Supporting Information

S1 Figure



Supporting Information Caption

S1. Cell treatment with only BAfA1 does not alter PtdIns3P. (A and B): HEK293 cells cultured in complete media grown to 90-100% confluence run in parallel with apilimod and BafA1+apilimod samples shown in Fig 5 were labeled for 25 h with 25 μ Ci/mL *mvo*-[2-³H]inositol. Cells were than treated in the same labeling medium with vehicle (control, 0.1% DMSO) or BafA1 (15 nM in DMSO) for 40 min when additional vehicle (0.1% DMSO) was added for 60 min. Lipids were extracted, deacylated and subjected to HPLC separation. Shown are representative HPLC [³H]GroPInsP profiles from control (left panel) and BafA1-treated (right panel) cells (A) and quantification of bafilomycin-dependent changes in steady-state levels of PtdIns3P, PtdIns4P, PtdIns5P, PtdIns $(3,5)P_2$ and PtdIns $(4,5)P_2$ from 3 independent experiments (B). Apparent is the lack of changes in steady-state levels of PtdIns3P, PtdIns4P, PtdIns(3,5)P₂ and PtdIns(4,5)P₂ by BafA1. For reasons that remain to be clarified in future studies, there was a 40% decrease in steady-state levels of PtdIns5P compared to the control that received only vehicle. Note that BafA1 did not alter low levels of PtdIns5P reduced upon apilimod treatment (see Fig 5AB).