



Supplementary figure 16. Ibuprofen suppresses cellular stemness in PDAC. (A-D) IC50 of ibuprofen in PANC-1 and BxPC-3 cell lines and PDX1# PDX2# primary cell lines were detected by CCK-8. 1mM ibuprofen was used in following experiments. (E) Adherent PDAC cell lines were pre-treated with 1mM ibuprofen for 48 hours (DMSO was used as control); and then cells were collected and cultured with serum-free medium for the following sphere formation assays. Ibuprofen abbreviated as Ibu in following experiments. Representative images were shown (left) and statistical analysis were shown (right). (F) Percentage of CD44⁺CD24⁺ population were detected in indicated cell lines treated with 1mM ibuprofen by flow cytometry. Representative dot plots were shown (left) and statistical analysis were presented (right). (G) Western blot for stemness markers (Sox9, Sox2, Nanog and Oct4) were performed on indicated cell lines treated with 1mM ibuprofen. (H) In vivo limited dilution assay was performed to determine the effects of ibuprofen on CSC self-renewal of PANC-1 cells. Tumor incidence and CSCs probabilities were shown. (I) Percentage of ALDH⁺ cells in harvested tumor tissues from panel H. PI was used to exclude dead cells and CD45 was used to exclude leukocytes and DEAB was used as negative control. Representative dot plots were shown (left) and statistical analysis were shown (right). Paired Student's t-test was used for statistical analysis of in vitro experiments

and non-paired Student's t-test was used for statistical analysis of in vivo murine experiments. **P<0.01, ***P<0.001 and n.s. means non-significant.