Supplementary Information

The Lin28b/Wnt5a axis drives pancreas cancer through crosstalk between cancer associated fibroblasts and tumor epithelium

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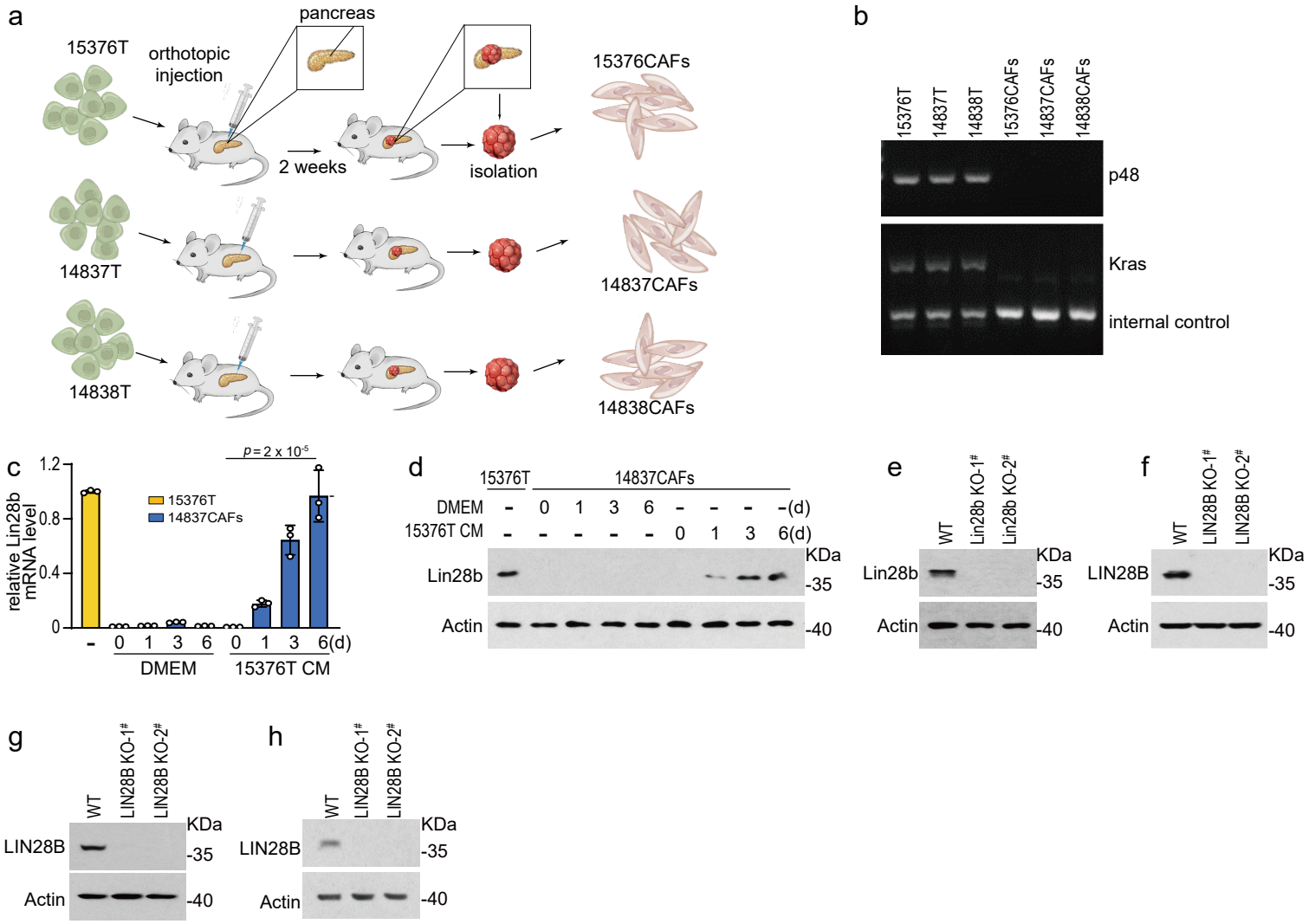
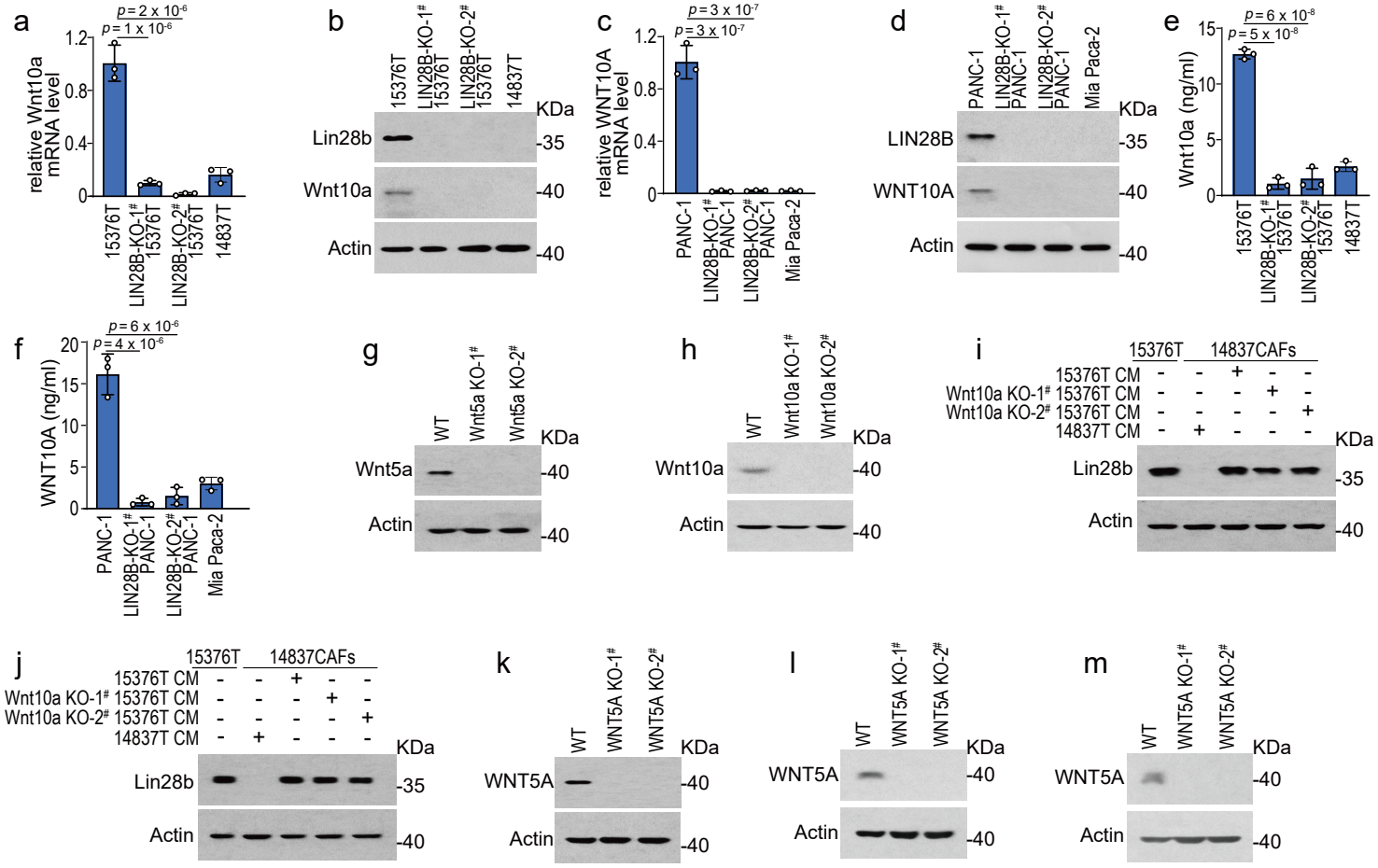


