Long Noncoding RNAs Preserve Pancreatic Cancer Identity and Resist Cell Fate Conversion

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Keywords: cancer cell identity; transcriptional memory; transcriptional rigidity; long noncoding RNA; master transcription factor-mediated cellular reprogramming; pancreatic cancer; pluripotency; plasticity; ATXN7L3-AS1; AC079921.2

Summary

Pancreatic ductal adenocarcinoma (PDAC), among the deadliest malignancies, is paradoxically both transcriptionally plastic and rigid, maintaining a therapy-resistant epithelial identity through poorly understood transcriptional memory. Although Yamanaka factors (OCT4, SOX2, KLF4, MYC; OSKM) can override identity in normal cells, PDAC resists reprogramming, indicating active epigenetic barriers preserving its memory. Long noncoding RNAs (lncRNAs) regulate chromatin state and cell identity, yet their role as enforcers of cancer-specific transcriptional memory has been unknown. Using CRISPR interference screens, we identified PDAC-enriched lncRNAs whose depletion suppresses malignant programs, enhances OSKM-mediated reprogramming, and reduces tumorigenicity in vivo. Two of the most potent—ATXN7L3-AS1 and AC079921.2—are minimally expressed in normal pancreas but upregulated in PDAC. ATXN7L3-AS1 harbors pathogenic PDAC variants and is linked to mitotic progression, potentially ensuring faithful inheritance of malignant programs, whereas AC079921.2 is associated with a mesenchymal-like, invasion-prone transcriptional state that resists suppression during OSKM reprogramming. These findings reveal a previously unrecognized role for cancer-associated lncRNAs as active gatekeepers of transcriptional memory and malignant identity in PDAC, providing a conceptual and therapeutic framework to reset the epigenetic state, erase cancer cell memory, and re-sensitize tumors to differentiation- or reprogramming-based therapies.

Introduction

Pancreatic ductal adenocarcinoma (PDAC) is one of the deadliest human cancers, emphasizing the urgent need for deeper biological understanding to develop new therapies[1]. Many cancer cells show remarkable transcriptional plasticity and heterogeneity[2-7]. Yet, their epigenetic landscape plays a crucial role in limiting this plasticity, maintaining a stable, cancer-specific identity[8-13]. PDAC exemplifies this paradox; it exhibits transcriptional plasticity with dynamic transitions between classical and basal subtypes, yet it keeps a stable core identity defined by oncogenic transcriptional programs and classical pancreatic epithelial signatures[14-20]. This coexistence of plasticity and rigidity contributes to therapeutic resistance and aggressive clinical behavior[14-20]. However, the molecular mechanisms that preserve malignant identity by constraining transcriptional memory remain poorly understood. Elucidating how this malignant identity is stabilized despite dynamic transcriptional states is critical for developing effective therapeutic strategies against one of the most lethal human cancers.

Experimental reprogramming through the forced expression of lineage-defining master transcription factors (TFs), such as pioneer factors, has demonstrated the capacity to override established cell identity[21-27]. The best-known example is the induction of pluripotency via Yamanaka factors (OCT4, SOX2, KLF4, and MYC: OSKM), which reprograms normal somatic cells into induced pluripotent stem cells (iPSCs)[26,27]. However, in solid tumors including PDAC, reprogramming is often impeded: reprogrammed cancer cells frequently retain epigenetic memory of their tissue of origin and resist full identity erasure[28-32]. This resistance is unexpected given the inherent plasticity of many cancers, suggesting the presence of robust, cancer-specific transcriptional memory mechanisms.

Cellular identity is maintained by tightly coordinated gene regulatory networks composed of TFs, cofactors, and chromatin regulators[33-36]. Although these networks can be reset during regeneration or experimental reprogramming, core networks encode a transcriptional memory preserved through mitotic bookmarking and chromatin state[35-40]. This memory stabilizes cell

identity and acts as a barrier to fate conversion[41-52]. Long noncoding RNAs (lncRNAs) have emerged as critical regulators of cell memory and exhibit far greater tissue specificity than mRNAs[53-56]. By scaffolding chromatin-remodeling complexes and guiding gene expression in trans and cis, lncRNAs orchestrate cell type-specific transcriptional programs[57-64]. They play essential roles in development, maintain pluripotency and differentiation in embryonic stem (ES) cells[64-68], and influence cellular reprogramming by modulating microRNAs that regulate core pluripotency factors[68,69]. Dysregulation of lncRNAs is increasingly recognized in cancer, where they contribute to disease progression and therapeutic resistance[70-73]. However, whether specific lncRNAs in solid tumors, such as PDAC, act as active gatekeepers that preserve malignant identity and impede cell fate conversion remains unknown.

Herein, we hypothesized that a subset of cancer-associated lncRNAs enforces transcriptional memory in PDAC, which preserves malignant identity and blocks OSKM-induced reprogramming. CRISPR interference (CRISPRi) screens in normal pancreatic and PDAC cells under OSKM induction identified lncRNAs whose suppression erased PDAC phenotypes, enabled pluripotent reprogramming, and reduced tumorigenicity in vivo. These findings establish cancer-associated lncRNAs as molecular gatekeepers of malignant identity and define a conceptual framework for reprogramming-based therapeutic strategies to reset the epigenetic landscape, erase malignant transcriptional memory, and re-sensitize PDAC cells to differentiation- or reprogramming-based interventions.

RESULTS

Restricted Plasticity of PDAC Cells Toward a Pluripotent State. Primary PDAC samples include a large proportion of normal cells that are more amenable to reprogramming (Table S1)[74]. To accurately assess the reprogramming of human PDAC cells, we first enriched these cells using patient-derived xenografts (PDXs)[74]. PDAC comprises two major transcriptional subtypes: classical and basal-like (also referred to as squamous)[14-16], and we fully characterized

PDX tumors representing both subtypes[74]. To assess the reprogramming potential across all PDAC subtypes, we employed PDX-derived PDAC cells and established PDAC cell lines representing both classical (CFPAC1, ST-00014490 PDX-derived PDAC) and basal (Panc1, ST-00007599 PDX-derived PDAC) subtypes, using BJ human fibroblasts and H6c7 human normal pancreatic ductal epithelial cells (hnPDECs) as controls (Fig. 1A)[74,75].

To enable transgene-free reprogramming, we utilized Sendai virus–mediated delivery of the polycistronic vector expressing OSKM[76]. Because the Sendai virus is a non-integrating RNA virus, it provides a clean platform for reprogramming without genomic integration[75]. This system achieved efficient and consistent transgene expression across all primary PDAC cells, comparable to that in H6c7 hnPDECs and BJ fibroblasts[75].

We first validated the Sendai virus–mediated OSKM delivery system using human BJ fibroblasts and human acinar cells isolated from pancreatitis tissue of PDAC patients (Fig. S1A-S1B). The reprogramming efficiency of acinar cells (0.4%) was comparable to that of fibroblasts (Fig. S1C). The resulting iPSC lines expressed the canonical pluripotency marker TRA-1-60[77,78], exhibited normal karyotypes, and successfully differentiated into all three germ layers (Fig. S1D-F). These results confirmed that Sendai virus–mediated OSKM delivery effectively reprogrammed human pancreatic acinar cells and fibroblasts into a pluripotent state.

In contrast to pancreatic acinar cells, H6c7 hnPDECs failed to express TRA-1-60 by day 30 post-OSKM induction, although many expressed SSEA-1, a marker associated with the pluripotency of murine cells but with the early embryonic differentiation in human cells[79,80] (Fig. S2A). TRA-1-60 expression emerged by day 60, indicating that hnPDECs retain stronger lineage memory than acinar cells or fibroblasts. After OSKM induction, classical-type PDAC cells expressed SSEA-1 but not TRA-1-60, consistent with stalled progression toward pluripotency (Fig. S2A). In basal subtype PDAC cells, TRA-1-60 and SSEA-1 were sporadically expressed and unaffected by OSKM induction, indicating resistance to transcriptional rewiring (Fig.S2A). Even after 60 days of OSKM induction, PDAC cells failed to achieve full

reprogramming. Altogether, these findings indicate that PDAC cells maintain stable, cancer-associated programs that act as a barrier to complete cell fate conversion.

A Subset of PDAC Identity Genes Can Be Overridden by OSKM, but Many Others Cannot. Early molecular events following OSKM induction are critical in determining cell fate during reprogramming[23,42,81-86]. To characterize early transcriptional responses in PDAC versus normal cells, we examined transcriptomic profiling of PDAC cells (ST-00007599, ST-00014490, Panc1, CFPAC1) and control cells (BJ fibroblasts and H6c7 hnPDECs) at day 7 post-OSKM induction (Fig. 1A). Notably, ectopic OSKM expression levels were comparable across all cell types (Fig. S2B), and PCA data show consistent patterns across technical replicates within each sample (Fig. S3A), enabling direct comparison of downstream transcriptional responses.

OSKM typically initiates reprogramming of normal cells by silencing the original lineage-specific gene programs[23,42,81-86]. We therefore hypothesized that OSKM might downregulate a subset of the PDAC-specific transcriptome, particularly those involved in the malignant identity. To test this, we first defined PDAC-unique genes by identifying transcripts significantly upregulated across all PDAC cells relative to both fibroblasts and hnPDECs before OSKM treatment (Fig. 1B, Table S2A). Only 7 out of 375 of these PDAC-unique genes were significantly downregulated on day 7 following OSKM induction (Fig. 1B, Table S2B), with enrichment in apoptosis and epithelial differentiation pathways (Table S2C). Notably, SPDEF, a driver of classical PDAC tumorigenesis[87], was among these few OSKM-responsive genes and its high expression correlated with poor prognosis (Fig. 1C-D).

We assessed transcriptional responses to OSKM on day 7 post-induction across cell types. BJ fibroblasts showed the greatest number of differentially expressed genes (DEGs), followed by H6c7 hnPDECs, whereas PDAC cells had markedly fewer DEGs overall (Fig. S3B, Table S2D). This reduced number may reflect inter-sample heterogeneity, as each PDAC sample exhibited more DEGs than H6c7 hnPDECs (Fig. S3B, Table S2E), indicating higher

intrinsic transcriptional plasticity in PDAC. Importantly, this plasticity was not accompanied by loss of malignant identity; rather, it was constrained by robust transcriptional memory that preserved PDAC-specific programs despite OSKM induction.

Collectively, these findings indicate that while OSKM can partially suppress tumor-associated gene programs, the core transcriptional identity of PDAC cells remains largely intact.

PRC2-Linked Lineage Genes Remain Active During OSKM Reprogramming in PDAC. Despite OSKM induction, PDAC cells maintained a stable malignant transcriptional state, suggesting the presence of transcriptional memory that actively resists reprogramming. To identify the molecular programs underlying this resistance, we compared OSKM-induced transcriptional changes in PDAC cells with those in normal controls (BJ fibroblasts, H6c7 hnPDECs) (Fig. 1E and S3C-D). We hypothesized that “OSKM-unresponsive genes” in PDAC act as “gatekeepers” preserving malignant identity, whereas “OSKM-aberrantly upregulated genes”—silenced or unchanged in normal cells—represent maladaptive, cancer-specific barriers that antagonize cell fate conversion. Such aberrant responses may not only impede reprogramming but also drive maladaptive PDAC plasticity.

To account for inter-sample heterogeneity, we developed a classification framework incorporating variability across PDAC (n=4) and normal (n=2) samples. DEGs before and after OSKM induction were identified using likelihood ratio testing (LRT)[88] and ranked by a weighted Hamming distance[89] relative to predefined patterns, including: (1) downregulated in normal cells but minimally changed in cancer (“OSKM unresponsive in PDAC”), and (2) unchanged or downregulated in normal cells but upregulated in cancer (“OSKM aberrantly upregulated in PDAC”) (Fig. 1E-F and S3C–D, Table S2F-H, method).

OSKM-unresponsive genes were enriched in cell adhesion pathways, while OSKM-aberrantly upregulated genes were associated with mesodermal and morphogenetic lineage programs (Fig. 1G–H). Both categories were predicted targets of the PRC2 core components

(SUZ12, EZH2) (Fig. 1G-H), and significantly overlapped with known SUZ12 targets in human and mouse embryonic stem (ES) cells (Fig. S3E). Notably, PRC2 subunit expression remained unchanged post-OSKM (Fig. S3F), suggesting that impaired PRC2 recruitment—rather than reduced subunit abundance—underlies the failure to suppress these programs.

In summary, OSKM downregulated a small subset of PDAC marker genes but left most PDAC-specific genes unchanged or upregulated. Many of these belonged to PRC2-regulated lineage programs, highlighting a key epigenetic barrier to successful reprogramming in PDAC.

Suppression of Cancer-Associated lncRNAs in PDAC Cells Significantly Enhances the Reprogramming Efficiency. Given the role of lncRNAs in directing PRC2 recruitment and maintaining transcriptional memory across diverse biological contexts[57,90-94], we hypothesized that a subset of cancer-associated lncRNAs sustains PDAC identity and that their depletion could weaken epigenetic barriers to OSKM-mediated reprogramming. Accordingly, we performed CRISPRi screens targeting 691 cancer-associated lncRNAs (10 gRNAs per lncRNA, plus controls, total 7087 gRNAs; Table S3A)[95,96]. The screens were conducted in the context of OSKM-mediated reprogramming of normal ductal cells and PDAC cells, using the H6c7 and HPAFII cells expressing catalytically inactive Cas9 (dCas9)-KRAB (henceforth referred to as H6c7-dCas9-gRNA and HPAFII-dCas9-gRNA; Fig. 2A, Fig. S3G).

By day 30 post-OSKM induction, TRA-1-60^{+} pluripotent-like colonies were rare in HPAFII dCas9 control cells, as expected. Strikingly, in cells transduced with the lncRNA-targeting gRNA pool, the fraction of TRA-1-60^{+} cells increased to levels comparable with TRA-1-60^{-} cells (Fig. 2B, Table S3B). The isolated TRA-1-60^{+} and TRA-1-60^{-} populations (hereafter referred to as dCas9-gRNA-OSKM-TP and dCas9-gRNA-OSKM-TN, respectively) remained phenotypically stable over multiple passages (Fig. 2C). In contrast, the same gRNA pool targeting lncRNAs did not enhance reprogramming of H6c7 hnPDEC cells into pluripotency (Fig.

S3H), suggesting that these lncRNAs act in a PDAC-specific manner to preserve malignant transcriptional memory.

To profile transcriptional changes, we performed single-cell RNA sequencing (scRNA-seq) on dCas9-gRNA-OSKM-TP and dCas9-gRNA-OSKM-TN populations, along with controls: dCas9, dCas9-gRNA, and dCas9-OSKM-TP (rare TRA-1-60^{+} cells from OSKM-reprogrammed HPAFII without lncRNA knockdown) (Fig. 3A, S4A–B). Louvain clustering revealed 15 distinct clusters (Fig. 3A–C). Control dCas9 cells localized mainly to tubular (Group 0) and invasive epithelial (Group 1) clusters, with several minor heterogeneous populations (Fig. 3B–C, Fig. S4C). As expected, OSKM-only cells (dCas9-OSKM-TP cells; green) were transcriptionally indistinguishable from control dCas9 cells (black), confirming OSKM alone was insufficient to reprogram PDAC cells (Fig. 3B).

Although dCas9-gRNA cells (light green, Fig. 3B) upregulated pyruvate metabolism genes and downregulated epithelial and oxidative phosphorylation genes compared to dCas9 cells (Fig. S4D), they remained restricted to Groups 0 and 1, consistent with persistent PDAC transcriptional identity (Fig. 3B–C).

In contrast, OSKM reprogrammed lncRNA-depleted PDAC cells (dCas9-gRNA-OSKM-TP; Red, Fig. 3B) were largely depleted from Groups 0 and 1 and instead clustered predominantly in Group 5, characterized by loss of pancreatic and germ layer markers and increased pluripotency gene expression (Fig.3B-J, S4E, Table S4A-C). PCA further confirmed that dCas9-gRNA-OSKM-TP cells were transcriptionally distinct from all other subpopulations (Fig. S4F), aligning most closely with early human embryo profiles and far from PDAC or normal pancreatic cells (Fig. 3K).

Together, these findings demonstrate that lncRNA knockdown enhances OSKM-induced reprogramming and enables the emergence of cells with pluripotent-like transcriptional features. In contrast, OSKM expression alone was insufficient to erase the transcriptional identity of PDAC cells.

Suppression of Cancer-Associated lncRNAs During OSKM Reprogramming Disrupts PDAC Programs and Restores Tumor Suppressor Genes. Next, we investigated whether the cell state transition induced by the combination of lncRNA depletion and OSKM induction was accompanied by transcriptional erasure of PDAC identity. Cell clusters corresponding to mucinous (Group 0) and invasive epithelial (Group 1) phenotypes were markedly depleted in both TP and TN populations (Fig. 3B–C), indicating broad disruption of cancer-specific programs, even in TRA-1-60^{-} cells.

To quantify PDAC identity suppression, we applied a curated PDAC-specific gene signature from published scRNA-seq datasets (Table S4D)[97,98]. These genes were significantly enriched among transcripts downregulated in OSKM-reprogrammed lncRNA knockdown cells, especially TP populations, compared with dCas9-gRNA controls (Fig. 3L). Neither OSKM nor lncRNA knockdown alone induced significant downregulation, confirming that both OSKM expression and lncRNA knockdown are required to effectively repress PDAC transcriptional programs.

