



Fumarate suppresses B-cell activation and function through direct inactivation of LYN

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Activated B cells increase central carbon metabolism to fulfill their bioenergetic demands, yet the mechanistic basis for this, as well as metabolic regulation in B cells, remains largely unknown. Here, we demonstrate that B-cell activation reprograms the tricarboxylic acid cycle and boosts the expression of fumarate hydratase (FH), leading to decreased cellular fumarate abundance. Fumarate accumulation by FH inhibition or dimethyl-fumarate treatment suppresses B-cell activation, proliferation and antibody production. Mechanistically, fumarate is a covalent inhibitor of tyrosine kinase LYN, a key component of the BCR signaling pathway. Fumarate can directly succinate LYN at C381 and abrogate LYN activity, resulting in a block to B-cell activation and function in vitro and in vivo. Therefore, our findings uncover a previously unappreciated metabolic regulation of B cells, and reveal LYN is a natural sensor of fumarate, connecting cellular metabolism to B-cell antigen receptor signaling.

B cells are key elements of adaptive immune responses that combat pathogen infections by producing highly specific antibodies¹. Pathogen capture and antigen engagement can lead to B-cell receptor (BCR) crosslinking and phosphorylation of immunoreceptor tyrosine activation motifs (ITAMs) that are present in the cytoplasmic tail of the BCR^{2,3}. ITAMs contain YxxL pairs for recognition and phosphorylation by Src family kinases, and in B cells, the kinase LYN is primarily responsible for ITAMs phosphorylation. In general, once ITAMs are phosphorylated, splenic tyrosine kinase (SYK) is recruited through its tandem Src homology 2 domain and phosphorylated by LYN, which initiates the activation of downstream signaling cascades such as phospholipase- $\text{C}\gamma 2$, phosphoinositide 3-kinase (PI3K) and Bruton's tyrosine kinase, ultimately leading to B-cell activation^{4,5}. Activation of PLC γ produces InsP and induces the release of endoplasmic reticulum calcium ions (Ca^{2+}), initiating Ca^{2+} entry into the cytoplasm⁶ and driving B-cell proliferation. In the germinal center (GC), fully activated B cells undergo class-switch recombination and somatic hypermutation⁷. GC B cells expressing the highest affinity are positively selected for clonal expansion, producing high-affinity antibodies for humoral immunization⁷. Notably, aberrant BCR signaling is closely associated with malignant B-cell proliferation as well as autoimmune diseases⁸. Therefore, studying the regulatory mechanisms of BCR signaling activation is essential for understanding B-cell immunity.

Metabolic alterations are critical for T lymphocyte activation, proliferation and effector functions^{9,10}. In contrast, our current knowledge of metabolic changes within B cells is very limited. Currently, we know that increased glucose uptake and glycolysis enhance activated B-cell proliferation and production of antibodies^{11–13}. On activation, B cells also increase their mitochondrial mass and protein content^{11,14}. And, restriction of glycogen synthase 3 on mitochondrial biogenesis, glycolysis and reactive oxygen species production can be observed in naïve recirculating B cells¹⁴. Although metabolic reprogramming may meet the bioenergetic or

biosynthetic requirements when B cells are activated, the metabolic regulation of B-cell activation signaling remains to be determined.

Several metabolites of the tricarboxylic acid (TCA) cycle, including acetyl CoA, α -ketoglutarate, succinate and fumarate, are endowed with signaling functions in tumorigenesis and certain immune cell-mediated immune responses by inhibiting enzymes or driving covalent modifications of proteins^{15–17}. For instance, modification of glycerol-3-phosphate dehydrogenase by fumarate inhibits its glycolysis in macrophages and some lymphocytes^{18,19}. Increased fumarate was also found to succinate gasdermin D and prevent macrophage pyroptosis²⁰. In fumarate hydratase (FH)-deficient mice, aconitase 2 (ACO2) is inhibited by fumarate-mediated succination²¹. Additionally, high levels of fumarate promote the nuclear factor (erythroid-derived 2)-like 2 (Nrf2)-mediated antioxidant response pathway by succinating Kelch-like ECH-associated protein 1 (KEAP1) in some FH-associated tumors^{22,23}. And, fumarate accumulation induced by *SDHA* mutation in persistent polyclonal B-cell lymphocytosis (PPBL) B cells leads to KEAP1–Nrf2 activation²⁴. In treatment of multiple sclerosis, dimethyl-fumarate (DMF) treatment is associated with suppressed memory T cells and pathogenic B-cell functions^{25–28}.

Here we report that fumarate can regulate B-cell activation and function by directly inactivating LYN. B-cell activation leads to increased expression of FH and a corresponding decrease in fumarate levels. Fumarate accumulation by FH inhibition or DMF treatment restrains B-cell activation and function in vitro and in vivo. Using bioorthogonal probes to enrich and identify the modified proteins, we found that LYN was succinated by fumarate on C381, leading to inhibition of LYN activity and signaling, resulting in blocked B-cell activation and response to pathogens. Moreover, mutations at the C381 site (for example, C381S) effectively block LYN phosphorylation as well as B-cell activation and BCR signaling. Thus, these findings reveal a role for LYN in sensing fumarate metabolism alteration and might provide new insights into the metabolic regulation of B-cell function.

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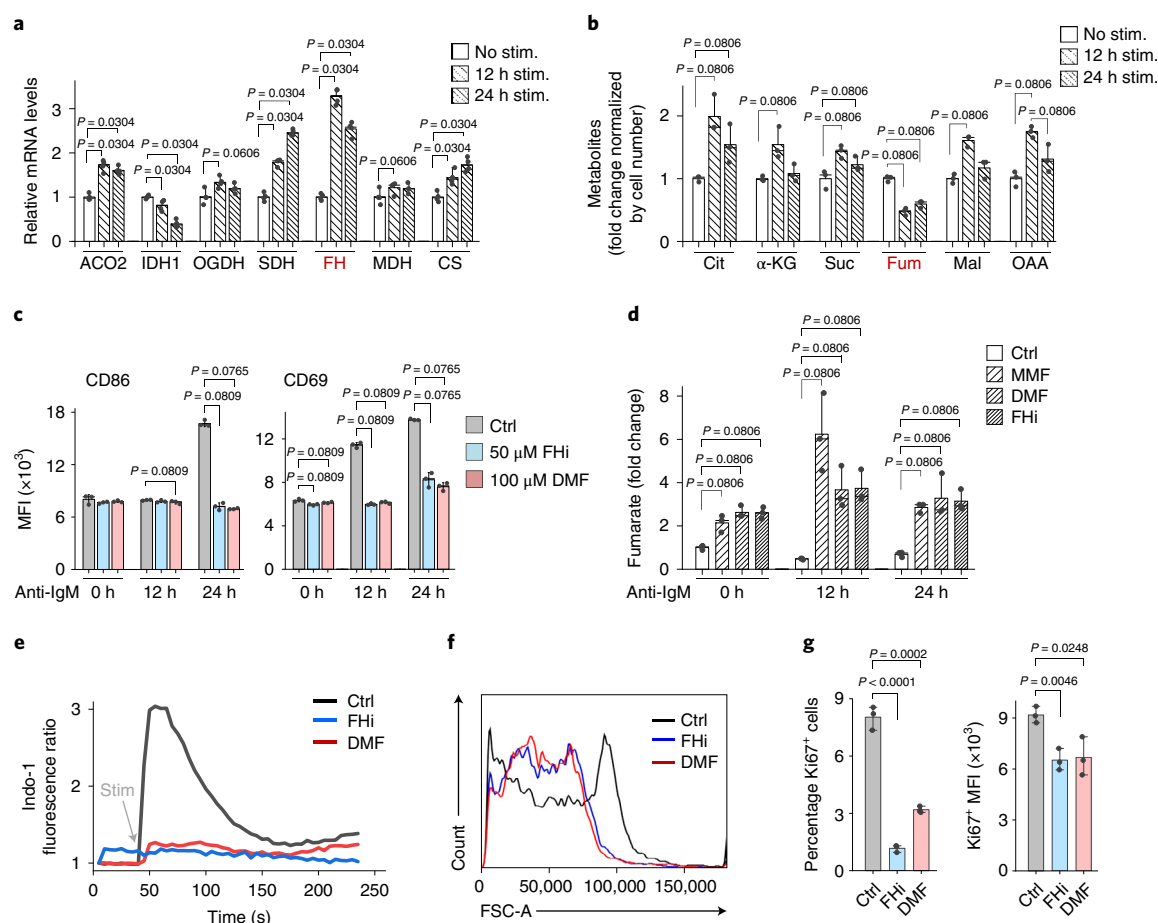


Fig. 1 | Identification of fumarate as a critical metabolic regulator of B-cell activation. **a, b**, Mouse naive B cells were stimulated with $10 \mu\text{g ml}^{-1}$ F(ab')_2 fragments of antimouse IgM antibody for 12 h, 24 h or left unstimulated (no stim.). The levels of mRNA (**a**) and metabolites (**b**) were measured by quantitative RT-PCR and LC-MS, respectively, and normalized to the unstimulated samples (set as 1.0). Cit, Citrate; α -KG, α -ketoglutarate; Suc, succinate; Fum, fumarate; Mal, malate; OAA, oxaloacetate. Stim stands for stimulation ($n=3$ independent wells). **c**, Mouse naive B cells were treated with DMSO (Ctrl), $50 \mu\text{M}$ FHI or $100 \mu\text{M}$ DMF for 1 h and then activated with $10 \mu\text{g ml}^{-1}$ F(ab')_2 fragments of antimouse IgM antibody (anti-IgM) for the indicated time. The MFI of CD86⁺ B cells and CD69⁺ B cells was measured by FACS analysis ($n=3$ independent wells). **d**, Mouse naive B cells were treated with MMF ($200 \mu\text{M}$), DMF ($100 \mu\text{M}$), FHI ($50 \mu\text{M}$) or DMSO (Ctrl) for 1 h, followed by stimulation with $10 \mu\text{g ml}^{-1}$ F(ab')_2 fragment of antimouse IgM antibody (anti-IgM) for the indicated times. The cellular levels of fumarate were analyzed by LC-MS and normalized to the unstimulated samples ($n=3$ independent wells). **e**, Mouse naive B cells pretreated with FHI ($50 \mu\text{M}$), DMF ($100 \mu\text{M}$) or DMSO (Ctrl) for 1 h were activated with $10 \mu\text{g ml}^{-1}$ F(ab')_2 fragments of antimouse IgM antibody (stim). Ca^{2+} flux was determined by indo-1-AM staining and FACS analysis. **f, g**, After 48 h of stimulation, cell size (**f**) and the percentage and MFI of Ki67⁺ B cells (**g**) were measured by FACS analysis. The data in **a** and **b** are normalized to the average of no anti-IgM stimulation samples. The data in **c** and **d** are normalized to the average of control samples. The data in **g** are normally distributed, and the differences were evaluated by using a two-tailed Student's *t*-test. The data in **a–d** are non-Gaussian distributed, and the differences were evaluated by using a two-tailed Mann-Whitney *U*-test. Data are the mean $\pm$ s.d. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, based on a Student's *t*-test or Mann-Whitney *U*-test.

Results

Fumarate is a metabolic regulator of B-cell activation. The TCA cycle is the central hub of oxidative metabolism (Extended Data Fig. 1a)²⁹. To investigate the regulatory role of TCA cycle in B cells, we first compared the expression levels of TCA cycle enzymes in naive and activating B cells triggered by F(ab')_2 fragments of antimouse IgM antibody. The messenger RNA levels of ACO2, SDH, FH and C381S were increased to different degrees during B-cell activation, while the upregulation of FH was the greatest (Fig. 1a). Consistent with this, the activation of B cells led to a strong increase in FH protein levels (Extended Data Fig. 1b). FH converts fumarate to malate. In keeping with the expression data (Fig. 1a and Extended Data Fig. 1b), results from liquid chromatograph–mass spectrometry (LC-MS) analysis showed most TCA cycle metabolites were increased except fumarate that declined notably (Fig. 1b and Extended Data Fig. 1c), demonstrating the distinctiveness of fumarate and arguing

for a role for it in B-cell activation. The increased levels of the other TCA cycle metabolites may be caused by metabolic compensation due to fumarate depletion. To further determine the relevance of this metabolic change to B-cell activation, we treated B cells with different TCA cycle metabolites as well as fumarate hydratase-IN-1, an FH inhibitor (FHI). Inhibition of FH strongly suppressed B-cell activation as indicated by the decreased mean fluorescence intensity (MFI) and percentage of activation markers (CD86- and CD69-positive cells), compared with minimal effects of other TCA cycling intermediates (Extended Data Fig. 1d). The inhibitory effect of FHI on B-cell activation was further confirmed by treatment of cells with different concentrations of FHI or with FHI for different times (Fig. 1c and Extended Data Fig. 1e–g). FH inhibition led to the accumulation of fumarate (Fig. 1d), raising the possibility that the increased fumarate mediates the blockade of B-cell activation by FHI. In support of this, treatment with exogenous fumarate

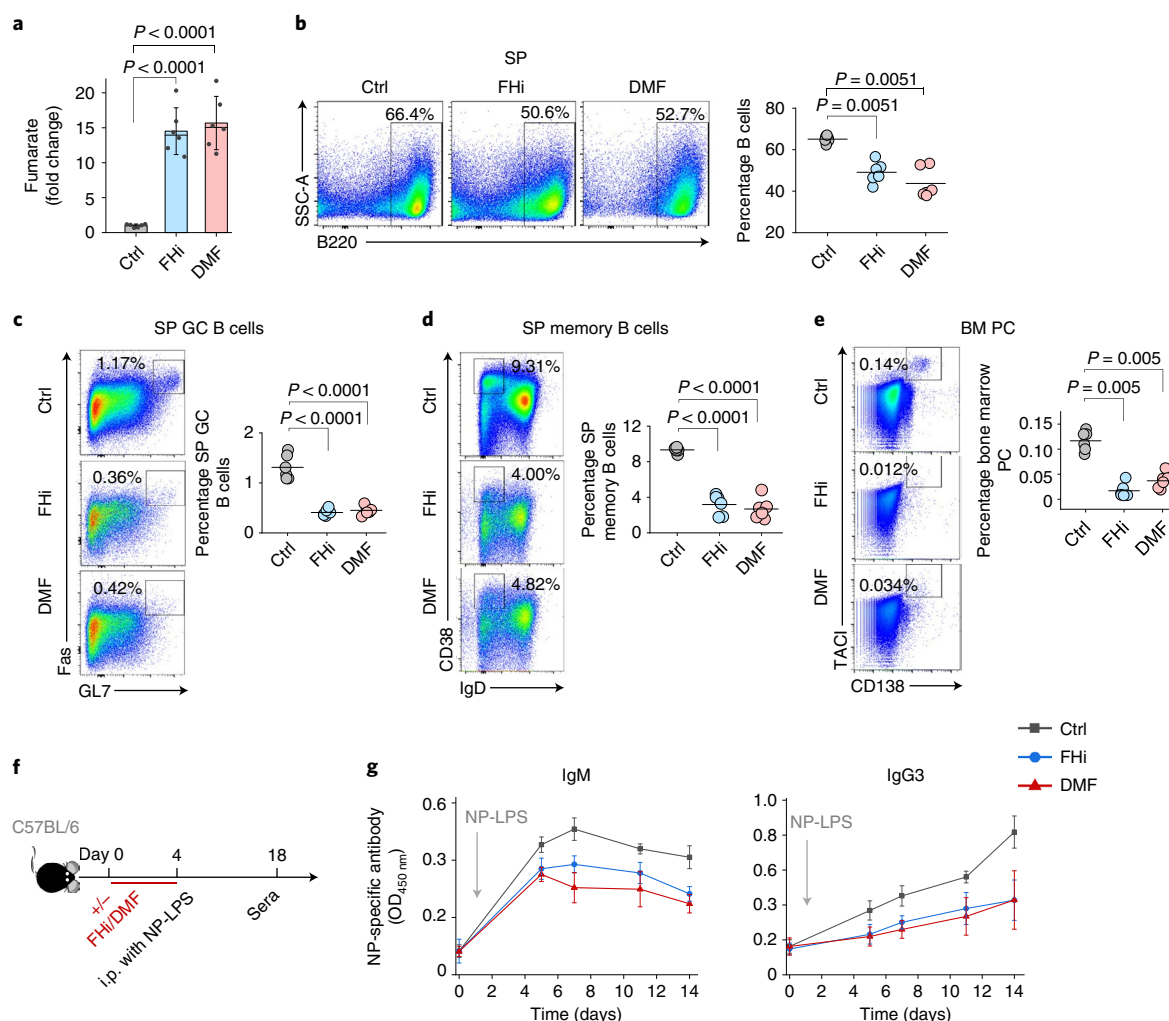


Fig. 2 | Fumarate inhibits B-cell differentiation and function in vivo. **a–e**, C57BL/6 mice were injected intraperitoneally (i.p.) with DMSO (Ctrl) or FHi (25 mg kg⁻¹ mouse), or treated with DMF (50 mg kg⁻¹ mouse) by oral gavage for 4 days ($n = 6$ mice per group). Then mice were immunized with NP-LPS (10 μg per mouse) for 7 days. The abundance of fumarate in blood was examined by LC-MS (**a**). Percentage of B220⁺ cells (**b**), GC B cells (**c**), memory B cells (**d**) in spleens and bone marrow plasma cells (**e**) was determined by FACS analysis. GC B cells (GL7⁺, Fas⁺) were pregated on B220⁺ cells. SP, spleen; PC, plasma cells; BM, bone marrow; Mem B, memory B cells. **f,g**, C57BL/6 mice were injected intraperitoneally (i.p.) with DMSO (Ctrl) or FHi (25 mg kg⁻¹ mouse), or treated with DMF (50 mg kg⁻¹ mouse) by oral gavage for 4 days ($n = 6$ mice per group). Mice were then immunized with NP-LPS (10 μg per mouse) for another 2 weeks. IgM and IgG3 responses in serum were determined by ELISA. The data in **a** are normalized to the average of control samples. The data in **a**, **c**, **d** and **g** are normally distributed, and the differences were evaluated by using a two-tailed Student's *t*-test. The data in **b** and **e** are non-Gaussian distributed, and the differences were evaluated by using a two-tailed Mann-Whitney *U*-test. Data are the mean $\pm$ s.d. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, based on a Student's *t*-test or Mann-Whitney *U*-test.

