

Table S1. Nucleotide sequences of CDR-H3 from homozygous *ΔD-iD* mice that contain an inverted D_H gene segment

ID	Fxn	VH	RF	JH	V	P-N-P	D	P-N-P	J	Hydrophobicity
DSP2.2										
iD										
RLS01523	B	1	i2	3	GCAAGACA	T	TCTACTATGATTACGA GTAGCTT C TACTATGATTACGATAAA	GA	GCTTAC	0.602
RLS01588	B	1	i1	3	GCAA	A	.TAGCTT C TACTATGATTACGAT...	CTCC	GGTTTGCTTAC	-0.190
RLS01540	B	18	i2	3	GCAA	AC	GTAGCTT C TACTATGATTACGATAAA	GGA	CTGGTTTGCTTAC	0.372
RLS01570	B	1	i2	3	GCAAGACA	GGGGC	..AGCTT C TACTATGATTACG.....	CCTC	GGTTTGCTTAC	0.105
GCI00149	C	9	i2	3	GCAAGA	GG	..AGCTT C TACTATG.....	GTTACAAGG G	CCTGGTTTGCTTAC	0.229
RLS00613	C	1	i1	3	GCgAGACA	T	GTAGCTT C TACTATGATTACGATAA.	GACGGG	C	-0.447
GCI00131	C	9	i2	1	GCAAGAC	CT	GTAGCTT C TACTATGATTACGATAA.	CTCCA	TGGTACTTCGATGTC	0.393
GCI00309	F	12	i1	1	GCAAGAG	GCCGA	..AGCTT C TACTATGATTACGATAAA	ACCTC	CTGGTACTTCGATGTC	-0.340
GCI00291	F	20	i2	1	GCAAGACA	AGAAACTT C TACTATGactACGATA...	GGGGGTCCGT	CTTCGATGTC	0.108
RLS00751	F	9	i1	1	GCAAGAC	GGGACTT C TACTATGATTACG.....	CCCCAAG	CTACTGGTACTTCGATGTC	-0.261
GCI00270	F	10	i2	3	GCAAGA	ACGGCTT C TACTATGATTACGAT...	C	TTTGCTTAC	0.583

ID, sequence identifier. Fxn, the bone marrow B lineage subpopulation according to the scheme of Hardy et al. (Hardy, R.R. and K. Hayakawa. 2001. *Annu. Rev. Immunol.* 19:595–621) from which the CDR-H3 sequence were cloned. VH, the V_H gene segment according to the IMGT classification (Lefranc, M.-P. 2003. *Nucleic Acids Res.* 31:307–310). RF, the D_H reading frame used by the inverted D. P-N-P, palindromic (P) and N region sequence. P sequences are positioned adjacent to their donor gene segment sequence. D, the D_H sequence component of CDR-H3. The coding sequence of DSP2.3 is bold. J, the J_H sequence component of CDR-H3. Hydrophobicity, the average, normalized Kyte-Doolittle hydrophobicity of the CDR-H3 loop (Kyte, J. and R.F. Doolittle. 1982. *J. Mol. Biol.* 157:105–132; Eisenberg, D. 1984. *Annu. Rev. Biochem.* 53:595–623).

Table S2. Predicted amino acid sequences of CDR-H3 from homozygous $\Delta D-iD$ mice that contain an inverted D_H gene segment

ID	Fxn	VH	RF	JH	Base	Loop	Base	Hydrophobicity
RLS01523	B	1	i2	3	AR	HVASTMITI	RAY	0.602
RLS01588	B	1	i1	3	AN	SFYYDYDLR	FAY	-0.190
RLS01540	B	18	i2	3	AN	VASTMITIKDW	FAY	0.372
RLS01570	B	1	i2	3	AR	QGAASTMITPR	FAY	0.105
GCI00149	C	9	i2	3	AR	GASTMVTRAW	FAY	0.229
RLS00613	C	1	i1	3	AR	HRSFYDYD	KTG	-0.447
GCI00131	C	9	i2	1	AR	PVASTMITITPWY	FDV	0.393
GCI00309	F	12	i1	1	AR	GRSFYYDYDKTSWY	FDV	-0.340
GCI00291	F	20	i2	1	AR	QETSTMOTTIGGPV	FDV	0.108
RLS00751	F	9	i1	1	AR	RDFYYDYAPSYWY	FDV	-0.261
GCI00270	F	10	i2	3	AR	TASTMITI	FAY	0.583

