INZ-701, a recombinant ENPP1-Fc protein, prevents ectopic mineralization in a mouse model of Pseudoxanthoma Elasticum (PXE)

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DISCLOSURE

- *The work reported is sponsored by Inozyme Pharma.*
- *ZC, KO, DT, YS are employees of Inozyme Pharma.*

Pseudoxanthoma Elasticum (PXE)

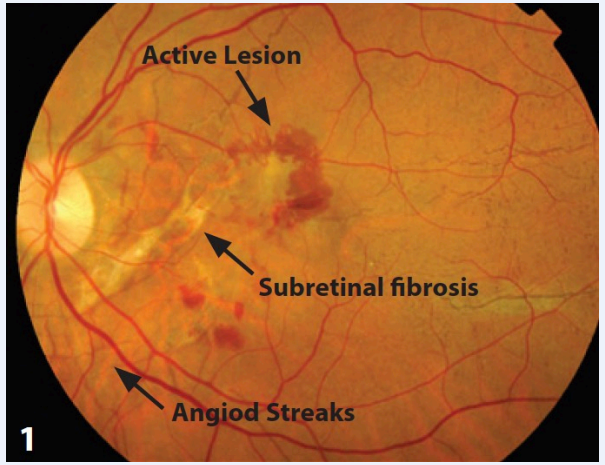
- Predominantly caused by mutations in ABCC6 gene
- Characterized by ectopic mineralization of the skin, eyes and the cardiovascular system
- Significant morbidity, including visual impairment and cardiovascular complications
- Some infants with ABCC6 mutations have severe vascular calcification, resembling Generalized Arterial Calcification of Infancy (GACI) caused by ENPP1 deficiency

Pathological Mineralization

Skin lesions

Borst et al. Trends in Biochemical Sciences, February 2019,

Retinal abnormalities



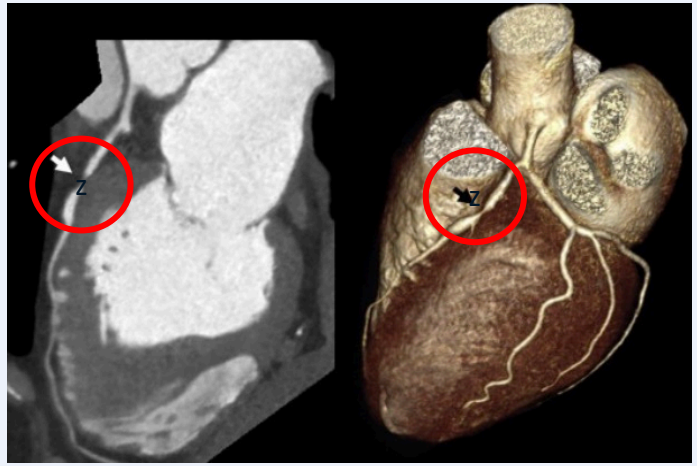
Zaria et al. eye news | OCTOBER/NOVEMBER 2015

Vascular Calcification

Kranenburg et al. Atherosclerosis, NOVEMBER 2016

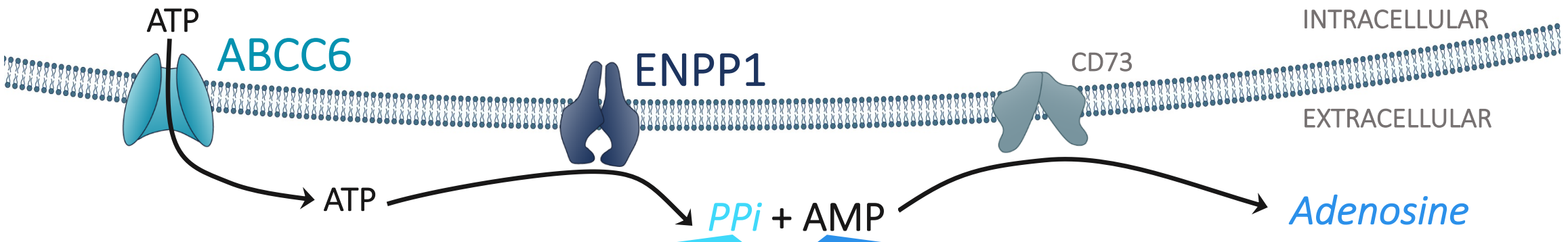
Arterial Stenosis

Neointimal Proliferation



Karam et al. J Cardio Comp Tomo 2015

The Biological Pathway that Regulates Mineralization and Neointimal Proliferation



Maintains Healthy Mineralization

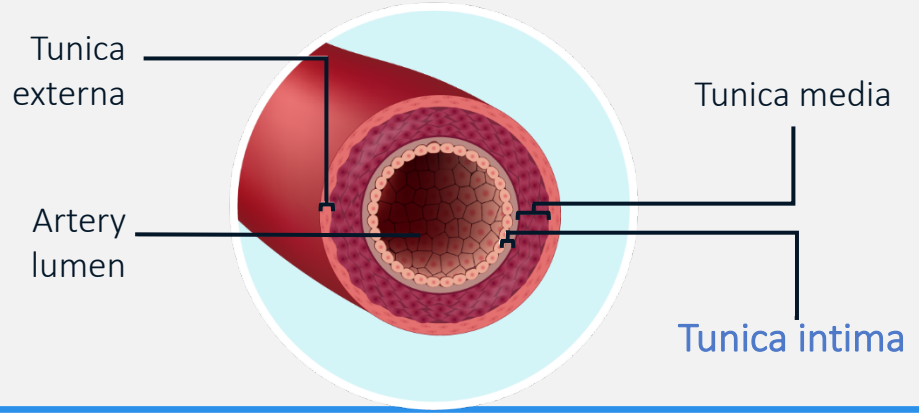
PPi inhibits growth and formation of hydroxyapatite, which results in:

