SUPPLEMENTARY MATERIAL

Figure S1. Endogenous Fetal Oocytes Express ΔPE *Oct4*-GFP at Low Intensity and Lack SSEA1 Expression

(A) *Oct4*-GFP was expressed at high intensity in transgenic ESCs, female/male genital ridge at e12.5, and e15.5 fetal testis but was expressed at low intensity in oocytes from post-committed e15.5 ovary by flow cytometry analysis. The high intensity GFP+ gate was based on transgenic ESC and e12.5 PGC intensity. The low intensity GFP+ gate was set below the high intensity gate and above non-transgenic GFP- cells (solid peak).

(B) Undifferentiated ESCs and PGCs from e12.5 genital ridge were SSEA1+, but e15.5 ovary and testis lacked robust SSEA1 expression. Solid peak represents ESCs stained with only secondary antibody. X-axis: fluorescence intensity. Y-axis: cell number.

Figure S2. Confirmation of *Oct4*-GFP+ ESC-derived Oocyte Maturation

(A) Transcripts expressed beginning in pre-meiotic germ cells (*Oct4, Stella, Nanos3, and Vasa*) were enriched in the double-positive population while markers of meiotic entry and oocyte maturation (*Stra8 and Gdf9*) were elevated in GFP+ / SSEA1- cells by quantitative RTPCR analysis. Somatic cell markers (*Kdr and Sox1*) were robustly detected in the double-negative population with minimal levels observed in GFP+ germ cells. *Hoxa1* was expressed in endogenous somatic and germ cells and was also detected in all ESC-derived populations.

(B) *Oct4*-GFP+ ESC-derived germ cell maturation was confirmed by single-cell quantitative RTPCR expression profiling of meiotic germ cell transcripts before *in vitro* differentiation and after 5 days of differentiation (-/+) FAC media. Meiotic transcripts

were elevated following differentiation in FAC media compared to ESCs and non-FAC treated cultures. Relative expression (2^(-ΔCt) relative to *Gapdh*).

Figure S3. **Single *Oct4*-GFP+ Cells are more likely to Express Germ Cell Marker Transcripts following Differentiation**

Although a fraction of the undifferentiated GFP+ ESCs expressed germ cell markers, a greater percentage of GFP+ cells expressed germ cell transcripts following differentiation by quantitative RTPCR analysis. Relative expression (2^(-ΔCt) relative to *Gapdh*).

Figure S4. Single *Oct4*-GFP+ Cells are enriched for Germ Cell Transcripts following Differentiation

Transcript levels were increased following 5 days of differentiation by quantitative RTPCR analysis, and were elevated with significance following differentiation in FAC media, compared to undifferentiated GFP+ ESCs. Transcript levels were normalized (2^(-ΔCt) relative to *Gapdh*), and single-cell values were averaged for each population. Significance of D5+FAC to D0 ESC ($p=0.0048$). Significance of D5+FAC to D5 ($p=0.0071$).

Figure S5. *Stra8*-GFP ESC-derived Meiotic Entry

(A and B) Similar to *Oct4*-GFP ESC flow cytometry analysis, *Stra8*-GFP is expressed in most cells before and throughout the differentiation time course with a reduction in

SSEA1 expression by day 14 of differentiation and by day 7 in FAC media. X-axis: GFP intensity. Y-axis: SSEA1 intensity.

(C) *Stra8*-GFP+ cells isolated from day 14 EBs by FACS were enriched for meiotic marker transcripts by RTPCR analysis (*Stra8, Scp3, Scp1, normalized to UbiquitinB*).

(D) *Stra8*-GFP+ ESC-derived germ cell meiotic entry and early progression demonstrated by immunofluorescence staining for SCP3 showing nuclear localization and partial SCP3 chromosomal alignment.

(E) Endogenous pachytene spermatocyte control illustrating complete SCP3 and SCP1 chromosomal alignment with telocentric CREST centromere staining.

(F) Endogenous leptotene-zygotene spermatocyte controls with nuclear SCP3 and punctate γ-H2AX expression.

(G) Endogenous pachytene spermatocyte controls showing complete SCP3 chromosomal alignment and γ-H2AX localization to the XY body.

