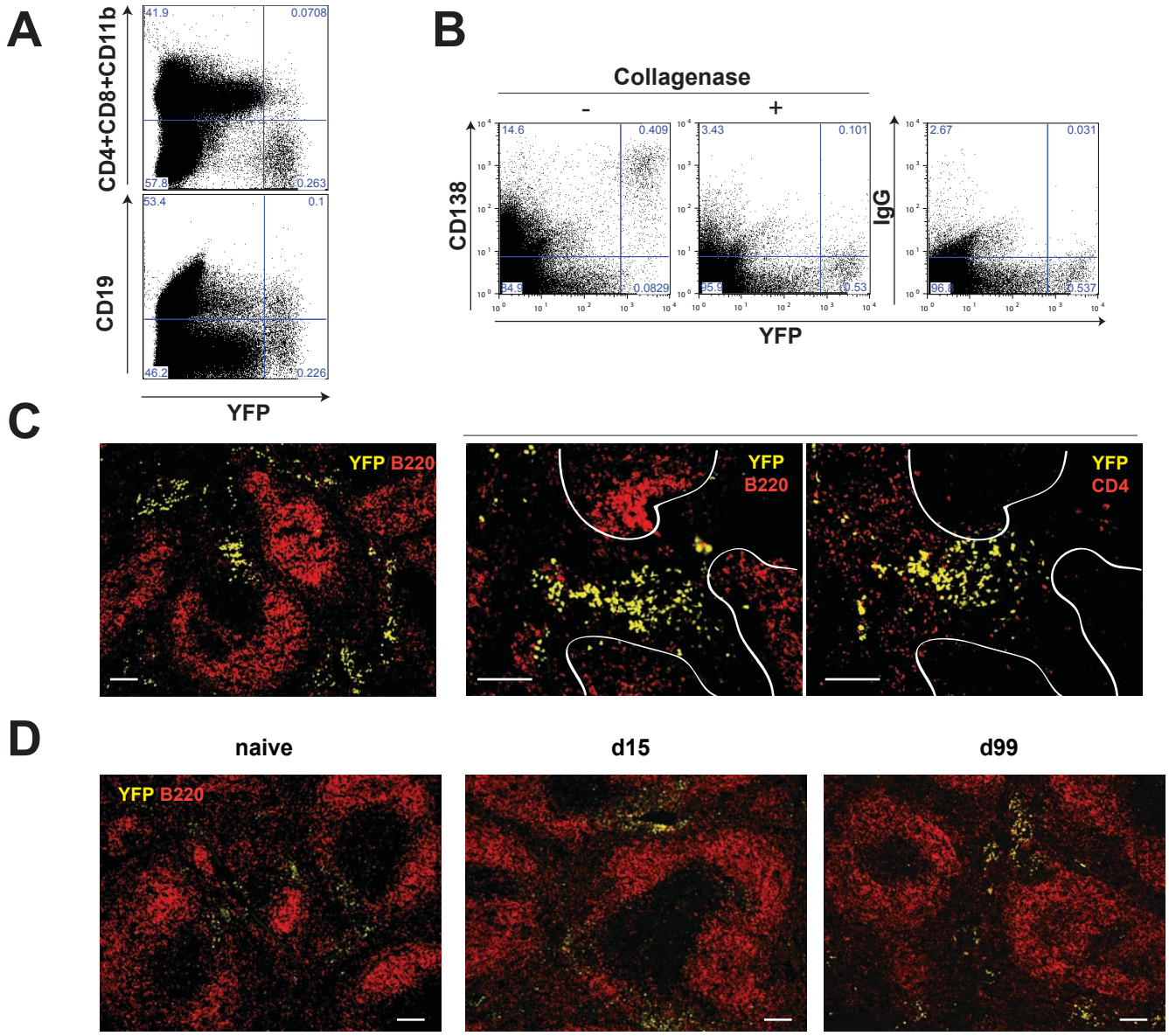
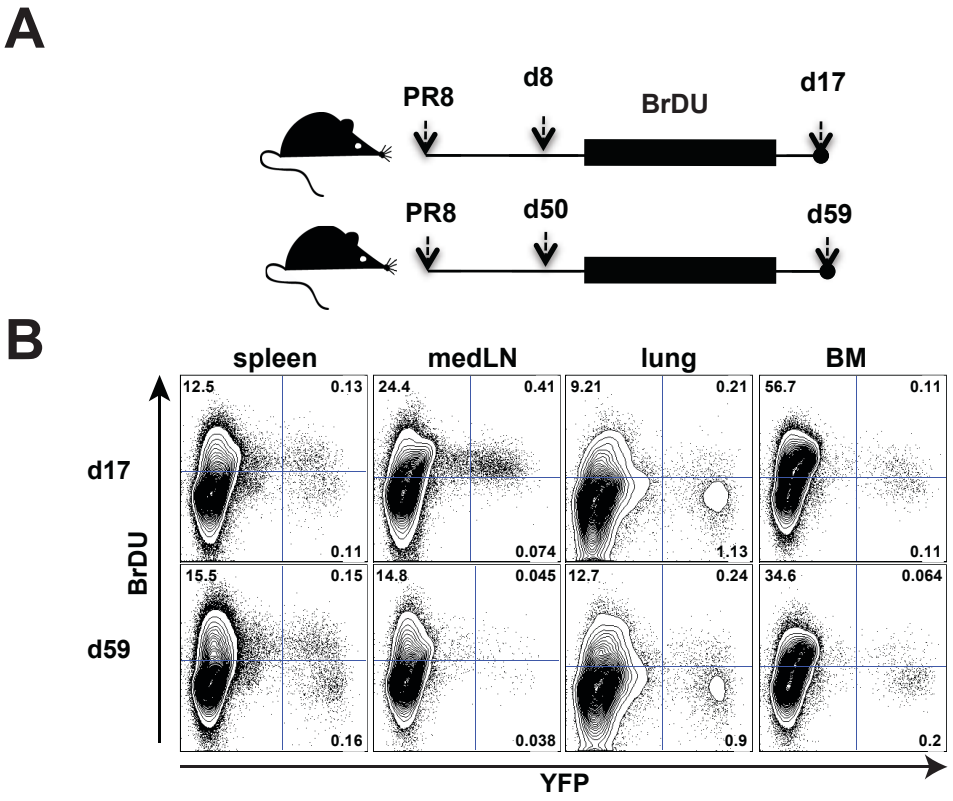


