ZF-TF mediated epigenetic repression of *SCN9A* gene as a therapeutic approach for painful peripheral neuropathies

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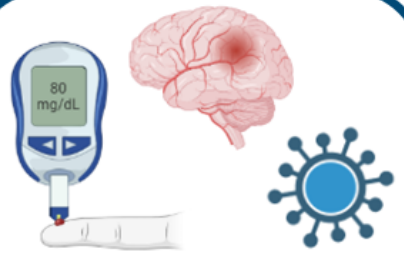
Disclosure

I am a full-time employee of Sangamo Therapeutics

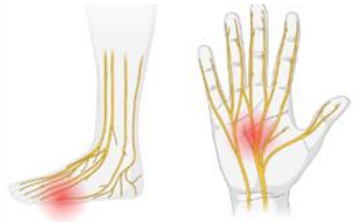
Neuropathic pain is one of the most difficult pain syndromes to manage

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

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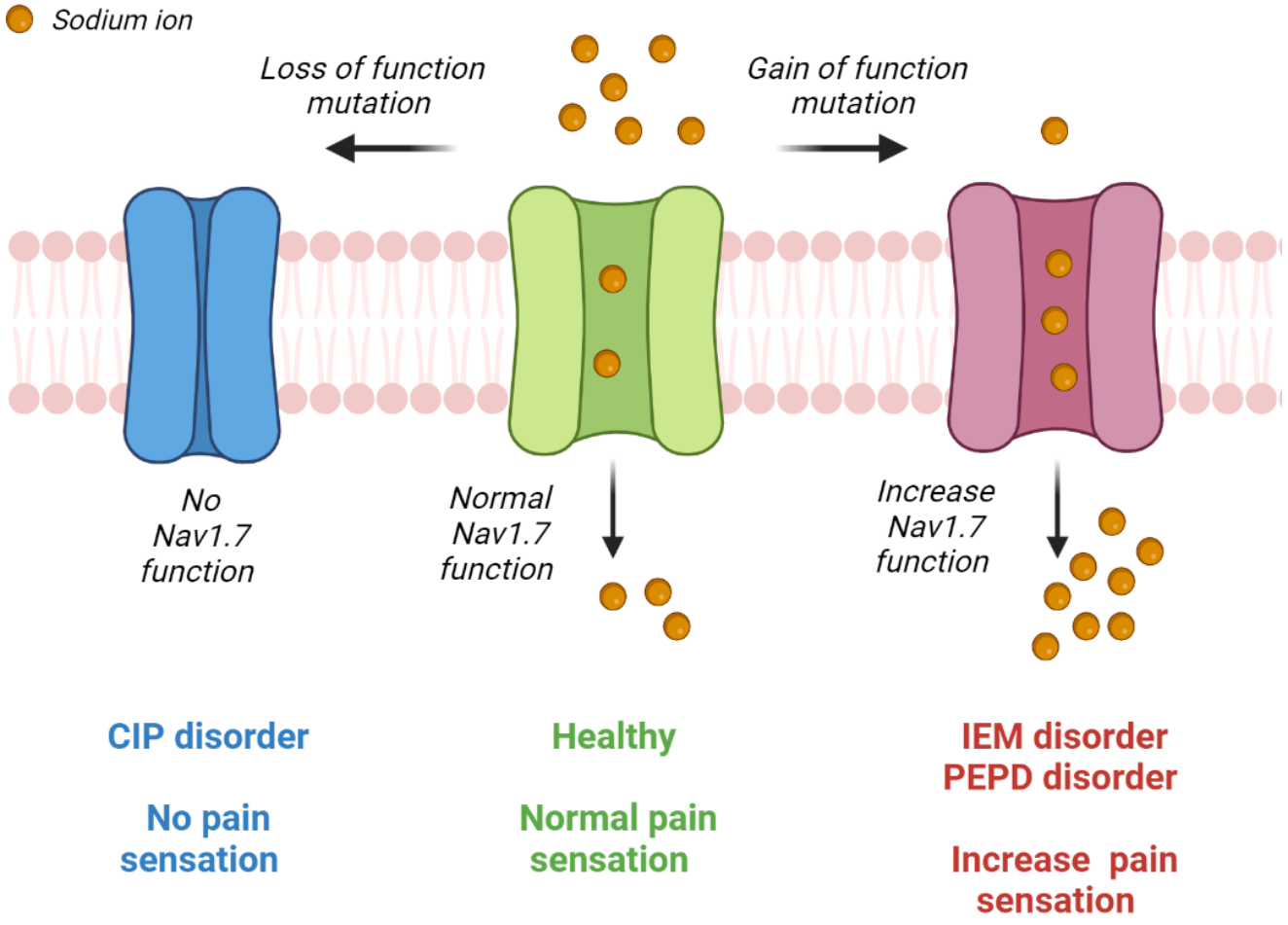
Mutations in the *SCN9A* gene (Nav1.7) are linked to inherited pain disorders



