

Supplementary Figure 1. Localization and dynamics of SMAD2/4 and *ctgf* response.

A) Time course immunofluorescence analysis of SMAD4 translocation in response to 5nM TGF- β stimulation.

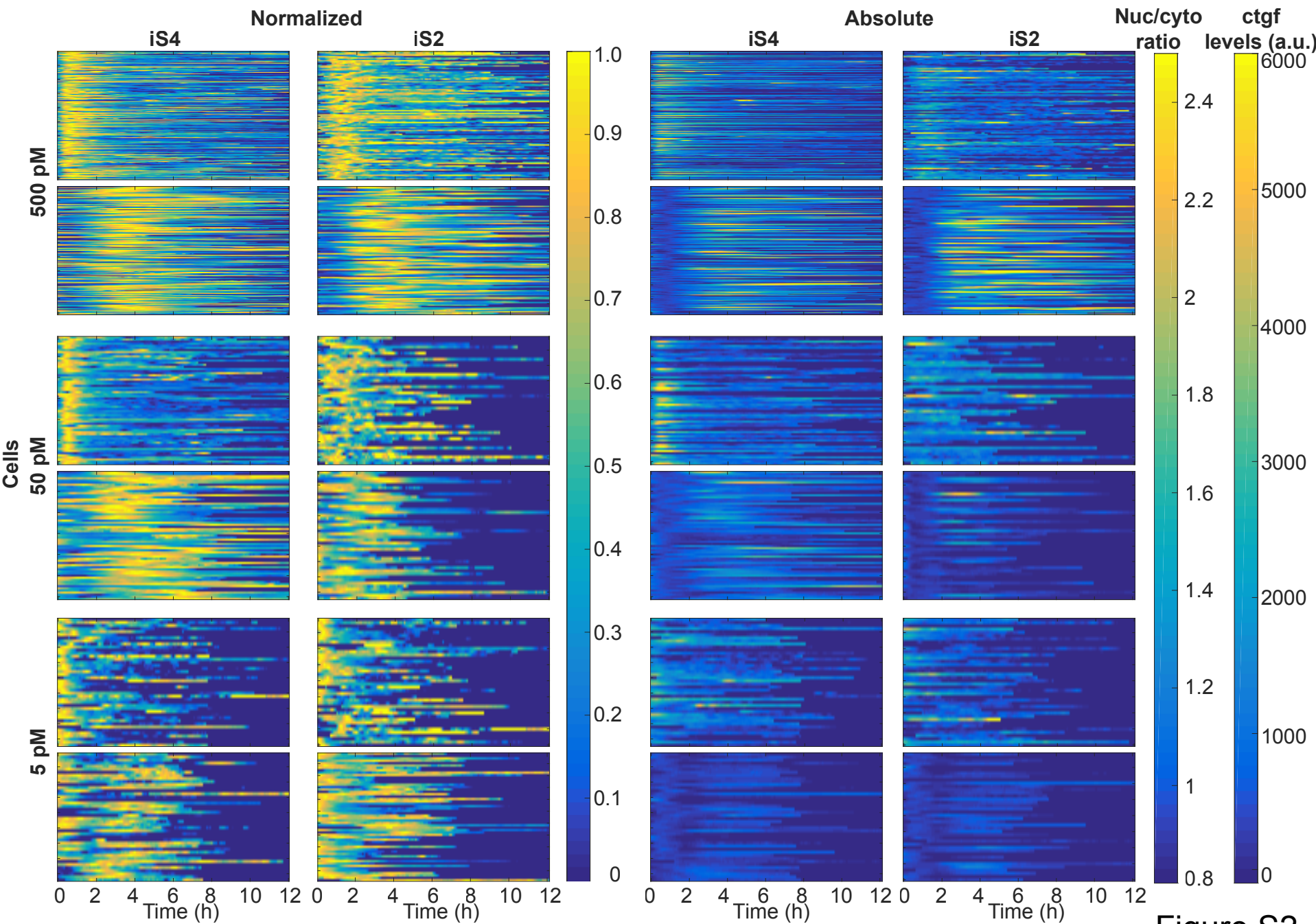
B) Western blots of pSMAD2 in total cell lysates after 5nM TGF- β stimulation. The quantification shows normalized band intensity with min-max scaling. The cropped blot is used in the figure and the full length Western blot scans for the cropped images are shown in Supplementary Figure 5.

