

Supplementary Figure 1: Analysis of lymphocyte phenotype and differentiation stratified by time post HSCT

PBMCs from healthy donors (n=22-24), untransplanted DOCK8-deficient patients (n=7-9), or DOCK8-deficient patients at 0-11 (n=10), 12-22 (n=6) or 23-43 months (n=3-4) following HSCT were labelled with mAbs against CD3, CD4, CD8, CD20, CD45RA, CCR7, CD10, CD27, CD28, CD56, CD57, CD127, PD1, TCR $\alpha\beta$, TCR $\gamma\delta$, TCRV α 24, TCRV β 11, CD161 and TCR α V.2.

Proportions of (A) CD3⁺ cells, CD4⁺ T cells (CD3⁺CD4⁺), CD8⁺ T cells (CD3⁺CD8⁺), B cells (CD20⁺); (B) transitional, naïve and memory B cell subsets; (C) CD4⁺ naïve, T_{CM} and T_{EM} cell subsets; (D) CD8⁺ naïve, T_{CM}, T_{EM} and T_{EMRA} cell subsets; (E) αβ and γδ TCR⁺ T cells; (F) MAIT cells; (G) NK cells; (H) NKT cells; (I) memory CD4⁺ T cell (CD4⁺CD8⁻CD45RA⁻CCR7[±]) and (J) CD8⁺ T_{EM} cell expression of PD1, CD57, CD27, CD28 and CD127 were then determined by flow cytometric analysis. Data are the mean ± SEM. Statistics performed using Prism unpaired t-test with Welch's correction *<0.05, **<0.01, ***<0.005, ****<0.001.

Supplementary Figure 2: Analysis of intracellular cytokine expression by DOCK8-deficient CD4⁺ T cells: effect of cell division

Naïve and memory CD4⁺ T cells were sort-purified from the peripheral blood of healthy donors (n=13-25), untransplanted DOCK8-deficient patients (n=2-7), or DOCK8-deficient patients following HSCT (DOCK8 pHST; n=8-18). The cells were labelled with CFSE and then cultured under Th0 (TAE beads; memory) or Th1 (+IL-12) conditions (naïve). After five days, cells were restimulated with PMA/ionomycin before permeabilization and intracellular staining to determine (A) proportions of memory CD4⁺ T cells expressing Th1 (IFNγ and TNFα) or Th2 cytokines (IL4 and IL13); (B) proportions of naïve CD4⁺ T cells expressing IFNγ and the proportions of (C) memory or (D) naïve CD4⁺ T cells expressing IFNγ and IL-21 in each division interval as determined by dilution of CFSE. The data represent the mean ± SEM of cytokine positive cells, or cytokine-expressing cells in each division. Statistics performed using Prism unpaired t-test with Welch's correction *<0.05, **<0.01, ***<0.005, ****<0.001.

Supplementary Figure 3: Changes observed in paired pre- and post-transplant DOCK8-deficient patients are consistent with those seen at the cohort level.

PBMCs from healthy donors (red columns), and DOCK8-deficient patients before (blue dots), or following HSCT (DOCK8 pHST) (green dots) were labelled with mAbs against CD3, CD4, CD8,

CD20, CD56, CD45RA, CCR7, CD10, CD27, IgG, IgA, TCR $\alpha\beta$, TCR $\gamma\delta$, TCR V α 24, TCR V β 11, CD161, TCR V α 7.2, PD1, CD57, CD27, CD28 and CD127. Proportions of:

(A) CD3⁺ cells, CD4⁺ T cells (CD3⁺CD4⁺), CD8⁺ T cells (CD3⁺CD8⁺), B cells (CD20⁺);

(B) transitional, naive and memory B cell subsets;

