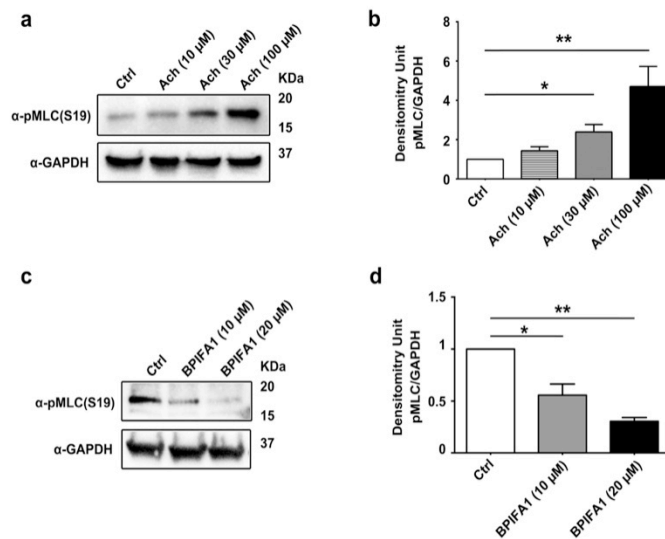
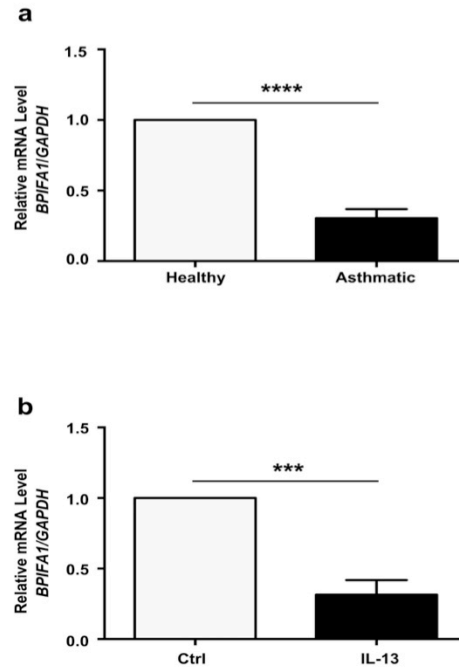


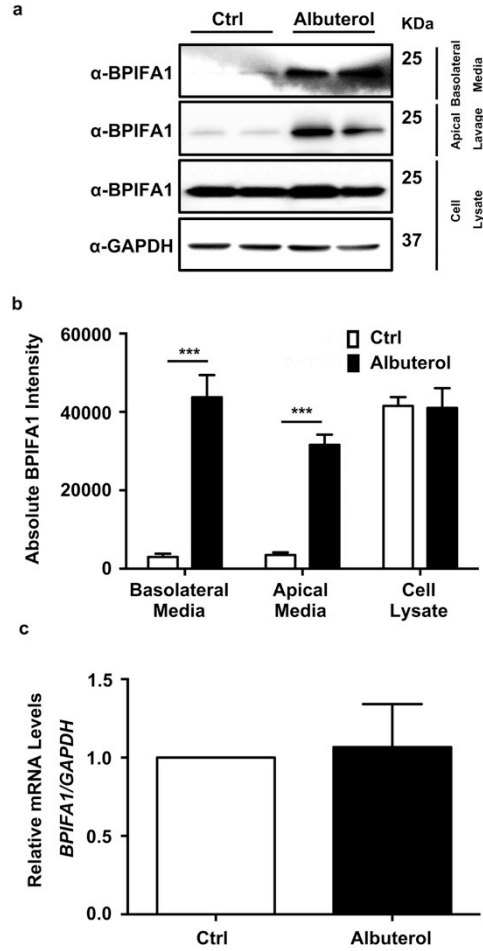
**Supplementary Figure 1. *Bpifa1*<sup>-/-</sup> murine tracheal smooth muscles show no sign of hypertrophy.** Quantification of smooth muscle from *Bpifa1*<sup>+/+</sup> and *Bpifa1*<sup>-/-</sup> mice tracheas was expressed as average surface areas (a) and tracheal wall thickness (b) in arbitrary units. Images were analyzed using ImageJ software. Two sections/mouse, 3 mice/genotype were analyzed. Data are mean±s.e.m and were analyzed using student's *t*-test.



