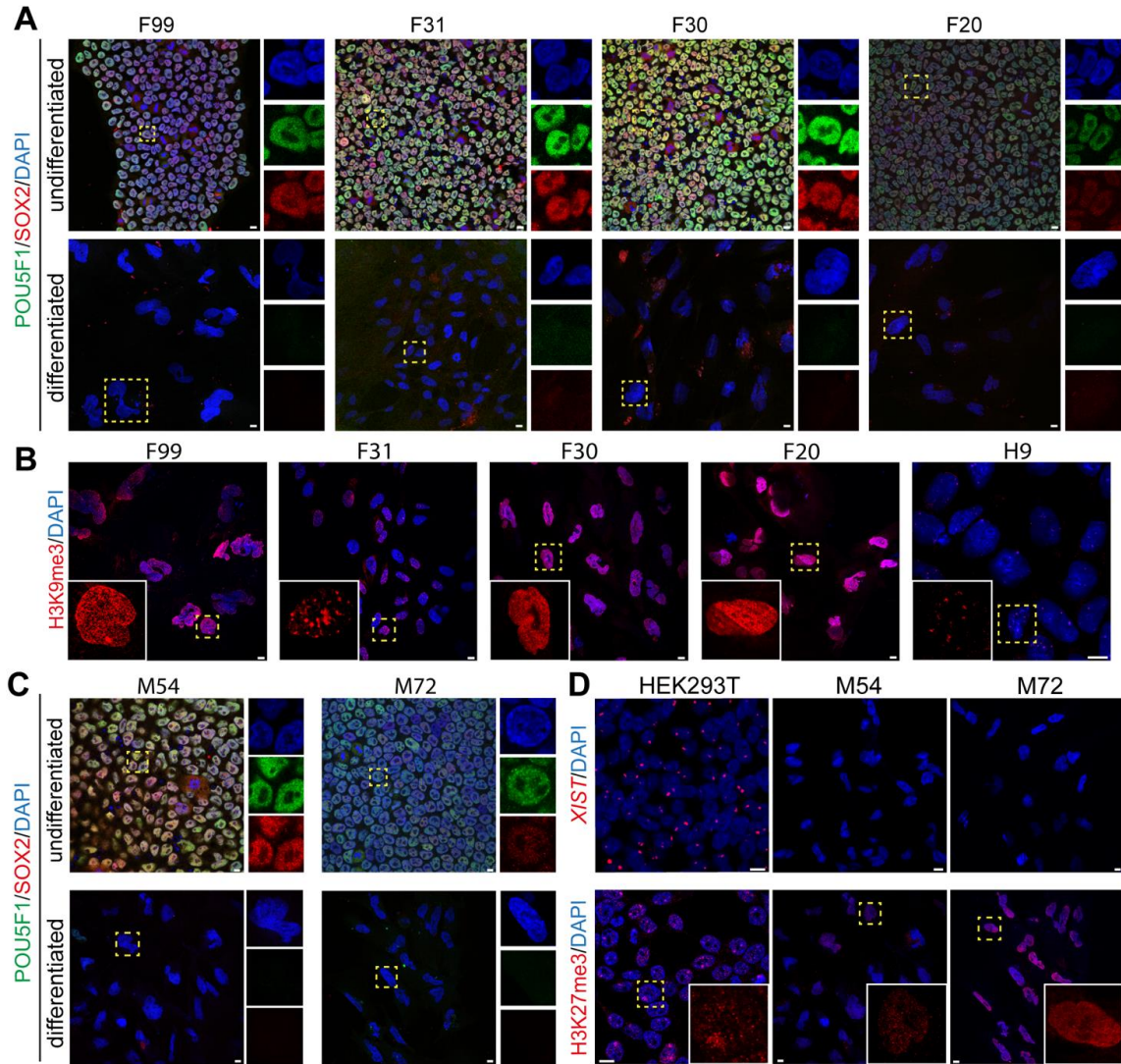
