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Supplementary information

Modelling post-implantation human development to yolk sac blood emergence

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Supplementary Information
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Modelling Post-Implantation Human Embryogenesis and Early Hematopoiesis via Engineering a Multilineage Yolk Sac Tissue Niche

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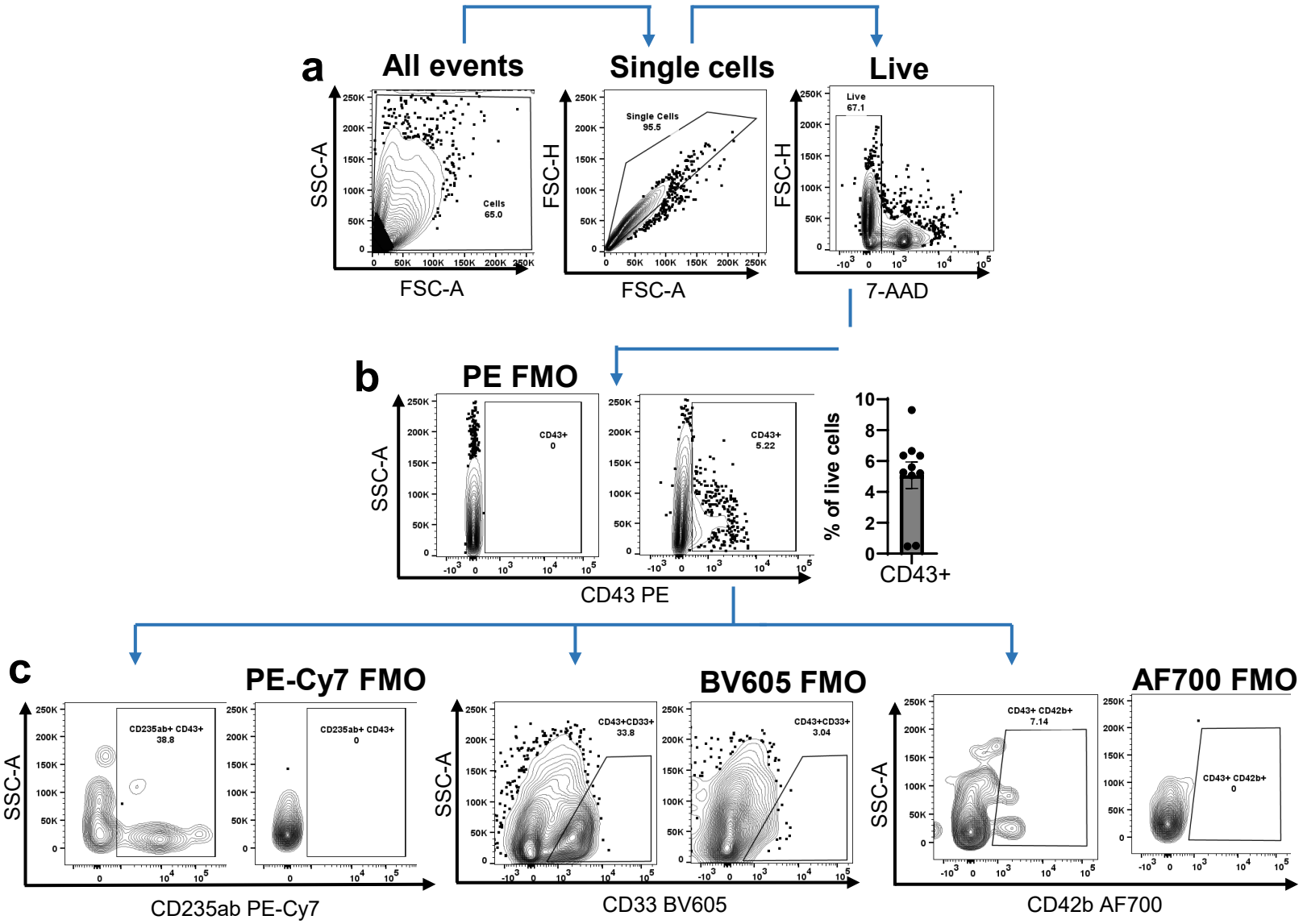
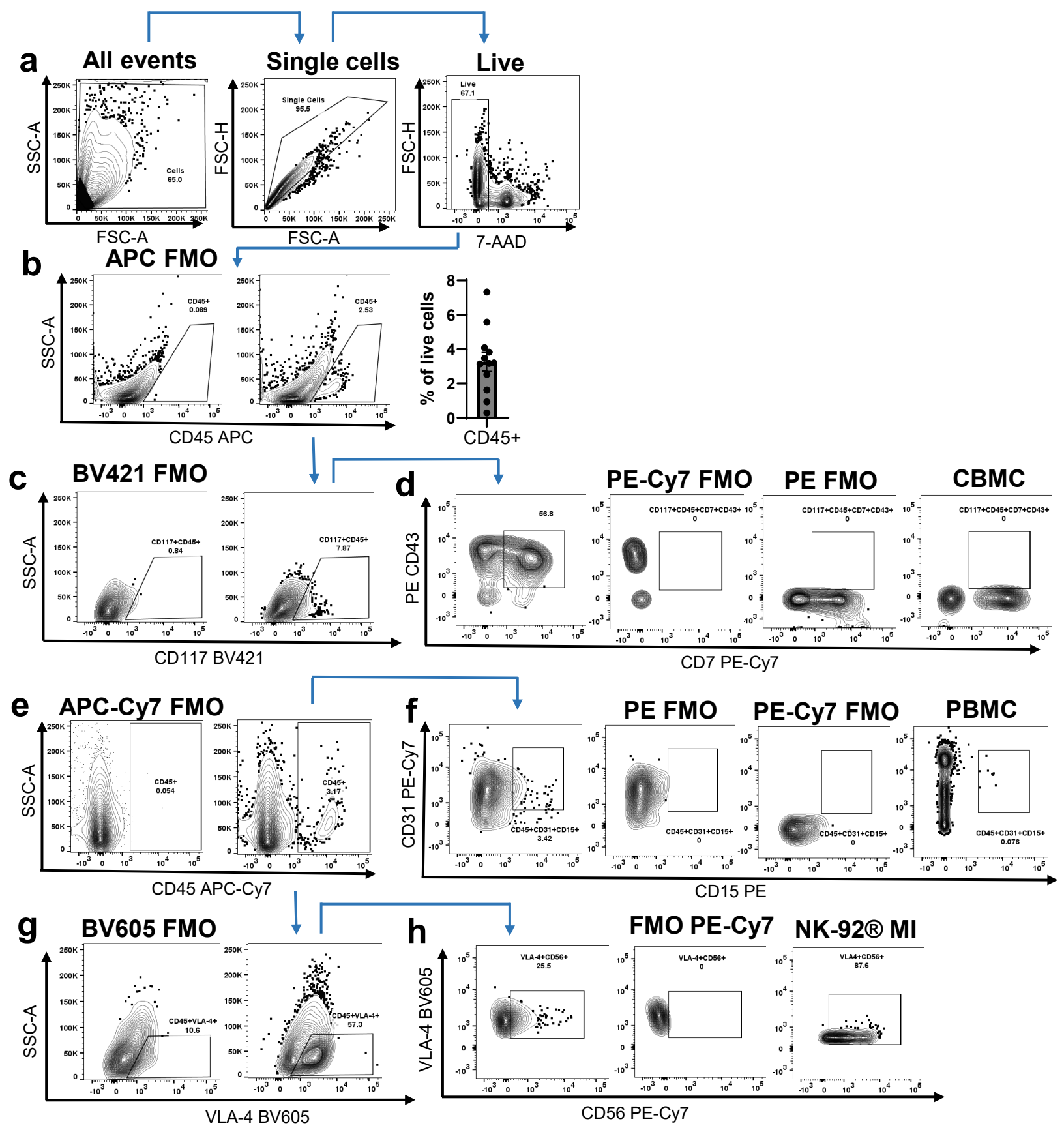
Flow cytometry backgatings and controls



