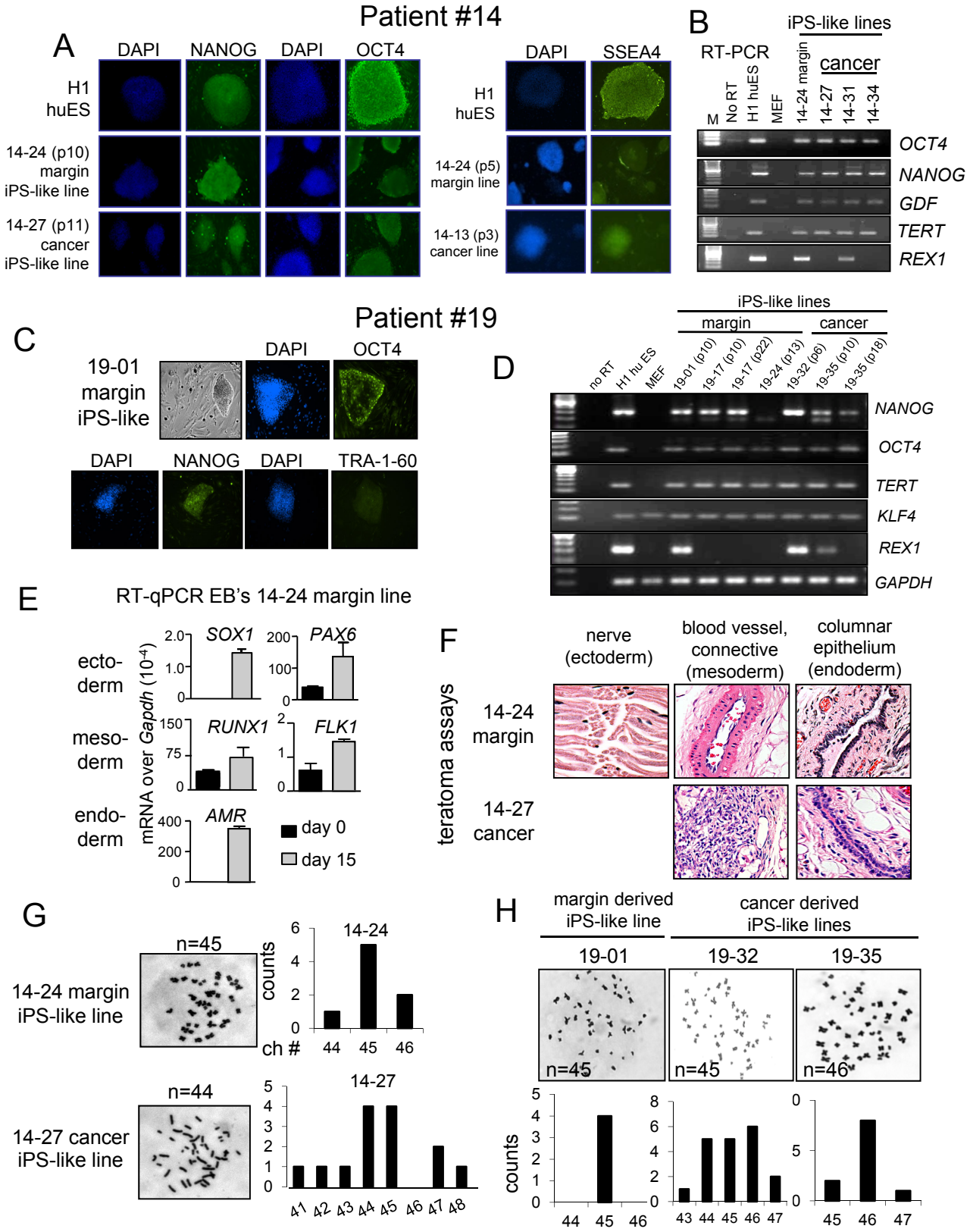
