Supplementary figure legends

Fig. E1. mRNA and protein expression of mutant IKAROS. (A) Relative quantification of *IKZF1* mRNA in B cells from healthy controls (C1-C3) and patients (P1, P2) normalized to the average of the three housekeeping genes (GAPDH, Actin, HPRT) and relative to C1 (two repeats in triplicate). **(B)** Representative western blot analysis of IKAROS expression in whole protein lysates from EBV-transformed B cells from P1, P2, C4 and C5. **(C)** Quantification of IKAROS protein from western blot in B, normalized to histone H1 (4 technical replicates).

Fig. E2 Representative EMSAs using FLAG-tagged expression plasmids. (A) Representative western blot of nuclear enriched fractions from HEK293T cells transfected with Flag-tagged plasmids containing 100% WT IKAROS, 100% L188V mutant IKAROS, WT:L188V IKAROS in a 50:50 ratio or 100% p.173-253del control (two repeats). **(B)** Western blot of the nuclear extracts in (A) using anti-Flag antibodies as in A but normalized to WT expression for use in EMSA (two repeats). **(C)** EMSAs were performed using two different probes: IK-bs4 and YSat8 as indicated. Lanes were loaded with nuclear extracts from untransfected HEK cells (Untr) or HEK cells transfected with 100% WT, 100% mutant L188V, WT:L188V in a 50:50 ratio or 100% p.173-253del control. A supershift was induced by adding anti-Flag antibody. **(D)** Lanes were loaded with nuclear extracts containing the same amount of WT IKAROS but increasing amount of mutant L188V, supplemented with empty vector (EV). The arrows mark the size of complexes containing IKAROS.

Fig. E3. Representative EMSAs using HA-tagged WT and Flag-tagged L188V expression plasmids. (A) Representative western blot of nuclear enriched fractions from HEK293T cells transfected with HA-tagged WT driven by SV40 promoter and Flag-tagged L188V mutant IKAROS driven by CAG promoter (three repeats to achieve normalization to housekeeping genes). **(B)** Lanes were loaded with nuclear extracts containing the same amount of HA-tagged WT IKAROS driven by the SV40 promotor and increasing amounts of Flag-tagged L188V mutant IKAROS driven by the CAG promoter. Relative quantification for each probe is shown on the far right (five independent experiments for IK-bs4, two for YSat8).

The arrows mark the size of complexes containing IKAROS.

Fig. E4. Mutant IKAROS feeds a pro-germinal center immunological phenotype. PBMCs were isolated from P1, P2 (together referred to as 'P' for patients), mother, brother and 3 healthy young adult controls (HC) to stain for a comprehensive B- and T cell panel (samples from two different time points denoted by circles or triangles). P1 and P2 are always indicated in blue and in red respectively. Error bars denote the standard error of the mean. **(A)** Total B cell numbers as a percentage of total lymphocytes for P1 and P2 compared to HC. **(B)** TREC and kappa-deleting recombination excision circles values. Pediatric controls (<18 yr) are indicated by triangles and adult controls by circles. **(C-G)** B cell subsets of HC and P as a percentage of CD19+ cells. **(H)** CD4+ and CD8+ T cells as a percentage of total lymphocytes in HC and P. **(I)** CD4/CD8 ratio**. (J-K)** Naïve T cells and CD45RA+ effector memory T cells (TEMRA) as a percentage of single positive CD8+ T cells in age-matched HC and P2. **(L-M)** Regulatory T cells (Treg) and follicular helper T cells (Tfh) as a percentage of single positive CD4+ cells in HC and P. **(N)** Follicular regulatory T cells (Tfr) in HC and P as a percentage of CD25+Foxp3+ Treg.

Fig. E5. Decreased naïve antigen receptor repertoire diversity in patients with L188V IKAROS. (A) V gene and **(B)** J gene usage in purified B cells from 10 healthy controls (grey) as well as P1 (blue) and P2 (red). **(C)** Junction characteristics of mutant IKAROS patients compared to 10 healthy controls (HC). Median number of total deletions, N-nucleotide additions and the CDR3 length of IGH rearrangements. **(D)** Diversity index of the naive B cell repertoire in mutant IKAROS patients and age-matched HC. **(E)** The subclass distribution of IGG and IGA transcripts with 7 age-matched controls for P1 and 6 age-matched controls for P2. **(F)** Median percentage of somatic hypermutation (SHM) is shown for P1 and P2 and age-matched controls. Mean±SEM. Experiments in (A-F) were performed once.

Fig. E6. Proliferative potential of mutant IKAROS B cells using a CFSE assay. CFSE-labeled B cells were negatively selected from P1, P2, mother, brother and 3 healthy controls (HC), and stimulated in culture for 5 days with 10µg/ml anti-IgM mix + 1µg/ml anti-CD40L +

1µg/ml CpG (two repeats). **(A)** Representative plots gated for percentage of proliferating cells for both patients and 2 out of 5 controls. **(B)** Percentage of proliferating B cells from HC versus P. **(C)** Proliferation index of HC versus P. **(D)** PBMCs stimulated with anti-IgM-IgA-IgG 10µg/ml or anti-IgM-IgA-IgG 10µg/ml + CD40L 1µg/ml for 24hrs were stained for Ki67. Percentage of Ki67+ cells in the CD19+ population (two repeats).