- Maintenance of healthy bones and teeth

- Inhibition of pathological ectopic mineralization (i.e., mineralization of arteries, organs, and joints)

Maintains Healthy Vessel Wall Thickness

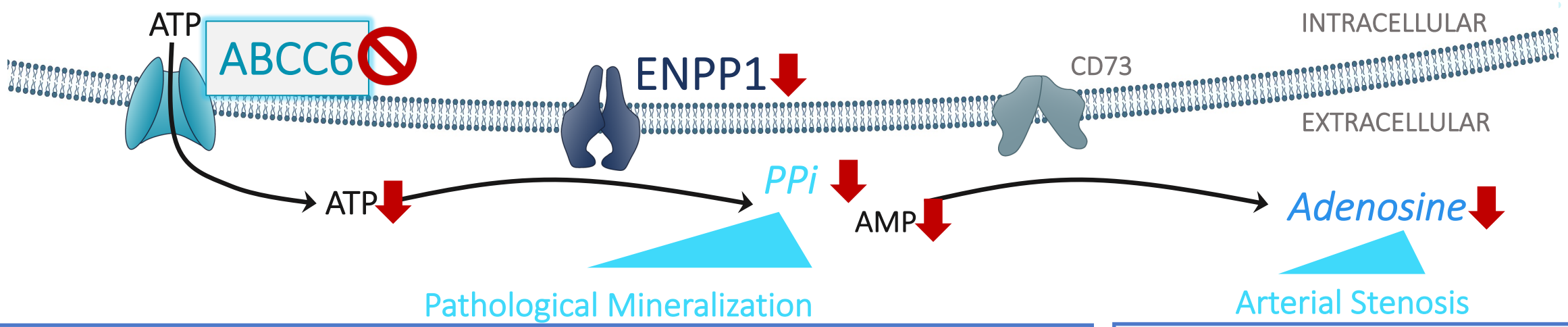
Adenosine inhibits neointimal proliferation



ABCC6 = Adenosine triphosphate binding cassette transporter protein subfamily C member 6; AMP=adenosine monophosphate; ATP=adenosine triphosphate; ENPP1 = ectonucleotide pyrophosphatase/phosphodiesterase 1; PPi = pyrophosphate

Sources: 1. Jansen RS, et al. *Arterioscler Thromb Vasc Biol*. 2014;34(9):1985-1989. **2.** Nitschke Y, et al. *Am J Hum Genet*. 2012;90(1):25-39. **3.** Nitschke Y, et al. *Exp Mol Med*. 2018;50(10):1-12.

ABCC6 Deficiency Leads to Pseudoxanthoma Elasticum (PXE), a Chronic Disease of High Morbidity



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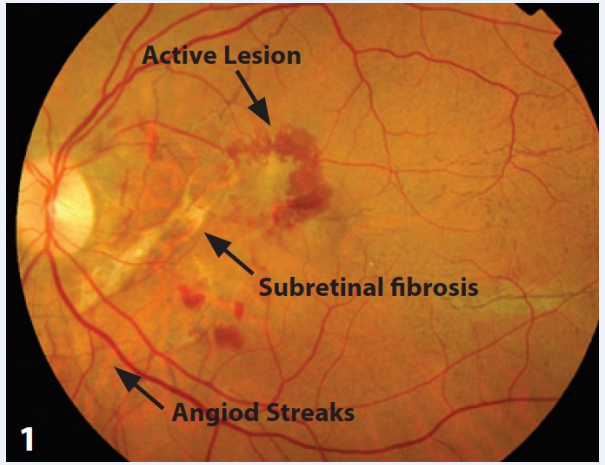

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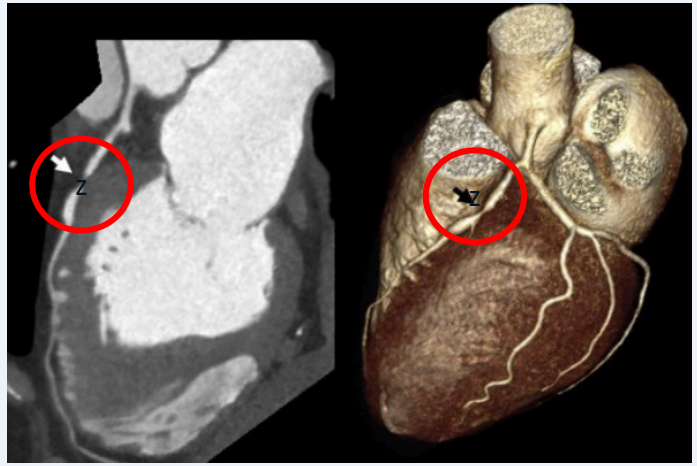
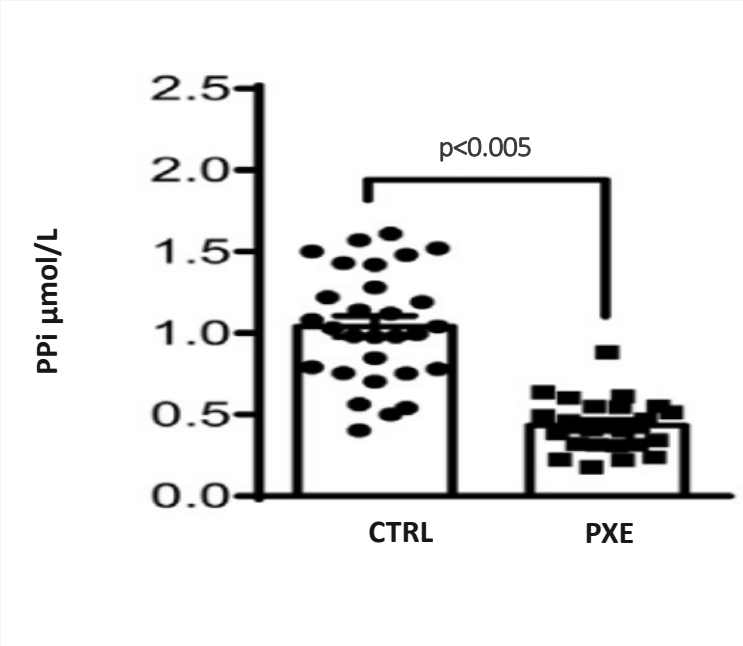
Neointimal Proliferation



