Zinc finger mediated epigenetic repression of *SCN9A* gene as a therapeutic approach for painful peripheral neuropathies

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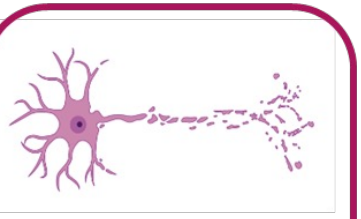
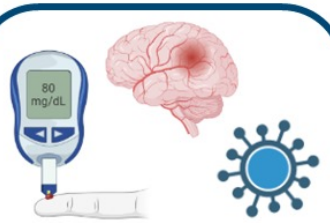
Disclosure

I am a full-time employee of Sangamo Therapeutics

Neuropathic pain is one of the most difficult pain syndromes to manage

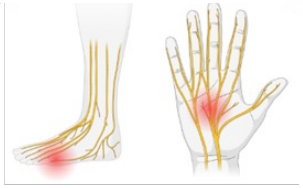


Damage or alterations to **sensory neurons**



Usually associated with **diabetes**, **stroke**, or **infection**

Large number of patients are affected globally



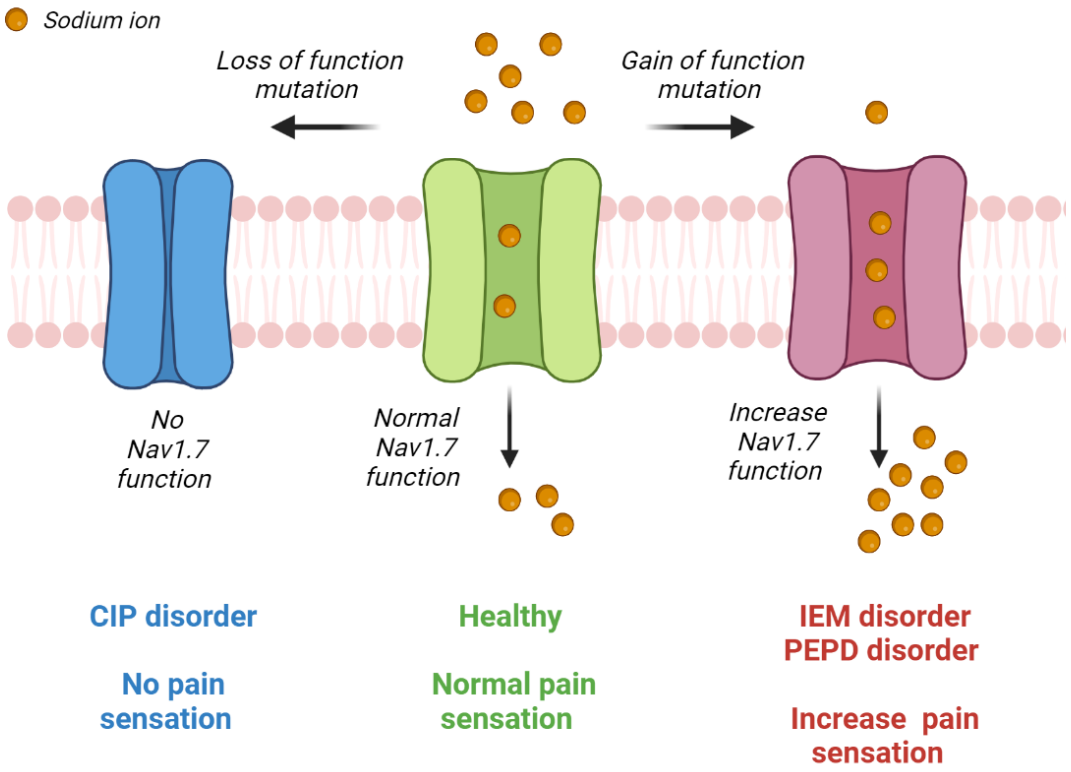
Manifests as burning and stabbing feeling in the **feet** and **hands**

Many patients are **refractory** to common pain medications

Given the high unmet need and lack of effective treatments, there is an urgent need to develop novel therapeutics for the treatment of chronic neuropathic pain

Images made with Biorender.com

Mutations in the *SCN9A* gene (Nav1.7) are linked to inherited pain disorders



- Nav1.7 is a voltage gated sodium channel expressed in the Dorsal Root Ganglion (DRG)
- Alterations in Nav1.7 activity directly regulate pain levels in several genetic disorders, validating Nav1.7 as a therapeutic target for pain
- Lowering Nav1.7 is expected to reduce pain without adversely affecting other sensory functions
- High structural similarities among Nav channels has made it challenging to develop Nav1.7 selective inhibitors

What is Sangamo solution for specifically targeting Nav1.7?

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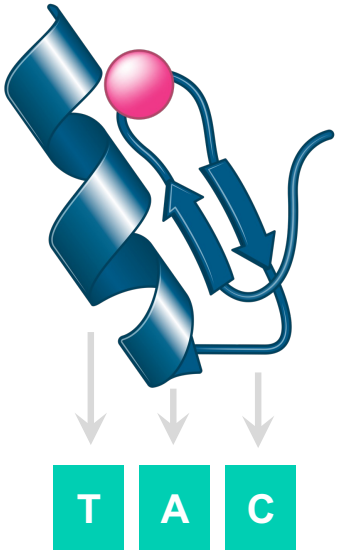
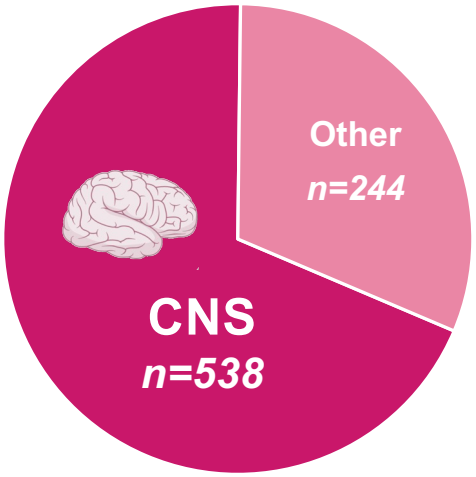
Images made with Biorender.com

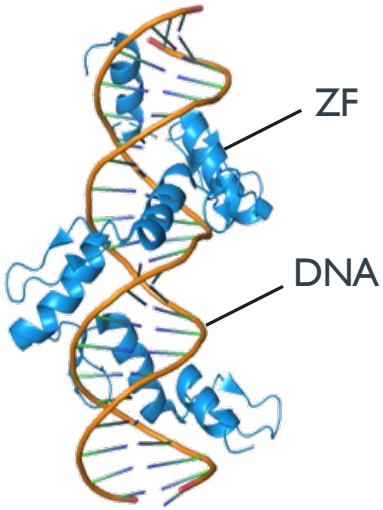
Zinc fingers (ZFs) are nature's solution for highly specific DNA binding



Zinc Fingers are
natural proteins
that bind DNA sequences
with high specificity



At least 782 human genes
encode for Zinc Fingers
Proteins



Most natural Zinc Finger
Proteins function to
regulate the epigenetic state
of other genes

