Electronic Supplementary Material (ESI) for Molecular BioSystems

Supplementary Information: Figures

Figure S1. Simulation of the effects of pancreatic cancer mutations on TGF-β signaling dynamics. Same simulations as in Figure 2 with the normal dynamics of the HaCaT case from Figures 2A and 2D and of the Colo-357 case from Figures 2B and 2E. (A) Results for nuclear phosphorylated Smad2 and (B) nuclear Smad4 species. Solid lines represent the normal dynamics of the model and dashed lines represent those of the model with the mutation described in the caption of Figure 2.

Figure S2. Simulation of the effects of endocytosis inhibitors on TGF-β signaling dynamics. Same simulations as in Figure 3 with the normal dynamics of the HaCaT case from Figure 3A and those with potassium depletion (-KCl) and Nystatin of Figures 3B and 3C, respectively. Results for the ratio of phosphorylated Smad2 to total Smad2 for (A) potassium depletion (-KCl) and (B) Nystatin treatment. Solid lines represent the normal dynamics of the model and dashed lines represent those of the model with the endocytosis inhibitors described in the caption of Figure 3.

Figure S3. Schematic illustration of the Smad-dependent TGF-β signaling pathway model with targetable processes highlighted. Same illustration as Figure 1 with reaction arrows colored if they are specifically targetable for the 'receptor downregulation' mutation (Figure 6). Colors correspond to the parameter colors in Figure 6.

Supplementary Information: Tables

Table S1. System of ODEs for each modeled species. Equations track the rate of change in concentration of each species in the model,[19] which are illustrated in Fig. 1. Overbars indicate receptor species internalized in the endosome. The prefix *p* denotes that a species is phosphorylated and the subscripts *c* and *n* represent cytoplasmic and nuclear species, respectively.

$$\frac{d[RII]}{dt} = k_{syn,RII} - k_{deg,RII}[RII_T] - k_{1a}[TGF\beta][RII_T] + k_{1d}[TGF\beta \bullet RII_T] + k_{17rec}\left([\overline{C_{1T}}] + [\overline{C_2}]\right) - k_{19int}[RII] + k_{19rec}[\overline{RII}]$$

$$\frac{d[TGF\beta \bullet RII]}{dt} = k_{1a}[TGF\beta][RII] - k_{1d}[TGF\beta \bullet RII] - k_{2a}[TGF\beta \bullet RII]\left([RI_1] + [RI_2]\right) + k_{2d}\left([C_1] + [C_2]\right)$$

$$\frac{d[RI_1]}{dt} = k_{syn,RI} - k_{deg,RI}[RI_1] - k_{2a}[TGF\beta \bullet RII][RI_1] + k_{2d}[C_1] + k_{17rec}[\overline{C_1}] - k_{18int}[RI_1] + k_{18rec}[\overline{RI_1}]$$

$$\frac{d[RI_2]}{dt} = k_{syn,RI} - k_{deg,RI}[RI_2] - k_{2a}[TGF\beta \bullet RII_T][RI_2] + k_{2d}[C_2] + k_{17rec}[\overline{C_2}] - k_{18int}[RI_2] + k_{18rec}[\overline{RI_2}]$$

$$\frac{d[C_1]}{dt} = k_{2a}[TGF\beta \bullet RII][RI_1] - k_{2d}[C_1] - k_{3int}[C_1] - k_{16deg}[C_1] - k_{20a,1}[C_1][S7]$$

$$\frac{d[C_2]}{dt} = k_{2a}[TGF\beta \bullet RII][RI_2] - k_{2d}[C_2] - k_{3int}[C_2] - k_{16deg}[C_2] - k_{20a,2}[C_2][S7]$$

$$\frac{d[\overline{C_1}]}{dt} = k_{3int}[C_1] - k_{4a}[\overline{C_1}][SI_c] + k_{4d}[\overline{C_1} \bullet SI_c] + k_{5phos}[\overline{C_1} \bullet SI_c] - k_{17rec}[\overline{C_1}]$$

$$\frac{d[\overline{C_2}]}{dt} = k_{3int}[C_2] - k_{4a}[\overline{C_2}][S2_c] + k_{4d}[\overline{C_2} \bullet S2_c] + k_{5phos}[\overline{C_2} \bullet S2_c] - k_{17rec}[\overline{C_2}]$$

