

**Title: ARID1A Maintains Differentiation of Pancreatic Ductal
Cells and Inhibits Development of Pancreatic Ductal
Adenocarcinoma in Mice**

Short title: *Arid1a* suppresses IPMN and PDAC formation

Authors: Yoshito Kimura¹, Akihisa Fukuda¹, Satoshi Ogawa¹, Takahisa Maruno¹,
Yutaka Takada¹, Motoyuki Tsuda¹, Yukiko Hiramatsu¹, Osamu Araki¹,
Munemasa Nagao¹, Takaaki Yoshikawa¹, Kozo Ikuta¹, Takuto Yoshioka¹, Zong
Wang², Haruhiko Akiyama³, Christopher V. Wright⁴, Kyoichi Takaori⁵, Shinji
Uemoto⁵, Tsutomu Chiba¹, Hiroshi Seno¹

¹Department of Gastroenterology and Hepatology, Kyoto University Graduate
School of Medicine, 54 Shogoin-Kawahara-cho, Sakyo-ku, Kyoto 606-8507
Japan.

²Department of Cardiac Surgery, Cardiovascular Research Center, University of
Michigan, 2800 Plymouth Road, An Arbor, MI 48109, USA.

³Department of Orthopaedics, Gifu University, 1-1 Yanagido, Gifu City 501-1194,

17 Japan.

18 ⁴Program in Developmental Biology and Department of Cell and Developmental
19 Biology, Vanderbilt University School of Medicine, Nashville, Tennessee 37240,
20 USA.

21 ⁵Division of Hepatobiliary-Pancreatic Surgery and Transplantation, Department
22 of Surgery, Kyoto University Graduate School of Medicine, 54
23 Shogoin-Kawahara-cho, Sakyo-ku, Kyoto 606-8507 Japan.

24 **Grant support:** This work was supported in part by Grants-in-Aid KAKENHI
25 (25112707, 26293173, 14J03460, 16K09394, 16K15427, and 17J05511); a
26 research program as part of the Project for Development of Innovative Research
27 on Cancer Therapeutics (P-Direct) from the Ministry of Education, Culture,
28 Sports, Science, and Technology and the Japan Society for the Promotion of
29 Science. It was also supported by the Naito Foundation (2013-007); Princess
30 Takamatsu Cancer Research Fund (13-24514); the Mochida Foundation
31 (2017bvAg); the Mitsubishi Foundation (281119); the Takeda Foundation
32 (201749741). It was also supported by an NIH grant (R01HL109054) to Zhong

33 Wang.

34 **Abbreviations:** AT rich interactive domain 1A (ARID1A), Brahma related gene 1
35 (BRG1), pancreatic intraepithelial neoplasia (PanIN), intraductal papillary
36 mucinous neoplasm (IPMN), pancreatic ductal adenocarcinoma (PDAC),
37 pancreatic ductal cells (PDCs)

38 **Correspondence:** Akihisa Fukuda

39 Department of Gastroenterology and Hepatology, Kyoto University Graduate
40 School of Medicine, 54 Shogoin-Kawahara-cho, Sakyo-ku, Kyoto 606-8507
41 Japan.

42 E-mail: fukuda26@kuhp.kyoto-u.ac.jp

43 Phone: +81-75-751-4319

44 Fax: +81-75-753-4303

45 **Disclosures:** The authors disclose no conflict of interest.

46 **Transcript Profiling:** GSE96926

47 **Author contributions:** Y.K. and A.F. conceived and designed the study. O.S.,
48 T.M., Y.T., M.T., K.I., T.Y., Y.H., O.A., M.N., and T.Y. performed the experiments

49 and analyzed the data. H.A. generated the *Sox9OE* mice. C.V.W. generated the
50 *Ptf1a-Cre^{ER}* mice. Y.K., T.M., M.T., K.T., and S.U. contributed
51 reagents/materials/analysis tools. Y.K. wrote the manuscript, and A.F., T.C., and
52 H.S. revised it.

53

54 **Abstract**

55 **Background & Aims:** The AT-rich interaction domain 1A gene (*ARID1A*)
56 encodes a protein that is part of the large ATP-dependent chromatin remodeling
57 complex SWI/SNF and is frequently mutated in human pancreatic ductal
58 adenocarcinomas (PDACs). We investigated the functions of ARID1A during
59 formation of PDACs in mice.

60

61 **Methods:** We performed studies with *Ptf1a*-Cre; *Kras*^{G12D} mice, which express
62 activated Kras in the pancreas and develop pancreatic intraepithelial neoplasias
63 (PanINs), as well as those with disruption of *Arid1a* (*Ptf1a*-Cre; *Kras*^{G12D};
64 *Arid1a*^{ff} mice) or disruption of *Brg1* (encodes a catalytic ATPase of the SWI/SNF
65 complex) (*Ptf1a*-Cre; *Kras*^{G12D}; *Brg1*^{ff} mice). Pancreatic ductal cells (PDCs)
66 were isolated from *Arid1a*^{ff} mice and from *Arid1a*^{ff}; SOX9OE mice, which
67 overexpress human SOX9 upon infection with an adenovirus expressing Cre
68 recombinase. Pancreatic tissues were collected from all mice and analyzed by
69 histology and immunohistochemistry; cells were isolated and grown in

2-dimensional and 3-dimensional cultures. We performed microarray analyses to compare gene expression patterns in intraductal papillary mucinous neoplasms (IPMNs) from the different strains of mice. We obtained 58 samples of IPMN and 44 samples of PDAC from patients who underwent pancreatectomy in Japan, and analyzed them by immunohistochemistry.

Results: *Ptf1a*-Cre; *Kras*^{G12D} mice developed PanINs, whereas *Ptf1a*-Cre; *Kras*^{G12D}; *Arid1a*^{fl/fl} mice developed IPMNs and PDACs; IPMNs originated from PDCs. ARID1A-deficient IPMNs did not express SOX9. ARID1A-deficient PDCs had reduced expression of SOX9 and dedifferentiated in culture. Overexpression of SOX9 in these cells allowed them to differentiate and prevented dilation of ducts. Among mice with pancreatic expression of activated *Kras*, those with disruption of *Arid1a* developed fewer PDACs from IPMNs than mice with disruption of *Brg1*. ARID1A-deficient IPMNs had reduced activity of the mTOR pathway. Human IPMN and PDAC specimens had reduced levels of ARID1A, SOX9, and phosphorylated S6 (a marker of mTOR pathway activation).

86 Levels of ARID1A correlated with levels of SOX9 and phosphorylated S6.

87

88 **Conclusions:** ARID1A regulates expression of SOX9, activation of the mTOR
89 pathway, and differentiation of PDCs. ARID1A inhibits formation of PDACs from
90 IPMNs in mice with pancreatic expression of activated KRAS, and is
91 downregulated in IPMN and PDAC tissues from patients.

92

93 **KEY WORDS:** pancreatic cancer, tumorigenesis, oncogene, transcriptional
94 **regulator**

95 351 words

96

97 **Introduction**

98 Pancreatic ductal adenocarcinoma (PDAC) is a lethal disease with an
99 extremely poor prognosis¹. Therefore, earlier diagnosis and more effective
100 therapies are required to improve outcomes, which require a better
101 understanding of the molecular mechanism of PDAC development.

102 Recent genome-wide sequencing studies have revealed that mutation of
103 the subunit genes of the switch/sucrose non-fermentable (SWI/SNF) chromatin
104 remodeling complexes are widespread across diverse human cancers^{2, 3}. In fact,
105 mutations, translocations, and deletions involving various subunits of the
106 SWI/SNF complexes have been found in approximately 20% of human cancers,
107 thus representing one of the most common molecular mechanisms in human
108 cancers^{2, 3}. Indeed, mutations of the subunit genes of SWI/SNF complexes have
109 been found in 12–23% of human PDAC cases²⁻⁴. The SWI/SNF complexes
110 remodel the chromatin structure using energy from ATP hydrolysis and thereby
111 modulate gene expression⁵. Using a mouse model, we recently demonstrated
112 that the pancreatic loss of *Brg1*, which encodes a catalytic ATPase of the
113 SWI/SNF complex, cooperates with oncogenic *Kras* to form intraductal papillary

114 mucinous neoplasm (IPMN), which subsequently developed into invasive
115 PDAC⁶. Furthermore, another study showed that *Brg1* promotes tumorigenesis
116 in full-blown PDAC by supporting a mesenchymal-like transcriptional landscape⁷.
117 Therefore, *Brg1* has both tumor-suppressive and oncogenic activities at distinct
118 stages of PDAC formation.

119 AT-rich interactive domain 1 (ARID1A) is the most frequently mutated
120 subunit in the SWI/SNF complexes in human PDAC^{2-4, 8}. ARID1A is assumed to
121 contribute to the specific recruitment of chromatin remodeling activity of the
122 SWI/SNF complex by binding to transcription factors⁹⁻¹². Furthermore, the
123 ARID1A–DNA interaction is considered to be essential for promoter occupancy
124 by the SWI/SNF complexes¹³. In mouse models, conditional knockout of *Arid1a*
125 promotes ovarian tumorigenesis by activating the phosphatidylinositol
126 3-kinase(PI3K)/AKT/mammalian target of rapamycin (mTOR) pathway^{14, 15}.
127 Moreover, intestinal *Arid1a* deletion *per se* results in colon cancer formation in
128 mice¹⁶. However, in spite of the frequent mutations of *ARID1A* in human PDAC,
129 the *in vivo* role of *ARID1A* in pancreatic tumorigenesis remains elusive.

130 Therefore, in this study, we aimed to investigate the impact of *Arid1a*
131 inactivation in the pancreas and clarify the functional role of *Arid1a* in the
132 specification of PDAC precursors and PDAC formation in mice.
133

134 **Material and Methods**

135 Detailed methods are described in the Supplemental Materials and Methods.

136 ***Mouse lines***

137 Experimental animals were generated by crossing *Ptf1a-Cre* (a gift from Y.
138 Kawaguchi, Kyoto University, Kyoto, Japan)¹⁷, *Ptf1a-Cre*^{ERT2}¹⁸, *Hnf1b-Cre*^{ERT2} (a
139 gift from Jorge Ferrer, Imperial College, London, UK)¹⁹, *Kras*^{G12D} (a gift from D.
140 Tuveson, Cold Spring Harbor Laboratory)²⁰, *Brg1*^{f/+} (a gift from D. Reisman,
141 University of Florida, with permission from P. Chambon)²¹, *Arid1a*^{f/+}²², *Trp53*^{R172H}
142 ²³, and *Sox9OE* mice²⁴. The mice were crossed in a mixed genetic background.

143 All experiments were approved by the animal research committee of Kyoto
144 University and performed in accordance with Japanese government regulations.

145

146 ***Pancreatic ductal cell (PDC) isolation and two-dimensional (2D) culture***

147 PDCs were isolated following previously described protocols^{25, 26}. The
148 pancreas was minced into pieces smaller than 1 mm³. Next, the pancreas was
149 digested with collagenase type V for 20 min and trypsinized for 5 min;

trypsinization was stopped using a soy trypsin inhibitor. The cells were filtered using a 40- μ m cell strainer. For 2D culture, the single-cell suspension was plated onto collagen-covered plates. The PDC full medium was replaced every two days until the cells achieved 80% confluence. After three passages, pure ductal cell lines were established.

Three-dimensional (3D) ductal cell culture

The 3D culture of PDCs was performed according to a previously described protocol²⁵. PDCs were mixed with collagen and placed on top of collagen-coated chamber slides. The PDC full medium was replaced every two days. PDC cysts were analyzed at 7 to 11 days after plating.

Human samples

Fifty-eight IPMN samples from 58 patients who underwent pancreatectomy for IPMN or IPMN-derived PDAC at the Kyoto University Hospital from 1991 to 2005, and 44 PDAC samples from 44 patients who underwent pancreatectomy

166 at the Kyoto University Hospital from 2000 to 2005 were included in this study.

167 Details of the cases are summarized in Supplementary Table 1.

168

Results

***Arid1a* inhibits dilation of the pancreatic ducts and maintains ductal structure in adult mice**

First, we examined the pattern of *Arid1a* expression in the murine pancreas, which was detected in the nuclei of all three types of pancreatic epithelial cells (ductal cells, acinar cells, and islet cells) in adult wild-type (WT) mice (Figure 1A). To investigate the functional role of *Arid1a* in pancreatic development and maintenance, we crossed the *Ptf1a-Cre* mice¹⁷ with *Arid1a*^{ff} mice²². *Ptf1a-Cre*; *Arid1a*^{ff} mice were born according to the predicted Mendelian ratio and were indistinguishable from *Ptf1a-Cre*; *Arid1a*^{f/+} and WT mice in appearance and body weight at 8 weeks of age (Figure 1B). Macroscopically, the *Ptf1a-Cre*; *Arid1a*^{ff} pancreata were indistinguishable from those of *Ptf1a-Cre*; *Arid1a*^{f/+} and WT mice at 8 weeks of age. Histologically, ARID1A expression was not detected in the acinar cells or a subset of ductal cells in the *Ptf1a-Cre*; *Arid1a*^{ff} pancreata, as expected according to the Cre activity pattern in *Ptf1a-Cre* mice^{17, 27}. However, at over 9 weeks of age, dilated pancreatic ducts were observed in *Ptf1a-Cre*;

185 *Arid1a*^{ff} mice. At 18 weeks of age, the dilated ducts formed cystic lesions
 186 composed of *Arid1a*-deficient duct-like epithelial cells positive for cytokeratin and
 187 acetylated tubulin, which are markers of PDCs (Figure 1C, 1D). These cells also
 188 expressed PDX1, which is expressed in the pancreatic progenitors and islet β
 189 cells in adulthood²⁸ (Figure 1D). This ectopic expression pattern of PDX1
 190 suggested that *Arid1a* deletion results in the dedifferentiation of pancreatic
 191 ductal cells. In addition, phosphorylated extracellular signal-regulated kinase
 192 (pERK1/2) was detected in these cells (Figure 1D). Thus, these data indicated
 193 that *Arid1a* is dispensable for pancreatic development but is required for
 194 maintenance of PDCs and the structure of pancreatic ducts.

195

196 ***Ptf1a*-Cre; *Kras*^{G12D}; *Arid1a*^{ff} mice develop multilocular fluid-filled cystic**
 197 **neoplasms**

198 To investigate the role of *Arid1a* in pancreatic tumorigenesis, we crossed
 199 *Ptf1a*-Cre; *Arid1a*^{ff} mice with *loxP-stop-loxP Kras*^{G12D} (*Kras*^{G12D}) mice. Similar to
 200 *Ptf1a*-Cre; *Kras*^{G12D} mice²⁰, *Ptf1a*-Cre; *Kras*^{G12D}; *Arid1a*^{f/+} mice developed

201 pancreatic intraepithelial neoplasias (PanIN) throughout the pancreata. In
202 contrast, *Ptf1a-Cre; Kras^{G12D}; Arid1a^{ff}* mice developed multilocular fluid-filled
203 cysts throughout the pancreata by 9 weeks of age (Figure 2A), and the
204 pancreata were enlarged due to the extensive cystic lesions (Figure 2B).
205 Histologically, the cystic lesions were composed of dysplastic cells with
206 abundant supranuclear mucin (Figure 2C). Immunohistochemical analysis
207 revealed that the epithelial cells of the neoplastic cysts observed in *Ptf1a-Cre;*
208 *Kras^{G12D}; Arid1a^{ff}* mice were positive for cytokeratin and acetylated tubulin²⁹ and
209 negative for amylase expression (Figure 2C), indicating that these neoplastic
210 cells showed a duct-like character. In addition, the absence of ARID1A
211 expression and activation of the mitogen-activated protein kinase (MAPK)
212 cascade were observed in these neoplastic cells, as expected (Figure 2D). The
213 proliferation activity was low in the cystic neoplasms based on the low positive
214 rate of Ki67 expression compared to that of the PanIN in *Ptf1a-Cre; Kras^{G12D}*
215 mice (Figure 2D), suggesting that expansion of the cystic neoplasms is not due
216 to aggressive cell growth but is most likely due to abundant intraductal mucin.

Moreover, acinar-to-ductal metaplasia (ADM) and PanIN were also observed in *Ptf1a-Cre; Kras^{G12D}; Arid1a^{flf}* mice (Supplementary Figure 1), suggesting that absence of *Arid1a* expression does not inhibit PanIN formation. Thus, these results indicated that deletion of *Arid1a* with mutant *Kras* results in formation of cystic neoplasms in the pancreas.

