Supplementary information

Postnatal lymph node expansion of stromal progenitors towards reticular and CD34$^+$ stromal cell subsets is determined by distinct transcriptional programs

Joern Pezoldt[1,2], Carolin Wiechers[1], Maria Litovchenko[2], Marjan Biocanin[2], Mangge Zou[1], Katarzyna Sitnik[3], Michael Beckstette[1,4], Wanze Chen[2], Vincent Gardeux[2], Stefan Floess[1], Maria Ebel[1], Julie Russeil[2], Panagiota Arampatzi[5], Ehsan Vafardanejad[6], Antoine-Emmanuel Saliba[6], Bart Deplancke[2,#], Jochen Huehn[1,7*,#]

[1]Department Experimental Immunology, Helmholtz Centre for Infection Research, Braunschweig, Germany

[2]Laboratory of Systems Biology and Genetics, École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland

[3]Department of Vaccinology, Helmholtz Centre for Infection Research, 38124 Braunschweig, Germany

[4]Department of Computational Biology for Individualised Medicine, Centre for Individualised Infection Medicine, Helmholtz Centre for Infection Research and Hannover Medical School, Hannover, Germany

[5]Core Unit Systems Medicine, University of Wuerzburg, 97080 Wuerzburg, Germany

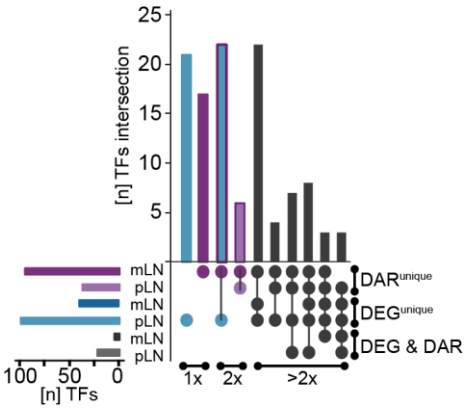
[6]Helmholtz Institute for RNA-based Infection Research, 97080 Wuerzburg, Germany

[7]Cluster of Excellence RESIST (EXC 2155), Hannover Medical School, Hannover, Germany