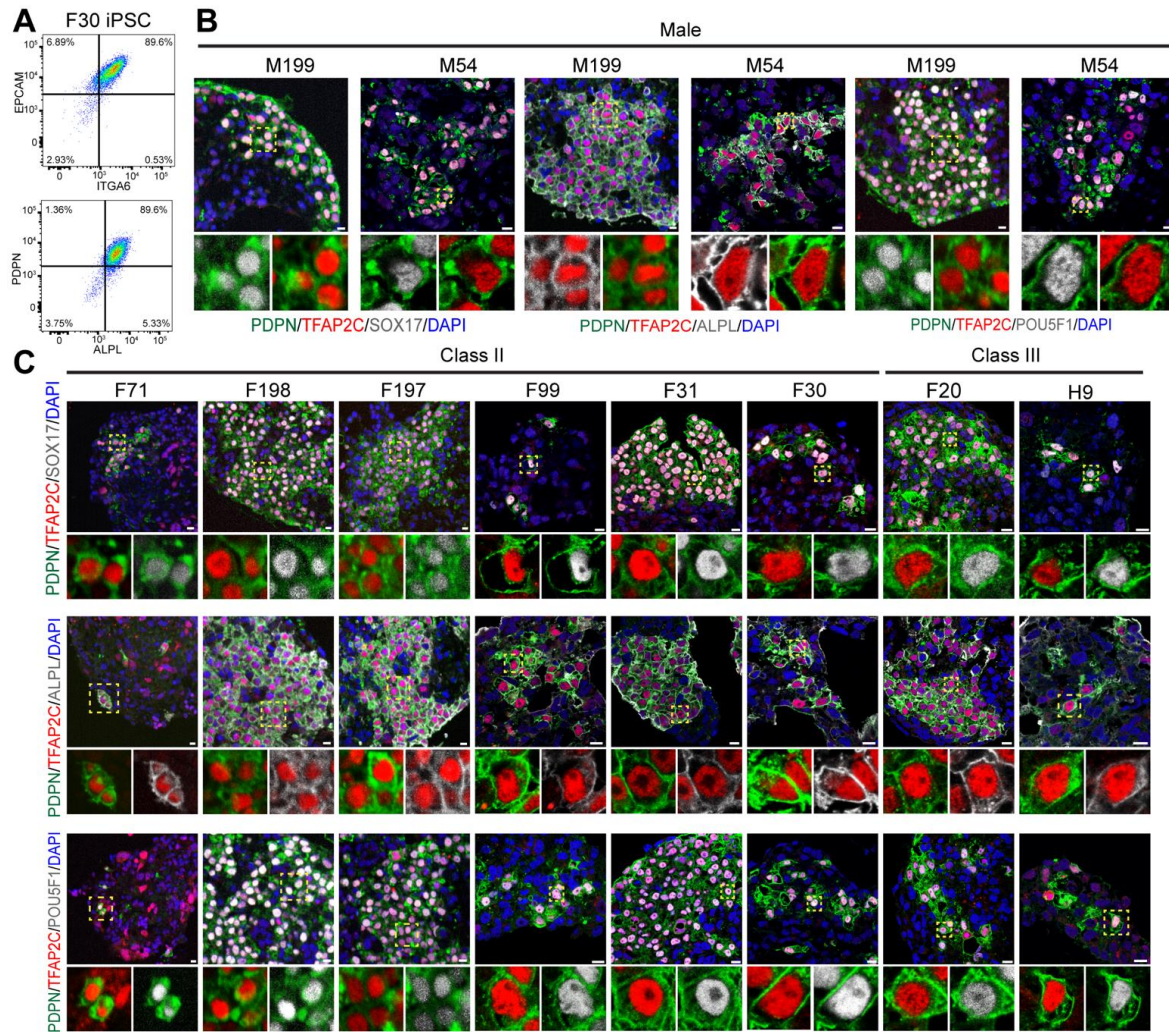


