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Supplemental Information

Generation of *foxn1*/*Casper* Mutant Zebrafish for Allograft and Xenograft of Normal and Malignant Cells

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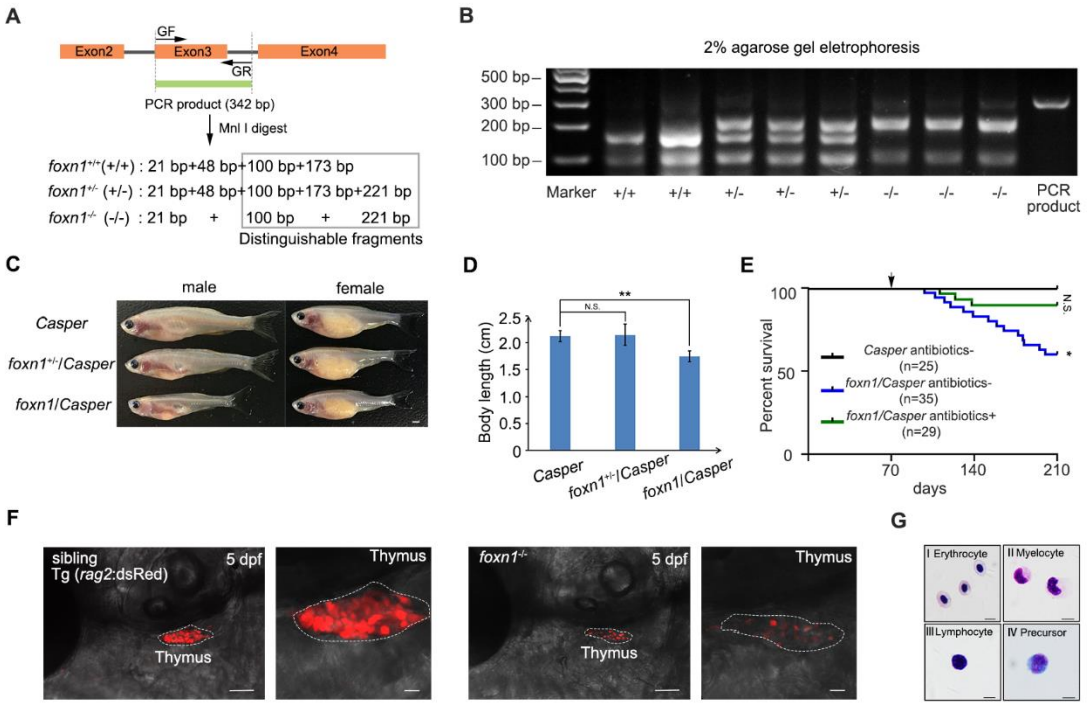

Figure S1

The Characterization of *foxn1/Casper* Mutant. Related to Figure 1

(A) Schematic representation of *foxn1* mutant genotyping. The mutated site is in 3rd exon. The designed genomic DNA PCR product length was 342 bp, and it can be digested by restriction enzyme Mnl1 into different fragments. 4 DNA fragments (21 bp, 48 bp, 100 bp, 173 bp) were obtained in wild type (WT), 5 DNA fragments (21 bp, 48 bp, 100 bp, 173 bp, 221 bp) were obtained in *foxn1*$^{+/-}$, while 3 fragments (21 bp, 100 bp, 221 bp) were obtained in *foxn1*$^{-/-}$.

(B) The 2% agarose gel electrophoresis analysis of different fragments. The 21 bp and 48 bp fragments were not shown in this gel image. The WT, *foxn1*$^{+/-}$ and *foxn1*$^{-/-}$ contain 2 fragments (100 bp and 173 bp), 3 fragments (100 bp, 173 bp and 221 bp) and 2 fragments (100 bp and 221 bp), respectively.

(C) The body shape of 3-month-old *Casper*, *foxn1*$^{+/-}$*/Casper* and *foxn1*/*Casper* mutant. The size of male mutant is smaller than *Casper* or *foxn1*$^{+/-}$*/Casper*, while female mutant is comparable to *Casper* or *foxn1*$^{+/-}$*/Casper*. Scale bar, 1 mm.

