

COS-7 cells were co-transfected with mCherry-myo10-CIB2 variant constructs (Baits, red) and GFP-whirlin truncated constructs (Prey, green). Single channels are shown. mCherry-myo10 construct was used as a negative control. Scale bar =  $10 \, \mu m$ .

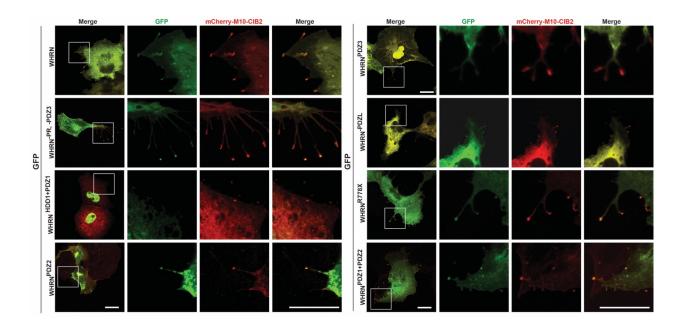


Fig. S2. Nanoscale pull down using CIB2 and truncated whirlin constructs COS-7 cells were co-transfected with mCherry-myo10-CIB2 $^{WT}$  constructs (Baits, red) and GFP-whirlin truncated constructs (Prey, green). Single channels are shown. Scale bar = 10  $\mu$ m.

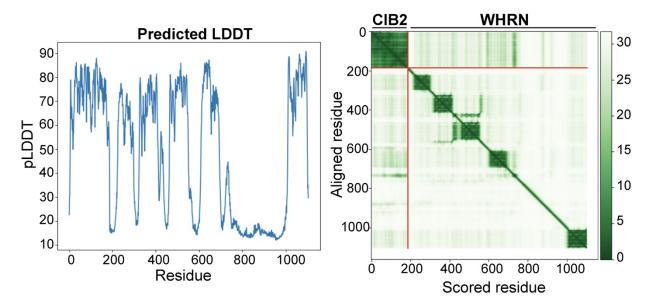


Fig. S3. Alphafold multimer prediction quality measures for the CIB2-whirlin complex, including predicted Local Distance Difference Test (pLDDT) (left) and Predicted Aligned Error (PAE) (right).