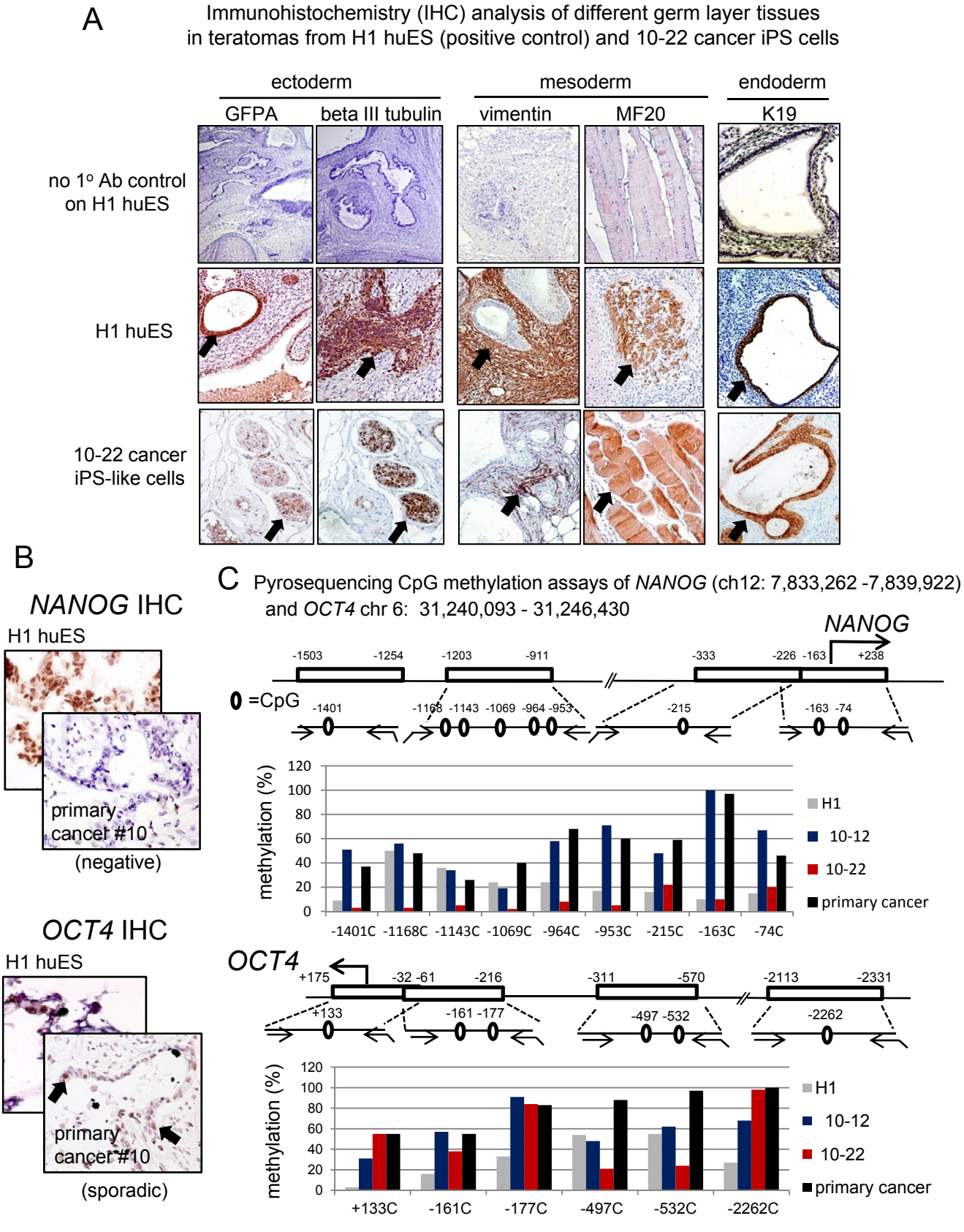
Kim/Zaret Supplementary Figure 1