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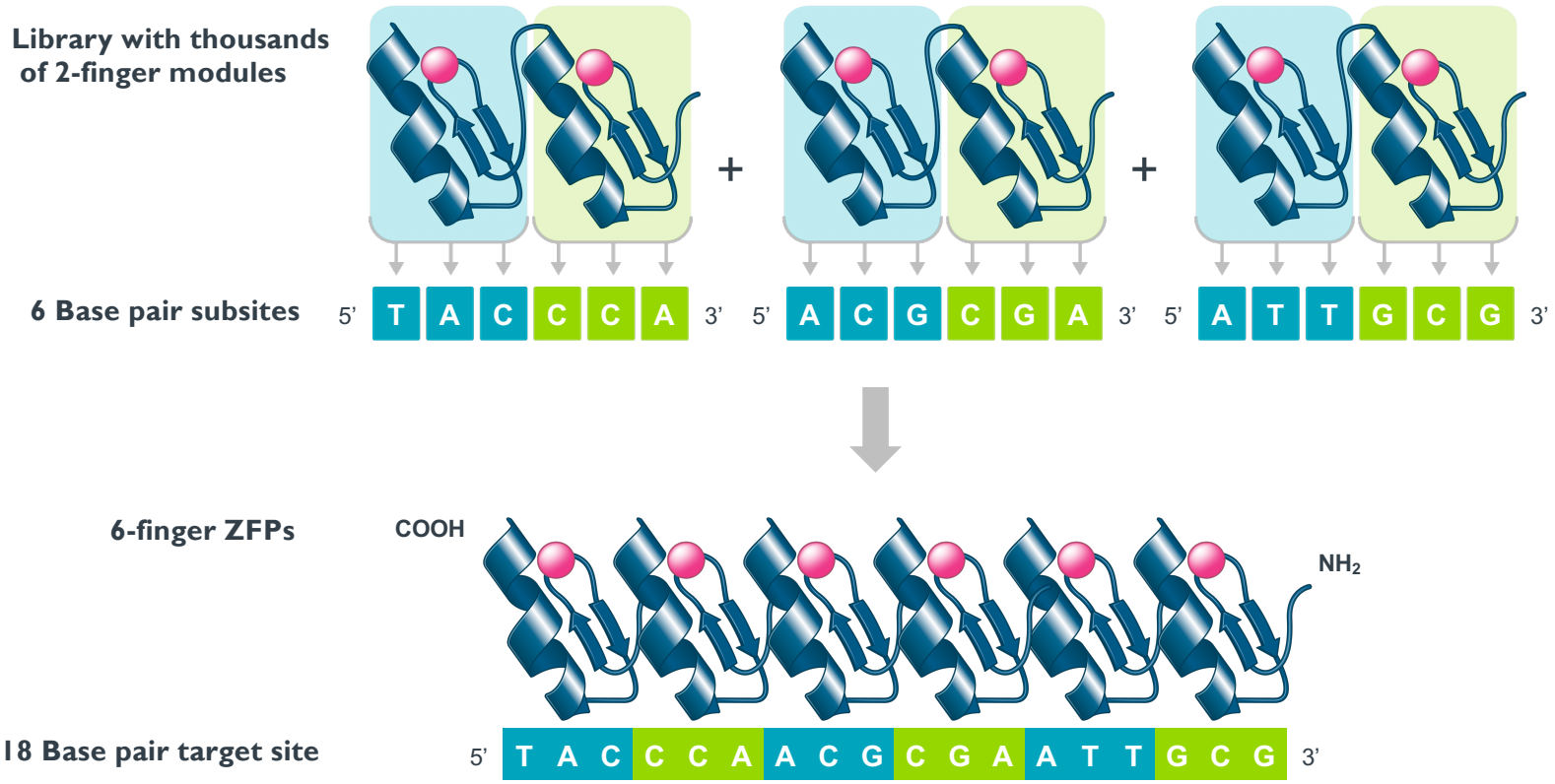
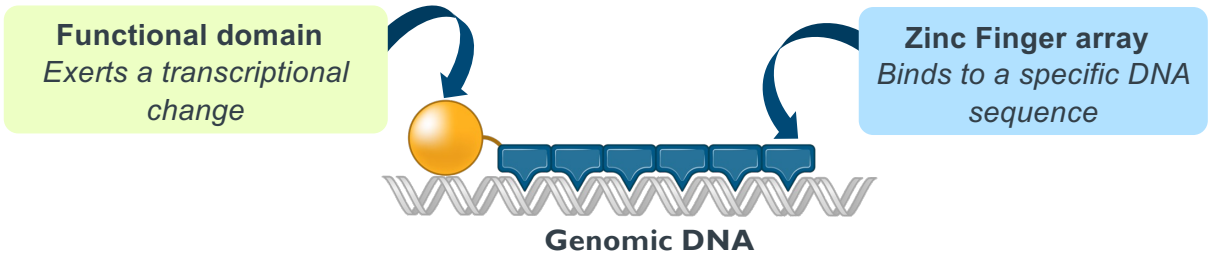
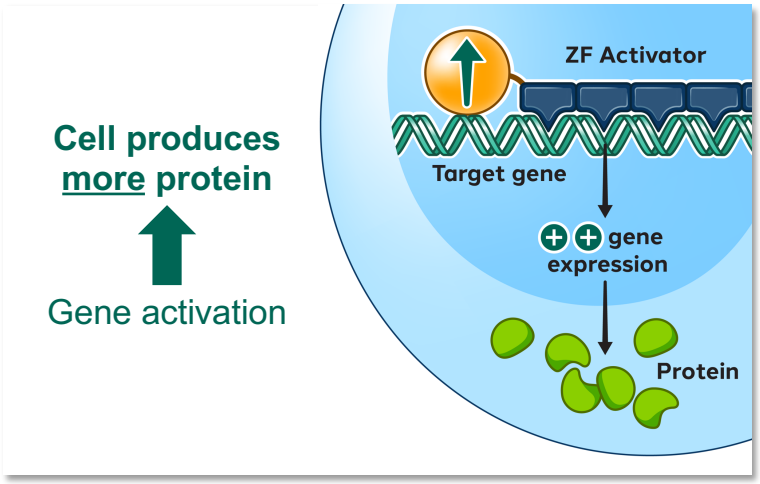
Zinc Finger proteins can be rapidly designed and engineered against any genomic sequence



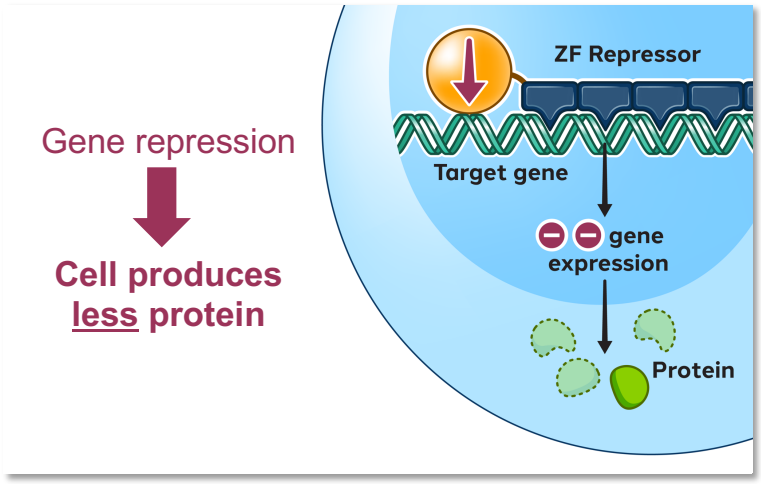
Epigenetic Regulation with Zinc Finger Activators and Repressors



Zinc Finger Activator (ZF-A)

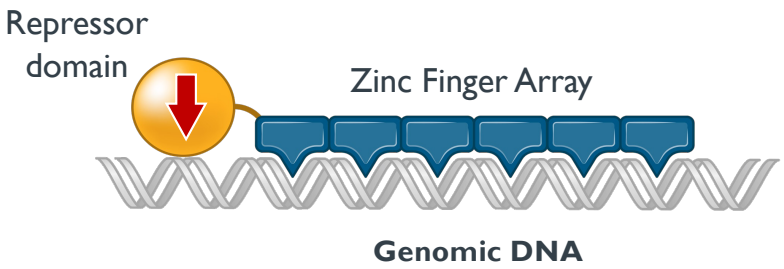


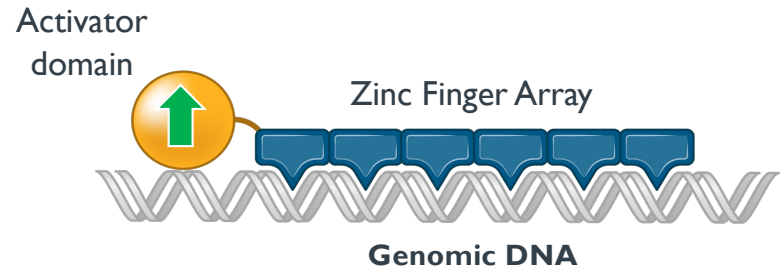
Zinc Finger Repressor (ZF-R)



