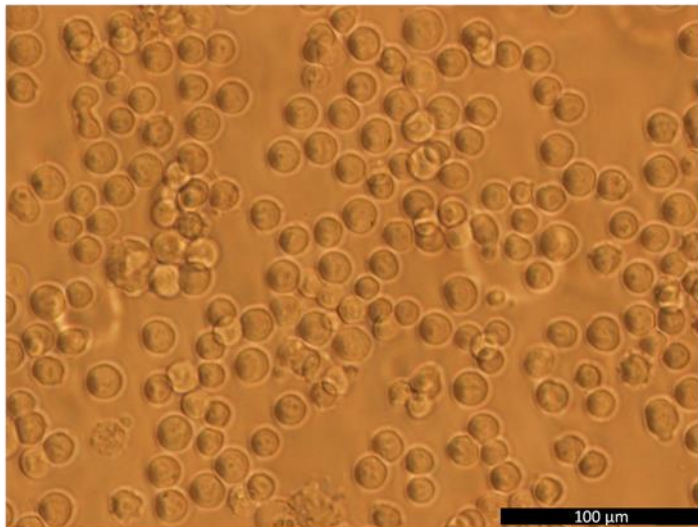
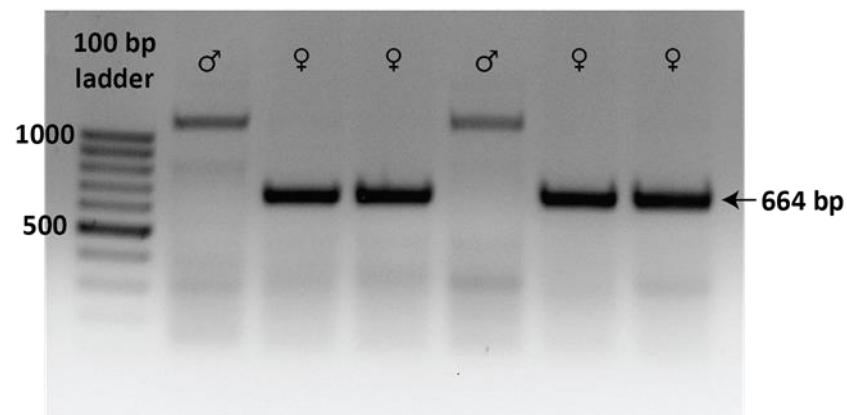


## Supplementary Information Appendix

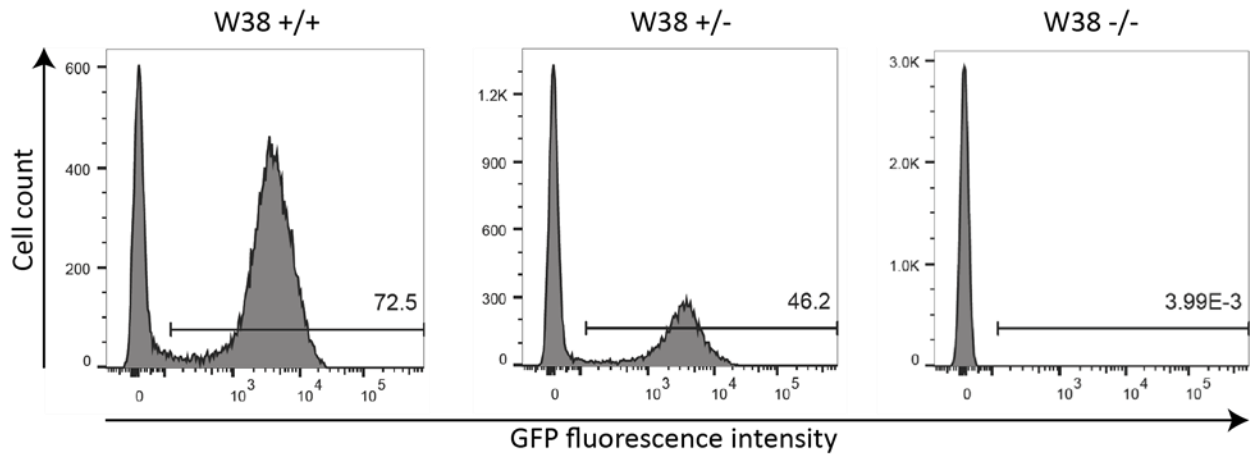
**A**



**B**



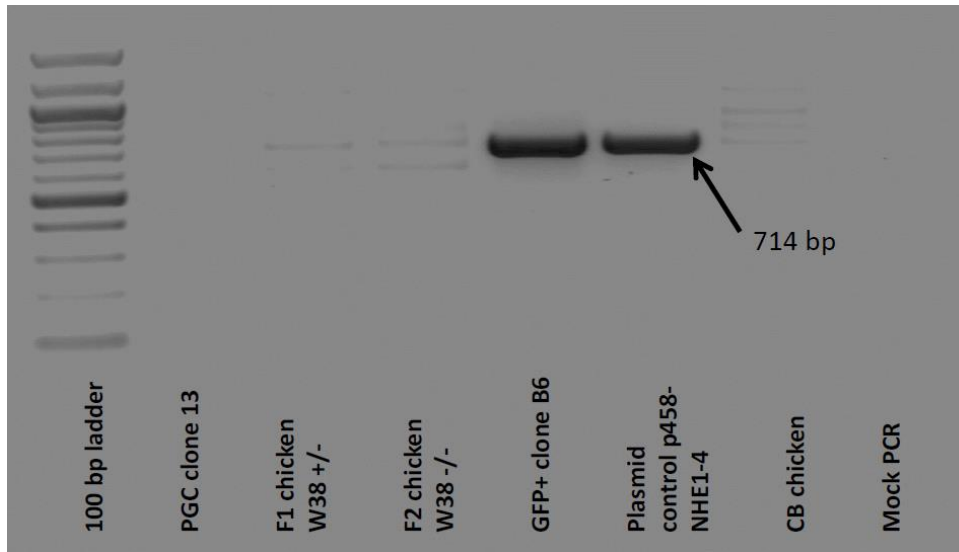
**Figure S1.** Derivation and sex determination of chicken PGCs. (A) Microscopic image of the PGC clone 13 showing the size, round shape, non-adherence, and low level of cell-to-cell clustering. (B) Detection of the W-chromosome-specific PCR product of 664 bp in several PGC lines derived in this study.



