Supporting Information

Park and Han 10.1073/pnas.1203823109

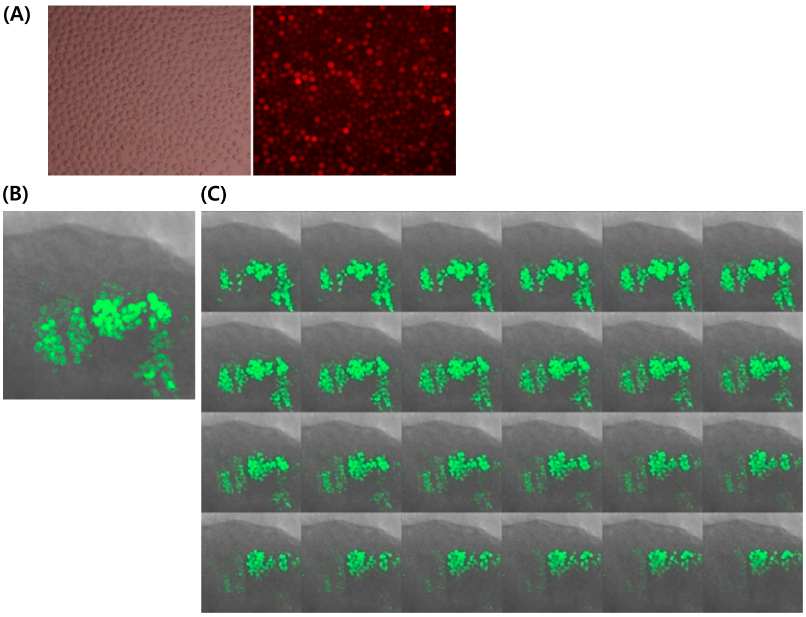


Fig. S1. (*A*) Transfection and G418-selection of SNUhp1 primordial germ-cll (PGC) line with a *piggyBac* CMV-DsRed expression vector (magnification, 200×). (*B* and *C*) Detection of GFP-expressing donor PGCs (SNUhp26 line) in testes of hatched germ-line chimera by confocal laser scanning microscope. (*B*) Merged image of all *z*-stack sections. (*C*) Serial *z*-stack images. (Magnification: 200×.) Many GFP$^+$ donor PGCs are distributed through seminiferous tubules of recipient testes.

