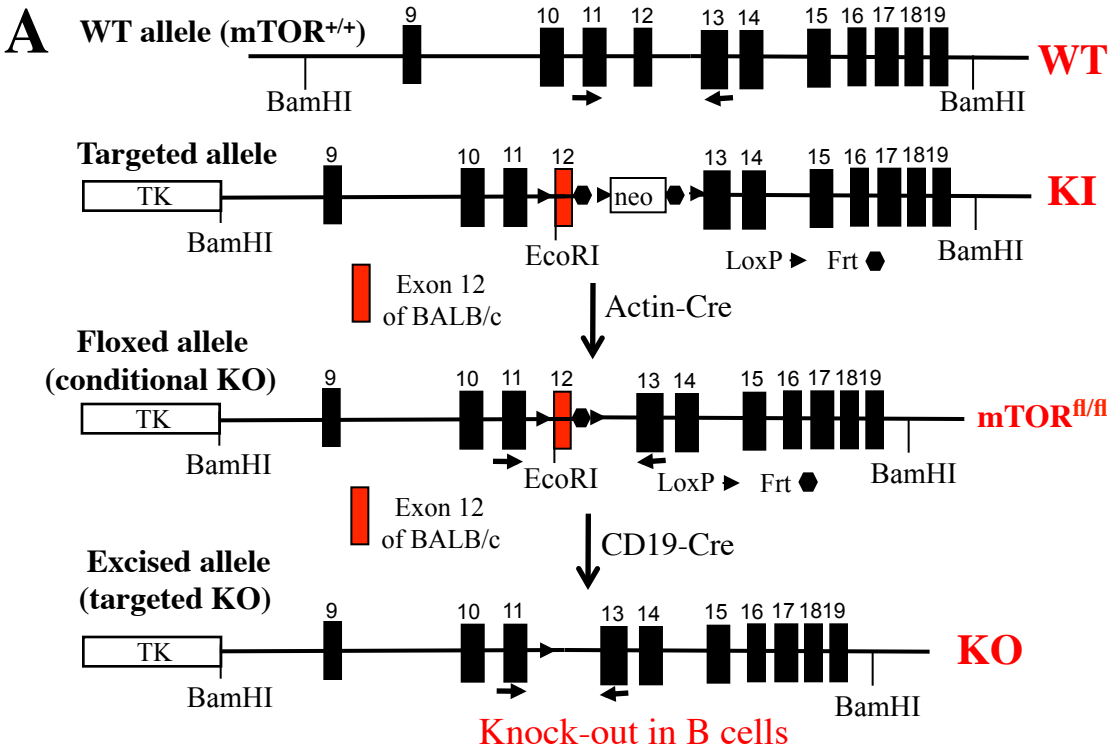
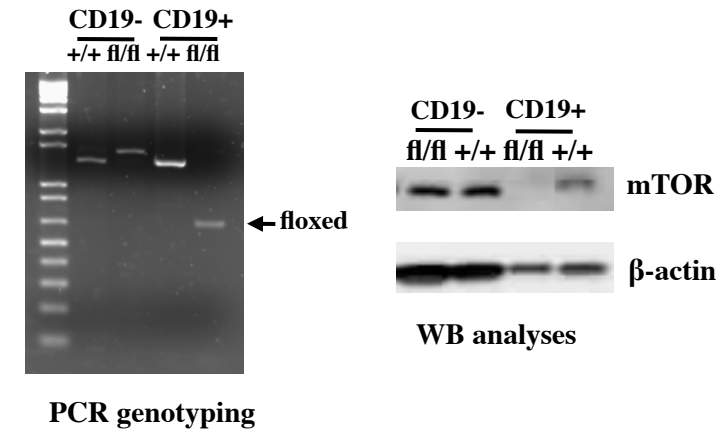
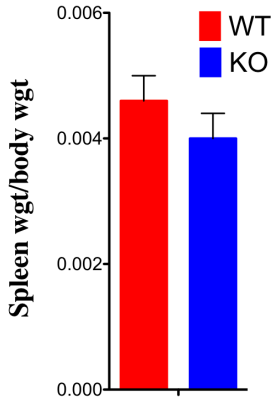
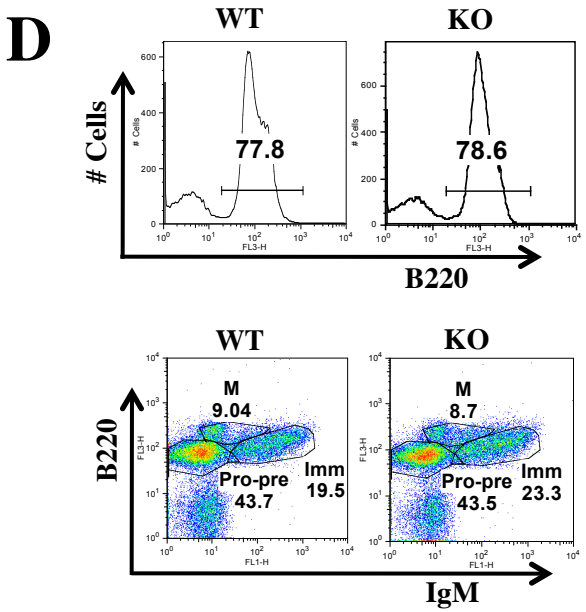

