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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, see <u>Authors & Referees</u> and the <u>Editorial Policy Checklist</u>.

### Statistics

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
	$\bigtriangledown$ The exact sample size ( <i>n</i> ) 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.
$\boxtimes$	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)
$\boxtimes$	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> .
$\boxtimes$	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
$\boxtimes$	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
$\boxtimes$	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 <u>availability of computer code</u>						
Data collection	Canto II, Aria II or III, FACS Diva ver.8;					
Data analysis	Flowjo 10, MATLAB (R2016b; The MathWorks, Natick, MA), Mathematica (version 11.2; Wolfram research, Champaign, Illinois)					
For monuscripts utilizing a	ustom algorithms or affluers that are control to the recearch but not used described in published literature, affluers must be made qualible to adjust (reviewers)					

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

Life sciences

Policy information about availability of data

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

All data is available as supplementary data. All codes are available at https://github.com/Q-bio-at-IIS/Kaneko2019CommBiol.

# Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

Behavioural & social sciences Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see <u>nature.com/documents/nr-reporting-summary-flat.pdf</u>

## Life sciences study design

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

Sample size	No statistical methods were done to predetermine sample size.
Data exclusions	There is no data exclusion.
Replication	All samples were done in biological and technical replicates. All experiments were replicated at least two times. All replications were similar results.
Randomization	No randomization was done.
Blinding	No blind test was done

### 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	$\boxtimes$	ChIP-seq
$\times$	Eukaryotic cell lines		Flow cytometry
$\boxtimes$	Palaeontology	$\boxtimes$	MRI-based neuroimaging
	Animals and other organisms		
$\boxtimes$	Human research participants		
$\times$	Clinical data		

### Antibodies

Antibodies used	PECy7-anti-CD4 (clone RAM4-4, used as x200 dilution), FITC-anti-CD4 (clone RAM4-4, x200 dilution), APCCy7-anti-CD8 (clone 53-6.7, x200), APCCy7-anti-CD45 (clone 30 F-11, x200), APCCy7-anti-TER119 (clone TER-119, x200), FITC-anti-EpCAM (BioLegend, clone G8.8, x400), PE-anti-CD80(clone 16-10A1, x400), Biotin-anti-mouse Ly-6G/Ly-6C(Gr-1) (x400),, Biotin anti-mouse/human CD45R/B220 (x400), Biotin anti-mouse TER-119/Erythroid cells (clone TER-119, x400), Biotin conjugated anti-mouse CD11b (x400), PE anti-mouse/human CD44 (clone IM7, x400), APC anti-mouse CD25 (clonePC61, x400), Streptavidin PE-Cyanine7 (x400), and Streptavidin-PECy7 (x400) were purchased from Biolegnd. UEA-biotin (x400) was from Vector laboratories (Burlingame, CA).
Validation	Antibodies were validated in the companies from which they were purchased

### Animals and other organisms

Policy information about <u>studies involving animals</u> ; <u>ARRIVE guidelines</u> recommended for reporting animal research					
Laboratory animals	Seven week-old female Balb/cA mice were used in this study.				
Wild animals	No wild animals were used.				
Field-collected samples	No field collected samples were used.				
Ethics oversight	All mice were handled in accordance with the Guidelines for the Institutional Animal Care and Use Committee (IACUC) of RIKEN Yokohama Branch.				

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

### Flow Cytometry

#### Plots

Confirm that:

 $\bigcirc$  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.

 $\bigotimes$  A numerical value for number of cells or percentage (with statistics) is provided.

### Methodology

Sample preparation	Each thymus was cut and gently agitated in 2 ml of RPMI-1640 (Sigma-Aldrich, St. Louis, MO, U.S.A.) to release thymocytes for the flow cytometric analysis. The rest of the thymic tissue was digested using Liberase in RPMI1640 (Wako) at 37 degree for 30 min. The thymic stroma rich-fraction was analyzed by flow cytometry to detect the TEC populations.
Instrument	FACS Aria and Canto II (BD).
Software	Flowjo
Cell population abundance	Not available
Gating strategy	Shown in Figure 1 and supplementary figure

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