ONLINE REPOSITORY

MATERIALS AND METHODS

Human subjects

All studies were completed under protocol 11-AR-0223, which was approved by the National Institute of Arthritis and Musculoskeletal and Skin Diseases/National Institute of Diabetes and Digestive and Kidney Diseases Institutional Review Board. Written informed consent and assent was obtained for conduct of research, use of photographic images, and publication of findings.

Genetic analysis

Whole human exome sequencing was performed (Otogenetics Corporation) on patient, parents, and unaffected sister peripheral leukocyte DNA using Agilent 51Mb Human Exome V5 (Agilent Technologies) capture and PE100-125 Illumina HiSeq2500 (Illumina) sequencing with a 50x average read coverage. A computational pipeline was developed to process the read data and perform tasks such as quality control (QC), variant discovery, annotation, and filtering. Briefly, sequence reads were aligned to human reference genome (GRC Build 37) with Burrows-Wheeler Aligner (BAM). BAM files were then processed to remove duplicate reads, refine alignment indels and

recalibrate base quality scores, according to the best practice guideline by the Genome Analysis Toolkit (GATK) from the Broad Institute. UnifiedGenotyper from GATK was used to make joint variant calls across multiple samples, followed by a variant quality score recalibration step by the GATK VQSR tool. Variants were annotated with functional impact and allele frequency in public databases and local datasets. Sex and sample kinship were analyzed to identify potential sample errors and false family relationships (i.e. erroneous maternity or paternity) using KING software. Possible disease-causing mutations were selected and prioritized based on quality score, allele frequency, functional impact, inheritance, and review of literature. While no autosomal recessive mutations were detected, *de novo* mutations in *MYD88*, *GFPT2*, and *WFIKKN2* were identified. Considering the critical role of *MYD88* in the immune response and its relevance to arthritis in animal models[E6-E8] and rheumatoid arthritis,[E10] further efforts were focused on *MYD88*. Presence (or absence) of *MYD88* S222R mutation was confirmed by Sanger sequencing (ACGT, Inc.) peripheral leukocyte DNA from all family members, as well as from isolated CD14+ monocyte, EBV-LCL, and cultured dermal fibroblast DNA from patient and mother.

Cell Culture

Human primary dermal fibroblasts were isolated from outgrowth of Dispase (StemCell Technologies) digested skin biopsies and maintained in DMEM supplemented with 10% FBS and 100 U/mL Penicillin/Streptomycin (P/S, Gibco). EBV-LCLs were generated from fresh whole blood and maintained in RPMI 1640 supplemented with 20%

FBS and 100 U/mL P/S. THP-1 cells were maintained in RPMI 1640 medium supplemented 10% FBS and 100 U/mL P/S. Both U2932 and SUDHL2 cell lines were maintained in RPMI 1640 RPMI 1640 supplemented with 20% Hyclone FBS (GE Healthcare) and 100 U/mL P/S. All cells listed grown at 37° C with 5% CO_2.

Flow cytometry

Peripheral blood from patient and controls were collected in sodium heparin BD Vacutainer tubes and used within 1 hour of collection. For immunophenotyping, red blood cells were lysed with BD Pharm Lyse lysing buffer and washed with PBS. Remaining cells were fixed with 4% paraformaldehyde, then incubated with specific antibodies for 1 hour at 4 °C, and then washed. After exclusion of dead cells using LIVE/DEAD Fixable Red Dead Cell Stain Kit (ThermoFisher Scientific), leukocytes were identified using the following antibodies (BD Biosciences unless otherwise specified): anti-CD3 (SK7), anti-CD4 (SK3), anti-CD8 (SK1), anti-CCR6 (11A9), anti-CD19 (SJ25C1), anti-CD20 (2H7), anti-CD56 (B159), anti-HLA-DR (G46-6), anti-CD16 (B73.1), anti-CD123 (7G3), anti-CD11c (B-ly6), anti-CD14 (MγP9), anti-CD1c (L161; Biolegend), anti-CD303 (201A; Biolegend), anti-CD203c (NP4D6; Biolegend), and anti-CD141 (AD5-14H12; Miltenyi). For phosphorylated-STAT3 analysis, peripheral blood was stained with cell subset-specific antibodies and stimulated with IL-6 50 ng/mL (PeproTech) for 20 minutes at 37°. Red blood cells were then lysed and remaining cells fixed for 10 minutes with BD Phosflow Lyse/Fix Buffer, then permeabilized with BD Phosflow Perm Buffer III. After washes, cells were stained with anti-pSTAT3 (4/P-

STAT3) overnight at 4° C. Cell surface markers and p-STAT3 intracellular staining were measured via BD LSR-Fortessa flow cytometer and analyzed with FlowJo software v10.1r5.

