Preclinical Development of a Zinc Finger Transcriptional Repressor Targeting the *SCN9A* Gene as a Novel Therapy for Peripheral Neuropathic Pain

Toufan Parman, PhD, DABT
Senior Director, Nonclinical Safety Evaluation
Sangamo Therapeutics

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Disclosure

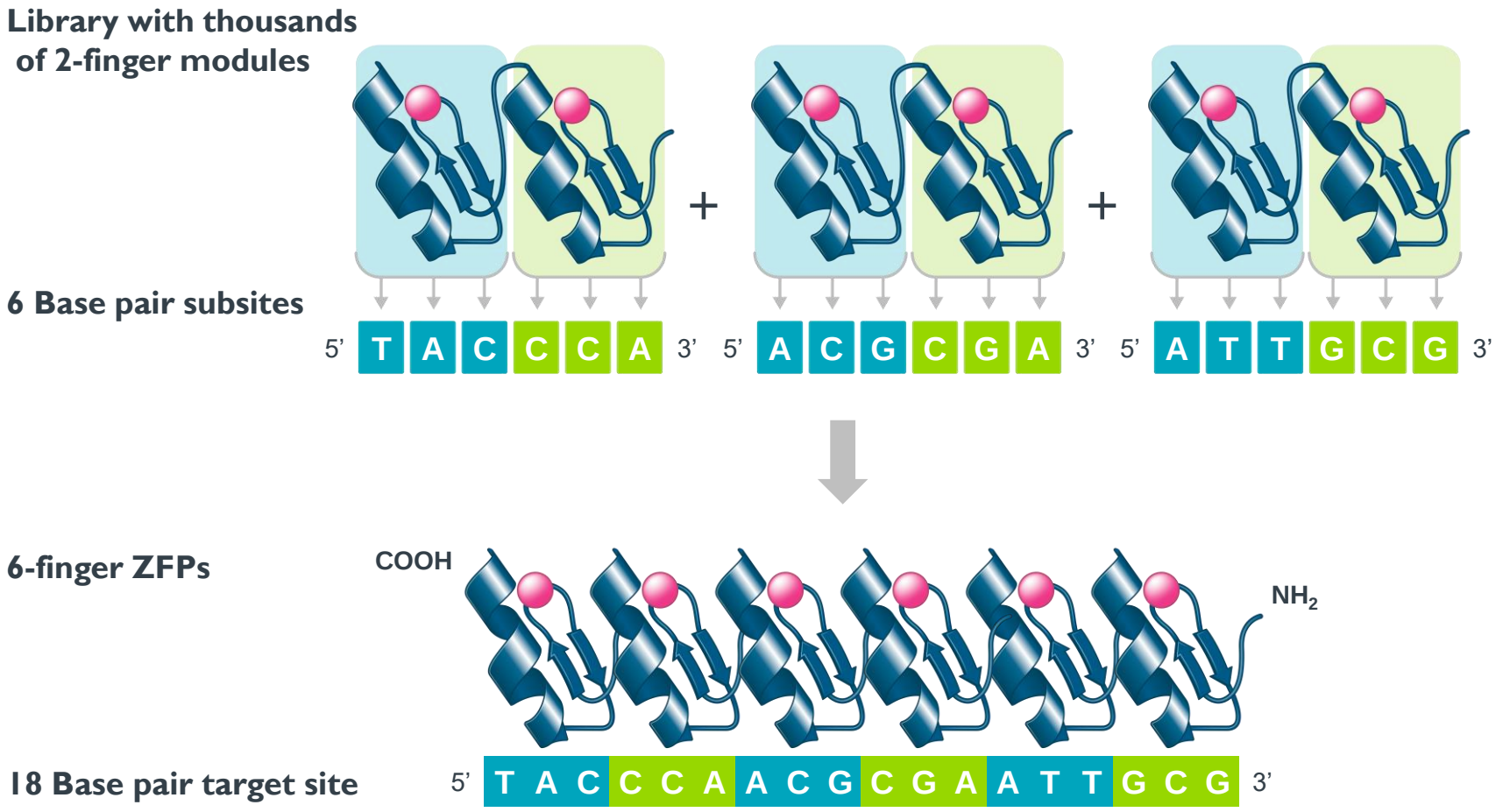
Sangamo is a genomic medicine company that aims to transform patients' lives by replacing today's symptomatic treatments with tomorrow's genomic cures. We are working with urgency to create new medicines and new hope for patients.

I am a full-time employee of Sangamo Therapeutics, Inc.

Zinc Finger Proteins

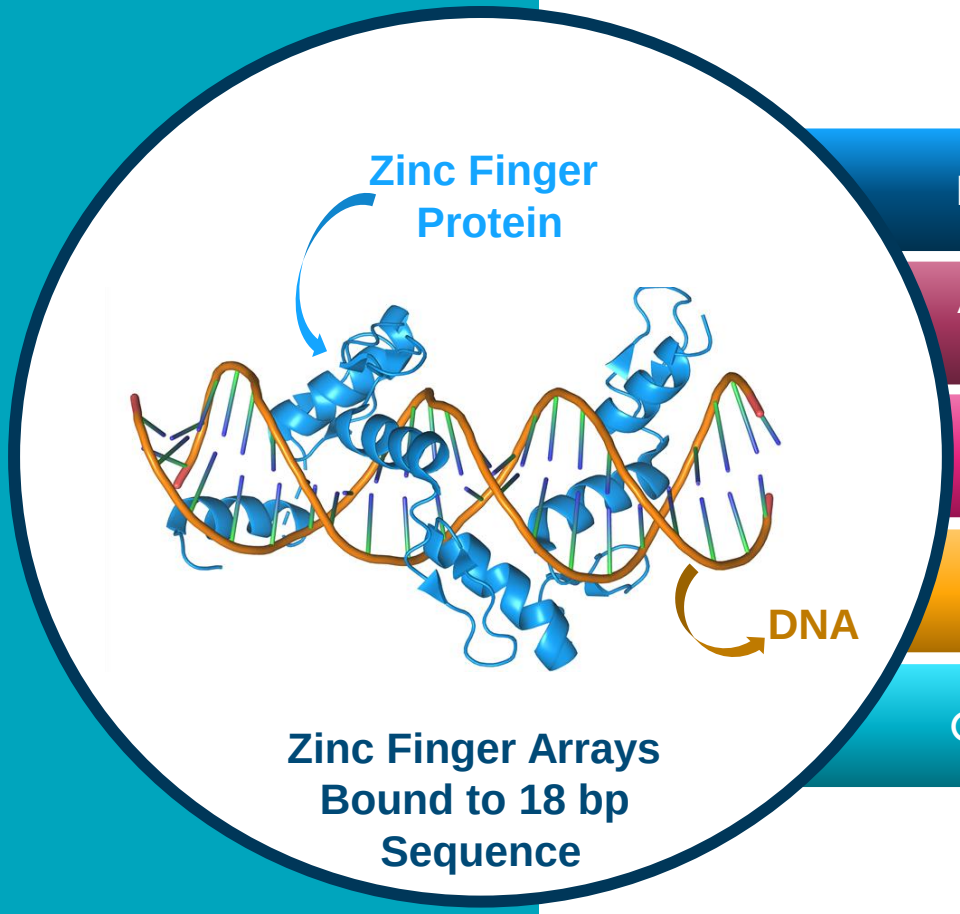
What are Zinc Finger Proteins (ZFPs)

- They are natural proteins that bind DNA sequences
- They are nature's choice for highly specific DNA binding
- The most natural function of ZFPs is to regulate the epigenetic state of other genes



Zinc Finger Platform Key Features

Zinc Finger based epigenetic regulation is the best-in-class approach for safe and effective genomic medicines



- Highly potent, exquisitely specific, and optimizable for any target
- All components are derived from naturally occurring human genes
- Tunable expression with no modifications or edits to the genome
- Requires binding just two DNA targets per cell to regulate RNA and protein levels
- Compact: Easily packaged into AAV, can be multiplexed

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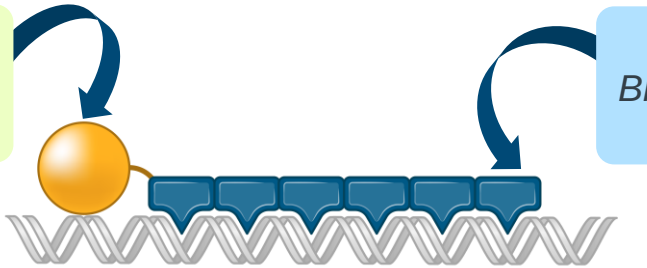
Epigenetic Regulation with Zinc Finger Activators and Repressors

Functional domain
Exerts a transcriptional change



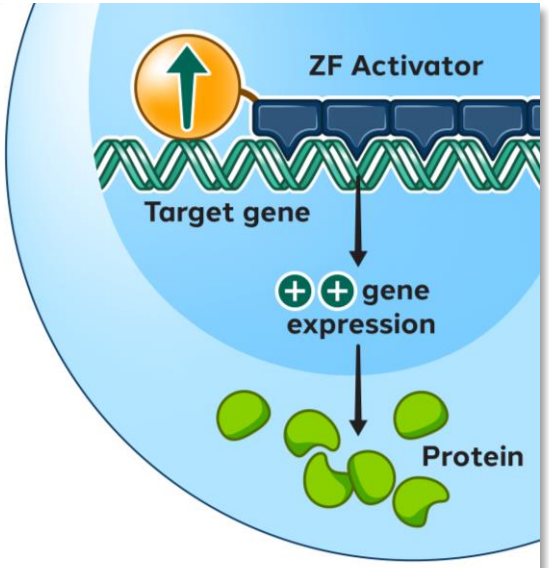
Zinc Finger array
Binds to a specific DNA sequence

Zinc Finger Activator (ZF-A)

