Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Zhang Q, Davis JC, Lamborn IT, et al. Combined immunodeficiency associated with *DOCK8* mutations. N Engl J Med 2009;361:2046-55. DOI: 10.1056/NEJMoa0905506.

SUPPLEMENTAL METHODS

Cells

Peripheral blood mononuclear cells (PBMC) were enriched from whole blood using Ficoll-Paque PLUS gradient density centrifugation (GE Healthcare) and ACK lysis buffer (Quality Biological). Cell counts were performed by trypan blue exclusion. Human pan T cell isolation kit II, CD8 T cell isolation kit, or CD4 T cell isolation kit II were used for T cell subset purifications by negative selection (Miltenyi Biotec). Cells at $2x10^6$/mL were stimulated with 1 μg/mL each of anti-CD3 (OKT3, Ortho Biotech) plus anti-CD28 (BD Biosciences), in RPMI medium supplemented with 10% fetal bovine serum (FBS). After three days, T cells were washed and maintained in 100 IU/mL recombinant human IL-2 (Aldesleukin, Novartis) with fresh medium every three days. Monocytes or B cells were purified from PBMC by negative selection using monocyte isolation kit II or B cell isolation kit II (Miltenyi Biotech). B lymphocytes were immortalized with Epstein-Barr Virus (EBV), and T lymphocytes with Herpes virus saimiri (HVS) according to standard protocols.

Comparative genomic hybridization (CGH) analyses

CGH analyses were performed using 244K arrays, as per manufacturer's instructions with modifications (Agilent Technologies). For each array, equal amounts of patient and reference DNA were prepared in parallel. Briefly, genomic DNA (2-3 μg) was digested with Rsa1 and Alu1 restriction enzymes (Promega). Digested DNA was labeled with either cyanine-5-dUTP (patient) or cyanine 3-dUTP (reference) by random priming, using Genomic DNA Labeling kit PLUS (Agilent), and then purified using Vivaspin 500 columns (Sartorius Stedim). Labeled patient and reference DNA were combined with human Cot-1 DNA (Invitrogen), using Agilent aCGH Hybridization kit. Samples were hybridized to arrays while rotating at 65°C for 40 h. Arrays were washed at room temperature for 5 minutes with 0.5x SSC (20x SSC contains 3M NaCl, 0.3 M sodium citrate pH 7.0), 0.005 % Triton X-102 (Sigma-Aldrich), followed by 5

min at 37 ºC with 0.1x SSC, 0.005 % Triton X-102. Washed arrays were immediately scanned using an Agilent scanner. Data extraction and analyses were performed using Agilent's Feature Extractor 10.1.1.1 and DNA Analytics 4.0 software packages. Deletions were searched against the Database of Genomic Variants Build 36 (http://projects.tcag.ca/variation)

DNA Sequencing

Genomic DNA was isolated from peripheral blood leukocytes, or EBV- or HVS-transformed lymphoblastoid cell lines by DNeasy kit (Qiagen), or was purchased (Coriell Institute). Genomic sequencing was performed after polymerase chain reaction (PCR) amplifying exons with their flanking intronic and/or untranslated regions. In some cases, total RNA was isolated from primary lymphocytes or cultured transformed cell lines by RNeasy kit, with DNaseI in-column digestion (Qiagen). Two μg of total RNA was reverse-transcribed with Superscript III first-strand synthesis supermix (Invitrogen). cDNA sequencing was performed after PCR amplifying across exons. Genomic DNA (25 ng) or cDNA (1/20 of volume of reverse-transcription reaction) was amplified with 35 cycles (95°C for 30 s, 60-64°C for 30 s, 72°C for 1 min) using FastStart PCR Master (Roche). PCR products were separated on 2% agarose gels in 1x TAE electrophoresis buffer (Tris-acetate-EDTA buffer contains 40 mM Tris–acetate, 1 mM EDTA) and visualized with ethidium bromide. Products were purified using MinElute 96 UF PCR Purification Kit (Qiagen). Primer and template were combined in ABI 96-well Optical Reaction plates, and sequencing reactions were set up using Applied Biosystems BigDye® Terminator v3.1 Cycle Sequencing Kit by adding 1 μl of BigDye® Terminator Ready Reaction Mix v3.1, 3 μl of 5x Sequencing Buffer, and 2 μl of water for a final volume of 10 μl. Cycle sequencing was performed for 27 cycles (96ºC for 10 s, 50ºC for 5 s, 60ºC for 4 min) using a Bio-Rad Tetrad 2 (Bio-Rad Laboratories) or ABI 9700 thermal cycler. Fluorescence-labeled extension products were purified using BigDye® XTerminator™ Purification protocol before processing on an ABI 3730xL DNA Analyzer. The FINCH data management system (Geospiza) was used to store sequence data for all subsequent downstream sequencing analysis

using Sequencher 4.9 software (Gene Codes). Primer sequences for amplification and sequencing are listed in Supplementary Appendix – Table 1. All point mutations detected were confirmed in a second PCR amplification reaction. For confirmation of frameshift mutations, purified PCR-amplified products were cloned using TOPO TA Cloning Kit for Sequencing (Invitrogen). PureLink 96 Plasmid Purification System was used to isolate plasmid DNA from transformed TOP10 *E. coli* colonies (Invitrogen). Sequencing of 44 clones was performed using M13 Forward and Reverse primers, and yielded expected frequencies in patient 7-1 (23 clones with the insertion mutation, 21 clones with the deletion mutation). SNPs were searched against NCBI's dbSNP Build 130 (http://www.ncbi.nlm.nih.gov/). Novel variants were sequenced in several control groups, including six *STAT3*-mutant autosomal dominant-HIES, 32 patients with other immunological diseases (non-HIES), 15 healthy donors of varied ethnicities, and 100 healthy Caucasian controls.

All genomic variants are described according to Human Genome Variation Society recommendations (http://www.hgvs.org/mutnomen/)[1] using GenBank Reference Sequences NC_000009.10 (DNA), NM_203447.2 (mRNA), and NP_982272.2 (protein) based on NCBI Build 36.3. Coding DNA variations are described with the A of the ATG translation initiation codon designated as nucleotide +1.

Quantitative real-time RT-PCR

Total RNA was isolated from primary lymphocytes or cultured transformed cell lines by RNeasy kit, with DNaseI in-column digestion (Qiagen). One μg of total RNA was reverse-transcribed with Superscript III first-strand synthesis supermix (Invitrogen). Appropriately diluted cDNA was analyzed by quantitative real-time PCR with SYBR Green dye on a 7500 Real Time PCR System (Applied Biosystems). Standard conditions of 40 cycles (95ºC for 15 s, 60ºC for 1 min) were used. The quantity of RNA was calculated from the cycle number by using primer-specific standard curves. Expression of DOCK8 was normalized to the housekeeping gene β-actin. Primer sets designed to span exon junctions are listed in Supplemental Table 1.

Immunoblotting

Cells were lysed in 1% NP-40, 150 mM NaCl, 50 mM Hepes pH 7.4, 1 mM EDTA, and complete protease inhibitor (Roche). Protein lysates (100-200 μg) in 5% β-mercaptoethanol were separated on 3-8% Tris acetate gels in Tris-acetate sodium dodecyl sulfate (SDS) buffer (Invitrogen). Samples were transferred to nitrocellulose overnight at 4ºC, blocked with 5% non-fat dry milk in PBS containing 0.1% Tween-20, incubated with polyclonal rabbit anti-DOCK8 (Sigma-Aldrich, or the kind gift of Dr. Ruusala, Ludwig Cancer Institute, Uppsala, Sweden[2]) or mouse anti-β-actin antibodies (Sigma-Aldrich) on the same blot. Following incubation with horseradish peroxidase-conjugated anti-rabbit Ig or anti-mouse IgG1 (Southern Biotechnology), signal was developed with Supersignal West chemiluminescent substrate (Thermo Scientific), and exposed to film.

Flow cytometry

Except where otherwise indicated, all antibodies and buffers were from BD Biosciences. Samples were acquired on BD FACSCanto or BD FACSCalibur instruments using FACSDiva or CellQuest Pro software (BD Biosciences). Analyses were performed using the FlowJo software package versions 8.7.3 or higher (TreeStar).

Standard flow cytometry methods were used for cell-surface marker staining. PE anti-CD25, PE-Cy5 anti-CD4, APC-Cy7- or APC- anti-CD8 antibodies were purchased from BD Biosciences. For carboxyfluorescein succinimidyl ester (CFSE) dilution assays, cells at 10^7/mL in PBS were labeled with 2.5 μM CFSE (Invitrogen) for 15 min in the dark, washed in the presence of FBS, and resuspended at $2x10^6$/mL. Cells were harvested for CFSE analysis after three days of culture under conditions of anti-CD3 plus anti-CD28 stimulation, or no stimulation.

For detection of intracellular cytokines, purified T cells were incubated at 37ºC overnight and resuspended at $2x10^6$ cells/ml in RPMI supplemented with 10% FBS. Cells were either left unstimulated, or stimulated with 10 ng/mL phorbol myristate acetate (PMA) plus 500 ng/ml

ionomycin (Calbiochem) at 37ºC for 2 h. GolgiPlug™ was added at a final concentration of 1:1000 for an additional 4 h. Cells were incubated with PE Cy5 anti-CD45RO and APC Cy7 anti-CD8 antibodies for 20 min at 4ºC, in the presence of 1% FCS, 0.09% sodium azide, followed by Cytofix/Cytoperm for 20 min at 4ºC. Washed cells were resuspended in Perm/Wash buffer and incubated with optimally titrated FITC anti-IFN-ɣ and PE Cy7 anti-TNF-α antibodies for 1 h at 4ºC in the dark. Cells were sequentially washed with perm/wash buffer and PBS, resuspended in PBS, and acquired on the flow cytometer.

