

## Supplementary information

### Production of Viable Chicken by Allogeneic Transplantation of Primordial Germ Cells Induced from Somatic Cells

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### **Supplementary Tables**

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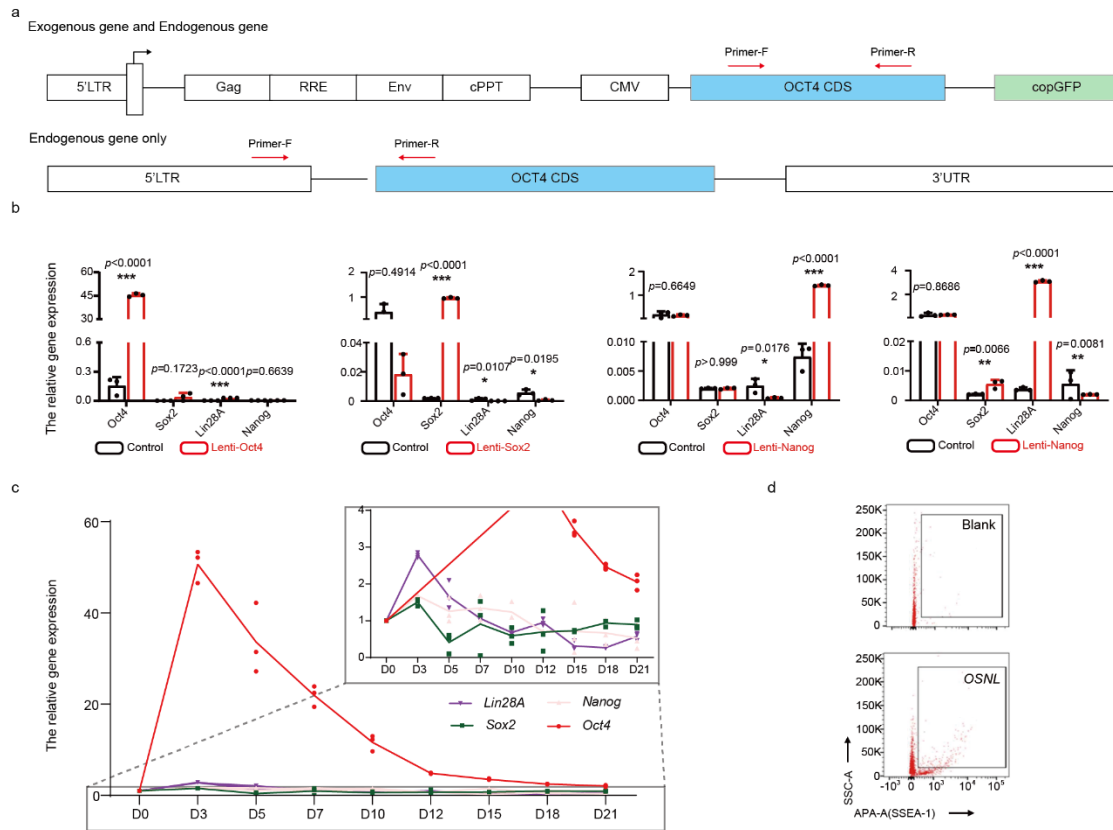
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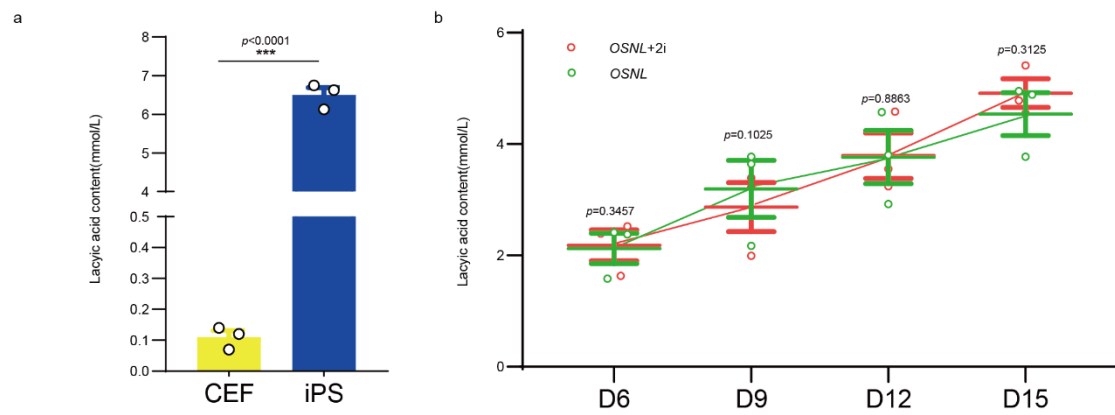
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## Supplementary Figure 1



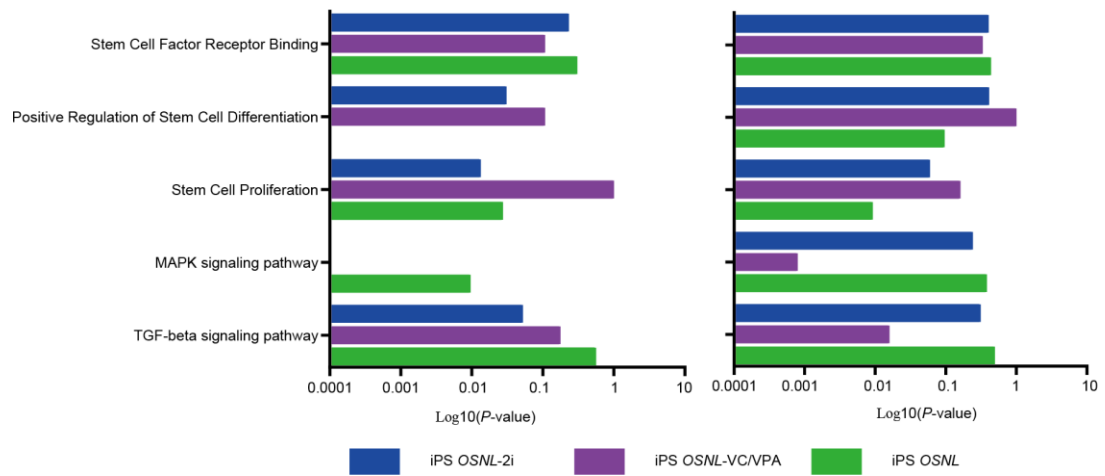
**Supplementary Figure 1. Evaluation of the OSNL vector function.** **a.** Schematic diagram of the primers designed for evaluation of the exogenous and endogenous *OSNL* gene expression by qRT-PCR. **b.** qRT-PCR analysis of *OSNL* gene (exogenous and endogenous) expression in the CEFs after transfection with *OSNL* vectors for 48 h (Data are shown as the mean $\pm$ SEM,  $n=3$  independent experiments, \*  $p<0.05$ , \*\*  $p<0.01$ , \*\*\*  $p<0.001$ , unpaired two-tailed  $t$ -test). **c.** qRT-PCR analysis of exogenous *OSNL* gene expression in the CEFs after transfection with *OSNL* vectors for 21 consecutive days (Data are shown as the mean $\pm$ SEM,  $n=3$  independent experiments). **d.** Flow cytometric analysis of the SSEA-1-positive cells in the *OSNL*-induced CEFs ( $n=3$  independent experiments).

