Supplemental methods:

Retroviral constructs transduction and adoptive transfer

The *cd19* coding sequences were amplified using primers (*CD19* 5' primer: agtcctcgagccaccatgccatctcctctccc and *CD19* 3' primer: gttaactcacgtggttccccaagtcc) and cloned into the retroviral vectors MIGR1 (MSCV-IRES-GFP). Retroviruses were packaged by transfection of 293T cells with the retroviral vectors along with plasmid pCLeco. Isolated bone marrow cells with depleted lineage positive cells were incubated with 2 ml IMDM medium (50μM 2-Mercaptoethanol, 1x Amp/Strep, 50 ng/ml SCF, 20 ng/ml TPO, and 15% FBS) in 12-well plates with $2x10^6$ Lin^- BM cells per well. Retrovirus medium was collected by 0.45 μm filter from the transfected 293T culture plate and transferred to the BM culture plate by adding ploybrene (8 μg/ml). After incubation for 30 minutes, the plate was spinned at 37 °C and 1000 *g* for 90 minutes. After incubation for 2 hours, the retrovirus medium was replaced with the stored medium and the plate was incubated for 24–48 hours. The cells were collected and washed 2 times by FACS buffer. The LSK ($Lin^-Sca1^+cKit^+$) cell number was counted to check the transduced efficiency. CD45.1 recipient mice were irradiated and rested for 4 hours and the same number of CD45.1 LSK cells was adoptively transferred to the mice.

Preparation of monobiotinylated Fab′ Ab and model antigens

Monobiotinylated Fab′ fragment of anti-mouse IgM+G Ab (mB-Fab′–anti-Ig) was generated from the $F(ab')_2$ fragment (Jackson ImmunoResearch Laboratories) using a published protocol[1]. The disulfide bond that links the two Fab′ was reduced using 20 mM 2-mercaptoethylamine, and the reduced cysteine was biotinylated by maleimide-activated

biotin (Thermo Scientific). Fab′ was further purified using Amicon Ultracentrifugal filters (Millipore). One biotin per Fab′ was confirmed by a biotin quantification kit (Thermo Scientific). Fab′ was labeled with Alexa Fluor (AF) 546 (Invitrogen). To activate B cells with sAg, splenic B cells were incubated with AF546–mB-Fab′–anti-Ig (2 µg/ml) mixed with mB-Fab′–anti-Ig (8 µg/ml) for 30 min and streptavidin (1 µg/ml) for 10 min at 4°C. As a control, streptavidin was omitted. The cells were washed and warmed up to 37°C for varying lengths of time. To activate B cells with mAg, cells were incubated with AF546–mB-Fab′–anti-Ig and mB-Fab′–anti-Ig tethered to planar lipid bilayers by streptavidin at 37°C for varying lengths of time. As a control for mAg, surface BCRs were labeled with AF546–Fab–anti-mouse IgM+G (2 µg/ml) at 4°C, washed, and then the B cells were incubated with transferrin (Tf)-tethered lipid bilayers where the molecular density of Tf on lipid bilayers was equivalent to that of AF546–mB-Fab′–anti-Ig.

Preparation of Ag-tethered planar lipid bilayers

The planar lipid bilayer was prepared as described previously [2,3]. Liposomes were made by sonicating 1,2-dioleoyl-*sn*-glycero-3-phosphocholine and 1,2-dioleoyl-*sn*-glycero-3-phosphoethanolamine-cap-biotin (Avanti Polar Lipids) in a 100:1 molar ratio in PBS at a lipid concentration of 5 mM. Aggregated liposomes were removed by ultracentrifugation and filtration. Coverslip chambers (Nalge Nunc International) were coated with the planar lipid bilayer by incubating with the liposomes (0.05 mM) for 10 min. After extensive washes, the coated coverslip chamber was incubated with 1 µg/ml streptavidin (Jackson ImmunoResearch Laboratories), followed by 2 µg/ml AF546-mB-Fab′–anti-Ig mixed

with 8 μg/ml mB-Fab′–anti-Ig Ab. For a non-antigenic control, surface BCRs were labeled by incubating with AF546-Fab–anti-Ig (2 μg/ml) on ice for 30 min. The labeled B cells were then incubated with biotinylated holo-transferrin (Tf; 16 μg/ml, which gave an equal molar concentration of 10 μg/ml mB-Fab′–anti-Ig; Sigma-Aldrich) tethered to lipid bilayers by streptavidin.