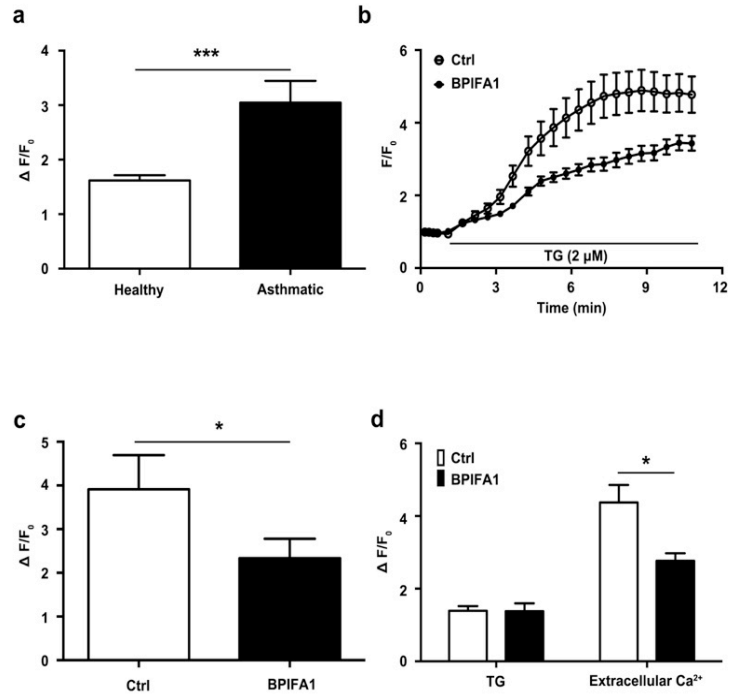
**Supplementary Figure 2. BPIFA1 decreases MLC phosphorylation in a dose-dependent fashion.** Immunoblots were probed with an antibody that recognizes phosphorylated MLC and normalized to GAPDH. **(a)** Representative immunoblot demonstrating that Ach increases MLC phosphorylation. **(b)** Intensity of immunoblots from (a) were quantified using Image J, normalized to GAPDH and expressed as relative intensity (n=3). **(c)** Representative immunoblot demonstrating that BPIFA1 increases MLC phosphorylation. **(d)** Bar graph of immunoblot intensity from (c) were quantified using Image J, normalized to GAPDH and expressed as relative intensity (n=3). Data in b and d are mean $\pm$ s.e.m. The data were analyzed using one-way ANOVA followed by Turkey post hoc analysis. \* and \*\* indicate P<0.01 and P<0.05 different to control respectively.



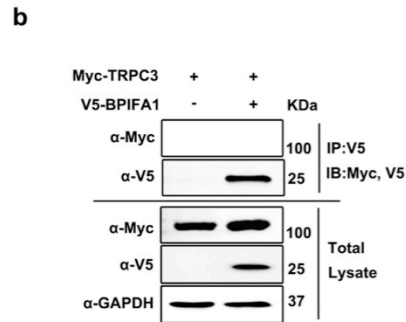
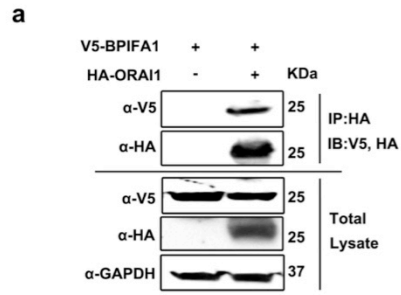
**Supplementary Figure 3. BPIFA1 mRNA is decreased in asthmatic (a) and IL-13 treated (b) human bronchial epithelial cultures (HBECS).** Total RNA from normal and asthmatic HBECS was extracted and mRNA levels of *BPIFA1* were measured by qRT-PCR (n=3/group). Data are mean±s.e.m and were analyzed using student's *t*-test. \*\*\* Indicates P<0.001 and \*\*\*\* indicates P<0.0001 different to control.



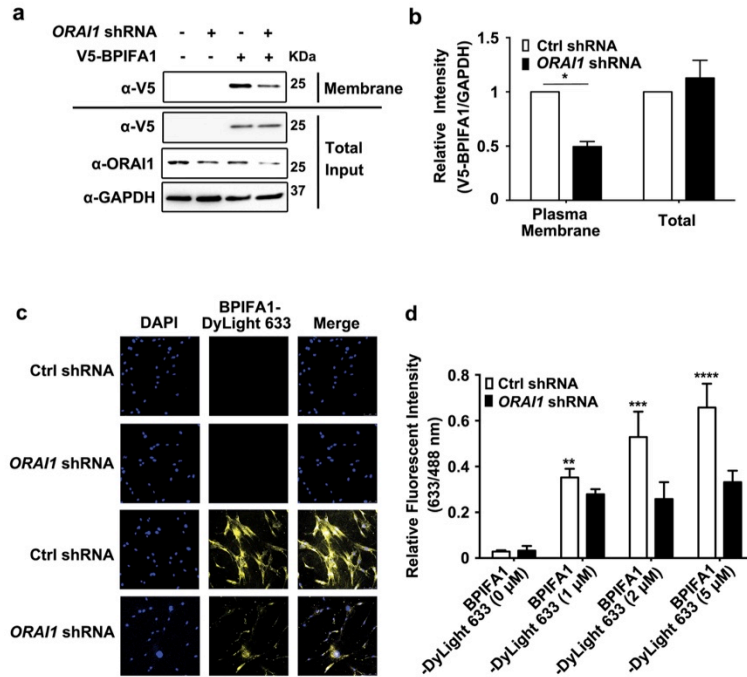
**Supplementary Figure 4. Albuterol increases BPIFA1 secretion but not *BPIFA1* expression in HBECS.** (a) Immunoblots demonstrating that BPIFA1 secretion in HBEC basolateral media and apical lavage is triggered by a 2 h exposure to albuterol (10  $\mu$ M). (b) Mean densitometry taken from (a). (c) Total RNA from normal and asthmatic HBECS was extracted and mRNA levels of *BPIFA1* were quantified by qRT-PCR (n=3/group). Data in b and c are mean $\pm$ s.e.m. The data were analyzed using student's *t*-test. \*\*\* indicates P<0.001 different to control.