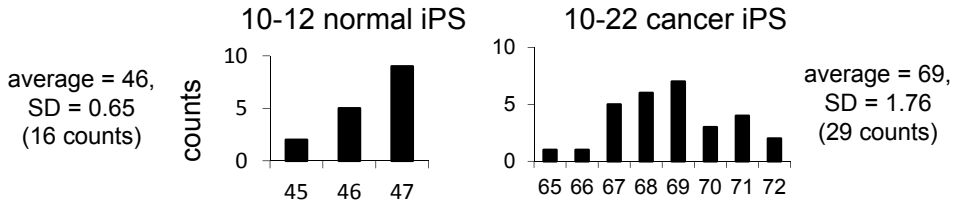


Kim/Zaret Supplementary Figure 2

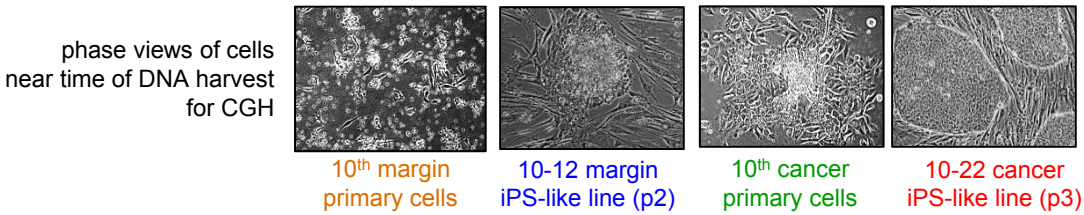


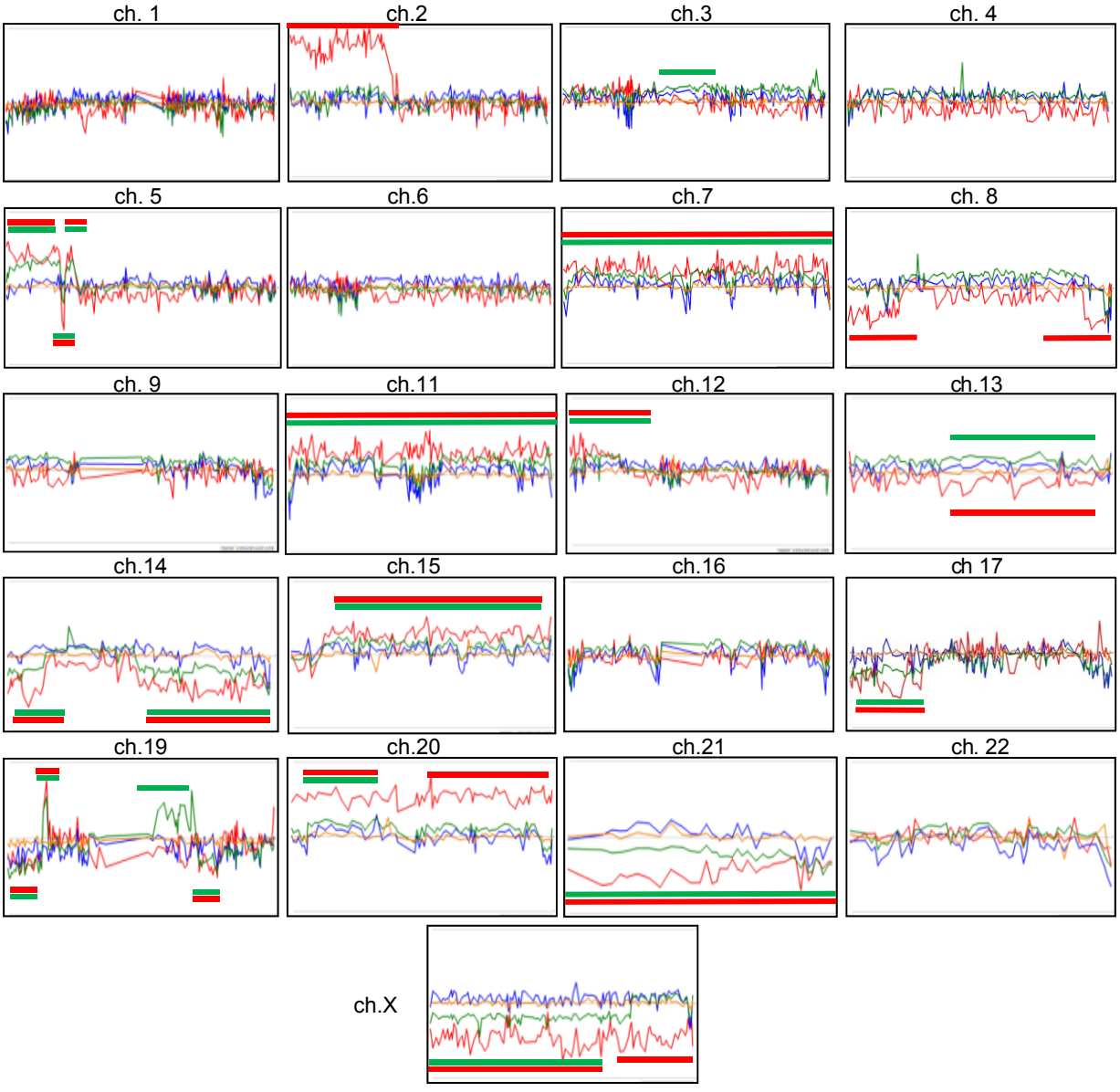
Kim/Zaret Supplementary Figure 3

A Karyotyping of 10th experiment clones

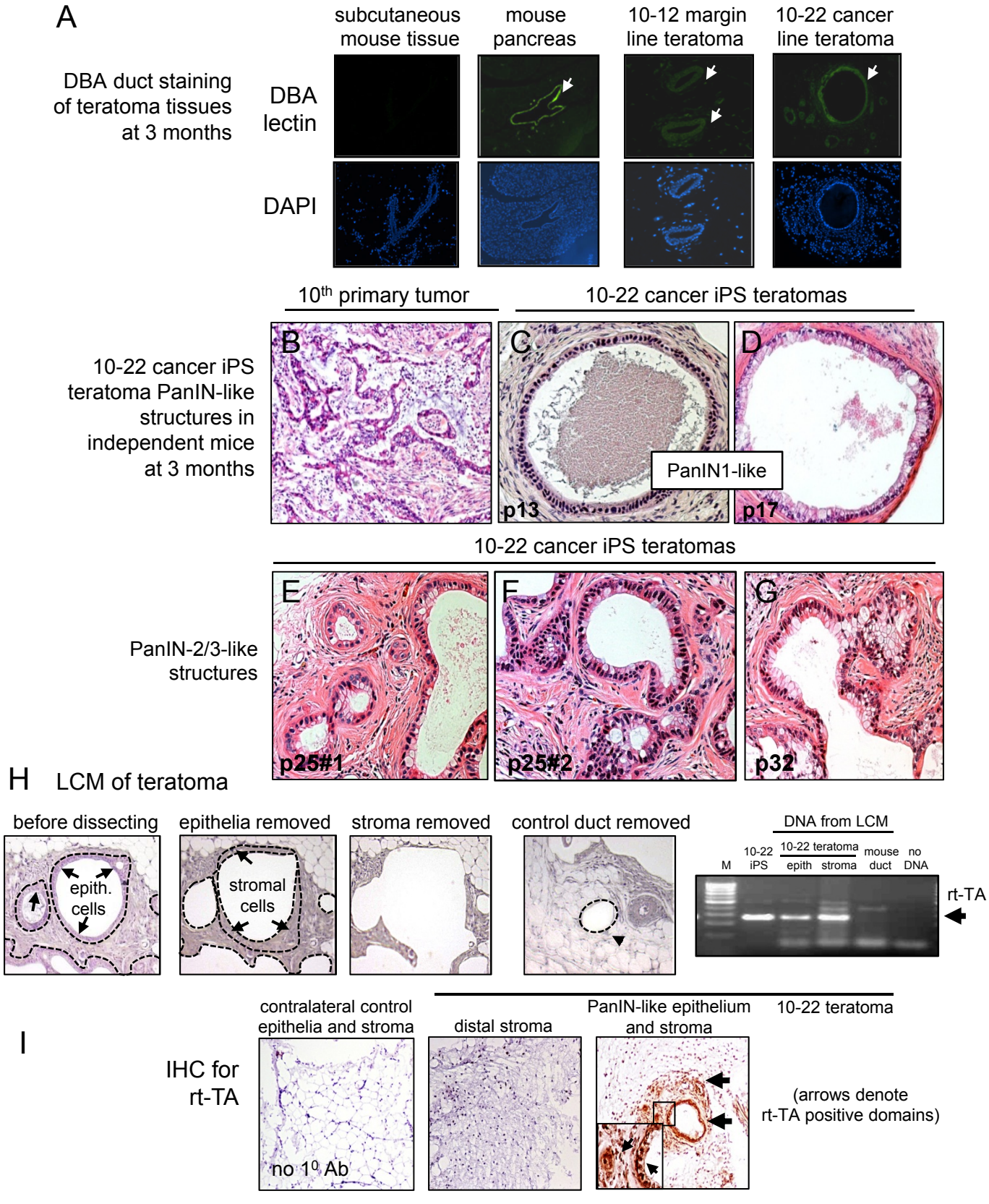


B Comparative Genomic Hybridization (CGH) of 10th clones

