Fig. S4

a

Stage	Embryos	Exp. (n)	Sample[a]	Neg[b]	Total
E8.0 (3-5 sp)	4	1	40	8	48
E8.5 (8-9 sp)	14	3	236	50	286
E9.0 (17-18 sp)	6	2	205	40	245
Total	24	6	481	98	579

[a]CD45⁻CD31⁺CD144⁺ sample cells from body
[b]CD45⁻CD31⁻CD144⁻ negative control cells from body

b

Cluster: vEC, pEC, pAEC, earlyAEC, lateAEC, HEC, HC, Neg

Stage: E8.0, E8.5, E9.0, E9.5, E10.0, E10.5, E11.0

Location: Body, DA, Neg

Cluster (E9.5-E11.0): vEC, earlyAEC, lateAEC, HEC, HC, Others

c

Genes: Pecam1, Cdh5, Ptprc, Nr2f2, Etv2, Gja5, Ltbp4, Runx1, Kit, Itga2b, Spn

Columns: vEC, pEC, pAEC, earlyAEC, lateAEC, HEC, HC, Neg

Average Expression: 6, 4, 2, 0

Percentage: 0, 25, 50, 75, 100

d

Component 2 vs Component 1

Pseudotime: 0, 10, 20, 30

e

Ordered by pseudotime

Pattern (# Gene)
- P1 (988)
- P2 (308)
- P3 (459)
- P4 (450)
- P5 (646)

Stage: E8.0, E8.5, E9.0, E9.5, E10.0, E10.5, E11.0

Cluster: pEC, pAEC, earlyAEC, HEC, T1 pre-HSC

Row scale (smoothed): 2, 1, 0, -1

f

GO:BP enrichment results

ribonucleoprotein complex biogenesis
rRNA processing
RNA localization
mitotic nuclear division
nucleoside monophosphate metabolic process
coenzyme metabolic process
actin filament organization
cell-cell junction organization
negative regulation of cellular component movement
small GTPase mediated signal transduction
cell-substrate adhesion
negative regulation of locomotion
angiogenesis
vasculogenesis
endothelium development
endothelial cell differentiation
endothelial cell migration
positive regulation of cell migration
lipid catabolic process
myeloid cell differentiation
response to purine-containing compound
negative regulation of ATP metabolic process
negative regulation of glycolytic process
response to organophosphorus
regulation of hemopoiesis
leukocyte activation involved in immune response
cell activation involved in immune response
myeloid leukocyte activation
T cell activation

Columns: P1, P2, P3, P4, P5

Adjusted P value: 0.02, 0.01

GeneRatio: 0.05, 0.10, 0.15

g

Expression level, log₂(TPM/10+1)

Genes: Etv2, Sox17, Sox4, Fos, Fosb, Hoxa10, Etv6, Gata2, Gfi1, Hlf, Hoxa5, Hoxa9, Meis1, Myb, Mycn, Pbx1, Rora, Runx1, Spi1, Erg, Gfi1b, Hoxb4, Lcor, Lmo2, Prdm5, Runx1t1, Tal1, Zfp37