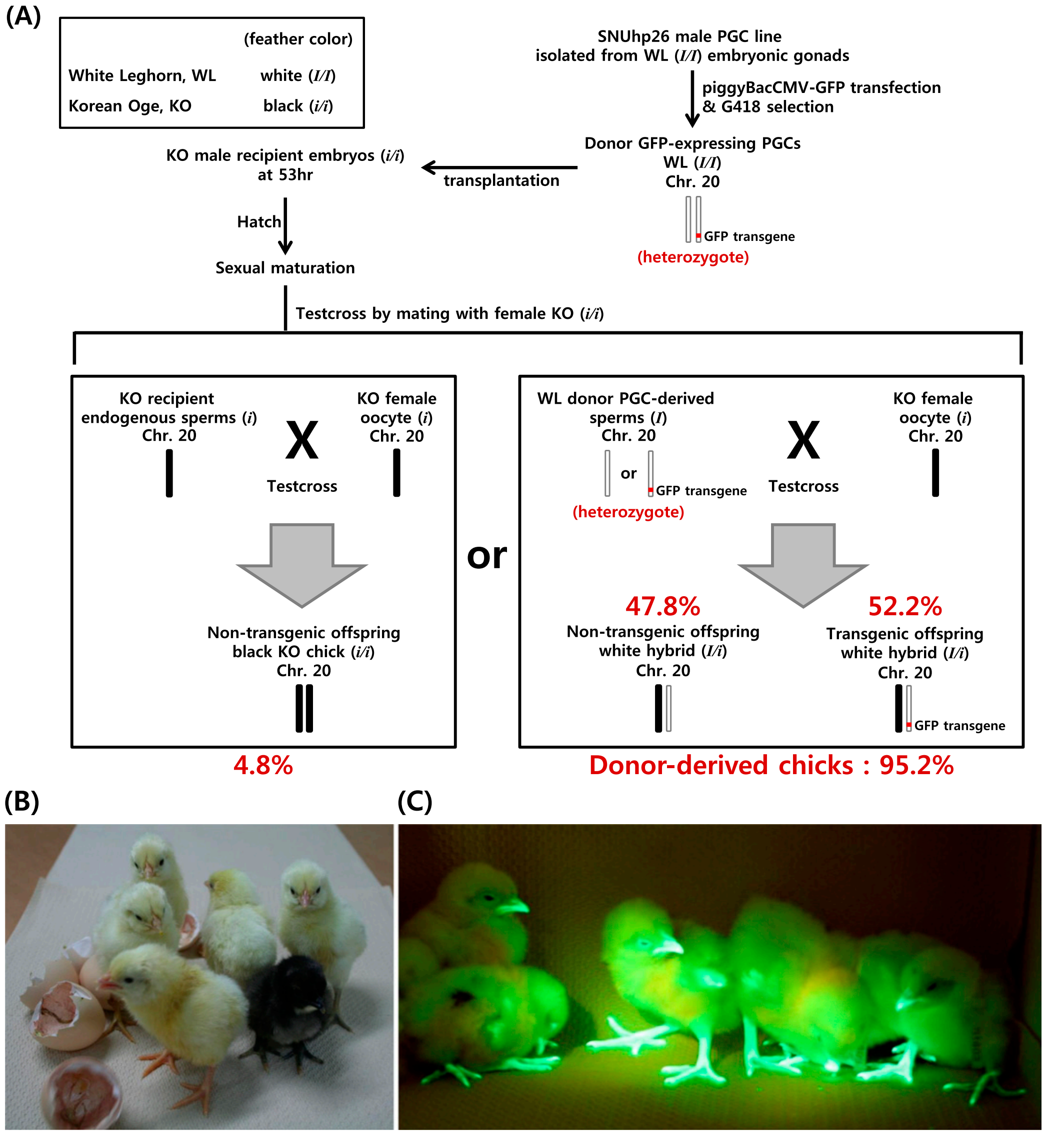


Fig. S2. (*A*) G418-selected and GFP-expressing donor PGCs, in which a single transgene integrated onto the distal end of chromosome 20 were transplanted into recipient embryos. Through test-cross analysis, the germ-line chimeras were identified by the phenotype of their offspring. Endogenous germ cells (sperm) in the recipient male chickens produce only black Korean Oge (KO) because of recessive pigmentation inhibitor gene (*i*/*i*), whereas White Leghorn (WL) donor-derived germ cells produce the white hybrids with *I*/*i*. The germ-line chimerism of donor-PGCs was 95.2%, and only 4.8% of progenies were derived from endogenous germ cells. Because GFP-expressing donor PGCs were heterozygous, in which a transgene was inserted into one chromosome 20, half of the donor-derived offspring (52.2%) were transgenic chicks and the other half (47.8%) were nontransgenic chicks derived from donor PGCs. (*B*) The phenotype of WL (*I*/*I*) or hybrid (*I*/*i*) between KO and WL is white, but that of KO (*i*/*i*) is black. (*C*) Generation of GFP-expressing transgenic chicks from germ-line chimeric founders through the test-cross. All of the transgenic progenies are strongly expressing GFP and their behavioral activity is energetic.