Karam et al. J Cardio Comp Tomo 2015

Reduced Levels of PPi and ENPP1 Lead to Pathological Mineralization in ABCC6 Deficiency

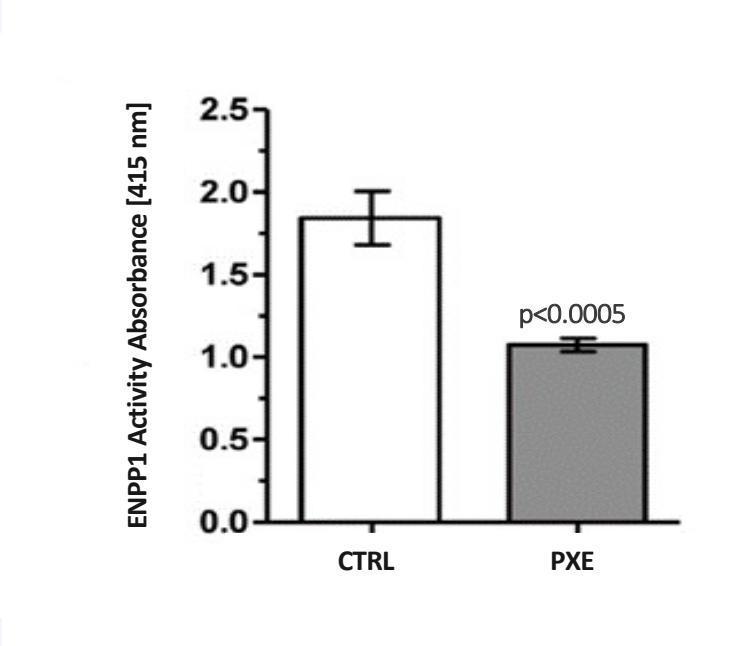
1 *Reduced plasma pyrophosphate levels in PXE patients*



