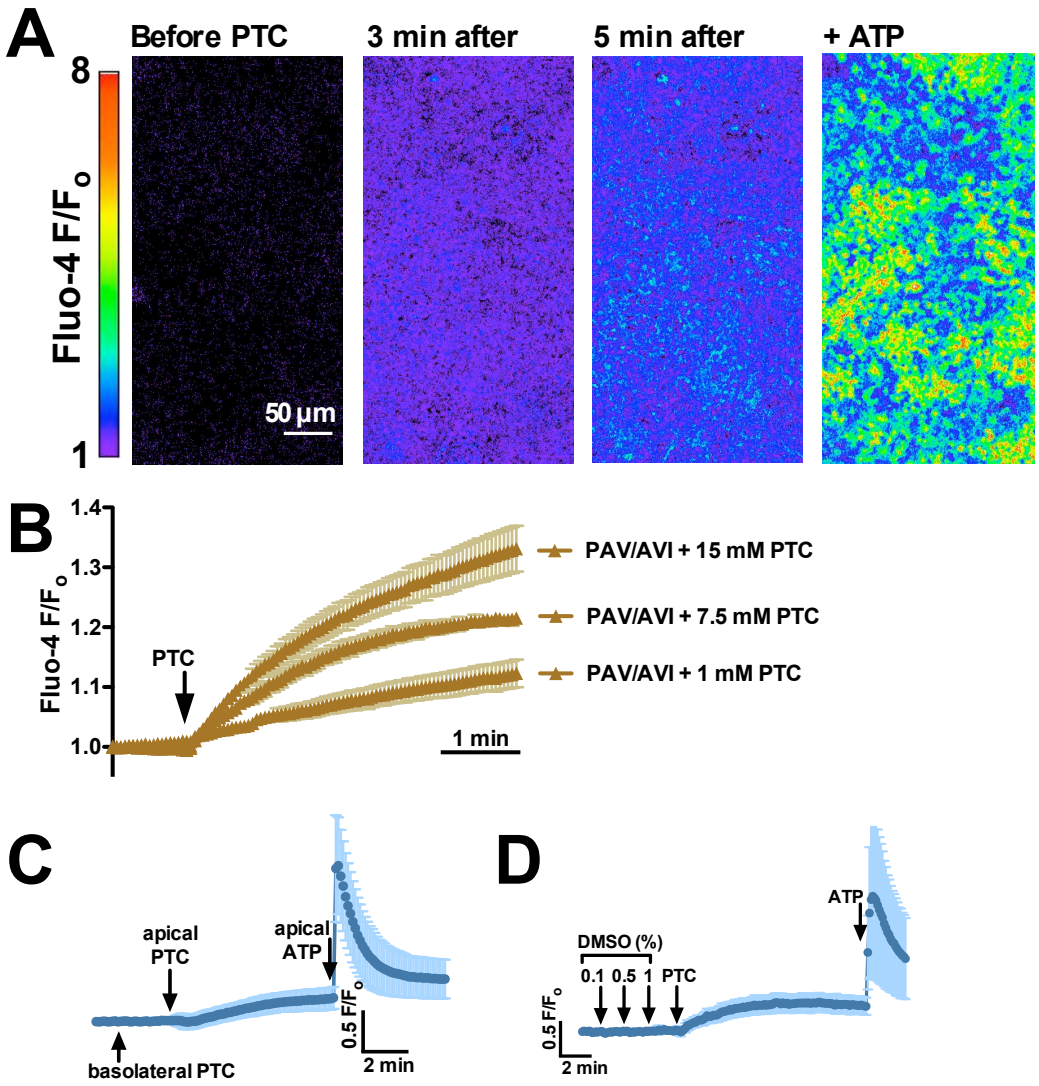
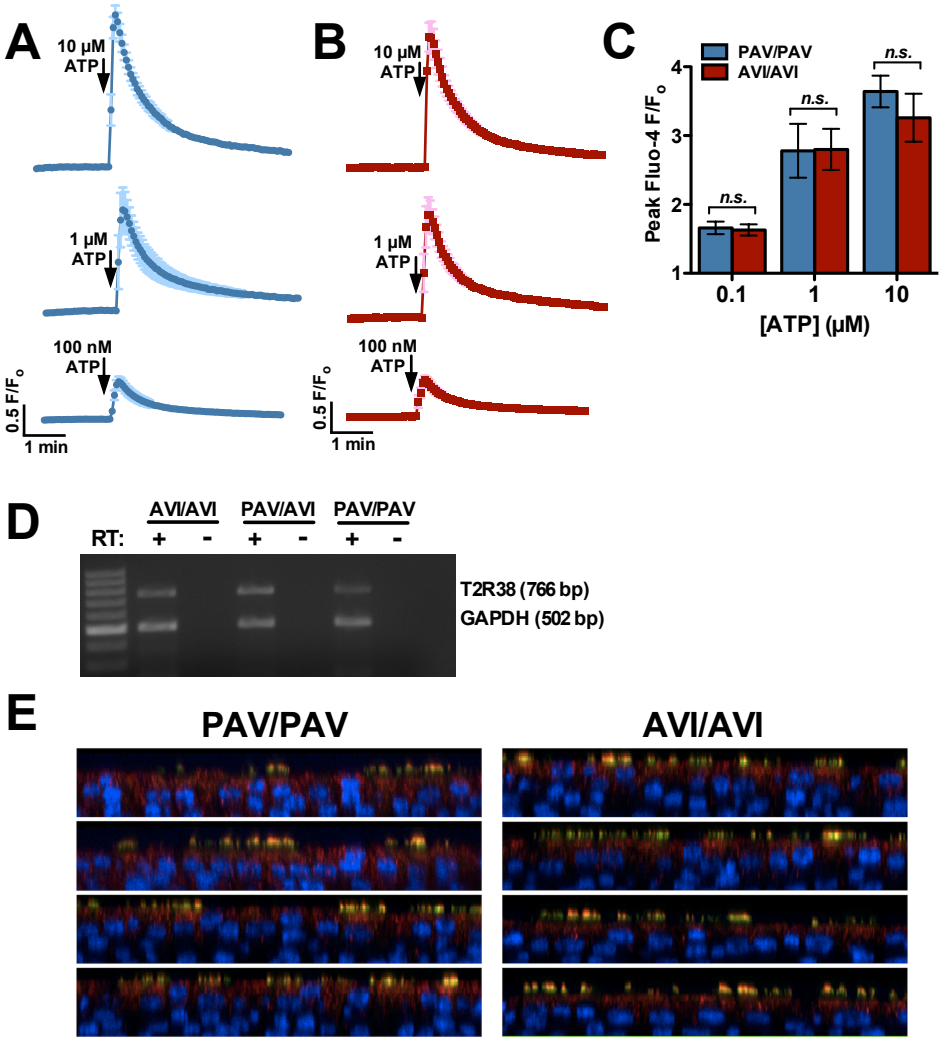
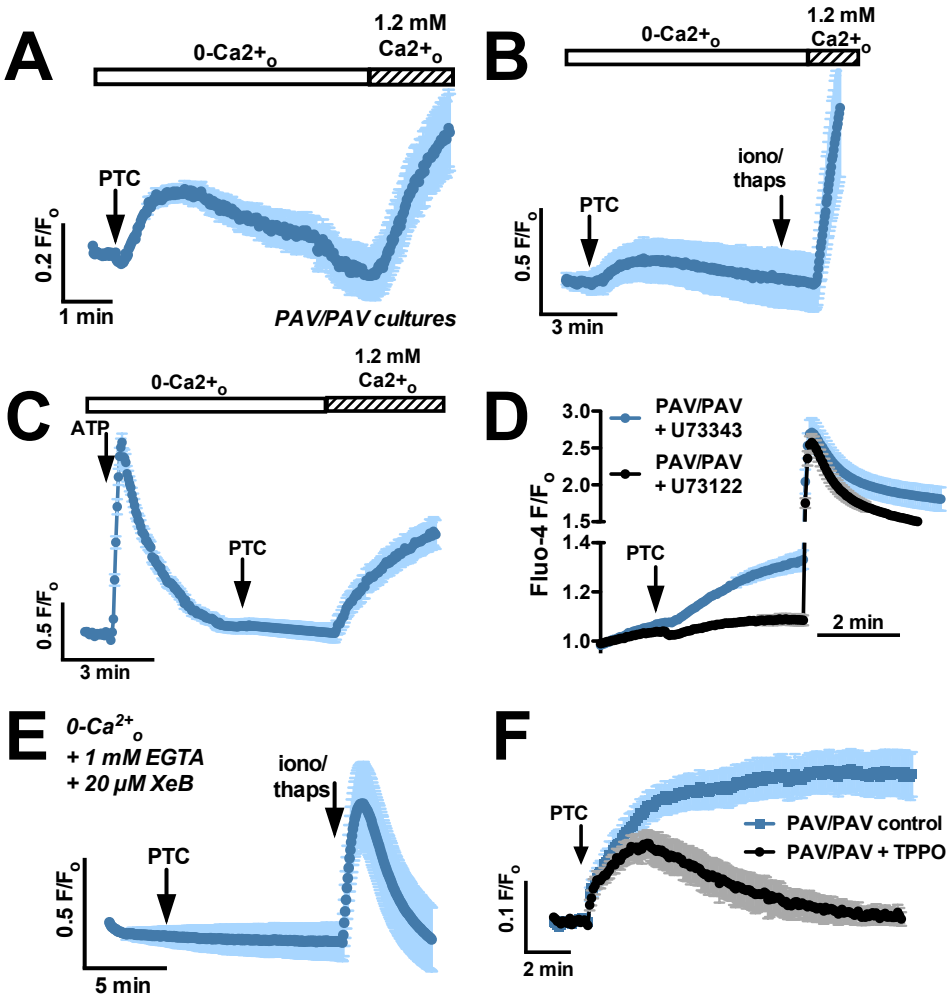
SUPPLEMENTARY MATERIAL



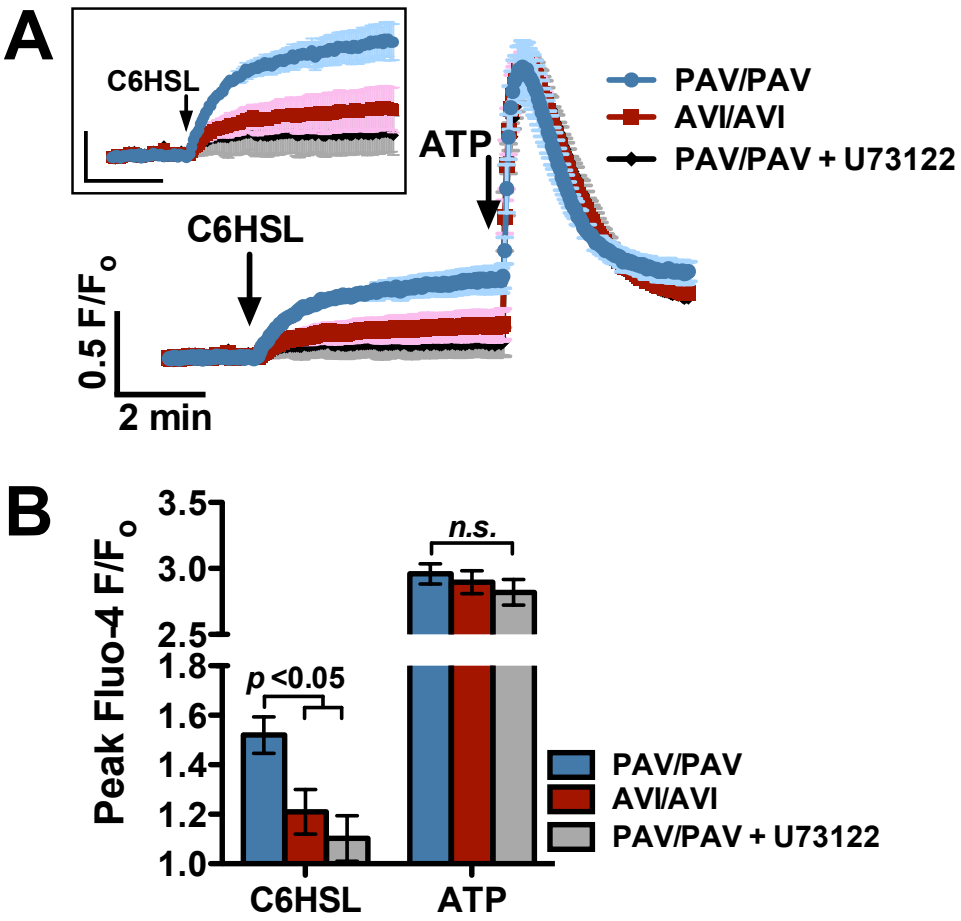
Supplementary Figure 1 Apical PTC application results in a dose-dependent elevation of $[Ca^{2+}]_i$ in the majority of cells within sinonasal ALI cultures. **(A)** Representative F/F_o image showing PTC-induced increase in Fluo-4 fluorescence in all loaded cells (PAV/PAV culture) in the field of view (loading determined by ATP response, which should activate all cells), agreeing with immunofluorescence data showing ubiquitous T2R38 expression in sinonasal ALI cultures (**Figure 1**). **(B)** Dose response curve of PTC-induced Ca^{2+} response in PAV/AVI (heterozygous) cultures. Peak Fluo-4 fluorescence in these experiments was 1.12 ± 0.02 (1 mM PTC; *n* = 3 cultures), 1.20 ± 0.01 (7.5 mM PTC; *n* = 4 cultures), and 1.32 ± 0.05 (15 mM PTC; *n* = 7 cultures). **(C)** Apical, but not basolateral, application of PTC induced Ca^{2+} elevations. Shown is one representative trace from 4 experiments using PAV/PAV taster cultures. **(D)** Apical application of vehicle (DMSO) had no effect on Ca^{2+} either at the concentrations used (0.1%-0.5%) or higher (1%). Shown is 1 representative trace from 3 PAV/PAV cultures tested.