Whole blood and dermal fibroblast cytokine/chemokine secretion assays

For whole blood cytokine secretion, peripheral blood from patient and controls were collected in sodium heparin BD Vacutainer tubes, diluted 1:2 with RPMI 1640 medium (Gibco), and incubated for 22 hours at 37° C within one hour of blood collection. Supernatants were then collected and stored at -80° C. To assess dermal fibroblast cytokine secretion, $1x10^5$ cells/well of patient and mother fibroblasts were seeded into 12-well plates. After resting overnight, cells were washed with PBS and media replaced. Supernatants were then collected 24 hours later and stored at -80° C. Concentrations of supernatant cytokines and chemokines from whole blood and fibroblasts were subsequently measured via multiplex immunoassay using ProcartaPlex Human Th1/Th2 & Chemokine Panel 1 20-plex (Affymetrix eBioscience) per manufacturer's instructions.

Neutrophil chemotaxis assay

Neutrophil chemotaxis was measured using CytoSelect 96-Well Cell Migration Assay 3 μm (Cell Biolabs, Inc.) per manufacturer's instructions. Briefly, patient and mother fibroblast conditioned supernatants were generated by seeding $2.5x10^5$ cells/well

into 6 well plates and allowing them to rest at 37° C overnight. The next day, media was removed and cells washed with PBS. 1 mL of FBS-free DMEM was then placed into wells and incubated at 37° for 24 hours. Resultant supernatants were then collected and stored at -80° C. On day of assay, thawed supernatants were placed into feeder wells in triplicate. Freshly elutriated healthy donor neutrophils were placed into upper chambers at density of $5x10^5$/well after propidium iodide exclusion viability testing. After 4 hours, migratory cells in feeder wells were lysed, CyQuant GR Fluorescent Dye added, and quantified using a fluorescence plate reader at 480nm/520nm.

Molecular dynamics studies

Four replicate 1.5 microsecond molecular dynamic calculations of the wild type MyD88 TIR domain (PDB code: 4DOM, Ref. 79) or S222R mutant was performed using Gromacs with the Charmm36 forcefield, explicit solvent and a 1fs time step. Using Visual Molecular Dynamics (VMD), 500 snapshots of the last 500 ns of each simulation were superposed based on the backbone atoms of the central beta sheet, and these superposed structures were clustered in 5 groups based only on the position of the alpha C-helix (residues 243-255) backbone atoms using an rmsd distance function with 1.5 Å cutoff. For cluster analysis of the alpha C helix, all four replicates were analyzed separately. In these analyses, the most populated clusters contain >60% of all clustered structures.

THP-1 Cell lines, retroviral transduction, and NF-κB reporter system

THP1-Dual KO-MyD Cells (Invivogen), where MyD88 gene expression is knocked-out via nuclease technology, was retroviral transduced sequentially with N-terminal MyD88-AU1, then C-terminal MyD88-GFP constructs.[4] Vectors were packaged into vesicular stomatitis virus envelope (VSV-G) containing retroviral particles (Alstem) and THP-1 cells transduced overnight at MOI=5. Successfully transduced cells were isolated via Ly-2 magnetic bead selection (Miltenyi) and purity of both MyD88-AU1 and MyD88-GFP cells were verified via flow cytometry (Ly-2) and immunoblot for MyD88. THP-1 clones produced for this study were MyD88 KO clones re-expressing equal amounts of WT MyD88 with two different tags (WT-MyD88-AU1 and WT-MyD88-GFP), WT MyD88 and S222R MyD88 with AU1 and GFP tags, respectively (WT-MyD88-AU1 and S222R-MyD88-GFP), WT MyD88 and S222R MyD88 with the alternative tags (S222R-MyD88-AU1 and WT-MyD88-GFP), and S222R MyD88 with two different tags (S222R-MyD88-AU1 and S222R-MyD88-GFP). THP1-Dual KO-MyD Cells used contain stable integration of secreted embryonic alkaline phosphatase (SEAP) reporter gene containing IFN-β minimal promoter fused to five copies of the NF-κB consensus response element and three copies of the c-Rel binding site. Baseline (unstimulated) SEAP over a 24-hour period was detected via enzyme-substrate driven colorimetry per manufacturer's instructions (Invivogen).

Proximity ligation assay (PLA)

THP-1 cells were attached to coverslips with phorbol 12-myristate 13-acetate (PMA, 10 ng/mL) for 24 hours and then allowed to rest in fresh complete RPMI media for at least 72 hours. PLA was performed on THP-1 double transduced clones (above)

using Duolink In Situ Orange Mouse/Rabbit kit (Sigma) per manufacturer's instructions. Briefly, cells were washed with HBSS, fixed with 4% paraformaldehyde (Electron Microscopy Sciences) in PBS for 15 minutes, and subsequently permeabilized/washed with 0.04% saponin (Calbiochem). After 1 hour blocking in 1% BSA (Sigma), 2% horse serum (Abcam), 3% donkey goat serum (Abcam), 0.04% saponin, and 0.01% sodium azide (Sigma), cells were incubated for 16 hours at 4° C with rabbit anti-GFP antibody (Abcam, ab290) and mouse anti-AU1 (Biolegend) both at 1:1000 in blocking solution. Next, cells were washed with 0.04% saponin in PBS and incubated with anti-rabbit PLUS and anti-mouse MINUS for 1 hour. Then, cells were subject to ligation for 30 minutes and then amplification for 100 minutes. Cells were mounted with the kit's DAPI-containing medium. Per cell clone, confocal images were taken from 4 randomly selected fields using 63x objective, then maximum projection images were produced from 14 z-slices. Identical imaging settings were used for all images. PLA events were quantitated using CellProfiler v2.2 Spot Detection pipeline (https://github.com/tischi/cellprofiler-practical-NeuBIAS-Lisbon-2017/blob/master/practical-handout.md) from maximum projection images collected. For each experiment of each clone, at least 65 cells were counted in each of 4 field-of-views, or at least 260 cells for all 4 fields-of-view total.