A
WT allele (mTOR+/+)
9 10 11 12 13 14 15 16 17 1819
BamHI
BamHI
WT
Targeted allele
TK
neo
BamHI
EcoRI
LoxP
Frt
BamHI
KI
Exon 12 of BALB/c
Actin-Cre
Floxed allele (conditional KO)
mTORfl/fl
EcoRI
LoxP
Frt
Exon 12 of BALB/c
CD19-Cre
Excised allele (targeted KO)
KO
Knock-out in B cells


B
CD19- CD19+
+/+ fl/fl +/+ fl/fl
floxed
PCR genotyping
CD19- CD19+
fl/fl +/+ fl/fl +/+
mTOR
β-actin
WB analyses


C
Spleen wgt/body wgt
0.006
0.004
0.002
0.000
WT
KO


D
WT
KO
Cells
77.8
78.6
B220
WT
KO
M
9.04
Pro-pre
43.7
Imm
19.5
M
8.7
Pro-pre
43.5
Imm
23.3
B220
IgM

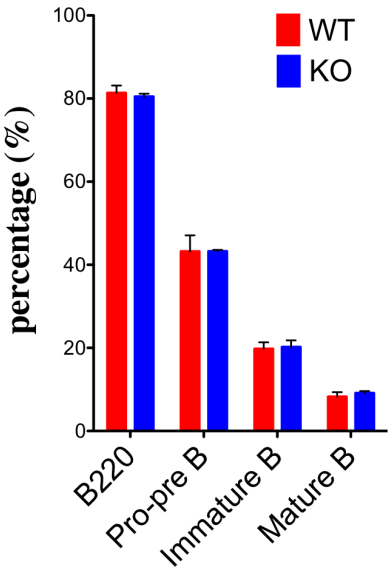

percentage (%)
100
80
60
40
20
0
WT
KO
B220
Pro-pre B
Immature B
Mature B

Figure S1. Strategy for generating mTOR conditional knockout mice with $CD19^{Cre}$.

