## Mutagenetix Phenotypic Mutation 'poppy'

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<td>Base Change (assembly)</td>
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<td>Syk</td>
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<tr>
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<td>Synonym(s)</td>
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<tr>
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<td>Institutional Source</td>
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<td>Science Writers</td>
<td>Eva Marie Y. Moresco, Anne Murray</td>
</tr>
<tr>
<td>Authors</td>
<td>Ying Wang, Hexin Shi, Ming Zeng, Bruce Beutler</td>
</tr>
<tr>
<td>Illustrators</td>
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<td>Last Updated</td>
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Cite this information as follows: Ying Wang, Hexin Shi, Ming Zeng, Eva Marie Y. Moresco, Anne Murray, Beutler B. Record for poppy, updated Sep 14, 2017. MUTAGENETIX (TM), B. Beutler and colleagues, Center for the Genetics of Host Defense, UT Southwestern Medical Center, Dallas, TX. URL: mutagenetix.utsouthwestern.edu

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Phenotypic Description

Figure 1. *Poppy* mice have a reduced number of B cells.

Figure 2. Flow cytometric analysis revealed that *poppy* mice have a reduced number of B cells, but an increase in neutrophils.
Figure 3. *Poppy* mice exhibit hypersensitivity to LPS as well as to the TLR agonists MALP2 and R848. Peritoneal macrophages from the *poppy* mice secrete increased amounts of TNFα in response to LPS, MALP2, and R848 compared to wild-type and unaffected mice.

The *poppy* phenotype was initially identified by flow cytometric analysis of peripheral blood from G3 mice carrying mutations induced by N-ethyl-N-nitrosourea (ENU). The index *poppy* mice (T2313, T2314, and T2315) were deficient in B cells; neutrophil numbers were increased (Figure 1 & 2). Peritoneal macrophages from *poppy* mice exhibited hypersensitivity to the Toll-like receptor (TLR) agonists LPS, MALP2 and R848 as determined by increased secretion of TNFα compared to wild type macrophages (Figure 3).
Whole exome HiSeq sequencing of the G1 grandsire identified 56 mutations. Seventeen G3 mice from the *poppy* pedigree were genotyped at all 56 mutation sites. Among six mice with the *poppy* phenotype, five were homozygous and one was heterozygous for a mutation in *Syk* on chromosome 13. Among eleven unaffected mice, eight were either heterozygous or wild type at the *Syk* locus, while three were homozygous for the *Syk* mutation, indicating that the *poppy* phenotype is recessive and incompletely penetrant. These data resulted in a LOD score of 5.30. The mutation is an A to G transition at base pair 52640733 (v38) on Chromosome 13 in the GenBank genomic region NC_000079 encoding *Syk*. The mutation corresponds to residue 1718 in the NM_011518 mRNA sequence and 1714 in the NM_001198977 mRNA sequence within exon 11 of 14 total exons.

NM_011518:

| 1702 CTGGTCACACAGCACAATGCCAAGATCGCGAT |
| 496 -L--V--T--Q--H--Y--A--K--I--S--D- |

NM_001198977:

| 1698 CTGGTCACACAGCACAATGCCAAGATCGCGAT |
| 496 -L--V--T--Q--H--Y--A--K--I--S--D- |

The mutated nucleotide is indicated in red. The mutation results in a tyrosine (Y) to cysteine (C) substitution at residue 501.

**Protein Prediction**

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Figure 4. Structure of Syk. Mouse Syk consists of two N-terminal Src-homology 2 (SH2) domains and a C-terminal kinase domain. The SH2 domains are connected by a linker known as interdomain A (IDA), while the region between the second SH2 and catalytic domains is known as interdomain B (IDB). Aspartic acid (D) 488 is the proton acceptor during the catalytic cycle. Several tyrosine (Y) residues are autophosphorylated following BCR stimulation. The *poppy* mutation causes a tyrosine to cysteine change at amino acid 501.

Figure 5. Crystal structure of the kinase domain of human Syk. Protein structure modeled from PDB: 1XBA and (40) using UCSF Chimera. Please see the text for more details about the structure. The location of the *poppy* mutation (Tyr507 in the human protein) is labeled. The active site is also labeled. Click the image to rotate.
Syk encodes spleen tyrosine kinase (Syk), one of two members of the Syk family of cytosolic protein kinases. The second Syk family member is zeta-chain-associated protein kinase 70 (Zap70) (see the records for murdock, trebia, wanna, and mrtless). Syk and Zap70 both have two Src homology 2 (SH2) domains and a C-terminal kinase region followed by a short C-terminal tail [Figure 4; reviewed in (1-3)]. Between the SH2 domains is interdomain A; between the C-terminal SH2 domain and the kinase domain is interdomain B [(4); reviewed in (1-3)].

The SH2 domains recognize and bind diphosphorylated immunoreceptor tyrosine-based activation motifs (ITAMs; (Asp/Glu)-X-X-Tyr-X-X-Leu/Iso-(X)_{6-12}-Tyr-X-X-Leu/Iso) or hemi-ITAMs (i.e., contain one Tyr) in a target protein as well as ? integrin (ITAM-independent) to initiate downstream signaling [reviewed in (1-3; 5; 6)]. The SH2 domains of Syk have a conformational flexibility that can recognize the relative distance between phosphotyrosines of an ITAM and adjust the orientation of the SH2 domains to fit that spacing [(7); reviewed in (5)]. The flexibility of the SH2 domains allows for Syk to interact with several different immunoreceptors (e.g., B- and T-cell antigen receptors and Natural Killer (NK)-cell receptors) and, possibly, some G-protein-coupled receptors [reviewed in (5)].