Cell lysis, antibodies, and immunoblotting

Cells were lysed in buffer containing 20 mM Tris-Cl pH 7.5, 100 mM NaCl, 10 mM EDTA, 1% triton x-100, protease inhibitor cocktail (Roche), 0.04% NaN_3, 0.5 mM PMSF, 1 mM Na_3VO_4, 10 mM NaF, and 10 mM sodium pyrophosphate dibasic. After Bradford protein concentration measurement and normalization of protein amounts,

samples were added to SDS loading buffer and heated to 95° C for 3 minutes. After PAGE (4-20% Criterion TGX, Bio-Rad), proteins were transferred to PVDF membranes (Bio-Rad), blocked with 5% BSA, and incubated overnight with primary antibodies at 4° C. Horseradish peroxidase (HRP) conjugated anti-rabbit IgG or anti-mouse IgG (R & D Systems) secondary antibodies were used with Pierce ECL or West Pico (Thermo Scientific) to detect protein expression and visualized with Amersham Hyperfilm (GE) or Bio-Rad ChemiDoc Imaging System. Primary antibodies used: mouse anti-vinculin (V284, Millipore), mouse anti-GAPDH (Santa Cruz Biotech, clone 6C5, sc-32233), mouse anti-beta actin (Abcam, ab8226), rabbit, anti-beta tubulin (Abcam, ab6046), rabbit anti-MYD88 (D80F5), rabbit anti-A20/TNFAIP3 (D13H3), rabbit anti-TIRAP (Abcam, ab17218), and rabbit anti-NF-κB p65 (D14E12) (all from Cell Signaling Technology unless otherwise noted). Quantification of immunoblots are densitometric values performed using ImageJ.

SUDHL2, U2932, and EBV B lymphoblastoid (EBV-LCL) p-STAT3 studies

SUDHL2, U2932, and EBV-LCLs used in this study were maintained at cell density of 0.8-1x10^6 cells/mL. Media changes for EBV B lymphoblastoids was performed by removing 50% of media and replacing it with an equal amount of fresh media every 3 days. Supernatant testing for cytokines in all cases was performed immediately before media changes. IRAK4 inhibition of EBV-LCLs was accomplished using 200 nM IRAK4 inhibitor AS2444697 (Sigma) for 16 hours, which had no effect on viability as determined by propidium iodide exclusion.

For conditioned-media P-STAT3-induction experiments, U2932 were pre-treated with their own growth media containing either nothing, human serum IgG (Sigma) 200 ng/mL, or tocilizumab 200 ng/mL (TCZ; Actemra, Genentech) for 2 hours. Next, cells were centrifuged and resuspended in their own warmed media (U2932-conditoned) or SUDHL2-conditioned media also containing nothing, human IgG, or TCZ. After 2 hours, cells were lysed for protein and probed for loading control GAPDH, STAT3 (Cell Signaling Technology, clone 124H6, #9139), and P-STAT3 (Y705) (Cell Signaling Technology, clone D3A7, #9145). Similarly, unrelated healthy control EBV-LCLs were pre-treated with their own growth media containing either nothing, human serum IgG 200 ng/mL, or TCZ 200 ng/mL for 2 hours. Next, cells were centrifuged and resuspended in either its own warmed media, mother's EBV-LCL conditioned media, or patient's EBV-LCL conditioned media also containing nothing, human IgG 200 ng/mL, or TCZ 200 ng/mL. After 2 hours, cells were lysed for protein and probed for loading control GAPDH, STAT3, and P-STAT3. For all STAT3 immunoblots, quantification of p-STAT3/STAT3 was calculated by dividing GAPDH-normalized p-STAT3 from one gel by GAPDH-normalized STAT3 from another gel. Protein levels were determined by densitometry using ImageJ.

RNA isolation, cDNA synthesis, and gene expression analysis

In all cases, total RNA was isolated via phenol-chloroform extraction (Life Technologies), and purity and quantity was assessed by NanoDrop 8000 Spectrophotometer (ThermoFisher Scientific). cDNA was produced via reverse transcription of 1000 ng of RNA using iScript cDNA Synthesis Kit (Bio-Rad). qPCR was

performed on QuantStudio 6 Plex (Applied Biosystems) using 40 ng cDNA template, oligonucleotide primers, and SsoFast EvaGreen Supermix (Bio-Rad). For qPCR, gene expression relative to the housekeeping genes *PPIA* and *HPRT1* was calculated using comparative Ct method. Oligonucleotide primer sequences are available upon request. NanoString gene expression analysis was performed on total RNA from isolated peripheral monocytes via both CD14 positive and negative selection, and cultured dermal fibroblasts, using nCounter Human Inflammation v2 pre-built CodeSet (NanoString Technologies) per manufacturer's instructions. Briefly, 200 ng total RNA was hybridized with Capture and Reporter probes in thermocycler at 65° C for 24 hours. Samples were then loaded onto nCounter Cartridge using nCounter Prep Station and RNA counted by the Digital Analyzer. After quality control checks, normalized RNA counts were generated by negative control subtraction and geometric mean of housekeeping genes *TUBB*, *PGK1*, *GUSB*, *HPRT1*, *CLTC*, and *GAPDH* using nSolver Analysis Software v2.5. Genes were considered not expressed if normalized RNA counts were less than twice the standard deviation of the negative control counts in both patient and controls.

