Supplementary Data

Supplementary Figures

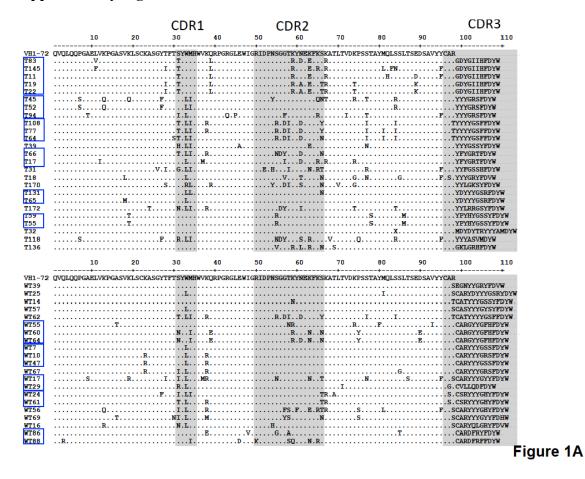


Figure 1A: Localization of amino acid substitutions in distinct clones encoding **VH1-72 heavy chains of anti-NP monoclonal antibodies obtained from wild type and TACI-deficient mice**. Shown are independent VH sequences compared to the germline VH1-72 (indicated above each group). The boxed sequences correspond to the same clone, defined by the V(D)J junction. CDR1, CDR2 and CDR3, defined according to Kabat are shadowed. Sequences shown are all IgG1.

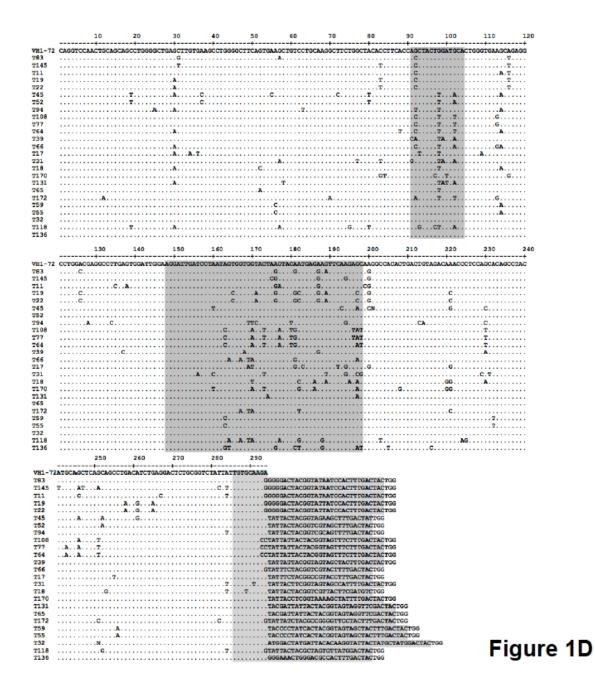
70 CDI	100	130	CDR2	190	220	250	CDR3
VL1 TCRSSTGAVI		+			+	+	
							FCALWYSNHS
							WVFGGGTKLXCPK
							WVFGGGTKLNCPK
							GWVFGGGTKLNCPNK
T11SG.			vv.	.v			D.WVFGGGTKLTVLK
							WVFGGGTKLXCPK
							WVFGGGTKLDCPK
							WVFGGGTKLDCPN
							WVFGGGTKLDCPK
T52		V.	P		т		WVFGGGTKLDCPK
							WVFGGGTKLXCPXN
							FIFGSGTKVHCPK
							WVFGGGTKLDCPK
							WVFGGGTKLDCPN
							T. YIFGGGTKVHCPN
							T.FVFGGGTKVTXLK T.WVFGGGTKLDCPK
VLZ							
70	100	130	160	190	220	250	280
VL1 TCRSSTGAVI							
							WVFGGGTKLDCPK
							WVFGGGTKLDCPK
							WVFGGGTKLNCPN
							WVFGGGTKLNCPK
WT64	.RS.		I.VS				VFWVFGGGTKRTCPK
							T.FVFGGGTXVHCPK
WT20							WVFGGGTKLDCPX TPWVFGGGTKRTVLK
WT13							
WT46							
WT47							
							WVFGGGTKLDCFN
							WVFGGGTKLNCPK
							WVFGGGTKLXCDK
							WVFGGGTKXTVL
WT12							
							WVFGGGTKLTVLX
WT17							WVFGGGTXVHCPX
WT27							
VL2			s	.v		D.M.	т
							Eigu

Figure 1B

Figure 1B: Localization of amino acid substitutions in distinct clones encoding Ig light chains of anti-NP monoclonal antibodies obtained from wild type and TACIdeficient mice. Shown are independent VL (V λ 1 or V λ 2) sequences compared to the germline (indicated above and below each group). CDR1, CDR2 and CDR3, defined according to Kabat are shadowed. The VL frequency of mutations was the same (1.7%) in clones derived from wt or from TACI-deficient mice.