We next examined whether this transcriptional reset de-represses tumor suppressor gene (TSG) using the OncoKB database (Table S4E)[99,100]. While parental dCas9 cells expressed few or no TSGs, OSKM or lncRNA knockdown alone reactivated limited subsets (Fig. 3M). Strikingly, reprogrammed pluripotent cells (dCas9-gRNA-OSKM-TP) exhibited the most robust and broad re-expression of TSGs (Fig. 3M), with TSG expression predominantly enriched in NANOG^{+} states (Fig. 3N-O).

Together, these results demonstrate that lncRNA depletion combined with OSKM reprogramming not only induces a pluripotent-like state but also erases PDAC identity and restores TSG expression—features consistent with reversal of malignant cell fate.

LncRNA Depletion in Combination with OSKM Erases PDAC Identity and Reduces Malignant Tumor Formation In Vivo. We next assessed whether the pluripotency and suppression of the PDAC identity driven by lncRNA depletion in combination with OSKM induction altered the tumorigenic potential in vivo.

To this end, we xenografted HPAFII-dCas9-gRNA-OSKM-TP and -TN cells into immunodeficient NOD-SCID-IL2Rγ^{null} (NSG) mice[101], using HPAFII, HPAFII-dCas9-gRNA, and HPAFII-dCas9-OSKM-TP cells as controls. In addition, we included human ES (huES) cells as a pluripotency control (Fig. 4A, S5A-E). As expected, HPAFII cells rapidly formed tumors within one-month post-injection (Fig. 4B–C). All control groups similarly formed tumors, although dCas9-gRNAs produced slightly smaller tumors compared to HPAFII cells. Strikingly, mice injected with dCas9-gRNA-OSKM-TP cells either failed to develop tumors or formed significantly smaller tumors than dCas9-gRNA-OSKM-TN and all controls ($p < 0.005$; Fig. 4B–C).

Histopathological analysis confirmed that tumors from all control groups displayed classical grade 2 adenocarcinoma morphology, with strong human-specific marker TRA-1-85, pancreatic ductal marker CK19, and proliferation marker Ki67 immunostainings and absence of apoptotic marker Cleaved Caspase-3 (CC3) immunostaining, indicating high proliferation and minimal apoptosis (Fig. 4D-G, 5A-P). Extensive central necrosis was observed, consistent with advanced PDAC pathology. In contrast, tumors derived from dCas9-gRNA-OSKM-TP cells lacked typical adenocarcinoma features (Fig. 4H) and showed robust CC3 immunostaining in addition to Ki67 immunostaining (Fig. 5Q-T), suggesting a transition toward a less aggressive, more benign-like phenotype.

Importantly, tumors from dCas9-OSKM-TP and dCas9-gRNAs cells retained classical adenocarcinoma histology (Fig. 4E-F, 5E-F, and 5I-J), underscoring that neither OSKM expression nor lncRNA knockdown alone was sufficient to reprogram the malignant phenotype. Interestingly, dCas9-OSKM-TP tumors contained more differentiated, non-epithelial cell types in the tumor center, reflecting partial but incomplete identity shifts.

Consistent with being pluripotent, human ES cells typically form fully mature teratomas within three months post-injection[102,103]. At the one-month point used for PDAC comparisons, ES cell-derived tumors were small and composed predominantly of neuronal epithelial cells, consistent with ectodermal differentiation upon early exit from pluripotency[104,105] (Fig. S5A-C). These early teratomas possess both proliferation and apoptotic markers, further distinguishing them from HPAFII-derived tumors (Fig. S5D-E).

Together, these findings demonstrate that lncRNA depletion in combination with OSKM expression effectively reprogrammed PDAC cells, resulting in reduced tumorigenicity, loss of classical adenocarcinoma identity, and reactivation of apoptotic programs, in vivo. Importantly, this phenotypic reversal was not achieved by OSKM expression or lncRNA knockdown alone, highlighting the requirement of both to overcome the malignant PDAC state.

PDAC-Specific lncRNAs Maintain Malignant Transcriptional Programs and Resist OSKM-Mediated Reprogramming. To identify lncRNAs that maintain PDAC gene expression and are selectively depleted during successful reprogramming, we sequenced genomic DNA from FACS-sorted dCas9–gRNA–OSKM-TN and TP cells, alongside dCas9–gRNA controls (Fig. 6A and S5F, Table S5A–E). gRNA counts from two independent cultures were normalized using Z-score and MAGeCK negative control normalization[106], and lncRNAs with Z-scores > 2 and positive β-scores ($p < 0.05$) in both cultures were retained (Fig. 6A and S5F, Table S5F–K, methods).

This stringent analysis identified several lncRNAs-targeting gRNAs enriched in dCas9-gRNA-OSKM-TP cells compared to dCas9-gRNA controls (Fig. 6A-B, S5G-I). Among these, *ATXN7L3-AS1* (ENSG00000267394), *AC079921.2* (ENSG00000249685), and *INTS4P2* (ENSG00000273024, within LINC03006:ENSG00000290926) were validated by qRT-PCR and found to be significantly downregulated in HPAFII-dCas9-gRNA-OSKM-TP cells compared to control HPAFII-dCas9 cells (Fig. 6C). Importantly, these lncRNAs were minimally expressed or

absent in normal pancreatic tissue[107] (Fig. 6D-F) and their expression remained unchanged after OSKM induction alone (Fig. S6A), indicating resistance to OSKM-mediated reprogramming.

To test their contribution to PDAC transcriptional identity and reprogramming, we expressed gRNAs targeting *ATXN7L3-AS1*, *AC079921.2*, or *INTS4P2* in PDAC HPAFII-dCas9 and normal H6c7-dCas9 cells. In HPAFII cells, knockdown of each lncRNA reduced expression of several PDAC-enriched genes, including basal (*KRT5*, *TP63*) and classical markers (*GATA6)* (Fig. 7A). *ATXN7L3-AS1* or *AC079921.2* knockdown modestly increased early pancreatic progenitor gene expression (*PDX1*, *FOXA2*, *SOX9*), whereas *INTS4P2* knockdown had minimal effect.

Consistent with these changes, *ATXN7L3-AS1* depletion drastically increased OSKM-mediated reprogramming efficiency in HPAFII cells, as measured by TRA-1-60^{+} pluripotent-like cells (Fig. 7B). In contrast, the same intervention had no effect on H6c7 cells, either on gene expression or reprogramming (Fig. S6B), highlighting a PDAC-specific role of *ATXN7L3-AS1* in maintaining malignant transcriptional memory and resisting OSKM reprogramming.

Supporting its disease relevance, *ATXN7L3-AS1* locus harbors multiple pathogenic somatic mutations in PDAC (OSMIC[108]; Fig. 7C) and showed positive correlation with genes involved in mitotic cell cycle progression in TCGA Pan-Cancer datasets[109] (Fig. S6C, Table S7A). Motif analysis[110,111] predicted UBTF as a potential upstream regulator of these genes (Fig. S6D), which also carried active enhancer marks (H3K27ac and H3K4me2)[55], features associated with transcriptional activity and memory (Fig. S6E).

In contrast, AC079921.2 expression correlated with genes involved in extracellular matrix (ECM) remodeling, cell adhesion, and migration—hallmarks of invasive PDAC (Fig. S6F, Table S7B). These correlated genes were predicted to be PRC2 (SUZ12) targets (Fig. S6G) and displayed promoter enrichment for H3K27me3 (Fig. S6H), suggesting AC079921.2 may counteract PRC2-mediated transcriptional silencing. Notably, genes aberrantly upregulated or

unresponsive to OSKM (Fig. 1F, Table S2F-S2H) significantly overlapped with AC079921.2–associated genes (Fig. 7D–E). This shared set was enriched for morphogenesis, migration, and ECM-related pathways (Fig. 7F–G) and also predicted to be SUZ12 targets (Fig. 7H–I). These findings indicate that *AC079921.2* reinforces malignant PDAC identity by sustaining pro-invasive, morphogenetic transcriptional programs that resist OSKM-mediated reprogramming.

Genes negatively correlated with ATXN7L3-AS1 or AC079921.2 were enriched for translational and metabolic pathways (Fig. S6I–J, Table S7C-D) and carried active histone marks (H3K36me3, H3K79me2) (Fig. S6K–L). Predicted upstream regulators included MYC, MAX, TAF1, and GABPA (Fig. S6M–N), suggesting these lncRNAs may suppress biosynthetic and proliferative programs while preserving malignant identity.

Collectively, these results identify ATXN7L3-AS1 and AC079921.2 as PDAC-specific, OSKM-resistant lncRNAs that sustain malignant gene programs through distinct regulatory networks and may act as barriers to OSKM-mediated reprogramming.

Discussion

PDAC exhibits remarkable transcriptional rigidity despite underlying plasticity, resisting lineage reprogramming and maintaining a stable malignant identity. Here, we performed CRISPRi screens during OSKM-induced reprogramming and identified a subset of cancer-associated lncRNAs as active enforcers of transcriptional memory, preserving malignant identity and impeding cell fate conversion. Depletion of these lncRNAs, in combination with OSKM, enabled efficient reprogramming of PDAC cells into pluripotent-like states, resulting in loss of adenocarcinoma identity and reduced malignant potential in vitro and in vivo. These findings reveal a previously unrecognized role for cancer-associated lncRNAs as molecular gatekeepers that preserve PDAC identity and constrain cell fate plasticity. Targeting them provides a conceptual and therapeutic framework for resetting the epigenetic landscape, erasing cancer

cell memory, and re-sensitizing PDAC cells to differentiation- or reprogramming-based therapies.

Are solid tumors truly resistant to reprogramming into pluripotency? This question is somewhat ironic given the prevailing view that cancers are highly plastic and often exhibit sporadic expression of pluripotency-associated TFs. Although solid tumors may express individual components of the OSKM pluripotency cocktail, this combination remains the most effective for initiating reprogramming in cancer cells; however, full reprogramming of solid tumors is rarely achieved[28-32]. Why?

Technical barriers, including tumor heterogeneity and inefficient gene delivery, have long confounded this question. To overcome these limitations, we optimized a high-efficiency, non-integrating gene delivery system[75], amplified primary PDAC cells[74], and developed an analytical framework to account for inter-sample heterogeneity.

Under these optimized conditions, it became clear that OSKM alone was insufficient to erase PDAC identity. While normal pancreatic ductal epithelial cells are inherently more resistant to reprogramming than fibroblasts or acinar cells, they could ultimately be reprogrammed with sustained OSKM expression. In contrast, PDAC cells—despite responding transcriptionally to OSKM—failed to reach a pluripotent state. This resistance arose not from insufficient OSKM-induced gene activation, but from the inability to repress a core set of malignant identity genes. Normally silenced in healthy epithelial cells, these genes remained aberrantly active, reflecting PDAC's paradoxical combination of high plasticity and entrenched transcriptional memory.

Many of these identity-maintaining genes are predicted PRC2 targets, suggesting that lncRNAs could contribute to their epigenetic maintenance. Because lncRNAs are often expressed at low levels, standard RNA-seq pipelines using conventional DESeq2 thresholds

can miss those functionally involved in this process. Whether oncogenic KRAS actively drives such epigenetic barriers remains an important question for future investigation.

ATXN7L3-AS1 and AC079921.2 as gatekeepers of PDAC identity. Cellular memory, the epigenetic inheritance of transcriptional states through cell divisions, is essential for maintaining stable cell identity. During mitosis, specific factors act as “bookmarks” to preserve lineage fidelity[37-40]. Similarly, lncRNAs can maintain transcriptional memory by shaping chromatin architecture and regulating gene expression through chromatin-modifying enzymes[53-64].

Among the lncRNAs identified in our screens, *ATXN7L3-AS1* and *AC079921.2* emerged as the most potent barriers to OSKM-mediated reprogramming. Knockdown of either lncRNA reduced PDAC marker expression and partially reactivated pancreatic progenitor genes, indicating a loosening of lineage constraints. Strikingly, *ATXN7L3-AS1* depletion robustly enhanced reprogramming toward pluripotency, positioning it as a central barrier to fate conversion in PDAC.

What underlies ATXN7L3-AS1’s repressive role? ATXN7L3-AS1 resides on chromosome 17, upstream of ATXN7L3 and UBTF, and is transcribed antisense (Fig. 7C). ATXN7L3 encodes a subunit of the SAGA histone acetyltransferase complex[112], while UBTF is an HMGB-box TF essential for Pol I/II activity and known to be oncogenic[112]. Upstream motif analysis predicts UBTF binding near ATXN7L3-AS1–correlated genes, suggesting that ATXN7L3-AS1 may reinforce a UBTF-driven transcriptional program. Although UBTF’s role in PDAC is unknown, it is oncogenic in other cancers[113]—for example, UBTF::ATXN7L3 fusions define a high-risk B-ALL subtype[114], and UBTF duplications recur in AML[115]. Notably, ATXN7L3-AS1 harbors pathogenic PDAC variants and is associated with mitotic progression in TCGA datasets (Fig. 7C, S6C), supporting a model in which it preserves malignant identity via mitotic bookmarking and transcriptional memory.

In contrast, *AC079921.2* appears to enforce a distinct but complementary program. Pan-cancer analyses revealed that *AC079921.2* expression correlates positively with genes involved in ECM remodeling, cell adhesion, and migration—hallmarks of mesenchymal-like and invasive tumor phenotypes (Fig. S6F). Notably, many of these genes are silenced in normal epithelial tissues by PRC2, a chromatin silencing complex comprising SUZ12 and EZH2. This suggests that *AC079921.2* may antagonize PRC2-mediated silencing. Supporting this model, genes aberrantly upregulated or unresponsive to OSKM reprogramming significantly overlap with *AC079921.2*–correlated genes in PDAC (Fig. 7D–I), indicating that *AC079921.2* sustains a transcriptional program resistant to OSKM-induced reprogramming. By maintaining ECM and morphogenetic gene expression, *AC079921.2* may reinforce a mesenchymal-like, invasion-prone transcriptional state, limit reprogramming potential while promoting adaptability to microenvironmental cues. Whether *AC079921.2* physically interacts with PRC2 to modulate its activity remains an open question—one that may yield new therapeutic insights. Targeting this lncRNA could offer a strategy to limit undesired cell-state transitions during therapy or reprogramming.

Intriguingly, levels of both *ATXN7L3-AS1* and *AC079921.2* are negatively correlated with genes involved in translation and metabolic pathways (Fig. S6I-N). This suggests a broader regulatory function whereby these lncRNAs repress biosynthetic and metabolic programs, possibly to enforce a quiescent stem-or senescence-like malignant state less amenable to reprogramming. Indeed, we previously observed that a subset of cancer iPS-like colonies entered senescence-like states that could not be maintained over time.

Together, these findings establish *ATXN7L3-AS1* and *AC079921.2* as gatekeepers of PDAC identity, acting through distinct molecular mechanisms to maintain transcriptional memory and stabilize malignant cell states. Their depletion not only enhances reprogramming efficiency but also provides a conceptual framework for targeting transcriptional rigidity in pancreatic cancer.

Combining lncRNA depletion with OSKM induction is required to erase PDAC identity. While the knockdown of *ATXN7L3-AS1*, *AC079921.2*, or *INTS4P2* moderately reduced PDAC marker expression (Fig. 7A), neither lncRNA depletion nor OSKM induction alone was sufficient to erase PDAC identity or suppress malignancy in vitro or vivo (Fig. 3-5). In xenografts, lncRNA depletion without OSKM modestly reduced tumor size, likely due to decreased proliferation without increased apoptosis, but did not induce reprogramming or erase the adenocarcinoma identity. Likewise, OSKM alone yielded tumors with more differentiated histology and reduced proliferation, yet these tumors retained core features of PDAC.

In contrast, combining lncRNA knockdown with OSKM induction (HPAFII-dCas9-gRNA-OSKM-TP cells) erased adenocarcinoma identity, reactivated apoptotic pathways, and generated NANOG^{+} pluripotent-like cells. The enrichment of TSG expression in NANOG^{+} pluripotent cells suggests that cell fate conversion to pluripotency is a prerequisite for TSG re-expression.

Toward a new reprogramming cocktail: lncRNA depletion sensitizes PDAC to OSKM-induced cell fate transitions. Our prior work demonstrated that full reprogramming into a pluripotent state was impeded, with partially reprogrammed states requiring sustained ectopic OSKM expression[28]. Although this model proved informative for biomarker discovery[116], its reliance on persistent OSKM expression limits its applicability in translational cancer therapies.

Herein, we propose that lncRNA depletion sensitizes PDAC cells to OSKM-mediated reprogramming by weakening the epigenetic barriers that preserve malignant identity. To enhance clinical relevance, we optimized a non-integrating, Sendai virus–mediated system for transient OSKM expression across multiple patient-derived PDAC cells, offering efficient reprogramming while minimizing the risks associated with genomic integration[75].

Beyond pluripotency, lineage reprogramming of cancer cells into alternative fates—such as dendritic cells—has been demonstrated[117-119]. It will be crucial to determine whether lncRNA depletion can similarly facilitate PDAC reprogramming toward alternative or non-permissive lineages, such as acinar cells, which are incompatible with oncogenic KRAS[120-122]. Targeting lncRNAs enforcing transcriptional memory could enhance responsiveness to reprogramming cues and improve lineage fidelity.

Conclusions. The combined depletion of cancer-associated lncRNAs and OSKM induction is required to erase the malignant identity of PDAC cells. These cancer-specific lncRNAs function as active enforcers of transcriptional memory, preserving malignant identity and resisting reprogramming even under potent reprogramming cues. By maintaining the epigenetic rigidity of PDAC cells, they may contribute to therapeutic resistance and disease recurrence. Targeting such lncRNA gatekeepers provides both a conceptual and therapeutic framework for resetting the epigenetic landscape, erasing cancer cell memory, and unlocking new vulnerabilities in pancreatic cancer**.**

Limitations of the Study. Our study focuses on PDAC-specific lncRNAs that sustain the cancer identity and uses OSKM to force reprogramming of PDAC toward pluripotency. However, lncRNAs that sustain the cancer identity may differ across cancer types, and the effective reprogramming into other lineages will likely require tailored combinations of lineage-specific transcription factors.

