Supplementary Figures

FIG E1. Defective TLR9-induced B-cell activation in CD19-deficient patients is B-cell specific and not caused by altered TLR9 expression levels. **A,** Immunoblot analysis of lysates from EBV-immortalized B-cell lines derived from a CD19-deficient patient and two unrelated healthy controls. **B,** Surface IgM expression levels on $CD20^+$ $CD10^-$ $CD21^+CD27^-$ mature naïve B cells from a CD19-def. patient and a healthy control were analyzed by flow cytometry. **C,** Surface expression of CD80 and CD86 on purified CD303+CD4+ plasmacytoid dendritic cells of a healthy control and a CD19-deficient patient after no stimulation or *in vitro* stimulation with the TLR9 ligand CpG or the TLR7 ligand Gardiquimod for two days was analyzed by flow cytometry.

FIG E2. Defective TLR7-induced B-cell activation in CD19-deficient patients. Surface expression of TACI, CD23, CD86 and CD80 on CD20+CD27- naïve B cells of a healthy family member (CD19-non carrier), a CD19-heterozygous carrier (CD19-het.) and a CD19-deficient patient (CD19-def.) after no stimulation or *in vitro* stimulation with the TLR7 ligand Loxorobine for two days.

FIG E3. TLR7 stimulation induces phosphorylation of CD19, BTK and AKT in human B cells. Phosphorylation of CD19, BTK, AKT and SYK was assessed by immunoblot in lysates from purified healthy control peripheral blood B cells stimulated or not for the indicated time with TLR7 ligand Gardiquimod.

FIG E4. *CD19* knock-down in Ramos B cells. Ramos B cells were transduced with lentiviral constructs containing no shRNA (pTRIP control) or an shRNA targeting CD19 (pTRIP CD19 shRNA) and GFP**.** GFP+CD19+ (control) or GFP+CD19- (CD19 knock-down) Ramos B cells were sorted and expanded in culture. CD19 expression was

determined by flow cytometry, dashed lines display the isotype control staining (MFI, mean fluorescence intensity). CD19 expression was also determined by immunoblot analysis of lysates from control or CD19 shRNA transduced Ramos B-cell lines.

FIG E5. Inhibition of PI3K, AKT or BTK mimics TLR7 induced B-cell activation defects observed in CD19-deficient B cells. Surface expression of TACI, CD23, CD86 and CD69 on purified $CD19^{+}CD27^{-}$ naive B cells of healthy individuals after *in vitro* stimulation with the TLR7 ligand Gardiquimod for two days with or without addition of PI3K-inhibitor (CAL-101), BTK-inhibitor (PCI32765) or AKT inhibitor (AKT-IV inhibitor) was analyzed by flow cytometry. Dot blots of a representative experiments are shown in **A** and the data of five independent experiments is summarized in **B**. Each bar represents the mean ± SEM frequency, horizontal dashed lines represent the mean of the unstimulated samples. (* $p<0.05$; ** $p<0.01$; *** $p<0.001$).

Supplemental Methods

Antibodies

The following monoclonal antibodies against human antigens were used for flow cytometry: anti-CD10 (HI10a), anti-CD19 (HIB19), anti-CD20 (2H7), anti-CD23 (EBVCS-5), anti-CD25 (BC96), anti-CD27 (O323), anti-CD69 (FN50), anti-CD80 (16-10A1), anti-CD86 (IT2.2) and anti-TACI (1A1; all from Biolegend) and anti-CD21 (B-ly4) and anti-IgM (G20-127; from BD Biosciences).

The following antibodies were used for immunoblot or immunoprecipitation: anti-CD19 (Cell Signaling, 3574), anti-CD19 phosphorylated at Y531 (Cell Signaling, 3571), anti-BTK (Cell Signaling, D3H5), anti-BTK phosphorylated at Y551 (abcam, EP267Y), anti-AKT (Cell Signaling, C67E7), anti-AKT phosphorylated at S473 (D9E), anti-SYK (abcam, ab3993), anti-SYK phosphorylated at Y352 (Cell Signaling, 65E4), anti-PYK2 (Cell Signaling, 5E2), anti-LYN (Cell Signaling, 2732), anti-MyD88 (Cell Signaling, D80F5) and anti-p85 PI3K (Cell Signaling, 19H8).

