Supplemental Information Inventory

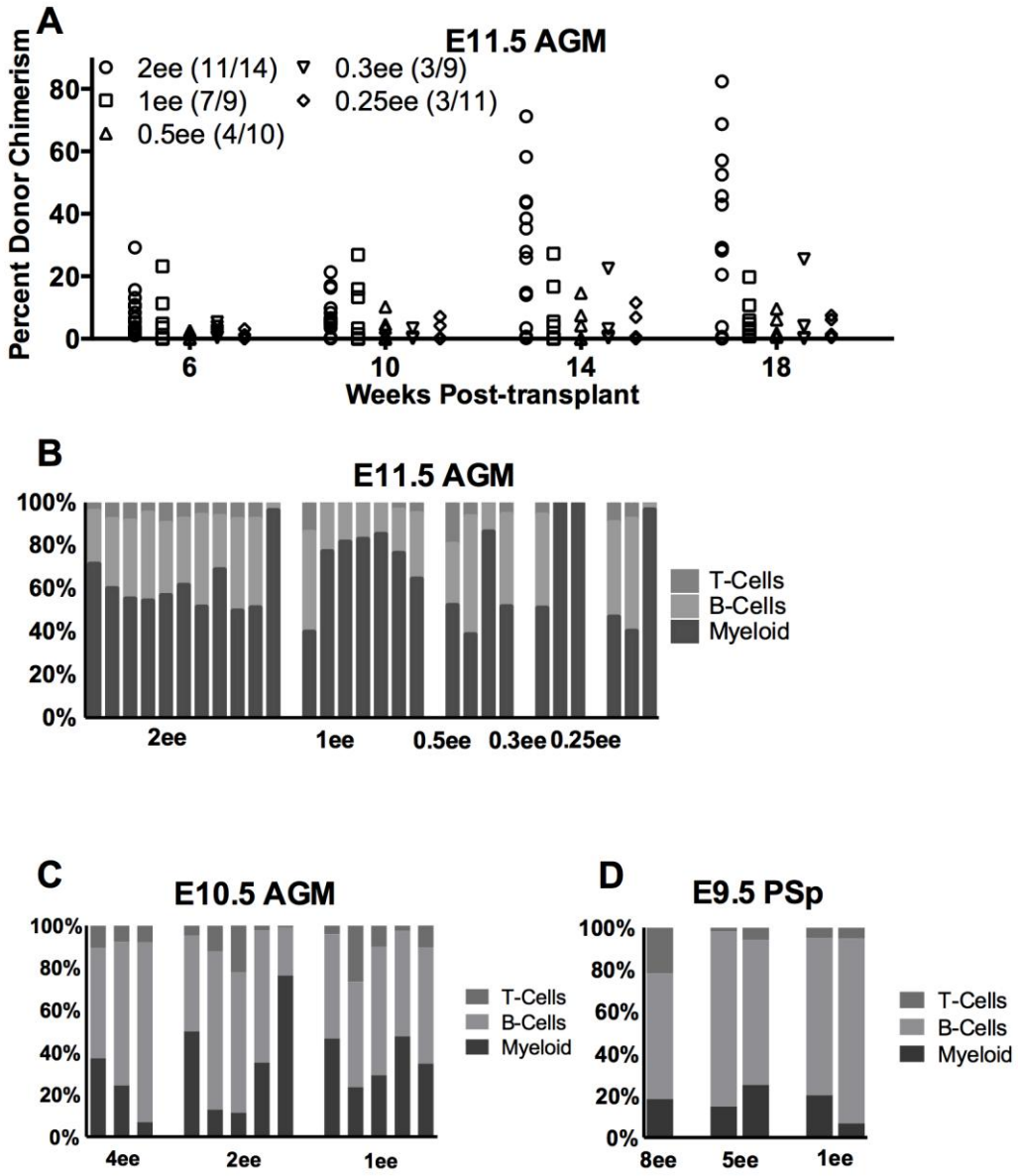

Figure S1: Multi-lineage reconstitution and self-renewal capacity of early embryonic HSCs, Related to Figure 1. A) Neonatal recipients were transplanted with limiting doses of whole E11.5 AGM. The numbers in the graph legend reflect the number of animals engrafted over the number of animals transplanted. **B)** Lineage breakdown of donor contribution in peripheral blood at 18 weeks post-transplantation for neonates engrafted with E11.5 AGM. Lineage breakdown of donor contribution in peripheral blood at 18 weeks post-transplantation for neonates engrafted with **C)** E10.5 AGM and **D)** E9.5 PSp.