$$\frac{d[\overline{C_1} \bullet SI_c]}{dt} = k_{4a}[\overline{C_1}][SI_c] - k_{4d}[\overline{C_1} \bullet SI_c] - k_{5phos}[\overline{C_1} \bullet SI_c]$$

$$\frac{d[\overline{C_2} \bullet S2_c]}{dt} = k_{4a}[\overline{C_2}][S2_c] - k_{4d}[\overline{C_2} \bullet S2_c] - k_{5phos}[\overline{C_2} \bullet S2_c]$$

$$\frac{d[SI_c]}{dt} = k_{syn,RS} - k_{deg,RS}[SI_c] - k_{4a}[SI_c][\overline{C_1}] + k_{4d}[\overline{C_{1T}} \bullet SI_c] - k_{12imp}[SI_c] + k_{12exp}[SI_n]$$

$$\frac{d[S2_c]}{dt} = k_{syn,RS} - k_{deg,RS}[S2_c] - k_{4a}[S2_c][\overline{C_2}] + k_{4d}[\overline{C_2} \bullet S2_c] - k_{12imp}[S2_c] + k_{12exp}[S2_n]$$

$$\frac{d[S4_c]}{dt} = k_{syn,S4} - k_{deg,S4}[S4_c] - k_{6a}[S4_c]\left([pSI_c] + [pS2_c]\right) + k_{6d}\left([pS1S4_c] + [pS2S4_c]\right) - k_{14imp}[S4_c] + k_{14exp}[S4_n]$$

$$\frac{d[pSI_c]}{dt} = k_{5phos}[\overline{C_1} \bullet SI_c] - k_{6a}[pSI_c][S4_c] + k_{6d}[pS1S4_c] - k_{13imp}[pSI_c]$$

$$\frac{d[pS2_c]}{dt} = k_{5phos}[\overline{C_2} \bullet S2_c] - k_{6a}[pS2_c][S4_c] + k_{6d}[pS2S4_c] - k_{13imp}[pS2_c]$$

$$\frac{d[pS1S4_c]}{dt} = k_{6a}[pSI_c][S4_c] - k_{6d}[pS1S4_c] - k_{7imp}[pS1S4_c]$$

$$\frac{d[pS2S4_c]}{dt} = k_{6a}[pS2_c][S4_c] - k_{6d}[pS2S4_c] - k_{7imp}[pS2S4_c]$$

$$\frac{d[pS1S4_n]}{dt} = k_{7imp}[pS1S4_c] - k_{8dp}[pS1S4_n] + k_{10a}[pSI_n][S4_n] - k_{10d}[pS1S4_n]$$

$$\frac{d[pS2S4_n]}{dt} = k_{7imp}[pS2S4_c] - k_{8dp}[pS2S4_n] + k_{10a}[pS2_n][S4_n] - k_{10d}[pS2S4_n]$$

$$\frac{d[S1S4_n]}{dt} = k_{8dp}[pS1S4_n] - k_{9d}[S1S4_n]$$

$$\frac{d[S2S4_n]}{dt} = k_{8dp}[pS2S4_n] - k_{9d}[S2S4_n]$$

$$\frac{d[SI_n]}{dt} = k_{9d}[S1S4_n] + k_{11dp}[pSI_n] + k_{12imp}[SI_c] - k_{12exp}[SI_n]$$

$$\frac{d[S2_n]}{dt} = k_{9d}[S2S4_n] + k_{11dp}[pS2_n] + k_{12imp}[S2_c] - k_{12exp}[S2_n]$$

$$\frac{d[S4_n]}{dt} = k_{9d}\left([S1S4_n] + [S2S4_n]\right) - k_{10a}[S4_n]\left([pSI_n] + [pS2_n]\right) + k_{10d}\left([pS1S4_n] + [pS2S4_n]\right) + k_{14imp}[S4_c] - k_{14exp}[S4_n]$$

$$\frac{d[pSI_n]}{dt} = -k_{10a}[pSI_n][S4_n] + k_{10d}[pS1S4_n] - k_{11dp}[pSI_n] + k_{13imp}[pSI_c] - k_{15deg}[pSI_n]$$

$$\frac{d[pS2_n]}{dt} = -k_{10a}[pS2_n][S4_n] + k_{10d}[pS2S4_n] - k_{11dp}[pS2_n] + k_{13imp}[pS2_c] - k_{15deg}[pS2_n]$$

