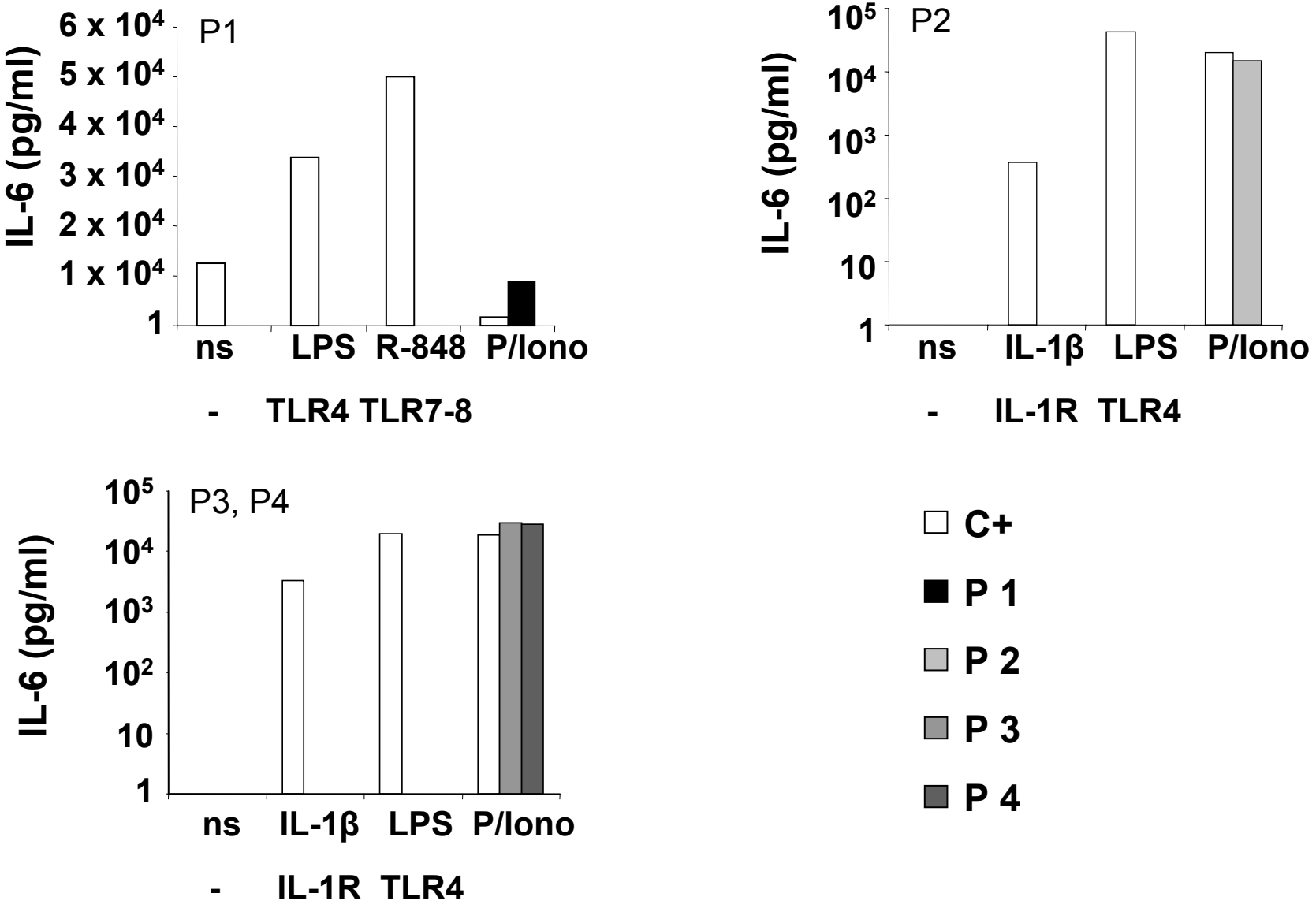
Supplementary Figure 1



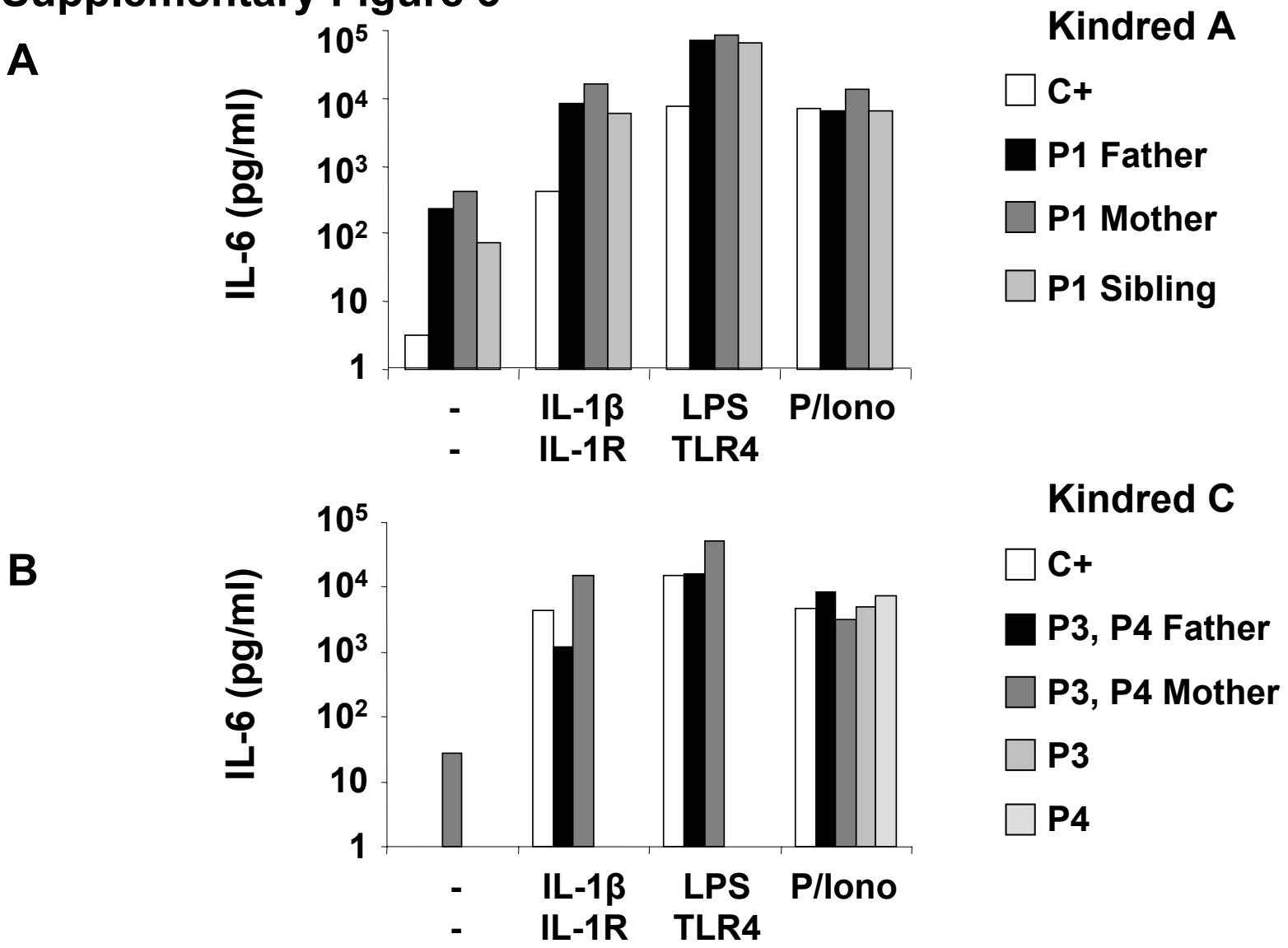
Supplementary Figure 1. Electrophoregrams showing the various mutations identified in exon 1 of *MYD88* for P1, P6, P8 and P9 (E52del), in exons 1 and 3 for P2 (L93P and R196C), and in exon 3 for P3 and P4 (R196C), compared to a healthy control (C+).

