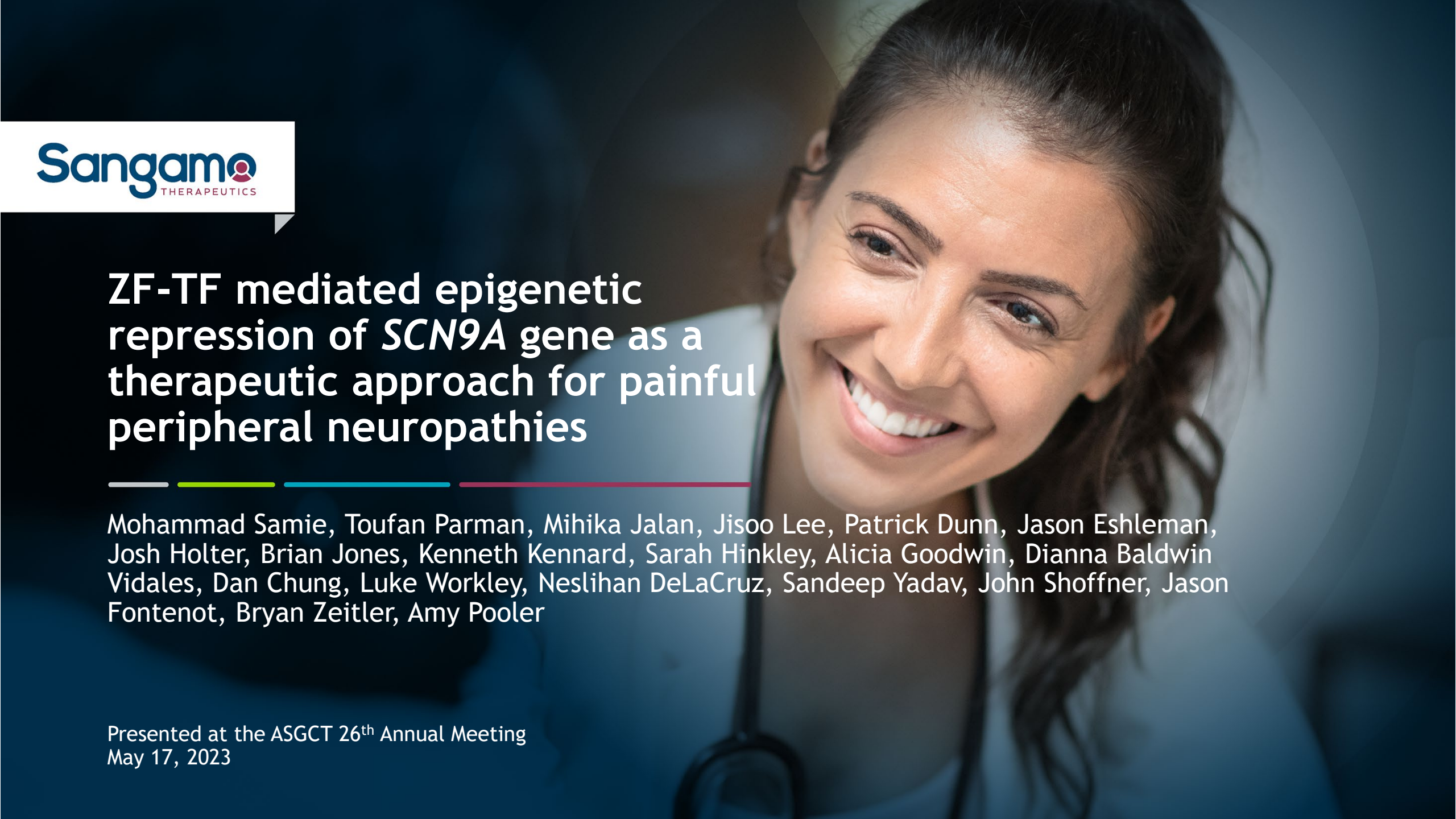


ZF-TF mediated epigenetic repression of *SCN9A* gene as a therapeutic approach for painful peripheral neuropathies

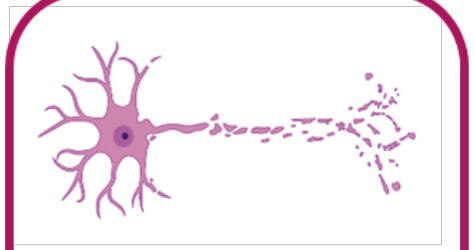
Mohammad Samie, Toufan Parman, Mihika Jalan, Jisoo Lee, Patrick Dunn, Jason Eshleman, Josh Holter, Brian Jones, Kenneth Kennard, Sarah Hinkley, Alicia Goodwin, Dianna Baldwin Vidales, Dan Chung, Luke Workley, Neslihan DeLaCruz, Sandeep Yadav, John Shoffner, Jason Fontenot, Bryan Zeitler, Amy Pooler