C) SMAD4 localization changes (top) and *ctgf* expression (bottom) without stimulation.

D) Individual cell traces (top) and population average (bottom) of *ctgf* expression levels in samples treated with the TGF β -receptor antagonist SB-431542 (dashed black line). Solid line: mean; shaded areas: SD.

E) Comparison of SMAD4 translocation (top) and *ctgf* expression (bottom) in unstimulated cells (n= 58, gray) or cells stimulated with 5nM TGF- β (red and blue). Solid line: mean; shaded areas: SD. Scale bar: 20 μ m.

F-G) Time series images of a tracked single cell of iS2 cell line on both Nluc (left) and Fluc (right) channel by luminescence microscopy. Time resolution: 5 minutes. Four individual traces are shown for both SMAD2 translocation and *ctgf* response.



Supplementary Figure 2. Heat maps of for single cell traces of SMAD4/2 translocation and *ctgf* responses to TGF- β stimulation.

Cells were treated with different doses of TGF- β (500-50-5 pM) at time $t=0$ and response traces for all cells are shown both in normalized (left) and absolute (right) terms. For each experiment, SMAD translocation (top) and *ctgf* response (bottom) are shown.

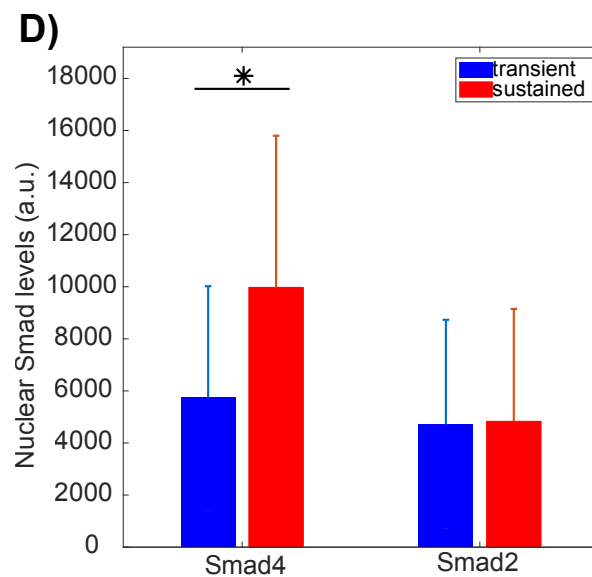
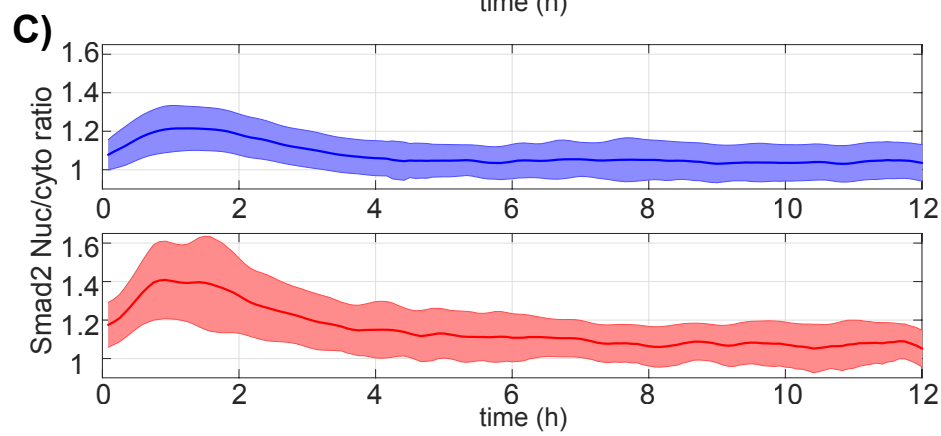
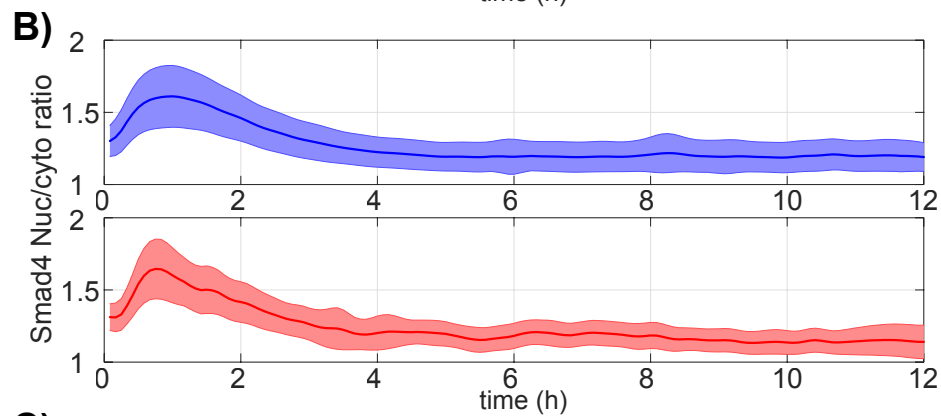
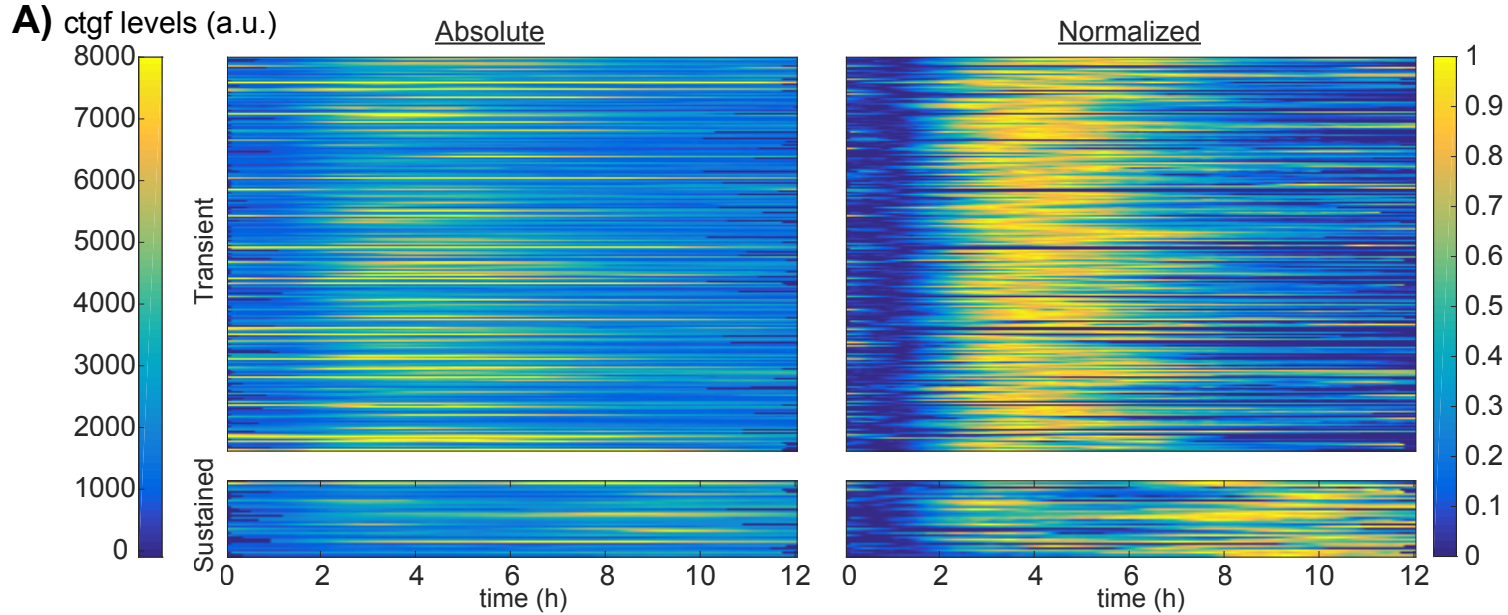


Figure S3

Supplementary Figure 3. SMAD shuttling dynamics and levels in cells responding in a transient and sustained manner TGF- β stimulation.

A) Decomposition of single cell *ctgf* responses into transient and sustained classes using k-means clustering in iS2 cell line upon 2ng/ml dox treatment and 5nM TGF- β stimulation. Heatmaps for *ctgf* traces belonging to transient (upper panels) and sustained (lower panels) are shown, quantified as both absolute (left) and normalized (right) levels (min-max scaled, 0 to 1).

B-C) Average SMAD nuclear to cytoplasmic ratio trajectories belonging to two classes identified with clustering (blue-transient, red-sustained), in iS4 (B) and iS2 (C) cell lines.