Supplementary Figure 2



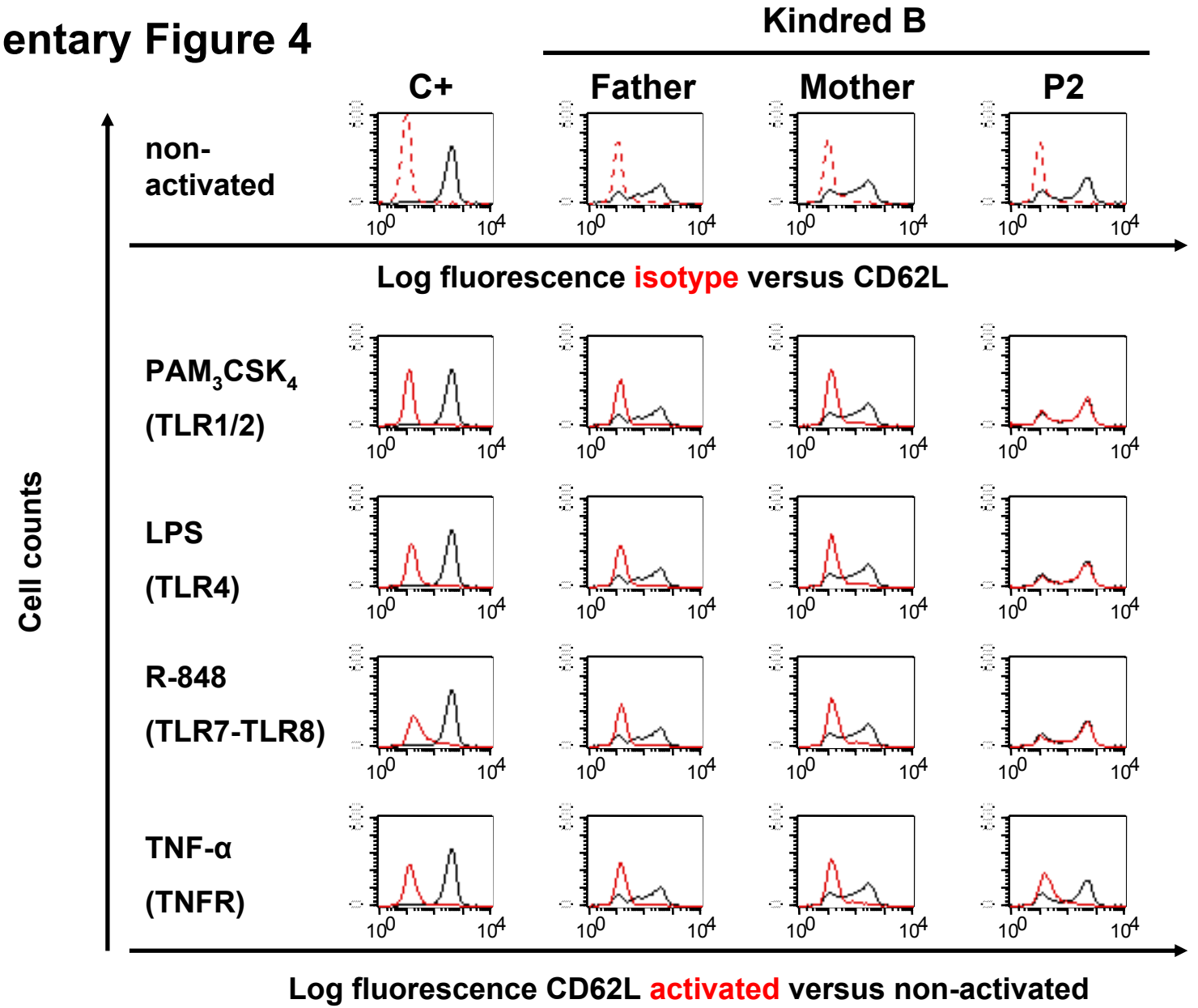
Supplementary Figure 2. IL-6 production by PBMCs (P1) and whole blood (P2, P3 and P4) upon activation. Healthy controls (C+) are shown in white, patients (P1 - P4) are shown in various shades of gray.