(monomethyl fumarate (MMF) or DMF) restrained B-cell activation in both concentration- and time-dependent manners (Fig. 1c and Extended Data Fig. 1e–g). Similar to FH inhibition by FHi, silencing of FH expression led to fumarate accumulation and correspondingly suppressed B-cell activation (Extended Data Fig. 1h,i). This regulation existed only during anti-IgM stimulation and not within activated B cells (Extended Data Fig. 2a), suggesting that fumarate regulates the transition of naive B cells into the active state, but not vice versa.

Activated B cells increase cytosolic Ca²⁺ mobilization and undergo cell growth and fast proliferation^{6,7}. We thus further assessed the effect of fumarate on B-cell activation by measuring Ca²⁺ mobilization on BCR crosslinking. Fumarate accumulation almost completely abolished cytosolic Ca²⁺ mobilization (Fig. 1e) and strongly dampened cell growth stimulated by anti-IgM antibody (Fig. 1f and Extended Data Fig. 2b–d). Moreover, B cells

proliferated to a lower extent in response to fumarate accumulation as indicated by Ki67 staining and cell counting, respectively (Fig. 1g and Extended Data Fig. 2e). In keeping with this, treatment with FHi or DMF induced cell death as indicated by increased percentage of 7-aminoactinomycin D (7AAD)-positive cells and 4,6-diamidino-2-phenylindole (DAPI) staining (Extended Data Fig. 2f–i). Collectively, these findings indicate that fumarate has a negative role in regulating B-cell activation, Ca²⁺ mobilization, B-cell growth and proliferation.

Fumarate represses B-cell differentiation and function in vivo. We next investigated the effect(s) of fumarate accumulation on B cells in vivo. 4-hydroxy-3-nitrophenylacetyl (NP)-lipopolysaccharide (LPS)-immunized mice were subjected to intraperitoneal injection of FHi or oral gavage administration of DMF. Compared to control animals, the FHi- or DMF-treated mice displayed increased levels

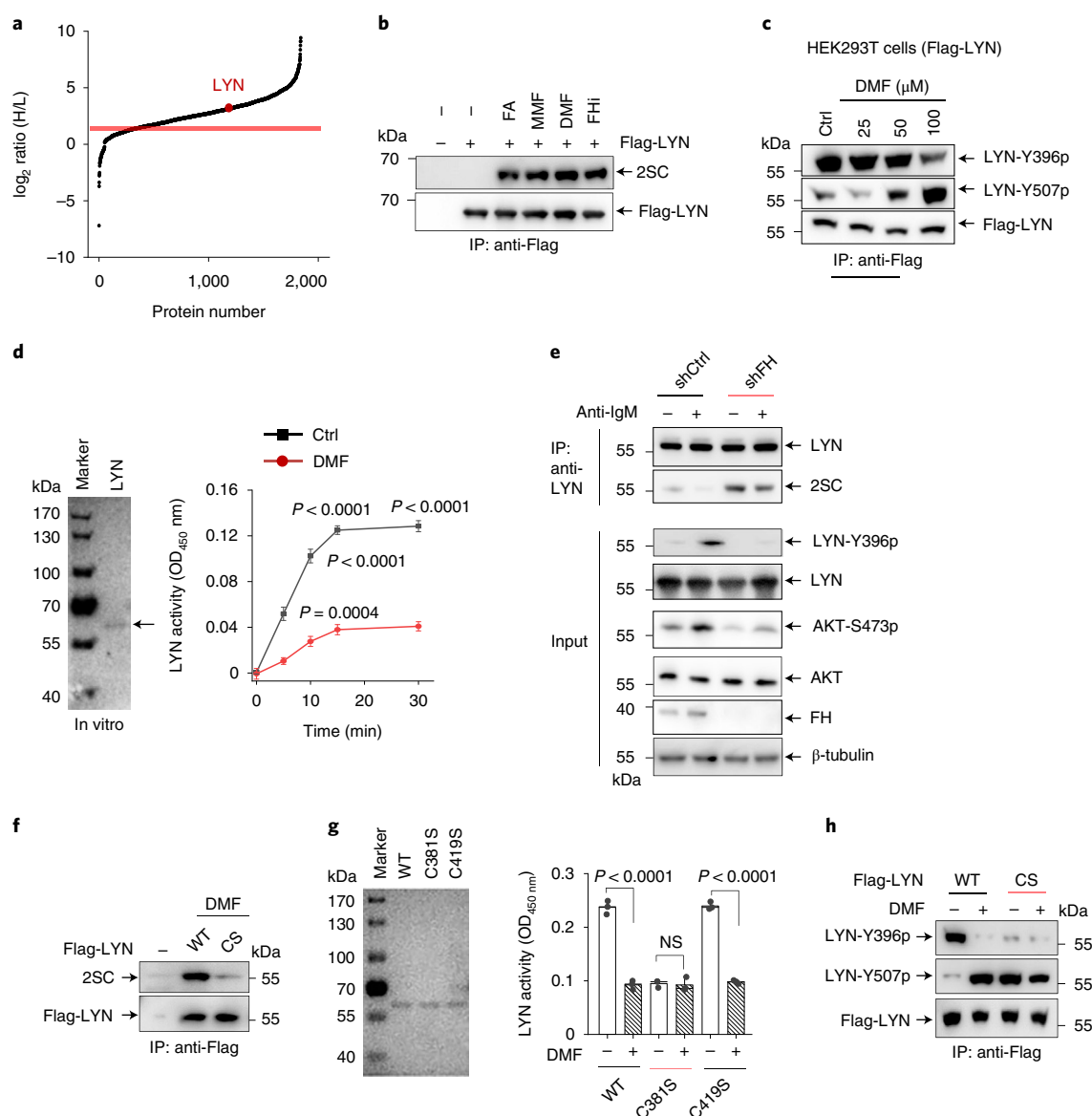


Fig. 3 | Fumaric esters succinate LYN and restrain its activity. **a**, B-cell lysates were labeled with IA-alkyne probe without (–) or with DMF (100 μ M) competition. Then the enriched proteins were digested for MS analysis as described in Extended Data Fig. 3a. Competition ratios of the DMF modified proteins from B cells are shown ($n = 3$ independent samples). **b,c**, Human embryonic kidney 293T (HEK293T) cells transfected with Flag-LYN were treated with 1 mM fumarate (FA), 200 μ M MMF, 100 μ M DMF or 50 μ M FHi for 2 days (**b**), or treated with DMSO (Ctrl) or increasing concentrations of DMF for 2 days (**c**). Flag-LYN protein was immunoprecipitated using anti-Flag beads and immunoblotted with indicated antibodies. **d**, Purified LYN proteins were analyzed by SDS-PAGE followed by Coomassie Blue staining (left panel). Recombinant LYN was treated with DMSO (Ctrl) or 100 μ M DMF for 30 min. The kinetics of LYN kinase activity was measured by ELISA using biotin-poly (Glu4:Tyr, 4:1) as described in the Methods (right panel). **e**, Mouse naive B cells were infected with lentivirus expressing FH shRNA (shFH) or scramble shRNA (shCtrl) for 48 h. Then cells were stimulated with 10 μ g ml $^{-1}$ F(ab') $_2$ fragments of antimouse IgM antibody (anti-IgM) for 5 min or left unstimulated (–). LYN protein was immunoprecipitated using anti-LYN antibody and immunoblotted with indicated antibodies. **f,h**, HEK293T cells transfected with WT or C381S (CS) mutated Flag-LYN were treated with DMSO (–) or 100 μ M DMF for 2 days. FLAG-LYN protein was immunoprecipitated using anti-Flag beads and immunoblotted with indicated antibodies. **g**, Purified WT LYN, LYN-C381S and LYN-C419S proteins were analyzed by SDS-PAGE followed by Coomassie Blue staining (left panel). The proteins were incubated with DMSO (Ctrl) or DMF (100 μ M) for 30 min and then LYN kinase activity was analyzed by ELISA (right panel). The data in **d** and **g** are normally distributed, and the differences were evaluated by using a two-tailed Student's *t*-test. Data are the mean $\pm$ s.d. Each experiment was performed three independent times. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, based on a Student's *t*-test.

of blood fumarate (Fig. 2a) and a decrease in the total number of B cells in the spleen as indicated by B220 staining (Fig. 2b). Notably, fumarate-accumulated mice showed a three- to fourfold reduction in the number of GC B cells (Fig. 2c), and an approximately two-fold decrease in the population of memory B cells relative to vehicle control mice (Fig. 2d). Moreover, a nearly fourfold decline in the

number of plasma cells was found in FHi- or DMF-treated mice (Fig. 2e). The reduction in B-cell differentiation, especially in GC cells and plasma cells, suggests a possible defect in antibody production capacity in fumarate-accumulated mice. Indeed, the NP-specific IgM and IgG3 antibody responses were strongly decreased in FHi- or DMF-treated mice compared to vehicle control mice (Fig. 2f,g).

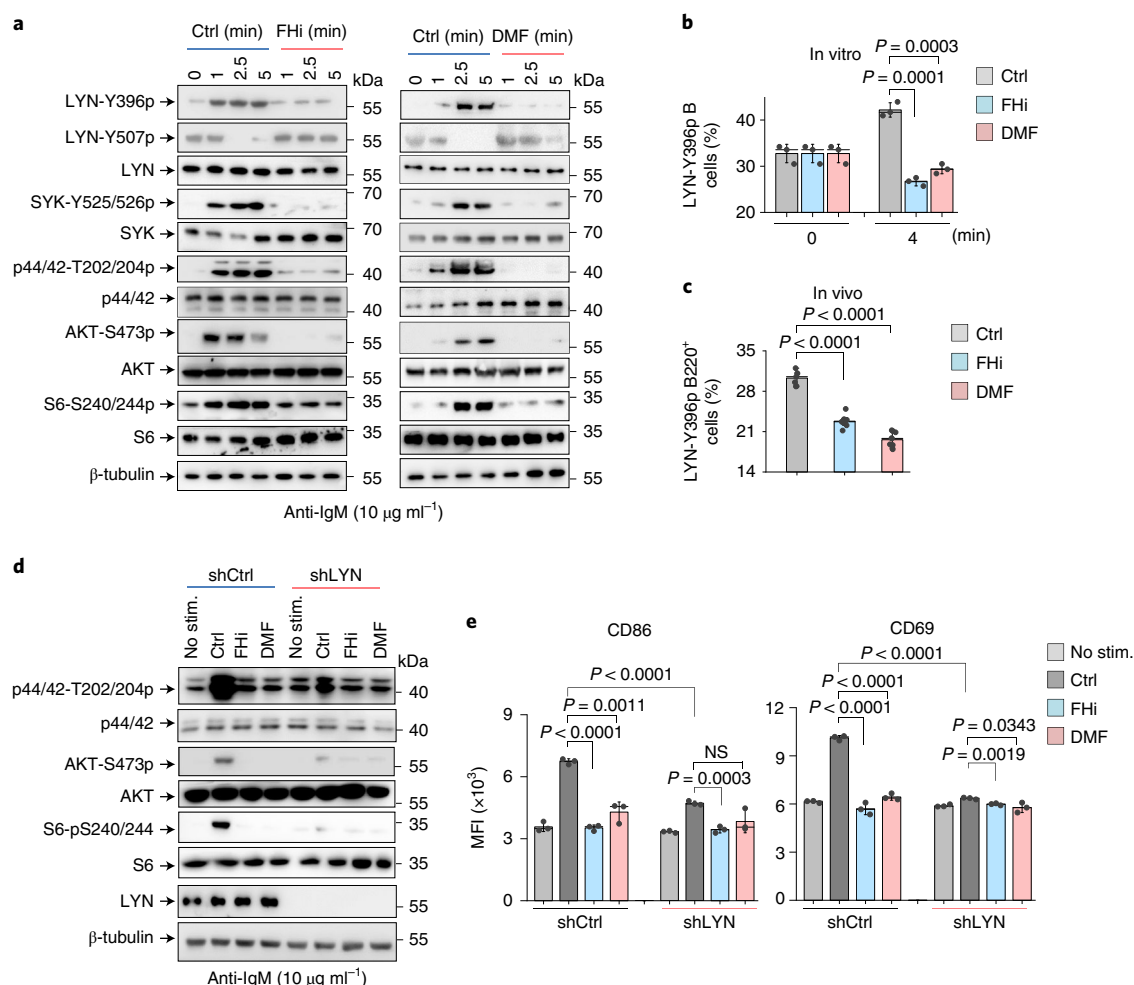


Fig. 4 | Fumarate suppression of LYN abolishes BCR signaling in vitro and in vivo. **a**, Mouse naive B cells were treated with DMSO (Ctrl), 50 μ M FHi (left panel) or 100 μ M DMF (right panel) for 1 h prior to stimulation with 10 μ g ml⁻¹ F(ab')₂ fragments of anti-mouse IgM antibody (anti-IgM) for the indicated times. Protein expression was determined by western blot using indicated antibodies. **b**, Mouse naive B cells treated with DMSO (Ctrl), 50 μ M FHi or 100 μ M DMF for 1 h before stimulated with 10 μ g ml⁻¹ F(ab')₂ fragments of anti-mouse IgM antibody for 0 or 4 min. The phosphorylation levels of LYN (Y396) were analyzed by FACS ($n = 3$ independent wells). **c**, C57BL/6 mice were injected intraperitoneally (i.p.) with DMSO (Ctrl) or FHi (25 mg kg⁻¹ mouse), or treated with DMF (50 mg kg⁻¹ mouse) by oral gavage for 4 days ($n = 7$ mice per group). Mice were then immunized with NP-LPS (10 μ g per mouse) for another 12 h. B220⁺ B cells in spleens were stained with anti-P-Y396LYN antibody and analyzed by FACS. **d**, Mouse naive B cells infected with lentivirus expressing LYN shRNA (shLYN) or scramble shRNA (shCtrl) were treated with 100 μ M DMF, or 50 μ M FHi for 1 h. Cells were then stimulated without (no stim.) or with 10 μ g ml⁻¹ F(ab')₂ fragments of anti-mouse IgM antibody for another 5 min. Protein expression was determined by western blot analysis using indicated antibodies. **e**, Mouse naive B cells were infected with lentivirus expressing LYN shRNA (shLYN) or scramble shRNA (shCtrl) were treated with 50 μ M FHi, or 100 μ M DMF for 1 h. Then cells were stimulated with 10 μ g ml⁻¹ F(ab')₂ fragments of anti-mouse IgM antibody for 1 h or left unstimulated (no stim.). The MFI of CD86⁺ B cells and CD69⁺ B cells was measured by FACS analysis respectively ($n = 3$ independent wells). The data in **b**, **c** and **e** are normally distributed, and the differences were evaluated by using a two-tailed Student's *t*-test. Data are the mean $\pm$ s.d. Each experiment was performed three independent times. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, based on a Student's *t*-test.

Taken together, these data demonstrate that increased fumarate may inhibit B-cell differentiation and antibody production.

Fumaric esters succinate LYN and restrain its activity. The above findings prompted us to further explore the underlying mechanism(s) for the effect of fumarate on B cells. Supplementation with TCA cycle metabolites except fumarate minimally affected B-cell activation (Extended Data Fig. 1d), indicating that the regulatory effect fumarate on B cells may not reply on metabolic reprogramming. Alternatively, as an electrophile, fumarate can react with nucleophilic cysteine residues on proteins and form S-(2-succinyl) cysteine (2SC) through a Michael addition reaction³⁰. This unique reactivity of fumarate suggests that its accumulation

may impart a succinated modification to certain proteins. By using iodoacetamide-alkyl (IA-alkyne) as an activity-based probe for competitive activity-based protein profiling followed by gene ontology analysis³¹ (Extended Data Fig. 3a,b and Supplementary Data 1), we found that LYN was enriched notably by fumarate ester modification, indicating that it might be a potential target for succination by fumarate (Fig. 3a and Extended Data Fig. 3c).

