Nuclear Receptor COUP-TFII Controls Pancreatic Islet Tumor Angiogenesis by Regulating VEGF/VEGFR-2 Signaling

Jun Qin, Xinpu Chen, Li-yuan Yu-Lee, Ming-Jer Tsai, and Sophia Y. Tsai

Supplemental Material and Methods:

Matrigel Plug Assay: To induce COUP-TFII inactivation in the adult, 2-month-old mice (F/F or Cre/+; F/F) were intraperitoneally injected with 0.5 mg Tam for 5 consecutive days. The Matrigel plug assay was then performed as previously described (1) using the BD Matrigel Matrix (Becton Dickinson). Briefly, 500 μl of liquified Matrigel containing 100ng/ml VEGF-A and FGF was subcutaneously injected into mice and plugs were removed for analysis 10 days later.

RNA isolation and qRT-PCR analysis: Total RNA was isolated from Matrigel implants or pancreatic tissue (Adjacent normal pancreatic tissue and Pancreatic islet tumor) using the RNeasy Total RNA Isolation kit (Qiagen). Reverse transcription was performed using random priming and reverse transcription reagent. Ang1 and VEGFR-1 quantities were normalized against 18S RNA or CD31. The primers/probes used in this study were purchased from Applied Biosystems.

Wound healing assay: HUVEC were seeded at high density in a cultured medium. Ten hours after transfection, wounding was carried out through scraping the confluent monolayer with a pipette tip.

Supplementary Figure Legend

Fig. 1 COUP-TFII is expressed in the endothelial cells and pericytes within the tumor microenvironment. (A) Immunofluorescence staining for pancreatic β tumor cell marker large T antigen (green) and COUP-TFII (red). **(B)** COUP-TFII deletion efficiency was assessed by western blot analysis of tumors from three independent control and mutant mice. **(C)** Immunofluorescence staining for the endothelial cell marker CD31 (upper panel), pericyte marker NG2 (lower panel) with COUP-TFII expression in the pancreatic tumor. Arrow indicates endothelial cell and arrowhead denotes perciyte.

Fig. 2 COUP-TFII is important for endothelial cell proliferation and migration. (A) DNA synthesis in control and COUP-TFII knockdown cells was measured by [^{3}H]-thymidine incorporation assays. **(B)** Wound healing assay was used to determine cell migration rate upon VEGF stimulation in control and COUP-TFII knockdown cells. ** $P<0.01$

Fig. 3 Up regulation of VEGFR-1 and sVGFR-1 in COUP-TFII depleted HUVEC cells, but not in primary LEC cells. qRT-PCR analysis showed that mRNAs for VEGFR-1 and soluble VEGFR-1 (sVEGFR-1) were increased in the COUP-TFII depleted HUVEC cells, but not in the COUP-TFII depleted primary lymphatic endothelial cells (LEC). The expression levels of COUP-TFII, VEGFR-1 and sVEGFR-1 in LEC cells were relatively set as one.

Fig. 4 Regulation of Ang1 expression by COUP-TFII in the normal pancreatic tissue and angiogenic islet tumors

