

1. Desired Needs

- Cx40 is downregulated in PAH and restoring its function rescues disease phenotypes in mice, but its expression, trafficking, and regulation remain poorly defined
- No framework exists to identify its regulatory proteins, engineer better-expressing constructs, or screen small molecule modulators

2. Constraints

- **Safety/Regulatory:** Standard lab safety protocols followed, Future work testing in vitro functionality requires FDA 21 CFR Part 58, scRNA-seq datasets were used following FAIR standards
- **Risks:** Cx40's low recombinant expression compressed signal-to-noise across all assays, limited funding and a two-quarter timeline required strict timelines
- **Global Impact:** PAH kills ~22,000 annually, existing therapies only manage symptoms without correcting the underlying Cx40 signaling deficit particularly affecting women
- **Manufacturability/QC:** Pipelines are open-source, flow cytometry expression normalized to WT, Pearson correlation selected after cross-dataset stability validation over Spearman and CS-Core

3. Engineering Standards

- AlphaFold3, ProteinMPNN, and PhosphositePlus use community-validated scoring matrices, FAIR governed scRNA-seq processing, this work could establish a standard pipeline for PDZ interactome screening and a generalizable IF puncta quantification metric

4. Ethical, Environmental, or Societal Concerns

- Public datasets used per data sharing agreements, future use requires clinical validation before patients, compute energy minimized by pre-filtering before AF3 runs, computational-first approach reduces early-stage animal testing

5. Active Teamwork and Leadership

- Clear sub-project segmentation (Nishant: permeability assay, Ben: IF imaging, Anirudh: BioEmu/AF3, Curtis: mutagenesis/flow cytometry, Satvik: ProteinMPNN, Aiden: scRNA-seq/kinase/PDZ), Gantt charts tracked milestones, team contract established clear expectations
- Weekly feedback from Dr. Kufareva drove iterative improvements to the ProteinMPNN scoring pipeline and resolved the co-expression bottleneck via bicistronic vector redesign

6. Motivating Factors

- **New knowledge/Self-initiating:** Independently identified the Cx40 PDZbm as an underexplored regulatory axis and built the full pipeline from scratch, learning AlphaFold3, BioEmu, PhosphositePlus, scRNA-seq, and quantitative IF imaging along the way

- **Persist:** Multiple failed construct iterations led to systematic exploration of PTM mutations and plasmid redesigns, moving from separate plasmids to a bicistronic vector for better co-expression

7. Innovative and/or Entrepreneurial Ideas

- Integrated pipeline combining AF3 co-folding (275 PDZ proteins), PhosphositePlus kinase scoring (303 kinases), and scRNA-seq coexpression nominated SNX27, MAST4, MY18A and CSNK1D, CSNK2A1, TGFBR2 as top candidates, ProteinMPNN, AlphaMissense and PMI scoring identified stabilizing mutations G230A, A216L, and F173L
- Automated IF puncta platform proved PDZbm is critical for junctional localization