For enumeration of regulatory T cells, Human Regulatory T cell Staining Kit #2 (eBiocscience) was used. Briefly, after cell surface staining with PE anti-CD4 and FITC anti-CD25, freshly isolated PBMC were treated with Fixation/Permeabilization buffer for 45 min at 4 ºC. After washing with Permeabilization buffer, cells were incubated with APC anti-FoxP3 for 1 hour at 4 ºC. After additional washes, cells were resuspended in PBS and acquired on the flow cytometer.

For detection of intracellular perforin, after cell surface staining with APC anti-CD8-antibodies, cells were incubated with 4% paraformaldehyde in PBS for 20 min at 4ºC. Fixed cells were washed with permeabilization buffer (0.1% saponin, 0.1% BSA, 1 mM $CaCl_2$, 1 mM $MgSO_4$, 0.05% sodium azide, 10 mM HEPES pH 7.4) and incubated with PE-anti-perforin antibodies or isotype control for 1 h at 4ºC in the dark. After additional washes, cells were resuspended in PBS and acquired on the flow cytometer.

For detection of degranulation, CD8 T cells were used as effectors after initial activation and culture for 14 days. P815 target cells were washed with PBS, labeled with 2.5 μM CFSE for 10 min at room temperature in the dark, and washed with medium containing FBS. Labeled target cells were pre-incubated with anti-CD3 antibodies for 20 min at room temperature before adding to effector cells. Target and effector cells were incubated together, each at a concentration of $5x10^5$ cells/mL. Cells were incubated in the presence of 100 ng/mL of anti-CD3 antibodies, 100 U/mL of IL-2, APC anti-CD107 antibodies, and GolgiStop™ at a final

concentration of 1:816, for 2 h at 37ºC. Cells were stained with FITC-anti-CD8 antibodies before being acquired on the flow cytometer.

Contributions

H.C.S. designed this study. The patients were recruited and studied under immunodeficiency and hyper-IgE screening and natural history protocols designed by S.M.H., and were also studied under a lymphocyte homeostasis screening protocol designed by H.C.S. as part of the Lymphocyte Molecular Genetics Unit (LMGU) program. Q.Z., J.C.D., I.T.L., A.F.F., H.J., A.J.F., H.F.M., J.D., G.U., S.M.H., and H.C.S. gathered the data. Q.Z., J.C.D., I.T.L., A.F.F., H.J., G.U., and H.C.S. analyzed the data. H.C.S. vouches for the data and the analysis. The initial draft of the paper was written by H.C.S with significant contribution from J.C.D. H.C.S. and S.M.H. decided to publish the paper. There were no confidentiality agreements between the authors and the NIH, the sponsor of the study. No outside editorial assistance was provided to the authors.

Supplemental figure legends

Figure S1. Regulatory T cells. Shown are flow cytometric profiles depicting the FoxP3^{+} CD25^{hi} subset in gated CD4 T lymphocytes for patients 1-1 (A) and 4-2 (B); percentages indicate T_{reg} cells as a proportion of the CD4 T cells. C, healthy control. Pt, patient. The percentages of T_{reg} as a proportion of lymphocytes in (A) were 3.3, 3.3, and 1.4 for C1, C2, and Pt 1-1, respectively; and in (B), 2.8, 3.3, 3.5, and 3.1 for C1, C2, C3, and Pt 4-2, respectively.

Figure S2. *DOCK8* molecular analyses. (A) Shown for each proband is a pictorial representation of Log_2 ratios (x-axis) of patient DNA/reference DNA after hybridization to probes at or near the DOCK8 locus, vs. genomic position (y-axis). Green crosses indicate probes where the signal of the log_2 ratio was less than zero, and the red crosses more than zero. Arrows pointing to the shaded regions indicate homozygous or heterozygous *DOCK8* deletions. (B) Sequence chromatograms for the point mutations in proband and corresponding normal sequence in a healthy control. A vertical arrow indicates the location where a base change(s) occurs in patient as compared to control. Horizontal arrow indicates beginning of exon 6. Horizontal bar for allele II in patient 7-1 indicates the inserted bases. The corresponding amino acid changes for the patients are shown below in red. (C) *DOCK8* structural variants and point mutations. Variants are aligned against the protein (NP_982272.1; grey, top) and genomic DNA (NC_000009.10; boxes indicate exons) sequences. Bars designate deletions, with coordinates taken from oligonucleotide probes or by sequencing across the deletion. Bars are dotted where a breakpoint occurs between two adjacent probes. Symbols designate point mutations, with predicted effects on protein coding indicated. CDS, coding sequence. DHR, DOCK homology region. Note that (C) is not drawn to scale.

Figure S3. *DOCK8* exons 10 through 23 are deleted in patient 1-1. (A) Exon-specific primers were used for PCR amplifications from genomic DNA isolated from peripheral T cells of healthy

controls (C1, C2), patient (1-1), or no template (-) control. PCR products were separated by gel electrophoresis. (B) A unique 1047 bp crossover PCR product spanning the deletion was amplified from genomic DNA isolated from EBV- or HVS- transformed patient cell lines, as well as peripheral T cells from parents (P1, P2), but not healthy control (C) or no template (-) control. (C) Sequence chromatogram showing exact breakpoints of deletion. A line identifies the overlapping region where intron 9 joins intron 23 in the PCR amplified product.

Figure S4. *DOCK8* exons 5 through 24 are deleted in patient 2-1. (A) Exon-specific primers were used for PCR amplifications from genomic DNA isolated from peripheral T cells of healthy controls (C1, C2), neutrophils of patient (2-1), or no template (water) control. PCR products were separated by gel electrophoresis. (B) A unique crossover PCR product spanning the deletion was amplified from genomic DNA isolated from patient 2-1, but not healthy control (C). (C) Sequence chromatogram showing exact breakpoints of deletion. A line identifies the overlapping region where intron 4 joins intron 24 in the PCR amplified product.

Figure S5. Abnormal DOCK8 splicing in patients 3-1 and 6-1. (A) Primers were used for PCR amplification from exon 5 to exon 8 of cDNA prepared from EBV lines of healthy controls (C1, C2), patient 3-1, patient 6-1, and a parent (P). PCR products were separated by gel electrophoresis. Both patient 3-1 and parent P carry the c.538-15T>G intron 5 splice mutation. Patient 6-1 carries the c.538-18C>G intron 5 splice mutation. (B) Sequence chromatograms showing splice junctions for the PCR amplified products. The 205 bp band found in patients 3-1 and 6-1 corresponds to an aberrantly spliced product in which exon 5 skips exon 6 and instead joins to exon 7, resulting in an out-of-frame premature termination. The corresponding amino acid changes for the patients are shown below in red. The 290 bp band corresponds to normally spliced product in which exons 5, 6, 7, and 8 are sequentially spliced together.

Figure S6. (A) DOCK8 mRNA expression in leukocyte subsets from healthy blood donors. Quantitative real-time RT-PCR was used to measure DOCK8 mRNA, normalized to β-actin, in various leukocyte subsets and cell lines: freshly isolated monocytes (95%, n=3), T cells (95%, n=3), CD8 T cells (81%, n=1), CD4 T cells (88%, n=1), and B cells (95%, n=4), at indicated purities; activated peripheral blood T cells expanded in IL-2 after stimulation with anti-CD3 plus anti-CD28, cultured for indicated number of total days (n=1); and EBV- (n=3) or HVS- (n=2) transformed cell lines. Standard deviations are shown where three or more samples were tested. (B) DOCK8 mRNA expression in lymphocytes from patients. Quantitative real-time RT-PCR utilizing primer set A or B was used to measure DOCK8 mRNA, normalized to β-actin, in primary T cells (previously activated with anti-CD3 plus anti-CD28 and expanded in culture with IL-2), HVS-transformed T cells, and EBV-transformed B cells. C, healthy control; Pt, patient. (C) Schematic diagram with bars showing locations on mRNA targeted by RT-PCR primer sets. CDS, coding sequence.

Figure S7. DOCK8 protein expression in patients 1-1, 2-1, 5-1, and 5-2. Immunoblotting was performed on lysates from control (C1, C2, C3), patients 2-1, 5-1, and 5-2 HVS-transformed T cells (A), or lysates from control or patient 1-1 primary T cells (B). Antibodies to DOCK8 or β-actin were used. Arrow indicates residual protein of altered mobility consistent with deletion A.

Figure S8. DOCK8 deficiency impairs CD8 T cell proliferation. (A) After activation of PBMC with anti-CD3 plus anti-CD28 for 3 days, T cells were expanded in culture with IL-2. Absolute numbers of CD8 T cells were calculated based upon flow cytometric determinations of their percentages. CD8 T cell numbers over time were graphed as fold-increases normalized to the pre-stimulated population. (B) CD4 T cell numbers from the experiments in (A) were similarly analyzed. (C, D) Flow cytometric plots of CFSE-labeled PBMC either unstimulated or stimulated as in (A). After 3 days, cells were stained for T cell subset markers and CFSE dilution assessed by flow cytometry. Shown are CFSE dilutions of gated CD8 T cells (C) or

gated CD4 T cells (C, D). Grey shaded peak, unstimulated; blue line, with stimulation. C, healthy control; P, parent. Results for family 1 are representative of two independent experiments; results for families 4, 5, and 8 represent one experiment each.

Figure S9. CD25 induction, cytokine production, and perforin expression by DOCK8-deficient CD8 T cells. (A) CD25 expression was evaluated on gated CD8 T cells by flow cytometry 3 days after stimulating PBMC with anti-CD3 plus anti-CD28 antibodies. Median Fluorescence Intensities (MFI) are indicated on plots. Grey shaded peak, unstimulated; blue line, with stimulation. C, healthy control; Pt, patient. Results for Patient 1-1 are representative of two experiments. Results for patient 8-2 are from one experiment. (B) Ex vivo intracellular IFN-γ or TNF-α expression in gated CD8 T cells, as assessed by flow cytometry after PMA plus ionomycin restimulation. Percent positive are indicated on plots. Similar results were obtained for patient 5-2. Grey shaded peak, isotype control; blue line, cytokine. (C) MFI for perforin expression in gated CD8 T cells after expansion in culture for 14 days, as assessed by flow cytometry. (D) CD107a surface expression after degranulation, showing percent positive in gated CD8 T cells. Line indicates geometric mean. Healthy control, ●; patient 5-1, ✖; patient 5-2, ▼; patient 6-1, ◆; patient 8-2, ▲.