- 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

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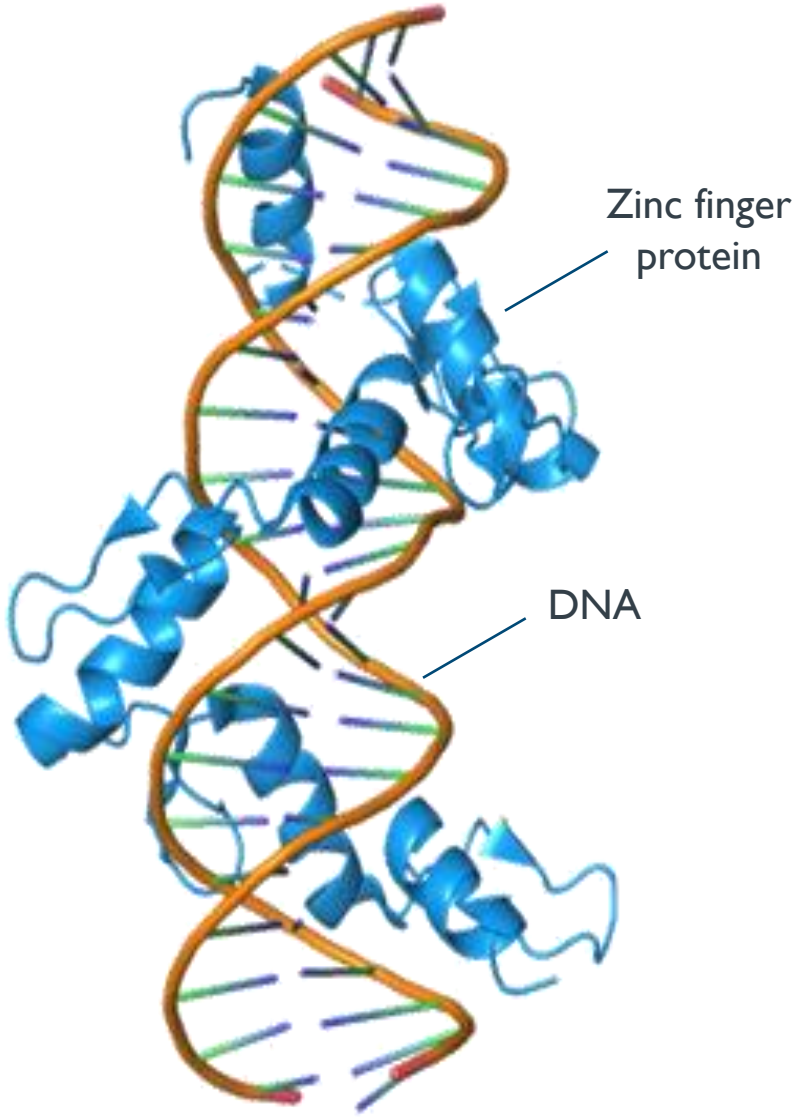
Zinc Finger based epigenetic regulation

- Require binding just two DNA targets per cell to regulate RNA and protein levels
- Highly potent, specific and optimizable
- Derived from naturally occurring human genes
- No modifications or edits to the genome



