

Supplementary Table 1. PDBePISA analysis of the type II interface (ActRIIB) of activin ternary models.

Type II (ActRIIB)		
Solvent-accessible (Å ²)		
Ligands	Interface	Total
ActB	738	7641
ActC	779	7635
ActE	751	7408
ActA (PDB: 7OLY)	680	7726

Supplementary Table 2. PDBePISA analysis of the fingertip region of the type I interface (ALK4 or ALK7) of activin ternary models.

Type I Fingertips (Chain A)		
Solvent-accessible (Å ²)		
Ligands	Interface	Total
ActB	401	7651
ActC	447	7635
ActE	375	7408
ActA (PDB: 7OLY)	346	7726

Supplementary Table 3. PDBePISA analysis of the prehelix/wrist region of the type I interface (ALK4 or ALK7) of activin ternary models.

Type I Prehelix/wrist (Chain B)		
Solvent-accessible (Å ²)		
Ligands	Interface	Total
ActB	785	7641
ActC	837	7599
ActE	781	7386
ActA (PDB: 7OLY)	617	7726

Supplementary Table 4. Root mean square difference (RMSD) measurements of the fingertip regions of the type I interfaces.

Fingertip Region (Chain A)			
	ActB	ActC	ActE
ActB	0	0.19	0.231
ActC	0.19	0	0.212
ActE	0.231	0.212	0
ActA (PDB: 7OLY)	0.364	0.401	0.396

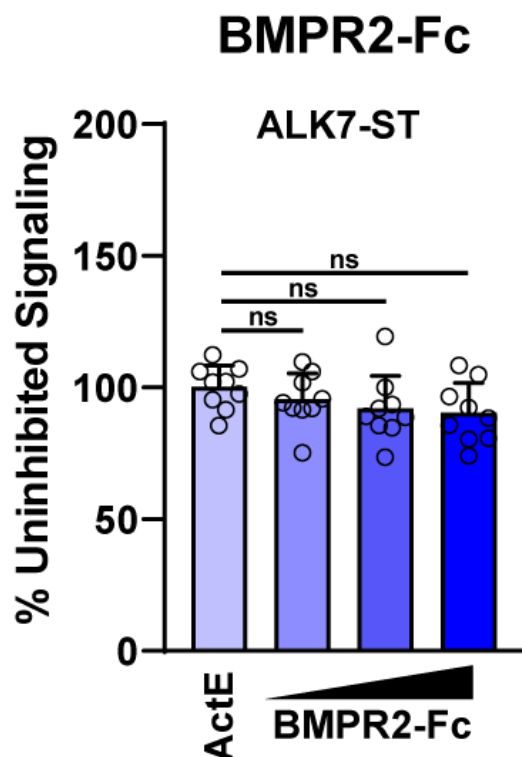
Supplementary Table 5. Root mean square difference (RMSD) measurements of the prehelix/wrist regions of the type I interfaces.

Prehelix/Wrist Region (Chain B)

