Aim: to determine the functionality of split-SCN1A constructs

Approach: Transfect HEK293 cells with the following plasmid combinations: 1) split-SCN1A-GFP(1-10), 2) split-SCN1A-GFP(11), 3) split-SCN1A-GFP(1-10) and split-SCN1A-GFP(11), or 4) split-SCN1A-GFP(1-10), split-SCN1A-GFP(11), and pCD8-IRES-hB1(SCN1B). Split-SCN1A sequences were amplified from a codon optimized SCN1A plasmids that was kindly provided by Dr. Gavazzo. pCD8-IRES-hB1 plasmid encoding beta1b subunit was kindly provided by Dr. Al George. Beta subunits functions with alpha subunit in promoting sodium channel trafficking to the plasma membrane. Beta subunit was included in this experiment to test whether co-expression of SCN1B could alleviate trafficking defects of reconstituted SCN1A). Cells were fixed and processed for confocal imaging 48 hours after transfection.

Procedure:

1. Coverslips (18mm round, #1.5) were covered and coated with 100ug/mL of poly-d-lysine coating solution for an hour at room temp.
2. 2x10^5 HEK293 cells (passage 87-88) were seeded on each coverslip (fit in 12-well plate) the day before transfection
3. Cells were transfected with Qiagen Effectene at the following ratios:
    a. 0.3ug of split-SCN1A-GFP(1-10)
    b. 0.3ug of split-SCN1A-GFP11
    c. 0.15ug of split-SCN1A-GFP(1-10) + 0.15ug of split-SCN1A-GFP11
    d. 0.1ug each of split-SCN1A-GFP(1-10), split-SCN1A-GFP11, and pCD8-IRES-hB1
4. 2.4uL of enhancer (1:8, DNA:enhancer) and 3.0uL of effectene (1:10, DNA:effectene) were used for each transfection reaction
5. Cells were fixed with 4% PFA for 15 minutes at room temperature.
6. Rinse and permeabilize with 0.01% PBT (PBS + 0.01% Triton)
7. Blocked with 2.5% NDS (normal donkey serum) dilute in 0.01% PBT for an hour
8. Incubate in primary antibodies for an hour at room temp.
9. Wash and incubate with secondary antibodies for an hour at room temp.
10. Cells were mounted and left to cure overnight in dark (at room temp)
11. Slides were stored at 4°C until imaging

Notes:

- Confocal images files are attached. Files contain metadata (acquisition speed, lasers, and settings) of the image.
- The following antibodies were used:
    - A to C: Rhodamine Phalloidin, Hoechst
    - D: anti-CD8 (clone YTC182.20, diluted at 1:500) and AF647 anti rat secondary (diluted at 1:1000)