Cell culture

The mpkCCDc14 line (RRID:CVCL_F596) is an immortalized mouse collecting duct principal cell line, which was cultured as described previously (Wu *et al.*, 2016). These cells were cultured in a 1:1 mixture of DMEM and Ham's F-12 medium (GIBCO) supplemented with 20 mM HEPES, 2 mM L-glutamine, 50 nM dexamethasone, 1 nM triiodothyronine, 2% heat-inactivated FBS, and 0.1% penicillin-streptomycin. The mpkCCD$_{c14}$ cells were plated at a density of 75,000 cells/cm and grown on permeable supports to maintain cell polarization (Costar Transwells; 0.4-μm pore, 24-mm diameter) and cultured for at least 7 days before the experiments. The mpkCCDc$_{14}$ cells were treated for 48 h with 5 μM lovastatin, 30 μg/ml cholesterol, or 5 μM lovastatin plus 30 μg/ml Cho, respectively.

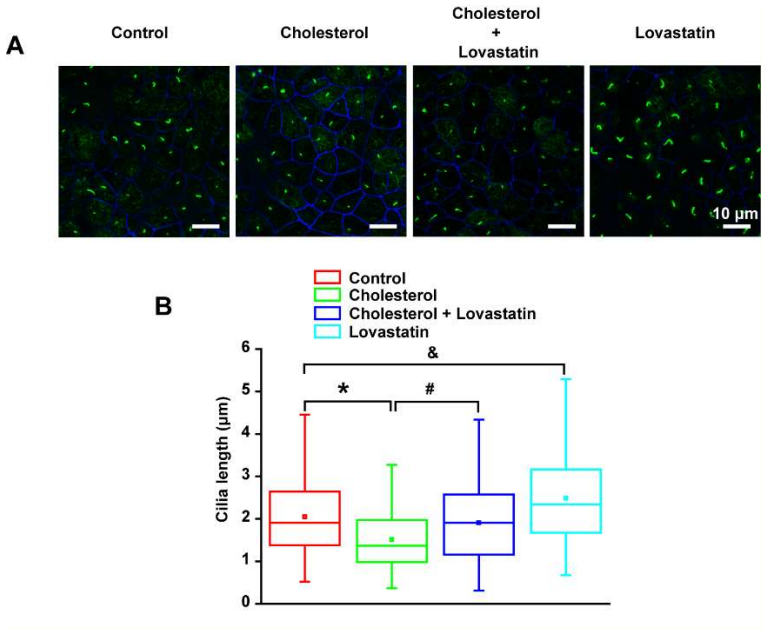
A6 cell line (RRID:CVCL_F714 ;Rockville, MD), which is originated from the distal nephron of Xenopus laevis, was purchased from American Type Culture Collection. Cells were cultured in plastic flasks in a modified DMEM/F12 media containing 100 mM NaCl, 20 mM $NaHCO_3$, 60 U/ml penicillin, 60 U/ml streptomycin, 2 mM L-glutamine, 10% fetal bovine serum (Invitrogen, Carlsbad, CA), and 1 μM aldosterone (Sigma-Aldrich, St. Louis, MO) at 26°C and 4% CO_2, as previously described (Ma, 2011). Cells were removed from the flasks and plated on polyester membrane attached to Snapwell inserts (Corning Costar, Pittsburgh, PA) at least 10 days to fully polarize before experiments. A6 cells were either under control conditions or treated for 24 hrs with 1 μM CsA or 1 μM CsA plus 5 μM lovastatin, respectively.

