Authentication of Key Biological and/or Chemical Resources

All key resources for this proposal will be authenticated to enhance the reproducibility of our results, as appropriate and according to NIH policy (Notice Number: NOT-OD-16-011). Key Biological Resources that will be utilized in this proposal include:

Cells: Primary pulmonary artery endothelial and smooth muscle cells will be purchased commercially (Lonza) and validated for expression of key cell type-specific genes by RT-qPCR. Additionally, primary endothelial lung cells from mice will be isolated. All endothelial cells will be characterized by flow cytometry for cell surface markers and by RNA analysis of endothelial vs. lymphocyte gene expression. Endothelial cells will be analyzed immediately and not sub-cultured, minimizing the risk for cross-contamination. To monitor for human cell contaminants from other experiments in the laboratory, we will incorporate primers for two human short tandem repeat markers into multiplex PCR assays to facilitate detection of contamination. In addition, when comparing results from primary cells of genetically modified mice, we routinely and randomly isolate DNA at endpoints to authenticate their genotype. Finally, although we use standard aseptic techniques, we will screen for contamination using commercially available kits (bacteria/yeast: Cell Culture Contamination Detection Kit, Invitrogen; mycoplasma: MycoSEQ, Applied Biosystems; virus: ViralSEQ, Applied Biosystems).

Genetic mouse strains: This proposal employs a mouse model (C57BL/6N strain) in which VE-Cadherin Cre-ER mice crossed with FXN flox/flox mice. These will be compared to littermate control FXN flox/flox mice that do not express Cre-ER. We will employ standard PCR genotyping protocols as we described[45]. We will also confirm independently by RT-qPCR that endogenous FXN expression is absent in conditional knockout mice used for this experimental protocol. We also recognize the need to validate genotypes and phenotypes at sacrifice to ensure accurate interpretation of our data. Finally, we will minimize naturally occurring genetic drift by systematically re-infusing our breeding colonies with pedigreed mice from. The Jackson Laboratory. Both males and females will be studied.

Viruses: This protocol proposes to use the HIV-based lentiviral strains for gene transduction to cultured cells. Their identity and function will be authenticated by PCR genotyping of key genes upon infection of recipient cells.

Others:

- Oligonucleotide siRNA inhibitors of FXN, ISCU
- Taqman primer for protein-coding genes and microRNAs
- Plasmids: FXN, GFP transgenes
- Chemicals: SU-5416 and monocrotaline

All of these reagents will be obtained commercially. Validation will entail expression analysis by RT-qPCR of target genes after delivery of these reagents to recipient cells. SU-5416 and monocrotaline will be authenticated by the ability to induce pulmonary hypertension in rodents, per published descriptions.

Antibodies: All antibodies used for the research will be obtained commercially and will be validated for specificity and reproducibility via immunoblotting of cellular lysates from cells in which the target gene is either depleted or overexpressed. For some antibodies, we will also validate their function for in situ staining of lung tissues where the target gene is depleted or overexpressed. We will also ensure reproducibility between assays and different lots of antibody obtained from the vendor.

All other standard reagents we anticipate using for the proposed work are commercially available and validated by the companies that provide them. Additional resources used in this proposal will be standard laboratory reagents. Should we need to generate or obtain additional unique resources in the course of this proposal, these will be authenticated using methods similar to those described above.