Henderson et al., http://www.jgp.org/cgi/content/full/jgp.201311102/DC1

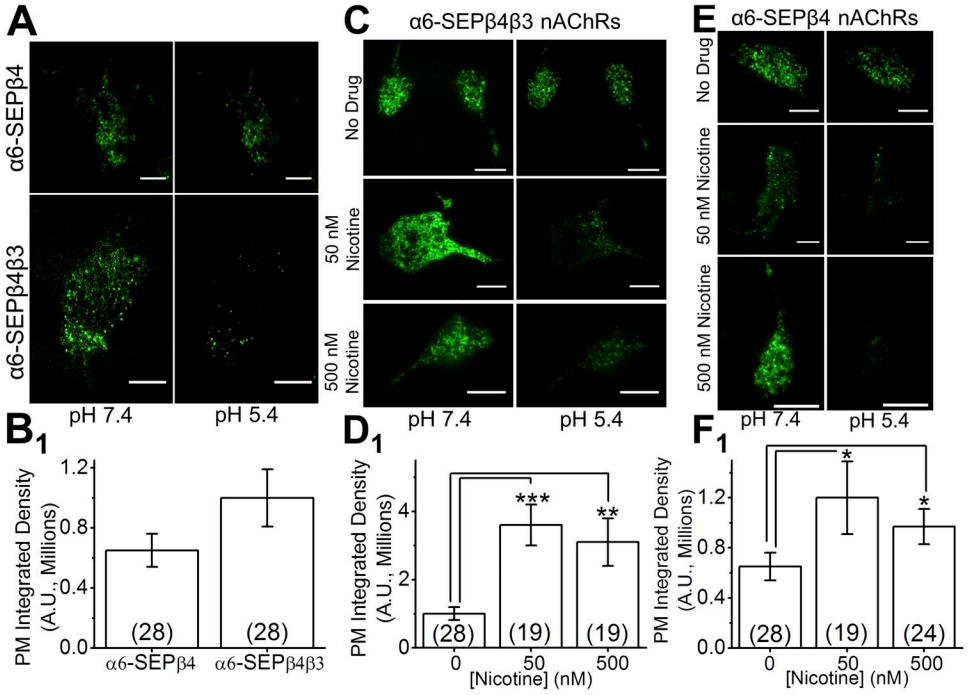

Figure S1. β3 subunits increase α6β4* nAChR density on PM. (A, C, and E) Representative TIRF images of Neuro-2a cells transfected with α6-SEP and a combination of β4 with or without β3 nAChR subunits at basic (pH 7.4) and acidic (pH 5.4) conditions. Nicotine was added at the listed concentrations (24 h). (B_1, D_1, and F_1) PMID for α6-SEP* nAChRs. Number of imaged cells is indicated in parentheses. Bars, 10 µm. Data are mean values ± SEM. n.s., not significant; *, $P < 0.05$; **, $P < 0.005$; ***, $P < 0.0001$.

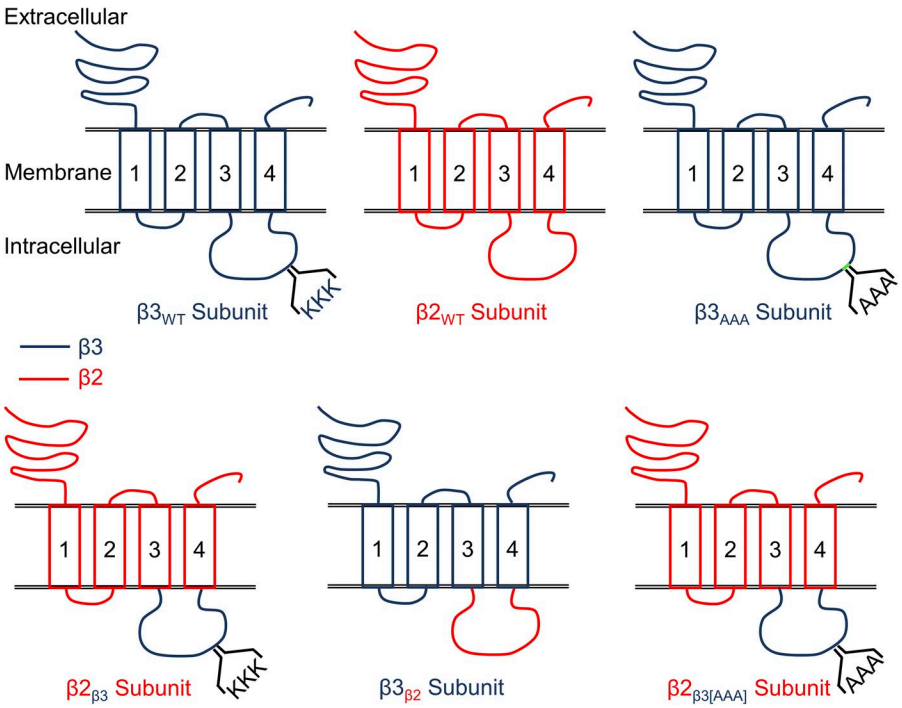

Figure S2. Schematic representation of nAChR chimeras and mutants. β3 and β2 nAChR subunits are designated blue and red, respectively. Numbers in diagrams correspond to transmembrane domains 1–4.

