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Supplemental Information

Tartrate-Resistant Acid Phosphatase 5/ACP5 Interacts with p53 to Control the Expression of SMAD3 in Lung Adenocarcinoma

Yinan Hu, Jun Yu, Qi Wang, Lei Zhang, Xueying Chen, Yong Cao, Jianping Zhao, Yongjian Xu, Dingsheng Jiang, Yi Wang, and Weining Xiong

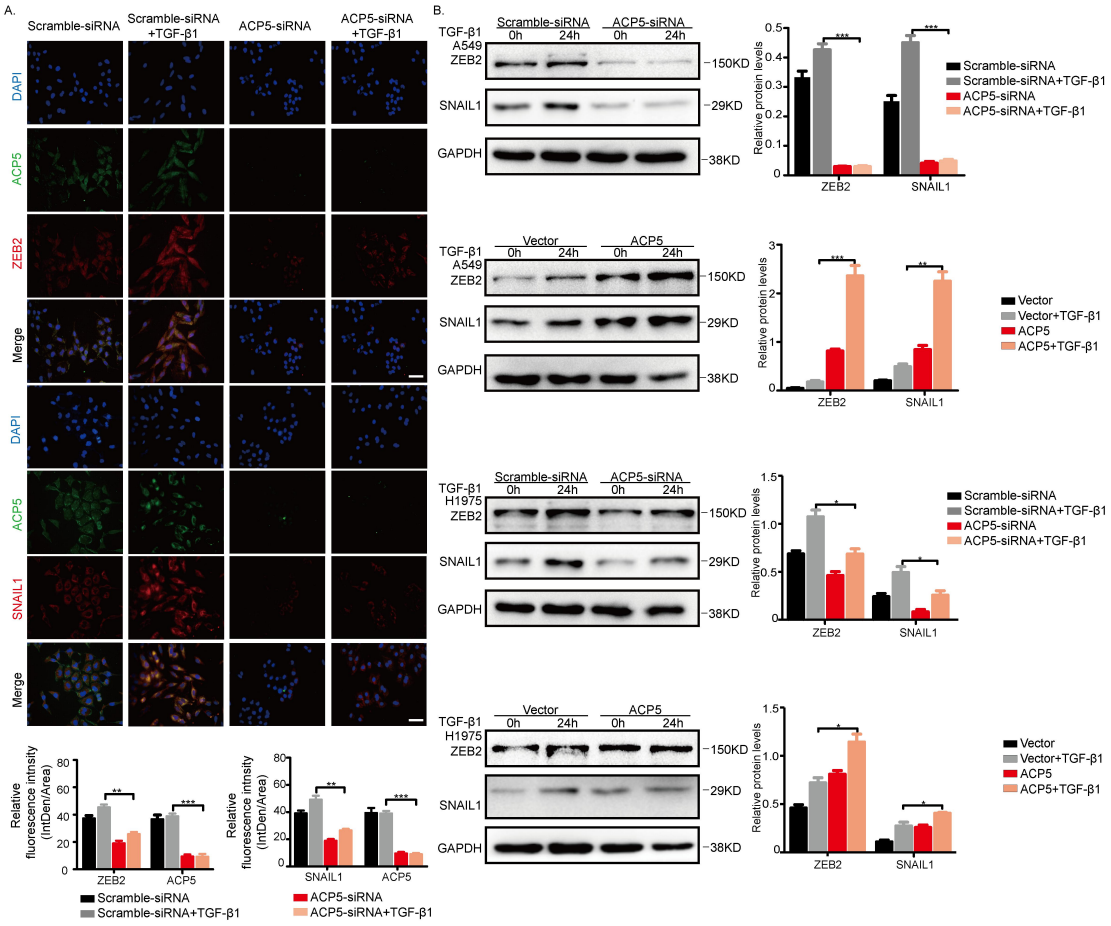

Figure S1. ACP5 regulated the transfactors in EMT. (A) An immunofluorescence staining assay showed the expression of ZEB2 and SNAIL1 in A549 cells with suppressed ACP5 expression. Cell nuclei were visualized by DAPI. (magnification 400×, scale bars=50 μm). (B) Western blot was used to analyze ZEB2 and SNAIL1 expression in A549 and H1975 cells. The results are summarized as the mean ± SEM of three independent experiments (*P<0.05; **P<0.01; and ***P<0.001, independent Student's t test).