Presented at the ASGCT 26th Annual Meeting
May 17, 2023

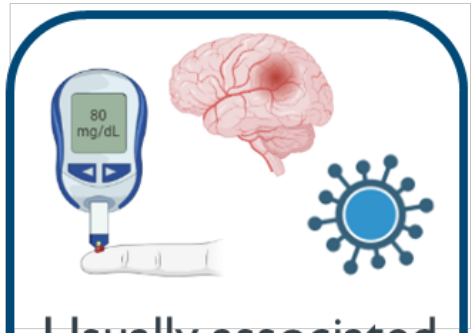
Disclosure

I am a full-time employee of Sangamo Therapeutics

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

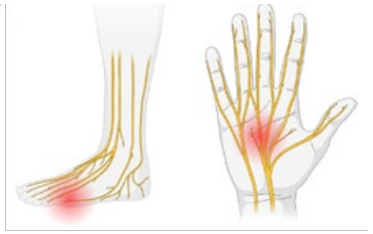


Damage or alterations to **sensory neurons**



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

Large number of patients are affected globally



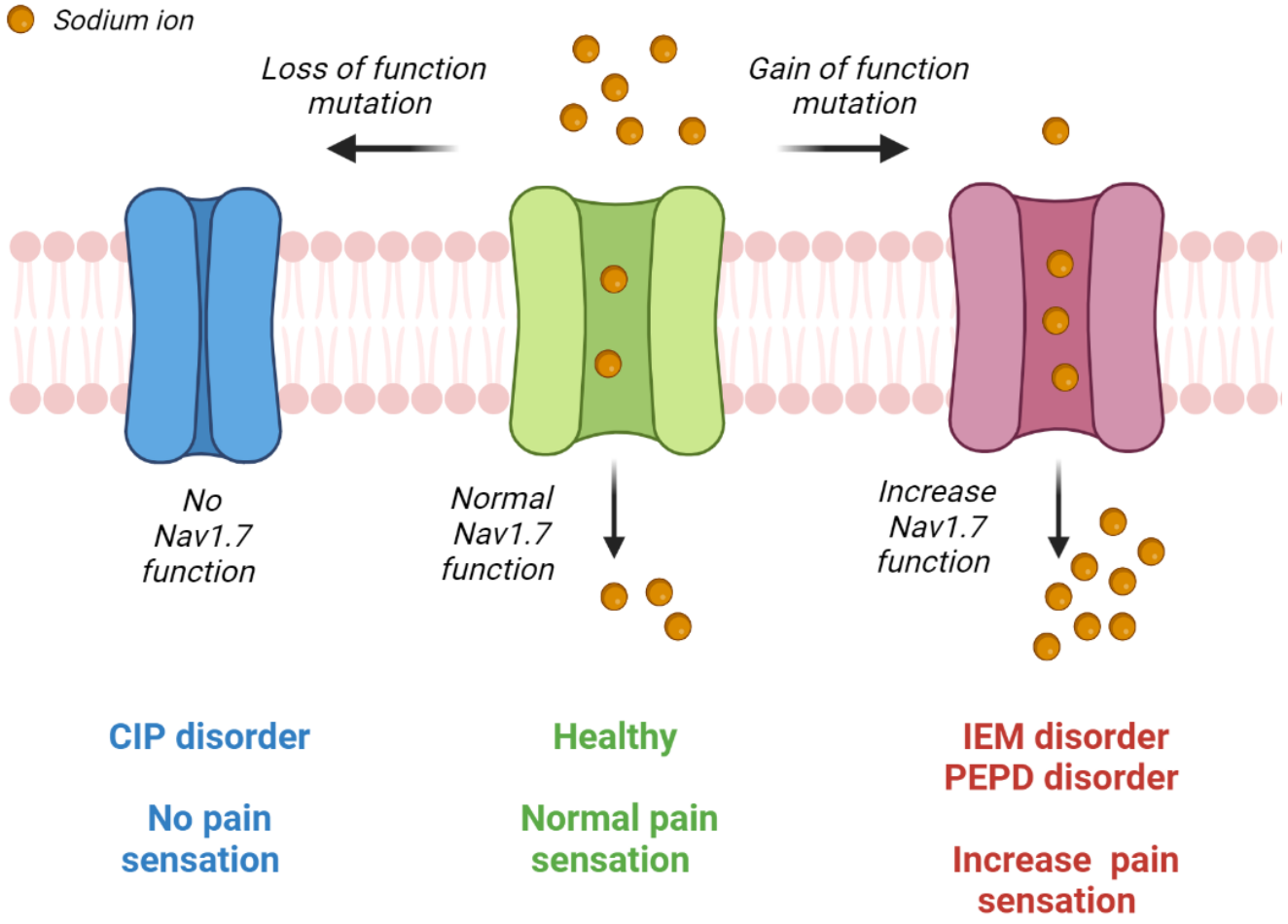
Manifests as burning and stabbing feeling in the **feet** and **hands**

