

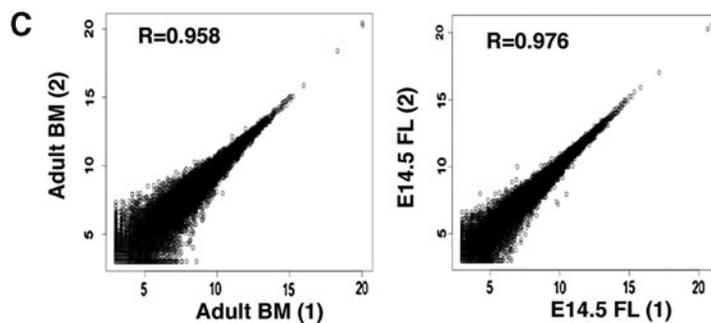
Supplementary Data

A

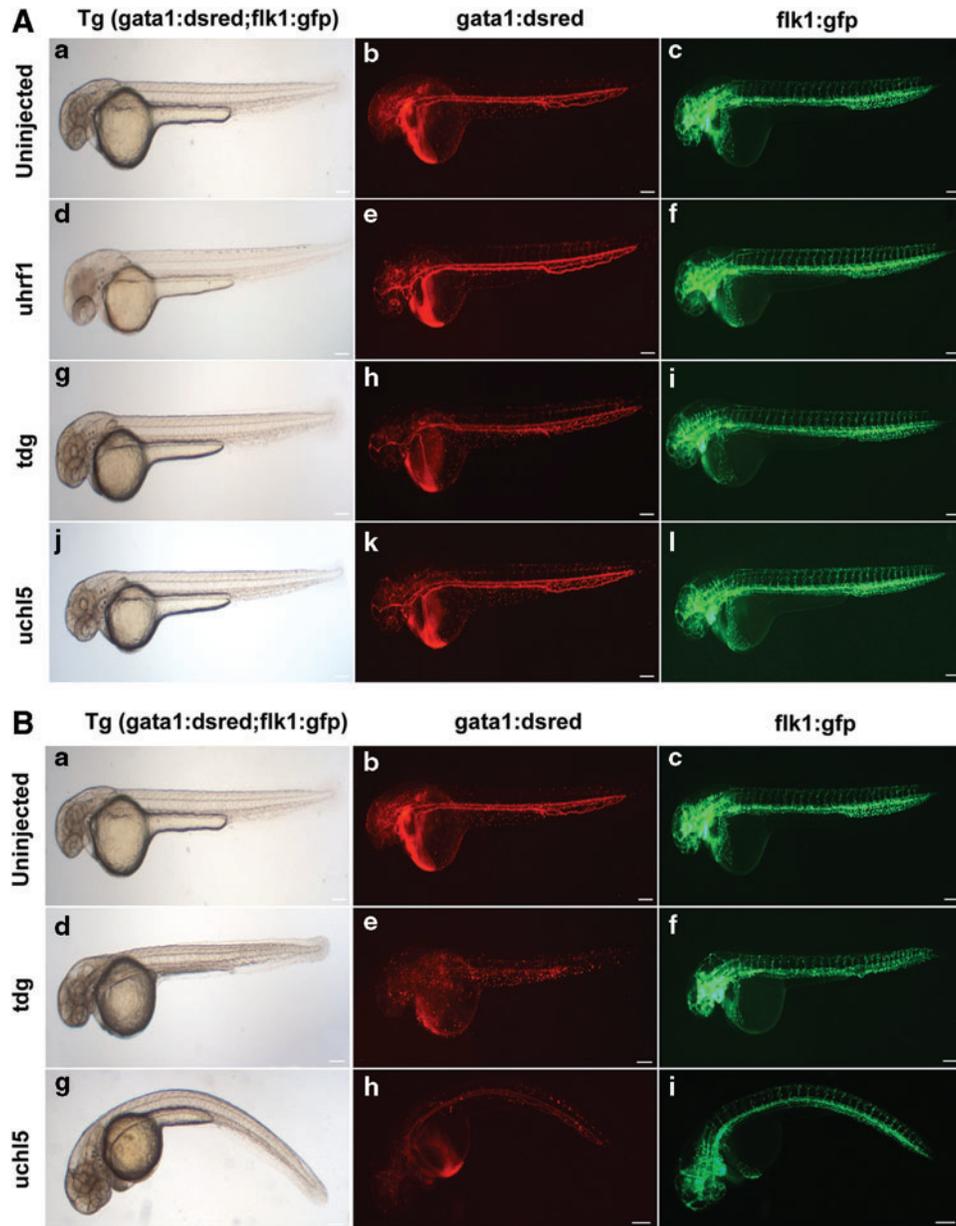
Sample	Total reads	Reads mapped to Genome			
		No. of reads	% of total reads	Unique match	% of total reads
FL14.5-1	39541158	34835501	88.10%	33049926	83.58%
FL14.5-2	38343180	34099745	88.93%	32464103	84.67%
BM1	39119372	35566226	90.92%	34248275	87.55%
BM2	38640484	34805547	90.08%	33451360	86.57%