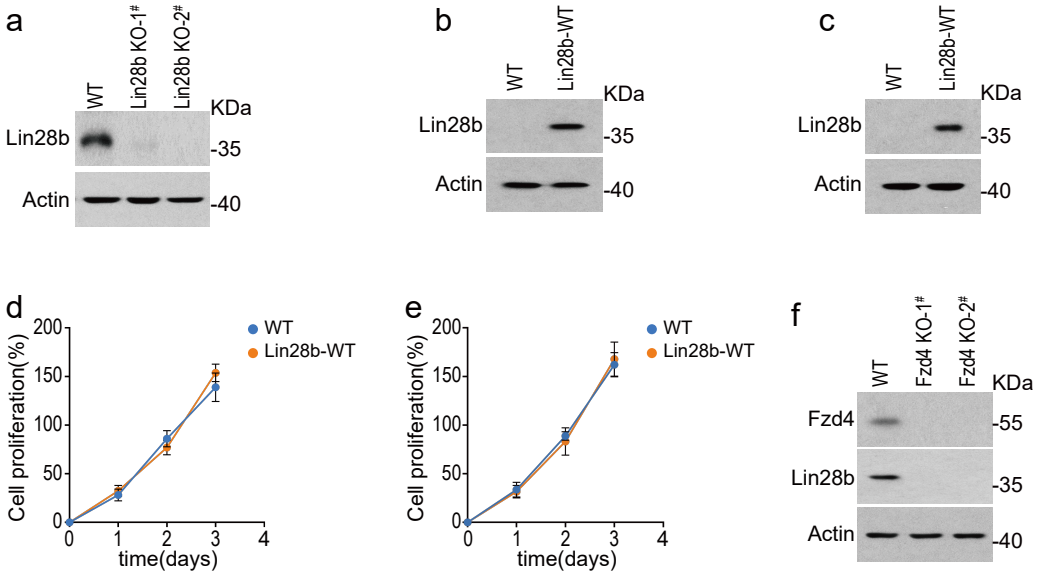
Supplementary Fig.1 Lin28b expression in CAFs is induced by conditioned medium from Lin28b^{high} tumor cells.