ID, sequence identifier. Fxn, the bone marrow B lineage subpopulation according to the scheme of Hardy et al. (Hardy, R.R. and K. Hayakawa. 2001. *Annu. Rev. Immunol.* 19:595–621) from which the CDR-H3 sequence was cloned. VH, the V_H gene segment according to the IMGT classification (Lefranc, M.-P. 2003. *Nucleic Acids Res.* 31:307–310). RF, the D_H reading frame used by the inverted D. P-N-P, palindromic (P) and N region sequence. Base, the predicted amino acid sequence of the CDR-H3 base. Loop, the predicted amino acid sequence of the CDR-H3 loop. Hydrophobicity, the average, normalized Kyte-Doolittle hydrophobicity of the CDR-H3 loop (Kyte, J. and R.F. Doolittle. 1982. *J. Mol. Biol.* 157:105–132; Eisenberg, D. 1984. *Annu. Rev. Biochem.* 53:595–623).

Table S3. Predicted amino acid sequences of CDR-H3 whose average Kyte-Doolittle hydrophobicity is less than -0.700

ID	Genotype	Fxn	VH	DH	RF	JH	Base	CDR-H3 Loop	Base	Hydrophobicity
RLS01543	ΔD-iD	B	16	iD	1	3	AR	DEKRNHRSRR	FAY	-0.911
RLS01596	ΔD-iD	B	?	iD	1	1	AR	HDRNHGREDWY	FDV	-0.800
RLS01545	ΔD-iD	B	1	iD	1	2	AR	QRDRNHSRSPYY	FDY	-0.743
RLS01551	ΔD-iD	B	10	iD	1	1	AR	PRNHSRSYERY	FDV	-0.720
RLS01547	ΔD-iD	B	13	iD	1	2	AR	HGRNHSRSYR	FDY	-0.716
RLS00587	ΔD-iD	B	6	iD	1	2	AA	YRNHSRS	LDY	-0.711
GCI00164	ΔD-iD	C	14	iD	1	2	AR	DKDRNHSR	IDY	-0.964
RLS00611	ΔD-iD	C	10	iD	1	2	AR	QRRDRNHSRSYN	FDY	-0.882
RLS00618	ΔD-iD	C	10	iD	1	2	AR	HDRNHS	RSS	-0.870
RLS00592	ΔD-iD	C	1	iD	1	3	AR	RDRNHSRSYR	FAY	-0.858
RLS00607	ΔD-iD	C	9T	iD	1	2	AR	HHRNHSRS	LDY	-0.816
RLS00595	ΔD-iD	C	9T	iD	1	1	AN	RNHSRRYWY	FDV	-0.732
RLS00603	ΔD-iD	C	10	iD	1	2	AR	SDRNHSRS	YAY	-0.726
GCI00143	ΔD-iD	C	1	iD	1	3	AR	HDRNHSRSYP	FAY	-0.726
RLS00658	ΔD-iD	D	10	iD	1	3	AR	QEDRNHSRSG	LAY	-0.768
RLS00659	ΔD-iD	D	10	iD	1	3	AR	HERNHSRSW	FAY	-0.751
RLS00665	ΔD-iD	D	9	iD	1	4	AR	HRNHSRSY	GDY	-0.736
GCI00209	ΔD-iD	D	9	iD	1	3	AR	PYRNHRG	DAY	-0.731
RLS00667	ΔD-iD	D	9T	iD	1	3	AP	NRNHSRSS	FAY	-0.726
RLS00629	ΔD-iD	D	14	iD	1	2	AR	QDYRNHSRSY	VDY	-0.725
GCI00197	ΔD-iD	D	12	iD	1	3	AR	EGDPYRNHSR	RTY	-0.722
GCI00240	ΔD-iD	E	2	iD	1	3	AR	DRNHRG	FAY	-0.913
GCI00251	ΔD-iD	E	17	iD	1	2	TR	DDHRNHSRSHY	FDY	-0.800
GCI00249	ΔD-iD	E	16	iD	1	3	AK	YRNHRG	FAY	-0.792
GCI00230	ΔD-iD	E	5	iD	1	2	AR	DDRNHSRSY	PDY	-0.776
GCI00253	ΔD-iD	E	20	iD	1	3	AR	QNHSRS	FAY	-0.735
GCI00244	ΔD-iD	E	12	iD	1	3	AR	EGDPYRNHSR	RTY	-0.722
RLS00693	ΔD-iD	E	2	iD	1	1	AR	DRGNYRNHSRSYRY	FDV	-0.721
RLS00702	ΔD-iD	E	2	No D		2	AR	D	HGD	-1.000
RLS00728	ΔD-iD	F	18	iD	1	4	AR	GDRHN	MDY	-0.863
RLS00741	ΔD-iD	F	9T	iD	1	1	AR	HERNHSRS	PDV	-0.828
RLS00727	ΔD-iD	F	10	iD	1	2	AR	HRNHSRS	FDY	-0.803
RLS00742	ΔD-iD	F	9T	iD	1	1	AR	QNRNHSRGNWY	FDV	-0.726
RLS00737	ΔD-iD	F	2	iD	1	1	AR	EEEDRNHSRSYWY	FDV	-0.722
GCI00296	ΔD-iD	F	10	iD	1	2	AR	YRNHSRS	FDY	-0.711
RLS00730	ΔD-iD	F	10	No D		2	AR	QRDY	FDY	-0.893
RLS00053	ΔD-DFL	C	10	DFL16.1	1	4	AR	HRH	YGS	-1.040
RLS00023	ΔD-DFL	C	10	No D		3	AR	R	FAY	-1.300
RLS00211	ΔD-DFL	E	10	No D		3	AR	QK	SAY	-1.050
RLS00207	ΔD-DFL	E	10	No D		4	AR	HE	RGY	-0.955
RLS00234	ΔD-DFL	F	2	DFL16.1	1	2	AR	HEGSRRNY	FDY	-0.731
RLS01658	WT	B	10	DQ52		3	AR	RD	PAY	-1.150
RLS01167	WT	B	9	DSP2.02	1	3	AR	RDY	SAY	-0.857
RLS01615	WT	B	12	DSP2.03	1	3	AR	GRREDYGYDE	GAY	-0.708
RLS01626	WT	B	5	DSP2.05	1	3	AR	HNGN	YTY	-0.720
RLS01104	WT	B	1	DSP2.11	2	2	AR	R	GND	-1.300
RLS01140	WT	B	1	No D		3	AR	QEKW	FAY	-0.810
RLS00357	WT	C	18	DQ52		2	AR	DDWD	FDY	-0.785
RLS01342	WT	C	1	DSP2.02	1	4	AK	RYDR	LDY	-0.968
III00196	WT	C	2	DSP2.02	1	3	AR	DRYDP	FAY	-0.788
RLS01256	WT	C	5	DSP2.02	1	4	AR	HRDRDY	DVD	-0.963