## Supplementary Figure 2



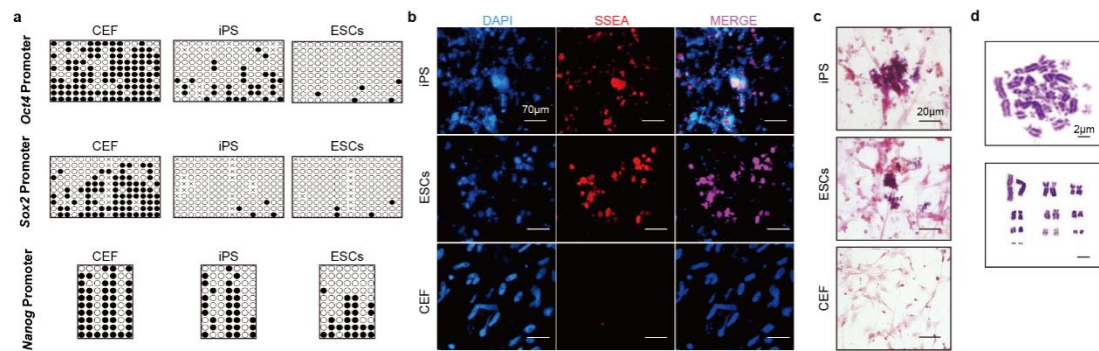
**Supplementary Figure 2. Evaluation of the glycolytic products in iPSCs and CEFs. a.** Lactic acid concentration was evaluated to reflect the glycolytic level in CEFs and iPSCs (Data are shown as the mean $\pm$ SEM,  $n=3$  independent experiments, \*\*\*  $p < 0.001$ , unpaired two-tailed  $t$ -test). **b.** The dynamic glycolytic levels in OSNL- and OSNL+2i-induced iPSC formation from days 1 to 15 (Data are shown as the mean $\pm$ SEM,  $n=3$  independent experiments, unpaired two-tailed  $t$ -test).

### Supplementary Figure 3



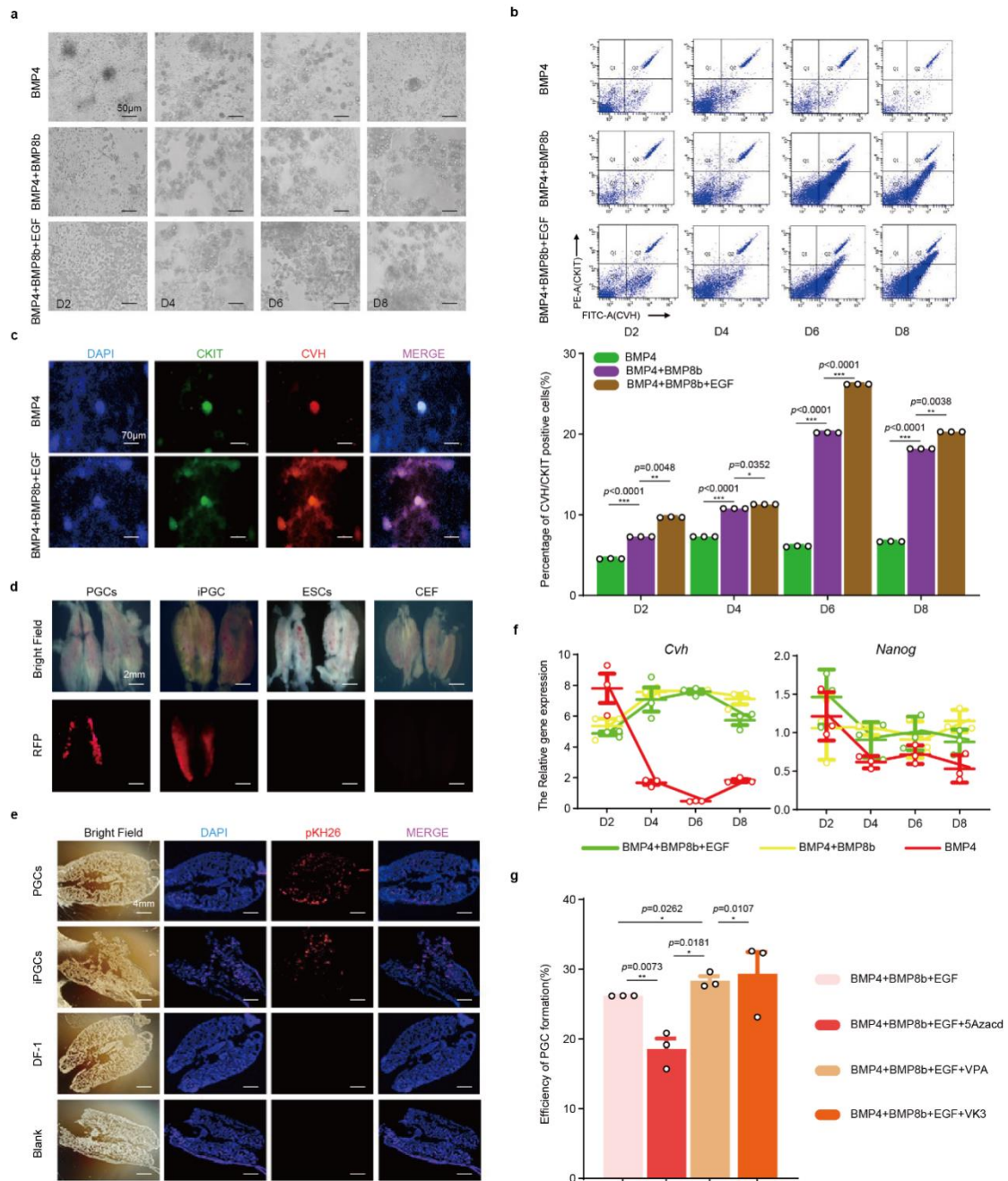
**Supplementary Figure 3. Transcriptome analysis of iPSCs derived from CEFs in different induction systems.** GO and KEGG analysis of DEGs between iPSCs vs CEFs or ESCs with different induction systems, including *OSNL*, *OSNL+2i* and *OSNL+VC/VPA*.

