Gain-of-function variants in SYK cause immune dysregulation and systemic inflammation in humans and mice

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Spleen tyrosine kinase (SYK) is a critical immune signaling molecule and therapeutic target. We identified damaging monoallelic SYK variants in six patients with immune deficiency, multi-organ inflammatory disease such as colitis, arthritis and dermatitis, and diffuse large B cell lymphomas. The SYK variants increased phosphorylation and enhanced downstream signaling, indicating gain of function. A knock-in (SYK-Ser544Tyr) mouse model of a patient variant (p.Ser550Tyr) recapitulated aspects of the human disease that could be partially treated with a SYK inhibitor or transplantation of bone marrow from wild-type mice. Our studies demonstrate that SYK gain-of-function variants result in a potentially treatable form of inflammatory disease.

Immunoreceptors, including B cell receptors, T cell receptors and Fc receptors, signal through cytosolic immunoreceptor tyrosine-based activation motifs that are rapidly tyrosine phosphorylated to recruit and activate kinases with tandem SH2 domains. These kinases are the ζ-chain-associated protein kinase of 70 kDa (ZAP70), which is primarily expressed in T cells and natural killer cells, and SYK, which is primarily expressed in mononuclear phagocytes (MNPs), B cells and, to a lesser extent, the intestinal epithelium. SYK is also involved in signaling cascades from receptors with single-copy tyrosine-based activation motifs (HemITAM) including C-type lectin receptors and receptors without immunoreceptor tyrosine-based activation motifs, such as Toll-like receptors (TLRs) and integrins. Global knockout of SYK in mice is perinatally lethal. Chimeric mice carrying a Syk-deficient hematopoietic system are protected from autoantibody-mediated arthritis and have a block in B cell development at the pro-B to pre-B cell transition. Loss-of-function biallelic variants in ZAP70 result in human immunodeficiency characterized by a selective T cell defect (MIM 269840), while pathogenic variation of SYK has not been described in humans. In this study, we identify monoallelic gain-of-function variants in SYK that result in immunodeficiency and systemic inflammatory disease in humans and show that the expression of one of these variants in a mouse model replicates major aspects of the human immunopathology.

Results

Clinical characteristics of two unrelated families with very early onset multi-organ inflammation. Patient 1, a female infant of Chinese ancestry born to healthy non-consanguineous parents, presented at 2 weeks of age with fever, whole-body rash and non-bloody diarrhea (5–8 times per day). Colonoscopy at 17 months of age revealed multiple ulcers in the colon (Fig. 1a) with histologic features consistent with chronic colitis (Fig. 1b). She also developed perianal fistulas (Fig. 1c) and arthritis indicated by joint pain and swelling of her hands (Fig. 1d). Laboratory tests showed episodes of elevated white blood cell (WBC) counts, elevated C-reactive protein (CRP), a high level of serum interleukin (IL)-6 and reduced serum levels of immunoglobulin M (IgM) and IgG (Fig. 1e and Supplementary Table 1). She had notable growth failure (Fig. 1f) and recurrent infections. The patient passed away before her third birthday owing to complications from an infection.

Patient 2, a female infant of reported Ashkenazi Jewish ancestry born to non-consanguineous parents, presented at 2 weeks of age with whole-body rash, generalized vasculitis (Fig. 1g) and diarrhea.

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Colonoscopy at 4 weeks of age revealed ulcers in the cecum with features of chronic inflammation. By 15 months of age, she developed arthritis (Fig. 1h) with worsening colitis and multiple episodes of elevated CRP and WBC counts (Fig. 1i and Supplementary Table 2). Her father (patient 3, age 35) had a similar disease that started at 2 weeks of life and was characterized by oral ulcers, fever, rash, diarrhea and failure to thrive. As a child, he was diagnosed with an undefined immunodeficiency with reduced CD4+ T cell counts and low immunoglobulin levels (Supplementary Table 3) and was advised against using live vaccines. Currently, he continues to have bowel and skin symptoms with recurrent joint swelling associated with elevated CRP. Patients 2 and 3 both have reduced memory bowel and skin symptoms with recurrent joint swelling associated with elevated CRP. Patients 2 and 3 both have reduced memory

Identification of monoallelic SYK variants in an immune dysregulation syndrome. Analysis of whole-exome sequencing of DNA samples from these two families did not identify any known monogenic inborn errors of immunity (Extended Data Fig. 1a,b). However, monoallelic variants affecting amino acid 550 in the SYK gene were nominated as a potential candidate in all three patients on the basis of the gene’s known biological function and damaging scores (Table 1; patient 1 de novo c.1649C>A, NM_003177.6; p.Ser550Tyr; patients 2 and 3 c.1649C>T, NM_003177.6; p.Ser550Phe; Fig. 2a,b). We next screened for additional SYK variants in a number of patient registries and found three additional patients with potential monoallelic damaging variants in evolutionarily conserved residues of SYK (p.Pro342Thr, p.Ala353Thr and p.Met450Ile). The p.Pro342Thr variant identified in patient 4 was de novo (Extended Data Fig. 1c and Supplementary Table 5); however, the inheritance patterns of patients 5 and 6 (SYK variants p.Ala353Thr and p.Met450Ile, respectively) were unknown as they were sequenced as singletons with no parental DNA available (Extended Data Fig. 1d,e).

We compared the clinical phenotypes of all six patients bearing SYK variants (detailed clinical history and laboratory values are available in Table 1, Supplementary Tables 1–5, Supplementary Note and Extended Data Fig. 2a–c). Patients 1–3 with the variants affecting Ser 550 presented as infants, while patients 4–6 had a later disease onset. All patients had recurrent infections and dysgammaglobulinemia, patients 2 and 3 had B cell defects, and patients 1 and 4 had decreased ratios of CD4+ and CD8+ T cells. All patients had inflammation in multiple tissues including bowel (patients 1–6), skin (patients 1–5), joints (patients 1–3 and 6), lung (patients 2, 5 and 6) and central nervous system (patients 4 and 5) and granulomatous liver disease (patient 6). Patients 5 and 6 developed diffuse large B cell lymphoma (DLBCL) years after their initial presentation of inflammatory disease (both germline SYK variants were confirmed by sequencing of buccal samples; Extended Data Fig. 1d,e). Taking these findings together, we conclude that all six individuals are manifesting an autosomal dominant syndrome of immune dysregulation, multi-organ inflammation and lymphoma predisposition, caused by monoallelic variants in SYK.

SYK variants demonstrate a gain of function. All of the putatively pathogenic SYK variants, validated by Sanger sequencing, were novel or very rare, located in highly conserved regions (Extended Data Fig. 2d) and predicted to be damaging with combined annotation-dependent depletion (CADD) scores7 greater than 25 (Table 1). While Ser 550 is located close to two essential activation loop tyrosine residues but distant from the ATP-binding pocket, Met 450 is located close to the ATP-binding pocket of SYK. The p.Pro342Thr and p.Ala353Thr variants are located in the linker region (interdomain B) that maintains SYK in an autoinhibited conformation (Fig. 2c,d). With the exception of Thr 342 in the moth Amyelois transistella, none of the identified missense amino acids is present in SYK homologs, suggestive of evolutionary exclusion (Extended Data Fig. 2e).

To determine the functional consequences of the SYK monoallelic variants, we first examined SYK phosphorylation in patient resting peripheral blood mononuclear cells (PBMCs), where monocytes and B cells are the primary cells expressing SYK. In the steady state, monocytes and B cells are not expected to have active, phosphorylated SYK (pSYK). In contrast to healthy family controls, PBMCs from patients 1 and 3 showed spontaneous tyrosine phosphorylation of SYK Tyr 525/526, tyrosine residues known to regulate autophosphorylation and activation (Fig. 2e,f), despite a decrease in SYK expression. Likewise, intestinal biopsy samples from patient 1 demonstrated increased pSYK (Extended Data Fig. 3a,b). These data pointed towards constitutive activation of the SYK Ser 550 variants.

To examine whether this is a feature of all the putative pathogenic alleles, we individually expressed the wild-type (WT) and each variant form of SYK at comparable levels by transfection into human embryonic kidney (HEK)293 cells that do not express the kinase endogenously. Using three independent methods (flow cytometry, western blotting and ELISA), we found that the p.Pro342Thr, p.Ala353Thr, p.Met450Ile, p.Ser550Tyr and p.Ser550Phe SYK variants all showed significantly increased phosphorylation of Tyr 525/526 compared to WT SYK and a p.Tyr525/526Phe inactive SYK negative control (Fig. 2g–j and Extended Data Fig. 4a–h). A putative gain-of-function variant identified previously in rat cells8 (p.Tyr323Phe) was used as a positive control and showed increased Tyr 525/526 phosphorylation of SYK in flow cytometry assays, although to a much lesser extent than the variants identified in our patients (Fig. 2i, Extended Data Fig. 4a–f and data not shown). Thus, the patient-identified SYK variants all lead to constitutive SYK activation, consistent with gain of function.

Expression of constitutively active SYK variants in HEK293 cells drove downstream signaling as revealed by increased phosphorylation of extracellular signal-regulated kinases (ERKs) and c-Jun amino-terminal kinases (JNKs), which was not observed in cells expressing the p.Tyr525/526Phe inactive or WT forms of SYK (Extended Data Fig. 4g,h). In addition, when stimulated with tumor necrosis factor (TNF) to engage the TNF receptor or phorbol-12-myristate-13-acetate (PMA) to activate protein kinase C, cells expressing p.Ser550Tyr SYK showed exaggerated AP-1 and NF-κB activity compared to WT SYK (Extended Data Fig. 4i). These results demonstrate that patient SYK variants caused elevated tonic (ligand-independent) signaling and augmented well-defined receptor-mediated signaling pathways.

Small-molecule inhibitors of SYK have been developed including fostamatinib. R406, the active metabolite of fostamatinib, attaches to SYK Tyr 525/526 phosphorylation and reduced downstream signaling including the phosphorylation of JNK and ERK (Extended Data Fig. 4j,k).

The hyperactivation of SYK caused by Ser 550 variants was not only observed in HEK293 cells. When expressed in human SW480 colonic epithelial cells, the p.Ser550Tyr SYK variant also showed increased phosphorylation at Tyr 525/526 (Extended Data Fig. 5a).

Human intestinal epithelial cells can respond to microbe-associated molecular patterns (MAMPs) including bacterial and fungal cell wall components (for example, β(1,3)-glucans; Extended Data Fig. 5b). MAMPs can be shared between pathogens and the normal microbiome, and therefore signaling responses must be tailored appropriately or otherwise lead to autoinflammation. Accordingly, intestinal epithelial cells express low levels of pattern recognition receptors such as dectin 1 at their surface that recognize the exposed β(1,3)-glucans.
of microbes. We therefore stimulated SW480 cells expressing WT SYK or the p.Ser550Tyr SYK variant with zymosan (prepared from the cell walls of the yeast *Saccharomyces cerevisiae*) or curdlan (derived from the cell walls of *Alcaligenes faecalis*, a gram-negative bacterium), which both contain exposed β(1,3)-glucans. We found that, in addition to increasing phosphorylation of SYK Tyr525/526 at baseline, the p.Ser550Tyr-expressing SW480 cells showed heightened, sustained and robust signaling in response to zymosan and curdlan (Extended Data Fig. 5c,d). The heightened response of the p.Ser550Tyr-expressing cells to β(1,3)-glucan was sufficient to heighten, sustain and robust signaling in response to zymosan and curdlan (Extended Data Fig. 5c,d). The heightened response of the p.Ser550Tyr-expressing cells to β(1,3)-glucan was sufficient to produce secondary alterations in the T cell compartment (Extended Data Figs. 6 and 7; gating strategies outlined in Supplementary Figs. 1–3). We found that in patient 1, the ratio of CD4+ to CD8+ T cell receptor-positive (αβ) T cells decreased at 1.5 years and 2 years of age (Extended Data Fig. 6a–d and Extended Data Fig. 7a). The peripheral CD8+ T cells largely consisted of bona fide effector/memory type cells in this patient (Extended Data Fig. 6a–d and Extended Data Fig. 7a). The peripheral CD8+ memory T cell frequencies in this patient were also increased relative to naive T cells (Extended Data Figs. 6g and 7e) and showed a high frequency of CD4+ memory T cell frequencies in this patient were also increased relative to naive T cells (Extended Data Figs. 6g and 7e) and showed a high frequency of CCR6+ and RORyt+ cells that produced elevated IL-17A, IL-22, IFNy and TNF but not IL-10 or IL-13 (Extended Data Fig. 6h–j and Extended Data Fig. 7f–j). Indeed, the peripheral blood T cells expressed in T cells, we postulated that multi-organ inflammation observed in patients with gain-of-function variants in SYK would produce secondary alterations in the T cell compartment (Extended Data Figs. 6 and 7; gating strategies outlined in Supplementary Figs. 1–3). We found that in patient 1, the ratio of CD4+ to CD8+ T cell receptor-positive (αβ) T cells decreased at 1.5 years and 2 years of age (Extended Data Fig. 6a–d and Extended Data Fig. 7a). The peripheral CD8+ T cells largely consisted of bona fide effector/memory type cells in this patient (Extended Data Fig. 6a–d and Extended Data Fig. 7a). The peripheral CD8+ memory T cell frequencies in this patient were also increased relative to naive T cells (Extended Data Figs. 6g and 7e) and showed a high frequency of CCR6+ and RORyt+ cells that produced elevated IL-17A, IL-22, IFNy and TNF but not IL-10 or IL-13 (Extended Data Fig. 6h–j and Extended Data Fig. 7f–j). Indeed, the peripheral blood T cells expressed in T cells, we postulated that multi-organ inflammation observed in patients with gain-of-function variants in SYK would produce secondary alterations in the T cell compartment (Extended Data Figs. 6 and 7; gating strategies outlined in Supplementary Figs. 1–3). We found that in patient 1, the ratio of CD4+ to CD8+ T cell receptor-positive (αβ) T cells decreased at 1.5 years and 2 years of age (Extended Data Fig. 6a–d and Extended Data Fig. 7a). The peripheral CD8+ T cells largely consisted of bona fide effector/memory type cells in this patient (Extended Data Fig. 6a–d and Extended Data Fig. 7a). The peripheral CD8+ memory T cell frequencies in this patient were also increased relative to naive T cells (Extended Data Figs. 6g and 7e) and showed a high frequency of CCR6+ and RORyt+ cells that produced elevated IL-17A, IL-22, IFNy and TNF but not IL-10 or IL-13 (Extended Data Fig. 6h–j and Extended Data Fig. 7f–j). Indeed, the peripheral blood T cells expressed in T cells, we postulated that multi-organ inflammation observed in patients with gain-of-function variants in SYK would produce secondary alterations in the T cell compartment (Extended Data Figs. 6 and 7; gating strategies outlined in Supplementary Figs. 1–3).
and amino acid alignment of the human SYK p.Ser550Tyr and the corresponding mouse SYK p.Ser544Tyr and Sanger validation). The heterozygous SYK-Ser544Tyr mice spontaneously developed arthritis as indicated by paw swelling (Fig. 3a), an increased arthritis clinical score (Fig. 3b), age-dependent reduced capacity in a functional grip assay (Fig. 3c) and bone erosion assessed by micro-computed tomography (micro-CT; Fig. 3d), whereas none of those phenotypes was observed in WT littermates. Consistent with our studies in cell lines, western blot analysis of ankle joint tissues showed increased SYK phosphorylation in the SYK-Ser544Tyr mice (Extended Data Fig. 8a). Furthermore, SYK-Ser544Tyr mice showed a significantly reduced tail length while the tail diameter was significantly increased.

