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

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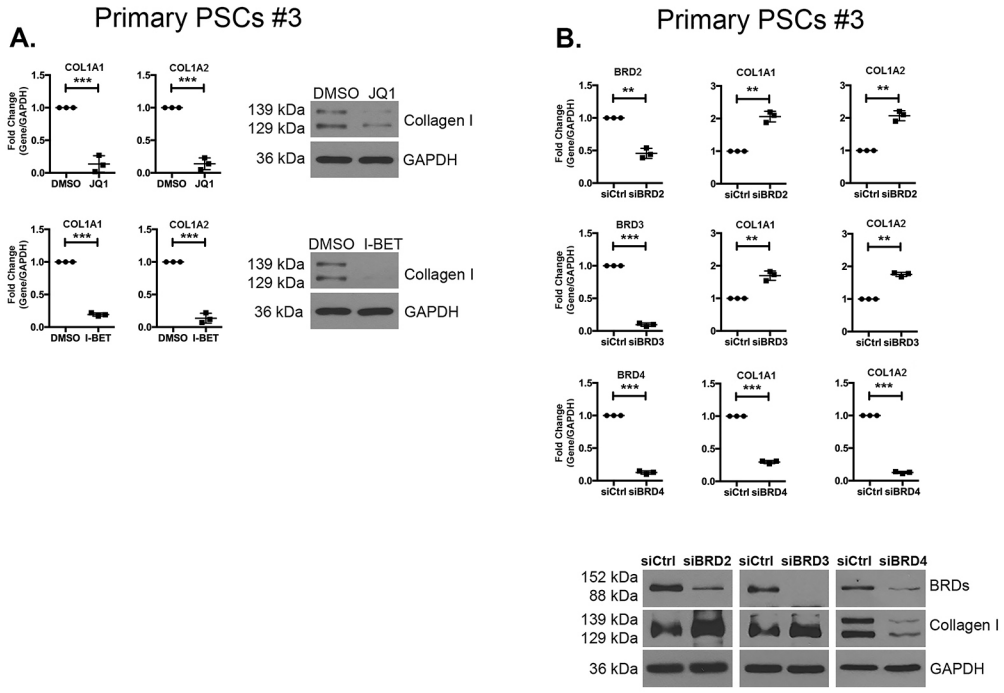
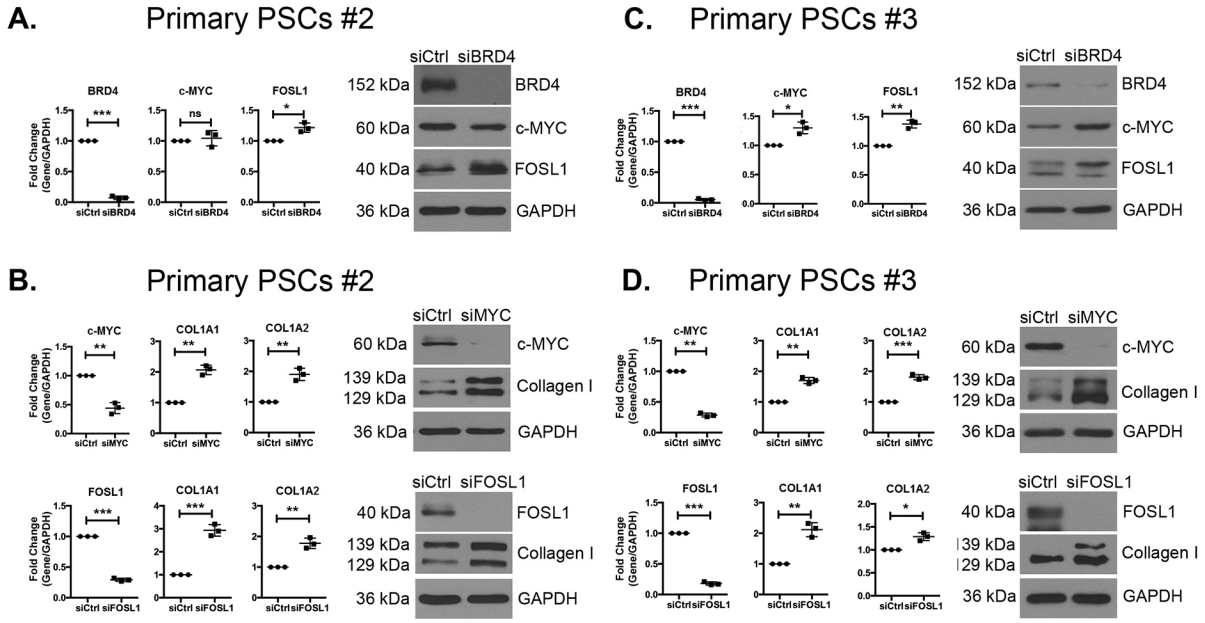
SUPPLEMENTAL DATA



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.