				40	50	60	70	80	90	100	110
	ACTOCACCA	COCTOCOCC	TGACCUTCTC	ABCCCTCCCC		COTOTOC	AACCOUNCE	TACACCTE			TGGGTGAAGCA
											193913AA3CA
										т	
									7	T	
										.T	G
					.						A
									A		
							.G			.T	G
							.G			.T	G
							.G				G
		T.						т		.T	A G
										.TA.	
									C	T	8
									-	T.	a
									A 7	T	
	130	140	150	160	170	180	190	200	210	220	230
	130		100	100	170	180	190	200	210	220	2.50
											TCCAGCACAGC
JIGGACG	AGGCCTIGA	STOCKTION	DISGATIGAT	CUINAIAUIO	GIGGIACIA	GIACANIGAG	ANGTICANGAG	AAGGCCAC	CIGACIGI	usicianico.	ICCASCACASCO
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						TT	G.AGC	.G		T	
.c			сс			TT	G.AGC	.G		T	
.c			сс			TT	G.AGC	.G.			
.c			cc	C			G.AGC	.G.		T	
.c			cc	C		TT	G.AGC	.G.			
.c			сс с	C. G.			G.AGC	.G.			
.c			cc	C			G.AGC	.G.			
.c	250		с	290			G.AGC	.G.			
.c	250		сс с	290			G.AGC	.G.			
.C.	250 CAGCAGCCT	260 GACATCTGA	270 3GACTCTGCG	280 GTCTATTAT		AGGAAATTA	G.AG.C	.G.	TCTGG		
.C.	250 CAGCAGCCT	260 GACATCTGA	270 3GACTCTGCG	280 GTCTATTAT		AGGAAATTA	G.AG.C	.G.	TCTGG		
.C.	250 CAGCAGCCT	G	270 3GACTCTGCG	290 GTCTATTAT	TT.T. .TA.T. 	AGGAAATTAG ACGATAATTAG	G.AGC 	.G.	rctog NCTOG 3G		
.C.	250 CAGCAGCCT	G	270 3GACTCTGCG	290 GTCTATTAT	TT.T. .TA.T. 	AGGAAATTAG ACGATAATTAG	G.AGC 	.G.	rctog NCTOG 3G		
C	250 CAGCAGCCT		270	290 GTCTATTAT	TT. T. TA. T. G. T. C. 290 TGTGCAAGA G. 	AGGGAAATTAG AGGGAAATTAG ATTACTACGGI ATTACTACGGI	G.AGK G CTACGGTCGGT TACGGTAGTA TAGTAGCTACT	.G.	rergg Kergg 3G 3G		
.C	250 CAGCAGCCT		270 GACTCTGCG	280 280 GTCTATTAT	TT.T. TA.T. G. T.C. 290 TGTGCAAGA G. CCT. CCT. T. T. T. T. T. T. T. T. T.	.TT. AGGGARATTA ACGATTATTA ATTACTACGG ATTACTACGG ATTACTACTAC	G.AG. C. A. CG. CTACGGTCGGT TACGGTAGTATA TATAGGTAGTAT TATAGGTAGT	G. CTTCGATG GTACGACT TGACTACT TGACTACT	rergg Kergg 3G 3G		
.C	250 CAGCAGCCT	260 GACATCTGAC	270 3GACTCTGCG	280 270 GTCTATTAT	TT.T. TA.T. G. T.C. 290 TGTGCAAGA 	.TT. AGGGAAATTAK ACGATTATTAK ATTACTACGG ATTACTACGG ATTATTACTAK GCTACTATGG GCTACTATGG	CTACGGTCGGT CTACGGTCGGT TTACGGTACTACT TTATAGCTACT TGTAGCTACT TGTAGCTTTG	.G. 	rergg Kergg 3G 3G		
.C	250 CAGCAGCCT		270 GGACTCTGCG	280 GTCTATTAT		AGGGAAATTAM ACGATTATTAG ATTACTACGGT ATTATTACTACGGT ATTATTACTACGG GCTACTATGGJ GCTACTATGGJ	CTACGGTCGGT TACGGTCGGT TACGGTAGTA FAGTAGCTACT CGGTAGTTCT ATTCCACTTTG	.G. 	rergg Kergg 3G 3G		
.C	250 CAGCAGCCT		270 270 GACTCTGCG G	280 GTCTATTAT	TT T. TA T. G. C. 290 TOTGCAAGA 	AGGGAAATTAM ACGATTATTAM ATTACTACGGT ATTACTACGGG ATTATTACTAG GCTACTATGGJ GCTACTATGGJ GCTACTATGGJ	G.AG. C 	.G. 	rergg Kergg 3G 3G		
.C.	250 CAGCAGCCT	260 GACATCTGAC	270 270 3GACTCTGCG G.G.	280 280 GTCTATTAT	TT T TA T G G C 290 TGTGCAAGA G C C C C C C C C C C C C C	.TT. AGGGAAATTA/ ACGATTATTA/ ACTACTACGG/ ATTACTACGG/ ATTATTACTAG GCTACTATGGJ GCTACTATGGJ GCTACTATGGJ GCTACTATGGJ ATTACTACGG/	G.AG.C A CG TACGGTAGTA TACGGTAGTA CACTAGCTACT CGTAGCTACT ATTCCACTTTG ATTCCACTTTG ATTCCACTTTG	.G. 	rergg Kergg 3G 3G		
.C. .GCAGCTO A. .A.	250 CAGCAGCCTY	G	270 3GACTCTGCG 	280 GTCTATTAT	TT T. TA T. G. C. 290 TGTGCAAGA 	.TT. MGGAAATTAK MGGATTATTAK ATTACTACGG ATTATTACTAC GCTACTATGG GCTACTATGG GCTACTATGG MTTACTACGG	G.AG. C 	.G.	rergg Kergg 3G 3G		
.C.	250 		270 270 GGACTCTGCG GG	280 27077777777	TT T TA T C TA T C T T C 290 C C C C C C C C C C C C C C C C C C C	.TT. AGGGAAATTAK ACGATTATAGG ATTACTACGG ATTACTACGG CTACTATGG GCTACTATGG CCTACTATGG ATTACTACGG ATTACTACGG ATTACTACGG	G.AG.C A CG CG CG CG. CG C	.G. 	rergg Kergg 3G 3G		
.C	250 CAGCAGCCT: 	260 260	270 270 3GACTCTGCG	280 37CTATTAT	TT T TA T G G TC 290 TGTGCAAGA G C C C C C C C C C C C C C	TT. AGGGAAATTAG AGGATATTAC ATTACTACGG ATTACTACGG ATTACTACGG GCTACTATGGI GCTACTATGGI ATTACTACGG ATTACTACGG ATTACTACGG	CTACGGTCGGT TACGGTCGGT TACGGTAGTA TGTAGGTAGTA TGTAGTAGTAGT TTCACTTTG AGTAGCTTTG AGTAGCTTTG AGTAGCTTTG AGTAGCTTTG	G	rergg Kergg 3G 3G		
GCAGCTC A.	250 DAGCAGCCT: 	260 260 	270 270 3045557666	280 220 27CTATTAT	. TT. T	.TT. AGGGAAATTAK ACGATTATTA ATTACTACGA ATTACTACGA GCTACTATGG GCTACTATGG GCTACTATGG GCTACTATGG ATTACTACGG ATTACTACGG ATTACTACGG ATTACTACGG	CTACGGTCGGT TACGGTCGGT TACGGTAGTA TGGTAGTACTACT TGTAGTACTACT TGTAGTACTACT TGTAGTACTTTG TGGTAGTTTG TGGTAGTTTG TAGTAGCTTTG TAGTAGCTTTG	.G. 	rergg Kergg 3G 3G		
