SUPPLEMENTAL INFORMATION

Supplemental Table 1. Primers used for qRT-PCR. Sequences of the forward and reverse primer pairs used in qRT-PCR experiments.

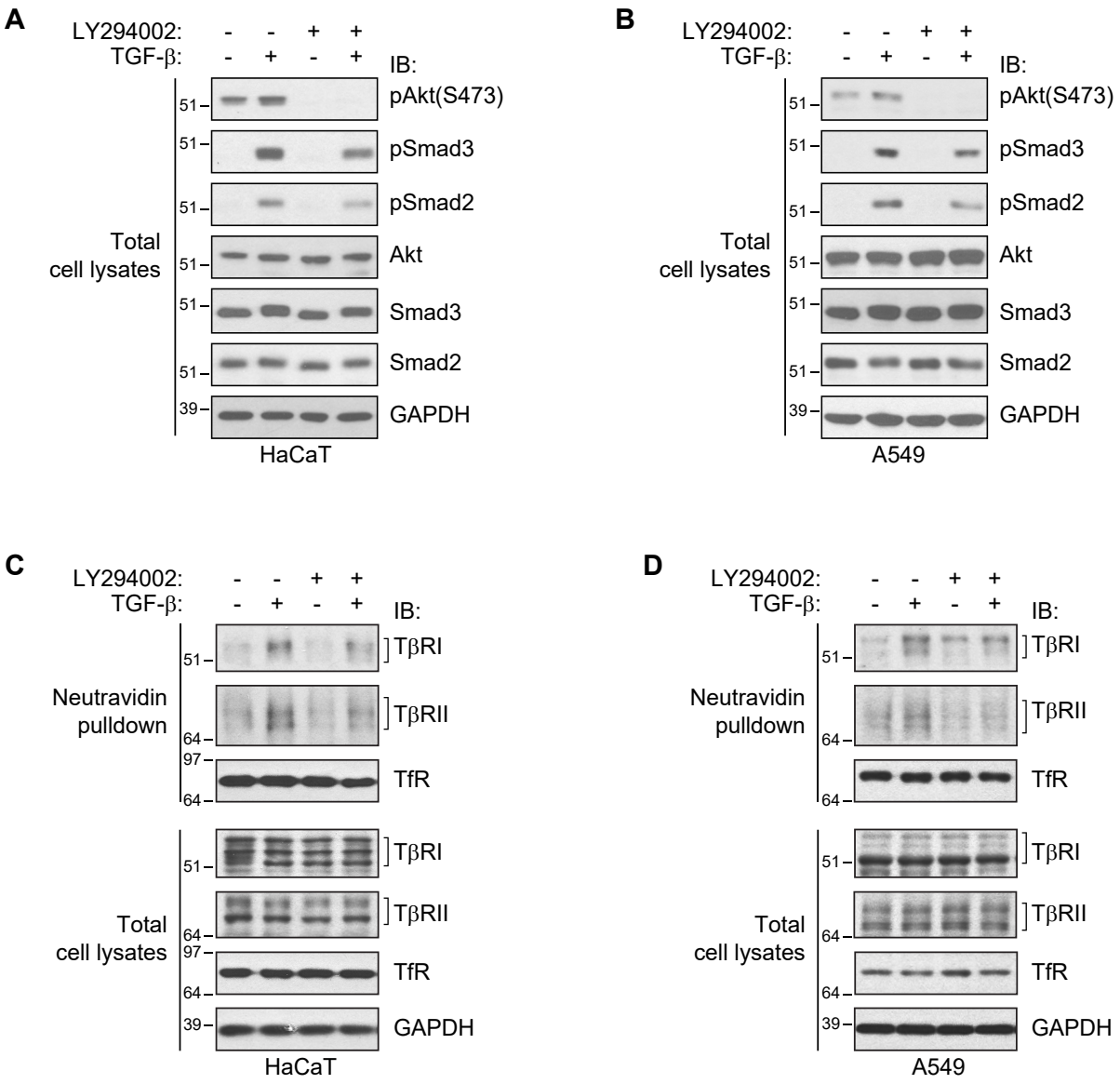
Figure S1. Inhibition of PI3K prevents Akt phosphorylation, and attenuates activation of Smad3 and Smad2 and upregulation of TGF-β receptors. (A-D) Effect of the PI3K inhibitor LY294002 on TGF-β-induced activation of Akt, Smad3, and Smad2, assessed by immunoblotting for phospho-Smad3, -Smad2, or -Akt (A, B), and TβRI and TβRII levels at the cell surface, assessed by cell surface protein biotinylation and immunoblotting (C, D), in HaCaT (A, C) or A549 (B, D) cells. In a manner similar to the Akt inhibitors AktVIII and MK2206, Ly294002 blocked basal and TGF-β-induced Akt activation, and attenuated Smad2 and Smad3 activation without affecting whole-cell levels of Akt, Smad2, or Smad3 (A, B). Furthermore, LY294002 attenuated the TGF-β-induced increase in cell surface TβRI and TβRII levels without affecting cell surface levels of TfR or whole-cell levels of TGF-β receptors. (C, D). GAPDH and TfR levels serve as loading controls. *Note: Figure S1A presents additional data from the same experiment that was shown in Figures 2A (right) and 5D. Figures S1B, S1C, and S1D present data obtained from the same experiments as shown in the right side panels of Figure 2B, 2C, and 2D, respectively.*