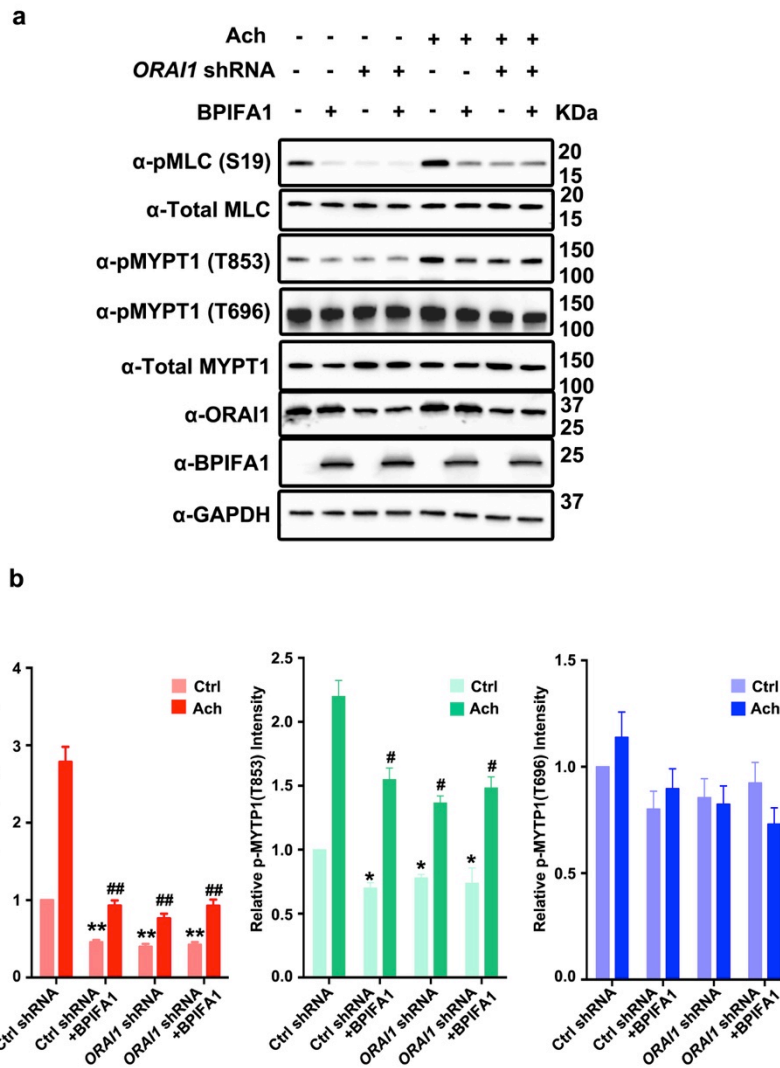
**Supplementary Figure 5. BPIFA1 suppresses thapsigargin (TG)-induced elevations in cytosolic  $Ca^{2+}$  levels.** (a) Summary of peak fluorescent ratio change in ASMCs incubated with media from healthy and asthmatic HBECs, respectively (n=3/group). (b) Average traces of fura-2 fluorescence as an indicator of cytosolic  $Ca^{2+}$  levels. ASMCs were co-incubated with fura-2 and either BPIFA1 or vehicle for 1 h. The fura-2 emission ratio was then recorded over time. (c) Summary of peak fluorescent ratio changes in fura-2 fluorescence with or without BPIFA1 (both n=3). (d) Summary of peak fluorescent ratio change in the presence of TG and extracellular  $Ca^{2+}$  (n=3). Data in a, c and d are mean $\pm$ s.e.m. The data were analyzed using student's t-test. \* indicates  $P < 0.05$  different to control. \*\*\* indicates  $P < 0.001$  different to control.



**Supplementary Figure 6. BPIFA1 does not interact with TRPC3.** Immunoprecipitation analysis was performed using cell lysates from HEK293T cells co-transfected with V5-BPIFA1 and either HA-ORAI1 (**a**) or Myc-TRPC3 (**b**). Data are representative of 3 individual blots per condition.

