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REVIEW

Genes and Immunity Focus

LCK'ed in: Inborn errors of immunity in LCK reveal how TCR signaling is calibrated

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TCR signaling must be precisely calibrated to guide thymic selection, lineage commitment, and immune homeostasis. LCK, the dominant proximal Src-family kinase in T cells, functions as a volume dial governing initial TCR signal amplitude. Human inborn errors of immunity affecting LCK (LCK-IEI) demonstrate that graded reductions in LCK translate into distinct developmental and clinical outcomes. Null or near-null variants silence αβ thymocyte output, resulting in profound immunodeficiency, whereas hypomorphs permit limited thymopoiesis but selectively impair regulatory T cell development, skew TCR repertoires, and drive autoimmunity. Notably, γδ T cells are preserved, underscoring lineage-specific signaling thresholds. Signaling defects downstream of LCK produce overlapping phenotypes, confirming T cell fate reflects signal strength, not molecular identity. Here, we synthesize insights from human LCK-IEI and emerging spatial views of thymic microenvironments to define how quantitative and contextual regulation of TCR signaling shapes selection, tolerance, and immune balance. We highlight unresolved questions and experimental strategies aimed at restoring immune sufficiency while avoiding immune dysregulation.

Introduction

Decades of work using both cell lines and mouse models have firmly established the Src-family kinase LCK as essential for T cell development, selection, differentiation, activation, and function, reviewed by Bommhardt et al. (2019), De Sanctis et al. (2024), Rudd (2021), and Zhang et al. (2023). The high degree of conservation between murine and human LCK—approximately 95% amino acid identity with complete preservation of all major functional domains and regulatory residues—makes these systems powerful tools for dissecting LCK biology (Perlmutter et al., 1988; Veillette et al., 1988; Zamoyska et al., 2003). Studies of *Lck* knockout mice (*Lck*$^{-/-}$), which show a near-complete block in αβ T cell development, were instrumental in defining early checkpoints of thymic selection (Denzel et al., 2003; Eberl et al., 1999; Hernández-Hoyos et al., 2000; Legname et al., 2000; Levin et al., 1993; Molina et al., 1992; Penninger et al., 1993; Rudd et al., 2006; Van Oers et al., 1992; van Oers et al., 1996c; Wei et al., 2020; Michel et al., 2012). Similarly, kinase-inactive and co-receptor–uncoupled Lck mutants have illuminated the importance of catalytic activity and CD4/CD8 association (Levin et al., 1993; Horkova et al., 2023; Van Laethem et al., 2007; Zhang et al., 2025; Seavitt et al., 1999). However, these experimental strategies—complete gene deletion, kinase-dead knock-in alleles, or introduction of transgenes—create signaling states that are often binary; that is, fully functional or WT versus complete loss-of-expression or function. These reductionist approaches incompletely model the spectrum of LCK function encountered in human disease, precluding insights into how LCK calibrates TCR signaling in T cells at different developmental stages, in distinct cellular contexts, and within discrete microenvironments across thymic and peripheral lymphoid organs.

Human LCK variants offer a nuanced way forward, occupying intermediate positions on the "volume dial." The biological stakes of this dial are high, as T cell development is exquisitely sensitive to the strength and duration of TCR signals—signals that are too weak result in death by neglect, while excessively strong signals trigger negative selection (Gascoigne et al., 2016). Between these extremes lies a narrow window in which TCR signal amplitude must be precisely calibrated to generate the diverse T cell subsets required for adaptive immunity while ensuring lack of self-reactivity. This calibration governs the bifurcation between αβ and γδ lineages and subsequently directs αβ thymocytes toward conventional CD4$^+$ or CD8$^+$ T cells, Foxp3$^+$

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regulatory T cells (Tregs), or innate-like lineages such as natural killer T cell (NKT) and mucosal-associated invariant T cell (MAIT) cells (Molina et al., 1992; Penninger et al., 1993; Van Laethem et al., 2007; van Oers et al., 1996b; Zhang et al., 2025; Wei et al., 2020). Importantly, optimal signaling thresholds differ across developmental checkpoints: positive selection, negative selection, Treg induction, and lineage diversification operate over partially overlapping but nonidentical signal ranges. When mutations lock the dial at a particular amplitude, only cell fates accessible at that set-point emerge—a restriction that can manifest as immunodeficiency, autoimmunity, or both (immune dysregulation). As naturally occurring mutations, these variants arise in their native genomic and developmental context, where thymocyte fate depends on sequential encounters with distinct stromal microenvironments. This complexity generates a range of dysfunctional signaling states that neither mouse knockouts (all or nothing) nor transformed cell lines (lacking tissue context) can replicate. Recently identified human inborn errors of immunity (IEI) affecting LCK (Hauck et al., 2012; Keller et al., 2023; Lanz et al., 2023; Lui et al., 2024) uniquely enable the dissection of proximal TCR signaling *in vivo* across an allelic series of varying severity (Table 1). These variants reveal that different T cell subsets exhibit distinct signaling thresholds and that modest residual LCK activity may be sufficient for limited thymic output while remaining inadequate for Treg differentiation and/or immune tolerance. Additionally, they also illustrate the different TCR signaling thresholds required for selection in different T cell subsets. These human genotypes/phenotypes underscore that LCK operates as an analog dial rather than a binary switch.

In this review, we synthesize classical models of LCK modulation with emerging findings from human inborn errors of LCK (LCK-IEI). Central to this synthesis is the recognition that LCK is the most proximal point at which TCR signal strength and microenvironmental context converge to shape T cell fate. We begin by exploring the molecular regulation of LCK and its relationship to other proximal signaling molecules, then examine how LCK-IEI reveal what happens when the volume dial is misset—altering TCR signal strength, which in turn disrupts selection thresholds, lineage decisions, and immune homeostasis. Throughout, we focus on the cellular and physiological consequences of LCK dysfunction in humans, illustrating how naturally occurring genetic mutations complement and refine insights learned from *in vitro* cell line and *in vivo* murine models.

Inside the volume dial: Molecular regulation of LCK

Molecular regulation of LCK occurs at multiple levels—transcriptional, posttranscriptional, and posttranslational—each offering distinct opportunities for calibration. Fig. 1 depicts the basic structure of the LCK protein, including specific residues that govern its function. For a comprehensive review of LCK biochemistry and signaling, see Bommhardt et al., 2019, De Sanctis et al., 2024, and Rudd, 2021.

At the transcriptional level, LCK expression is regulated by two developmentally controlled and conserved promoters—a distal (D) and a proximal (P) promoter—that drive alternative first exons and distinct 5′ UTRs, generating multiple annotated transcripts (Brenner et al., 2002; Shimizu et al., 2001; Yamada et al., 2001). Expression from these promoters is shaped by lineage-specific transcription factors and chromatin accessibility that varies with T cell developmental stage (Brenner et al., 2002; Shimizu et al., 2001). This differential expression pattern supports LCK's role in T cell development, as its actions modulate TCR signal strength to determine T cell fate. In mice and humans, proximal promoter activity predominates in early thymocytes coinciding with TCR upregulation during positive selection (Wang et al., 2001; Finco et al., 1998), whereas the distal promoter is upregulated at later thymic stages and in mature T cells. Beyond promoter-driven mRNA diversity, alternative splicing generates additional LCK isoforms. The most well-characterized is LckΔexon7, a naturally occurring splice variant detected in human and murine cell lines (Germani et al., 2003) and in at least one human severe combined immunodeficiency (SCID) patient (Goldman et al., 1998). This isoform exhibits reduced kinase activity and acts as a negative regulator of full-length p56LCK (Germani et al., 2003). Altered ratios of LCK splice variants have also been reported in certain tumors, suggesting context-dependent functional relevance (Rouer et al., 1993).

LCK function is regulated by a variety of posttranslational modifications that control membrane localization and enzymatic activity. Myristoylation at Gly2 and dual palmitoylation at cysteines 3 and 5 in the N-terminal SH4 domain enable membrane/lipid-raft targeting and influence cytoplasmic sequestration (Kabouridis et al., 1997; Yurchak and Sefton, 1995; Rossy et al., 2013; Ventimiglia and Alonso, 2013). Enzymatic activity is determined by posttranslational tyrosine phosphorylation events that induce conformational changes in the protein. At rest, LCK is maintained in an inactive conformation (closed) by phosphorylation of the inhibitory tyrosine residue Y505 by C-terminal Src kinase (Csk), which stabilizes intramolecular binding between its SH2 domain and the phosphorylated C-terminal tail (Abraham and Veillette, 1990). Dephosphorylation of Y505 by the receptor-like tyrosine phosphatase CD45 relieves this inhibitory interaction, partially activating the kinase (Ostergaard et al., 1989). Full activation requires autophosphorylation of the activation loop tyrosine Y394, stabilizing the active (open) conformation and maximizing catalytic activity (Turner et al., 1990; Veillette et al., 1988). Phosphorylation of Y192 in the SH2 domain also inhibits LCK activity by preventing it from adopting an open active conformation (Courtney et al., 2017). Finally, CBL E3 ubiquitin ligases target active LCK for proteasomal degradation, both directly and via adaptor networks, thereby modulating its half-life and availability at the immunological synapse (Nath and Isakov, 2024; Rao et al., 2002).

LCK operates at the most proximal step of the TCR signaling cascade, as a free molecule or bound to the co-receptors CD4 and CD8 (Barber et al., 1989; Veillette et al., 1988) or to CD3ε itself (Hartl et al., 2020) (Fig. 2). Murine Lck can also function as an adaptor that mediates kinase-independent signal transduction (Fukushima et al., 2006; Legname et al., 2000; Briones et al., 2024; Xu and Littman, 1993). Accumulating evidence supports

Table 1. **Summary of human LCK-IEI**

Reference		LCK		Cellular			Clinical			
Citation	**Mutation**	**Protein expression**	**Functionality**	**T cells**	**B cells**	**Immunoglobulins**	**Age onset**	**Infectious**	**Noninfectious**	**Treatment and outcome**
Hauck et al., 2012	c.1022T>C p.L341P	Decreased	Catalytically inactive	↓ Total T ↓ $CD4^+$ T ↔ $CD8^+$ T ↓ Treg ↑ γδT (ratio) ↑ TCM $CD4^+$ T ↑ TEM $CD8^+$ T	↓ Total B	↔ IgG ↔ IgA ↑ IgM ↔IgE	18 mo	Upper/lower respiratory; pneumonia; pneumatocele	Chronic diarrhea; nodular skin lesions; arthritis; inflamed hypodermis; retinal vasculitis; pericarditis; serositis; $dsDNA^+$	HSCT; died at age 30 mo, 7 days after HSCT
Li et al., 2016	Intronic c.188-2A>G ΔExon 3	ND	ND	↓ $CD4^+$ T	ND	ND	23 years 18 years 15 years	Childhood onset pneumonia; bacterial infections; HPV infections (EV-specific HPV and genital HPV)	Not observed; pityriasis	Unknown; one died – cranial tumor; one died – unknown reasons; one alive (ATR)
Lanz et al., 2023	c.1393T>C p.C465R	Decreased	Limited activity ↓ Ca^{2+}-flux ↓ pZAP70 ↓ pERK	↓ Total T ↓ $CD4^+$ T ↓ $CD8^+$ T ↑ Treg ↑ γδT (ratio) ↑ TEM ↓ Naive	↑ Total B	↔ IgG ↔ IgA ↓ IgM ↔ IgE	6 mo	Oral and perianal candidiasis; EBV; ADV; CMV; respiratory infections	Not observed	Died at age 12 mo, respiratory failure
Keller et al., 2023	c.1129dupA p.S377KTer14	Decreased Truncated (lacks kinase domain)	Kinase dead	↓ Total T ↓ $CD4^+$ T ↓ $CD8^+$ T Treg (NL) ↑ γδT ↓ Naive	↔ B	Hypogammaglobulinemia	11 mo	Recurrent pneumonia; otitis media; skin infections	Not reported	HSCT Alive (ATR)
					↑ Naive ↓ Class-switched ↓ Transitional B	↓ IgG IgA ? IgM ? IgE ?	42 mo	Recurrent pneumonia; otitis media; stool positive for cryptosporidium antigen; oral candidiasis; CMV; EBV	Recurrent erythematous and ulcerative plaque skin lesions; cutaneous and subcutaneous lesions	IVIG, HSCT; died at age 3.5 years, 20 days after HSCT
Lui et al., 2024	c.1318C>T; P440S	Decreased	Limited activity ↓ Ca^{2+}-flux ↓ Phosphorylation of LCK Y394, CD3ζ, ZAP70	↓ Total T ↓ $CD4^+$ T ↓ $CD8^+$ T Treg (ND) ↑ γδT ↓ Naive	↔ Total B ↑ Naive B ↓ Memory B (ratio)	↓ IgG ↓ IgA ↔ IgM	Birth	Recurrent viral and fungal respiratory and gastrointestinal infections; HSV stomatitis; oral candidiasis; cryptosporidiosis; *Norovirus*; *Salmonella* enteritis	Chronic diarrhea	IVIG, HSCT; died at age 6.5 years, 3 mo after HSCT, pneumonia
						ND	1.5 mo			IVIG, HSCT; age 3 years, alive (ATR)

ADV, Adenoviruses; ATR, At the time of report; EV, Epidermodysplasia verruciformis; HPV, human papillomavirus; IVIG, Intravenous immunoglobulin; ND, Not determined; TEM, T-effector memory.

