Heterozygosity for Roquin<sup>san</sup> leads to angioimmunoblastic T-cell lymphoma-like tumors in mice

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Introduction

Angioimmunoblastic T-cell lymphoma (AITL) is the second most common peripheral T-cell lymphoma with unusual clinical and pathologic features and a poor prognosis despite intensive chemotherapy. Recent studies have suggested AITL derives from follicular helper T (TFH) cells, but the causative molecular pathways remain largely unknown. Here we show that approximately 50% of mice heterozygous for the "san" allele of Roquin develop tumors accompanied by hypergammaglobulinemia by 6 months of age. Affected lymph nodes displayed the histologic features diagnostic of AITL, except for the presence of expanded FDC networks. Accumulation of TF<sub>H</sub> cells preceded tumor development, and clonal rearrangements in the TCR-β genes were present in most tumors. Furthermore, TF<sub>H</sub> cells exhibited increased clonality compared with non-TF<sub>H</sub> cells from the same lymph nodes, even in the absence of tumors. Genetic manipulations that prevent TF<sub>H</sub> development, such as deletion of ICOS, CD28, and SAP, partially or completely abrogated tumor development, confirming a TF<sub>H</sub>-derived origin. Roquin<sup>san/san</sup> mice emerge as a useful model to investigate the molecular pathogenesis of AITL and for preclinical testing of therapies aimed at targeting dysregulated TF<sub>H</sub> cells or their consequences. (Blood. 2012; 120(4):812-821)
lupus-like disease characterized by lymphadenopathy, splenomegaly, hypergammaglobulinemia, and the development of glomerulonephritis. Interestingly, mice heterozygous for the Roquin \textit{\textit{san}} allele (Roquin\textsubscript{sanroque}+) also have dysregulated T\textsubscript{FH} cells but do not develop the full-blown lupus-like autoimmunity. Instead, a proportion of mice develop asymmetric lymphadenopathy.

Here we report that enlarged lymph nodes from Roquin\textsuperscript{sanroque}+ mice display T-cell oligoclonality and many histopathological features consistent with human AITL. Furthermore, tumor development was found to be dependent on T\textsubscript{FH} cells, suggesting that, in human AITL, T\textsubscript{FH} cells may be the neoplastic driver of disease. These findings suggest that Roquin\textsuperscript{sanroque}+ mice may provide a good model for human AITL and thereby promote further understanding of disease mechanisms and help in the discovery of novel therapeutic targets.

Methods

Mice and tumor characterization

Roquin\textsuperscript{san}, Roquin\textsuperscript{san} Sap\textsuperscript{−/−}, Roquin\textsuperscript{san} Cd28\textsuperscript{−/−}, and Roquin\textsuperscript{san} Ecos\textsuperscript{−/−} mice were bred and maintained under pathogen-free conditions at the National University Animal Ethics and Experimentation Committee. Lymph nodes examined for tumor development were cervical nodes, axillary nodes, brachial nodes, and inguinal nodes. Size of lymph nodes was visually inspected and classified as tumorous when they were at least 5 times the size of normal lymph nodes from wild-type mice. Mice were characterized as tumorous if there was at least 1 enlarged lymph node among the aforementioned inspected nodes.

Antibodies

All antibodies and streptavidin conjugates used for flow cytometry were from BD Bioscience unless otherwise indicated: anti–mouse B220 PE-Cy7, CD20 PerCP, CD4 APC, CD8 FITC, CXCR5-biotin, GL-7 FITC, FAS PE, and PD-1 PE (eBioscience), mouse anti-Bcl6 A647, B220 PerCP-Cy5.5, and streptavidin PE Cy7. For immunohistochemistry, the primary antibodies used were goat anti–mouse CD3e (Santa Cruz Biotechnology), goat anti–mouse Pax-5 (Santa Cruz Biotechnology), and rat anti–mouse F4/80 (BMA Biomedicals).

ELISA

ELISA was performed to analyze total IgG levels in mouse serum as previously described.

