HUMAN KCNQ2 BASE EDITING GENE THERAPY PRODUCES DURABLE ANALGESIA IN PRECLINICAL NEUROPATHIC PAIN

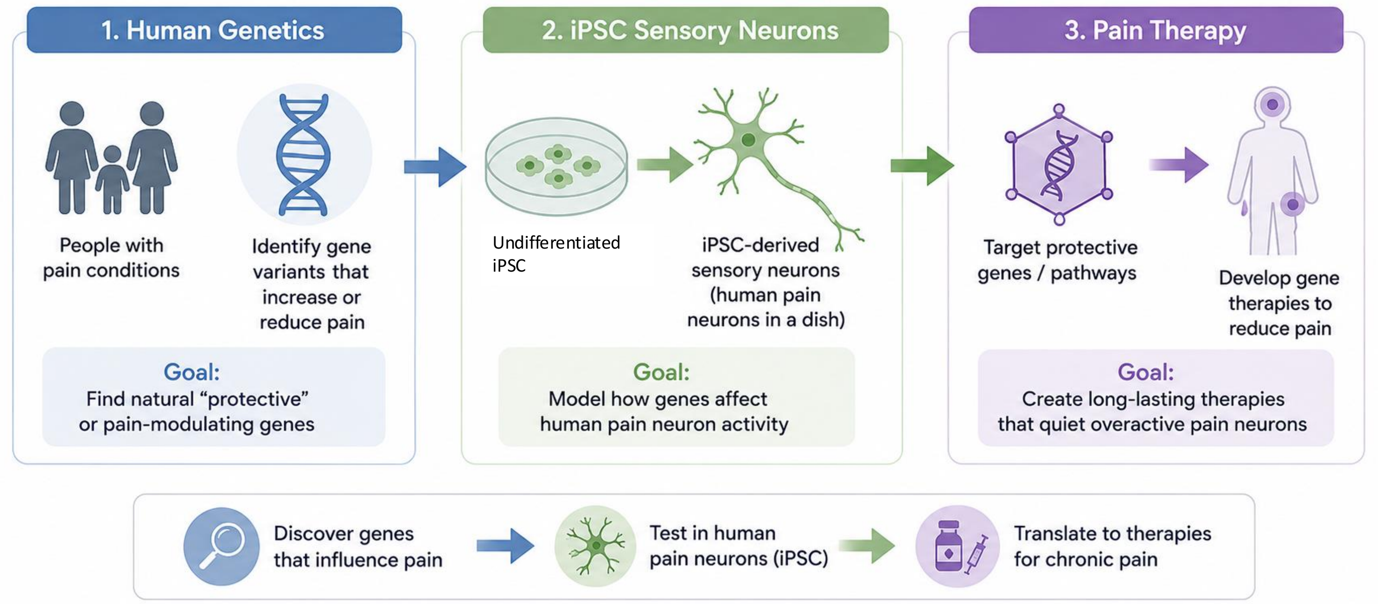
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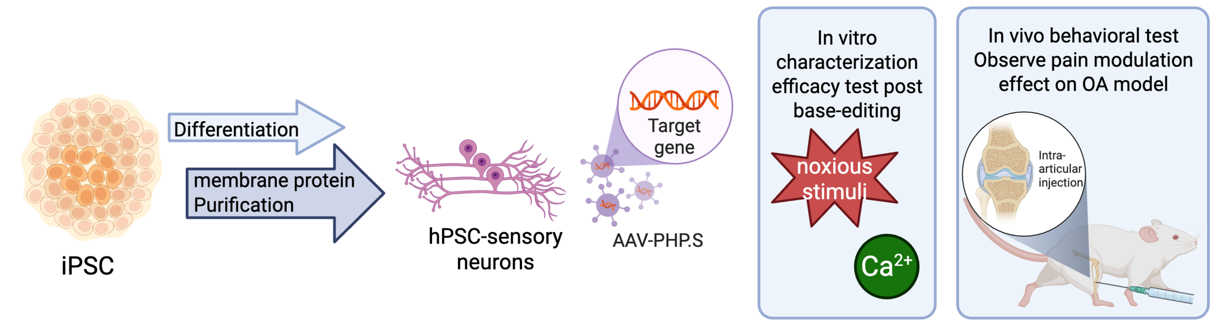
Introduction



- Human genetics reveals natural protection against pain.
- iPSC-derived sensory neurons enable human-relevant functional validation.
- SN102 targets KCNQ2/KV7.2 to quiet hyperexcitable sensory neurons.
- A durable, non-opioid gene therapy for trigeminal neuralgia.

