Supporting information for

Spatiotemporal Control of TGF-β Signaling with Light

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List of Supporting Information

Supplementary Figures

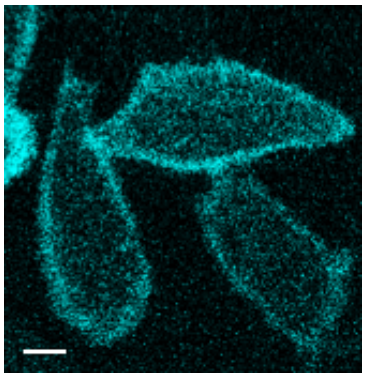

Figure S1: Subcellular localization of mCerulean tagged Myr-cytTβRI-CIBN protein in HeLa cells. The expression of the membrane-anchored cytTβRI protein fused with CIBN domain and mCerulean fluorescence tag (Myr-cytTβRI-CIBN-mCer) shows the plasma membrane localization in HeLa cells. Scale bar: 10 μm.

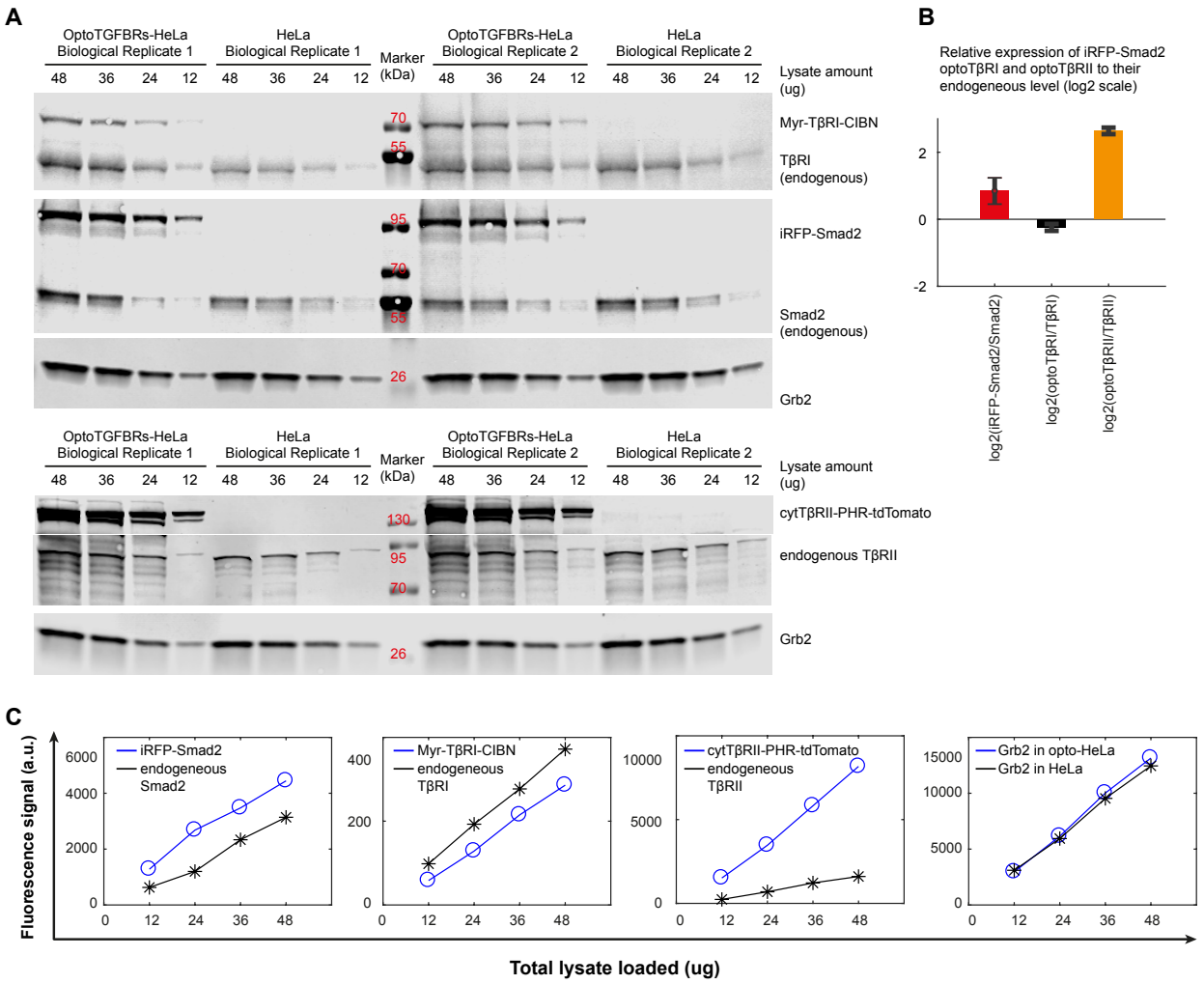

Figure S2: The expression of iRFP-Smad2, Myr-TβRI-CIBN and cytTβRII-PHR-tdTomato proteins in the optoTGFBRs-HeLa cells.