Total internal reflection fluorescence microscopy

Images were acquired using a Nikon A1R confocal and TIRF system on an inverted microscope (Nikon Eclipse Ti-PFS), equipped with a 100×, NA 1.49 Apochromat TIRF objective (Nikon Instruments), an iXon EM-CCD camera (Andor), and three solid-state lasers of wavelengths 405, 488 and 546 nm.

To image intracellular molecules, B cells were incubated with an Ag-tethered lipid bilayer at 37°C for varying lengths of time. Cells were then fixed with 4% paraformaldehyde, permeabilized with 0.05% saponin, and stained for phosphotyrosine (pY) (Millipore), phosphorylated Mst1 (pMst1, T183; Abcam), Btk (pBtk, Y551; BD Biosciences), SHIP-1 (pSHIP, Y1020; Cell Signaling Technology) and CD19 (pCD19, Y531; Abacam). The B cell contact area was determined using IRM images and NIS-Elements AR 3.2 software. The mean fluorescence intensity (MFI) of each staining in the B cell contact zone were determined using NIS-Elements AR 3.2 software. Background fluorescence generated by Ag tethered to lipid bilayers in the absence of B cells or secondary Ab controls was subtracted. For each set of data, >20 individual cells from two or three independent experiments were analyzed.

Immunoblot analysis

Primary B cells were activated with or without F(ab′) -anti-Ig plus streptavidin for indicated times and lysed. Cell lysates were analyzed with SDS-PAGE and Western blot, and probed for pMob1, Mob1, pYap, Yap (all from Cell Signaling), CD19 (Abacam), Btk and TEAD2 (Santa Cruz) respectively. The blots were stripped and reprobed with anti-mouse actin (Sigma-Aldrich) for establishing loading controls [4].

Phos flow

B cells were incubated with monobiotinylated Fab' fragment of anti-human IgG+M (mB-Fab'-anti-Ig) plus streptavidin at 37°C for varying lengths of time. Cells were fixed with Phosflow Lyse/Fix buffer, followed by permeabilization with Phosflow Perm buffer III (BD Biosciences) and staining with the following antibodies: PE-anti-pErk (T202/Y204, BD Biosciences), PE-anti-pBtk (Y551, BD Biosciences), PE-anti-pSyk (Y348, BD Biosciences).

Luciferase assay

The promoter region and 3'UTR or 3'UTR mutant of *cd19* were apmlified according to the designed primers (Supplemental table Ⅰ) and cloned into pGL3-basic vector together, named *cd19*-3'UTR or *cd19*-3'UTR-M. *cd19*-3'UTR or *cd19*-3'UTR-M were transfected together with pcDNA3.1-tead2 and internal control plasmid-pRL-TKB into 293 cells with 70% confluence in a 6-well plate by using TransIT®-293 transfection reagent (Mirus Bio Corporation). After 24

hrs transfection, cells were lysed and luciferase assay was performed by using firefly luciferase reporter gene assay kit (Beyotime).

Calcium Flux

The calcium flux was examined by flow cytometry using the calcium-sensitive dyes Fluo4 AM and Fura Red (Life) using manufacturer protocols. The relative levels of intracellular calcium were determined by a ratio of Fluo4 to Fura Red emission using FlowJo software (Tree Star, Inc., Ashland, OR)[5].

Quantitative RT-PCR

For comparison of gene expression in B cells from WT and Mst1 KO mice, total RNA was extracted with RNAPURE kit (RP1202; BioTeke) and reverse-transcribed with a PrimeScript™ RT reagent Kit (RR037A; Takara). The resulting cDNA was analyzed for the expression of various genes with SsoAdvancedTM SYBR® Green supermix (Bio-Rad) on a CFX96 Touch Real-Time System (Bio-Rad) and the appropriate primers for ‘test genes’.