(D) The body length (centimeter, cm) of *Casper*, *foxn1*$^{+/-}$*/Casper and foxn1*/*Casper* mutant. The body length of male *foxn1*/*Casper* mutant is significantly shorter than that of *Casper* and *foxn1*$^{+/-}$*/Casper* (mean ± SD, one-way ANOVA, Tukey's; ***P* <0.01, N.S.=non-significant, n=6 in each group).

(E) Survival rates of *foxn1*/*Casper* under non-antibiotics and antibiotics-supplemented conditions. Black short arrow denotes the genotyping of *fonx1* mutant at 70-80 dpf. Broken lines from top to bottom indicate the *Casper* under non-antibiotics condition, *foxn1*/*Casper* under antibiotics-supplemented condition and *foxn1*/*Casper* under non-antibiotics condition, respectively (one-way ANOVA, Tukey's; N.S.=non-significant, **P* <0.05, n=10).

(F) The representative confocal images of T cells in 5 dpf control and *foxn1*$^{-/-}$ in Tg (*rag2*:dsRed) background. The white dashed line denotes the thymus region, and the number of *rag2*$^{+}$ cells are decreased in *foxn1*$^{-/-}$. Magnification: left panel in each group (20×), Scale bar, 50 μm; right panel in each group (20×, zoom 4×), Scale bar, 10 μm.

(G) Representative Wright-Giemsa staining pictures of different blood lineages based on Figure 1I. Scale bar, 5 μm.

