SUPPLEMENTAL MATERIAL

Kolhatkar et al., http://www.jem.org/cgi/content/full/jem.20150585/DC1

Table S1. Summary of sequence data obtained from 5′-RACE 454-pyrosequencing of bulk and sorted splenic B cell subsets from WT, *Was*$^{-/-}$, *Was*$^{fl/fl}$*Mb-1*^{cre}, and *Was*$^{-/-}$*Myd88*$^{-/-}$ mice

Genotype	Subtype	Number of total functional sequences analyzed	Number of unique clonotypes
WT	Bulk	54,604	14,637
WT	MZ	49,945	22,107
Was$^{-/-}$	Bulk	49,063	14,835
Was$^{-/-}$	MZ	42,622	12,255
Was$^{fl/fl}$*Mb-1*^{cre}	Bulk	29,813	8,940
Was$^{-/-}$*Myd88*$^{-/-}$	MZ	30,482	4,956

Data are representative of at least two experiments with five to six mice pooled in each experiment.

Table S2. WAS subject and HC subject information

Subjects	Age at draw	*WAS* mutation	WASp sequence	Clinical problems
Young WAS subjects				
WAS 1A	3 mo	c.361-1G>C	p.D121Tfs*106	Thrombocytopenia, eczema, petechiae
WAS 1B	8 mo	" "	" "	" "
WAS 1C	11 mo	" "	" "	" "
WAS 2	9 mo	c.397 G>A	p.E133K	Thrombocytopenia, eczema, petechiae
WAS 3	19 yr	c.1124C>T	p.R364Stop	Asthma, rhinitis, tubulointerstitial nephritis with interstitial fibrosis
WAS 4*	5 mo	c.361-1G>C		Thrombocytopenia, eczema, lymphopenia, eosinophilia, high IgE
WAS 7*	17 yr	c.257G>A	p.R86H	Thrombocytopenia, splenectomy at 3 yr old
WAS 8*	6 mo	c.100C>T	p.R34X	Thrombocytopenia, eczema, high IgE
WAS 10*	8 yr	c.755G>A	p.W252X	Thrombocytopenia, infections, vasculitis, chronic pericarditis, lymphadenopathy
WAS 11*	23 mo	c.134C>T	p.T45M	thrombocytopenia
Adult WAS subjects				
WAS5	20 yr	c.1453G>A	p.Asp485Asn	Thrombocytopenia, one episode of Arthritis
WAS6	28 yr	c.256C>T	p.Cys256Thr	s/p splenectomy, sepsis
WAS14	31 yr	c.116T>C	p.Leu39Pro	s/p splenectomy, seminoma, AIHA, autoimmune thrombocytopenia
WAS21	19 yr	c.256C>T	p.Cys256Thr	s/p splenectomy, adenopathy, sinusitis
Young control subjects				
HC 1	4.31 yr			
HC 2	15 mo			
HC 3	2 yr 9 mo			
HC 4	1 yr			
HC 5	5 yr			
HC 6	10 yr			
HC 7	8 yr			
HC 8	5 yr			
Adult control subjects				
HC 9	24 yr			
HC 10	23 yr			
HC 11	28 yr			

Age of blood draw, *WAS* mutation, protein change, clinical symptoms, and disease score of each WAS and HC subject. *, evaluated only using FACS 9G4 staining.

Table S3. Human VDJ sequencing information data

Sample	B cell subpopulation	Total number of productive sequences	Total number of unique sequences
Young WAS patients (age range: 3 mo to 19 yr)			
WAS1c	Transitional	224,245	4,280
WAS2	Transitional	326,541	9,400
WAS3	Transitional	34,694	486
WAS1c	Naive	660,470	24,825
WAS2	Naive	259,866	31,559
WAS3	Naive	429,045	13,363
WAS1c	IgM memory	27,657	1,676
WAS2	IgM memory	47,451	435
WAS3	IgM memory	1,766	109
WAS2	Switched memory	235,747	2,708
WAS3	Switched memory	4,159	524
WAS NIH adult patients (age range: 19–31 yr)			
WAS5	Naive	389,819	31,443
WAS6	Naive	254,551	6,162
WAS14	Naive	319,509	29,058
WAS21	Naive	1,327,676	49,013
Young controls (age range: 1–10 yr)			
HC 1	Transitional	355,934	5,493
HC 3	Transitional	551,979	11,978
HC 4	Transitional	911	225
HC 5	Transitional	70,992	2,396
HC 1	Naive	340,406	8,046
HC 2	Naive	104,124	2,239
HC 3	Naive	378,488	33,557
HC 4	Naive	399,149	26,611
HC 5	Naive	367,098	22,993
HC 2	IgM memory	83,111	804
HC 3	IgM memory	71,773	4,015
HC 4	IgM memory	15,897	273
HC 5	IgM memory	94,993	888
HC 3	Switched memory	138,023	2,548
HC 4	Switched memory	46,976	638
HC 5	Switched memory	87,973	796
Adult controls (age range: 23–28 yr)			
HC 9	Naive	122,608	28,335
HC 10	Naive	331,441	43,827
HC 11	Naive	322,315	20,970

Total number of productive clonotypic sequences generated by Illumina-based sequencing (Adaptive Biotechnologies) for each subject sample.