Immunization

For experiments involving antigen-induced GC B cell response, mice were immunized as described [6].

Supplementary Table 1. Primer sequence

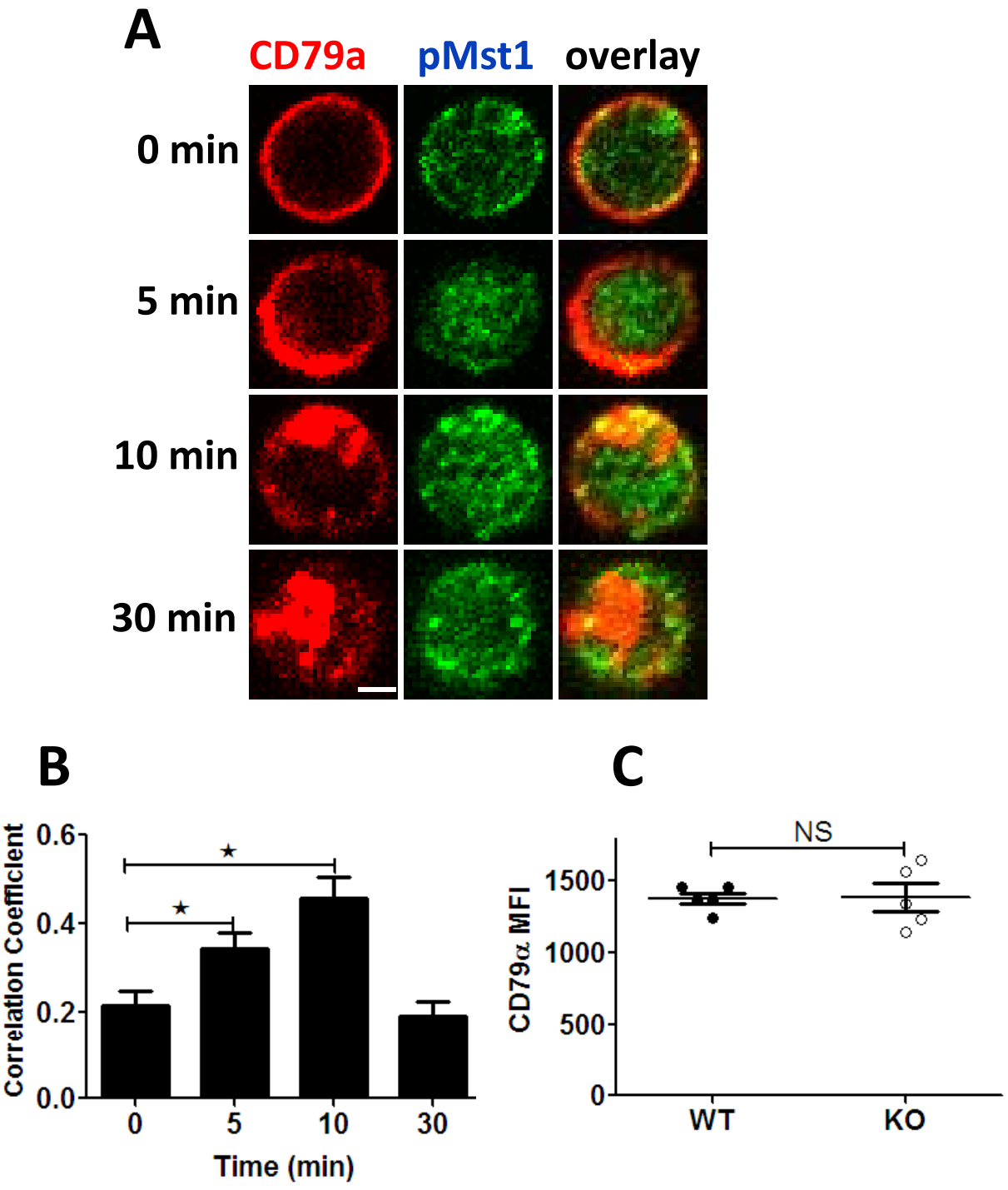
Primers used in gene expression		
Gene symbol	**5' primer**	**3' primer**
Cd19	5'-ggacagtgaacgtggaggat	3'-gggcacatacaggctttgtt
Btk	5'-cgccattacgttgtgtgttc	3'-tagaaggcgcgtttttgttt
Cr2	5'-aattgcaaatggtggtcaca	3'-gatcggggcaatgagttaga
Tead1	5'-ggaggccctggctatctatc	3'-tgcttcctggtccttgtctt
Tead2	5'-accatcctccaggttgtgac	3'-tactgggctcctcgttcact
Tead3	5'-cgaacgctttcttccttgtc	3'-cgtactcggtctccaccttc
Tead4	5'-gagcccggagaacatgatta	3'-tctcatagcgggcatactcc
Mob1a	5'-gaggctcacctcaacacctc	3'-attcctgaagtggtgccaac
Yap	5'-aaggctggaccctcgtttt	3'-ttcaaccgcagtctctcctt
Primers used in luceferase assay		
Gene location	**5' primer**	**3' primer**