LYN is rapidly phosphorylated and activated on BCR crosslinking³² and the lack of LYN in mice results in reduced B-cell numbers and impaired B-cell function³³. To determine whether LYN is a direct succination substrate for fumarate, we performed an in vitro succination assay. Notably, treatment with fumaric ester (fumarate, MMF or DMF) promoted LYN succination as detected by 2SC

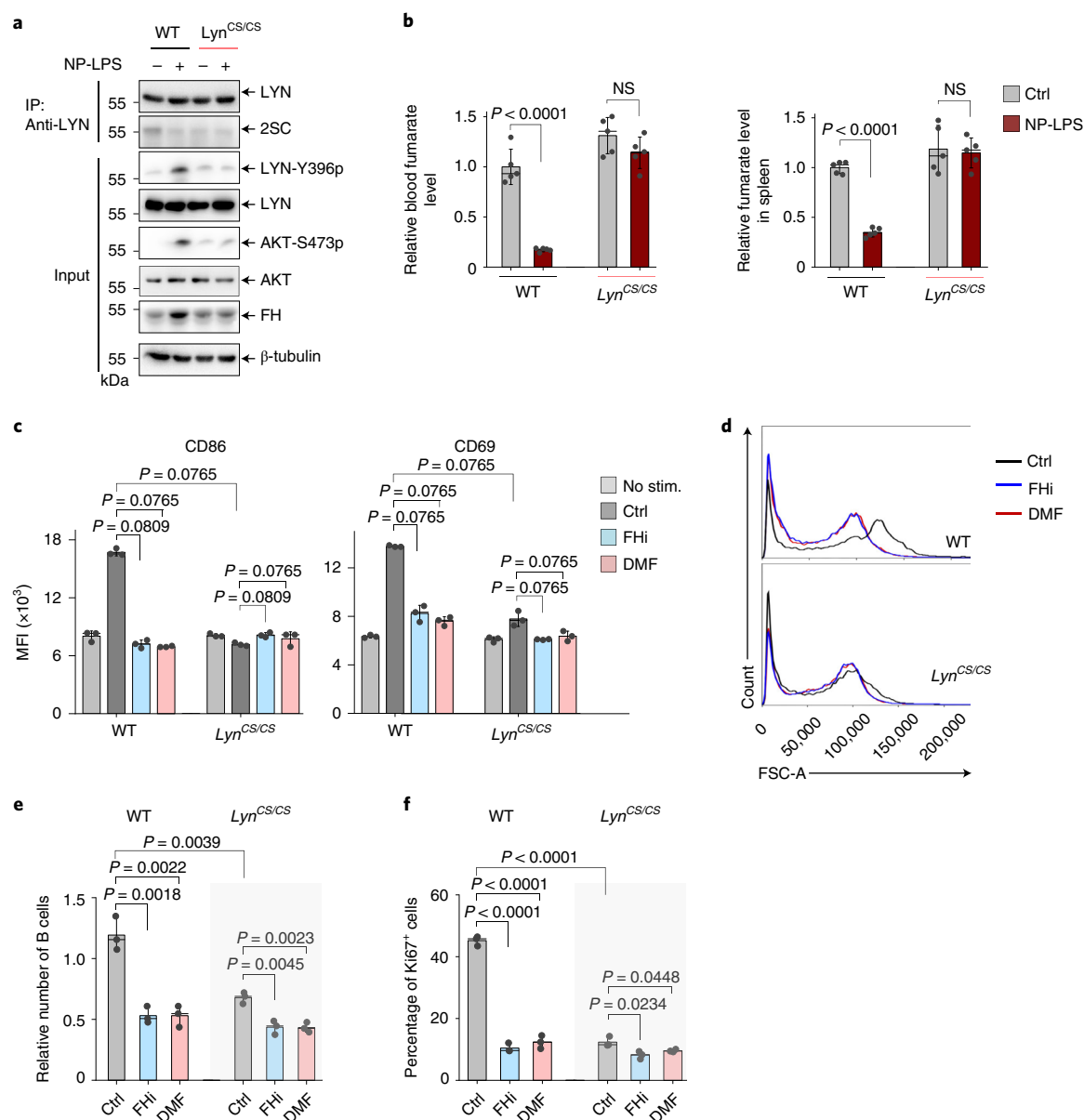


Fig. 5 | LYN-C381 is critical for BCR signaling and B-cell activation in vivo. **a,b**, $Lyn^{WT/WT}$ and $Lyn^{CS/CS}$ ($Lyn^{C381S/C381S}$) mice were immunized with NP-LPS (10 μ g per mouse) for 12 hours. **a**, Mouse B cells were enriched and isolated from $Lyn^{WT/WT}$ and $Lyn^{CS/CS}$ ($Lyn^{C381S/C381S}$) mice and were subjected to immunoprecipitation using anti-LYN antibody and immunoblotted with indicated antibodies. **b**, The abundance of fumarate in blood (left panel) and spleens (right panel) was examined by LC-MS and normalized to the unimmunized WT mice ($n=5$ mice per group). **c**, Mouse naive B cells enriched and isolated from $Lyn^{WT/WT}$ and $Lyn^{CS/CS}$ ($Lyn^{C381S/C381S}$) mice were treated with DMSO (Ctrl), FHi (50 μ M) or DMF (100 μ M) for 1 h. After stimulation without (no stim.) or with 10 μ g ml $^{-1}$ F(ab') $_2$ fragments of antimouse IgM antibody for 24 hs, the MFI of CD86 $^+$ B cells and CD69 $^+$ B cells was measured by FACS analysis ($n=3$ independent wells). **d-f**, Mouse naive B cells enriched and isolated from $Lyn^{WT/WT}$ and $Lyn^{CS/CS}$ ($Lyn^{C381S/C381S}$) mice were treated with DMSO (Ctrl), FHi (50 μ M) or DMF (100 μ M) for 1 h and then stimulated with 10 μ g ml $^{-1}$ F(ab') $_2$ fragments of antimouse IgM antibody. **d**, Cell size was measured by FACS analysis after 48 hours of activation. **e,f**, Cell number was counted (**e**) and the amount of Ki67 $^+$ B cells was determined by FACS analysis (**f**) after 72 h of activation ($n=3$ independent wells). The data in **b** are normalized to the average of WT no NP-LPS treatment samples. The data in **e** are normalized to the average of control samples. The data in **b**, **e** and **f** are normally distributed, and the differences were evaluated by using a two-tailed Student's *t*-test. The data in **c** are non-Gaussian distributed, and the differences were evaluated by using a two-tailed Mann-Whitney *U*-test. Data are the mean $\pm$ s.d. Each experiment was performed three independent times. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, based on a Student's *t*-test or Mann-Whitney *U*-test.

blotting (Fig. 3b). Generally, LYN activity can be promoted by Tyr396 phosphorylation and inhibited by Tyr507 phosphorylation, while both sites can undergo spontaneous and stoichiometric autophosphorylation³⁴. Consistent with this, DMF incubation resulted in decreased Tyr396 phosphorylation and increased Tyr507 phosphorylation (Fig. 3c), indicating succination may lead to a

block to LYN activity. Indeed, results from in vitro kinase assays indicated that DMF incubation can reduce LYN activity in both concentration- and time-dependent manners (Fig. 3d and Extended Data Fig. 3d). These findings were also observed in cells. Ablation of FH caused a strong increase in LYN succination and dampened the phosphorylation of LYN on Tyr396 (Fig. 3e).

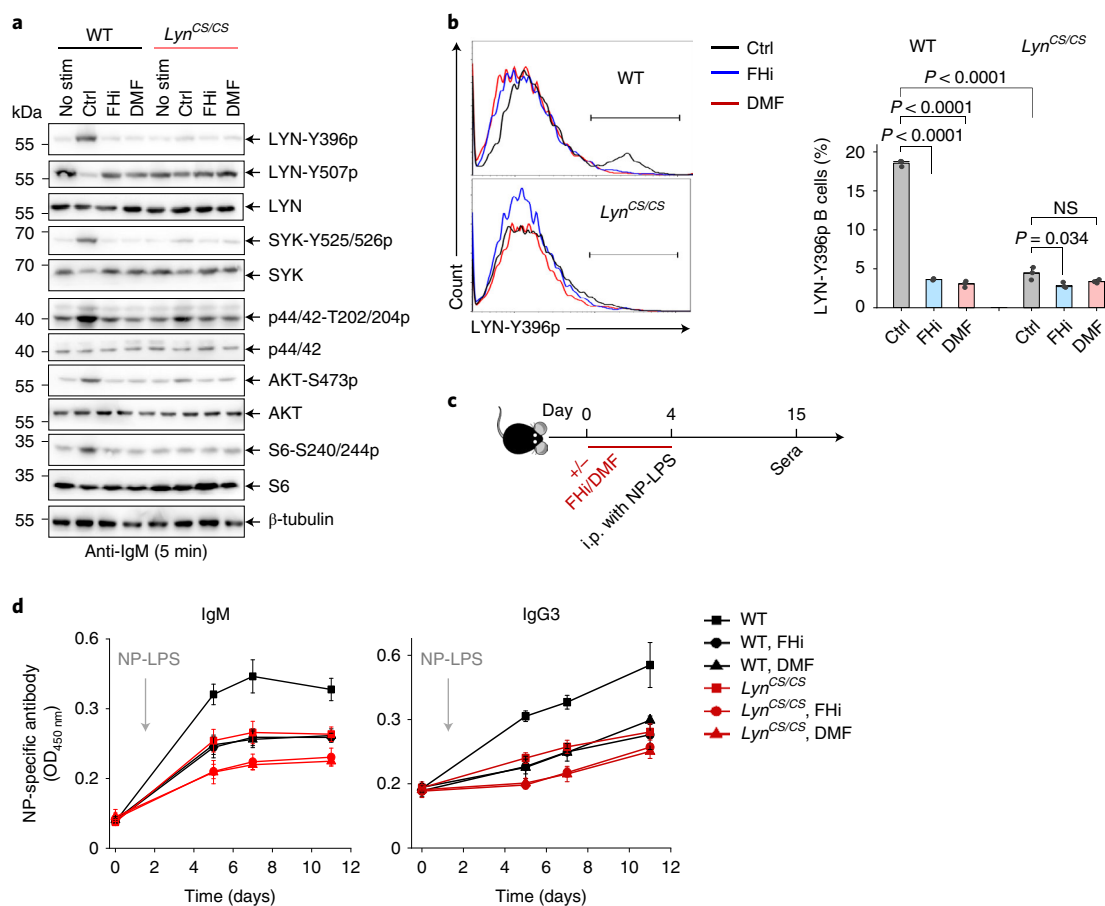


Fig. 6 | *Lyn*^{C381S/C381S} transgenic mice display suppressed LYN activity and B-cell function. **a,b**, Mouse naive B cells enriched and isolated from *Lyn*^{WT/WT} and *Lyn*^{C381S/C381S} (*Lyn*^{C381S/C381S}) mice were treated with DMSO, FHi (50 μ M) or DMF (100 μ M) for 1 h. After 10 μ g ml⁻¹ F(ab')₂ fragments of antimouse IgM antibody (anti-IgM) stimulation for 5 min, cells were subjected to immunoblotting with the indicated antibodies (**a**), or fixed and penetrated for FACS analysis of LYN-Y396 phosphorylation (**b**) ($n = 3$ independent wells). **c,d**, *Lyn*^{WT/WT} and *Lyn*^{C381S/C381S} (*Lyn*^{C381S/C381S}) mice were injected intraperitoneally (i.p.) with DMSO or FHi (25 mg kg⁻¹ mouse), or treated with DMF (50 mg kg⁻¹ mouse) by oral gavage for 4 days ($n = 6$ mice per group). **c**, Then mice were immunized with NP-LPS (10 μ g per mouse) as indicated times. **d**, IgM and IgG3 responses in serum were determined by ELISA. The data in **b** are normally distributed, and the differences were evaluated by using a two-tailed Student's *t*-test. The data in **d** are non-Gaussian distributed, and the differences were evaluated by using a two-tailed Mann-Whitney *U*-test. Data are the mean $\pm$ s.d. Each experiment was performed three independent times. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, based on a Student's *t*-test or Mann-Whitney *U*-test.

By MS analysis, we further identified several potential succination sites for fumaric esters (Extended Data Fig. 3e,f). Notably, changing the cysteine on 381 to serine (CS) almost completely abolished the succination of LYN, suggesting that the cysteine residue C381 on LYN may be the main site of succination by fumarate (Fig. 3f). Therefore, we next focused on studying C381. Kinase activity studies showed that while, as a control, the C419 mutation to serine(S) had almost no effect, the C381S mutation led to strongly reduced LYN activity (Fig. 3g,h and Extended Data Fig. 3g). In this case, further inhibition of LYN by DMF treatment was not observed (Fig. 3g and Extended Data Fig. 3g).

C381 is located in the protein kinase structural domain (247–501) and close to the DFG motif (residues 385–387), the orientation of which shapes the activity of the LYN protein^{35,36} (Extended Data Fig. 4a,b). By analyzing the structure change of DFG motif of LYN-C381S using a reported molecular dynamic simulation method^{35–38}, we found that C381S mutation altered the structure of DFG motif (Extended Data Fig. 4c,d). Moreover, Ramachandran plot analysis further showed that the dihedral angle scattering of the D site in LYN-C381S was broader, suggesting that the ADFG motif in LYN-C381S is more unstable compared to that in LYN (Extended

Data Fig. 4e). Thus, the C381S mutation seems to reduce the stability of the DFG motif, which leads to the inhibition of LYN activity.

Mutation of C381 to other 13 amino acids including polar or nonpolar amino acids also resulted in strong suppression of LYN activity, and DMF treatment was unable to further inhibit these mutants (Extended Data Fig. 5a–c). Consistent with this, results of molecular dynamics simulations showed that mutation of C381 to any of these 13 amino acids would change the structure of the DFG motif (Extended Data Fig. 5d). Therefore, the cysteine at position 381 appears to be important for the activation of LYN, and mutations at this site can lead to inhibition of LYN activity.

Fumarate-mediated inhibition of LYN abolishes BCR signaling.

BCR crosslinking rapidly induces activation of LYN and its downstream signaling through a cascade of phosphorylation, including SYK, phosphoinositide 3-kinase/AKT, mTORC1, S6 and extracellular signal-regulated kinase (ERK) (Extended Data Fig. 6a)^{39,40}. Notably, treatment with FHi or DMF, which could accumulate endogenous or exogenous fumarate, resulted in a remarkable decrease in the phosphorylation of LYN (Y396) and an increase in Y507 phosphorylation in both concentration- and time-dependent

manners, indicating an intracellular suppression of LYN activity by fumarate accumulation (Fig. 4a,b and Extended Data Fig. 6b). Consistent with this, the phosphorylation of LYN substrate SYK, as well as downstream AKT, S6 and ERK were all decreased when cells were treated with FHi or DMF (Fig. 4a and Extended Data Fig. 6b). Similar findings were also observed in vivo. In NP-LPS-immunized mice, FHi or DMF treatment led to a decrease in the phosphorylation levels of LYN-Y396 in B cells (Fig. 4c). We further used fluorescence-activated cell sorting (FACS) analysis and high-resolution imaging to confirm the regulation of downstream signaling of LYN by fumarate. While AKT and ERK phosphorylation increased on BCR crosslinking, this effect was strongly inhibited by fumarate accumulation induced by FHi or DMF (Extended Data Fig. 6c–f). To further confirm the effect of LYN on fumarate-mediated suppression of BCR signaling, we knocked down the expression of LYN. Supplementation with FHi or DMF could strongly reduce the phosphorylation of p44/42, AKT and S6 in shCtrl cells, but not in shLYN cells (Fig. 4d). Consistent with this, LYN-ablated B cells showed suppressed activation regardless of the treatment with FHi or DMF (Fig. 4d,e and Extended Data Fig. 6g). Together, these findings suggest fumarate-mediated inhibition of LYN impedes BCR signaling.

It seems that the regulation of B cells by fumarate is independent on T cells, as treatment of FHi or DMF still led to a notable reduction in B-cell development in NP-LPS stimulated and CD4-depleted mice (Extended Data Fig. 7a–d). Consistent with above findings, fumarate accumulation by FHi or DMF treatment resulted in increased LYN succination, and impaired activation of AKT and p44/p42 (Extended Data Fig. 7e). Like B cells, macrophages also express LYN and DMF treatment resulted in elevated LYN succination in these cells (Extended Data Fig. 7f), suggesting that fumarate succination of LYN may be a common event in immune cells.

LYN-C381 controls BCR signaling activity and B-cell function.

As mentioned above, the C381 seems to be vital for the activation of LYN (Extended Data Fig. 5a–d). To directly assess the regulatory role of LYN-C381 in BCR signaling and B-cell function, we generated *Lyn^{C381S/C381S}* transgenic mice (Extended Data Fig. 8a). In keeping with the findings that activating B cells boosted FH expression (Fig. 1a and Extended Data Fig. 1b), Spleen B cells from wild-type (WT) mice immunized with NP-LPS displayed increased expression of FH (Fig. 5a), and correspondingly reduced levels of fumarate in blood and spleens were observed (Fig. 5b). Consistent with this, a decrease in LYN succination was observed on NP-LPS challenge in these mice (Fig. 5a). By contrast, *Lyn^{C381S/C381S}* transgenic mice had impaired BCR signaling activity as indicated by reduced phosphorylation of LYN and AKT, suggesting C381S mutation, which leads to inhibition of LYN (Figs. 3h and 5a), may abolish B-cell activation in vivo. Indeed, B cells from *Lyn^{C381S/C381S}* mice displayed noticeably decreased expression of CD86 and CD69 compared to those from WT control mice (Fig. 5c). Therefore, in this case, we did not observe an increase in FH1 expression, as well as a decrease in fumarate levels (Fig. 5a,b).

Notably, the C381S mutation inhibited B-cell activation, and FHi or DMF treatment did not alter this effect (Fig. 5c and Extended Data Fig. 8b), demonstrating the importance of LYN-C381 in B-cell activation. FHi and DMF treatment to some extent reduced the expression of CD86 and CD69 in *Lyn^{C381S/C381S}* B cells, indicating that fumarate may also regulate B-cell activation via LYN-independent mechanism(s). Nevertheless, further cell size and proliferation analysis revealed that *Lyn^{C381S/C381S}* B cells were smaller and proliferated slower than their WT control counterparts (Fig. 5d–f). Moreover, increased *Lyn^{C381S/C381S}* B-cell death was found (Extended Data Fig. 8c). Again, these phenotypes were not altered in B cells of *Lyn^{C381S/C381S}* mice by the accumulation of fumarate (Fig. 5c–f and Extended Data Fig. 8b,c).

The phosphorylation of LYN-Y396 was substantially lower in *Lyn^{C381S/C381S}* B cells compared to WT cells (Fig. 6a,b). In accordance, downstream AKT and ERK phosphorylation levels declined in *Lyn^{C381S/C381S}* B cells (Fig. 6a and Extended Data Fig. 8d–f). Moreover, treatment with FHi and DMF led to a sharp decrease in the phosphorylation of these proteins in WT B cells, but not in *Lyn^{C381S/C381S}* B cells (Fig. 6a and Extended Data Fig. 8d–f). We further examined B-cell function in WT and *Lyn^{C381S/C381S}* mice. Measurement of NP-specific IgM and IgG3 antibody production from NP-LPS immunized mice serum showed *Lyn^{C381S/C381S}* mice have strongly decreased responses compared to WT mice (Fig. 6c,d). Together, these results demonstrate that *Lyn^{C381S/C381S}* mutant blocks BCR signaling efficiently.