Table S4. Complete VH gene usage in sorted naive B cells from WAS (n = 3) and HC (n = 5) subjects

VH gene	HC mean	HC SE	WAS mean	HC SE
IGHV01-02	2.8	1.1	3.2	0.9
IGHV01-03	1.3	1.1	0.6	0.5
IGHV01-08	0.9	0.5	1.3	0.4
IGHV01-14	0.0	0.0	0.0	0.0
IGHV01-17	0.1	0.0	0.1	0.0
IGHV01-18	2.3	0.6	2.4	0.5
IGHV01-24	0.8	0.2	0.5	0.1
IGHV01-45	0.0	0.0	0.0	0.0
IGHV01-46	1.3	0.2	1.1	0.1
IGHV01-58	0.2	0.1	0.3	0.2
IGHV01-67	0.0	0.0	0.0	0.0
IGHV01-68	0.0	0.0	0.0	0.0
IGHV01-69	3.0	1.1	3.3	0.7
IGHV01-c	0.0	0.0	0.0	0.0
IGHV01-f	0.2	0.2	0.1	0.1
IGHV01-or15_02	0.0	0.0	0.0	0.0
IGHV01-or15_04	0.0	0.0	0.0	0.0
IGHV02-05	1.7	0.4	1.5	0.4
IGHV02-10	0.0	0.0	0.0	0.1
IGHV02-26	0.7	0.5	1.1	0.4
IGHV02-70	1.7	0.9	1.0	0.7
IGHV03-09	1.7	1.3	2.2	0.7
IGHV03-11	0.3	0.2	0.5	0.2
IGHV03-13	0.3	0.2	0.4	0.1
IGHV03-15	3.0	1.2	2.3	0.2
IGHV03-19	0.0	0.0	0.0	0.0
IGHV03-20	0.2	0.1	0.4	0.2
IGHV03-21	0.0	0.0	0.0	0.0
IGHV03-22	0.1	0.0	0.1	0.0
IGHV03-23	5.8	2.1	6.8	1.4
IGHV03-25	0.0	0.0	0.0	0.0
IGHV03-30	0.0	0.0	0.0	0.0
IGHV03-33	0.0	0.0	0.0	0.0
IGHV03-35	0.0	0.0	0.0	0.0
IGHV03-37	0.0	0.0	0.0	0.0
IGHV03-41	0.0	0.0	0.0	0.0
IGHV03-43	1.1	0.5	0.7	0.1
IGHV03-47	0.0	0.0	0.0	0.0
IGHV03-48	0.9	0.6	0.8	0.7
IGHV03-49	1.3	0.6	1.0	0.3
IGHV03-52	0.0	0.0	0.0	0.0
IGHV03-53	1.1	0.4	1.3	0.7
IGHV03-64	1.0	0.2	0.5	0.4
IGHV03-65	0.1	0.1	0.0	0.0
IGHV03-66	0.7	0.9	0.5	0.4
IGHV03-71	0.0	0.0	0.0	0.0
IGHV03-72	0.3	0.1	0.1	0.0
IGHV03-73	0.8	0.4	0.6	0.1
IGHV03-74	1.5	0.6	1.0	0.2
IGHV03-76	0.0	0.0	0.0	0.0
IGHV03-d	0.0	0.0	0.0	0.0
IGHV03-or02_adap22	0.0	0.0	0.0	0.0
IGHV03-or16_07_1	0.0	0.0	0.0	0.0
IGHV03-or16_08	0.0	0.0	0.0	0.0
IGHV03-or16_09	0.0	0.0	0.0	0.0
IGHV04-28	0.0	0.0	0.0	0.0
IGHV04-30_2	0.1	0.1	0.1	0.1
IGHV04-30_4	0.0	0.0	0.0	0.0
IGHV04-31	0.0	0.0	0.0	0.0
IGHV04-34	5.8	1.2	9.1	2.1
IGHV04-39	1.0	0.8	0.8	0.7
IGHV04-55	0.2	0.1	0.3	0.1
IGHV04-59	0.0	0.0	0.0	0.0
IGHV04-61	0.0	0.0	0.0	0.0
IGHV04-80	0.0	0.0	0.0	0.0
IGHV04-b	0.1	0.2	0.0	0.0
IGHV04-or15_8	0.0	0.0	0.0	0.0
IGHV05-51	2.4	0.3	1.9	0.5
IGHV05-78	0.0	0.0	0.0	0.0
IGHV05-a	1.4	0.4	0.6	0.6
IGHV06-01	0.8	0.1	0.6	0.2
IGHV07-04_1	0.4	0.5	0.0	0.0
IGHV07-27	0.0	0.0	0.0	0.0
IGHV07-40	0.0	0.0	0.0	0.0
IGHV07-56	0.0	0.0	0.0	0.0
IGHV07-81	0.0	0.0	0.0	0.0
IGHV_II-15_1	0.0	0.0	0.0	0.0
IGHV_II-22_1	0.0	0.0	0.0	0.0
IGHV_II-28_1	0.0	0.0	0.0	0.0
IGHV_II-30_1	0.0	0.0	0.0	0.0
IGHV_II-33_1	0.0	0.0	0.0	0.0
IGHV_II-49_1	0.0	0.0	0.0	0.0
IGHV_III-02_1	0.0	0.0	0.0	0.0
IGHV_III-05_2	0.0	0.0	0.0	0.0
IGHV_III-26_1	0.0	0.0	0.0	0.0
IGHV_III-38_1	0.0	0.0	0.0	0.0
IGHV_III-47_1	0.0	0.0	0.0	0.0
IGHV_III-67_3	0.0	0.0	0.0	0.0
IGHV_III-82	0.0	0.0	0.0	0.0
IGHV_III-or02_adap26	0.0	0.0	0.0	0.0
IGHV_IV-44_1	0.0	0.0	0.0	0.0