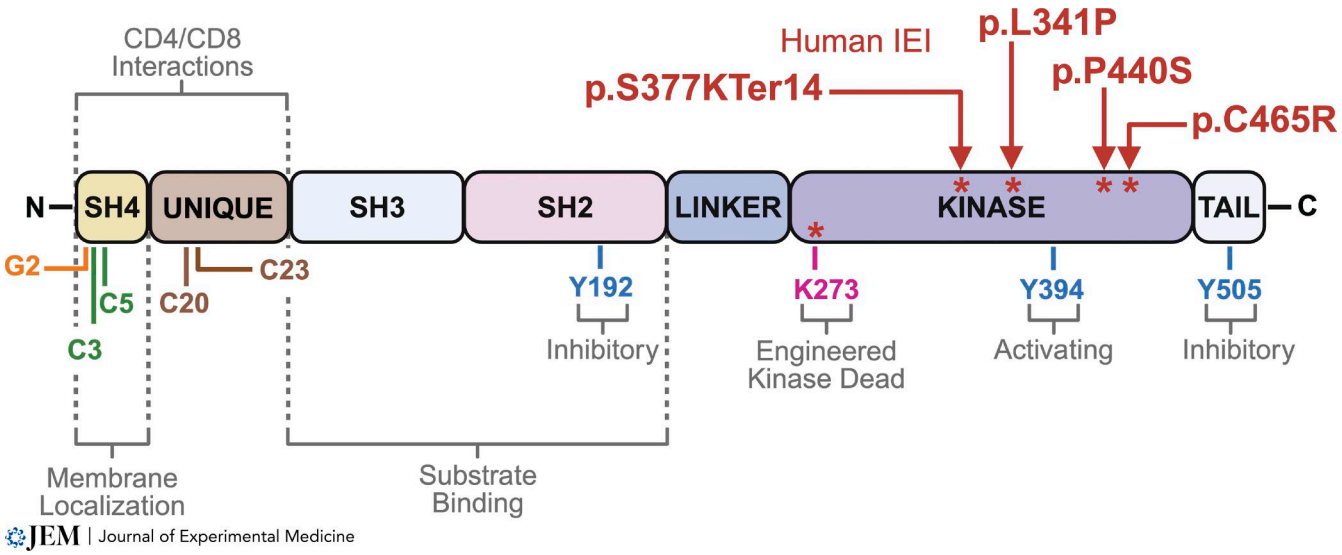


Figure 1. **Domain organization of human LCK and positions of pathogenic variants.** The schematic illustrates LCK structural domains: SH4 and unique regions (N-terminal membrane localization), SH3 and SH2 domains (substrate binding), linker region, kinase domain, and C-terminal tail. Key regulatory residues are indicated: Y192 and Y505 (inhibitory), Y394 (activating), and K273 (engineered kinase-dead). Reported human disease–associated variants (red asterisks) are mapped to their respective domains. SH, Src homology; Y, tyrosine; K, lysine.

a model in which TCR signaling is initiated by a pool of enzymatically active LCK that is not constitutively tethered to CD4 or CD8, with co-receptor–associated LCK primarily modulating TCR signal efficiency and sensitivity and thereby T cell lineage specification (Casas et al., 2014; Hui and Vale, 2014; Nika et al., 2010; Wei et al., 2020). Classical studies demonstrated that CD3 engagement can induce tyrosine phosphorylation and downstream signaling in the absence of CD4 (Granja et al., 1994). A substantial fraction of LCK (up to 40%) is phosphorylated at its activating tyrosine (Y394) in resting T cells, and half of this pLCK is not bound to CD4/CD8 (Nika et al., 2010). More recently, single-molecule and super-resolution imaging approaches have revealed that active LCK resides in dynamic nanoclusters and undergoes rapid transitions between free and confined diffusion states, enabling transient access to engage TCR complexes prior to stable co-receptor recruitment (Hilzenrat et al., 2020; Mørch et al., 2022; Roh et al., 2015; Rossy et al., 2013). Biochemical separation of free versus co-receptor–bound LCK has further shown that the free pool is enriched for catalytically active kinase, whereas CD4/CD8-associated LCK is comparatively restrained (Wei et al., 2020). LCK's catalytic output is also modulated by the opposing actions of CD45 and CSK (Brdicka et al., 2000; Donovan and Koretzky, 1993; Rheinländer et al., 2018; Veillette et al., 1988) (Fig. 2 A). Notably, CD45 is excluded from the immunological synapse due to the large size of its extracellular domain (Chang et al., 2016; Davis and van der Merwe, 2006) and cytoplasmic sequestration (Nath and Isakov, 2024). Finally, a zinc-clasp interface—formed by coordination of a zinc ion with LCK cysteines 20 and 23 and specific cysteines in the CD4 (420) or CD8 (421) cytoplasmic tails—tethers LCK and positions it adjacent to the signaling machinery (Kim et al., 2003; Turner et al., 1990). Engagement of TCR with the major histocompatibility complex (MHC) concentrates and stabilizes LCK at the synapse (Kabouridis et al., 1997; Kim et al., 2003) (Fig. 2 B).

Upon antigen engagement of TCR, activated proximal LCK (free or bound) phosphorylates immunoreceptor tyrosine-based activation motifs (ITAMs) in the cytoplasmic domains of CD3ε, ζ, δ, and γ chains (Wu et al., 2020; Chan et al., 1994; Straus and Weiss, 1992; van Oers et al., 1996a; van Oers et al., 1996b). The phosphorylated ITAMs serve as docking sites for zeta chain–associated protein kinase 70 (ZAP-70), a Syk-family kinase essential for downstream TCR signaling. LCK phosphorylates CD3-bound ZAP-70, activating its full enzymatic activity (van Oers et al., 1996a). ZAP-70 propagates the signal via its phosphorylation of adaptor proteins—linker for activation of T cells (LAT) and SH2-domain-containing leukocyte protein of 76 kDa (SLP-76) (Fig. 2 B). These proteins coordinate crucial downstream events, including calcium mobilization, MAPK activation, and transcriptional programs dependent on nuclear factor κ-light chain enhancer of activated B cells (NF-κB)/nuclear factor of activated T cells (Finco et al., 1998). LCK also phosphorylates CD28 and other co-stimulatory molecules that provide secondary signals (signal 2) required for T cell activation (Raab et al., 2001; Acuto and Michel, 2003; Holdorf et al., 1999). LCK's dual role in both initiating and amplifying TCR signals positions it as a critical control point where small changes in LCK action can profoundly influence T cell fate decisions.

Collectively, the highlighted studies demonstrate that precise calibration of TCR signal amplitude is largely governed by coordinated regulation of LCK expression, phosphorylation state, co-receptor coupling, localization to membrane microdomains, and ubiquitin-mediated protein degradation. These layered regulatory mechanisms, briefly summarized above and reviewed by Bommhardt et al. (2019), De Sanctis et al. (2024), Rudd (2021), Zhang et al. (2023), and Mohapatra et al. (2013), ensure that LCK is available, enzymatically active, and in the proper location to "dial-in" the right TCR signal amplitude at the right developmental stage. When any of these layers is disrupted by

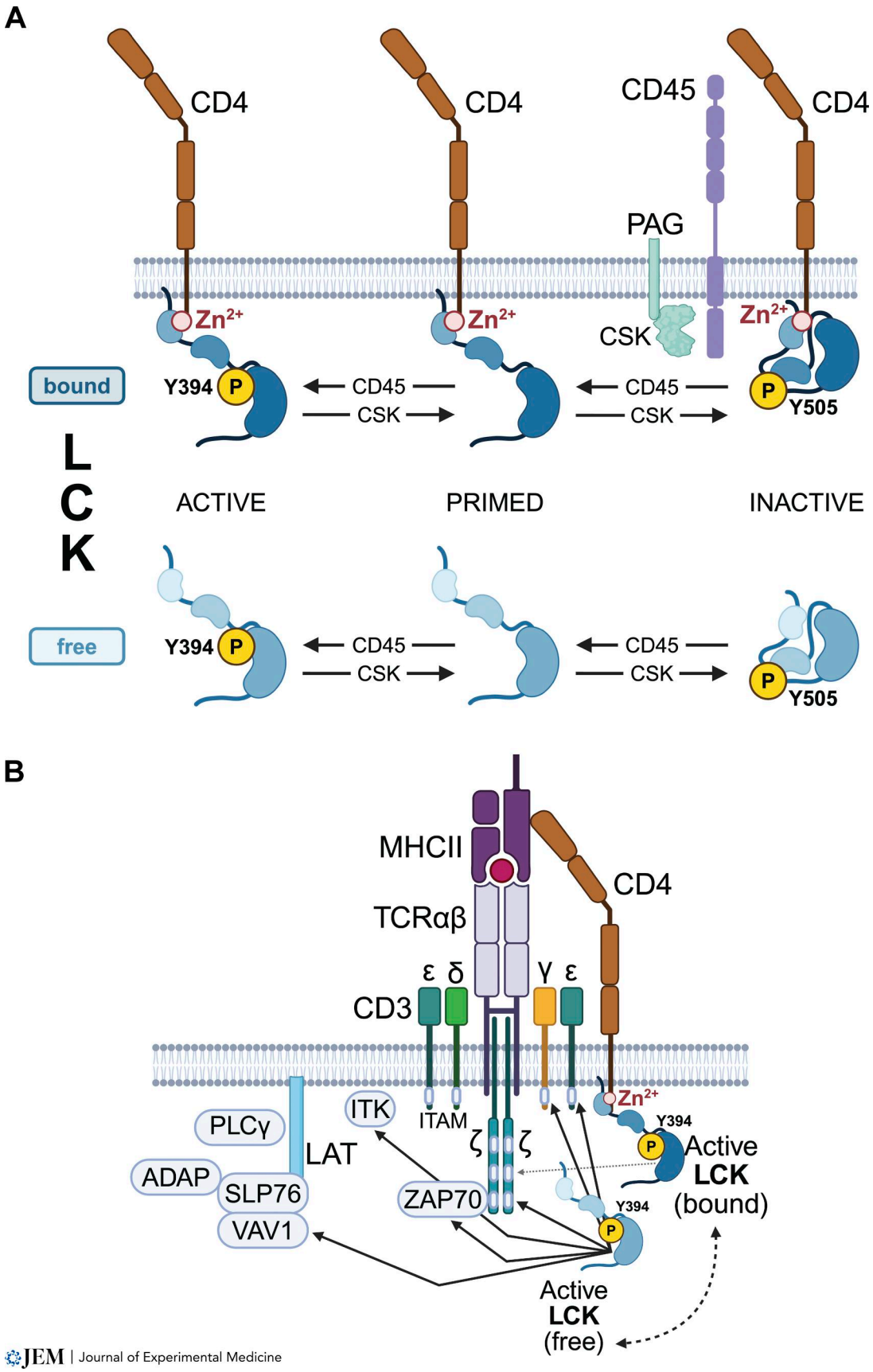


Figure 2. **Regulation and signaling functions of LCK in proximal TCR signaling. (A)** LCK exists in co-receptor–bound and free pools, each cycling among active, primed, and inactive conformations. CD45 dephosphorylates the inhibitory Y505, promoting activation, whereas CSK (recruited via PAG) phosphorylates

Y505 to maintain the inactive state. Autophosphorylation of Y394 stabilizes the active conformation. Zn^{2+} coordinates LCK binding to CD4/CD8 co-receptors. **(B)** Upon TCR engagement, the free, active pool of LCK initiates TCR signaling via phosphorylation of ITAMs within CD3 chains, while co-receptor–bound LCK modulates sensitivity, efficiency, and lineage calibration. ITAM phosphorylation enables ZAP-70 recruitment and activation of downstream effectors, including LAT, SLP-76, and ITK.

genetic mutation, the consequences for T cells and human health can be profound—as revealed by the growing catalog of human LCK-IEI and other proximal TCR genetic defects.

Damaged at the factory: Human LCK-IEI

The five autosomal-recessive LCK-IEI described to date are all homozygous for distinct mutations in the kinase domain, three as missense mutations and one as a frameshift mutation that results in a premature stop codon (Table 1 and Fig. 1 B). Together with the intronic exon-3–skipping variant described by Li et al. (2016), these mutations exemplify the concept of allelic heterogeneity—one gene, many phenotypes. Complete loss of LCK, whether through lack of expression (p.C465R) or abolished catalytic function (p.L341P; p.S377KTer14), causes SCID-like disease dominated by infections (Lanz et al., 2023; Hauck et al., 2012; Keller et al., 2023). Partial reduction of LCK expression, function, or location (p.P440S; c.188-2A>G) produces "leaky" or incomplete SCID-like disease with infections and prominent immune dysregulation (Li et al., 2016; Lui et al., 2024). To date, no gain-of-function mutations have been reported in humans; however, a transgenic mouse expressing constitutively active Lck (Y505F) arrests thymopoiesis at the $CD4^+CD8^+$ double-positive (DP) stage due to premature TCR activation (Seavitt et al., 1999; Abraham et al., 1991), demonstrating that excessive signal amplitude can also be disruptive. A summary of comparisons and contrasts between mouse and human mutation in LCK are summarized in Table S1.

Hauck et al. described the first LCK-IEI in a child with a homozygous missense mutation (L341P) in exon 9 that rendered LCK catalytically inactive despite residual protein expression (Hauck et al., 2012). *In vitro*, the L341P allele behaves as kinase-null, failing to restore phosphorylation of proximal or distal signaling intermediates in LCK-deficient cell lines. Yet in the patient, this apparently null allele permits limited thymic output, likely via LCK's adaptor functions and/or compensatory actions of other SRC-family kinases, e.g., FYN (Fukushima et al., 2006; Legname et al., 2000; Briones et al., 2024; Xu and Littman, 1993). This results in reduced but detectable $CD3^+$ T cells with a skewed peripheral profile marked by $CD4^+$ T cell lymphopenia, relatively preserved $CD8^+$ T cell numbers, decreased Treg counts, enrichment of memory-phenotype cells ($CD4^+$ central memory (TCM) and $CD8^+$ terminally differentiated effector memory (TEMRA), and increased $TCR\gamma\delta^+$ T cell frequency. Clinically, the patient exhibited both susceptibility to infection and striking autoinflammation/autoimmunity. This pathological convergence illustrates a recurring theme in LCK-IEI. Residual TCR signaling above a minimum threshold seems to preferentially support effector over regulatory lineages, enabling escape of autoreactive clones while failing to generate adequate Tregs.