(A) Cell lysates were loaded at different amount to optimize the range where antibody signal is linear. **(B)** The relative expressions of iRFP-Smad2 to endogenous Smad2, optoTβRI to endogenous TβRI, and optoTβRII to endogenous TβRII were estimated from the average of two biological replicates. **(C)** The antibodies' fluorescence signal has a linear relationship with the amount of lysate loaded when measured with the LI-COR odyssey CLx imaging system.

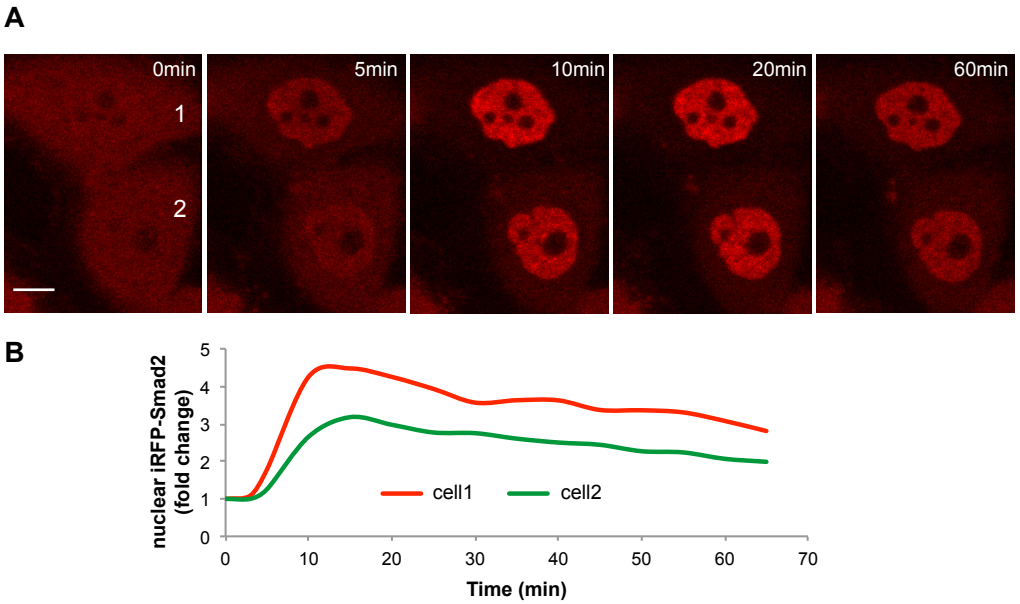

Figure S3: The optoTGFBRs system can be activated by two-photon excitation. (A) optoTGFBRs-HeLa cells were excited with two-photon illumination at 860 nm to induce translocation of iRFP-Smad2 to the nucleus. Scale bar: 10 μm. (B) Quantification of nuclear Smad2 signaling dynamics shown in panel A.