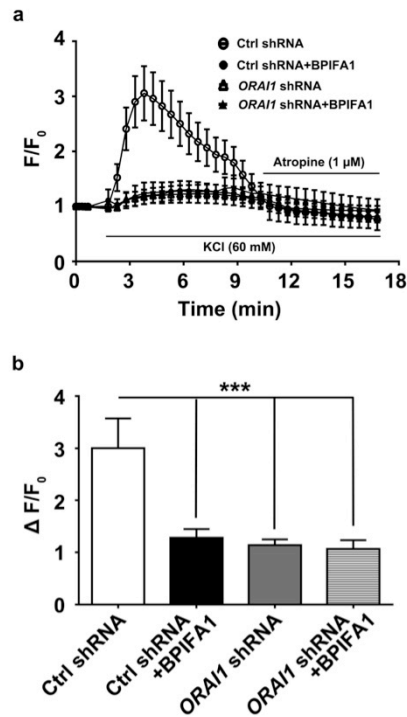


**Supplementary Figure 7. Knockdown of *ORAI1* using shRNA decreases BPIFA1 binding to ASMC plasma membrane.** (a) Human ASMCs were transfected with scrambled shRNA (control) and *ORAI1* shRNA respectively for 72 h. Cells were incubated in the presence or absence of BPIFA1, followed by surface biotinylation and immunoblot using indicated antibodies. (b) Intensity of immunoblots from (a) were quantified using Image J, normalized to GAPDH and expressed as relative intensity (all n=3). (c) Human ASMCs were transfected with scrambled control shRNA and *ORAI1* shRNA respectively for 72 h. BPIFA1 was labeled with DyLight-633 (yellow). 72 h post-transfection, cells were treated with or without BPIFA1-DyLight 633 for 1 h, then washed 5 times with ice-cold Ringer's solution. Cells were then fixed with ice-cold methanol and counter stained with DAPI (blue). Fluorescent images were taken using a Leica SP8 confocal microscope. Scale bar indicates 75  $\mu$ m. (d) Bar graph showing BPIFA1-DyLight 633 binding to ASMCs as detected using a fluorescence plate reader. Cells were also stained with calcein to determine cell number per well. Relative fluorescence intensity was calculated by normalizing Dylight-633 to calcein emission. Data in b and d are mean $\pm$ s.e.m. The data were analyzed using student's *t*-test in b, and two-way ANOVA followed by Sidak corrected post hoc analysis in d. \* indicates  $P < 0.05$ , \*\* indicates  $P < 0.01$ , \*\*\* indicates  $P < 0.001$ , and \*\*\*\* indicates  $P < 0.0001$  different to control.

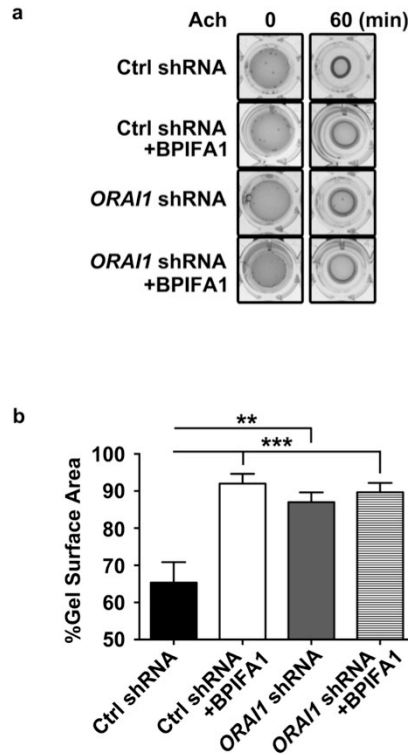


**Supplementary Figure 8. Either BPIFA1 or *ORAI1* knockdown suppresses MLC(S19) and MYTP1(T853) phosphorylation but not MYTP1 (T696) phosphorylation. (a)** Immunoblots showing phosphorylation of MLC(S19), MYTP1(T853) and MYTP1 (T696) under both basal and induced conditions. **(b)** Mean densitometry taken from (a) (n=3). Data in b are mean $\pm$ s.e.m. The data were analyzed using two-way ANOVA followed by Sidak corrected post hoc analysis. \* and # indicate  $P < 0.05$ , \*\* and ## indicates  $P < 0.01$  different to control.