Charged CDR-H3 intervals impair B cell function

RLS00296	WT	C	10	DSP2.05	1	3	AR	DGNYR	FAY	-0.708
RLS01237	WT	C	11	DSP2.05	1	1	AR	DDGRN	FDV	-0.854
III00226	WT	C	1	DSP2.09	1	2	AR	QEDD	GYP	-1.000
RLS00349	WT	C	10	DSP2.11	1	2	AR	RDYRY	DQG	-0.828
GCI00035	WT	C	18	DSP2.11	1	1	AK	RDYRYEY	FDV	-0.773
RLS00347	WT	C	13	DST4		3	AR	HREE	FAY	-1.053
RLS01230	WT	C	2	No D		4	AR	EK	EVH	-1.050
RLS00317	WT	C	9	No D		4	AR	RDP	MDY	-0.890
RLS00332	WT	C	10	No D		3	AG	H	DSY	-0.910
RLS00324	WT	C	10	No D		3	AR	HERS	FAY	-0.828
III00278	WT	D	6	DFL16.2	1	2	AR	QRNYG	FDY	-0.708
GCI00065	WT	D	9	DSP2.02	1	2	TR	DQGD	MTY	-0.743
RLS00417	WT	D	10	DSP2.02	1	2	AR	HNDRDPP	FDY	-0.850
RLS01408	WT	D	2	DSP2.09	3	2	AR	DRW	GDY	-0.813
RLS00421	WT	D	9	DSP2.11	1	3	AI	YRYE	FAY	-0.710
RLS01401	WT	D	6	DST4		3	AR	RRQGW	SAY	-0.742
RLS00440	WT	D	11	DST4		3	AR	DR	GAY	-1.150
III00497	WT	D	1	No D		3	AR	HEGETR	FAY	-0.708
III00275	WT	D	2	No D		3	AR	DR	PAY	-1.150
III00296	WT	D	2	No D		3	AR	DR	FAY	-1.150
III00491	WT	D	5	No D		4	AR	RS	MDY	-0.700
RLS01400	WT	D	6	No D		3	AR	RR	FAY	-1.300
GCI00089	WT	E	10	DQ52		3	AR	HENWEP	FAY	-0.737
RLS00477	WT	E	10	DSP2.05	1	3	AR	QDGNRG	FAY	-0.707
III00340	WT	E	6	No D		3	AR	HD	RAY	-0.955
III00387	WT	E	10	No D		4	AR	H	MDY	-0.910
RLS00505	WT	E	10	No D		3	AR	H	AAY	-0.910
RLS00528	WT	F	9	DSP2.02	1	4	AR	RDDD	LDY	-1.075