A) The strategy used to generate Cre-mediated mTOR deletion in $CD19^{+}$ B cells. Schematic diagram of wild-type (WT), knock-in (KI) mTOR (targeting allele), floxed and excised alleles after Cre recombination of the mTOR gene. **B)** PCR and Western Blot analyses of floxed (fl) and wild-type (+) mTOR in $CD19^{-}$ and $CD19^{+}$ cells from $mTOR^{+/+}CD19^{Cre/+}$ (WT) and $mTOR^{fl/fl}CD19^{Cre/+}$ (KO) mice. **C, D)** mTOR conditional knockout in $CD19^{+}$ B cells did not alter the **C)** relative weight of spleen to body weight, or the **D)** development of B cells in bone marrow (BM). The age of mice ranged from 8 to 12 weeks (n=10). Data are presented as mean $\pm$ SEM. Cells from BM of WT and KO mice **(D)** were stained with antibodies to B220, IgM, and IgD. Sub-populations of B cells were analyzed by FACS and FlowJo (M: mature B cell; Pro-pre: pro- and pre-B cells; Imm: immature B cells). Data (n=6) are presented as mean ± SEM.

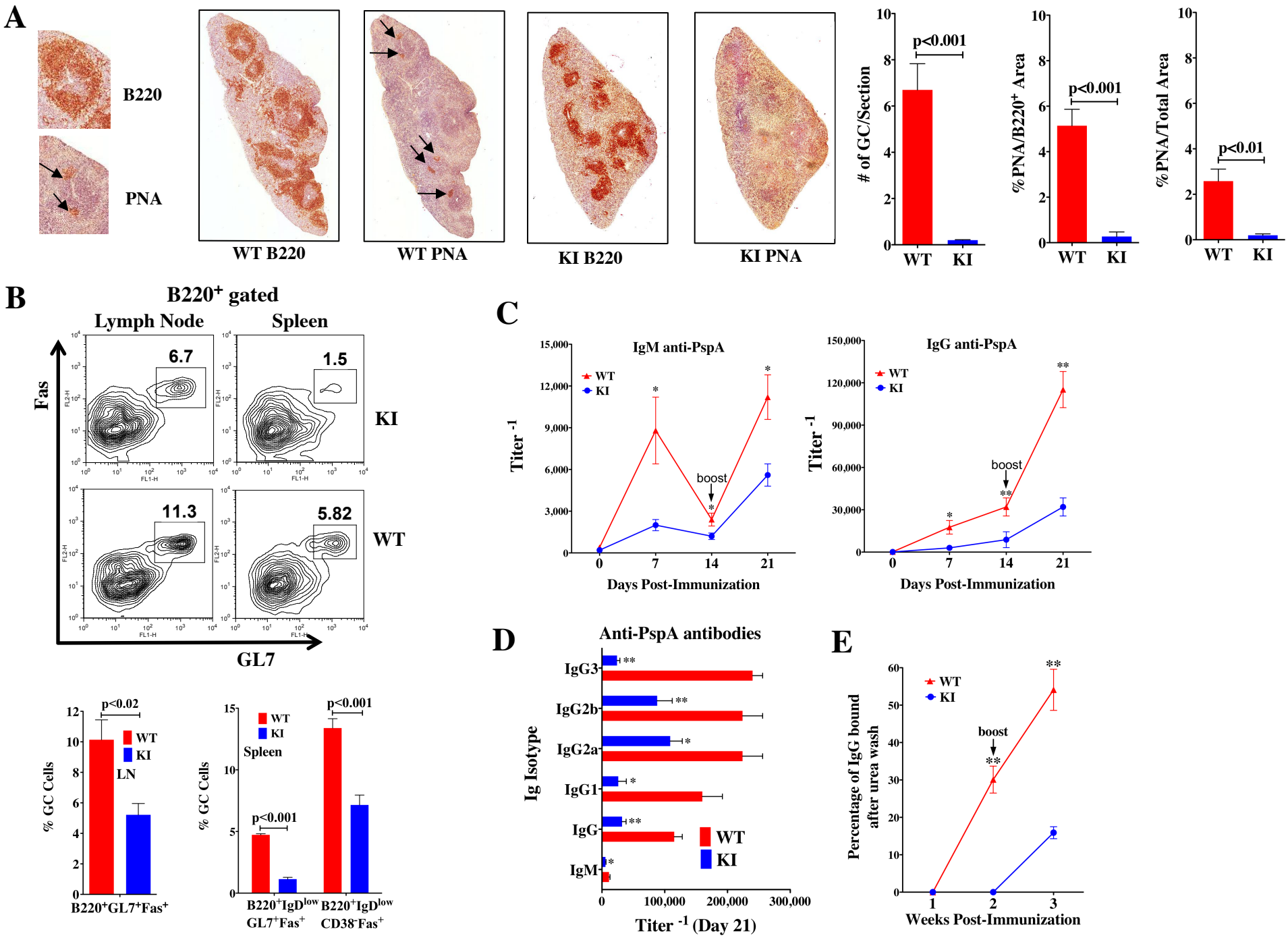
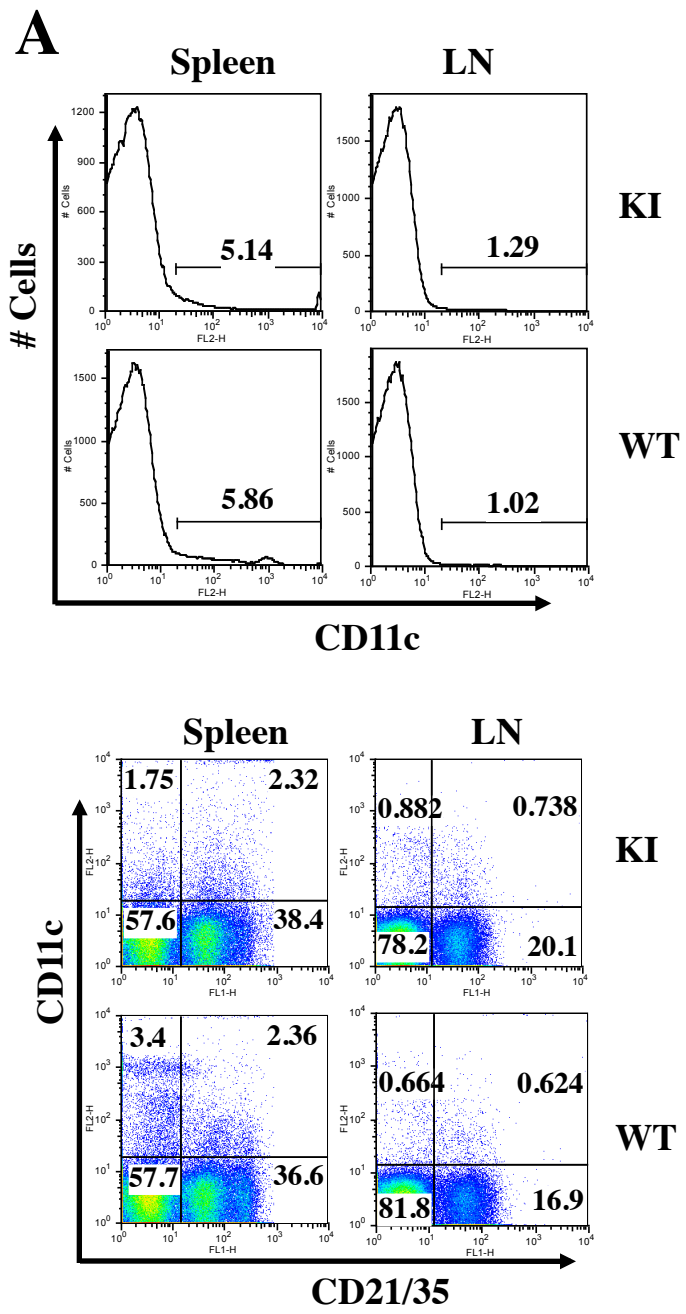

