### Lemon

<table>
<thead>
<tr>
<th><strong>Allele</strong></th>
<th><strong>Mutation Type</strong></th>
<th><strong>Chromosome</strong></th>
<th><strong>Coordinate</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Lemon</td>
<td>missense</td>
<td>4</td>
<td>3,746,768 bp (GRCm38)</td>
</tr>
</tbody>
</table>

**Gene**
- **Lyn**
- **LYN proto-oncogene, Src family tyrosine kinase**
- **Hck-2**

**Accession Number**
- NCBI RefSeq: NM_0011111096, NM_010747; MGI: 96892

**Base Change (assembly)**
- A → G

**Chromosomal Location**
- 3,678,115-3,813,122 bp (+)

**Phenotypic Category**
- FACS IgD+ B cell percentage - decreased
- FACS IgM+ B cells - decreased
- FACS neutrophils - increased

**Penetrance**
- All alleles (11)
- Chemically induced (ENU) (3)
- Targeted (8)

**Lab Alleles**

**Mode of Inheritance**
- Autosomal Semidominant

**Local Stock**
- Live Mice

**Repository**
- MMRRC: 37578

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**Illustrators**
- Peter Jurek, Katherine Timer

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Figure 1. Lemon mice exhibit increased frequencies of peripheral neutrophils. Flow cytometric analysis of peripheral blood was utilized to determine neutrophil frequency. Raw data are shown. Abbreviations: WT, wild-type; REF, homozygous reference mice; HET, heterozygous variant mice; VAR, homozygous variant mice. Mean (\( \mu \)) and standard deviation (\( \sigma \)) are indicated.

Figure 2. Lemon mice exhibit decreased frequencies of peripheral B cells. Flow cytometric analysis of peripheral blood was utilized to determine B cell frequency. Normalized data are shown. Abbreviations: WT, wild-type; REF, homozygous reference mice; HET, heterozygous variant mice; VAR, homozygous variant mice. Mean (\( \mu \)) and standard deviation (\( \sigma \)) are indicated.
The Lemon phenotype was identified among G3 mice of the pedigree R0079, some of which showed an increased frequency of neutrophils (Figure 1) and a reduced frequency of B cells (Figure 2) including a reduced percentage of IgD⁺ (Figure 3) and IgM⁺ B cells (Figure 4), all in the peripheral blood.
Whole exome sequencing of the G1 grandsire (R0079) identified 63 mutations. All of the above anomalies were linked by continuous variable mapping to a mutation in \textit{Lyn}: an A to G transition at base pair 3,746,768 (v38) on chromosome 4, or base pair 68,648 in the GenBank genomic region NC\_000070. The strongest association was found with an additive (semi-dominant) model of linkage to the reduced frequency of B cells, wherein 10 affected variant homozygotes and 22 heterozygous mice departed phenotypically from 14 homozygous reference mice with a P value of $1.048 \times 10^{-6}$ (Figure 5). A semidominant effect was observed in most of the assays; in no assay was a purely dominant effect observed. The mutation corresponds to residue 794 in the mRNA sequence NM\_001111096 within exon 7 of 13 total exons as well as to residue 731 in the mRNA sequence NM\_010747 within exon 7 of 13 total exons.

Genomic numbering corresponds to NC\_000070. The mutated nucleotide is indicated in red. Alternative splicing of exon 2 of \textit{Lyn} produces two Lyn isoforms, Lyn$^{p56}$ and Lyn$^{p53}$, that differ at the N-terminus (1;2); Lyn$^{p56}$ contains an additional 21 amino acids compared to Lyn$^{p53}$ (1;2; reviewed in (3)). The mutation results in a histidine (H) to arginine (R) substitution at position 182 (H182R) in the Lyn$^{p56}$ (ENSMUSP00000038838) isoform as well as a H to R substitution at position 162 (H162R) in the Lyn$^{p53}$ (ENSMUSP00000100075) isoform. The mutation is strongly predicted by Polyphen-2 to cause loss of function (probably damaging; score = 1.000).