### Table 1 Genetic and clinical characteristics of patients with monoallelic SYK variants

| Variant annotation | Patient 1 (p.Ser550Tyr) | Patient 2 (p.Ser550Phe) | Patient 3 (p.Ser550Phe) | Patient 4 (p.Pro342Thr) | Patient 5 (p.Met450Ile) | Patient 6 (p.Ala353Thr) |
|---------------------|--------------------------|-------------------------|-------------------------|--------------------------|--------------------------|--------------------------|
| Chromosome          | 9                        | 9                       | 9                       | 9                        | 9                        | 9                        |
| Position (GRCh38)   | 90,887,816                | 90,887,816              | 90,887,816              | 90,874,692               | 90,877,739               | 90,874,725               |
| nt ref              | C                        | C                       | C                       | G                        | G                        | G                        |
| nt alt              | A                        | T                       | T                       | A                        | A                        | A                        |
| dbSNP151            | rs1304839707              | rs200167353             |                         |                          |                          |                          |
| pos (GRCh37)        | 93,650,098                | 93,650,098              | 93,650,098              | 93,636,974               | 93,640,021               | 93,637,007               |
| 1000Gp3_AF          |                          |                         |                         |                          |                          |                          |
| ESP6500_AA_AF       |                          |                         |                         |                          |                          |                          |
| ExAC_AF             | 2.47 × 10⁻⁵              |                         |                         |                          |                          |                          |
| gnomAD_exomes_AF    | 7.95 × 10⁻⁶              | 1.99 × 10⁻⁵             |                         |                          |                          |                          |
| COSMIC              |                          |                         |                         |                          |                          |                          |
| CADD_value          |                          |                         |                         |                          |                          |                          |
| SIFT_pred           | Damaging                 | Damaging                | Damaging                | Damaging                 | Damaging                 | Tolerated                |
| Polyphen2 richness_hDIV_pred | Damaging | Damaging | Damaging | Damaging | Damaging | Damaging |
| LRT_pred            | Damaging                 | Damaging                | Damaging                | Damaging                 | Damaging                 | Damaging                 |
| MutationTaster_pred | Damaging                 | Damaging                | Damaging                | Damaging                 | Damaging                 | Damaging                 |
| PROVEAN_pred        | Damaging                 | Damaging                | Damaging                | Damaging                 | Damaging                 | Tolerated                |
| MetaSVM_pred        | Damaging                 | Damaging                | Damaging                | Damaging                 | Damaging                 | Tolerated                |
| M-CAP_pred          | Damaging                 | Damaging                | Damaging                | Damaging                 | Damaging                 | Damaging                 |
| fathmm-MKL_coding_preds | Damaging | Damaging | Damaging | Damaging | Damaging | Damaging |

| Clinical presentation | | | | | | |
|-----------------------| | | | | | |
| Age of diagnosis      | 2 weeks | 2 weeks | 2 weeks | 12 years | 34 years | 44 years |
| Intestinal inflammation | +   | +   | +   | +   | +   | +   |
| Skin inflammation     | +   | +   | +   | +   | +   | -   |
| Joint inflammation    | +   | +   | +   | -   | -   | +   |
| Lung inflammation     | -   | +   | -   | -   | +   | +   |
| Central nervous system inflammation | -   | -   | -   | +   | +   | -   |
| Liver inflammation    | -   | -   | -   | -   | -   | +   |
| Recurrent infections  | +   | +   | +   | +   | +   | +   |
| Hypogammaglobulinemia | +   | +   | +   | +   | +   | +   |
| DLBCL                 | -   | -   | -   | -   | +   | +   |

| Therapy (in chronological order first used) | | | | | |
|---------------------------------------------| | | | | |
| ABX                                         | ANAK | ABX | IVIG | IVIG | IVIG |
| SASA                                        | CSTD | IVIG | CSTD | CSTD | ABX |
| CSTD                                        | TACR | RITUX | CYCA | RITUX | RITUX |
| IVIG                                        | RITUX | AZA | CHOP | CHOP |
| THAL                                        | TRIAM | RITUX | ABX |
| VEDO                                        | | | | | |

*Patient 3 had documented hypogammaglobulinemia as a child. *Patient 6 was diagnosed at 44 years of age but had lifelong infections. Nt, nucleotide; ref, reference strand; alt, alternate strand; pos, position; AF, allele frequency; pred, prediction; ABX, antibiotics; ANAK, anakinra; SASA, 5-aminosalicylic acid-based therapy; AZA, azathioprine; CHOP, cyclophosphamide, doxorubicin hydrochloride (hydroxydaunorubicin), vincristine sulfate (Oncovin) and prednisone; CSTD, corticosteroid (intravenous and/or oral); CYCA, ciclosporine A; IVIG, intravenous immunoglobulin; RITUX, rituximab; TACR, oral tacrolimus; THAL, thalidomide; TRIAM, intra-articular triamcinolone; VEDO, vedolizumab.
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cells were slightly decreased in circulation, their frequencies were increased in tissue, consistent with a defect in immunoglobulin generation. CD3+ T cell frequencies were increased in both circulation while their frequencies were increased as a result of pro-inflammatory cytokines (Extended Data Fig. 8f).

Next, we analyzed the cellular composition of immune cell infiltrates in the affected ankles of SYK-Ser544Tyr mice. We determined the frequencies of CD45+ cells, B220+ B cells, CD3+ T cells, CD4+ T cells, regulatory T cells (CD3+CD4+Foxp3+CD25+), CD11b+F4/80- and CD11b+F4/80+ MNP and macrophages (CD11b+F4/80+) in the ankles as compared to blood (flow cytometry gating strategy outlined in Supplementary Figs. 6 and 7). Interestingly, while CD45+ cells were slightly decreased in circulation, their frequencies were markedly increased in ankle tissue of SYK-Ser544Tyr mice compared to WT mice (Extended Data Fig. 9a). B cell frequencies and normalized counts were significantly reduced in both circulation and tissue, consistent with a defect in immunoglobulin generation. CD3+ T cell frequencies were increased in blood but reduced in tissue, while their normalized counts increased in both compartments. CD4+ T cells instead showed a proportional and normalized absolute increase in blood and tissue. Regulatory T cells showed reduced frequencies in blood but increased frequencies in inflamed tissue. MNP (CD11b+F4/80- and CD11b+F4/80+) and macrophages were significantly increased in inflamed ankles of SYK-Ser544Tyr mice (Extended Data Fig. 9b–f). Full blood counts revealed reduced lymphocyte frequencies and counts in SYK-Ser544Tyr mice. Monocyte and neutrophil counts did not show significant differences in circulation while their frequencies were increased as a result of proportional changes in lymphocytes, in particular the reduction in B cells (Extended Data Fig. 10). While some of the cytokines that we found elevated in SYK-Ser544Tyr mouse have specific functions linked to the TCR1 and TCR17 subsets (for example, IL-10/β, IFNγ, IL-17A, IL-17F and GM-CSF), they also influence the polarization and inflammatory phenotypes of innate immune cells.

To investigate the cause of the pronounced defect in bone homeostasis observed in ankles and tails of SYK-Ser544Tyr mice, we next analyzed the presence of osteoclasts. To detect osteoclasts, we stained the ankle joints for tartrate-resistant acid phosphatase (TRAP), a metalloprotein enzyme expressed by osteoclasts and involved in bone degradation. The joints of SYK-Ser544Tyr mice showed significantly increased signals for TRAP+ cells, suggestive of an accumulation of osteoclasts at the site of inflammation (Fig. 3g,h). Osteoclasts can be differentiated ex vivo by culturing bone marrow-derived macrophages in receptor activator of nuclear factor-κB ligand (RANKL). We found that SYK-Ser544Tyr mice generated 2.5-fold higher numbers of TRAP+ multinucleated osteoclasts that ultimately covered a 5-fold greater surface area in such experiments (Fig. 3i,j). These results suggest a central role of dysregulated osteoclast differentiation and potentially their hyperactivation in the development of the erosive arthritis observed in the SYK gain-of-function mice and patients.

Treatment of SYK-Ser544Tyr ameliorates the disease phenotype.

The SYK gain-of-function mouse model allowed us to establish an experimental system to test compounds that could ameliorate disease. We treated the SYK-Ser544Tyr mice with the SYK-specific inhibitor R406 and found that treatment reduced symptoms of arthritis including ankle thickness and disease activity scores in young (1 month old; Fig. 4a) and adult (3 months old; Fig. 4b) animals. As our data suggest that SYK plays a role in both mucosa and innate immune compartments, we performed bone marrow transplantation experiments to determine whether the disease was driven by cells of the hematopoietic lineage. Indeed, when irradiated SYK-Ser544Tyr mice were transplanted with WT bone marrow, there was a dramatic resolution of disease symptoms (Fig. 4c–e). Conversely, irradiated WT mice transplanted with SYK-Ser544Tyr bone marrow developed severe erosive arthritis as assessed by micro-CT (Fig. 4c,f–h).

Discussion

SYK is a critical signaling molecule and a therapeutic target for autoimmune disease and cancer. Here we report causal SYK variants in humans with immune dysregulation and inflammation. Functional studies demonstrate that the observed monoallelic variants exhibit gain of function, causing constitutive activation of SYK and downstream signaling associated with an augmented response to immunoreceptor engagement. In patients, this constitutive SYK activation results in immune defects and varying degrees of intestinal, skin, joint, liver and nervous system inflammation, as well as lymphoma predisposition. SYK plays a complex role in a number of cellular processes including adaptive and innate immunity in both bone marrow-derived and epithelial cells. While we provide functional insight into the role of SYK gain-of-function variants in human disease, specifically joint disease, the underlying function of activated SYK in the pathogenesis of disease in other tissues and/or disease processes is not yet fully understood.