Li et al. (2016) reported three young adults with an intronic splice-site mutation (c.188-2A>G). This mutation causes exon 3 skipping, which shifts the reading frame and triggers nonsense-mediated mRNA decay, markedly reducing mature transcript levels. The predicted 75-amino acid product should lack both the N-terminal unique domain—which tethers LCK to CD4/CD8 co-receptors—and the kinase domain, yielding a protein that is both uncoupled and catalytically dead (Li et al., 2016). Importantly, work by Horkova et al. (2023) in engineered mice ($Lck^{CA/KR}$) established that co-receptor coupling and kinase activity make separable contributions to LCK function, helping explain why the Li mutation, which eliminates both on a single protein, might produce a severe phenotype even if some truncated product escapes mRNA decay. Clinically, all three patients presented with $CD4^+$ T cell lymphopenia, recurrent respiratory infections, broad human papillomavirus susceptibility, and atypical epidermodysplasia verruciformis, indicative of impaired T cell–mediated antiviral and barrier immunity. Although protein expression and detailed T cell subset analyses were not performed on peripheral blood mononuclear cells (PBMC) from the c.188-2A>G patients, their clinical phenotype combined with the predicted molecular defects is consistent with findings from the $Lck^{CA/KR}$ mouse model demonstrating that spatial positioning of LCK relative to the TCR/CD3 complex and enzymatic activity each contribute to TCR signal amplitude.

In 2023–2024, three additional kinase-domain variants were reported, collectively illustrating how different degrees of LCK disruption produce distinct perturbations in T cell compartments (Keller et al., 2023; Lanz et al., 2023; Lui et al., 2024). Keller et al. identified an exon 11 frameshift variant (p.S377KTer14) in two siblings, which generates a truncated protein lacking the kinase domain but retaining the unique, SH3, and SH2 domains—potentially preserving co-receptor binding and adaptor function, as proposed for the Hauck variant (Keller et al., 2023; Hauck et al., 2012). Both children presented with leaky SCID characterized by profound immunodeficiency (viral and fungal infections) alongside immune dysregulation with mucosal manifestations (skin in Keller; gastrointestinal in Lui; Table 1) (Keller et al., 2023; Lui et al., 2024). The patients exhibited $CD4^+$ and $CD8^+$ T cell lymphopenia, a marked reduction in naive $CD4^+$ and $CD8^+$ populations, relative enrichment of $TCR\gamma\delta^+$ cells, and hypogammaglobulinemia with minimal class-switched memory B cells. The near-absence of naive $CD4^+$ helper T cells, coupled with contraction of class-switched memory B cells, underscores how severe LCK insufficiency collapses both thymic output and T cell–dependent B cell maturation.

The p.C465R missense mutation reported by Lanz et al. lies in exon 13 and causes near-complete loss of LCK protein (Lanz et al., 2023). This expression-null variant produces the most severe infectious clinical symptoms described to date—global $CD3^+$ T cell lymphopenia with reduced $CD4^+$ and $CD8^+$ T cells,

severe naive T cell depletion, and relative γδ T cell enrichment. The infant succumbed to overwhelming viral and fungal infections before inflammatory complications could manifest. This implies that when TCR signal amplitude falls below the threshold required for sustained positive selection and peripheral homeostasis, the clinical picture is dominated by immunodeficiency rather than immune dysregulation.

By contrast, the hypomorphic P440S mutation described by Lui et al. reduces but does not abolish LCK protein expression and function (Lui et al., 2024). Patients presented with leaky SCID—recurrent viral and fungal infections alongside chronic diarrhea and pulmonary disease suggestive of immune dysregulation. Whether gut and lung pathology arose from recurrent infection or mutation-intrinsic immune dysregulation could not be resolved clinically. Comparison of knockout ($Lck^{-/-}$) and hypomorphic ($Lck^{P440S/P440S}$) mice provided mechanistic insight, indicating that residual LCK expression and/or activity drive the gastrointestinal inflammation. This paired research strategy—human reveals, mouse dissects—allows the field to move beyond binary models and begin to map graded TCR signaling thresholds across cell types and tissue niches, clarifying how signal amplitude shapes both antimicrobial defense and tolerance in humans.

Patients expressing the Lui hypomorphic P440S mutation displayed immunophenotypic features similar to those in the Keller and Hauck cases, including decreased $CD4^+$ and $CD8^+$ T cell numbers, with effector memory skewed phenotype, oligoclonal TCR repertoires, and preservation or enrichment of γδ T cells. Importantly, Lui et al. (2024) demonstrated that although both $Lck^{-/-}$ and $Lck^{P440S/P440S}$ mice show reduced T conventional (Tconv) and Treg numbers, Tconv effector function and proliferative capacity persisted only in the hypomorph (albeit at reduced levels as compared with WT), whereas Treg function was compromised in both. Together with the pronounced gastrointestinal tissue inflammation, these data indicate that an intermediate level of TCR signal amplitude suffices for limited thymic output but remains inadequate for robust central tolerance and peripheral regulatory control.

These functional perturbations may be compounded by a molecular one. Across reports, LCK dysfunction consistently destabilizes CD4 and CD8 surface expression (Hauck et al., 2012; Keller et al., 2023; Lanz et al., 2023; Lui et al., 2024; Van Laethem et al., 2013). Stable CD4 expression (and to a lesser extent CD8) depends on association with LCK (Horkova et al., 2023). Whether this reflects loss of LCK kinase activity, impaired co-receptor binding, or both remains unclear, but data from both murine models and human variants indicate that either defect reduces surface CD4. In four patients, CD8 expression was also diminished, though to a lesser degree, demonstrating *in vivo* that CD8 can better maintain TCR synapse stability despite weaker LCK binding. Notably, heterozygous family members of the Lanz patient (LCK-null) showed subtle reductions in CD4 surface expression; the families of other LCK-IEI patients have not been tested. Together, these findings reveal an allelic hierarchy in which CD4 stability is exquisitely sensitive to LCK quantity and/or quality.

Strikingly, these LCK-IEI also share the preservation—or expansion—of γδ T cells. Unlike αβ T cells, γδ T cells largely bypass canonical LCK-dependent TCR signal amplification during thymic development, with many subsets undergoing agonist selection via innate-like, ligand-restricted interactions rather than graded TCR signal strength (Contreras and Wiest, 2020; Hayes et al., 2005; Alizadeh et al., 2023; Marin et al., 2017). Consequently, when αβ T cell development collapses below a critical threshold, γδ lineages fill the resulting homeostatic vacuum (van Oers et al., 1996b; Salmond et al., 2011). This relative enrichment reflects not only their reduced dependence on LCK, but likely their capacity to expand in response to epithelial stress and inflammatory cytokines (e.g., IL-7 and IL-15) (Michel et al., 2012; Durum et al., 1998; Huck et al., 2009). Expansion of γδ T cells intersects with mucosal disease prominent in hypomorphic LCK-IEI. Many γδ subsets, particularly which reside in gut and skin, serve as first responders that sense epithelial damage, dysbiosis, and barrier disruption (Alizadeh et al., 2023). In LCK hypomorphism, insufficient Tregs and limiting numbers of naive $CD4^+$ T cells fail to support balanced mucosal immunity, often allowing γδ T cells to become disproportionately activated and adopt inflammatory effector programs (IL-17A, GM-CSF, and IFN-γ) (Lui et al., 2024). Without adequate regulatory containment, these responses amplify epithelial injury, propagate barrier breakdown, and sustain a feed-forward inflammatory circuit. Thus, the enriched γδ compartment is not merely a byproduct of αβ lymphopenia; it plausibly contributes to mucosal autoimmunity—including colitis—through heightened responsiveness to epithelial distress signals and unchecked effector differentiation.

The convergence of deficient Treg and naive $CD4^+$ (LCK-dependent) selection, skewed αβ T cell homeostatic expansion toward effector phenotypes, and hyperreactive γδ responses at mucosal barriers creates a permissive environment for chronic inflammation. This mechanistic triad may explain why patients with hypomorphic LCK alleles (Hauck et al., 2012; Lui et al., 2024) frequently develop early-onset colitis and mucocutaneous disease despite having relatively preserved γδ T cell numbers, demonstrating why loss of LCK-modulated signal fidelity affects both systemic tolerance and barrier-site immune homeostasis (Hauck et al., 2012; Lui et al., 2024). Similar immunological disturbances namely altered αβ:γδ ratios and Treg dysfunction, occur in inflammatory bowel disease, even without monogenic etiology (Chandwaskar et al., 2024). Finally, while αβ TCR repertoire analysis in LCK-IEI reveals oligoclonality, the γδ TCR repertoire remains largely unexplored, yet distinct mutations may shape γδ repertoire composition, tissue-specific differentiation, and functional fates in ways that account for why autoinflammatory and immune complications vary widely across these variants.

In summary, LCK-IEI demonstrate that LCK does not operate as a binary switch, but rather as a finely adjustable dial that modulates TCR signal amplitude to differentially shape $CD4^+$, $CD8^+$, Treg, and γδ T cell compartments. At very low effective TCR signal strength (Lanz et al., 2023), thymic αβ T cell output is greatly reduced, producing profound $CD4^+/CD8^+$ lymphopenia and overwhelming infection without time for development of autoimmunity (Lanz et al., 2023). At intermediate LCK levels—whether from reduced catalytic function (Lui et al., 2024) or

kinase-dead with preserved co-receptor binding and adaptor capacity (Hauck et al., 2012; Keller et al., 2023)—residual LCK function supports limited thymic export but skews the repertoire toward oligoclonal, antigen-experienced, or homeostatically expanded cells; hallmarks of almost all leaky SCID patients (Bosticardo et al., 2025; Lui et al., 2024; Hauck et al., 2012; Keller et al., 2023). Hypomorphic levels of TCR signaling support T effector homeostatic proliferation, including activation and cytokine production, but fails to support Treg function (Lui et al., 2024). LCK-independent signaling, likely mediated by FYN, permits some $CD8^+$ and residual $CD4^+$ effector-memory populations to emerge even when LCK is functionally absent (Hauck et al., 2012; Keller et al., 2023; Lui et al., 2024). Although not yet demonstrated in humans, experiments in mice show that Fyn can partially support thymocyte development in the absence of Lck (Zamoyska et al., 2003; Groves et al., 1996; van Oers et al., 1996b). In these patients, impaired Treg output combined with leaky positive selection results in immune dysregulation and autoimmunity alongside infection. Each LCK mutation thus shapes the TCR signal landscape differently, exposing the distinct amplitude thresholds required for thymic output, subset balance, and tolerance.

Alterations in subset balance are a fundamental feature of LCK-IEI. Rather than uniformly reducing all T cells, each allelic variant resets multiple ratios: $CD4^+$:$CD8^+$, naive:memory, αβ:γδ, and Treg:Teffector. Just as adjusting bass, mid, and treble changes the character of a radio broadcast without changing the station, altering LCK rebalances these cellular compartments while preserving some overall T cell presence. Null-leaning alleles flatten the thymic output entirely, nearly eliminating naive $CD4^+$ cells and Treg and leaving only sparse effector and γδ cells. Hypomorphs, on the other hand, preferentially depress Treg and naive $CD4^+$ output while relatively sparing $CD8^+$ and γδ subsets. These distorted ratios help explain why some patients present predominantly with infection (insufficient naive helper output), others with autoimmunity/inflammation (disproportionate Treg loss and oligoclonal effector expansion), and many with both.

Despite these insights, a comprehensive understanding of LCK deficiency in humans remains incomplete. Published reports have focused primarily on αβ T cell defects, leaving critical gaps regarding γδ T cells, NKT cells, MAIT cells, NK cells, and B cell subsets *in vivo*. A coordinated collaborative effort to studying LCK-IEI patients (and those with other genetic defects that affect thymic T cell development, selection, and differentiation) is urgently needed, incorporating deep immunophenotyping (cell numbers, subset ratios, and activation markers), multi-omic profiling (transcriptomics and proteomics), TCR and BCR repertoire sequencing, and, where feasible, tissue biopsies to assess lymphoid architecture and resident immune populations. Such characterization would clarify LCK's role in human immune development and identify molecular and cellular defects amenable to targeted intervention. Hematopoietic stem cell transplant (HSCT) is currently the only curative therapy for these patients, but given its morbidity and mortality, defining these lesions more precisely could reveal less invasive therapeutic strategies. Small-molecule kinase modulators, gene therapy approaches, or pathway-specific immunomodulation may be able to restore immune function without the risks that accompany transplantation. Hence, understanding the immunological consequences of naturally occurring LCK mutations advances our fundamental knowledge of human immunity and enables development of precision therapeutics for affected individuals and those facing similar immunological challenges due to other TCR signaling proximal defects (reviewed below).

Faulty amplifiers: IEI in downstream modulators of TCR signaling

The phenotypic hierarchy observed across LCK-IEI—from profound immunodeficiency (Lanz et al., 2023) through leaky SCID with immune dysregulation (Hauck et al., 2012; Keller et al., 2023; Lui et al., 2024)—is not unique to LCK. Similar genotype–phenotype gradients emerge from IEI affecting other proximal TCR signaling components, including ZAP70, CD3 chains, LAT, SLP-76, and ITK (Table 2 and Fig. 2 B). In each case, complete loss of function typically produces SCID, while hypomorphic variants generate phenotypes characteristic of intermediate TCR signaling amplitudes (impaired Treg development, narrow naive $CD4^+$ pool, and skewing toward memory-biased or unconventional populations). Notably, these IEI each manifest with a distinct immune profile and clinical presentation often reflective of its position in the signaling cascade (Bosticardo et al., 2025). Collectively, these naturally occurring mutations provide what artificial knockout and transgenic models cannot: a graded, physiologically relevant readout of how quantitative changes in TCR signal amplitude shape human immune development. Understanding this pathway through the lens of human genetics not only clarifies mechanism but also identifies nodes where therapeutic modulation—rather than complete blockade—might restore immune balance.