Advantages of ZF based technology

- **Nuclease-free**
  ZF-transcription factors regulate gene expression without DNA breaks
- **Tunable**
  Achieve specified gene regulation level
- **Human origin**
  ZFP and the functional domains are derived from human genes
- **High potency**
  2 target sites per cell
- **Compact**
  Easily packaged into AAV or lentivirus
- **Multiplexing**
  Can combine several ZFPs for multiplex gene regulation





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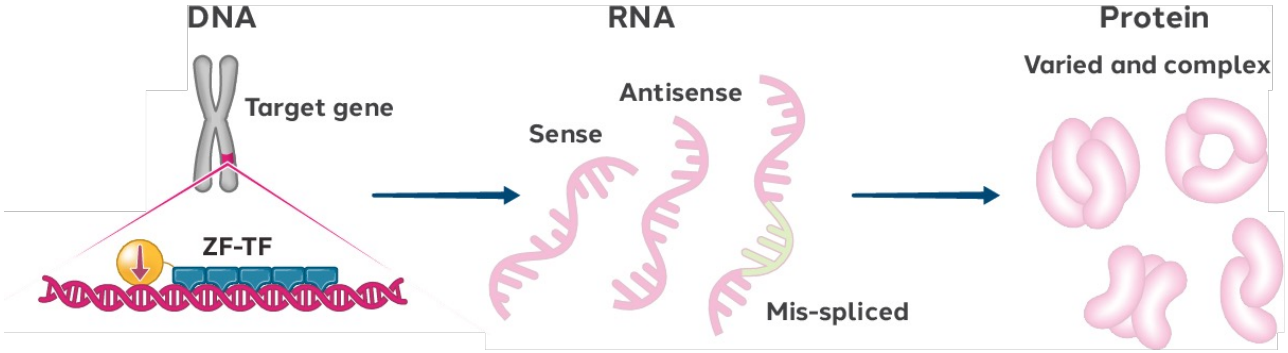
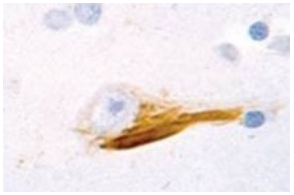
For neurodegenerative diseases, gene regulation with zinc fingers affords an opportunity to intervene upstream of complexities in RNA and protein biology



TAUOPATHIES



Tau

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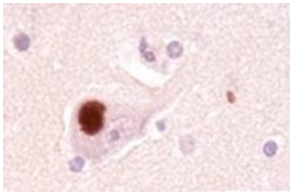
PARKINSON'S DISEASE



α-Synuclein

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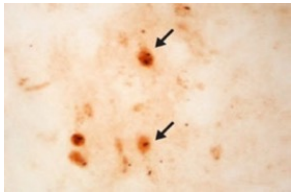
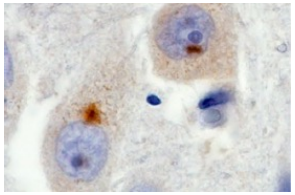
HUNTINGTON'S DISEASE



Huntingtin

Takeda

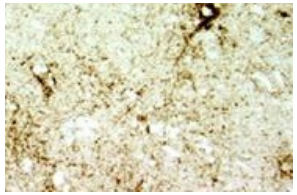
ALS



C9orf72

Pfizer

PRION DISEASE



Prion

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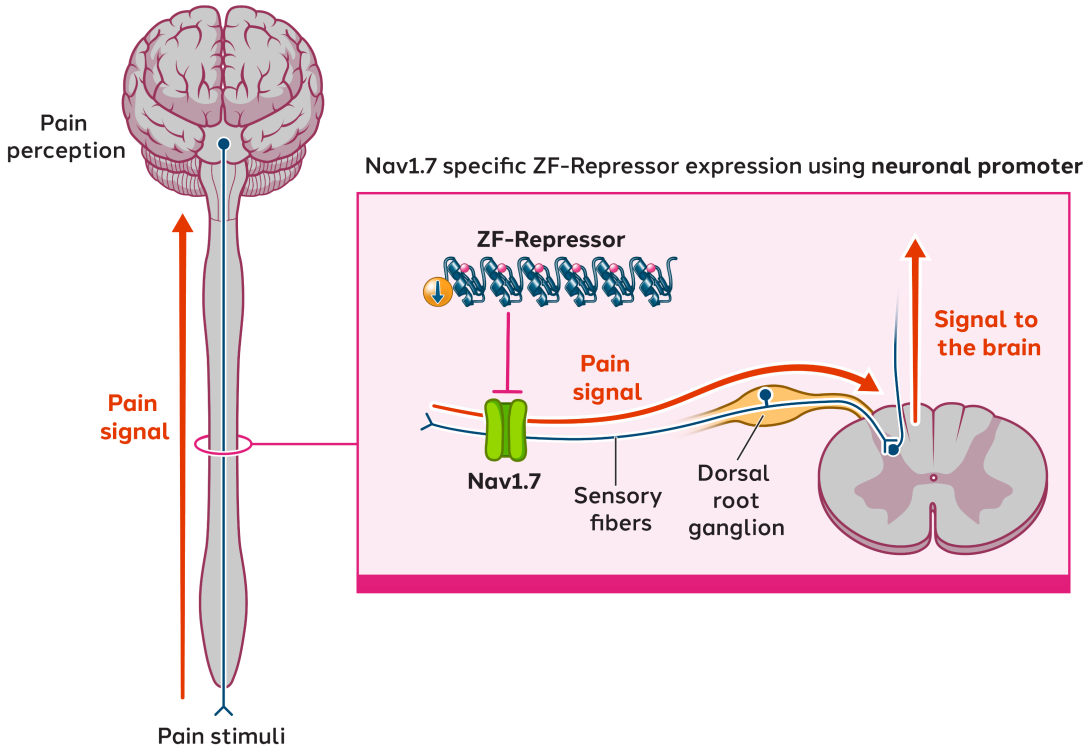
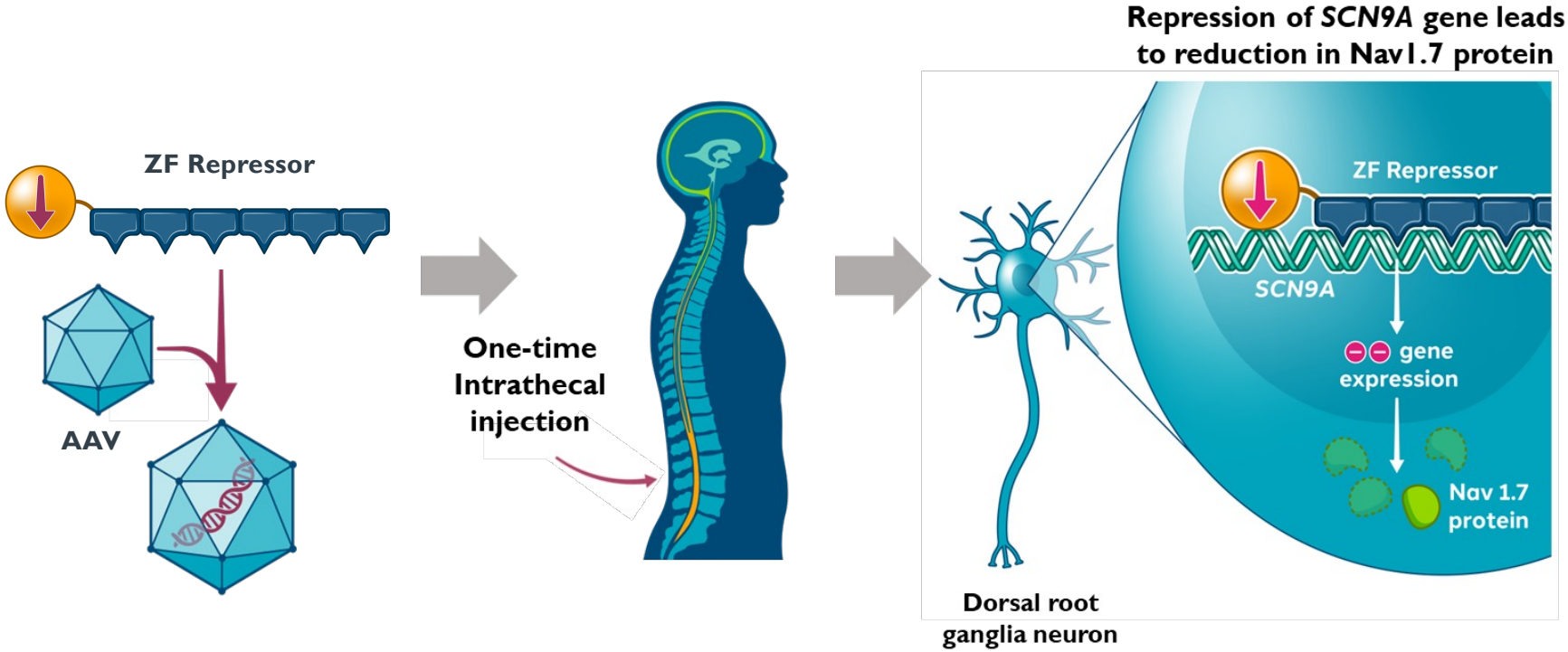
Hill et al., 2003 | Jucker & Walker 2013 | Irwin et al., 2015 | Waldvogel et al., 2014

