

# Activins as dual specificity TGF- $\beta$ family molecules: SMAD-activation via activin- and BMP-type I receptors

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## Supplementary Material

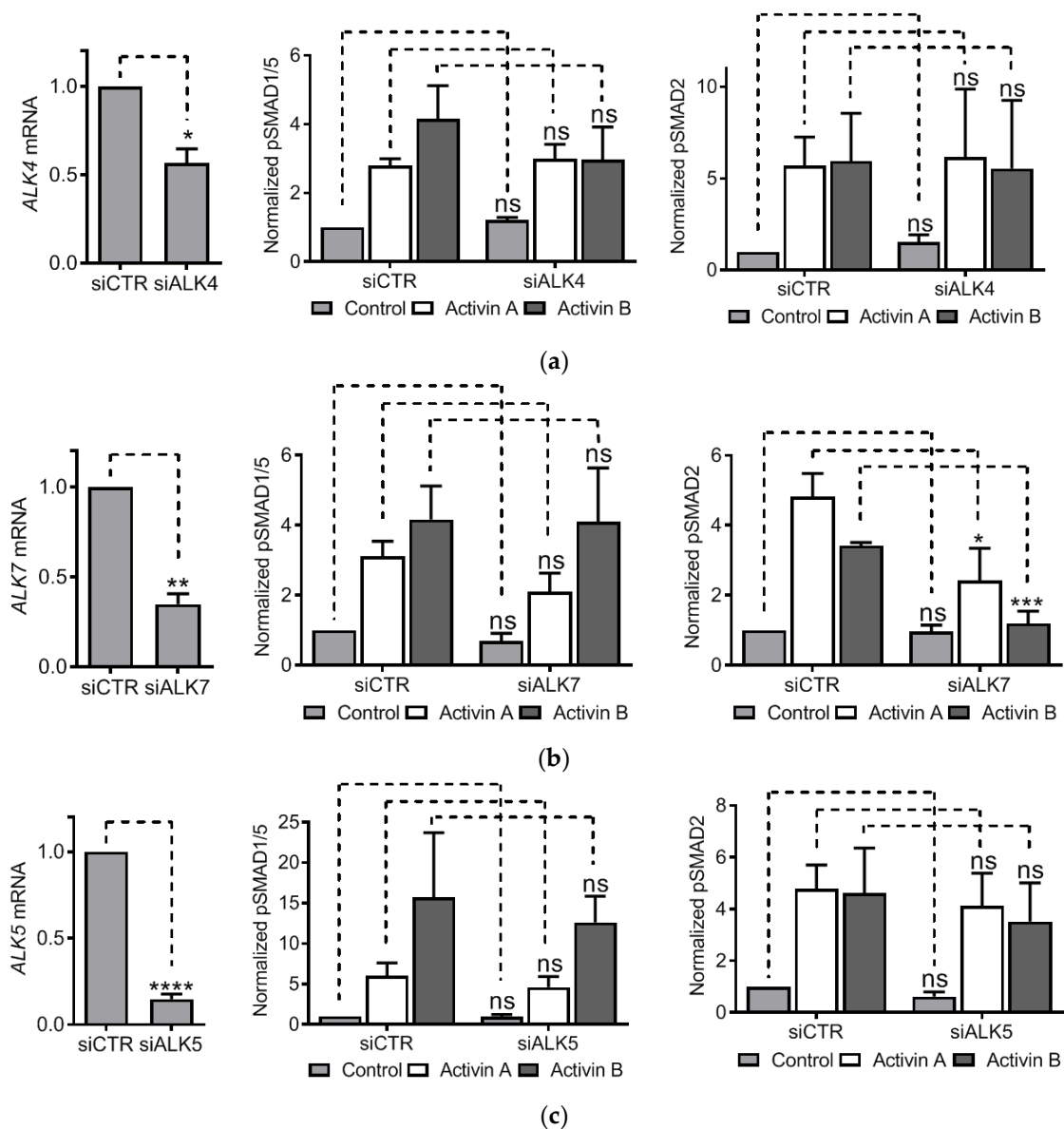
Figure S1. Effects of knockdown of activin/TGF- $\beta$  type 1 receptors in INA-6 myeloma cells