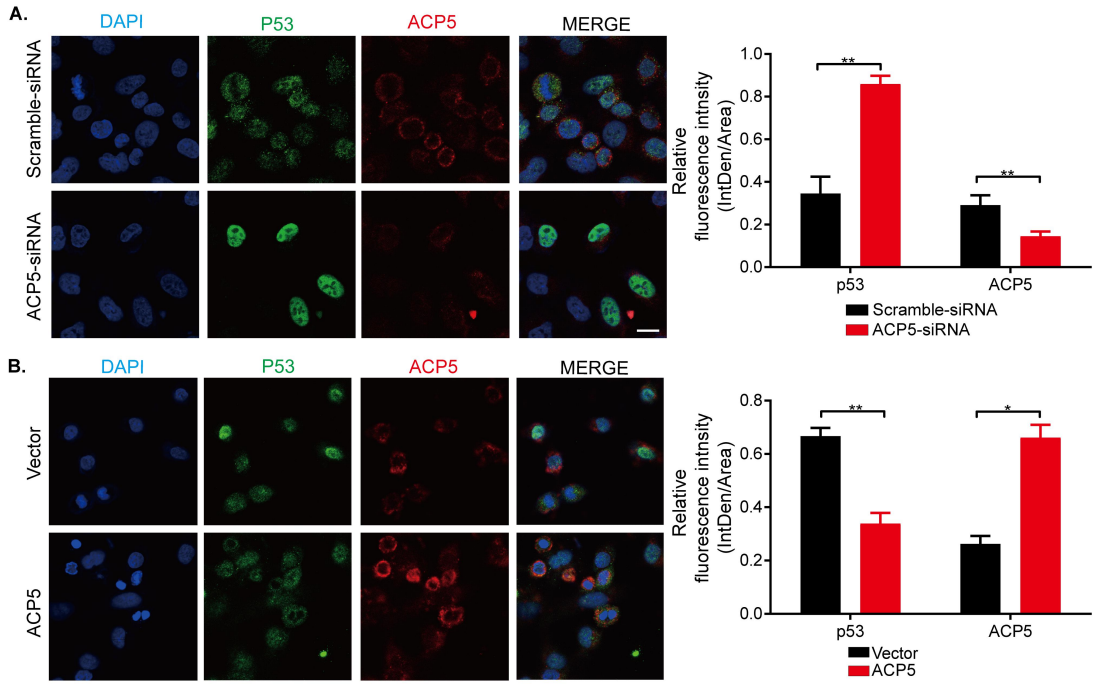

Figure S2. Confocal microscopy detected ACP5 and p53 cellular localization of A549 cell line by immunofluorescence assay. The A549 cell line was transfected with ACP5 siRNA (A) or overexpressing ACP5 plasmid (B) for 24 hours. Images were obtained using an Olympus FV1000 Spectral confocal microscope and overlayed to assess protein localization. The results are summarized as the mean ± SEM of three independent experiments (*P<0.05; **P<0.01; and ***P<0.001, independent Student's t test).

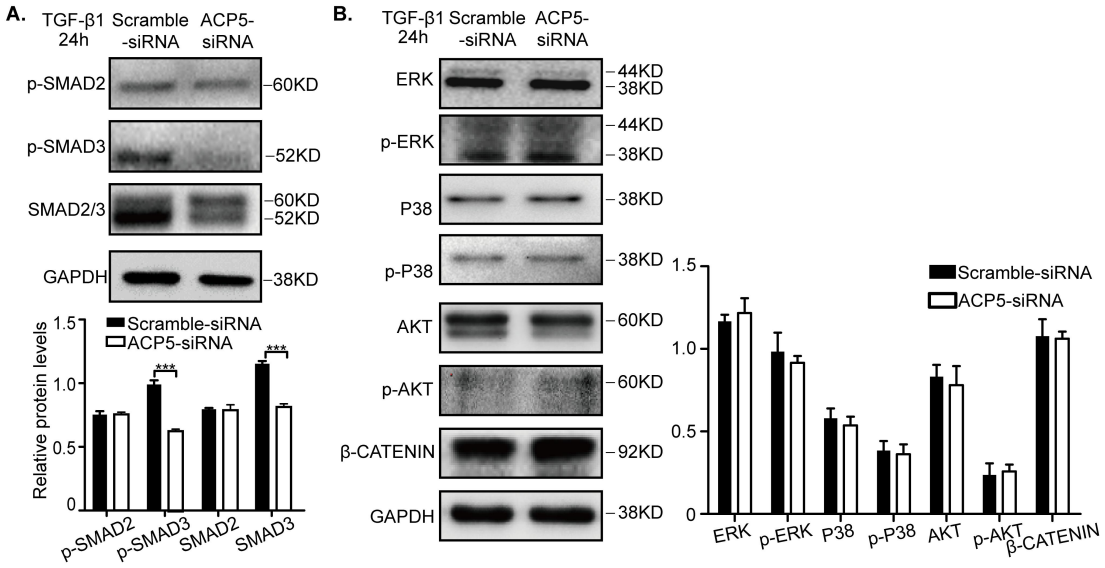

Figure S3. The representative Western blot results for the TGF-β-related signaling pathway proteins at 24h of TGF-β1 stimulation. (A) Knockdown the expression of ACP5 attenuated the levels of SMAD3 and p-SMAD3. (B) Silencing ACP5 expression might not impact other TGF-β-related signaling pathway molecules. The results are summarized as the mean ± SEM of three independent experiments (*P<0.05; **P<0.01; and ***P<0.001, independent Student's t test).