Fig. E7. Hyperresponsive patient EBV transformed B cells. (A) Total Erk1/2 MFI on EBV transformed B cells from P2 and unrelated HC (one repeat). **(B)** EBV transformed B cells stimulated with 10µg/ml anti-IgM-IgA-IgG were stained for pErk. Shaded area marks one standard deviation above and below the mean of the HC (Representative of two repeats). **(C)** Baseline MFI of pErk on EBV transformed B cells from P2 and unrelated HC normalized to average of HC (two repeats).

Fig. E8. IKAROS binding to the CD22 locus. (A) Signal tracks of IKAROS ChIP-seq data from human GM12878 B-lymphocytes and human pre-B ALL. Location of the CD22 EMSA probes are indicated. **(B)** Representative CD22 EMSAs performed using two different probes: CD22.1 and CD22.2 as indicated. Lanes were loaded with nuclear extracts from untransfected HEK cells (Untr) or HEK cells transfected with 100% WT, 100% mutant L188V, WT:L188V in a 50:50 ratio or 100% p.173-253del control. The arrows mark the size of complexes containing IKAROS. A supershift was induced by adding anti-Flag antibody (three repeats). **(C)** ChIP-qPCR using IgG input from EBV transformed B cells of P2, HC and GM12878 human B lymphocytes (three repeats).

Supplementary tables

Table E1. Laboratory values of P1 and of P2 at age 12 years

Parameter	P1 (Age 49y)	P2 (Age 12y)	
		Before treatment	After treatment*
Lymphocytes (count/μL)	2300 (1200-3600)	2000 (1500–2000)	1600 (1000-5300)
$CD19^+$ (% of total lymphocytes)	16.8 (5.0 -20.0)	6.9 (8.0-24.0)	5.0 (8.0-24.0)
$CD19^+$ (count/μL of lymphocytes)	381 (82-476)	138 (200-600)	81 (200-600)
$CD3^+$ (% of total lympohocytes)	70.0 (58.0-84.0)	86.6 (52.0-78.0)	82.7 (52.0-78.0)
$CD3^+$ (count/μL of lymphocytes)	1594 (798-2823)	1733 (800-3500)	1340 (800-3500)
Lupus anticoagulant Ab	Positive	Very strongly positive	Very strongly positive
Anticardiolipin Ab (GPL/ml)	4 (≤20)	NA	NA
Anticardiolipin IgG	NA	9.4 (≤2.5)	4.8 (≤2.5)
Anticardiolipin IgM	NA	6.4 (≤3.5)	2.1 (≤3.5)
	NA	Negative	Negative
ANA**	Positive: nuclear,	Positive: nuclear,	Positive: nuclear,
	fine speckled 1/160	homogeneous 1/1280	homogeneous 1/1280
Anti-dsDNA Ab by Farr (IU/ml)	≤7	40.5	23.5
IgG (g/L)	8.85 (7.51-15.60)	9.38 (5.76-12.65)	2.94 (5.76-12.65)
IgA (g/L)	1.12 (0.82-4.53)	0.60 (0.81-2.32)	0.17 (0.81-2.32)
IgM (g/L)	0.42 (0.46-3.04)	0.90 (0.30-1.59)	0.69 (0.30-1.59)
Antibody response			
Pneumococcal polysaccharide	/	Normal	/
Hepatitis B surface Ab (mIU/ml)	/	0.0 (≥10.0)	/
Mumps IgG	/	Negative	/
Measles IgG (mIU/ml)	/	430 (≥10.0)	/
Rubellavirus IgG (IU/ml)	/	6.3 (≥10.0)	/

* Current treatment four months after initiation comprised of 4mg corticosteroids, hydroxychloroquine, oral anticoagulation and prophylactic amoxicilline.

** Other autoantibodies tested in P2 were negative: Ro60, SSB/La, U1-RNP, RNP-70, Sm, Scl-70 sensitive, Jo-1, Ro52

ANA = anti-nuclear antibodies, Ab = antibodies, anti-dsdNA = anti-double stranded DNA, IgG/IgA/IgM = immunoglobulin G/A/M

