Recurrent GNAQ mutation encoding T96S in natural killer/T cell lymphoma

Zhaoming Li1,2,9, Xudong Zhang1,2,9, Weili Xue1,3,9, Yanjie Zhang1,3,9, Chaoping Li1,3, Yue Song1,3, Mei Mei1,3, Lisha Lu1,3, Yingjun Wang1,3, Zhiyuan Zhou1,3, Mengyuan Jin1,3, Yangyang Bian4, Lei Zhang1,2, Xinhua Wang1,2, Ling Li1,2, Xin Li1,2, Xiaorui Fu1,2, Zhenchang Sun1,2, Jingjing Wu1,2, Feifei Nan1,2, Yu Chang1,2, Jiaqin Yan1,2, Hui Yu1,2, Xiaoyan Feng1,2, Guannan Wang5, Dandan Zhang5, Xuefei Fu6, Yuan Zhang7, Ken H. Young8, Wencai Li5 & Mingzhi Zhang1,2

Natural killer/T cell lymphoma (NKTCL) is a rare and aggressive malignancy with a higher prevalence in Asia and South America. However, the molecular genetic mechanisms underlying NKTCL remain unclear. Here, we identify somatic mutations of GNAQ (encoding the T96S alteration of Gαq protein) in 8.7% (11/127) of NKTCL patients, through whole-exome/targeted deep sequencing. Using conditional knockout mice (Ncr1-Cre-Gnaqfl/fl), we demonstrate that Gαq deficiency leads to enhanced NK cell survival. We also find that Gαq suppresses tumor growth of NKTCL via inhibition of the AKT and MAPK signaling pathways. Moreover, the Gαq T96S mutant may act in a dominant negative manner to promote tumor growth in NKTCL. Clinically, patients with GNAQ T96S mutations have inferior survival. Taken together, we identify recurrent somatic GNAQ T96S mutations that may contribute to the pathogenesis of NKTCL. Our work thus has implications for refining our understanding of the genetic mechanisms of NKTCL and for the development of therapies.
Natural killer/T cell lymphoma (NKTCL) is an aggressive subtype of non-Hodgkin’s lymphoma that is rare overall but shows a predilection for Asian and South American populations. NKTCLs are almost exclusively extranodal involving the nasal and/or paranasal area, with a strong association with Epstein–Barr virus (EBV) infection. Despite a multimodality chemotherapy and radiotherapy treatment approach, the survival of patients with NKTCL is poor.

The molecular pathogenesis of NKTCL currently remains elusive. In the past decade, however, several oncogenic pathways, including Janus kinase (JAK)—signal transducer and activator of transcription (STAT), mitogen-activated protein kinase (MAPK), AKT, and NF-kB signaling pathways, have been identified in the development of NKTCL by gene expression profiling. Recently, DDXX, STAT3, JAK3, BCOR, and TP53 have been revealed as novel genes mutated in NKTCL by high-throughput sequencing studies.

In this study, we sought to identify additional oncogenic drivers and altered pathways that contribute to NKTCL tumorigenesis in 127 patients with NKTCL through whole-exome/targeted deep sequencing. In addition to frequently mutated genes reported previously, somatic mutations of GNAQ (encoding the T96S alteration of Gαq protein) were identified in 8.7% (11/127) of the patients with NKTCL. Experiments using conditional knockout mice demonstrated that Gαq deficiency enhanced the survival of natural killer (NK) cells. We also found that Gαq suppressed NKTCL tumor growth via inhibition of the AKT and MAPK signaling pathways. Furthermore, the Gαq T96S mutant might act in a dominant negative manner to promote tumor growth in NKTCL. In addition, we observed that patients with GNAQ T96S mutations had inferior survival.

To our knowledge, the present study includes one of the largest series of NKTCL patients ever described and defines in detail the genetic landscape of mutations. In particular, recurrent GNAQ T96S mutations were detected in our NKTCL patients.

**Results**

**Whole-exome sequencing of NKTCL.** Whole-exome sequencing was performed on paired normal and tumor DNA from 28 patients with NKTCL (Supplementary Fig. 1). The demographics and clinical features of the patients are summarized in Supplementary Table 1. The mean sequencing depth was 84.67×, and a mean of 99.38% of the target sequence was covered to a depth of at least 20× (Supplementary Table 2). A total of 2642 nonsilent mutations, including 2374 missense, 114 nonsense, 105 splice site, and 7). The mean coverage of the target genes was 1408× (a minimum of 1011×), and a mean of 99.38% of the target sequence was covered to a depth of at least 100× (Supplementary Tables 6 and 7).

To identify the frequently mutated genes in NKTCL, we combined the sequencing data of the discovery cohort and the validation cohort. After excluding implausible genes, such as the genes encoding olfactory receptors and extremely large proteins, and genes with very long introns, we identified the following 14 genes: DDXX (16/101), KMT2D (also known as ML2L, 13/101), STAT3 (12/101), GNAQ (11/101), FAT1 (10/101), MSN (9/101), ATRID (9/101), TET2 (8/101), USP8 (6/101), TP53 (6/101), PTPN13 (6/101), CD93 (5/101), ATRX (5/101), and TRAM1L1 (5/101). Each of these gene mutations was present in at least five NKTCL samples (Fig. 2a).

Notably, 15 point mutations in GNAQ were identified in 11 NKTCL samples: 9 mutations encoding T96S, 4 mutations encoding Y101X, 1 mutation encoding p.K57T, and 1 mutation encoding p.M59L. Mutations in T96S cooccurred with Y101X and p.M59L in three cases and one case, respectively (Fig. 2b and Supplementary Fig. 3). The frequently mutated genes were further validated in another independent cohort of NKTCL patients without matched normal tissue by performing whole-exome sequencing (n = 26, Supplementary Table 8). Interestingly, GNAQ T96S mutations were found in 2 of the 26 examined cases, and both were subsequently validated by Sanger sequencing (Supplementary Fig. 4). In this study, GNAQ T96S mutations were identified in 8.7% (11/127) of the NKTCL cases.

**Gαq deficiency leads to enhanced NK cell survival.** NKTCL is a mature NK cell neoplasm. However, the function of GNAQ in normal NK cells is unclear. To address this question, we generated Ncr1-Cre-Gnaqfl/fl mice that express Cre recombinase under control of the Ncr1 promoter, which allowed NK cell-specific ablation of Gnaq (Fig. 3a, b). Ncr1-Cre-Gnaqfl/fl mice were born at the expected Mendelian ratio without any visible alterations in organ morphology or overt pathology.

