Gene	Variant Type	CADD Score	# in ExAC	Description	Mutation Pos. Family Members
RELA	**Nonsense**	**37**	**0**	**NF-kB family member p65**	**None / De-Novo**
IKBKE	Missense	33	8	Serine/Threonine kinase capable of phosphorylating and inactivating inhibitors of NF-kB	Father
IKBIP	Missense	16.5	0	IkB kinase (IKK) interacting protein	Father
LAG3	Missense	26.3	0	Lymphocyte activating protein	Mother
FGR	**Missense**	**25.4**	**0**	**Tyrosine protein-kinase; proto-oncogene, known role in the immune system**	**Mother**
KLHL42	Missense	25.7	0	Substrate speicfic adapter of BCR E3 ubiquitin ligase complex; involved in class I MHC antigen processing and presentation	Father
ADRA1B	**Missense**	**24.9**	**0**	**Adrenergic receptor, GPCR signaling through calcium-phosphatidylinositol second messenger system**	**Father/Sister**
LPIN2	Missense	<1	0	Involved with metabolism of fatty acids; associated with Majeed syndrome (autosomal recessive autoinflammatory bone disorder)	Father/Sister
PPL	Missense	23.3	0	Component of desmosomes in keratinocytes; associated with pemphigus (autoimmune disease)	Mother/Sister
RAB44	Missense	22.7	0	Ras oncogene family protein (small G protein); involved in neutrophil degranulation	Mother
RGS8	Arg89His	20.7	0	Regulates signaling through GPCRs	Mother/Sister
DLK2	Missense	14	0	Notch ligand; regulates adipogenesis; p38-MAPK related signaling pathway	Father/Sister
BANK1	Missense	3.7	0	B-cell scaffolding protein, polymorphisms associated with lupus	Mother
CYBA	Missense	2.9	0	Cytochrome B-245 alpha chain; associated with chronic granulamtous disease (CGD)	Father/Sister
STK10	Missense	1.6	0	Serine/threonine kinase; negatively regulates IL-2 expression in T cells through MAPK pathway	Mother
JUN	**Missense**	**<1**	**0**	**Subunti of AP-1 transcription factor, proto oncogene**	**Father**
UHRF1BP1L	In-frame	N/A	0	No info	Father
FANCA	Frameshift deletion	N/A	0	Associated with Fanconi anemia	Father/Sister
TUBB3	Frameshift deletion	N/A	0	Member of beta tubulin family ; associated with cortical dysplasia	Mother/Sister
MC1R	Frameshift deletion	N/A	0	Protein receptor for melanocyte stimulating hormone	Mother/Sister
GEMIN7	Frameshift deletion	N/A	0	Plays a role in assembly of small nuclear ribonucleaproteins	Father
IGLON5	Missense	29.9	0	Associated with sleep disorders	Mother
MAPK8IP2	Missense	29.1	0	MAPK8 interacting protein; associated with cervical vrrocous carcinoma	Mother/Sister
MYH16	Essential splice site	28.5	0	Pseudogene	Father/Sister
GSG1L	Missense	28.2	0	Component of AMPA receptor	Father/Sister
KIAA1324L	Missense	28.1	0	No info	Father/Sister
ADAT2	Missense	27.9	0	tRNA specific adenosine deaminase	Father/Sister
HSPA12B	Missense	26.4	0	Heat shock protein	Mother
GFM2	Missense	26.3	0	Mitochondrial translational elongation factor; associated with Leigh syndrome	Father/Sister
AGBL4	Missense	25.7	0	ATP/GTP Binding Protein Like 4, associated with macular degeneration	Father
LIFR	Missense	25.3	0	Receptor for the polyfunctional cytokine leukemia inhibitory factor; associated with bent-bone dysplasias	Mother/Sister
PCDH17	**Missense**	**25.3**	**0**	**Protocadherin**	**Father/Sister**
METTL17	Missense	25.3	0	Methyltransferase like protein	Father
TAS2R8	**Missense**	**24.9**	**0**	**Taste receptor**	**Mother/Sister**
ZNF45	Missense	24.8	0	Zinc finger protein	Mother
BMP6	Missense	24.7	0	Protein ligand of TFG-beta superfamily of proteins; associated with hemachromatosis and bone marrow necrosis	Father
AQP6	Missense	24.7	0	Aquaporin	Mother
TGM3	Missense	24.2	0	Transglutaminase; associated with dermatitis and ALS	Father/Sister
PITX1	**Missense**	**23.9**	**0**	**Homeobox protein; associated with developmental disorders**	**Father/Sister**

