

Signaling lymphocytic activation molecule (SLAM)/SLAM-associated protein pathway regulates human B-cell tolerance

Laurence Menard, PhD,^a Tineke Cantaert, PhD,^a Nicolas Chamberlain, BS,^a Stuart G. Tangye, PhD,^b Sean Riminton, MD, PhD,^c Joseph A. Church, MD,^d Amy Klion, MD,^e Charlotte Cunningham-Rundles, MD,^f Kim E. Nichols, MD,^g and Eric Meffre, PhD^a *New Haven, Conn, Darlinghurst and Sydney, Australia, Los Angeles, Calif, Bethesda, Md, New York, NY, and Philadelphia, Pa*

Background: Signaling lymphocytic activation molecule (SLAM)–associated protein (SAP) can mediate the function of SLAM molecules, which have been proposed to be involved in the development of autoimmunity in mice.

Objective: We sought to determine whether the SLAM/SAP pathway regulates the establishment of human B-cell tolerance and what mechanisms of B-cell tolerance could be affected by SAP deficiency.

Methods: We tested the reactivity of antibodies isolated from single B cells from SAP-deficient patients with X-linked lymphoproliferative disease (XLP). The expressions of SAP and SLAM family members were assessed in human bone marrow–developing B cells. We also analyzed regulatory T (Treg) cell function in patients with XLP and healthy control subjects.

Results: We found that new emigrant/transitional B cells from patients with XLP were enriched in autoreactive clones, revealing a defective central B-cell tolerance checkpoint in the absence of functional SAP. In agreement with a B cell–intrinsic regulation of central tolerance, we identified SAP expression in a discrete subset of bone marrow immature B cells. SAP colocalized with SLAMF6 only in association with clustered

B-cell receptors likely recognizing self-antigens, suggesting that SLAM/SAP regulate B-cell receptor–mediated central tolerance. In addition, patients with XLP displayed defective peripheral B-cell tolerance, which is normally controlled by Treg cells. Treg cells in patients with XLP seem functional, but SAP-deficient T cells were resistant to Treg cell–mediated suppression. Indeed, SAP-deficient T cells were hyperresponsive to T-cell receptor stimulation, which resulted in increased secretion of IL-2, IFN- γ , and TNF- α .

Conclusions: SAP expression is required for the counterselection of developing autoreactive B cells and prevents their T cell–dependent accumulation in the periphery. (J Allergy Clin Immunol 2013;■■■:■■■–■■■.)

Key words: SLAM-associated protein, signaling lymphocytic activation molecule, B-cell tolerance, regulatory T cells

From ^athe Department of Immunobiology, Yale University School of Medicine, New Haven; ^bthe Immunology Program, Garvan Institute of Medical Research, and St Vincent's Clinical School, University of New South Wales, Darlinghurst; ^cthe Department of Immunology, Concord Hospital, Sydney; ^dthe Divisions of Clinical Immunology and Allergy, Children's Hospital Los Angeles and Keck School of Medicine, University of Southern California, Los Angeles; ^ethe Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda; ^fthe Department of Medicine, Immunology Institute, Mt Sinai School of Medicine, New York; and ^gthe Division of Oncology, Children's Hospital of Philadelphia.

Supported by National Institutes of Health (NIH)/National Institute of Allergy and Infectious Diseases (NAID) grants AI061093, AI071087, AI082713, AI095848 (to E.M.), and T32 AI089704 (to L.M.); the Division of Intramural Research, NIH/NAID (to A.K.); and a Rubicon grant from The Netherlands Organization for Scientific Research (to T.C.).

Disclosure of potential conflict of interest: L. Menard has received research support from the National Institutes of Health (NIH; T32 AI089704). S. G. Tangye has been supported by one or more grants from the National Health and Medical Research Council of Australia and from Cancer Council NSW; has received support for travel from ESID, Keystone Symposia, and the Jeffrey Modell Foundation; and has provided expert testimony in a patent dispute. E. Meffre has received research support from the NIH/National Institute of Allergy and Infectious Diseases (AI071087, AI082713, AI061093, and AI095848) and from AbbVie, Biogen, and Merck Serono. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication March 20, 2013; revised August 28, 2013; accepted for publication October 15, 2013.

Corresponding author: Eric Meffre, PhD, Yale University School of Medicine, 300 George Street, New Haven, CT 06511. E-mail: eric.meffre@yale.edu.

0091-6749

<http://dx.doi.org/10.1016/j.jaci.2013.10.051>

X-linked lymphoproliferative disease (XLP) 1 is a primary immunodeficiency caused by mutations/deletions in the *SH2D1A* gene, which encodes the signaling lymphocytic activation molecule (SLAM)–associated protein (SAP).^{1–3} SAP is a single SH2 domain–containing molecule that plays a crucial role in the signaling of SLAM molecules. It might function as an adaptor for the Src family tyrosine kinase Fyn, as well as a competitor for phosphatases, such as Src homology domain 2–containing protein tyrosine phosphatase (SHP) 1 and SHP-2, thereby modulating the function of SLAM family members.⁴ The SAP/SLAM pathway has been implicated in the development of autoimmunity. The mouse *Sle1b* locus, which has been linked to lupus susceptibility, contains genes encoding members of the SLAM family.⁵ In the lupus-prone mouse strain NZM2410, the expression of the *Ly108.1* isoform leads to altered central B-cell tolerance mechanisms, including B-cell anergy, receptor editing, and deletion.⁶ Although polymorphisms in SLAM family genes have been linked to lupus and rheumatoid arthritis in human subjects,^{7,8} a direct role of the SAP/SLAM pathway in the control of B-cell tolerance in human subjects has not yet been demonstrated.

In healthy human subjects most developing autoreactive B cells are removed at 2 discrete steps.⁹ First, a central tolerance checkpoint in the bone marrow between early immature and immature B cells removes most of the developing B cells that express highly polyreactive antibodies. Then a peripheral B-cell tolerance checkpoint further counterselects autoreactive new emigrant B cells before they enter the mature naive B-cell compartment.⁹ The central B-cell tolerance checkpoint seems to be mostly regulated by B cell–intrinsic pathways. Alterations of the B-cell receptor (BCR) signaling pathway in patients lacking functional

Abbreviations used

ANA:	Anti-nuclear antibody
APC:	Allophycocyanin
BAFF:	B cell-activating factor of the TNF family
BCR:	B-cell receptor
CFSE:	Carboxyfluorescein succinimidyl ester
FOXP3:	Forkhead box protein 3
IPEX:	Immune dysregulation, polyendocrinopathy, enteropathy, X-linked
NKT:	Natural killer T
PE:	Phycoerythrin
SAP:	SLAM-associated protein
SHP:	Src homology domain 2-containing protein tyrosine phosphatase
SLAM:	Signaling lymphocytic activation molecule
TCR:	T-cell receptor
TLR:	Toll-like receptor
Treg:	Regulatory T
Tresp:	Responder T
XLP:	X-linked lymphoproliferative disease

Bruton tyrosine kinase (BTK) or in healthy subjects carrying the *C1858T PTPN22* risk allele result in failure to counterselect developing autoreactive B cells in the bone marrow.¹⁰⁻¹² In addition, mutations in genes encoding molecules, such as IL-1 receptor-associated kinase 4, myeloid differentiation primary response gene-88, UNC-93B, and adenosine deaminase, which mediate and regulate the functions of Toll-like receptors (TLRs), potentially sensing self-antigens, also interfere with the establishment of central tolerance, especially toward nucleic acid-containing antigens.^{11,13,14} Although showing normal central B-cell tolerance, CD40 ligand (CD40L)- and MHC class II-deficient patients display specific defects in the peripheral B-cell tolerance checkpoint characterized by high frequencies of autoreactive mature naive B cells correlating with low numbers of circulating CD4⁺CD25⁺CD127^{lo} forkhead box protein 3 (FOXP3)-positive regulatory T (Treg) cells.¹⁵ The essential role of Treg cells in regulating the peripheral B-cell tolerance checkpoint was demonstrated in FOXP3-deficient patients with immune dysregulation, polyendocrinopathy, enteropathy, X-linked (IPEX) syndrome, who display nonfunctional Treg cells and harbor severe defects in the counterselection of autoreactive peripheral B cells.¹⁶

To determine the role of the SAP/SLAM pathway in the establishment of human B-cell tolerance checkpoints, we analyzed the repertoire and reactivity of antibodies expressed by single new emigrant/transitional and mature naive B cells from SAP-deficient patients with XLP. We found that SAP is expressed by a discrete population of developing immature B cells and is required for central B-cell tolerance. We also found that SAP expression in T cells likely prevents the accumulation of autoreactive mature naive B cells, further suggesting the importance of B-cell/T-cell interactions for the establishment of peripheral B-cell tolerance.

METHODS**Patients**

Information from patients with XLP is included in Table E1 in this article's Online Repository at www.jacionline.org. Healthy donors were previously reported.^{9,10,12-17} None of the patients with XLP experienced accelerated

phases if they encountered EBV, and none of the 4 patients with XLP analyzed for antibody reactivity displayed the *C1858T PTPN22* risk allele, which by itself interferes with the counterselection of developing autoreactive B cells.¹⁰⁻¹² All samples were collected in accordance with institutional review board-reviewed protocols.

Cell staining and sorting, cDNA, RT-PCR, antibody production, ELISAs, and indirect fluorescence assays

Single CD21^{lo}CD10⁺⁺IgM^{hi}CD27⁻ new emigrant/transitional and CD21⁺CD10⁻IgM⁺CD27⁺ peripheral mature naive B cells from patients and control donors were sorted on a FACSVantage (Becton Dickinson, Mountain View, Calif) into 96-well PCR plates, and antibody reactivities were tested as previously described.⁹ The following antibodies were used for flow cytometric staining: CD19-allophycocyanin (APC)-Cy7, CD19-Pacific Blue, CD27-PerCP-Cy5.5, CD10-phycoerythrin (PE), CD10-PE-Cy7, IgM-fluorescein isothiocyanate, CD21-APC, CD4-APC-Cy7, CD25-PE-Cy7, CD127-PerCP-Cy5.5, CD45RO-Pacific Blue, CD48-fluorescein isothiocyanate, CD150-PE, CD352-PE, CD319-PE, CD244-APC (all from BioLegend, San Diego, Calif), CD3-eFluor605NC (eBioscience, San Diego, Calif), CD21-BDHorizonV450, and CD84-PE (Becton Dickinson). The anti-SAP mAb (clone 1C9; Abnova, Taipei, Taiwan) was biotinylated with the Fluoreporter Mini-biotin-XX protein labeling kit (Molecular Probes, Eugene, Ore). Intracellular staining for FOXP3-Alexa Fluor 488 (clone PCH101, eBioscience) and Helios-AlexaFluor647 (BioLegend) was performed with the FOXP3/Transcription Factor Staining Buffer Set (eBioscience), according to the manufacturer's instructions. For intracellular cytokine detection, CD4⁺ responder T (Tresp) cells activated for 4 days *in vitro* were then stimulated with 30 nmol/L phorbol 12-myristate 13-acetate and 200 nmol/L ionomycin for 4 hours in the presence of GolgiStop (BD Biosciences), and intracellular staining of cytokines (IFN- γ and TNF- α) was performed with FOXP3 staining buffers (eBioscience) and the following antibodies: IFN- γ (clone 4S.B3, eBioscience) and TNF- α (clone Mab11, eBioscience).

Real-time RT-PCR analysis

CD19⁺ cells from fetal liver, fetal bone marrow, or peripheral blood of healthy donors were enriched with CD19 magnetic beads (Miltenyi Biotec, Bergisch Gladbach, Germany). Peripheral mature naive B cells were further sorted to exclude the presence of CD3⁺ T cells, which represent the majority of circulating lymphocytes. Total RNA was then extracted with the Absolutely RNA Microprep Kit (Agilent Technologies), and 150-ng RNA samples were reverse transcribed by using random hexamers (Applied Biosystems, Santa Clara, Calif) and the SuperScript III Reverse Transcriptase kit (Invitrogen, San Diego, Calif). For mRNA gene expression assays, probes were purchased from Applied Biosystems (Foster City, Calif; *SH2D1A* [*SAP*]: Hs00158978_m1, *CD3E*: Hs01062241_m1, and *HPRT1*: Hs02800695_m1), and reactions were run on a 7500 Real-Time PCR system (Applied Biosystems) in duplicate. Values are represented as the difference in cycle threshold values normalized to *HPRT1* for each sample before comparisons between fetal samples and peripheral B and T cells.

Immunofluorescence

PBMCs depleted from CD20⁺ B cells, purified naive B cells (EasySep Naive B cell isolation kit), or pre-enriched CD34⁻CD19⁺ bone marrow B cells were washed in PBS 1 $\times$ and deposited on poly-L-lysine-coated glass slides (Sigma-Aldrich, St Louis, Mo) in a cytospin centrifuge (Thermo, Waltham, Mass). Acetone-fixed cells were stained with mouse anti-IgM (Santa Cruz Biotechnology, Santa Cruz, Calif), anti-Ig κ (BD Biosciences), anti-Ig λ (BD Biosciences), VpreB (BioLegend), or anti-SLAMF6/CD352 (BioLegend); rat anti-SAP-PE (clone 1D12, Santa Cruz Biotechnology) or rat isotype control-PE (eBioscience); or 4'-6-diamidino-2-phenylindole dihydrochloride or rabbit anti-Ig κ and anti-Ig λ (DAKO, Glostrup, Denmark). Primary antibodies were revealed with donkey anti-mouse Alexa Fluor 488

(Invitrogen, Molecular Probes); goat anti-rat-PE (Santa Cruz Biotechnology), followed by donkey anti-goat PE (Jackson ImmunoResearch, West Grove, Pa); and donkey anti-rabbit Alexa Fluor 350 (Invitrogen, Molecular Probes). The percentage of cells presenting a clustered pattern of either IgM or Igκ/Igλ was estimated by counting the number of cells with clustered versus diffuse/nonclustered IgM or Igκ/Igλ cells in the SAP⁺ and SAP⁻ cells. Slides were visualized under a Zeiss AxioObserver.Z1 microscope (Carl Zeiss, Oberkochen, Germany).

In vitro Treg cell suppression assay

CD4⁺ T cells were enriched with the EasySep Human CD4⁺ T cells kit (STEMCELL Technologies, Vancouver, British Columbia, Canada) and either stained for fluorescence-activated cell sorting of CD4⁺CD25^{hi}CD127^{lo/-} Treg cells or depleted from CD25⁺ cells with anti-human CD25 microbeads (Miltenyi Biotec) to obtain the Tresp cell fraction. CD4⁺CD25⁻ Tresp cells were labeled with CellTrace carboxyfluorescein succinimidyl ester (CFSE; InvivoGen, San Diego, Calif). Cocultures of Treg and Tresp cells at a 1:1 ratio with or without the addition of cytokines at 50 ng/mL concentration (PeproTech, Rocky Hills, NJ) were stimulated with the Treg Suppression Inspector Human kit (Miltenyi Biotec). Proliferation of the viable Tresp cells was analyzed by means of CFSE dilution at day 4.5.

Cytokine detection

Serum B cell-activating factor of the TNF family (BAFF) concentrations were determined by using ELISA, according to the manufacturer's instruction (R&D Systems, Minneapolis, Minn). Cytokines (IL-2, IL-4, IL-6, IL-7, IL-10, IL-13, IFN-γ, TNF-α, and GM-CSF) in plasma or culture supernatants were measured with the High Sensitivity Human Cytokine Magnetic Bead kit (Millipore, Temecula, Calif) by using a Luminex200. IL-21 was measured with the Human IL-21 ELISA Ready-Set-Go kit (eBioscience).

κ-Deleting recombination excision circle assay

The ratio of κ-deleting recombination excision circle joints (signal joint) to the Jκ-Cκ recombination genomic joints (coding joint) was determined, as previously described.^{18,19} The number of cell divisions was calculated by subtracting the cycle threshold of the PCR detecting the coding joint from that of the PCR detecting the signal joint.

Statistical analysis

Differences between patients and healthy donors were analyzed for statistical significance with unpaired Student *t* tests by using GraphPad Prism (GraphPad Software, La Jolla, Calif).

RESULTS

Defective central B-cell tolerance checkpoint in patients with XLP

Most developing B cells that express polyreactive and anti-nuclear antibodies (ANAs) are removed at a central B-cell tolerance checkpoint in the bone marrow. To assess whether central tolerance is functional in SAP-deficient patients with XLP, we cloned antibodies expressed by single CD19⁺CD10⁺⁺CD21^{lo}IgM^{hi}CD27⁻ new emigrant/transitional B cells from 8 patients with XLP (see Tables E1-E9 in this article's Online Repository at www.jacionline.org) and analyzed their immunoglobulin gene repertoire. Although the immunoglobulin heavy chain gene segment repertoires showed no differences between healthy donors and patients with XLP (see Fig E1 in this article's Online Repository at www.jacionline.org), we found that both Igκ and Igλ variable gene uses were biased in new emigrant/transitional B cells from patients with

XLP (see Fig E2 in this article's Online Repository at www.jacionline.org). D reading frames favoring hydrophobic amino acids have been suggested to favor self-reactivity.^{10,20} Remarkably, the D6-6 gene is the only D gene that was virtually never found using its hydrophobic reading frame in new emigrant B cells from 11 healthy donors (see Fig E3 in this article's Online Repository at www.jacionline.org). In contrast, D6-6 was used as frequently in its hydrophobic and hydrophilic reading frame in B-cell counterparts from patients with XLP, a feature that we previously observed in other patients with defective central B-cell tolerance.^{10,21} In addition, antibodies with long immunoglobulin heavy chain and Igλ but not Igκ CDR3s were frequently expressed in new emigrant/transitional B cells from patients with XLP, another feature that favors self-reactivity normally counterselected in the bone marrow, suggesting an altered central B-cell tolerance checkpoint in these patients (Fig 1, A-C).⁹ In agreement with these observations, we found that the frequency of polyreactive clones was significantly higher in all SAP-deficient patients with XLP (average of 26.3%) compared with that seen in healthy control subjects (average of 7.3%, *P* < .0001; Fig 1, D and E). However, the frequency of ANA clones was not increased, which suggests that ANA-expressing B cells are properly removed in the bone marrow of patients with XLP (Fig 1, F). In conclusion, we found that the central B-cell tolerance checkpoint is impaired in the absence of functional SAP.