Protein Prediction

Figure 6. Domain structure of Lyn. Please see the text for details about the domains. The \textit{Lemon} mutation (H182R) is indicated in red. The image is interactive; click to view other Lyn mutations.
Lyn is a member of the Src family of tyrosine kinases (SFKs), which also includes Src, Yes, Fgr, Fyn, Lck (see the record for iconoclast), Hck, Blk, and York. The members of the SFKs share highly conserved domains including a Src-homology 3 (SH3) domain (amino acids 66-122 in Lyn), an SH2 domain (amino acids 127-217), a tyrosine kinase domain (amino acids 247-497), and a C-terminal regulatory region [Figure 6; reviewed in (4-6)]. A ‘unique’ domain of 50-70 amino acids between the N-terminus and the SH3 domain varies among the members of the SFKs (6). The function of the unique domain in Lyn is unknown; in Lck it mediates protein-protein interactions between Lck and the cytoplasmic tails of the T-cell coreceptors CD4 and CD8 (7,8). The unique domain of Lyn undergoes myristoylation at Gly2 after removal of the N-terminal methionine as well as palmitoylation at Cys3 [reviewed in (3-4)]. Myristoylation and palmitoylation of Lyn are essential for anchoring Lyn to the plasma membrane and for its localization into lipid rafts, respectively [(9); reviewed in (3)].

The SH3 and SH2 domains of Lyn mediate protein-protein interactions between Lyn and Pro-rich motifs and with phosphotyrosine-containing proteins, respectively [(10); reviewed in (3)]. The SH3 domain consists of five anti-parallel β-strands that fold into two anti-parallel α-sheets that are packed at almost right angles to form a ?-barrel (11). The β-strands are connected by three loops and by a helical turn connecting strands 4 and 5 (11). A hydrophobic core is formed by the non-polar amino acids (i.e., Val13, Ala15, Leu27, Phe29, Met35, Val37, Ala47, Gly56, Iso58, Pro59, and Val63) of the β-strands and one of the loops that connects the β-strands (11).

The kinase domain has N- and C-terminal lobes that flank an ATP- and substrate-binding cleft [Figure 7; PDB: 2SRC; (12-15); reviewed in (3)]. The N-terminal lobe is involved in anchoring and orienting ATP, while the C-terminal lobe is primarily responsible for substrate binding and initiating phosphotransfer (16). The N-terminal lobe of mouse Lyn is comprised of a 5-stranded anti-parallel twisted β-sheet and a single large α-helix, termed the αC helix (17). The two most N-terminal β-strands in the N-terminal lobe form a flexible β-strand-turn-β-strand glycine-rich structure called the G-loop (alternatively, the ATP phosphate-binding P-loop) that extends from the N-lobe (16). The side chains of Lys252 and Glu260 within the G-loop form a salt bridge that stabilizes the G-loop in active SFKs and anchors non-transferable ATP β-phosphates to allow for the optimal ATP orientation for β-phosphoryl transfer (12;14;16;18-20). Mutation of residues Lys252 or Glu260 to glycines reduces the catalytic activity of Lyn (16). The C-terminal lobe of the kinase domain is comprised predominantly of α-helices and also contains a 2-stranded anti-parallel β-sheet (17). The two lobes are connected by a loop that borders the cleft that forms the ATP-binding pocket; the loop forms a hinge that makes the overall structure flexible (17).

[Flash Content]

Figure 7. Crystal structure of inactive Src. The unique domain is not part of the structure. UCSF Chimera structure is based on PDB: 2SRC, Xu et al, Mol. Cell 3, 629-638 (1999). Click on the 3D structure to view it rotate.