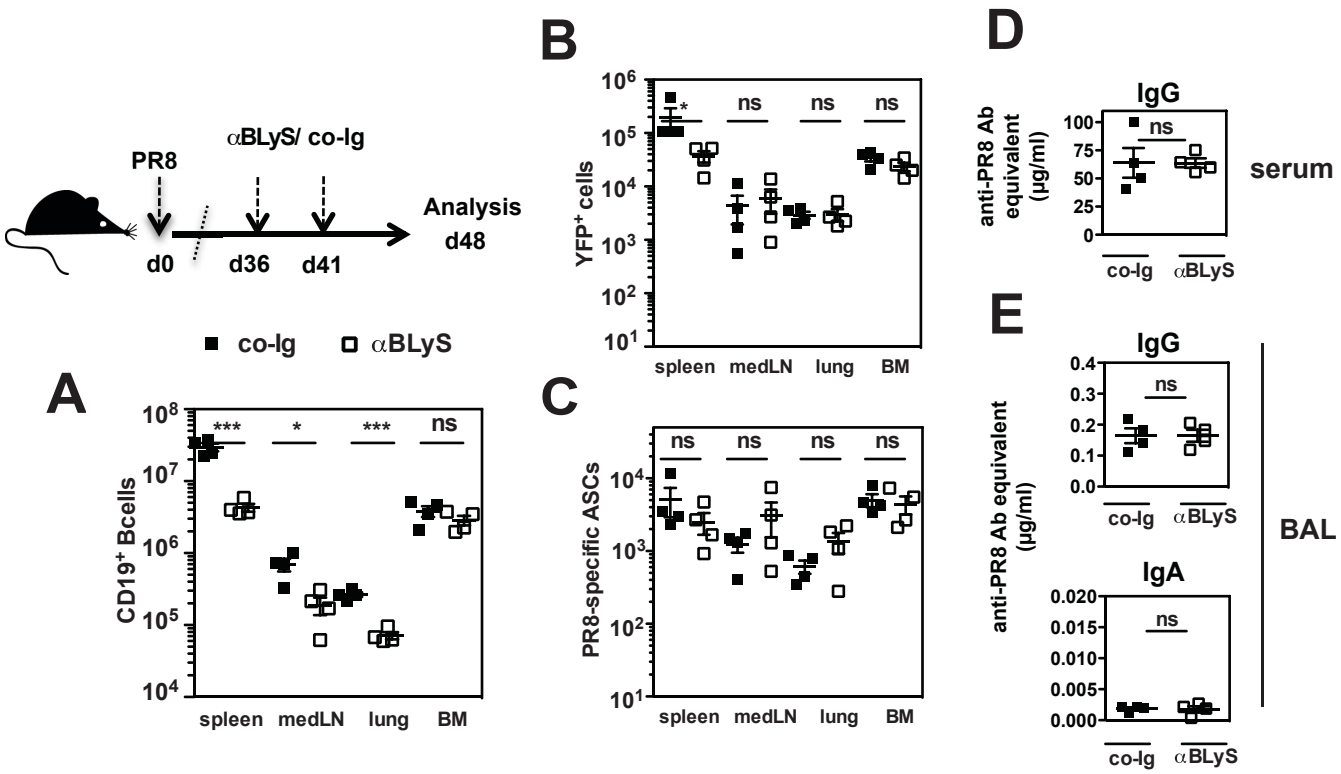
Supplemental Figure S1: YFP⁺ antibody secreting cells in BLIMP-1-YFP mice