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

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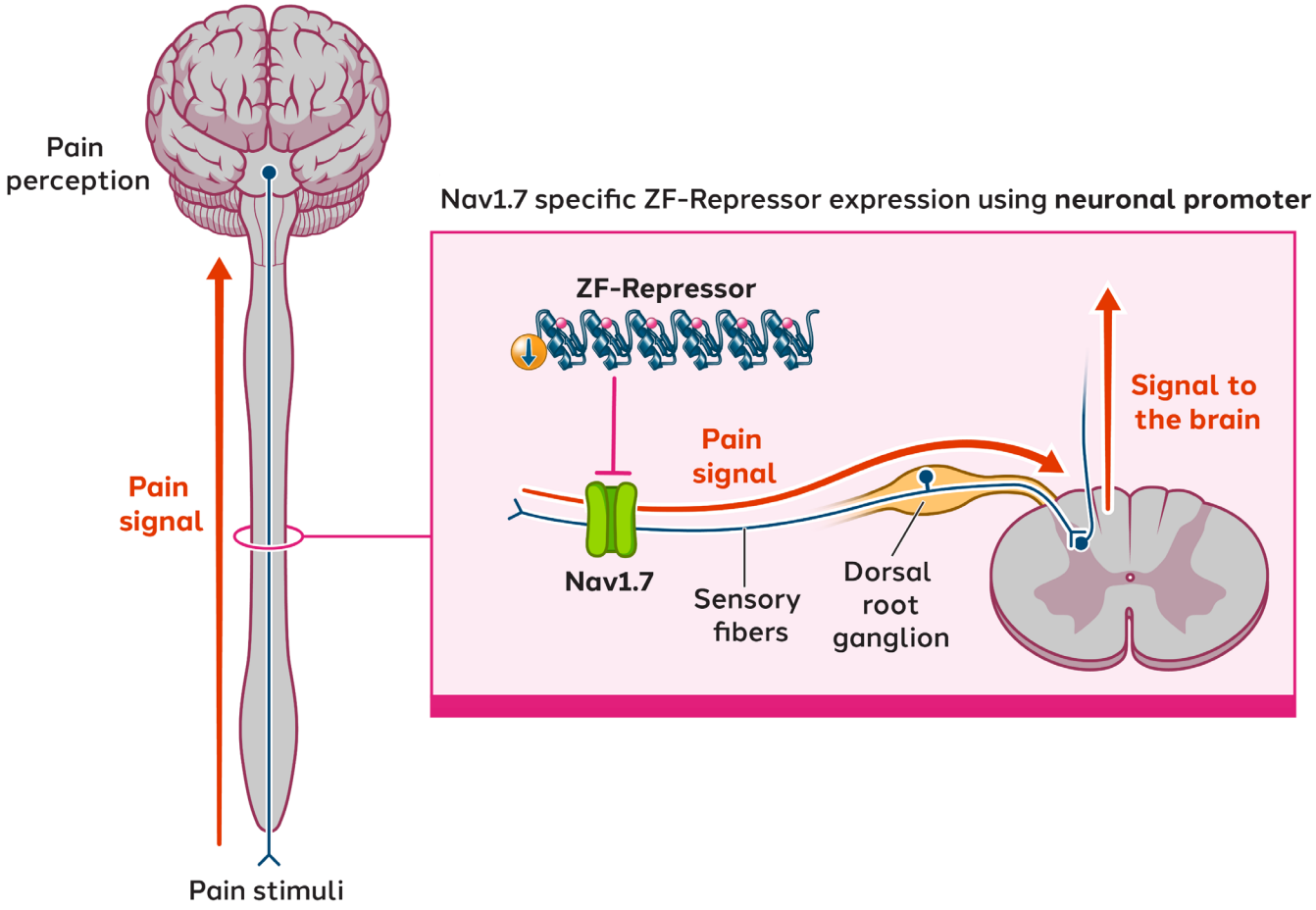
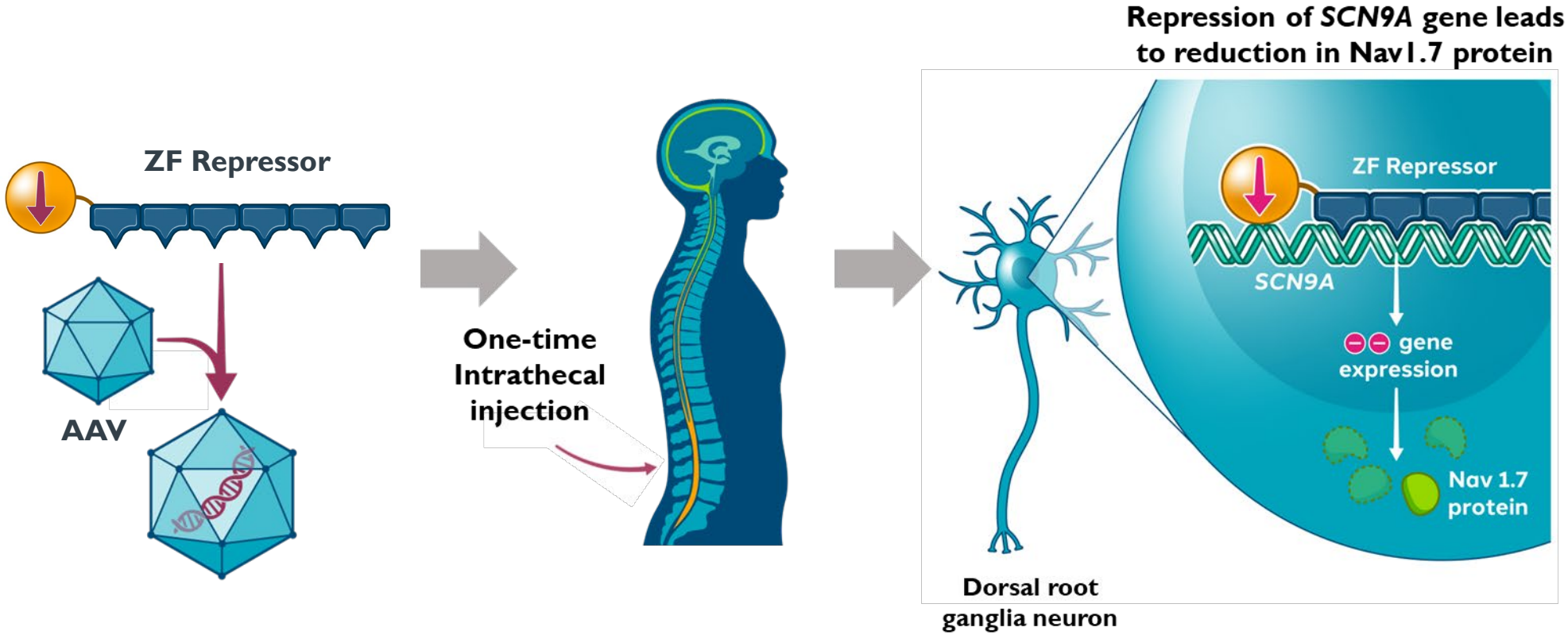
Blocking pain transmission to the brain has the potential to treat multiple pain indications