SAP is expressed in a discrete population of immature B cells

Central B-cell tolerance is believed to be regulated by intrinsic B-cell factors, including BCRs and TLRs, which sense and bind to self-antigens at the immature B-cell stage in bone marrow.¹¹ SAP expression in B cells is controversial and might be restricted to a subset of activated germinal center B cells.²²⁻²⁴ However, SAP expression in bone marrow B cells has not been characterized. Purified CD19⁺ precursor B cells from fetal or adult bone marrow showed SAP expression in approximately 10% of IgM⁺ cells, whereas SAP expression was not detected in peripheral blood B cells and only found in CD3⁺ T cells (Fig 2, A and B, and see Fig E4, A, in this article's Online Repository at www.jacionline.org).^{23,24} SAP expression was restricted to Igκ⁺/Igλ⁺ immature B cells and not found in VpreB⁺ pre-B cells (Fig 2, B, and see Fig E4, B and C). In addition, SAP⁺ immature B cells from fetal and adult bone marrow preferentially showed a pattern of clustered IgM or Igκ/Igλ, suggesting that these BCRs were cross-linked by self-antigens (Fig 2, C, and see Fig E4, B and C). In contrast, SAP⁻ immature B cells displayed nonclustered BCRs, suggesting that these B cells do not bind self-antigens (Fig 2, C, and see Fig E4, B and C). To further identify SAP expression in human B-cell precursors, we assessed the presence of SAP transcripts in fetal liver and fetal bone marrow CD19⁺ B-cell precursors and mature naive B cells that were further sorted to exclude any T-cell contamination (Fig 2, D). We found that SAP transcripts could be detected in CD19⁺ B-cell precursors from fetal liver and bone marrow but not in peripheral mature naive B cells (Fig 2, E). The absence of CD3E transcripts in all these CD19⁺ fractions further attested to the absence of T-cell contamination (Fig 2, F). SAP expression was 100-fold less in CD19⁺ fetal liver and bone marrow cells than in T cells but is consistent with SAP being only expressed in a discrete population (10%) of

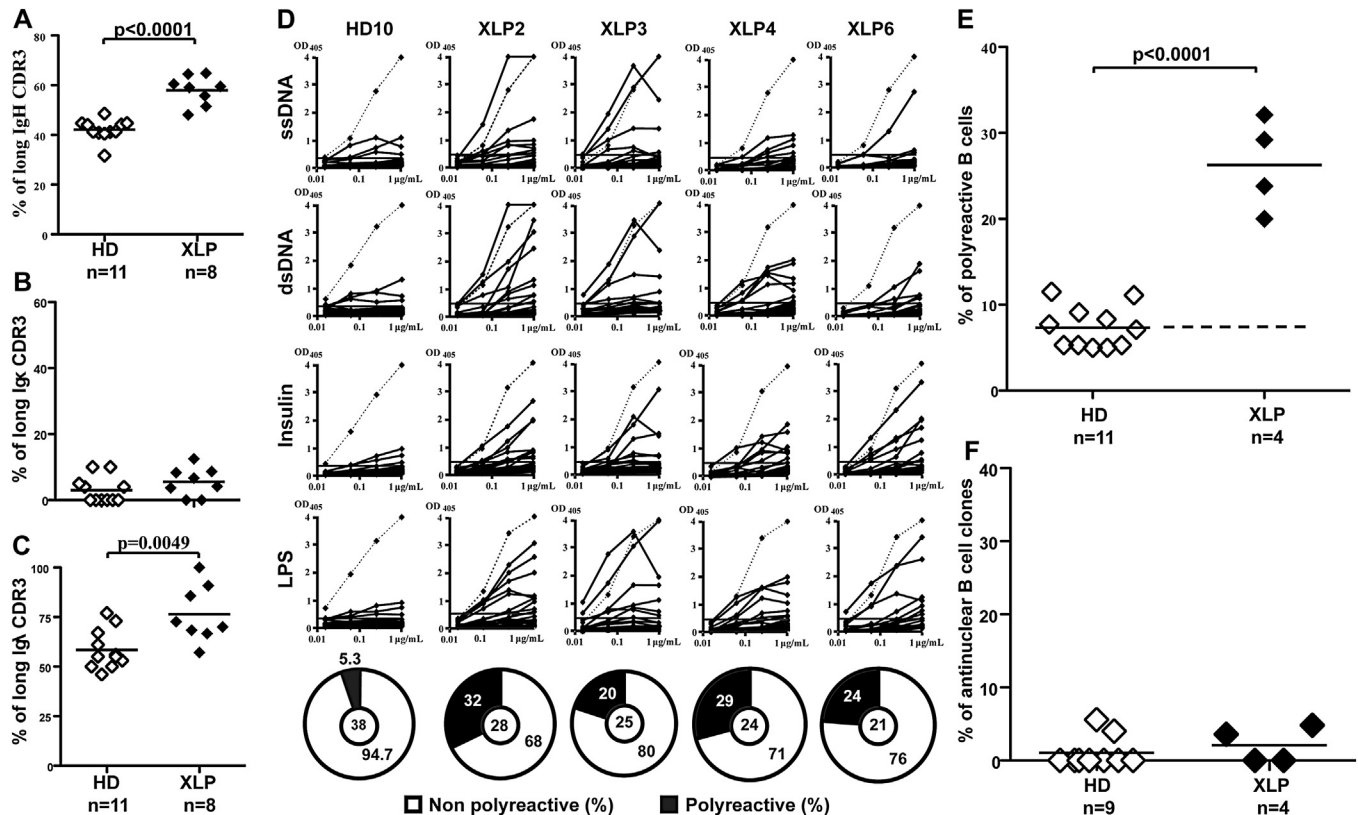


FIG 1. The central B-cell tolerance checkpoint is not functional in patients with XLP. The frequencies of long IgH (≥ 15 amino acids; **A**) or long Igκ (**B**) or Igλ (**C**; ≥ 11 amino acids) in new emigrant/transitional B cells are represented for 11 healthy donors and 8 patients with XLP. Each diamond represents a subject. The average is shown with a bar. **D-F**, Antibodies from new emigrant/transitional B cells from patients with XLP were tested by means of ELISA for reactivity against single-stranded DNA (ssDNA), double-stranded DNA (dsDNA), insulin, and LPS (Fig 1, D), and the frequencies of polyreactive and anti-nuclear clones are displayed in Fig 1, E and F, respectively. Dotted line, ED38 positive control; horizontal line, cutoff OD₄₀₅ for positive reactivity. For each subject, the frequency of polyreactive (solid area) and nonpolyreactive (open area) clones is summarized in pie charts, with the total number of clones tested indicated in the center.

CD19⁺IgM⁺Igκ⁺/Igλ⁺ immature B cells, which itself only represents approximately 20% to 30% of CD19⁺ cells (Fig 2, E, and see Fig E4, D). Hence SAP is expressed by a discrete subset of developing immature B cells, which are likely autoreactive and undergoing central selection.

SLAMF6 colocalizes with SAP when segregating with autoreactive BCRs

We assessed the expression during B-cell differentiation of SLAM family members that might require SAP to mediate their function.⁴ SLAMF1/CD150, SLAMF2/CD48, and SLAMF6/CD352 (human NTB-A/mouse Ly108) expression increased with B-cell maturation (low in pre-B cells, intermediate in immature B cells in the bone marrow, and high in mature naive B cells in peripheral blood), whereas SLAMF5/CD84 expression seems to be increased at the immature B-cell stage (Fig 3, A). SLAMF4/CD244 and SLAMF7/CD319 (CRACC) are not expressed or expressed at very low levels, respectively, suggesting that these SLAM members are unlikely to play a role in central B-cell selection (Fig 3, A). Because *Slamf6* (mouse Ly108) is required for proper central B-cell tolerance mechanisms in mice⁶ and because SLAMF6 (human NTB-A) seems to be one of the SLAM family members expressed at the highest level on

immature B cells, we analyzed its expression using immunofluorescence in SAP⁺Igκ/Igλ⁺ immature B cells. We found in all analyzed cells that SLAMF6/CD352 and SAP only associated with each other when cosegregating with clustered BCRs (Fig 3, B, middle and bottom). In contrast, SLAMF6/CD352 and SAP never colocalized in immature B cells with diffuse Igκ/Igλ expression (Fig 3, B, top). We conclude that SAP might be recruited by SLAMF6/CD352 to BCR clusters in autoreactive immature B cells and might regulate BCR signaling and the induction of central B-cell tolerance mechanisms in human bone marrow.

Defective peripheral B-cell tolerance checkpoint in patients with XLP

To analyze the effect of SAP deficiency on the peripheral B-cell tolerance checkpoint, using ELISA, we tested the reactivity of recombinant antibodies from CD19⁺CD10[−]CD21⁺IgM⁺CD27[−] mature naive B cells from patients with XLP (see Tables E10-E13 in this article's Online Repository at www.jacionline.org) against HEp-2 cell lysates (Fig 4, A). We found that the frequency of mature naive B cells that expressed HEp-2-reactive antibodies was significantly increased in all 4 patients with XLP tested (38% to 64%) compared with

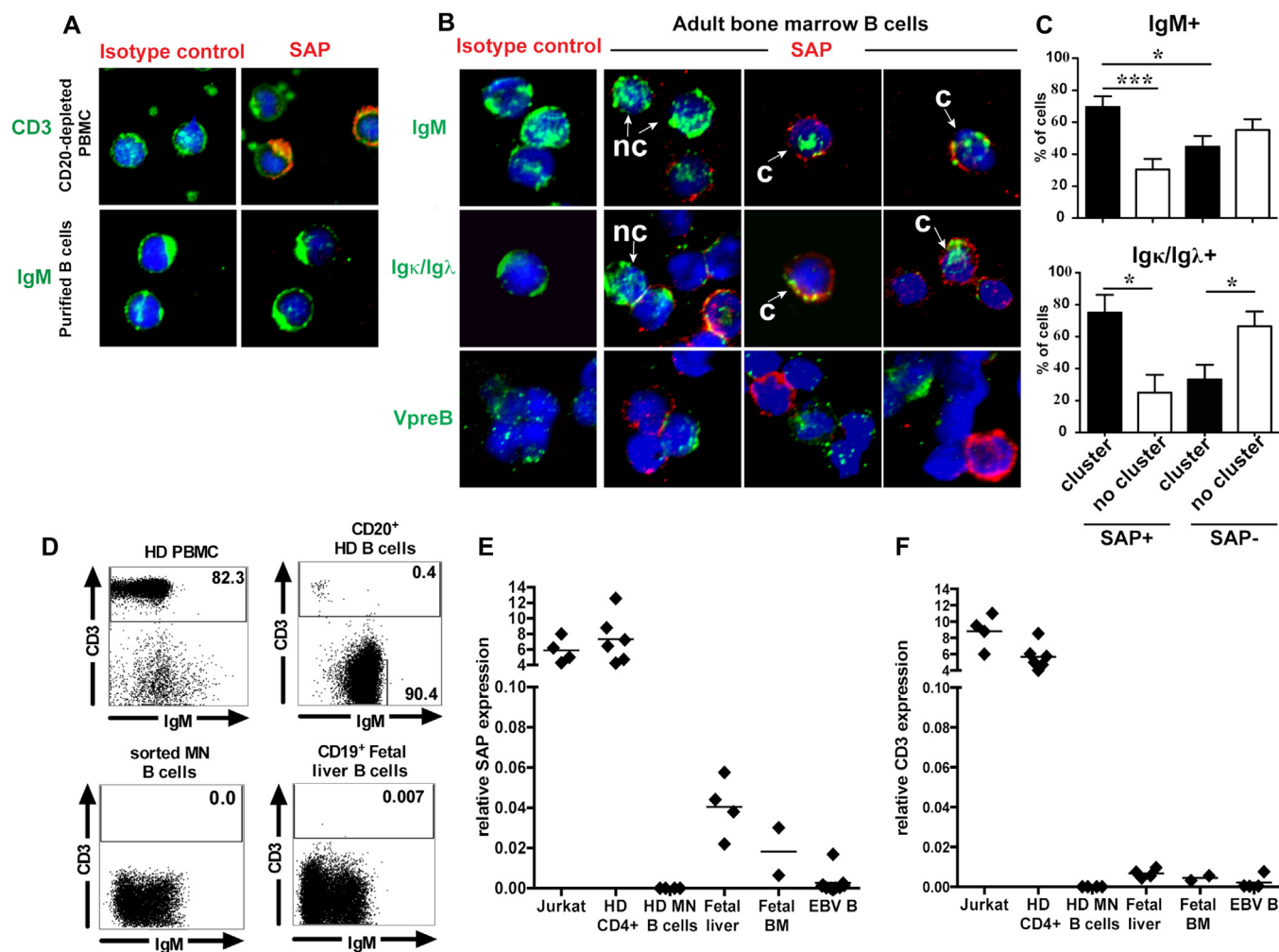


FIG 2. SAP is expressed by some immature B cells. **A**, PBMCs depleted from CD20⁺ B cells or purified naive B cells were stained for CD3 or IgM (green) or SAP or isotype control (red). **B**, CD19⁺ adult bone marrow cells were stained for IgM, Igκ and Igλ, or VpreB (green) and SAP or isotype control (red). **C**, Proportion of cells in SAP⁺ versus SAP⁻ cells that displayed a clustered versus not clustered IgM and Igκ/Igλ staining pattern. **P* < .05, ***P* < .01, and ****P* < .001. **D**, Dot plots show the proportion of CD3⁺ T cells in PBMCs from healthy donors (HD PBMC), CD20-enriched B cells using magnetic beads, sorted mature naive (MN) B cells, and CD19⁺ fetal liver cells. **E** and **F**, SAP (Fig 2, E) and CD3E (Fig 2, F) gene expression was assessed by using quantitative RT-PCR in mature naive B cells, CD19⁺ fetal liver and bone marrow cells, and EBV cell lines. Jurkat and healthy donor (HD) CD4⁺ T cells were used as positive controls for both SAP and CD3E expression.

that seen in healthy donors (17% to 26%, *P* < .0001; Fig 4, B). Consistent with these data, the frequency of mature naive polyreactive B cells was higher in patients with XLP than in healthy donors (*P* < .0001; Fig 4, C). However, there was no increase in ANA clones in the mature naive B-cell compartment of patients with XLP (Fig 4, D). Thus the high frequencies of HEP-2-reactive and polyreactive mature naive B cells in patients with XLP reveal a defective peripheral B-cell tolerance checkpoint in the absence of functional SAP expression.

Patients with XLP display normal serum BAFF concentration and Treg cell frequency

Defects in the human peripheral B-cell tolerance checkpoint seem to correlate with increased serum BAFF concentrations and

decreased Treg cell frequencies.¹¹ However, more recent analyses revealed that these defects can occur in the absence of BAFF dysregulation.^{15,16} Indeed, we found that patients with XLP displayed normal serum BAFF concentrations comparable with those of healthy donors, revealing that BAFF does not account for the defective peripheral B-cell tolerance checkpoint in patients with XLP (Fig 5, A). Treg cells play an essential role in preventing the accumulation of autoreactive mature naive B cells in the periphery and might control their homeostatic expansion, as is demonstrated by FOXP3-deficient patients with IPEX syndrome who display a defective peripheral B-cell tolerance checkpoint.¹⁶ SAP-deficient naive B-cell subsets showed a normal proliferative history, suggesting normal Treg cell functions in patients with XLP (Fig 5, B). In agreement with this observation, normal Treg cell frequencies and phenotypes were found in patients with XLP, as evidenced

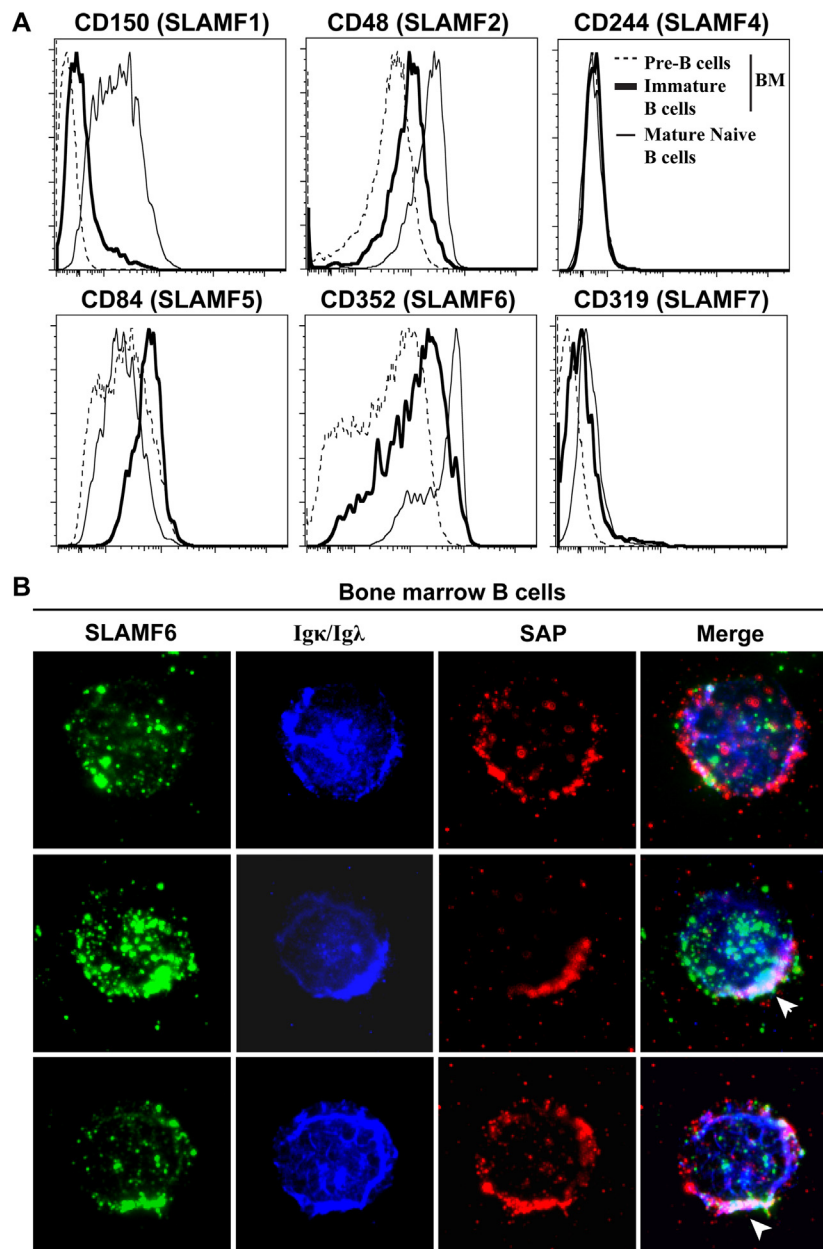


FIG 3. SAP and SLAMF6 colocalize in B cells with aggregated BCRs. **A**, Overlays display the expression of SLAM family members by CD19⁺CD27[−]CD10⁺IgM[−] pre-B cells (dotted line) and CD19⁺CD27[−]CD10⁺IgM⁺ immature B cells (bold line) from bone marrow (BM) and peripheral CD19⁺CD27[−]CD10[−]IgM⁺ mature naive B cells (thin line). **B**, CD19⁺ enriched bone marrow B cells were stained for SLAMF6 (green), Igκ/Igλ (blue), and SAP (red). Representative cells without (top row) or with (middle and bottom rows) costaining of SLAMF6, Igκ/Igλ, and SAP are shown. Costaining of clustered Igκ/Igλ, SLAMF6, and SAP appears white as the superposition of green, red, and blue and is indicated by an arrow.

by the normal expression of Helios, CD25/IL-2 receptor α , CD127/IL-7 receptor α , and CD45RO (Fig 5, C-I). In contrast, non-Treg cell FOXP3[−]Helios[−]CD4⁺ populations from patients with XLP displayed a dysregulated phenotype characterized by an increased expression of CD127/IL-7 receptor α (patients with XLP: mean fluorescence intensity = 1012 vs healthy donors: mean fluorescence intensity = 744; $P = .0006$) and increased frequencies of CD45RO⁺ memory cells (Fig 5, F-I). Hence SAP-deficient Treg cells display a normal phenotype, suggesting that these T cells might be functional in patients with XLP,

whereas non-Treg CD4⁺ T cells show an altered phenotype in these patients.

SAP-deficient T cells are resistant to *in vitro* suppression by Treg cells

Control and SAP-deficient Treg cells were then tested *in vitro* for their ability to suppress Tresp cell proliferation. CD3⁺CD4⁺CD25^{hi}CD127^{−/lo} Treg cells were sorted by using flow cytometry and cultured with CFSE-labeled

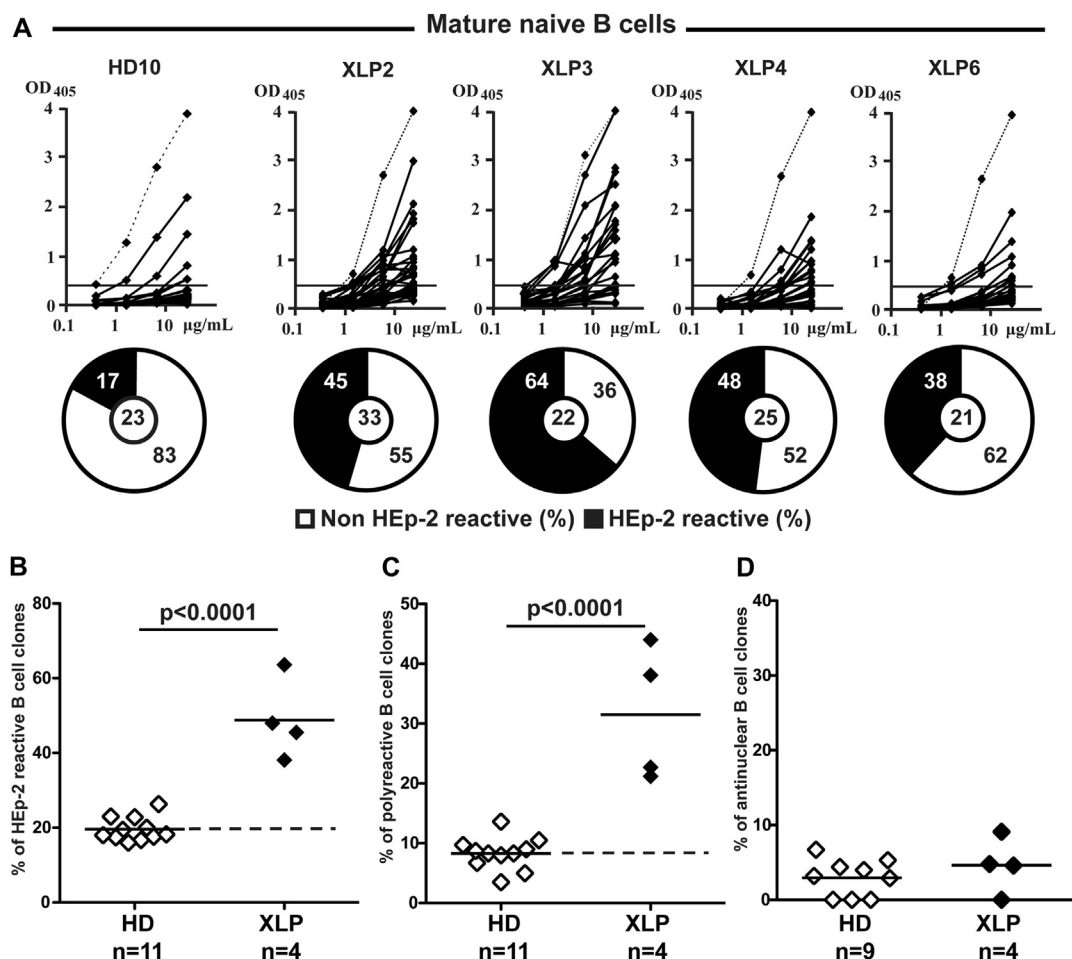


FIG 4. Defective peripheral B-cell tolerance checkpoint in patients with XLP. **A**, Antibodies from mature naive B cells from patients with XLP were tested by using ELISA for anti-HEp-2 cell reactivity. **B-D**, Frequencies of HEp-2-reactive (Fig 4, B), polyreactive (Fig 4, C), and anti-nuclear mature (Fig 4, D) naive B cells. Dotted line, ED38 positive control; horizontal lines, cutoff OD₄₀₅ for positive reactivity. For each subject, the frequency of HEp-2-reactive (solid area) and non-HEp-2-reactive (open area) clones is summarized in pie charts, with the total number of clones tested indicated in the centers. HD, Healthy donors.

