BET inhibitors block pancreatic stellate cell collagen I production and attenuate fibrosis in vivo

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SUPPLEMENTAL DATA
Supplemental Figure 1: BET inhibitors and BRD4 knockdown decrease collagen I production by primary PSCs isolated from human PDAC tumors. (A) Early passage primary PSCs, which were isolated from a de-identified human PDAC specimen (Tumor #3) using the outgrowth assay, were treated with the BET inhibitors JQ1 (1 μM) and I-BET151 (1 μM). The effects on COL1A1 and COL1A2 mRNAs were determined by qRT-PCR (n=3). ***P < 0.001. Data were analyzed by two-tailed unpaired Student’s t test. The effects on collagen I protein expression were determined by Western Blotting. These results are representative of three (n=3) independent experiments. (B) Primary cancer-associated PSCs isolated from a human PDAC specimen (Tumor #3) were transfected with control siRNA or with siRNAs against BRD2, BRD3 or BRD4. The effects on the individual BRD mRNAs and COL1A1 and COL1A2 mRNAs were determined by qRT-PCR (n=3). **P < 0.01, ***P < 0.001. Data were analyzed by two-tailed unpaired Student’s t test. The effects on individual BRD proteins and collagen I protein expression were determined by Western Blotting. These results are representative of three (n=3) independent experiments. BET, bromodomains and extra-terminal; COL, collagen; PDAC, pancreatic ductal adenocarcinoma; PSCs, pancreatic stellate cells.
Supplemental Figure 2: FOSL1 is repressed by BRD4 in stellate cells and negatively regulates collagen I expression. (A and C) BRD4 was knocked down in primary PSCs isolated from human PDAC tumors (Tumors #2 and #3) and the effects on c-MYC and FOSL1 mRNA were determined by qRT-PCR (n=3). *P < 0.05, **P < 0.01, ***P < 0.001, ns, not significant. Data were analyzed by two-tailed unpaired Student’s t test. The effects on c-MYC and FOSL1 protein expression were determined by Western blotting. These results are representative of three (n=3) independent experiments. (B and D) c-MYC and FOSL1 were individually knocked down in primary cancer-associated PSCs and the effects on COL1A1 and COL1A2 mRNA were determined by qRT-PCR (n=3). *P < 0.05, **P < 0.01, ***P < 0.001. Data were analyzed by two-tailed unpaired Student’s t test. The effect on collagen I protein expression was determined by Western blotting. These results are representative of three (n=3) independent experiments. COL, collagen; PSCs, pancreatic stellate cells.