Supplementary references

1. Van Eyck L, De Somer L, Pombal D, Bornschein S, Frans G, Humblet-Baron S, et al. Brief report: IFIH1 mutation causes systemic lupus erythematosus with selective IgA deficiency. *Arthritis Rheumatol*. 2015;67(6):1592-1597.
2. Kircher M, Witten DM, Jain P, O'Roak BJ, Cooper GM, Shendure J. A general framework for estimating the relative pathogenicity of human genetic variants. *Nat Publ Gr*. 2014;46(3):310-315.
3. Itan Y, Shang L, Boisson B, Patin E, Bolze A, Moncada-Vélez et al. The human gene damage index as a gene-level approach to prioritizing exome variants. *Proc Natl Acad Sci U S A*. 2015;112(44):13615-13620.
4. Neitzel H. A routine method for the establishment of permanent growing lymphoblastoid cell lines. *Hum Genet*. 1986;Aug(73(4)):320-326.
5. Molnár A, Georgopoulos K. The Ikaros gene encodes a family of functionally diverse zinc finger DNA-binding proteins. *Mol Cell Biol*. 1994;14(12):8292-8303.
6. Sun L, Liu A, Georgopoulos K. Zinc finger-mediated protein interactions modulate Ikaros activity, a molecular control of lymphocyte development. *EMBO J*. 1996;15(19):5358-5369.
7. van der Burg M, Kreyenberg H, Willasch A, Barendregt BH, Preuner S, Watzinger F, et al. Standardization of DNA isolation from low cell numbers for chimerism analysis by PCR of short tandem repeats. *Leuk Off J Leuk Soc Am Leuk Res Fund, UK*. 2011;25(9):1467-1470.
8. Driessen GJ, Ijspeert H, Weemaes CMR, Haraldsson Á, Trip M, Warris A, et al. Antibody deficiency in patients with ataxia telangiectasia is caused by disturbed B- and T-cell homeostasis and reduced immune repertoire diversity. *J Allergy Clin Immunol*. 2013;131(5).
9. IJspeert H, Wentink M, van Zessen D, Driessen GJ, Dalm VA, van Hagen, MP, et al. Strategies for B-cell receptor repertoire analysis in primary immunodeficiencies: from severe combined immunodeficiency to common variable immunodeficiency. *Front Immunol*. 2015;6(April):157.
10. van Dongen JJ, Langerak AW, Brüggemann M, Evans PA, Hummel M, Lavender FL,

et al. Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: report of the BIOMED-2 Concerted Action BMH4-CT98-3936. *Leukemia*. 2003;17(October):2257-2317.

11. Moorhouse MJ, van Zessen D, IJspeert H, Hiltemann S, Horsman S, van der Spek PJ, et al. ImmunoGlobulin galaxy (IGGalaxy) for simple determination and quantitation of immunoglobulin heavy chain rearrangements from NGS. *BMC Immunol*. 2014;15(1):59.

12. IJspeert H, van Schouwenburg PA, van Zessen D, Pico-Knijnenburg I, Stubbs AP, van der Burg M. Antigen Receptor Galaxy: A User-Friendly, Web-Based Tool for Analysis and Visualization of T and B Cell Receptor Repertoire Data. *J Immunol*. 2017.

13. Alamyar E, Duroux P, Lefranc MP ,Giudicelli V. IMGT(®) tools for the nucleotide analysis of immunoglobulin (IG) and T cell receptor (TR) V-(D)-J repertoires, polymorphisms, and IG mutations: IMGT/V-QUEST and IMGT/HighV-QUEST for NGS. *Methods Mol Biol*. 2012;(882):569-604.

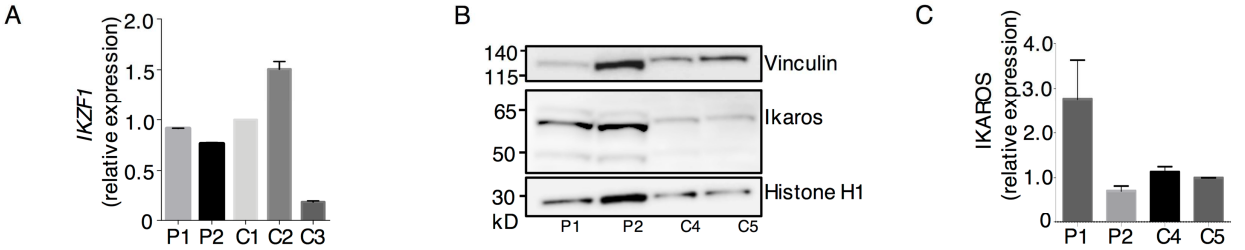
14. IJspeert H, van Schouwenburg PA, van Zessen D, Pico-Knijnenburg I, Driessen GJ, Stubbs AP, et al. Evaluation of the Antigen-Experienced B-Cell Receptor Repertoire in Healthy Children and Adults. *Front Immunol*. 2016;7(October):410.

