Supporting Material

Binding properties of the TGF-beta co-receptor betaglycan: proposed mechanism for potentiation of receptor complex assembly and signaling

Maria Villarreal, Sun Kyung Kim, Lindsey Barron, Ravi Kodali, Jason Baardsnes, Cynthia S. Hinck, Troy Krzysiak, Morkos A. Henen, Olga Pakhomova, Valentín Mendoza, Maureen D. O'Connor-McCourt, Eileen M. Lafer, Fernando López-Casillas, and Andrew P. Hinck

Figure S1

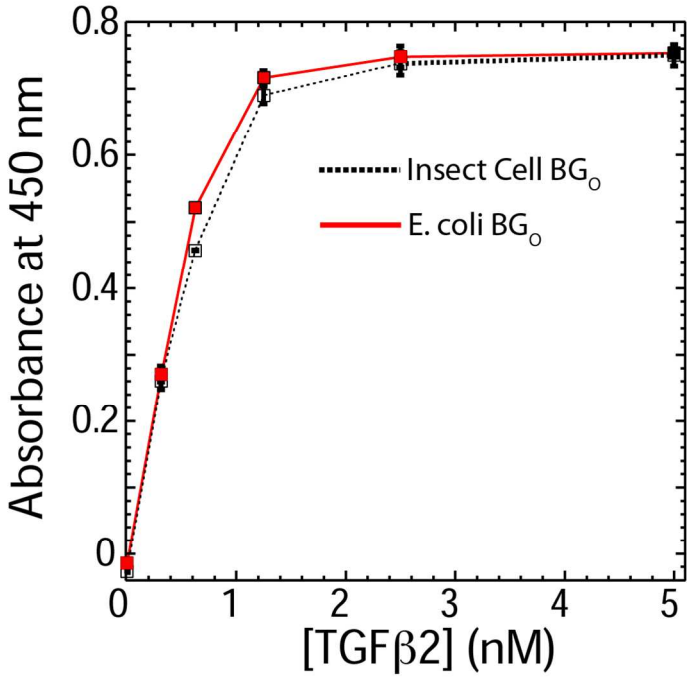

Figure S1. Amount of TGF-β2 detected in a sandwich ELISA experiment in which 5 μg of insect cell or *E. coli* recombinant BG_O was coated into the well of an absorbant ELISA plate. Data points shown represent the average of triplicate measurements and correspond to the difference between blank wells with no receptor coated and ones in which receptor (BG_O) was coated. Other experimental details can be found in Qian, et al [Qian, et al, J. Biol. Chem., 271, 30656-62 (1996)].

Figure S2

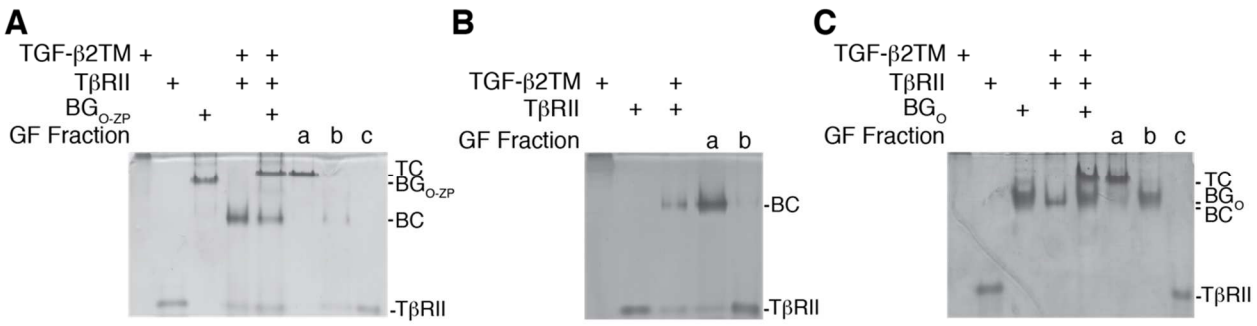

Figure S2. Analysis of complexes eluted from size exclusion chromatography runs by native gel electrophoresis. A. Analysis of complexes eluted from the SEC run with TGF-β2TM, TβRII, and BG_{O-ZP} (shown in Fig. 4A in the main text) by native gel electrophoresis. Isolated complexes from the SEC column (a, b, and c) are run alongside the mixtures of the individual components as shown on the left side of the panel (+ corresponds to the addition of 1 μg of the component indicated). B-C. Analysis of complexes eluted from the SEC runs with TGF-β2TM and TβRII and TGF-β2TM, TβRII, and BG_O (shown in Fig. 4B and 6D in the main text) by native gel electrophoresis. Other details are as in panel A, except + corresponds to 4 μg BG_O in panel C.