(D, F and G) Blue is DAPI. (D) to (G) magnification is 630X.

Figure S6. ESC and Ovary Immunostaining Controls

(A and B) TRA98 immunofluorescence stain of undifferentiated ESCs (A) and newborn ovary (B) showing robust oocyte-specific expression.

(C and D) GFP and SSEA1 co-immunofluorescence stain of transgenic *Oct4*-GFP undifferentiated ESCs (C) and adult ovary (D) confirming a lack of SSEA1 expression by oocytes. Blue is DAPI. Magnification is 200X.

Figure S1.

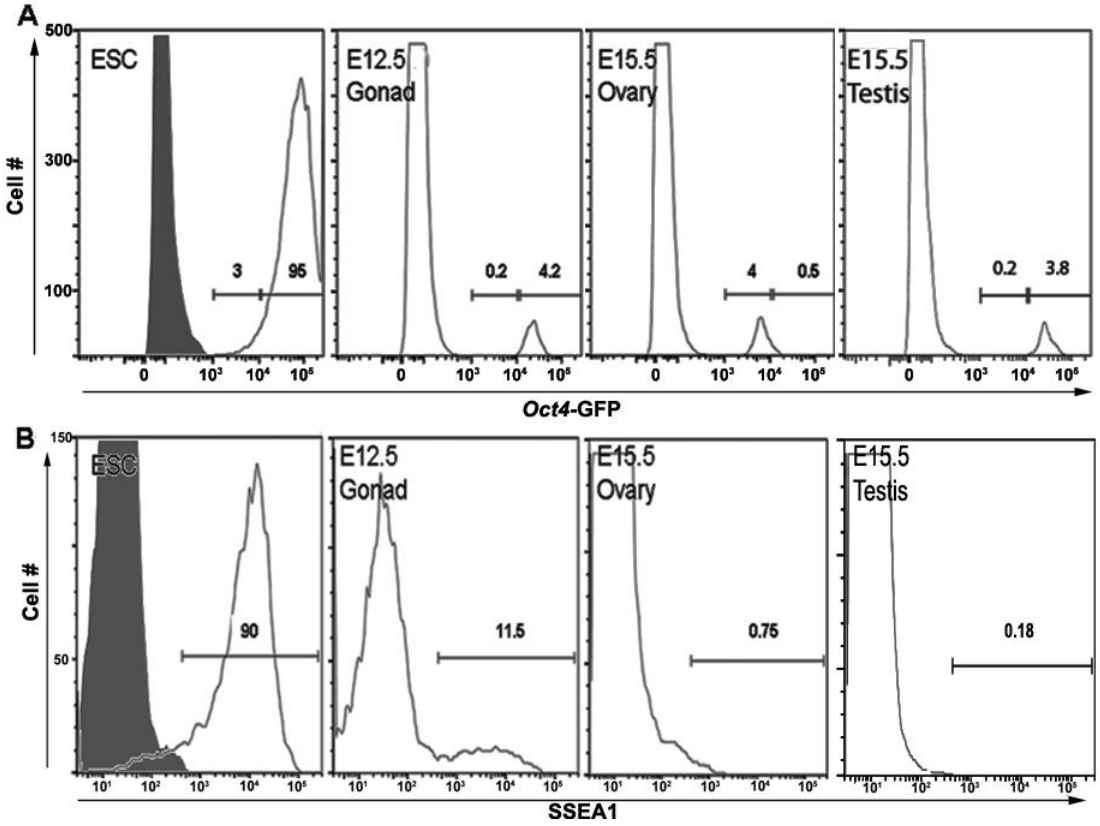
A
ESC
E12.5 Gonad
E15.5 Ovary
E15.5 Testis
Cell #
500
300
100
3
95
0.2
4.2
4
0.5
0.2
3.8
0
10³
10⁴
10⁵
Oct4-GFP
B
ESC
E12.5 Gonad
E15.5 Ovary
E15.5 Testis
Cell #
150
50
90
11.5
0.75
0.18
10¹
10²
10³
10⁴
10⁵
SSEA1

Figure S2.

