Fusion transcripts FYN-TRAF3IP2 and KHDRBS1-LCK hijack T cell receptor signaling in peripheral T-cell lymphoma, not otherwise specified

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Peripheral T-cell lymphoma (PTCL) is a heterogeneous group of non-Hodgkin lymphomas with poor prognosis. Up to 30% of PTCL lack distinctive features and are classified as PTCL, not otherwise specified (PTCL-NOS). To further improve our understanding of the genetic landscape and biology of PTCL-NOS, we perform RNA-sequencing of 18 cases and validate results in an independent cohort of 37 PTCL cases. We identify FYN-TRAF3IP2, KHDRBS1-LCK and SIN3A-FOXO1 as new in-frame fusion transcripts, with FYN-TRAF3IP2 as a recurrent fusion detected in 8 of 55 cases. Using ex vivo and in vivo experiments, we demonstrate that FYN-TRAF3IP2 and KHDRBS1-LCK activate signaling pathways downstream of the T cell receptor (TCR) complex and confer therapeutic vulnerability to clinically available drugs.
Peripheral T-cell lymphoma (PTCL) arises from post-thymic mature T lymphocytes. PTCL comprises 10–20% of non-Hodgkin lymphomas, depending on geographical variation. With the exception of ALK- and DUSP22-translocated anaplastic large cell lymphoma (ALCL), the outcome with current therapies is unsatisfactory. Up to 30% of PTCL cases lack distinctive features and are classified as PTCL, not otherwise specified (PTCL-NOS). Five-year overall survival for PTCL-NOS has stagnated at 30% and guidelines for first-line treatment recommend to treat patients with PTCL-NOS in the context of a clinical trial. Although efforts have been made to study the genetic landscape of PTCL-NOS, the disease biology remains poorly characterized, which hampers the rational design of new clinical trials.

Engagement of immunoreceptors elicits a brisk proliferative and metabolic response in B cells and T cells. This response is intensely intertwined with the Nuclear Factor Kappa light chain enhancer of activated B cells (NF-kB) pathway. Oncogenic activation of the NF-kB pathway by somatic single nucleotide variants and copy number variants is frequent in activated B-cell-like diffuse large B-cell lymphoma. With the notable exception of translocations involving MALT1 in mucosa-associated lymphoid tissue lymphoma and an IKT-SYK gene fusion in peripheral T-cell lymphoma with a T follicular helper phenotype (PTCL-TFH), activation of the NF-kB pathway by gene fusions is rare. On the whole, gene fusions are recurrent disease drivers in hematological malignancies of both lymphoid and myeloid origin. A subset of gene fusions gives rise to chimeric proteins with altered kinase activity or specificity, altered transcriptional regulation or altered post-translational regulation. Chimeric transcripts are distinctive disease features with a pivotal role in oncogenesis and have also been identified in PTCL. These features render them ideal candidates for therapeutic intervention.

In this study, we identify two novel chimeric transcripts in a cohort of PTCL-NOS that hijack signaling pathways downstream of the T cell receptor (TCR) complex. We describe a recurrent FYN-TRAF3IP2 fusion transcript in PTCL-NOS and PTCL-TFH and a KHDRBS1-LCK fusion transcript in PTCL-NOS. Furthermore, we show that both transcripts confer therapeutic vulnerability to clinically available drugs.

Results
Transcriptome sequencing identifies fusion transcripts in PTCL-NOS. To identify fusion events in PTCL-NOS, we performed high coverage, paired-end RNA sequencing (RNA-seq) on a cohort of 15 PTCL-NOS (Supplementary Table 1) cases and 3 PTCL-TFH. Gene expression analysis indicated expression of T follicular helper (T<sub>F</sub>) markers in the PTCL-TFH cases (Supplementary Fig. 1a). In agreement with the histopathological diagnosis, a subset of PTCL-NOS cases expressed TNFRSF8 (CD30). Expression of the ALCL marker genes TNFRSF8 and SIN3A-FOXO1 (case PTCL12) and TMOD1 clearly separated the PTCL-NOS cases from ALCL cases (Supplementary Fig. 1b).

In 6 cases, we detected fusion transcripts, including the VAV1-MYOIF and TBL1XR1-TP63 fusions (Supplementary Table 1) that were reported previously in PTCL-NOS and further confirm the validity of this cohort. In addition, we identified 3 novel in-frame fusion transcripts: FYN-TRAF3IP2 (2 cases), KHDRBS1-LCK (1 case) and SIN3A-FOXO1 (1 case) (Supplementary Fig. 1c). We found evidence for a gene fusion between the neighboring FYN and TRAF3IP2 genes in 1/15 PTCL-NOS (case PTCL2) and 1/3 PTCL-TFH (case PTCL4). In the PTCL-NOS case, exon 7 of FYN was fused to exon 3 of TRAF3IP2 (Fig. 1a). To exclude that such fusion was the result of transcriptional read-through, we investigated the genomic region by long-range PCR and we identified an intrstitial deletion of 92597 bp on chromosome 6 (Fig. 1b). In the PTCL-TFH case, exon 8 of FYN was fused to exon 3 of TRAF3IP2 (Fig. 1a). In both variants of the fusion protein, the FYN moiety contained the membrane localization motif (SH4 domain) and the SH3 domain of FYN, but lacked the FYN tyrosine kinase domain. The TRAF3IP2 moiety consisted of the almost complete open reading frame, only lacking the first 7 amino acids and thus retaining the TNF receptor-associated factor 6 (TRAF6) binding domain in the fusion protein. Additionally, we discovered a KHDRBS1-LCK gene fusion that was caused by an intrastitial deletion on chromosome 1 in 1/15 PTCL-NOS (case PTCL17). This gene fusion generated a chimeric transcript in which exon 3 of KHDRBS1 was fused to exon 3 of LCK, resulting in the fusion of the QUA1 and K homology (KH) dimerization domains of KHDRBS1 to the SH3, SH2 and kinase domains of LCK (Fig. 1c).

To validate the importance of these fusion genes, we tested the presence of the FYN-TRAF3IP2 and the KHDRBS1-LCK fusion genes with RT-PCR in an independent validation cohort of 37 PTCL cases (30 PTCL-NOS, 6 PTCL-TFH, 1 Adult T-cell leukemia/lymphoma (ATLL)). This screen confirmed that the FYN-TRAF3IP2 fusion is highly recurrent as it was detected (and verified by Sanger sequencing) in 6/37 (3 PTCL-NOS, 3 PTCL-TFH) samples (Supplementary Fig. 1d, e, Supplementary Table 2). We detected no additional cases of the KHDRBS1-LCK fusion in the independent validation cohort.

FYN-TRAF3IP2-dependent signaling intersects with TCR signaling. To study the oncogenic properties of the FYN-TRAF3IP2 fusion protein, we cloned the open reading frame (ORF) derived from patient cDNA (case PTCL2) in a bicistronic pMSCV vector with an IRES-GFP reporter (pMIG). As controls, full-length TRAF3IP2 and the N-terminal fragment of FYN containing the membrane localization motif, the SH3 domain and a truncated SH2 domain (FYN<sup>1–232</sup>) were cloned in the pMIG vector (Supplementary Fig. 2a). Only retroviral transduction of interleukin-3 (IL-3) dependent Ba/F3 cells with the FYN-TRAF3IP2 ORF conferred IL-3 independent growth in vitro, indicating that the FYN-TRAF3IP2 fusion protein is endowed with oncogenic properties and that the combined signaling properties of both fusion partners are required (Fig. 2a).