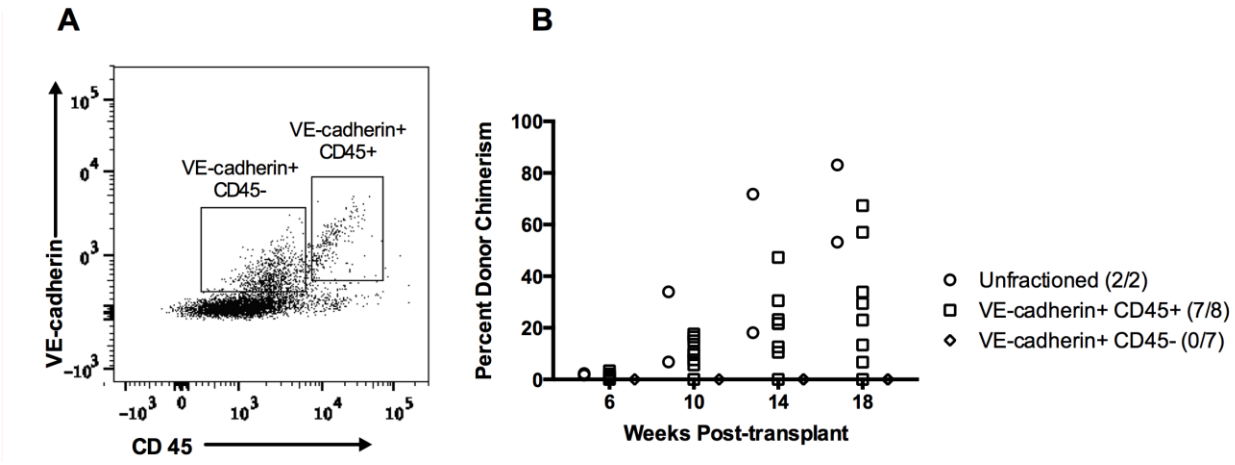

Figure S2: Adult-engrafting subpopulations also engraft neonatal recipients, Related to Figure 1. A) FACS plot of E11.5 AGM fractionated by VE-Cadherin and CD45. **B)** Neonatal recipients transplanted with unfractionated, VE-Cadherin $^{+}$ CD45^{+}, or VE-Cadherin^{+} CD45^{-} cells from E11.5 AGM. The numbers in the graph legend reflect the number of animals engrafted over the number of animals transplanted.

