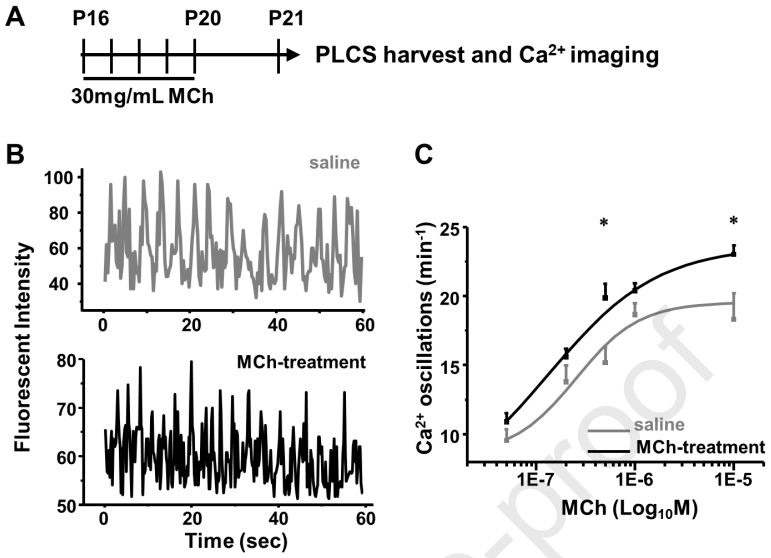

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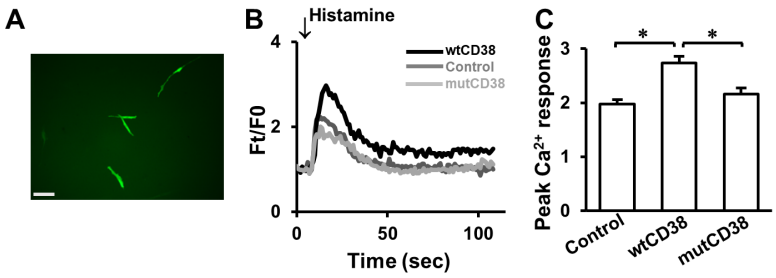
Supplementary Fig. E1



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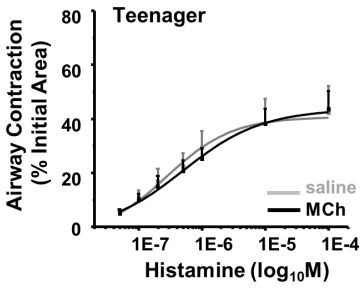
Supplementary Fig. E2



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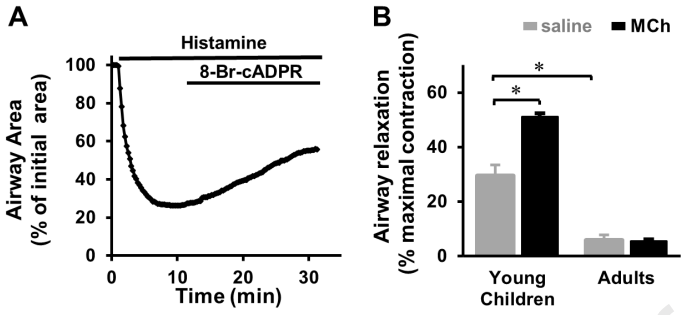
Supplementary Fig. E3



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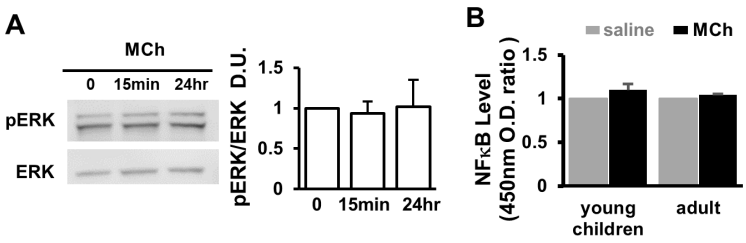
Supplementary Fig. E4



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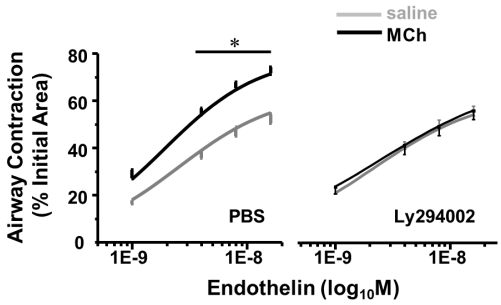
Supplementary Fig. E5



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Supplementary Fig. E6

Supplementary Material and Methods:

Nebulization of mouse pups with methacholine: Mouse pups were nebulized with 30 mg/ml of MCh (A2251; Sigma-Aldrich) for 10 minutes per day for 5 consecutive days between P16 and P20. Control mice were nebulized with saline. Mice were euthanized at P21 for the preparation of PCLSs and Ca^{2+} imaging.

Plasmid transfection of primary HASM cells: For CD38 overexpression, primary human ASM cell cultures at around 80% confluency were transfected with a pEGFP-N1-CD38 plasmid containing full-length CD38 cDNA with an N-terminal EGFP tag. pEGFP-sCD38 with a point mutation (226 Glu to Gln) was the control. Transfection was performed using lipofectamine 2000 (Invitrogen, 11668-019) following the manufacturer's protocol. The transfected cells were identified by the expression of EGFP after 48 hours and subjected to Ca^{2+} imaging assay after 4-hour recovery.