## Supplementary Figure 4



**Supplementary Figure 4. iPSC identification.** **a.** DNA methylation status evaluated by bisulfite sequencing for *Oct4*, *Sox2* and *Nanog* promoter regions of iPSCs, CEFs and ESCs, Black dots represent methylated sites, and white dots represent unmethylated sites, and ‘×’ represents undetected sites ( $n=10$  repeats). **b.** Fluorescence staining of iPSCs with SSEA-1. CEFs and ESCs were used as the negative controls and positive controls, respectively. Scale bar: 70  $\mu\text{m}$ . ( $n= 3$  independent experiments). **c.** Alkaline phosphatase staining of iPSCs. CEFs and ESCs were used as the negative controls and positive controls, respectively. Scale bar: 20  $\mu\text{m}$ . ( $n= 3$  independent experiments). **d.** Chromosome karyotype analysis of iPSCs showing normal diploid karyotypes. Scale bar: 2  $\mu\text{m}$ . ( $n= 3$  independent experiments).

## Supplementary Figure 5

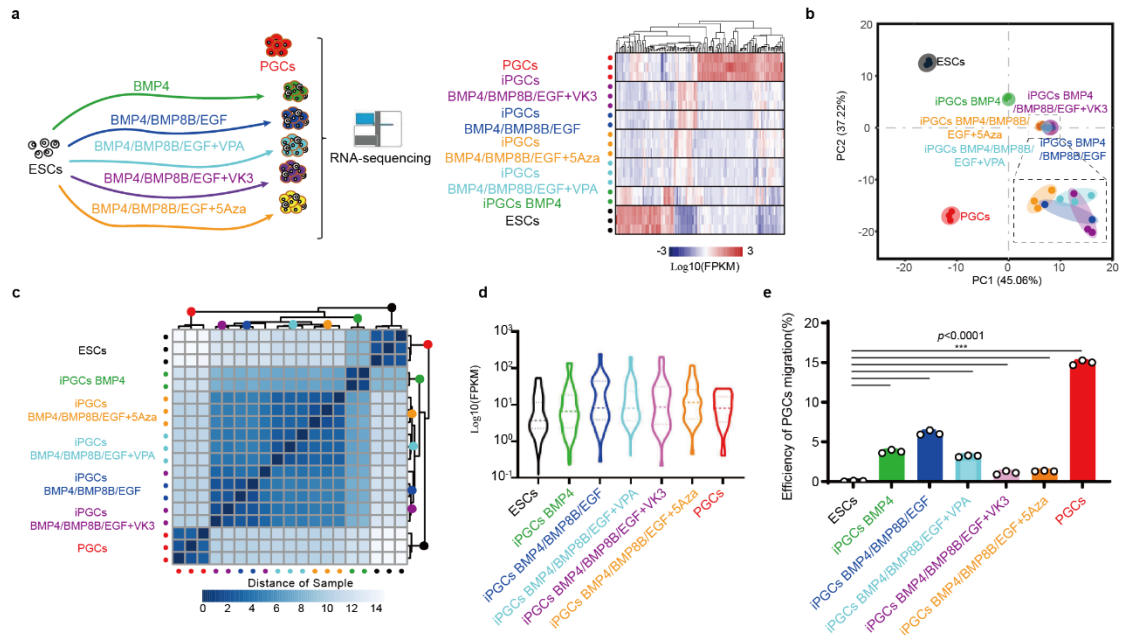


**Supplementary Figure 5. Optimization of the iPGC induction system.** **a.** Morphological observations of ESCs induced to differentiate into iPGCs. Scale bar: 50  $\mu\text{m}$ . ( $n=3$  independent experiments). **b.** Flow cytometric analysis of CKIT- and CVH-positive cells. (Data are shown as the mean $\pm$ SEM,  $n=3$  independent experiments, \*\*\*  $p<0.001$ , one-way ANOVA). **c.** CKIT and CVH immunofluorescence staining of iPGCs induced by BMP4 or BMP4/BMP8a/EGF on day 6, Scale bar: 70  $\mu\text{m}$ . ( $n=3$  independent experiments). **d.** PGCs, iPGCs, ESCs and CEFs stained with pKH26 were injected into the embryo vessels. The red fluorescence was monitored by real-time

fluorescence in day 4.5 chicken embryo genital ridges, Scale bar: 2 mm. ( $n= 3$  independent experiments). **e.** PGCs, iPGCs and CEFs stained with pKH26 were injected into embryo vessels. Genital ridges from day 4.5 chicken embryos were sectioned for red fluorescence and observed to track the migration of the injected cells, Scale bar: 4 mm. ( $n= 3$  independent experiments). **f.** qRT-PCR analysis of *Nanog* and *Cvh* gene expression during ESC induction to iPGCs with different components. ( $n= 3$  independent experiments). **g.** The induction efficiency of iPGCs by different combinations, including BMP4/BMP8B/EGF/VK3, BMP4/BMP8b/EGF/5Aza-cd and BMP4/BMP8b/EGF, was evaluated by flow cytometry. (Data are shown as the mean $\pm$ SEM,  $n=3$  independent experiments, \*  $p<0.05$ , \*\*  $p<0.01$ , one-way ANOVA).