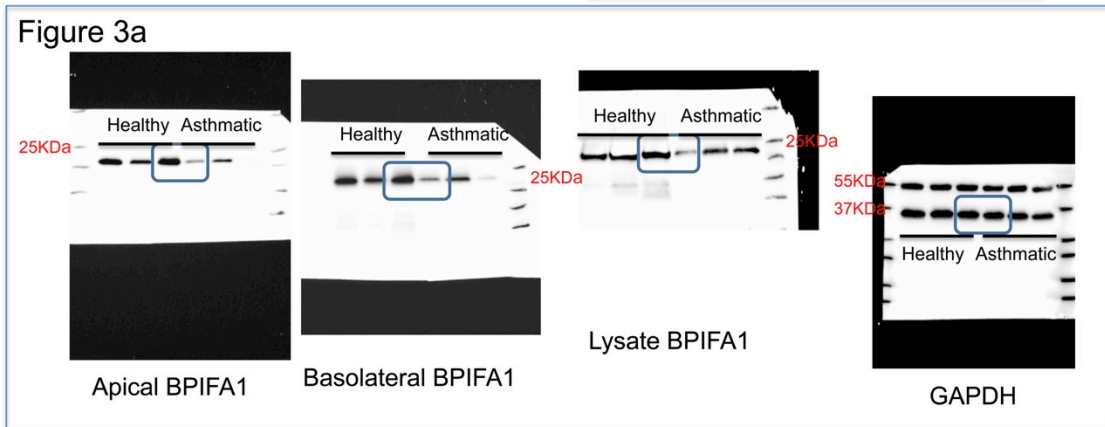
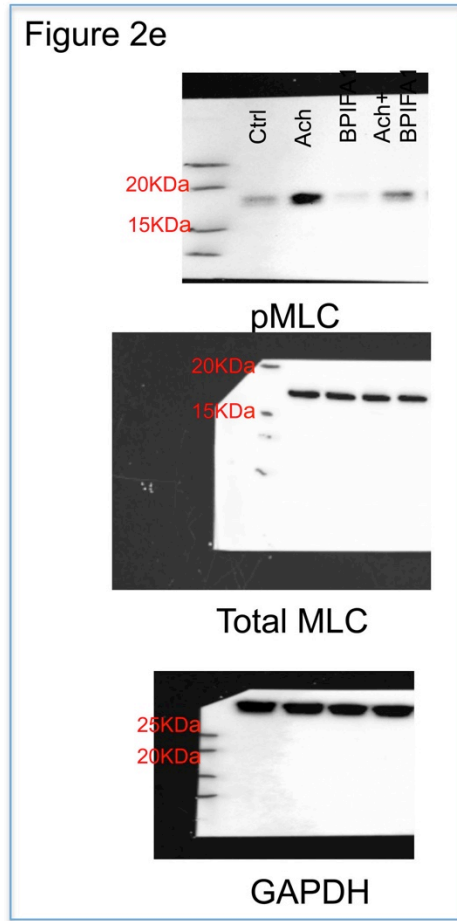
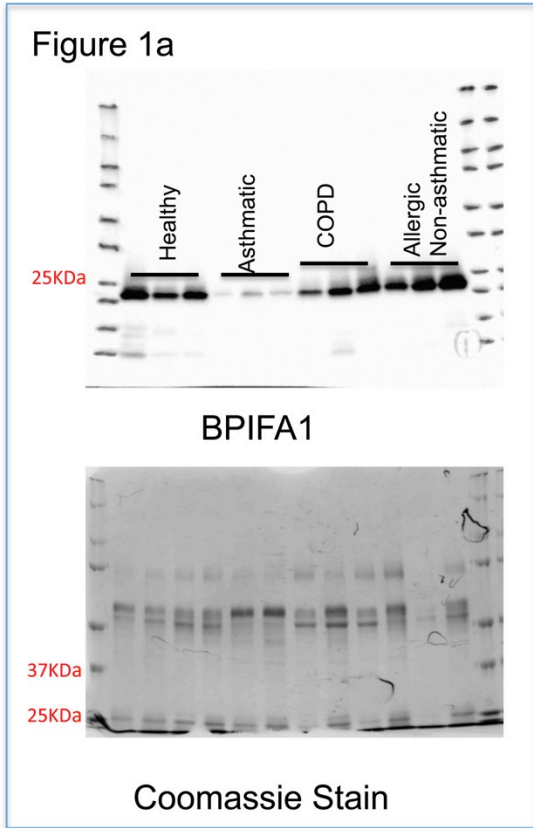