While we observed a robust reduction in malignancy and activation of apoptosis in tumors derived from HPAFII-dCas9-gRNA-OSKM-TP cells, it remains unclear whether the erasure of malignancy is permanent. Long-term xenograft studies will be required to assess the durability of reprogramming-induced tumor suppression.

RESOURCE AVAILABILITY

Lead Contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Jungsun Kim (kimjungs@ohsu.edu).

Materials availability

- Plasmids, PDX tumors, and reprogrammed cancers generated in this study will be available upon request.
- This study did not generate new unique reagents.

Data and code availability

- Raw data and codes for sequencing data have been deposited at NCBI GEO and are publicly available as of publication. Accession numbers will be listed in the key resource table. The lead contact, upon request, will share any raw data reported in this paper.
- Codes for sequencing data have been deposited on GitHub and are publicly available as of publication.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

ACKNOWLEDGMENTS We thank Drs. Ken Zaret and Theresa Lusardi for comments on the manuscript. We thank Dr. Jeffrey Drebin for providing de-identified resected tumor specimens for the initial phase of this study, which was exempt from IRB review. We thank the Brenden-Colson Center for Pancreatic Care, the Oregon Pancreas Tissue Registry, the OHSU Knight Biostatistics Shared Resource and Biolibrary (NCI P30CA69533), and the Molecular Virology Core at the Oregon National Primate Research Center (NIH P51OD011092). The IRB protocol is the Oregon Pancreas Tissue Registry (IRB #3609). The work was funded by the CRUK-

OHSU Project Award (2018-CRUK-OHSU-001 to JK), MRF New Investigator Grant (GCNCR1042A to JK), and Knight CEDAR (68182-906-000, 68182-933-000, 68182-939-000, 68182-954-000, Manuscript Prep 2024-1978) from the Cancer Early Detection Advanced Research Center at OHSU Knight Cancer Institute. This article was edited by Life Science Editors.

AUTHOR CONTRIBUTIONS

Conceptualization, JK; Methodology, DG, SL, CMF, SR, MB, AH, TE, EM, JT, MCC, JL, and WY; Validation, DG, SL, CMF, SR, SF, TM, and WY; Formal Analysis, DG, SL, CMF, SR, AH, SF, TM, WY, EF, ST, and JK; scRNAseq analysis supervision, ST and JK; Investigation, D.G., SL, CMF, SR, MB, SF, TM, WY, EF, ST, and JK.; Resources, JL, DK, BCS, EF, and RCS; Data Curation, DG, SL, CMF, SR, AH, SF, and ST; Writing-original draft, JK; Writing-Review & Editing, DG, SL, CMF, SR, and JK; Visualization, DG, CMF, SR, AH, SF, and ST, Supervision, JK; Project Administration, JK; Funding Acquisition, JK.

DECLARATION OF INTERESTS

The authors declare no competing interests.

STAR METHODS

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Derivation of Patient-Derived Xenografts (PDX) and Tissue Culture. De-identified human PDAC specimens were obtained under the University of Pennsylvania study (IRB-exempted) or the Oregon Pancreas Tissue Registry protocol (IRB00003609). Written informed consent was obtained from all participants. All experimental protocols were approved by the OHSU Institutional Review Board. All methods were carried out in accordance with relevant guidelines and regulations. All animal work for PDX tumors was performed with the OHSU Institutional Animal Use and Care Committee (IACUC) approval. The derivation, expansion, culture, and characterization of PDAC PDXs, are described in our protocol paper[74]. Culture conditions for H6c7 and other PDAC cell lines are described[74]. BJ6 fibroblasts were cultured in DMEM supplemented with 10% fetal bovine serum (FBS)[23].

Reprogramming of Pancreatic Cancer Cells and Normal Controls. Reprogramming was performed using the CytoTune-iPS Sendai Reprogramming Kit (Invitrogen), which delivers the non-integrating Sendai virus encoding OCT4, SOX2, KLF4, and c-MYC (OSKM)[123]. Viral titers for Sendai virus and lentivirus were described in our earlier publication[75]. On day 7 post-transduction, cells were harvested for RNA sequencing and re-seeded onto irradiated mouse embryonic fibroblasts (MEFs) to support further reprogramming.

CRISPR interference (CRISPRi) Screen. We performed CRISPRi–based screens targeting 691 cancer-associated lncRNAs using a pooled lentiviral library containing 7,087 gRNAs (10 gRNAs per lncRNA, plus non-targeting controls, Millipore, Table S3A)[95,96]. The HPAFII and H6c7 cells expressing dCas9-KRAB were generated by transducing HPAFII and H6c7 cells with

lentiviruses carrying the dCas9-Bsr gene at an MOI of 1, followed by selecting cells for Blastidin. The CRISPRi efficiency was assessed in the clonal HPAFII and H6c7 cells expressing dCas9 with positive controls. The clonal HPAFII-dCas9 and H6c7-dCas9 were subsequently transduced with a lentiviral library of 7087 sgRNAs at an MOI of 0.1-0.3 with 300- 500x coverage to maintain an average 300-500-fold representation of each sgRNA. After puromycin selection, cells were collected for reference controls (300x, 500x). HPAFII and H6c7 cells were reprogrammed with OSKM Sendai viruses using the OSKM Sendai viruses[123]. The CRISPRi-targeted cells were always maintained at 300-500 coverage throughout the procedure.

METHOD DETAILS

Tissue immunohistochemistry (IHC), cell immunofluorescence (IF) staining, and qRT-PCR. Tissue IHC, IF, and qRT-PCR were performed as previously described in our published work[28].

QUANTIFICATION AND STATISTICAL ANALYSIS

RNA-seq Data Processing and Normalization. Bulk RNA-seq was performed as previously described[75,124]. Raw FASTQ files were processed using fastp to generate clean reads by removing adapter sequences, reads containing poly-N, and low-quality reads. During this step, quality metrics such as Q20, Q30, and GC content were also calculated. All downstream analyses were conducted using the high-quality clean reads. The reference genome and gene annotation files were downloaded directly from the genome database. The genome index was built using HISAT2 (v2.0.5), and paired-end clean reads were aligned to the reference genome using HISAT2 (v2.0.597)[125]. FeatureCounts (v1.5.0-p3) was used to quantify the number of reads mapped to each gene. FPKM (Fragments Per Kilobase of transcript per Million mapped reads) values were then calculated for each gene based on its length and the number of mapped

reads as needed. Differential expression analysis was performed using the DESeq2 R package (v1.20.0), which employs statistical routines based on the negative binomial distribution to identify DEGs from count data[88]. P-values were adjusted for multiple testing using the Benjamini-Hochberg method to control FDR. Genes with an adjusted P-value ≤ 0.05 were considered significantly differentially expressed. RNA-seq data are available on NCBI GSE247243.

Identification of PDAC Gene Signatures Repressed by OSKM. To identify PDAC-specific gene categories, we first performed differential expression analysis using DESeq2 to compare each PDAC cell to normal controls (BJ fibroblasts and H6c7 pancreatic epithelial cells). Genes that were significantly upregulated in PDAC cells (FDR < 0.05, $\log_2$ FC > 1) were selected. We then intersected the upregulated gene sets across all four PDAC samples to define a core set of PDAC-upregulated genes (Table S2A). To assess the impact of OSKM induction, we compared gene expression profiles before and after OSKM treatment and identified differentially expressed genes using the same thresholds (FDR < 0.05, $\log_2$FC> 1 or < –1, Table S2B).

Pattern Classification Following OSKM and Gene Ranking by Weighted Hamming Distance. DEGs before and after OSKM induction (day 7 vs. day 0) were identified for each cell type using the LRT implemented in DESeq2[88]. To establish robust thresholds for DEG calling, we evaluated how closely observed DEG statistics matched the expected null hypothesis distribution. Specifically, we constructed empirical quantile-quantile (Q-Q) plots comparing DEG statistics from genes presumed to be non-differentially expressed (those with simultaneously high adjusted p-values and low log2(FC)) against their expected null distribution. We applied Huber regression to these Q-Q plots and measured the mean absolute deviation (MAD) between the fitted regression line and the identity line over the range of observed statistics. Higher MAD values indicate greater divergence from the null distribution, reflecting genuine

biological signals. MAD values were computed across a range of adjusted p-values and log2(FC) threshold combinations and visualized as a heatmap. Based on this analysis, genes with log2(FC) > 0.2 and FDR < 0.05 were considered significantly differentially expressed, as these thresholds corresponded to a clear elevation in MAD.

To classify gene expression responses to OSKM induction, we analyzed DEGs across six cell types—two normal controls and four PDAC cells ([normal#1][normal#2][cancer#1][cancer#2][cancer#3][cancer#4])—using six predefined expression patterns: i) Downregulated in normal, unchanged in cancer: [-1][-1][0][0][0][0], ii) Downregulated in normal, upregulated in cancer: [-1][-1][1][1][1][1], iii) Downregulated in normal, upregulated or unchanged in cancer: [-1][-1][0/+1][0/+1][0/+1][0/+1], iv) Unchanged in normal, upregulated in cancer: [0][0][1][1][1][1], v) Upregulated in normal, downregulated in cancer: [1][1][-1][-1][-1][-1], vi) Upregulated in normal, unchanged in cancer: [1][1][0][0][0][0]. These patterns were used to stratify gene responses to OSKM and identify cancer-specific regulatory behaviors. We classified genes in Class I ([-1][-1][0][0][0][0]) as OSKM-unresponsive genes, and genes in Class II ([-1][-1][1][1][1][1]), III ([-1][-1][0/+1][0/+1][0/+1][0/+1]), and IV [0][0][1][1][1][1] as OSKM-aberrantly upregulated genes (Table S2F-H).

To rank genes within each predefined expression pattern category, we used a weighted Hamming distance metric[89] relative to the query vector. Let q denote the query vector representing a specific regulation profile (e.g., upregulated in normal with minimal change in cancer samples), and let cat(S) denote the categorical expression vector of a gene. The weighted Hamming distance d is calculated as: $d = \sum(i=1 \text{ to } N)\ w_i \times \mathrm{Ind}(q_i \neq \mathrm{cat}(S)_i)$ where N is the number of conditions or replicates, w_i is the weight assigned to the *i*th condition, and Ind is the indicator function that returns 1 if the query and gene vector differ at position *i*, and 0 otherwise. This metric penalizes deviations from the target pattern, with weights chosen to balance representation across biological conditions and to reflect the hierarchical structure of replicates within each condition. Genes with lower values of the weighted Hamming distances

are considered better matches to the specified query. For each query of interest, genes were ranked in ascending order based on their weighted Hamming distance (Table S2F-H).

Single-cell RNAseq analysis. Single-cell RNA sequencing (scRNA-seq) was performed using the Chromium Next GEM Single Cell 5' Kit (v2.0, 10x Genomics). All scRNA-seq samples were processed using Seurat v5. Following quality control, cells were normalized, scaled, batch-corrected, and filtered in accordance with best practices outlined in the Seurat v5 vignette[126]. Cells expressing gRNA were filtered for BFP. After filtering out low-quality cells with >10% mitochondrial gene content and regressing out cell cycle–associated transcripts, we retained high-quality transcriptomes for downstream analysis (Fig. S4A-B). Clustering was performed using the Seurat integration workflow with the CCA (Canonical Correlation Analysis) method, at a resolution of 0.5 using the Louvain algorithm. Harmony integration was also performed to assess potential differences compared to the Seurat CCA-based integration. scRNAseq data set was extracted from human PDAC (n=6, GSE212966[97]), human and mouse normal pancreas (n=4, GSE84133[98]), human early embryo before the three germ layers formed (D6-D14, n=555, GSE136447[127]), and iPSC derived from Parkinson's patients' fibroblasts (n=12, GSE183248[128]). "PDAC-specific signature genes" were curated from published scRNA-seq datasets by identifying transcripts enriched in PDAC cells but expressed in fewer than 20% of normal pancreatic cells (Table S4D)[97,98]. Tumor suppressor genes (TSG) were curated from the OncoKB database[100] (Table S4E). NT clusters were manually defined based on NANOG and TRA-1-60 expression profiles. Cells were grouped according to their expression of these markers, with clusters curated based on predefined thresholds rather than unsupervised clustering algorithms.

Next-Generation Sequencing for Identification of Depleted gRNAs in Reprogrammed Cells (Table S5). Genomic DNA from each group was subjected to nested PCR amplification

to generate NGS libraries, enabling identification of enriched or depleted sgRNAs in TRA-1-60^{+} reprogrammed cells compared to either reference controls or TRA-1-60^{-} cells. These analyses were performed on two independently passaged biological cultures, separated by 3–6 passages. The hit count corresponding to each sgRNA was computed from raw FastQ files using Millipore's custom pipelines. The raw hit counts were normalized by Z-score for exploratory analysis. The MAGeCK package with negative control sgRNA normalization was used to analyze the enriched lncRNAs[106]. The MAGeCK algorithm first performs normalization and then uses information borrowing across genes to estimate variance[106]. A negative binomial model is then fit and used to test for differential hits on lncRNAs across samples. Significance testing used permutations. We used the MAGeCK mle module, since there were more than two conditions. The MAGeCKFlute R package was used with R version 4.4 for data analysis and visualization[129]. To ensure robustness, raw gRNA counts from both experiments were merged, scaling the second dataset to correct for batch differences (Exp1/Exp2 ≈ 1.86), with counts rounded up to the nearest integer (Table S5J). gRNAs consistently enriched from two independent samples, as defined by either Z-scores > 2 and positive beta scores ($p < 0.05$), were retained (Table S5K).

Gene Ontology (GO) and Upstream Analysis. GO analysis was performed using Enrichment Analysis[130] or the ToppGene Suite[131]. To determine whether gene sets of interest are enriched for putative TF targets, we performed ChIP-X enrichment analysis using datasets from ENCODE[132] or ChEA[111], accessed through Enrichr[133] or X2K[110]. ChIP-X refers to genome-wide TF binding data obtained from ChIP-ChIP, ChIP-Seq, ChIP-PET, or DamID experiments[111]. The p-value is computed using Fisher's exact test or the hypergeometric test. This is a binomial proportion test that assumes a binomial distribution and independence for the probability of any gene belonging to any set. The q-value is an adjusted p-value using the Benjamini-Hochberg method for correction for multiple hypothesis testing. The combined score is calculated by taking the logarithm of the p-value from the Fisher exact test and multiplying it by the Z-score, which

reflects the deviation from the expected rank. The odds ratio (used for rank-based enrichment) is derived by performing the Fisher exact test across numerous random gene sets to estimate the mean and standard deviation of the expected rank for each term in the gene-set library. A Z-score is then computed to quantify the deviation of the observed rank from expectation.

Identification of Disease-Associated Somatic Variants. Disease-associated somatic variant data for ATXN7L3-AS1 were collected from the COSMIC[108] and ClinVar[134] databases via LncBook 2.0[135]. We retained variants labeled as "confirmed somatic mutations" in COSMIC. Variants were classified as pathogenic if they were annotated as having a significant impact on disease in ClinVar or if they had a FATHMM-MKL score greater than 0.7, a threshold previously shown to predict pathogenic potential of noncoding variants[136] (Table S6A-B).

Expression of lncRNAs in normal Tissues and TCGA pan-cancer cohorts. The expression profiles of lncRNAs in normal tissues were obtained from the GTEx database, and those in the TCGA Pan-Cancer dataset were obtained through The Atlas of Non-coding RNAs in Cancer (TANRIC)[109] (Table S7A-D).

ADDITIONAL RESOURCES. In vivo amplification and characterization of patient-derived xenograft (PDX) tumors have been described in STAR Protocols[74]. Detailed protocols for transduction of PDAC cells and subtype characterization of PDX tumors were published in Heliyon[75].