Discussion

The activation, cell growth and proliferation, as well as effector functions of immune cells require metabolic alteration to provide energy and building blocks for macromolecule biosynthesis⁴¹. We recently found that asparagine, a nonessential amino acid, improves T cell immune responses by directly acting on LCK signaling⁴². Currently it still remains unclear whether existence of signaling metabolites in B cells. In this work, we discovered that the TCA cycle intermediate fumarate has a role in regulating B-cell activation, proliferation and function via LYN, which is a succination substrate of fumarate and can be succinated at C381 (Extended Data Fig. 8g). In vitro and in vivo study revealed that C381S mutation strongly impeded LYN activity, and replacement of C381 with other amino acids all resulted in a substantial decrease in the activity of LYN, indicating that this residue has a decisive effect on the activity of LYN and that alteration of the amino acid structure at this site might lead to impairment of the activity of LYN. Because of this, whether succination of C381 is responsible for the effects observed after DMF treatment remains not entirely clear and requires further investigations.

LYN is required for the initiation of the BCR signaling. Besides of this, LYN seems to have both positive and negative effects on BCR signaling. While LYN-deficient B cells have exaggerated BCR-induced ERK signaling and intracellular calcium flux at certain transitional stages during maturing^{43–45}, sustained activation of LYN also boosts the BCR signaling and calcium flux in response to BCR crosslinking⁴⁶. A potential explanation for these observations is that, the bidirectional functions of LYN might be context-dependent and mediated by different downstream recognition and mediating mechanisms. In line with this, our findings suggest that fumarate accumulation-induced succination might confer a gain-of-function to LYN to suppress BCR signaling.

B cells play important roles in autoimmune diseases, including multiple sclerosis. B-cell depletion is an effective therapy for multiple sclerosis⁴⁷. DMF has been approved for the treatment of psoriasis⁴⁸ and relapsing multiple sclerosis⁴⁹ due to its immunomodulatory potential⁴⁹. DMF treatment leads to a decline in the absolute B-cell number in patients with multiple sclerosis⁵⁰. However, the exact mechanism of DMF has not been explained so far. Our study identifies LYN as a target for DMF and BCR signaling can be inhibited by DMF treatment, providing mechanistic insight and therapeutic implications for DMF immunotherapy. This work also highlights the importance of Cys381 residue on LYN, which may serve a potential target for B-cell-associated immune diseases.

Online content

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Methods

Antibodies and reagents. The antibodies that were used in this work are listed in Supplementary Table 1. The following reagents were purchased from the indicated sources: Fluo-4 (Life, F14217), Fixation/Permeabilization Diluent (Invitrogen, 00-5223-56), fixation/Permeabilization Concentrate (Invitrogen, 00-5123-43), FH-IN-1 (MCE, HY-100004). NP-LPS (N-5065-5 BIOSEARCH TECHNOLOGIES), Alexa Fluor 488-goat antirabbit IgG (Life Technologies, A11008), Fluo-4, AM, cell permeant (Life, F14217), 96-well Clear Flat Bottom Polystyrene High Bind Microplate (Corning, 9018), enzyme-linked immunosorbent assay (ELISA) stop solution (Solarbio, C1058) TMB Substrate Set (Biollegend, 421101), 7AAD (BD Biosciences, 559925). IA-alkyne (Bide, BD324491), tris(2-carboxyethyl)phosphine (TCEP) (Beyotime, ST049), tris-(benzyltriazolylmethyl)amine (TBTA) (Topsience, 7086), biotin-azide (Click Chemistry Tools, 1265) and streptavidin agarose resin (Pierce, 20353).

The following reagents were purchased from Sigma: disodium fumarate, MMF, DMF, citrate, α -ketoglutarate, succinate, malate, oxaloacetate, FLAG peptide, anti-Flag M2 affinity gel, streptavidin, trichloroacetic acid and DAPI.

Animals. Six- to eight-week-old C57BL/6 mice were purchased from Vital River Laboratory Animal Technology for B-cell isolation or serum immunogen experiments. KI mutation was recombined in oosphere of C57BL/6J background mice by using the CRISPR-Cas9 system. All of the mice used in this study have been accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International and Institutional Animal Care and Use Committee (IACUC) of Tsinghua University. All animals were maintained in groups of 5–6 on a 12/12-h light/dark cycle at 20–26°C with around 40–70% humidity, and give ad libitum access to standard food and water. All the mice were maintained in pathogen-free facilities and used strictly in accordance with the protocols approved by the IACUC of Tsinghua University. The study is compliant with all of the relevant ethical regulations regarding animal research.

Cell lines. The 293T cells and A20 cells were purchased from the American Type Culture Collection. A20 cells and primary B cells were cultured in RPMI-1640 medium (Macgene, CM10041) containing 10% fetal bovine serum and 100 U ml⁻¹ penicillin/streptomycin. Primary B cells were stimulated with F(ab')₂ fragments of antimuscle IgM antibody. The 293T cells and Lenti-X 293T cells (Takara, 632180) were cultured in DMEM medium (Gibco, C11995) supplemented with 10% FBS. All the cell lines that were used have been authenticated and were tested for mycoplasma contamination.

DNA constructs and lentivirus production. shFH was generated using a pair of primers (forward primer: 5'-CCGGGCTAATGAATGAGTCTTTAATCTC GAGATTAAAGACTCATTTCATTAGCTTTTG-3'; reverse primer: 5'-AATTC AAAAGCTAATGAATGAGTCTTTAATCTCGAGATTAAAGACTCATTCA TTAGC-3'). shLYN was generated using a pair of primers (forward primer: 5'-CCGGCGGAGATCGAAGATAAAGCTGAGTTATCTCGA TGACTCTCGCGTTTTTG-3'; reverse primer: 5'-AATTCAAAAACGCGAGAG TCATCGAAGATAAAGCTGAGTTATCTCGATGACTCTCGCG-3'). The primers were cloned into plasmid pLKO.1-TRC (Addgene 10878). To produce short-hairpin RNA (shRNA) lentivirus, Lenti-X 293T cells were transfected with a combination of plasmids containing PTK, pMDLg-pRRE, pRSV-rev and pMD2.G.

Lentiviral transduction. Here, 293T cells were transfected with the shRNA plasmid together with the lentiviral packaging vectors pMD2.G (Addgene 12259) and psPAX2 (Addgene 12260). 48- or 72-h after transfections, the culture supernatant containing the released viral particles was collected and a final concentration of 8 μ g ml⁻¹ of polybrene (Sigma AL-118) was added. After 6–10 h, the medium was replaced with fresh virus-free medium for further growth until use.

Protein expression and purification. The Flag-LYN vector was constructed by cloning LYN complementary DNA or LYN mutants into the pCHN1 vectors. After transfection of Flag-LYN into 293T cells, cells were collected and lysed. The supernatants were incubated with FLAG M2 beads, which were washed three times with wash buffer (PBS, 150 mM NaCl and 1% Triton X-100), followed by competitive elution with Flag peptides.

Reverse transcription and PCR (RT-PCR) analysis. Total RNA and cDNA were prepared using TRIzol (Invitrogen) and quantitative PCR (qPCR) (Vazyme R223-01). PCR was performed according to the manufacturer's instructions (Vazyme Q121-03). Real-Time PCR Analysis Software CFX Maestro Software 2.3 was used for Real-Time PCR signal collection. The expression levels of the genes are normalized by the expression of the housekeeping *Actb* gene by using a 2(-Delta Delta C(T)) method. The following primer pairs were used for real-time qPCR: Mus *Actb* (forward): 5'-TGACGTTGACATCCGTAAAGA-3', Mus *Actb* (reverse): 5'-GCCGACTCATCTGACTCC-3'; Mus *Idh1* (forward): 5'-GTGGG CGTCAAGTGTGCTA-3', Mus *Idh1* (reverse): 5'-CCACCCAGAATGTTTCG GATG-3'; Mus *Aco2* (forward): 5'-ATCGAGCGGGAAAGACATAC-3', Mus *Aco2* (reverse): 5'-TGATGGTACAGCCACCTTAGG-3'; Mus *Ogdh* (forward): 5'-AGGGCATATCAGATACGAGGG-3', Mus *Ogdh* (reverse): 5'-CTGTGGA

TGAGATAATGTCAGCG-3'; Mus *Sdha* (forward): 5'-GGAACACTCCAAAA CAGACCT-3', Mus *Sdha* (reverse): 5'-CCACCACTGGGTATTGAGTAGAA-3'; Mus *Fh1* (forward): 5'-GAATGGCAAGCCAAAATTCCTT-3', Mus *Fh1* (reverse): 5'-TCTTACGGTCTGAGCACCATAA-3'; Mus *Mdh* (forward): 5'-GAA CCAATCAGAGTCCCTTGTGAC-3', Mus *Mdh* (reverse): 5'-GGCACAGTCTTGC AGTTCCA-3'; Mus *Cs* (forward): 5'-GGACAATTTTCCAACCAATCTGC-3', Mus *Cs* (reverse): 5'-AGTCAATGGCTCCGATATGC-3'.

Flow cytometry analyses. Single-cell suspensions were obtained from the spleens or bone marrow of mice and treated with red blood cell lysis solution for 5 min. Then cells were stained with antibodies for 1 h. Information pertaining to all antibodies used in this study is shown in Supplementary Table 1. BD FACSDiva Software v.8.0 was used for FACS signal collection. Data analysis was carried out using FlowJo (v.10.0.7, FlowJo, LLC). The amount of GC B cells (pregated on B220⁺ cells, GL7⁺, Fas⁺), memory B cells (CD38⁺, IgD⁺) and plasma cells (B220-CD138⁺ or TACI⁺ CD138⁺) were analyzed by FACS analysis. The FACS gating strategies are described in Supplementary Fig. 1.

Western blot analysis. Cell extracts were separated on 8% SDS-PAGE gels, transferred to PC membranes, blocked with Tris-buffered saline/Tween 20 (TBST) containing 5% skim milk for 1 h at room temperature, and then probed with the indicated primary antibody at 4°C overnight. After three washes, the membranes were incubated with a 1:5,000 dilution of HRP-conjugated secondary antibodies for 1 h at room temperature. The immunoblots were detected using ECL according to the manufacturer's protocol. Certain western blot bands were quantified using Image J software v.1.37.

LYN kinase activity assays by western blot or ELISA. To measure the phosphorylation of LYN, recombinant LYN proteins were pre-incubated with a buffer containing 60 mM Tris-HCl (pH 7.5), 20 mM MgCl₂, 2 mM MnCl₂, 0.1 mM NaVO₃, 2 mM dithiothreitol and with or without FHI or DMF were incubated at 37°C for 30 min. ATP was then added to the mixtures to a final concentration of 500 μ M and incubated for 15 min at 37°C. The reaction mixtures were boiled for 10 min at 98°C and transferred to western blot to detect the phosphorylation of LYN.

For the determination of LYN activity by substrate phosphorylation of biotin-poly (Glu4:Tyr, 4:1), LYN proteins (1 μ g ml⁻¹) were incubated with 5 μ g ml⁻¹ poly (Glu4:Tyr, 4:1)-biotin in a buffer containing 60 mM Tris-HCl (pH 7.5), 20 mM MgCl₂, 2 mM MnCl₂, 0.1 mM NaVO₃, 2 mM dithiothreitol with or without FHI or DMF. After an incubation of 30 min at 37°C, ATP was added and incubated for another 15 min, followed by boiling at 98°C. The ELISA plate was then loaded with 50 μ g ml⁻¹ streptavidin, blocked with 5% BSA and washed three times with PBS with Tween (PBST). The LYN reaction mixtures were then transferred into the plate wells (100 μ l per well) and incubated for 15 min. Anti-phospho-Tyr (100 μ l, 1:5,000) was then added to the wells for 1 h incubation at 37°C, followed by washing with PBST and incubation with 100 μ l of goat antimouse IgG-HRP for another 1 h. After addition of TMB buffer and subsequently TMB stop buffer according to the manufacturer's manual (BioLegend, 421101), the LYN activity was measured and calculated the absorbance at 450 nm using a microplate reader (Molecular Devices, SpectraMAX M2e). Softmax Pro v.6.3 was used to collect fluorescent and absorption data for enzyme assay.

Calcium mobilization assay. Calcium mobilization were measured in indo-1-labeled cells. Primary B cells were negatively enriched and then cells were incubated with 4 μ M indo-1 at 37°C for 30 min. Then cells were incubated with or without FHI/DMF at 37°C for 30 min. After washing with PBS, cells were stimulated with antimuscle IgM F(ab')₂ fragments (10 μ g ml⁻¹) at 37°C, continuous fluorescent measurements of free calcium were detected at 516 nm by flow cytometry immediately. Curve fitting was performed using OriginPro SR1 v.b9.5.1.195 software.

Immunogen isotype ELISA and immunizations. Total serum Ig titers were determined by ELISA using goat antimouse IgG-HRP as a capture reagent and detected with TMB buffer and subsequently TMB stop buffer. For NP-specific immunizing, NP-LPS (10 μ g per mouse, Biosearch Technologies, N-5065-5) was administered by intraperitoneal injection. NP25-BSA (Biosearch Technologies, N-5050H-10) coated plates blocked with 1% BSA in PBS before serum incubation. Bound Abs were detected with horseradish peroxidase coupled antimouse IgM, IgG1, IgG2b, IgG2c and IgG3.

Confocal immunofluorescence imaging. Andor SpinningDisk (Dragonfly) Confocal fluorescence microscope consisting of an inverted microscope (TIE2, Nikon) equipped with and Nikon UPLSAPO $\times$ 100 numerical aperture 1.49 oil immersion lens was used to collect the fluorescence signal. Briefly, primary B cells were treated with or without FHI/DMF for 30 min. Then cells were stimulated with antimuscle IgM F(ab')₂ fragments (10 mg ml⁻¹) at 37°C for the indicated time. The cells were then fixed with 4% paraformaldehyde (PFA) and 0.1% Triton X-100 for 1 h at 4°C, followed by 5% BSA blocking for 30 min at 37°C. The cells were gently washed three times with PBS and stained overnight with the indicated antibody at 4°C. After three washes with PBS, the cells were incubated with

the anti-rabbit IgG-488 for 1 h at 4 °C and washed three times with PBS before imaging. The images were analyzed using ImarisViewer $\times 64$ v.9.5.1 and quantified by Image J software v.1.37.

Metabolite extraction and MS-based metabolomics analysis. Analyses of TCA cycle metabolites were performed using standard procedures^{51,52}. Briefly, metabolites were extracted from primary B cells with cold 0.18 M aqueous trichloroacetic acid. Insoluble materials in lysates were centrifuged at 12,000g for 15 min, and the resulting supernatants were freeze-dried. Samples were resuspended in 30 μ l of ultrapure water for liquid chromatography-high-resolution mass spectrometry. The concentrations of the extracted metabolites were normalized to the cell numbers. Compounds were separated on an XBridge Amide column (100 $\times$ 4.6 mm, 3.5 μ m; Waters). Buffer A comprised 20 mM ammonium acetate (pH 9.4) in water, and buffer B was acetonitrile (LC-MS grade). The retention times were based on standard metabolites. Xcalibur Software (v.2.2.0) was used for metabolic signal collection, data acquisition and processing. Data are presented as fold changes and the mean $\pm$ s.d., and were compared to the WT or control samples.

Quantitative profiling of succination target proteins. The A20 cells were lysed with 0.1% Triton X-100/PBS buffer containing EDTA-free protease inhibitor cocktail. After sonication and centrifugation at 4 °C, the lysates were diluted to 2 mg ml⁻¹ with PBS according to the results of BCA protein quantification. Then 1 ml lysates were incubated with 2 mM DMF or DMSO for 12 h, and 100 μ M IA-alkyne for 45 min. The excessive probes were removed by adding 4 ml of methanol, 1 ml of chloroform and 3 ml of water, and the precipitated proteins were washed twice with cold methanol and resuspended with 1 ml of 0.4% SDS or PBS. CuAAC was performed by adding the final concentrations of 1 mM CuSO₄, 100 μ M TBTA, 1 mM TCEP and 100 μ M biotin-azide. The mixture was incubated at room temperature for 1 h, and the proteins were precipitated with methanol/chloroform, washed twice with cold methanol, and resuspended with 1 ml of 1.2% SDS or PBS. Next, 100 μ l of streptavidin beads were washed three times with 1 ml PBS, and resuspended with 5 ml of PBS. The beads were mixed with protein samples, incubated at 29 °C for 4 h and washed three times with 5 ml of PBS and three times with 5 ml of water. The resulting beads were incubated with 500 μ l of PBS containing 6 M urea and 10 mM DTT at 65 °C for 20 min. A final concentration of 20 mM iodoacetamide was added into the beads and incubated at room temperature for 30 min in the dark. The beads were collected by centrifugation, and was resuspended with 200 μ l of 100 mM triethylammonium bicarbonate containing 1 mM CaCl₂ and 10 ng μ l⁻¹ trypsin, and the proteins were digested at 37 °C with rotation overnight.

Next, 8 μ l of 4% light formaldehyde (CH₂O) and heavy formaldehyde (CD₂O) was added into DMF-treated sample and DMSO-treated sample, respectively. Then 8 μ l of 600 mM sodium cyanoborohydride was added into the beads and the samples were incubated at room temperature for 1 h, followed by the addition of 32 μ l of 1% NH₃·H₂O and 16 μ l of 5% formic acid. The resulting peptides was collected by centrifugation and the beads were washed three times with 100 μ l of water. The light and heavy labeled peptides were combined and fractionated using C18 SPE cartridge under high pH conditions (10 mM NH₄HCO₃, pH 10). Briefly, 6, 9, 12, 15, 18, 21, 25, 30 and 35% of acetonitrile were added for peptide elution, and the nine eluted samples were combined into six samples (6 + 25%, 9 + 30%, 12 + 35, 15, 18, 21%), which were dried for LC-tandem MS (MS/MS) detection.