Cd19-3'UTR +5498 to +6423	5'-agtc tctaga ctcccaagtgactaggtgag	3'-agtcgtcgacttagaaagtataaaggcagatttattgagagc
Cd19-3'UTR+5498 to +6423 Mutant	5'-ccagccgctaacccttatggtgtcccacaagggag	3'-gggacaccataagggttagcggctggacatatttgcatc
Cd19-promoter-383 to +35	5'-agtcgctagcaggtgtaaaataaggtcagagcctgg	3'-agtc aagctt ggtagccaggctccctgg
Primers used in CHIP		
Genome location	**5' primer**	**3' primer**
Cd19 +14K	5'-gagaaggggaaggaatgaa	3'-agaggcaaaccatgtgaggt
Cd19 +9K	5'-tggtaggctcaggctcagat	3'-ctagcaccttgcctcctctg
Cd19 +8.6K	5'-tggctctggttgtattggtg	3'-cattggtcttcctcacagca
Cd19 +6K	5'-gggacaccatggaatgtagc	3'-ctgagtcacccagaggaagg
Cd19 -2.2K	5'-gcagaccccacttgcttaac	3'-tggaggaatgaatggagagc
Cd19 -4.1K	5'-agctgtctgggatgcagatt	3'-aggattcccctcccctaact
Cd19 -8.3K	5'-gaggcaatggttgagagcat	3'-tggtctgagaaagtcccaatg
Cd19 -11K	5'-ttgaggcagggtcttgctat	3'-gtcagcagttgcagtggtgt
Cyr61-postive control	5'-ctggcatctccacacgagttac	3'-tgccctttttaggctgctg