Goals

1. Generate purifiable populations of TRPV1+, SCN9A+, and MRGPRX1+ sensory neurons from hPSC-sensory neurons.
2. Identify target gene base edit sequence for gene therapy.
3. Functional test on purified hPSC-nociceptor and in vivo pain model.



Methods/Results

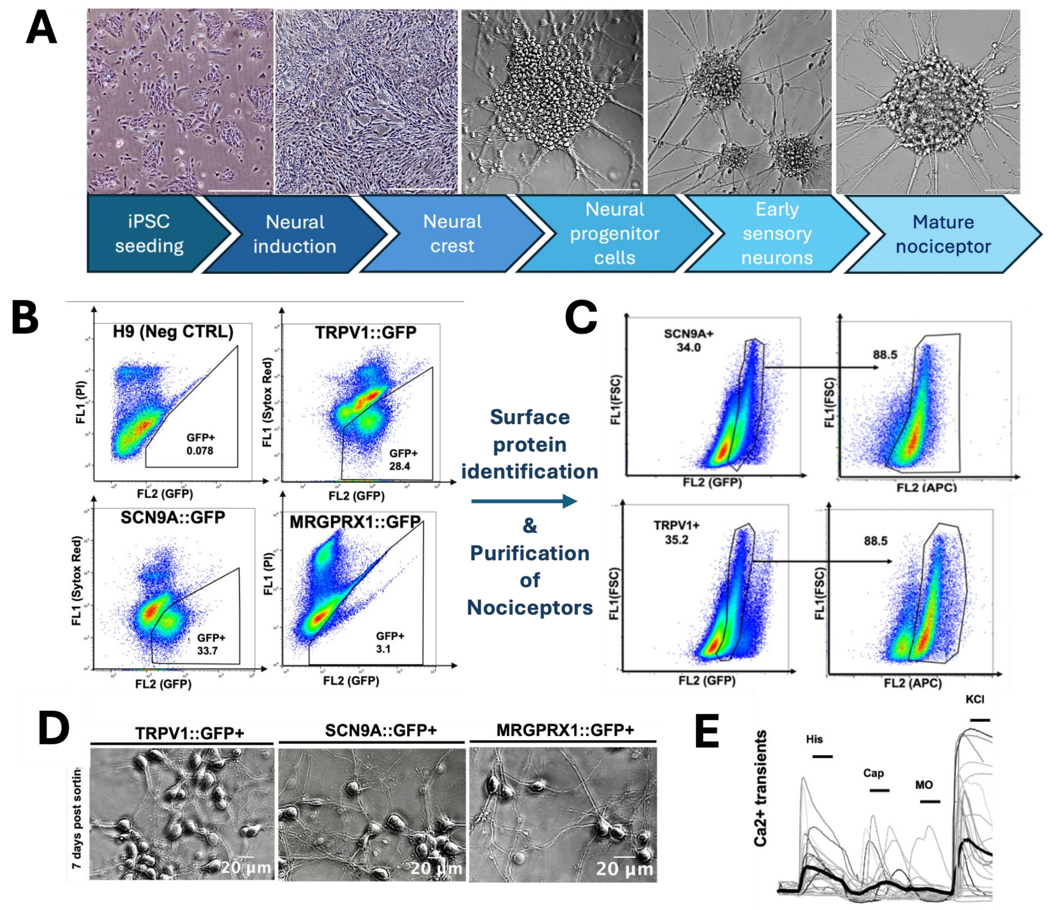


Figure 1. Differentiation of hPSC to sensory neuronal cells. **(A)** Representative images of each stages of differentiation and stepwise sensory neural differentiation to mature nociceptor cells. **(B)** TRPV1, SCN9A, and MRGPRX1 expression of hPSC-derived sensory neurons. **(C)** Identified specific cell surface marker for isolation of sensory neuron without GFP tagged genetic modification allows purification of nociceptor at >85% purity. **(D)** FACS purified sensory neurons with morphological maturation. **(E)** Response of FACS-purified iPSC-SNs to histamine (His), mustard oil (MO) and capsaicin (Cap). Functional polymodality of TRPV1+, SCN9A+, and MRGPRX1+ hPSC sensory neurons.