Histology and immunohistochemistry

Hematoxylin and eosin-stained sections from a series of 19 Roquin\textsuperscript{sanroque}+ and 4 Roquin\textsuperscript{sanroque} formalin-fixed, paraffin-embedded lymph nodes were morphologically reviewed. Five lymph nodes belonging to Roquin\textsuperscript{sanroque}+ littermates were examined as controls. Immunohistochemistry for mouse CD3e, Pax5, and F4/80 was performed on formalin-fixed, paraffin-embedded sections as follows: tissue sections were deparaffinized, rehydrated, and treated for antigen retrieval. After quenching of endogenous peroxidase and blocking in normal serum, tissues were incubated with primary antibody overnight at 4°C, followed by incubation with biotinylated secondary antibody. Specific interactions were visualized using the Envision System (Dako North America) following the manufacturer’s instructions. Slides were counterstained with hematoxylin, dehydrated, and mounted. Slides were visualized on a Leica DMD108 microsystem at 25°C and acquired with LAS-DMD Version 1.3.1 software (Leica).

Flow cytometry

Single-cell suspensions of lymph nodes were prepared in FACS buffer (PBS/2% BSA/0.05% NaN\textsubscript{3}) by sieving and gently pipetting through 70-μm nylon mesh filters. After red blood cell lysis, 3 × 10\textsuperscript{6} cells were incubated with each antibody or conjugate layer for 30 to 60 minutes on ice. Samples were run on a FACSCalibur (BD Biosciences). Analysis was performed using FlowJo Version 7.2.5 (TreeStar).

B- and T-cell clonality studies

PCR was performed to clone the expansion of T and B cells.

Loss of heterozygosity

Using flow cytometry, 1, 10, or 100 T\textsubscript{FH} and naive T cells were sorted directly into the wells of a 96-well plate and digested with proteinase K (QIAGEN) to isolate DNA. A primary PCR was performed for the region of Roquin approximately 200 bp either side of the \textit{san} mutation. A secondary PCR was performed using fluorescently labeled primers to detect the Roquin\textsuperscript{san} and Roquin\textsuperscript{−} alleles, as regularly used to genotype the sanroque mouse strain. Primer sequences are available on request.

Statistics

Statistical analysis was performed in Prism Version 5 software (GraphPad Software) with a Mann-Whitney test unless otherwise stated.

Results

Roquin\textit{sanroque}+ mice develop asymmetrically enlarged lymph nodes and hypergammaglobulinemia

We have previously reported that Roquin\textit{sanroque}+ mice develop generalized lymphadenopathy, splenomegaly, and T\textsubscript{FH} dysregulation. In these mice, accumulation of T\textsubscript{FH} cells causes a lupus-like pathology. Initial inspection of Roquin\textit{sanroque}+ lymph node histopathology showed large polymorphic infiltrates and the presence of atypical T cells characteristic of human AITL (Figure 1). Given that reactive nodes commonly accompany systemic autoimmunity and this may confound the diagnosis of AITL, we investigated heterozygous Roquin\textsuperscript{sanroque}+ mice instead: initial observations suggested that a proportion Roquin\textsuperscript{sanroque}+ mice developed lymphadenopathy in the absence of overt autoimmunity.
To assess lymph node pathology in Roquin<sup>−/−</sup> mice, groups of mice were killed at monthly intervals from 4 months of age. All the major lymph nodes, including cervical, axillary, brachial, and inguinal, were examined. None of the Roquin<sup>−/−</sup> mice developed the generalized symmetric lymph node enlargement that is observed in 100% of Roquin<sup>−/+</sup> mice by 8 weeks of age. Instead, 53% of mice (100 of 188 mice studied) developed 1 to 4 enlarged lymph nodes, whereas other lymph nodes remained normal (Figure 2A; Table 1). This lymphadenopathy was not lethal, because of the tumors only being externally palpable once they had reached a considerable size, 4 of 11 mice (36%) investigated at 4 to 5 months of age had already developed tumors, suggesting some tumors are likely to develop before 4 months of age.

In addition to lymphadenopathy, many AITL patients present with splenomegaly. To Roquin<sup>−/−</sup> mice displayed a slight but significant (P < .05) increase in spleen weight compared with control Roquin<sup>−/+</sup> mice (Figure 2B-C). No significant difference in spleen size was observed between Roquin<sup>−/−</sup> mice with or without tumors, suggesting that splenomegaly does not correlate with, and may precede, lymphadenopathy. This is consistent with observations that nontumor lymph nodes from Roquin<sup>−/−</sup> mice are often

![Figure 1. AITL-like pathology in Roquin<sup>−/−</sup> mice.](image)

![Figure 2. Roquin<sup>−/−</sup> mice develop asymmetric lymphadenopathy and splenomegaly.](image)

