Supplementary Data

Supplementary Figures

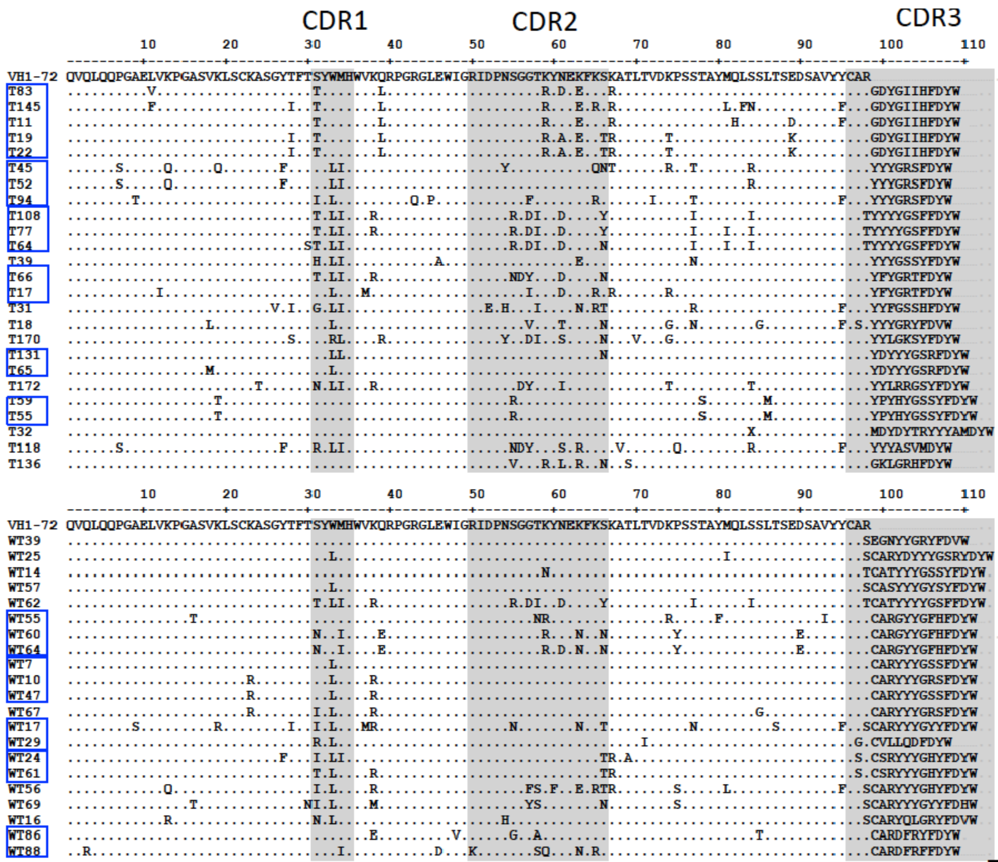

Figure 1A

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.