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Human TRA-1-85/CD147 Antibody	R&D Systems	Cat # MAB3195
PE Mouse anti-Human TRA-1-85 Antigen	BD Biosciences	Cat # 563021

human Cytokeratin 19 (Abcam, BA-17)	Abcam	Cat # ab7755
Ki67	Cell Signaling Technology,	Cat # 9449
Cleaved Caspase-3	Cell Signaling Technology	Cat # 12692
FITC conjugate, Anti-TRA-1-60 Antibody, clone TRA-1-60, FITC conjugate	Millipore-Sigma	FCMAB115F
TRA-1-60 Alexa Fluor™ 488 Conjugate Kit for Live Cell Imaging	Thermo Scientific	Cat # A25618
Human/Mouse SSEA-1 Alexa Fluor« 594-conjugated Antibody	R&D systems	IC2155T-050UG
GloLIVE Human/Mouse SSEA-1 NL557 Live Cell Antibody	R&D Systems	Cat # NLLC2155R
Sendai virus HN Monoclonal Antibody (1A6), Alexa Fluor™ 647, eBioscience™	Invitrogen	Cat # 51-6494-82
OCT4 Antibody (OTI9B7) [Alexa Fluor« 405]	Novus Biologicals	NBP2-71225AF405
Anti-Oct4 antibody - ChIP Grade	Abcam	Cat# ab19857
Biological samples		
CF-1 MEFS 2 MILLION	Thermo Fisher Scientific	A34180
Dr4 Mouse Embryonic Fibroblasts, irradiated	Thermo Fisher Scientific	A34966
Chemicals, peptides, and recombinant proteins		
Iscript™ Advanced cDNA Synthesis Kit	Bio-Rad	1725037
RNase-Free DNase Set (50)	Qiagen	79254
Applied Biosystems TaqMan Fast Advanced Master Mix	Thermo Fisher Scientific	44-445-57
SPRIselect, Liquid	Beckman Coulter	B23318
Keratinocyte-SFM (1X)	Invitrogen	17005042
Primocin™	Thermo Fisher Scientific	ant-pm-05
Agilent RNA 6000 Pico Reagents	Agilent Technologies	5067-1514
ACCUMAX™ Cell Detachment Solution, Sterile, Liquid	Thermo Fisher Scientific	07921
InSolution™ Y-27632	Calbiochem	688001-500UG
Epidermal Growth Factor, human, animal component free,	Millipore-Sigma	E5036
Gibco fgf Basic (aa 10 155) Recombinant Human Protein	Invitrogen	PHG0021
Knockout™ Serum Replacement	Invitrogen	10828-028
L Glutamine (200mM)	Invitrogen	25030081
Eagle's Minimum Essential Medium (EMEM)	ATCC	30-2003
0.1% Gelatin in Water	STEMCELL Technologies	07903
Embryonic Stem Cell FBS, Qualified, One Shot™ Format, US Origin	Thermo Fisher Scientific	16141002
Human TruStain FcX™ (Fc Receptor Blocking Solution) 50 tests	Bio Legend	422301
Cholera Toxin from Vibrio cholerae	Thermo Fisher Scientific	C8052-.5MG
Blasticidin, Sterile, ≥95%, 10 mg/mL, Liquid	InvivoGen	ANTBL05
Puromycin, 100 mg (10 x 1 ml tubes)	InvivoGen	ANTPR1
DEFINED KERATINOCYTE-SFM, COMBO COMBINATION	Invitrogen	10744019
Protein Free, Thermo Scientific, Pierce™ Protein-free T20 (PBS) blocking buffer	Pierce	PI37573
Bovine Pituitary Extract (BPE)	Invitrogen	13028014

PowerTrack™ SYBR Green Master Mix	Applied Biosystems	A46109
Collagen I Rat Protein, Tail	Gibco	A1048301
FIX VIABILITY STAIN 660 100UG	Bd Biosciences	BDB564405
Cytotune™ EmGFP Sendai Fluorescence Reporter	Life Science	A16519
Critical commercial assays		
CytoTune™-iPS 2.0 Sendai Reprogramming Kit	*Invitrogen*	A16518
Human CRISPRi Common Cancer Cell Lines Kit	Millipore-Sigma	CRISPRIS10
Human CRISPR Control Kit	Millipore	CRISPRICON-1KT
QIAamp UCP DNA Micro Kit	Qiagen	56204
Lenti-X™ Provirus Quantitation Kit	Takara	631239
Deposited data		
Raw and analyzed data	This paper	GEO: GSE247243
Table S3A. List of gRNAs targeting cancer-associated lncRNAs (CRISPRIL17_hg38).	This paper, Supplementary Table	Table S3A.
Experimental models: Cell lines		
Human: BJ fibroblast cells	ATCC	CRL-2522
Human: H6c7 normal pancreatic ductal cells	KeraFast	ECA001-FP
Human: hTERT-HPNE,	ATCC	CRL-4023
Human: Passage 40 H1 and H9 ES cells	Wicell	WA01
Human: HPAFII PDAC cells	ATCC	CRL-1997
Human: Panc1 PDAC cells	ATCC	CRL-1469
Human: CFPAC1 cells	ATCC	CRL-1918
Human: PDAC cells from PDX (ST-00014490)	Star Protocols[74]	n.a.
Human: PDAC cells from PDX (ST-00007599)	Star Protocols[74]	n.a.
Experimental models: Organisms/strains		
Mouse: NOD-SCID-IL2Rgc null (NSG) (4-6 weeks old, female/male)	The Jackson Laboratory	JAX: #005557
Oligonucleotides		
Primer: ATXN7L3-AS1	IDT	Forward primer: TCTCCAAGAGCC CCAAAGTC Reverse primer: GCGTCCTGTCCT GATAACCT
Primer: AC079921.2 #2	IDT	Forward: AGAGCATGGGGTG AAAGACC Reverse: AGAGACTTTCTCAG GGTGGC

Primer: INTS4P2 #1:	IDT	Forward: AGCAGCAAAACAG ACTTTACCC Reverse: TGTCCACAAAGCT GTCCTGC
PrimerINTS4P2 #2	IDT	Forward: AGATACGGACCCT GGAGCAT Reverse: GAACCACCCAAGA GCCATCA
RAB1A	Life Technologies	Hs00366313_m1
GAPDH	Life Technologies	Hs02758991_g1
GATA6	Life Technologies	Hs00232018_m1
SOX9	Life Technologies	Hs00165814_m1
KRT19	Life Technologies	Hs00761767_s1
Foxa2	Life Technologies	Hs05036278_s1
Hnf1b	Life Technologies	Hs01001602_m1
Onecut1	Life Technologies	Hs00413554_m1
FUT4	Life Technologies	Hs01106466_s1
MNX1	Life Technologies	Hs00907365_m1
MNX1	Life Technologies	Hs03454395_m1
PDX1	Life Technologies	Hs00426216_m1
Sendai-CMYC	Life Technologies	Mr04269876_mr
Sendai-OCT3-4	Life Technologies	Mr04269878_mr
Sendai-KLF4	Life Technologies	Mr04421256_mr
SEV-SOX2	Life Technologies	Mr04269881_mr
Sendai	Life Technologies	Mr04269880_mr
Recombinant DNA		
Software and algorithms		
DESEQ2	Love et al.,[88]	https://bioconductor.org/packages/devel/bioc/vignettes/DESeq2/inst/doc/DESeq2.html
MAGeCK	Li et al., 2014[106] and Wang e al., 2019[129]	https://github.com/liulab-dfci/MAGeCK
Other		

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MAIN FIGURE TITLES and LEGENDS

Figure 1. OSKM Reprogramming Partially Suppresses PDAC-Unique Genes, Leaving Others Unresponsive or Aberrantly Induced. A. Schematic diagram of reprogramming of normal controls (BJ fibroblast and H6c7 hnPDEC) and human classical (CFPAC1, ST-00014490) and basal (Panc1, ST-00007599) subtypes of PDAC cells using Sendai virus-mediated expression of OSKM factors. Cells were transduced with OSKM-expressing Sendai virus on day 0. For transcriptomic analysis, cells were harvested on days 0 and 7. To assess pluripotency and early differentiation, cells were fixed on day 30 and immunostained for TRA-1-60 and SSEA1, respectively. B. Each Venn diagram shows PDAC-specific genes that are upregulated relative to normal controls on day 0 ($\log_2$[PDAC/normal controls] ≥ 1, FDR < 0.05). The heatmap displays the expression profiles of 375 PDAC-specific genes consistently upregulated across all PDAC samples, with Z-scores indicated by the color key. Seven of the 375 PDAC-unique genes were significantly downregulated by OSKM across all PDAC cells ($\log_2$[$OSKM^+$/$OSKM^-$] < –1, FDR < 0.05). C-D. Example of an OSKM-induced downregulated PDAC-specific gene. (C) *SPDEF* mRNA levels in the indicated cells without and with OSKM induction. (D) Kaplan–Meier survival analysis of *SPDEF* expression in the TCGA PDAC cohort (n = 150). Patients were stratified by SPDEF expression quartiles (Low: <7.611; High: >9.675), based on $\log_2$-transformed normalized RSEM values. P-value was calculated using the log-rank test (χ^2, rho = 0). The high *SPDEF* expression in PDAC is associated with poor overall survival of patients with PDAC. E. Identification of aberrantly increased or unchanged genes in PDAC following OSKM induction. The empirical cumulative distribution function (ECDF) displays the cumulative probability distribution of $\log_2$ fold-change (FC) values across samples, before and after OSKM induction (day 7 vs. day 0). The X-axis indicates $\log_2$(FC), and the Y-axis indicates the cumulative fraction of genes. The plot is zoomed in to the range of -1 to 1 to highlight subtle shifts in gene expression. Genes with $\log_2$(FC) > 0.2 or < –0.2 and FDR < 0.05 were defined as

DEGs in each sample, based on inspection of the Mean Absolute Deviation (MAD) heatmap as an empirical measure of deviation from the null hypothesis (Fig. S3C; see Methods). Genes were then queried against pre-defined patterns of expression changes across the samples. A weighted Hamming distance was used to measure how well a gene's pattern of expression changes across samples matched with each query (see Methods). For each query, genes with weighted Hamming distances no greater than 0.35 were selected as hits for downstream analysis. F. Violin plots showing expression levels for OSKM-unresponsive PDAC genes (left: distance < 0.375, n = 1,466; Table S2F) and OSKM-aberrantly upregulated genes (middle: distance < 0.25, n = 1,815; right: distance = 0, n = 82; Tables S2G–S2H; see Methods). G-H. Gene Ontology (GO)[131] and X2K upstream regulator[110,111] analyses for genes that were either unresponsive to OSKM in PDAC (G, Table S2F) or aberrantly upregulated following OSKM induction (H, Table S2G). The red dashed line indicates the significance threshold (FDR or p-value < 0.05).

Figure 2. CRISPRi of Cancer-Associated lncRNAs Enhances Reprogramming Efficiency in PDAC Cells. A. Schematic diagram of reprogramming HPAFII PDAC and H6c7 hnPDEC cells by CRISPRi screening. The reprogramming efficiency was measured by immunostaining for TRA-1-60 and SSEA1. The TRA-1-60+ pluripotent stem cell-like cells were quantified and sorted by flow cytometry at 30 days. B. Flow cytometry–based quantification of TRA-1-60^{+} and TRA-1-60^{-} fractions in HPAFII-dCas9 control cells and HPAFII-dCas9 cells expressing lncRNA-targeting gRNAs (BFP^{+}) at day 30 after OSKM induction. For each condition, the proportion of TRA-1-60^{+} versus TRA-1-60^{-} cells was compared within the same cell type. In dCas9 control cells, TRA-1-60^{+} cells were significantly less frequent than TRA-1-60^{-} cells (*q = 1.60E-05, n = 4, unpaired Welch t-test), whereas no significant difference between TRA-1-60^{+} and TRA-1-60^{-} fractions was observed in dCas9+gRNA cells (Table S3B). C. Representative images and

quantification summary of TRA-1-60^{+} and SSEA1^{+} cells on plates after multiple passages (Scale bar: 284 μm).

Figure 3. Repression of PDAC Programs and De-repression of Pluripotency and Tumor Suppressor Signatures in PDAC Cells Reprogrammed with OSKM and lncRNA Depletion. A. Schematic diagram of the scRNA-seq workflow. B-J. UMAP visualizations showing distinct clusters by sample (B), 15 Louvain-defined clusters (C), cells expressing BFP (D), pancreatic ductal marker KRT19 (E), *PODXL* encoding TRA-1-60 (F), *NANOG* (G), pluripotency-associated genes (H, Table S4A), endodermal and PDAC-related genes (I, Table S4B), and ectodermal/mesodermal lineage markers (J, Table S4C). Each dot represents a cell, and the color intensity indicates the normalized UMI counts (scaled by the total UMI per cell). K. Principal component analysis (PCA) of HPAFII-dCas9-gRNA-OSKM-TP cells alongside publicly available scRNA-seq datasets from human PDAC (n=6, GSE212966[97]), human and mouse normal pancreas (n=4, GSE84133[98]), human early embryo before the three germ layers formed (D6-D14, n=555, GSE136447[127]), and iPSC derived from Parkinson's disease patients' fibroblast (n=12, GSE183248[128]). L. Volcano plots of DEGs between dCas9-gRNA and either dCas9-gRNA-OSKM-TN (left) or dCas9-gRNA-OSKM-TP (right). PDAC-specific signature genes are annotated (n=332, Table S4D). M. Heatmap showing expression of 318 TSGs across the indicated cell populations (Table S4E). The plot shows z-scores of the mean TSG expression across cells in the sample. The distinct subsets show that each sample has a set of TSGs more highly expressed in that sample than in others. N-O. Heatmap (N) and quantification (O) of TSG expression in the dCas9-gRNA-OSKM-TP cells. (N) Each column represents an individual cell, each row corresponds to a TSG, and expression levels are shown as Z-scores normalized across all cells for each gene. TSGs were grouped into three distinct clusters based on their expression profiles using unsupervised k-means clustering. (O) Expression levels of TSGs in each cluster are shown across the indicated subpopulations based on NANOG and TRA-1-60

expressions-NANOG$^-$/TRA-1-60$^-$ (N$^-$/T$^-$), NANOG$^-$/TRA-1-60$^+$ (N$^-$/T$^+$), NANOG$^+$/TRA-1-60$^-$ (N$^+$/T$^-$), and NANOG$^+$/TRA-1-60$^+$ (N$^+$/T$^+$). The Y-axis indicates Z-score.

Figure 4. Xenograft Tumor Growth Is Reduced in PDAC Cells Reprogrammed with OSKM and lncRNA Depletion. A. Schematic diagram of xenograft assay of TRA-1-60$^+$ and TRA-1-60$^-$cells derived from OSKM-reprogrammed HPAFII-dCas9/gRNA, along with controls. Tumors were harvested from NSG mice one month after being subcutaneously injected with dCas9-gRNA-OSKM-TN and dCas9-gRNA-OSKM-TP, along with controls: Parental HPAFII, huES, dCas9-gRNA, dCas9-OSKM-TP (n=4-8 for each cell type). B. Representative images of tumors from two independent experiments; two to four mice for each cell type were used in each experiment. C. Comparison of tumor size among different cell types. The differences in tumor sizes (width x height) were tested pairwise using a non-parametric Mann-Whitney test. Only tumor sizes between parental HPAFII and dCas9-gRNA-OSKM-TP are significantly different (*** p=0.0002, n=8 each, Mann-Whitney test). D-H. Representative H&E images of the indicated xenografted tumor samples (D; HPAFII, E; dCas9-gRNA, F; dCas9-OSKM-TP, G; dCas9-gRNA-OSKM-TN, H; dCas9-gRNA-OSKM-TP) from three independent mice. Mouse identifiers (e.g., #1-1, #1-2, #2-1, #2-2) denote individual animals associated with each cell type. The scale bar indicates 50 μm.

Figure 5. Xenograft Tumors from OSKM/lncRNA-Depleted PDAC Cells Lose Ductal Identity and Express Apoptotic Markers. Representative images of TRA-1-85 human specific marker, human CK19 pancreatic ductal marker, Cleaved Caspase-3 apoptotic marker, and Ki67 proliferation marker staining in xenograft tumors derived from parental HPAFII cells (A–D), dCas9-gRNA cells (E–H), dCas9-OSKM-TP cells (I–L), dCas9-gRNA-OSKM-TN cells (M–P), and dCas9-gRNA-OSKM-TP cells (Q–T). Mouse identifiers (e.g., #1-1, #1-2, #2-1, #2-2) denote

individual animals associated with each cell type. Arrows indicate positive brown signals. Scale bar: 50 μm.

Figure 6. Identification of *ATXN7L3-AS1*, AC079921.2, and INTS4P2 as Candidate Barriers to Reprogramming in OSKM/lncRNA-Depleted PDAC Cells. A. Schematic flow chart of CRISPRi screening. B. Significantly depleted lncRNAs in HPAFII-dCas9-gRNA-OSKM-TP cells in comparison to the HPAFII-dCas9-gRNA reference sample are visualized in the volcano plot. The enrichment score of gRNAs is shown as the "β-scores." Positive and negative β-scores indicate positive and negative selections of gRNAs in the TRA-1-60$^+$ compared to the reference condition, respectively. The X-axis shows the β-score of TRA-1-60$^+$ compared to the reference. The Y-axis displays –log10 (p-values). The p-value was calculated by ranking lncRNAs with a skewed distribution compared to a uniform null model and permuting to calculate the significance of the skew in distribution. β-score and p-values were calculated separately from two independent experiments and combined to display the top-ranked lncRNAs in one volcano plot. The horizontal and vertical lines indicate the cutoff threshold. The red dots indicate lncRNAs with a β-score over zero and a permutation p-value <0.05. The candidate lncRNAs that were validated by qRT-PCR were highlighted with names. C. The expression of candidate lncRNAs in normal pancreatic cells and cancers, and HPAFII-dCas9-gRNA-OSKM-TN and TP cells (n=3, y-axis indicates relative expression over GAPDH, error bars=SD). D-F. Expression level of candidate lncRNAs in human normal tissues (GTEx)[107]. Expression values are in TPM (Transcripts per Million), calculated from a gene model with isoforms collapsed to a single gene. The X-axis shows $\log_{10}$(TPM+1). Box plots are shown as median and 25th and 75th percentiles; points are displayed as outliers if they are above or below 1.5 times the interquartile range. Pancreatic tissues (n = 362) are highlighted in red.

