**Fig. S3**

### a

**Neur3**

**Cluster**

- HC
- EC
- aEAC
- bEAC
- NEC
- HEC
- PK44

### b

| Stage            | Region | Exp (n) | Population in EC (CD44+CD31+CD45+) | Cell dose* | Clusters* |
|------------------|--------|---------|------------------------------------|------------|-----------|
| E9.5-E10.0 (30-34 sp) Caudal half | 5      | CD44*Neur3-EGFP$^+$ | 1.5-2.5 ee$^2$ | 6/6 (100%) |
|                  |        | CD44*Neur3-EGFP$^+$ | 1.5-5.0 ee$^2$ | 3/6 (50%)  |

*Cell dose per recipient. *Hematopoietic cluster positive wells/total wells. *Somite pairs.

### c

**Pearson correlation analysis**

- $R = 0.33, P = 0.036$
- $R = 0.35, P = 0.027$

### d

**Cell cycle phase**

- G0
- G1
- Quiescent

### e

**Neur3**

**Cluster**

- HC
- EC
- aEAC
- bEAC
- NEC
- HEC
- PK44

**Frequency in CD44+ ECs (%)**

- HSC-competence
- 8.5
- 35.8

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**Notes:**

- CD44$^+$CD31$^+$CD45$^+$Neur3-EGFP$^+$ cells.
- *Only endothelial tube positive wells/total wells.
- **Only haematopoietic progeny positive wells/total wells.
- *Somite pairs.
- *Independent experiments, totally 32 embryos were used.
- *Independent experiments, totally 33 embryos were used.
**Supplementary Figure 3.** Identification of the HSC-competent HECs marked by Neurl3-EGFP reporter. 

a. Expression of Neurl3 exhibited on t-SNE plot. The same t-SNE plot with the clusters of interest (HC, vEC, earlyAEC, lateAEC, HEC, and PK44) mapped on it is shown to the right. 

b. Detailed information of the co-culture/transplantation assays performed with the caudal half cells from E9.5-E10.0 Neurl3EGFP/+ embryos. 

c. Boxplot showing the transcriptional expression level of EGFP in NE+ cell population, NE+, CD41-CD43 CD45 CD31*CD44*Neurl3-EGFP+ population from AGM region of E10.0 Neurl3EGFP/+ embryos. 

d. Scatter plots showing correlation of the expression of EGFP with that of Neurl3 and Runx1, respectively. Fitted line and 95% confidence interval are shown in red. Pearson correlation coefficients and P values are also shown in blue text. 

e. Expression of Neurl3 exhibited on t-SNE plot. The same t-SNE plot with the clusters of interest (HC, vEC, earlyAEC, lateAEC, tif-HEC, and NE+) mapped on it is shown to the right. 

f. Stacked bar chart showing the constitution of different cell cycle phases in the indicated clusters. 

g. Representative immunostaining on cross sections at AGM region of E10.0-E10.5 (left column) and E11.0 (right three panels) Neurl3EGFP/+ embryos. Arrow indicates Neurl3-EGFP+ aortic ECs; Yellow arrowheads indicate Neurl3-EGFP+ bulging and bulged cells and also IAHCs. White pink arrowheads indicate CD44+Runx1*Neurl3-EGFP- hematopoietic cells distributed outside the aorta. Images in yellow boxes show the corresponding high magnification views of those in Fig. 5a. nt, neural tube; DA, dorsal aorta. Scale bars, 100 μm. 

h. Graph showing the frequencies of the NE+ (CD41-CD43-CD45-CD31*CD44*Neurl3-EGFP+) cells in CD44+ ECs in E9.5 caudal half (CH) or E10.0-E10.5 AGM region of Neurl3EGFP/+ embryos. Data are means ± s.d.. Data are from 8, 5, and 6 independent experiments for E9.5, E10.0, and E10.5 embryos, respectively. 

i. Representative FACS plots and the corresponding diagram to the right showing the relationship between PK44 and NE+ populations in E10.0 AGM of Neurl3EGFP/+ embryos. 

j. Detailed information of endothelial-hematopoietic dual-potential induction assays performed with cells from E9.5 caudal half and E10.0-E10.5 AGM region of Neurl3EGFP/+ embryos.