Treatment with an AAV vector expressing ENPP1-Fc prevents ectopic tissue calcification and restores bone parameters in Enpp1 deficient mice

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Inozyme Pharma

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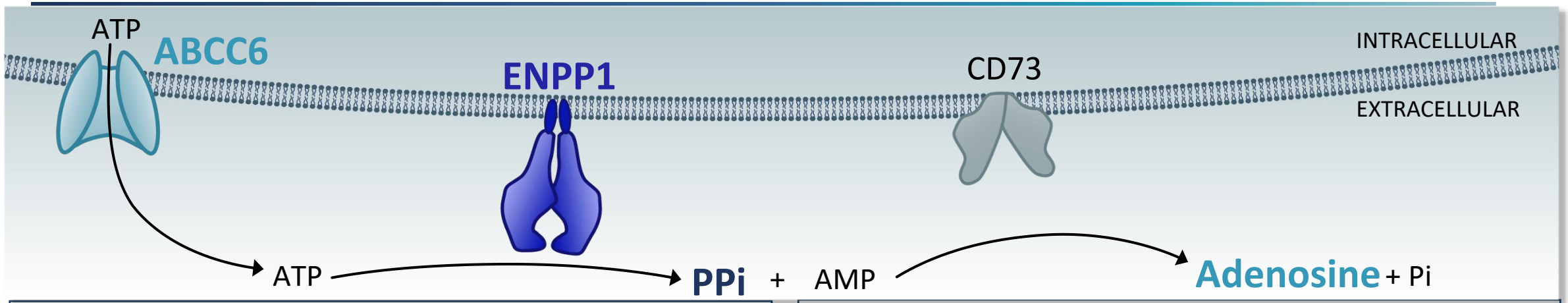
DISCLOSURE

- *The authors are employees of Inozyme Pharma.*
- *The work reported is sponsored by Inozyme Pharma.*

ENPP1 Plays A Critical Role In The Regulation Of Mineralization



PPi

Maintains healthy mineralization

by inhibiting ectopic growth and formation of hydroxyapatite

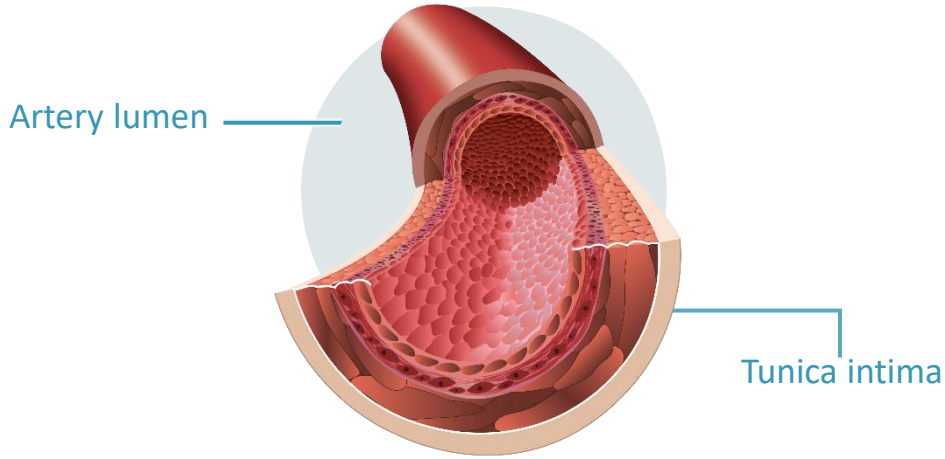
Inhibits mineralization of arteries, organs, and joints

Maintains healthy bones and teeth

Adenosine

Maintains healthy vessel wall thickness

by inhibiting neointimal hyperplasia



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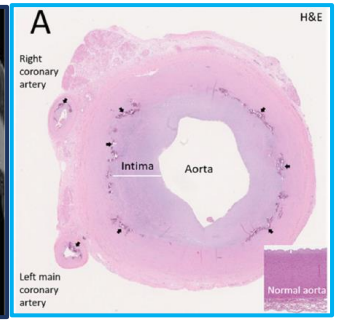
ENPP1 Deficiency: Heterogeneous Clinical Manifestations Across The Age Spectrum

INFANCY



CHILDHOOD/ ADOLESCENCE

ADULTHOOD

Signs/Symptoms

- Severe cardiovascular complications
- >50% mortality by 6 months of age

- Bone deformities
- Impaired growth
- Hearing loss

- Bone and joint pain
- Mobility impairment

Diagnosis

Generalized Arterial Calcification of Infancy (GACI)

Autosomal Recessive Hypophosphatemic Rickets Type 2 (ARHR2)

Signs/ symptoms of arterial calcification and/or neointimal proliferation can be seen across the age spectrum

Ferreira CR, et al. *J Bone Miner Res*. Published online August 5, 2021. doi:10.1002/jbmr.4418; Rutsch F, et al. *Circ Cardiovasc Genet.* 2008;1(2):133-140; Ferreira CR et al. *Genet Med*. 2021;23(2):396-407.; Boyce AM et al. *Curr Osteoporos Rep*. 2020;18(3):232-241.; Kotwal A et al. J Bone Miner Res. 2020 Apr;35(4):662-670.