siRNA-mediated protein knockdown

Protein knockdown was performed using Amaxa Nucleofector II with Human Dermal Fibroblast Nucleofector Kit (Lonza) per manufacturer's protocol. Briefly, after cell dissociation using Accutase (Life Technologies), 6-7x10^5 patient or mother dermal fibroblasts were resuspended in 100 μL Human Dermal Fibroblast Nucleofector Solution, siRNA added, nucleofected using program U-023, and then divided into 3 wells of a 6-

well plate containing pre-warmed DMEM supplemented with 10% FBS. After 80 hours, cells were lysed for protein and RNA analysis. siRNA (Ambion) and final concentrations used are as follows: *MYD88* (s9138), 30 nM; *TNFAIP3* (s14260), 1000 nM; *RELA* (s11914), 1000 nM; *TIRAP* (s195607), 1000 nM; and Negative Control (4390843), 1000 nM. Percent knockdown compared to negative control siRNA was calculated by densitometry of patient fibroblast protein expression using ImageJ (NIH).

Statistical analysis

GraphPad Prism 6 software was used for all statistical calculations. Statistical significance was determined using unpaired Student's t test. Differences with p-values < 0.05 (*), < 0.01 (**), <0.001 (***), and <0.0001 (****) were considered significant.

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TABLE E1. Summary of patient clinical laboratory values and peripheral leukocyte immunophenotyping. Subject laboratory values over span of 3 years given as a range. Reference range defined as range of normal values according to National Institutes of Health Clinical Center Department of Laboratory Medicine. Immunophenotyping data presented as mean (range of 3 values over span of 27 months).

FIG E1. Genotype of pedigree and patient CD14+ monocytes, dermal fibroblasts, and EBV-LCLs. **A**, Sanger sequencing chromatograms of peripheral leukocyte DNA. P, patient; M, mother; F, father; S, sister. **B**, Sanger sequencing chromatograms demonstrating the presence of c.666T>G mutation in patient CD14+ monocytes, dermal fibroblasts, and EBV B lymphoblastoids.

FIG E2. Immunophenotyping of peripheral blood dendritic cells. CD1c (BDCA-1), CD303 (BDCA-2), CD141 (BDCA-3), and CD203c surface expression for CD123+CD11c- (Q1), CD123+CD11c+ (Q2), and CD123-CD11c+ (Q3) dendritic cells. Positive control CD203c+ cells were gated from HLA-DR+ myeloid fraction of peripheral leukocytes.

FIG E3. Molecular dynamics modeling and effect of S222R on MyD88 self-association. **A**, Molecular dynamics cluster analysis of wild type (left) and S222R (right) MyD88 TIR domains. Images show superposition of the representative structure for the most populated cluster for each of 4 independent replicate simulations. **B**, Representative

immunoblot showing relative expression of MyD88-AU1 and MyD88-GFP in THP-1 transductants, with vinculin as a loading control. **C**, NF-κB activity measured by secreted alkaline phosphatase activity as reporter gene. Activity is relative to cells containing WT-MyD88-AU1 & WT-MyD88-GFP. Each data point represents the average of the median of 3 replicates from 3 independent experiments. **D**, Representative confocal images of proximity ligation assay (PLA). Image 1, MyD88-KO; 2, WT-MyD88-AU1 and WT-MyD88-GFP; 3, WT-MyD88-AU1 and S222R-MyD88-GFP; 4, S222R-MyD88-AU1 and WT-MyD88-GFP; and 5, S222R-MyD88-AU1 and S222R-MyD88-GFP. Blue, DAPI and red, PLA event using anti-AU1 and anti-GFP antibodies. **E**, Average PLA events per cell from 3 independent experiments**.** Error bars represent ± SD. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.