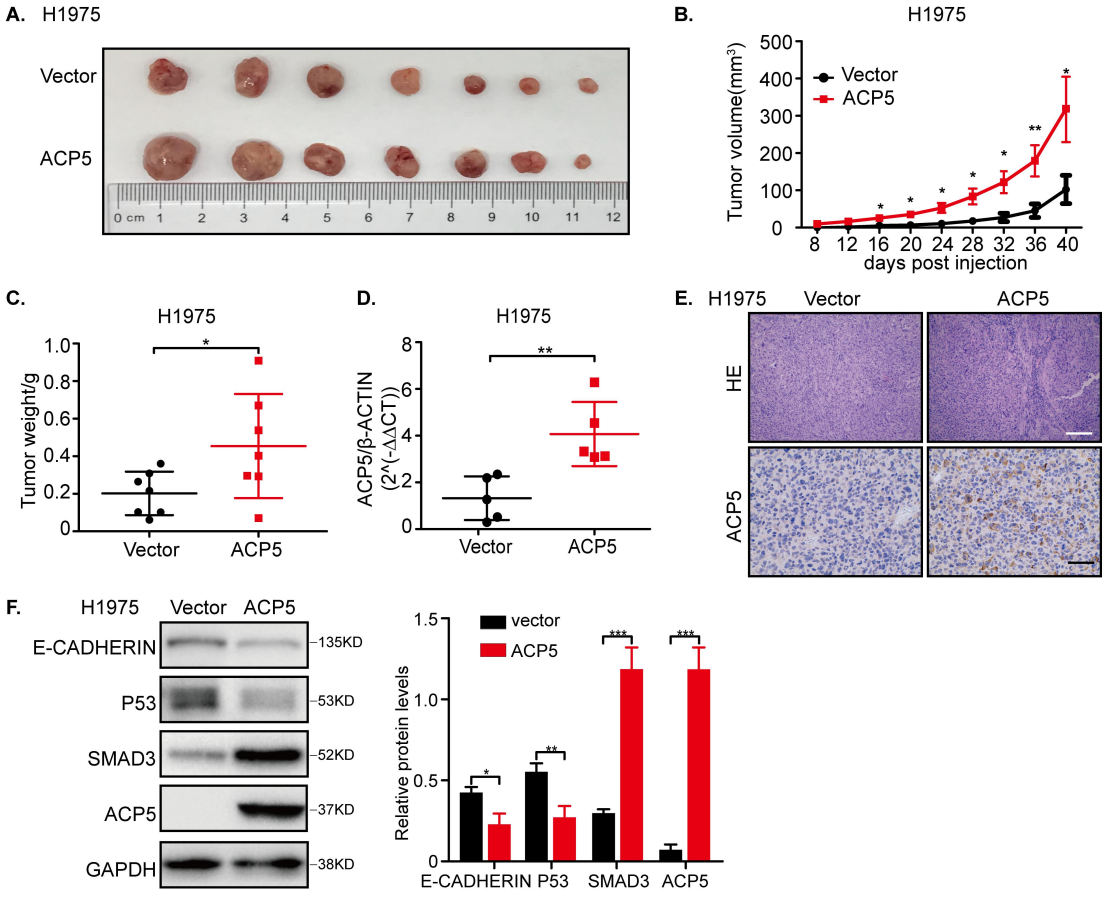

Figure S4. Nude mice were subcutaneously injected with NCI-H1975 cells stably overexpressed for ACP5 (H1975-ACP5) or control (H1975-Vec) cells. (A) Images of the tumor lumps from the indicated groups at the endpoint of the experiment. n = 7 mice per group. (B) Tumor formation in nude mice treated with H1975-ACP5 or H1975-Vec cells was monitored at the indicated time points. (C) Tumor weights were measured at the last time point. (D) RT-PCR for *ACP5* expression in xenograft tumor tissues of H1975-Vec (n=5) and H1975-ACP5 groups (n=5). (E) Representative H&E staining images (top panel) (magnification 100×, scale bars=200 μm) and IHC images of ACP5 staining (bottom panel) (magnification 400×, scale bars=50 μm) in sections of xenograft tumor of H1975 cells. (F) The expression of E-cadherin, P53, SMAD3 and ACP5 was detected by Western blot in xenograft tumors from the H1975-Vec (n=5) and H1975-ACP5 (n=5). The results were expressed as the mean ± SD of three independent experiments (*P<0.05; **P<0.01; and ***P<0.001, independent Student's t test).