Supplementary Figures

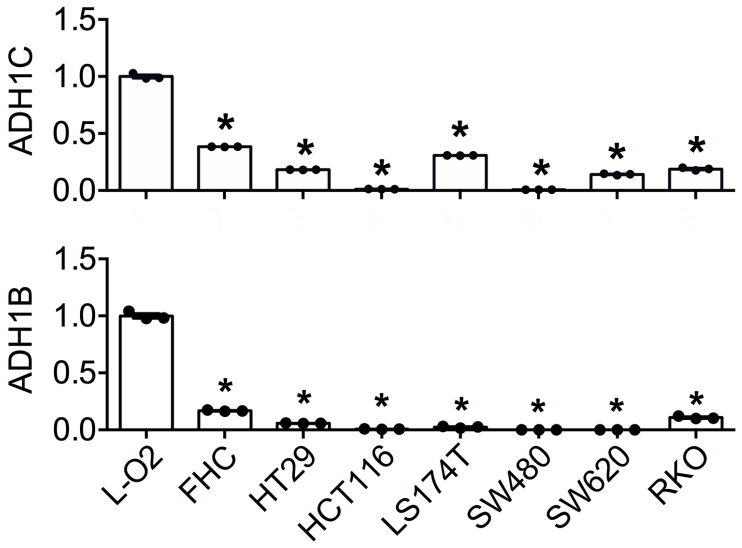

Figure S1, related to Figure 3. The expressions of both ADH1C and ADH1B are extensively decreased in CRC cell lines. Quantitative real-time PCR assay for ADH1C, data were normalized to GAPDH and L-O2 cell line. Bars were expressed as mean ± SEM. $^{*}P<0.05$, vs L-O2.