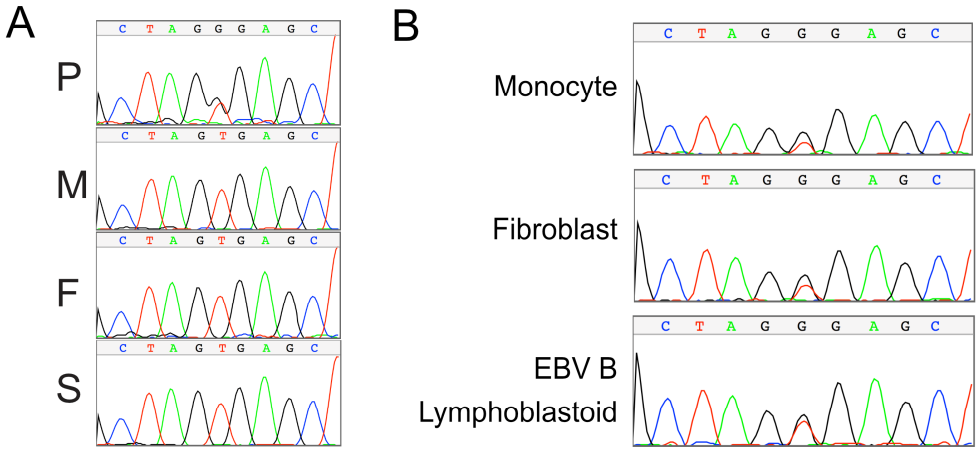
FIG E4. Dermal fibroblast neutrophil-attracting chemokine secretion and function. **A**, unstimulated patient and control fibroblast secretion of CXCL1 and IL-8 over 24 hours. **B**, unrelated health donor neutrophil chemotaxis over 4 hours in response to patient and mother fibroblast-conditioned media. P, patient; M, mother. RLU, relative light units. For **A** and **B**, each data point represents the median of 3 replicates, with results from 3 independent experiments (n=3) shown. Error bars represent ± SD. *, $p<0.05$; ****, $p<0.0001$.

FIG E5. SUDHL2 and patient EBV-LCL IL-6 and IL-8 expression, and STAT3 phosphorylation. **A**, Chemokine/cytokine gene and protein expression from U2932 and SUDHL2 cell lines. **B**, Chemokine/cytokine protein and gene expression from EBV-

LCLs, without (Control) and with AS2444697 (200 nM for 16 hours) as indicated. P, Patient; M, mother; HC, unrelated healthy control. **C**, Levels of P-STAT3 and total STAT3 in SUDHL2 and U2932 cells. Representative immunoblot (left) with quantification (right), from 4 independent experiments. Due to marked overexpression of total STAT3 in SUDHL2 cells, blot was run using 1:10 dilution of SUDHL2 protein lysate. **D**, Levels of P-STAT3 and total STAT3 in EBV-LCLs from patient (P) and mother (M). **E**, STAT3 phosphorylation in U2932 cells in response to 2 hour incubation with conditioned media from SUDHL2 or U2932 cells. Conditioned media was used alone, or after human IgG (IgG) or tocilizumab (TCZ; 200 ng/mL) pre-treatment. Relative STAT3 phosphorylation (P-STAT3) was determined as for **D**. **F**, STAT3 phosphorylation in EBV-LCLs from healthy control (HC) in response to conditioned media from HC, mother (M), or patient (P). Conditioned media was used alone or after IgG or TCZ pre-treatment, and relative STAT3 phosphorylation determined as described in **E**. For **D**, **E**, and **F**, GAPDH used as a loading control. Error bars represent ± SD. *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$.

TABLE E1. Summary of subject's laboratory values and peripheral leukocyte immunophenotyping