ZF-R mediated repression of Nav1.7 at the DRG blocks pain transmission to the brain



- Blocking Nav1.7 in the DRG is expected to prevent the **transmission of nociceptive pain signals** to the brain
- This allows us to target **multiple neuropathic pain indications**, regardless of the cause of the pain
- Reducing pain by inhibiting Nav1.7 is not predicted to be associated with **any CNS adverse effects**

Zinc finger-mediated repression of Nav1.7 as a potent and specific therapeutic avenue for neuropathic pain



Developmental path to identify mouse and human ZF-repressors targeting the Nav1.7 gene

MOUSE ZF-Repressors (mZF-R)

Mouse Nav1.7 gene (*Scn9a*)

500+ 6-finger ZF-Rs designed & screened in neurons

Efficacy in pain mouse model

***In vivo* proof of concept**

HUMAN ZF-Repressors (hZF-R)

Human/NHP Nav1.7 gene (*SCN9A*)

600+ 6-finger ZF-Rs designed & screened in neurons

On-target engagement & safety in nonhuman primates (NHPs)

Clinical candidate

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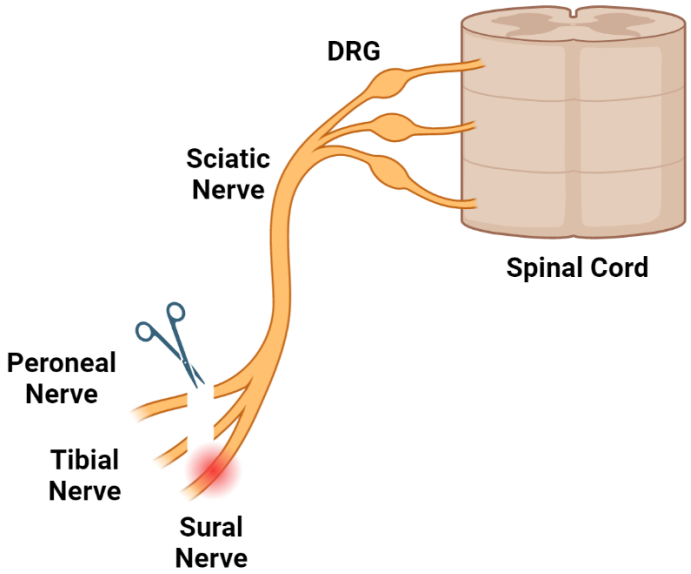
The efficacy of mZF-R was evaluated in the Spared Nerve Injury (SNI) neuropathic pain model

SNI model
C57BL/6
Mouse

- SNI is the most validated mouse neuropathic pain model ("Gold standard")
- Surgically induced hypersensitivity to pain



- **Mechanical** and **cold** induced pain were measured before (baseline) and 4 weeks after ZF-R treatment
- *Scn9a* repression in DRG was evaluated at the bulk and single-cell (nociceptor) level
- Gabapentin was used as a positive control and administered one hour before the pain measurements

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Images made with Biorender.com