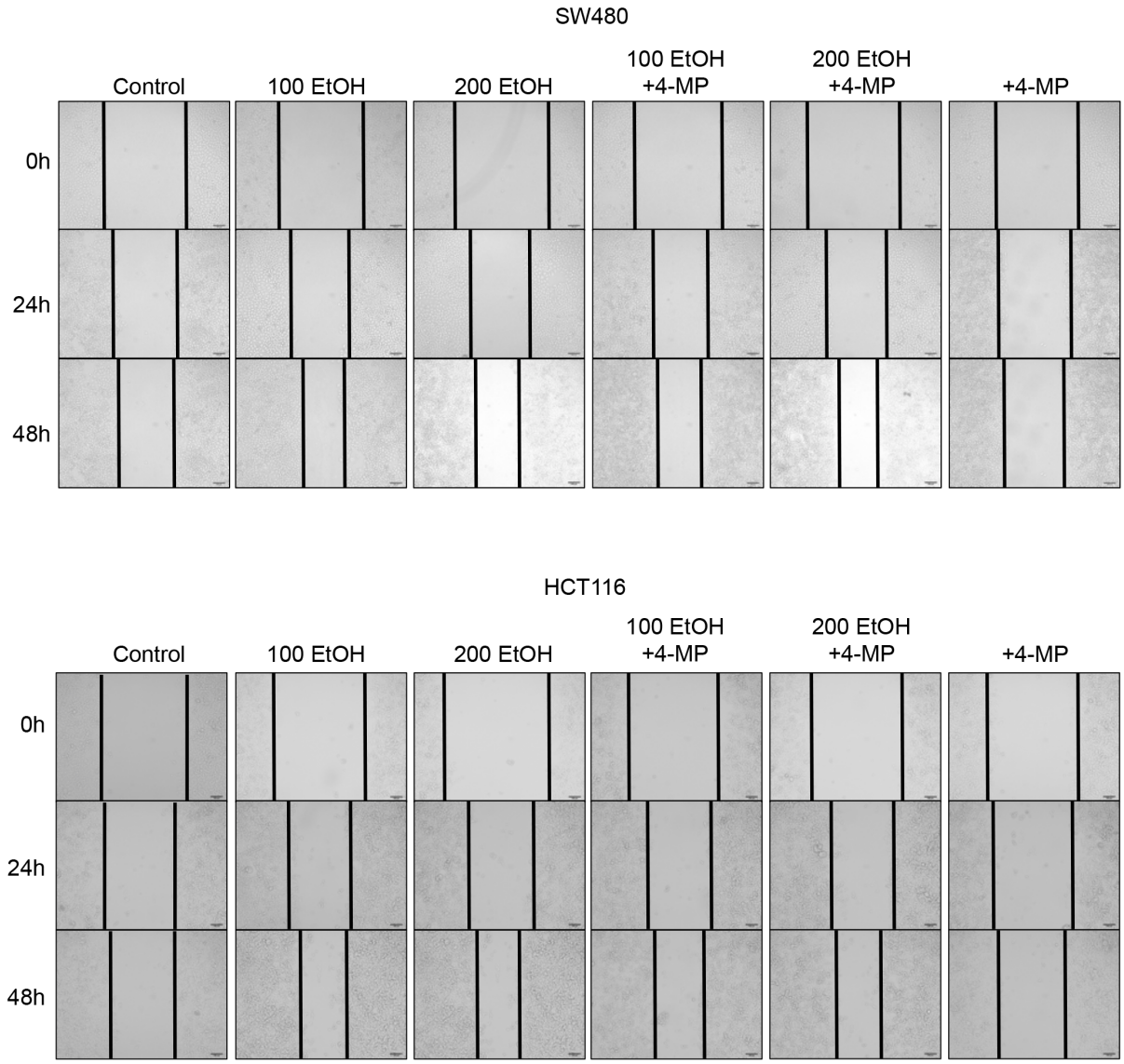

Figure S2, related to Figure 3E. Ethanol promotes the motility of CRC cells. The representative figures of wound healing assay for SW480 and HCT116 cells that were treated with PBS control, 100 mg/dl or 200 mg/dl ethanol, and/or 4-MP. The wounds were measured under a microscope in three randomly selected fields. Representative figures are shown.

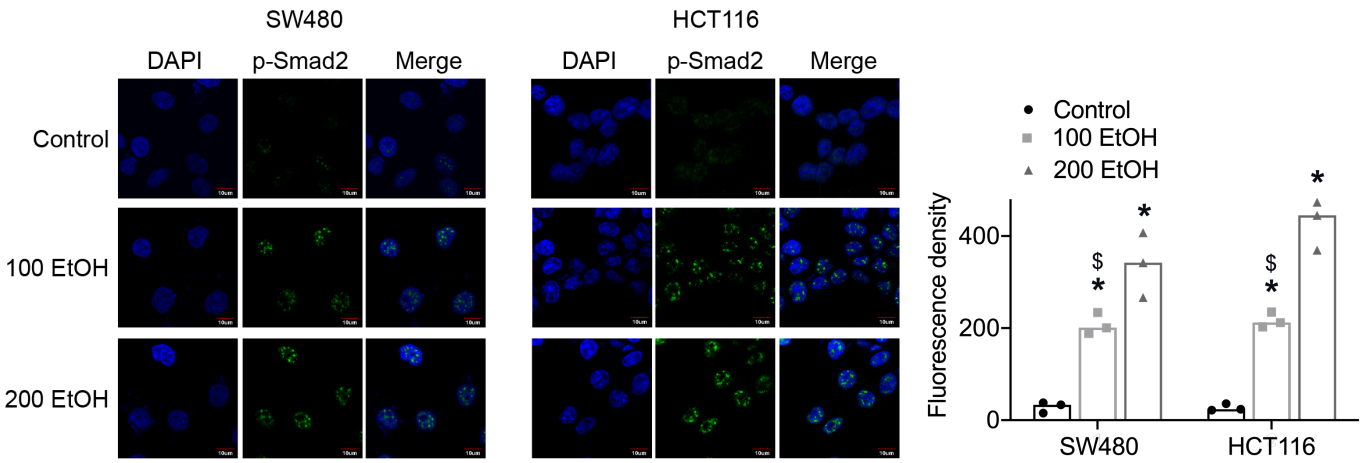

Figure S3, related to Figure 4C. Ethanol activates TGF-β/Smad signalling pathway. Immunofluorescence assay showed the expression of phosphorylated-Smad2 proteins in the SW480 and HCT116 cells. Representative figures are shown. Scale bars represent 10 μm.