References:

1. Peluso P, Wilson DS, Do D, et al. Optimizing antibody immobilization strategies for the construction of protein microarrays. *Anal Biochem*. 2003;312(2):113-124.
2. Sohn HW, Tolar P, Pierce SK. Membrane heterogeneities in the formation of B cell receptor-Lyn kinase microclusters and the immune synapse. *J Cell Biol*. 2008;182(2):367-379.
3. Grakoui A, Bromley SK, Sumen C, et al. The immunological synapse: a molecular machine controlling T cell activation. *Science*. 1999;285(5425):221-227.
4. Sharma S, Orlowski G, Song W. Btk regulates B cell receptor-mediated antigen processing and presentation by controlling actin cytoskeleton dynamics in B cells. *J Immunol*. 2009;182(1):329-339.
5. Liu C, Bai X, Wu J, et al. N-wasp is essential for the negative regulation of B cell receptor signaling. *PLoS Biol*. 2013;11(11):e1001704.

6. Kang SG, Liu WH, Lu P, et al. MicroRNAs of the miR-17 approximately 92 family are critical regulators of T(FH) differentiation. *Nat Immunol*. 2013;14(8):849-857.

Fig.S1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CD79a
pMst1
overlay
0 min
5 min
10 min
30 min
B
Correlation Coefficient
0.6
0.4
0.2
0.0
0
5
10
30
Time (min)
C
NS
CD79α MFI
1500
1000
500
0
WT
KO

Fig.S1 The recruitment of Mst1 to CD79α aggregates in B cells stimulated by sAg. To mimic sAg, splenic B cells were incubated with anti-CD79α for 10 min at 4° C to label the CD79α . Then, the cells were either incubated with $F(ab)_2'$–anti-Ig or with the medium alone (0 min) as a control at 37° C for varying lengths of time. After fixation and permeabilization, the cells were stained for pMst1 and analyzed using confocal microscopy (CFm) (A). Images were quantitatively analyzed to determine the correlation coefficients between the CD79α and pMst1 (B). Flow cytometry analysis of the MFI of CD79α in WT and Mst1 KO B cells (C). Shown are representative images and mean values (±SD) from three independent experiments where over 50 cells were individually analyzed using NIS-Elements AR 3.2 software. Scale bars, 2.5 μm. ∗, p <0.01.

Fig.S2

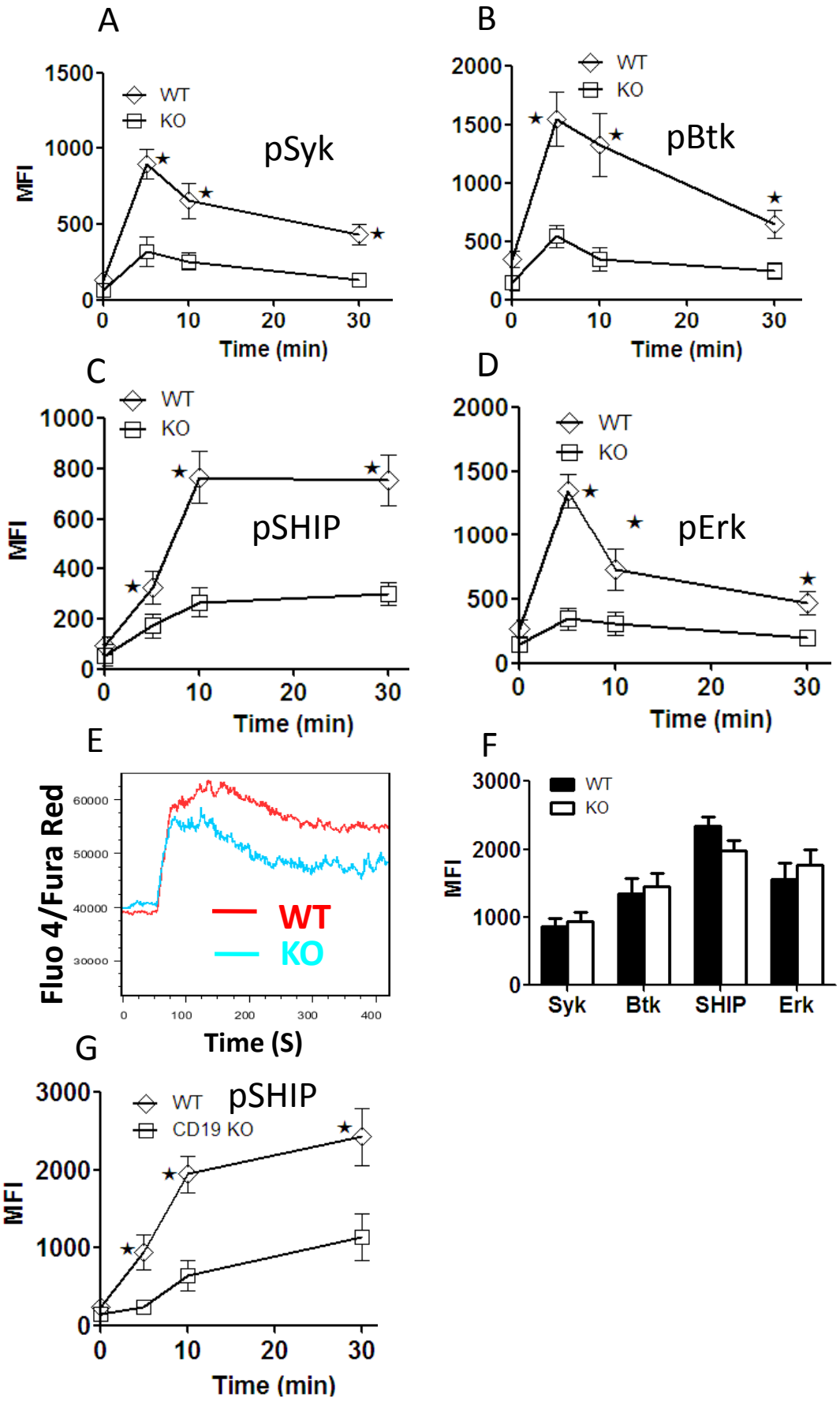
A
B
C
D
E
F
G
pSyk
pBtk
pSHIP
pErk
WT
KO
CD19 KO
MFI
Time (min)
Fluo 4/Fura Red
Time (S)
Syk
Btk
SHIP
Erk