Constitutive phosphorylation and activation of SYK has been observed in autoimmune disease*. Our data support the role of...
of SYK activation in the initiation of arthritis, as we found that SYK-Ser544Tyr gain-of-function mice developed severe erosive arthritis. SYK-Ser544Tyr mice had increased counts of multinucleated osteoclasts following in vitro differentiation from bone marrow-derived macrophages in the presence of M-CSF and RANKL. These data are complementary to a recent report on the

**Patient 1**

WT/c.1649C>A  
p.Ser550Tyr

**Patient 2**

WT/c.1649C>T  
p.Ser550Phe

**Patient 3**

WT/c.1649C>T  
p.Ser550Phe

**WT SYK amino acids 15–631 (PDB ID: 4FL2)**

- SH2 domains
- Interdomain regions
- Kinase domain
- Gain-of-function variant sites
- Phosphoregulatory tyrosines
- ATP analog

**SYK phosphorylation**

- WT SYK
- p.Ser550Tyr
- p.Ser550Phe
- p.Met450Ile
- p.Ala353Thr
- p.Pro342Thr
- p.Tyr323Phe

**pSYK (Tyr 525/526)**

- ELISA
- Western blot
- Phosphorylation
- Ubiquitination

**Control**

WT  
p.Thr525/526Phe  
p.Ser550Tyr  
p.Ser550Phe  
p.Met450Ile  
p.Ala353Thr  
p.Pro342Thr

**WT**

p.Tyr525/526Phe  
p.Ser550Tyr  
p.Ser550Phe  
p.Met450Ile  
p.Ala353Thr  
p.Pro342Thr
Fig. 3 | Phenotype of the SYK-Ser544Tyr mouse model. a, Photographs of the hind limb of WT (top, n = 6) and SYK-Ser544Tyr (bottom, n = 6) mice at the age of 3 months. b, Quantification of arthritis severity by clinical score at the indicated age (n = 7). c, Percentages of mice that were able to hold on to the grid at the indicated time points after the grid was flipped over. The experiment was repeated 15–20 times per mouse (n = 5). d, Representative micro-CT images of the ankle joints of WT (left, n = 6) and SYK-Ser544Tyr (right, n = 6) mice at the age of 3 months. The outlined areas are shown to the right at a higher digital magnification. e, Photograph (left) and quantitative analysis (middle and right) of WT (left in photograph) and SYK-Ser544Tyr (right in photograph) mouse tails at the age of 3 months. WT, n = 6; SYK-Ser544Tyr, n = 9. Mann–Whitney test. f, Micro-CT images of the tails of WT (left, n = 3) and SYK-Ser544Tyr (right, n = 3) mice at the age of 3 months. g, Representative images of immunofluorescence-based analysis of TRAP expression in WT (top) and SYK-Ser544Tyr (bottom) mouse ankle tissue. h, Quantification of the TRAP+ area in WT (top) and SYK-Ser544Tyr (bottom) mouse ankle tissue according to g (n = 3; unpaired t-test). i, Representative TRAP staining of bone marrow-derived macrophages from WT and SYK-Ser544Tyr mice cultured with M-CSF (10 ng ml⁻¹) and RANKL (50 ng ml⁻¹) for 8 days. j, Quantification of counts of in vitro-generated TRAP+ osteoclasts according to i. Mature osteoclasts were identified as multinucleated (>3 nuclei) TRAP+ cells. WT, n = 8; SYK-Ser544Tyr, n = 7. Mann–Whitney test. b, c, Mean ± s.d. e, h, j, Quartiles and median.
role of osteoclasts in erosive arthritis where osteoclast-specific and hematopoietic-specific knockout of Syk resulted in a reduction of bone resorption and increased trabecular bone mass in mice\(^1\). In SYK-Ser544Tyr mouse ankles, we found a marked infiltration of integrin alpha M\(^+\) (CD11b\(^+\)) MNP s that may contribute to the substantial increase in the number of osteoclasts in the joints of the SYK-Ser544Tyr gain-of-function mice and provide a potential mechanism for erosive arthritis. Indeed, CD11b has been identified of integrin alpha M\(^+\) (CD11b\(^+\)) MNP s that may contribute to the substantial increase in the number of osteoclasts in the joints of the SYK-Ser544Tyr gain-of-function mice and provide a potential mechanism for erosive arthritis. Indeed, CD11b has been identified
as an osteoclastogenesis-promoting factor through SYK-dependent signaling in MNPs.16
In addition to SYK's role in MNPs and osteoclasts, we also found that the inflamed joints of SYK-Ser544Tyr mice showed a marked cellular infiltration of macrophages and T lymphocytes, suggesting that these immune cells contribute to disease. The mechanism by which constitutive activation of SYK in macrophages and monocytes leads to erosive arthritis in mice and patients is not clear. Given the function of SYK in signaling from immunoreceptors, integrins and TLRs1,2, it is possible that elevated signaling from TLRs in phagocytes of the joints leads to a feed-forward mechanism whereby elevated inflammatory cytokine production subsequently recruits monocytes to the joint fluid.

The majority of patients with SYK gain-of-function variants had mild and/or intermittent intestinal disease. Similarly, SYK-Ser544Tyr mice did not develop spontaneous intestinal inflammation. These findings would suggest that while active SYK in the intestinal epithelium can prime inflammatory responses, it may not be sufficient to cause colitis. Instead, intestinal dysbiosis combined with SYK hypersensitivity may drive the inflammatory disease as suggested by our human intestinal epithelial cell experiments using MAMPs from both bacterial and fungal cell wall components. Interestingly, we found an extreme expansion of CD4+ Treg17 and T11 and CD8+ effector/memory cells in patient 1 that may reflect and contribute to this patient's severe disease course. Owing to the lack of comparable samples in other patients with gain-of-function variants in SYK, this finding awaits replication. While the original driver of intestinal inflammation remains to be identified, the SYK inhibitor fostamatinib does lead to reduced disease severity of intestinal inflammation in rodent models of colitis9,20, suggesting that it may be a treatment for at least a subset of patients with SYK-driven colitis.

SYK-knockout studies in mice have revealed a critical role of SYK in normal embryonic development14 and function as a pro-survival factor in B cells during pro- to pre-B cell transition and mature B cell generation17. We demonstrate that SYK gain-of-function variants result in reduced B cell levels and dysgammaglobulinemia in mice and humans. Hyperactivation of SYK in murine B cells has been associated with terminal differentiation and apoptosis by suppression of anti-apoptotic BCL-2 and induction of BLIMP110. Importantly in B cell malignancy, SYK hyperactivation, in combination with anti-apoptotic factors such as BCL-215 or MYC18, suggests that SYK may act as a tumor promoter. Indeed, we identified DLBCLs at a comparatively young age in two of the four adult patients (patients 5 and 6) with pathogenic SYK variants. Whereas SYK hyperphosphorylation has been identified as a key therapeutic checkpoint in patients with lymphoma, mutations in SYK are rare and occur in less than 3% of patients with this disease19. However, in DLBCL, CD79B and MYD88 mutations in the B cell receptor and TLR signaling pathways are frequently observed. These mutations drive pro-survival signaling via hyperphosphorylation of SYK19,20. Furthermore, the SYK variant identified in patient 6 (p.Ala353Thr) is present in the Catalogue Of Somatic Mutations In Cancer (COSMIC) database16 (8 counts: 5 large intestine, 2 liver, 1 prostate) and reported in 7 out of 122 tumor cell lines, making it one of the most prevalent tyrosine kinase mutants observed21.

The loss of SYK in myeloid cells (LysMCre; Syk−/−) has also been shown to protect against fungus-induced colorectal cancer19, indicating that the divergent roles of SYK in cancer pathogenesis20 are likely due to cell-specific SYK signaling and potential secondary hits. Overall, these data suggest that patients with suspected SYK gain-of-function variants should be regularly screened for hematologic malignancies.

We showed that treating SYK-Ser544Tyr mice with the SYK inhibitor R406 reduced ankle thickness and clinical arthritis scores. Although fostamatinib failed in an initial trial of patients with rheumatoid arthritis who were not responsive to a biologic agent, a subsequent meta-analysis concluded that fostamatinib was effective21,22. In patients with polygenic forms of T11-17 and T11-driven tissue inflammation, including arthritis12-14, our findings support the pharmacological inhibition of SYK. Our data also point to alternative treatment strategies that target molecules downstream of SYK signaling12-14. Finally, the mouse data presented here indicate that allogeneic haematopoietic stem cell transplantation is a potential treatment option for patients with SYK gain-of-function variants. However, as SYK has known epithelial expression and the SYK variants identified here were shown to result in hyperactivated epithelial SYK signaling, tissue-specific inflammation may not be amenable to transplant.
Overall, our study highlights that the steady-state control of SYK phosphorylation is critical to maintaining immune homeostasis.

Online content
Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41588-021-00803-4.

Received: 25 April 2020; Accepted: 27 January 2021; Published online: 29 March 2021

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Genomics England Research Consortium

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Methods
Study participants/ethics approval. Experiments were carried out with Research Ethics Board approval from the Children’s Hospital of Fudan University, the Hospital for Sick Children, the Hanen Children’s Hospital, the Oxford IBD cohort study (Rare disease subproject), the Ludwig Boltzmann Institute for Rare and Undiagnosed diseases and the Genomics England Research Consortium. Informed consent written to participate in research was obtained from patient/families and controls.

Whole-exome sequencing and variant analysis. Whole-exome sequencing was performed as previously described28,29, with minor changes. Briefly, reads were aligned to GRCh37, variants were called following the GATK best practice pipeline and VEP was used for annotation. Common variants were filtered using maf >0.01 in 1000 Genomes, gnomAD (v.2.1) and dbSNP build 149. Internal cohort frequency was used to remove recurrent variants.

DNA purification from buccal swabs for patients 5 and 6. Genomic DNA was purified from buccal swab samples according to the QIamp DNA Mini Kit (Qiagen) and amplified by PCR (35 cycles) using Phusion High-Fidelity Taq polymerase (Thermo Fisher Scientific).

 Constructs. The p3xFlag-CMV-14 plasmid from Addgene (catalog no. E4901) was used for the expression of SYK variants and purchased from ACGT Canada and validated by sequencing.

Cell culture and transfection. HEK293 cells were cultured at 37 °C in DMEM supplemented with 10% fetal bovine serum (FBS; Gibco), penicillin and streptomycin (100 IU/ml and 100 µg/ml) (Sigma–Aldrich). HEK293 cells were seeded in 24-well plates (luciferase assay) or 12-well plates (immunoblotting) and cultured for 16–24 h before transfection using PolyJet (Polyplus Transfection) and equal amounts of DNA.

PBS was isolated by gradient Ficoll centrifugation (Sigma–Aldrich). For the analysis of SYK and pSYK by immunoblotting, PBMCs were cultured overnight in RPMI-1640 supplemented with 20% FBS.

For ex vivo T cell phenotyping, PBMCs were cultured in RPMI-1640 with 10% human serum (NHS Blood Centre Oxford), 1% sodium-pyruvate, 1% non-essential amino acids (Gibco) and 1% penicillin/streptomycin (Gibco).

Generation of SYK K.Ser550Tyr stably expressing intestinal epithelial cells. SW480 human colon epithelial cells (ATCC) were seeded in 6-well plates and the next day transfected with human WT SYK expression vector pWLZL-NcoI-Flag-SYK, the SYK K.Ser550Tyr derivative, as well as a GFP-carrying vector, to assess transfection, using Lipofectamine 3000 (Thermo Fisher Scientific). At 48 h post-transfection, G418 (Enzo, 1.1 mg/ml) was added to select for stable expression. Resistant cells were maintained in the presence of G418. All cells were grown in RPMI medium, supplemented with 10% FBS, 100 units/ml penicillin G and 100 µg/ml streptomycin (Biological Industries) and maintained in a humidified incubator at 37 °C with 5% CO2.

Luciferase reporter assays. HEK293 cells were transfected with 200 ng of expression plasmid, and 10 ng NF-κB or 100 ng AP-1 reporter plasmid (Promega). To normalize the transfection efficiency, 10 ng pRL-TK Renilla luciferase reporter plasmid was used. After 18 h transfection, cells were stimulated with 20 ng/ml TNF or PMA (InvivoGen), and 2µM SYK inhibitor R406 (InvivoGen) for 16 h before measurement. Cells were collected using passive lysis buffer (Promega) and luciferase assays were carried out using a dual-specific luciferase reporter assay system (Promega) according to the instructions. Each experiment was performed in triplicate.

Immunoblotting (PBMCs and HEK293). Cell lysates were collected after washing with ice-cold PBS and then lysed with ice-cold lysis buffer (150 mM NaCl, 50 mM HEPES, 1% Triton X-100, 10% glycerol, 1.5 mM MgCl2, 10 mM EGTA) supplemented with protease and phosphatase inhibitors (100 mM NaF, 1 mM Na3VO4, 1 mM phenylmethylsulfonyl fluoride, 1:1,000 aprotinin, 1:1,000 leupeptin and 1:1,000 pepstatin). Lysates were separated on 4%–20% SDS–PAGE gels (Bio-Rad Laboratories), and then transferred to polyvinylidene difluoride membranes using the Trans-Blot Turbo Blotting System (Bio-Rad Laboratories). Membranes were blocked with 5% skimmed milk diluted in PBST for 1 h before incubation while shaking in individual primary antibodies overnight at 4 °C. For detection, blots were incubated with anti-mouse or goat anti-rabbit antibodies conjugated with horseradish peroxidase (HRP) for 1 h at room temperature and visualized using Immobilon Forte western HRP substrate (Millipore Sigma) and the Odyssey Fc imaging system (LI-COR) (see the Nature Research Reporting Summary for antibody details).

Cell lysis and western blot analysis of SW480 cells. Confuent cells were solubilized in lysis buffer (50 mM HEPES (pH 7.5), 150 mM NaCl, 10% glycerol, 1% Triton X-100, 1 mM EDTA (pH 8), 1 mM EGTA (pH 8), 1.5 mM MgCl2, 1 mM Na2VO4), and a protease inhibitor cocktail (539131, Calbiochem). Lysates were cleared by centrifugation (21,000 g for 10 min at 4 °C), sample buffer was added, and lysates were boiled for 5 min. Proteins were resolved on 4–20% gradient NuSep Tris-glycine gels and transferred to a nitrocellulose membrane. Membranes were blocked for 1 h in TBST buffer containing 6% non-fat milk and incubated with primary antibodies according to the manufacturer’s instructions: rabbit monoclonal anti-SYK phosphorylated on Tyr 525/526, rabbit polyclonal anti-SYK phosphorylated on Tyr 323, mouse monoclonal anti-SYK, anti-GAPDH, and rabbit polyclonal anti-β-actin. A 1:1,000 dilution of fluorescent secondary antibodies (IRDye 680RD goat anti-mouse and 800CW goat anti-rabbit) was performed at room temperature in TBST. Signals were visualized using an Odyssey imager.