Kauffenstein et al., 2018

Previously demonstrated in a mouse model by Jansen, et al., 2014

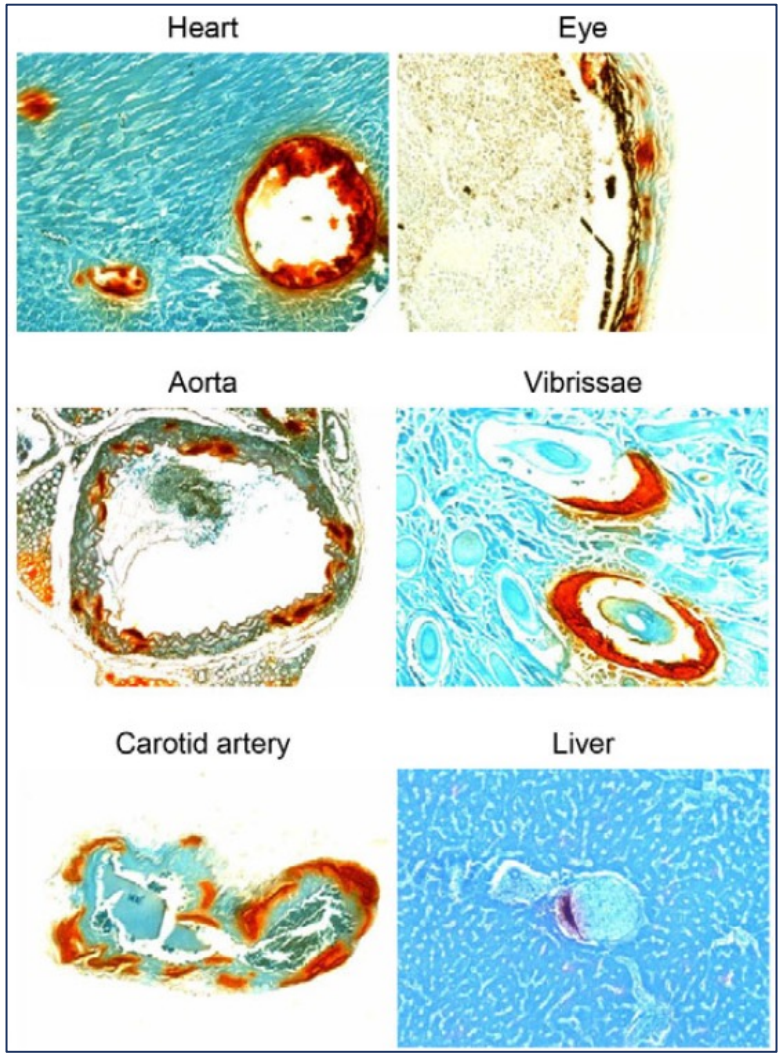
2 *Reduced ENPP1 in PXE patients*



Dabisch-Ruthe et al., 2014

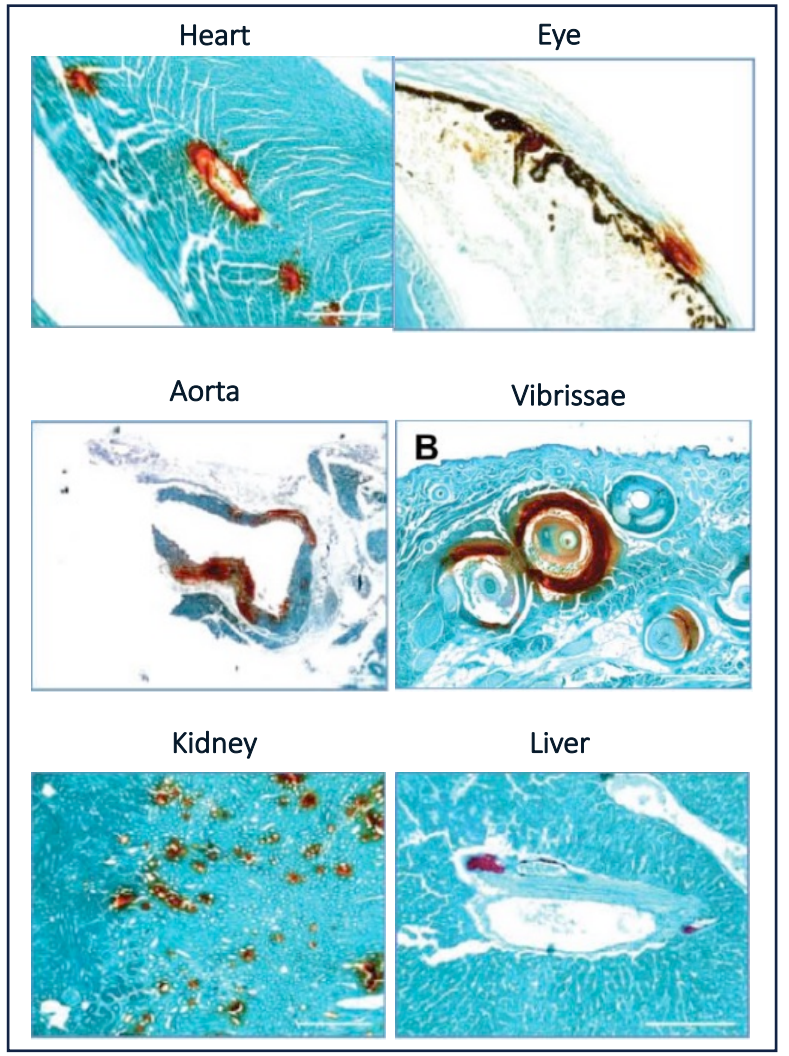
ABCC6 Deficiency have Phenotypic Overlap with ENPP1 Deficiency in Patients and Animal Models