Peptide samples were detected by LC-MS/MS on Orbitrap mass spectrometers coupled with nano-LC under positive ionization mode. Full scan mass spectra were acquired from m/z 350 to 1,800 with the resolution of 70,000. MS/MS fragmentation was performed using the high-collision dissociation mode by selecting the top 20 most intense ions with the resolution of 17,500. The isolation window was 2.0 m/z , charge was 2+ and 3+, collision energy was 28% and dynamic exclusion was 15 s.

The LC-MS/MS data were analyzed by Proteome Discoverer (v.2.4) with variable modification of carbamidomethyl (Cys, C₂H₃NO, +57.02 Da) and oxidation (Met, O, +15.99 Da), and isotopic modifications of light labeled (Lys, N terminal, C₂H₄, +28.03 Da) and heavy labeled (Lys, N terminal, C₂D₄, +32.06 Da). The mass accuracy for precursors and fragment ions were set as 10 ppm and 0.02 Da, respectively. Xcalibur Software (v.2.2.0) was used for metabolic and proteomic signal collection, data acquisition and processing. The ratios of demethylation were quantified only when the protein was detected in at least two of the three independent experiments. Microsoft Excel 2016MSO (v.2202 Build 16.0.14931.20118) was used for demethylation ratio quantification and statistical analysis. DAVID bioinformatics database was used for gene ontology analysis.

LYN verification from probe enriched proteome. The A20 cells were lysed through the same procedure mentioned above. Then 1 ml of lysates (2 mg ml⁻¹) were incubated with 2 mM DMF (stocked in DMSO) or DMSO for 12 h and 100 μ M IA-alkyne or DMSO for 45 min. The excessive probes were removed by adding 4 ml of methanol, 1 ml of chloroform and 3 ml of water, and the precipitated proteins were washed twice with cold methanol and resuspended with 1 ml of 0.4% SDS or PBS. CuAAC was performed by adding the final concentrations of 1 mM CuSO₄, 100 μ M TBTA, 1 mM TCEP and 100 μ M biotin-azide. The mixture was incubated at room temperature for 1 h, and the proteins were precipitated with

methanol and chloroform, washed twice with cold methanol and resuspended with 1 ml of 1.2% SDS or PBS. 30 µl of the samples was saved as 'input'. Then 100 µl of streptavidin beads were washed three times with 1 ml of PBS, and resuspended with 5 ml of PBS. The beads were mixed with protein samples, incubated at 29 °C for 4 h, and washed three times with 5 ml of PBS and three times with 5 ml of water. The resulting beads were added with 40 µl of loading buffer, followed by heating at 95 °C for 10 min and centrifugation, which were saved as 'enriched'. The 'input' and 'enriched' samples were resolved on SDS-PAGE gels and analyzed by western blot.

Identification of succination sites of LYN. FLAG-LYN containing cell lysates were incubated with FLAG M2 beads and washed three times with wash buffer. The beads were resuspended with PBS containing 1 mM FA, 200 µM MMF, or 100 µM DMF at 37 °C for 12 h, and were washed three times with PBS. The beads were incubated with 500 µl of PBS containing 6 M urea and 10 mM DTT at 65 °C for 20 min. A final concentration of 20 mM iodoacetamide was added into the beads and incubated at room temperature for 30 min in dark. The beads were collected by centrifugation, and was resuspended with 200 µl of PBS containing 2 M urea, 1 mM CaCl₂ and 10 ng µl⁻¹ trypsin, and the proteins were digested at 37 °C with rotation overnight. The resulting peptides were collected by centrifugation and the beads were washed three times with 200 µl of water. The peptide samples were purified

with C18 SPE cartridges before LC-MS/MS analysis. LC-MS/MS parameters were set as mentioned above. The succinated peptide AANVLVSESLMCK [2+, 3+] was detected by tandem MS for verification of the modification by FA, MMF and DMF.

The LC-MS/MS data were analyzed by Mascot (v.2.3.02) with variable modification of carbamidomethyl (Cys, C₂H₃NO, +57.02 Da), succinated (Cys, C₄H₄O₄, +116.01 Da), monomethyl succinated (Cys, C₅H₆O₄, +130.03 Da), dimethyl succinated (Cys, C₆H₈O₄, +144.04 Da) and oxidation (Met, O, +15.99 Da). The mass accuracies for precursors and fragment ions were set at 20 ppm and 0.8 Da, respectively.

Molecular dynamics simulation. The initial structure of LYN was built based on the LYN crystal structure (Protein Data Bank ID 5XY1). The missing loop in the crystal structure was modeled with ModLoop^{37,38}. The ligand in the crystal structure was removed. The structure of mutated LYN was obtained with Pymol (v.2.5.2). The protein was prepared with force field amber99sb-ildn and solvated in water. Ions were added to neutralize the system. The system containing the protein was first annealed from 425 to 300 K in 7 ns and then went through a 50 ns molecular dynamics simulation at 300 K to collect the information of the structure. The dihedral angle of the ADFG motif was analyzed. The molecular dynamics simulation procedures from the preparation to analysis were done with GROMACS (v.2021.3).

Statistical analysis. The experiments in this study were set up using 3–10 samples or animals per independent group, condition or repeat. Statistical analysis was carried out as described previously^{53,54}, by using Origin Pro2018 software 2018C b9.5.0.193. The normality of distribution was assessed by Shapiro–Wilk test. When the data were normally distributed, Student's *t*-tests for two groups comparison were used. When the data were non-Gaussian distributed, we used a Mann–Whitney *U*-test to evaluate differences. All tests were performed using two-tailed analysis. Most graphs provide mean values with standard deviation (s.d.) unless otherwise stated. The *P* values are indicated in the related figures; **P* < 0.05, ***P* < 0.01, ****P* < 0.001 and NS, not significant (*P* > 0.05).

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

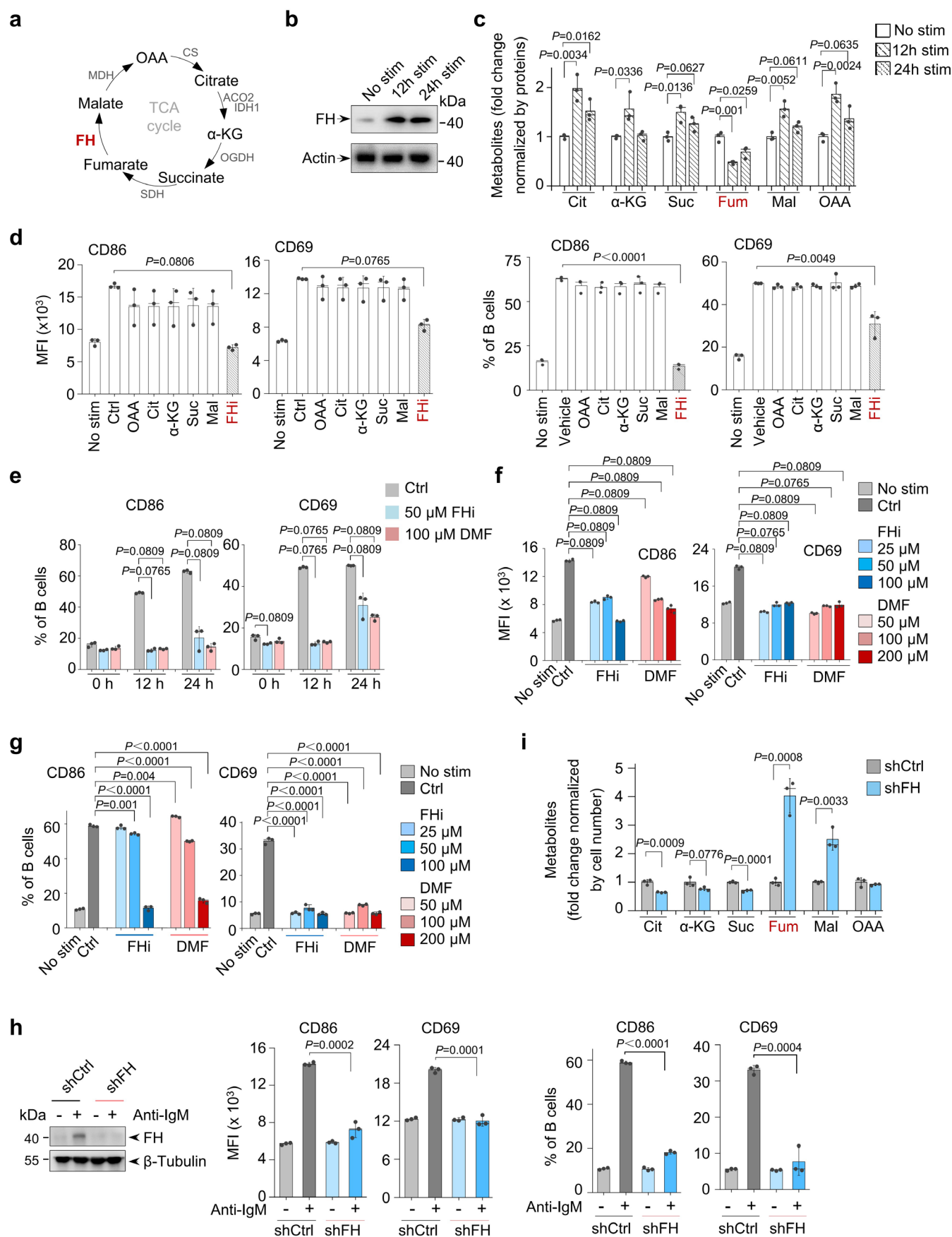
The source data underlying Figs. 1–6 as well as Extended Data Figs. 1–8 are provided as a source data file with this paper. All the other data supporting the findings of this study are available within the article and its supplementary information files, and from the corresponding author on reasonable request. A reporting summary for this article is available as a Supplementary Information file. The FACS gating strategies are described in Supplementary Fig. 1. LYN crystal structure (PDB ID 5XY1) is available on the RCSB PDB. Proteome database (mouse, *Mus musculus*, UP000000589) is available on the UniProt (<https://www.uniprot.org/proteomes/UP000000589>).

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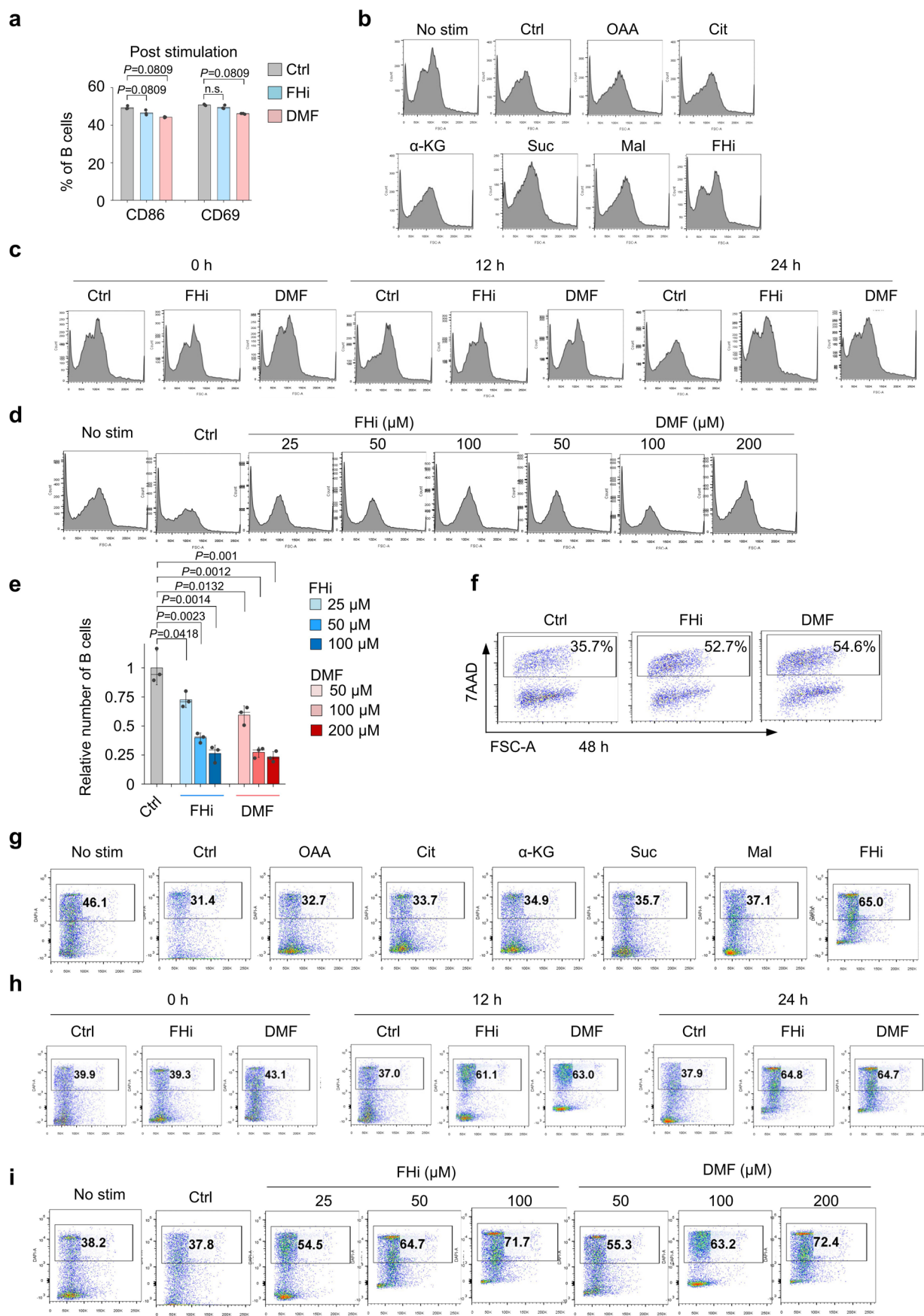
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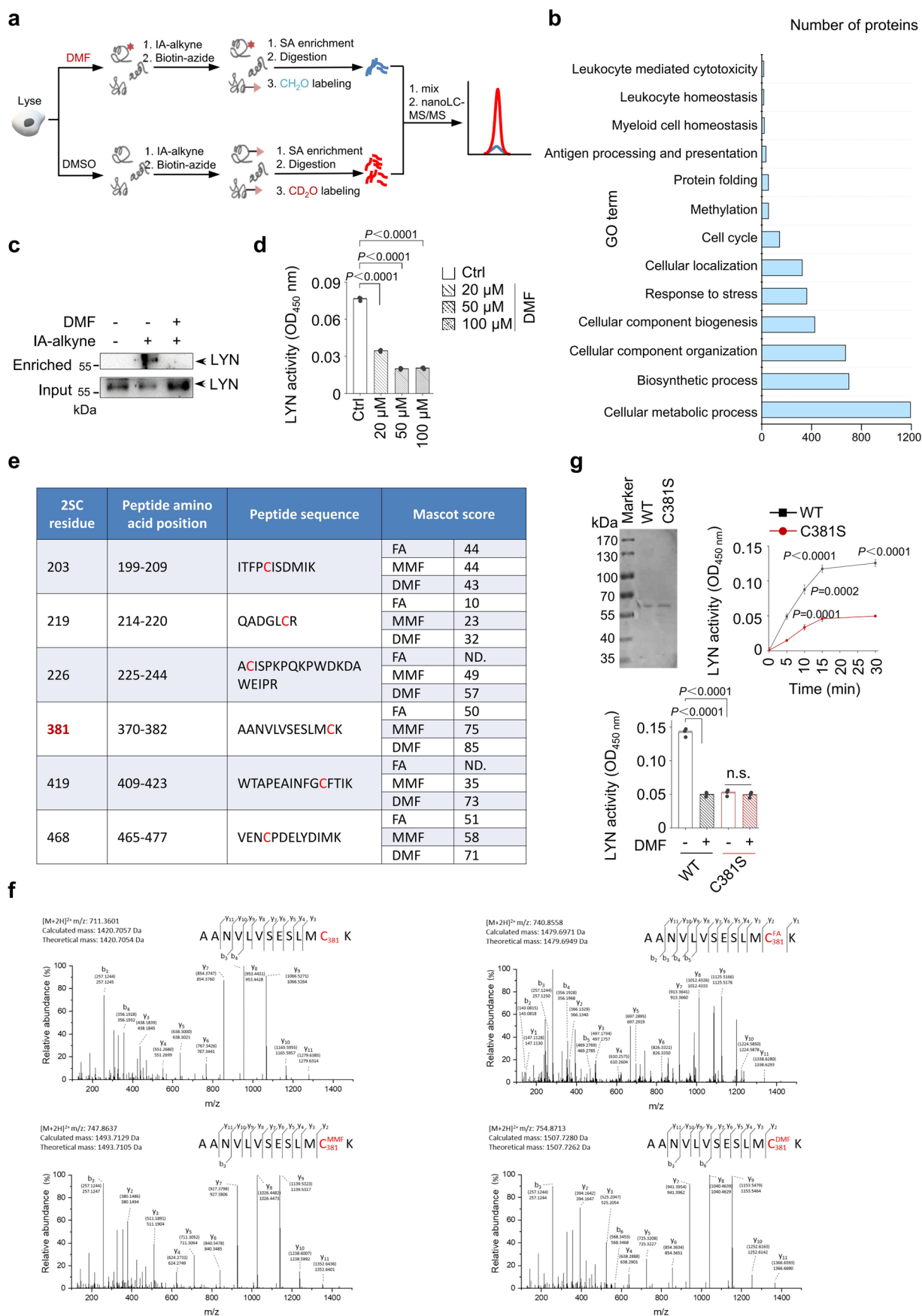
Extended Data Fig. 1 | See next page for caption.