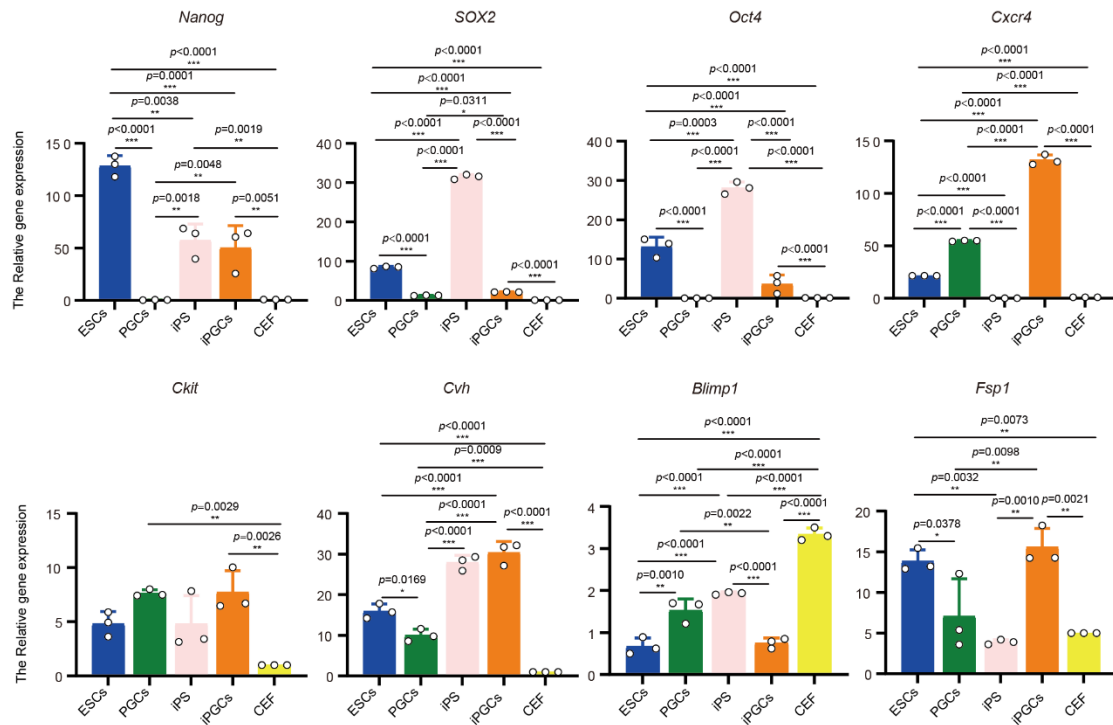


## Supplementary Figure 6



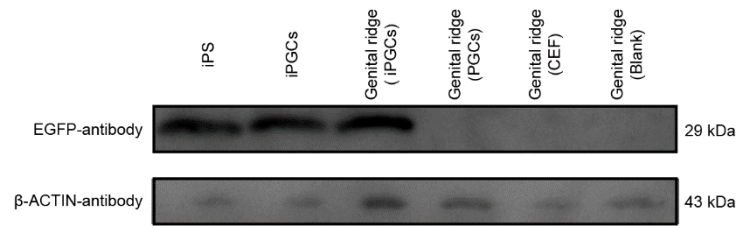
**Supplementary Figure 6. Optimization of the iPGC induction system. a.** Schematic diagram of RNA-seq for iPGCs induced from ESCs by different components. ESCs and PGCs were used as the negative and positive controls, respectively. Unsupervised hierarchical clustering based on PGC development was applied to analyze the similarities among the ESCs, PGCs and iPGCs induced by different systems. The heatmap shows the selected gene expression profile. The color key from blue to red indicates low to high gene expression. White cells represent ESCs, red cells represent PGCs, cells with other colored represent iPGCs derived from different induction conditions. Dots with different colors represent independent samples of corresponding cells for RNA-seq. **b, c.** PCA (b) and correlation analysis (c) of ESCs, PGCs and iPGCs induced by different systems based on the genes selected in unsupervised hierarchical clustering analysis. The color key from blue to white indicates short to long distances between samples. The colored dots represent sequencing data from individual cell samples. **d.** Violin plot of PGC marker gene expression from the RNA-seq data of iPGCs from different groups, ESCs and PGCs. The solid lines at each end of the violin diagram represent the maximum and minimum values, respectively. The three dotted lines in the middle of the violin diagram represent the 75% percentile, the mean, and the 25% percentile in turn. **e.** iPGCs induced with different components, as well as ESCs and PGCs, were stained with pKH26 and injected into the recipients. The migration of the injected cells was evaluated by flow cytometric analysis of the pKH26-positive cells in isolated genital ridges from different groups. ESCs are used as control. (Data are shown as the mean±SEM,  $n=3$  independent experiments, \*\*\*  $p<0.001$ , one-way ANOVA)

## Supplementary Figure 7



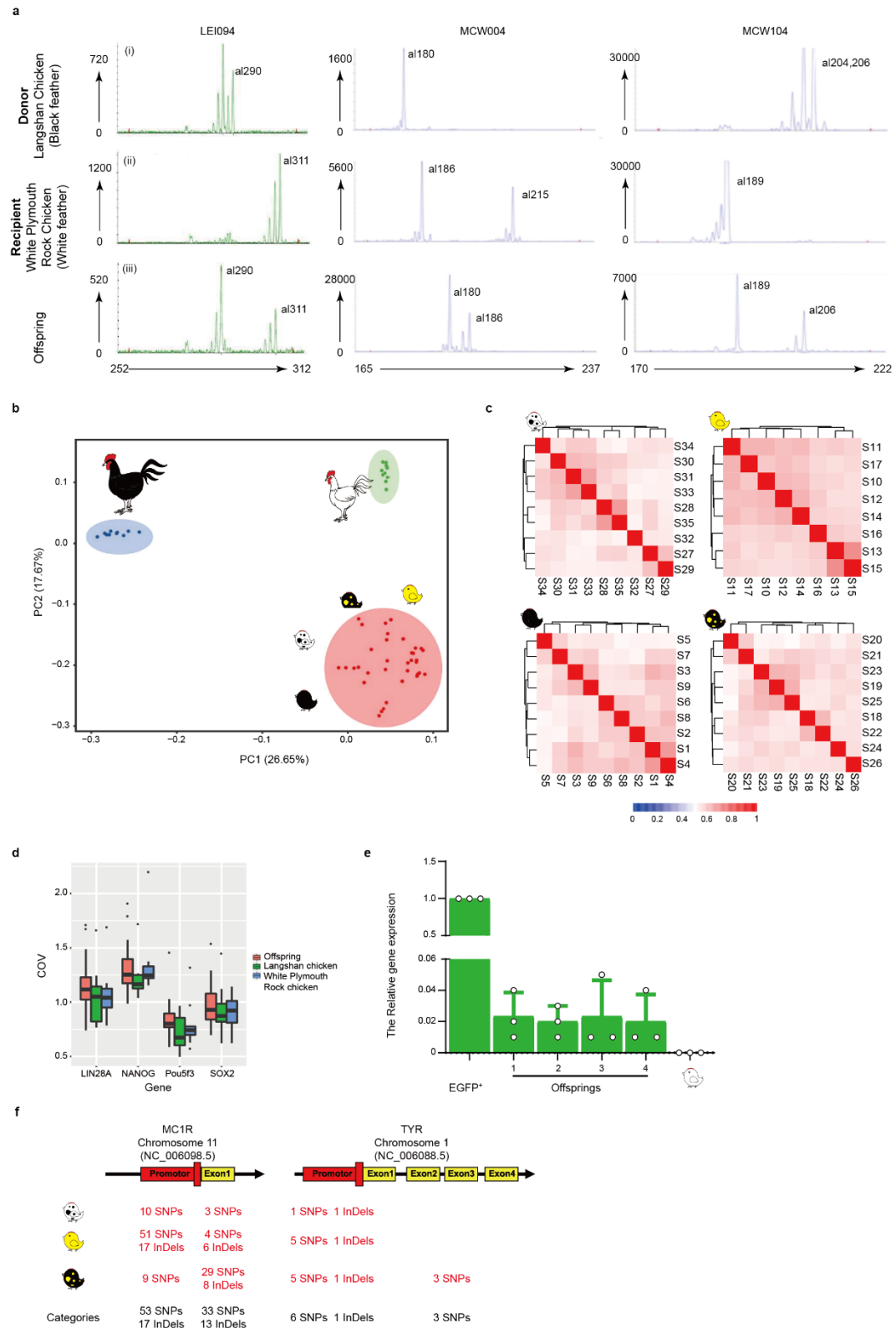
**Supplementary Figure 7. PGC marker gene expression in ESCs, PGCs, iPSCs and iPGCs.** The expression of *Nanog*, *Sox2*, *Oct4*, the migration-related gene *Cxcr4*, the reproductive marker genes *c-kit* and *Cvh*, the PGC marker gene *Blimp1* and the CEF marker gene *Fsp1* in ESCs, PGCs, iPSC, iPGCs and CEFs was evaluated by qRT-PCR. (Data are shown as the mean±SEM,  $n=3$  independent experiments, \*  $p<0.05$ , \*\*  $p<0.01$ , \*\*\*  $p<0.001$ , one-way ANOVA)