Trans-activation domain

Cell produces more protein

Gene activation

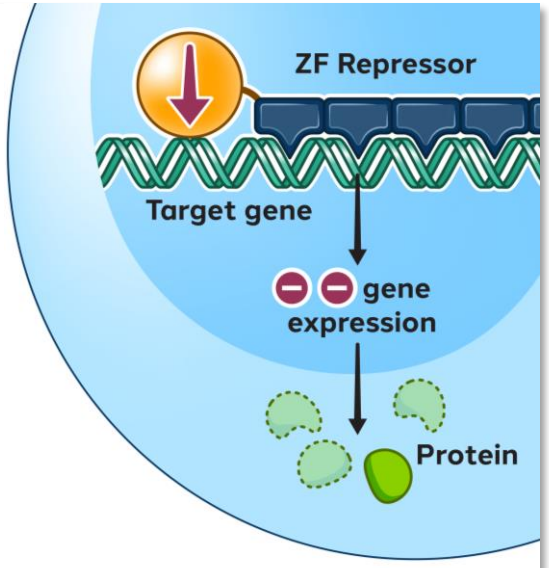


Zinc Finger Repressor (ZF-R)

KRAB (Krüppel Associated Box) transcription repression domain

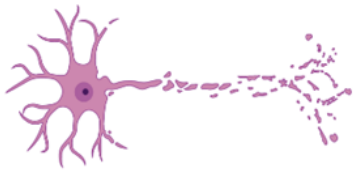
Gene repression

Cell produces less protein

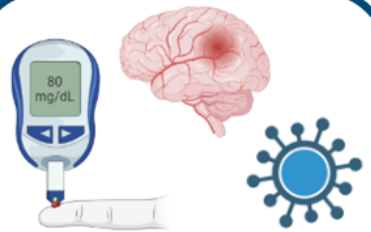


Neuropathic Pain and Role of Nav1.7

Neuropathic Pain: A Debilitating Condition with a High Unmet Need

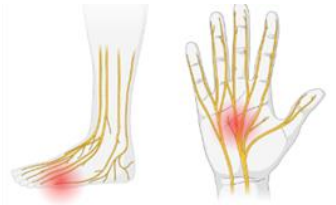


Damage or alterations to **sensory neurons**



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

Large number of patients are affected globally

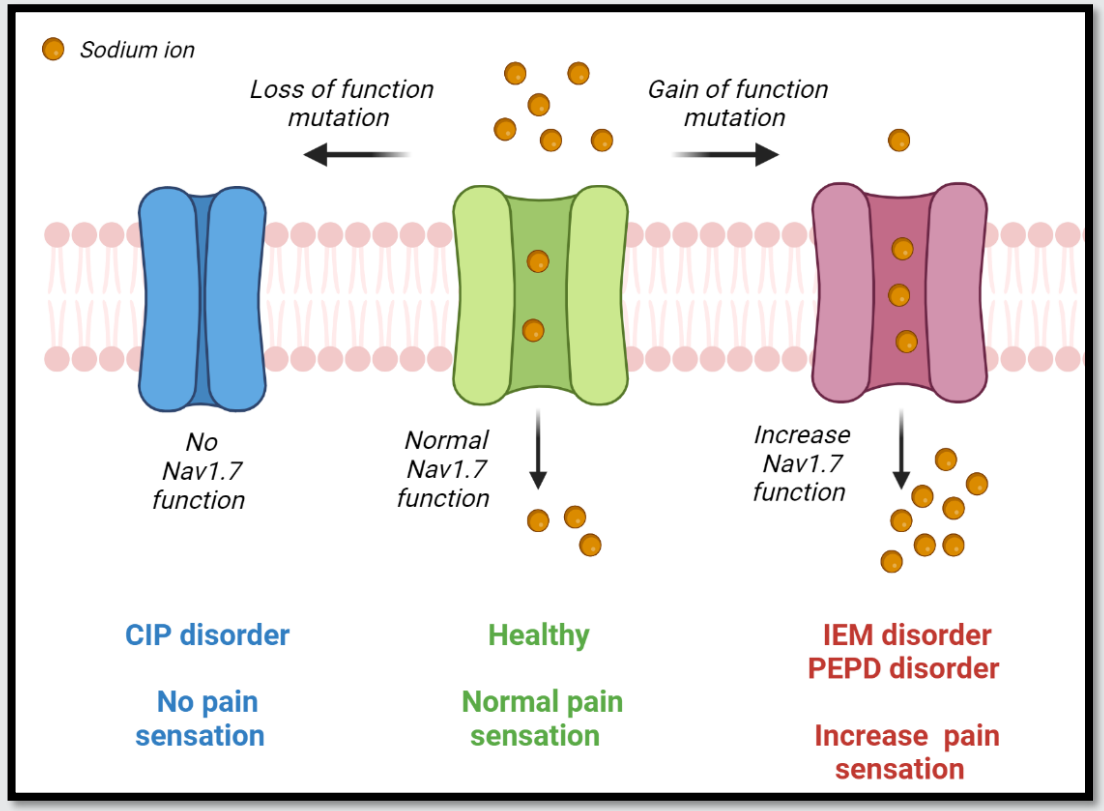


Burning and stabbing feeling in the **feet** and **hands**

Many patients are **refractory** to common Treatments

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

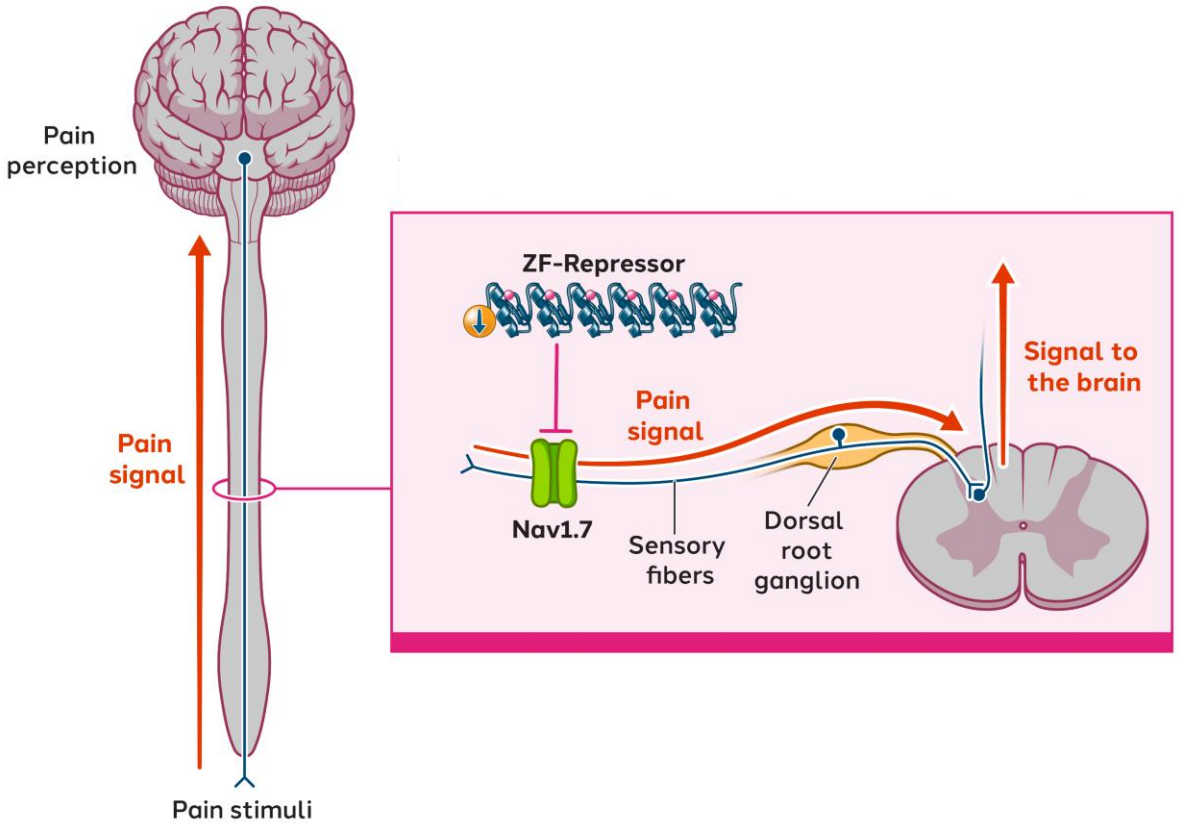
Role of *SCN9A* (Nav1.7) Gene in Inherited Pain Disorders



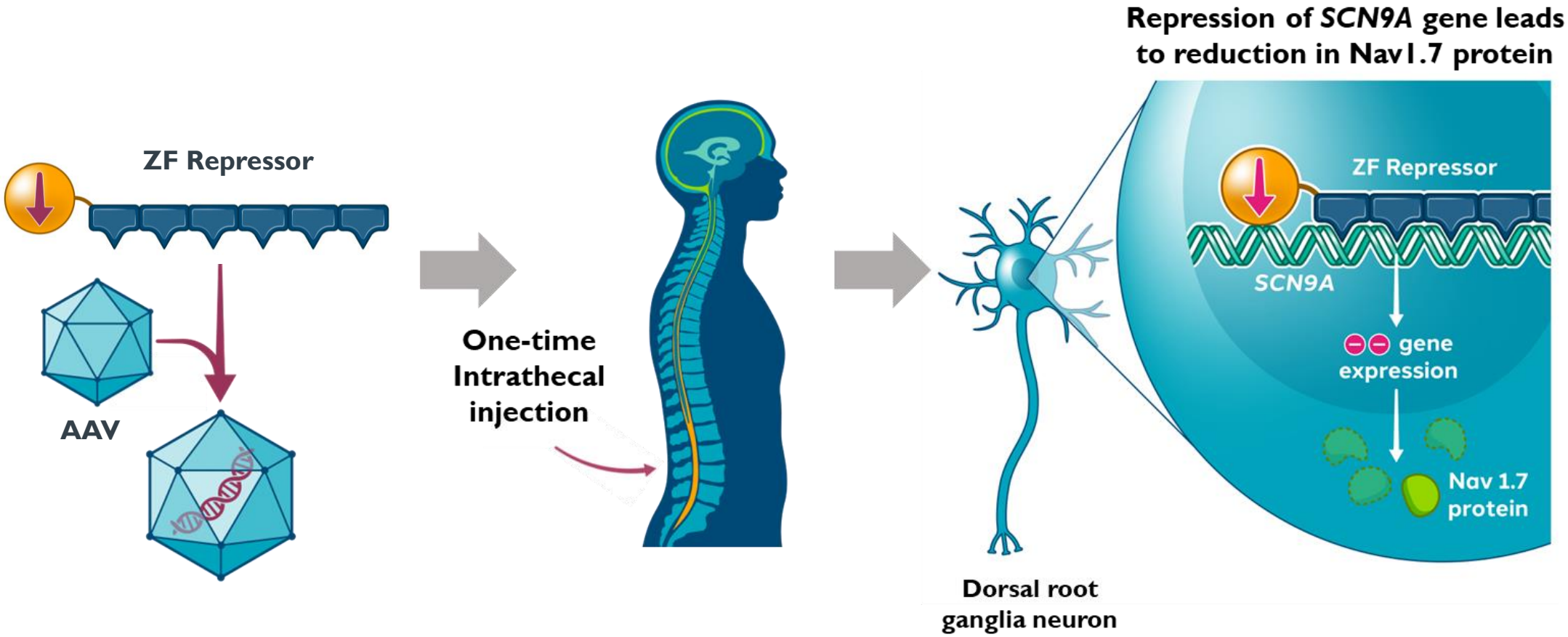
- Nav1.7 is a voltage gated sodium channel encoded by *SCN9A* gene and is expressed in the Dorsal Root Ganglion (DRG) sensory neurons
- Mutations in the *SCN9A* gene are linked to inherited pain disorders
  - Alterations in Nav1.7 activity directly regulate pain levels in several genetic disorders, validating 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