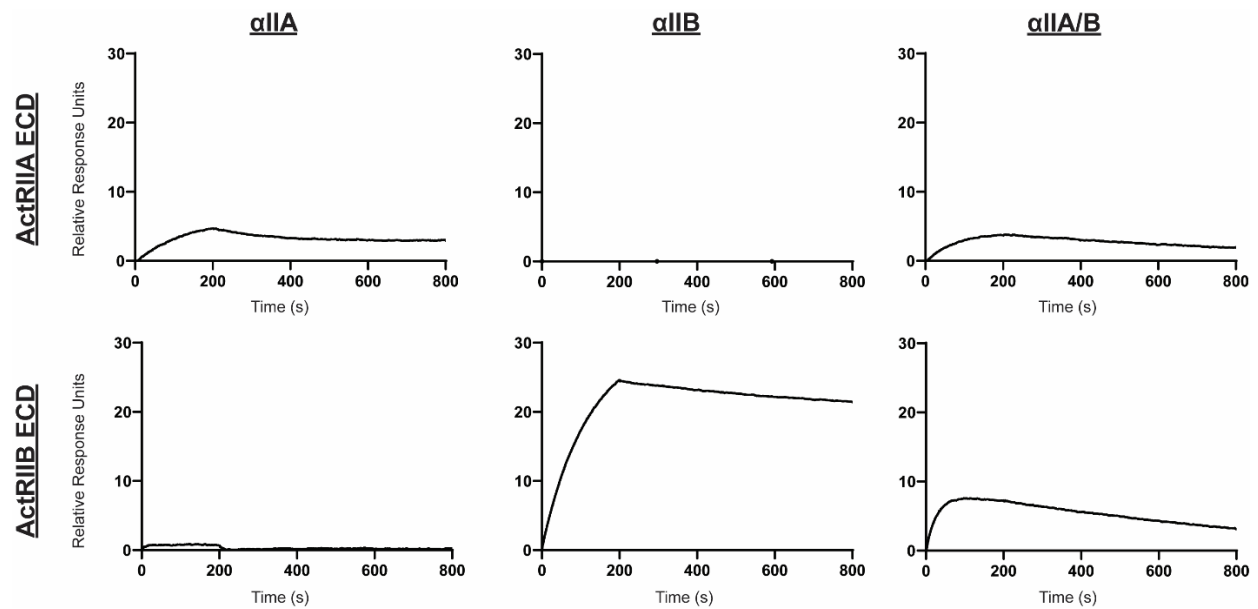
	ActB	ActC	ActE
ActB	0	0.86	0.808
ActC	0.86	0	0.195
ActE	0.808	0.195	0
ActA (PDB: 7OLY)	1.047	0.659	0.761

Supplementary Table 6. PDBePISA analysis of the type II interface (ActRIIA) of activin ternary models.

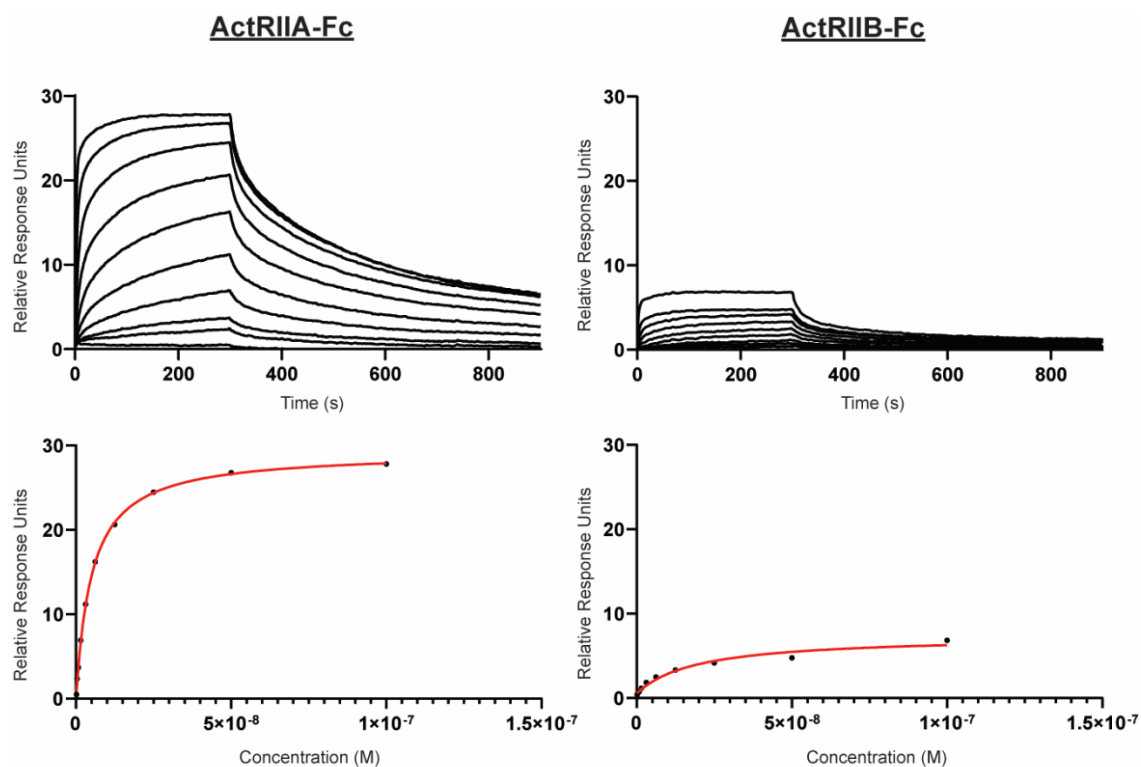
Type II (ActRIIB)		
Solvent-accessible (Å ²)		
Ligands	Interface	Total
ActB	749	7785
ActC	755	7729
ActE	752	7492
ActA	775	7711



