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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 Authors & Referees and the Editorial Policy Checklist .

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	
☐	☒	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. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	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 *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	QuantStudio 12K Flex Software (v1.2.4), BD FACSDiva software (LSR: v.8.0.1, Aria II: v.8.0.2), Living Image (v4.0), Kronos control software (v2.3, Atto), Metamorph (v7.6, Molecular Devices), NIS-Elements AR (v4.20.00)
Data analysis	cutadapt-1.14, HISAT2 (v2.1.0), Cufflinks package (v2.2.1), HTSeq (v0.6.1), edgeR (v3.18.1), ARSER (v2.0), DAVID web tools (DAVID 6.8) (https://david.ncifcrf.gov/), IPA (QIAGEN Inc., https://www.qiagenbioinformatics.com/products/ingenuitypathway-analysis), HCS (v2.0.10), RTA (v1.17.21.3), CASAVA (v1.8.2), Picard (v2.0.1), ANNOVAR (v2016Mar30), Sequencher (v5.1), Snapgene (v3.1.4 to v5.0.4), GWASTools (v1.16.1), PennCNV (v1.0.3), cnvPartition (v3.2.0), MAD (v1.0.1), Metamorph (v7.6, Molecular Devices), Matlab (v2018b, MathWorks), Fiji (v1.52p, ImageJ), Excel (Microsoft Office 2011/2016), R (v3.2.5, v3.3.1, v3.4.2, v3.5.1), FlowJo (v10.6.1)

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 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 RNA sequencing data utilized for this study have been deposited in Gene Expression Omnibus (https://www.ncbi.nlm.nih.gov/geo/) under the accession number GSE116935. SNP array data in the current publication have been deposited in and are available upon application from the dbGaP database under accession phs001975.v1.p1 (http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs001975.v1.p1) and their use is limited to health/medical/biomedical purposes. Utilized computational codes & scripts are available at GitHub (https://github.com/mebisuya/SegmentationClock) and from the corresponding authors upon request. Source Data for Figs. 1-4 and Extended Data Figs. 1, 2, 5-12 are available in the online version of the paper.

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.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

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

Life sciences study design

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

Sample size	No statistical method was used to predetermine the sample size. All experiments were performed (if not otherwise stated) with at least two (most of the time three) independent experiments, yielding similar and reproducible results.
Data exclusions	No samples/data were excluded.
Replication	Each experiment was reproduced and performed at least two times, with multiple biological and/or technical replicates if not otherwise stated. See figure legends and methods section for details.
Randomization	No particular randomization method was utilized. Animals used for experiments were randomly allocated.
Blinding	The investigators were not blinded during data collection and analysis.

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

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☐	☒ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

All antibodies used in this study are commercially available antibodies, which were validated by suppliers and other researchers in the field. Details on used antibodies are listed in Supplementary Table 8.

Primary antibodies used for immunocytochemistry
BRACHYURY, R&D Systems, #:AF2085, Lot: KQP0618021, Polyclonal Goat IgG, Dilution 1:100
COL1, Southern Biotech, #:1310-01, Lot: B2918-T858, Polyclonal Goat IgG, Dilution 1:200
COL2, Southern Biotech, #:1320-01, Lot: J0513-S328, Polyclonal Goat IgG, Dilution 1:200
FOXC2, DSHB, #:1B6, Lot: 8/17/17, Monoclonal Mouse IgG1, Dilution 1:10
HNA, Merck, #:MAB1281, Lot: 2366521, Clone: 235-1, Dilution 1:50
MESP2, DSHB, #:1D4, Lot: 6/1/17, Monoclonal Mouse IgG2b, Dilution 1:10
MYH, Abcam, #:ab91506, Lot: GR11678-3, Polyclonal Rabbit IgG, Dilution 1:2000
MYOSIN, DSHB, #:MF20-s, Lot: 12/7/17, Monoclonal Mouse IgG2b, Dilution 1:20
PAX7, DSHB, #:PAX7-s, Lot: 3/8/18, Monoclonal Mouse IgG1, Dilution 1:10
PRRX1, Sigma-Aldrich, #:HPA051084, Lot: G114643, Polyclonal Rabbit IgG, Dilution 1:100
SAA, Abcam, #:ab9465, Lot: GR266197-2, Clone: EA-53, Dilution 1:1000
TBX6, R&D Systems, #:AF4744, Lot: CAPT0217111, Polyclonal Goat IgG, Dilution 1:100
TCF15, Abcam, #:ab204045, Lot: GR268168-3, Polyclonal Rabbit IgG, Dilution 1:50