Nav 1.7 Repression as Potential Therapy for Various Pain Indication

- Repressing Nav1.7 in the DRG sensory neurons is expected to prevent the transmission of nociceptive pain signals to the brain
- This allows targeting multiple neuropathic pain indications, regardless of the cause of pain
- Reducing pain by repressing Nav1.7 is not predicted to be associated with any CNS adverse effects



Zinc Finger-Mediated Gene Regulation for Neurological Diseases



Preclinical Development Strategy

MOUSE: *Using Surrogate ZF-Rs targeting mouse SCN9a*

HUMAN: *Using ZF-Rs targeting human SCN9A*

Top 3 leads selected

Pre-IND Received FDA Responses

FDA Recommendations Incorporated

Clinical candidate selected

CC = Clinical Capsid
CP = Clinical Product

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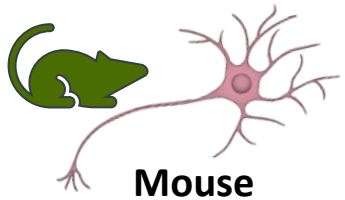
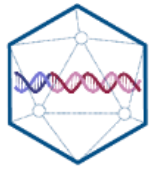
Mouse Surrogate ZF-R Screening and Efficacy Study

Screening and Selection of Surrogate ZF-Rs Targeting Mouse *Scn9a* Gene



Mouse neurons

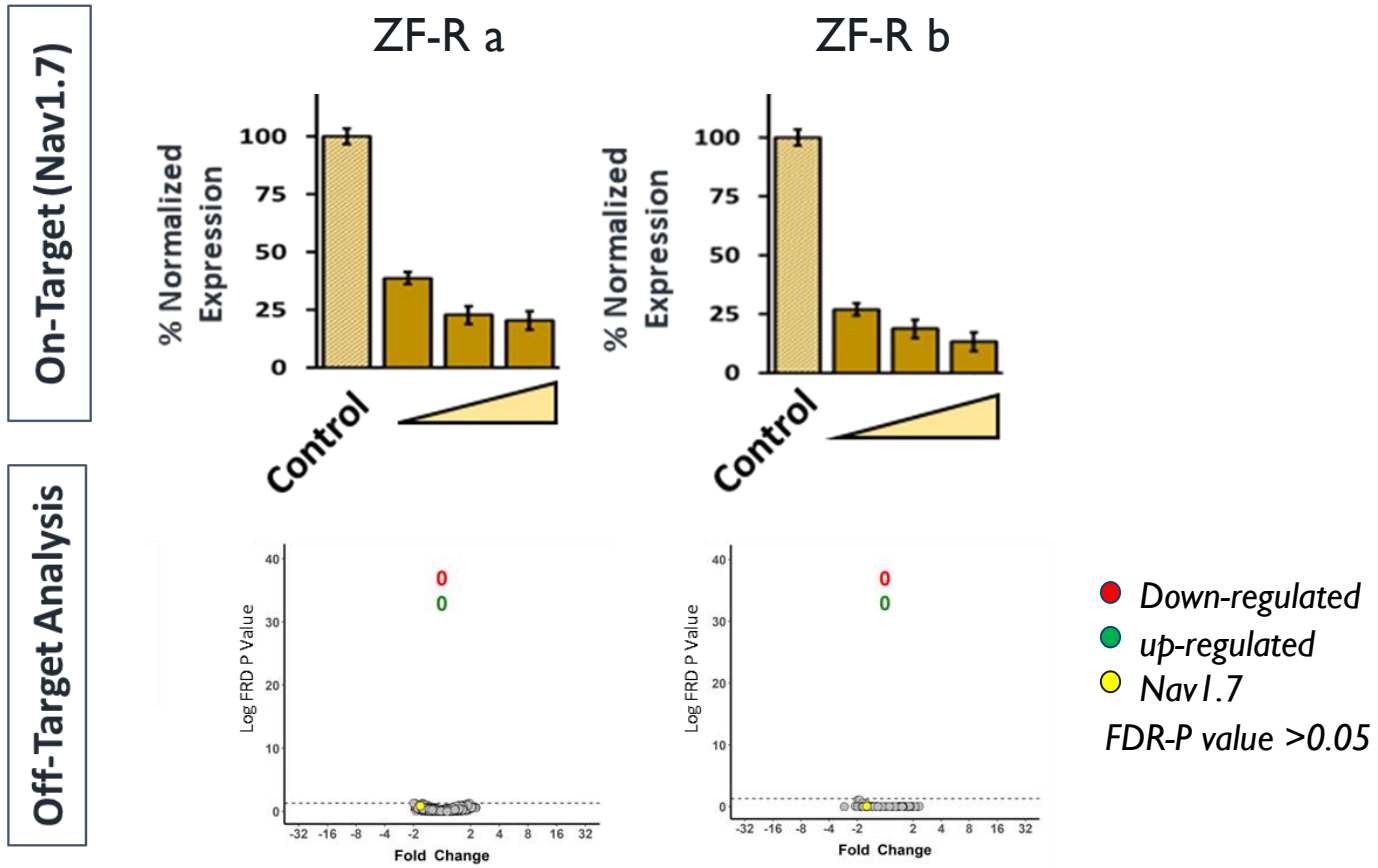
+



AAV-ZF-R

Incubation

- RT-qPCR
- Microarray



- Screened ~ 800 Surrogate ZF-Rs in vitro
- Selected top two Surrogate ZF-Rs with dose dependent Nav 1.7 mRNA repression
- The selected ZF-Rs showed exquisite selectivity in mouse neurons

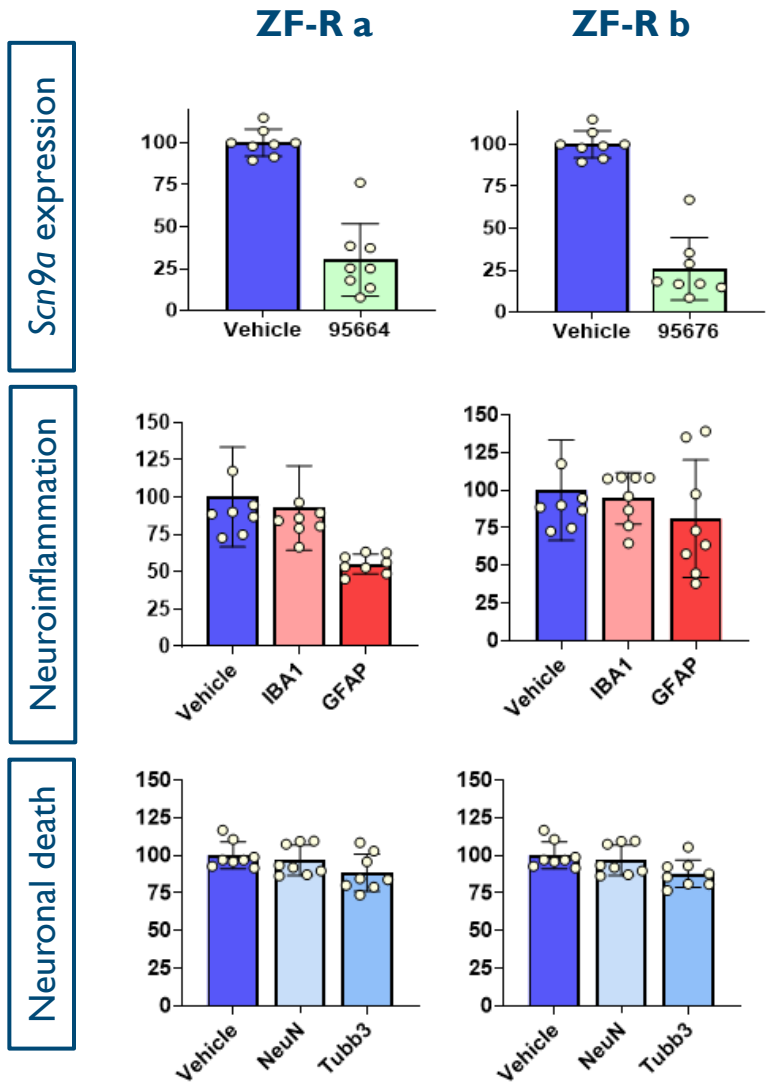
4-Week Target Engagement Study after a Single Dose in Mice

ROA – IT (intrathecal)

AAV (clinical capsid)

