Figure S1

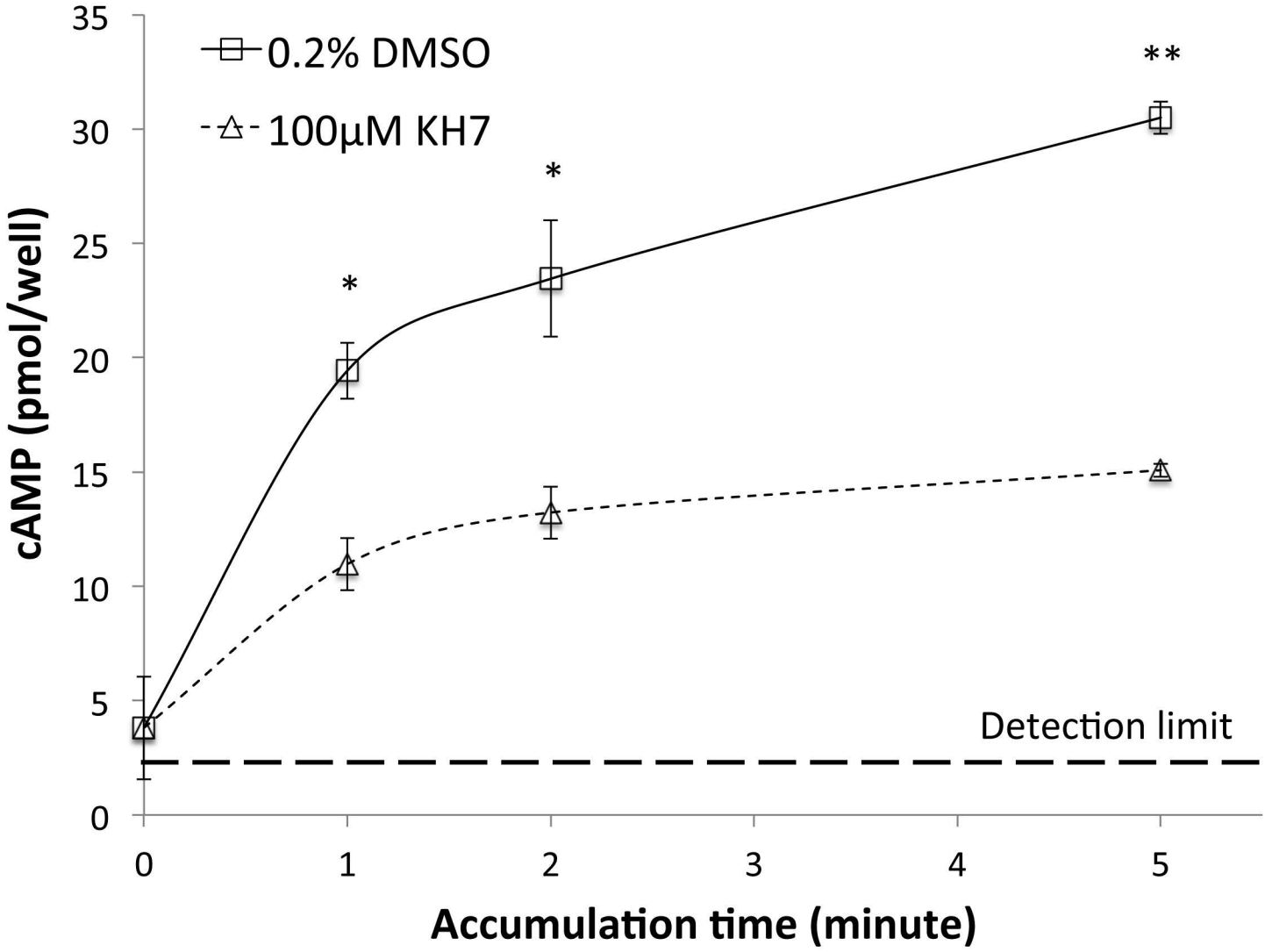

0.2% DMSO
100μM KH7
cAMP (pmol/well)
Accumulation time (minute)
Detection limit
*
*
**
35
30
25
20
15
10
5
0
0
1
2
3
4
5

