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Supplemental Data

Evaluation of the Therapeutic Potential of a CNP Analog in a *Fgfr3* Mouse Model Recapitulating Achondroplasia

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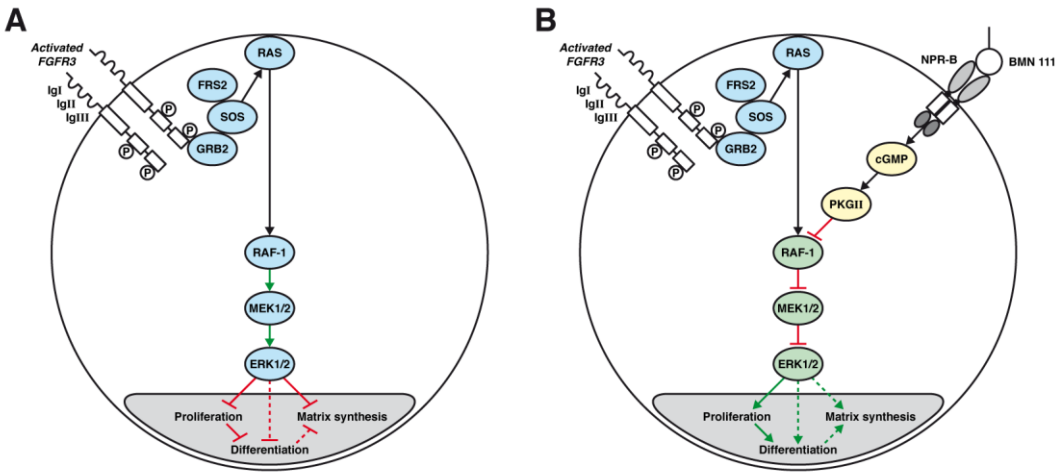

Figure S1. Crosstalk between Activated FGFR3 and NPR-B Signaling in Growth-Plate Chondrocytes

(A) Activated FGFR3 inhibits chondrocyte proliferation and differentiation and disturbs matrix synthesis.

(B) BMN 111 is a 39 amino acid CNP pharmacological analogue that inhibits FGFR3 downstream signaling at the level of Raf-1 in the growth plate and induces chondrocyte proliferation and differentiation.

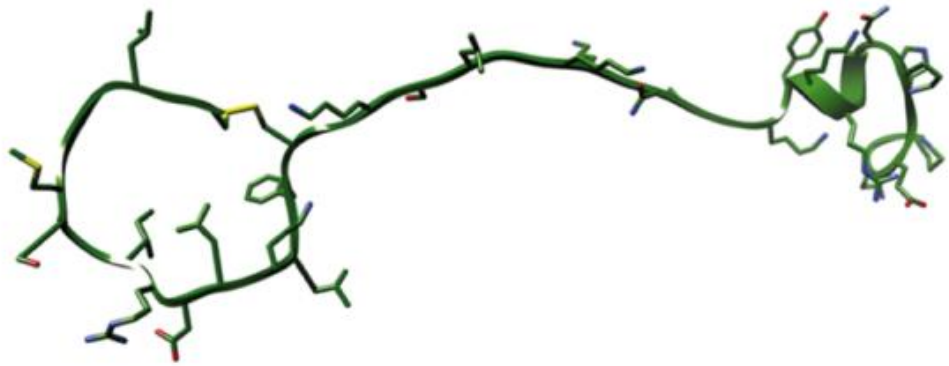

Figure S2. BMN 111 Amino Acid Sequence

BMN 111 is a 39 amino acid CNP pharmacological analogue. Its sequence is 1 PGQEHPNARK YKGANKKGLS KGCFGLKLDR IGSMSGLGC 39.