Results

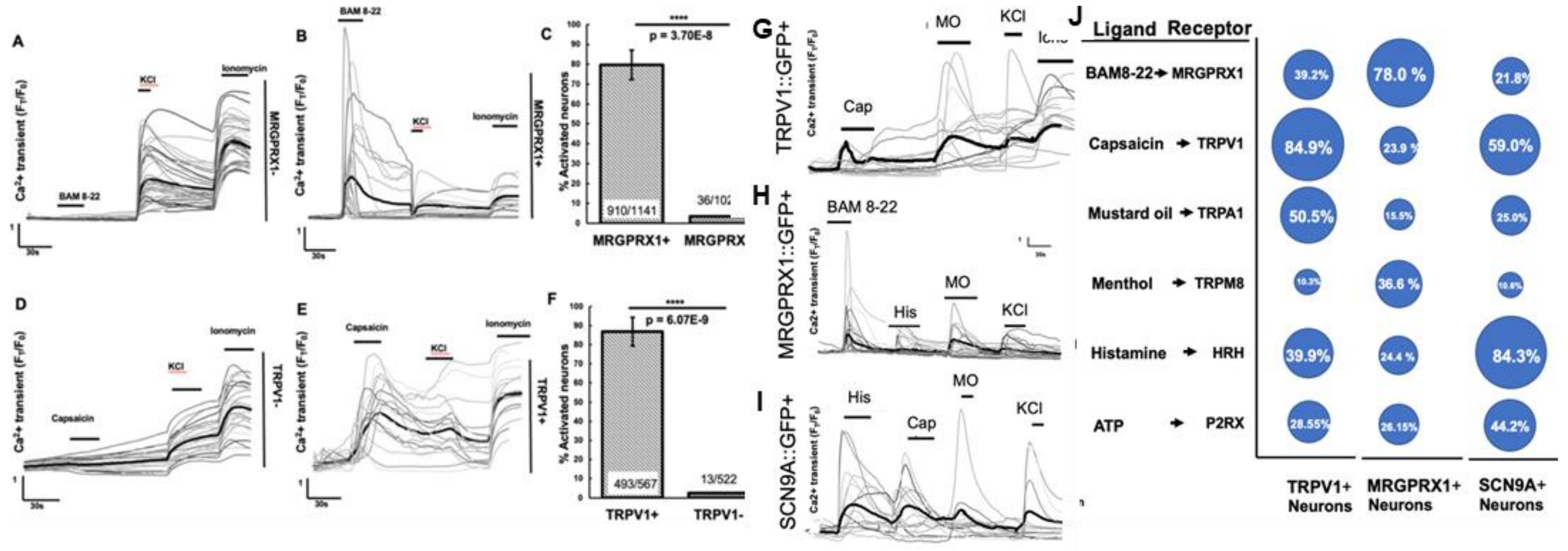
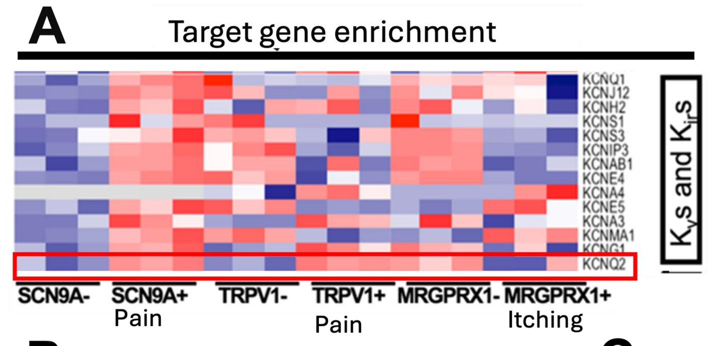


Figure 2. FACS-purified MRGPRX1+, SCN9A+ and TRPV1+ iPSC-nociceptors respond to various pain- and itch-inducing ligands. **(A-C)** MRGPRX1- (A) and MRGPRX1+ (B) neurons (N=1141) displayed differential responses to BAM 8-22 (10μM) (C). **(D-F)** TRPV1- cells (D) and TRPV1+ neurons (N=567) (E) displayed significantly different responses to capsaicin (10μM) (F). Cell counts were pooled from N = 5 experiments. **(G-I)** Polymodality of iPSC-derived sensory neurons (N=459 TRPV1, 665 MRGPRX1 and 575 SCN9A neurons) were characterized by their response profiles to capsaicin (10μM), mustard oil (100μM), BAM 8-22, histamine (50μM), menthol (100μM), and ATP (50μM). **(J)** Percentages of cells expressing MRGPRX1, TRPV1, TRPA1, TRPM8, HRH, and P2RX receptors that respond after stimulation. F&C: Data are represented as mean ± s.e.m.



