Soft-tissue sarcomas represent a heterogeneous group of cancer, with more than 50 histological subtypes\(^1,2\). The clinical presentation of patients with different subtypes is often atypical, and responses to therapies such as immune checkpoint blockade vary widely\(^3,4\). To explain this clinical variability, here we study gene expression profiles in 608 tumours across subtypes of soft-tissue sarcoma. We establish an immune-based classification on the basis of the composition of the tumour microenvironment and identify five distinct phenotypes: immune-low (A and B), immune-high (D and E), and highly vascularized (C) groups. In situ analysis of an independent validation cohort shows that class E was characterized by the presence of tertiary lymphoid structures that contain T cells and follicular dendritic cells and are particularly rich in B cells. B cells are the strongest prognostic factor even in the context of high or low CD8\(^+\) T cells and cytotoxic contents. The class-E group demonstrated improved survival and a high response rate to PDI blockade with pembrolizumab in a phase 2 clinical trial. Together, this work confirms the immune subtypes in patients with soft-tissue sarcoma, and unravels the potential of B-cell-rich tertiary lymphoid structures to guide clinical decision-making and treatments, which could have broader applications in other diseases.
**Fig. 1** The SICs exhibit strongly different TMEs. This figure refers to the TCGA SARC cohort (n = 213). a, Composition of the TCGA SARC cohort by SIC, and histology. b, Composition of the TME by SIC as defined by the MCP-counter Z-scores. NK cells, natural killer cells. c, Expression of gene signatures related to the functional orientation of the immune TME by SIC. d, Expression of genes related to immune checkpoints by SIC. Adjusted P-values are obtained from Benjamini–Hochberg correction of two-sided Kruskal–Wallis tests P-values.

**Immune classification of STS**

The TME compositions from four independent discovery primary STS datasets (TCGA SARC, Gene Expression Omnibus (GEO) accessions GSE21050, GSE21122 and GSE30929) (Extended Data Table 1) with publicly available gene expression profiles were analysed by MCP-counter, a gene-expression-based TME deconvolution tool. An immune-based classification of STS was developed from this analysis (Extended Data Fig. 1). Methods) and tumours were assigned to one of five sarcoma immune classes (SICs), labelled A, B, C, D and E, with highly distinct profiles (Fig. 1).

We compared the SIC distribution across histological subtypes and found that most LMS tumours were classified to SICs A and B (Fig. 1a). DDLPS accounted for half of SIC C tumours. Tumours classified as SICs D and E were more evenly distributed across histological subtypes. Application of the predictor of the immune classes (Methods) to other STS histologies from French Sarcoma Group (FSG) cohort (Extended Data Table 1) revealed that all SICs could be identified in each histology (Extended Data Fig. 2a).

The TME composition differs significantly between SICs (Fig. 1b). Three SICs showed homogeneous profiles. SIC A, ‘immune desert’, was characterized by the lowest expression of gene signatures related to immune cells, as well as low vasculature. SIC C, ‘vascularized’, was dominated by a high expression of endothelial-cell-related genes. SIC E, ‘immune and TLS high’, was characterized by the highest expression of genes specific to immune populations such as T cells, CDS+ T cells, natural killer cells and cytotoxic lymphocytes. Notably, a key determinant of SIC E was the high expression of the B lineage signature (P = 1.8 × 10−25). SICs B and D were characterized by heterogeneous but generally immune-low and immune-high profiles, respectively.

The expression of genes associated with T cell or myeloid cell chemo-taxis, T cell activation and survival, major histocompatibility complex class I, and regulatory gene signatures was high in SICs D and E, intermediate in SICs B and C, and very low in SIC A (Fig. 1c). Expression of the lymphoid-structures-associated B-cell-specific chemokine CXCL13 was notably high in E tumours, moderate in D tumours, generally low in B and C tumours, and negligible in A tumours.

The expression of immune-checkpoint-related genes (Fig. 1d) followed that of immune infiltrates, with high expression of the genes encoding PD1, PDL2, CTLA4 and TIM3 (PDCD1, PDCD1LG2, CTLA4 and HAVCR2, respectively) in SIC E followed by SIC D tumours, and low-to-moderate expression in SIC C, B and A tumours. CD274 (which encodes PDL1) was heterogeneously expressed across SICs, whereas LAG3 was expressed at high levels only in SIC E tumours, and its expression was low in all other classes. The above findings were consistent across the four discovery cohorts (Extended Data Fig. 3).
**SICs are associated with patient survival**

After confirmation that the two cohorts with available survival data (TCGA SARC, \(n = 213\); GSE21050, \(n = 283\)) exhibited similar survival patterns (data not shown), the cohorts were pooled to study the clinical outcome of the five SICs (Fig. 2a). Patients with SICA exhibited the shortest overall survival compared with group D or E patients (\(P = 0.048\) and \(P = 0.025\), respectively). Similarly, among the other STS histologies from the FSG cohort, patients with SICA had a shorter overall survival than patients with SICE (Extended Data Fig. 2b). In a multivariate model with classical prognostic factors (Fig. 2b), SICs were found to be significantly associated with prognosis, independent of other clinical parameters (as compared with SICA, \(P = 0.011\) and \(P = 0.029\), for SICs D and E, respectively). Tumours were separated between high and low expression of CD8+ T cells, cytotoxic lymphocytes and B lineage signatures based on the observation of the MCP-counter scores distribution (Extended Data Fig. 4). Detailed analysis of the effect of these immune cell population signatures revealed that whereas neither CD8+ T cells (\(P = 0.277\)) (Fig. 2c) nor cytotoxic lymphocytes (\(P = 0.0513\)) (Extended Data...
The overall tumour mutational burden was low across the studied cohort of 93 STS cases (NTUH cohort) (Extended Data Table 1). Seventy-three samples passed quality control for transcriptomic analysis using Nanostring nCounter technology. We classified this cohort into the same five SICs (Methods) with the following distribution: A, 16 (21.9%); B, 19 (26.0%); C, 10 (13.7%); D, 17 (23.3%); and E, 11 (15.1%). The NTUH cohort samples exhibited gene-expression-based TME profiles that were similar to that of TCGA SARC and GSE21050 datasets (Extended Data Fig. 7a).