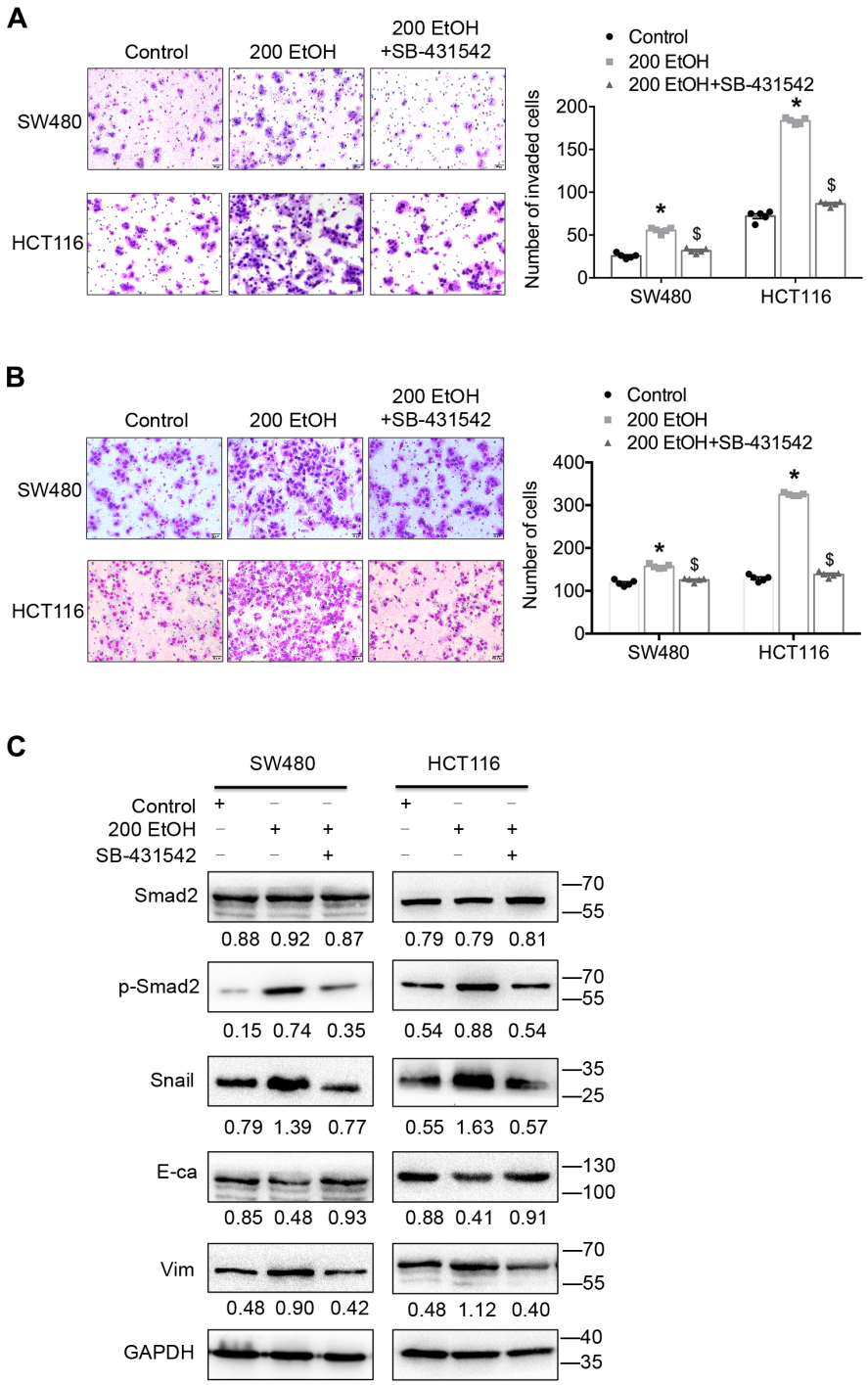

Figure S4. related to Figure 5. Blocking TGF-β/Smad signaling pathway eliminated the promotion of ethanol on EMT. (A) Transwell assay with matrigel for investigating the invasive property of CRC cells. Representative figures were shown in left panel. (B) Transwell assay without matrigel for investigating the migration property of CRC cells. Representative figures were shown in left panel. (C) **.** Western blot analysis was performed to detect the expression of EMT and TGF-β signaling associated proteins. Representative figures are shown. Values under the bands represent the expression of proteins normalized to reference gene GAPDH expression. Bars were expressed as mean ± SEM. "*" indicated $P<0.05$, *vs* control.

