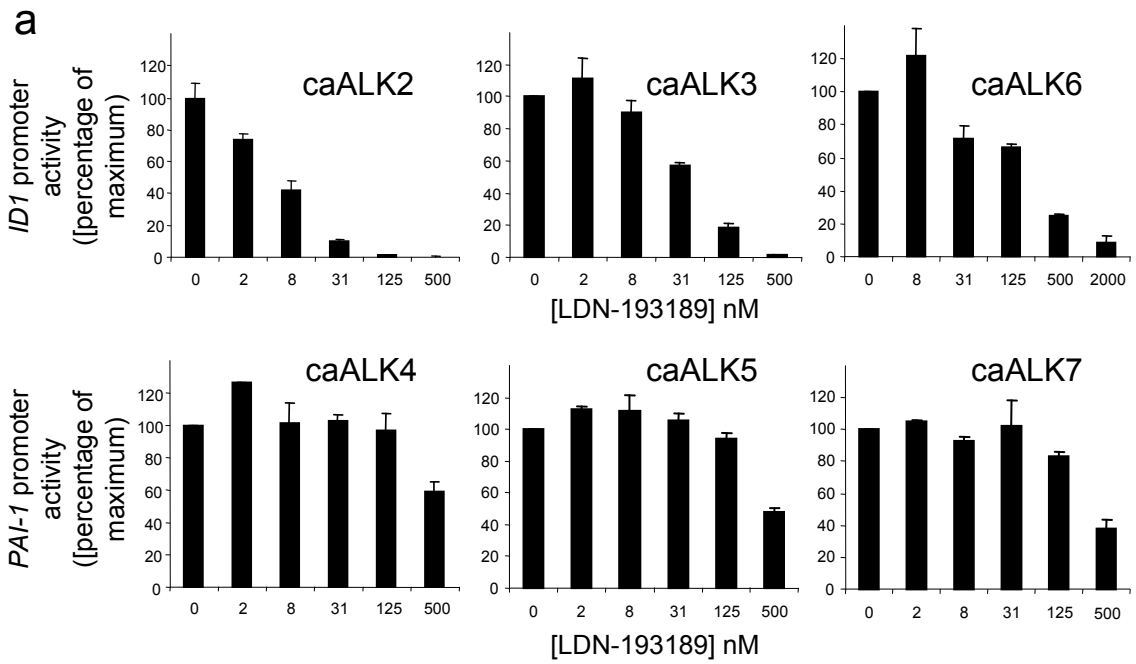
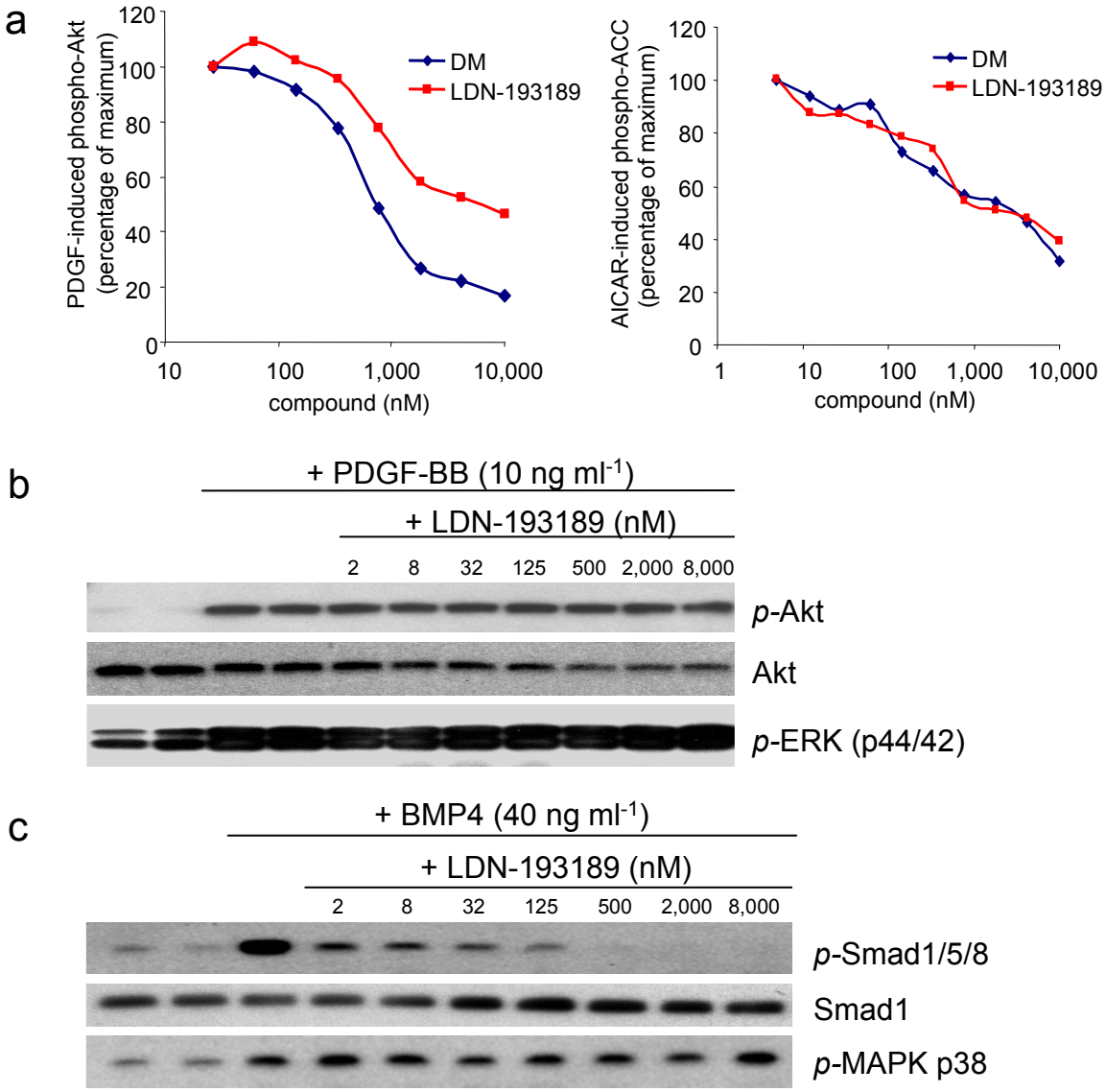
Yu PB, et al. "BMP type I receptor inhibition prevents ectopic ossification in a mouse model of fibrodysplasia ossificans progressiva"