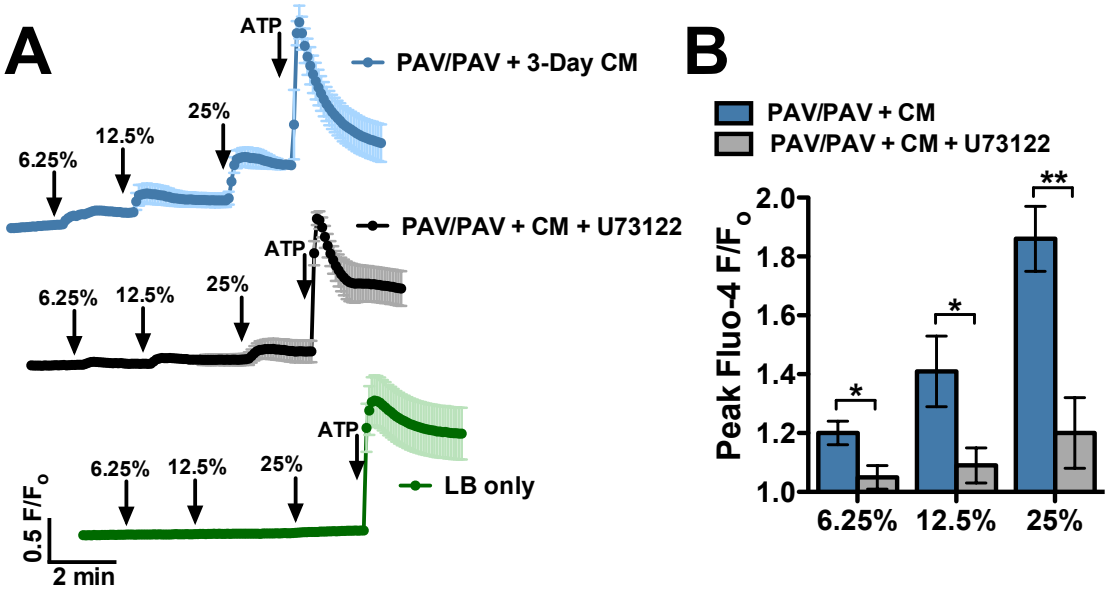
Supplementary Figure 2 Differences between PAV/PAV and AVI/AVI cultures are not likely due to intrinsic defects in Ca^{2+} signaling or T2R38 expression. **(A-B)** Three concentrations of the purinergic agonist ATP were tested, applied apically to naïve cultures that had not been previously exposed to PTC. Shown are average traces from PAV/PAV (**A**) and AVI/AVI (**B**) cultures (*n* = 4 cultures from 4 pts of each genotype for each ATP concentration). No differences were observed in the magnitude or kinetics of the responses. **(C).** Bar graph showing peak Fluo-4 fluorescence during ATP stimulation. Fluo-4 fluorescence was 1.66 ± 0.09 (PAV/PAV taster) and 1.63 ± 0.08 (AVI/AVI nontaster) during 100 nM ATP stimulation, 2.78 ± 0.39 (PAV/PAV) and 2.80 ± 0.30 (AVI/AVI) during 1 μM ATP stimulation, and 3.64 ± 0.23 (PAV/PAV) and 3.26 ± 0.35 (AVI/AVI) during 10 μM ATP stimulation. As indicated, no values were significantly different between genotypes, determined by 2-tailed unpaired Student's *t*-test. **(D)** Agarose gel (1.5%; 0.5 μg/ml EtBr stained) showing results from reverse transcription (rt) PCR of RNA isolated from ALI cultures. Expression of T2R38 and GAPDH (control) mRNA were both observed (766 and 502 bp, respectively). Primer sequences for human T2R38 were (forward) 5'-ACAGTGATTGTGTGCTGCTG-3' and (reverse) 5'-GCTCTCCTCAACTTGGCATT-3'; primer sequences for GAPDH were (forward) 5'-ATCTTCCAGGAGCGAGATCC-3' and (reverse) 5'-ACCACTGACACGTTGGCAGT-3'. **(E)** Representative immunofluorescence images of PAV/PAV and AVI/AVI cultures showing similar expression patterns and fluorescence intensity levels between the two genotypes.



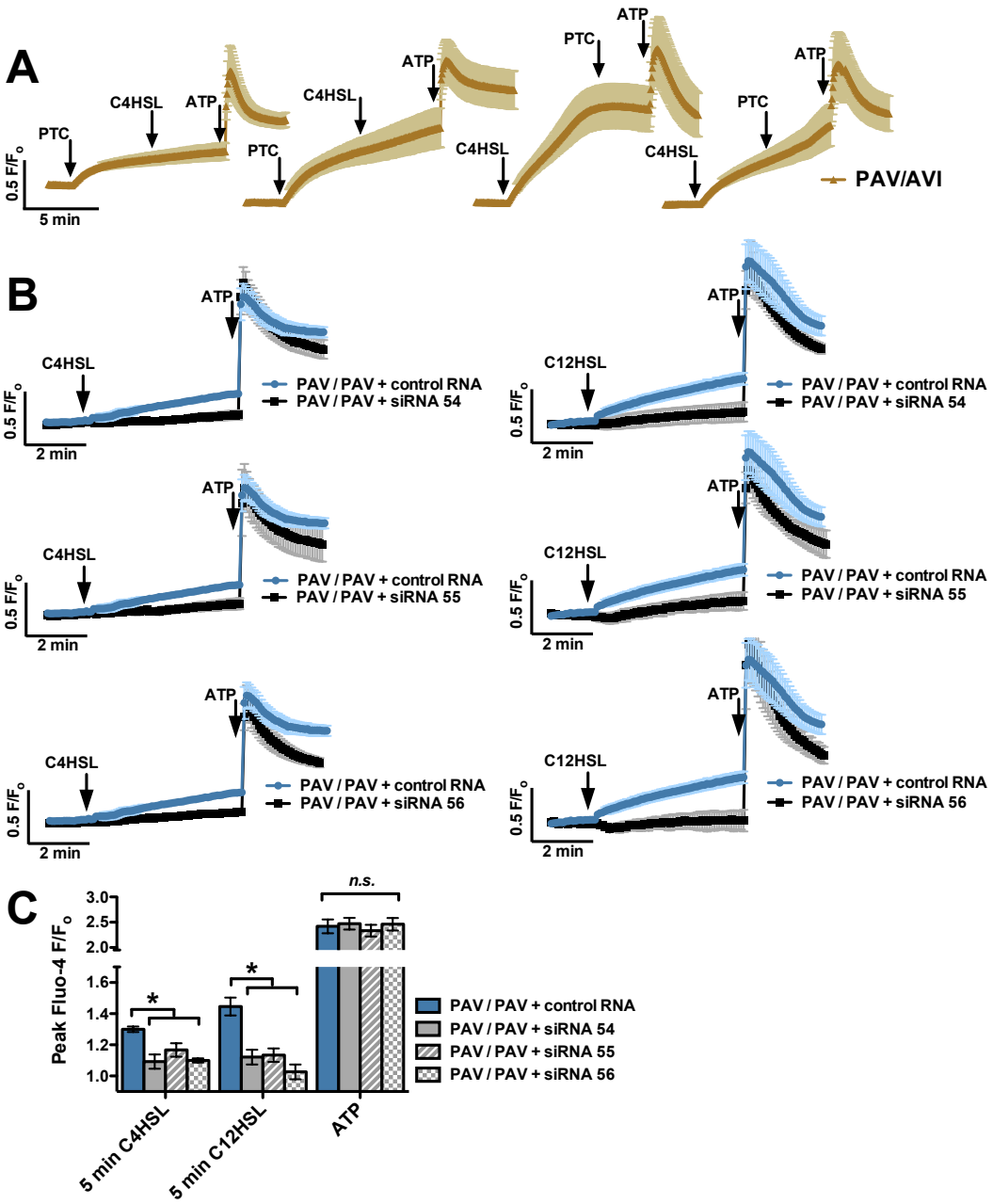
Supplementary Figure 3 PTC-induced Ca^{2+} responses involve PLC, IP_3 receptor, and TRPM5 function. All experiments performed with PAV/PAV cultures. **(A)** In the absence of extracellular Ca^{2+} (0-Ca^{2+}_o solution), 5 mM PTC induced transient Ca^{2+} elevations, suggesting PTC causes Ca^{2+} release from intracellular stores while sustained responses require Ca^{2+} influx. Representative trace from 4 cultures. **(B)** After 5 mM PTC in 0-Ca^{2+}_o, subsequent stimulation with ionomycin and thapsigargin (10 µg/ml each) revealed depletion of intracellular Ca^{2+} stores; Fluo-4 fluorescence did not increase until Ca^{2+} was added back to the extracellular solution. Representative trace from 4 cultures. **(C)** Prior Ca^{2+} store depletion by stimulation with ATP in 0-Ca^{2+}_o solution resulted in no subsequent PTC (5 mM)-induced response. Representative trace from 4 cultures. **(D)** PTC (7.5 mM)-induced Ca^{2+} responses were blocked by the PLCβ2 inhibitor U73122 (5 µM; 10 min apical-side pre-incubation) but not by the inactive U73343. Responses to ATP were unaffected by the U73122 concentration used. **(E)** The Xestospongin-B (IP_3 receptor inhibitor; 20 µM; 20 min apical pre-incubation) blocked PTC-induced Ca^{2+} responses in 0-Ca^{2+}_o solution, suggesting IP_3 receptor function is required for PTC-induced Ca^{2+} release. Subsequent stimulation with ionomycin/thapsigargin in continued 0-Ca^{2+}_o confirmed that intracellular Ca^{2+} stores were intact. Representative trace from 4 cultures. **(F)** Incubation with 80 µM triphenylphosphine oxide, a TRPM5 blocker (Ref. 1) (TPPO; black trace; average of 6 cultures) resulted in transient Ca^{2+} elevation in response to 1 mM PTC. Blue trace shows control (no TPPO) responses from 6 cultures from the same patients.



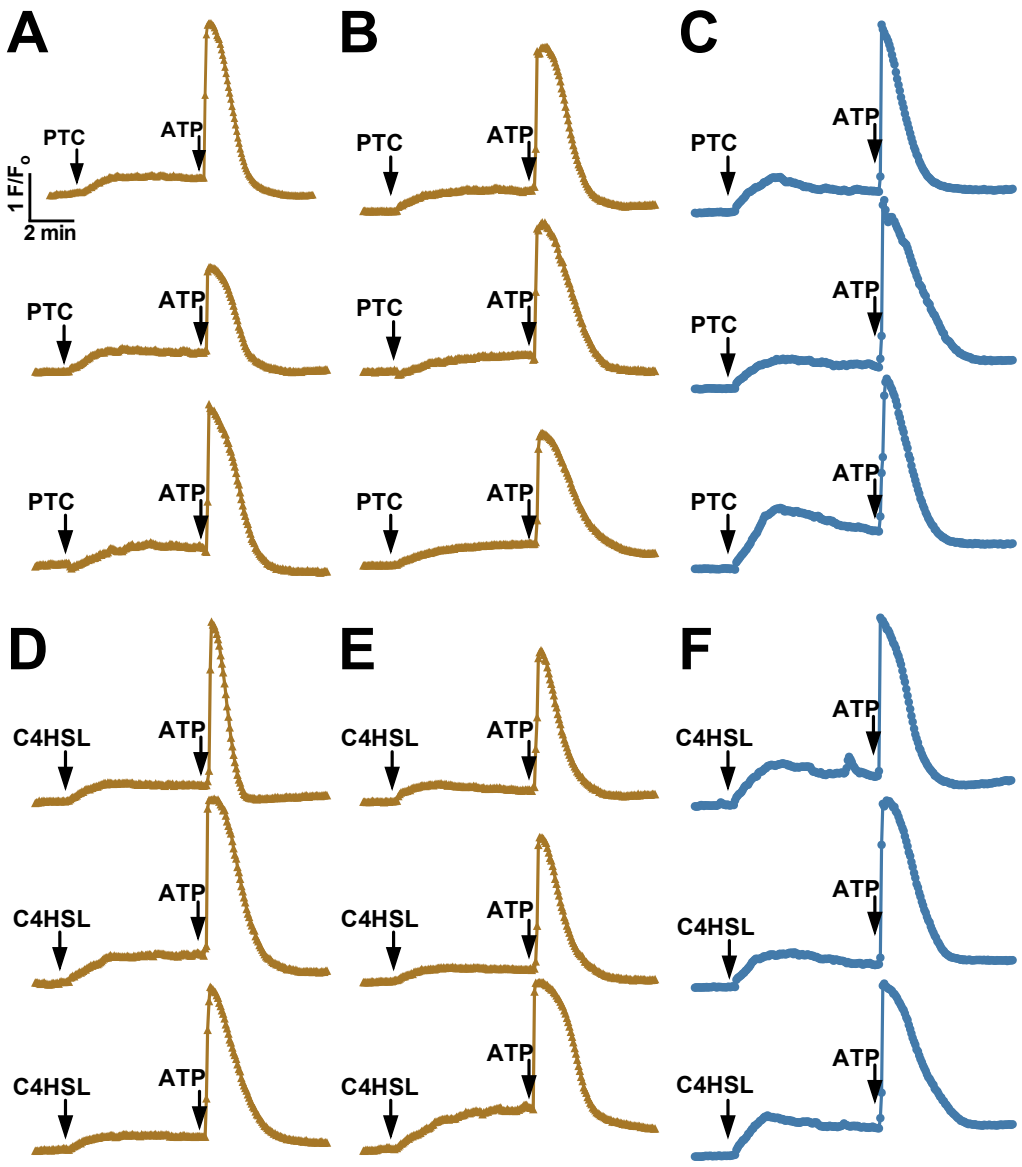
Supplementary Figure 4 C6HSL (200 μM) induces T2R38- and PLCβ2-dependent Ca^{2+} signals. **(A)** Average traces from 6 PAV/PAV cultures (2 each from 3 patients) , 6 AVI/AVI cultures (2 each from 3 patients), and 4 PAV/PAV cultures (1 each from 4 patients) treated with U73122. **(B)** Bar graph showing results from **A**. Average peak Fluo-4 F/F_o after 5 min stimulation with C6HSL was 1.52 ± 0.074 (PAV/PAV), 1.10 ± 0.09 (PAV/PAV + U73122; *P* <0.05 vs PAV/PAV), and 1.21 ± 0.09 (AVI/AVI; *P* <0.05 vs PAV/PAV). Peak Fluo-4 F/F_o during 10 μM ATP stimulation was 2.96 ± 0.08 (PAV/PAV), 2.82 ± 0.1 (PAV/PAV + U73122; *n.s.* vs PAV/PAV), and 2.90 ± 0.09 (AVI/AVI; *n.s.* vs PAV/PAV). Significance derived from 1-way ANOVA with Tukey-Kramer post-test.