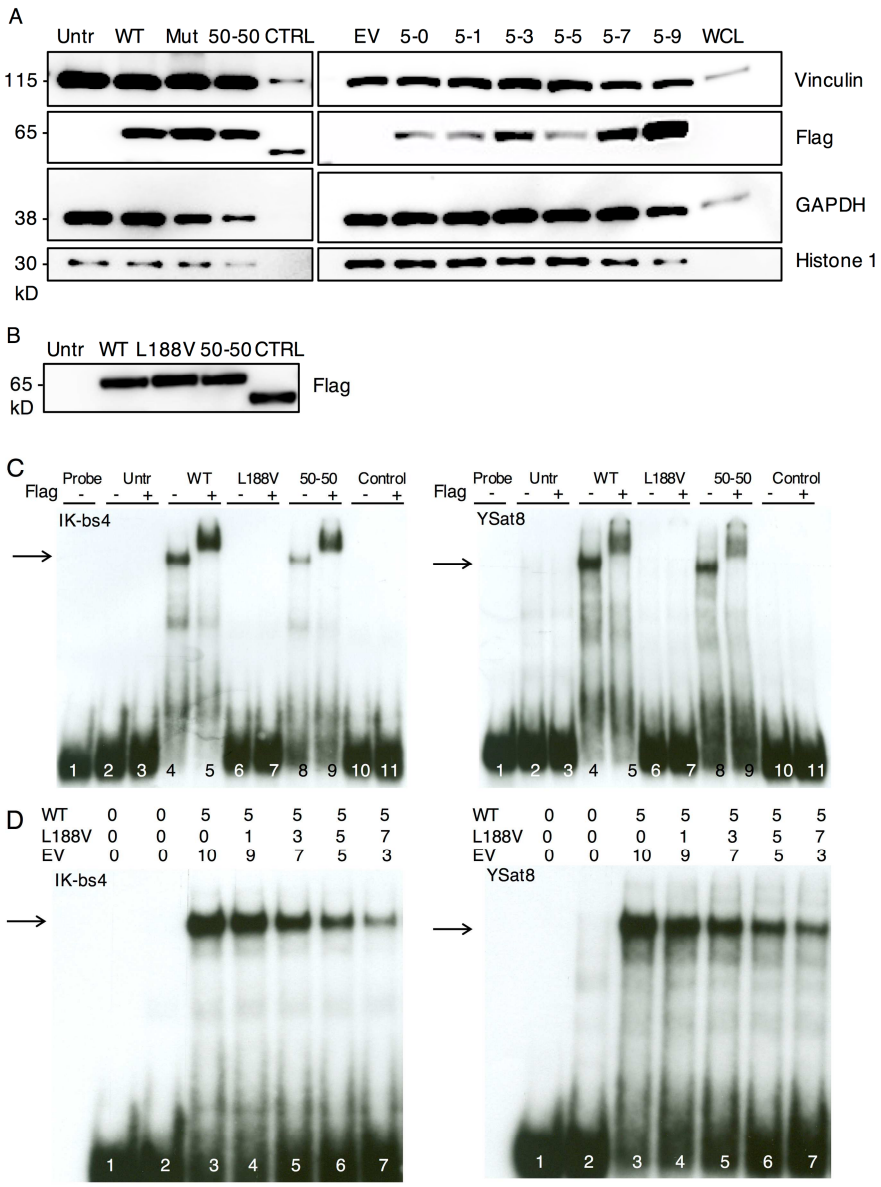
15. Tiller T, Meffre E, Yurasov S, Tsuiji M, Nussenzweig MC, Wardemann H. Efficient generation of monoclonal antibodies from single human B cells by single cell RT-PCR and expression vector cloning. *J Immunol Methods*. 2008;329(1-2):112-124.

16. Berkowska MA, Schickel JN, Grosserichter-Wagener C, de Ridder D, Ng YS, van Dongen JJ, et al. Circulating Human CD27-IgA+ Memory B Cells Recognize Bacteria with Polyreactive Igs. *J Immunol*. 2015;195(4):1417-1426.

17. Schjerven H, Ayongaba EF, Aghajanirefah A, McLaughlin J, Cheng D, Geng H, et al. Genetic analysis of Ikaros target genes and tumor suppressor function in BCR-ABL1+ pre–B ALL. 2017:1-22.

18. Levy C, Fusil F, Amirache F, Costa C, Negre D. Baboon envelope pseudotyped lentiviral vectors efficiently transduce human B cells and allow active factor IX B cell secretion in vivo in NOD/SCIDγc-/- mice. 2016:2478-2492.


A
IKZF1
(relative expression)
2.0
1.5
1.0
0.5
0
P1 P2 C1 C2 C3
B
140
115
65
50
30
kD
P1 P2 C4 C5
Vinculin
Ikaros
Histone H1
C
IKAROS
(relative expression)
4.0
3.0
2.0
1.0
0
P1 P2 C4 C5


