Single-cell immune profiling reveals thymus-seeding populations, T cell commitment, and multilineage development in the human thymus

Cordes, Martijn; Canté-Barrett, Kirsten; van den Akker, Erik B.; Moretti, Federico A.; Kiełbasa, Szymon M.; Vloemans, Sandra A.; Garcia-Perez, Laura; Teodosio, Cristina; van Dongen, Jacques J.M.; Pike-Overzet, Karin

DOI
10.1126/sciimmunol.ade0182

Publication date
2022

Document Version
Final published version

Published in
Science immunology

Science Immunology
AAAS

Supplementary Materials for

Single-cell immune profiling reveals thymus-seeding populations, T cell commitment, and multilineage development in the human thymus

Martijn Cordes *et al.*

Corresponding author: Frank J.T. Staal, f.j.t.staal@lumc.nl

Sci. Immunol. **7**, eade0182 (2022)
DOI: 10.1126/sciimmunol.ade0182

The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

Supplementary Table S1. List of antibodies

Target (Hu)	Fluorochrome	Clone	Vendor
CD34	APC	581	BD
CD1a	PE	HI149	BD
CD38	PE-Cy7	HB-7	BD
CD8	FITC	SK1	BD
CD4	AF405	S3.5	Thermo Fisher
CD3	APC	SK7	BD
CD26	PE	M-A261	BD
CD56	PE	MY31	BD
CD13	PE	WM15	BD
CD33	PE	WM53	BD
CD19	PE	HIB19	BD
CD34	PE	581	BD
CD38	BUV395	HB-7	BD
CD8	BUV496	RPA-T8	BD
CD11c	BUV661	b-ly6	BD
CD4	BUV805	SK3	BD
CD141	BV4211	1A4	BD
CD20	SB436	2H7	eBioscience
CD36	PacB	FA6.152	Beckman Coulter
CD123	BV480	7G3	BD
CD15	BV510	W6D3	BD
CD16	BV570	3G8	BioLegend
CD10	BV605	HI10a	BioLegend
CD45	BV650	HI30	BD
HLA-DR	BV711	G46-6	BD
CD11b	BV750	M1/70	BioLegend
CD64	BV786	10.1	BD
CD14	Qdot800	TUK4	Thermo Fisher
FcERI	FITC	AER-37	eBioscience
CD7	AF532	124-1D1	eBioscience
CD105	PE	266	BD
CD34	PE-CF594	581	BD
CD3	AF594	UCHT1	BioLegend
CD56	PE-Cy5	B159	BD
CD117	PE-Cy7	104D2D1	Beckman Coulter
CD19	AF647	SJ25C1	BioLegend
CD71	AF700	M-A712	BD
CD13	APC	WM15	BD
CD300e	APC-Vio770	UP-H2	Miltenyi Biotec
CD45	BV650	HI30	BD
TCRab	PerCP-Cy5.5	IP26	BioLegend
CD3	AF700	UCHT1	BD

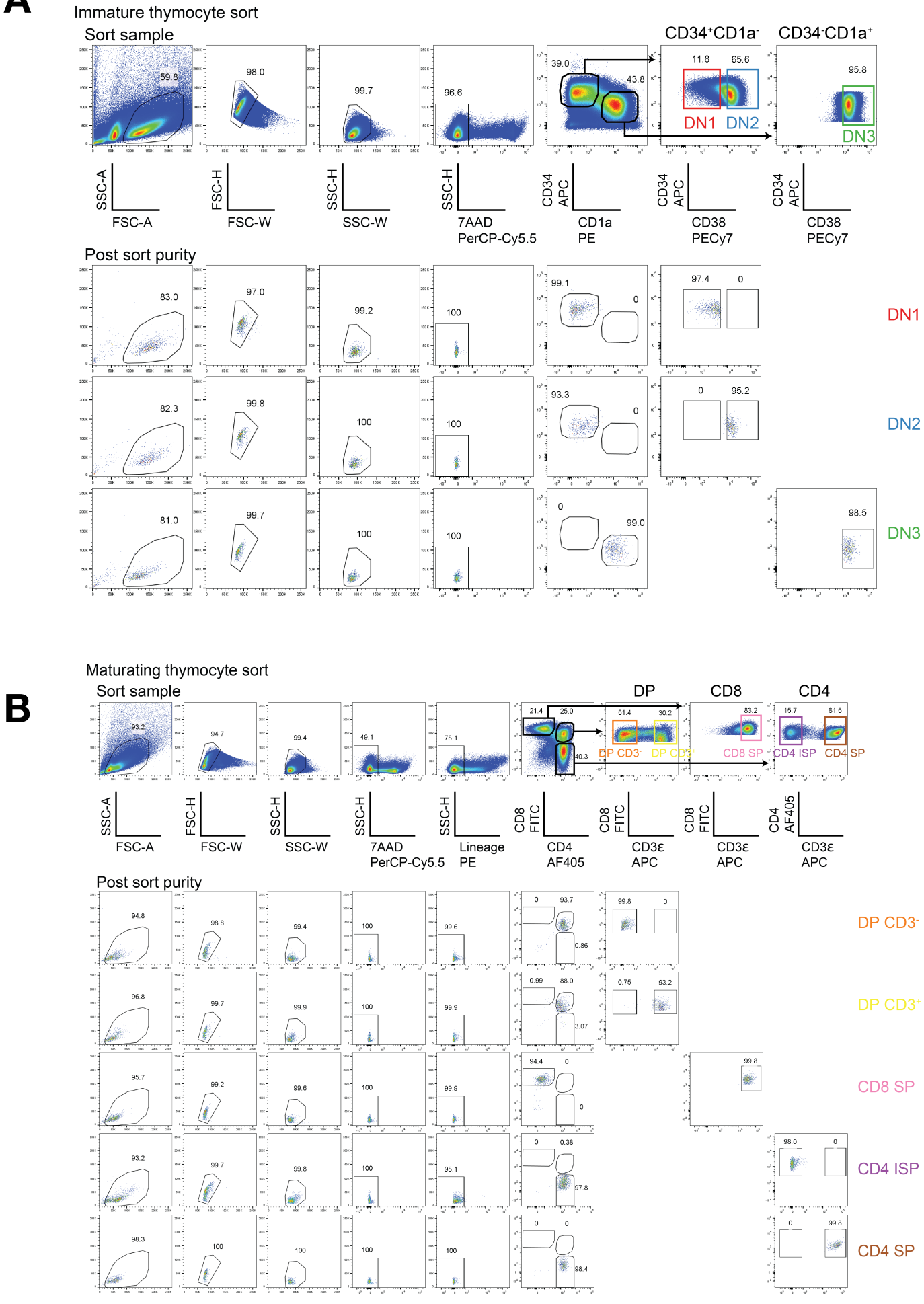
CD4	PE	SK3	BD
CD8	PE-Cy7	SK1	BD
CD123	BV480	7G3	BD
CD14	APC	HCD14	BioLegend
CD19	FITC	4G7	BD
CD56	APC-Cy7	HCD56	BioLegend
CD3	FITC	SK7	BD
TCRab	FITC	WT31	BD
CD33	PE-Cy5	WM53	BioLegend
Zombie	NIR		BioLegend