CD3⁺CD4⁺CD25⁻ Tresp cells in the presence of anti-CD2-, anti-CD3-, and anti-CD28-coated beads. The proliferation of healthy donor Tresp cells was inhibited by the addition of Treg cells, either from healthy control subjects or patients with XLP (Fig 6, A and C). Thus SAP-deficient Treg cells have normal suppressive functions. However, the proliferation of XLP Tresp cells was not inhibited by either Treg cells from healthy donors or patients with XLP, thereby showing that Tresp cells from patients with XLP are resistant to *in vitro* suppression by Treg cells (Fig 6, A and C). Tresp cells from patients with XLP were hyperresponsive to stimulation because 47% of Tresp cells from patients with XLP had undergone 4 divisions after 4.5 days in culture versus only 17% of Tresp cells from healthy control subjects (Fig 6, B). This increased proliferation of SAP-deficient Tresp cells was linked to increased concentrations of cytokines, such as IL-2, IFN- γ , TNF- α , and GM-CSF, which were detected in the supernatants of these *in vitro* cultures in the presence of Treg cells from either healthy donors or patients with XLP (Fig 6, D). These cytokines originate from Tresp cells themselves, as shown by the increased production of the T_H1 cytokines IL-2, IFN- γ , TNF- α , and GM-CSF, as well as the

T_H2 cytokine IL-13, by *in vitro*-stimulated Tresp cells from patients with XLP compared with healthy donor control cells (Fig 6, E). Increased production of IFN- γ and TNF- α by Tresp cells from patients with XLP activated for 4.5 days *in vitro* was further evidenced by intracytoplasmic detection of these cytokines by means of flow cytometry (see Fig E5 in this article's Online Repository at www.jacionline.org). Sera of patients with XLP displayed increased concentrations of several cytokines, further reflecting the hyperactive T-cell phenotype of SAP deficiency. Although we did not detect increased serum IL-2 and IFN- γ levels, TNF- α and IL-7 concentrations were higher in patients with XLP (Fig 7). In addition, the sera of patients with XLP were especially enriched in IL-4, IL-6, IL-10, and IL-21, which favor B-cell survival and activation (Fig 7, A). Moreover, we found that IL-4, IL-7, and IL-21 and, to a lesser extent, IL-6 and TNF- α blocked *in vitro* Treg cell suppression by themselves (Fig 7, B and C). When all the cytokines with levels found to be increased in patients with XLP (XLP cytokine mix) were added to the Treg cell assay, we observed an inability for Treg cells to suppress refractory Tresp cell proliferation similarly to Treg-Tresp cells isolated from patients with XLP

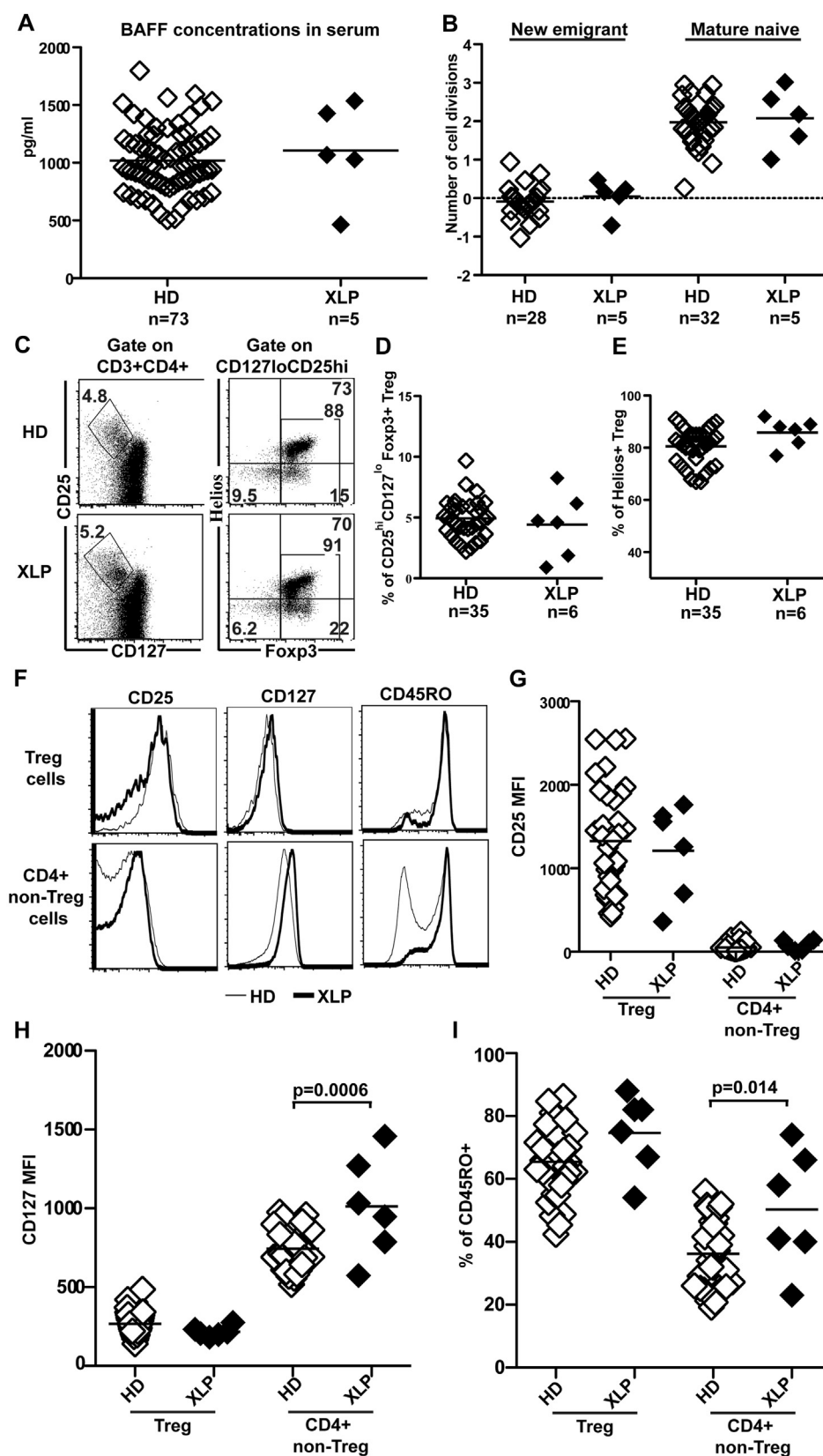


FIG 5. Normal serum BAFF concentrations and Treg cell frequencies in patients with XLP. **A**, Serum BAFF concentrations (in picograms per milliliter) in healthy donors (n = 73) and patients with XLP (n = 5). **B**, Evaluation of the number of cell divisions undergone *in vivo* by using κ -deleting recombination excision circle analysis on new emigrant and mature naive B cells of healthy donors (n = 28 and n = 32, respectively)

(Figs 6, C, and 7, C). Hence SAP deficiency affects peripheral CD4⁺ T cells, which are hyperresponsive to T-cell receptor (TCR) stimulation and prone to secrete many T_H1 and T_H2/T_{FH} cytokines, rendering these T cells resistant to Treg cell suppression.

DISCUSSION

We report here that SAP/SLAMs play a major role in the establishment of central and peripheral B-cell tolerance in human subjects. Many transitional B cells that recently emigrated from the bone marrow of patients with XLP expressed BCRs characterized by an abnormal immunoglobulin repertoire associated with self-reactivity, thereby demonstrating a defective central B-cell tolerance checkpoint in the absence of functional SAP. Central B-cell tolerance is normally regulated by intrinsic B-cell pathways involved in sensing the binding of self-antigens and inducing tolerance mechanisms, such as receptor editing, anergy, or deletion.¹¹ Defective BCR and TLR signaling and function result in a failure to remove developing autoreactive B cells in the bone marrow.¹⁰⁻¹² We identified SAP expression in a discrete population of B-cell precursors that expressed both heavy and light chains and therefore belonged to the immature B-cell compartment in which the reactivity of newly synthesized BCRs is assessed for binding to self-antigens. In addition, the preferential expression of SAP in immature B cells displaying aggregated BCRs likely binding self-antigens suggests that SAP expression might be mostly restricted to autoreactive clones. SAP expression in immature B cells might therefore be important for the establishment of central B-cell tolerance by potentially regulating the BCR signaling threshold and modifying SLAM functions. In T cells the SAP/Ly108 pathway enhances the signaling strength of the TCR to reach the threshold required for restimulation-induced apoptosis and cytolytic function.^{25,26} Indeed, in the absence of SAP, many SLAMs bind SHP-1, which is a negative regulator of TCR signaling.²⁷ When SAP is expressed, SAP competes with and displaces SHP-1 binding to SLAMs and switches off Ly108/SLAMF6 inhibitory function.^{27,28} Interestingly, we found that SLAMF6 is expressed in immature B cells and colocalized with SAP in BCR clusters. Moreover, Ly108, the mouse homologue to SLAMF6, regulates mechanisms of central B-cell tolerance by modulating the strength of the BCR signaling.⁶ Hence SAP expression at the immature B-cell stage might allow SLAM proteins, including SLAMF6, to modulate BCR signaling strength and thereby participate in the counterselection of autoreactive B cells. We conclude that SLAM/SAP play an essential role in mediating central B-cell tolerance in human subjects, potentially by favoring the induction of apoptosis in immature autoreactive B cells, as reported in B cell–derived cell lines.²⁹

In contrast to central tolerance, the establishment of peripheral B-cell tolerance seems to rely on B cell–extrinsic factors. Indeed, defects in the expression of functional CD40L and FOXP3 mostly restricted to the T-cell lineage result in a specific abnormal peripheral B-cell tolerance checkpoint.^{15,16} The failure in patients with XLP to counterselect autoreactive B cells between the new emigrant/transitional and mature naive B-cell stages at which SAP is no longer expressed in B cells further supports the B cell–extrinsic regulation of peripheral B-cell tolerance. Defects in the peripheral B-cell tolerance checkpoint often correlate with decreased Treg cell frequencies and increased serum BAFF concentrations.¹⁵ Recently, we identified similar peripheral B-cell tolerance abnormalities in FOXP3-deficient patients with IPEX syndrome, who do not have functional Treg cells and display normal serum BAFF concentrations. This observation further suggests that defective Treg cell functions in these patients, but not serum BAFF concentrations, are responsible for the impairment in preventing the accumulation of autoreactive B cells in the mature naive B-cell compartment.¹⁶ The normal serum BAFF concentrations in most patients with XLP who display defective peripheral B-cell tolerance also suggest that BAFF is not responsible for the impaired peripheral selection of autoreactive B cells. However, we did not observe any decrease in Treg cell frequencies in SAP-deficient patients or any unusual phenotype of these T cells that could infer compromised functions. In addition, we found that Treg cells retained their *in vitro* suppressive function in the absence of functional SAP. It has been proposed that Treg cells can directly interact with autoreactive B cells by recognizing self-antigens presented by these B cells through MHC class II, leading to their elimination.³⁰ Interestingly, examination of SAP-deficient mice revealed that functional interactions between B and T cells do not form in the absence of SAP, whereas T cell–dendritic cell interactions are not affected.^{27,31-33} Hence the defective peripheral B-cell tolerance checkpoint in patients with XLP might be due to the inability of T cells and potentially Treg cells to interact directly with autoreactive B cells and mediate their removal in the absence of functional SAP independently of dendritic cells and other antigen-presenting cells.

Alternatively, increased cytokine secretion by SAP-deficient T cells might be responsible for a break in peripheral B-cell tolerance. Enhanced cytokine secretion in the absence of functional SAP detected *in vitro* in activation experiments, as well as *in vivo* directly in the patients' sera, contrasted with the hyporesponsive phenotype reported in SAP-deficient T-cell lines obtained from 2 patients with XLP or in engineered Jurkat cells.^{34,35} However, these artificial models might not be representative of polyclonal responses induced in freshly isolated T cells from patients with XLP. Indeed, enhanced T-cell responses in patients with XLP were recently reported in 2 asymptomatic

and patients with XLP (n = 5). **C**, Representative CD25 and CD127 (left) and Helios and FOXP3 (right) staining on CD3⁺CD4⁺ T cells from a healthy donor and a patient with XLP. **D**, CD3⁺CD4⁺CD25^{hi}CD127^{lo}FOXP3⁺ Treg cell frequencies in 35 healthy donors and 6 patients with XLP. **E**, Frequencies of FOXP3⁺Helios⁺ cells in the CD3⁺CD4⁺CD25^{hi}CD127^{lo}FOXP3⁺ population in 35 healthy donors and 6 patients with XLP. **F**, Representative staining of CD25, CD127, and CD45RO expression in a patient with XLP (bold line) and a healthy donor (thin line) for FOXP3⁺Helios⁺ Treg cells and FOXP3⁺Helios⁺CD4⁺ T cells. **G-I**, Mean fluorescence intensity (MFI) of CD25 (Fig 5, G) and CD127 (Fig 5, H) and frequency of CD45RO⁺ cells (Fig 5, I) for Helios⁺FOXP3⁺ Treg cells and Helios⁺FOXP3⁺CD4⁺ non-Treg cells from healthy donors and patients with XLP. **HD**, Healthy donors.

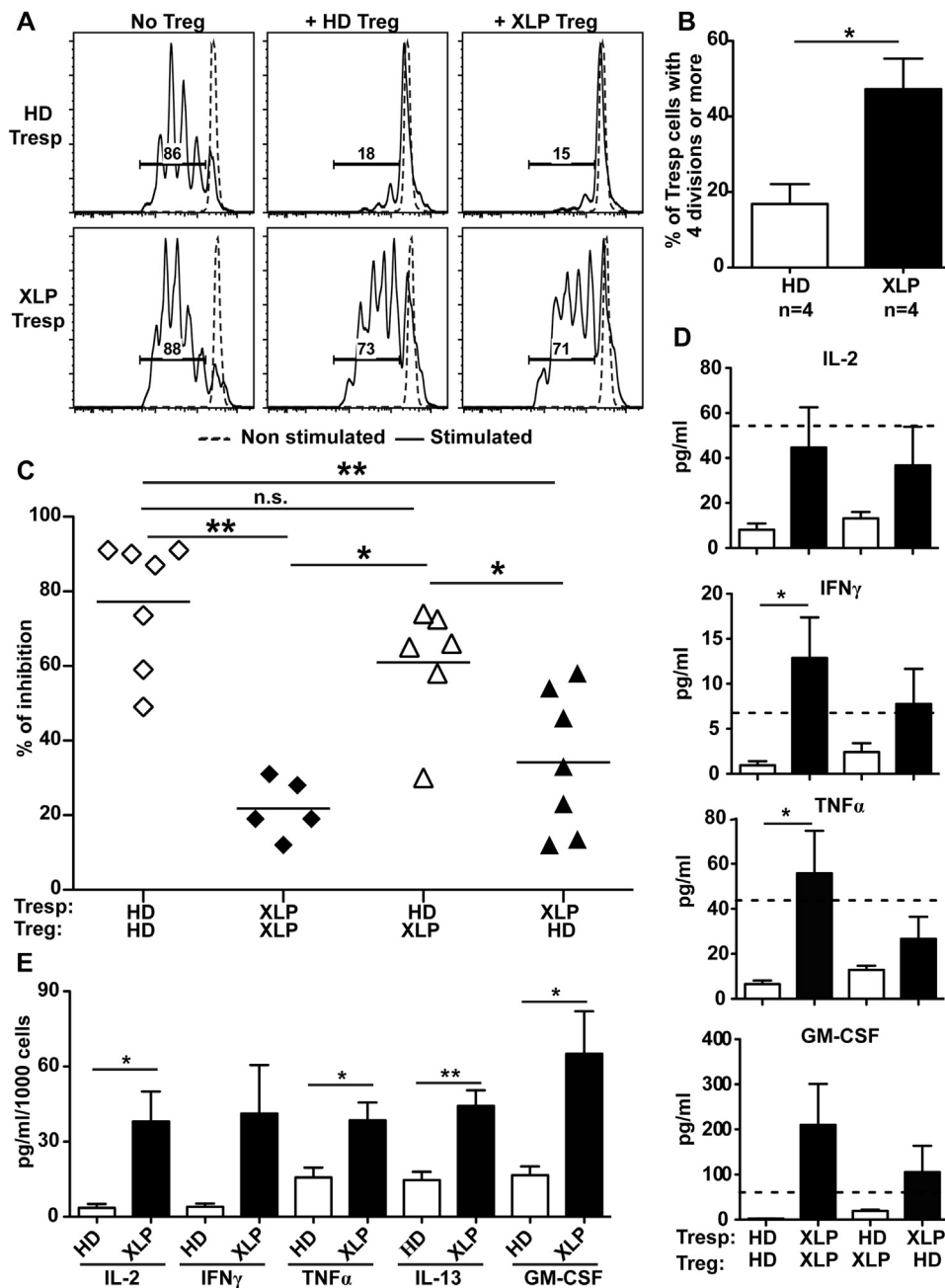


FIG 6. Increased resistance of SAP-deficient responder CD4⁺ T cells to suppression by Treg cells. **A**, Representative histograms of Treg cell-mediated suppression of autologous and heterologous CFSE-labeled Tresp cells on day 4.5 from a patient with XLP compared with a healthy donor. The *dashed line* displays nonstimulated Tresp cells. **B**, Increased percentage of Tresp cells that divided 4 times or more in patients with XLP compared with healthy donors in the "Tresp only" culture after 4.5 days. **C**, Autologous and heterologous suppressive activities of Treg cells from healthy donors and patients with XLP. **D**, Increased concentrations of IL-2, IFN- γ , TNF- α , and GM-CSF in supernatants of XLP "Tresp+Treg" cocultures. The *dotted line* represents the level of each cytokine in the supernatant of the stimulated "HD Tresp only" well. **E**, Concentrations of the indicated cytokines in stimulated healthy donors or patients with XLP CD4⁺ T-cell monocultures after 4 days. * $P < .05$ and ** $P < .01$. HD, Healthy donors.

patients with XLP.³⁶ In addition, increased IFN- γ secretion has been reported during acute EBV infection in patients with XLP and in SAP-deficient mice infected with lymphocytic choriomeningitis virus or *Toxoplasma gondii*.³⁷⁻³⁹ The increased IL-2, IFN- γ , and TNF- α secretion might account for the ineffective suppression of Tresp cell proliferation by Treg cells,

as suggested by several reports.⁴⁰⁻⁴² In agreement with this hypothesis, we found that cytokines, such as IL-4, IL-7, and IL-21 and, to a lesser extent, IL-6 and TNF- α , were able to inhibit by themselves *in vitro* Treg cell suppression. SAP deficiency recapitulates defective suppressive scenarios observed in patients with type 1 diabetes and multiple sclerosis. Indeed, patients with

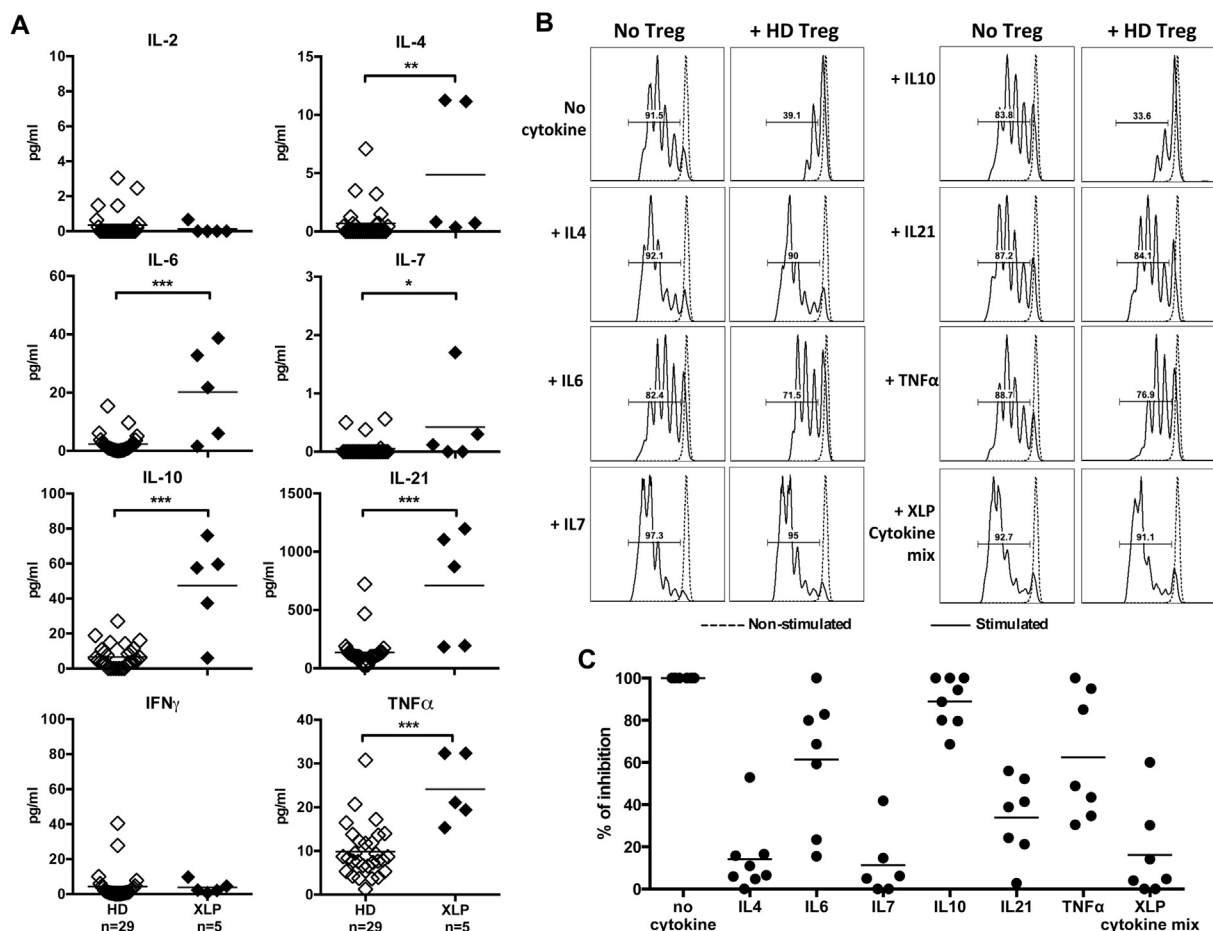


FIG 7. Increased T_H2/T_H cytokine concentrations in sera of patients with XLP. **A**, IL-2, IFN- γ , TNF- α , IL-7, IL-4, IL-6, IL-10, and IL-21 cytokine concentrations in sera of 5 patients with XLP and 29 healthy donors were measured by using Luminex or ELISA. * $P < .05$, ** $P < .01$, and *** $P < .0001$. **B**, Representative histograms of Treg cell-mediated suppression of autologous CFSE-labeled Tresp cells on day 4.5 from a healthy donor in the presence of the indicated cytokines. The dashed line displays nonstimulated Tresp cells. **C**, Suppressive activities of Treg cells in the presence of diverse cytokines. The XLP cytokine mix includes IL-4, IL-6, IL-7, IL-10, IL-21, and TNF- α , levels of which were found to be increased in patients with XLP.

type 1 diabetes also display Tresp cells that are refractory to Treg cell suppression *in vitro* associated with a defective peripheral B-cell tolerance checkpoint.^{12,43} IFN- γ secretion by Treg cells from patients with multiple sclerosis results in a loss of *in vitro* suppressive activity and correlates with an abnormal peripheral B-cell tolerance checkpoint in these patients.^{19,40} A defective suppression of T cells by Treg cell is also consistent with the overwhelming and uncontrolled B-cell and T-cell responses to EBV infection in patients with XLP.³⁹ In addition to rendering Tresp cells refractory to Treg cells and perhaps directly affecting Treg cell suppressive function, T_H2/T_H cytokines, including IL-4, IL-6, IL-10, and IL-21, favor B-cell activation, isotype switch, and plasma cell development.^{42,44} Paradoxically, patients with XLP display severely decreased isotype-switched B-cell frequencies, likely resulting from defective interactions with CD4⁺ T cells.^{23,24,45} Nonetheless, SAP deficiency could also interfere with the establishment of peripheral B-cell tolerance by favoring the survival of autoreactive B cells through enhanced serum cytokine concentrations.