(a) 14837T, 14838T, or 15376T cells were orthotopically injected into C57BL/6J mice. After 2 weeks, mCAFs (14837CAFs, 14838CAFs and 15376CAFs) were isolated from the indicated tumors. (b) The genotype of 14837T, 14838T, 15376, 14837CAFs, 14838CAFs and 15376CAFs. (c-d) 14837CAFs were cultured with DMEM or 15376T-CM for 6 days. Then, Lin28b levels were measured by real-time qPCR (c) and western blotting (d). Lin28b levels in 15376T were included as a positive control. Representative of n=3 independent experiments (d). (e-h) Western blotting was used to detect the knockout efficiency of Lin28b in 15376T (e), PANC-1 (f), PANC03.27 (g) and hPDAC1# (h). Representative of n=2 independent experiments.Three biologically independent experiments were performed (c). Data are shown as mean ± s.d. P-value were determined by one-way ANOVA with Tukey's multiple comparison test (c).



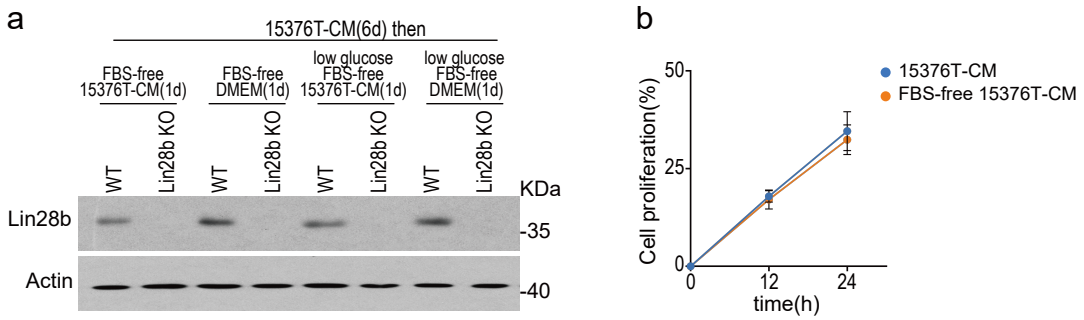
Supplementary Fig.2 Lin28b^{high} PDAC cells secret Wnt5a to induce Lin28b expression in CAFs.