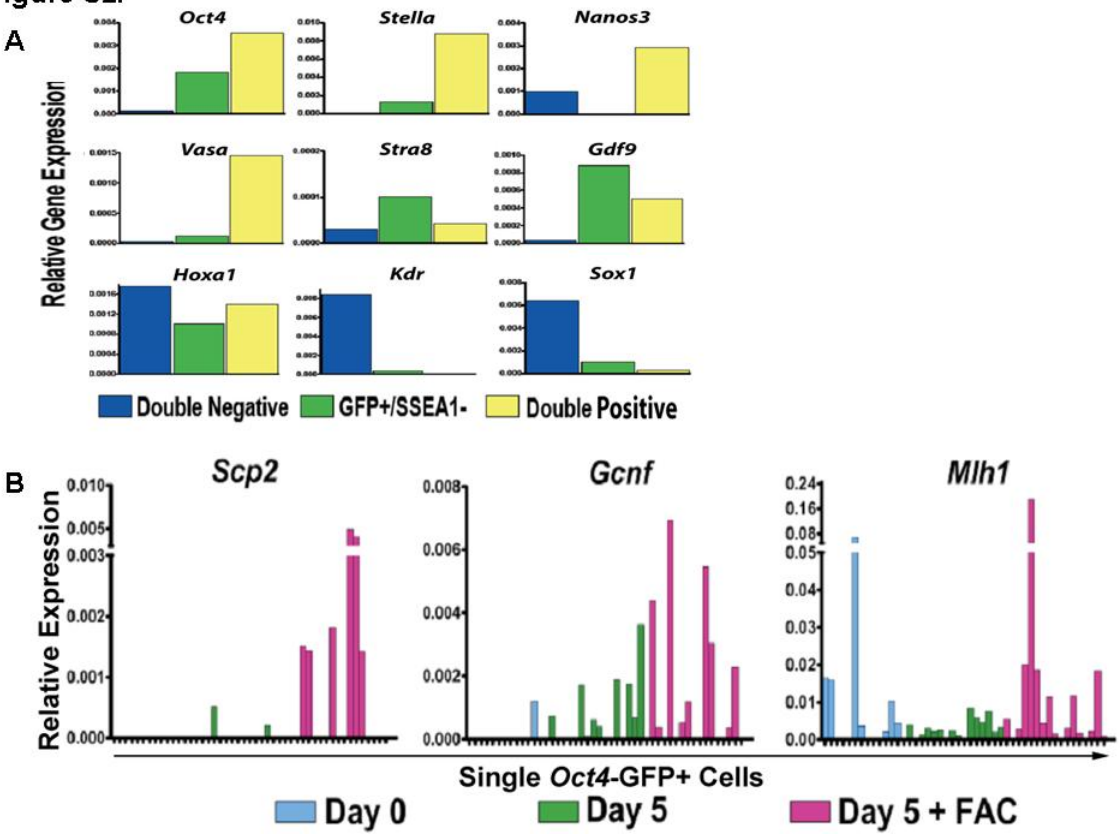
A
Oct4
Stella
Nanos3
Vasa
Stra8
Gdf9
Hoxa1
Kdr
Sox1
Relative Gene Expression
Double Negative
GFP+/SSEA1-
Double Positive
B
Scp2
Gcnf
Mlh1
Relative Expression
Single Oct4-GFP+ Cells
Day 0
Day 5
Day 5 + FAC

Figure S3.