At the most upstream structural level, mutations in *TCRA* reduce the very capacity of thymocytes to assemble productive αβ TCR complexes, thereby attenuating the "input signal" before LCK engagement. Reported patients with TCRA defects exhibit impaired surface TCR expression, restricted repertoire diversity, and combined immunodeficiency with variable autoimmunity (Garkaby et al., 2022; Materna et al., 2025; Morgan et al., 2011; Rawat et al., 2021). Rather than eliminating signaling entirely, these lesions effectively compress the dynamic range of TCR engagement, leading to reduced positive selection and oligoclonal expansion—features that parallel hypomorphic LCK states and reinforce the concept that quantitative receptor signaling strength sets the ceiling for downstream amplification.

Immediately downstream of LCK, the CD3 complex provides the ITAM scaffold required for ZAP-70 recruitment and signal propagation. Biallelic defects in CD3D produce classic $T^-B^+NK^+$ SCID or Omenn-like phenotypes due to failure of TCR assembly or surface expression (Al-Hammadi et al., 2020; Dadi et al., 2003; de Saint Basile et al., 2004; Gil et al., 2011; Sonmez et al., 2025; Takada et al., 2005; Vignesh et al., 2020). Similarly, CD3G deficiency spans a broad clinical spectrum—from profound immunodeficiency to immune dysregulation—often with selective Treg instability and autoimmunity, illustrating that

Table 2. **Summary of human proximal TCR signaling defects**

TCR SS	Gene	Mutation	Protein	T cells			B cells		Clinical presentation	Autoimmunity	Infection	Citation
				Relative numbers	Responsiveness	Repertoire	Relative numbers	Antibodies				
– –	*TCRA*	Homozygous p.Ser116*	Null or truncate	↓ Total CD3⁺ ↓ CD4⁺ T ↓ CD8⁺ T ↓ Naive ↑ CD45RO⁺	↓ Proliferation after PHA	Abnormal Vβ	NL	NR	SCID-like disease	NR	NR	Garkaby et al., 2022
– –	*TCRA*	Homozygous: p.Trp65* (Pt1) Homozygous: p.Arg121* (Pt2)	Null or complete LOF	↓ Total CD3⁺ ↓ CD4⁺ TCRαβ ↓ CD8⁺ TCRαβ ↓ Naive ↑ γδT	None (TCR complex does not form)	Mostly γδT, few αβ, limited TRAV	NL	↑ IgG (Pt1) ↑ IgA (Pt1, 2, 3) ↔ IgM (Pt1, 2, 3) ↑ IgE (Pt1, 2, 3)	SCID	NR	CMV (Pt1) BCG (Pt1, 2) Salmonella and oral thrush (Pt2)	Materna et al., 2025
–/+	*TCRA*	Homozygous: p.Thr107LeufsX56	Partial deficiency	↓ CD4⁺ TCRαβ ↓ CD8⁺ TCRαβ ↓ Naive ↑ γδT	↓ Proliferation after PHA, anti-CD3, or PWM (mislocalized TCR)	Mostly γδT, few αβ	NL	NL (total Ig subtypes) NL (tetanus, pneumo, and Hib) ↑ IgE-Pt1	CID: Recurrent respiratory tract infections, otitis media, candidiasis, diarrhea, and failure to thrive	Hypereosinophilia, low-titer antinuclear antibodies (ANA), vitiligo, and alopecia areata (Pt1) Hypereosinophilia, eczema, autoimmune hemolytic anemia, anti-lymphocyte antibodies, anti-TTG antibodies, low-titer ANA, and pityriasis rubra pilaris (Pt2)	*Cryptosporidium* *Staphylococcus aureus* *Streptococcus pneumoniae* *Pseudomonas* Rotavirus *Salmonella enterica* Varicella zoster	Morgan et al., 2011
–/+	*TCRA*	Homozygous: p.Thr107LeufsX56	Partial deficiency	↓ Total CD3⁺ ↓ CD4⁺ TCRαβ ↓ CD8⁺ TCRαβ ↓ Naive ↑ γδT	NR Mislocalized TCR	Mostly γδT, few αβ, and limited TRAV	↓ B	↑ IgG (Pt1) ↑ IgA (Pt1–3) ↔ IgM (Pt1–3) ↑ IgE (Pt1–3)	CID: Recurrent lower respiratory tract infections, lymphoproliferation, warts, and mimics DOCK8 deficiency	No autoimmunity	Lower respiratory tract infections and EBV (Pt3)	Rawat et al., 2021
– –	*CD3D*	Homozygous: p.R68X	Null or complete LOF	↓ Total CD3⁺ ↓ CD4⁺ TCRαβ ↓ CD8⁺ TCRαβ ↓ Naive ↑ γδT	↓ Proliferation after PHA	NR	NL	NL Total Ig	SCID: Fever, tachypnea, tachycardia, respiratory arrest, chronic diarrhea, respiratory distress, lethargy, and jaundice	NR	Adenovirus (Pt2) CMV (Pt3)	Dadi et al., 2003
– –	*CD3D*	Homozygous: p.Trp43* (with BTK LOF mutation)	Null	↓ Total CD3⁺ ↓ CD4⁺ T ↓ CD8⁺ T	NR	NR	NL	Hypogammagloblunemia due to concurrent BTK mutation	SCID	NR	Rotavirus BCGosis due to vaccination Oral thrush (Pt2)	Al-Hammadi et al., 2020
– –	*CD3D*	Homozygous: p.C93X (Pt1) Homozygous: p.R68X (Pt2)	Truncate	↓ Total CD3⁺ ↓ CD4⁺ T ↓ CD8⁺ T	NR (Pt1) ↓ Proliferation (Pt2)	NR	NL	NR	SCID ($T^-B^+NK^+$)	NR	NR	de Saint Basile et al., 2004
– –	*CD3D*	Homozygous: c.56-1G>T Exon 1 skipping	Truncate	↓ Total CD3⁺ ↓ CD4⁺ T ↓ CD8⁺ T	NR	NR	NL	↓ IgG ↓ IgA ↓ IgM	SCID: Failure to thrive	NR	Recurrent sinopulmonary infections and candidiasis, BCG lymphadenitis	Sonmez et al., 2025

Table 2. **Summary of human proximal TCR signaling defects (*Continued*)**

TCR SS	Gene	Mutation	Protein	T cells			B cells		Clinical presentation	Autoimmunity	Infection	Citation
				Relative numbers	Responsiveness	Repertoire	Relative numbers	Antibodies				
– –	*CD3D*	Homozygous: p.S53X (Pt1) Homozygous: c.IVS2-2A>G (Pt2)	Truncate or null (Pt1) Truncate (Pt2)	↓ Total CD3⁺ ↓ CD4⁺ TCRαβ ↓ CD8⁺ TCRαβ ↓ Naive ↑ γδT	NR	NR	NL (Pt1) ↑ B (Pt2)	↓ IgG (Pt1) ↓ IgA (Pt1) ↑ IgE (Pt2)	SCID: Failure to thrive	NR (Pt1) Eczema (Pt2) Omen syndrome (Pt2)	Recurrent pneumonia Septicemia *Candida* (Pt2)	Vignesh et al., 2021
– –	*CD3D*	Homozygous: c.IVS2-2A>G mRNA lacks exon3	Truncate	↓ Total CD3⁺	NR	NR	NL (Pt1) ↑ B (Pt2)	↓ IgG (Pt1) ↓ IgA (Pt1) ↑ IgE (Pt2)	SCID	NR	CMV, pneumonitis (Pt1); oral thrush (Pt2)	Takada et al., 2005
–	*CD3D*	Homozygous: c.IVS2+5G>A G>A at position +5 in the 5′ splice donor site of intron 2 (in frame exon 2 deletion)	Truncate	↓ Total CD3⁺ ↓ CD4⁺ TCRαβ ↓ CD8⁺ TCRαβ ↓ Naive ↑ γδT	↓ CD25/CD69 upregulation upon activation	NR	NL	↔ IgG ↔ IgA ↑ IgE (Pt1) Antibody response to protein antigens impaired (Pt1) Hypogammagloblunemia (Pt2)	Leaky SCID: Failure to thrive, severe diarrhea, prostrating diarrhea, respiratory distress, discrete lymphopenia, severe hypogammaglobulinemia, and protein-losing enteropathy	Hyper-IgE Eosinophilia Atopic dermatitis (all Pt1)	Pt1: Bronchopneumonia *Salmonella*, *Campylobacter*, *Cryptosporidium*, oral candidiasis (Pt1) Urine CMV, nasal adenovirus (Pt2)	Gil et al., 2011
–	*CD3G*	Homozygous: c.80-1G>C Splice-acceptor variant causing aberrant splicing with a 17-bp exonic deletion and frameshift (IVS2-1G>C)	Null	↓ CD3 T NL CD4⁺ T ↓ CD8⁺ T ↓ Naive T ↑ Memory T	NR	NR	NR	Selective Ig2 deficiency (Pt1) NL Antibody responses to protein antigens	SCID (with features of autoimmunity): Failure to thrive, intractable diarrhea, autoimmune hemolytic anemia, mild respiratory distress, asthma	Serum autoantibodies against mitochondria, smooth muscle, and intestinal epithelial cells Severe autoimmune hemolytic anemia and respiratory distress Autoimmune enteropathy with gut epithelial cell autoantibodies (Pt1) Asthma, vitiligo, and atopic eczema (Pt2)	Repeated gram-positive and negative bacterial infections, viral pneumonia, parainfluenza 3 (Pt1), Hecht's pneumonia (Pt2)	Arnaiz-Villena et al., 1992; Allende et al., 2000
–/+	*CD3G*	Homozygous: c.1A > G p.translation start change	Null or complete LOF	NL Total CD3⁺ ↓ CD4⁺ TCRαβ ↓ Naive ↑ Memory T ↓ Treg	↓ Proliferation after PHA, Con-A, or anti-CD3	Reduced diversity	↓ All memory B	Hypogammaglobulinemia Impaired vaccination response to *Haemophilus influenzae* type B and Pneumococcus	CID with autoimmunity	GLILD Autoimmune enteropathy AIHA	EBV *Clostridioides difficile* *Klebsiella pneumoniae*	Delmonte et al., 2021
+	*CD3G*	Homozygous: c.del213A p.Asn71Metfs*110	Null or complete LOF	NR Treg NL	NL Proliferation after PHA	NR	↓ Switched memory B ↓ CD40L	Hypogammaglobulinemia ↓ IgG ↓ IgA ↓ IgM ↓ IgE	CVID: Without recognizable autoimmunity	NR	Recurrent sinopulmonary infections without opportunistic infections	Lee et al., 2019
–	*CD3G*	Homozygous: p.K69X	Complete LOF	Mild αβ and γδ T lymphocytopenia	↓ Proliferation after PHA or antigens (low CD3 expression)	NR	NL	NL	SCID: Chronic diarrhea (Pt1, 2)	Psoriasis, low titer microsomal and thyroglobulin autoantibodies (Pt3)	Pulmonary infections, recurrent otitis media, oral thrush, severe diaper dermatitis, and perianal fistula (Pt1) Oral thrush, perianal fistula (Pt2)	Recio et al., 2007

Table 2. **Summary of human proximal TCR signaling defects (*Continued*)**

TCR SS	Gene	Mutation	Protein	T cells			B cells		Clinical presentation	Autoimmunity	Infection	Citation
				Relative numbers	Responsiveness	Repertoire	Relative numbers	Antibodies				
+	***CD3G***	Homozygous: p.K69X	Truncate	↓ Total $CD3^+$ ↓ $CD4^+$	NR low CD3 expression	NR	NL	NL Ig ↓ IgA	CID with autoimmunity	Psoriasis Asthma	Upper/lower respiratory tract infections VZV infection	Sonmez et al., 2025
+	***CD3G***	Homozygous: p.Lys71fs	Truncate	↓ Total $CD3^+$ NL $CD4^+$ ↓ $CD8^+$ ↓ Naive ↑ Memory ↓ Treg	NR	NR	↑ Naive B ↑ Memory B	Positive ANA, high IgE	Lupus-like disease: With immunodeficiency	Autoimmune thyroiditis AIHA ITP Asthma	Recurrent respiratory infections, sinusitis, and latent tuberculosis infection	Lin et al., 2024
+ +	***CD3G***	Homozygous: c.80-1G>C	Splice variant-reduced expression	↓ Total $CD3^+$ ↓ $CD4^+$ ↓ $CD8^+$ ↓ Naive (except Pt1) ↓ γδT	NR	NR	NL	↑ IgG (Pt1, 4) ↓ IgA (Pt3, 4) ↓ IgM (Pt4) ↑ IgE (Pt1, 2) Low anti-HBs titer (Pt4)	CID ($T^-B^+NK^+$)	Diffuse vitiligo (Pt1) Autoimmune thyroiditis (Pt1–5) Atopic dermatitis/ pityriasis alba (Pt2) Autoimmune hepatitis (Pt4) Autoimmune hemolytic anemia (Pt4, 5) Immune thrombocytopenic purpura (Pt4)	Recurrent upper and lower respiratory tract infections VZV *Candida albicans* Giardia intestinalis	Gokturk et al., 2014; Tokgoz et al., 2013
+ +	***CD3G***	Homozygous: c.1A>G (Pt1) Homozygous: c.80-1G>C (Pt2–5) Compound: c.1A>G /c.80-1G>C (Pt6)	Reduced surface expression	NL (Pt1, 4, 5, 6) ↓ Total $CD3^+$ (Pt2, 3) NL $CD4^+$ NL $CD8^+$ ↑ Memory ↑ TEMRA ↑ Exhausted $CD8^+$ ↓ Treg	↓ Proliferation after PHA ↓ CD25 after anti-CD3/28	Skewed use of TRBV and TRBJ	NR	NL for most ↓ IgG (Pt4) ↓ IgA (Pt4)	Immunodeficiency with autoimmunity	Autoimmune enteropathy, GLILD, AIHA (Pt1) Hypothyroidism (Pt2, 3, 4, 6) AIHA, ITP, nephropathy, ALPS (Pt4) Thyroiditis (Pt5) Vitiligo (Pt6)	*Klebsiella pneumoniae*, MSSA, severe EBV (Pt1) Acute bronchitis (Pt2) RRTI, bronchiectasis (Pt4) Soft tissue abscesses, viral meningitis (Pt6)	Rowe et al., 2018
- -	***CD3E***	c.fs43 Creates stop 13 residues downstream p.extracellular domain only	Null	↓ Total $CD3^+$ ↓ $CD4^+$ ↓ $CD8^+$	NR	NR	NL	↓ IgA (Pt2) ↓ IgM (Pt2)	SCID	NR	Pneumonitis, oral candidiasis, disseminated CMV infection	de Saint Basile et al., 2004
- -	***CD3E***	NR	Null	↓ Total $CD3^+$ ↓ $CD4^+$	Absence of T cell proliferation, following PHA anti-CD3 and anti-CD2 antibody-triggered T cell activation	NR	NR	NR	SCID	NR	Recurrent lung infections	Thoenes et al., 1990; Thoenes et al., 1992
- -	***CD3E***	Homozygous: p.Y96X (Pt1) Homozygous: c.352 + 1G>A (Pt2)	Truncate (Pt1) Splice variant (Pt2)	↓ Total $CD3^+$ (Pt1)	NR	NR	↑ B	↓ IgG (Pt1, 2) ↓ IgA (Pt1, 2) ↓ IgM (Pt1)	SCID: Persistent diarrhea	NR	Recurrent pneumonia Disseminated BCGosis (Pt1) Tuberculosis and *Pseudomonas* (Pt2)	Vignesh et al., 2021

