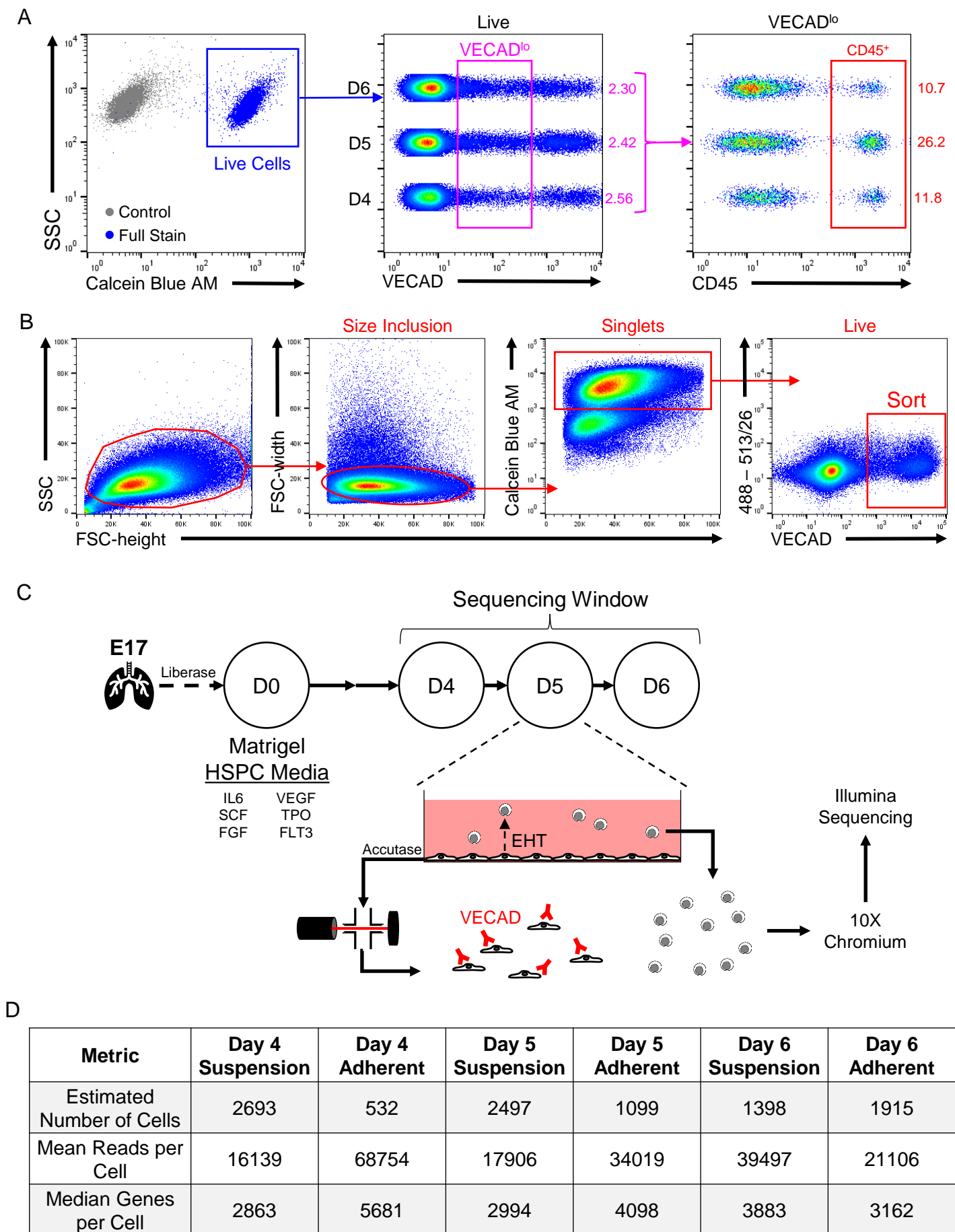
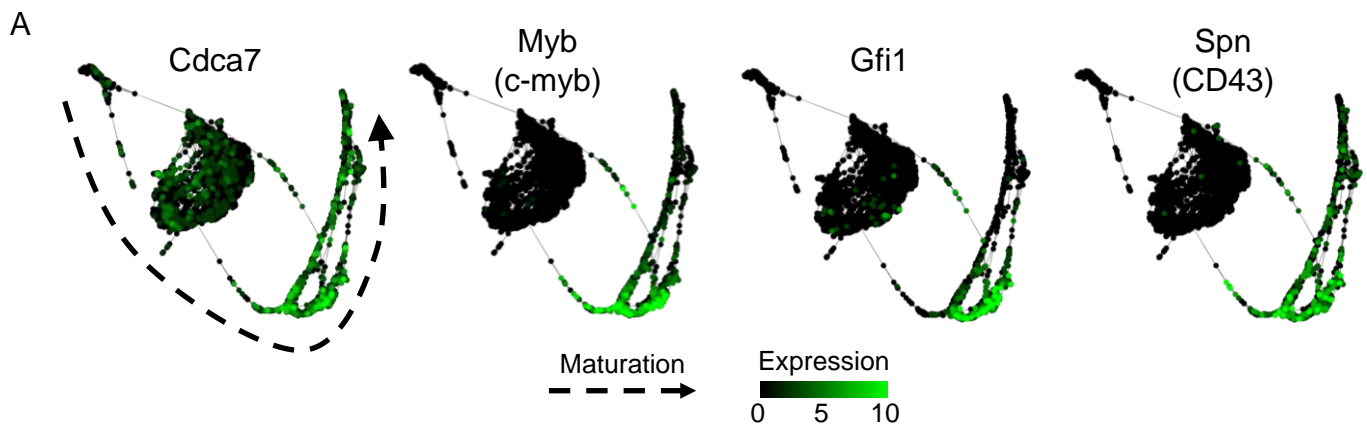