First, we verified Gq expression in sorted NK cells isolated from Ncr1-Cre-Gnaqfl/fl mice and their littermate controls that harbored loxp-flanked Gnaq alleles but lacked Ncr1-Cre (Gnaqfl/fl mice). As expected, splenic NK cells from Ncr1-Cre-Gnaqfl/fl mice showed significantly reduced Gq expression compared with NK cells from control mice; however, there was no difference in Gq expression on non-NK cells between the two groups (Fig. 3c). Therefore, Gnaq was deleted exclusively in NK cells.
Next, we determined whether Gnaq influences the distribution of NK cells within different compartments. However, no significant differences in NK cell frequency were detected among tissue types (bone marrow, spleen, peripheral blood, liver, and lymphoma node) in Ncr1-Cre-Gnaqfl/fl mice and their littermate controls (Fig. 3d).

Finally, we evaluated whether Gnaq is involved in the regulation of NK cell expansion or survival. The expansion of splenic NK cells was assessed by an in vivo BrdU incorporation assay. No differences in DNA replication were observed in splenic NK cells was assessed by an in vivo BrdU incorporation assay. No differences in DNA replication were observed in splenic NK cells between Ncr1-Cre-Gnaqfl/fl mice and their littermate controls (Fig. 3e). We further investigated whether Gnaq regulates NK cell survival. Purified splenic NK cells were cultured in media without IL-2 for 6 h, and cell viability was monitored by Annexin V staining. Surprisingly, NK cells from Ncr1-Cre-Gnaqfl/fl mice exhibited a significant survival advantage compared with NK cells from their corresponding littermate controls (Fig. 3f). Collectively, these data suggested that NK cells from mice lacking Gnaq have an intrinsic survival advantage over normal NK cells.

**Tumor-suppressive role of Gαq in NKTCL.** We next sought to determine the functional role of Gαq in NKTCL. First, we examined the expression of GNAQ in NK cells isolated from

---

**Fig. 1** Whole-exome sequencing in 28 patients with NKTCL. a The number and type of nonsilent somatic mutations identified by whole-exome sequencing. b The spectrum of mutations in NKTCL, with 30 curated mutational signatures defined by the COSMIC database. c, d Three dominant signatures identified by combined nonnegative matrix factorization clustering and correlation in NKTCL, with 30 curated mutational signatures defined by the COSMIC database. e The correlation analysis of nonsilent somatic mutations and the age of the NKTCL patients (n = 28; R² = 0.196, P = 0.018). f The association of the somatic nonsilent mutation burden with overall survival in NKTCL (log-rank P = 0.397).
normal and neoplastic tissues. A pooled analysis of previously published datasets\textsuperscript{15} and our own RNA sequencing data showed that \textit{GNAQ} expression was significantly lower in neoplastic NK cell lines and tumor samples compared with primary human NK cells (Fig. 4a). This result was further confirmed by western blot analysis of \textit{Gaq} protein expression in primary human NK cells and different neoplastic NK cell lines. In particular, loss of \textit{Gaq} expression was observed in a novel NK leukemia cell line (KHYG1) (Fig. 4b). Chromosome 9q deletion has been reported in KHYG1, which may account for the loss of \textit{Gaq} in this cell line\textsuperscript{32,33}. Additionally, somatic copy number alteration analysis was performed in the 28 exome cases, and somatic copy number loss of \textit{GNAQ} was found in one case (Supplementary Fig. 5).

Second, we generated stable cell lines (YT and NKYS) expressing vector control or \textit{GNAQ}. Both cell lines carried wild-type \textit{GNAQ} according to the RNA sequencing data\textsuperscript{15}. As shown in Fig. 4c, forced expression of \textit{Gaq} significantly suppressed tumor cell viability in comparison with the vector control in both cell lines. We further examined the effect of \textit{Gaq} on cell apoptosis and proliferation by Annexin V staining and EdU incorporation assay, respectively. In vitro studies demonstrated that overexpression of \textit{Gaq} significantly enhanced cell apoptosis but had little effect on NK cell proliferation (Fig. 4d).

Finally, YT cells stably expressing \textit{Gaq} or vector control cells were injected subcutaneously into two groups of NOD/SCID mice \((n = 5\) each). The tumor size was measured every 2 days with calipers, and tumor burden was monitored 6 weeks after cell implantation. As expected, in vivo fluorescence imaging of xenograft mice showed that the fluorescence intensity \((p^\text{-}1\text{sec}^\wedge-1\text{cm}^\wedge-2\text{sr}^\wedge-1)\) of tumors derived from the \textit{Gaq} group was significantly lower than those from the control group \((P = 0.011; \text{Fig. 4e})\). Consistently, the mean tumor weight of the \textit{Gaq} group at the end of the experiment was significantly lower \((0.81 \pm 0.24\, \text{g})\) than that of the control group \((0.21 \pm 0.12\, \text{g}; \text{Fig. 4e})\).

We further addressed the question of whether \textit{Gaq} affected cell survival in vivo. The number of apoptotic cells was significantly higher in the \textit{Gaq} group than in the vector control group, as determined by the TDT-mediated dUTP nick end labeling (TUNEL) assay \((P = 0.031; \text{Fig. 4f})\). In addition, the YT cells expressing \textit{Gaq} were significantly less proliferative based on Ki67 staining and analysis of tumor sections (Supplementary Fig. 6). This finding indicated that overexpression of \textit{Gaq} affects the survival and/or proliferative capacity of YT cells in the tumor microenvironment, which appears to be the mechanism by which \textit{Gaq} can suppress tumor growth in vivo.

\textbf{Gaq T96S mutants act in a dominant negative manner in NKTCL.} We then attempted to characterize the functional role of the \textit{Gaq} T96S mutant in NKTCL. Because all \textit{Gaq} mutation-positive cases of NKTCL were heterozygous for this mutation, we established tetracycline-inducible cell lines (KHYG1 and YT) that could simultaneously express equal levels of wild-type and mutant \textit{Gaq}. The expression levels of wild-type and \textit{Gaq} T96S mutant were verified by western blot (Fig. 5a).

