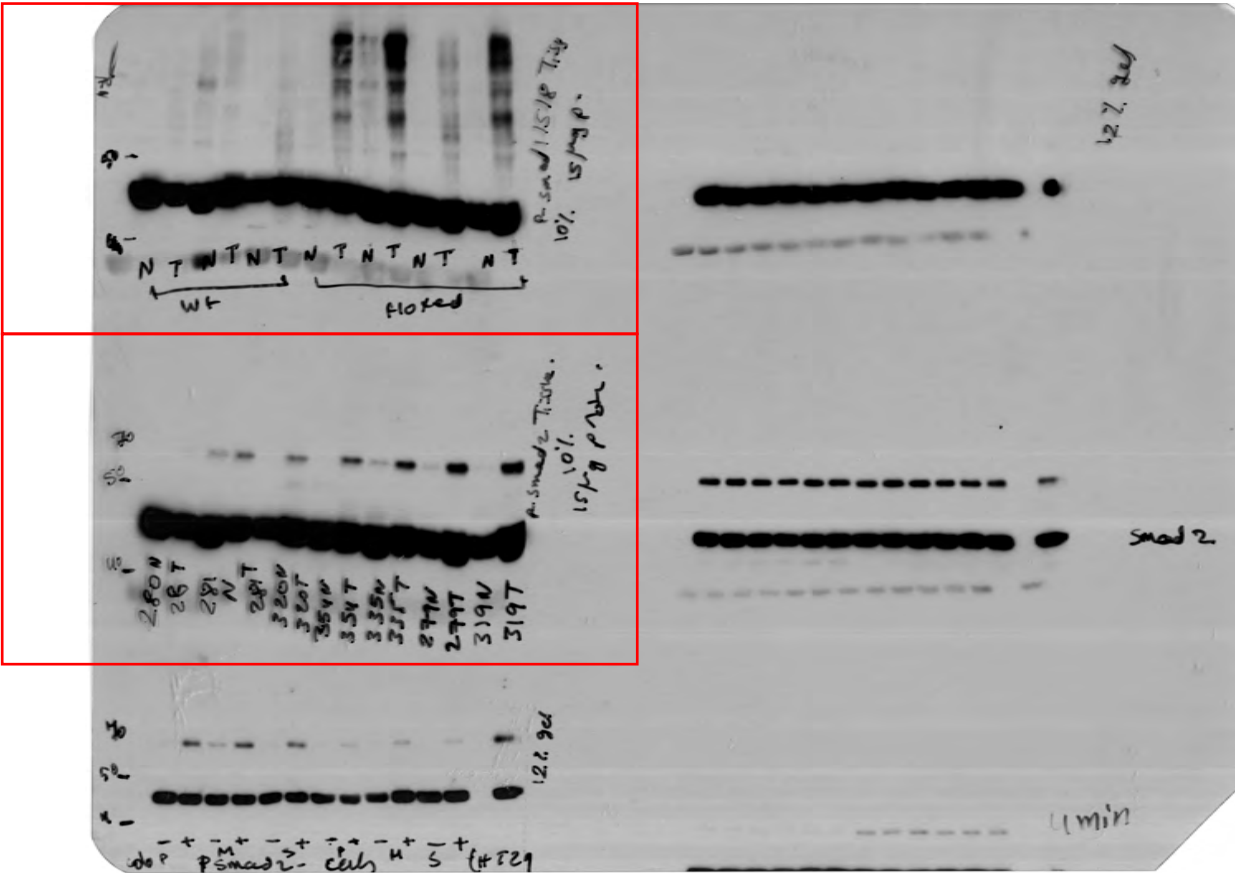


***Smad4* and TGF- β 1 dependent gene expression signatures in conditional intestinal adenoma, organoids and colorectal cancer.**

