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Supplemental information

Megakaryocyte production is sustained by direct differentiation from erythromyeloid progenitors in the yolk sac until midgestation

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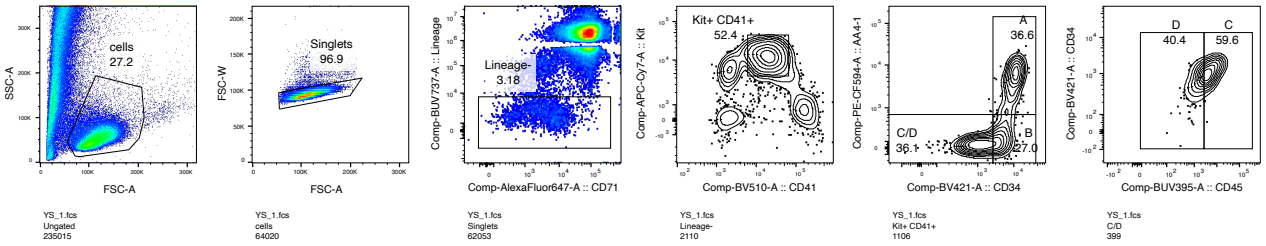
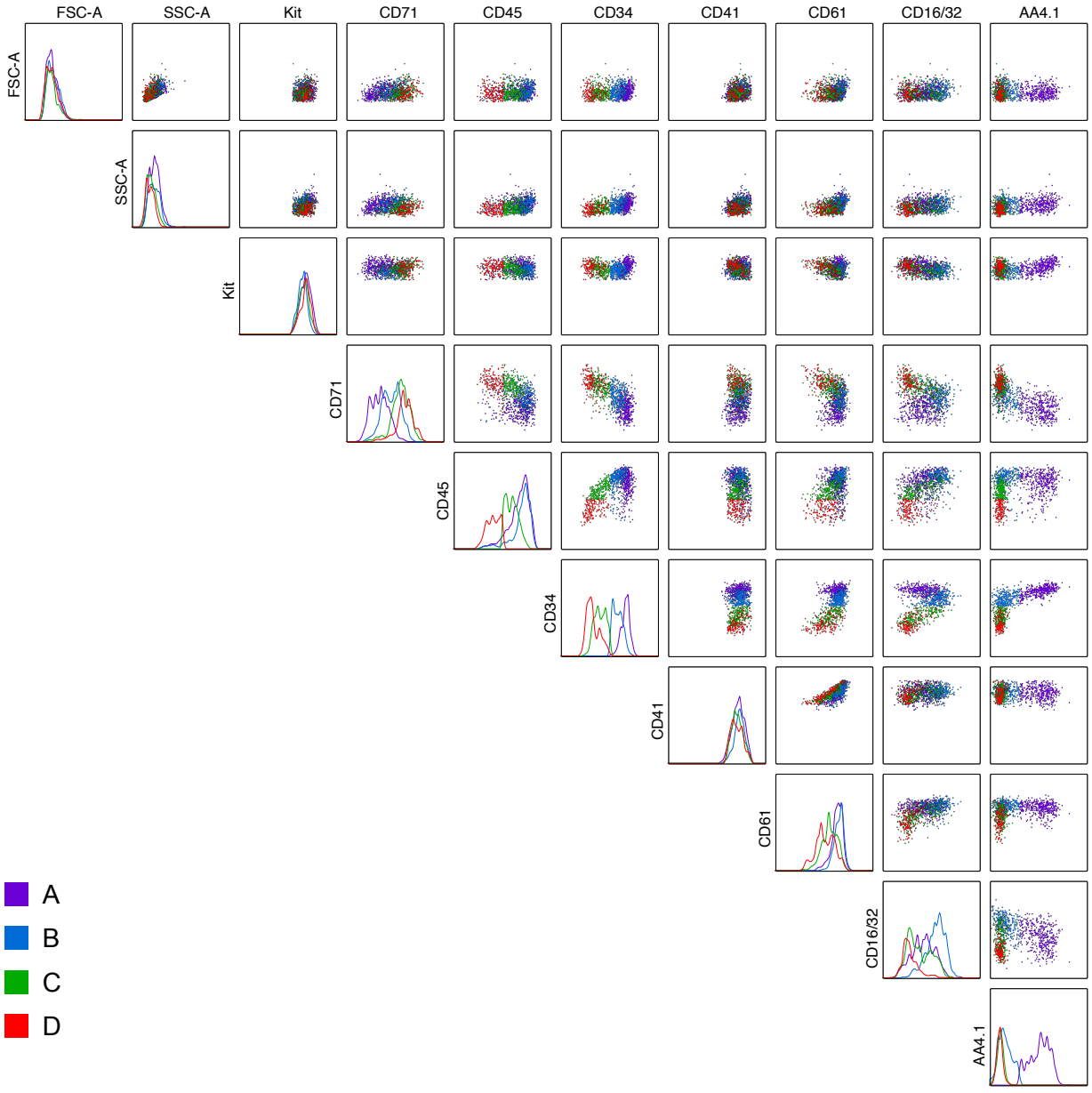
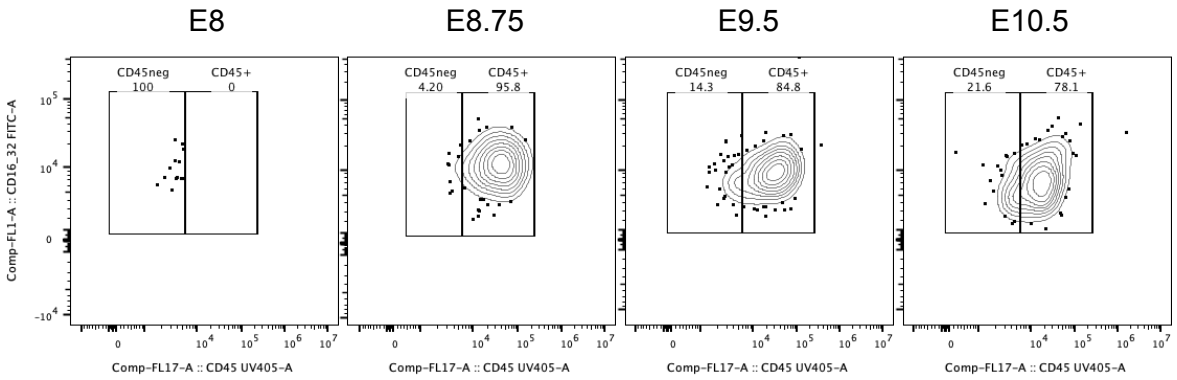
A Gating strategy for EMPs in the yolk sac