Figure S2. Effects of ALK2 knockdown in HepG2 cells

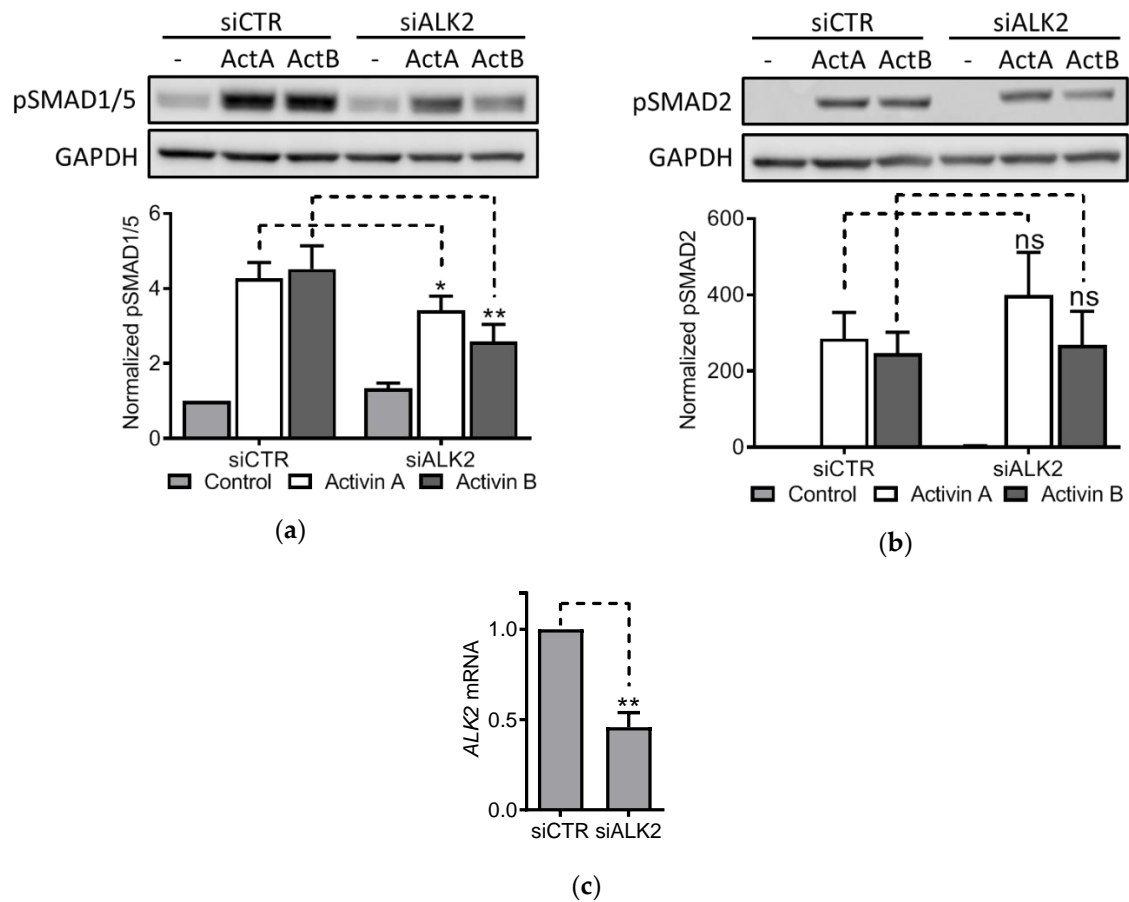
Figure S3. Activin-induced SMAD activity was not caused by an autocrine TGF- $\beta$  loop