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Src family kinases are held in a closed, inactive conformation by intramolecular interactions between a phosphorylated tyrosine in the C-terminal tail (Y508 in Lyn) and the SH2 domain [Figure 7; (13;15;17;21;22); reviewed in (3)]. This interaction couples the SH2 domain to the C-terminal tail, and also holds the SH2 and SH3 domains in a rigid conformation that disfavors kinase activation (23). In addition to the SH2-C-terminal tail interaction, the closed conformation of Src family kinases is also maintained by the docking of the SH3 domain onto an internal polyproline type II helical sequence formed by the linker between the SH2 and kinase domains (13;15;21). This polyproline helix is sandwiched between the SH3 domain and the back surface of the N-terminal lobe of the catalytic domain. Binding of the SH2 domain to the phosphorylated tail segment has been proposed to be important for correctly positioning the SH2-kinase domain linker for interaction with the SH3 domain. When the activation loop tyrosine (Tyr397 in Lyn) is dephosphorylated, the C helix rotates outward, assuming a conformation unable to coordinate the \(-\) and \(^{\gamma}\)-phosphates of ATP within the catalytic cleft. Dephosphorylation of Tyr508 by the tyrosine phosphatase CD45 results in a conformation change in Lyn, subsequently promoting Lyn autophosphorylation of Tyr397 and an increase in kinase activity (24;25); reviewed in (3). Phosphorylation of the activation loop also increases the accessibility of the SH3 domain for ligands, and it has been proposed that Src activity may generally control the availability of its regulatory domains (17;26); reviewed in (3). Together, phosphorylation of the tyrosine in the C-terminal tail and dephosphorylation of the activation loop tyrosine promote a closed, inactive conformation in which the lobes of the kinase domain are closely apposed and the C helix is shifted outwards.

The Lemon mutation (His182Arg) is within the SH2 domain of both Lyn isoforms.

**Expression/Localization**

Lyn is expressed in all blood cells (except T lymphocytes) as well as in the brain, prostate cells, and colon cells ([1;2;27;28]; reviewed in [29]). Within the brain, Lyn is highly expressed in the telencephalon, cerebellum striatum, cortex, and thalamus (28;30;31).

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The SFKs interact with immune cell receptors, growth factor receptors, integrins, and G protein-coupled receptors to regulate cell migration, adhesion, phagocytosis, cell survival, differentiation, DNA synthesis, and proliferation through the phosphorylation of signaling intermediates ([32]; reviewed in [29; 33]). Lyn can act as both a positive and negative signaling molecule in several cell types including hematopoietic progenitors, mature myeloid cells (neutrophils, macrophages, monocytes, eosinophils, and dendritic cells), platelets, erythrocytes, and osteoclasts. Lyn can phosphorylate either immunoreceptor tyrosine-based activation motifs (ITAMs) or immunoreceptor tyrosine-based inhibitory motifs (ITIMs) within the cytoplasmic domains of several receptors including the B-cell receptor (BCR), the high-affinity IgE receptor (FcεRI), CD40 (see the record for walla), the mast/stem cell growth factor receptor (c-KIT; see the record for Pretty2), the thrombopoietin receptor (c-Mpl), the erythropoietin receptor (EpoR), and the high-affinity IgG receptor (FcγRI) [Figure 8; (34-39); reviewed in (3;29)]. Lyn also phosphorylates several signaling substrates including PI3-kinase (PI3K; see the record for stinger), phospholipase C gamma 2 (PLCγ2; see the record for queen), and signal transducer and activator of transcription 5 (STAT5) (17). As a result, Lyn regulates several cellular functions including proliferation, degranulation, cytokine production, adhesion, activation, migration, and survival. A summary of Lyn functions in hematopoietic cells is highlighted in Table 1. Several of these functions are described in more detail, below.

Table 1. Summary of Lyn functions in hematopoietic cells. Figure adapted from (29)

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Ligand</th>
<th>Cell type</th>
<th>Function</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Negative role</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type I cytokine receptors</td>
<td>GM-CSF, G-CSF, IL-3, IL-6, IL-4, RANKL, thrombopoietin</td>
<td>Myeloid precursors, macrophages, dendritic cells, mast cells, megakaryocytes</td>
<td>Proliferation, survival, differentiation</td>
<td>(35;40-42)</td>
</tr>
<tr>
<td>Immunoglobulin superfamily receptors</td>
<td>M-CSF, SCF</td>
<td>Myeloid precursors, macrophages, mast cells</td>
<td>Proliferation</td>
<td>(41-43)</td>
</tr>
<tr>
<td>Fc?RI</td>
<td>IgE</td>
<td>Mast cells</td>
<td>Proliferation, degranulation, cytokine production</td>
<td>(36;44-46)</td>
</tr>
<tr>
<td>Integrin</td>
<td>Extracellular matrix proteins, counter receptors</td>
<td>Macrophages, neutrophils, myeloid precursors</td>
<td>Adhesion, activation, survival</td>
<td>(47-49)</td>
</tr>
<tr>
<td><strong>Positive role</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Type I cytokine receptors</td>
<td>IL-5, G-CSF, GM-CSF, Epo</td>
<td>Neutrophils, eosinophils, erythroblasts</td>
<td>Survival, proliferation, differentiation</td>
<td>(41;42;50; 51)</td>
</tr>
<tr>
<td>G protein-coupled receptors</td>
<td>Chemokines</td>
<td>Neutrophils, macrophages, myeloid precursors, mast cells</td>
<td>Activation, migration</td>
<td></td>
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</table>
Following BCR ligation, Lyn phosphorylates the ITAMs of the Igα/Igβ BCR subunits. These phosphotyrosines then act as docking sites for the SH2 domains of Syk. In pre-BCR signaling, Syk phosphorylates many substrates 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.