Laboratory Test	Subject's Value Range	Reference Range
Total Leukocyte Count	6.05-7.25	3.98-10.04 $x10^3$/uL
Neutrophil %	53.8-57.3	34-71.1%
Lymphocyte %	30.5-36.3	19.3-51.7%
Monocyte %	7.8-10.2	4.7-12.5%
Eosinophils	1.0-2.9	0.7-5.8%
Basophils	0.1- 0.5	0.1-1.2%
Erythrocyte Sedimentation Rate (ESR)	5-13	0-42 mm/hr
C-reactive Protein (CRP)	1.10-7.00	0-4.99 mg/L
Osteocalcin	113.8-201.6	7.3 – 38.5 ng/mL
Anti-Nuclear Antibodies (ANA)	Negative	Negative < 0.9 EU
Anti-ENA Screen (Anti-RNP, Anti-Sm, Anti-SS-A, Anti-SS-B, Anti-Jo-1, Anti-Scl-70)	Negative	Negative < 20 EU
Anti-Histone / Anti-dsDNA	Negative / Negative	Negative < 1 U / Negative < 30 U
Rheumatoid Factor / Anti-Cyclic Citrullinated Peptide	Negative / Negative	Negative <15 U / Negative <20 U
Serum C3/C4	128.4/33.5	90-180/10-40 mg/dL
Serum IgG/IgM/IgA	619-874/20-39/120-235	716-1711/15-188/47-249 mg/dL
IgG Subclasses, IgG1/IgG2/IgG3/IgG4	367/140/27/3	289-934/82-516/20-103.2/0.7-121.7 mg/dL
Isohemagglutinins, anti-A/anti-B	Positive/Positive	n/a
EBVCA-IgG/IgM/EBNA	Negative/Negative/Negative	Negative < 18 / <36 / <18
CMV Ab IgG/IgM	2.30/Negative	Positive > 0.70 U/mL
Anti-Rubella IgG, Random	29.4	Reactive > 10 IU/L
Mumps Ab IgG, Random	Negative	Negative < 0.8 AI
Anti-Rubeola IgG, Random	Negative	Negative < 25 AU/mL
Anti-Diphtheria Ab, Random	> 1.00	Positive > 0.01 IU/mL
Anti-Haemophilus Influenza Ab, Random	0.14 (Negative)	Positive > 0.15 mg/L
Anti-Tetanus IgG, Random	0.18 (Positive)	Positive > 0.01 IU/mL