Values were averaged among individual sequencing data with standard deviation values listed.

Table S5. Summary of experimental assays in this study in both the murine model of WASp deficiency and WAS subjects

Parameter	Purpose of experiment	Results/observations
Murine *Was*$^{-/-}$ model		
Repertoire analysis	Evaluate repertoire of the naive B cell compartment, specifically VH family gene usage and HCDR3 characteristics via 5′-RACE 454-pyrosequencing	Increased VH14 usage and decreased VH9 and VH7 in *Was*$^{-/-}$ and *Was*$^{fl/fl}$*Mb-1*^{cre} total splenic B cells
		Increased VH10 usage and decreased VH9 and VH7 usage in *Was*$^{-/-}$ MZ B cells; Myd88 deficiency results in reduction in selection of VH10-expressing BCRs
	Evaluate reactivity of the naive MZ compartment from WT and *Was*$^{-/-}$ mice	Enrichment of BCRs reactive to sm-RNP antigens in *Was*$^{-/-}$ MZ compartment; self-reactive *Was*$^{-/-}$ MZ BCRs are of lower affinity compared with WT MZ BCR reactivity
Peripheral B cell selection	Determine effect of WASp deficiency on LC usage	Increased λ-LC usage in T2, FM, MZp, and MZ B cell compartments in *Was*$^{-/-}$ and *Was*$^{fl/fl}$*Mb-1*^{cre} B cells
	Determine whether negative selection mechanisms are altered or impaired in *Was*$^{-/-}$ mice	Clonal deletion and induction of anergy are intact in HEL Tg model and no significant differences observed in rates of κ^{+} LC editing in *Was*$^{-/-}$ mice
	Evaluate transitional B cell expansion and turnover kinetics	Increased BrdU^{+} and Ki67 labeling in T1 and T2 *Was*$^{-/-}$ and *Was*$^{fl/fl}$*Mb-1*^{cre} B cells
	Evaluate effect of WASp deficiency on selection of autoreactive specificities	*Was*$^{-/-}$ M167 Tg mice exhibit reduced selection for high-affinity PC-specific BCRs as well as reduced expansion of the Id^{+} M167 T2 BCRs in *Was*$^{-/-}$ mice
	Determine the role of antigen-dependent signaling in driving positive selection of transitional B cells using Nur77-GFP Tg	*Was*$^{-/-}$ Nur77-GFP Tg mice exhibit increased T2-GFP^{hi} population compared with WT Nur77-GFP Tg mice, indicating enhanced antigen-driven activation and proliferation
Human WAS experiments		
Repertoire analysis	Evaluate repertoire of the transitional, naive, and memory compartment via Illumina-based sequencing	Both pediatric (n = 3) and adult (n = 4) WAS subjects exhibit increased selection of VH4-34 transitional to the naive and IgM memory B cell compartment compared with age-matched HC (n = 4 and 4, respectively)
	Assess the reactivity of the naive B cell compartment by cloning monoclonal BCRs from pediatric HC (n = 2) and WAS (n = 1) subjects	WAS naive B cells exhibit increased reactivity to phosphorylcholine, dsDNA, MDA-LDL, and antinuclear antigens, as shown by immunofluorescence
Peripheral B cell selection	Track selection of 9G4^{+} (VH4-34) B cells throughout development in pediatric and adult WAS (n = 9 and 4) and HC (n = 8 and 4) subjects, respectively	Both pediatric and adult WAS subjects exhibit increased selection of VH4-34–expressing BCRs from the transitional to the naive and within the IgM memory B cell compartment compared with age-matched HC