**Hematopoietic cells**

- **B cells**

Following BCR ligation, Lyn phosphorylates the ITAMs of the Igα/Igβ BCR subunits [Figure 8 & 9, (52-54)]. These phosphotyrosines then act as docking sites for the SH2 domains of Syk (see the record for *poppy*), resulting in Syk phosphorylation and activation. Syk phosphorylates a number of downstream targets including B cell linker (BLNK; see the record for *busy*), PLCγ2, and protein kinase Cζ (PKCζ; 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 (Bruton’s tyrosine kinase), facilitating membrane recruitment of the kinase. Phosphorylated BLNK also provides docking sites for Btk, as well as PLCγ2, which results in the additional phosphorylation and activation of PLCγ2 by Btk leading to the hydrolysis of phosphatidylinositol-3,4,5-triphosphate (PIP3) to inositol-1,4,5-trisphosphate (IP3) and diacylglycerol (DAG) (55). 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 (56)]. 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 *finlay*, *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 (57)]. Lyn is essential for the terminal differentiation of peripheral B cells as well as the elimination of autoactive B cells (58); however, Lyn, Fyn, and Blk have redundant functions in pre-B cell expansion and BCR signaling initiation.

Lyn has a non-redundant role in negative regulation of BCR signaling (52). Lyn phosphorylates the ITIMs of the BCR associated co-receptors CD22 (see the record for *well*), Fc receptor gamma IIb (FcγRIIb), and paired immunoglobulin-like receptor-B (PIR-B) (34;59-63). Inhibitory signaling involves the activation of the protein tyrosine phosphatase SHP-1 (see the record for *spin*), and SH2-containing 5′-inositol phosphatase (SHIP-1; see the record for *styx*) (16). SHIP-1 is recruited to receptor-associated signaling complexes via adaptors (e.g., Shc, Grb2, Dok3), scaffold proteins like Gab1, or directly via its SH2 domain. After recruitment to the plasma membrane, SHIP can then hydrolyze PIP3. Hydrolysis of PIP3 inhibits recruitment of PH domain containing kinases like Akt, Btk, and PLCγ to the plasma membrane and thus limits the activity of several different PI3K effectors that promote cell survival, migration, differentiation, or proliferation.

- **Neutrophils**
with a tethered Jak kinase that phosphorylates tyrosine residues in the receptor cytoplasmic domain upon cytokine binding to the receptor [reviewed in (64)]. Jak-mediated phosphorylation of the receptor provides a docking site for members of the STAT family of transcription factors (e.g., STAT5, STAT3, and STAT1; see the record for domino for information on STAT1). The STAT proteins subsequently dimerize and translocate to the nucleus to regulate gene expression. Lyn is essential for regulation of neutrophil granulopoiesis, apoptosis, and adhesion. Lyn has a positive role in the regulation of calcium influx, MAPK activation and actin polymerization in response to cytokines [reviewed in (29)]. GM-CSF-mediated inhibition of neutrophil apoptosis and the regulation of the pro-survival effect of LPS are dependent on Lyn (42;65). Lyn phosphorylates caspase-8 during resting conditions as well as after LPS stimulation, making it resistant to activation cleavage and subsequently inhibiting the progression of apoptosis (65). Other studies have shown that Lyn is an activator of accelerated apoptosis (66). Lyn recruits SHIP-1 to the plasma membrane, which then blocks pro-survival integrin-induced Akt activation by reducing PIP$_3$ levels. The role of Lyn in G-CSF-induced signaling is unclear. While some studies have proposed that Lyn is negative regulator of G-CSF signaling, others have shown that Lyn is a positive transducer of G-CSF responses [reviewed in (29)]. Scapini et al. proposed that Lyn may act as a negative regulator of G-CSF responses in neutrophil precursors and may act as a positive regulator in more differentiated cells [reviewed in (29)]. Lyn is also a negative regulator of integrin-dependent responses in neutrophils (47-49).

