Mechanisms determining a differential threshold for sensing Src family kinase activity by B and T cell antigen receptors

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Although signal transduction by immunoreceptors such as the T cell antigen receptor (TCR), B cell antigen receptor (BCR), and Fc receptors uses the same schematic and similar molecules, the threshold and the fine-tuning are set differently for each receptor. One manifestation of these differences is that inhibition of Src family kinases (SFK) blocks TCR but not BCR signaling. SFKs are key kinases phosphorylating immunoreceptor tyrosine-based activation motifs (ITAM) in both these receptors. However, it has been proposed that in B cells, downstream kinase SYK can phosphorylate ITAM sequences independently of SFK, allowing it to compensate for the loss of SFK activity, whereas its T cell paralog ZAP-70 is not capable of this compensation. To test this proposal, we examined signaling in SYK- and ZAP-70-deficient B and T cell lines expressing SYK or ZAP-70. We also analyzed signal transduction in T cells expressing BCR or B cells expressing part of the TCR complex. We show that when compared with ZAP-70, SYK lowered the threshold for SFK activity necessary to initiate antigen receptor signaling in both T and B cells. However, neither SYK nor ZAP-70 were able to initiate signaling independently of SFK. We further found that additional important factors are involved in setting this threshold. These include differences between the antigen receptor complexes themselves and the spatial separation of the key transmembrane adaptor protein LAT from the TCR. Thus, immunoreceptor sensing of SFK activity is a complex process regulated at multiple levels.

Evolution of receptors and associated signaling pathways has generated a range of different signal transduction systems. However, among this variety, groups of receptors can be distinguished which utilize common schemes of signal transmission. The multiple usage of similar molecules in similar arrangements is typical for immunoreceptor signaling, including signaling by TCR, BCR, Fc receptors, NK cell receptors, and several others (1–4). The signaling is initiated by Src family kinases (SFK), which phosphorylate immunoreceptor tyrosine-based activation motifs (ITAM), located within intracellular domains of receptor-associated transmembrane adaptor proteins. Phosphorylated ITAM motifs serve as docking sites for SYK family kinases. Their activation is a crucial step in subsequent signal transduction. It results in the recruitment and assembly of a signalosome activating Ca\(^{2+}\) response (increase of intracellular concentration of free Ca\(^{2+}\) ions) (1–4).

Several studies have questioned this basic scheme of immunoreceptor signaling by suggesting that under some circumstances BCR signaling can be triggered independently of SFKs (5–8). We and others have demonstrated that SFK inhibition by widely used inhibitor PP2 (9, 10) abolished TCR signaling in T cells but was unable to block signaling by BCR in B lymphocytes (8, 11). Similar observation was later also reported by another group, showing the same effect at the level of SLP65 and SLP76 adaptor protein phosphorylation (12). It has been proposed that SFK-independent signaling in B cells can be explained by unique features of SYK. SYK family of kinases contains only two members, SYK and ZAP-70. They show different expression patterns. B cells and myeloid cells express mainly SYK and T cells mainly ZAP-70 (13, 14). Both kinases are in the steady state in the cytoplasm in a closed inactive conformation and upon receptor activation, they are recruited to the phosphorylated ITAM motifs. Binding to ITAMs via their tandem SH2 domains leads to their transition to active conformation (15–18). However, to become fully active and/or to stabilize interaction with ITAMs, SYK family kinases must be further phosphorylated. For ZAP-70 this phosphorylation seems to be more dependent on SFKs than for SYK, which shows higher propensity to autophosphorylate during this step (13, 17–23). Furthermore, it has been proposed that intense stimulation of B cells with multivalent ligands can trigger SYK to phosphorylate ITAM motifs independently of SFKs; ZAP-70 has been thought to lack this ability. However, the data directly demonstrating the SYK-mediated phosphorylation of ITAM motifs were all generated using either recombinant proteins and peptides * in vitro or overexpression in nonhematopoietic cells, such as S2, 293T, or COS cells (5, 8, 23, 24).

In this study, we have re-evaluated these data using human B and T cell lines. We show that SYK-mediated ITAM phosphorylation is rather limited and that what may appear as SFK-independent signaling by SYK in studies employing SFK inhibitors can be largely explained by incomplete inhibition of SFKs, coupled to a low threshold for signaling initiation in B cells. Importantly, multiple components of TCR and BCR signaling apparatuses, including antigen receptors themselves, Src and Syk family kinases, and LAT, appear to be parts of a complex mechanism differentially setting this threshold for signaling by BCR and TCR.