SAP is essential for natural killer T (NKT) cell development.^{46,47} Consequently, patients with XLP lack NKT cells. NKT cells express an invariant TCR that recognizes lipid self-antigens and are able to provide cognate help to B cells in a SAP-dependent manner potentially, preventing the activation of autoreactive B cells.⁴⁸ Lower NKT numbers and function have been linked to the development of type 1 diabetes and lupus in NOD mice with genetic defects of *slamf1* and *slamf6* combined with altered SLAM expression.⁴⁹ Therefore we cannot exclude the involvement of NKT cells in the control of human peripheral B-cell tolerance. The absence of NKT cells might contribute to the improper removal of autoreactive clones from the mature naive B-cell compartment of SAP-deficient patients with XLP.

In conclusion, we found that SAP is required for the establishment of B-cell tolerance in human subjects. Transient SAP expression in bone marrow–developing B cells is required for functional central B-cell tolerance, whereas in the periphery SAP expression in T cells is likely necessary for preventing the accumulation of autoreactive mature naive B cells by ensuring

stable B-cell/T-cell interactions and repressing uncontrolled cytokine secretion by human T cells.

We thank Dr S. Rudchenko, L. Devine, and C. Wang for cell sorting.

Key messages

- SAP expression in human autoreactive immature B cells is required for the establishment of central B-cell tolerance in bone marrow.
- SAP expression by T cells prevents the accumulation of autoreactive mature naive B cells in the periphery.

REFERENCES

- Nichols KE, Harkin DP, Levitz S, Krainer M, Kolquist KA, Genovese C, et al. Inactivating mutations in an SH2 domain-encoding gene in X-linked lymphoproliferative syndrome. *Proc Natl Acad Sci U S A* 1998;95:13765-70.
- Coffey AJ, Brooksbank RA, Brandau O, Oohashi T, Howell GR, Bye JM, et al. Host response to EBV infection in X-linked lymphoproliferative disease results from mutations in an SH2-domain encoding gene. *Nat Genet* 1998;20:129-35.
- Sayos J, Wu C, Morra M, Wang N, Zhang X, Allen D, et al. The X-linked lymphoproliferative-disease gene product SAP regulates signals induced through the co-receptor SLAM. *Nature* 1998;395:462-9.
- Cannons JL, Tangye SG, Schwartzberg PL. SLAM family receptors and SAP adaptors in immunity. *Annu Rev Immunol* 2011;29:665-705.
- Wandstrat AE, Nguyen C, Limaye N, Chan AY, Subramanian S, Tian XH, et al. Association of extensive polymorphisms in the SLAM/CD2 gene cluster with murine lupus. *Immunity* 2004;21:769-80.
- Kumar KR, Li L, Yan M, Bhaskarabhatla M, Mobley AB, Nguyen C, et al. Regulation of B cell tolerance by the lupus susceptibility gene Ly108. *Science* 2006;312:1665-9.
- Cunningham Graham DS, Vyse TJ, Fortin PR, Montpetit A, Cai YC, Lim S, et al. Association of LY9 in UK and Canadian SLE families. *Genes Immun* 2008;9:93-102.
- Suzuki A, Yamada R, Kochi Y, Sawada T, Okada Y, Matsuda K, et al. Functional SNPs in CD244 increase the risk of rheumatoid arthritis in a Japanese population. *Nat Genet* 2008;40:1224-9.
- Wardemann H, Yurasov S, Schaefer A, Young JW, Meffre E, Nussenzweig MC. Predominant autoantibody production by early human B cell precursors. *Science* 2003;301:1374-7.
- Ng YS, Wardemann H, Chelnis J, Cunningham-Rundles C, Meffre E. Bruton's tyrosine kinase is essential for human B cell tolerance. *J Exp Med* 2004;200:927-34.
- Meffre E. The establishment of early B cell tolerance in humans: lessons from primary immunodeficiency diseases. *Ann N Y Acad Sci* 2011;1246:1-10.
- Menard L, Saadoun D, Isnardi I, Ng YS, Meyers G, Massad C, et al. The PTPN22 allele encoding an R620W variant interferes with the removal of developing autoreactive B cells in humans. *J Clin Invest* 2011;121:3635-44.
- Isnardi I, Ng YS, Srdanovic I, Motaghedi R, Rudchenko S, von Bernuth H, et al. IRAK-4- and MyD88-dependent pathways are essential for the removal of developing autoreactive B cells in humans. *Immunity* 2008;29:746-57.
- Sauer AV, Morbach H, Brigida I, Ng YS, Aiuti A, Meffre E. Defective B cell tolerance in adenosine deaminase deficiency is corrected by gene therapy. *J Clin Invest* 2012;122:2141-52.
- Herve M, Isnardi I, Ng YS, Bussell JB, Ochs HD, Cunningham-Rundles C, et al. CD40 ligand and MHC class II expression are essential for human peripheral B cell tolerance. *J Exp Med* 2007;204:1583-93.
- Kinnunen T, Chamberlain N, Morbach H, Choi J, Kim S, Craft J, et al. Accumulation of peripheral autoreactive B cells in the absence of functional human regulatory T cells. *Blood* 2013;121:1595-603.
- Isnardi I, Ng YS, Menard L, Meyers G, Saadoun D, Srdanovic I, et al. Complement receptor 2/CD21- human naive B cells contain mostly autoreactive unresponsive clones. *Blood* 2010;115:5026-36.
- van Zelm MC, Szczepanski T, van der Burg M, van Dongen JJ. Replication history of B lymphocytes reveals homeostatic proliferation and extensive antigen-induced B cell expansion. *J Exp Med* 2007;204:645-55.
- Kinnunen T, Chamberlain N, Morbach H, Cantaert T, Lynch M, Preston-Hurlburt P, et al. Specific peripheral B cell tolerance defects in patients with multiple sclerosis. *J Clin Invest* 2013;123:2737-41.
- Meffre E, Schaefer A, Wardemann H, Wilson P, Davis E, Nussenzweig MC. Surrogate light chain expressing human peripheral B cells produce self-reactive antibodies. *J Exp Med* 2004;199:145-50.
- Meyers G, Ng YS, Bannock JM, Lavoie A, Walter JE, Notarangelo LD, et al. Activation-induced cytidine deaminase (AID) is required for B-cell tolerance in humans. *Proc Natl Acad Sci U S A* 2011;108:11554-9.
- Shlapatska LM, Mikhalap SV, Berdova AG, Zelensky OM, Yun TJ, Nichols KE, et al. CD150 association with either the SH2-containing inositol phosphatase or the SH2-containing protein tyrosine phosphatase is regulated by the adaptor protein SH2D1A. *J Immunol* 2001;166:5480-7.
- Ma CS, Pittaluga S, Avery DT, Hare NJ, Maric I, Klion AD, et al. Selective generation of functional somatically mutated IgM+CD27+, but not Ig isotype-switched, memory B cells in X-linked lymphoproliferative disease. *J Clin Invest* 2006;116:322-33.
- Veillette A, Zhang S, Shi X, Dong Z, Davidson D, Zhong MC. SAP expression in T cells, not in B cells, is required for humoral immunity. *Proc Natl Acad Sci U S A* 2008;105:1273-8.
- Snow AL, Marsh RA, Krummey SM, Roehrs P, Young LR, Zhang K, et al. Restimulation-induced apoptosis of T cells is impaired in patients with X-linked lymphoproliferative disease caused by SAP deficiency. *J Clin Invest* 2009;119:2976-89.
- Zhao F, Cannons JL, Dutta M, Griffiths GM, Schwartzberg PL. Positive and negative signaling through SLAM receptors regulate synapse organization and thresholds of cytotoxicity. *Immunity* 2012;36:1003-16.
- Kageyama R, Cannons JL, Zhao F, Yusuf I, Lao C, Locci M, et al. The receptor Ly108 functions as a SAP adaptor-dependent on-off switch for T cell help to B cells and NKT cell development. *Immunity* 2012;36:986-1002.
- Palendira U, Low C, Chan A, Hislop AD, Ho E, Phan TG, et al. Molecular pathogenesis of EBV susceptibility in XLP as revealed by analysis of female carriers with heterozygous expression of SAP. *PLoS Biol* 2011;9:e1001187.
- Nagy N, Matskova L, Kis LL, Hellman U, Klein G, Klein E. The proapoptotic function of SAP provides a clue to the clinical picture of X-linked lymphoproliferative disease. *Proc Natl Acad Sci U S A* 2009;106:11966-71.
- Zhao DM, Thornton AM, DiPaolo RJ, Shevach EM. Activated CD4+CD25+ T cells selectively kill B lymphocytes. *Blood* 2006;107:3925-32.
- Qi H, Cannons JL, Klauschen F, Schwartzberg PL, Germain RN. SAP-controlled T-B cell interactions underlie germinal center formation. *Nature* 2008;455:764-9.
- Cannons JL, Qi H, Lu KT, Dutta M, Gomez-Rodriguez J, Cheng J, et al. Optimal germinal center responses require a multistage T cell:B cell adhesion process involving integrins, SLAM-associated protein, and CD84. *Immunity* 2010;32:253-65.
- Deenick EK, Chan A, Ma CS, Gatto D, Schwartzberg PL, Brink R, et al. Follicular helper T cell differentiation requires continuous antigen presentation that is independent of unique B cell signaling. *Immunity* 2010;33:241-53.
- Baldanzi G, Pighini A, Bettio V, Rainero E, Traini S, Chianale F, et al. SAP-mediated inhibition of diacylglycerol kinase α regulates TCR-induced diacylglycerol signaling. *J Immunol* 2011;187:5941-51.
- Sanzone S, Zeyda M, Saemann MD, Soncini M, Holter W, Fritsch G, et al. SLAM-associated protein deficiency causes imbalanced early signal transduction and blocks downstream activation in T cells from X-linked lymphoproliferative disease patients. *J Biol Chem* 2003;278:29593-9.
- Coraglia A, Felippo M, Schierloh P, Malbran A, de Bracco MM. CD4+ T Lymphocytes with follicular helper phenotype (TFH) in patients with SH2D1A deficiency (XLP). *Clin Immunol* 2011;141:357-64.
- Czar MJ, Kersh EN, Mijares LA, Lanier G, Lewis J, Yap G, et al. Altered lymphocyte responses and cytokine production in mice deficient in the X-linked lymphoproliferative disease gene SH2D1A/DSHP/SAP. *Proc Natl Acad Sci U S A* 2001;98:7449-54.
- Wu C, Nguyen KB, Pien GC, Wang N, Gullo C, Howie D, et al. SAP controls T cell responses to virus and terminal differentiation of TH2 cells. *Nat Immunol* 2001;2:410-4.
- Nichols KE, Ma CS, Cannons JL, Schwartzberg PL, Tangye SG. Molecular and cellular pathogenesis of X-linked lymphoproliferative disease. *Immunol Rev* 2005;203:180-99.
- Dominguez-Villar M, Baecher-Allan CM, Hafler DA. Identification of T helper type 1-like, Foxp3+ regulatory T cells in human autoimmune disease. *Nat Med* 2011;17:673-5.

41. Valencia X, Stephens G, Goldbach-Mansky R, Wilson M, Shevach EM, Lipsky PE. TNF downmodulates the function of human CD4+CD25hi T-regulatory cells. *Blood* 2006;108:253-61.
42. Ruprecht CR, Gattorno M, Ferlito F, Gregorio A, Martini A, Lanzavecchia A, et al. Coexpression of CD25 and CD27 identifies FoxP3+ regulatory T cells in inflamed synovia. *J Exp Med* 2005;201:1793-803.
43. Schneider A, Rieck M, Sanda S, Pihoker C, Greenbaum C, Buckner JH. The effector T cells of diabetic subjects are resistant to regulation via CD4+ FOXP3+ regulatory T cells. *J Immunol* 2008;181:7350-5.
44. Peluso I, Fantini MC, Fina D, Caruso R, Boirivant M, MacDonald TT, et al. IL-21 counteracts the regulatory T cell-mediated suppression of human CD4+ T lymphocytes. *J Immunol* 2007;178:732-9.
45. Crotty S, Kersh EN, Cannons J, Schwartzberg PL, Ahmed R. SAP is required for generating long-term humoral immunity. *Nature* 2003;421:282-7.
46. Nichols KE, Hom J, Gong SY, Ganguly A, Ma CS, Cannons JL, et al. Regulation of NKT cell development by SAP, the protein defective in XLP. *Nat Med* 2005;11:340-5.
47. Pasquier B, Yin L, Fondaneche MC, Relouzat F, Bloch-Queyrat C, Lambert N, et al. Defective NKT cell development in mice and humans lacking the adapter SAP, the X-linked lymphoproliferative syndrome gene product. *J Exp Med* 2005;201:695-701.
48. Wermeling F, Lind SM, Jordo ED, Cardell SL, Karlsson MC. Invariant NKT cells limit activation of autoreactive CD1d-positive B cells. *J Exp Med* 2010;207:943-52.
49. Baev DV, Caielli S, Ronchi F, Coccia M, Facciotti F, Nichols KE, et al. Impaired SLAM-SLAM homotypic interaction between invariant NKT cells and dendritic cells affects differentiation of IL-4/IL-10-secreting NKT2 cells in nonobese diabetic mice. *J Immunol* 2008;181:869-77.

1 ONLINE REPOSITORY

2

3 **Signaling Lymphocyte Activated Molecule (SLAM)/SLAM-associated**
4 **Protein (SAP) Regulates Human B-cell Tolerance**

5

6 **Running title:** SAP is essential for human B-cell tolerance

7

8 Laurence Menard, PhD¹, Tineke Cantaert, PhD¹, Nicolas Chamberlain, BS¹, ,
9 Stuart G. Tangye, PhD², Sean Riminton, MD, PhD³, Joseph A. Church, MD⁴,
10 Amy Klion, MD⁵, Charlotte Cunninham-Rundles, MD⁶, Kim Nichols, MD⁷, and
11 Eric Meffre, PhD¹

12

13 ¹Department of Immunobiology, Yale University School of Medicine, New Haven,
14 CT, USA; ²Immunology Program, Garvan Institute of Medical Research, and St
15 Vincent's Clinical School, University of NSW, Darlinghurst, NSW, Australia;

16 ³Department of Immunology, Concord Hospital, Sydney, NSW, Australia,

17 ⁴Divisions of Clinical Immunology and Allergy, Children's Hospital Los Angeles
18 and Keck School of Medicine, University of Southern California, Los Angeles,
19 CA, USA; ⁵Laboratory of Parasitic Diseases, National Institute of Allergy and

20 Infectious Diseases, National Institutes of Health, Bethesda, MD, USA;

21 ⁶Department of Medicine, The Immunology Institute, Mt. Sinai School of
22 Medicine, New York, New York, USA; ⁷Division of Oncology, Children's Hospital
23 of Philadelphia, Philadelphia, PA, USA.

24 **Corresponding author:** Eric Meffre, Yale University School of Medicine, 300
25 George Street, New Haven, CT 06511, USA, tel: 203-737-4535; Fax: 203-785-
26 7903; e-mail: eric.meffre@yale.edu

Figure E1. New emigrant/transitional B cells from XLP patients display a heavy chain gene segment repertoire similar to their counterparts in healthy donors (HD). **(A)** Usage frequencies of VH genes in HD and XLP patients. **(B)** Usage frequencies of VH, D and JH family genes in HD and XLP patients. 362 sequences from 11 HD and 265 sequences from 8 XLP patients were analyzed.

Figure E2. New emigrant/transitional B cells from XLP patients display a different Variable (V) light chain gene repertoire compared to their counterparts in healthy donors (HD). **(A)** Usage frequencies of V κ genes in HD and XLP patients. **(B)** Usage frequencies of V κ and J κ family genes in HD and XLP patients. **(C)** Usage frequencies of V λ genes in HD and XLP patients. **(D)** Usage frequencies of V λ and J λ family genes in HD and XLP patients. 362 sequences from 11 HD and 265 sequences from 8 XLP patients were analyzed. *: p-value <0.05, **: p-value <0.01

Figure E3 . Reading frame usages for frequently used D genes in new emigrant/transitional B cells from healthy donors (HD) and XLP patients. 362 sequences from 11 HD and 265 sequences from 8 XLP patients were analyzed. The abnormal hydrophobic reading frame usage of D6-6 in XLP patients suggests a defective central B cell tolerance checkpoint.

Figure E4: SAP is expressed by some developing immature fetal B cells. **(A), (B)** CD19+ fetal bone marrow B cell precursors were stained for IgM, Ig κ and Ig λ , VpreB (green) and SAP (red). Arrow in **(A)** points to a SAP+IgM+ cell. **(C)** % of cells in SAP+ vs SAP- cells from staining in **(B)** that displayed a clustered vs not clustered IgM and Ig κ /Ig λ staining pattern. *:p-value<0.05; **: p-value<0.01. **(D)** Representative CD10 and IgM staining on CD19+ gated cells from fetal liver and bone marrow samples.

Figure E5: SAP-deficient CD4+T cells express more IFN γ and TNF α cytokines. **(A)** Representative dot-plots of IFN γ and TNF α expression measured by intracellular cytokine staining in HD and XLP CD4+ T cells stimulated for 4.5 days. Percentage of IFN γ + **(B)** and TNF α + **(C)** T cells after 4.5 days of stimulation.