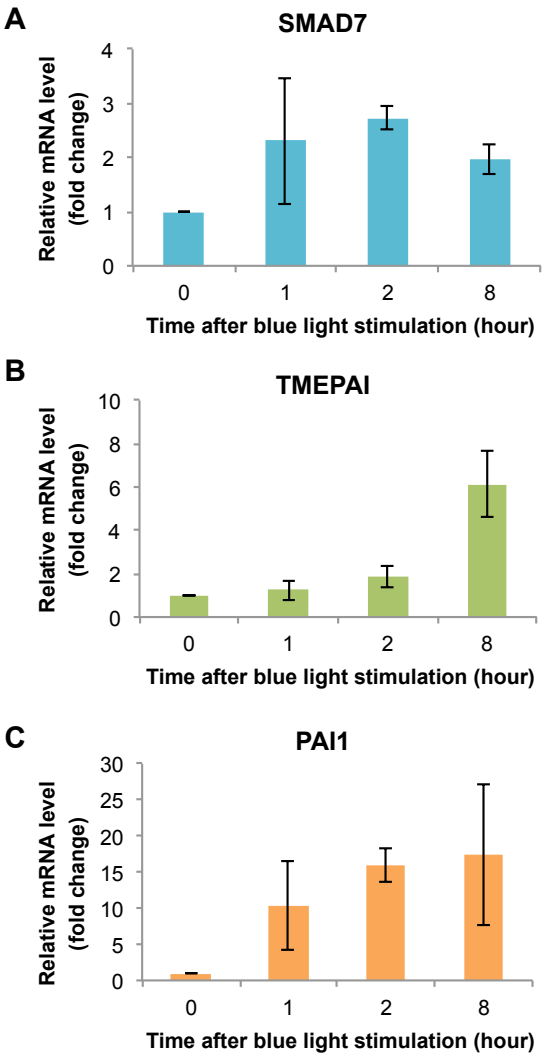

Figure S4: The optoTGFBRs system can induce the expression of TGF-β responsive genes. Quantitative PCR assay for the expression of **(A)** Smad7, **(B)** TEMPAI and **(C)** PAI1 genes in the optoTGFBRs-HeLa cells at 0, 1, 2 and 8 hours after blue light illumination (488 nm, 4 mW/cm^2) in LED box. The averages and standard deviations from three replicates are shown.

