# natureresearch

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# **Reporting Summary**

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, seeAuthors & Referees and theEditorial Policy Checklist.

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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.
n/a	Confirmed
	$\blacksquare$ 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
	The statistical test(s) used AND whether they are one- or two-sided Only common tests should be described solely by name; describe more complex techniques in the Methods section.
×	A description of all covariates tested
×	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
	For null hypothesis testing, the test statistic (e.g. <i>F</i> , <i>t</i> , <i>r</i> ) with confidence intervals, effect sizes, degrees of freedom and <i>P</i> value noted <i>Give P values as exact values whenever suitable.</i>
×	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
×	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
×	Estimates of effect sizes (e.g. Cohen's <i>d</i> , Pearson's <i>r</i> ), indicating how they were calculated
	Our web collection on <u>statistics for biologists</u> contains articles on many of the points above.

#### Software and code

Policy information about availability of computer code

Data collection

BD FACSDiva software - Fortessa flow cytometer (BD Biosciences) - BD FACS ARIA II cell sorter (BD Biosciences)

Data analysis

FlowJo V10, Prism 8.0 software (GraphPad), R studio Version 1.2.1335 - TCGA-BRCA

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

Policy information about <u>availability of data</u>

All manuscripts must include a <u>data availability statement</u>. 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  $% \left( 1\right) =\left( 1\right) \left( 1\right) \left($
- A description of any restrictions on data availability

The RNA-seq data are available at https://www.ebi.ac.uk/arrayexpress/experiments/E-MTAB-9112 - TCR-seq data that support the findings of this study have been deposited in NCBI Gene Expression Omnibus with the GSE115545 accession code: [https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE115545] - TCGA-BRCA (Breast Cancer) - Legacy -https://portal.gdc.cancer.gov/legacy-archive - mRNA gene expression and quantification HT\_HG-U133A

### Field-specific reporting

## Life sciences study design

All studies must disclose on these points even when the disclosure is negative.				
Sample size	No sample size calculation was performed. We used all the samples available in each dataset.			
Data exclusions	No exclusions. All experimental results were included.			
Replication	All experiments were performed at least twice and reproducible results were obtained.			
Randomization	No randomisation was used. Since we mainly perform pair-wise comparisons, variables like age, and genetic differences do not confound our analyses. In order to avoid batch effects, the matched organs were always processed in the same batch.			
Blinding	The investigators handling the samples were not blinded during data collection and processing as the nature of the organ (TDLNs and tumor) was visibly different.			

# 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	Involved in the study	n/a Involved in the study	
	x Antibodies	ChIP-seq	
×	Eukaryotic cell lines	Flow cytometry	
×	Palaeontology	MRI-based neuroimaging	
x	Animals and other organisms	·	
	Human research participants		
x	Clinical data		

#### **Antibodies**

Antibodies used

CD32 IV.3 purified mouse IgG2b, κ Stem Cell 2B4 C1.7 APC mouse IgG1, κ BD Biosciences CD27 O323 BV605 mouse IgG1, κ BD Biosciences CD3 OKT3 BV650 mouse IgG2a, κ BD Biosciences CD4 OKT4 BV785 mouse IgG2b, κ BD Biosciences PD1 EH12.2H7 BV711 mouse IgG1, к BD Biosciences TIM3 F38-2E2 BV421 mouse IgG1, κ BD Biosciences GITR DT5D3 APC mouse IgG1 Miltenyi Biotec CD4 M-T466 APC mouse IgG1 Miltenyi Biotec TIM-3 RMT3-23 APC mouse IgG1k Miltenyi Biotec KLRG1 REA261 APC-Vio770 human IgG1 Miltenyi Biotec CD8 3B5 Alexa 700 mouse IgG2a, κ ThermoScientific EPCAM 1B7 PERCP e710 mouse IgG1, κ eBioscience ICOS ISA-3 PERCP e710 mouse IgG1, κ eBioscience CD8 2ST8.5H7 ECD mouse IgG2a Beckman Coulter CD56 N901 PE-Cy5 mouse IgG1 Beckman Coulter CD3 UCHT1 PE-Cy7 mouse IgG1, к Beckman Coulter CD3 UCHT1 Alexa 700 mouse IgG1, к BD Biosciences CD45 2D1 APC Cy7 mouse IgG1, κ BD Biosciences CD19 HIB19 Alexa 700 mouse IgG1,  $\kappa$  BD Biosciences CD45RA HI100 Alexa 700 mouse IgG2b, к BD Biosciences CD45RA HI100 PE-Cy5 mouse IgG2b, к BD Biosciences OX40 ACT35 PECy5 mouse IgG1, κ BD Biosciences CD25 M-A251 PE mouse IgG1, к BD Biosciences CD25 M-A251 APC Cy7 mouse IgG1, κ BD Biosciences CD4 RPA-T4 PE-CF594 mouse IgG1, κ BD Biosciences CCR6 G034E3 BV605 mouse IgG1, к BioLegendTM CXCR5 51505 APC mouse IgG2b, κ R&D