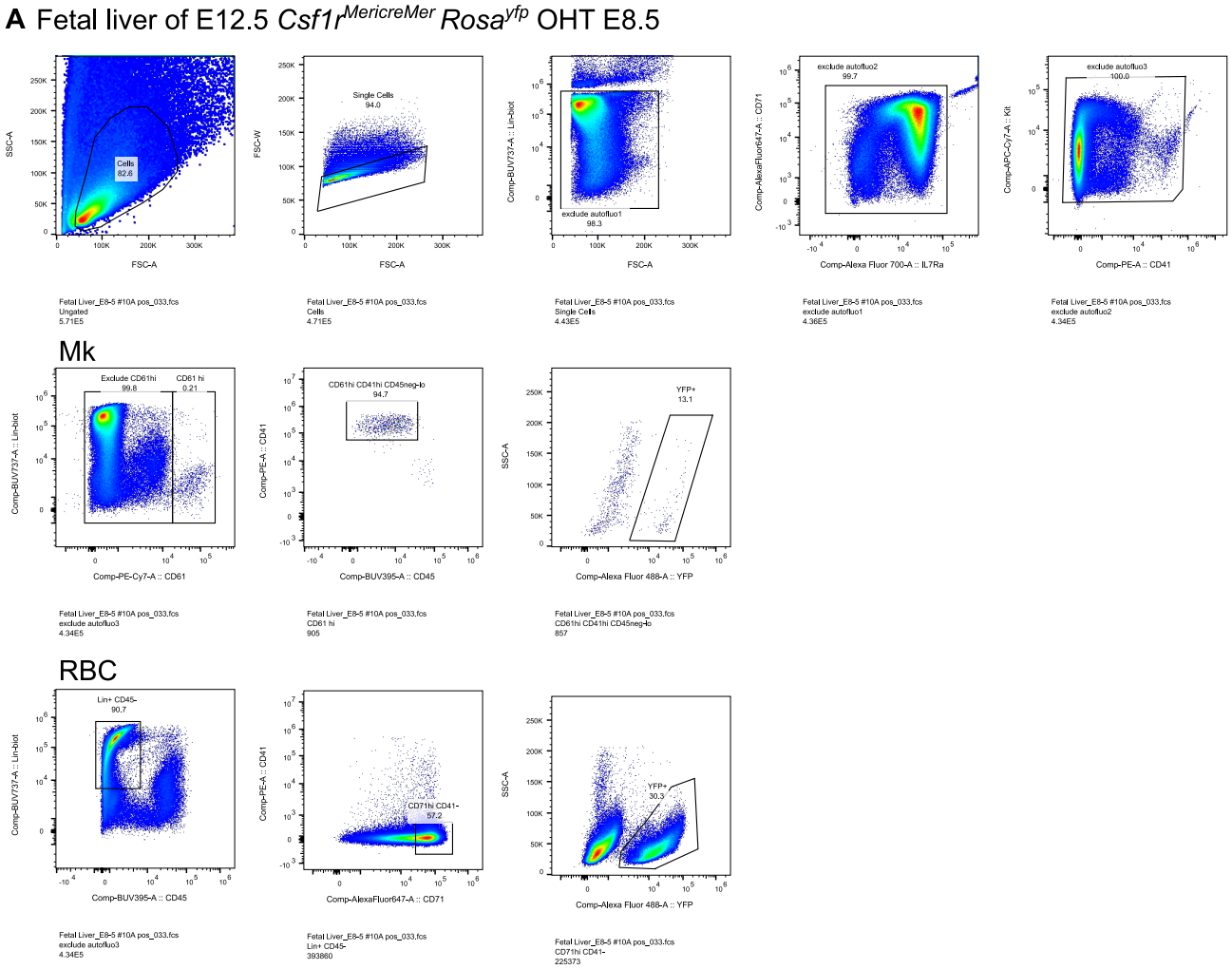
B Phenotype of Kit+ CD41+ at E10.5



C Expression of CD45 in A



Supplementary Figure 1. (Related to Figure 1). (A) Gating strategy for EMP subsets in the E10.5 yolk sac. Cells were gated based on size (FSC-A) and scatter (SSC-A). After doublet exclusion, lineage negative cells were selected and EMPs were gated using expression of Kit and CD41. Among Kit^{+} CD41^{+} cells, A was defined as AA4.1^{+} CD34^{+} and B as AA4.1^{neg} CD34^{+}. In the AA4.1^{neg} CD34^{neg} gate (DN), C and D were identified based on CD45 expression. (B) Immunophenotype of E10.5 yolk sac Kit^{+} CD41^{+} cells with the markers used for the t-SNE analysis. Histogram of individual markers and dot plots with overlay of A (purple), B (blue), C (green) and D (red) populations. (C) Expression of CD45 on A (Kit^{+} CD41^{+} AA4.1^{+} CD34^{+}) cells in the yolk sac over time, from E8 to E10.5.