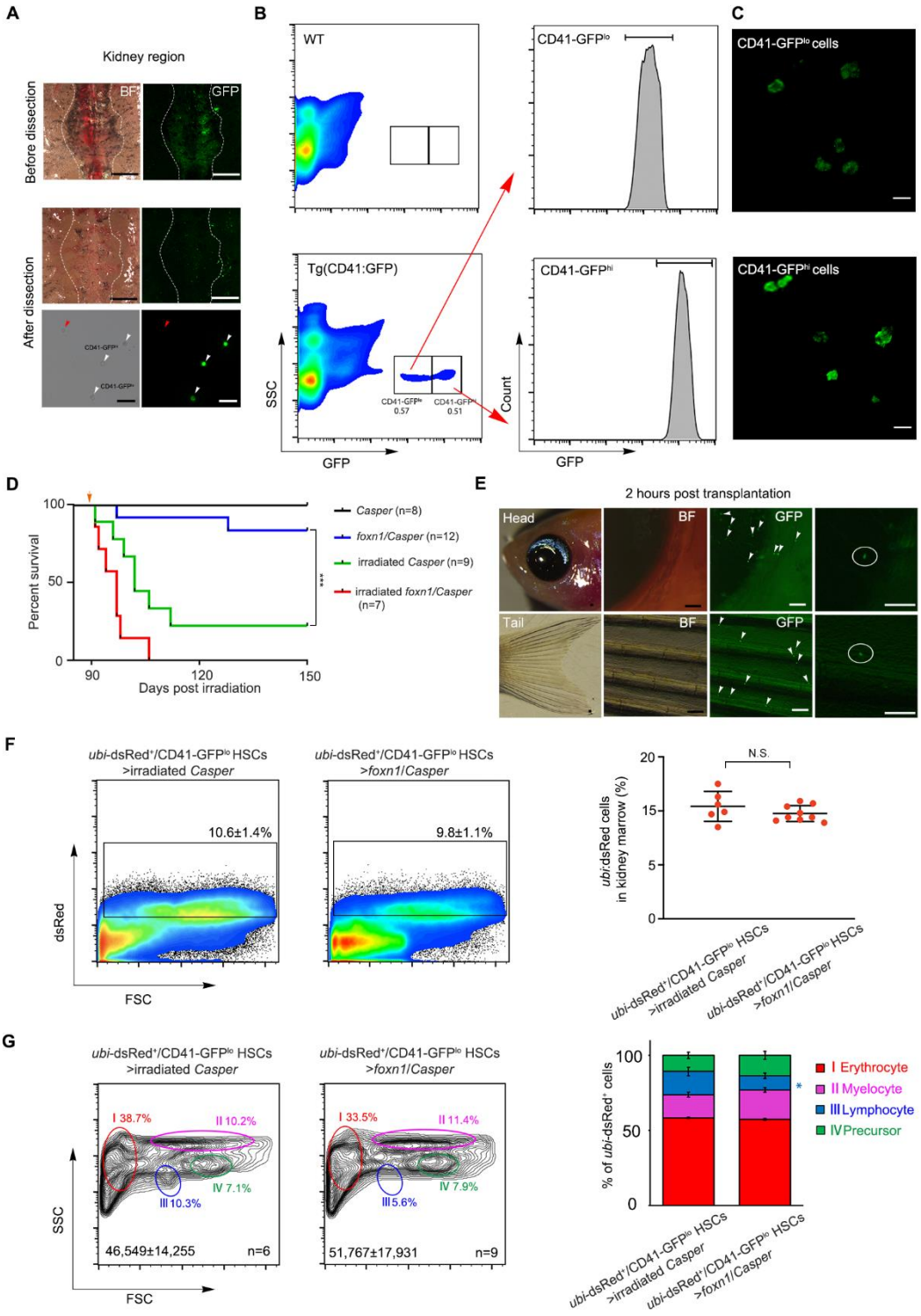

Figure S2

***Foxn1*/*Casper* Mutant Permits Nonconditioned HSCT. Related to Figure 2**

(A) The kidney marrow (KM) was obtained from Tg (CD41:GFP) adult zebrafish. The upper panels denote anatomical ventral view of KM, and middle panels indicate kidney was taken out. Scale bar, 500 μm. The lower panels indicate dissected single cells. The white arrows denote CD41-GFP^{+} cells

and the red arrow denotes CD41-GFP^{-} cells. Scale bar, 50 μm. The left row was shown in bright field (BF) and right in green fluorescent protein (GFP) field.

(B) Representative FACS images of CD41-GFP^{+} cells. CD41-GFP^{+} cells could be separated into GFP-low (CD41-GFP^{lo}) and GFP-high (CD41-GFP^{hi}) clusters, and the proportion of them in KM were 0.57% and 0.51%, respectively.

(C) Fluorescent microscopy of sorted CD41-GFP^{lo} and CD41-GFP^{hi} cells. Scale bar, 10 μm.

(D) Survival rates of *Casper*, *foxn1*/*Casper*, irradiated *Casper* and irradiated *foxn1*/*Casper* under antibiotics-supplemented condition. Orange short arrow denotes the 3-month-old zebrafish irradiated at 90 dpf. Broken lines from top to bottom indicate the *Casper*, *foxn1*/*Casper*, 25 Gy irradiated *Casper* and 15 Gy irradiated *foxn1*/*Casper*, respectively (n (*Casper*)=8, n (*foxn1*/*Casper*)=12, n (irradiated *Casper*)=9, and n (irradiated *foxn1*/*Casper*)=7).

(E) Imaging of the head and tail regions of *foxn1*/*Casper* mutant at 2 hours post transplantation. The left panels (BF, 4×), middle-left (BF, 10×), middle-right (GFP, 10×), right (GFP, 20×) are head and tail regions, respectively. Each white arrow and circle denote one CD41-GFP^{lo} cell. Bright field (BF), Green fluorescent protein (GFP) field. Scale bar, 100 μm.