Cystic neoplasms in *Ptf1a-Cre; Kras^{G12D}; Arid1a^{flf}* mice resemble human IPMN but not mucinous cystic neoplasms (MCN)

There are two types of pancreatic mucus-producing cystic neoplasms in humans: IPMN and MCN³⁰. IPMN are characterized by connection to the pancreatic ductal system, whereas MCN are characterized by dense ovarian-like stroma^{30, 31}. Given that the cystic neoplasms in *Ptf1a-Cre; Kras^{G12D}; Arid1a^{flf}* mice had abundant mucin, we investigated whether these cystic neoplasms more closely resembled human IPMN or MCN. Histological examination revealed that the cystic neoplasms were connected to the pancreatic ductal system (Figure 2C) and lacked ovarian-like stroma, as indicated by staining for

estrogen receptor and progesterone receptor (Supplementary Figure 2). In addition, most of the cystic neoplasms lacked the MCN-typical dense stroma exhibiting wavy nuclei. These findings indicated that the cystic neoplasms in *Ptf1a-Cre; Kras^{G12D}; Arid1a^{ff}* mice resembled human IPMN but not MCN. Human IPMN can be further classified into four types according to the directions of differentiation of the neoplastic cells and mucin expression patterns: gastric, intestinal, pancreatobiliary, and oncocytic type^{31, 32}. The IPMN-like cells expressed MUC1 and MUC5AC but lacked MUC2 expression in *Ptf1a-Cre; Kras^{G12D}; Arid1a^{ff}* mice, indicating that the mucin expression pattern was similar to that of human pancreatobiliary- or oncocytic-type IPMN (Figure 2E).

Molecular characterization of IPMN-like cystic neoplasms

Because human IPMN represent a form of PDAC precursor lesions, we examined certain markers of the signaling pathways known to play a critical role in pancreatic carcinogenesis. Phospho-STAT3, which is a critical signaling pathway for PDAC formation³³, was upregulated in the IPMN of *Ptf1a-Cre;*

249 *Kras*^{G12D}; *Arid1a*^{ff} mice but was weaker than the phospho-STAT3 expression
250 detected in the PanIN of *Ptf1a-Cre*; *Kras*^{G12D} mice (Supplementary Figure 3A).
251 PDX1 expression was positive in the mouse IPMN (Supplementary Figure 3B).
252 The expression levels of p21, p16 (INK4A), and p53 were reduced or completely
253 absent in the IPMN of *Ptf1a-Cre*; *Kras*^{G12D}; *Arid1a*^{ff} mice, as observed in
254 *Ptf1a-Cre*; *Kras*^{G12D}; *Brg1*^{ff} mice⁶, whereas SMAD4 expression was maintained
255 in the *Arid1a*-deficient IPMN (Supplementary Figure 3C–F).

256

257 **Mouse IPMN gradually develop into PDAC**

258 A previous report indicated that the IPMN in *Ptf1a-Cre*; *Kras*^{G12D}; *Brg1*^{ff}
259 mice frequently progressed to PDAC by 18 weeks of age⁶. Similar to these mice,
260 at 18 weeks of age, the *Ptf1a-Cre*; *Kras*^{G12D}; *Arid1a*^{ff} mice developed IPMN with
261 varying grades of dysplasia (Figure 3A); however, PDAC was not observed (n =
262 13). Even at 24 weeks of age, PDAC did not develop in *Ptf1a-Cre*; *Kras*^{G12D};
263 *Arid1a*^{ff} mice (n = 15). At 48 weeks of age, PDAC developed in 3 of 15 (20%)
264 *Ptf1a-Cre*; *Kras*^{G12D}; *Arid1a*^{ff} mice (Figure 3B, C), one of which was carcinoma

265 *in situ*, and the others were invasive adenocarcinoma (Figure 3C).
266 Immunohistochemical analysis revealed that the PDAC cells in *Ptf1a-Cre*;
267 *Kras^{G12D}; Arid1a^{ff}* mice were negative for ARID1A and positive for pERK1/2
268 expression, as expected. The frequent positive expression of Ki67 indicated
269 vigorous growth of the PDAC cells, and PDX1 expression was detected in the
270 PDAC cells (Figure 3D). Given that the incidence of PDAC formation in
271 *Ptf1a-Cre; Kras^{G12D}* and *Pdx1-Cre; Kras^{G12D}* mice was previously estimated to
272 be less than 10% around 50 weeks of age²⁰ and that no PDAC was observed in
273 our control *Ptf1a-Cre; Kras^{G12D}* (n = 8) and *Ptf1a-Cre; Kras^{G12D}; Arid1a^{f/+}* (n = 16)
274 mice at 44–48 weeks of age (Figure 3B), these data suggested that *Arid1a*
275 inhibits IPMN-derived PDAC formation and functions as a tumor suppressor in
276 the murine pancreas.

277 Although deletion of *Arid1a* promotes IPMN and IPMN-derived PDAC
278 formation, there was no significant difference in survival between *Ptf1a-Cre*;
279 *Kras^{G12D}; Arid1a^{f/+}* and *Ptf1a-Cre; Kras^{G12D}; Arid1a^{ff}* mice (Supplementary
280 Figure 4). This was most likely due to the slow progression of PDAC from

281 *Arid1a*-deficient IPMN.

282 Moreover, we performed immunohistochemical staining of ARID1A in
283 PanIN-derived PDAC sections from *Ptf1a-Cre; Kras^{G12D}; Trp53^{R172H}* (KPC) mice
284 (n = 8), and found positive ARID1 expression in all sections examined (data not
285 shown).

286

287 **Mouse IPMN originate from PDCs but not from pancreatic acinar cells**

288 Although PanIN showed a duct-like characteristic, studies with mouse
289 models have shown that the cellular origin of PanIN is the acinar compartment^{34,}
290 ³⁵. Moreover, the cellular origin of human IPMN has been considered to be the
291 ductal compartment, because human IPMN are found in ductal structures. In
292 some mouse models, the cellular origin of IPMN was reported to be the ductal
293 compartment or duct glands^{6, 36}. To investigate the cellular origin of mouse
294 *Arid1a*-deficient IPMN, we used two tamoxifen-inducible Cre lines, *Ptf1a-Cre^{ERT2}*
295 and *Hnf1b-Cre^{ERT2}*, which enabled Cre recombination in adult pancreatic acinar
296 cells and PDC with tamoxifen treatment, respectively^{19, 35}. First, we crossed

297 *Ptf1a-Cre^{ERT2}* mice with *Kras^{G12D}*; *Arid1a^{ff}* mice (Figure 4A). *Ptf1a-Cre^{ERT2}*;
 298 *Kras^{G12D}*; *Arid1a^{f/+}* and *Ptf1a-Cre^{ERT2}*; *Kras^{G12D}*; *Arid1a^{ff}* mice were treated with
 299 tamoxifen and caerulein and were examined as presented in Figure 4B. Both
 300 mouse lines developed multiple PanIN but no IPMN (Figure 4C). This indicated
 301 that *Arid1a* is not required for PanIN formation from acinar cells and that the
 302 acinar compartment is not the cellular origin of IPMN in this mouse model.

303 We next crossed *Hnf1b-Cre^{ERT2}* mice with *Kras^{G12D}*; *Arid1a^{ff}* mice (Figure
 304 4D). *Hnf1b-Cre^{ERT2}*; *Kras^{G12D}*; *Arid1a^{f/+}* and *Hnf1b-Cre^{ERT2}*; *Kras^{G12D}*; *Arid1a^{ff}*
 305 mice were treated with tamoxifen and examined as presented in Figure 4E.
 306 Although *Hnf1b-Cre^{ERT2}*; *Kras^{G12D}*; *Arid1a^{f/+}* mice showed no signs of duct atypia,
 307 atypical PDCs were observed in all three *Hnf1b-Cre^{ERT2}*; *Kras^{G12D}*; *Arid1a^{ff}* mice.
 308 Furthermore, one of the six *Hnf1b-Cre^{ERT2}*; *Kras^{G12D}*; *Arid1a^{ff}* mice developed
 309 cystic lesions composed of a single layer of columnar cells with supranuclear
 310 mucin. We observed the absence of expression and activation of the MAPK
 311 cascade in the epithelial cells of these cystic lesions (Figure 4F). The mucin
 312 expression pattern of the cystic lesions in *Hnf1b-Cre^{ERT2}*; *Kras^{G12D}*; *Arid1a^{ff}* mice

was similar to that of the IPMN in *Ptf1a-Cre; Kras^{G12D}; Arid1a^{ff}* mice (Figure 4F).

These data indicated that *Arid1a* inhibits the dysplasia of adult PDCs and IPMN transformation from adult PDCs in the context of activated KRAS.

***Arid1a* loss leads to the dedifferentiation of PDCs and pancreatic ductal dilation via suppression of SOX9 expression**

To determine the underlying molecular mechanism by which IPMN are formed from PDCs in the absence of *Arid1a*, we performed immunostaining for SOX9, a key transcriptional factor for the differentiation of PDCs^{37, 38}. Notably, immunohistochemical analysis revealed that SOX9 expression was absent in the dilated ducts of *Ptf1a-Cre; Arid1a^{ff}* mice and in the IPMN of *Ptf1a-Cre; Kras^{G12D}; Arid1a^{ff/+}* and *Hnf1b-Cre^{ERT2}; Kras^{G12D}; Arid1a^{ff}* mice (Figure 5A). Thus, we hypothesized that the neoplastic transformation of PDCs was mediated by the downregulation of SOX9 expression in the absence of *Arid1a*. To test this hypothesis, we isolated and cultured PDCs from *Arid1a^{ff}* mice^{25, 26}, and then induced genetic recombination by infecting the PDCs with an adenovirus

329 expressing both Cre recombinase and GFP (Ad-Cre). We used an adenovirus
330 expressing GFP (Ad-GFP) as a control (Figure 5B). Ad-Cre-infected PDCs
331 showed markedly lower expression levels of *Arid1a*, as expected, and formed
332 cysts of significantly larger size in 3D culture than control Ad-GFP-infected PDCs
333 isolated from the same mouse (Figure 5C, 5E). Furthermore, Ad-Cre-infected
334 PDCs exhibited lower expression levels of mouse *Sox9* (*mo-Sox9*) and higher
335 expression levels of pancreatic progenitor markers, including *Gata4* and *Gata6*,
336 than control PDCs (Figure 5E). These findings indicated that loss of *Arid1a*
337 expression in PDCs results in suppression of *Sox9* expression, dedifferentiation
338 of PDCs, and disturbance of the pancreatic ductal structure *ex vivo*.

339 To investigate whether these effects of *Arid1a* deficiency in PDCs were due
340 to suppression of SOX9 expression, we next isolated and cultured PDCs from
341 *Arid1a^{ff}; Sox9OE* mice in which *Arid1a* was deleted and human *Sox9* (*hu-Sox9*)
342 was simultaneously overexpressed after Cre recombination. We then induced
343 genetic recombination by Cre recombinase, similar to the procedure described
344 above for *Arid1a^{ff}* mice (Figure 5B). Ad-Cre-infected PDCs from *Arid1a^{ff}*;

345 Sox9OE mice showed lower expression levels of *Arid1a* and *mo-Sox9* but
346 extremely higher expression levels of *hu-Sox9* than the control cells, as
347 expected (Figure 5E). Notably, these *Arid1a*-deficient and Sox9-overexpressed
348 PDCs expressed equal levels of pancreatic progenitor markers, including *Gata4*
349 and *Gata6*, and formed cysts of equal size in 3D culture compared to WT control
350 PDCs (Figure 5D, 5E). These data indicated that overexpression of Sox9
351 canceled out the effect of *Arid1a* deletion on the dedifferentiation of PDCs and
352 ductal dilation *ex vivo*.

353 To investigate whether the effects of *Arid1a* deletion in PDCs on pancreatic
354 ductal dilation were mediated by downregulation of SOX9 expression *in vivo*, we
355 next generated *Ptf1a-Cre; Arid1a^{ff}; Sox9OE* mice. Histologically, pancreatic
356 ductal dilation was not observed in *Ptf1a-Cre; Arid1a^{ff}; Sox9OE* mice at 9-20
357 weeks of age (n = 3) (Supplementary Figure 5A, C), indicating that Sox9
358 overexpression offsets the effect of *Arid1a* deletion on ductal dilation *in vivo*.

359 To investigate whether Sox9 overexpression also prevents development of
360 IPMN from *Arid1a*-deficient PDCs *in vivo*, we next generated *Ptf1a-Cre;*

361 *Kras*^{G12D}; *Arid1a*^{ff}; *Sox9OE* mice. At 4-8 weeks of age, IPMN was formed in
362 *Ptf1a-Cre*; *Kras*^{G12D}; *Arid1a*^{ff}; *Sox9OE* mice (n = 4) similarly to *Ptf1a-Cre*;
363 *Kras*^{G12D}; *Arid1a*^{ff} mice (Supplementary Figure 5B, C), indicating that only Sox9
364 overexpression does not cancel out the effect of combination of *Arid1a* deletion
365 and oncogenic *Kras* in terms of IPMN development. Therefore, we concluded
366 that *Arid1a* prevents the dedifferentiation of PDCs and maintains the pancreatic
367 ductal structure through regulation of *Sox9* expression *in vivo*.

368 Furthermore, we performed a global microarray analysis of *Arid1a*-deficient
369 PDCs to investigate the specific pathways affected by *Arid1a* deletion in PDCs,
370 leading to IPMN development. The pathway analysis revealed that
371 Ras-associated pathways (the Ras signaling pathway and PI3K-AKT signaling
372 pathway) were most strongly affected by *Arid1a* deletion in PDCs. Moreover, GO
373 enrichment analysis revealed that genes encoding membrane proteins were
374 suppressed by *Arid1a* loss (Supplementary Figure 6). Therefore, these
375 pathways might also play roles in the development of IPMN from
376 *Arid1a*-deficient PDCs.

377

378 **The mTOR pathway is activated during the progression of IPMN to PDAC**

379 Although IPMN was generally formed in *Ptf1a-Cre; Kras^{G12D}; Arid1a^{ff}* mice,
380 similar to *Ptf1a-Cre; Kras^{G12D}; Brg1^{ff}* mice at the same age, the incidence of
381 PDAC formation was much lower in the *Ptf1a-Cre; Kras^{G12D}; Arid1a^{ff}* mice
382 compared with that in *Ptf1a-Cre; Kras^{G12D}; Brg1^{ff}* mice⁶. BRG1 expression was
383 observed in the IPMN of *Ptf1a-Cre; Kras^{G12D}; Arid1a^{ff}* mice, whereas ARID1A
384 expression was retained in the IPMN of *Ptf1a-Cre; Kras^{G12D}; Brg1^{ff}* mice (Figure
385 6A), indicating that BRG1 and ARID1A expression are independently regulated
386 in the absence of *Arid1a* and *Brg1*, respectively.

387 To better understand the mechanism driving the progression of IPMN to
388 PDAC in the absence of *Arid1a*, we next compared the gene expression
389 patterns between *Ptf1a-Cre; Kras^{G12D}; Arid1a^{ff}* and *Ptf1a-Cre; Kras^{G12D}; Brg1^{ff}*
390 mice. We performed microarray analysis of mRNA obtained from the IPMN in
391 mice of these two genotypes by laser micro-dissection. GSEA^{39, 40} revealed that
392 seven gene sets associated with Notch, the PRC2 complex, mTOR pathway,

393 KRAS, and embryonic stem cells were differentially expressed in
394 *Arid1a*-deficient IPMN compared to *Brg1*-deficient IPMN (Figure 6B,
395 Supplementary Figure 7). Although mutations in the mTOR pathway have not
396 been commonly observed in human PDAC^{41, 42}, this pathway, which can be
397 activated by Kras, is important for growth, metastasis, and chemotherapy
398 tolerance during PDAC⁴³⁻⁴⁶. To validate the correlative relationship between
399 *Arid1a* expression and the mTOR pathway in the IPMN of these mice, we next
400 performed immunohistochemistry for pS6 at serine 235/236. Consistent with the
401 microarray data, pS6 was barely expressed in the *Arid1a*-deficient IPMN,
402 whereas pS6 was strongly expressed in the *Brg1*-deficient IPMN (Figure 6C).
403 Notably, pS6 was expressed in IPMN-derived *Arid1a*-deficient PDAC (Figure
404 6C), indicating that the mTOR signaling pathway is activated during the
405 progression of IPMN to PDAC in *Ptf1a-Cre; Kras^{G12D}; Arid1a^{fl/fl}* mice. These
406 results suggested that the mTOR signaling pathway appears to be a key
407 signaling pathway for the progression of IPMN to PDAC in the absence of *Arid1a*
408 *in vivo*. Moreover, the lower frequency of PDAC formation could be attributed to

the lower activity of the mTOR signaling pathway in *Arid1a*-deficient IPMN compared to *Brg1*-deficient IPMN.

Next, we investigated whether *Arid1a* deletion could alter the mTOR pathway activity in PDCs *ex vivo*. PDCs isolated from *Arid1a^{ff}* mice were transfected with Ad-GFP and Ad-Cre as shown in Figure 5B. qPCR analysis revealed that expression levels of *Pik3ca*, *Akt3*, and *mTor* were downregulated in *Arid1a*-deficient PDCs compared with the control, indicating that *Arid1a* regulates activity of the mTOR pathway by supporting the expression of components of this pathway in PDCs (Figure 6D).

Absence of ARID1A expression in subsets of human IPMN and PDAC

Mutations of *ARID1A* are frequently observed in human PDAC^{2-4, 8}. Therefore, we next examined ARID1A expression in human IPMN and PDAC samples. Immunohistochemical analysis revealed that ARID1A expression was absent in 13 out of 58 surgically resected human IPMN samples and in 16 out of 44 surgically resected human PDAC samples (Figure 7A, Supplementary Table

1). As presented in Supplementary Table 1, patients with ARID1A-negative IPMN were indistinguishable from those with ARID1A-positive IPMN with respect to sex, age, clinical subtype, and incidence of PDAC. The frequency of oncocytic-type IPMN was significantly higher and that of gastric-type IPMN was significantly lower in cases of ARID1A-negative IPMN than in cases of ARID1A-positive IPMN.