The oncogenic signaling properties of the FYN-TRAF3IP2 fusion protein could not be related to tyrosine kinase signaling of the FYN kinase domain since it was not retained in the fusion protein. Instead, it was only the N-terminal part of FYN that was fused to the majority of the TRAF3IP2 protein. Under physiological circumstances, TRAF3IP2 is essential for IL-17 signaling. Upon engagement of the heterodimeric IL-17RA/IL-17RC complex, TRAF3IP2 translocates to the juxtmembrane compartment via homotypic SEFIR domain interactions and relays signals to the canonical NF-kB pathway and mitogen-activated protein kinase (MAPK) pathways. IL-17RA is ubiquitously expressed, but IL-17RC expression is more restricted and defines the cellular response to IL-17. Neither T cells from healthy volunteers (Supplementary Fig. 2b), nor any of the lymphoma samples in our cohort (Supplementary Fig. 2c) expressed IL17RC. Accordingly, the levels of TRAF3IP2-regulated transcripts in naive CD4<sup>+</sup> T cells transduced with empty pMIG vector (EV), pMIG-TRAF3IP2 or pMIG-FYN-TRAF3IP2 were primarily determined by expression of FYN-TRAF3IP2. Stimulation with IL-17 had an additive rather than a synergistic effect on the expression TRAF3IP2-regulated transcripts (Fig. 2b). Combined, these data strongly suggest that the
Given that TCR signaling is a nexus in the integrated control of proliferation and survival in healthy and malignant T cells and TRAF3IP2 activates NF-κB signaling and MAPK downstream of the IL-17 receptor, we hypothesized that aberrant FYN-TRAF3IP2 phosphorylation of the NF-κB signaling would intersect with NF-κB signaling downstream of the TCR. We transduced the Jurkat T cell line to measure NF-κB transcriptional activity. Cells transduced with pMSCV-FYN-TRAF3IP2 had increased NF-κB transcriptional activity in resting conditions and after TCR stimulation with an agonistic CD3 antibody compared to Jurkat cells transduced with an empty vector (Supplementary Fig. 3a). Intracellular flow cytometry indicated increased activation of canonical NF-κB signaling by means of increased Inhibitor of NF-κB alpha (IκBα) degradation and increased Ser536 phosphorylation of p65 in FYN-TRAF3IP2-expressing cells (Fig. 2f). There was no evidence for enhanced activation of proximal TCR signaling (ZAP70 Tyr318 phosphorylation) or increased MAPK signaling (Fig. 2g).

In summary, these data show that FYN-TRAF3IP2 activates canonical NF-κB signaling independent of IL-17.

**FYN-TRAF3IP2 localizes to the cell membrane and activates the NF-κB pathway.** Ligation of the TCR engenders the formation of a multiprotein signalosome at the membrane-cytosol interface. The Src-family kinases FYN and LCK are critical for proximal TCR signaling and are anchored to the plasma membrane by acylation of the unique N-terminal SH4 domains. FYN is myristoylated at Gly2, followed by palmitoylation at Cys319. IL-17 dependent recruitment of TRAF3IP2 to the plasma membrane initiates signaling downstream of TRAF3IP2. Taken together, we reasoned that acylation of FYN-TRAF3IP2 would anchor the fusion protein to the plasma membrane and mediate chronic active TRAF3IP2-dependent signaling.

To this end, we mutated the N-terminal Gly2 of FYN to Ala (FYNG2A-TRAF3IP2) to abolish acylation of the N-terminal FYN domain. Immunofluorescence imaging of 293T cells transfected with either wild-type TRAF3IP2, FYN-TRAF3IP2 or FYNG2A-TRAF3IP2, confirmed that wild-type TRAF3IP2 resided in the cytosol, FYN-TRAF3IP2 segregated to the plasma membrane and FYNG2A-TRAF3IP2 resulted in the redistribution of the fusion protein (FYNG2A-TRAF3IP2) to abolish acylation of the N-terminal FYN domain.
protein to the cytosol (Fig. 3a). The subcellular compartmentalization of FYN-TRAF3IP2 and FYNG2A-TRAF3IP2 was confirmed in Ba/F3 cells (Supplementary Fig. 3b) and Ba/F3 cells transduced to express FYNG2A-TRAF3IP2, were no longer able to grow out in the absence of IL-3 (Fig. 3b).

Next, we collected cytosolic and membrane fractions of Jurkat cells with ectopic expression of TRAF3IP2, FYN-TRAF3IP2 or FYNG2A-TRAF3IP2 and confirmed that disruption of FYN myristoylation impeded incorporation of the fusion protein in the plasma membrane (Fig. 3c). Delocalization from the plasma membrane impaired the ability of FYNG2A-TRAF3IP2 to activate NF-κB transcriptional activity compared to FYN-TRAF3IP2 (Fig. 3d). Likewise, immunofluorescence analysis of CD4+ T cells displayed association of FYN-TRAF3IP2 but not TRAF3IP2 or FYNG2A-TRAF3IP2 with the cell membrane (Fig. 3e). Exclusion of FYNG2A-TRAF3IP2 from the membrane...
Fig. 2 FYN-TRA3IP2-dependent signaling intersects with TCR signaling. a Outgrowth of Ba/F3 cells transduced with empty pMIG vector, pMIG-FYN, pMIG-TRA3IP2 or pMIG-FYN-TRA3IP2 after withdrawal of IL-3. n = 3 biological replicates per condition. b qRT-PCR for IL-17-regulated transcripts in naïve CD4+ T cells transduced with empty pMIG vector, pMIG-TRA3IP2 or pMIG-FYN-TRA3IP2. n = 3 biological replicates per condition. p values were calculated with Tukey’s post-hoc multiple comparisons test. c Western blot analysis for canonical and non-canonical NF-kB activation in Jurkat cells transduced with empty pMIG vector, pMIG-TRA3IP2 or pMIG-FYN-TRA3IP2. d NF-kB GFP reporter signal intensity in Jurkat cells with empty control vector or expression of FYN, TRA3IP2 or FYN-TRA3IP2 after stimulation with an agonistic anti-CD3ε antibody or exposure to control immunoglobulin (Ig). n = 3 biological replicates per condition for Ig control, n = 4 for anti-CD3ε. p values were calculated with Tukey’s post-hoc multiple comparisons test. e Western blot analysis for activation of MAPK pathways in Jurkat cells transduced with empty pMIG vector, pMIG-TRA3IP2 or pMIG-FYN-TRA3IP2. f Intracellular flow cytometry for IkBα (top row) or phospho-p65 (bottom row) in CD4+ T cells treated with control immunoglobulin (Ig) or agonistic anti-CD3ε antibody. n = 3 biological replicates per condition. p values were calculated with Šidák’s multiple comparisons test (two-sided). g Intracellular flow cytometry proximal TCR signaling and MAPK pathway activation in CD4+ T cells treated with control immunoglobulin (Ig) or agonistic anti-CD3ε antibody. n = 3 biological replicates per condition. p values were calculated with Šidák’s multiple comparisons test (two-sided). All data are represented as mean ± standard deviation (SD).