Ca^{2+} imaging: The Ca^{2+} imaging of ASM in the mouse PLCS has been described in detail previously[1]. Briefly, PCLSs were incubated with the HBSS containing 20 μmol/L Oregon Green BAPTA-1-AM (Life Technologies, O-6807), 0.1% Pluronic F-127 (Thermo Fisher Scientific, P6867), and 200 μmol/L sulfobromophthalein (Sigma-Aldrich, S0252) at 30°C for 1 hour and then 100 μmol/L sulfobromophthalein for additional 30 minutes for fluorescent dye de-esterification. The Ca^{2+} signaling of ASM was detected with a custom-built, video-rate, scanning 2-photon laser microscope at a rate of 15 frames per second and analyzed by Video Savant software (IO Industries, London, Canada). The EGFP plasmid transfected primary ASM cells were loaded with 5μM Rhod-3 (Life Technologies, R10145) in HBSS for 1 hour at 37°C and applied for Ca^{2+} signaling assay as described in the previous session.

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Airway relaxation assay: Human PCLSs were pre-treated with 1 μM histamine for 10 minutes to induce a stable airway contraction prior to exposure to 100uM 8-Br-cADPR for 20 minutes in the presence of histamine. The increase in airway lumen area (indicative of relaxation) following 8-Br-cADPR exposure was measured by NIH imaging J and normalized to luminal constriction induced by histamine.

ELISA assay of NFκB level: Following the MCh or saline treatment, primary human ASM cells (~2X10^6) were collected, from which nuclear protein was obtained using a Nuclear Extraction Kit (EMD Millipore, 2900). The nuclear protein solution was then applied to detect the NFκB level with an ELISA Kit (Cayman Chemical Company, 10007889). Finally, the NFκB level of MCh-treated ASM cells was expressed as absorbance (450nm) ratio compared to saline-treated ASM cells in the same age group.

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References:

1 Ressmeyer AR, Bai Y, Delmotte P, Uy KF, Thistlethwaite P, Fraire A, Sato O, *et al.* Human airway contraction and formoterol-induced relaxation is determined by Ca2+ oscillations and Ca2+ sensitivity. *American journal of respiratory cell and molecular biology* **43**, 179-191, doi:10.1165/rcmb.2009-0222OC (2010).

Supplementary Table and Figures:

Supplementary Table E1: Demographic information of human lung donors in each age group.

Supplementary Figure E1. Early life MCh exposure in mice enhances the Ca2+ response in ASM cells. (A) Scheme of MCh treatment in neonatal mice followed by PCLS preparation and Ca^{2+} imaging. (B) Representative traces showing Ca^{2+} oscillations in ASM in PCLSs from saline- (grey) or MCh- (black) treated mice at P21 in response to 1μM MCh. (C) The dose curve of Ca^{2+}

oscillation frequency in response to MCh in PCLSs prepared from saline (grey) or MCh (black) exposed mouse pups. Each point represents 10-16 ASM cells from 3 mice in the saline group or 16-21 ASM cells from 4 mice in the MCh group, * $p<0.05$ by two-way ANOVA followed by Tukey's post-hoc test.

Supplementary Figure E2. CD38 overexpression augments the Ca2+ response of ASM. (A) Representative image showing primary ASM cells transfected with the pEGFPN1-CD38 plasmid. (B) Representative fluorescence traces and (C) peak Ca2+ response to 10μM histamine in untransfected ASM cells (control) and ASM cells with wild type (wt) or mutant (mut) CD38 plasmid transfection. Each column represents mean ± SEM of 6-8 cells in each experiment. Scale bars, 50 μm. * $p<0.05$ by unpaired Student's t-test.

Supplementary Figure E3. The impact of MCh treatment on airway contraction in PCLSs from adolescent donor lungs. The dose-response of saline- or MCh-treated human airways to histamine stimulation in PCLSs from teenage donor lungs. Each point represents the average ± SEM of 5 PCLSs from 2 adolescent donors.

Supplementary Figure E4. Blockade of the CD38 pathway causes more prominent airway relaxation in PCLSs from young children than adults. (A) Representative trace showing the relaxation of a 1μM histamine pre-contracted human airway in response to 100μM 8-Br-cADPR. (B) The relaxant effect of 100μM 8-Br-cADPR on pre-contracted airways in PCLSs from young children and adults. PCLSs were treated with MCh or saline, contracted by 1 μM histamine, and then exposed to 8-Br-cADPR. After 20 minutes, the reduction in contraction, measured as the increase in the airway luminal area, was quantified by normalizing to the maximum contraction

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induced by histamine. Each column represents 4-8 airways from 2 human donors. *p<0.05 by unpaired Student's t-test.

Supplementary Figure E5. MCh exposure has no age-different impact on the phosphorylation of Erk or NFκB level in human ASM cells. (A) Western blot assay (left panel) and densitometric quantification (right panel) of 0, 15-minute, and 24-hour MCh exposure-induced ERK phosphorylation in ASM cells from young children. Each column represents 3 independent experiments from 2 donors. (B) NFκB level of ASM cells following 2-hour MCh exposure in young children and adult groups by ELISA assay. Each column represents data from 2 donors.

Supplementary Figure E6. PI3K inhibition opposes MCh-induced airway hypercontraction in neonatal mice. Airway contraction in P21 mouse PCLSs in response to endothelin. Mouse PCLSs were treated with saline or MCh ±10 μM Ly294002. Each point represents the result of 12-15 PCLSs from 4 mice at P21. * p<0.05 by two-way ANOVA followed by Tukey's post-hoc test.

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Table E1. Demographic information of lung donors

Age groups	Donor Demographics	
	age	gender
Young children	5 days	female
	6 months	male
	16 months	female
Teenagers	13 years old	female
	14 years old	male
Adults	57 years old	male
	36 years old	female
	59 years old	male
	48 years old	male

*** All donors have been ruled out for smoking history, chronic lung disease including asthma, COPD, ILD, acute pulmonary infection or lung injuries identified by abnormal chest images, or arterial blood gas.**

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