Table 2. **Summary of human proximal TCR signaling defects (*Continued*)**

TCR SS	Gene	Mutation	Protein	T cells			B cells		Clinical presentation	Autoimmunity	Infection	Citation
				Relative numbers	Responsiveness	Repertoire	Relative numbers	Antibodies				
- -	***CD3E***	Homozygous: c.fs*9 p.L58H	Truncate	↓ Total $CD3^+$ ↓ $CD4^+$ ↓ $CD8^+$ ↓ Treg	NR	NR	↑ B	↓ IgG (Pt1, 2) ↓ IgA (Pt1, 2) ↓ IgM (Pt1)	SCID ($T^-B^+NK^+$): ARDS, diarrhea	NR	Sepsis CMV (Pt1) Pneumonitis, oral candidiasis (Pt2)	Firtina et al., 2017
- -	***CD3E***	Homozygous: c.567 + 42_567 + 73del	Null-mRNA stability compromised	↓ Total $CD3^+$ ↓ $CD4^+$ ↓ $CD8^+$	NR	NR	NL	↓ IgG (Pt1) ↓ IgA (Pt1, 2) ↓ IgM (Pt1)	SCID: Fever	NR (Pt1) Autoimmune hepatitis (Pt2)	Oral candidiasis, lung infection (Pt1) Persistent candidiasis, CMV, pneumonia (Pt2)	Sonmez et al., 2025
-	***CD3E***	Homozygous: c.IVS7DS, T-C, +2 p.W59X	Truncate-reduced expression	↓ Total $CD3^+$ ↓ $CD4^+$ ↓ $CD8^+$	Absence of T cell proliferation, CD25 expression, and calcium flux after anti-CD3 and anti-CD2	NR	NL	NL Total IgA Defective poliovirus or isohemagglutinin IgA	CID: Recurrent pneumonia	NR	*Hemophilus influenzae* Recurrent otitis media	Soudais et al., 1993; Le Deist et al., 1991
- -	***CD3Z***	Homozygous: p.D138fsX272 eliminates ITAM3	Null	↓ Total $CD3^+$ ↓ $CD4^+$ ↓ $CD8^+$	↓ Proliferation after PHA, Con A, PWM, *Candida*, anti-CD3ε, autologous cells, or allogeneic cells	Oligoclonal	NL	Pre-IVIG values not available	SCID ($T^-B^+NK^+$): Failure to thrive, chronic cough, chronic mild rash, gastroenteritis	No definitive autoimmunity, but some symptoms	CMV *Salmonella* gastroenteritis Recurrent otitis media	Roberts et al., 2007
+ +	***CD3Z***	Heterozygous: p.Y152X and p.Q101X ITAM3 eliminated	Dominant negative	↓ Total $CD3^+$ ↓ $CD4^+$ (Pt1, 2) ↓ $CD8^+$ (Pt1, 2) ↑ Memory (Pt1, 3)	NL Proliferation after anti-CD3	NR	↑ B (Pt1) NL for others Pts	↓ IgG (Pt1) ↓ IgA (Pt1) ↓ IgM (Pt1)	No overt immunodeficiency, but low TRECs (Pt1) Only autoimmunity (Pt2) Autoimmunity (Pt3)	NR (Pt1) Autoimmune hypothyroidism, alopecia areata, anti-thyroglobulin antibodies (Pt2) NR (Pt3) Rheumatoid factor high (Pt4) NR (Pt5) Immune thrombocytopenic purpura (Pt6)	CMV (Pt6)	Briones et al., 2024
-	***CD3Z***	Homozygous: p.Q70X No transmembrane or intracellular domains	Truncated-mosaicism, 10% of cells revertant in other allele	↓ Total $CD3^+$ ↓ $CD4^+$ ↓ $CD8^+$	↓ Proliferation after PHA, anti-CD3, or tetanus	NR	NL	↑ Total, IgG, A, M, E ↓ Tetanus, diphtheria, polio response	SCID	Autoantibodies against erythrocytes and neutrophils detected	*Pseudomonas aeruginosa*, Herpes simplex virus, *C. albicans*, *Streptococcus pneumoniae*	Rieux-Laucat et al., 2006
-	***CD3Z***	Homozygous: p.M1T No extracellular domain	Truncate-0.2% of T cells had revertant somatic mosaicism (p.Q70L/W/Y somatic variants)	↓ Total $CD3^+$ ↓ $CD4^+$ ↓ $CD8^+$ ↓ Naive ↓ γδT ↑ DN ($CD4^-CD8$)	↓ Proliferation (short-term) after anti-CD3 antibody ↓ CD69 upregulation ↓ ZAP-70 ↓ ERK phosphorylation	Reduced TCRβ clonality	↓ Total B ↓ Naive ↑ Memory ↑ DN ($CD27^-IgD^-$)	↑ IgG	SCID, extremely low surface TCR levels	NR	CMV	Marin et al., 2017; Vales-Gomez et al., 2016; Blazquez-Moreno et al., 2017; Briones et al., 2025

Table 2. **Summary of human proximal TCR signaling defects (*Continued*)**

TCR SS	Gene	Mutation	Protein	T cells			B cells		Clinical presentation	Autoimmunity	Infection	Citation
				Relative numbers	Responsiveness	Repertoire	Relative numbers	Antibodies				
- -	*ZAP70*	Heterozygous: Kinase domain 3 aa (LEQ) insertion (Pt1) Heterozygous, compound: p.S518R (Pt2) Affects kinase domain	Null	NL Total CD3$^+$ NL CD4$^+$ ↓ CD8$^+$ ↓ Treg	↓ Proliferation (short-term) after PHA or anti-CD3 ↓ ZAP-70 ↓ ERK phosphorylation ↓ Ca^{2+} flux	NR	NL	NL or increased total Ig Impaired tetanus antibody levels	SCID-like disease	NR	Upper respiratory infections, CMV (Pt1) CMV, rotavirus (Pt2) Otitis media with perforation, *Pneumocystis carinii* pneumonia (Pt3) Oral ulcerations, *P. carinii* pneumonia (Pt4)	Chan et al., 1994; Monafo et al., 1992
-	*ZAP70*	Homozygous: p.3 aa (LEQ) insertion in kinase domain	Null	NL Total CD3$^+$ NL CD4$^+$ ↓ CD8$^+$ ↓ Treg	↓ Proliferation after PHA or anti-CD3 ↓ PLCγ phosphorylation ↓ Ca^{2+} flux	NR	NL	NL	CID	NR	*P. carinii* pneumonia CMV retinitis Parainfluenza virus Chronic viral enteritis	Arpaia et al., 1994; Poliani et al., 2013; Roifman et al., 1989
- -	*ZAP70*	Homozygous: c.13-bp deletion (nucleotides 1,719–1,731) p.Expected to cause a translational frameshift after amino acid 503; kinase dead	Null	↑ Total CD3$^+$ ↑ CD4$^+$ ↓ CD8$^+$	Unresponsive to PHA and anti-CD3	NR	NL	NL	SCID-like disease	NR	NR	Elder et al., 1994
- -/-	*ZAP70*	Homozygous: p.Ala507Val (Pt1) Homozygous: p.Leu337Arg (Pt2) Homozygous: p.Cys564Arg (Pt3)	Null (Pt1, 2) Reduced expression (Pt3)	NL Total CD3$^+$ ↑ or NL CD4$^+$ ↓ CD8$^+$ (Pt1–3)	↓ Proliferation after PHA	NR	NL	↓ IgG (Pt1) ↓ IgA (Pt1) ↓ IgM (Pt1) ↓ IgE (Pt1)	SCID (Pt1): Classic symptoms Failure to thrive (Pt2): Wheezing, recurrent gastroenteritis Omenn-like syndrome (Pt3): Eczematous skin lesions, atopic dermatitis with eosinophilia, elevated IgE	NR (Pt1, 2) Omenn-like syndrome with widespread exfoliative dermatitis and subcutaneous nodules (Pt3)	Lower respiratory tract infections, *Pneumocystis jirovecii*, CMV, *Mycoplasma pneumoniae*, *Mycobacterium* (Pt1) Lower respiratory tract infections, and oral thrush (Pt2) Recurrent pneumonia and oral thrush (Pt3)	Turul et al., 2009
-	*ZAP70*	Homozygous: p.Pro502ArgfsX43 (Pt1) Homozygous: p.Leu337Arg (Pt2) Homozygous: p.Ile398Ser (Pt3)	Null/kinase dead (Pt1) Expression NA (Pt2)	NL Total CD3$^+$ ↑ or NL CD4$^+$ ↓ CD8$^+$ (Pt1–3)	NR	NR	NL	NL Total Ig	CID: Chronic diarrhea and infections (Pt1) Edema around the eyes and in the lower extremities, infections (Pt2)	NR (Pt1) Coombs-positive hemolytic anemia (Pt2)	*Mycobacterium tuberculosis*, *Campylobacter* spp., recurrent oral thrush, encephalomalacias (Pt1) CMV (Pt2)	Akar et al., 2015
-	*ZAP70*	Variable (n = 49)	Variable	↓ CD8$^+$ (97.9%) ↓ CD4$^+$ (12%)	↓ Proliferation after PHA (95% of cases)	NR	↓ B (11% of cases)	Defective antibody production (57%)	73% SCID, 15% CID, 9% ZAP70-deficient phenotype, 3% EBV lymphoproliferative disease	Autoimmunity (19.4% of cases)	Recurrent respiratory infections (81.8% of cases)	Sharifinejad et al., 2020

Table 2. **Summary of human proximal TCR signaling defects (*Continued*)**

TCR SS	Gene	Mutation	Protein	T cells			B cells		Clinical presentation	Autoimmunity	Infection	Citation
				Relative numbers	Responsiveness	Repertoire	Relative numbers	Antibodies				
-/+	*ZAP70*	Homozygous: p.R170C (Pt1) Heterozygous, compound: p.R170C + c.13bp deletion in kinase domain Homozygous: p.R192W (Pt3, 4)	Catalytically inactive (Pt1) Reduced kinase activity (Pt2) SH2 interaction impaired (Pt3, 4)	↓ Total CD3$^+$ (Pt1, 3) ↓ CD4$^+$ (Pt3) ↓ CD8$^+$ (Pt1–4)	↓ Proliferation after PHA or anti-CD3 ↓ PLCγ phosphorylation ↓ ERK phosphorylation	NR	NL (Pt1, 2, 4) ↓ B (Pt3)	↓ IgG (Pt1, 3, 4) ↓ IgA (Pt3, 4) ↓ IgM (Pt3, 4) No titers to diphtheria, tetanus, and pneumococcal vaccine (Pt3)	CID	Alopecia areata and papules (Pt1) Maculopapular rash on face/extremities with unusual clustering, palatal ulcers, bilateral hand arthritis, with inflammation at the proximal, intermediate and distal interphalangeal joints (Pt3)	*Pneumocystis* pneumonia, mycobacterial infection (Pt1) *P. jirovecii* pneumonia mycobacterial infections, recurrent respiratory and gastrointestinal infections (Pt2) Pneumonitis and hepatitis due to MMR vaccine (Pt3)	Mongellaz et al., 2023
+	*ZAP70*	Compound: p.R192W/R360P	Reduced binding to phosphorylated ζ-chain (R192W) Hyperactive kinase (GOF) disrupted autoinhibitory mechanism (R360P)	↓ Total CD3$^+$ (Pt1) ↓ CD4$^+$ T (Pt1) ↓ CD8$^+$ T (Pt1, 2) ↓ Naive (Pt2) ↑ Memory (Pt2)	↓ Proliferation after PHA	NR	↓ B (Pt1) NL (Pt2)	↓ IgM (Pt1, 2) ↓ Pneumococcal IgG, Type 3, 8, 12	Early onset, severe autoimmunity	Nephrotic syndrome, hemarthrosis, inflammatory colitis, bullous pemphigoid, colitis, and proteinuria (Pt1) Bullous pemphigoid, inflammatory colitis, proteinuria (Pt2)	No infection history	Chan et al., 2016
+ +	*ZAP70*	Homozygous: c.836 + 121G>A (intronic)	Reduced expression of alternatively spliced cDNA No protein data shown	↓ Total CD3$^+$ ↓ CD4$^+$ T ↓ CD8$^+$ T ↑ Memory T	↓ Proliferation after PHA or anti-CD3/CD28 ↓ LAT ↓ PLCγ ↓ Akt phosphorylation ↓ Ca^{2+} flux	NR	NL B ↑ Memory B	NL Total Ig ↑ IgE (Pt1) ↓ Anti-poliovirus, anti-tetanus	Healthy: T cells functionally impaired	NR	No serious infection history	Picard et al., 2009
- -	*LAT*	Homozygous: p.Leu16AlafsX28	Expression null	↓ Total CD3$^+$ (Pt1) ↓ CD4$^+$ T (Pt1–3) ↓ CD8$^+$ T (Pt1–3)	↓ Proliferation after PHA ↓ CD69 upregulation ↓ Vav ↓ SLP-76/LAT phosphorylation ↓ Ca^{2+} mobilzation	NR	NL	IgG was present (likely maternal transfer) (Pt2, 3, 5) IgA (below level of detection) (Pt2, 3, 5) ↓↓ IgM (Pt2, 3, 5)	SCID (T$^-$B$^+$NK$^+$): Severe recurrent infections and failure to thrive	NR	Recurrent infections	Bacchelli et al., 2017
-	*LAT*	Homozygous: p.Y207fsTer33	Truncate	↓ Total CD3$^+$ ↓ CD4$^+$ T ↓ CD8$^+$ T	↓ Proliferation after PHA	NR	NL	NL	Leaky SCID (T$^-$B$^+$NK$^+$): Fever, BCGosis, axillary and supraclavicular lymphadenopathy, failure to thrive	NR	BCG	Alizadeh et al., 2023