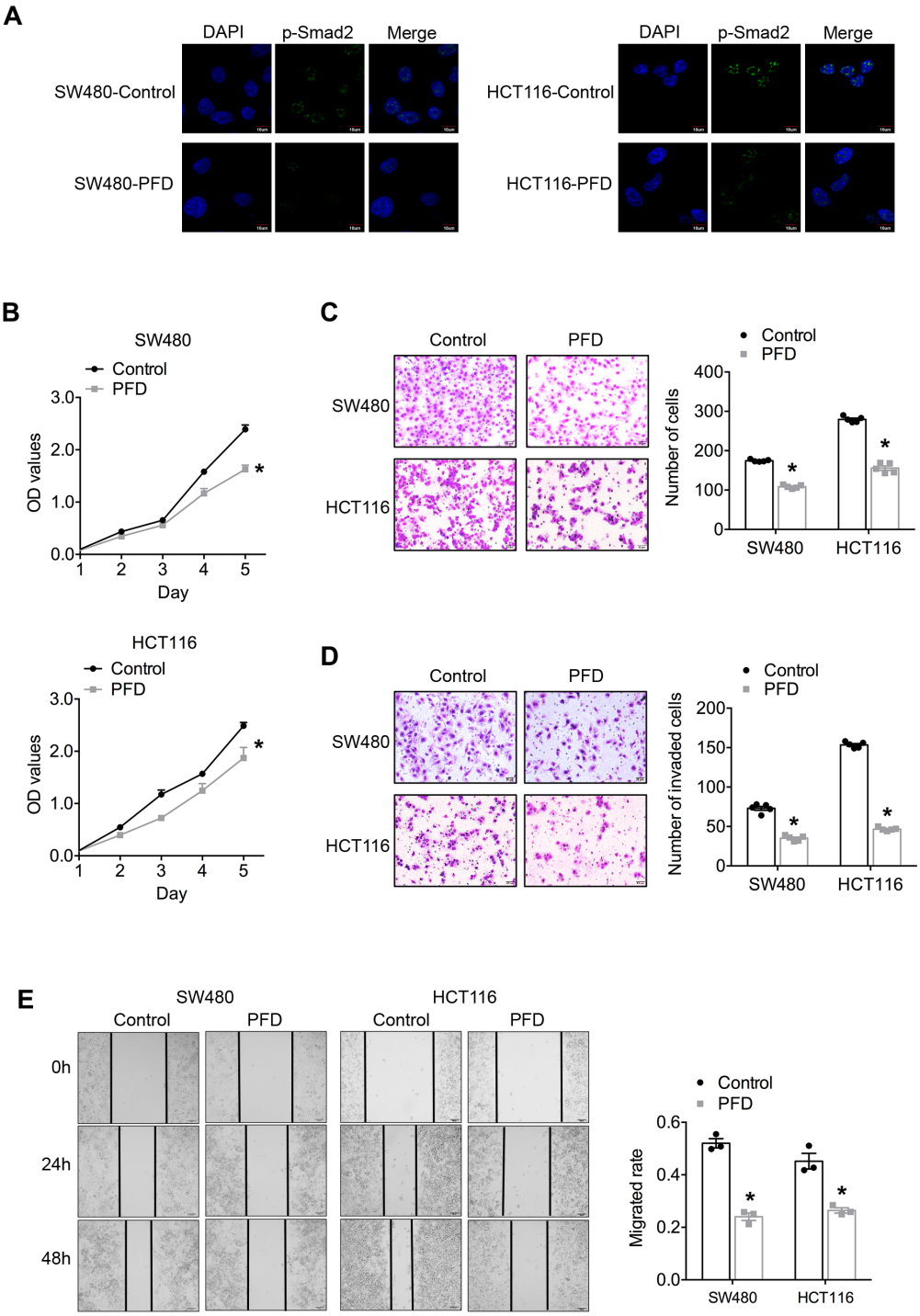

Figure S5, Pirfenidone suppresses the aggressiveness of CRC cells by targeting TGF-β signaling. (A) Representative figures of immunofluorescence assay for the expression of p-Smad2 in treated cells. Scale bars represent 10 μm. (B) CCK-8 assay for cell proliferation. (C) Transwell assay without matrigel for investigating the migration property of CRC cells. Representative figures were shown in left panel. (D) Transwell assay with matrigel for investigating the invasive property of CRC cells. Representative figures were shown in left panel. (E) Would-healing assay for investigating the motility of CRC cells. Representative figures were shown in left panel. Bars were expressed as mean ± SEM. "*" indicated $P<0.05$, *vs* control.

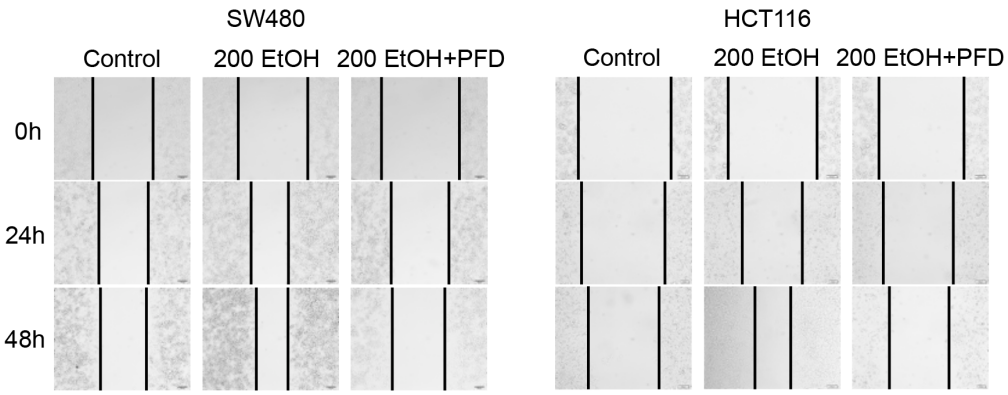

Figure S6, related to Figure 5B. Blocking TGF-β/Smad signaling pathway eliminated the promotion of ethanol on cell motility. The representative figures of wound healing assay for SW480 and HCT116 cells that were treated with ethanol or/and Pirfenidone (PFD). The wounds were measured under a microscope in three randomly selected fields. Representative figures are shown.

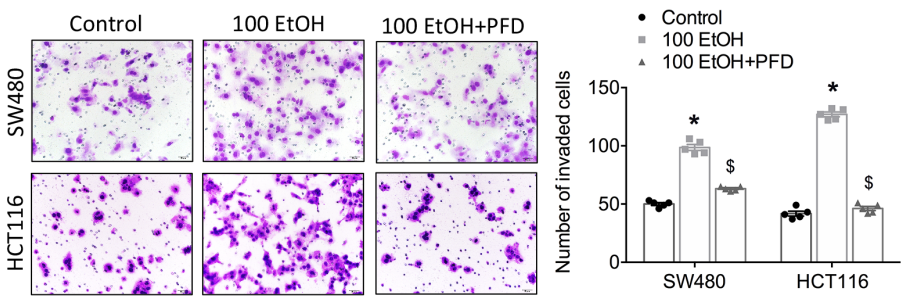

Figure S7, related to Figure 5. Blocking TGF-β/Smad signaling pathway eliminated the promotion of ethanol on cell invasion. The representative figures of invasion assay for SW480 and HCT116 cells that were treated with ethanol and/or pirfenidone. Bars in the right panel were expressed as mean ± SEM. The cells were counted under a microscope in five randomly selected fields.