Secondary antibodies used for immunocytochemistry
Alexa Fluor® 488 Donkey Anti-Rabbit IgG (H+L), Invitrogen, #:A-21206, Lot: 2072687, Dilution 1:500
Alexa Fluor® 488 Goat Anti-Mouse IgG (H+L) Invitrogen, #:A-10680, Lot: 1917945, Dilution 1:500

Alexa Fluor® 555 Donkey Anti-Goat IgG H&L Abcam, #:ab150130, Lot: GR226396-1, Dilution 1:500
Alexa Fluor® 555 Goat Anti-Mouse IgG (H+L) Invitrogen, #:A-21422, Lot: 1180091, Dilution 1:500
Alexa Fluor® 647 Donkey Anti-Mouse IgG (H+L) Invitrogen, #:A-31571, Lot: 2045337, Dilution 1:500
Donkey Anti-Rabbit IgG Cy3 Merck, #:AP182C, Lot: 2984232, Dilution 1:500
Alexa Fluor® 647 Phalloidin Invitrogen, #:A-2228, Lot: 2101947, Dilution 1:100

Primary antibodies used for flowcytometry
BRACHYURY-PE, R&D Systems, #:IC2085P, Lot: LVB0213071, Polyclonal Goat IgG Dilution 1:50
DLL1-APC, R&D Systems, #:FAB1818A, Lot: AAYU0216061, Clone: 251127, Dilution 1:200
FOXA2-PE, BD Biosciences, #:561589, Lot: 7090765, Clone: N17-280 , Dilution 1:50
NANOG-Alexa Fluor® 488, BD Biosciences, #:560791, Lot: 7110923, Clone: N31-355 Dilution 1:25
NCAM-BV421, BioLegend, #:318328, Lot: B241630, Clone: HCD56, Dilution 1:25
OCT3/4-Alexa Fluor® 647, BD Biosciences, #:560329, Lot: 7201923, Clone: 4O/Oct3, Dilution 1:25
PAX6-Alexa Fluor® 488, BD Biosciences, #:561664, Lot: 7174912, Clone: O18-1330, Dilution 1:25
SOX2-BV421, BioLegend, #:656114, Lot: B208882, Clone: 14A6A34, Dilution 1:50
SOX17-Alexa Fluor® 647, BD Biosciences, #:562594, Lot: 7104800, Clone: P7-969, Dilution 1:25
Anti-TBX6, R&D Systems, #:AF4744, Lot: CAPT0217111, Polyclonal Goat IgG, Dilution 1:25

Secondary antibodies & isotype controls used for flowcytometry
Alexa Fluor® 488 Anti-Goat IgG, Abcam, #:ab150129, Lot: GR246088-1, Polyclonal Goat IgG, Dilution 1:50
APC-conjugated Mouse IgG2b,κ, BD Biosciences, #:555745, Lot: B163785, Clone: MG2b-57, Dilution 1:200
PE-conjugated Goat IgG, R&D Systems, #:IC108P, Lot: LVD0811021, Polyclonal Goat IgG, Dilution 1:50
Unconjugated Goat IgG, R&D Systems, #:AB108C, Lot: ES4119031, Polyclonal Goat IgG, Dilution 1:25
Alexa Fluor® 647-conjugated Mouse IgG1,κ, BioLegend, #:400130, Lot: B205347, Clone: MOPC-21, Dilution 1:100
Alexa Fluor® 488-conjugated Mouse IgG1,κ, BioLegend, #:400129, Lot: B277964, Clone: MOPC-21, Dilution 1:25
Alexa Fluor® 488-conjugated Mouse IgG2a,κ, BioLegend, #:400233, Lot: B286502, Clone: MOPC-173, Dilution 1:25
PE-conjugated Mouse IgG1,κ, BioLegend, #:400112, Lot: B244597, Clone: MOPC-21, Dilution 1:50
BV421-conjugated Mouse IgG1,κ, BioLegend, #:400158, Lot: B243837, Clone: MOPC-21, Dilution 1:50

Validation

Besides initial validation of utilized (commercial) primary antibodies by manufacturers/suppliers, antibodies were validated/ tested for possible signals at unrelated stages/controls; only antibodies with reproducible (differentiation stage) specific signals were used.

Primary antibodies used for immunocytochemistry

BRACHYURY, R&D Systems, AF2085 (https://www.rndsystems.com/products/human-mouse-brachyury-antibody_af2085)
Anti-BRACHYURY antibody was validated by the manufacturer by using human embryonic stem cells. There are 60 citations.