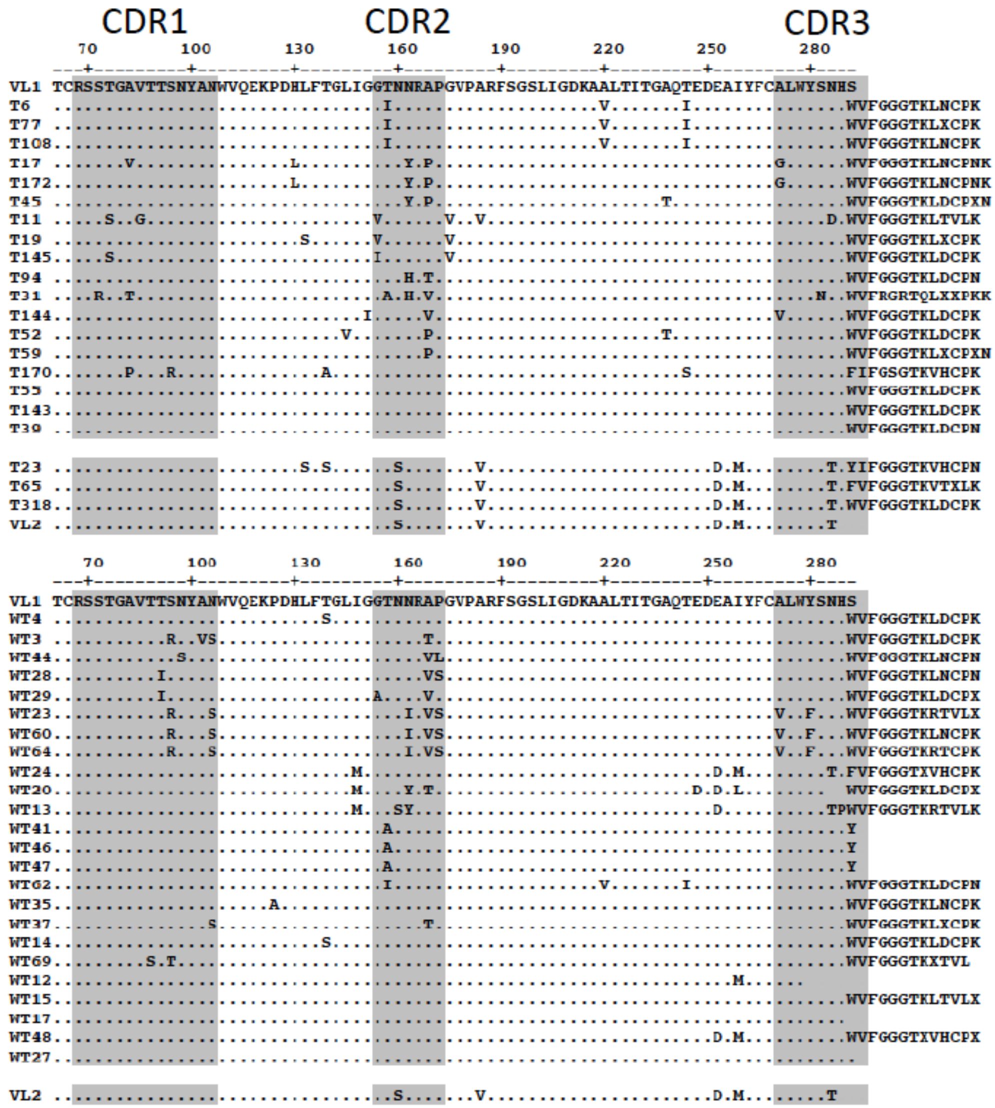

Figure 1B

Figure 1B: Localization of amino acid substitutions in distinct clones encoding Ig light chains of anti-NP monoclonal antibodies obtained from wild type and TACI-deficient mice. Shown are independent VL (Vλ1 or Vλ2) 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.