(A) Splenocytes from BLIMP-1-YFP mice were stained with a combination of αCD4, αCD8, αCD11b and αCD19. YFP⁺ ASCs are negative for CD4, CD8, and CD11b and express CD19 to varying degrees. **(B)** Staining for CD138 on YFP⁺ ASCs is collagenase-sensitive. Splenocytes from BLIMP-1-YFP mice were digested with or without Collagenase D (400U) for 30 min at 37°C, stained with αCD138 or isoptype control Ab and analyzed by flow cytometry. **(C)** Serial sections from a spleen of a BLIMP-1-YFP mouse stained with αB220 and αCD4. Distribution of YFP⁺ cells in extrafollicular foci in the white pulp (10x magnification, left). Middle and Right: Same area in consecutive sections (20x magnification). White line was drawn to outline B220⁺ area. **(D)** Location of YFP⁺ ASCs in the spleen of naive and PR8-infected BLIMP-1-YFP mice. B220 staining is shown in red. Original magnification 10x. Scale bar: 100 μm.



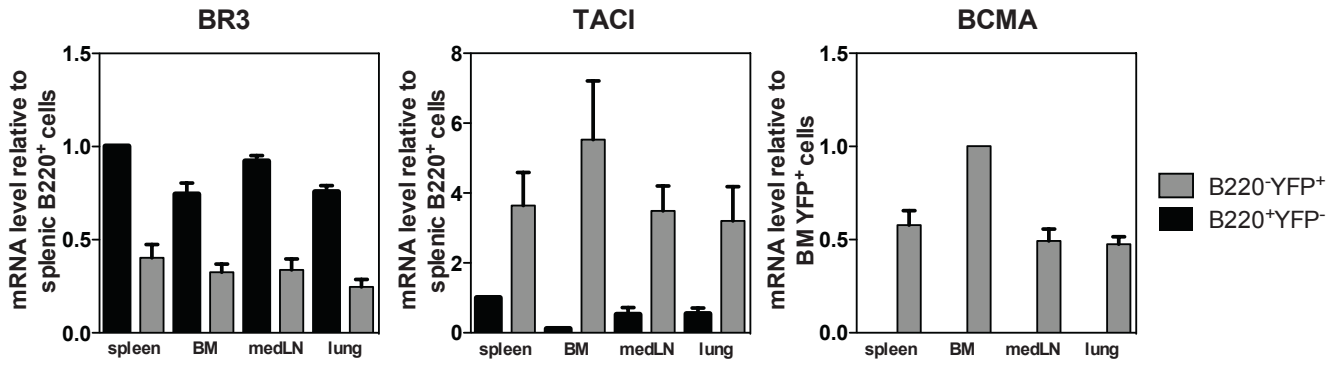
Supplemental Figure S2: Majority of lung ASCs do not proliferate.

(A and B) BLIMP-1-YFP mice received BrdU in their drinking water (0.8 mg/ml) for 8 days starting on day 8 or day 50 after infection with influenza virus PR8. 24h later, incorporation of BrdU was determined. **(B)** Representative flow cytometry dot plots of BrdU staining in $CD4^{-}CD8^{-}CD11b^{-}$ cells from spleen, medLN, lungs and BM from n=3 mice.