Figure 7. *ATXN7L3-AS1* and *AC079921.2* Preserve PDAC Identity and Associate with Pro-Invasive Gene Programs and Somatic Mutations. A. Expression of basal subtype markers (*KRT5, TP63*), classical ductal TFs (*GATA6, HNF1B, ONECUT1*), and pancreatic progenitor markers (*PDX1, FOXA2, and SOX9*) in HPAFII cells following CRISPRi-mediated knockdown of *ATXN7L3-AS1, AC079921.2*, or *INTS4P2*. Y-axis indicates relative expressions normalized to GAPDH and scaled to non-targeting controls (NT); error bars represent SD. N=2-3. N.E. indicates no expression. B. Representative images of TRA-1-60^{+} colonies in HPAFII-dCas9–*ATXN7L3-AS1* gRNA cells at day 30 after OSKM reprogramming. HPAFII-dCas9-NT gRNA cells serve as a control. Bar graphs show the percentage of colonies positive for TRA-1-60 (green) or SSEA1 (red) among total colonies counted. C. UCSC genome browser view of the *ATXN7L3-AS1* locus (chr17:44,198,841–44,234,868; hg38), located between the *ATXN7L3* and *UBTF* genes. Pathogenic somatic variants identified in PDAC from the COSMIC database are displayed along the locus. D-E. Overlap of *AC079921.2*–positively correlated genes in pan-TCGA tumors with OSKM-aberrantly upregulated (D) or unresponsive (E) genes in PDAC reprogramming. The hypergeometric test was used for significance. F-G. GO analysis of overlapping genes from (D) and (E), enriched for ECM organization, muscle contraction, cell adhesion, and migration pathways. H-I. Upstream regulator analysis highlights SUZ12 and chromatin-associated complexes as candidate regulators of *AC079921.2*–associated transcriptional programs.

SUPPLEMENTAL FIGURE TITLES and LEGENDS

Figure S1 (Relevant to Figure 1). Transgene-free reprogramming of human normal fibroblast and pancreatic acinar cells into pluripotency using Sendai-virus mediated expression of OSKM master TFs. A. Immunostaining of pluripotency markers in iPSCs established from BJ6 human normal fibroblast cells. B. Verification of expression of pancreatic acinar marker amylase and lack of pancreatic ductal marker K19 on pancreatic acinar cells using immunofluorescence (IF). C. Schematic diagram of reprogramming pancreatic acinar cells into iPSC using Sendai-OSKM. Initial numbers of cells and emerged TRA-1-60^{+} cells after 26 days were counted to determine the reprogramming efficiency. Three clones were established after five passages. D. Confirmation of expression of pluripotency surface markers TRA-1-60 and SSEA4 in established acinar iPS lines through flow cytometry. E. Representative image of cytogenetic analysis on 20 G-banded metaphase cells derived from pancreatic acinar-derived iPSC. F. Confirmation of functional pluripotency of acinar-derived iPSC clones. Their embryoid bodies (EB) differentiated into three germ layer lineages, which were verified by staining for three germ layer markers using flow cytometry.

Figure S2 (Relevant to Figure 1). Reprogramming of human normal pancreatic ductal and cancer cells. A. Representative images of colonies stained for TRA-1-60 and SSEA-1 in indicated cells. Scale bars indicate 100 μm. B. Expression of OSKM, pluripotency, and PDAC genes in the indicated cells before and 7 days after OSKM induction.

Figure S3 (Relevant to Figures 1-2). A-F. RNA-seq of classical (CFPAC1 and ST-00014490) and basal (Panc1 and ST-00007599) subtypes of PDAC and normal controls (human BJ6 fibroblast and H6c7 hnPDECs) before and 7 days after OSKM induction. (A) PCA plot. (B) Proportion of genes changed upon OSKM induction among a total of 302,383 genes in normal cells (BJ, H6c7) and PDAC (CFPAC1, ST-00014490, Panc1, and ST-00007599). A chi-squared

test was used to compare the proportion of genes exhibiting expression changes following OSKM induction in PDAC cells relative to other normal cell types. The Y-axis indicates the proportion of DEGs relative to the total number of genes. *** indicates p-value < 2.2×10^{-13}. Black and blue bars indicate comparison between PDAC cells and BJ or H6c7 cells, respectively (See Table S2D-E). (C) Mean Absolute Deviation (MAD) heatmap of Q–Q statistics across FDR cut-off points and $\log_2$ (FC) truncated thresholds. The heatmap displays the MAD between observed Q–Q statistics and a truncated standard normal distribution (N(0,1)), computed across combinations of FDR cutoffs (x-axis) and $\log_2$ FC thresholds (y-axis). The color key indicates MAD of Q-Q (stat) vs truncated N (0,1). Lower MAD values (dark blue) indicate better agreement with the expected null distribution, suggesting more reliable gene selection. Red contours mark regions of increasing deviation. Black horizontal and vertical lines indicate $\log_2$(FC) = 0.2 and FDR 0.05 thresholds, respectively. (D) Density and Empirical Cumulative Distribution Function (ECDF) plots of log2 FC values across all samples. Red dashed lines indicate |log2 FC|=0.2. (E) Intersection of aberrantly upregulated PDAC genes (downregulated in normal but upregulated in PDAC) and SUZ12 target genes in human and mouse ES cells (hypergeometric p-value). F. Expressions of genes encoding PRC2 components (SUZ12 and EZH2) and REST transcriptional repressor in indicated cells before and after OSKM induction. G-H. CRISPRi against cancer-associated lncRNAs. (G) Schematic diagram of KRAB-dCas9-Blastidin and gRNA-EF1a-Puro2a-BFP vectors. (H) Flow cytometry analysis of TRA-1-60$^+$ cells at day 30 after OSKM induction in H6c7-dCas9 cells expressing gRNAs targeting lncRNAs.

Figure S4 (Relevant to Figure 3) scRNAseq of HPAFII-dCas9-gRNA-OSKM-TP cells along with controls. A. Quality control density plots showing the distribution of cells by key metrics: percentage of mitochondrial gene expression, complexity (features per count), total transcripts per cell, and number of detected genes per cell. B. Expression levels of *BFP*, *OSKM*, and *NANOG* across samples (dCas9 only, dCas9-gRNA, dCas9-gRNA-OSKM-TN, dCas9-gRNA-

OSKM-TP, dCas9-OSKM-TP). The X-axis represents individual samples, and the Y-axis shows normalized UMI counts. C. Top GO terms enriched in genes from Group 0 and Group 1 clusters. D. Top GO terms associated with DEGs in dCas9-gRNA cells compared to parental dCas9 cells. E. Violin plots showing normalized UMI counts for genes in cells from groups 0, 1, and 5 across samples. F. PCA projection of each sample.

Figure S5 (Relevant to Figure 4-6) A-E. Xenograft of huES cells; (A) Representative images of immunostaining of H&E, (B) human-specific marker TRA-1-85, (C) human ductal marker CK19, (D) apoptotic marker Cleaved Caspase-3, and (E) proliferation marker Ki67. F-I. Identification of candidate gRNAs reproducible from two independent experiments; (F) Detailed diagram of selecting candidate lncRNAs. (G) Z-score of top 20 gRNAs. (H) The estimated β-score plots of TRA-1-60^{+} and TRA-1-60^{-} cells compared to references (HPAFII-dCas9-gRNA). The β scores of all gRNAs are normalized based on the negative control normalization. (I) Volcano plot of top-ranked lncRNAs showing β scores enriched in TRA-1-60^{+} cells compared to the reference control. The Y-axis displays $-\log_{10}$ (p-values). The p-value is calculated by ranking genes with a skewed distribution compared to a uniform null model and permuting to calculate the significance of the skew in distribution. The X-axis indicates the β scores in TRA-1-60^{+}. Red indicates positive enrichment (beta score >0) and blue indicates negative enrichment (beta score <0).

Figure S6 (Relevant to Figure 6-7) A. Expression of candidate lncRNAs before and after OSKM induction. B. Expression of basal subtype markers (*KRT5, TP63*), classical ductal TFs (GATA6, HNF1B, ONECUT1), and pancreatic progenitor markers (*PDX1, FOXA2, and SOX9*) in H6c7 cells following CRISPRi-mediated knockdown of *ATXN7L3-AS1*, *AC079921.2*, or *INTS4P2*. Y-axis indicates relative expression normalized to *GAPDH* and scaled to non-targeting controls (NT); error bars represent SD. N=2~3. N.E. indicates no expression. C-H.

Association of *ATXN7L3-AS1* with mitotic cell cycle progression and *AC079921.2* with cell adhesion and migration in the TCGA Pan-cancer dataset (TANRIC[109]). C–E. Top GO terms (C), potential regulators (D), and histone modifications (E) enriched upstream of genes positively correlated with ATXN7L3-AS1 mRNA levels in TCGA pan-cancer (Spearman correlation > 0.4; n = 2,887, Table S7A). F–H. Top GO terms (F), potential regulators (G), and histone modifications (H) enriched upstream of genes positively correlated with AC079921.2 in TCGA pan-cancer (Spearman correlation > 0.4; n = 2,305, Table S7B). I–N. Top GO terms (I, J), histone modifications upstream (K, L), and potential upstream regulators (M, N) of genes negatively correlated with *ATXN7L3-AS1* and *AC079921.2* in TCGA pan-cancer, respectively (Spearman correlation < –0.4; *ATXN7L3-AS1*, n = 1,928, Table S7C; *AC079921.2*, n = 667, Table S7D).

SUPPLEMENTAL TABLE TITLES and LEGENDS

Table S1. (*Related to Figure 1*) KRAS genotyping in iPS cells

Table S2. (*Related to Figure 1*) OSKM-mediated reprogramming is not sufficient to override PDAC identity.

- **Table S2A.** Regularized log_2-transformed DESeq2 expression values for 375 PDAC-unique genes.
- **Table S2B.** Regularized log_2-transformed DESeq2 expression values for 7 PDAC-unique genes regulated by OSKM induction.
- **Table S2C.** Gene ontology (GO) analysis of 7 PDAC-unique genes downregulated by OSKM induction.
- **Table S2D.** Chi-square test comparing the proportion of genes with expression changes in pooled PDAC vs. normal cells following OSKM induction.
- **Table S2E.** Chi-square test comparing the proportion of genes with expression changes in each PDAC and normal sample following OSKM induction.
- **Table S2F.** List of OSKM-unresponsive PDAC genes (signature: [-1][-1][0][0][0][0], outliers removed, n=1,466, distance < 0.375; related to Fig. 1F).
- **Table S2G.** List of OSKM-aberrantly upregulated PDAC genes (signature: [-1][-1][0/+1][0/+1][0/+1][0/+1], outliers removed, n=1,815, distance < 0.25; related to Fig. 1F).
- **Table S2H.** List of strongly OSKM-aberrantly upregulated PDAC genes (signature: [-1/0][-1/0][1][1][1][1], n=82, distance = 0; related to Fig. 1F).

Table S3. (*Related to Figure 2*) Depletion of cancer-associated lncRNAs enhances reprogramming of PDAC

- **Table S3A.** List of gRNAs targeting cancer-associated lncRNAs (CRISPRIL17_hg38).
- **Table S3B.** Quantification of TRA-1-60$^+$ and TRA-1-60$^-$ cells by flow cytometry.

Table S4. (*Related to Figure 3)* Single-cell RNA-seq reveals transcriptional reprogramming toward pluripotency, suppression of PDAC identity, and reactivation of tumor suppressor genes (TSGs)

- **Table S4A.** List of pluripotency marker genes.
- **Table S4B.** List of endodermal and PDAC marker genes.
- **Table S4C.** List of ectodermal and mesodermal marker genes.
- **Table S4D.** List of "PDAC-specific signature genes" (n=332)
- **Table S4E**. List of OncoKB-annotated tumor suppressor genes (total: n=379; excluding oncogenes: n=318).

Table S5. (*Related to Figure 6)* Identification of depleted gRNAs in HPAFII-dCas9-gRNA-OSKM-TP cells.

- **Table S5A.** Sample barcodes and Illumina adaptor sequences.
- **Table S5B.** Read counts aligned to gRNAs (Experiment 1).
- **Table S5C.** Read counts aligned to gRNAs (Experiment 2).
- **Table S5D.** Raw hit counts (Experiment 1).
- **Table S5E.** Raw hit counts (Experiment 2).
- **Table S5F.** Z-scores of each gRNA (Experiment 1).
- **Table S5G.** Z-scores of each gRNA (Experiment 2).
- **Table S5H.** MAGeCK-MLE output (aggregated reference x500, Experiment 1).
- **Table S5I.** MAGeCK-MLE output (Experiment 2).
- **Table S5J.** MAGeCK-MLE output (combined Experiment 1 and 2).
- **Table S5K.** Final list of selected candidate gRNAs.

Table S6. *(Related to Figure 7)* COSMIC Pathogenic somatic variants in ATXN7L3-AS1 (GRCh38).

- **Table S6A**. Pathogenic somatic variants in ATXN7L3-AS1 identified in pan-cancers

- **Table S6B**. Pathogenic somatic variants in ATXN7L3-AS1 identified in pancreatic cancer

Table S7. (Related to Figures 7 and S6) TCGA Pan-Cancer Genes Correlated with ATXN7L3-AS1 and AC079921.2.

- **Table S7A.** TCGA Pan-Cancer Genes Positively Correlated with ATXN7L3-AS1 (Spearman correlation > 0.4)
- **Table S7B.** TCGA Pan-Cancer Genes Positively Correlated with AC079921.2 (Spearman correlation > 0.4)
- **Table S7C.** TCGA Pan-Cancer Genes Negatively Correlated with ATXN7L3-AS1 (Spearman correlation <- 0.4)
- **Table S7D.** TCGA Pan-Cancer Genes Negatively Correlated with AC079921.2 (Spearman correlation <- 0.4)

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Figure 1

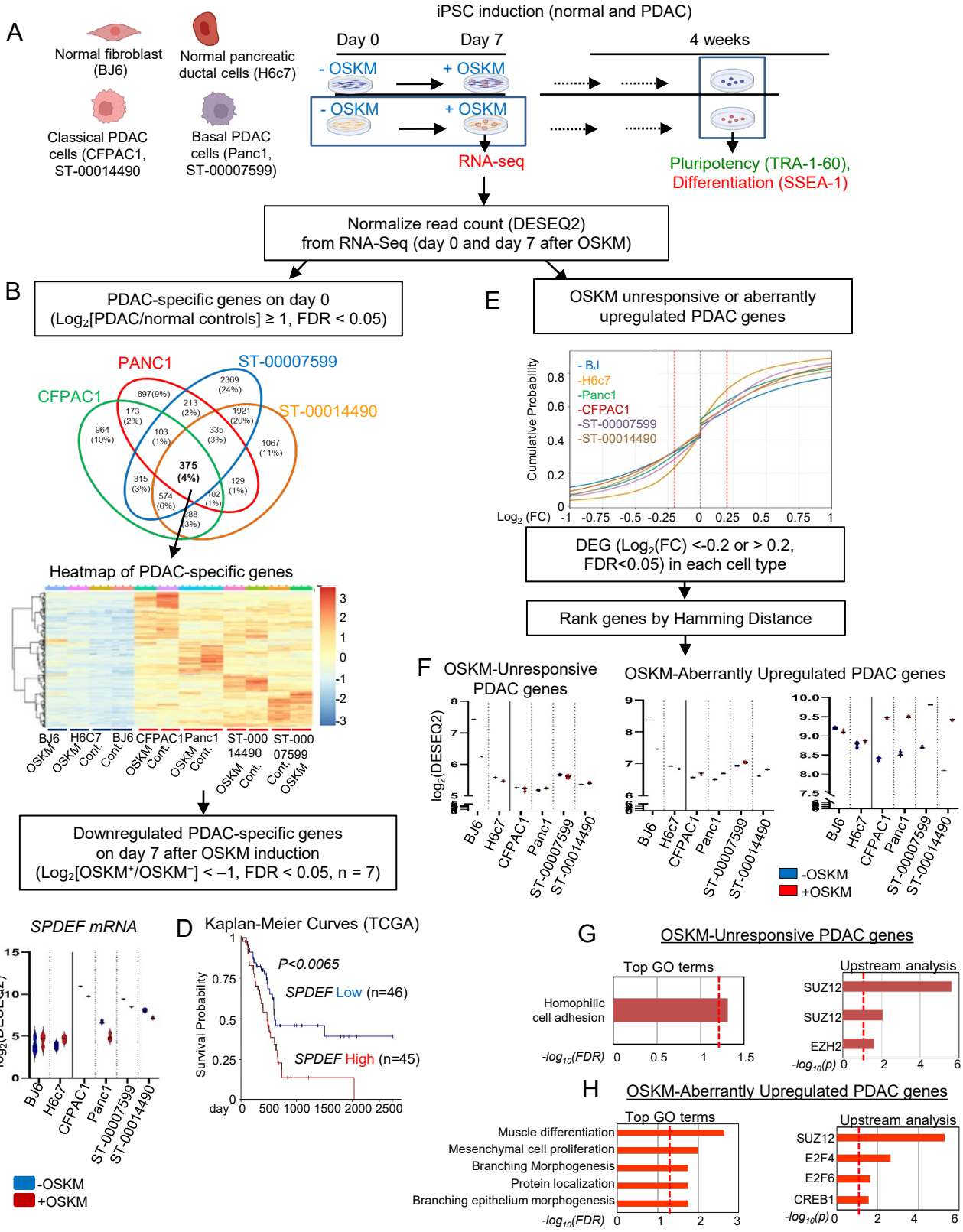

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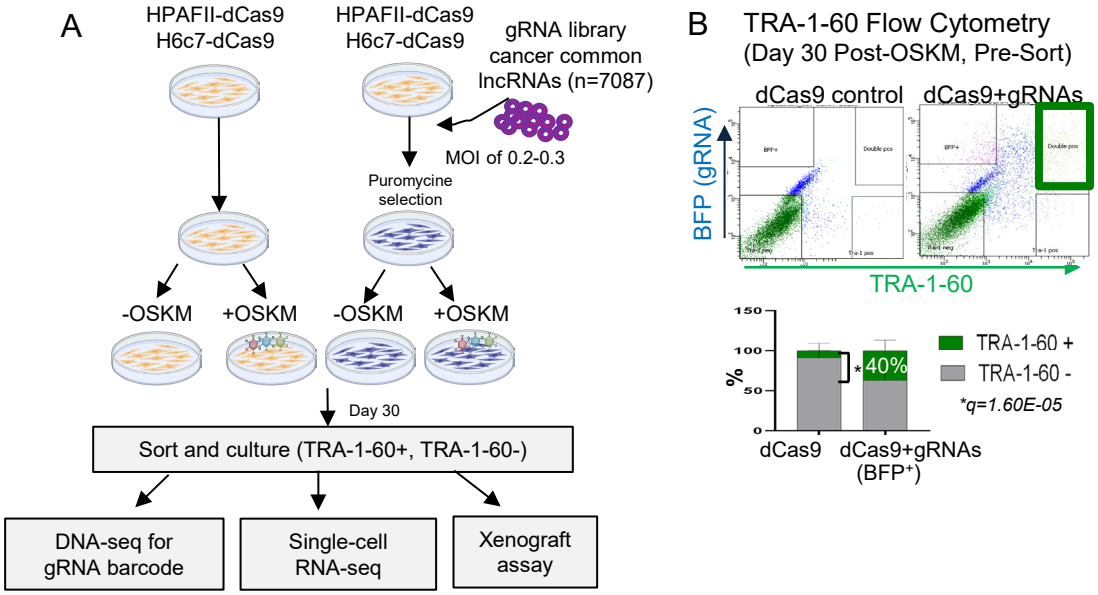
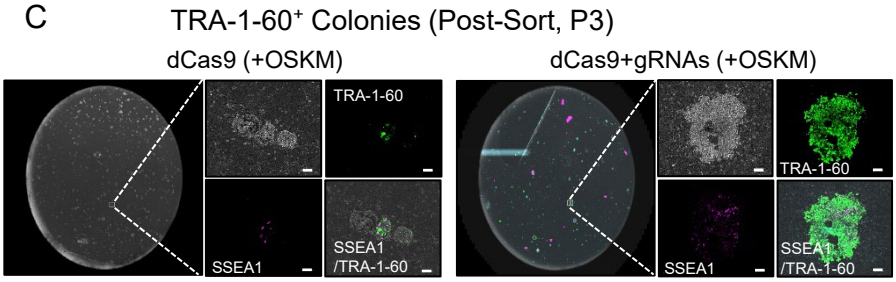
Figure 2