ZBED1	Missense	23.7	0	Transcription factor; associated with laryngostenosis	Father
CPXM1	Missense	23.4	0	Carboxypeptidase X	Father/Sister
HNRNPUL2	Missense	23.2	0	RNA Binding	None / De-Novo
PARVA	Missense	23.2	0	Actin binding protein; associated with metagonimiasis	Father/Sister
ZNF578	Missense	23.2	0	Zinc finger protein	Mother
SIK1	Missense	23.1	0	Salt inducible kinase associated with epileptic encephalopathy	Mother
HYI	Missense	22.1	0	Metabolic enzyme	Father
TMEM8B	Missense	21.8	0	Putative tumor suppressor	Father
PDSS1	Missense	21.5	0	Metabolic enzyme; associated with CoQ10 defiency	Father/Sister
SCIN	Missense	21.3	0	Ca2+-dependent actin severing and capping protein involved in endocytosis regulation	Father
HIST1H1T	Missense	20.7	0	Histone	Father/Sister
EAF1	Missense	20.6	0	Transcriptional transactivator; associated with eosinophilic angiocentric fibrosis	Father/Sister
CTNNAL1	Missense	19.4	0	Scaffold for RhoGEF	Mother/Sister
ANKRD11	Missense	19	0	Inhibitor of transcription; associated with kgb syndrome	Mother/Sister
NAA30	Missense	15.5	0	Associated with encephalitis	Father/Sister
R3HDM1	Missense	15	0	Nucleic acid binding protein	Mother
ZNF287	Missense	14.6	0	Zinc finger protein	Mother
LMNB1	Missense	14.4	0	Protein part of nuclear lamina; associated with dominant leukodystrophy	Mother
ARID4A	Missense	13.2	0	Binds to pRb; associated with retinoblastoma	Father/Sister
COL13A1	Missense	12.5	0	Collagen type XIII Alpha 1 chain	Father
FLJ14816	Missense	11.9	0	No info	Father
CEP135	Missense	11.2	0	Centrosomal protein; associated with microcephaly (recessive)	Father
PPARGC1B	Missense	10.9	0	Stimulates activity of other transcription factors; allelic variants associated with obesity	Mother
OPA3	Missense	10.7	0	Outer mitochondrial membrane lipid metabolism regulator	Father
FASTKD1	Missense	9.8	0	Nucleic acid binding/protein kinase activity	Mother/Sister
SLC22A13	Missense	7.9	0	Transmembrane protein involved in small molecule transport	Mother
RP11-725M22.1	Missense	4.6	0	RNA gene	Mother/Sister
LMO7	Missense	2.1	0	Involved with protein-protein interactions	Mother
AGMAT	Missense	<1	0	Enzyme involved in proline and arginine metabolism	Father/Sister
ZNF687	Missense	<1	0	Zinc finger protein associated with bone disease	Father
LGSN	Missense	<1	0	Associated with retinitis pigmentosa (lens protein)	Mother
ZNF90	Missense	<1	0	Zinc finger protein	Mother

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RELA haploinsufficiency in CD4 lymphoproliferative disease with autoimmune cytopenias.

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Figure Legends

Electronic Repository Figure 1: Normal patient T cell death in response to multiple stimuli.