Dose – 2.00E 11 vg/animal

4 weeks in-life phase



- Both ZF-Rs were well tolerated
- Surrogate ZF-Rs resulted in 60%-75% reduction in mouse Nav1.7 in lumbar DRG ($P \leq 0.0001$)
- No neuronal loss or neuroinflammation was observed in the lumbar DRG

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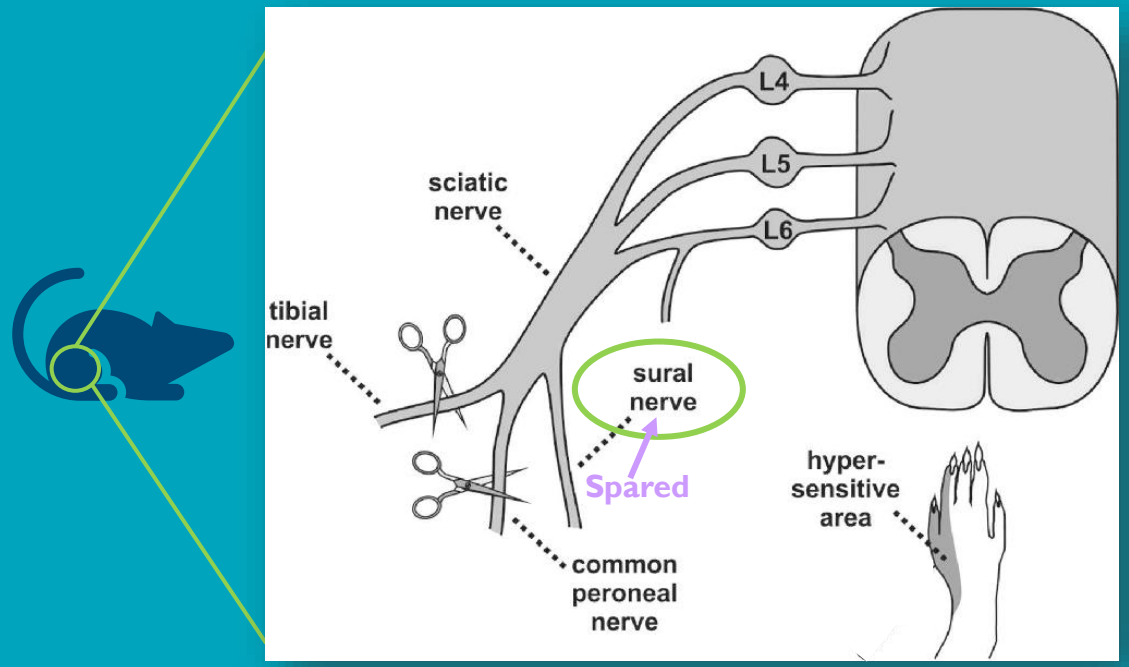
Spared Nerve Injury (SNI) Mouse Model of Neuropathic Pain

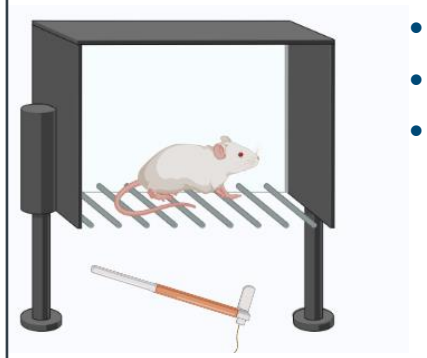
SNI mouse model generation

- Gold standard model of chronic neuropathic pain
- Surgically generated
- Increased pain response in the affected hind paw
- Pain monitored as nocifensive behavior
  - Paw withdrawal, licking paw, and shaking paw



Pain assessment using SNI model

Mechanical induced pain



- Von Frey assessment
- Response is measured in Force (g)
- Pressure is applied from the bottom of the cage to the affected paw

Cold induced pian

- Cold plate measurement
- Paw withdrawal responses are measured in seconds (s)

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Efficacy Study Design in SNI Mouse Model of Neuropathic Pain

ROA – IT (intrathecal)

AAV (clinical capsid)

Dose – 8.00E 11 vg/animal

4 weeks in life

SNI in C57BL/6

Group	Treatment	Total Dose (vg/mouse)	Dose mg/kg	No. of Mice
1	Sham Operated*	0	-	8M/8F
2	Vehicle Control (Buffer for ZF-R)	0	-	8M/8F
3	Gabapentin (GBP)	-	50**	8M/8F
4	ZF-R a	8.12E+11	-	8M/8F
5	ZF-R b	7.99E+11	-	8M/8F

* Animals had mock surgery and did not receive ZF-TF or Vehicle

** 50 mg/kg given one hour before assessment on each assessment day

Analysis

In-life

- Clinical and necropsy observation

Tissue

- RT-qPCR for Nav 1.7 transcript in DRG neurons

Pain

- Mechanical induced pain
- Cold induced pain

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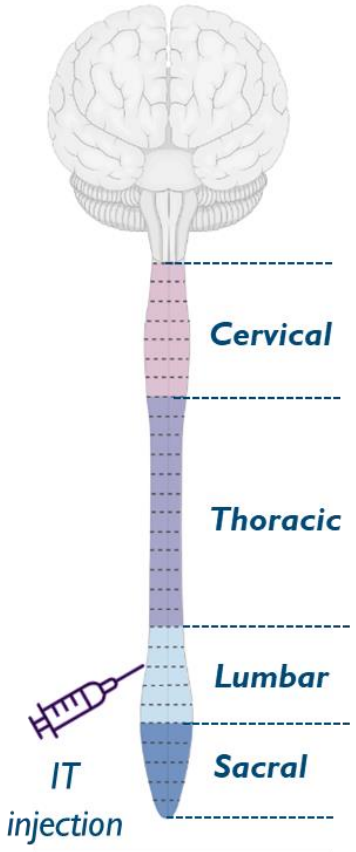
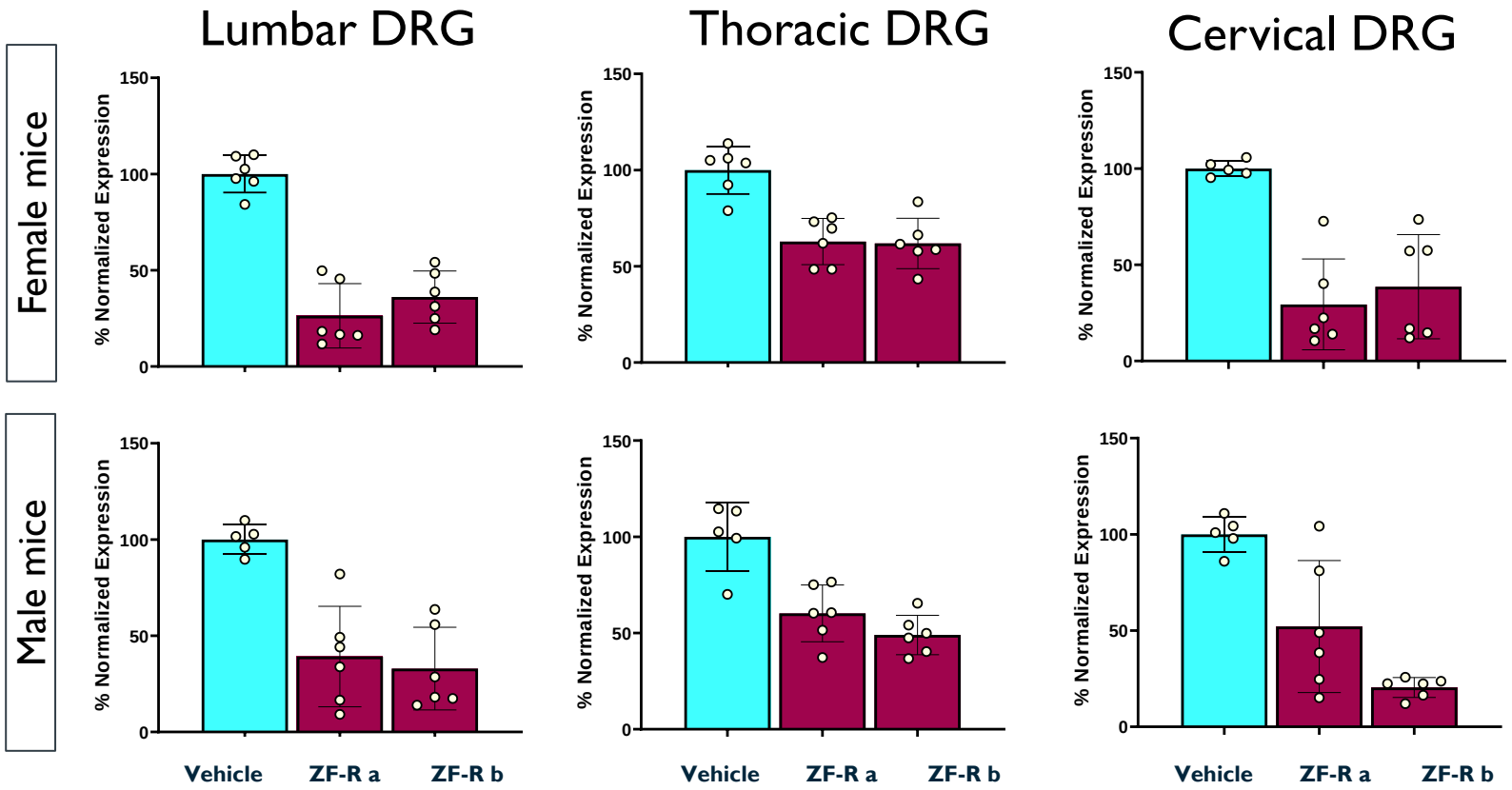
Effect of ZF-Rs on Mouse Nav1.7 in DRGs

SNI model



Nav1.7 expression (RT-qPCR)



Both ZFRs repressed Nav1.7 in Lumbar and Cervical DRGs better than in Thoracic DRG