Supplementary Table S2. List of primers and probes

Target	Forward primer (5'-3')	Reverse primer (5'-3')	Probe (5'-3')
TCRD Dδ2-Dδ3	Dδ2: CAAGGAAAGGGAAAAAGGAAGAA	Dδ3: TTGCCCCTGCAGTTTTTGTAC	Dδ3: FAM-ATACGCACAGTGCTACAAAACCTACAGAGACCT-TAMRA
TCRD Dδ2-Jδ1	Dδ2: AGCGGGTGGTGATGGCAAAGT	Jδ1: TTAGATGGAGGATGCCTTAACCTTA	Jδ1: FAM-CCCGTGTGACTGTGGAACCAAGTAAGTAACTC-TAMRA
ALBUMIN	GCTGTCATCTCTTGTGGGCTGT	ACTCATGGGAGCTGCTGGTTC	VIC-CCTGTCATGCCCACACAAATCTCTCC-TAMRA

SUPPLEMENTARY FIGURES

Supplementary Figure 1

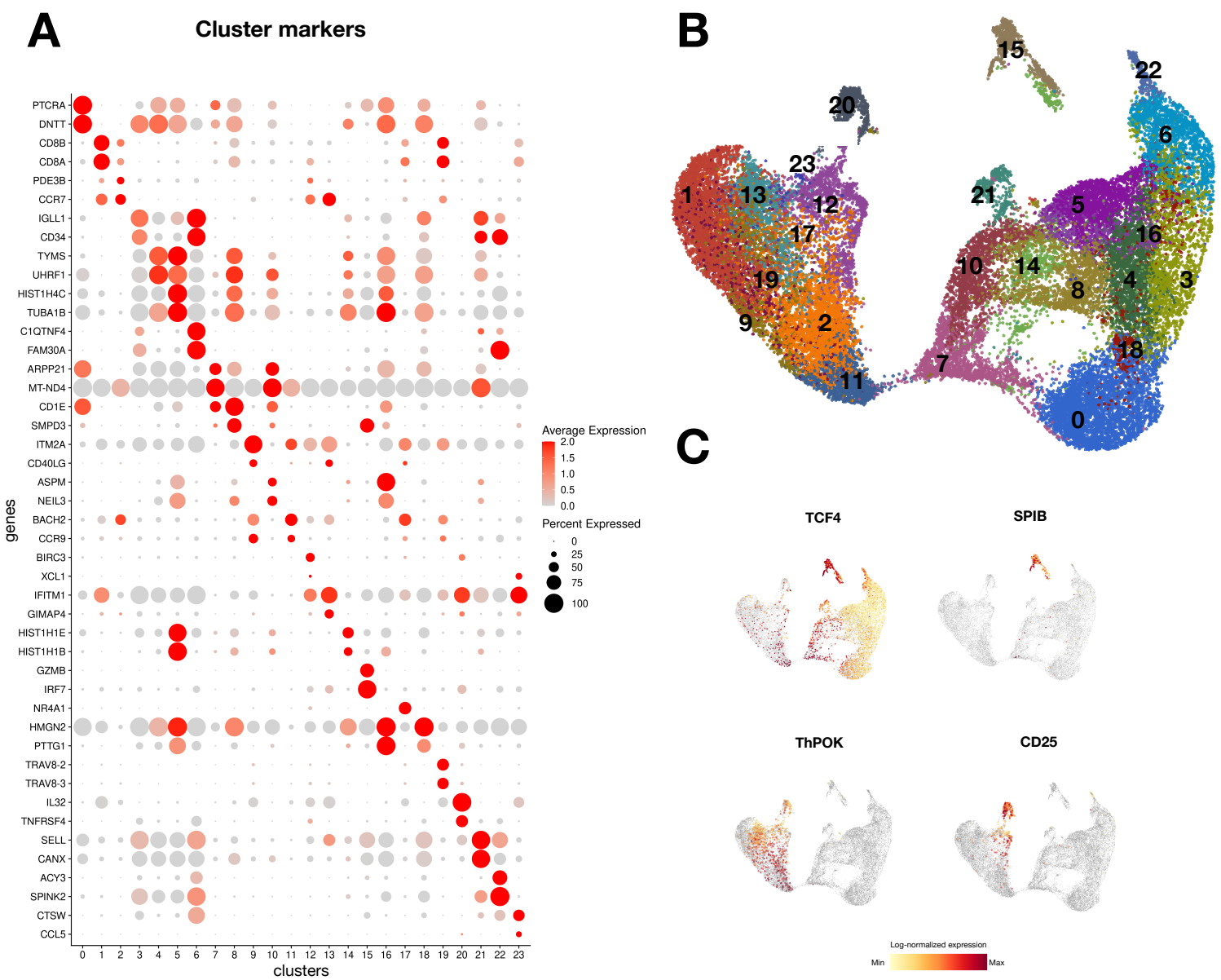


Suppl. Fig. S1. Cell sorting strategies and populations purities.

(A) Sorting strategies and population purities of immature DN1, DN2, and DN3 thymocytes. Prior to sorting, thymocytes were enriched for CD34 expression.

(B) Sorting and purities of more mature DP $CD3^-$, DP $CD3^+$, CD8 SP, CD4 ISP, and CD4 SP thymocytes.

Supplementary Figure 2



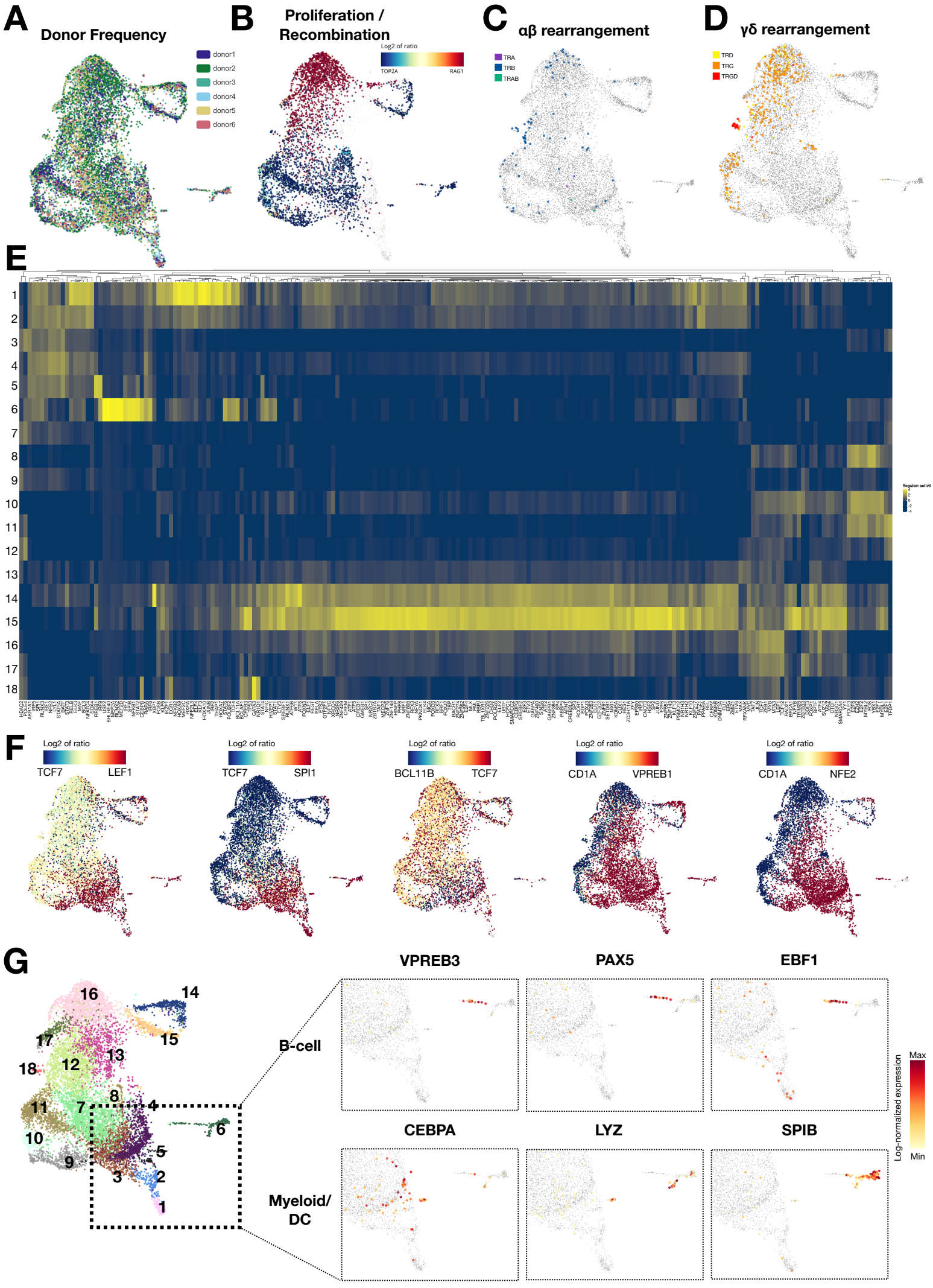
Suppl. Fig. S2. Cluster analysis of the total thymocyte UMAP.

