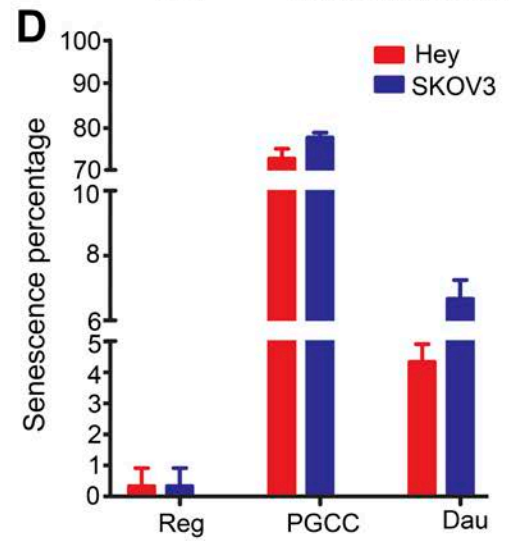
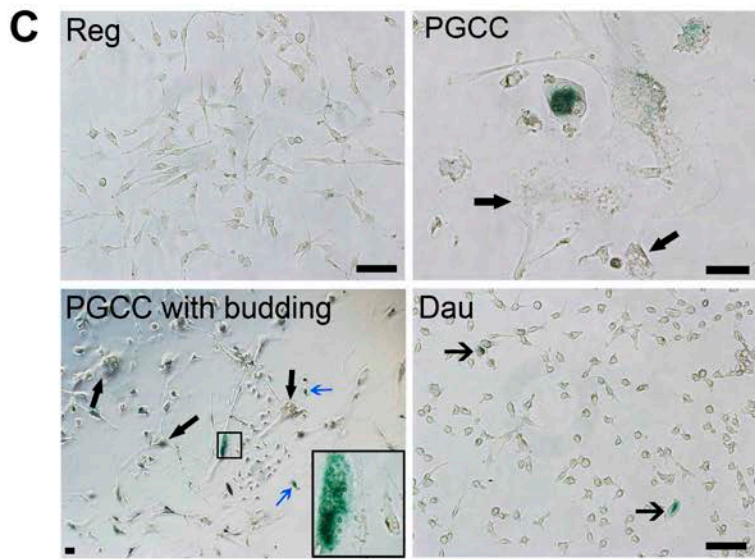
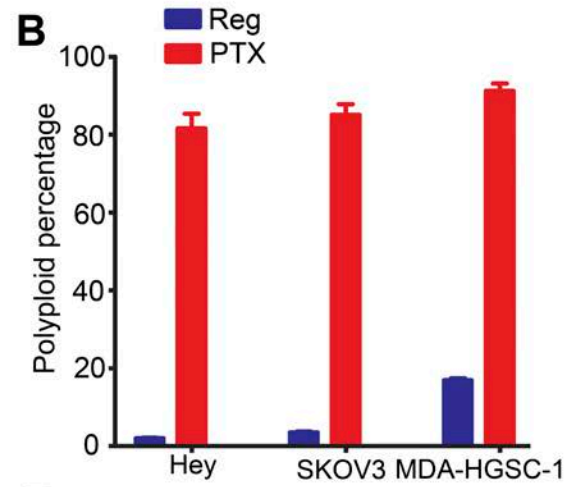
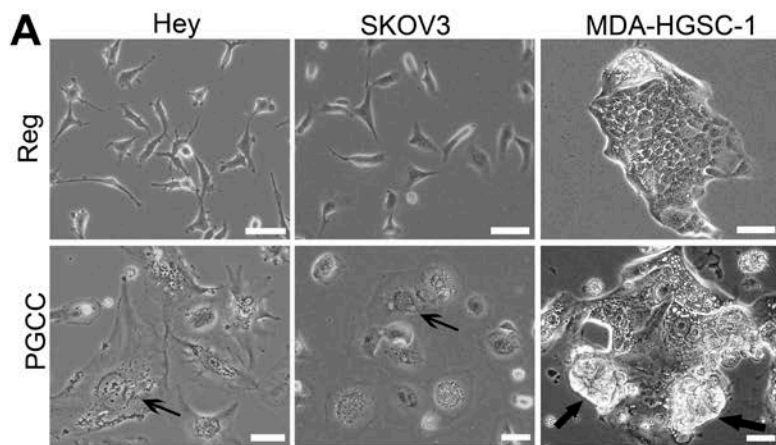


