Supplementary Figures and Tables

A non-pump function of sodium iodide symporter in thyroid cancer via crosstalk with PTEN signaling

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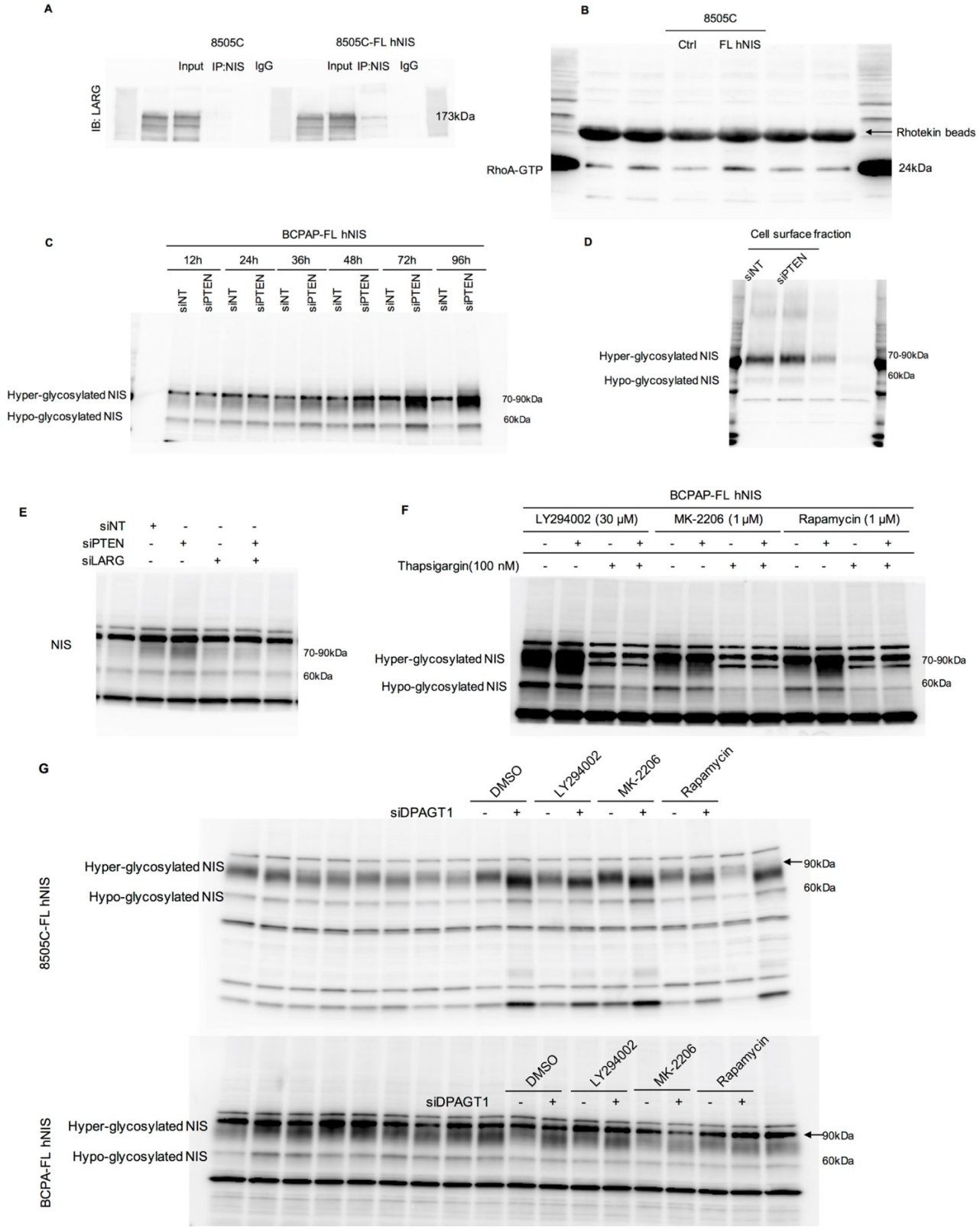
Supplementary Table S1. NIS-thyroid cell line models

Name	Cancer type	NIS expression	Tag	*PTEN* status
BCPAP	PTC	Stable	-	WT
8505C	ATC	Stable	-	WT
FTC-133	FTC	Stable	Flag	Null

