Authentication of Key Resources

The research proposed in this application will involve transgenic mice mammalian cell lines, antibodies, cDNA constructs, and chemical reagents. Our approaches to validate each are as follows.

1) **Transgenic mouse strains.** During this project, we will use WT rat, and C3 PMP22 overexpressing transgenic mice, purchased from Jackson Laboratory (stock #030052, B6.Cg-Tg(PMP22)C3Fbas/J). We confirmed the transgene in these mice by genotyping and over-expression of PMP22, western blot for protein, and Q-RTPCR for mRNA. Before analysis, we will continue to validate these transgenic animals by standard genotyping methods.

2) **Mammalian cells.** We developed the stable PMP22-expressing cell lines used in this work in-house, starting with mammalian tissue culture cell lines acquired directly from ATCC. All our cell lines are authenticated periodically at the Vanderbilt VANTAGE (Vanderbilt Technologies for Advanced Genomics) core by short tandem repeat (STR) profiling. We will conduct routine testing of the cells for contamination by mycoplasma and bacteria using standard detection kits. We will continuously monitor the cells for doubling times and morphology and only use cells passaged less than 20 times.

   For experiments using primary neurons and Schwann cells, we will isolate them from rats or mice. Routinely identifying the cells by immunostaining for TuJ1, a neuron marker, or S100, a Schwann cell glial marker, will be standard practice. The Schwann cells generated using CRISPR-Cas9 expressing mutant PMP22 will be confirmed by genotyping with primers identifying both the mutant and wild-type endogenous allele. We routinely genotype the Trembler-J mice to confirm the mutation in PMP22. In addition, by staining for PMP22, we can ensure that cells from the Trembler-J mice do not express significant amounts of PMP22 on the surface but retain it intracellularly.

   Testing RNAi cell lines by expression analysis using quantitative PCR will ensure the correct gene(s) of interest knockdown.

3) **Antibodies.** We utilize commercially available antibodies for biochemical or immunofluorescence experiments. When we need to purchase a new antibody against a protein we have not worked with before, we first evaluate the published literature to determine which antibodies others have successfully used for that particular application as a starting point. We use at least two independent antibodies to detect the same protein whenever possible. Typically we will choose one mouse monoclonal antibody and one rabbit polyclonal antibody to provide flexibility for dual labeling experiments. Comparing the labeling obtained by the two antibodies also serves as a convenient internal control.

   When we first receive a new primary antibody, we conduct several control experiments to confirm that it behaves as expected. These include 1) immunostaining cells to ensure that the staining pattern matches the expected localization of the protein; 2) performing Western blotting to ensure that it recognizes a band of the correct molecular weight and to assess its specificity; 3) confirming that it recognizes overexpressed GFP-tagged or epitope-tagged versions of the protein by immunofluorescence microscopy and by Western blotting; 4) when possible, performing immunofluorescence labeling and Western blotting of knockout or knocked down cells to ensure that no cross-reactive staining. If an antibody passes our internal quality control checks, it is deemed fit for further use. Using positive and negative controls in subsequent experiments will provide internal checks that the antibody is working as expected.

   Routine validation of secondary antibodies used in our experiments enables us to assess whether there is non-specific labeling by the secondary antibody in a given experiment. Additionally, all antibodies are registered with the Resource Identification Portal, which authenticates biological resources to support transparency and rigor in biomedical science.

4) **cDNA constructs.** We typically obtain cDNA constructs through Addgene or request them directly from investigators who initially reported them in the literature. We also generate or mutagenize some constructs in-house or through commercial cloning services using standard molecular biology techniques. Upon obtaining a new construct, we confirm its identity by sequencing. When appropriate, we also demonstrate it yields a DNA fragment of the expected size after cleavage with restriction enzymes. We next express the construct in cells by transient transfections and perform control experiments to confirm that the expressed protein is localized as expected using immunofluorescence staining or live cell imaging. We also ensure that it runs at the correct molecular weight by Western blotting using antigen-specific antibodies. For proteins reported to have a specific cellular function, we routinely perform positive control experiments in which we reproduce that function before carrying out more detailed experiments.

5) **Plasmids.** We typically obtain cDNA constructs through Addgene or request them directly from investigators who initially reported them in the literature. We also generate or mutagenize some constructs in-house or through commercial cloning services using standard molecular biology techniques. Upon obtaining a new construct, we confirm its identity by sequencing. When appropriate, we also demonstrate it yields a DNA fragment of the expected size after cleavage with restriction enzymes. We next express the construct in cells by transient transfections and perform control experiments to confirm that the expressed protein is localized as expected using immunofluorescence staining or live cell imaging. We also ensure that it runs at the correct molecular weight by Western blotting using antigen-specific antibodies. For proteins reported to have a specific cellular function, we routinely perform positive control experiments in which we reproduce that function before carrying out more detailed experiments.

6) **Chemical reagents.** Chemical resources used in this proposal will be standard laboratory reagents commercially available and validated by the companies that provide them. We will purchase adequate quantities at the highest purity available from reputable vendors (e.g., Sigma), keep records of the batch information when available, and verify the results by purchasing a second aliquot as needed. Purchased chemicals from commercial vendors with Certificates of Analysis will be used without further characterization. The identity of all other chemicals will be confirmed by liquid chromatography-mass spectrometry (LC-MS). Any newly synthesized probes for affinity purification, cross-linking, and protein labeling will be characterized by chemical synthesis. Then probes are subjected to LC-MS, 1H/13C/DEPT NMR analysis, HRMS, IR, and synthetic protocols documented with characterization data. Final products are registered by batch within the VU compound registration system.