Interdomain A facilitates the proper conformation of the SH2 domains to promote binding to phosphorylated ITAMs using a helical coiled-coil structure [reviewed in (1; 2)]. In addition, the C-terminal portion of interdomain A is critical for calcium mobilization via B cell receptor (BCR) signaling (8).
Mutagenetix Phenotypic Mutation 'poppy'

proteins (e.g., phospholipase C? 1 (PLC? 1), Vav1 and c-Cbl) as well as regulate the ability of Syk to bind to ITAMs [(9-13); reviewed in (1,2,14)]. Interdomain B also contains a non-canonical nuclear localization signal that permits Syk to shuttle between the nucleus and cytoplasm (15;16). A splice variant of Syk, SykB, lacks 23 amino acids of interdomain B, a sequence necessary for maximal ITAM binding by Syk [(12,15;17)]. SykB has comparable enzymatic activity to that of Syk, but is less efficient in coupling to phosphorylated ITAMs such as those of the Fc?RI receptor of mast cells [(12); reviewed in (1;14)].

Syk Phosphorylation
Full-length Syk has ten autophosphorylation sites [out of 32 total phosphoacceptor sites (18)] with distinct functions (19):

1. Tyr130 within interdomain A is autophosphorylated upon BCR activation. Mutation of Tyr130 to a phenylalanine (Y130F) increased Syk ITAM binding and decreased the basal activity of Syk; substitution of Tyr130 to a glutamic acid (Y130E) decreased ITAM binding and increased kinase activity (20). Taken together, it is proposed that phosphorylation of Tyr130 mediates Syk activation and its subsequent release from the BCR (20).

2. Phosphorylation of Tyr290, within interdomain B, has no known function. Site-directed mutagenesis of Tyr290 to a phenylalanine (Y290F) resulted in lower expression levels of Syk when the mutant was expressed in RBL-2H3 cells, a basophilic leukemia cell line; Y290F Syk protein levels in BI-141 T cells were comparable to wild-type levels (12). In the RBL-2H3 cells, the Y290F mutant could rescue Fc?RI-mediated degranulation in vitro to a similar extent as wild-type Syk (12). In the BI-141 cells, Y290F Syk responded at comparable levels to wild-type Syk in an antigen stimulation assay (12). Furthermore, analyses showed that T cell receptor (TCR)-induced protein tyrosine phosphorylation in the BI-141 cells expressing the Y290F mutant was indistinguishable from that in cells expressing wild-type Syk (12).

3. Autophosphorylation of another site within interdomain B, Tyr317, is proposed to function in the negative regulation of Syk activity by serving as a binding site for c-Cbl, a protein that ubiquitinates Syk and targets it for proteasome-mediated degradation (11,21-23). Signal transduction in B cells and mast cells is negatively regulated upon Tyr317 phosphorylation (10,23). Expression of a Tyr317 mutant (Y317F) resulted in an increase in Fc?RI-induced degranulation as well as an increase in PLC?1 and PLC?2 phosphorylation (23). The corresponding residue in Zap70 (Tyr292) also binds c-Cbl (24) and is a negative-regulatory phosphorylation site [i.e., dephosphorylation of Tyr292 is required for Zap70 activation] (21,25). Cells expressing a Zap70 Tyr292 mutant (Y292F) exhibit a hyperactive phenotype in T-cell receptor signaling (26).

4. Autophosphorylation of Tyr342 mediates the binding of Syk to the SH2 domain of Vav1, facilitating Syk-mediated phosphorylation of Vav1, a guanine nucleotide exchange factor for the Rho/Rac GTPases (27). Tyr342 and/or Tyr346 are required for the interaction of Syk and PLC?1 via adaptor molecules such as LAT, SLP-76, or BLNK in immune cells (9,13,28,29). In addition to binding PLC?1 in vitro, autophosphorylated Tyr346 has also been shown to bind to the SH2 domain of the P13K regulatory subunit, p85 (30). In vitro expression of a Syk mutant with mutations of both Tyr342 and Tyr346 (Y342F and Y356F, respectively) abrogated Fc?RI-induced degranulation of mast cells, calcium flux, phosphorylation of PLC?1, PLC?2, LAT, SLP76, and Vav1 as well as AKT and ERK activation (11). Comparison of mast cells expressing either the Y342F or Y346F mutant determined that ERK and AKT activation is more dependent on Tyr346 than Tyr342 (11). Mutant Syk (Y342F, Y346F) was not constitutively active in an IgM-BCR-expressing B-cell line, but could be activated by the BCR (31). In contrast, a mutant Syk with both Tyr342 and Tyr346 mutated to glutamic acid was more active in phosphorylating SLP-65 than wild-type Syk (31).

5. In vitro analysis determined that Tyr358 is autophosphorylated, although the function is unknown (19).
phosphorylated ITAMs. Similar Tyr residues are found in activation loops of other protein tyrosine kinases, including Zap70 and the insulin receptor. In the case of the insulin receptor, dephosphorylated Tyr1162 within the activation loop physically blocks ATP binding to the active site (32). Phosphorylation of Tyr1162 relieves the inhibition, allowing for kinase activation (32). Phosphorylation of Tyr519 and Tyr520 is required for Syk signal transduction including, although not absolutely required for Syk kinase activity (33,34). Activation loop autophosphorylation sustains Syk signaling after transient ITAM phosphorylation ends (35).