1.5 × 10<sup>6</sup> cells, whereas the normal lymph node in the same mouse or in Roquin<sup>−/+</sup> mice without tumors consisted of approximately 1.0 × 10<sup>6</sup> cells (data not shown). The prevalence of tumors was 1.6 times higher in female mice (65%) than in male mice (41%; P = .001) regardless of age (Table 1). The sex distribution ratio for human AITL cases has been reported to be 1:1.25.26

Although the age of onset could not be accurately determined because of the tumors only being externally palpable once they had reached a considerable size, 4 of 11 mice (36%) investigated at 4 to 5 months of age had already developed tumors, suggesting some tumors are likely to develop before 4 months of age.
larger than lymph nodes from Roquin<sup>+/−</sup> control mice (data not shown). Hypergammaglobulinemia is another frequent finding in AITL, with 50% of patients displaying increased IgG in the serum.<sup>3,6</sup> Interestingly, although 4-month-old Roquin<sup>+/−</sup> mice do not develop overt lupus-like autoimmunity, Roquin<sup>+/−</sup> mice, both with and without tumors, developed hypergammaglobulinemia, showing that total serum IgG levels increased 2- to 3-fold above Roquin<sup>−/−</sup> mice (Figure 2D). A moderate (1.5-fold) but statistically significant (P < .05) increase in total serum IgG was observed in Roquin<sup>−/−</sup> mice that had developed tumors compared with mice of the same genetic background without tumors. Investigation of serum IgG levels over time suggested that, even at 6 weeks of age, Roquin<sup>+/−</sup> mice exhibit increased serum IgG titers compared with age-matched controls; however, this did not reach significance until 15 weeks of age (supplemental Figure 1, available on the Blood Web site; see the Supplemental Materials link at the top of the online article).

### Histopathology of Roquin<sup>+/−</sup> lymph nodes is reminiscent of AITL

Diagnosis of AITL is based on histologic analysis. The 5 main diagnostic criteria consist of: (1) effaced lymph node architecture, (2) prominent arborization of epithelioid venules, (3) extrafollicular meshwork of FDCs, (4) presence of atypical T cells with T<sub>FH</sub> phenotype, and (5) scattered large CD20<sup>+</sup> B cells.<sup>3</sup> Histopathologic examination of hematoxylin and eosin-stained sections of enlarged lymph nodes from Roquin<sup>−/−</sup> mice showed features reminiscent of AITL, including effacement of the nodal architecture, prominent vascularization, atypical T cells, and large B cells (Figure 3).

In addition to these features, there was a striking increase in the number of macrophages within the paracortex as shown by F4/80 staining with occasional foci of myeloid metaplasia (Figure 3B). Dense aggregates of small PAX5-positive lymphocytes were visible all over the parenchyma forming follicles without germinal centers (GCs), although a few large pale lymphoid cells were always found among them (Figure 3C-D). The same reactive B-blasts were also found in the interfollicular areas of the lymph node. Small clusters of mature plasma cells were also seen. A polymorphic infiltrate composed of small- to medium-sized proliferating CD3-positive lymphocytes was found in the interfollicular compartment, some of them showing cytologic atypia and increased size (Figure 3E). Moreover, occasionally T cells formed rosettes around large blasts (Figure 3F), which have been reported in AITL. Sinusoidal dilatation was also evident, as well as vessels filled with T cells. A prominent feature present in most AITL cases, but absent in the tumors of Roquin<sup>−/−</sup> mice, is prominent expansion of the FDC network,<sup>3,7,8,27</sup> as determined by an absence of CD21<sup>+</sup> dendritic cells in lymph node sections (data not shown). Together, this histologic appearance is highly reminiscent of AITL.

In contrast, histologic examination of nonenlarged lymph nodes from tumor-bearing Roquin<sup>−/−</sup> mice revealed that nodal architecture was preserved (Figure 3G). These lymph nodes displayed both primary and secondary follicles with reactive GCs as well as a marked increase of mature plasma cells in the interfollicular area (Figure 3H).

### Analysis of tumor composition

In AITL, the neoplastic T cells account for only a small fraction of the lymphoid infiltrate and are admixed with a large number of reactive immune cell types, including small lymphocytes, eosinophils, histiocytes, plasma cells, and large B cells. To quantify tumor composition, we enumerated T, B, and myeloid cells subsets by flow cytometry.