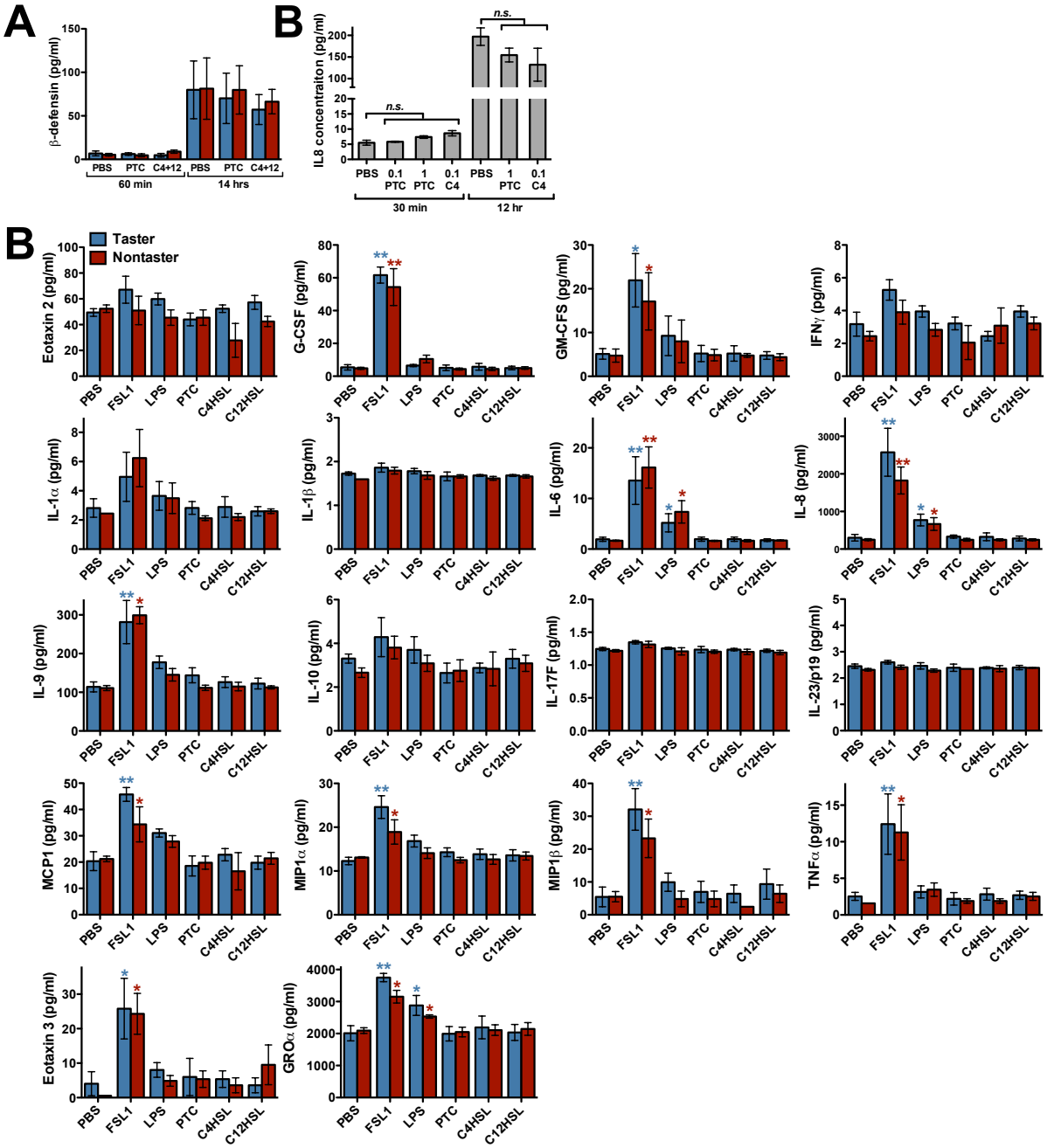
Supplementary Figure 5 *Pseudomonas*-conditioned medium (CM) and C6HSL activate PLCβ2-dependent Ca^{2+} responses. **(A)** Shown are average traces from 4 cultures from 4 taster patients for each condition. Cultures were either untreated or treated with 5 μM U73122 for 20 min before stimulation with filter sterilized PAO1 medium from a 3-day culture (diluted with PBS to the indicated concentration). No response was observed with LB alone. **(B)** In the absence of U73122, peak Fluo-4 F/F_o was 1.2 ± 0.04 (6.25% CM), 1.41 ± 0.12 (12.5% CM), and 1.86 ± 0.11 (25% CM). In the presence of U73122, peak Fluo-4 F/Fo was 1.05 ± 0.04 (6.25% CM) 1.09 ± 0.06 12.5% CM) and 1.2 ± 0.12 (25% CM). Significance is indicated in the bar graph and was determined by 2-tailed Student's *t*-test; * = P <0.05 and ** = P <0.01.



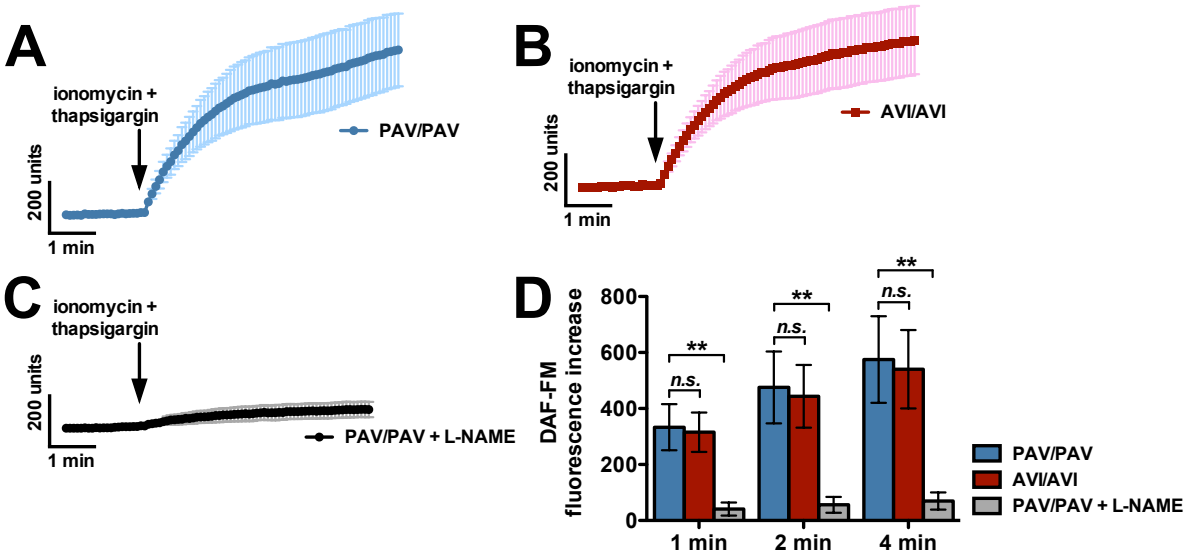
Supplementary Figure 6 Sequential addition experiments and siRNA knockdown experiments suggest that PTC and C4HSL activate Ca^{2+} signaling through a common T2R38-dependent pathway. **(A)** Addition of a high concentration of C4HSL (400 μM) after addition of a high concentration of PTC (15 mM) resulted in no further enhancement of Ca^{2+}signaling. Likewise, addition of PTC after C4HSL had little effect on Ca^{2+} signaling. In either case, subsequent addition of 10 μM ATP, which acts through a purinergic receptor-dependent T2R-independent pathway, resulted in a further increase in Ca^{2+} concentration. Shown are average Fluo-4 traces from >80 cells in 1 culture, each ± SEM. **(B)** Three separate siRNA duplexes directed against different regions of the *Tas2R38* mRNA sequence inhibited Ca^{2+} elevations in response to C4HSL (left) and C12HSL (right; n = 4 cultures from 2 patients for each condition). Responses to C4HSL and C12HSL in cells transfected with control RNA are reproduced on each graph for each agonist. Purinergic receptor-dependent Ca^{2+} responses were unaffected. **(C)** Bar graph showing peak Ca^{2+} responses after 5 min stimulation with C4HSL (1.30 ± 0.02, control; 1.09 ± 0.05, siRNA 54; 1.11 ± 0.05, siRNA 55; 1.10 ± 0.01, siRNA 56; all siRNA values P <0.05 vs control), C12HSL (1.45 ± 0.05, control; 1.12 ± 0.05, siRNA 54; 1.13 ± 0.04, siRNA 55; 1.03 ± 0.05, siRNA 56; all siRNA values P <0.05 vs control), and ATP (2.42 ± 0.33, control; 2.47 ± 0.28, siRNA 54; 2.33 ± 0.32, siRNA 55; 2.46 ± 0.35, siRNA 56; all values *n.s.*).



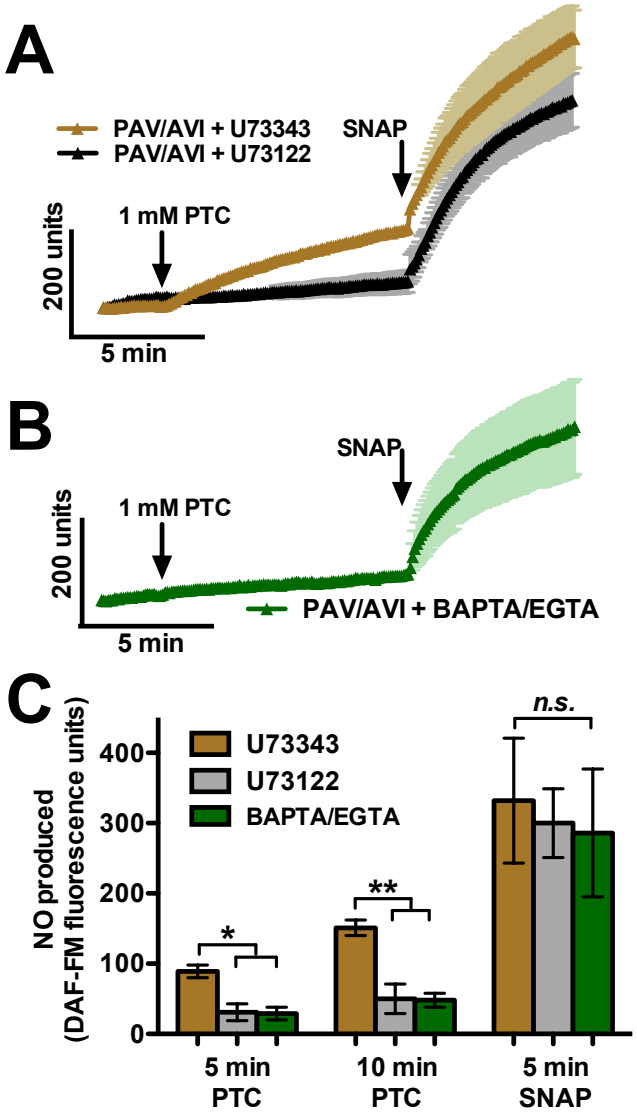
Supplementary Figure 7 Ca^{2+} responses to PTC (1 mM) and C4HSL (200 μM) in acutely dissociated nasal ciliated epithelial cells. **(A-E)** After tissue was treated with protease for cell isolation, as small aliquot was washed and plated on glass coverslips coated with Bovine Type I collagen and human fibronectin. Cells were incubated in culture medium for 12-36 hrs to allow for cell adhesion. Cells were then washed 3 times with HBSS and loaded with Fluo-4 (10 μM Fluo-4AM for 10-15 min). Single adherent ciliated cells were then identified under differential interference contrast (40x; 0.8 NA UPlanFLN objective) and imaged as described in the methods. **(A-C)** Responses to PTC were observed in at least 3 dissociated ciliated cells from 3 different patients who were subsequently genotyped for *TAS2R38* (brown traces are from PAV/AVI cultures; blue traces are from PAV/PAV cultures). **(D-E)** Responses to C4HSL were likewise observed in ciliated cells from the same patients. Scales of all traces in **A-F** are identical.