	Total colonies #	Total SSEA1+	Total TRA-1-60+	TRA-1-60+ SSEA1+	TRA-1-60+ only	SSEA1+ only
dCas9+OSKM	809	29 (3.6%)	35 (4.3%)	20 (2.5%)	15 (1.9.%)	9 (1%)
dCas9+gRNAs	2665	23 (1%)	0	0	0	23 (1%)
dCas9+gRNAs+OSKM	212	47 (22.2%)	96 (45.3%)	32 (15.1%)	64 (30.2%)	15 (7.1%)

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Figure 3

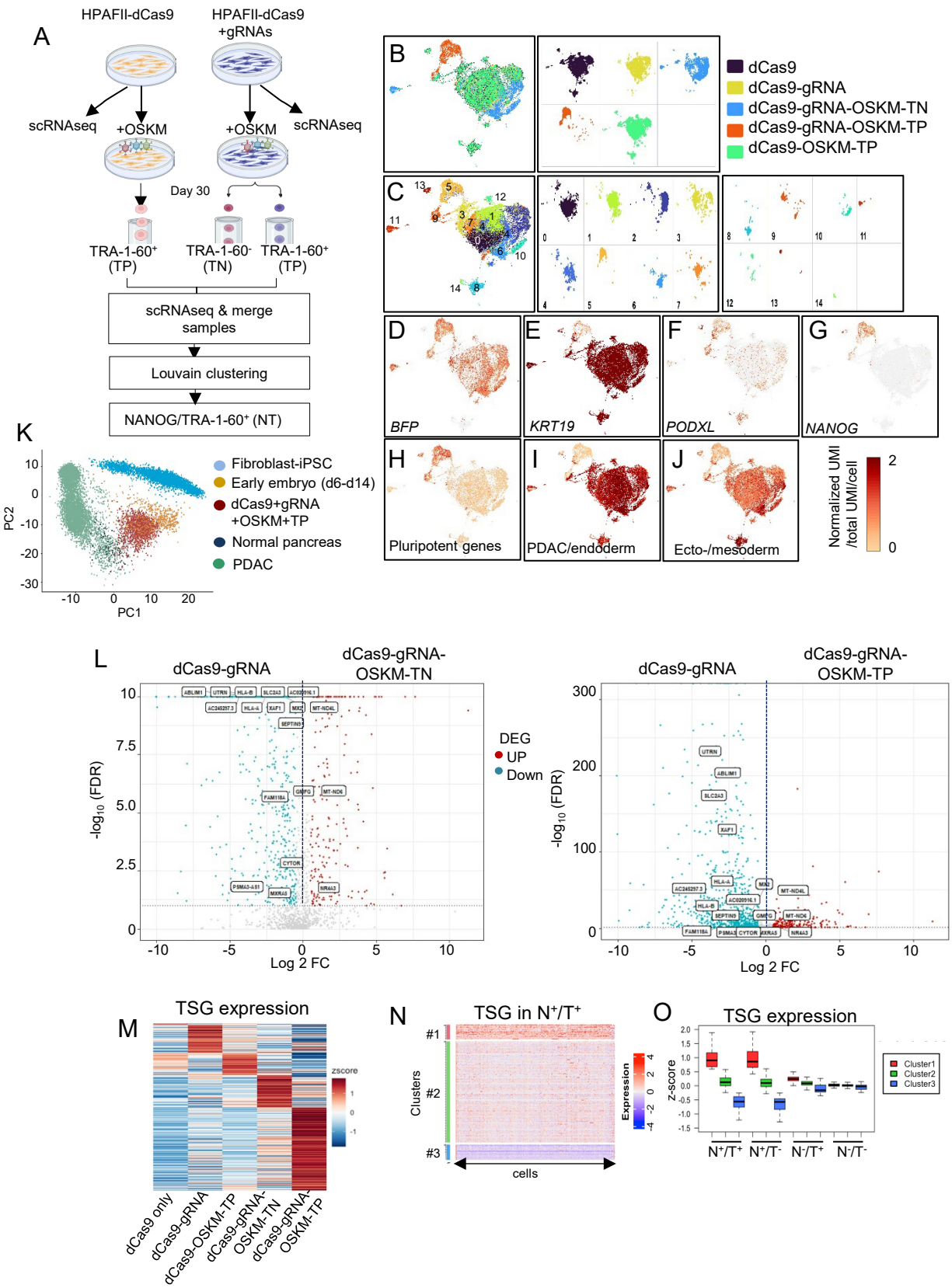

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Figure 4

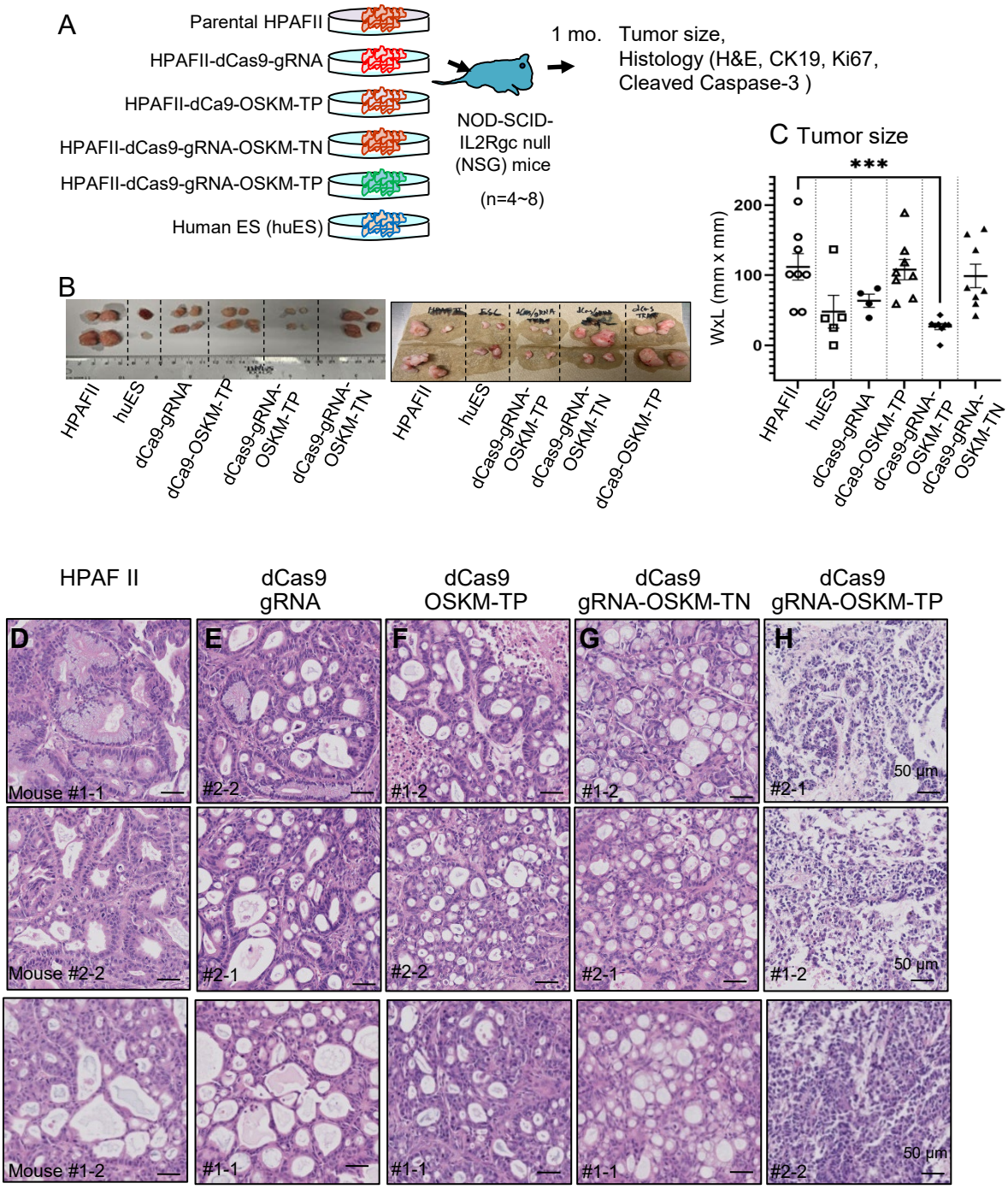

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Figure 5

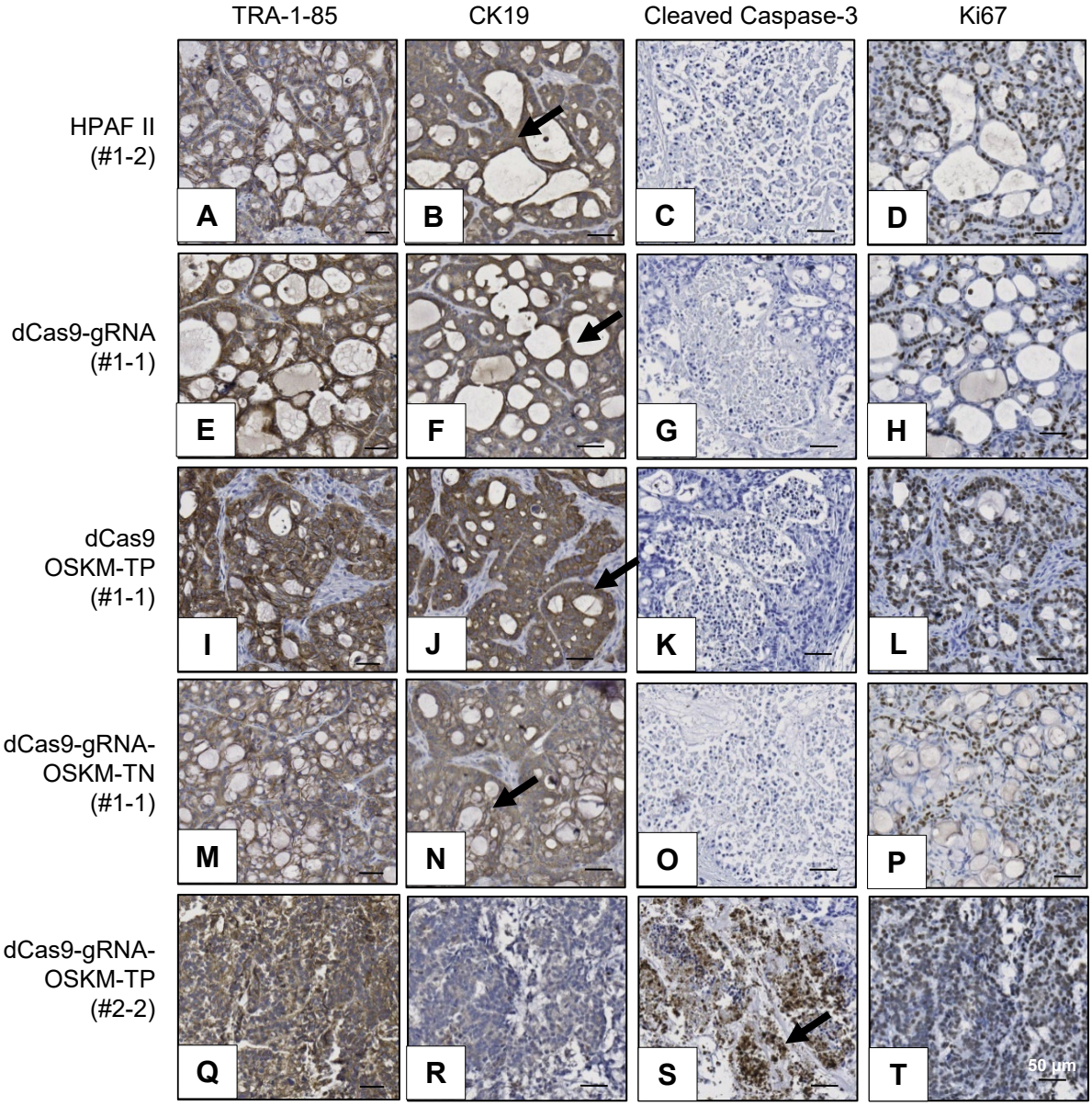

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Figure 6

A

Schematic flow chart of CRISPRi screening

DNA sequencing : HPAFII-dCas9-gRNA controls, TRA-1-60⁻ and TRA-1-60⁺ from OSKM-reprogrammed HPAFII-dCas9-gRNAs (x500)

↓

gRNA Read Mapping
Align trimmed reads using BowTie2→
Filter out ambiguous hits

↓

Normalization & Analysis
Z-score & MAGeCK-MLE
(negative sgRNA normalization)

↓

Differential Abundance
Identify enriched lncRNAs
(Negative Binomial distribution,
p<0.05, permutation test)

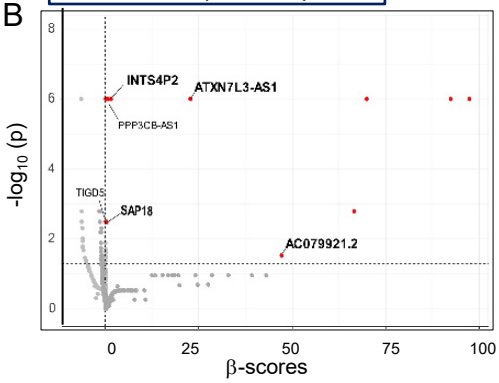
↓

Select Reproducible Candidates
Enriched in TRA-1-60⁺:
Z-score> 2 or β-score>0, p<0.05, n=2)

↓ ← Validation of qRT-PCR

Final candidates to test the functional test: ***ATXN7L3-AS1, AC079921.2 INTS4P2 (LINC03006)***

B



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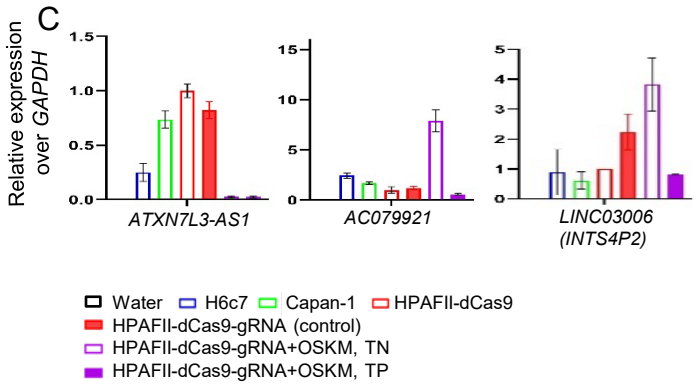

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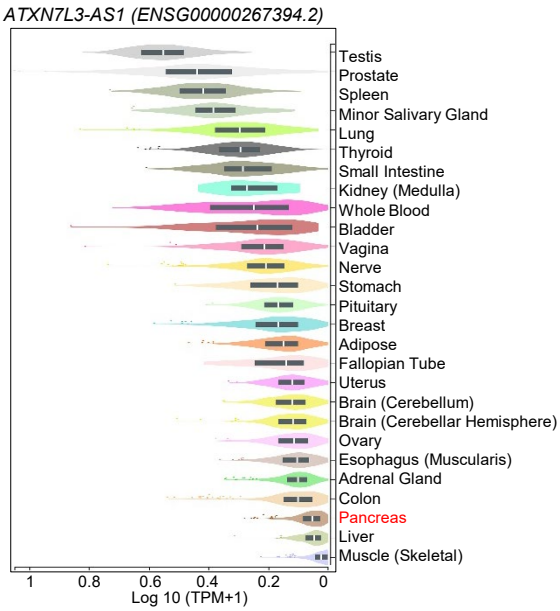