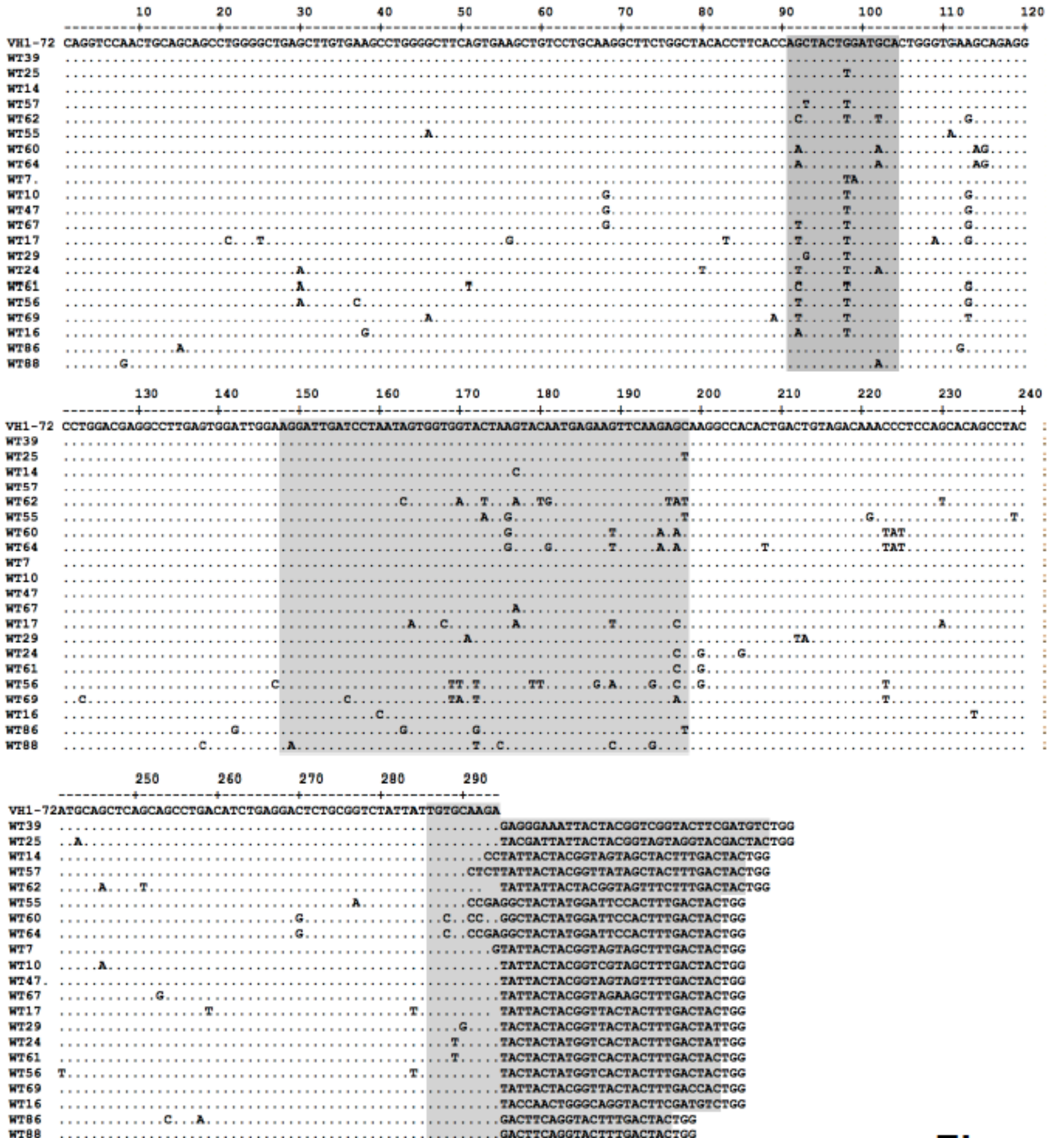

Figure 1C

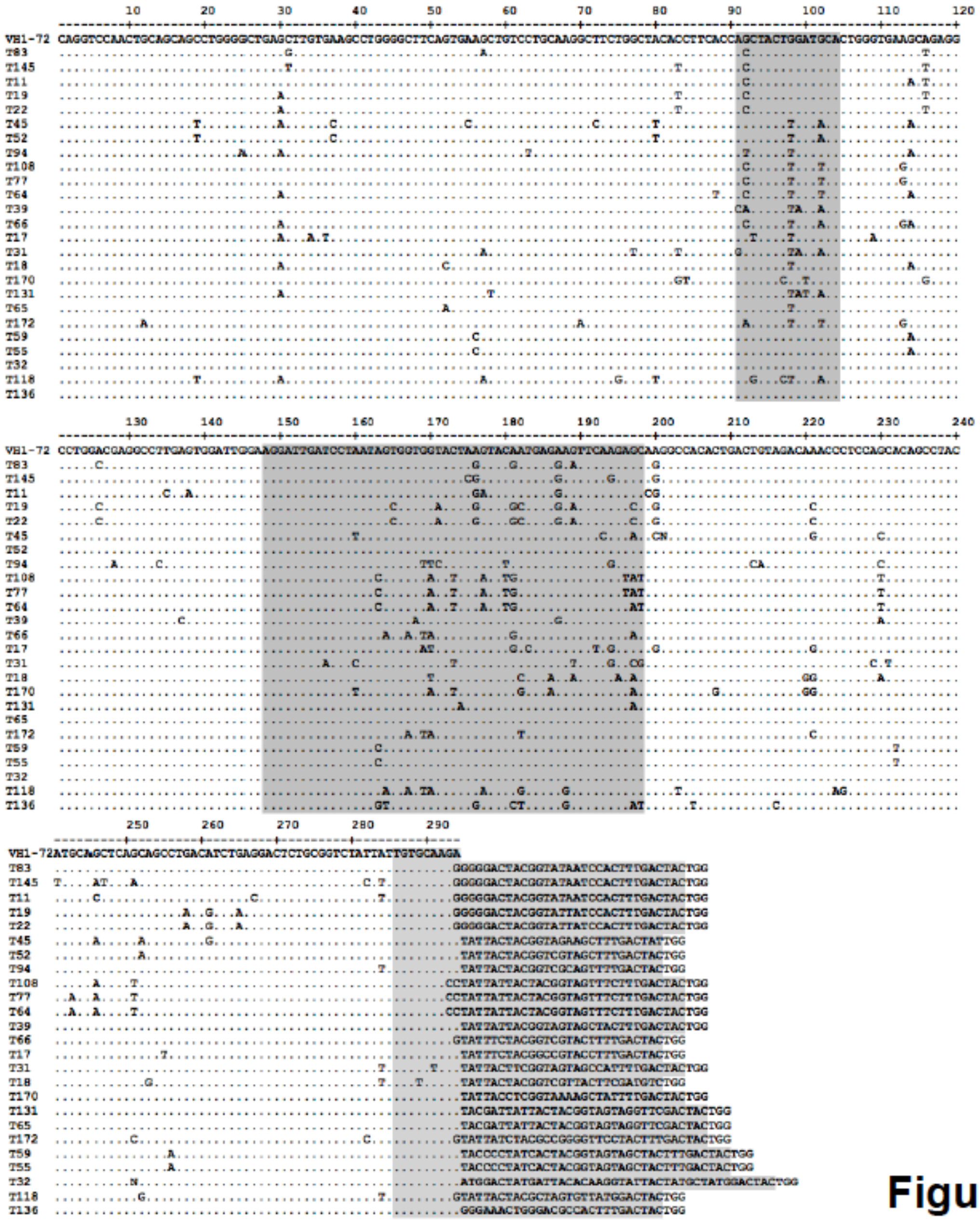

Figure 1D

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.

