

Figure S1 related to Figure 1 and 3.

(A). Multi-lineage long-term (18 weeks) contribution of HSCs generated in culture from E11.5 AGM region, extraembryonic vessels (EEV), placenta (PI), head (H), yolk sac (YS). Each column represents an individual recipient (preHSC_LDA assay). % of specialised cell lineages in donor derived blood cells.

(B). E10 (30-34sp) pre-HSC are exclusively VC+CD45-

(C). E12 (51-54sp) pre-HSCs are exclusively VC+CD45+

(B,C) AGM cells were dissociated and sorted for 4 populations. Each population were co-aggregated with op9 and after maturation in culture injected in recipient mice. Embryonic stage and dose injected (e.e.) are indicated at the top of each graph. Donor-derived peripheral blood chimerism is shown by diamonds.

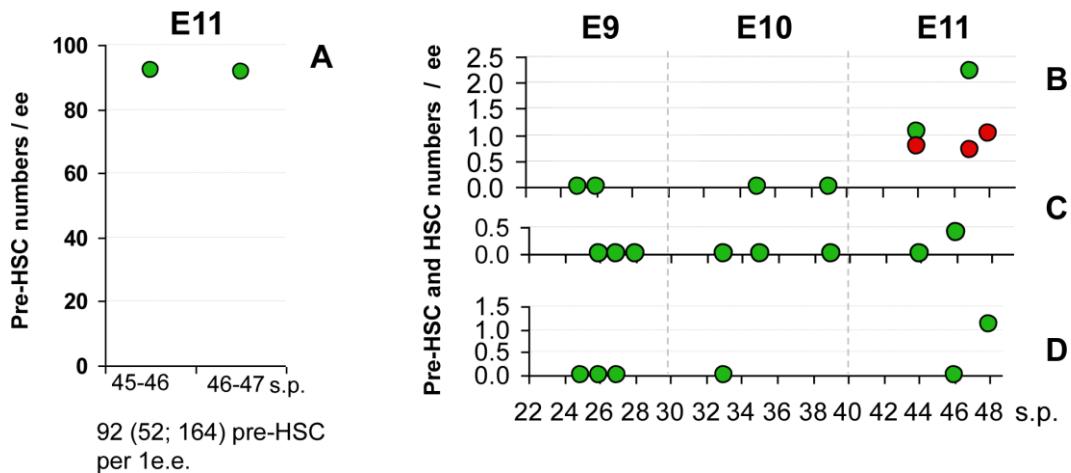


Figure S2 related to Figure 2. Quantitation of pre-HSCs and dHSCs in embryonic tissues

(A) E11.5 caudal part + extraembryonic vessels together contain ~92 pre-HSC (with 95% confidential intervals, 52; 164, lower and upper limits) (n = 2 experiments).

(B-D). E9 -11.5 tissues: placenta (B), yolk sac (C) and head (D) (n = 6 experiments).

Note that each symbol represents pre-HSC or HSC number in an individual experiment (not in an individual animal) calculated by preHSC_LDA (in green) or dHSC (in red; as assayed by direct transplantations of fresh tissues, for methodology see Fig 1A and 1B, respectively).

Table S1. Related to figure 3B. Ratio of preHSC-I and II in E11 AGM estimated by preHSC-LDA.

Type I and II pre-HSCs populations were sorted and assayed by preHSC-LDA to determine pre-HSC numbers. For this purpose limited doses of sorted cells were co-aggregated and cultured with OP9 cells. Each co-aggregate was injected into an individual recipient. 1 e.e. of sorted cells for co-aggregation was adjusted in accordance with losses of dead cells. Pre-HSC numbers (95% confidential intervals with lower and upper limits) are shown ($n = 6$ independent experiments).

The ratio between type I and II pre-HSCs is shown below the main table, for methodology see Fig 1B.

E11.5 (41-45sp)	PreHSC-I population (VC+CD45-)		PreHSC-II population (VC+CD45+)	
	Mice repopulated/total	Sorted ee dose /co-aggregate /mouse	Mice repopulated/total	Sorted ee dose/ co-aggregate /mouse
Exp.1	2/8	0.30	6/14	0.20
Exp.2	2/9	0.30	8/9	0.22
Exp.3	2/8	0.26	5/11	0.20
Exp.4	2/6	0.50		
Exp.5	2/4	0.50		
Exp.6	1/4	0.50		
Pre-HSCs	0.99 (0.58; 1.66)		3.94 (2.50; 6.17)	
preHSC-I/II ratio in E11 AGM is 0.99 : 3.94 (~ 1:4)				

Suppl. Table 2A Level of recipient blood chimerism (%) and pre-HSC number estimation by ELDA software. **AGM cells** (to Fig 2A).