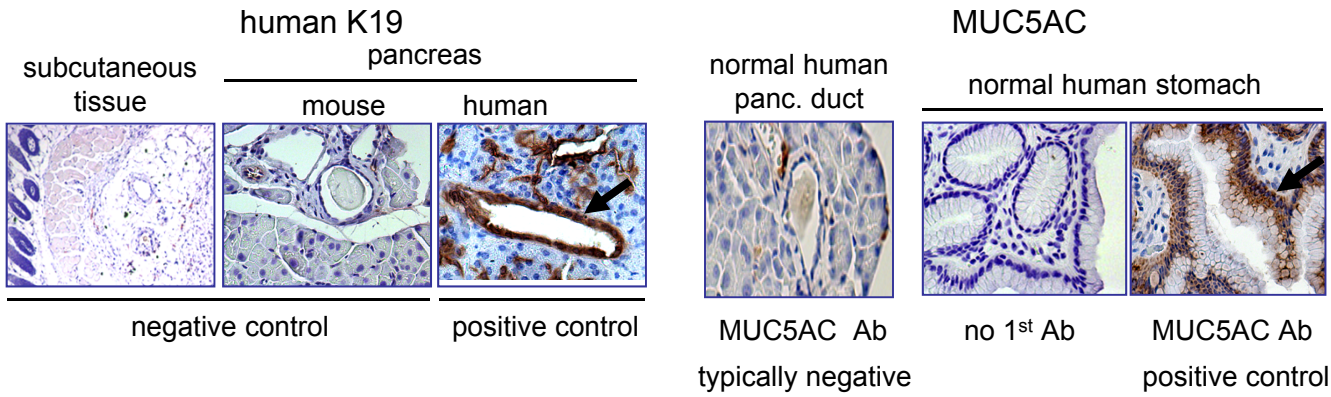


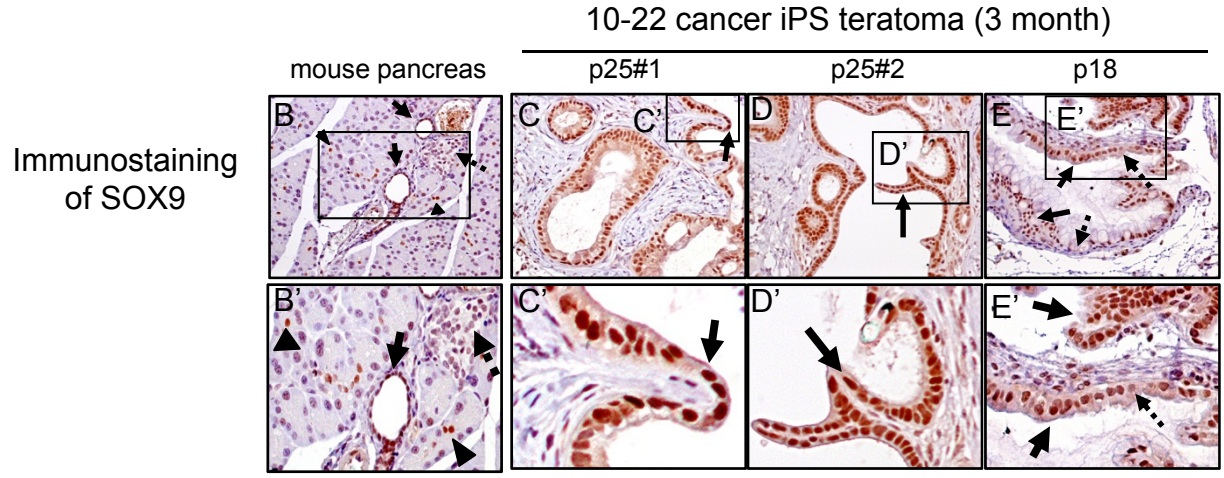


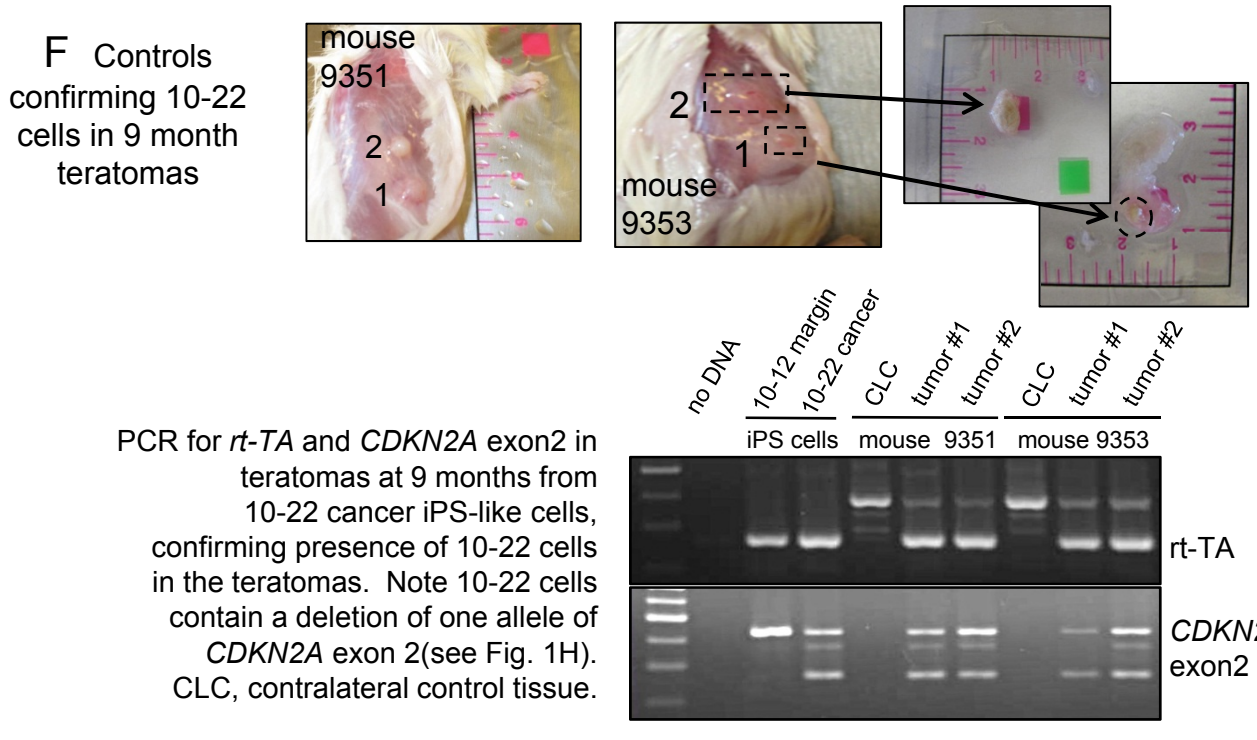
Kim/Zaret Supplementary Figure 4



Kim/Zaret Supplementary Figure 5



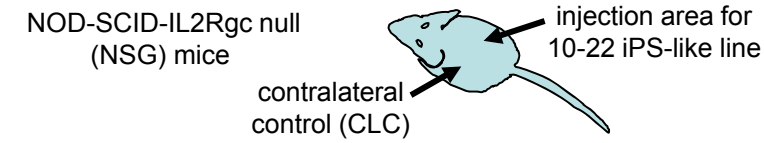




PCR for *rt-TA* and *CDKN2A* exon2 in teratomas at 9 months from 10-22 cancer iPS-like cells, confirming presence of 