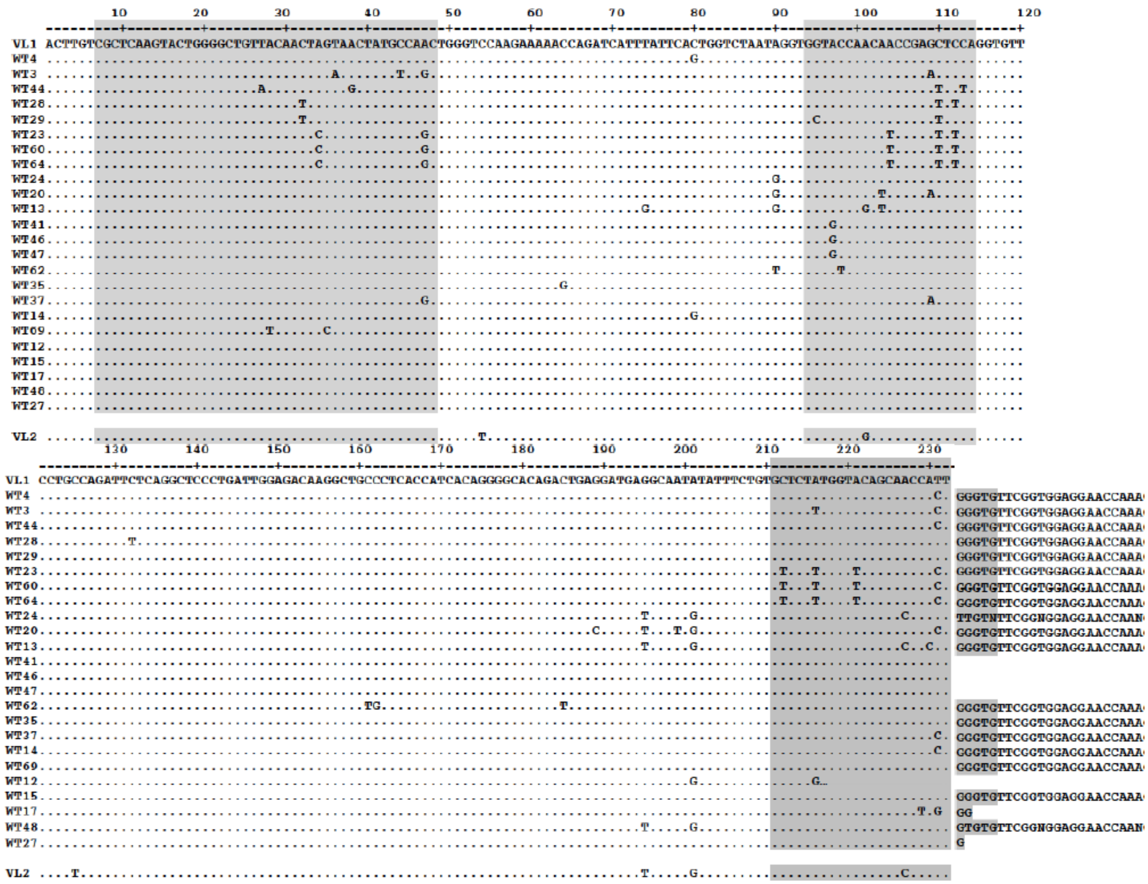

Figure 1E

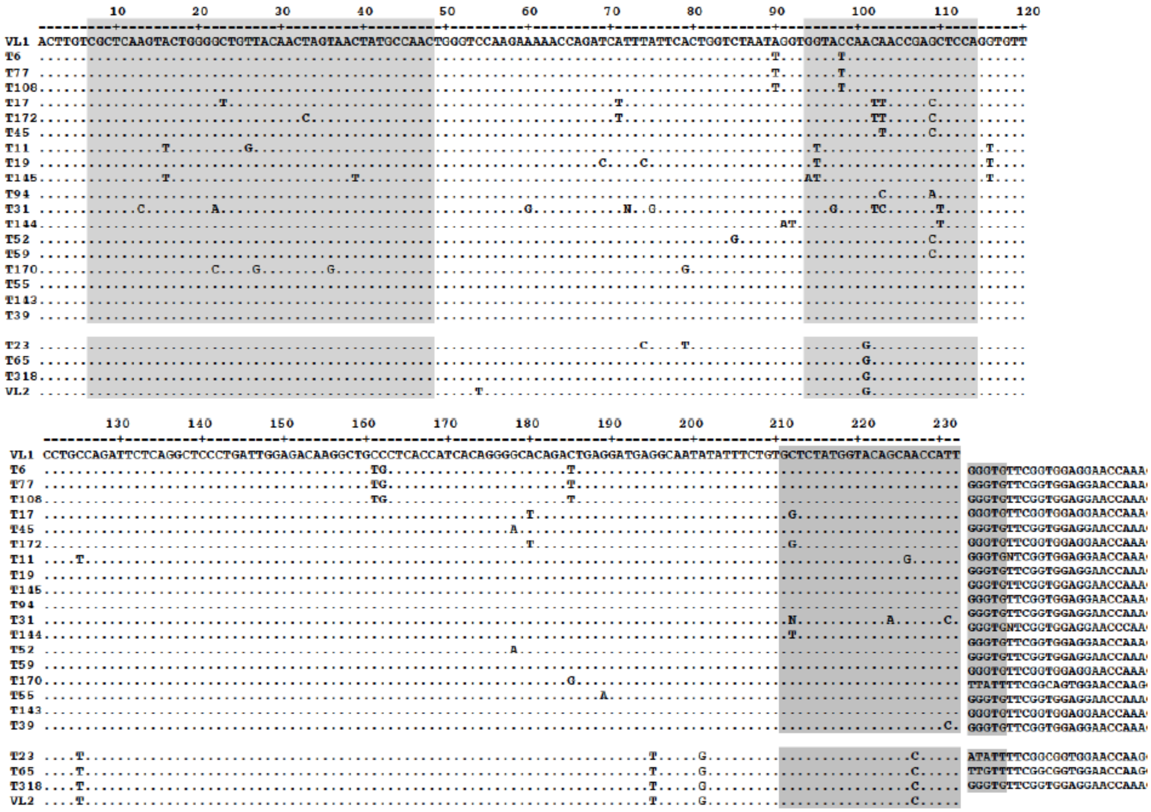

Figure 1F

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.

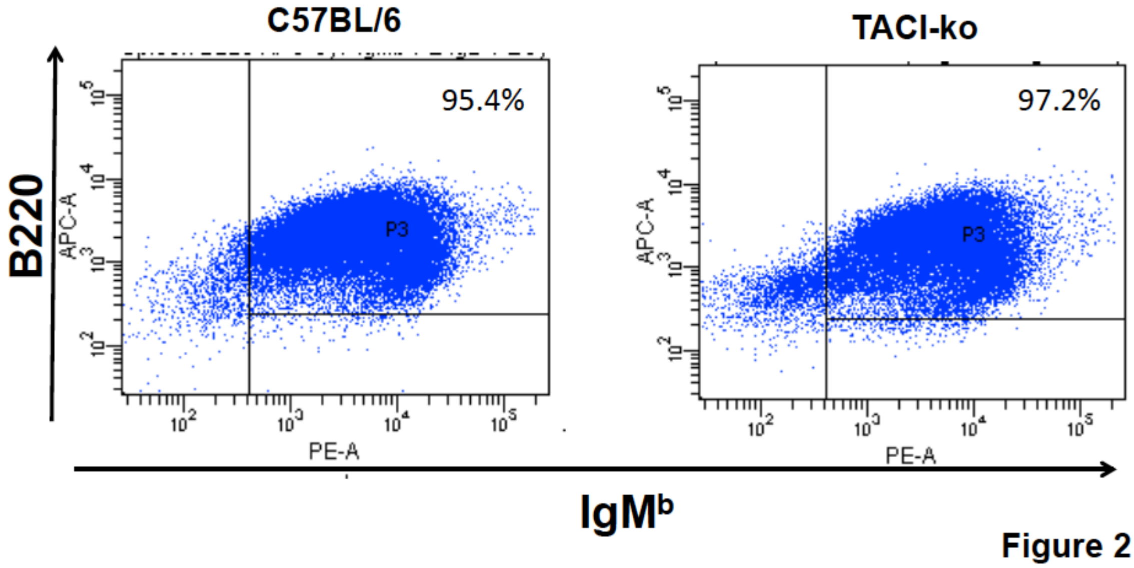

Figure 2: Flow Cytometer analysis of splenocytes obtained from wild type or TACI-deficient mice 10 days after re-infection with C. rodentium. Y-axis depicts staining with anti-B220 antibody; the X-axis depicts staining with anti-IgM^b antibody. Since IgM^b is the IgM allotype expressed by B cells of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