Enpp1$^{asj/asj}$ mice



Li, 2013

Abcc6$^{-/-}$ mice

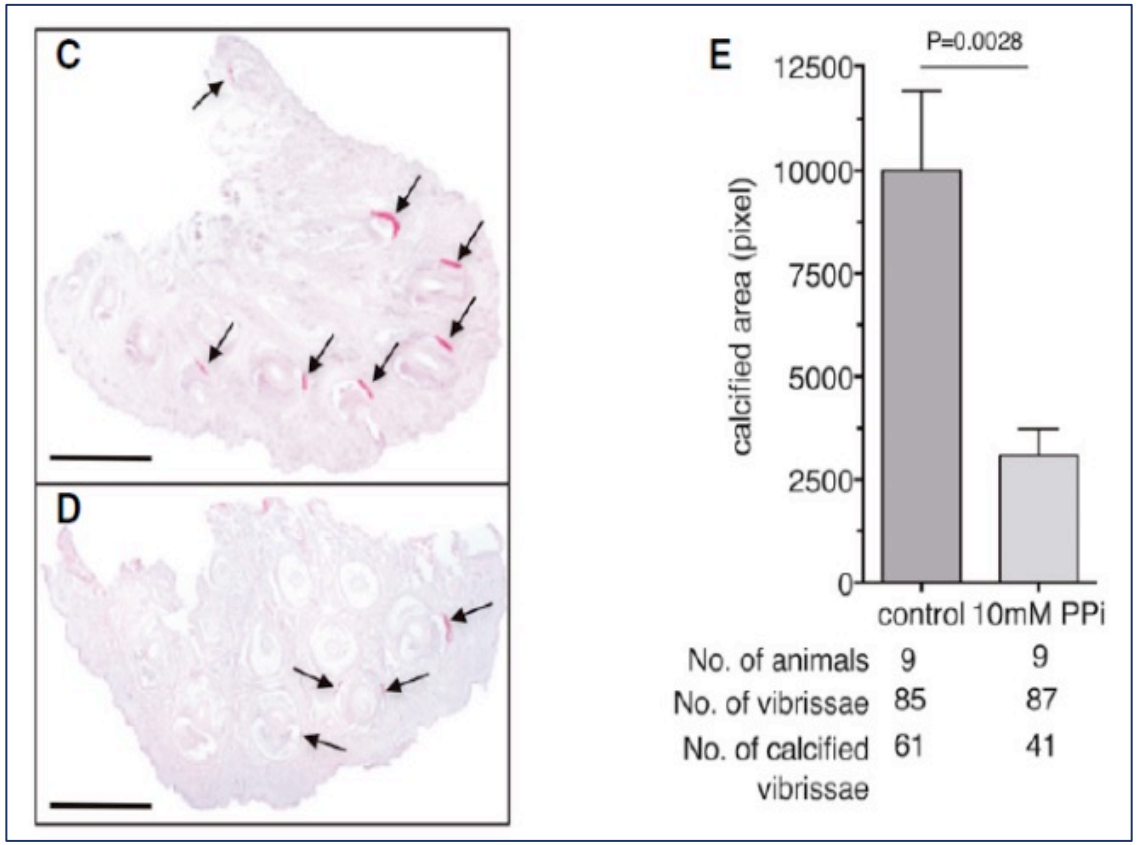


Li, 2015

Calcium deposits appear maroon with Alizarin Red staining

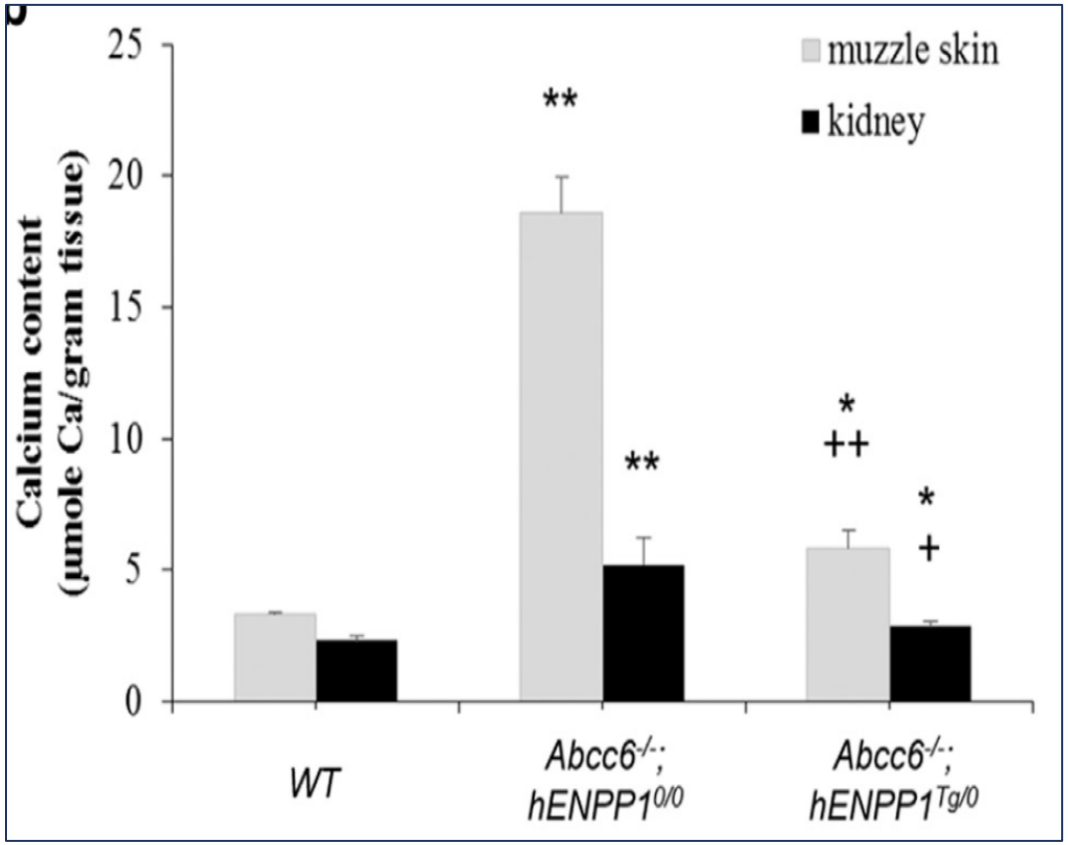
PPi Treatment And ENPP1 Overexpression Have Shown Reduced Tissue Calcification In *Abcc6* $^{-/-}$ Mice