Immunofluorescence analysis on biopsy samples. Intestinal tissue samples from patients and controls were processed as previously described37. Paraffin was removed using xylene and samples were dehydrated in ethanol at graded concentrations. Antigen retrieval was performed using high pressure cooking in 1 mM EDTA buffer at pH 9.0. Tissue sections were blocked for 1 h at room temperature with 5% BSA and 15% goat serum in 1X PBS. Slides were incubated with primary antibody overnight at 4 °C. The next day, the sections were washed three times in PBS for 10 min. Tissue slides were incubated with secondary antibody for 1 h in darkness at room temperature. The following primary antibodies were used: rabbit anti-SYK, anti-pSYK (Tyr 525/526) and anti-β-catenin. Alexa Fluor 488 goat anti-mouse and Alexa Fluor 568 goat anti-rabbit were used as secondary antibodies. Nuclei were stained using Hoechst 33342 fluorescence stain (Thermo Fisher Scientific). Finally, mounting of stained sections was performed using Vectashield mounting medium (Vector Laboratories). Images of stained slides were taken on an Olympus spinning-disk confocal microscope.

Alternatively, tissue sections were collected, dewaxed and subjected to antigen retrieval using a microwave and Target Antigen Retrieval Solution according to the manufacturer’s instructions (Dako). Tissue sections were blocked with 10% (v/v) normal goat serum before being incubated with monoclonal mouse anti-CDB and polyclonal rabbit anti-CDB in combination or mouse anti-CDB8 and polyclonal rabbit anti-pSYK (Tyr 525/526) in combination. Following incubation, primary antibody labeling was detected with goat anti-mouse Alexa Fluor 647 (Life Technologies) and goat anti-rabbit Alexa Fluor 555 (Life Technologies). Nuclei were stained with Hoechst 33342 pentahydrate (bis-benzimide) (Life Technologies) and slides were mounted with glycerol/PBS containing N-propyl galate. Images were collected on an Olympus BX51 microscope.

Immunohistochemistry. Formalin-fixed paraffin-embedded tissues were sectioned at 5µm and collected onto Superfrost glass slides. Tissue sections were dewaxed in xylene and rehydrated through graded alcohol to water. Endogenous peroxidase activity was blocked with 3% (v/v) hydrogen peroxide before masked antigens were retrieved by microwaving in Target Retrieval Solution (Dako). Endogenous avidin and biotin were blocked (Vector Laboratories) and the tissue sections were blocked with 10% (v/v) normal horse serum for mouse primary antibodies or normal goat serum (Sigma–Aldrich) for stained sections performed in rabbit. Tissue sections were incubated overnight at 4 °C in a humidified environment with mouse anti-CDB3, monoclonal mouse anti-CDB8, polyclonal rabbit anti-CDB4 or polyclonal rabbit anti-pSYK (Tyr 525/526).

Following incubation, primary antibody labeling was detected with a biotinylated horse anti-mouse IgG or a biotinylated goat anti–rabbit IgG secondary antibody (Vector Laboratories). Tissue sections were incubated with streptavidin–HRP (Vector Laboratories) and signal was detected using diaminobenzidine (Vector Laboratories). Tissue sections were counterstained with Mayer’s hematoxylin (Sigma–Aldrich) before being dehydrated through graded alcohol to xylene and mounted with DPX and coverslips applied. Images were collected on an Olympus BX51 microscope.

Stimulation of chemokine secretion and quantification by ELISA. Cells were seeded in 12-well plates at a density of 7.5 x 105 (SW480) cells per well. After overnight incubation, the medium was replaced with fresh medium and zymosan (thl-zyn, InvivoGen) or curdlan (C7821 Sigma–Aldrich). To avoid possible contamination with endotoxin, we used endotoxin-free (<0.001 EU) zymosan or curdlan. Where indicated the SYK inhibitors [3-[(1-methyl-1H-indol-3-yl)-methylene]-2-oxo-2,3-dihydro-1H-indole-5-sulfonylamide] (no. 574711, CAS 622387-85-3 Calbiochem, Merck-Killipore) and R406 (InvivoGen) were added 1 h before the addition of zymosan. All treatments were performed in duplicate/triplicate. After 20h, supernatants were collected, and maintained at −70 °C until analysis. IL-8, CCL2, CXCL1 and GM-CSF concentrations were determined using ELISA (DY208, DY271, DY215 and DY275, respectively, from R&D Systems) according to the manufacturer’s instructions.

Ex vivo T cell phenotyping and analysis of cytokine production. Expression of surface markers was analysed by staining for 15 min at room temperature in PBS supplemented with 0.5% (v/v) human serum using the following fluorophore-conjugated antibodies: anti-CD3, anti-αβTCCR, anti-CD4, anti-CD8, anti-CD14, anti-CD19, anti-CD25, anti-CD45RA, anti-CD56, anti-CCR4, NATURE GENETICS | www.nature.com/naturegenetics
anti-CCR6, anti-CCR7 and anti-CCXCR3. For the exclusion of dead cells during the analysis, cells were stained before fixation using Fixable Viability Dye eFluor 780 (eBioscience) according to the manufacturer's instructions.

For ex vivo intracellular cytokine staining, total PBMCs were stimulated for 5 h with PMA (0.2 μM; Sigma–Aldrich) and ionomycin (1 μg ml⁻¹; Sigma–Aldrich) in the presence of brefeldin A (10 μg ml⁻¹; Sigma–Aldrich) for the final 2.5 h of culture.

Cells were fixed and permeabilized with Cytofix/Cytoperm (BD Biosciences) according to the manufacturer's instructions. For the exclusion of dead cells during the analysis, cells were stained before fixation using Fixable Viability Dye eFluor 780 (eBioscience) following the manufacturer's instructions. Fluorochrome-conjugated anti-cytokine antibodies used for analysis include anti-IL-10, anti-IL-13, anti-IL-17A, anti-IL-22, anti-IFN-γ and anti-TNF.

For the analysis of transcription factor expression, unstimulated PBMCs were stained for surface markers and fixed (1 h at room temperature) and permeabilized (1 h at room temperature) using the Transcription Factor Staining Buffer Set (eBioscience). Cells were subsequently stained (1 h at room temperature) for ROSt. Data were acquired on a Becton Dickinson and Company (BD) LSR II or BD LSRFortessa and analyzed using FlowJo v10.7.1 (BD).

For gating strategies, see Supplementary Figs. 1–3. Flow cytometry data were collected using BD FACs Diva software (v8.0.2) on a BD LSR II and analyzed using FlowJo v10.7.1 (BD).

SYK ELISA. At 24–48 h after transfection of equal amounts of plasmid (400 ng), the pSYK (panTyr) Sandwich ELISA Kit (no. 7298, CST) was used to assess SYK phosphorylation. Absorbance at 450 nm was measured using a plate reader ( Molecular Devices, LLC) – 1 min after the addition of stop solution.

Phosphoflow analysis. HEK293 cells were plated in 96-well flat bottom plates at a density of 25,000 cells per well. After 24 h, cells were transfected with the ratio 0.2 μg DNA/0.4 μl Lipofectamine2000 per well in Opti-MEM medium (Gibco). At 24 h post transfection, cells were serum starved and DMEM for 2 h. To exclude dead cells, cells were washed in PBS (Sigma–Aldrich) and stained on ice using the Fixable Viability Dye eFluor 780 (eBioscience) for 15 min. Cells were washed with PBS plus 0.5% BSA (Sigma–Aldrich) and fixed by incubation in 3.7% formaldehyde (Sigma–Aldrich) at 37 °C for 20 min. Permeabilization was performed on ice for 30 min in –20 °C 90% methanol (Merck). Staining was performed for 1 h at room temperature in PBS with 0.5% BSA using the PE-conjugated antibody pSYK (Tyr525/526) and biotinylated SYK antibody. For detection of total SYK, streptavidin–phycoerythrin–Cy7 (PE–Cy7) was used. Flow cytometry data were collected using BD FACs Diva software (v8.0.2) on a BD LSR II and analyzed using FlowJo v10.7.1 (BD).

Structural model. The molecular structure of human SYK was retrieved from the Protein Data Bank (https://www.rcsb.org/) code PDB 4FL2 (ref. 7). Graphics were generated using UCSF Chimera (v1.10).

SYK conservation analysis. The conservation of SYK across species was analyzed using the ConSurfServer6-8 (vConSurf – 2016). For the multiple sequence alignment shown in Extended Data Fig. 2c, the EMBL-EBI Clustal Omega online tool (v1.2.2) was used.

Generation of SYK-Ser544Tyr mouse model. Ser 550 in human SYK is equivalent to mouse SYK Ser 544 (Supplementary Fig. 4). At the serine codon level (Supplementary Fig. 5), a p.Ser544Tyr model was generated by microinjection of Cas9 messenger RNA, single guide RNA (see Supplementary Methods) and single-stranded oligodeoxynucleotides (see Supplementary Methods) into zygotes as described previously15. Two weeks after birth, genomic DNA from the tail of mature osteoclasts were identified as multinucleated (≥3 nuclei) and measured by Dakowei Co. (Shanghai).

Bone marrow transplantation. Mice were irradiated with 7.5 Gy and received donor bone marrow cells (3 × 10⁶) by tail vein injection. X-rayed mice that received no bone marrow cells died in a week.

Measurement of serum and ankle inflammatory factors. Orbital blood was collected and kept at room temperature for 15 min, and then centrifuged at 12,000 × g for 5 min, before serum collection. Inflammatory factors were measured by Dakowe Co. (Shanghai).

Generation of bone marrow-derived dendritic cells and macrophages. Murine bone marrow-derived dendritic cells were generated as described previously17. The medium was changed every other day. Lipopolysaccharide (LPS, 200 ng ml⁻¹ Sigma) was added on day 6 to induce dendritic cell maturation. The SYK inhibitor R406 (Selleck, S1533) was added 30 min before LPS stimulation. Mature dendritic cells were harvested after 6 days. For the exclusion of dead cells during the analysis, cells were fixed in 4% PFA overnight, decalcified in 0.5 M EDTA/PBS for 3 weeks, and then resuspended in wash buffer (1% FBS in PBS) before analysis using a LSRFortessa and FlowJo v10.7.1 (BD).

For gating strategies, see Supplementary Figs. 6 and 7. Flow cytometry data were collected using BD FACs Diva software (v8.0.2) on a BD LSRFortessa and analyzed using FlowJo v10.7.1 (BD).

Assessment of arthritis. Arthritis severity was assessed weekly for 24 weeks from 2 weeks after birth. The severity of arthritis was assessed in each mouse using an established semi-quantitative scoring system of 0–4. Each limb was scored as follows: 0, normal; 1, mild redness and swelling of the ankle, or apparent redness and swelling limited to individual digits, reduced digits of number of affected digits; 2, moderate redness and swelling of the ankle; 3, redness and swelling of the entire paw including digits; 4, maximally inflamed limb with involvement of multiple joints (maximum possible score 16). Arthritis clinical scores were recorded every 2 days using a Vernier caliper. Photographs were taken at weeks and 3 months. To assess articular function, mice were placed on a wire grid that was flipped upside down, and the length of time the mice held on during a 20-s assessment period was recorded. This test was performed 15–20 times at the age of 4 weeks and 3 months.

Pharmacologic inhibition of SYK in vivo. One- and three-month-old SYK-Ser544Tyr mice were treated by intragastric administration with 100 μl R406 (dissolved in 5% dimethylsulfoxide + 95% corn oil) at 2 mg ml⁻¹ twice a day (~10 mg kg⁻¹ per day), while mice in the WT group were treated with 100 μl vehicle (5% dimethylsulfoxide + 95% corn oil) daily. R406 treatment was carried out for 28 days. Arthritis clinical scores were recorded every 2 days using the ankle thickness and the semi-quantitative scoring system described above.

Bone marrow transplantation. Mice were irradiated with 7.5 Gy and received donor bone marrow cells (3 × 10⁶) by tail vein injection. X-rayed mice that received no bone marrow cells died in a week.

Measurement of serum and ankle inflammatory factors. Orbital blood was collected and kept at room temperature for 15 min, and then centrifuged at 12,000 × g for 5 min, before serum collection. Inflammatory factors were measured by Dakowe Co. (Shanghai).

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For gating strategies, see Supplementary Figs. 6 and 7. Flow cytometry data were collected using BD FACs Diva software (v8.0.2) on a BD LSRFortessa and analyzed using FlowJo v10.7.1 (BD).

Ex vivo osteoclast differentiation and TRAP staining. Osteoclasts were differentiated from bone marrow cells as described previously42,44. A TRAP and alkaline phosphate double-staining kit (TaKaRa, MK300) was used to detect TRAP positive multinucleated osteoclasts according to the manufacturer’s protocol. Mature osteoclasts were identified as multinucleated (>3 nuclei) TRAP⁺ cells.