7. Phosphorylated Tyr624 binds to the SH2 domain of SLP-65, an association essential for BCR signaling and B cell development (31).

8. Tyr623, Tyr624, and Tyr625 maintain Syk in an inactive form (36;37). Expression of a Syk mutant in which these three sites were mutated to phenylalanines led to increased TCR signaling in Jurkat T cells (36;37). Upon autophosphorylation, the Tyr-mediated autoinhibitory interactions are disrupted, resulting in an open, active conformation (19;37).

The Syk kinases are maintained in a closed, inactive conformation by interactions between residues in interdomain A, interdomain B, and the kinase domain (36;38;39). The C-terminal region of interdomain A contains residues important for maintaining the resting state ([8]; reviewed in (2)]. In the inactive conformation, the SH2 domains are not aligned for ITAM binding and the kinase domain cannot perform the phospho-transfer reaction (36). Either phosphorylation of interdomain A/B tyrosines or binding of phosphorylated ITAMs to SH2 domains can release the inhibitory interactions that hold Syk in an inactive state, thereby permitting ATP and substrates access to the active site and stimulating Syk kinase activity (36;37;39).

The crystal structure of the kinase domain of human Syk (amino acids 353-635) has been solved [Figure 5; PDB: 1XBA; (40)]. The N-terminal lobe of the kinase domain is composed of a five-stranded β-sheet and a single α-helix, while the C-terminal lobe is largely α-helical with three short β-strands; the active site is between the two lobes (40). Unphosphorylated Syk kinase domain forms a loop-out conformation similar to what is seen in active, phosphorylated protein kinases, indicating that activation loop phosphorylation is not required for Syk kinase activity. A recent study crystalized full-length human Syk at 2.2 Å [Figure 6; PDB: 4FL3; (41)]. In this structure, the topology of the Syk kinase domain is similar to inactive kinase domains. The interdomain linkers (helices 1, 2, and 3) bind to helices E and I of the kinase domain, consistent with previous data supporting an autoinhibited conformation maintained by interactions between interdomains A and B, and the kinase domain. The SH2 domains each consist of a β-sheet flanked by two α-helices and the linkers consist of three α-helices to form a helical coiled coil (41).

The poppy mutation is a tyrosine to cysteine substitution at amino acid 501 within the C-terminal kinase domain.
Syk is highly expressed in hematopoietic cells including B cells, immature T cells, mast cells, dendritic cells, basophils, osteoclasts, leukocytes, neutrophils, macrophages, and platelets [reviewed in (1-3)]. Syk is also expressed in non-hematopoietic cells including fibroblasts (embryonic and nasal), epithelial cells (breast and airway), hepatocytes, neuronal cells, and vascular endothelial cells, red blood cells, and synoviocytes (4).

In B cells, Syk is localized to both nuclear and cytoplasmic compartments [reviewed in (1)]. The mechanism by which Syk is transported between the nucleus and cytoplasm is unknown, but interdomain B is essential for this to occur (16). Upon BCR aggregation, Syk is recruited to the plasma membrane [(42); reviewed in (1)].

Reduction in Syk mRNA and protein expression has been documented in several types of cancers including breast, gastric, melanoma, squamous cell carcinoma, pro-B cell acute lymphoblastic leukemia (ALL), acute myeloid leukemia (AML), and chronic lymphocytic leukemia (CLL) [(43-47); reviewed in (48)].

Background

[Flash Content]

Figure 7. Pre-BCR and BCR signaling. Pre-BCR and BCR engagement result in the activation of Syk. In pre-BCR signaling, Syk phosphorylates many substrates (see Table 1) and triggers signaling pathways that are involved in both proliferation and differentiation of pre-B cells. Syk-mediated signaling downstream from the BCR regulates cell proliferation, differentiation, and apoptosis as well as the secretion of antigen-specific antibodies. Please see the text for more details on these signaling pathways.
Syk plays a major role in B cell development as a component of the pre-BCR and BCR signaling pathways, and no mature B cells are found in mice with targeted null mutations of Syk (49-51). In BCR signaling, following the aggregation of BCR molecules, the ITAMs in the tails of Igα and Igβ become phosphorylated by Src family kinases (typically Lyn) [Figure 7; (52;53)]. These phosphorytrosines then act as docking sites for the SH2 domains of Syk, resulting in Syk phosphorylation and activation. Syk phosphorylates a number of downstream targets including BLNK (see the record for busy), PLCβ2 (see the record for queen), and protein kinase Cα (PKCα; see the record for Untied). BCR stimulation also activates phosphatidylinositol 3 kinase (PI3K) resulting in the generation of PIP3, which binds selectively to the pleckstrin homology domain of Btk, facilitating membrane recruitment of the kinase. Phosphorylated BLNK also provides docking sites for Btk, as well as PLCβ2, which results in the additional phosphorylation and activation of PLCβ2 by Btk leading to the hydrolysis of phosphatidylinositol-3,4-diphosphate (PIP2) to inositol-1,4,5-trisphosphate (IP3) and diacylglycerol (DAG) (54). The recruitment of Vav1, Nck and Ras by BLNK to the BCR activates MAP kinase cascades such as JNK, p38 and extracellular signal regulated kinase (ERK) [reviewed in (55)]. Together, these signals allow the activation of multiple transcription factors, including nuclear factor of activated T cells (NF-AT), NF-κB (see the records for puff, xander and panr2) and AP-1, which subsequently regulate biological responses including cell proliferation, differentiation, and apoptosis as well as the secretion of antigen-specific antibodies [reviewed in (56)]. Other molecules that play important roles in BCR signaling include Bcl10, mucosa-associated lymphoid tissue translocation gene 1 (Malt1), and caspase recruitment domain family, member 11 (CARMA1, alternatively Card11; see the record for king), which are involved in NF-κB activation along with PKCα (57-60).