Figure S3

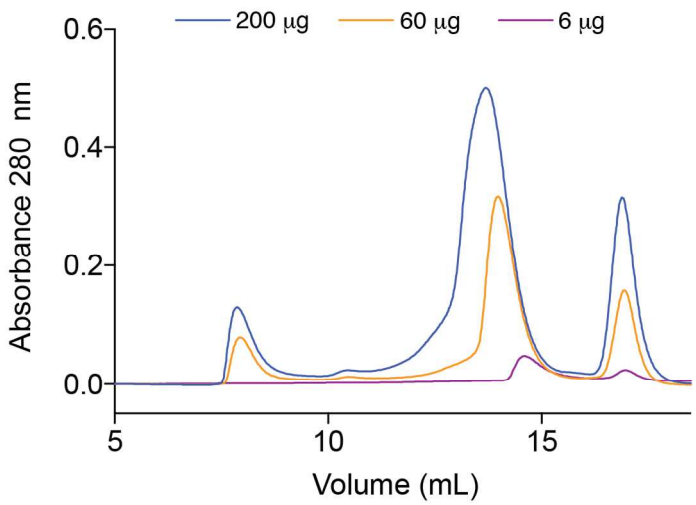

Figure S3. Elution profiles for the TGF-β2TM:TβRII binary complex on a 1 x 30 cm Superdex 200 Increase 10/300 GL size exclusion column (GE Healthcare, Piscataway, NJ) as a function of the amount of material loaded (blue trace – 200 μg, orange trace - 60 μg, and purple trace – 6 μg). Samples were injected in a volume of 100 μL and each run was performed at a flow rate of 0.5 mL per minute.

