Supplemental Figures and Table

Figure S1. Germ cell marker expression and optimization of PGCLC induction from hESCs.

Figure S2. Generating ITGA6/EPCAM//TNAP/cKIT positive PGCLCs.

Figure S3. PGCLC induction from 18 pluripotent hESC lines derived at UCLA.

Figure S4. Evaluation of *T* and *EOMES* in different combination of cytokines and inhibitors and molecular information of *EOMES* mutant alleles.

Supplemental Table S1. List of antibodies used in this study

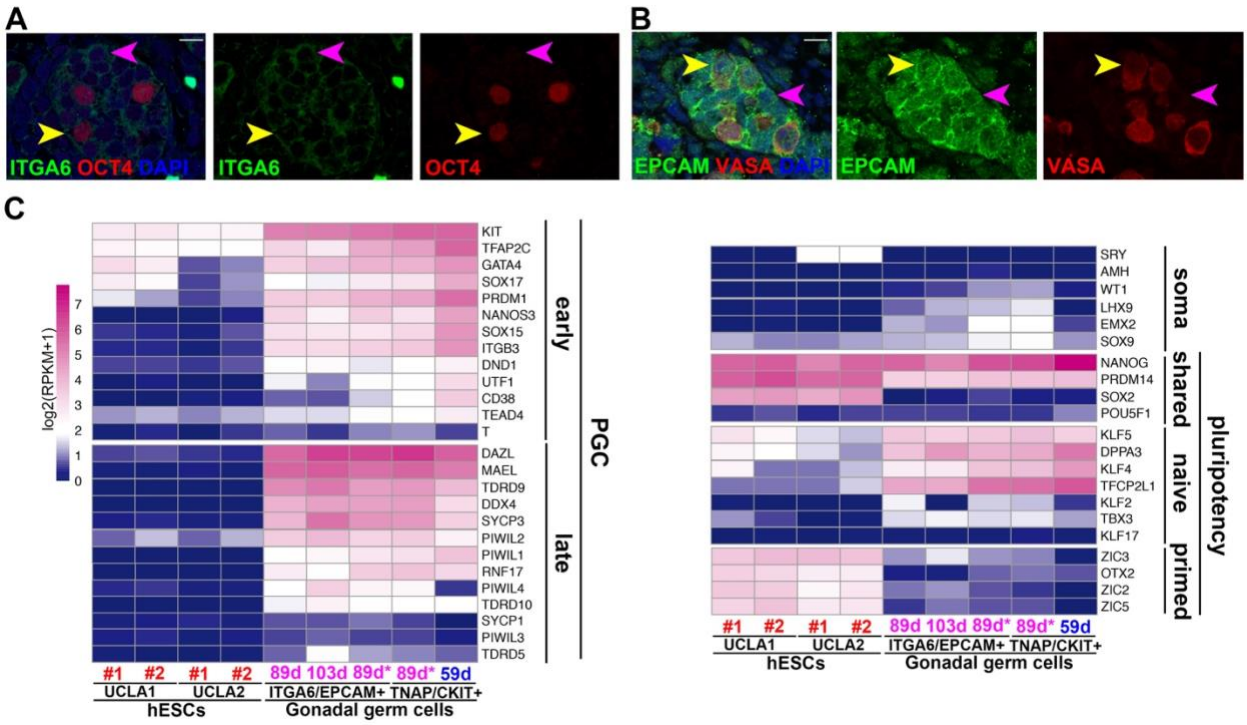

Figure S1. Germ cell marker expression and optimization of PGCLC induction from hESCs.

(A) Human embryonic testis at day (d) 115 section stained with ITGA6 (green), OCT4 (red), and DAPI (blue) by immunofluorescence. Yellow arrowhead points to a OCT4-positive germ cell that is also positive for ITGA6. Purple arrowhead points to a ITGA6-positive germ cell that is negative for OCT4. Scale bar: 10μm.

(B) Human embryonic testis at day (d) 72 section stained with EPCAM (green), VASA (red) and DAPI (blue) by immunofluorescence. Yellow arrowhead points to a VASA-positive germ cell that is also positive for EPCAM. Purple arrowhead points to a EPCAM-positive germ cell that is negative for VASA. Scale bar: 10μm.