Syk couples pre-BCR and BCR activation to downstream signaling pathways that mediate B cell development, proliferation, and survival (Figure 7). Pre-BCR engagement results in the activation of Syk, which together with Src-family kinases (Lyn, Fyn, Blk), phosphorylates many substrates (see Table 1) and triggers signaling pathways that are involved in both proliferation and differentiation of pre-B cells (52,53). These include activation of phosphoinositide 3 kinase (PI3K) by phosphorylating the coreceptor CD19 and/or the adaptor protein B-cell PI3K adaptor (BCAP; see the record for sothe) (61). PI3K activation results in the generation of phosphatidylinositol-3,4,5-triphosphate (PIP3), which recruits plekstrin-homology domain signaling molecules to the membrane including the serine threonine protein kinase B (PKB) and its activating kinase 3-phosphoinositide-dependent protein kinase 1 (PDK1). Signaling through this pathway suppresses recombination-activating gene 1 (RAG1; see the record for maladaptive) and RAG2 expression (in VDJ recombination in pro-B cells), blocks Igα? (the kappa chain of the immunoglobin light chain) gene recombination, and induces cell proliferation. Syk also phosphorylates SH2-domain containing leukocyte protein of SLP65, resulting in the organization of a molecular complex that includes Bruton’s tyrosine kinase (BTK) and PLCβ2 (54). This complex controls downregulation of Ig-5, a component of the surrogate light chain (SLC), and upregulates the expression of RAG proteins and the interferon-regulatory factor 4 (IRF4). IRF4 positively regulates Igα? recombination. SLP65 also modulates PKB activity either directly or by altering the activity of Syk, CD19, or PI3K. Alternatively, SLP65 may regulate PKB activity by activating lipid phosphatases such as SH2-domain containing inositol-5-phosphate (SHIP; see the record for styx) and altering PIP3 levels.

Table 1. Select substrates of Syk* [modified from (1)]

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<th>Substrate</th>
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## Enzymes

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<tr>
<td>P85 subunit of PI3K</td>
<td>Generates membrane-associated PIP(_3) as well as mediates Akt activity in B cells after BCR engagement and in B cells exposed to oxidative stress</td>
<td>(30;62)</td>
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<tr>
<td>c-Cbl</td>
<td>Ubiquitinates Syk, promoting Syk degradation</td>
<td>(21;22;63)</td>
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<td>PLC?2</td>
<td>Activates MAP kinase cascades such as JNK, p38 and ERK in BCR-coupled signaling and in platelet activation</td>
<td>(64-66)</td>
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<td>Btk</td>
<td>Controls downregulation of l-5, a component of the surrogate light chain, and upregulates the expression of RAG proteins and the interferon-regulatory factor 4 (IRF4) in B-cell development, differentiation, and signaling</td>
<td>(28;65;67;68)</td>
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<tr>
<td>Vav1</td>
<td>Activates MAP kinase cascades such as JNK, p38 and ERK in BCR-coupled signaling</td>
<td>(27;69)</td>
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<td>MAPKs (JNK, p38 MAPK, p44/p42 MAPK)</td>
<td>TNF-coupled signaling; cytoskeletal function (Rac/Rho pathway); (Ca^{2+}) entry and activation of PKC (IP3 and DAG pathway)</td>
<td>(64;70)</td>
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<tr>
<td>Phospholipase D (PLD)</td>
<td>BCR-associated signaling to mediate B cell survival and proliferation</td>
<td>(65)</td>
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## Adaptor molecules