A
B220
PNA
WT B220
WT PNA
KI B220
KI PNA
# of GC/Section
p<0.001
% PNA/B220+ Area
p<0.001
% PNA/Total Area
p<0.01
WT
KI
B
B220+ gated
Lymph Node
Spleen
Fas
GL7
6.7
1.5
11.3
5.82
KI
WT
% GC Cells
p<0.02
LN
p<0.001
Spleen
B220+IgDlow GL7+Fas+
B220+IgDlow CD38-Fas+
C
IgM anti-PspA
IgG anti-PspA
Titer -1
boost
Days Post-Immunization
D
Anti-PspA antibodies
Ig Isotype
IgG3
IgG2b
IgG2a
IgG1
IgG
IgM
Titer -1 (Day 21)
E
Percentage of IgG bound after urea wash
Weeks Post-Immunization

Figure S2. Constitutive reductions in mTOR impair GC formation and antibody responses to intact *Streptococcus pneumoniae* Pn14. mTOR-compromised KI and WT mice (N=5/group) were immunized i.p. with $2x10^8$ CFU/mouse heat-killed Pn14 in saline and boosted on day 14. Spleens and lymph nodes (LN) were collected 21 days post-immunization for IHC staining and FACS analysis. **A)** The numbers of GCs, the area of PNA staining and the area of B220+ staining were evaluated from scans of spleen sections (N =5) stained with B220 or PNA using color deconvolution analysis software (Aperio Technologies). **B)** Cells from LNs and spleens were stained with B220, GL7, Fas, CD38, and IgD antibodies and analyzed by FACS. Data are presented as mean ±SEM; p-values are indicated. **C)** KI and WT (N = 5/group) mice were immunized intraperitoneally with $2x10^8$ CFU/mouse equivalents of heat-killed Pn14 in saline and boosted on day 14. KI mice had decreased anti-protein (PspA) responses to intact Pn14, relative to WT littermate controls. **D)** Antigen-specific IgM and IgG isotype titers were determined from sera collected at the indicated days. Data are presented as mean $\pm$ SEM; *significance $p<0.05$;**significance $p<0.01$. **E)** Affinity maturation of antibodies in response to primary and secondary challenge with Pn14 was impaired in KI mice. The percent of anti-PspA IgG antibody bound after urea wash (to measure avidity) from d21 sera of KI and WT mice (N = 5/group) is shown. Data are presented as mean $\pm$ SEM;**significance $p<0.01$.


A
Spleen
LN
KI
WT
Cells
CD11c
5.14
1.29
5.86
1.02
1.75
2.32
57.6
38.4
0.882
0.738
78.2
20.1
3.4
2.36
57.7
36.6
0.664
0.624
81.8
16.9
CD21/35