(a-b) The levels of Wnt10a in 15376T, Lin28b-KO 15376T and 14837T were measured by real-time qPCR (a) and western blotting (b). Representative of n = 2 independent experiments. (c-d) The levels of WNT10A in PANC-1, LIN28B-KO PANC-1 and Mia Paca-2 were measured by real-time qPCR (c) and western blotting (d). Representative of n = 2 independent experiments. (e) Wnt10a levels in the supernatants of 15376T, Lin28b-KO 15376T and 14837T were examined by ELISA. (f) WNT10A levels in the supernatants of PANC-1, LIN28B-KO PANC-1 and Mia Paca-2 were examined by ELISA. (g-h) Western blotting was used to detect the knockout efficiency of Wnt5a (g) and Wnt10a (h) in 15376T. Representative of n = 2 independent experiments. (i-j) 14837CAFs (i) or 15376CAFs (j) were cultured with 15376T-CM or Wnt10a-KO 15376T-CM for 6 days. Then, Lin28b levels were measured by western blotting. Lin28b level in 15376T were included as a positive control. Representative of n = 3 independent experiments. (k-m) Western blotting was used to detect the knockout efficiency of WNT5A in PANC-1 (k), PANC03.27 (l) and hPDAC1# (m). Representative of n = 2 independent experiments.Three biologically independent experiments were performed (a, c, e, f). Data are shown as mean ± s.d. P-value were determined by one-way ANOVA with Tukey's multiple comparison test (a, c, e, f).



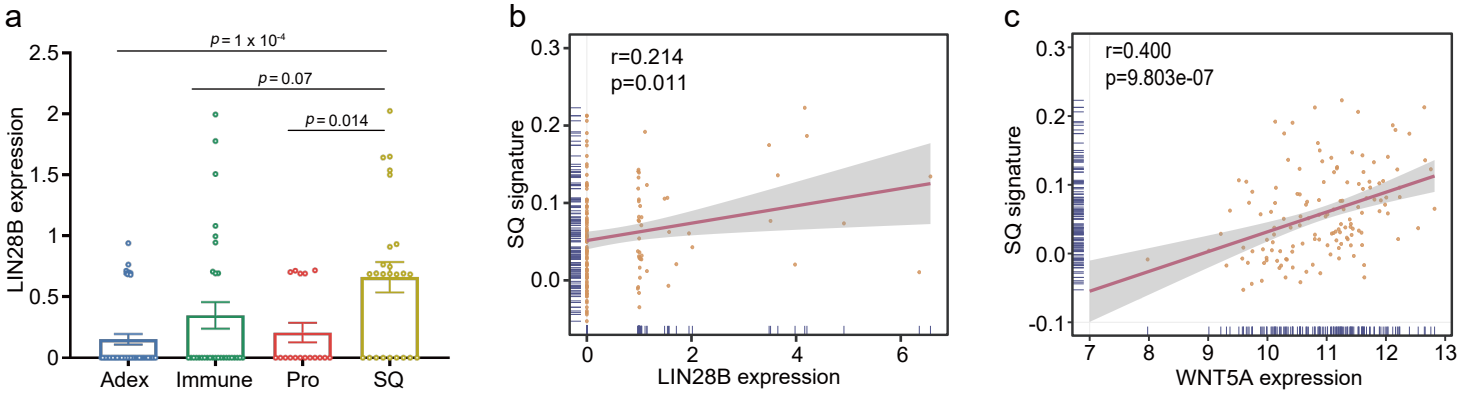
Supplementary Fig.3 Lin28b-positive CAFs promote growth of pancreatic cancer.

Before the experiment started, CAFs were cultured with 15376T-CM for 6 days to induce Lin28b expression. CM for CAFs culture was replaced daily (a, d). (a) Western blotting was used to detect the knocking-out efficiency of Lin28b in 15376CAFs. Representative of n = 2 independent experiments. (b-c) Lin28b-WT was stable expressed in 14837CAFs (b) and 15376CAFs (c). Western blotting was used to detect Lin28b expression efficiency. Representative of n=2 independent experiments. (d) 14837CAFs or Lin28b-WT 14837CAFs were cultured for 1, 2, or 3 days. The cells were counted to calculate the cell proliferation. (e) 15376CAFs or Lin28b-WT 15376CAFs were cultured for 1, 2, or 3 days. The cells were counted to calculate the cell proliferation. (f) Western blotting was used to detect the knocking-out efficiency of Fzd4 in 15376CAFs. Representative of n = 2 independent experiments.Three biologically independent experiments were performed (d, e). Data are shown as mean ± s.d. P-value were determined by one-way ANOVA with Tukey's multiple comparison test (d, e).



