

Fig. S1. R124H NIS is not targeted to the plasma membrane. Immunofluorescence analysis of total and plasma membrane (PM) WT or R124H NIS expression in permeabilized (P) and non-permeabilized (NP) transiently transfected COS-7 cells. Immunostaining was performed with an anti-HA antibody directed against an extracellular HA tag engineered at the amino terminus of NIS, followed by anti-rat Alexa 488-conjugated antibody. Nuclei stained with DRAQ5 are shown in blue. White scale bar = 20 μ m. PM: plasma membrane.

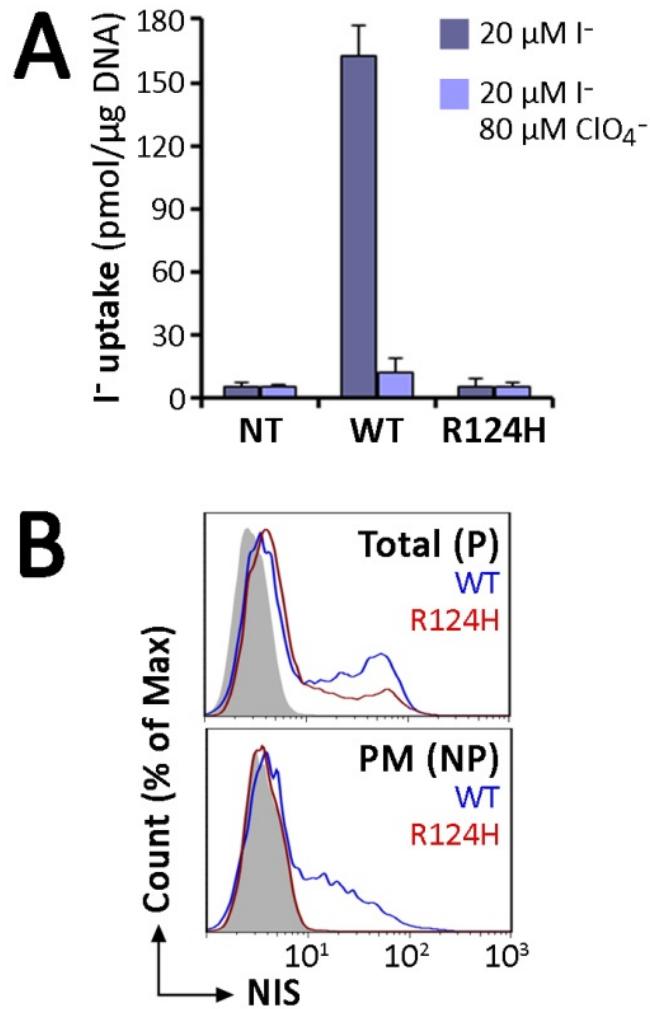


Fig. S2. R124H NIS expression and activity in MDCK-II cells. **A.** Steady state I⁻ transport assays in non-transfected (NT), wild-type (WT)-, or R124H NIS cDNA- permanently transfected MDCK-II cells. Cells were incubated with 20 μM I⁻ in the absence (blue bars) or presence (light blue bars) of 80 μM ClO₄⁻. Results are expressed in pmol I⁻/μg of DNA ± SD. Values are representative of 3 independent experiments; each experiment was performed in triplicate. **B.** Representative flow cytometry analysis of total (upper panel) and plasma membrane (lower panel - PM) NIS expression in permeabilized (P) and non-permeabilized (NP) MDCK-II cells non-transfected (solid grey) or expressing WT (blue line) or R124H NIS (red line). Cells were stained with anti-NIS VJ1 monoclonal Ab, followed by Alexa-488-conjugated anti-mouse Ab.

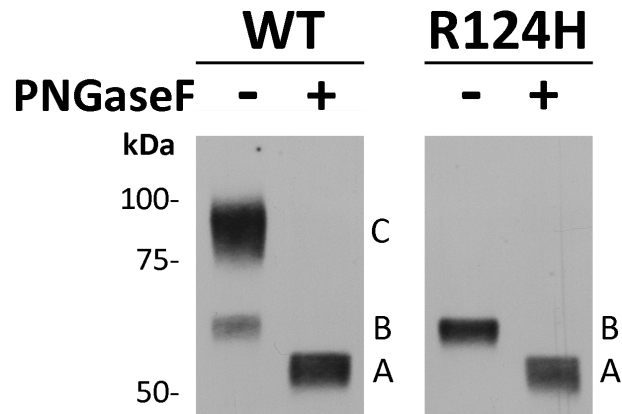


Fig. S3. Immunoblot analysis of R124H NIS protein treated with PNGase F. Membrane fractions prepared from WT or R124H NIS permanently expressing MDCK-II cells were treated with (+) or without (-) peptide-N-glycosidase F (PNGase F) (New England Biolabs) in 50 mM sodium phosphate buffer (pH 7.5) containing 1% NP-40 for 2 h at 37 C. Samples were resolved by SDS-PAGE and immunoblotted with polyclonal anti-human NIS Ab. Lines on the right side of the blot indicate the relative electrophoretic mobilities of non-glycosylated (band A) ~55 kDa, immaturely glycosylated (band B) ~60 kDa B, and fully glycosylated (band C) ~100 kDa NIS polypeptides.

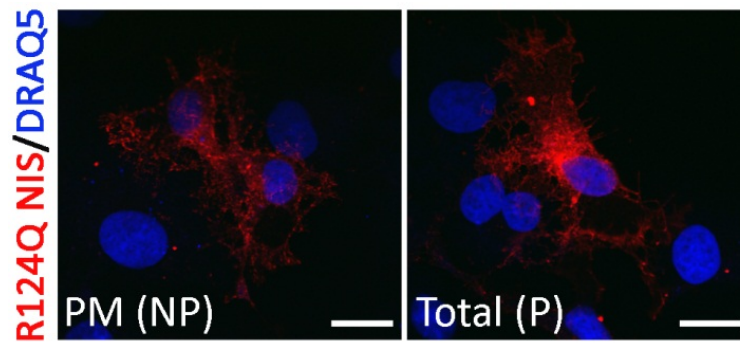


Fig. S4. R124Q targets to the plasma membrane. Immunofluorescence analysis of total and plasma membrane (PM) R124Q NIS expression in permeabilized (P) and non-permeabilized (NP) COS-7 cells. Immunostaining was performed with anti-NIS VJ1 mouse monoclonal Ab, followed by anti-mouse Alexa 555-conjugated antibody. Nuclei stained with DRAQ5 are shown in blue. White scale bar = 20 μm .