Mouse specific mZF-R induced up to 70% bulk repression of Scn9a in DRGs

SNI model

mZF-R

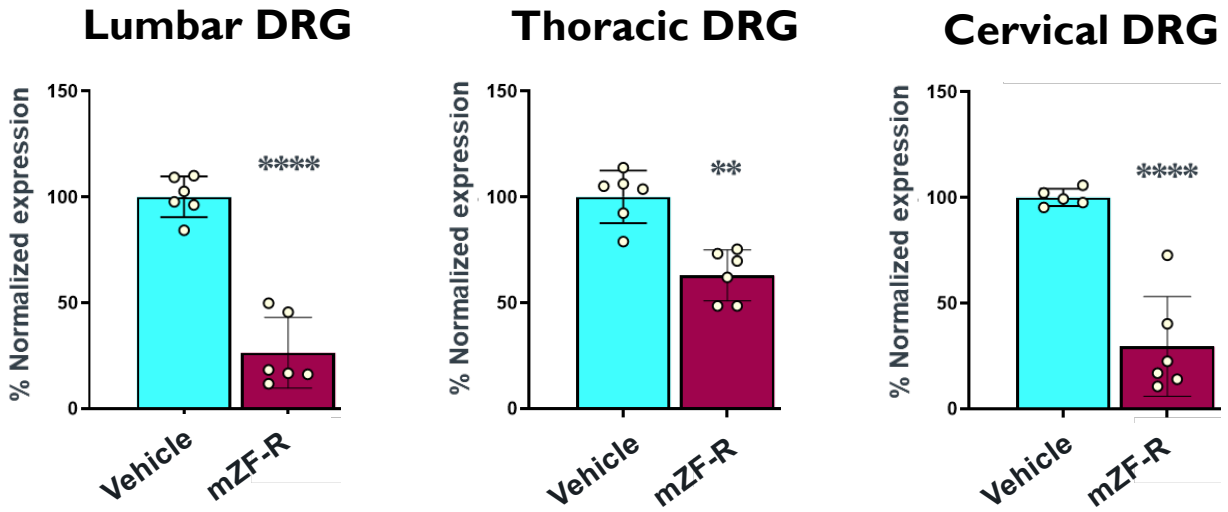
AAV

8E11 vg/mouse

Intrathecal-Lumbar injection

4 weeks

***Scn9a* (Nav1.7) expression (RT-qPCR)**



** P < 0.01 *** P < 0.001 **** P < 0.0001 Compared with Vehicle

One-way ANOVA

±SEM

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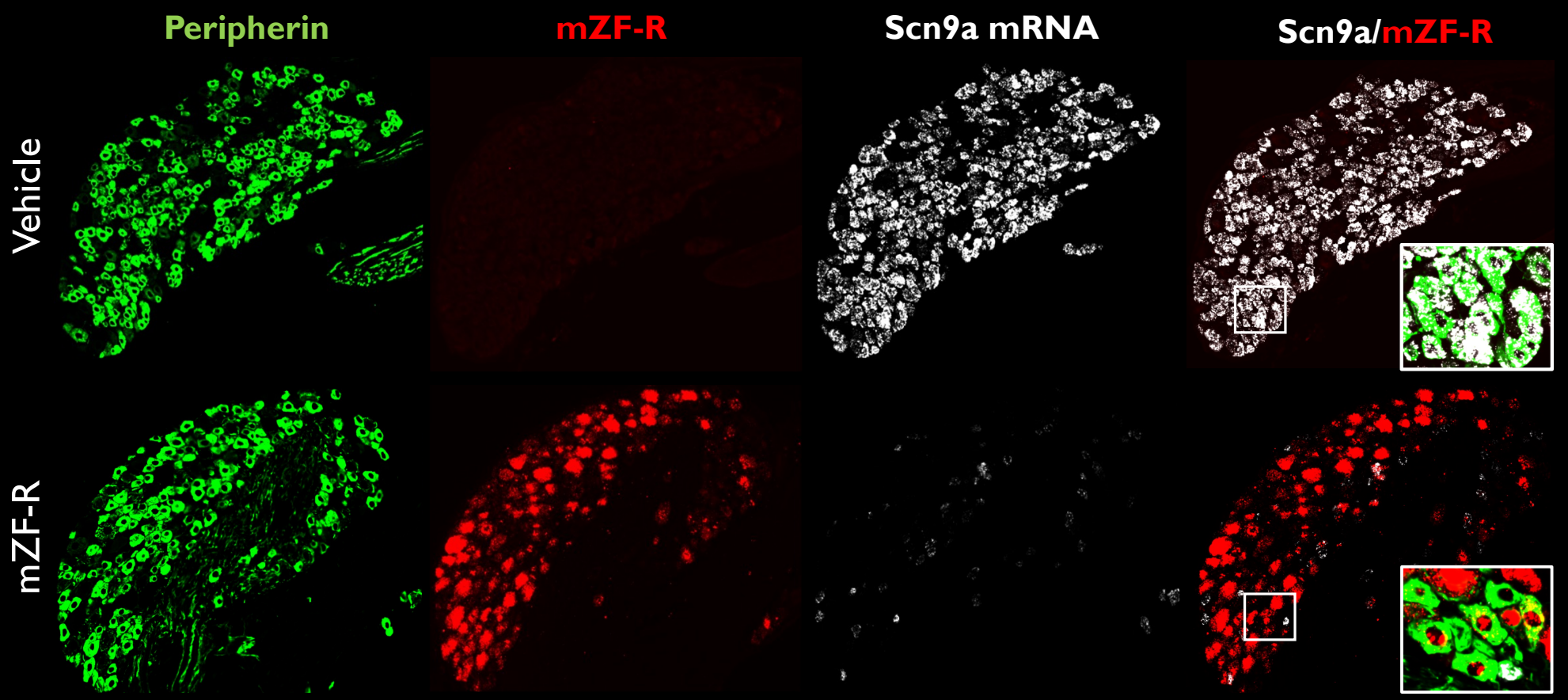
Potent repression of *Scn9a* mRNA in nociceptors of mouse lumbar DRG

SNI model



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No changes were observed in the Lumbar DRG for molecular markers of neuroinflammation or neuronal loss

SNI model

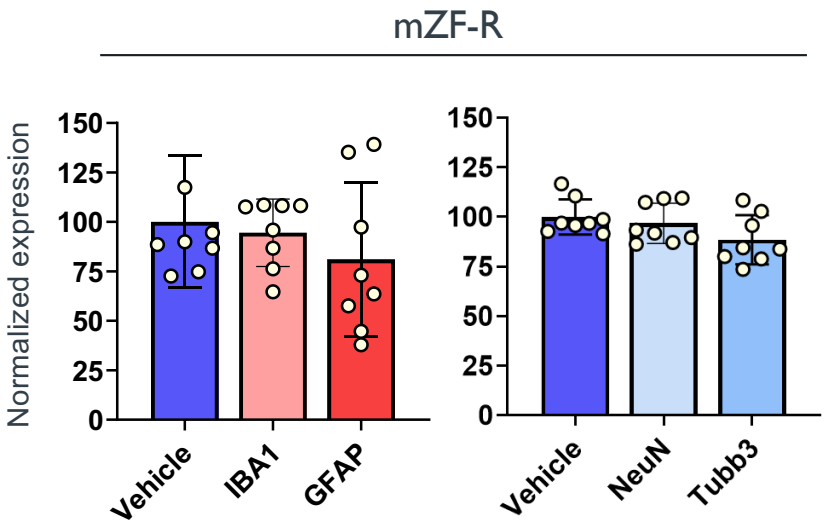
mZF-R

AAV

8E11 vg/mouse

Intrathecal-Lumbar injection

4 weeks

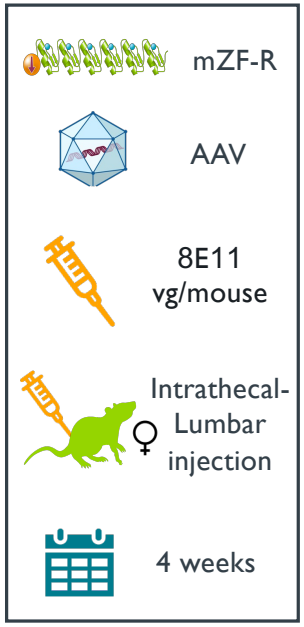


IBA1: marker for macroglia

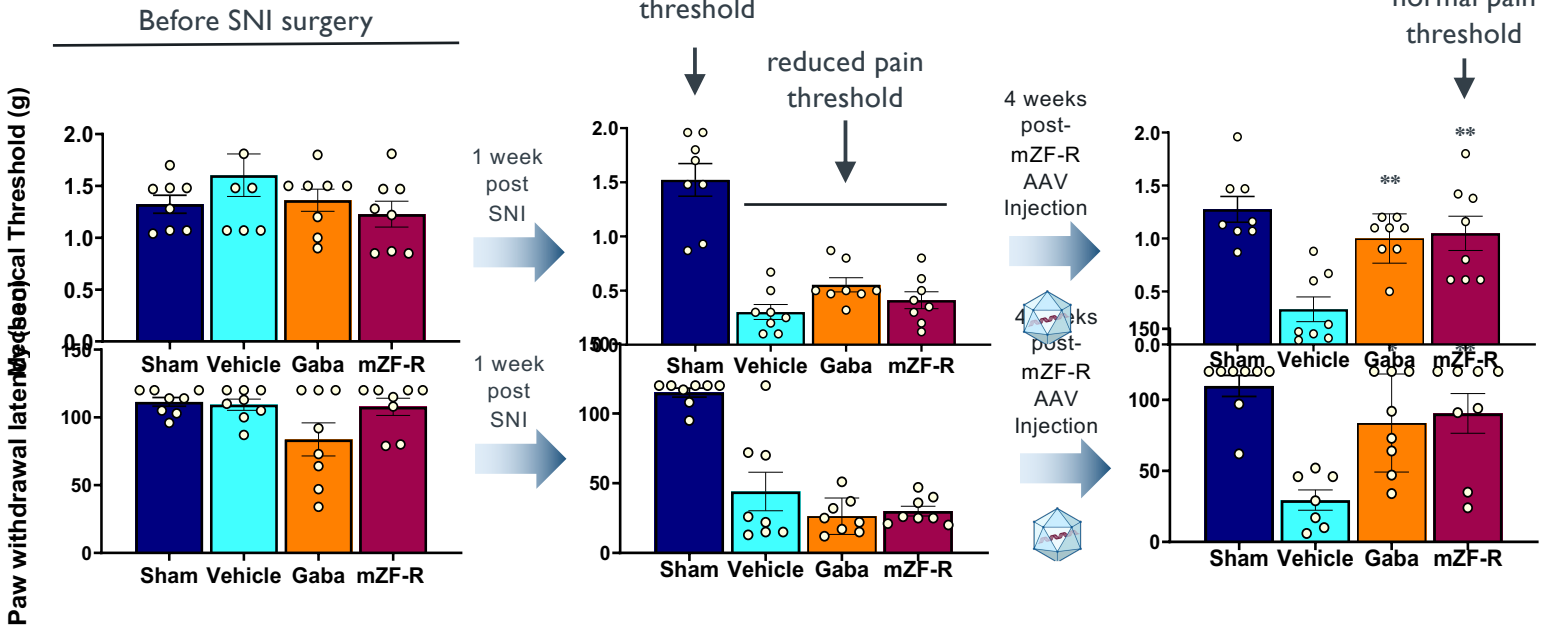
GFAP: marker for astrocytes

NeuN and Tubb3: Neuronal markers

In vivo repression of mouse *Scn9A* reverses pain hypersensitivity in a mouse model of neuropathic pain