By quantitative immunohistochemistry, immune-desert SIC A was characterized by very low densities of CD3+ T cells, CD8+ or CD20+ B cells, whereas immune-and-TLS-high SIC E exhibited high densities of these cells (pairwise comparison, \( P = 4.01 \times 10^{-6} \), \( P = 6.64 \times 10^{-6} \) and \( P = 9.90 \times 10^{-6} \), respectively). The vascularized SIC C exhibited a moderate infiltration by immune cells and a high density of CD34+ endothelial cells (Extended Data Fig. 7b, c).

**TLSs are a feature of SIC E tumours**

The CXCL13 chemokine, which is associated with the presence of TLSs, was strongly expressed in SIC E tumours (Fig. 1c, Extended Data Fig. 2c). Expression of CXCL13 was highly correlated with that of the TSL-associated I2-chemokine signature (Extended Data Fig. 8a), which suggests that TLSs could be a marker of SIC E. TLSs were defined as a CD20+ B-cell follicle juxtaposed to a CD3+ T-cell aggregate containing at least one DC-LAMP+ (also known as LAMP3) mature dendritic cell18,19 (Fig. 3a, left). A strong association between SICs and the presence of TLSs was identified (\( P = 3.13 \times 10^{-10} \)) (Fig. 3c). No TLSs were observed in tumours from SICs A, C and D, and only one tumour from SIC B had one TLS. By contrast, nine out of eleven (82%) SIC E tumours exhibited one or more TLS. All TLSs were intratumoural (Extended Data Fig. 8b), and found at the periphery and in the centre of the tumour in all histologies (Extended Data Fig. 8c, d).

We observed the presence of CD3+PD1+ T cells (Fig. 3a, right) in the germinal centre of TLSs with characteristics of follicular T helper cells (positive for CD4, PD1 and the CXCL13 receptor CXCR5) (Fig. 3b, left), CD23+CD21+ cells with reticular morphology characteristic of follicular dendritic cells, and peripheral node addressin (PNAd)-positive structures with high endothelial venules morphology (Fig. 3b, right). Germinal centres are a hallmark of secondary follicle-like TLSs (SFL-TLS), the final maturation step of TLS; the earlier steps being early TLSs (E-TLS) and primary follicle-like TLSs (PFL-TLS)19,20. E-TLS, PFL-TLS and SFL-TLS represented 60.5%, 21.1% and 18.3%, respectively, of all TLSs analysed (Extended Data Fig. 8e, f). This differed between histologies (\( P = 7.76 \times 10^{-4} \)), with UPS having only 16.7% of E-TLS.

Tumours with TLSs (11.8%, 11 out of 93) had significantly higher densities of tumour-infiltrating CD3+ T cells (\( P = 4.0 \times 10^{-6} \)), CD8+ T cells (\( P = 1.8 \times 10^{-6} \)) and CD20+ B cells (\( P = 1.5 \times 10^{-6} \)) (Fig. 3d). This association persisted even if T and B cells within TLSs were excluded from the analysis (\( P = 1.5 \times 10^{-3} \), \( P = 3.8 \times 10^{-6} \) and \( P = 7.9 \times 10^{-6} \), respectively) (Fig. 3d), which suggests that high immune cell infiltration is not limited to TLSs.
**SICs predict patient response to PD1 blockade**

We examined whether SICs can predict the patient response to checkpoint blockade therapy. We obtained 47 pre-treatment STS metastasis biopsies from patients enrolled in the SARC028 clinical trial and its expansion cohort\(^4\) (Extended Data Table I), which evaluated the efficacy of the anti-PD1 monoclonal antibody pembrolizumab in patients with metastatic STS. Of these 47 patients, I achieved a complete response, 9 a partial response, 17 stable disease and 20 had progressive disease (Fig. 4a). Pre-treatment tumours were classified into SICs based on gene-expression data. The objective response rate (ORR) (which accounts for complete and partial responses) as evaluated by response evaluation criteria in solid tumours (RECIST) criteria was 21.2% in the overall cohort. SICs, however, showed substantial variation in ORR, with SIC E patients exhibiting the highest ORR (50%, 5 out of 10), followed by SIC D (25%, 3 out of 12) and SIC C (22%, 2 out of 9) (Fig. 4a). A complete response was found only in SIC E, as well as one patient who had a 100% change in target lesions but a non-complete response. This SIC E patient was found to respond better to PD1 blockade therapy, although this should be validated on a larger cohort.

**Discussion**

This study is, to our knowledge, the most comprehensive analysis of the STS immune TME and the first to evaluate the prognostic effect of immune infiltrates by simultaneously integrating several immune cell populations and malignant cell characteristics. Previous studies have examined the immune profile of STS tumours, but the importance of B cells and TLSs was not investigated. The clinical effect of CD8\(^+\) T cells and PD1 expression has yielded controversial results\(^11\)-\(^13\). Here, we found the CD8\(^+\) T cell signature and PD1 were expressed in class D and E SICs, which are associated with favourable outcomes, providing high infiltration of B cells. The integrative analysis demonstrates that infiltration by B cells is a key discriminative feature of a group of patients with improved survival. This B-cell-high group was found to respond better to PD1 blockade therapy, although this should be validated on a larger cohort.

The field of immuno-oncology is rapidly expanding, and is crucial to accurately identify patients who are likely to respond. Here, we propose a classification for STS that is immune-centric with prognostic effect. It defines a group of patients with a better response to anti-PD1 therapy marked by B cells and TLSs. This finding may have broad applications. Sarcomas are considered immune-quiescent tumours, with a low mutational burden. Nevertheless, our data show that some STSs are immunogenic and that this is driven by B cells. Further work is needed to extend these findings to all STS histologies and other cancers. Similarly, the underlying mechanisms require further investigation, but a possible explanation is that TLSs are sites at which anti-tumoral immunity is generated, with B cells instructing T cells—in particular CD8\(^+\) T cells—to recognize tumour-associated antigens\(^14\). It is noteworthy that TLS-rich tumours are more infiltrated by CD8\(^+\) T cells. These T cells can become exhausted, explaining the correlation of the expression of immune checkpoints (such as PD1 and LAG3) with TLSs, and why treatment with checkpoint inhibitors may allow productive anti-tumour immunity in TLS-rich tumours. Overall, our findings lay the foundation for a tool to risk-stratify patients with STS and identify those who may be more likely to benefit from immunotherapies, and may be broadly applicable to other malignancies\(^16\)-\(^18\).