-Macrophages
Phagocytosis occurs upon recognition of microbes by macrophage cell surface receptors including the Fc?Rs, complement receptors, integrins, and scavenger receptors. The Fc?Rs recognize the Fc domains of IgGs to promote engulfment of foreign particles by phagocytosis. The Fc?Rs have ITAM domains that can be phosphorylated by Hck and Lyn (67;68). Fc?R phosphorylation recruits Syk as well as proline-rich tyrosine kinase-2 (Pyk-2), which facilitate actin reorganization. Class A scavenger receptors (SR-As) interact with several ligands including low-density lipoproteins (LDLs), lipoteichoic acid (LTA), and poly I:C to facilitate gene transcription and TNF-?, IL-1?, and IL-6 release. Lyn is a positive regulator of macrophage adhesion through the SR-As (69). Lyn also phosphorylates ITIMs in Fc?R and signal-regulatory protein alpha (SIRP?) in macrophages to negatively regulate phagocytosis through the recruitment of SHP-1 and SHP-2 [reviewed in (29;70)].

-Mast cells
Fc?RI, an antigen receptor found on mast cells, is phosphorylated by Lyn at ITAMs in the cytoplasmic tail of the receptor. The Fc?RI signaling pathway shares homology with the BCR and Fc?R signaling pathways. In mast cells, Lyn has a positive functional role in SCF-induced chemotaxis, a redundant positive role in Fc?RI-stimulated responses, and a negative regulatory role in Fc?RI-stimulated responses (i.e., degranulation, proliferation and cytokine production) [(36;44-46); reviewed in (29)].

-Dendritic cells
Lyn is involved in the regulation of dendritic cell (DC) GM-CSF-stimulated maturation, proliferation, and survival (71;72). In Lyn$^{-/-}$ mice, loss of Lyn expression resulted in enhanced DC expansion from bone marrow precursors as well as accelerated differentiation of DC progenitors; however the bone marrow-derived DCs exhibited a less mature phenotype and a reduced capacity to stimulate antigen-specific T cells (71). In addition, Lyn$^{-/-}$ DCs had higher survival rates after differentiation than wild-type cells (71). Lyn$^{-/-}$ DCs were unable to mature appropriately in response to innate stimuli, leading to DCs that had lower levels of MHC class II and costimulatory molecules (71). IL-12 production and antigen-specific T cell activation were reduced in Lyn$^{-/-}$ DCs after maturation, leading to impaired T helper type 1 (Th1) responses (71). Diminished IL-12 synthesis in the DCs was due to hyperactivation of the PI3K pathway (72). DC-specific Lyn (DC-Lyn) knockout mice exhibited spontaneous activation of B and T cells, the development of autoantibodies, severe nephritis (73). DCs from the DC-Lyn mice were hyperactivated and hyperresponsive to TLR agonist, GM-CSF, and IL-1? stimulation (73). After LPS or IL-1? stimulation, Lyn-deficient DCs exhibited increased phosphorylation and degradation of I?B?, indicating that NF-?B signaling was increased.

-Eosinophils
Lyn associates with the IL-5 receptor (IL-5R) alpha (IL-5R?) subunit and phosphorylates ITAMs in both the IL-5R? and ? subunits to positively regulate IL-5-induced differentiation, proliferation, survival, and activation in eosinophils [(72;74-76); reviewed in (29)]. Lyn also has a positive role in IL-3/GM-CSF-induced survival (74;75) and in Fas-mediated cell death (77); see the record for rigrand for more information on Fas/Fasl-mediated cell death.

-Erythroblasts
Lyn is essential for both (EpoR)-induced early erythroid cell expansion and late-stage maturation and cell survival; loss of Lyn expression prevented erythroid differentiation in response to erythropoietin (37;78-80). In EpoR signaling, activated Lyn phosphorylates STAT5 (50;79). Lyn also stimulates pathways that downregulate EpoR signaling (41).