(A) UMAP projection of 24 clusters identified by cluster analysis.

(B) Dot plot depicting the genes that identify clusters based on differential gene expression.

(C) Gene expression of selected genes projected on the total thymocyte UMAP.

Supplementary Figure 3



Suppl. Fig. S3, related to Fig. 3. DN1, DN2, DN3 UMAPs with TCR rearrangements and visualization of transcription factor activity and using SCENIC.

(A) DN1, DN2, DN3 UMAP visualizing the donor distribution.

(B) Proliferation versus recombination activity depicted by expression of TOP2A (blue) versus RAG1 (red).

(C) Cells with rearranged TCRα (purple), TCRβ (blue), or fully rearranged TCRαβ (green).

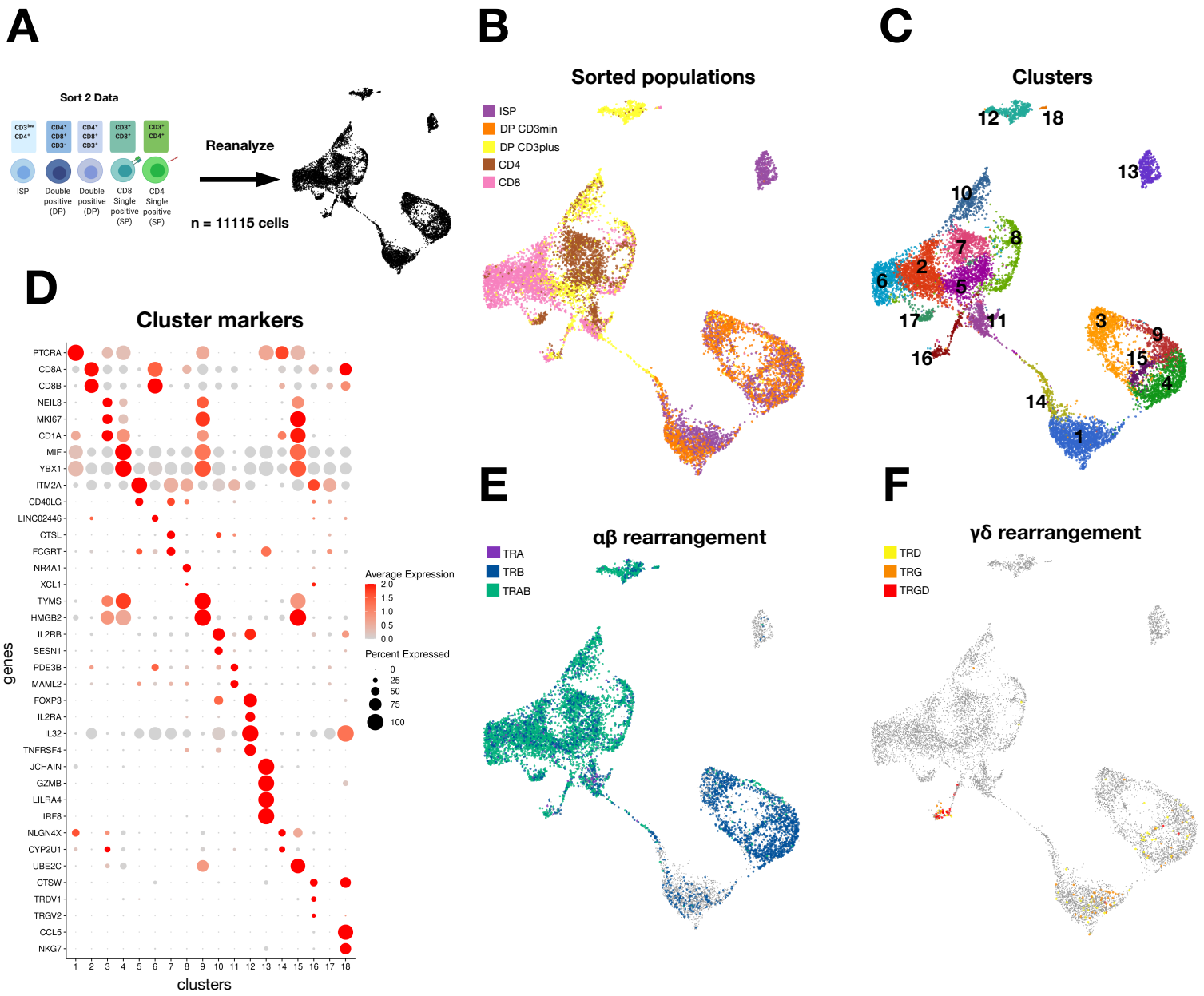
(D) Cells with rearranged TCRγ (orange), TCRδ (yellow), or fully rearranged TCRγδ (red).