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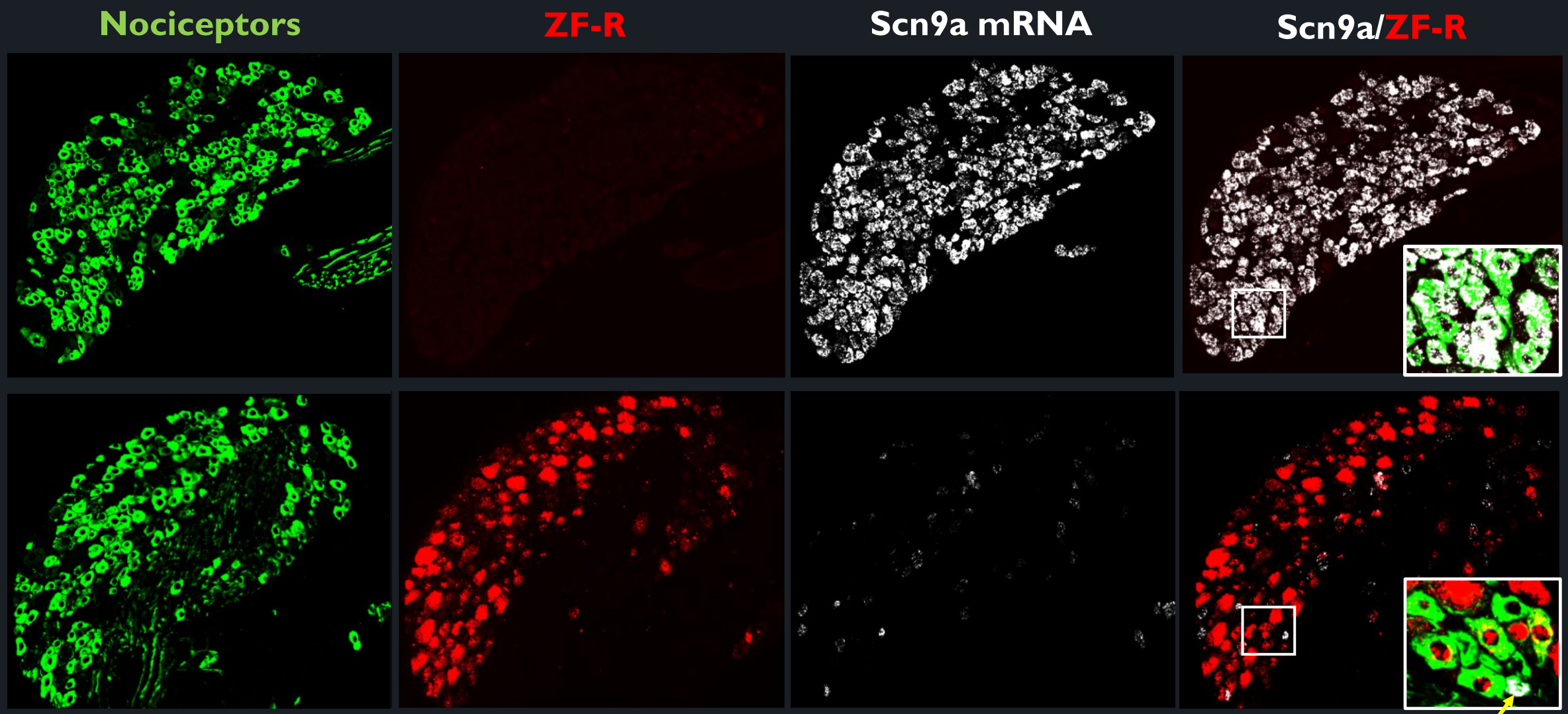
Potential Repression of *Scn9a* mRNA in Nociceptors of Mouse Lumbar DRG

SNI model



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Efficacy of Mouse Surrogate ZF-Rs in SNI Model of Neuropathic Pain (Females)

SNI model

Gabapentin was administered one hour before the measurement

- ZF-R
- AAV
- 8E11 vg/mouse
- Intrathecal-Lumbar injection
- 4 weeks

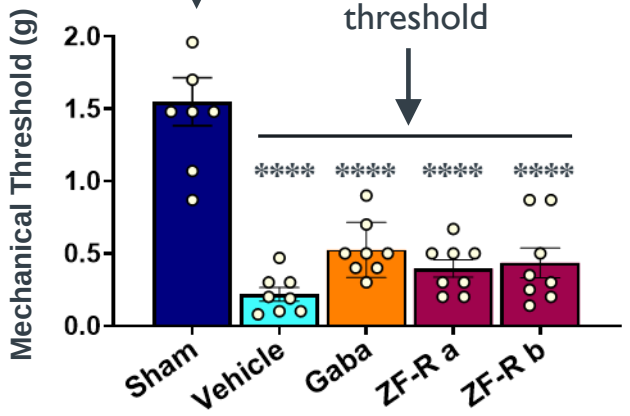
Mechanical induced pain

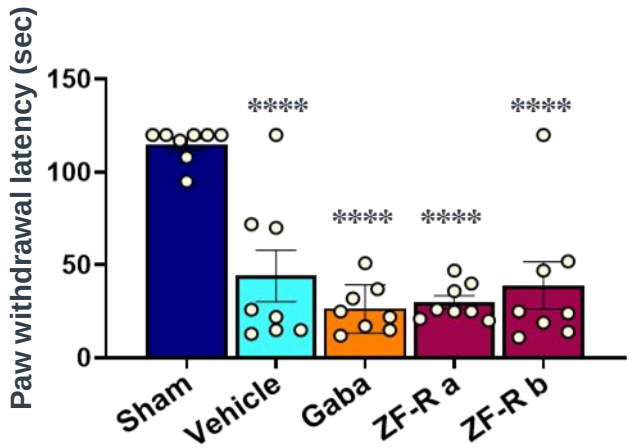
Cold induced pian

Before any Treatment

Normal pain threshold

reduced pain threshold



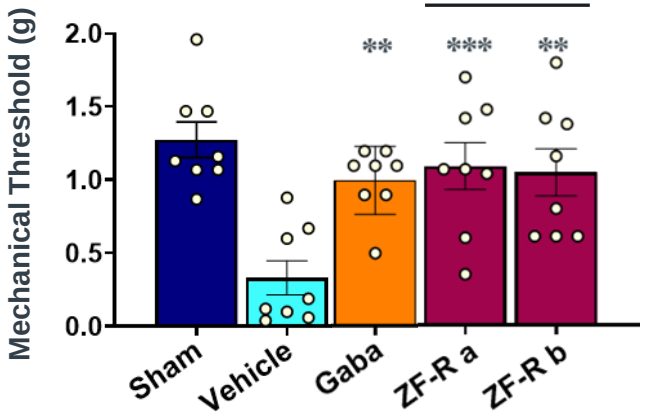


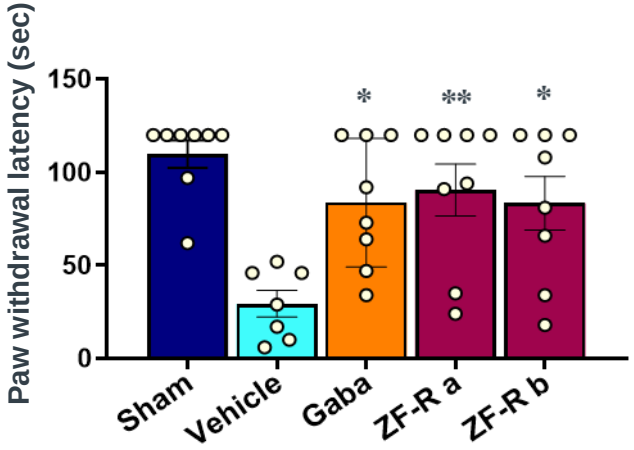
ZF-R AAV

4 weeks

After Treatment

Recovery to normal pain threshold





**** $P < 0.0001$ Compared to Sham
One-way ANOVA

** $P < 0.01$ *** $P < 0.001$ *Compared with Vehicle*
One-way ANOVA

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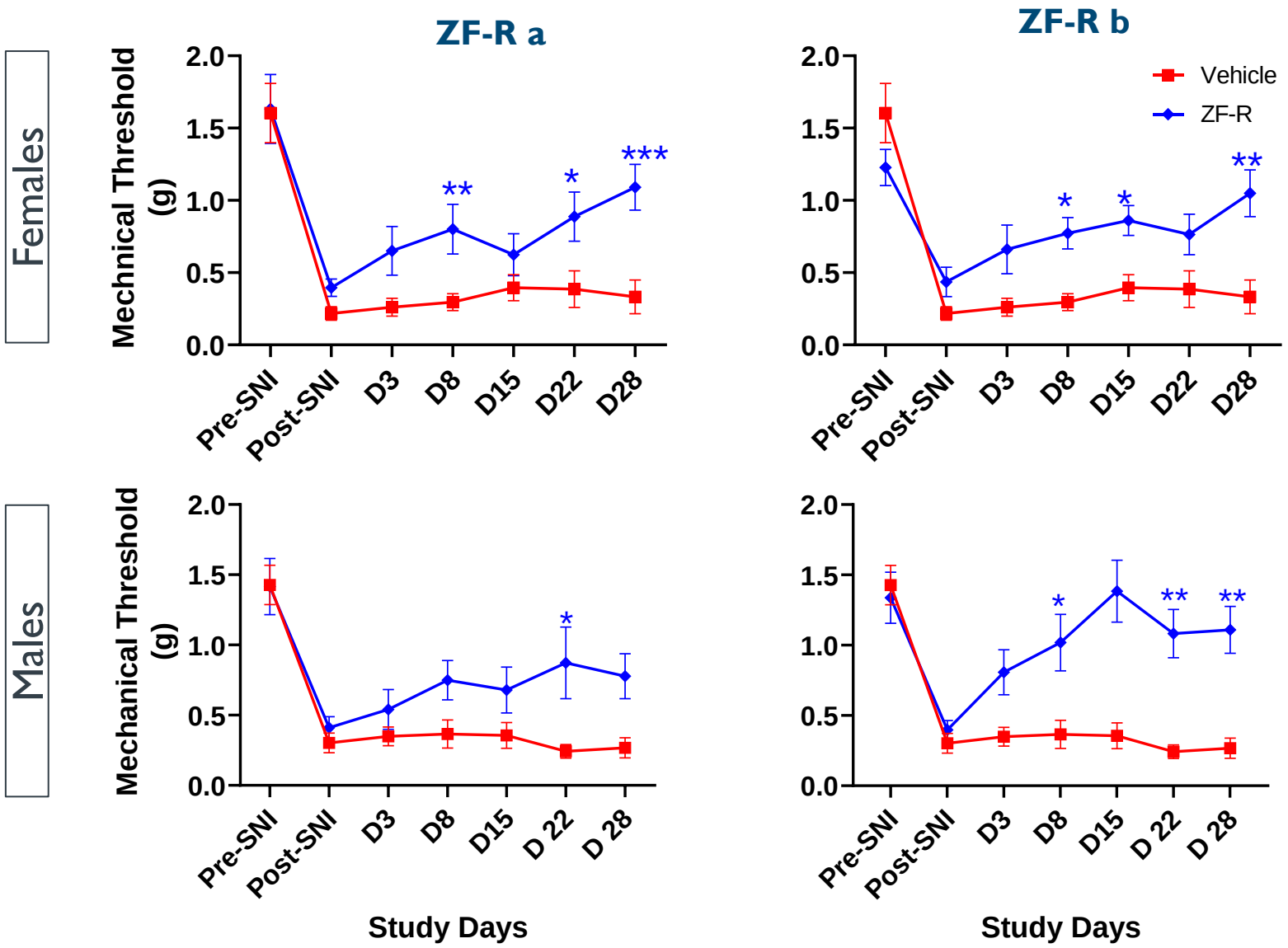
Time Course of Pain Phenotype Rescue after Mouse ZF-R Administration



