Promotion of Cx26 mutants located in TM4 region for membrane translocation successfully rescued hearing loss

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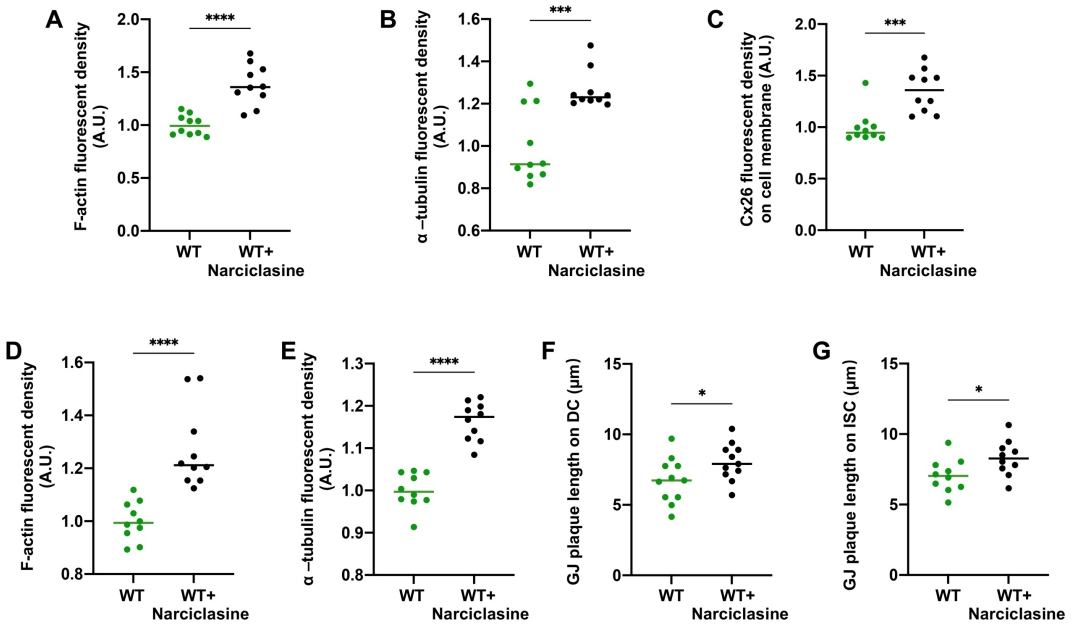

Figure S1 Narciclasine promotes the development of cytoskeleton and increases the membrane localization of WT-Cx26 in vitro and in vivo. (A-C) Quantification of the F-actin fluorescence intensity (A), acetylated α-tubulin fluorescence intensity (B), and Cx26 fluorescence intensity on plasma membrane (C) from the control group and Narciclasine-treated group in vitro. **(D-G)** Quantification of the F-actin fluorescence intensity (D), acetylated α-tubulin fluorescence intensity (E), and the lengths of GJPs on DCs (F) and ISCs (G) from the control group and the Narciclasine-treated group in vivo. *P < 0.05, **P < 0.005, ***P < 0.001, ****P < 0.0001.

