SUPPLEMENTAL METHODS

Single cell calcium measurement

HASM derived from two normal donors were grown to confluence, serum starved for 24 hr, then treated with vehicle (DMSO), CAL-101, or LY294002 (1 μM). Cells were then loaded with Fluo-8 calcium sensing dye purchased from Abcam (Cambridge, MA). Single cell calcium transients were measured over 120 seconds. Peak calcium flux (relative fluorescence) and area under the curve were measured for 10-20 cells/condition and compared to buffer control.

Measurement of cAMP levels

HASM cells were grown to 80% confluence, serum starved overnight, then stimulated with LY294004 or CAL-101 (10 μM) for 18 hr. Cells were then stimulated with isoprenaline (10 μM) for 10 min. Cells were lysed and cAMP levels assessed using cAMP-Screen System from Applied Biosystems (Foster City, CA).

RNAseq expression data

We obtained RNA-Seq results for genes of interest corresponding to a previously published study[1]. Briefly, primary ASM cells were isolated from white non-smoking donors, six who died of fatal asthma and twelve with no chronic illness or medication use. ASM cell cultivation was described previously [2, 3]. Passages 4 to 7 ASM cells maintained in Ham's F12 medium supplemented with 10% FBS, $CaCl_2$, buffered with HEPES, penicillin/streptomycin, primocin, and additional L-glutamine were used in all experiments. The F12 medium was used for culture because it provides Ca^{2+} levels that are consistent with seeing contractility of muscles in that media. Total RNA was extracted from cells using the miRNAeasy mini kit (Qiagen Sciences, Inc., Germantown, MD). Approximately 1 μg of RNA from each sample was used to generate RNA-Seq cDNA libraries for sequencing using the TruSeq RNA Sample Prep Kit v2 (Illumina, Inc., San Diego, CA). Sequencing of 75 bp paired-end reads was performed with an Illumina HiSeq 2000 instrument at Partners Personalized Medicine (Boston, MA). Taffeta scripts (https://github.com/blancahimes/taffeta) were used to analyze RNA-Seq data, which included trimming of adapters using trimmomatic (v.0.32) [4] and using FastQC [5] (v.0.11.2) to obtain overall QC metrics. Trimmed reads for each sample were aligned to the reference hg19 genome using TopHat [6] (v.2.0.11), while constraining mapped reads to be within reference hg19. For each sample, Cufflinks [7] (v.2.2.1) was used to quantify hg19 transcripts based on reads that mapped to the provided reference files. The RNA-Seq data is available at the Gene Expression Omnibus Web site (http://www.ncbi.nlm.nih.gov/geo/) under accession GSE58434. Differential expression of genes and transcripts was obtained using Cuffdiff

[7] (v.2.2.1) with the quantified transcripts computed by Cufflinks (v.2.2.1), while applying bias correction for all samples. The CummeRbund [8] R package (v.2.0.0) was used to measure significance of differentially expressed genes and create plots of the results. The reported q-values are false-discovery rate adjusted p-values according to the implementation in Cuffdiff and CummeRbund that take into account the large number of comparisons made.

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Fig. 1. Time course of airway dilation to CAL-101 and Y27632. Airways in lung slices were preconstricted to carbachol, then dilated to CAL-101 or Y27632 (60 μM), 0-30 min. Data is representative of mean ± SEM for 4-15 slices/condition.

Supplemental Fig. 2. PI3K p110δ (A) and γ (B) mRNA expression in airway smooth muscle assessed by RNAseq. PI3K inhibition has little effect on expression of total protein levels (C) of MYPT1 (D), AKT (E), and MLC (F). Data is representative of n=5-8 (A and B) or n=2 (C-F) HASM cell lines.

Supplemental Fig. 3. PI3K inhibition has little effect on agonist-induced calcium mobilization. HASM were stimulated with 10 μM carbachol following a 30 min pretreatment with vehicle (DMSO), CAL-101 or LY294002 (1 μM) and single cell calcium transients measured using Fluo-8 calcium sensing dye. (A) Time course tracings of calcium flux. Both peak calcium (B) and area under the curve (C) for each treatment were plotted as mean ± SD.

Supplemental Fig. 4. siRNA knockdown of PI3K p110γ had little effect on carbachol-induced (10 min, 10 μM) phosphorylation of MLC (pMLC), MYPT1, or Akt in HASM cells. (A) Immunoblot analyses of HASM cells transfected with PI3K p110γ siRNA or scrambled siRNA.

(B) Slight inhibition of carbachol-induced phosphorylation of Akt (pAkt) and MYPT1 (pMYPT1) in HASM cells by PI3K p110δ siRNA. Data are representative of five independent experiments (n = 5, mean + SEM); *P < 0.05.

Supplemental Fig. 5. PI3K inhibition has little effect alone to induce or augment cAMP levels. Data are representative of 3 donors and each condition in triplicate, with bars representing mean ± SEM with a P value of < 0.05 considered significant.

Supplemental Table 1. Donor demographics of asthma and non-asthma HASM cell donors.

Supplemental Table 2. Donor demographics of non-asthma hPCLS donors.

Supplemental Table 3. Pharmacologic properties of the inhibitors utilized in this study. *In vitro* kinase assay results, IC_{50} values in cell based assays, and the corresponding literature citations are shown for CAL-101, LY294002, and Y27632.

Supplemental Movie 1. Formoterol-induced relaxation of bradykinin contracted HASM. Link: https://youtu.be/51oJeQ73LEA

Supplemental Movie 2. CAL-101-induced relaxation of bradykinin contracted HASM. Link: https://youtu.be/2H3DKMqI-GU

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