**Online content**

Any methods, additional references, Nature Research reporting summaries, source data, extended data, supplementary information, acknowledgements, peer review information; details of author contributions and competing interests; and statements of data and code availability are available at https://doi.org/10.1038/s41586-019-1906-8.

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Methods

Ethics and patients
Patients diagnosed with DDLPS, LMS and UPS were identified and the pathology diagnosis was confirmed by a certified pathologist in National Taiwan University Hospital. The research was approved by the Research Ethics Committee of NTUH (201605061RNA) for this retrospective study. Formalin-fixed paraffin-embedded (FFPE) blocks were retrieved and 4–5-µm-thick slides were taken for immunohistochemistry staining and RNA extraction for Nanostring testing. Other cohorts were previously published.8,31–33

Establishing the immune classification of STS
To establish a robust immune classification of STS, publicly available transcriptomic data from TCGA data portal and the GEO repository representing four large and independent patient cohorts were included. Only tumours from the most common histologies of genomically complex STS were included: LMS, UPS and DDLPS. We analysed data from the TCGA SARC8 (n = 213), GSE210508 (n = 283), GSE211228 (n = 72) and GSE309298 (n = 40) cohorts.

Public transcriptomic data pre-processing
Transcriptomic data were downloaded from the TCGA data portal (SARC cohort) and GEO (accessions GSE21050, GSE21122 and GSE30929). TCGA SARC was restricted to complex genomics sarcomas (UPS, DDLPS and LMS). Normalized TCGA SARC RNA-sequencing data were log2-transformed. Microarray data were normalized using frozen-RMA and LMS. Normalized TCGA SARC RNA-sequencing data were log2-transformed of samples in each cohort was performed based on the metagene scores of each class were computed and transformed. Microarray data were normalized using frozen-RNA method34 from the R package frma. Batch effect was corrected across series using ComBat35, with histology as covariate.

Estimation of the TME composition
The TME composition of each tumour was assessed with the MCP-counter tool, which provides abundance scores for eight immune (T cells, CD8+ T cells, cytotoxic lymphocytes, natural killer cells, B cell lineage, monocytic lineage, myeloid dendritic cells and neutrophils), and two stromal populations (endothelial cells and fibroblasts). The scores are based on analysis of transcriptomic markers—that is, transcriptomic features that are strongly, specifically and stably expressed in a unique cell population. These scores are proportional to the abundance of each cell population in the tumour, therefore allowing intersample comparison and large cohort analyses.36 The MCP-counter signature compositions are as follows: T cells: CD28, CD3D, CD3G, CD5, CD6, CHRM3-AS2, CTLA4, FIT3LG1, ICOS, MAL, PBX4, SIRPQ, THEMIS, TNFRSF25 and TRAT1; CD8+ T cells: CD8B, cytotoxic lymphocytes: CD56, EOMES, FGFBP2, GNYL, KLR3C, KLR3C4 and KLRD1; B lineage: BANK1, CD19, CD22, CD79A, CR2, FCR2, IGKC, MS4A1 and PAX5; natural killer cells: CD160, KIR2DL1, KIR2DL3, KIR2DL4, KIR3DL1, KIR3DS1, NCR1, PTGDR and SH2D1B; monocytic lineage: ADAP2, CSFIR, FPR3, KYNU, PLA2G7, RASSF4 and TFEC; myeloid dendritic cells: CD1A, CD1B, CD1E, CLEC10A, CLEC2 and WFDC2IP; neutrophils: CA4, CEACAM3, CXCR1, CXCR2, CYP4F3, FGR3B, HAI1, KCNJ5, MEGF9, SLC25A37, STEAP4, TECPR2, TLE3, TNFSFR10C and VNN3; endothelial cells: ACVR1L, APLN, BCL6B, BMP6, BMX, CDH5, CLEC14A, Cxorf36 (also known as DIPK28), EDN1, ELTD1, EMCN, ESAM, ESM1, FAM124B, HECV2, HHIP, KDR, MMRN1, MMRN2, MYCT1, PALMD, PEAR1, PGF, PLXNA2, PTPRB, ROBO4, SPD, SHANK3, SHE, TEC, TIE1, VEPHI and VWF.

Intracohort immune classifications
The fibroblasts immune signature was removed from this analysis as all STS tumours exhibited high and homogeneous scores for this cell population, which is consistent with the mesenchymal origin of STS. The signature for CD8 T cells was removed from the analysis for GSE21050, GSE21122 and GSE30929 as it showed very small variation across all samples in these microarray-based cohorts. Unsupervised clustering of samples in each cohort was performed based on the metagene Z score for the included populations of MCP-counter (Extended Data Fig. 9a–d) using R software, with the Euclidian distance and Ward’s linkage criterion, using the gplots package. The TCGA SARC, GSE21050, GSE21122 and GSE30929 cohorts were separated into 6, 9, 7 and 6 groups, respectively. The number of clusters was chosen empirically following the dendrograms shown in Extended Data Fig. 9a–d. Analysis of the intersample variance revealed that much of the explainable variance was already attained at the chosen number of clusters as visualized in Extended Data Fig. 9e–h.

Pan-cohort immune classes
To aggregate the above four intracohort classifications, the transcriptome matrix of each cohort was independently zero-centred for each gene across all samples. Then, we computed the centroids of each class over the whole transcriptome and analysed the Pearson correlations between all the centroids on the set of genes shared across the four cohorts (Extended Data Fig. 9i). From these correlations, we deduced five SICs. The tumours from six remaining cohort-specific clusters shared intermediate/weak correlation patterns to other clusters and were temporarily labelled as ‘unclassified’. Prediction of the immune classes
Centroids of SICs were computed on MCP-counter intraseries Z-scores for T cells, cytotoxic lymphocytes, B cell lineage, natural killer cells, monocytic lineage, myeloid dendritic cells, neutrophils and endothelial cells, on all cohorts. To predict de novo the immune classes of each of the cohorts, MCP-counter Z-scores were computed, and each sample was assigned to the closest immune class based on its Euclidian distance to the related centroids. The SICs labels used are the ones predicted using this method. Principal component analysis of the 608 samples on the MCP-counter scores shows that the intra-SIC homogeneity was improved by this prediction step (Extended Data Fig. 9j, k), as confirmed by supervised tests across SICs (Extended Data Fig. 9l, m).