Abbreviations: PTC, papillary thyroid cancer; ATC, anaplastic thyroid cancer; FTC, follicular thyroid cancer; WT, wildtype

Supplementary Figure S1. Uncropped versions of cropped immunoblots



A. Immunoblot of LARG in BCPAP cells in Fig. 1C.

B. Immunoblot of RhoA from RhoA-GTP assay in 8505C cells in Fig. 1D.

C. Immunoblot of NIS in BCPAP-FL hNIS after PTEN knockdown in Fig. 2A.

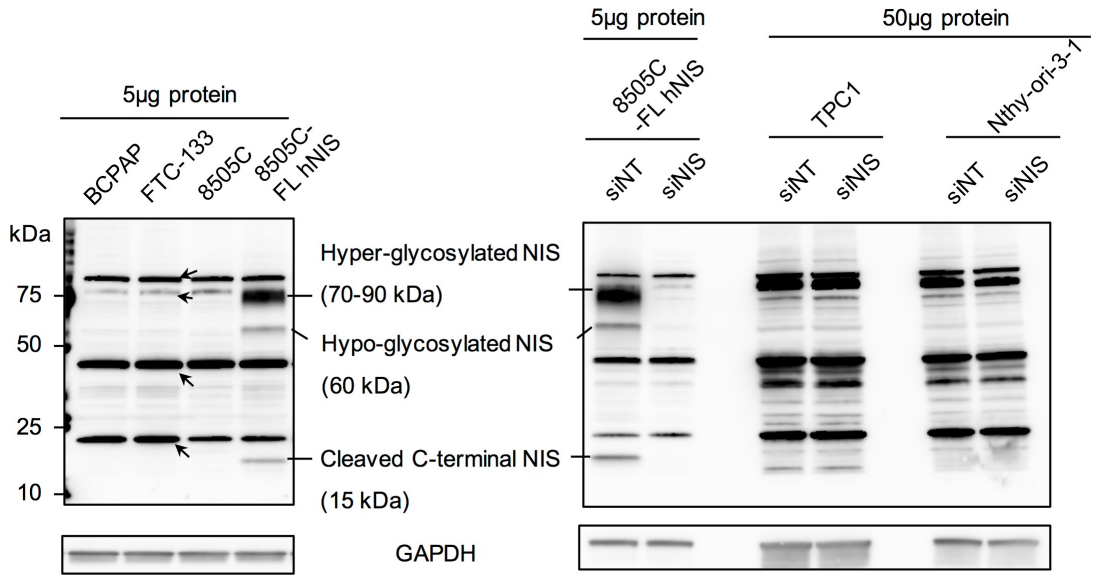
D. Immunoblot of NIS by using cell membrane fraction in BCPAP-FL hNIS after PTEN knockdown in Fig. 2B.