	Log_2 Fold Change	FDR-adjusted p-value
SCN9A+ vs. SCN9A-	2.05	9E-12
TRPV1+ vs. TRPV1-	2.81	3.9E-22
MRGPRX1+ vs. MRGPRX1-	-3.29	6.76E-29

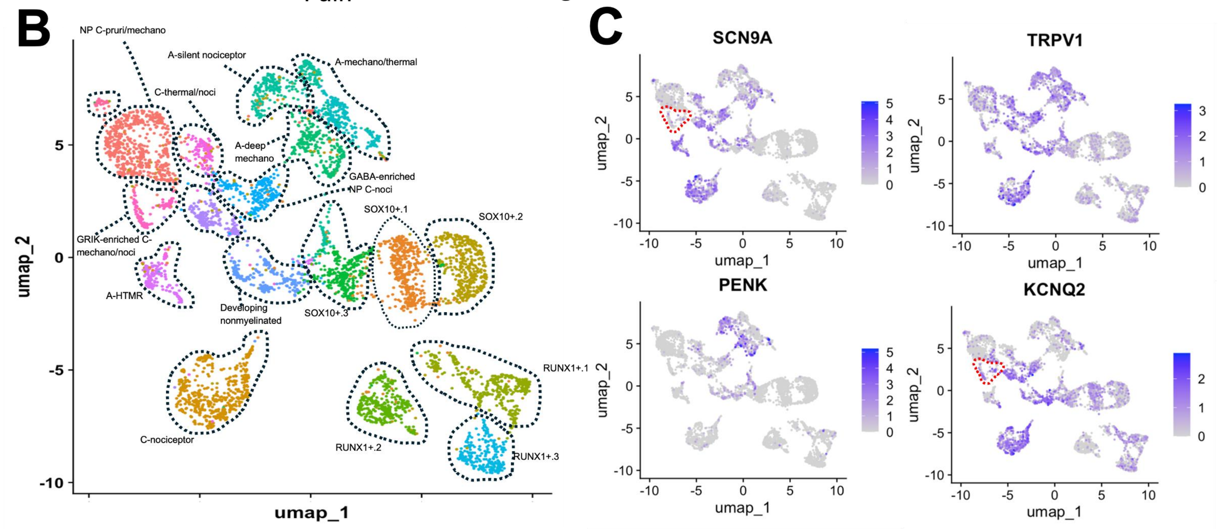


Figure 3. Identification of the target gene confers robust pain-resilience effects in purified iPSC-nociceptors. **(A)** Bulk RNA-seq shows enrichment of target gene *[Ref 3, 4]* in SCN9A and TRPV1 pain sensing neurons but not in MRGPRX1 itching sensing neurons. Fold change and p-values of the target genes in nociceptive and pruriceptive neurons. **(B)** scRNA-seq data shown the diverse sub-populations of sensory neurons. **(C)** The nociceptive neurons of SCN9A and TRPV1 cluster with the target gene of interest but not with proenkephalin (PENK) that produces peptides binds to opioid receptors.

