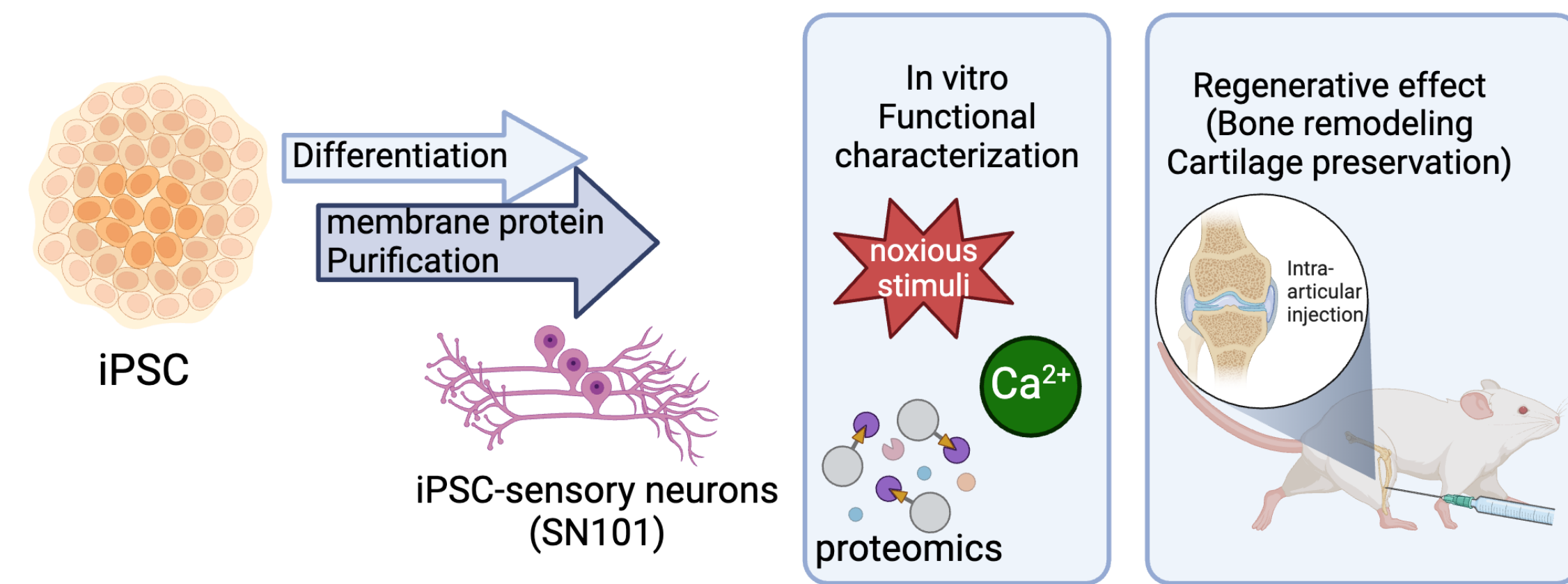


Introduction

- Over one-third of the world's population experiences pain, but current pain drugs often cause side effects by targeting non-specific pathways.
- Nociceptive neurons are diverse, with different ion channels and receptors, making it hard to understand pain mechanisms.
- Human pluripotent stem cells (hPSCs) offer potential for targeted therapies, but challenges remain in defining and purifying specific neuron subtypes.

Goals

- Generate purifiable populations of TRPV1+, SCN9A+, and MRGPRX1+ sensory neurons from hPSC-sensory neurons (SN101).
- Construct a transcriptomic continuum of developing hPSC-derived primary afferents and validate molecular signatures of SN101.
- Identify a novel surface marker for nociceptors, enabling purification without genetic modification.