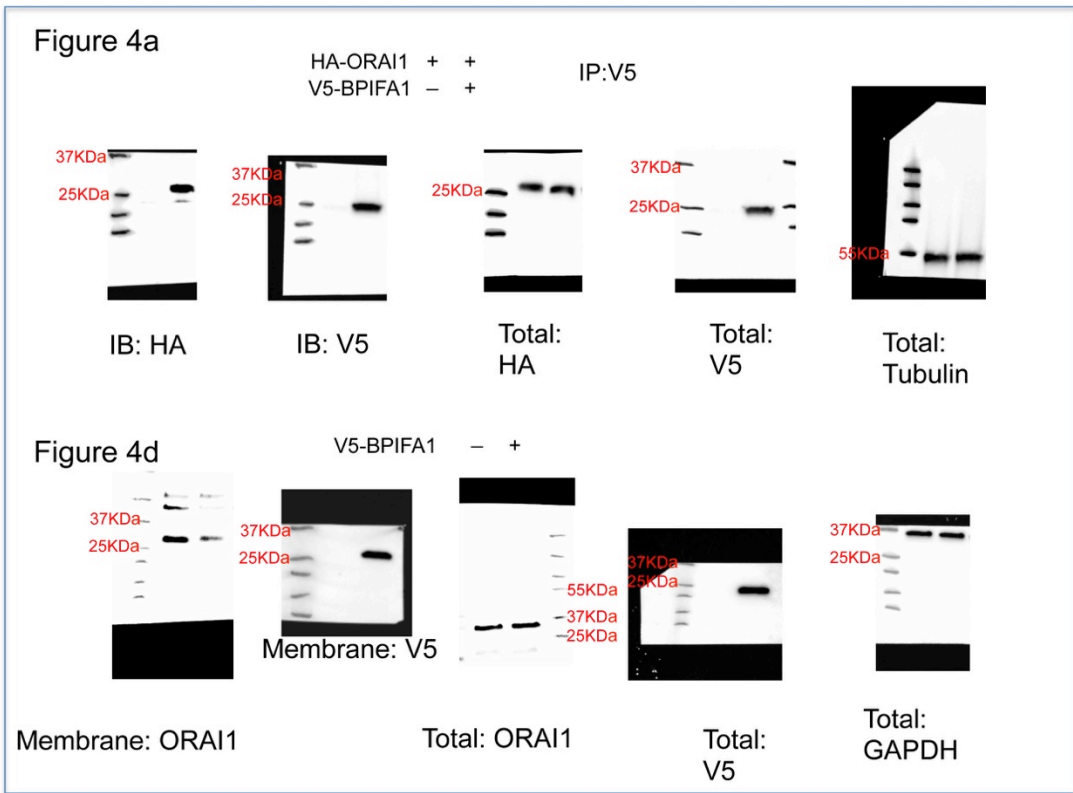
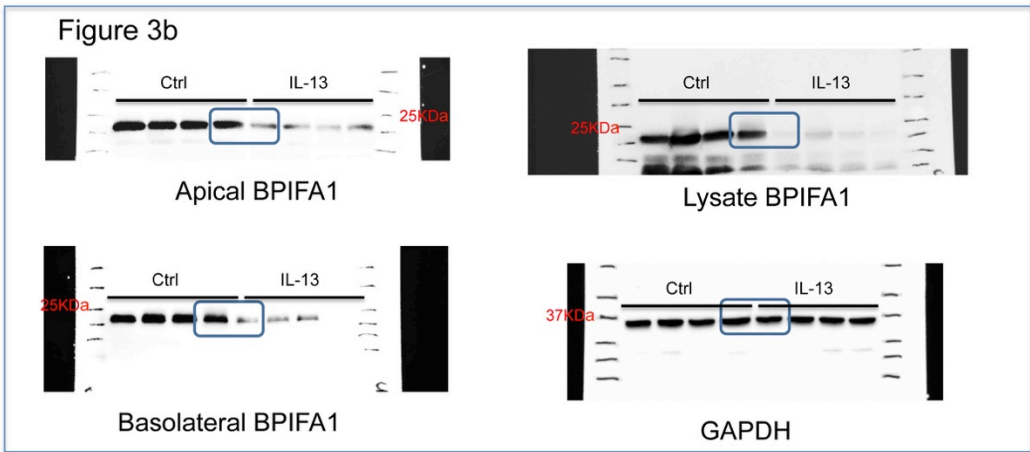
**Supplementary Figure 9. BPIFA1 or *Orai1* knockdown blocks KCl induced Ca<sup>2+</sup> influx.** (a) Representative traces of Ca<sup>2+</sup> imaging using fura-2. Human ASMCs were transfected with control and *Orai1* shRNA respectively for 72 h. After incubating with or without 10 μM BPIFA1 for 1 h, the fura-2 emission ratio was then recorded in the presence of 60 mM KCl, and 1 μM atropine was added at indicated time point. (b) Summary of peak fluorescent ratio changes in the presence of TG (n=3/group). Data in b are mean±s.e.m. The data were analyzed using one-way ANOVA followed by Turkey post hoc analysis. \*\*\* indicates P<0.001 different to control.



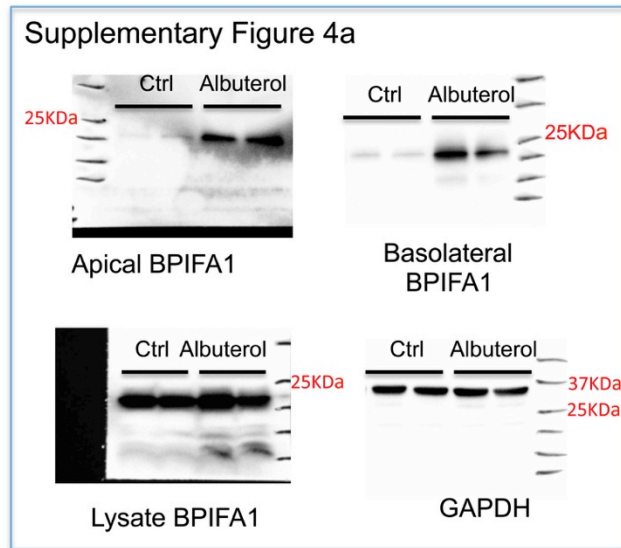
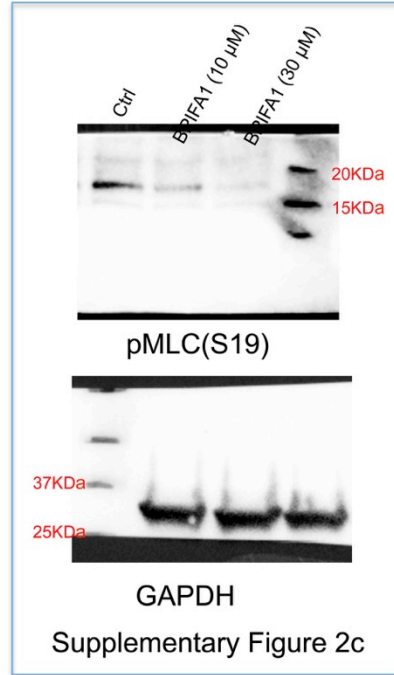
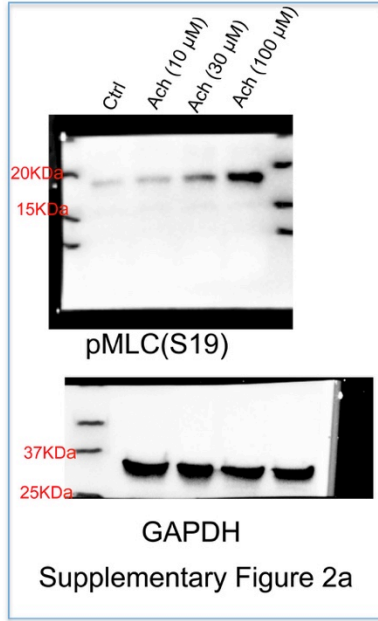
**Supplementary Figures 10. *ORAI1* knockdown decreases ASMC contractility *in vitro*.** (a) Human ASMCs were transfected with control or *ORAI1* shRNA respectively for 72 h and then grown in a type I collagen matrix in 24 well plates. ASMC were incubated with BPIFA1 (10  $\mu$ M) or vehicle control for 1 h and the gel contraction was performed  $\pm$  Ach (100  $\mu$ M). Representative images of the gel contraction assay at indicated time points. (b) Summary of contraction data expressed as decrease in gel surface area (%) at 60 min (n=3/group). Data in b are mean $\pm$ s.e.m. The data were analyzed using one-way ANOVA followed by Turkey post hoc analysis. \* indicates P<0.05 and \*\* indicates P<0.01 different to control.