Figure S2

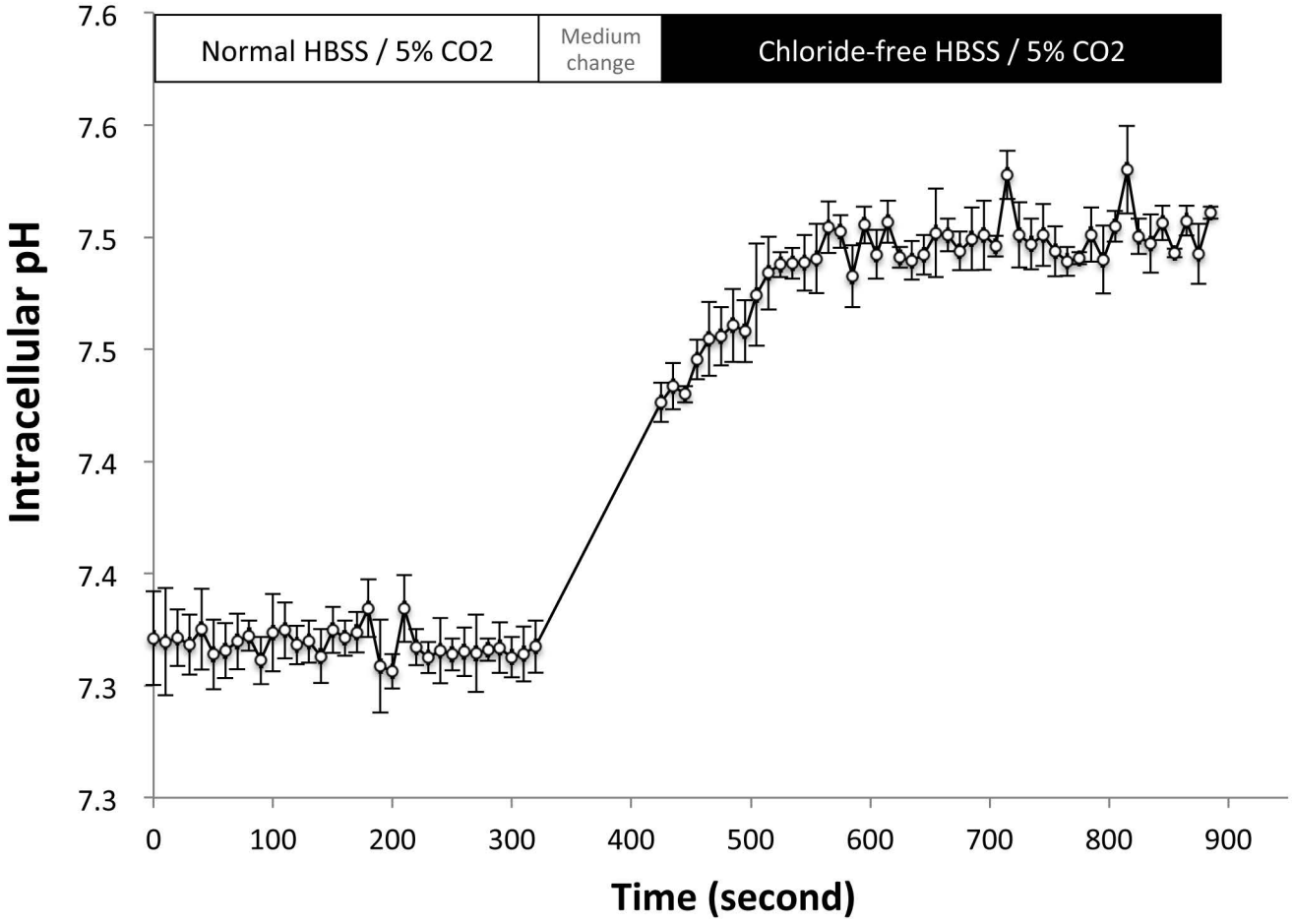

Normal HBSS / 5% CO2
Medium change
Chloride-free HBSS / 5% CO2
Intracellular pH
7.6
7.6
7.5
7.5
7.4
7.4
7.3
7.3
0
100
200
300
400
500
600
700
800
900
Time (second)

