Connexins are a family of proteins that form gap junction channels (GJCs) that mediate intercellular communication and allow for the passage of ions and small molecules. Connexin40 (Cx40) is downregulated in pulmonary arterial hypertension (PAH), and restoring its functions rescues the disease phenotype in mice, making Cx40 a potential therapeutic target for PAH. However, the mechanisms that mediate Cx40 trafficking, GJC formation, and intercellular permeability are not well understood. Via bioinformatic and structural analysis, we developed a predict–validate workflow to identify determinants of Cx40 structure, trafficking, and GJC function. AlphaFold3 structural predictions integrated with PhosphoSitePlus identified candidate phosphorylatable residues within the C-terminal PDZ-binding motif (PDZbm), while endothelial scRNA-seq coexpression analyses nominated PDZ domain–containing kinases and scaffolding proteins as potential Cx40 trafficking partners. In parallel, ProteinMPNN analysis nominated mutations predicted to improve the structural stability of the Cx40 sequence, and BioEmu simulations were used to explore the conformational landscape of Cx40 and characterize structural differences between open and closed channel states. These computational predictions are experimentally validated through targeted mutagenesis and functional assays. PDZbm phosphomimics/knockouts are evaluated using immunofluorescence microscopy with quantification to assess gap junction plaque formation at cell–cell interfaces, demonstrating that PDZbm deletion significantly disrupts junctional localization and trafficking. Separately, ProteinMPNN-predicted stabilizing mutants are being experimentally validated using flow cytometry–based assays to quantify expression levels. Together, this framework links computational prediction with experimental validation, establishing a scalable pipeline for identifying mutations that modulate Cx40 structure and channel function.