PPi treatment



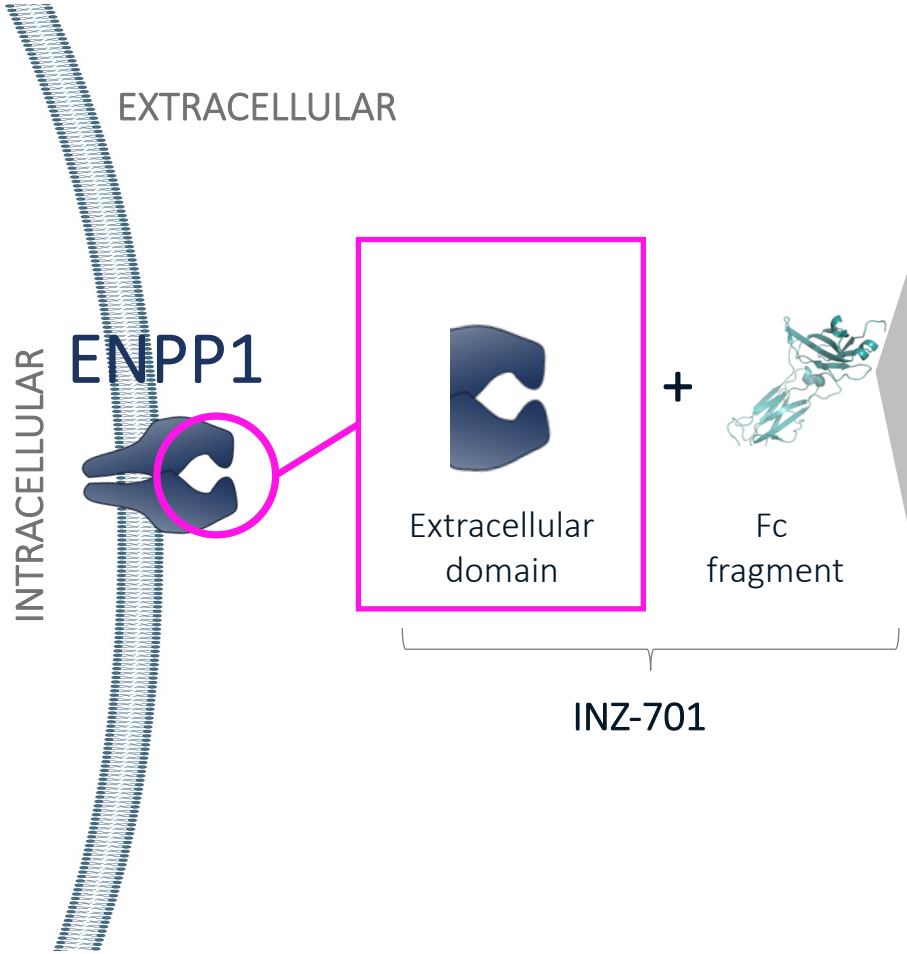
Dedinszki 2017

Overexpression of human ENPP1

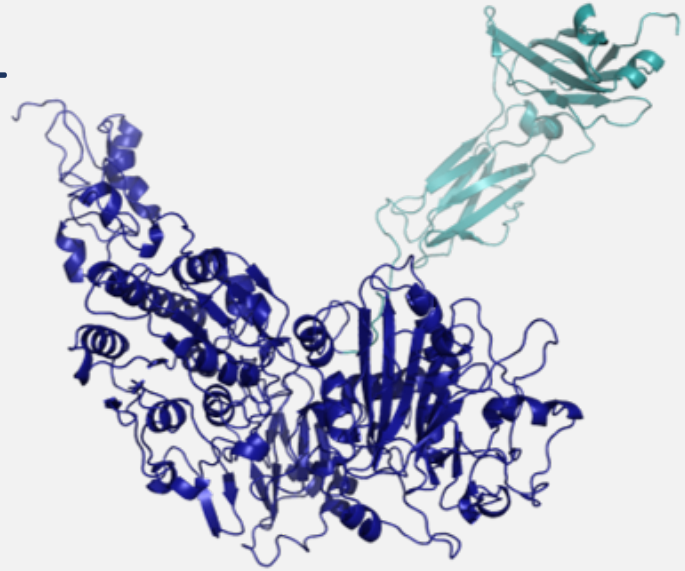


Zhao 2017

INZ-701 (hENPP1-Fc) is an ERT in development for ENPP1 deficiency and ABCC6 deficiency

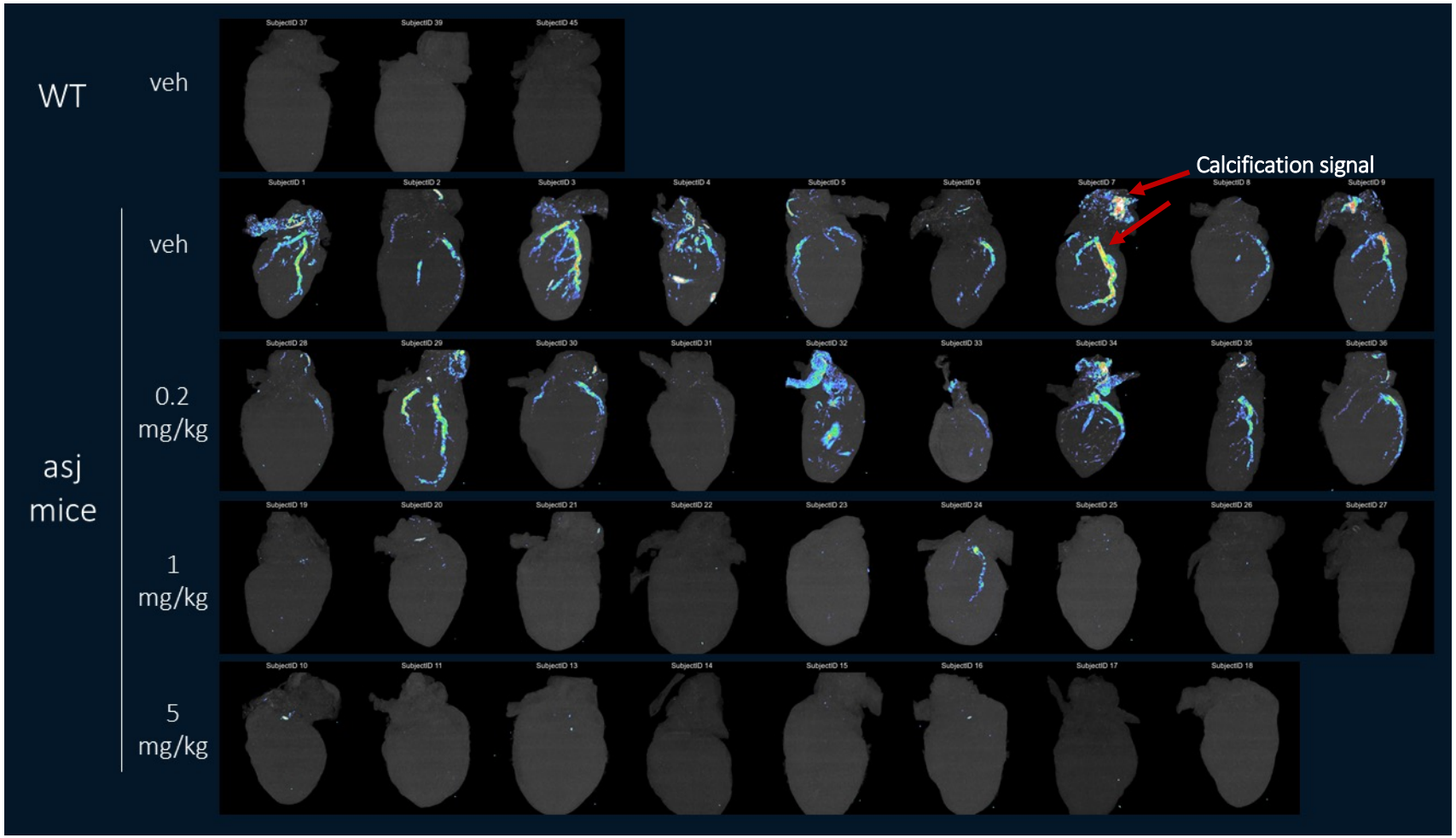


INZ-701



- **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)