Supplementary Figure 3



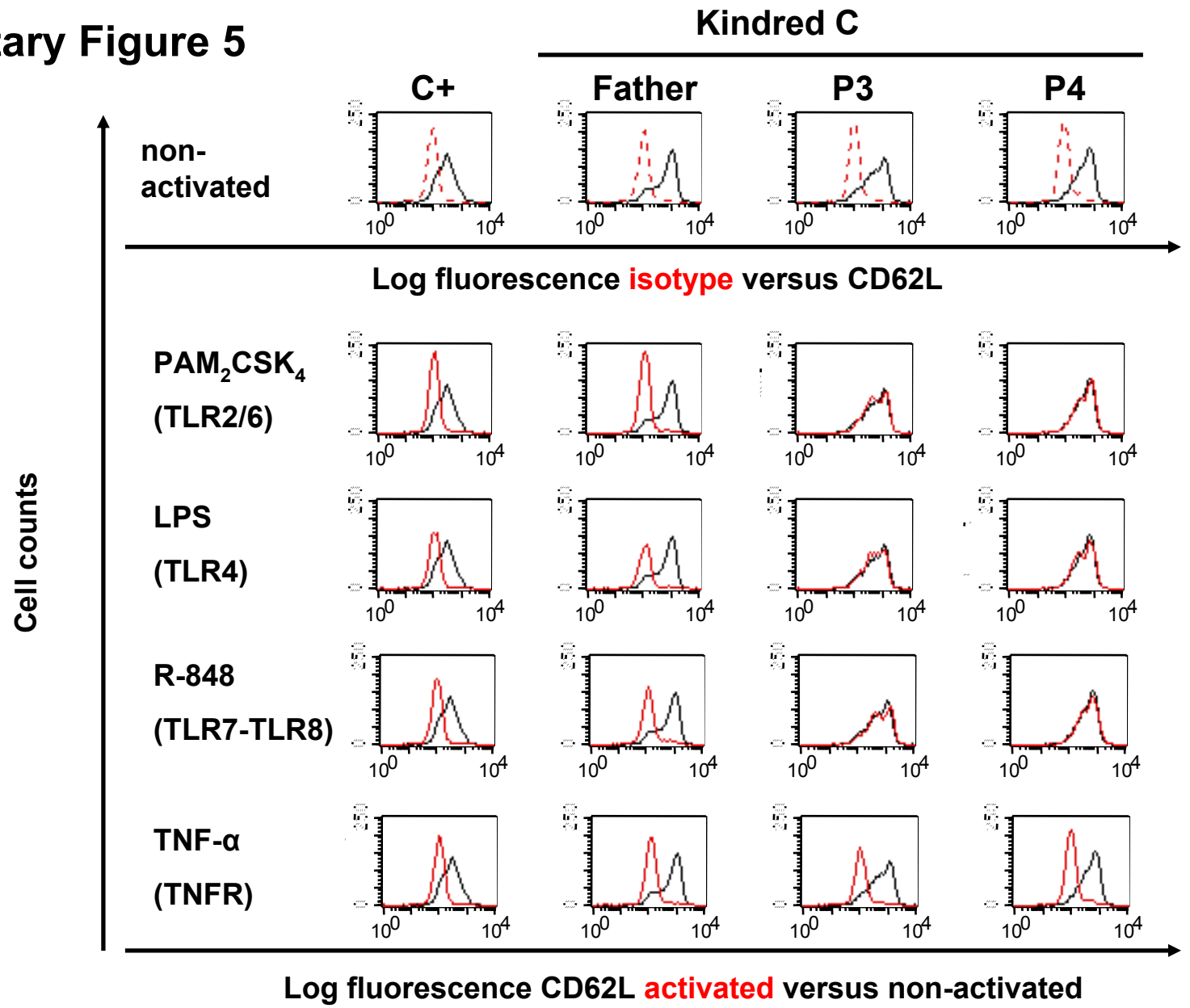
Supplementary Figure 3. Whole blood IL-6 production (A) for all members of kindred A (Father, Mother and Sibling of P1). **(B)** for the parents of kindred C (Father and Mother of P3 and P4, P3 and P4) upon activation. IL-6 production by cells from healthy controls is shown in white, and that by cells of members of affected families is shown in black and various shades of gray.