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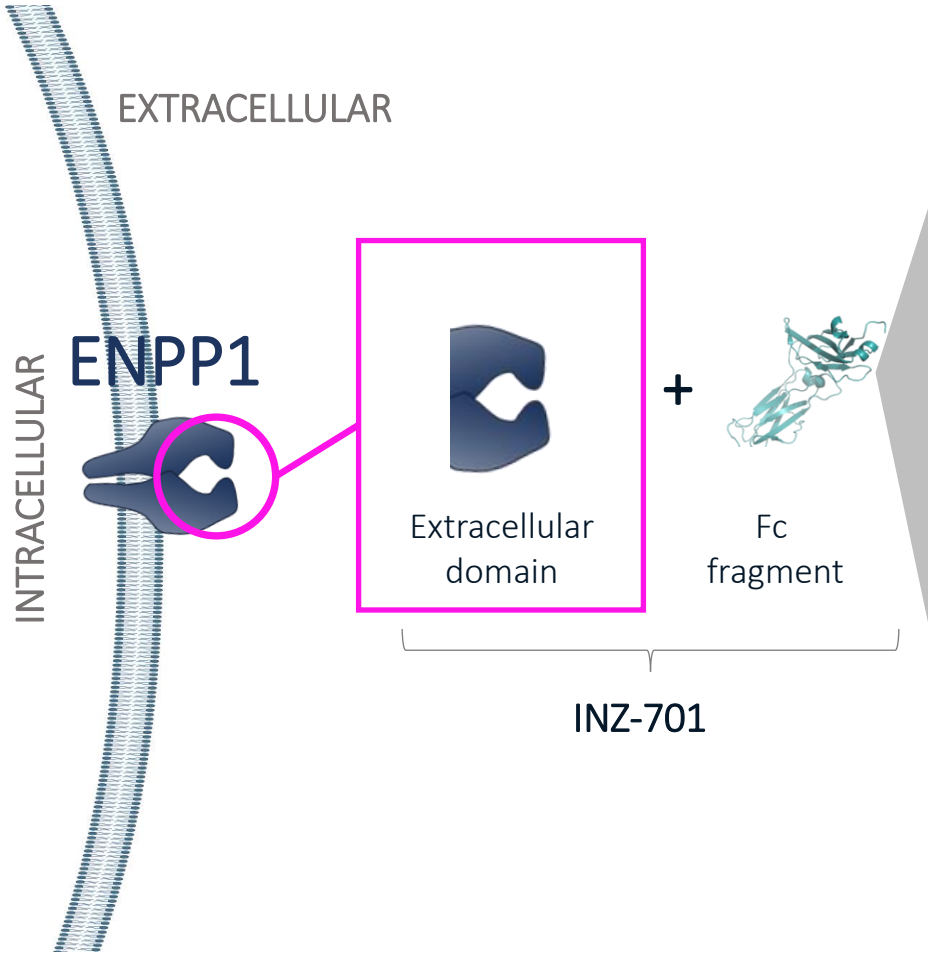
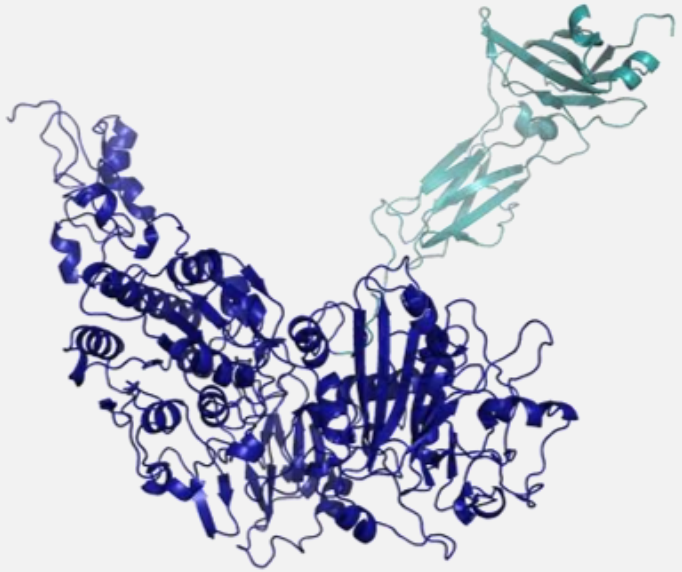
INZ-701 (hEnpp1-Fc) Is An ERT In Development For ENPP1 Deficiency And ABCC6 Deficiency