(E) Heatmap of regulon activity as determined by transcription factor activity using SCENIC, within each cluster.

(F) Projection of expression of two selected genes (red and blue) per UMAP. Yellow indicates both genes are expressed.

(G) Selected gene expression of factors related to B cell and myeloid/pDC differentiation, projected on a partial UMAP with the most immature subclusters.

Supplementary Figure 4



Suppl. Fig. S4, related to Fig. 3. Reanalysis of more mature thymocytes.

(A) Reanalysis of ISP, DP, and SP cells.

(B) UMAP projection of ISP (purple), DP (orange and yellow), CD4 SP (brown), and CD8 SP (pink) cells.

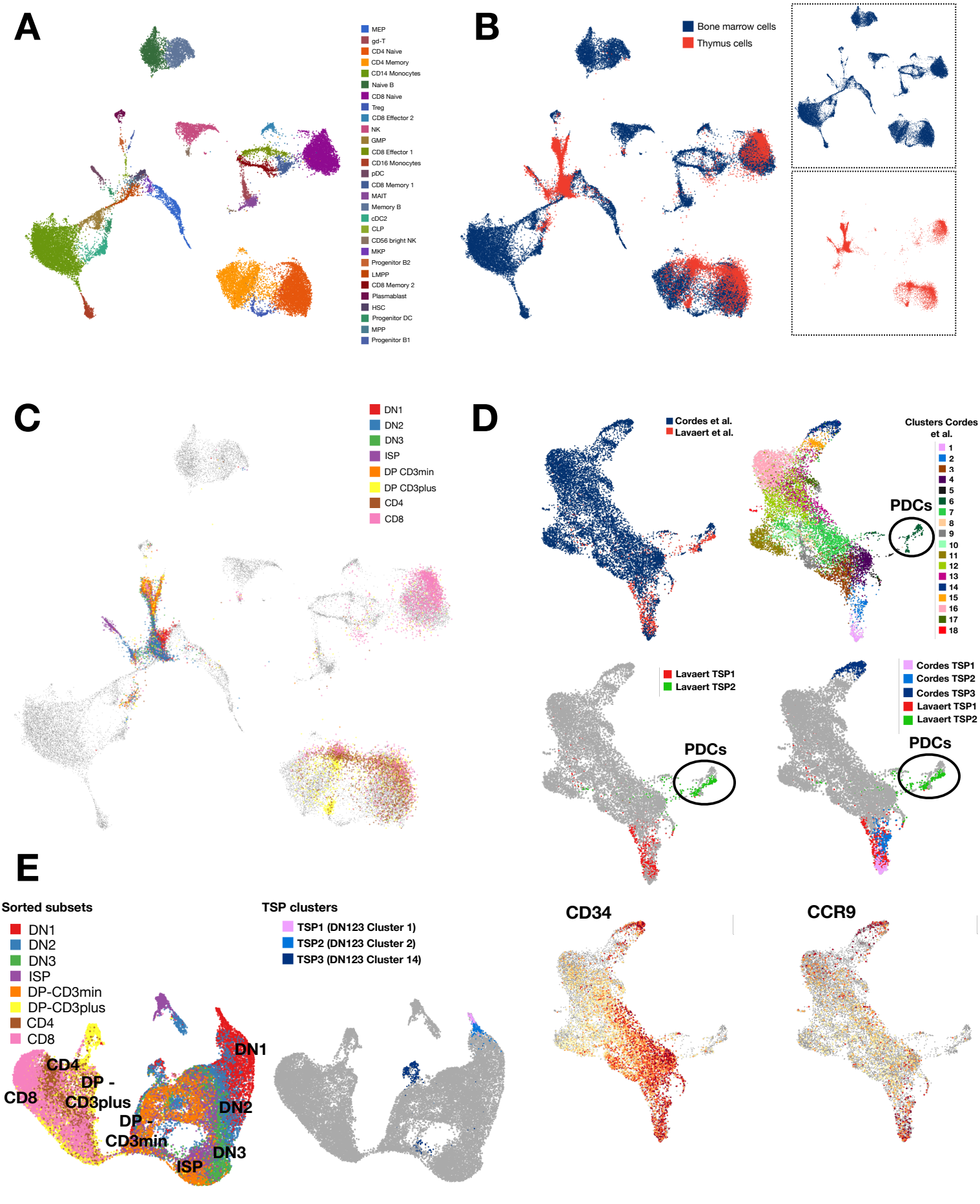
(C) UMAP with 18 clusters identified by cluster analysis.

(D) Dot plot depicting the genes that identify clusters based on differential gene expression.

(E) Cells with rearranged TCRα (purple), TCRβ (blue), or fully rearranged TCRαβ (green).

(F) Cells with rearranged TCRγ (orange), TCRδ (yellow), or fully rearranged TCRγδ (red).

Supplementary Figure 5



Suppl. Fig. S5, related to Fig. 4. and Fig. 5.