### Supplementary Figure 8



**Supplementary Figure 8. EGFP expression in chicken embryo genital ridges analyzed by western blots.** EGFP protein expression was evaluated in isolated embryo genital ridges from embryos transplanted with iPGCs, PGCs and CEFs. iPSCs and iPGCs were used as the positive controls.  $\beta$ -Actin was used as internal control of EGFP. ( $n=3$  independent experiments).

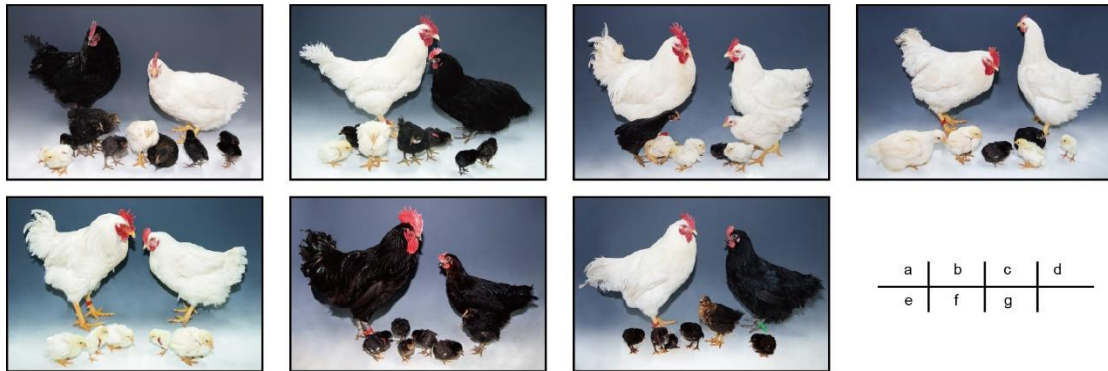
## Supplementary Figure 9



**Supplementary Figure 9. The genetic relationship between somatic cell-derived chickens and donors/recipients. a.** Microsatellite analysis of the LEI094 site. The length of the LEI094 site in black feathered Langshan chickens was 290 (i) and in White Plymouth Rock chickens was 311 (ii).

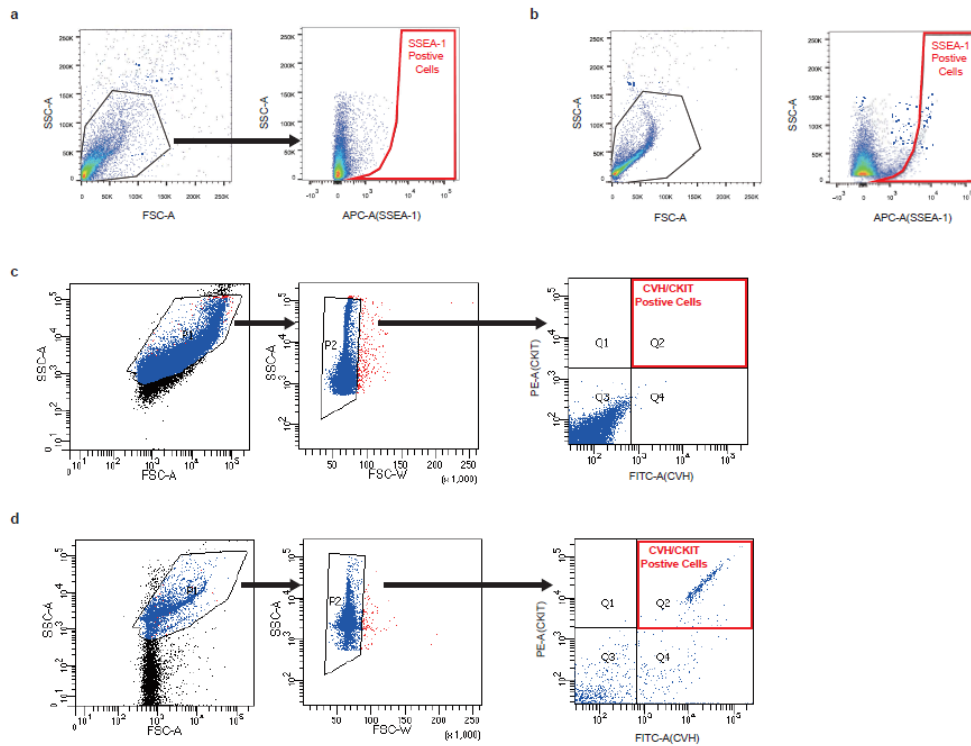
Lengths of both 290 and 311 were detected in the heterozygote offspring. The length of the MCW004 site was 180 in black feathered Langshan chickens and 186/215 in White Plymouth Rock chickens. Lengths of 180 and 186 were detected simultaneously in the heterozygotes. The length of the MCW104 site was 204/206 in black feathered Langshan chickens and 189 in White Plymouth Rock chickens. Lengths of 189 and 206 were detected simultaneously in the heterozygotes. ( $n=61$  independent individuals). **b.** PCA of somatic cell-derived chickens, black feathered Langshan chickens and White Plymouth Rock chickens. Different colored dots represent different individuals. **c.** Correlation analysis of the offspring with different feather color phenotypes, including black feathered, black-white feathered, yellow feathered and black-yellow feathered phenotypes, the color key from blue to red indicates the similarities from low to high between samples. **d.** The copy number of *OSNL* genes in the offspring. The boxplot contains the maximum values, the quarterback, the median, lower quartile and the minimum values in turn, the black dots represent outliers. **e.** qRT-PCR evaluation of EGFP expression in the somatic cell-derived chickens. (Data are shown as the mean $\pm$ SEM,  $n=3$  independent experiments). **f.** SNP variations in two feather color-related genes, *MC1R* and *TYR*, in individuals with different feather color phenotypes.