**Figure S2.** *In vitro* infection of fibroblasts derived from W38 +/+, W38 +/-, and W38 -/- embryos with GFP-transducing J subgroup-specific retrovirus RCASBP(J)GFP. Examples of FACS histograms of GFP-positive cells for each of W38 genotypes.



**Figure S3.** *In vivo* tumor induction by a transforming virus pseudotyped with J subgroup envelope. An example of the progressively growing tumor being dissected from the wing web of W38 +/+ chicken.



**Figure S4.** Amplification and detection of the GFP-specific PCR product of 714 bp in the CB PGC clone 13 (at the time of orthotopic transplantation), F<sub>1</sub> and F<sub>2</sub> chickens derived from the CB PGC clone 13 (genotypes W38 +/- and W38 -/-, respectively), Jurkat cell clone B6 contained single integrated copy of GFP gene per diploid genome, and plasmid control (p458-NHE1-4 CRISPR/Cas9 construct). Intact CB chicken and mock PCR were used as negative controls.

**Table S1.** Partial sequence of chNHE1 around the W38-encoding TGG triplet in PGC clones derived by CRISPR/Cas9-mediated homologous recombination.

NHE1 wt	CGCGTGTCTCCGAGCCCACC <b>TGGG</b> AGCAGCCGTGGGGAGAGCCCAGGGG
ssODNΔW38	CGCGGGTCTCCGAGCCCACC---GAGCAGCCGTGGGGAGAGCCCAGGGG
PGC clone 1	CGCGTGTCTCCGAGCCCACC---GAGCAGCCGTGGGGAGAGCCCAGGGG
PGC clone 2	CGCGTGTCTCCGAGCCCACC---GAGCAGCCGTGGGGAGAGCCCAGGGG
PGC clone 3 <sup>a</sup>	CGCGGGTCTCCGAGCCCACC---GAGCAGCCGTGGGGAGAGCCCAGGGG
PGC clone 4	CGCGGGTCTCCGAGCCCACC---GAGCAGCCGTGGGGAGAGCCCAGGGG
PGC clone 5	CGCGKGTCTCCGAGCCCACC---GAGCAGCCGTGGGGAGAGCCCAGGGG
PGC clone 6	CGCGKGTCTCCGAGCCCACC---GAGCAGCCGTGGGGAGAGCCCAGGGG
PGC clone 7	CGCGKGTCTCCGAGCCCACC---GAGCAGCCGTGGGGAGAGCCCAGGGG
PGC clone 8	CGCGKGTCTCCGAGCCCACC---GAGCAGCCGTGGGGAGAGCCCAGGGG
PGC clone 9	CGCGGGTCTCCGAGCCCACC---GAGCAGCCGTGGGGAGAGCCCAGGGG
PGC clone 10	CGCGTGTCTCCGAGCCCACC---GAGCAGCCGTGGGGAGAGCCCAGGGG
PGC clone 12	CGCGGGTCTCCGAGCCCACC---GAGCAGCCGTGGGGAGAGCCCAGGGG
PGC clone 13 <sup>a,b</sup>	CGCGGGTCTCCGAGCCCACC---GAGCAGCCGTGGGGAGAGCCCAGGGG
PGC clone 14	CGCGGGTCTCCGAGCCCACC---GAGCAGCCGTGGGGAGAGCCCAGGGG
PGC clone 15	CGCGGGTCTCCGAGCCCACC---GAGCAGCCGTGGGGAGAGCCCAGGGG
PGC clone 16	CGCGGGTCTCCGAGCCCACC---GAGCAGCCGTGGGGAGAGCCCAGGGG
PGC clone 17	CGCGTGTCTCCGAGCCCACC---GAGCAGCCGTGGGGAGAGCCCAGGGG
PGC clone 18	CGCGKGTCTCCGAGCCCACC---GAGCAGCCGTGGGGAGAGCCCAGGGG
PGC clone 19	CGCGGGTCTCCGAGCCCACC---GAGCAGCCGTGGGGAGAGCCCAGGGG
PGC clone 20	CGCGKGTCTCCGAGCCCACC---GAGCAGCCGTGGGGAGAGCCCAGGGG
PGC clone 21	CGCGGGTCTCCGAGCCCACC---GAGCAGCCGTGGGGAGAGCCCAGGGG
PGC clone 25	CGCGGGTCTCCGAGCCCACC---GAGCAGCCGTGGGGAGAGCCCAGGGG
PGC clone 41	CGCGGGTCTCCGAGCCCACC---GAGCAGCCGTGGGGAGAGCCCAGGGG
PGC clone 42	CGCGTGTCTCCGAGCCCACC-----GCCGTGGGGAGAGCCCAGGGG
PGC clone 45	CGCGTGTCTCCGAGCCCACC-----GGGG
PGC clone 46 <sup>a</sup>	CGCGGGTCTCCGAGCCCACC---GAGCAGCCGTGGGGAGAGCCCAGGGG
PGC clone 47 <sup>a,b</sup>	CGCGGGTCTCCGAGCCCACC---GAGCAGCCGTGGGGAGAGCCCAGGGG
PGC clone 49	CGCGGGTCTCCGAGCCCACC---GAGCAGCCGTGGGGAGAGCCCAGGGG
PGC clone 50	CGCGGGTCTCCGAGCCCACC---GAGCAGCCGTGGGGAGAGCCCAGGGG
PGC clone 52	CGCGTGTCTCCGAGCCCACC---GCAGCCGTGGGGAGAGCCCAGGGG
PGC clone 53	CGCGGGTCTCCGAGCCCACC---GAGCAGCCGTGGGGAGAGCCCAGGGG
PGC clone 54	CGCGGGTCTCCGAGCCCACC---GAGCAGCCGTGGGGAGAGCCCAGGGG
PGC clone 55	CGCGGGTCTCCGAGCCCACC---GAGCAGCCGTGGGGAGAGCCCAGGGG
PGC clone 56	CGCGGGTCTCCGAGCCCACC---GAGCAGCCGTGGGGAGAGCCCAGGGG
PGC clone 59	CGCGTGTCTCCGAGCCCACC--GGAGCAGCCGTGGGGAGAGCCCAGGGG

<sup>a</sup>Expanded PGC clones. <sup>b</sup>PGC clones used for orthotopic transplantation. Yellow background denotes the silent mutation T96G, K on the grey background denotes the T96G mutation in heterozygous state.