**FYN-TRA3IP2 activates TRAF6 independent of the CBM signaling.** Activation of the NF-kB pathway downstream of the TCR is initiated by protein kinase C θ (PKCθ)-dependent assembly of a signalosome composed of CARD11, BCL10 and MALT1 (CBM signalosome). The CBM signalosome recruits TRAF6, which leads to lysine 63-linked (K63-linked) polyubiquitination of TRAF6. K63-linked polyubiquitinated TRAF6 acts as a scaffold for the 1xK Kinase (IKK) complex and Transforming-growth-factor-β-Activated Kinase 1 (TAK1) complex which will ultimately lead to the release of NF-kB transcription factors from IkBα. The TRA3IP2 protein comprises an N-terminal TRAF6 binding motif and has a U-box domain with E3 ubiquitin ligase enzymatic activity through which it catalyzes K63-linked polyubiquitination of TRAF6. The interaction of the FYN-TRA3IP2 fusion protein with TRAF6 was found to be independent of the CBM signalosome to activate NF-kB.

To test this hypothesis, we substituted the acidic amino acid residues in the N-terminal TRAF6 binding motif (PVEVDE) with Ala residues (PVAVAA). While wild-type TRA3IP2 and FYN-TRA3IP2 co-immunoprecipitated with TRAF6, mutation of the TRAF6 binding motif (FYN-TRA3IP2ΔT6) abrogated the interaction with TRAF6 and led to a decrease in K63-linked polyubiquitination of TRAF6 (Fig. 1a). In the opposite direction, co-immunoprecipitation of TRAF6 – including polyubiquitinated TRAF6 – with FYN-TRA3IP2ΔT6 was attenuated (Fig. 1b). Contrary to Ba/F3 cells with expression of FYN-TRA3IP2, Ba/F3 cells with ectopic expression of FYN-TRA3IP2ΔT6 (Supplementary Fig. 3c) did not grow out after withdrawal of IL-3 (Fig. 1c).

Expression of FYN-TRA3IP2ΔT6 in Jurkat cells or primary CD4+ T cells (Supplementary Fig. 3c) impaired activation of the canonical NF-kB pathway (Fig. 4d, e). To investigate whether FYN-TRA3IP2 could activate the NF-kB pathway independent of CARD11, we generated CARD11 knock-out Jurkat cells with CRISPR/Cas9 genome editing (Supplementary Fig. 3d, e). Expression of FYN-TRA3IP2, but not TRA3IP2 or FYN-TRA3IP2ΔT6 augmented NF-kB transcriptional activity in resting conditions in both wild-type and CARD11 knock-out Jurkat cells. Activation of PKCθ with phorbol 12-myristate 13-acetate (PMA) and ionomycin increased NF-kB transcriptional activity in wild-type Jurkat cells, but significantly more in Jurkat cells with expression of FYN-TRA3IP2. CARD11 deficiency impaired NF-kB activation in response to PMA and ionomycin, but CARD11 knock-out Jurkat cells with FYN-TRA3IP2 expression remained significantly more responsive to PMA/ionomycin stimulation than empty vector control or cells with expression of TRA3IP2 or FYN-TRA3IP2ΔT6. This proves that FYN-TRA3IP2 does not require the CBM signalosome, but suggests an interaction – direct or indirect – between FYN-TRA3IP2 and PKCθ.

Collectively, these results indicate that FYN-TRA3IP2 directly interacts with TRAF6 and activates TRAF6 and the NF-kB pathway without intervention of the CBM signalosome.

**FYN-TRA3IP2 expression causes PTCL-NOS-like disease in vivo.** To test the oncogenic potential of the fusion protein in vivo, we transduced lineage negative hematopoietic stem and progenitor cells (HSPC) from wild-type mice with either the empty pMIG vector or the pMIG vector containing FYN-TRA3IP2 or FYNΔ2A-TRA3IP2. Subsequently, we injected the transduced cells (Supplementary Fig. 4a) intravenously in sublethally irradiated syngeneic recipient mice. Engraftment rates were comparable (Supplementary Fig. 4b). As early as 7 weeks after transplantation, GFP+ cells in the peripheral blood of mice transplanted with HSPC expressing FYN-TRA3IP2 skewed towards CD4+ cells (Supplementary Fig. 4c). Mice with expression of FYN-TRA3IP2 in hematopoietic cells, started losing weight 8 weeks after transplantation and succumbed within 16 weeks after transplantation (Fig. 5a). These mice had splenomegaly (Fig. 5b) and generalized lymphadenopathy. In contrast, mice transplanted with cells expressing mutant FYNΔ2A-TRA3IP2 did not develop disease (Fig. 5a, b). Lymph nodes from mice with FYN-TRA3IP2-expressing cells displayed an effaced lymph node architecture with paracortical expansion and loss of germinal centers. The lymph nodes contained medium-sized and large cells with irregular, pleomorphic nuclei and multiple prominent nucleoli. Malignant cells were surrounded by a morphologic infiltrate with numerous eosinophils and histiocytes (Fig. 5c). FYN-TRA3IP2-driven lymphomas were invariably CD44+ (Fig. 5d). These cells were also found in the spleens (Supplementary Fig. 4d) and infiltrated the livers (Fig. 5e, f). FYN-TRA3IP2 and FYNΔ2A-TRA3IP2 were expressed in lymph nodes (Fig. 5g) and FYN-TRA3IP2 expression was restricted to GFP+ cells (Supplementary Fig. 4e). Analysis of Tcrb locus rearrangements with RT-PCR corroborated the clonal nature of the disease (Fig. 5h).

Because we identified FYN-TRA3IP2 gene fusions in both PTCL-NOS and PTCL-TFH, we determined the immunophenotype of tumour FYN-TRA3IP2-driven lymphomas. Malignant cells were positive for PD-1 and ICOS, but negative for the Tfh marker CXCR5 (Fig. 6a). The BCL6 transcription factor orchestrates TH1 symptom.
Fig. 3 Membrane anchoring of FYN-TRAF3IP2 is required to activate NF-κB signaling. 

a Immunofluorescence images of 293T cells transfected with TRAF3IP2, FYN-TRAF3IP2 and FYNG42A-TRAF3IP2 stained for TRAF3IP2. Scalebars represent 10 µm. 

b Outgrowth of Ba/F3 cells transduced with pMIG-FYN-TRAF3IP2 or pMIG-FYN42A-TRAF3IP2 after withdrawal of IL-3. n = 3 biological replicates per condition. 

c Western blot analysis for TRAF3IP2 on cytoplasmic and membrane fractions of Jurkat cells transduced with pMIG-TRAF3IP2, pMIG-FYN-TRAF3IP2 and pMIG-FYN42A-TRAF3IP2. 

d NF-κB GFP reporter signal intensity in Jurkat cells with empty control vector or expression of TRAF3IP2, FYN-TRAF3IP2 or FYNG42A-TRAF3IP2 after stimulation with an agonistic anti-CD3ε antibody or exposure to control immunoglobulin (Ig). n = 3 biological replicates per condition for Ig control, n = 4 for anti-CD3ε. p values were calculated with Tukey’s post-hoc multiple comparisons test. 

e Immunofluorescence images (left) of TRAF3IP2 staining on CD4+ T cells transduced to express TRAF3IP2, FYN-TRAF3IP2 or FYNG42A-TRAF3IP2. Scalebars represent 5 µm. Quantification (right) of TRAF3IP2 signal intensity in the outer cell bin, expressed as fraction of signal intensity over the entire cell. Each dot represents a cell (n = 201 for TRAF3IP2, n = 192 for FYN-TRAF3IP2 and n = 179 for FYNG42A-TRAF3IP2). Horizontal line and whiskers represent median and interquartile range respectively. p values were calculated with Games-Howell’s multiple comparisons test. 