Figure S2. Anti-TGF-β neutralizing antibody prevents activation of Akt, Smad3, and Smad2. Effect of the neutralizing anti-TGF-β antibody 1D11 on activation of Akt, Smad3, and Smad2 in HaCaT cells in response to TGF-β, assessed by immunoblotting for phospho-Akt(S473), phospho-Smad3, and phospho-Smad2. Cells were incubated with control or 0.125 or 0.25 ng/mL TGF-β for 30 minutes, in the presence or absence of 1D11. Addition of 1D11 inhibited activation of Akt and blocked Smad3 and Smad2 phophorylation in response to either concentration of TGF-β, without affecting whole-cell levels of Akt, Smad3, or Smad2. GAPDH served as loading control.

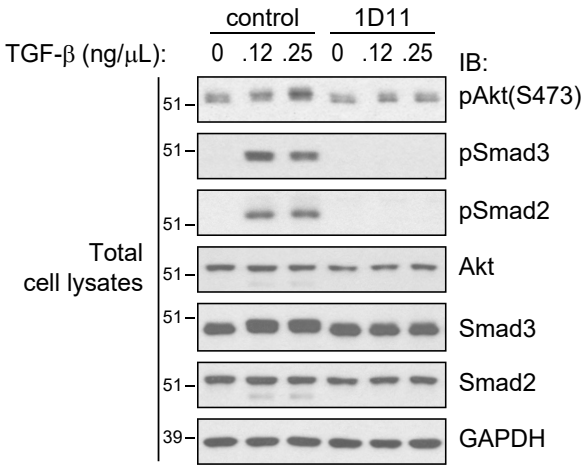
Figure S3. Quantification of key Western blot data. Results from quantification of immunoblot data for select experiments. Bands from (n=3) separate repeats of each experiment were quantified using ImageJ and normalized to control. The graphs show mean ±S.D. (*error bars*) of relative band intensity quantification, and are titled to indicate the corresponding figure in the manuscript.

Supplemental Table 1. qRT-PCR primer sequences.

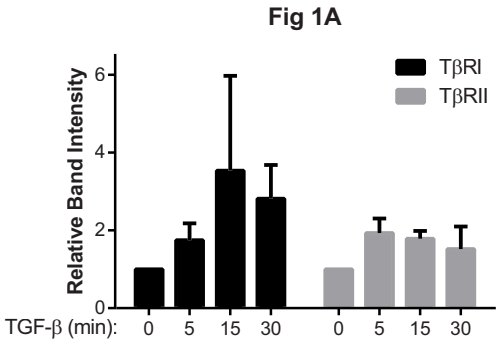
SERPINE1	5′-GGCTGACTTCACGAGTCTTTCA-3′ (forward)	5′-ATGCGGGCTGAGACTATGACA-3′ (reverse)
SNAI2	5′-TGTGACAAGGAATATGTGAGCC-3′ (forward)	5′-TGAGCCCTCAGATTTGACCTG-3′ (reverse)
SMAD7	5′-TGCTGTGAATCTTACGGGAAG-3′ (forward)	5′-AATCCATCGGGGTATCTGGAG-3′ (reverse)
ID1	5′-CTGCTCTACGACATGAACGG-3′ (forward)	5′-GAAGGTCCCTGATGTAGTCGAT-3′ (reverse)
ID3	5′-CATTCGTCTACATTCTCGACCTG-3′ (forward)	5′-TCCTTTTGTCGTTGGAGATGAC-3′ (reverse)
RPL19	5′-ATGTATCACAGCCTGTACCTG-3′ (forward)	5′-TTCTTGGTCTCTTCCTCCTTG-3′ (reverse)