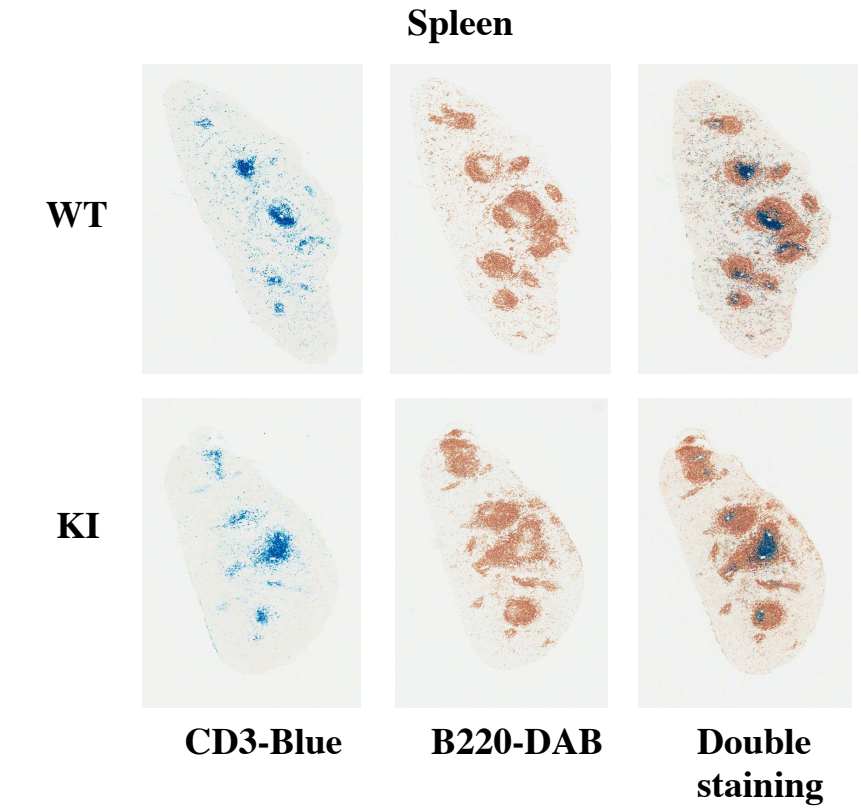

B
Spleen
WT
KI
CD3-Blue
B220-DAB
Double staining

Figure S3. Immunohistochemical stains of spleens and lymph nodes of mTOR KI mice. A) Splenic and LN cells isolated from WT and KI mice (N=2/grp) were stained with CD11c and CD21/35 antibodies; $CD11c^{+}$ and $CD21/35^{+}$ cells were analyzed by FACS with FlowJo. **B)** Representative splenic sections from WT and KI mice (N = 5/group) stained with B220 and CD3 antibodies.