Fig.S2 BCR signaling is reduced in Mst1 KO or CD19 KO B cells. B cells from WT and Mst1 KO or CD19 KO mice were stained with anti-B220 antibody at 4℃ for 30 minutes. And then cells incubated with soluble Fab′–anti-IgG+M plus streptavidin at 37° C for indicated times. Cells were fixed, permeablized and stained with anti-pSyk (A), anti-pBtk (B), anti-pSHIP (C) ,anti-pErk (D) , antibodies for the total proteins above described (F) and anti-pSHIP antibodies in CD19 KO mice (G) . Then samples were analyzed by flow cytometry. Ca^{2+} flux analysis of splenic B cells activated with soluble mB-Fab′–anti-Ig plus streptavidin using flow cytometry (E). Shown are the average MFI (±SD) from three independent experiments. *$p < 0.01$.

Fig.S3

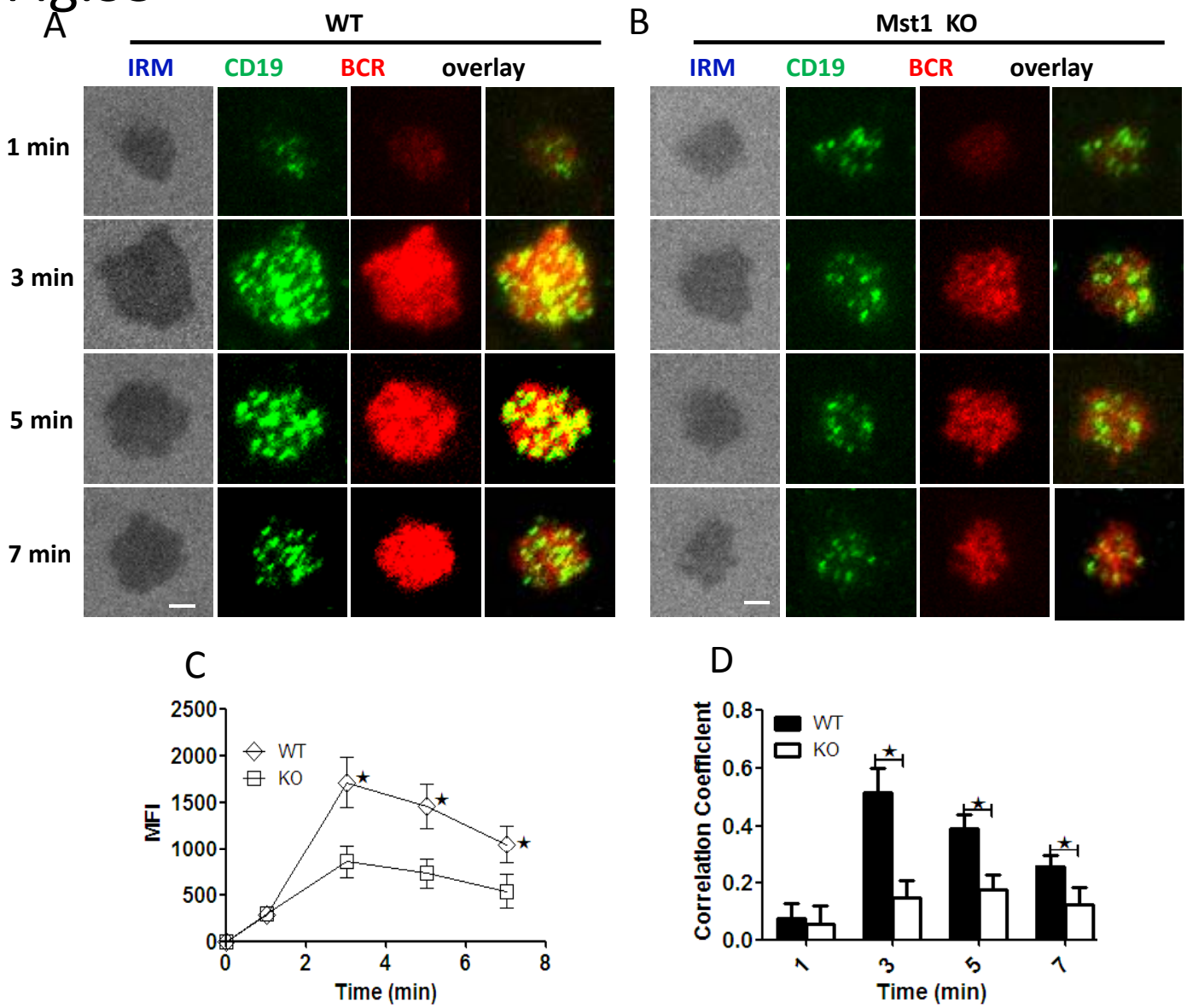
A
WT
IRM
CD19
BCR
overlay
1 min
3 min
5 min
7 min
B
Mst1 KO
IRM
CD19
BCR
overlay
C
MFI
WT
KO
Time (min)
D
Correlation Coefficient
WT
KO
Time (min)

Fig.S3 CD19 recruitment is decreased in Mst1 KO B cells. Splenic B cells from WT and Mst1 KO mice were incubated with AF546-mB-Fab′–anti-Ig tethered to lipid bilayers at 37° C for indicated times. Cells