Gabapentin was administered one hour before the measurement



mZF-R mediated pain efficacy is evident as early as day 3 post injection

SNI model

mZF-R

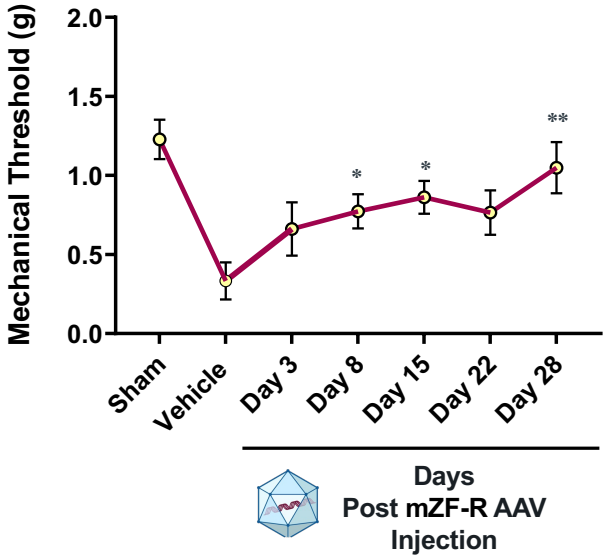
AAV

8E11 vg/mouse

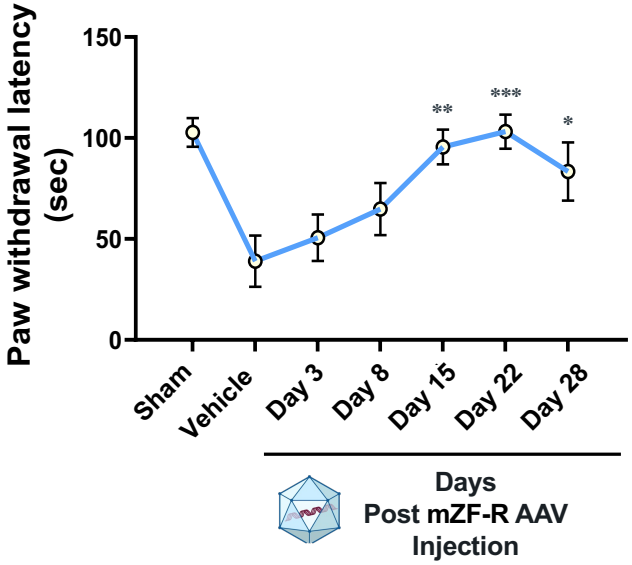
Intrathecal-Lumbar injection

4 weeks

Mechanical induced pain



Cold induced pian



*** P < 0.01 *** P < 0.001 **** P < 0.0001 Compared to Vehicle at each day*

One-way ANOVA
±SEM

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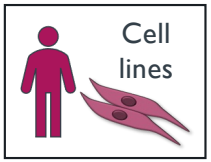
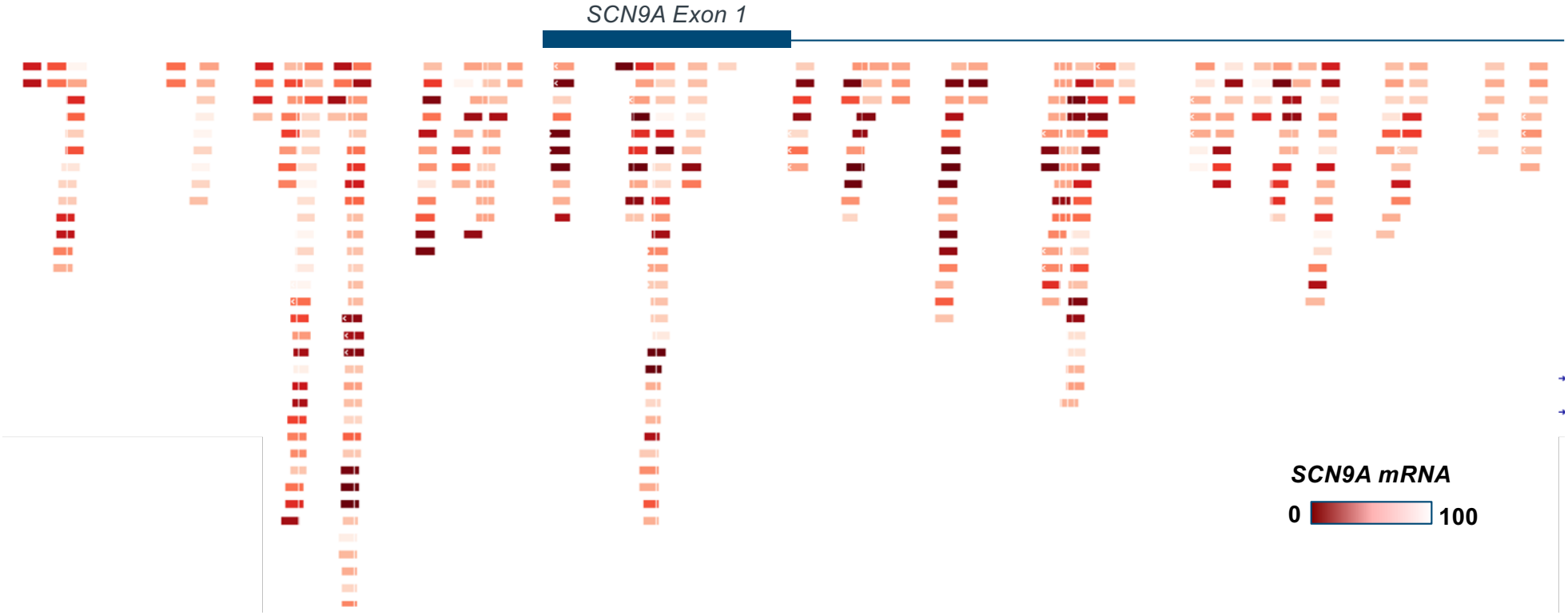
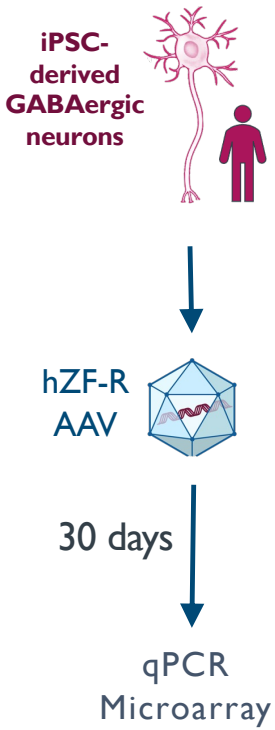
Rapid identification of potent ZF-Rs targeting human and nonhuman primates *SCN9A* gene

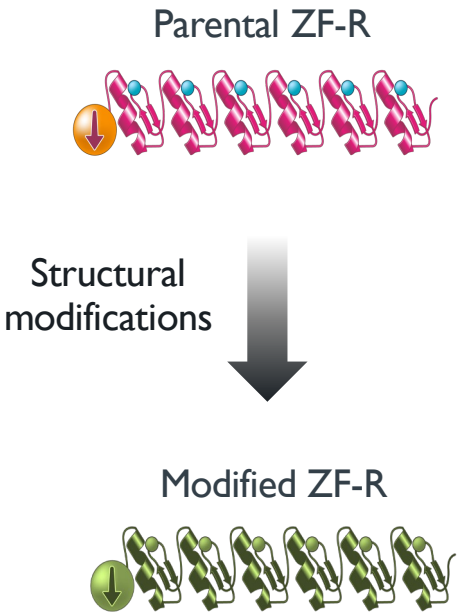


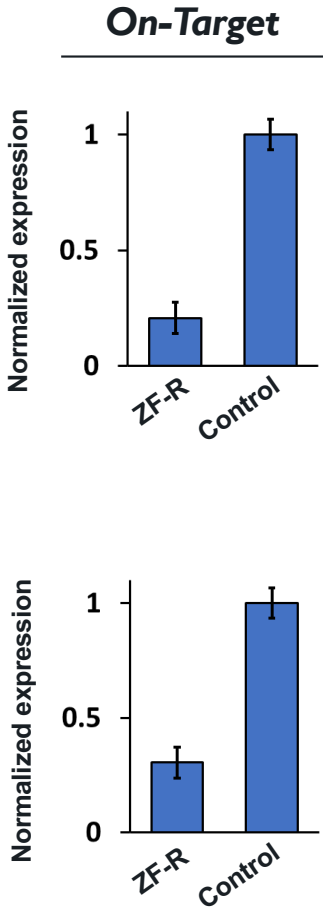


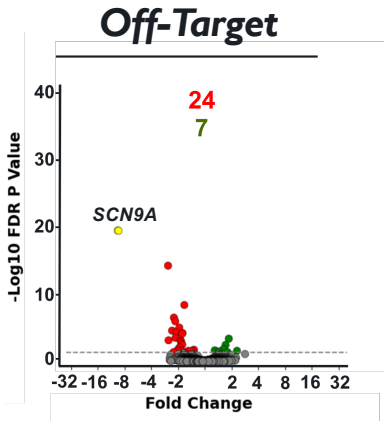
SKNMC cells harvested 24h after transfection (heat map, ZF-R dose 100ng mRNA);
RT-qPCR data normalized to mean of ATP5b, EIF4a2.