Extended Data Fig. 1 | Fumarate depletion by FH upregulation is essential for B-cell activation. **a**, Schematic representation of TCA cycle. **b** and **c**, Mouse naive B cells were stimulated by 10 $\mu\text{g}/\text{ml}$ (unless otherwise indicated) F(ab')_2 fragments of antimouse IgM antibody (hereafter referred to as anti-IgM) for indicated times or left unstimulated (no stim). Cells were analyzed by western blot (**b**) and LC-MS for metabolites (**c**). Cit, Citrate; $\alpha\text{-KG}$, alpha-ketoglutarate; Suc, succinate; Fum, fumarate; Mal, malate; OAA, oxaloacetate. Stim stands for stimulation ($n=3$ independent wells). **d**, Mouse naive B cells were treated with DMSO (Ctrl), indicated metabolites (1 mM) or 50 μM fumarate hydratase-IN-1 (FHi) for 1 hour prior to 24 hours of anti-IgM antibody treatment or left untreated (no stim). Surface expression of CD86^+ and CD69^+ on B cells were measured by FACS analysis ($n=3$ independent wells). **e**, Mouse naive B cells were treated with DMSO, 50 μM FHi or 100 μM DMF as indicated for 1 hour before activation for indicated times. The percentage of CD86^+ and CD69^+ B cells was measured ($n=3$ independent wells). **f** and **g**, Mouse naive B cells were treated with DMSO or increasing amounts of FHi or DMF for 1 hour prior to stimulation without (no stim) or with anti-IgM antibody for 24 hours. The MFI (**f**) and percentage (**g**) of CD86^+ B cells and CD69^+ B cells were measured ($n=3$ independent wells). **h** and **i**, Mouse naive B cells expressing shFH or shCtrl were stimulated with anti-IgM antibody. The levels of metabolites (**i**), MFI and percentage of CD86^+ and CD69^+ B cells, and FH expression were measured (**h**). $n=3$ independent wells. The data are normalized to the average of no stimulation samples (**c**) or the average of control samples (**i**). The data in **c**, **g**, **h**, and **i** are normally distributed, and the differences were evaluated by using 2-tailed Student's *t* test. The data in **d**, **e**, and **f** are non-Gaussian distributed, and the differences were evaluated by using 2-tailed Mann-Whitney U test. Data are the mean $\pm$ SD. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, based on Student's *t*-test or Mann-Whitney U test.



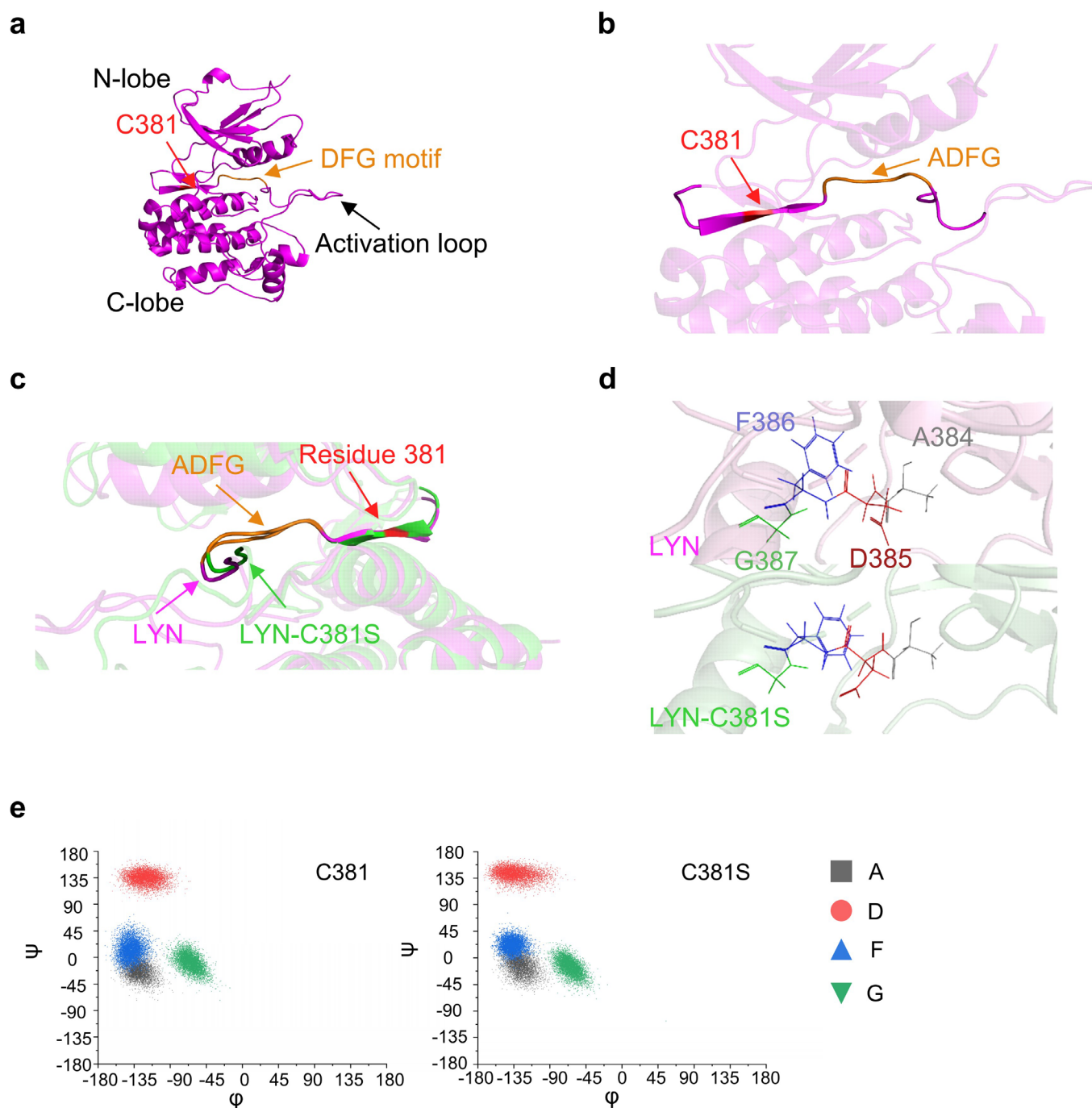
Extended Data Fig. 2 | See next page for caption.

Extended Data Fig. 2 | Effect of exogenous fumarate on B-cell death during activation. **a**, Mouse naive B cells were stimulated with 10 $\mu\text{g}/\text{ml}$ F(ab')_2 fragments of anti-mouse IgM antibody for 1 hour and then treated with DMSO (Ctrl), FHi (50 μM) or DMF (100 μM) for another 24 hours. The percentage of CD86^+ B cells and CD69^+ B cells was measured by FACS analysis ($n=3$ independent wells). Stim stands for stimulation. **b**, Mouse naive B cells were treated as in Extended Data Fig. 1d. Cell size was measured by FACS analysis. **c**, Mouse naive B cells were treated as in Fig. 1c. Cell size was measured by FACS analysis. **d**, Mouse naive B cells were treated as in Extended Data Fig. 1f. **e**, Cell size was measured by FACS analysis. **e**, Mouse naive B cells treated with DMSO (Ctrl) or increasing amounts of FHi, DMF or for 1 hour were stimulated with 10 $\mu\text{g}/\text{ml}$ F(ab')_2 fragments of anti-mouse IgM antibody for another 48 hours, and cell number was counted ($n=3$ independent wells). **f**, Mouse naive B cells treated with FHi (50 μM), DMF (100 μM) or DMSO (Ctrl) for 1 hour were activated with 10 $\mu\text{g}/\text{ml}$ F(ab')_2 fragments of anti-mouse IgM antibody for 48 hours. Cell death was determined by 7AAD staining and FACS analysis. **g**, Mouse naive B cells were treated as in Extended Data Fig. 1d. Cell were stained with DAPI and analyzed by FACS. **h**, Mouse naive B cells were treated as in Fig. 1c. Cell were stained with DAPI and analyzed by FACS. **i**, Mouse naive B cells were treated as in Extended Data Fig. 1f. **g**, Cell were stained with DAPI and analyzed by FACS. The data in **e** are normalized to the average of control samples. The data in **e** are normally distributed, and the differences were evaluated by using 2-tailed Student's *t* test. The data in **a** are non-Gaussian distributed, and the differences were evaluated by using 2-tailed Mann-Whitney U test. Data are the mean $\pm$ SD. Each experiment was performed three independent times. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, based on Student's *t*-test or Mann-Whitney U test.



Extended Data Fig. 3 | See next page for caption.

Extended Data Fig. 3 | Identification of LYN as a succination target for fumarate. **a**, The schematic representation of proteome labelling, enrichment, and digestions to provide probe-labelled peptides for MS analysis. LC-MS/MS, liquid-chromatography-MS/MS. **b**, Gene Ontology (GO) terms enrichment of functional pathways by competitive activity-based protein profiling ($P < 0.05$). **c**, B cell lysates were labeled with IA-alkyne probe without (-) or with DMF (100 μ M) competition. Then the enriched proteins as well as input proteins were immunoblotted with LYN antibody. **d**, Related to Fig. 3d. Purified LYN proteins was treated with DMSO (Ctrl) or increasing concentrations of DMF for 30 minutes and LYN kinase activity was measured by ELISA. **e** and **f**, Recombinant LYN with DMSO (up left panel), FA (1 mM), MMF (200 μ M), or DMF (100 μ M) treatment for 30 minutes was digested with trypsin and analyzed by LC-MS/MS for the position(s) of succination (**f**). Sequences for all the six detected peptide species covering the succinated residues are shown (**e**). The MASCOT score obtained for each peptide is listed accordingly. **g**, Purified wild-type (WT) LYN and LYN-C381S proteins were analyzed by SDS-PAGE followed by Coomassie Blue staining (left panel). The proteins were incubated with DMSO (-) or DMF (100 μ M) for 30 minutes to assay LYN kinase activity by ELISA (right and bottom panels). The data in **d** and **g** are normally distributed, and the differences were evaluated by using 2-tailed Student's *t* test. Data are the mean $\pm$ SD. Each experiment was performed three independent times. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, based on Student's *t*-test.



Extended Data Fig. 4 | Structural analysis of possible conformational change caused by C381S mutation in LYN. **a**, Structure of the kinase domain containing C381 and ADFG motif of LYN. **b**, Site C381 (red) is close in sequence to ADFG (residues 384–387, orange). **c**, Superimposition of LYN (magenta) and LYN-C381S (green). Mutation of C381 (red) may affect the conformation of ADFG motif (orange). **d**, Comparison of ADFG motif conformations in LYN (magenta) and LYN-C381S (green). **e**, Ramachandran plot of LYN and LYN-C381S. The dihedral angle scattering of the D site in LYN-C381S is broader than that in LYN. More information please see Methods.

Author contributions

J.C. and P.J. designed the experiments. J.C. and J.Y. performed most of the experiments. Y.L. performed the identification of fumarate succination target(s) and measurement of metabolites under the supervision of X.Z. L.Z., Y.Z., X.M. and Yining Chen provided technical assistance. X.S., Yunan Chen and Y.L. performed the molecular dynamic simulation under the supervision of X.Z. and Y.Z. J.C. organized and analyzed the data. P.J. supervised the research. J.C. and P.J. wrote the manuscript. All authors commented on the manuscript.

Competing interests

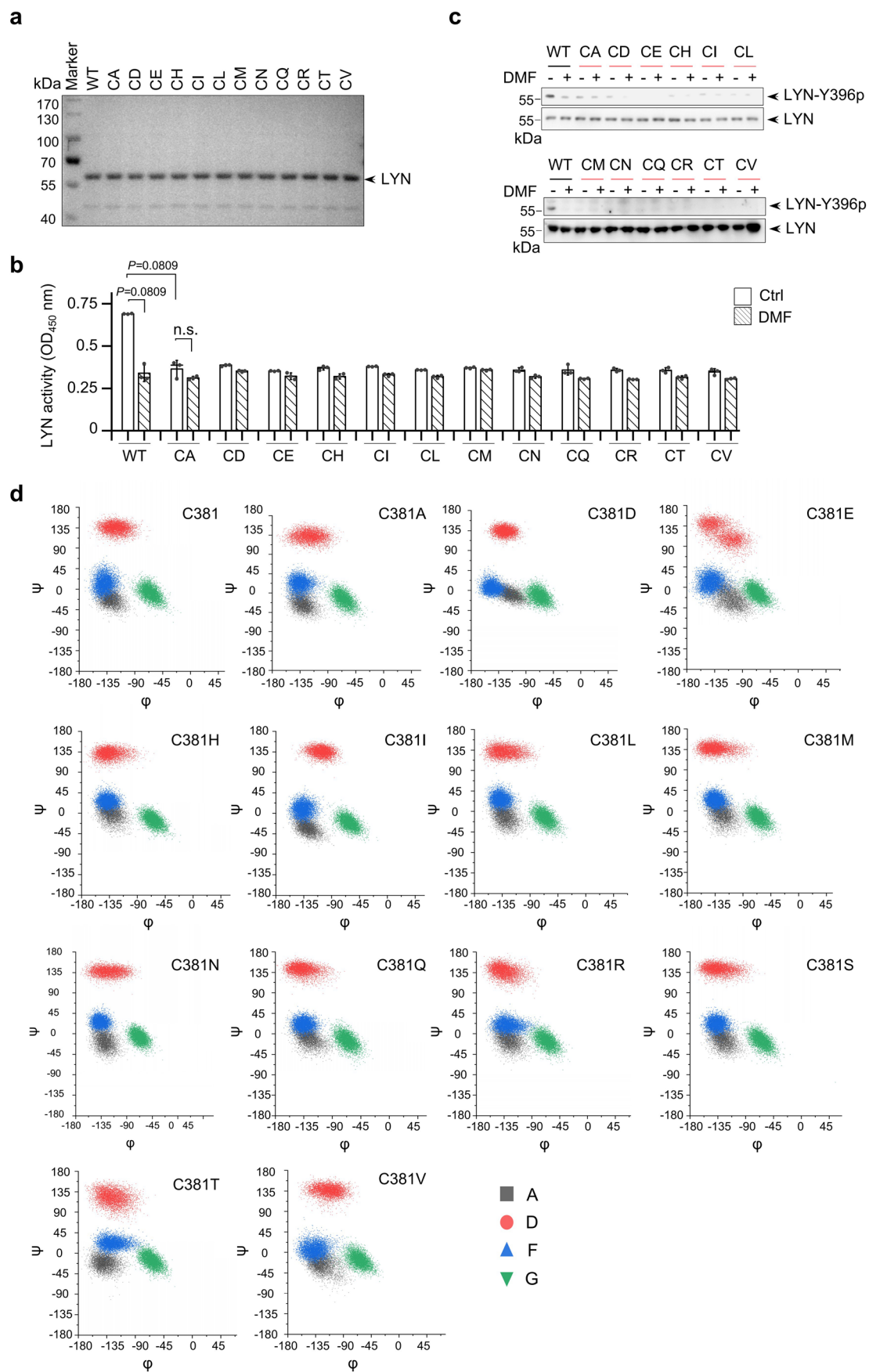
The authors declare no competing interests.

Additional information

Extended data are available for this paper at <https://doi.org/10.1038/s41589-022-01052-0>.

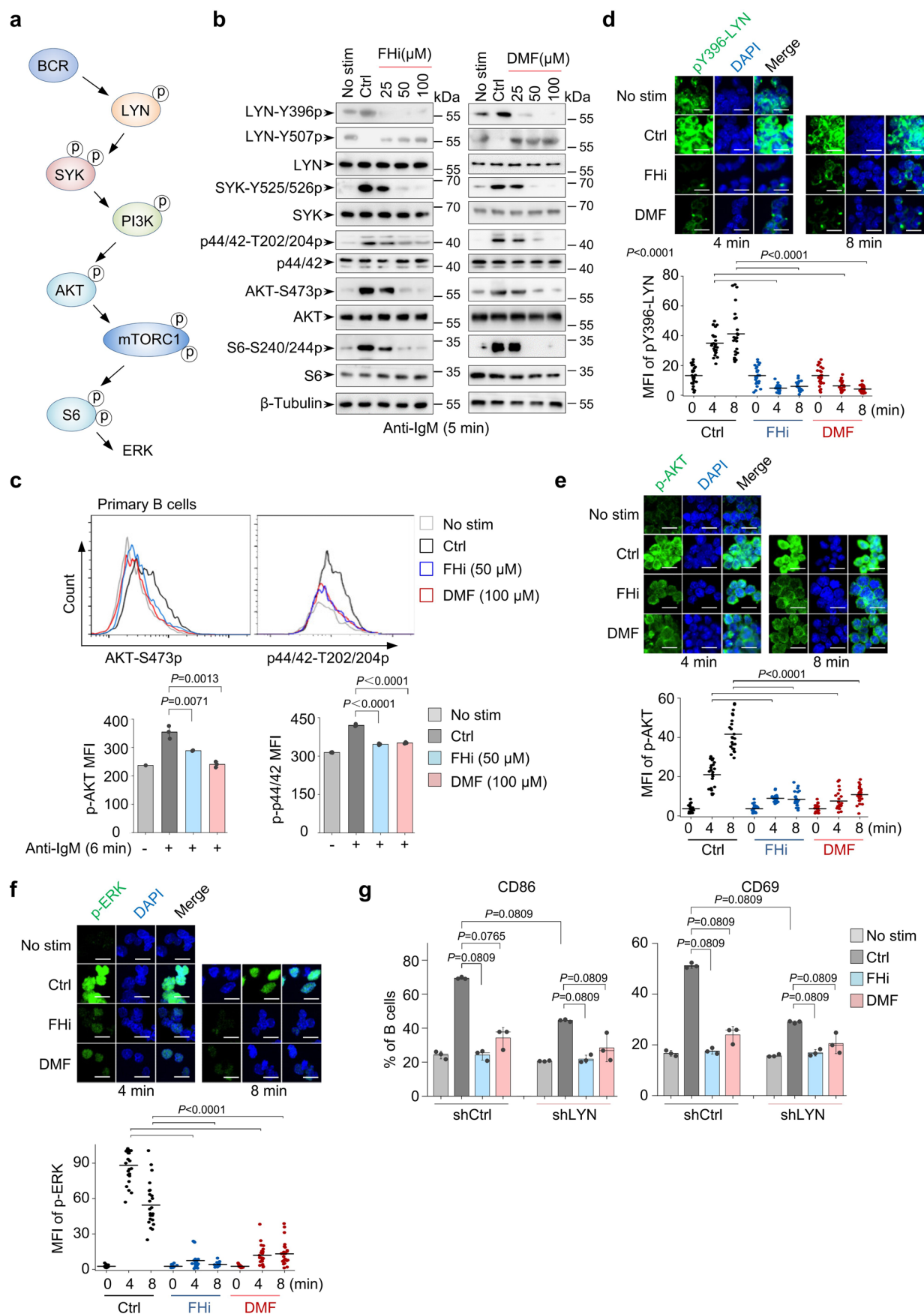
Supplementary information The online version contains supplementary material available at <https://doi.org/10.1038/s41589-022-01052-0>.