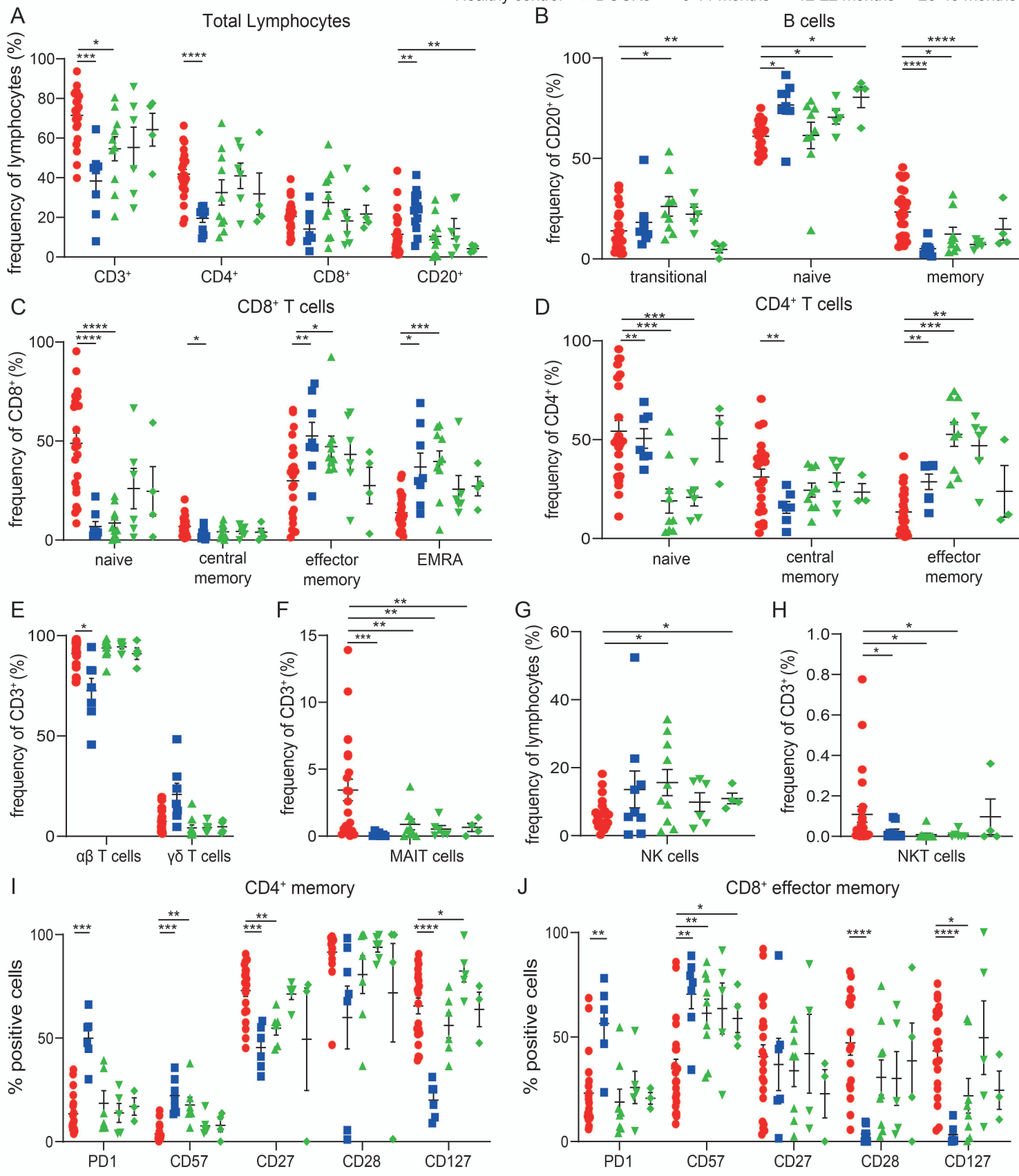
(C) CD4⁺ and (D) CD8⁺ naïve, T_{CM}, T_{EM} and T_{EMRA} cell subsets; and