E. Immunoblot of NIS in BCPAP-FL hNIS cell after LARG and/or PTEN knockdown in Fig. 3B.

F. Immunoblot of NIS in BCPAP-FL hNIS cells after treatment with PI3K/AKT/mTOR inhibitors in Fig. 4B.

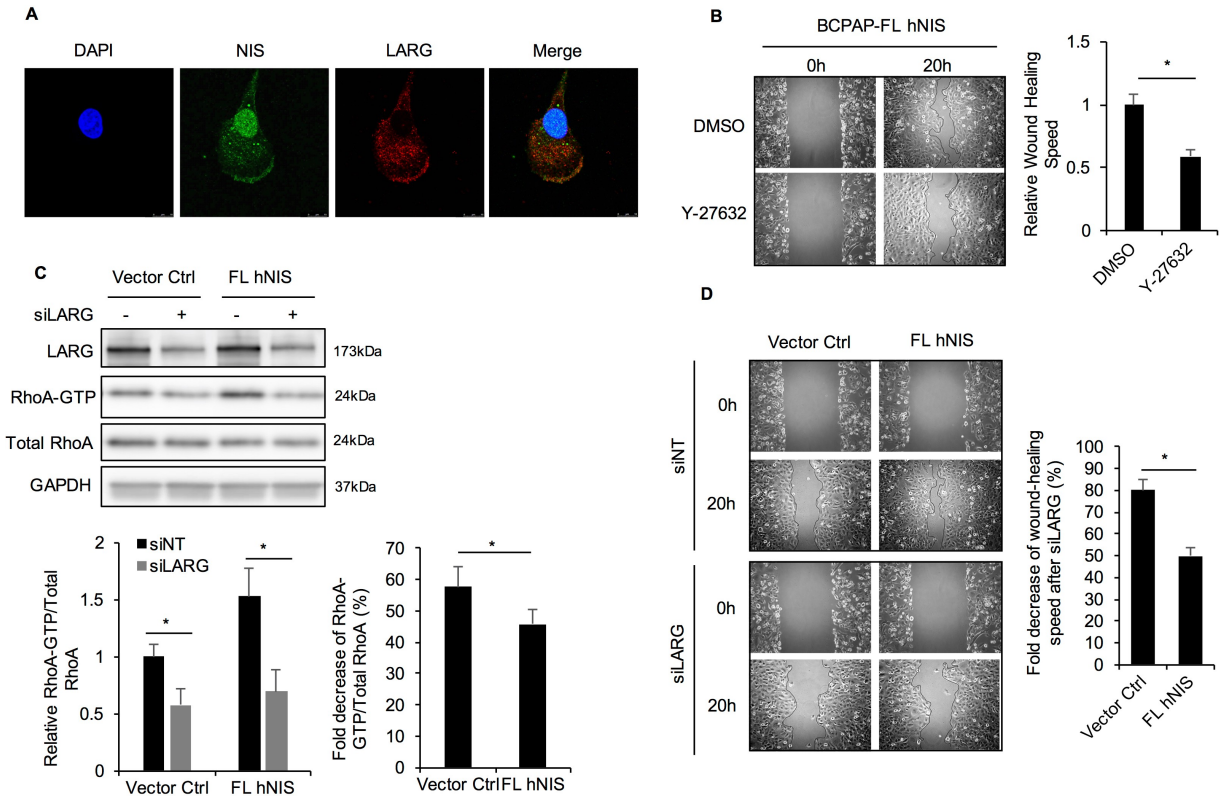
G. Immunoblot of NIS in BCPAP- and 8505C-FL hNIS cells after DPAGT1 knockdown and treatment with PI3K/AKT/mTOR inhibitors in Fig. 6A.

Supplementary Figure S2. Endogenous NIS is not detected in thyroid and thyroid cancer cell lines



Immunoblots of NIS in BCPAP, FTC-133, 8505C, Nthy-ori-3-1, TPC1 and 8505C-FL hNIS cell lines were performed using non-denaturing lysates with 5µg or 50µg protein. Exposure time is 2 minutes. The molecular weight of hyper-glycosylated NIS is about 70-90 kDa and that of the hypo-glycosylated NIS is about 60 kDa. Cleaved C-terminal NIS is about 15 kDa. Endogenous NIS cannot be detected in all of these cell lines except when NIS is exogenously expressed. NIS siRNA knockdown for 72 hours in 8505C-FL hNIS, Nthy-ori-3-1 and TPC1 was set as a control. Arrows point at non-specific bands.

Supplementary Figure S3. NIS interacts with LARG, activates RhoA and enhances cell migration in thyroid cancer cell lines



A. Confocal microscopy of BCPAP-FL hNIS after immunofluorescence staining of NIS and LARG. Data are representative of 3 sets of independent experiments. Scale bar, 10 µm.

B. Wound-healing assay of BCPAP-FL hNIS cells treated Rho kinase (ROCK) inhibitor Y27632. We added 30µM Y-27632 or DMSO into cell culture media immediately after wound scratches were performed. Microscopic images were taken at 0 and 20 hours after scratches were performed. Bar charts, relative wound healing rates of BCPAP-FL hNIS cells in the presence or absence of Y-27632. Data represent means ±SEM of 3 independent experiments.