**Supplementary Figure 10**



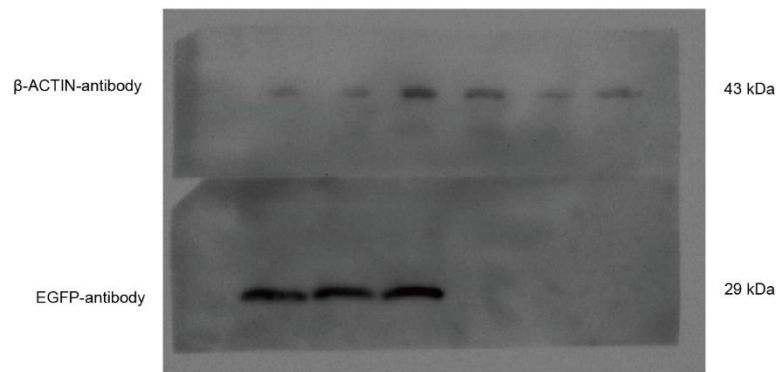
**Supplementary Figure 10. Chickens produced by allogeneic transplantation of PGCs. a.** White hen × black cock (recipient of allogeneic transplantation of white chicken PGCs) produced positive white feathered offspring; **b.** White cock × black hen (recipient of allogeneic transplantation of white chicken PGCs) produced positive white feathered offspring; **c.** White hen (recipient of allogeneic transplantation of black chicken PGCs) × white cock produced positive black feathered offspring; **d.** White hen × white cock (recipient of allogeneic transplantation of black chicken PGCs) produced positive black feathered offspring. **e.** White hen × white cock produced white feathered offspring; **f.** black hen × black cock produced black feathered offspring; **g.** black hen × white cock produced black feathered offspring. (Note: The gene encoding white feather is recessive.)

## Supplementary Figure 11



**Supplementary Figure 11. Strategy for FACS sequential gating.** **a, b.** Gating strategy for detection SSEA-1 positive cells from the unstain sample(**a**) and experiment sample(**b**) by flow cytometry analysis. Flowjo VX software package was used for flow cytometry data analysis. Select the gate tool to circle the area of majority cells to eliminate the interference from cell debris or background noise via FSC-A (forward scattering light area) and SSC-A (side scattering light area). APC-A (allophycocyanin area) was the fluorescent channel of SSEA-1 Alexa fluor 594 binding antibody to circle an area on the right side of the cell to detected the SSEA-1 positive cells within sample (the red circumscribe) (applied for Fig3b, Supplementary Figure 1c). **c, d.** Gating strategy for detection CVH/CKIT positive cells from the unstain sample(**c**) and experiment sample(**d**) by flow cytometry analysis. Flowjo VX software package was used for flow cytometry data analysis. Select the gate tool to circle the area of majority cells to eliminate the interference from cell debris or background noise via FSC-A and SSC-A. Moreover, the FSC-W (forward scattering light width) applied for improve the correlator output. FITC-A (fluorescein isothiocyante area) and PE-A (Phycoerythrin area) was the fluorescent channel of anti-CKIT and anti-CKIT, respectively. Absolute number of CKIT and CVH positive cells within sample was circle an area on the red circumscribe (applied for Fig5d, e; Supplementary Figure 5b).

**Supplementary Figure 12**



**Supplementary Figure 12. Uncropped western blotting images for Supplementary Figure 8.**



**Supplementary Table 1**

**Supplementary Table 1 Detailed information the samples for RNA-seq**

Group	Cell Name	Treatment	cell type
iPS( <i>OSNL</i> )-1	induced pluripotent stem cells	<i>OSNL</i>	iPS induced from CEF
iPS( <i>OSNL</i> )-2	induced pluripotent stem cells	<i>OSNL</i>	iPS induced from CEF
iPS( <i>OSNL</i> )-3	induced pluripotent stem cells	<i>OSNL</i>	iPS induced from CEF
iPS( <i>OSNL</i> +2i)-1	induced pluripotent stem cells	<i>OSNL</i> +2i	iPS induced from CEF
iPS( <i>OSNL</i> +2i)-2	induced pluripotent stem cells	<i>OSNL</i> +2i	iPS induced from CEF
iPS( <i>OSNL</i> +2i)-3	induced pluripotent stem cells	<i>OSNL</i> +2i	iPS induced from CEF
iPS( <i>OSNL</i> +VC+VPA)-1	induced pluripotent stem cells	<i>OSNL</i> +VC+VPA	iPS induced from CEF
iPS( <i>OSNL</i> +VC+VPA)-2	induced pluripotent stem cells	<i>OSNL</i> +VC+VPA	iPS induced from CEF
iPS( <i>OSNL</i> +VC+VPA)-3	induced pluripotent stem cells	<i>OSNL</i> +VC+VPA	iPS induced from CEF
CEF-1	chicken embryo fibroblasts		CEF
CEF-2	chicken embryo fibroblasts		CEF
CEF-3	chicken embryo fibroblasts		CEF
ESC-1	embryonic stem cells		ESCs
ESC-2	embryonic stem cells		ESCs
ESC-3	embryonic stem cells		ESCs
iPGC(BMP4)-1	primordial germ-like cells	BMP4	iPGCs induced from ESCs
iPGC(BMP4)-2	primordial germ-like cells	BMP4	iPGCs induced from ESCs
iPGC(BMP4+BMP8b+EGF)-1	primordial germ-like cells	BMP4+BMP8b+EGF	iPGCs induced from ESCs
iPGC(BMP4+BMP8b+EGF)-3	primordial germ-like cells	BMP4+BMP8b+EGF	iPGCs induced from ESCs
iPGC(VPA)-1	primordial germ-like cells	BMP4+BMP8b+EGF+VPA	iPGCs induced from ESCs
iPGC(VPA)-2	primordial germ-like cells	BMP4+BMP8b+EGF+VPA	iPGCs induced from ESCs
iPGC(VPA)-3	primordial germ-like cells	BMP4+BMP8b+EGF+VPA	iPGCs induced from ESCs
iPGC(VK3)-1	primordial germ-like cells	BMP4+BMP8b+EGF+VK3	iPGCs induced from ESCs
iPGC(VK3)-2	primordial germ-like cells	BMP4+BMP8b+EGF+VK3	iPGCs induced from ESCs
iPGC(VK3)-3	primordial germ-like cells	BMP4+BMP8b+EGF+VK3	iPGCs induced from ESCs
iPGC(5Aza)-1	primordial germ-like cells	BMP4+BMP8b+EGF+5Aza	iPGCs induced from ESCs
iPGC(5Aza)-2	primordial germ-like cells	BMP4+BMP8b+EGF+5Aza	iPGCs induced from ESCs
iPGC(5Aza)-3	primordial germ-like cells	BMP4+BMP8b+EGF+5Aza	iPGCs induced from ESCs
iPGC(iPS-derived)-1	primordial germ-like cells	BMP4+BMP8b+EGF	iPGCs induced from iPS
iPGC(iPS-derived)-2	primordial germ-like cells	BMP4+BMP8b+EGF	iPGCs induced from iPS
iPGC(iPS-derived)-3	primordial germ-like cells	BMP4+BMP8b+EGF	iPGCs induced from iPS
PGC-1	primordial germ cells		PGCs
PGC-2	primordial germ cells		PGCs
PGC-3	primordial germ cells		PGCs