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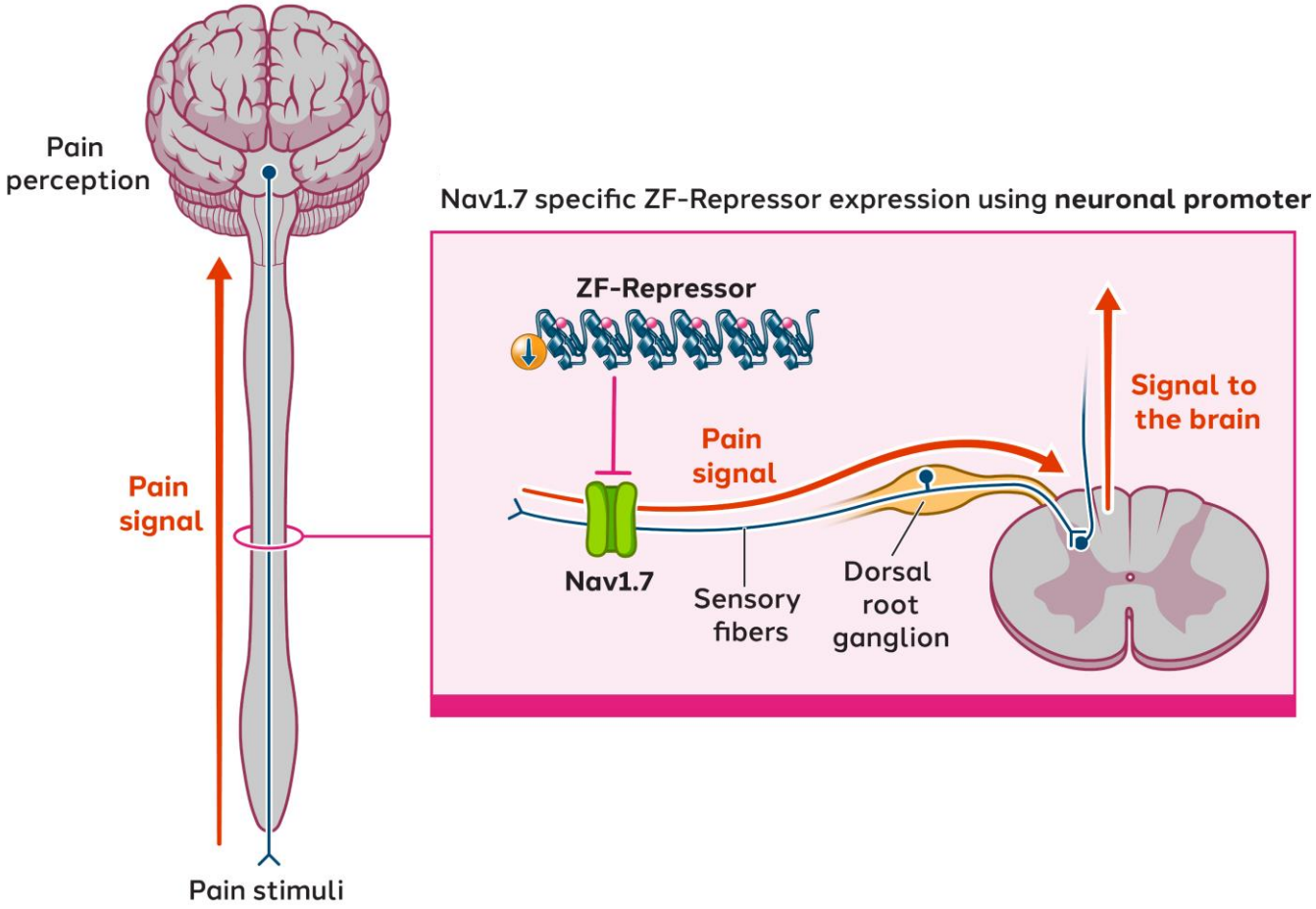
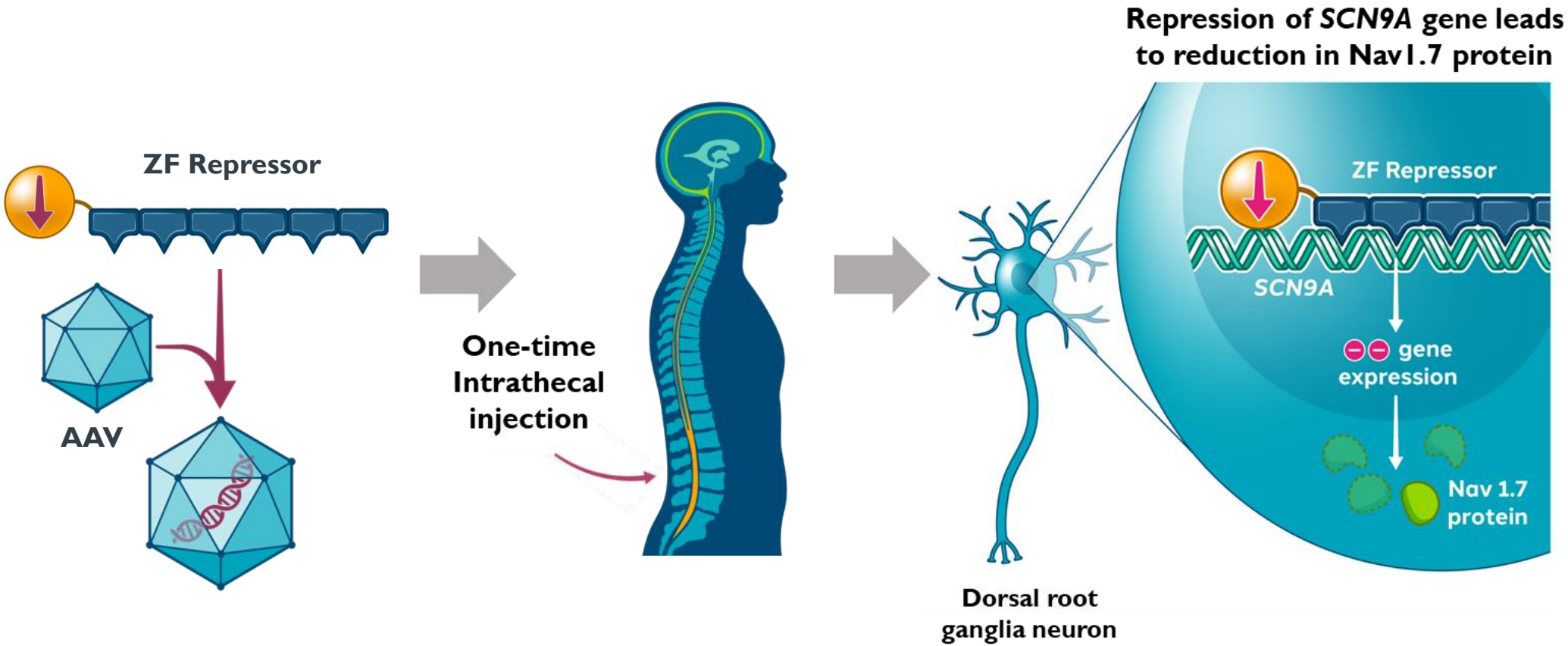
Blocking pain transmission to the brain has the potential to treat multiple pain indications



- 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 (ZF-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 (ZF-R)