There was a significantly increased proportion of B cells in tumor lymph nodes compared with normal lymph nodes from either Roquin<sup>+/−</sup> or Roquin<sup>−/−</sup> mice (Figure 4A). Analysis of T<sub>FH</sub> cells revealed that the frequency of these cells was increased in “normal” Roquin<sup>+/−</sup> lymph nodes compared with wild-type Roquin<sup>+/−</sup> mice (Figure 4B,D-E), and this was accompanied by a significant increase in frequency of GC B cells (Figure 4C). In contrast, the tumor lymph node displayed frequencies similar to Roquin<sup>+/−</sup> mice. Comparing an enlarged tumor node with its contralateral nonenlarged lymph node, although the proportion of T<sub>FH</sub> cells identified as either PD-1<sup>+</sup>CXCR5<sup>+</sup> (Figure 4B; P < .001)
or PD-1hiBcl-6+ (Figure 4D-E; not significant) within CD4+ T cells was decreased, the total T<sub>FH</sub>-cell number was significantly increased (Figure 4F; P < .05). This supports the hypothesis that, in tumor lymph nodes, an initial expansion of T<sub>FH</sub> cells is diluted because of a large reactive infiltrate resulting in lymphadenopathy. This is also consistent with the observation that T<sub>FH</sub> cells contribute to only a small fraction of the cellularity of humanAITL-affected lymph nodes. Analysis of expression of the proliferation marker, Ki-67, by flow cytometry indicated that approximately 30% the T<sub>FH</sub> cells in the tumor lymph nodes of Roquin<sup>san/+</sup> mice were proliferating (Figure 4G). This was significantly higher (P < .05) than the proportion of proliferating T<sub>FH</sub> cells in either the non tumor lymph node from tumor-bearing Roquin<sup>san/+</sup> mice or lymph nodes from Roquin<sup>−/−</sup> mice. These results are also consistent with the abnormal CD3ε+ proliferating cells observed in the histologic sections (Figure 3E). In contrast, GC B cells in the tumor lymph node appeared to be less proliferative compared with both the Roquin<sup>san/+</sup> and Roquin<sup>−/−</sup> controls (Figure 4H), although the differences were not significant.

An increased frequency of myeloid cells and dendritic cells was also detected by flow cytometry (Figure 5A), with monocyte-derived dendritic cells being the most dysregulated subset with a 2- to 3-fold expansion (P = .0535) in the tumor lymph node compared with wild-type mice (Figure 5B). Monocyte-derived dendritic cells were expanded to an intermediate level (P = .1099) in the contralateral nontumor lymph nodes (Figure 5A-B). In contrast, CD8+ and CD8− dendritic cells were proportionally decreased, significantly and nonsignificantly, respectively, in both tumor and nontumor lymph nodes from Roquin<sup>san/+</sup> mice (Figure 5B).

Together, these data suggest that dysregulation of T<sub>FH</sub> cells and subsequent GC expansion are an obligatory consequence of the Roquin<sup>san</sup> mutation and preclude tumor development. As the lymph node disorganization progresses, it is probable that GCs collapse and neoplastic T<sub>FH</sub> cells are diluted amid large numbers of reactive cells. This scenario is consistent with the histologic data showing hyperplastic expansion of GCs in the nonenlarged lymph nodes leading to complete effacement of the nodal architecture in enlarged tumor lymph nodes.

### Tumors from Roquin<sup>san/+</sup> mice exhibit T-cell clonality

Expansion of 1 or several T-cell clones is a common feature of AITL tumor development. We examined clonality of both T and B cells in tumor lymph nodes by PCR amplification of TCR-β and IgH genes, respectively. Clonal rearrangements were found in the TCR-β gene in 12 of 15 cases (Table 2). In contrast, a clonal peak of the IgH gene was found in only 1 case (Table 2). Five cases were also examined for clonal rearrangements in the TCR-γ gene; however, no PCR products were amplified (data not shown).