PreHSC_LDA analysis		13.5-18 weeks post transplantation (donor-derived blood chimerism, %)												Dose transplanted	Mouse numbers		ELDA results	
exp #	Somites pairs	mouse 1	mouse 2	mouse 3	mouse 4	mouse 5	mouse 6	mouse 7	mouse 8	mouse 9	mouse 10	mouse 11	mouse 12	ee /co-aggregated with op9 and injected after culture	injected	repopulated	pre-HSC/ee	95% confidence interval
exp1	59sp	0.0	0.0	2.0	4.6	0.2	0.0	0.0	0.0	82.0				0.01	9	1	11.8	2.9-47.2
	59sp	0.0	0.0	0.1	0.0	0.1	8.0	0.2	0.0	0.1				0.01	9	1		
exp2	55sp	0.0	64.0	0.0	0.0	0.2	0.0	0.1	0.1	0.0	0.0			0.01	10	1	10.0	3.3-27.9
		0.0	0.0	32.0	0.1	0.0	0.0	0.0	0.0	0.1	0.1			0.01	10	1		
exp3	52sp	0.0	94.0	0.1	0.0	84.0	27.0	2.0	0.0	0.0				0.01	9	3	40.5	13.0-126.6
exp4	51sp	79.0	92.0	10.0	93.0	49.0	4.0	81.0	1.0	0.0	86.0			0.02	10	7	60.2	27.4-132.5
exp5	50sp	30.0	2.0	92.0	90.0	77.0	84.0	63.0	74.0	63.0	1.0			0.025	10	8	64.5	29.8-139.1
exp6	49sp	93.0	16.0	75.0	95.0	92.0	95.0	93.0	4.0	86.0	92.0			0.06	10	9	38.3	17.1-86.2
exp7	45sp	82.0	0.4	0.2	75.0	81.0	87.0	90.0	0.1	94.0	0.3			0.01	20	8	48.8	28.5-83.2
	45sp	0.2	0.0	0.0	0.0	0.5	0.0	0.1	33.0	85.0	0.2							
exp7a	45sp	0.1	92.0	0.5	0.4	92.0	79.0	95.0	82.0	2.0	64.0			0.02	10	6		
exp8	44sp	74.0	75.0	3.0	79.0	34.0	84.0	89.0	92.0	78.0	83.0			0.025	10	9	60.2	33.7-87.0
exp9	40sp	0.1	74.0	76.0	84.0	85.0	72.0	85.0	0.0	0.3	0.0			0.017	9	6	53.8	23.5-123.5
exp10	38sp	0.9	94.0	2.0	11.0	91.0	0.1	0.8	0.0	93.0	0.7	13.0		0.025	11	5	24.3	10.0-59.2
exp11	37sp	70.0	4.8	0.1	57.0	0.7	11.0	90.0	89.0	50.0	90.0							
		92.0	74.0	31.0	89.0	84.0	0.2	0.4	0.0	92.0	0.4			0.033	20	12	27.8	15.5-50.0
exp12	35sp	59.0	43.0	73.0	76.0	80.0	92.0	79.0	72.0	82.0	0.2			0.1	10	9		
		0.2	0.0	87.0	28.0	64.0	86.0	58.0	90.0	63.0	86.0			0.1	10	8	17.3	10.4-28.0
exp13	35sp	16.0	10.0	0.7	95.0	39.0	85.0	68.0	90.0	0.5	0.0			0.1	10	7	11.3	5.3-23.2
exp14	33sp	32.0	1.0	54.0	64.0									0.25	4	3	5.4	1.6-17.2
exp15	30sp	4.4	17.3	8.4	3.3	22.7	69.5							0.25	6	4	4.4	1.6-12.3
exp16	29sp	0.1	28.0	0.2	51.0									0.25	4	2	2.7	0.7-10.8
exp17	28sp	0.1	76.0	45.0	1.0									0.25	4	2	2.7	0.7-10.8
exp18	27sp	0.2	10.0	0.0	0.1	0.0								0.2	5	1	1.1	0.2-7.9
exp19	26sp	0.3	0.0	0.1	0.5	12.4	3.7							0.25	6	1	0.7	0.1-5.2
exp20	26sp	12.0	0.2	1.0	0.1	0.0	0.1	0.1	16.0	0.1	0.1	0.4	0.1	0.5	12	2	0.4	0.1-1.5
	25sp	27.2	0.0	0.0	0.2									0.25	4	1		
exp21	25sp	0.1	0.0	0.0	51.4									0.5	4	1	0.8	0.2-3.1
	24sp	10.3	0.0	0.5	0.1	7.1	2.0							0.333	6	2		
exp22		30.0	0.0	1.0	2.0	0.3	0.0	21.0	0.2	0.1	0.4			0.5	10	2	0.7	0.2-1.7
exp23	23sp	0.0	13.3	0.0	0.2	2.0	0.2	0.0	0.7	0.2	70.4			0.5	10	2	0.4	0.1-1.8