A
Untr WT Mut 50-50 CTRL EV 5-0 5-1 5-3 5-5 5-7 5-9 WCL
115
65
38
30
kD
Vinculin
Flag
GAPDH
Histone 1
B
Untr WT L188V 50-50 CTRL
65
kD
Flag
C
Probe Untr WT L188V 50-50 Control
Flag - - + - + - + - + - +
IK-bs4
YSat8
1 2 3 4 5 6 7 8 9 10 11
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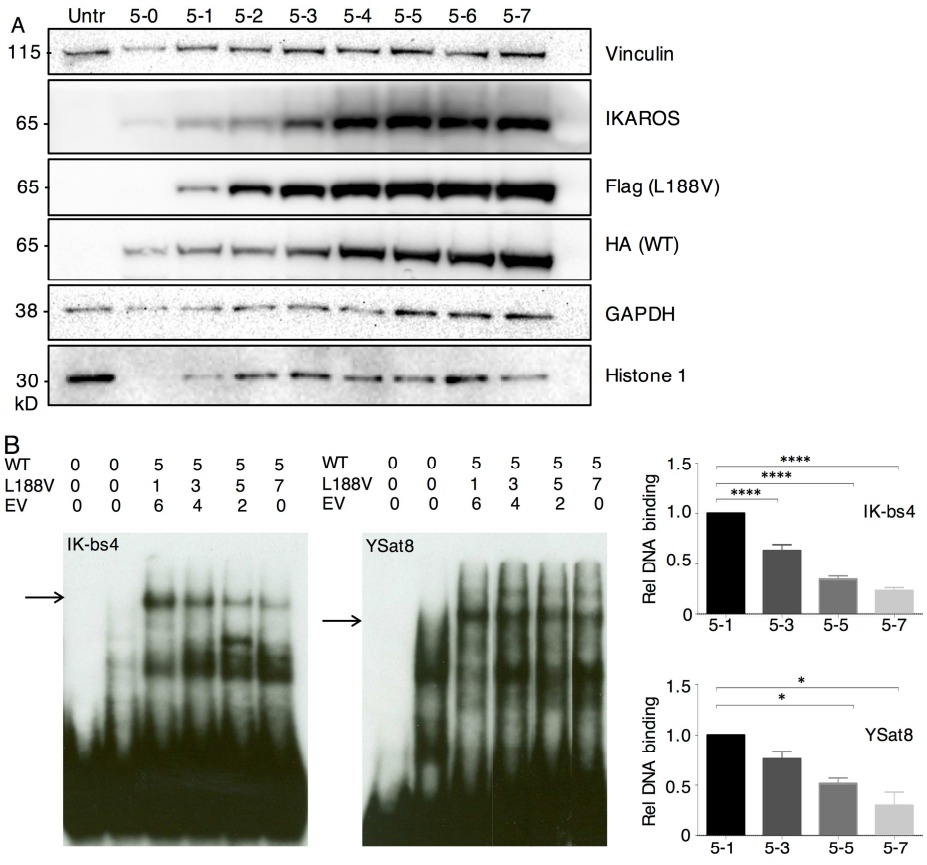
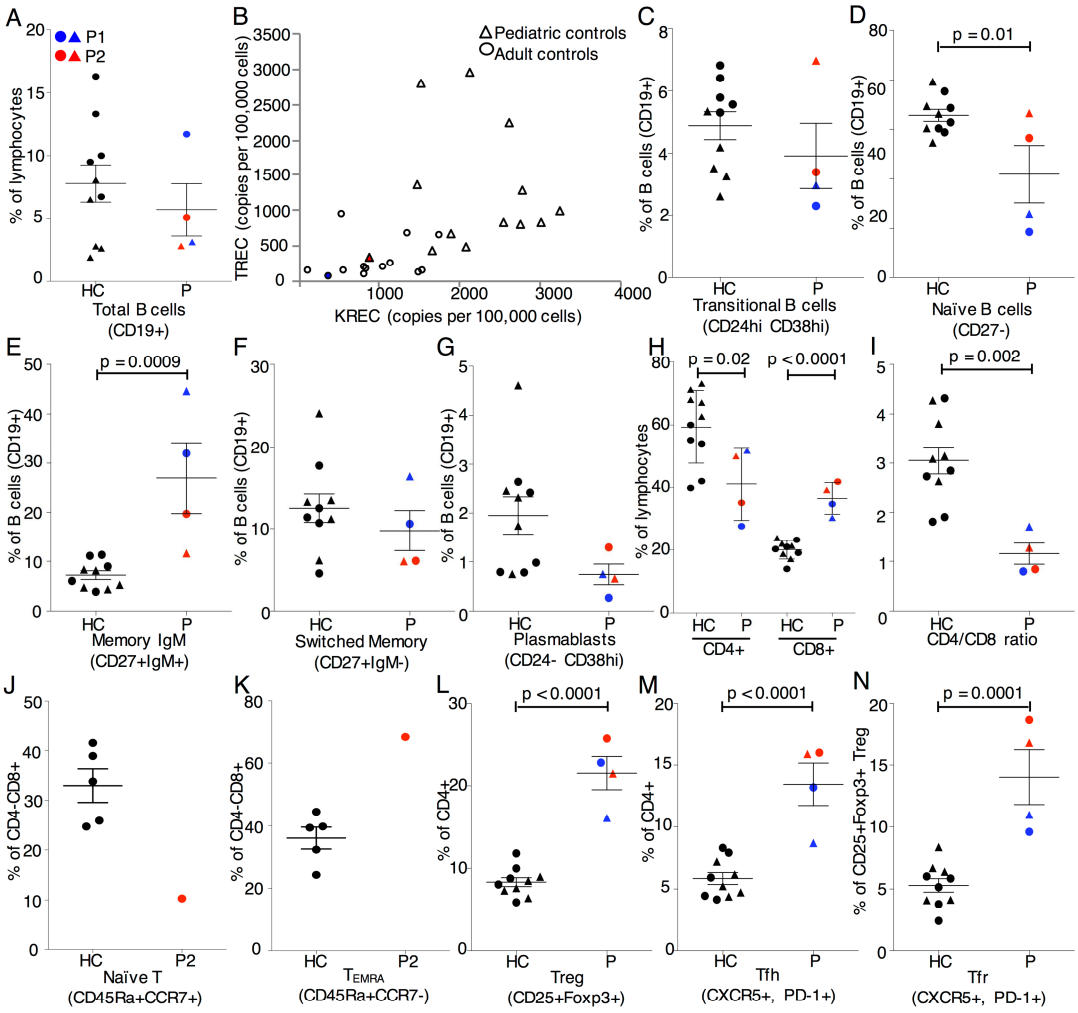
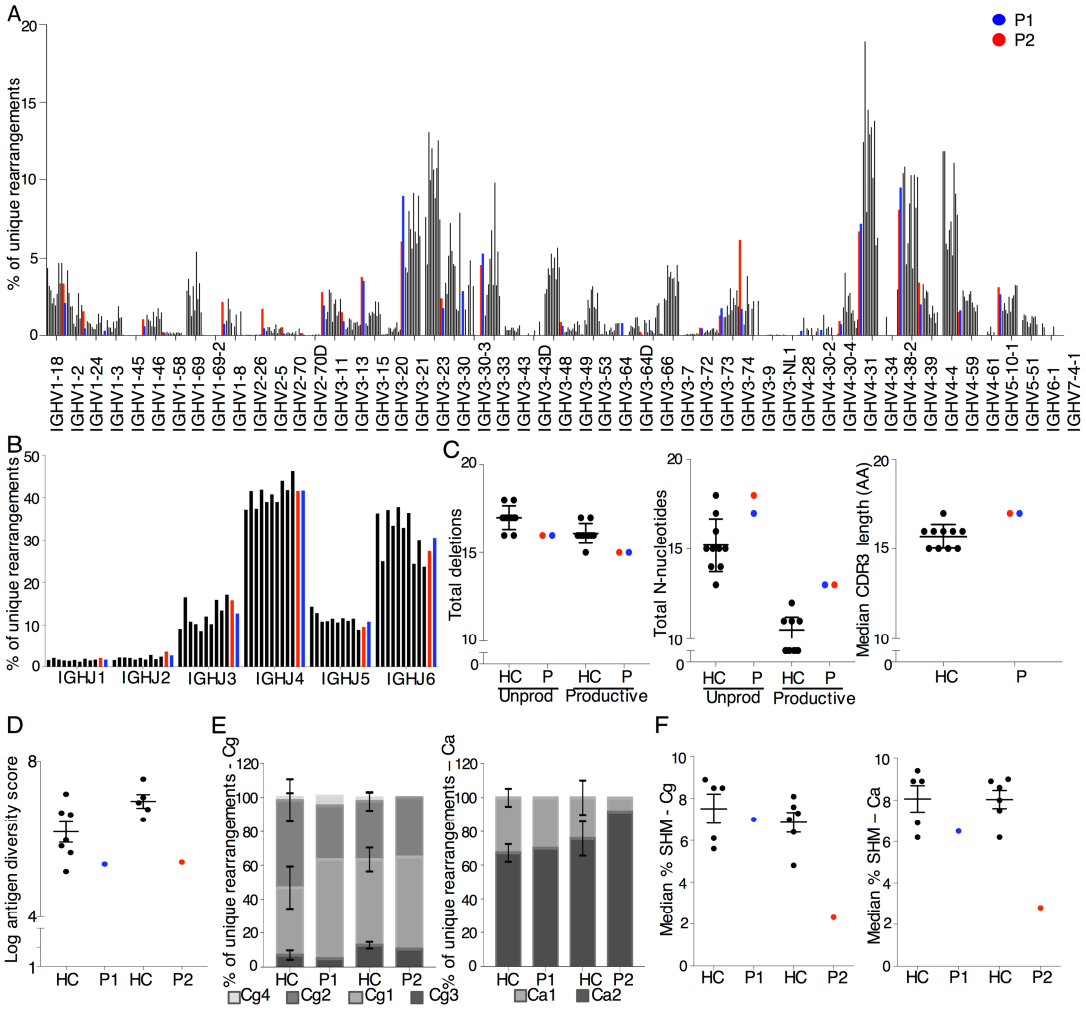
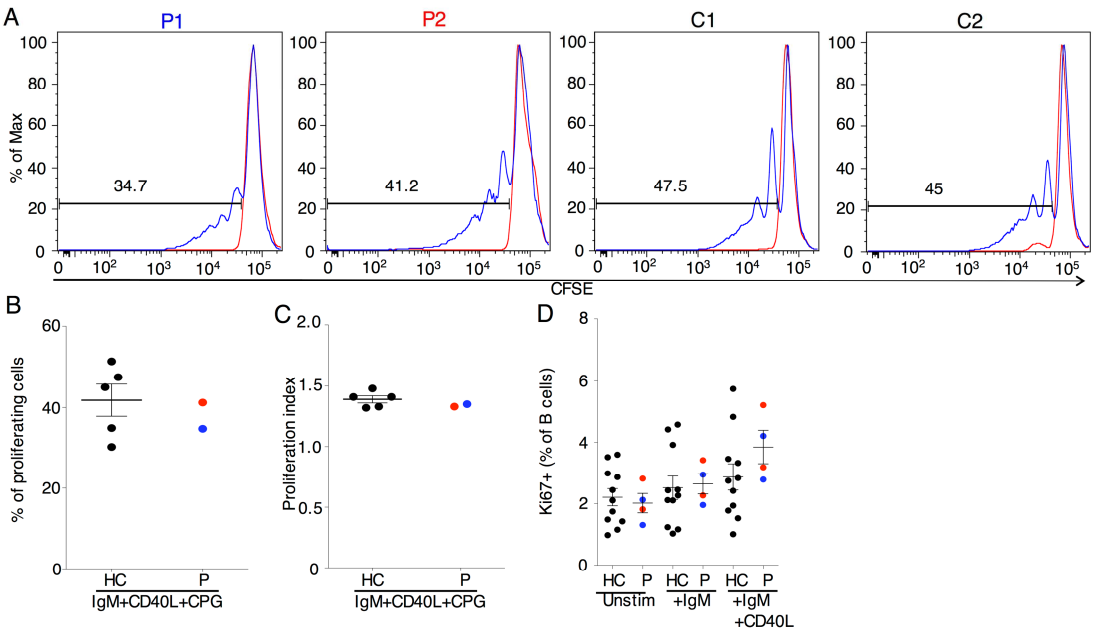
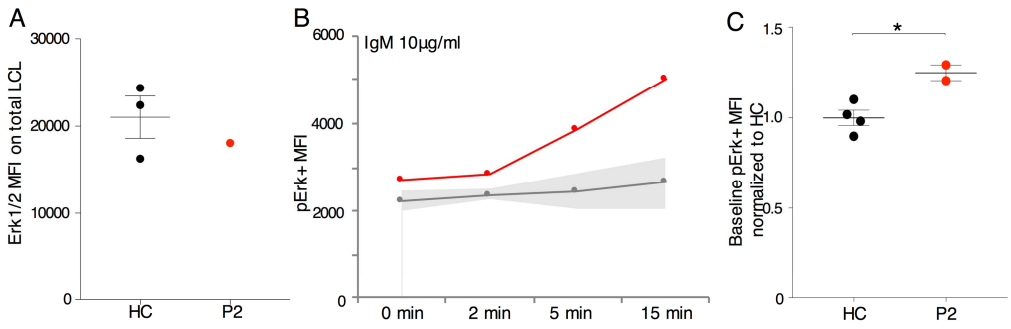
EV 0 0 10 9 7 5 3
IK-bs4
YSat8
1 2 3 4 5 6 7