GCAGCTC A. .A.	250 CAGCAGCCT T		270 270 36ACTCTGC6	280 270 37CTATTAT	TT. T. TA. T. G. T. C. 290 TGTGCAAGA G. C. CTCTT. C. CCGAGG C. CC. GG C. C. CCAGG C. CCCAGG CCCAGG C. CCCAGG C CCCAGG C. CCCAGG C. CCCAGG C. CCCAGG C. CCCAGG C. CCCAGG C. CCCAGG C. CCCAGG C CCCAGG C. CCCAGG C CCCAGG C CCCAGG C CCCAGG CC	TT. AGGGAANTTAK ACGATTATTAK TTACTACGG TTACTACGG TTACTACGG CCTACTATGG CCTACTATGG CCTACTATGG ATTACTACGG TTACTACGG TTACTACGG TTACTACGG TTACTACGG	G.A. G. C TACGGTCGGT TACGGTAGTA TACGGTAGTA TACGGTAGTA TACAGTAGTACT TATCCACTTG TACTACTTG CGTAGCTTG CGTAGCTTG TACTACTTG TACTACTTG	CTTCGATG JGTACGACT TGACTACT TGACTACT TGACTACT TGACTACT GCTACTGG CTACTGG CTACTGG CTACTGG CTACTGG CTACTGG CTACTGG CTACTGG	rergg Kergg 3G 3G		
GCAGCTC	250 CAGCAGCCT T		270 270 3045557666	280 270 37CTATTAT	TT. T. TA. T. G. T. C. 290 TGTGCAAGA G. C. CTCTT. C. CCGAGG C. CC. GG C. C. CCAGG C. CCCAGG CCCAGG C. CCCAGG C CCCAGG C. CCCAGG C. CCCAGG C. CCCAGG C. CCCAGG C. CCCAGG C. CCCAGG C. CCCAGG C CCCAGG C. CCCAGG C CCCAGG C CCCAGG C CCCAGG CC	TT. AGGGAANTTAK ACGATTATTAK TTACTACGG TTACTACGG TTACTACGG CCTACTATGG CCTACTATGG CCTACTATGG ATTACTACGG TTACTACGG TTACTACGG TTACTACGG TTACTACGG	CTACGGTCGGT TACGGTCGGT TACGGTAGTA TGGTAGTACTACT TGTAGTACTACT TGTAGTACTACT TGTAGTACTTTG TGGTAGTTTG TGGTAGTTTG TAGTAGCTTTG TAGTAGCTTTG	CTTCGATG JGTACGACT TGACTACT TGACTACT TGACTACT TGACTACT GCTACTGG CTACTGG CTACTGG CTACTGG CTACTGG CTACTGG CTACTGG CTACTGG	rergg Kergg 3G 3G		
GCAGCTC A	250 ************************************	260 	270 270 36ACTCTGC6	280 07CTATTAT	TT T T T T T T T T T T T T T T T T T T	.TT. AGGGAAATTAK ACGATTATTATTA THTACTACGG ATTACTACGG ATTACTACGG ATTACTACGG ATTACTACGG ATTACTACGG ATTACTACGG ATTACTACGG ATTACTACGG ATTACTACGG ATTACTACGG ATTACTACGG ATTACTACGG	G.A. G. C TACGGTCGGT TACGGTAGTA TACGGTAGTA TACGGTAGTA TACAGTAGTACT TATCCACTTG TACTACTTG CGTAGCTTG CGTAGCTTG TACTACTTG TACTACTTG	G. CTTCGATG GTACGACT TTGACTACT TTGACTACT CTACTGG CTACTGG CTACTGG CTACTGG CTACTGG CTACTGG CTACTGG CTACTGG CTACTGG CTACTGG CTACTGG CTACTGG CTACTGG	rergg Kergg 3G 3G		
GCAGCTC A.	250 		270 270 36ACTCTGCG	280 280 GTCTATTAT	TT T TA T G G TT T C C C C C C C C C C C C C C C C C	TT. AGGGAANTTAK ATACTACGG7 ATTACTACGG7 ATTACTACGG ATTATTACTACGG GCTACTATGG GCTACTATGG GCTACTATGG ATTACTACGG7 ATTACGG7 ATTACTACGG7 ATTAC	CTACGGTCGGT TACGGTCGGT TACGGTAGTA TTACGGTAGTA TTACAGTAGTAGTAGTAGTAGTAGTAGTAGCACTTG AGTAGCTTG TACTAGTTTG TACTAGTTTG TACTAGTTTG TACTAGTTTG	G. COTTOGATG CACTACACT TTGACTACT TTGACTACT TTGACTACT CTACTGG CCTACTGG CCTACTGG CCTACTGG CCTACTGG CCTACTGG CCTACTGG CCTACTGG CCTACTGG CCTACTGG	rergg Kergg 3G 3G		
GCAGCTC A.	250 + - - - - - - - - - - - - - - - - - -	260 260	270 270 270 264 277 264 277 270 264 270 270 270 270 270 270 270 270 270 270	280 GTCTATTAT	TT T. T	AGGGAAATTAM AGGGAAATTAM ACGATTATTATTA THTACTACGG ATTACTACGG ATTACTACGG GCTACTATGG GCTACTATGG GCTACTATGG GCTACTATGG ATTACTACGG ATTACTACGG ACTACTATGG ACTACTATGG ACTACTATGG	G.A.G.C TACGGTCGGT TACGGTAGTA TACGGTAGTA TATAGGTAGT TATAGGTAGT TATAGCTACT TATAGCTACT TAGTAGCTACT TAGTAGCTTTG TAGTAGCTTTG TAGTAGCTTTG TAGTAGCTTTG TACTACTTTG CACTACTTTG CACTACTTTG	G. CTTCGATG GTTCGACTACT TTGACTACT TTGACTACT CTACTGG CTACTGG CTACTGG CTACTGG CTACTGG CTACTGG CTACTGG CTACTGG CTACTGG CTACTGG CTACTGG CTACTGG CTACTGG	rergg Kergg 3G 3G		
GCAGCTC A.	250 DAGCAGCCT: 	260 260	270 270 30ACTCT0CC	280 2207ATTAT A.	. TT. T	.TT. AGGGAAATTAK ACGATTATTA ATTACTACGA ATTACTACGA GUACTATGG GUACTATGG GUACTATGG GUACTATGG GUACTATGG ATTACTACGG ATTACTACGG ACTACTATGG ACTACTATGG ACTACTATGG ACTACTATGG	CTACGGTCGGT TACGGTCGGT TACGGTAGTA TAGTAGTACTACT TATAGTACTACT TATAGTACTACT TGTAGTACTACT TGTAGTACTTTG TAGTAGCTTTG TAGTAGCTTTG TACTACTTTG TACTACTTTG TCACTACTTTG TCACTACTTTG	G	rergg Kergg 3G 3G		
.c	250 CAGCAGCCT T	260 260 	270 270 304557666	280 277777777777777777777777777777777777	TT. T. TA. T. C. C. C. C. C. C. C. C. C. C	AGGGAAATTAK AGGGAAATTAK ACGATTATTA THACTACGG THTACTACGG THTACTACGG GCTACTATGG GCTACTATGG GCTACTATGG GCTACTATGG ACTACTACGG ACTACTATGG ACTACTACGG ACTACTATGG ACTACTATGG ACTACTATGG	CTACGGTCGGT TACGGTCGGT TACGGTAGTA GGTAGTAGTAGTAG TGGTAGTAGTAGTAG TTCCACTTG AGTAGCTTG TACTAGCTTG TACTACTTTG TACTACTTTG TACTACTTTG CACTACTTTG CACTACTTTG CACTACTTTG	G.	rergg Kergg 3G 3G		