To determine whether the observed clonality corresponded to expanded T<sub>FH</sub>-cell clones, we performed additional studies on T<sub>FH</sub> (CD4+CD<sup>+</sup>PD-1<sup>+</sup>CD<sup>+</sup>CXCR5<sup>+</sup>) and CD4+ non-T<sub>FH</sub> (CD4+CD<sup>+</sup>PD-1<sup>+</sup>CD<sup>+</sup>CXCR5<sup>−</sup>) purified by flow cytometry. T<sub>FH</sub> cells from Roquin<sup>−/−</sup> mice with and without tumors were found to be more clonal than T<sub>FH</sub> cells from Roquin<sup>−/−</sup> mice (Figure 6A; Table 3). Furthermore, T<sub>FH</sub> cells from Roquin<sup>−/−</sup> mice exhibited a more restricted TCR-β repertoire with the total number of TCR-β chains detected in each sample being much less than T<sub>FH</sub> cells from Roquin<sup>−/−</sup> mice (Table 3).

Of particular interest, T<sub>FH</sub> cells also exhibited more clonality than CD4+ non-T<sub>FH</sub> cells from the same lymph nodes (Figure 6B; Table 3). Importantly, the clonal peaks detected in the T<sub>FH</sub> samples often (68%) composed at least 5% of the total TCR-β repertoire, and each sample had at least 1 clone that accounted for more than...
10% of the repertoire, suggesting that this clone is highly overexpressed. In contrast, in the non-TFH samples, only 19% of clonal peaks in CD8+ T cells had a frequency more than or equal to 5%, and only 1 of 5 samples had a clone that accounted for more than 10% of the repertoire. Together, these data indicate that lymph nodes from Roquin+/− mice typically display expanded TFH-cell clones, and this may possibly precede tumor development.

**TFH cells drive AITL-like tumors of Roquin−/− mice**

To further investigate a possible role of TFH cells in driving or maintaining AITL, we introduced genetic manipulations that reduce the number and/or function of TFH cells, and evaluated the tumor incidence in these mice. Reduction or abrogation of TFH cells in Roquin−/− mice results in AITL-like disease.

Table 2. Summary of clonality of TCR-β receptors in lymph nodes from Roquin−/− mice

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C indicates clonal; P, polyclonal; and NA, not amplified.

**Discussion**

AITL is an aggressive T-cell malignancy with characteristic autoimmune-like manifestations related to B-cell reactivity that make it a complex disease with no appropriate mouse model to
date. Here we have described that heterozygosity for Roquinsan recapitulates most of the clinical, histologic, and cellular features associated with AITL, including lymphadenopathy, hypergamaglobulinemia, increased TFH-cell numbers,9,10 and clonal expansion of T cells.28-30 Thus, this work establishes Roquinsan/+/H11001 mice as a potentially useful mouse model of this peculiar type of peripheral T-cell lymphoma.

Notably, AITL is characterized by a large histopathologic spectrum with 3 patterns described: hyperplastic GCs and no increase in FDC (pattern 1), depleted follicles and loss of normal lymph node architecture (pattern 2), and complete effacement of lymph node architecture, with prominent FDCs (pattern 3) in full-blown AITL. All 3 patterns display polymorphic infiltrates and vascular proliferation. The majority of patients present with pattern

Figure 6. Tumors from Roquinsan mice exhibit T-cell clonality. TFH (A) and non-TFH cells (B) isolated from the lymph nodes of Roquinsan mice with or without tumors and lymph nodes from Roquin/H11001 mice. In mice with tumors, a nonenlarged lymph node was also analyzed for clonality. Cells were purified by flow cytometry. TCRV-β chains were amplified by PCR and clonality determined by CDR3 spectratyping. #Mouse number, as in Table 3. Analysis of non-TFH cells from Roquin/H11001 mice was not performed. Panels show 5 representative TCRV-β chains selected on the basis of those expressed in most samples. ND indicates not detected. All mice were 26 to 40 weeks of age.
2 or 3 (consequently known as classic AITL). Although these different morphologic variants do not appear to influence survival, progression from pattern 1 to pattern 2 or 3 is well documented, suggesting that pattern 1 is an early phase of the disease. \(^{11,31,32}\) Roquinsan/ Incidence of tumors in mice crossed to genetic backgrounds known to be associated with several FDC tumors. \(^{41,42}\) The role of EBV in AITL remains to be determined. Alternatively, it is possible that EBV infection and/or FDC expansion in humans may already be displaying the early form of disease (pattern 1). The proportion of Ki67 \(^{+}\) TFH cells increased from Roquin \(^{+/-}\) to nontumor lymph nodes and again in tumor lymph nodes from the same mouse, suggesting that increased proliferation correlates with tumor development.