COL1, Southern Biotech, 1310-01 (https://www.southernbiotech.com/?catno=1310-01&type=Polyclonal#&panel1-5&panel2-1)
Anti-COL1 antibody was validated by the manufacturer by using rat kidney section postuninephrectomy. There are 35 citations.

COL2, Southern Biotech, 1320-01 (https://www.southernbiotech.com/?catno=1320-01&type=Polyclonal#&panel1-1&panel2-1)
Anti-COL2 antibody was validated by the manufacturer by using newborn mouse rib section, mouse tibial growth plate section, and mouse cartilage section. There are 34 citations.

FOXC2, DSHB, 1B6 (https://dshb.biology.uiowa.edu/PCRP-FOXC2-1B6)
Anti-FOXC2 antibody was validated by the manufacturer by using human samples. There is 1 citation.

HNA, Merck, MAB1281 (http://www.merckmillipore.com/JP/en/product/Anti-Nuclei-Antibody-clone-235-1,MM_NF-MAB1281)
Anti-HNA antibody was validated by the manufacturer by using using human neural stem cells. There are 126 citations.

MESP2, DSHB, 1D4 (https://dshb.biology.uiowa.edu/PCRP-MESP2-1D4)
Anti-MESP2 antibody was validated by the manufacturer by using human samples. There is 1 citation.

MYH, Abcam, ab91506 (https://www.abcam.co.jp/fast-myosin-skeletal-heavy-chain-antibody-ab91506.html)
Anti-MYH antibody was validated by the manufacturer by using sheep muscle tissue frozen section. There are 24 citations.

MYOSIN, DSHB, MF20-s (https://dshb.biology.uiowa.edu/MF-20)
Anti-MYOSIN antibody was validated by the manufacturer by using samples of amphibian, avian, chicken, fish, human, lizard, mammal, snake, xenopus, zebrafish. There are 121 citations.

PAX7, DSHB, PAX7-s (https://dshb.biology.uiowa.edu/PAX7)
Anti-PAX7 antibody was validated by the manufacturer by using samples of amphibian, avian, bovine, canine, fish, goat, human, mouse, ovine, porcine, rat, turtle, xenopus, zebrafish. There are 73 citations.

PRRX1, Sigma-Aldrich, HPA051084 (https://www.sigmaaldrich.com/catalog/product/sigma/hpa051084?lang=en&region=US)

Anti-PRRX1 antibody was validated by the manufacturer by using human malignant glioma. There are 4 citations.

SAA, Abcam, ab9465 (https://www.abcam.co.jp/sarcomeric-alpha-actinin-antibody-ea-53-ab9465.html)
Anti-SAA antibody was validated by the manufacturer by using mouse heart tissue, H9 hESC and CBiPSC6.2 cells. There are more than 100 citations.

TBX6, R&D Systems, AF4744 (https://www.rndsystems.com/products/human-tbx6-antibody_af4744)
Anti-TBX6 antibody was validated by the manufacturer by using embryonic mouse mesoderm (E9.5) and JOY6 human induced pluripotent stem cells undifferentiated and differentiated into mesoderm. There are 3 citations.

TCF15, Abcam, ab204045 (webpage is closed due to discontinuation of antibody.)
Anti-TCF15 antibody was validated by the manufacturer by using human lateral ventricle tissue.

Antibodies used for flowcytometric analysis

BRACHYURY-PE, R&D Systems, IC2085P (https://www.rndsystems.com/products/human-mouse-brachyury-pe-conjugated-antibody_ic2085p)
BRACHYURY-PE was validated by the manufacturer by using D3 mouse cell line by flowcytometry. There are 3 citations.

DLL1-APC, R&D Systems, FAB1818A (https://www.rndsystems.com/products/human-dll1-apc-conjugated-antibody-251127_fab1818a)
DLL1-APC was validated by the manufacturer by using T98G human glioblastoma cell line by flowcytometry. There are 13 citations.

FOXA2-PE, BD Biosciences, 561589 (https://www.bdbiosciences.com/us/applications/research/intracellular-flow/intracellular-antibodies-and-isotype-controls/anti-human-antibodies/pe-mouse-anti-human-foxa2-n17-280/p/561589)
FOXA2-PE was validated by the manufacturer by using definitive endoderm derived from H9 human embryonic stem (ES) cells. There are 6 citations.