Supplementary Figure 4



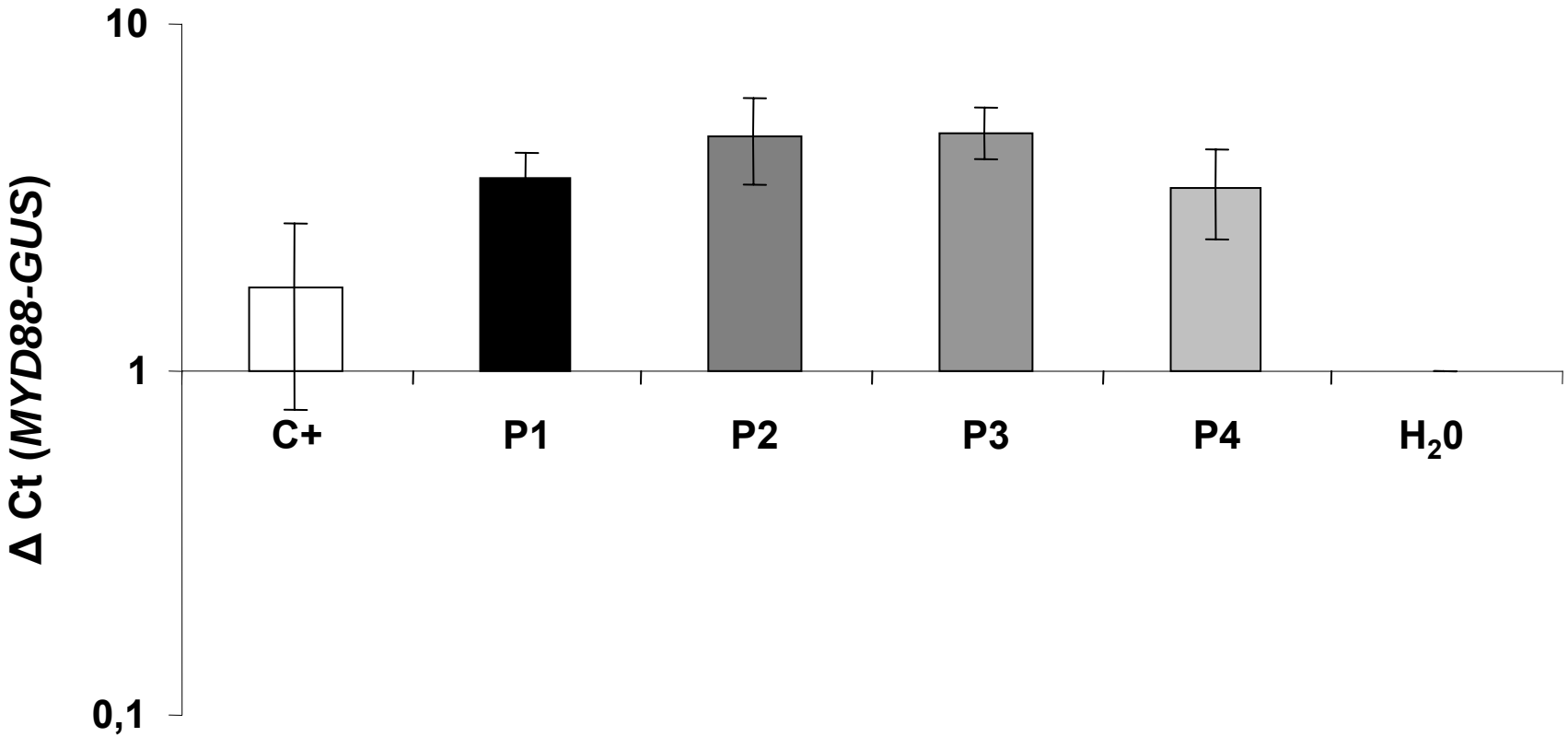
Supplementary Figure 4. Cleavage of CD62 ligand (CD62L) at the surface of granulocytes from a healthy control (C+), and all members of kindred B (Father, Mother, and P2). The red line shows CD62L expression after one hour of activation with various TLR agonists (induced CD62L shedding). The red dotted line shows the signal for the corresponding isotype control.