Figure 1C



Figures 1C and 1D: Nucleotide sequences of VH1-72 heavy chains of anti-NP monoclonal antibodies obtained from wild type and TACI-deficient mice. Shown are independent VH sequences compared to the germline VH1-72 (indicated above each group, 1C, TACI-ko, 1D, wt). CDR1, CDR2 and CDR3, defined according to Kabat are shadowed. Sequences shown correspond to the aminoacid sequences shown in figure 1A.

	10	20	30	40	50	60	70	80	90	100	110	120
	+	+	+	+	+	+	+	+	+	+	+	+
			TACAACTAGTA									
			A.									
			A									
			A									
			T									
			c									
0			c		3						T.T	
			c									
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			.т С									
7												
	130	140	150	160	170 T.	180	190	200	210	G	230	
	-+	140		-+	+	+	+	+	+	220	+	
CCTGCCAG	ATTCTCAGGC	140 +	150	TGCCCTCAC	CATCACAGGG	GCACAGACTG	AGGATGAGGC	AATATATTC	TGTGCTCTATO	220	CCATT	GTTCGGTGGAGGA
CCTGCCAG	+ FATTCTCAGGC	140 + TCCCTGATI	150 	TGCCCTCAC	CATCACAGGG	GCACAGACTG	AGGATGAGGC	аатататттс	TGTGCTCTATO	220 + GTACAGCAA	CCATT C. GGGT C. GGGT	GTTCGGTGGAGGA GTTCGGTGGAGGA
CCTGCCAG		140 	150 H GGAGACAAGGO	TGCCCTCAC	LATCACAGGG	GCACAGACTG	AGGATGAGGC	ATATATTTC	TGTGCTCTATC	220 + GTACAGCAA	CCATT C. GGGT C. GGGT	
CCFGCCAG	-+ SATTCTCAGGC	140 + TCCCTGATI	150 	TGCCCTCAC	CATCACAGGG	GCACAGACTG	AGGATGAGGC	алтататтс	TGTGCTCTATC	220 + GTACAGCAA	CCATT C. GGGT C. GGGT C. GGGT GGGT	GTTCGGTGGAGGAJ GTTCGGTGGAGGAJ GTTCGGTGGAGGAJ
CEFGCCAG	ATTCTCAGGC	140 TCCCTGATI	150 	TGCCCTCAC	CATCACAGGG	GCACAGACTG	AGGATGAGGC	аатататттс	TGTGCTCTATO	220 + HGTACAGCAAC	CCATT C. GGGT C. GGGT C. GGGT GGGT	GTTCGGTGGAGGAA GTTCGGTGGAGGAA GTTCGGTGGAGGAA GTTCGGTGGAGGAA
CCEGCCAG	T.	140 ++ TCCCTGATI	150 	TGCCCTCAC	+ CATCACAGGG	-+ GCACAGACTG	AGGATGAGGC			220 + GGTACAGCAAC	CCATT C. GGGT C. GGGT C. GGGT GGGT GGGT	GTTCGGTGGAGGAA GTTCGGTGGAGGAA GTTCGGTGGAGGAA GTTCGGTGGAGGAA GTTCGGTGGAGGAA
CCTGCCAG	T	140 _+ TCCCTGATT	150 	TGCCCTCAC		-+ GCACAGACTG	AGGATGAGGC	AATATATTTC		220 + GGTA CAGCAAO 	CCATT C. GGGT C. GGGT GGGT GGGT GGGT C. GGGT	GTTCGGTGGAGGAJ GTTCGGTGGAGGAJ GTTCGGTGGAGGAJ GTTCGGTGGAGGAJ GTTCGGTGGAGGAJ GTTCGGTGGAGGAJ
CCTGCCAG	T	140 -+ TCCCTGAT	150 HIGGAGACAAGGO	TGCCTCAC	-+ CATCACAGGG	-+ SCACAGACTG	AGGATGAGGC	AATATATTTC		220 + GTACAGCAAC 	CCATT C. GGGT C. GGGT C. GGGT GGGT C. GGGT C. GGGT C. GGGT	GTTCGGTGGAGGAA GTTCGGTGGAGGAA GTTCGGTGGAGGAA GTTCGGTGGAGGAA GTTCGGTGGAGGAA GTTCGGTGGAGGAA GTTCGGTGGAGGAA
CCTGCCAG	T.	140 	150 	TGCCTCAC	+ CATCACAGGG	-+ SCACAGACTG	AGGATGAGGC	G		220 + GGTACAGCAAO T. T. T. C.	CCATT C. GGGT C. GGGT GGGT GGGT C. GGGT C. GGGT C. GGGT C. GGGT	GTTCGGTGGAGGAJ GTTCGGTGGAGGAJ GTTCGGTGGAGGAJ GTTCGGTGGAGGAJ GTTCGGTGGAGGAJ GTTCGGTGGAGGAJ NTTCGGNGGAGGAJ
CCTGCCAG	artcrcagge	140 TCCCTGATI	150 	TGCCCTCAC	CATCACAGGG	GCACAGACTG	AGGATGAGGC	G	TGTGCTCTATO 	220 ++ #GTACAGCAAG 	CCATT C. GGGT C. GGGT C. GGGT C. GGGT C. GGGT C. GGGT C. GGGT C. GGGT C. GGGT	GTTCGGTGGAGGAJ GTTCGGTGGAGGA GTTCGGTGGAGGA GTTCGGTGGAGGAJ GTTCGGTGGAGGAJ GTTCGGTGGAGGAJ MTTCGGTGGAGGAJ GTTCGGTGGAGGAGAJ