Transduction

In detail, the pTRIP-Ubi-GFP lentiviral vector has been used for short hairpin RNA (shRNA) delivery. For the construction of pTRIP-shCD19, a DNA fragment containing the H1 promoter and a CD19 shRNA sequence was generated by double digestion of pSUPER-shCD19 plasmid (made by inserting a shRNA targeting human CD19 cDNA sequence (5′-GGATATGAGAGGAATCCTG-3′) into pSUPER plasmid) and was subcloned within the 3′ long terminal repeat of pTRIP-Ubi-GFP vector. Lentiviral particles were produced by transient transfection of 293T cells. Viruses were then used to transduce $3x10^5$ Ramos B cells in the presence of polybrene (Sigma).

the following inhibitors were used: PP2 (SFK inhibitor, 1 μM, Sigma), AG-17 (PYK2 inhibitor, 1.0 μM, Selleckchem), LY294002 (PI3K inhibitor, 7.5 μM, Selleckchem), CAL-101 (PI3K p110λ inhibitor, 250nM), PCI32765 (BTK inhibitor, 0.5 μM, Selleckchem), LFM-A13 (BTK inhibitor, 2.5 μM), AKT Inhibitor IV (1 μM EMD Chemicals) or MK-2206 (AKT inhibitor, 250 nM).

Figure E1

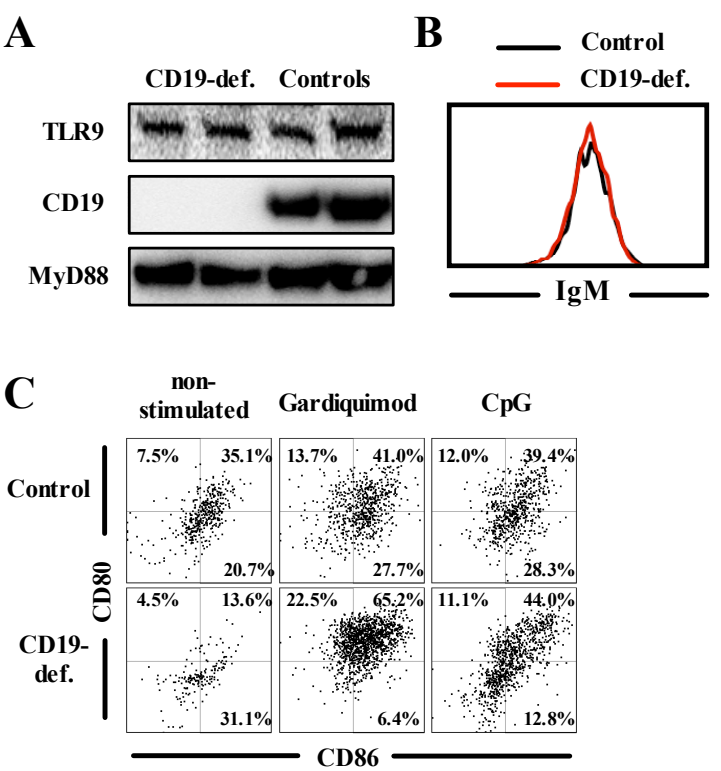

A
CD19-def.
Controls
TLR9
CD19
MyD88
B
Control
CD19-def.
IgM
C
non-stimulated
Gardiquimod
CpG
Control
CD19-def.
CD80
CD86
7.5%
35.1%
20.7%
13.7%
41.0%
27.7%
12.0%
39.4%
28.3%
4.5%
13.6%
31.1%
22.5%
65.2%
6.4%
11.1%
44.0%
12.8%