Expression of wild-type \textit{Gaq} led to a significant decrease in the viability of NK cells. In contrast, the expression of the \textit{Gaq} T96S mutant could compromise the ability of wild-type \textit{Gaq} to impair NK cell viability (Fig. 5b). However, no significant difference between the \textit{Gaq} T96S mutant and control groups was present, possibly due to the absent or extremely low expression of endogenous \textit{Gaq} in these two NK cell lines. Similarly, forced expression of wild-type \textit{Gaq} enhanced cell apoptosis of NK cells, and this proapoptotic effect of wild-type \textit{Gaq} could be attenuated by the \textit{Gaq} T96S mutant (Fig. 5c), suggesting a dominant
negative nature of Gaq T96S. Moreover, we examined the effect of the Gaq T96S mutant on NKTL tumorigenesis in vivo. Consistent with the above findings, wild-type Gaq significantly reduced the tumor-formation ability compared with the vector control, whereas the Gaq T96S mutant impaired the tumor-suppressor activity of wild-type Gaq. The mean weights of the tumors in the vector control, wild-type, Gaq T96S, and wild-type + Gaq T96S groups were (0.46 ± 0.08), (0.09 ± 0.02), (0.41 ± 0.06), and (0.37 ± 0.07) g, respectively (wild-type versus vector, \( P = 0.002 \); Gaq T96S versus vector, not significant; Fig. 5d).

Furthermore, we identified protein binding partners of wild-type or Gaq T96S mutants using immunoprecipitation coupled to mass spectrometry. Significance analysis of INTeractome (SAINT) expression was utilized to calculate the probability of protein–protein interaction from background, nonspecific interactions\(^ {34-37} \). Using a SAINT probability threshold of \( \geq 0.8 \), we identified 17 high-confidence interacting partners of Gaq (Fig. 5e and Supplementary Table 9). G\( \beta 1 \) (GNB1), a known direct binding partner of Gaq, appeared to have higher affinity for the Gaq T96S mutant than its wild-type. Furthermore, immunoprecipitation assay confirmed that the Gaq T96S mutant bound G\( \beta 1 \) more tightly than wild-type Gaq in YT cells (Fig. 5f). It is well known that the G\( \alpha \) mutant can exert a dominant negative effect in the heterotrimeric complex
by sequestration of the Gβγ subunits. It is therefore suggested that sequestering the Gβγ subunits may be a possible mechanism by which the Gqα T96S mutant exerts its dominant negative effect on wild-type Gqα in NK cells, which should be further investigated. Altogether, these data suggested that the Gqα T96S mutant might contribute to the pathogenesis of NKTCL through the inhibition of wild-type Gqα in a dominant negative manner.

**Gqα suppresses AKT and MAPK signaling pathways in NK cells.** To examine the effect of Gqα on gene expression in NK cells, we performed RNA sequencing analysis using RNA prepared from NKTCL tumor samples. Gene set enrichment analysis (GSEA) demonstrated that both MAPK and T cell receptor (TCR) signaling pathways were significantly enriched in cells expressing the vector control compared with wild-type Gqα cells (Fig. 6a and Supplementary

### Fig. 4 Tumor-suppressive role of Gqα in NKTCL

**a** GNAQ mRNA expression in normal NK cells, neoplastic NK cells, and tumor samples. The GNAQ expression values were obtained from previously published data and our RNA sequencing data and normalized to GAPDH. Resting NK: >95% CD56+CD3− NK cells isolated from peripheral blood lymphocytes. PBNK48h: 48-h IL-2-activated NK cells. PMIG, NK92 and PRDM1, NKTCL: NK92 cells transduced with PMIG and PRDM1, respectively. **b** GNAQ protein expression in normal and neoplastic NK cells. Forced expression of Gqα suppressed cell viability in YT (left) and NKTCL (right) cells. YT and NKTCL cells were stably transfected with vector control or wild-type GNAQ, and cell viability was assessed using a CCK-8 assay. Cell viability is presented by the absorbance value at OD 450 nm, which was measured with a Multiskan FC microplate reader (Thermo Scientific, Waltham, MA, USA). The value is directly proportional to the number of viable cells in the culture medium. Data are representative of three independent experiments. **c** Overexpression of Gqα significantly enhanced cell apoptosis but had little effect on NK cell proliferation. Cell apoptosis and cell proliferation were assessed by Annexin V staining (upper panel) and EdU incorporation assay (lower panel), respectively. Data are representative of at least three independent experiments. **d** NOD/SCID mice were inoculated subcutaneously with YT cells stably transfected with vector control or wild-type Gqα (n = 5 in each group). The tumor burden was monitored by utilizing the IVIS Spectrum system (Perkin Elmer, Beaconsfield, UK) after 6 weeks. Representative images and quantitative data for each group are shown in the upper panel. Representative images of xenograft tumors and tumor weights for each group are shown in the lower panel. **e** Representative images (left) and quantitative data (right) (n = 5 in each group) for TUNEL staining of the xenograft tumor tissues. Scale bars, 50 μm. All data are expressed as the mean ± s.e.m.; NS, not significant. *P < 0.05, **P < 0.01, ***P < 0.001, unpaired two-tailed Student’s t-test. Source data are provided as Source Data file.
**Fig. 5** Gaq T96S mutant acts in a dominant negative manner to promote tumor growth of NKTCL. a Western blot shows that tetracycline-inducible cell lines (KHYG1 and YT) express equal amounts of wild-type and mutant Gaq simultaneously. b Viability of YT and KHYG1 cells with inducible expression of vector, wild-type, T96S or wild-type plus T96S. Cell viability is presented as the absorbance value at OD 450 nm. Data are representative of three independent experiments. c Annexin V-positive cells (%). The wild-type and T96S groups show significantly higher expression of phospho-ERK (p-ERK) than their control littermates (**P < 0.01, **P < 0.001, unpaired two-tailed Student’s t-test). Data are provided as a Source Data file.

Table 10). Consistently, NK cells from Ncr1-Cre-GNAQ^{fl/fl} mice showed significantly higher expression of phospho-ERK (p-ERK) and phospho-AKT (p-AKT) than their control littermates (Fig. 6b). These results indicated that Gaq could suppress AKT and MAPK signaling pathways in NK cells, consistent with previous studies reporting that these two pathways could be suppressed by Gaq in mice B and T cells^{39,40}.

These findings were further validated in NKTCL cells. As shown in Fig. 6c, compared with the vector control group, the phosphorylation of ERK and AKT was suppressed in the presence of wild-type Gaq. However, unlike wild-type Gaq, the Gaq T96S mutant was unable to reduce the phosphorylation of ERK and AKT. In contrast, it could affect the suppressive function of wild-type Gaq on the two signaling pathways, underlining a dominant negative nature of Gaq T96S. Similar results were obtained from immunohistochemistry analysis of mouse xenograft tumors (Fig. 6d). Moreover, phospho-ERK and phospho-AKT were detected in human NKTCL samples, with significantly higher levels in patients with the mutant GNAQ T96S genotype than in patients with the wild-type Gaq genotype using two immunohistochemistry scoring methods (Fig. 6e and Supplementary Fig. 7).