Cilia staining

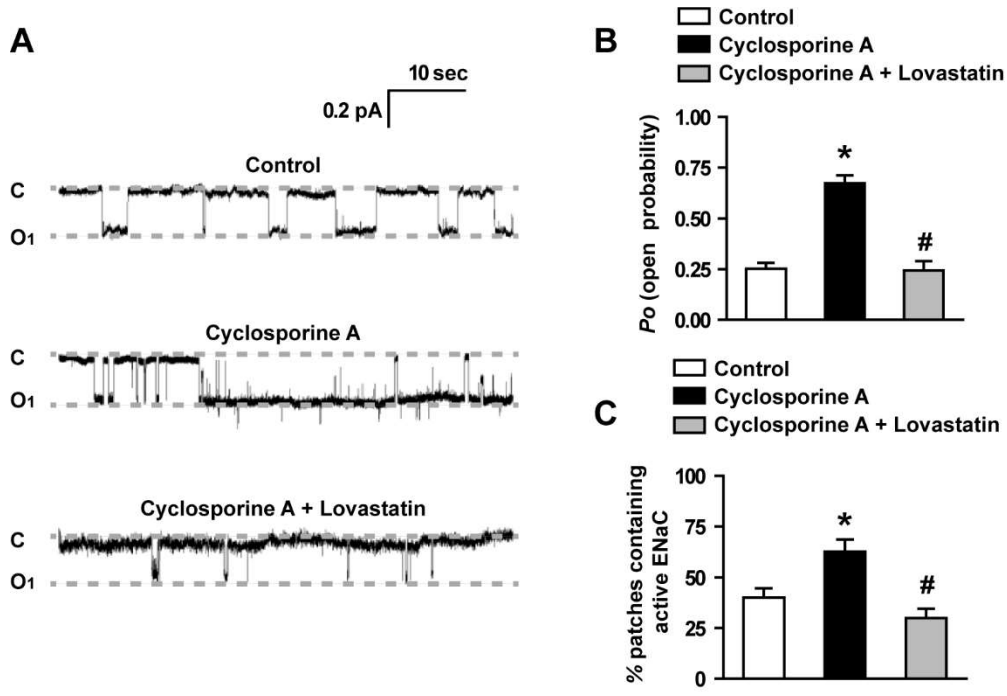
To identify primary cilia, the mpkCCDc$_{14}$ cells were stained using an anti-acetylated-α-tubulin antibody (shown in green). Optical sections were set at or near the apical membrane according to where the tight junction protein ZO-1 was localized (shown in blue). Ciliary length was analyzed using Olympus Fluoview FV1000 version 3.1 software.

Single-channel Recordings of Patch Clamp Technique

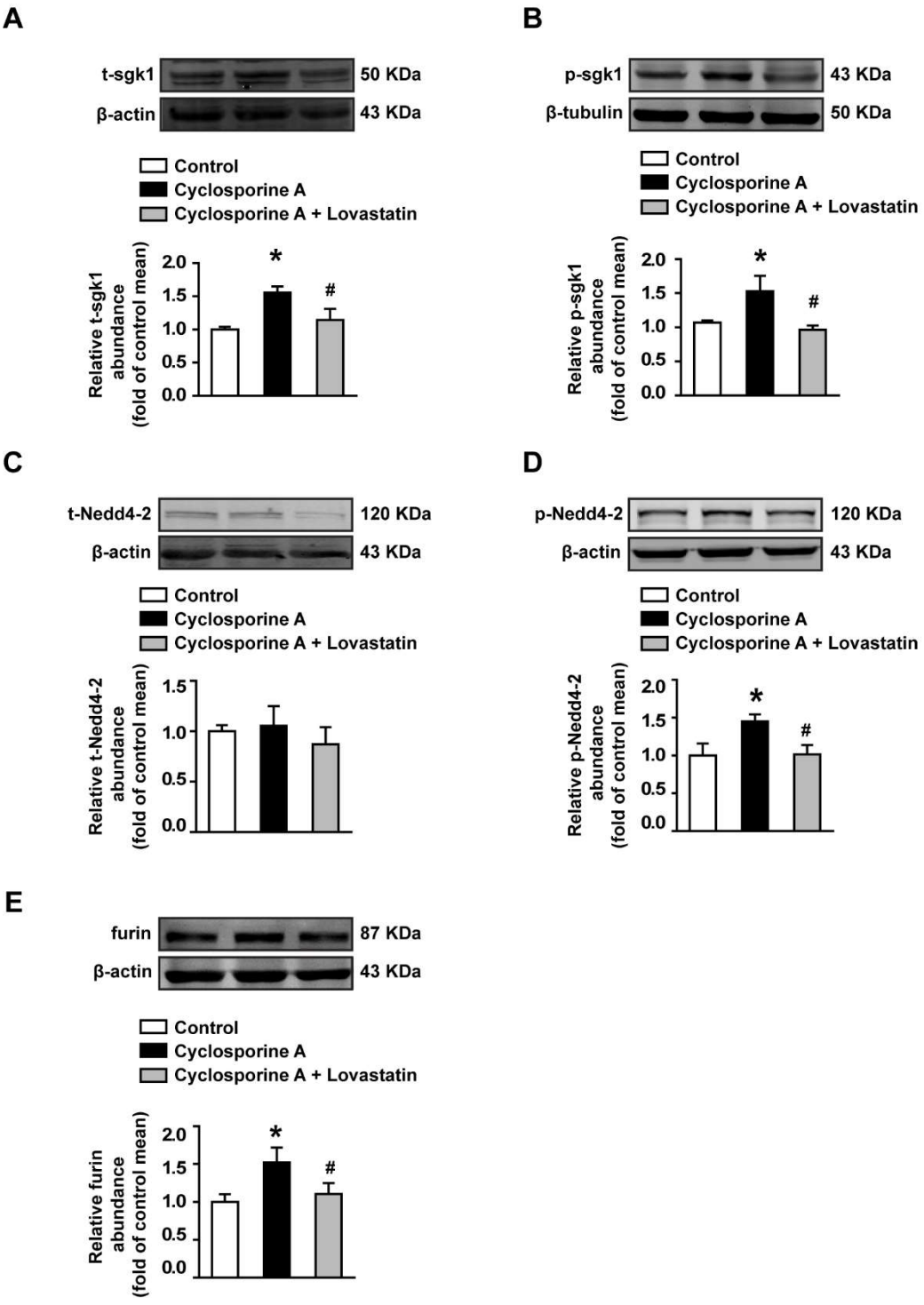
Cell-attached patch-clamp recordings of ENaC single-channel currents were carried out using an Axopatch 200B amplifier (Molecular Devices, Sunnyvale, CA). As previously described (Wang *et al.*, 2009), prior to the experiments, A6 cells cultured on the polyester membrane of Snapwell inserts were thoroughly washed with NaCl solution containing (in mM) 100 NaCl, 3.4 KCl, 1 $CaCl_2$, 1 $MgCl_2$, and 10 HEPES; pH was adjusted to 7.4 with NaOH. The glass micropipette was filled with NaCl solution (the pipette resistance was ranged from 7 to 10 MΩ). All experiments were conducted at room temperature (22–24 °C). The data were acquired by application of 0 mV pipette potential and were sampled at 5 kHz and low-pass filtered at 1 k Hz with Clampex 10.2 software (Molecular Devices, Sunnyvale, CA, USA). Prior to analysis, the single-channel traces were further filtered at 30 Hz. The open probability (P_O) of ENaC, which is percent open time, was calculated using Clampfit 10.2 (Molecular Devices, Sunnyvale, CA, USA).