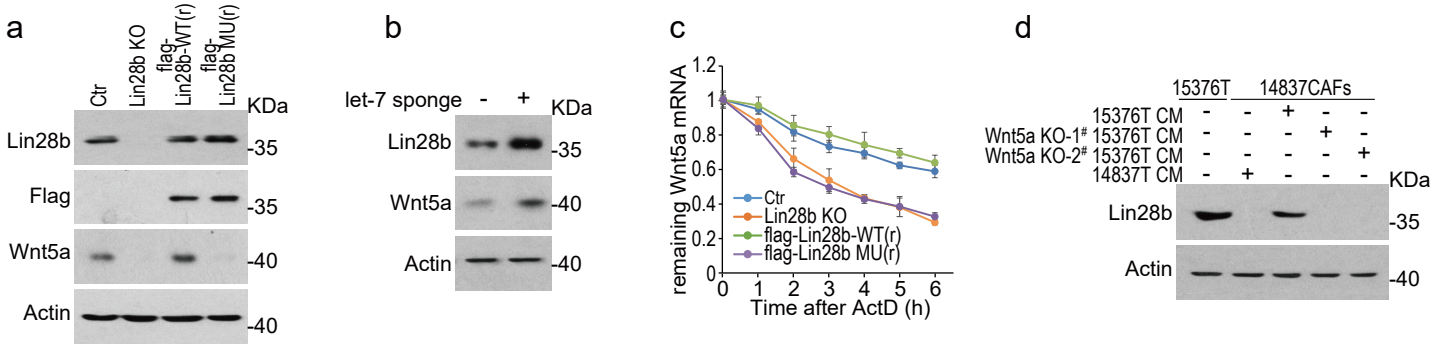
Supplementary Fig.4 Pcsk9 secreted by CAFs promotes PDAC growth.

(a) 15376CAFs and Lin28b-KO 15376CAFs were cultured with 15376T-CM for 6 days. Then, they were cultured with FBS-free 15376T-CM, FBS-free DMEM, low glucose FBS-free 15376T-CM or low glucose FBS-free DMEM. After 1 day, the levels of Lin28b were measured by western blotting. Representative of n = 2 independent experiments. (b) 15376CAFs were cultured with 15376T-CM or FBS-free 15376T-CM for 24 h. The cells were counted to calculate the cell proliferation. Three biologically independent experiments were performed (b). Data are shown as mean ± s.d. P-value were determined by one-way ANOVA with Tukey's multiple comparison test (b).



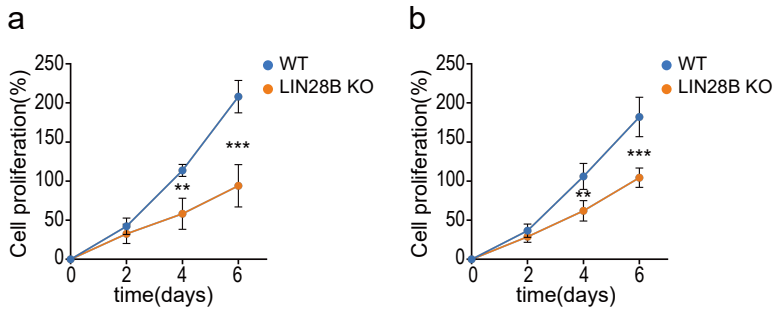
Supplementary Fig.5 Correlation between Lin28b-Wnt5a axis and squamous subtype tumors.

(a) LIN28B expression among human PDAC subtypes in PDAC RNA-seq dataset (dbGaP Study Accession: phs001287); n = 140 patients. ADEX, aberrantly differentiated endocrine exocrine subtype; Immu, immunogenic subtype; Pro, progenitor subtype; SQ, squamous subtype. P-value were determined by one-way ANOVA with Tukey's multiple comparison test. (b) Correlation between LIN28B expression and squamous subtype signature in PDAC RNA-seq dataset (dbGaP Study Accession: phs001287); n = 140 patients. (Pearson product-moment correlation test; r=0.214, p=0.011). (c) Correlation between WNT5A expression and squamous subtype signature in PDAC RNA-seq dataset (dbGaP Study Accession: phs001287); n = 140 patients. (Pearson product-moment correlation test; r=0.400, p=9.803e-07).