NANOG-Alexa Fluor® 488, BD Biosciences, 560791 (https://www.bdbiosciences.com/eu/applications/research/intracellular-flow/intracellular-antibodies-and-isotype-controls/anti-human-antibodies/alexa-fluor-488-mouse-anti-human-nanog-n31-355/p/560791)
NANOG-Alexa Fluor® 488 was validated by the manufacturer by using H9 human embryonic stem cells. There are 7 citations.

NCAM-BV421, BioLegend, 318328 (https://www.biolegend.com/en-us/products/brilliant-violet-421-anti-human-cd56-ncam-antibody-7143)
NCAM-BV421 was validated by the manufacturer by using Human peripheral blood lymphocytes. There are 13 citations.

OCT3/4-Alexa Fluor® 647, BD Biosciences, 560329 (https://www.bdbiosciences.com/us/applications/research/stem-cell-research/cancer-research/human/alexa-fluor-647-mouse-anti-oct34-40oct-3/p/560329)
OCT3/4-Alexa Fluor® 647 was validated by the manufacturer by using H9 human embryonic stem (ES) cells. There are 6 citations.

PAX6-Alexa Fluor® 488 BD Biosciences, 561664 (https://www.bdbiosciences.com/us/applications/research/intracellular-flow/intracellular-antibodies-and-isotype-controls/anti-human-antibodies/alexa-fluor-488-mouse-anti-human-pax-6-o18-1330/p/561664)
PAX6-Alexa Fluor® 488 was validated by the manufacturer by using neural induction of H9 human embryonic stem (ES) cells. There are 4 citations.

SOX2-BV421, BioLegend, 656114 (https://www.biolegend.com/en-us/search-results/brilliant-violet-421-anti-sox2-antibody-12705)
SOX2-BV421 was validated by the manufacturer by using NCCIT cells. There are 6 citations.

SOX17-Alexa Fluor® 647, BD Biosciences, 562594 (https://www.bdbiosciences.com/us/applications/research/intracellular-flow/intracellular-antibodies-and-isotype-controls/anti-human-antibodies/alexa-fluor-647-mouse-anti-human-sox17-p7-969/p/562594)
SOX17-Alexa Fluor® 647 was validated by the manufacturer by using definitive endoderm derived from H9 human embryonic stem (ES) cells. There are 5 citations.

TBX6, R&D Systems, AF4744 (https://www.rndsystems.com/products/human-tbx6-antibody_af4744)
Anti-TBX6 was validated by the manufacturer by using embryonic mouse mesoderm (E9.5) and JOY6 human induced pluripotent stem cells undifferentiated and differentiated into mesoderm. There are 3 citations.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)	Human induced pluripotent stem (iPS) cell lines derived from healthy donors, i.e. 1231A3 (derived from commercially available peripheral blood) and 201B7 (derived from commercially available human fibroblasts) were used for majority of

	experiments and obtained from/provided by the Center for iPS Cell Research and Application (CiRA). Additionally, human luciferase iPSC-reporter lines (625-A4 and 625-D4), which were utilized for xeno-transplantation experiments, were obtained from the Center for iPS Cell Research and Application (CiRA). Human iPSC (GCaMP) reporter line (Gen1C), which was used for calcium imaging experiments, was originally established by the Conklin Lab at the Gladstone Institute and shared with/provided by the Center for iPS Cell Research and Application (CiRA). SCDP1 patient sample was obtained from the NIGMS Human Genetic Cell Repository at the Coriell Institute for Medical Research (GM13539) and SCDP2 patient sample was obtained from a collaborating researcher at Meijo Hospital, Nagoya, Japan. Patient-derived iPSC lines were generated following strict guidelines of and approval by Kyoto University Graduate School and Medical Faculty, and Meijo Hospital, Japan. Mouse Epiblast Stem Cells (EpiSCs) were obtained from the RIKEN BioResource Research Center (RIKEN BRC) (#AES0204).
Authentication	Identity of cells generated/utilized in the lab/institute are commonly confirmed by multiple STR analyses using PowerPlex 16 HS System (Promega). For SCD patient and rescue iPSC clones SNP array analysis was also performed (see Methods section for details). Patient-like (knock-out) and patient-derived iPSC lines were also tested for presence of line-specific mutations via iPSC genotyping (see Methods section for details).
Mycoplasma contamination	All cell lines were tested negative for mycoplasma infection.
Commonly misidentified lines (See ICLAC register)	No commonly misidentified lines were used.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	NOD/ShiJic-scid Jcl (NOD/SCID) male mice were purchased from CLEA Japan and utilized for experiments at six weeks of age.
Wild animals	No wild animals were used.
Field-collected samples	No field-collected samples were used.
Ethics oversight	Animal experiments were approved by the institutional animal research committee of the Center for iPS Cell Research and Application (CiRA), Kyoto University and performed following the guidance of Regulation on Animal Experimentation at Kyoto University