Supplemental Figure 1. Lovastatin increased, but Cho decreased, the cilia length of mpkCCDc14 cells. (A) Representative confocal microscopy images of mpkCCD$_{c14}$ cells stained with anti-acetylated-α-tubulin antibody (shown in green), tight junction protein ZO-1 was localized (shown in blue). (B) Summary plots of normalized cilia length in mpkCCD$_{c14}$ cells after each indicated treatment. (Each experiment was repeated 5 times; $^{*}P < 0.05$, $^{\#}P < 0.05$, $^{\&}P < 0.05$; Kruskal-Wallis followed by Dunns post hoc test).



Supplemental Figure 2. Lovastatin attenuates CsA-induced increases in ENaC activity in A6 cells. (A) Representative single-channel recordings of ENaC activity from cell-attached patches from a control cell, a cell treated with 1 μM CsA alone for 24 hrs, or a cell treated with 1 μM CsA plus 5 μM lovastatin for 24 hrs. (B) Summary plot of ENaC P_O under each indicated conditions (*$P < 0.05$, $^{\#}P < 0.05$; control cell n = 16, cell treated with 1 μM CsA n= 25, cell treated with 1 μM CsA plus 5 μM lovastatin n = 12, which cells containing active ENaC channels; One-way ANOVA followed by Bonferroni post hoc test). (C) Summary plot of percent patches containing active ENaC channels (*$P < 0.05$, $^{\#}P < 0.05$; n = 40 in each group from five separate experiments; One-way ANOVA followed by Bonferroni post hoc test).



Supplemental Figure 3. Lovastatin attenuates CsA-induced increases in ENaC activity by inhibiting furin expression and Sgk1/Nedd4-2 signaling. (A and B) Western blots of t-Sgk1 and p-Sgk1 expression from control A6 cells, cells treated with 1 μM CsA alone for 24 hrs, or

cells treated with 1 μM CsA plus 5 μM lovastatin for 24 hrs. Summary plots of Western blots, showing t-Sgk1 and p-Sgk1 expression levels from A6 cells under each indicated conditions (*$P < 0.05$, $^{\#}P < 0.05$; n = 5 in each group; One-way ANOVA followed by Bonferroni post hoc test). (C and D) Western blot of t-Nedd4-2 and p-Nedd4-2 expression from control A6 cells, cells treated with 1 μM CsA alone for 24 hrs, or cells treated with 1 μM CsA plus 5 μM lovastatin for 24 hrs. Summary plots of Western blots, showing t-Nedd4-2 and p-Nedd4-2 expression level from A6 cells under each indicated conditions (*$P < 0.05$, $^{\#}P < 0.05$; n = 5 in each group; One-way ANOVA followed by Bonferroni post hoc test). (E) Western blot of furin expression from control A6 cells, cells treated with 1 μM CsA alone for 24 hrs, or cells treated with 1 μM CsA plus 5 μM lovastatin for 24 hrs. Summary plots of Western blots, showing furin expression level from A6 cells under each indicated conditions (*$P < 0.05$, $^{\#}P < 0.05$; n = 5 in each group; One-way ANOVA followed by Bonferroni post hoc test).

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