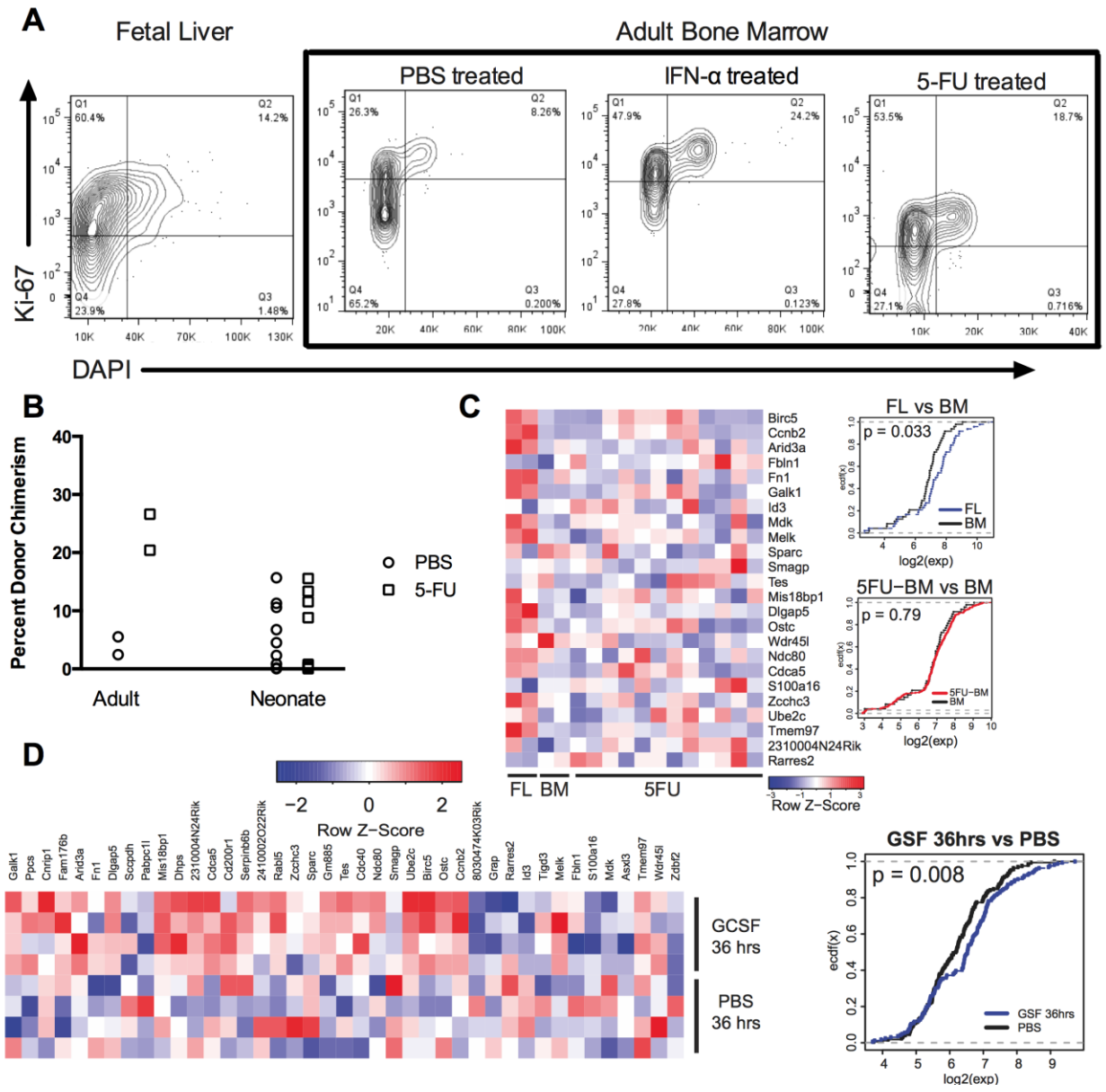

Figure S3: Proliferating adult-like LT-HSCs, Related to Figure 2. A) FACS plots for Ki-67 and DAPI expression on E14.5 FL LT-HSCs and sorted BM LT-HSCs from IFNα treated, 5-FU treated, or PBS control adult mice. **B)** Neonatal and adult recipients were transplanted with 100 BM LT-HSCs from adult mice treated with 5-FU (squares) or PBS (circles). Data shown are at 14 weeks post-transplantation. The commonly up-regulated genes from Figure 3 C were used in a gene-set enrichment analysis using two-sample Kolmogorov–Smirnov test in the dataset from **C)** Venezia *et al*. with 5-FU treated HSCs and **D)** Schuettpelz *et al*. with G-CSF treated HSCs.

Table S1: Neonatal engraftment from early embryonic HSCs, Related to Table 1.

Donor		Recipient	Irradiation Dose (Gy)	Number of Animals Engrafted	Number of Animals Transplanted	Percent Animals Engrafted	Average Percent Donor Chimerism	p-value
E11.5	1 ee	Adult	3.5	2	6	33.3	3.4	0.1357
	1 ee	Adult	6.5	3	6	50	2.1	0.1223
	1 ee	Adult	10	1	8	12.5	34.2	0.015
	1 ee	Neonate	3.5	7	9	77.8	6.9	
E10.5	1 – 7 ee	Adult	10	0	20	0	0	0.0018
	1 ee	Neonate	3.5	5	10	50	11	
E9.5		Adult	10	ND	ND	ND	ND	ND
	1 – 8 ee	Neonate	3.5	5	53	9.4	5.7	
Adult BM	100 Cells	Adult	10	5	5	100	25.4	0.033
	100 Cells	Neonate	3.5	5*	14	35.7	31.4	
E14.5 FL	100 Cells	Adult	10	6	6	100	77.9	1.44E-6
	100 Cells	Neonate	3.5	10	16	62.5	22.7	
Neonatal BM	50 Cells	Neonate	3.5	4	8	50	17.8	0.058
Neonatal Liver	50 Cells	Neonate	3.5	6	6	100	31.0	

The threshold for engraftment was drawn at 1%. Percent engraftment was calculated using only engrafted mice. P-values are between neonatal and adult recipients transplanted with the same donor population and calculated using Fisher's exact test, except the p-values for FL HSC transplants, neonatal HSC transplants, and E11.5 AGM transplanted into adult recipients conditioned with 650 rad were calculated using the student's t-test. *3/5 animals had robust engraftment that was not seen at any other time point up to 23 weeks post-transplant leading us to conclude it may have been a spurious experimental artifact. ND – not determined.