Supplemental References

1. den Dunnen JT, Antonarakis SE. Mutation nomenclature extensions and suggestions to describe complex mutations_ A discussion. Hum Mutat 2000;15:7-12.

2. Ruusala A, Aspenstrom P. Isolation and characterisation of DOCK8, a member of the DOCK180-related regulators of cell morphology. FEBS Lett 2004;572:159-66.

Supplemental Table 1

	5' Primer Sequence		3' Primer Sequence
Genomic Sequencing (PCR and Sequencing Primers)			
DOCK8exon1F	ACTTCGAAACCACTTCTGCC	DOCK8exon1R	TGGCATGGAAAGTTACCTCC
DOCK8exon2F	TTGCCTTTGTGCTTTTACCAG	DOCK8exon2R	GGCATCTCCCCTACCCAC
DOCK8exon3F	TTGGAATGATTGGGCAAGAAC	DOCK8exon3R	TGTGTGTGATATGGAGTGGGG
DOCK8exon4F	TCTAATGGTTTGCCGCTCTC	DOCK8exon4R	CCCTGGGCAGAAGAGCC
DOCK8exon5F	CCCAAATTGGAGCAGTCTTG	DOCK8exon5R	AATAATGAACAACACAATTGTCCAC
DOCK8exon6F	TTAACTTGAGCCGTGGAGGG	DOCK8exon6R	GAAGAATGACCATCAAGAAGTTGG
DOCK8exon7F	TCCAAATATTTCGGGAAACTGC	DOCK8exon7R	TCAAATCTGATAGGTTCTTGCTGC
DOCK8exon8F	TTTAAACCATGAATGCAACAGG	DOCK8exon8R	TGTGCTGGGAACAACACAGC
DOCK8exon9F	AGCTTCCTATGTCATCAAATATTGTC	DOCK8exon9R	GGGACACATTAGGGACTAAAGCC
DOCK8exon10F	AGGCAGTTGACTTGGTGCTG	DOCK8exon10R	AGTAAGCTGTGGACCTGGGC
DOCK8exon11F	TGGCCATATTCAGTGTTCCTG	DOCK8exon11R	ATCCTCCTCCTGTGGGTTCC
DOCK8exon12F	TCCCAGAAATCGTTTGTCCC	DOCK8exon12R	TGTGTCTAACGATAAGCAGTTTTGG
DOCK8exon13F	CTGCTAGCAATGCAGGGAAG	DOCK8exon13R	ACGGTTTTCCAAGAACCTGC
DOCK8exon14F	AAGCACCCCATGAATGGAAG	DOCK8exon14R	TCCCGGACTTGTGTGAAGTG
DOCK8exon15F	ACATCCTGTTGGCCTGATG	DOCK8exon15R	AAAACCAGGAGATGATGAAACC
DOCK8exon16F	GGCTGTGGCGTTTTACAATC	DOCK8exon16R	CTGCAGGGACCTCAGATGC
DOCK8exon17F	TTGCATTTGATTATTGCGAGC	DOCK8exon17R	CCACATGGGAATGGAAAGTG
DOCK8exon18F	GGGCTCCTGACTCCAAGAAC	DOCK8exon18R	AGAGGTTTGCATGTCCCCAC
DOCK8exon19F	CTTCTGGTCTGAAACGCTGG	DOCK8exon19R	GCACACATCATCAGTGGAAAGG
DOCK8exon20F	AAACTTCCACCTCAGGCTCC	DOCK8exon20R	ACTGCAGTGGGGTGGAGG
DOCK8exon21F	GCTTTTCATCCACCCTATCCC	DOCK8exon21R	GTGGGCAGCAGTAGTGGTTG
DOCK8exon22F	TCCAGATGTCTGGCTTACCTTTC	DOCK8exon22R	CACAGTGTGAGGGCATGAGG
DOCK8exon23F	GAACATCTAAGACACATGCTTCAGG	DOCK8exon23R	CAGCAGGGGCAACTCAGTG
DOCK8exon24F	GCACCACCAGCACAGATAAAG	DOCK8exon24R	CCACATCAGGGTCCAGTCC
DOCK8exon25F	CCCACCAGTGACCTGGAAG	DOCK8exon25R	CGAACATCACATTATATGAGAAGGAAC
DOCK8exon26F	GCCAACCTCAACTCCACTTACC	DOCK8exon26R	GCTAGTGGTGTAACTTCAGGGTAGC
DOCK8exon27F	TGGCCATCGCTATTTTCATTC	DOCK8exon27R	TTTACCAAGTGTGAGAAGCTAGACTG
DOCK8exon28F	TGCTTGGTTTTCACAGTCACC	DOCK8exon28R	CAGCACTGGAACGAATGACAG
DOCK8exon29F	GTAGGGGACATGGGGAAATG	DOCK8exon29R	GCTGAGGCATGAAGAACCC
DOCK8exon30F	GATCTCCAGCCTAGCAGTGATG	DOCK8exon30R	CTCCATGGCCCAAACAAAG
DOCK8exon31F	TTAGCTGGCATCACTGTGGAG	DOCK8exon31R	GGACATTCCTCCCCACAAAC
DOCK8exon32F	TTCTGGTTATCTTGGAGGGTTTC	DOCK8exon32R	TCAGTAATACAAGCAGCCTGGG
DOCK8exon33F	CCATCATGGGAACCTGGC	DOCK8exon33R	TGGGATCACATTTATGTCTTTCAC
DOCK8exon34F	CACTGGACATGGAACATCAGC	DOCK8exon34R	CTGTGACTTTTGGTCCACCTG
DOCK8exon35F	ATTTCAACGGTCCAGAAAGTG	DOCK8exon35R	TTTTCGCAGCTGATGCTTTAC
DOCK8exon36F	TGGCCATGCTGCTTTCAG	DOCK8exon36R	CATACACAATAGTTGGCAGATCCC
DOCK8exon37F	AATCCTAACTCTTCACCTGGGAC	DOCK8exon37R	AGTGACAAATCCTCGACCCC
DOCK8exon38F	AAAAGGTCACACAAAGTAGAAGAACAG	DOCK8exon38R	GACAAAATCGCCCCAGTTG
DOCK8exon39F	ATTCGGGGTTCCTGTGGTC	DOCK8exon39R	CCAGCACCCCAAGTCCAG
DOCK8exon40F	GGACAATGACCTCTGGTTGC	DOCK8exon40R	ATCTGTAGGACAGGGTCGCC
DOCK8exon41F	CACAATGAGAGACCCCTGCC	DOCK8exon41R	TGGTGATGACCCACTCTAACTTG
DOCK8exon42F	TCACTTCCAAGAAGACAAAGAGTTG	DOCK8exon42R	TTCTTGGGTAGAGAACGAAGGG
DOCK8exon43F	TCCATTGCGTCAGGGATG	DOCK8exon43R	GTTTGTGGGTCCTCCTGGG
DOCK8exon44F	TGGCCTCTCTGTCATAGCTCC	DOCK8exon44R	AAGGAAGAAGGGTGCAGACG
DOCK8exon45F	TGAGATGTCCAAGACCTTCCTT	DOCK8exon45R	GGTGCCTGAGACTTTGTCTTTC
DOCK8exon46F	AGGTGATCCCGATATGCCAC	DOCK8exon46R	TGACTGTGGGACCCTCTTCC
DOCK8exon47-1F	TGATCTTTTCTTGCTTCTGTACGC	DOCK8exon47-1R	ACTCCAATAAATTCAAATATGGGC
DOCK8exon47-2F	TCCACAATGTACCAAACAAGGC	DOCK8exon47-2R	TCCATTTAAGTGAAAGCAGTATCTGTG
RT-PCR			
DOCK8rt-A-F	TCAGCCTCTGTGGGTAGACA	DOCK8rt-A-R	CCGCACAAAGAGATTTTGGA
DOCK8rt-B-F	ACGCGCCGTGTAACTGTGAA	DOCK8rt-B-R	CCCCGAGCTCCTGGGCAA
Cross-PCR for Pt 1-1			
Pt1-1cross-1F	GCAAGGCCACCCCCTGTTGT	Pt1-1cross-1R	TTGCCCCTACCCCAGCTCCC
Cross-PCR for Pt 2-1			
DOCK8300919i4.10F	TGTCAAAACAATATGGCAAAGC	DOCK8388330i24.2R	GGGACCTGATAAAGAGGTTGC
cDNA Splice Site for Pt 3-1 and Pt 6-1			
DOCK8 RTex6_1F	GGACTTTGAGAAGCAGAACG	DOCK8 RTex6_2R	GCAGAAATCCTTTGAACTGG
Controls			
Beta actin F	CCTCACCCTGAAGTACCCCA	Beta actin R	TCGTCCCAGTTGGTGACGAT