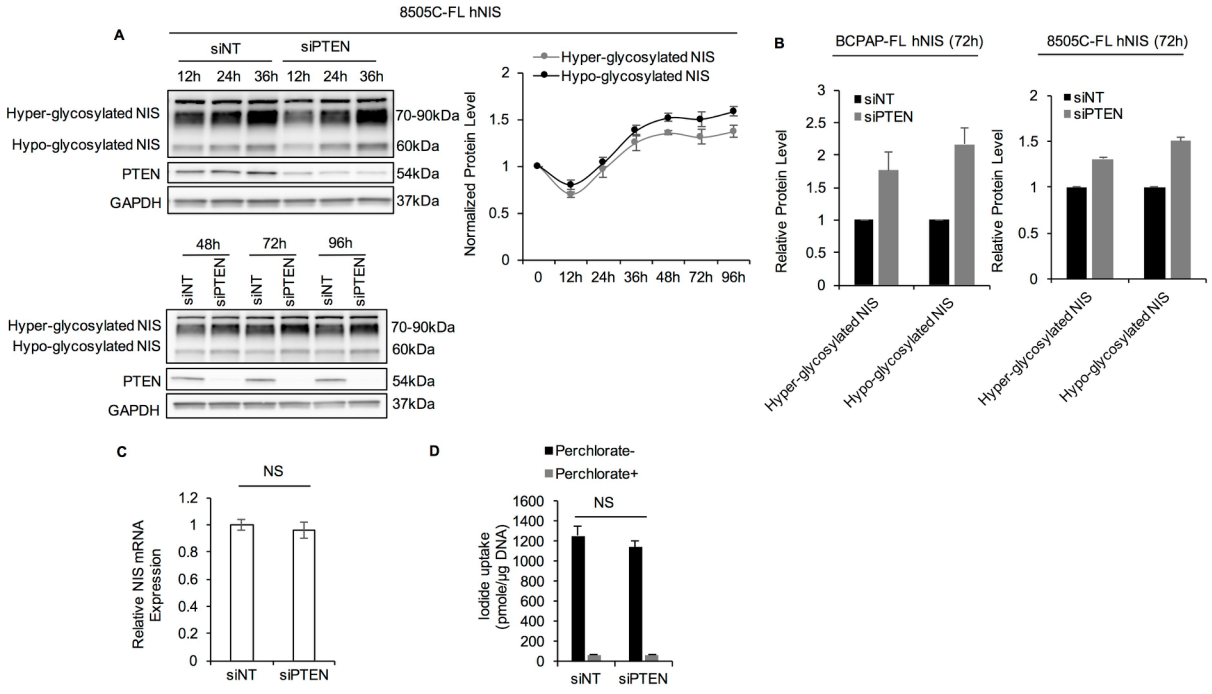
C. Immunoblots of RhoA and LARG from total cell lysates and RhoA after GST-Rhotekin pull-down from BCPAP-vector control and -FL hNIS cells with or without siLARG knockdown. Bar charts, normalized ratios of active to total RhoA levels and fold changes of activated RhoA-GTP

in BCPAP cells with or without FL hNIS after siLARG knockdown. GAPDH is used as a loading control for normalization. Data represent means ± SEM of 3 independent experiments.

D. Wound-healing assays of BCPAP-vector control and -FL hNIS cells after siLARG knockdown. Microscopic images were taken at 0 and 20 hours after wound scratch formation. Bar chart, fold changes of wound healing rates in BCPAP cells with or without FL hNIS after siLARG knockdown. Data represent means ± SEM of 3 independent experiments.

*P<0.05, **P<0.01.

Supplementary Figure S4. PTEN siRNA knockdown increases NIS protein levels in 8505C-FL hNIS cells



A. Immunoblot of NIS and PTEN using total lysates from 8505C-FL hNIS cells transiently transfected with siNT or siPTEN over different time points. The chart shows relative protein levels of hyper- and hypo-glycosylated NIS after PTEN knockdown normalized to control at each time point. Data represent means ± SEM of 3 independent experiments.

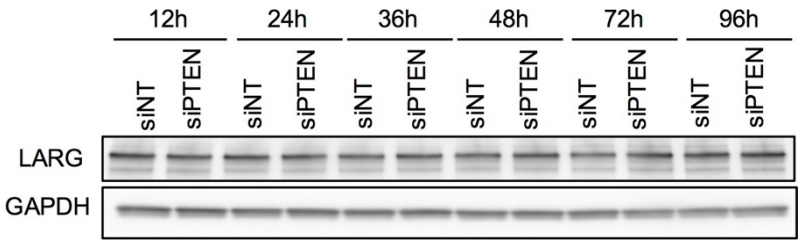
B. Increase of hypo-glycosylated NIS levels was more prominent than that of hyper-glycosylated NIS after PTEN knockdown. After PTEN knockdown for 72 hours, fold increases of hyper-glycosylated NIS and hypo-glycosylated NIS were 1.77 versus 2.17 (P=0.008) in BCPAP-FL hNIS and 1.31 versus 1.51 (P=0.040) in 8505C-FL hNIS cells. Data represent means ± SEM of 3 independent experiments.