**Table S2.** Extensive data on the tumor induction in  $\Delta$ W38 chickens.

Chicken No.	W38 genotype	Age of chicken at virus inoculation (days)	Tumor weight (g) 16 days p.i.	
			500 FFU	250 FFU
561	-/-	56	0	0
569	-/-	34	0	0
588	-/-	20	0	0
596	-/-	13	0	0
562	+/-	48	1.5	0.8
563	+/-	48	8.7	5.2
566	+/-	40	4.8	4.2
570	+/-	34	9.3	5.5
571	+/-	34	12.3	10.2
578	+/-	27	5.4	2.1
586	+/-	20	8.2	5.6
587	+/-	20	15.6	12.2
597	+/-	13	32.1	20.7
598	+/-	13	21.3	10.4
599	+/-	13	16.8	10.3
564	+/+	48	27.4	22.7
565	+/+	48	7.4	4.7
567	+/+	40	12.8	10.8
579	+/+	27	11.5	6.7
600	+/+	13	12.7	10.2

**Table S3.** List of oligonucleotide primers and ssODN used in this study<sup>a</sup>.

Primer name	Primer sequence (5' to 3')
W4 forward	GCACAGATGGAGACAAAAGC
W4 reverse	TTGGACACTTCAGGCTACAG
gRNA1	CACCGCCCCACGGCTGCTCCCAGGT
gRNA2	AAACACCTGGGAGCAGCCGTGGGGC
3'cDNA synthesis primer	AAGCAGTGGTATCAACGCAGAGTACT <sub>30</sub> VN <sup>b</sup>
ECL1 forward	CGGCTCCCTCCGCCATG
ECL2 reverse	TCATCAGGCAGGCCAGCAGGAT
J subgroup <i>env</i> forward	CACTGATAAGGTTATTTGGGTACC
J subgroup <i>env</i> reverse	GCACACAAGTATCATTTGAAAGAA
ssODN $\Delta$ W38	GCCCGCTGCTGCCCCGGCCAGCGCTTGCAGGCCGACGCCA CGCGGGTCTCCGAGCCCACCGAGCAGCCGTGGGGAGAGC CCGGGGGTATCACCGCCGCCCGCTGGCCACGGCCCAGG AGGTGCACCCGCTGAACAAACAGCACCACAACCACTC
GFP-F	GTGAGCAAGGGCGAGGAG
GFP-R	CTTGTACAGCTCGTCCATGC

<sup>a</sup>Oligonucleotides used for amplification of the potential off-target sites are given in SI Appendix (Table S4B).

<sup>b</sup>VN means the random sequence of V (ACG) and N (ACGT).

**Table S4.** Analysis of off-target mutations.

A. List of potential off-target sites with highest CFD score and core sequence of gRNA (at least 10 consecutive matched nucleotides close to protospacer adjacent motif). The mismatches between gRNA and off-target site are in bold, the core sequences are underlined, and the protospacer adjacent motifs are in italics.

OFF-TARGET NUMBER	SEQUENCE	CFD SCORE
NHE1	CCCCACGGCTGCTCCCAGGT <i>GGG</i>	1.00
1	<b>GCACA</b> <u>AGCTGCTCCCAGGT</u> <i>CGG</i>	0.73
2	<b>GCCCAT</b> <u>TCGT</u> <u>TGCTCCCAGGT</u> <i>GGG</i>	0.51
3	<b>ATCCACT</b> <u>GCTGCTCCCAGGT</u> <i>AGG</i>	0.51
4	<b>TCCCCT</b> <u>GACTGCTCCCAGGT</u> <i>GGG</i>	0.46
5	<b>GCACAC</b> <u>GGCTGCTCCCAGGA</u> <i>GGG</i>	0.44
6	<b>CTCCAG</b> <u>GGAT</u> <u>TGCTCCCAGGT</u> <i>GGG</i>	0.39
7	<b>CCTCAT</b> <u>TCGC</u> <u>AGCTCCCAGGT</u> <i>GGG</i>	0.37
8	<b>TCCC</b> <u>CAGC</u> <u>CTGCTCCCAGGT</u> <i>AGG</i>	0.28

**B. Specification of potential off-target sites with the position of primers used for PCR amplification (underlined) and the sequence of gRNA complementary site (in bold)**

**OFF-TARGET 1**

mm4\_intergenic\_MVP|ENSGALT00000060508.1/ENSGALT00000062859.1\_chr28\_80996

mm4\_intergenic\_MVP|ENSGALT00000060508.1/ENSGALT00000062859.1\_chr28\_71655

mm4\_intergenic\_MVP|ENSGALT00000060508.1/ENSGALT00000062859.1\_chr28\_61901

mm4\_intergenic\_MVP|ENSGALT00000060508.1/ENSGALT00000062859.1\_chr28\_52071

GACACAGAAGAGCCTCCAGGCAGCAAGGGAGGGCCACACATGAAGCCCTTCCACAGCGGGACGTCAGGCTGCG  
ACTTCCACAGAGACCCGAGCACACCGCGGCAGCCGTCCCAACAGCGGCGCCAGCCTGG**GCACAAAGCTGCT**  
**CCAGGTCGGT**CCATCAGCAGAACCATAATCTCCACAGCCCCACCCTCAGTGTGCATAGGAACAGCCGTGTGCCA  
ATGACACCTGCTCAACTTGGTCAACATCAGCCAAGACAATAAATCCCACTCATTTACACCAGCTTCCGTTCCCTC  
GTCTTTTCCTG

1F GACACAGAAGAGCCTCCAGG

1R CAGGAAAAGACGAGGGGAAC

**OFF-TARGET 2**

mm4\_intergenic\_NCK2|C2orf40\_chr1\_135467756

CTGAGGGTGGTTAGCTGCTCCCATGCCATCGTTGCTCCAGGTGGGAATGACTACAGCTCTTTAACCAGAGTA  
TTTGTTCGATCAAACCTGGGCAGTAACTGTGTGCTGTCTTCAAAGTCATTCAAACCTGAATTAATGGAGTGCTCTTGT  
AAGCTGAAGCATGGGGAGGCAAGTAAATCCCTTTCCCAAGTCTCAGAGGAGCTGTTGTTTCCCTGCAGCATGCA  
GGGACTCACACGTGTCTAAAATGTCACATATCAGTCTCAGAGCCCACGCTGCTGTTCCAGGTAATACCTTGGTAAG  
CACACAGTTTTTGTCCCTCTCGACCTAGCAATGTGTGTGCCATGTCATCATCTCTCTTTTTGTTTTAATAGGGACT  
GGTTATGCCGCGCAGCATGACAATTAG