Gene signatures for the functional orientation
The signatures used to determine the functional orientation of the TME were derived from the literature8,36. The signatures were the following: immunosuppression (CXCL12, TGFB1, TGFB3 and LGALS1), T cell activation (CXCL9, CXCL10, CXCL16, IFNG and IL15), T cell survival (CD70 and CD27), regulatory T cells (FOXP3 and TNFRSF18), major histocompatibility complex class I (HLA-A, HLA-B, HLA-C, HLA-E, HLA-F, HLA-G and B2M), myeloid cell chemotaxis (CCL2), and tertiary lymphoid structures (CXCL13). For each signature, scores were computed as the geometric mean signature expression.

De novo prediction of the immune classes of additional cohorts and other platforms
The predictor described above was adapted to analyse new and independent samples, from Nanostring-analysed FFPE samples. In a first step, SICs were estimated on the NTUH cohort by sorting samples on the B lineage signature, T cells signature then endothelial cell signature and assigning each sample according to the SIC it resembled the most. Similar to as described above, centroids of each SIC on Nanostring data MCP-counter scores Z-scores were computed and samples were reassigned to the SIC they were closest to the centroid of. For new samples from the SARC028 cohort, MCP-counter scores for T cells, cytotoxic lymphocytes, B lineage and endothelial cells were computed and transformed as Z-scores. Distances with Nanostring-defined centroids presented above were computed with Euclidian metric, and samples were assigned to the SIC with the lowest distance.

RNA extraction from FFPE tumours
Human FFPE tumour specimens were cut into 3-µm-thick sections and were reviewed under microscope for tumour histology. Non-tumour tissues were excluded and tumour tissues were deparaffinized by
deparaffinization solution (Qiagen 19093) and RNA were extracted by RNasey FFPE kit (Qiagen 73504) according to the manufacturer’s protocol. RNA quality and size distribution were determined by the Agilent 2100 Bioanalyzer with RNA analysis kits (RNA 6000 nano kit 5067-1511, RNA 6000 nano reagent 5067-1512. RNA 6000 nano ladder 5067-1529, RNA 6000 pico kit 5067-1513. RNA 6000 pico reagents 5067-1514, RNA 6000 pico ladder 5067-1535) for cohorts NTUH core and NTUH whole, and by the Agilent RNA ScreenTape assay (catalogue: RNA ScreenTape 5067-5576, RNA ScreenTape sample buffer 5067-5577, RNA ScreenTape ladder 5067-5578) and Agilent 2200 TapeStation for cohort SARC028. The samples from SAR028 were separately quality-controlled by the sarcoma pathology group at MD Anderson Cancer Center.

Nanostring nCounter analysis
The RNA was analysed using the nCounter Technology (Nanostring Technologies) as per the manufacturer’s protocol. Data were normalized using the nSolver software (Nanostring Technologies).

Enzymatic and fluorescent multiplexed immunohistochemistry
The FFPE human tumour and control specimens were cut into 3-µm-thick sections. Human FFPE tonsil sections were used as positive controls for CD3, CD4, CD8, CD20, CD21, CD23, CD34, CCR5, DC-LAMP, PD1, PD1 and PNAδ, placenta sections were used in addition for PDL1 and cerebral cortex tissue was used as a negative control. The specificity of all antibodies was tested by the manufacturers and the specificity of anti-PD1 antibodies was validated in our laboratory on overexpressing cells pellets as previously reported44. Antigen retrieval was carried out on a PT-link (Dako) using the EnVision FLEX Target Retrieval Solutions at High pH (Dako, K8004) or Low pH (Dako, K8005S). Endogenous peroxidase activity and non-specific Fc receptor binding were blocked with H2O2 3% (Gifrer, 10603051) and Protein Block (Dako, X0909) respectively. The primary and secondary antibodies used for immunohistochemistry and immunofluorescence are summarized in Extended Data Table 2. Immunohistochemistry and immunofluorescence images were independently analysed blindly by three observers (L.L., C.S.-F. and G.L.).

Enzymatic immunohistochemistry
The stainings were performed with an Autostainer Link 48 (Dako). Chromogenic detection was performed using 3,3'-diaminobenzidine (Dako, K3468) for CD8, CD20, CD21, PDL1 and PNAδ; 3-amino-9-ethylcarbazole (Enzo, AD1-9300-0030) and Protein Block (Dako, X0909) respectively. The primary and secondary antibodies used for immunohistochemistry and immunofluorescence are summarized in Extended Data Table 2. Immunohistochemistry and immunofluorescence images were independently analysed blindly by three observers (L.L., C.S.-F. and G.L.).

Fluorescent multiplexed immunohistochemistry
For the PD1, CD20 and CD3 3-plex staining, a tyramide system amplification (TSA) was used. The stainings were performed with a Leica Bond RX. The incubation with TSA reagent was performed after the incubation of the horseradish peroxidase (HRP)-conjugated polymer and was followed by antibody stripping at 97 °C for 10 min. This protocol was repeated for the second and third primary antibodies and corresponding polymer incubations. The dilutions used for the TSA are 1:400 for TSA AF488, 1:800 for TSA AF594 and 1:200 for TSA AF647, as per the manufacturer’s recommendations. For the CXCRA, CD4 and PD1 3-plex staining, we used a conventional fluorescent-dye conjugated secondary antibody system performed manually (all secondary antibodies were diluted at 1:100). For all the fluorescent stainings, the nuclei were stained with DAPI Solution (Thermo Fisher, 62248) at 2 µg ml⁻¹ for 10 min. After mounting with ProLong TM Gold Antifade Mountant (ThermoFisher, P36934), the slides were scanned with a Zeiss Axio Scan.Z1.

Statistical analysis
All statistical analyses were performed using the R software (v.3.4.4) and the packages survival, gplots, dunn.test and FactoMineR. The relationship between two categorical variables was estimated with the χ²-squared test. The relationship between a categorical variable and a quantitative variable was estimated with the Mann–Whitney U test (two categories) or the Kruskal–Wallis test (three or more categories). All tests were two-sided. In cases with three or more categories, pairwise comparisons were carried out with Dunn tests. The relationship between two quantitative variables was estimated with the Pearson correlation. When appropriate, P values were corrected for multiple hypothesis testing with the Bonferroni or Benjamini–Hochberg methods, as specified in the text or figure legends. Survival was analysed with Kaplan–Meier estimates and log-rank tests. No statistical methods were used to predetermine sample size. The experiments were not randomized, and investigators were not blinded to allocation during experiments and outcome assessment unless stated otherwise.