Supplemental Table 2. Characteristics of Patients

Variable	Patient 1-1	Patient 2-1	Patient 3-1	Patient 4-1	Patient 4-2	Patient 5-1	Patient 5-2	Patient 6-1	Patient 7-1	Patient 8-1	Patient 8-2
Age (yr)	6	21†	18†	17	14	21	14	14	13†	18†	16
Sex	female	male	female	male	female	female	female	male	female	female	male
Ethnicity	Yemeni	Lebanese	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Caucasian	Mexican	Mexican
Atopy											
Atopic dermatitis	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Allergies	Food - beef, cow's milk, egg, sesame; Environmental - Bermuda grass, mountain cedar	Food - avocado, banana, beef, cantaloupe, carrot, cow's milk, cucumber, egg, kiwi, lamb, lentil, mango, mustard, pea, pineapple, pistachio, pomegranate, salmon, sesame, shrimp, watermelon, wheat, yeast*; Environmental - seasonal rhinitis to Alternaria, ragweed, trees	Food- catfish, eggs, peanuts, shellfish*	Drug - cefaclor	Drug - penicillin	Food - apple, corn, cow's milk, egg, fish, legume, peanut, shellfish, soy, tree nuts, wheat; Drug - penicillin, sulfa; Environmental - grass, trees, pets	Food - cow's milk, egg, legumes, peanuts, tree nuts*; Drug - clarithromycin, penicillin, sulfa; Environmental - cat, grass	Food - crab, egg, tree nuts*; Environmental - seasonal rhinitis	Food - bananas, beans, cow's milk, goat's milk, onion, peanut, soy, tuna*; Drug - sulfa	Food - beans, beef, chicken, cow's milk, egg, fish, peanut, pork, tree nuts, tomato*; Drug - Cefipime, Lactinex, Propofol*; Environmental - dust, dog, grasses, mold	Food - beef, chicken, cow's milk, egg, peanut, pork*; Environmental - dust, dog, mold
Asthma	(-)	(+)	(+)	(+)	(+)	(+)	(+)	(-)	(-)	(-)	(-)
Infections											
Skin and soft tissue	Diaper cellulitis	*Staphylococcus aureus* skin abscesses, axillary lymphadenitis, otitis externa	*Staphylococcus aureus* skin abscesses, otitis externa	(-)	(-)	*Staphylococcus aureus* skin abscesses	*Staphylococcus aureus* impetigo and skin infections, otitis externa	Skin abscesses, impetigo	*Staphylococcus aureus* skin abscesses, otitis externa	*Staphylococcus aureus* skin infections, otitis externa	*Staphylococcus aureus* skin infections, *Acinetobacter baumanii* otitis externa
Respiratory tract	Recurrent otitis media, pneumonias¶§	Recurrent otitis media, sinusitis, adenoviral pneumonia¶§	*Streptococcus pneumoniae*, non-typeable *Haemophilus influenzae*, RSV pneumonias; otitis media	Recurrent otitis media, sinusitis, bronchitis, croup¶§	Recurrent otitis media, sinusitis, pneumonias, bronchitis¶§	Recurrent otitis media, sinusitis, mastoiditis, pneumonias including *Pneumocystis jirovecí*¶§	Recurrent otitis media, sinusitis, *Pneumocystis jiroveci* and *Haemophilus influenzae* pneumonias¶	Recurrent otitis media, sinusitis¶	Recurrent pneumonias, mastoiditis	(-)	Otitis media, sinusitis, pneumonia
Viral infections	Recurrent orolabial HSV•	Diffuse flat warts, herpes zoster	HSV - keratitis, eczema herpeticum, recurrent genital infections; diffuse molluscum contagiosum	Diffuse flat warts, diffuse molluscum contagiosum, severe primary varicella•	Recurrent orolabial HSV, diffuse flat warts, diffuse molluscum contagiosum•	Diffuse molluscum contagiosum, verrucous warts on fingers, recurrent herpes zoster•	Diffuse flat warts, persistent orolabial HSV	Flat and verrucous warts on face, trunk, extremities•	HSV - keratitis, eczema herpeticum, recurrent orolabial, eyelid, ear canal, and genital infections; Verrucous warts on fingers•	HSV keratitis, eczema herpeticum, herpes zoster	HSV keratitis and groin infection, eczema herpeticum, diffuse molluscum contagiosum•
Other	Oral candidiasis, tooth decay	Pericarditis	Salmonella enteritis, giardiasis, *Staphyloccocus aureus* osteomyelitis, vaginal candidiasis	(-)	Salmonella enteritis	Oral candidiasis	Oral candidiasis	*Staphylococcus aureus* osteomyelitis, nail candidiasis	Vaginal candidiasis	*Haemophilus influenzae* and cryptococcal meningitis; recurrent *Staphylococcus aureus* and *Acinetobacter baumanii* sepsis	(-)
Malignancies	(-)	Metastatic anal squamous cell carcinoma	Paranasal and vulvar squamous cell carcinomas; cutaneous T-cell lymphoma	(-)	(-)	(-)	(-)	(-)	Vulvar squamous cell carcinoma	(-)	(-)
Additional history	Poor growth, high forehead, thinning hair	Eosinophilic esophagitis, eosinophilic dermatitis, eosinophilic lung disease, bronchiectasis, hypospadius	Poor growth	Cavernous angioma	(-)	Eosinophilic esophagitis, bronchiectasis and lung cyst, high-arched palate, hyperextensibility	High-arched palate, minimal trauma fracture	Bronchiectasis, scoliosis, high-arched palate	Retained primary teeth, pneumatocele, poor growth	Delayed puberty	Retained primary teeth
HIES score°	40	Not done	Not done	27	30	33	62	42	61	54	42

† deceased
* anaphylaxis
¶ tympanostomy tubes
§ sinus surgery
• IFN-α treatment
° of maximal score of 111 (without age correction); scoring criteria according to Grimbacher B, Schäffer AA, Holland SM, et al. Genetic linkage of hyper-IgE syndrome to chromosome 4. Am J Hum Genet 1999;65:735-44.

Supplemental Table 3. Specific antibody function of patients.

Specific antibodies to	Patient 1-1	Patient 2-1	Patient 3-1	Patient 4-1	Patient 4-2	Patient 5-1	Patient 5-2	Patient 6-1	Patient 7-1	Patient 8-1	Patient 8-2
Tetanus toxoid	(-)	(-)	(-)	(+)	(+)	(-)	(-)	(-)	(+)	(-)	(-)
Diphtheria		(-)	(-)	(+)	(+)	(-)	(-)	(+)	(-)	(+)	(+)
Pneumococcal†	20/23		1/3	21/23	20/23	1/23	23/23	7/23		5/23	10/23
Rubella	(+)		(+)	(+)	(+)	(+)	(+)	(+)			(+)
Haemophilus influenzae type B	(+)	(-)		(+)	(+)	(-)	(+)	(-)		(-)	(+/-)
Varicella-zoster virus	(+)		(+)			(+)	(+)				
Measles			(-)							(-)	
Poliovirus	(-)		(-)								
Bacteriophage øX174				(-)	(-)						
Mumps			(-)								
Hepatitis B virus									(+)		(-)
Started on IVIG	no	yes	no	yes*	yes*	yes	yes	no	no	yes	no

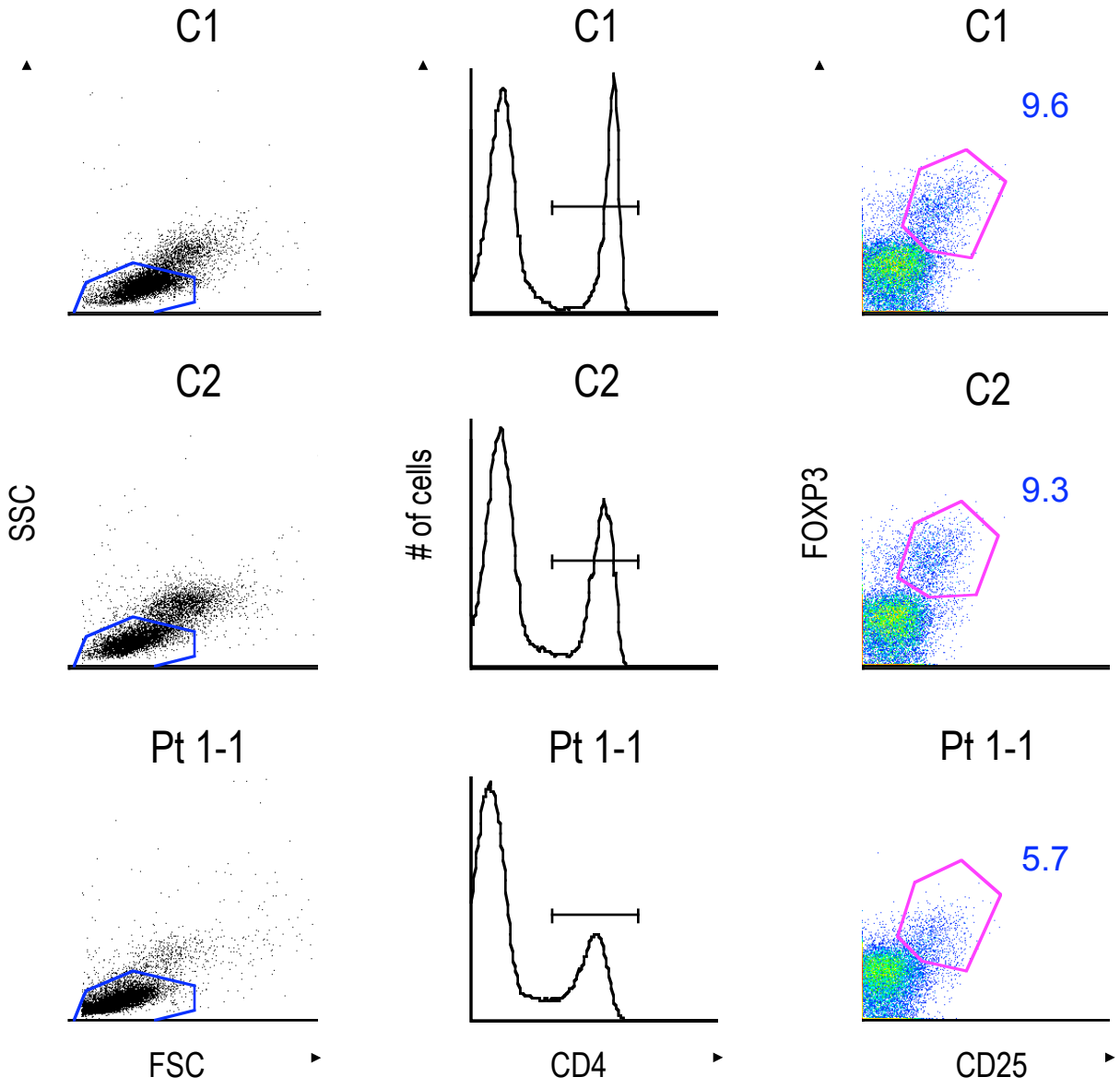
(-), (+), (+/-) indicate whether protective levels of antibodies were absent, present, or indeterminate, respectively