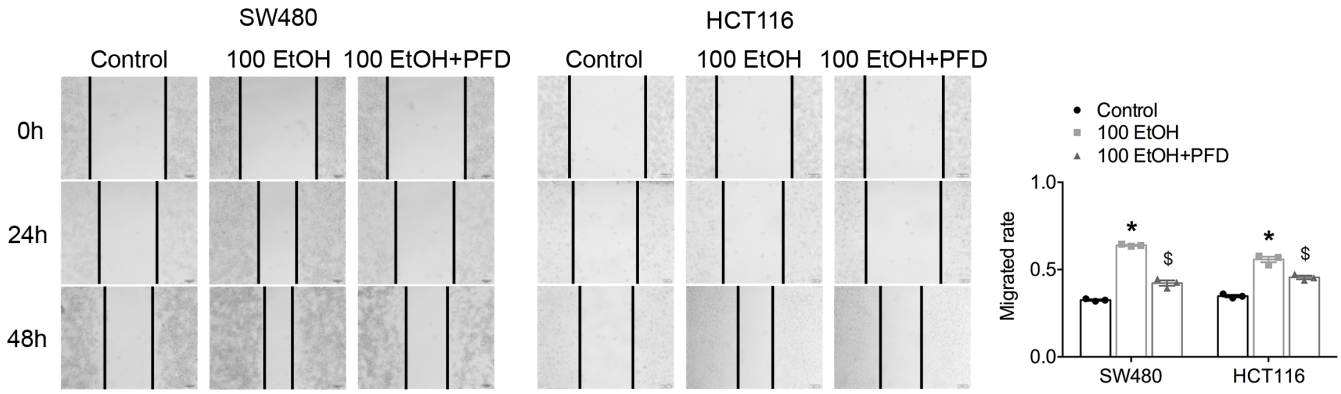

Figure S8, related to Figure 5. Blocking TGF-β/Smad signaling pathway eliminated the promotion of ethanol on cell motility. The representative figures of wound healing assay for SW480 and HCT116 cells that were treated with ethanol or/and Pirfenidone (PFD). Bars in the right panel were expressed as mean ± SEM. The wounds were measured under a microscope in three randomly selected fields.

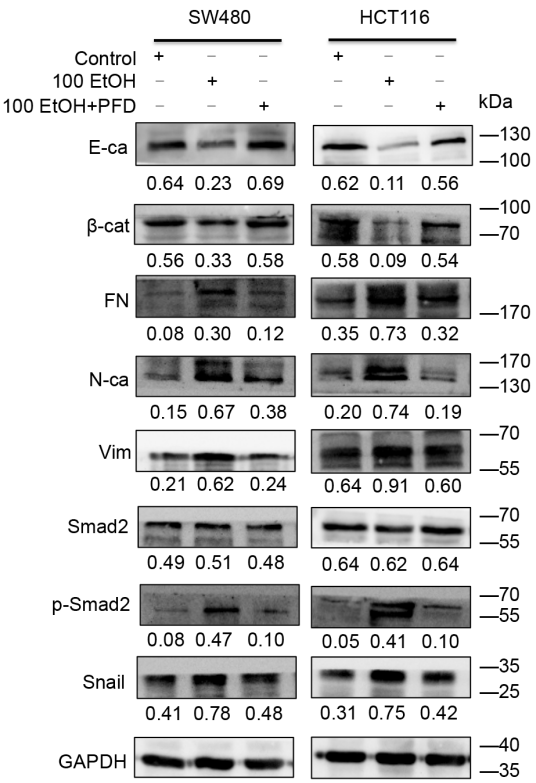

Figure S9, related to Figure 5. Blocking TGF-β/Smad signaling pathway eliminated the promotion of ethanol on EMT. Western blot analysis was performed to detect the expression of EMT and TGF-β signaling associated proteins. Representative figures are shown. Values under the bands represent the expression of proteins normalized to reference gene GAPDH expression.