B

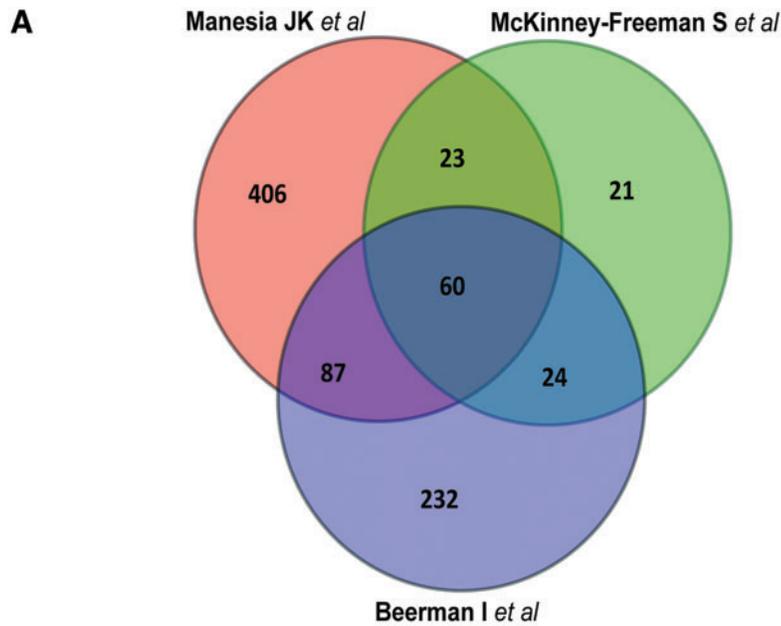
Sample	Total reads	Reads mapped to Genes			
		No. of reads	% of total reads	Unique match	% of total reads
FL14.5-1	39541158	15014048	37.97%	14527732	36.74%
FL14.5-2	38343180	15000015	39.12%	14517561	37.86%
BM1	39119372	12021107	30.73%	11611807	29.68%
BM2	38640484	12544338	32.46%	12143640	31.43%



SUPPLEMENTARY FIG. S1. Mapping statistics and correlation analysis after RNA-Seq for LT-HSC from murine FLs and BM. **(A)** Percentages of reads mapped to reference genome after RNA-seq analysis performed on murine FL E14.5 and ABM LT-HSCs. **(B)** A percentage of reads mapped to genes after RNA-seq analysis performed on FL E14.5 and ABM LT-HSCs. **(C)** The correlation coefficients between replicates of different samples derived from FL E14.5 and ABM LT-HSCs. ABM, adult bone marrow; FL, fetal liver; LT-HSC, long-term hematopoietic stem cell.



SUPPLEMENTARY FIG. S2. Morpholino knockdown of target genes in Tg:*gata1*;DsRed/*flk1*;EGFP zebrafish line. **(A)** Representative fluorescence images of embryos (48hpf): **(a–c)** uninjected control; after injection of 5 bp mismatch control MOs against *uhrf1* **(d–f)**, *tdg* **(g–i)**, and *uchl5* **(j–l)**. **(B)** Representative fluorescence images of embryos (48hpf): **(a–c)** uninjected control; after injection of splice-blocking MOs against *tdg* **(d–f)** and *uchl5* **(g–i)**. MOs, morpholino antisense oligonucleotides.



B

Overlapping of differential expressed genes (DEG)			
	Manesia JK <i>et al</i>	McKinney-Freeman S	Overlap
No. of DEG	576	128	83
	Manesia J <i>et al</i>	Beerman I <i>et al</i>	Overlap
No. of DEG	576	403	147

Threshold: Log₂FC ≥ 2, FDR ≤ 0.05

C

HSC source	Population	Cell surface phenotype	Reference	Accession NO.
Adult BM	LT-HSC	Lin-Sca-1+c-kit+CD150+CD34-	McKinney-Freeman S <i>et al</i> , 2012	GSE37000
FL E14.5	LT-HSC	Lin-Sca-1+c-kit+CD150+CD48-		
Adult BM	LT-HSC	Lin-Sca-1+c-kit+ CD34- Flk2- Il7ra-	Beerman I <i>et al</i> , 2014	GSE55525
FL E14.5	FL HSC	Lin-Sca-1+c-kit+ CD150+CD48-		

SUPPLEMENTARY FIG. S3. Cross comparison of RNA-seq with published microarray dataset. **(A)** Comparison of differentially expressed genes in this study with results from McKinney-Freeman *et al.* [6] and Beerman *et al.* [33]. Venn diagram depicting the overlap of differentially regulated genes in these three independent studies. Threshold used is: Log₂FC ≥ 2, false discovery rate ≤ 0.05. **(B)** Comparison of differentially expressed genes in this study with results from McKinney-Freeman *et al.* [6] and Beerman *et al.* [33]. Depicted is the number of genes differentially expressed between FL E14.5 and BM in the three independent studies and overlap estimated after cross comparison of differential gene expression. **(C)** Description of cell surface markers used for purification of LT-HSC from E14.5 FL and ABM in McKinney-Freeman *et al.* [6] and Beerman *et al.* [33].