INZ-701

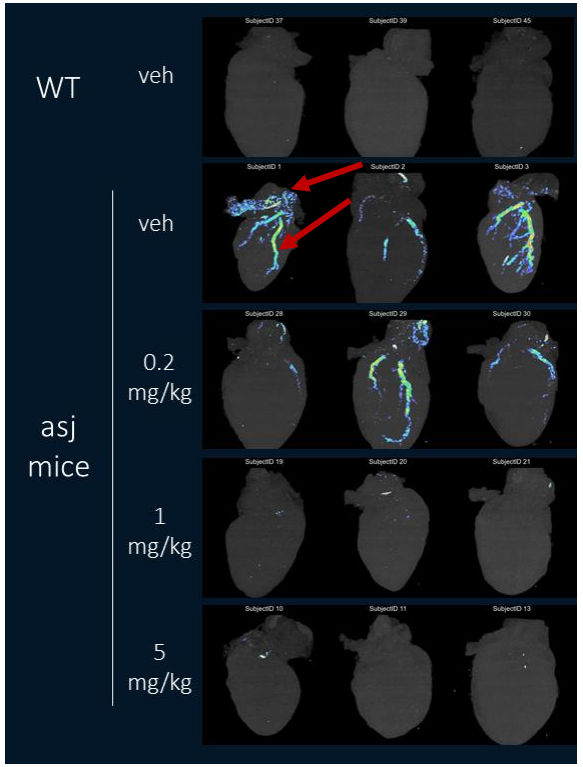


- **Protein:** Recombinant human ENPP1 (Ectonucleotide pyrophosphatase/phosphodiesterase 1)
- **Construct:** Recombinant Fc fusion protein with soluble extracellular domain of ENPP1
- **Dosing:** SC ; 2x/week in Ph. 1/2 for ENPP1 deficiency
- **Enzymatic Properties:** High catalytic efficiency (Kcat/Km)

- Evaluation of Safety, Tolerability, and Efficacy of INZ-701 in Adults With ENPP1 Deficiency (NCT04686175)
- Evaluation of Safety, Tolerability, and Efficacy of INZ-701 in Adults With ABCC6 Deficiency Causing PXE (NCT05030831)

We have previously shown that ERT (INZ-701) Prevents Tissue Calcification and Corrects Bone Phenotype

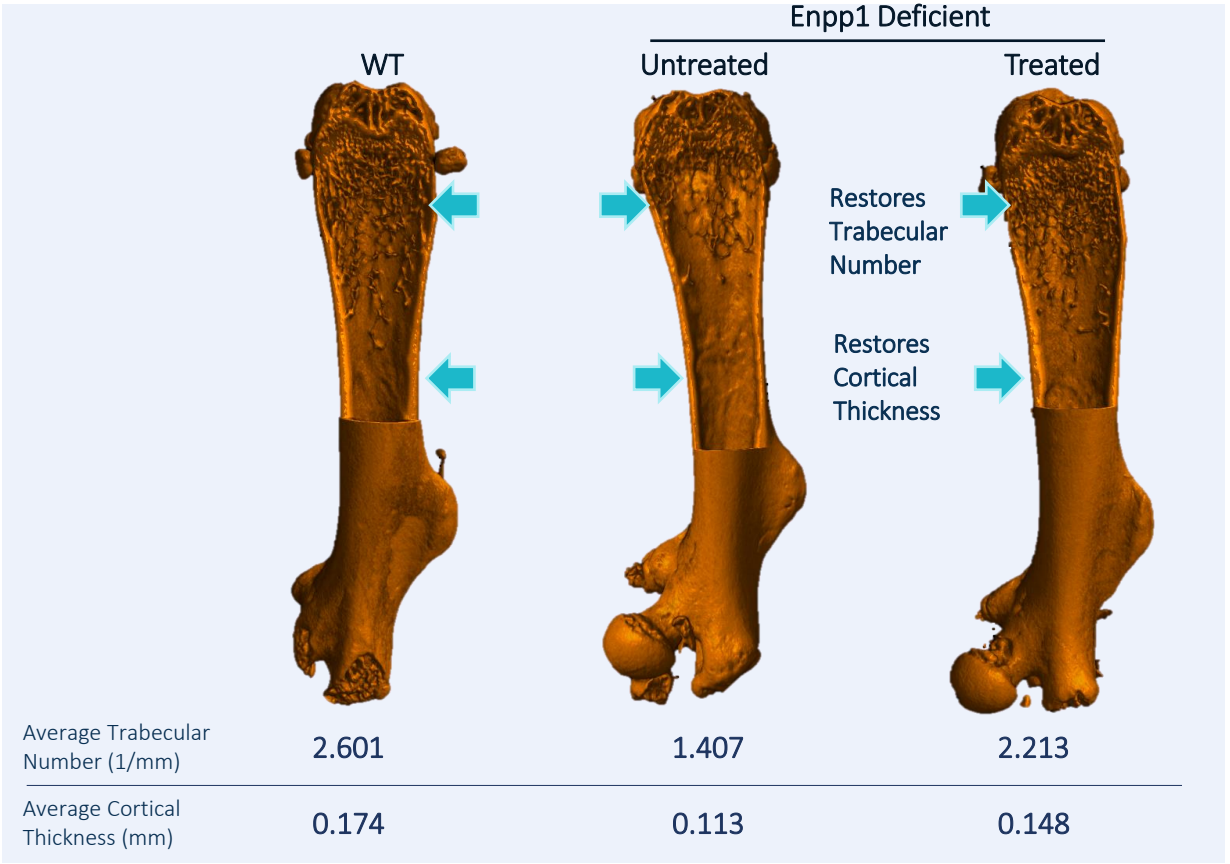
Prevented Soft Tissue Calcification



INZ-701 also prevents pathological calcification in:

- Heart, Lung, Spleen, Liver, Aorta, Kidneys

Corrected bone defects

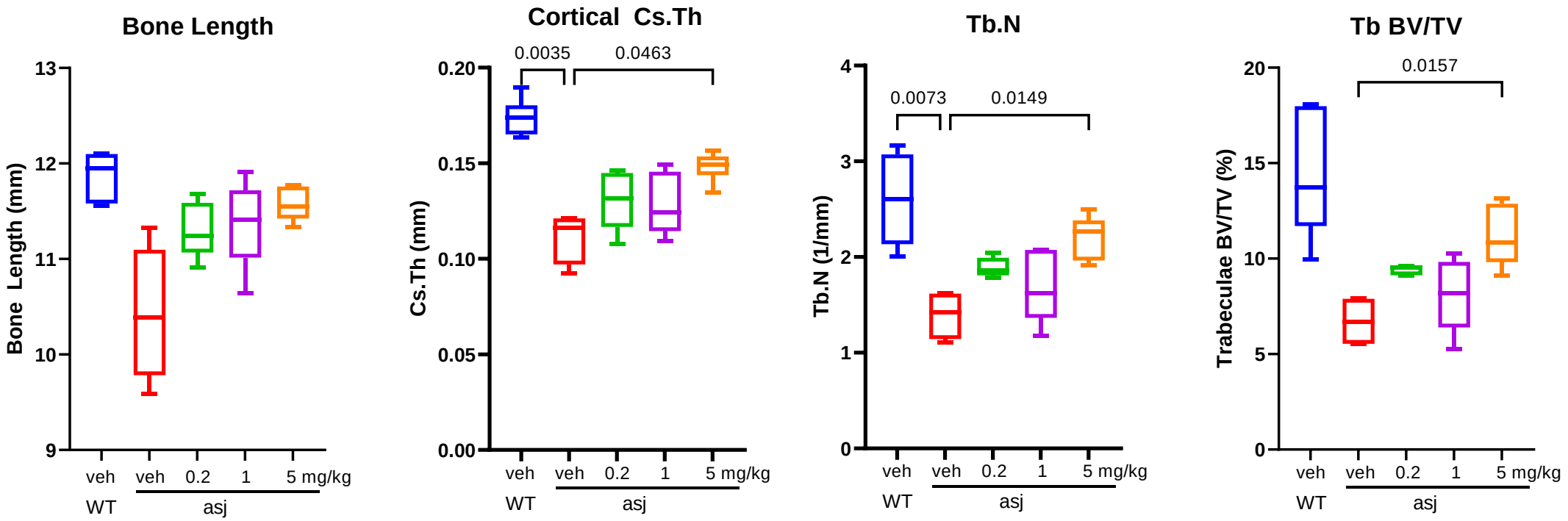


	WT	Enpp1 Deficient Untreated	Enpp1 Deficient Treated
Average Trabecular Number (1/mm)	2.601	1.407	2.213
Average Cortical Thickness (mm)	0.174	0.113	0.148

ENPP1 deficient mice (asj) recapitulate the clinical features of the disease

Adapted from Cheng. et.al., JBMR, 2021

INZ-701 Corrects Bone Defects in ENPP1 Deficient Mice



Cheng. et.al., JBMR, 2021

Goal: Develop a Gene Therapy For ENPP1 Deficiency

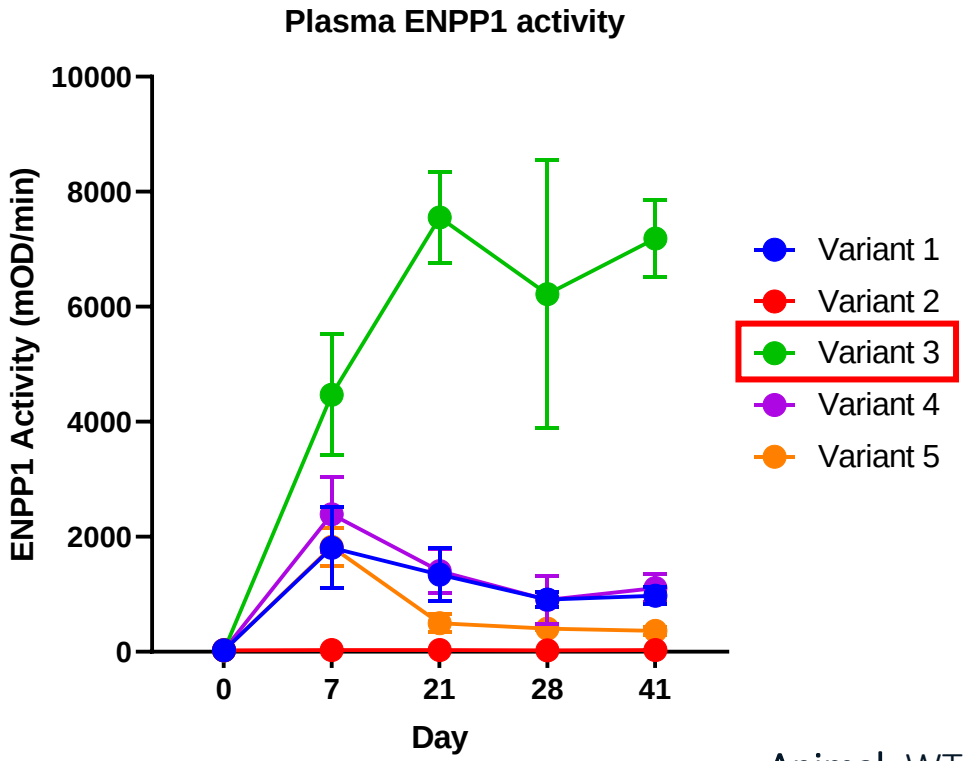
- Can we build on the success of ENPP1 ERT and develop a one-dose gene therapy to treat ENPP1 Deficiency?