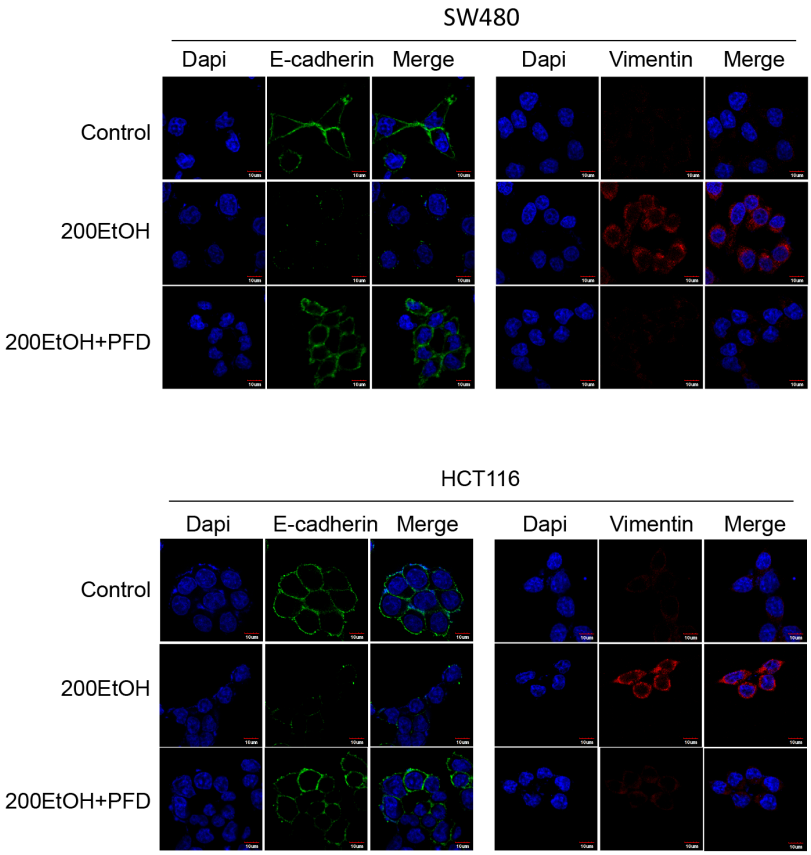

Figure S10, related to Figure 5D. Blocking TGF-β/Smad signaling pathway recovered the expression of EMT markers. Immunofluorescence assay showed the expression of EMT-associated proteins in the SW480 and HCT116 cells treated with ethanol and/or pirfenidone (PFD). Representative figures are shown. Scale bars represent 10 μm.

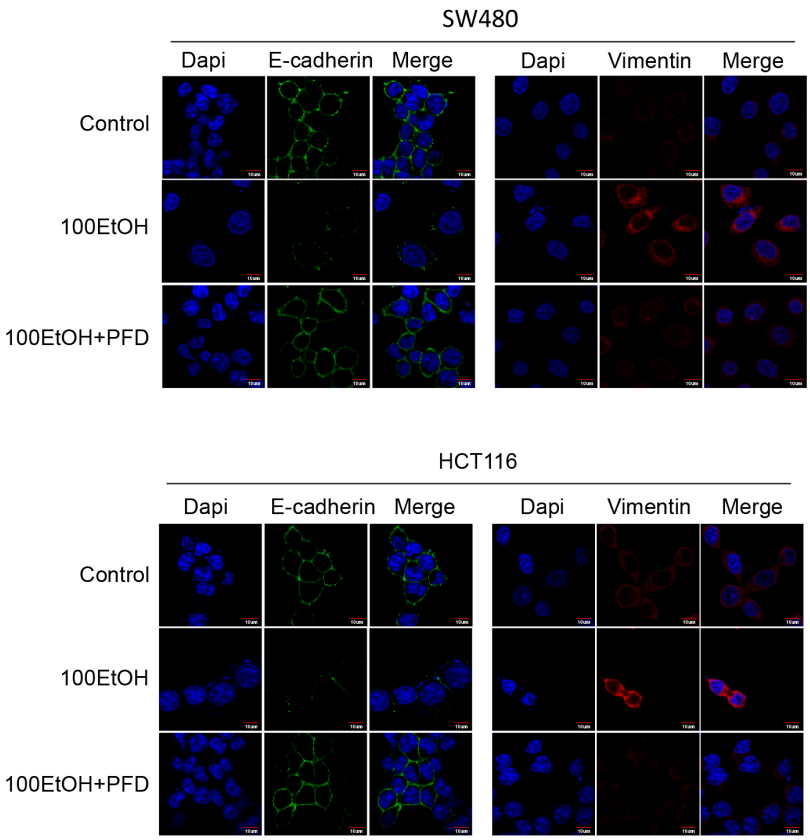

Figure S11, related to Figure 5. Blocking TGF-β/Smad signaling pathway recovered the expression of EMT markers. Immunofluorescence assay showed the expression of EMT-associated proteins in the SW480 and HCT116 cells treated with ethanol and/or pirfenidone (PFD). Representative figures are shown. Scale bars represent 10 μm.

