SUPPLEMENTAL MATERIAL

Locci et al., http://www.jem.org/cgi/content/full/jem.20081773/DC1

Flow cytometry. The following mAbs were used for surface staining: anti-CD19 (1D3), anti-CD11c (HL3), and anti-CD1d (1B1).

Immunization and antigen-specific antibody titer measurement. Mice were immunized on days 0 and 15 by i.p. injection of TNP-KLH antigen in PBS (20 μg per dose; Biosearch Technologies, Inc.) alone or with α-GalCer (1 μg per dose). Circulating antigen-specific antibodies were measured by endpoint ELISA as previously described (Galli, G., P. Pittoni, E. Tonti, C. Malzone, Y. Uematsu, M. Tortoli, D. Maione, G. Volpini, O. Finco, S. Nuti, et al. 2007. *Proc. Natl. Acad. Sci. USA*. 104:3984–3989).

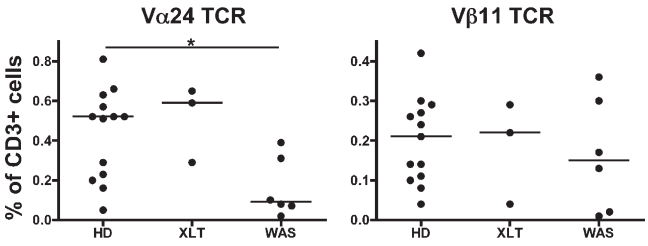

Fig. S1. TCR-Vα24 and TCR-Vβ11 expression. TCR-Vα24 and TCR-Vβ11 expressions were analyzed gating on $CD3^+$ lymphocytes obtained from 13 HD, 3 XLT, and 6 WAS patients. The graph shows the percentage of TCR-Vα24 and -Vβ11 single-positive cells, excluding DP ($TCR\text{–}V\alpha24^+$ $V\beta11^+$) cells. Bars represent the median value of each group. *, $P < 0.05$

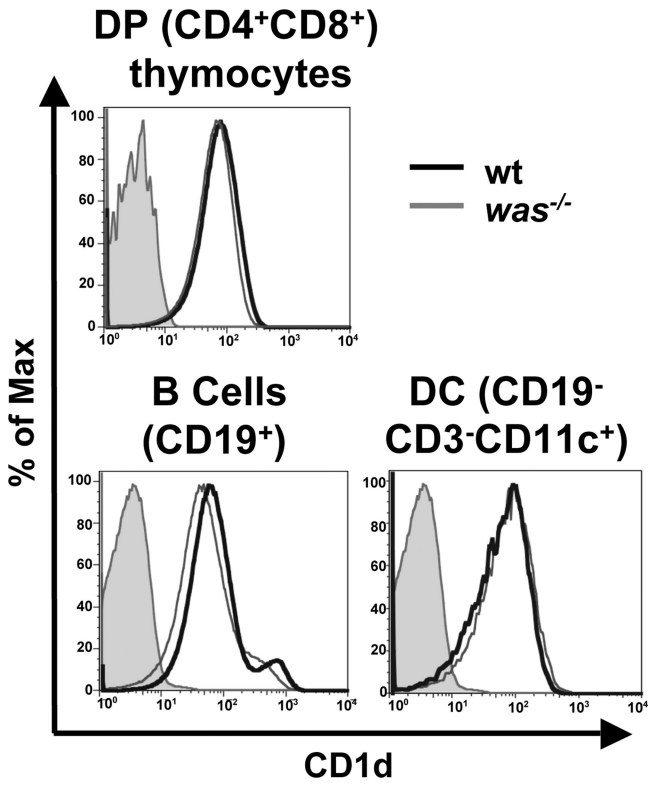

Fig. S2. Normal CD1d expression in *was*$^{-/-}$ mice. CD1d expression was analyzed in thymic CD4^{+} CD8^{+} DP lymphocytes (top) and in splenic CD19^{+} B cells and CD19^{-} CD3^{-} CD11c^{+} DC in WT (thick line) and *was*$^{-/-}$ (thin line) mice. Data are representative of six mice per group from two independent experiments.