ID, sequence identifier. Genotype, D-iD – homozygous *D-iD* mice, D-DFL – homozygous *D-DFL* mice, WT – BALB/c wild-type controls. Fxn, bone marrow B lineage Hardy-Fraction (Hardy, R.R. and K. Hayakawa. 2001. *Annu. Rev. Immunol.* 19:595–621). V_H, V_H7183 gene segment No D, no identifiable D_H. RF, the D_H reading frame used by the members of the DFL and DSP D_H families. Base, predicted amino acid sequence of the CDR-H3 base. Loop, predicted amino acid sequence of the CDR-H3 loop. Hydrophobicity, the average, normalized Kyte-Doolittle hydrophobicity of the CDR-H3 loop (Kyte, J. and R.F. Doolittle. 1982. *J. Mol. Biol.* 157:105–132; Eisenberg, D. 1984. *Annu. Rev. Biochem.* 53:595–623).

Table S4. Predicted amino acid sequences of CDR-H3 with average Kyte-Doolittle hydrophobicity is greater than +0.700

ID	Genotype	Fxn	VH	DH	RF	JH	Base	CDR-H3 Loop	Base	Hydrophobicity
RLS00572	ΔD-iD	B	1	iD	3	2	AR	RIVIIVEVY	FDY	0.814
RLS00586	ΔD-iD	B	9T	iD	2	4	AR	LIVIIRNA	MDY	0.821
RLS01533	ΔD-iD	B	12	iD	2	2	AR	VVIP	FDY	1.133
RLS00583	ΔD-iD	B	1	iD	2	2	AR	HFIVIIV	ADY	1.213
RLS01579	ΔD-iD	B	2	iD	2	3	AR	VIVIIVP	FAY	1.361
RLS00616	ΔD-iD	C	10	iD	2	2	AR	HVVIIIVEALYY	SDY	0.720
RLS00597	ΔD-iD	C	1	iD	2	2	AR	RVVIIVEAL	FDY	0.897
GCI00206	ΔD-iD	D	12	iD	2	2	AR	EGALIVIIVEAP	FDY	0.742
GCI00210	ΔD-iD	D	12	iD	2	2	AR	GGALIVIIVEAP	FDY	0.828
RLS00646	ΔD-iD	D	9	iD	2	2	AR	LIVIIDY	IDY	1.600
RLS00032	ΔD-DFL	C	12	DFL16.1	2	2	AR	PTVVA	FDY	0.706
RLS00103	ΔD-DFL	C	10	DFL16.1	2	2	AR	FITTVVAH	FDY	0.715
RLS00091	ΔD-DFL	C	12	DFL16.1	2	2	AR	GFITTVVG	RGY	0.740
RLS00131	ΔD-DFL	D	18	No D	2	2	AR	I	GDY	1.700
RLS00195	ΔD-DFL	E	9T	DFL16.1	2	2	AR	PFITTVVA	PDY	0.783
RLS00210	ΔD-DFL	E	13	DFL16.1	2	3	AR	FLTVVAGG	PAY	0.808
RLS01148	WT	B	18	DFL16.1	3	4	AR	VLPLYA	MDY	0.755
RLS01297	WT	C	9	DFL16.1	3	2	AR	HLLL	ADY	0.823
III00229	WT	C	10	DFL16.2	2	2	AR	LFIT	NDY	1.033
RLS00376	WT	C	12	DFL16.x	3	4	AR	GLLLYA	MDY	0.788
RLS01314	WT	C	9	DSP2.02	2	3	AR	YMI	KAY	0.743
III00217	WT	C	9	DSP2.02	2	2	AR	VITGL	RDY	0.932
RLS00327	WT	C	6	DSP2.02	2	2	AS	LSMI	ADY	0.950
RLS01285	WT	C	12	DSP2.09	3	4	AR	VPPVSLL	VDY	0.737
III00200	WT	C	1	No D	3	AR	HIFL	FAY	0.823	
RLS01325	WT	C	16	No D	2	AA	F	EGY	1.100	
RLS00434	WT	D	1	DSP2.05	2	3	AR	LTMVTM	FAY	0.743
III00326	WT	D	10	DSP2.09	3	2	AR	WLLF	FDY	0.940
RLS01424	WT	E	N	DFL16.1	2	1	TR	LLFT	ADV	0.958
III00386	WT	E	9	DQ52	2	AE	LG	QDY	0.715	
III00349	WT	E	3	DSP2.02	2	4	AR	ILMITYA	MDY	0.861
GCI00090	WT	E	16	DSP2.02	3	1	AS	L	LLF	1.400
MCZ00019	WT	F	14	DFL16.1	2	2	AS	LITTV	VDY	0.912