Anti-HBs Antibody, Random	Negative	n/a
Anti-Varicella-Zoster Virus IgG, pre-re-vaccination	Negative	Negative < 135 Index
Anti-Varicella-Zoster Virus IgG, post-re-vaccination	> 4000	Positive > 165 Index
Positive anti-Pneumococcal Ab serotypes, Random	1, 22F	n/a
Negative anti-Pneumococcal Ab serotypes, Random	2, 3, 4, 5, 8, 9N, 12F, 14, 17F, 19F, 20, 22F, 23F, 6B, 10A, 11A, 7F, 15B, 18C, 19A, 9V, 33F	n/a
Peripheral leukocyte immunophenotyping		
CD3 (% lymphocytes)	73.5% (65.2-80.3)	72.9% (69.6-70.3)
CD4 (% CD3+)	51.6% (37.8-60.2)	57.1% (40-72.3)
CD8 (% CD3+)	41.4% (34.7-46.2)	35.9% (26.5-48.3)
CD19 (% lymphocytes)	11.2 (9.94-12.6)	11.7% (6.97-19.5)
CD20+CD19+IgD-CD27+ Memory B Cells (% CD19+ B Cells)	4.1% (2.41-5.15)	23.3% (22.7-23.8)
CD20+CD19+IgD+CD27- Naive B Cells (% CD19+ B Cells)	84.1% (79.5-87.2, 85.4)	56.8% (53.7-58.7)
CD19+CD20-CD27+CD38+ Plasmablasts (% CD19+CD20- B Cells)	2.34% (1.64-2.86)	40.7% (23.1-58.7)
CD19+CD24+CD38+ Transitional B Cells (% CD19+ B Cells)	5.21% (4.51-6.18)	4.05% (3.33-4.91)
CD14++CD16- Monocytes (% total monocytes)	81.1% (76.8-86.1)	75.7% (68.1-80.8)
CD14+CD16+ Monocytes (% total monocytes)	0.0022% (0.0038-0.057)	4.93% (2.61-8.66)
CD14-CD16+ Monocytes (% total monocytes)	0.0132% (0.0096-0.015)	6.19% (3.78-8.51)
CD123+CD11c- Plasmacytoid DCs (% total DCs)	12.7% (2.27-23.5)	37.9% (3.01-56.5)
CD123-CD11c+ Myeloid DCs (% total DCs)	43.6% (19.1-88.5)	45.3% (34.4-61.1)