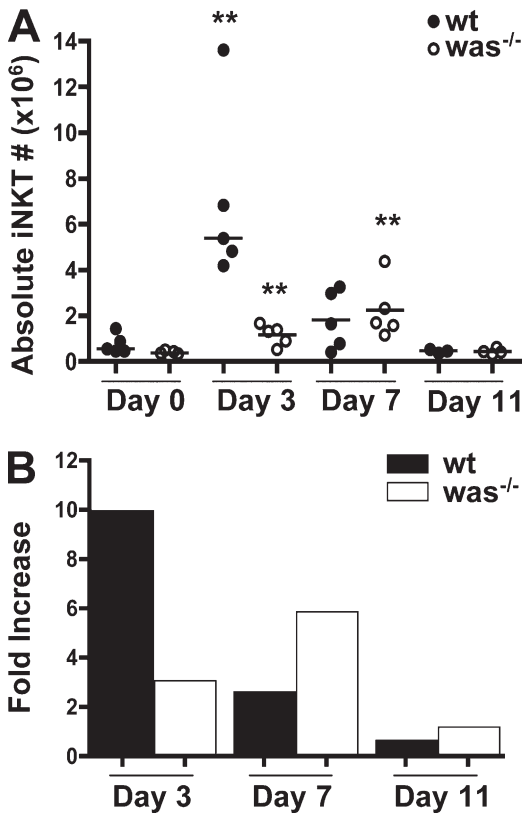

Fig. S3. Delayed and reduced *was*$^{-/-}$ iNKT cell in vivo expansion. (A) WT and *was*$^{-/-}$ mice were injected with α-GalCer, and iNKT in vivo expansion was measured by flow cytometry at days 3, 7, and 11 after the injection. To evaluate iNKT cell expansion, hepatic leukocytes were stained with anti-B220 and anti-CD3 mAbs and with α-GalCer–loaded CD1d tetramers. iNKT cell absolute number was determined as described in the Fig. 2 legend. The graph shows iNKT cell number of five WT and five *was*$^{-/-}$ mice per group, with the exception of day 11 (three WT and four *was*$^{-/-}$ mice). Statistic significance is referred to as the difference between each group and its relative untreated control. **, $P < 0.005$ for each group compared with untreated mice (day 0). (B) Fold increase of iNKT cell number at days 3, 7, and 11 was calculated as a ratio of, respectively, the mean value of iNKT cell numbers at days 3, 7, or 11 and the mean value of iNKT cell numbers from untreated mice. Data were obtained in two independent experiments.

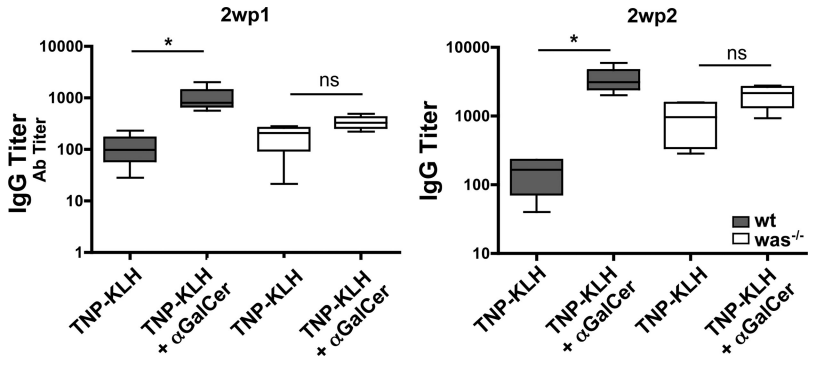

Fig. S4. Impaired ability of activated *was*$^{-/-}$ iNKT cell to support specific B cell responses. WT and *was*$^{-/-}$ mice were immunized twice with TNP-KLH alone or with α-GalCer. The figure shows the antigen-specific IgG titer at 2 wk after the second immunization (2wp2). Results are from one experiment representative of two, in which four to five mice were tested. ns, $P > 0.05$; *, $P < 0.05$.