CCTGCCAG	ATTCTCAGGC	140 TCCCTGATT	150 	PGCCTCAC	CATCACAGGG	GCACAGACTG	AGGATGAGGC	аатататттс 	TGTGCTCTATC	220 ++ 	CCATT C. GGGT 	GTTCGGTGGAGGAJ GTTCGGTGGAGGAJ GTTCGGTGGAGGAJ GTTCGGTGGAGGAJ GTTCGGTGGAGGAJ GTTCGGTGGAGGAJ NTTCGGNGGAGGAJ
CCTGCCAG	.T.	140 TCCCTGATT	150 NGGAGACAAGGG	TGCCCTCAC	CATCACAGGG	SCACAGACTG	AGGATGAGGC		TOTO TANK	220 # # # # # # # # # # # # # # # # # #	CCATT CCATT CC. GGGT CC. GGGT CC. GGGT CC. GGGT CC. GGGT CC. GGGT CC. GGGT	GTTCGGTGGAGGAJ GTTCGGTGGAGGA GTTCGGTGGAGGA GTTCGGTGGAGGAJ GTTCGGTGGAGGAJ GTTCGGTGGAGGAJ MTTCGGTGGAGGAJ GTTCGGTGGAGGAGAJ
CEFGCCAG	.T.	140 + TCCCTGATT	150 GGAGACAAGGO	TGCCCTCAC	CATCACAGGG	GCACAGACTG			TOTGCTCTAT	220 ++ HGTACAGCAAG T. T. T. T. C.	CCATT CCATT CC. GGGT CC. GGGT CC. GGGT CC. GGGT CC. GGGT CC. GGGT CC. GGGT	GTTCGGTGGAGGAJ GTTCGGTGGAGGA GTTCGGTGGAGGA GTTCGGTGGAGGAJ GTTCGGTGGAGGAJ GTTCGGTGGAGGAJ MTTCGGTGGAGGAJ GTTCGGTGGAGGAGAJ
CCEGCCAG	.T.	140 	150 ISGAGACAAGG	TECCETCAC	ATCACAGGG	GACAGACTG	AGGATGAGGC		TOTOCTATION	220 	CCATT CCATT CCATT CCAGGT CCAGGT CCAGGT CCAGGT CCAGGT CCAGGT CCAGGT	GTTCGGTGGAGGAJ GTTCGGTGGAGGAJ GTTCGGTGGAGGAJ GTTCGGTGGAGGAG GTTCGGTGGAGGAG GTTCGGTGGAGGAJ GTTCGGTGGAGGAGJ GTTCGGTGGAGGAJ
CCPGCCAG	.T.	140	150 GGAGACAAGG	TGCCCTCAC	CATCACAGGG	T	асбатбаббс т. т. т.		TOTGCICTAI	220 ++ KGTACAGCAAG T. T. T. C. C.		GTTCGGTGGAGGAJ GTTCGGTGGAGGA GTTCGGTGGAGGA GTTCGGTGGAGGAJ GTTCGGTGGAGGAJ GTTCGGTGGAGGAJ MTTCGGTGGAGGAJ GTTCGGTGGAGGAGAJ
CCPGCCAG		140 100 100 100 100 100 100 100 100 100	150 PGGAGACAAGGC	TGCCCTCAC	CATCACAGGG	T.	асбатбаббс т.		TOTGCICTAT	220 	CCATT CCATT C. GGGT C. GGGT	GTTCGGTGGAGGAI GTTCGGTGGAGGAI GTTCGGTGGAGGAG GTTCGGTGGAGGAI GTTCGGTGGAGGAI GTTCGGTGGAGGAG GTTCGGTGGAGGAG GTTCGGTGGAGGAG GTTCGGTGGAGGAG GTTCGGTGGAGGAG
CCPGCCAG	T.	140	150 Peckgacaage	TGCCCTCAC	TATCACAGGG		C. T.		T	220 GTACAGCAAC T	CCATT 	errceregances, errceres, errceres, errceres, errceres, errceres, errceres, errceres, errceres, errceres, erre
CCPGCCAG	.T.	140	150 PGGAGACAAGGC	.TG.	CATCACAGG	5CACAGACTG	C. T.		T. T	220 GTACAGCAAC T	CCATT 	etrcogregadea etrcogregadea etrcogregadea etrcogregadea etrcogregadea etrcogregadea etrcogregadea etrcogregadea etrcogregadea etrcogregadea etrcogregadea
CCPGCCAG	T.	140	150 PGAGACAAGGC	.TG.	CATCACAGOG	SCACAGACEG	AGGATGAGGC			220 	CATT C. GGT C. GGGT C. GGGT	errceregances, erreceregances, erreceres, er
CERGECEAG	.T.	140	150 Regagacaaged	-t- TRECCITCAC	CATCACAGOG	SCACAGACYG			T. C. T. C. C.	220 3077ACAGCAAC 	CCATT C. GGGT C. GGGT	errceregances, errceres, errceres, errceres, errceres, errceres, errceres, errceres, errceres, errceres, erre
CEPGCCAG	T.	140	150	.TG.	CATCACAGOG	SCRCAGACYG	асалтолосси т. т. т.			220 3077ACAGCAAC 	CATT 	ertcoregances, ertcor
CERGCCAG		140	150 Regagacaage	- FG.	CATCACAGOG	SCACAGACYG			T. C. C.	220 3677ACAGCAA T T C. C	CATT C. GGGT C. GGGT	errceregances, erreceregances, erreceres, er
CEPGCCAG		140	150	- FG.	CATCACAGOG	SCACAGACYG			T. C. C.	220 3077ACAGCAAC 	CATT C. GGGT C. GGGT	ertcoregances, ertcor
CERGCCAG		140	150 Regagacaage	. PG.	CATCACAGOG	SCACAGACYG			T. C. C.	220 3677ACAGCAA T T C. C	CCATT CCATT C. GGGT C.	ertcoregances, ertcor