Given that *Arid1a* deletion resulted in the suppression of Sox9 expression in our mouse model, we further examined the correlation between ARID1A and SOX9 expression in human IPMN (Figure 7A, Supplementary Table 1). Thirty-eight percent of the ARID1A-negative human IPMN samples (5/13) were positive for SOX9, whereas 84.4% of the ARID1A-positive IPMN samples (38/45) were positive for SOX9. Similarly, 56.3% of the ARID1A-negative PDAC samples (9/16) were positive for SOX9, whereas 96.4% of the ARID1A-positive PDAC samples (27/28) were positive for SOX9 (Supplementary Table 1). Thus, absence of ARID1A expression was observed in subsets of human IPMN and PDAC, and a significant positive correlation was observed between the

expression of ARID1A and SOX9 in human IPMN and PDAC.

To examine whether activity of the mTOR pathway was associated with ARID1A expression in human IPMN and PDAC, we next performed immunohistochemistry for pS6. We found a significant positive correlation between the expression of ARID1A and pS6 in human IPMN: pS6 was observed in 30% of the ARID1A-negative human IPMN samples (4/13), whereas it was expressed in 66.7% of the ARID1A-positive human IPMN samples (30/45) (Figure 7A, Supplementary Table 1). Similarly, 37.5% of the ARID1A-negative PDAC samples (6/16) were positive for pS6, whereas 60.7% of the ARID1A-positive PDAC samples (17/28) were positive for pS6 (Supplementary Table 1). Thus, activation of the mTOR pathway appears to be reduced in cases of ARID1A-negative human IPMN and PDAC.

Furthermore, consistent with the mouse data, publicly available mRNA expression data of human PDAC from The Cancer Genome Atlas (TCGA) revealed that the level of *ARID1A* expression is closely correlated with that of the components of the mTOR pathway, including *mTOR*, *PIK3CA*, *AKT3*, and

457 *RICTOR* (Spearman rank correlation coefficient = 0.63, 0.556, 0.463, 0.501,
458 respectively; Figure 7B, C). These data further support the conclusion that
459 ARID1A regulates mTOR pathway activity by supporting the expression of
460 components of the mTOR pathway in human PDAC.
461

462 Discussion

463 Recent genome-wide sequencing studies have revealed that human PDAC
464 mutations are classified into ten core signaling pathways⁴⁷. The SWI/SNF
465 chromatin remodeling complex is one of these pathways, and 12–23% of all
466 human PDACs show inactivated mutations in the SWI/SNF complexes,^{2-4, 47}
467 including *ARID1A* as the most frequently mutated gene^{2-4, 8}. However, the
468 mechanism by which *ARID1A* inactivation contributes to pancreatic
469 tumorigenesis *in vivo* remains unknown. In the present study, we revealed five
470 novel findings about the role of *Arid1a* in pancreatic tumorigenesis in mice.

471 First, we showed that pancreatic *Arid1a* deletion collaborates with mutant
472 *Kras* to form IPMN and IPMN-derived PDAC, demonstrating that *Arid1a* acts as
473 a tumor suppressor in the murine pancreas in the presence of mutant *Kras*.
474 Similarly, we previously showed that the pancreatic loss of *Brg1*, encoding the
475 ATPase subunit of the SWI/SNF complex, results in formation of IPMN and
476 IPMN-derived PDAC in the presence of mutant *Kras*⁶. *Arid1a*-deficient IPMN had
477 similar histological features with *Brg1*-deficient IPMN. These findings suggested

478 that *Arid1a* and *Brg1* cooperate to suppress IPMN formation in the murine
479 pancreas. Mechanistically, our findings, together with those of previous studies,
480 suggest that *Arid1a* and *Brg1* inhibit the dedifferentiation of PDCs and thus
481 IPMN formation through regulation of genes that sustain pancreatic duct cell
482 identity, including *Sox9*⁷. In contrast, *Brg1* was shown to promote tumorigenesis
483 in full-blown PDAC by supporting a mesenchymal-like transcriptional landscape⁷.
484 Therefore, similar to transforming growth factor-beta and Wnt signaling^{48, 49},
485 BRG1 plays both tumor-suppressive and promotive functions at distinct stages
486 of PDAC in a context-dependent manner. In line with the context-dependent
487 roles of BRG1 in PDAC, loss of *Brg1* shows opposing effects on the progression
488 of lung cancer at distinct stages of the disease⁵⁰. Furthermore, our data suggest
489 that *Arid1a* appears to have a tumor-promotive function in established IPMN
490 through positive regulation of the mTOR pathway. These context-dependent
491 roles of the SWI/SNF complex are most likely due to their multiple cellular
492 functions regulated by a wide range of target genes. Of interest, a recent study
493 demonstrated that patients with ARID1A-negative PDAC had a better prognosis

494 with respect to survival compared to those with ARID1A-positive PDAC⁸.
495 Therefore, future studies are required to clarify precisely how *Arid1a* inactivation
496 contributes to the progression of established PDAC *in vivo*.

497 Second, regarding the cellular origin of IPMNs, we showed that mouse
498 *Arid1a*-deficient IPMN originate from the ductal compartment but not from the
499 acinar compartment. In line with this finding, the cellular origin of mouse
500 *Brg1*-deficient IPMN was reported to be the PDCs⁶. However, a recent study
501 showed that the cellular origin of mouse IPMN induced by loss of trefoil factor
502 family 2 (TFF2) was pancreatic duct gland (PDG) cells³⁶. Given that enlargement
503 of PDGs was not observed in the *Ptf1a-Cre; Kras^{G12D}; Arid1a^{ff}* mice and that the
504 expression of SMAD4, which is essential for the IPMN suppressive role of TFF2
505 ^{36, 51}, was maintained in the *Arid1a*-deficient IPMNs, the present study suggests
506 that the IPMN induced by loss of *Arid1a* or *Brg1* have a different cellular origin
507 from those induced by the loss of *Smad4/Tff2*.

508 Third, we showed that *Arid1a* suppresses the dedifferentiation of PDCs and
509 thus the formation of IPMN from PDCs in part through positive regulation of

510 Sox9 expression, which is required for ductal cell differentiation and
511 maintenance³⁹. Consistent with our results, *Brg1* inhibited the dedifferentiation of
512 PDCs and IPMN formation through regulation of Sox9 expression. When Sox9
513 was inactivated during the embryonic period, a complete tissue-penetrating
514 pancreatic cystic phenotype was observed. Similar to embryonic inactivation,
515 conditional Sox9 deletion in adult mice resulted in a polycystic pancreas³⁹.
516 Therefore, Sox9 appears to be one of the key downstream effectors of the
517 SIW/SNF complex in maintenance of the pancreatic ductal structure to inhibit the
518 development of IPMN from PDCs.

519 Fourth, we found that activity of the mTOR pathway is relatively lower in
520 *Arid1a*-deficient IPMN compared to *Brg1*-deficient IPMN. The mTOR pathway
521 promotes pancreatic carcinogenesis⁴³⁻⁴⁶. Recently, Kopp JL et al. reported that
522 activation of Kras and AKT-mTor pathway leads to IPMN and PDAC formation⁵².
523 This is in line with the finding that the incidence of PDAC formation in
524 *Ptf1a-CreKras^{G12D}; Arid1a^{ff}* mice was markedly lower than that in *Ptf1a-Cre*;
525 *Kras^{G12D}; Brg1^{ff}* mice despite the histological similarities between

526 *Arid1a*-deficient and *Brg1*-deficient IPMN. Thus, activation of the mTOR
527 pathway appears to be important for the progression to PDAC from IPMN in the
528 absence of *Arid1a*.

529 Finally, we found that ARID1A expression is absent in subsets of human IPMN
530 and PDAC. Furthermore, we found a positive correlation between the expression
531 of ARID1A and SOX9/components of the mTOR pathway in human IPMN and
532 PDCA. Considering the strong correlation between ARID1A and the mTOR
533 pathway in human PDAC, the expression level of ARID1A might affect the
534 effectiveness of mTOR inhibitors for human PDAC. In addition, synthetic lethality
535 of *Arid1a*-mutated cancers was recently demonstrated by targeting EZH2 or ATR.
536 Therefore, it would be of value to investigate whether EZH2 or ATR might be
537 effective for the treatment of ARID1A-negative PDAC. Although we showed that
538 AIRD1A expression is absent in subsets of human IPMN and revealed that the
539 loss of ARID1A contributes to pancreatic tumorigenesis in this study, it remains
540 unclear precisely how ARID1A expression is reduced or lost in human IPMN.
541 Therefore, future studies are required to clarify the underlying mechanism.

In conclusion, we revealed that *Arid1a* inhibits duct cell-derived IPMN and subsequent PDAC formation in the context of oncogenic *Kras*, demonstrating a tumor-suppressive role of *Arid1a* in the pancreas *in vivo*. *Arid1a* prevents the dedifferentiation of PDCs and maintains pancreatic ductal structure in part through regulation of *Sox9* expression, providing mechanistic insight into IPMN formation. Expression of ARID1A and SOX9/components of the mTOR pathway was positively correlated in human IPMN and PDCA. Our data point to *Arid1a* as a cell-type specific mediator of *Kras*-driven tumorigenesis in the pancreas and underscore that the SWI/SNF chromatin remodeling complex is a critical determinant for the distinct route of PDAC formation.

Acknowledgements

The authors thank Yoshiya Kawaguchi for sharing the *Ptf1a-Cre* mice.

556 **References**

- 557 1. Hidalgo M, Cascinu S, Kleeff J, et al. Addressing the challenges of
558 pancreatic cancer: future directions for improving outcomes.
559 Pancreatology 2015;15:8-18.
- 560 2. Shain AH, Pollack JR. The spectrum of SWI/SNF mutations, ubiquitous in
561 human cancers. PLoS One 2013;8:e55119.
- 562 3. **Kadoch C, Hargreaves DC**, Hodges C, et al. Proteomic and
563 bioinformatic analysis of mammalian SWI/SNF complexes identifies
564 extensive roles in human malignancy. Nat Genet 2013;45:592-601.
- 565 4. **Witkiewicz AK, McMillan EA, Balaji U**, et al. Whole-exome sequencing
566 of pancreatic cancer defines genetic diversity and therapeutic targets. Nat
567 Commun 2015;6:6744.
- 568 5. Wilson BG, Roberts CW. SWI/SNF nucleosome remodellers and cancer.
569 Nat Rev Cancer 2011;11:481-92.
- 570 6. von Figura G, Fukuda A, Roy N, et al. The chromatin regulator Brg1
571 suppresses formation of intraductal papillary mucinous neoplasm and
572 pancreatic ductal adenocarcinoma. Nat Cell Biol 2014;16:255-67.

- 573 7. **Roy N, Malik S**, Villanueva KE, et al. Brg1 promotes both
574 tumor-suppressive and oncogenic activities at distinct stages of
575 pancreatic cancer formation. *Genes Dev* 2015;29:658-71.
- 576 8. Sausen M, Phallen J, Adleff V, et al. Clinical implications of genomic
577 alterations in the tumour and circulation of pancreatic cancer patients. *Nat*
578 *Commun* 2015;6:7686.
- 579 9. Wu JN, Roberts CW. ARID1A mutations in cancer: another epigenetic
580 tumor suppressor? *Cancer Discov* 2013;3:35-43.
- 581 10. Dallas PB, Cheney IW, Liao DW, et al. p300/CREB binding protein-related
582 protein p270 is a component of mammalian SWI/SNF complexes. *Mol*
583 *Cell Biol* 1998;18:3596-603.
- 584 11. Nie Z, Xue Y, Yang D, et al. A specificity and targeting subunit of a human
585 SWI/SNF family-related chromatin-remodeling complex. *Mol Cell Biol*
586 2000;20:8879-88.
- 587 12. Trotter KW, Archer TK. Reconstitution of glucocorticoid
588 receptor-dependent transcription in vivo. *Mol Cell Biol* 2004;24:3347-58.

- 589 13. Chandler RL, Brennan J, Schisler JC, et al. ARID1a-DNA interactions are
590 required for promoter occupancy by SWI/SNF. Mol Cell Biol
591 2013;33:265-80.
- 592 14. Guan B, Rahmanto YS, Wu RC, et al. Roles of deletion of Arid1a, a tumor
593 suppressor, in mouse ovarian tumorigenesis. J Natl Cancer Inst
594 2014;106.
- 595 15. **Chandler RL, Damrauer JS**, Raab JR, et al. Coexistent ARID1A-PIK3CA
596 mutations promote ovarian clear-cell tumorigenesis through
597 pro-tumorigenic inflammatory cytokine signalling. Nat Commun
598 2015;6:6118.
- 599 16. Mathur R, Alver BH, San Roman AK, et al. ARID1A loss impairs
600 enhancer-mediated gene regulation and drives colon cancer in mice. Nat
601 Genet 2017;49:296-302.
- 602 17. Kawaguchi Y, Cooper B, Gannon M, et al. The role of the transcriptional
603 regulator Ptf1a in converting intestinal to pancreatic progenitors. Nat
604 Genet 2002;32:128-34.

- 605 18. Pan FC, Bankaitis ED, Boyer D, et al. Spatiotemporal patterns of
606 multipotentiality in Ptf1a-expressing cells during pancreas organogenesis
607 and injury-induced facultative restoration. *Development* 2013;140:751-64.
- 608 19. **Solar M, Cardalda C, Houbracken I**, et al. Pancreatic exocrine duct cells
609 give rise to insulin-producing beta cells during embryogenesis but not
610 after birth. *Dev Cell* 2009;17:849-60.
- 611 20. Hingorani SR, Petricoin EF, Maitra A. Preinvasive and invasive ductal
612 pancreatic cancer and its early detection in the mouse. *Cancer Cell*
613 2003;4:437-450.
- 614 21. Sumi-Ichinose C, Ichinose H, Metzger D, et al. SNF2beta-BRG1 is
615 essential for the viability of F9 murine embryonal carcinoma cells. *Mol*
616 *Cell Biol* 1997;17:5976-86.
- 617 22. Gao X, Tate P, Hu P, et al. ES cell pluripotency and germ-layer formation
618 require the SWI/SNF chromatin remodeling component BAF250a. *Proc*
619 *Natl Acad Sci U S A* 2008;105:6656-61.
- 620 23. **Li B, Murphy L**, Laucirica R. A transgenic mouse model for mammary

- 621 carcinogenesis. *oncogene* 1998;16:997-1007.
- 622 24. Kim Y, Murao H, Yamamoto K, et al. Generation of transgenic mice for
623 conditional overexpression of Sox9. *J Bone Miner Metab* 2011;29:123-9.
- 624 25. Reichert M, Takano S, Heeg S, et al. Isolation, culture and genetic
625 manipulation of mouse pancreatic ductal cells. *Nat Protoc*
626 2013;8:1354-65.
- 627 26. Reichert M, Rhim AD, Rustgi AK. Culturing primary mouse pancreatic
628 ductal cells. *Cold Spring Harb Protoc* 2015;2015:558-61.
- 629 27. Heiser PW, Lau J, Taketo MM, et al. Stabilization of beta-catenin impacts
630 pancreas growth. *Development* 2006;133:2023-32.
- 631 28. **Ahlgren U, Jonsson J**, Jonsson L, et al. β -cell-specific inactivation of the
632 mouse *lpl1/Pdx1* gene results in loss of the beta-cell phenotype and
633 maturity onset diabetes. *Genes Dev* 1998;12:1763-8.
- 634 29. Seeley ES, Carriere C, Goetze T, et al. Pancreatic cancer and precursor
635 pancreatic intraepithelial neoplasia lesions are devoid of primary cilia.
636 *Cancer Res* 2009;69:422-30.