2F CTGAGGGTGGTTAGCTGCTC

2R CTAATTGTCATGCTGCCGGC

**OFF-TARGET 3**

mm3\_intron\_DIP2C\_chr2\_10160780

GCGAAGGCAGAAAACACGAGTAAGATTCAGAAAACCAACAAAAAAGTTGGAATTATGTTCCAAATGTGCTCCAGA  
CCCCTACCAGCTTTGTTGCCCTCTCTGAACGCACTCCATGGCCTCAATGTCTTTCTTGTAGTCCTTACACATCTGT  
GAAAACATACAT**CCACTGCTGCTCCAGGTAGG**ACAAAGGAGCGACAAAGCTCAGTCTCCCCAGGTCAGTCCACA  
CCCTATTTTCTGAGAACACTAAAAGTCAAATAAATCAATTCCTGCAATCCTAAGTGAGTTGTTTTGACCTAAGACT  
TATTGCAGTTTAAATGCCAACAAAACCAACCTTTTCTATTGCCGCTATAAATTCGGTGTCTGCTGCACCACCTCA  
GGACCTCAAC

3F GCGAAGGCAGAAAACACGAG

3R GTTGAGGTCTGAGGTGTGG



**OFF-TARGET 4**

mm4\_intron\_NDUFA10\_chr7\_6550501

CGTAGCCCTGTCCTTCCTTGCCCCAGCTTGTTCTGCTCCAGCTTCTTCTTTTCTGGTTCCTCCCCGAGGTTTTACC  
TCCCCAGAGAGGCTCTGCCACTCCTGGAAACCTCCTTAGTCACCTTTTTGCCTTCATACCTGAACAAGGTAA  
AACTGCTGGTGTACAAAATTCCACATGCTATAGTAACTTCATTTAATTACCTTTCCTGTTCTATTTGCTTTCACAAAT  
GAACCCTCACAAGGATTATGAGAAGATAGTGCTCTTCTTCTAAAGTTCTCATTTTAAAGGGTGTGAGGCAAACA  
GATGTTTTGGAATCAGGCAGTACCCCCAGCAC**CCACCTGGGAGCAGTCAGGGGA**AGTTTGCTGCAACAGCTGG  
TAGGACA

4F CGTAGCCCTGTCCTTCCTTG  
4R TGTCTACCAGCTGTTGCAG

**OFF-TARGET 5**

mm3\_intron\_CHRN2\_chr25\_1733917

AGGGATGTTACACCCACACCATGGGATCCCTGCCTGGCCACGTCCCCAGGAGCCCCCAGCCCCATATCTGACC  
CCTTCCCCATCAGCAC**CGGCTGCTCCAGGAGGG**CTGACCCACGCAGCCGCTGCCGCAGCACCGTGAGGCTGC  
AGATTTGAGGCAGCCTGGGATGGGACACAACCCAGCGTGGCTGAGCAGCGAGATGAGTTTCTGCACTCAGC  
AGAGGAGCTGCCAGGGCAGGGATGAGCCAGGGAGCCGCAGTGCTGAGGGTGCAGATCTGGGGCTCCGCAGGA  
TAAGAGGGCCAAGAGCTGAACCCCCCTGAGCTGACCAAACCCATCCCCGGCCCCACGCAGGTGAGCGAGGACTG  
GAAGTACGTGGCCATGGTCA

5F AGGGATGTTACACCCACAC  
5R TGACCATGGCCACGTACTTC

**OFF-TARGET 6**

mm3\_intergenic\_SLC2A1|ENSGALT00000066529.1/ENSGALT00000060478.1\_chr21\_6489988

CAATAGCCCCAAATTGCCGGCTCAGGCCAGGGCTTGTCCTCCTGTTGCAATACCCTGGCTCTGCTCTGGCATGCT  
GGGTGCAGTGGCCACGTCCCAGCTGGCCCACGATGCCGATCTGAGCTCCGTGTGGGGTTTGGGGCACAGAGA  
GCTCCTTCTATGCGAGGACCAGGAGGGTCCCTCCCTCCTGCAGCACCTCATCCCATCTCCACCT**CCAGGGATG**  
**CTCCAGGTGGG**TTCCCCCTCCTGGTTCCGACTGGGGTGGGAAGCACAGGCGACAGACACGTGTCTTTGCATAC  
TGACGGCTCAATCCATCCATCTGCTCATTGTTCTTGAGAGATGTAGGGCTGATTTCTTGTTGCCCGTGCCTCAGT  
TTCCCCACTCGCAGGACCCACCCAGCACTGCAGCCGAGCAGAGAACTCTGTGCTCCCT

6F CAATAGCCCCAAATTGCCGG  
6R AGGGGAGCACAGAGTTCTCT

**OFF-TARGET 7**

mm4\_intergenic\_IKZF3|ZPBP2\_chr27\_4696804

CAGACGTTTACCCGGACACAAAACGAGGGTTCCGTATTTCTCGTTTTCAATACCATCTACAGCTCAGAGCCCATT  
GCCCTCTCCATTGCGACCCCTCTGCGGTAGCACTGATGGAGCAGAAAGCACAGGAAGCGCCACAGCCCCTCTTC  
CACAGCCAAGGACAGACCTTGTGGCCCTTACAGGATCCGTTGGCACTGACACGGGTCCAATT**CCACCTGGGAGC**  
**TGCGATGAGG**ATCCCATGGGATGCTGGAGCCTGCAGAGCCCTGCAGCTGCAGGCTGGCTTCCGGGCTTACCCTC  
CTCACGCTGTACAGCCGTCCGCTTCTTCTCCTTCTCTTTCTCTTTTCTCTTTTTTTTTTTTTTTTTAAAC