**Figure S1. Characterization of XCI hiPSC lines.** (A) RNA-FISH for *XIST* and *HPRT* in female hPSCs. Representative cells indicated by the dashed boxes are shown in zoomed pictures with individual channels displayed separately. Scale bars are 10  $\mu$ m. (B) Male hPSC lines M54 and M72 showing RNA-FISH for *XIST* and *HPRT* (top panels), immunofluorescence for H3K27me3 (middle panels) and H3K9me3 (bottom panels). The cell in the dashed box is magnified below and shows DAPI, DAPI+*HPRT* and DAPI+*XIST*. (C) Female iPSCs showing immunofluorescence for H3K27me3 and EZH2. Representative cells indicated by the dashed boxes are shown in zoomed pictures with individual channels displayed separately. Scale bars are 10  $\mu$ m. (D) Female hPSC lines F99, F31, F30, F20 and H9 showing immunofluorescence for H3K9me3. The cell in the dashed box is magnified below and shows DAPI, H3K9me3 and DAPI+H3K9me3. Scale bars are 10  $\mu$ m.

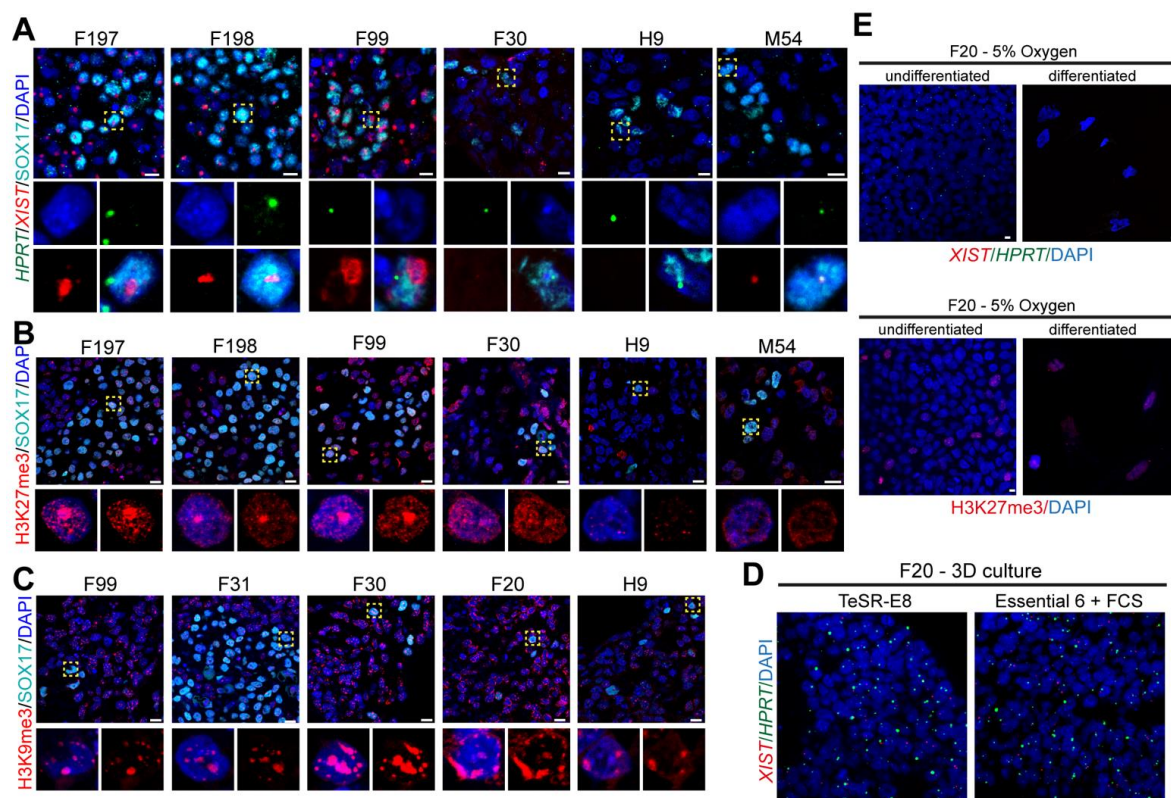


**Figure S2. Differentiation controls and characterization of FCS-induced monolayer differentiated cells.** (A) Immunofluorescence for pluripotency markers POU5F1 and SOX2, before and after differentiation in female hPSC lines. Dashed box indicates zoomed in cells with color channel separation. (B) Immunofluorescence for H3K9me3 in FCS-induced monolayer differentiated female hPSC lines. Dashed box indicates zoomed in cells showing H3K9me3. (C) Immunofluorescence for pluripotency markers POU5F1 and SOX2, before and after differentiation, in male hiPSC lines M54 and M72. Dashed box indicates zoomed in cells with color channel separation. (D) RNA-FISH for *XIST*, and immunofluorescence for H3K27me3 in differentiated M54, M72 and in HEK293T cells. Dashed box indicates zoomed in cells showing H3K9me3. Scale bars: 10  $\mu$ m.