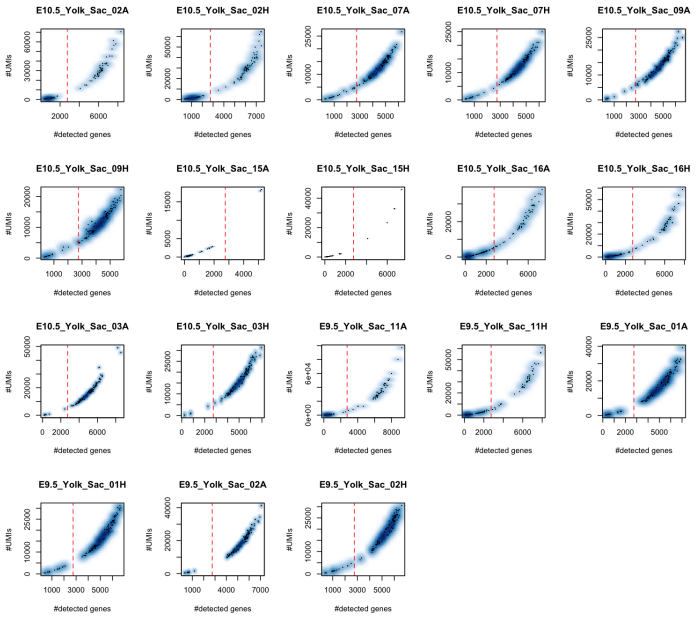


Supplementary Figure 2. (Related to Figure 2). (A) Gating strategy for Megakaryocytes (Mks) and red blood cells (RBC) in the fetal liver of E12.5 *Csf1r*^{MericreMer} *Rosa*^{yfp} embryos pulsed at E8.5. Cells were gated based on size (FSC-A) and scatter (SSC-A). After exclusion of doublets and autofluorescent debris, CD61^{high} cells were selected and Mks were gated using expression of CD45 and CD41. For RBC, CD71^{high} CD41^{neg} cells were gated among Lineage^{+} CD45^{neg} cells. YFP labelling was then analyzed in Mks (CD61^{high} CD41^{+} CD45^{neg}) and RBC (Lineage^{+} CD71^{high} CD45^{neg} CD41^{neg}).

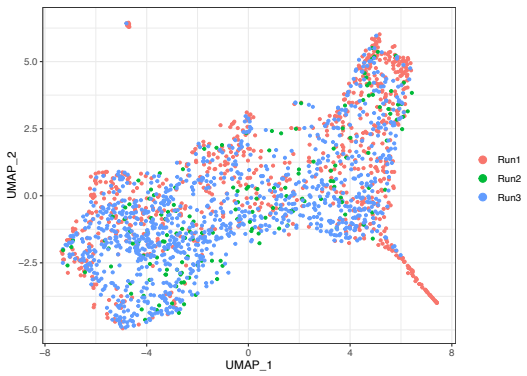
A

Sequencing run	Stage	Plate	Halfplate	Cells after QC
1	E10.5	E10.5_02	A	44
			H	58
	E10.5	E10.5_07	A	170
			H	175
	E10.5	E10.5_09	A	179
			H	168
2	E9.5	E10.5_11	A	46
			H	38
	E10.5	E10.5_15	A	discarded
			H	discarded
	E10.5	E10.5_16	A	42
			H	38
3	E10.5	E10.5_03	A	180
			H	179
	E9.5	E9.5_01	A	167
			H	179
	E9.5	E9.5_02	A	182
			H	169