(F) Flow cytometric analysis of engrafted *ubi*-dsRed^{+} cells in the recipient KM at 60 dpt (mean ± SD, n (*ubi*-dsRed^{+}/CD41-GFP^{+} HSCs>irradiated *Casper*)=6, n (*ubi*-dsRed^{+}/CD41-GFP^{+} HSCs>*foxn1*/*Casper*)=9). Statistical analysis (mean ± SD, *t* test; N.S.=non-significant)

(G) Multi-lineage differentiation potential of engrafted *ubi*-dsRed^{+}/CD41-GFP^{+} HSCs in recipients. Each circle represents different cell lineages (I: erythrocyte, II: myelocyte, III: lymphocyte and IV: precursor). Total cell numbers of whole KM were indicated by mean ± SD. The statistical analysis (mean ± SD, *t* test; **P* <0.05, n (*ubi*-dsRed^{+}/CD41-GFP^{+} HSCs>irradiated *Casper*)=6, n (*ubi*-dsRed^{+}/CD41-GFP^{+} HSCs>*foxn1*/*Casper*)=9).

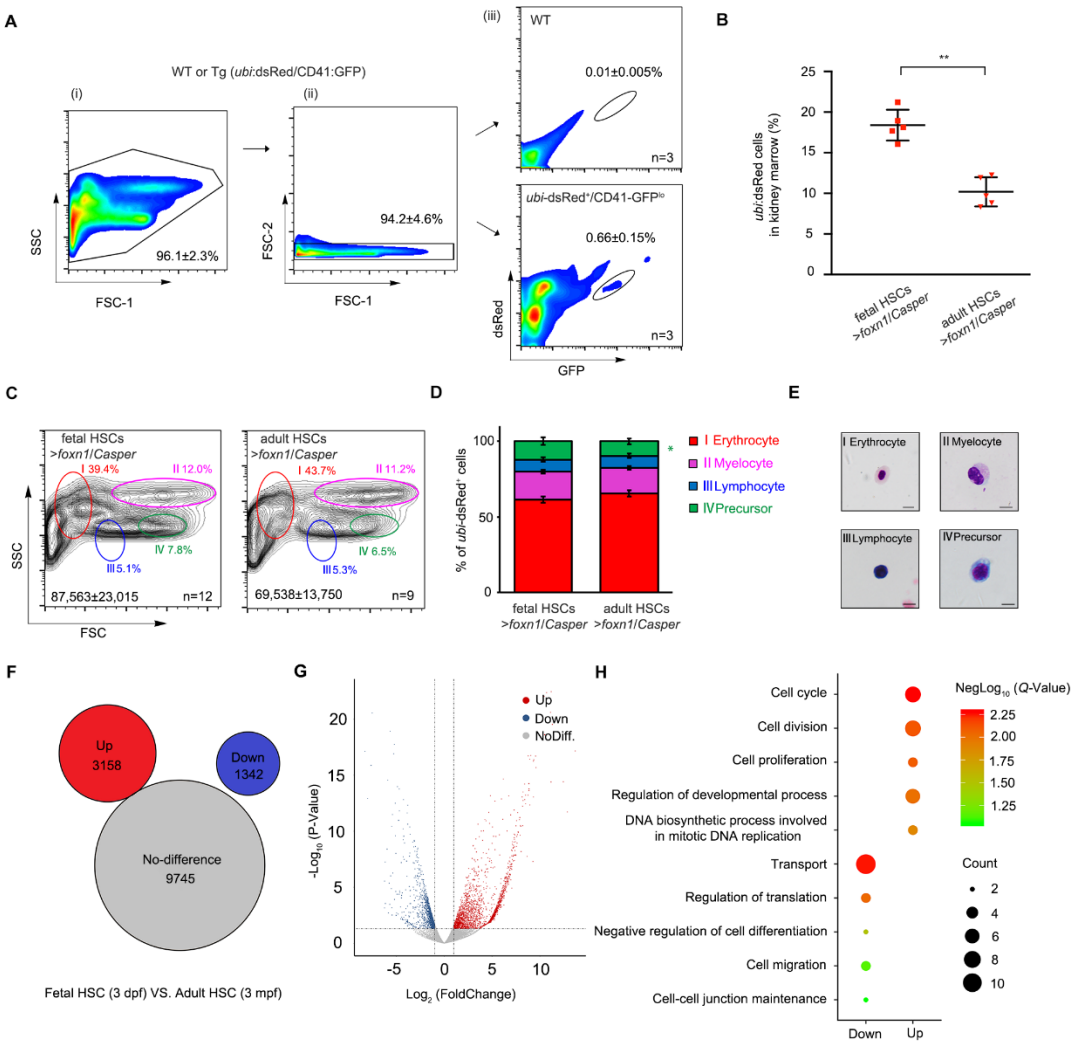

Figure S3

FACS and RNA-seq Data Analysis of Zebrafish Fetal and Adult HSCs. Related to Figure 3

(A) Representative FACS images of negative control. Live cells were gated in the first diagram, then single cell were gated for distinguish dsRed^{+}/GFP^{+} cells (mean ± SD, n (WT)=3, n (Tg (*ubi*:dsRed/CD41-GFP)=3).

(B) Statistical analysis of the engraftment efficiency of fetal and adult HSC at 90 dpt. (mean ± SD, *t* test; ***P* <0.01, n (fetal HSCs>*foxn1/Casper*)=5, n (adult HSCs>*foxn1*/*Casper*)=5).

(C) Multi-lineage differentiation potential of engrafted fetal and adult HSCs in *foxn1*/*Casper* recipients. Each circle represents different cell lineages (I: erythrocyte, II: myelocyte, III: lymphocyte and IV: precursor). Total cell numbers of whole KM were indicated by mean ± SD.

(D) The statistical analysis of (B) (mean ± SD, *t* test; **P* <0.05, n (fetal HSCs>*foxn1/Casper*)=12, n (adult HSCs>*foxn1*/*Casper*)=9).

(E) Representative Wright-Giemsa staining pictures of different blood lineages based on (C). Scale

bar, 5 μm.

(F) The bulk RNA-seq data of fetal HSCs and adult HSCs show that 3,158 and 1,342 genes were upregulated and downregulated, respectively. HSCs were sorted from Tg (CD41:GFP) zebrafish line and 2 replicates were performed in each group.

(G) Volcano analysis of gene expression level. Red, green and grey dots denote the upregulated, downregulated and no differential expressed transcripts, respectively.

(H) Gene ontology analysis of differentially expressed genes. Most of the upregulated genes were enriched in "cell cycle" and "cell division" terms. The heat bar denotes the significant difference and the size of circle denotes the number of differential genes in each term.