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<td>LAT</td>
<td>Activates PLC?1 and Ras pathways in the T cell; regulates PLC?2 in platelet activation via CLEC2</td>
<td>(29;66;71)</td>
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<td>SLP-76 (alternatively, LCP2)</td>
<td>Regulates blood and lymphatic vascular separation, bone reabsorption by osteoclasts, and platelet activation</td>
<td>(72-74)</td>
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<td>BLNK (alternatively, SLP-65)</td>
<td>Facilitates binding of Vav1, Btk, and PLC?2 to Syk in B cell activation</td>
<td>(28;75;76)</td>
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<td>BCAP and CD19</td>
<td>Activates the PI3K pathway to control proliferation and B cell survival</td>
<td>(61;77)</td>
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<td>HS1 (alternatively, Hcls1)</td>
<td>Facilitates actin assembly to lipid rafts and antigen presentation in B cells as well as platelet activation after GPVI stimulation</td>
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<td>Card9</td>
<td>Links receptor-mediated recognition of fungal pathogens and the NF-?B pathway in macrophages and dendritic cells; functions in Syk-mediated pro-IL1-? synthesis by the Nlrp3 inflammasome (see the record for (ND1))</td>
<td>(81;82)</td>
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<td>CARMA1 (alternatively, Card11)</td>
<td>Regulates NF-?B activation by antigen receptors in lymphocytes</td>
<td>(83)</td>
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<td>Shc</td>
<td>Activates the Ras/MAPK cascade after Fc?RI mast cell receptor activation</td>
<td>(84)</td>
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<td>CD3?</td>
<td>Mediates neuronal morphogenesis via the ephrin A pathway (see the record for (frog))</td>
<td>(85)</td>
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<td>3BP2 (alternatively, Sh3bp2)</td>
<td>Mediates BCR-mediated activation of nuclear factor of activated T cells (NF-AT)</td>
<td>(86;87)</td>
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<td>MyD88</td>
<td>Recruits c-Cbl resulting in attenuation of CD11b-integrin TLR-triggered responses</td>
<td>(88)</td>
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Syk is involved in ITAM-dependent, hemi-ITAM-dependent, and ITAM-independent signaling to mediate several cellular responses. Syk associates with many receptors in varied cell types. These include CLEC2, which regulates the separation of blood and lymphatic vessels during embryonic development. The TREM2-DAP12 complex mediates bone resorption and osteoclast differentiation via PLCγ1. Dectin-1 on dendritic cells, triggered by fungal antigens, signals through the CARD9 complex (CARD9-Bcl10-Malt1) to activate NF-κB. In addition, Dectin-1 (independently of Syk) can induce the activation of the MAPK pathway through Raf activation to mediate cytokine release in dendritic cells and macrophages. Syk can associate with MyD88 and TRIF in the TLR4 pathway that initiates inflammatory responses to LPS. Syk can mediate ITAM-dependent and ITAM-independent signaling through integrins in platelets, osteoclasts, and myeloid cells. Upon integrin association with FcεRI, Syk promotes signaling that will result in cytoskeletal reorganization. Syk can also directly bind β3-integrins independent of ITAMs to facilitate cytoskeletal reorganization. c-Cbl-mediated polyubiquitination and subsequent degradation of Syk can negatively regulate Syk function (shown here in the FcεRI-integrin pathway). This figure is not comprehensive: some intermediate signaling steps have not been included to facilitate clarity. Table 1 describes several of the substrates depicted here. Please see the text for more details on these pathways as well as Table 2 for a description of the multiple functions of Syk. The protein structures are schematic representations based on several sources, including solved crystal structures (when available): TLR4, PDB:3FXI & PDB:2J67; CLEC2, PDB:2C6U, ; TREM2, PDB:1Q8M; Dectin-1, PDB:2YHF; integrins, PDB:1JV2, PDB:2K9J; MAPK, 3O17; IRAK1/4, PDB:2NRU; c-Cbl, PDB:2Y1M; FAK, PDB:1MP8.

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Syk in other immune cell ITAM pathways
In addition to its role in BCR signaling, Syk also functions in pre-TCR signaling in thymocytes during the transition from the DN3 to the DN4 stage of development (89), and during TCR signaling in mature T cell populations (intraepithelial ?? T cells and naïve ?? T cells) (Table 2) (90;91). However, Zap70 is the major Syk family kinase in TCR signaling.

Syk functions in ITAM-associated signaling in other immune cells including natural killer cells downstream from the Fc?RIII receptor (92), in mast cells downstream from the Fc?RI receptor (11;13), and in neutrophils and macrophages downstream from Fc?R signaling (Figure 8 and Table 2; 93;94]). Although ligand recognition differs among these receptors, the intracellular pathways are highly conserved: activated Src family kinases (e.g., Hck, Fgr, Lyn, or Src) recruit Syk to the receptor-associated ITAMs (reviewed in (3;95)]. Activated Syk subsequently phosphorylates a number of proteins including SLP76, SLP65, Vav1, PLC?, and the p85? subunit of PI3K (see Table 1). These proteins activate downstream signaling components to trigger several cellular responses including proliferation, differentiation, cell survival, mast cell degranulation, reactive oxygen species (ROS) production, phagocytosis, and cytokine release (reviewed in (2;3)).

A role for Syk in numerous non-immune cells has also been demonstrated (Table 2).

Syk signaling via hemi-ITAMs
Syk can also bind to hemi-ITAMs (i.e., ITAM motifs containing one YxxL motif) in C-type lectin receptors including the C-type lectin-like protein type 2 (CLEC2) receptor, CLEC9A, and Dectin-1 (96). CLEC2 functions in the developmental separation of blood and lymphatic vessels (97;98), while Dectin-1 induces intracellular signaling upon recognition of fungal pathogens (99). The C-type lectin and BCR signaling pathways share many of the same downstream components, including PLC?2 (96).