**Supplemental Figure 1. Assessment of EHT and pre-HSC markers on fetal liver and fetal lung explants.** (A) Comparison of EHT and pre-HSC markers between fetal liver and fetal lung explants. (B) Comparison of EHT and pre-HSC markers between populations differentiated by VECAD and CD45 expression.



**Supplemental Figure 2. Optimization of scRNA-Seq protocol and analysis.** (A) Comparison of the fraction of VECAD<sup>lo</sup> cells that are CD45<sup>+</sup>. (B) Sorting strategy for scRNA-Seq of adherent cells from fetal lung explants. (C) Schematic of scRNA-Seq procedure. (D) Table showing baseline metrics of scRNA-Seq analysis.



**B**

Cluster	Index	Name	p-value
Endothelial	1	regulation of angiogenesis (GO:0045765)	3.55E-08
	2	apical junction assembly (GO:0043297)	2.14E-06
	3	bicellular tight junction assembly (GO:0070830)	2.58E-05
	4	blood vessel morphogenesis (GO:0048514)	2.58E-05
	5	tight junction assembly (GO:0120192)	2.58E-05
	6	glomerulus vasculature development (GO:0072012)	2.58E-05
	7	cell-cell junction assembly (GO:0007043)	4.79E-05
	8	maintenance of blood-brain barrier (GO:0035633)	5.71E-05
	9	extracellular structure organization (GO:0043062)	5.71E-05
	10	external encapsulating structure organization (GO:0045229)	5.71E-05
Transitional	1	extracellular matrix organization (GO:0030198)	3.99E-05
	2	skin morphogenesis (GO:0043589)	0.000173
	3	muscle contraction (GO:0006936)	0.000173
	4	extracellular structure organization (GO:0043062)	0.000173
	5	external encapsulating structure organization (GO:0045229)	0.000173
	6	skin development (GO:0043588)	0.000222
	7	supramolecular fiber organization (GO:0097435)	2.69E-04
	8	collagen fibril organization (GO:0030199)	2.82E-04
	9	regulation of membrane protein ectodomain proteolysis (GO:0051043)	1.95E-03
	10	negative regulation of dendritic cell differentiation (GO:2001199)	4.07E-03
Hematopoietic	1	neutrophil degranulation (GO:0043312)	2.92E-13
	2	neutrophil activation involved in immune response (GO:0002283)	2.92E-13
	3	neutrophil mediated immunity (GO:0002446)	2.92E-13
	4	defense response to bacterium (GO:0042742)	2.61E-09
	5	leukocyte aggregation (GO:0070486)	0.000104
	6	defense response to Gram-negative bacterium (GO:0050829)	0.000112
	7	neutrophil migration (GO:1990266)	1.25E-04
	8	innate immune response (GO:0045087)	7.64E-04
	9	regulation of phagocytosis (GO:0050764)	8.85E-04
	10	myeloid cell activation involved in immune response (GO:0002275)	8.85E-04