†Number of serotypes showing positive response of those tested

*IVIG trial not continued

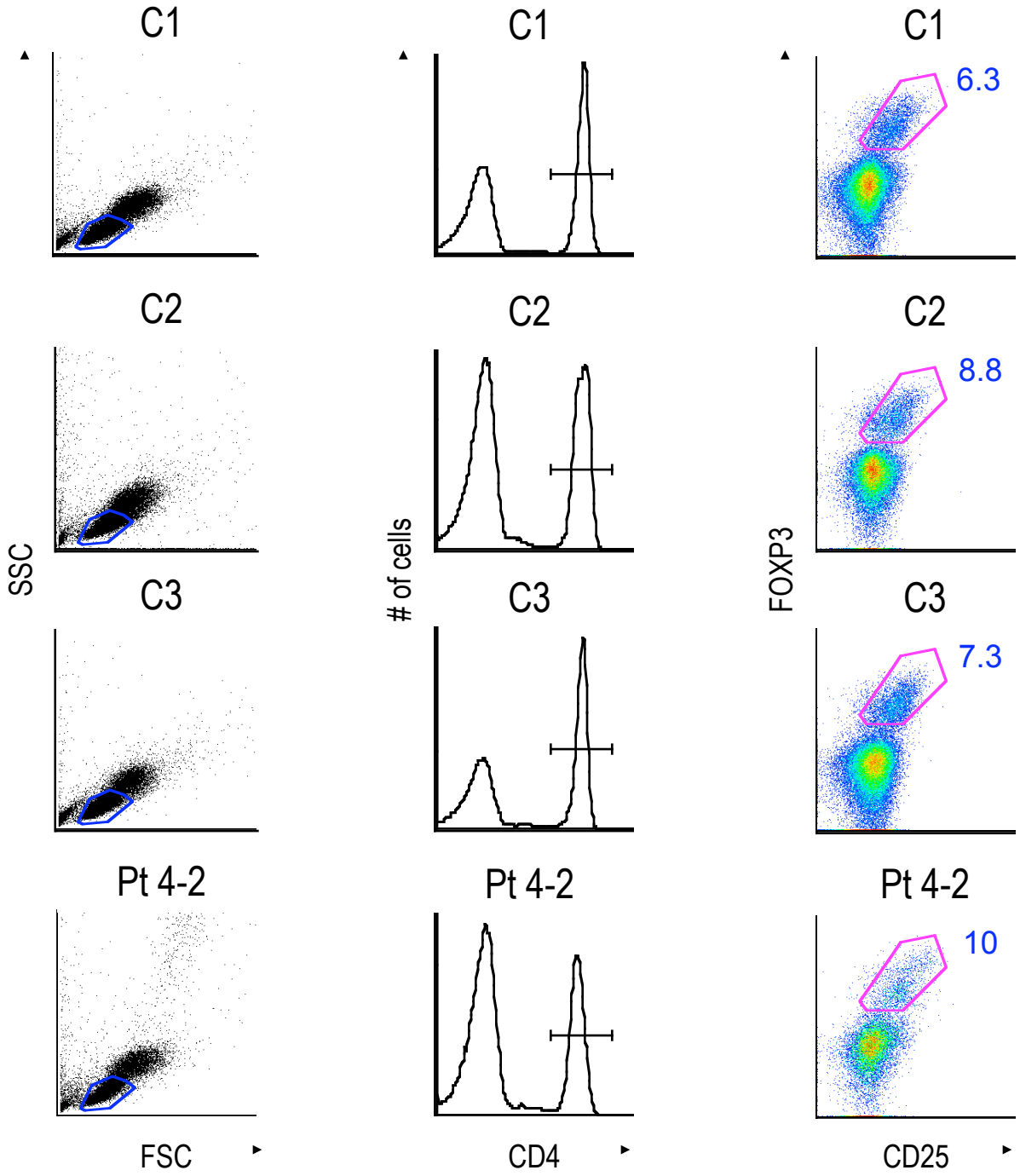
Supplemental Figure 1

A



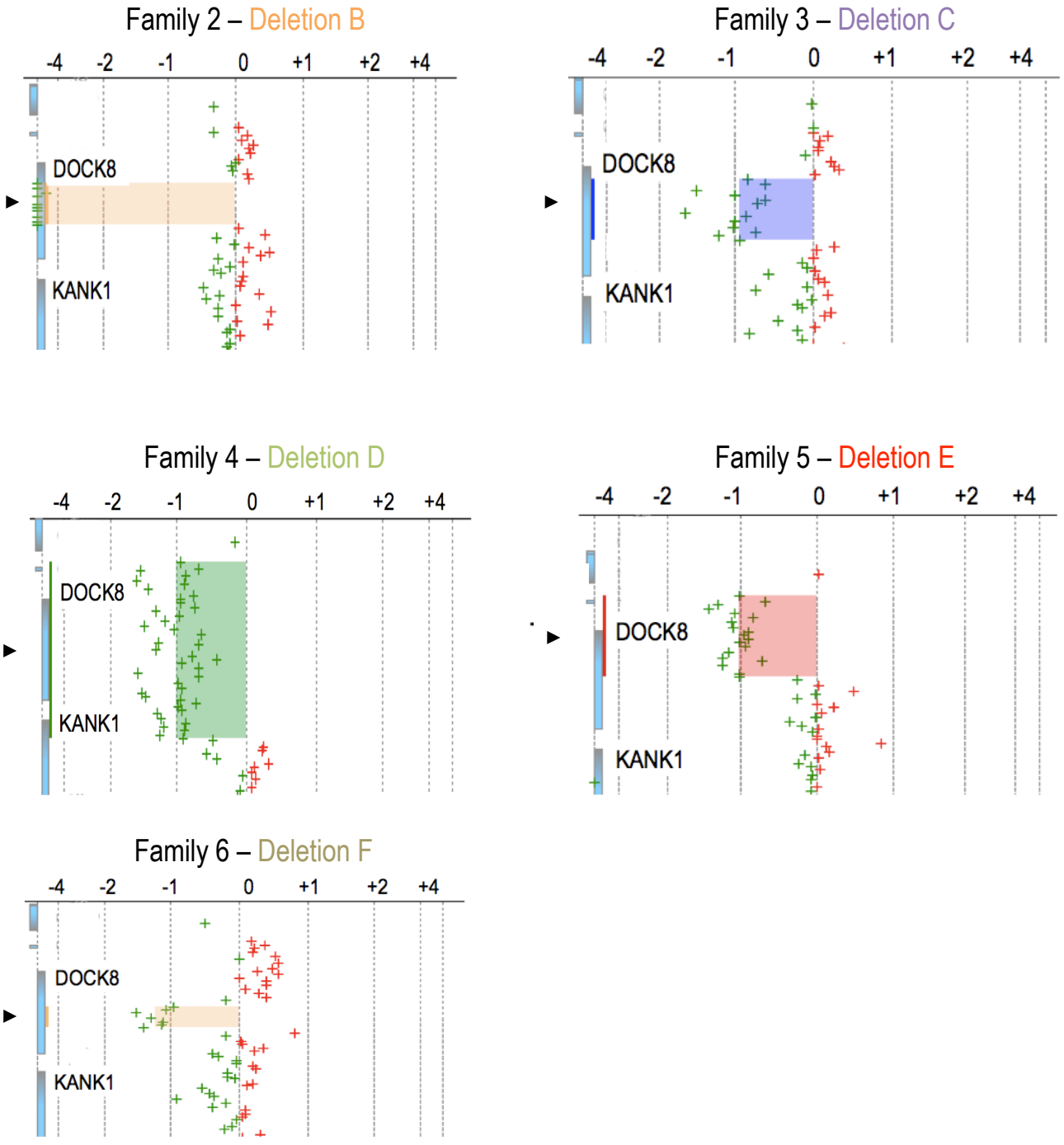
B

Supplemental Figure 1



Supplemental Figure 2

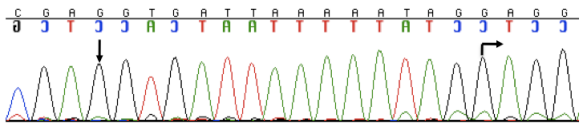
A



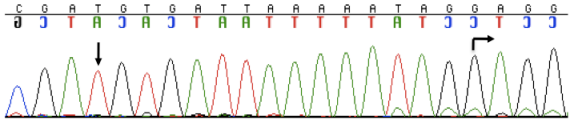
B

Supplemental Figure 2

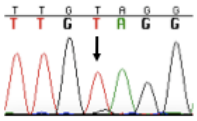
Pt 3-1



Control

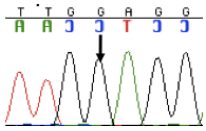


Pt 4-1



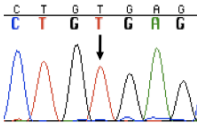
L X

Control



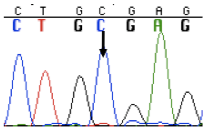
L E

Pt 5-1



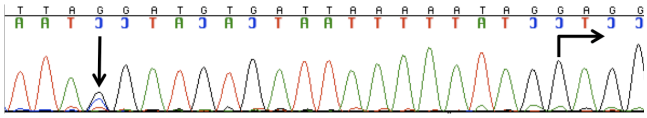
L X

Control

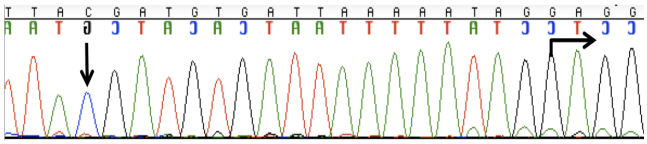


L R

Pt 6-1

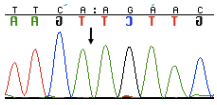


Control



Pt 7-1

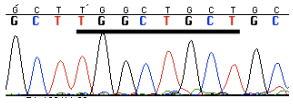
Allele I



T T CA_AGA A C

F K N

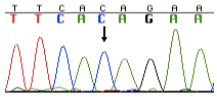
Allele II



G C T TGGCTGCTGC

A W L L

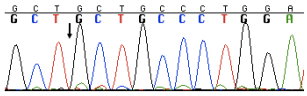
Control



T TC ACAG A A

F T E