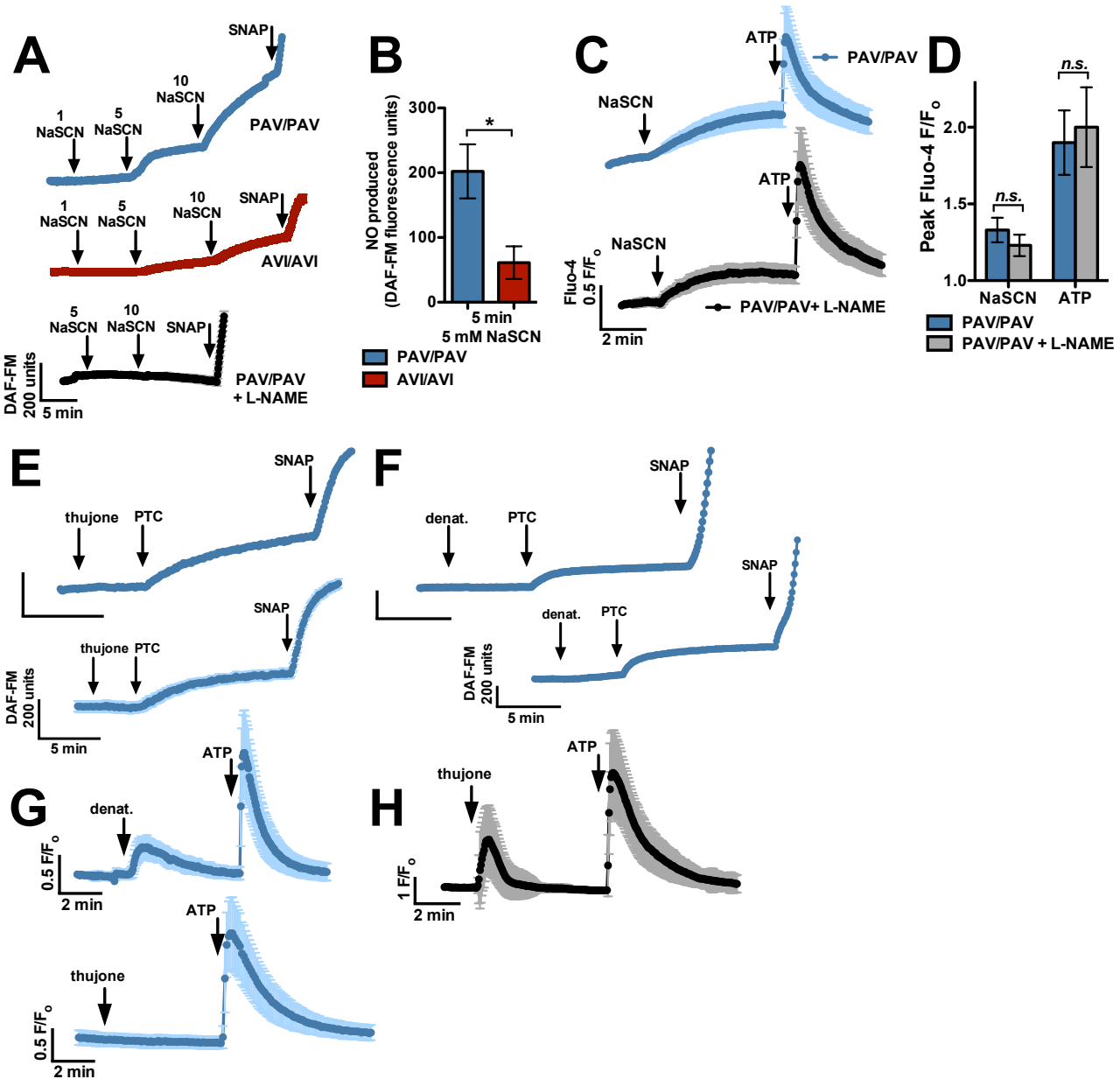
Supplementary Figure 8 T2R38 does not activate β-defensin or cytokine secretion. **(A)** Apical surface of ALI cultures were washed with PBS; afterward, 30 µL of PBS or PBS + 1mM PTC or 100µM each C4HSL and C12HSL. ASL was harvested after 60 min or 14 hrs at 37°C. Basal β-defensin secretion occurred over 14 hrs, but no significant differences were observed between stimulated and unstimulated or PAV/PAV (blue bars) and AVI/AVI (red bars) cultures. **(B)** PAV/PAV cultures were incubated in the presence of PBS or PBS + 0.1 or 1 mM PTC or 0.1 mM C4HSL on the apical side. Basolateral media was serum-free BEBM/DMEM. Samples of basolateral media were taken at 30 min and 12 hr time-points to assay for IL-8 secretion. While basal secretion occurred over 12 hrs, no significant differences were observed between stimulated and unstimulated or PAV/PAV and AVI/AVI cultures. **(C)** Secretion of 18 cytokines was measured via Luminex multiplex assay from media as in **B**. No significant increase in any cytokine was observed upon stimulation with 1 mM PTC, 100 µM C4HSL, or 100 µM C12HSL. FLS-1 stimulated significant elevation of G-CSF, GM-CSF, IL-6, IL-8, IL-9, MCP1, MIP1α, MIP1β, TNFα, Eotaxin 3, and GROα. Pseudomonas LPS stimulated significant elevation of IL-6, IL-8, IL-9, and GROα**.** None of these values were significantly different between PAV/PAV or AVI/AVI cultures (4 cultures each from 4 patients). Asterisks represent significance compared with isogenic PBS condition; **P* <0.05, ***P* <0.01 (ANOVA; Tukey-Kramer post-test).



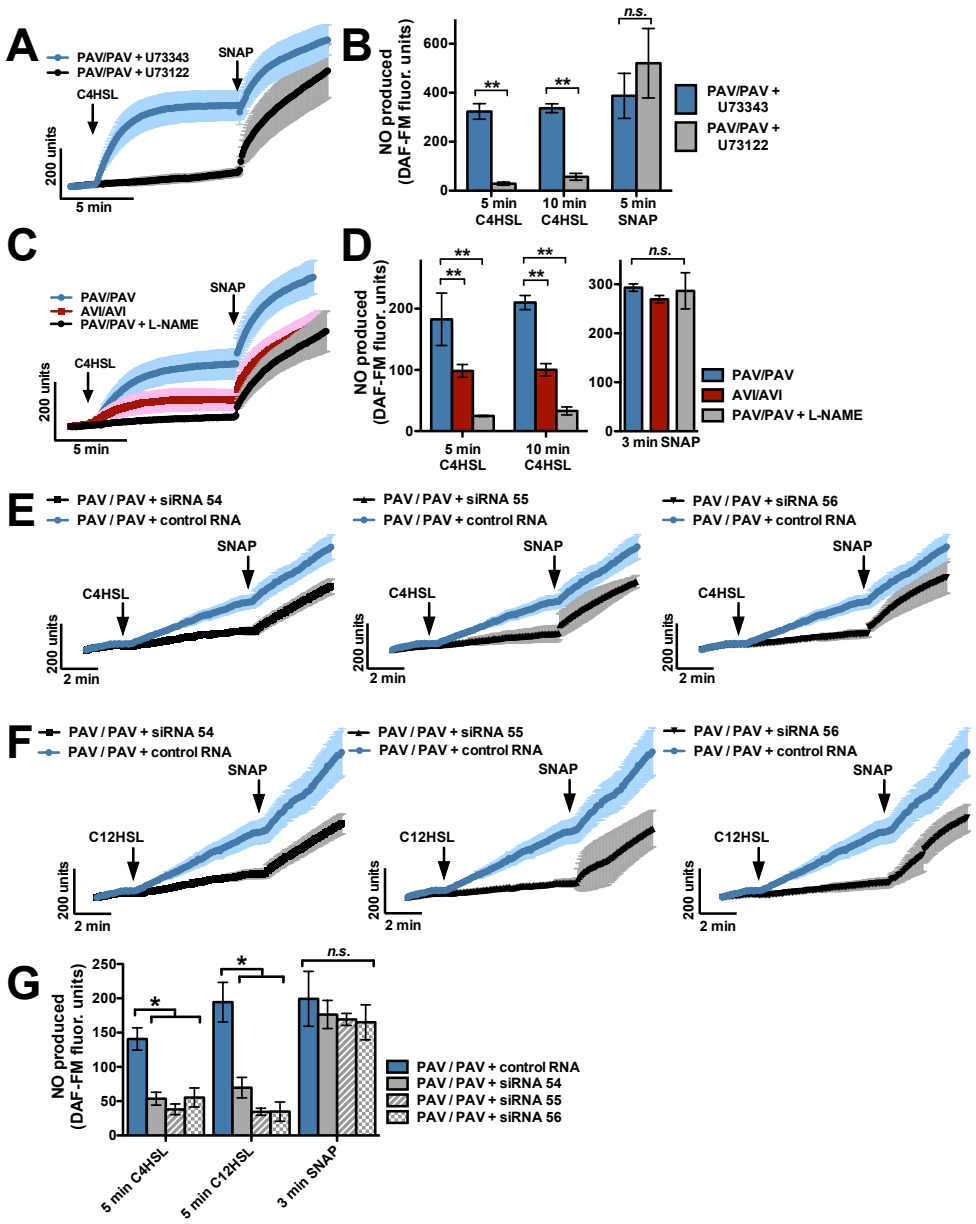
Supplementary Figure 9 NO-production during sustained intracellular Ca^{2+} elevation is independent of the *TAS2R38* genotype. **(A-B)** Average traces of DAF-FM fluorescence in PAV/PAV and AVI/AVI cultures (4 each from 2 patients per genotype) treated with ionomycin and thapsigargin (10 µg/ml each; effective concentrations in these cultures based on Supplementary Figure 3) in the presence of extracellular Ca^{2+} to stimulate global, receptor independent, sustained Ca^{2+} elevation. **(C)** Pretreatment of PAV/PAV cultures with L-NAME blocked the response to ionomycin + thapsigargin. **(D)** Summary of data from **a-c**, showing net DAF-FM fluorescence increase over baseline at 1, 2 and 4 min after application of ionomycin + thapsigargin. DAF-FM fluorescence increase was 333 ± 82 (PAV/PAV), 316 ± 70 (AVI/AVI; *n.s.* vs PAV/PAV), and 41 ± 23 (PAV/PAV + L-NAME; *P* <0.01 vs PAV/PAV control) at 1 min, 475 ± 128 (PAV/PAV), 444 ± 112 (AVI/AVI; *n.s.* vs PAV/PAV), 56 ± 28 (PAV/PAV + L-NAME; *P* <0.01 vs PAV/PAV control) at 2 min, and 575 ± 155 (PAV/PAV), 540 ± 140 (AVI/AVI; *n.s.* vs PAV/PAV), and 70 ± 31 (PAV/PAV + L-NAME; *P* <0.01 vs PAV/PAV control). ** *P* <0.01 by ANOVA with Tukey-Kramer post-test.



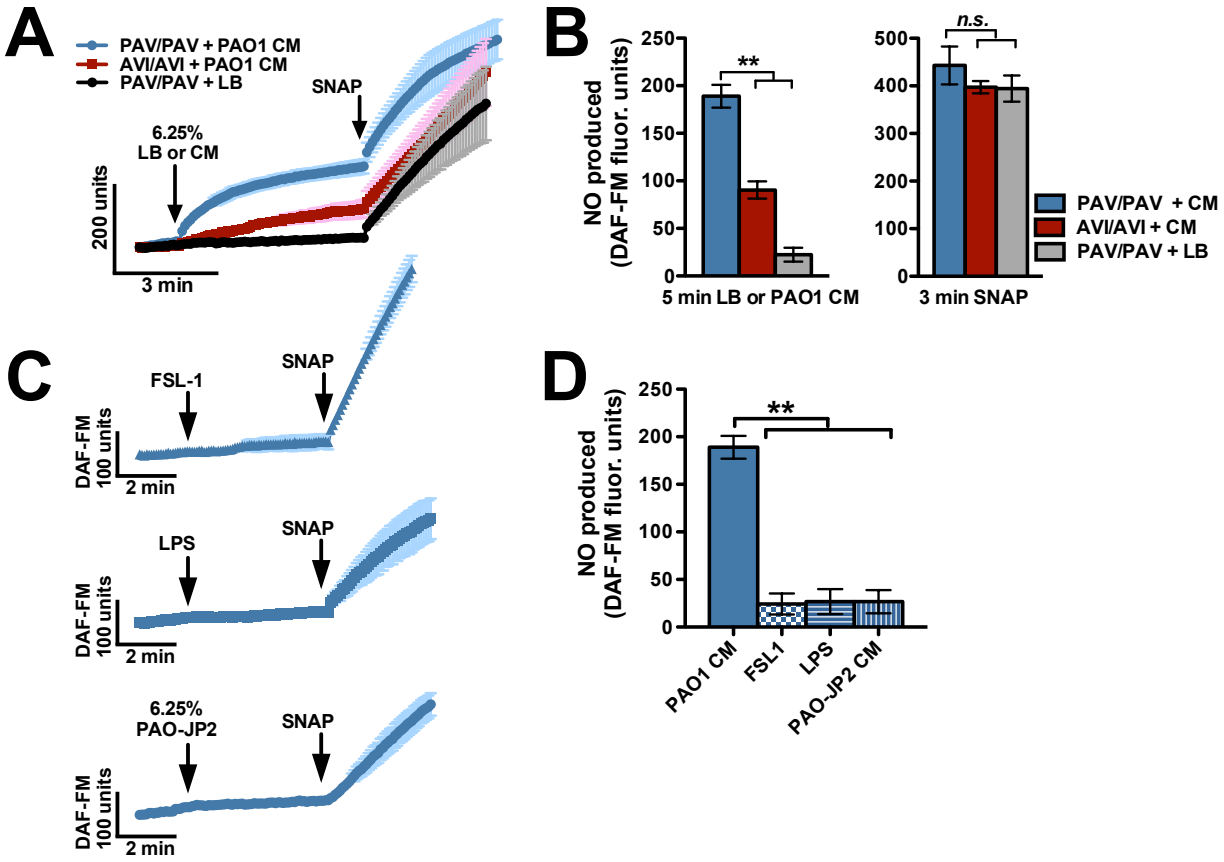
Supplementary Figure 10 PTC-induced NO generation is blocked by inhibitors of PLCβ2-dependent Ca^{2+} signaling. **(A-B)** Average DAF-FM traces (*n* = 4-7 cultures; 4 PAV/AVI pts for each condition) of cultures stimulated with 1 mM PTC in the presence of U73122 (PLCβ2 inhibibitor; blue trace; **A**) or U73343 (inactive analogue; brown trace; A) or after loading with BAPTA-AM in the presence of 1 mM EGTA (red trace; **B**). **(C)** Bar graph of data from A and B. After stimulation with 1 mM PTC, DAF-FM fluorescence increases were 89 ± 9 (U73343) vs 31 ± 12 (U73122; *P* <0.05 by ANOVA) vs 29 ± 9 (BAPTA/EGTA; *P* <0.05 by ANOVA) after 5 min and 151 ± 11 (U73343) vs 50 ± 21 (U73122; *P* <0.01) vs 48 ± 10 (BAPTA/EGTA; *P* <0.01). No significant difference was observed after stimulation with 100 μM SNAP for 5 min; DAF-FM fluorescence increase was 332 ± 89 (U73343) vs 300 ± 49 (U73122) vs 286 ± 91 (BAPTA/EGTA).