Figure E2

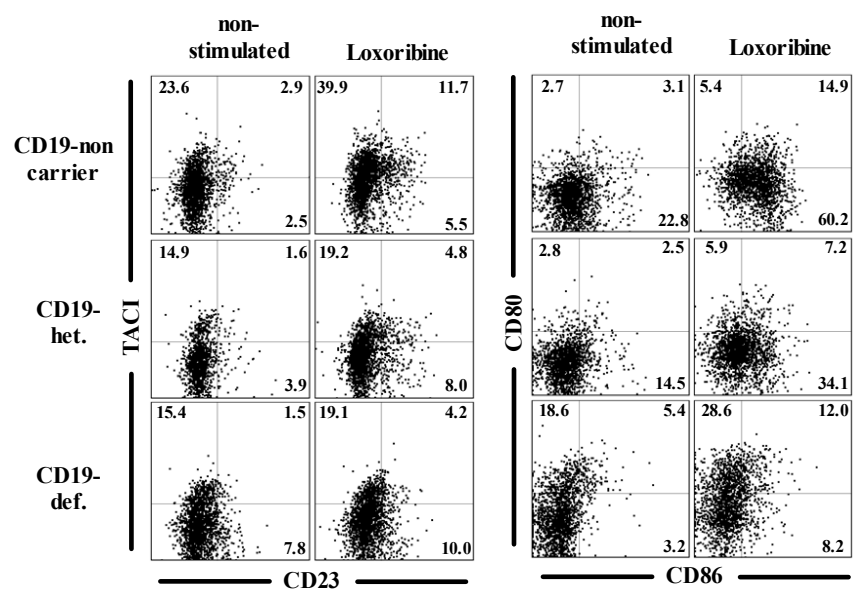

non-
stimulated
Loxoribine
non-
stimulated
Loxoribine
CD19-non
carrier
CD19-
het.
CD19-
def.
TACI
CD80
CD23
CD86
23.6
2.9
2.5
39.9
11.7
5.5
14.9
1.6
3.9
19.2
4.8
8.0
15.4
1.5
7.8
19.1
4.2
10.0
2.7
3.1
22.8
5.4
14.9
60.2
2.8
2.5
14.5
5.9
7.2
34.1
18.6
5.4
3.2
28.6
12.0
8.2