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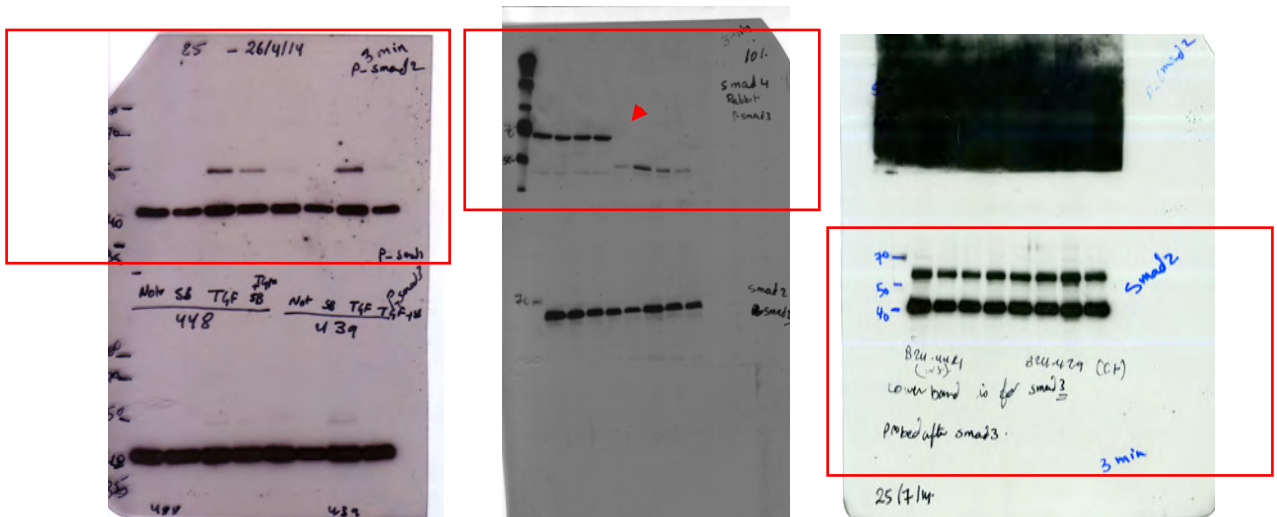
Supplementary Information Western Blots

Figure 1d
Western blot for anti-pSmad 1/5/8 and anti-pSmad3 and beta-actin



Western blot of adenoma (T) and normal tissue (N) from *Apc^{fl/fl}Smad4^{+/+}* and *Apc^{fl/fl}Smad4^{f/fl}* with anti-phospho-Smad2 (p-Smad2) and anti-phospho-Smad1/5/8 antibodies.

Figure 2c: Western blots for anti-pSmad2, anti-Smad2 and beta actin



Western blots showing p-Smad2 (upper left), Smad4 (middle) and Smad2/ beta-actin (right). Lower left replicate for p-Smad3 in Fig 2c. The order of the samples from left to right as follow: Lane 1-4 is Smad4 *Apc Δ/Δ Smad4 $^{+/+}$* treated with vehicle, SB-505124, TGF- β 1, and SB-505124, TGF- β 1. And Lane 5-8 *Apc Δ/Δ Smad4 Δ/Δ* treated with vehicle, SB-505124, TGF- β 1, and SB-505124, TGF- β 1.

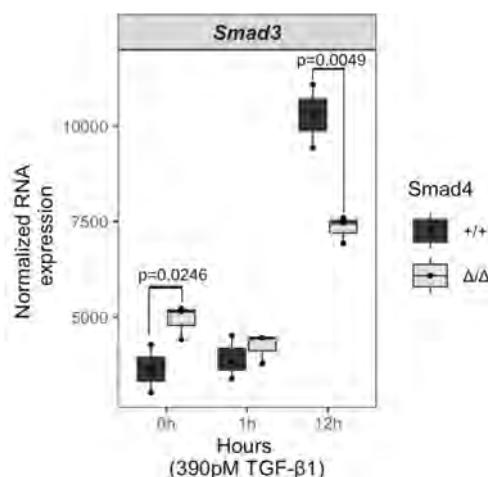
Three panels of gel electrophoresis images showing EMSA results for p-Smad3 and Smad3.

Left Panel: Shows p-Smad3 and Smad3 bands. The p-Smad3 band is labeled "p-Smad3" and the Smad3 band is labeled "Smad3".