-Osteoclasts
Lyn is a negative regulator of osteoclastic bone resorption and differentiation of osteoclast precursors in response to receptor activator of NF-kappaB (RANK) ligand (RANKL); Lyn forms a complex with RANK, SHP-1, and Grb2-associated binder 2 (Gab2). See the record for xander for more information on the function of RANK in the non-canonical NF-?B pathway. Osteoclasts from Lyn$^{-/-}$ mice exhibited increased Gab2 phosphorylation as well as increased JNK and NF-?B activity. As a result, Lyn$^{-/-}$ mice showed accelerated osteclastogenesis and bone loss in response to RANKL (81).

Lyn function in the brain

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stimulation. Following Lyn activation, the MAPK pathway regulates the expression of brain-derived neurotrophic factor (BDNF) to mediate fast synaptic transmission \(82\). Lyn negatively regulates the N-methyl-D-aspartate (NMDA) pathway in controlling striatum- and nucleus accumbens-regulated motor activity \(30\). In NMDA signaling, Lyn inhibits PKC activity, which is responsible for the phosphorylation of the NR1 regulatory subunit of the NMDA receptor \(83\). \(Lyn^{-/-}\) mice did not move normally in a new environment due to an enhancement in NMDA signaling upon the loss of Lyn expression; the dopaminergic pathway was normal \(30\).

Leukemia and Tumors

In the mouse, Lyn is a proposed tumor suppressor; this function is affected by the genetic background of mouse models and by other environmental factors including chronic infection and the spectrum of commensal flora \([29]\). \(Lyn^{-/-}\) mice older than one year of age can develop large aggregates of myelomonocytic cells on the tail, ears, and legs due to hyperresponsiveness to cytokine stimulation \([41]\); reviewed in \([29]\). In cell lines established from patients with chronic myelogenous leukemia (CML) and acute myeloid leukemia (AML), Lyn is hyperactivated \([84]\); reviewed in \([29]\). Lyn is essential in maintaining AML cell proliferation and anti-apoptotic pathways \([85]\). Lyn also regulates the activation of the Akt anti-apoptotic pathway in colon carcinoma cells \([86]\). In prostate cancer cell lines, inhibition of Lyn resulted in reduced cell proliferation \([87]\).

Putative Mechanism

The Lemon phenotype is similar to that observed in the \(Lyn^{-/-}\) and \(Lyn^{weeb}\) (WeeB) mouse models indicating that LynLemon has reduced function. Lyn knockout \((Lyn^{-/-}; Lyn^{tm1Sor}, Lyn^{tm1Tya}, and Lyn^{tm1Sor})\) mice exhibit normal B lineage compartments through to the transitional stage in the spleen \((34;58;88;89)\). Lyn\(^{-/-}\) transitional 1 (T1) B cells develop normally, but T2 B cells develop primarily from the T1 subset in the spleen and fail to develop from immature B cells in the bone marrow \((52;90)\). Transitional and maturing B cells in the \(Lyn^{-/-}\) mice undergo increased death rates in the T2 and mature subsets; most dying cells do not pass through the anergic T3 stage \((52)\). Lyn\(^{-/-}\) mice exhibit normal frequency of pro-B cells, indicating that Lyn does not control pro-B cell proliferation or survival and/or that it plays a redundant role therein \((34;58;88;91)\). Lyn\(^{-/-}\) mice exhibit progressive splenomegaly and enlargement of lymph nodes, reduced numbers of mature follicular B cells, absence of marginal zone B cells, produce large quantities of anti-nuclear antibodies, and develop glomerulonephritis as early as 5 months of age \((34;58;88;89)\). B cells from \(Lyn^{-/-}\) mice are both hyperresponsive to BCR ligation and resistant to the inhibitory signals from Fc\(R\)IIb and CD22 \((34;59-61)\). Peritoneal IgM\(^+\) B220\(^+\) B cell numbers were significantly lower in \(Lyn^{-/-}\) mice at 2 months of age compared to wild-type mice and the size of the Peyer’s patches were reduced \((58;89)\). As a result, CD5\(^+\) B220\(^{high}\) conventional B cells and B1 cells were also reduced \((89)\). WeeB mice, have an ENU-induced mutation within the kinase domain resulting in perturbed BCR signaling \((16)\). The WeeB mice have ~50% reduced peripheral blood B cell numbers. The numbers of all splenic B cell subsets and of mature B220\(^{high}\)IgM\(^+\) recirculating B cells were reduced in the WeeB mice at less severe amounts than in the \(Lyn^{-/-}\) mice. With age, the WeeB mice develop enlarged spleens with an accumulation of lymphoblast-like and plasma cells, reduced B cell numbers, and disrupted B cell zone organization. The WeeB mice exhibit a later glomerulonephritis onset (12-14 months) than \(Lyn^{-/-}\) mice (~8 months).
Lemon 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**
Lemon(F): 5’- GGCCAGGTCATACTTCCTGTTTTGTT -3’
Lemon(R): 5’- TGAAACTGTGCCTACACAACCAACC -3’