D) Average SMAD4/2 expression level during the 12 hours following 5nM TGF- β stimulation, belonging to the transient and the sustained classes from samples treated with all doses of dox. Error bars: SD; *p<0.005 (t-test).

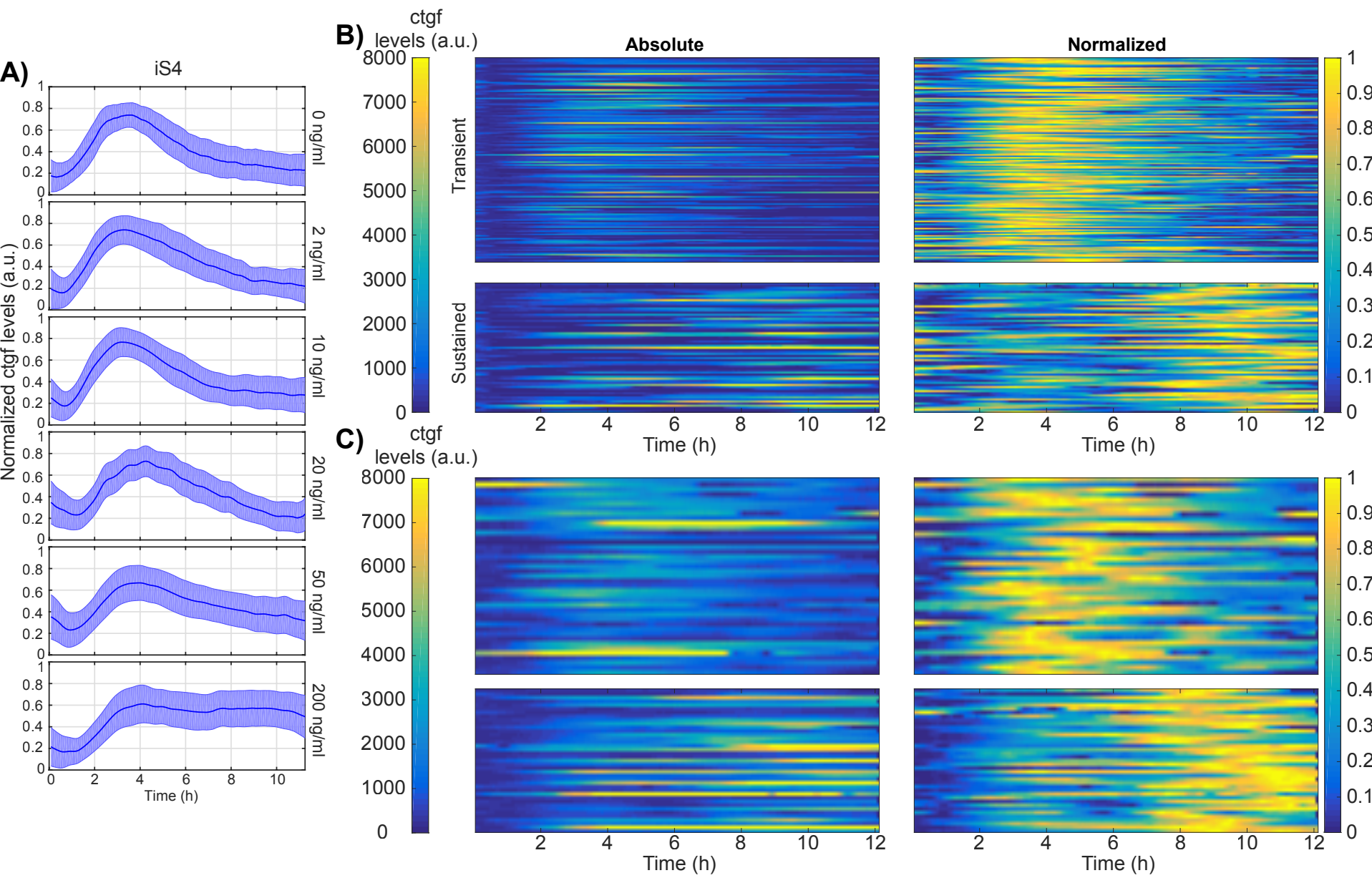


Figure S4

Supplementary Figure 4. TGF- β -induced SMAD signaling and *ctgf* response in iS4 cell line treated with different doses of Dox.