C57BL/6

Clone	IgH chain	Strain	Junction
BL6_E08	Musmus IGHV1-18*01 F	C57BL/6	CARITYNSREFPYW
BL6_H07	Musmus IGHV1-18*01 F	C57BL/6	CARWRNGYFDVW
BL6_C02	Musmus IGHV1-31*01 F	C57BL/6	CARSITTAPFDYW
BL6_C07	Musmus IGHV1-54*01 F	C57BL/6	CARSFMITKAMDYW
BL6_D03	Musmus IGHV1-64*01 F	C57BL/6	CARRGLYGSSPYYFDYW
BL6_F03	Musmus IGHV1-7*01 F	C57BL/6	CARGDGYYPPYYAMDYW
BL6_G01	Musmus IGHV1-7*01 F	C57BL/6	CARGYYYGSSYGYFDVW
BL6_H09	Musmus IGHV1-72*01 F	C57BL/6	CTRGFYDGFSSWFAYW
BL6_E02	Musmus IGHV1-81*01 F	C57BL/6	CARVYYAMDYW
BL6_E06	Musmus IGHV1-81*01 F	C57BL/6	CAREDGSSSWFAYW
BL6_A06	Musmus IGHV1-81*01 F	C57BL/6	CAREGDYSFAYW
BL6_B02	Musmus IGHV1-81*01 F	C57BL/6	CARWTAQATCYW
BL6_G11	Musmus IGHV1-81*01 F	C57BL/6	CARDSAGQAWFAYW
BL6_H08	Musmus IGHV1-82*01 F	C57BL/6	CARSGSSYYYYAMDYW
BL6_H02	Musmus IGHV1-82*01 F	C57BL/6	CARSIYYGIYYAMDYW
BL6_B09	Musmus IGHV1-9*01	C57BL/6	CARCSNLYYFDYW
BL6_F09	Musmus IGHV1-9*01 F	C57BL/6	CARGSNLYYFDYW
BL6_D09	Musmus IGHV1-9*01 F	C57BL/6	CARSTFDYW
BL6_E12	Musmus IGHV1-9*01 F	C57BL/6	CARRGGAYW
BL6_C09	Musmus IGHV10-1*01 F	C57BL/6	CVRPSLYDGYLGGFAYW
BL6_G09	Musmus IGHV10-3*02 F	C57BL/6	CVRHYGSYYYAMDYW
BL6_B01	Musmus IGHV14-2*01 F	C57BL/6	CALYGYDRAWFAYW
BL6_H11	Musmus IGHV14-4*01 F	C57BL/6	CTTSTTVVASDYW
BL6_G02	Musmus IGHV3-6*01 F	C57BL/6	CARGRSGPFAYW
BL6_H04	Musmus IGHV5-17*01 F	C57BL/6	CARSLIFSFAYW

TACI-ko

Clone	IgH chain	Strain	Junction
CI_D09	Musmus IGHV1-19*01 F	C57BL/6	CALGSSSFDYW
CI_E02	Musmus IGHV1-19*01 F	C57BL/6	CARSFAYW
CI_A09	Musmus IGHV1-19*01 F	C57BL/6	CARDSSGYAYW
CI_D01	Musmus IGHV1-19*01 F	C57BL/6	CATPDGYYGYFDVW
CI_C01	Musmus IGHV1-20*01 F	C57BL/6	CARTGTTYFDYW
CI_D04	Musmus IGHV1-39*01 F	C57BL/6	CARTLYYFDYW
CI_H02	Musmus IGHV1-50*01 F	C57BL/6	CARYDGYLGYFDYW
CI_H12	Musmus IGHV1-52*01 F	C57BL/6	CARWGLRRSYAMDYW
CI_C12	Musmus IGHV1-52*01 F	C57BL/6	CARFHYWYFDVW
CI_E11	Musmus IGHV1-53*01 F	C57BL/6	CARGGVITTVVARYWYFDV
CI_H10	Musmus IGHV1-53*01 F	C57BL/6	CARTAQATWGVFDYW
CI_F03	Musmus IGHV1-64*01 F	C57BL/6	CARGYGSIYWYFDVW
CI_F11	Musmus IGHV1-64*01 F	C57BL/6	CARKGLYYRNYVGVAYW
CI_E04	Musmus IGHV1-64*01 F	C57BL/6	CARRWLLRSDYW
CI_B02	Musmus IGHV1-69*01 F	C57BL/6	CARWNYDFDYW
CI_C10	Musmus IGHV1-72*01 F	C57BL/6	CVRWGYFDVW
CI_E05	Musmus IGHV1-80*01 F	C57BL/6	CARFLTTVVATDYYAMDYW
CI_A06	Musmus IGHV1-81*01 F	C57BL/6	CTLDVPFAYW
CI_D06	Musmus IGHV1-81*01 F	C57BL/6	CARYYYGSSFFDYW
CI_D02	Musmus IGHV1-85*01 F	C57BL/6	CARGGIYYGITGAMDYW
CI_G12	Musmus IGHV1-85*01 F	C57BL/6	CXXXGIYXGIXGAXXYX
CI_F07	Musmus IGHV10-3*01 F	C57BL/6	CVRGNAMDYW
CI_B12	Musmus IGHV14-2*01 F	C57BL/6	CARYPEAYW
CI_A04	Musmus IGHV14-2*01 F	C57BL/6	CALEGGFAYW
CI_C05	Musmus IGHV2-2*01 F	C57BL/6	CARKGDGYYDAMDYW
TACI_B09	Musmus IGHV3-6*01 F	C57BL/6	CAREGNWEAMDYW
TACI_H07	Musmus IGHV3-6*01 F	C57BL/6	CAKGAARGFAYW
TACI_G01	Musmus IGHV5-17*01 F	C57BL/6	CAMGGYSAWFAYW
TACI_G02	Musmus IGHV5-17*01 F	C57BL/6	CARNWVDYW
TACI_B10	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 TACI-deficient mice. CDN, amplification and sequencing were done according to (1).

