Expanded View Figures

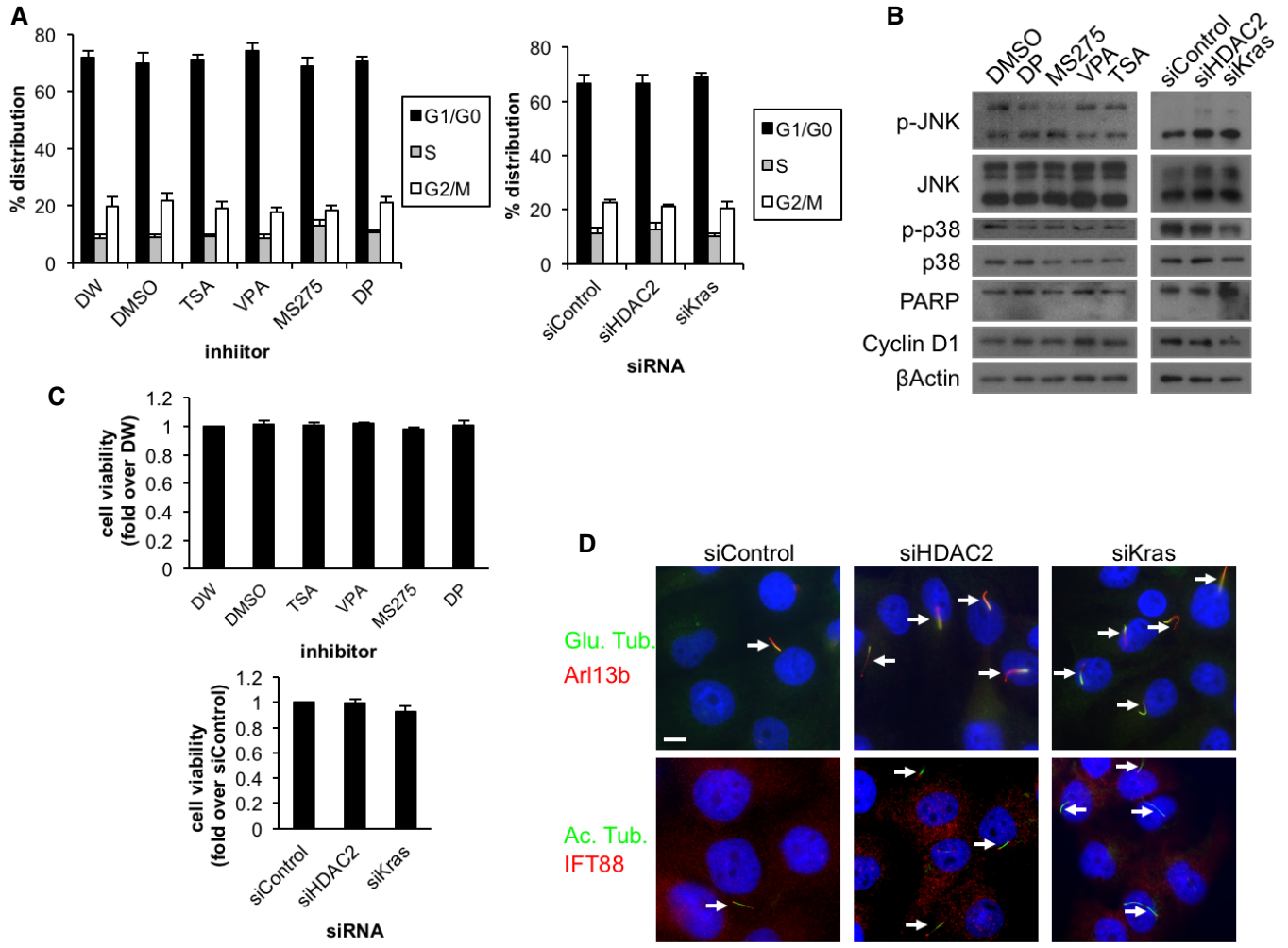

Figure EV1. Depletion or inhibition of HDAC2 does not impact on cell cycle, cell growth, and expression of various signaling markers in serum-starved PDAC cells.

A–C Panc1 cells were treated with the indicated inhibitors or siRNAs and cultured in serum-starved medium for 48 h. (A) FACS analysis. Average of five to six (inhibitor) or three (knockdown) independent experiments is shown. (B) Cell extracts were immunoblotted with antibodies against phospho-JNK, JNK, phospho-p38, p38, PARP, and cyclin D1. β-Actin was used as a loading control. (C) MTT cell viability analysis. Average of three (inhibitor) or five (knockdown) independent experiments is shown. Error bars represent SEM.