(C) Heat map showing the expression of germ cell, somatic cell and pluripotency genes in transcriptomes shown in Figure 1E.

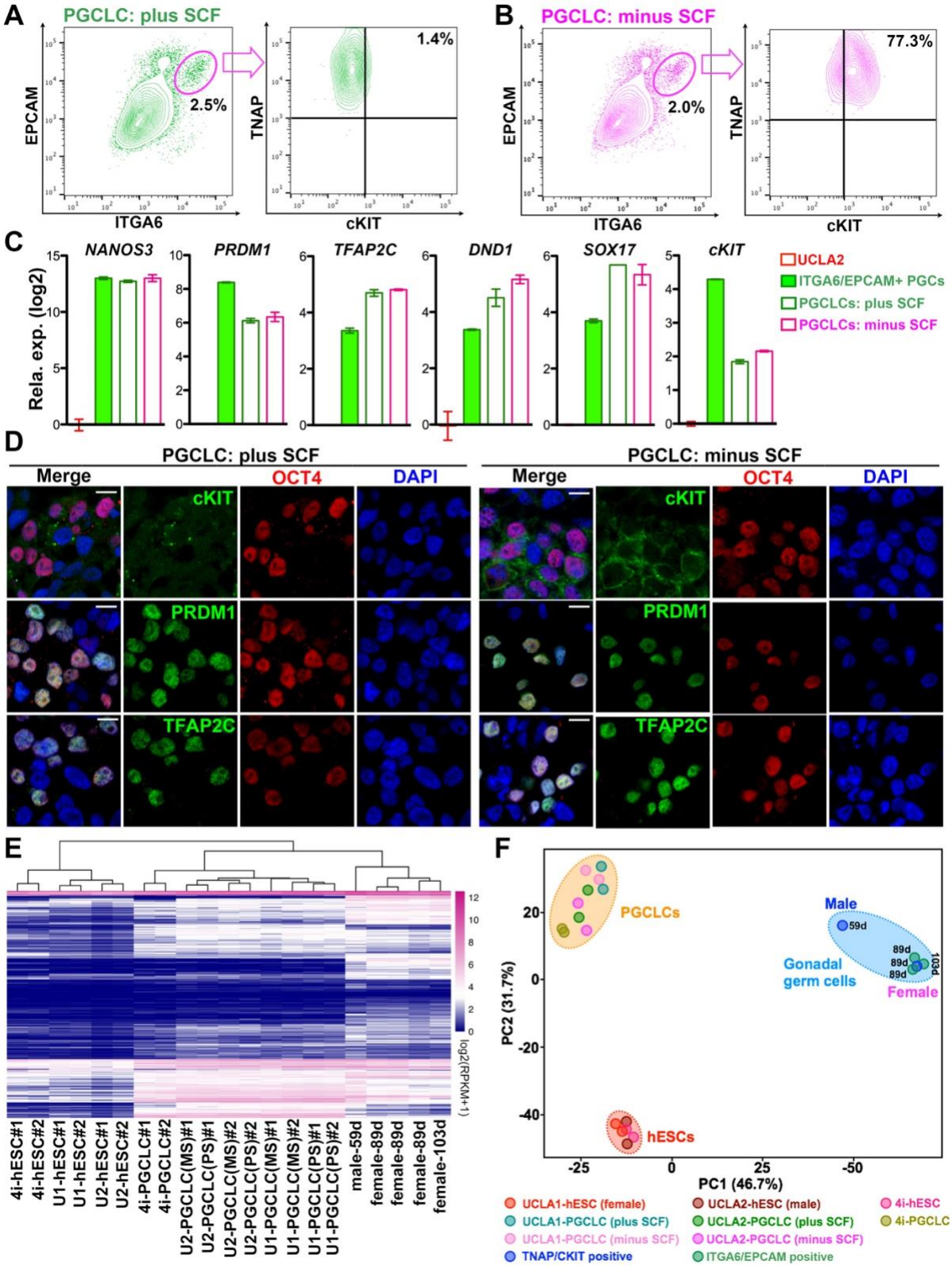

Figure S2. Generating ITGA6/EPCAM//TNAP/cKIT positive PGCLCs.