CD123+CD11c+ DCs (% total DCs)	34.4% (8.69-54.3)	0.73% (0.62-0.88)
CD3+CD4+CCR6+ (Th17) (% CD4 Cells)	14.3% (9.19-19.7)	8.39% (4.66-12.7)
CD3+CD4+CD25+CD127- Treg (% CD4+ T Cells)	7.25% (6.26-8.15)	9.79% (8.49-11.1)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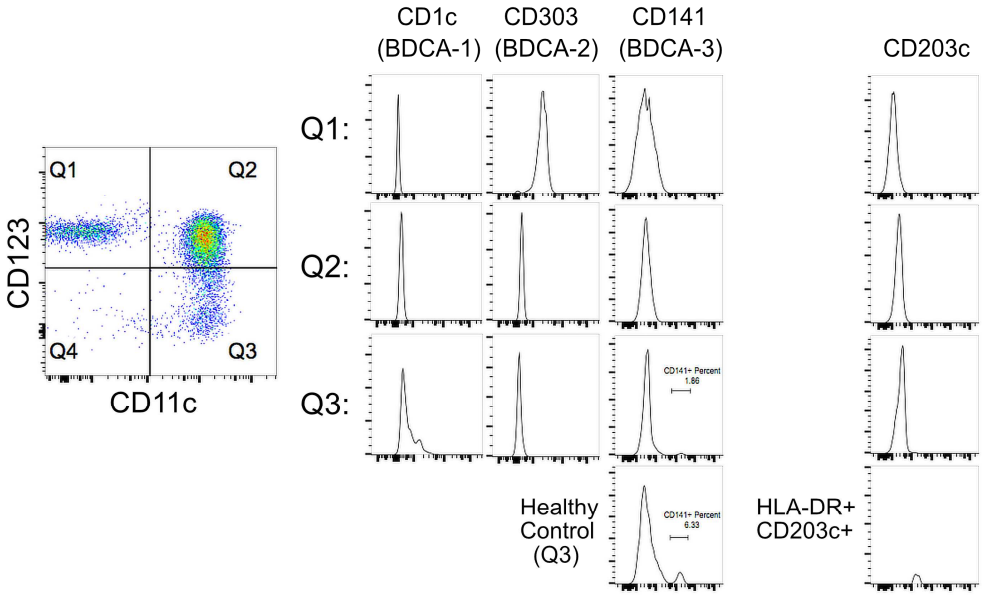
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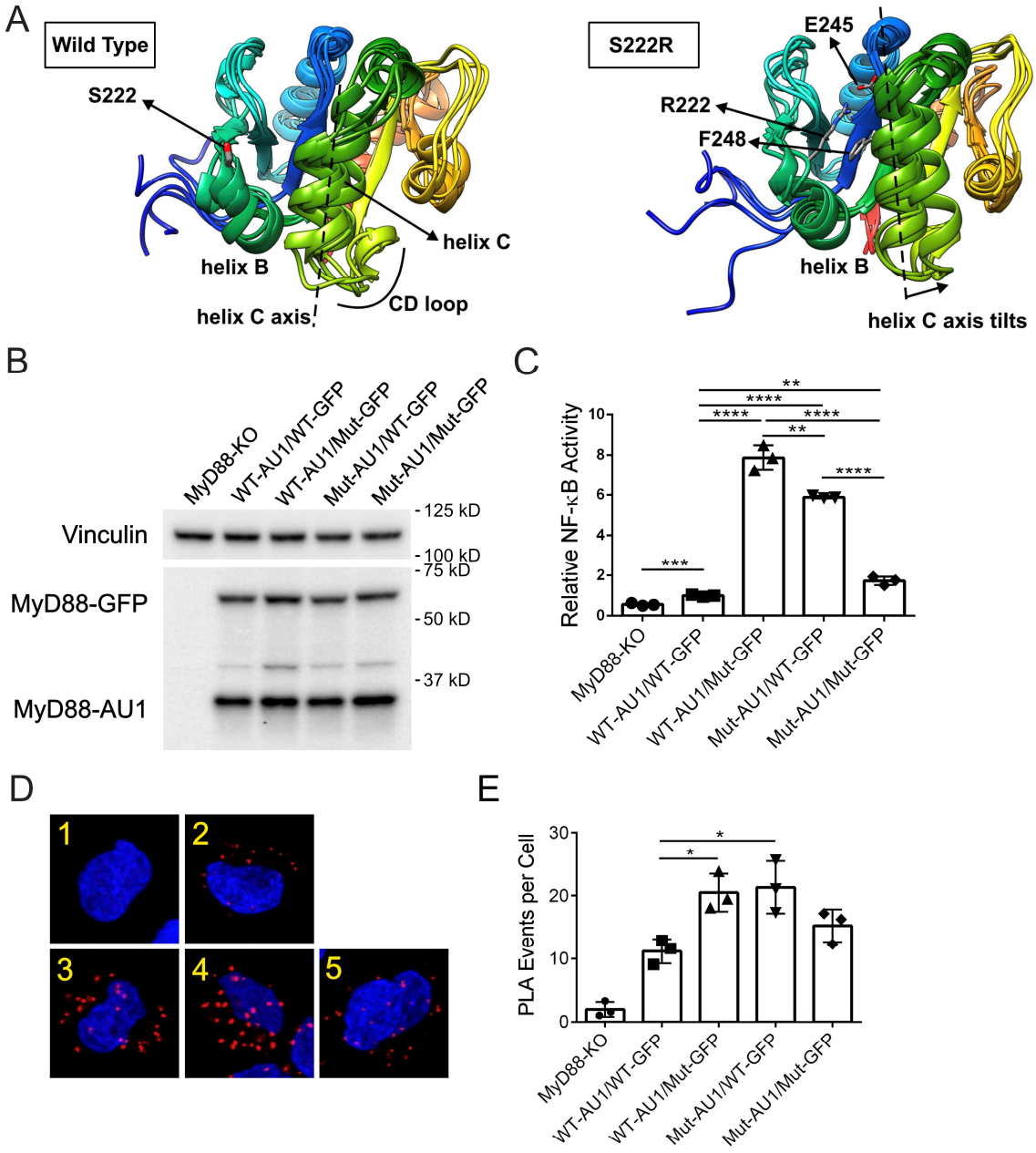
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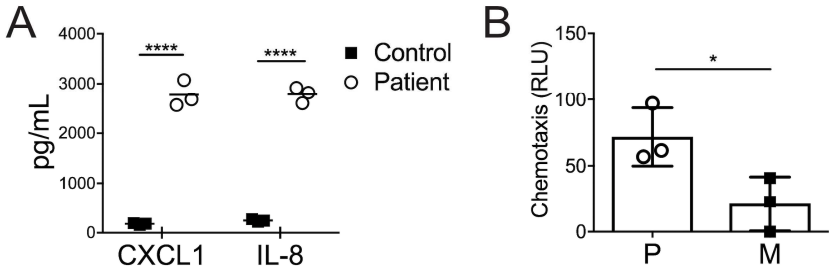
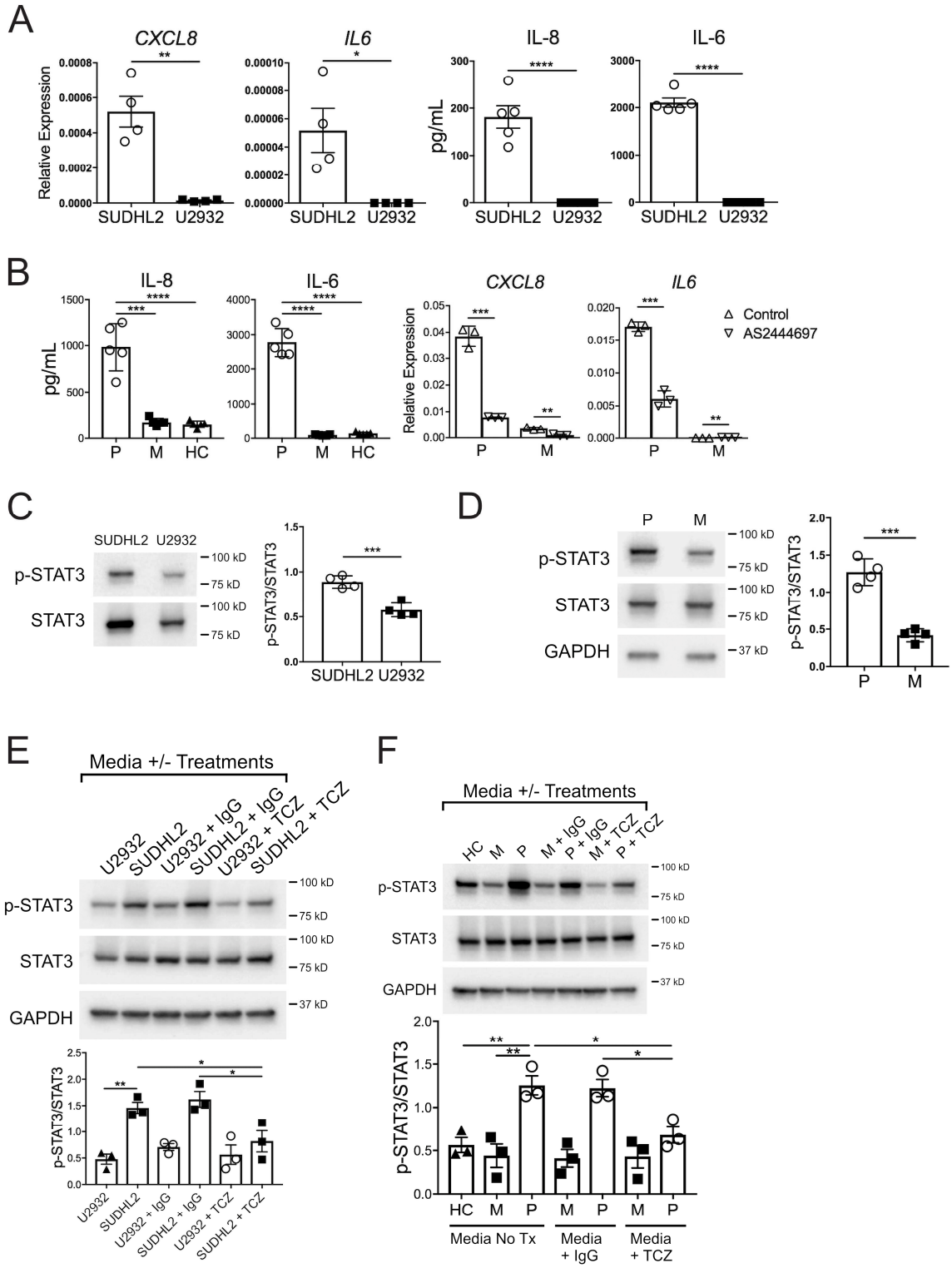
helix C
helix B
helix C axis
CD loop
S222R
E245
R222
F248
helix B
helix C axis tilts
B
MyD88-KO
WT-AU1/WT-GFP
WT-AU1/Mut-GFP
Mut-AU1/WT-GFP
Mut-AU1/Mut-GFP
Vinculin
MyD88-GFP
MyD88-AU1
125 kD
100 kD
75 kD
50 kD
37 kD
C
Relative NF-κB Activity
D
1
2
3
4
5
E
PLA Events per Cell


A
4000
3000
2000
1000
0
pg/mL
****
****
Control
Patient
CXCL1
IL-8
B
150
100
50
0
Chemotaxis (RLU)
*
P
M


A
CXCL8
IL6
IL-8
IL-6
Relative Expression
pg/mL
SUDHL2
U2932
B
IL-8
IL-6
CXCL8
IL6
pg/mL
Relative Expression
P
M
HC
Control
AS2444697
C
SUDHL2 U2932
p-STAT3
STAT3
100 kD
75 kD
p-STAT3/STAT3
D
P
M
p-STAT3
STAT3
GAPDH
100 kD
75 kD
37 kD
p-STAT3/STAT3
E
Media +/- Treatments
U2932
SUDHL2
U2932 + IgG
SUDHL2 + IgG
U2932 + TCZ
SUDHL2 + TCZ
p-STAT3
STAT3
GAPDH
100 kD
75 kD
37 kD
p-STAT3/STAT3
F
Media +/- Treatments
HC
M
P
M + IgG
P + IgG
M + TCZ
P + TCZ
p-STAT3
STAT3
GAPDH
100 kD
75 kD
37 kD
p-STAT3/STAT3
HC M P M P M P
Media No Tx
Media + IgG
Media + TCZ