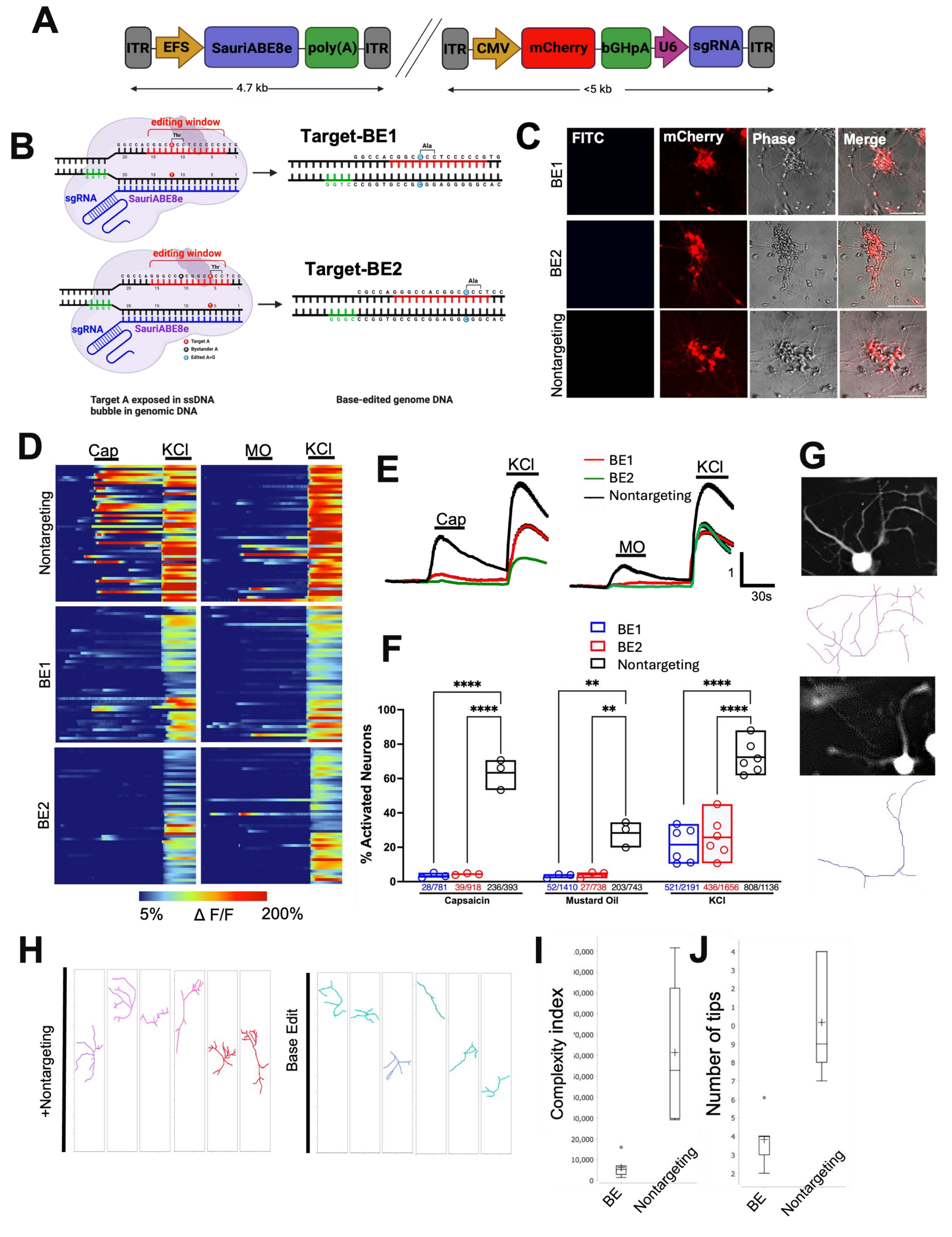


Figure 4. Target gene base editor (BE) AAV PHP.S design for the specificity and functional output on iPSC-nociceptors. **(A)** A dual-AAV PHP.S *[Ref 5, 6]* delivery system to install target gene in purified nociceptors. **(B)** Two CRISPR base-editors (BE) of target gene editors at protospacer position 11 and 6 of the editing windows. **(C)** BE1 and BE2 transduction visualized via mCherry in nociceptive neurons. **(D)** Heatmap to represent Ca2+ transient of neurons harboring nontargeting sgRNA, BE1 and BE2. (n=50) **(E)** Reduced activation in BE1 and BE2 nociceptors when measured upon the noxious stimuli of capsaicin (Cap) and mustard oil (MO). Shown in quantified data in **(F).** **(G)** Representative image to measure the neurite extension of nontargeting (top panel, magenta) vs base edited nociceptor (bottom panel, blue) (H) 6 nociceptive neurons with nontargeting sgRNA vs base edited shown for measurement of **(I)** complexity index and **(J)** number of neurite tips.