(A-B): Flow cytometry of day 4 aggregates made with SCF (A) or without SCF (B) from UCLA2 (p13) and stained for ITGA6/EPCAM/TNAP/cKIT.

(C) Real time PCR of the sorted ITGA6/EPCAM positive PGCLCs made from UCLA2 (p13, p14) with SCF (green open columns) or without SCF (purple open columns) and compared to gene expression in ITGA6/EPCAM PGCs at day 72 post-fertilization (Figure 1A-B) (green solid columns). Fold change is calculated relative to expression levels of each gene in the UCLA1 hESC line, which was given a value of 1.0.

(D) Immunofluorescence of day 4 PGCLC aggregates from UCLA2 (p14) to examine co-localization of OCT4 (red), with cKIT, PRDM1 and TFAP2C (all green). Scale bar: 10μm.

(E) UHC of primed undifferentiated hESCs (UCLA1 p14, p15 and UCLA2 p13, p14), day 4 PGCLCs sorted by FACS using ITGA6/EPCAM (made from UCLA1 p14, p15 and UCLA2 p13, p14) with (plus) and without (minus) SCF, undifferentiated 4i cultured hESCs sorted with TNAP (WIS2) and day 4 PGCLCs sorted by FACS using TNAP/NANOS3-mCherry) (made from WIS2). UHC was based on the expression of DEGs between hPGCs and H9 hESCs defined by Irie et al., 2015 and Sasaki et al., 2015. U1 and U2 indicate UCLA1 and UCLA2, respectively. Gonadal germ cell libraries analyzed here are the same in Figure 1E. MS = minus SCF. PS = plus SCF.

(F) PCA of transcriptomes in (E).