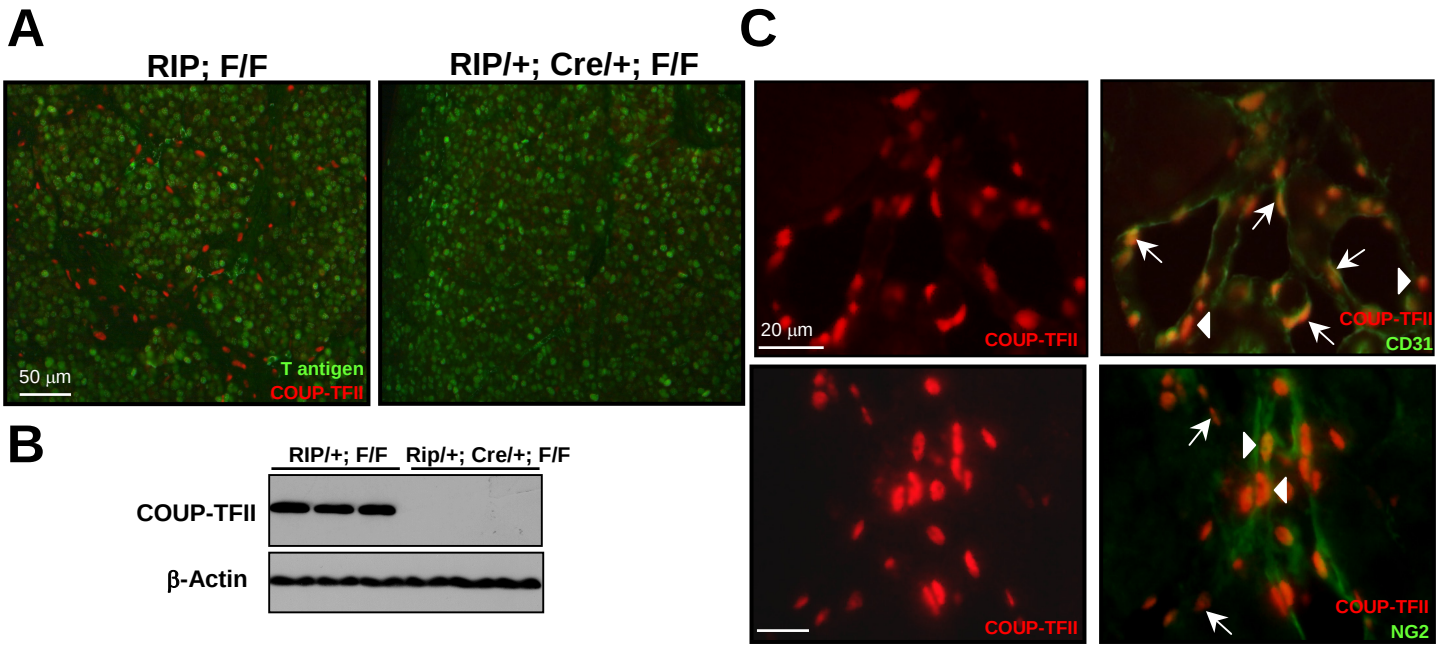
qRT-PCR analysis of Ang1 expression in adjacent normal pancreatic tissue and angiogenic islet tumors from control and COUP-TFII mutant mice (N=6). The expression of Ang1 is decreased in the adjacent normal pancreatic tissue from mutant mice compared with control mice. However, there is no statistical significance in Ang1 expression in islet tumors between control and mutant mice. * $P<0.05$

Fig. 5 COUP-TFII regulates the expression of VEGFR-1 in adult neoagiogenesis.

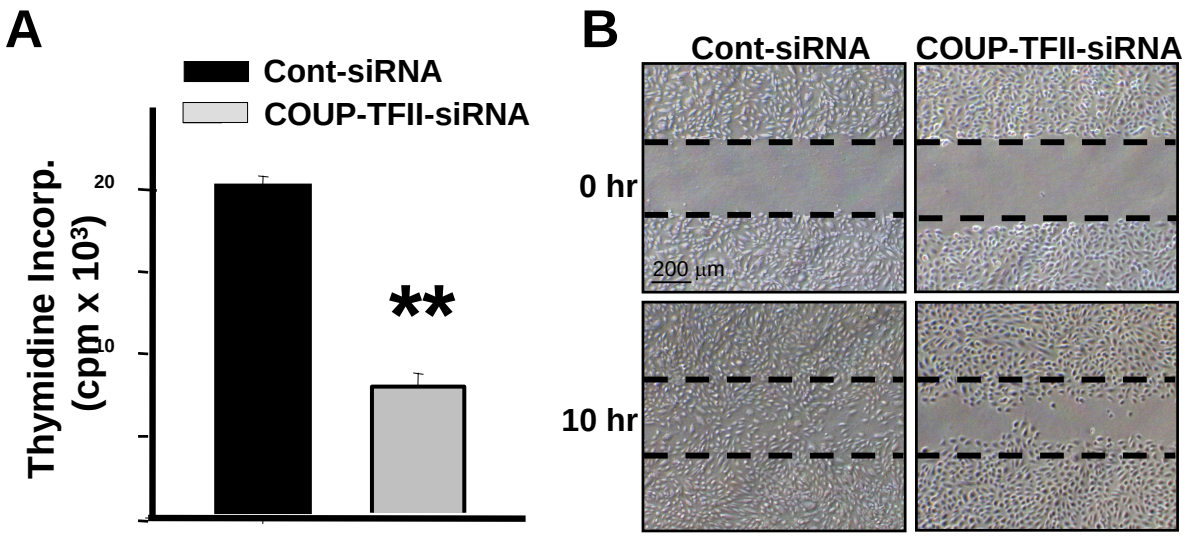
(A) Matrigel plugs containing VEGF-A and FGF were implanted subcutaneously and the plugs were removed and photographed 10 days subsequent to implantation. Photographs of representative plugs excised from control and mutant mice are shown. **(B)** qRT–PCR analysis of VEGFR-1 transcripts in the Matrigel plug from Control (F/F, Tamoxifen) and COUP-TFII mutant (Cre/+; F/F, Tamoxifen) mice. VEGFR-1 quantities were normalized against CD31 (N=6). * $P<0.05$

Supplemental Reference:

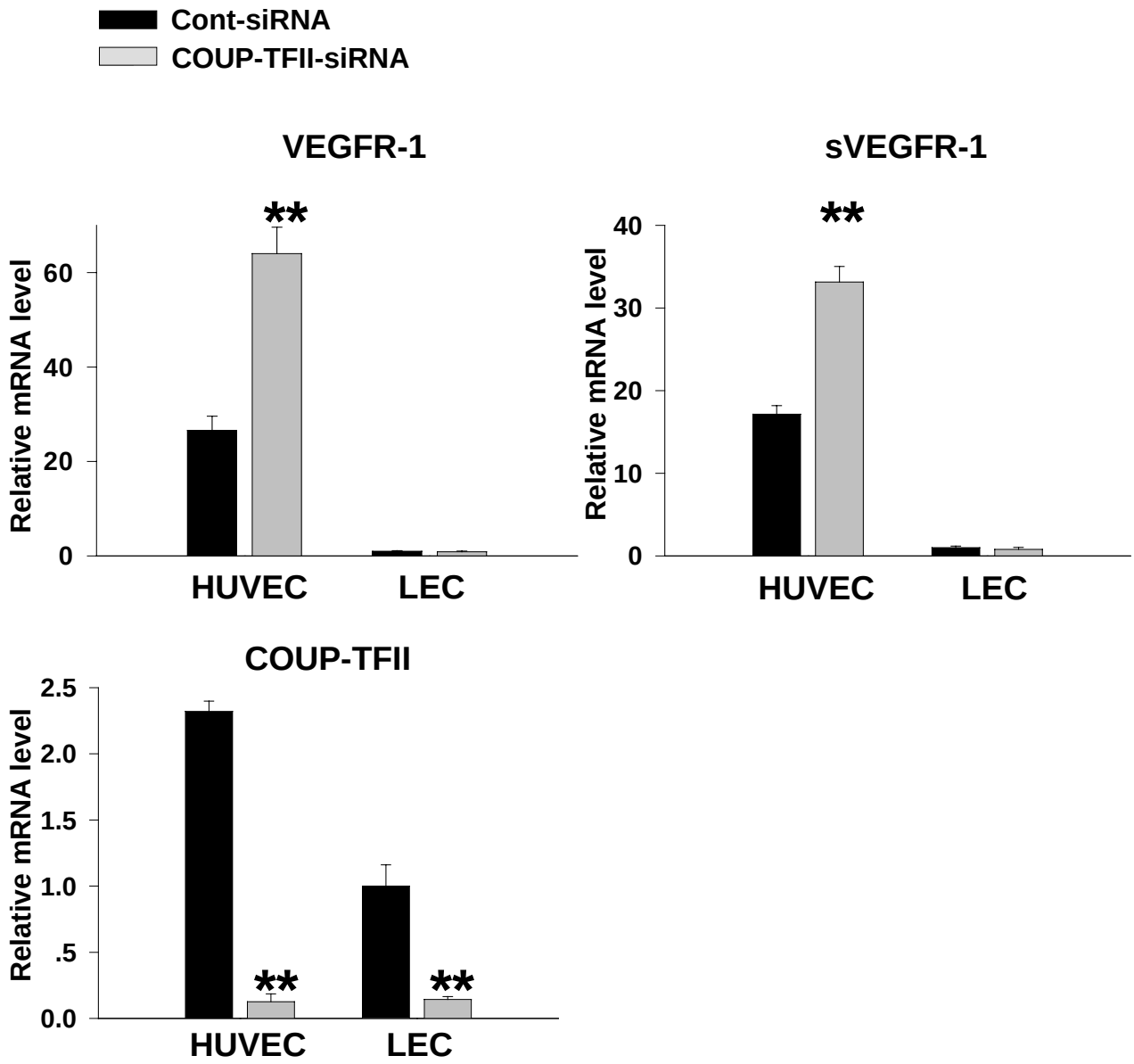
1. Qin J, Chen X, Xie X, Tsai MJ, Tsai SY. COUP-TFII regulates tumor growth and metastasis by modulating tumor angiogenesis. Proc Natl Acad Sci U S A. 2010;107:3687-92.