Supplemental Table 1: XLP patients

patients	age	clinical phenotype	current status	infected by EBV	mutation	ref.
XLP2*	41	FIM, Hypog	Stable, on IV-Ig	Yes	Missense F87S, reduced protein stability and reduced affinity for SLAMF1 and FYN	<i>Ma et al, J Clin Invest, 2005</i>
XLP3*	46	FIM, Hypog	Stable, on IV-Ig	Yes	Missense F87S, reduced protein stability and reduced affinity for SLAMF1 and FYN	<i>Ma et al, J Clin Invest, 2005</i>
XLP4	17	CNS Vasculitis	Stable	No	Deletion of <i>SH2D1A</i> , no SAP	<i>C. Cunningham-Rundles</i>
XLP5	31	Psoriasis, Hypog Splenectomy	Stable, no treatment	Yes	Deletion of <i>SH2D1A</i> , no SAP	<i>Milone et al, Blood, 2005</i>
XLP6	31	Eosinophilia, Vasculitis Splenectomy	Stable, no treatment	No	Missense C163T	<i>Talaat et al, Pediatr Blood Cancer, 2009</i>
XLP7	30	Pulmonary lymphoma vs lymphomatoid granulomatosis	Stable, no treatment	EBV-negative at 10 yo	STOP codon, truncated SAP	<i>J. Church, unpublished</i>
XLP8#	37	Asymptomatic	Stable, no treatment		Deletion of <i>SH2D1A</i> exon2, no SAP	<i>J. Church, unpublished</i>
XLP9#	22	Abdominal non-Burkitt's lymphoma	Stable, no treatment	Yes	Deletion of <i>SH2D1A</i> exon2, no SAP	<i>J. Church, unpublished</i>
XLP10	26	FIM	Unknown	yes (3 yo)	Missense T53I, loss of interaction with CD48, reduced affinity for SLAMF1, loss of interaction with nonphosphorylated SLAMF1	<i>K. Nichols</i>

*** and #: brothers**

None of the patients developed an accelerated phase

FIM : fulminant infectious mononucleosis

Hypog: hypogammaglobulinemia

Supplemental Table 2: Repertoire and reactivity of antibodies from new emigrant B cells of XLP2

Ig	HEAVY						LIGHT				REACTIVITY		
	VH	D	RF	JH	CDR3 (aa)	Length	Vκ	Jκ	CDR3 (aa)	Length	Poly	HEp-2	Staining
neXLP2 01	4-39	3-9	2	2	VLRGVNYDILTGYYHDYWFYDL	22	1-5	4	QQYNSYPLT	9	+	+	+(F)
neXLP2 02	4-39	3-22	2	4	QKGKDYDSSGYLPYYFDY	18	4-1	2	QQYYSTPYT	9	-	-	-
neXLP2 04	4-34	3-22	2	6	ARGHYDSSGYFPLGYYMDV	21	1-5	4	QQYNSYPLT	9	+	+	-
neXLP2 06	3-23	3-10	2	4	DGHGSGSYRPKYFDY	16	1-33	4	QQYDNLPLT	9	-	-	-
neXLP2 07	4-4	1-26	2	4	SSGSYYNFDY	10	3-20	3	QQYGSSPLFT	10	-	+	-
neXLP2 08	4-59	3-10	2	4	AWANYYGSGVGTYFFDY	17	1-5	4	QQYNSYRLT	9	-	+	-
neXLP2 10	3-30-3	2-2	3	5	GADIVVPAASPSASWFDP	19	1-12	3	QQANSFPHT	9	-	-	-
neXLP2 12	3-30-3	2-2	2	5	DHAYCSSTSCYFEGDNWFDP	20	1-12	4	QQANSFPLT	9	-	-	-
neXLP2 13	3-33	5-24	3	4	DSVEMATILDDFDY	14	3-15	1	QQYNNWPPWT	10	-	-	-
neXLP2 14	4-39	2-2	2	6	HGCSSTSCSYVYYYMDV	18	3-11	1	QQRSNWWT	8	+	+	-
neXLP2 17	3-33	6-13	2	6	DRGYSSSWSYYYYYMDV	19	1-13	4	QQFNSYLLT	9	+	+	-
neXLP2 18	3-23	3-9	2	5	DCANGGYDILTGYYNGFYPPGWFDP	27	3-20	1	QQYGSSPPVWT	11	+	+	-
neXLP2 20	3-9	3-22	2	3	GLVTYYYDSSGYYPV	15	3-20	2	QQYGSSPT	8	-	+	-
neXLP2 21	3-30-3	3-22	2	4	DGGYYDSSGPVDY	14	3-20	3	QQYGSSPLFT	10	-	-	-
neXLP2 23	4-31	6-6	3	4	TPGYRAARPFYD	12	2-28	1	MQALQTWT	8	+	+	-
neXLP2 28	3-30-3	5-12	2	6	VFIEKSGDGGYDLDDYYYMDV	22	3-11	3	QQRSNWPLFT	11	-	-	-
neXLP2 36	3-23	3-9	1	4	GYFDWLTYDLDY	12	1-5	2	QQYNSYSYT	9	+	+	+(M)
neXLP2 37	3-9	3-22	2	4	DIGINYDSSGYYYDEVGIDY	21	3-20	4	QQYGSSLT	8	-	-	-
neXLP2 38	4-30-4	2-2	3	4	GIVVPAATLTIFYD	15	1-5	1	QQYNSYSWT	9	-	-	-
neXLP2 41	4-30-4	3-9	2	6	GSYDILTGYYSVVRTPLSFYMDV	23	1-5	1	QQYNSYSGT	9	+	+	+(C)
neXLP2 43	3-33	3-22	3	4	EGPRGTMDIRAGDFDY	17	1-8	2	QQYYSTPYT	9	+	+	-
neXLP2 44	4-61	4-17	2	4	DRYGDYFFDY	10	3-20	4	QQYGSSPRLT	10	-	+	-
neXLP2 45#	3-11	7-27	3	6	LPLTGEDVYYYYYMDV	17	2-24	4	MQATQFPLT	9			
neXLP2 48	4-34	/	/	5	GKSRGNWFDP	10	1-8	4	QQYYSTPQLT	10	-	+	+(N)
	VH	D	RF	JH	CDR3 (aa)	Length	Vλ	Jλ	CDR3 (aa)	Length	Poly	HEp-2	Staining
neXLP2 19	3-7	5-5	3	3	DRVVTYDI	8	1-51	3	GTWDSSLSAGV	11	-	-	-
neXLP2 30	3-30-3	6-19	2	4	GAAYSSGWYYFDY	13	3-21	2	QVWDSSSDHVV	11	-	-	-
neXLP2 31#	1-2	5-5	1	5	EQLWHMFDP	9	1-44	3	AAWDDSLNGWV	11			
neXLP2 33	1-8	3-22	2	6	RGTYDSSGYYYPYYYYMDV	20	1-44	3	AAWDDSLNGPV	11	-	+	-
neXLP2 35	4-34	2-2	2	6	VAYGYCSSTSCPNYYYYMDV	21	1-51	2	GTWDSSLSAGV	11	-	+	-
neXLP2 47	4-34	/	/	5	EMKARNWFDP	10	1-51	1	GTWDSSLSVYV	11	-	-	-
	VH	D	RF	JH	CDR3 (aa)	Length							
neXLP2 34	4-34	3-3	2	5	GYSNENQTYDFWGSYYPSGFGWFDP	27							

RF, reading frame; #, antibody failed to be expressed

-, non-reactive; +, reactive; +2, highly reactive

C, diffuse cytoplasmic staining; A, actin staining; G, Golgi staining; M, mitochondria staining; MS, mitotic spindle staining; N, nuclear staining; F, cytoplasmic fibers

Supplemental Table 3: Repertoire and reactivity of antibodies from new emigrant B cells of XLP3

Ig	HEAVY					LIGHT					REACTIVITY			
	VH	D	RF	JH	CDR3 (aa)	Length	Vκ	Jκ	CDR3 (aa)	Length	Poly	Hep-2	Staining	
neXLP3 08	3-21	3-10	1	3	IAQLLWFGGEYAFDI	14	1-6	4	LQDYNYPILT	9	-	+	-	
neXLP3 10	4-59	5-5	3	6	DTAMAKTYYYYYMDV	15	2-28	1	MQALQTPKKT	9	-	-	-	
neXLP3 12	1-58	6-13	3	5	EIPAAAGTGWFDP	13	1-39	4	QQSYSTPRKT	9	-	+	-	
neXLP3 13	3-7	6-13	3	5	DTPPVLNLVPRRNIAAAGTGWFDP	24	1-8	1	QQYYSYPQKT	9	-	-	-	
neXLP3 19	4-34	3-3	2	6	GPRDDFWSGYGDSEYYYYYYMDV	25	1-39	2	QQSYSTPT	8	+	+	-	
neXLP3 20	3-23	3-10	2	4	GPYSGEDY	9	1-16	2	QQYNSYPYT	9	-	-	-	
neXLP3 23	1-69	5-24	2	4	SRRVGYNCLDY	11	1-33	1	QQYDNLPPWT	10	-	+	-	
neXLP3 29	3-30	6-13	3	4	VDSKAAAGTSKY	12	1-27	4	QKYNASPLT	9	-	-	-	
neXLP3 30	3-20	3-16	3	3	GAMITFGGVIVIQGDAFDI	20	1-27	4	QKYNASLRT	9	-	-	-	
neXLP3 32	4-34	2-2	3	6	GRGLGRRRAIGLYYYMDV	18	4-1	4	QQYYSTPLT	9	+	+	-	
neXLP3 39	4-34	3-22	2	4	YYYDSSGYRY	10	2-28	4	MQALQTPILT	9	-	-	-	
neXLP3 52#	3-30	6-19	2	4	ANSSGWYTLDY	11	1-8	2	QQYYSYPYT	9				
neXLP3 54	4-34	1-26	3	6	GPSIVGAKKDYYYGMDV	17	3-20	2	QQYGSSPYT	9	-	-	-	
neXLP3 59	1-69	2-2	3	3	VGFVVPAATLYAFDI	16	3-20	1	QQYGSSPPNPWTWT	13	+	+	-	
neXLP3 61	1-69	3-22	2	6	GIYDPIYYYYYGMVDV	16	3-20	3	QQYGSSQFT	9	+	+	-	
neXLP3 63	3-30	3-10	1	3	GDKLWFGELWVAFDI	15	3-20	1	QQYRT	5	-	-	-	
neXLP3 65	3-30	6-19	3	6	DMSGRAVAGTLDWYYYGMDV	21	1-33	4	QQYDNLPLT	9	-	-	-	
neXLP3 66	3-30	5-5	3	4	DLRRAAMVTPYYFDY	15	2-29	4	MQSIQLPLT	9	-	-	-	
neXLP3 70	3-48	3-22	2	6	VTYYYDSSGYSQYMDV	16	3-20	1	QQYGSSPWTWT	11	-	-	-	
neXLP3 72	4-34	/	/	5	GIPHVRVDTARGETNWFDV	18	3-11	2	QQRSNWPPYT	10	-	-	-	
neXLP3 84	5-51	3-10	2	6	HFYGSQTVTPHYYYYGMDV	20	2-28	2	MQALQTPPEYT	11	-	+	-	
neXLP3 35							2-28	5	MQALQTPVT	9				
neXLP3 51							3-11	2	QQRSNWPPYT	10				
neXLP3 55							1-5	2	QQYNSYLYT	9				
neXLP3 62							1-9	4	QQLNSYPPLT	10				
neXLP3 89							3-11	5	QQRSNWPPIT	10				
neXLP3 90							1-33	5	QQYDNLPPIT	10				
neXLP3 93							3-11	2	QQRSNWPSYT	10				
neXLP3 94							1-5	2	QQYNSLMYT	9				
neXLP3 95							1-39	2	QQSYSTSRNT	10				
	VH	D	RF	JH	CDR3 (aa)	Length	Vκ	Jκ	CDR3 (aa)	Length	Poly	Hep-2	Staining	
neXLP3 07	4-39	4-17	2	4	LYGSLNYFDF	10	1-40	1	QSYDSSLGSGV	11	-	-	-	
neXLP3 09#	3-38	4-4	2	4	ETNGPDYKVMRSYFDY	16	1-44	3	AAWDDSLNGWV	11				
neXLP3 16#	4-39	1-1	2	5	PGYNWNDEYNWFDP	15	3-21	1	QVWDSSSDHYV	11				
neXLP3 21	1-69	3-10	1	5	ETPLWFGELLMWRGNWFDP	19	1-44	2	AAWDDSLNGVV	11	+	+	-	
neXLP3 22#	1-69	3-22	2	5	DRYYYDSSGGGWFDV	15	1-44	1	AAWDDSLNGFYV	12				
neXLP3 28	1-69	3-22	2	5	DKISYDSSGYYYGWFDV	17	3-21	3	QVWDSSSDQGV	11	-	+	-	
neXLP3 36#	1-24	2-2	3	5	IVVPAINQNDWFDP	16	3-9	3	QVWDSSSTAWV	10				
neXLP3 38#	1-69	2-15	3	5	DLVVVAATRWFDV	14	3-25	2	QSADSSVV	8				
neXLP3 57	3-23	5-12	2	4	APTASGYDYFDY	12	3-25	2	QSADSSGYTVV	11	-	-	-	
neXLP3 92	3-9	4-17	2	6	GDYGDYSYYGMDV	14	3-1	2	QAWDSSTVV	9	-	-	-	
neXLP3 02							3-1	2	QAWDSSTSVV	10				
neXLP3 36							3-9	3	QVWDSSSTAWV	10				
neXLP3 46							1-44	2	AAWDDSLNGPV	11				
neXLP3 53							2-8	3	SSYAGSNNLV	10				
neXLP3 60							2-14	2	SSYTSSSTLL	10				
neXLP3 74							3-25	1	QSADSSGTYHYV	12				
neXLP3 79							2-8	2	SSYAGSNNFVV	11				
	VH	D	RF	JH	CDR3 (aa)	Length								
neXLP3 05	1-18	2-2	2	6	SHCSSTSCYVGENYYYYGMDV	21								
neXLP3 06	3-21	5-24	3	4	VSIEMATDDRTGGGY	15								
neXLP3 27	1-18	6-13	3	4	GIAAAGTSGY	10								
neXLP3 49	3-23	2-2	3	4	DRDIVVPAIAIYYFDY	17								
neXLP3 77	4-61	/	/	5	FTAPIWEGWFDV	12								
neXLP3 85	4-59	6-13	2	4	LAPYSSSWARGLDY	14								
neXLP3 96	3-11	6-13	3	4	VSRSAQFVYFDY	13								

RF, reading frame; #, antibody failed to be expressed

-, non-reactive; +, reactive; +2, highly reactive

C, diffuse cytoplasmic staining; A, actin staining; G, Golgi staining; M, mitochondria staining; MS, mitotic spindle staining; N, nuclear staining; F, cytoplasmic fibers

Supplemental Table 4: Repertoire and reactivity of antibodies from new emigrant B cells of XLP4

Ig	HEAVY					LIGHT					REACTIVITY			
	VH	D	RF	JH	CDR3 (aa)	Length	Vκ	Jκ	CDR3 (aa)	Length	Poly	HEp-2	Staining	
neXLP4 02	3-23	6-13	3	4	AAAAGTGSFDY	11	4-1	2	QQYYSTLYS	9	-	-	-	
neXLP4 04	3-30-3	2-15	3	6	EKVVGDYYYYYGMDV	15	3-15	3	QQYNNWPPGIT	11	+	+	-	
neXLP4 06	3-15	/	/	6	DHTSGFNYYYYGMDV	16	3-11	1	QQRSNWPPWT	10	+	-	-	
neXLP4 08	4-31	6-13	1	4	EPEKQLGADY	10	1-27	1	QKYNSAPRT	9	-	+	-	
neXLP4 13	4-39	3-16	1	4	DKDGRLLRGLSLSF	15	3-11	4	QQRSNWPGALT	11	+	+	-	
neXLP4 17	4-39	3-22	2	3	DRGYDSSGYPDAFDI	16	1-5	3	QQYNSYSGT	9	-	-	-	
neXLP4 20#	1-69	4-17	1	5	DLGYQVRTLRFANWFD	17	3-15	4	QQYNNWPLT	9				
neXLP4 22	4-4	3-10	2	4	GVGRTYGSGSYLDY	15	3-20	1	QQYGSSPKT	9	-	+	-	
neXLP4 23	3-74	2-21	3	6	DHSPQIVVTAIPEDYYYYGMDV	24	2-28	1	MQALQTPWT	9	-	-	-	
neXLP4 26	4-59	1-26	3	4	ASRIVGATTFDY	12	1-8	1	QQYYSYPWT	9	+	+	-	
neXLP4 27	3-7	5-5	2	4	GGYSYGSHFDY	12	1-5	2	QQYNSYSGS	9	-	-	-	
neXLP4 31	3-30	1-26	2	4	EPPGSGYGYFDY	13	3-15	1	QQYNNWPPWT	10	-	-	-	
neXLP4 33#	1-69	/	/	6	PDTEWGDYYYGMDV	15	3-20	1	QQYGSSPPWT	10				
neXLP4 35	3-23	6-19	2	5	EYSSGWYVSGWFDP	14	3-11	4	QQRSNWPRT	9	+	-	-	
neXLP4 38#	4-39	6-13	2	4	DYHPSSSWYVFLTILDY	18	1-33	5	QQYDNLRLT	9				
neXLP4 39	3-15	4-17	3	3	GFPTTVTTAGVHAFDI	16	3-15	3	QQYNNWPL	8	-	-	-	
neXLP4 41	3-21	5-12	3	4	ESIVATAGIFDY	12	3-11	4	QQHSNWPLT	10	-	-	-	
neXLP4 42	3-20	1-26	2	2	DTASGSYPNWFYDL	14	3-15	1	QQYNNWPRT	9	-	-	-	
neXLP4 44	1-2	5-12	2	6	YGGYDFRFTSSYGMDV	17	3-11	5	QQHSNWAIT	9	+	+	-	
neXLP4 47#	4-39	2-15	3	3	GDVVVVAATVAFDI	14	1-12	3	QQANSFPGT	9				
neXLP4 48#	1-3	3-10	3	4	DRITMVQGVIIRVTAFDY	18	3-20	5	QQYGSSPPGIT	11				
neXLP4 11							1-5	2	QQYNSYYS	8				
neXLP4 40							1-39	1	QQSYSTPLT	9				
neXLP4 45							3-20	3	QQYGSSPIFT	10				
	VH	D	RF	JH	CDR3 (aa)	Length	Vλ	Jλ	CDR3 (aa)	Length	Poly	HEp-2	Staining	
neXLP4 01	4-39	2-15	2	4	DPIYCSGGSCYGGKNSYYFDY	23	6-57	2	QSYDSSNHVVV	11	-	-	-	
neXLP4 10#	1-69	2-2	2	6	VFSDCSSTSCLYYYGMDV	19	3-21	1	QVWDSSSDHYV	11				
neXLP4 15	4-39	2-21	2	5	LYCGGDCYFLHFDP	15	1-40	1	QSYDSSLGFIYV	12	-	-	-	
neXLP4 18	4-59	2-15	3	3	GGTMVVGGSFDI	12	3-21	1	QVWDSSSDHYV	11	-	-	-	
neXLP4 25	4-59	2-15	2	4	LDGYCSGGSCAWGV	16	1-44	3	AAWDDSLNGHWV	12	-	-	-	
neXLP4 29#	3-7	3-3	2	5	EYYDFWSGSQHTGGWFD	18	1-40	1	QSYDSSLGSGV	11				
neXLP4 30	3-74	3-22	3	6	DMVITLGVGSHNYLYYYGMDV	22	2-14	1	SSYTSSSTLV	10	+	+	-	
neXLP4 32	1-8	6-13	3	6	GQKAAVENYYYGMDV	15	3-1	2	QAWDSSLV	9	-	-	-	
neXLP4 36	3-15	/	/	6	AYLVYYYGMDV	12	1-51	3	GTWDSLSADVV	12	-	-	-	
neXLP4 46	1-2	/	/	6	GVSGLTGYGMDV	13	2-23	2	CSYAGSSTLV	10	-	+	+(M)	
neXLP4 16							1-51	2	GTWDSLSAVV	11				
	VH	D	RF	JH	CDR3 (aa)	Length								
neXLP4 07	3-21	4-17	2	4	DYGETYYFDY	10								
neXLP4 21	5-51	/	/	4	RRDRISPVDY	10								
neXLP4 34	4-59	/	/	3	RQYAPWAFDI	10								

RF, reading frame; #, antibody failed to be expressed

-, non-reactive; +, reactive; +2, highly reactive

C, diffuse cytoplasmic staining; A, actin staining; G, Golgi staining; M, mitochondria staining; MS, mitotic spindle staining; N, nuclear staining; F, cytoplasmic fibers

Supplemental Table 5: Repertoire and reactivity of antibodies from new emigrant B cells of XLP5