f Intracellular flow cytometry for IkBα (top row) or phospho-p65 (bottom row) in CD4+ T cells treated with control immunoglobulin (Ig) or agonistic anti-CD3ε antibody. n = 3 biological replicates per condition. p values were calculated with Tukey’s post-hoc multiple comparisons test. All data are represented as mean ± SD unless stated otherwise.
identity\textsuperscript{23}, but was not expressed in FYN-TRA3IP2-induced lymphomas (Fig. 6b). B cell proliferation with an expansion of germinal center (GC) B cells and plasma cells frequently accompanies Tfh-related neoplasms. In contrast, we observed a reduction in B cells in FYN-TRA3IP2-associated lymphomas (Fig. 6c) without an increased proportion of GC B cells (Fig. 6d). The presence of plasma cells was more variable, but overall, not significantly altered (Fig. 6e). Consistent with the histologic finding of an increased number of histiocytes and eosinophils, the proportion of CD11b\textsuperscript{+} myeloid cells was increased in FYN-TRA3IP2-driven lymphomas (Supplementary Fig. 4f). The fraction of CD4\textsuperscript{+} cells was increased in FYN-TRA3IP2-driven lymphomas and there were no changes in the number of CD8\textsuperscript{+} cells (Supplementary Fig. 4f). Finally, there was no proliferation of high
endothelial venules (Fig. 6f). These results are compatible with PTCL-NOS rather than PTCL-TF.

Collectively, these in vivo data indicate that FYN-TRAF3IP2 is a disease driver in PTCL-NOS and that the oncogenic potential of the FYN-TRAF3IP2 fusion protein is critically dependent on its association with the plasma membrane because lymphomagenesis was abolished in FYNG2A/TRAF3IP2-expressing cells.

FYNG2A-driven lymphomas have active NF-κB signaling and are sensitive to inhibition of BCL-XL. We compared the gene expression profile of sorted CD4+GFP+ lymphoma cells with naive CD4+ T cells, CD4+GFP- non-malignant stromal T cells from the lymphoma and CD4+GFP+ FYNG2A-TRAF3IP2-expressing T cells. Consistent with the neoplastic nature of the disease, numerous cell-cycle-associated genes were among the most significantly upregulated genes in FYN-TRAF3IP2-expressing lymphoma cells (Supplementary Fig. 5a). Overexpression of Icos and Pdcd1 was congruent with the immunophenotype determined with flow cytometry. Additionally, lymphoma cells overexpressed Runx2 and Id2. Both genes oppose the Tαb phenotype and are repressed by Bcl26, in support of a non-Tαb origin of the lymphomas (Supplementary Fig. 5a). We analyzed the cis-regulatory features associated with differentially expressed genes to identify the transcription factors driving these phenotypes with i-cisTarget. This analysis consistently retrieved the NF-κB1 motif and the PU.1 motif as the most significantly enriched cis-regulatory features associated with overexpressed genes in FYN-TRAF3IP2-expressing lymphoma cells (Fig. 7a). Gene set enrichment analysis (GSEA) for different p65 (RelA) target gene sets reaffirmed increased expression of canonical NF-κB target genes in FYN-TRAF3IP2-expressing cells (Fig. 7b) and nuclear translocation of p65 was most pronounced in FYN-TRAF3IP2-expressing cells (Fig. 7c). As expected, malignant cells expressed Relb—a known direct transcriptional target of p50/p6523 (Supplementary Fig. 5b). However, there was no enrichment of RelB target genes in malignant cells (Supplementary Fig. 5c) and RelB was sequestered in the cytosol of FYN-TRAF3IP2-expressing cells (Supplementary Fig. 5b). The transcription factor PU.1 (also known as SPI1) has been associated with the Tαb phenotype. The cytokine profile of FYN-TRAF3IP2-expressing lymphoma cells was compatible with a Tαb phenotype25 (Supplementary Fig. 5d). Consistent with in vitro assays, there was no univocal activation of MAPK signaling in FYN-TRAF3IP2-expressing cells. The AP-1 motif was associated with cis-regulatory features of upregulated genes in lymphoma cells versus naive CD4+ T cells and downregulated genes in lymphoma cells versus FYNG2A-TRAF3IP2-expressing CD4+ T cells (Fig. 7a). Likewise, GSEA for c-Jun target genes yielded inconsistent results (Supplementary Fig. 5e).

Targeting NF-κB directly for clinical purposes remains elusive because of on-target toxicities. This prompted us to examine vulnerabilities downstream of NF-κB. Promoting cell survival through induction of target genes, is one of the best-documented functions of NF-κB26. Indeed, pro-survival and to a lesser extent pro-apoptotic factors were upregulated in FYN-TRAF3IP2-expressing cells (Fig. 8a). We reasoned that inhibition of pro-survival signals could tilt the balance towards apoptosis. BCL-XL (encoded by the Bcl2l1 gene) was consistently overexpressed by lymphoma cells at both the RNA level and protein level in our mouse model (Fig. 8a, b). BCL-XL expression was also confirmed in case PTCL2 (Fig. 8c).

Compared to naive CD4+ T cells cultured under identical conditions, FYN-TRAF3IP2-expressing cells were >30-fold more sensitive to inhibition of BCL-XL, BCL-W and BCL2 with ABT-263 treatment (Fig. 8d).

These results demonstrate that FYN-TRAF3IP2 activates NF-κB signaling in vivo and that this confers malignant cells with vulnerability to inhibition of BCL-XL.

KDHRB1-LCK mediates chronic active TCR signaling. We cloned the open reading frame of the KDHRB1-LCK fusion transcript derived from patient cDNA in the pMIG vector to study the signaling properties of the KDHRB1-LCK fusion protein. Here, the kinase domain of LCK was fused to the KDHRB1 dimerization domain. It thus lacks the nuclear localization signal of KDHRB1 and the membrane localization motif of LCK (Fig. 1c), suggesting that this protein functions in the cytosol. Indeed, in primary T cells with ectopic expression of KDHRB1-LCK, the fusion protein accumulated in the cytosol when compared to T cells with ectopic expression of LCK (Fig. 9a). Retroviral transduction of IL-3 dependent Ba/F3 cells with KDHRB1-LCK (Supplementary Fig. 6a) led to IL-3 independent growth in vitro and this was abolished by an inactivating LCK kinase domain mutation (KDHRB1-LCKK273R) (Fig. 9b). Likewise, inhibition of LCK kinase activity with dasatinib could efficiently block the proliferation of transformed Ba/F3 cells at low nanomolar concentrations (Fig. 9c). KDHRB1-LCK but not KDHRB1-LCKK273R was constitutively active (Tyr394 phosphorylation) in unstimulated Jurkat cells (Fig. 9d). Likewise, proximal TCR signaling was significantly enhanced in primary T cells with expression of KDHRB1-LCK in resting conditions and after TCR stimulation compared to primary T cells transduced with empty pMIG vector or LCK (Fig. 9e). Inhibition of LCK kinase activity with dasatinib reverted these differences (Fig. 9f).

Collectively, these data show that KDHRB1-LCK is a constitutively active tyrosine kinase that leads to chronic active TCR signaling.