Figure 1E

10	20	30	40	50	60	70	80	90	100	110	120
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T6									T		
T77 T108											
T108											
T172											
T45											
T11	т	G						т			T
T19						cc.		т			T
T145	T		T					AT			T
T94											
T31C											
T52 T59											
T170											
T55											
T143											
T39											
T23						c.	T		G		
T65									G		
T318									G		
VL2				т					G		
130	140	150	160	170	180	190	200	210	220	230	
				+	+	+		+	+	+	
VL1 CCTGCCAGATTC	CAGGCTCCCTG	TTGGAGACAAG	GCTGCCCTCA	CCATCACAGG	GGCACAGAG	TGAGGATGAG	GCAATATATTT	TGTGCTCT	ATGGTACAGC	AACCATT	200808900000000000000000000000000000000
	CAGGCTCCCTG	+ ATTGGAGACAA	GGCTGCCCTCA	CCATCACAGG	GGCACAGAG	TGAGGATGAG	GCAATATATTT	+ CTGTGCTCT	TGGTACAGC	AACCATT	3GGTGTTCGGTGGAGGAACCAAA SGGTGTTCGGTGGAGGAACCAAA
VL1 CCTGCCAGATTC T6	+ CAGGCTCCCTG	+ ATTGGAGACAAG	GCTGCCCTCA	CCATCACAGG	GGCACAGAC	+ TGAGGATGAG	GCAATATATTT	+ CTGTGCTCT	+ ATGGTACAGC	AACCATT	366Tettcs6T66A66Aaccaaa 366Tettc66T66A66Aaccaaa 366Tettc65T66A66Aaccaaa
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VLI CCTGCCAGATTC T6 T77 T108 T17 T45 T172 T11 T11 T19 T19	CAGGCTCCCTGA	ITTGGAGACAA(5GCTGCCCTCA TG. TG. TG.	CCATCACAGG	GGCACAGAC T T T 	TGAGGATGAG	GCARTATATTT	G.	TGGTACAGCI	AACCATT	3GGTGTTCGGTGGAGGAACCAAA 3GGTGTTCGGTGGAGGAACCAAA 3GGTGTTCGGTGGAGGAACCAAA 3GGTGTTCGGTGGAGGAACCAAA 3GGTGTTCGGTGGAGGAACCAAA
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VL1 CCTGCCAGATTC7 T6 T77 T108 T45 T17 T45 T11 T19 T18 T04 T04 T31 T144	CAGGCTCCCTG	TTGGAGACAA	GCTGCCCTCA TG TG TG TG	CCATCACAGG	GGCACAGAG 	TGAGGATGAG	GCARTATATT		ATGGTACAGCI	GC.	SUF OTTOGFTGAGAGAACCAAN SUF OTTOGFTGAGAGAACCAAN SUF OTTOGFTGAGAGAACCAAN SUF OTTOGFTGAGAGAACCAAN SUF OTTOGFTGAGAGAACCAAN SUF OTTOGFTGAGAGAACCAAN SUF OTTOGFTGAGAGAACCAAN SUF OTTOGFTGAGAGAACCAAN SUF OTTOGFTGAGAGAACCAAN
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VL1 CCTGCCAGATTC T6	CAGGOTCCCTG	ATTGGAGACAR(GGCTGCCCTCAA TQ. TG. TG. TG.	CCATCACAGG	GGCACAGAG 	TGAGGATGAG	GCARTATATT	.G. .N. .T.	ATGGTACAGC	GC.	SUF OTTOGFTGAGAGAACCAAN SUF OTTOGFTGAGAGAACCAAN SUF OTTOGFTGAGAGAACCAAN SUF OTTOGFTGAGAGAACCAAN SUF OTTOGFTGAGAGAACCAAN SUF OTTOGFTGAGAGAACCAAN SUF OTTOGFTGAGAGAACCAAN SUF OTTOGFTGAGAGAACCAAN SUF OTTOGFTGAGAGAACCAAN
VL1 CCTGCCAGATCS T6	CAGGCTCCCTG	ATTGGAGACARG	GGCTGCCCTCA TO TG. TG. TG.	CCATCACAGG	GGCACAGAG 	TGAGGATGAG	GCARTATATT	G	ATGGTACAGC	gC.	Sof of the Carles and the Carles and Carles
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VL1 CCTGCCAGATCS T6 T77 T108 T17 T45 T172 T11 T19 T165 T04 T31 T144 T52 T59 T170 T15 T170 T165 T170 T170 T185 T170 T185 T170 T185 T170 T185 T170 T185 T170 T185 T170 T185 T170 T185 T170 T185 T170 T185 T170 T185 T170 T185 T170 T185 T170 T185 T170 T185 T170 T185 T170 T185 T185 T170 T185 T170 T185 T185 T185 T170 T185	CAGGCTCCCTG	ATTGGAGACAAG	GGCTGCCCTCA TQ TG TG TG.	CCATCACAGG	GCACAGAC 	TGAGGATGAG	GCARTATATTY	G	ATGGTACAGC	g	SOF OTTCG/TGGAGAACCAAA SOF OTTCG/TGGAGAACCAAA SOF OTTCG/TGGAGAACCAAA SOF OTTCG/TGGAGAACCAAA SOF OTTCG/TGGAGGAACCAAA SOF OTTCG/TGGAGGAACCAAA SOF OTTCG/TGGAGGAACCAAA SOF OTTCG/TGGAGGAACCAAA SOF OTTCG/TGGAGGAACCAAA SOF OTTCG/TGGAGGAACCAAA SOF OTTCG/TGGAGGAACCAAA SOF OTTCG/TGGAGGAACCAAA SOF OTTCG/TGGAGGAACCAAA SOF OTTCG/TGGAGGAACCAAA
VL1 CCTGCCAGATCS T6 T77 T108 T17 T45 T17 T18 T19 T14 T52 T59 T170 T52 T31 T52 T170 T52 T23	CAGGCTCCCTG	ATTGGAGACAAG	GCTGCCCTCA TQ. TG. TG. TG.	CCATCACAGG	GCACAGAGA 	TGAGGA TGAG	GCARTATATTY	G.	ATGGTACAGC	BACCATT C.	SUF OFTCOFTCARAGARCCAAR SUF OFTCOFTCARAGARCCAAR
VL1 CCTGCCAGATTC7 T6 T77 T108 T17 T145 T172 T11 T13 T145 T144 T52 T59 T170 T144 T52 T170 T143 T39 T23 T T T T T T T	CAGGCTCCCTG	ATTGGAGACAAG	GGCTGCCTCAA TQ TG TG TG.	CCATCACAGG	BGCAAGAA 		GCARTATATTY	G.	ATGGTACAGC	G	SUF OTTCGFTGAGAGAACCAA SUF OTTCGTGAGAACCAAA SUF OTTCGTGAGAACCAAA SUF OTTCGFTGAGAGAACCAAA SUF OTTCGFTGAGAGAACCAAA SUF OTTCGFTGAGAGAACCAAA SUF OTTCGFTGAGAGAACCAAA SUF OTTCGFTGAGAGAACCAAA SUF OTTCGFTGAGAGAACCAAA SUF OTTCGFTGAGAGAACCAAA SUF OTTCGFTGAGAGAACCAAA SUF OTTCGFTGAGGAGAACCAAA SUF OTTCGFTGAGGAGAACCAAA SUF OTTCGFTGAGGAGAACCAAA SUF OTTCGFTGAGGAGAACCAAA SUF OTTCGFTGAGGAGAACCAAA
VL1 CCTGCCAGATCS T6 T77 T108 T17 T45 T17 T18 T19 T14 T52 T59 T170 T52 T31 T52 T170 T52 T23	CAGGCTCCCTG	ATTGGAGACAAG	GGCTGCCCTCA TQ. TG. TG. TG.	CCATCACAGG	56CACAGAG 		GCARTATATTY GCARTATATTY G. G. G.	G. G. N. T.	ATGGTACAGC	G	SUF OFTCOFTCARAGARCCAAR SUF OFTCOFTCARAGARCCAAR