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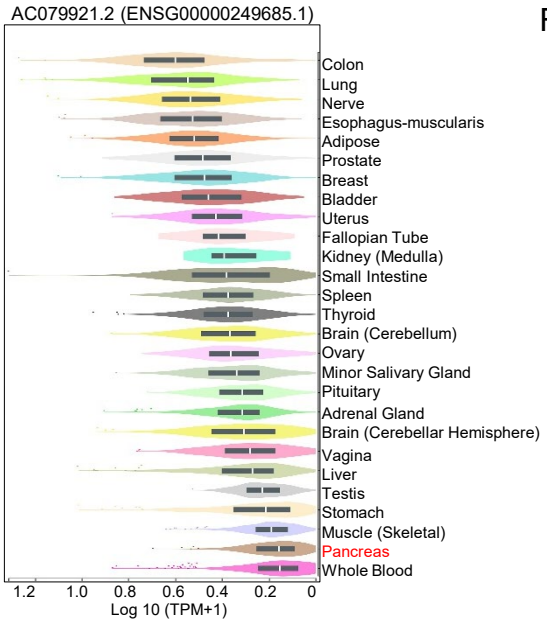

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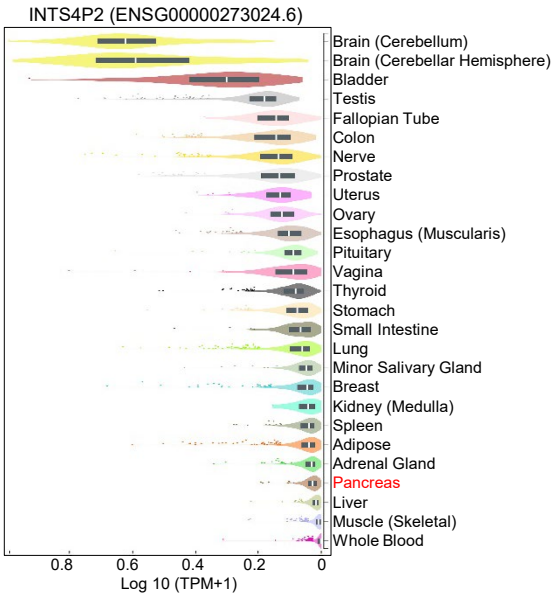

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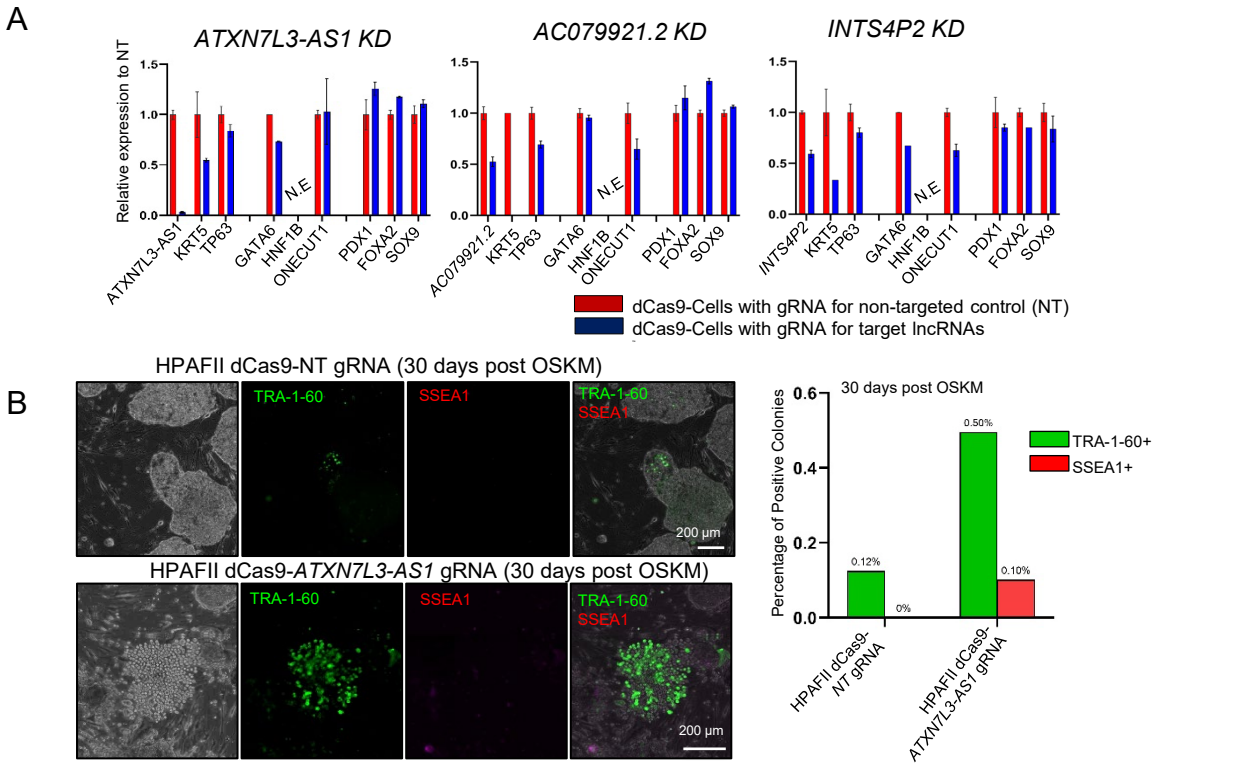
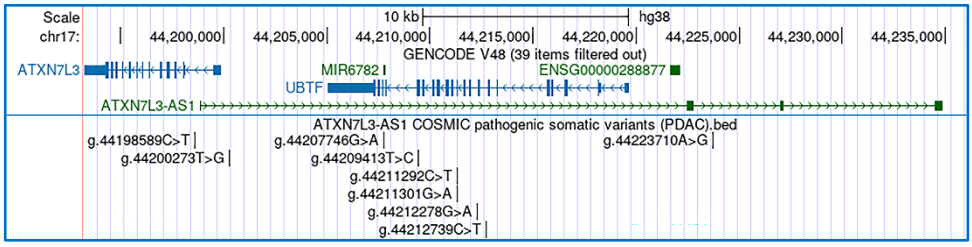
Figure 7





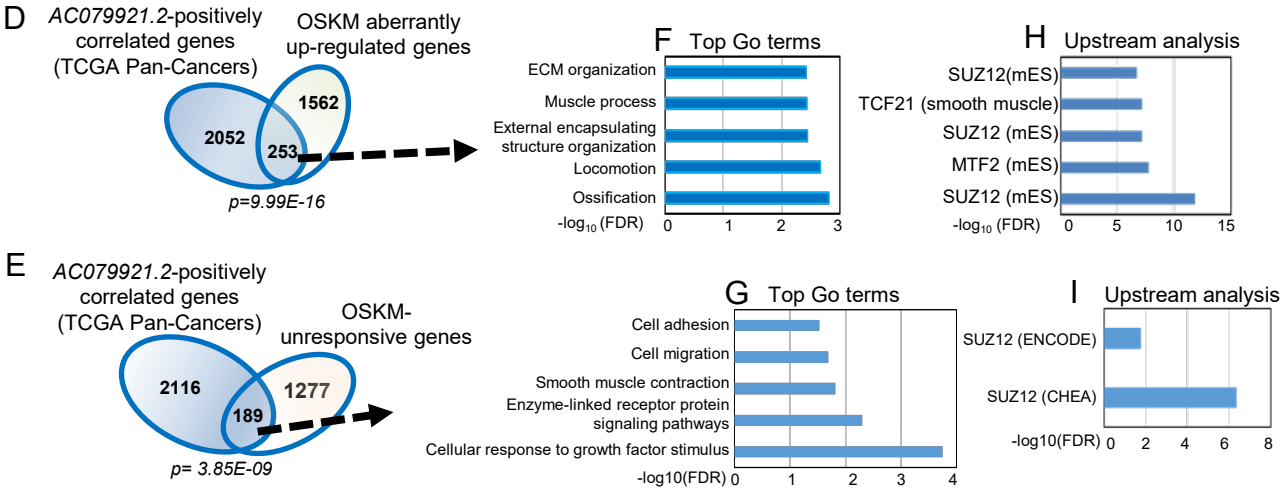


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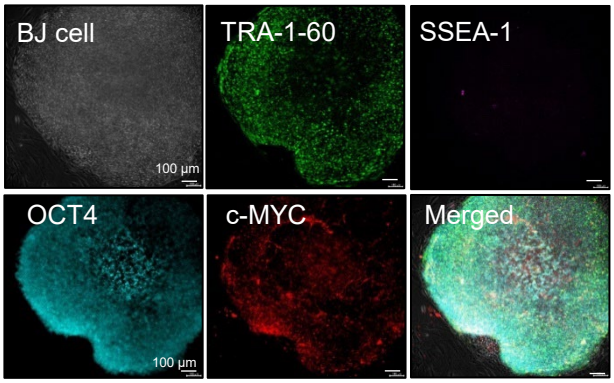
Figure S1

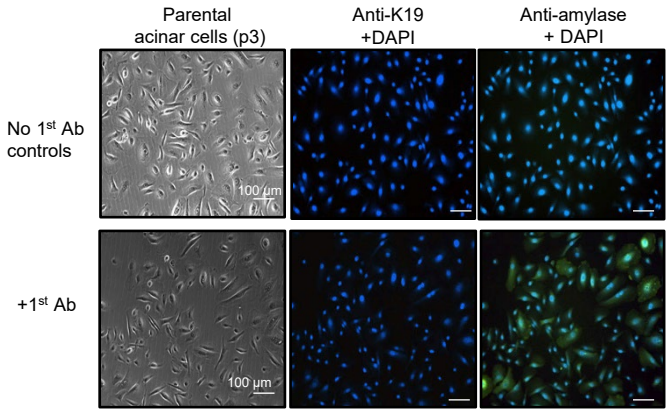
A

Immunostaining of pluripotency markers in BJ6 established iPSC



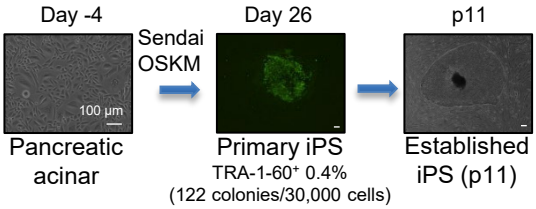
B

Immunostaining of acinar and ductal markers in patient-derived acinar cells



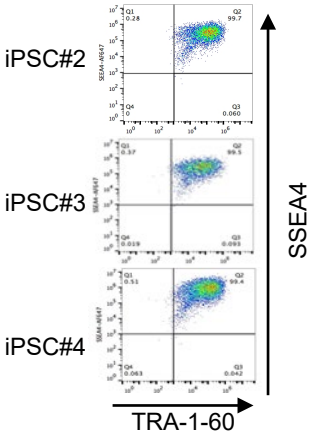
C

Reprogramming of pancreatic acinar cells



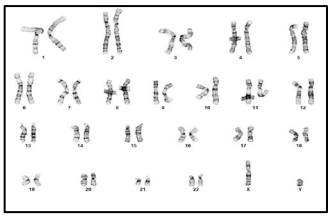
D

Pluripotency



E

iPSC#2



46, XY

F

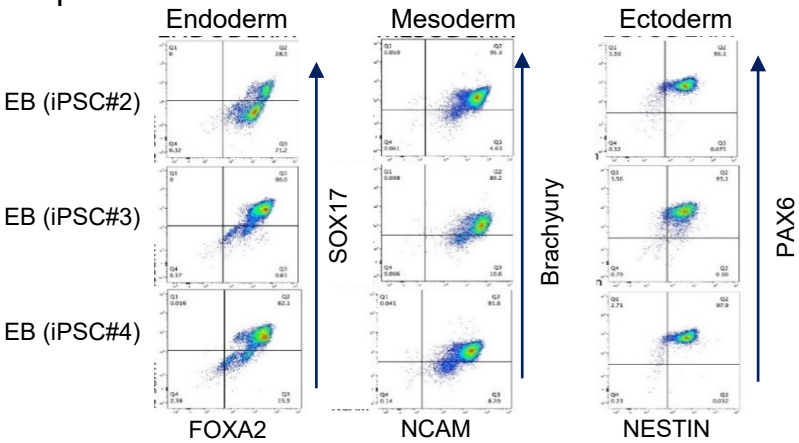


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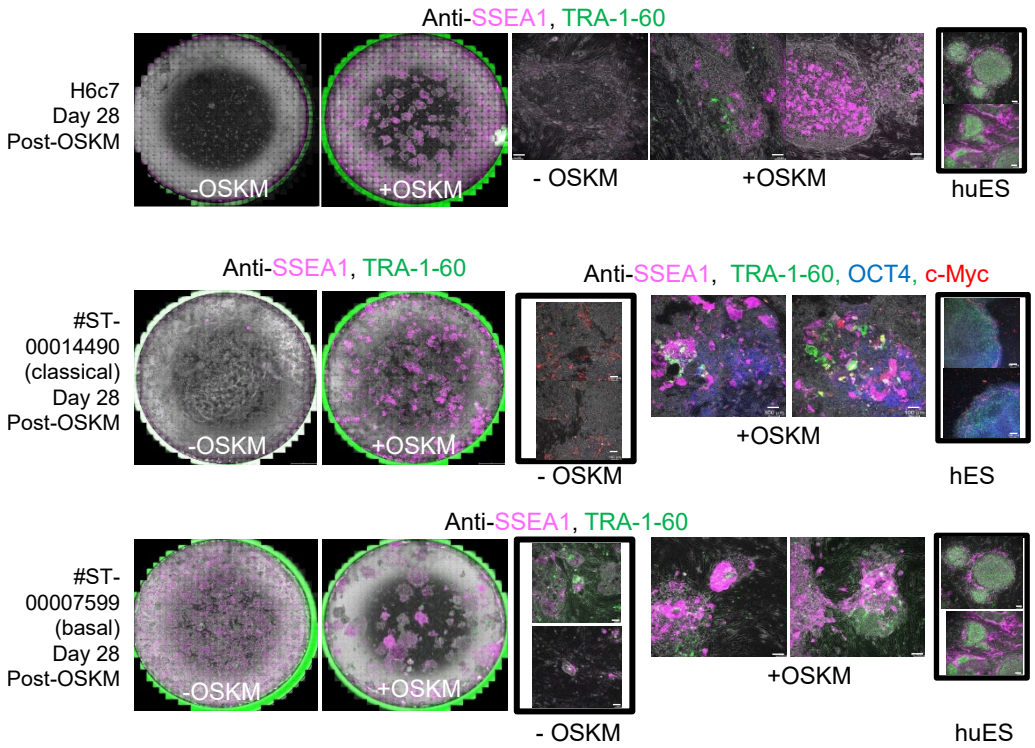
Figure S2