$$\frac{d[\overline{RI_1}]}{dt} = k_{18int}[RI_1] - k_{18rec}[\overline{RI_1}]$$

$$\frac{d[\overline{RI_2}]}{dt} = k_{18int}[RI_2] - k_{18rec}[\overline{RI_2}]$$

$$\frac{d[\overline{RII}]}{dt} = k_{19int}[RII] - k_{19rec}[\overline{RII}]$$

$$\frac{d[S7]}{dt} = \frac{k_{syn,S7} + k_{lip,1}K_{A,1}[pS1S4_n] + k_{lip,2}K_{A,2}[pS2S4_n]}{1 + K_{A,1}[pS1S4_n] + K_{A,2}[pS2S4_n]} - k_{deg,S7}[S7] - k_{20a,1}[C_1][S7] - k_{20a,2}[C_2][S7]$$

Table S2. Parameter values used in the simulations. They have been obtained from references [10, 19, 22, 56-64], as described in reference [19].

Name	Description	HaCaT	BAEC	C2C12	Unit
$k_{syn,RII}$	Constitutive production of type II receptors	8.00	8.00	8.00	molec min^{-1}
$k_{deg,RII}$	Constitutive degradation of type II receptors	2.78×10^{-2}	2.78×10^{-2}	2.78×10^{-2}	min^{-1}
$k_{syn,RI}$	Constitutive production of type I receptors	8.00	8.00	8.00	molec min^{-1}
$k_{deg,RI}$	Constitutive degradation of type I receptors	2.78×10^{-2}	2.78×10^{-2}	2.78×10^{-2}	min^{-1}
$k_{syn,RS}$	Constitutive production of R-Smads	2.74×10^{1}	2.74×10^{1}	2.74×10^{1}	molec min^{-1}
$k_{deg,RS}$	Constitutive degradation of R-Smads	6.46×10^{-4}	6.46×10^{-4}	6.46×10^{-4}	min^{-1}
$k_{syn,S4}$	Constitutive production of Smad4	5.00×10^{1}	5.00×10^{1}	5.00×10^{1}	molec min^{-1}
$k_{deg,S4}$	Constitutive degradation of Smad4	1.20×10^{-3}	1.20×10^{-3}	1.20×10^{-3}	min^{-1}
$k_{syn,S7}$	Constitutive production of Smad7	1.51×10^{2}	0	0	molec min^{-1}
$k_{deg,S7}$	Constitutive degradation of Smad7	3.88×10^{-3}	3.88×10^{-3}	3.88×10^{-3}	min^{-1}
k_{1a}	Association of ligand and type II receptor	6.60×10^{-3}	1.20×10^{-2}	6.60×10^{-4}	molec^{-1} min^{-1}
k_{1d}	Dissociation of ligand/type II receptor complex	2.98×10^{-1}	2.98×10^{-1}	2.98×10^{-1}	min^{-1}
k_{2a}	Association of ligand/type II receptor and type I receptor	6.60×10^{-3}	1.44×10^{-3}	3.76×10^{-2}	molec^{-1} min^{-1}
k_{2d}	Dissociation of receptor complex	2.98×10^{-1}	2.98×10^{-1}	2.98×10^{-1}	min^{-1}
k_{3int}	Internalization of receptor complex	3.95×10^{-1}	3.11×10^{-1}	3.02	min^{-1}
k_{4a}	Association of receptor complex and R-Smad	1.50×10^{-4}	2.12×10^{-4}	1.92×10^{-5}	molec^{-1} min^{-1}
k_{4d}	Dissociation of receptor complex and R-Smad	9.71×10^{-1}	9.61	9.68	min^{-1}
k_{5phos}	Phosphorylation of R-Smad	4.48×10^{4}	4.48×10^{4}	4.48×10^{4}	min^{-1}