Immunofluorescence of mouse ankle tissue. Ankle of 3-month-old mice were fixed in 4% PFA overnight, decalcified in 0.5 M EDTA/PBS for 3 weeks, dehydrated, paraffin wax embedded and sectioned to 5 μm. ImageJ was used to calculate TRAP⁺ area.
tests as described in the figure legends. P values <0.05 were considered statistically significant. The figures present only statistically significant P values.

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

**Data availability**

The identified SYK variants have been submitted to the ClinVar database ([https://www.ncbi.nlm.nih.gov/clinvar/](https://www.ncbi.nlm.nih.gov/clinvar/)) with the IDs SCV001450452 [c.1649C>A, p.Ser550Tyr], SCV001450453 [c.1649C>T, p.Ser550Phe], SCV001450454 [c.1024C>A, p.Pro342Thr], SCV001450455 [c.1350G>A, p.Met450Le] and SCV001450456 [c.1057G>A, p.Ala353Thr]. The whole-exome sequencing data will not be made publicly available as they contain information that could compromise research participant privacy/confidentiality. Information on the whole-exome sequencing raw data supporting the findings of this study is available from the corresponding authors upon request. Publicly available datasets/databases used in this study include the COSMIC database ([https://cancer.sanger.ac.uk/cosmic](https://cancer.sanger.ac.uk/cosmic)), Kinase.com ([http://www.kinase.com/human/kinome/](http://www.kinase.com/human/kinome/)) and the RCSB Protein Databank (PDB, [https://www.rcsb.org/](https://www.rcsb.org/)). Source data are provided with this paper.

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**Acknowledgements**

We thank the patients, their families and all healthy individuals who actively participated in this study. We thank K. Fiedler for assistance with patient-related materials and H.-B. Shu (Wuhan University, China) for providing plasmids. We acknowledge the contribution to this research through access to the data and findings generated by the 100,000 Genomes Project. The 100,000 Genomes Project is managed by Genomics England Limited (a wholly owned company of the Department of Health). The 100,000 Genomes Project and/or research infrastructure are funded by the National Institute for Health Research, the National Health Service England, the Wellcome Trust, Cancer Research UK and the Medical Research Council. The 100,000 Genomes Project uses data provided by patients and collected by the National Health Service as part of their care and support. We acknowledge the contribution of the Oxford IBD cohort study and the Gastrointestinal Illness Biobank, which are supported by the NIHR Biomedical Research Centre, Oxford. We acknowledge the contribution of the VEOBBD.org Consortium, D.L., Z.Z., X.C. and Liren Wang are funded by the National Key R&D Program of China (2019YFA0110802). Lin, Wang and Y.H. are funded by the Helmhaus Charitable Trust. Lin, Wang and X.C. are supported by a Chinese Government Scholarship. C.K. and D.K. are funded by the German Research Foundation CRC1054 Project A05 and the Care-For-Rare Foundation. H.U.U. and D.A. are supported by the NIHR Biomedical Research Centre, Oxford. Q.L. and D.L. were supported by a Crohn’s and Colitis Canada, Canadian Association of Gastroenterology, and Canadian Institutes of Health Research (CIHR) Fellowships. K.B. is supported by a European Research Council Consolidator Grant (DynChart, ERC grant agreement number 820074). B.G. receives support through the DFG SFB1160/2 B5, under Germany’s Excellence Strategy (CIBSS – EXC-2189 – Project ID 390939844, and RESIST – EXC-2155 – Project ID 390874248); through the E-rease program of the EU, managed by the DFG, grant code GR1617/4-1/1-PID; and through the ’Netzwerke Seltenes Erkrankungen’ of the German Ministry of Education and Research (BMBF), grant code GAIN_01GM1910A. WH.A.K was supported by operating grants from the CIHR (PJ1-15695 and PJ1-153168). S.H., K.R.E. and H.H.G were funded by the Wellcome Trust (207556_Z_17_Z). A.M.M., C.K., S.B.S., H.U.U., I.D. and S.C.N. are funded by the Leona M. and Harry B. Helmsley Charitable Trust. A.M.M., C.K. and S.B.S. are funded by an NIH (RC2DK122532) grant. A.M.M. is funded by a Canada Research Chair (Tier 1) in Pediatric IBD, a CIHR Foundation grant and NIDDK NIH (RC2DK118640). D.L., Z.Z., X.C. and Liren Wang are funded by the National Key R&D Program of China (2019YFA0110802) and National Natural Science Foundation of China (no. 32025023).

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**Competing interests**

The authors declare no competing interests.

**Additional information**

Extended data is available for this paper at [https://doi.org/10.1038/s41588-021-00803-4](https://doi.org/10.1038/s41588-021-00803-4).

**Supplementary information**

The online version contains supplementary material available at [https://doi.org/10.1038/s41588-021-00803-4](https://doi.org/10.1038/s41588-021-00803-4).

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**Peer review information**

Nature Genetics thanks Iwona Aksentijevich, Geert van Loo and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

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Extended Data Fig. 1  | Genetic analysis pipeline and validation of heterozygous variants by sanger sequencing. Variant identification pipeline and Sanger sequencing validation of WES data for (a) Patient 1 (p.S550Y), (b) Patient 2 (p.S550F) and Patient 3 (p.S550F) and (c) Patient 4 (p.P342T). (d) Chronologic disease course and Sanger sequencing validation for Patient 5 (p.M450I) and (e) Patient 6 (p.A353T).
Extended Data Fig. 2 | Patient 4 phenotype summary and SYK variants conservation analyses. **a**, Patient 4's laboratory tests revealed normal white blood cell (WBC) counts. **b**, Cervical and thoracic spine MRI of Patient 4 taken at 26 years of age showing a signal enhancing intramedullary space-occupying lesion through nearly the entire length of the cervical and thoracic spinal cord. One representative image out of 6 acquired images is shown. All images revealed signs of inflammation. **c**, Spinal cord biopsy with prominent granulomatous inflammation (clinical data, n = 1). **d**, Species conservation of SYK amino acids p.p342, p.A353, p.M450, and p.S550. **e**, Summary of the residue variety in % for each identified variant in SYK across 124 homologues across species following ClustalW-based multiple sequence alignment using the ConSurf server (see Methods). Human wild-type SYK residues are indicated in blue and human SYK variants in red.
Extended Data Fig. 3 | SYK hyperphosphorylation in human epithelial cells. **a,** SYK hyper-phosphorylation in intestinal tissue from Patient 1 compared to healthy controls. Double immunostaining of normal and p.S550Y SYK variant colon biopsy sections for pSYK (red), β-catenin (green) and merged dual labeling (yellow). The normal case demonstrated a distinctive glandular apical expression of pSYK. The fine apical signal almost reaches to the microvilli. Scattered infiltrated cells show pSYK staining in lamina propria. Unstained patches of glandular epithelium represent goblet cells. Immunostaining of β-catenin as a structural membrane marker indicates an organized glandular architecture in the normal colon section. Staining for pSYK was evident at the glandular epithelial base (membrane and cytoplasm) in colon sections of the patient. The β-catenin labeling in patient 1 intestinal biopsies presented a disorganized glandular architecture compared to the normal control. One representative image is shown out of 3 total images acquired. **b,** Double immunostaining of pSYK and the myeloid marker CD68 in rectal and duodenal biopsy sections of Patient 1 illustrating strong pSYK expression in intestinal epithelial cells with moderate overlap with CD68+ myeloid cells. One representative image is shown out of 6 total images acquired from rectal biopsies and 4 images acquired from duodenal biopsies.
Extended Data Fig. 4 | See next page for caption.
Extended Data Fig. 4 | Comparative assays of human SYK variants and reduction of SYK phosphorylation by R406 SYK inhibitor treatment. a, Flow cytometry comparison of percent phosphorylated SYK (Y525/S526) normalised to ectopic SYK expression in HEK293 cells. b, Non-normalised data presentation of percent phosphorylated SYK (Y525/S526). c, Percent ectopic SYK expression in HEK293 cells following transfection. d, Relative mean fluorescence intensity (rMFI) of phosphorylated SYK(Y525/S526) normalised to ectopic SYK expression. e, Non-normalised rMFI of phosphorylated SYK(Y525/S526) and f, rMFI of ectopic SYK (a-f: the dotted line indicates WT median; a-c: quartiles and median; d-f: median and interquartile range and minimum to maximum range; n of independent experiments/n of cell culture replicates: eV(8/30), WT(8/24), p.S550Y(8/28), p.S550F(6/21), p.M450I(8/28), p.A353T(8/30), p.P342T(8/30), p.Y323F(3/12); Mann-Whitney test). g, Western blot analysis of SYK phosphorylation (Y525/S526), total SYK expression, JNK phosphorylation (T183/Y185), total JNK expression and GAPDH expression in HEK293 cells after transfection with control, p.Y525F/Y526F mutated SYK, wild-type SYK and the range of different identified variants in SYK (p.S550Y, p.S550F, p.A353T, p.P342T and p.M450I). h, Quantification of pJNK normalised to total JNK according to (g) quartiles and median; n of independent experiments: WT(8), p.Y525/S526 F(8) p.S550Y(8), p.S550F(8), p.M450I(3), p.A353T(3), p.P342T(6); Mann-Whitney test.). i, Analysis of AP-1 (n = 4) or NF-κB (n = 3) activity by luciferase reporter assay in HEK293 cells following co-transfection with reporter plasmids and empty vector (Control). WT SYK or p.S550Y SYK and 24 hours stimulation with TNF (20 ng/ml) or PMA (20 ng/ml) (Min-max and median; unpaired t-test). j, Western blot analysis of SYK phosphorylation (Y525/S526), total SYK expression, JNK phosphorylation (T183/Y185), total JNK expression, ERK phosphorylation (T202/Y204), total ERK expression and GAPDH expression in HEK293 cells after transfection with wild-type SYK, p.S550Y SYK and p.S550F SYK in presence or absence of R406 SYK inhibitor (2 μM). k, Quantification of pSYK (Y525/S526) (n = 7), pJNK (T183/Y185) (n = 6), pERK (T202/Y204) (n = 6) western blot signals normalised to the respective total protein expression according to (j) quartiles and median; Mann-Whitney test). Representative western blot panels have been assembled from individual gels. Raw data are presented in the respective source data.
Extended Data Fig. 5 | SYK response to stimulation in intestinal epithelial cells. **a**, Western blot analysis of SYK and pSYK (Y525/526) in SW480 cells transfected with plasmid expressing p.S550Y SYK or WT SYK. **b**, Time-course of SYK phosphorylation upon zymosan (200 µg/mL) stimulation by western blot analysis of SYK and pSYK (Y525/526) in SW480 cells for the indicated time (NT = untreated cells). **c**, Western blot analysis of SYK, pSYK (Y525/526) and pSYK (323) in SW480 cells transfected with plasmid expressing p.S550Y SYK or WT SYK left either untreated (-) or stimulated with 20 µg/mL or 100 µg/mL zymosan. **d**, Western blot analysis of SYK and pSYK (Y525/526) SW480 cells transfected with plasmid expressing p.S550Y SYK or WT SYK left either untreated (NT) or stimulated with curdlan (100 µg/mL) for the indicated time. **e, f**, Western blot analysis of SYK and pSYK (Y525/526) in SW480 or in SW480 cells transfected with plasmid expressing p.S550Y SYK, upon stimulation with zymosan (200 µg/mL) for 1 hour, in the presence or absence of SYK inhibitor (5 µM, Calbiochem, #574711) added 1 hour prior to zymosan stimulation. **g**, IL-8, CXCL1, GM-CSF and CCL2 secretion by 20 hours zymosan-stimulated SW480 cells expressing WT SYK or p.S550Y SYK. SYK inhibitor (5µM, Calbiochem, #574711) was added 1 hour prior to zymosan stimulation and analysis performed by ELISA. Median and interquartile range and minimum to maximum range; n of independent experiments/ n of cell culture replicates: iL-8: (3/3-10); CXCL1, GM-CSF, CCL2: (2-3/2-7); Mann-Whitney test. **h**, IL-8, CXCL1, GM-CSF and CCL2 secretion by SW480 cells expressing WT SYK or p.S550Y SYK cells incubated with/without r406 (5 µM) for 60 mins prior to the addition of curdlan and analyzed by ELISA 20 hours following stimulation. Median and interquartile range and minimum to maximum range; n of independent experiments/n of cell culture replicates: IL-8: (3/3-10); CXCL1, GM-CSF, CCL2: (2-3/2-7); Mann-Whitney test. Representative western blot panels have been assembled from individual gels. Raw data are presented in the respective source data.
Extended Data Fig. 6 | See next page for caption.
Extended Data Fig. 6 | Phenotypic and functional analysis of Patient 1T cells. a, Dot plot presentation of CD4⁺, CD8⁺ and double negative (DN) T cell frequencies among CD3⁺αβTCR⁺ cells as determined by surface staining and flow cytometry of PBMC from Patient 1 at 2 years of age and a 3 year-old healthy donor (HD). b, Kinetics of CD4/CD8 ratio based on clinical laboratory measurements. c, CD4/CD8 ratio as estimated from patient intestinal biopsy sections stained for CD3 and CD8 by immunohistochemistry. d, Intraepithelial CD8⁺ T cell counts in patient intestinal tissue sections manually counted based on CD4 and CD8 immunofluorescence staining. e, Dot plot presentation of CD3⁺αβTCR⁺CD8⁺CD25⁻ naïve and memory T cell frequencies as assessed by surface staining of CD45RA and CCR7. f, Dot plot presentation of TNF and IFN-γ producing CD3⁺αβTCR⁺CD8⁺CD25⁻ memory T cell frequencies as assessed by intracellular cytokine staining. g, CD45RA and CCr7 naïve and memory T cell frequencies as assessed by surface staining of CD45RA and CCR7 and flow cytometry analysis. h, Dot plot presentation of CD3⁺αβTCR⁺CD4⁺CD25⁻ naïve and memory T cell frequencies as assessed by combined surface and intracellular staining. i, Presentation of IL-17A, IL-22 and IFN-γ producing CD3⁺CD4⁺CD25⁻ memory T cell frequencies. j, Summary of IL-17A-, IL-22- and IFN-γ-producing CD3⁺CD4⁺CD25⁻ memory T cell frequencies as assessed by intracellular cytokine staining following 5 hrs PMA (0.2 μM) and ionomycin (1 μg/mL) stimulation. Grey areas indicate healthy donor control ranges.
Extended Data Fig. 7 | Phenotypic and functional analysis of Patient 1T cells. a, Summary of CD3^+CD8^+ T cell frequencies of adult healthy donors (HD) (HD adult: n = 26) and 9 months to 14 years old HD (HD 9 months to 14 years: n = 17) and of Patient 1 at 1.5 years and 2 years of age (n = 2, each 3 technical replicates) expressed as percent of live CD3^+ T cells. b, Summary of CD3^+CD8^+CD25^- memory T cell frequencies as assessed by surface staining of CD45RA and CCr7. c,d, Summary of IFN-γ- (c) and TNF-producing (d) CD3^+CD8^+CD25^- memory T cell frequencies as assessed by intracellular cytokine staining following 5 hours PMA (0.2μM) and ionomycin (1μg/mL) stimulation. e, Summary of CD3^+CD4^+CD25^- memory T cell frequencies as assessed by surface staining of CD45RA and CCr7. f, Summary of CD3^+CD4^+CD25^-CCR6^- memory T cell frequencies as assessed by surface staining and flow cytometry. g, Summary of CD3^+CD4^+CD25^-RORyt^+ memory T cell frequencies as assessed by combined surface and intracellular staining. h,j, Summary of TNF- (h), IL-10- (i) and IL-13-producing (j) CD3^+CD4^+CD25^- memory T cell frequencies as assessed by intracellular cytokine staining following 5 hours PMA (0.2μM) and ionomycin (1μg/mL) stimulation. k, CCr6^+CCR4^+CXCR3^- (Th17-enriched) and CCr6^+CXCR3^- CCR4^- (Th1/Th17-enriched) CD3^+CD4^+CD25^- memory T cell frequencies as assessed by surface staining and flow cytometry.
Extended Data Fig. 8 | See next page for caption.
Extended Data Fig. 8 | Phenotypic and functional characterization of SYK<sup>S544Y</sup> mice. a, Immunoblot analysis of SYK and pSYK protein levels in ankle of 3 months old mice (wt: n = 3; SYK<sup>S544Y</sup>: n = 3). b, Hematoxylin and eosin stain of tail tissue sections from 3 months old wild-type and SYK<sup>S544Y</sup> mice showing bone erosion and immune cell infiltration (wt: n = 3; SYK<sup>S544Y</sup>: n = 3). c, Hyper-phosphorylation of SYK in intestinal tissue from SYK<sup>S544Y</sup> mice compared to wild-type mice (wt: n = 3; SYK<sup>S544Y</sup>: n = 3). d, Western blot analysis of wild-type and SYK<sup>S544Y</sup> bone marrow derived dendritic cells SYK phosphorylation (Y519/S520), total SYK expression, JNK1 (T183/Y185) phosphorylation, total JNK1 expression, ERK1 phosphorylation (T203/Y205) and total ERK1 expression treated or not treated with R406 SYK inhibitor (2 μM, R406 was added 30 minutes prior to LPS (200 ng/mL) stimulation and samples collected after 24 hours) (wt: n = 3; SYK<sup>S544Y</sup>: n = 3). e, Analysis of IgG and IgM in serum from wild-type and SYK<sup>S544Y</sup> mice by ELISA at indicated age (n = 3; Unpaired t-test). f, RT-qPCR analysis of Il17f (wt: n = 3; SYK<sup>S544Y</sup>: n = 6), Csf2 (wt: n = 3; SYK<sup>S544Y</sup>: n = 9), Ifng (wt: n = 3; SYK<sup>S544Y</sup>: n = 3), Il17a (wt: n = 6; SYK<sup>S544Y</sup>: n = 6) and Il4 (wt: n = 3; SYK<sup>S544Y</sup>: n = 3) expression in blood at the age of 3 months. Unpaired t-test. g, Serum cytokine concentrations in wild-type and SYK<sup>S544Y</sup> mice at 3 months of age measured by ELISA (n = 4; unpaired t-test). Violin plots indicate quartiles and median. Representative western blot panels have been assembled from individual gels. Raw data are presented in the respective source data.
Extended Data Fig. 9 | See next page for caption.
Extended Data Fig. 9 | Phenotype of immune cells in circulation and tissue of a CRISPR-Cas9-knock-in SYK<sup>S544Y</sup> mouse model. 