(A) Annotated multimodal BM reference (*46*)

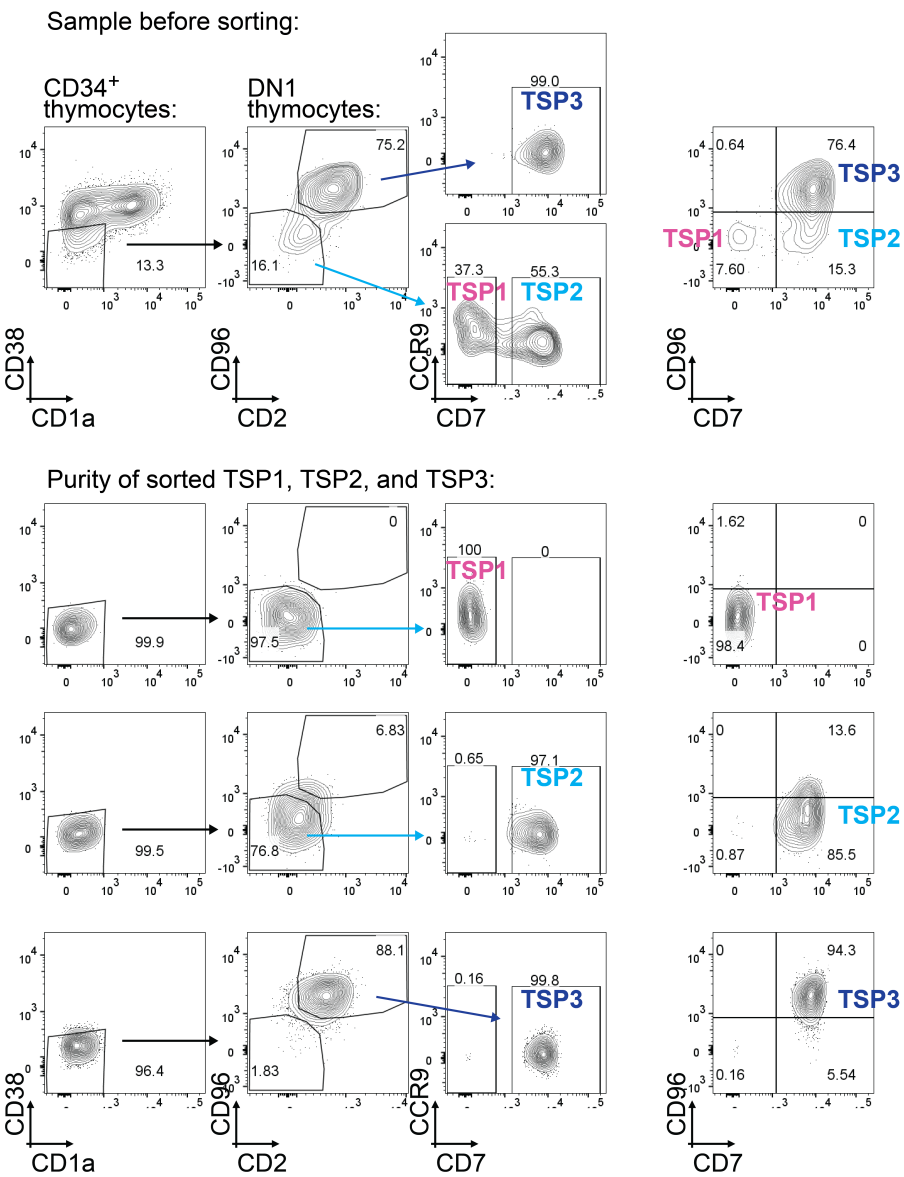
(B) Total thymocytes (red) mapped onto BM reference (blue).

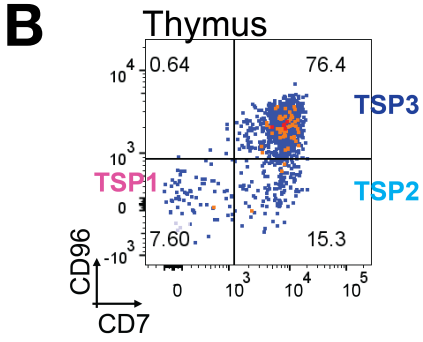
(C) Mapped thymocytes identified by the eight flow-sorted subsets.

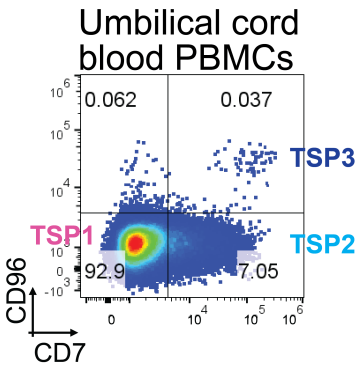
(D) Integration of TSP populations from Lavaert et. al. with DN123 data.

(E) Positioning of TSP 1, 2 and 3 in UMAP from complete dataset (Figure 2).