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Results

**Differences between SYK and ZAP-70 cannot fully explain differential sensitivity of TCR and BCR signaling to SFK inhibition**

First, we established our experimental system using model T cell line Jurkat and B cell line Ramos. In Jurkat T cells, 2 μM concentration of PP2 was sufficient to completely inhibit Ca²⁺ response initiated by TCR crosslinking with anti-TCR antibody C305 (Fig. 1A). Consistent with previously published data (8,11), Ca²⁺ response in B cell line Ramos stimulated with anti-BCR antibody was resistant to at least 20 μM PP2, although it exhibited a delay dependent on the PP2 dose (Fig. 1B). The difference can be explained by two alternative hypotheses. First, that downstream B cell kinase SYK but not its T cell parologue ZAP-70 can initiate receptor signaling by phosphorylating ITAMs independently of SFKs. Second, it is also possible that SFKs are incompletely inhibited by PP2 and their residual activity is sufficient to initiate signaling by BCR but not by TCR.

To start addressing these questions we employed Jurkat-derived T cell line P116, which lacks expression of SYK and ZAP-70 (25). We reconstituted these cells with SYK, ZAP-70, or empty vector (all containing IRES-CD90.1 reporter) and stimulated these cells with anti-TCR antibody C305. To select the cells with expression of SYK and ZAP-70 similar to their

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**Figure 1. Differences between SYK and ZAP-70 only partially explain higher resistance of BCR signaling to SFK inhibition.** A and B, flow cytometry analysis of Ca²⁺ response in TCR-stimulated Jurkat T cell line (A) or BCR-stimulated Ramos B cell line (B) in the presence or absence of different concentrations of PP2 (n ≥ 3). C, flow cytometry analysis of SYK and ZAP-70 expression in P116 cell line transduced with constructs coding for SYK or ZAP-70 followed by IRES-C90.1 reporter. D, Ca²⁺ signaling in P116 SYK or ZAP-70 cells stimulated with anti-TCR antibody C305 (n = 3). See main text for explanation of the gating strategy. E, quantification of Ca²⁺ response from (D). The data are plotted as area under the curve after subtraction of the steady state fluorescence. The data are normalized to PP2 nontreated cells (-fold change). F, Western blot analysis of SYK expression in Ramos and R.SYK<sup>ko</sup> cells. Beta actin staining serves as a loading control. G, Ca²⁺ signaling in Ramos and R.SYK<sup>ko</sup> cells stimulated with anti-BCR antibody. H, flow cytometry analysis of SYK and ZAP-70 expression in R.SYK<sup>ko</sup> cells transduced with construct coding for SYK or ZAP-70-IRES-C90.1. I, Ca²⁺ response in R.SYK<sup>ko</sup> SYK- or ZAP-70-expressing cells stimulated with anti-BCR antibody (n = 3). J, quantification of the data from (I). Data are plotted and analyzed similarly as in (E). A, B, D, G, I, black arrows indicate the time of the stimulation. All data from Ca²⁺ measurements are depicted as medians of relative Ca²⁺ concentration in the cells.
native expression in Jurkat and Ramos cells, we have determined the level of reporter gene CD90.1, at which the amount of transduced SYK or ZAP-70 was similar to the endogenous levels and then we gated on these cells using only the reporter (Fig. 1C and Fig. S1). Interestingly, SYK expression somewhat increased TCR signaling resistance to SFK inhibition and these cells responded even in the presence of 5 μM PP2. However, 10 μM concentration, a standard dose widely used to inhibit SFK activity, completely abolished Ca^{2+} response in SYK expressing P116 cells (Fig. 1, D and E). This result suggested that although it contributes to the resistance of the signaling to SFK inhibition, SYK activity alone cannot explain it.