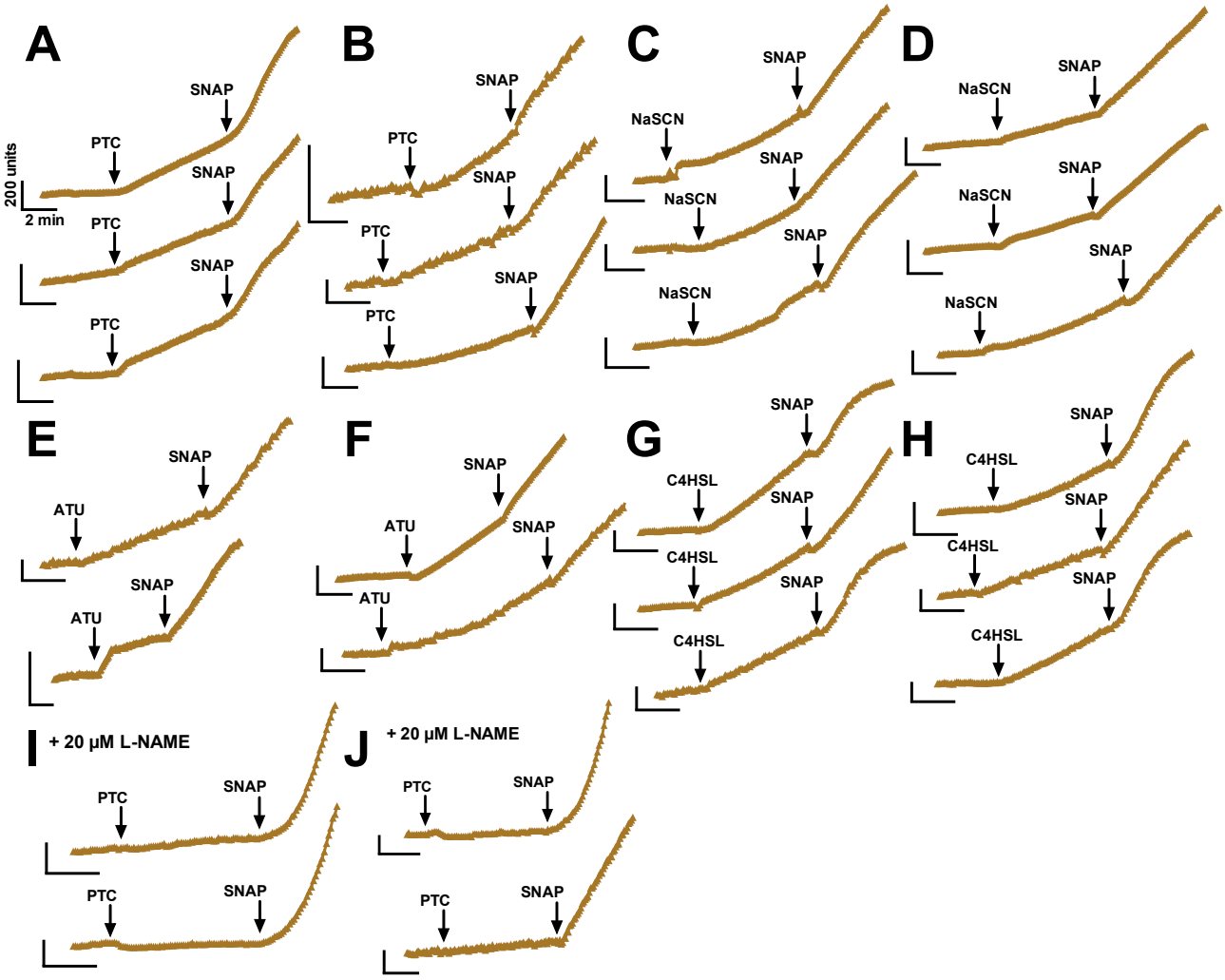
Supplementary Figure 11 The T2R38 agonist sodium thiocyanate (NaSCN) activates NO generation in sinonasal ALIs, whereas 2 non–T2R38-activating bitter agonists have no effect. **(A)** Representative traces (each from ~100 cells from a single ALI; SEM smaller than symbol size; representative of 6 experiments each) of DAF-FM response to NaSCN. **(B)** Bar graph showing NO production in tasters and non-tasters (n = 3 pts [2 cultures each] for each genotype). When averaged by patient, DAF-FM fluorescence increase after 5 min NaSCN was 202 ± 38 units (PAV/PAV) vs 61 ± 23 units (AVI/AVI; $P = 0.016$ by Student's *t*-test; *P <0.05) **(C)** Representative traces showing Ca^{2+} responses to NaSCN and ATP in the presence (black) or absence (blue; control) of L-NAME (50 μM). **(D)** After stimulation with 5 mM NaSCN, F/F_o was increased to 1.33 ± 0.08 (control) and 1.24 ± 0.07 (+ L-NAME; *n.s.* by Student's *t*-test). Peak F/F_o during stimulation with 1 μM ATP was 1.9 ± 0.21 (control) and 2.0 ± 0.26 (L-NAME; n.s. by Student's *t*-test). L-NAME had no significant effect on Ca^{2+} signaling at the concentration used, thus L-NAME block of DAF-FM fluorescence increase likely reflects inhibition of NOS. **(E-F)** Neither the T2R10 and T2R14 specific agonist thujone (5 mM) nor the T2R4, T2R8, T2R10, T2R13, T2R39, T2R43, T2R46, and T2R47-specific agonist denatonium benzoate (10 mM) had any effect on NO production. Shown are 2 representative traces for each agonist from 2 cultures from different PAV/PAV patients (4 patients tested). **(G)** Ca^{2+} responses to denatonium but not thujone were observed in sinonasal ALI cultures. **(H)** As a control for thujone activity, Ca^{2+} responses to thujone were observed in human bronchial epithelial (HBE) cells.



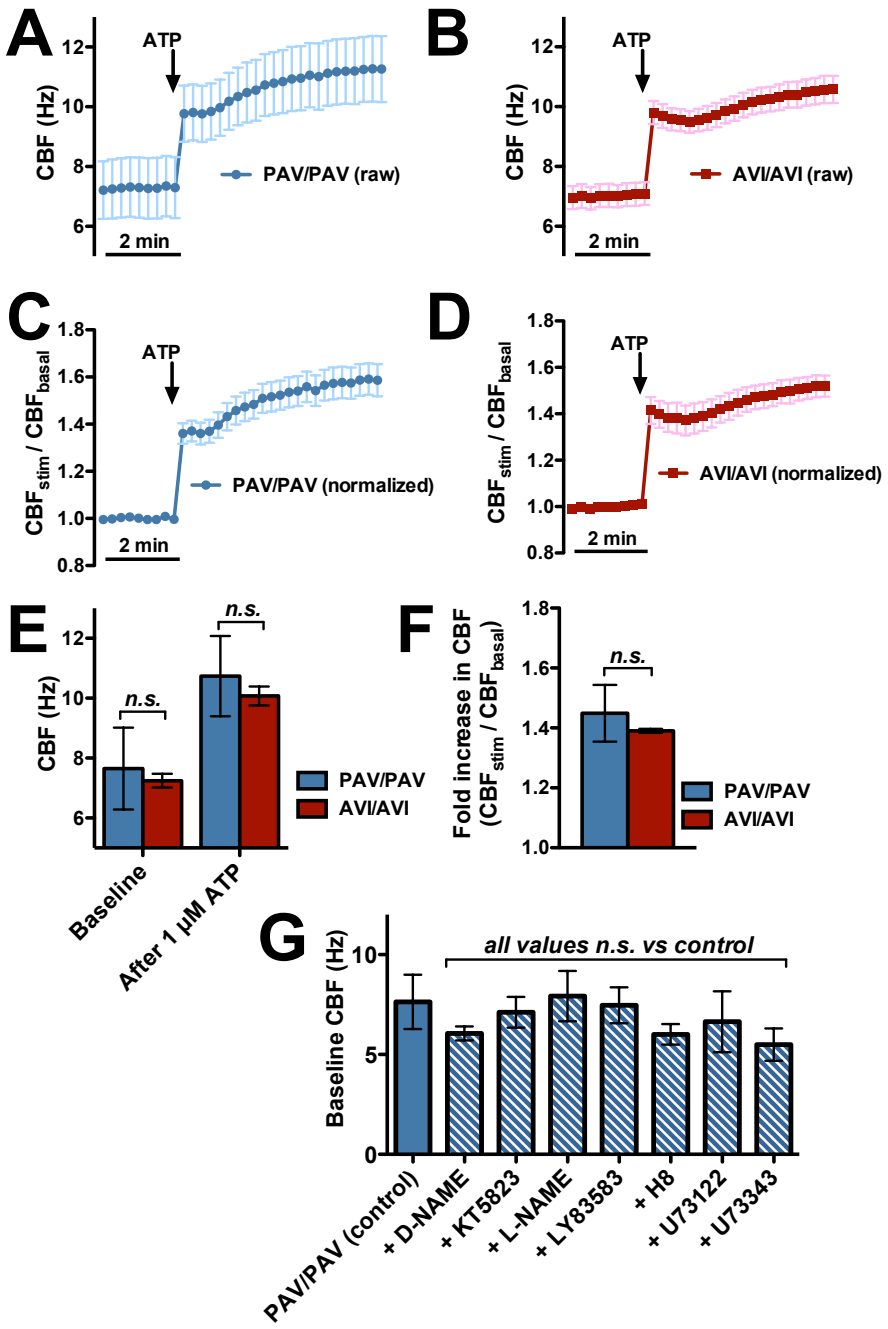
Supplementary Figure 12 C4HSL-induced NO production is blocked by PLCβ2 and NOS inhibition as well as siRNA knockdown of *Tas2R38* expression. **(A)** Average DAF-FM traces (left; 6 cultures from 3 PAV/PAV pts for each condition) stimulated with 200 μM C4HSL in the presence of U73122 or U73343 (5 μM each). **(B)** Bar graph of data from **A** averaged by patient (n = 3 each). After 5 min stimulation with C4HSL, DAF-FM fluorescence increase was 323 ± 10 (U73343) vs 29 ± 4 (U73122) units (*P* <0.001). After 10 min, fluorescence increase was 336 ± 10 (U73343) vs 56 ± 8 (U73122) units (*P* <0.01). No significant difference was observed after stimulation with SNAP (420 ± 32 and 487 ± 62 in U73343 and U73122-treated cultures, respectively). **(C)** Average DAF-FM traces (left; 6-10 cultures; 3 pts each) with C4HSL. **(D)** Results from **C** averaged by patient. Fluorescence increases with C4HSL were 183±43 (PAV/PAV), 99±1 (AVI/AVI), and 25±4 (PAV/PAV + L-NAME) after 5 min and 210±12 (PAV/PAV), 100±10 (AVI/AVI), and 33±7 (PAV/PAV + L-NAME) after 10 min. Increases after SNAP were not significantly different. **(E-F)** NO production in response to C4HSL (**E**) and C12HSL (**F**) was inhibited by siRNAs targeted against *Tas2R38* expression (n = 4 cultures from 2 patients for each condition). Responses observed in cultures transfected with control RNA were reproduced for each graph in **E** and **F**. NO production in response to SNAP application was not different between control and siRNA transfected cultures. **(G)** Bar graph showing DAF-FM fluorescence increases after 5 min stimulation with C4HSL (141 ± 16, control; 54 ± 9, siRNA 54; 38 ± 7.5, siRNA 55; 55 ± 14, siRNA 56; all siRNA values *P* <0.05 vs control) or C12HSL (194 ± 32, control; 70 ± 15, siRNA 54; 35 ± 5, siRNA 55; 35 ± 14, siRNA 56; all siRNA values *P* <0.05 vs control) or 3 min stimulation with SNAP (199 ± 60, control; 176 ± 30, siRNA 54, 169 ± 13, siRNA 55; 165 ± 36; siRNA 56; all values *n.s.*).



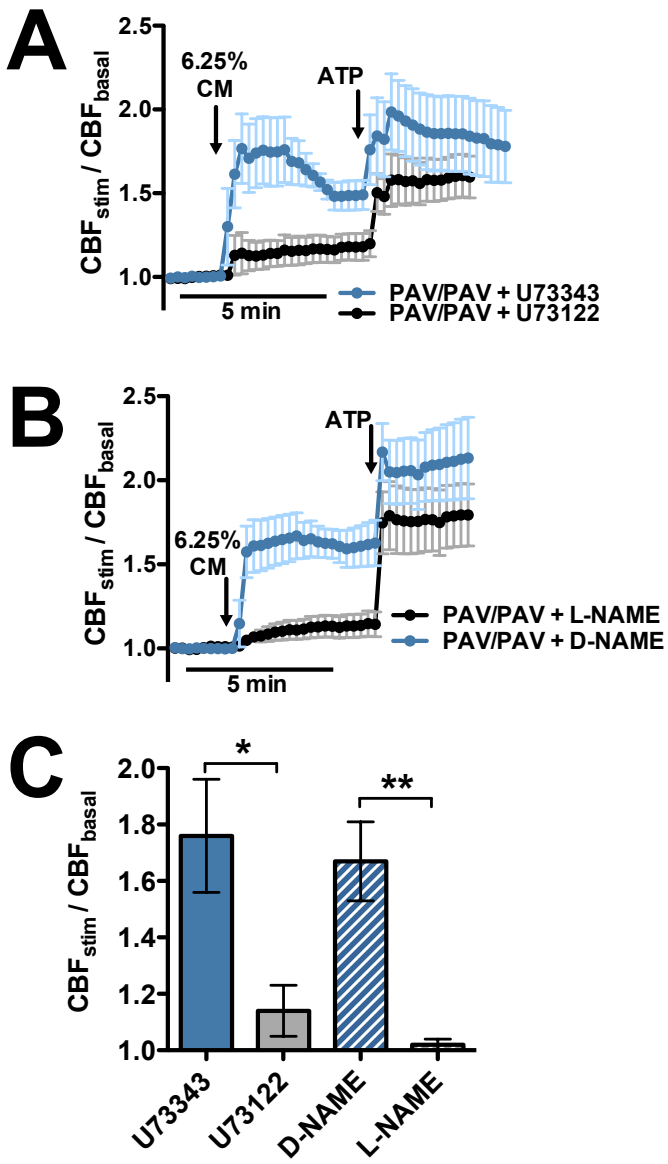
Supplementary Figure 13 Dilute (6.25%) *Pseudomonas*-conditioned-medium activates T2R38-dependent NO production **(A)** Average DAF-FM traces (9 cultures from 3 pts for each condition) stimulated with 6.25% *Pseudomonas* (3-day)-conditioned medium (CM) or LB (control). **(B)** Bar graph of results from C averaged by patient (n = 3 each). After 5 min stimulation with CM, DAF-FM fluorescence increase was 188 ± 12 units (PAV/PAV) and 90 ± 9 units (AVI/AVI; *P* <0.01); after stimulation with LB, DAF-FM fluorescence increased by 22 ± 7 units (PAV/PAV; *P* <0.01 PAV/PAV with CM). No significant difference was observed when cultures were stimulated with 100 μM SNAP. DAF-FM fluorescence increase was 443 ± 40 (PAV/PAV after CM), 388 ± 20 (AVI/AVI after CM), and 362 ± 48 (PAV/PAV after LB). **(C)** Conditioned media from PAO-JP2 as well as the TLR agonists LPS and FSL-1 did not stimulate NO * = *P* <0.05, and ** = *P* <0.01.