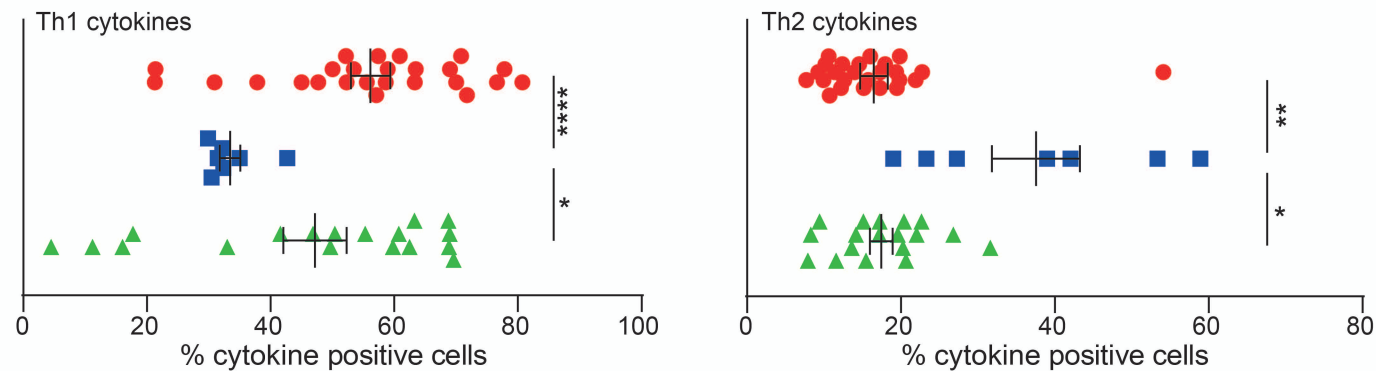
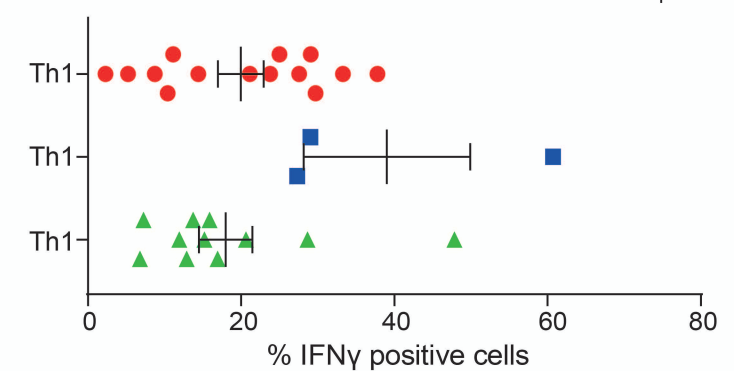
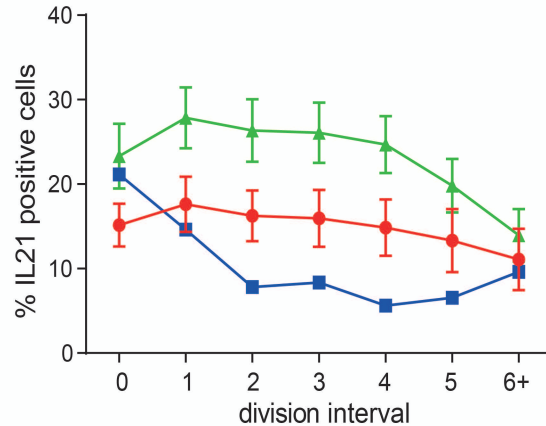
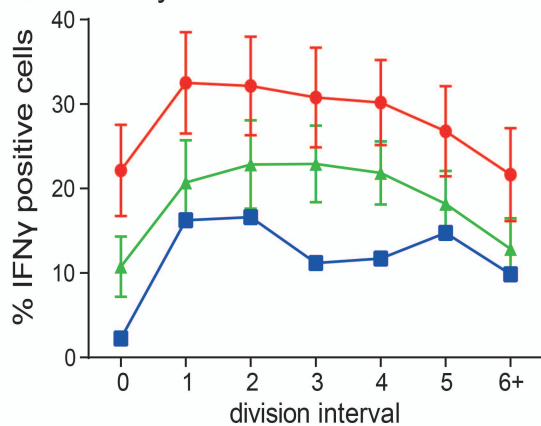