Correspondence and requests for materials should be addressed to Xinxiang Zhang or Peng Jiang.



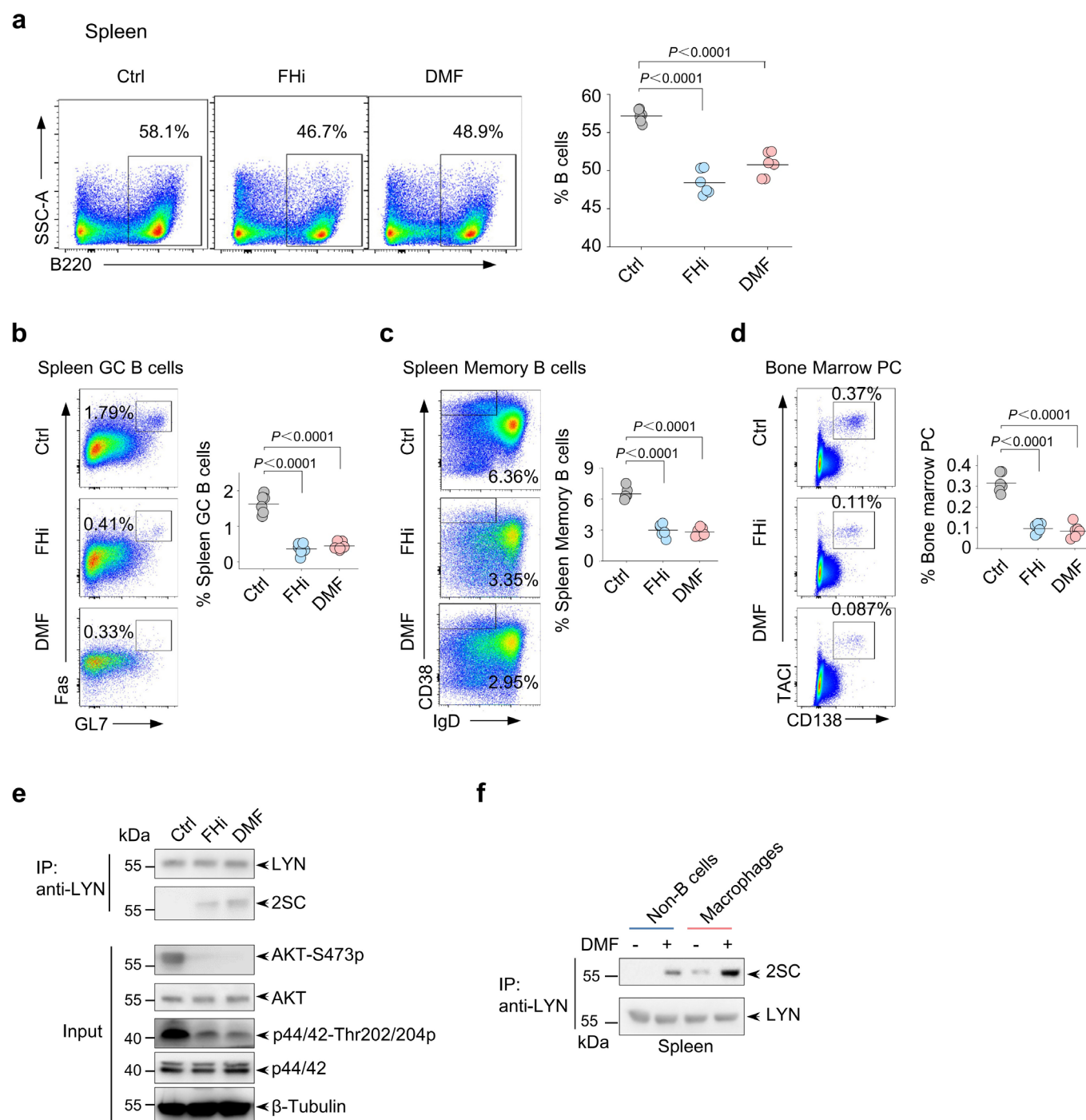
Extended Data Fig. 5 | See next page for caption.

Extended Data Fig. 5 | C381 residue structurally controls LYN activity. a-c, Purified WT and mutant LYN were analyzed by SDS-PAGE followed by Coomassie Blue staining (**a**). The LYN proteins were incubated with DMSO (Ctrl) or with DMF (100 μ M) for 30 minutes. LYN kinase activity was analyzed by ELISA (**b**), and levels of LYN and phosphorylated LYN-Y396 were determined by western blot (**c**). **d,** Ramachandran plot of various LYN-C381 mutants. The data in **b** is non-Gaussian distributed, and the differences were evaluated by using 2-tailed Mann-Whitney U test. Data are the mean $\pm$ SD. Each experiment was performed three independent times. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, based on Mann-Whitney U test.



Extended Data Fig. 6 | See next page for caption.

Extended Data Fig. 6 | Fumarate accumulation attenuates BCR signaling. **a**, The BCR signaling pathway is illustrated. **b**, Mouse naive B cells were treated with DMSO (Ctrl), or increasing amounts of FHi or DMF for 1 hour followed by stimulation with 10 $\mu\text{g}/\text{ml}$ of F(ab')_2 fragment of anti-mouse IgM antibody (anti-IgM) for 5 minutes or no stimulation (no stim). Cell lysates were prepared and used for immunoblotting with indicated antibodies. **c**, Mouse naive B cells pretreated with DMSO (Ctrl), 50 μM FHi or 100 μM DMF for 1 hour were stimulated with 10 $\mu\text{g}/\text{ml}$ F(ab')_2 fragments of anti-mouse IgM antibody (anti-IgM) for 6 minutes or left unstimulated (no stim). The phosphorylation levels of AKT-S473 and p44/42-T202/204 were analyzed by FACS ($n = 3$ independent wells). **d-f**, Mouse naive B cells pretreated with DMSO (Ctrl), FHi (50 μM) or DMF (50 μM) for 30 minutes were stimulated with 10 $\mu\text{g}/\text{ml}$ F(ab')_2 fragments of anti-mouse IgM antibody for indicated times, or left unstimulated (no stim). Then cells were subjected to immunostaining with the indicated antibodies followed by confocal immunofluorescence imaging. Scale bars, 30 μm . **g**, Related to Fig. 4e. Mouse naive B cells were infected with lentivirus expressing LYN shRNA (shLYN) or scramble shRNA (shCtrl) were treated with DMSO (Ctrl), 100 μM DMF, or 50 μM FHi for 1 hour. Then cells were stimulated with 10 $\mu\text{g}/\text{ml}$ F(ab')_2 fragments of anti-mouse IgM antibody for 24 hours or left unstimulated (no stim). The percentage of CD86⁺ B cells and CD69⁺ B cells was measured by FACS analysis ($n = 3$ independent wells). The data in **c** are normally distributed, and the differences were evaluated by using 2-tailed Student's *t* test. The data in **d-g** are non-Gaussian distributed, and the differences were evaluated by using 2-tailed Mann-Whitney U test. Data are the mean $\pm$ SD. Each experiment was performed three independent times. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, based on Student's *t*-test or Mann-Whitney U test.

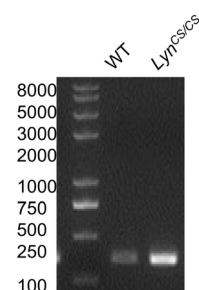
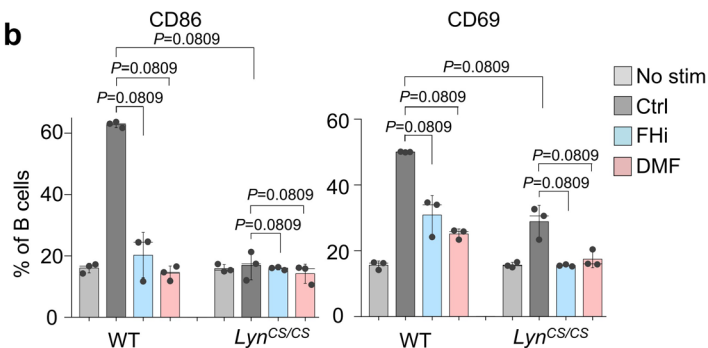
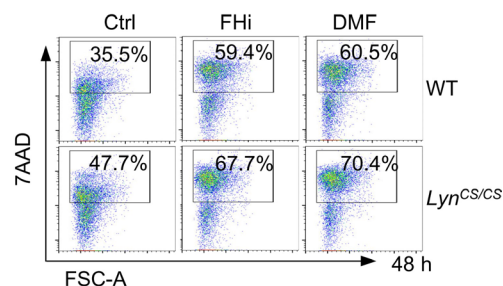
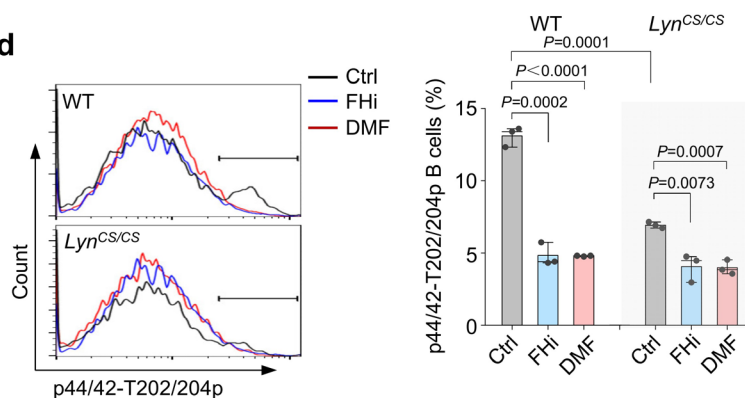
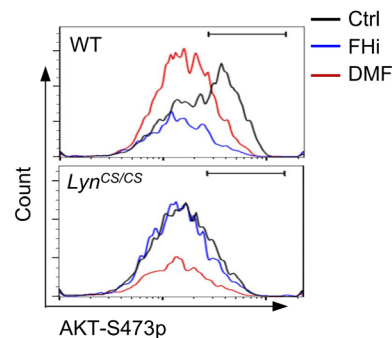
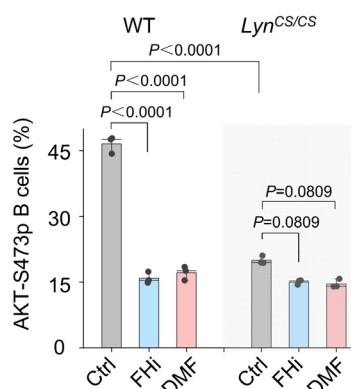
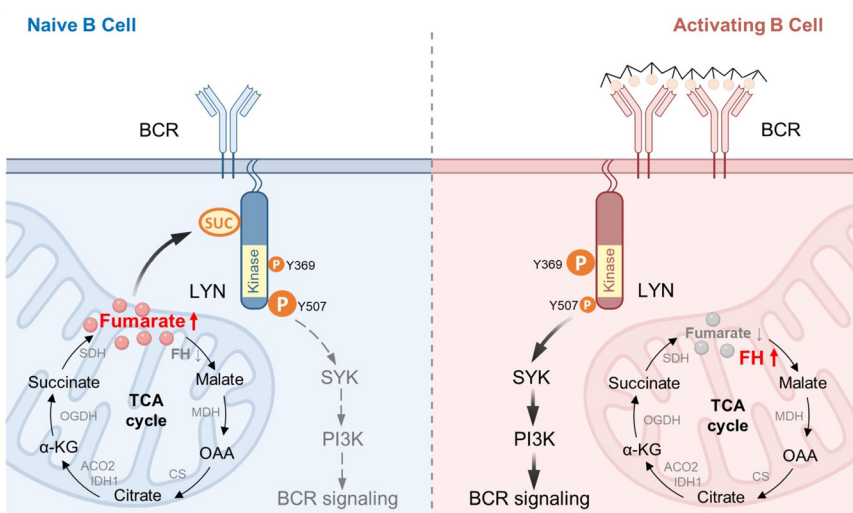


Extended Data Fig. 7 | Fumarate accumulation impairs B-cell development in CD4-depleted mice and induces LYN succination in myeloid cells. a-d, C57BL/6 mice were intraperitoneally (i.p.) injected with anti-CD4 antibody (clone GK1.5, 200 μ g/mouse, twice a week) to deplete CD4⁺ T cells. The mice were then injected intraperitoneally (i.p.) with DMSO (Ctrl) or FHi (25 mg/kg mouse), or treated with DMF (50 mg/kg mouse) by oral gavage for 4 days ($n=6$ mice per group), followed by immunization with NP-LPS (10 μ g/mouse) for another 7 days. Percentage of B220⁺ cells (**a**), germinal center (GC) B cells (**b**), and memory B cells (**c**) in spleens, and bone marrow plasma cells (**d**) was analyzed by FACS analysis. GC B cells (GL7⁺, Fas⁺) were pregated on B220⁺ cells. SP, spleen; PC, plasma cells; BM, bone marrow; GC, germinal center; Mem B, memory B cells. **e**, Mouse naive B cells pretreated with DMSO (Ctrl), FHi (50 μ M) or DMF (50 μ M) for 1 hour were stimulated with 10 μ g/ml F(ab')₂ fragments of anti-mouse IgM antibody for 5 minutes. LYN protein was immunoprecipitated using anti-LYN antibody and immunoblotted with indicated antibodies. **f**, Mouse non-B cells were enriched from spleens of C57BL/6J mice and treated without (-) or with DMF (50 μ M) for 1 hour. Mouse bone marrow-derived macrophages (BMDMs) were isolated from C57BL/6J mice and cultured with 10 ng/mL M-CSF for 7 days before treated without (-) or with DMF (50 μ M) for 1 hour. LYN proteins in non-B cells and BMDMs were immunoprecipitated using anti-LYN antibody and immunoblotted with indicated antibodies. The data in **a-d** are normally distributed, and the differences were evaluated by using 2-tailed Student's *t* test. Data are the mean $\pm$ SD. Each experiment was performed three independent times. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, based on Student's *t*-test.

a

Lyn-C381S (mutation residues are indicated in green color)

122456 AAAGAT GCACAA ACACAC ACATAC ACATAT GAACAC ACACAG AGACAT ACAGAT AATAAA
 122516 ACCTTC TTGAA GTTATC ATCATT GTAGAT GTTAGG GACAGA TTTTAG AAAGGA TTCTAA
 122576 GGGTGA CTTTAG CACTAT CTTAAA AATGTG AACAAC AGGAAT AATAAT ACACAT GAAAGA
 122636 ATCTCA AAAGCT CAAGTT CTGTGT TTGTGT TTGTTT TGTITT TCTTGA TGGCCA ACCATG
 122696 ACAGCC TATCTA GAATAC AAGCCT GAGCCC CTTCTA TTGCA GATTGC AGAAGG CATGGC
 122756 GTACAT CGAGCG GAAGAA CTACAT CCACCG TGATCT GCGAGC TGCTAA CGTCCT CGTCAG
 122816 CGAGTC ACTCAT GAGCAA GATTGC AGACTT TGGCCT CCGCAG AGTCAT CGAAGA TAACGA
 122876 GTACAC AGCAAG GGAAGG TATGTG TGCTGG GCCACA GAGGCC ATCTCA CTGCAG GCTTGT
 122936 GCTCTG CTTTCC AACTTT TGACCC CCGTGC CTGATG CCCTCC TGTTC TCCACC CCCAAT
 122996 TC T C T G C A A T C T T G C T C A T G A G T G

**b****c****d****e****f****g**

Extended Data Fig. 8 | See next page for caption.