Direct transplantation																		
exp #	Somites pairs	13.5-18 weeks post transplantation (donor-derived blood chimerism, %)												Dose transplanted	Recipient numbers		ELDA results	
		mouse 1	mouse 2	mouse 3	mouse 4	mouse 5	mouse 6	mouse 7	mouse 8	mouse 9	mouse 10	mouse 11	mouse 12	ee/ mouse	injected	repopulated	dHSC/ee	95% confidence interval
exp24	57sp	0	2	0	25	0	0.2	43						0.25	7	2	1.3	0.3-5.4
exp2	55sp	21	0	0.1	0.1									0.25	4	1	1.2	0.2-8.2
exp25	47sp	66	70	12	11	25	0	27	0	0.4	0.1	7		1	11	7	1.0	0.5-2.2
exp26	46sp	4	19	0	74	0	0.1	39	4	69	0	70	0.1	1	12	5	0.5	0.2-1.3
exp7	45sp	0	17	0	28	0	29	0	42	59				1	9	5	0.8	0.3-2.0
exp8	44sp	0	6	0	0.2	0	0.5	10	0.3	0.1	48	64	82	1	12	5	0.5	0.2-1.3
exp27	42sp	4	19	0	74	0	0.1	39	4	69	0	70	0.1	1	12	5	0.5	0.2-1.3
exp11	37sp	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0				2	8	0	0.0	-

pre-HSC/dHSC numbers depending at each embryo age

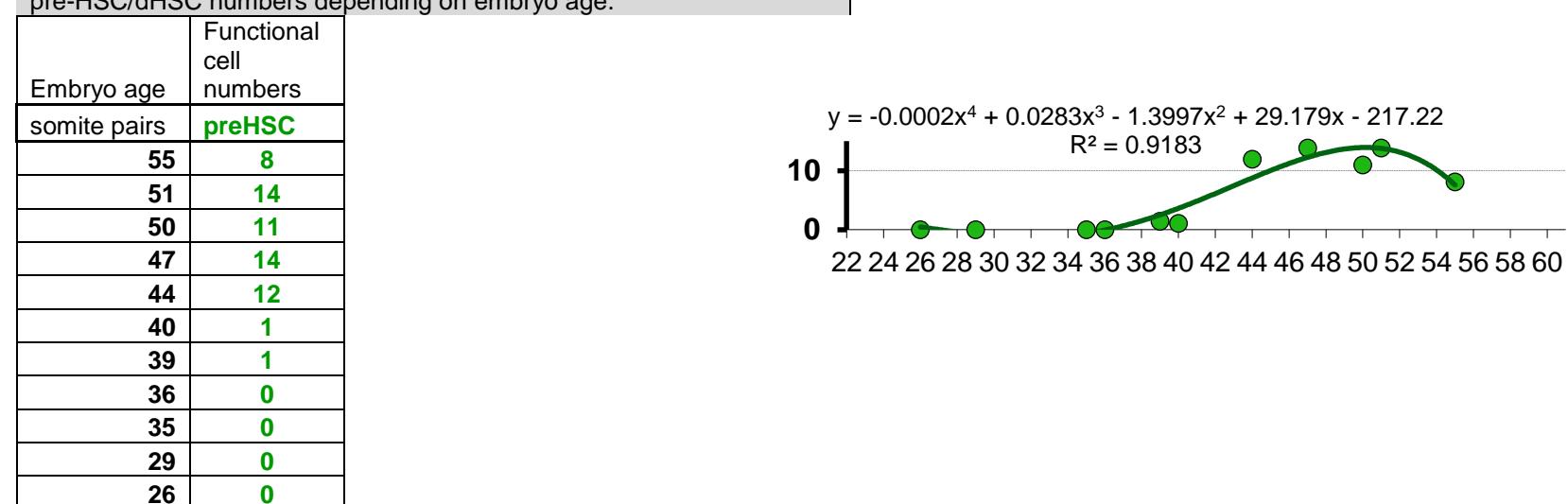
Embryo age	Functional cell numbers	
somite pairs	preHSC	dHSC

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Supplementary table 2B Level of recipient blood chimerism (%) and pre-HSC number estimation by ELDA software. Extraembryonic vessels cells (to Fig 2B).