Supplemental Figure 1. Activin E signaling does not require the type II receptor, BMPR2, to signal. (CAGA₁₂)-luciferase HEK293T cells transfected with ALK7-ST receptor constructs and treated with ActE conditioned media (20x), and 10 μ M SB-431542 in the presence of increasing amounts (6.25, 12.5, 25 nM) of BMPR2-Fc (ns = not significant).



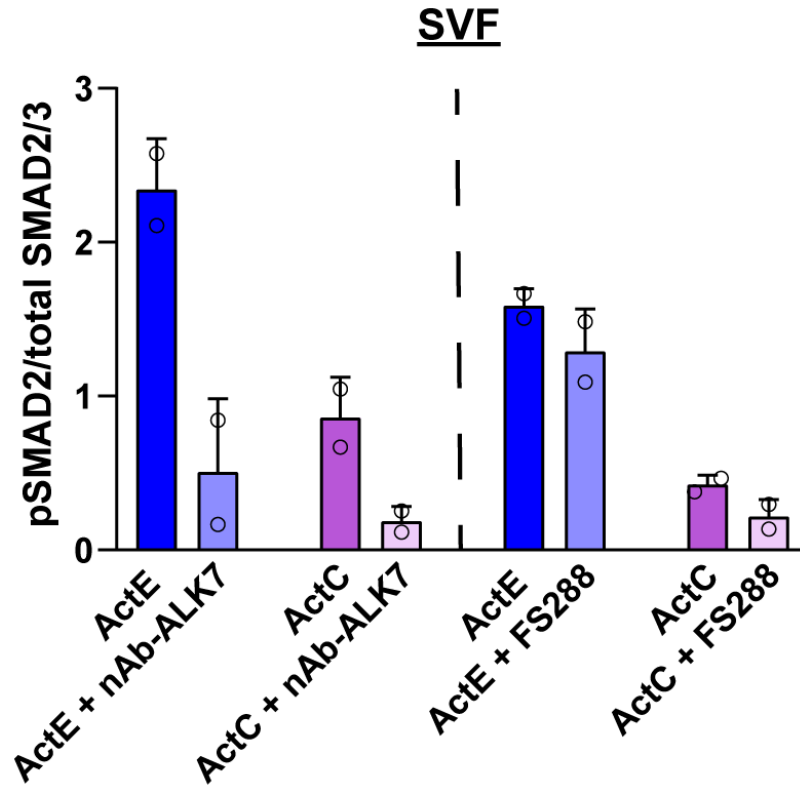
Supplemental Figure 2. Binding specificity of the activin type II neutralizing antibodies. Representative surface plasmon resonance (SPR) sensorgrams of the ActRIIA ECD (25nM) or ActRIIB ECD (25nM) binding to protein A captured anti-ActRIIA nAb, anti-ActRIIB nAb, or anti-ActRIIA/ActRIIB nAb.