Figure S1. Formation of PGCCs and spheroids

(A) Morphology of regular (Reg) Hey cells and PGCCs induced by paclitaxel (PTX) 500 nM at recovery day 7. PGCCs were as large as 300 μm in diameter and had single or multiple nuclei (small black arrows). MDA-HGSC-1 cells grew as spheroids rather than individual attached cells in the absence of PTX and grew as spheroids with multiple giant cells (black arrows) following PTX treatment. Bars, 50 μm .

(B) Polyploid percentage in each cell line detected by flow cytometry at recovery day 7 following PTX (500 nM) treatment. Hey: Regular (Reg), $2.0 \pm 0.1\%$; PTX, $81.6 \pm 3.7\%$. SKOV3: Regular (Reg), $3.5 \pm 0.2\%$; PTX, $85.1 \pm 2.7\%$. MDA-HGSC-1: Regular (Reg), $16.9 \pm 0.5\%$; PTX, $91.3 \pm 1.9\%$.

(C) Senescent cancer cells detected by β -gal staining in regular Hey cells, PGCCs induced by PTX treatment of Hey cells, and PGCC-derived daughter cells. Blue arrows indicate positive β -gal staining (signals were dark green in cytoplasm); black arrows indicate non-senescent PGCCs.

(D) Percentage of senescent cells based on β -gal staining. Hey: Regular (Reg), $0.4 \pm 0.5\%$; PGCC, $73.0 \pm 3.1\%$; Daughter (Dau), $4.7 \pm 0.5\%$. SKOV3: Regular (Reg), $0.4 \pm 0.5\%$; PGCC, $78.7 \pm 2.0\%$; Daughter (Dau), $7.1 \pm 0.8\%$.

(E) Spheroid formation from a single PGCC induced by PTX treatment of MDA-HGSC-1 cells. Early-stage development was similar to the early development of the human embryo, including the zygote (spheroid day 1 [sD1]), 2-cell (sD2), 4-cell (sD5), and morula (sD7) stages.

(F) Three-dimensional confocal scanning showed inner cell cluster in early-stage spheroid, indicated by yellow arrows; a to c, three different images of same spheroids from different rotation angles. Cells were labeled with green fluorescent protein-ubiquitin, and nuclei were stained with Hoechst 33342.

Bars, 50 μm .