- 637 30. **Matthaei H, Schulick RD, Hruban RH**, et al. Cystic precursors to
638 invasive pancreatic cancer. *Nat Rev Gastroenterol Hepatol*
639 2011;8:141-50.
- 640 31. Shi C, Hruban RH. Intraductal papillary mucinous neoplasm. *Hum Pathol*
641 2012;43:1-16.
- 642 32. Takasu N, Kimura W, Moriya T, et al. Intraductal papillary-mucinous
643 neoplasms of the gastric and intestinal types may have less malignant
644 potential than the pancreatobiliary type. *Pancreas* 2010;39:604-10.
- 645 33. **Fukuda A, Wang SC**, Morris JPt, et al. Stat3 and MMP7 contribute to
646 pancreatic ductal adenocarcinoma initiation and progression. *Cancer Cell*
647 2011;19:441-55.
- 648 34. Habbe N, Shi G, Meguid RA, et al. Spontaneous induction of murine
649 pancreatic intraepithelial neoplasia (mPanIN) by acinar cell targeting of
650 oncogenic Kras in adult mice. *Proc Natl Acad Sci U S A*
651 2008;105:18913-8.
- 652 35. **Kopp JL, von Figura G**, Mayes E, et al. Identification of Sox9-dependent

- 653 acinar-to-ductal reprogramming as the principal mechanism for initiation
 654 of pancreatic ductal adenocarcinoma. *Cancer Cell* 2012;22:737-50.
- 655 36. Yamaguchi J, Mino-Kenudson M, Liss AS, et al. Loss of Trefoil Factor 2
 656 From Pancreatic Duct Glands Promotes Formation of Intraductal Papillary
 657 Mucinous Neoplasms in Mice. *Gastroenterology* 2016;151:1232-1244
 658 e10.
- 659 37. **Delous M, Yin C, Shin D**, et al. Sox9b is a key regulator of
 660 pancreaticobiliary ductal system development. *PLoS Genet*
 661 2012;8:e1002754.
- 662 38. Shih HP, Kopp JL, Sandhu M, et al. A Notch-dependent molecular
 663 circuitry initiates pancreatic endocrine and ductal cell differentiation.
 664 *Development* 2012;139:2488-99.
- 665 39. Subramanian A, Tamayo P, Mootha VK, et al. Gene set enrichment
 666 analysis: a knowledge-based approach for interpreting genome-wide
 667 expression profiles. *Proc Natl Acad Sci U S A* 2005;102:15545-50.
- 668 40. Mootha VK, Lindgren CM, Eriksson KF, et al. PGC-1alpha-responsive

- 669 genes involved in oxidative phosphorylation are coordinately
670 downregulated in human diabetes. *Nat Genet* 2003;34:267-73.
- 671 41. Okami K, Wu L, Riggins G, et al. Analysis of PTEN/MMAC1 alterations in
672 aerodigestive tract tumors. *Cancer Res* 1998;58:509-11.
- 673 42. Samuels Y, Velculescu VE. Oncogenic mutations of PIK3CA in human
674 cancers. *Cell Cycle* 2004;3:1221-4.
- 675 43. Asano T, Yao Y, Zhu J, et al. The PI 3-kinase/Akt signaling pathway is
676 activated due to aberrant Pten expression and targets transcription
677 factors NF-kappaB and c-Myc in pancreatic cancer cells. *Oncogene*
678 2004;23:8571-80.
- 679 44. Asano T, Yao Y, Zhu J, et al. The rapamycin analog CCI-779 is a potent
680 inhibitor of pancreatic cancer cell proliferation. *Biochem Biophys Res*
681 *Commun* 2005;331:295-302.
- 682 45. Lim KH, Counter CM. Reduction in the requirement of oncogenic Ras
683 signaling to activation of PI3K/AKT pathway during tumor maintenance.
684 *Cancer Cell* 2005;8:381-92.

- 685 46. **Bruns CJ, Koehl GE**, Guba M, et al. Rapamycin-induced endothelial cell
686 death and tumor vessel thrombosis potentiate cytotoxic therapy against
687 pancreatic cancer. Clin Cancer Res 2004;10:2109-19.
- 688 47. Bailey P, Chang DK, Nones K, et al. Genomic analyses identify molecular
689 subtypes of pancreatic cancer. Nature 2016;531:47-52.
- 690 48. Ikushima H, Miyazono K. TGF β signalling: a complex web in cancer
691 progression. Nat Rev Cancer 2010;10:415-424.
- 692 49. John P, Morris I, Sam C. KRA, Hedgehog, Wnt and the twisted
693 developmental biology of pancreatic ductal adenocarcinoma. Nat Rev
694 Cancer 2010;10:683-695.
- 695 50. Walter D, Venancio O, Buza E. Systematic in vivo inactivation of
696 chromatin-regulating enzymes identifies Setd2 as a potent tumor
697 suppressor in lung adenocarcinoma. Cancer Res 2017;77:1719-1729.
- 698 51. Izeradjene K, Combs C, Best M, et al. Kras(G12D) and Smad4/Dpc4
699 haploinsufficiency cooperate to induce mucinous cystic neoplasms and
700 invasive adenocarcinoma of the pancreas. Cancer Cell 2007;11:229-43.

701 52. Kopp JL, Dubois CL, Schaeffer DF. Loss of PTEN and Activation of Kras
702 Synergistically Induce Formation of Intraductal Papillary Mucinous
703 Neoplasia From Pancreatic Ductal Cells in Mice. Gastroenterology 2018;
704 doi: 10.1053/j.gastro.2017.12.007.

705 Author names in bold designate shared co-first authorship.

706

707 **Figure legends**

708 **Figure 1. *Arid1a* loss in the murine pancreata results in the formation of**
 709 **cysts.**

710 (A–C) From the top, a macroscopic image, hematoxylin and eosin (H&E) and
 711 ARID1A staining. (D, pancreatic duct; broken line, islet; D1, ARID1A-positive
 712 duct; D2, ARID1A-negative duct; C, pancreatic cyst) (A) Wild-type (n = 8)

713 (B) *Ptf1a-Cre; Arid1a^{f/+}* pancreata (n = 10) showed no abnormalities. (C)

714 *Ptf1a-Cre; Arid1a^{ff}* pancreata (n = 21) developed cystic formations. (D)

715 Molecular characterization of the cystic lesions in the *Ptf1a-Cre; Arid1a^{ff}*
 716 pancreata. All white scale bars = 1 cm. All black scale bars = 50 μ m.

717

718 **Figure 2. *Arid1a* deletion collaborates with mutant *Kras* to form neoplastic**
 719 **cystic lesions that resemble human IPMN.**

720 (A) Macroscopic image of control *Ptf1a-Cre; Kras^{G12D}; Arid1a^{f/+}* (duodenum +
 721 pancreas + spleen) (n = 10) and *Ptf1a-Cre; Kras^{G12D}; Arid1a^{ff}* mice (pancreas +
 722 spleen) (n = 43).

723 (B) Relative pancreatic weight (PW) to body weight (BW) ratio of *Ptf1a-Cre*;

724 *Kras^{G12D}; Arid1a^{f/+}* (n = 6) and *Ptf1a-Cre; Kras^{G12D}; Arid1a^{f/f}* (n = 6) mice.

725 (C) From the top, hematoxylin & eosin, cytokeratin, amylase, and alcian blue

726 staining of *Ptf1a-Cre; Kras^{G12D}; Arid1a^{f/+}* and *Ptf1a-Cre; Kras^{G12D}; Arid1a^{f/f}* mice.

727 The arrowhead denotes a connection between the cystic lesion and pancreatic

728 ductal system. (D) From the top, ARID1A, phospho-p44/42 MAPK

729 (Thr202/Tyr204) (pERK1/2), Ki67 staining of control *Ptf1a-Cre; Kras^{G12D};*

730 *Arid1a^{f/+}* and *Ptf1a-Cre; Kras^{G12D}; Arid1a^{f/f}* mice.

731 (E) Mucin expression pattern of IPMN in *Ptf1a-Cre; Kras^{G12D}; Arid1a^{f/f}* mice.

732 White scale bars = 1 cm. Black scale bars = 50 μ m.

733

734 **Figure 3. IPMN in *Ptf1a-Cre; Kras^{G12D}; Arid1a^{f/f}* mice exhibit varying grades**

735 **of dysplasia and progress to PDAC**

736 (A) IPMN with low- and high-grade dysplasia in *Ptf1a-Cre; Kras^{G12D}; Arid1a^{f/f}*

737 mice at 18 weeks of age. (B) Incidence of PDAC formation in *Ptf1a-Cre;*

738 *Kras^{G12D}; Arid1a^{f/f}* (n = 43), *Ptf1a-Cre; Kras^{G12D}* (n = 8), and *Ptf1a-Cre; Kras^{G12D};*

739 *Arid1a*^{ff} (n = 16) mice at indicated time points. (C) Two images on the left show
 740 IPMN with severe-grade dysplasia at 48 weeks of age. Two images on the right
 741 show invasive adenocarcinoma developed from IPMN in *Ptf1a-Cre; Kras*^{G12D};
 742 *Arid1a*^{ff} mice at 48 weeks of age. (D) ARID1A, pERK1/2, Ki67, and PDX1
 743 staining in PDAC cells in *Ptf1a-Cre; Kras*^{G12D}; *Arid1a*^{ff} mice.
 744 Scale bars = 50 μm.

745

746 **Figure 4. *Arid1a*-deficient IPMN originates from the pancreatic ductal cells**
 747 **but not from acinar cells.**

748 (A) Schema of recombination in *Ptf1a-Cre*^{ERT2}; *Kras*^{G12D}; *Arid1a*^{ff} mice.

749 (B) Tamoxifen and caerulein administration schema for experiments using

750 *Ptf1a-Cre*^{ERT2}; *Kras*^{G12D}; *Arid1a*^{f/+} (n = 5) and *Ptf1a-Cre*^{ERT2}; *Kras*^{G12D}; *Arid1a*^{ff}

751 mice (n = 5).

752 (C) *Ptf1a-Cre*^{ERT2}; *Kras*^{G12D}; *Arid1a*^{f/+} and *Ptf1a-Cre*^{ERT2}; *Kras*^{G12D}; *Arid1a*^{ff} mice

753 developed multiple PanIN but not IPMN.

754 (D) Schema of recombination in *Hn1fb-Cre*^{ERT2}; *Kras*^{G12D}; *Arid1a*^{ff} mice.

755 (E) Tamoxifen administration schema for experiments using *Hn1b-Cre^{ERT2}*;

756 *Kras^{G12D}*; *Arid1a^{f/+}* (n = 5) and *Hn1b-Cre^{ERT2}*; *Kras^{G12D}*; *Arid1a^{ff}* mice (n = 6).

757 (F) The upper figures show that *Arid1a* deletion in the presence of mutant *Kras*

758 in PDC resulted in a cystic neoplastic lesion. The lower figures show that the

759 mucin expression patterns of the cystic lesions in *Hnf1b-Cre^{ERT2}*; *Kras^{G12D}*;

760 *Arid1a^{ff}* mice were similar to those in *Ptf1a-Cre*; *Kras^{G12D}*; *Arid1a^{ff}* mice.

761 Scale Bar = 50 μ m

762

763 **Figure 5. *Arid1a* deletion leads to reduction in Sox9 expression,**

764 **dedifferentiation of PDCs, and duct dilation, and ectopic expression of**

765 **Sox9 prevents the dedifferentiation of PDCs and duct dilation ex vivo.**

766 (A) SOX9 staining in wild type (WT), *Ptf1a-Cre*; *Arid1a^{ff}*, *Ptf1a-Cre*; *Kras^{G12D}*;

767 *Arid1a^{ff}*, and *Hnf1b-Cre^{ERT2}*; *Kras^{G12D}*; *Arid1a^{ff}* mice.

768 (B) Schemas of adenovirus infection experiments. +Ad-GFP, control GFP

769 expressing adenovirus transmission; +Ad-Cre, Cre recombinase and GFP

770 expressing adenovirus transmission.

771 (C, D) Images of 3D culture of PDCs isolated from *Arid1a^{ff}* and *Arid1a^{ff}; Sox9OE* mice. Diameters of the cysts formed by PDCs are shown in bar graphs.

772

773 (E) q-PCR analysis of components of the SWI/SNF (*Arid1a* and *Brg1*)

774 complexes, duct cell markers [mouse *Sox9* (moSox9), human *Sox9* (huSOX9),

775 *Krt19*, and *Hnf1b*], and progenitor cell markers (*Gata4*, *Gata6*, and *Hes1*) in

776 cultured PDCs isolated from *Arid1a^{ff}* (n = 3) and *Arid1a^{ff}; Sox9OE* mice (n = 3)

777 and infected by adenoviruses.

778 Scale Bar = 50 μ m

779 $P < 0.05$ and $P < 0.01$ were represented with single and double asterisks,

780 respectively.

781

782 **Figure 6. The mTOR pathway activity is lower in *Arid1a*-deficient IPMN**

783 **compared to that in *Brg1*-deficient IPMN.**

784 (A) BRG1 expression in *Ptf1a-Cre; Kras^{G12D}; Arid1a^{ff}* mice (n = 6) and ARID1A

785 expression in *Ptf1a-Cre; Kras^{G12D}; Brg1^{ff}* mice (n = 6).

786 (B) Schema of microarray analysis of IPMN in *Ptf1a-Cre; Kras^{G12D}; Arid1a^{ff}* (n =
 787 2) and *Ptf1a-Cre; Kras^{G12D}; Brg1^{ff}* (n = 3) mice. Seven gene sets were
 788 significantly upregulated in *Ptf1a-Cre; Kras^{G12D}; Arid1a^{ff}* mice compared to those
 789 in *Ptf1a-Cre; Kras^{G12D}; Brg1^{ff}* mice. The AKT_UP_MTOR_DN.V1_UP gene set
 790 contains genes upregulated by everolimus in mouse prostate tissue
 791 transgenically expressing the human *AKT1* gene versus untreated controls.

792 (C) Phosphorylated S6 (pS6) was barely observed in IPMN in *Ptf1a-Cre;*
 793 *Kras^{G12D}; Arid1a^{ff}* mice, whereas it was generally observed in PanIN in
 794 *Ptf1a-Cre; Kras^{G12D}* mice, IPMN in *Ptf1a-Cre; Kras^{G12D}; Brg1^{ff}* mice, and PDAC
 795 in *Ptf1a-Cre; Kras^{G12D}; Arid1a^{ff}* mice.

796 (D) q-PCR analysis of the components of the mTor pathway in PDCs with *Arid1a*
 797 deletion.

798 Scale Bar = 50 μ m

799 $P < 0.05$ and $P < 0.01$ were represented with single and double asterisks,
 800 respectively. $P > 0.05$ was represented by a number sign.

801

802 **Figure 7. Absence of ARID1A expression is observed in subsets of human**
803 **IPMN, and ARID1A expression is positively correlated with expression of**
804 **SOX9 and components of the mTOR pathway in human IPMN and PDAC.**

805 (A) Histological analysis of human IPMN (n = 58) by ARID1A, SOX9, and pS6
806 staining.

807 (B) Spearman's rank correlation coefficient between *ARID1A* expression and
808 components of the mTOR pathway in human PDAC.

809 (C) Plots of mRNA expression of *ARID1A* and components of the mTOR
810 pathway.