SNI pain model in C57BL/6 mouse

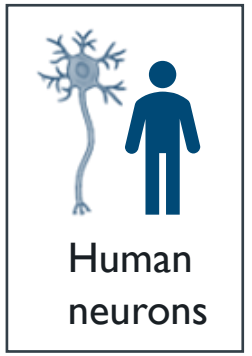
ZF-Rs rescue pain phenotype as early as Day 3 post administration

Conclusion - Mouse ZF-R Summary

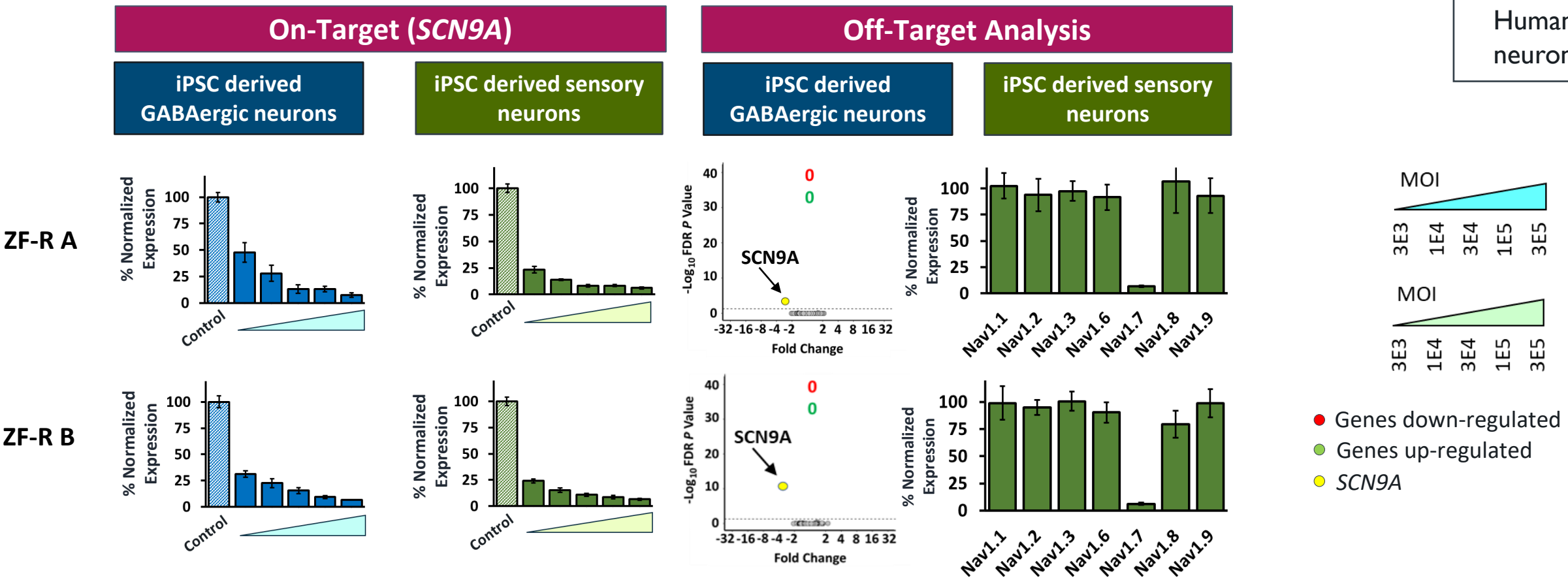
- More than 500 ZF-Rs targeting mouse Nav1.7 were designed and screened
- The top ZF-Rs were selected based on on-target *in vitro* efficacy and minimal off-target in mouse neurons
- Mouse ZF-Rs were well tolerated in vivo and did not result in neuroinflammation or neuronal loss
- ZF-Rs were able to significantly repress *Nav1.7* gene in the lumbar DRG one month after injections
  - Bulk level
  - Single-cell level
- ZF-Rs significantly increased pain threshold in a SNI mouse model (Gold Standard model of neuropathic pain)
- Pain threshold was increased longitudinally as early as Day 3 post ZF-R single IT administration

Human ZF-R Screening and Clinical Candidate Selection

Screening and Selection of Human ZF-Rs Targeting Human *SCN9A* Gene

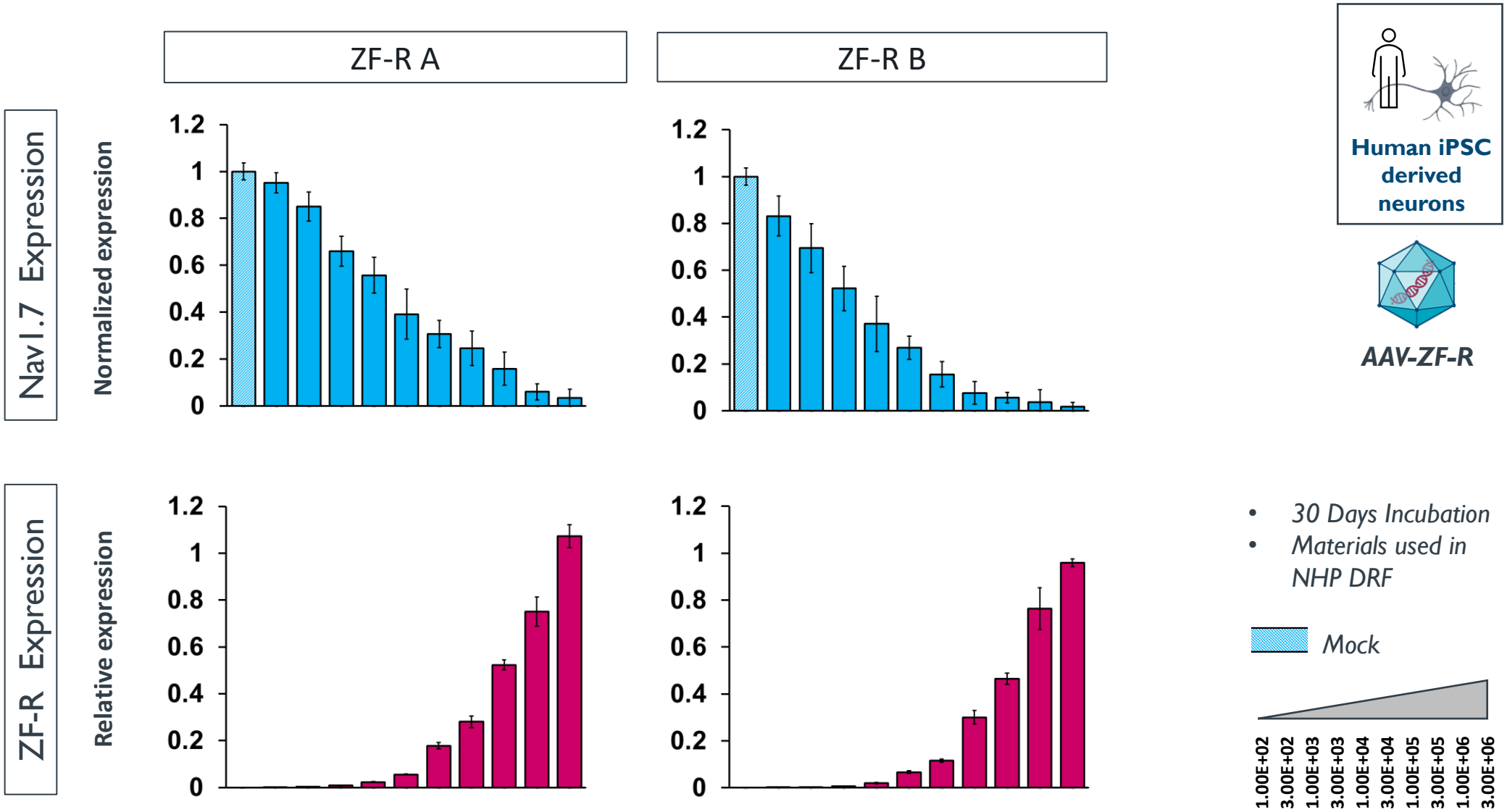


After screening of ~800 ZF-Rs:



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

Potency of Manufactured AAV-ZF-Rs in Human iPSC Derived Neurons



Single Dose Range-Finding Toxicology Study: Objectives and Design

Objectives

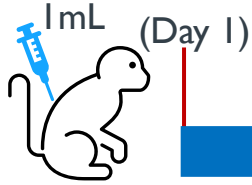
- Several objectives to satisfy regulatory requirements
- Key objective
  - Selection of a clinical candidate from three lead ZF-TFs