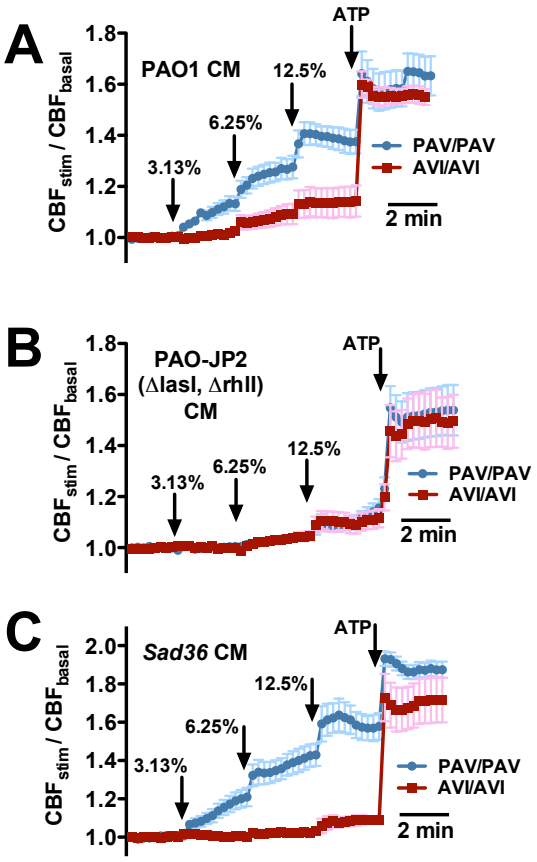
Supplementary Figure 14 Traces of T2R38-induced NO production in acutely dissociated ciliated epithelial cells. **(A-J)** Cells were isolated from nasal polyps removed from 2 patients (both PAV/AVI patients). DAF-FM was loaded by 15 min incubation with 10 μM DAF-FM diacetate in HBSS containing 20 μM cPTIO. After 4 washes with HBSS (no cPTIO), cells were imaged and DAF-FM fluorescence was recorded. **(A-F)** DAF-FM fluorescence was observed to increase in response to PTC (5 mM; A and B), NaSCN (5 mM; C and D), acetylthiourea (ATU, 2 mM; E and F). All three T2R38 agonists activated NO production. SNAP was included at the end as a positive control for dye loading. **(G-H)** C4HSL (100 μM) likewise induced an increase in DAF-FM fluorescence reflecting NO production. **(I-J)** PTC-induced, but not SNAP-induced DAF-FM fluorescence increases were blocked by L-NAME, demonstrating that the DAF-FM fluorescence increases specifically reflect NOS-mediated NO production in dissociated ciliated cells. Together, these data suggest that responses observed in sinonasal cultures recapitulate the responses of ciliated epithelial cells *in vivo*.



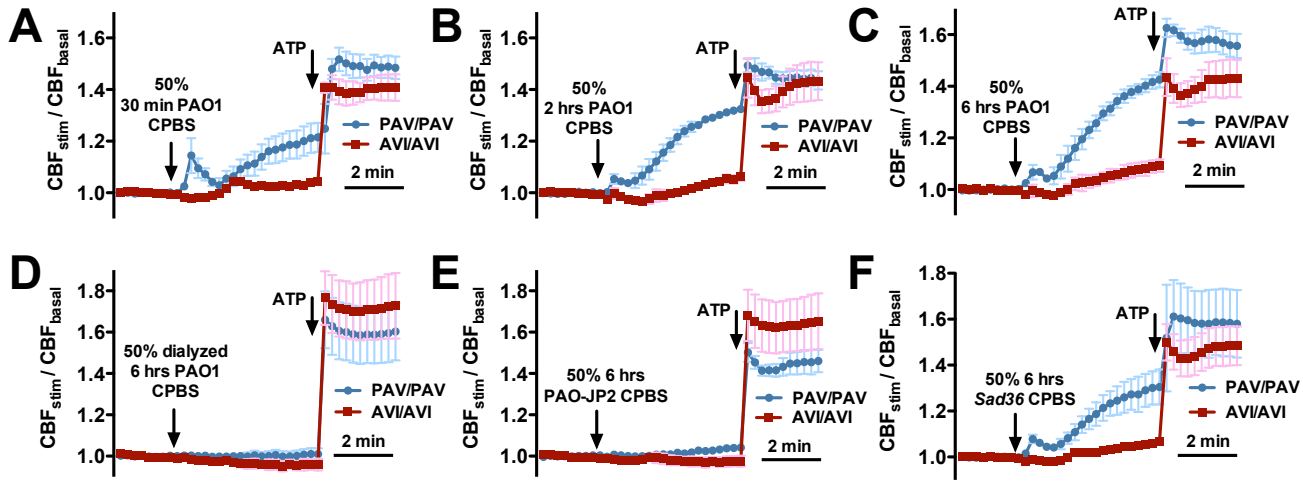
Supplementary Figure 15 No intrinsic differences in baseline and ATP-stimulated CBF were observed in AVI/AVI and PAV/PAV cultures. **(A-D)** Representative traces of raw (**A-B**) and normalized (**C-D**) CBF showing baseline and 1 µM ATP-stimulated CBF in PAV/PAV (**A,C**) and AVI/AVI (**B,D**) cultures. **(E)** Bar graph showing raw baseline and ATP-stimulated CBF averaged by patient (n = 2 cultures each from 4 patients for each genotype). Baseline CBF was 7.7 ± 1.4 Hz and 7.2 ± 0.2 Hz in PAV/PAV and AVI/AVI cultures, respectively (*n.s.* by Student's *t*-test). CBF after stimulation with 1 µM ATP was 10.7 ± 1.3 Hz and 10.1 ± 0.3 Hz in PAV/PAV and AVI/AVI cultures, respectively (*n.s.* by Student's *t*-test). **(F)** Bar graph showing normalized mean ATP-stimulated increase in CBF, which was 1.5 ± 0.09 and 1.4 ± 0.01 in PAV/PAV and AVI/AVI cultures, respectively (*n.s.* by Student's *t*-test). **(G)**. Bar graph showing raw resting CBF in untreated (control) PAV/PAV cultures as well as PAV/PAV cultures treated with the indicated inhibitors. Resting CBF was 7.7 ± 1.4 Hz in control cultures, 6.1 ± 0.34 Hz in D-NAME treated cultures, 7.9 ± 1.3 Hz in L-NAME-treated cultures, 7.1 ± 0.77 in KT5823-treated cultures, 7.5 ± 0.90 Hz in LY83583-treated cultures, 6.0 ± 0.51 Hz in H8-treated cultures, 6.6 ± 1.5 Hz in U73122-treated cultures, and 5.5 ± 0.81 Hz in U73343-treated cultures.



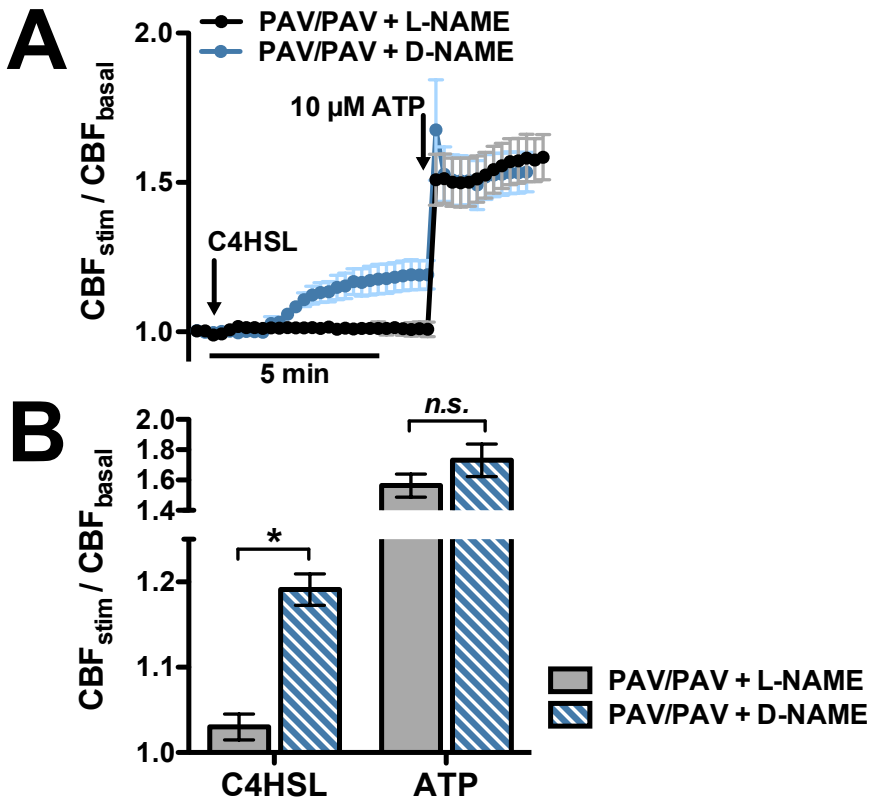
Supplementary Figure 16 *Pseudomonas*-conditioned medium-stimulated CBF is blocked by inhibition of PLCβ2 or NOS. **(A)** Average traces showing normalized CBF in PAV/PAV cultures stimulated with 6.25% Pseudomonas CM in the presence of U73122 or U73343 (6 cultures each from 3 patients each). Peak CBF was 1.14 ± 0.09 with U73122 vs 1.76 ± 0.2 with U73343 (P = 0.02 vs U73122 by Students' t-test; $P < 0.05$ vs U73122 and *n.s.* vs untreated cultures [**b-c**] by ANOVA). **(B)** Trace showing inhibition of CM-induced CBF increase by the NOS inhibitor L-NAME but not its inactive analogue, D-NAME. Cultures were pretreated with L-NAME or D-NAME (50 μM applied to the apical side only) for 15 min before CBF was measured (n = 6 culture from 3 pts for L-NAME and 9 cultures from 3 pts for D-NAME). Peak CBF was 1.02 ± 0.02 in the presence of L-NAME and 1.67 ± 0.14 in the presence of D-NAME ($P < 0.01$ vs L-NAME by Student's t-test). **(C)** Bar graph showing peak CM-activated CBF increase from experiments in **A-B**; *P <0.05 and **P <0.01.



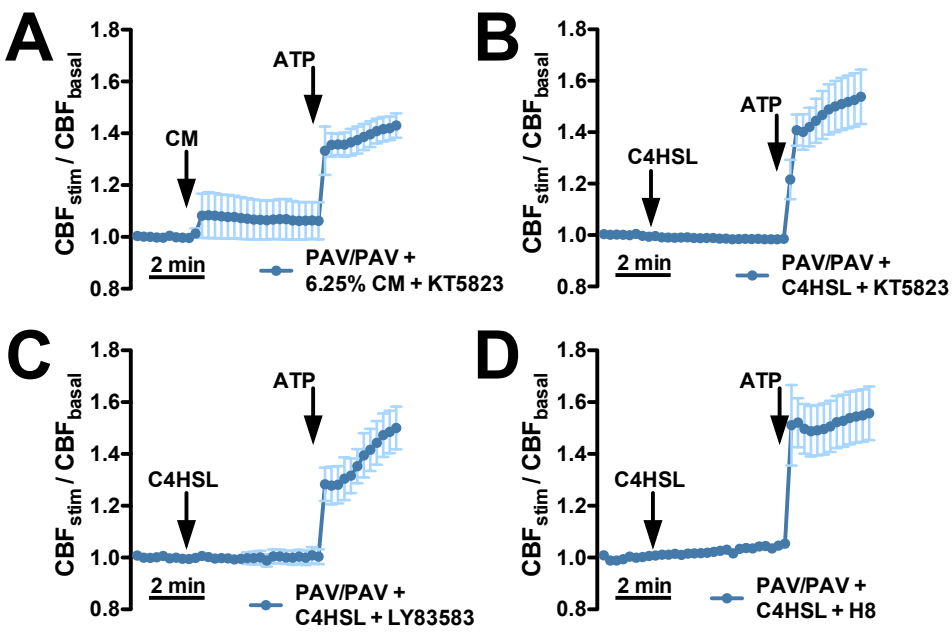
Supplementary Figure 17 Dilute conditioned medium (CM) from planktonic *Pseudomonas* cultures stimulates a HSL-dependent CBF increase. Eight cultures from 4 PAV/PAV patients and 6 cultures from 3 AVI/AVI patients were used for each condition. **(A)** PAO-1 conditioned medium elevated CBF by 1.15 ± 0.03 (PAV/PAV) vs 1.03 ± 0.01 (AVI/AVI) at 3.13%, 1.267 ± 0.04 (PAV/PAV; P <0.05) vs 1.092 ± 0.03 (AVI/AVI; P <0.01) at 6.25%, and 1.41 ± 0.05 (PAV/PAV) vs 1.14 ± 0.06 (AVI/AVI; P <0.05) at 12.5%. **(B)** PAO-JP2 medium elevated CBF by 1.01 ± 0.007 (PAV/PAV) vs 1.006 ± 0.005 (AVI/AVI; *n.s.*) at 3.13%, 1.04 ± 0.02 (PAV/PAV) 1.04±0.03 (AVI/AVI; *n.s.*) at 6.25%, and 1.16 ± 0.03 (PAV/PAV) and 1.12 ± 0.04 (AVI/AVI; *n.s.*) at 12.5%. **(C)** *Sad36* medium elevated CBF by 1.21 ± 0.05 (PAV/PAV) vs 1.003 ± 0.003 (AVI/AVI; P <0.05) at 3.13%, 1.43 ± 0.06 (PAV/PAV) vs 1.03 ± 0.02 (AVI/AVI; P <0.01) at 6.25%, and 1.59 ± 0.07 (PAV/PAV) vs 1.09 ± 0.02 (AVI/AVI; P <0.01) at 12.5%.