C. Relative expression of NIS mRNA in 8505C-FL hNIS and vector control cells transiently transfected with siNT or siPTEN for 72 hours. Data represent means ± SEM of 3 independent experiments.

D. Iodide uptake from 8505C-FL hNIS after PTEN siRNA knockdown for 72 hours. Data represent means ± SEM of 3 independent experiments.

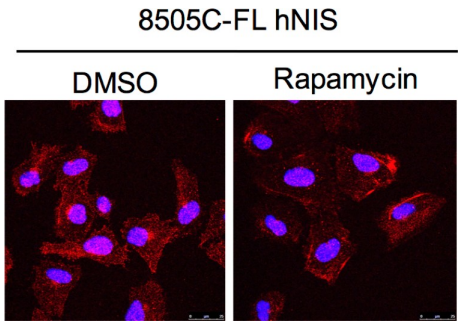
NS = not significant.

Supplementary Figure S5



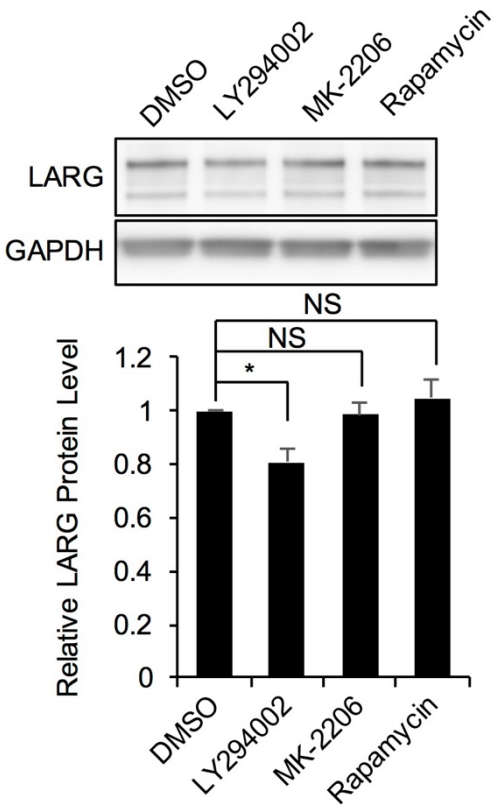
Immunoblot of LARG in BCPAP-FL hNIS cells transiently transfected with siNT or siPTEN over different time points. GAPDH is used as a loading control. Data are representative of 3 sets of independent experiments.

Supplementary Figure S6



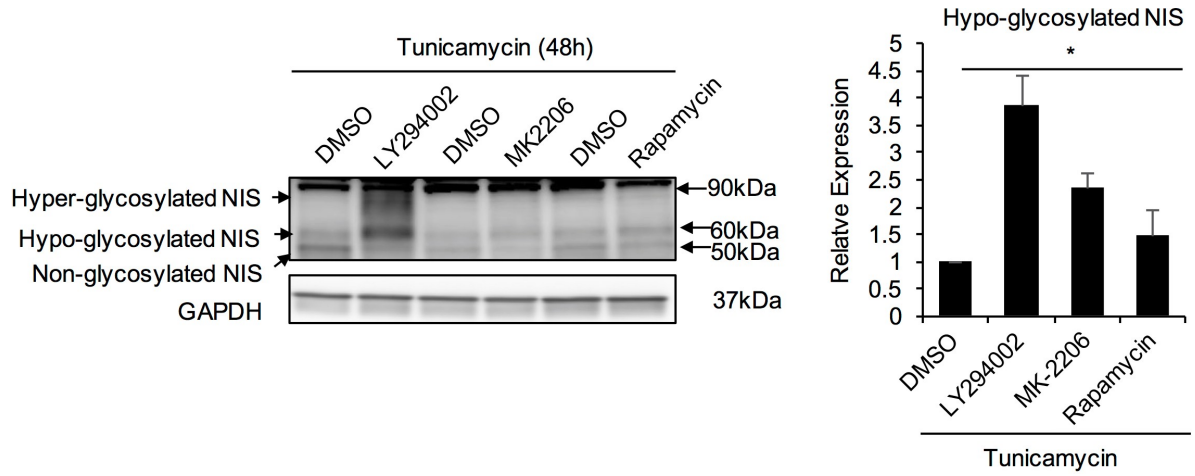
Immunofluorescence staining of NIS in 8505C-FL hNIS treated with DMSO or rapamycin. Scale bar, 25 μm. Data are representative of 3 sets of independent experiments.