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

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

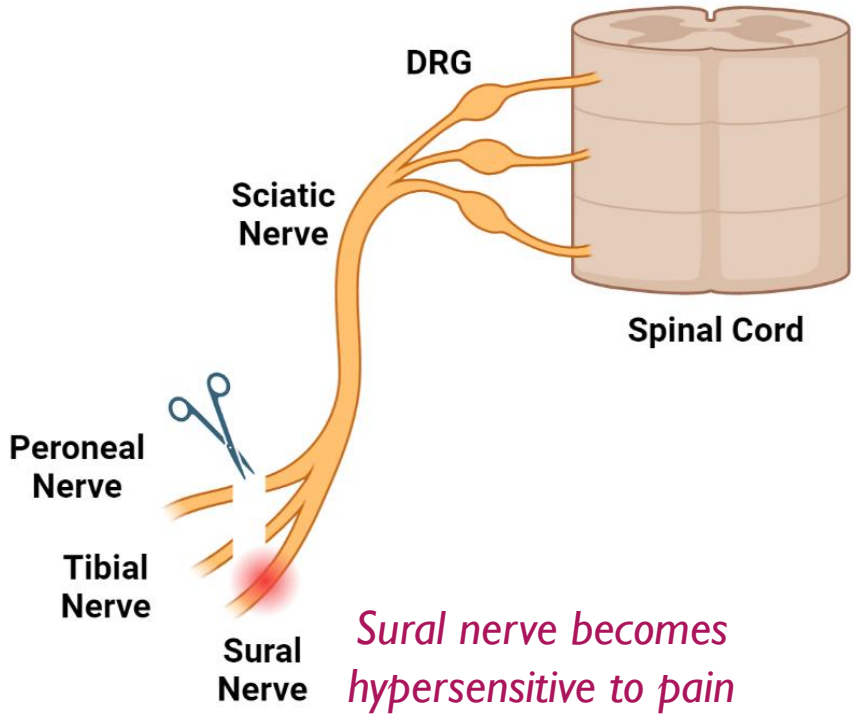
On-target engagement & safety in nonhuman primates (NHPs)

Clinical candidate

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

SNI model

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



Sural nerve becomes hypersensitive 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 measurement on day 28

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Mouse specific ZF-Rs induced up to 70% bulk repression of Scn9a in DRGs