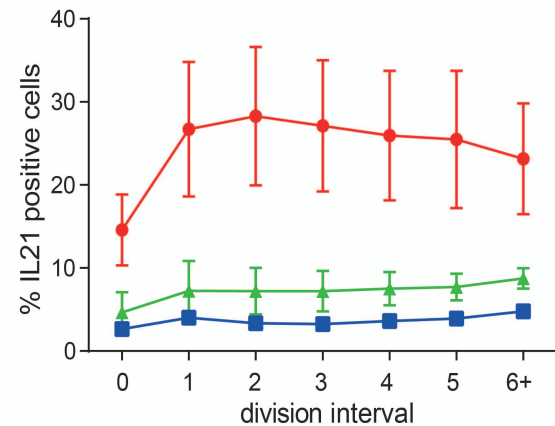
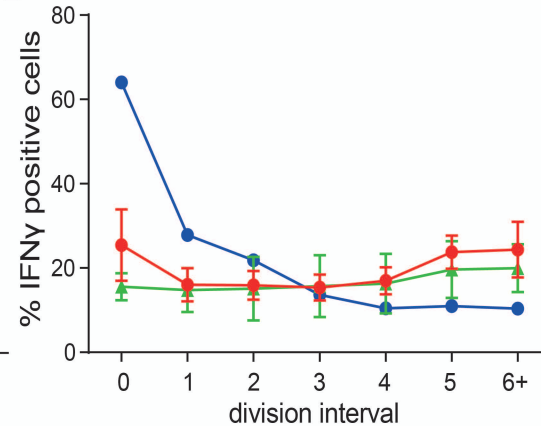
(E) memory CD4⁺ T cells and (F) T_{EM} CD8⁺ T cells expressing PD1, CD57, CD27, CD28 and CD127 were determined.