Figure 1F

Figures 1E and 1F: Nucleotide sequences of Light chains of anti-NP monoclonal antibodies obtained from wild type and TACI-deficient mice. The VL sequences compared to the germline V (indicated above each group, 1E, TACI-ko, 1F, wt). CDR1, CDR2 and CDR3, defined according to Kabat are shadowed. Sequences shown correspond to the aminoacid sequences shown in figure 1B.

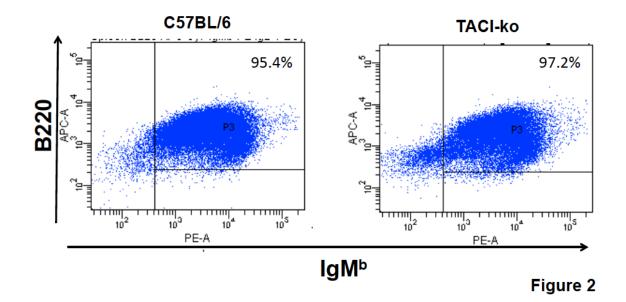


Figure 2: Flow Cytometer analysis of splenocytes obtained from wild type or TACIdeficient mice 10 days after re-infection with C. rodentium. Y-axis depicts staining with anti-B220 antibody; the X-axis depicts staining with anti-IgM^b antibody. Since IgM^b is the IgM allotype expressed by B cells of C57BL/6 mice, the figure indicates that B cells express the C57BL/6 IgM allotype.

Germinal center B cell clones from	C,	. rodentium infected mice
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C57BL/6

TACI-ko

Clone	lgH chain	Strain	Junction	Clone	lgH chain	Strain	Junction
BL6_E08	Musmus IGHV1-18*01 F	C57BL/6	CARITYNSREFPYW	CI D09	Musmus IGHV1-19*01 F	C57BL/6	CALGSSSFDYW
BL6_H07	Musmus IGHV1-18*01 F	C57BL/6	CARWRNGYFDVW	CI E02		C57BL/6	CARSFAYW
BL6_C02	Musmus IGHV1-31*01 F	C57BL/6	CARSITTAPFDYW	CI_A09	Musmus IGHV1-19*01 F	C57BL/6	CARDSSGYAYW
BL6_C07	Musmus IGHV1-54*01 F	C57BL/6	CARSFMITKAMDYW	CI_D01	Musmus IGHV1-19*01 F	C57BL/6	CATPDGYYGYFDVW
BL6_D03	Musmus IGHV1-64*01 F	C57BL/6	CARRGLYGSSPYYFDYW	CI_C01	Musmus IGHV1-20*01 F	C57BL/6	CARTGTTYFDYW
BL6_F03	Musmus IGHV1-7*01 F	C57BL/6	CARGDGYYPPYYAMDYW	/ CI_D04	Musmus IGHV1-39*01 F	C57BL/6	CARTLYYFDYW
BL6_G01	Musmus IGHV1-7*01 F	C57BL/6	CARGYYYGSSYGYFDVW	CI_H02	Musmus IGHV1-50*01 F	C57BL/6	CARYDGYLGYFDYW
BL6_H09	Musmus IGHV1-72*01 F	C57BL/6	CTRGFYDGFSSWFAYW	CI_H12	Musmus IGHV1-52*01 F	C57BL/6	CARWGLRRSYAMDYW
BL6_E02	Musmus IGHV1-81*01 F	C57BL/6	CARVYYAMDYW	CI_C12	Musmus IGHV1-52*01 F	C57BL/6	CARFHYWYFDVW
BL6_E06	Musmus IGHV1-81*01 F	C57BL/6	CAREDGSSSWFAYW	CI_E11	Musmus IGHV1-53*01 F	C57BL/6	CARGGVITTVVARYWYFDV
BL6_A06	Musmus IGHV1-81*01 F	C57BL/6	CAREGDYSFAYW	CI_H10	Musmus IGHV1-53*01 F	C57BL/6	CARTAQATWGVFDYW
BL6_B02	Musmus IGHV1-81*01 F	C57BL/6	CARWTAQATCYW	CI_F03	Musmus IGHV1-64*01 F	C57BL/6	CARGYGSIYWYFDVW
BL6_G11	Musmus IGHV1-81*01 F	C57BL/6	CARDSAGQAWFAYW	CI_F11	Musmus IGHV1-64*01 F	C57BL/6	CARKGLYYRNYVGVAYW
BL6_H08	Musmus IGHV1-82*01 F	C57BL/6	CARSGSSYYYYAMDYW	CI_E04	Musmus IGHV1-64*01 F	C57BL/6	CARRWLLRSDYW
BL6_H02	Musmus IGHV1-82*01 F	C57BL/6	CARSIYYGIYYAMDYW	CI_B02	Musmus IGHV1-69*01 F	C57BL/6	CARWNYDFDYW
BL6_B09	Musmus IGHV1-9*01	C57BL/6	CARCSNLYYFDYW	CI_C10	Musmus IGHV1-72*01 F	C57BL/6	CVRWGYFDVW
BL6_F09	Musmus IGHV1-9*01 F	C57BL/6	CARGSNLYYFDYW	CI_E05	Musmus IGHV1-80*01 F	C57BL/6	CARFLTTVVATDYYAMDYW
BL6_D09	Musmus IGHV1-9*01 F	C57BL/6	CARSTFDYW		Musmus IGHV1-81*01 F	C57BL/6	CTLDVPFAYW
BL6_E12	Musmus IGHV1-9*01 F	C57BL/6	CARRGGAYW		Musmus IGHV1-81*01 F	C57BL/6	CARYYYGSSFFDYW
BL6_C09	Musmus IGHV10-1*01 F	C57BL/6	CVRPSLYDGYLGGFAYW		Musmus IGHV1-85*01 F	C57BL/6	CARGGIYYGITGAMDYW
BL6 G09	Musmus IGHV10-3*02 F	C57BL/6	CVRHYGSYYYAMDYW		Musmus IGHV1-85*01 F	C57BL/6	CXXXGIYXGIXGAXXYX
BL6_B01	Musmus IGHV14-2*01 F	C57BL/6	CALYGYDRAWFAYW			C57BL/6	CVRGNAMDYW
BL6_H11	Musmus IGHV14-4*01 F	C57BL/6	CTTSTTVVASDYW		Musmus IGHV14-2*01 F	C57BL/6	CARYPEAYW
BL6 G02	Musmus IGHV3-6*01 F	C57BL/6	CARGRSGPFAYW		Musmus IGHV14-2*01 F	C57BL/6	CALEGGFAYW
BL6 H04	Musmus IGHV5-17*01 F	C57BL/6	CARSLIFSFAYW		Musmus IGHV2-2*01 F	C57BL/6	CARKGDGYYDAMDYW
-				IACI_B09	Musmus IGHV3-6*01 F	C57BL/6	CAREGNWEAMDYW
					Musmus IGHV3-6*01 F	C57BL/6	CAKGAARGFAYW
					Musmus IGHV5-17*01 F	C57BL/6	CAMGGYSAWFAYW
					Musmus IGHV5-17*01 F	C57BL/6	CARNWVDYW
					Musmus IGHV5-4*01 F	C57BL/6	CARNDYRCFYDAXDYXGQ
				TACI_B01	Musmus IGHV5-6*01 F	C57BL/6	CARLAYYSNYGWYFDVW
							Figure 3