INZ-701 Prevented Tissue Calcification In *Enpp1*$^{asj/asj}$ Mice



INZ-701 prevented pathological calcification in:

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

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Can INZ-701 Prevent Ectopic Mineralization in ABCC6 Deficiency?

INZ-701 efficacy study plan in *Abcc6* $^{-/-}$ mice, an animal model of PXE

Study	Genotype	Dose (s.c., q.o.d)	Terminal Point	Endpoint Readouts
Efficacy Study	WT	Vehicle	Day 56	• ENPP1 activity • PPi level • Vibrissae calcium • Histology
	Abcc6-/-	Vehicle	Day 56	
	Abcc6-/-	INZ-701, 2mg/kg,	Day 56	
	Abcc6-/-	INZ-701, 10mg/kg	Day 56	

- All animals on normal diet
- Duration: 8 weeks from ~5 wk of age to ~13 wk of age

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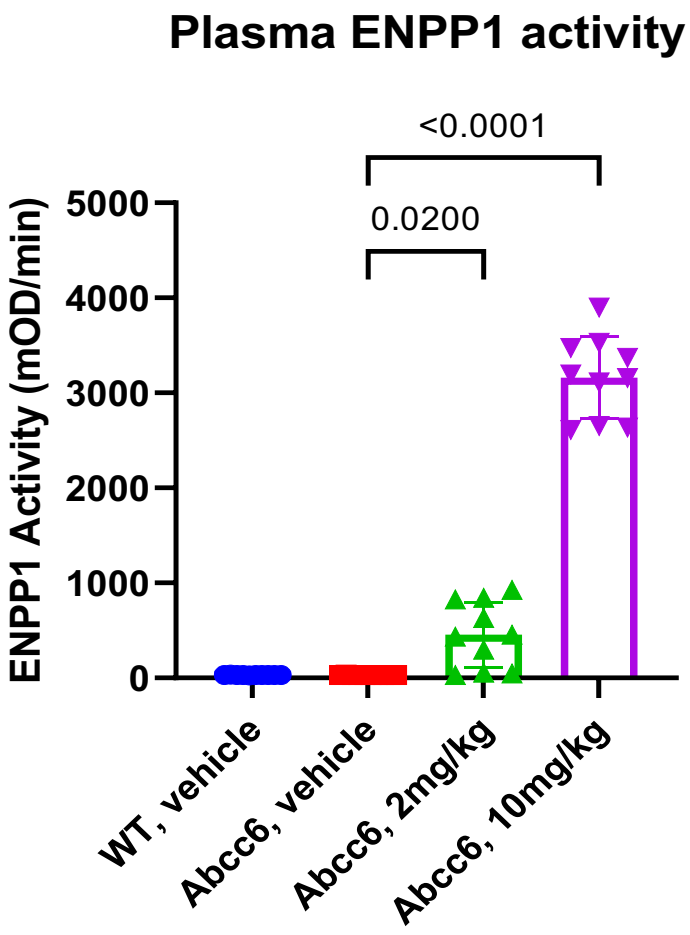
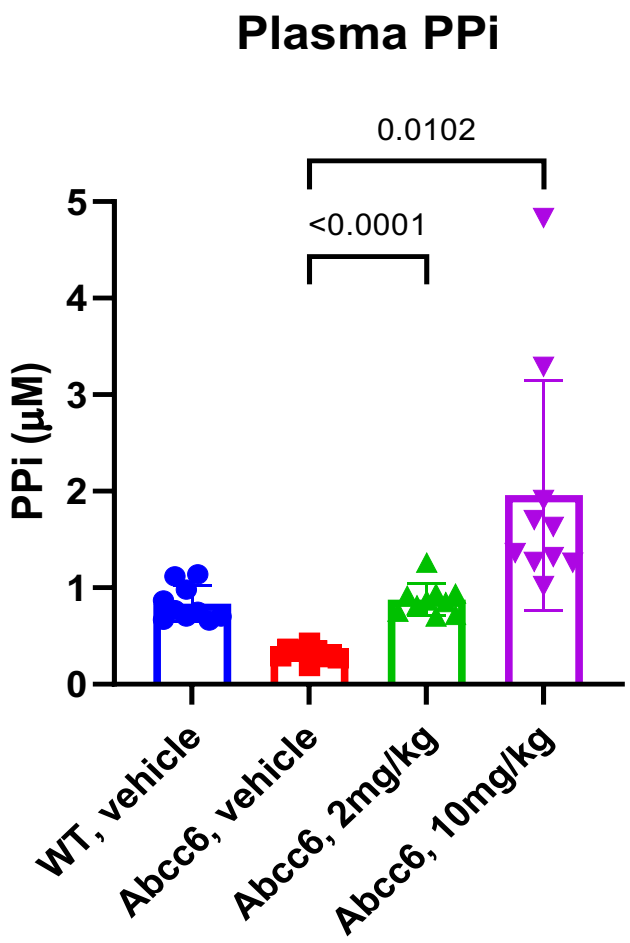
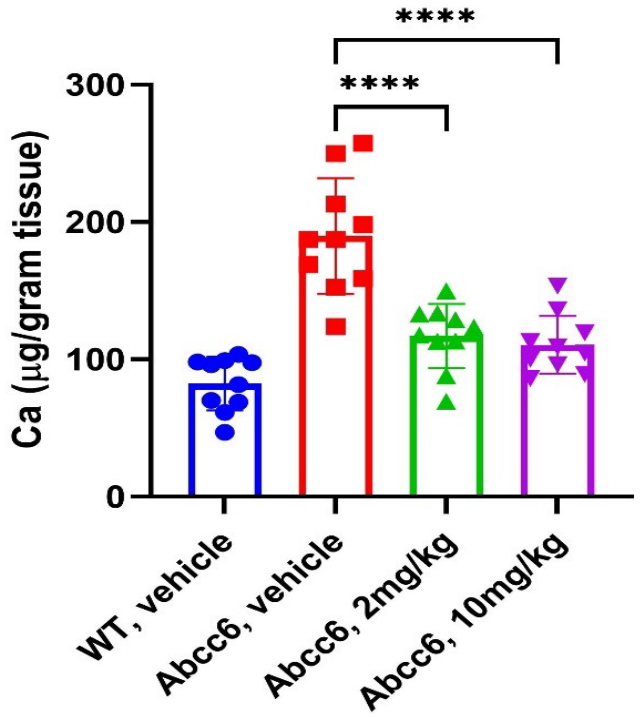
INZ-701 Increased Plasma PPi levels In *Abcc6* $^{-/-}$ Mice