Figure S3

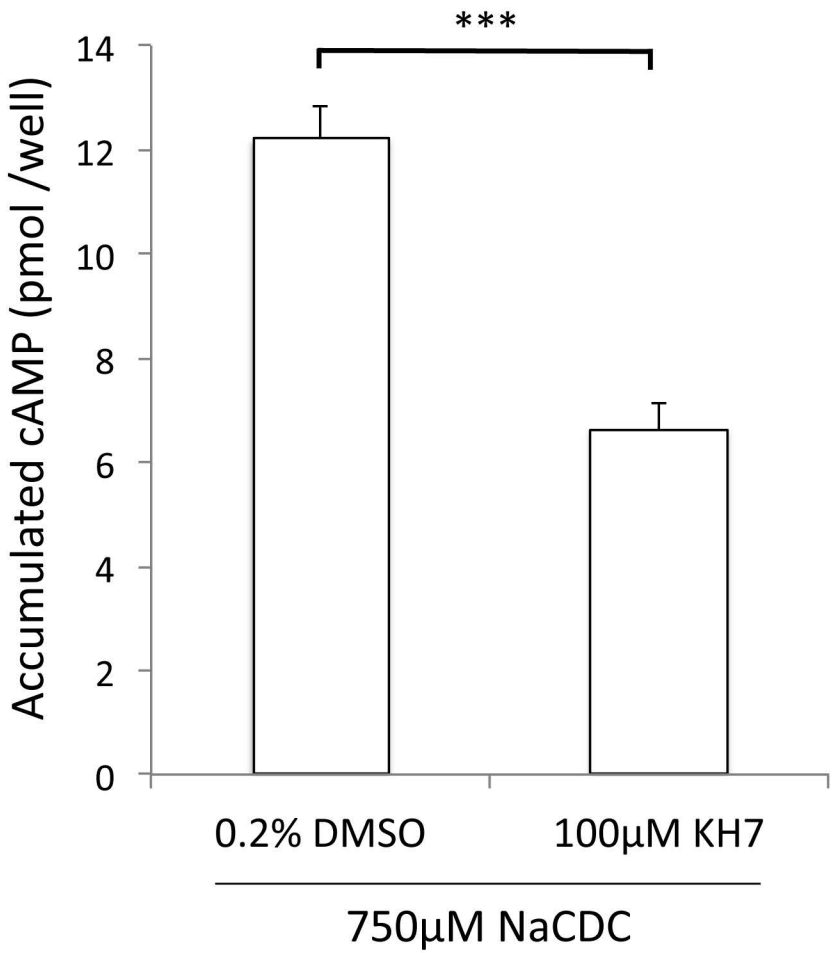

***
14
12
10
8
6
4
2
0
Accumulated cAMP (pmol /well)
0.2% DMSO
100μM KH7
750μM NaCDC

Figure S4

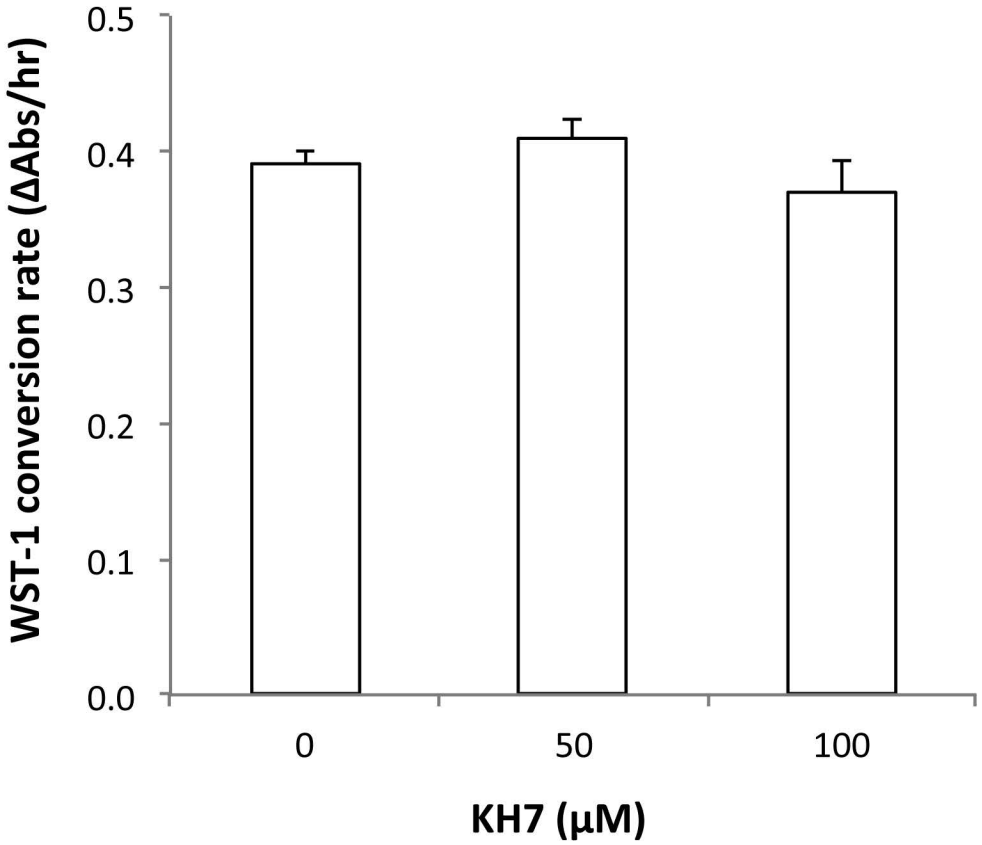


Figure S5

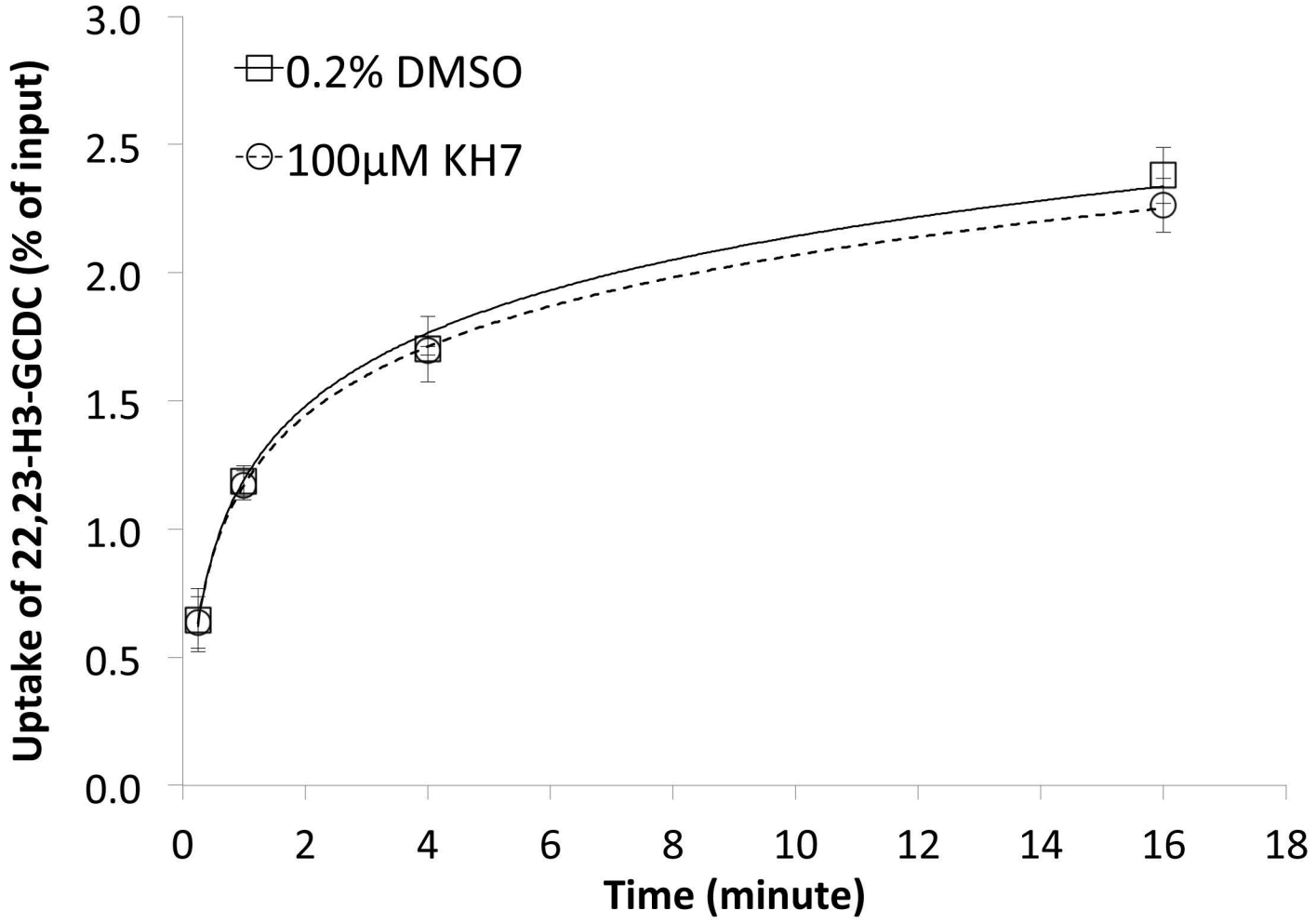

0.2% DMSO
100μM KH7
Uptake of 22,23-H3-GCDC (% of input)
Time (minute)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
0
2
4
6
8
10
12
14
16
18