KDHRB1-LCK instigates PTCL-NOS in vivo. To test the oncogenic potential of KDHRB1-LCK in vivo, we transduced HSPC from wild-type mice with MSCV retrovirus with either an empty pMIG vector or a pMIG vector containing the
**KHDRBS1-LCK ORF.** We observed incomplete disease penetrance (Fig. 10a). One mouse developed a lymphoproliferative disorder with generalized lymphadenopathy, moderate splenomegaly, pleural effusion and thymic enlargement, but no leukocytosis (Supplementary Fig. 6b). Normal tissue architecture was effaced. Residual follicles were pushed to the border of the lymph nodes. The expanded paracortex was dominated by a monotonous proliferation of medium-sized lymphocytes with irregular nuclei with 1 or 2 nucleoli and an increased number of blood vessels (Fig. 10b). Malignant cells were CD4−CD8+ and the majority expressed surface TCRβ (Fig. 10c, Supplementary Fig. 6c). Bone marrow infiltration was limited. Negativity for TdT ruled out a lymphoblastic lymphoma/leukemia (Fig. 10d). Lymphomas expressed the KHDRBS1-LCK fusion protein, which was highly phosphorylated (Fig. 10e). Interestingly, also endogenous LCK was highly phosphorylated in lymphomatous spleens compared to spleens from empty pMIG vector mice despite comparable LCK protein levels, hinting at activation of endogenous LCK by KHDRBS1-LCK.

**Discussion**
Recent advances on the genetics of PTCL-NOS have not led to tangible results for the development of novel therapeutic strategies. On the other hand, a handful of therapies (e.g., romidepsin\(^{28}\)) is successful in a minority of patients with PTCL-NOS, but insight in their mechanism of activity is lacking. In this
study, we have identified two novel gene fusions in PTCL-NOS that could function as targets for therapy. We have studied their activity in T-cell models, both in vitro and in vivo, and we were able to show that both gene fusions confer therapeutic vulnerability to clinically available compounds.

We identify and characterize here a recurrent FYN-TRAF3IP2 gene fusion in PTCL-NOS and PTCL-TFH. Despite the apparent heterogeneity of PTCL-NOS, the FYN-TRAF3IP2 gene fusion ranks as one of the most recurrent events identified to date in this disease. To gain insight in the biology of malignant cells, efforts have been made to establish a cell of origin for different T-cell lymphomas. In our mouse model there was evidence for a Th9-related signature of the Th2-related transcription factor GATA3 during neoplasms. It was not identified to date in this disease. Several outstanding questions remain to be answered. First, the FYN-TRAF3IP2 gene fusion appears specific for mature T cell neoplasms. It was not identified in recent large-scale studies of mature B cell neoplasms, 36–38. A concurrent report confirms that the FYN-TRAF3IP2 gene fusion is a T-cell lymphoma exclusive event. In our mouse model all mice invariably developed CD4+ T-cell lymphomas despite constitutive expression of the fusion protein in all hematopoietic lineages. T cell specificity could be related to interactions of the FYN-TRAF3IP2 protein with T cell-specific partners. Indeed, the SH4 domain of FYN can interact directly with CD3ζ. Therefore, it is plausible that the FYN-TRAF3IP2 fusion protein preferentially partitions to TCR cluster regions in the plasma membrane, facilitating the activation of downstream pathways. Our data suggest that the FYN-TRAF3IP2-initiated signaling bypasses proximal TCR components such as ZAP70. Further downstream in the TCR cascade, FYN-TRAF3IP2-expressing cells display enhanced responsiveness to activation of PKCθ by PMA/ionomycin, but do not require the CBM signalosome to activate TRAF6. Rather, FYN-TRAF3IP2 interacts directly with TRAF6 to activate canonical NF-κB signaling. From these observations, it is reasonable to assume that FYN-TRAF3IP2 requires proximity to the TCR complex to activate NF-κB. FYN-TRAF3IP2-dependent signaling appears to bypass much of the TCR cascade, but our findings could point at a crosstalk between both pathways at the levels of the TCR complex and PKCθ. Putative interactions between FYN-TRAF3IP2 and the TCR complex and PKCθ warrant further exploration.

In all experiments, we observed comparably lower levels of the FYN-TRAF3IP2 fusion protein than mutant variants of the fusion protein. This could not be related to lower mRNA expression, because the fusion protein was expressed along with GFP from a bicistronic vector and we observed no decrease of the GFP domain of ITK directs the ITK-SYK fusion protein to the plasma membrane leading to constitutive SYK kinase activity and chronic active TCR signaling in PTCL-TFH. Unlike ITK-SYK, FYN-TRAF3IP2 does not have kinase activity, but acts as a signaling adapter and has E3 ubiquitin ligase activity. Together with the Ubc13-Uev1A E2 complex, FYN-TRAF3IP2 will catalyze polyubiquitination of TRAF6. Polyubiquitinated TRAF6 will activate the IKK and TAK1 kinase complexes. Phosphorylation of these complexes, will mark IkB for proteasomal degradation thereby releasing NF-κB transcription factors. These are now free to translocate to the nucleus and initiate transcription. Importantly, our data suggest that the direct interaction between FYN-TRAF3IP2 and TRAF6 will bypass most of the paradigmatic TCR cascade, including the CBM signalosome.
protein (Fig. 5g). These observations suggest that levels of the FYN-TRAF3IP2 fusion protein are likely controlled by post-transcriptional regulation and/or post-translational regulation. Persistent activation of TRAF3IP2 by IL-17 desensitizes cells through degradation of TRAF3IP2 orchestrated by Skp1-cullin-1-F-box-type (SCF) E3 ubiquitin ligase complexes41. It is unknown whether levels of active FYN-TRAF3IP2 protein are regulated similarly by SCF-type E3 ubiquitin ligase complexes. Secondary

Fig. 6 Characterization of FYN-TRAF3IP2-driven lymphomas in mice. a Gating strategy for Tfh cells on lymph node cell suspensions from mice with empty-vector-transduced cells (top). Representative flow cytometry plot for the immunophenotype of FYN-TRAF3IP2-driven lymphomas (bottom, n = 9). b Histograms for BCL6 protein in Tfh cells (representative for n = 2), sorted viable CD4⁺GFP⁺ splenocytes from FYN-TRAF3IP2-driven lymphomas (n = 5) and PD-1⁺CXCR5⁺CD4⁺ T cells (representative for n = 2). Representative flow cytometry plots (left) and quantification (right) of CD19⁺B220⁺ B cells (c), CD95⁺GL7⁺ germinal center (GC) B cells (gated on CD19⁺B220⁺ B cells) (d) and CD19⁻B220⁻CD138⁺CD11b⁺ plasma cells (e) in lymph node suspensions from mice transplanted with empty-vector-transduced (EV) cells, FYN-TRAF3IP2-transduced (FT) cells or FYNG2A-TRAF3IP2-transduced cells (FG2AT). n = 5 mice per group. p values were calculated with Tukey’s post-hoc multiple comparisons test. f Representative pictures of CD31 immunohistochemistry on lymph node sections of mice transplanted with HSPC transduced with empty pMIG vector, pMIG-FYN-TRAF3IP2 or pMIG-FYNG2A-TRAF3IP2. n = 5 mice per group. Scalebars represent 50 µm. Data are represented as mean ± SD.
lesions which impact FYN-TRAF3IP2 protein turnover, could enhance lymphomagenesis. The number of sequenced samples in this study did not allow statistical inference to identify events that co-occur with a FYN-TRAF3IP2 gene fusion, but this will be of interest for future research.