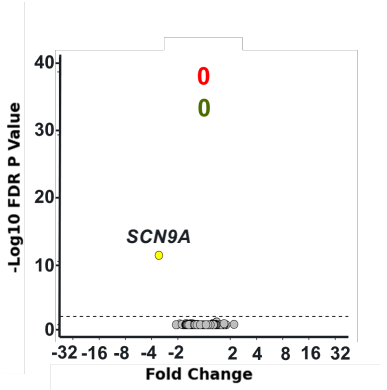
Structural modifications enable highly specific ZF-Rs











Repressed genes
Activated genes
FDR P <0.05

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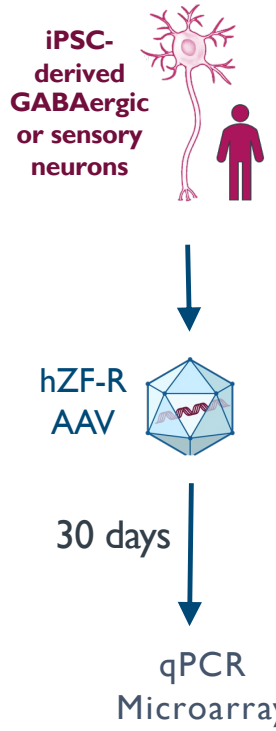
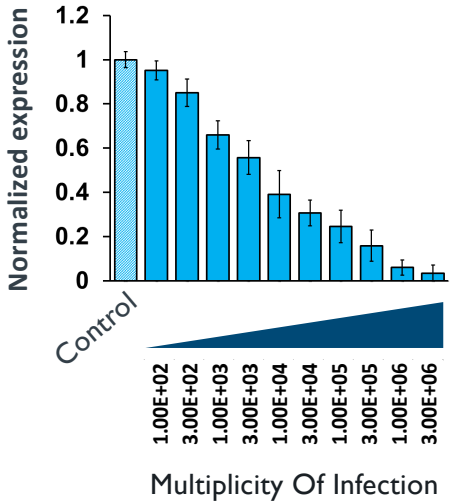
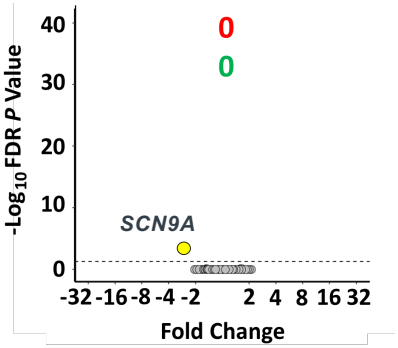
The human ZF-R candidate specifically repressed *SCN9A* by more than 90% over a wide dose range

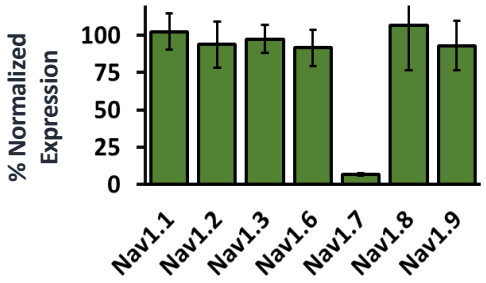


Potency



Specificity





- Affymetrix analysis of 20,000 genes showed presence of no off-target suggesting high selectivity of hZF-R
- No repression of any other Nav channels was observed suggesting high specificity of hZF-R

Specificity was analyzed at 1.00E+05 MOI

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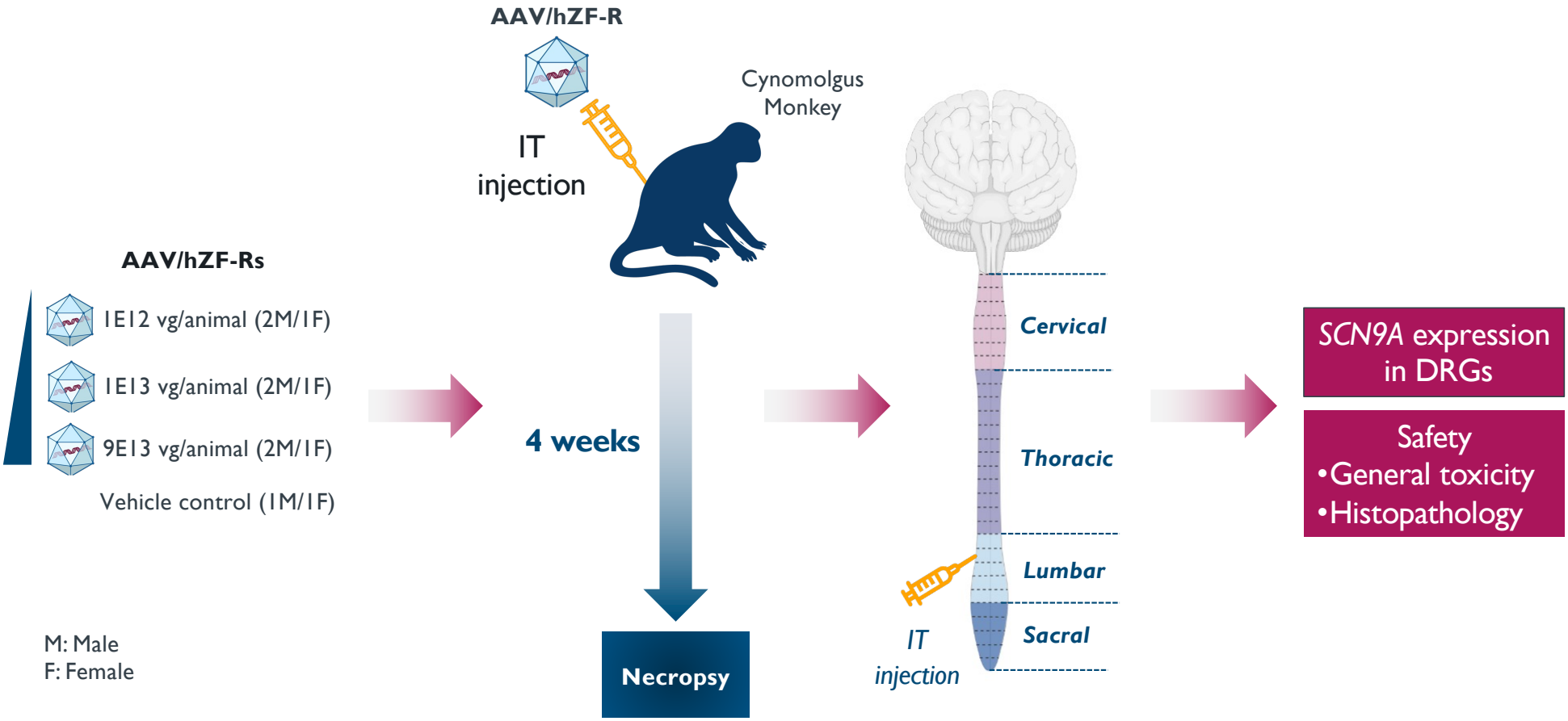
The potency and safety of the ZF-R candidate was evaluated in nonhuman primates



M: Male
F: Female

Images made with Biorender.com

Clinical candidate ZF-R repressed *SCN9A* by up to 40-60% at the bulk tissue level across a 100-fold dose range