Syk-associated signaling via ITAM-signaling adaptors
Syk can also signal through ITAM-containing adaptors (e.g., DNA activation protein-12 (DAP12) and FcR?) that associate with immunoglobulin superfamily-containing proteins [e.g., Fc receptors, PIR-A, OSCAR, GPVI, or triggering receptor expressed on myeloid cells (TREM) (100;101)], C-type lectin receptors [e.g., Dectin-2, Mincle, or MDL-1 (102;103)], and cytokine receptors [e.g., macrophage colony-stimulating factor (M-CSF) (104;105)], as well as integrins (106;107). DAP12, FcR?, and their associated receptors are found in several cell types including neutrophils, macrophages, monocytes, dendritic cells, basophils, eosinophils, mast cells, NK cells, microglia, osteoclasts, megakaryocytes, and platelets (reviewed in (108)). Signaling via the ITAM-containing adaptors and their associated receptors is similar to ITAM receptor signaling. Syk-mediated activation of PLC?2 and CARD9 leads to the activation of NF-??B and MAPKs (reviewed in (109)). Signaling via the ITAM-signaling adaptors mediates phagocytosis (110) and osteoclast development (111) as well as cell proliferation and survival (105).

The ITAM-associated receptors and TLRs activate distinct signaling pathways, but the two pathways can cross-talk to activate NF-??B and the MAPKs. For example, Syk activation and association with DAP12-TREM can either amplify or dampen TLR-induced signals in a ligand-dependent manner during an inflammatory response, possibly through the CARD complex or the activation of ERK (112).

ITAM-independent signaling

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in platelets, activation of \( \alpha IIb \beta 3 \) integrin (in association with the ITAM-containing receptor Fc\( \gamma \)RIIA) can activate Syk, subsequently leading to platelet activation through SLP76 and PLC\( \gamma 2 \) [reviewed in (3)]. However, some evidence suggests that integrins activate Syk in an ITAM-independent manner, through direct binding between Syk and the integrin \( \alpha \) chain [reviewed in (3)].

**Table 2. Syk is a multi-functional kinase** [modified from (5)]
<table>
<thead>
<tr>
<th>Cell type</th>
<th>Syk-associated receptor</th>
<th>Signaling adaptor</th>
<th>Cellular activity supported by Syk</th>
<th>Refs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Effector T cells</td>
<td>TCR</td>
<td>CD3?</td>
<td>Self-antigen presentation to B cells</td>
<td>(113)</td>
</tr>
<tr>
<td>NK cells</td>
<td>Fc? RII; Fc? RIII; Ig? and Ig?</td>
<td>DAP12</td>
<td>Surveillance of genotoxic stress/ transformation and mitotic cells as well as elimination of antibody coated cells</td>
<td>(114)</td>
</tr>
<tr>
<td>B cells</td>
<td>BCR or FcgRIIB</td>
<td>Ig? and Ig?</td>
<td>Pre-B cell development and activation via the BCR; inhibition of B cell activation via the FcgRIIB receptor</td>
<td>(49;115; 116)</td>
</tr>
<tr>
<td>Red blood cells</td>
<td></td>
<td></td>
<td>Phosphorylation of band 3 protein; cell removal from circulation, glycolysis, cell shape, membrane transport</td>
<td>(113)</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>Fc? R1; Fc? RII; Fc? R1; Fc? RIII; integrins</td>
<td>FCR?</td>
<td>Cell adhesion and phagocytosis as well as degranulation and cell spreading in response to proinflammatory stimuli</td>
<td>(93;107; 117)</td>
</tr>
<tr>
<td>Basophils</td>
<td>Fc? RI</td>
<td>FcR?; FcR?</td>
<td>Degranulation</td>
<td>(118;119)</td>
</tr>
<tr>
<td>Eosinophils</td>
<td>Fc? R; Fc? R</td>
<td>FcR?</td>
<td>Degranulation and reactive oxygen intermediates generation</td>
<td>(120)</td>
</tr>
<tr>
<td>Macrophages</td>
<td>FcgR1; Fc? RII; Fc? R1; integrins</td>
<td>DAP12; FcR?</td>
<td>Cell adhesion and phagocytosis as well as degranulation and cell spreading in response to proinflammatory stimuli</td>
<td>(93;94)</td>
</tr>
<tr>
<td>Mast cells</td>
<td>Fc? RI</td>
<td>FcR?; FcR?</td>
<td>Degranulation and cytokine production</td>
<td>(121;122)</td>
</tr>
<tr>
<td>Dendritic cells</td>
<td>FcRs</td>
<td>DAP12</td>
<td>Antigen internalization, presentation, cell maturation</td>
<td>(123)</td>
</tr>
<tr>
<td>Osteoclasts</td>
<td>RANK; TREM2</td>
<td>DAP12; FcR?</td>
<td>Osteoclastogenesis; bone resorption</td>
<td>(106;111)</td>
</tr>
<tr>
<td>Platelets</td>
<td>GpVI; CLEC2; ? IIb? 3 integrin</td>
<td>FcR?</td>
<td>Spreading, aggregation, and serotonin secretion</td>
<td>(124-126)</td>
</tr>
<tr>
<td>Embryonic fibroblasts</td>
<td>G protein</td>
<td></td>
<td>Differentiation to adipocytes</td>
<td>(4)</td>
</tr>
<tr>
<td>Nasal fibroblasts</td>
<td></td>
<td></td>
<td>LPS-induced RANTES production; IL-1 induced chemokine production</td>
<td>(4;127)</td>
</tr>
<tr>
<td>Synoviocytes</td>
<td>TNFR</td>
<td>TNF-? induced JNK activation, metalloprotease 3 gene expression, matrix degradation and synovial fluid regulation</td>
<td>(128)</td>
<td></td>
</tr>
<tr>
<td>Breast epithelial cells</td>
<td></td>
<td></td>
<td>Controls cell division</td>
<td>(4;47;48)</td>
</tr>
<tr>
<td>Airway epithelial cells</td>
<td>? 1-integrin</td>
<td></td>
<td>Expression of ICAM-1 and IL-6</td>
<td>(129)</td>
</tr>
<tr>
<td>Hepatocytes</td>
<td>AT1; AT2</td>
<td>G protein</td>
<td>ERK activation; glycogen granule accumulation</td>
<td>(4;130)</td>
</tr>
<tr>
<td>Smooth muscle</td>
<td>AT1; AT2</td>
<td>G protein</td>
<td>Protein synthesis; cell migration</td>
<td>(4)</td>
</tr>
<tr>
<td>Vascular endothelial cells</td>
<td></td>
<td></td>
<td>Cell growth, migration, survival; separation of lymphatic vessels from blood vessels</td>
<td>(4;131)</td>
</tr>
<tr>
<td>Neuronal</td>
<td>CD3??</td>
<td></td>
<td>Neuronal differentiation and neurite extension</td>
<td>(4)</td>
</tr>
<tr>
<td>Melanocytes</td>
<td></td>
<td></td>
<td>Metastatic behavior regulation</td>
<td>(132)</td>
</tr>
</tbody>
</table>
Syk in human disease
Syk signaling affects several tumor cell activities including cell division, cell survival, 3-D cell outgrowth, cell migration, neuron-like cell differentiation, phagocytosis, and cell adhesion (47;48;133;134). Syk is expressed in human breast tissue and benign breast tumors, but is undetectable or expressed at low levels in invasive breast tumors and cell lines (47). Syk transfection into a Syk-negative breast cancer cell line resulted in inhibition of tumor growth and metastasis in athymic mice (47). In addition, overexpression of a kinase-deficient Syk in a Syk-expressing breast cancer cell line increased the incidence and growth of tumors (47). Thus, Cooper et al. propose that Syk functions as a tumor suppressor in human breast carcinomas (47). Consistent with this hypothesis, reduced amounts of SYK mRNA expression in breast cancer tissue relative to adjacent non-cancerous tissue were correlated with increased incidence of distant metastasis, translating to poor prognosis (135). Syk has been shown to regulate mammalian target of rapamycin (mTOR) and MAPK signaling in AML (44) and in B cell lymphoma (48;49). Syk is activated in CLL (45;46), a condition characterized by the expansion of monoclonal, mature B cells [reviewed in (136)], and regulates the survival of CLL B cells (137). Syk inhibition can suppress CLL B cell activation and migration (43).