Direct targeting of NF-κB signaling with IKK inhibitors has shown promise in preclinical models, but to date no IKK inhibitors are in clinical use. On one hand, they exhibit limited efficacy. On the other hand, they provoke prohibitive on-target toxicities related, but not limited, to the ubiquitous activity of the NF-κB pathway in both the innate and adaptive immune system42. Therefore, we shifted our focus to downstream effectors of the NF-κB pathway. The ability of NF-κB to promote cell survival through induction of target genes is well-described26. With the notable exception of Mcl1, we observed a global upregulation of pro-survival factors in murine lymphomas induced by FYN-TRAF3IP2. The expression of pro-apoptotic factors Bax, BakI, BH3-only activators and BH3-only sensitizers was elevated...
to a lesser extent (Fig. 8a). Bcl2l1 was one of the most significantly upregulated pro-survival genes. Expression of its gene product BCL-XL was confirmed in malignant cells in murine lymphomas and in a clinical specimen with a FYN-TRAF3IP2 gene fusion. Given the increased expression of pro-apoptotic factors in FYN-TRAF3IP2-driven murine lymphomas, we reasoned that these cells are primed for apoptosis. Indeed, FYN-TRAF3IP2-dependent lymphoma cells display exquisite sensitivity to the BCL-XL, BCL2 and BCL-W (encoded by Bcl2l2) inhibitor ABT-263. ABT-263 is in clinical development for the treatment of novel treatment regimens for PTCL cases with these fusion oncogenic fusion proteins and was able to activate LCK in an insertion mutagenesis screen. We show that KHDTRS1-LCK kinase activates different branches of the TCR signaling cascade. We provide evidence that targeting LCK kinase activity with dasatinib has potent inhibitory effects on KHDTRS1-LCK-driven signaling, paving the way for therapeutic intervention in a clinical setting. Early-stage clinical trials suggest activity of dasatinib in PTCL-NOS and TFH-related PTCL. With the identification of the KHDTRS1-LCK gene fusion, we provide an additional biological basis to repurpose dasatinib for the treatment of PTCL-NOS.

Together, our data provide genetic and biologic insights in PTCL-NOS and PTCL-TFH as well as a rationale for the design of novel treatment regimens for PTCL cases with these fusion genes. Overall, these data warrant further study of abnormal TCR signaling in PTCL.

**Methods**

**Patient samples.** Patient samples for the discovery cohort were collected retrospectively from the tumor banks of the University Hospitals Leuven and the CHU Mont-Godinne and prospectively in the University Hospitals Leuven. For prospectively obtained samples, we obtained informed consent from all patients. All cases were reviewed by two hematopathologists (LM and TT).

Patient samples for the validation cohort were obtained from the T-cell lymphoma biobank (TENOMIC) of the Lymphoma Study Association (LYSA) and were reviewed by two hematopathologists (PG en LdL). The study was approved by the Ethics Committee UZ/KU Leuven.
**Fig. 9 KHDRBS1-LCK drives chronic active TCR signaling.** a Quantification (left) and representative immunofluorescence images (right) of LCK and CD3ε staining on primary T cells with ectopic expression of LCK (n = 268 cells) or KHDRBS1-LCK (n = 242 cells). Each dot represents a cell. Horizontal line and whiskers represent median and interquartile range respectively. p values were calculated with a two-sided Mann–Whitney test. Scalebars represent 10 μm. 

b Outgrowth of Ba/F3 cells transduced with empty pMIG vector, pMIG-KHDRBS1-LCK or pMIG-KHDRBS1-LCKK273R after withdrawal of IL-3. n = 3 biological replicates per condition. 

c Growth inhibition of Ba/F3 cells transformed by KHDRBS1-LCK or Ba/F3 cells transduced with empty pMIG vector after a 24-h treatment with dasatinib. n = 3 replicates per condition. 

d Western blot for total LCK and phospho-LCK (Tyr394) in Jurkat cells transduced with empty pMIG vector, pMIG-KHDRBS1-LCK or pMIG-KHDRBS1-LCKK273R. 

e Quantification (top) and representative flow cytometry histograms (bottom) for phospho-ZAP70 in primary T cells treated with control immunoglobulin (Ig) or agonistic anti-CD3ε antibody in the absence or presence of dasatinib. n = 3 biological replicates per condition. p values were calculated with Tukey’s post-hoc multiple comparisons test. 

f Intracellular flow cytometry quantification of phospho-ERK1/2 levels in primary T cells treated with control immunoglobulin (Ig) or agonistic anti-CD3ε antibody in the absence or presence of dasatinib. n = 3 biological replicates per condition. p values were calculated with Tukey’s post-hoc multiple comparisons test. All data are represented as mean ± SD unless stated otherwise.

**Mice.** We used 6- to 10-week-old C57BL/6j mice for the isolation of primary T cells and bone marrow transplant experiments. Mouse experiments were approved and supervised by the KU Leuven ethical committee and conducted according to EU legislation (Directive 2010/63/EU).

Mice were housed in individually ventilated cages with a temperature between 18 and 23 °C and humidity between 40 and 60%. No more than 5 mice were housed in a single cage. The room had a programmed 12-h light and 12-h dark cycle.

**RNA extraction, cDNA synthesis and qRT-PCR.** RNA from clinical samples was extracted from 4 cryosections, each 10 μm thick. Briefly, sections were resuspended in Trizol (Thermo Fisher Scientific). After addition of chloroform, RNA was precipitated from the aqueous phase with 100% ethanol. RNA was washed and eluted with the RNeasy Mini Kit (Qiagen) according to the manufacturer’s instructions. Concentrations and purity were measured with the NanoDrop 2000 (Fisher Scientific). RNA integrity was measured with the Bioanalyzer 2100 system using the RNA 6000 Nano Kit (both from Agilent). Samples with an RNA integrity
number >8 were retained for RNA sequencing. cDNA synthesis was performed using SuperScript IV (Thermo Fisher Scientific). To detect fusion transcripts, cDNA was amplified with GoTaq polymerase (Promega) using the primers listed in Supplementary Table 3.

Mouse tissue was disrupted in Trizol (Thermo Fisher Scientific) with the Tissuelyser LT (Qiagen) at 50 Hz for 4 min. Supernatants were collected. After addition of chloroform, RNA was precipitated from the aqueous phase with 100% ethanol. RNA was washed and eluted with the RNeasy Mini Kit (Qiagen) according to the manufacturer’s instructions. Concentrations and purity were measured with the NanoDrop 2000 (Fisher Scientific). cDNA synthesis was performed using GoScript (Promega). T cell receptor clonality was verified on cDNA with GoTaq polymerase (Promega) using the primers listed in Supplementary Table 3. PCR products were loaded on the QIAxcel System (Qiagen).

RNA was extracted from cultured cells using the Maxwell RSC simplyRNA Cells purification kit (Promega). cDNA synthesis was performed using GoScript (Promega) and qRT-PCR was performed using the GoTaq qRT-PCR master mix (Promega) on the ViaA7 Real Time PCR system (Applied Biosystem). Primers used for qRT-PCR are listed in Supplementary Table 3. qRT-PCR data were analyzed with the geNorm algorithm in the R package SQuaPcR v1.54.0 (https://doi.org/10.18129/B9.bioc.SQuaPcR) in R version 4.0.

RNA sequencing. For clinical samples, libraries were made with the TruSeq Stranded mRNA Library Prep Kit (Illumina). We used 1 µg RNA as input. Libraries were prepared per manufacturer’s instructions. Libraries were pooled and sequenced on an S2 flow cell on a NovaSeq 6000 system (both Illumina) in 2 × 100 bp mode to an average depth of 200 million reads per sample.