10-22 cells in the teratomas. Note 10-22 cells contain a deletion of one allele of *CDKN2A* exon 2(see Fig. 1H). CLC, contralateral control tissue.

Kim/Zaret Supplementary Figure 6

A Scheme for generating organoid cultures from teratomas of 10-22 iPS-like cells



Day 0 Tissue was minced and dissociated in liberase T flex (1.3W/ml) at 37 °C for 30 min, then cells embedded into Matrigel in serum-free medium

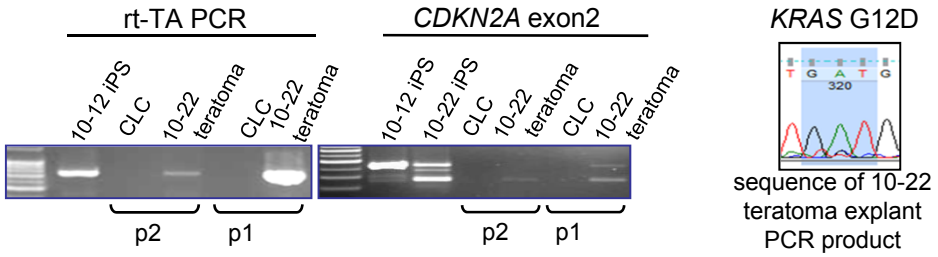
Day 6 Collect conditioned media for proteomic analysis

Day 9 To passage, cells were dissociated and re-embedded onto Matrigel

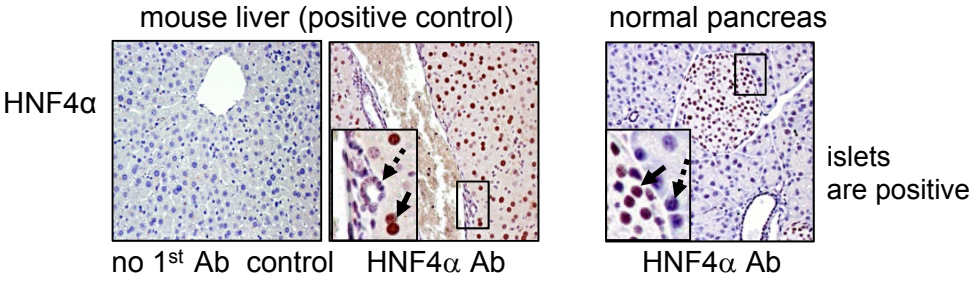
Day 19 Cells harvested for DNA and PCR analysis at 2nd passage (see B below)