A
Untr 5-0 5-1 5-2 5-3 5-4 5-5 5-6 5-7
115
Vinculin
65
IKAROS
65
Flag (L188V)
65
HA (WT)
38
GAPDH
30
kD
Histone 1
B
WT 0 0 5 5 5 5
L188V 0 0 1 3 5 7
EV 0 0 6 4 2 0
IK-bs4
YSat8
Rel DNA binding
1.5
1.0
0.5
0
5-1 5-3 5-5 5-7
****
*


A
P1
P2
% of lymphocytes
HC
P
Total B cells
(CD19+)
B
Pediatric controls
Adult controls
TREC (copies per 100,000 cells)
KREC (copies per 100,000 cells)
C
% of B cells (CD19+)
Transitional B cells
(CD24hi CD38hi)
D
p = 0.01
Naïve B cells
(CD27-)
E
p = 0.0009
Memory IgM
(CD27+IgM+)
F
Switched Memory
(CD27+IgM-)
G
Plasmablasts
(CD24- CD38hi)
H
p = 0.02
p < 0.0001
CD4+
CD8+
I
p = 0.002
CD4/CD8 ratio
J
% of CD4-CD8+
Naïve T
(CD45Ra+CCR7+)
K
TEMRA
(CD45Ra+CCR7-)
L
p < 0.0001
% of CD4+
Treg
(CD25+Foxp3+)
M
Tfh
(CXCR5+, PD-1+)
N
% of CD25+Foxp3+ Treg
Tfr
(CXCR5+, PD-1+)