Ig	HEAVY						LIGHT			
	VH	D	RF	JH	CDR3(aa)	Length	Vk	Jk	CDR3(aa)	Length
neXLP5 04	3-30-3	2-2	2	4	DLMSSTSCYANY	13	4-1	1	QQYYSTPPT	9
neXLP5 15	3-15	3-22	2	4	DYYYGSSGYYTGPSDY	16	3-15	2	QQYNNWPL	8
neXLP5 17	3-21	2-15	2	4	DGQYCSGGSCYSYD	14	1D-8	1	QQYYSFPRT	9
neXLP5 22	5-51	/	/	4	PSVDYFDY	8	3-15	1	QQYNNWPRWT	10
neXLP5 26	3-11	3-10	2	4	DRRGPYMGSGSYPRDYFDY	19	1-5	1	QQYNSYGT	8
neXLP5 27	3-15	/	/	4	GSPQTPNGHEAY	12	4-1	1	QQYYSTLS	8
neXLP5 29	3-21	/	/	3	SWSDDAFDI	9	4-1	1	QQYYSTPPWT	10
neXLP5 30	1-24	2-2	2	4	EGPGPYCSTSCRKPLDY	18	3-20	4	QQYGSSPPT	9
neXLP5 32	3-33	2-21	3	4	DGPVVVTAPSFVFDY	16	4-1	2	QQYYSTPYT	9
neXLP5 33	4-4	3-10	3	4	DRGVTPYFDY	10	3-11	1	QQRSNWPRT	9
neXLP5 34	3-30-3	5-12	2	4	QPMMLNSGYGGNYFDY	16	3-20	4	QQYGSSLT	8
neXLP5 39	3-30	1-26	3	3	SSEAGATKDAFDI	13	1-5	1	QQYNSYWT	8
neXLP5 40	3-20	6-13	2	4	DWVGYSSSWSATPHFDY	17	1-13	5	QQFNSYPIT	9
neXLP5 43	3-11	2-15	3	6	GSVVVAATDYYYYGMDV	17	3-15	1	QQYNNWPWT	9
neXLP5 46	4-59	2-21	3	6	VDVVVTATPPLGYGMDV	17	2-28	3	MQALQTTF	9
neXLP5 48							4-1	4	QQYYSTPLT	9
neXLP5 06							4-1	1	QQYYSTPWT	9
neXLP5 07							3-20	3	QQYGSSPRGFT	11
neXLP5 08							3-15	2	QQYNNWPRYT	10
neXLP5 16							3D-15	2	QQYNNWPPYT	10
neXLP5 19							1-5	1	QQYNSYPS	8
neXLP5 28							1-33	4	QQYDNLQVT	10
neXLP5 31							3-11	1	QQRSNWRGT	9
neXLP5 35							1-12	4	QQANSPAT	8
neXLP5 36							3-11	2	QQRSNWPYT	9
neXLP5 37							4-1	1	QQYYSTPLT	9
neXLP5 41							1-17	4	LQHNSYPR	8
neXLP5 44							1-17	1	LQHNSYPRT	9
	VH	D	RF	JH	CDR3(aa)	Length	Vλ	Jλ	CDR3(aa)	Length
neXLP5 13	3-30	6-13	2	4	SDGSSWLFYD	10	4-69	2	QTWGTGIGV	9
neXLP5 20	3-7	3-10	2	6	DPRKYYYGSGSPAAYYYGMDV	22	1-51	2	GTWDSLSAVV	11
neXLP5 21	3-53	3-10	1	5	VWFGGWFDP	10	3-21	2	QVWDSSSDHPRTV	13
neXLP5 12							2-11	3	CSYAGSYTFWV	11
neXLP5 41							1-47	1	AAWDDSLSGPNYV	13
neXLP5 45							1-51	3	GTWDSLSAWV	11
	VH	D	RF	JH	CDR3(aa)	Length				
neXLP5 02	4-34	6-6	3	5	ALPIAAREGWFD	13				
neXLP5 05	3-33	4-17	3	6	DHLLMTTVSHYYYYYGMV	19				
neXLP5 14	3-21	2-15	2	6	PPDCSGGSCPLLGGMDV	18				
neXLP5 11	3-15	3-10 1-26	2 2	4	GAGSSGSPDFDY	13				
neXLP5 18	1-46	3-22	2	6	DHVPNKITYYDSSGFYYGMDV	22				
neXLP5 23	3-33	3-16	1	4	GEDGRSDY	8				
neXLP5 24	3-53	/	/	6	DSGGFSGYYYGMDV	14				
neXLP5 42	3-64	/	/	4	WVPTAYYFDY	10				
neXLP5 45	3-30-3	1-26	3	6	EPLGMGASYYYYYGMV	17				

RF, reading frame; #, antibody failed to be expressed

-, non-reactive; +, reactive; +2, highly reactive

C, diffuse cytoplasmic staining; A, actin staining; G, Golgi staining; M, mitochondria staining; MS, mitotic spindle staining; N, nuclear staining; F, cytoplasmic

Supplemental Table 6: Repertoire and reactivity of antibodies from new emigrant B cells of XLP6

Ig	HEAVY					LIGHT					REACTIVITY		
	VH	D	RF	JH	CDR3 (aa)	Length	Vκ	Jκ	CDR3 (aa)	Length	Poly	HEp-2	Staining
neXLP6 03	3-9	3-10	2	4	GVSGGGDFDY	10	3-11	5	QQRSNWPLT	9	-	-	-
neXLP6 06	3-66	3-22	2	5	DPNYDSSGAP	11	3-20	1	QQYGSSRWT	9	-	+	-
neXLP6 08	3-74	4-17	2	2	AFYGDYWFYDL	11	3-20	5	QQYGSSPPT	10	-	-	-
neXLP6 10	3-23	/	/	4	EGWDFDY	7	2-24	2	MQATQFPYT	9	-	-	-
neXLP6 14	3-30	6-13	3	4	GETIAAADTLDY	12	1-8	4	QQYYSYPLT	9	-	-	-
neXLP6 18	3-74	1-26	3	4	VPTRGIVGATLDY	13	1-5	1	QQYNSYPWT	9	+	+	-
neXLP6 19	3-48	6-13	3	4	GPTEFGIAAAGTDY	14	3-11	5	QQRSNWPPIT	10	-	-	-
neXLP6 21	3-11	3-22	2	6	DLTGYYDSSGYLRGYGMDV	20	1-33	4	QQYDNLTLT	9	-	-	-
neXLP6 22	3-48	6-19	3	4	QALAGTSVDY	10	1D-8	1	QQYYSFPRT	9	-	-	-
neXLP6 23#	1-8	3-10	2	4	YIGSGSYHLNQYFYDY	16	1-13	4	QQFNNTY	7			
neXLP6 26	3-23	/	/	4	DVDY	4	1-5	1	QQYNSYSRT	9	-	-	-
neXLP6 29#	3-21	1-26	2	3	VEGRGSAGSYGKGAFDI	18	1-5	1	QQYNSYSWT	9			
neXLP6 30#	3-48	2-21	2	3	YTPTYCGGDCPPESGAFDI	19	1-27	4	QKYNAPLT	9			
neXLP6 31	3-49	3-22	3	4	DFPFTMIVVRYIRDY	16	1-9	3	QQLNSYPLFT	10	+	+	+(N)
neXLP6 36κ	3-11	3-22	2	3	VRDSSGYYPYDAFDI	16	3-20	2	QQYGSSSYT	9	-	-	-
neXLP6 38	4-39	6-19	3	1	RVIAVADPRAEYFQH	15	3-11	5	QQRSNWPIT	9	+	+	-
neXLP6 39	1-24	3-3	2	4	SPGRSGYYTSFGPVSGFYFDY	21	1-13	4	QQFNYSYPLT	9	-	-	-
neXLP6 40	4-39	3-10	3	4	HGPITMVRGVFDY	13	2D-29	4	MQSIQLPLT	9	-	-	-
neXLP6 41#	4-61	3-10	3	3	ADHPIMVRGVIVDAFDI	17	4-1	4	QQYYSTPLT	9			
neXLP6 42	3-30	3-10	1	4	DPGEATWDPWYFYDY	14	3-11	4	QQRSNWPLT	9	-	-	-
neXLP6 45#	3-43	5-5	3	4	DIRGTAMVGYFDY	13	3-15	1	QQYNNWWT	8			
neXLP6 48#	3-23	3-22	2	4	DFEVVWGPTYDSSGYYSYDY	20	1-8	3	QQYYSYPLT	9			
neXLP6 17							1-5	4	QQYNSYWLT	9			
	VH	D	RF	JH	CDR3 (aa)	Length	Vλ	Jλ	CDR3 (aa)	Length	Poly	HEp-2	Staining
neXLP6 07	3-13	5-12	1	2	ELRSAWYFDL	10	2-14	2	SSYTSSSTPHV	12	-	-	-
neXLP6 12	3-23	2-21	3	4	NLRENGVTALSSWERPYFYDY	21	3-21	2	QVWDSSSDHV	11	-	-	-
neXLP6 13	4-61	3-3	3	6	GITIFGVIIAPLDV	15	1-44	3	AAWDDSLNGEV	11	+	+	-
neXLP6 20#	4-39	6-19	3	3	QIWGFTDVIAVAGINAFDI	19	2-8	1	SSYAGSTFYV	10			
neXLP6 24	1-18	3-22	2	3	RAPYYYDSSGYSSQEAFDI	19	2-23	2	CSYAGSSTSYV	12	+	+	-
neXLP6 28	3-9	1-26	3	4	DIGAMGATIPDY	12	2-14	2	SSYTSSSTLV	10	-	-	-
neXLP6 35#	4-39	/	/	4	GPPGAATDHY	10	1-44	3	AAWDDSLNAWV	11			
neXLP6 36λ#					see kappa		1-44	1	AAWDDSLNGYV	11			
neXLP6 37#	3-13	6-13	3	4	GPGAAAGERGGFVDY	15	6-57	2	QSYDSSNVV	9			
	VH	D	RF	JH	CDR3 (aa)	Length							
neXLP6 15	3-23	2-15	3	4	DILYFGASGQDIVVVAARPYFYDY	25							
neXLP6 44	3-30	5-12	2	4	DFSGYSGYFLDYFYDY	15							
neXLP6 46	4-61	/	/	5	APSKYNWFDP	10							

RF, reading frame; #, antibody failed to be expressed

-, non-reactive; +, reactive; +2, highly reactive

C, diffuse cytoplasmic staining; A, actin staining; G, Golgi staining; M, mitochondria staining; MS, mitotic spindle staining; N, nuclear staining; F, cytoplasmic fibers

Supplemental Table 7: Repertoire and reactivity of antibodies from new emigrant B cells of XLP7

Ig	HEAVY						LIGHT				
	VH	D	RF	JH	CDR3(aa)	Length	Vκ	Jκ	CDR3(aa)	Length	
neXLP7 02	1-18	2-2	3	6	DGSVQVVDSPAITHIYYGMDV	22	1-39	1	QQSYSTPRT	9	
neXLP7 03	3-11	3-10	1	6	GRGWFGFIYYYYGMDV	17	1-39	2	QQSYSTPRT	9	
neXLP7 09	1-3	1-26	3	4	DGEVGATRYFDYFDY	15	4-1	4	QQYYSTPLT	9	
neXLP7 10	3-7	6-19	3	6	PAQSIAGVFHHYGMVDV	17	1-5	3	QQYNSYSFFT	10	
neXLP7 14	3-23	3-3	2	6	DNDFWSGYDGFSDYGMVDV	18	3-11	1	QQRSNWSWT	9	
neXLP7 16κ	1-2	6-13	3	5	EGSFVIAAGWDETGWFDP	19	3-20	4	QQYGSSPGT	9	
neXLP7 17	4-59	5-24	1	5	VPPLKRWLQLAGWFDP	16	3-15	1	QQYNNWPPWT	10	
neXLP7 21	3-23	/	/	4	EGSLMDPPDY	11	1-5	1	QQYNSYSRT	9	
neXLP7 23	3-64	6-19	2	4	DVHERRYSSGWYGFYD	16	1-39	3	QQSYSTPPV	9	
neXLP7 25	4-34	3-10	3	4	VQRVRGVNPEGFDY	14	1D-8	1	QQYYSFPRT	9	
neXLP7 26	3-53	6-19	3	4	MGIAVAEI	8	1-17	1	LQHNSYPWT	9	
neXLP7 29	3-30	4-17	2	4	DADYGDYVGKLGFDY	15	1-12	1	QQANSFPQT	9	
neXLP7 31	3-15	2-2	2	4	DLVRCSSGTCYNEGAY	16	3-20	1	QQYGSSSPRT	9	
neXLP7 33	3-23	2-15	2	4	DHLSWGGSCDVFDY	14	1-12	2	QQANSFPPT	9	
neXLP7 35	3-66	3-10	2	6	DWVDYYGSGSSHVSFLDV	18	1-39	1	QQSYSTPPR	9	
neXLP7 38	3-7	4-4	2	6	KGDYYSNYPNYYYGMDV	17	1-8	3	QQYYSYPVT	9	
neXLP7 40κ	3-30	2-8	2	6	DQYCTGGVCSEGTTRYYYYGMDV	23	3-20	1	QQYGSSPPQT	10	
neXLP7 43	3-48	3-3	2	4	AKDFWSGYLVFDY	13	3-15	5	QQYNNWPRVT	10	
neXLP7 44	3-53	4-17	2	6	DKAPIYGELAPYGMVDV	16	3-11	3	QQRSNWPPEFT	11	
neXLP7 45	1-2	/	/	5	DGNKLFDP	8	3-20	3	QQYGSPT	8	
neXLP7 46	4-39	5-5	3	4	YYDTAMAPRAGLDY	14	2-28	1	MQALQTPWT	9	
neXLP7 47κ	3-30	3-22	2	1	DESYWDYDSSGPEYFQH	18	1-39	1	QQSYSTPWT	9	
neXLP7 48	3-13	6-13	1	6	VLRQLALYYYYGMDV	16	2-28	5	MQALQTPST	9	
neXLP7 42							3-11	3	QQRSNWPS	8	
	VH	D	RF	JH	CDR3(aa)	Length	Vλ	Jλ	CDR3(aa)	Length	
neXLP7 06	4-59	5-12	2	4	GRGYDSSFYD	10	1-44	1	AAWDDSLNGRYV	12	
neXLP7 07	3-15	4-17	3	4	PMTTVTTSY	10	1-47	3	AAWDDSLSGWV	11	
neXLP7 08	1-69	3-22	2	6	HPHYDSSGGMDV	13	2-14	2	SSYTSSSTLV	10	
neXLP7 11	1-8	5-24	1	2	PVTRWPFPAWYFDL	14	2-23	2	CSYAGSSTLV	10	
neXLP7 12	4-39	1-26	2	4	HHFSGSYVRN	11	1-40	2	QSYDSSLGSGVV	12	
neXLP7 16λ					see kappa		1-44	2	AAWDDSLNGPV	11	
neXLP7 18	3-15	3-22	2	4	GWTYYYDSSGYSPFDY	17	2-23	3	CSYAGSSTPYWV	12	
neXLP7 19	3-49	1-26	3	4	IVGATAGPFYD	11	3-21	3	QVWDSSSDHPV	11	
neXLP7 22	3-30	2-2	2	4	ELLYGCSSTSCFAPQGPSDY	21	2-23	3	CSYAGPWV	8	
neXLP7 24	1-46	6-6	2	6	DLYSSSSYYYYGMDV	15	6-57	2	QSYDSSNHVV	10	
neXLP7 27	4-39	4-17	2	4	RIYGDYPFDY	10	2-23	2	CSYAGSSTLV	10	
neXLP7 28	3-43	3-3	2	5	SYDFWWSGYLWFDP	15	2-14	2	SSYTSSSTHVV	11	
neXLP7 32	3-11	3-22	2	5	DGGYYDSSGYWDYGLTRPLFDP	23	1-47	3	AAWDDSLSGPGV	12	
neXLP7 34	1-18	4-17	2	6	DAPPLSPGDYESIYYGMDV	20	2-14	2	SSYTSSSTI	9	
neXLP7 36	3-7	5-5	1	6	AGPIQLWTFVYYGMDV	17	1-51	2	GTWDSSLSVGV	11	
neXLP7 37	5-a	6-13	1	4	HKALWQLEFDY	12	2-23	3	CSYAGSSTWV	10	
neXLP7 39	3-7	6-13	2	6	DHYKYSSWSGQPGRRTYGMVDV	22	2-8	2	SSYAGSNNLV	10	
neXLP7 40λ					see kappa		3-1	1	QAWDSSTVV	9	
neXLP7 41	1-18	2-8	3	4	DHEQIVLVYASEGYFDY	18	1-51	2	GTWDSSLSAVV	11	
neXLP7 47λ					see kappa		2-14	2	SSYTSSSTPHVV	12	
neXLP7 15							3-21	2	QVWDSSSDHVV	11	
	VH	D	RF	JH	CDR3(aa)	Length					
neXLP7 04	4-4	4-17	3	4	IASTTVTTDYFDY	13					
neXLP7 30	1-2	/	/	6	DDPPDDYYYYGMDV	14					

RF, reading frame; #, antibody failed to be expressed

-, non-reactive; +, reactive; +2, highly reactive

C, diffuse cytoplasmic staining; A, actin staining; G, Golgi staining; M, mitochondria staining; MS, mitotic spindle staining; N, nuclear staining; F, cytoplasmic fibers

Supplemental Table 8: Repertoire and reactivity of antibodies from new emigrant B cells of XLP9

Ig	HEAVY						LIGHT					
	VH	D	RF	JH	CDR3 (aa)	Length	Vκ	Jκ	CDR3(aa)	Length		
neXLP9 49	1-69	6-25	3	6	GVAALPGYYYY MDV	15	3-15	2	QQYNNWPHT	9		
neXLP9 53	4-59	2-15	2	4	VRGAGARYCSGGSCYSPGYFDY	22	1-5	1	QQYNSISDET	10		
neXLP9 57	3-15	4-4	2	4	APGDDYSNYDRSPSC	15	1-16	2	QQYNSYPYT	9		
neXLP9 60	4-30-2	5-5	2	3	GLLGGYTRGDDAFDI	15	1-39	5	QQSYSTPLIT	10		
neXLP9 61	3-23	3-22	2	3	PKSPSPWDSSGYLRFDAFDI	21	1-8	4	QQYYLSLT	9		
neXLP9 66	3-23	2-21	2	3	CGDLDAFDI	9	1-5	1	QQYNSYPWT	9		
neXLP9 67	1-69	1-7	3	6	GLTGTTSNGPDYYYYYMDV	19	3-11	3	QQRSNWPPPT	9		
neXLP9 74	3-64	3-3	3	4	QLAVPVDY	8	3-11	4	QQRSNFYT	8		
neXLP9 75	3-23	3-10	2	4	YYGSGSARFDY	12	3-11	5	QQRSNWPPIT	10		
neXLP9 79	4-34	2-2	3	5	GLKRAPSVPAAYNWFDP	18	3-20	2	QQYGSSPYT	9		
neXLP9 80	3-64	3-22	2	4	VEDQYYDSSGYHFDY	15	3-11	1	QQRSNWPPWT	10		
neXLP9 83	3-48	6-13	3	6	DGIAAADTSPKYYYYYYMDV	21	3-11	4	QQRSNWFLT	9		
neXLP9 84	4-34	2-8	2	6	GKHSIRYCTNGVCSPIQIPNYYYYMDV	27	1-5	1	QQYNSYSRT	9		
neXLP9 86	3-33	6-19	3	6	DLIAVAGNYYYYYMDV	16	1-39	5	QQSYSTPLIT	10		
neXLP9 88	4-59	1-26	1	1	AGWELAWIQH	10	4-1	1	QQYYSTPWT	9		
neXLP9 91	3-21	2-15	2	6	DSCSGGSCYASPPKYYYYYMDV	23	3-20	4	QQYGSSPPKIT	11		
neXLP9 93	4-39	3-22	2	4	NYVDSSGYPTFDY	15	1-39	2	QQSYSTPLYT	10		
neXLP9 94	4-39	4-17	1	4	HLFVRWLLGPALYYFDY	18	3-11	5	QQRSNWLDIT	10		
neXLP9 95	1-3	6-19	2	4	GEILYNIGWGRAAYYFDY	18	3-15	2	QQYNNWPPRYT	11		
neXLP9 52							3-11	3	QQRSNWPFPT	9		
neXLP9 63k							3-20	2	QQYGSSPGT	9		
neXLP9 85							1-39	1	QQSYSTPVT	9		
neXLP9 92							1-39	1	QQSYSTPWT	9		
neXLP9 96							1-5	2	QQYNSYSYT	9		
	VH	D	RF	JH	CDR3 (aa)	Length	Vλ	Jλ	CDR3(aa)	Length		
neXLP9 50	4-39	6-19	2	4	HGGGGSGWYWVS	12	1-40	2	QSYDSSLGSPVV	12		
neXLP9 51	4-39	4-17	2	5	SYGDYVWGNPWFFDP	15	2-14	2	SSYTSSSTLDVV	12		
neXLP9 56	5-51	3-10	2	3	RLGWDYYGSGSYRGDAFDI	21	2-14	2	SSYTSSSTPHVV	12		
neXLP9 59	5-51	/	/	6	SMTNENYYYY MDV	14	1-40	2	QSYDSSLSGSV	11		
neXLP9 62	3-15	6-19	3	5	GFSIGVMAGTLWFDP	15	2-8	1	SSYAGSNNYV	10		
neXLP9 64	3-23	3-3	1	4	DILRFLEWSPPDY	13	3-21	3	QVWDSSSDHWV	11		
neXLP9 65	5-51	2-15	3	5	LVVVAATLEAWFFDP	15	3-1	2	QAWDSSTAV	9		
neXLP9 68	3-23	/	/	6	DQWEDYYYY MDV	13	1-40	2	QSYDSSLGSLVV	13		
neXLP9 70	4-34	5-5	1	4	RIQLHGFMGY	10	2-14	1	SSYTSSSTLPFV	12		
neXLP9 71	3-23	4-4	1	4	MRGTRLHEWFFDH	14	1-40	3	QSYDSSLSGWV	11		
neXLP9 73	1-69	3-10	3	4	TVGTMVRGVPEGFDY	15	3-1	2	QAWDSSTSVV	10		
neXLP9 76	1-3	3-22	3	4	GHPMIVVVPFDY	12	1-51	2	GTWDDSSLAVV	11		
neXLP9 81	3-21	6-13	3	6	DIAAAGSYYYYY MDV	16	3-1	2	QAWDSSTVV	9		
neXLP9 87	4-39	1-20	2	5	QGTIDNWNPPGRWFDP	15	2-14	1	SSYTSSSTLDV	11		
neXLP9 89λ	3-33	6-19	3	4	PGEKVAGSGQFDY	13	3-10	1	YSTDSSGNRGV	11		
neXLP9 58							1-51	3	GTWDDSSLAYWV	11		
neXLP9 63λ							2-23	2	CSYAGSVV	8		
neXLP9 72							1-44	2	AAWDDSLNGVV	11		
neXLP9 77							2-11	2	CSYAGSYTSV	10		
	VH	D	RF	JH	CDR3 (aa)	Length						
neXLP9 54	3-15	3-10	2	2	YYGSGSYTYEYFDL	16						
neXLP9 69	4-4	6-19	1	6	APTPQWLVAQYYY MDV	17						
neXLP9 90	4-34	6-19	2	4	SSGWYDGIGY	10						