PreHSC LDA analysis		13.5-18 weeks post transplantation (donor-derived blood chimerism, %)										Dose transplanted	Mouse numbers		ELDA results	
exp #	Somites pairs	mouse 1	mouse 2	mouse 3	mouse 4	mouse 5	mouse 6	mouse 7	mouse 8	mouse 9	mouse 10	ee /co-aggregated with op9 and injected after culture	injected	repopulated	pre-HSC/ee	95% confidence interval
Exp2	55sp	90.0	0.0	0.0	0.0	34.0	0.0					0.05	6	2	8.1	2.0-32.8
Exp4	51sp	67.0	0.0	78.0	40.0	0.0	60.0	0.0	0.3			0.05	8	4	13.9	5.1-37.7
Exp5	50sp	82.0	76.0	0.0								0.1	3	2	11.0	2.6-47.2
Exp7	47sp	0.2	80.0	92.0	92.0							0.1	4	3	13.9	4.1-47.2
Exp8	44sp	48.0	1.5	0.6	75.0	34.0	67.0	0.0	52.0	0.0	4.7	0.05	20	9	12.0	6.2-23.2
		0.0	70.0	0.0	0.0	51.0	0.0	47.0	0.0	0.0	26.0					
exp9	40sp	0.0	21.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	10	1	1.1	0.1-7.5
exp10	39sp	0.0	10.0	0.1	0.0							0.2	4	1	1.4	0.2-10.3
exp11	36sp	0.3	0.3	0.3	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.2	10	0	0.0	-
exp13	35sp	0.0	0.0	0.1								0.33	3	0	0.0	-
exp16	29sp	0.0	0.0	0.0								0.33	3	0	0.0	-
exp19	26sp	0.0	0.0	0.3	0.0							4	4	0	0.0	-

pre-HSC/dHSC numbers depending on embryo age.



Supplementary table 2C

Level of recipient blood chimerism (%) and pre-HSC number estimation by ELDA software. **Fetal liver cells** (to Fig 2C).

PreHSC LDA analysis		13.5-18 weeks post transplantation (donor-derived blood chimerism, %)											Dose transplanted		Mouse numbers		ELDA results	
exp #	Somites pairs	mouse 1	mouse 2	mouse 3	mouse 4	mouse 5	mouse 6	mouse 7	mouse 8	mouse 9	mouse 10	ee /co-aggregated with op9 and injected after culture	injected	repopulated	pre-HSC/ee	95% confidence interval		
expF1	60sp	11.0	10.0	0.0	11.0	8.0	9.0	19.0	0.0	0.2	20.0	0.01	10	7	120	54.9-264.6		
expF2	58sp	6.0	15.0	0.2	0.0	4.0	0.8	10.0	25.0	19.0	40.0	0.01	10	6	92	40.0-209.6		
expF3	57sp	0.8	0.0	2.0	76.0	1.0	80.0	5.0	65.0	15.0	3.0	0.005	10	4	102	37.9-275.5		
expF4	55sp	6.0	0.2	2.0	78.0	2.0	79.0	0.0	0.0	87.0	0.5	0.005	10	3	71	22.9-222.7		
exp25	47sp	0.0	0.0	0.0	0.0							2	4	0	0	-		
exp26	46sp	0.0	0.0	0.0	0.0	0.0						1	5	0	0	-		
exp7	45sp	0.0	0.0	0.0	0.0							1	4	0	0	-		
exp8	44sp	0.0	0.0	0.0								1	3	0	0	-		
Direct transplantation																		
exp #	Somites pairs	13.5-18 weeks post transplantation (donor-derived blood chimerism, %)										Dose transplanted	Recipient numbers		ELDA results			
		mouse 1	mouse 2	mouse 3	mouse 4	mouse 5	mouse 6	mouse 7	mouse 8	mouse 9	mouse 10	ee/ mouse	injected	repopulated	dHSC/ee	Upper/Low 95% confidence interval		
expF1	60sp	23	59	77	1	4	46	42	65	87	0	0.01	10	7	120	54.9-264.6		
expF2	58sp	4	3	74	80	57	0	2	57	78	60	0.01	10	6	92	40.0-209.6		
expF3	57sp	74	2	81	88	84	72	88	80	0	2	0.01	10	7	120	54.9-264.6		
expF4	55sp	2.5	0.3	84	86	0	40					0.01	6	3	69	21.8-219.8		
expF5	52sp	1.2	0.5	39	29	1	42	15	0	0	0.1	0.017	10	4	30	11.2-80.6		
exp25	47sp	0	0	0	0							2	4	0	0	-		
exp26	46sp	0	0	0	0							1	4	0	0	-		
exp8	44sp	0	0	0								1	3	0	0	-		

pre-HSC/dHSC numbers depending at each embryo age.

Embryo age	Functional cell numbers	
somite pairs	preHSC	dHSC
60	120	120
58	92	92
57	102	120
55	71	69
52		30
47	0	0
46	0	0
45	0	
44	0	0