**Supplementary Table 2**

**Supplementary Table 2 Results of microsatellite analysis**

Number	Nameplate	Color of feather	MCW004		MCW104		Type
1	2001	black-white	180	186	202	221	Heterozygote
2	2002	black	180	186	189	206	Heterozygote
3	2006	black-white	180		190	206	Heterozygote
4	2007	black-white	186		189	204	Heterozygote
5	2011	black-white	182	186	194	206	Heterozygote
6	2012	black-white	182	186	206	214	Heterozygote
7	2016	black-white	182	186	186	189	Heterozygote
8	2019	yellow	180	182	206		Homozygous
9	1853	yellow	168		184	188	Heterozygote
10	1854	black	180	186	189	202	Heterozygote
11	1858	yellow	180	186	189	206	Heterozygote
12	1981	yellow	184	186	189	206	Heterozygote
13	1984	black-white	180	182	214	221	Heterozygote
14	1987	yellow	180	186	189		Heterozygote
15	3051	yellow	182	186	189	214	Heterozygote
16	3080	yellow	180	186	189		Heterozygote
17	3512	black-white	180	186	189	221	Heterozygote
18	3513	black-yellow	184	186	189	207	Heterozygote
19	3519	black-white	180	186	189		Heterozygote
20	3700	black-yellow	179	186	195		Heterozygote
21	3704	yellow	180	186	189	192	Heterozygote
22	3707	yellow	179	186	188		Heterozygote
23	3719	black-white	180	186	189		Heterozygote
24	3724	yellow	180	186	189		Heterozygote
25	3736	black-white	180	186	189		Heterozygote
26	3739	black	184	186	202	214	Heterozygote
27	3962	black-yellow	180	186	202	206	Heterozygote
28	3968	yellow	180	186	189	202	Heterozygote
29	3971	black-white	184	186	206	221	Heterozygote
30	3975	black-white	180	186	189	206	Heterozygote
31	3984	black-white	180	186	189		Heterozygote
32	3992	yellow	180	182	189	194	Heterozygote
33	3995	black	182	186	192		Heterozygote
34	4000	black	184	186	189		Heterozygote
35	4005	yellow	180	186	189	206	Heterozygote
36	4011	black	180		189	221	Homozygous
37	4013	yellow	182	186	189	192	Heterozygote
38	4017	black	180	186	189	194	Heterozygote
39	0556	yellow	180		189		Heterozygote
40	1986	yellow	179	186	189	202	Heterozygote

41	0539	yellow	179	186	189		Heterozygote
42	3970	yellow	179	186	189	221	Heterozygote
43	3977	yellow	179	181	214	221	Homozygous
44	1994	black-yellow	179		189	221	Heterozygote
45	3728	black-yellow	179	186	189	206	Heterozygote
46	3735	black-yellow	182	186	214	221	Homozygous
47	3517	black-yellow	184	186	189	202	Heterozygote
48	3960	black-yellow	179	186	189	221	Heterozygote
49	3972	black	184	186	189	202	Homozygous
50	3978	black	179	182	189	194	Homozygous
51	0560	yellow	179	186	206	221	Heterozygote
52	0562	black	179	186	189	202	Heterozygote
53	0573	black	179	186	189		Heterozygote
54	3073	black-white	179		206	221	Heterozygote
55	3074	yellow	184	186	202		Homozygous
56	3524	yellow	184	186	189	214	Heterozygote
57	3526	black	184	186	189	221	Heterozygote
58	3964	yellow	179	186	189	221	Heterozygote
59	3071	black-yellow	179	186	202	221	Heterozygote
60	3969	black-white	179	181	189	214	Heterozygote
61	3505	black-yellow	179	186	189		Heterozygote

**Supplementary Table 3****Supplementary Table 3 Sample list for whole genome resequencing**

Number	Feather color	Chicken number
1	black	2002
2	black	3995
3	black	5488
4	black	5495
5	black	1851
6	black	5964
7	black	5484
8	black	3728
9	black	0634
10	yellow	3051
11	yellow	5498
12	yellow	2019
13	yellow	4005
14	yellow	5451
15	yellow	3714
16	yellow	5386
17	yellow	5447
18	black-yellow	5969
19	black-yellow	1994
20	black-yellow	3962
21	black-yellow	5229
22	black-yellow	5967
23	black-yellow	5965
24	black-yellow	5973
25	black-yellow	5441
26	black-yellow	5976
27	black-white	2012
28	black-white	1855
29	black-white	2016
30	black-white	0003
31	black-white	0005
32	black-white	0012
33	black-white	0013
34	black-white	0016
35	black-white	0021