k_{6a}	Association of cytosolic phospho-R-Smad and Smad4	6.00×10^{-3}	8.94×10^{-3}	5.31×10^{-2}	molec^{-1} min^{-1}
k_{6d}	Dissociation of cytosolic phospho-R-Smad–Smad4	1.46×10^{3}	1.46×10^{3}	1.46×10^{3}	min^{-1}
k_{7imp}	Nuclear import of phospho-R-Smad–Smad4	8.10×10^{-1}	2.70×10^{-1}	7.52	min^{-1}
k_{8dp}	Dephosphorylation of nuclear phospho-R-Smad–Smad4	2.52×10^{-2}	2.52×10^{-2}	2.52×10^{-2}	min^{-1}
k_{9d}	Dissociation of nuclear R-Smad–Smad4	1.01×10^{-1}	9.60×10^{-1}	1.01	min^{-1}
k_{10a}	Association of nuclear phospho-R-Smad and Smad4	1.67×10^{-4}	3.08×10^{-5}	1.37×10^{-3}	molec^{-1} min^{-1}
k_{10d}	Dissociation of nuclear phospho-R-Smad–Smad4	9.09×10^{-1}	2.50	3.83	min^{-1}
k_{11dp}	Dephosphorylation of phospho-R-Smad	2.52×10^{-2}	2.52×10^{-2}	2.52×10^{-2}	min^{-1}
k_{12imp}	Nuclear import of R-Smad	1.62×10^{-1}	1.62×10^{-1}	1.62×10^{-1}	min^{-1}
k_{12exp}	Nuclear export of R-Smad	3.48×10^{-1}	3.48×10^{-1}	3.48×10^{-1}	min^{-1}
k_{13imp}	Nuclear import of phospho-R-Smad	5.03×10^{-1}	5.03×10^{-2}	4.85	min^{-1}
k_{14imp}	Nuclear import of Smad4	2.01×10^{-2}	2.31×10^{-2}	1.45×10^{-1}	min^{-1}
k_{14exp}	Nuclear export of Smad4	1.74×10^{-1}	1.74×10^{-1}	1.74×10^{-1}	min^{-1}
k_{15deg}	Constitutive degradation of nuclear phospho-R-Smad	5.40×10^{-3}	2.00×10^{-3}	4.33×10^{-2}	min^{-1}
k_{16deg}	Constitutive degradation of receptor complex	2.78×10^{-2}	2.78×10^{-2}	2.78×10^{-2}	min^{-1}
k_{17rec}	Recycling of receptor complex	3.95×10^{-2}	3.95×10^{-2}	3.95×10^{-2}	min^{-1}
k_{18int}	Internalization of type II receptor	3.95×10^{-1}	3.11×10^{-1}	3.02	min^{-1}
k_{18rec}	Recycling of type II receptor	3.95×10^{-2}	3.95×10^{-2}	3.95×10^{-2}	min^{-1}
k_{19int}	Internalization of type I receptor	3.95×10^{-1}	3.11×10^{-1}	3.02	min^{-1}
k_{19rec}	Recycling of type I receptor	3.95×10^{-2}	3.95×10^{-2}	3.95×10^{-2}	min^{-1}
$k_{20a,1}$	Association of Smad7 and C_1	8.72×10^{-4}	9.38×10^{-4}	8.72×10^{-4}	molec^{-1} min^{-1}
$k_{20a,2}$	Association of Smad7 and C_2	2.96×10^{-5}	1.50×10^{-6}	1.50×10^{-6}	molec^{-1} min^{-1}
$k_{lip,1}$	Ligand-induced production of Smad7 through $pS1S4_n$	0	4.26×10^{2}	0	molec min^{-1}
$k_{lip,2}$	Ligand-induced production of Smad7 through $pS2S4_n$	8.53×10^{3}	0	5.54×10^{2}	molec min^{-1}
$K_{A,1}$	Association constant of $pS1S4_n$ with Smad7 promoter	0	7.22×10^{-5}	0	molec^{-1}
$K_{A,2}$	Association constant of $pS2S4_n$ with Smad7 promoter	1.03×10^{-6}	0	1.51×10^{-3}	molec^{-1}