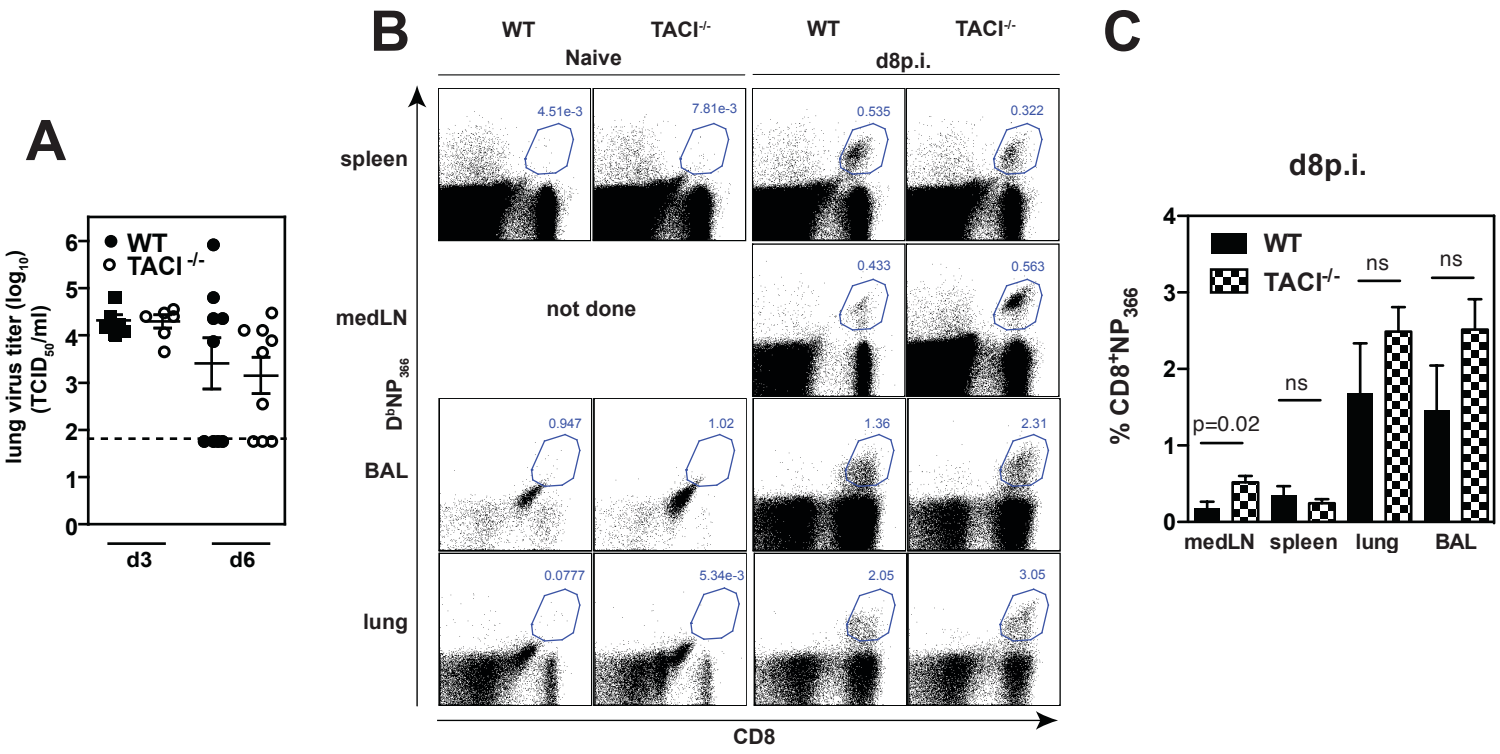
Supplemental Figure S3: Inhibition of BLyS in BLIMP-1-YFP mice one month after influenza virus infection does not reduce virus-specific antibody secreting cells.

(A-E) BLIMP-1-YFP mice (n=4/group) were treated with 100 μg αBLyS (10F4) or rat IgG1 control Ab (co-Ig) at days 36 and 41 after infection with influenza virus PR8 and analyzed at day 48 p.i. **(A)** Cells from spleen, medLN, lungs and BM were stained with αCD19 and enumerated. **(B)** YFP$^+$ ASCs were enumerated by flow cytometry and **(C)** PR8-specific ASCs were determined by ELISPOT. **(D)** PR8-specific IgG Ab titer in serum and **(E)** Ab titers of PR8-specific IgG and IgA in BAL were measured by ELISA. All data with mean±SEM represent one out of two independent experiments.



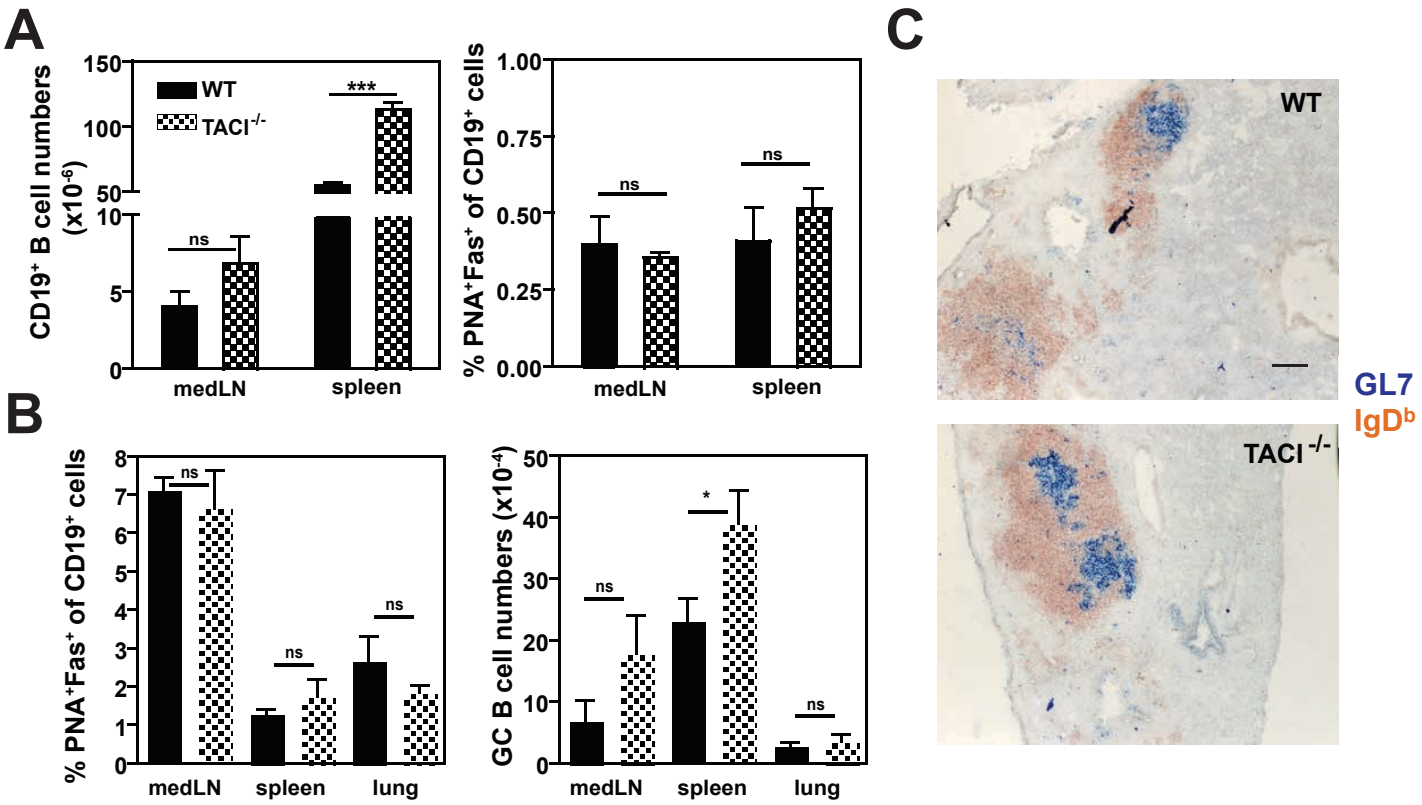
Supplemental Figure S4: Expression of TACI, BR3 and BCMA on YFP$^+$ ASCs in influenza virus-infected BLIMP-1-YFP mice