Fas induced apoptosis of patient and control $CD4^+$ T cell blasts following crosslinking of the FAS receptor for 18hrs (A). Restimulation-induced cell death of patient and control $CD4^+$ T cell blasts following ligation of the TCR with the indicated dose of anti-TCR antibody for 18hrs (B). IL-2 withdrawl induced cell death of patient and control cells (C). Live cells were counted as those that excluded AnnexinV and propidium iodide (PI) dyes. Results are representative of two different experiments.

Electronic Repository Figure 2: Enhanced T cell differentiation into Th1 effectors and decreased iTreg formation in patient cells.

Flow cytometric plots showing staining of ex-vivo $CD4^+$ T cells for the chemokine receptors CXCR3 and CCR6. Expression of these chemokine receptors denote the indicated effector type cell (A). Flow cytometric plots showing staining of ex vivo $CD4^+$ T cells for CD25 and CD127. The gate is drawn to show the frequency of nTregs in the patient compared to controls (B). FOXP3 expression on day 5 post-activation of naïve $CD4^+$ T cells grown with the indicated amounts of TGFβ, representative of three different experiments (C). Quantification of FOXP3 induction in cells stimulated as in C, % of FOXP3 positive cells were normalized to the maximum response of 8 different controls at 10 ng/mL of TGFβ for combined analysis. Pooled from three different experiments (D).

Electronic Repository Figure 3: Normal B cell development and antibody responses in patient B cells

Flow cytometric analysis of ex-vivo B cell populations in patients compared to controls (A). Levels of IgM, IgG, and IgA measured in patient serum during repeated clinical evaluation, red dashed lines indicate the normal range ($5\text{-}95^{th}$ percentiles) (B, C, and D, respectively).

Electronic Repository Figure 4: Patient T cells express normal levels of NF-κB subunits other than p65 and have altered p65 containing dimers

Western blot showing levels of NF-κB subunits in NP-40 cytosolic fractions and in p65 co-immunoprecipitations, representative of 3 different experiments (A). Densitometry quantification of NF-κB subunits in IP lanes from three different experiments (B). Densitometry quantification of NF-kB subunits in IP lanes, normalized to control levels of each subunit (B). Densitometry quantification of NF-kB subunits in IP lanes, normalized to p65 levels immunoprecipitated from the same sample (C).

Electronic Repository Table 1: Rare or novel genetic variants in patient identified by WGS

Very rare or novel coding variants found in whole genome sequencing of the patient. Highlighted fields denote variants in genes known to play a role in the immune response and bold text indicates variants in genes denoted as missense (Z Score above 3.873) constrained in the ExAC database. Red text indicates genes known to play a role in regulation of NF-κB signaling.

Materials and Methods

Human Subjects

Written informed consent was provided by all human subjects (or their legal guardians) in accordance with Helsinki principles for enrollment in research protocols that were approved by the Institutional Review Board of the National Institute of Allergy and Infectious Diseases, National Institutes of Health (NIH). Patient and healthy control blood was obtained at the NIH under approved protocols. As described previously, mutations will be automatically archived by Online Mendelian inheritance in Man (OMIM) at time of publication, and whole-genome data will be submitted in dbGaP.

Genetic Analysis Methods

Genomic DNA (gDNA) was obtained from probands and family members by isolation and purification from peripheral blood mononuclear cells (PBMCs) using Qiagen's DNeasy Blood and Tissue Kit. DNA was then submitted for Whole Genome Sequencing (WGS). Whole Genome Sequencing was performed by massively parallel sequencing by Illumina HiSeq sequencing system on the collected DNA. For individual samples, WGS produced 30-60x average sequence coverage. All sequenced DNA reads were mapped to the hg19 human genome reference by Burrows-Wheeler Aligner with default parameters. Single nucleotide variant and indel calling were performed using the Genome Analysis Toolkit version 3.4 (the Broad Institute). Variants were then annotated by VEP and prioritized by GEMINI (GEnome MINIng) based on population allele frequency, functional prediction and potential genetic models (autosomal recessive or de novo models).