were fixed, permeabilized, and stained for CD19 using a specific mAb and AF488-conjugated secondary Ab. Cells were analyzed using TIRFm (A and B). The MFI of CD19 staining in the B-cell contact zone was quantified (C). Shown are representative images and TIRFM analysis of the spatial relationship of BCR with CD19 in the contact zone of splenic B cells incubated with membrane-tethered Fab′–anti-Ig. The correlation coefficients between BCR and CD19 staining were determined using NIS-Elements AR 3.2 software (D).

Fig.S4

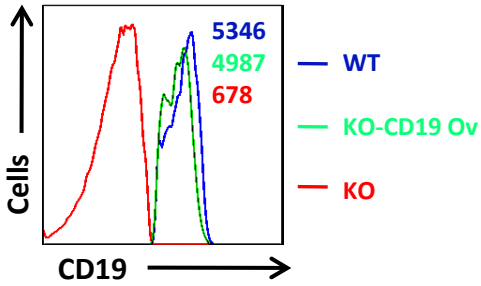

Fig.S4 Overexpression of CD19 recovers the level of CD19 in Mst1 KO B cells. WT Bone marrow cells were transduced with GFP tagged retroviral vector only (WT). Mst1 KO bone marrow B cells were transduced with GFP tagged retroviral vector expressing with (KO-CD19 Ov) or without CD19 (KO) then transferred into CD45.1 mice recipients together with CD45.1 WT bone marrow B cells. After 8 weeks reconstitution, $CD45.2^{+}$ GFP^{+} donor derived splenic B cells were stained with CD19 antibody to check the surface expression of CD19 after bone marrow reconstitution.