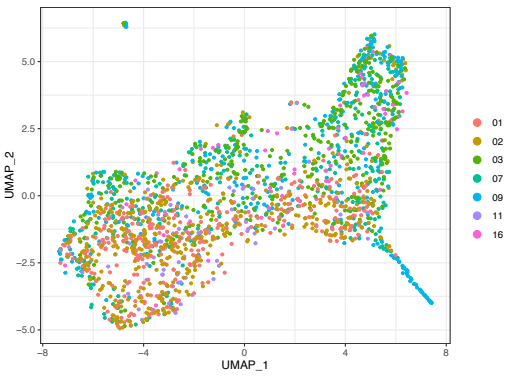
B



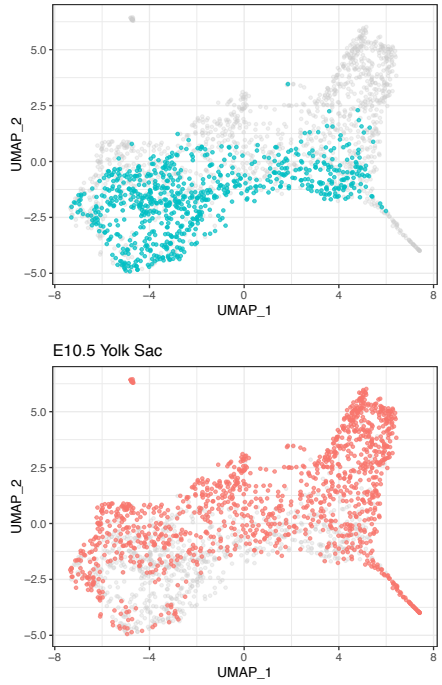
C



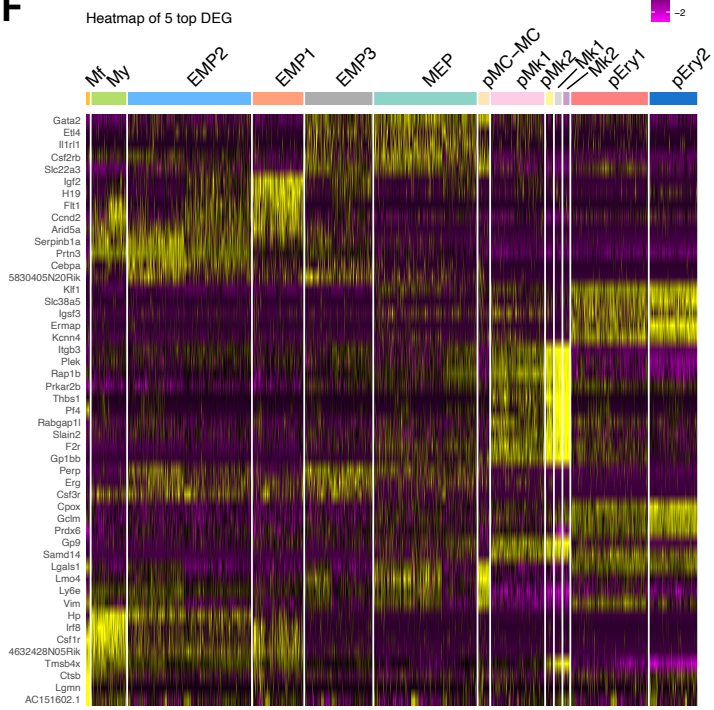
D



E

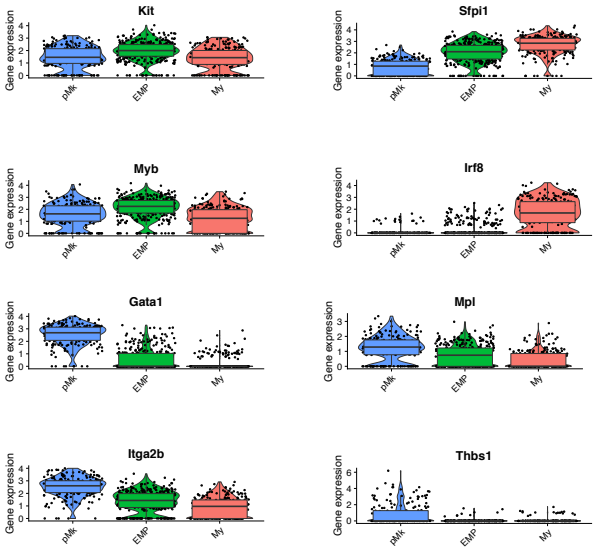


F

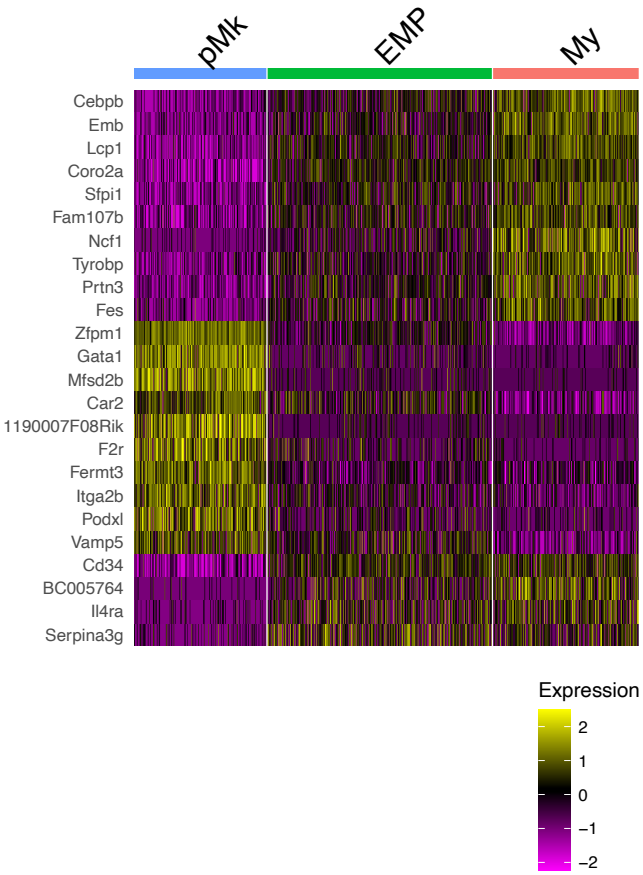


Supplementary Figure 3. (Related to Figure 3). (A) Table summarizing the sequencing runs, sorted 384-well plates, separately processed half-plates and the number of cells that passed the quality control in each half plate. (B) Graphical representation of the number of UMI per number of detected genes in each cell from each processed half-plate and with the threshold at 2750 detected genes for the QC (red dotted line). (C) Representation