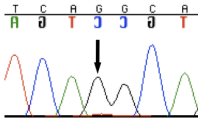
Control



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

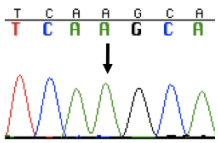
A A A L

Pt 8-1



F R

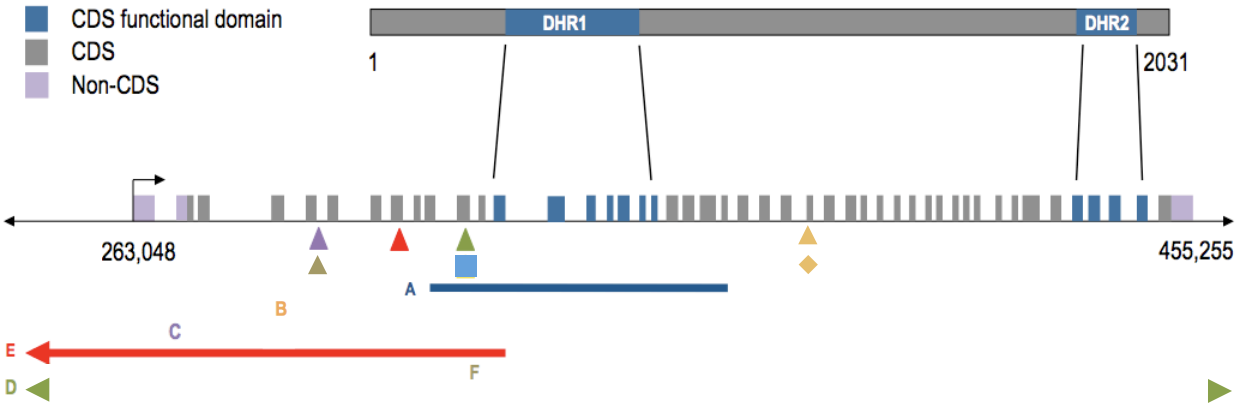
Control



F K

C

Supplemental Figure 2

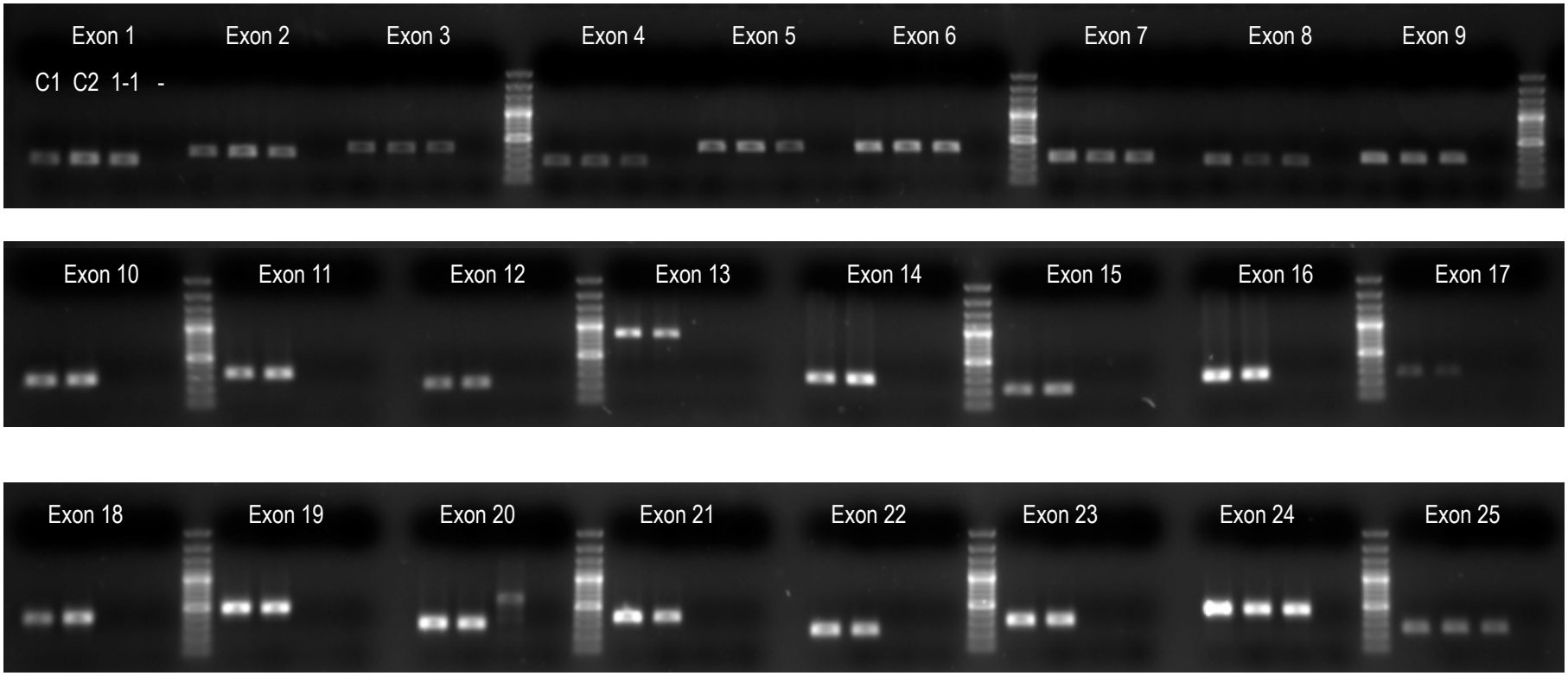


Symbol	Mutation
A	chr9:g.323,830_384,034del ➔ c.921_2765del ➔ p.308S_922Ldel (Pt 1-1)
B	chr9:g.301,734_388,139del ➔ c.426_3017 ➔ p.109A_972Kdel (Pt 2-1)
C	chr9:g.(278,220_285,346)_(395,056_406,292)del (Pt 3-1)
D	chr9:g.(153,190_194,193)_(528,588_534,450)del ➔ none (Pts 4-1, 4-2)
E	chr9:g.(153,190_194,193)_(351,777_360,184)del ➔ none (Pts 5-1, 5-2)
F	chr9:g.(330,142_346,079)_(395,056_406,292)del (Pt 6-1)

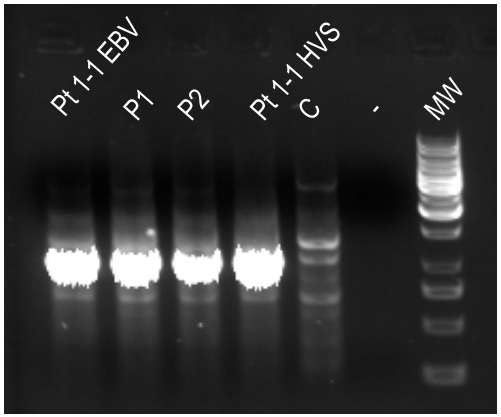
Symbol	Mutation
▲	c.538-15T>G ➔ p.E180VfsX4 (Pt 3-1)
▲	c.1153G>T ➔ p.E385X (Pts 4-1, 4-2)
▲	c.745C>T ➔ p.R249X (Pts 5-1, 5-2)
▲	c.538-18C>G ➔ p.E180VfsX4 (Pt 6-1)
▲	c.3290delC ➔ p.T1097KfsX29 (Pt 7-1)
◆	c.3303_3304insTGGCTGCT ➔ p.A1102WfsX27 (Pt 7-1)
■	c.1214A>G ➔ p.K405R (Pts 8-1, 8-2)