Table S1. Approved HUGO gene names for TGF- $\beta$  family receptors

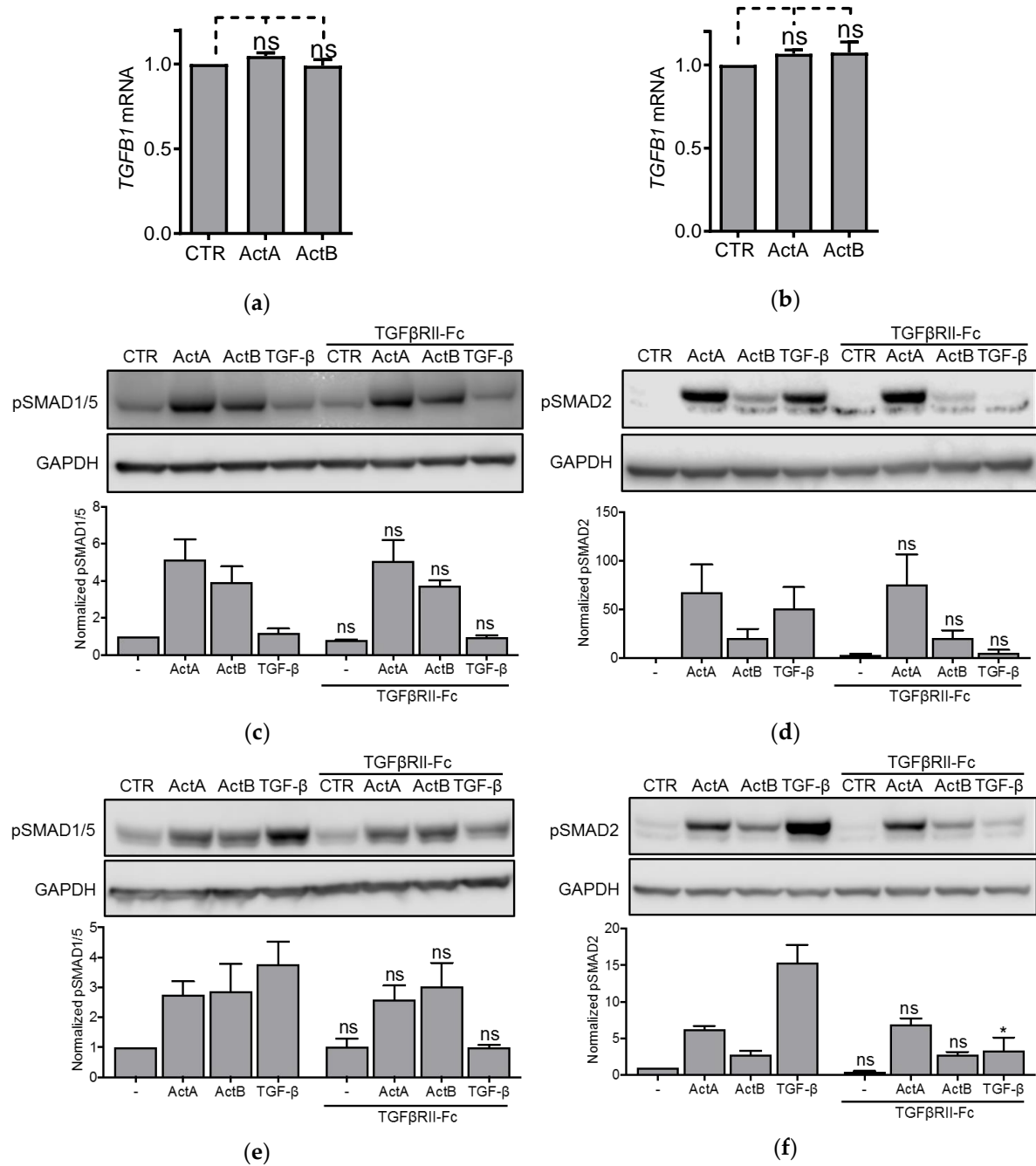
Table S2. TGF- $\beta$ /SMAD inhibitors; reported inhibitory potential



**Figure S1.** Effects of knockdown of activin/TGF- $\beta$  type 1 receptors in INA-6 myeloma cells. To check for a possible involvement of the activin/TGF- $\beta$  type 1 receptors ALK4, ALK7, or ALK5 in activin-induced activation of the two different SMAD branches, we did transient knockdown with siRNA targeting these receptors in INA-6 myeloma cells. The cells were treated 2 days post transfection with activin A (50 ng/mL) or activin B (10 ng/mL) for 2 hours. ALK4, ALK7 or ALK5 mRNA levels (a-c, left) were measured by PCR using the comparative Ct method and *GAPDH* as housekeeping gene. The graph represents mean $\pm$ s.e.m. of n=3 independent experiments. Two-tailed, paired *t*-test was performed (\* $P$  $\leq$ 0.05, \*\* $P$  $\leq$ 0.01, \*\*\*\* $P$  $\leq$ 0.0001). The effect of reduced ALK4, ALK7, or ALK5 mRNA on relative activin A- or activin B-induced activation of SMAD1/5 (a-c, middle) or SMAD2 (a-c, right) was calculated based on signal intensities of the SMADs and *GAPDH* for normalization. The graphs represent mean $\pm$ s.e.m. of n=3 independent experiments. Two-way ANOVA, Bonferroni's multiple comparisons test was performed (\* $P$  $\leq$ 0.05, \*\*\*\* $P$  $\leq$ 0.001, ns (not significant)  $P$ >0.05).