Figure S6

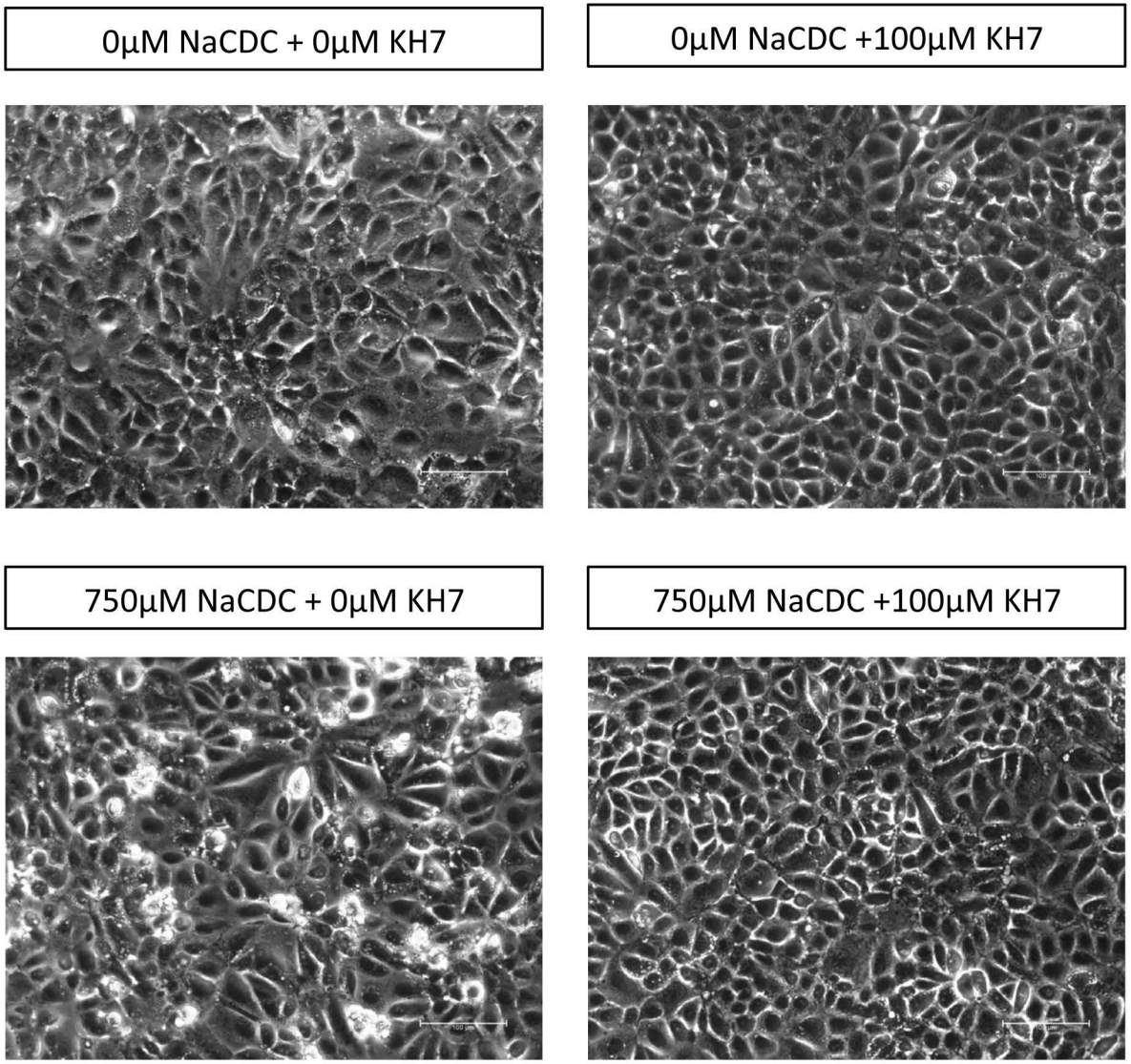
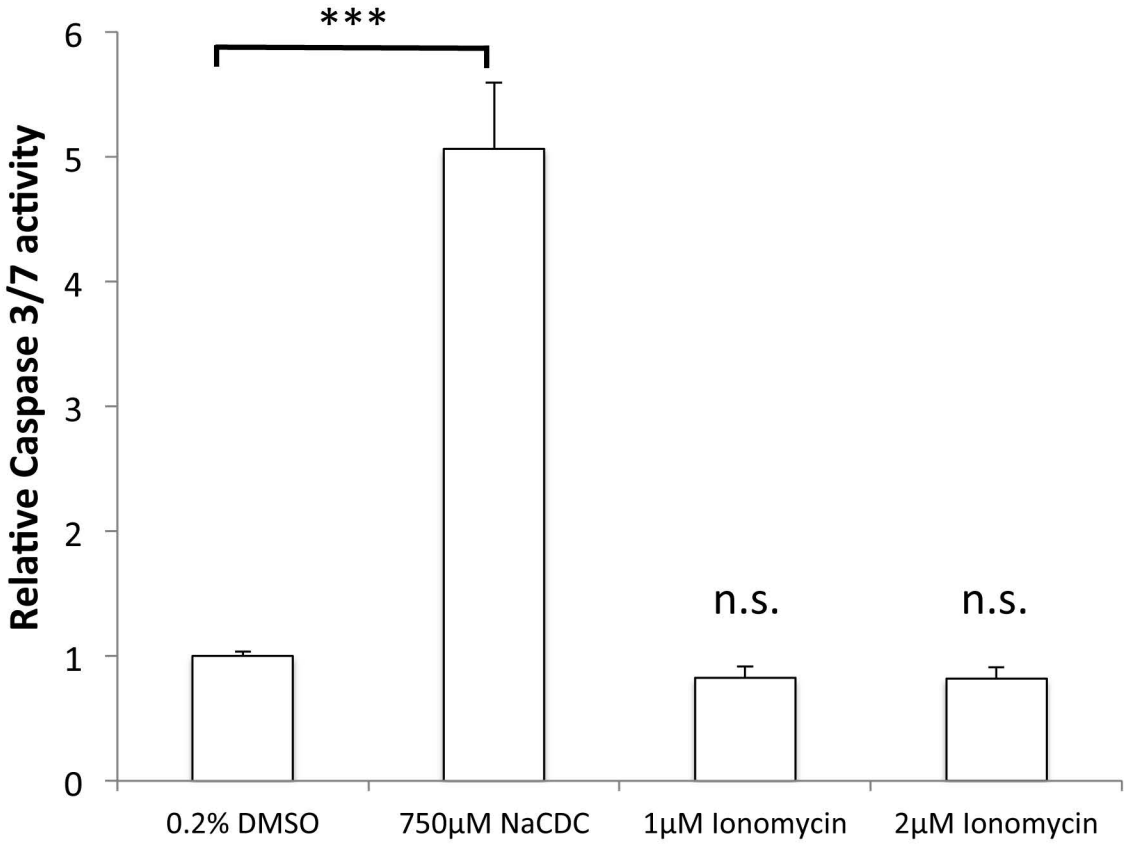

0μM NaCDC + 0μM KH7
0μM NaCDC +100μM KH7
750μM NaCDC + 0μM KH7
750μM NaCDC +100μM KH7