Middle Panel: Shows p-Smad3 and Smad3 bands. The p-Smad3 band is labeled "p-Smad3" and the Smad3 band is labeled "Smad3". The treatments are: not, SB, TGF β , TGF β + SB, and TGF β + 5 μ . The gel is labeled "gel 10%".

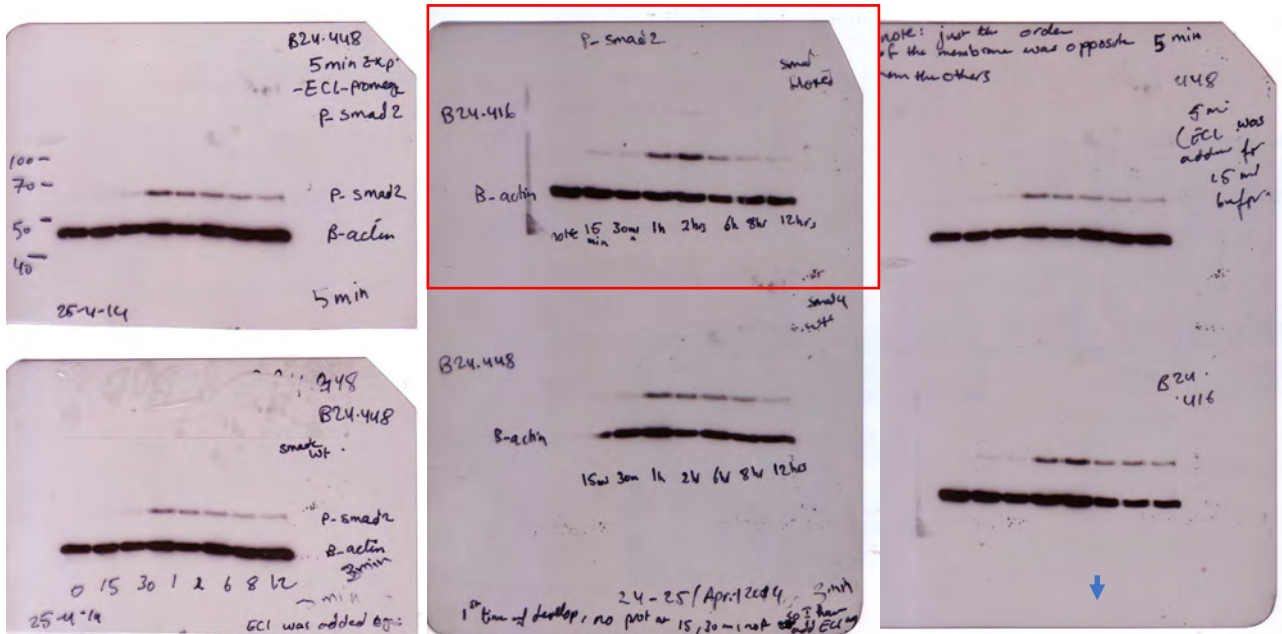
Right Panel: Shows p-Smad3 and Smad3 bands. The p-Smad3 band is labeled "p-Smad3" and the Smad3 band is labeled "Smad3". The treatments are: not, SB, TGF β , TGF β + SB, and TGF β + 5 μ . The gel is labeled "gel 10%". A note at the bottom says "Reprobed with 1 $^{\circ}$ A over night (Amersham kit was used 0.5 μ l)".

Western blots showing p-Smad3 (upper band) and Smad3 (Lower panel). The order of the samples from left to right as follow: Lane 1-4 is Smad4 *Apc*^{Δ/Δ}*Smad4*^{+/+} treated with vehicle, SB-505124, TGF-β1, and SB-505124, TGF-β1. And Lane 5-8 *Apc*^{Δ/Δ}*Smad4*^{Δ/Δ} treated with vehicle, SB-505124, TGF-β1, and SB-505124, TGF-β1. Middle and right panel replicates, with blot in Fig2c panel an additional replicate.