Supplementary Figure 6





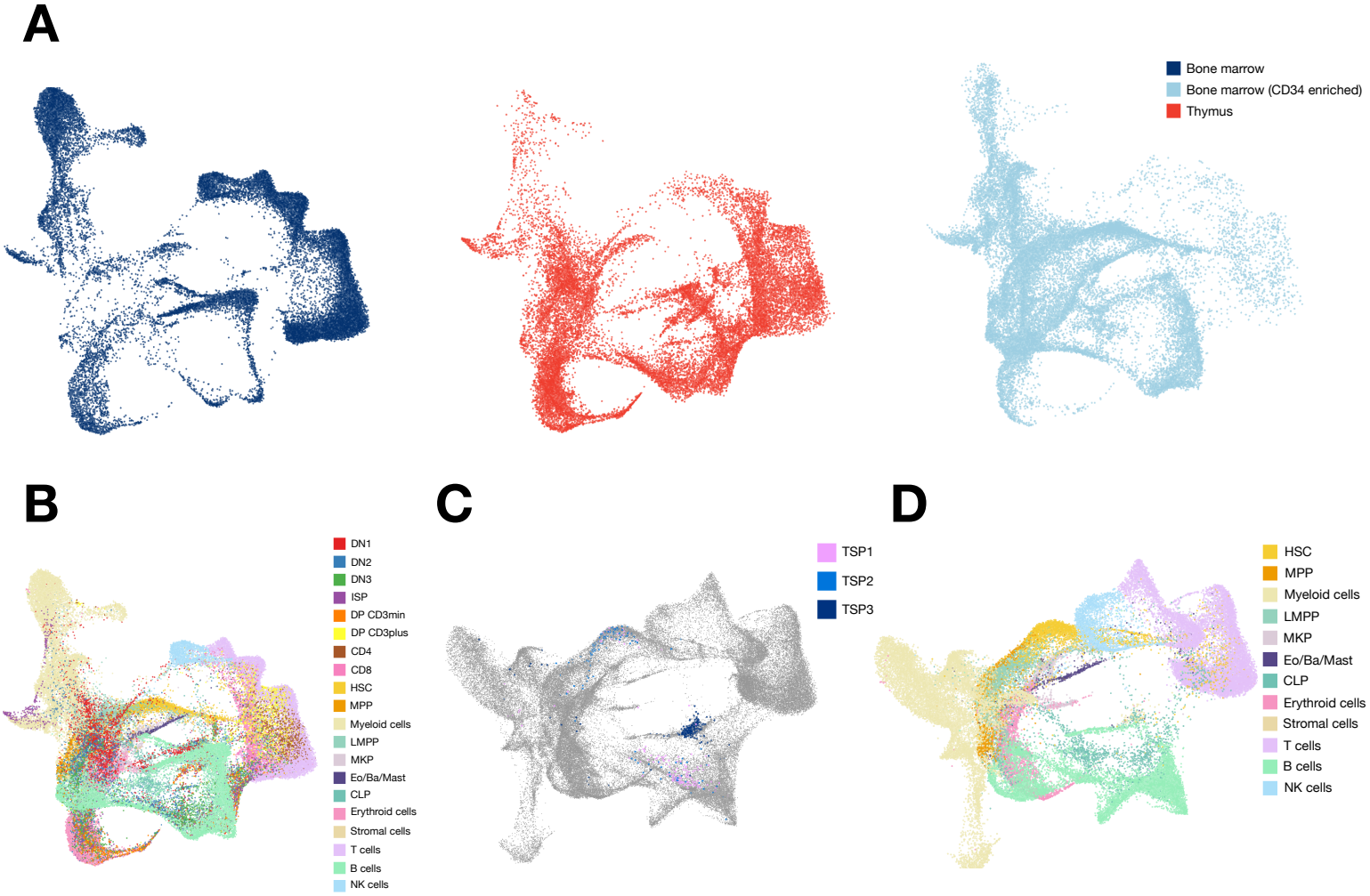


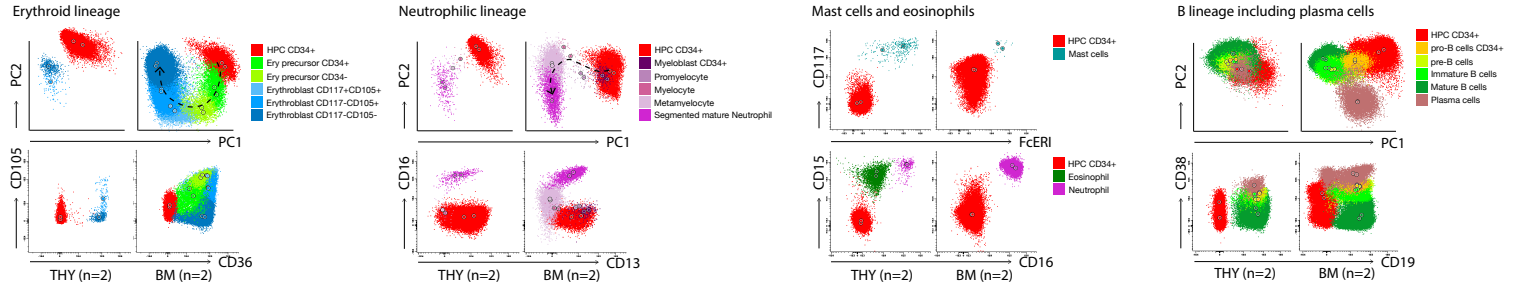
Suppl. Fig. S6, related to Fig. 5C-E.

