Supplementary Figure S1: IC87114 does not affect basal Ca\(^{2+}\) level nor nicotine-induced Ca\(^{2+}\) influx. (a) Bovine chromaffin cells were loaded with Fluo-4AM (1 μM) in buffer A containing 0.02% of pluronic acid for 30 min at room temperature. Cells were washed with buffer A containing 2 mM CaCl\(_2\) and imaged by time-lapse confocal microscopy before and during IC87114 (1 μM) application (arrow). (b) Cells pre-treated with or without IC87114 were imaged before and after addition of nicotine (100 μM). Time-lapse movies were captured by confocal microscopy. The Ca\(^{2+}\) variations measured using Fluo-4AM fluorescence intensity in the indicated conditions, taken from one representative experiment, are shown in the bottom panels (n=10 cells/condition, experiment carried out 3 times). Scale bar, 50 μm.
Supplementary Figure S2: Change in PtdIns(4,5)P2 levels in PI3Kδ knockout chromaffin cells and in response to acute IC87114 inhibition. $^{32}$P-labelled chromaffin cells were treated with vehicle (DMSO) or IC87114 (1 μM) for 5 min before lysing the adherent cells. Phosphoinositides were extracted, deacylated and analyzed on a SAX-HPLC column as described in the methods section. (a) WT littermate or p110 $\delta^{D910A/D910A}$ mice chromaffin cells were isolated, phosphoinositides extracted as described in Fig. 2 and analyzed for the amount of PtdIns(4,5)P2 levels by TLC. (b) Representative chromatography profile of PtdIns(4,5)P2 peaks in the indicated conditions are shown. (c) PtdIns(4,5)P2 levels were quantitated and normalized (n=3 independent experiments). One representative TLC overlay blot is shown. Bar graph shows the quantification of the level of PtdIns(4,5)P2 from 4 independent experiments. Data are expressed as mean ± SEM. (Student’s t-test, *p<0.05).
Supplementary Figure S3: IC87714-induced PtdIns(4,5)P<sub>2</sub> rise is prevented by sequestration of PI(3,4,5)P<sub>3</sub>. Bovine chromaffin cells co-expressing either (a) Akt-PH-GFP or (b) Btk-PH-GFP and PH-PLC<sub>δ1</sub>-mRFP were imaged by TIRF microscopy at 1 frame/second before and during IC87114 (1 μM) treatment. Representative images shown are taken before (left) and after (right) IC87114 application. Time-course of the fluorescence intensity in the indicated conditions is shown in the lower panel (n=4). Data are expressed as mean ± SEM. Scale bar, 10 μm.
Supplementary Figure S4: IC87714-induced PtdIns(4,5)P$_2$ rise is blocked by the Akt kinase inhibitor, A6730. (a) Control and (b) A6730 (1 μM for 20 min)-treated bovine chromaffin cells expressing PH-PLC$_{δ1}$-EGFP were treated with IC87114 (1 μM) were examined by TIRF at 1 frame/second. (c) Time-course variation of PH-PLC$_{δ1}$-EGFP fluorescence in the indicated conditions is shown (n=4). (d) Bar graph showing the average peak fluorescence intensity change of PH-PLC$_{δ1}$-EGFP in the indicated conditions (n=3-14 regions of interest from 3 independent experiments). Data are expressed as mean ± SEM. Scale bar, 10 μm.
Supplementary Figure S5: IC87114-induced secretory vesicle translocation occurs without significantly affecting the position of the plasma membrane. (a) The plasma membrane of live chromaffin cells expressing NPY-Cherry was labeled with WGA-488 for 10 min at room temperature. Cells were examined by TIRF microscopy at 1 frame/second before and after addition of IC87114 (1 μM). (b) Time-course variation of WGA-488 and NPY-Cherry fluorescence intensities upon IC87114 treatment (indicated by an arrow) on the (selected) plasma membrane regions of interest (n=3–4 ROI). (c) Average peak fluorescence intensity change of WGA-488 and NPY-Cherry in the indicated conditions expressed as mean ± SEM (n=5 independent experiments). (Student’s t-test, *p<0.05). Scale bar, 10 μm.
Supplementary Figure S6: IC87114-induced secretory vesicle translocation occurs without vesicle fusion. Bovine chromaffin cells co-expressing NPY-Cherry and VAMP2-pHluorin were treated with (a) vehicle or (b) IC87114 alone (arrow), or (c) pre-treated with IC87114 (for 20 min) before being stimulated (arrow) with barium (2 mM). Time-course variation of the NPY-Cherry and VAMP2-pHluorin fluorescence was shown in the right panel (n=4). Average peak fluorescence intensity changes in (d) NPY-Cherry or (e) VAMP2-pHluorin were expressed as mean ± SEM (n=13-15 from 4 independent experiments). Data are expressed as mean ± SEM. Scale bar, 10 μm.
Supplementary Figure S7: IC87114 promotes the activation of Cdc42 on sub-domains of the plasma membrane. (a) Ratio images of bovine chromaffin cells expressing CBD-YFP and mCherry. The insets showed region of the plasma membrane that exhibits highest Cdc42 activity. (b) Relative recruitment (Rm/Rc) of CBD-YFP on the selected regions of interest of the plasma membrane to the cytosol (mCherry) as a function of time (n=3 ROI)