# Supplemental Figure 1



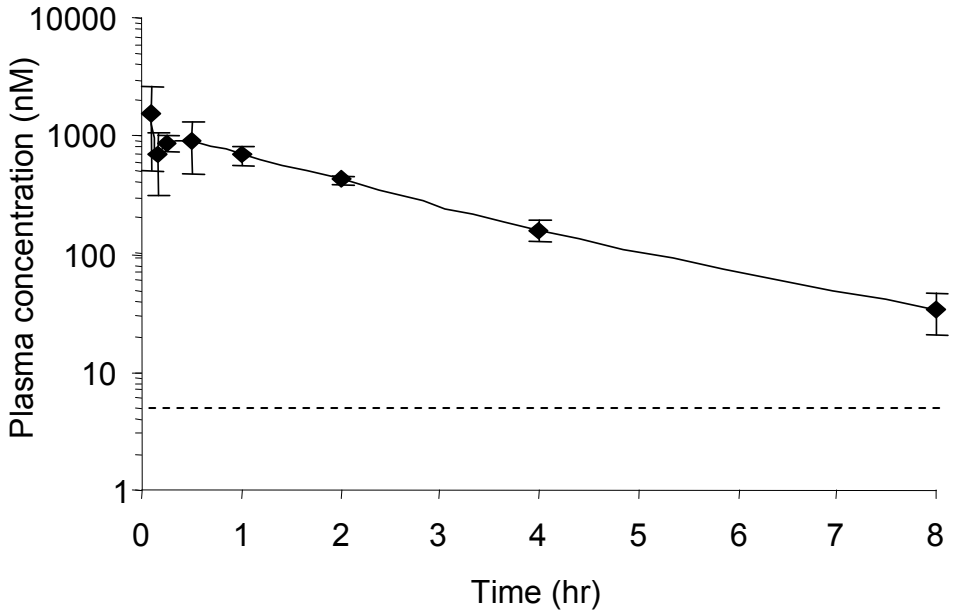

**Supplemental Fig. 1. LDN-193189 preferentially inhibits the activity of BMP type I receptors over Activin and TGF-β type I receptors.** Top panel: *ID1* promoter activity, expressed as a percent of full activity induced by transfection with constitutively-active (ca-)ALK2, caALK3, and caALK6 transfection, was inhibited by LDN-193189 in a dose-dependent fashion (IC50 ~ 5 nM, 30 nM, and 150 nM, respectively, *n* = 3 measurements, results expressed as mean ± s.d.). Bottom panel: *PAI-1* promoter activity, expressed as a percent of full activity induced by caALK4, caALK5, and caALK7 transfection, was tested with varying concentrations of LDN-193189, showing partial inhibition at concentrations ≥ 500 nM (*n* = 3 measurements, results expressed as mean ± s.d.).