(G) Memory CD4⁺ T cells were sort-purified from healthy donors, untransplanted DOCK8-deficient patients, or DOCK8-deficient patients following HSCT, and cultured with TAE beads. After five days, the proportions of cells expressing Th1 (IFN γ , TNF α) and Th2 (IL-4, IL-13) cytokines, and the Th2/Th1 ratio were determined.

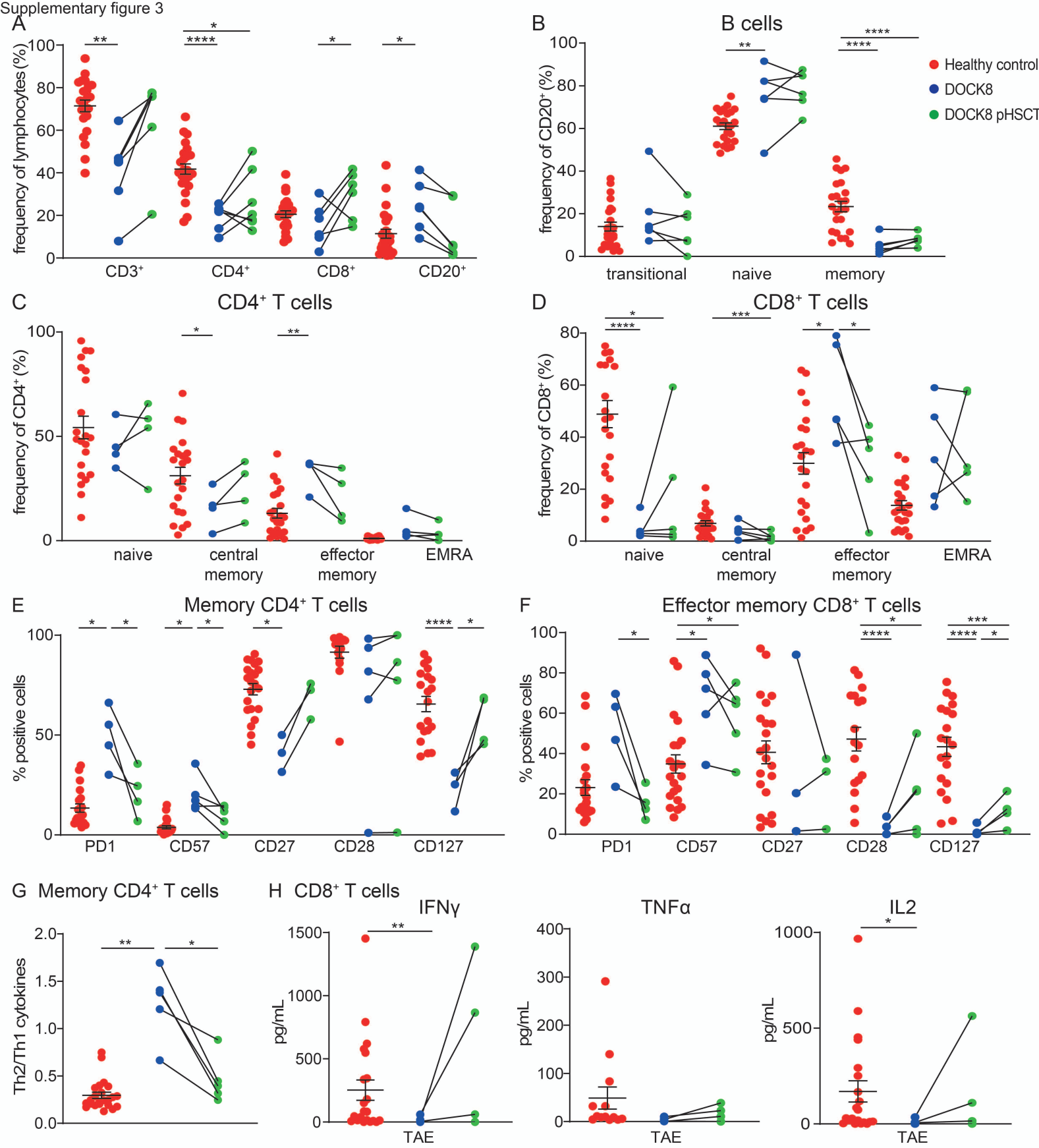
(H) CD8⁺ T cells were sorted from healthy donors, untransplanted DOCK8-deficient patients, or DOCK8-deficient patients following HSCT and cultured with TAE beads. Secretion of IFN γ , TNF α , and IL-2 was determined after 5 days by cytometric bead arrays.

Data presented is from healthy controls (n=21-25) and patients with both a pre- and post-transplant sample (n=4-6), with data from individual patients joined by a line. Statistics performed using Prism paired t-test or unpaired t-test with Welch's correction *<0.05, **<0.01, ***<0.005, ****<0.001.



A Memory CD4⁺ T cellsB Naive CD4⁺ Th1 cellsC Memory CD4⁺ T cellsD Naive CD4⁺ Th1 cells