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

Data availability
The transcriptomic datasets analysed in this study can be accessed on the GDC Portal (portal.gdc.cancer.gov, cohort TCGA SARC) and the GEO repository under accession numbers GSE21122 and GSE30929. FSG cohort data are publicly available from ArrayExpress for gastrointestinal tumour with accession code E-MTAB-373, and from the GEO for synovial sarcomas with accession number GSE40021. Myxoid liposarcomas from the FSG cohort are available from the corresponding authors upon reasonable request. Immunohistochemistry and gene expression data related to the NTUH cohort (Fig. 3, Extended Data Figs. 7, 8) are available upon reasonable request to W.H.F. (herve.fridman@crc.jussieu.fr). The data that support the findings related to Fig. 4 are available from SARC but restrictions apply to the availability of these data, which were used under license for the study. Data are, however, available from H.A.T. (htawbi@mdanderson.org) upon reasonable request and with permission of SARC.

Code availability
All code used in this study is available from the corresponding author upon reasonable request.
Acknowledgements This work was supported by the Institut National de la Santé et de la Recherche Médicale, the Université de Paris, Sorbonne University, the Programme Cartes d’Identité des Tumeurs (CIT) from the Ligue Nationale Contre le Cancer, grants from Institut National du Cancer (HTE-INSERM plan cancer, C16082DS), Association pour la Recherche sur le Cancer (ARC), Cancer Research for Personalized Medicine programme (CARPEM TIB), French Sarcoma Group, the European Connective Tissue Cancer Network (CONTICANET, FP6-018806), ‘FONCER contre le cancer’ programme, Labex Immuno-Oncology (LAX62_9UMR972 FRIDMAN), the National Institutes of Health (E.Z.K. is supported by T32CA0095969) and the Moon Shot programme at MD Anderson Cancer Center. Grants from the Ministry of Education (NTU-107L9014) and Ministry of Science and Technology (MOST 107-3017-F-002 -002), Taiwan and from the National Taiwan University (YongLin Chair Grant S-01 and S-03) also supported this study. SARC028 was jointly funded by Merck, Inc.; SARC, Sarcoma Foundation of America, and the QuadW Foundation. F.P. supported by CARPEM doctorate fellowship. C.L.R is recipient of the Paul Calabresi Clinical Oncology Award (K12 CA088084). The slides stained for immunofluorescence were scanned and analysed at the Centre d’Histologie, d’Imagerie et de Cytométrie (CHIC), Centre de Recherche des Cordeliers UMR5138 (Paris, France). CHIC is a member of the Sorbonne University Flow Cytometry Network (RECYF). We thank C. Klein, K. Garbin and E. Devreve for their support with the imaging. The Nanostring analysis of the NTUH core cohort was performed by the Plateforme Génomique of the Institut Curie (Paris, France). We thank D. Gentien and E. Henry for their support. We acknowledge the help of H. Yan and B. Singh.

Author contributions F.P., W.H.F., C.S.-F., A.d.R., TW-W.C., H.A.T. and A.I. designed the study and experiments. F.P., A.d.R., C.L. and Y.L. performed the bioinformatics analysis. L.L., G.L., I.N., L.-P.H., A.B., M.M. and F.P. carried out the immunohistochemistry experiments. J.C., Y.-M.J. and J.A. performed anatomic-pathology revision on the samples. E.Z.K., C.-M.S., W.-L.W. and K.M.W. performed the RNA extraction and Nanostring experiments. TW-W.C., A.I., M.T. and H.A.T. provided clinical guidance. TW-W.C., M.T., A.I., E.Z.K., A.I.L., C.L.R., M.A.B., V.B., D.R. and H.A.T. cared for the patients and provided patient materials or clinical data. F.P., W.H.F., C.S.-F., H.A.T., A.d.R., E.Z.K., C.L.R., A.I.L., TW-W.C., C.-M.S., J.A.W. and A.I. discussed the data and wrote the text. W.H.F., C.S.-F., A.d.R. and H.A.T. supervised the study and all authors commented on the manuscript and approved the submission.

Competing interests W.H.F. is a consultant for AstraZeneca, Novartis, Servier and Pierre Fabre. A.I. serves in the advisory board of Bayer, Daiichy, Epizyme, Lilly, Novartis, Roche and Springworks, and received research funding from Astra Zeneca, Bayer, Chugai, Merck, MSD, Novartis and Pharmamar. J.A. is a consultant for AstraZeneca, Bayer, CMS, MSD and Roche and received research funding from MSD, Pfizer and Pierre Fabre. J.A.W. participated on advisory boards for Merck, BMS, Novartis, Astra Zeneca, Roche Genentech and Illumina. M.A.B. is a consultant for EMD Serono, Immune Design, Eisai. H.A.T. serves on advisory boards and receives consulting fees from BMS, Merck and Genentech, and received research funding from BMS, Merck, Celgene, GSK, and Genentech. T.W.-W.C. participated in advisory boards for Eisai and Lilly and received research funds from Eisai. C.L.R. received research funding from BMS. The other authors declare no conflict of interest.

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Correspondence and requests for materials should be addressed to H.A.T or W.H.F.