of the three sequencing runs in the UMAP. (D) Representation of the seven independently sorted plates in the UMAP. (E) Representation of the two developmental time points (E9.5, turquoise, and E10.5, red) in the UMAP. (F) Heatmap representing the top 5 differentially expressed genes (DEG) out of each pairwise cluster comparison among the 13 clusters identified in the scRNA-seq analysis.

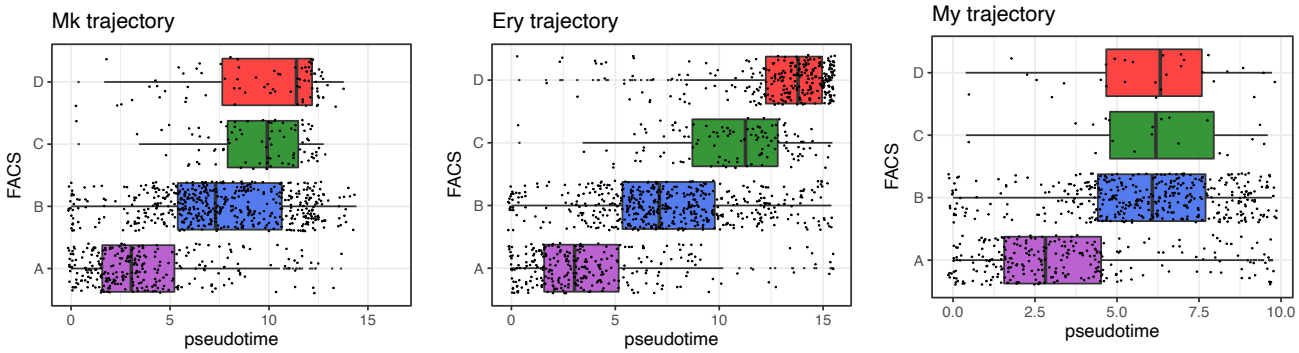
A Expression of lineage genes in E9.5 clusters



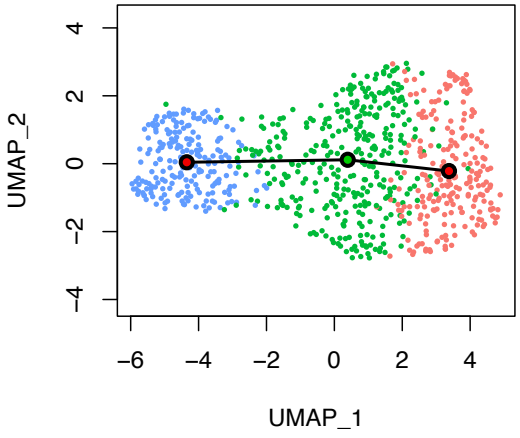
B Top 10 DEG in E9.5 clusters



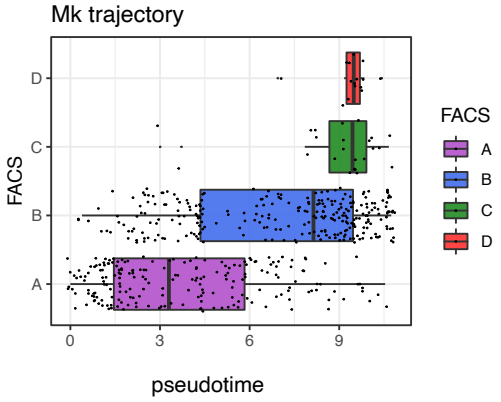
C FACS progenitor annotation along trajectories