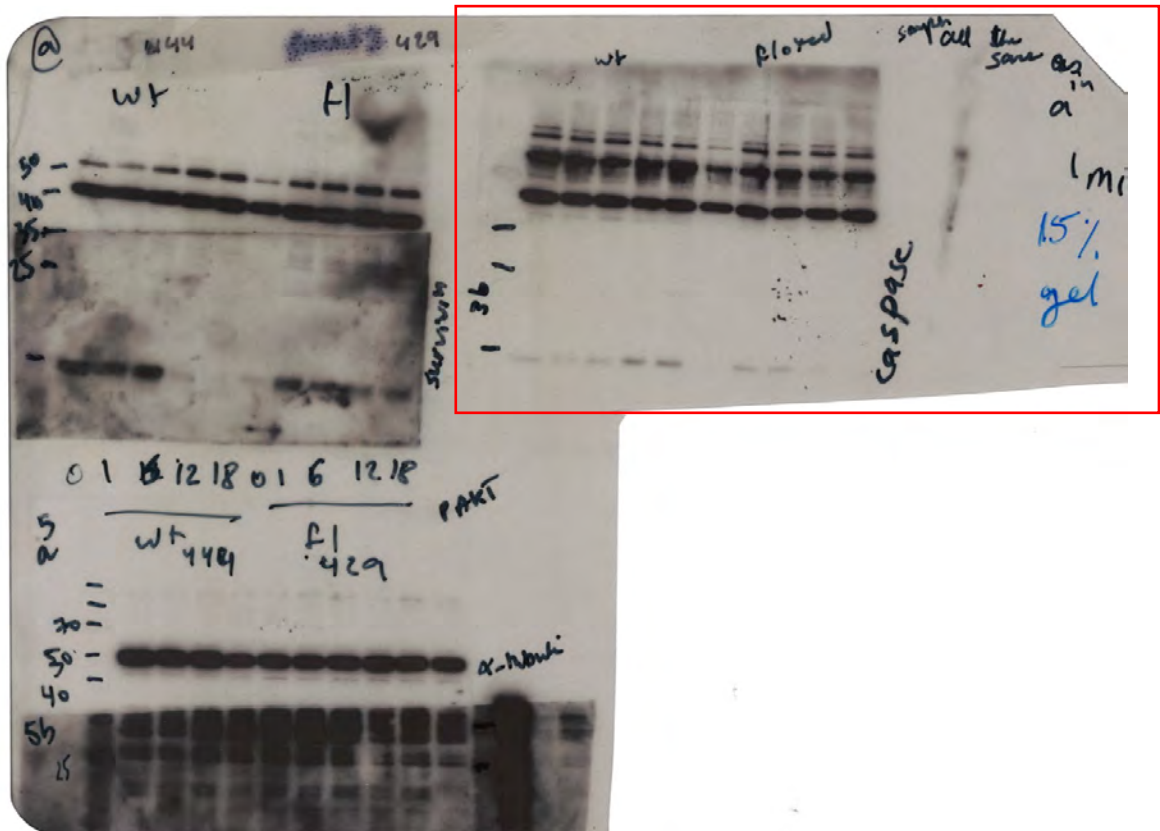
Effects of TGF- β 1 exposure on Smad3 mRNA expression (as in Figure 3h).

Figure 2d
Western blot for anti-pSmad2 and beta-actin



Associated western immunoblots of p-Smad2 at different time points (h, annotated) following TGF- β 1 (390pM), densitometry relative to β -actin.