Figure 3: Identification of VH genes and V(D)J junctions of B cell clones isolated from the spleen of mice infected with *C. rodentium* 10 days earlier. Figure shows that all VH genes isolated match best to C57BL/6 germline alleles. Germinal center B cells (CD19+, GL7+ and Fas+) were isolated by single cell sorting from wt or TACIdeficient mice. CDN, amplification and sequencing were done according to (1).

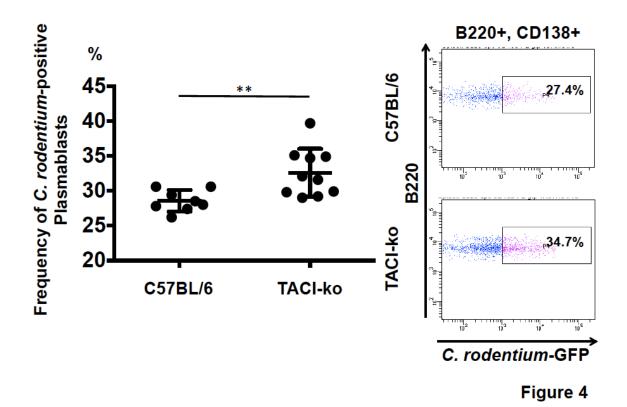


Figure 4: Identification of *C. rodentium* binding B cells. The figure shows flow cytometry analysis of splenocytes obtained from mice infected twice with *C. rodentium* with a month interval. Cells were analyzed 10 days after second infection. The left panel shows the frequency (Y-axis) of plasmablasts that bind to GFP-expressing *C. rodentium*. Panels on the right show representative dot plots (from N=10/strain) of splenocytes stained with anti-B220 antibody (Y-axis), anti-CD138 antibody and incubated with GFP-expressing *C. rodentium* (X-axis). Cells shown are both B220+ and CD138+ (plasmablasts). Rectangles depict the population that stains positively (above background) with the GFP-labeled *C. rodentium*.

Supplementary methods:

Primers for real-time PCR:

CTATTACGTGGATCGCAATGATGA(cIAP1-F),TCTCCAGGGCCAAAATGCACCACT(cIAP1-R);CGGGACATCTTGACGGAC(BCL6-F),CAGGGCTGATTTCAGGATCTA(BCL6-R);CATTCTGCCCCAAAGATCAGT (TACI-F), TGGTGCCTTCCTGAGTTGTCT (TACI-R);AGCCCATCACCATCTTCCAGGAG(GAPDH-F),CCTCTCGCCACAGCTTTCCA (GAPDH-R)

Primers for sequencing:

Forward Primers:

V1-72 (IMGT) (2, 3)) specific forward primer

(CTTGACCCAGATGTCCCTTCTTCTCCAGCAGG);

Universal forward primers (AGGTSMARCTGCAGSAGTCWGG),

(GGGAATTCGAGGTGCAGCTGCAGGAGTCTGG) and

(SARGTNMAGCTGSAGSAGTC);

Reverse primers:

J4 reverse primer (TTCCTTGACCCCAGTAGTCCA).

Nested PCR:

The nested PCR was done using the same forward primer and one of several reverse

primers located on J1-J2 intron (ATGGCCTGACATGGGGAGATCTG), J2-J3 intron

(GGGTCTAG AGGTGTCCCTAGTCCTTCATGACC) or J3-J4 intron (GGGTCTAG AGGTGTCCCTAGTCCTTCATGACC).

Amplification and sequencing of VH and VL exons from single sorted germinal center B cells isolated from mice infected with *C. rodentium* were done according to (1). Primers for amplifying and sequencing $\lambda 1$ and $\lambda 2$ light chain exons from hybridomas were according to (1)

References:

- 1. Tiller T, Busse CE, and Wardemann H. Cloning and expression of murine Ig genes from single B cells. *Journal of Immunological Methods*. 2009;350(1-2):183-93.
- 2. Giudicelli V, Chaume D, and Lefranc MP. IMGT/GENE-DB: a comprehensive database for human and mouse immunoglobulin and T cell receptor genes. *Nucleic Acids Res.* 2005;33(Database issue):D256-61.
- 3. Alamyar E, Giudicelli V, Li S, Duroux P, and Lefranc MP. IMGT/HighV-QUEST: the IMGT(R) web portal for immunoglobulin (IG) or antibody and T cell receptor (TR) analysis from NGS high throughput and deep sequencing. *Immunome Res.* 2012;8(1):26.