Table 2. **Summary of human proximal TCR signaling defects (*Continued*)**

TCR SS	Gene	Mutation	Protein	T cells			B cells		Clinical presentation	Autoimmunity	Infection	Citation
				Relative numbers	Responsiveness	Repertoire	Relative numbers	Antibodies				
-/+	*LAT*	Homozygous: c.268_269delGG exon 5 premature stop	Truncate Contains an intact extracellular and transmembrane region but a shortened intracellular region, eliminating the known major phosphorylation sites Y132, Y171, Y191, and Y226	↓ Total $CD3^+$ (Pt1) ↓ $CD4^+$ T (Pt1–3) NL $CD8^+$ T ↓ Naive (Pt2) ↑ Memory (Pt2) ↑ γδT (Pt2, 3) ↓ Treg (Pt2)	↓ Proliferation NL ZAP70, ITK phosphorylation Absent PLCγ1 phosphorylation ↓ ERK phosphorylation ↓ CD69	NR	NL (Pt2) ↓ B (Pt1, 3)	Progressive hypogammaglobulinemia (Pt1, 2) Hypergammagloblunemia (Pt3) Hepatitis B^-, $measles^-$, $mumps^-$, $rubella^-$ (Pt1) $Rubella^+$, $measles^-$, EBV^+, CMV^- (Pt3)	CID: Lymphoproliferation, and life-threatening autoimmune disease since early infancy	$Coombs^+$ AIHA, ITP, autoimmune neutropenia (Pt1) $Coombs^+$ AIHA, ITP (Pt2) Anti-ADAMTS13$^+$ microangiopathic hemolytic anemia (Pt3)	Recurrent pneumonia, EBV/CMV viremia, CMV pneumonia (Pt1) Congenital toxoplasmosis, recurrent pneumonia, VZV, CMV viremia, *Candida* pneumonia with adenovirus (Pt2) Recurrent pneumonia, urinary infections, gastroenteritis, CMV viremia (Pt3)	Keller et al., 2016
-	*SLP76*	Homozygous: c.957 + 1G>A p.K309FSx17	Expression null (*in vivo*) or Truncate (in Jurkat)	↓ Total $CD3^+$ (Pt1) ↓ $CD4^+$ T (Pt1–3) ↑ $CD8^+$ T ↓ Naive (Pt2) ↑ Memory ↑ γδT (Pt2, 3) NL Treg	↓ Proliferation after PHA or anti-CD3/CD28 Cytokine defect upon TCR activation ↓ CD69, CD25, and CD98 upregulation ↓ PLCγ and ERK activation	Restricted TRG and TRB repertoire	↓ Class switched memory B ($IgM^-IgD^-CD19^+CD27^+$) ↓ Transitional B ($IgM2^+$ $CD19^+CD382^+$) ↑ Naive ($IgD^+CD27^-CD19^+$) ↑ Immature B ($IgD^+CD38^+CD19^+$	Pre-IVIG data not available	CID: Early-onset life-threatening infections, T and B cell immunodeficiency, severe neutrophil function defect, impaired platelet aggregation, recurrent skin abscesses, skin rash	Coombs-positive hemolytic anemia	*Aspergillus fumigatus*, BCG, CMV	Lev et al., 2021
-	*SLP76*	Homozygous: c.991del.C p. Q331Sfs*6	Truncate (in Jurkat)	↓ Total $CD3^+$ ↓ $CD4^+$ T ↓ $CD8^+$ T	↓ Proliferation after PHA or anti-CD3/CD28 Cytokine defect upon TCR activation ↓ CD69 upregulation ↓ ERK activation ↓ Ca^{2+} flux	Skewed/ restricted TCR-Vβ	NL	Pre-IVIG data not available	CID with EBV-related lymphoma	Pancytopenia, lymphoproliferation, thrombocytopenia	Enterovirus, PJP, EBV	Lev et al., 2023
--/+	*SLP76*	Compound: p.P190R + p.R204W	Reduced in B, $CD4^+$, $CD8^+$ T, and NK cells	All NL ↓ TemRO	↓ S6 and PLCγ1 phosphorylation	NR	↓ Unswitched memory B ↓ Class-switched memory B	↓ Serum IgA Poor response to Prevenar13 and Pneumovax23	CID: Early-onset immune dysregulation	Specific antibody deficiency, autoimmunity, and inflammatory bowel disease, autoimmune hemolytic anemia	Recurrent oral mucocutaneous candidiasis Recurrent chest infections: *Morganella morganii*, *Raoultella planticola*, *Enterobacter cloacae* complex, *Staphylococcus aureus*, *P. aeruginosa*, *C. albicans*, *Serratia marcescens*, *Klebsiella oxytoca*, and *Citrobacter koseri*, *Stenotrophomonas* sp., *C. albicans*, methicillin-resistant *S. aureus*, and *Pseudomonas* sp.	Edwards et al., 2023

Table 2. **Summary of human proximal TCR signaling defects (*Continued*)**

TCR SS	Gene	Mutation	Protein	T cells			B cells		Clinical presentation	Autoimmunity	Infection	Citation
				Relative numbers	Responsiveness	Repertoire	Relative numbers	Antibodies				
-/+	*ITK*	Homozygous: p.R335W SH2 domain mutation	Expression null (in Jurkat)	↓ Total $CD3^+$ ↓ $CD4^+$ T ↓ $CD8^+$ T ↓ Treg ↓ NKT	NL PHA response ↓ Proliferation after anti-CD3/CD28 ↓ Ca^{2+} flux	NR	↑ B	Hypogammaglobulinemia NL vaccine response (diphtheria, tetanus, strep, and Hib)	CID with EBV-associated lymphoproliferative disorder and immune dysregulation	Anemia, thrombocytopenia (Pt1)	Aphthous stomatitis, candida stomatitis, EBV, BK polyomavirus infection, *P. jirovecii* pneumonia (Pt1) EBV (Pt2)	Huck et al., 2009
-/+	*ITK*	Homozygous: p.Thr110ArgfsTer155	Nonsense mediated decay (no protein data shown)	↓ Total $CD3^+$ ↓ Th17 ↑ Th1 ↑ Treg	↓ Proliferation after anti-CD3/CD28	NR	↑ B	NR	Hodgkin's lymphoma	NR	EBV, pneumonia	Eken et al., 2019
-/+	*ITK*	Homozygous: p.Y588X Predicted to produce kinase dead truncate	Undetectable protein	↓ Total $CD3^+$ ↓ $CD4^+$ T (Pt2) ↑ $CD8^+$ T (Pt2) ↓ $CD8^+$ T (Pt3) ↓ Naive (Pt2) ↑ Memory (Pt2) ↓ Treg ↓ NKT (Pt3)	↓ Ca^{2+} flux	NR	↑ B	Hypogammaglobulinemia	Hodgkin's lymphoma	Glomerulonephritis, Tubulointerstitial nephritis (Pt2)	Persistent infectious mononucleosis, including recurrent febrile episodes, lymphadenopathies, extremely high EBV viral load	Stepensky et al., 2011
+	*ITK*	p.R29H (Pt1) p.D500T, F501L, M503X (Pt2)	Reduced membrane targeting (Pt1) Reduced protein (Pt2)	↓ Total $CD3^+$ ↓ $CD4^+$ T (Pt1, 2) ↓ $CD8^+$ T ↓ Naive (Pt1, 2) ↑ Memory (Pt2) ↓ NKT (Pt1, 2)	↓ Ca^{2+} flux	NR	↑ B	Hypogammaglobulinemia (Pt1)	B cell lymphoma Proliferative disease (Pt1) Large B cell lymphoma (Pt2): Lymphomatoid granulomatosis	AIHA, ITP (Pt1)	EBV	Linka et al., 2012

"- -" indicates absent signal strength, correlating with SCID; "-" indicates near-absent signal strength, correlating with leaky SCID; "-/+" indicates severely reduced signal strength, correlating with CID with significant infections; "+" indicates low but present signal strength, correlating with autoimmunity-predominant CID; "+ +" indicates mildly reduced signal strength, correlating with mainly autoimmunity or mild immunodeficiency; "+ + +" indicates near-normal signal strength, correlating with healthy or minimal clinical impact.

AIHA, autoimmune hemolytic anemia; ALPS, xxx; ARDS, acute respiratory distress syndrome; BCG, Bacillus Calmette-Guérin; GLILD, granulomatous lymphocytic interstitial lung disease; GOF, gain-of-function; HBs, Hepatitis B surface Antigen; Hib, *Haemophilus influenzae* type b; ITP, immune thrombocytopenia purpura; PJP, *P. jirovecii* pneumonia; LEQ, Leucine-Glutamate-Glutamine; LOF, loss-of-function; MSSA, methicillin-susceptible *Staphylococcus aureus*; NR, not reported; NL, normal; Pt, patient; PWM, pokeweed mitogen; RRTI, recurrent respiratory tract infections; TRAV, T cell Receptor Alpha Variable; TREC, T-cell receptor excision circles.

partial ζ/γ chain signaling can permit thymic escape of autoreactive clones (Arnaiz-Villena et al., 1992; Delmonte et al., 2022; Gokturk et al., 2014; Lee et al., 2019; Briones et al., 2024; Recio et al., 2007; Rowe et al., 2018; Sonmez et al., 2025; Tokgoz et al., 2013) (Delmonte et al., 2021). Defects in CD3E similarly disrupt TCR complex stability and produce SCID or leaky SCID phenotypes depending on residual protein expression (de Saint Basile et al., 2004; Le Deist et al., 1991; Sonmez et al., 2025; Soudais et al., 1993; Thoenes et al., 1990; Thoenes et al., 1992; Vignesh et al., 2020). Finally, *CD3Z* (CD247) mutations impair ITAM density and ZAP-70 docking despite intact LCK function, yielding combined immunodeficiency with defective signal propagation and abnormal activation states (Blazquez-Moreno et al., 2017; Briones et al., 2024; Briones et al., 2025; Marin et al., 2017; Roberts et al., 2007; Vales-Gomez et al., 2016). Collectively, CD3 defects demonstrate that when the ITAM scaffold is weakened, LCK may be functional but cannot write its signal onto a compromised substrate—the volume dial turns, but the signal has nowhere to go.

ZAP-70 deficiency provides the clearest parallel to LCK-IEI. Initial studies identified autosomal-recessive ZAP-70 deficiency as a cause of SCID, demonstrating that complete absence of ZAP-70 abolishes effective TCR signal propagation despite intact CD3 assembly and ITAM phosphorylation by LCK (Chan et al., 1994; Monafo et al., 1992; Roifman et al., 1989). Independently, Arpaia and colleagues showed that humans lacking functional ZAP-70 exhibit selective absence of $CD8^+$ T cells, with residual $CD4^+$ T cells that are numerically preserved but functionally impaired—reflecting subthreshold signaling is insufficient for $CD8^+$ positive selection yet permissive for partial $CD4^+$ development (Arpaia et al., 1994). Subsequent identification of hypomorphic ZAP-70 variants revealed that intermediate reductions in kinase activity produce some T cells resulting in a leaky SCID phenotype with immune dysregulation and autoimmunity (Akar et al., 2015; Ashouri et al., 2022; Chan et al., 2016; Mongellaz et al., 2023; Picard et al., 2009; Poliani et al., 2013; Sharifinejad et al., 2020; Turul et al., 2009). These human observations are reinforced by SKG mice, which carry a partial loss-of-function ZAP-70 mutation that lowers TCR signaling thresholds, alters thymic selection, and drives systemic autoimmunity (Sakaguchi et al., 2003). Notably, the SKG model—derived from a spontaneous mutation rather than an engineered deletion—demonstrates that mouse models mimicking the graded defects seen in human IEI can reveal pathogenic mechanisms invisible to binary knockout approaches. Together, human and murine ZAP-70 studies establish that intermediate signal amplitude is uniquely pathogenic, a principle that closely parallels hypomorphic LCK-IEI such as the P440S Lui variant.