To further dissect the role of SYK, we complemented the experiment in T cells with a similar experiment in B cell line Ramos. Using CRISPR-Cas9 technology, we have obtained several clones of Ramos B cells, which lack expression of SYK (Fig. 1F). None of the tested clones was responsive to BCR stimulation and all the clones had comparable expression of BCR (Fig. 1G and data not shown). For further experiments, we have used one of these clones, hereafter termed R.SYK^{KO}. To avoid clonal bias, we always used SYK reconstituted cells as a control. We transduced R.SYK^{KO} cells with SYK, ZAP-70, and empty vector (Fig. 1HF) and stimulated the cells with anti BCR antibody in the presence of different doses of PP2. SYK-expressing cells were similarly resistant to PP2 inhibition as original Ramos cells. Importantly, ZAP-70 expressing cells were also resistant to 10 μM PP2 and the signaling was only inhibited by 20 μM concentration (Fig. 1, I and J). Such a high resistance of ZAP-70–expressing cells suggests that the differences between ZAP-70 and SYK account only for part of the resistance of BCR signaling to SFK inhibition and additional mechanisms substantially contribute to this phenomenon.

**Resistance of BCR signaling to SFK inhibition cannot be explained by SYK-mediated ITAM phosphorylation**

To test if SYK ability to phosphorylate ITAMs contributes to the BCR resistance to SFK inhibition, we have analyzed tyrosine phosphorylation of CD79a immunoprecipitated from BCR-stimulated R.SYK^{KO} cells reconstituted (or not) with SYK or ZAP-70. We also probed the phosphorylation of tyrosine 182 of CD79a in the whole cell lysates of the same cells to complement immunoprecipitation with an independent method (Fig. 2A). Expression of both SYK and ZAP-70 increased ITAM phosphorylation in activated cells detected by CD79a immunoprecipitation followed by phosphotyrosine staining. However, only in the case of ZAP-70 the difference was statistically significant (Fig. 2, A and B). Staining for phosphotyrosine 182 of CD79a in the whole cell lysates did not reveal any significant effects, although the results in general followed a similar trend with slight increase of tyrosine 182 phosphorylation in the presence of SYK or ZAP-70 (Fig. 2, A and C). These data do not support the hypothesis that SYK can potentiate ITAM phosphorylation more efficiently than ZAP-70. They rather suggest that ZAP-70 is at least as efficient as SYK if not better. Despite this, SYK was better able to restore the resistance of BCR signaling to SFK inhibition (Fig. 1, I and J). These observations led to the conclusion that any potentially unique ability of SYK to phosphorylate ITAM motifs is not the reason why BCR better tolerates SFK inhibition. Importantly, increase in ITAM phosphorylation induced by BCR stimulation could be observed even after treatment with 20 μΜ PP2 in the absence of SYK and presence of ZAP-70 or in the complete absence of SYK or ZAP-70 (Fig. 2, A–C), demonstrating that even high dose of PP2 is not able to completely inhibit ITAM phosphorylation by SFKs. Collectively, these data show that SYK ability to phosphorylate ITAMs does not explain increased resistance of SYK-expressing cells to SFK inhibition. In addition, they also show that there still is a residual SFK activity in PP2-treated cells, which is probably sufficient to trigger BCR-mediated signaling.

**Structural features of antigen receptors contribute to their differential sensitivity to SFK inhibition**

To address the question whether TCR per se is inherently less sensitive than BCR to SFK activity, we transduced our T and B cell lines with chimeric protein composed of extracellular domain of CD16 and full-length TCRζ (Fig. 3, A and B). Importantly, CD16 is not expressed in any of the cell lines used in our experiments (Fig. 3C). Crosslinking of CD16-ζ on T cells induced strong Ca^{2+} response similar to the one elicited by TCR stimulation (Fig. 3D). Crosslinking of CD16-ζ on B cells induced relatively strongly but already delayed Ca^{2+} response, which was inhibited by 10 μΜ concentration of PP2 (Fig. 3, E and F), indicating that antigen receptor composition is important for the resistance to SFK inhibition. To further explore this possibility, we transduced Jurkat T cells with the BCR complex, including IgH, Igo, and Igβ (Fig. 4A). We stimulated these cells with anti-TCR or anti-BCR antibody and treated with different doses of PP2. Interestingly, and analogously to CD16-ζ–induced signaling in B cells, BCR signaling in T cells was delayed compared with TCR signaling even in PP2 nontreated cells (Fig. 4B). However, despite this, BCR signaling was still more resistant to PP2 inhibition than TCR signaling in the same cells (Fig. 4, B–D), demonstrating that structural features of BCR contribute to the higher resistance of BCR signaling to SFK inhibition.