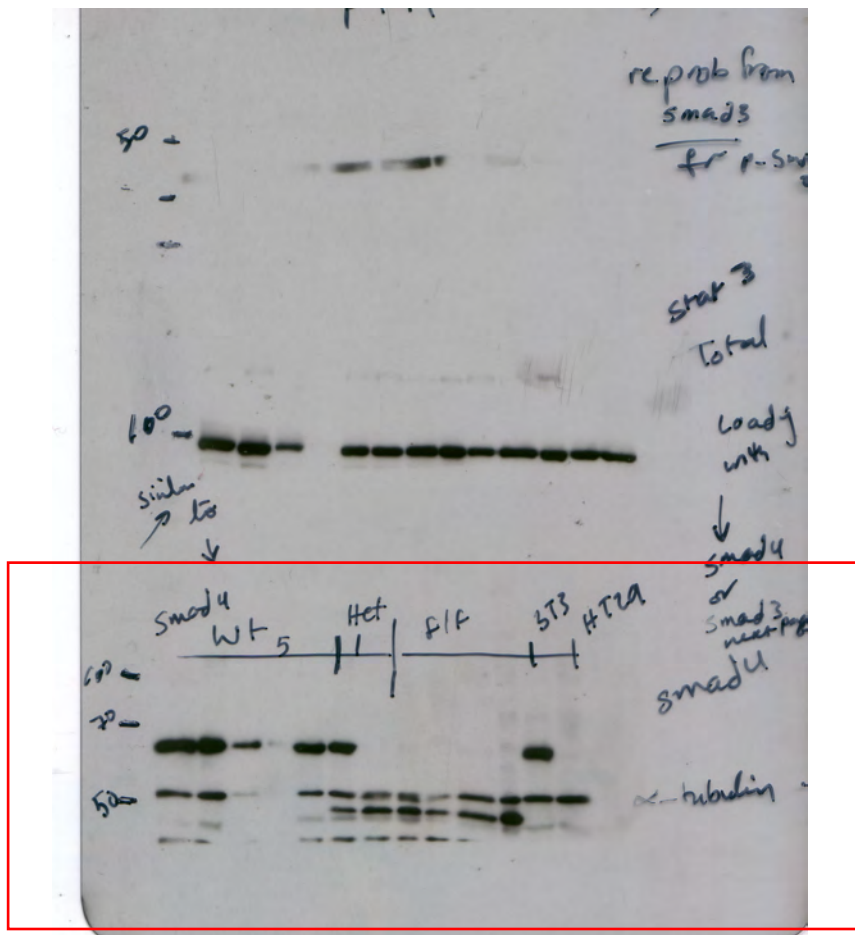
Figure 2g
Western blot for anti-Cleaved Caspase 3 (CC3)



Associated western immunoblots of CC3 at different time points (h, annotated) following TGF- β 1 (390pM), densitometry relative to β -actin.