- 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**

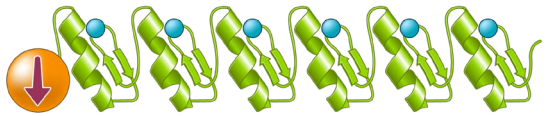
Zinc finger-mediated repression of Nav1.7 as a potent and specific therapeutic avenue for neuropathic pain



ZF-repressors (ZF-Rs) are derived from human transcription factors that reduce target gene expression without inducing DNA breaks

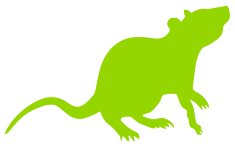
Developmental path to identify mouse and human ZF-repressors targeting the Nav1.7 gene

MOUSE ZF-Repressors (ZF-R)



Mouse Nav1.7 gene (*Scn9a*)

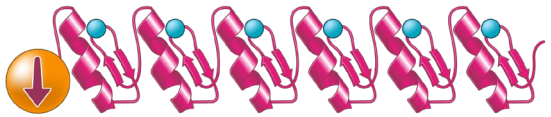
500+ 6-finger ZF-Rs designed & screened in neurons



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

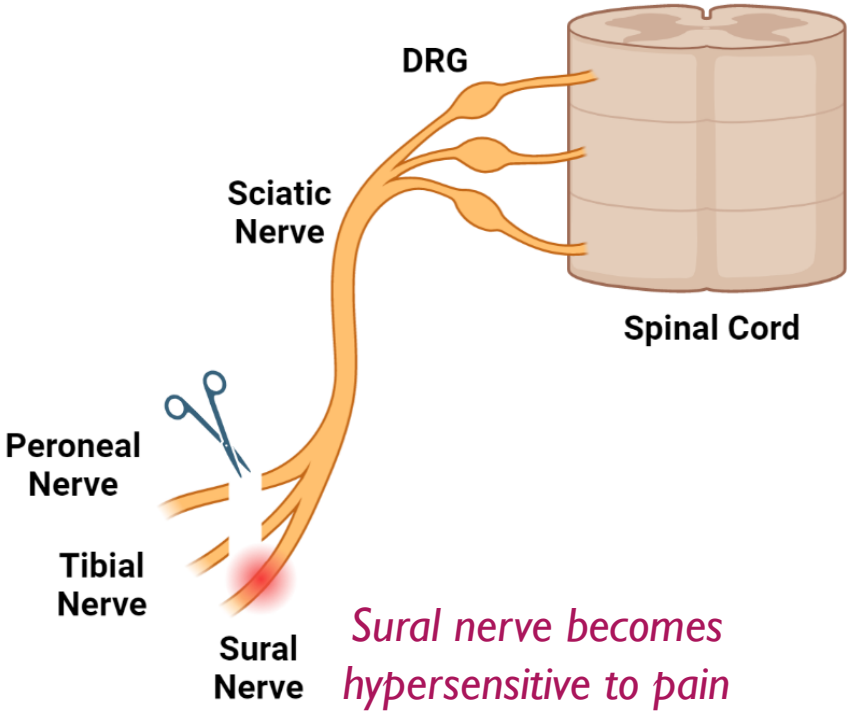
On-target engagement & safety in nonhuman primates (NHPs)