A. Immunostaining of pluripotency and early-differentiation markers



B. Expression of OSKM, pluripotency, and PDAC genes on day 7 after OSKM induction

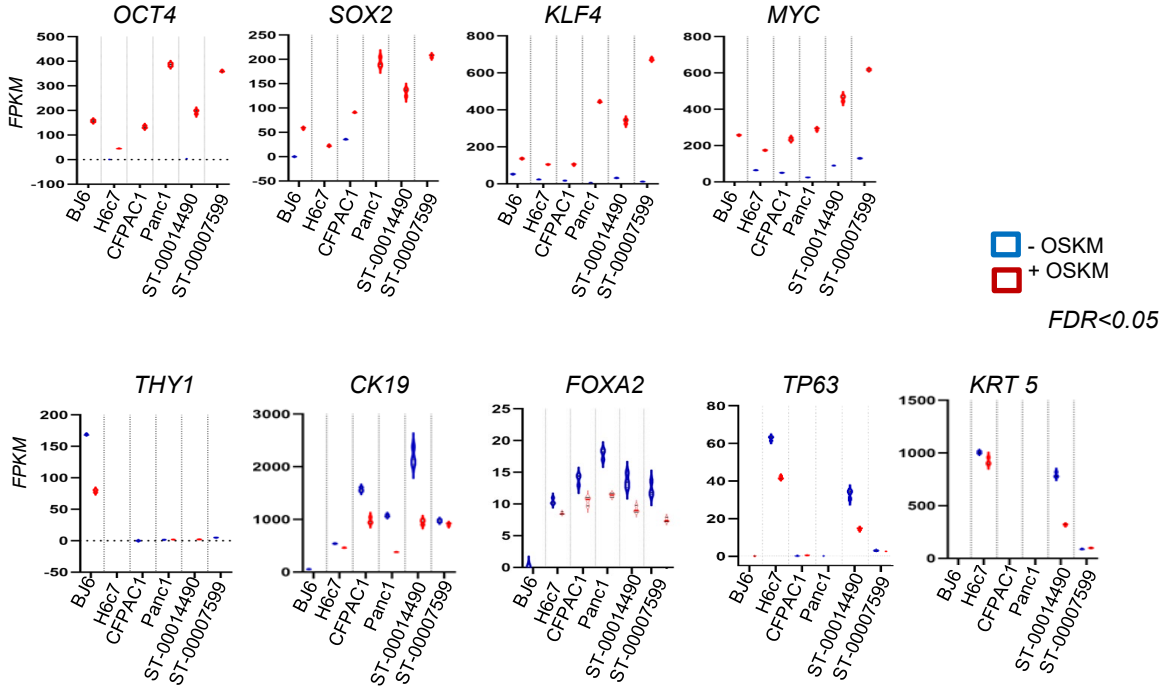


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Figure S3

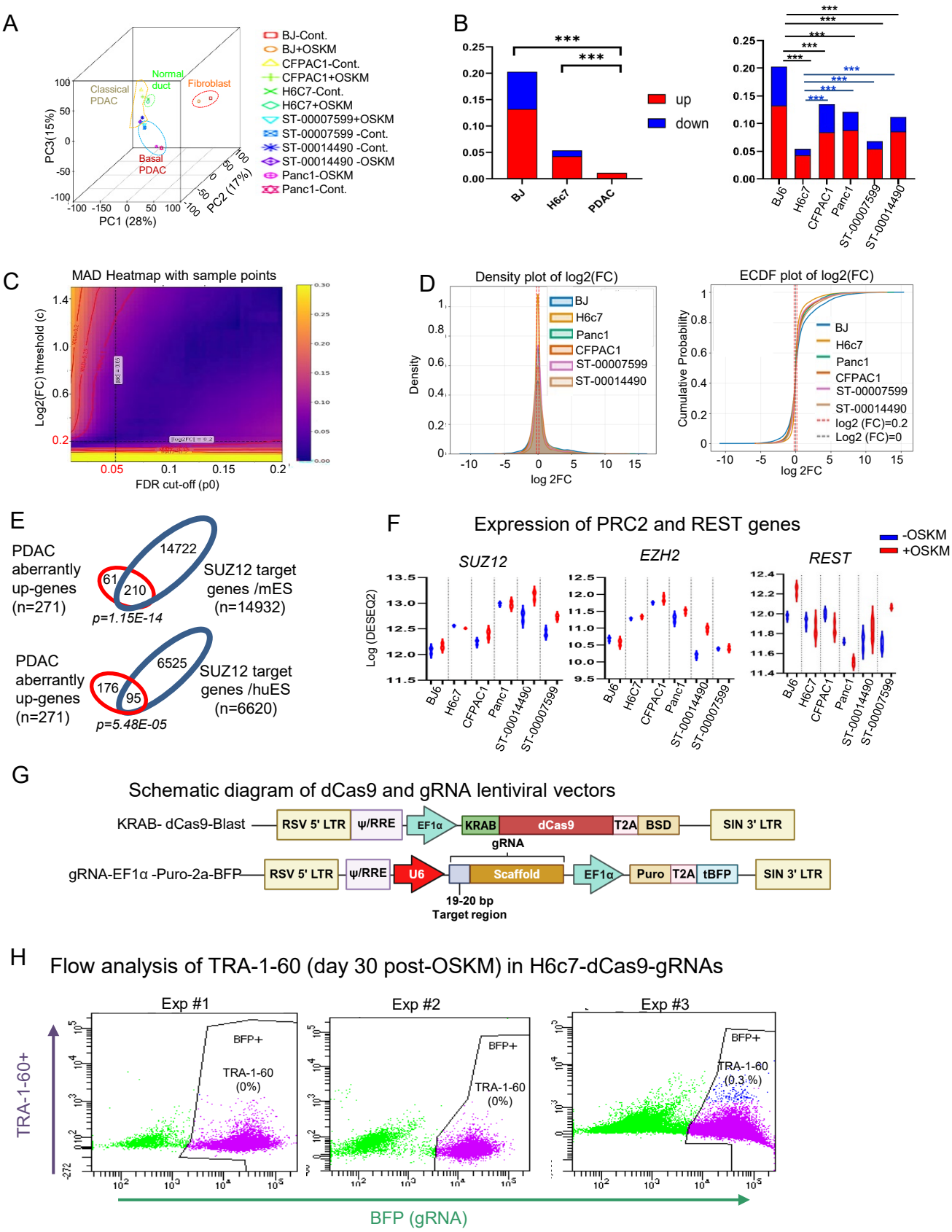

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Figure S4

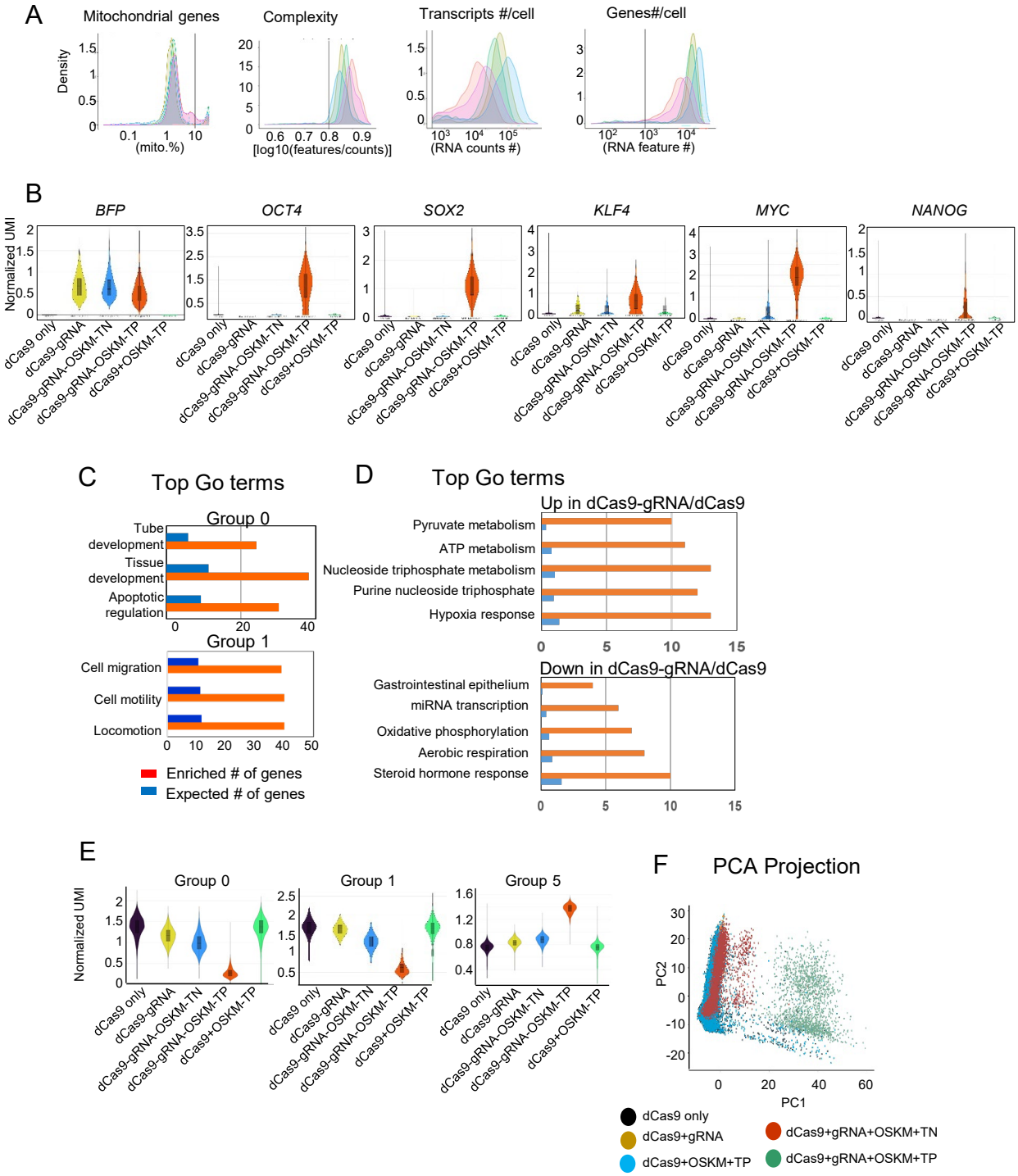

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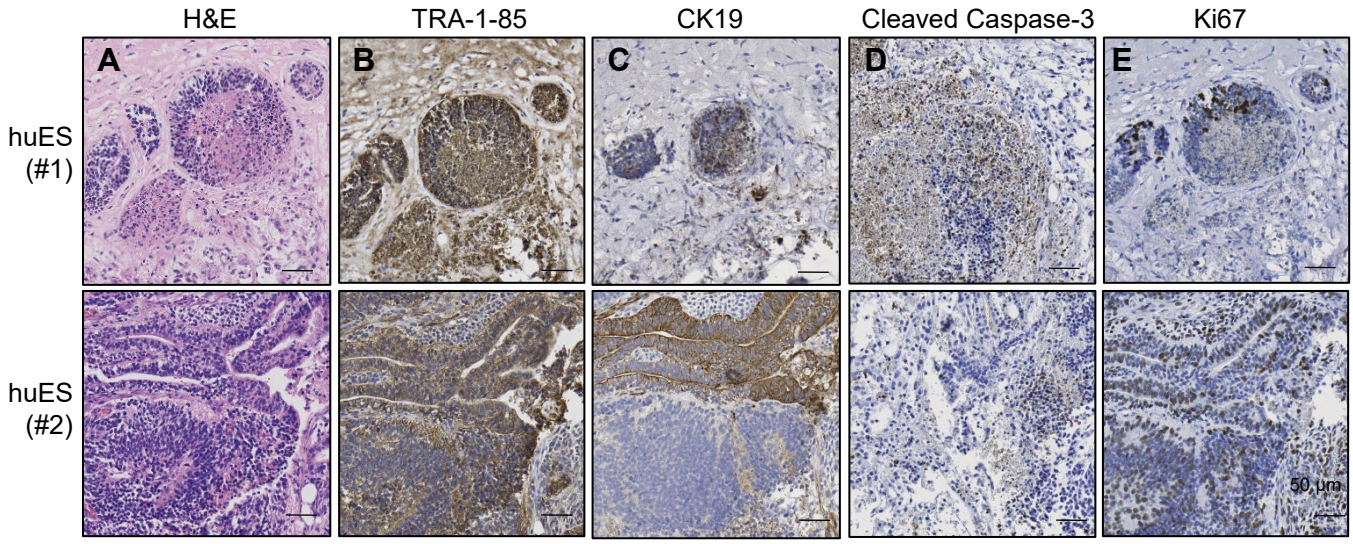
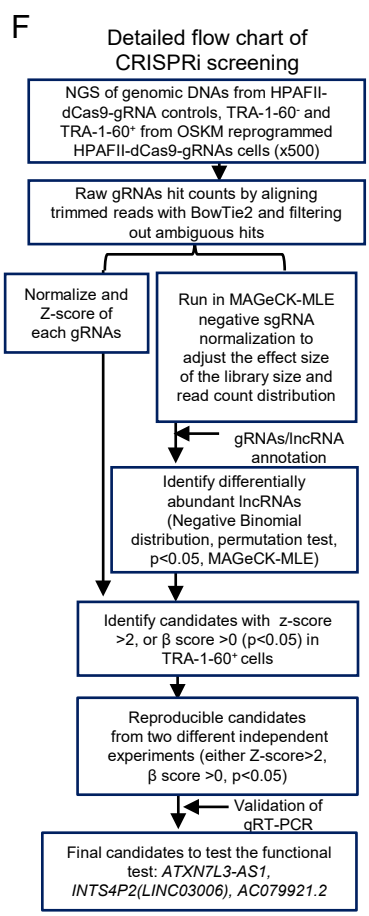
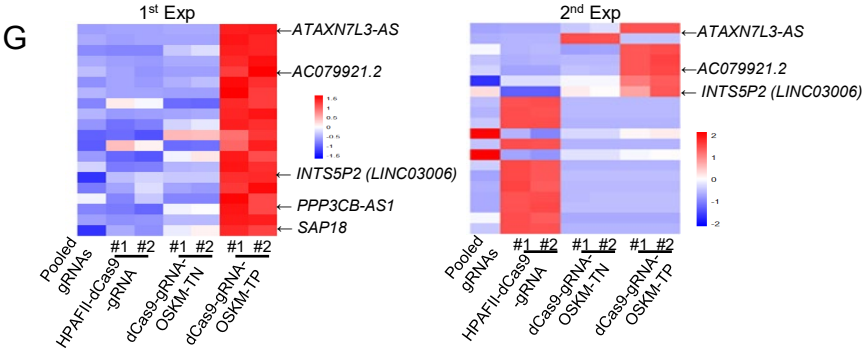
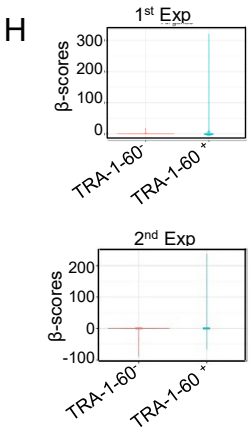
Figure S5









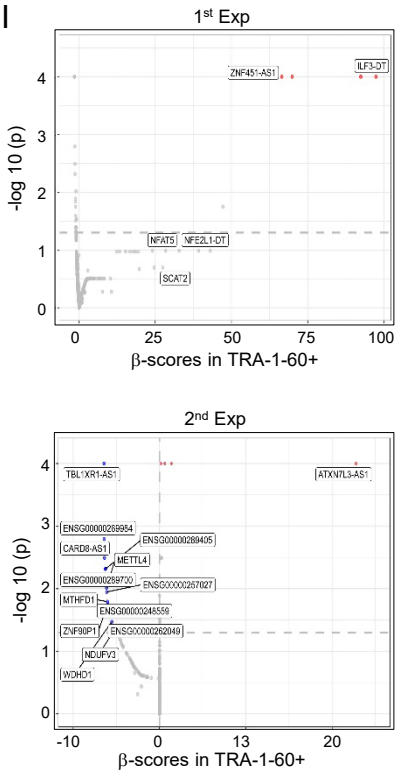
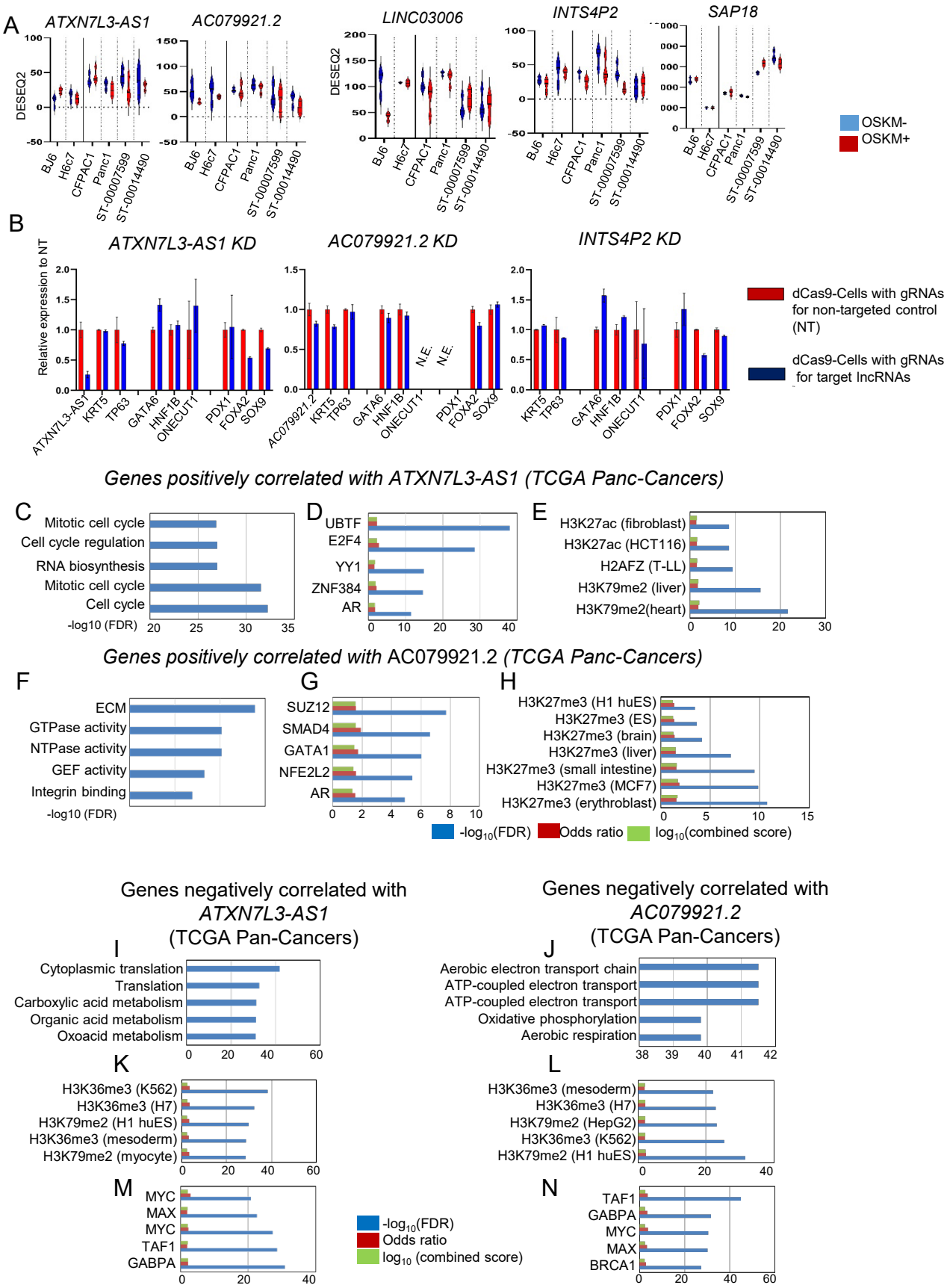


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Figure S6