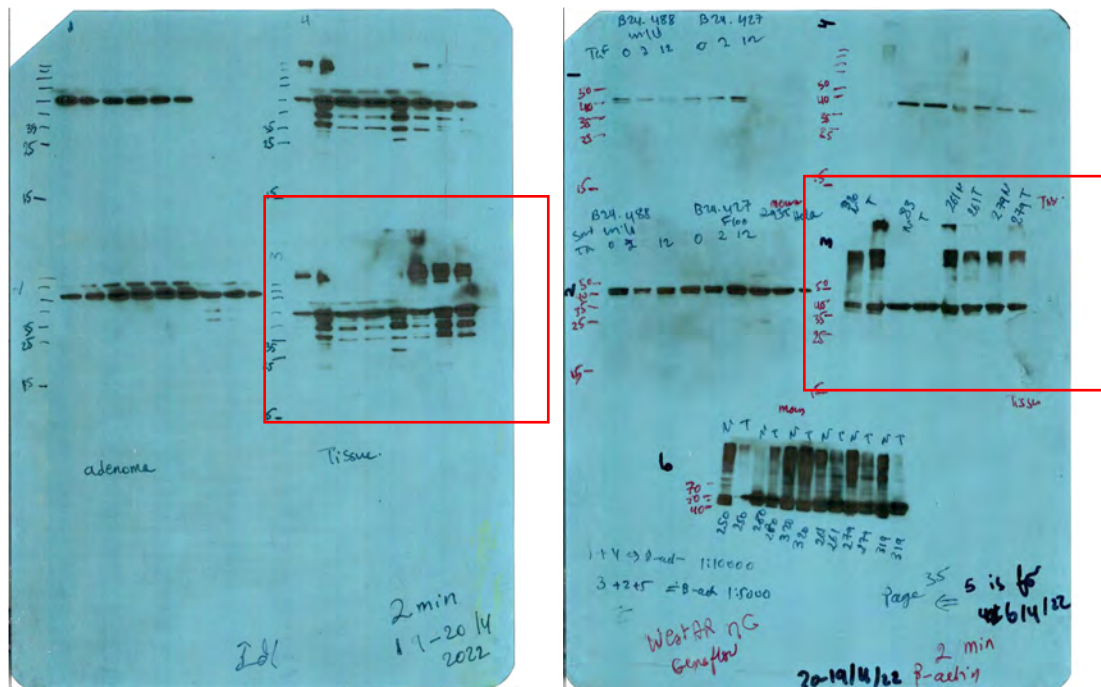
Supplementary Figure 2c

Western blot for anti-Smad4 and anti-tubulin



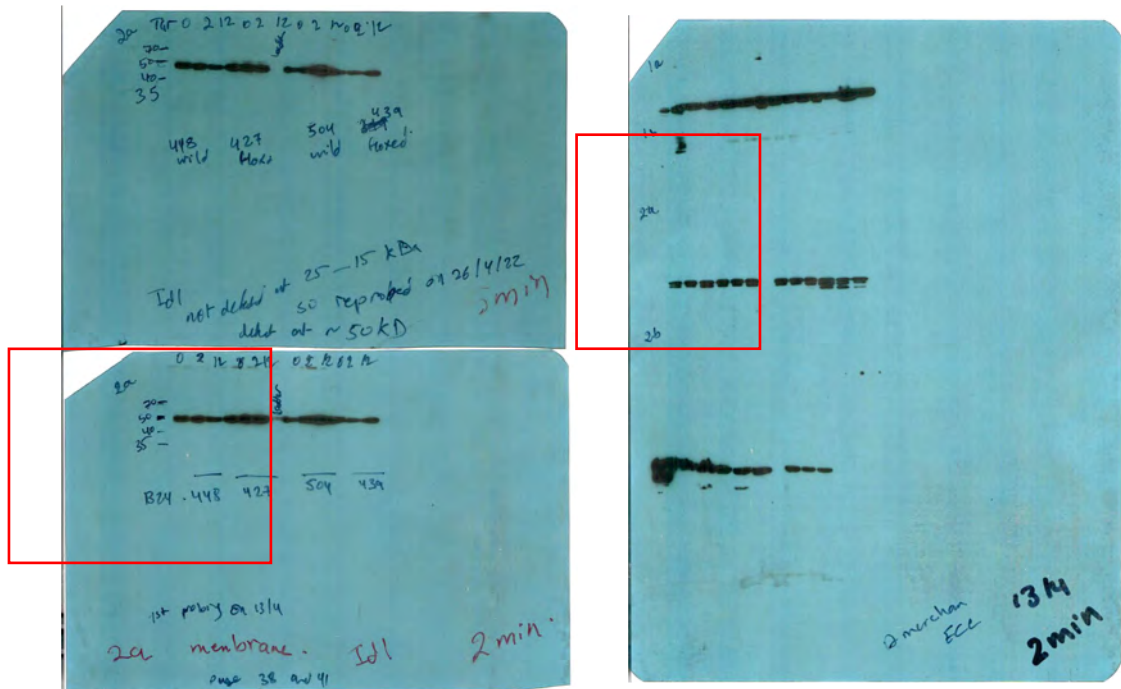
Western blots (left panel) showing Smad4 protein of 61kDa from *Apc^{Δ/Δ}Smad4^{+/+}* and *Apc^{Δ/Δ}Smad4^{Δ/+}* adenomas, and a truncated Smad4 protein of 43 kDa in *Apc^{Δ/Δ}Smad4^{Δ/+}* and *Apc^{Δ/Δ}Smad4^{Δ/Δ}*. Controls: 3T3 fibroblast cells with intact *Smad4* (positive control). HT29 CRC cells have *Smad4* mutation (C931 C>T) in the MH2 domain (negative control).