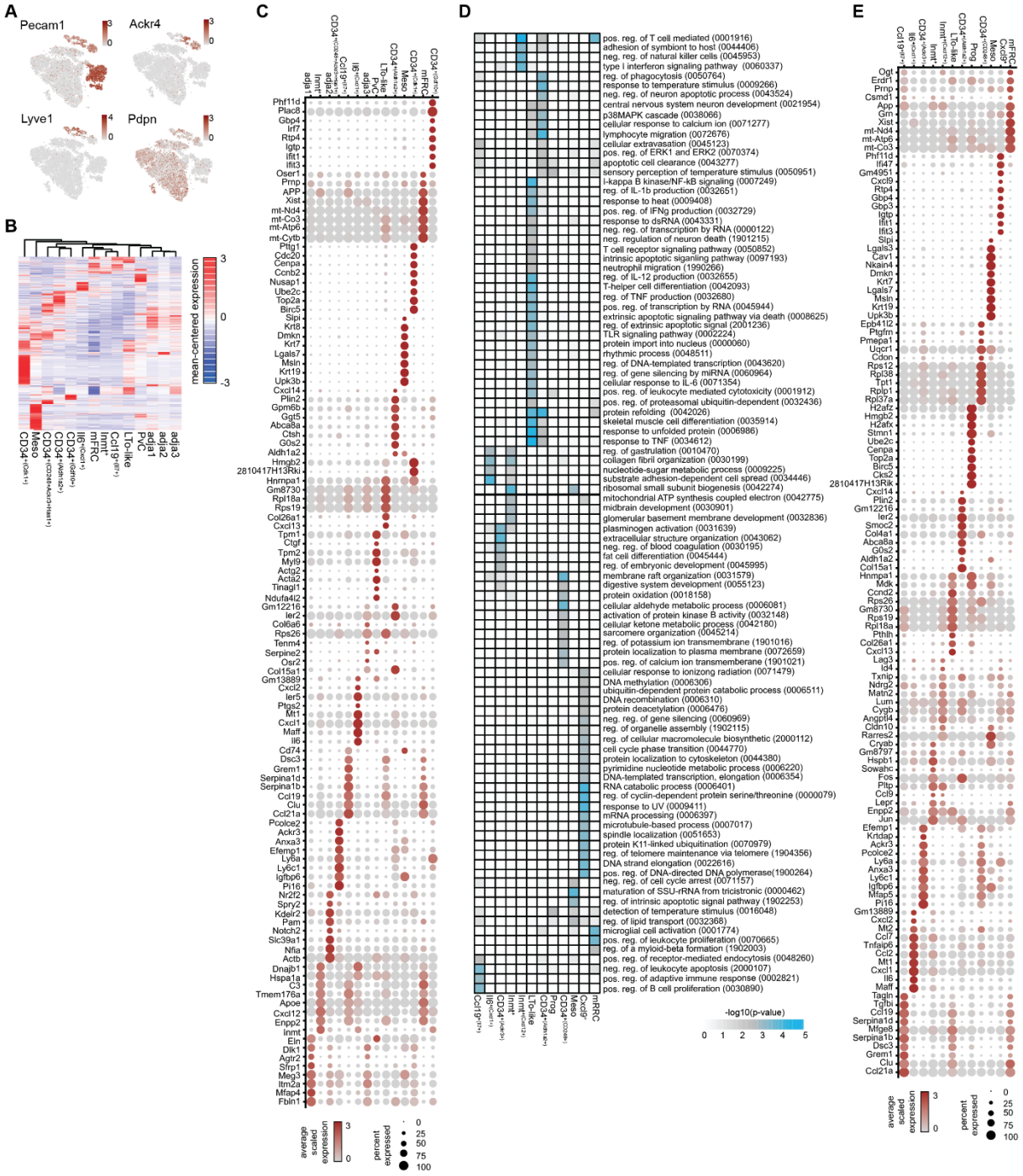
[#]Equally contributing senior authors

*Corresponding author:

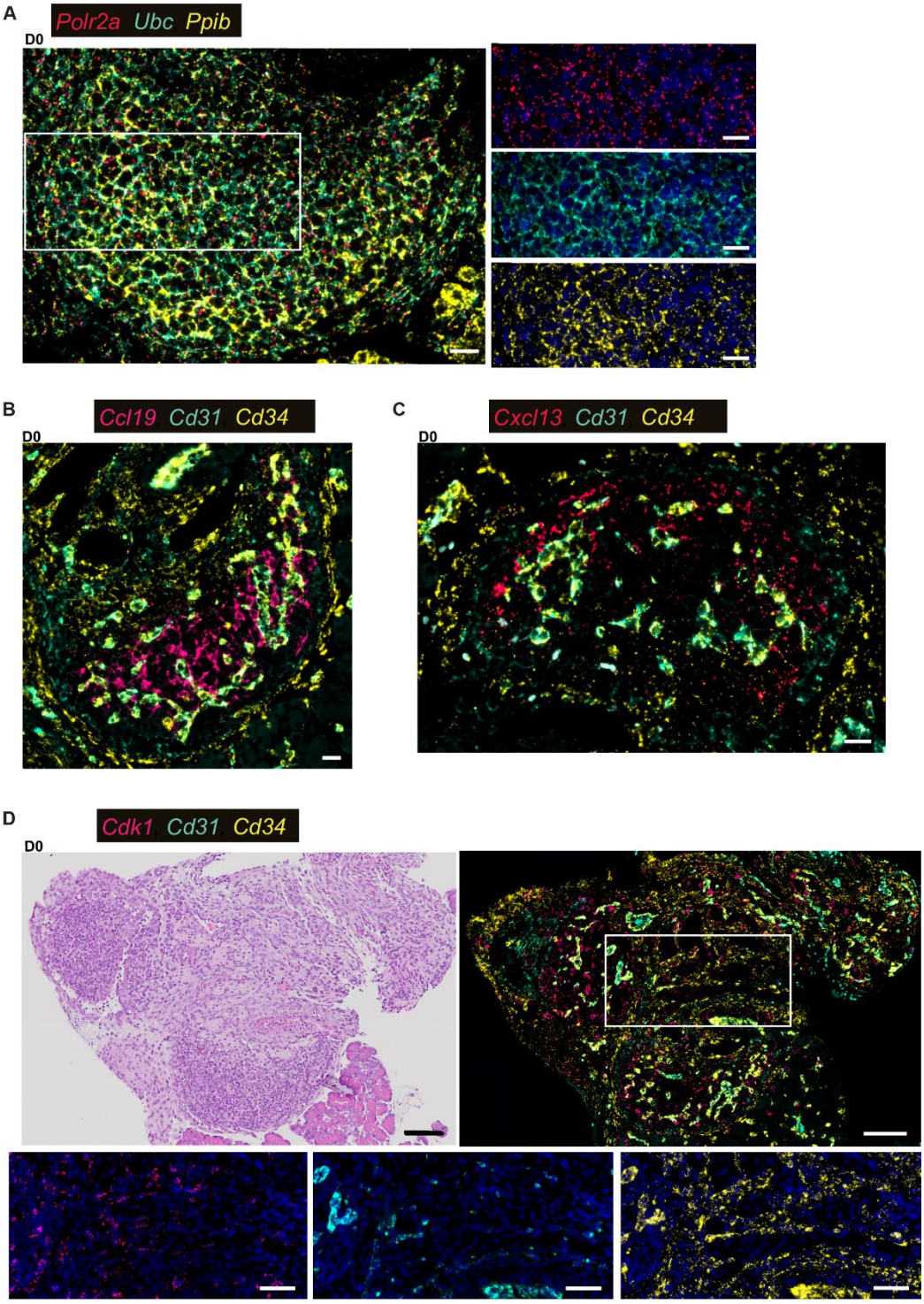
Jochen Huehn (Jochen.Huehn@helmholtz-hzi.de)