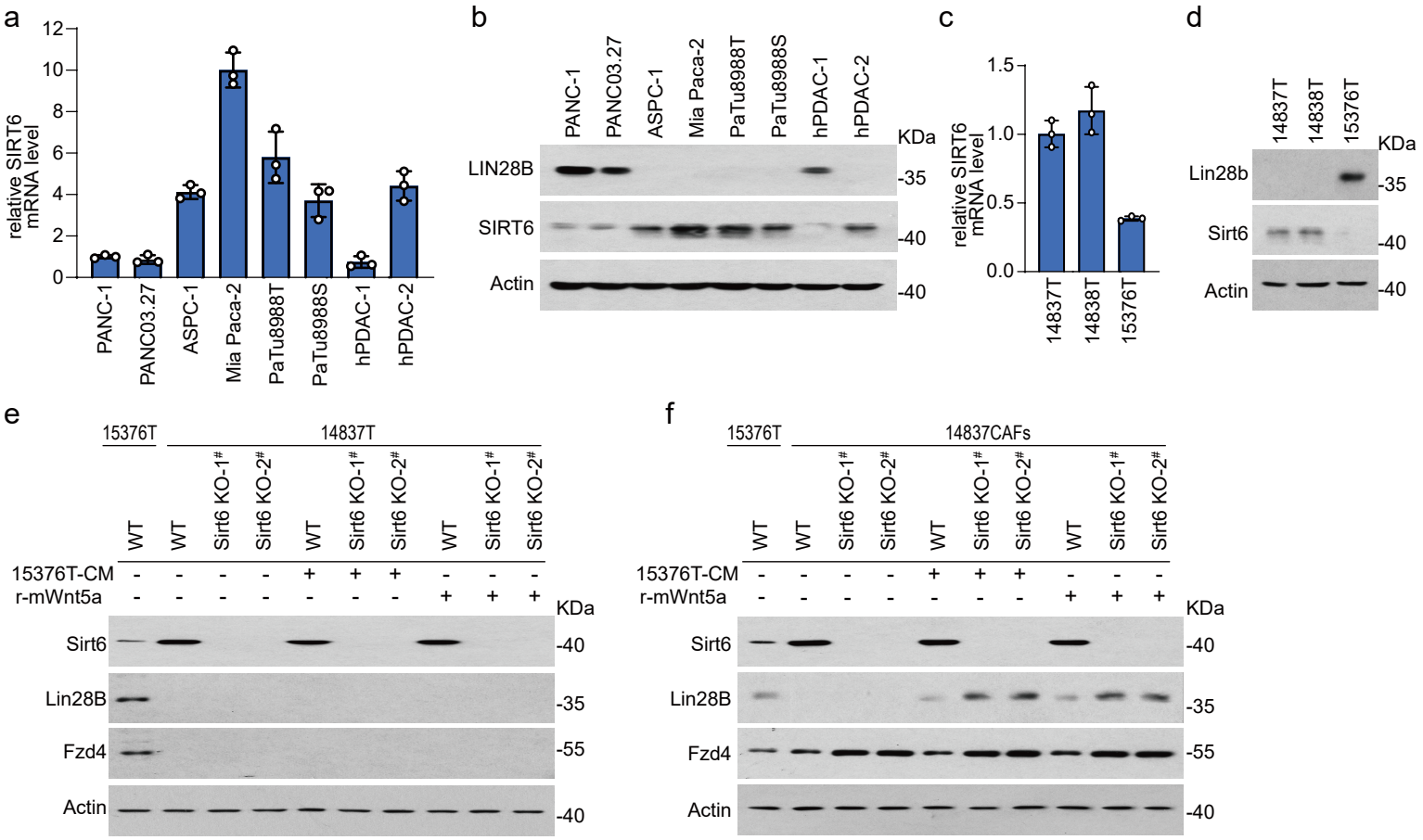
Supplementary Fig.6 Wnt5a was regulated by Lin28b/let-7 pathway.

(a) The levels of Wnt5a in 15376T, Lin28b-KO 15376T, flag-Lin28b-WT(r)-expressing 15376T and flag-Lin28b-MU(r)-expressing 15376T were measured by western blotting. Representative of n = 3 independent experiments. (b) 15376T were transfected with let-7 sponge vector. The protein levels of Lin28b and Wnt5a were measured by western blotting. Representative of n=3 independent experiments. (c) The mRNA stability of Wnt5a in 15376T, Lin28b-KO 15376T, flag-Lin28b-WT(r)-expressing 15376T and flag-Lin28b-MU(r)-expressing 15376T were measured by real-time qPCR (n = 3 independent experiments). Data are shown as mean ± s.d. P-value were determined by one-way ANOVA with Tukey's multiple comparison test. (d) 14837CAFs were cultured with 15376T-CM or Wnt5a-KO 15376T-CM under low glucose (2mM glucose) for 2 days. Then Lin28b levels were measured by western blotting. Lin28b levels in 15376T were included as a positive control. Representative of n = 3 independent experiments.