NHP

hZF-R

AAV

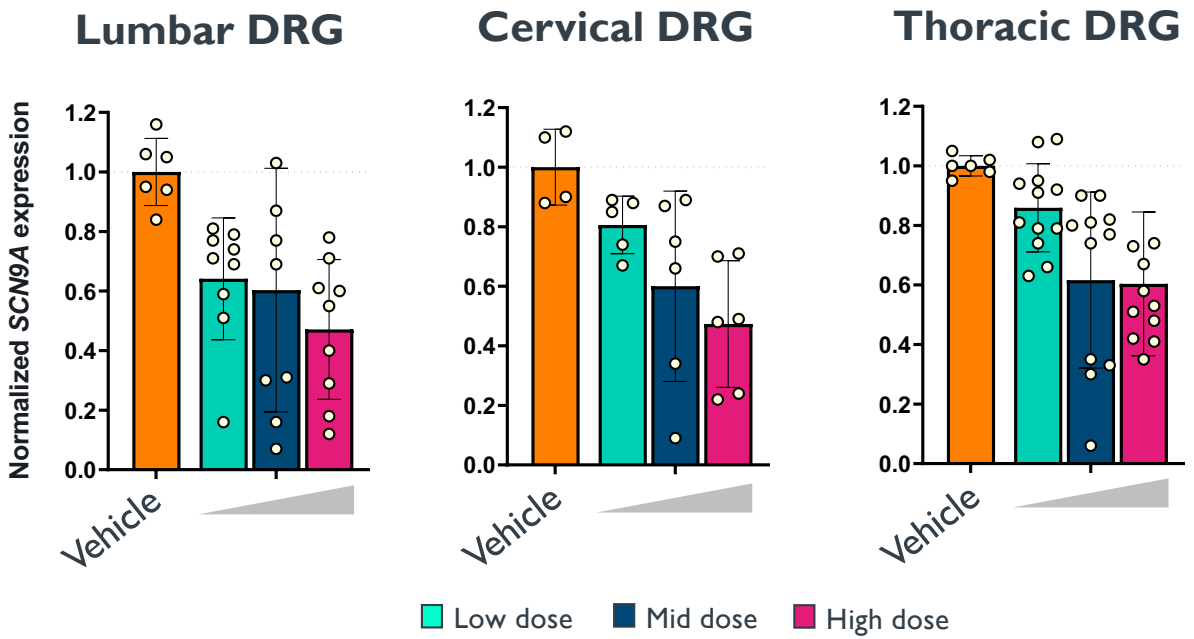
1E12 vg/NHP

1E13 vg/NHP

9E13 vg/NHP

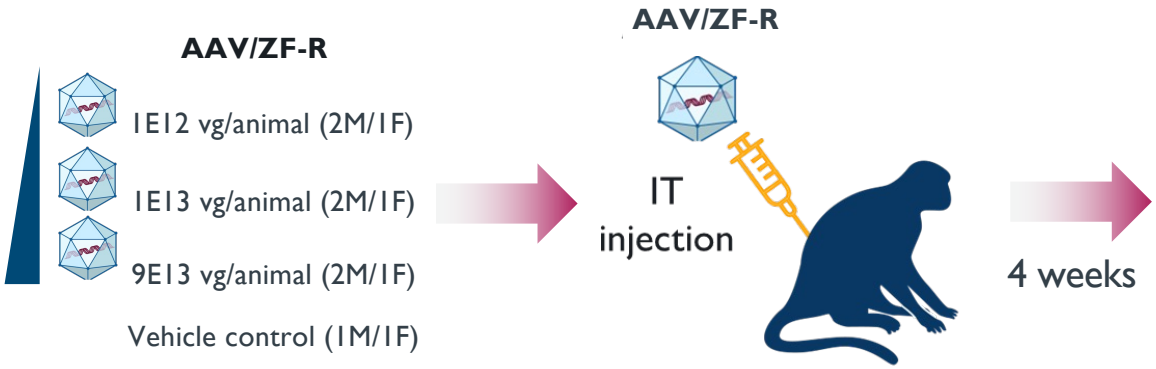
Intrathecal-Lumbar injection

4 weeks



- Multiple DRGs were evaluated for each level per animal

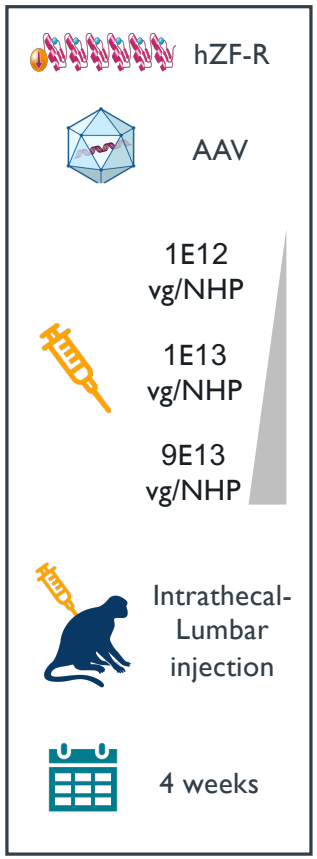
Clinical candidate ZF-R was well tolerated in nonhuman primates at all doses



- No Mortality/Morbidity
- No clinical signs
- No change in body weights
- No clinical pathology including:
  - Hematology
  - Clinical Chemistry (including liver panel)
  - Coagulation
- No necropsy observations
- No change in organ weights

Major Internal Organs exhibited normal pathology

NHP



Tissues evaluated:

- Brain
- Adrenal Gland
- Epididymis
- Heart
- Kidney
- Intestine Large (Jejunum)
- Intestine Small (Duodenum)
- Liver
- Lung
- Lymph Node (mandibular)
- Ovary
- Pancreas
- Skeletal Muscle
- Testes
- Thymus
- Uterus/cervices
- Spleen
- Stomach

Current Findings

Scoring system used by the pathologist

Grade	Percent of tissue affected
Normal	0
Minimal	<5%
Mild	5-20%
Moderate	20-40%
Marked	>50%

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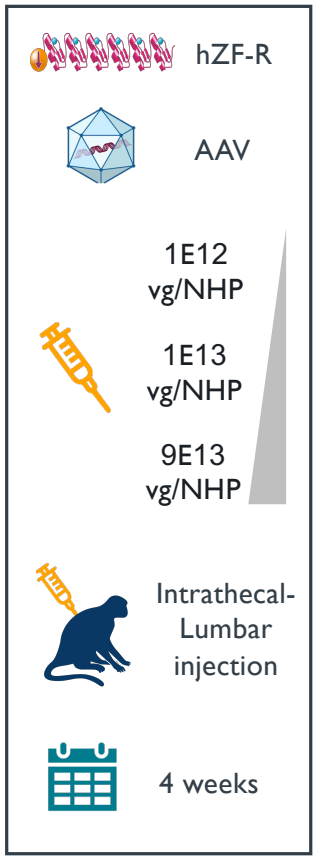
Minimal pathology findings were observed mainly in peripheral nervous system

NHP



- Tissues affected:
  - DRGs, spinal cord, sciatic nerve, and trigeminal ganglion
- Types of findings:
  - **Minimal** Mononuclear Cell Infiltration (MN)
  - **Minimal** Axonal Degeneration (AD)
  - **Minimal** Single Neuronal Degeneration (SDN)

Current Findings →

Scoring system used by the pathologist

Grade	Percent of tissue affected
Normal	0
Minimal	<5%
Mild	5-20%
Moderate	20-40%
Marked	>50%

A score below **moderate** in the DRG and associated tissues is not considered dose-limiting for AAV gene therapy

These results supported the initiation of IND-enabling GLP Toxicology study

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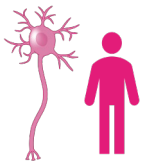
Conclusion

- Mouse ZF-R potently repressed *Scn9a* at the bulk and single-cell level in mouse DRG
- IT-L administered ZF-Rs reversed pain hypersensitivity in the SNI model of neuropathic pain



- Human ZF-R potently repressed *SCN9A* >90% in human iPSC-derived neurons
- ZF-Rs were highly specific with no off-target activity detected, including no repression of any other Nav channels

- Human ZF-R repressed *SCN9A* by up to 40-60% at all DRG levels in NHP
- ZF-Rs were well tolerated at all doses tested with no adverse findings
- These results support the continued progression to IND-enabling nonhuman primate study

Sangamo THERAPEUTICS

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AfaSci
(Mouse studies)
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Thank you