811 Scale Bar = 50 μ m.

Figure1

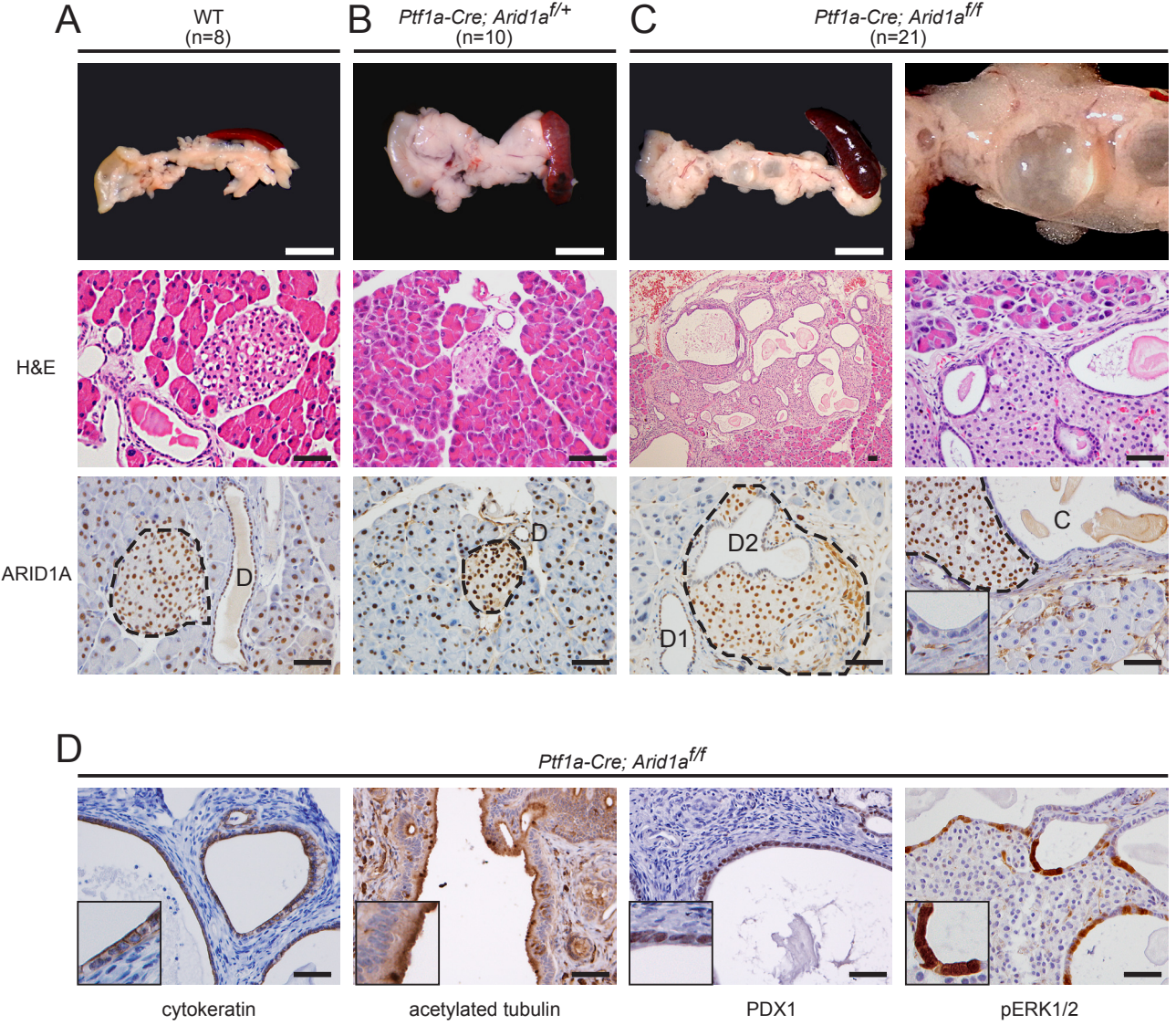


Figure2

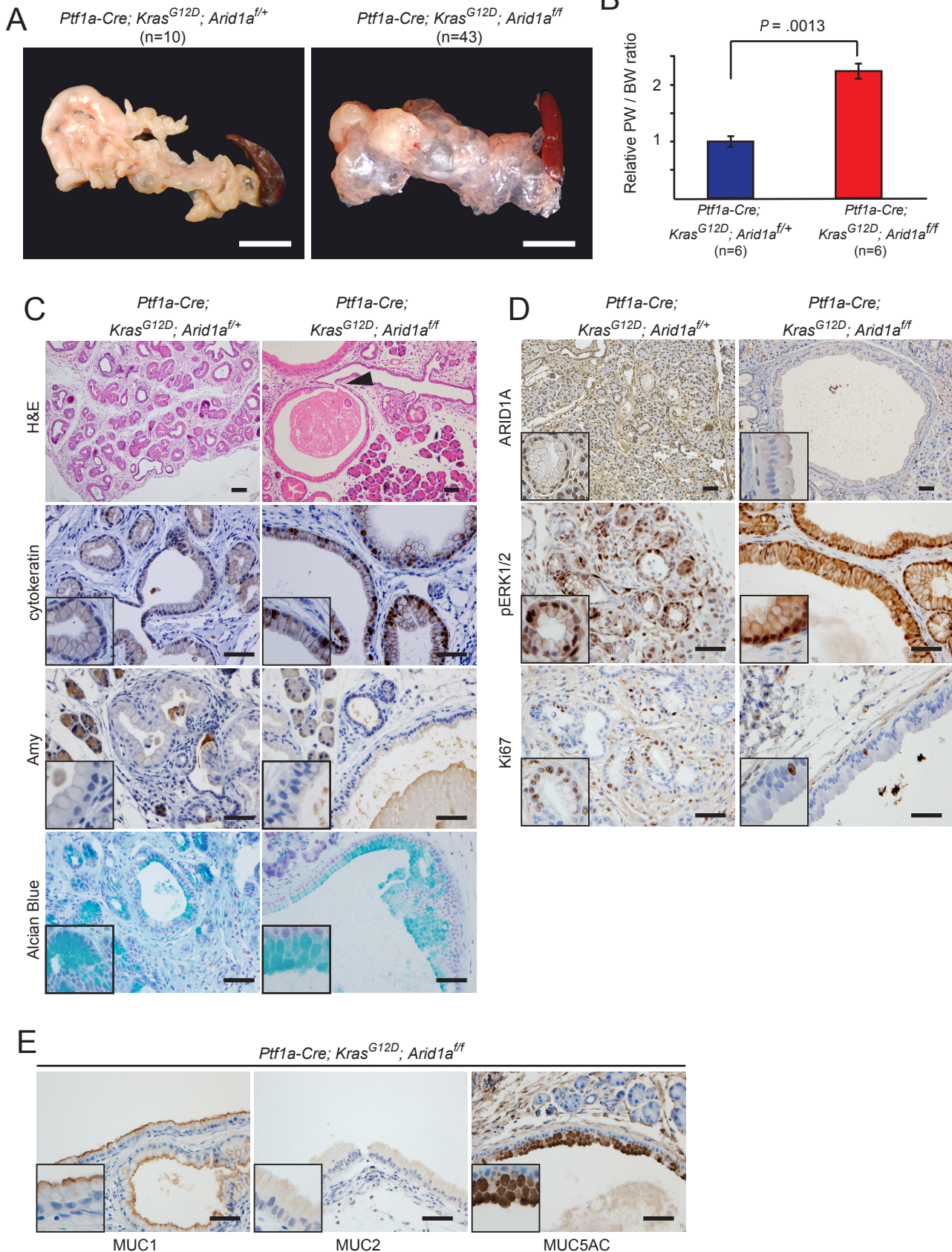
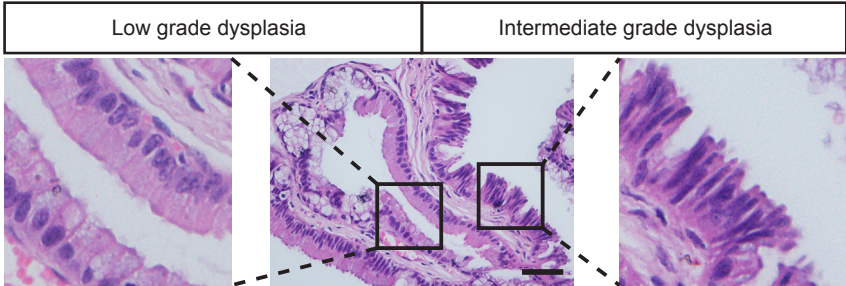


Figure3

A

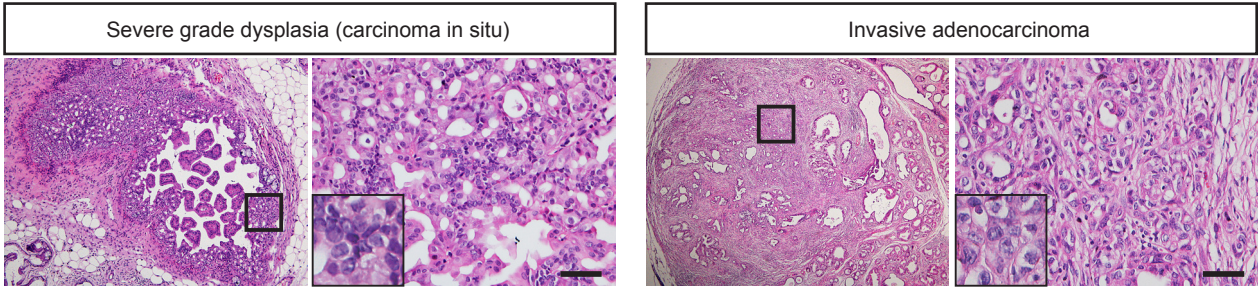


B

Incidence of adenocarcinoma

	<i>Ptf1a-Cre; Kras^{G12D}; Arid1a^{fl/fl}</i> (n=43)	<i>Ptf1a-Cre; Kras^{G12D}</i> (n=8) OR <i>Ptf1a-Cre; Kras^{G12D}; Arid1a^{fl/+}</i> (n=16)
14-18 weeks	0% (n=13)	0% (n=8)
20-24 weeks	0% (n=15)	
44-48 weeks	20% (n=15)	0% (n=16)

C



D

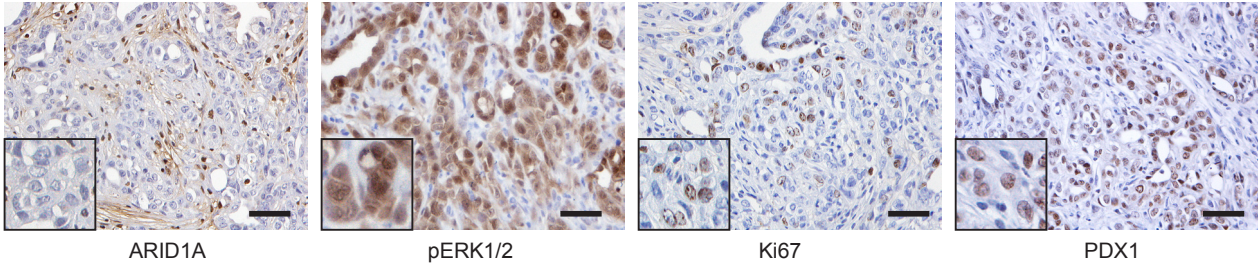


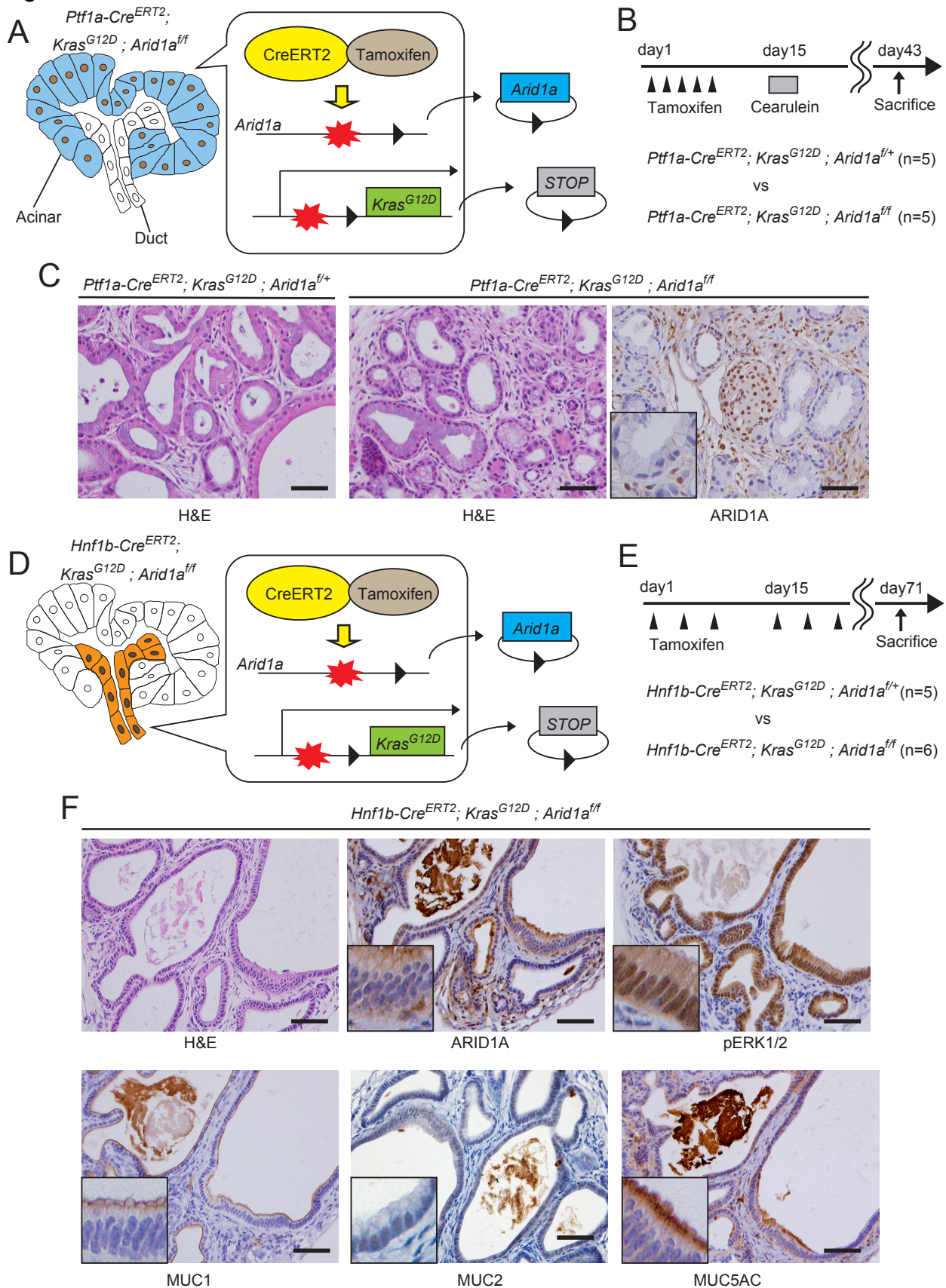
Figure4

Figure5

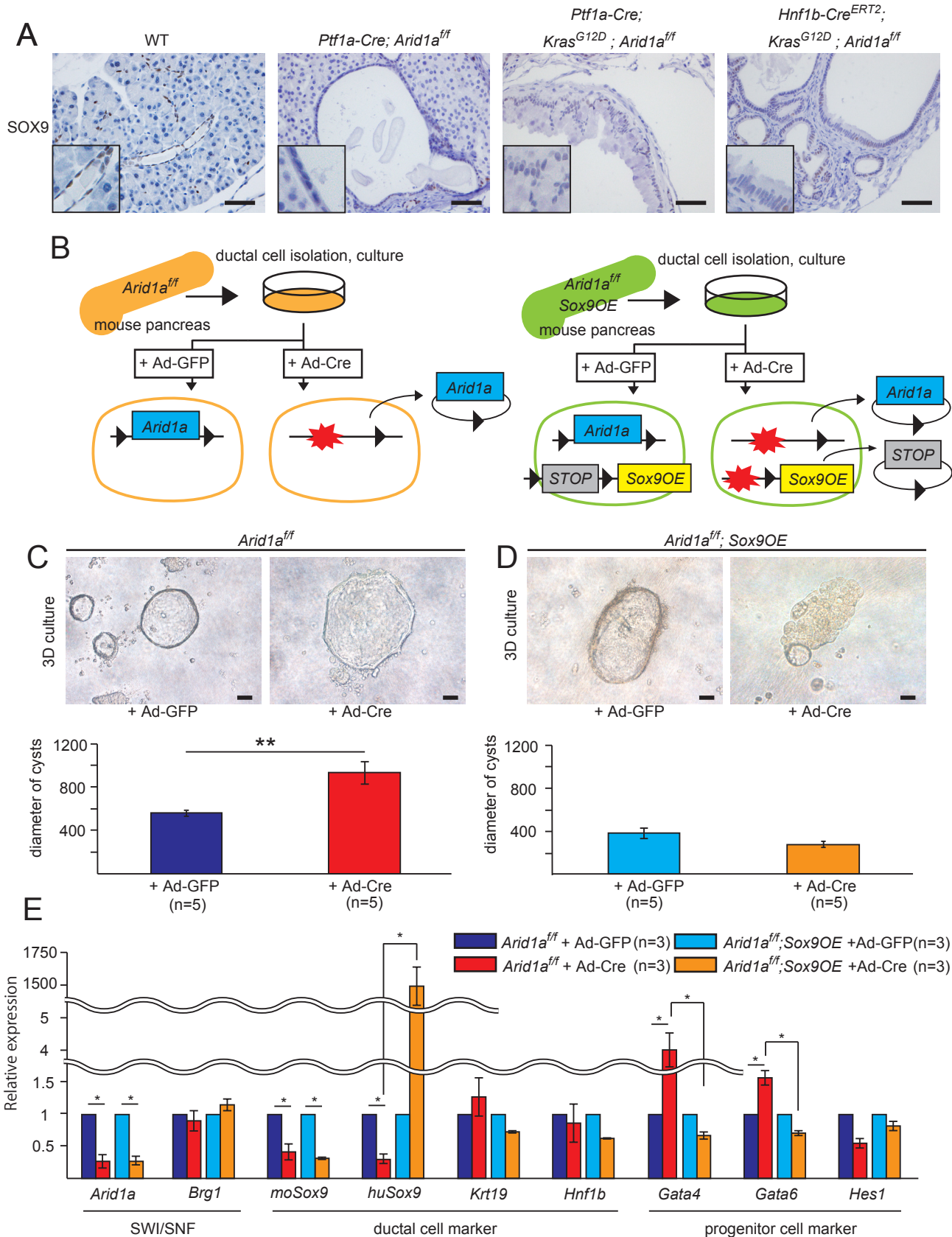
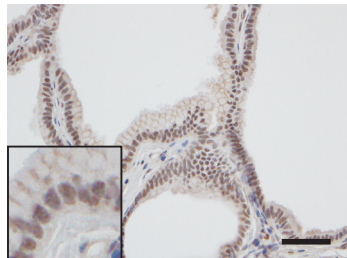


Figure 6

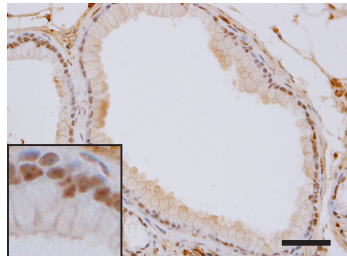
A

Ptf1a-Cre; Kras^{G12D}; Arid1a^{ff} (n=6)



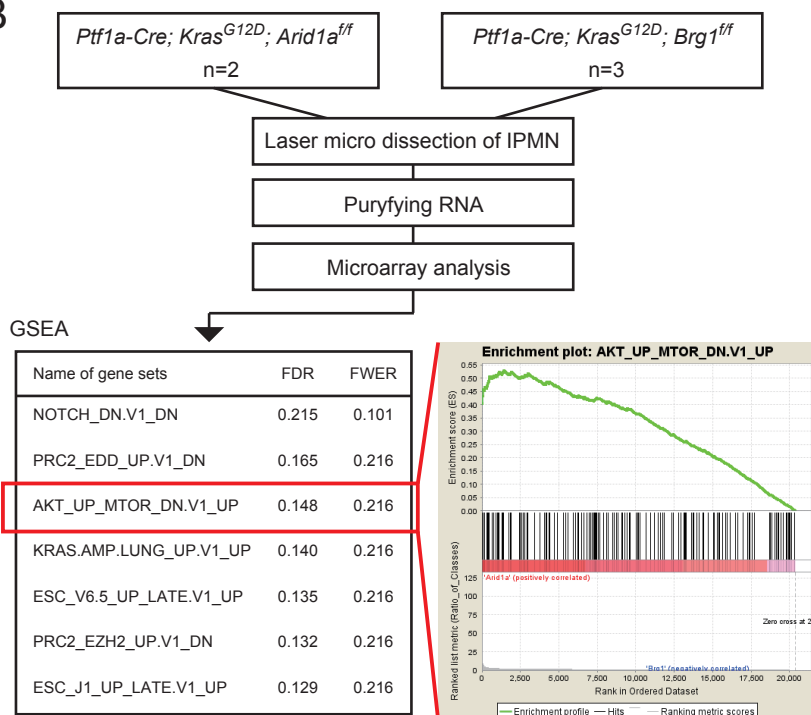
BRG1

Ptf1a-Cre; Kras^{G12D}; Brg1^{ff} (n=6)