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

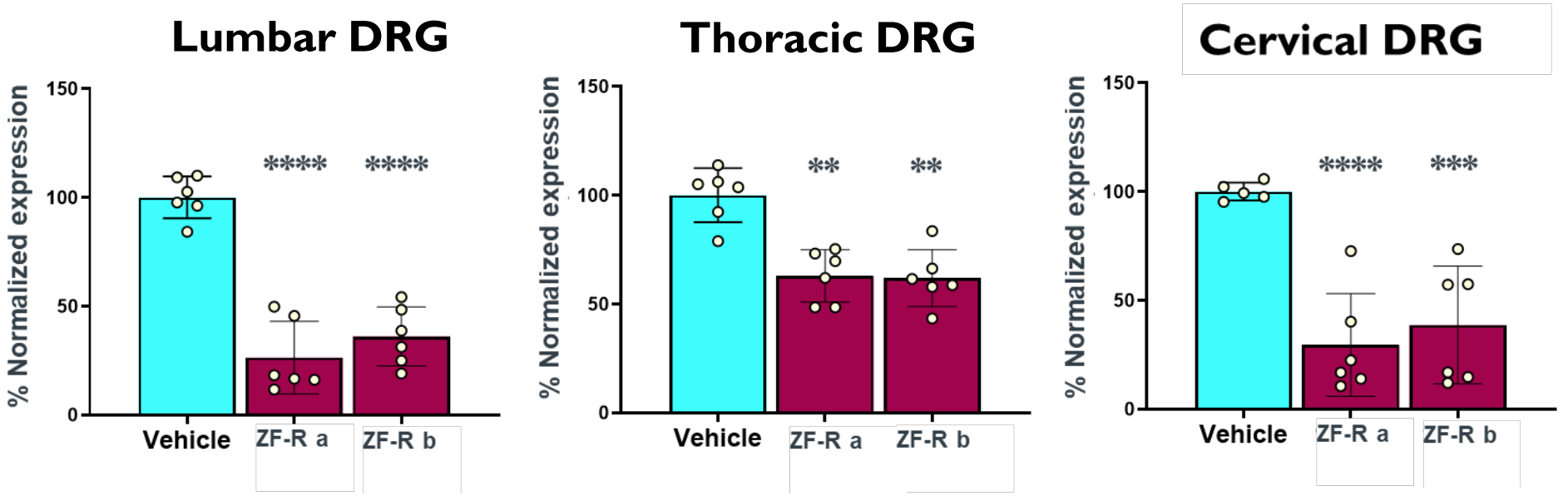
AAV

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

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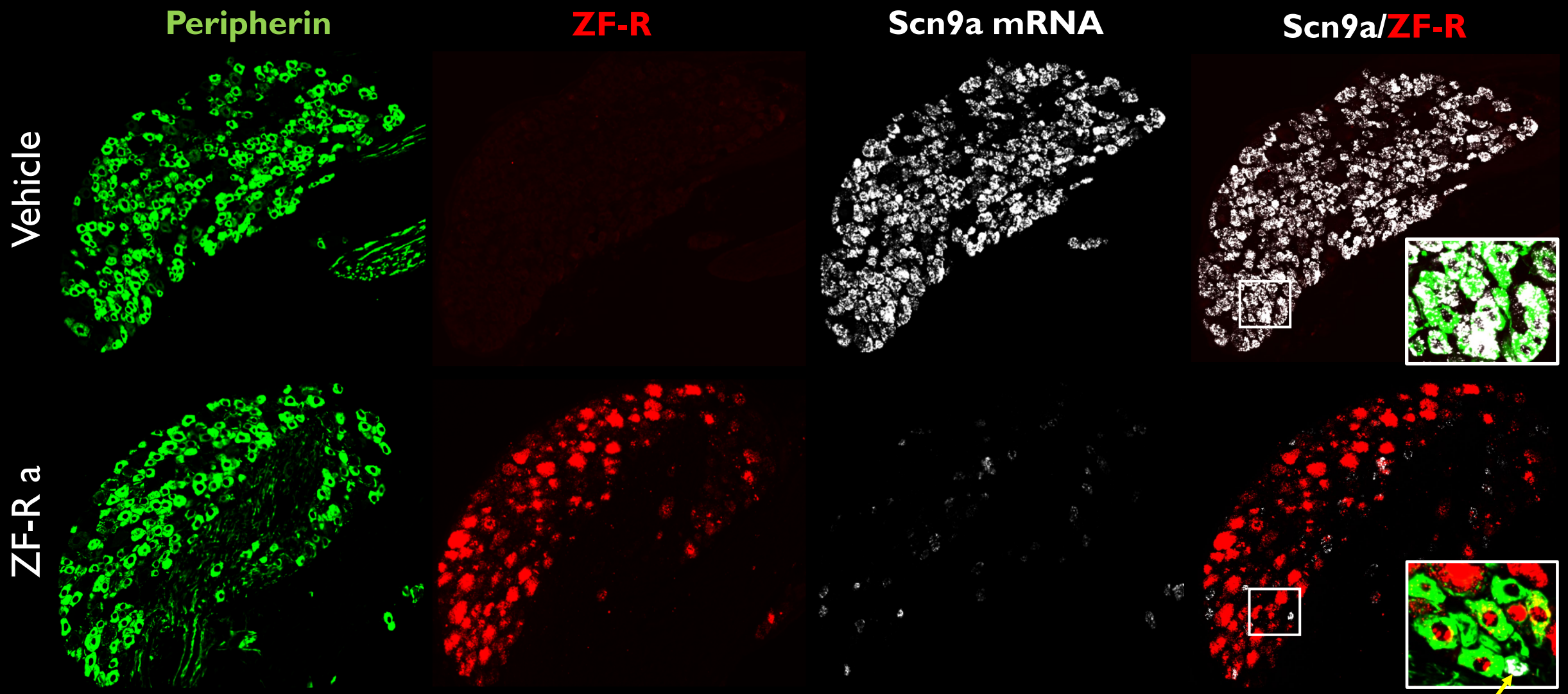
Potent repression of *Scn9a* mRNA in nociceptors of mouse lumbar DRG