**GNAQ T96S mutations predict a worse prognosis.** We investigated the relationship between GNAQ T96S mutations and clinical characteristics. In this analysis, only patients with
matched tumor/normal sample pairs were included, two individuals with non-T96S GNAQ mutations (Y101X in #8188 and K57T in #36) were excluded from the survival analysis. We found that GNAQ T96S mutations were significantly associated with advanced disease stage ($P = 0.025$) and a higher fraction of Ki67-positive cells (>50%) in the tumor samples ($P = 0.035$; Supplementary Table 11).

Univariate analysis suggested that patients with GNAQ T96S mutations ($n = 9$) had a worse prognosis than individuals with wild-type GNAQ ($n = 90$). The estimated 3-year OS rates of patients with GNAQ T96S mutations and wild-type GNAQ were 33.3 ± 15.7% and 72.3 ± 5.0%, respectively ($P = 0.006$, FDR = 0.048; Fig. 7a). The estimated 3-year progression-free survival (PFS) rates for subjects from the GNAQ T96S and wild-type GNAQ groups were 22.2 ± 13.9% and 57.3 ± 6.2%, respectively ($P = 0.005$, FDR = 0.059; Fig. 7b). Moreover, when a Cox multivariate regression model was applied, the T96S mutation status of GNAQ remained an independent prognostic marker for poor outcomes after adjusting for B symptoms, the International Prognostic Index (IPI), the Ki67 index (>50%), and the primary site of the tumor (OS: HR = 3.31, CI = 1.33–8.24, $P = 0.010$; and PFS: HR = 2.85, CI = 1.27–6.37, $P = 0.011$; Supplementary Table 12). Altogether, these results suggested that GNAQ T96S mutations were positively correlated with advanced tumor stage and poor clinical outcomes in NKTCL.

In addition, mutations in DDX3X or TP53 were associated with a worse prognosis of patients with NKTCL, consistent with previous studies. In contrast, patients without mutations in DDX3X, TP53, or GNAQ (T96S) showed a more favorable prognosis than those harboring the mutations (Fig. 7c, d). These observations indicated that the mutational status of DDX3X, TP53 and GNAQ genes might represent a promising marker for therapeutic stratification of patients with NKTCL, which needs to be validated in future large, prospective studies.

**Discussion**

NKTCL is a rare, malignant neoplasm characterized by a highly aggressive clinical course. Although considerable advances in our understanding of the mechanisms involved in NKTCL have been made in recent years, the rare nature of NKTCL and its heterogeneity limit the ability to standardize therapy. Somatic mutation analysis represents a useful tool in selecting personalized therapy. In this study, we undertook large-scale screening in a cohort of NKTCL patients and identified recurrent somatic GNAQ mutations encoding a p. T96S alteration in 8.7% (11/127) of NKTCL samples. In addition to GNAQ, mutations in multiple genes involved in G protein-coupled receptor (GPCR) signaling pathways, including GPR98, GPR125, GNAS, GNAI2, GNAI2, and PLCB3, were also detected in our
study. These mutations appeared to be mutually exclusive and affected 24.8% (25/101) of total NKTCL cases (Supplementary Fig. 8), suggesting that deregulated GPCR signaling plays a major role in the tumorigenesis of NKTCL.

Gaq is a member of the q class of Ga subunits that mediates signals between GPCRs and downstream effectors48,49. Although the oncogenic potential of Gaq has been widely studied in human tumors, its role in NK cells remains unclear49. Using newly generated conditional knockout mice (Ncr1-Cre-GNAQf/f), we found that NK cells lacking GNAQ have an intrinsic survival advantage over normal counterparts, which is consistent with previous studies in B and T lymphocytes39,40. Further functional studies in neoplastic NK cells revealed a tumor-suppressive role of Gaq in NKTCL. Gaq suppressed the activation of AKT and ERK in the same way it did in B and T lymphocytes39,40. Therefore, the dual function of Gaq in different types of tumors suggests that it may potentially act either as a tumor suppressor or as an oncogene depending on the cellular context. This type of functional duality based on cellular context has been observed for Ga13 (encoded by GNA13), which is another member of the Ga protein family49,50. Ga13 has been previously linked to cellular transformation and characterized as growth promoting in epithelial cancer models51,52. However, recent studies have identified a Ga13-dependent pathway that exerts dual actions in suppressing growth and blocking dissemination of germinal center B cells, which are frequently disrupted in germinal center B-cell-derived lymphomas53–55.

According to the COSMIC database29, there are four mutation hotspots in GNAQ: Q209, R183, T96 and Y101. The frequency of these mutation hotspots within GNAQ appears to exhibit significant differences between solid and hematopoietic tumors. Q209 mutations comprise the most frequently mutated hotspot in solid tumors, followed by R183 mutations. In striking contrast, T96 is the predominantly mutated hotspot in hematopoietic tumors, followed by Y101 (Supplementary Fig. 9). This pattern was also verified in NKTCL patients in the present study. This work thus suggests the intriguing possibility that different GNAQ mutations may have different functional consequences, and the properties crucial for their oncogenic functions vary depending on the tissue of origin.

However, this study has some limitations that must be noted. First, the Y101X mutation harbors a premature termination codon. In contrast to T96S, cell line transduction experiments showed that the Y101X mutation could disrupt truncated Gaq protein expression (Supplementary Fig. 10). We speculated that instability of truncated Gaq protein or nonsense-mediated
mRNA decay might account for the disruption of truncated Gaq protein expression, which needs to be further investigated. We did not conduct functional analysis of the non-hot spot mutants (K57T and M59L) in the present study, given that these two mutants occurred in only 1% of the NKTCL cases. However, the functions of the two mutants need to be addressed in future studies. Second, germline DNA was unavailable in 26 specimens among the 127 NKTCL cases. Although a strict filter was applied for variant calling, the somatic nature of these mutations identified in this cohort could not be confirmed. We therefore excluded these cases in the subsequent survival analysis. Third, further studies are warranted to clarify the molecular mechanisms mediated by Gaq in suppressing AKT and MAPK signaling in NK cells.

Taken together, we identified recurrent somatic GNAQ T96S mutations, which may contribute to the pathogenesis of NKTCL. These findings have implications for refining our understanding of the genetic mechanisms of NKTCL and for treatment development.

Methods

Patients and samples. The demographics and clinical features of the patient cohort are summarized in Supplementary Table 1. In total, 127 formalin-fixed, paraffin-embedded (FFPE) tumor tissues from NKTCL patients were obtained at the time of diagnosis at the First Affiliated Hospital of Zhengzhou University. All cases were reviewed and interpreted independently by three experienced pathologists, and diagnoses were made according to the current World Health Organization classification criteria. The experimental design is depicted in Supplementary Fig. 1. The study was conducted in accordance with the Declaration of Helsinki and with approval from the Institutional Review Board of the First Affiliated Hospital of Zhengzhou University. Signed informed consent was obtained from these patients.