Supplementary Figure 5



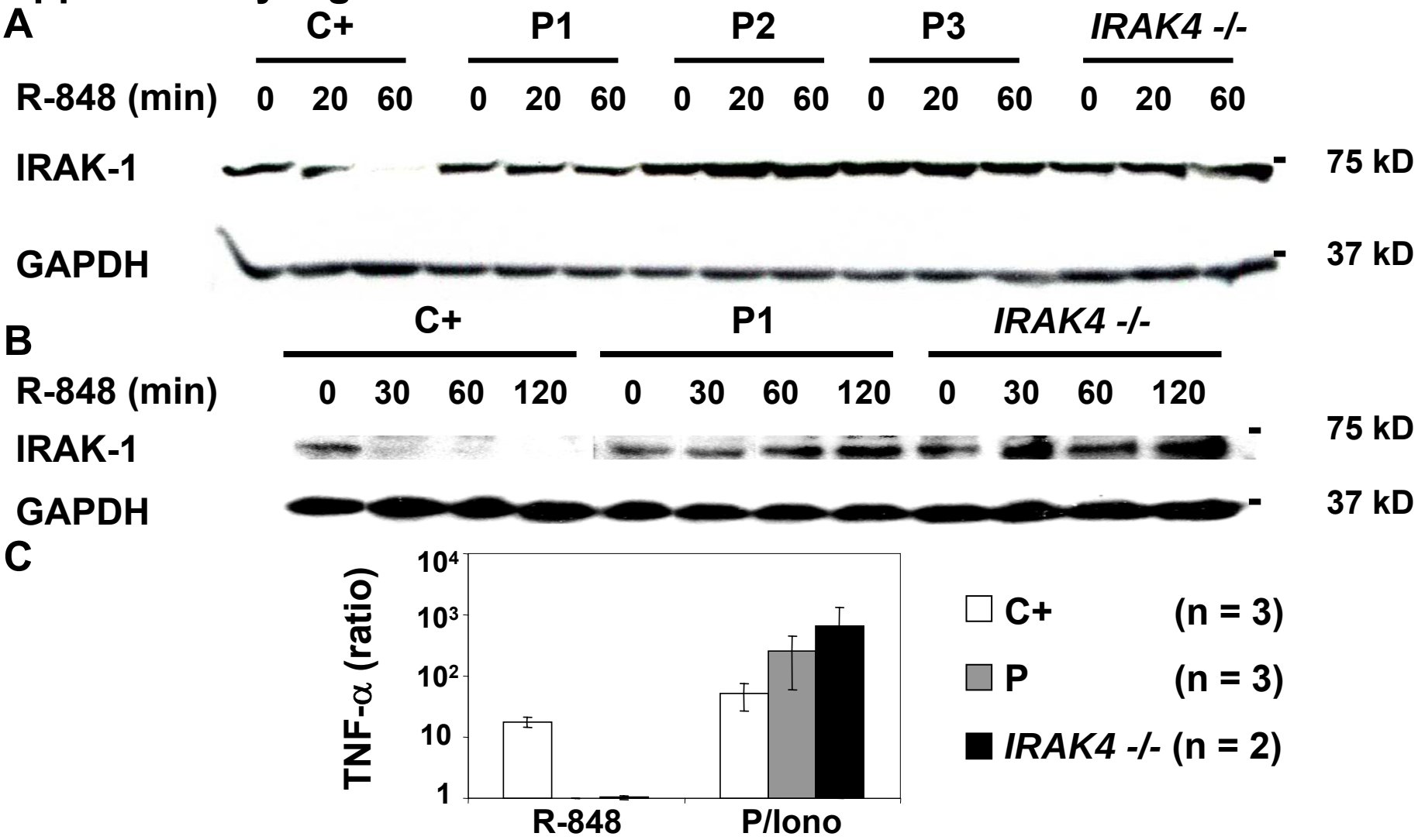
Supplementary Figure 5. Cleavage of CD62 ligand (CD62L) at the surface of granulocytes from a healthy control (C+), the father and both patients of kindred C (P3 and P4). The red line shows CD62L expression after one hour of activation with various TLR agonists (induced CD62L shedding). The red dotted line shows the signal of the corresponding isotype control.