Study Design

Two AAV/ZF-Rs Tested (ZF-R A or ZF-R B)

- 1E12 vg/animal (2M/1F)
- 1E13 vg/animal (2M/1F)
- 9E13 vg/animal (2M/1F)
- Vehicle control (1M/1F)

Route: Intrathecal-Lumbar (IT-L)
Species: Cynomolgus Monkey (NHP)
Age: 2-3yrs



Observation Period: 4wks

Necropsy (Day 28)

Tissue Collection

Expression (RT-qPCR)

Pathology (H&E)

Used for Candidate Selection

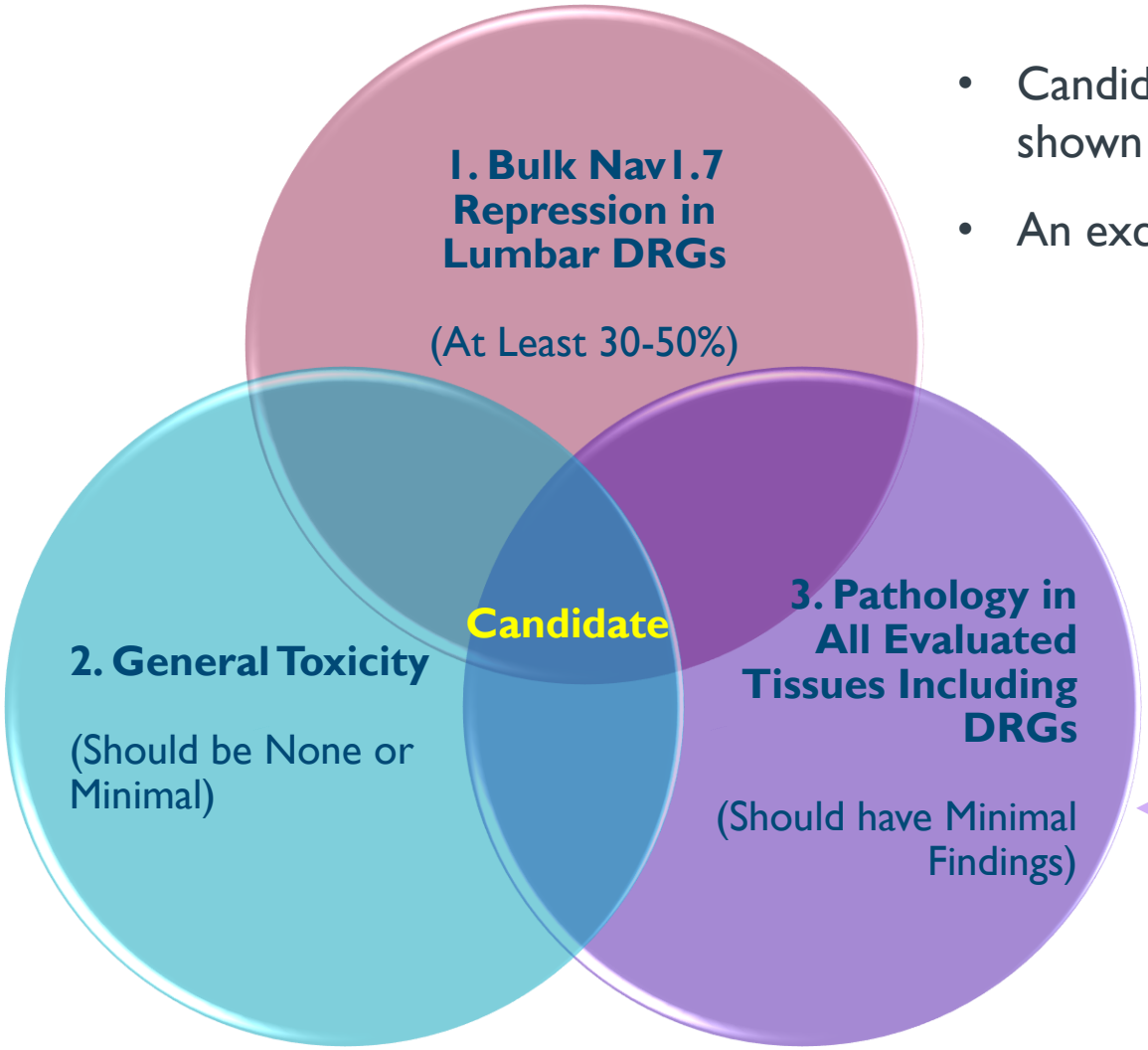
Biodistribution (qPCR)

Pharmacokinetic (qPCR)

Single Cell (scRNA-Seq / ISH)

Data not available

Clinical Candidate Selection Criteria

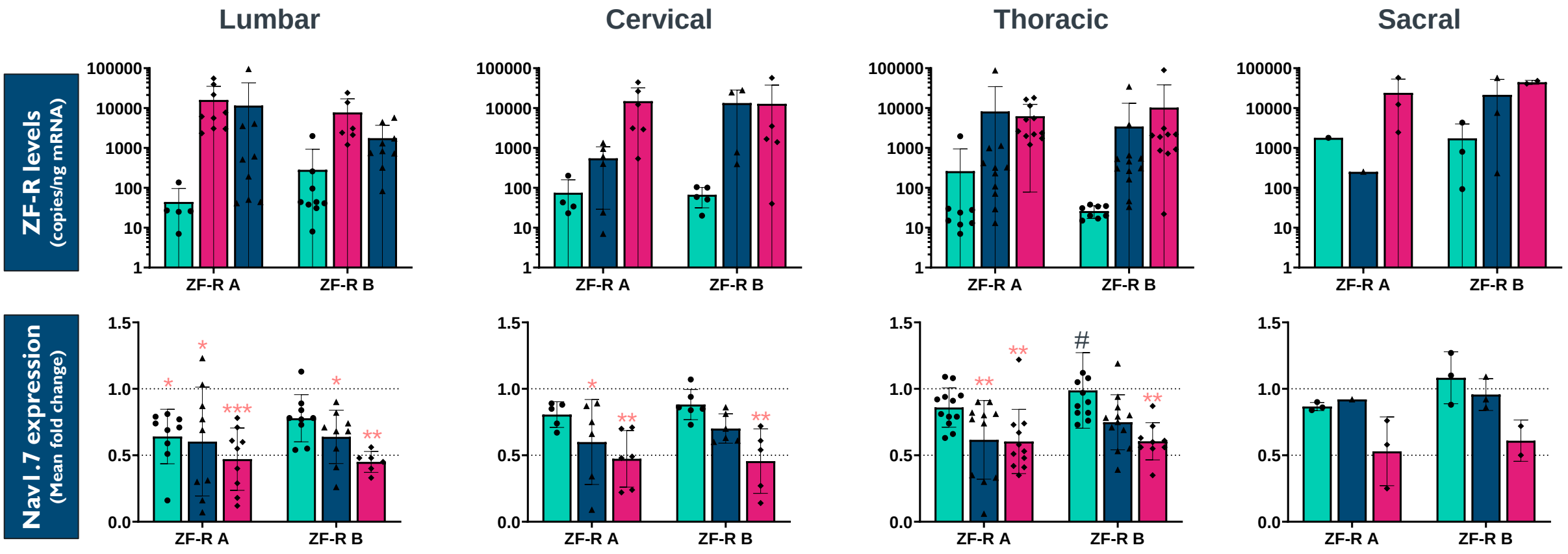


- Candidate selection was based on the 3 major criteria shown in the Venn diagram
- An excellent candidate should fulfill all three criteria

- This is a very high bar, particularly for DRG neurons
  - As per the published literature most findings are of high severity at low doses particularly after IT-L administration*

* Hordeaux J, et al. (2020) Human Gene Ther; 31(15-16): 808-18.

Pharmacology of Human ZF-Rs in Nonhuman Primates



ZF-Rs repressed *SCN9A* gene bulk mRNA levels from 30-55% depending on dose and tissue type

- - - Basal level Nav1.7 expression is set to 1.0 on the y-axis.

 # A single outlier >1.5 not displayed for ZF-R B at low dose.

** P ≤ 0.05, ** P ≤0.001, *** P ≤ 0.0005 (Compared with the control group)*

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General Toxicology

- **Endpoints evaluated:**
  - Mortality/Morbidity
  - Clinical Signs
  - Body Weights
  - Clinical Pathology
    - Hematology
    - Clinical Chemistry (including liver panel)
    - Coagulation
  - Necropsy Observations
  - Organ Weights
  - Histopathology

- **Evaluation Outcome:**

For both ZF-Rs:

- ZF-R-related toxicity was not present in any of the endpoints evaluated except for histopathology
- Histopathology findings related to ZF-Rs were noted

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Pathology: Tissues Evaluated and Scoring System

Major Internal Organs Evaluated:

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

Central and Peripheral Nervous System Tissues Evaluated:

- Brain
- DRGs (left and right)
  - Cervical: C2 and C4
  - Thoracic: T2, T4, and T5
  - Lumbar: L3, L4, and L7
  - Sacral: S2
- Olfactory Bulb
- Sciatic Nerve
- Spinal Cord
  - Cervical: C2, and C4
  - Thoracic: T2, T4, and T5
  - Lumbar: L3, L4, and L7
- Trigeminal Ganglia

Scoring System

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

Minimal and Mild findings are **not** considered dose-limiting for AAV gene therapy

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Histopathology: Findings and Scores

Tissues Affected

- All evaluated tissues were normal except for the following tissues:
  - DRGs (S, L, T, C)
  - Spinal cord (L, T, C)
  - Sciatic nerve
  - Trigeminal ganglia