DNA extraction. Genomic DNA from whole blood and FFPE samples was extracted using a BioTea Cell Culture DNA Kit (Quagen, Hilden, Germany) and a QIAamp DNA FFPE Tissue Kit (Quagen), respectively. The quality and yield of purified DNA were assessed by fluorimetry (Qubit, Invitrogen), Nanodrop 1000 spectrophotometry (Thermo Scientific, Wilmington, DE, USA), and gel electrophoresis.

Whole-exome sequencing and bioinformatics analysis. To identify somatic genomic variants associated with NKTCL, we performed WES on 28 tumor samples and their matched whole-blood samples. Genomic DNA (1–1.5 μg) was fragmented with a Covaris ultrasonicator targeting peak sizes ranging from 180 to 280 bp. The fragment ends were blunted and 5′-phosphorylated with T4 poly-nucleotide kinase, T4 DNA polymerase, and Klenow Large Fragment (New England Biolabs). The fragmented DNA was purified with Agencourt AMPure XP beads and enriched by PCR using the Illumina PCR primers InPE1.0 and InPE2.0 and PCR primer indices. Pooled, indexed libraries were captured using the Agilent SureSelect Human All Exome 50 Mb Kit (Agilent Technologies) according to the manufacturer’s protocol and sequenced on an Illumina HiSeq 2500 instrument.

Sequencing reads were aligned to the human reference genome hg19 (downloaded from the UCSC Genome Browser http://genome.ucsc.edu/) using the Burrows-Wheeler Aligner (BWA)28 version 0.5.8 with default parameter settings. SAMtools was used to convert the SAM files into BAM files and to pile up sequences from local alignments29. The Picard command (http://picard.sourceforge.net/projects/picard/files/picard-tools/) was used to remove PCR duplications. Next, insertion or deletion (InDel) realignment and base quality score recalibration were performed with the Genome Analysis Toolkit (GATK) version 2.6.30. For tumor–normal tissue sample pairs, somatic single-nucleotide variations (SNVs) and somatic InDels were called using MuTeC31 and STrelka32, respectively. Control-FRECC was used to detect copy number alterations in tumors compared with normal cells33. Somatic copy number alteration (Supplementary Table 14) and loss of heterozygosity (LOH, Supplementary Table 15) analyses were performed in the 28 exome cases. For the 26 non-paired tumor samples with no matched germline DNA, probable somatic variants were detected by SomVariUS with the default settings34. Only variants from the 26 non-paired tumor samples were further filtered to remove those that were present in dbSNP v135 (http://www.ncbi.nlm.nih.gov/SNP/), the 1000 Genomes Project (http://browser.1000genomes.org), or an in-house database containing germline variants identified in ~300 Chinese exomes. Variants were also visually inspected with the Integrative Genome Viewer (IGV, http://www.broadinstitute.org/igv/) to exclude probable sequencing artifacts. Gene mutation annotation of the identified variants was carried out using ANNOVAR35. The impact of the SNVs on protein function was predicted by PolyPhen-2, SIFT, or MutationTaster. Whole-exome sequencing data were deposited into the NCBI Sequence Read Archive under accession code SRP107053.

Targeted deep sequencing. We designed a custom panel of 221 genes (Supplementary Table 5) using the SureDesign Tool (Agilent Technologies). Sequencing libraries were prepared from DNA extracted from 73 paired tumor and normal samples using the SureSelect XT2 Target Enrichment System for the Illumina Multiplexed Sequencing Platform (Illumina) according to the manufacturer’s instructions. Target-enriched libraries were then sequenced on an Illumina HiSeq 2500 sequencing platform. A bioinformatics analysis was performed as described for the exome sequencing analysis. All candidate variants were manually inspected in the IGV to exclude false positives.

Sanger sequencing. Selected SNVs detected by whole-exome sequencing were validated by Sanger sequencing. Primers specific to the gene markers were designed and synthesized by Sangon Biotech (Shanghai, China, Supplementary Table 4). PCR was performed using standard procedures followed by direct sequencing on an ABI 3730xl automatic sequencer (PE Applied Biosystems, Foster City, CA, USA).

Mice and genotyping. Ncr1-Cre-Gnaq+/− mice (C57BL/6) were generated by crossing Gnaq+/− mice with Ncr1-Cre mice. Gnaq+/− mice were generated by Viewold Biotech (Beijing, China). Briefly, loxP sites flanking exon 6 of Gnaq were introduced by CRISPR/Cas9. This locus of Gnaq has been previously successfully used to generate conditional Gnaq−/− mice36. In vitro-synthesized sgRNA, Cas9 mRNA, and donor vectors were injected into mouse zygotes and then transferred to pseudopregnant mice. Neonatal mutant mice were identified by genotyping and sequencing. Genomic DNA samples were prepared from tail biopsies of 5-day-old mice. PCR genotyping was performed. The primer pairs used to identify correct insertion of LoxP flanking exon 6 of Gnaq and Cre are provided in Supplementary Table 13. The relevant Animal Ethics and Experimentation Committee of the First Affiliated Hospital of Zhengzhou University approved animal experiments according to the guidelines of Zhengzhou University. Both female and male mice aged between 8 and 15 weeks of age were used in this study. Age and sex matching was performed for each independent experiment (n ≥ 4 mice per genotype). All procedures were conducted in accordance with the Animal Care and Use Committee guidelines of Zhengzhou University.

To isolate cells from the spleen, spleens were minced and treated with 2 mg/ml collagenase D in Hanks balanced salt solution with CaCl2/MgCl2 for 30 min at room temperature followed by filtering through 70-μm nylon cell strainers. The livers of mice were perfused with PBS to remove blood for the isolation of liver-resident cells, and single-cell suspensions were subsequently generated as described for the spleen. Single-cell suspensions from lymph nodes were generated by meshing the organs through 70-μm nylon cell strainers. Contaminating erythrocytes in cell suspensions were lysed using ACK lysis buffer (Thermo Scientific, Waltham, MA, USA) or removed by density-gradient centrifugation through Lymphoprep (St Louis, MO, USA). Remaining contaminating cells were washed and subjected to subsequent analysis and functional assays. Purified NK cells were obtained by negative selection using NK Cell Isolation kits (Miltenyi Biotec). The purity as assessed by flow cytometry was 90–95%.