**Figure S2.** Effects of ALK2 knockdown in HepG2 cells. To check for a possible involvement of the BMP type 1 receptor ALK2 in activin-induced activation of the two different SMAD branches, we did transient knockdown with siRNA targeting ALK2 in HepG2 cells. The cells were treated the day after transfection with activin A (20 ng/mL) or activin B (60 ng/mL) for 1 hour. The effect of reduced ALK2 mRNA on relative activin A- or activin B-induced activation of SMAD1/5 (a) or SMAD2 (b) was calculated based on signal intensities of the SMADs and GAPDH for normalization. The graphs represent mean $\pm$ s.e.m. of n=5 independent experiments. Two-way ANOVA, Bonferroni's multiple comparisons test was performed (\* $P\leq 0.05$ , \*\* $P\leq 0.01$ , ns (not significant)  $P>0.05$ ). (c) ALK2 mRNA levels were measured by PCR using the comparative Ct method and GAPDH as housekeeping gene. The graph represents mean $\pm$ s.e.m. of n=5 independent experiments. A two-tailed, paired *t*-test was performed (\*\* $P\leq 0.01$ ).



**Figure S3.** Actin-induced SMAD activity was not caused by an autocrine TGF- $\beta$  loop. IH-1 (a) and INA-6 (b) cells were treated with activin A (20 ng/mL for IH-1 and 50 ng/mL for INA-6) and activin B (4 ng/mL for IH-1 and 10 ng/mL for INA-6) for 4 h and *TGF $\beta$ 1* mRNA was measured by PCR using the comparative Ct method and *GAPDH* as housekeeping gene. The graphs represent mean  $\pm$  s.e.m. of  $n=3$  independent experiments. One-way ANOVA, Dunnett's multiple comparisons test was performed (ns (not significant)  $P>0.05$ ). IH-1 cells were treated for 1 h with activin A (20 ng/mL), activin B (4 ng/mL) or TGF- $\beta$  (5 ng/mL) with or without soluble TGF $\beta$ RII-Fc (10  $\mu$ g/mL) to look for a possible contribution of TGF- $\beta$  activity on activation of SMAD1/5 (c) or SMAD2 (d). INA-6 cells were treated for 1 h with activin A (50 ng/mL), activin B (10 ng/mL) or TGF- $\beta$  (0.5 ng/mL) with or without soluble TGF $\beta$ RII-Fc (10  $\mu$ g/mL) to look for a possible contribution of TGF- $\beta$  activity on activation of SMAD1/5 (e) or SMAD2 (f). The graphs represent mean  $\pm$  s.e.m. of  $n=3$  independent experiments. Two-way ANOVA, Bonferroni's multiple comparisons test was performed (\* $P\leq 0.05$ , ns (not significant)  $P>0.05$ ).

**Table S1.** Approved HUGO gene names for TGF- $\beta$  family receptors

<b>Alias name</b>	<b>Approved symbol</b>	<b>Approved name</b>
ALK1	ACVRL1	activin A receptor like type 1
ALK2	ACVR1	activin A receptor type 1
ALK3	BMPR1A	bone morphogenetic protein receptor type 1A
ALK4	ACVR1B	activin A receptor type 1B
ALK5	TGFB1	transforming growth factor beta receptor 1
ALK6	BMPR1B	bone morphogenetic protein receptor type 1B
ALK7	ACVR1C	activin A receptor type 1C
ActRIIA	ACVR2A	activin A receptor type 2A
ActRIIB	ACVR2B	activin A receptor type 2B
BMPRII	BMPR2	bone morphogenetic protein receptor type 2
TGF $\beta$ RII	TGFB2	transforming growth factor beta receptor 2
AMHRII	AMHR2	anti-Mullerian hormone receptor type 2

Gene symbols and names of TGF- $\beta$  family receptors as approved by the HUGO gene nomenclature committee (HGNC, <https://www.genenames.org/>).

**Table S2.** TGF- $\beta$ /SMAD inhibitors; reported inhibitory potential.

Name	BMP Type 1 receptors				Activin/TGF- $\beta$ Type 1 receptors		
	ALK1	ALK2	ALK3	ALK6	ALK4	ALK7	ALK5
K02288	++++	++++	+++	++++	++		++
ML347	+++	+++	-				
LDN-193189		++++	+++	++	++		++
SB431542					+	+	++
RepSox							++++
ZC-47-C95	-	-	-	-			++

The information on IC<sub>50</sub> values for K02288, ML347, LDN-193189, SB431542, and RepSox was retrieved from various sources.(1-5) ZC-47-C95 was resynthesized compound 18a (6) retrieved from Novartis. "+" indicates IC<sub>50</sub> values between 1-10  $\mu$ M, "++"; between 0.1-1  $\mu$ M, "+++"; between 0.01-0.1  $\mu$ M, "++++"; between 0.001-0.01  $\mu$ M, and "-" >10  $\mu$ M.

## References

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