Primary cells

Patient or control blood was subjected to a Ficoll density gradient centrifugation, after which peripheral blood mononuclear cells (PBMCs) were collected from the interface. Naïve $CD4^+$ T cells were isolated to at least 95% purity by negative selection using the Naive $CD4^+$ T Cell Isolation Kit II (Miltenyi).

Media

Human T cells were grown in RPMI 1640 (Gibco / Thermo Fisher) supplemented with 10% heat inactivated FBS (Gibco), 1% Penicillin / Streptomycin (Gibco), 1% L-Glutamine (Gibco), 50uM 2-Mercaptoethanol (Sigma-Aldrich), and 100 units/mL IL-2 (complete RPMI).

Antibodies and Reagents

The rabbit-anti N and C-terminal p65 antibodies were from Biolegend and Santa Cruz, rexpectively. The mouse-anti-p65 for flow cytometry was from biolgend. Flow cytometry antibodies (IL2-APC, IFNγ-FITC, CD45RA-PE-Cy7, CCR7-PE, CD8-APC-CY7, CD3-BV421, CD25-PE, CD127-APC, CD45RO-APC-Cy7, FOXP3 PE and FOXP3 APC, CD20-APC-Cy7, CD27 PE-Cy7, CD19-PerCP-Cy5.5 were all from biolegend. CFSE was from Biolegend. All secondary antibodies for flow cytometry and western blotting were from Invitrogen. C-REL, RELB, and NF-κB antibodies were from Cell Signaling Technologies and the NF-κB1 antibody was from Biolgend.

Flow Cytometry

Suspension cells were collected from culture following mixture of the culture by repeat pipetting. Cells were washed and resuspended in FACS buffer (1% FBS, 0.05% sodium azide, and 5mM EDTA in PBS) at $2x10^6$ cells/ml in the presence of human TruStain FcX (biolgend). Staining or isotype antibodies were added at 1:200 final dilutions and incubated with cells at 4^oC for 30 minutes to 1hr. Cells were then washed 3X and resuspended in FACS buffer with 1% PFA and analyzed by flow cytometry. For intracellular staining of cytokines or p65 cells were collected, washed 2X in FACS buffer and fixed in 4% PFA in PBS for 20minutes. Fixed cells were washed three times and permeabilized for 5minutes in 0.2% Triton X-100. Flow files were analyzed on FlowJo version 9.9 and all flow plots are presented on a log10 axis and histograms are presented as % of max expression for the Y-axis.

T Cell culture and activation

$CD4^+$ Naïve T cells were isolated from PBMCs of patients and controls using the Naive $CD4^+$ T Cell Isolation Kit II (Miltenyi) and expanded in complete RPMI following activation with CD3/28 stimulatory Dynabeads (invitrogen). Cells were passaged every 2-3 days and maintained below $1x10^6$ cells/mL. For iTreg differentiation assay 10nM all-trans retinoic acid (Sigma-Aldrich) and the indicated amount of TGFβ was added to culture conditions and cells were analyzed for FOXP3 expression on Day 6 of culture by flow cytometry. For IFNy/ IL-2 analysis cells were taken within the second week of culture, removed from the stimulatory beads by magnetic seperation and rested in basal RPMI for 3hrs. After the three-hour rest cells were or weren't stimulated with PMA (10ng/mL) and Ionomycin (1μg/mL) in the presence of Monensin (Biolegend) for 4-5hrs. Intracellular cytokine accumulation was then analyzed by flow cytometry. To access antigen driven proliferation $CD4^+$ T cell blasts were rested down to a non-proliferating state by daily halving of the amount of IL-2 in the culture until 1unit/mL was reached. At this point cells appeared small and round. Cells were then collected and live cells were enriched by ficoll purification. Cells were then labeled with 1μM CFSE for 5 minutes at room temperature and washed 3X in complete RPMI (5 units/mL IL2). Cells were then stimulated for 3-4 days in 96 well plates that had been previously coated with 1μg/mL anti-CD28 and the indicated dose of anti-CD3. Cells were then collected and analyzed by flow cytometry for CFSE dilution.