Methods/Results

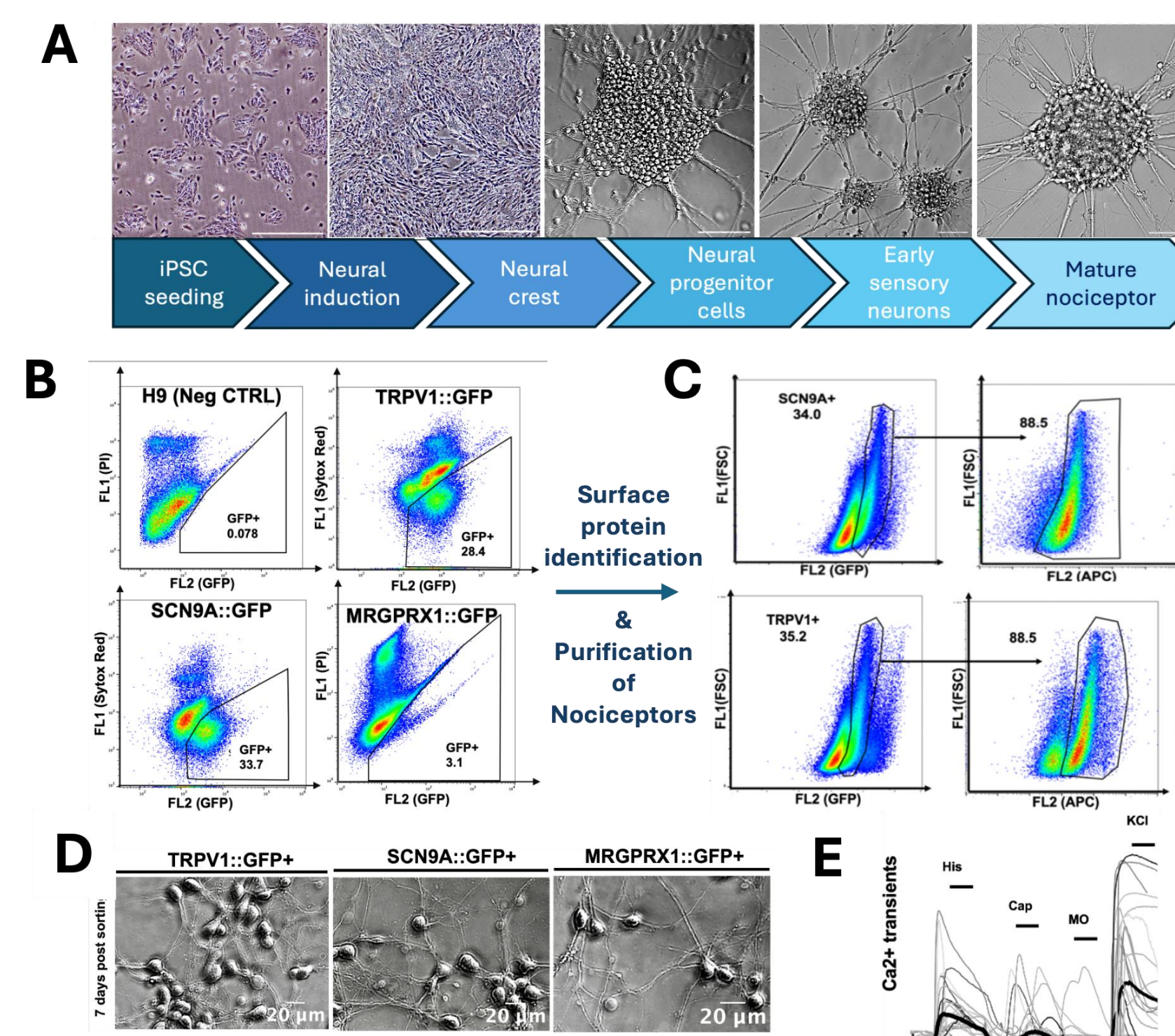


Figure 1. Differentiation of hPSC to sensory neuronal cells. (A) Representative images of each stages of differentiation and stepwise sensory neural differentiation to mature nociceptor cells. (B) TRPV1, SCN9A, and MRGPRX1 expression of hPSC-derived sensory neurons. (C) Identified specific cell surface marker for isolation of sensory neuro 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 iPS-SNs to histamine (His), mustard oil (MO) and capsaicin (Cap). Functional polymodality of TRPV1+, SCN9A+, and MRGPRX1+ hPSC sensory neurons.