Extended Data Fig. 8 | Generation of *Lyn*^{CS/CS} (*Lyn*^{C381S/C381S}) KI mice and effect of C381S mutation on BCR signaling. **a**, Generation of *Lyn*^{CS/CS} (*Lyn*^{C381S/C381S}) KI mice. KI mutation was recombined in oosphere of C57BL/6JGpt background mice by using CRISPR/Cas9 system. After amplification of mice genome, PCR products were analyzed by southern blot (right panel) and sequenced (left panel). **b**, Mouse naive B cells were treated as in Fig. 5c. the percentage of CD86⁺ B cells and CD69⁺ B cells was measured by FACS analysis (n=3 independent wells). **c**, Mouse naive B cells enriched and isolated from *Lyn*^{WT/WT} and *Lyn*^{CS/CS} (*Lyn*^{C381S/C381S}) mice were treated with DMSO (Ctrl), FHi (50 μM) or DMF (100 μM) for 1 hour. After stimulation with F(ab')₂ fragments of anti-mouse IgM antibody for 48 hours, cell death was determined by 7AAD staining and FACS analysis. **d-f**, Related to Fig. 6b. Mouse naive B cells enriched and isolated from *Lyn*^{WT/WT} and *Lyn*^{CS/CS} (*Lyn*^{C381S/C381S}) mice were treated with DMSO (Ctrl), FHi (50 μM) or DMF (100 μM) for 1 hour. After stimulation with 10 μg/ml F(ab')₂ fragments of anti-mouse IgM antibody for 5 min, cells were fixed and penetrated for FACS analysis of the phosphorylation of p44/42-T202/204 (**d**) and AKT-S473 (**e, f**). **g**, A working model illustrating succination of LYN by fumarate suppressing B-cell activation and function. Increased fumarate in naive B cells due to low expression of FH directly succinates LYN at C381 and inhibits LYN activity, resulting in a block to BCR signaling activation. However, during B-cell activation, FH expression is strongly upregulated and consequently intracellular fumarate is reduced, leading to a decrease in LYN succination and activation of LYN and BCR signaling. The data in **d** and **f** are normally distributed, and the differences were evaluated by using 2-tailed Student's t test. The data in **b** is non-Gaussian distributed, and the differences were evaluated by using 2-tailed Mann-Whitney U test. Data are the mean ± SD. Each experiment was performed three independent times. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, based on Student's *t*-test or Mann-Whitney U test.

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- ☒ ☐ A description of all covariates tested
- ☐ ☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- ☐ ☒ A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- ☐ ☒ For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
Give P values as exact values whenever suitable.
- ☒ ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- ☒ ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- ☒ ☐ Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated

Our web collection on [statistics for biologists](#) contains articles on many of the points above.

Software and code

Policy information about [availability of computer code](#)

Data collection

Real-Time PCR Analysis Software CFX Maestro Software 2.3 was used for Real-Time PCR signal collection.
Xcalibur Software (version 2.2.0) was used for metabolic and proteomic signal collection.
BD FACSDiva Software v8.0 was used for FACS signal collection.
Softmax pro 6.3 was used to collect fluorescent and absorption data for enzyme assay.
Andor SpinningDisk (Dragonfly) Confocal fluorescence microscope consisting of an inverted microscope (TIE2, Nikon) equipped with and Nikon UPLSAPO 100 x NA 1.49 oil immersion lens was used to collect the confocal fluorescence signal.
Pymol (version 2.52) and Gromacs (version 2021.3) was used for molecular dynamic simulation.

Data analysis

OriginPro 2018C b9.5.0.193 was used for data plotting and statistical analysis.
Xcalibur Software (version 2.2.0) was used for metabolic and proteomic data acquisition and processing.
Mascot (version 2.3.02) was used for protein modification analysis.
Flowjo v10.0.7 was used for FACS data analyzing.
Proteome Discoverer (version 2.4) was used for quantitative proteomics data analysis.
Microsoft Excel 2016MSO (version 2202 Build 16.0.14931.20118) was used for demethylation ratio quantification and statistical analysis.
Certain western blot bands were quantified using Image J software version 1.37.
DAVID bioinformatics database was used for gene ontology analysis.
ImarisViewer x64 9.5.1 was used for confocal immunofluorescence imaging data analysis and data were then quantified by Image J software version 1.37.
Pymol (version 2.52) and Gromacs (version 2021.3) was used for molecular dynamic simulation.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The source data underlying Fig. 1-6 as well as Extended Data Fig. 1-8 are provided as a Source Data file. All the other data supporting the findings of this study are available within the article and its supplementary information files and from the corresponding author upon reasonable request. A reporting summary for this article is available as a Supplementary Information file. The FACS gating strategies are described in Extended Data Fig. 9. LYN crystal structure (PDB ID:5xy1) is available on the RCSB Protein Data Bank (<https://www.rcsb.org/structure/5XY1>, PDB DOI: 10.2210/pdb5XY1/pdb). Proteome database (mouse, Mus musculus, UP000000589) is available on the UniProt (<https://www.uniprot.org/proteomes/UP000000589>).

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Statistical methods were not used to predetermine sample size. All sample size was at least three replicates. Animal sample size was at least five mice and determined by experimental feasibility and sample availability to demonstrate certain results.
Data exclusions	No data were excluded from the analyses.
Replication	Biological replications and statistics were indicated in the legends. All attempts at replication were successful based on replications on different days showing comparable significance level for biological comparison.
Randomization	Lyn knock in mice samples were allocated into experimental groups by the confirmed Lyn genetic mutation. This design does not allow for randomization, as the origin of samples is critical. However, whenever possible samples were analyzed in a randomized order (i.e. mouse experiments with FHi or DMF treatment, or when run on liquid-chromatography mass spectrometry).
Blinding	The researchers were blinded to group allocation during animals research data collection and analysis, experiments apart from animal studies, and data analysis.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology and archaeology
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data
☒	☐ Dual use research of concern

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	Phospho-AKT (Ser473), Cell Signaling Technology 4060, 1:1000 for WB; AKT, Cell Signaling Technology 9272S, 1:1000 for WB; S6, Cell Signaling Technology 2217, 1:1000 for WB; Phospho-S6 (Ser240/244), Cell Signaling Technology 5364, 1:1000 for WB;
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Phospho-Tyrosine Mouse mAb (P-Tyr-100), Cell Signaling Technology 4g10, 1:1000 for WB, 1:5000 for ELISA ;
 Phospho-Src Family (Tyr416), Cell Signaling Technology 2101S, 1:1000 for WB;
 Phospho-Lyn (Tyr507) Cell Signaling Technology 2731S, 1:1000 for WB;
 Phospho-Syk (Tyr525/526) Cell Signaling Technology 2711S, 1:1000 for WB;
 SYK Cell Signaling Technology 2712T, 1:1000 for WB;
 PT202/T204-ERK1/2 Cell Signaling Technology 4370S, 1:1000 for WB;
 ERK Cell Signaling Technology 9102, 1:1000 for WB;
 Lyn (H-6) Santa Cruz sc-7274, 1:1000 for WB, 1:200 for IP;
 ACTB Santa Cruz sc-1616, 1:2000 for WB;
 Beta-tubulin Santa Cruz sc-55529, 1:2000 for WB;
 GAPDH Santa Cruz sc-47724, 1:2000 for WB;
 FH GeneTex GTX109877, 1:1000 for WB;
 HRP-conjugated Goat anti-mouse IgG (H+L) antibody, Jackson ImmunoResearch Laboratories, 115-035-003, 1:5000 for WB;
 HRP-conjugated Goat anti-rabbit IgG (H+L) antibody, Jackson ImmunoResearch Laboratories, 115-035-045, 1:5000 for WB;
 AffiniPure F(ab')₂ Fragment Goat Anti-Mouse IgG + IgM (H+L) Jackson ImmunoResearch Laboratories, 115-006-068, 10 mg ml⁻¹ for stimulation;
 anti-CD4 clone GK1.5 antibody, Thermo 14-0041-86, 200 mg per mice for CD4⁺ T cells depletion;
 mTACI-PE antibody, Biolegend 133404, 1:200 for FACS ;
 CD69-BV421 Biolegend 104528, 1:200 for FACS;
 FITC anti-mouse Ki-67 Biolegend 652410, 1:200 for FACS;
 PE anti-mouse CD86 Biolegend 105106, 1:200 for FACS;
 APC-B220 Biolegend 103212, 1:200 for FACS;
 anti mouse IgM-FITC Biolegend 406506, 1:200 for FACS;
 FITC-IgD EBIOSCIENCE 11-5993-82, 1:200 for FACS;
 IgM-HRP Southernbiotech 1021-05, 1:500 for ELISA;
 IgG1-HRP Southernbiotech 1070-05, 1:1000 for ELISA;
 IgG2b-HRP Southernbiotech 1091-05, 1:1000 for ELISA;
 IgG2a-HRP Southernbiotech 1081-05, 1:1000 for ELISA;
 IgG3-HRP Southernbiotech 1100-05, 1:1000 for ELISA.

Validation

All antibodies used in this work were purchased from companies, and validated by the manufacturers and by extensive use in published work. All western blot, IP, FACS, and ELISA antibodies in the manuscript had been validated. Each antibody has been validated for the species and application on the manufacturer's website.

Antibodies used in WB were validated in mouse:

AKT (Cell Signaling Technology 9272S, <https://www.cellsignal.com/products/primary-antibodies/akt-antibody/9272>), Phospho-AKT (Ser473) (Cell Signaling Technology 4060, <https://www.cellsignal.com/products/primary-antibodies/phospho-akt-ser473-d9e-xp-rabbit-mab/4060>), S6 (Cell Signaling Technology 2217, <https://www.cellsignal.com/products/primary-antibodies/s6-ribosomal-protein-5g10-rabbit-mab/2217>), Phospho-S6 (Ser240/244) (Cell Signaling Technology 5364, <https://www.cellsignal.com/products/primary-antibodies/phospho-s6-ribosomal-protein-ser240-244-d68f8-xp-rabbit-mab/5364>), Phospho-Tyrosine Mouse mAb (P-Tyr-100) (Cell Signaling Technology 4g10, <https://www.cellsignal.com/products/primary-antibodies/phospho-tyrosine-4g10-mouse-mab/96215?site-search-type=Products&N=4294956287&Ntt=4g10&fromPage=plp>), Phospho-Src Family (Tyr416) (Cell Signaling Technology 2101S, https://www.cellsignal.com/products/primary-antibodies/phospho-src-family-tyr416-antibody/2101?site-search-type=Products&N=4294956287&Ntt=2101s&fromPage=plp&_requestid=4671612), Phospho-Lyn (Tyr507) (Cell Signaling Technology 2731S, https://www.cellsignal.com/products/primary-antibodies/phospho-lyn-tyr507-antibody/2731?site-search-type=Products&N=4294956287&Ntt=2731s&fromPage=plp&_requestid=4671938), Phospho-Syk (Tyr525/526) (Cell Signaling Technology 2711S, https://www.cellsignal.com/products/primary-antibodies/phospho-syk-tyr525-526-antibody/2711?site-search-type=Products&N=4294956287&Ntt=2711s&fromPage=plp&_requestid=4672029), SYK (Cell Signaling Technology 2712T, https://www.cellsignal.com/products/primary-antibodies/syk-antibody/2712?site-search-type=Products&N=4294956287&Ntt=2712t&fromPage=plp&_requestid=4672150), PT202/T204-ERK1/2 (Cell Signaling Technology 4370S, https://www.cellsignal.com/products/primary-antibodies/phospho-p44-42-mapk-erk1-2-thr202-tyr204-d13-14-4e-xp-rabbit-mab/4370?site-search-type=Products&N=4294956287&Ntt=4370s&fromPage=plp&_requestid=4672225), ERK (Cell Signaling Technology 9102, <https://www.cellsignal.com/products/primary-antibodies/p44-42-mapk-erk1-2-antibody/9102>), Lyn (H-6) (Santa Cruz 100704, <https://www.scbt.com/p/lyn-antibody-h-6>), ACTB (Santa Cruz sc-1616, <https://www.scbt.com/p/actin-antibody-i-19?requestFrom=search>), Beta-tubulin (Santa Cruz sc-55529, <https://www.scbt.com/p/beta-tubulin-antibody-g-8?requestFrom=search>), GAPDH (Santa Cruz sc-47724, <https://www.scbt.com/p/gapdh-antibody-0411?requestFrom=search>), FH (GeneTex, GTX109877, <https://www.genetex.cn/Product/Detail/Fumarate-hydratase-antibody/GTX109877>), HRP-conjugated Goat anti-mouse IgG (H+L) (Jackson ImmunoResearch Laboratories, 115-035-003, <https://www.jacksonimmuno.com/catalog/products/115-035-003>), HRP-conjugated Goat anti-rabbit IgG (H+L) (Jackson ImmunoResearch Laboratories, 111-035-045, <https://www.jacksonimmuno.com/catalog/products/111-035-045>), AffiniPure F(ab')₂ Fragment Goat Anti-Mouse IgG + IgM (H+L) (Jackson ImmunoResearch Laboratories, 115-006-068, <https://www.jacksonimmuno.com/catalog/products/111-035-045>).

Antibody for IP was validated in mouse: Lyn (H-6) (Santa Cruz 100704, <https://www.scbt.com/p/lyn-antibody-h-6>).

Antibodies for FACS were validated in mouse:

BV421 anti-mouse CD69 (Biolegend 104528 1:200 for FACS, <https://www.biolegend.com/en-us/products/brilliant-violet-421-anti-mouse-cd69-antibody-7358>), FITC anti-mouse Ki-67 (Biolegend 652410 1:200 for FACS, <https://www.biolegend.com/en-us/products/fits-anti-mouse-ki-67-antibody-8573>), PE anti-mouse CD86 (Biolegend 105106 1:200 for FACS, <https://www.biolegend.com/en-us/products/pe-anti-mouse-cd86-antibody-435>), APC anti-mouse B220 (Biolegend 103212 1:200 for FACS, <https://www.biolegend.com/en-us/products/apc-anti-mouse-human-cd45r-b220-antibody-442>), FITC anti-mouse IgM (Biolegend 406506 1:200 for FACS, <https://www.biolegend.com/en-us/products/fits-anti-mouse-igm-2334>), FITC anti-mouse IgD (EBIOSCIENCE 11-5993-82 1:200 for FACS, <https://www.thermofisher.cn/cn/zh/antibody/product/IgD-Antibody-clone-11-26c-11-26-Monoclonal/11-5993-82>), PE anti-mouse TACI (Biolegend 133404 1:200 for FACS, <https://www.biolegend.com/en-us/products/pe-anti-mouse-cd267-taci-antibody-5807>).

Antibodies for ELISA were validated in mouse:

IgM-HRP (Southernbiotech 1021-05 1:500 for ELISA, <https://www.southernbiotech.com/goat-anti-mouse-igm-hrp-1021-05>), IgG1-HRP (Southernbiotech 1070-05 1:1000 for ELISA, <https://www.southernbiotech.com/goat-anti-mouse-igg1-human-ads-hrp-1070-05>),

IgG2b-HRP (Southernbiotech 1091-05 1:1000 for ELISA, <https://www.southernbiotech.com/goat-anti-mouse-igg2b-hrp-1091-05>), IgG2a-HRP (Southernbiotech 1081-05 1:1000 for ELISA, <https://www.southernbiotech.com/goat-anti-mouse-igg2a-hrp-1081-05>), IgG3-HRP (Southernbiotech 1100-05 1:1000 for ELISA, <https://www.southernbiotech.com/goat-anti-mouse-igg3-human-ads-hrp-1100-05>). Anti-CD4 (clone GK1.5) used for CD4+ T cells depletion was validated in mouse (Thermofisher 14-0041-86 200 mg per mice, <https://www.thermofisher.cn/antibody/product/14-0041-86.html>).

Eukaryotic cell lines

Policy information about [cell lines](#)

Cell line source(s)	293T cells and A20 cells were ordered from ATCC. Lenti-X 293T cells were ordered from Takara (632180). All cell lines were cultured for no more than 2 months and their morphology was confirmed periodically to avoid crosscontamination or misuse of cell lines.
Authentication	To authenticate cell lines, we used short tandem repeat (STR) profiling method as exhaustively described by Dr. Asadi Jahanbakhsh and his/her colleagues (Khosravi Ayyoob et al, Tumor Biol. (2016) 37:3197-3204).
Mycoplasma contamination	All cells used have been tested negative for mycoplasma contamination.
Commonly misidentified lines (See ICLAC register)	No such cell lines were used in this work. Furthermore, we have authenticated all the cell lines used in our lab.

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals	Six- to eight-week-old male C57BL/6 mice were purchased from Vital River Laboratory Animal Technology for B cell isolation or serum immunogen experiments. Six- to eight-week-old C57BL/6 Lyn knockin mice were generated according to Method and used for B cell isolation or serum immunogen experiments. All animals were maintained in groups 5-6 animals on a 12/12-h light/dark cycle at 20-26 °C with around 40-70% humidity, and give ad libitum access to standard food and water.
Wild animals	The study did not involve wild animals.
Field-collected samples	The study did not involve field-collected samples
Ethics oversight	All of the mice used in this study have been accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International and Institutional Animal Care and Use Committee (IACUC) of Tsinghua University. All of the mice were maintained in pathogen-free facilities and used strictly in accordance with the protocols approved by the IACUC of Tsinghua University. The study is compliant with all of the relevant ethical regulations regarding animal research.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Flow Cytometry

Plots

Confirm that:

- ☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- ☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- ☒ All plots are contour plots with outliers or pseudocolor plots.
- ☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Mouse primary B cells were isolated from spleen by negative selection kit. Cells were cultured in 1640 medium as described in Methods. For surface staining, cells were harvested and washed by PBS for 2 times, then stained in PBS containing 2% FBS with antibodies at the recommended dilution on ice for 30 minutes in the dark and then washed twice with PBS prior to Flow Cytometry analysis. For intracellular of mouse primary B cells to measure Ki67, cells were harvested and washed for 2 times with PBS, fixed, permeabilized and stained with transcription factor staining buffer set (eBioscience, 00-5223-56 and 00-5123-43) according to manufacturer's protocol. Stained cells were washed 2 times with PBS prior to FACS analysis.
Instrument	Cells were sorted on BD Accuri C6 flow cytometer (BD Biosciences, San Jose, CA, USA).

Software

FACSDiva software (BD) version 8.0 for FACS data collection and Flowjo V10 for FACS data analysis.

Cell population abundance

Cell purity was 99.3% determined by post-sort purity checks of representative samples.

Gating strategy

Cells were first gated using FSC/SSC characteristics to exclude debris, followed by gating FSC-A and FSC-H, then SSC-A and SSC-H to eliminate nonsinglets.
Then target cells were gated the population of interest by specific stain.
The details of the gating strategy was provided in the extended Figure 10.

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.