Supplemental Figure 3

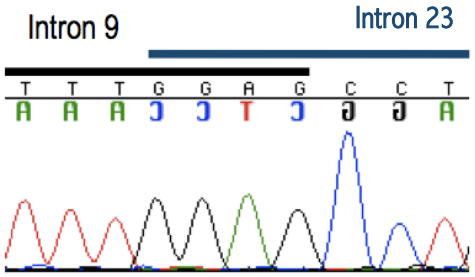
A



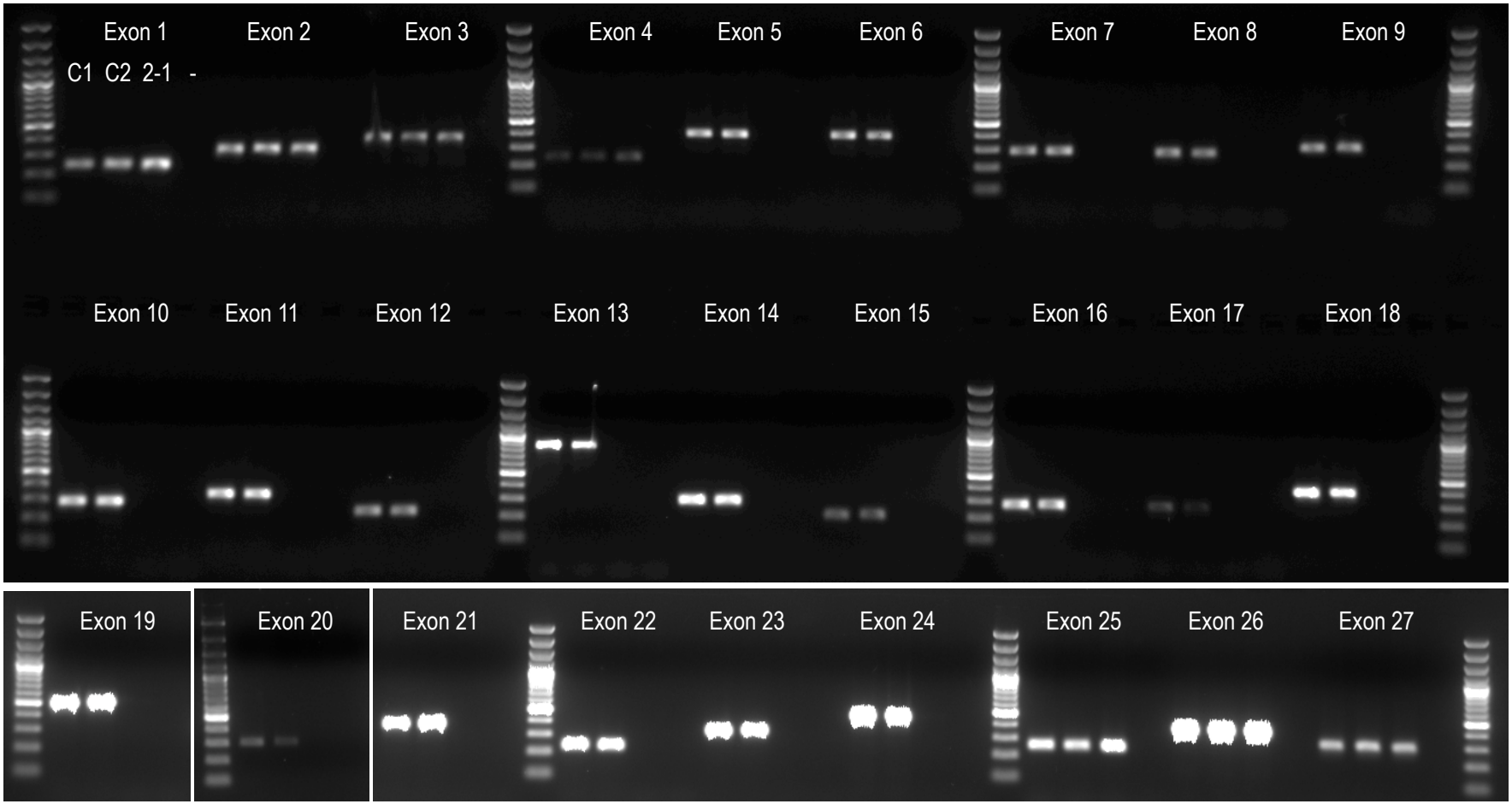
B



C

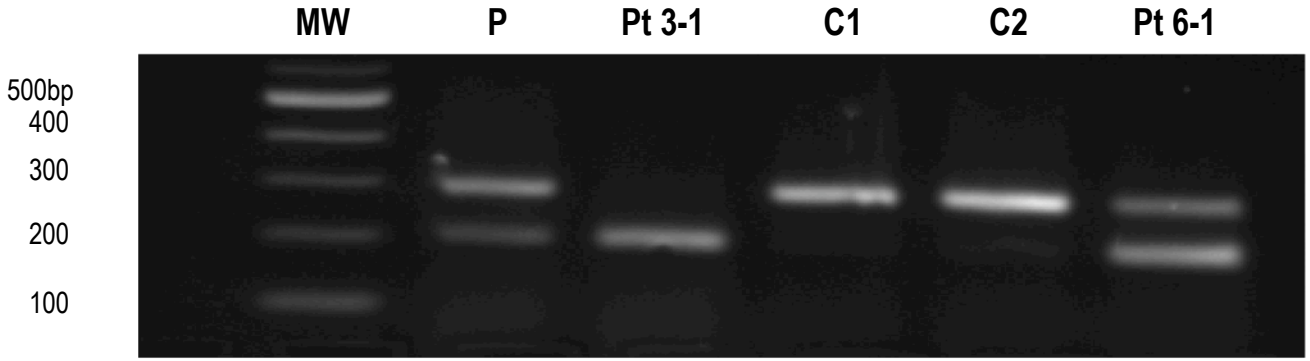


Supplemental Figure 4

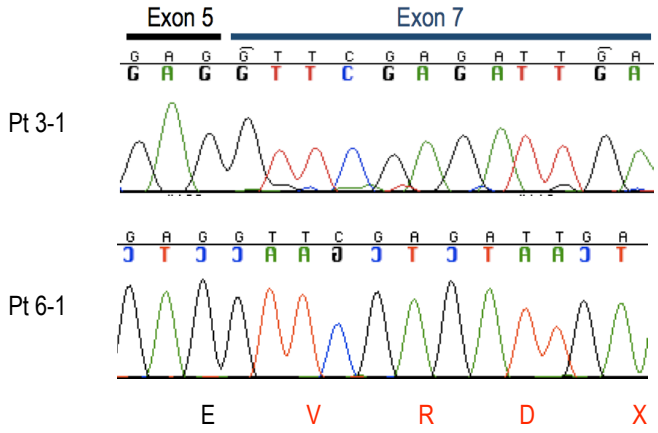


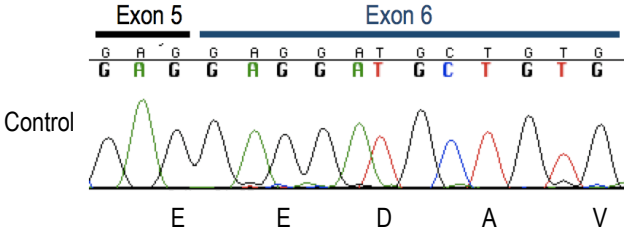
Supplemental Figure 5

A

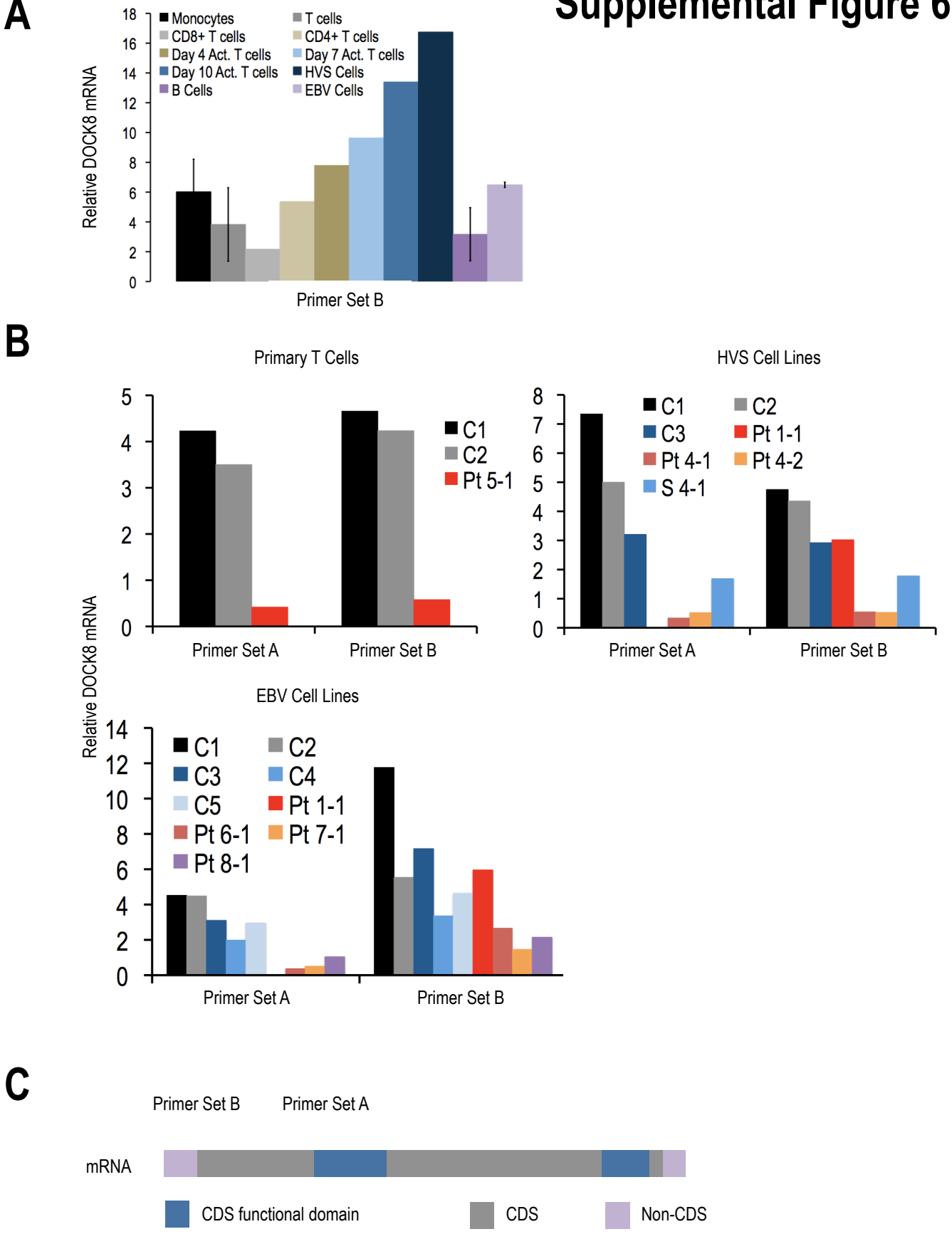


B



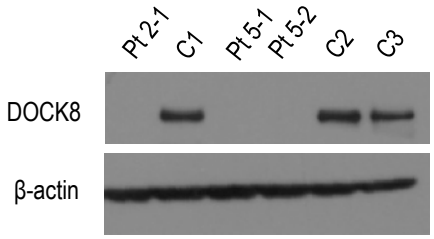