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

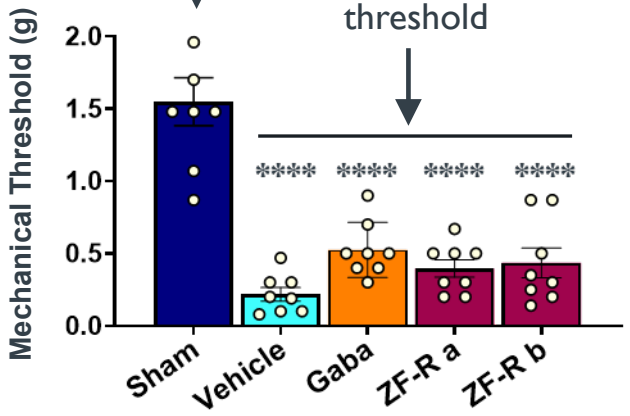
Mechanical induced pain

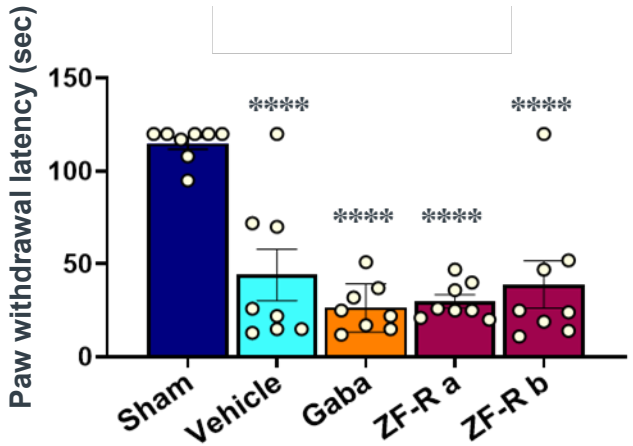
Cold induced pian

Before any Treatment

Normal pain threshold

reduced pain threshold





**** $P < 0.0001$ Compared to Sham

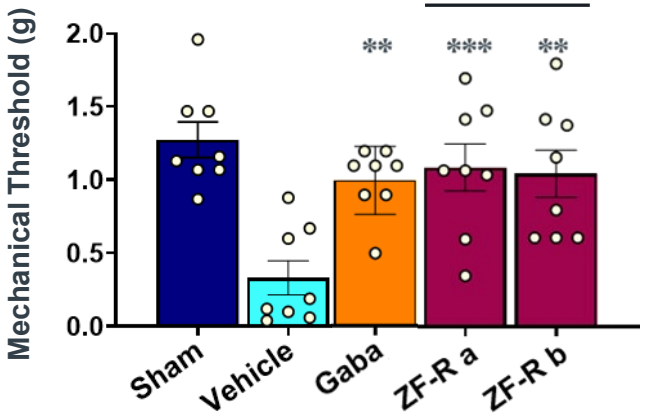
One-way ANOVA

ZF-R AAV

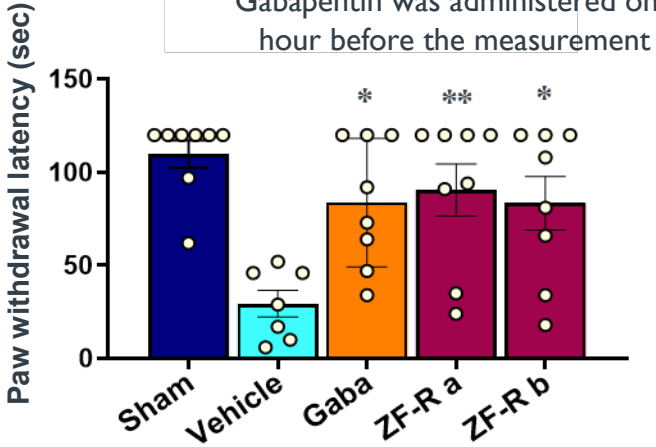
4 weeks

After Treatment

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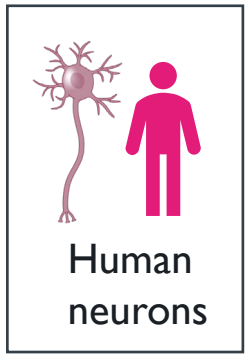
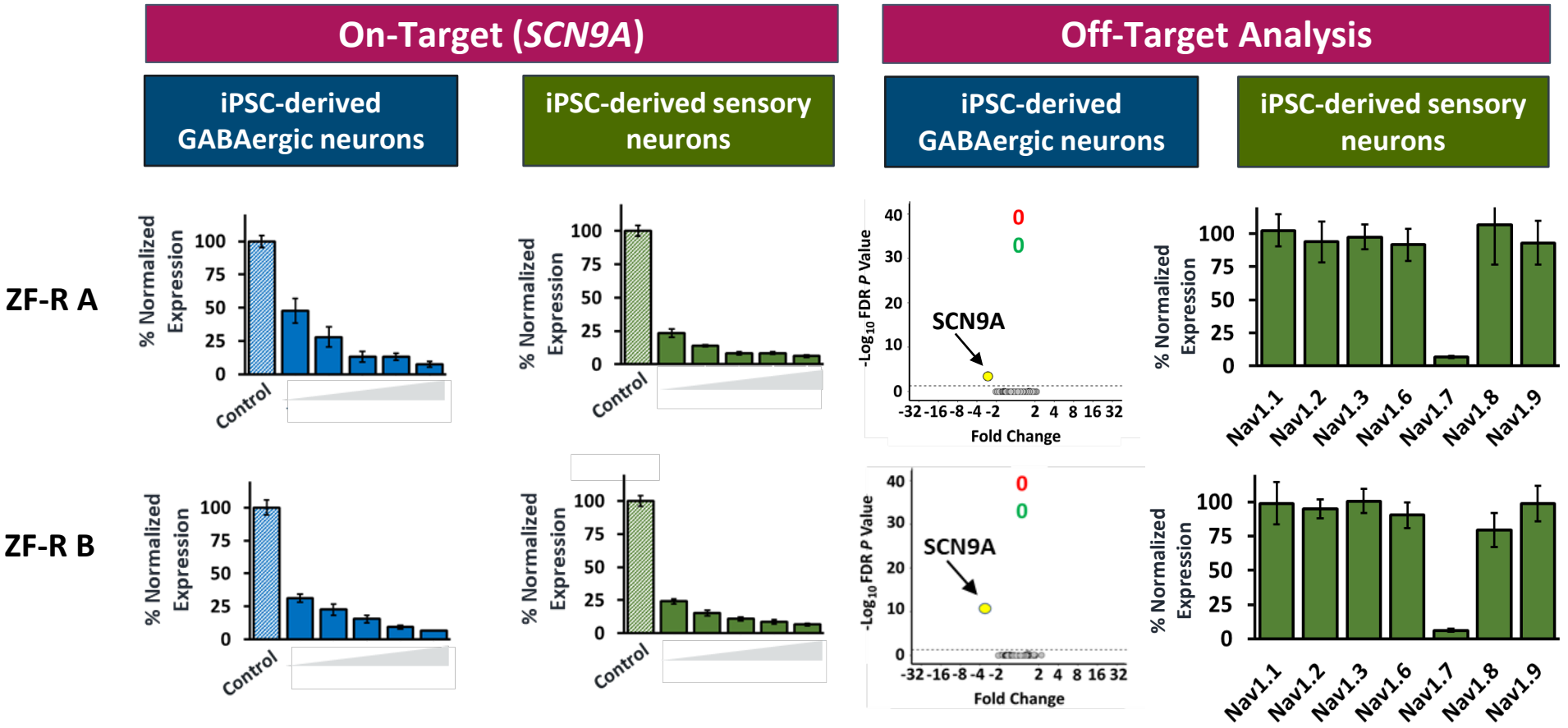
ZF-Rs repressed *SCN9A* by more than 90% over a wide dose range with no detectable off-target activity