Ordered by pseudotime

Pattern: P1, P2, P3, P4, P5
Cluster: pEC, pAEC, earlyAEC, HEC, T1 pre-HSC
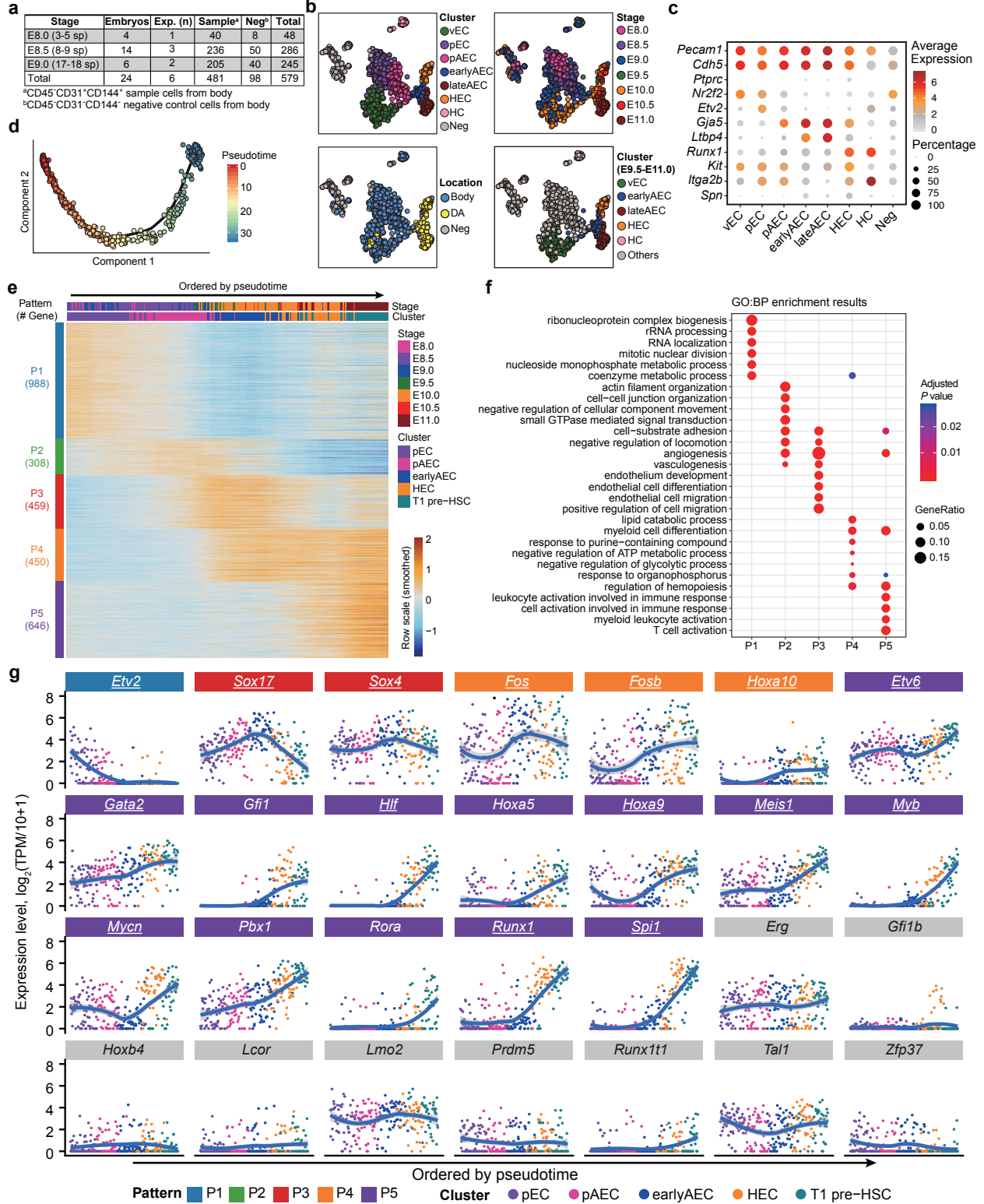
Supplementary Figure 4. Molecular programs from primitive vascular ECs to HSC-primed HECs. **a.** Embryo, independent experiment, and cell number information for additional scRNA-seq. sp, somite pairs. **b.** t-SNE plots with clusters (upper left), sampling locations (lower left), embryonic stages (upper right) and clusters previously defined (lower right) mapped onto it. **c.** Dot plot showing the average and percentage expression of selected marker genes in the indicated clusters. **d.** Pseudotemporal ordering of the cells involved in HEC specification, including those in pEC, pAEC, earlyAEC, HEC, and T1 pre-HSC, inferred by monocle 2, with pseudotime mapped to it. **e.** Heatmap showing smoothed and scaled expression levels of 2 851 pattern genes. Genes are ordered by patterns. Cells are ordered by pseudotime. **f.** Dot plot showing the top six enriched Gene Ontology biological process (GO:BP) terms for each pattern. Dot color indicates statistical significance of the enrichment and dot size represents the fraction of genes annotated to each term. **g.** Scatter plots showing the expression levels of the TF genes previously reported to be functional in HSPC regeneration along the pseudotemporal order with loess smoothed fit curves and 95% confidence interval indicated. The patterns to which the genes belong are indicated by different fill colors. The core TFs of the significantly overlapped regulons are underlined.