nature portfolio

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Reporting Summary

Nature Portfolio 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 Portfolio policies, see our <u>Editorial Policies</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

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||ig || 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

 \propto 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 <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	No software was used.			
Data analysis	For scRNA-seq data analysis, Cell Ranger 7.0 was used to generate the gene expression matrix. The subsequent analysis was performed using Seurat v5.0.17, Harmony, and clusterProfiler in R 4.3.2. For high-dimensional flow cytometry data analysis, Peak-based selection of high quality cytometry data (PeacoQC, 1.14.0) algorithm and Flow Self-Organizing Maps (FlowSOM, 2.12.0) algorithm were used. For TCRseq analysis, MiXCR 4.3.2 and Immunarch 1.0.0 were used.			

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 and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Policy information about availability of data

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 description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

Raw FASTQ files of the TCR-seq data has been deposited at the NCBI SRA under the BioProject: PRJNA1040021. scRNAseq data are accessible through this link: https://zenodo.org/uploads/11243058.

Research involving human participants, their data, or biological material

Policy information about studies with <u>human participants or human data</u>. See also policy information about <u>sex, gender (identity/presentation)</u>, <u>and sexual orientation</u> and <u>race, ethnicity and racism</u>.

Reporting on sex and gender	Gender is N.A. Sex is summarized in the corresponding tables 1-3.
Reporting on race, ethnicity, or other socially relevant groupings	N.A.
Population characteristics	Our study focused on investigating immunological changes in children who had undergone cardiac surgery of CHD with concomitant thymectomy and enrolled 5–12-year-old children after surgery (Table 1) and neonates before surgery (Table 3). The children and neonates were diagnosed with a wide spectrum of critical and/or complex CHDs, such as simplex d-TGA (dextro-transposition of great arteries), complex TGA, TAPVR (total anomalous pulmonary vein return), and Shone's variant (Supplementary Table S1, Supplementary Table S2), in some cases requiring multiple surgical and/or interventional procedures.
Recruitment	For the post-surgical study population children with CHD (aged 5–12 years), who visited the pediatric cardiology outpatient clinic for a routine follow up appointment, were eligible for the study. These study participants had underwent partial or complete thymectomy within the first 6 weeks of life performed during a palliative or corrective heart surgery (n = 18, Supplementary Table S1). Age-matched, generally healthy children who had a blood sample taken before planned minor surgery at the Department of Pediatric Surgery, Hannover Medical School, Hannover, Germany, were included in the study as non-CHD controls.
Ethics oversight	Hannover Medical School (10198_BO_S_2022)

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

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.

Ecological, evolutionary & environmental sciences

Life sciences

Behavioural & social 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	The sample size was determined based on patients who visited the hospital.			
Data exclusions	No data was excluded.			
Replication	Replication was done by different methods, namely FACS phenotyping, in vitro stimulation, and single cell RNA sequencing.			
Randomization	Patients were selected based on the disease.			
Blinding	The initial data analysis of all FACS data was blinded.			

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 Antibodies Γ \boxtimes ChIP-seq Eukaryotic cell lines Flow cytometry Palaeontology and archaeology \boxtimes MRI-based neuroimaging \boxtimes Animals and other organisms \boxtimes Clinical data Dual use research of concern \boxtimes \boxtimes Plants

Antibodies

Antibodies used

CD3 BL	JV661, UCHT1, BD, Cat 612964, Lot 0311377;
	(570, RPA-T4, BioLegend, Cat 300534, Lot B346965;
	3UV805, SK1, BD, Cat 612889, Lot 2140241;
	E-Cy, HIB1, eBioScience, Cat 25-0199-42, Lot 2527485;
	·B700, MφP, BD, Cat 566465, Lot 1055172;
	PE, REA591, Miltenyi, Cat 130-113-512, Lot 5240102466;
	oGreen, REA173, Miltenyi, Cat 130-110-512, Lot 5240102400,
	r-CP-Vio700, REA771, Miltenyi, Cat 130-111-014, Lot 5230405826;
	C, REA470, Miltenyi, Cat 130-125-204, Lot 5231106985;
	SUV496, 3G8, BD, Cat 612944, Lot 2272394;
	SUV563, NCAM16.2, BD, Cat 612928, Lot 0325598;
	7.2 BUV615, OF-5A12, BD, Cat 751162, Lot 2147028;
	APC-Cy7, HP-3G10, BioLegend, Cat 339928, Lot B399062;
	A BV605, HI100, BioLegend, Cat 304134, Lot B346157;
	V785, G043H7, BioLegend, Cat 353230, Lot B340912;
	E Fire 700, M-A251, BioLegend, Cat 356146, Lot B376207;
CD31 F	E-Cy5, WM59, BioLegend, Cat 303150, Lot B338135;
CD27 A	lexa Fluor F700, O323, BioLegend, Cat 302814, Lot B316073;
CD28 A	lexa Fluor 647, CD28.2, BioLegend, Cat 302954, Lot B350959;
CD38 A	.PC-Fire810, HIT2, BioLegend, Cat 303550, Lot B359420;
PD-1 B	V421, EH12.2H7, BioLegend, Cat 329920, Lot B398161;
CD57 F	B, HNK-1, BioLegend, Cat 359608, Lot B331659;
NKG2A	PE-Vio615, REA110, Miltenyi, Cat 130-120-035, Lot 5221010545;
CD69 E	UV737, FN50, BD, Cat 612817, Lot 2122353;
HLA-DI	R BV570, L243, BioLegend, Cat 307638, Lot B363164;
νδ2 Αβ	C-Vio770, REA771, Miltenyi, Cat 130-111-013, Lot 5231009636;
IFN-γ P	E-Cy7, B27, BioLegend, Cat 506518, Lot B326674;
GZMB	APC, GB12, Invitrogen, Cat MHGB05, Lot 2690614;
	AF700, MAb11, BioLegend, Cat 502928, Lot B347121;
	ITC, S21011A, BioLegend, Cat 387303, Lot B400042;
	532, SK7, eBioscience, Cat 58-0038-42, Lot 2338602;
	APC, CB9, BioLegend, Cat 507220, Lot B316004;
	AF647, GB11, BioLegend, Cat 515406, Lot B341642;
	PC-Fire810, HIB19, BioLegend, Cat 302272, Lot B375854;
	3UV563, RPA-T8, BD, Cat 612914, Lot 20322344;
	750, SK3, BioLegend, Cat 344643, Lot B355409;
	BV650, AO1905, BioLegend, Cat 351326, Lot B359697;
	BV650, AO1905, BloLegend, Cal 351326, Lot B579697; BV711, BER-ACT8, BD, Cat 563162, Lot 2033635;
	E-Cy5, CD28.2, BioLegend, Cat 302910, Lot B336927
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Validation

N.A.

Plants

Seed stocks	Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.
Novel plant genotypes	Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor
Authentication	was applied. Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.

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	PBMCs were thawed in 37°C water bath and stained with antibodies.
Instrument	Spectral flow cytometer Cytek Aurora using Spectroflo software (Cytek Biosciences)
Software	FCS Express, FlowJo, Peak-based selection of high quality cytometry data (PeacoQC) algorithm and Flow Self-Organizing Maps (FlowSOM) algorithm
Cell population abundance	Purity of FACS-sorted T cell subset was determined by short re-analysis by FACS. The purity was above 95% for all samples.
Gating strategy	All antibodies were tested by titration and if neccessary by FMO controls. Gating strategy was based on the positive and negative signals.

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