D Panc1 cells transiently transfected with control, HDAC2#2, or Kras siRNA were cultured in serum-starved medium for 48 h and immunostained with antibodies to (upper row) glutamylated tubulin (green) and Arl13b (red), and (lower row) acetylated tubulin (green) and IFT88 (red). DNA was stained with Hoechst (blue). Scale bar, 10 μm. Arrows indicate primary cilia.

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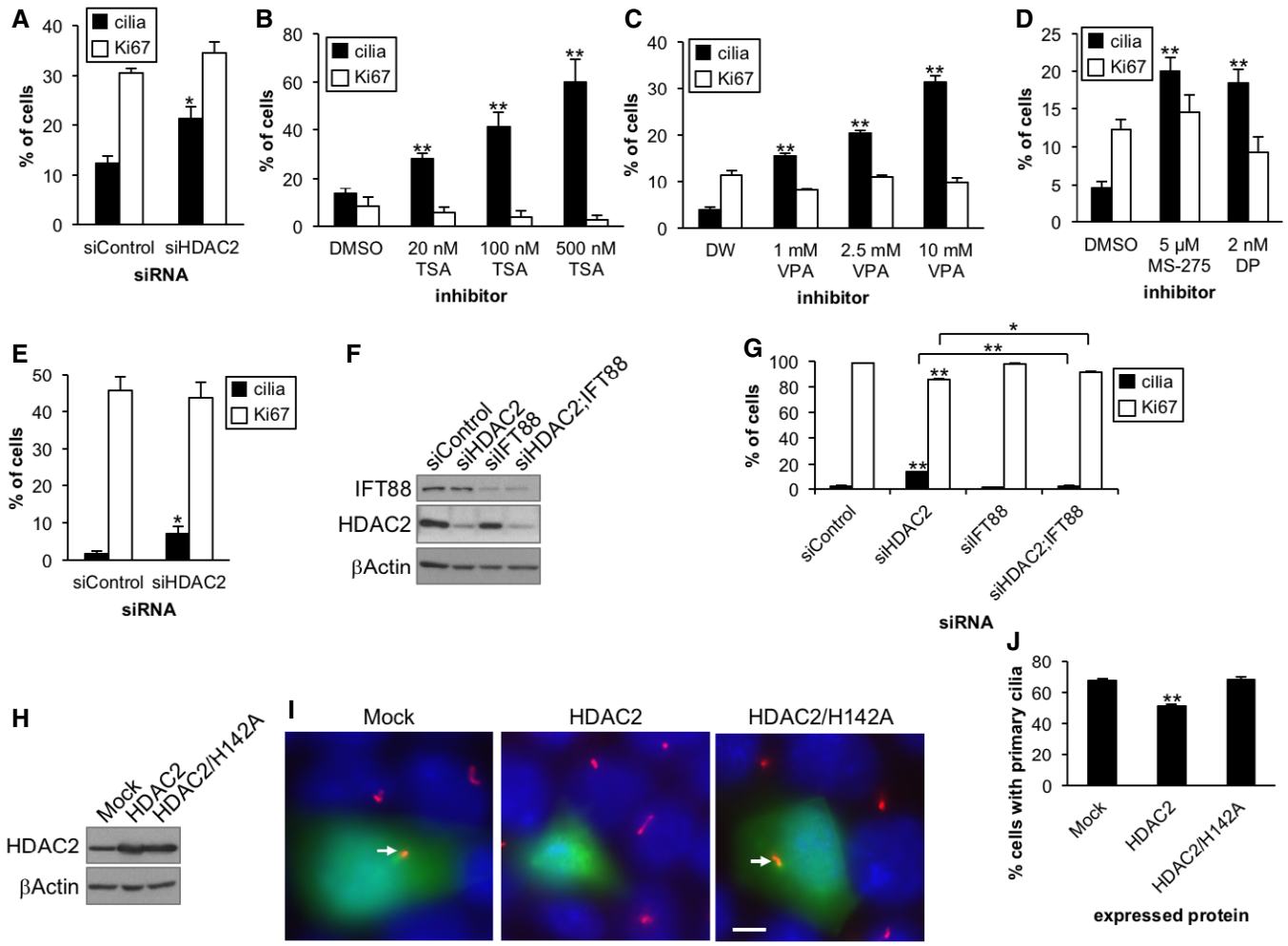

Figure EV2. Depletion or inhibition of HDAC2 restores primary cilia in PDAC cells.