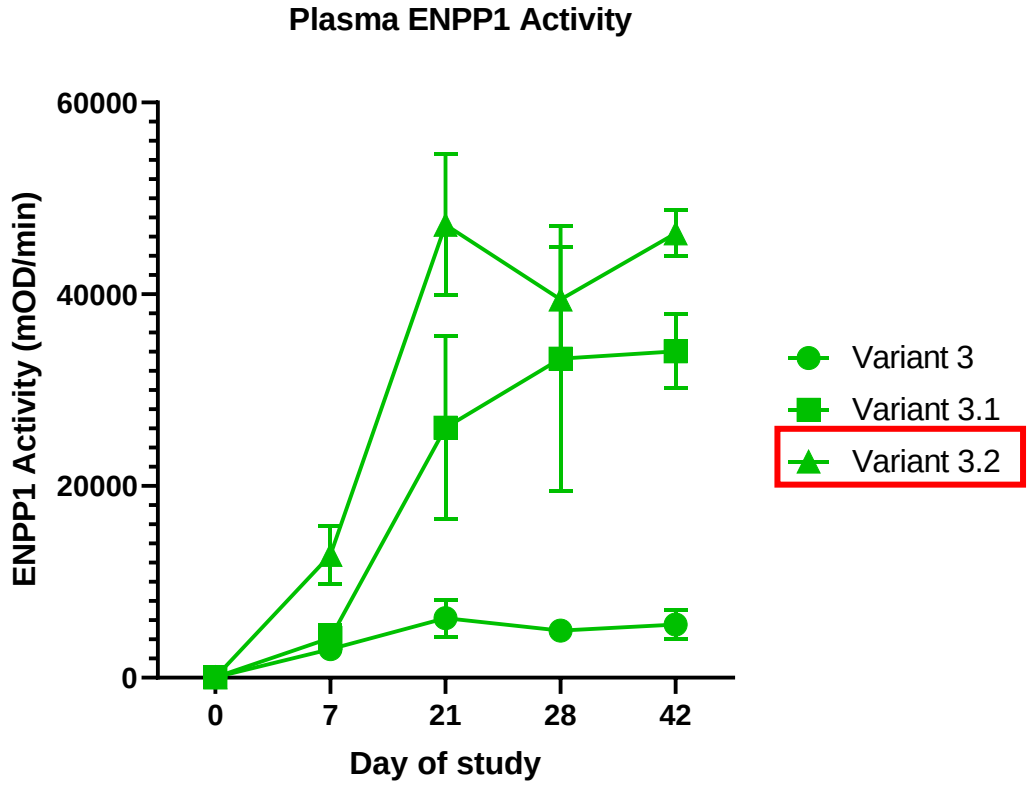
  - An AAV vector that can produce efficacious and durable amount of soluble ENPP1-Fc proteins after a single dose.

AAV Vector Optimization Leads To ~40-fold Increase In Expression

ENPP1-Fc sequence optimization

Promoter Selection





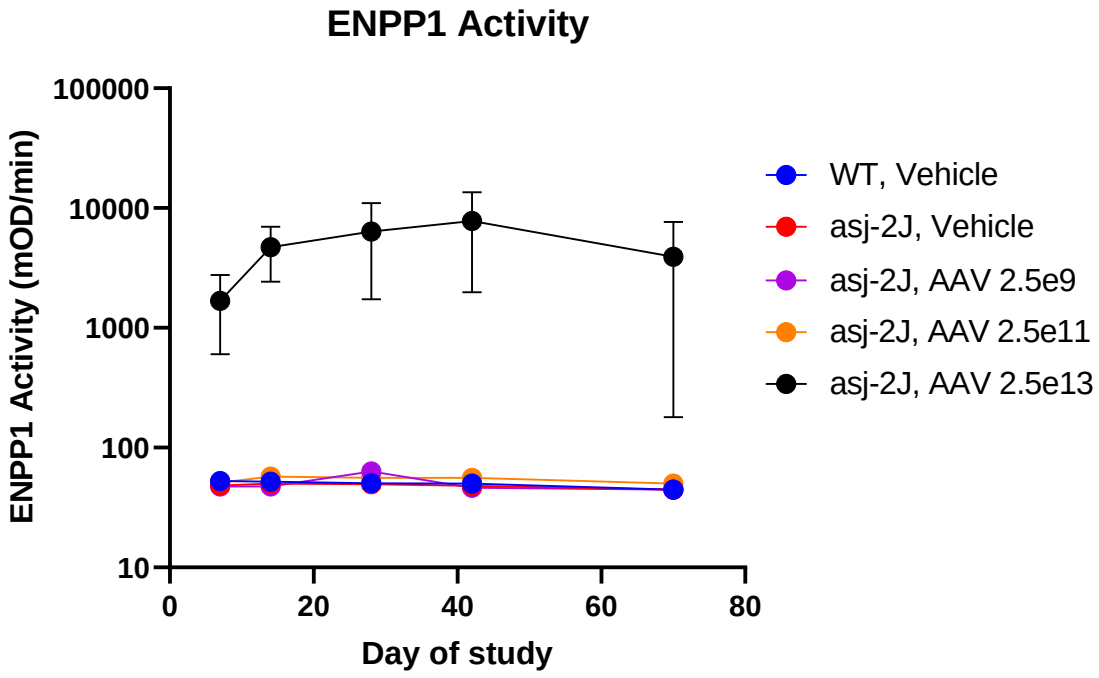
Animal: WT C57BL/6 mice.
Dose: Single i.v. dose at $1x10^{14}$ vg/kg