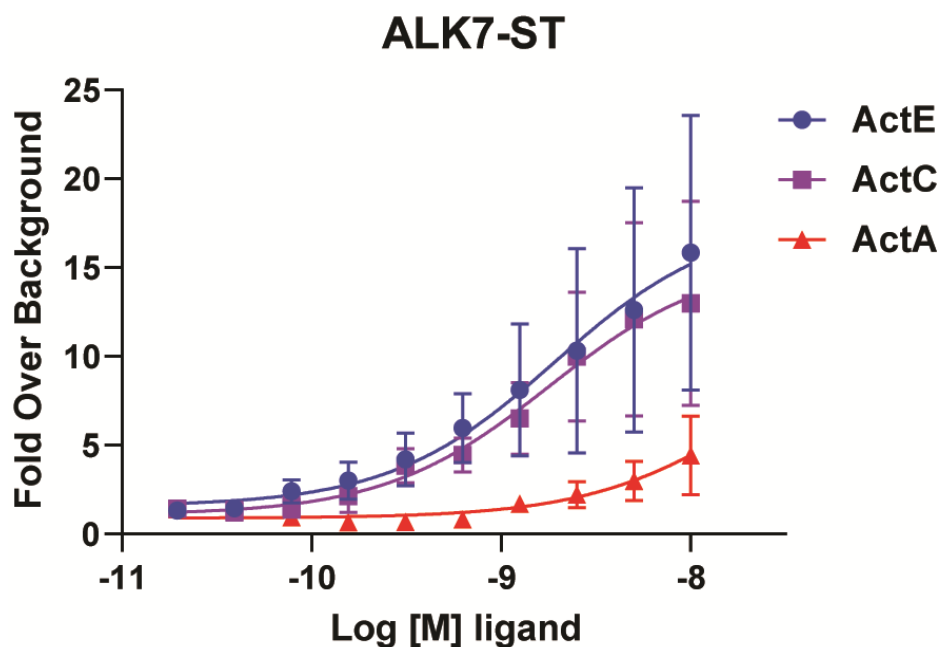
Receptor	KD (M)	Chi ² (RU ²)
ActRIIA-Fc	5.0E-09	0.066
ActRIIB-Fc	2.0E-08	0.17

Supplemental Figure 3. Activins E binds to the activin type II receptors with low affinity.

Representative surface plasmon resonance (SPR) sensorgrams of ActE ([R&D Systems](#)) binding to ActRIIA-Fc or ActRIIB-Fc capture on a protein A sensor chip. The top represents the unfit SPR curves while the bottom depicts the steady analysis of the top experiments.



Supplementary Figure 4. Quantitation of SVF western blot images. Quantitative analysis of western blot images in used in Figure 7 for changes in signaling following treatment with either ActE conditioned media (20x) or ActC in the presence or absence of an ALK7 neutralizing antibody or FS 288. Images were quantified using ImageJ software.



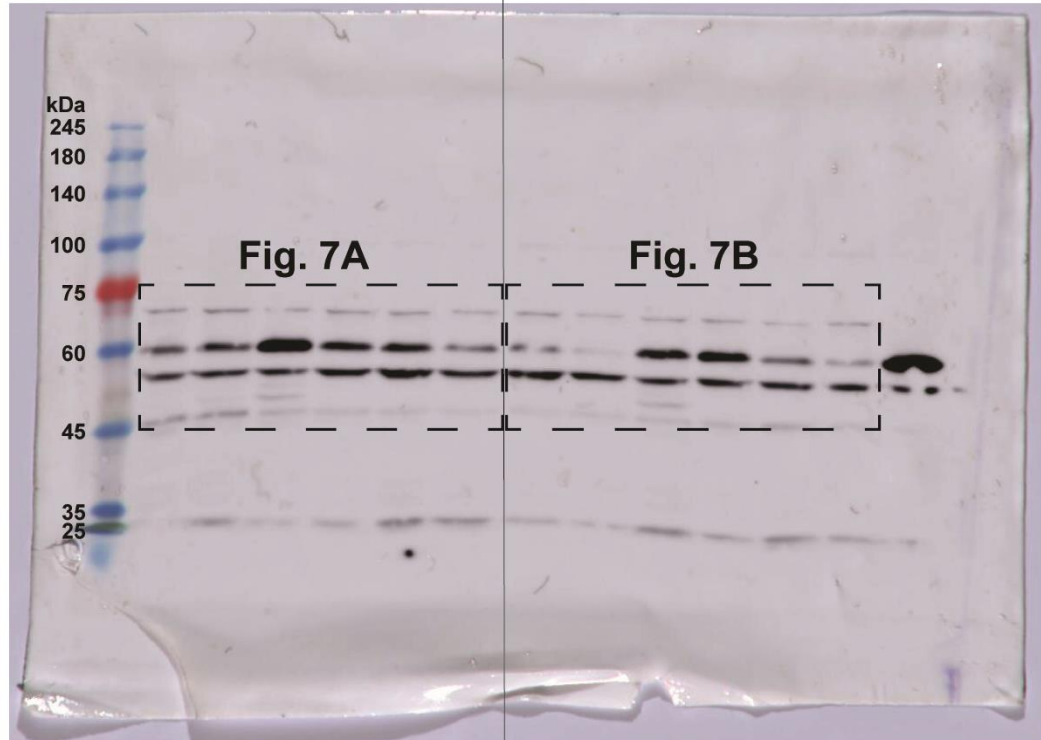
Ligand	EC50 (M)	SD	95% CI
ActE	2.0E-09	1.6E-10	7.8e-010 to 5.3e-009
ActC	1.6E-09	8.9E-10	9.5e-010 to 3.5e-009
ActA	NA	NA	NA