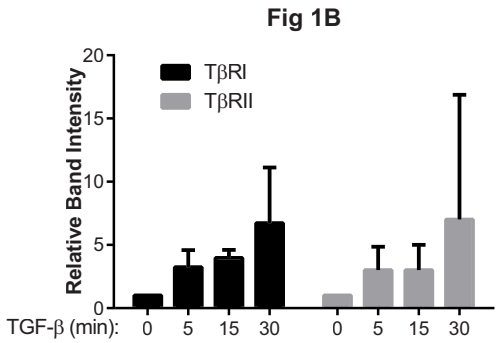


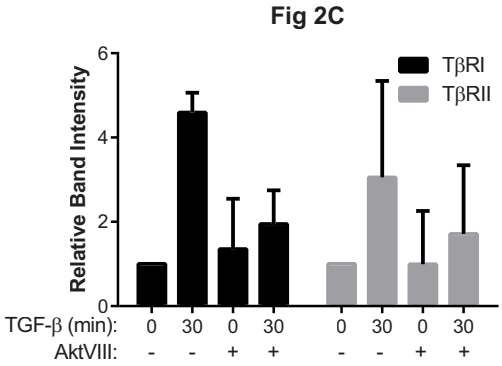
Duan and Derynck. Figure S1

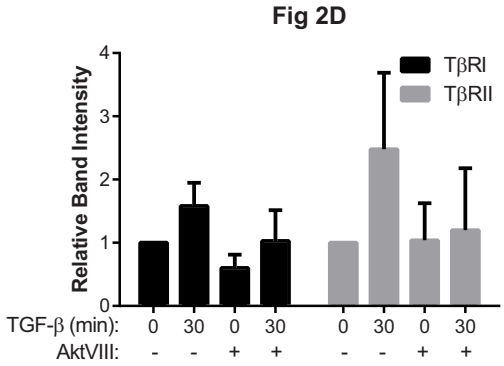


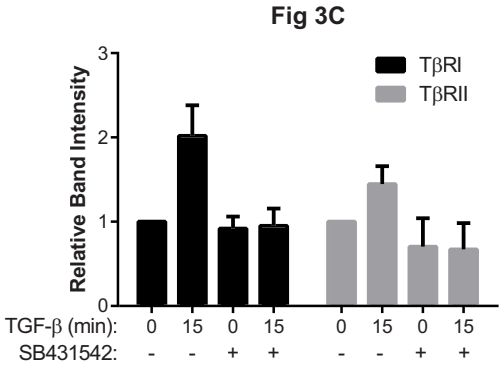
Duan and Derynck. Figure S2

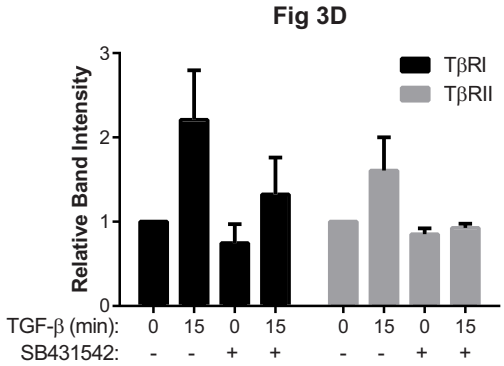


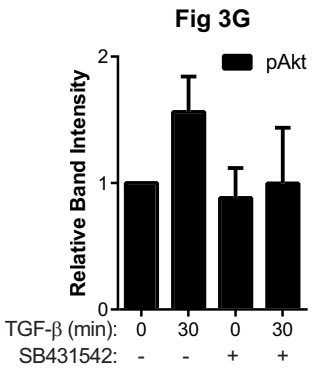


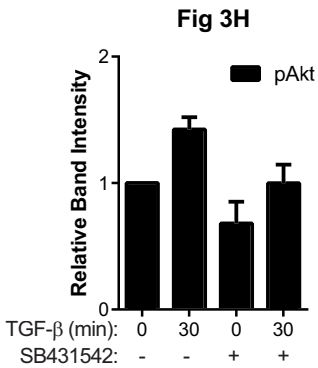


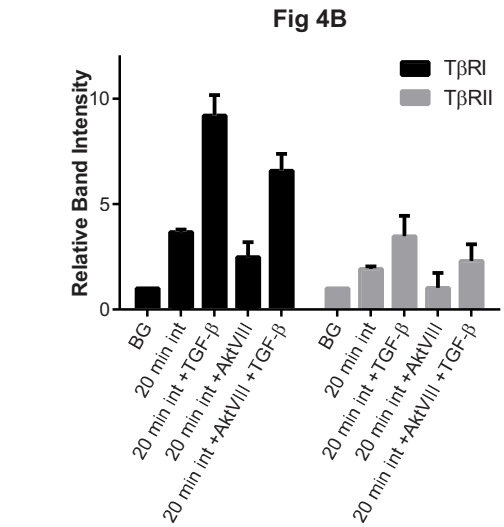


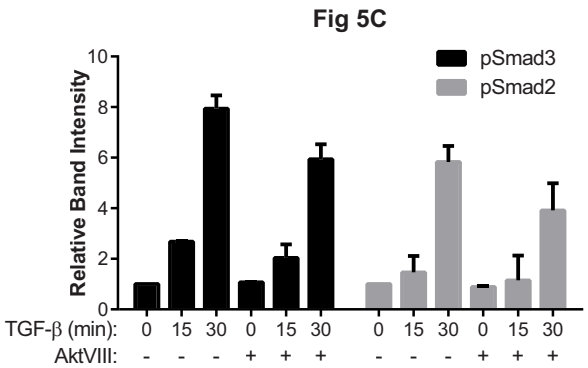












Duan and Derynck. Figure S3