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

Genes down-regulated
Genes up-regulated
SCN9A

MOI
3E3 1E4 3E4 1E5 3E5

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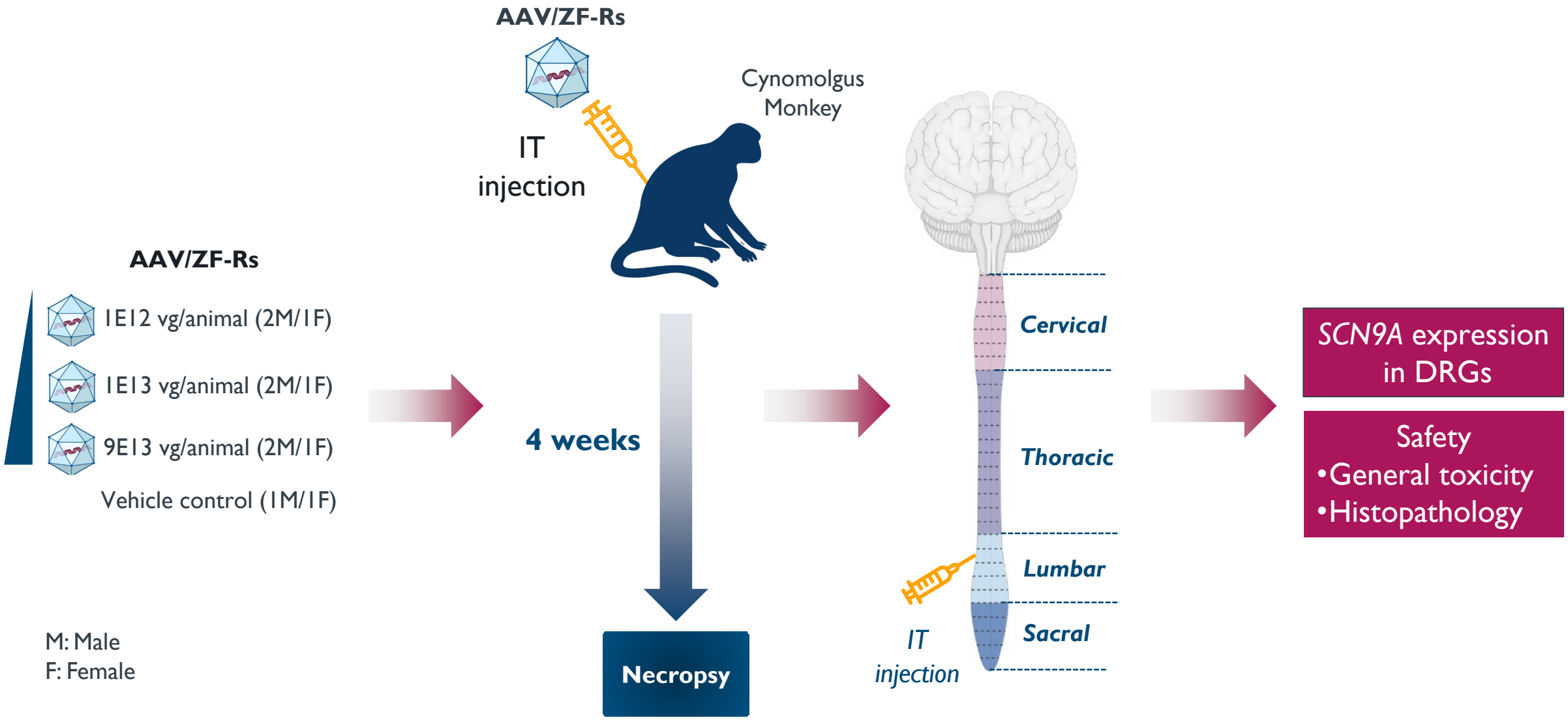
The potency and safety of the human ZF-R candidates were evaluated in nonhuman primates (NHPs)