AGATCTGAACATCTTTTCTCTGCTTTGCACACAACCCAGGAGCGGCTCCAGGAGGGACAGAGCCCATTCCCACTGG  
TTTGGT

7F CAGACGTTTACCCGGACACA

7R ACCAAACCAGTGGGAATGGG

**OFF-TARGET 8**

mm4\_intron\_DYM\_chrZ\_1166318

GGCCAAACACTGTGCAAGAGCAGGACATGTCGATCTGACCTGCTGATCAAGGGCCAGATTAACCAAAAATTCTG  
AAATTAATAATGTGCAAAAGTGATGAAATTAGAATTTGCCAACCGCTTCATTAATTTCTTAACGTTGGCAATAATG  
AGACGTAATTGCTGGCTCGAAACTCCCCAGCCTGCTCCAGGTAGGAAGATGAATGTGTCCCTCCCCTACTGTGGTT  
TGCACTGATCGTTCTGGTTTTCTGCTTCTTTGTCCCTTTGATGTTCAGGTGCTTTTGGGCAGCCCTGTAGCATCCA  
GCTTCCTGTGCAGCAGTGGGGCAGTTGGTGCAGCCAAGGTAACCAAATCAAAGGAATTTCTTAGGGAGAAAGAC  
AGGGAAAAATGGTCAGGTTGCTGCAGAAA

8F GGCCAAACACTGTGCAAGAG

8R TTTCTGCAGCAACCTGACCA

**C. Alignments of the potential off-target site sequences of the CB inbred chicken line, the W38 -/- PGC clone 13, and NCBI chicken genome. Sequencing primers (either forward or reverse) are highlighted with green color, the gRNA complementary sequence with red color.**

**OFF-TARGET 1**

```

NCBI chicken   GACACAGAAGAGCCTCCAGG CAGCAAGGGAGGGCCACACATGAAGCCCTTCCACAGCGG
CB             -----TTCCACAGCGG
PGC clone 13   -----TTCCACAGCGG
                *****

NCBI chicken   GACGTCAGGCTGCGACTTCCCACAGAGACCCGAGCACACCCGGCAGCCGGTCCCAACAG
CB             GACGTCAGGCTGCGACTTCCCACAGAGACCCGAGCACACCCGGCAGCCGGTCCCAACAG
PGC clone 13   GACGTCAGGCTGCGACTTCCCACAGAGACCCGAGCACACCCGGCAGCCGGTCCCAACAG
                *****

NCBI chicken   CGGCGGCCAGCCTGGG GCACAAAGCTGCTCCCAGGTCGGTCCATCAGCAGAACCATAATCT
CB             CGGCGGCCAGCCTGGGCACAAAGCTGCTCCCAGGTCGGTCCATCAGCAGAACCATAATCT
PGC clone 13   CGGCGGCCAGCCTGGGCACAAAGCTGCTCCCAGGTCGGTCCATCAGCAGAACCATAATCT
                *****

NCBI chicken   CCACAGCCCGACCCTCAGTGTGCATAGGAACAGCCGTGTGCCAATGACACCTGCTCAACT
CB             CCACAGCCCGACCCTCAGTGTGCATAGGAACAGCCGTGTGCCAATGACACCTGCTCAACT
PGC clone 13   CCACAGCCCGACCCTCAGTGTGCATAGGAACAGCCGTGTGCCAATGACACCTGCTCAACT
                *****

NCBI chicken   TGGTCAACATCAGCCAAGACAATAAATCCCACACTCATTTACACCAGCTTCGTTCCCTC
CB             TGGTCAACATCAGCCAAGACAATAAATCCCACACTCATTTACACCAGCTTC-----
PGC clone 13   TGGTCAACATCAGCCAAGACAATAAATCCCACACTCATTTACACCAGCTTC-----
                *****

NCBI chicken   GTCTTTTCCTG
CB             -----
PGC clone 13   -----

```

**OFF-TARGET 2**

```

NCBI chicken   CTGAGGGTGGTTAGCTGCTCCCAT GCCCATCGTTGCTCCCAGGTGGCAATGACTACAGC
CB             -----CCCATGCCATCGTTGCTCCCAGGTGGGAATGACTACAGC
PGC clone 13   -----CCCATGCCATCGTTGCTCCCAGGTGGGAATGACTACAGC
                *****

NCBI chicken   TCTTTTAACCAGAGTATTTGTTTCGATCAAACCTGGGCAGTAACTGTGTGCTGTCTTCAAAG
CB             TCTTTTAACCAGAGTATTTGTTTCGATCAAACCTGGGCAGTAACTGTGTGCTGTCTTCAAAG
PGC clone 13   TCTTTTAACCAGAGTATTTGTTTCGATCAAACCTGGGCAGTAACTGTGTGCTGTCTTCAAAG
                *****

NCBI chicken   TCATTCAAACCTGAATTAATGGAGTGTCTTGTAAGCTGAAGCATGGGGAGGCAAGTAAA
CB             TCATTCAAACCTGAATTAATGGAGTGTCTTGTAAGCTGAAGCATGGGGAGGCAAGTAAA
PGC clone 13   TCATTCAAACCTGAATTAATGGAGTGTCTTGTAAGCTGAAGCATGGGGAGGCAAGTAAA
                *****

NCBI chicken   TCCCTTTCCCAAGTCTCAGAGGAGCTGTTGTTTCCCTGCAGCATGCAGGGACTCACACG
CB             TCCCTTTCCCAAGTCTCAGAGGAGCTGTTGTTTCCCTGCAGCATGCAGGGAGTCAACG

```

```

PGC clone 13      TCCCTTTCCCAAGTCTCAGAGGAGCTGTGTTCCCTGCAGCATGCAGGGAGTCACAG
*****
NCBI chicken     TGTCTAAAAATGTCACATATCAGTCTCAGAGCCCACGCTGCTGTTTCAGGTAATACCCTTGG
CB               TGTCTAAAAATGTCACATATCAGTCTCAGAGCCCACGCTGCTGTTTCAGGTAATACCCTTGG
PGC clone 13     TGTCTAAAAATGTCACATATCAGTCTCAGAGCCCACGCTGCTGTTTCAGGTAATACCCTTGG
*****
NCBI chicken     TAAGCACACAGTTTTTGTCCCTCTCGACCTAGCAATGTGTGTGCCATGTCATCATCATCT
CB               TAAGCACACAGTTTTTGTCCCTCTCGACCTAGCAATGTGTGTGCCATGTCATCATCATCT
PGC clone 13     TAAGCACACAGTTTTTGTCCCTCTCGACCTAGCAATGTGTGTGCCATGTCATCATCATCT
*****
NCBI chicken     CCTTTTTGTTTTAATAGGACTGGGTATGCCGGCAGCATGACAATTAG
CB               -----
PGC clone 13     -----