# Supplemental Figure 2



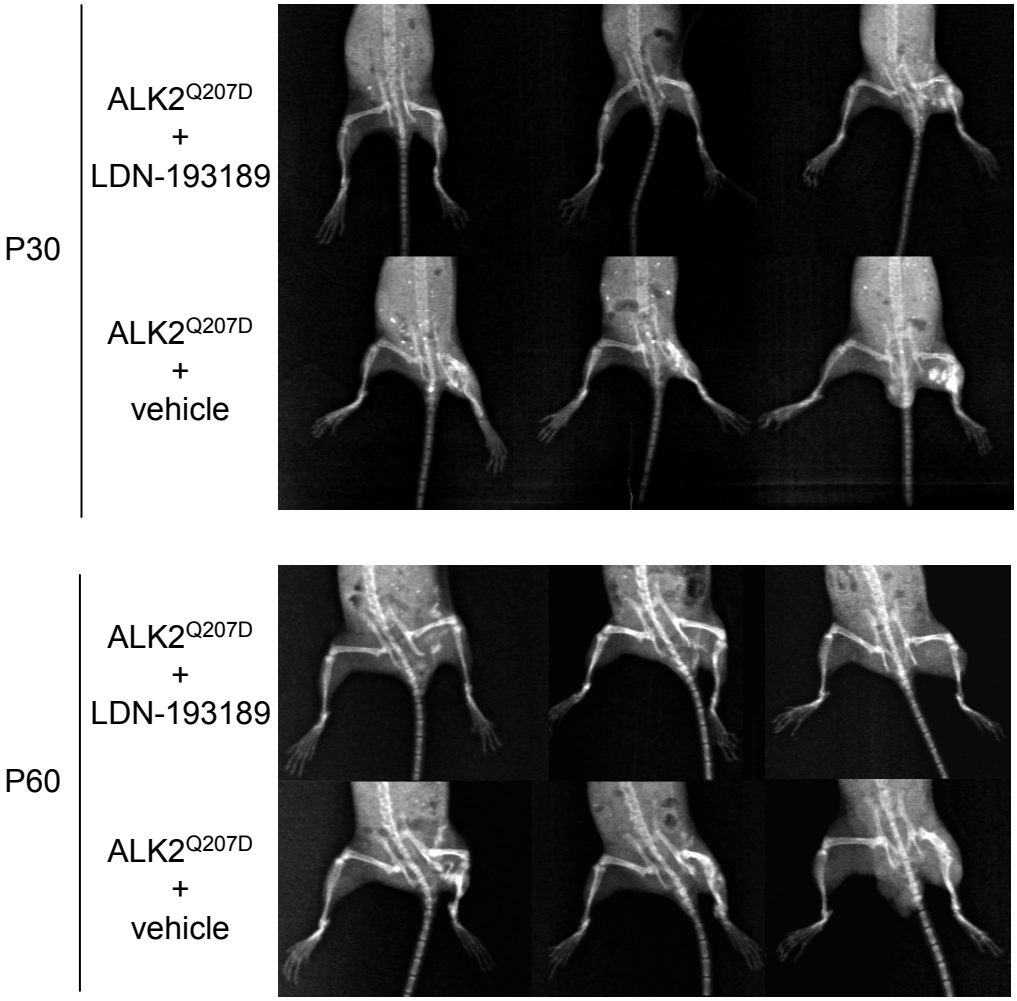

**Supplemental Fig. 2. LDN-193189 is a selective inhibitor of BMP signaling.** (**a**) Left panel: PDGFR signaling activity in PASMC, measured by the ability of PDGF-BB (10 ng $mL^{-1}$) to induce phosphorylation of Akt using a cell-based ELISA[6], was moderately inhibited by dorsomorphin (IC50 ~ 800 nM) and substantially less so by LDN-193189 (IC50 > 10 μM). Right panel: AMP-activated kinase activity, measured by the ability of AICAR (0.5 mM) to induce phosphorylation of Acetyl-CoA carboxylase (ACC) using a cell-based ELISA, was moderately inhibited by dorsomorphin and LDN-193189 (IC50 ~ 2 μM). (**b**) PASMCs were stimulated with PDGF-BB (10 ng $mL^{-1}$), and phosphorylation of Akt and MAP kinase ERK (p44/42) were analyzed by immunoblot. LDN-193189, at concentrations up to 8 μM, did not inhibit the activation of Akt or ERK by PDGF-BB. (**c**) PASMCs were stimulated with BMP4 (10 ng $mL^{-1}$), and phosphorylation of Smad1/5/8 and MAPK p38 were assessed by immunoblot. BMP4-induced activation of MAPK p38 was not affected by LDN-193189 at concentrations up to 8 μM.