Supplementary Figure S7



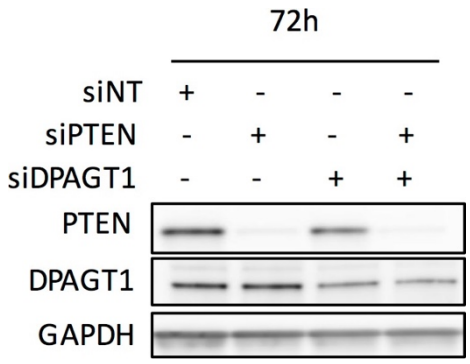
Immunoblot of LARG in BCPAP-FL hNIS cells treated with LY294002, MK-2206, rapamycin or DMSO. Bar chart, LARG protein levels normalized to GAPDH. Data represent means ± SEM of 3 independent experiments. **P*<0.05, NS = not significant.

Supplementary Figure S8



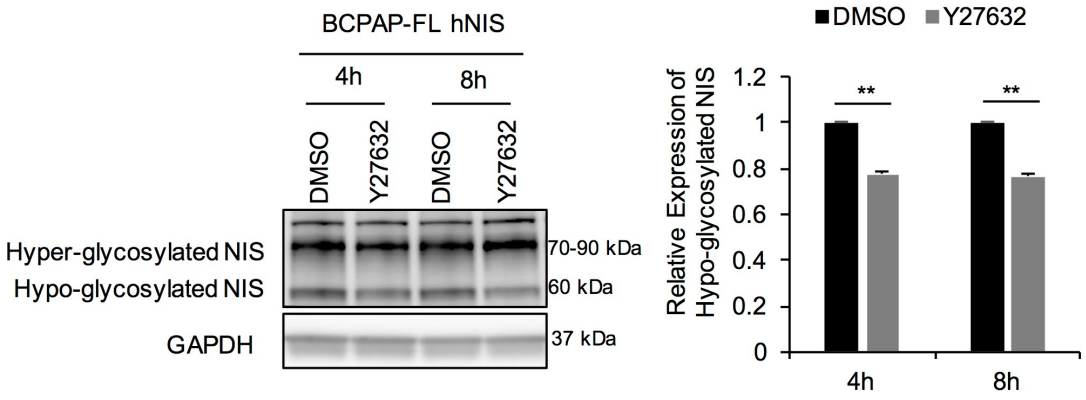
PI3K/AKT/mTOR inhibitors partly reversed the inhibition of tunicamycin on NIS glycosylation. Left panel, Immunoblot of NIS in BCPAP-FL hNIS cells treated with LY294002, MK-2206, rapamycin or DMSO and co-treated with tunicamycin for 48 hours. Bar charts, intensity of hypo-glycosylated NIS bands normalized to GAPDH and then normalized to DMSO control. All of these drugs increased hypo-glycosylated NIS significantly whereas the non-glycosylated form of NIS was similar to untreated controls. Data represent means ± SEM of 3 independent experiments. *P<0.05.

Supplementary Figure S9



Immunoblot of PTEN and DPAGT1 in BCPAP-FL hNIS cells transiently transfected with siNT, siPTEN, siDPAGT1 or both siPTEN and siDPAGT1 for 72 hours. Data are representative of 3 sets of independent experiments.

Supplementary Figure S10



Immunoblots of NIS in BCPAP-FL hNIS cells treated with ROCK inhibitor Y27632 (30 μM) or DMSO for 4h and 8h. GAPDH is used as a loading control. Data represent means ± SEM of 3 independent experiments. ***P*<0.01.