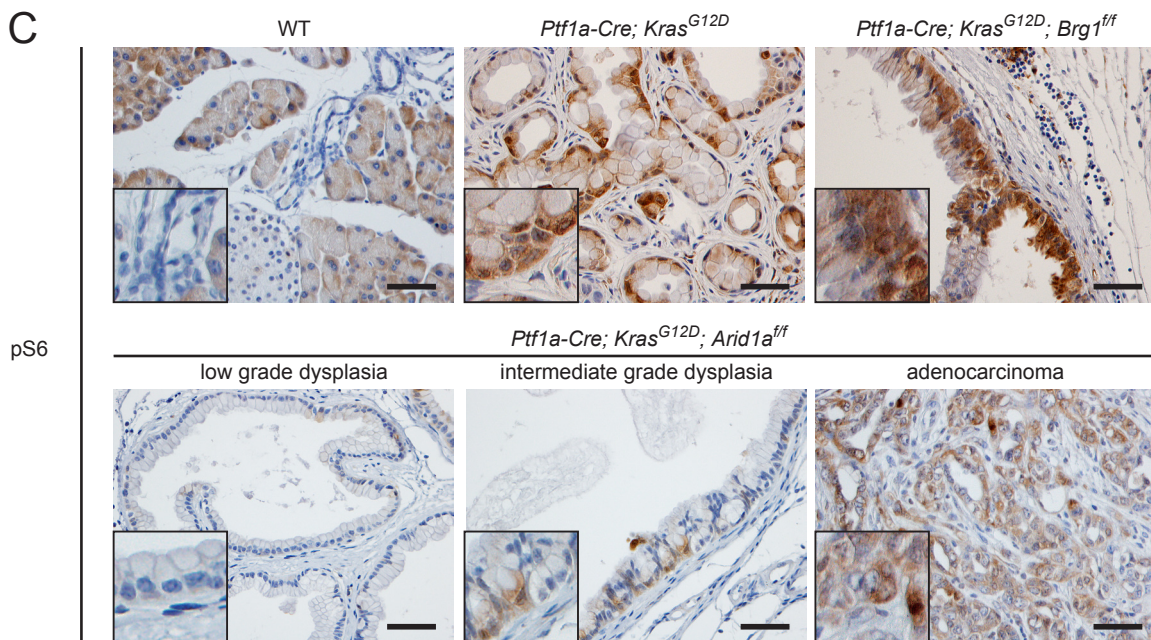


ARID1A

B



C



D

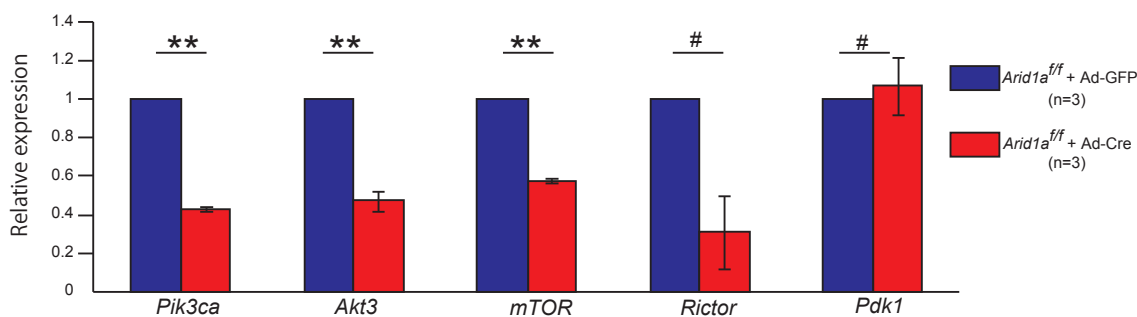
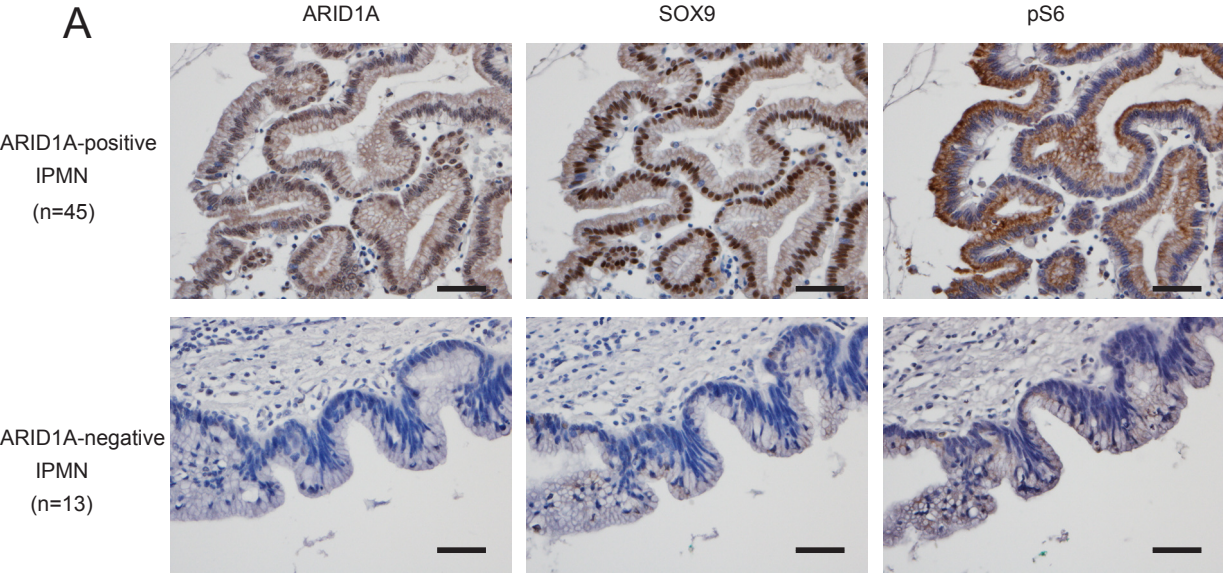


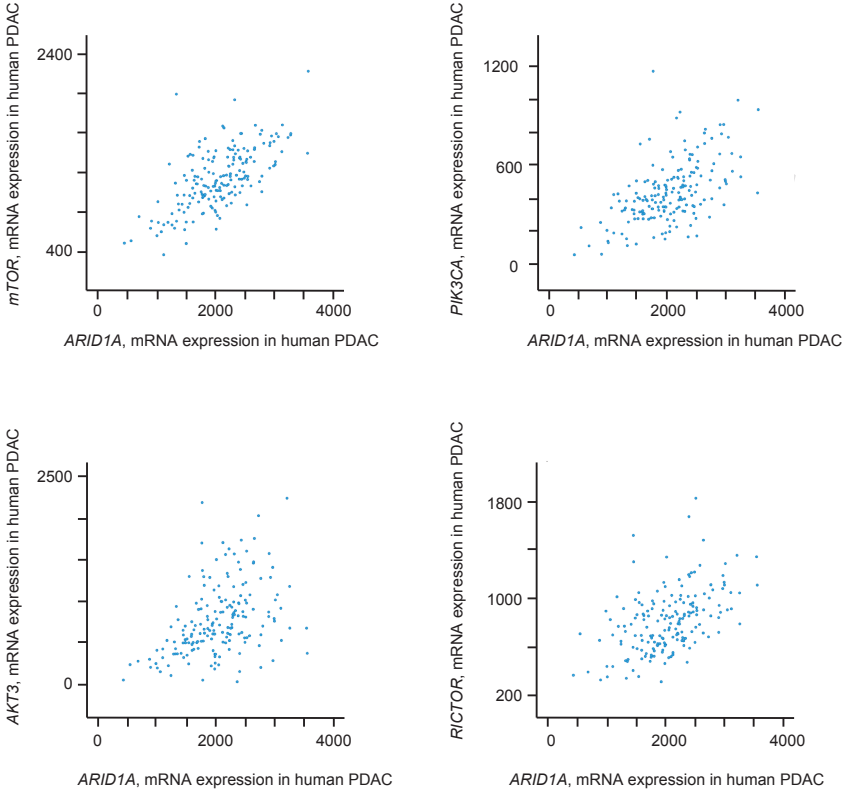
Figure7



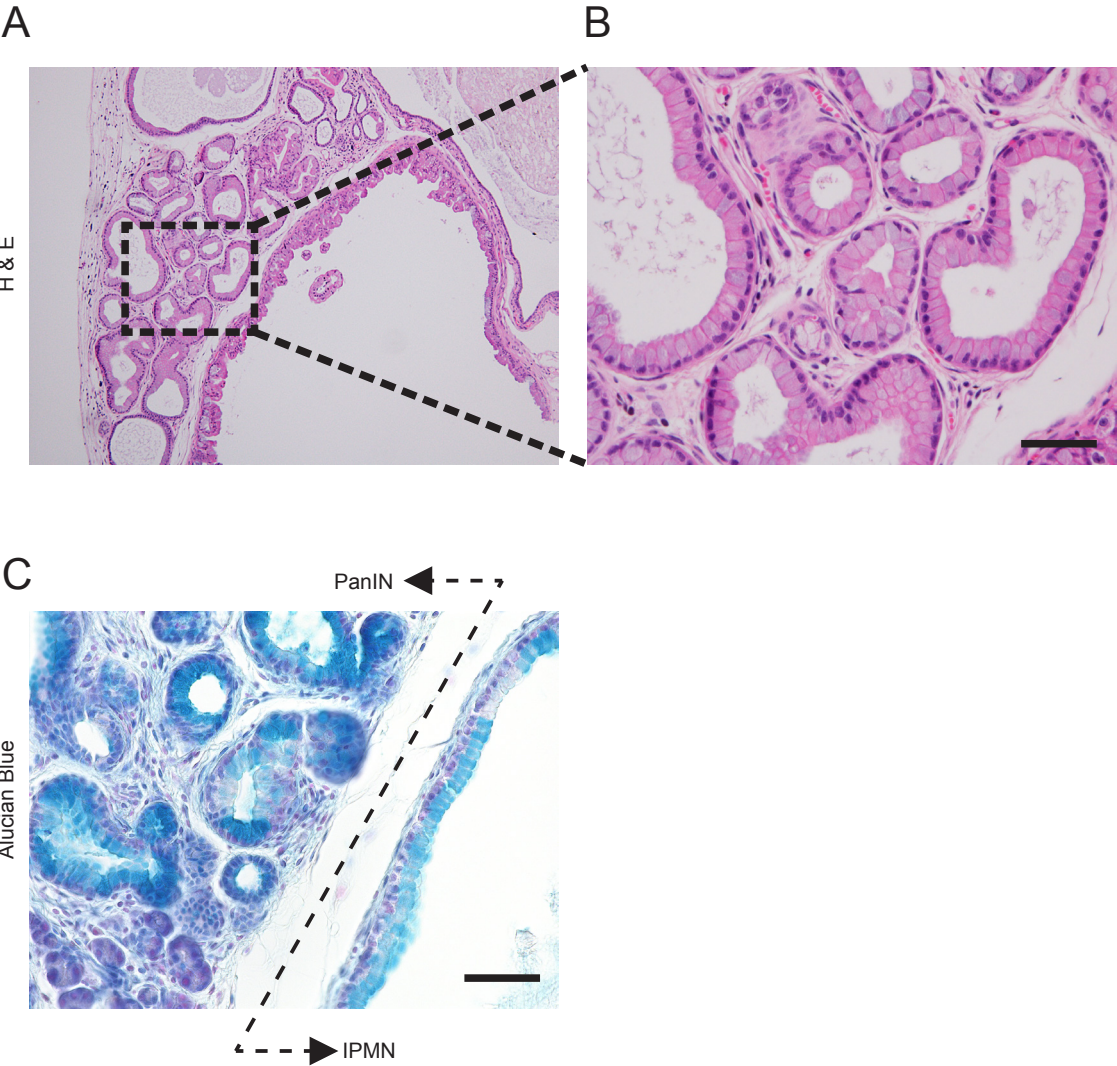
B

gene	spearman
<i>PIK3CA</i>	0.556
<i>PTEN</i>	0.305
<i>PDK1</i>	0.073
<i>PDK2</i>	-0.005
<i>PDK3</i>	0.158
<i>AKT1</i>	-0.113
<i>AKT2</i>	0.028
<i>AKT3</i>	0.463
<i>TSC1</i>	0.298
<i>AKT1S1</i>	-0.409
<i>RHEB</i>	-0.319
<i>RPTOR</i>	0.087
<i>RICTOR</i>	0.501
<i>MTOR</i>	0.63
<i>RPS6KB1</i>	0.225
<i>RPS6</i>	-0.285