**Supplemental Figure 3. SPRING and DGE analysis of fetal lung scRNA-Seq.** (A) SPRING plots showing the expression of key hematopoietic commitment markers. The dotted arrow represents the trajectory of maturation. (B) Extended list of Gene Ontology biological processes enrichment analysis. Full enrichment analysis can be found in Supplemental\_Data.

Marker	Species	Conjugate	Clone	Vendor	Catalog. #	Dilution
CD235a	Human	PE	HIR2	BD Biosciences	555570	1:1000
CD34	Human	BV421	581	BD Biosciences	562577	1:100
CD41	Human	BV421	HIP8	Biologend	303730	1:200
CD41	Human	Unconjugated	HIP8	BD Biosciences	BDB555465	1:100
CD42b	Human	APC	HIP1	BD Biosciences	551061	1:200
CD45	Human	APC	2D1	Biologend	368512	1:200
CD45	Human	PE-Cy7	2D1	Biologend	368532	1:200
EPCR (CD201)	Human	APC	RCR-401	Biologend	351906	1:100
GPI80	Human	PE	3H9	MBL	D087-5	1:50
KDR	Human	PE	89106	BD Biosciences	560494	1:200
KDR	Human	APC	89106	BD Biosciences	560871	1:200
Vascular Endothelial Cadherin (VECAD/CD144)	Human	AF647	55-7H1	BD Biosciences	561567	1:100
Vascular Endothelial Cadherin (VECAD/CD144)	Human	PE	55-7H1	BD Biosciences	561714	1:100
Vascular Endothelial Cadherin (VECAD/CD144)	Human	Unconjugated	Polyclonal	R&D Systems	AF938	1:100
CD44	Human/Murine	BV711	IM7	Biologend	103057	1:800
CD45	Human/Murine	Unconjugated	Polyclonal	Invitrogen	PIPA587427	1:100
Epithelial Cadherin (E-Cadherin)	Human/Murine	Unconjugated	DECMA-1	Santa Cruz Biotechnology	sc-59778	1:50
CD150	Murine	BV711	TC15-12F12.2	Biologend	115941	1:400
CD41	Murine	BV421	MWReg30	Biologend	133911	1:400
CD43	Murine	FITC	S11	Biologend	143204	1:100
CD45	Murine	BUV395	30-F11	BD Biosciences	564279	1:200
CD48	Murine	BV605	HM48-1	Biologend	103441	1:100
EPCR	Murine	APC	eBio1560	eBioscience	50-151-23	1:200
Kit	Murine	BV510	ACK2	Biologend	135119	1:400
Kit	Murine	APC	2B8	Biologend	105812	1:200
Lineage Cocktail	Murine	FITC	Cocktail	Invitrogen	22777072	1:5
Sca-1 (Ly-6A/E)	Murine	PE	D7	eBioscience	50-110-49	1:200
Vascular Endothelial Cadherin (VECAD/CD144)	Murine	Biotin	BV13	Biologend	138008	1:200
Streptavidin	N/A	APC-Cy7	N/A	Biologend	405208	1:200
Calcein Blue AM	Viability Dye	N/A	N/A	Invitrogen	C1429	1:1000
Live/Dead Fixable Blue	Viability Dye	N/A	N/A	Invitrogen	L34961	1:800

**Supplemental Table 1. Antibodies used for flow cytometry and immunofluorescent microscopy.**