Supplementary Figure 7b
Western blot for anti-Id1 and anti-beta-actin



7b Western blot of Id1 (50kDa) from adenoma (T) and adjacent normal tissue (N) from *Apc^{fl/fl}Smad4^{+/+}* and *Apc^{fl/fl}Smad4^{fl/fl}*. β -actin (43kDa) is a loading control.

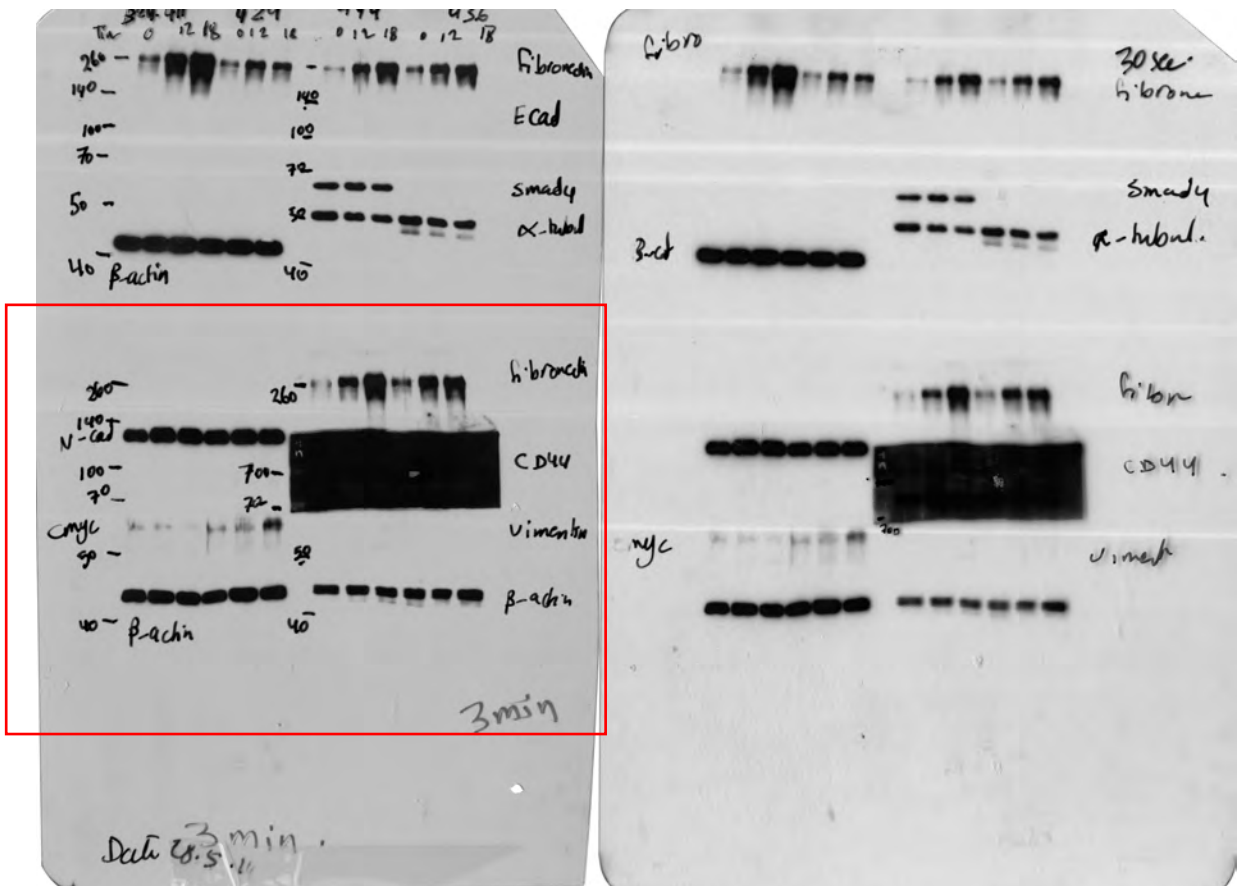
Western blot for anti-Id1 and anti-beta-actin



7c Western blot of Id1 from in *Smad4*^{Δ/Δ} and *Smad4*^{+/+} adenoma organoids at t=0, t=2 and t=12hr of 390pM TGF-β1 treatment.