Figure S7


***
Relative Caspase 3/7 activity
6
5
4
3
2
1
0
n.s.
n.s.
0.2% DMSO
750µM NaCDC
1µM Ionomycin
2µM Ionomycin

Supplementary Methods

cAMP accumulation assay

To demonstrate bicarbonate responsiveness (Figure 1), confluent H69 cholangiocyte monolayers in 96-well plates were washed twice with Hank's balanced salt solution (HBSS) and incubated with bicarbonate-free HBSS in a 37°C water bath in ambient air for 30 minutes. Bicarbonate-free HBSS was aspirated and HBSS modified for 5% CO_2 with or without chloride was added (see *Supplementary Table 1* for formulation of modified HBSS), with 50μM KH7 or vehicle, in the presence of 250μM 3-isobutyl-1-methylxanthine (IBMX), a pan-phosphodiesterase inhibitor. Cells were incubated in 5% CO_2 stove for 30 minutes. Total cAMP was assayed with cAMP Direct Biotrak EIA kit (GE healthcare Life Sciences) according to the instruction manual. Results were corrected for protein content of individual wells by bicinchoninic acid (BCA) assay. For sAC activity in resting state (Figure S1) or during bile salt treatment (Figure S3), H69 cholangiocytes were cultured in 24-well plates. Experiments were performed in DMEM with 10% FBS. cAMP accumulated in the presence of 500μM IBMX for 2 minutes and stopped by 0.1M HCl and 1% Triton X-100.

cAMP was measured by Direct cAMP ELISA kit (Enzo Life Sciences).

Caspase 3/7 activity assay

Confluent H69 cholangiocyte monolayers in 96-well plates were treated with sodium chenodeoxycholate and KH7 in either normal DMEM or calcium-free DMEM (USBiological) supplemented with 1.5g/L sodium bicarbonate and 20mM HEPES-NaOH (pH 7.4) at 37°C in a 5% CO_2 incubator. Calcium-free DMEM was supplemented with 0.5mM EGTA to chelate the free Ca^{2+} in FBS. Apoptosis induced by sodium glycochenodeoxycholate was performed in DMEM supplemented with 0.375g/L sodium bicarbonate and 20mM HEPES-NaOH (pH6.8) in at 37°C in a 5% CO_2 incubator. Primary mouse cholangiocytes were cultured for 1 week on thick collagen gel in 96-well plate. Cells were treated with sodium chenodeoxycholate and KH7 (Tocris Bioscience) under identical conditions as the H69 cells. Caspase 3/7 activity was measured with artificial peptide substrate $(Ac\text{-}DEVD)_2$-Rh110 by SensoLyte Homogeneous Rh110 Caspase 3/7 Assay Kit (AnaSpec Inc., Fremont, CA) according to instruction manual. The kinetics of fluorophore

Rh110 release, as a result of cleavage by caspase 3 and caspase 7, was measured by NOVOstar microplate reader (BMG Labtech GmbH, Offenburg, Germany) at λ_{ex} / λ_{em} = 480 / 510 nm. The slope in the linear window of the kinetic reading was calculated and defined as the caspase 3/7 activity. For experiments involving different cell lines, caspase 3/7 activities were normalized to protein content in a parallel plate by BCA assay.

Cell fractionation for cytochrome c release and Bax translocation assays

All solutions used were ice-cold. Confluent monolayers of H69 in 6-well plate were washed twice with phosphate-buffered saline. Monolayers were permeabilized with 50µg/mL digitonin in 400µL intracellular buffer (ICB: 120mM KCl, 10mM NaCl, 0.5mM KH_2PO_4, 1.5mM $MgSO_4$, 5mM Na_2-succinate, 20mM HEPES-KOH, pH 7.4) supplemented with 2mM EGTA, 1mM DTT and cOmplete EDTA-free protease inhibitor cocktail (Roche). Plates were incubated on orbital shaker at 100 rpm for 15 minutes at 4°C. The supernatants were harvested as the cytosolic fraction and the permeabilized monolayers were immediately gently washed twice with 1mL ICB and lysed

with 400µL RIPA buffer (150mM NaCl, 1% (w/v) Triton X-100, 0.5%(w/v) sodium deoxycholate, 0.1% SDS, 20mM Tris-HCl, pH 8.0) supplemented with 5mM EDTA and protease inhibitor cocktail. The digitonin-permeabilized cell layers contained intact mitochondria, nuclei and plasma membrane, and was used here as the mitochondrial fraction. Equal volume of cytosolic and mitochondrial fractions were subjected to SDS-PAGE and immunoblotting.

Intracellular Ca^{2+} chelation by BAPTA

H69 cholangiocyte monolayers in 96-well plates were washed twice with FBS-free DMEM and incubated with 2.5µM, 5µM, or 10µM BAPTA-AM (Molecular Probes) in FBS-free DMEM at 37°C in a 5% CO_2 incubator for 30 minutes. BAPTA-AM was dissolved in DMSO and an equivalent amount of DMSO (0.1%) was added to the control incubations. Monolayers were then washed twice with FBS-free DMEM and treated with 750µM sodium chenodeoxycholate for 1 hour. Caspase 3/7 activity was measured as described above.糰

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Lentiviral transduction for shRNA-mediated knockdown

Lentiviral constructs for shRNA-mediated knockdown of were obtained from MISSION® TRC shRNA library (Sigma-Aldrich, St. Louis, MO): AE2 (TRCN0000043862), MCU (TRCN0000133861), shRNA control (SHC002). Production of Lentivirus was performed as described previously[1]. Stable knockdown H69 cholangiocytes cell lines were made by Lentiviral transduction and selected with 2.5µg/mL puromycin. All transformants used in experiments were polyclonal.

Cloning of tetracycline inducible sAC knockdown vector

Tet-pLKO-puro was a gift from Dmitri Wiederschain (Addgene plasmid # 21915). Target sequence for sAC was generated at The RNAi Consortium (TRC) public portal (http://www.broadinstitute.org/rnai/public/seq/search). Cloning of shRNA expressing vectors was performed as previously described[2]. Briefly, Tet-pLKO-puro was digested with *Age*I and *EcoR*I and gel purified. Sense and anti-sense oligo's for sAC and SHC (*Supplementary Table 2*) were

annealed and ligated into the open vector. Lentivirus production and transduction were performed as described above.