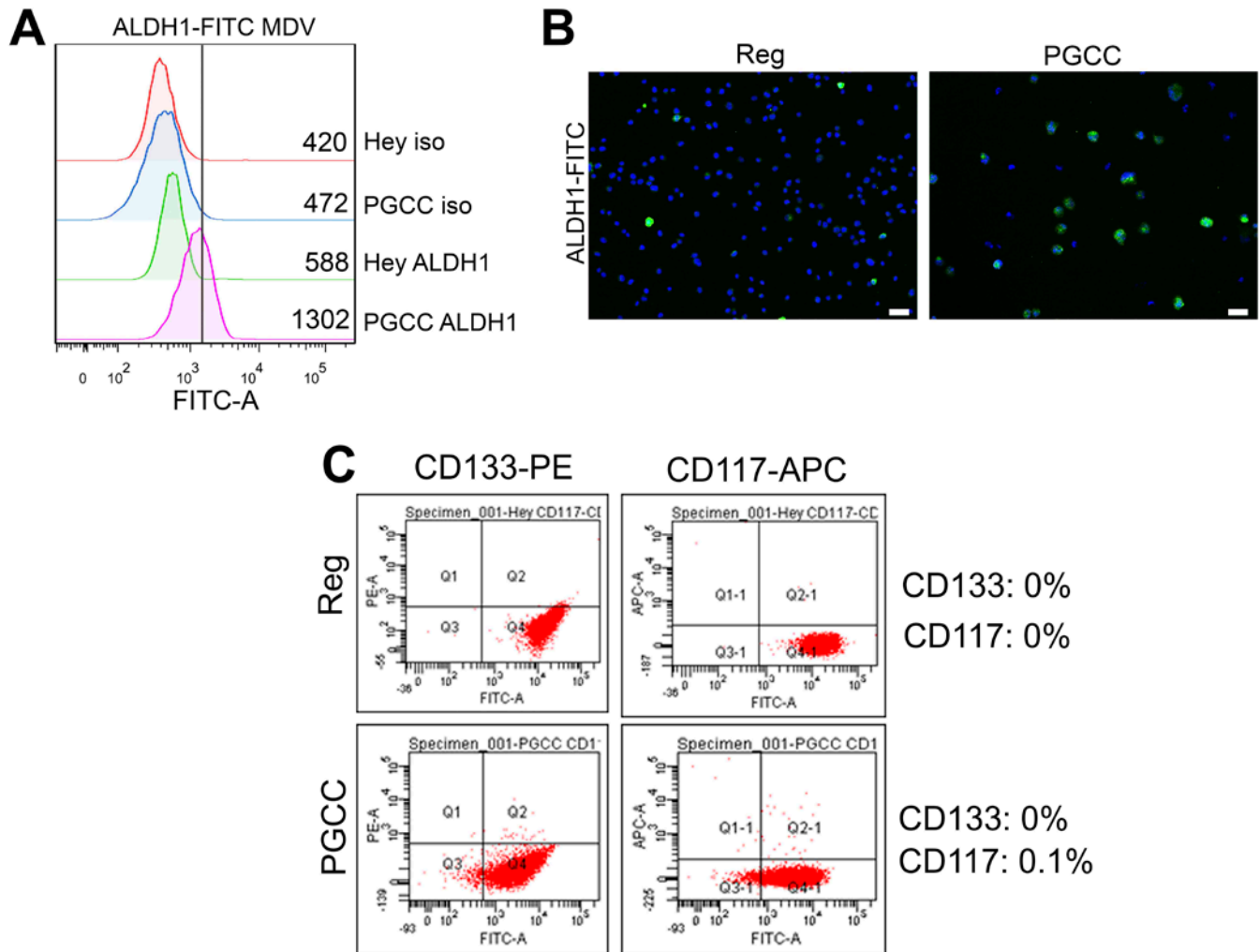


Figure S2. Expression of cancer stem cell markers of PGCCs

(A, B) Expression of ALDH1 in PGCCs from Hey cells compared with isotype control (iso) detected by FACS (A) followed by direct observation of cell smear (B). MDV, mean density value. Bars, 50 μ m.

(C) Expression of cancer stem cell markers (CD133, CD117, and CD44) analyzed by FACS. Expression of CD117 (0.1%) was detected in PTX-treated cells but not in untreated control cells.