Peer review information Nature thanks Naiyer Rizvi and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

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Extended Data Fig. 1 | Diagram of analytic workflow. The drawing of the syringe in the bottom left corner originates from Servier Medical Art (https://smart.servier.com), and is distributed under a CC-BY 3.0 Attribution license (https://creativecommons.org/licenses/by/3.0/).
Extended Data Fig. 2 | SICs in various STS histologies. a, Repartition of the SICs in various histologies of TCGA SARC and GSE21030 (LMS, UPS and DDLPS), and FSG cohort (synovial sarcoma, myxoid liposarcoma, gastrointestinal stromal tumour (GIST)). b, Survival of patients from the FSG cohort (n = 136) according to SIC classification. Patients with synovial sarcoma, myxoid liposarcoma and gastrointestinal stromal tumour were pooled. Analysis was performed with Kaplan–Meier estimates and two-sided log-rank tests.
Extended Data Fig. 3 | The SICs exhibit strongly different TMEs. This figure refers to the GSE21050 cohort (n = 283). a, Composition of the GSE21050 cohort by SIC, histology and site of disease. b, Composition of the TME by SIC as defined by the MCP-counter Z-scores. c, Expression of gene signatures related to the functional orientation of the immune TME by SIC. d, Expression of genes related to immune checkpoints by SIC. Adjusted P-values are obtained from Benjamini–Hochberg correction of two-sided Kruskal–Wallis test P-values. These observations stand for cohorts GSE21122 and GSE30929 (not shown).
Extended Data Fig. 4 | Distribution of MCP-counter scores. a–e, MCP-counter scores in TCGA SARC (n = 213) (a, c, e) and GSE21050 (n = 283) (b, d, e), for CD8$^+$ T cells (a, b), cytotoxic lymphocytes (c, d) and B lineage cells (e, f). The blue line indicates the density curve. The red dotted line indicates the cut-off chosen to segregate high or low values, set at the median for CD8$^+$ T cells and at the third quartile for cytotoxic lymphocytes and B lineage, in each cohort. These values were chosen because the CD8 T cells scores present a normal distribution, whereas the cytotoxic lymphocytes and B lineage scores distribution exhibit a long right tail.
Extended Data Fig. 5 | B cell infiltration of STS is the key factor associated with overall survival. This figure refers to TCGA SARC and GSE21050 pooled cohorts (n = 496). a, Overall survival of patients with STS according to MCP-counter scores for cytotoxic lymphocytes. b, Overall survival of patients based on the infiltration level of their tumours by B lineage cells and cytotoxic lymphocytes. c–e, Overall survival of patients based on degree of tumour infiltration by B lineage cells and expression of PDCD1 (c), CD274 (d) and FOXP3 (e). The analyses were performed with the Kaplan–Meier estimates and two-sided log-rank tests. Tumours were considered high for expression of PDCD1, CD274 and FOXP3 if their expression was above median, and high for B lineage and cytotoxic lymphocytes if the MCP-counter score was above the third quartile.
Extended Data Fig. 6 | The mutational landscape of STS tumours does not vary significantly between SICs. This figure refers to the TCGA SARC cohort (n = 213). a, Mutational burden according to the SIC of the tumours, expressed in number of non-silent mutations. P value was computed with a Kruskal–Wallis test. Box plots as in Fig. 3d. b, Mutation frequency of all genes that are mutated in greater than 2.5% of tumours. c, Mutation frequency for genes that are mutated in more than 5% of tumours, according to SICs in the TCGA SARC cohort. The dashed lines indicate the overall mutation frequency. P values were obtained through one-sample two-sided t tests, corrected for multiple testing with the Bonferroni method. This was applied only to samples that had mutations on the considered genes (TP53: n = 75; ATRX: n = 34; TTN: n = 21; RB1: n = 19; MUC16, n = 17; PCLO, n = 13; DNAH5, MUC17 and USH2A: n = 11; PTEN, n = 6; KRAS, n = 2; BRAF, n = 1).
Extended Data Fig. 7 | Validation of SIC profiles by immunohistochemistry. This figure refers to the NTUH cohort. **a**, SIC attribution as defined by gene expression using the MCP-counter Z-scores in 73 cases. **b**, Cell density counts showing the differences in TME composition according to SIC identification of the 73 cases (SIC A: n = 16; SIC C: n = 10; SIC E: n = 11). P values are determined by two-sided Kruskal–Wallis (KW) tests. Pairwise comparisons are derived from the Dunn test. Box plots are as in Fig. 3d. **c**, Representative images of CD3 (green), CD20 (pink), CD8 (brown) and CD34 (green) expression by immunohistochemistry of SIC A, C and E tumours. The same area of the tumour is represented (0.05 mm$^2$) in each image. Similar results were observed on the other tumours from the same SICs (SIC A: n = 16; SIC C: n = 10; SIC E: n = 11).
Extended Data Fig. 8 | See next page for caption.
Extended Data Fig. 8 | Location and maturation of TLSs. 

a, Pearson correlation between the expression of CXCL13 and the 12-chemokine signature of TLS in TCGA SARC cohort (n = 213). Samples are coloured according to SICs. 
b, Intratumoural location of TLSs in three different examples from the NTUH cohort—DDLPS, UPS and LMS, respectively. TLSs are observed by the presence of CD20⁺ B cells aggregates (brown, surrounded by blue shapes). The red line delineates the tumoral zone. Similar findings were observed on the 11 tumours with TLS. 
c, Definition of peripheral, medium and central zones, accounting for 25%, 25% and 50% of the total tumour area, respectively. 
d, Distribution of TLSs in the various zones. Each bar represents one tumour. The letters above bars indicate the SIC of the tumour when the sample passed quality control of Nanostring nCounter hybridization. Dots indicate tumours in which SIC could not be determined because of RNA quality control. Similar images were observed for 66 E-TLS, 23 PFL-TLS and 20 SFL-TLS. 
e, Illustration of diverse degrees of TLS maturation in STS tumours. Consistent with maturation events occurring in secondary lymphoid organs, three maturation steps have been described for TLS: E-TLS (bottom), PFL-TLS (middle) and SFL-TLS (top), which differ in the presence of follicular dendritic cells (FDC) and their markers. E-TLS contain aggregates of CD20⁺ B cells and CD3⁺ T cells without FDC, PFL-TLS contain CD21⁺ FDC (red dotted zones) and SFL-TLS contain a germinal centre, notably visible through the presence of CD21⁺CD23⁺ follicular dendritic cells (yellow dotted zone). DAPI staining is shown in white. DAPI-negative green dots correspond to fluorescent erythrocytes. 
f, Distribution of TLS maturation steps in a subset of tumours. Each bar represents one tumour. Differences between the number of TLSs observed here and in other figures can be explained by use of non-consecutive slides or a different tumour block for some samples.
Extended Data Fig. 9 | See next page for caption.
Extended Data Fig. 9 Pan-cohort immune classification. This figure refers to the four discovery cohorts: TCGA SARC (n = 213), GSE21050 (n = 283), GSE21122 (n = 72) and GSE30929 (n = 40). a–d, Heat map and unsupervised hierarchical clustering of the MCP-counter scores describing the tumour microenvironment. Each of the population is represented by the Z-scores of the signature. a, TCGA SARC. b, GSE21050. c, GSE21122. d, GSE30929. e–h, Evolution of the variance explained by the clusters as a function of the number of clusters. Red dots indicate the number of clusters that was retained in this study. Each graph corresponds to the heat map on its left. i, Heat map of the Pearson correlation of centroids from each SIC class of discovery cohorts (TCGA SARC, GSE21050, GSE21122 and GSE30929, n = 608), with five immune classes and two groups of unclassified samples. j, k, Principal component analysis of samples from the four discovery cohorts (n = 608), based on their normalized and merged MCP-counter scores. j is coloured according to the original classes, k is coloured according to the predicted immune classes, showing a heightened homogeneity within each SIC class. l, m, Composition of the TME with classes defined as in j and k for the four discovery cohorts (n = 608), expressed in cohort-specific row Z-scores.
## Extended Data Table 1 | Clinicopathological composition of the cohorts included in this study