For mouse cells, libraries were made with the QuanSeq 3’ mRNA-Seq Library Prep Kit (Lexogen). We used 20–100 ng RNA as input. Libraries were prepared per manufacturer’s instructions. Libraries were pooled and sequenced on a HiSeq 4000 system (Illumina) in 1 × 50 bp mode.

Bioinformatics. Raw sequencing data from the Illumina sequencer was demultiplexed and converted to the fastq file format with illumina’s bc2fastq v2.20. software. For anaplastic large cell lymphoma samples12 and normal lymph nodes31, we used data from publicly accessible repositories. Quality of fastq files was assessed with FastQC v0.11.9. RNA-sequencing data was first cleaned (i.e., removal of adapters and low-quality reads) with fastq-MCF (ca-utils v1.1.2), again followed by quality control with FastQC. Chimeric reads were identified with FusionCatcher v2.1.0. Reads were aligned with HISAT2 v2.1.0 to their respective genomes, either Homo sapiens (GRCh38/hg38) or Mus musculus (mm10) and
coordinate-sorted with SMARTools v1.10.54. HTSeq-count v0.9.1 was used to count the number of reads per gene. Differential gene expression analysis was performed with the DESeq2 R package, v2.40.0, using version 4.0. Gene set enrichment analysis was performed with the Broad Institute GSEA software. Analysis of cis-regulatory features associated with co-expressed genes was performed with i-cisTarget.

Genomic DNA. Genomic DNA from clinical samples was extracted with the DNaseasy Kit (Qiagen) according to the manufacturer’s instructions. PCR on genomic DNA was performed with Q5 polymerase (New England Biolabs) and the primers listed in Supplementary Table 3.

Plasmids and vectors. RNA from patient samples and Jurkat cells were retro-transcribed with SuperScript IV reverse transcriptase (Thermo Fisher Scientific) using oligo(dT)15 primers (Promega).

Cell culture. Ba/F3 cells were cultured in RPMI 1640 supplemented with 10% fetal bovine serum (FBS) and IL-3 (3 ng/ml; Peprotech). For transformation assays, cells were cultured in RPMI 1640 with 10% FBS at a density of 10^5 cells/ml. For virus production and transduction, cells were seeded at a density of 10^6 cells/ml and incubated on ice for 15 min prior to stimulation with anti-CD3 antibody.

ZAP70, after 15 min for all other analyses). For experiments with dasatinib, T cells were cultured in RPMI 1640 with 10% FBS at a density of 10^5 cells/ml. Cell proliferation was measured at 12°C for 15 min prior to stimulation with anti-CD3 antibody.

CARD11 knock-out Jurkat cells were reproduced in clones 4 and 6 (Supplementary Fig. 3d).

Virus production and transduction. Ecotropic retrovirus was produced in 293T cells transfected with the pK6.1MVC.europac packaging plasmid using GeneJuice transfection reagent (Merck Millipore). 24 h after transfection, the medium was changed to RPMI 1640 with 10% FBS. 24 h later, the supernatant with viral particles was harvested. One ml of viral supernatant was added to 20% FBS, IL-3 (3 ng/ml; Peprotech), IL-2 (10 ng/ml; Peprotech), SCF (50 ng/ml; Peprotech), and Primocin (Invitrogen). The following day, cells were transfected by spinoculation (90 min at 2000 g) with viral supernatant and 8 µg/ml polybrene and syngeneic female recipient mice were subcutaneously irradiated (5 Gy). The following day, the cells were washed in PBS and injected (1 x 10^6 cells/0.5 ml) into the lateral tail vein of sublethally irradiated syngeneic female recipient mice. Mice were housed in individually ventilated cages and monitored daily.

Immunoblotting and co-immunoprecipitation. Cells were lysed in Cell Lysis buffer (Cell Signaling). Tissues were homogenized with a TissueLyserLT (Qiagen). Membrane protein extraction kit (Thermo Fisher Scientific) and transferred to PVDF membranes. Subsequent Western blot analysis was performed using antibodies listed in Supplementary Table 4, diluted in 1% BSA in TBS. Blots were exposed to the ChemiDocXRS system (Bio-Rad) for visualization. For immunoprecipitation of TRAF3IP2, 50 µl of Dynabeads sheep anti-rat IgG (Thermo Fisher Scientific) were mixed with lysis buffer and T cells were incubated at 4°C overnight. The next day, the samples were centrifuged at 12,000 × g for 5 min and the supernatant was collected. Protein A/G agarose beads (Thermo Fisher Scientific) were incubated with the supernatant at 4°C for 1 h. The samples were centrifuged at 12,000 × g for 5 min, and the supernatant was collected. The samples were subjected to immunoprecipitation with the indicated antibody, followed by washing with PBS and SDS loading buffer (Laemmli buffer). The samples were subjected to Western blot analysis using antibodies against TRAF3IP2, Traf6, and NF-κB p65.

Infection with luciferase reporter lentivirus was performed in 293T cells transfected with pGreenFire1-NF-κB luciferase/GFP plasmid, pSPAK (Addgene #12260) and pCMV-VSV-G plasmid (Addgene #8454) using Genejuice (Merck Millipore). After 6 h, the medium was changed to RPMI 1640 with 20% FBS. Supernatant carrying viral particles was harvested. Jurkat cells were transduced on retroenon-coated plates (Takara) by spinfection. Luciferase/GFP dual NF-κB reporter lentivirus was produced in 293T cells transfected with pGreenFire1-NF-κB luciferase/GFP plasmid, pSPAK (Addgene #12260) and pCMV-VSV-G plasmid (Addgene #8454) using Genejuice (Merck Millipore). After 6 h, the medium was changed to RPMI 1640 with 20% FBS. Supernatant carrying viral particles was harvested 48 h after transfection and concentrated on a 20% sucrose gradient by ultracentrifugation at 112,500 × g for 2 h. Jurkat cells were transduced on retroenon-coated plates (Takara) by spinfection (1 h at 2000 × g).

Murine bone marrow transplantion. C57BL/6 mice were purchased from Charles River Laboratories. Bone marrow cells were harvested from femur and tibia of male mice. Lineage negative cells were enriched (EasySep Mouse Hematopoietic Progenitor Cell Isolation Kit, STEMCELL Technologies) and cultured overnight in RPMI 1640 supplemented with 10% FBS at a density of 10^6 cells/ml with or without PMA/ionomycin (50 nM PMA, 13.4 µM ionomycin; Thermo Fisher Scientific). 1640 supplemented with 10% FBS at a density of 10^5 cells/ml. Cell proliferation was measured at 1°C for 15 min prior to stimulation with anti-CD3 antibody.

Drug treatment. Ba/F3 cells, MACS-sorted naive CD4+ T cells or FACS-sorted CD4+ GFP+ lymphoma cells were seeded in 96-well plates. Ba/F3 cells were cultured in RPMI 1640 with 10% FBS with or without IL-3. T cells were cultured in T cell medium. Compounds were dissolved in DMSO and added in a randomized fashion using a D300e digital dispenser (Tecan). Cell proliferation was measured at 12°C for 15 min prior to stimulation with anti-CD3 antibody.

