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Supplemental Data

The Effect of BAFF Inhibition on Autoreactive B-Cell Selection in Murine Systemic Lupus Erythematosus

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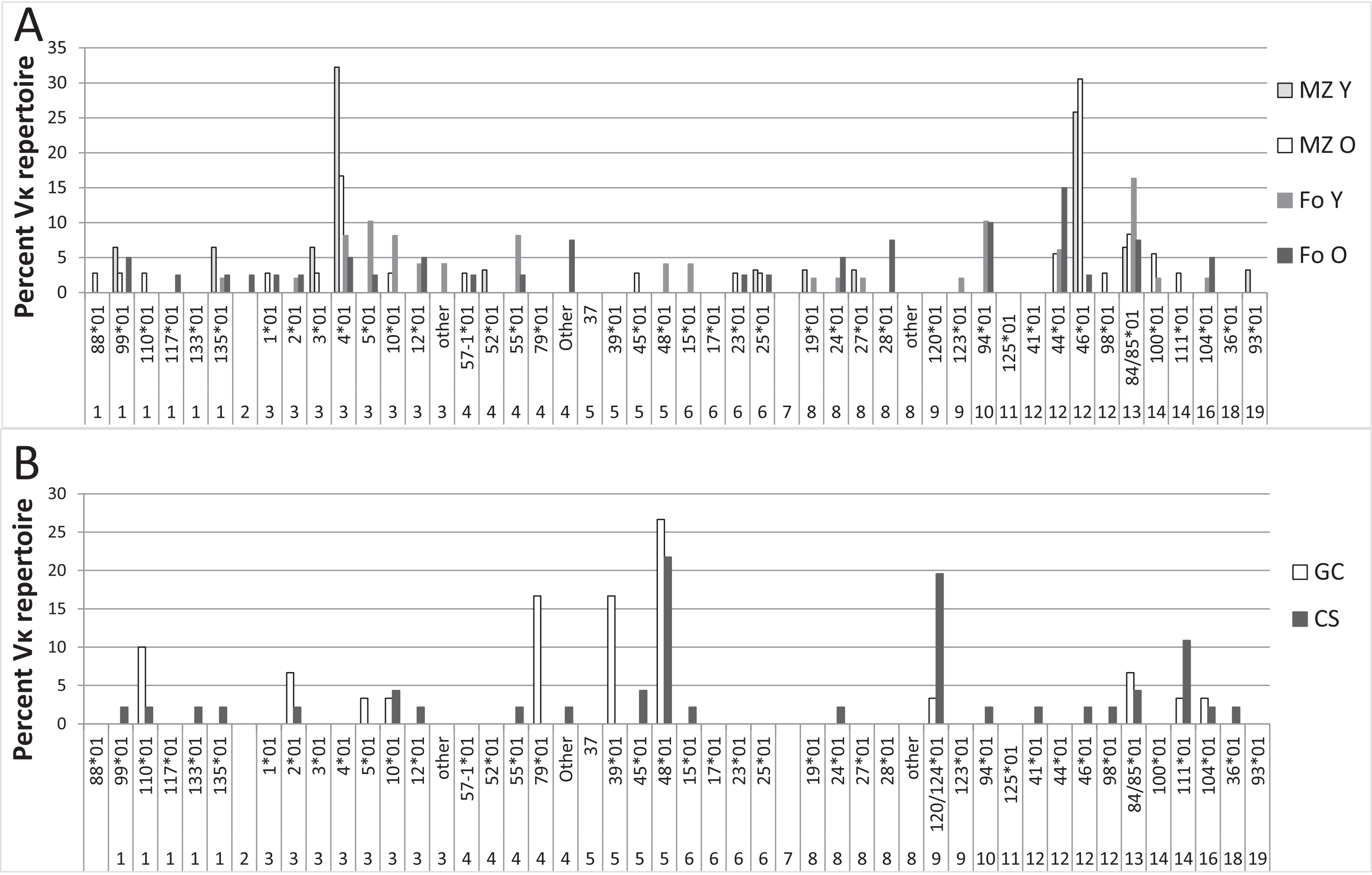
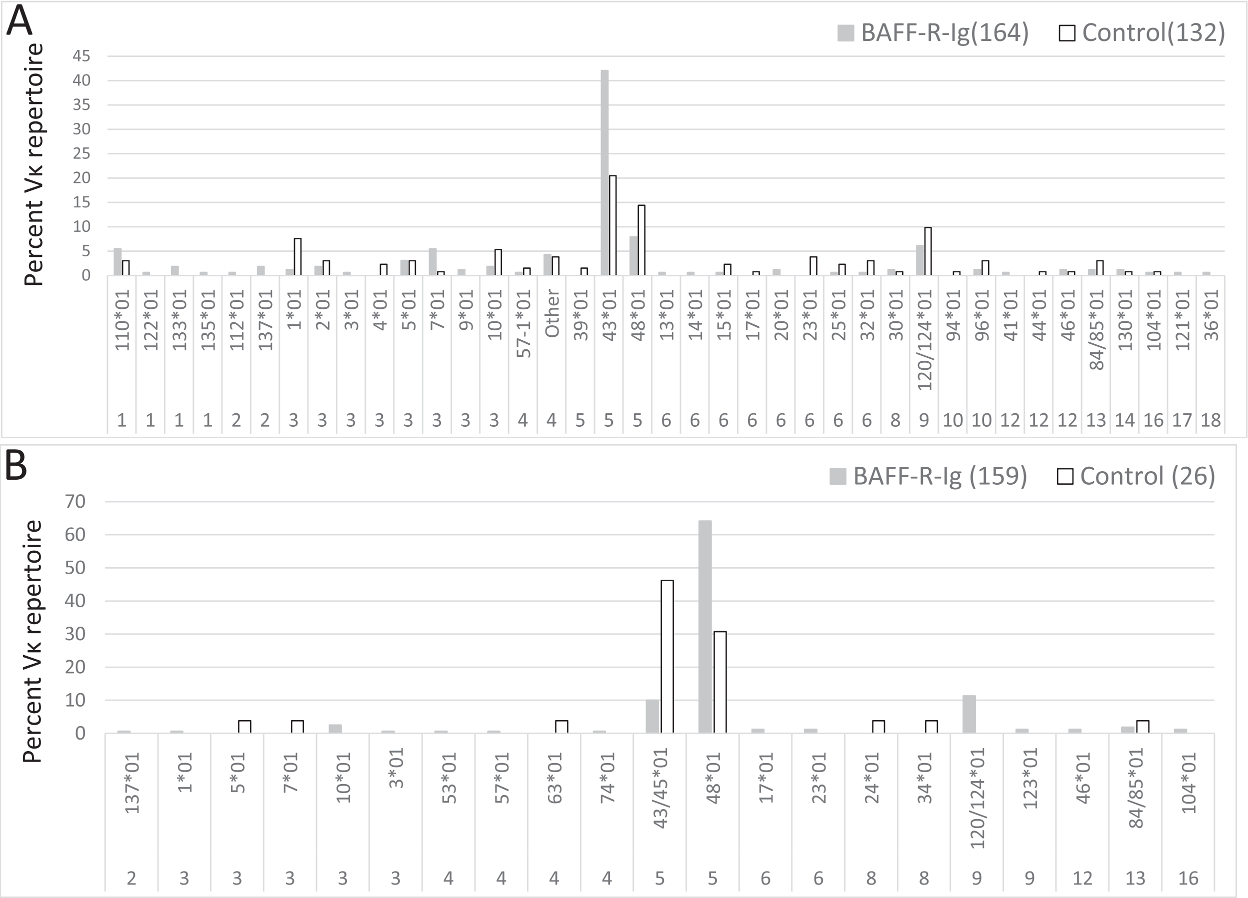