For in vivo bromodeoxyuridine (BrdU) incorporation assays, mice were injected intraperitoneally with 2 mg of BrdU (in 200 μl). After 12 h, spleens were isolated, stained, fixed, permeabilized, and treated with DNAse. Analysis of BrdU incorporation was performed using a BrdU Flow Kit (BD Pharmingen). Survival was assessed by culturing freshly isolated splenic NK cells for 6 h at a density of 3 × 105 cells per well in 100 μl of RPMI 1640 medium plus FBS (without IL-2) in 96-well plates. Live and dead NK cells were scored with propidium iodide and Annexin V–FITC (BD Pharmingen). Stained samples were analyzed using FACS Coul6 and FACS Diva software Version 6.1.2 (BD Biosciences).

Cell culture. Three NK cell lines (YT85, KHYG1, and NKYS80) were obtained from Dr. Wing C. Chan (City of Hope Medical Center). The NK92 cell line was purchased from the American Type Culture Collection (ATCC), and the NK92 Flp-InT2 cell line was purchased from the cell bank of the Ren Culture Collection (Beijing, China). The SNK6 cell line was kindly provided by Dr. Norio Shimizu and Yu Zhang of Chiba University. Cells were cultured in RPMI 1640 medium (Invitrogen, California, USA) supplemented with 10% heat-inactivated FBS (Sigma-Aldrich, St Louis, MO, USA), 2 mM glutamine, 100 U/ml penicillin, and 100 μg/ml streptomycin (Invitrogen, California, USA) in 5% CO2 atmosphere at 37 °C. In addition, all cell lines except the YT line were interleukin-2 (150 IU/ml, PeproTech, Rocky Hill, NJ, USA)-dependent. The identity of these cell lines was confirmed by short tandem repeat (STR) profiling or high-throughput sequencing, and they both tested negative for mycoplasma contamination using a Lonza MycoAlert Mycoplasma Detection Kit.

Mutagenesis and constructions. Full-length human GNAQ cDNA was purchased from Sino Biological Inc. (Beijing, China). Mutagenesis to create constructs...
encoding the T96S or Y101X mutants was carried out using a Quick-Change Site-Directed Mutagenesis Kit (Agilent Technologies, Santa Clara, CA) according to the manufacturer’s instructions. These constructs encoding wild-type and mutant GNAQ were subcloned into the lentivirus-based expression vector pCDH-CMV-MCS-EF1-copGFP (System Biosciences, #CD511B-1). In addition, 3x-Flag-tagged GNAQ T96S was subcloned into the tetracycline-inducible pLVX-TetOne-Puro vector (Clontech, Takara, #631849). All cDNA sequences were confirmed by Sanger sequencing.

**Lentivirus production and generation of stable cell lines.** For overexpression of wild-type GNAQ and mutants, 12 µg of purified plasmids pCDH-CMV-MCS-EF1-copGFP-vector, pCDH-CMV-MCS-EF1-copGFP-wild-type, pCDH-CMV-MCS-EF1-copGFP-T96S, or pCDH-CMV-MCS-EF1-copGFP-Y101X was cotransfected with 8 µg of pSPAX2 packaging plasmid and 4 µg of pHDLG envelope plasmid into HEK293T cells using Lipofectamine 2000 (Invitrogen) according to the manufacturer’s protocol. The viruses were harvested 72 h posttransfection, and cells were infected with the viruses in the presence of 8 mg/ml polybrene. Infected cells expressing GFP were sorted using a flow fytometer (FACSCompTII, BD Bioscience, San Jose, CA, USA), and the purity of the sorted cell fractions consistent exceeded 95%.

To establish cell lines stably expressing equal amounts of wild-type and T96S simultaneously, KHYG1 and YT cells were infected with pCDH-CMV-MCS-EF1-copGFP-vector or pCDH-CMV-MCS-EF1-copGFP-wild-type lentivirus. The stably transfected clones were selected by GFP. These cells were subsequently infected with pLVX-TetOne-Puro vector or pLVX-TetOne-Puro-3X-Flag-T96S copGFP, and selected with 4 µg/ml puromycin (Sigma Chemical Co., St Louis, MO, USA) to establish tetracycline-inducible cell lines. The tetracycline-inducible cell lines were maintained in RPMI 1640 medium with 0.25 µg/ml puromycin, and the cells were incubated with 20 ng/ml doxycycline (Sigma-Aldrich, Shanghai, China) for up to 48 h to induce T96S mutant expression.

**Cell viability assay.** Cells were seeded in 96-well plates in triplets at a density of 5 × 10³ cells/well in 100 µl of cell culture medium. Cell viability was evaluated using a Cell Counting Kit-8 (CCK-8, Dojindo, Tokyo, Japan) at the indicated days according to the manufacturer’s instructions. In brief, 10 µl of CCK-8 reagent was added to each well. The cells were incubated at 37 °C in an atmosphere containing 5% CO₂ for 2 h. Cell viability was presented by the absorbance value at 450 nm, which was measured with a Multiskan FC microplate reader (Thermo Scientific, Waltham, MA, USA). The value is directly proportional to the number of viable cells in the culture medium. Independent experiments were repeated at least three times.

**Flow cytometric analysis.** Cell proliferation was assessed by BrDU incorporation assays with a BrDU Flow Kit (BD Pharmingen) or an EDU Click Proliferation Kit (BD Phramingen). Cell apoptosis was analyzed with a PE-Anexin V Kit (BD Pharmingen). The following antibodies were purchased from BD Biosciences: CD122-PE (TM-Bta1), CD19-FITC (1D3), CD335/NKp6-Pe (29A1.4), CD3e-FITC (145-2C11), CD3e-PerCP-Cy5.5 (145-2C11), CD4-FITC (RM4-5), CD49b (DX5) Pan-NK Cells-BV421, CD8a-PE (53-6.7), Erythroid Cells-FITC (TER-119), (BD Pharmingen). Cell apoptosis was analyzed with a PE-Annexin V Kit (BD Pharmingen) which was measured with a Multiskan FC microplate reader (Thermo Scientific, Waltham, MA, USA). Lastly, the libraries were applied to an Illumina Hiseq 2000 platform, and 100-bp paired-end reads were generated. Bowtie (v2.2.3) was used to build the reference genome index, and TopHat (v2.0.12) was used to align the clean paired-end reads to the reference genome. The read numbers mapped to each gene were counted by HTSeq (v0.6.1), and the expected number of fragments per kilobase of transcript sequence per million base pairs sequenced (FPKM) was calculated. GSEA (version 2.2.4) and gene set collection of KEGG pathways were used for enrichment analysis. The statistical significance of signature enrichment was assessed using 1000-gene-set permutations. The raw sequencing data are available from the NCBI and are archived under the accession number SRP180943.