YFP$^+$ ASCs (grey) and B220$^+$ YFP$^-$ cells (black) were sorted from indicated organs of BLIMP-1-YFP mice 28 days after infection, RNA extracted and expression of BR3, TACI and BCMA determined by RT-PCR. Data with mean±SEM of two independent experiments.



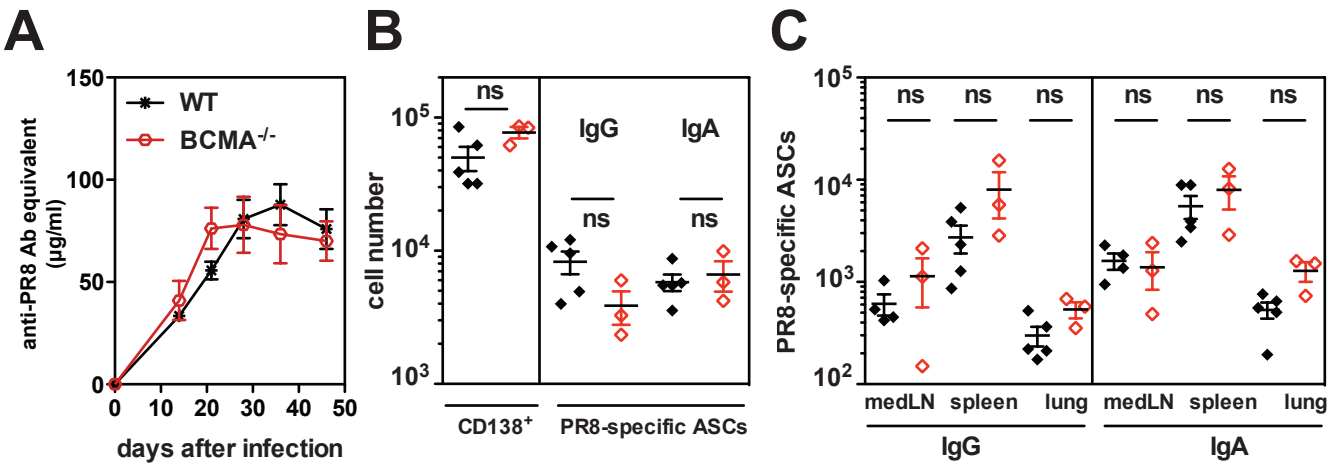
Supplemental Figure S5: Course of viral infection and influenza virus-specific CD8⁺ T cell response is similar in WT and TACI$^{-/-}$ mice.

(A) WT and TACI$^{-/-}$ mice were infected with influenza virus PR8. On day 3 and day 6 p.i., lungs from WT and TACI$^{-/-}$ mice (n=6-9 mice/group) were harvested and viral titers determined. Dotted line indicates the limit of detection of the assay. Data with mean±SEM of two independent experiments. **(C)** Cells in medLN, spleen, lungs and BAL from naive WT and TACI$^{-/-}$ mice or 8 days p.i. were stained for CD8 T cells and D^bNP_{366} tetramer and analyzed by flow cytometry. Representative dot plots of one out of two independent experiments are shown. **(C)** The percentage of CD8⁺ T cells positive for NP-tetramer staining at day 8 p.i. was quantified. Data shown are represented as mean±SEM from two combined independent experiments with n=4-6 mice/group.