Attempts to transplant AITL-like tumors were unsuccessful consistent with the reported poor transplantability of most peripheral T-cell tumors and B-cell lymphomas of GC origin. \(^{34-37}\) The difficulty in transplanting these tumors probably relates to the specialized environment of the GC, containing unique niches and stromal elements that may be required to support tumor growth. \(^{1-3}\) The absence of an expanded FDC network was arguably the one discordant feature between the tumors of Roquin \(^{+/-}\) mice and AITL. Most AITL patients are infected by EBV, which does not infect mice. It is therefore possible that the FDC expansion is a consequence of EBV infection. \(^{39}\) Although EBV infection is normally associated with B cells, FDCs express high levels of CD21, the surface receptor for this virus, and to the fact that terminally differentiated TFH cells do not recirculate because of the down-regulation of CCR7. \(^{38}\)

The role of EBV in FDC expansion in AITL remains to be determined. Alternatively, it is possible that EBV infection and/or FDC expansion in humans may drive/support TFH growth and/or survival, providing the right environment for neoplasia to develop. Indeed, in human AITL samples, neoplastic T cells are intimately associated with FDC networks. \(^{33}\) In contrast, the cell-autonomous accumulation of TFH cells in the presence of the Roquin "san" mutation may bypass the requirement of an expanded FDC network.

### Table 3. TCR BV clonality and frequency in TFH and non-TFH cells

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<th>Nontumor Roquin (^{+/-}) (mouse 4)</th>
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Values are the percentage of total TCR- \(\mu\)V chain mRNA.

C indicates clonal; O, oligoclonal; P, polyclonal; NA, not amplified (mRNA product not detected); and IN, inconclusive (mRNA detected, but CDR3 spectratyping inconclusive).

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Figure 7. TFH cells are the drivers of tumor development in Roquin \(^{+/-}\) mice. Incidence of tumors in Roquin \(^{+/-}\) mice crossed to genetic backgrounds known to reduce TFH cell numbers and function. All mice were 26 to 40 weeks of age.
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Increased clonality among TFH cells compared with non-TFH cells from the same lymph node, even in nontumor lymph nodes from Roquin<sup>ind/</sup> mice, is an intriguing finding. It suggests that ongoing antigen-specific responses to either self or foreign antigens are probably driving expansion of nonmalignant T<sub>FH</sub> clones, some of which may subsequently become neoplastic, thus mimicking early stages of AITL.

Our data provide experimental evidence in support of the notion emerging from histopathologic studies that T<sub>FH</sub> cells may be the cellular counterparts of AITL, and, indeed, blockade in T<sub>FH</sub>-cell development completely prevented AITL-like tumor development. AITL patients do not respond well to cytotoxic chemotherapeutic treatment, and alternative strategies are needed to improve patient outcome. Novel therapies that neutralize or deplete T<sub>FH</sub> cells by targeting ICOS, PD-1, SAP, or the PI3K signaling pathway may prove to be more specific and effective and improve prognosis. Because AITL neoplastic cells represent only a small proportion of the tumor, conventional T-cell lymphoma mouse models, in which disease is the result of uncontrolled malignant cell proliferation, may not be useful in testing new therapeutic compounds. In contrast, the Roquin<sup>ind/</sup> model of AITL is useful for preclinical testing of these or other novel therapeutic compounds.

In addition, Roquin<sup>ind/</sup> mice may help further understanding of AITL disease development mechanisms and pathways. Despite all Roquin<sup>ind/</sup> mice exhibiting dysregulated T<sub>FH</sub> cells, only 53% of mice develop AITL-like disease. This suggests that additional, possibly mutagenic, events are required for neoplastic transformation. Dissecting the pathway(s) that lead to lymphadenopathy and AITL-like disease in Roquin<sup>ind/</sup> mice may thereby provide important insights into human disease development and progression.

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Authorship

Contribution: J.I.E. performed and analyzed experiments and wrote the manuscript; T.C., S.-M.R.-P., J.L.M., X.H., M.N.-G., J.F.G., and S.-M.-M. and performed and analyzed experiments; M.-H.D.-L. and P.G. provided critical analysis of the manuscript and clinical interpretation of the data; G.W. designed experiments and interpreted data; M.C.C. helped conceive the original study and had intellectual input; and M.A.P. and C.G.V. conceived the study, analyzed data, provided intellectual input, and revised the manuscript.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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Heterozygosity for Roquin<sup>San</sup> leads to angioimmunoblastic T-cell lymphoma-like tumors in mice