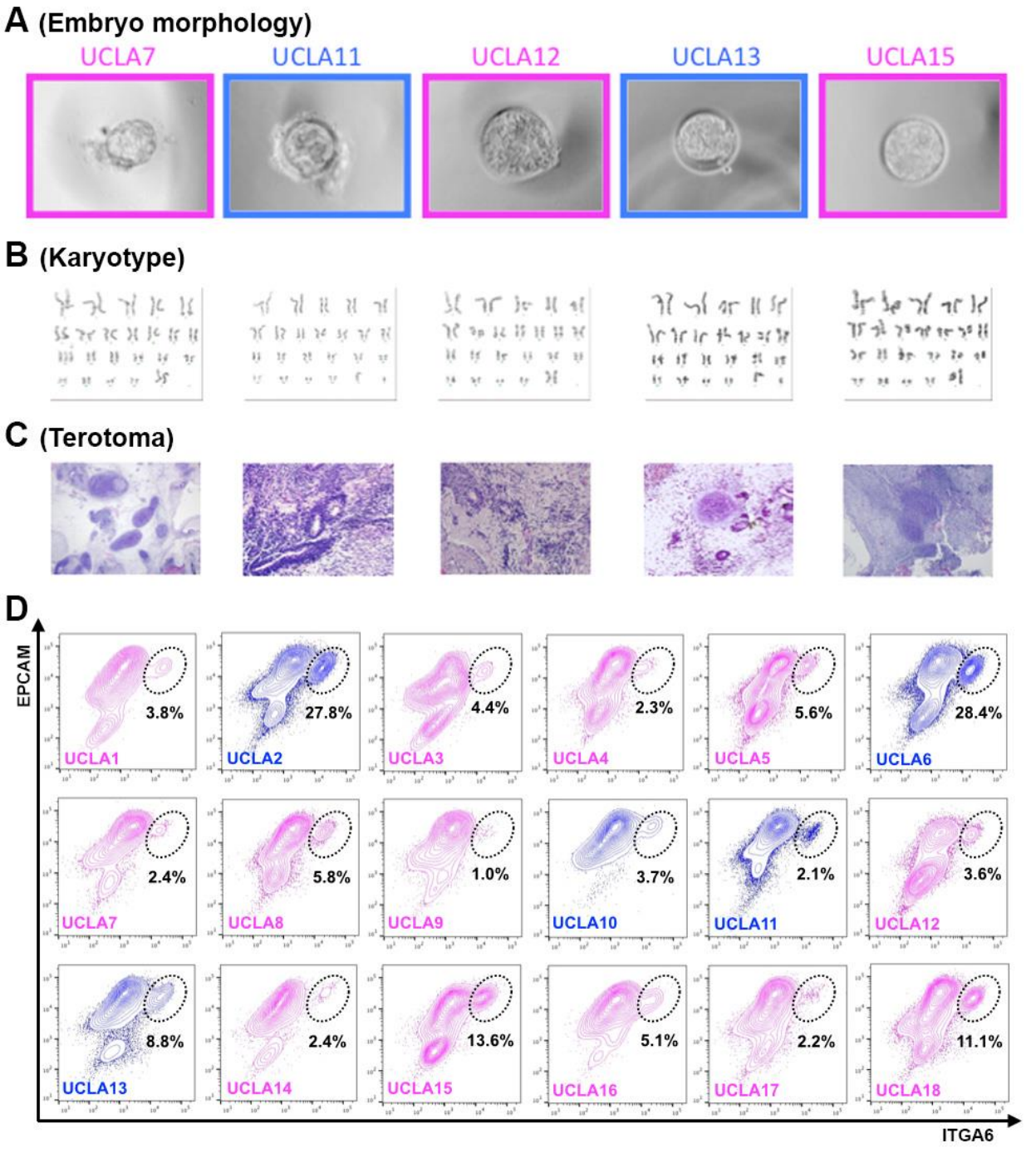

Figure S3. PGCLC induction from 18 pluripotent hESC lines derived at UCLA.

(A) Morphology of human embryos used for derivation of hESC lines UCLA7, UCLA11, UCLA12, UCLA13, and UCLA15. All other hESC lines are reported elsewhere.

(B) Karyotypes of hESC lines UCLA7, UCLA11, UCLA12, UCLA13, and UCLA15 (from left to

right). All other hESC lines are reported elsewhere.

(C) Representative images showing teratomas formed by injection of hESC lines UCLA7, UCLA11, UCLA12, UCLA13, and UCLA15 (from left to right) into the testes of SCID-beige mice. All other hESC lines are reported elsewhere.

(D) FACS plots of day 4 PGCLCs (sorted with ITGA6/EPCAM) induced from 18 hESC lines through 24 hours of iMeLC differentiation.