Defects in Syk-associated signaling are linked to autoimmune and allergic diseases such as rheumatoid arthritis, asthma, and allergic rhinitis [(138;139); reviewed in (140)].

Syk null mouse models
Syk deficiency in homozygous Syk knockout mouse models (MGI:1857421, MGI:2384078, and MGI: 3690645) leads to the absence of mature B cells due to abnormal B cell differentiation (49-51). Homozygotes exhibited early postnatal lethality (49; 51) with a concomitant defect in blood vessel morphology, dilated vasculature, and systemic hemorrhage in the embryo (49; 51;98;141). Further observation of homozygous Syk^tm1Tyb^ mice (MGI:2384078) found that the model exhibited defective osteoclast differentiation (111), decreased bone resorption (111), decreased TNF secretion from neutrophils after 16-hour culture with S. aureus or E. coli (142), impaired lymphatic vessel morphology in the lungs and brain [i.e., there is a connection between the vasculature and lymphatic systems; (98)], impaired neutrophil degranulation in response to S. aureus and E. coli (142), impaired neutrophil phagocytosis of bacteria (142), reduced T cell number in the skin (49), and inefficient CD8^+^ dendritic cell antigen cross-presentation (143).
Consistent with an essential role for Syk in BCR signaling, B cell proliferation, and B cell differentiation (49;50;144), homozygous poppy mice are B cell-deficient. However, homozygous poppy mice are born at expected Mendelian ratios and survive to adulthood, indicating that the poppy allele retains sufficient functionality to support postnatal survival.

The role of Syk in TLR-mediated signaling is unclear. The poppy mutation results in an increase in macrophage TNF-? secretion upon treatment with the TLR agonists MALP2 (TLR2/6) and R848 (TLR7). Studies have documented elevated TLR4-, TLR3-, and TLR9-mediated signaling in Syk-deficient macrophages, leading to a subsequent increase in TNF production relative to wild type macrophages (145). However, the role of Syk in TLR2/6, TLR7 or TLR8 signaling has not been documented.

Some evidence suggest that TLR or TNF receptor activation leads to integrin activation ([88;146]; reviewed in (109]), subsequently causing the integrins to assume a conformation that promotes high-affinity ligand-binding in a process known as ‘inside-out’ signaling (147;148). Inside-out signaling promotes increased adhesion to the extracellular matrix, cell spreading and enhanced cell-cell interactions. The increased integrin ligand affinity also results in high-avidity ligation of integrins and the subsequent generation of an integrin-mediated ‘outside-in’ signal. The outside-in signal of ?2 and ?3 integrins is propagated by DAP12 and Fc?Rs, which recruit Syk to their phosphorylated ITAMs.