A CFPAC1 cells transiently transfected with control or HDAC2#2 siRNA were cultured in serum-starved medium for 48 h. The percentages of cells with primary cilia or Ki67-positive nuclei were determined as described in Fig 1. Average of three independent experiments is shown.

B–D KrasPDEC cells in serum-starved medium were incubated with the indicated concentration of (B) TSA, (C) VPA, and (D) MS-275 or depsipeptide for 24 h. The percentages of cells with primary cilia or Ki67-positive nuclei were determined as described in Fig 1. Average of three to four independent experiments is shown.

E U87-MG cells transiently transfected with control or HDAC2#2 siRNA were cultured in serum-starved medium for 48 h. The percentages of cells with primary cilia or Ki67-positive nuclei were determined as described in Fig 1. Average of three independent experiments is shown.

F, G RPE1 cells transiently transfected with control, HDAC2#2, IFT88 or HDAC2#2, and IFT88 siRNA were cultured in serum-containing medium for 48 h. (F) Cell extracts were immunoblotted with antibodies against IFT88 and HDAC2. β-Actin was used as a loading control. (G) The percentages of ciliated or Ki67-positive cells were determined as described in Fig 1. Average of three independent experiments is shown.

H–J IMCD3 cells transiently transfected with plasmids expressing GFP and mock, HDAC2 or HDAC2/H142A were cultured without serum for 72 h. (H) Cell extracts were immunoblotted with an antibody against HDAC2. β-Actin was used as a loading control. (I) Cells were immunostained with an anti-Arl13b antibody (red). DNA was stained with Hoechst (blue). Arrows indicate primary cilia in GFP-positive cells. Scale bar, 5 μm. (J) The percentages of GFP-positive IMCD3 cells with primary cilia were determined. Average of three independent experiments is shown.

Data information: Error bars represent SEM. *$P < 0.05$, **$P < 0.01$ compared with siControl (A, E, G), DMSO (B, D), DW (C), or mock (J) (two-tailed Student's *t*-test).

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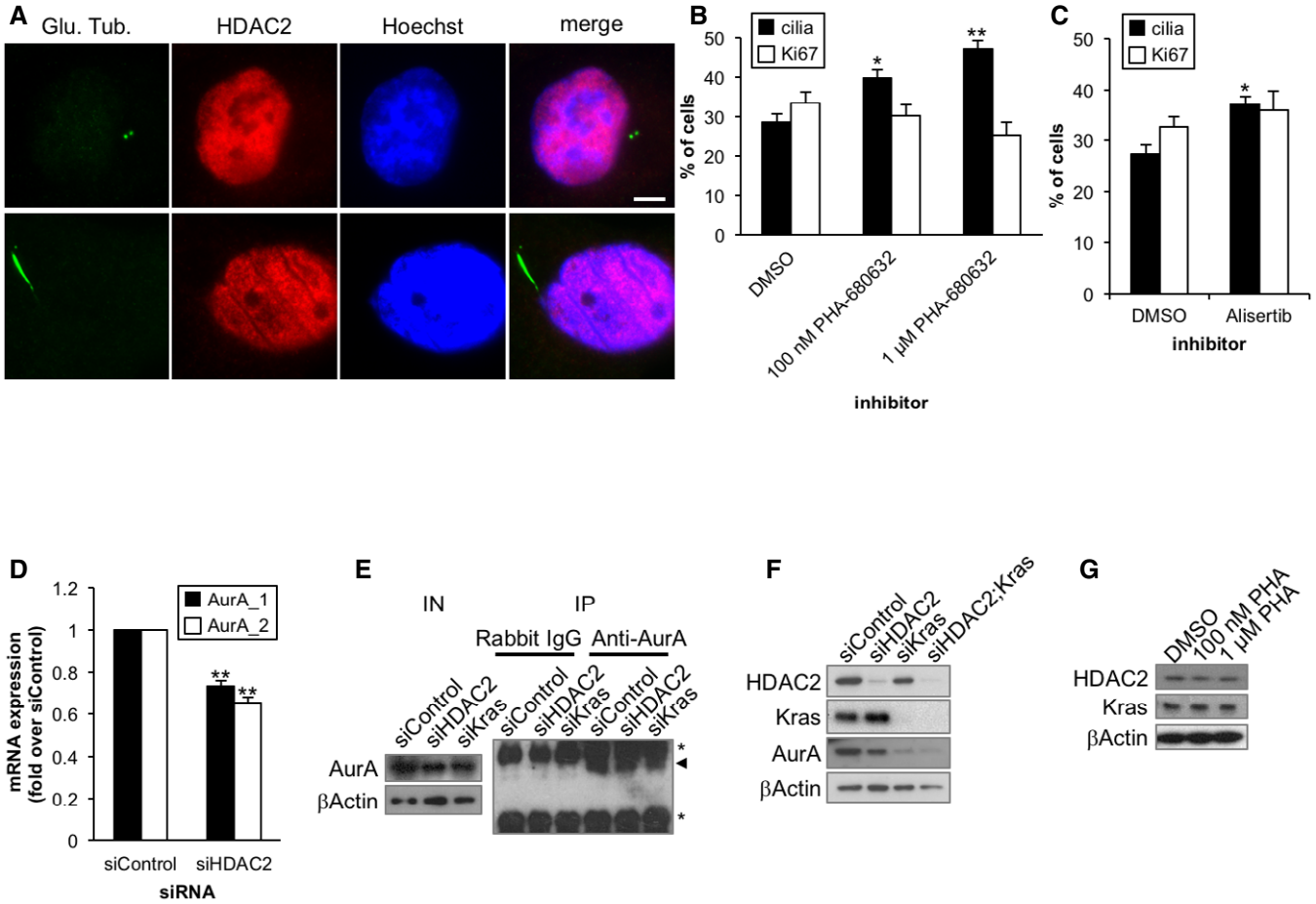

