Supplementary methods

Activation of T-cell signaling and phospho-specific fluorescence flow cytometry

The samples were thawed, and 1 million cells per sample were used for flow cytometry based live/dead discrimination and immunophenotyping. Activation of signaling and cytokine production was performed in the remaining sample. For TCR-induced signaling, the cells (10 x 10^6/mL in RPMI 1640 supplemented with 10% FCS) were allowed to rest at 37°C for 4 h, before redistribution at 200 µL per well into v-bottomed 96 well plates and given another 20 minutes rest. For functional studies over time, cells were cultured for 48 h at 37 °C, at 2.5 x 10^6/mL in CellGro DC (CellGenix) supplemented with 5% human serum (Diaserve Laboratories) and 20 U/mL IL-2 (Chrion) before redistribution into 96-well plates as described above. TCR signaling was activated by anti-CD3 biotin (5 µg/mL) and anti-CD28 biotin (5 µg/mL) for 2 minutes followed by avidin (50 µg/mL) crosslinking for 1, 4 or 10 minutes. Signaling was stopped by adding paraformaldehyde (PFA) at a final concentration of 1.6% for 5 minutes at RT, followed by centrifugation and permeabilization in >90% freezer-cold methanol. After rehydrating the cells by centrifugation twice in PBS, the cells were stained with antibodies, or "barcoded" prior to staining with antibodies as previously described (1). The samples were then collected on a LSR II flow cytometer (BD Biosciences). Data was analyzed using Cytobank Software (https://community.cytobank.org), as previously described (2). Briefly, relative phosphorylation changes are calculated using arcsinh transformation of median fluorescence intensity (MFI) of the cell population of interest. Activation-induced phosphorylation levels were calculated as follows: Fold change = arcsinh (MFI of phospho-protein in activated cells/scale argument) - arcsinh (MFI of phospho-protein in unstimulated cells/scale argument). The scale argument numbers are channel-specific.

Immunophenotyping and viSNE

Three antibody panels were established, all including the same eight lineage markers in addition to three antibodies against inhibitory receptors that differed between the panels. The computational tool viSNE was used for visualization (3). The viSNE analysis included data from the three panels for $CD3^+$ cells from 2 tonsils and 4 FL samples, and was based on the expression of six overlapping lineage markers. In the viSNE maps, position of cells along the unitless t-sne axes represents similarity of the six lineage markers (CD4, CD8, ICOS, CXCR5, CCR7 and CD45RA), and color represents intensity of the indicated marker in each map.

Gene expression analysis

Raw gene expression data from in total 238 FL pre-treatment biopsies were downloaded from the Gene Expression Omnius website (accession nr GSE53820) and the Lymphoma/Leukemia Molecular Profiling Project (LLMPP) website (https://llmpp.nih.gov/FL/). The CEL-files were background corrected and normalized with the rma function from the *affy* R-package (4), and annotated with annotation files respective to the microarray platforms (HG-U133_Plus_2.na36.annot.csv for GSE53820, HG-U133A.na36.annot.csv and HG-U133B.na36.annot.csv for LLMPP). Probes matching the same genes were collapsed calculating the average signal.

References

1. Myklebust JH, Irish JM, Brody J, Czerwinski DK, Houot R, Kohrt HE, *et al.* High PD-1 expression and suppressed cytokine signaling distinguish T cells infiltrating follicular lymphoma tumors from peripheral T cells. Blood 2013;121(8):1367-76.
2. Irish JM, Myklebust JH, Alizadeh AA, Houot R, Sharman JP, Czerwinski DK, *et al.* B-cell signaling networks reveal a negative prognostic human lymphoma cell subset that emerges during tumor progression. Proc Natl Acad Sci U S A 2010;107(29):12747-54.
3. Amir el AD, Davis KL, Tadmor MD, Simonds EF, Levine JH, Bendall SC, *et al.* viSNE enables visualization of high dimensional single-cell data and reveals phenotypic heterogeneity of leukemia. Nat Biotechnol 2013;31(6):545-52.
4. Gautier L, Cope L, Bolstad BM, Irizarry RA. affy--analysis of Affymetrix GeneChip data at the probe level. Bioinformatics 2004;20(3):307-15.