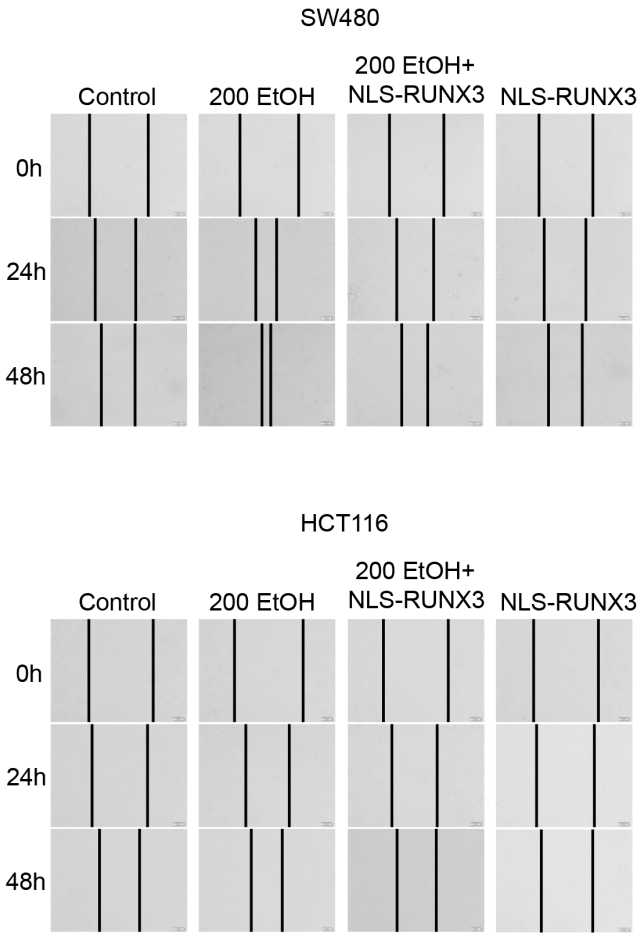

Figure S12, related to Figure 7C. Re-expression of nuclear RUNX3 reduced the promotion of ethanol on cell motility. The representative figures of wound healing assay, SW480 and HCT116 cells that were treated with 200mg/dl or/and NLS-RUNX3. The wounds were measured under a microscope in three randomly selected fields.

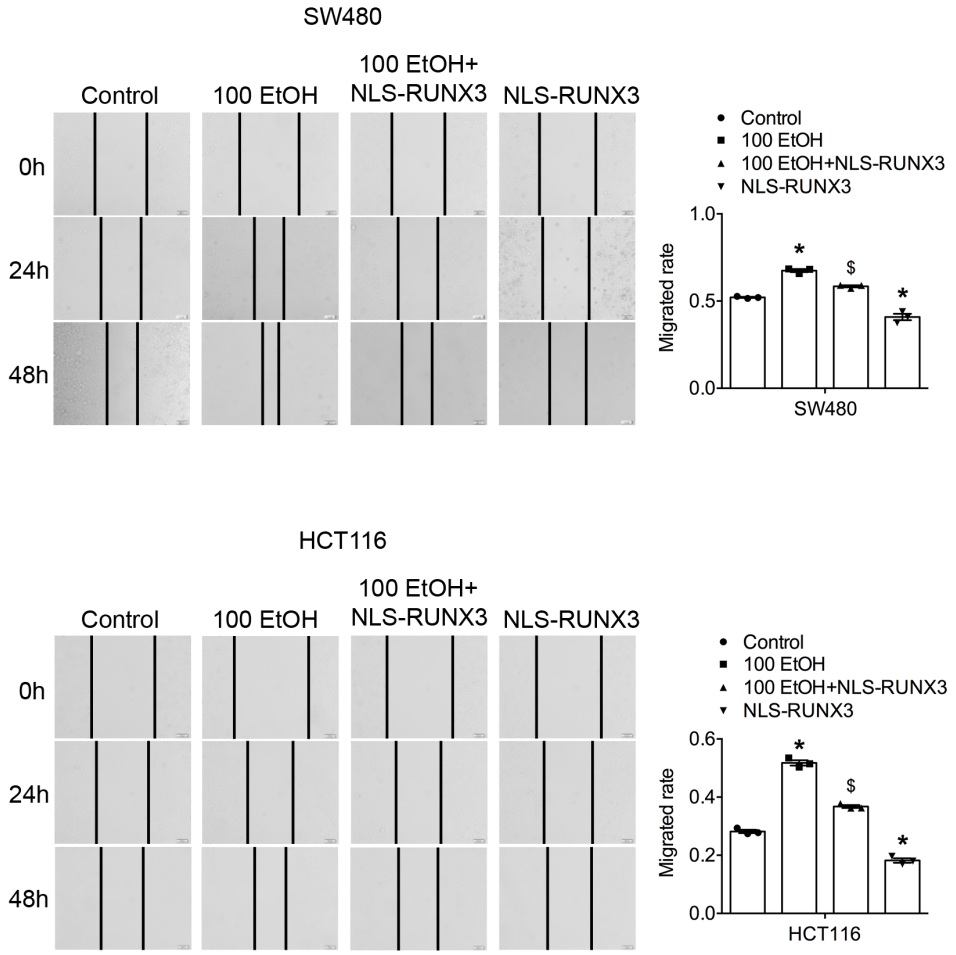

Figure S13, related to Figure 7. Re-expression of nuclear RUNX3 reduced the promotion of ethanol on cell motility. The representative figures of wound healing assay, SW480 and HCT116 cells that were treated with 100mg/dl and/or NLS-RUNX3. Bars in the right panel were expressed as mean ± SEM. The wounds were measured under a microscope in three randomly selected fields.