S = Sacral; L= Lumbar; T = Thoracic; C = Cervical
DRGs = Dorsal Root Ganglia

Types of Findings

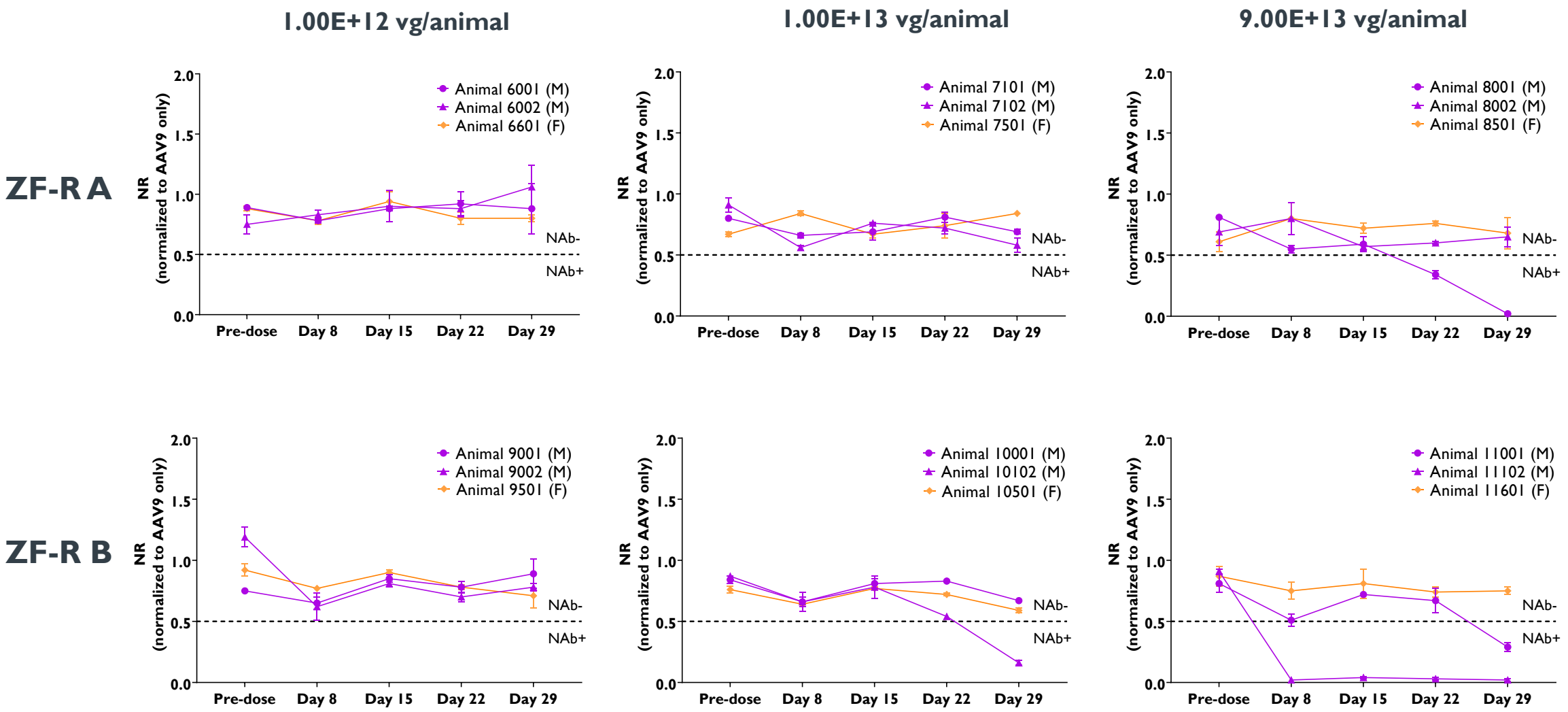
- Mononuclear cell infiltration (MN)
  - Due to inflammatory response
  - Recruitment of lymphocytes and monocytes into the tissue
- Axonal Degeneration (AD)
- Single Neuronal Degeneration/Necrosis (SDN)

Finding Scores

- Majority of findings were **minimal** for both ZF-Rs at all dose levels
- Few **mild** findings were noted only for one ZF-R B at the high-dose

Minimal and Mild findings in DRG, SC, TG and SN neurons are not considered dose-limiting for AAV gene therapy

Anti-AAV Neutralizing Antibody in CSF after a Single ZF-R IT-L Administration

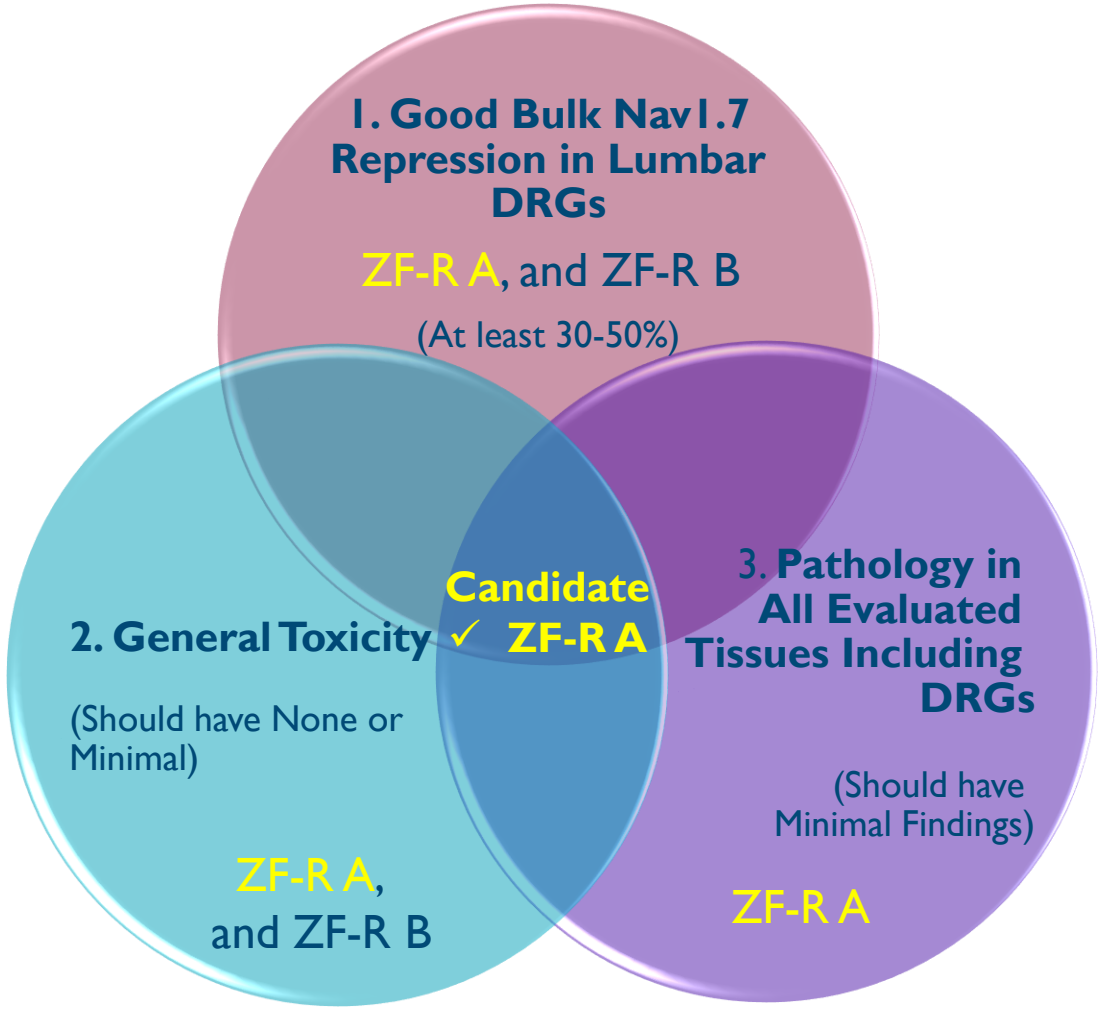


Selection of the Clinical Candidate Based on the Three Selected Criteria

- Selected ZF-R A as the clinical candidate for the following reasons:

  ✓ Demonstrated an excellent profile and fulfilled all three criteria at all dose levels including the **highest dose** administered (9.00E+13)

    ✓ No general toxicity
    ✓ Minimal tissue score for pathology
    ✓ Good Nav1.7 gene repression



Conclusion - Human ZF-R Selection

- The neutralizing antibody formation in serum and CSF pre- and post-treatment did not impact safety or level of repression

- No ZF-R related safety findings in other general toxicity endpoints

- Both ZF-Rs were well tolerated and no dose-limiting toxicity in DRG, SC, TG or sciatic nerve even at the highest dose administered

➢ Clinical candidate ZF-R A is selected

➢ GLP study in NHPs to initiate in 2023 and IND submission anticipated in 2024

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Pathology: Incidence Summary by Group

Test Article	Dose Level	Animal #	Sciatic Nerve	TGG (R)	DRG (C)	DRG (T)	DRG (L)	DRG (S)	SP (C)	SP (T)	SP (L)
ZF-R A	Low	M (6001)		MN				MN			MN
		M (6002)					MN				
		F (6601)		MN	MN	MN			AD	AD	AD
	Mid	M (7102)				MN	MN SDN	MN SDN	AD	AD	MN
		F (7501)								AD	MN
	High	M (8001)					MN		AD	AD	AD
		M (8002)								AD	MN
		F (8501)		MN		MN	MN SDN				
ZF-R B	Low	M (9001)					MN	MN SDN			
		M (9002)		MN	MN		MN	MN			
		F (9501)		MN	MN		MN	MN			
	Mid	M (10001)			MN	MN SDN					
		M (10102)					MN				
		F (10501)					MN SDN				AD
	High	M (11001)	MN AD				MN SDN	MN SDN		AD	MN AD
		M (11102)	AD	MN			MN SDN	MN			
		F (11601)	MN AD		MN SDN	MN SDN	MN SDN	MN SDN		AD	

Incidence and frequency of mild findings:

- **ZF-R B**: In the high-dose group one animal had mild findings in lumbar DRG, and thoracic spinal cord; 2 animals had mild findings in lumbar and one in sacral DRGs
- **ZF-R A**: no animal had mild findings at any dose level.

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

C = Cervical; T = Thoracic; L = Lumbar; S = Sacral; R = Right; TGG = Trigeminal Ganglia; DRG = Dorsal Root Ganglia; SP = Spinal Cord