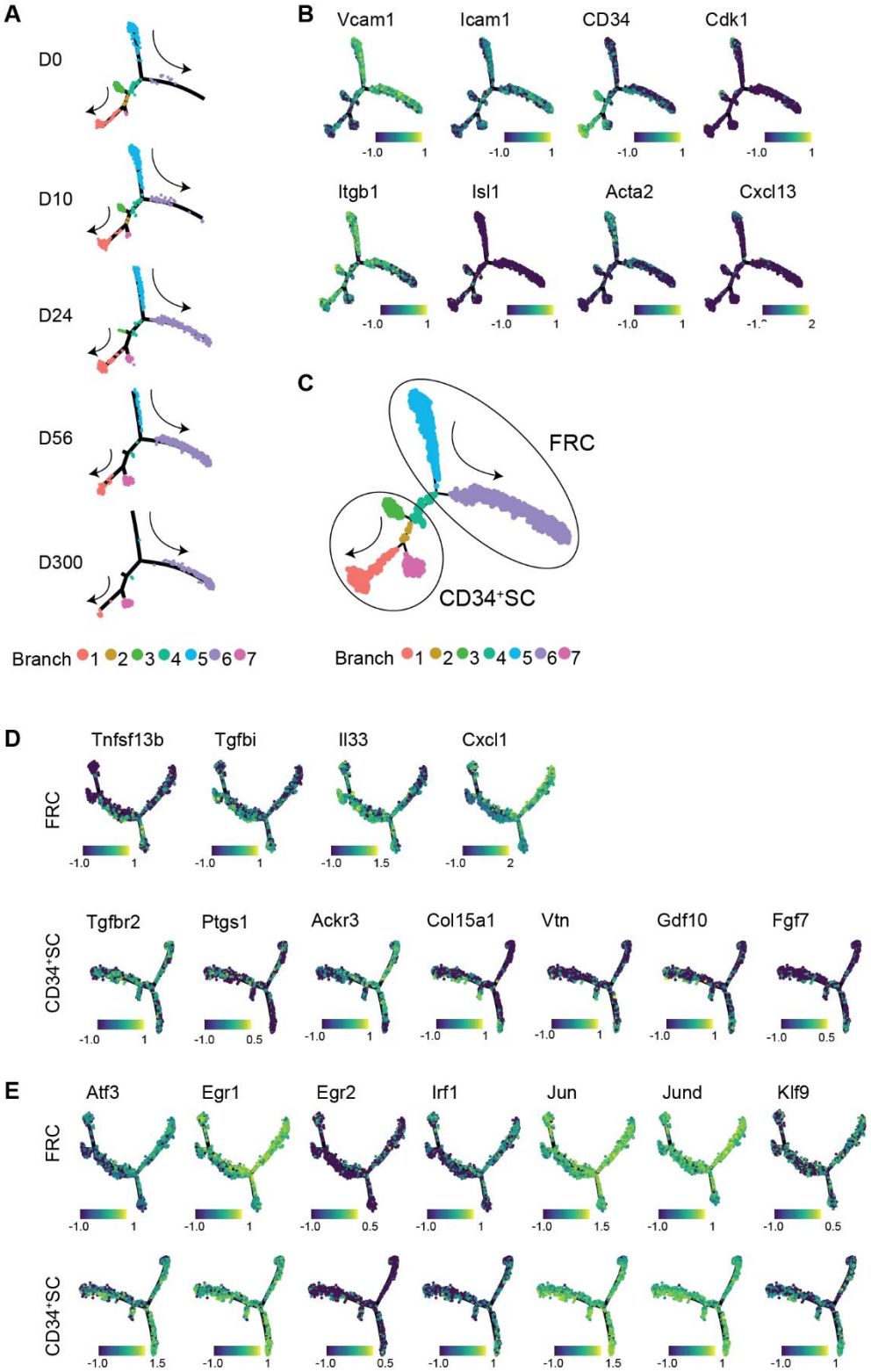
Supplementary Figure 1. Overlap of TFs identified from the epigenomic landscape and transcribed genes. *Related to Figure 2.* CD45$^-$CD24$^-$CD31$^-$Pdpn$^+$ SCs were isolated from mLNs and pLNs of SPF mice. Subsequently, RNA-seq or ATAC-seq analyses were performed. DEGs and DARs were identified in mLN vs. pLN pairwise comparisons. Matrix layout for all intersections of putative TFs identified (bar graph left). Circles in the matrix indicate sets that are part of the intersection and the size of the intersection (all intersections with >= 2 are depicted) is indicated in the top bar graph. ATAC-seq, assay for transposase accessible chromatin sequencing; DAR, differentially accessible region; DEG, differentially expressed gene; mLN, mesenteric lymph node; pLN, skin-draining lymph node; SPF, specific pathogen-free; SC, stromal cell; TF, transcription factor.