Supplementary Figure 8

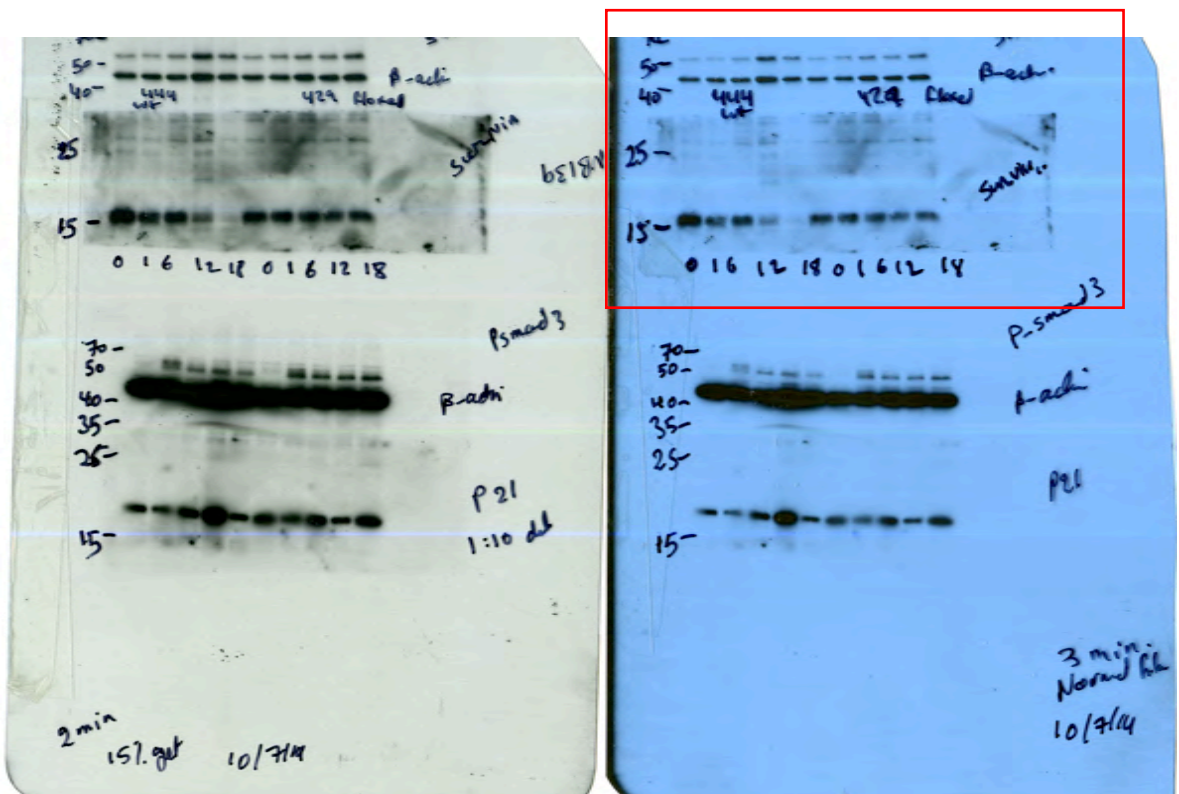
Western blots for anti-fibronectin, anti-N-cadherin, anti-Smad4, anti-c-Myc and anti-beta-actin



Western blot used for Suppl Figure 8 for fibronectin, N-cadherin with β -actin, also included C-myc and Smad4.

Supplementary Figure 9

Western blots for anti-survivin and anti-beta-actin



Western immunoblots showing the downregulation of Survivin within 12h TGF- β 1 treatment predominantly in Smad4^{+/+} adenomas.

Representative western blots showing for β -catenin, E-cadherin and c-Myc at different time points following TGF- β 1 exposure. β -catenin at 92 kDa (arrow) with multiple bands suggesting degradation. E-cadherin at 130 kDa with multiple bands suggesting degradation. c-Myc at 67 kDa.