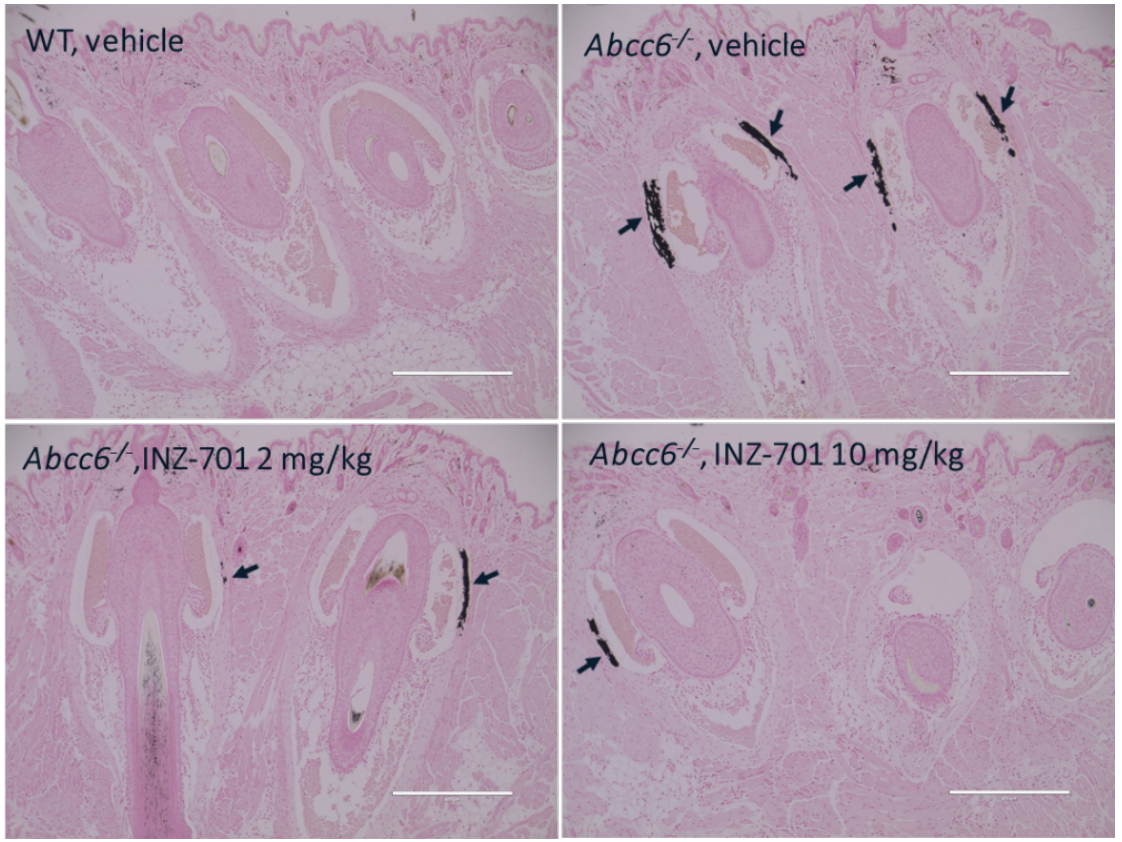


INZ-701 Prevented Tissue Calcification In *Abcc6* $^{-/-}$ Mice

Muzzle Skin Calcification



Von Kossa Stain of Muzzle Skin



Summary And Next Steps

- **ABCC6 deficiency and ENPP1 deficiency** share overlapping clinical manifestation, due to the same pathophysiology of hypopyrophosphatemia (low plasma PPi).
- **INZ-701, a hENPP1-Fc protein,** increased plasma pyrophosphate levels and prevented ectopic calcification in an Abcc6 deficient mouse model, demonstrating the potential of treating PXE patients.

Next steps:

- Long-term efficacy study in *Abcc6* $^{-/-}$ mice to investigate the effect of INZ-701 on other clinically relevant endpoints.
- Mechanism of Action study to understand ABCC6 function and calcification regulation.

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