RF, reading frame; #, antibody failed to be expressed

-, non-reactive; +, reactive; +2, highly reactive

C, diffuse cytoplasmic staining; A, actin staining; G, Golgi staining; M, mitochondria staining; MS, mitotic spindle staining; N, nuclear staining; F, cytoplasmic fibers

Supplemental Table 9: Repertoire and reactivity of antibodies from new emigrant B cells of XLP10

Ig	HEAVY						LIGHT			
	VH	D	RF	JH	CDR3(aa)	Length	Vκ	Jκ	CDR3(aa)	Length
neXLP10 01	1-3	5-5	3	6	DTAMVNGYYYYGMDV	15	3-11	4	QQRSNWPLT	9
neXLP10 07	4-39	/	/	6	QQGGTDTVCGMDV	13	1-33	3	QQYDNLPLT	9
neXLP10 09	1-18	1-7	3	5	GITGTTKTPMGPSEGFD	18	2-30	1	MQGTHWPLT	9
neXLP10 10	3-49	3-22	3	4	DSMIVVPRDY	11	1-33	1	QQYDNLPR	9
neXLP10 12	3-30	3-16	1	4	DRSRELIGSSSDY	13	1-39	2	QQSYSTPYS	9
		6-6	2							
neXLP10 17	4-4	6-13	3	4	GAGIAAGSSVDY	13	1-5	1	QQYISYSKT	9
neXLP10 18	4-39	3-22	2	6	RADYYDSSGYYYYGMDV	19	2-28	4	MQALQTLLT	9
neXLP10 21	4-30-4	2-21	2	6	LTTCGGDPMGYYYGMDV	17	1D-13	4	QQFNRYRV	9
neXLP10 22	3-15	6-19	2	4	DVVWYSSGWYGGY	13	3-20	1	QQYGSSPRT	9
neXLP10 24	3-21	5-5	3	3	IADTAPGAFDI	11	4-1	2	QQYYSTPYS	9
neXLP10 30	4-59	3-22	2	2	KPGYDSSGYYSWYFDL	16	3-11	4	QQRSNWPLT	9
neXLP10 34	3-7	1-26	3	4	VGDEVGATTFDY	12	1-5	1	QQYNSYSRT	9
neXLP10 35	3-30	3-22	2	6	DPGSDSSGYYYYGMDV	18	2-29	1	MQGIHLPLT	9
neXLP10 36	3-23	/	/	4	DIAAVRDAQPIDY	13	1-16	2	QQYNSYPQS	9
neXLP10 43	3-49	2-15	3	3	GGADIVVVAATADAFDI	18	2-28	1	MQALQTPWT	9
neXLP10 45	1-18	/	/	6	WKTSLGGYYYYGMDV	15	2-28	2	MQALQTLYS	9
neXLP10 33							3-20	4	QQYGSFLT	8
neXLP10 44							2-29	2	MQGIHLLYS	9
neXLP10 47							1-13	3	QQFNSYLRYT	10
	VH	D	RF	JH	CDR3(aa)	Length	Vλ	Jλ	CDR3(aa)	Length
neXLP10 11	4-4	2-2	2	6	DQIQGYCSSTSCYKTYYGMDV	22	1-47	1	AAWDDSLSGLYV	12
neXLP10 23	1-2	3-22	2	4	GYDSSGYHAPGDY	15	7-43	3	LLYYGAWV	9
neXLP10 42	3-30	2-2	2	6	DPSAPYCSSTSCSYYYGMDV	22	3-10	1	YSTDSSGNHMGVV	13
neXLP10 48	1-69	3-22	2	6	AYYYDKYHYYYGMDV	15	1-51	2	GTWDSSLSAVV	11
neXLP10 04							1-40	2	QSYDSSLSGPV	11
neXLP10 37							2-8	2	SSYAGSNNFVV	11
neXLP10 41							1-51	3	GTWDSSLSAWV	11
	VH	D	RF	JH	CDR3(aa)	Length				
neXLP10 05	5-51	3-16	2	6	QLRFPGDYVWGSSYYGMDV	21				
neXLP10 27	3-33	3-22	2	4	LPYDSSGYYS	12				
neXLP10 46	3-49	3-22	2	4	MEYYDSSGYYPGYFDY	18				

RF, reading frame; #, antibody failed to be expressed

-, non-reactive; +, reactive; +2, highly reactive

C, diffuse cytoplasmic staining; A, actin staining; G, Golgi staining; M, mitochondria staining; MS, mitotic spindle staining; N, nuclear staining; F, cytoplasmic fibers

Supplemental Table 10: Repertoire and reactivity of antibodies from mature naive B cells of XLP2

Ig	HEAVY					Length	LIGHT				Length	REACTIVITY		
	VH	D	RF	JH	CDR3 (aa)		Vκ	Jκ	CDR3 (aa)	Poly		HEp-2	Staining	
mXLP2 07#	3-33	6-19	3	5	GLTPKTRAIIVAGP	14	3-15	2	QQYNNWPYT	9				
mXLP2 09	3-30-3	1-26	1	4	WELLDDY	7	1-27	1	QKYNAPWIT	9	-	+		-
mXLP2 10	4-39	6-6	2	5	LSSAGYWFD	10	2-40	4	MQRIEFPLT	9	-	+		+(N)
mXLP2 12	3-72	/	/	4	VFMGLDY	7	3-20	1	QQYGSSPPGT	10	-	-		-
mXLP2 13	3-33	/	/	4	AGGEGYYFDY	10	1-39	1	QQSYSTLRWT	10	-	-		-
mXLP2 14	4-34	3-9	2	6	GYFDWLDYYYYMDV	15	3-15	2	QQYNNWPYPYT	10	+	+		-
mXLP2 15	4-4	6-19	2	4	GRGYSSGWLRGDFDY	16	1-33	2	QQYDNSGYT	9	-	+		-
mXLP2 16	3-30-3	3-10	3	3	DFFLVTMGAFDI	12	1-6	3	LQDYNYPG	8	+	+		-
mXLP2 20	1-8	5-5	2	4	GPGYSGYAFDY	12	3-20	2	QQYGSSPPYT	10	-	-		-
mXLP2 25	3-15	3-22	2	4	AYYYDSSGYLPWAY	15	2-40	2	MQRIEFPYT	9	-	-		-
mXLP2 26	3-49	1-26	2	6	DRSYLYYYYYMDV	14	3-15	4	QQYNNWPLT	9	+	+		+(C+N)
mXLP2 28	1-69	6-6	3	2	HDMGGAARIYYFDL	15	1-13	4	QQFNSYPPT	9	+	+		-
mXLP2 30	4-31	/	/	4	EGNTGKFDY	9	1-39	1	QQSYSTPWT	9	-	-		-
mXLP2 31	3-21	5-5	2	4	DPGYSYGLNWFD	13	1-5	1	QQYNSYST	8	+	+		+(M)
mXLP2 36	3-48	1-26	3	4	GLGATFPVAYSDFDY	15	2-30	1	MQGTHWPWT	9	+	+		-
mXLP2 38	4-61	3-10	2	3	EGKYSGSGAFDI	13	1-8	1	QQYYSYPR	9	-	+		-
mXLP2 41κ	3-33	4-4	2	4	ELGYSNYAIDY	11	1-39	2	QQSYSTPMYT	10	-	-		-
mXLP2 43	3-15	2-15	2	4	SPTLNCSGGSCYYFDY	16	3-11	2	QQRSNWWYT	9	-	-		-
mXLP2 46#	4-31	3-10	3	4	AGTMVRGVITYYFDY	16	1-39	3	QQSYSTLFT	9				
mXLP2 48	4-59	4-23	2	4	ALGGNPIFDY	10	3-11	4	QQRSNWPLT	9	-	-		-
mXLP2 32							3-15	1	QQYNNWPPT	9				
mXLP2 42							1-33	3	QQYDNLFLT	9				
	VH	D	RF	JH	CDR3 (aa)	Length	Vλ	Jλ	CDR3 (aa)	Length	Poly	HEp-2		Staining
mXLP2 02	4-39	3-22	2	4	PAYDSSGYRGYYFDY	15	2-14	3	SSYTSSSTWV	10	-	+		-
mXLP2 04	3-30-3	6-13	2	3	DRGVVEYSSSWYSGGAFDI	19	3-21	1	QVWDSSSDHYV	11	-	-		-
mXLP2 05	4-61	2-2	3	5	VVVPAIGGWFD	14	1-51	2	GTWDSLSAVV	11	-	-		-
mXLP2 06	3-33	3-22	2	4	DGPSSGYYYFDY	12	3-1	2	QAWDSSPVV	9	-	+		+(C+N)
mXLP2 17	3-73	1-1	2	3	RGYNWNDDTRLDAFDI	16	3-21	2	QVWDSSSDHEV	11	-	-		-
mXLP2 18	4-61	4-23	3	6	TVVTLYMDV	10	1-44	2	AAWDDSLKGV	10	-	-		-
mXLP2 21	3-30-3	3-3	2	4	GRPSYDFWGSYDY	13	2-14	2	SSYTSSSTVV	10	-	+		-
mXLP2 23	3-21	1-7	3	4	DQTGTDFDY	10	3-21	3	QVWDSSSDHWV	11	-	-		-
mXLP2 27	3-33	6-6	3	4	GFSIAARNLDY	11	2-14	1	SSYTSSSTLYV	11	-	+		-
mXLP2 33	1-2	5-12	2	4	VRYSGDYTFDY	12	2-14	2	SSYTSSSTVV	10	-	-		-
mXLP2 34	3-23	/	/	4	AIRHQLGYDY	10	3-21	1	QVWDSSSDHYV	11	-	-		-
mXLP2 35	3-33	1-26	2	3	DFLDGSYGTDAFDI	14	2-11	2	CSYAGSYTWV	10	-	-		-
mXLP2 39	4-4	2-2	2	6	EVRCSSSTSCYWFPEVRYMDV	20	1-40	2	QSYDSSLSGSHVV	13	+	+		-
mXLP2 41λ#					see kappa		3-1	3	QAWDSSSTAV	9				
mXLP2 44	3-33	3-3	1	3	ERFLYAFDI	9	3-21	2	QVWDSSSDHPVV	12	-	-		-
mXLP2 47	3-15	6-13	1	6	DPQQQLVRSYYYYMDV	17	2-23	2	CSYAGSSTLV	10	-	+		-
mXLP2 03							1-47	3	AAWDDSLRV	9				
	VH	D	RF	JH	CDR3 (aa)	Length								
mXLP2 08	1-69	4-17	2	5	DCYGDYIGWFD	12								
mXLP2 19	1-18	1-26	3	4	AGWMSVVGATRGTFDY	16								
mXLP2 37	3-30	6-19	3	4	DSRIAVALYYFDY	13								

RF, reading frame; #, antibody failed to be expressed

-, non-reactive; +, reactive; +2, highly reactive

C, diffuse cytoplasmic staining; A, actin staining; G, Golgi staining; M, mitochondria staining; MS, mitotic spindle staining; N, nuclear staining; F, cytoplasmic fibers

Supplemental Table 11: Repertoire and reactivity of antibodies from mature naive B cells of XLP3

Ig	HEAVY					LIGHT					REACTIVITY		
	VH	D	RF	JH	CDR3 (aa)	Length	Vκ	Jκ	CDR3 (aa)	Length	Poly	HEp-2	Staining
mXLP3 01	4-34	3-10	2	4	FRPRPYGSGGGLRGPYYFDY	21	1-39	2	QQSYSTPPYT	10	-	+	-
mXLP3 13	3-21	6-13	2	4	DLGGSSWYFFDY	12	3-15	2	QQYNNWPPYT	10	-	-	-
mXLP3 19	4-34	6-6	3	6	CKARRDYYYYYMDV	15	3-20	2	QQYGSSPPAYT	11	-	+	+(C)
mXLP3 24	4-4	3-22	2	4	DRGEGYDDSSGYFDY	16	1-5	1	QQYNSYPWT	9	-	-	-
mXLP3 28	4-34	6-13	2	4	SSSFFHYFDY	10	2-28	1	MQALQTPKT	9	-	-	-
mXLP3 37#	5-51	3-10	2	6	RSGSGYYYYYGMDV	14	2-28	5	MQALQTFT	8			
mXLP3 41	3-74	5-24	3	4	GMATNK	6	4-1	2	QQYSTPYT	9	-	+	-
mXLP3 46#	3-74	3-3	2	4	DPAYYDFWSGYCYFDY	16	1-39	5	QQSYSSIT	8			
mXLP3 56	1-69	/	/	4	LDY	3	3-20	1	QQYGSSPPWT	10	+	+	-
mXLP3 64	4-34	2-2	3	5	RVTLVPAATTNWFD	16	4-1	4	QQYSTPLT	9	-	-	-
mXLP3 68	1-18	4-17	2	4	DKVRGDYGYVSFDY	14	3-15	2	QQYNNWPPYT	10	-	-	-
mXLP3 70	7-4-1	3-10	2	6	EASRYYGSGSYTPYYYYGMDV	22	2-28	2	MQALQTPT	9	-	+	-
mXLP3 84#	1-2	3-22	2	3	RRTSTYYYDSSGYSSNAFDI	20	3-15	1	QQYNNWPPWT	10			
mXLP3 85#	4-34	3-22	2	3	GRGRGHYDSSGYYYASHKNDAFDI	25	1-8	1	QQYSSYPRT	9			
mXLP3 88#	4-34	3-9	2	6	GHYDILTGYYISRKPYYYYGMDV	23	1-39	1	QQSYSTPPT	9			
mXLP3 91	3-53	4-23	3	6	GLLTTVDGMDV	11	3-15	1	QQYNNWPPWT	10	-	+	-
mXLP3 93	3-9	6-6	3	6	DALNIADIIYYYYMDV	16	3-15	2	QQYNNWPMYT	10	-	+	-
mXLP3 95	7-4-1	6-19	3	6	KSVAGHASSYYYYGMDV	17	1-33	4	QQYDNPLT	9	-	+	-
mXLP3 96	7-4-1	2-15	2	6	LSSWEDCSGGSCYSGPRYYYYGMDV	26	1-9	3	QQLNSYPT	8	-	+	-
	VH	D	RF	JH	CDR3 (aa)	Length	Vλ	Jλ	CDR3 (aa)	Length	Poly	HEp-2	Staining
mXLP3 02	1-2	3-10	3	5	VMAMVRGRSHNWFD	15	1-51	1	GTWSSLSAGV	11	-	+	+(N)
mXLP3 07	3-33	3-9	2	4	GGLTGYPY	8	3-1	2	QAWDSSTVV	9	+	+	-
mXLP3 11	1-46	7-27	2	6	DDWGYRYYYGMDV	13	3-25	2	QSADSSGTYYV	11			
mXLP3 25#	7-4-1	3-9	2	4	VGILTALDY	9	3-1	2	QAWDSSTVV	9			
mXLP3 26	1-46	/	/	2	DRGLPGWYFDL	11	2-8	2	SSYAGSNTLVV	11	-	-	-
mXLP3 35	4-34	6-13	3	5	DIAAAGRNDWFD	13	1-47	3	AAWDDSLSGWV	11	-	-	-
mXLP3 48	4-31	3-22	2	3	SPDYDSSGYNHDAFDI	17	1-51	1	GTWSSLSAGPYV	13	-	+	+(C)
mXLP3 59	1-58	6-13	2	6	VPYSSSWYGAVIHMDV	17	3-25	2	QSADSSVV	8			
mXLP3 69	4-34	/	/	6	DLKGHPYYYYYYMDV	16	2-23	3	CSYAGSSTFV	10	+	+	-
mXLP3 82	1-8	3-22	2	5	GQNKAVYYDSSSLRRSGGFDP	21	2-23	1	CSYAGSSTYV	10	-	-	-
mXLP3 94	1-8	3-9	1	3	GQPLRYFDWLPIGGWEDAFDI	21	2-14	3	SSYTSSSPWV	10	+	+	-
mXLP3 08							1-51	3	GTWSSLSAGPV	12			
mXLP3 33							2-8	1	SSYAGSNPYV	10			
mXLP3 42							1-44	3	AAWDDSLNGWV	11			
mXLP3 60							2-14	1	SSYTSSSTLEKV	12			
mXLP3 71							3-10	3	YSTDSSGNHPNWV	13			
mXLP3 73							2-8	2	SSYAGSNNFGVV	12			
mXLP3 78							2-14	2	SSYTSSSTLEV	12			
mXLP3 87							1-47	2	AAWDDSLSAL	10			
mXLP3 92							2-11	1	CSYAGSYTYV	10			
	VH	D	RF	JH	CDR3 (aa)	Length							
mXLP3 09	3-21	3-22	2	4	TTAAANWYYDSSGGYDY	18							
mXLP3 61	3-33	2-2	3	4	DDGGDIVVPGYYFDY	16							
mXLP3 65	4-59	4-17	2	5	GYGDYWFDP	9							
mXLP3 67	4-39	3-3	1	6	VLRFLWLLPYYYYMDV	17							
mXLP3 77	7-4-1	3-10	2	5	DGSGSYNNADNWFD	15							
mXLP3 79	3-15	6-13	2	4	DPLGSSSWYFVDY	13							
mXLP3 83	4-34	3-10	2	6	GSYYYGSGYYKRAYYYGMDV	22							
mXLP3 89	1-2	3-22	2	4	APPYYDSSGGYGGKLDY	16							

RF, reading frame; #, antibody failed to be expressed

-, non-reactive; +, reactive; +2, highly reactive

C, diffuse cytoplasmic staining; A, actin staining; G, Golgi staining; M, mitochondria staining; MS, mitotic spindle staining; N, nuclear staining; F, cytoplasmic fibers

Supplemental Table 12: Repertoire and reactivity of antibodies from mature naive B cells of XLP4

Ig	HEAVY						LIGHT				REACTIVITY		
	VH	D	RF	JH	CDR3 (aa)	Length	Vκ	Jκ	CDR3 (aa)	Length	Poly	HEp-2	Staining
mXLP4 01	1-2	2-8 6-13	3 3	4	EPRGVLVYAIIPSEGAAGDY	21	3-20	1	QQYGSSPWWT	10	+	+	-
mXLP4 07	4-b	2-15	2	3	DLGKLGYSGGSCSDDAFDI	20	1-12	4	QQANSFPLT	9	+	-	-
mXLP4 08	4-39	4-17	2	6	PKGYGDSPIYYYGMDV	16	1-5	1	QQYNSYPWT	9	+	+	-
mXLP4 14	3-23	3-3	2	4	GHYDFWGSDDY	11	1-12	2	QQANSFPYS	10	+2	+	-
mXLP4 16	1-18	6-13	3	3	DRAGAKIAAADINAFDI	17	3-11	5	QQHSNWPPRVT	11	-	-	-
mXLP4 20	3-21	3-22	2	4	DEGGSSGYPDFDY	14	1-39	1	QQSYSTPWT	9	-	-	-
mXLP4 27	4-59	/	/	4	GEGYRLLRH	9	3-15	1	QQYNNWPPWT	10	+	+	-
mXLP4 28	3-30	3-3	2	5	ERADYDFWSGYSDLIRGNWFDP	22	3-15	3	QQYNNWPS	8	+	+	-
mXLP4 30κ#	4-59	3-3	2	4	EGQGDFWTTNPTYFYDY	17	3-11	4	QQHSNWPP	8			
mXLP4 32	3-23	6-19	3	3	DRGAVDAFDI	10	1-5	2	QQYNSYSEYS	10	+	+	-
mXLP4 33	4-34	3-3	2	4	GFNFWSGYYSIRFDY	15	1-39	1	QQSYSTPRT	9	+	+	-
mXLP4 34	3-9	6-19	2	4	GRGPYSSGLFDY	12	3-11	4	QQHSNWPL	8	-	+	-
mXLP4 36	3-23	/	/	5	VGRWERYGNWFDP	13	1-5	2	QQYNSYLYT	9	+	+	-
mXLP4 38	3-30	6-13	1	4	SFSFQQLVDY	11	3-11	1	QQHSNWPPWT	9	-	-	-
mXLP4 41	1-18	1-26	3	1	VLVGTPPAYFQH	12	3-11	3	QQRSNWRVT	9	-	+	-
mXLP4 44	3-23	1-26	3	3	LVGATTLDAFDI	12	3-11	4	QQHSNWPLT	9	-	-	-
mXLP4 48	4-34	3-3	1	4	TLSVLPQSGYFDY	13	1-5	1	QQYNSYPAT	9	-	-	-
	VH	D	RF	JH	CDR3 (aa)	Length	Vλ	Jλ	CDR3 (aa)	Length	Poly	HEp-2	Staining
mXLP4 04	4-59	3-22	2	4	QAGDSSGYPIDY	12	3-21	2	QVWDSSSDVV	10	-	-	-
mXLP4 05	3-30	/	/	3	DWDWTHAFDI	10	2-11	1	CSYAGSYNV	9	-	-	-
mXLP4 06	4-59	3-16	1	6	DTRRLGEFGISYYYGMDV	19	2-14	3	SSYTSSSIL	9	+	+	-
mXLP4 18	4-31	6-13	1	4	VIQQQLAHFDY	11	1-51	3	GTWDSSLSAGV	11	-	-	-
mXLP4 29	4-30-4	5-12	3	5	DRRGVATMFNWFDP	14	2-23	2	CSYAGSSTFVV	11	-	-	-
mXLP4 30λ					see kappa		1-44	3	AAWDDSLNGV	10	+	-	-
mXLP4 31	3-9	6-13	3	4	DMVGRAGIAAAGMDY	15	2-14	2	SSYTSSSTFVV	11	-	-	-
mXLP4 45	1-2	/	/	3	YATNAFDI	8	1-51	1	GTWDSSLSAEV	11	-	-	-
mXLP4 47	5-51	6-19	3	5	RGSSAVADLRYNWFDP	16	3-1	2	QAWDSSTAFHVV	12	-	+	-
mXLP4 24							1-36	2	AAWDDSLNGVV	11			