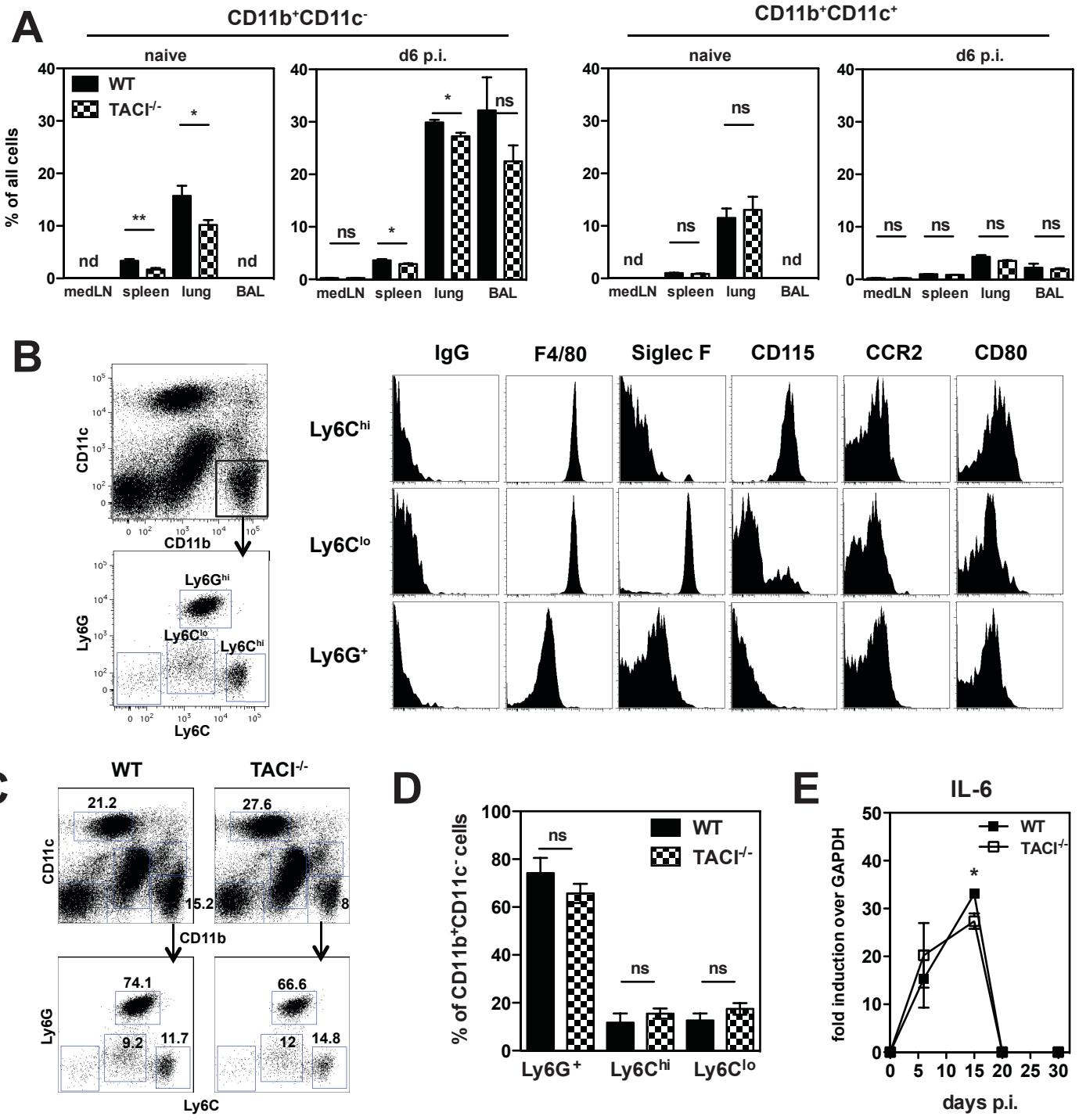
Supplemental Figure S6: Germinal center numbers and formations are similar in WT and TACI$^{-/-}$ mice.

(A) MedLN and spleen from WT and TACI$^{-/-}$ mice were harvested at day 6 after infection. Analysis was performed by flow cytometry for CD19^{+} B cells and germinal center B cells (PNA^{+}FAS^{+}CD19^{+} cells). Data are representative of n=9 mice/group from at least two independent experiments. **(B)** Analysis of germinal center B cells at day 32 p.i. in medLN, spleen and lungs from WT and TACI$^{-/-}$ mice (n=5 mice/group). Data with mean±SEM of at least two independent experiments. **(C)** Lung sections from WT and TACI$^{-/-}$ mice at day 32 p.i. were stained for germinal center cells (GL7, blue) and mature naive B cells (IgD, brown). Representative picture from n=3 mice/group. Original magnification 10x, Scale bar: 100 µm.