| Cohort  | TCGA SARC | GSE21050 | GSE21122 | GSE30929 | FSG | NTUH | SARC028 |
|---------|----------|----------|----------|----------|-----|------|---------|
| n       | 213      | 283      | 72       | 40       | 168 | 93 (StC: 73) | 47      |
| Age (median, range) | 63 (33-90) | 63 (15-92) | ND | ND | 36 (1-83) | 58 (9-94) | 57 (25-83) |
| Gender (n, %) | Male 98 (46%) | 131 (48.7%) | ND | ND | 99 (58.9%) | 37 (39.8%) | 34 (72.3%) |
|            | Female 114 (54%) | 138 (51.3%) | ND | ND | 69 (41.1%) | 56 (60.2%) | 13 (27.7%) |
| STS histology (n, %) | DDLPS 58 (27.2%) | 62 (21.9%) | 46 (63.9%) | 40 (100%) | 0 (0%) | 30 (32.3%) | 19 (40.4%) |
|            | LMS 104 (48.8%) | 85 (30%) | 26 (36.1%) | 0 (0%) | 0 (0%) | 31 (33.3%) | 6 (12.8%) |
|            | UPS 51 (23.9%) | 136 (48.1%) | 0 (0%) | 0 (0%) | 0 (0%) | 32 (34.4%) | 19 (40.4%) |
|            | Synovial sarcoma 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 58 (34.5%) | 0 (0%) | 3 (6.4%) |
|            | Myxoid liposarcoma 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 50 (29.8%) | 0 (0%) | 0 (0%) |
|            | GIST 0 (0%) | 0 (0%) | 0 (0%) | 0 (0%) | 60 (35.7%) | 0 (0%) | 0 (0%) |
| SIC (n, %) | A 55 (25.8%) | 65 (23%) | 14 (19.4%) | 8 (20%) | 37 (22%) | 16 (21.9%) | 5 (10.6%) |
|            | B 52 (24.4%) | 78 (27.6%) | 22 (30.6%) | 13 (32.5%) | 37 (22%) | 19 (26%) | 11 (23.4%) |
|            | C 35 (16.4%) | 39 (13.9%) | 8 (11.1%) | 6 (15%) | 26 (15.5%) | 10 (13.7%) | 9 (19.1%) |
|            | D 33 (15.5%) | 57 (20.1%) | 21 (29.2%) | 7 (17.5%) | 26 (15.5%) | 17 (23.3%) | 12 (25.5%) |
|            | E 38 (17.8%) | 44 (15.5%) | 7 (9.7%) | 6 (15%) | 42 (25%) | 11 (15.1%) | 10 (21.3%) |

For cohort GSE21050, sex information could not be retrieved for 14 patients. For cohort NTUH, SIC could be determined for 73 patients only. NA, not available.
## Extended Data Table 2 | Antibodies used for immunohistochemistry and immunofluorescence

| Antibody | References | Species | Clone | Source | Concentration (μg/mL) | Antigen Retrieval | Secondary Antibody | Tertiary Reagents |
|----------|------------|---------|-------|--------|----------------------|------------------|--------------------|-------------------|
| **Immunohistochemistry** | | | | | | | | |
| CD3      | 790-4341   | Rabbit  | 2Gv6  | Roche  | 0.4                  | pH9              | Alkaline Phosphatase Goat Anti-Rabbit IgG | Alkaline Phosphatase Blue Substrate |
| CD8      | M7103      | Mouse IgG1 | C6/144B | Agilent | 1.57                  | pH9              | EnVision+ System-HRP, Labelled Polymer (Mouse) | DAB |
| CD20     | M0755      | Mouse IgG2a | L26   | Agilent | 0.6                   | pH6              | EnVision+ System-HRP, Labelled Polymer (Mouse) | DAB |
| CD21     | M0784      | Mouse IgG1 | 1F8   | Agilent | 6.6                   | pH6              | EnVision+ System-HRP, Labelled Polymer (Mouse) | DAB |
| CD23     | ab16702    | Rabbit  | SP23  | Abcam  | 0.5                   | pH9              | EnVision+ System-HRP, Labelled Polymer (Mouse) | Permanent HRP Green |
| CD34     | M7165      | Mouse IgG1 | Qbend-10 | Agilent | 0.48                  | pH6              | EnVision+ System-HRP, Labelled Polymer (Mouse) | Permanent HRP Green |
| DC-Lamp  | DDX0191    | Rat IgG2a | 1010E1.01 | Dendritics | 5                     | pH9              | Biotin Donkey Anti-Rat IgG | AEC Peroxidase (HRP) Substrate |
| PD-L1    | 13684      | Rabbit  | E1L3N | Cell Signaling | 1.25                  | pH9              | EnVision+ System-HRP, Labelled Polymer (Mouse) | DAB |
| PNAδ     | 553663     | Rat IgM | MECA-79 | BD | 5                     | pH6              | ImmPRESS HRP Anti-Rat (Peroxidase) Polymer Detection Kit | DAB |
| **Immunofluorescence** | | | | | | | | |
| CD3      | 790-4341   | Rabbit  | 2Gv6  | Roche  | 0.4                  | pH9              | EnVision+ System-HRP, Labelled Polymer (Mouse) | Alexa Fluor™ 647 Tyramide Reagent |
| CD4      | AC-0173    | Rabbit  | EP204 | Epitomics | 0.17                  | pH9              | Cy3 Donkey Anti-Rabbit IgG | NA |
| CD20     | M0755      | Mouse IgG2a | L26   | Agilent | 0.6                   | pH6              | EnVision+ System-HRP, Labelled Polymer (Mouse) | Alexa Fluor™ 594 Tyramide Reagent |
| CD21     | M0784      | Mouse IgG1 | 1F8   | Agilent | 7.5                   | pH6              | Cy3 Goat Anti-Mouse IgG2a | NA |
| CD23     | ab16702    | Rabbit  | SP23  | Abcam  | 2                     | pH6              | FITC Donkey Anti-Rabbit IgG | NA |
| CXCR5    | MAB190-100 | Mouse IgG2b | 51505 | R&D | 5                     | pH9              | FITC Rat Anti-Mouse IgG2b | NA |
| PD-1     | NA         | Mouse IgG2a | EH33  | CoStim | 3                     | pH6              | EnVision+ System-HRP, Labelled Polymer (Mouse) | Alexa Fluor™ 488 Tyramide Reagent |
|          |            |         |       |        | 6                     | pH9              | Cy5 Goat Anti-Mouse IgG2a | NA |
Reporting Summary