Supplementary Figure 6



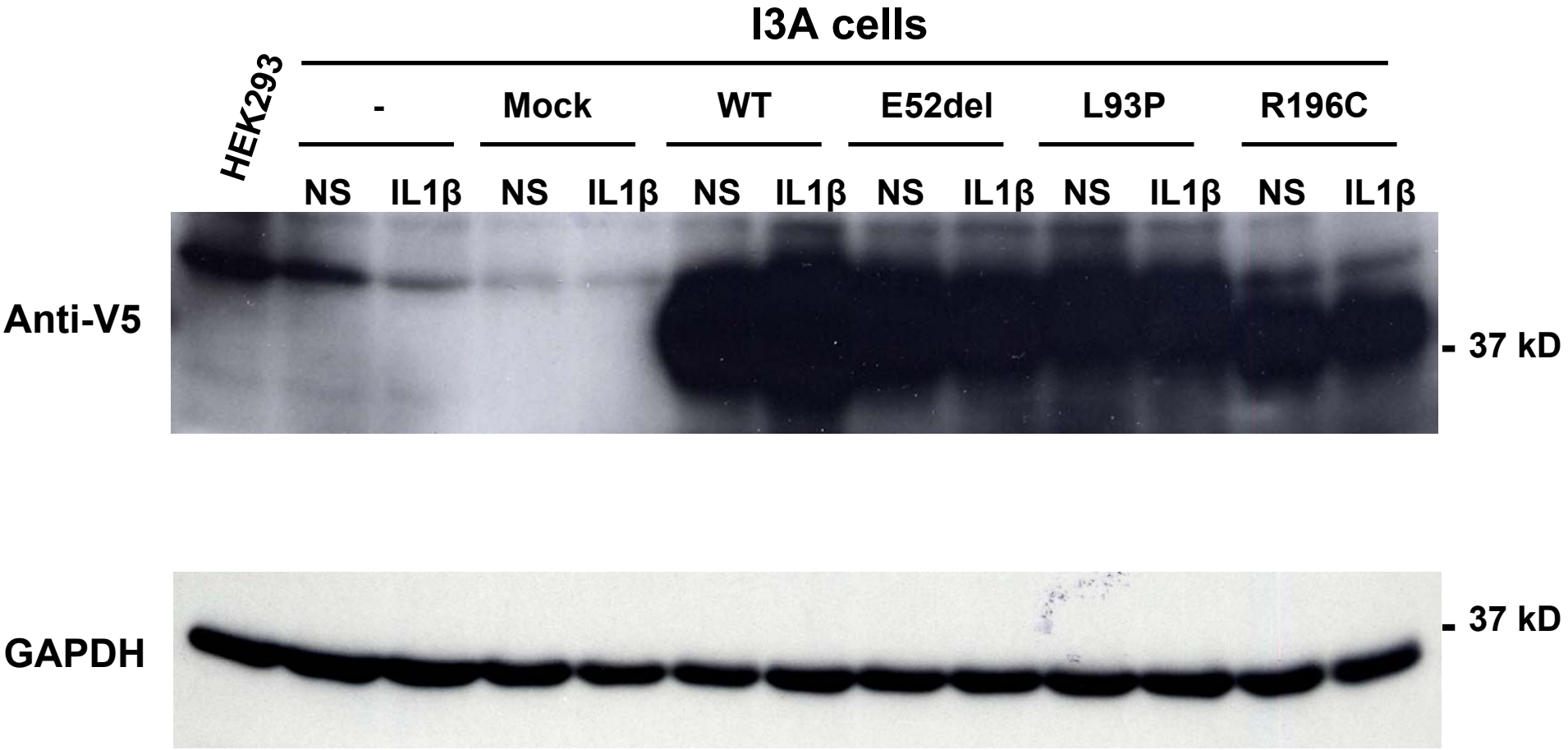
Supplementary Figure 6. Quantitative RT-PCR for *MYD88* transcripts in SV40-fibroblasts from controls and patients (P1 –P4). *MYD88* transcript levels were quantified as the difference between mRNA levels for *MYD88* and *GUS*, a housekeeping gene for the SV40-transformed fibroblast cell lines. The figure shows the results of two independent experiments.

Supplementary Figure 7



Supplementary Figure 7. (A) IRAK-1 degradation in EBV-transformed B cells from a healthy control, three MyD88-deficient patients, representing the three mutated genotypes identified — *MYD88E52del* (P1), L93P/R196C (P2), R196C (P3) — and an IRAK-4-deficient patient (*IRAK4-/-*) upon stimulation with R-848**. (B) IRAK-1 degradation** in EBV-transformed B cells from a healthy control (C+), MyD88-deficient P1 and an IRAK-4-deficient patient (*IRAK4-/-*) upon stimulation with R-848 (TLR-7/8) over a time course from 30 min to 120 min. **(C) TNF-α production** in EBV-transformed B cells. Healthy controls (C+) are shown in white, MyD88-deficient patients (P1 to P3) are shown in gray, and IRAK-4-deficient patients (*IRAK4-/-*) are shown in black.

Supplementary Figure 8



Supplementary Figure 8. Western blotting using anti-V5 specific antibody to verify the transfection of I3A-cells, in non activated (-) and IL-1β-activated cells (+).