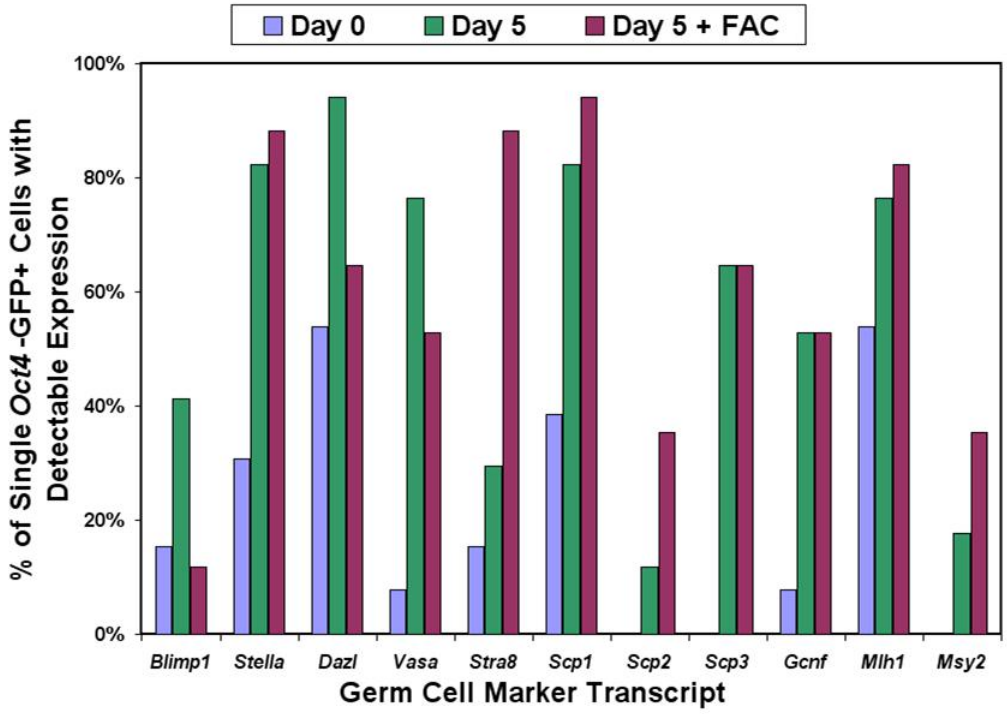
Day 0
Day 5
Day 5 + FAC
100%
80%
60%
40%
20%
0%
% of Single Oct4 -GFP+ Cells with Detectable Expression
Blimp1
Stella
Dazl
Vasa
Stra8
Scp1
Scp2
Scp3
Gcnf
Mlh1
Msy2
Germ Cell Marker Transcript

Figure S4.

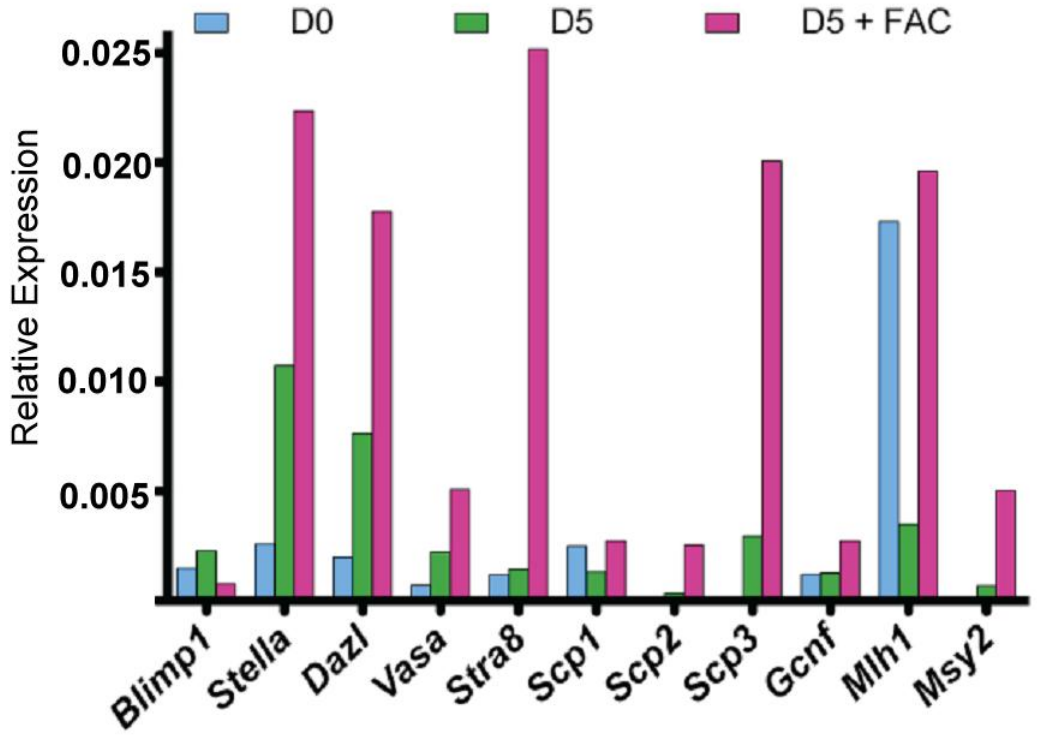
D0
D5
D5 + FAC
Relative Expression
0.025
0.020
0.015
0.010
0.005
Blimp1
Stella
Dazl
Vasa
Stra8
Scp1
Scp2
Scp3
Gcnf
Mlh1
Msy2

Figure S5.