Table S1. Molecular and clinical data of WAS patients

Patient	Age[a]	Mutation type	genomic DNA mutation	Protein change	Clinical score[c]	Thrombo-cytopenia	T cell lymphopenia	Infections	Tumor
WAS1	26 yr	Splice intron 9	IVS9 + 2 del tgag	ND	WAS (5)	Yes	No	Severe HSV, pneumonia	Yes
WAS26	21 mo	Missense exon 4	431 G>A	E133K	XLT (2)	No[d]	No	Recurrent fevers	No
WAS27	22 yr	Missence exon 1	150T>C	L39P	XLT (2)	Yes[d]	No	Recurrent influenza	No
WAS28	29 yr	Splice intron 2	IVS2 + 4 T>C	ND	WAS (3)	Yes	No	Bronchiectasis, mild recurrent infection	No
WAS29	6 mo	Nonsense exon 1	155 C>T	R41X	WAS (3)	Yes	Yes	Recurrent upper respiratory tract infections, otitis media, and urinary tract infections	No
WAS30	19 yr	Missense exon 12	1487 G>A	D485N	XLT (2)	Yes	No	Few upper respiratory tract infections	No
WAS31	13 mo	Splice	NR[b]	No expression	WAS (3)	Yes[e]	Yes	Mild recurrent infections	No
WAS32	32 mo	Insertion exon 10	C 1238-1239 Ins C	P402X949	WAS (5)	Yes[e]	No	Persistent CMV	No
WAS33	5 yr	Entire gene missing	Entire gene missing	No expression	WAS (4)	Yes[e]	No	Persistent CMV	No

del, deletion; IVS, intervening sequence (intron); NR, not reported.
[a]Age refers to the time of blood sampling.
[b]Mutation is going to be published separately.
[c]Disease score is given according to the classification reported previously (Imai, K., T. Morio, Y. Zhu, Y. Jin, S. Itoh, M. Kajiwara, J. Yata, S. Mizutani, H.D. Ochs, and S. Nonoyama. 2004. *Blood.* 103:456–464).
[d]Splenectomized.
[e]Undergoing platelet transfusion.

Table S2. TCR-Vβ repertoire of WAS patients

Vβ	HD mean[a,b]	HD min[a,b]	HD max[a,b]	WAS 1[b]	WAS 28[b]	WAS 30[b]	WAS 33[b]
Vβ1	3.53	1.89	11.7	3.18	1.73	8.31	0.8
Vβ2	8.30	4.03	23.48	4.30	2.44	6.87	1.19
Vβ3	4.68	0.52	15.71	0.15	0.31	1	0.54
Vβ4	1.91	0.79	3.26	0.07	1.19	1.05	0.22
Vβ5.1	5.45	3.19	14.93	3.34	1.26	3.98	0.56
Vβ5.2	1.33	0.49	4.98	1.24	4.86	1.63	1.17
Vβ5.3	1.08	0.37	2.98	0.03	1.82	5.08	0.67
Vβ7.1	2.56	0.64	20.01	0.65	3.9	2.09	0.38
Vβ7.2	1.47	0.05	5.45	1.46	1.35	3.29	0.22
Vβ8	4.68	2.26	29.47	6.09	3.93	8.52	0.95
Vβ9	3.13	1.1	9.3	0.28	4.55	1.55	0.2
Vβ11	1.04	0.25	5.11	0.25	2.24	3.56	0.49
Vβ12	1.66	1	4.76	1.89	1.84	1.07	1.10
Vβ13.1	3.83	1.62	8.16	2.02	6.07	2.23	0.23
Vβ13.2	2.80	0.8	5.28	0.27	3.37	2	1.29
Vβ13.6	1.86	0.84	8.8	8.03	0.26	1.09	1.58
Vβ14	3.49	1.33	8.03	0.95	1.88	4.63	13.44
Vβ16	0.92	0.42	1.9	1.40	1.62	2.07	1.69
Vβ17	5.15	2.28	12.61	1.62	2.37	5.16	0.54
Vβ18	1.49	0.58	5.23	0.94	1.06	1.77	0.25
Vβ20	2.52	0	9.73	0.9	0.79	0.94	1.10
Vβ21.3	2.38	1.08	5.97	0.9	7.50	1.03	0.5
Vβ22	3.84	1.99	9.89	2.02	1.37	2.88	14.57
Vβ23	0.85	0.28	4.76	8.16	1.82	1.37	0.58

Min, minimum value obtained; max, maximum value obtained. TCR-Vβ repertoire was performed on fresh whole blood from XLT and WAS patients using the IOTest β Mark (Beckman Coulter).

[a]Healthy donor values are reported in the quick reference card of the TCR-Vβ Repertoire kit (Beckman Coulter).

[b]All values represent the percentage of $CD3^+$ lymphocytes.