Day 26 Collect organoids for IHC for MUC5AC and K19 staining (see Fig. 5A-C)

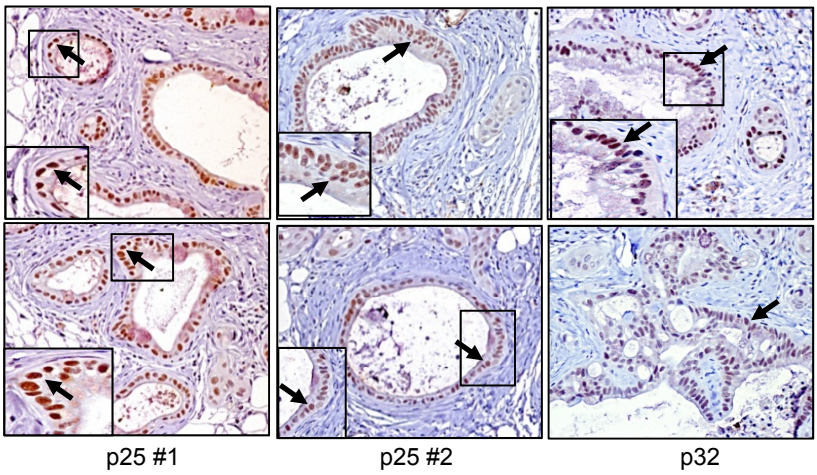
B



C control tests of HNF4α immunostaining



D immunostaining of HNF4α on 10-22 cancer iPS-like teratomas (3 months)



Supplementary Figure Legends

Supplementary Figure 1. A-B. Generating iPS-like lines from patient #14. Pancreatic margin and cancer iPS-like lines were generated from patient #14 and characterized by immunostaining for NANOG, OCT4, SSEA4 (A) and qRT-PCR for pluripotency genes (B). C-D. Pancreatic margin and cancer iPS-like lines were generated from patient #19, characterized by immunostaining for OCT4, NANOG, and TRA-1-60 (C), RT-PCR for pluripotency (D). E. Embryoid bodies were generated from 14-24 margin iPS-like line for 15 days and checked for the expression of differentiation markers using qRT-PCR. Expression levels are relative to *Gapdh*. Error bars are the mean ± SD (E). F. Teratoma assays showed the differentiation of 14-24 margin and 14-27 cancer derived iPS-like lines into multilineage cells; but note that in limited experiments with the 14-27 line, we did not detect neural derivatives. G-H, Karyotype analysis showed that 14-24 margin and 14-27 cancer iPS-like lines had subdiploidy (G) and 19th lines showed diploidy (H).

Supplementary Figure 2. A. Immunohistochemistry (IHC) analysis of different germ layer tissues on 10-22 cancer iPS teratoma tissue after 3 months. H1 huES teratoma tissue was used as a positive control. Glial Fibrillary Acidic protein (GFAP) and beta III tubulin were used for ectoderm, vimentin and MF20 were for mesoderm, and K19 was used for endoderm. B. Immunohistochemical staining for NANOG (upper panels) and OCT4 (lower panels), with the H1 huES line as a positive control and the primary tumor #10 experimental sample, analyzed at the same time. The data indicate that NANOG is not expressed in the primary #10 tumor and that OCT4 is expressed weakly in sporadic cells.

The absence of NANOG expression is consistent with the hypothesis that the primary tumor #10 was not composed largely of pancreatic cancer stem cells (Lonardo et al. 2011). C. Analysis of the CpG methylation state of 10-12 margin and 10-22 cancer iPS-like lines at the designated CpG sites (ovals, below position with respect to the transcription start) in the 5' upstream of the human *NANOG* and *OCT4* genes by bisulfite pyrosequencing. Genomic DNA from H1 huES cells was used for positive control and genomic DNA from parental primary tumor of patient #10 was used for a negative control. The schematic diagram of upstream region of *NANOG* and *OCT4* examined in this study. The regions amplified by PCR are enlarged. The graphs show the percent methylated C's in the designated CpG positions. At *NANOG*, all 9 CpG sites tested were methylated in the parental #10 tumor DNA and demethylated in the 10-22 iPS-like line, comparable to that seen in the H1 human ES control cells. At *OCT4*, C's at positions -497 and -532 exhibited marked demethylation in the 10-22 iPS-like line, while at position -161 there was modest demethylation. The H1 control was not markedly demethylated at the -497 and -532 sites, but was at other sites tested. Methylation of the *NANOG* locus provides further evidence that the primary tumor #10 was not composed of pancreatic cancer stem cells.