1) Supplementary Figure 1: Flow cytometry backgating strategies

A. Representative flow cytometry plots showing the gatings from all events to live cells. This gating strategy has been used for all flow cytometry data analysis.
B. Representative flow cytometry plots showing the gatings of $CD43^{+}$ compared to FMO control. This gating is used for flow cytometry plots in main figure 5J. Error bars are ± SEM. 10 biological replicates were used to make the bar plot.
C. Representative flow cytometry plots showing the CD235ab $^{+}$, CD33 $^{+}$ and CD42b $^{+}$ cells pre-gated for CD43 $^{+}$ with their respective FMO controls (attributed to the main figure 5J).

Flow cytometry backgatings and controls



2) Supplementary Figure 2: Flow cytometry gating strategies

A. Representative flow cytometry plots showing the gatings from all events to live cells. This gating strategy has been used for all flow cytometry data analysis.
B. Representative flow cytometry plots showing the gating of $CD45^+$ compared to FMO control. This gating is used for initial gating after live cells gating for flow cytometry plot in main figure 5L. Error bars are ± SEM. 12 biological replicates was used for making the bar plot.
C. Representative flow cytometry plots showing the gating for $CD117^+$ cells pre-gated on $CD45^+$ cells in panel B. (attributed to the main figure 5L)
D. Representative flow cytometry plots showing the gating for $CD7^+/CD43^+$ cells pre-gated on $CD117^+/CD45^+$ cells in panel C. CBMC: Cord blood mononuclear cells. (attributed to the main figure 5L)
E. Representative flow cytometry plots showing the gating of $CD45^+$ compared to FMO control. This gating is used for initial gating after live cells gating for flow cytometry plot in main figure 5K and 5M.
F. Representative flow cytometry plots showing the gating for $CD31^+/CD15^+$ cells pre-gated on $CD45^+$ cells in panel E. PBMC: Peripheral blood mononuclear cells. (attributed to the main figure 5K)
G. Representative flow cytometry plots showing the gating for VLA-4^+ cells pre-gated for $CD45^+$ cells shown in panel E. (attributed to the main figure 5M)
H. Representative flow cytometry plots showing the gating for CD56+ cells pre-gated for $CD45^+$ /VLA-4^+ cells shown in panel G. (attributed to the main figure 5M)

Primer sequences used for qRT-PCR

Gene Target	Sequence	Source
HBE	Forward: 5'-TCTGGCTACTCACTTTGGCAAGGA-3' Reverse: 5'-TCACAGGAACACCTGCAAACTGGA-3'	Atkins et al. 2023 (reference 57)
HBG	Forward: 5'-TGGGAAATGTGCTGGTGACCGTTT-3' Reverse: 5'-TCACAGGAACACCTGCAAACTGGA-3'	Atkins et al. 2023 (reference 57)
RNA18S5	Hs.PT.39a.22214856.g	IDT technologies

Supplementary Table 1: Oligonucleotides used for qRT-PCR