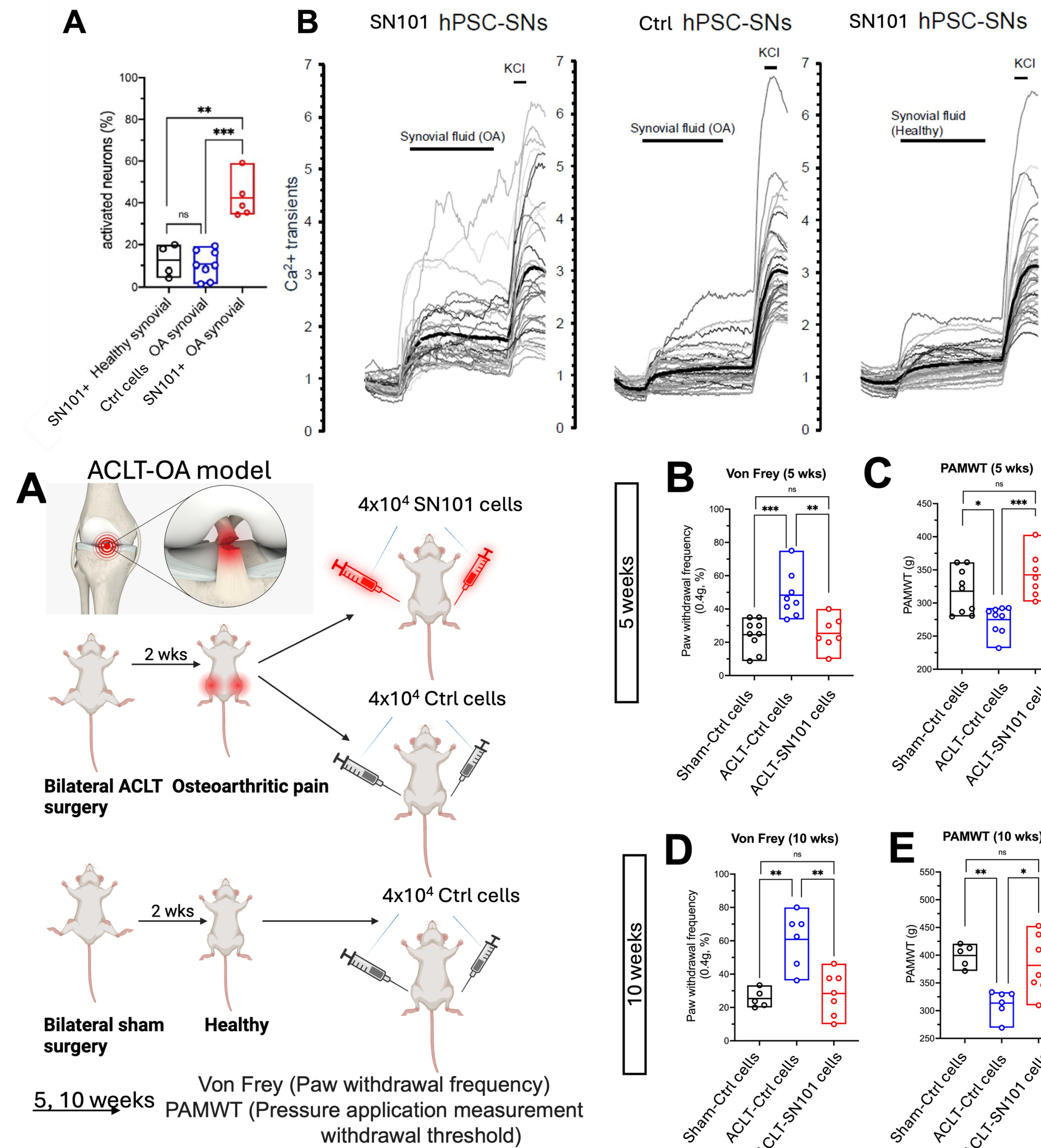


Figure 2. hPSC-sensory neurons are activated by human OA synovial fluid. (A) SN101 (Red) and counterpart of control cells (Blue) were administered with OA synovial or healthy controls (Black, far left). (B) Representative Ca²⁺ traces from SN101+OA synovial fluid, control cells + OA synovial fluid, and SN101 + healthy synovial fluid.