```

**OFF-TARGET 3**

```

NCBI chicken     GCGAAGGCAGAAAACACGAGTAAGATTAGAAAACCAACAAAAAGTTGGAATTATGTTG
CB               -----AAAAGTTGGAATTATGTTG
PGC clone 13     -----AAAAGTTGGAATTATGTTG
*****

NCBI chicken     CAAATGTGCTCCAGACCCCTCACCAGCTTTGTTGCCCTCTCTGAACGCACTCCATGGCC
CB               CAAATGTGCTCCAGACCCCTCACCAGCTTTGTTGCCCTCTCTGAACGCACTCCATGGCC
PGC clone 13     CAAATGTGCTCCAGACCCCTCACCAGCTTTGTTGCCCTCTCTGAACGCACTCCATGGCC
*****

NCBI chicken     TCAATGTCTTTCTTGTAGTCCTTACACATCTGTGAAAACATACATCCACTGCTGCTCCCA
CB               TCAATGTCTTTCTTGTAGTCCTTACACATCTGTGAAAACATACATCCACTGCTGCTCCCA
PGC clone 13     TCAATGTCTTTCTTGTAGTCCTTACACATCTGTGAAAACATACATCCACTGCTGCTCCCA
*****

NCBI chicken     GCTAGGACAAAGGAGCGACAAAGCTCAGTCTCCCAGGTCAGTCCACACCCTATTTTCTG
CB               GGTAGGACAAAGGAGCGACAAAGCTCAGTCTCCCAGGTCAGTCCACACCCTATTTTCTG
PGC clone 13     GGTAGGACAAAGGAGCGACAAAGCTCAGTCTCCCAGGTCAGTCCACACCCTATTTTCTG
*****

NCBI chicken     AGAACACTAAAAGTCAAAATAAATCAATTCCTGCAATCCTAAGTGAGTTGTTTTGACCTA
CB               AGAACACTAAAAGTCAAAATAAATCAATTCCTGCAATCCTAAGTGAGTTGTTTTGACCTA
PGC clone 13     AGAACACTAAAAGTCAAAATAAATCAATTCCTGCAATCCTAAGTGAGTTGTTTTGACCTA
*****

NCBI chicken     AGACTTATTGCAGTTTAAATGCCAACAAAACCAACCTTTTCTATTGCCGCTATAAATTCC
CB               AGACTTATTGCAGTTTAAATGCCAACAAAACCAACCTTTTCTATTGCCGCTATAGATTCC
PGC clone 13     AGACTTATTGCAGTTTAAATGCCAACAAAACCAACCTTTTCTATTGCCGCTATAGATTCC
*****

NCBI chicken     GTGTCTGCTGCACCACACCTCAGGACCTCAAC
CB               GGGTCTGCTGC-----
PGC clone 13     GGGTCTGCTGC-----
* *****

```

**OFF-TARGET 4**

```

NCBI chicken     CGTAGCCCTGTCCTTCCTTGCCCCCAGCTTGTTCCCTGCTCCAGCTTCTTCCTTTTCTGGT
CB               -----TTTTCTGGT
PGC clone 13     -----TTTTCTGGT
*****

```

NCBI chicken CB PGC clone 13	TCCTCCCCGAGGTTTTACCTCCCCCAGAGAGGCTCTGCCACTCCTGGAAACCTCCTCTTA TCCTCCCCGAGGTTTTACCTCCCCCAGAGAGGCTCTGCCACTCCTGGAAACCTCCTCTTA TCCTCCCCGAGGTTTTACCTCCCCCAGAGAGGCTCTGCCACTCCTGGAAACCTCCTCTTA *****
NCBI chicken CB PGC clone 13	GTCACCTCTTTTTGCCTTCATACCTGAACAAGGTAAAAGTCTGGTGTACAAAATTCCA GGCACCTCTTTTTGCCTTCATATCTGAACAAGGTAAAAGTCTGGTGTACAAAATTCCA GGCACCTCTTTTTGCCTTCATATCTGAACAAGGTAAAAGTCTGGTGTACAAAATTCCA * *****
NCBI chicken CB PGC clone 13	CATGCTATAGTAACTTCATTTAATTACCTTTCCTGTTCTATTTGCTTTCACAAATGAACC CATGCTATAGTAACTTCATTTAATTACCTTTCCTGTTCTATTTGCTTTCACAAATGAACC CATGCTATAGTAACTTCATTTAATTACCTTTCCTGTTCTATTTGCTTTCACAAATGAACC *****
NCBI chicken CB PGC clone 13	CTCACAAGGATTATGAGAAGATAGTGCCTTCTTCCCTAAAGTTCTCATTTTAAAGGGTGC CTCACAAGGATTATGAGAAGATAGCGCTCTTCTTCCCTAAAGTTCTCATTTTAAAGGGTGC CTCACAAGGATTATGAGAAGATAGCGCTCTTCTTCCCTAAAGTTCTCATTTTAAAGGGTGC *****
NCBI chicken CB PGC clone 13	TGAGGCCAAACAGATGGTTTGAATCAGGCAGTACCCCCAGCAC <b>CCCACCTGGGAGCAGTC</b> TGAGGCCAAACAGATGGTTTGAATCAGGCAGTACCCCCAGCACCCCACCTGGGAGCAGTC TGAGGCCAAACAGATGGTTTGAATCAGGCAGTACCCCCAGCACCCCACCTGGGAGCAGTC *****
NCBI chicken CB PGC clone 13	<b>AGGGGA</b> AGTTTGCTGCAACAGCTGGTAGGACA AGGGGAAGTTG----- AGGGGAAGTTG----- *****