**Figure S3. Expression of canonical hPGC markers in PGCLC-EBs.** (A) FACS analysis showing EPCAM/ITGA6 and PDPN/ALPL in hiPSC line F30. (B) Immunofluorescence for PDPN, TFAP2C and SOX17 (left panels), PDPN, TFAP2C and ALPL (middle panels) and PDPN, TFAP2C and POU5F1 (right panels) in PGCLC-EBs generated from indicated male hPSC lines. The cell in the dashed box is magnified below and shows PDPN+SOX17 and PDPN+TFAP2C (left panels), ALPL+TFAP2C and PDPN+TFAP2C (middle panels) and PDPN+POU5F1 and PDPN+TFAP2C (right panels). (C) Immunofluorescence for PDPN, TFAP2C and SOX17 (top panels), PDPN, TFAP2C and ALPL (middle panels) and PDPN, TFAP2C and POU5F1 (bottom panels) in PGCLC-EBs generated from indicated female hPSC lines. The cell in the dashed box is magnified below and shows PDPN+TFAP2C and PDPN+SOX17 (top panels), PDPN+TFAP2C and ALPL+TFAP2C (middle panels) and PDPN+TFAP2C and PDPN+POU5F1 (bottom panels).



**Figure S4. Characterization of XCI state in hPGCLCs and in Class III (F20) cells under Hypoxic and pluripotent 3D conditions.** (A) RNA-FISH of *XIST* and *HPRT*, co-stained for SOX17 in PGCLCEBs of indicated PSC lines. Dashed boxes highlight PGCLCs depicted with separated and merged color channels. (B) Immunofluorescence for H3K27me3 and SOX17 in PGCLC-EBs generated from the indicated hPSC lines. Dashed boxes highlight PGCLCs showing H3K27me3+DAPI and H3K27me3. (C) Immunofluorescence for H3K9me3 and SOX17 in PGCLC-EBs generated from the indicated PSC lines. Dashed boxes highlight PGCLCs showing H3K9me3+DAPI and H3K9me3. Scale bars: 10  $\mu$ m. (D) RNA-FISH for *XIST* and *HPRT* in F20 3D-aggregates cultured either in TeSR-E8 (pluripotent) or FCS-containing medium (differentiating) conditions. Scale bars: 10  $\mu$ m. (E) Undifferentiated and monolayer-differentiated female hPSC F20 cultured under hypoxia conditions (5% O<sub>2</sub>) showing RNA-FISH for *XIST* and *HPRT* (top panels) and showing immunofluorescence for H3K27me3 (bottom panels). Scale bars: 10  $\mu$ m.

**Table S1.** List of antibodies used in this study.

<b>Primary antibodies</b>	<b>Catalogue #</b>	<b>Manufacturer</b>	<b>Species</b>	<b>Dilution</b>
H3K27me3	07-449	Sigma- Aldrich	Rabbit	1:500
H3K9me3	07-442	Sigma- Aldrich	Rabbit	1:500
EZH2	612666	BD Biosciences	Mouse	1:200
POU5F1 (OCT4)	sc-5279	Santa Cruz	Mouse	1:100
POU5F1 (OCT4)	sc-8628	Santa Cruz	Goat	1:200
SOX2	sc-17319	Santa Cruz	Goat	1:200
PDPN (podoplanin)	ab77854	Abcam	Mouse	1:500
ALPL (TNAP)	AF2910	R&D System	Goat	1:200
SOX17	AF1924	R&D systems	Goat	1:500
TFAP2C	sc-8628	Santa Cruz	Rabbit	1:200
<b>Secondary antibodies</b>				
Donkey anti-Mouse IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 488	A-21202	Thermo Fisher	Donkey	1:500
Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor 555	A-31572	Thermo Fisher	Donkey	1:500
Donkey anti-Goat IgG (H+L) Cross-Adsorbed Secondary Antibody, Alexa Fluor 647	A-21447	Thermo Fisher	Donkey	1:500
Peroxidase AffiniPure Donkey Anti-Goat IgG (H+L)	705-035-003	Jackson ImmunoResearch	Donkey	1:500
<b>Conjugated antibodies for FACS</b>				
Alexa Fluor 488 conj. Alkaline Phosphatase (ALPL)	561495	BD Biosciences	Mouse	1:100
Brilliant Violet 421 conj. CD49f (ITGA6)	313623	Biolegend	Mouse	1:100
APC conj. CD326 (EPCAM)	324207	Biolegend	Mouse	1:100
PE conj. Podoplanin (PDPN)	12-9381-80	eBioscience	Rat	1:3000