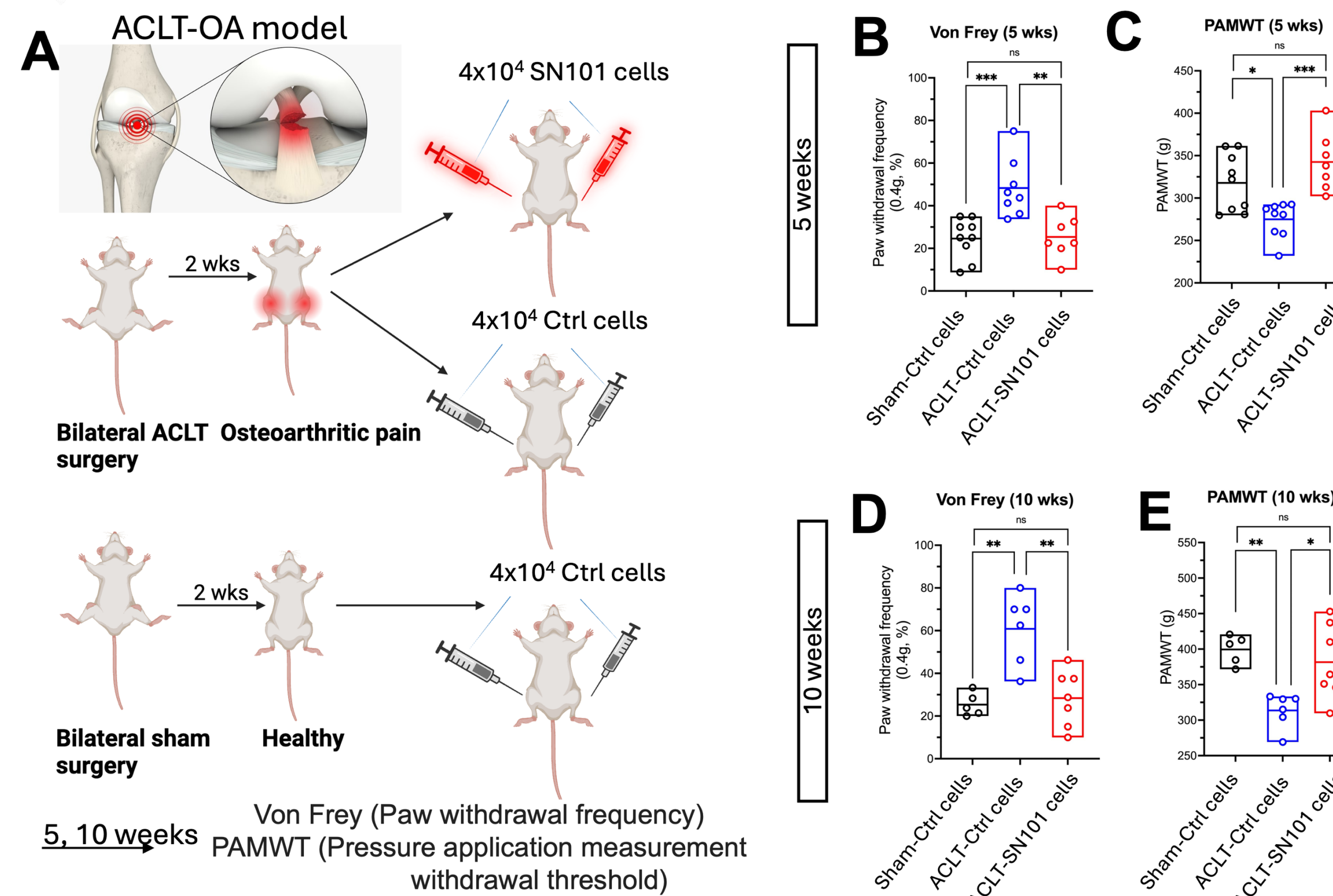


Figure 3. SN101 therapeutic effects in anterior cruciate ligament tear (ACLT)-OA mouse model. (A) Diagram shown the tear of ACL causing OA pathophenotype. Two weeks after ACLT surgery was performed to immune deficient mouse, SN101 was injected intra-articular space. (B,C) 5- and (D,E) 10-weeks post injection, behavioral tests shown by Von Fray (B,D), PAMWT (C,E) demonstrating that the ACLT-SN101 cell group has reduced pain behavioral compatible to the sham-surgery control group.