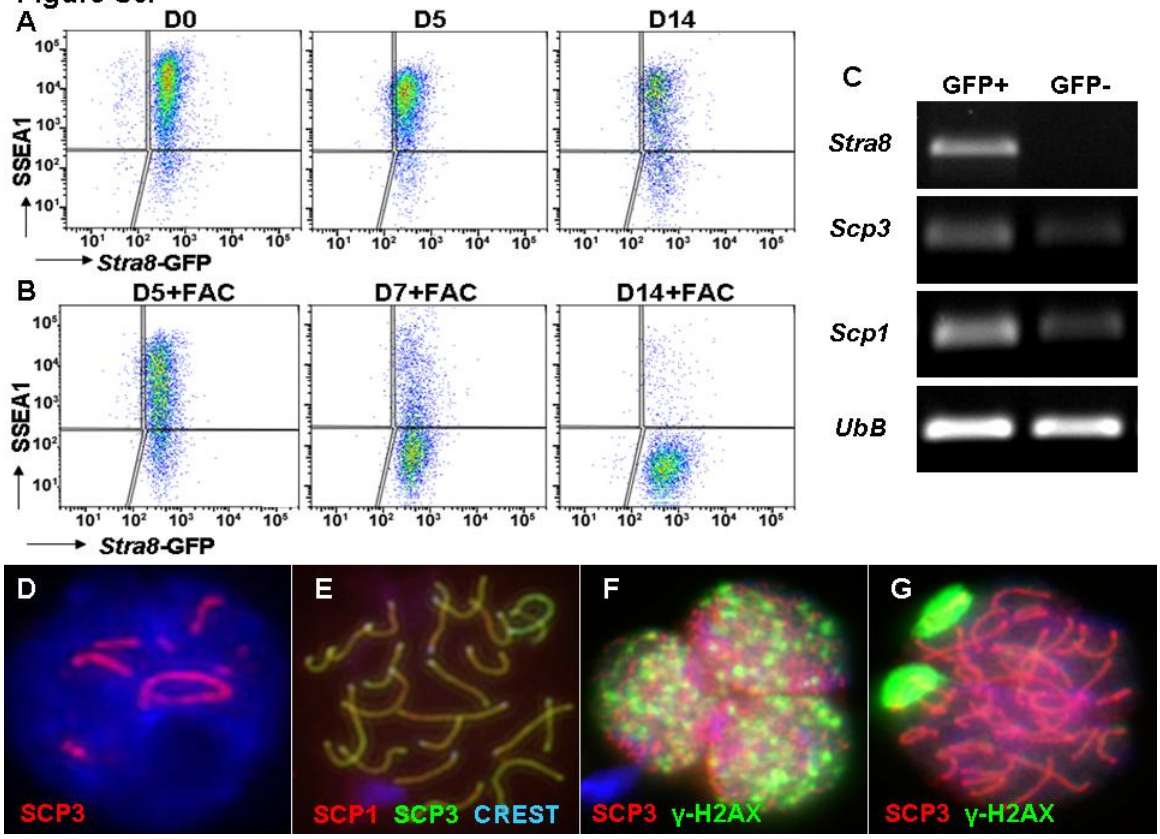
A
D0
D5
D14
SSEA1
Stra8-GFP
B
D5+FAC
D7+FAC
D14+FAC
SSEA1
Stra8-GFP
C
GFP+
GFP-
Stra8
Scp3
Scp1
UbB
D
SCP3
E
SCP1 SCP3 CREST
F
SCP3 γ-H2AX
G
SCP3 γ-H2AX

Figure S6.