Published data currently support two distinct mechanisms by which Syk may negatively regulate TLR signaling. First, Syk interacts with, and induces the phosphorylation of, the TLR adapters MyD88 and TRIF (88). Phosphorylation of MyD88 and TRIF subsequently results in c-Cbl-induced ubiquitination and degradation of Syk (88). Second, ?2 integrins and Fc?Rs in macrophages signal via the ITAM-containing adaptor DAP12 and Syk to inhibit TLR signaling through the induction of IL-10 and the TLR signaling inhibitors SOCS3, ABIN-3, and A20 (149). Notably, DAP12-deficient macrophages also displayed elevated TNF production in response to TLR ligands (145), supporting a DAP12 negative regulatory signaling cascade involving Syk that inhibits TLR signaling.

These findings contrast with data supporting a positive role for Syk in TLR signaling. In one study, Syk phosphorylation was induced in THP1 human monocytes upon TLR9 activation, subsequently leading to cell migration, adhesion, and secretion of IFN-? and IL-6 (150). In another study, LPS stimulation of murine macrophages induced Syk phosphorylation and the expression of inflammatory genes (151). Piceatannol, a Syk inhibitor, as well as Syk inhibition by antisense oligonucleotides resulted in attenuation of the LPS-induced events in macrophages (151;152). Lin et al. examined the role of Syk in TLR9, -3, and -4-mediated signaling by using Syk inhibitors (SykI and BAY61-3606) in TLR-stimulated RAW 264.7 macrophages and primary bone marrow-derived macrophages (BMDM) (153). They found that pharmacological inhibition of Syk in the RAW 264.7 macrophages resulted in an inhibitory effect on TLR-induced JNK activation and the attenuation of inflammatory gene expression (iNOS and COX2); IKK, p38 and ERK activation were not changed (153). However, siRNA-mediated knockdown of Syk in RAW 264.7 cells followed by treatment with LPS, CpG, or poly(I:C) did not result in changes to JNK activation although there was an observed increase in iNOS and COX-2 expression (153). Chaudhary et al. treated human monocyte THP-1 cells with piceatannol and found that Syk activity is necessary for TLR4 tyrosine phosphorylation upon LPS stimulation and that piceatannol treatment results in an inhibition of IL-10 and IL-12 release from the THP-1 cells upon LPS stimulation (154).
**Mutagenetix Phenotypic Mutation 'poppy'**

*Poppy* genotyping is performed by amplifying the region containing the mutation using PCR, followed by sequencing of the amplified region to detect the single nucleotide transition. The same primers are used for PCR amplification and for sequencing.

**PCR Primers**
- Poppy(F): 5’- CCGCCGTCCACTCTTTGGTAATG-3’
- Poppy(R): 5’- TGGTTTCAAGCAAGTTGACGCAAGC-3’

**Sequencing Primer**
- Poppy_seq(F): 5’- CAGGTTTCCATGGGGATGAA-3’

**PCR program**
1) 94°C 2:00  
2) 94°C 0:30  
3) 55°C 0:30  
4) 72°C 1:00  
5) repeat steps (2-4) 40X  
6) 72°C 10:00  
7) 4°C ?

The following sequence of 797 nucleotides (from Genbank genomic region NC_000079 for the linear DNA sequence of *Syk*) is amplified:

```
57130  cgccgtccac tctttgtaat gatgtcgtgt gattctaggc acattaagga
57181  taagaacatc atagagcttg ttcaccaggt ttccatgggg atgaaatatt tggagagag
57241  cacagcagtct cggctcgccg gaacgtcttt cttgccacac agcactatgc
57301  caagatcagc gatttcggtc tttccaaagc cctgcgtgct gatgaaaact actacaaggt
57361  agagcgccttc ggtcagactc acacagagatc tggtggctgc gaacgtgctt ctggtcacac
57421  gacgaggctg agagctcagt acacatggcg ctctcctgag agggacacgc ttccatgcat
57481  gttaaggttt gcaggtttata aagatataag aagataaatg tgtgtcctct gtatctttatc
57541  atgttagaaa ttccatctgt aaccttgagc gagaatattc ttaagatcga ttctgctcat
57601  caagaacac aacccccaaa tactgaatgt ttctcctaat ggaatctttt tcagactagg
57661  atcttatggt gatatataata atatatatat tgtgtggtgcc tgggttcctttttt tttttttttt ttcgttttttac
57721  atttaacttta tgtctttcgg gtatgagagt atgtgtgccc tagtggctta atagagagca
57781  gaggacaact tagagaagt tttctcctcc atctcactgt ctatcaggag gaatcgaact
57841  ctgatccttgg gattcgccag caggcaccct acccatggag ccacatgctg gcctctaaa
57901  tggctgcgtgc aacctgtgctt gaaaca
```

Primer binding sites are underlined and the sequencing primer is highlighted; the mutated A is shown in red text.

**References**


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Connects B Cell Receptor to Phosphoinositide 3-Kinase Activation. Immunity. 13, 817-827.
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5327-5332.
Mutagenetix Phenotypic Mutation 'poppy'


Cite this information as follows: Ying Wang, Hexin Shi, Ming Zeng, Eva Marie Y. Moresco, Anne Murray, Beutler B. Record for poppy, updated Sep 14, 2017. MUTAGENETIX (TM), B. Beutler and colleagues, Center for the Genetics of Host Defense, UT Southwestern Medical Center, Dallas, TX. URL: mutagenetix.utsouthwestern.edu

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