Antigens Clones Fluorochromes Isotypes Manufacturers

CXCR3 1C6/CXCR3 PE-CF594 mouse IgG1, κ BD Biosciences

CCR4 1G1 PE-Cy7 mouse IgG1, к BD Biosciences

CCR7 GO43H7 BV421 mouse IgG2a, к BioLegendTM

CRTh2 BM16 FITC rat IgG2A, k BioLegendTM

CD127 A019D5 BV650 mouse IgG1, к BioLegendTM

CCR10 FAB3478P PE rat IgG2A R&D

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Antigens Clones Fluorochromes Manufacturers

FOXP3 236A/E7 Alexa 488 mouse IgG1, κ eBioscience

FOXP3 236A/E7 PE mouse IgG1, κ eBioscience

FOXP3 259D/C7 PE-CF594 mouse IgG1, K BD Biosciences

GranzymeB GB11 Alexa647 mouse IgG1, κ BD Biosciences

CTLA-4 Goat polyclonal PE R&D

IL17 BL168 BV711 mouse IgG1, κ BioLegendTM

IFNy B27 V450 mouse IgG1, κ BD Biosciences

CD80 L307.4 BV786 mouse lgG1k BD Biosciences

IgG1k clone X40 BV786 BD Biosciences

LIVE/DEAD Fixable Aqua ThermoScientific

Validation

All the antibodies have been validated by the manufacturer and then titrated in house.

### Human research participants

Policy information about studies involving human research participants

Population characteristics

TDLNs and tumors were collected from 54 patients with an average of 57 years with luminal breast cancer having undergone standard-of-care surgical resection. The clinical and pathological characteristic are described in Supplementary Table 1.

Recruitment

The study cohort included volunteer patients for whom samples of the NI and I TDLNs and the primary tumor were available at the institut Curie Hospital (Paris, France).

Ethics oversight

TDLNs and tumors were collected at the Institut Curie Hospital (Paris, France), in accordance with institutional ethical guidelines and informed consent was obtained. The protocol was approved by the Ethical Committee of Curie Institut ( "Comité de la Recherche Institutionnel", CRI-0804-2015).

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

### Flow Cytometry

### Plots

Confirm that:

- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- | All plots are contour plots with outliers or pseudocolor plots.
- 🗶 A numerical value for number of cells or percentage (with statistics) is provided.

### Methodology

Sample preparation

Samples were obtained within a few hours after the primary surgery, cut into small fragments, and digested with 0.1 mg/ml Liberase TL (Roche) in the presence of 0.1 mg/ml DNase (Roche) for 20 min before the addition of 10 mM EDTA. Cells were filtered on a 40-µm cell strainer (BD Biosciences).

Instrument

BD FACSDiva software - Fortessa flow cytometer (BD Biosciences) - BD FACS ARIA II cell sorter (BD Biosciences)

Software

FlowJo V10

Cell population abundance

Expressed as a frequency of the selected population

Gating strategy

The FMOs were used for determining the division point for negative and positive population

🕱 Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.