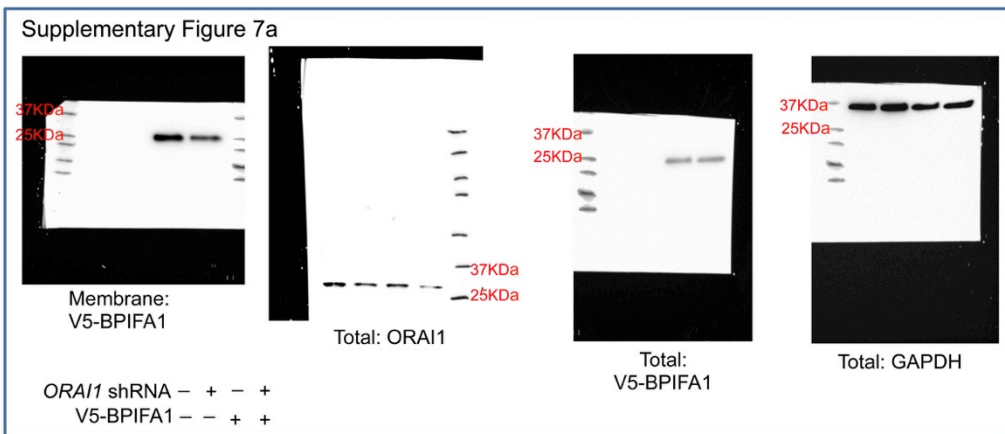
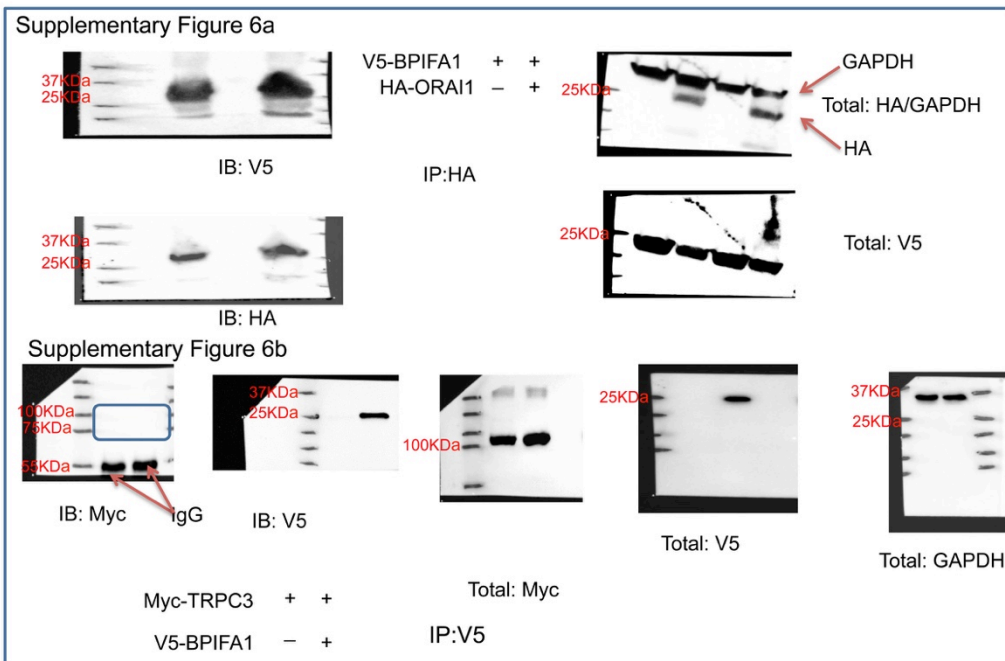
## Supplementary Figure 11