**Supplementary Table 4****Supplementary Table 4 Specific SNPs from Black Langshan chicken and White Plymouth****Rock Chicken in offspring**

sample	LS	PR	het	miss	LS_perc	PR_perc	het_perc	miss_perc
S1	63783	90615	66160	762	28.8194	40.943	29.8934	0.344298
S2	43849	106260	70722	489	19.8125	48.0119	31.9546	0.220947
S3	45979	116348	58362	631	20.7749	52.57	26.37	0.285108
S4	56504	98381	65734	701	25.5305	44.4519	29.7009	0.316736
S5	56336	97861	66555	568	25.4545	44.217	30.0718	0.256642
S6	44219	89317	87266	518	19.9797	40.3565	39.4298	0.23405
S7	53636	87504	79618	562	24.2346	39.5373	35.9742	0.253931
S8	51116	87459	82344	401	23.096	39.517	37.2059	0.181186
S9	52019	111044	57623	634	23.504	50.1735	26.0361	0.286463
S10	51216	111698	57634	772	23.1412	50.469	26.041	0.348816
S11	32623	105394	82880	423	14.7402	47.6206	37.448	0.191126
S12	53675	108945	57939	761	24.2522	49.2251	26.1788	0.343846
S13	34592	112435	74083	210	15.6299	50.802	33.4733	0.0948852
S14	40313	116174	64382	451	18.2148	52.4914	29.09	0.203777
S15	35781	101934	83392	213	16.1671	46.0573	37.6794	0.0962407
S16	35520	109422	76125	253	16.0492	49.4406	34.3959	0.114314
S17	38693	105269	77163	195	17.4828	47.5642	34.8649	0.0881077
S18	49541	113287	58155	337	22.3843	51.187	26.2764	0.152268
S19	44083	114038	62906	293	19.9182	51.5263	28.4231	0.132387
S20	42412	83114	95586	208	19.1632	37.5538	43.189	0.0939816
S21	57738	103360	59899	323	26.088	46.7016	27.0644	0.145943
S22	37417	103796	79883	224	16.9063	46.8986	36.0939	0.101211
S23	38810	105496	76772	242	17.5357	47.6667	34.6882	0.109344
S24	36121	106427	78489	283	16.3207	48.0874	35.464	0.127869
S25	37810	101504	81567	439	17.0839	45.863	36.8548	0.198355
S26	51897	106244	62584	595	23.4489	48.0047	28.2776	0.268841
S27	50690	93237	76988	405	22.9035	42.1277	34.7858	0.182993
S28	56727	103770	59998	825	25.6312	46.8869	27.1092	0.372763
S29	58528	104903	57286	603	26.445	47.3988	25.8838	0.272456
S30	61481	93004	66223	612	27.7792	42.0224	29.9218	0.276523
S31	56089	94273	70388	570	25.3429	42.5958	31.8037	0.257546
S32	58767	94776	67208	569	26.553	42.8231	30.3669	0.257094
S33	44511	81461	94982	366	20.1116	36.8069	42.9161	0.165371
S34	53219	113082	54328	691	24.0462	51.0943	24.5473	0.312218
S35	50201	80775	89983	361	22.6825	36.4969	40.6574	0.163112

Note: LS represented Langshan Feather chicken, and PR represented White Plymouth Rock Chicken.

**Supplementary Table 5**

**Supplementary Table 5 Specific SNP sites in the offspring of different feather color phenotypes**

Group	offspring			
	black	black-white	yellow	black-yellow
Specific SNPs	519	674	698	966

**Supplementary Table 6**

**Supplementary Table 6 Primers for qRT-PCR, Bisulfite Sequencing and Microsatellite**

**Primers**

<b>qRT-PCR Primers</b>		
<b>Primer Name</b>	<b>Forward Primer 5' -&gt; 3'</b>	<b>Reverse Primer 5' -&gt; 3'</b>
<i>Oct4 UTR</i>	CGGGATCTCCATGAACAACAG	CTGGCCCCAGGCAGGTAA
<i>Sox2 UTR</i>	GTTCCAGGCTAAAGTAGTTTGA	CGGGCTGTTCTTCTGGTTGT
<i>SSEA-1 UTR</i>	GCCACCTACCTGAAGTTCCTCG	GCTGATTCCCTGCCGTCTT
<i>Nanog UTR</i>	GTATGCAACCAGCTCACC	TAGTAGTGTCCGCACCTAAC
<i>Lin28 UTR</i>	AAAGCCAATGCCAAGTGA	CAAACAAACCCAAAGATACG
<i>Klf4</i>	ATGCACAGGATGCTGCAACACG	TGGTGTGCGCCAGGATGAAGTC
<i>Rps17</i>	ACACCCGTCTGGGCAACGAC	CCCCTGGATGCGCTTCATC
<i>SALL4</i>	GTCCACTGCGGACCCCAACG	GGTGGAGAAGGCACGGCCAC
<i>TRIM7</i>	CATCGTGGCTGACCGCAGCA	CGACGATCCGGCGTGAGACG
<i>Nanog</i>	TGGTGTGCGCCAGGATGAAGTC	TGCTGGGTGTTGCAGCTTGTTT
<i>Cvh</i>	TTCTTGTTGGCAACTTCGG	AACTTCTGCTGGGCTTC
<i>Sox2</i>	AAACCAAGACCCTGATGAAGA	ATCCCATAGCCTCCGTTG
<i>Oct4</i>	TGCAATGCAGAGCAAGTGCTGG	ACTGGGCTTCACACATTTGCGG
<i>C-kit</i>	GCATCCAGCAATGGTGAC	AAGTTGCGTTGGGTCTAT
<i>Blimp1</i>	AAGAATCTGGTGAAGGGAG	GCAGTTTGATGCGTATTTG
<i>Cxcr4</i>	GCCATTCTGGTCTGTGGATG	GGCATGGACTATTGCCAGGT
<i>FSP1</i>	CTTCTCCGTCAACGTCTCAG	GTTCGGCTTGGTGTATCC
<i>Actin</i>	CAGCCATCTTTCTGGGTAT	CTGTGATCTCTTCTGCATCC
<b>Bisulfite Sequencing Primers</b>		
<i>Oct4</i>	TTAAGAATAATAAATTAAGGGGAAGG	ACATCAAACAAAAAATACAACACC
<i>Sox2</i>	GGTTTTTTTTGTTTTGTTTTTTTATG	TATCAACTCTAAACTCCAAAAATTTAATT
<i>Lin28A</i>	AAAGAGTTGTTTGGTTTAGTAGAGA	AACTTTAAAATCCCCAAAAAATAT
<i>Nanog</i>	GGGAAGTTTTGTTAGTAAAGGGATT	CAAATACTATCTTACCCTAAAACAC
<b>Microsatellite Primers</b>		
<i>LEI094</i>	AGGATGGCTGTTATGCTTCCA	GACCATACTTCTGGAACAAG
~290 in Black Langshan Chicken/~311 in White Plymouth Rock Chicken		
<i>MCW104</i>	TAGCACAACCTCAAGCTGTGAG	CAGACTTGCACAGCTGTGACC
~180 in Black Langshan Chicken /~186 or 215 in White Plymouth Rock Chicken		
<i>MCW-004</i>	GGATTACAGCACCTGAAGCCACTAG	AAACCAGCCATGGGTGCAGATTGG
~204 or 206 in Black Langshan Chicken /~189 in White Plymouth Rock Chicken		