**a.** Frequencies of live CD45<sup>+</sup> cells in blood (top) and ankles (bottom) of wild-type and SYK<sup>S544Y</sup> mice (blood: n = 4, ankle: n = 3; unpaired t-test) at the age of 3 months. 

**b-f.** Frequencies and normalised counts of B220<sup>+</sup> B cells, CD3<sup>+</sup> T cells, CD4<sup>+</sup> T cells, Foxp3<sup>+</sup>CD25<sup>+</sup> regulatory T cells (T reg), CD11b<sup>+</sup>F4/80<sup>+</sup> mononuclear phagocytes, CD11b<sup>+</sup>F4/80<sup>-</sup> mononuclear phagocytes and CD11b<sup>+</sup>F4/80<sup>+</sup> macrophages (M<sub>ϕ</sub>) in blood (top) and ankles (bottom) of wild-type and SYK<sup>S544Y</sup> mice at the age of 3 months (blood: n = 4, ankle: n = 3; T reg-blood: n = 3, T reg-ankle (wt): n = 2, T reg-ankle (SYK<sup>S544Y</sup>): n = 4; unpaired t-test). Normalised counts were calculated as counts/1*10<sup>5</sup> live CD45<sup>+</sup> cells relative to the average change in live CD45<sup>+</sup> cells comparing wild-type and SYK<sup>S544Y</sup> mice. Violin plots indicate quartiles and median.
Extended Data Fig. 10 | Full blood counts of wild-type and CRISPR-Cas9-knock-in SYK<sup>S544Y</sup> mice. Full blood counts of wild-type (n = 5) and SYK<sup>S544Y</sup> (n = 6) mice; Mann-Whitney test. Box plots and whiskers indicate median and interquartile range and minimum to maximum range. MCV: mean corpuscular volume; MCH: mean corpuscular haemoglobin; MCHC: mean corpuscular haemoglobin concentration; MPV: mean platelet volume.
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Software and code

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Data collection

Flow Cytometry:
Flow Cytometry Data were collected using BD FACS Diva software (v8.0.2) on a BD LSRII or BD LSRFortessa.

Micro-CT:
Micro-CT (Skyscan1272)
NRecon software (v1.7.0.4)
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Data analysis

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Flow cytometry data were analysed with FlowJo (Tree Star), version 10.7.1 or later.

Statistical analysis:
Statistical analyses were run on GraphPad Prism 8 or Microsoft Excel for Mac (v15.32).

Structural model and conservation analysis:
UCSF Chimera (v1.10)
ConSurf Server: (https://consurf.tau.ac.il/); version: ConSurf - 2016
EMBL-EBI Clustal Omega online tool: (https://www.ebi.ac.uk/Tools/msa/clustalo/); version: 1.2.2

Exome Sequencing Analysis:
Variant calling was performed using the TCAG pipeline and the Hg19/GRCh37 reference genome. Variant annotation and filtering was conducted using the VarSeq Suite version 2.1 (Golden Helix) analysis package.

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The identified SYK variants are submitted to the ClinVar database (https://www.ncbi.nlm.nih.gov/clinvar/) with IDs, SCV001450452 [c.1649C>A, p.S550Y], SCV001450453 [c:1649C>T, p.S550F], SCV001450454 [c.1024C>A, p.P342T], SCV001450455 [c.1350G>A, p.M450I], and SCV001450456 [c.1057G>A, p.A353T]. The whole exome sequencing data will not be made publicly available as they contain information that could compromise research participant privacy/consent. Information on the whole-exome sequencing raw data supporting the findings of this study is available from the corresponding authors HHU, YH, KB, and AMM upon request. Publicly available datasets/databases used in this study include the Catalogue Of Somatic Mutations In Cancer (COSMIC) database (https://cancer.sanger.ac.uk/cosmic), Kinase.com (http://www.kinase.com/human/kinome/) and the RCSB Protein Databank (PDB, https://www.rcsb.org/).

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  - Sample size was not predetermined by statistical methods. For rare patient samples the number of experiments performed reflects the sample availability. Experiments that were not limited by sample availability were replicated at least 3 independent times.

- Data exclusions
  - No data were excluded from analyses.

- Replication
  - Experiments were repeated at least 3 times independently. In the case of rare patient samples we aimed to present data from at least 3 technical replicates. Biological and technical replicates are described in figure legends.

- Randomization
  - Randomization was not applied. Where possible, age matched individuals were compared.

- Blinding
  - Data collection and analyses of samples from healthy donors and patients was performed at the same time, pair-matched and not blinded. Blinding was not possible because of the need to compare patients and controls.

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| □ | Human research participants | □ | |
| □ | Clinical data | □ | |

Antibodies

Antibodies used

- Immunofluorescence (# Cat. Number and/or clone; company/dilution):
  - anti-SYK (#13198; CST/1:100)
  - anti-phospho SYK (Y525/526) (#2710/C87C1; CST/1:100)
  - anti-β-catenin (#610154; BD Transduction Labs/1:200)
  - anti-CD8 (C8/144B; Biolegend/1:50)
  - anti-CD4 (EP66855; AbCam/1:100)
anti-CD68 (PG-M1; Dako/1:100)
anti-TRAP (32694; polyclonal; SAB/1:200)

Immunohistochemistry (# Cat. Number and/or clone; company/dilution):
- anti-CD3 (NCL-L-CD3-565; Novocastra/1:50)
- anti-CD8 antibody (Clone C8/144B; Biolegend/1:50)
- anti-CD4 (EPR6855; AbCam/1:100)
- anti-pSYK (Tyr 525/526) (#2710/C87C1; CST/1:100)
- anti-pSYK (Tyr 323) (#2715, CST/1:100)
- Biotinylated Horse anti-Mouse IgG (BA-1400-2.1; Vector Laboratories/1:200)
- Biotinylated Goat anti-Rabbit IgG (BA-1300-2.2; Vector Laboratories/1:200)

Immunoblotting (# Cat. Number and/or clone; company/dilution):
- anti-SYK (#13198; CST/1:1000)
- anti-phospho-SYK (Y525/526) (#2710; CST/1:1000)
- anti-pSYK (Tyr 323) (#2715, CST/1:1000)
- anti-JNK (#9252; CST/1:1000)
- anti-phospho-JNK (T183/Y185) (#9251; CST/1:1000)

Flow cytometry:

Human Surface antigens (company; clone; fluorochromes used/dilution):
- anti-CD3 (BD Biosciences; UCHT1; Bv711/1:100; PE-Cy5/1:100)
  [Online resource](http://www.bdbiosciences.com/us/applications/research/t-cell-immunology/th-1-cells/surface-markers/human/bv711-mouse-anti-human-cd3-ucht1-also-known-as-ucht-1-ucht-1/p/563725)
- anti-CD4 (Biolegend; RPA-T4; Bv510/1:50, Bv605/1:50)
  [Online resource](https://www.biolegend.com/de-de/products/brilliant-violet-510-anti-human-cd4-antibody-9598)
- anti-CD8 (Biolegend; SK1; AF700/1:100)
  [Online resource](https://www.biolegend.com/de-de/products/alexa-fluor-700-anti-human-cd8-antibody-9062)
- anti-CD8 (BD Biosciences; R-PA8-T; PE-CF594/1:100)
  [Online resource](http://www.bdbiosciences.com/us/reagents/research/antibodies-buffers/immunochemistry-reagents/anti-human-antibodies/cellsurface-antigens/pe-cf594-mouse-anti-human-cd8-rpa-8t/p/562282)
- anti-CD14 (Biolegend; M5E2; Bv650/1:200)
  [Online resource](https://www.biolegend.com/de-de/products/brilliant-violet-650-anti-human-cd14-antibody-7819)
- anti-CD16 (Biolegend; 3G8; PE-Cy7/1:300, Bv510/1:300)
  [Online resource](https://www.biolegend.com/de-de/products/pe-cy7-anti-human-cd16-antibody-1905)
- anti-CD19 (BD Biosciences; SJ25C1; Bv711/1:100, PE-Cy5/1:100)
  [Online resource](http://www.bdbiosciences.com/us/applications/research/clinical-research/oncology-research/blood-cell-disorders/surface-markers/human/bv711-mouse-anti-human-cd19-sj25c1-also-known-as-sj25-c1/p/563036)
- anti-CD19 (BD Biosciences; HIB19; Bv711/1:100)
  [Online resource](http://www.bdbiosciences.com/us/reagents/research/antibodies-buffers/immunochemistry-reagents/anti-human-antibodies/cellsurface-antigens/bv711-mouse-anti-human-cd19-hib19/p/740774)
- anti-CD25 (Biolegend; M-A251; PE, Bv785/1:10)
  [Online resource](https://www.biolegend.com/de-de/products/pe-anti-human-cd25-antibody-8389)
- anti-CD45RA (Biolegend; HI100; Bv650/1:200)
  [Online resource](https://www.biolegend.com/de-de/products/brilliant-violet-650-anti-human-cd45ra-antibody-7662)
- anti-CD56 (BD Biosciences; NCAM16.2; Bv510/1:50)
  [Online resource](http://www.bdbiosciences.com/us/reagents/research/antibodies-buffers/immunochemistry-reagents/anti-human-antibodies/cellsurface-antigens/bv510-mouse-anti-human-cd56-ncam162-also-known-as-ncam-16/p/563041)
- anti-CD56 (Biolegend; MEM-188; PE-Cy5/1:50)
  [Online resource](https://www.biolegend.com/de-de/products/pe-cy5-anti-human-cd56-nacam-antibody-1606)
- anti-CCR4 (BD Biosciences; 1G1; PE-Cy7/1:100)
  [Online resource](http://www.bdbiosciences.com/us/applications/research/t-cell-immunology/th-2-cells/surface-markers/human/pe-cy7-mouseanticd194-1g1/p/561034)
- anti-CCR6 (Biolegend; G034E3; Bv605/1:100)
  [Online resource](https://www.biolegend.com/en-gb/products/brilliant-violet-421-anti-human-cd196-ccr6-antibody-7547)
- anti-CCR7 (Biolegend; G043H7; Bv421/1:100; Bv711)
  [Online resource](https://www.biolegend.com/en-gb/products/brilliant-violet-421-anti-human-cd197-ccr7-antibody-7497)
  [Online resource](https://www.biolegend.com/en-gb/products/brilliant-violet-711-anti-human-cd197-ccr7-antibody-7948)
Human Intracellular antigens (company; clone; fluorochromes used/dilution):

- **anti-CXCR3** (BD Biosciences; 1C6; PE-Cy5/1:30)
  [https://www.bdbiosciences.com/us/applications/research/t-cell-immunology/th-1-cells/surface-markers/human/pe-cy5-mouseanti-human-cd183-1c6cxcr3-also-known-as-1c6-is177-1c6/p/551128](https://www.bdbiosciences.com/us/applications/research/t-cell-immunology/th-1-cells/surface-markers/human/pe-cy5-mouseanti-human-cd183-1c6cxcr3-also-known-as-1c6-is177-1c6/p/551128)

- **anti-IL-4** (Biolegend; MP4-25D2; BV605/1:50)
  [https://www.biolegend.com/de-de/products/brilliant-violet-421-anti-human-il-4-antibody-7224](https://www.biolegend.com/de-de/products/brilliant-violet-421-anti-human-il-4-antibody-7224)

- **anti-IL-6** (eBioscience; MQ2-13A5; AF700/1:30)
  [https://www.thermofisher.com/antibody/product/IL-6-Antibody-clone-MQ2-13A5-Monoclonal/56-7069-42](https://www.thermofisher.com/antibody/product/IL-6-Antibody-clone-MQ2-13A5-Monoclonal/56-7069-42)

- **anti-IL-10** (Biolegend; JES3-9D7; PE-Cy7/1:30)
  [https://www.biolegend.com/de-de/products/pe-cy7-anti-human-il-10-antibody-6113](https://www.biolegend.com/de-de/products/pe-cy7-anti-human-il-10-antibody-6113)

- **anti-IL-13** (eBioscience: 85BRD; FITC/1:50)
  [https://www.thermofisher.com/antibody/product/IL-13-Antibody-clone-85BRD-Monoclonal/11-7319-82](https://www.thermofisher.com/antibody/product/IL-13-Antibody-clone-85BRD-Monoclonal/11-7319-82)

- **anti-IFN-γ** (eBioscience; 4S.B3; FITC, eF450/1:100)
  [https://www.thermofisher.com/antibody/product/IFN-gamma-Antibody-clone-4S-B3-Monoclonal/11-7319-82](https://www.thermofisher.com/antibody/product/IFN-gamma-Antibody-clone-4S-B3-Monoclonal/11-7319-82)

- **anti-TNF** (Biolegend; MAb11; BV605/1:150)
  [https://www.biolegend.com/de-de/products/brilliant-violet-605-anti-human-tnf-alpha-antibody-7679](https://www.biolegend.com/de-de/products/brilliant-violet-605-anti-human-tnf-alpha-antibody-7679)

- **anti-ROGt** (BD Biosciences; Q21-559; PE/1:10)
  [https://www.bdbiosciences.com/us/applications/research/t-cell-immunology/th17-cells/intracellular-markers/cell-signallingand-transcription-factors/human/pe-mouse-anti-human-rot-q21-559/p/563081](https://www.bdbiosciences.com/us/applications/research/t-cell-immunology/th17-cells/intracellular-markers/cell-signallingand-transcription-factors/human/pe-mouse-anti-human-rot-q21-559/p/563081)

- **anti-pSYK** (CST; C87C1; PE/1:50)
  [https://www.cellsignal.co.uk/products/antibody-conjugates/phospho-syk-tyr525-526-c87c1-rabbit-mab-pe-conjugate/6485?site-search-type=Products](https://www.cellsignal.co.uk/products/antibody-conjugates/phospho-syk-tyr525-526-c87c1-rabbit-mab-pe-conjugate/6485?site-search-type=Products)

- **anti-SYK** (CST; D3Z1E; Biotinylated/1:500)
  [https://www.cellsignal.co.uk/products/antibody-conjugates/syk-d3z1e-xp-rabbit-mab-biotinylated/97818?_=1587142297864&Ntt=SYK&tahead=true](https://www.cellsignal.co.uk/products/antibody-conjugates/syk-d3z1e-xp-rabbit-mab-biotinylated/97818?_=1587142297864&Ntt=SYK&tahead=true)

**Mouse surface antigens (company; clone):**

- **anti-CD45** (BioLegend; 104; PerCP-Cy5.5)
  [https://www.biolegend.com/en-us/search-results/percp-cyanine5-5-anti-mouse-cd452-antibody-4271](https://www.biolegend.com/en-us/search-results/percp-cyanine5-5-anti-mouse-cd452-antibody-4271)

- **anti-CD3** (BioLegend; 17A2; APC-Cy7)
  [https://www.biolegend.com/en-us/products/apc-cyanine7-anti-mouse-cd3-antibody-6068](https://www.biolegend.com/en-us/products/apc-cyanine7-anti-mouse-cd3-antibody-6068)

- **anti-CD4** (BioLegend; GK1.5; FITC, PE)
  [https://www.biolegend.com/en-gb/products/fitc-anti-mouse-cd4-antibody-248](https://www.biolegend.com/en-gb/products/fitc-anti-mouse-cd4-antibody-248)

- **anti-CD25** (BioLegend; 3C7; FITC)
  [https://www.biolegend.com/en-gb/products/fitc-anti-mouse-cd25-antibody-4511](https://www.biolegend.com/en-gb/products/fitc-anti-mouse-cd25-antibody-4511)

- **anti-B220** (BioLegend; RA3-6B2; FITC, PE, PE-Cy7)
  [https://www.biolegend.com/en-gb/products/pe-anti-mouse-human-cd45r-b220-ab conjugate/447](https://www.biolegend.com/en-gb/products/pe-anti-mouse-human-cd45r-b220-ab conjugate/447)

- **anti-CD11c** (BioLegend; N418; APC)
  [https://www.biolegend.com/en-gb/products/apc-anti-mouse-cd11c-antibody-1813](https://www.biolegend.com/en-gb/products/apc-anti-mouse-cd11c-antibody-1813)

- **anti-MHCII** (BioLegend; M5/114.15.2; PE-Cy7)
  [https://www.biolegend.com/en-gb/products/pe-cy7-anti-mouse-i-a-i-e-antibody-6136](https://www.biolegend.com/en-gb/products/pe-cy7-anti-mouse-i-a-i-e-antibody-6136)

- **anti-CD11b** (BioLegend; M1/70; BV510; FITC)
  [https://www.biolegend.com/en-gb/products/brilliant-violet-510-anti-mouse-human-cd11b-antibody-7993](https://www.biolegend.com/en-gb/products/brilliant-violet-510-anti-mouse-human-cd11b-antibody-7993)

- **anti-F4/80** (BioLegend; BM8; APC)
  [https://www.biolegend.com/en-gb/products/apc-anti-mouse-f4-80-antibody-4071](https://www.biolegend.com/en-gb/products/apc-anti-mouse-f4-80-antibody-4071)

- **anti-LY-6C** (BioLegend; HK1.4; AF488)
  [https://www.biolegend.com/en-gb/products/alexa-fluor-488-anti-mouse-ly-6c-antibody-6756](https://www.biolegend.com/en-gb/products/alexa-fluor-488-anti-mouse-ly-6c-antibody-6756)

- **anti-FOXP3** (Invitrogen; FJK1-6s; eF660)
  [https://www.thermofisher.com/antibody/product/FOXP3-Antibody-clone-FJK-16s-Monoclonal/50-5773-82?imageId=92387](https://www.thermofisher.com/antibody/product/FOXP3-Antibody-clone-FJK-16s-Monoclonal/50-5773-82?imageId=92387)
All antibodies used in this study have been validated by the manufacturer. All flow cytometry antibodies were titrated for optimal detection and positive/negative gating was defined using isotype controls, fluorescence-minus-one controls and unstimulated cells in the case of inducible protein expression.

Antibody; Validated Application; Validated Species; Validation Statement

anti-B220; F; H, M, F; Clone RA3-6B2 has been described to react with an epitope on the extracellular domain of the transmembrane CD45 glycoprotein which is dependent upon the expression of exon A and specific carbohydrate residues.

anti-CCR4; F; H; The 1G1 monoclonal antibody specifically binds to CD194, also known as the human CC Chemokine Receptor type 4 (CCR4).

anti-CCR6; F; H, Ag, Bb, Cb Rh; Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis. For flow cytometric staining, the suggested use of this reagent is 5 μg per million cells in 100 μl staining volume or 5 μl per 100 μl of whole blood.

anti-CCR7; F; H; Ag, Bb, Cn Rh; Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis. For flow cytometric staining, the suggested use of this reagent is 5 μl per million cells in 100 μl staining volume or 5 μl per 100 μl of whole blood.

anti-CD11b; F; H, M, Mk, Rb; Clone M1/70 has been verified for immunocytochemistry and frozen immunohistochemistry.

anti-CD11c; F; M; Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis.

anti-CD14; F; H, Ag, CM, B, Ch, Mt, Dg, Rh, PM, SM; Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis. For flow cytometric staining, the suggested use of this reagent is 5 μl per million cells in 100 μl staining volume or 5 μl per 100 μl of whole blood.

anti-CD16; F, IHC, CyT, IP; H; Ag, Bb CM, Ch, Cn, Mt, PM, Rh, SM; Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis. For flow cytometric staining, the suggested use of this reagent is 2.0 μg per million cells in 100 μl volume. It is recommended that the reagent be titrated for optimal performance for each application.

anti-CD19; F; H; BV711 is a tandem fluorochrome of BD Horizon BV421 and an acceptor dye with an emission maximum at 711 nm. This dye offers a very bright choice for the violet laser. Due to the excitation and emission characteristics of the acceptor dye, there may be moderate spillover into the Alexa Fluor® 700 and PerCP-Cy5.5 detectors. BV711 will also have moderate spillover into the BD Horizon Brilliant™ Violet 786 detector.

anti-CD25; F; H, Bb, Cn, Rh; Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis. For flow cytometric staining using the μg size, the suggested use of this reagent is 70.4 μg per million cells in 100 μl volume. For flow cytometric staining using tests sizes, the suggested use of this reagent is 5 μl per million cells in 100 μl staining volume or 5 μl per 100 μl of whole blood.

anti-CD3; IHC; H; Clone LN10 is specific for the non-glycosylated epsilon chain of the human CD3 molecule. Clone LN10 recognizes T cells in thymus, bone marrow, peripheral lymphoid tissue and blood and is a pan T cell marker.

anti-CD3; F, H, Rh, Cn, Bb; Clone SP34-2 is routinely tested using Purified Mouse IgG1, clone MOPC-21 (Cat. No. Cat. No 556648), as the isotype control. Alternate isotype controls specific for the lambda light chain, such as clones S1-68.1 (Cat No. 553452) and A111-3 (Cat No. 553485), are not routinely tested for flow cytometry application.

anti-CD4; F, IHC, IF; M; clone GK1.5, Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis.

anti-CD4; W, IHC, F; H; clone EPR68SSS, This product is a recombinant monoclonal antibody, which offers several advantages including: High batch-to-batch consistency and reproducibility, Improved sensitivity and specificity.