**OFF-TARGET 5**

NCBI chicken CB PGC clone 13	AGGGATGTTACACCCACACCATGGGATCCCTGCCTGGCCACGTCCCCAGGAGCCCCCCC -----CATGGGGTCCCTGCCTGGCCATGTCCCCAGGAGCCCCCCC -----CATGGGGTCCCTGCCTGGCCATGTCCCCAGGAGCCCCCCC *****
NCBI chicken CB PGC clone 13	AGCCCCATATCTGACCCCTTCCCCATCA <b>SCACACGGCTGCTCCCAGGAGGCC</b> TGACCCA AGCCCCATATCTGACCCCTTCCCCATCAGCACACGGCTGCTCCCAGGAGGGCTGACCCA AGCCCCATATCTGACCCCTTCCCCATCAGCACACGGCTGCTCCCAGGAGGGCTGACCCA *****
NCBI chicken CB PGC clone 13	CGCAGCCGCTGCCGCAGCACCGTGAGGCTGCAGATTTGAGGCAGCCTGGGATGGGGACAC CGCAGCCGCTGCCGCAGCACCGTGAGGCTGCAGATTTGAGGCAGCCTGGGATGGGGACAC CGCAGCCGCTGCCGCAGCACCGTGAGGCTGCAGATTTGAGGCAGCCTGGGATGGGGACAC *****
NCBI chicken CB PGC clone 13	AACCCAGCGTGGCTGAGCAGCGAGATGAGTTTCTGCACTCAGCAGAGGAGCTGCCAGG AACCCAGCGTGGCTGAGCAGCGAGATGAGTTTCTGCACTCAGCAGAGGAGCTGCCAGG AACCCAGCGTGGCTGAGCAGCGAGATGAGTTTCTGCACTCAGCAGAGGAGCTGCCAGG *****
NCBI chicken CB PGC clone 13	GCAGGGATGAGCCAGGGAGCCGAGTGCTGAGGGTGCAGATCTGGGGCTCCGCAGGATA GCAGGGATGAGCCAGGGAGCCGAGTGCTGAGGGTGCAGATCTGGGGCTCCGCAGGATA GCAGGGATGAGCCAGGGAGCCGAGTGCTGAGGGTGCAGATCTGGGGCTCCGCAGGATA *****
NCBI chicken CB	AGAGGGCCAAGAGCTGAACCCCTGAGCTGACCAAACCCATCCCCGGCCCCACGCAGGT AGAGGGCCAAGAGCTGAACCCCTGAGCTGACCAAACCCATCCCCG-----

PGC clone 13 AGAGGGCCAAGAGCTGAACCCCTGAGCTGACCAAACCCATCCCCG-----  
\*\*\*\*\*

NCBI chicken GAGCGAGGACTG **GAAGTACGTGGCCATGGTCA**  
CB -----  
PGC clone 13 -----

**OFF-TARGET 6**

NCBI chicken **CAATAGCCCCAAATTGCCGG**CTCAGGCCAGGGCTTGTCCCTCCTGTTGCAATACCCTGGC  
CB -----TTGCAATACCTTGGC  
PGC clone 13 -----TTGCAATACCTTGGC  
\*\*\*\*\*

NCBI chicken TCTGCTCTGGCATGCTGGGTGCAGTGGCCACGTCCCAGCTGGCCACGATGTCCCATCT  
CB TCTGCTCTGGCATGCTGGGTGCAGTGGCCACGTCCCAGCTGGCCACGATGTCCCATCT  
PGC clone 13 TCTGCTCTGGCATGCTGGGTGCAGTGGCCACGTCCCAGCTGGCCACGATGTCCCATCT  
\*\*\*\*\*

NCBI chicken GAGCTCCGTGTGGGGTTTGGGGCACAGAGAGCTCCTTTCTATGCGAGGACCAGGAGGGT  
CB GAGCTCCGTGTGGGGTTTGGGGCACAGAGAGCTCCTTTCTATGCGAGGACCAGGAGGGT  
PGC clone 13 GAGCTCCGTGTGGGGTTTGGGGCACAGAGAGCTCCTTTCTATGCGAGGACCAGGAGGGT  
\*\*\*\*\*

NCBI chicken CCCTCCCTCCTGCAGCACCTCATCCCCATCTCCAC **CTCCAGGGATGCTCCCAGGTGGGT**  
CB CCCTCCCTCCTGCAGCACCTCGTCCCCATCTCCACCTCCAGGGATGCTCCCAGGTGGGT  
PGC clone 13 CCCTCCCTCCTGCAGCACCTCGTCCCCATCTCCACCTCCAGGGATGCTCCCAGGTGGGT  
\*\*\*\*\*

NCBI chicken TCCCCCTCCTGGTTTCCGACTGGGGTGGGAAGCACAGGCGACAGACACGTGTCTTTGCAT  
CB TCCCCCTCCTGGTTTCCGACTGGGGTGGGAAGCACAGGCGACAGACACGTGTCTTTGCAT  
PGC clone 13 TCCCCCTCCTGGTTTCCGACTGGGGTGGGAAGCACAGGCGACAGACACGTGTCTTTGCAT  
\*\*\*\*\*

NCBI chicken ACTGACGGCTCAATCCATCCATCTGCTCATTTGTTCCCTTGAGAGATGTAGGGCTGATTTCT  
CB ACTGACGGCTCAATCCATCCATCTGCTCATTTGTTCCCTTGAGAGATGTAGGGCTGATTTCT  
PGC clone 13 ACTGACGGCTCAATCCATCCATCTGCTCATTTGTTCCCTTGAGAGATGTAGGGCTGATTTCT  
\*\*\*\*\*

NCBI chicken TGTGTCCCCGTGCCTCAGTTTCCCCACTCGCAGGACCCACCCAGCACTGCAGCCGAGCA  
CB TGTGTCCCCGTGCCTCAGTTTCCCCACTCGCAGGACCCACCCAGCACTGCAGCCGAGC-  
PGC clone 13 TGTGTCCCCGTGCCTCAGTTTCCCCACTCGCAGGACCCACCCAGCACTGCAGCCGAGC-  
\*\*\*\*\*

NCBI chicken GAGAACTCTGTGCTCCCCT  
CB -----  
PGC clone 13 -----

**OFF-TARGET 7**

NCBI chicken **CAGACGTTTACCCGGACACA**AAACGAGGGGTTCCGTATTTCTCGCTTCAATACCATCTA  
CB -----ATACCATCTA  
PGC clone 13 -----ATACCATCTA  
\*\*\*\*\*