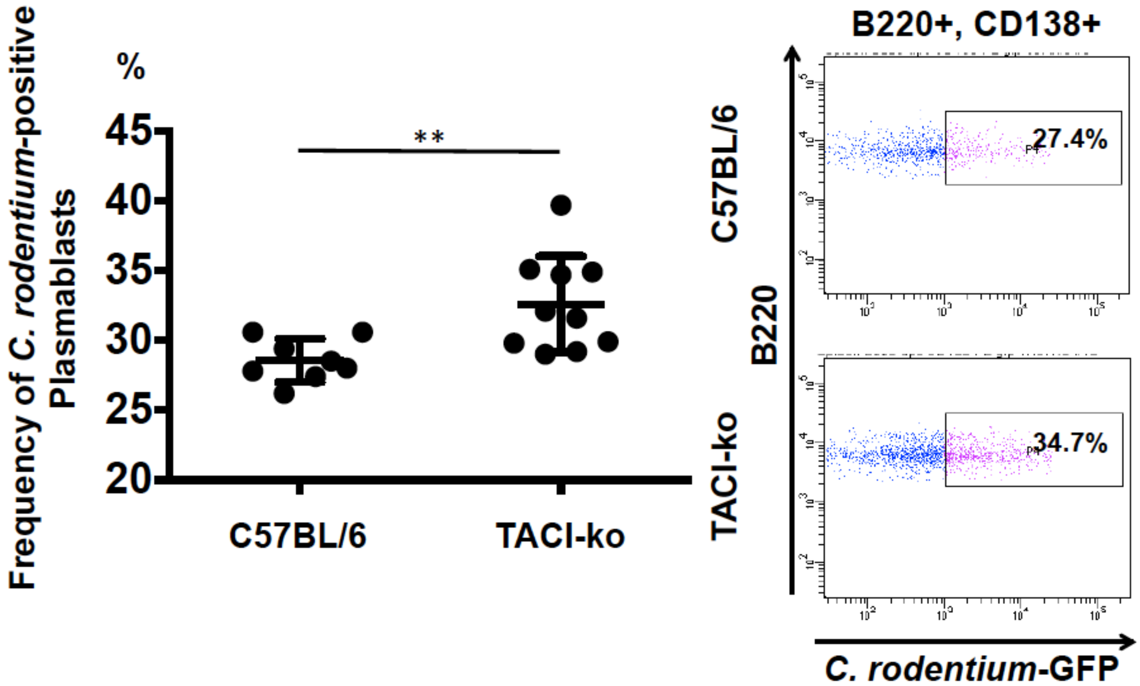

Figure 4

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 λ1 and λ2 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.