anti-CD4; F, H, Ch; clone RPA-T4, each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis. For flow cytometric staining using the μg size, the suggested use of this reagent is 0.5 μg per million cells in 100 μl volume. It is recommended that the reagent be titrated for optimal performance for each application. For flow cytometric staining using the test sizes, the suggested use of this reagent is 5 μl per million cells in 100 μl staining volume or 5 μl per 100 μl of whole blood.
of whole blood.

anti-CD45; F; M; Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis.

anti-CD45RA; F; H; Ch; Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis. For flow cytometric staining, the suggested use of this reagent is 5 µl per million cells in 100 µl staining volume or 5 µl per 100 µl of whole blood.

anti-CD56; F; H; clone NCAM16.2, The antibody was conjugated to BD Horizon™ BV510 which is part of the BD Horizon™ Brilliant Violet™ family of dyes. With an Ex Max of 405-nm and Em Max at 510-nm, BD Horizon™ BV510 can be excited by the violet laser and detected in the BD Horizon™ V500 (525/50-nm) filter set. BD Horizon™ BV510 conjugates are useful for the detection of dim markers off the violet laser.

anti-CD56; F; H, B, Pg; clone MEM-188, Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis. For flow cytometric staining, the suggested use of this reagent is 5 µl per million cells in 100 µl staining volume or 5 µl per 100 µl of whole blood.

anti-CD68; IHC; H; Our Abpromise guarantee covers the use of ab783 in the following tested applications. The application notes include recommended starting dilutions; optimal dilutions/concentrations should be determined by the end user. Perform enzymatic antigen retrieval before commencing with IHC staining protocol. (use ABC method). We suggest an incubation period of 30-60 minutes at room temperature.

anti-CD8; W, IHC, F, A; H, M, R; clone C8, Each lot of this antibody is quality control tested by formalin-fixed paraffin-embedded immunohistochemical staining.

anti-CD8; F, H, Ag, Ch, Cn, PM, Rh, SM; clone SK1, Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis. For flow cytometric staining, the suggested use of this reagent is 5 µl per million cells in 100 µl staining volume or 5 µl per 100 µl of whole blood.

anti-CD8; F, H, Bb, Cn, Rh; clone RPA-T8, BD Horizon™ PE-CF594 is a tandem conjugate, developed exclusively by BD Biosciences, that combines PE and CF594. PE-CF594 is a brighter alternative to PE-Texas Red®, with improved spectral characteristics. PE-CF594 reagents exhibit very consistent spillover values lot to lot, making them an ideal choice for the PE-Texas Red® detector (610/20 nm).

anti-CXCR3; F; H, Rh, Cn, Bb; The 1G6/CXCR3 monoclonal antibody specifically binds to human CD183, also known as the CXCR3 chemokine receptor.

anti-ERK; W, IP, IHC; H, M, R, Hm, Mk, Mk, Z, B, Pg, Sc; p44/42 MAPK (Erk1/2) Antibody detects endogenous levels of total p44/42 MAP kinase (Erk1/2) protein. In some cell types, this antibody recognizes p44 MAPK more readily than p42 MAPK. The antibody does not recognize either JNK/SAPK or p38 MAP kinase.

anti-F4/80; F; M; Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis.

anti-FOXP3; W, IP, IHC, F; B, Dg, F, M, Pg, R; The FJK-16s antibody reacts with mouse, rat, porcine, bovine and cat Foxp3 also known as FORKHEAD BOX P3, SCURFIN, and JM2; cross reactivity of this antibody to other proteins has not been determined.

anti-GAPDH; W, IP, IF, IHC; H; GAPDH (0411) is recommended for detection of GAPDH of human origin by Western Blotting (starting dilution 1:200, dilution range 1:100-1:1000), immunoprecipitation [1-2 µg per 100-500 µg of total protein (1 ml of cell lysate)], immunofluorescence (starting dilution 1:50, dilution range 1:50-1:500) and immunohistochemistry (including paraffin-embedded sections) (starting dilution 1:50, dilution range 1:50-1:500); not recommended for detection of GAPDH of mouse or rat origin.

anti-IFN-gamma; F; H, M, Mk; The 4S.B3 monoclonal antibody reacts with interferon-gamma (IFN gamma).

anti-IL-10; F; H, Mk; The JES3-9D7 antibody reacts with human and viral interleukin-10 (IL-10).

anti-IL-13; F; H; The B5BRD monoclonal antibody reacts with human interleukin-13 (IL-13). IL-13, also known as NC30, is a 12.5 kDa protein secreted by activated T helper cells, CD8+ T cells, and NK cells.

anti-IL-17A; F, IF, IHC; H, R, Hm; The eBio64DEC17 antibody reacts with human IL-17A.

anti-IL-4; F; H, Mk, Pg; The fluorochrome-labeled MP4-25D2 antibody is useful for intracellular immunofluorescent staining and flow cytometric analysis to identify IL-4 -producing cells within mixed cell populations.

anti-IL-6; F, IF, H; The MQ2-13A5 antibody reacts with human interleukin-6 (IL-6), a 21-28 kDa cytokine secreted by a variety of cell types.

anti-JNK; W, H, M, R, Hm, Mk, Z, B, Sc; SAPK/JNK Antibody detects endogenous levels of total JNK1, JNK2 or JNK3 protein.*

anti-LY-6C; F; M; Each lot of this antibody is quality control tested by immunofluorescent staining with flow cytometric analysis.

anti-MHCII; F; M; The MS/114.15.2 antibody reacts with a polymorphic determinant shared by the I-Ab, I-Ad, I-Aq, I-Ed, and I-Ek MHC class II alloantigens from mice carrying H-2p,r,q,b,d,u haplotypes.
anti-phospho-ERK; W, IP, IF, F; H, M, R, Hm, MK, Mt, Dm, Z, B, Pg, Ce; Phospho-p44/42 MAPK (Erk1/2) (Thr202/Tyr204) Antibody detects endogenous levels of p44 and p42 MAP Kinase (Erk1 and Erk2) when phosphorylated either individually or dually at Thr202 and Tyr204 of Erk1 (Thr185 and Tyr187 of Erk2). The antibody does not cross-react with the corresponding phosphorylated residues of either JNK/SAPK or p38 MAP Kinase, and does not cross-react with non-phosphorylated Erk1/2.

anti-phospho-JNK; W, IP; H, M, R, Hm, Dm, B, Sc; Phospho-SAPK/JNK (Thr183/Tyr185) Antibody detects endogenous levels of p46 and p54 SAPK/JNK dually phosphorylated at threonine 183 and tyrosine 185. This antibody does not recognize unphosphorylated SAPK/JNK. This antibody may slightly cross-react with phospho-Erk1/2 or -p38 phosphorylated at the homologous residues. It will also react with SAPK/JNK singly phosphorylated at Thr183 and singly phosphorylated at Tyr185.

anti-phospho-SYK; W, IP, IHC, F; H, pM, pR; Phospho-Syk (Tyr525/526) (C87C1) Rabbit mAb detects endogenous levels of Syk protein only when phosphorylated at Tyr525/526 of human Syk or Tyr519/520 of mouse Syk. It also detects Syk protein when singly phosphorylated at Tyr526 of human Syk or Tyr520 of mouse Syk. It does not cross-react with other tyrosine-phosphorylated protein tyrosine kinases.

anti-pSYK; F; H, M; The Q21-559 monoclonal antibody recognizes ROR gamma t (ROR?t), an isoform of ROR gamma (ROR?).

anti-SYK; W, IP, IHC, F; H, M, R; Syk (D3Z1E) XP® Rabbit mAb recognizes endogenous levels of total Syk protein.

anti-TNF; F; H, F; The fluorochrome-labeled MAb11 antibody is useful for intracellular and membrane-bound immunofluorescent staining and flow cytometric analysis to identify TNF-a-producing cells within mixed cell populations.

anti-TRAP; W, IF, IHC; H, M, R; The antibody detects endogenous levels of total ACP5 protein.

anti-TRAP; F; H, F; The antibody detects endogenous level of total ACPS protein.

anti-alpha-tubulin; W, IF, IHC, F; H, M, R; Detects a band of approximately 50 kDa. Excellent as a protein loading control antibody.

anti-beta-actin; W, IF, IHC; H, M, R, B; The immunogen used for this product shares 77% homology with Gamma actin/actin cytoplasmic 2. Cross-reactivity with this protein has not been confirmed experimentally.

anti-beta-catenin; W, IP, IHC, IF; H, M, R, Dg; Each lot of this antibody is quality control tested by formalin-fixed paraffin embedded immunohistochemical staining.

Legend - Application
A - Activation
CyT - CyTOF
F - Flow Cytometry
IF - Immunofluorescence
IHC - Immunohistochemistry
IP - Immunoprecipitation
W - Western

Legend - Species
Bb - Baboon
B - Bovine
C - Chicken
Ce - C. elegans
Ch - chimpanzee
CM - Capuchin Monkey
Cn - Cynomolgus
Dg - Dog
Dm - D. melanogaster
F - feline
H - Human
Hm - Hamster
Hr - Horse
Mt - Marmoset
Mi - Mink
Mk - Monkey
M - Mouse
p - predicted
Pg - Pig
Pm - Pigtailed Macaque
Rb - rabbit
Rh - Rhesus
R - Rat
Sc - S. cerevisiae
SM - Squirrel Monkey
X - Xenopus
**Eukaryotic cell lines**

Policy information about [cell lines](#)

| Cell line source(s) | HEK293 (293 [HEK-293] (ATCC® CRL-1573))  
| | SW480 (SW480 [SW-480] (ATCC® CCL-228)) |
| Authentication | None of the cell lines used have been authenticated. |
| Mycoplasma contamination | All the cell lines were negative for mycoplasma contamination. |
| Commonly misidentified lines | No commonly misidentified cell lines were used. |

**Animals and other organisms**

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

| Laboratory animals | Animals. SYK (S544Y) and wild-type mice of C57BL/6j genetic background between 1-7 months age were used as indicated in the manuscript. 4-6 week-old female wild-type C57BL/6j mice and 8-12 week-old female wild-type ICR mice were used as embryo donors and foster mothers, respectively, to generate the SYK (S544Y) point variant mouse model. C57BL/6j and ICR mouse strains purchased from Shanghai Laboratory Animal Center were housed in standard cages in a specific pathogen-free facility at temperatures of 20°C-26°C and relative humidity of 40% to 70% on a 12 h light/dark cycle with ad libitum access to food and water. All procedures involving mice and experimental protocols conformed to the regulations drafted by the Association for Assessment and Accreditation of Laboratory Animal Care in Shanghai and were approved by the Experimental Animal Ethics Committee of East China Normal University. |
| Wild animals | No wild animals were used in this study. |
| Field-collected samples | No field collected samples have been used in this study. |
| Ethics oversight | All procedures involving mice and experimental protocols conformed to the regulations drafted by the Association for Assessment and Accreditation of Laboratory Animal Care in Shanghai and were approved by the Experimental Animal Ethics Committee of East China Normal University. |

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

**Human research participants**

Policy information about [studies involving human research participants](#)

| Population characteristics | 6 patients from 5 families, 2 males, 4 females and age matched healthy controls. |
| Recruitment | Patients conforming to a clinical presentation of multiple tissue inflammation and/or CID of unknown cause, genetic diagnosis of rare variation in SYK and experimental confirmation of gain of function of the spleen tyrosine kinase. Experiments were carried out with Research Ethics Board (REB) approval from the Hospital for Sick Children, the Henan Children’s Hospital, the Oxford IBD cohort study (Rare disease subproject), Ludwig Boltzmann Institute for Rare and Undiagnosed Diseases, Genomics England Research Consortium Members and all centers involved. Informed written consent to participate in research was obtained from patients and controls. |
| Ethics oversight | Studies were carried out with local REB/IRB approval at all participating institutions (the Hospital for Sick Children, the Henan Children’s Hospital, the Oxford IBD cohort study (Rare disease subproject), Ludwig Boltzmann Institute for Rare and Undiagnosed Diseases, Genomics England Research Consortium Members and all centers involved). |

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

**Flow Cytometry**

Plots

- Confirm that:
  - The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
  - The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a ‘group’ is an analysis of identical markers).
  - All plots are contour plots with outliers or pseudocolor plots.
  - A numerical value for number of cells or percentage (with statistics) is provided.
| Methodology          |                                                                                           |
|---------------------|-------------------------------------------------------------------------------------------|
| Sample preparation  | Sample preparations for flow cytometry measurements are described in detail in the respective Material and Methods sections. |
| Instrument          | All data were collected on a BD LSRII or BD Fortessa.                                     |
| Software            | Flow Cytometry Data were collected using BD FACS Diva software (v8.0.2) on a BD LSRII or BD LSRFortessa. Flow cytometry data were analysed with FlowJo (Tree Star), version 10.6.1 or later. |
| Cell population abundance | No FACS-based enrichment of cell populations was performed.                                |
| Gating strategy     | All gating strategies are described in detail in the Material and Methods section and outlined in the Supplemental note Appendix Figures 1-5. Total leukocytes and lymphocytes were identified by cell size in a FSC-A vs. SSC-A plot. Single cells were discriminated in a FSC-A vs. FSC-H and SSC-A vs. SSC-W plot. Dead cells were excluded from analysis as described in the Material and Methods section. |

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