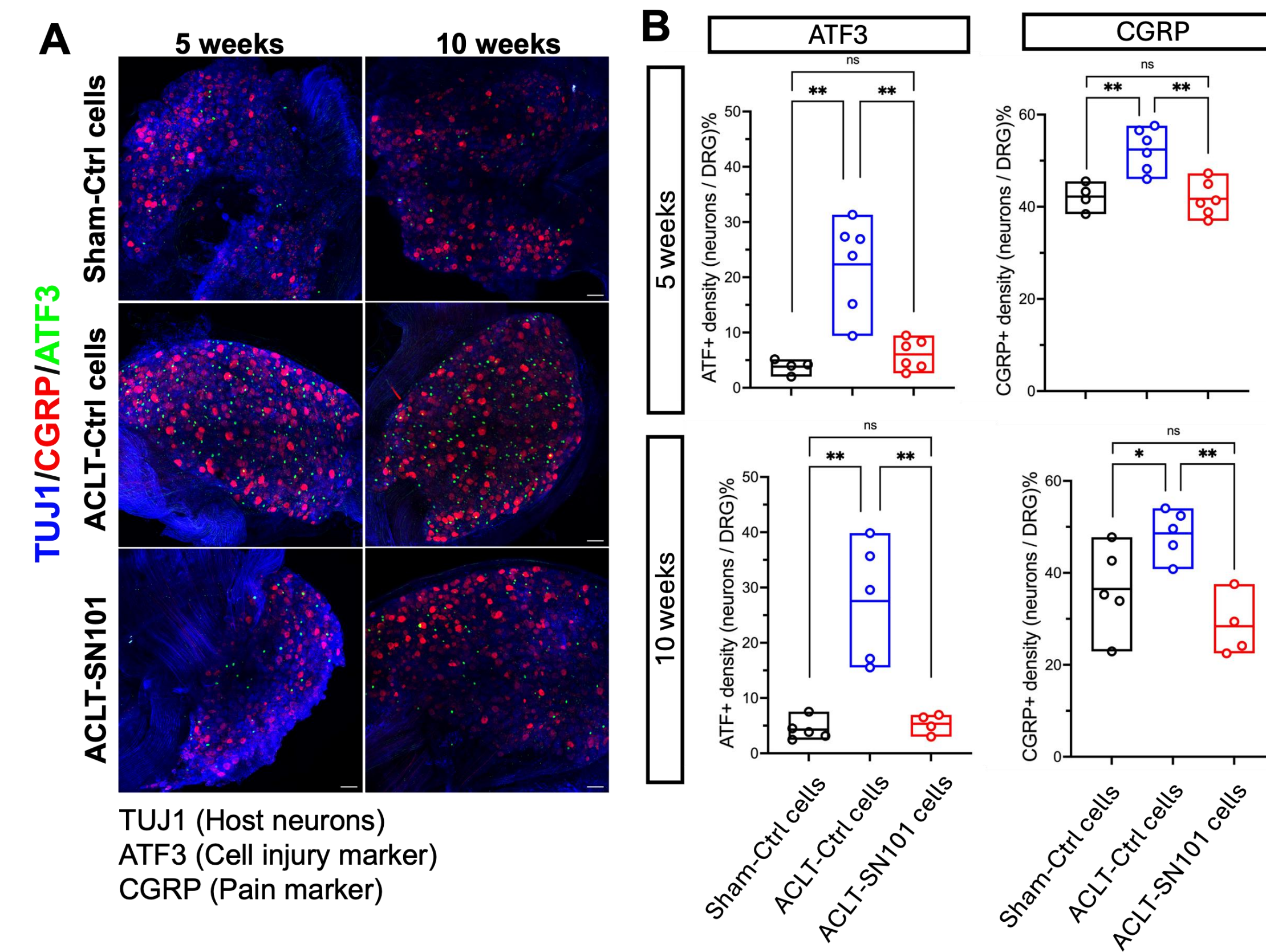


Figure 4. SN101 therapeutic effects in ACLT-OA mouse model. (A) Representative images of cross sectional view of dorsal root ganglion (DRG) region stained with TUJ1 (Blue, neurons), CGRP (Red, pain marker), and ATF3 (Green, cell injury marker). Scale bars are 10 μ m. (B) Quantification of ATF3 and CGRP at 5- and 10-week post SN101 or counterpart control cell injection demonstrated reduced pain and injury signals in DRG region. (C) Schematic image of DRG region where the neuronal signals received from knee joint innervated exons. Dashed line indicate the cross-sectional view of (A).