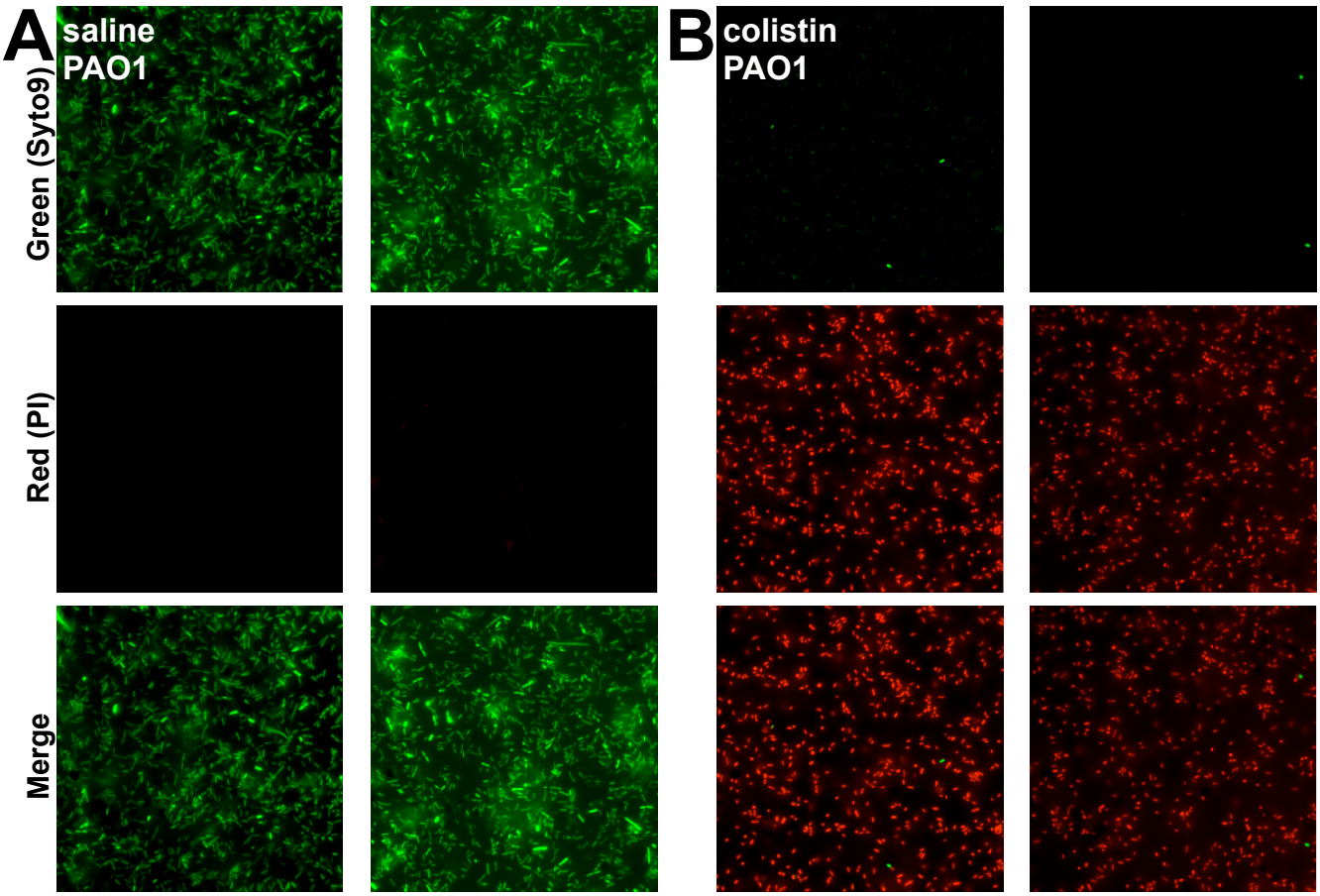
Supplementary Figure 18 *Pseudomonas*-conditioned PBS (CPBS) activates an HSL-dependent increase in CBF. **(A-C)** Average traces showing CBF response to CPBS that had been exposed to PAO1 *Pseudomonas* for 30 min (**A**), 2 hrs (**B**), and 6 hrs (**C**) showing marked T2R38-dependent CBF increase (n = 4-7 cultures from 3 patients for each condition for each genotype). Peak CBF increases were [30 min CPBS] 1.22 ± 0.06 (PAV/PAV) vs 1.04 ± 0.01 (AVI/AVI; P <0.05), (2 hrs CPBS) 1.32 ± 0.01 (PAV/PAV) vs 1.06 ± 0.02 (AVI/AVI; P <0.01), and [6 hrs CPBS] 1.43 ± 0.03 (PAV/PAV) vs 1.09 ± 0.02 (AVI/AVI; P <0.01). (**D**) Dialysis of 6 hrs CPBS using 3,500 MWCO dialysis membrane resulted in a complete loss of CBF stimulatory activity. Cell viability was confirmed with ATP stimulation. (**E**) Conditioned PBS exposed to approximately the same amount (OD 0.1) of HSL-deficient *Pseudomonas* PAO-JP2 did not stimulate an increase in CBF (n = 4 cultures from 4 patients per genotype). (**F**) CPBS (6 hrs) from the flagella mutant *Sad36* exhibited a robust T2R38-dependent CBF increase (n = 3 cultures from 3 patients for each genotype). CBF increases were 1.30 ± 0.08 (PAV/PAV; *n.s.* vs PAO-1 CPBS PAV/PAV) and 1.07 ± 0.01 (AVI/AVI; *P* <0.01 vs Sad36 PAV/PAV).



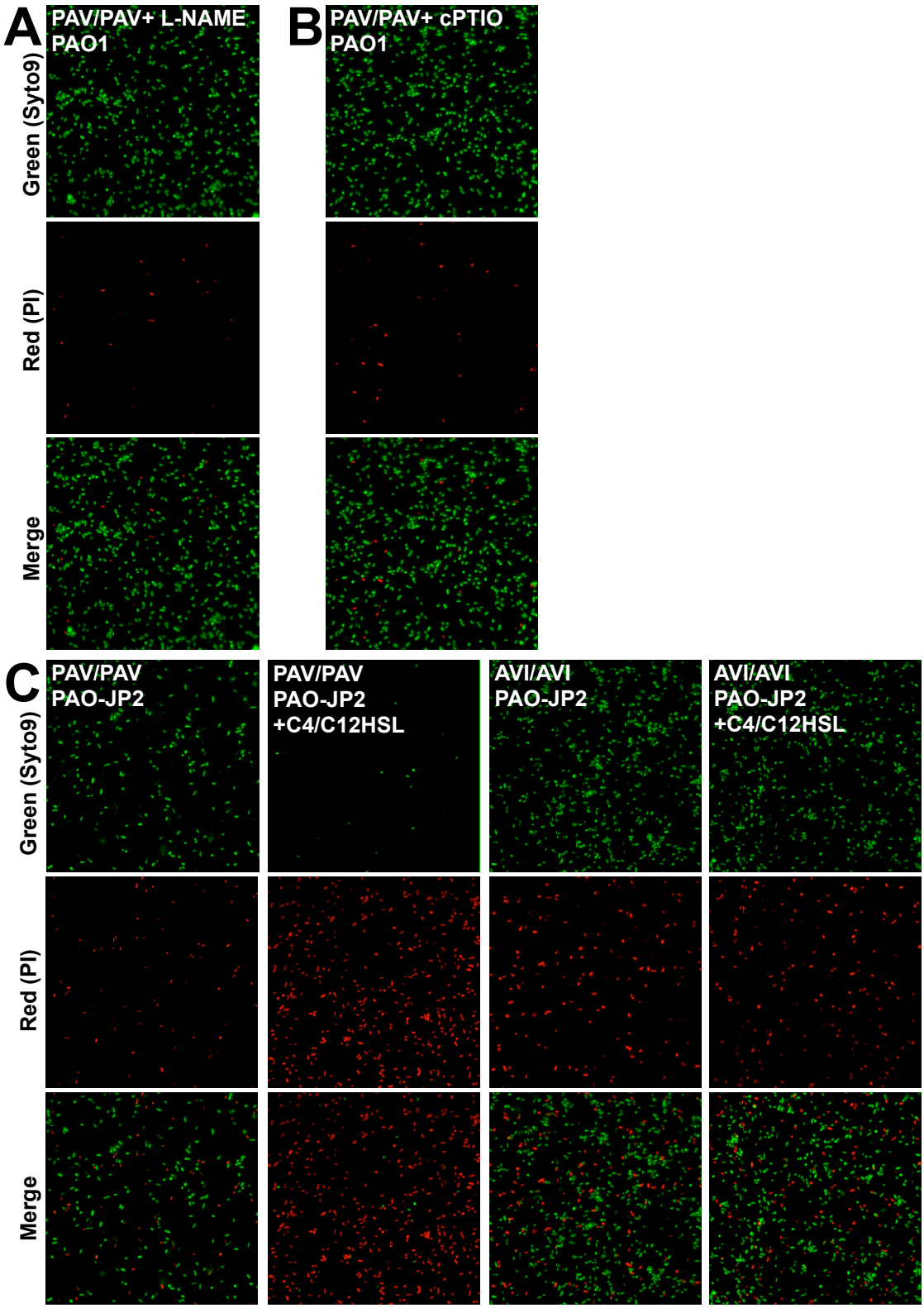
Supplementary Figure 19 C4HSL-activated CBF increase is blocked by inhibition of NOS. **(A)** In PAV/PAV cultures in the presence of D-NAME (*n* = 8 cultures from 4 patients), peak C4HSL-stimulated CBF was 1.19 ± 0.05. In the presence of L-NAME (middle panel; *n* = 6 cultures from 3 patients), the peak CBF was reduced to 1.01 ± 0.02 (*P* <0.05 vs D-NAME by Student's *t*-test). **(B)** Bar graph of data from **A** taking the peaks from each experiment then averaging results from each patient (3-4 each; * = *P* <0.05). In response to C4HSL, peak CBF was 1.03 ± 0.02 (PAV/PAV + L-NAME; *P* <0.05 vs PAV/PAV without L-NAME [**Figure 4E**] by ANOVA), and 1.19 ± 0.02 (PAV/PAV + D-NAME; *n.s.* vs PAV/PAV control [**Figure 4E**] and *P* <0.05 vs PAV/PAV + L-NAME by ANOVA). In response to ATP-stimulation, peak CBF increase was 1.57 ± 0.08 (PAV/PAV + L-NAME) and 1.73 ± 0.11 (PAV/PAV + D-NAME; *n.s.* compared with each other or with values in **Figure 4E** by ANOVA).



Supplementary Figure 20 Conditioned medium (CM)-stimulated and C4HSL-stimulated CBF increases were blocked by inhibitors of cGMP/PKG signaling. **(A)** Average trace of normalized CBF in PAV/PAV cultures stimulated with 6.25% CM in the presence of the PKG inhibitor KT5823 (2 μM; n = 6 cultures from 3 patients). **(B)** Average trace of CBF during C4HSL (200 μM) stimulation in the presence of KT5823 (n = 6 cultures from 3 patients). **(C)** Average trace of CBF during stimulation with C4HSL in the presence of the soluble guanylyl cyclase inhibitor LY83583 (50 μM; *n* = 5 cultures from 2 pts). **(D)** Average trace of CBF during stimulation with C4HSL in the presence of the PKG inhibitor H8 (5 μM; *n* = 6 cultures from 3 pts).



Supplementary Figure 21 Validation of Live/Dead staining technique. **(A-B)** *Pseudomonas* (PAO1) were incubated on transwell filters (no human cells) as described in the text in either saline **(A)** or saline + colistin **(B)**. Saline-exposed bacteria exhibited nearly universal green fluorescence while colistin-exposed bacteria exhibited nearly universal red fluorescence. As demonstrated by the images, signal bleed-through was minimal between red and green channels. Quantification of staining is presented in the Figure 7.



Supplementary Figure 22 T2R38-dependent antibacterial activity is dependent upon NO and HSLs; **(A-B)** Inhibition of NOS using L-NAME (applied to the basolateral side) or NO scavenging with cPTIO (applied to the apical side along with the bacteria) increased the number of viable (green) PAO1, suggesting NO is required for maximal bacterial killing. **(C)** Minimal killing was observed with HSL-deficient PAO-JP2. However, addition of 10 μM C4HSL and 10 μM C12HSL when PAO-JP2 was added to the cultures resulted in near-complete killing in PAV/PAV but not AVI/AVI cultures. Quantification of staining is presented in the Figure 7.