Figure E3

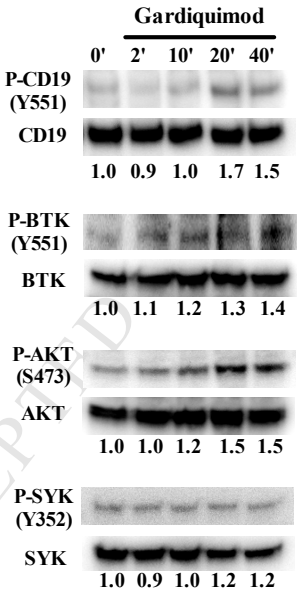


Figure E4

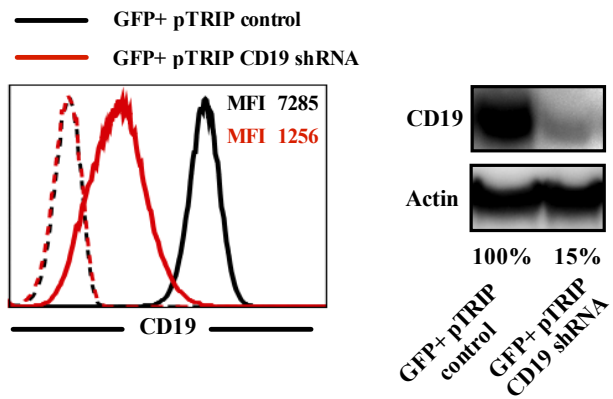
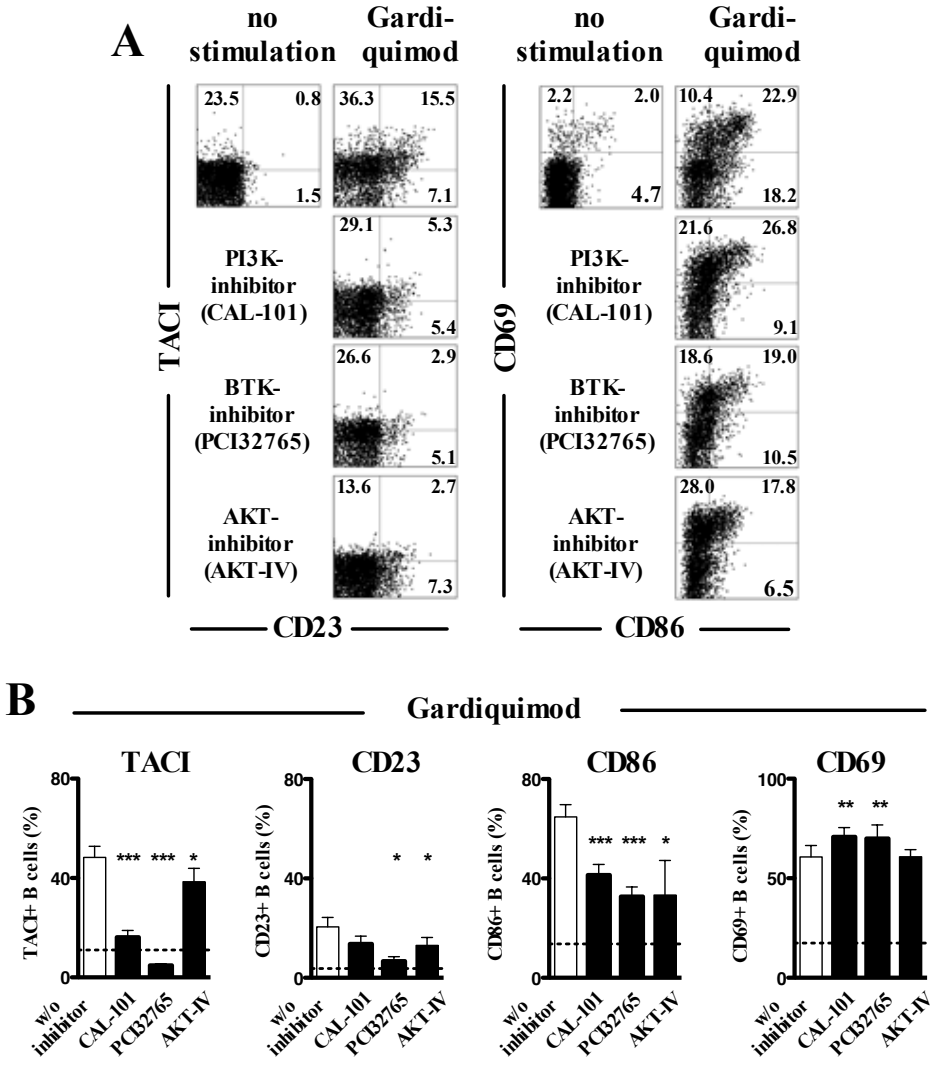

GFP+ pTRIP control
GFP+ pTRIP CD19 shRNA
MFI 7285
MFI 1256
CD19
CD19
Actin
100%
15%
GFP+ pTRIP control
GFP+ pTRIP CD19 shRNA

Figure E5


A
no stimulation
Gardi-quimod
no stimulation
Gardi-quimod
TACI
CD69
PI3K-inhibitor (CAL-101)
BTK-inhibitor (PCI32765)
AKT-inhibitor (AKT-IV)
23.5
0.8
1.5
36.3
15.5
7.1
29.1
5.3
5.4
26.6
2.9
5.1
13.6
2.7
7.3
2.2
2.0
4.7
10.4
22.9
18.2
21.6
26.8
9.1
18.6
19.0
10.5
28.0
17.8
6.5
CD23
CD86
B
Gardiquimod
TACI
CD23
CD86
CD69
TACI+ B cells (%)
CD23+ B cells (%)
CD86+ B cells (%)
CD69+ B cells (%)
w/o inhibitor
CAL-101
PCI32765
AKT-IV