RNA isolation, reverse transcription and real-time quantitative PCR (RT-qPCR)

H69 cholangiocytes were cultured in 6-well plate until confluent. Total RNA was isolated using TriPure Isolation Reagent (Roche) or TRI Reagent (Sigma). 2µg total RNA was treated with DNase I (Promega) and then reversely transcribed into cDNA with 5 pmole of gene-specific primer and 100U RevertAid reverse transcriptase (Thermo Scientific) in a reaction volume of 20µL. cDNA was diluted with water to 100µL. 2µL of diluted cDNA were used as template for RT-qPCR using SYBR Green I Master (Roche) or SensiFAST SYBR No-ROX kit (Bioline). Initial fluorescent values were calculated by LinRegPCR[3] (version 2013.0, Academic Medical Center, Amsterdam). Expression levels were normalized to reference gene *36B4*. Please refer to *Supplementary Table 3* for primer sequences used in reverse transcription and RT-qPCR.

WST-1 viability assay

Viability of H69 cholangiocytes after sAC inhibition by KH7 was determined by conversion of WST-1 to formazan. Confluent H69 cholangiocyte monolayers in 96-well plates were incubated with 0, 50, or 100μM KH7 in culture medium for 1 hour. Monolayers were washed twice with culture medium and incubated with WST-1 (Roche, 1:10 dilution in culture medium) for one hour in 5% CO_2 incubator. Empty wells with WST-1 served as blank. Absorbance at 450nm (formazan) and 690nm (reference wavelength) was measured by Synergy HT Multi-Mode Microplate Reader (BioTek) every 30 minutes for 2 hours. The conversion rate was calculated from the slope.

DNA fragmentation assay

Isolation of fragmented genomic DNA was performed as described by Herrmann et al[4] with modification. Confluent H69 cholangiocyte monolayers were treated with 750μM sodium chenodeoxycholate and increasing concentrations of KH7 for 1 hour at 37°C in 5% CO_2 incubator. Media were

harvested and centrifuged at 20,000g to pellet apoptotic bodies. The attached cells were harvested by adding 285µL lysis buffer (1% NP-40, 20mM EDTA, 50mM Tris-HCl, pH 8.0) and pooled with the apoptotic body pellets. Fifteen µL 10% SDS and 3µL proteinase K (20mg/mL in 20mM $CaCl_2$, 50% glycerol and 10mM Tris-HCl, pH 7.5) were added to the lysates and incubated for 48 hours in a 37°C water bath with shaking. Proteinase K was then heat-inactivated at 75°C for 15 minutes before digesting RNA with 2µL RNase A (1mg/mL in water) at 37°C overnight. To precipitate protein, 100µL 10M ammonium acetate was added and the mixture centrifuged at 20,000g for 20 minutes. The supernatants (300µL) were harvested and DNA was precipitated by adding 2.5 volumes of ethanol and centrifuged at 20,000g for 10 minutes. The DNA pellets were washed once with 1mL 80% ethanol, dried by heating at 55°C for 10 minutes, dissolved in 25µL DNA loading buffer with 0.1% SDS and resolved on a 1.5% agarose gel.

Membrane protein extraction

Control and AE2 knockdown H69 cholangiocytes were cultured on 60 mm

culture dish until confluent. Confluent monolayers were permeabilized with 1.2mL 50µg/mL digitonin in intracellular buffer (ICB: 120mM KCl, 10mM NaCl, 0.5mM KH_2PO_4, 1.5mM $MgSO_4$, 5mM Na_2-succinate, 20mM HEPES-KOH, pH 7.4) supplemented with 2mM EGTA, 1mM DTT and cOmplete EDTA-free protease inhibitor cocktail (Roche). Plates were incubated on orbital shaker at 100 rpm for 15 minutes at 4°C. Supernatants were discarded. The permeabilized monolayers were immediately washed once with 3mL ice-cold ICB and collected in 1mL ICB by scraping. The homogenates were centrifuged at 20,000g, 4°C for 10 minutes. Supernatants were discarded and the pellets extracted with 0.45% (w/v) NP-40 in ICB for 15 minutes on ice and centrifuged again at 20,000g, 4°C for 10 minutes. The supernatants containing the extracted membrane protein from both plasma membrane and organelles were subjected to SDS-PAGE and immunoblotting.

SDS-PAGE and Immunoblotting

Except for cytochrome c release and Bax translocation assay, whole cell lysates in RIPA buffer or membrane protein extracts were quantified by BCA

assay. Equal amounts of protein (40 to 50µg per lane) were subjected to SDS-PAGE, transferred to PVDF membranes by semi-dry blotting and blocked overnight in 5% non-fat milk / PBST (phosphate-buffered saline with 0.05% (w/v) Tween 20) at 4°C. For immunodetection, the PVDF membranes were incubated with primary antibody for 1 hour, washed 3 times with TBST (Tris-buffered saline with 0.05% (w/v) Tween 20), incubated with horseradish peroxidase-conjugated secondary antibody for 1 hour, washed again 3 times with TBST. All antibodies were diluted in 1% non-fat milk-TBST and incubation was performed at room temperature. The PVDF membrane was developed with homemade enhanced chemiluminescence reagents (100mM Tris-HCl pH 8.5, 1.25mM luminol, 0.2mM p-coumarin and freshly added 3mM H_2O_2) and detected by ImageQuant LAS 4000 (GE Healthcare Life Sciences). Densitometry was performed by software ImageJ[5]. Please refer to *Supplementary Table 4* for the list of primary antibodies and dilution.