Cell death assays

Patient or control $CD4^+$ T cell blasts, between 2-3 weeks post initial activation, were collected and resupended in fresh IL-2 containing media. Cells were then added to 96 well plates in triplicate and stimulated with either the indicated amount of anti-Fas antibody (APO-1-3) with 1/10 the amount of Protein-A or the indicated amount of plate adsorbed anti-CD3 (HIT3a) (Biolegend) for 18hrs. Cells were then collected and stained with AnnexinV-APC and Propidium Iodide (PI) and analyzed by flow cytometry. For IL-2 withdrawal, cells were collected and resupended in media with or without 100units/mL IL-2. The percentage of live cells was determined by AnnexinV and PI staining for both populations and expressed as a percentage of cells lost from media that did not contain IL-2.

Quantitative RT-PCR

Total RNA was isolated with the RNeasy kit (Qiagen). cDNA was synthesized from 1ug of total RNA using the SuperScript III First-Strand Synthesis System (Thermo Fisher Scientific). cDNA was brought to a total volume of 100μL in double distilled (DD)H_2O and either immediately used or stored at -80℃ for future use. Quantitative RT-PCR for RELA and GAPDH was performed using the Power Syber green (Thermo Fisher) method on a 7900HT machine (ABI) using 2.5uL of cDNA reaction product. Results were analyzed by the $\Delta\Delta C_t$ method and normalized to the first healthy normal donor values. GAPDH Primers: Fwd-GATGACATCAAGAAGGTGGTG, Rev- ACCACCTGGTGCTCAGTGTAG. RELA Primers: Fwd- AACAACCCCTTCCAAGTTCCT, Rev- GATCTTGAGCTCGGCAGTGT.

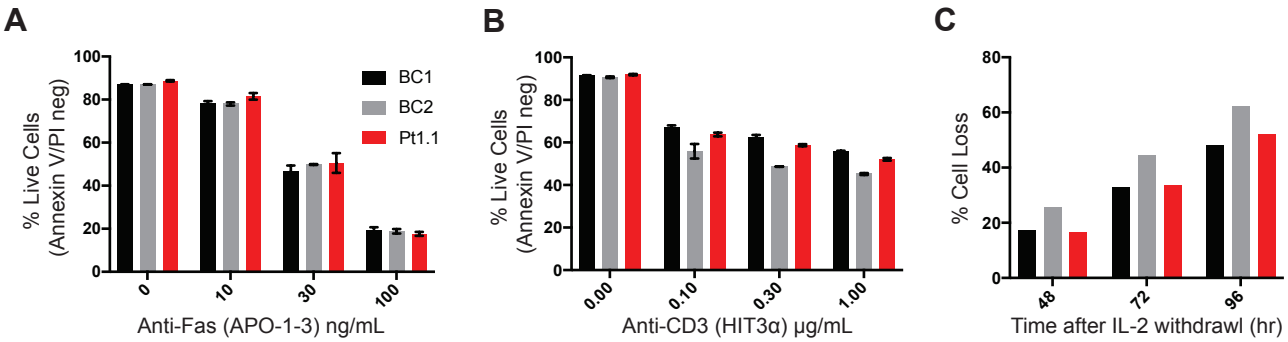
Immunoprecipitation and Western Blotting