**Immunoprecipitation.** The immunoprecipitation assay was performed using the Pierce™ Classic Magnetic IP/Co-IP Kit (Thermo Scientific, Waltham, MA, USA) according to the manufacturer’s protocol. Briefly, cells (1.0 × 10⁶) were lysed in cold Pierce IP Lysis buffer and Halt™ Protease and Phosphatase Inhibitor Cocktail (Thermo Scientific, Waltham, MA, USA) for 15 min on ice. Cell debris was removed by centrifugation at 13,000g for 10 min. Cell lysate (1000 µg) was combined with 10 µg of Flag antibody (Thermo Scientific, Waltham, MA, USA) and incubated overnight at 4 °C with rotation. The antigen sample/antibody mixture was added to the tube containing prewashed magnetic beads (50 µl) and incubated at room temperature for 1 h with mixing. The beads were collected with a magnetic stand and washed twice with IP lysis/wash buffer and once with purified water. The antigen/antibody complex was then eluted.

**Mass spectrometry analysis.** The eluted proteins were digested according to the SISPROT protocol, as described in a previous study70. The desalted peptides were lyophilized under vacuum and kept at −20 °C until mass spectrometry analysis. The desalted sample was dissolved in solvent A and analyzed via reverse-phase high-pressure liquid chromatography electrospay ionization tandem mass spectrometry (RP-HPLC-ESI-MS/MS) using a TripleTOF 5600+ mass spectrometer (SCIEX, Concord, Ontario, Canada) coupled to a NanoSpray III ion source and a nanoLC Eksigent 415 system (SCIEX, Concord, Ontario, Canada). Nano-scale reverse-phase liquid chromatography (RPLC) was performed with a trap-and-elution configuration using a Nano C18 (200 µm × 0.5 mm × 0.5 cm 3.5 µm A) and a nano-scale analytical column (75 µm × 15 cm ChromXP C18-A 3 µm 120 Å). Solvents A and B were 2% and 98% acetonitrile in water supplemented with 0.1% formic acid. The sample was loaded in the trap column at a flow rate of 3 µl/min for 7 min using 100% solvent A. For each sample, a stepwise gradient of 60 min (0–5 min, 95–92% A; 0.5–38 min, 92–20% A; 38–44 min, 75–65% A; 44–60 min, 60–20% A; 60–95 min, 20–95% A; 95–100 min, 95% A) was employed. The raw sequencing data are available from the NCBI and are archived under the accession number SRP180943.
rolling collision energy, and the CES was set to 5. The following parameters were used: dynamic excluded time, 18 s; ignore peaks within 6 Da; exclude after occurrences; 1; mass-to-charge ratio, 50 ppm. The original .wiff files were converted to .mgf files using PeakView software (SCIEX, Concord, Ontario, Canada), and the resulting files were subjected to database searches using Mascot 2.3 (Matrix Science, London, United Kingdom). The following parameters were used for database search: trypsin, KR/P; max cleavage sites, 2; fixed modification, carbamidomethylation (C); variable modification, oxidation (M), deamidated (NQ), acetyl (N-term); mass tolerance, 0.05 Da for the precursor ion and 0.1 Da for the fragment ion. The searches were conducted in a UniProt Swiss-Prot database (downloaded in May 2017, with 20201 reviewed and canonical protein sequence entries) containing whole Homo sapiens proteins and the same number of reversed protein sequences. Three independent IP-Mass experiments were performed. SAINT express (v3.6.3) was the statistical tool utilized to calculate the probability of protein-protein interaction from background, nonspecific interactions with a SAINT probability threshold of ≥0.8 (ref. 34–37).

Western blot analysis. Cells were lysed in cold RIPA lysis buffer and Halt Protease and Phosphatase Inhibitor cocktail (Thermo Scientific, Waltham, MA, USA) for 20 min on ice. Protein extracts (150 μg) were resolved by SDS-PAGE and transferred onto nitrocellulose membranes (Amer sham Biosciences, Piscataway, NJ, USA). The membranes were blocked in TBS-T buffer (20 mM Tris-HCl pH 7.5, 150 mM NaCl, and 0.05% Tween-20) containing 5% (w/v) non-fat milk at room temperature for 1 h. Membranes were incubated at 4 °C overnight with primary antibodies to detect: phospho-ERK1/2 (clone c127E7, 1:1000), phospho-akt (Ser473) (clone D9E, 1:1000), phospho-p44/p42 MAPK (ERK1/2) (Thr202/Tyr204) (clone D13.14.4E, 1:1000), p44/p42 MAPK (ERK1/2) (clone 137F5, 1:2000) from Cell Signaling Technology (Boston, MA, USA); glyceraldehyde-3-phosphate dehydrogenase (GAPDH, clone a1b4; 1:5000) from Abcam (Cambridge, MA, USA); DYKDDDDK Tag Monoclonal Antibody (FG4R, MA1-91878, 1:1000) from Thermo Scientific (Waltham, MA, USA), and GAPDH (clone 60004-1-lg, 1:2000) from ProteinTech (Chicago, IL, USA). Detection was carried out with the SuperSignal West Femto Maximum Sensitivity Substrate Trial kit (Pierce, Rockford, IL, USA). The band images were digitally captured and quantified with a ChemiDoc™ XRS+ system (Bio-Rad Laboratories, Hercules, CA, USA).

Immunohistochemistry. Immunohistochemistry was performed as described previously. The FFPE sections were immunostained using the Dako EnVision™ Flex+ System (K8012; Dako, Glostrup, Denmark). Deparaffinization and epitope unmasking were carried out in a PT Link instrument using an EnVision™ Flex system (K8012) for 20 min. Proteins (10–25 μg) were resolved by SDS-PAGE and transferred onto nitrocellulose membranes (Amer sham Biosciences, Piscataway, NJ, USA). The membranes were blocked in TBS-T buffer (20 mM Tris-HCl pH 7.5, 150 mM NaCl, and 0.05% Tween-20) containing 5% (w/v) non-fat milk at room temperature for 1 h. Membranes were incubated at 4 °C overnight with primary antibodies to detect: phospho-ERK1/2, (clone c127E7, 1:1000), phospho-akt, (Ser473) (clone D9E, 1:1000), phospho-p44/p42 MAPK (ERK1/2) (Thr202/Tyr204) (clone D13.14.4E, 1:1000), p44/p42 MAPK (ERK1/2) (clone 137F5, 1:2000) from Cell Signaling Technology (Boston, MA, USA); glyceraldehyde-3-phosphate dehydrogenase (GAPDH, clone a1b4; 1:5000) from Abcam (Cambridge, MA, USA); DYKDDDDK Tag Monoclonal Antibody (FG4R, MA1-91878, 1:1000) from Thermo Scientific (Waltham, MA, USA), and GAPDH (clone 60004-1-lg, 1:2000) from ProteinTech (Chicago, IL, USA). Detection was carried out with the SuperSignal West Femto Maximum Sensitivity Substrate Trial kit (Pierce, Rockford, IL, USA). The band images were digitally captured and quantified with a ChemiDoc™ XRS+ system (Bio-Rad Laboratories, Hercules, CA, USA).