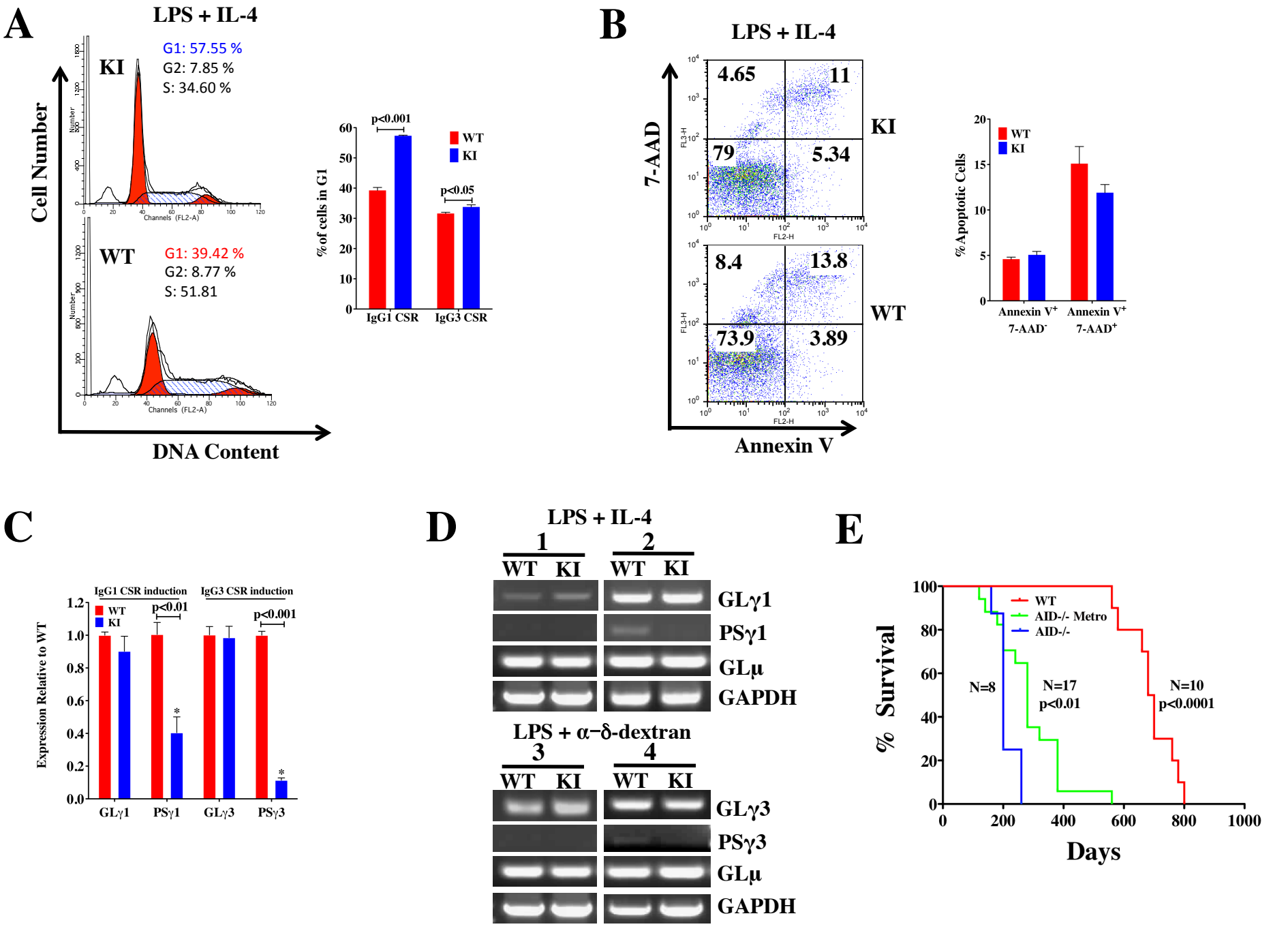

A
LPS + IL-4
KI
G1: 57.55 %
G2: 7.85 %
S: 34.60 %
WT
G1: 39.42 %
G2: 8.77 %
S: 51.81
Cell Number
DNA Content
Channels (FL2-A)
% of cells in G1
p<0.001
p<0.05
WT
KI
IgG1 CSR
IgG3 CSR
B
LPS + IL-4
7-AAD
Annexin V
4.65
11
79
5.34
8.4
13.8
73.9
3.89
% Apoptotic Cells
Annexin V+ 7-AAD-
Annexin V+ 7-AAD+
C
IgG1 CSR induction
IgG3 CSR induction
Expression Relative to WT
p<0.01
p<0.001
GLγ1
PSγ1
GLγ3
PSγ3
D
LPS + IL-4
LPS + α−δ-dextran
GLγ1
PSγ1
GLμ
GAPDH
GLγ3
PSγ3
E
% Survival
Days
AID-/- Metro
AID-/-
N=8
N=17
p<0.01
N=10
p<0.0001

Figure S4. Constitutive reductions in mTOR affect cell cycle, apoptosis and post switch transcripts in IgG_1 positive B cells. $CD43^-$ resting B cells purified from spleens of KI and WT mice (N=3/group) were stimulated with LPS and IL4 (IgG_1 induction) or LPS and α-δ-dextran (IgG_3 induction) for 48 hours. Cells were stained with PI for cell cycle analysis **(A)** and annexin V and 7-AAD for apoptosis **(B).** Stained cells were analyzed by FACS using FlowJo and ModFit LT. Data are presented as mean ± SEM; significance p-values are indicated. Constitutive reductions in mTOR decrease post switch transcripts of IgG_1 and IgG_3 after CSR induction. **C, D)** Real-time and RT-PCR of germline (GL, containing I and C_H exons of isotype) and post-switch transcripts (PS, containing Iμ and each of C_H exons). RT-PCR of germline and post-switch transcripts from $CD43^-$ resting B cells from spleens of WT or KI control (panels 1,3) or stimulated with LPS and IL4 (IgG_1 induction) (panel 2) or stimulated with LPS and α-δ-dextran (IgG3 induction) (panel 4) for 48h. The results **(C)** are displayed as relative fold changes of mRNA expression in KI compared with WT, upon normalization by 18S RNA (n = 3*)*. Statistically significant changes were analyzed for WT compared with KI, Student's t-test. Data are presented as mean ± SEM; p-values are indicated. **E)** The survival of AID KO mice in non-SPF conditions. Survival curves for 10 wild-type (red), 8 AID KO (blue) and 17 AID KO (green) mice given metronidazole and maintained in filter-top cages. Significance values are based on Log-rank (Mantel-Cox) $\chi 2$ values.