Farther along the pathway, study of autosomal-recessive mutations in LAT, SLP-76 (LCP2), and ITK reveal that adaptor and kinase defects also modulate TCR signal amplitude in nonbinary ways (Edwards et al., 2023; Delmonte et al., 2022; Ogishi et al., 2023; Ghosh et al., 2018; Huck et al., 2009). LAT deficiency results in combined immunodeficiency with impaired downstream signal propagation and abnormal lymphocyte activation states (Alizadeh et al., 2023; Bacchelli et al., 2017; Keller et al., 2016). SLP-76 deficiency can cause combined immunodeficiency with thrombocytopenia, impaired calcium flux, and defective MAPK activation, producing a phenotype in which some T cells develop but fail to amplify TCR signals effectively (Edwards et al., 2023; Kim et al., 2009; Keller et al., 2023; Delmonte et al., 2022; Ogishi et al., 2023). ITK deficiency, by contrast, often presents with EBV-driven lymphoproliferation, $CD4^+$ lymphopenia, skewed effector $CD8^+$ populations, and variable NK and NKT cell abnormalities (Eken et al., 2019; Ghosh et al., 2018; Huck et al., 2009; Linka et al., 2012). In ITK-deficient patients, proximal TCR signaling through LCK, CD3, and ZAP70 remains intact, but PLCγ1 activation and downstream calcium signaling are selectively blunted (Ogishi et al., 2023). The result is a T cell repertoire with disproportionate expansion of EBV-specific or activated $CD8^+$ T cells and impaired T helper function, again reflecting attenuated TCR signal rather than complete silence.

Taken together, human IEI affecting *LCK*, *TCRA*, *CD3D/G/E/Z*, *ZAP70*, *LAT*, *SLP-76*, and *ITK* trace the proximal TCR signaling axis, with lesions at each node imposing distinct constraints on T cell development and function. The result is node-specific distortion of $CD4^+$, $CD8^+$, Treg, and unconventional T cell compartments—further evidence that graded reductions in TCR signal amplitude guide thymic output, repertoire selection, and tolerance. LCK occupies the apex of this cascade—serving as the most proximal "volume control knob." Its allelic variants determine not only whether ITAMs can be phosphorylated but also the dynamic range over which downstream components operates. Characterizing these IEI in even greater detail will be essential for defining the quantitative thresholds that govern human T cell development. For affected patients, this knowledge informs restorative approaches such as HSCT and gene therapy. For the broader field, these naturally occurring hypomorphs caution that therapeutic modulation of TCR signaling must restore immune sufficiency without inducing autoimmunity. Importantly, while HSCT restores the hematopoietic compartment and T cell progenitor pool, it does not repair a thymic environment already distorted due to the abnormal thymocyte–thymic epithelial cell (TEC) cross talk. The role of this cross talk in health and disease—and how LCK and other TCR proximal defects perturb it—is discussed in the following section. Understanding this interplay has direct therapeutic relevance. Recognition that thymocyte–TEC cross talk is essential for T cell development, selection, and differentiation underpins the recent Food and Drug Administration (FDA) approval of cultured thymus tissue implantation (CTTI). While currently approved only for congenital athymia (CA), CTTI holds promise as adjunctive or primary therapy for genetic defects in which T cell lesions secondarily disrupt thymic architecture. Beyond this, the mechanistic clarity that rare monogenic disorders provide may guide therapeutic strategies for reshaping effector and Treg balance in far more prevalent autoimmune and oncologic conditions.

Setting the dial: Signal amplitude across thymic microenvironments

Recent advances in spatial and single-cell mapping have reframed thymopoiesis as a spatially organized process rather than

a uniform selection arena (Fig. 3 A). High-resolution thymic atlases demonstrate that cortical, medullary, and mesenchymal compartments generate distinct antigenic landscapes, cytokine gradients, and cellular neighborhoods that guide thymocyte migration and fate decisions along the corticomedullary axis (Yayon et al., 2024; Gustafsson et al., 2025). TECs—divided into cortical and medullary subtypes—provide thymocytes with essential developmental signals via IL-7, Notch ligands (DLL1 and DLL4), and self-peptide–MHC complexes (Kadouri et al., 2020). In turn, developing thymocytes reciprocally deliver TCR-dependent signals required for TEC maturation and spatial organization (Lopes et al., 2015; Stephen et al., 2012). Together with foundational work by Yayon and colleagues, these studies substantiate that TCR signal strength, dwell time, and spatial context jointly shape both thymocyte fate and thymic structure itself (Yayon et al., 2024). Not surprisingly, perturbation of this critical bidirectional cross talk can result in variety of clinical affects, from mild to severe.

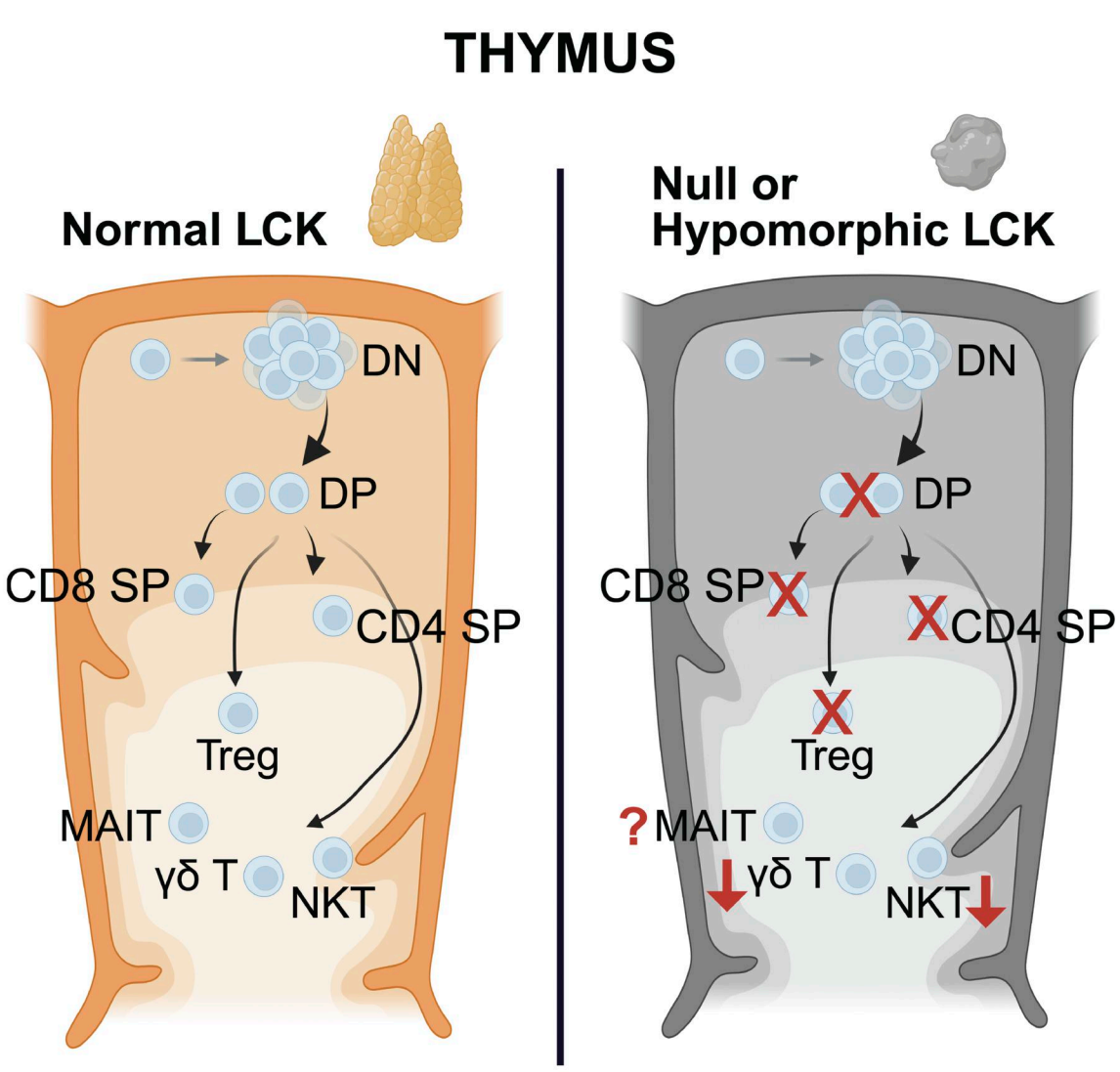
LCK essentially functions as a molecular interpreter in T cell development, translating TCR engagement into graded intracellular responses that must be correctly matched to the local thymic microenvironment. A key determinant of LCK's interpretive capacity is its dynamic stoichiometry with CD4/CD8 co-receptors. Developmental shifts in co-receptor expression from early thymocytes to mature peripheral T cells govern the balance between free and co-receptor–bound LCK pools, thereby modulating both TCR signal amplitude and duration at each stage (Douglass and Vale, 2005; Nika et al., 2010). Human LCK variants that reduce LCK protein abundance, disrupt co-receptor coupling, or impair catalytic activity lock TCR signal amplitude, preventing thymocytes from both giving and receiving appropriate microenvironmental-specific cues. Thus, T cell fate depends not only on the amplitude at which the dial locks but also on which developmental stages and thymic niches that set-point can sustain (Granja et al., 1994; Irles et al., 2003; Van Laethem et al., 2007).

Human LCK-IEI reveal what happens when LCK interpretation of TCR engagement fails in patients. As illustrated in Fig. 3 A, right, individuals with LCK-IEI exhibit thymic atrophy, profound depletion of cortical $CD4^+CD8^+$ (DP thymocytes and reduced mature single-positive (SP) populations, consistent with a developmental block at the positive selection checkpoint (Lui et al., 2024). Impaired LCK function flattens TCR signaling gradients, blurring the distinction between weak self-peptide: MHC interactions that normally permit $CD4^+$ and $CD8^+$ maturation and stronger signals that enforce central tolerance. The consequence is not merely reduced thymic output, but a qualitatively distorted TCR repertoire, with preferential loss of intermediate signal–dependent Treg differentiation, erosion of diverse $CD4^+$ lineages, and relative preservation of clones selected under low-signal or homeostatic conditions. While conventional αβ T cells and Tregs are severely affected—often with differential impacts on subset number and function—consequences for unconventional T cell populations (γδ T, NKT, and MAIT) and the thymus itself remain insufficiently characterized. Further, thymic architectural abnormalities observed in CD3δ-deficient patients demonstrate that disruption of TCR signaling can result in thymic niche disorganization (de Saint Basile et al., 2004). Although detailed assessment of human thymic tissue in LCK-IEI has not been performed, this finding suggests that similar secondary niche disorganization may occur in LCK deficiency and other disorders characterized by aberrant TCR signaling.

Murine models illustrate this principle—that TCR signal strength and microenvironmental context converge to shape both T cell fate and thymic integrity—with more granularity (Fig. 3 B). *Lck*$^{-/-}$ mice exhibit a partial block at the DN3–DN4 transition and impaired positive selection at the DP stage, resulting in pronounced thymic atrophy with a sparsely populated cortex and severely diminished medulla (Molina et al., 1992). Mutations in other proximal signaling components produce comparable effects, with architectural consequences tied to the stage of developmental arrest. CD3ε-overexpressing mice arrest at DN1, the earliest double negative (DN stage), and display disturbed cortical epithelial architecture (Holländer et al., 1995). *Rag1*$^{-/-}$ and *Rag2*$^{-/-}$ mice arrest at DN3, retaining cortical structure but exhibiting defective medullary development. *Zap70*$^{-/-}$ and *Tcrα*$^{-/-}$ mice similarly accumulate DP thymocytes that fail positive selection, showing cortical expansion or no change and medullary collapse (Negishi et al., 1995; Philpott et al., 1992; Surh et al., 1992). Finally, Cd3ζ$^{-/-}$ mice display impaired β-selection with incomplete DN–DP blockade, yielding small thymuses with markedly reduced DP thymocytes, near-absent mature SP cells, and diminished medullary regions (Ardouin et al., 1998; Love et al., 1993; Malissen et al., 1993). In each of these models, thymic abnormalities almost certainly arise from impaired thymocyte development rather than intrinsic stromal abnormalities, as expression of these proteins is restricted to lymphoid lineage cells (Heng et al., 2008; Liu et al., 1993). Notably, thymic damage caused by thymocytes with defective TCR-signaling is reversible in mice. Transplantation of T cell–depleted bone marrow into SCID models restores thymic architecture, while transfer of mature T cells alone promotes medullary recovery (Shores et al., 1991; Surh et al., 1992). However, timing is important—medullary restoration early after HSCT is critical for re-establishing tolerance (Takahama, 2022).

These murine findings have clinical implications for patients with LCK-IEI. If prolonged LCK dysfunction has damaged the thymic medulla before transplant, HSCT may not fully restore architecture—potentially compromising reconstitution and predisposing to autoimmunity even after successful engraftment. Clinical observations support this concern. Patients with hypomorphic LCK defects who underwent HSCT—including one diagnosed in infancy—failed to achieve sustained T cell reconstitution or a diverse TCR repertoire (Manfred Hoenig, personal communication). Findings in other IEI patients with TCR signaling defects reinforce this pattern. Among 60 patients with hypomorphic RAG1/RAG2 mutations, 15% developed new-onset autoimmunity after HSCT, preexisting autoimmunity persisted in approximately half, and resolution occurred in only 75% (Schuetz et al., 2023). Delmonte et al. further showed that poor TCR β diversity early after HSCT predicts reconstitution failure in patients with leaky SCID—consistent with the hypothesis that distorted, rather than absent, T cell development inflicts thymic

A



B

MUTATION	DEVELOPMENTAL ARREST	THYMIC ARCHITECTURE
Lck-/-	Partial block at DN3-DN4 (β-selection) impaired positive selection at DP stage; severe DP reduction	Pronounced thymic atrophy, sparsely populated cortex, severely diminished medulla; cellularity 1-5% of normal
CD3ε overexpression Tgε26	DN1 (CD44+CD25-)	Disturbed cortical epithelium meshwork, very small thymus
Rag1-/- Rag2-/-	DN3 (CD44-CD25+)	Cortical structure retained; defective medullary development
Zap70-/-	DP stage (fail positive selection) DP accumulation; no SP	Cortical expansion with medullary collapse
TCR α-/-	DP stage (fail positive selection) DP accumulation; no SP	Cortical epithelium unaltered; medulla considerably reduced/absent
CD3 ζ-/-	Partial block at DN-DP; DP reduction	Pronounced thymic atrophy, hypocellular/attenuated cortex, severely diminished medulla; cellularity 3-50% of normal

Figure 3. **Impact of proximal TCR signaling defects on thymic development. (A)** Left: Spatial organization of thymic microenvironments from cortex to medulla, with zones of positive and negative selection indicated. Right: Comparison of normal versus null/hypomorphic LCK thymi (in cross section). LCK deficiency impairs αβ lineage output (CD4 SP, CD8 SP, and Treg), while γδ T and NKT cells are reduced but preserved; MAIT cell status remains uncertain. **(B)** Mouse models of proximal TCR signaling defects, showing stage of developmental block and resulting thymic architecture. CMJ, corticomedullary junction.

damage that hematopoietic stem cell replacement alone cannot repair (Delmonte et al., 2022). At present, HSCT remains the only definitive therapy for LCK-IEI, albeit with limited and unpredictable success (Hauck et al., 2012; Keller et al., 2023; Lanz et al., 2023; Lui et al., 2024). In some SCID/CID settings, successful HSCT can restore thymic output and sustain long-term immune competence (Berteloot et al., 2021; Marrella et al., 2014; Patel et al., 2000). Yet peripheral immune reconstitution does not necessarily equate to restoration of thymic niche architecture or durable tolerance, particularly when preexisting damage is substantial.