● Healthy control
 ■ DOCK8
 ▲ DOCK8 pHSCT



Supplementary Table 1: Antibodies and reagents used

Target-Fluorochrome	Clone	Supplier
CCR7-FITC	150503	R&D Systems
CCR7-PECy7	G043H7	BioLegend
CD107a-AF647	H4A3	BioLegend
CD10-APC	HI10a	Becton Dickson Biosciences
CD10-BUV737	HI10a	Becton Dickson Biosciences
CD127-BV421	A019D5	BioLegend
CD127-BV650	A019D5	BioLegend
CD161-PerCPCy5.5	HP-3G10	eBioscience
CD20-BUV395	2H7	Becton Dickson Biosciences
CD20-PE	L27	Becton Dickson Biosciences
CD25-FITC	2A3	Becton Dickson Biosciences
CD25-PE	M-A251	Becton Dickson Biosciences
CD25-PECy7	M-A251	Becton Dickson Biosciences
CD27-BV786	L128	Becton Dickson Biosciences
CD27-PECy7	M-T271	Becton Dickson Biosciences
CD28-PerCPCy5.5	CD28.2	Becton Dickson Biosciences
CD3-BV421	UCHT1	Becton Dickson Biosciences
CD3-BV786	UCHT1	Becton Dickson Biosciences
CD45RA-BV605	HI100	Becton Dickson Biosciences
CD45RA-PerCPCy5.5	HI100	eBioscience
CD4-APCCy7	RPA-T4	Becton Dickson Biosciences
CD4-BUV395	SK3 (Leu3a)	Becton Dickson Biosciences
CD4-BUV737	SK3 (Leu3a)	Becton Dickson Biosciences
CD4-PacBlue	OKT4	eBioscience
CD56-BV605	HDC56	BioLegend
CD57-FITC	NK-1	Becton Dickson Biosciences
CD8-APC	2ST8.5H7	Becton Dickson Biosciences
CD8-BUV395	RPA-T8	Becton Dickson Biosciences
CD8-PECy7	RPA-T8	Becton Dickson Biosciences
CD8-PerCPCy5.5	RPA-T8	Becton Dickson Biosciences
CD95-PECF594	DX2	Becton Dickson Biosciences
DOCK8	G-2	Santa Cruz Biotechnology
GranzymeB-AF700	GB11	Becton Dickson Biosciences
IFN γ -BV605	B27	Becton Dickson Biosciences
IgD-PE	IA6-2	Becton Dickson Biosciences
IgG1 κ isotype control	P3.6.2.8.1	eBioscience
IgG-APC	G18-145	Becton Dickson Biosciences
IgM-FITC	G20-127	Becton Dickson Biosciences
IL13-BV421	JES10-5A2	Becton Dickson Biosciences
IL17A-APCCy7	BL168	BioLegend
IL17F-BV786	O33-782	Becton Dickson Biosciences

IL21-ef660	eBio3A3-N2	eBioscience
IL2-BV650	MQ1-17H12	Becton Dickson Biosciences
IL4-PECy7	8D4-8	eBioscience
PD1-Biotin	eBioJ105	eBioscience
Streptavidin-BV421	N/A	BioLegend
TCR $\alpha\beta$ -PECy7	IP26	BioLegend
TCR $\gamma\delta$ -PerCPe710	B1.1	eBioscience
TCRV α 24-FITC	C15	Beckman Coulter
TCRV α 7.2-BV421	3C10	BioLegend
TCRV β 11-PE	C21	Beckman Coulter
TNF α -BUV395	MAb11	Becton Dickson Biosciences
anti-mouse IgG1-PE	G18-145	Becton Dickson Biosciences
Reagent		Supplier
ABTS		Sigma-Aldrich
anti-human IgM/IgG/IgA antisera		Southern Biotech
Brefeldin A		Sigma-Aldrich
calibrite beads		Becton Dickson Biosciences
Carboxyfluorescein succinimidyl ester (CFSE)		Invitrogen
CD40L		R&D Systems
CpG 2006		Sigma-Aldrich
cytometric bead array (CBA)		Becton Dickson Biosciences
HA peptide		R&D Systems
HRP-streptavidin		Jackson ImmunoResearch
Ig standards		Sigma-Aldrich
Ionomycin		Sigma-Aldrich
monensin		eBioscience
Paansorbin cells (SAC-Cowan I strain)		Millipore
Phorbol 12-myristate 13-acetate (PMA)		Sigma-Aldrich
Prostaglandin E2		Sigma-Aldrich
recombinant human IL12		R&D Systems
recombinant human IL-1 β		PeptoTech
recombinant human IL2		Millipore
recombinant human IL-21		PeptoTech
recombinant human IL-23		PeptoTech
recombinant human IL-4		Dr Rene de Waal Malfyt (DNAX Research Institute)
recombinant human IL-6		PeptoTech
recombinant human TGF- β		PeptoTech
saponin		Sigma-Aldrich
T-cell activation and expansion (TAE) beads		Miltenyi Biotech
Zenon mouse IgG1 labeling kit		Thermo Fischer Scientific
Zombie aqua fixable dye		BioLegend

AF, Alexa fluor; APC, allophycocyanin; BUV, brilliant ultra violet; BV, brilliant violet; FITC, fluorescein isothiocyanate; PE, phycoerythrin; PerCP, peridinin-chlorophyll-protein complex.