Intracellular pH measurement by

2',7'-Bis-(2-Carboxyethyl)-5-(and-6)-Carboxyfluorescein (BCECF)

H69 human cholangiocytes were cultured in 96-well black plate with clear bottom (Corning, Costar 3606) until confluence. Normal and chloride-free HBSSs for 5% CO2 were equilibrated overnight in 5% CO2, 37°C incubator and pH confirmed on day of experiment to be 7.4. H69 monolayers were loaded with 5μM BCECF•AM in bicarbonate-free HBSS at room temperature for 45 minutes. Excessive dye was removed and incubated for additional 30 minutes in bicarbonate-free HBSS at room temperature. Cells were refreshed with HBSS modified for 5% CO2 and incubated at 37°C, 5% CO2 in CLARIOstar (BMG LABTECH, Ortenberg, Germany) under atmospheric control. Medium was refreshed again before assay started. Duo-excitation ratiometric fluorescence of BCECF was measured every 2 minutes at $\lambda_{ex}/\lambda_{em}$= 440/535 and 490/535 nm. After signals stabilized, measurement was paused and medium replaced by chloride-free HBSS for 5% CO2. Fluorescence measurement was resumed and continued until signals were stable. To

calibrate, cells were incubated in high potassium buffer (140.85 mM KCl, 1.27 mM CaCl2, 0.81 mM MgSO4, 0.44mM KH2PO4, 0.34mM K2HPO4, 5.55mM glucose, 30mM HEPES) with 10μM Nigericin (Cayman Chemical) and pH titrated to 5.59, 6.32, 6.90, 7.39, 7.75, 7.86 and 7.91 by 5M KOH. Fluorescence of unloaded cells was recorded for background correction. After background correction, Fluorescence ratios ($R= F_{490/535} / F_{440/535}$) were calculated. Standard curve was fitted to the equation $pH=pK_a+C*\log[(R-F_{min})/(F_{max}-R)]$ by least square regression using Solver Add-in in Microsoft Excel Spreadsheet[6]. pK_a, C, F_{min} and F_{max} were fitted to be 7.221, 0.988, 0.349 and 1.742 respectively. R^2 of the fitted curve is 0.9999.

Bile salt uptake assay

Bile salt uptake assay was performed with H69 cholangiocyte monolayer in 24-well plate at 37°C in ambient air. H69 monolayers were equilibrated for 15 minutes with bicarbonate-free HBSS supplemented with 20mM HEPES-NaOH, pH 7.4 for 15 minutes. Medium was removed and 200μL HEPES-buffered bicarbonate-free HBSS with 22,23-^{3}H-GCDC (a generous gift from Dr. Alan F.

Hofmann. Average total activity per well ≈ 2.87 kBq.) and 750µM unlabeled NaGCDC. Bile salts were removed after 0.25, 1, 4 and 16 minutes. Monolayers were washed twice with ice-cold HBSS and lysed in 200µL RIPA buffer. Radioactivity of the RIPA lysates was detected by liquid scintillation counter Tri-carb 2900TR (Perkin Elmer, Groningen, The Netherlands). Results were normalized to total input activity.

Morphological study of apoptosis

H69 cholangiocytes were treated with (combinations of) sodium chenodeoxycholate, KH7 and pan-caspase inhibitor Q-DV-OPh for 1 hour at 37°C and 5% CO_2. Overnight treatment with 50µM etoposide served as positive control. Cells were fixed with 4% paraformaldehyde in PBS at room temperature for 15 minutes. Apoptotic blebbing was studied by phase contrast microscopy on a DMi8 microscope (Leica). To visualize nuclear morphology, cells were permeabilized with 0.1% Triton X-100 for 15 minutes, mounted in Vectashield / 4',6-diamidino-2-phenylindole (Vector Laboratories) and imaged on a DMi8 microscope (Leica).

Isolation and culture of primary mouse cholangiocytes

Primary mouse cholangiocytes were isolated from a 10-week old C57BL/6J wild type female mouse[7, 8]. Animal experiment was approved by institutional animal experiment committee. Briefly, the mouse was anesthetized with FFD mix (11.76mg/kg fluanisone, 0.37mg/kg fentanyl citrate and 5.88mg/kg diazepam) and perfused via portal vein first with Hanks A solution (120mM NaCl, 5mM KCl, 0.4mM KH_2PO_4, 0.2mM Na_2HPO_4, 2.5mM EGTA, 25mM $NaHCO_3$, pre-equilibrated with 5% CO_2, 95% O_2 carbogen) supplemented with 4U/mL heparin and subsequently Hanks B solution (120mM NaCl, 5mM KCl, 0.4mM KH_2PO_4, 0.2mM Na_2HPO_4, 25mM $NaHCO_3$, 0.4mM $MgSO_4$, 0.5mM $MgCl_2$ and 3mM $CaCl_2$, pre-equilibrated with 5% CO_2, 95% O_2 carbogen) supplemented with 0.25mg/mL type IV collagenase and 5µg/mL soybean trypsin inhibitor. The Glisson's capsule and digested liver parenchyma were removed carefully, leaving the intact intrahepatic biliary tree. The biliary tree was then finely minced and digested in 37°C water bath with shaking in DMEM/F-12 medium with 1mg/mL bovine serum albumin, 0.25mg/mL type IV

collagenase, 0.34mg/mL pronase, 0.06mg/mL DNase, 100U/mL penicillin and 100µg/mL streptomycin. The digested tissues were serial filtered though 100µm and 40µm Falcon cell strainers (BD biosciences). The trapped fractions were digested in DMEM/F-12 medium with 1mg/mL bovine serum albumin, 0.25mg/mL type IV collagenase, 0.26mg/mL hyaluronidase, 0.06mg/mL DNase, 100U/mL penicillin and 100µg/mL streptomycin and serial filtered though 100µm and 40µm filters. The fraction trapped by the 40µm cell strainer was collected, washed 5 times with phosphate-buffered saline and cultured on a thick collagen gel made of type I collagen from rat tail (Corning, #354236) in the described full hormone-supplemented medium[9].

Supplementary Reference

Reference List

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Supplementary tables

Supplementary Table 1. Formulation of standard and modified HBSS

Concentration (mM)	Standard HBSS	Bicarbonate-free HBSS	Normal HBSS for 5% CO_2	Chloride-free HBSS for 5% CO_2
NaCl	136.89	141.34	119.78	—
Na gluconate	—	—	—	119.88
$CaCl_2$	1.27	1.27	1.27	—
Ca gluconate	—	—	—	1.27
KCl	5.37	5.37	5.37	—
K gluconate	—	—	—	5.37
KH_2PO_4	0.44	0.44	0.44	0.44
Na_2HPO_4	0.34	0.34	0.34	0.34
$MgSO_4$	0.81	0.81	0.81	0.81
$NaHCO_3$	4.17	—	21.43	21.43
Glucose	5.55	5.55	5.55	5.55
HEPES-NaOH, pH 7.4	20	20	20	20

Supplementary Table 2. Oligo sequences for Tet-pLKO-puro cloning (5'→3')

Target		Oligo Sequences†
Short hairpin control	*Sense*	CCGG**CAACAAGATGAAGAGCACCAA**CTCGAGTTGGTGCTCTTCATCTTGTTG*TTTTT*
	Antisense	AATT*AAAAA*CAACAAGATGAAGAGCACCAACTCGAGTTGGTGCTCTTCATCTTGTTG
Soluble adenylyl cyclase	*Sense*	CCGG**GAATGTGCTATGGATATATTT**CTCGAGAAATATATCCATAGCACATTC*TTTTT*
	Antisense	AATT*AAAAA*GAATGTGCTATGGATATATTTCTCGAGAAATATATCCATAGCACATTC

† Sequence annotations: **bolded**, target sequence; underlined, *Age*I site; double underlined, *EcoR*I site; dotted line, hairpin loop with *Xho*I restriction site;

italicized, termination signal.