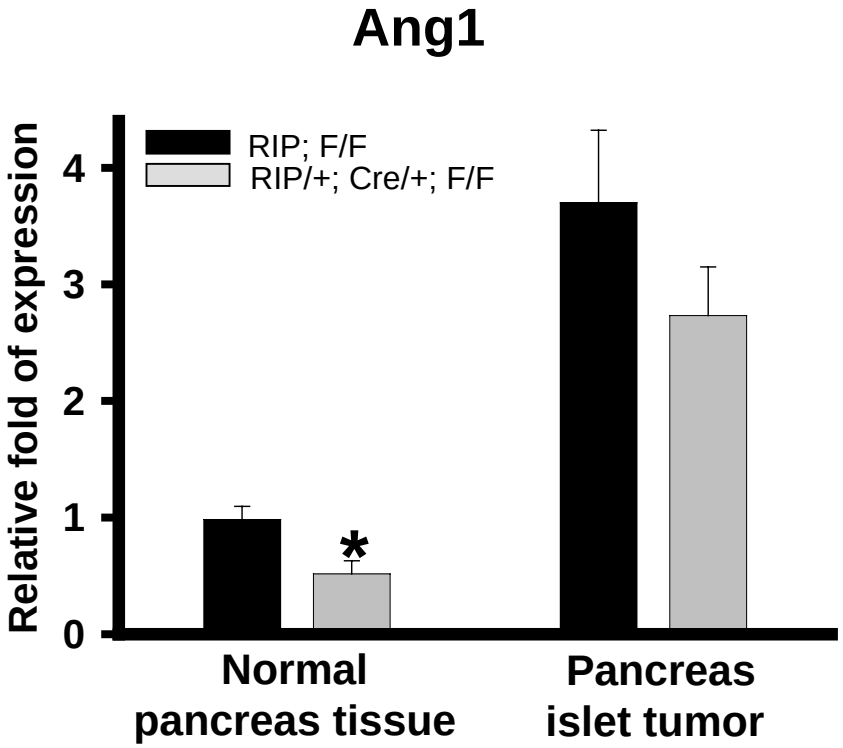
Supplementary Fig. 1 Qin et. al.



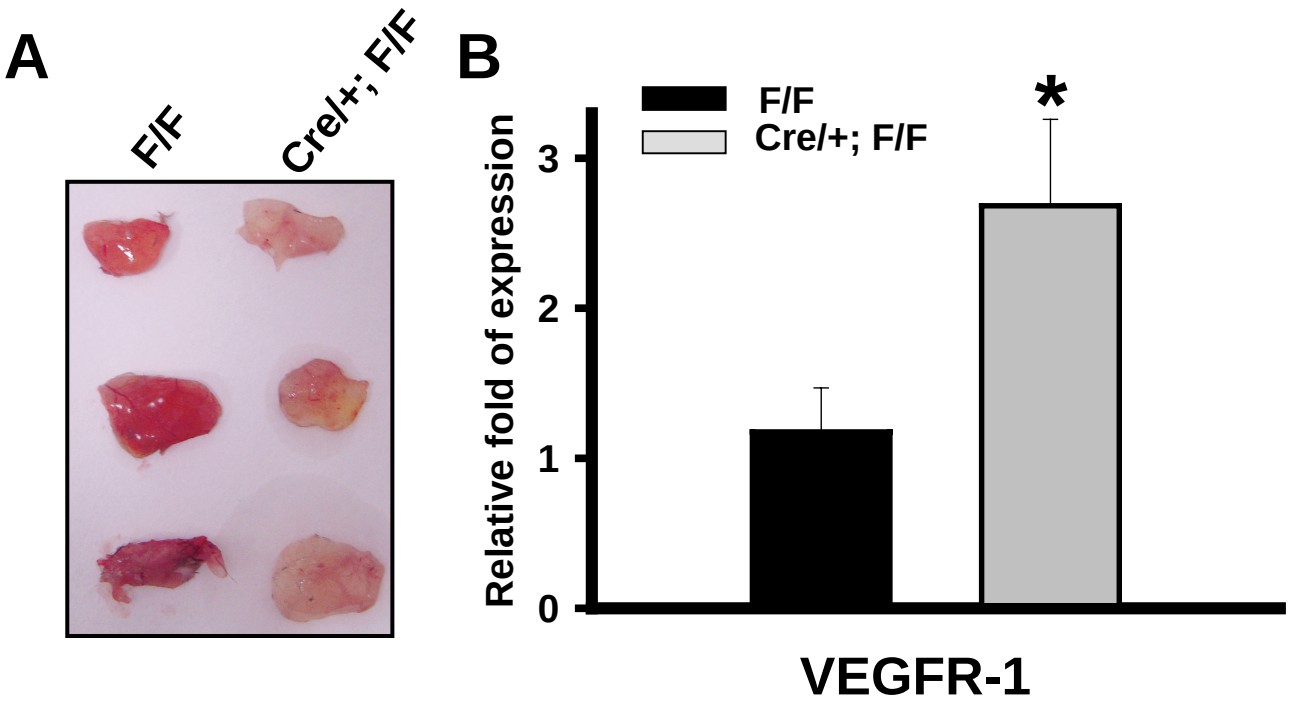
Supplementary Fig. 2 Qin et. al.



Supplementary Fig. 3 Qin et. al.



Supplementary Fig. 4 Qin et. al.



Supplementary Fig. 5 Qin et. al.