SUPPLEMENTARY METHODS

Reagents. Eagle’s minimal essential medium (EMEM) containing Earle’s salts but not L-glutamine, the calcium free media S-MEM, and L-glutamine were purchased from Life Technologies (Rockville, MD, USA). FBS, DMEM and the DMEM/F12 were obtained from Mediatech Inc. (Herndon, VA, USA). Antibody to CaR was obtained from Affinity Bioreagents (Golden, CO, USA), antibody to human CD104 was from BD Biosciences (San Jose, CA, USA), while antibodies to villin and human GAPDH was from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Alexa fluor 555 anti-rabbit IgG (for CaR), Alexa fluor 488 anti-goat IgG (for villin), the Histostain-Plus Kit and DAPI were obtained from Invitrogen (Carlsbad, CA, USA). Fugene was purchased from Roche Diagnostics Co. (Indianapolis, IN, USA). Rp-8-pCPT-cGMP, Rp-cAMPS, and L-cis-diltiazem were from Biomol International L.P. (Plymouth Meeting, PA, USA), while 8-br-PET-cGMPS was purchased from EMD Biosciences Inc. (La Jolla, CA, USA). Native ST and the inactive analog TJU 1-103 were prepared as described (reference 9 in manuscript). The West Pico Chemiluminescent Substrate was obtained from Pierce Biotechnology (Rockford, IL, USA). BAPTA-AM, 3-isobutyl-1-methylxanthine (IBMX), 8-br-cGMP, 8-pCPT-cGMP, Ca$^{2+}$, Mg$^{2+}$, Gd$^{3+}$, spermine and all other chemicals were from Sigma Chemical Co. (St. Louis, MO, USA).

Cell Culture. Caco-2 (passage 30-50), T84 (passage 50-70) and SW480 (passage 100-120) human colon carcinoma cells were obtained from the American Type Culture Collection (Manassas, VA, USA). Cell cultures were maintained at 37°C (5% CO$_2$) in DMEM (Caco-2 and SW480) or DMEM/F12 (T84) containing 100 IU/ml penicillin, 100 μg/ml streptomycin, and 10% FBS. Cells were fed every third day and split when subconfluent.
For experiments, tumor cells were synchronized in EMEM for 48 h, and then washed (3 times with PBS) and stimulated to proliferate with 10 mM l-glutamine in S-MEM or an *ad hoc* minimal essential medium (112 mM NaCl, 1 mM MgCl₂, 5.33 mM KCl, 10 mM NaH₂PO₄, 5.56 mM glucose, 25 mM NaHCO₃, 0.5 mM Na-pyruvate, 15 mM Na-HEPES, MEM amino acids, MEM vitamins) in the presence of the indicated concentration of Ca²⁺ and reagents. Calcium-free S-MEM or the *ad hoc* minimal essential medium were employed for experiments with ≤5 mM Ca²⁺, whereas for experiments employing ≥10 mM Ca²⁺ only the *ad hoc* minimal essential medium, which contains magnesium chloride in place of magnesium sulfate, was used to permit sustained high Ca²⁺ levels into solution.
LEGENDS TO SUPPLEMENTARY FIGURES

Supplementary Fig. 1. Regulation of CaR expression by ST in human colon carcinoma cells. (A) Time-course of ST (1 μM) effects on CaR expression in total cell lysates from T84 cells (left panels). GAPDH, the loading control; PBS, the vehicle control. *Lower left panel* represents relative (to the 0 h PBS) CaR levels, normalized to the respective loading control (GAPDH), from 4 independent experiments as quantified by densitometry. *, p<0.05, versus respective parallel control (PBS) condition. In the right panel, CTR (control) reflects anti-CaR immunoblots employing human parathyroid CaR in the absence (+) or presence (-) of the specific blocking peptide. (B) Selective expression of specific markers GAPDH and villin in cytosol and membrane extracts from T84 cells. (C) Relative (to the 0 h PBS) CaR levels, normalized to the respective loading control (villin), in T84 membrane fractions from 3 independent experiments as quantified by densitometry. *, p<0.05, versus respective parallel control (PBS) condition. (D) Relative (to the respective CaR in total cell lysate) CaR protein levels in distinct T84 cell fractions by densitometry. The percentage of cytosolic CaR to the total CaR in cell lysates, normalized to respective GAPDH controls, was examined to determine the amount of CaR translocation. Membrane CaR protein was calculated as [100-(Cytosolic CaR/total CaR X 100)]. Results are mean ± SEM from 3 independent experiments. Means are also reported in table.

Supplementary Fig. 2. GCC signaling does not alter CD104 expression in colon cancer cell surfaces. Membranes of non-permeabilized T84 cells were stained (brown) with mouse anti-human CD104, a specific intestinal epithelial cell marker. Tumor cells were treated for 24 h with PBS (vehicle control), ST (1 μM) or 8-br-cGMP (5 mM) in the presence of 5 mM Ca^{2+}. In blue, staining of nuclei with hematoxylin. Magnification, 40X.
Supplementary Fig. 3. Antisense-mediated inhibition of CaR expression in colon cancer cells. Anti-CaR immunoblots from T84 cells stably expressing the CaR sense vector (S-CaR) or the CaR antisense vector (AS-CaR). GAPDH, loading control.

Supplementary Fig. 4. $\text{Ca}^{2+}_o$ inhibits DNA synthesis of human colon cancer cells preconditioned with ST by targeting CaRs. Synchronized T84 cells were stimulated to proliferate with 10 mM L-glutamine for 21 h in the presence of the vehicle control (PBS) or 1 µM ST. Then, tumor cells were washed (3 times) with PBS and DNA synthesis examined by $^3\text{H}$-thymidine incorporation for 3 h in the presence of the indicated concentrations of $\text{Ca}^{2+}_o$. 
Pitari_Supplementary Figure 1

A

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CTR

PBS ST PBS ST PBS ST PBS ST

B

GAPDH

Villin

C

0 h

3 h

D

0 h

3 h

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Pitari_Supplementary Figure 2

CD104

PBS

ST

8-br-cGMP
Pitari_Supplementary Figure 3

![Supplementary Figure 3](image-url)
DNA Synthesis (CPM, x1,000)

- PBS
- ST

Ca^{2+}_o
10 mM

Ca^{2+}_o
1 mM

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