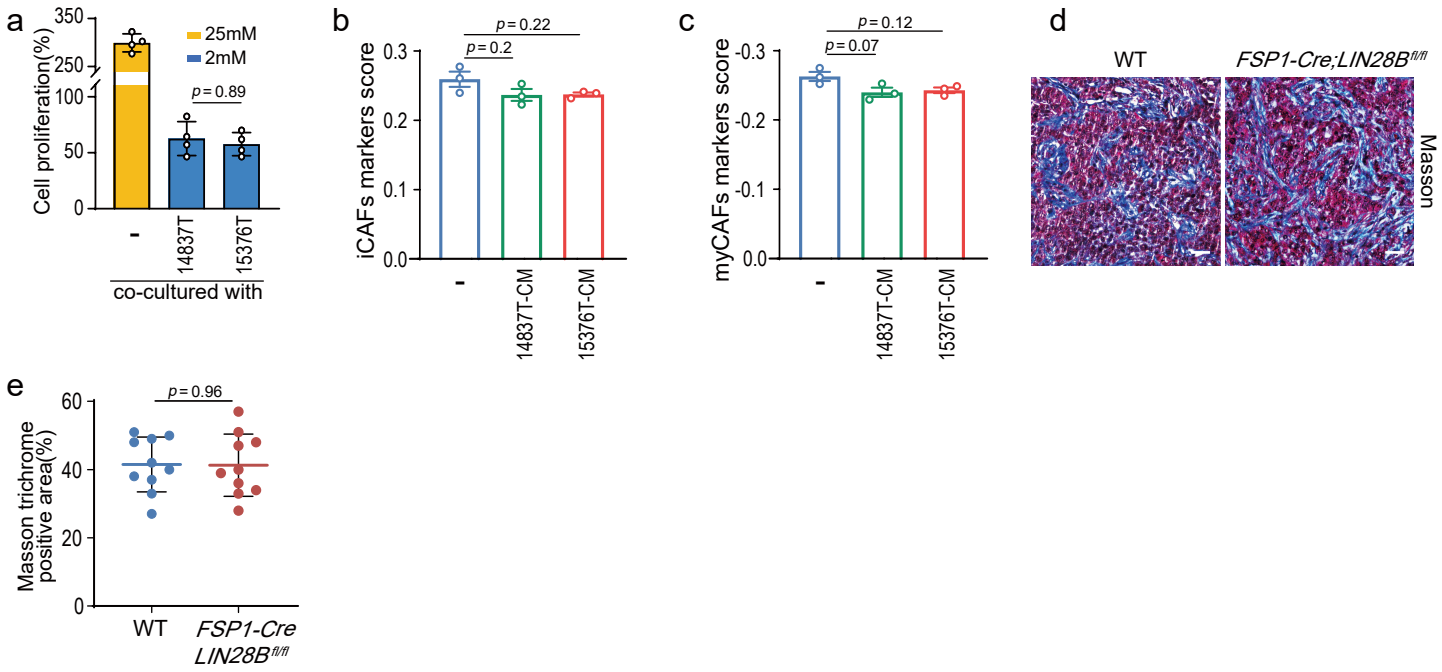
Supplementary Fig.7 Lin28b expression promoted human PDAC cell growth.

(a) PANC-1 or Lin28b-KO PANC-1 were cultured for 2, 4, or 6 days. The cells were counted to calculate the cell proliferation. (b) PANC03.27 or Lin28b-KO PANC03.27 were cultured for 2, 4, or 6 days. The cells were counted to calculate the cell proliferation. Three biologically independent experiments were performed (a, b). Data are shown as mean ± s.d. Significance levels are denoted as *$p<0.05$; **$p<0.01$; ***$p<0.001$.P-value were determined by one-way ANOVA with Tukey's multiple comparison test (a, b).