RF, reading frame; #, antibody failed to be expressed

-, non-reactive; +, reactive; +2, highly reactive

C, diffuse cytoplasmic staining; A, actin staining; G, Golgi staining; M, mitochondria staining; MS, mitotic spindle staining; N, nuclear staining; F, cytoplasmic fibers

Supplemental Table 13: Repertoire and reactivity of antibodies from mature naive B cells of XLP6

Ig	HEAVY					LIGHT					REACTIVITY		
	VH	D	RF	JH	CDR3 (aa)	Length	Vκ	Jκ	CDR3 (aa)	Length	Poly	HEp-2	Staining
mXLP6 02	3-9	3-22	2	3	TLRAYDSSGYYSDAFDI	18	1-27	2	QKYNLSALMYT	10	-	-	-
mXLP6 03	3-66	6-19	3	4	DVTMAVAES	9	1-39	4	QQSYSTPLT	9	-	-	-
mXLP6 05	3-30	2-15	2	4	GSEGYCSGGSCYEDY	15	1-33	3	QQYDNLPLA	9	-	-	-
mXLP6 09	3-53	6-19	3	4	AGPGIAVAGTGYFDY	15	3-15	1	QQYNNWWT	8	-	-	-
mXLP6 12#	1-8	3-10	1	5	GRLLWFGDNNWFDP	14	1-17	1	LQHNSYPWT	9			
mXLP6 14	3-53	/	/	4	GTKPLGPLMTY	11	4-1	1	QQYYSTPPWT	10	+	+	+(N)
mXLP6 15	4-39	3-22	2	6	PYYYDSSGYMDV	12	1-33	4	QQYDNLQVVT	10	-	-	-
mXLP6 16	3-7	3-22	2	4	SSYYDDPKYYFDY	13	1-5	1	QQYNSYPWT	9	-	-	-
mXLP6 18	3-21	6-19	3	4	TGIAVAGPRY	10	1-33	4	QQYDNLPLT	9	+	+	-
mXLP6 19	4-39	3-22	2	2	PMDYYDSSGYHPSQTNWYFDL	21	3-11	2	QQRSNWPPG	9	-	-	-
mXLP6 20	3-21	4-17	2	4	EHGETPGDSDY	11	1-17	2	LQHNSYPHT	9	-	-	-
mXLP6 22	4-59	3-22	2	4	GYDDSSGYYY	10	2-28	3	MQALQTPFT	9	-	-	-
mXLP6 25	3-49	3-22	1	3	VLGLLPVPDAFI	13	3-15	4	QQYNNWPLT	9	+	+	-
mXLP6 29#	4-39	2-21	3	3	RNGGPLGAIVVTATNAFDI	20	1-33	1	QQYDNLST	9			
mXLP6 30	3-53	3-10	1	6	HTELWFGELPKLSHYYYGMDV	21	1-9	5	QQLNSYPIT	9	+	+	-
mXLP6 32#	4-4	6-19	3	5	AWGSIAGTASWFDP	16	3-15	4	QQYNNWPLT	10			
mXLP6 33	3-21	3-16	2	6	DSSYDYVWGSYPVYYYGMDV	21	3-11	2	QQGGT	5	+	+	-
mXLP6 34	4-59	3-16	1	6	HPFEGRLRGLSPPPHYYYGMDV	24	2-28	3	MQALQTPHGGFT	12	+	-	-
mXLP6 35	3-23	5-12	3	4	LGGATTFDY	9	1-8	4	QQYYSYPRGLT	11	+	+	-
mXLP6 40#	4-59	/	/	4	DVGSSRDY	8	3-20	2	QQYGSSPLT	9			
mXLP6 41#	3-23	3-22	2	4	DPGTHYYDSTGRYFDY	16	3-11	4	QQRSNWPPRA	10			
mXLP6 43#	3-15	3-22	2	4	VYYYDSSGYYPWGY	15	1-33	2	QQYDNLPLYT	9			
mXLP6 45#	3-23	3-10	2	4	DALPMHHSGRYFDY	14	3-20	4	QQYGSSPLT	9			
mXLP6 10							2-28	1	MQALQWT	8			
mXLP6 28							1-39	4	QQSYSTPPT	9			
mXLP6 37							1-5	1	QQYNSYSTWT	10			
mXLP6 42							1-39	1	QQSYSTPK	8			
	VH	D	RF	JH	CDR3 (aa)	Length	Vλ	Jλ	CDR3 (aa)	Length	Poly	HEp-2	Staining
mXLP6 07	3-30	3-10	2	4	EGGGYGSGSALDY	13	2-14	2	SSYTSSSTQ	9	-	-	-
mXLP6 11#	3-48	3-10	2	4	APPYYYGSGSYGRFCDY	17	3-1	1	QAWDSSTLYV	10			
mXLP6 21	3-30	3-10	3	6	GYMVRGPYYYGMDV	15	3-1	1	QAWDSSTGGV	10	-	+	-
mXLP6 24#	3-64	3-10	1	3	DRQEYDLSLGFPHAPDAFI	21	3-12	3	QVWDSSTDHPGV	12			
mXLP6 38#	3-7	3-22	2	4	DRRTYYYDSSGIPVDY	16	3-21	2	QVWDSSTDHVV	11			
mXLP6 39	4-61	3-9	2	6	VTSKYDILTGYMDYYYGMDV	23	1-44	3	AAWDDSLNGPV	11	+	+	-
mXLP6 44	4-31	3-10	2	4	DYYGSGRHHGLDY	13	3-1	2	QAWDSSTVV	9	-	-	-
mXLP6 48	4-61	3-10	2	5	EDYYSNWFDP	11	1-47	3	AAWDDSLSGHWV	12	-	-	-
	VH	D	RF	JH	CDR3 (aa)	Length							
mXLP6 31	3-21	1-26	2	4	SRPGDSGSYFDY	12							

RF, reading frame; #, antibody failed to be expressed

-, non-reactive; +, reactive; +2, highly reactive

C, diffuse cytoplasmic staining; A, actin staining; G, Golgi staining; M, mitochondria staining; MS, mitotic spindle staining; N, nuclear staining; F, cytoplasmic fibers

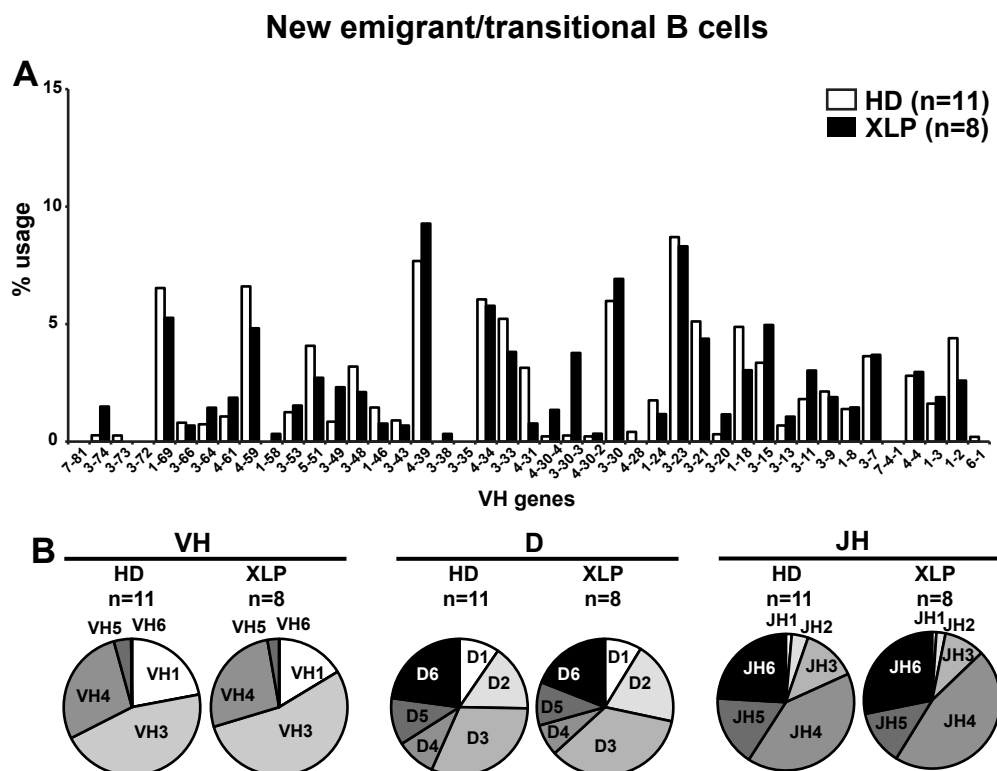


Figure E1. New emigrant/transitional B cells from XLP patients display a heavy chain gene segment repertoire similar to their counterparts in healthy donors (HD). (A) Usage frequencies of VH genes in HD and XLP patients. (B) Usage frequencies of VH, D and JH family genes in HD and XLP patients. 362 sequences from 11 HD and 265 sequences from 8 XLP patients were analyzed.

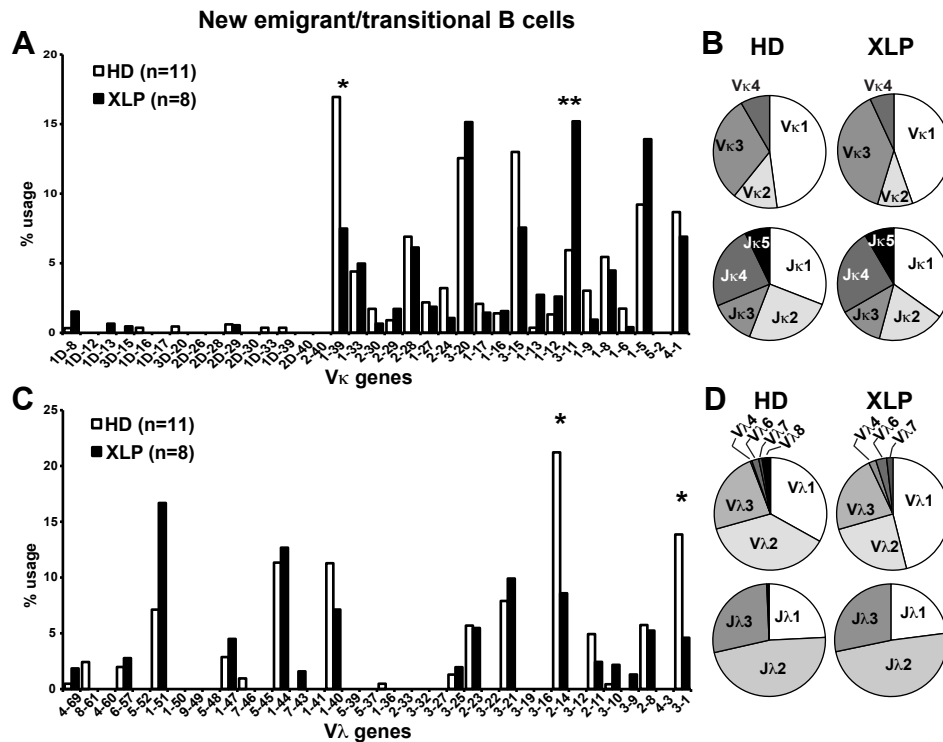


Figure E2. New emigrant/transitional B cells from XLP patients display a different Variable (V) light chain gene repertoire compared to their counterparts in healthy donors (HD). (A) Usage frequencies of V_{κ} genes in HD and XLP patients. (B) Usage frequencies of V_{κ} and J_{κ} family genes in HD and XLP patients. (C) Usage frequencies of V_{λ} genes in HD and XLP patients. (D) Usage frequencies of V_{λ} and J_{λ} family genes in HD and XLP patients. 362 sequences from 11 HD and 265 sequences from 8 XLP patients were analyzed. *: p-value <0.05, **: p-value <0.01

New emigrant/transitional B cells

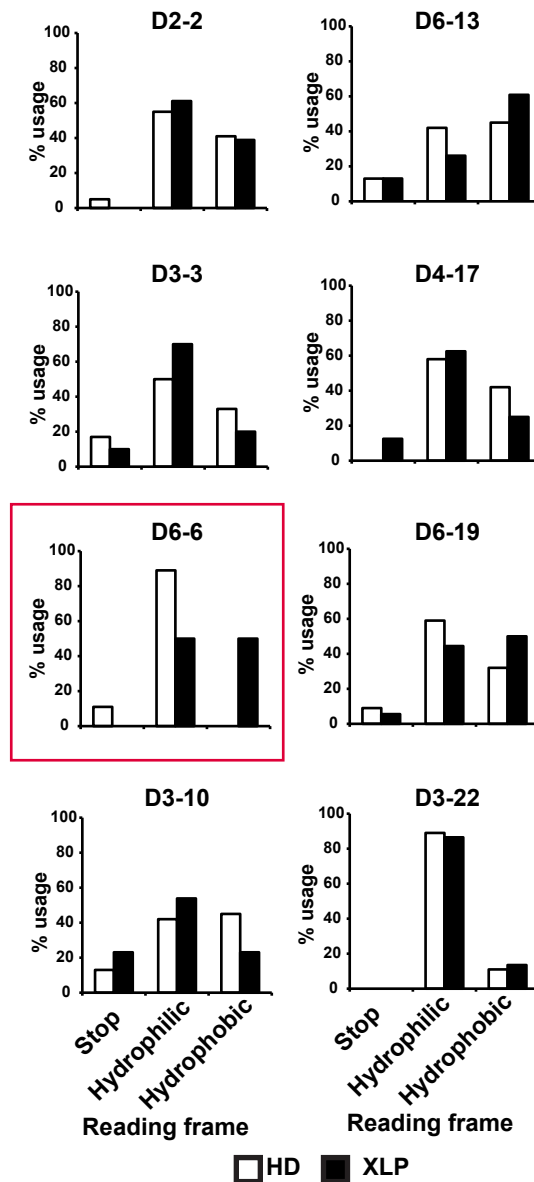


Figure E3. Reading frame usages for frequently used D genes in new emigrant/transitional B cells from healthy donors (HD) and XLP patients. 362 sequences from 11 HD and 265 sequences from 8 XLP patients were analyzed. The abnormal hydrophobic reading frame usage of D6-6 in XLP patients suggests a defective central B cell tolerance checkpoint.

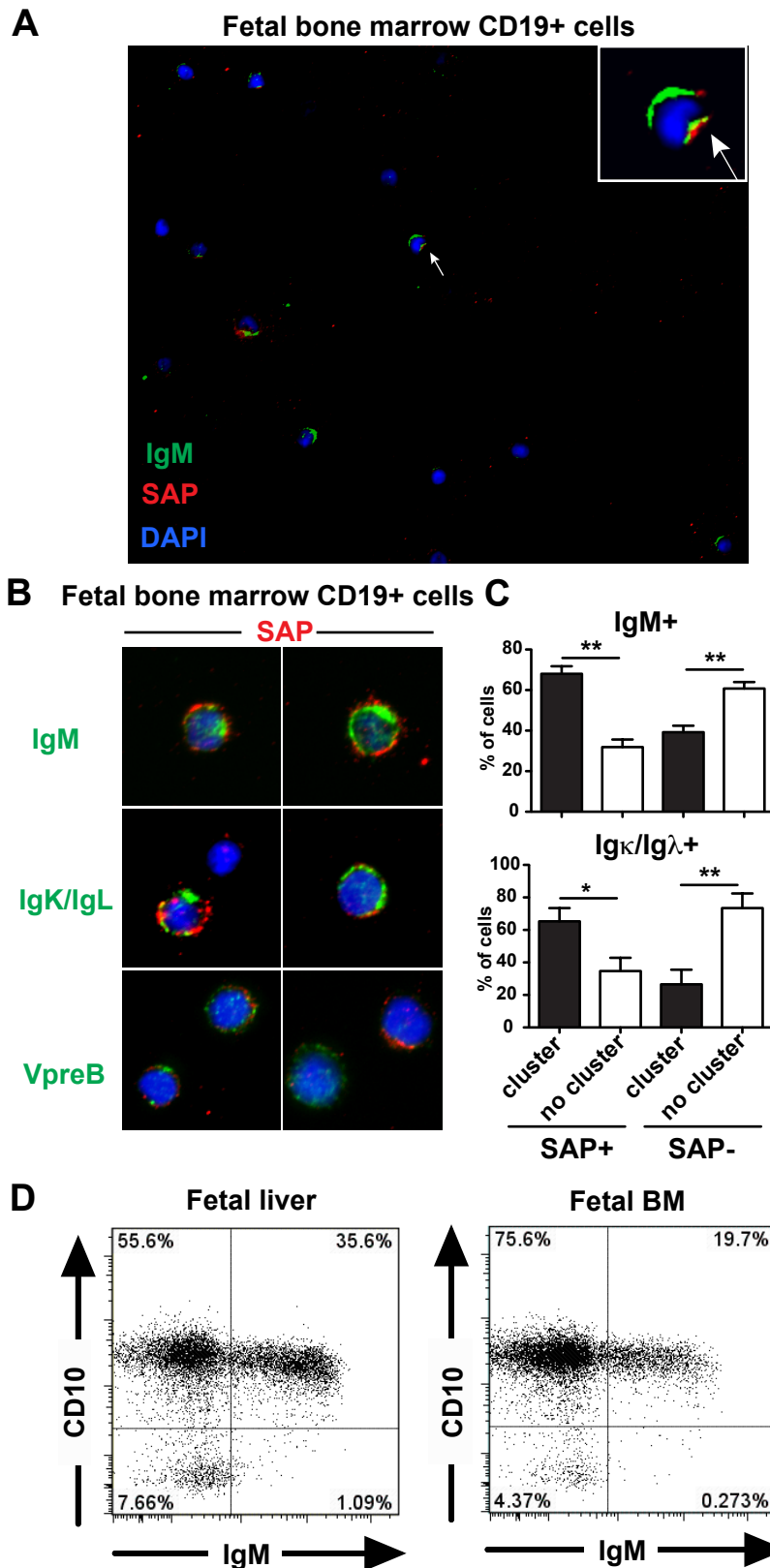


Figure E4: SAP is expressed by some developing immature fetal B cells. (A), (B) CD19⁺ fetal bone marrow B cell precursors were stained for IgM, Igk and Igλ, VpreB (green) and SAP (red). Arrow in (A) points to a SAP⁺IgM⁺ cell. (C) % of cells in SAP⁺ vs SAP⁻ cells from staining in (B) that displayed a clustered vs not clustered IgM and Igk/Igλ staining pattern. *:p-value<0.05; **: p-value<0.01. (D) Representative CD10 and IgM staining on CD19⁺ gated cells from fetal liver and bone marrow samples.

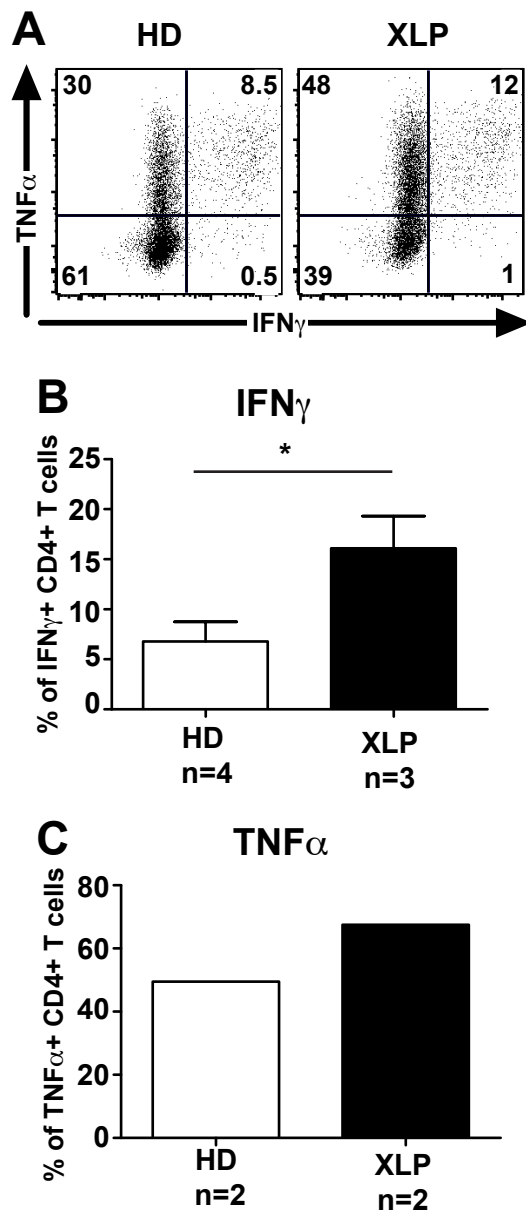


Figure E5: SAP-deficient CD4+T cells express more IFN γ and TNF α cytokines. (A) Representative dot-plots of IFN γ and TNF α expression measured by intracellular cytokine staining in HD and XLP CD4+ T cells stimulated for 4.5 days. Percentage of IFN γ + (B) and TNF α + (C) T cells after 4.5 days of stimulation.