SNI model

ZF-R

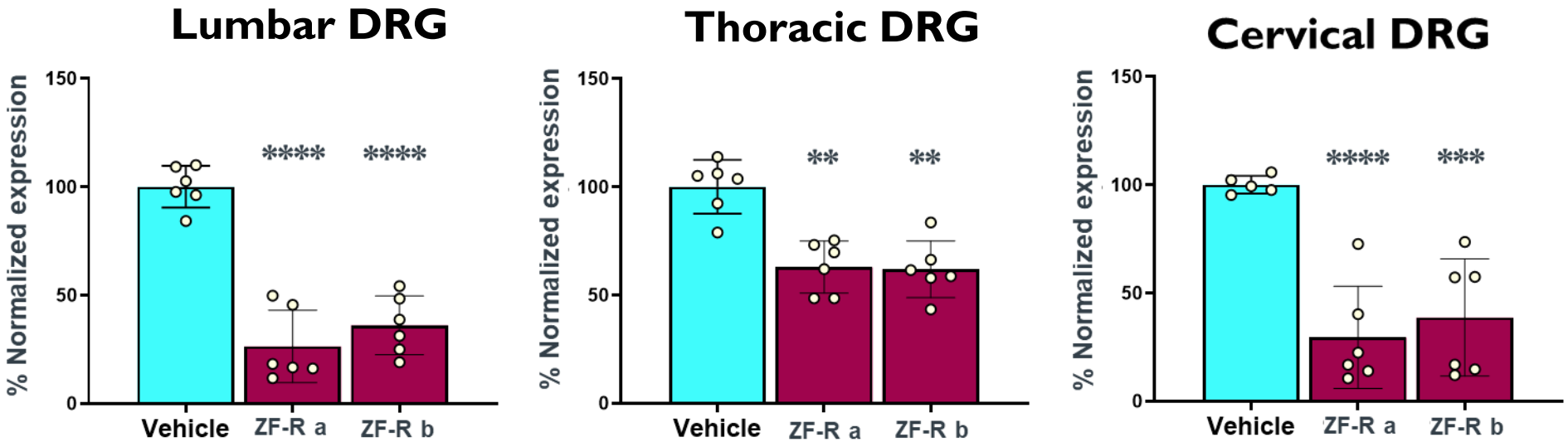
AAV

8E11 vg/mouse

Intrathecal-Lumbar injection

4 weeks

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

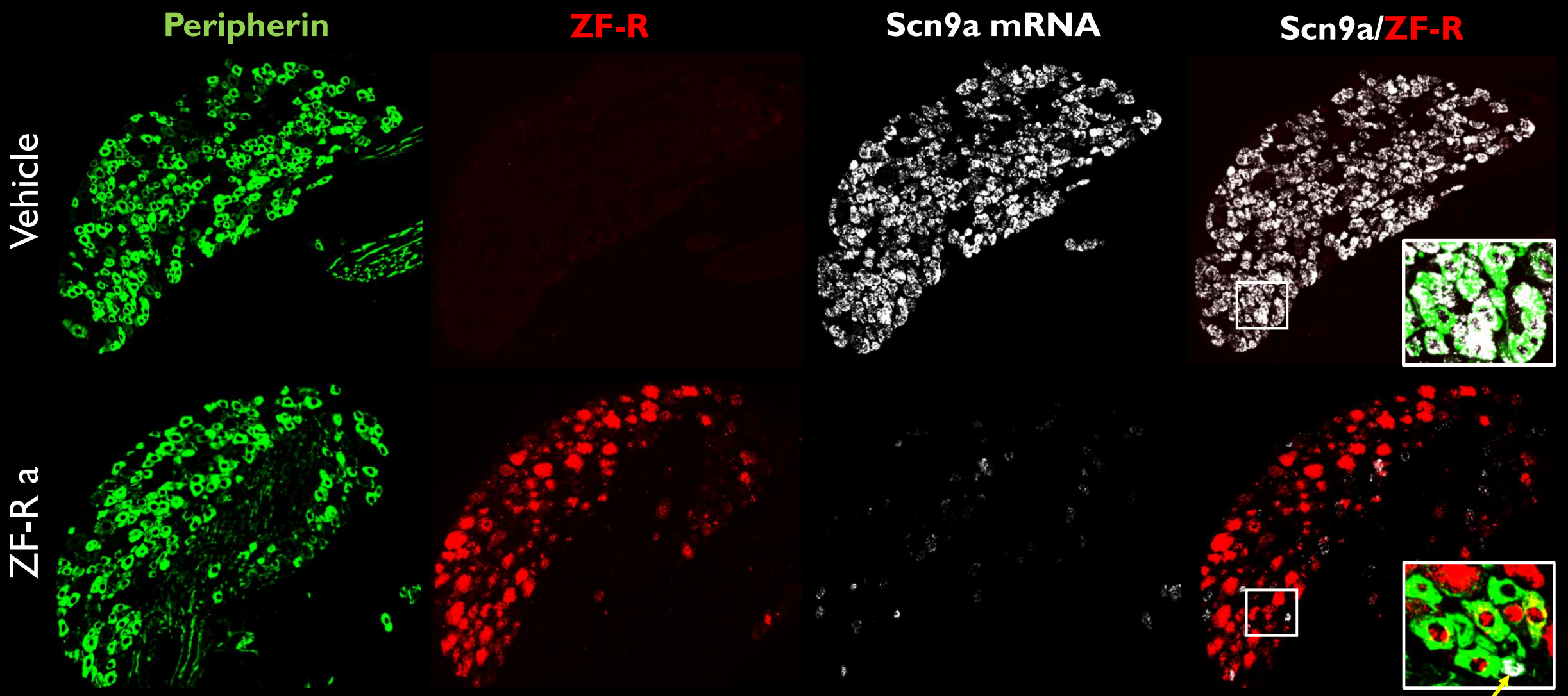


- No changes were observed in the DRG for molecular markers of neuroinflammation or neuronal loss