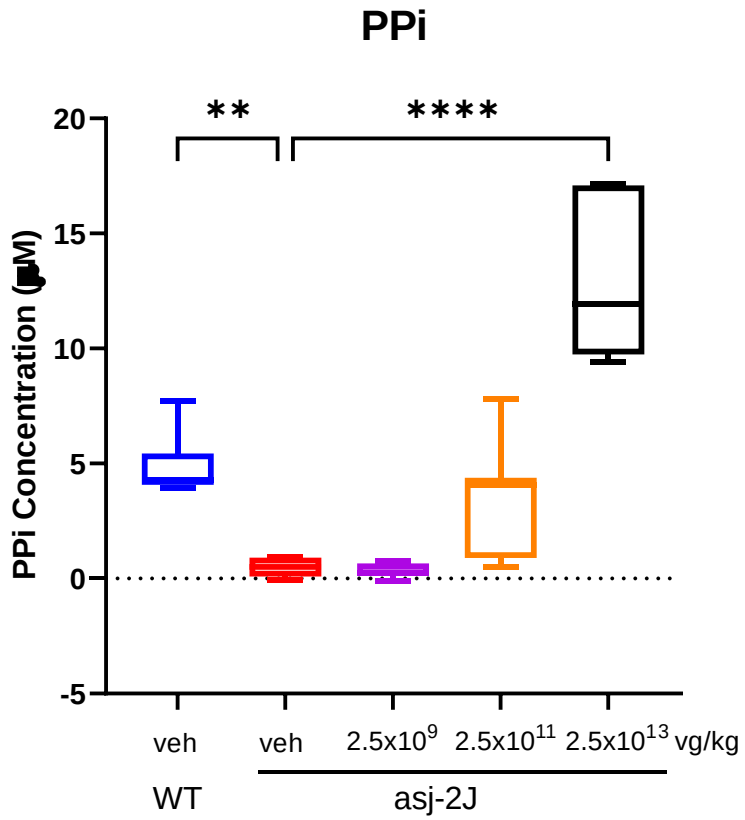
Sources: *Internal, Unpublished Data.*

AAV Dose Response Study design

Animal	Test Article	Dosing	Start	End	Readouts
WT	vehicle	i.v. Single dose on D1	~2wk of age (D1)	~12wk of age (D70)	• Enpp1 activity • Plasma PPi • Tissue calcium • Bone parameters
Enpp1$^{asj\text{-}2J/asj\text{-}2J}$	vehicle				
Enpp1$^{asj\text{-}2J/asj\text{-}2J}$	AAV-ENPP1-Fc $2.5x10^{9}$ vg/kg				
Enpp1$^{asj\text{-}2J/asj\text{-}2J}$	AAV-ENPP1-Fc $2.5x10^{11}$ vg/kg				
Enpp1$^{asj\text{-}2J/asj\text{-}2J}$	AAV-ENPP1-Fc $2.5x10^{13}$ vg/kg				