Supplemental Figure 5. Recombinant ActE signals through ALK7. Titration of recombinant mature ActA, ActC, or ActE [from R&D Systems](#) (10 nM) in (CAGA₁₂)-luciferase HEK293T cells transfected with SB-431542 resistant ALK7-ST. EC50 values were calculated using non-linear dose response curve fitting in Prism. The ActA curve was not fit to a non-linear regression (NA=Not Applicable).

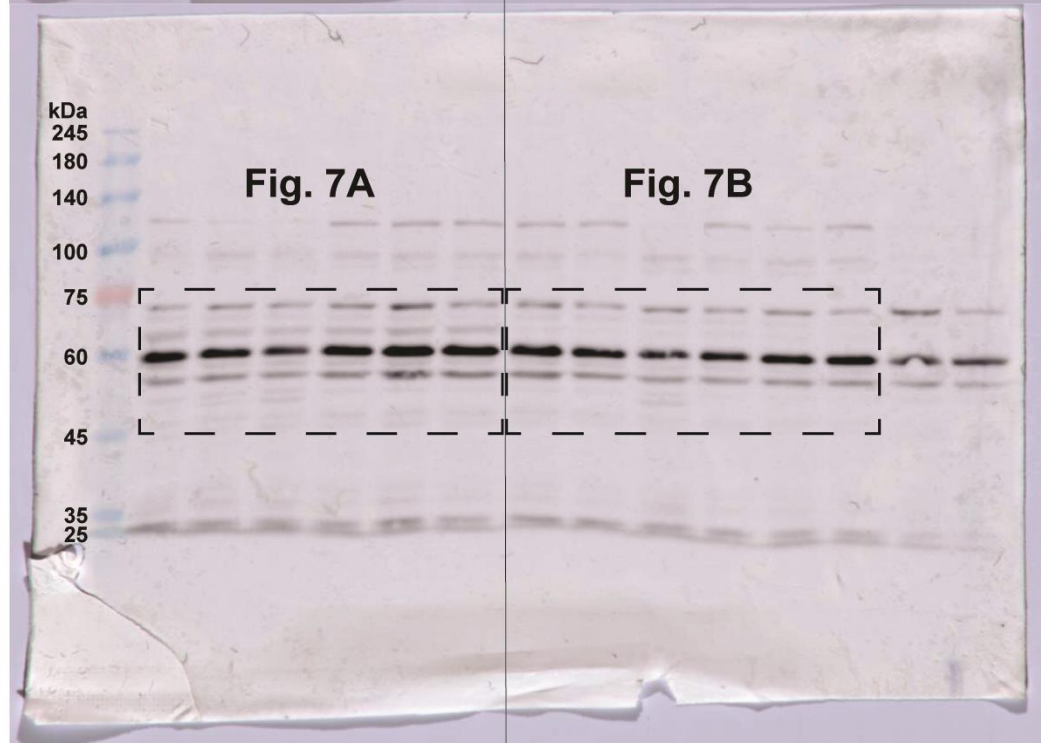
SVF

EV	+	+	-	-	-	-	+	+	-	-	-	-
ActE	-	-	+	+	-	-	-	-	+	+	-	-
ActC	-	-	-	-	+	+	-	-	-	-	+	+
nAb-ALK7	-	+	-	+	-	+	-	-	-	-	-	-
FS288	-	-	-	-	-	-	-	+	-	+	-	+

pSMAD2



SMAD2/3



Supplemental Figure 6. Raw pSMAD2 and SMAD2/3 western blots images. Supplemental western blot images for blots shown in Figure 7. Dashed boxes depict where the images were cropped for figure generation.