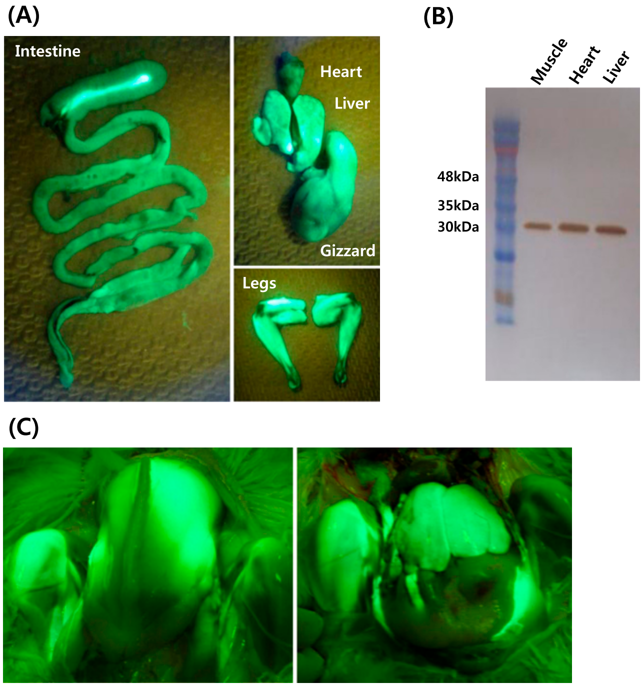
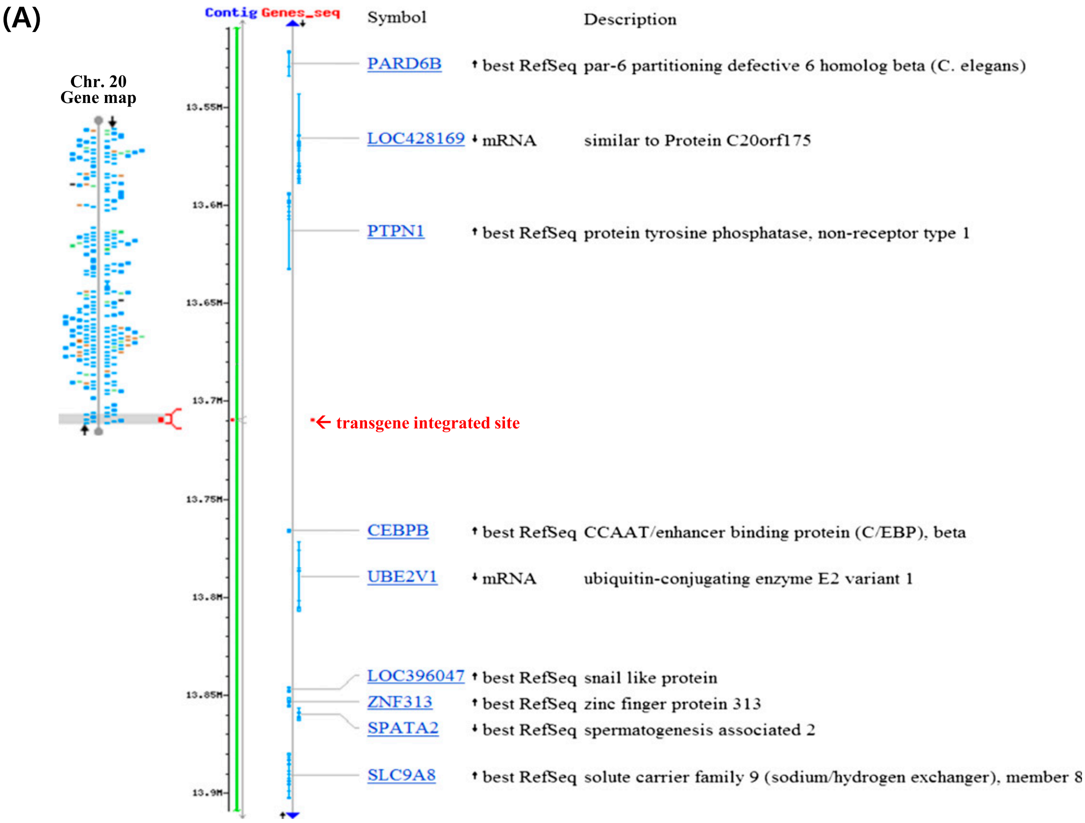


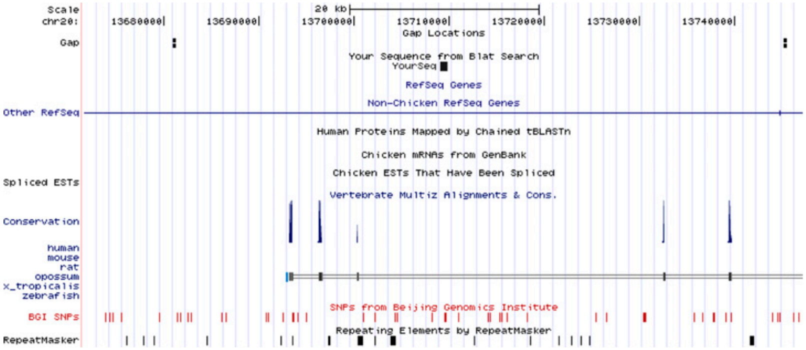
Fig. S3. (*A*) GFP expression in organs of transgenic chicken 7 d after hatch. All organs examined (intestine, heart, liver, and gizzard) in transgenic chickens showed strong GFP expression. (*B*) Western blotting of GFP in muscle, heart and liver of transgenic chickens. GFP was clearly detected in all tissues. (*C*) GFP expression of 20-wk-old transgenic chicken.