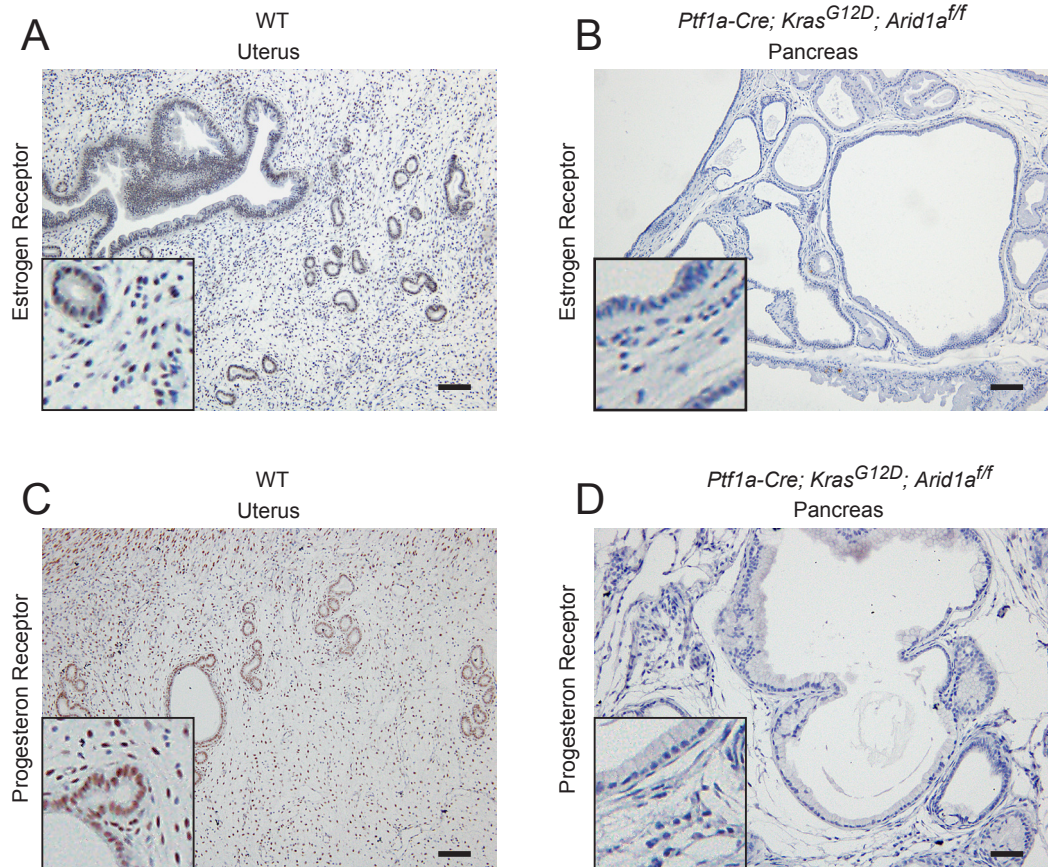
C



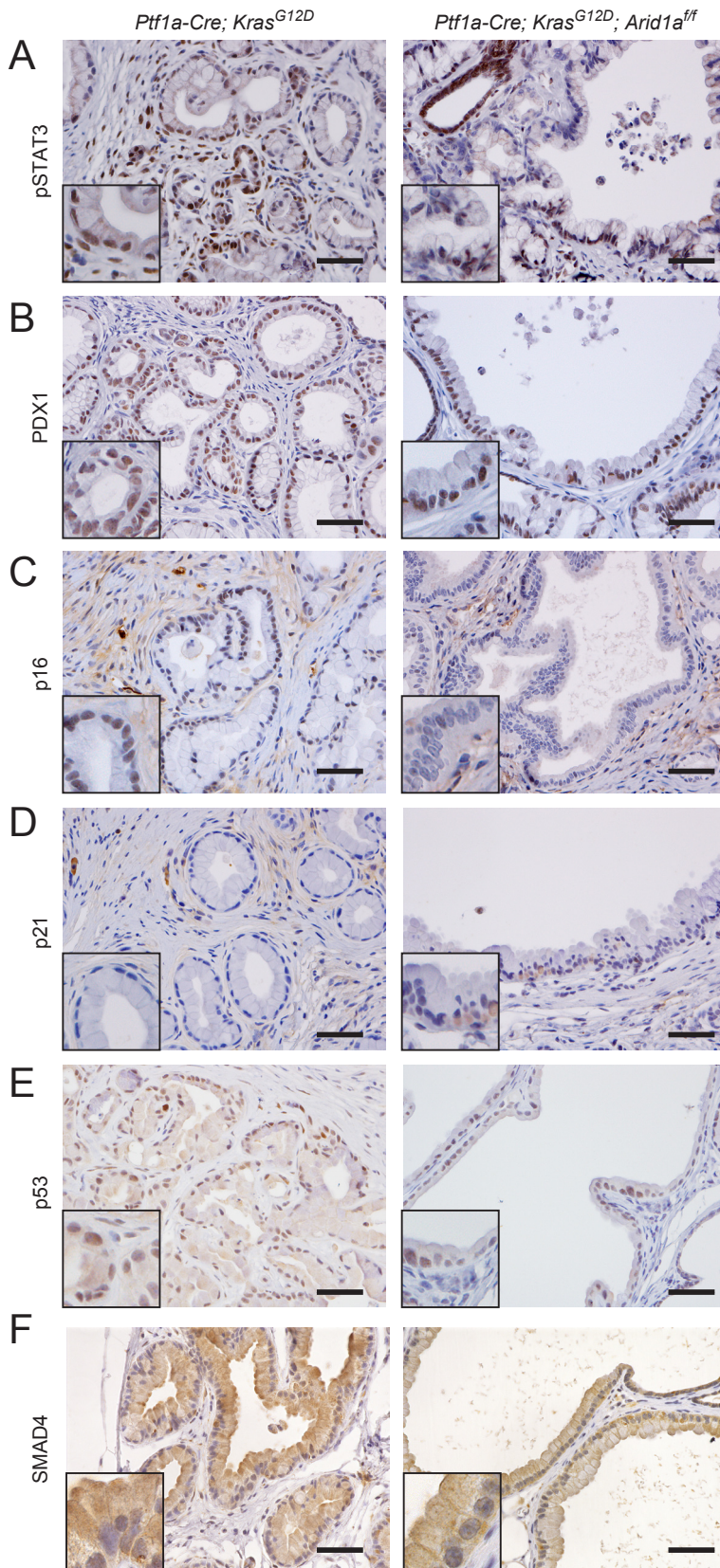
Supplementary Figure1



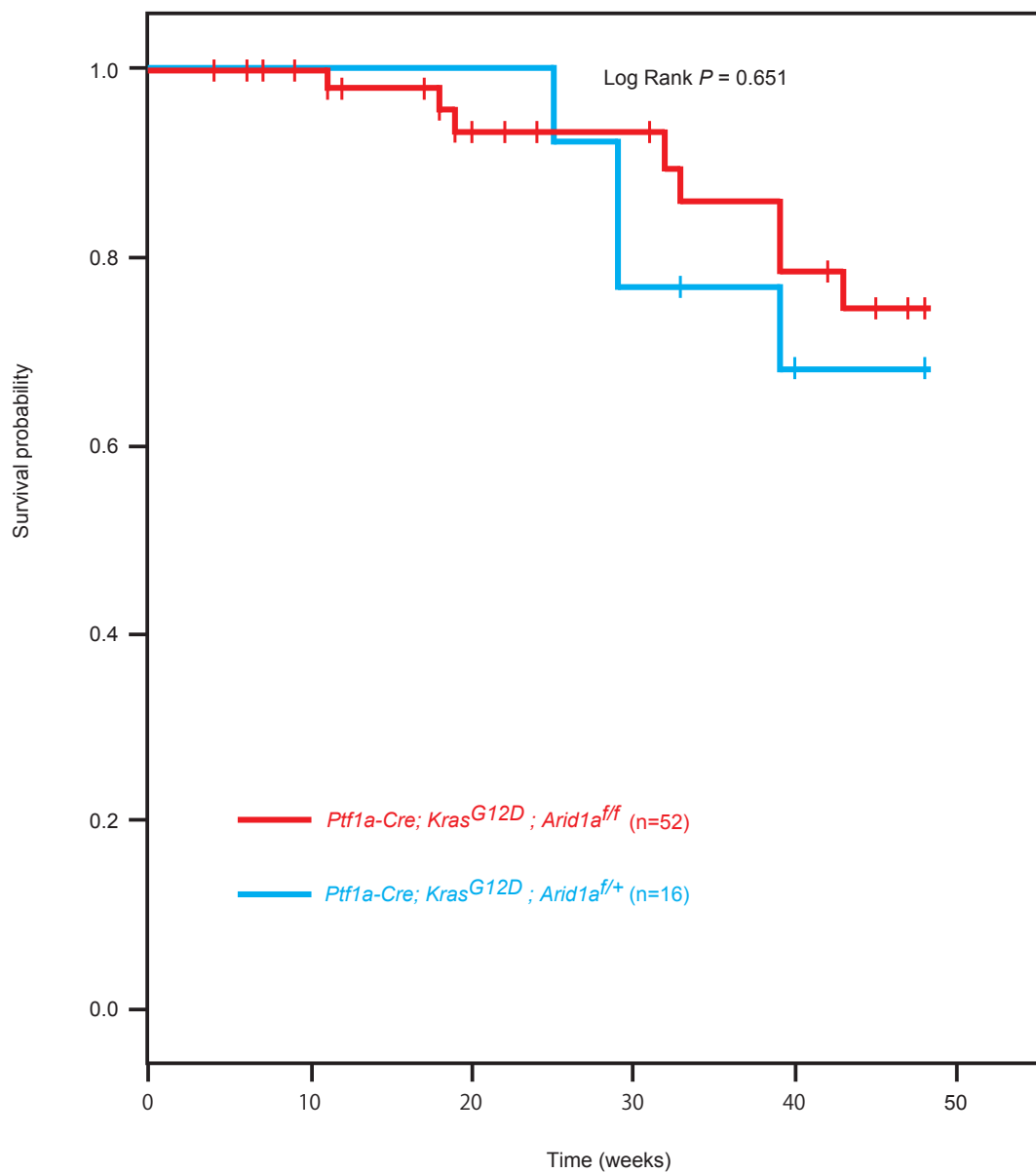
Supplementary Figure2



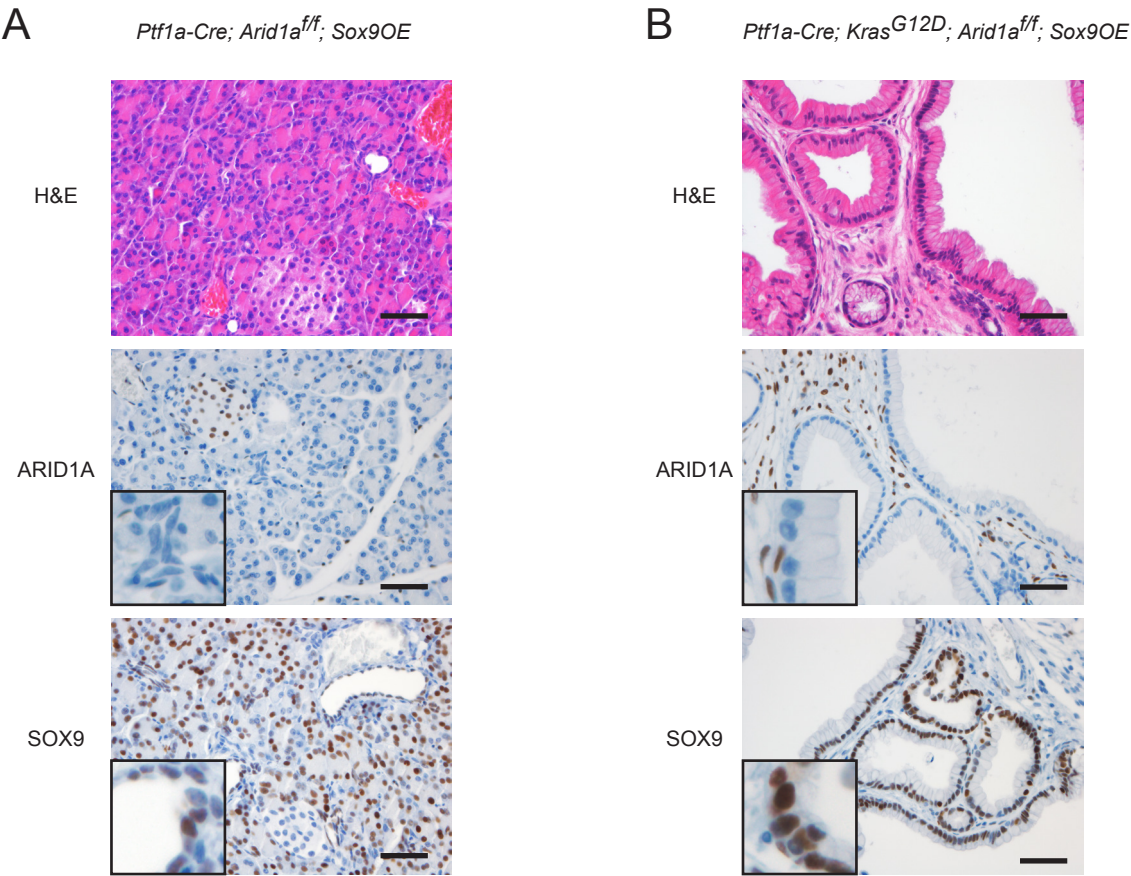
Supplementary Figure3



Supplementary Figure4



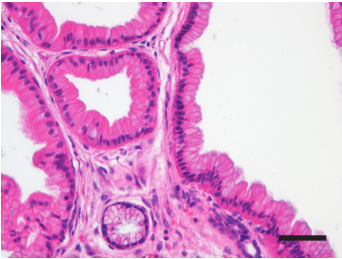
Supplementary Figure5



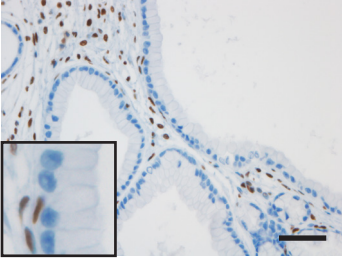
B

Ptf1a-Cre; Kras^{G12D}; Arid1a^{ff}; Sox9OE

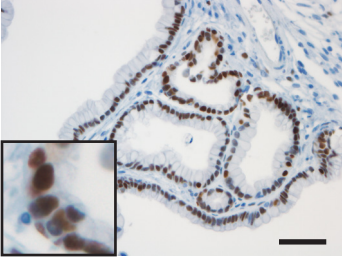
H&E



ARID1A



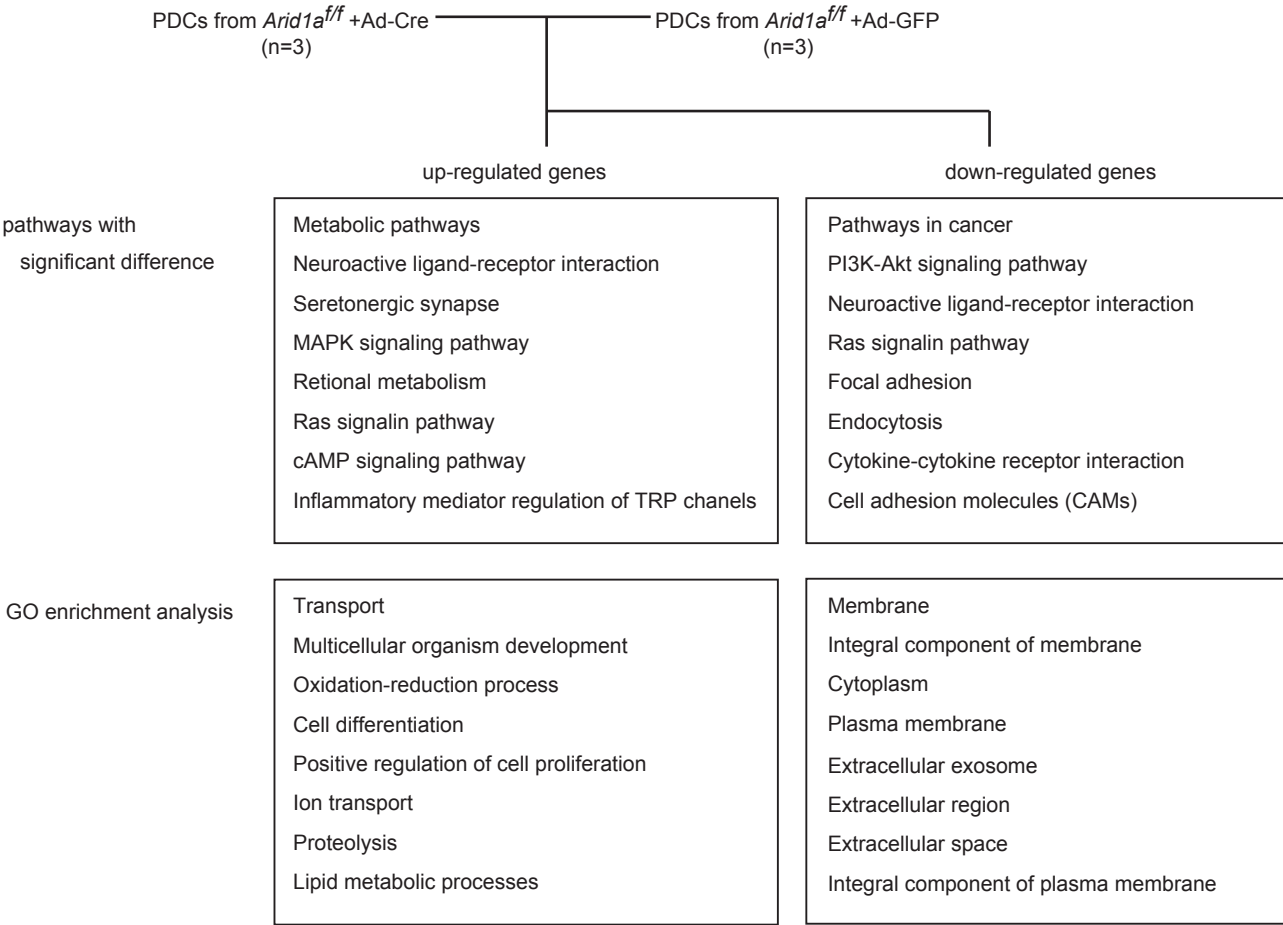
SOX9



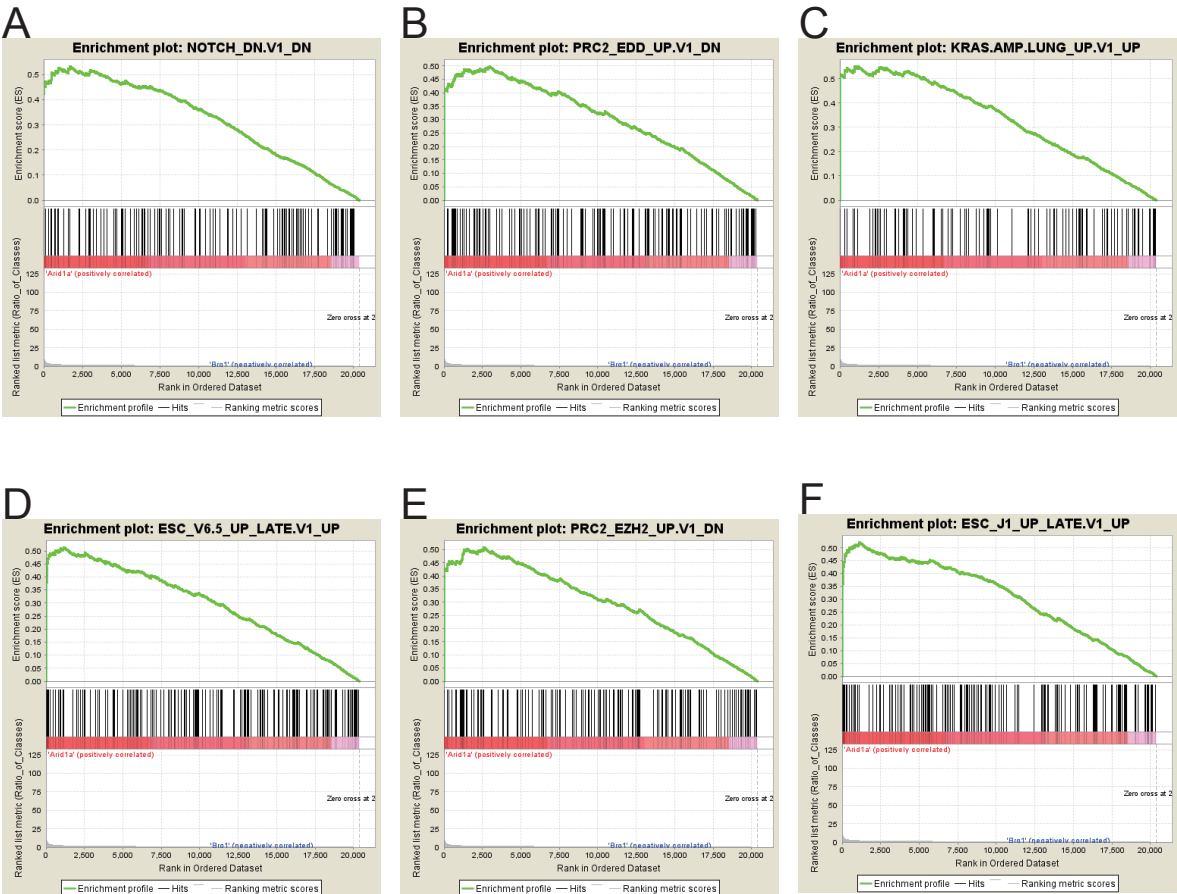
C

	<i>Ptf1a-Cre; Arid1a^{ff}</i>	<i>Ptf1a-Cre; Arid1a^{ff} ; Sox9OE</i>	<i>Ptf1a-Cre; Kras^{G12D} ; Arid1a^{ff}</i>	<i>Ptf1a-Cre; Kras^{G12D} ; Arid1a^{ff}; Sox9OE</i>
Dilation of ducts	+	-	+	+
IPMN	-	-	+	+