For analysis of total p65 levels $CD4^+$ T cell blasts were washed in PBS and lysed in 1X RIPA buffer with complete protease inhibitor cocktail (Roche), and phosphatase inhibitor cocktails (Sigma-Aldrich) on ice for 20 minutes. The lysates were then cleared at 15,000 g at 4℃ for 15 min. For co-immunoprecipitation $20x10^6$ $CD4^+$ T cell blasts were lysed in 200uL NP-40 Hepes buffer (1%NP-40, 1mMEDTA, 50mM Hepes, 150mM NaCl) with protease inhibitor cocktail for 20minutes on ice and lysates were cleared at 15,000 g at 4℃ for 15 min. Some lysate was saved for analysis of the input. 100uL of cleared lysate was then combined with 50uL of washed and BSA blocked ProteinA dynabeads (Invitrogen) and 2ug of Rbt anti-p65 for 1hr at 4C with end-over-end rotation. Beads were then separated from the flowthrough by magnetic separation and washed 3X with 500uL of ice-cold lysis buffer. Flowthrough was saved for analysis of p65 depletion efficiency. Beads were then reconstituted in 100uL of ice-cold lysis buffer. Lysates and IP samples were then diluted with 2X SDS sample buffer (Quality Biologicals) supplemented with 10% BME. Approximately $2x10^6$ cell equivalents were separated by SDS-PAGE on 4-20% precast gels (Invitrogen) and transferred to a nitrocellulose membrane (invitrogen). Membranes were blocked with 3% BSA in Tris-buffered saline (TBS) with 0.01% Tween-20 (TBST) for 30minutes at room temperature before incubating with primary antibody overnight at 4℃. After 3x 5 minutes washes with TBST at room temperature with rocking, fluorescent secondary antibody was added for two hours at room temperature. After 5 x 5 minute washes in TBST and 1 wash in PBS membranes were analyzed on the LI-COR Odyssey imaging system.

Statistical Analysis

Flow plots are representative of at least three different experiments and graphs and standard deviations are generated by averaging at least three different experiments. Comparative statistics were not performed as patient samples represent biological repeats, not independent samples.

Electronic Repository Figure E1



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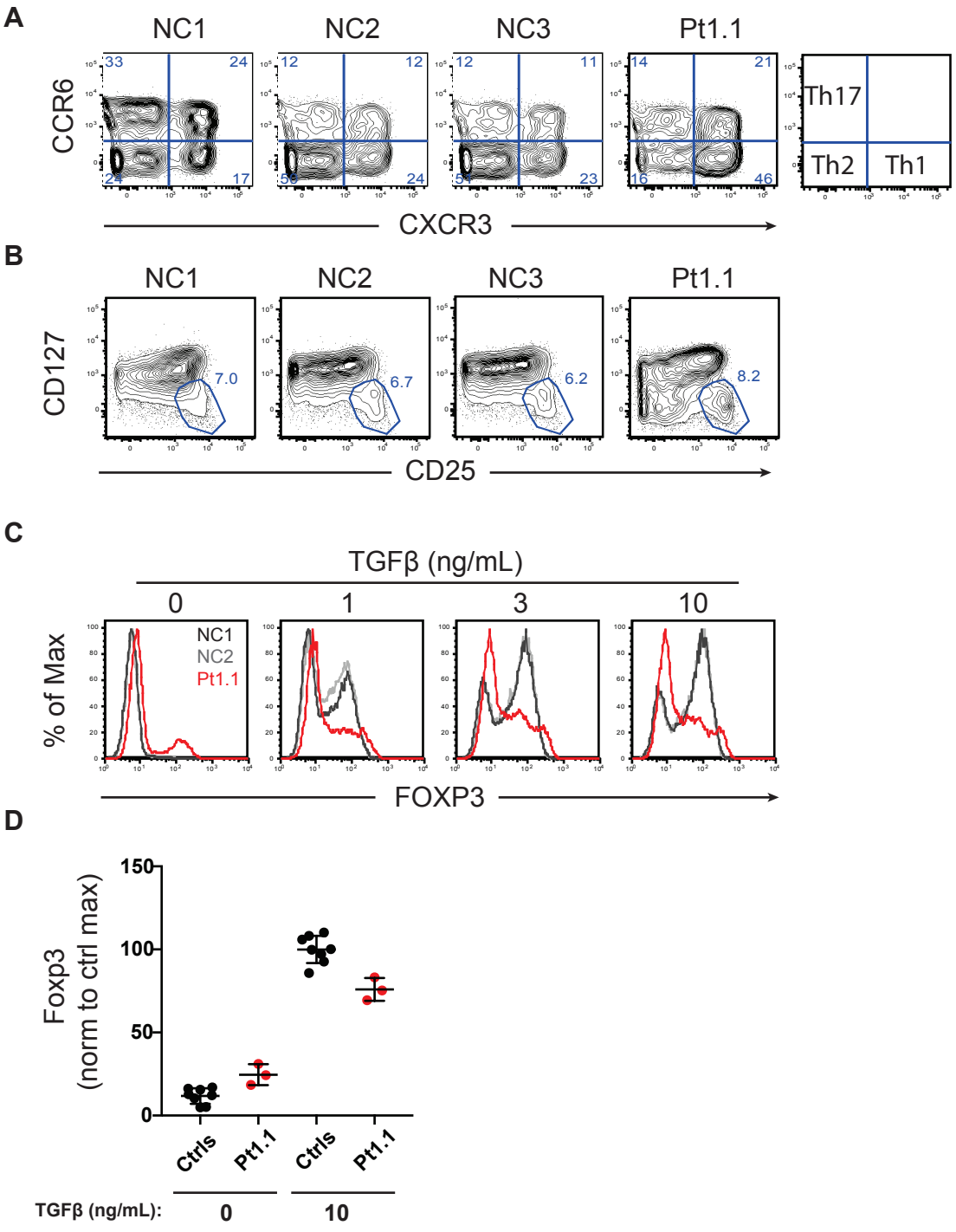