Figure EV3. Aurora A contributes to suppress primary ciliogenesis in PDAC cells.

A Panc1 cells were visualized with antibodies against glutamylated tubulin (GT335)(green) and HDAC2 (red). DNA was stained with Hoechst (blue). Scale bar, 5 μm.

B, C Panc1 cells in serum-starved medium were treated with the indicated concentration of PHA-680632 (B) or 10 nM alisertib (C) for 48 h. The percentages of cells with primary cilia or Ki67-positive nuclei were determined as described in Fig 1. Average of three independent experiments is shown.

D CFPAC1 cells transiently transfected with control or HDAC2#2 siRNA were cultured in serum-starved medium for 48 h. Relative amount of Aurora A mRNA was determined using quantitative PCR and GAPDH was used as a control. Average of three independent experiments is shown.

E Cell extracts and immunoprecipitated fractions were immunoblotted with an anti-Aurora A antibody. Asterisks and arrowhead indicate IgG and Aurora A, respectively. β-Actin was used as a loading control.

F Panc1 cells transiently transfected with control, HDAC2#2, Kras, or HDAC2#2 and Kras siRNA were cultured in serum-starved medium for 48 h. Cell extracts were immunoblotted with antibodies against Kras, HDAC2, and Aurora A. β-Actin was used as a loading control.

G Panc1 cells in serum-starved medium were treated with the indicated concentration of PHA-680632 for 48 h. Cell extracts were immunoblotted with antibodies against Kras and HDAC2. β-Actin was used as a loading control.

Data information: Error bars represent SEM. *$P < 0.05$, **$P < 0.01$ compared with DMSO (B, C) or siControl (D) (two-tailed Student's *t*-test).