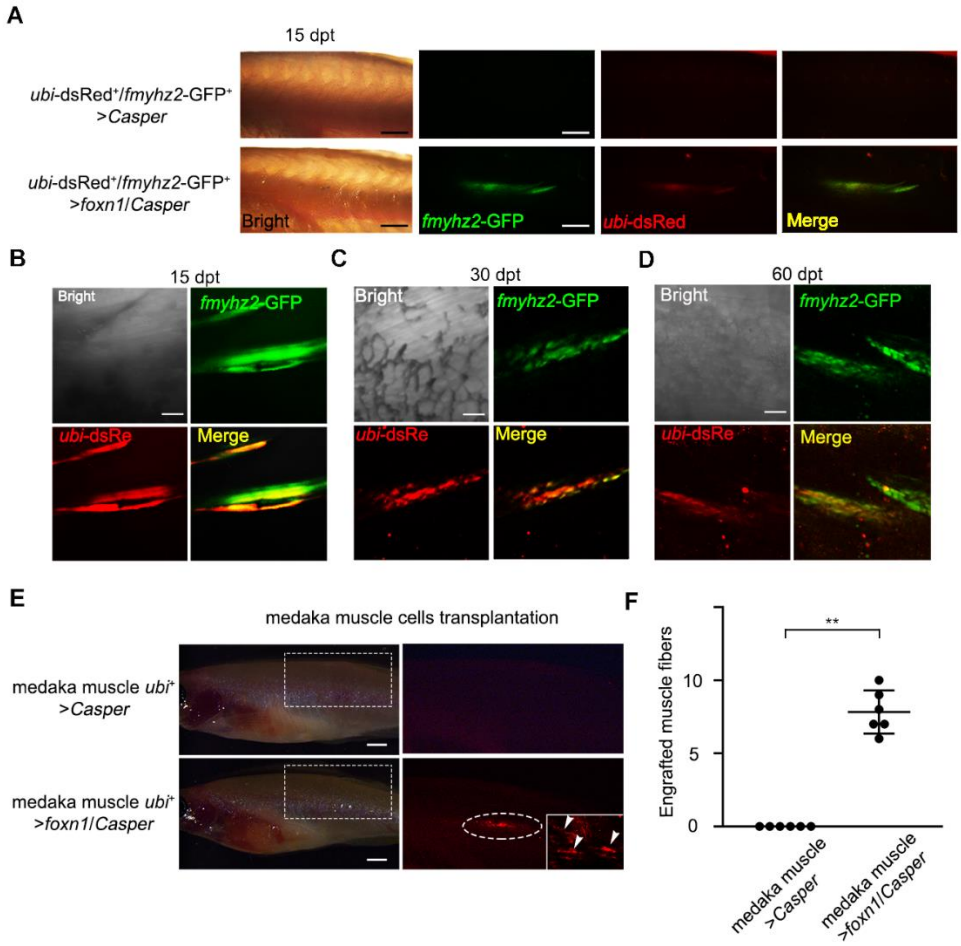

Figure S4

Allogeneic and Xenogeneic Muscle Cell Transplantation with Zebrafish Tg (*ubi*:dsRed/*fmyhc2*:GFP) and Medaka Tg (*ubi*:mCherry)

(A) The observation of engrafted muscle cells at 15 dpt. The upper panels denote *Casper* recipient, the lower panels denote *foxn1/Casper* recipient. n (*Casper*)=0/10, n (*foxn1/Casper*)=12/16. Scale bar, 1 mm.

(B and C) Confocal imaging of muscle cells at 15 and 30 dpt. Magnification: 20×. Scale bar, 100 μm.

(D) Confocal imaging of muscle cells at 60 dpt. Magnification: 20×. Scale bar, 100 μm.

(E) Confocal imaging of engrafted medaka muscle cells at 30 dpt. The *ubi*-mCherry^{+} cells could be visualized in *foxn1/Casper* recipients, not in the *Casper* recipients. n (*Casper*)=0/5, n (*foxn1/Casper*)=6/12. Scale bar, 1 mm.

(F) The statistical analysis of muscle fibers in each engrafted recipient (mean ± SD, *t* test; $^{**}P<0.01$, n (medaka muscle *ubi*$^{+}$>*Casper*)=6, n (medaka muscle *ubi*$^{+}$>*foxn1/Casper*)=6).

Table S1. The Statistical Analysis of Adult Kidney Marrow in WT, *foxn1*$^{+/-}$ and *foxn1*$^{-/-}$. Related to Figure 1

	Wild type sibling (n=4)	*foxn1* heterozygous sibling (n=5)	*foxn1* mutant (n=3)
I Erythrocytes	42.4±8.6%	53.6±7.2	58.8±6.3%
II Myelocytes	15.4±3.5%	16.1±4.1	12.4±5.2%
III Lymphocytes	13.8±2.8%	11.5±3.2	5.3±1.6% **
IV Precursors	6.4±2.3%	7.2±2.5	4.8±2.1% *

Note: data represented as mean ± SD (%) of each blood lineage in kidney marrows, *t* test (wild-type vs. mutant); *P <0.05, **P <0.01.

Table S2. Transplantation with flow cytometry sorted CD41-GFP^{lo} cells from Tg (CD41:GFP). Related to Figure 2

10,000 CD41-GFP^{lo} cells/recipients	Survival (%)	Engrafted (%)
Casper	10/10 (100)	0/10 (0)
foxn1/*Casper*	15/19 (79)	8/15 (53)
Irradiated *Casper* (25 Gy)	13/23 (56)	8/13 (62)
Irradiated *foxn1*/*Casper* (15 Gy)	4/21 (19)	4/4 (100)

Note: The proportion of CD41-GFP^{+} > 0.1% in recipient KM was set as the cut off for positive engraftment.