Supplemental Figure 6

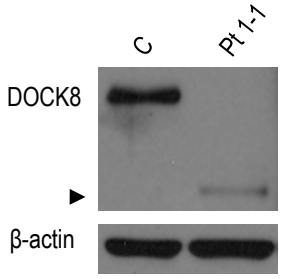


Supplemental Figure 7

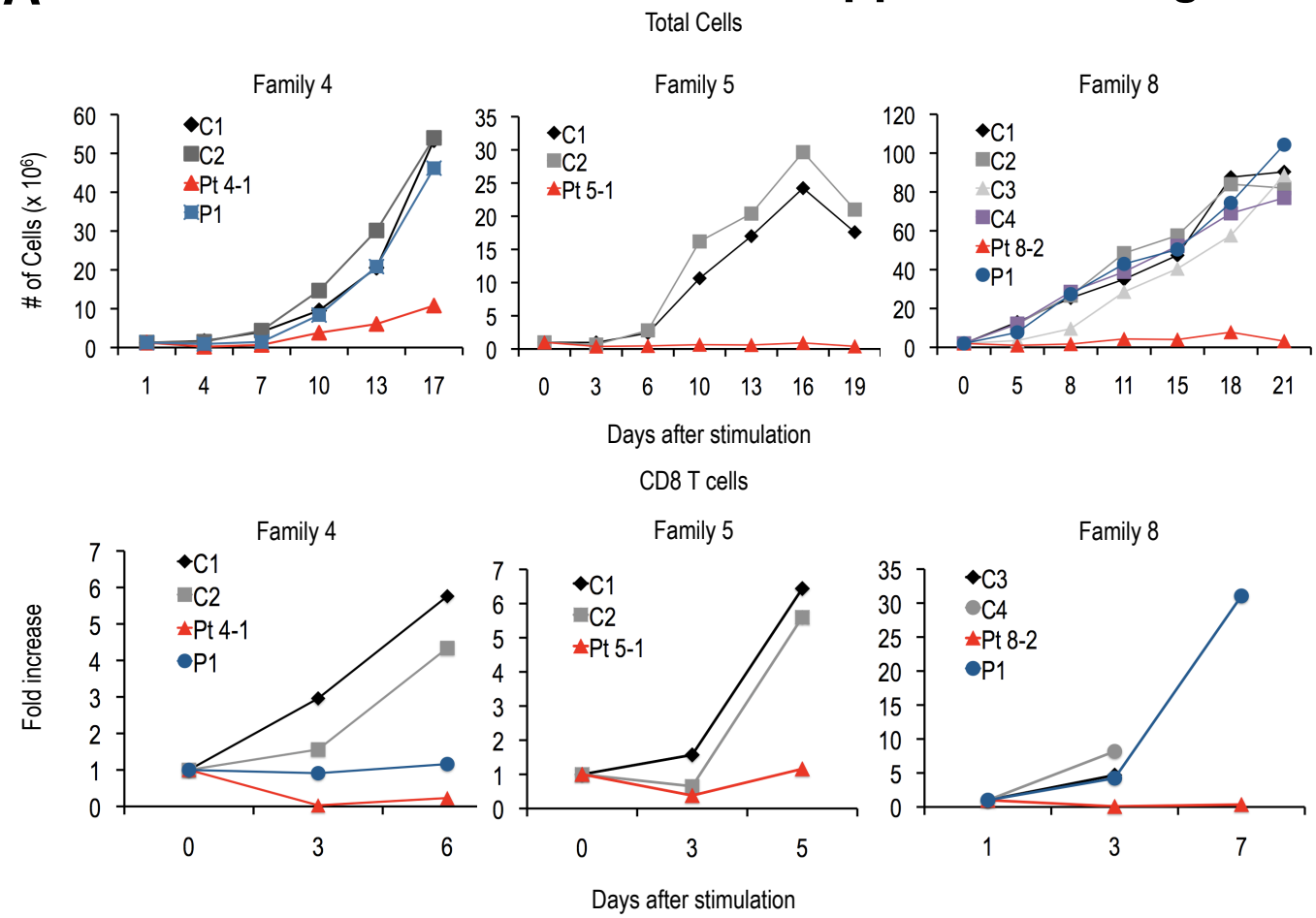
A

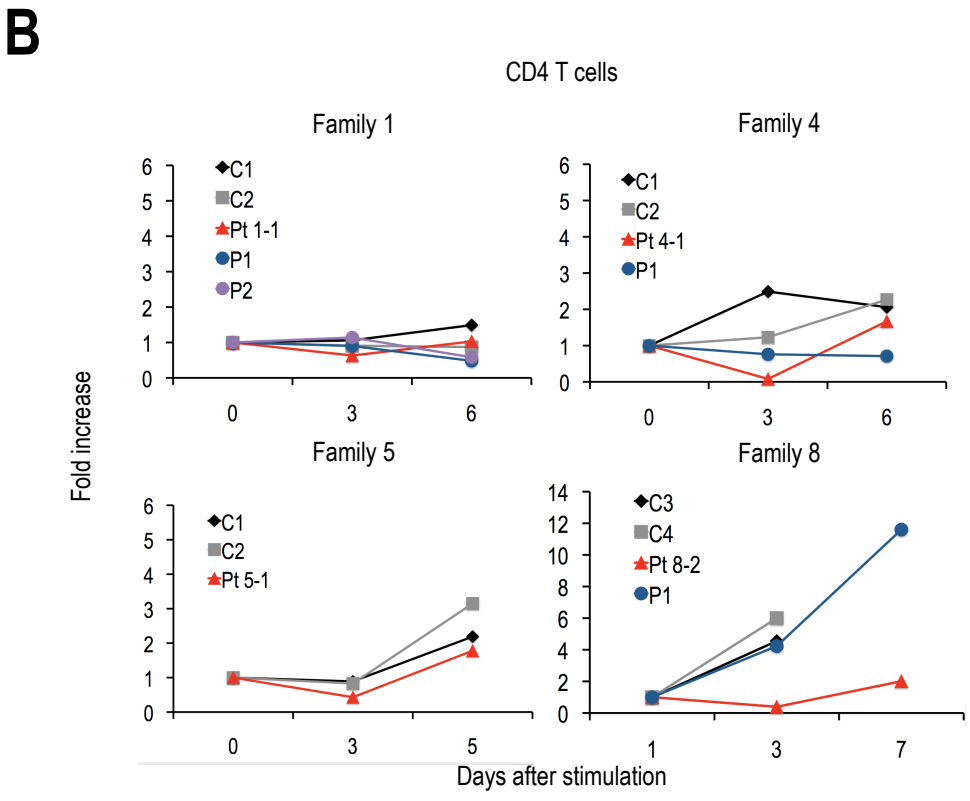


B



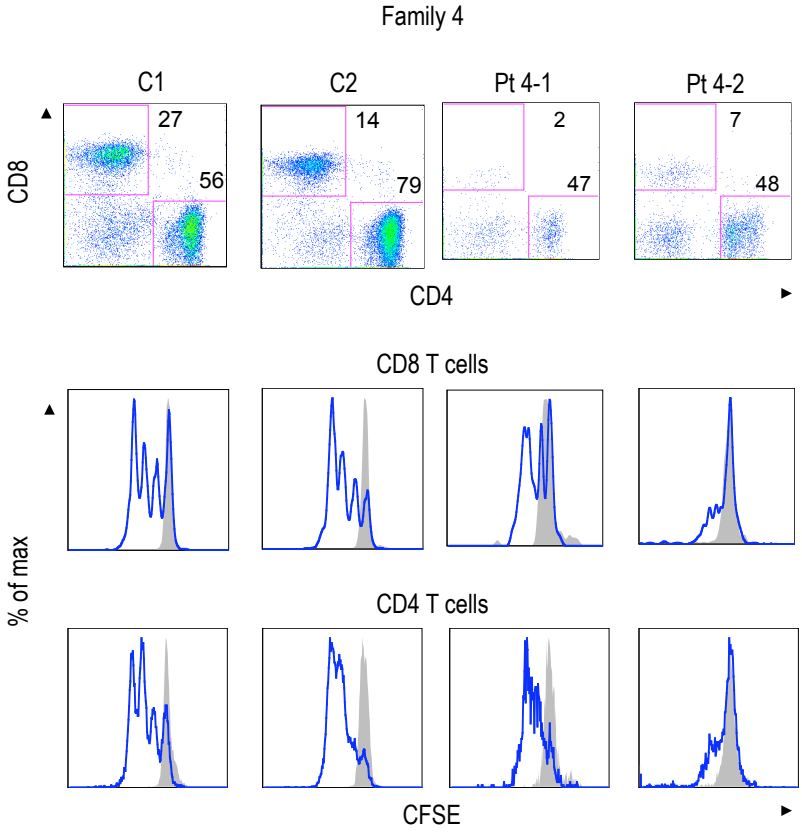
Supplemental Figure 8



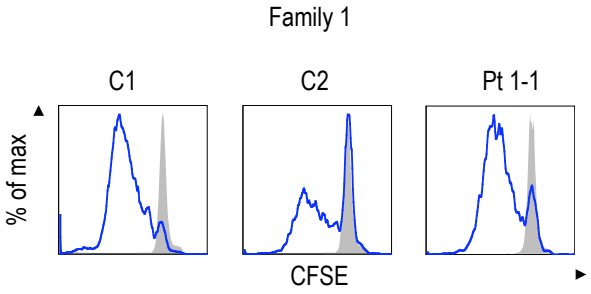


Supplemental Figure 8

C



D



Supplemental Figure 9