Supplemental Figure 3



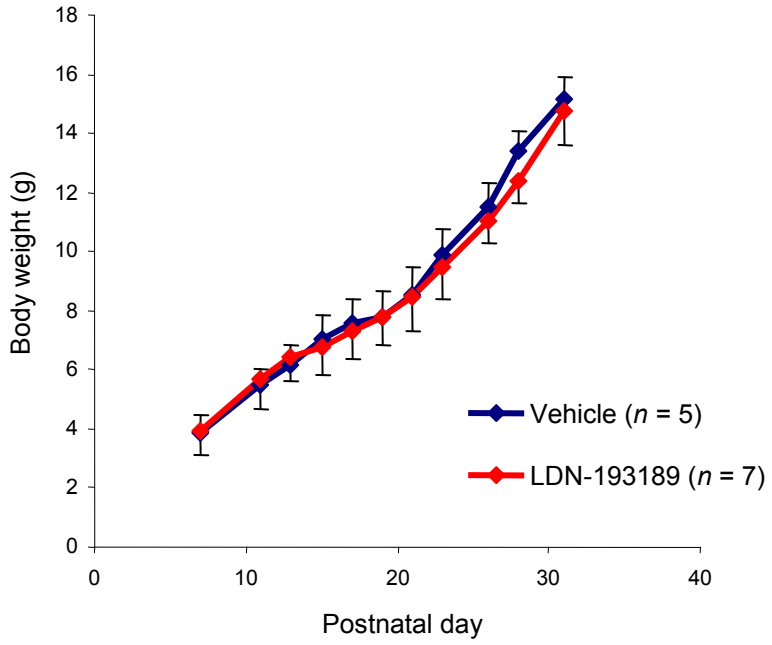

**Supplemental Fig. 3. Plasma LDN-193189 levels in mice after IP injection.** C57BL/6 mice received a single 3 mg $kg^{-1}$ IP dose of LDN-193189, and plasma levels of LDN-193189 were serially measured in individual mice at varying intervals after injection by LC-MS/MS (*n* = 3 mice each point, mean ± s.d.). At 8 h following injection, plasma levels were 5-fold greater than the *in vitro* IC50- of LDN-193189 for BMP4-mediated activation of Smad1/5/8 (indicated by the dashed line).

Supplemental Figure 4



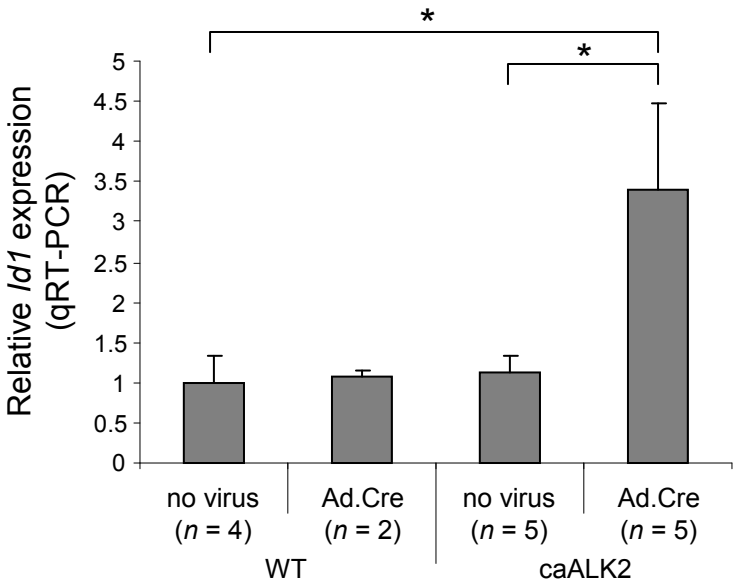

**Supplemental Fig. 4**. **Phenotypic penetrance of ectopic calcification in Ad.Cre-injected conditional caALK2 mice, and rescue with LDN-193189.** Mutant (**ALK2$^{Q207D}$**) transgenic mice treated with drug (3 mg kg$^{-1}$ IP every 12 h) following Ad.Cre injection exhibited varying degrees of rescue from ectopic calcification at P30 (upper panel), with two-thirds of mice exhibiting normal skeletal morphology and no calcifications, and the remainder exhibiting mild calcification, with preserved joint structure. In comparison, all vehicle-treated mice exhibited dense calcification and evidence of knee and hip joint fusion. At P60 (lower panel), one-third of mice treated with LDN-193189 had no evidence of ectopic calcification, while the remainder developed mild to moderate calcifications while preserving joint structure. All vehicle-treated mice exhibited fusion of hip, knee and ankle joints and dense calcification. Data shown are representative of 6 independent experiments, each performed with 3 to 5 mice per experimental group.