**Ectopic expression of Lyn in T cells modulates the resistance to SFK inhibition**

Even though BCR signaling in Jurkat T cells was more resistant to PP2 inhibition than TCR signaling in the same cells, it still could be completely inhibited by a relatively low dose of PP2, suggesting that other factors are contributing too. We have shown above that SYK activity is one of these factors. Next we wanted to test whether differences between SFK members in T cells and B cells can also be responsible. Therefore, we transduced the Jurkat BCR cells with Lyn, a major B cell member of Src family (Fig. 5A). Interestingly, in Jurkat BCR cells, Lyn expression accelerated Ca^{2+} response both in the absence and in the presence of PP2, which demonstrates certain level of nonredundancy between the T cell and B cell SFK and suggests that Lyn expression also slightly increases the resistance of BCR signaling to PP2. Moreover, the fact that Lyn was able to alter signaling output significantly more in the presence of PP2 further supports the argument that signaling in PP2-treated cells is still initiated by SFKs (Fig. 5, B and C).
**Src-family kinases in BCR and TCR signaling**

**Bridging LAT to TCR induces strong resistance to SFK inhibition**

The data described above suggested that multiple members of antigen receptor signaling apparatus cooperate to set the threshold for the amount of SFK activity required for receptor activation. This threshold appears to be lower in B cells than in T cells, allowing B cells to respond even to the residual SFK activity surviving in the presence of PP2. When considering the differences between BCR and TCR signaling at the level of organization of the molecules potentially involved in regulating this threshold, we realized that one of the most apparent differences is in the usage of receptor-independent transmembrane adaptor LAT by TCR signaling machinery, which serves as a docking site for assembly of PLCγ containing signalosome. It was recently published that bridging the TCR to LAT requires adaptor function of Lck, which depends on Lck-mediated phosphorylation of ZAP-70 (18, 20, 26). We speculated that because of its relative complexity, maintaining this bridge may require comparatively high SFK activity. Moreover, when functioning as a mere adaptor, Lck cannot employ enzymatic amplification...
and, thus, more Lck molecules in open active conformation are required to transduce the signal. To test this possibility, we wanted to bring LAT to the proximity of TCR independently of Lck-mediated bridging mechanism. To achieve this, we prepared chimeric protein composed of extracellular domain of CD16 and full-length LAT (Fig. 6 A). It enabled us to co-crosslink TCR with LAT and thus probe the resistance of TCR signaling to SFK inhibition independently of LAT-bridging step.

**Figure 3.** Signaling initiated by crosslinking of CD16-tagged TCRζ molecules (CD16-ζ) in B cells can be inhibited by PP2. A, schematic representation of chimeric CD16-ζ molecule. B, flow cytometry analyses of CD16-ζ expression in Jurkat and Ramos cell line transduced with construct coding for CD16-ζ-ires-LNGFR (reporter gene LNGFR is a truncated (inactive) version of human low-affinity nerve growth factor receptor) (42). C, flow cytometry analyses of CD16 expression in human granulocytes (gated on SSA-high cells), Ramos, Jurkat, and P116 cells. D, Ca²⁺ response in Jurkat CD16-ζ cells stimulated with anti-CD16 antibody or anti-TCR antibody (C305). The experiment was performed similarly as in Fig. 1A (n = 3). E, Ca²⁺ response in Ramos CD16-ζ cells stimulated with anti-CD16 antibody or anti-BCR antibody in the presence or absence of 10 μM concentration of PP2. Experiment was performed similarly as in Fig. 1A. D and E, black arrows indicate the time of the stimulation. F, quantification of Ca²⁺ response from (E). The data were plotted as area under the curve after the subtraction of steady state fluorescence. Data were normalized to PP2 nontreated cells (-fold change) (n = 3).
We transduced ZAP-70 or SYK expressing P116 T cells with chimeric construct coding for CD16-LAT (Fig. 6B). Strikingly, co-crosslinking of LAT to TCR resulted in resistance of the TCR signaling to 10 mM concentration of PP2, which is the highest level of resistance we were so far able to achieve in T cells. As expected, the resistance was more profound in case of SYK-expressing cells (Fig. 6, C–F). Although the resistance was still not as strong as in the case of B cells, the results support...
the conclusion that LAT bridging step is regulated by SFKs and that it is an important part of the mechanism by which TCR signaling apparatus sets the threshold for the amount of SFK activity required for signal propagation.