Supplementary Figure S1. The repertoire of Vκ light chains associated with the 3H9 heavy chain in NZB/W 3H9 transgenic mice: The y-axis shows percent of the total repertoire for each subset. (A) the repertoire of marginal zone (MZ) and follicular (Fo) B cell subsets sorted from young (Y) 8-10-wk-old and Old (O) 52-wk-old 3H9.NZB/W spleens. Note the expansion of Vκ3-4*01 and Vκ12-46*01 in the MZ B cells compared with the diverse repertoire of Fo cells. (B) The repertoire of germinal center (GC) and class switched (CS) B cells from 52-wk-old 3H9.NZB/W spleens. Note that the repertoire of these cells is more restricted than that of Fo cells with predominance of Vκ5 encoded light chains. Three to five spleens and 35-60 sequences were analyzed for each subset.



Supplementary Figure S2. The repertoire of Vκ light chains associated with the 3H9 heavy chain in plasma cells from NZW/BXSB (A) and NZB/W (B) chimeras. The y-axis shows percent of the total repertoire for each subset. Three to six spleens were analyzed for each subset (see Supplementary Table S2 for sequences and Supplementary Table S3 for statistical analysis).

Supplementary Table S1. Donor strains and chimeras.

Chimera name	Donor 3H9		Recipient	Harvest (days after transplant)
M W/B WT	M	-	M W/B	86 +/- 16
M W/B 3H9	M	+	M W/B	95 +/- 31
M W/B 3H9 BAFF-R-Ig	M	+	M W/B	119 +/-19
F WT B/W	F	-	F B/W	178
F 3H9 B/W	F	+	F B/W	188 +/- 50
F 3H9 B/W BAFF-R-Ig	F	+	F B/W	229 +/- 13

Supplementary Table S2. 3H9 associated Vκ usage of B cell subsets from chimeric mice.

Follicular B cells NZW/BXSB				Follicular B cells NZB/W			
Vκ gene	V allele	BAFF-R-Ig	Control	Vκ gene	V allele	BAFF-R-Ig	Control
1	88*01	0	1	1	99*01	3	1
1	99*01	3	3	1	110*01	1	3
1	110*01	1	5	1	117*01	0	1
1	117*01	1	0	1	133*01	0	2
1	135*01	2	2	1	135*01	5	3
2	all	2	1	2	137*01	1	4
3	1*01	4	5	3	1*01	14	1
3	2*01	6	8	3	2*01	4	4
3	3*01	3	0	3	3*01	0	4
3	4*01	7	21	3	4*01	4	13
3	5*01	7	8	3	5*01	3	5
3	7*01	1	0	3	7*01	1	1
3	10*01	8	19	3	9*01	2	0
3	12*01	9	9	3	10*01	13	22
4	53*01	0	0	3	12*01	6	7
4	55*01	2	10	4	57*01	2	0
4	57*01	0	1	4	55*01	0	3
4	57-1*01	3	8	4	59*01	1	3
4	58*01	1	1	4	61*01	1	0
4	59*01	0	0	4	63*01	1	2
4	61*01	1	0	4	74*01	2	0
4	72*01	1	0	4	80*01	1	0
4	74*01	1	0	4	83*01	1	1
4	78*01	1	0	5	39*01	1	0
4	79*01	1	0	5	43/45*01	4	3
4	80*01	1	0	5	48*01	8	16
4	91*01	0	1	6	17*01	1	1
5	39*01	1	4	6	23*01	2	1
5	43/45*01	1	1	6	25*01	0	5
5	48*01	0	2	8	24*01	1	3
6	14*01	1	1	8	27*01	1	1
6	15*01	2	1	8	28*01	2	2
6	17*01	3	3	8	30*01	1	1
6	23*01	6	4	9	120/124*01	1	0
6	25*01	2	2	10	94*01	3	0
6	32*01	1	1	12	38*01	2	2
8	19*01	0	4	12	41*01	0	1
8	21*01	0	1	12	44*01	4	4
8	24*01	3	0	12	46*01	0	3
8	27*01	2	1	12	98*01	0	1
8	28*01	6	6	13	84*01 85*01	18	11
8	30*01	5	4	14	111*01	0	2
9	120/124*01	1	2	14	130*01	0	1
9	123*01	0	7				
10	94*01	3	4				
12	38*01	2	2				
12	44*01	6	4				
12	46*01	5	11				
12	98*01	1	0				
13	84/85*01	17	27				
14	100*01	1	1				
16	104*01	4	2				
19	93*01	0	2				
	Total	139	200		Total	115	138

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Supplementary Table S2. ***Continued.***

Germinal center B cells NZW/BXSB				Germinal center B cells NZB/W			
Vκ gene	V allele	BAFF-R-Ig	Control	Vκ gene	V allele	BAFF-R-Ig	Control
1	110*01	10	3	1	99*01	0	1
1	117*01	1	1	1	115*01	2	0
1	133*01	0	1	1	117*01	1	0
1	135*01	2	2	1	88*01	1	0
1	88*01	0	1	3	1*01	1	1
1	99*01	1	0	3	4*01	0	1
2	137*01	2	2	3	5*01	0	3
3	1*01	0	4	3	7*01	0	3
3	12*01	8	1	3	10*01	1	0
3	2*01	2	2	3	12*01	2	0
3	3*01	0	2	4	57*01	4	8
3	4*01	2	8	4	68*01	0	1
3	5*01	1	2	4	72*01	0	1
4	50*01	2	0	4	74*01	0	1
4	55*01	3	0	4	79*01	1	4
4	52*01	0	1	5	39*01	3	1
4	57-1*01	3	9	5	43/45*01	67	43
4	58*01	1	0	5	48*01	31	30
4	59*01	2	1	6	15*01	0	2
4	69*01	1	0	6	17*01	0	1
4	91*01	1	0	6	25*01	2	0
4	74*01	1	1	9	120/124*01	22	6
4	79*01	3	0	9	123*01	1	0
4	61*01	0	2	9	124*01	2	0
4	63*01	0	2	10	94*01	2	2
4	73*01	1	3	13	84/85*01	4	10
4	80*01	0	1	14	100*01	2	0
5	39*01	0	1	14	111*01	1	0
5	43*01	115	52	16	104*01	3	0
5	48*01	18	35				
6	15*01	12	12				
6	17*01	0	1				
6	20*01	1	0				
6	23*01	0	4				
6	25*01	0	1				
8	21*01	0	11				
8	24*01	3	0				
8	27*01	1	0				
8	30*01	4	2				
9	120/124*01	19	19				
9	123*01	1	1				
10	94*01	0	2				
12	38*01	0	1				
12	44*01	3	0				
12	46*01	2	1				
13	84/85*01	3	3				
14	100*01	1	1				
14	111*01	6	1				
16	104*01	9	3				
	Total	245	200		Total	153	119