	Microbiology Report	T2R38 Genotype	TAS2R19 rs10772420	TAS2R46 rs2708381	TAS2R30 rs2599404	Gender	Age	Site	Indication for surgery	# Prior FESS	Polyps	Co-Morbids	Prior Medical Management
	No Growth												
1	Rare Staphylococcus species (Coagulase Negative)	AVI/PAV	A/G	C/T	A/C	F	61	Ethmoid	CRS	0	No	Asthma, AR, GERD, HTN, DM	Amoxicillin/Clavulanic acid, Nasal steroids, saline irrigations
2	Few Mixed Upper Respiratory Flora	AVI/PAV	A/G	C/C	A/C	M	51	Ethmoid	CRS	3	Yes	Asthma, AR, GERD, ASA intolerance	Levofloxacin, Prednisone, Nasal steroids, saline irrigations
3	No Growth	AVI/PAV	G/G	C/C	A/A	F	72	Ethmoid	CRS	1	No	None	Ciprofloxacin, Nasal steroids, saline irrigations
4	Few Staphylococcus species (Coagulase Negative)	AVI/PAV	A/A			M	64	Sphenoid	PT	0	No	AR, HTN	Nasal steroids
5	Few Staphylococcus species (Coagulase Negative)	AVI/PAV	A/G	C/C	A/C	F	24	Ethmoid	CRS	0	No	Asthma, AR	Minocycline, Nasal steroids, saline irrigations
6	Moderate Staphylococcus species (Coagulase Negative)	AVI/PAV		C/T	A/C	M	44	Ethmoid	CRS	0	Yes	AR, GERD	Nasal steroids, saline irrigations
7	Few Staphylococcus species (Coagulase Negative)	AVI/PAV		T/T	C/C	M	25	Ethmoid	CRS	0	No	AR, HTN, DM	Clindamycin, Prednisone, saline irrigations
8	Moderate Staphylococcus species (Coagulase Negative)	AVI/PAV				M	37	Ethmoid	CRS	4	Yes	Asthma, AR, GERD	Prednisone, Nasal steroids, saline irrigations
9	Few Staphylococcus species (Coagulase Negative)	AVI/PAV		C/C		M	54	Ethmoid	CRS	2	Yes	Asthma, ASA intolerance	Nasal steroids, saline irrigations
10	No Growth	AVI/PAV		C/C	CNV	F	46	turbinate	TH	0	No	non-allergic rhinitis	Nasal steroids, ipratropium bromide, saline irrigations
11	No Growth	AVI/PAV				M	39	turbinate	TH	0	No	non-allergic rhinitis	Nasal steroids, ipratropium bromide, saline irrigations
12	No Growth	AVI/AVI	A/G	C/C	A/C	F	29	Ethmoid	CRS	0	No	AR	Levofloxacin, Prednisone, saline irrigations
13	Few Mixed Upper Respiratory Flora	AVI/AVI	A/A	C/T	C/C	M	38	Ethmoid	CRS	3	Yes	Hx of Polyps	Prednisone, Nasal steroids, saline irrigations
14	Few Mixed Upper Respiratory Flora	AVI/AVI	A/G	C/T	A/C	F	41	Ethmoid	CRS	1	Yes	AR, GERD	Prednisone,Nasal steroids, saline irrigations
15	No Growth	AVI/AVI	A/G	C/C	A/C	M	33	Ethmoid	CRS	0	Yes	AR	Cirprofloxacin, Prednisone, Nasal steroids, saline irrigations
16	Few Staphylococcus species (Coagulase Negative)	AVI/AVI	A/G	C/T	A/C	M	56	Ethmoid	CRS	1	Yes	AR, HTN	Prednisone, Nasal steroids, saline irrigations
17	Few Staphylococcus species (Coagulase Negative)	AVI/AVI	A/G	C/C	A/C	M	51	Ethmoid	CRS	0	No	Asthma, AR	Nasal steroids, saline irrigations
18	Few Mixed Upper Respiratory Flora	AVI/AVI	A/G	C/T	A/C	M	41	Sphenoid	PT	0	No	None	None
19	Few Staphylococcus species (Coagulase Negative)	AVI/AVI	A/G	C/T	A/C	F	75	Ethmoid	CRS	5	Yes	Asthma, ASA intolerance,, AR	Prednisone, Nasal steroids, saline irrigations
20	Few Staphylococcus species (Coagulase Negative)	AVI/AVI	A/G	C/C	A/C	F	25	Ethmoid	CRS	4	Yes	GERD, HTN	Ciprofloxacin, Prednisone, Nasal steroids, Bactroban nasal irrigations
21	Few Staphylococcus species (Coagulase Negative)	AVI/AVI		C/C	A/C	M	57	Ethmoid	CRS	1	Yes	AR, GERD, HTN	Prednisone,Nasal steroids, saline irrigations
22	Few Staphylococcus species (Coagulase Negative)	AVI/AVI		C/T	C/C	F	56	Sphenoid	PT	0	No	HTN, DM	None
23	Rare Staphylococcus species (Coagulase Negative)	AVI/AVI		C/T	A/C	F	36	Ethmoid	CRS	0	Yes	Asthma, AR	Nasal steroids, saline irrigations
24	No Growth	AVI/AVI				M	31	turbinate	TH	0	No	non-allergic rhinitis, HTN	Nasal steroids, saline irrigations
25	No Growth	PAV/PAV	G/G	C/C	A/A	M	39	Sphenoid	PT	0	No	AR, GERD	None
26	No Growth	PAV/PAV	A/G	C/T	A/C	F	38	Ethmoid	CRS	2	Yes	AR	Nasal steroids, saline irrigations
27	Few Staphylococcus species (Coagulase Negative)	PAV/PAV	A/G	C/C	A/C	M	59	Ethmoid	CRS	1	No	None	Levofloxacin, Prednisone, Nasal steroids, saline irrigations
28	No Growth	PAV/PAV	G/G	C/C	A/A	F	34	Ethmoid	CRS	2	Yes	Asthma	Levofloxacin, Prednisone, Nasal steroids, saline irrigations
29	Few Staphylococcus species (Coagulase Negative)	PAV/PAV				M	78	Frontals	CRS	1	No	AR, HTN, GERD, DM	Nasal steroids, saline irrigations
30	No Growth	PAV/PAV	A/A	C/T	C/C	M	42	Ethmoid	CRS	3	Yes	Asthma, AR, GERD	Levofloxacin, prednisone, Nasal steroids, saline irrigations
31	Moderate Mixed Upper Respiratory Flora	PAV/PAV		C/T	A/C	M	44	Sphenoid	PT	0	No	GERD	None
32	No Growth	PAV/PAV	A/A	T/T	C/C	M	75	Ethmoid	CRS	2	Yes	AR	Prednisone, Nasal steroids, saline irrigations
33	Few Mixed Upper Respiratory Flora	PAV/PAV		T/T	C/C	M	64	Ethmoid	CRS	1	Yes	None	Prednisone, Nasal steroids, saline irrigations
34	Few Staphylococcus species (Coagulase Negative)	PAV/PAV		C/C	A/A	M	36	Ethmoid	CRS	2	Yes	Asthma, AR, GERD, DM	Prednisone, Nasal steroids, saline irrigations
35	Few Staphylococcus species (Coagulase Negative)	PAV/PAV		C/C	A/C	M	66	ethmoid	CRS	0	No	None	Amoxicillin/Clavulanic acid, Prednisone, Nasal steroids, saline irrigations
	Gram Negative												
36	Moderate Pseudomonas aeruginosa; Few Staphylococcus species (Coagulase Negative)	AVI/PAV	A/A	C/T	C/C	M	50	Ethmoid	CRS	3	No	Asthma, AR	Clindamycin, Prednisone, Nasal steroids, saline irrigations
37	Moderate Pseudomonas aeruginosa; Few Escherichia coli	AVI/PAV	A/A	C/T	C/C	M	54	Ethmoid	CRS	6	Yes	Asthma, AR	Levofloxacin, Pulmicort irrigations
38	Moderate Serratia marcescens; Few Haemophilus influenzae; Rare Mixed Upper Respiratory Flora	AVI/PAV	G/G	C/C	A/A	F	45	Ethmoid	CRS	3	Yes	Asthma, AR	Prednisone, Nasal steroids, saline irrigations
39	Many Pseudomonas aeruginosa	AVI/PAV	A/G	C/T	A/C	F	65	Ethmoid	CRS	1	No	GERD	Amoxicillin/Clavulanic acid, Nasal steroids, saline irrigations
40	Many Pseudomonas aeruginosa, Non-Mucoid Strain; Moderate Pseudomonas aeruginosa, Mucoid Strain; Moderate Staphylococcus species (Coagulase Negative)	AVI/PAV	A/G	C/C	A/C	F	64	Ethmoid	CRS	2	No	HTN, DM	Levofloxcin, Prednisone, Nasal steroids, saline irrigations
41	Pseudomonas aeruginosa	AVI/PAV	A/A	C/C	C/C	F	45	Maxillary	CRS	0	No	None	Amoxicillin/Clavulanic acid, Prednisone, Nasal steroids, saline irrigations
42	Few Pseudomonas aeruginosa	AVI/PAV	G/G	C/C	A/A	M	53	Ethmoid	CRS	1	Yes	GERD, HTN	Prednisone, Nasal steroids, saline irrigations
43	Many Pseudomonas aeruginosa	AVI/PAV	A/A	C/C	C/C	M	34	Ethmoid	CRS	0	No	AR, GERD	Prednisone, Nasal steroids, saline irrigations
44	Few Moraxella catarrhalis; Rare Staphylococcus aureus; Rare Streptococcus pneumoniae; Few Staphylococcus species (Coagulase Negative)	AVI/PAV	G/G	C/C	A/A	M	35	Ethmoid	CRS	2	Yes	Asthma, AR, GERD	Prednisone, Nasal steroids, saline irrigations
45	Moderate Pseudomonas aeruginosa; Moderate Staphylococcus aureus	AVI/PAV		CNV	A/C	F	21	Ethmoid	CRS	0	Yes	None	Nasal steroids, saline irrigations
46	Moderate Enterobacter aerogenes; Escherichia coli; Few Staphylococcus species (Coagulase Negative)	AVI/PAV	A/G	C/T	A/C	M	70	Ethmoid	CRS	0	No	Asthma	Prednisone, Nasal steroids, saline irrigations
47	Moderate Pseudomonas aeruginosa; Moderate Staphylococcus aureus; Moderate Streptococcus agalactiae (Group B)	AVI/PAV	A/G	C/C	A/C	F	44	Ethmoid	CRS	2	No	AR, GERD	Nasal steroids, saline irrigations
48	Few Citrobacter koseri	AVI/PAV				F	52	Ethmoid	CRS	2	Yes	Asthma	Nasal steroids, saline irrigations
49	Many Enterobacter aerogenes	AVI/PAV	G/G	C/C	A/A	F	35	Ethmoid	CRS	3	Yes	Asthma, ASA intolerance, AR	Prednisone, Nasal steroids, saline irrigations
50	Many Pseudomonas aeruginosa	AVI/AVI				F	63	Ethmoid	CRS	5	No	None	Moxifloxacin, Prednisone, Nasal steroids, saline irrigations
51	Many Pseudomonas aeruginosa	AVI/AVI	A/G	C/T	A/C	F	45	Ethmoid	CRS	5	Yes	Asthma, AR, GERD	Ciprofloxacin, Prednisone, Nasal steroids, saline irrigations
52	Moderate Pseudomonas aeruginosa, Mucoid Strain; Few Staphylococcus species (Coagulase Negative)	AVI/AVI	G/G	C/C	A/A	F	29	Ethmoid	CRS	2	Yes	AR	Prednisone, Nasal steroids, saline irrigations
53	Moderate Pseudomonas aeruginosa, Mucoid Strain; Moderate Achromobacter denitrificans;	AVI/AVI	A/A	C/T	C/C	F	38	Ethmoid	CRS	2	Yes	Asthma, ASA intolerance, AR, GERD	Amoxicillin/Clavulanic acid, Prednisone, Nasal steroids, saline irrigations
54	Many Methicillin-Resistant Staphylococcus aureus; Moderate Pseudomonas aeruginosa; Few Morganella morganii ssp. morganii	AVI/AVI	G/G	C/C	A/A	M	59	Ethmoid	CRS	0	Yes	Asthma, AR, GERD	Nasal steroids, saline irrigations
55	Many Pseudomonas aeruginosa	AVI/AVI		C/C	A/C	F	46	Ethmoid	CRS	0	Yes	Asthma, AR, GERD, HTN	Prednisone, Nasal steroids, saline irrigations
56	Moderate Serratia marcescens; Few Staphylococcus species (Coagulase Negative)	AVI/AVI				M	58	Ethmoid	CRS	0	No	Asthma, AR, GERD	Clindamycin, Prednisone, Nasal steroids, saline irrigations

Supplementary Table 1 Microbiology reports and patient information. Genotypes of 4 T2Rs (38, 19, 46, and 30) and correlated microbiology reports from patients utilized in Table 1. Additionally, patient sex, age, site of tissue acquisition, indication for surgery, number of prior sinus surgeries, presence of sinonasal polyps and medications used at least one month prior to surgery. In the "no growth" group there were 35 patients of whom 66% were male, average age was 47.5 years with a range of 24 - 78 years. These patients on average had 1.1 prior sinonasal procedures and 51% had sinonasal polyps. In those patients with positive cultures for gram-negative bacteria, there were 21 patients of whom 38% were male, average age was 47.9 years with a range of 21-79. In this group, patients had on average 1.9 prior sinonasal procedures (p=0.059) and 57% had sinonasal polyps. [CNV = putative copy number variants, FESS = functional endoscopic sinus surgery, CRS = chronic rhinosinusitis, PT = pituitary tumor, TH = inferior turbinate hypertrophy]

REFERENCE

1. Palmer, R.K., Atwal, K., Bakaj, I., Carlucci-Derbyshire, S., Buber, M.T., Cerne, R., Cortes, R.Y., Devantier, H.R., Jorgensen, V., Pawlyk, A., et al. 2010. Triphenylphosphine oxide is a potent and selective inhibitor of the transient receptor potential melastatin-5 ion channel. *Assay Drug Dev Technol* 8:703-713.