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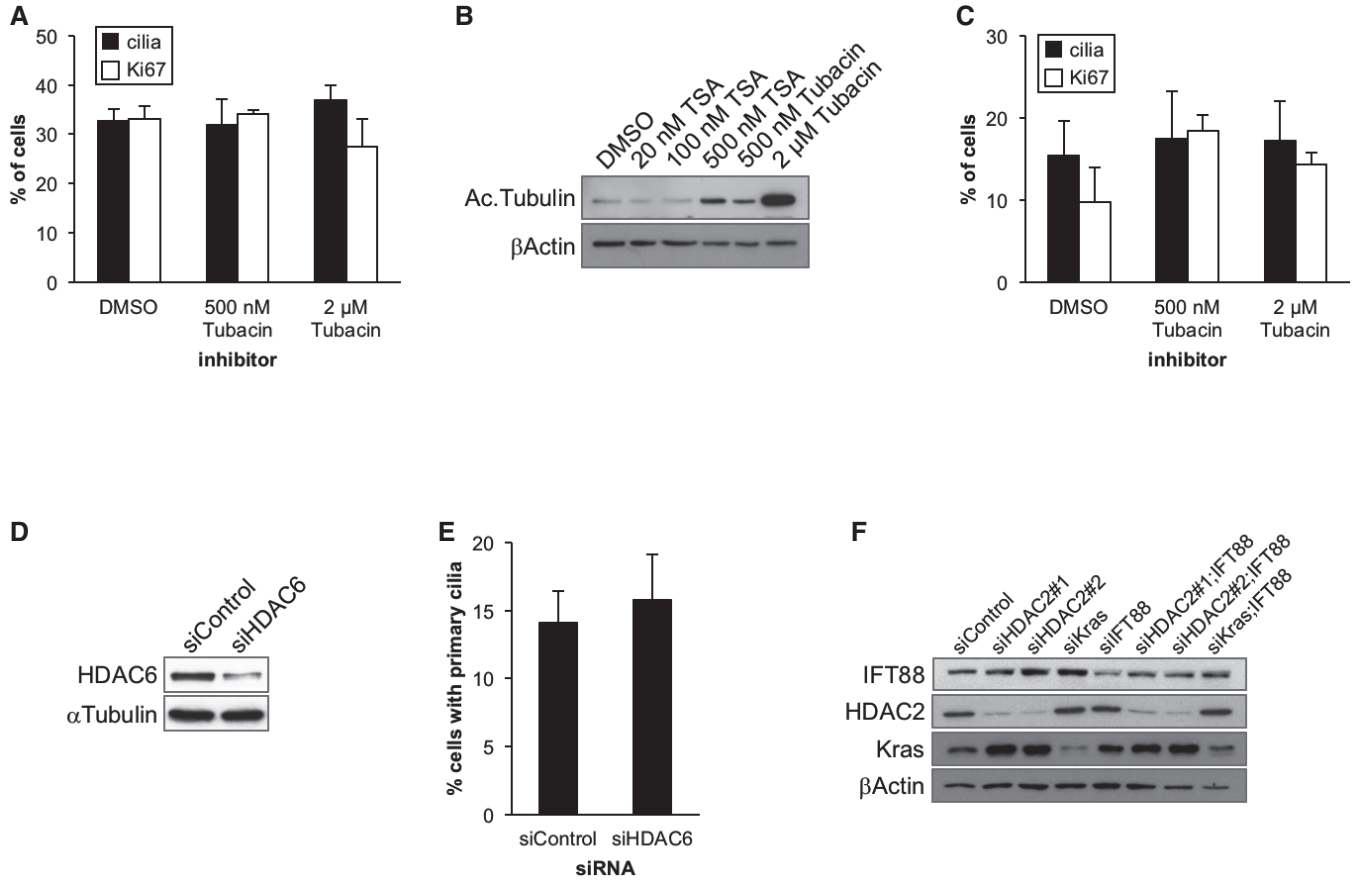

Figure EV4. HDAC6 is not involved in primary cilia formation in PDAC cells.

A, B Panc1 cells in serum-starved medium were treated with the indicated inhibitors for 48 h. (A) The percentages of cells with primary cilia or Ki67-positive nuclei were determined as described in Fig 1. Average of three independent experiments is shown. (B) Cell extracts were immunoblotted with an anti-acetylated tubulin antibody. β-Actin was used as a loading control.

C KrasPDEC cells in serum-starved medium were treated with the indicated concentration of tubacin for 24 h. The percentages of cells with primary cilia or Ki67-positive nuclei were determined as described in Fig 1. Average of three independent experiments is shown.

D, E Panc1 cells transiently transfected with control or HDAC6 siRNA were cultured in serum-starved medium for 48 h. (D) Cell extracts were immunoblotted with an anti-HDAC6 antibody. α-Tubulin was used as a loading control. (E) The percentages of cells with primary cilia were determined as described in Fig 1. Average of three independent experiments is shown.

F Panc1 cells transiently transfected with control, HDAC2#1, HDAC2#2, Kras, IFT88, HDAC2#1 and IFT88, HDAC2#2 and IFT88, or Kras and IFT88 siRNA were cultured in serum-containing medium for 48 h. Cell extracts were immunoblotted with antibodies against IFT88, HDAC2, and Kras. β-Actin was used as a loading control.

Data information: Error bars represent SEM.

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