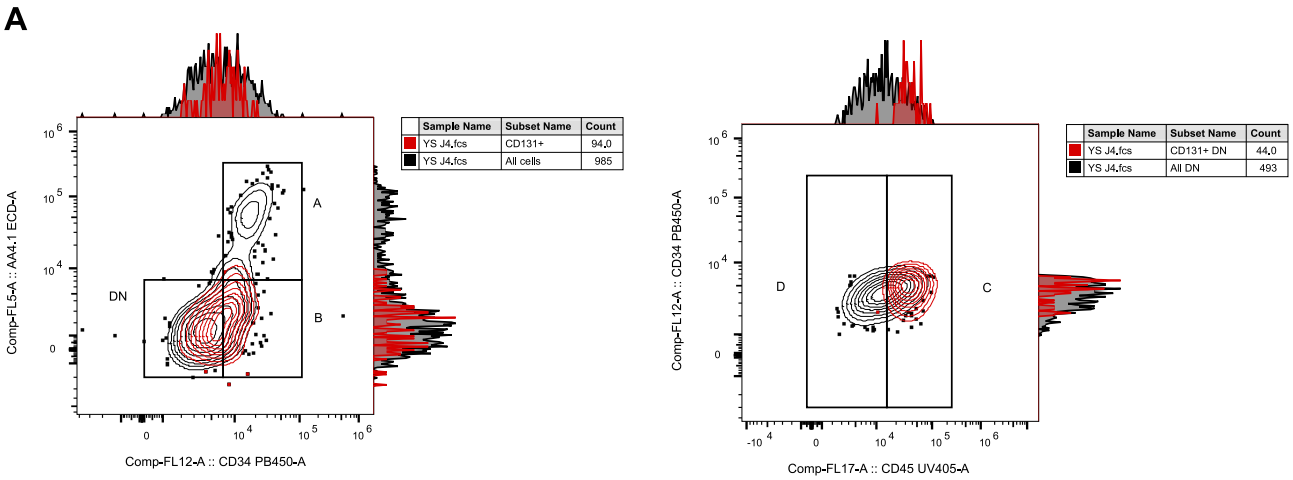
D Slingshot parameters in E9.5 dataset



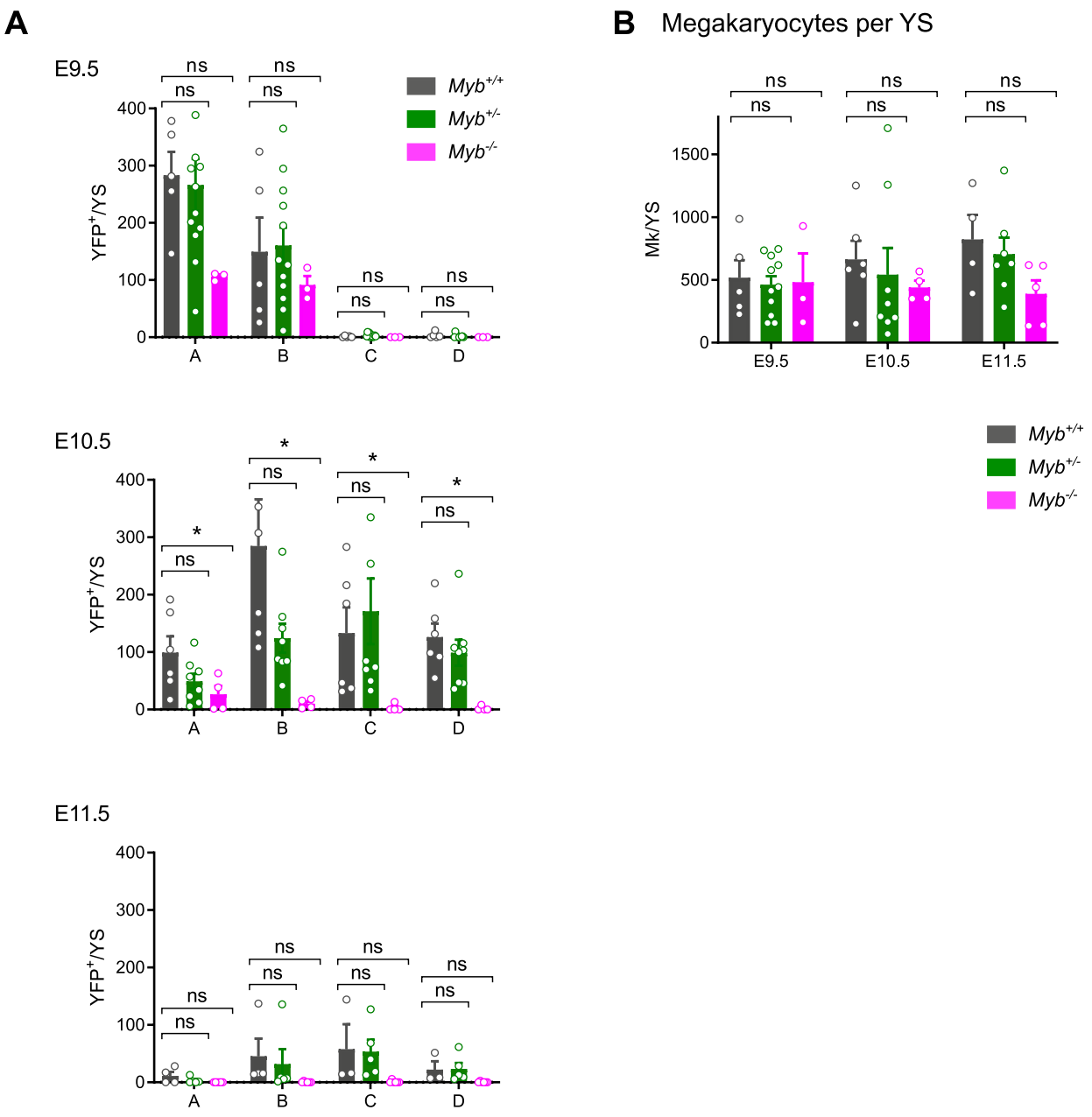
E FACS progenitor annotation in E9.5 dataset



Supplementary Figure 4. (Related to Figure 4). (A) Violin plot representing expression of key transcription factors (*Kit*, *Myb*, *Gata1*, *Spf1* (Pu.1), *Irf8*) and Mk-associated genes (*Itg2b*, *Mpl*, *Thbs1*) by the 3 hematopoietic clusters at E9.5. (B) Heatmap representing the 10 top differentially expressed genes (DEG) among the 3 clusters identified at E9.5. pMk, Mk progenitor; My, Myeloid progenitor. (C) Representation of the progenitor phenotype (A, purple; B, blue; C, green; D, red) of the cells belonging to each of the three trajectories along the pseudotime value. (D) UMAP representation of YS E9.5 *Csf1r*^{icre} *Rosa*^{yfp} YFP^{+} Kit^{+} cells and the three clusters used for trajectory analysis with Slingshot. (E) Representation of the progenitor phenotype (A, purple; B, blue; C, green; D, red) of the cells belonging to the megakaryocyte trajectory in E9.5 dataset alone along the pseudotime value.



Supplementary Figure 5. (Related to Figure 5). (A) Phenotype of CD131^{+} cells in the E10.5 yolk sac in regards to progenitor subsets A-D. CD131^{+} cells (red) are overlaid on top of all CD41^{+} Kit^{+} progenitors.



Supplementary Figure 6. (Related to Figure 6). (A) Number of YFP$^+$ cells per yolk sac in each EMP subset (A-B-C-D) in *Myb*$^{+/+}$, *Myb*$^{+/-}$ and *Myb*$^{-/-}$ *Csf1r*^{MericreMer} *Rosa*^{yfp} embryos pulsed at E8.5 and analysed at E9.5, E10.5 and E11.5. Results of 3 independent litters. Bars represent mean± sem; ns, not significant; *, p<0.1. (B) Number of Mk cells per yolk sac in *Myb*$^{+/+}$, *Myb*$^{+/-}$ and *Myb*$^{-/-}$ at E9.5, E10.5 and E11.5. Bars represent mean± sem.