**Data availability**

The data supporting the main findings of this study are available within the article and its Supplementary Information and Supplementary Data files. Whole-exome sequencing data are publicly accessible and are available in the NCBI Sequence Read Archive under accession code SRP107053 (https://www.ncbi.nlm.nih.gov/sra). The raw RNA sequencing data are available from NCBI and are archived under accession number SRP180943 (https://www.ncbi.nlm.nih.gov/sra). The raw LC/MS data have been deposited to the ProteomeXchange Consortium via the PRIDE partner repository with the dataset identifier PXD013285 (www.ebi.ac.uk/pride). The source data underlying Figs. 3b–f, 4d, 5a–d, and 6h, c and supplementary Fig. 6 are provided as a Source Data file. Any other data are available from the authors upon reasonable request.

Received: 16 November 2017 Accepted: 16 August 2019 Published online: 16 September 2019

**References**

1. Aviles, A., Diaz, N. R., Neri, N., Cleto, S. & Talavera, A. Angiogenic natural T/natural killer cell lymphoma: a single centre study of prognostic factors in 108 patients. Clin. Lab. Haematol. 22, 215–220 (2000).
2. Barrionuevo, C. et al. Extranodal NK/T-cell lymphoma, nasal type: study of clinicopathologic and prognosis factors in a series of 78 cases from Peru. Appl. Immunohistochem. Mol. Morphol. 15, 38–44 (2007).
3. Kwong, Y. L. Natural killer-cell malignancies: diagnosis and treatment. Leukemia 19, 2186–2194 (2005).
4. Gaulco, G. et al. Clinicopathologic and molecular features of 122 Brazilian cases of nodal and extranodal NK/T-cell lymphoma, nasal type, with EBV subtyping analysis. Am. J. Surg. Pathol. 35, 1195–1203 (2011).
5. Li, S. et al. Extranodal NK/T-cell lymphoma, nasal type: a report of 73 cases at MD Anderson Cancer Center. Am. J. Surg. Pathol. 37, 14–23 (2013).
6. Peng, R. J. et al. Genomic and transcriptomic landscapes of Epstein-Barr virus in extranodal natural killer T-cell lymphoma. Leukemia 33, 1451–1462 (2019).
7. Kawa, K. [Clinical characteristics of Epstein-Barr virus-associated natural killer cell lymphoma/leukemia]. Nikho Rinsho Ipn. J. Clin. Med. 55, 424–428 (1997).
8. Nava, V. E. & Jaffe, E. S. The pathology of NK-cell lymphomas and leukemias. Adv. Anat. Pathol. 12, 27–34 (2005).
9. Laurini, J. A. et al. Classification of non-Hodgkin lymphoma in Central and South America: a review of 1028 cases. Blood 120, 4795–4801 (2012).
10. Iaccard, A. et al. Efficacy of L-asparaginase with methotrexate and dexamethasone (AspaMetDex regimen) in patients with refractory or relapsing extranodal NK/T-cell lymphoma, a phase 2 study. Blood 117, 1834–1839 (2011).
11. Tse, E. & Kwong, Y. L. How I treat NK/T-cell lymphomas. Blood 121, 4997–5005 (2013).
12. Lim, S. T. et al. Comparative analysis of extra-nodal NK/T-cell lymphoma and peripheral T-cell lymphoma: significant differences in clinical characteristics and prognosis. Eur. J. Haematol. 80, 55–60 (2008).
13. Chan, J. Y. & Lim, S. T. Novel findings from the Asian Lymphoma Study Group: focus on T and NK-cell lymphomas. Int. J. Hematol. 107, 413–419 (2018).
14. de Mel, S. et al. The genomics and molecular biology of natural killer/T-cell lymphoma: opportunities for translation. Int. J. Mol. Sci. 19, 1931 (2018).
15. Kucuk, C. et al. Activating mutations of STAT3B and STAT3 in lymphomas derived from gammadelta-T or NK cells. Nat. Commun. 6, 6025 (2015).
16. Huang, Y. et al. Gene expression profiling identifies emerging oncogenic pathways operating in extranodal NK/T-cell lymphoma, nasal type. Blood 115, 1226–1237 (2010).
17. Ng, N. B. et al. Dysregulated microRNAs affect pathways and targets of biologic relevance in nasal-type natural killer/T-cell lymphoma. Blood 118, 4919–4929 (2011).
18. Ng, N. B. et al. Activated oncogenic pathways and therapeutic targets in extranodal nasal-type NK/T-cell lymphoma revealed by gene expression profiling. J. Pathol. 223, 496–510 (2011).
19. Ishaj, J. et al. Natural killer-cell lymphoma shares strikingly similar molecular features with a group of non-hepatosplenic gammadelta T-cell lymphoma and is highly sensitive to a novel aurora kinase A inhibitor in vitro. Leukemia 25, 348–358 (2011).
Hospital of Zhengzhou University (to M.Z.). The authors gratefully acknowledge Dr. Tian Tian for critical review and language editing of the manuscript.

**Author contributions**
Z.L., W.L. and M.Z. designed and performed the research, analyzed the data, and wrote the paper; Z.L., X.Z., W.X. and Yanjie Zhang executed the experiments and interpreted the data; C.L., Y.S., M.M., Lisha Lu, Y.W., Z.Z., M.J., Y.B., L.Z., X.W., Ling Li, X.L., Xiaoqiu Fu, Z.S., J.W., F.N., Y.C., Xiaoyan Feng, J.Y. and H.Y. executed the experiments; W.L., G.W., D.Z. and K.H.Y. contributed to the IHC analysis; Xuefei Fu contributed to the sequencing and bioinformatics analysis; Z.L., W.L., M.Z. and Yuan Zhang contributed to the experimental design and analysis.

**Additional information**
Supplementary Information accompanies this paper at https://doi.org/10.1038/s41467-019-12032-9.

Competing interests: The authors declare no competing interests.

Reprints and permission information is available online at http://npg.nature.com/reprintsandpermissions/

Peer review information Nature Communications thanks Philippe Gaulard, Ryan Morin and other, anonymous, reviewer(s) for their contribution to the peer review of this work.

Publisher’s note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.