Discussion

In our work, we analyzed the mechanisms that make TCR signaling sensitive and BCR signaling resistant to the inhibition of SFK. This phenomenon has been observed in previous works. Similar difference in the sensitivity of antigen receptor signaling to PP2-mediated inhibition has been observed in primary mouse T cells and B cells (8, 11). It was also described in cell lines where SFKs were inhibited genetically by membrane-targeted Csk (11). Delayed but still robust Ca$^{2+}$ response has also been observed in LYN-deficient DT-40 cell line (7). Differences in the sensitivity to SFK inhibition were ascribed either to the unique ability of SYK to phosphorylate ITAMs and thus initiate the signaling independently of SFKs or to the residual activity of SFKs which initiated signaling in B cells (8, 11). Here, we re-evaluated these results using SYK- and ZAP-70–deficient B and T cell lines reconstituted with SYK or ZAP-70.

Our observation of increased ITAM phosphorylation in SYK-deficient B cells even in the presence of high concentration of PP2 suggested that even high concentration of PP2 does not completely inhibit SFK activity. On the other hand, we have also observed enhanced resistance to SFK inhibition in both B and T cells expressing SYK, when compared with ZAP-70. However, it is important to note that ZAP-70–expressing B cells retained high resistance to SFK inhibition, whereas SYK-expressing T cells were still inhibited by relatively low dose of PP2, which indicated that SYK is only a part of the mechanism responsible for the resistance of BCR signaling to SFK inhibition. Moreover, SYK ability to phosphorylate ITAMs could not explain increased resistance of SYK-expressing cells to SFK inhibition, because the phosphorylation of ITAMs was more enhanced in ZAP-70 than in SYK-expressing cells, where only insignificant increase was observed. Interestingly, enhanced ITAM phosphorylation in the presence of ZAP-70 was also reported previously (27, 28). The mechanism does not necessarily involve the direct phosphorylation of ITAM motifs by SYK or ZAP-70. It is equally possible, that SH2 domains of SYK and ZAP-70 protect BCR and TCR ITAMs from dephosphorylation and in this way increase the observed phosphorylation (28, 29). Regardless of the mechanism, our data show that the ability of SYK to keep ITAMs phosphorylated is not better than that of ZAP-70 and so the mechanism by which SYK increases the resistance to SFK inhibition must be different. It is plausible that trans-autophosphorylation of SYK (21–23), its ability to bind hemi ITAMs (30), or its higher kinase activity give it an advantage over ZAP-70 (31).

Because SYK compensates only partially for the loss of SFK kinase activity we continued to search for other mechanisms responsible for the resistance. We show that TCR$\zeta$ signaling in B cells can be inhibited by standard dose of PP2 and that BCR signaling in T cells, compared with TCR signaling, slightly enhances the resistance to SFK inhibition, which suggest that...
differences in structure and composition of TCR and BCR play an additional role in the mechanism of the resistance. General composition of BCR and TCR is very similar. They use surface receptor for antigen recognition and ITAMs containing transmembrane adaptor proteins associated with the receptors for signaling. However, TCR uses an additional accessory adaptor protein LAT not present in B cells, which does not contain ITAM motifs but serves as a docking platform for PLCγ-containing signalosome (32). Moreover, recent articles provide evidence that LAT phosphorylation by ZAP-70 is the rate-limiting step in TCR activation (33) and that bridging of LAT to TCR depends on phosphorylated ZAP-70 and adaptor function of Lck (26, 34). Whether SFK kinase activity plays any direct or indirect role in LAT bridging process was not clear. Here we show that direct bridging of LAT to TCR highly reduces TCR sensitivity to SFK inhibition. It suggests that recruiting LAT to the TCR proximity is highly dependent on SFK kinase activity. These data have interesting implications in understanding of affinity discrimination. Current models of TCR activation, including kinetic proofreading model, posit that there is a threshold which has to be overcome to allow signal propagation. It is required for the discrimination between antigens of varying affinity (35–40). Even in our relatively crude model of antibody-mediated TCR activation this threshold could be observed and investigated. Our data suggest that multiple components of the TCR signaling apparatus cooperate to set its level, including structure of antigen receptor itself and unique properties of Lck and ZAP-70.
Replacing any of these components with their B cell counterparts lowers this threshold. In addition, our data suggest that another important component of this mechanism is LAT and its separation from TCR signaling machinery. Bringing LAT to the proximity of TCR complex also substantially lowers this threshold. Thus, these thresholds in TCR and BCR signaling are not set by a single protein but rather by multiple components of antigen receptor signaling apparatuses, which are designed to work together to properly set their level.