Table S3. Transplantation with flow cytometry sorted fetal and adult HSCs from Tg (*ubi*:dsRed/CD41:GFP) . Related to Figure 3

10,000 *ubi*-dsRed^{+}/CD41-GFP^{lo} cells/recipient		Survival (%)	Engrafted (%)
Fetal (3 dpf) derived HSCs	*Casper*	6/6 (100)	0/6 (0)
	foxn1/*Casper*	20/24 (83)	12/20 (60)
Adult (3 mpf) derived HSCs	*Casper*	7/8 (88)	0/7 (0)
	foxn1/*Casper*	15/20 (75)	9/15 (60)

Note: The proportion of *ubi*-dsRed^{+} > 1% of recipient KM was set as the cut off for positive engraftment.

Table S4. Quantitative PCR Primer Pairs

Gene	Forward Primer (5'-3')	Reverse Primer (5'-3')
foxn1	CATTAGGCACTGATGTGGAGAC	ACTGGTGTTGTAGAGGACTG
rag1	AGAGGACAGTGGGTAAAGA	ATGGGTTCAGGTGTTGGTT
rag2	TGAGACTCAGAAGCGCATGG	ACCAAGTACGACTGTGGCTG
lck	GCCTCCAGTCAGTCAGAATTT	TTGTATATGGCCACCACCAG
tcra	ACCAAGTGGGAAACTCATGC	TGCCCAGTGACAAGAAGTTG
tcrb	CCGAAGGGATTGAAGCTGGT	ACCAGTGCATACAGGAAGCTC
igm	GTTTCCTCAGCTCAACCA	AGTATAATCTCCTTCCTTCCC
igz	AAAGCAACGATACCAAAGTG	AACAGCTTGCAAGACAATTC
pax5	TCTGGCTGGCGGATTG	TGTGGAGTAACTGCCTTGTC
cd37	TGTTGACGGTTCTGCTC	TTCCTGCCTCTGAATGTA
il2rb	GAAGCCAGTTGAGCCG	TTTGGGTGAATCCTCTTTT
lyz	GTGAAAATGGACGGGCTGAA	CTTTGTTTGCGCTGCTCACA
mpo	CCGTGGATTGATTGGTCGTC	CACCACAGCCAATTCTTGCT
ccna1	TGGCTCAGGGTCATTTATGGA	ATTCTTCGCCAACTTCCACC
ccnb1	GGCGTTAAGGTTGTGTCTGAG	TTCACTGCAAAGCATGGGA
ccne	CTGGCTAATGGAGGTTTGTGAG	GGCAGCTATAAAGAGACAGGAG
ccnd1	CGGCGAATTATTGCAAATGGA	AGAGGGCCACAAAGGTCTG
ccnh	TTGATCTGAAGACCAGGTACCC	ATCCATCTTGAGGCCAGCAC
cdk1	GTTGTACGCCTGCTAGATGTG	CCCTCCAGGATCTGATAAAGGT
cdk2	GAATCTCCTCATCAACGCTCAG	GCCCTCCGAGTAATCATTTCAG
cdk7	AGTTGTCACAAGATGGTATCGG	GATCTCATCTGTTGGTGTTCCC
cdc20	TACGCACCAGAGGGTTATCAG	TGTCTCCTTCACCAGCATCC
pcna	GCATTCCAGAGCAGGAATACAG	TGATGTTGCCTGTGCCCA
cdkn1a	TGCAGAAGCTCAAAACATATTGTC	ACGCAAAGTCGAAGCTCCAG
cdkn1b	GTCTCGCTCATCGTGTCAAA	TATGTGGGTGTCGGACTCAA
foxn1	CATTAGGCACTGATGTGGAGAC	ACTGGTGTTGTAGAGGACTG