*** P < 0.01 *** P < 0.001 ****P <0.0001*
Compared with Vehicle
One-way ANOVA

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

SNI model



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

SNI model

ZF-R

AAV

8E11 vg/mouse

Intrathecal-Lumbar injection

4 weeks

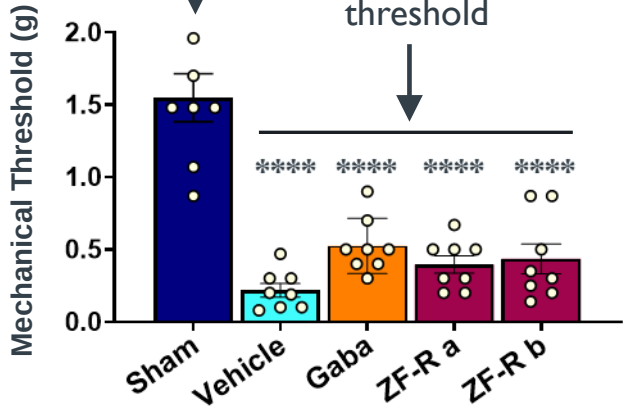
Mechanical induced pain

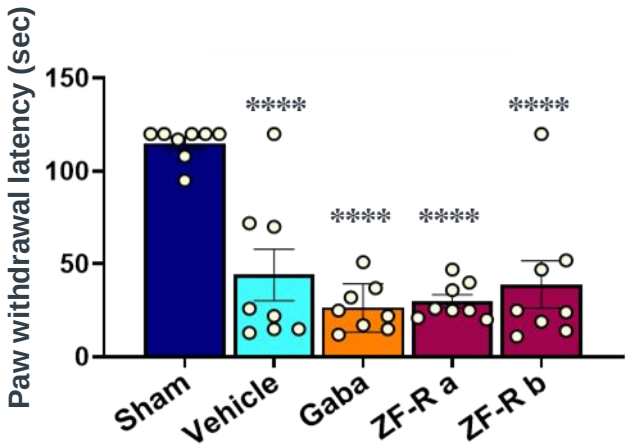
Cold induced pian

Post SNI surgery
Before any Treatment

Normal pain threshold

reduced pain threshold



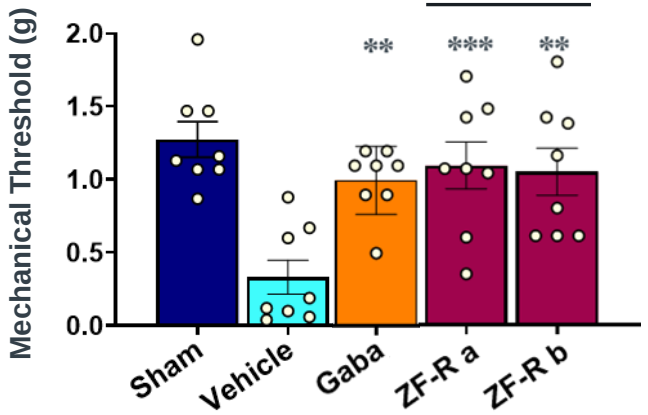


**** $P < 0.0001$ Compared to Sham
One-way ANOVA

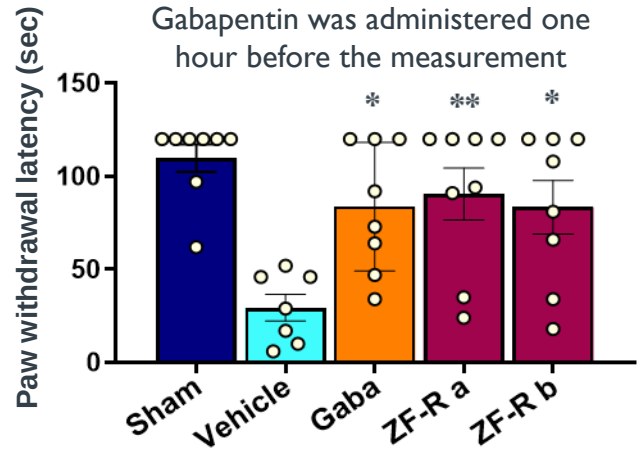
ZF-R AAV

4 weeks

Recovery to normal pain threshold



Gabapentin was administered one hour before the measurement



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

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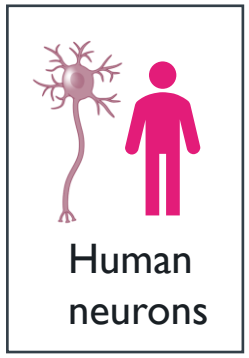
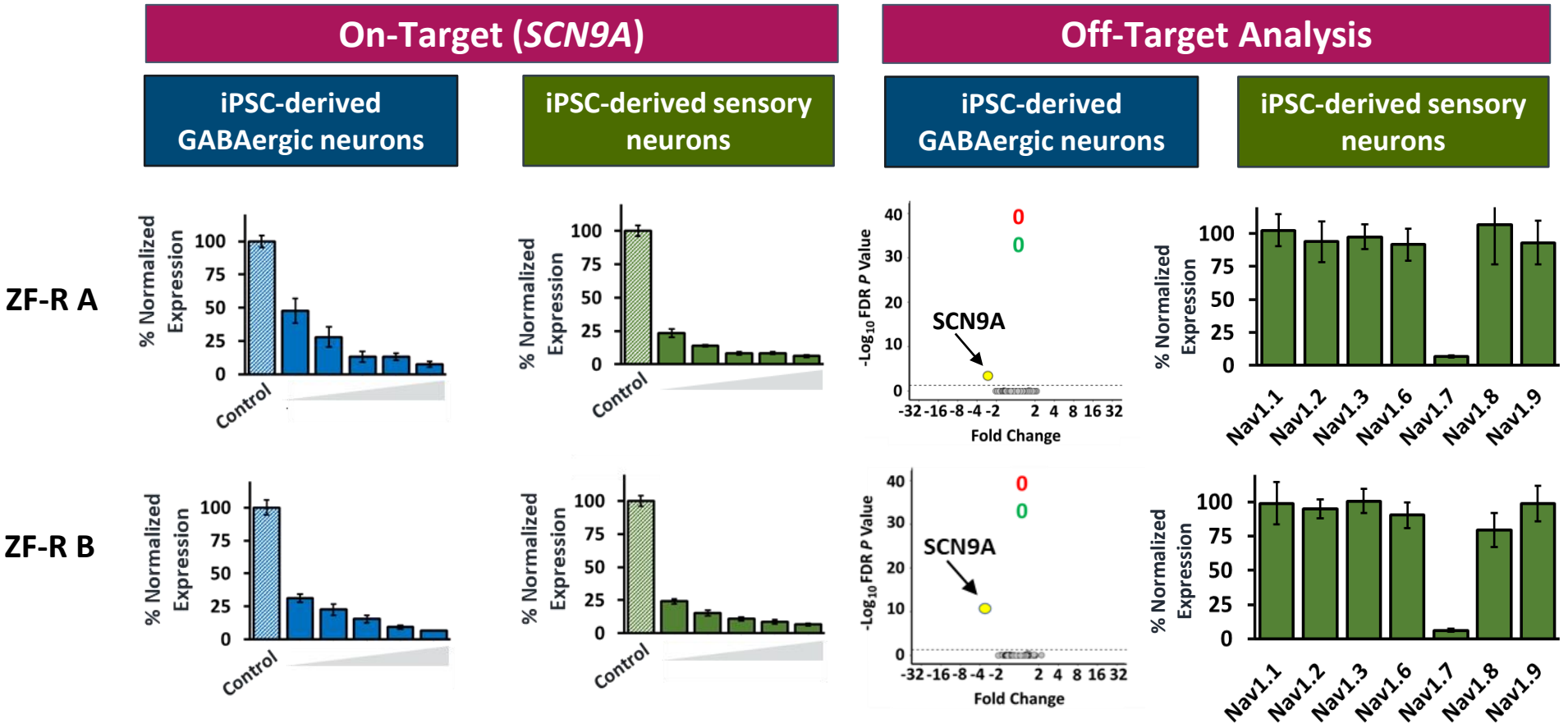
ZF-Rs repressed *SCN9A* by more than 90% over a wide dose range with no detectable off-target activity