Symbol		Description
PARD6B	best RefSeq	par-6 partitioning defective 6 homolog beta (C. elegans)
LOC428169	mRNA	similar to Protein C20orf175
PTPN1	best RefSeq	protein tyrosine phosphatase, non-receptor type 1
CEBPB	best RefSeq	CCAAT/enhancer binding protein (C/EBP), beta
UBE2V1	mRNA	ubiquitin-conjugating enzyme E2 variant 1
LOC396047	best RefSeq	snail like protein
ZNF313	best RefSeq	zinc finger protein 313
SPATA2	best RefSeq	spermatogenesis associated 2
SLC9A8	best RefSeq	solute carrier family 9 (sodium/hydrogen exchanger), member 8

Fig. S4. (Continued)

(B)



(C)

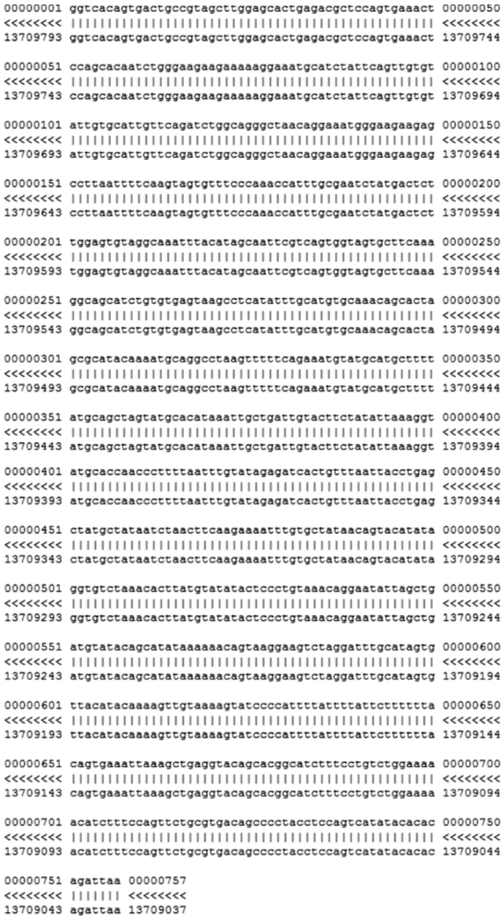


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Fig. S4. (*A*) Alignment and comparison of the transgene-flanking sequences from DNA walking analysis was conducted using the University of California at Santa Cruz Genome Browser (http://genome.ucsc.edu). A *piggyBac* GFP transgene was integrated into chromosome 20 (reverse direction). (*B*) No functional gene or transcript was observed in the flanking regions from the transgene integration site. This result is consistent with the BLAST Assembled Genome Database (http://blast.ncbi.nlm.nih.gov). (*C*) Alignment of sequences from DNA walking with chicken genomic sequences using UCSC genome browser.

Table S1. RT-PCR primer sequences and cycle conditions for sexing, germ-cell–, or stem-cell–specific genes

Gene	Sequences	Annealing temperature (°C)	Cycles
USP	F5′-CTATGCCTACCACATTCCTATTTGC-3′ R5′-AGCTGGACTTCAGACCATCTTCT-3′	60	35
CPE15	F5′-AAGCATAGAAACAATGTGGGAC-3′ R5′-AACTCTGTGTGGAAGGACTT-3′		
Qsex	F5′-CTATGCCTACCACATTCCTATTTGC-3′ R5′-AGCTGGACTTCAGACCATCTTCT-3′	66	35
Vasa	F5′-GCTCGATATGGGTTTTGGAT-3′ R5′-TTCTCTTGGGTTCCATTCTGC-3′	65	40
Dazl	F5′-GCT TGC ATG CTT TTC CTG CT-3′ R5′-TGC GTC ACA AAG TTA GGC A-3′	65	35
PouV	F5′-AAATGTGTGAAGCCCAGTCC-3′ R5′-TTGTGGAAAGGTGGCATGTA-3′	65	30
Nanog	F5′-GGTGAGAGTGGGACAAGGAA-3′ R5′-CCAGATACGCAGCTTGATGA-3′	60	30
GAPDH	F5′-GGTGGTGCTAAGCGTGTTAT-3′ R5′-ACCTCTGCCATCTCTCCACA-3′	65	30