ID, sequence identifier. Genotype, D-iD – homozygous *D-iD* mice, D-DFL – homozygous *D-DFL* mice, WT – BALB/c wild-type controls. Fxn, bone marrow B lineage Hardy-Fraction (Hardy, R.R. and K. Hayakawa. 2001. *Annu. Rev. Immunol.* 19:595–621). VH, the V_H7183 gene segment. No D, no identifiable D_H. RF, the D_H reading frame used by the members of the DFL and DSP D_H families. Base, predicted amino acid sequence of the CDR-H3 base. Loop, predicted amino acid sequence of the CDR-H3 loop. Hydrophobicity, the average, normalized Kyte-Doolittle hydrophobicity of the CDR-H3 loop (Kyte, J. and R.F. Doolittle. 1982. *J. Mol. Biol.* 157:105–132; Eisenberg, D. 1984. *Annu. Rev. Biochem.* 53:595–623).

Table S5. Distribution of unique, in-frame sequences by Hardy fraction

Fraction	Previous WT	$\Delta D-ID$ WTLM	Total WT	$\Delta D-DFL$	$\Delta D-ID$
B	78	66	144	14	71
C	276	43	319	83	50
D	182	37	219	57	63
E	175	23	198	46	82
F	156	38	194	42	76
Total	867	207	1074	242	342

Number of $V_{H}7183$ VDJC μ transcripts analyzed. Previous WT, wild-type unique in-frame open previously reported CDR-H3 sequences from homozygous, wild-type BALB/c mice (Ivanov, I.I., R.L. Schelonka, Y. Zhuang, G.L. Gartland, M. Zemlin, and H.W. Schroeder, Jr. 2005. *J. Immunol.* 174:7773–7780; Schelonka, R.L., I.I. Ivanov, D. Jung, G.C. Ippolito, L. Nitschke, Y. Zhuang, G.L. Gartland, J. Pelkonen, F.W. Alt, K. Rajewsky, and H.W. Schroeder, Jr. 2005. *J. Immunol.* 175:6624–6632). $\Delta D-ID$ WTLM, sequences from homozygous wild-type littermates of the $\Delta D-ID$ mice. Total WT, sequences from homozygous wild-type littermate controls plus previously reported sequences from wild-type BALB/c mice. $\Delta D-DFL$, sequences from homozygous $\Delta D-DFL$ mice (Schelonka, R.L., I.I. Ivanov, D. Jung, G.C. Ippolito, L. Nitschke, Y. Zhuang, G.L. Gartland, J. Pelkonen, F.W. Alt, K. Rajewsky, and H.W. Schroeder, Jr. 2005. *J. Immunol.* 175:6624–6632). $\Delta D-ID$, sequences from homozygous $\Delta D-ID$ mice.