A) Population averages of *ctgf* responses upon 5nM TGF- β stimulation in samples treated with varying doses of doxycycline in the iS4 cell line.

B-C) Clustering of single cell *ctgf* responses into transient and sustained classes in iS4 cell line treated with 50ng/ml (B) and 200ng/ml (C) of dox.

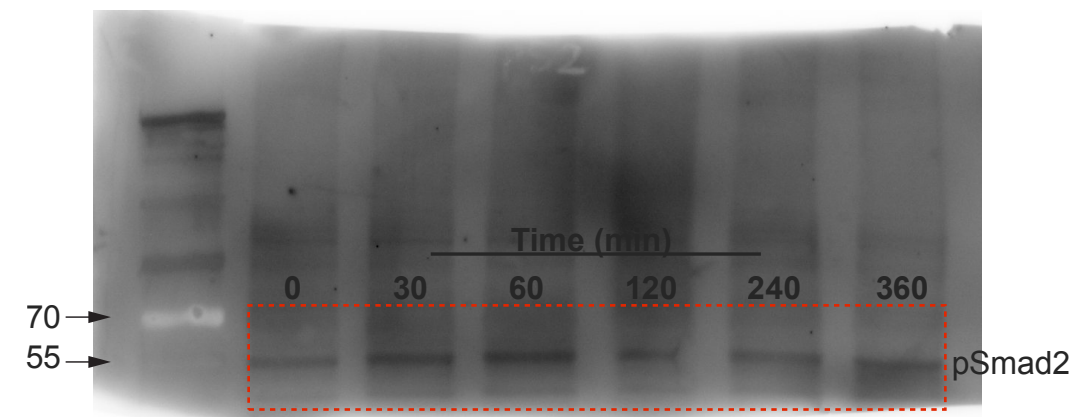
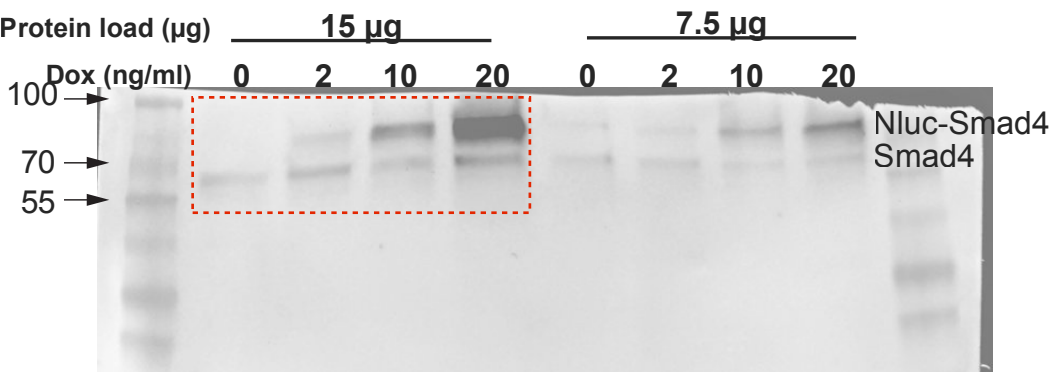
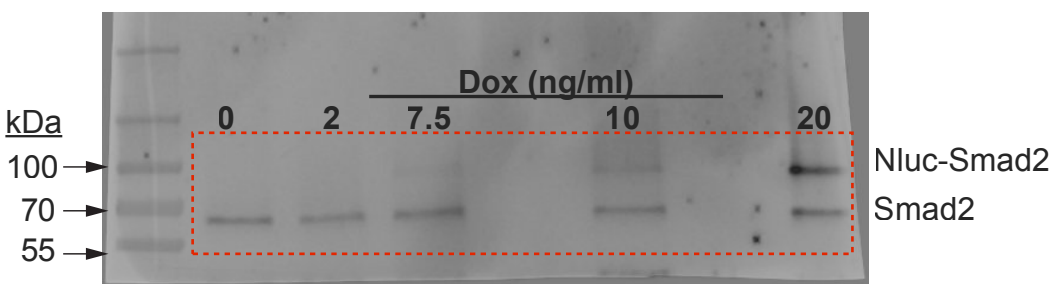


Figure S5. Full images of Western blots shown in Figure 1 and Supplementary Figure 1. Red dotted lines indicate the cropping locations. Brightness was adjusted during processing of these blots.