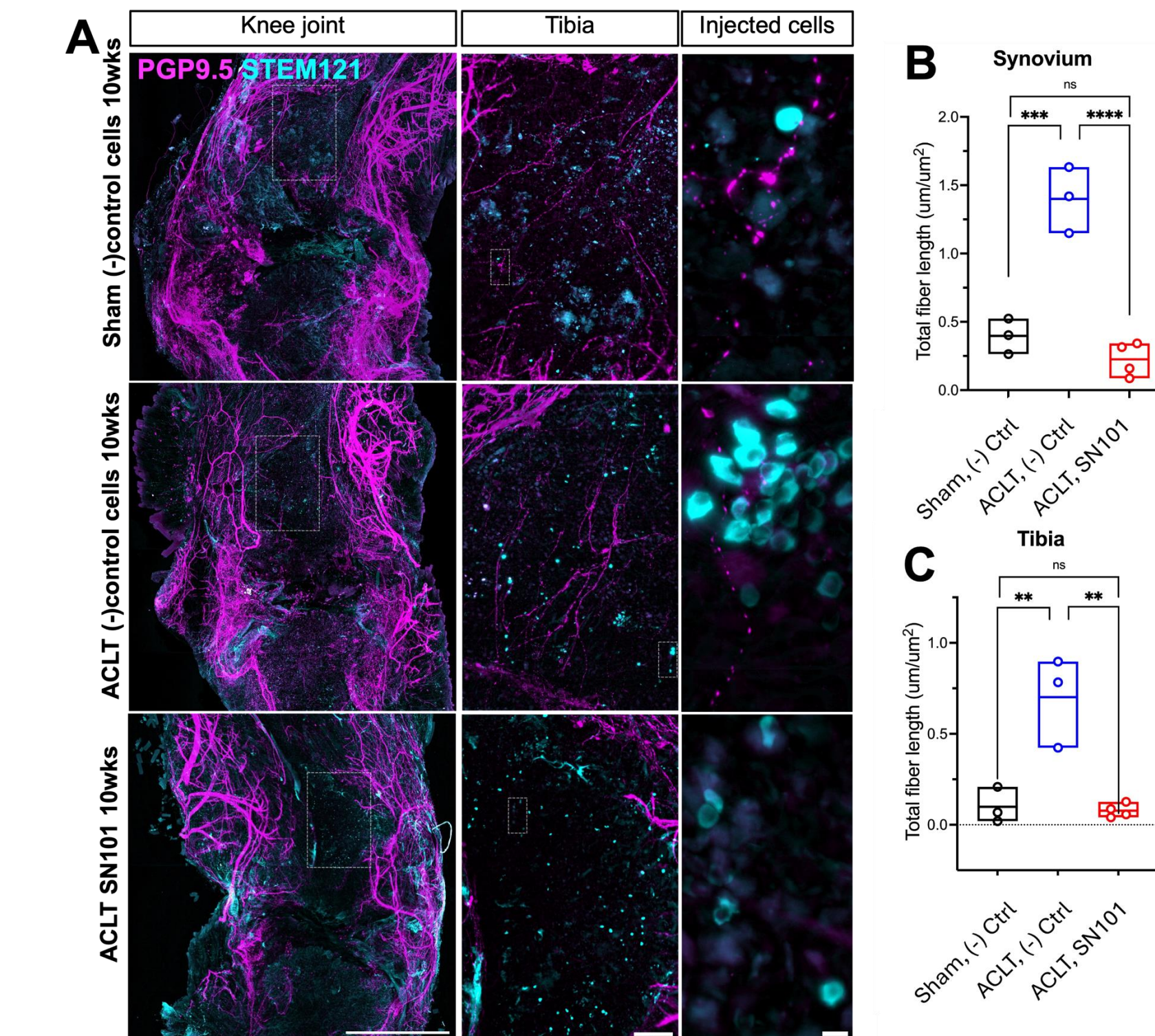


Figure 5. SN101 reduces innervation after 10 weeks post transplantation. (A) Light sheet staining of total knee after injection of SN101 or counterpart cells or (-) control cells in ACLT OA model. The host neurons are shown in PGP9.5 (Magenta) and human cells in STEM121 (Cyan). Scale bars are 1000 μ m (knee joint image), 100 μ m (Tibia) and 10 μ m (injected cells). The total fiber length were quantified in synovium (B) and tibia (C).