Supplemental Figure 5



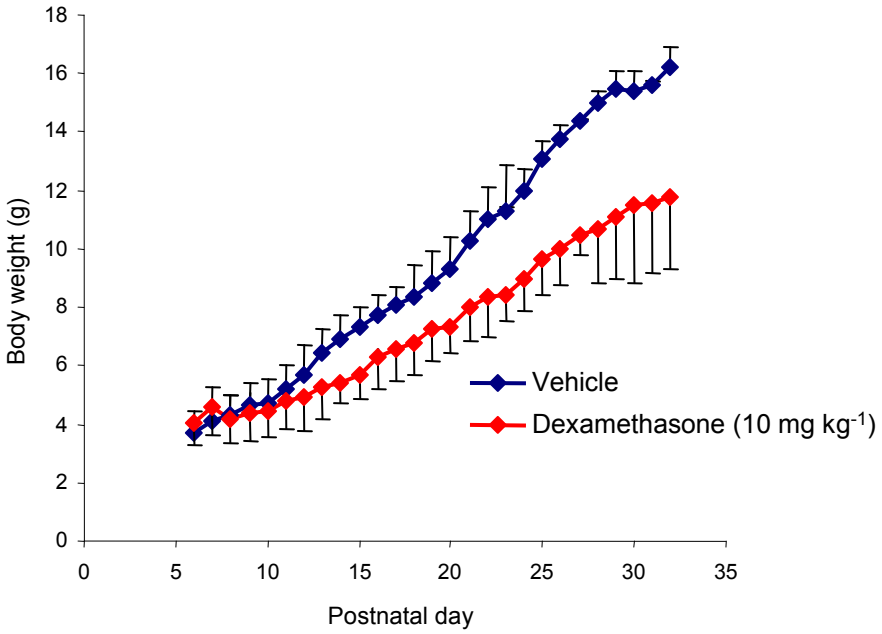

**Supplemental Fig. 5. LDN-193189 treatment of wild-type mice does not affect body weight during early postnatal development.** Wild-type mice were treated with LDN-193189 (3 mg $kg^{-1}$ IP every 12 h) or vehicle alone starting on postnatal day 7 through day 31 ($n$ = 5 and $n$ = 7, each treatment group, data are shown as mean ± s.d.). No significant differences in body weight between vehicle- and LDN-193189-treated mice were observed.

Supplemental Figure 6




**Supplemental Fig. 6. Ad.Cre injection of conditional caALK2 mice increases BMP transcriptional activity.** Wild-type and conditional caALK2 mice were either left untreated or injected with Ad.Cre (1 x $10^8$ pfu) into the left hindlimb at P7. At P10, total mRNA was isolated from left hamstring, gastrocnemius and soleus muscles. By quantitative RT-PCR, the ratios of *Id1* to *18S* mRNA levels were greater in mutant mice injected with Ad.Cre than in wild-type mice or uninjected mutant mice (*$P < 0.05$).

Supplemental Figure 7




**Supplemental Fig. 7. Dexamethasone treatment of caALK2 mutant mice impairs weight gain during postnatal development**. Conditional caALK2 mice injected with Ad.Cre (1 x $10^8$ pfu, P7) were treated with dexamethasone (10 mg $kg^{-1}$ IP daily) and body weights assessed from P7 through P33. Decreased growth, as assessed by weight gain, was seen as a result of corticosteroid therapy.

**Supplementary Movie Files**

**Movie 1a.** A wild-type mouse injected with Ad.Cre in its left hindlimb on P7 is shown at P60. No deficits in limb function or gait are apparent.

**Movie 1b.** A conditional caALK2 mouse injected with Ad.Cre in its left hindlimb on P7 is shown at P60. Severe fixed extension of the left hindlimb prevents use of the limb during ambulation.

**Movie 2a.** A conditional caALK2 mouse injected with Ad.Cre in its left hindlimb on P7, and then treated with vehicle is shown at P15. Early changes in left hindlimb function are evident at this time point, with moderate impairment of gait due to limited range-of-motion and mild fixed extension of the knee and hip joints.

**Movie 2b.** The vehicle-treated, Ad.Cre-injected caALK2 mouse shown at P30 exhibits more severe gait impairment due to severely fixed extension of knee, hip and ankle joints.

**Movie 2c.** A conditional caALK2 mouse injected with Ad.Cre on P7, and treated with LDN-193189 (3 mg $kg^{-1}$ every 12 h) is shown at P15. No impairment of gait or left hindlimb range-of-motion is apparent at this interval.

**Movie 2d.** An LDN-193189 treated, Ad.Cre-injected caALK2 mouse shown at P30 exhibits gait abnormality due to mild fixed extension of knee and hip joints, but substantially less impairment than vehicle-injected controls at this interval.