Figure S4

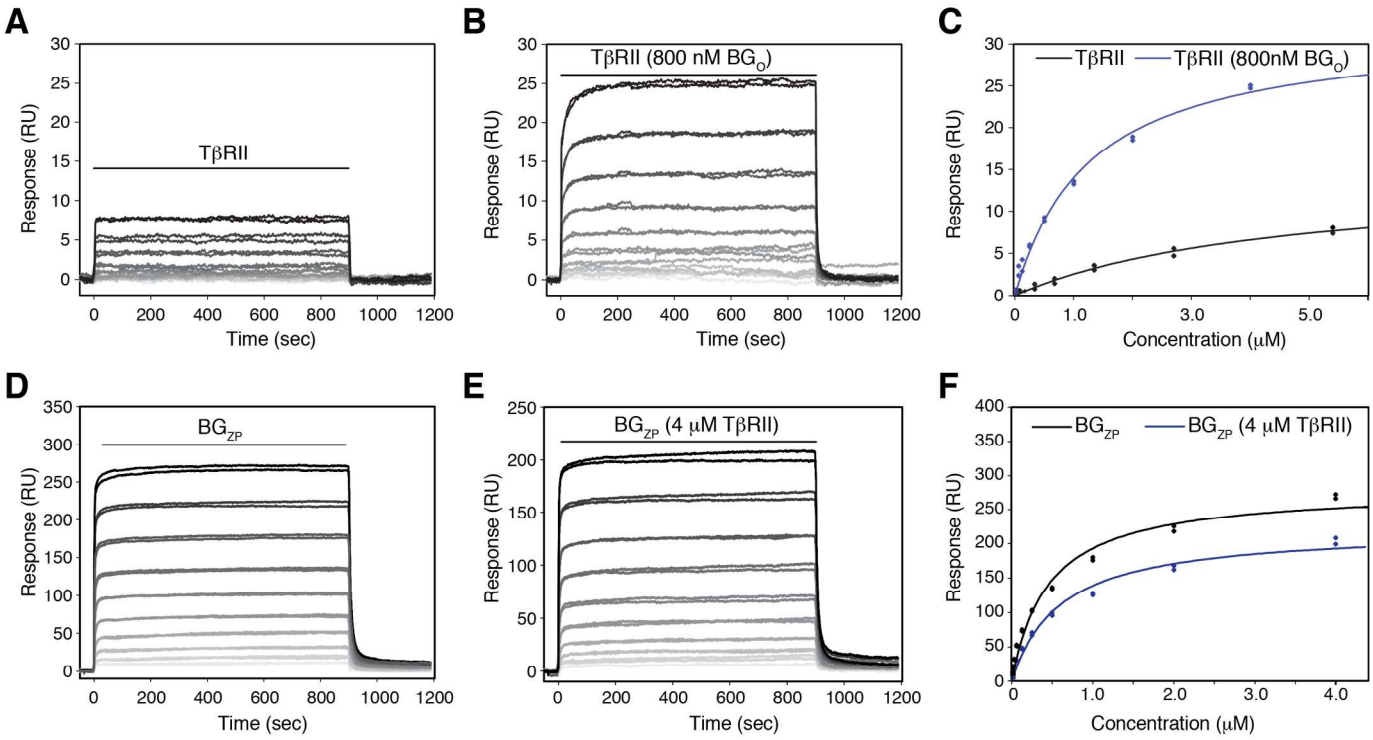

Figure S4. Effect of BG_O and BG_{ZP} on binding of TβRII to TGF-β2. A-B. SPR sensograms for binding of TβRII binding to TGF-β2 in the absence (A) and presence (B) of 800nM BG_O. Black lines over sensograms denote the period of injection of a two-fold dilution series of TβRII from 5.4 μM to 0.011 μM (A) or 4 μM to 0.008 μM (B). C. Plot of the equilibrium response for TβRII binding to TGF-β2 in the absence (black) or presence (blue) of 800 nM BG_O. Equilibrium binding constants were obtained by fitting the equilibrium response as a function of concentration to a standard binding isotherm. Fitted curve is shown by continuous line, solid black or blue in the absence or presence of BG_O, respectively. D-E. SPR sensorgrams for binding of BG_{ZP} to TGF-β2 in the absence (D) or presence (E) of 2 μM TβRII. Black lines over sensograms denote the period of injection of a two-fold dilution series of BG_{ZP} from 4 μM to 0.008 μM. Other details are as in panels A-B. F. Plot of the equilibrium response for BG_{ZP} binding to TGF-β2 in the absence (black) or presence (blue) of 4 μM TβRII. Other details are as in panel C.

Figure S5

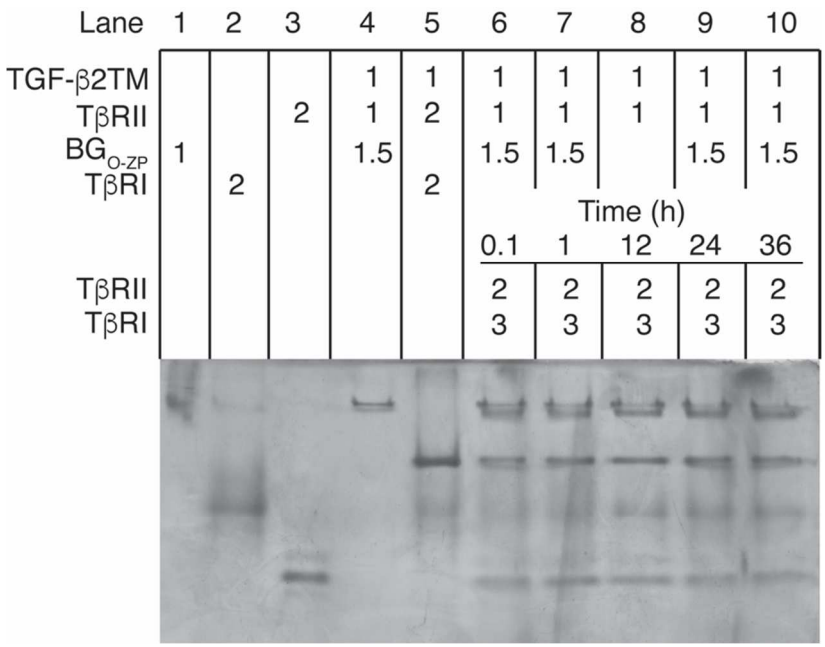

Figure S5. Native gel analysis to assess the ability of TβRI to displace betaglycan in the context of a TGF-β2TM:TβRII:BG$_{O-ZP}$ complex to form the TGF-β2TM:TβRII:TβRI ternary signaling complex. Number shown in the upper part of the lane loading diagram indicate relative molar amounts added. Numbers shown for TβRI and TβRII in the lower part of the loading diagram indicate relative molar amounts added to the pre-formed TGF-β2TM:TβRII:BG$_{O-ZP}$ complex specified in the upper part of the loading diagram – samples prepared in this way were incubated at 25 °C for the time period shown before loading onto the native gel (see *Materials and Methods* for other details pertaining to the native gels).

Table 1. SPR binding constants for TGF-β to betaglycan in the presence of CHAPS*

Surface	Analyte	CHAPS	k_{on} ($M^{-1}s^{-1}$)	k_{off} (s^{-1})	K_D (μM)	R_{max} (RU)
TGF-β2	$BG_{O\text{-}ZP}$	0	2.42×10^5	0.00102	0.0042	14.3
	$BG_{O\text{-}ZP}$	15 mM	2.68×10^5	0.00391	0.0146	43.9
	$BG_{O\text{-}ZP}$	30 mM	6.60×10^5	0.0170	0.0258	50.6
TGF-β2	BG_O	0	2.48×10^4	0.0224	0.902	51.2
	BG_O	15 mM	1.68×10^4	0.0593	3.52	12.6
	BG_O	30 mM	6.10×10^2	0.0143	23.4	17.7
TGF-β2	$BG_{ZP\text{-}C}$	0	2.88×10^4	0.0604	2.10	39.7
	$BG_{ZP\text{-}C}$	15 mM	5.38×10^4	0.251	4.67	29.7
	$BG_{ZP\text{-}C}$	30 mM	9.19×10^3	0.036	3.89	5.00

*These SPR measurements were performed using TGF-β2 biotinylated in a different manner relative to that used for all other SPR measurements (TGF-β2 for these experiments was biotinylated on lysine residues, while the TGF-βs used for all other experiments were biotinylated on aspartate or glutamate residues). The K_Ds measured here for BG_O an $BG_{ZP\text{-}C}$ are lower than those reported in for BG_O and $BG_{ZP\text{-}C}$ in other experiments, and is therefore likely due to the different manner of biotinylation.