Supplementary Table 3. Primers for reverse transcription and RT-qPCR (5'→3')

Target	Primer for reverse transcription	Forward Primer for RT-qPCR	Reverse Primer for RT-qPCR
sAC	ATCTGCAACACCTTTCATAA	GCTCTCAAGTCCACAAAATCCAAAC	CCAATGACTGTGTACTCGTGTCTC
AE2	GACATAATCAGCTCCGACAC	ACCCTCATGTCAGACAAGCAA	TCCTCTCGCTTCTTGAGCATC
MCU	GCTCCATGATGTCCCAGGAA	CCTGTGATATCTGTGAGGCTACC	GCGAACACCATCTGGTGAATAG
36B4	TAATCCGTCTCCACAGACAA	TGTGGGAGCAGACAATGTGG	TGAGGCAGCAGTTTCTCCAG

Supplementary Table 4. Primary antibodies used in immunoblotting

Primary antibody	Company	Catalog No.	Dilution
Anti-sAC (R21)	Gift from prof. Lonny Levin and Prof. Jochen Buck, Weill Medical College, Cornell University		1:3000
Anti-AE2 (N-12)	Santa Cruz	sc-46710	1:200
Anti-ATP1A1	Gift from Dr. Jan B. Koenderink		1:1000
Anti-MCU	Sigma	HPA016480	1:500
Anti-β-Actin	Sigma	A5441	1:5000
Anti-PARP	Roche	11835238001	1:2000
Anti-cleaved caspase 3	Cell Signaling	#9661	1:1000
Anti-JNK	Cell Signaling	#9252	1:1000
Anti-pJNK (Thr183 / Tyr185)	Cell Signaling	#4668	1:1000
Anti-Mcl-1 (S-19)	Santa Cruz	sc-819	1:500
Anti-Bax	Cell Signaling	#2772	1:1000
Anti-pBax (Thr167)	Assay Biotech	A0733	1:500

Supplementary figure legends

Figure S1. sAC accounted for a significant portion of total cAMP pool in H69 cholangiocytes. The total cellular cAMP pool consists of the sAC-dependent cAMP pool and various tmAC-dependent pools. To distinguish sAC-dependent cAMP pool in H69 cholangiocytes from those of tmACs, cAMP accumulation assay was performed in the absence or presence of sAC-specific inhibitor KH7. Cells were pretreated with 0.2% DMSO or 100μM KH7 for 5 minutes. Accumulation of cAMP was initiated by the addition of 500μM IBMX and stopped after 1, 2 and 5 minutes. The amount of accumulated cAMP was determined by ELISA. Baseline total cAMP assayed before the addition of IBMX lies about the detection limit (dashed line) of the assay as a result of breakdown by phosphodiesterases. A representative duplicate assay is shown. Two-tailed Student's t-test, * $P < .05$; ** $P \leq .01$.

Figure S2. Removal of extracellular chloride resulted in intracellular alkalinization in H69 human cholangiocytes. Intracellular pH was measured with pH probe BCECF under 5% CO_2 at 37°C as described in Supplementary

Method. After baseline intracellular pH was stable, extracellular chloride was replaced with gluconate. Average of triplicate assay with standard deviation is shown.

Figure S3. sAC-specific inhibitor KH7 reduced total cellular cAMP during BSIA. Confluent H69 monolayers were pre-incubated with 750μM NaCDC in the presence or absence of 100μM KH7 in 5% CO_2, 37°C incubator for 10 minutes before addition of 500μM IBMX. cAMP was accumulated for 2 minutes. A representative triplicate assay was shown. Two-tailed Student's t-test, ** $P \leq .01$.

Figure S4. sAC-specific inhibitor KH7 did not cause acute cytotoxicity in H69 cholangiocytes. H69 cholangiocytes were incubated with 0, 50, 100μM KH7 for 1 hour. WST-1 conversion rate was measured as a viability index. One-way ANOVA, p=0.0714.

Figure S5. sAC-specific inhibitor KH7 did not affect bile salt uptake by H69 human cholangiocytes. Confluent H69 human cholangiocyte

monolayers were incubated with 2.87kBq 22,23-^{3}H-glycochenodeoxyholate and 750μM sodium glycochenodeoxycholate in bicarbonate-free HBSS at 37°C in ambient air. After 0.25, 1, 4, and 16 minutes, cells were washed twice糎 ice-cold bicarbonate-free HBSS and lysed in RIPA buffer. Radioactivity of cell lysates was determined by liquid scintillation counter. Results were normalized to input.

Figure S6. Bile salt-induced apoptotic membrane blebbing was prevented by sAC inhibition. H69 cholangiocytes were treated with combination of 750μM NaCDC and 100μM KH7 for 1 hour in 5% CO_2, 37°C incubator. Cells were fixed with 4% paraformaldehyde and apoptotic membrane blebbing was examined by phase contrast microscope.糎

Figure S7. Increasing intracellular free Ca^{2+} by ionomycin did not induced apoptosis in H69 human cholangiocytes. H69 human cholangiocyte monolayers were treated with 750μM NaCDC, 1μM and 2μM ionomycin for 1 hour in 5% CO_2, 37°C incubator. Caspase 3/7 activity was

determined. Shown here average of a triplicate assay. Results were normalized to the vehicle-treated group.糠wo-tailed Student’s t-test (compared with vehicle-treated group), *** $P \leq .001$; n.s. not significant.糠