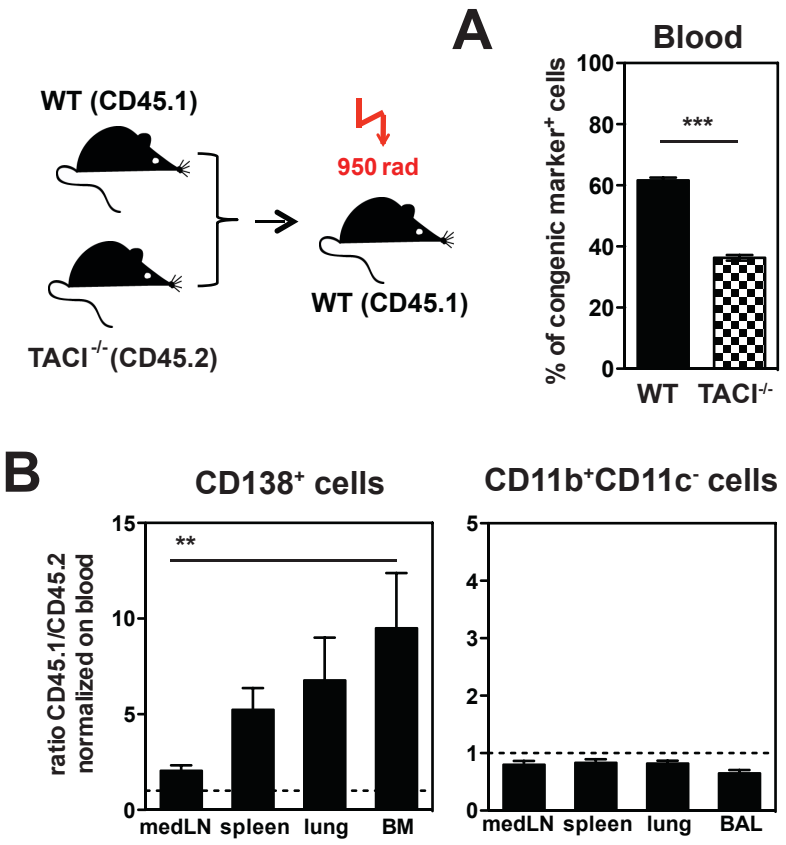
Supplemental Figure S7: BCMA is not required for Ab responses to influenza virus.

WT and BCMA$^{-/-}$ mice (n=3-5 mice/group) were infected with influenza virus PR8. **(A)** Blood samples were collected prior to (day 0) and days 14, 21, 28, 36 and 46 after infection and PR8-specific IgG Ab titer determined by ELISA. **(B-C)** At day 54 p.i., WT and BCMA$^{-/-}$ mice were analyzed for ASCs. **(B)** BM was harvested from tibia of mice, cells stained for CD138^{+} ASCs and analyzed by flow cytometry. PR8-specific IgG and IgA ASCs were determined by ELISPOT in BM **(B)** and medLN, spleen and lungs **(C)**. All data with mean±SEM from one experiment.



Supplemental Figure S8: Characterization of myeloid subsets in WT and TACI$^{-/-}$ mice

Naive and influenza virus PR8-infected WT and TACI$^{-/-}$ mice were analyzed for CD11b^{+} cell subsets. **(A)** MedLN, spleen, lungs and BAL were harvested from naive and infected (day 6 p.i.) WT and TACI$^{-/-}$ mice (n=6 mice) and the frequency of CD11b^{+}CD11c^{-} cells and CD11b^{+}CD11c^{+} cells determined. nd: not done. **(B)** Representative dot plot of CD11b^{+}CD11c^{-} cells in the lungs of a WT mouse day 35 p.i. harboring cell populations distinguishable by expression of Ly6G and Ly6C. Phenotypic characterization of Ly6G^{+}, Ly6C^{hi} and Ly6C^{lo} cells based on staining with isotype control Ab and Abs against F4/80, Siglec F, CD115, CCR2 and CD80. **(C)** Representative dot plots and **(D)** frequencies of myeloid cell subsets in the lungs of WT and TACI$^{-/-}$ mice (n=8-9 mice/group) at days 34-35 p.i. Data with mean±SEM represent at least two independent experiments. **(E)** RT-PCR for IL-6 expression in whole lung tissue homogenates from WT and TACI$^{-/-}$ mice (n=3 mice/group) following infection. Data with mean±SEM from one experiment shown.