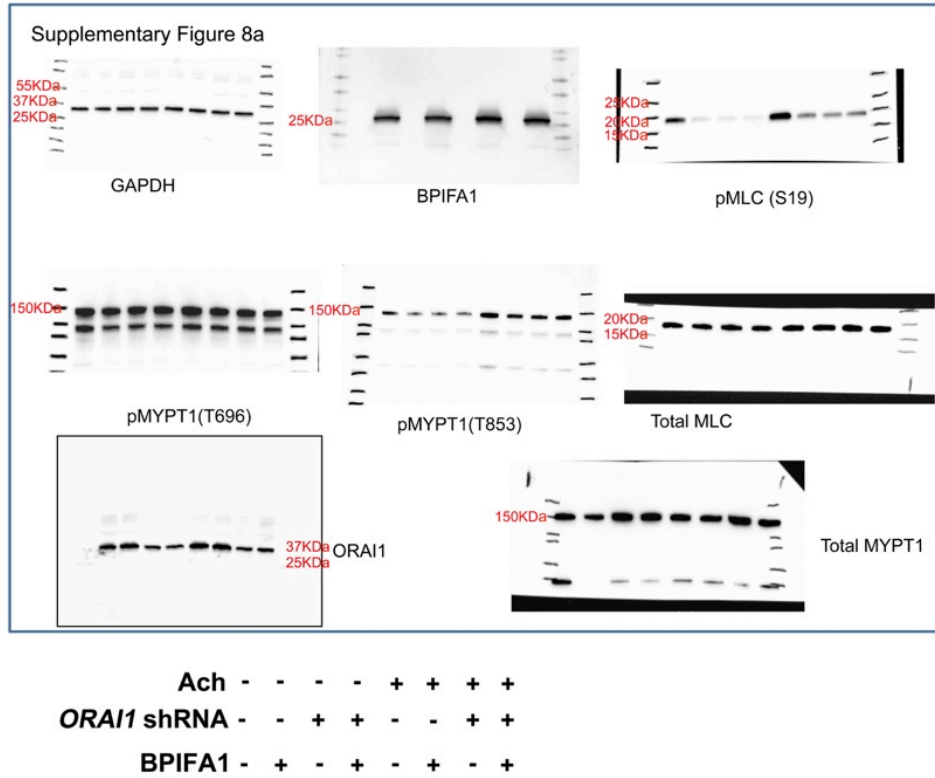
**Supplementary Figure 11**



**Supplementary Figure 11**



**Supplementary Figure 11**



### Supplementary Figure 11

**Supplementary Figures 11.** Original un-cropped images of all Western blots shown in the Figures and Supplementary Figures. We used protein ladders (Bio-Rad #161-0374) to identify the molecular weight of the proteins of interest.

Donor Disease Status	Age	Gender	FVC	FEV <sub>1</sub>	FVC %PRED	FEV <sub>1</sub> %PRED
Nonasthmatic /nonallergic/nonsmoker	21	M	6.48	5.25	117	110
Nonasthmatic /nonallergic/nonsmoker	23	F	4.2	3.25	117	105
Nonasthmatic /nonallergic/nonsmoker	20	F	3.64	3.08	84	84
Nonasthmatic /nonallergic/nonsmoker	23	M	6.05	4.77	106	96
Nonasthmatic /nonallergic/nonsmoker	28	F	4.6	3.44	115	102
Nonasthmatic /nonallergic/nonsmoker	20	M	5.15	4.1	107	99
Asthmatic/nonsmoker	22	F	3.76	2.93	101	91
Asthmatic/allergic	21	F	4.27	3.26	103	90
Asthmatic/allergic	36	M	5.85	4.66	102	102
Asthmatic/allergic	31	F	3.52	2.8	105	98
Asthmatic/allergic	21	F	4.84	3.65	122	106
Asthmatic/allergic	26	F	4.74	3.32	119	96
COPD	62	F	2.01	1.14	74	54
COPD	46	F	3.82	2.92	101	96
COPD	67	M	3.92	1.82	48	48
COPD	70	F	2.06	1.12	72	51
COPD	52	M	3.4	1.66	72	45
COPD	58	M	4.21	1.2	84	32
Healthy/allergic	37	F	3.53	3.07	103	108
Healthy/allergic	25	F	4.23	3.47	115	111
Healthy/allergic	22	F	2.89	2.59	94	97
Healthy/allergic	28	M	4.89	4.09	87	87
Healthy/allergic	27	M	4.89	3.86	98	92
Healthy/allergic	30	M	5.02	4.18	105	104

**Supplementary Table 1. Demographic information of sputum donors in Figure 1.**



<b>Donor disease status</b>	<b>Age</b>	<b>Gender</b>
Healthy/nonasthmatic/non-smoker	19	M
Healthy/nonasthmatic/non-smoker	45	M
Healthy/nonasthmatic/non-smoker	49	M
Asthmatic/smoker	50	F
Asthmatic/smoker	25	M
Asthmatic/smoker	38	F

**Supplementary Table 2. Demographic information of lung donors pertinent to Figures 3(a), (c) and Supplementary Figure 3(a).**