CTTI offers a complementary approach that directly addresses the thymic niche and provides a powerful platform for investigating how LCK-calibrated TCR signals influence stromal architecture. CTTI, currently FDA-approved only for CA, demonstrates that hematopoietic progenitors can undergo thymopoiesis on an allogeneic thymic scaffold and generate functional, self-tolerant T cells (Hale et al., 2026). In CTTI recipients, host-derived thymocytes are selected on donor thymic epithelium, re-establishing a diverse αβ TCR repertoire capable of supporting antimicrobial immunity, vaccine responses, and T cell help—despite persistent lymphopenia and MHC mismatch (Markert et al., 2022). Importantly, the variable emergence of autoimmunity following CTTI mirrors the unpredictability observed after HSCT in LCK-IEI and leaky SCID/CID, underscoring that restoration of thymic output alone is insufficient for durable tolerance (Hauck et al., 2012; Keller et al., 2023; Lanz et al., 2023; Lui et al., 2024). Instead, tolerance appears to depend on successful reconstitution of medullary niches that enforce appropriate TCR signal thresholds—particularly those intermediate-strength signals required for Treg differentiation. Viewed through the lens of LCK-dependent signal interpretation, CTTI may enable a biological "reset" of thymic spatial organization. For patients with LCK-IEI specifically, CTTI raises the possibility that providing a functional thymic niche—rather than simply replacing hematopoietic progenitors—may be necessary to overcome the architectural damage inflicted by prolonged TCR signaling dysfunction.

Together, these observations establish LCK as a regulator not only of thymocyte-intrinsic signaling thresholds but also of how developing T cells engage, traverse, and are instructed by spatially organized thymic niches. HSCT and CTTI represent complementary natural experiments revealing that immune reconstitution and immune tolerance can be uncoupled—and that LCK-dependent signal interpretation may be central to both. Future investigations integrating human LCK genetics with spatially resolved thymic analyses (spatial transcriptomics, multiplexed imaging, and microdomain-resolved single-cell mapping) will be essential to define which niches fail in each LCK mutant allele and how impaired LCK alters thymocyte migration, residence time, and signal decoding. CTTI recipients offer a unique opportunity for such studies, as thymic tissue can be directly analyzed before implantation and T cell output monitored longitudinally thereafter. Such investigations will clarify how LCK and thymic microenvironments collaborate to establish the signaling thresholds that govern T cell fate, self-tolerance, and immune homeostasis—and may point toward therapeutic strategies that restore not just thymic output but the architectural integrity on which durable tolerance depends.

Scanning for new stations: Emerging questions in LCK biology

Despite decades of research establishing the critical role of LCK in TCR signaling, many important questions await resolution. Although phosphorylation/dephosphorylation of the core regulatory sites—Y394 (activating) and Y505 (inhibitory)—are well defined, how membrane/lipid raft localization, co-receptor association, and other posttranslational modifications (ubiquitination, palmitoylation, and acetylation) jointly fine-tune LCK function is incompletely understood (Nath and Isakov, 2024). Advances in proteomics, base-editing screens, and high-resolution structural analyses are beginning to reveal heterogeneity in LCK activation states (Salter et al., 2018; Walsh et al., 2025). Future work integrating single-cell phosphoproteomics and quantitative modeling will be critical to uncover how stochastic differences in LCK state drive tolerance, exhaustion, or robust effector responses. Further, the recognition of LCK as an analog modulator of TCR signaling—rather than a binary switch—reframes long-standing questions in T cell biology and opens a new set of conceptual and experimental frontiers. Insights from human LCK-IEI, together with advances in spatial, single-cell, and systems immunology, now compel a shift from asking if LCK is required to how, where, and to what extent LCK modulates TCR signaling across developmental stages, tissue niches, and immune contexts. Of particular importance, in addition to what is happening in the thymus, is whether LCK signaling is modulated differently in tissue-resident memory T cells compared with circulating effector or central memory subsets. Given the metabolic and spatial distinctions of these compartments, determining whether LCK phosphorylation dynamics, half-life, or co-receptor dependence differ across memory populations represents an important direction for defining context-dependent immune responses. Addressing these gaps will refine our mechanistic knowledge and guide translational applications.

What are the precise LCK signaling thresholds for distinct thymic fates?

Human LCK-IEI reveal that different T cell lineages operate within partially overlapping but nonidentical signaling amplitudes. Minimal residual LCK activity may suffice for limited αβ thymopoiesis, whereas higher thresholds appear necessary for stable CD4 lineage commitment and, critically, for Treg differentiation and function. In contrast, γδ T cell development is relatively preserved—or even expanded—across a wide range of LCK dysfunction, underscoring their reduced dependence on LCK. Defining these thresholds will require allele-series modeling that moves beyond knockout versus WT comparisons. CRISPR-engineered hypomorphic alleles, allelic replacement systems, and patient-derived induced pluripotent stem cell–based thymopoiesis models offer opportunities to titrate LCK expression and/or function across a continuous range and directly link TCR signal amplitude to fate decisions. Coupling such systems with quantitative readouts of proximal signaling, e.g.,

ITAM, ZAP-70, LAT phosphorylation, and downstream transcriptional activation (via single-cell RNA sequencing) will enable more precise mapping of developmental checkpoints as a function of TCR signal strength.

How does thymic spatial context shape LCK-mediated signaling and vice versa?

As discussed, human T cell development unfolds within highly structured thymic microenvironments along the corticomedullary axis, with distinct stromal, epithelial, and myeloid niches. These niches differ in cytokine availability, antigen presentation, and co-stimulatory landscapes—factors that are likely to modulate LCK localization, activation state, and dwell time at the TCR. Limited access to human thymus tissue (a constraint that does not apply to mouse models) restricts research in this area and obscures where along the spatially partitioned landscape T cell development arrests. Current LCK-IEI studies rely almost entirely on peripheral blood as a proxy, capturing only the output of thymic selection, not the process itself. Fortunately, clinical thymus implantation programs may change this by providing a feasible source of human thymic tissue for spatially resolved studies that pinpoint where LCK dysfunction disrupts T cell development.

While HSCT alone restores hematopoiesis but not thymic architecture, thymus implantation provides the stromal scaffold necessary for physiologic selection. For LCK deficiency and related IEI where repertoire distortion, Treg insufficiency, and impaired central tolerance are dominant drivers of pathology, adjunct thymus implantation could, in principle, correct the site of selection failure, not only the hematopoietic input. This approach could help restore a diverse naive $CD4^+/CD8^+$ pool, normalize selection thresholds, and re-establish Treg development—addressing defects that HSCT alone may not fully repair. Maximizing this therapeutic potential will require defining niche-specific signaling thresholds. Spatial phosphoomics could provide that resolution, revealing whether a locked LCK set-point yields constant TCR signal strength across niches or shifts depending on stromal context.

What determines subset-specific vulnerability to LCK perturbation?

Across LCK-IEI, $CD4^+$ T cells—particularly naive and regulatory subsets—are consistently more affected than $CD8^+$ T cells, suggesting differential dependence on LCK dosage, co-receptor coupling, or compensatory Src-family kinase usage. Whether this reflects stronger reliance on CD4-associated LCK, distinct tonic signaling requirements, or lineage-specific thresholds for survival and proliferation remains unresolved. Our understanding of LCK function remains heavily skewed toward conventional αβ T cells, with unconventional/innate-like T cells—γδ, NKT, and MAIT cells—poorly characterized in humans despite evidence that LCK activity influences lineage commitment during thymic development. Recently described autosomal-recessive LCK mutants clinically present along a continuum from SCID to combined immunodeficiency with autoimmunity and immune dysregulation (Hauck et al., 2012; Keller et al., 2023; Lanz et al., 2023; Lui et al., 2024; Li et al., 2016), yet fundamental questions about unconventional T cell development and function in these patients are unaddressed. Do γδ T cells, NKT cells, and MAIT cells successfully exit the thymus in LCK-deficient individuals, and if so, at what frequencies and in what ratios compared with healthy controls? For those subsets that do develop, what are their functional capabilities—can they respond to cognate antigens, produce cytokines, mediate cytotoxicity, and contribute to immune surveillance? Unconventional T cell subsets, γδ T, NKT, and MAIT, account for ~10% of circulating T cells and can comprise the bulk of tissue resident cells, particularly in the liver and barrier tissues such as the skin, gut, and lungs (Constantinides and Belkaid, 2021). Although the basic requirement for LCK for proximal signaling events in human γδTCR signaling is expected based on the studies in murine γδ T cells, intriguingly, across reported human LCK deficiency cases, patients exhibit profound αβ T cell lymphopenia yet show relative enrichment of γδ T cells in peripheral blood, consistent with the differential LCK dependence observed in mice and implying altered selection thresholds when LCK is globally limiting (Hauck et al., 2012; Keller et al., 2023; Lanz et al., 2023; Lui et al., 2024). However, comprehensive characterization of human γδ T cells in LCK-IEI patients, including TCR repertoire analysis, detailed functional studies comparing tissue-resident subsets across anatomical sites and absolute γδ counts, remains incomplete. Emerging evidence places γδ T cells at the nexus of mucosal immune surveillance and pathology in colitis as well as skin. In the human gut, γδ T cells reside in the epithelial and lamina propria compartments, where they regulate barrier integrity, epithelial repair, and microbial interactions. Studies of human LCK-IEI have shown that altered TCR signaling skews T cell development and function, suggesting that even subtle perturbations in LCK action could modify γδ T cell fate decisions. Defining how LCK-dependent signaling thresholds govern γδ T cell homeostasis may reveal mechanisms driving their pathogenic versus reparative roles in colitis and guide therapeutic strategies that selectively target inflammatory γδ T subsets while preserving tissue-protective ones. Dissecting these differences will benefit from thymic organoid systems that allow controlled manipulation of stromal composition, cytokine gradients, and co-receptor engagement while preserving key aspects of thymic architecture. Organoids seeded with allele-defined progenitors can be used to test how graded LCK activity intersects with lineage-specific cues, providing a reductionist but physiologically grounded complement to *in vivo* studies.

How do graded defects in LCK reshape immune tolerance and dysregulation?

One of the most striking lessons from LCK-IEI is that partial—not complete—loss of LCK function is most permissive for immune dysregulation. Intermediate TCR signaling levels support effector differentiation and homeostatic expansion while failing to enforce central and peripheral tolerance, particularly through impaired Treg development and function. This raises broader questions that extend beyond monogenic disease: do subtle reductions in proximal TCR signaling—whether genetic,

epigenetic, or environmentally induced—contribute to common autoimmune and inflammatory disorders? Addressing this will require integrative approaches linking thymic selection to peripheral tissue immunity, including paired thymus and PBMC analyses with longitudinal immune profiling in patients and model systems with graded signaling defects.

Can thymocyte TCR signaling be restored or recalibrated therapeutically?

Finally, conceptualizing LCK as a volume dial rather than a binary switch has direct translational implications. Rather than global inhibition or activation, future therapeutic strategies may aim to re-establish appropriate signaling bandwidths. Approaches such as CTTI, co-transplantation of thymic tissue with hematopoietic progenitors, or niche-targeted modulation of stromal support cells offer potential avenues to restore TCR diversity and tolerance in settings of disrupted thymic architecture. In parallel, precision therapies informed by allele-specific signaling defects—such as stabilizing hypomorphic LCK proteins, modulating co-receptor coupling, or enhancing compensatory pathways—may allow selective correction of immune insufficiency without precipitating autoimmunity. Human genetic variation thus provides not only mechanistic insight, but also a roadmap for therapeutic recalibration of TCR signaling.

Moving forward, LCK research must bridge mechanistic studies in conventional αβ T cells with investigations in unconventional T cells, tissue-resident subsets, and human genetic immunodeficiencies. A combined approach integrating mouse genetics, patient cohorts, thymic organoid systems, and translational immunotherapy models will be required to fully define the breadth of LCK's roles in immunity and disease. Ultimately, fine-tuning TCR signaling remains the goal, with LCK representing one critical lever among many. Human LCK-IEI offer what reductionist models could not—a window into how graded dysfunction at one node ripples through the entire signaling network as signal amplitude and tissue context converge to shape immune outcomes. With the field poised to move from signal detection to signal design, these naturally occurring variants will help show us not just how to read the volume dial but how to adjust it. Stay tuned.

Online supplemental material

Table S1 shows a comparison of LCK genetic variants (mouse versus human).

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Supplemental material

Provided online is Table S1. Table S1 shows a comparison of LCK genetic variants (mouse versus human).