AAV-Enpp1-Fc Increases Plasma ENPP1 Activity And Plasma PPi Level

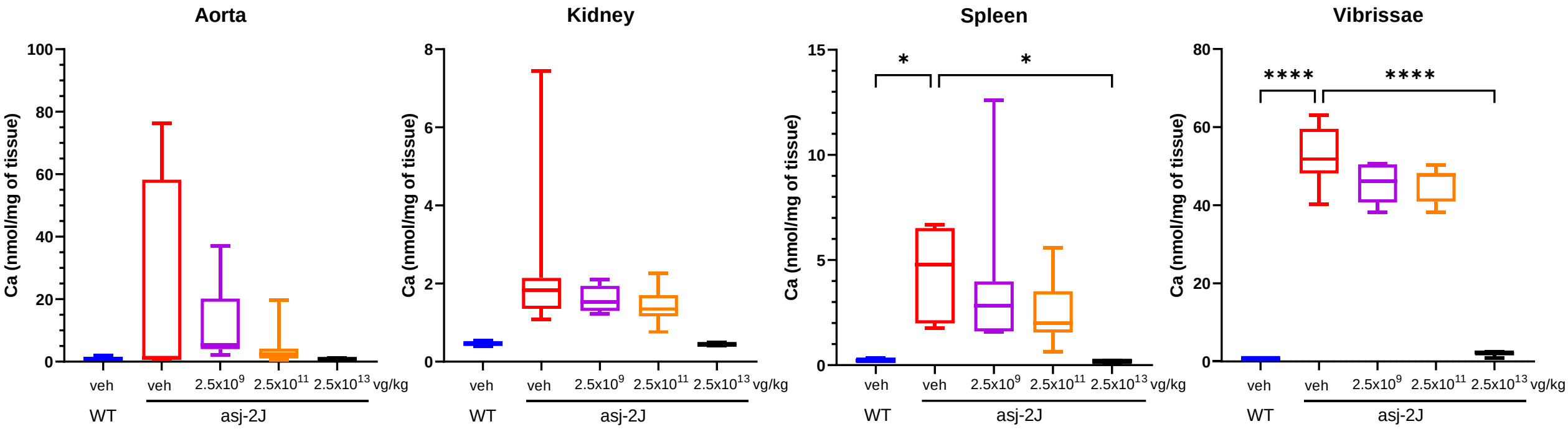




** p ≤ 0.01, **** p ≤ 0.0001

Sources: *Internal, Unpublished Data.*

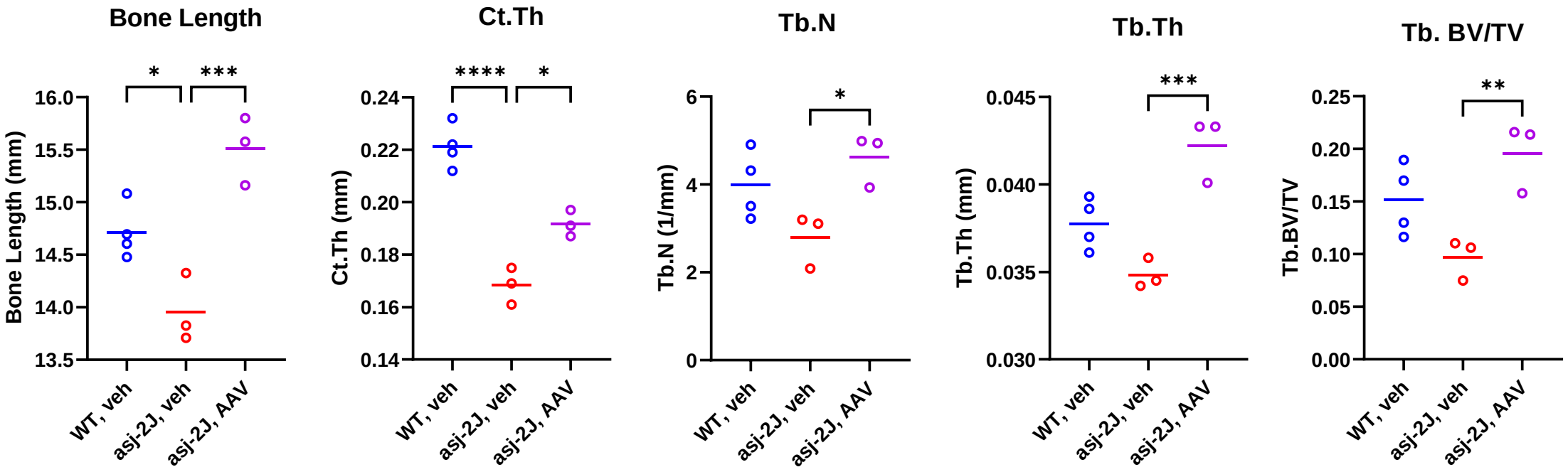
AAV-ENPP1-Fc Prevents Tissue Calcification



Sources: *Internal, Unpublished Data.*

* $p \leq 0.05$, **** $p \leq 0.0001$.

AAV-ENPP1-Fc Corrects Bone Defects



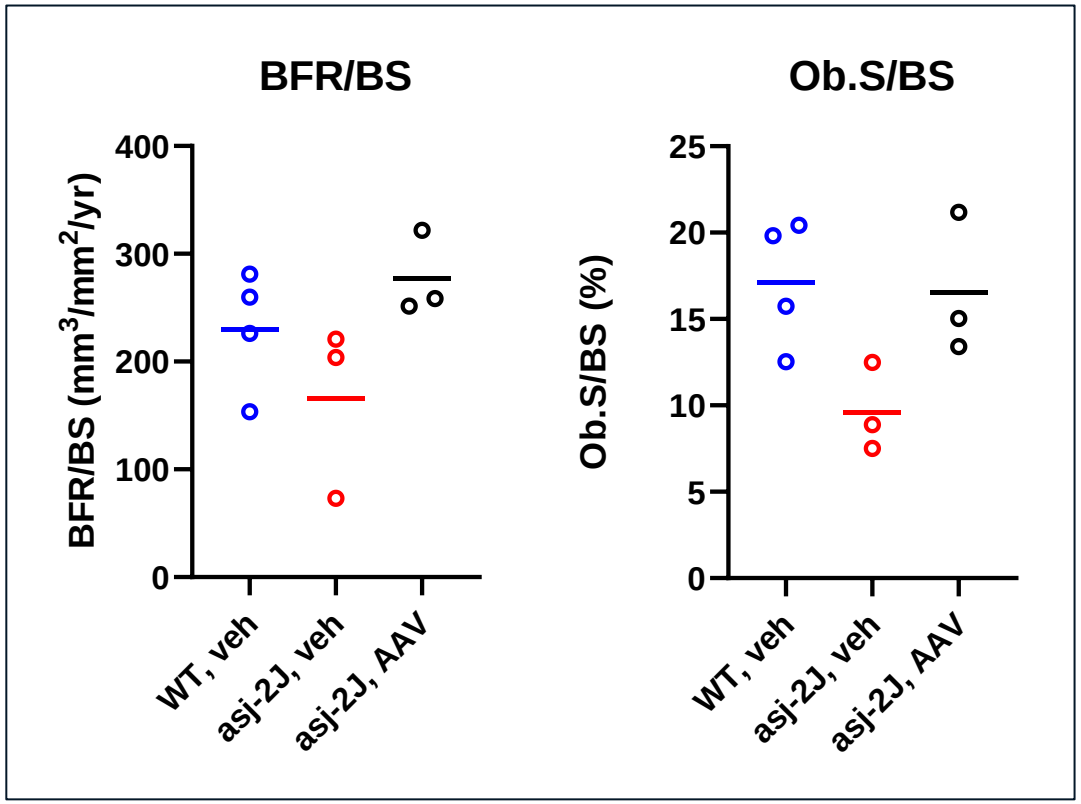
- Micro-CT analysis of femora from female animals.
- AAV dose $2.5x10^{13}$ vg/kg.