Supplementary Figure6



Supplementary Figure7



Supplementary Table 1. Summary of clinical and histological characteristics of human IPMN and PDAC

	IPMN			PDAC		
	ARID1A negative	ARID1A positive	<i>p</i>	ARID1A negative	ARID1A positive	<i>p</i>
number of case	13	45		16	28	
age (mean)	65.6	67.2		69.1	68.9	
sex (male/female)	3.33	1.14		3	1.8	
historological subtype						
gastric	2 (15.4%)	22 (48.9%)	*			
intestinal	7 (53.8%)	12 (26.7%)				
pancreatobiliary	1 (7.7%)	9 (20.0%)				
oncocytic	3 (23.1%)	0 (0%)	**			
clinical subtype						
branch-duct	3 (23.1%)	11 (24.4%)				
main-duct	6 (46.2%)	16 (35.6%)				
mixed	4 (30.8%)	18 (40.0%)				
adenocarcinoma	10 (76.9%)	23 (51.1%)				
immunohistochemical analysis						
SOX9 positive	5 (38.5%)	38 (84.4%)	**	9 (56.3%)	27 (96.4%)	**
pS6 positive	4 (30.8%)	30 (66.7%)	*	6 (37.5%)	17 (60.7%)	

* $p < 0.05$ ** $p < 0.01$

Supplementary Table 2. Conditions and primary antibodies for immunohistochemistry

Antibody	Supplier	Catalog number	IHC dilution
acetylated tubulin	Sigma	T7451	1:200
Amylase	Abcam	ab21156	1:1000
ARID1A	Abnova	H0000829-M02	1:200
BRG1	Santa cruz	sc-10768	1:1000
wide spectrum cytokeratin	Abcam	ab9377	1:200
Estrogen receptor α	Millipore	06-935	1:400
Ki67	Dako	M7249	1:25
MUC1	Abcam	ab15481	1:200
MUC2	Santa cruz	sc-15334	1:100
MUC5ac	Abcam	ab3649	1:100
pERK 1/2 (phospho-p44/42)	Cell Signaling	4370	1:200
PDX1	Abcam	ab47308	1:1000
progesterone receptor	Thermo	RM-9102-S0	1:1000
pSTAT3	Cell Signaling	9145	1:200
pS6 (Ser235/236)	Cell Signaling	2211	1:400
p16	Santa cruz	sc-1661	1:500
p21	Abcam	ab109199	1:250
p53	Vector Lab	VP-P956	1:500
SOX9	Millipore	AB5535	1:10000

Supplementary Table 3. Sequences of primer sets for quantitative RT-PCR

primer set	Forward	Reverse
<i>Gapdh</i>	AGGTCGGTGTGAACGGATTTG	TGTAGACCATGTAGTTGAGGTCA
<i>Arid1a</i>	GACCCCTCAGTCATCCAGTC	GAGTATGGGTAGTCCCACCA
<i>Brg1</i>	CAAAGACAAGCATATCCTAGCCA	CACGTAGTGTGTGTTAAGGACC
<i>mo-Sox9</i>	AGTACCCGCATCTGCACAAC	ACGAAGGGTCTCTTCTCGCT
<i>hu-Sox9</i>	AGCGAACGCACATCAAGAC	CTGTAGGCGATCTGTTGGGG
<i>Krt19</i>	GGGGGTTTCAGTACGCATTGG	GAGGACGAGGTACGAAGC
<i>Hnf1b</i>	GTTACTGCCGTCCCCGAATTT	CGGCTTGGTGTGCGAGATCC
<i>Gata4</i>	CCCTACCCAGCCTACATGG	ACATATCGAGATTGGGGTGTCT
<i>Gata6</i>	TTGCTCCGGTAACAGCAGTG	GTGGTCGCTTGTGTAGAAGGA
<i>Hes1</i>	ATAGCTCCCGGCATTCCAAG	GCGCGGTATTTCCCCAACA
<i>Pik3ca</i>	CCACGACCATCTTCGGGTG	ACGGAGGCATTCTAAAGTCACTA
<i>Akt3</i>	TGGGTTCAGAAGAGGGGAGAA	AGGGGATAAGGTAAGTCCACATC
<i>mTor</i>	CAGTTCGCCAGTGGACTGAAG	AGACTCCTCCTGACTCATCTCT
<i>Rictor</i>	GCTGCGCTATCTCATCCAAGA	GGGTTCTGAAGTGCTAGTTCAC
<i>Pdk1</i>	GGACTTCGGGTCAGTGAATGC	TCCTGAGAAGATTGTCGGGGA

Supplementary Materials and Methods

Histological analysis

Mouse tissues were fixed with 4% buffered paraformaldehyde solution, embedded in paraffin, and cut into 5- μ m sections. Sections were deparaffinized, rehydrated, and stained with hematoxylin and eosin (H&E) or alcian blue and counterstained with Nuclear Fast Red (Kirkegaard & Perry Laboratories). For immunohistochemistry, antigen retrieval was performed by boiling the sections in primarily 10 mM citric acid buffer with pH 6.0 for 15 min. The sections were washed with phosphate-buffered saline. Blocking was performed using Protein Block Serum-Free (Dako, catalog no. X0909). For primary antibodies, incubation was performed overnight at 4 °C in a humidified chamber; secondary antibody incubation was performed for 1 h at room temperature. Immunoperoxidase labeling was performed with the Vectastain ABC kit (Vector Laboratories, catalog no. PK-6102), and the sections were then subjected to chromophore treatment with diaminobenzidine (DAB) substrate (Dako, catalog no. K3468) and were counterstained with hematoxylin. Primary antibodies are listed in Supplementary Table 2.

18

19 ***Tamoxifen and caerulein treatment***

20 Tamoxifen (Sigma-Aldrich, St Louis, MO, USA) was administered by
21 subcutaneous injection to *Ptf1a-Cre^{ERT2}* mice at a dose of 4 mg/body weight per
22 injection and by oral gavage to *Hnf1b-Cre^{ERT2}* mice at a dose of 10 mg/body
23 weight per gavage. Time courses and the amount of injections/gavages are
24 outlined in Figure 4. Acute pancreatitis was induced by intraperitoneal injection of
25 50 µg/kg caerulein (diluted in saline; Sigma-Aldrich) on two consecutive days
26 once every hour for 8 h each day.

27

28 ***Adenoviral infection***

29 The single-cell suspension of PDCs was prepared by digesting the 2D-cultured
30 PDCs and the collagen layer with collagenase type V followed by trypsinization.
31 For adenoviral infection, PDCs were incubated at 37 °C with green fluorescent
32 protein (GFP)-expressing adenovirus (Ad-GFP) or Cre recombinase and GFP-
33 expressing adenovirus (Ad-Cre) at a multiplicity of infection of 50 on the collagen-

coated plates overnight. The PDC full medium was replaced every two days.

RNA isolation from cultured cells and quantitative RT-PCR (qPCR)

RNA was isolated using the RNeasy Mini kit (Qiagen, Valencia, CA, USA).

Single-stranded cDNA was prepared using the ReverTra Ace qPCR RT kit

(Toyobo, Osaka, Japan). qPCR was performed with a SYBR Green-based gene

expression assay using the LightCycler 480 system (Roche, Madison, WI, USA).

Expression levels were normalized to *GAPDH* levels using the ΔC_t method.

Primer sequences are described in Supplementary Table 3. All reactions were

performed in triplicates.

Laser capture micro-dissection and RNA extraction

The resected pancreas was immersed in the RNAlater solution (Ambion)

overnight at 4 °C and then embedded in Frozen Section Compound (Leica) and

stored at -80 °C. Frozen specimens were cut into 10- μ m sections using a

CM1950 cryostat (Leica) and embedded on frame slides (Leica). The sections

were fixed in a 19:1 ethanol and acetic acid mixture, incubated for 3 min on an ice bath, and then washed in ultra-pure distilled water (Invitrogen) for 1 min. For each mouse, 5–6 lesions were selected, and their RNA was extracted using the RNeasy Micro kit (Qiagen, Valencia, CA, USA). After drying, the sections were dissected using the LMD-3500 (Leica) laser-capture micro-dissection system, as previously described¹. Total RNA was extracted from the dissected tissue using the RNeasy Micro Kit (Qiagen).

Microarray analysis

The quality of RNA extracted from adenovirus-infected PDCs and epithelial cells of IPMNs was examined with an Agilent 2100 bioanalyzer (Agilent Technologies). The epithelial cells of IPMNs were obtained using a laser-capture micro-dissection system (Leica). The RNA integrity numbers of the RNA samples used in the microarray analysis were above 7. The RNA samples were labeled with SureTag Complete DNA Labeling Kit (Agilent Technologies) and hybridized to the SurePrint G3 Mouse GE 8x60K Microarray Kit (Agilent Technologies) using the

Gene Expression Hybridization Kit (Agilent Technologies). The raw data were quantified by Agilent Feature Extraction software (Agilent Technologies). Quantified data were normalized by Gene Spring 12.5 software (Agilent Technologies). Unnamed genes were excluded. As previously described, gene expression data of the epithelial cells of IPMNs were analyzed using Gene Set Enrichment Analysis (GSEA) software and the Molecular Signature Database (MSigDB), which is provided by the Broad Institute of MIT and Harvard^{2, 3}. The pathway analyses and Gene Ontology (GO) enrichment analyses of the gene expression data of adenovirus-infected PDCs were performed using DAVID 6.8.⁴

⁵

Statistical analysis

Data are presented as means $\pm$ SEM. To determine P-values for the qPCR analysis of ductal cell culture experiments, Welch's t-test was performed. To determine P-values for other experiments, Student's t-test was performed. A P-value <0.05 was considered as statistically significant. All statistical analyses

82 were performed with either Graph-Pad Prism software (version 6.0, GraphPad,

83 San Diego, CA, USA) or Microsoft Excel.

84

85 **Supplementary References**

- 86 1. Emmert-Buck MR, Bonner RF, Smith PD, et al. Laser capture
87 microdissection. Science 1996;274:998-1001.
- 88 2. Subramanian A, Tamayo P, Mootha VK, et al. Gene set enrichment
89 analysis: a knowledge-based approach for interpreting genome-wide
90 expression profiles. Proc Natl Acad Sci U S A 2005;102:15545-50.
- 91 3. Mootha VK, Lindgren CM, Eriksson KF, et al. PGC-1alpha-responsive
92 genes involved in oxidative phosphorylation are coordinately
93 downregulated in human diabetes. Nat Genet 2003;34:267-73.
- 94 4. Huang D, Sherman B, Lempicki R. Systemic and integrative analysis of
95 large gene lists using DAVID Bioinformatics Resources. Nature Protoc
96 2009;4:44-57.
- 97 5. Huang D, Sherman B, Lempicki R. Bioinformatics enrichment tools: paths
98 toward the comprehensive functional analysis of large gene lists. Nuclei
99 Acids Res 2009;37:1-13.

101 **Supplementary Figure 1.**

102 ***Arid1a* deletion does not suppress PanIN formation.**

103 (A) PanIN lesions were frequently observed between IPMN in *Ptf1a-Cre*;

104 *Kras*^{G12D}; *Arid1a*^{ff} mice.

105 (B) Magnified image of (A).

106 (C) Alcian blue staining

107 Scale Bar = 50 μm

108

109 **Supplementary Figure 2.**

110 **Cystic lesions in *Ptf1a-Cre*; *Kras*^{G12D}; *Arid1a*^{ff} mice do not resemble those**

111 **of human MCN.**

112 (A) Estrogen receptor staining in the uterus isolated from wild type (WT) mice.

113 (B) Estrogen receptor staining in the pancreas isolated from *Ptf1a-Cre*; *Kras*^{G12D};

114 *Arid1a*^{ff} mice.

115 (C) Progesterone receptor staining in the uterus isolated from WT mice.

116 (B) Progesterone receptor staining in the pancreas isolated from *Ptf1a-Cre*;

117 *Kras^{G12D}*; *Arid1a^{ff}* mice.

118 Scale Bar = 50 μ m

119

120 **Supplementary Figure 3.**

121 **Histological analysis of IPMN in *Ptf1a-Cre*; *Kras^{G12D}*; *Arid1a^{ff}* mice and**

122 **PanIN in *Ptf1a-Cre*; *Kras^{G12D}* mice.**

123 (A) Phospho-Stat3, (B) Pdx1, (C) p16, (D) p21, (E) p53, and (F) Smad4 staining.

124 Scale Bar = 50 μ m

125

126 **Supplementary Figure 4.**

127 **Overall survival analysis of *Ptf1a-Cre*; *Kras^{G12D}*; *Arid1a^{ff}* and *Ptf1a-Cre*;**

128 ***Kras^{G12D}*; *Arid1a^{ff}*/+ mice**

129 Overall survival analysis of the indicated mouse genotypes. Kaplan-Meier survival

130 curves and the log-rank test showed no significant difference in the survival rates

between *Ptf1a-Cre; Kras^{G12D}; Arid1a^{ff}* mice (red, n = 52) and *Ptf1a-Cre; Kras^{G12D}; Arid1a^{ff/+}* mice (blue, n = 16).

Supplementary Figure 5.

Sox9 overexpression offsets the effect of *Arid1a* deletion on pancreatic ductal dilation *in vivo*.

(A) Histological analysis of *Ptf1a-Cre; Arid1a^{ff}; Sox9OE* mice at the age of 9-20 weeks (n = 3). H&E staining and immunohistochemistry for Arid1a and Sox9.

(B) Histological analysis of *Ptf1a-Cre; Kras^{G12D}; Arid1a^{ff}; Sox9OE* mice at the age of 4-8 weeks (n = 4). H&E staining and immunohistochemistry for Arid1a and Sox9.

(C) Summary of the phenotypes in terms of pancreatic ductal dilation and IPMN development of *Ptf1a-Cre; Arid1a^{ff}*, *Ptf1a-Cre; Arid1a^{ff}; Sox9OE*, *Ptf1a-Cre; Kras^{G12D}; Arid1a^{ff}* and *Ptf1a-Cre; Kras^{G12D}; Arid1a^{ff}; Sox9OE* mice.

Scale Bar = 50 μ m

147 **Supplementary Figure 6.**

148 **Schema of the microarray analysis of *Arid1a*-deficient and wild type (WT)**
149 **pancreatic ductal cells.**

150 Pathway analysis and GO enrichment analysis using DAVID 6.8 revealed
151 up/down-regulated pathways or functions by knockdown of *Arid1a* in pancreatic
152 ductal cells. The top eight pathways or GO terms that were most affected by
153 knockdown of *Arid1a* are listed in the boxes.

154

155 **Supplementary Figure 7.**

156 **Gene set enrichment analysis revealed sets of genes that were differently**
157 **expressed in *Arid1a*-deficient IPMN compared with *Brg1*-deficient IPMN.**

158 (A) NOTCH_DN.V1_DN; genes downregulated in MOLT4 cells (T-ALL) by DAPT
159 [PubChem ID: 16219261], an inhibitor of the NOTCH signaling pathway.

160 (B) PRC2_EDD_UP.V1_DN; genes downregulated in TIG3 cells (fibroblasts) on
161 knockdown of embryonic ectoderm development (EED).

162 (C) KRAS.AMP.LUNG_UP.V1_UP; genes upregulated in epithelial lung cancer

163 cell lines overexpressing KRAS.

164 (D) ESC_V6.5_UP_LATE.V1_UP; genes upregulated during the late stages of

165 differentiation of embryoid bodies from V6.5 embryonic stem cells.

166 (E) PRC2_EZH2_UP.V1_DN; genes downregulated in TIG3 cells (fibroblasts) on

167 knockdown of enhancer of zeste homolog 2 (EZH2).

168 (F) ESC_J1_UP_LATE.V1_UP; genes upregulated during the late stages of

169 differentiation of embryoid bodies from J1 embryonic stem cells.

170

171

172 **Supplementary Table 1**

173 **Summary of clinical and histological characteristics of human IPMN and**

174 **PDAC**

175

176 **Supplementary Table 2**

177 **Conditions and primary antibodies for immunohistochemistry**

178

179 **Supplementary Table 3**

180 **Sequences of primer sets for quantitative RT-PCR**