Materials and methods

Cell culture

Ramos, Jurkat, and P116 cells were cultured in RPMI (Thermo Fisher Scientific) and Phoenix cells were cultured in DMEM (Thermo Fisher Scientific). Media were supplemented with 10% FBS (Thermo Fisher Scientific) and antibiotics, further referred to as complete RPMI.

Flow cytometry

For extracellular staining, cells were incubated on ice for 30 min in PBS, washed twice with PBS, and analyzed. For intracellular staining, cells were fixed for 20 min in room temperature with 4% formaldehyde in PBS (Thermo Fisher Scientific). Alternatively, cells were stained for surface markers as described above. After fixing and/or staining, excess of formaldehyde was washed with PBS and cells were simultaneously blocked and permeabilized for 40 min using 0.3% Triton X-100 (Merck) and 5% BSA (Merck) in PBS. Next, the cells were incubated on ice for 1 h with primary antibody in 0.3% Triton X-100, 1% BSA in PBS, washed with PBS, and analyzed. For Ca\(^{2+}\) response measurement (relative intracellular concentration of free Ca\(^{2+}\)), cells in concentration of 1 \(\times\) 10\(^7\) per ml were loaded with 4 µg of Fura Red (Thermo Fisher Scientific) dissolved in DMSO (Merck) for 30 min in complete RPMI at 37°C, washed, and resuspended in complete RPMI in concentration of 6 \(\times\) 10\(^6\) cells per ml and placed on ice. Before stimulation, 0.5 ml of the cell suspension was pre-warmed at 37°C with or without PP2. Cells were then measured for 30 s without stimulation and then stimulated with 0.5 ml of complete RPMI containing anti-TCR antibody (C305, produced as supernatant in-house, 10 µg/ml final concentration) and incubated for 3 min. Next, the cells were chilled on ice for 1 min, centrifuged, and lysed for 1 h in 500 µl of lysis buffer (50 mM Tris, pH 7.4, 150 mM NaCl, 1% Nonidet P-40 substitute (AppliChem Gmbh), 10 mM chloroacetamide (Merck), phosphatase inhibitor tablets (PhosSTOP, Roche), protease inhibitor mixture (Roche), 2 mM EDTA (Merck)). Nuclei and insoluble debris were removed by centrifugation and supernatants were incubated overnight with indicated antibodies. Next day, antibodies were isolated from the lysates using protein A/G agarose beads (Santa Cruz Biotechnology), washed, and eluted with SDS-PAGE sample buffer.

Cloning, transfection, transduction, cell sorting

Sequences were amplified by PCR using Q5-polymerase (New England Biolabs) from human PBMC-derived cDNA or from Ramos cell–derived cDNA. DNA fragments were cloned into plasmids using Invitrogen enzymes as recommended by the manufacturer. Plasmids, inserts, and cloning strategies are summarized in Table S1. Sequences of all inserts were verified using Sanger sequencing (Eurofins Genomics). For virus production, Phoenix amphi cells were transected using Lipofectamine 2000 (Invitrogen). 2 days after transfection, virus containing supernatant was collected, supplemented with Polybrene (2 µg/ml for Jurkat or P116 cells, 8 µg/ml for Ramos cells) (Merck) and centrifuged (1260 \(\times\) g, 30°C, 90 min) onto the cells. Subsequently, infected cells were sorted based on reported gene expression on Influx sorter (BD Biosciences).

Antibodies

Antibodies used for Western blotting and flow cytometry are listed in Tables S2 and S3.

Statistical analysis

The p-values were calculated using one-way analysis of variance (repeated measures) and Tukey’s post test. The only exceptions were Fig. 2, B and C (only PP2 nontreated cells), where repeated measures analysis was not possible because of sample loss during the experiment, and Fig. 3F where paired t test was employed. Western blot quantifications and flow cytometry area under the curve quantifications were for the purpose of statistical analysis transformed as log (x + 1) (after the normalization described in figure legends).

Data availability

Representative experiments are shown in the figures. For any additional information, please contact the corresponding author.

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Abbreviations—The abbreviations used are: TCR, T cell antigen receptor; BCR, B cell antigen receptor; SFK, Src family kinases; ITAM, immunoreceptor tyrosine-based activation motifs; SYK, spleen tyrosine kinase; Lck, lymphocyte-specific protein tyrosine kinase; Lyn, Lck/Yes-related novel protein tyrosine kinase.

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