Supplementary methods

Primary FL cell culture. Fresh tissues from lymph nodes were dissociated using the gentleMACS™ Octo Dissociator (Miltenyi, Paris, France). Cell suspensions were frozen in 4% human albumin (VIALEBEX 40 mg/ml, LFB Biomedicaments)/10% DMSO until FL diagnosis. After diagnosis, cells were thawed in complete medium and their phenotypes were analyzed by Fortessa X20 (BD Biosciences Le Pont de Claix, France) after staining by fluorochrome-labelled antibodies (see flow cytometry section).

PDLS generation. 25 000 cells in 100 µL of enriched medium supplemented with cytokines (IMDM medium + 10% HiClone serum, 5.10^{-5}M 2-ME, 50 µg/ml gentamicin, 40 µg/ml apotransferrin, 1 mM sodium pyruvate, 1X nonessential amino acids and 20 mM HEPES, 0.2 µM ODN (Invivogen, Toulouse, France), 15 ng/mL IL-15 (Biolegend, Amsterdam, The Netherlands), 10 ng/mL IL-2 (Biolegend), 50 ng/mL IL-4 (Biolegend), 50 ng/mL CD40L (Biolegend) were seeded in 96-well round bottom ULA plates (Corning, Samois sur Seine, France), centrifuged 10 minutes at 1 000 rpm and cultured at 37 °C in a humidified 5% CO_2 atmosphere. At day 3 of culture, 100 µL of fresh enriched medium containing or not treatments were added and PDLS were cultured at 37 °C in a humidified 5% CO_2 atmosphere until the different time points.

Flow cytometry analyses. Composition of FL samples at day 0 (after thawing) and at day 3 or day 6 of 3D culture was determined by flow cytometry analyses based on the staining with combinations of fluorochrome-labelled antibodies (see supplementary table 3). Immune cell composition of PDLS was determined as follows: for T cell populations ($CD3^+CD4^+$ cells for $CD4^+$ T cells, $CD3^+CD8^+$ for $CD8^+$ T cells, $CD56^+CD3^-$ for NK cells, $gamma9^+CD3^+$ for gamma delta T cells, $CD3^+CD4^+CXCR5^+ICOS^+$ for TFh/TFr, $CD3^+CD4^+CXCR5^-ICOS^-$ for non-TFh), for B cell populations ($CD10^+CD19^+$ for B tumoral cells, $CD10^-CD19^+$ for healthy B cells), immune checkpoints on T cells (CD39, CD73, PD-1, BTLA, LAG-3, TIGIT, TIM-3) and immune checkpoints on B cells (CD39, CD73, PDL1, PDL2, PD-1). B cell depletion was determined by flow cytometry and normalized by untreated condition percentage. 5 µl of 7-aminoactinomycin D (7AAD, BD Biosciences, Le Pont de Claix, France) were then added according to the manufacturer's instructions and dissociated cells were analyzed on a Fortessa X20 flow cytometer (BD Biosciences). Dead cells ($7AAD^+$) were excluded from the analyses with Cytobank. Cytokine release was determined by measuring TNFα, IFNγ, Granzyme B, IL-6, IL-8, IL-10 concentrations in the supernatant of PDLS at day 6 of 3D culture using a BD cytometric bead array (CBA) human soluble protein master kit following provider's instructions (BD Biosciences).

PDLS immunohistochemistry. PDLS were fixed at day 6 of culture directly in the wells with 4% PFA (Alfa Aesar, Haverhill, MA, USA) overnight at 4 °C. PDLS were then rinsed with PBS and included in 1% low-melting agarose (Life Technologies, Villebon sur Yvette, France), quickly labelled with China ink before being included in paraffin. Automated classical immunohistochemistry (IHC) was performed using the Benchmark ULTRA (Roche, Ventana Medical Systems, Innovation Park Drive Tucson, Arizona, USA) on FFPE tissue sections (3 µm). After dewaxing, tissue slides were heat pre-treated using a CC1 (pH8) buffer (Roche) at 98°C. The slides were blocked for endogenous peroxidase activity and incubated with primary antibodies (see suppl. Table 2). The target was then visualized using the OptiView DAB detection kit (Roche). The tissue slides were counterstained using hematoxylin (Roche) for 8 minutes followed by post-coloration using Bluing reagent for 4 minutes at room temperature (Roche). The slides were then dehydrated (ethanol and xylene) and mounted using xylene-based mounting.

PDLS characterization by 3D imaging. PDLS were fixed directly in the wells with 4% PFA overnight at 4 °C and rinsed with PBS. Nuclei were labelled with 10 µg/mL propidium iodide (PI) (Life technologies) for 4 hours at room temperature under agitation. PDLS were then rinsed with PBS and included in 1% low-melting agarose (Life Technologies). 8 mm disks were punched and cleared with the methanol-benzyl alcohol/benzyl benzoate (BABB) technique.(28) Acquisitions were performed with an 880 confocal microscope (Zeiss, Oberkochen, Germany) at 10X magnification. IMARIS 7 software (BitPlane, South Windsor, CT, USA) was used for PDLS 3D representations. Image analyses (volume, sphericity, eccentricity and roundness quantification) were performed using the processing pipeline described in (27).

3'RNA sequencing. After thawing, cells from FL biopsies were cultured in suspension overnight in RPMI medium supplemented with FBS and the next day RNA was extracted (Direct-zol RNA Minipep kits – Zymo Research). In parallel, cells from the same sample were cultured in 3D in an enriched medium supplemented with cytokines and after 3 days, PDLS were mechanically dissociated and RNA extraction was performed. Libraries were prepared with 500 ng of RNA using the QuantSeq 3'mRNA-Seq Library Prep Kit-FWD (Lexogen, Vienna, Austria) and UMI Second Strand Synthesis Module (Lexogen) following the manufacturers' instructions. 13 cycles of library amplification were performed. The libraries were quantified using the Qubit™ dsDNA HS Assay Kit (Invitrogen, Life Technologies) and equimolar pooling was performed at 8nM. The pooled libraries were sequenced on single read 75 pb run, on an Illumina NextSeq550DX instrument (Illumina). Expected read depth was 15 millions of uniquely mapped reads per sample. The data analyses were performed by the pipeline on the BlueBee® Genomics Platform.

Correlogram & correlation curves. Correlogram was obtained with Open source Rstudio (RStudio Team (2020), PBC, Boston, MA URL http://www.rstudio.com/) and corrplot package. For each variable, the values of the 7 PDLS were used to calculate side-by-side correlation coefficients and correlation matrix (non-parametric Spearman's correlation). Associated Correlation curves were generated based on the same data set with GraphPad Prism 9.