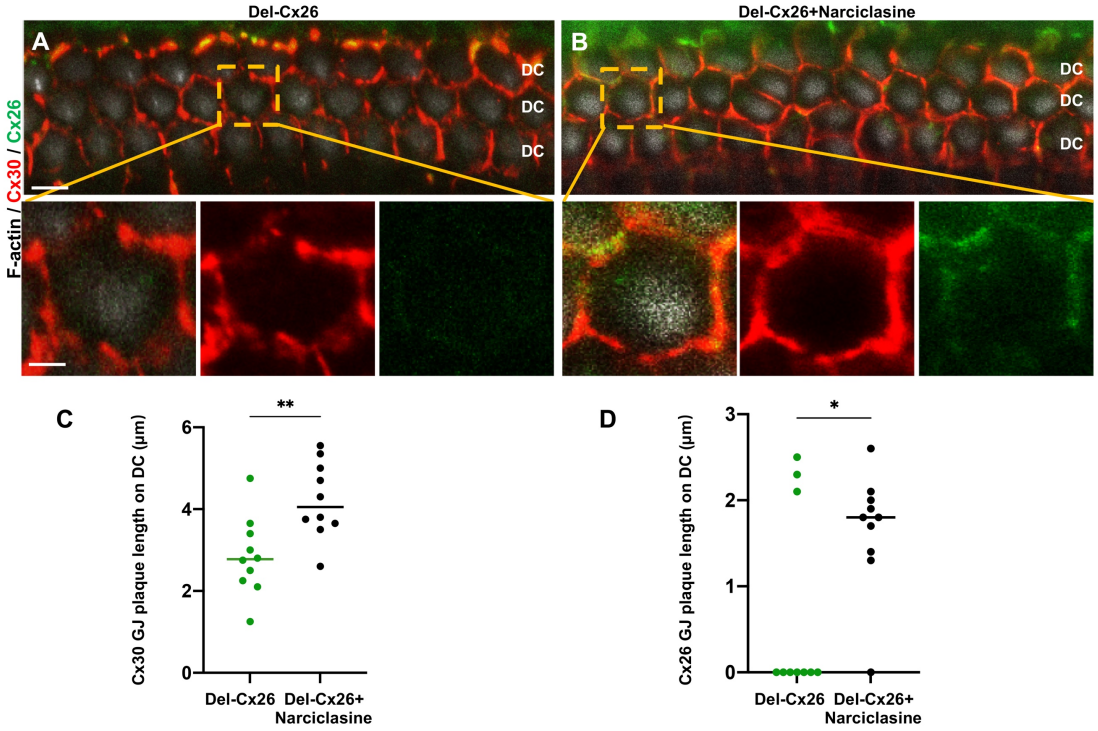

Figure S2 Narciclasine treatment increases membrane localization of Cx30 and Cx26 in DCs in Del-Cx26 mice. (A-B) Immunofluorescence staining of Cx30 (Red), Cx26 (Green), and F-actin (White) in the Del-Cx26 mice (A) and in the Del-Cx26 mice treated with Narciclasine (B). **(C-D)** Quantification of the lengths of Cx30 GJPs (C) and Cx26 GJPs (D) from the Del-Cx26 mice and the Del-Cx26 mice treated with Narciclasine on DCs at P30. Scale bars: 20 µm (panels A-B), and 7 µm (partial enlargement in panels A-B).*P < 0.05, **P < 0.005.

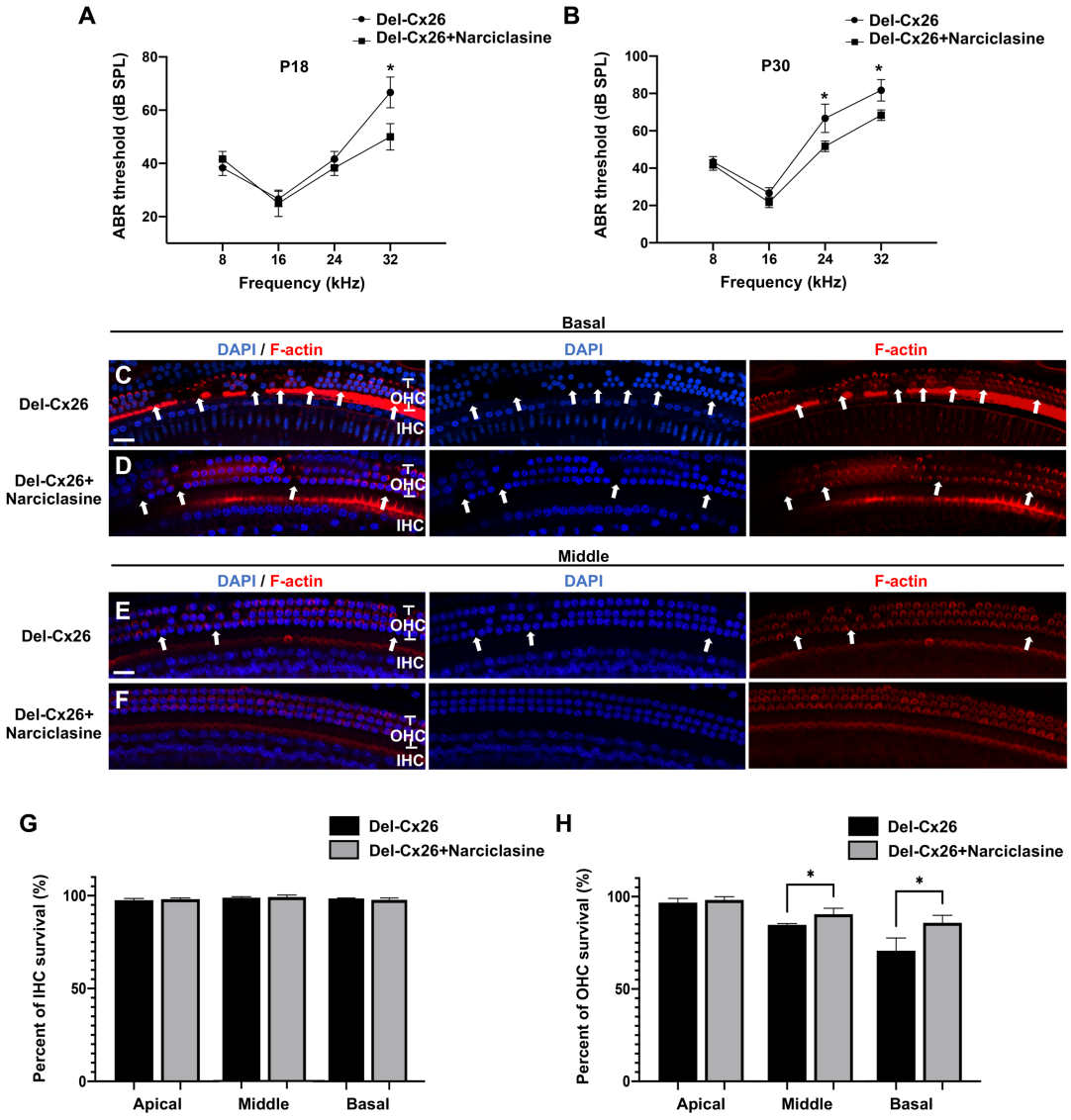

Figure S3 Intraperitoneal injection of Narciclasine rescues hearing loss and hair cell degeneration in Del-Cx26 mice. (A-B) Changes in ABR thresholds at different frequencies in the Del-Cx26 mice and in the Del-Cx26 mice treated with Narciclasine at P18 (A) and P30 (B) (*N*=5). **(C-F)** Representative images of IHCs and OHCs of different turns in the Del-Cx26 mice (C, E) and in the Del-Cx26 mice treated with Narciclasine (D, F) at P30. **(G-H)** Percent of IHCs (G) and OHCs (H) survival in the Del-Cx26 mice and in the Del-Cx26 mice treated with Narciclasine. Scale bars: 20 μm (panels C-F). *P < 0.05.