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Supplementary Table S2. ***Continued.***

Plasma cells NZW/BXSB				Plasma cells NZB/W			
Vκ gene	V allele	BAFF-R-Ig	Control	Vκ gene	V allele	BAFF-R-Ig	Control
1	110*01	9	4	2	137*01	1	0
1	88*01	0	1	3	1*01	1	0
1	122*01	1	0	3	5*01	0	1
1	133*01	3	0	3	7*01	0	1
1	135*01	1	0	3	10*01	4	0
2	112*01	1	0	3	3*01	1	0
2	137*01	3	0	4	53*01	1	0
3	1*01	2	10	4	57*01	1	0
3	10*01	3	7	4	63*01	0	1
3	2*01	3	4	4	74*01	1	0
3	3*01	1	0	5	43/45*01	16	12
3	4*01	0	3	5	48*01	102	8
3	5*01	5	4	6	17*01	2	0
3	7*01	9	1	6	23*01	2	0
3	9*01	2	0	8	24*01	0	1
4	57-1*01	1	2	8	34*01	0	1
4	Other	7	5	9	120/124*01	18	0
5	39*01	0	2	9	123*01	2	0
5	43/45*01	69	27	12	46*01	2	0
5	48*01	13	19	13	84/85*01	3	1
6	13*01	1	0	16	104*01	2	0
6	14*01	1	0				
6	15*01	1	3				
6	17*01	0	1				
6	20*01	2	0				
6	23*01	0	5				
6	25*01	1	3				
6	32*01	1	4				
8	30*01	2	1				
9	120/124*01	10	13				
10	94*01	0	1				
10	96*01	2	4				
12	41*01	1	0				
12	44*01	0	1				
12	46*01	2	1				
13	84/85*01	2	4				
14	130*01	2	1				
16	104*01	1	1				
17	121*01	1	0				
18	36*01	1	0				
	Total	164	132			159	26

Supplementary Table S3. Pairwise comparisons of Vκ repertoires of FO, GC and PC subsets.*

B cell subset comparison		NO. Vκ genes	No. genes in top 50%[†]	Top gene[‡] (% λ^2)	Individual genes contributing > 5% to the λ^2 analysis						
FNZB/W	FO	43	8	10.7	3-1	6-25	10-94	3-3	3-4	13-84/85	4-55
FNZB/W	GC	30	8	11.4	9-120/124	13-84/85	3-5	4-79	3-7	6-15	4-57
FNZB/W	PC	21	7	31.73	5-43/45	8-34	8-24	4-63	3-7	3-5	5-48
MNZW/BXSB	FO	51	10	8.6	9-123	3-3	8-24	3-4	4-55		
MNZW/BXSB	GC	49	7	12.7	8-21	5-43/45	5-48				
MNZW/BXSB	PC	40	7	14.5	5-43/45	3-1	6-23	3-7	3-4		

*See reference 3

[†] Number of genes contributing the top 50% to the overall λ^2

[‡] Percent contribution of the top gene to the overall λ^2

Supplementary Table S4. Mutation analysis of 3H9 associated Vκ5 light chains.

				Framework		CDR	
Subset	Chimera	Number sequences	Mutations/ sequence	S*	R	S	R
Vκ5-43	3H9 W/B	28	3.5	45	30	4	19
	3H9 W/B BAFF-R-Ig	26	3.6	20	45	6	22
	3H9 B/W	16	6.3	10	45	3	43
	3H9 B/W BAFF-R-Ig	18	4.0	13	39	3	17[†]
Vκ5-48	3H9 B/W	19	4.7	28	48	0	29
	3H9 B/W BAFF-R-Ig	18	8.8	41	72	7	38

*S: silent; R: replacement

[†] p<0.01 compared with 3H9 B/W