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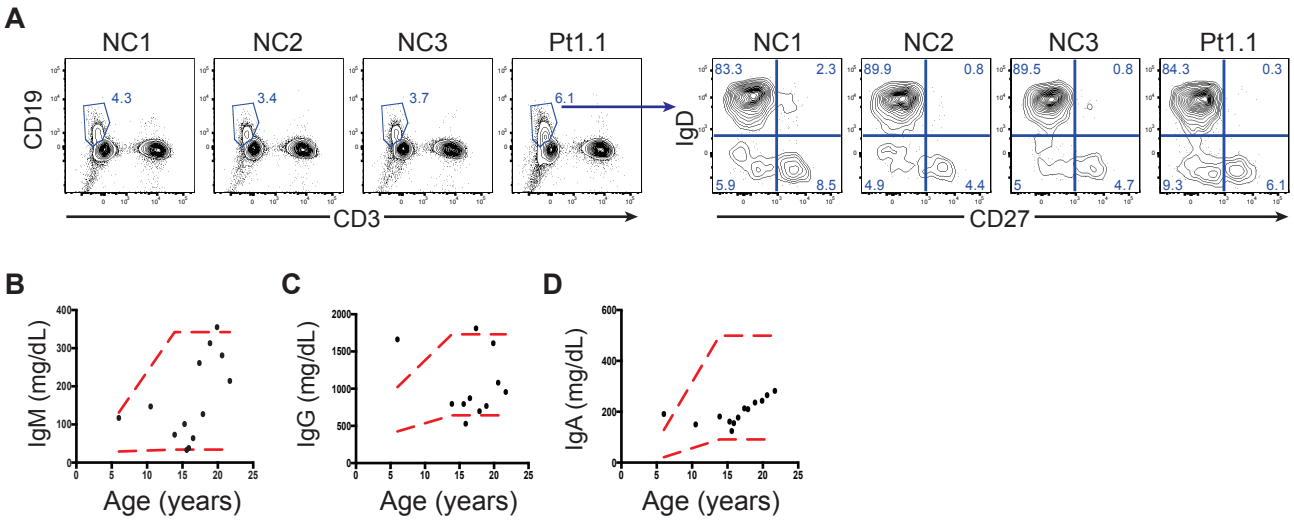

Electronic Repository Figure E4