- *SCN9A* was the only significantly regulated transcript out of >20,000 genes analyzed
- No repression of any other Nav channel was observed

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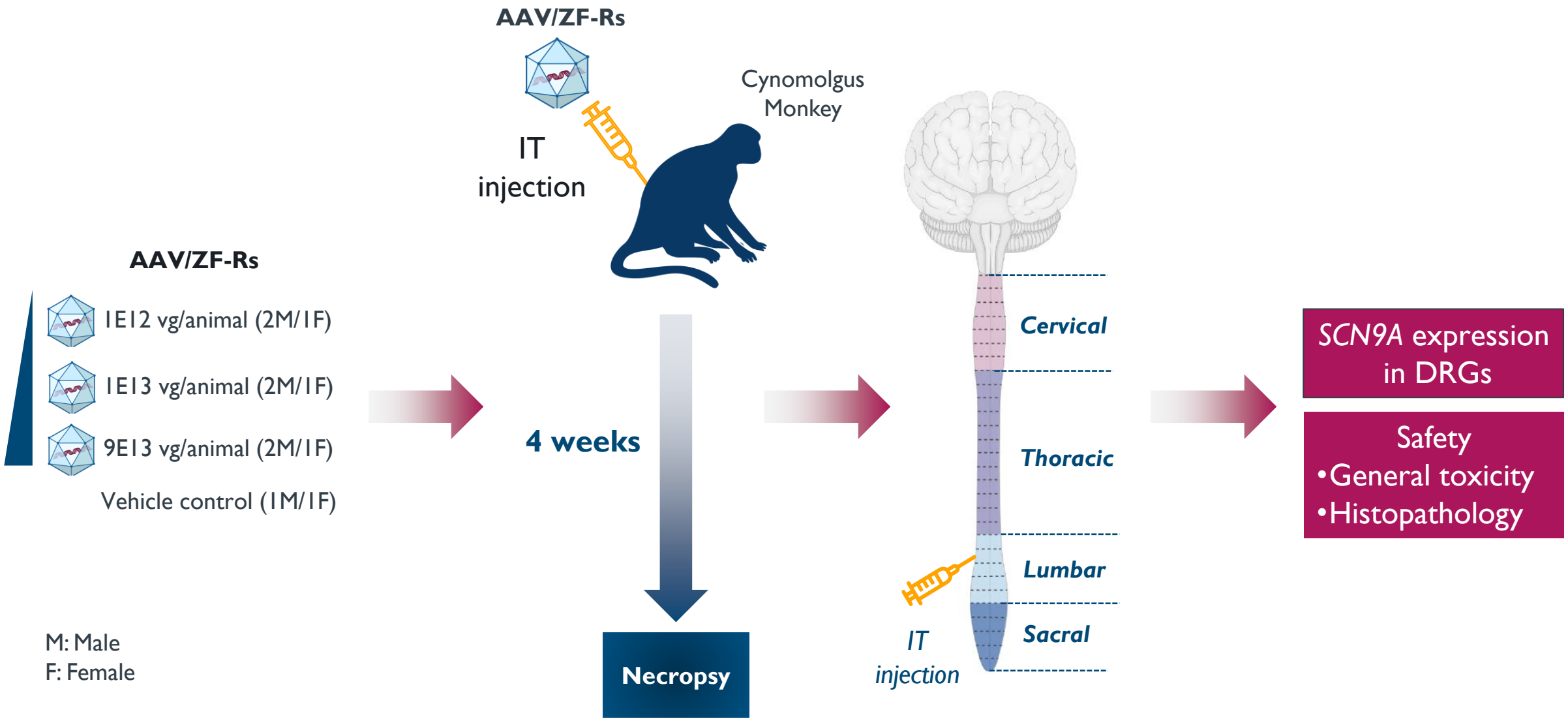
The potency and safety of the human ZF-R candidates were evaluated in nonhuman primates



M: Male
F: Female

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ZF-Rs repressed the expression of *SCN9A* in a dose-dependent manner in multiple DRG levels