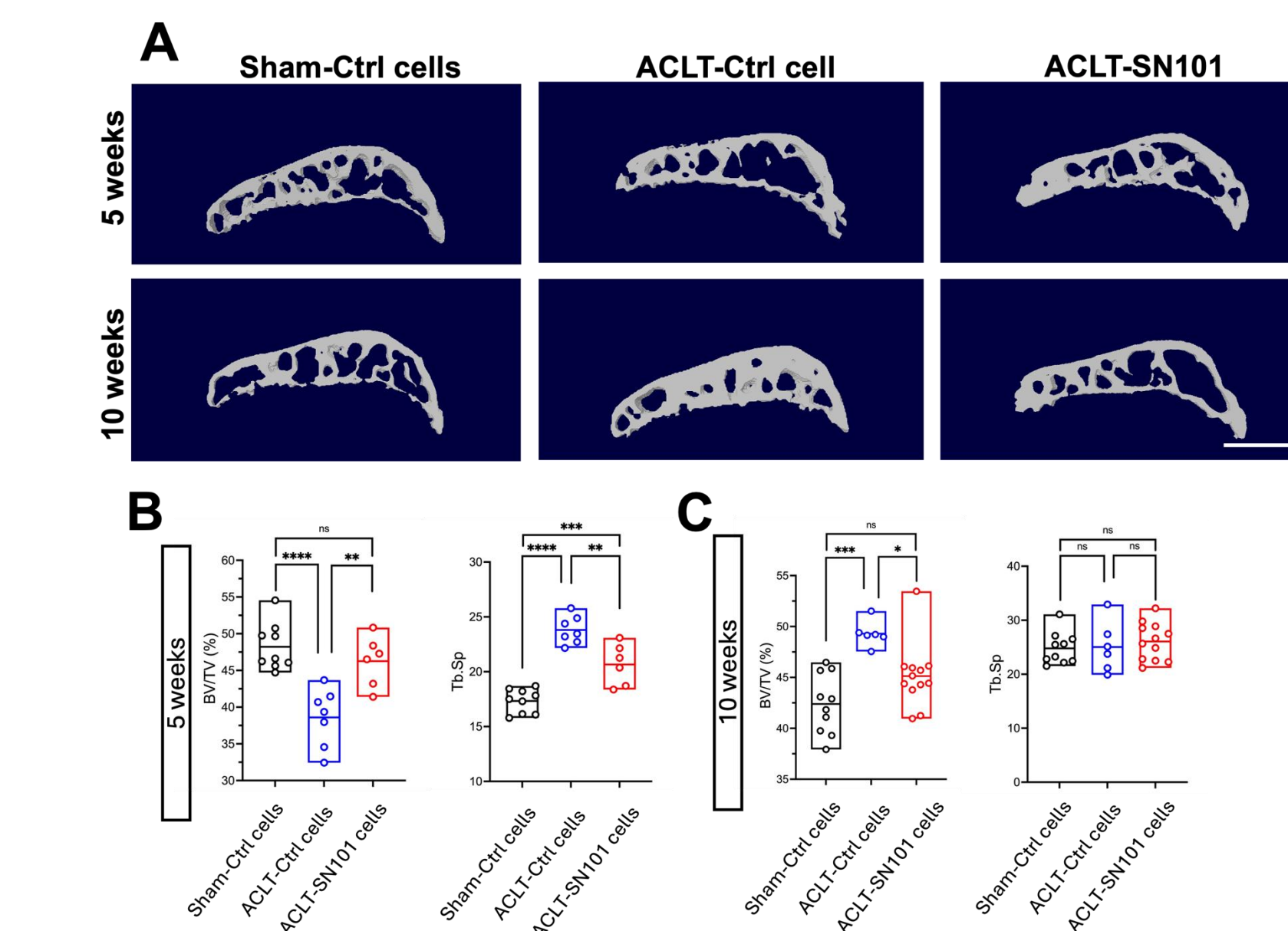
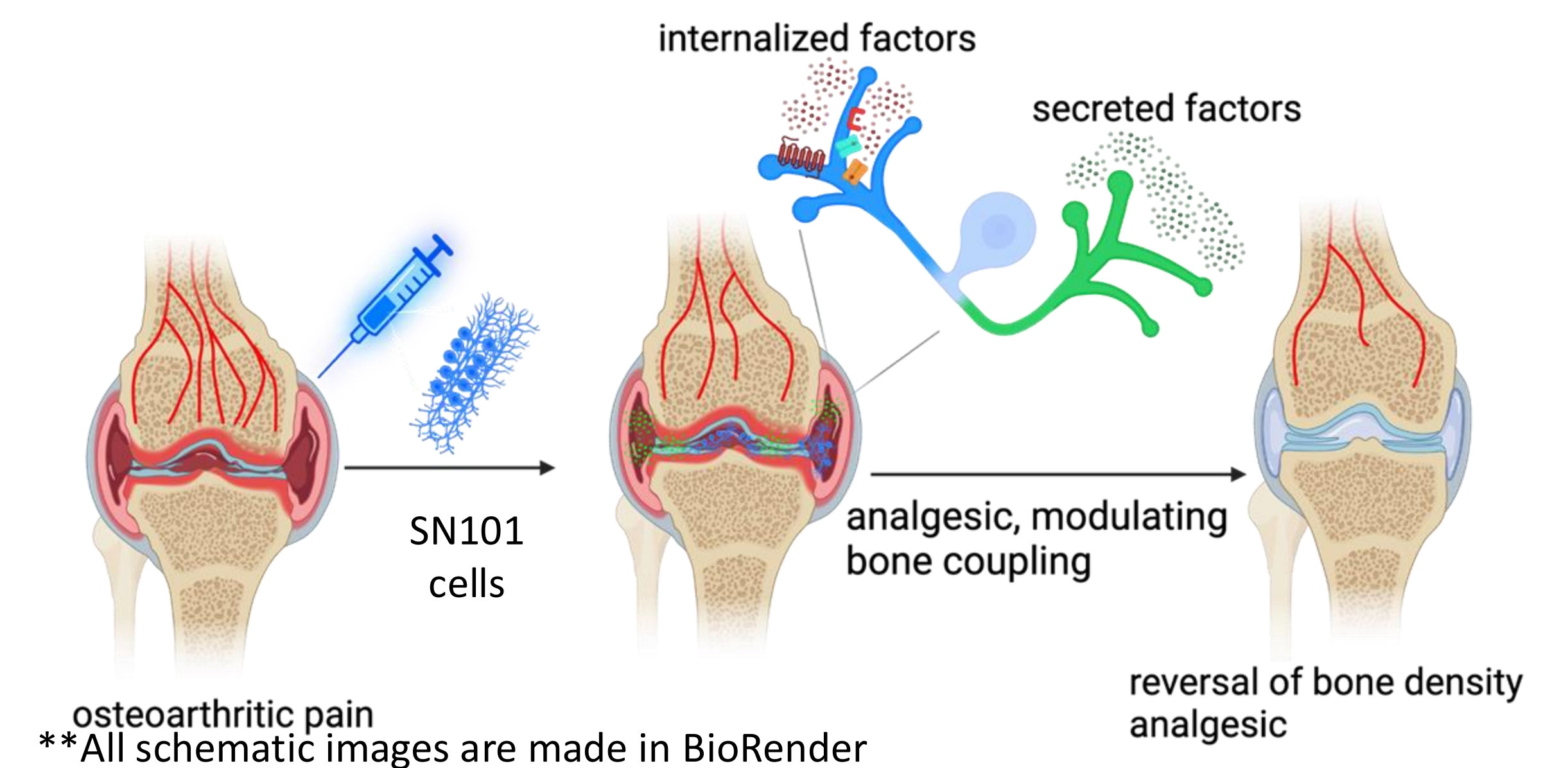


Figure 6. Bone remodeling effect of SN101. (A) micro-CT images shown trabecular bone post injection 5 and 10 weeks. (B,C) SN101 cell injected animal in OA model (ACLT-SN101) demonstrated improved knee joint bone parameters by trabecular bone volume (BV/TV) and trabecular separation (Tb.Sp).

Conclusions

Our results build a foundation to develop SN101 cell therapy for knee OA chronic pain as well as provide a discovery platform for discovering other therapeutic candidates for persistent pain/itch conditions.



osteoarthritic pain
*All schematic images are made in BioRender