Supplemental Figure S9: Mixed bone marrow chimeras do not show a TACI-intrinsic defect in CD11b^{+}CD11c^{-} cells

Mixed BM chimeras were generated by injection of 1:1 mixture of BM from congenic CD45.1 WT and TACI$^{-/-}$ CD45.2 mice into lethaly irradiated CD45.1 WT hosts. **(A)** Six weeks after reconstitution, mice were bled and the frequency of CD45.1^{+} (WT) and CD45.2^{+} cells (TACI$^{-/-}$) in the blood determined by flow cytometry. **(B)** Chimeric mice were infected with influenza virus PR8 six weeks after reconstitution and analyzed 31 days later. **(C)** Cells from medLN, spleen, lung, BM and BAL were stained for CD138^{+} ASCs and CD11b^{+}CD11c^{-} cells and the ratio of CD45.1^{+} and CD45.2^{+} was graphed based on the ratio of BM-donor cells in blood (see **A**). The dotted line at 1 represents an equal ratio of WT- toTACI$^{-/-}$-derived cells. Statistical significance was calculated with an One-way ANOVA. All data with mean±SEM represent one experiment with n=6-8 mice.

Supplemental Methods

Mice

15-week old female BCMA$^{-/-}$ mice on C57BL/6 background were obtained from Dr. Loren Erickson. C57BL/6 WT control mice were 10-week old females purchased from the National Cancer Institute (NCI). For generation of bone marrow chimeras, congenic CD45.1 (Ly5.2/Cr) mice were purchased from NCI. All experiments were performed in accordance with Institutional Animal Care and User Committee guidelines at the Wistar Institute.

Treatment with BrDU

BLIMP-1-YFP mice received 0.8mg/ml BrDU (Sigma) in their drinking water for 8 days starting either at day 8 or day 50 after infection with influenza virus PR8. Incorporation of BrDU was determined by flow cytometry using the APC BrDU Flow Kit according to the manufacturer's instructions (BD Biosciences).

In vivo neutralization of BLyS

To neutralize BLyS, BLIMP-1-YFP mice were injected intraperitoneally with 100 μg αBLyS (10F4) or rat IgG1 control Ab (BD Biosciences) at days 36 and 41 p.i. and mice were analyzed at day 48p.i.. These experiments were performed in the laboratory of Dr. M. Cancro with αBLyS provided by Human Genome Sciences, Inc..

Real time PCR for BR3, TACI and BCMA expression

$B220^+$ YFP^- and $B220^-$ YFP^+ cells from medLN, spleen, lungs and BM were sorted from BLIMP-1-YFP mice 4 weeks after infection. Extraction of RNA, reverse transcription and RT-PCR for BR3, TACI and BCMA were performed as described (1).

Histochemistry of germinal centers in the lungs

Lungs were snap-frozen in OCT. Sections were fixed in acetone and stained with GL7-FITC and biotinylated IgD^b (BD Biosciences). Streptavidin AP (Southern Biotech) and anti-FITC HRP (Millipore) were used as secondary Abs. All histology was examined and recorded on an upright Nikon E600 Microscope with the image software Image Pro (Media Cybernetics).

Phenotypic characterization of myeloid cell subsets

For phenotypic characterization of myeloid subsets, cells were first incubated with Fc-block (αCD32/αCD16), then incubated with the following Abs purchased from eBioscience if not indicated otherwise: CD3ε-FITC, NK1.1-PerCPcy5.5, Ly6C-APC, Ly6G-Alexa Fluor 450 (BioLegend), CD11c-Biotin and CD11b-PE-cy7 (BD Biosciences), B220- PE-TexasRed (Caltag), Streptavidin-Qdot655 (Invitrogen) and PE-conjugated Abs for F4/80, CCR2 (R&D Systems), CD115 (BioLegend), Siglec F, CD80 and isotype control IgGs (BD Biosciences). Analysis was performed on a LSRII (BD Biosciences). Data were processed using FlowJo software (Tree Star).

Generation of mixed bone marrow chimeras

CD45.1 mice were irradiated with 950 rad. Within 24h, BM was prepared from $TACI^{-/-}$

mice (CD45.2) and congenic WT mice (CD45.1) and mixed in a 1:1 ratio prior to injection of $10x10^6$ cells into irradiated sex-matched CD45.1 hosts. Mice received neomycin in their drinking water for 2 weeks after irradiation. Six weeks later, mice were bled, stained with Abs to CD45.1 and CD45.2 and the frequency of BM-derived donor cells determined.

Statistical Analyses

Data are presented as mean±standard error of the mean (SEM). Statistical significance between two groups was calculated using the unpaired Student's t-test and between multiple groups using a One-way ANOVA with post testing (Kruskal-Wallis test with Dunn's multiple comparison). All statistical tests were performed using Prism software (GraphPad Software). P-values are depicted as follows: NS, $p>0.05$; *, $p<0.05$; **, $p<0.01$ and ***, $p<0.001$.

References for Supplemental Methods

1. Stadanlick, J.E., Kaileh, M., Karnell, F.G., Scholz, J.L., Miller, J.P., Quinn, W.J., 3rd, Brezski, R.J., Treml, L.S., Jordan, K.A., Monroe, J.G., et al. 2008. Tonic B cell antigen receptor signals supply an NF-kappaB substrate for prosurvival BLyS signaling. *Nat Immunol* 9:1379-1387.