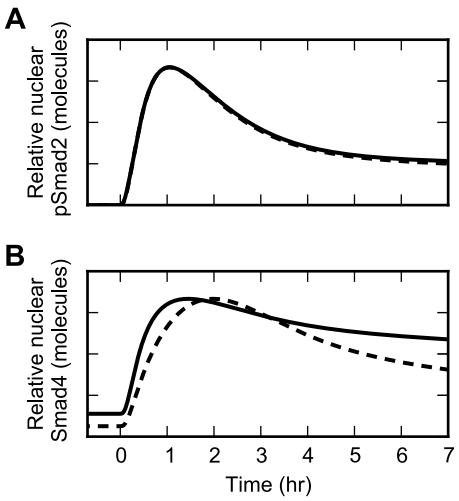

Figure S1

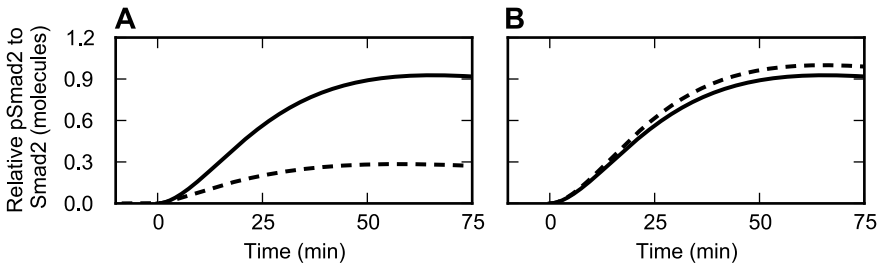

Figure S2

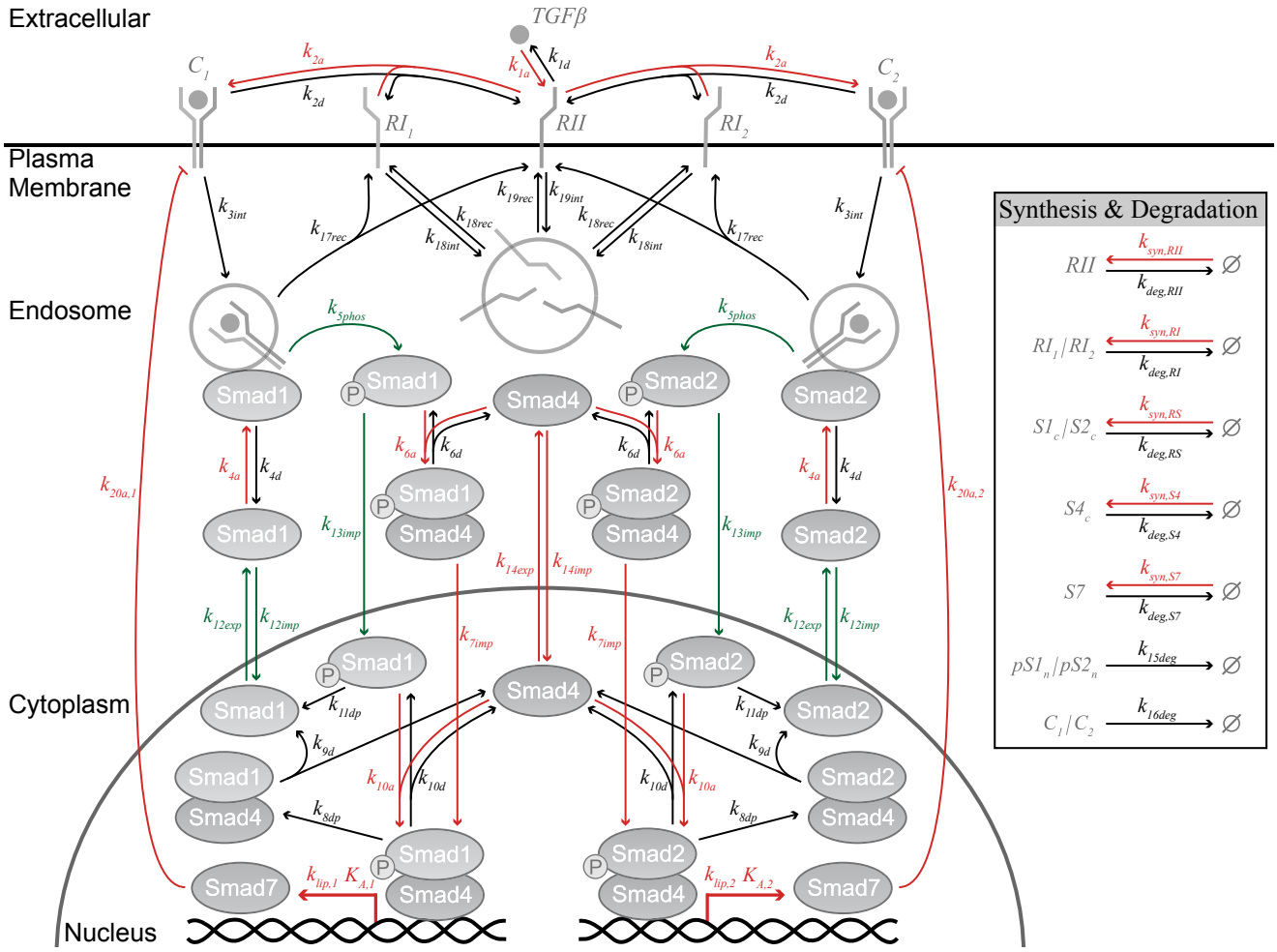

Figure S3