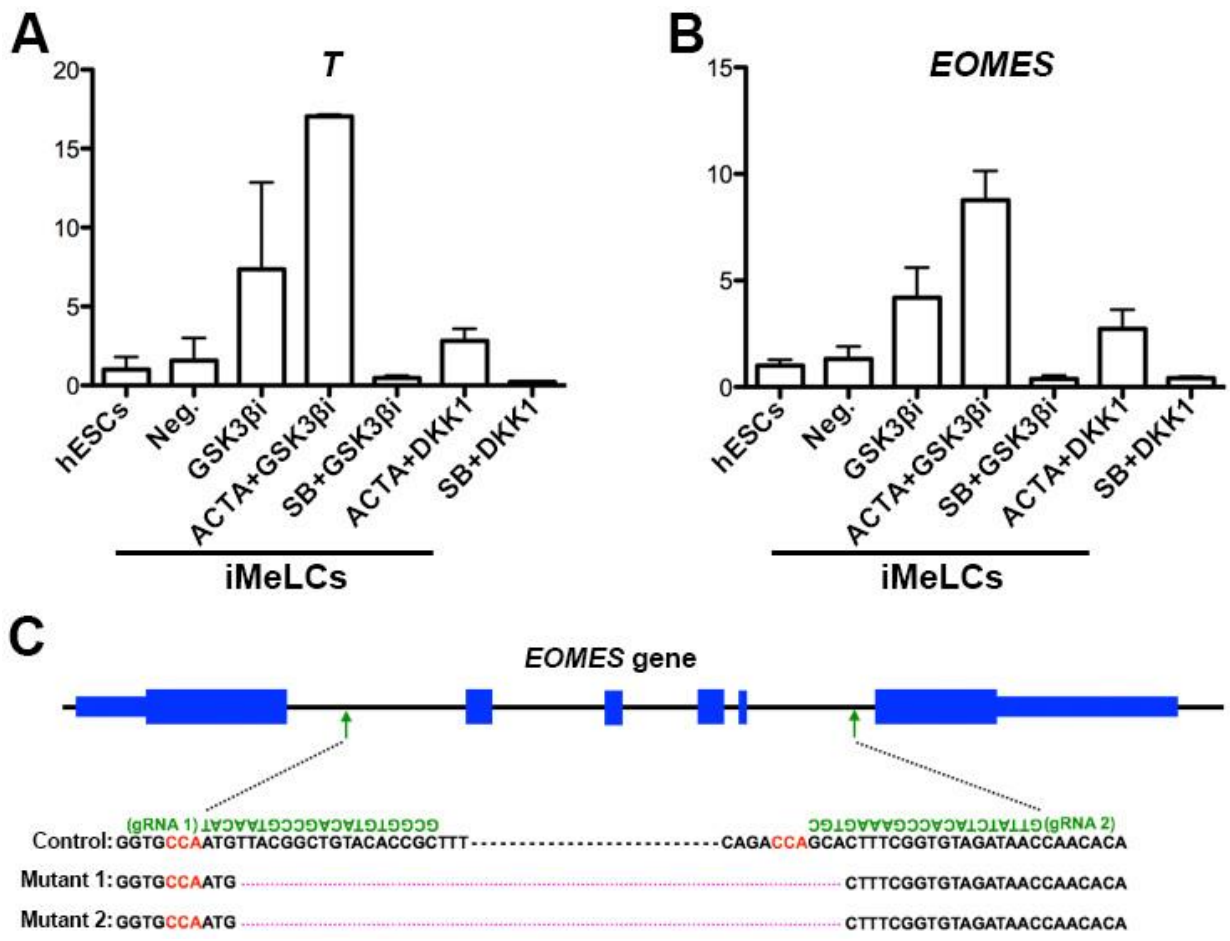

Figure S4. Evaluation of *T* and *EOMES* in different combination of cytokines and inhibitors and molecular information of *EOMES* mutant alleles.

(A) *T* expression in the iMeLCs with different combinations of cytokines and signaling inhibitors.

(B) *EOMES* expression in the iMeLCs with different combinations of cytokines and signaling inhibitors.

(C) Molecular information of gRNAs for targeting *EOMES* and the resulting *EOMES* mutant alleles in the subline used in this study.

Supplemental Table S1
List of antibodies used in this study

Name	Catalog number	Purpose	Dilution
BV421 anti-human/mouse ITGA6	BioLegend Cat# 313624	FACS	1:60
488 anti-human EPCAM	BioLegend Cat# 324210	FACS	1:60
APC anti-human EPCAM	BioLegend Cat# 324208	FACS	1:60
PE anti-human TNAP	BD Pharmingen Cat# 561433	FACS	1:60
APC anti-human cKIT	BD Pharmingen Cat# 550412	FACS	1:60
Rabbit anti-human EPCAM	Abcam Cat# ab71916,	Immunofluorescence	1:50
Goat anti-human VASA	R&D Systems, Cat# AF2030	Immunofluorescence	1:20
Rabbit anti-human cKIT	DAKO Cat# A4502	Immunofluorescence	1:100
Goat anti-human OCT4	Santa Cruz Biotechnology Cat# sc-8628x	Immunofluorescence	1:100
Rabbit anti-human PRDM1	Cell Signaling Technology Cat# 9115	Immunofluorescence	1:100
Mouse anti-human PRDM1	R&D Systems Cat# MAB36081SP	Immunofluorescence	1:100
Rabbit anti-human TFAP2C	Santa Cruz Biotechnology Cat# sc-8977	Immunofluorescence	1:100
Mouse anti-human TFAP2C	Santa Cruz Biotechnology Cat# sc12762	Immunofluorescence	1:100
Rat anti-human ITGA6	Santa Cruz Biotechnology Cat# sc-80554	Immunofluorescence	1:100
Goat anti-human T	R&D Systems Cat# AF2085	Immunofluorescence	1:100
Goat anti-human SOX17	Neuromics Cat# GT15094	Immunofluorescence	1:100
Rabbit anti-mouse EOMES	Abcam Cat# ab23345	Immunofluorescence	1:100
Rabbit anti-human β-CATENIN	Cell Signaling Technology Cat# 9582	Immunofluorescence	1:100
Rabbit anti-human pSMAD2/3	Cell Signaling Technology Cat# 8828	Immunofluorescence	1:100