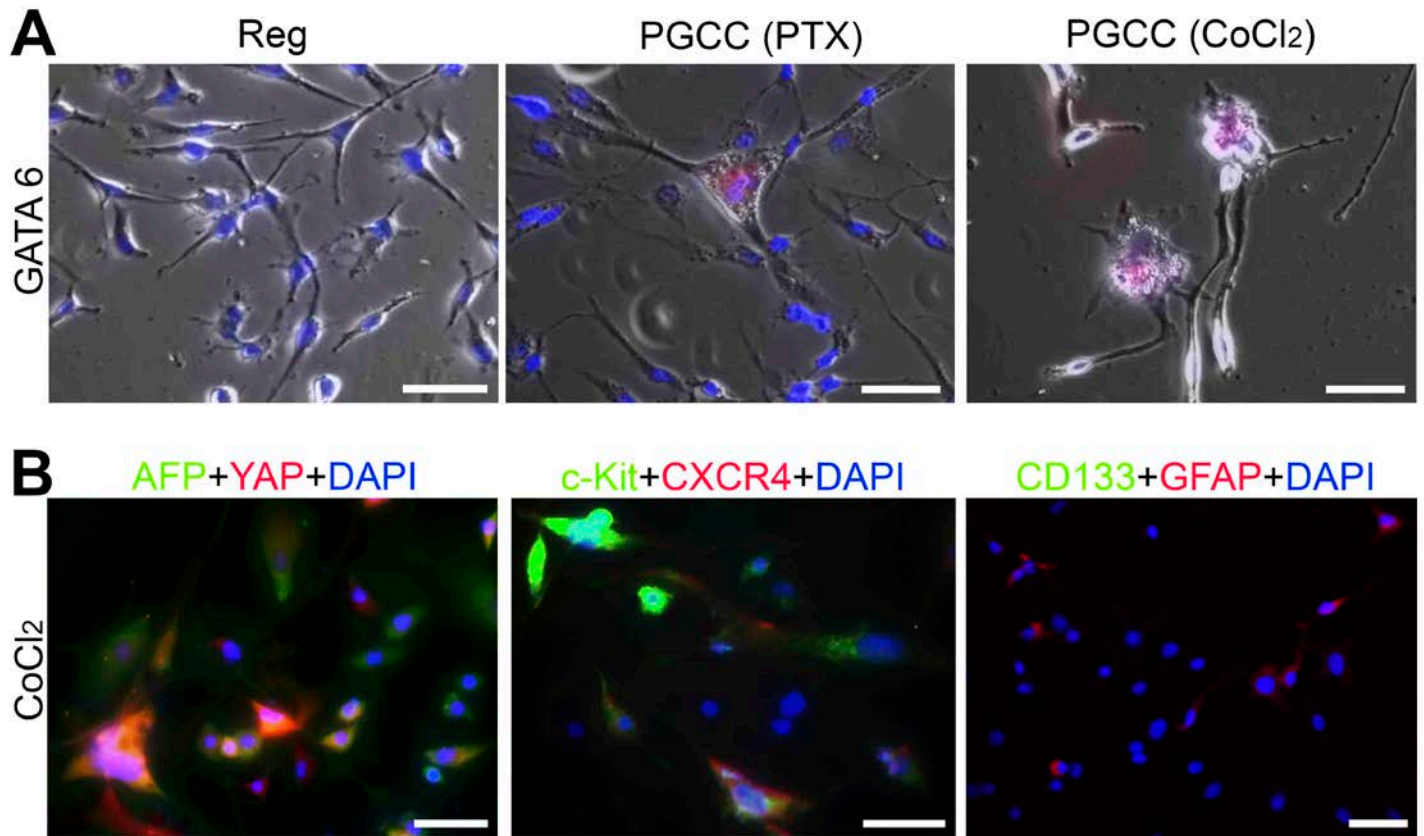


Figure S3. Expression of GATA6 in PGCCs and endoderm differentiation

(A) Activation of GATA6 in PGCCs induced by paclitaxel (PTX) (500 nM) and CoCl₂ (450 μ M). Bars, 50 μ m.

(B) Endoderm differentiation of PGCC spheroids induced by CoCl₂ (450 μ M), indicated by positive expression of AFP, c-Kit, or CXCR4. Bars, 50 μ m.