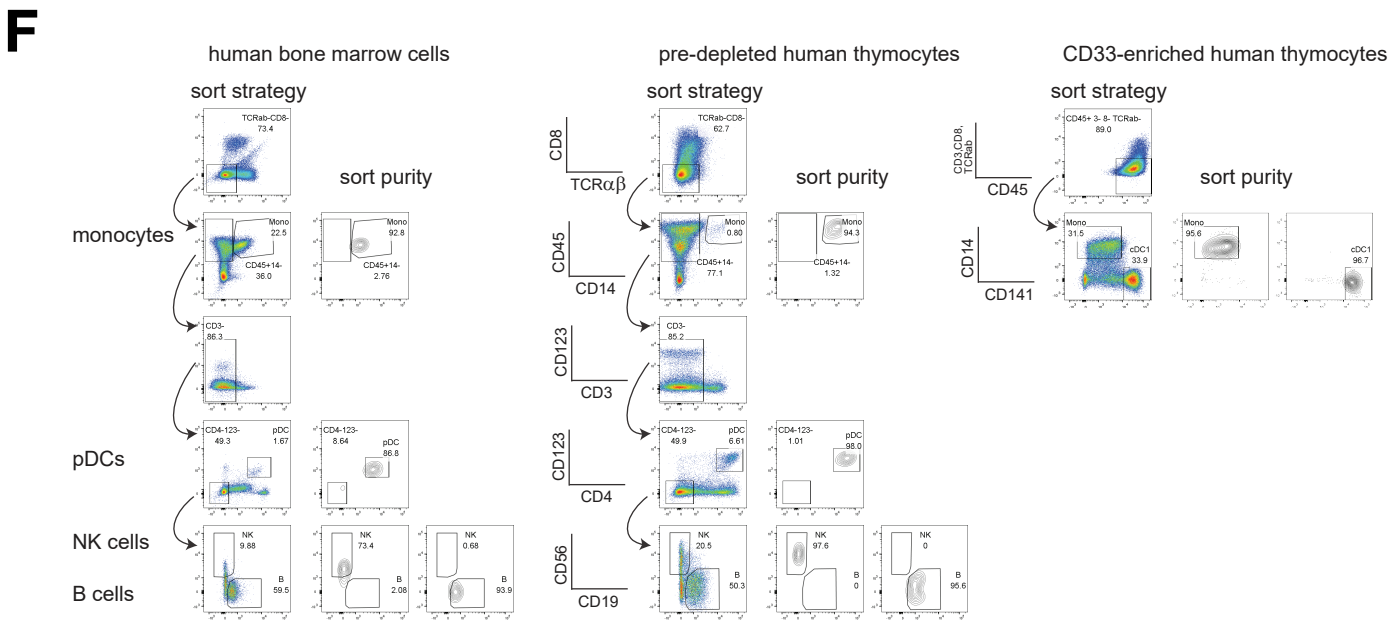
(A) Flow-sorting strategy and purity of sorted TSP1, TSP2, and TSP3. Prior to sorting, thymocytes were pre-enriched for CD34.

(B) TSP1, TSP2, and TSP3 in human thymocytes (top) and umbilical cord blood (bottom).

Supplementary Figure 7







Suppl. Fig. S7, related to Fig. 6. Intrathymic development of alternative lineages.
(A) UMAP projection of each individual dataset after integration of mature BM cells (*51*) with $CD34^+$ BM (*52*) and our complete thymus dataset (red).
(B) UMAP projection of the complete integration of BM and thymus, with annotated BM labels and sorted thymocyte populations.
(C) UMAP projection (turned 90 degrees in relation to UMAP in B with Bioturing BBrowser (*53*)to demonstrate positioning of TSP1,2,3.
(D) BM annotation of cells in the 90-degrees turned UMAP projection.
(E) Principal component analyses and example flow plots of human thymocytes using 28-color spectral flow cytometry to indicate mature non-T cell types (erythoid cells, neutrophils cells, mast cells, eosinophils, and plasma B cells) and their progenitors, where applicable.
(F) Sorting strategies and population purities of monocytes, NK cells, B cells, pDCs, and cDC1s from human bone marrow and thymus. Prior to sorting, thymocytes were pre-depleted from the majority of T cells using CD8 and TCRαβ (middle panel), or pre-enriched for myeloid cells using CD33 (right panel).

SUPPLEMENTARY MOVIES

Movie S1
Complete Thymus 3D UMAP (Belongs to Figure 2): https://youtu.be/0Pk5bM313h4

Movie S2
Integration of Bone marrow CD34+ cells and mature Bone marrow cells (belongs to Figure 6A): https://youtu.be/gdvxN67na0o

Movie S3
T cell development can be followed also when integrated with BM datasets (belongs to Figure 6C & S7): https://youtu.be/gs5v1LelsO0

Movie S4
B cell development in integrated Bone Marrow datasets (belongs to Figure 6E and D): https://youtu.be/w0qSVO-cp9M

Movie S5
DN1 cells differentiating into Myeloid cells, NK cells and B cells (belongs to Figure 6D and E): https://youtu.be/fb14x9Zm6ko

Movie S6
DN2 and ISP cells differentiating into PDCs (belongs to Figure 6D and E): https://youtu.be/Edv5zE6zVYk