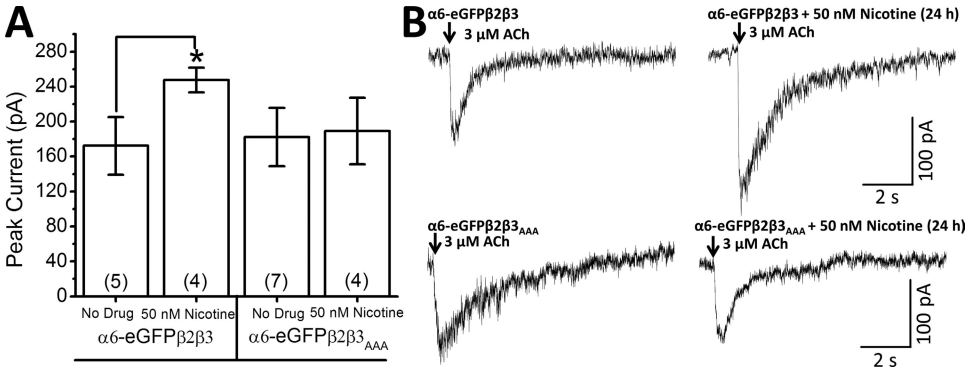

Figure S3. Functional up-regulation is observed in WT α6β2β3 nAChRs but not $\alpha6\beta2\beta3_{AAA}$ nAChRs. (A) α6-eGFPβ2β3 and $\alpha6\text{-eGFP}\beta2\beta3_{AAA}$ nAChR currents elicited by 3 μM ACh in the absence or presence of nicotine treatment (50 nM nicotine, 24 h). (B) α6-eGFPβ2β3 nAChRs displayed a functional up-regulation of peak currents when treated chronically with nicotine, but $\alpha6\text{-eGFP}\beta2\beta3_{AAA}$ nAChRs did not display a functional up-regulation after chronic treatment with nicotine. *, $P < 0.05$.

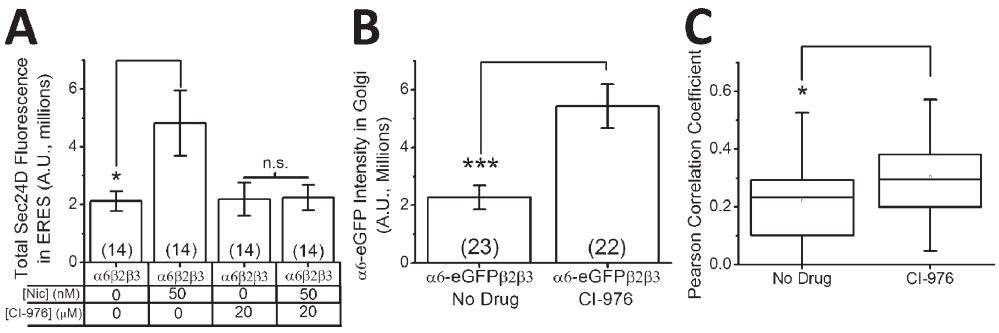

Figure S4. Blocking Golgi–ER cycling with CI-976 produces an increase in Golgi without an increase in ER export. Quantification of Sec24D fluorescence in ERES (A), α6-eGFP* intensity in Golgi cell sections (B), and Pearson correlation coefficients between α6-eGFP* and GalT-mCherry (Golgi marker) for α6-eGFPβ2β3 nAChRs (C).

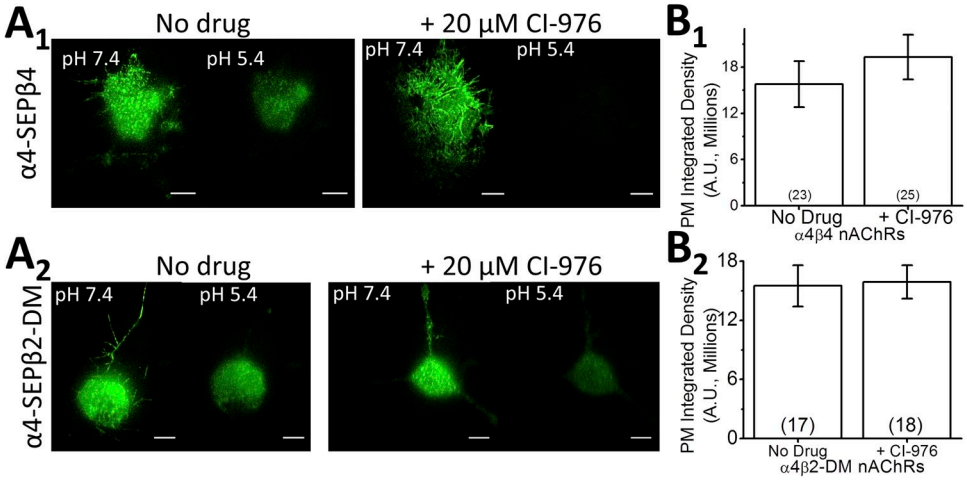

Figure S5. Cl-976 does not affect basal PM density of high ER export nAChRs. (A_1 and A_2) Representative TIRF images of Neuro-2a cells transfected with α4-SEP and cotransfected with either β4 or β2-DM nAChR subunits. 20 μM Cl-976 was added 24 h before imaging. Cells were imaged at basic (pH 7.4) and acidic (pH 5.4) conditions. Bars, 10 μm. (B_1 and B_2) PMID for nAChRs. Numbers of imaged cells is indicated in parentheses.