* $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$

Sources: *Internal, Unpublished Data.*

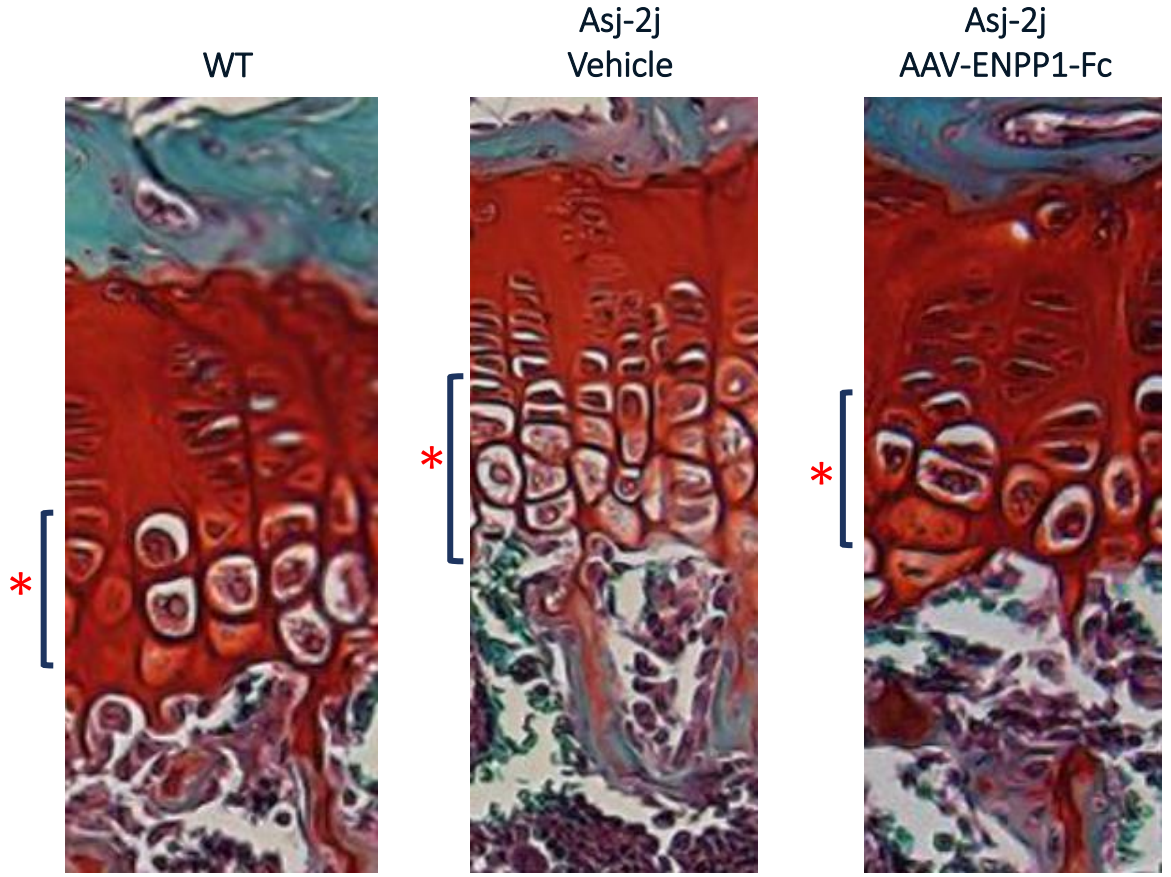
AAV-ENPP1-Fc Maintains Normal Osteoblast Function and Growth Plate Structure

AAV-ENPP1-Fc maintains normal bone formation rate and osteoblast surface



- Histomorphometric analysis of femora from female animals.
- AAV dose $2.5x10^{13}$ vg/kg.

AAV-ENPP1-Fc prevents the rachitic phenotype



*: Hypertrophic chondrocytes

Sources: *Internal, Unpublished Data.*

Summary

Administration of a single dose of an AAV vector expressing modified ENPP1-Fc at $2.5x10^{13}$ vg/kg in young ENPP1 deficient mice lead to:

- Increases plasma ENPP1 activity for >10 weeks
- Increases plasma pyrophosphate levels
- Prevents soft tissue calcification
- Corrects bone defects

Ongoing: Dose response study to establish lowest efficacious dose.

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