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For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

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☑ | The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
☑ | A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
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☑ | Estimates of effect sizes (e.g. Cohen's d, Pearson's r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection
- Immunohistochemistry images were analysed with HALO 10 software (IndicaLab). Immunofluorescence data were obtained with AxioScan (Zeiss)

Data analysis
- Data was analysed with R software (version 3.4.4) and packages gplots, survival and FactoMineR. Custom code was produced in R for the analysis.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

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All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The transcriptomic datasets analysed in this study can be accessed on the GDC Portal (TCGA SARC) and the Gene Expression Omnibus repository (accession numbers GSE21050, GSE21122, GSE30929). Immunohistochemistry, gene expression and clinical-related to NTUH cohorts (Fig. 3, Extended Data Figs. 7 and 8) are available from the corresponding author on reasonable request. The data that support the findings related to Fig. 4 are available from SARC but restrictions apply to the availability of these data, which were used under license for the study. Data are however available upon reasonable request to HAT (HTawbi@mdanderson.org) and with permission of SARC. All code used in this study is available from the authors upon reasonable request.
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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | TCGA SARC: n=213, GSE211050: n=283, GSE21122: n=72, GSE30929: n=40, FSG: n=168, NTUH n=93, SARCO28: n=47. Total: n=916. |
|-------------|-----------------------------------------------------------------------------------------------------------------------------------|
| Data exclusions | 20 tumours from the NTUH cohort were excluded from gene expression (end SIC) analysis due to low quality of the extracted RNA. |
| Replication | No replication was done, but validation cohorts were analysed. |
| Randomization | Randomization is only relevant to the SARCO28 cohort, which was previously published. |
| Blinding | All image and data analysis were performed blindly, independently of sample knowledge. |

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

| Materials & experimental systems | Methods |
|----------------------------------|---------|
| n/a | n/a |
| ☑ Antibodies | ☑ ChIP-seq |
| ☑ Eukaryotic cell lines | ☑ Flow cytometry |
| ☑ Palaeontology | ☑ MRI-based neuroimaging |
| ☑ Animals and other organisms | |
| ☑ Human research participants | |
| ☑ Clinical data | |

Antibodies

Antibodies used

CD3: 2GV6, Roche ; DC-Lamp: 1010E1.01, Dendritics ; CD20: L26, Agilent ; CD8: C8/144B, Agilent ; CD21 :1F8, Agilent ; CD23 : SP23, Abcam ; CD34: Qbend-10, Agilent ; PD-L1: E1L3N, Cell Signaling ; PD-1: EH33, CoStim Pharmaceuticals ;

Validation

The specificity of anti-CD3, anti-CD4, anti-CD8, anti-CD20, anti-CD21, anti-CD23, anti-CD34, anti-CXCR5 and anti-DC-Lamp antibodies, and MECA-79 (PNAd) was validated on FFPE tonsil sections as positive control. For anti-CD20, certified manufacturing facilities from the company guarantee full quality control including western blot and studies using COS-1 cells transfected with cDNA encoding the CD20 molecule indicate that the antibody labels an intracytoplasmic epitope localized on the CD20 molecule. For anti-CD8, certified manufacturing facilities from the company guarantee full quality control including western blot and indicate that the antibody recognizes the cd8alpha chain. For anti-CD34, certified manufacturing facilities from the company guarantee full quality control. For anti-CD21, certified manufacturing facilities from the company guarantee full quality control including western blotting of the immunogen, and that the antibody labels cells or cell lines known to express CD21 (Raji, NC 37, tonsil cells), whereas no labeling is observed in the CD21-negative Jurkat cells (T-cell line) and human erythrocytes. For anti-CD23, certified manufacturing facilities from the company guarantee full quality control including western blotting, IHC on human tonsils and flow cytometry on Raji cells. For anti-CXCR5, certified manufacturing facilities from the company guarantee full quality control using human CXCR5 transfectants by flow cytometry and lack of cross reactivity with human CXCR2, CXCR3, or CXCR4 transfectants. For PNAd, certified manufacturing facilities from the company guarantee full quality control including western blotting, IHC and flow cytometry. For anti-PD-L1, specificity was validated by the company using immunohistochemical analysis of paraffin-embedded human placenta using PD-L1 (E1L3N®) XP® Rabbit mAb in the presence of control peptide or antigen-specific peptide. Specificity was verified by using FPE sections from placenta as positive control and cerebral cortex tissue as negative control. Anti-PD-1 (Freeman GJ and col.) was obtained from CoStim Pharmaceuticals and validated as described in Fig. S1 of Giraldo et al., Clinical Cancer Research, 2015. Tonsil, placenta and cerebral cortex slides were obtained from Geneticist Inc.
Human research participants

Policy information about studies involving human research participants

| Population characteristics | All available characteristics are reported in Extended Data Table 1. |
|-----------------------------|---------------------------------------------------------------------|
| Recruitment                 | Patients were recruited prior to the study and were not selected on specific criteria other than their pathology. |
| Ethics oversight            | The research was approved by the Research Ethics Committee of NTUH (201605061RINA). |

Note that full information on the approval of the study protocol must also be provided in the manuscript.