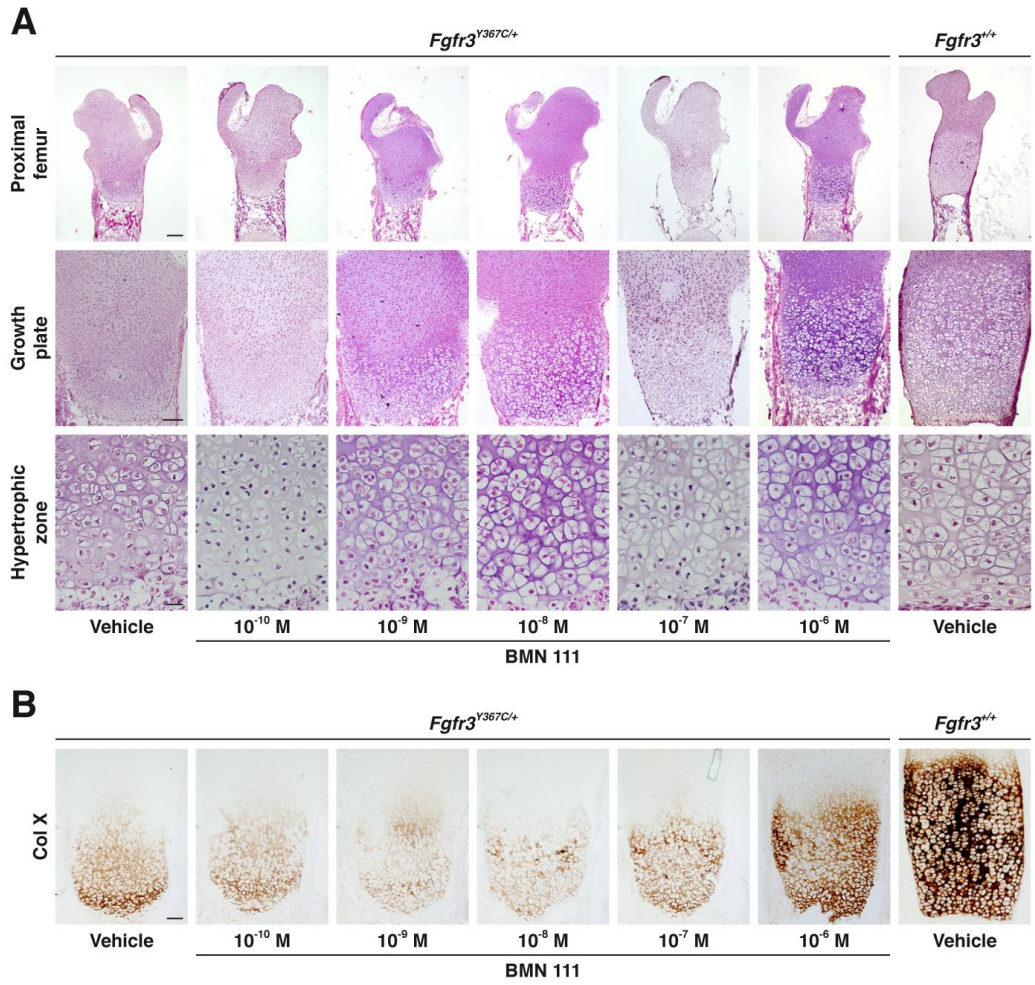

Figure S3. Restoration of Chondrocyte Proliferation and Differentiation in *Fgfr3*$^{Y367C/+}$ Femur Explants upon BMN111 Coincubation

BMN 111 rescued the size and growth plate defect of *Fgfr3*$^{Y367C/+}$ femur explants in an *ex vivo* culture model.

(A) Analysis of H&E stained longitudinal sections of *Fgfr3*$^{Y367C/+}$ femurs co-incubated with BMN 111 (10^{-6}M to 10^{-10}M) revealed a concentration-dependent increase in the height of the proliferative and hypertrophic zones of the growth plate along with larger and more spherical hypertrophic chondrocytes. No growth plate modification was visible at BMN 111 10^{-10}M.

(B) Type X collagen immunohistochemical staining of the hypertrophic zone of *Fgfr3*$^{Y367C/+}$ femurs co-incubated with BMN 111 (10^{-6}M to 10^{-10}M) demonstrated a concentration-dependent restoration of the height of the hypertrophic zone. Type X collagen is a marker of chondrocyte differentiation.

Table S1. Phenotypic Characterization of the *Fgfr3* Mouse Model at 17 Days of Age

Parameters at 17 Days of Age	WT (*Fgfr3*$^{+/+}$)		*Fgfr3*$^{Y367C/+}$		
	Mean ± SD	N	Mean ± SD	N	% of WT Mean
Body Weight (g)	10.2 ± 1.8	16	4.9 ± 0.7	14	47
Tail Length (mm)	48 ± 3	9	25 ± 3	9	52
Naso-Anal Length (mm)	69.08 ± 2.20	9	45.14 ± 1.43	9	65
Head Anterior-Posterior Length (mm)	20.25 ± 0.86	9	14.18 ± 0.50	9	70
Lumbar Vertebrae L4-L6 Length (mm)	5.83 ± 0.43	8	4.16 ± 0.30	9	71
Femur Length (mm)	9.84 ± 0.17	17	5.31 ± 0.15	18	54
Tibia Length (mm)	12.99 ± 0.56	18	5.41 ± 0.40	18	42
Foramen Magnum (C0)					
Sagittal Diameter (mm)	3.78 ± 0.11	8	2.95 ± 0.22	4	78
Lateral Diameter (mm)	4.89 ± 0.43	8	3.16 ± 0.22	4	65

All mean values for WT mice were significantly larger ($p<0.001$) than the corresponding values for *Fgfr3*$^{Y367C/+}$ mice using a two-tailed Student's T-test for statistical comparison. Data were obtained from WT and *Fgfr3*$^{Y367C/+}$ mice treated SC once daily with vehicle starting at 7 days of age.

Table S2. Summary of the Gains in the Length of *Fgfr3*$^{Y367C/+}$ and *Fgfr3*$^{+/+}$ Femur Explants after Coincubation with Vehicle or BMN 111 (10^{-10} to 10^{-6}M) for 6 Days

Mouse Femurs	Gain in Length					
	Vehicle	BMN 111 10^{-6}M	BMN 111 10^{-7}M	BMN 111 10^{-8}M	BMN 111 10^{-9}M	BMN 111 10^{-10}M
Fgfr3$^{Y367C/+}$	390 ± 106	661 ± 166**	709 ± 106**	606 ± 218**	542 ± 30*	387 ± 75
(µm, Mean ± SD)	(n=42)	(n=13)	(n=10)	(n=8)	(n=5)	(n=6)
Fgfr3$^{+/+}$	981 ± 194	1389 ± 320**	1549 ± 193**	1341 ± 326**	1144 ± 118*	1014 ± 249
(µm, Mean ± SD)	(n=47)	(n=15)	(n=9)	(n=8)	(n=7)	(n=8)

Whole femurs from *Fgfr3*$^{Y367C/+}$ and *Fgfr3*$^{+/+}$ littermates were collected at E16.5 and cultured for 6 days with BMN 111 (10^{-10} to 10^{-6} M) or vehicle. A concentration-dependent increase in longitudinal growth was observed in *Fgfr3*$^{Y367C/+}$ and *Fgfr3*$^{+/+}$ femurs co-incubated with BMN 111 in comparison to vehicle-treated *Fgfr3*$^{Y367C/+}$ and *Fgfr3*$^{+/+}$ femurs. *$p<0.05$; **$p<0.001$ using a two-tailed unpaired Student's T-Test comparing BMN 111-treated vs. vehicle-treated femur explants.