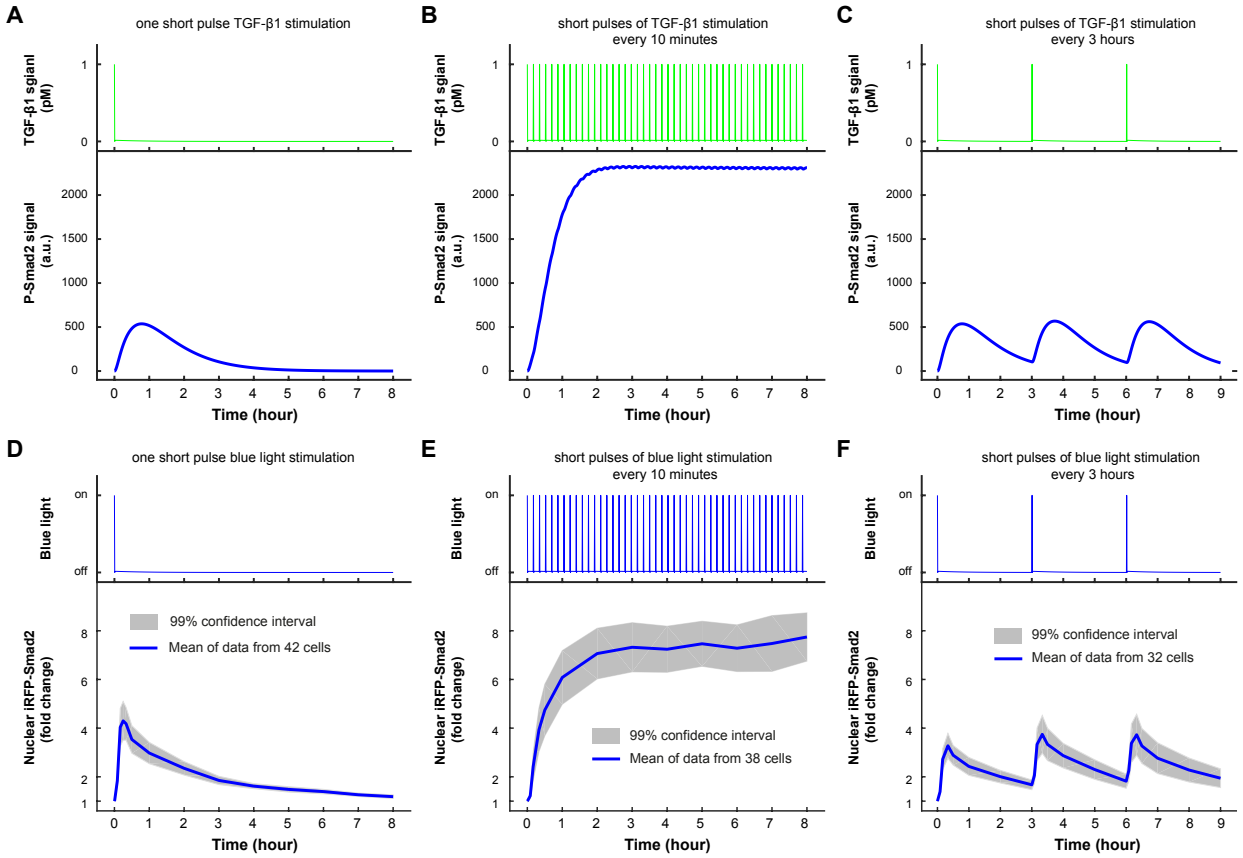

Figure S5: Dynamics of Smad2 signaling to pulses of TGF-β and blue light stimulations. (A-C) Predicted dynamics of P-Smad2 response to different TGF-β pulse stimulations using a published mathematical model (Zi *et al.* Mol Syst Biol, 2011, Reference 32). (D-F) Quantification of Smad2 signaling responses to similar patterns of blue light stimulations in optoTGFBRs-HeLa cells. The 99% confidence interval is based on Student's t-distribution.

Tables S1: Summary of initial screen results with different combinations of optoTβRI and optoTβRII constructs

Combinations of constructs	Smad2 nuclear translocation upon blue light stimulation	Basal Smad2 signaling without blue light stimulation
TβRI-CIBN-mCer TβRII-PHR-mCit	No	No
Myr-cytTβRI -CIBN-mCer Myr-cytTβRII -PHR-mCit	Yes	High
Myr-cytTβRI-PHR-mCit Myr-cytTβRII-CIBN-mCer	No	No
Myr-cytTβRI-PHR-mCit Myr-cytTβRII-PHR-mCit	Yes	High
Myr-cytTβRI-CIBN Myr-cytTβRII-PHR	Yes	High
cytTβRI-CIBN-mCer cytTβRII-PHR-mCit	Yes	High
Myr-cytTβRI-CIBN-mCer cytTβRII-PHR-mCit	Yes	Low
cytTβRI-CIBN-mCer Myr-cytTβRII-PHR-mCit	Yes	High
cytTβRI-CIBN-mCherry cytTβRII-PHR-mCherry	Yes	High
Myr-cytTβRI-CIBN cytTβRII-PHR-mCherry	Yes	Low
Myr-CIBN-cytTβRI cytTβRII-PHR-mCherry	No	No
cytTβRI-CIBN-mCherry Myr-cytTβRII-PHR	Yes	High
Myr-CIBN PHR-cytTβRII-Tdtomto	No	No
Myr-CIBN Tdtomto-PHR-cytTβRII	No	No
Myr-CIBN-cytTβRI PHR-cytTβRII-Tdtomto	No	No
Myr-CIBN-cytTβRI Tdtomato-PHR-cytTβRII	No	No
Myr-cytTβRI-CIBN Tdtomato-PHR-cytTβRII	No	No
Myr-cytTβRI-CIBN PHR-cytTβRII-Tdtomato	No	No
Myr-cytTβRI-CIBN cytTβRII-PHR-Tdtomato	Yes (final selected construct)	Low