M: Male
F: Female

Images made with Biorender.com

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

NHP

ZF-R

AAV

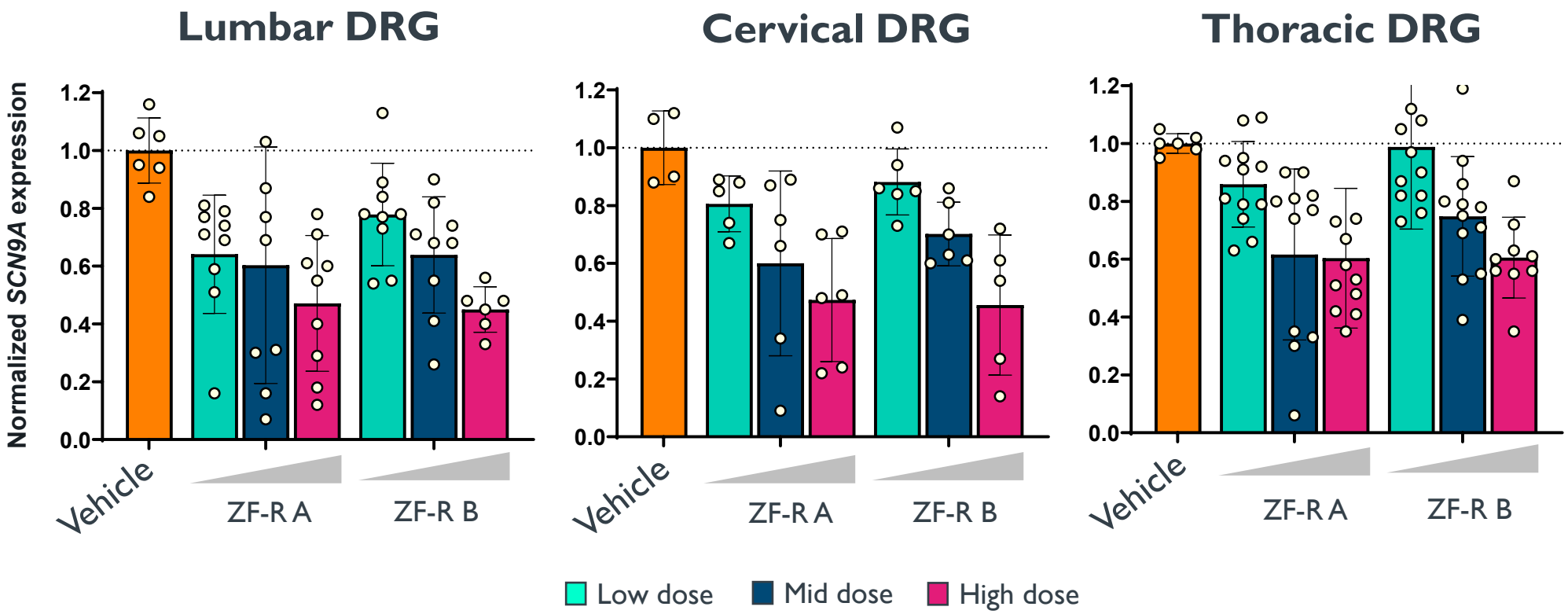
1E12 vg/NHP

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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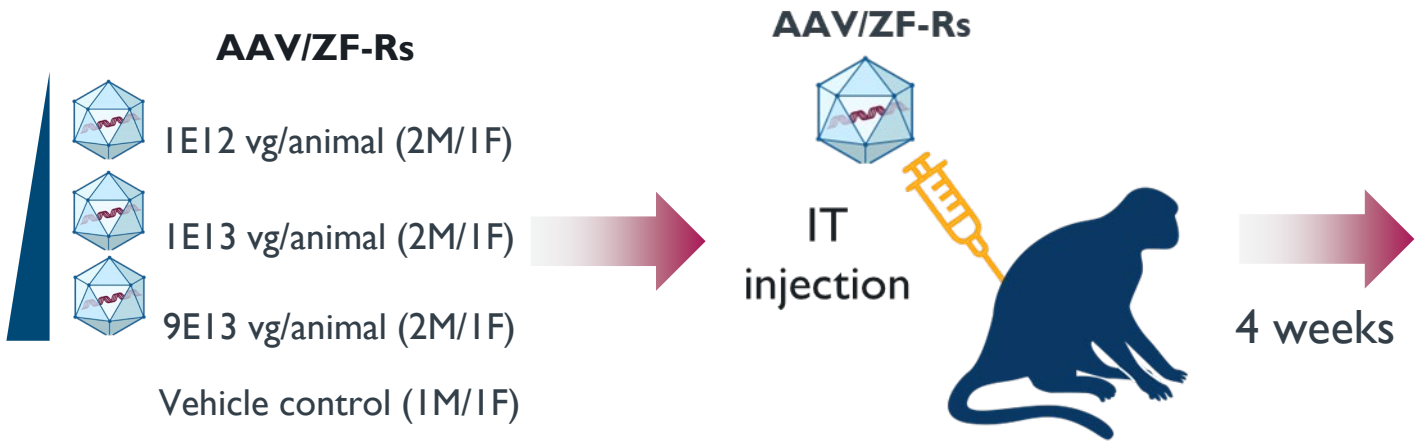
ZF-Rs were well tolerated in nonhuman primates



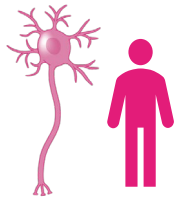
- ZF-Rs were well tolerated at all doses
- No overt toxicity was observed
- No adverse macroscopic or microscopic findings were observed

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

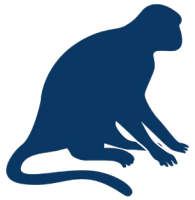
Additional NHP pharmacology, safety, and biodistribution results will be presented at a future meeting

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

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Associate Director, Neuroscience

Sangamo Therapeutics Inc.