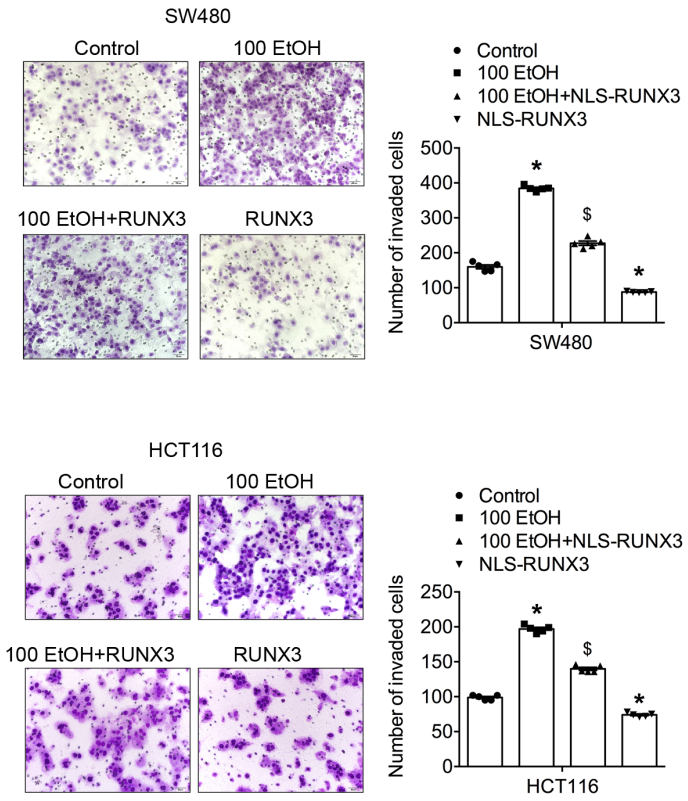

Figure S14, related to Figure 7. Re-expression of nuclear RUNX3 reduced the promotion of ethanol on cell invasion. The representative figures of transwell assay for SW480 and HCT116 cells that were treated with 100mg/dl and/or NLS-RUNX3. Bars in the right panel were expressed as mean ± SEM. The cells were counted under a microscope in five randomly selected fields.

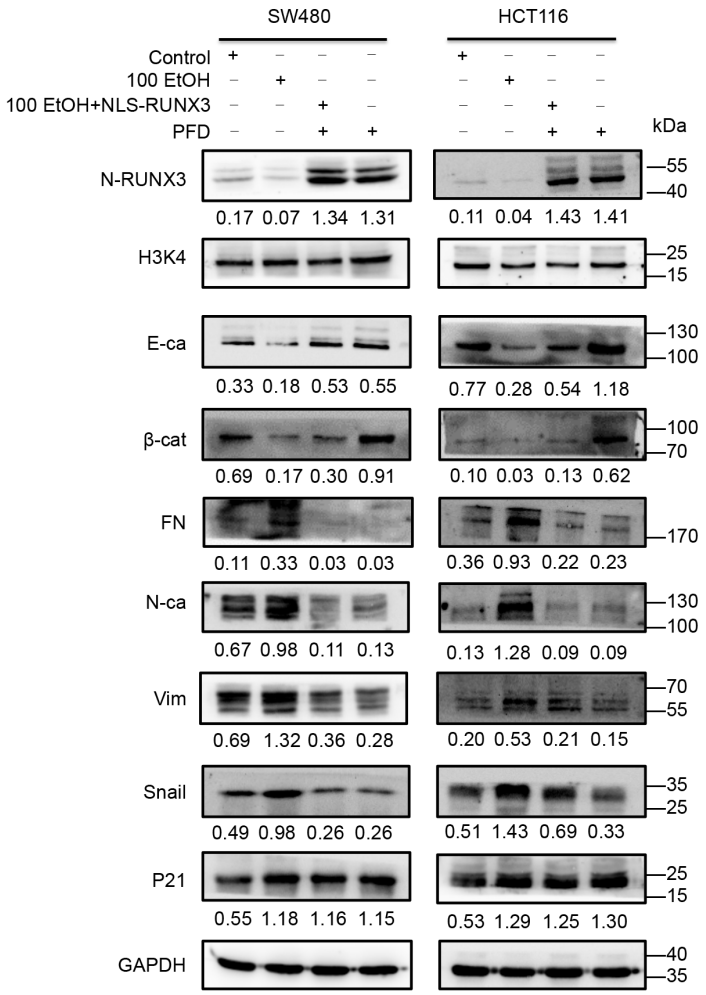

Figure S15, related to Figure 7. Re-expression of nuclear RUNX3 eliminated the promotion of ethanol on EMT by targeting Snail. Western blot analysis was performed to detect the expression of EMT and TGF-β signaling associated proteins. Representative figures are shown. Values under the bands represent the expression of proteins normalized to reference gene H3K4 or GAPDH expression.