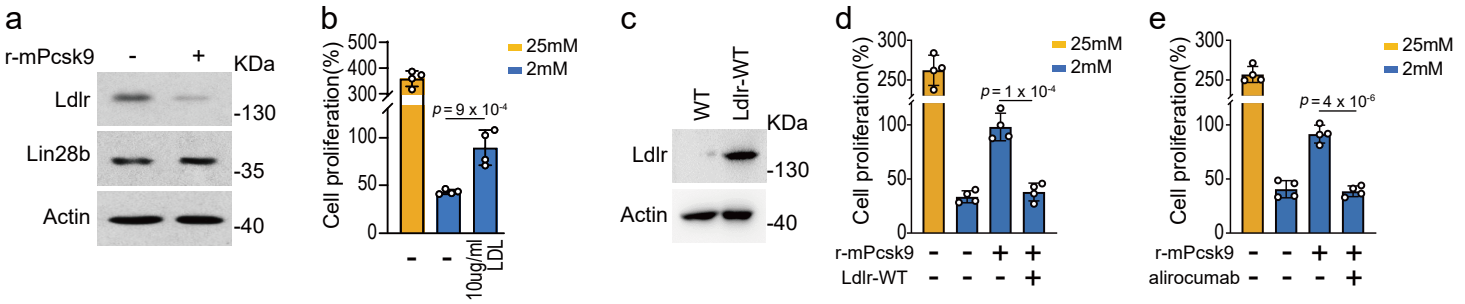
Supplementary Fig.8 The effect of Sirt6 on Lin28b expression.

(a-b) The levels of SIRT6 in human PDAC cell lines were measured by real-time qPCR (a) and western blotting (b). Representative of n = 3 independent experiments (b). (c-d) The levels of Sirt6 in mouse PDAC cell lines were measured by real-time qPCR (c) and western blotting (d). Representative of n = 3 independent experiments (d). (e-f) 14837T (e) or 14837CAFs (f) were treated with 15376T-CM or 100ng/ml r-mWnt5a for 6 days. Then, the levels of Sirt6, Lin28b and Fzd4 were measured by western blotting. Representative of n = 3 independent experiments. Three biologically independent experiments were performed (a, c). Data are shown as mean ± s.d. P-value were determined by one-way ANOVA with Tukey's multiple comparison test (a, c).



Supplementary Fig.9 Lin28b expression in tumors did not affect the survival and subtype switch of CAFs.

(a) 15376CAFs were co-cultured with 14837T or 15376T in a transwell chamber for 2 days. The cells were counted to calculate the cell proliferation. (b-c) 15376CAFs treated with 14837T-CM or 15376T-CM. iCAFs markers score (b) and myCAFs markers score (b) were shown. (d-e) 15376T cells were orthotopically injected into WT or FSP-Cre;Lin28b$^{fl/fl}$ mice (n=6 mice). After 2 weeks, the tumors were analyzed by Masson trichrome staining. Scale bars: 30 μM (d). Quantification of Masson trichrome-positive area in WT or FSP-Cre;Lin28b$^{fl/fl}$ mice (n=10 views per group) (e). Four biologically independent experiments were performed (a). Three biologically independent experiments were performed (b, c). Data are shown as mean ± s.d. P-value were determined by one-way ANOVA with Tukey's multiple comparison test (a-c) or two-tailed unpaired Student's t-tests (e).



Supplementary Fig.10 Pcsk9 decreased the protein level of Ldlr.

(a) Tumors were treated with 100ng/ml r-mPcsk9 for 2 days. Then, the levels of Ldlr and Lin28b were measured by western blotting. Representative of n = 3 independent experiments. (b) 15376T were treated with 10ug/ml LDL under low glucose (2 mM) for 2 days. The cells were counted to calculate the cell proliferation. (c) Ldlr-WT was stable expressed in 15376T. Western blotting was used to detect Ldlr expression efficiency. Representative of n = 2 independent experiments. (d) 15376T and Ldlr-WT 15376T were treated with 100ng/ml recombinant Pcsk9 (r-mPcsk9) under low glucose (2 mM) for 2 days. The cells were counted to calculate the cell proliferation. (e) 15376T were treated with 100ng/ml recombinant Pcsk9 (r-mPcsk9) in the presence or absence of alirocumab (200 nM) under low glucose (2 mM) for 2 days. The cells were counted to calculate the cell proliferation. Four biologically independent experiments were performed (b, d, e). Data are shown as mean±s.d. P-value were determined by one-way ANOVA with Tukey's multiple comparison test (b, d, e).