NCBI chicken CAGCTCAGAGCCCATTGCGCCTCTCCATTGCGCACCCCTCTGCGGTAGCACTGATGGAGC  
CB CAGCTCAGAGCCCATTGCGCCTCTCCATTGCGCACCCCTCTGCGGTAGCACTGATGGAGC  
PGC clone 13 CAGCTCAGAGCCCATTGCGCCTCTCCATTGCGCACCCCTCTGCGGTAGCACTGATGGAGC  
\*\*\*\*\*

NCBI chicken CB PGC clone 13	AGAAAGCACCAGGAAGCGCCACAGCCCCCTCTCCACAGCCAAGGACAGACCTTGTGGCCC AGAAAGCACCAGGAAGCGCCACAGCCCCCTCTCCACAGCCAAGGACAGACCTTGTGGCCC AGAAAGCACCAGGAAGCGCCACAGCCCCCTCTCCACAGCCAAGGACAGACCTTGTGGCCC *****
NCBI chicken CB PGC clone 13	TTACAGGATCCGTTGGCACTGACACGGGTCCAATT <b>CCCACCTGGGAGCTGCGATGAGG</b> AT TTACAGGATCCGTTGGCACTGACACGGGTCCAATTCCCACCTGGGAGCTGCGATGAGGAT TTACAGGATCCGTTGGCACTGACACGGGTCCAATTCCCACCTGGGAGCTGCGATGAGGAT *****
NCBI chicken CB PGC clone 13	CCCATGGGATGCTGGAGCCTGCAGAGCCCTGCAGCTGCAGGCTGGCTTCCGGGCTTCACC CCCATGGGATGCTGGAGCCTGCAGAGCCCTGCAGCTGCAGGCTGGCTTCCGGGCTTCACC CCCATGGGATGCTGGAGCCTGCAGAGCCCTGCAGCTGCAGGCTGGCTTCCGGGCTTCACC *****
NCBI chicken CB PGC clone 13	CTCCTCACGCTGTACAGCCGTCCGCTTCCTTCCTCCTTCCTTTCCTCTTTCTCTTTTTC CTCCTCACGCTGTACAGCCGTCCGCTTCCTTCCTCCTTCCTTTCCTCTTTCTCTTTTTC CTCCTCACGCTGTACAGCCGTCCGCTTCCTTCCTCCTTCCTTTCCTCTTTCTCTTTTTC *****
NCBI chicken CB PGC clone 13	CTCTTTTTTTTTTTTTTTTTTAAACAGATCTGAACATCTTTTCTCTGCTTTGCACACAACC CTCTTTTTTTTTTTTTTTTTTAAACAGATCTGAACATCTTTTCCCGGTTTTGCACACAACC CTCTTTTTTTTTTTTTTTTTTAAACAGATCTGAACATCTTTTCCCGGTTTTGCACACAACC ***** ** * * * * * * * * * * * * * * * * * *
NCBI chicken CB PGC clone 13	CAGGAGCGGCTCCAGGAGGGACAGAGCCCATTCCCACTGGTTTGGT CAGGASGGGCCCCGGGAGGAAAAA----- CRGGAGGGGCCCCGGGGGGAAAAA----- * * * * * * * * * * * * * * * * * *

**OFF-TARGET 8**

NCBI chicken CB PGC clone 13	<b>GGCCAAACTGTGCAAGAG</b> CAGGACATGTCGATCTGACCTGCTGATCAAGGGCCAGATT -----AGGGCCAGATT -----GCCAGATT *****
NCBI chicken CB PGC clone 13	AAACCAAAAATTCTGAAATTAATAATGTGCAAAAGTGATGAAATTAGAATTTGCCAACCG AAACCAAAAATTCTGAAATTAATAATGTGCAAAAGTGATGAAATTAGAATTTGCCAACCG AAACCAAAAATTCTGAAATTAATAATGTGCAAAAGTGATGAAATTAGAATTTGCCAACCG *****
NCBI chicken CB PGC clone 13	CTTCATTAATTTCTTAACGTTGGCAATAATGAGACGTAATTGCTGGCTCGAAAC <b>TCCCG</b> CTTCATTAATTTCTTAACGTTGGCAATAATGAGACGTAATTGCTGGCTCGAAACTCCCC CTTCATTAATTTCTTAACGTTGGCAATAATGAGACGTAATTGCTGGCTCGAAACTCCCC *****
NCBI chicken CB PGC clone 13	<b>AGCCTGCTCCAGGTAGG</b> AAGATGAATGTGTCCCTCCCCACTGTGGTTTGCACTGATCGT AGCCTGCTCCAGGTAGGAAGATGAATGTGTCCCTCCCCACTGTGGTTTGCACTGATCGT AGCCTGCTCCAGGTAGGAAGATGAATGTGTCCCTCCCCACTGTGGTTTGCACTGATCGT *****
NCBI chicken CB PGC clone 13	TCTGGTTTTCTGCTTCTTTGTGCCCTTTGATGTTCAAGTGCTTTTGGGCAGCCCTGTAG TCTGGTTTTCTGCTTCTTTGTGCCCTTTGATGTTCAAGTGCTTTTGGGCAGCCCTGTAG TCTGGTTTTCTGCTTCTTTGTGCCCTTTGATGTTCAAGTGCTTTTGGGCAGCCCTGTAG *****
NCBI chicken CB	CATCCAGCTTCCTGTGCAGCAGTGGGGCAGTTGGTGCAGCCAAGGTAACCAAAATCAAAGG CATCCAGCTTCCTGTGCAGCAGTGGGGCAGTTGGTGCAGCCAAGGTAACCAAAATCAAAGG

PGC clone 13      CATCCAGCTCCTGTGCAGCAGTGGGGCAGTTGGTGCAGCCAAGGTAACCAAATCAAAGG  
\*\*\*\*\*

NCBI chicken      AATTTCTTAGGGAGAAAGACAGGGAAAAATGGTCAGGTTGCTGCAGAAA  
CB                    AATTTCTTAGGGAGAAAGACAGGGAAAAA-----  
PGC clone 13      AATTTCTTAGGGAGAAAGACAGGGAAAAA-----  
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