**Sequencing Primers**
Lemon_seq(F): 5’- GATTCCCCTGCTTCTGCC -3’
Lemon_seq(F): 5’- TGTTCATCCCCAGCCAAG -3’

**PCR program**
1) 94°C 3:00
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 974 nucleotides (from Genbank genomic region NC_000070 for linear DNA sequence of Lyn) is amplified:

68171 ggcaggtca tacctcctgt tttgttgtg tgtattatgaaa gtgaatccta
68221 agtgtcaaga ggaaactcaa aataattgtt ctattcagct aaatgatttt ggaaaactac
68281 ggtgaagaag aaaagtgata tattgcctac ttgtctagca aacaaattgc tattgtcatt
68341 tatcaagtca aaaatttaaa gtaccttgta tttttcctcc gaggccttgg ctagcctggt
68401 attagcata ggattcccctgc ttctgcc tcc cacgtgcctg ggctaaaatc atcactgcac
68461 aaggctgcag agctttcatg tgatgaggtt gtatttgtat gtttcatggt aaaatggtta
68521 tcacagtgtg gtataatgta gaccttatta ataacatata aacatgcctt ctcttaactg
68581 ttttccacag gaagcttctc tctttctgtc agagattatg accctatgca tggtgatgtc
68641 attaagcact acaaaattag aagttcgagc aatgttgacct attacatctc tctctgcatc
68701 acttttcccct gcatcagtga catgattaag cattaccaaa gtaagtcaaaa actgaaggcc
68761 gggagaggca gattcatcctt ataagttgta gtaagccaag gcataagcctt attacatcctt
68821 atttggccct tcaagtttaag ccaggggtag tactctcataa gatgcttctaa cttattgtag
68881 atactcccttg acctccaggt agcaattcag tcatgactgt gatgtgcctt gtaagtgctt
68941 taagttgaaag atacaattcga ttccgcctat cttgctqaca ccctqactct acttatagcc
69001 caggtgtgac gcctagcctt ctaacagctt gcctagcttt gccctcgctt atgaagcatt
69061 aacctctgttg tcgctgtgct ctacagcagc ggcaggtca tttgttgtggt attggttgtg

**FASTA sequence**

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

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85. Vitro and in Vivo.
83. Differentiation of J2E Erythroid Cells.
82. myelo/erythropoiesis in Lyn-Deficient Mice is Similar to that in Mice Lacking the Inhibitory Phosphatases SHP-1 and SHIP-1.
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80. Eosinophilic Inflammation.
79. Apoptosis by Cytokines in Human Eosinophils.
78. 168
77. 1593-1604
76. 3901-3910.
75. 1407-1414
74. 196, 1593-1604.
73. 168, 1029-1034.
72. 1407-1414
71. 92, 1058-1066
70. 3901-3910.
69. 1029-1034.
68. 1593-1604.
67. 2469-2478.
66. 72-76.
65. 49-78.
64. 1867-1875.
62. 855-861.
61. 397-401.
60. 111, 2269-2279.
59. 5275-5283.
58. 1407-1414
57. 1867-1875.
56. 1593-1604
55. 262-271.
54. 33, 49-78.
53. 946-949.
52. 163, 2325-2330.
51. 72-76.
50. 397, 72-76.
49. 9.72-76.
34. Proc Natl Acad Sci USA 106, 2325-2330.
17. Proc Natl Acad Sci USA 106, 2325-2330.

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