NHP

ZF-R

AAV

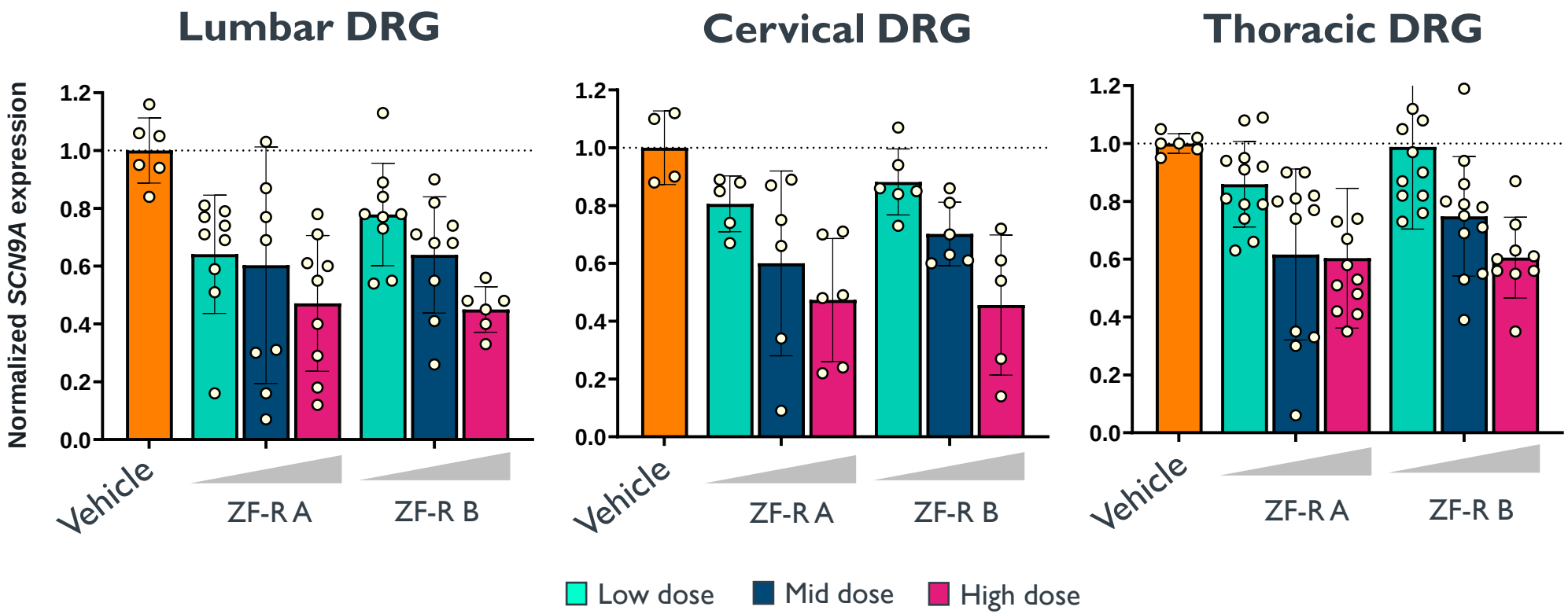
1E12 vg/NHP

1E13 vg/NHP

9E13 vg/NHP

Intrathecal-Lumbar injection

4 weeks



Clinical candidate ZF-Rs repressed *SCN9A* by up to 40-60% across a 100-fold dose range one month after intrathecal-lumbar treatment

- Comparable data were obtained in sacral DRG
- Multiple DRGs were evaluated for each level per animal

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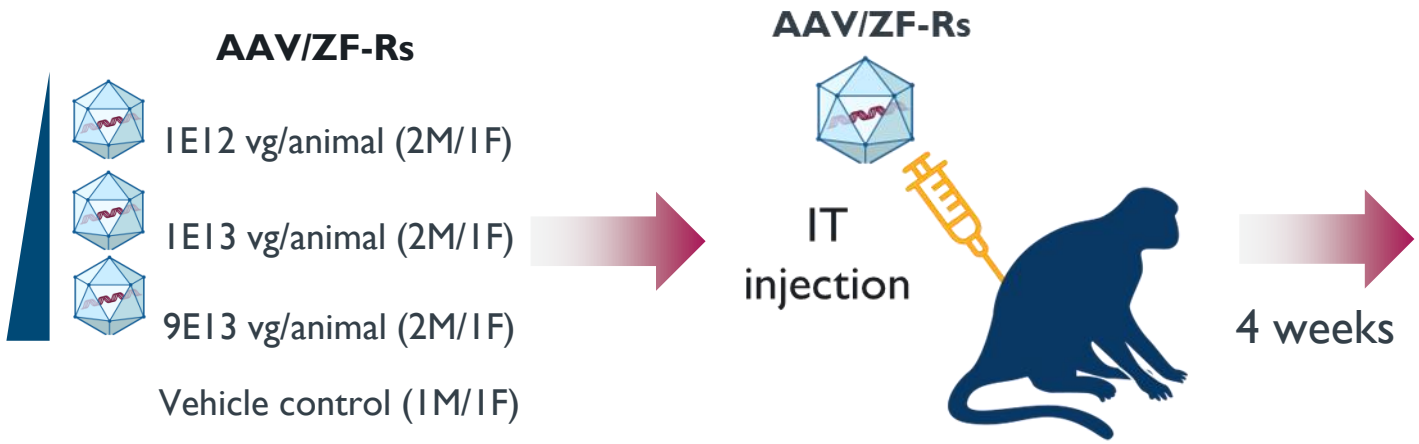
ZF-Rs were well tolerated in nonhuman primates

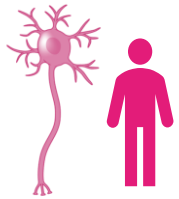


- ZF-Rs were well tolerated at all doses
- No clinical signs of toxicity
- No adverse macroscopic or microscopic findings were observed

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

Conclusion

- Mouse ZF-Rs 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-Rs 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-Rs 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 related to treatment
- These results support the continued progression to IND-enabling nonhuman primate study

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Thank you

Mohammad Samie

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Sangamo Therapeutics Inc.