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

Human research participants

Policy information about studies involving human research participants

Population characteristics	SCDP1 | Patient with mutations in MESP2; M-SDV-G (SCD2) SCDP2 | Patient with mutation in DLL3; M-SDV-G (SCD1)
Recruitment	Patient samples utilized in this study were either obtained from the NIGMS Human Genetic Cell Repository at the Coriell Institute for Medical Research (SCDP1) or Meijo Hospital, Japan (SCDP2) based on provided clinical/radiological data indicating a patient with segmentation defects of the vertebrae (SDV). SCDP1 | Patient with mutations in MESP2; M-SDV-G (SCD2): Patient was diagnosed with spondylothoracic dysostosis, malsegmentation of the spine, numerous hemivertebrae, "crab thorax" and lordosis. Primary tissue samples utilized for derivation of patient iPSCs (SCDP1) were provided by Coriell Institute for Medical Research (GM13539). SCDP2 | Patient with mutation in DLL3; M-SDV-G (SCD1): Patient was diagnosed with spondylothoracic dysostosis, segmentation defects of the vertebrae with involvement of the entire spine (sacrum to C1), bilateral fusion of ribs posteriorly, with fanning out in a “crab-like” appearance, mild scoliosis and marked reduction of thoracic lordosis. Primary tissue samples utilized for derivation of patient iPSCs (SCDP2) were provided by Meijo Hospital, Nogoya, Japan.
Ethics oversight	All experiments followed relevant guidelines and regulations and were approved by Ethics committees of the Kyoto University Graduate School and Faculty of Medicine, Kyoto, Japan and Meijo Hospital, Nagoya, Japan. Informed consent was obtained from legal guardians of patients by relevant institutions.

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	Cells were washed with PBS and dissociated using Accutase (Life Technologies) and centrifuged. Cells were resuspended (1.0 × 10^7 cells/ml) in FACS buffer (0.1% BSA in PBS) and stained with allophycocyanin (APC)-conjugated DLL1 antibody for 30 minutes at 4°C. Then, cells were stained with DAPI to eliminate dead cells after washing with FACS buffer once and finally strained through a filter mesh. As for the co-staining of intracellular molecules TBX6 and BRACHYURY with DLL1, cells were fixed with 4% paraformaldehyde (PFA) for 20 minutes at 4°C after initial staining with DLL1 antibody and washed twice with staining medium, which contained PBS with 2% fetal bovine serum (FBS). Samples were permeabilized with BD Perm/Wash buffer (BD Biosciences) for 15 minutes at room temperature and stained with TBX6 primary antibody or phycoerythrin (PE)-conjugated BRACHYURY antibody for 60 minutes at room temperature and washed with BD Perm/Wash buffer twice. The cells stained with TBX6 antibody were stained with Alexa Fluor® 488-conjugated secondary antibody for 60 minutes at room temperature. The samples were washed with BD Perm/Wash buffer twice and suspended into staining medium. For FACS-based evaluation of undifferentiated PSCs and their differentiation capacity into three germ layers, cells (1.0 × 10^6 cells each) were fixed with 4% paraformaldehyde phosphate buffer solution (4% PFA/PBS) for 20 minutes at 4°C and washed twice with staining medium, which contained PBS with 2% fetal bovine serum (FBS). Samples were permeabilized with BD Perm/Wash buffer (BD Biosciences) for 15 minutes at room temperature and stained with fluorescence-conjugated primary antibodies listed in Supplementary Table 8.3. The samples were washed with BD Perm/Wash buffer twice and suspended into staining medium.
Instrument	Flow cytometric analysis was performed using LSR or BD FACSAria II cell sorter (BD Biosciences).
Software	FACS data was analyzed and graphs were generated using FlowJo software (FlowJo LLC, version 10.6.1).
Cell population abundance	Abundance of distinct cell populations of interest was determined using appropriate negative controls.
Gating strategy	Standard gating settings commonly utilized at FACS core facility of the institute were used. Besides using appropriate isotype controls, negative (control) cell samples were utilized to set appropriate gates and determine true positive cell populations.

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