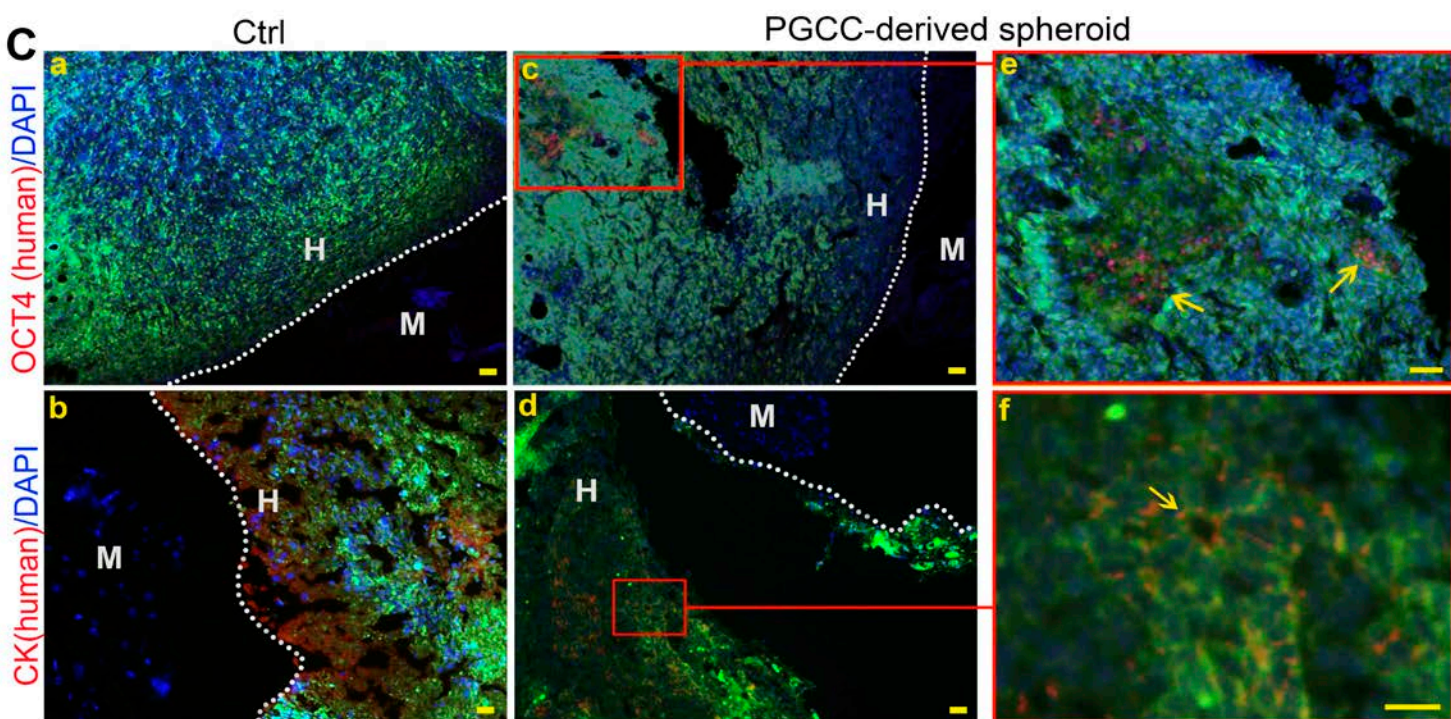
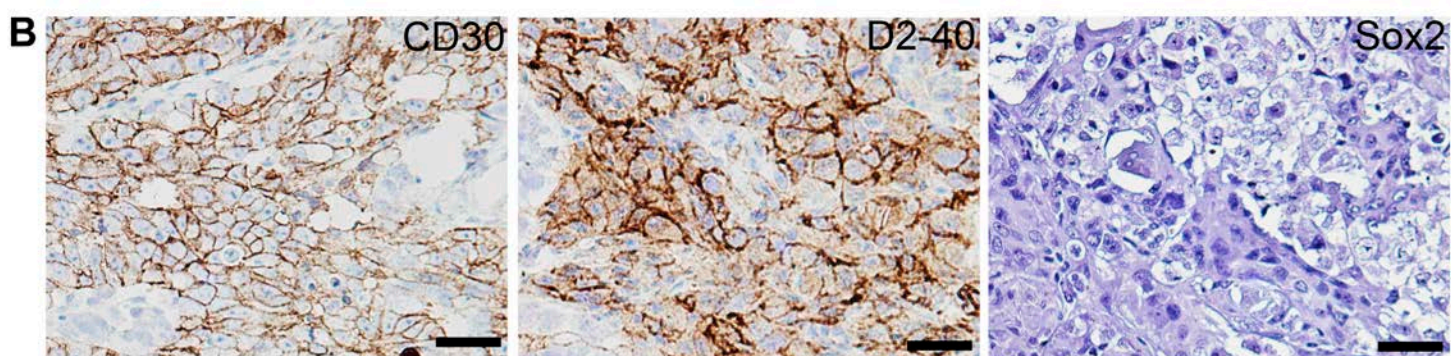
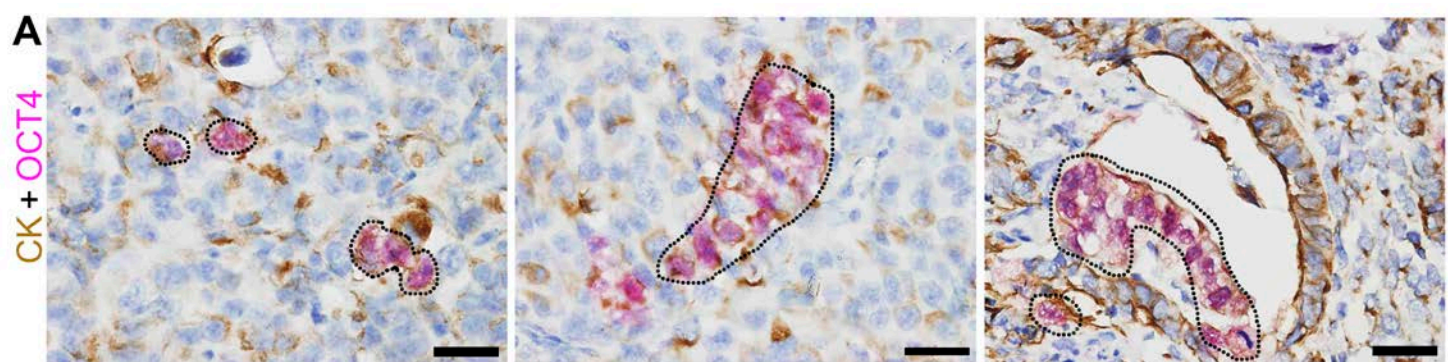


Figure S4. IHC and immunofluorescence on xenografts generated by PGCC spheroids

(A) Expression of OCT4 and cytokeratin (CK) in tumors from PGCC-derived spheroids. Single or clustered germ cells (black dotted lines) or partial epithelial glands stained positive for OCT4. Bars, 50 μ m.

(B) Embryonic carcinoma also stained positive for CD30 (marker for embryonic carcinoma), and dysgerminoma stained positive for D2-40 (marker for dysgerminoma) and negatively for SOX2. Bars, 50 μ m.

(C) Human origin of germ cell tumors. Hey cells were labeled with ubiquitin-enhanced green fluorescent protein. OCT4- and CK-positive populations (yellow arrows) were detected in xenograft derived from PGCC spheroids. a, Mice injected with control regular Hey spheroids. All of the green cells were dispersed within the nodule and were negative for OCT4. b, Positive for human-specific CK. c and e, Single human cancer cells or human cancer cell clusters (green) were positive for OCT4. d and f, Gland-like structures stained positive for cytokeratin. H, human; M, mouse. Bars, 50 μ m.