A
P1
P2
% of unique rearrangements
IGHV1-18
IGHV1-2
IGHV1-24
IGHV1-3
IGHV1-45
IGHV1-46
IGHV1-58
IGHV1-69
IGHV1-69-2
IGHV1-8
IGHV2-26
IGHV2-5
IGHV2-70
IGHV2-70D
IGHV3-11
IGHV3-13
IGHV3-15
IGHV3-20
IGHV3-21
IGHV3-23
IGHV3-30
IGHV3-30-3
IGHV3-33
IGHV3-43
IGHV3-43D
IGHV3-48
IGHV3-49
IGHV3-53
IGHV3-64
IGHV3-64D
IGHV3-66
IGHV3-7
IGHV3-72
IGHV3-73
IGHV3-74
IGHV3-9
IGHV3-NL1
IGHV4-28
IGHV4-30-2
IGHV4-30-4
IGHV4-31
IGHV4-34
IGHV4-38-2
IGHV4-39
IGHV4-4
IGHV4-59
IGHV4-61
IGHV5-10-1
IGHV5-51
IGHV6-1
IGHV7-4-1
B
% of unique rearrangements
IGHJ1
IGHJ2
IGHJ3
IGHJ4
IGHJ5
IGHJ6
C
Total deletions
Total N-nucleotides
Median CDR3 length (AA)
HC
P
Unprod
Productive
D
Log antigen diversity score
HC
P1
P2
E
% of unique rearrangements - Cg
% of unique rearrangements - Ca
Cg4
Cg2
Cg1
Cg3
Ca1
Ca2
F
Median % SHM - Cg
Median % SHM – Ca


A
P1
P2
C1
C2
% of Max
34.7
41.2
47.5
45
CFSE
B
% of proliferating cells
HC
P
IgM+CD40L+CPG
C
Proliferation index
HC
P
IgM+CD40L+CPG
D
Ki67+ (% of B cells)
HC P HC P HC P
Unstim +IgM +IgM +CD40L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Erk1/2 MFI on total LCL
30000
20000
10000
0
HC
P2
B
IgM 10µg/ml
pErk+ MFI
6000
4000
2000
0
0 min
2 min
5 min
15 min
C
Baseline pErk+ MFI normalized to HC
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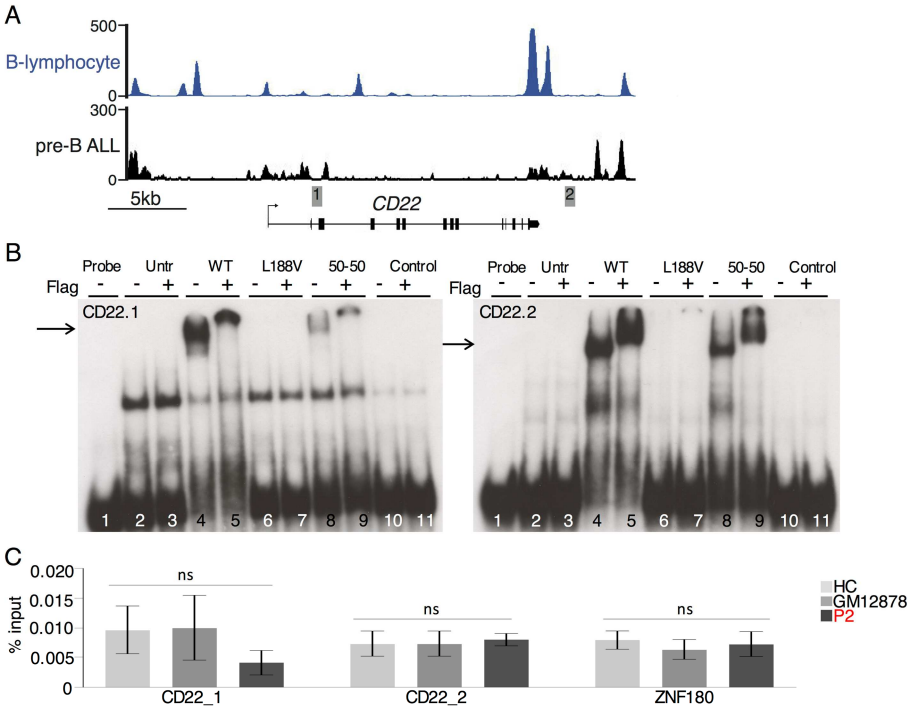
1.0
0.5
0
*
HC
P2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B-lymphocyte
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300
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0
5kb
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CD22
2
B
Probe
Untr
WT
L188V
50-50
Control
Flag
CD22.1
CD22.2
1 2 3 4 5 6 7 8 9 10 11
C
0.020
0.015
0.010
0.005
0
% input
ns
CD22_1
CD22_2
ZNF180
HC
GM12878
P2