Supplementary Figure 2: The LNSC compartment can be dissected into distinct cellular subsets using scRNA-seq. *Related to Figure 3.* CD45$^-$CD24$^-$ cells were isolated from mLNs of day 0, 10, 24, 56 and 300 old SPF-housed mice and subjected to scRNA-seq. **(A)** t-SNE plot of merged 15,925 endothelial and non-endothelial mLN SCs. Expression of indicated genes is superimposed on the t-SNE map. **(B)** Heatmap of the top 40 DEGs across all non-endothelial SC subsets including PvC and adjacent tissue cell subsets (adja). **(C)** Expression dot plot of top 8 DEGs, which were selected by foldchange, per subset comparing all remaining non-LEC and non-BEC mLN SCs. **(D)** GO analysis of biological processes was performed on DEGs per non-endothelial SC subsets. **(E)** Expression dotplot of top 10 DEGs, which were selected by foldchange, per subset comparing all non-endothelial SC subsets. DEG, differentially expressed gene; GO, gene ontology; Meso, mesothelial; mLN, mesenteric lymph node; Prog, progenitor; PvC, perivascular cells; SC, stromal cell; SPF, specific pathogen-free.



Supplementary Figure 3: Expression of key marker genes in neonatal LN and adjacent tissues. *Related to Figure 3.* Sections (3 µm) of early postnatal mLN were stained with the indicated RNAscope probes and imaged by fluorescence microscopy. Nuclei were counter-stained with DAPI (blue). "White squares" indicate regions of interest. Representative tissue sections = 2-3. **(A)** Positive control probes for the genes *Polr2a*, *Ppib* and *Ubc* (scale bars = 20 µm). **(B)** Overview of mLN anlagen for RNA-probes specific for *Ccl19, Cd31* and *Cd34* (scale bar = 20 µm). **(C)** Overview of mLN anlagen for RNA-probes specific for *Cxcl13, Cd31* and *Cd34* (scale bar = 20 µm). **(D)** ***Upper left*** Haematoxylin-Eosin (HE) overview staining of D0 mLN embedded in surrounding tissue (scale bar = 100 µm). ***Upper right*** Overview of mLN anlagen for RNA-probes specific to *Cdk1, Cd31* and *Cd34* (scale bar = 100 µm). ***Lower*** Zoom-ins for RNA-probes specific to *Cdk1, Cd31* and *Cd34* (scale bar = 50 µm). HE, Haematoxylin-Eosin; mLN, mesenteric lymph node.



Supplementary Fig. 4. Bifurcation of LNSC development. *Related to Figure 4.* CD45$^-$CD24$^-$ cells were isolated from mLNs of day 0, 10, 24, 56 and 300 old SPF-housed mice and subjected to scRNA-seq. Target SCs were identified as non-LECs, non-BECs and non-PvCs. **(A)** Pseudotemporal trajectory of non-endothelial SCs plotted for the indicated age. Arrows indicate increasing pseudotime. **(B)** Gene expression on the pseudotime trajectory. **(C)** Applied electronic gating for re-embedding CD34$^+$ SCs and FRCs. **(D)** Expression of selected DEGs along FRC and CD34$^+$ SC. **(E)** Expression of common TFs for both FRCs and CD34$^+$ SCs. DEGs, differentially expressed genes; FRC, reticular fibroblastic stromal cell; PvC, perivascular cell; SC, stromal cell; SPF, specific pathogen-free; TF, transcription factor.