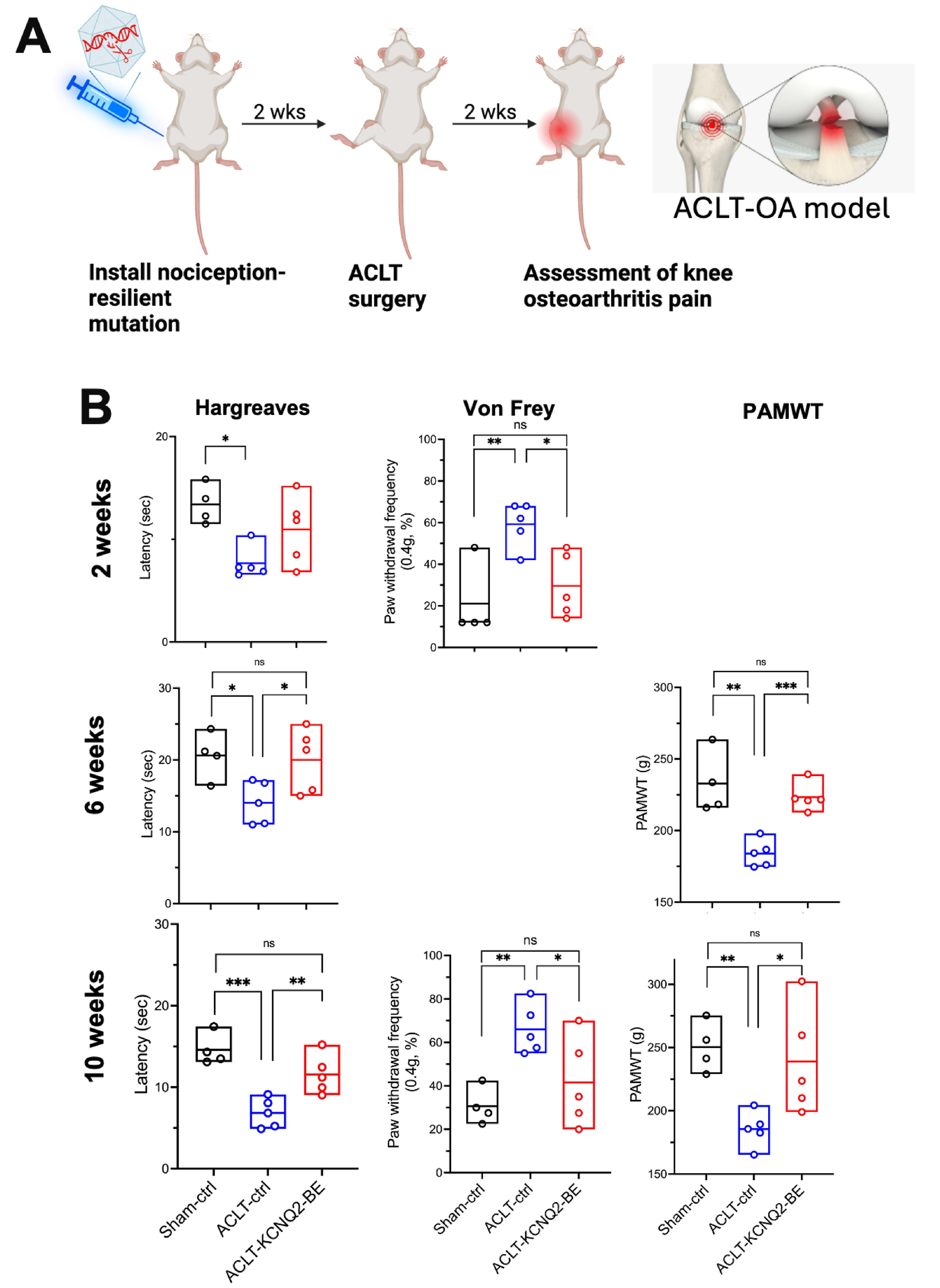


Figure 5. SN102 reduces pain and improved the behavioral test in murine knee pain model. **(A)** The schematic figure to introduce in vivo experiments. AAV PHP.S delivered to intra articular space before anterior cruciate ligament transection (ACLT) osteoarthritis pain model. **(B)** Two-, six-, and ten-week post ACRT surgery, the pain was analyzed by Hargreaves (thermal pain sensitivity), Von Frey (touch and mechanical sensitivity), and PAMWT (Paw Angle Measurement While Walking, pain related change related posture and gait) demonstrating that the SN102 treated animal functionally compatible to the sham surgery control animal.

Conclusions

- We established a nociceptor platform using hPSC-derived sensory neurons that faithfully recapitulate key molecular and functional features of human DRG subtypes.
- Transcriptomic and functional profiling identified target gene to introduce CRISPR-Cas9–mediated of a protective mutation via dual-AAV PHP.S conferred robust pain resilience in vitro and in a mouse osteoarthritis model.
- This work highlights a subtype-targeted gene therapy approach for chronic pain, offering a translational path toward precision analgesia.
- Future direction: In vivo TN murine model.

References

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