Supplementary Figure 3. A. Karyotype analysis of 10-12 margin and 10-22 cancer iPS-like clones. B. Comparative genomic hybridization assay of 10-12 margin, 10-22 cancer iPS-like clones. Of the 23 chromosomal aberrations seen in the epithelial cell cultures of the 10^{th} primary cancer, 20 aberrations were present in the 10-22 cancer iPS-like line, as seen by the green and red double bars. Single green and red bars denote aberrations unique to the primary cancer or cell line, respectively. See Fig. 1I for ch10 and ch18. Note

that the chromosomal aberrations seen in the 10th primary cancer cultures (green CGH traces), which were composed mainly of the cancer epithelial cells but also contained stromal cells, are amplified in the 10-22 iPS line (red CGH traces), a clone from the cancer culture.

Supplementary Figure 4. A. 10-12 margin and 10-22 cancer teratoma ductal structures at three months expressed DBA lectin. Contralateral control subcutaneous fat tissue was used as a negative control and mouse pancreas was used as a positive control. B-G. The PanIN-like structures derived from teratomas from the 10-22 cancer iPS-like line at three months in independent NSG mice. Compared to the poorly differentiated structures in the parental primary tumor of patient #10 (B), the 10-22 cancer iPS-like lines generated PanIN stages in independent mice regardless passage number of initial 10-22 cancer iPS-like line (C-G). H. PCR for rt-TA in LCM-dissected 10-22 cancer iPS teratoma tissue at three months. PCR for rt-TA on laser capture microdissected PanIN-like duct, stroma, and mouse duct. We removed all epithelial cells from the region prior to removing stromal cells, to avoid possible cross-contamination. I. Immunostaining of rt-TA on PanIN-like epithelial and stroma cells derived from 10-22 cancer iPS teratoma tissue at three months. The fibroblast distal region was negative for rt-TA. The data show that at least part of the stroma surrounding the PanIN-like epithelium in the teratoma is derived from the 10-22 human iPS-like cell line.

Supplementary Figure 5. A. Validation of antibodies against K19 and MUC5AC. Species-specificity of anti-human K19 was tested on mouse subcutaneous tissue, mouse

and human pancreatic tissue at 1:2000 dilution ratios. The specificity of the MUC5AC antibody was tested in normal human pancreas and normal human stomach. B-E Immunostaining of SOX9 in PanIN-like ducts of 10-22 cancer iPS teratoma arising at 3 months in three different NSG mice. As a positive control, mouse pancreas was stained with SOX9. Subsets of duct (arrows) and centroacinars (arrowheads) in mouse pancreas moderately expressed SOX9 (B-B', brown). Islet cells didn't express SOX9 (B-B', dashed arrows). PanIN-like ducts expressed SOX9 (brown, arrows, C-E). F. Human origin of 9 month 10-22 teratoma arose from NSG mice injected with 10-22 cancer iPS-like lines was confirmed by PCR for rt-TA and *CDKN2A*. Four tumors derived from two different mice preserved rt-TA sequence and deletions of *CDKN2A*. DNA from contralateral control (CLC) tissue was used for negative control.

Supplementary Figure 6. A. Scheme for in vitro explants of three month teratomas from 10-22 cancer iPS-like lines. Three independent teratoma tissues were explanted for in vitro culture (#7761, 9223, and 9225). Contralateral control tissue and 10-22 teratoma tissue generated spheres. B. Human origin of 10-22 teratoma in vitro explants was confirmed by RT-PCR for rt-TA and *CDKN2A*, and sequencing for the *KRAS* G12D mutation in the 10-22 iPS-like cells (see Fig. 1F-H). C. Control tests of HNF4α immunostaining on mouse liver tissue and normal pancreas. HNF4α was expressed only in islets and not in acinar or ductal cells in the mouse normal pancreas. Solid arrow indicates the positive cells and dashed arrow indicates the negative cells. D. HNF4α staining on additional 10-22 cancer iPS teratoma tissue derived from independent mice at 3 months. Solid arrow indicates the positive cells.