CRISPR/Cas9 genome editing. To generate CARD11 knock-out Jurkat cells, we cloned oligos complementary to the target site (Supplementary Table 3) in the pX330 vector (Addgene #42230) after digestion with BbsI (Thermo Fisher Scientific). We electroporated the pX330 plasmid in Jurkat cells in serum-free RPMI 1640 medium using the Gene Pulser Xcell™ system (Biorad). Immediately after electroporation, cells were washed in PBS and lysed in 30 µl passive lysis buffer (Promega) with gentle agitation. After 20 min, 20 µl of lysate was transferred to a new plate and loaded on a Vector multilabel plate reader (PerkinElmer). With the
dispenser, 100 μl of Luciferase Assay Reagent II (Promega) was added and luciferase signal was measured well per well.

**Flow cytometry.** For surface staining, single cell suspensions were blocked with unconjugated CD16/32 antibody and stained with Fixable viability dye in staining buffer (PBS with 2% FBS) for 10 min protected from light at room temperature. Next, cells were washed with stain buffer. Cells were resuspended in staining buffer with the appropriate antibody dilutions for 30 min at 4 °C protected from light. For the detection of cytosolic proteins, cells were fixed with room-temperature IC fixation buffer and permeabilized with eBioscience Permeabilization buffer (BD) from Thermo Fisher Scientific. For the detection of phosphorylated cytosolic proteins, cells were fixed with room-temperature IC fixation buffer and permeabilized with ice-cold methanol. For the detection of phosphorylated transcription factors, cells were prepared with the Transcription Factor Buffer set (BD Biosciences) according to the manufacturer’s protocol. Antibodies and dilutions are listed in Supplementary Table 5. Data were acquired on Fortessa X-20, Becton Dickinson Imaging. Data were analyzed with FlowJo version 10.6 (BD Biosciences).

**Histology and immunofluorescence staining.** For the membrane staining of FYN-TRAF3IP2 in 293T cells, we coovered covers with poly-L-lysine. 293T cells were seeded on top of the cover slips in RPMI 1640 with 10% FBS. After 6 h, cells were transfored with empty plMIG vector or plMIG constructs encoding TRAF3IP2, FYN-TRAF3IP2 or FYNΔA-TRAF3IP2 using GeneJuice transfection reagent (Merck Millipore). After 12 h, the medium was replaced with RPMI 1640 with 10% FBS. 36 h later, cells were fixed for 20 min with 4% paraformaldehyde in PBS at room temperature. Next, cells were blocked in staining buffer (4% BSA, 0.1% digitonin in PBS) for 2 h at room temperature. After blocking, cover slips were carefully removed from the plates and stained over night at 4 °C with primary anti-TRAF3IP2 antibody (Thermo Fisher Scientific, #14-4040-82) diluted 1:200 in staining buffer. The next day, cover slips were washed with staining buffer followed by staining with Alexa Fluor 647-conjugated donkey anti-rat antibody (Thermo Fisher Scientific, A-21247) diluted 1:50 in staining buffer for 1 h at room temperature. Finally, cover slips were washed with PBS, counterstained with DAPI and mounted with ProLong Gold Antifade mounting solution (Thermo Fisher Scientific, P36930).

For the membrane staining of TRAF3IP2 and LCK on GFP-sorted primary T cells, 400,000 sorted cells were loaded in cytosin columns (Hettich) and spun down for 6 min at 400 × g on glass slides. Cells were fixed with 4% paraformaldehyde in PBS for 20 min at room temperature. Further processing was identical to the membrane staining protocol in 293T cells. For LCK and CD3E staining, cells were incubated over night at 4 °C with anti-LCK antibody (Cell Signaling, #27875) diluted 1:200 and anti-CD3E antibody (BioLegend, #100223) diluted 1:500 in staining buffer for 1 h at room temperature. Finally, cover slips were washed with PBS, counterstained with DAPI and mounted with ProLong Gold Antifade mounting solution (Thermo Fisher Scientific, P36930).

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**Image analysis.** All image analysis was done with CellProfiler v4.0.6 software.1 For all analyses, images were segmented based on the DAPI channel. For analysis of membrane staining, cells were divided in bins and the integrated signal intensity was measured in every bin and expressed as a fraction of integrated signal intensity over the entire cell mask. For the analysis of nuclear RELA accumulation, cells were classified as GFP-positive or GFP-negative based on the GFP signal in each individual segment. Next, we measured the NF-κB signal intensity within the DAPI masked region, shrunk by 2 pixels to avoid interference from the cytoplasmic signal at the nuclear rim. We used the lowest quartile intensity signal to classify cells based on their nuclear NF-κB signal. For the analysis of nuclear RELB accumulation, cells were classified as RELB-positive or RELB-negative based on the RELB signal within each individual segment. Subsequently RELB-positive cells were classified as GFP-positive or -negative. RELB integrated signal intensity was measured over the nuclear mask and the cell mask after subtraction of the background signal (mean RELB signal in the inverted cell mask). For the quantification of BCL-XL, BCL-XL integrated signal intensity was measured over the cell mask after subtraction of the background signal and normalized to cell area.

**Statistics and reproducibility.** Statistical analysis and graphical representation were performed with Prism version 9 (GraphPad) and the R language (version 4.0). The Western blot in Fig. 10e was performed once. Figures 2b, c, e–g, 3a, c–f, 4a–f, 5g, 8d, 9a, d–f and Supplementary Figs. 2a, 3a–e, 6a are representative of 2 independent experiments with similar results. Figures 2a, d, 3b, 9c, b are representative of 3 independent experiments with similar results. Unless stated differently, all graphs use the mean as a measure of central tendency and the standard deviation as a measure of variation. For in vitro assays, we analyzed at least 3 biological replicates per condition. For qRT-PCR data, every data point represents the average of 3 technical replicates of a single biological replicate. Boxplots were made with the R package ggplot2 v3.3.2. Statistical tests to calculate p values are always indicated in the figure legends. A Sidak’s multiple comparisons test was always two-sided and used α = 0.05. Tukey’s multiple comparisons test and Games-Howell’s multiple comparisons test were always calculated at α = 0.05.

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

**Data availability.** Raw sequence data from clinical specimens has been deposited in the European Genome-phenome Archive (accession EGAS00001001466 for 3 PTCL-TFH cases, accession EGAS00001005015 for 15 PTCL-NOS cases). Access to the raw sequencing data from clinical specimens requires the completion of an application form, which will be reviewed by the appointed Data Access Committee, and requesters have to comply with the Data Access Policy. Raw sequence data from murine CD4+ T cells is available in the European Nucleotide Archive (accession P49E42764). Sequence data from anaplastic large cell lymphoma was obtained from the Sequence Read Archive (accession SRP044708) and sequence data from healthy lymph node was obtained from ArrayExpress (accession E-MTAB-2836). Expression data for immune cells in healthy individuals used in Supplementary Fig. 2b was obtained from DICE. The source data underlying Figs. 2, 3, 4, 5, 6, 7, 8, 9, 10 and Supplementary Figs. 1–6 are provided as a Source Data file. All the other data supporting the findings of this study are available within the article and its supplementary information files and from the corresponding author upon reasonable request. Source data are provided with this paper.

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Author contributions
K.D. conceived the project, collected clinical samples, performed bioinformatic analysis, planned and performed experiments, interpreted data and wrote the manuscript; L.Ma., M.v.B., N.M., O.G., K.J. and M.B. performed experiments; S.D. performed bioinformatic analysis; G.G., L.Mi., C.G. and I.W. provided clinical samples; P.G., L.d.L. and T.T. provided clinical samples and reviewed histopathology; J.C. and D.D. supervised the project, planned experiments, interpreted data and wrote the manuscript.

Competing interests
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