β2-adrenoceptor Activation Stimulates IL-6 Production via PKA, ERK1/2, Src, and Beta-arrestin2 Signaling Pathways in Human Bronchial Epithelia

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Research

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Abstract

Background: β 2 -adrenoceptor agonists are widely used to treat asthma because of their bronchial-dilation effects. However, a recent study describing a side effect of aggravating eosinophilic inflammation in the mouse airway epithelia by β 2 -adrenoceptor agonists could impact the future clinical use of these bronchodilators. We previously reported that isoprenaline, via the apical and basolateral β 2 -adrenoceptor, induced Cl - secretion by activating cyclic AMP (cAMP)-dependent pathways in human bronchial epithelia. Despite these results, whether and how the β 2 -adrenoceptor-mediated cAMP-dependent pathway contributes to pro-inflammatory cytokine release in human bronchial epithelia remains poorly understood.

Methods: We investigated β 2 -adrenoceptor-mediated signaling pathways involved in the production of two pro-inflammatory cytokines, interleukin (IL)-6 and IL-8, in 16HBE14o- human bronchial epithelia. The effects of isoprenaline or formoterol were assessed in the presence of protein kinase A (PKA), exchange protein directly activated by cAMP (EPAC), Src, and extracellular signal-regulated protein kinase (ERK)1/2 inhibitors. The involvement of b-arrestin2 was examined using siRNA knockdown.

Results: Both isoprenaline and formoterol (both β 2 agonists) induced IL-6, but not IL-8, release, which could be inhibited by ICI 118551 (β 2 antagonist). The PKA-specific inhibitor, H89, partially inhibited IL-6 release. Another intracellular cAMP receptor, EPAC, was not involved in IL-6 release. Isoprenaline-mediated IL-6 secretion was attenuated by dasatinib, a Src inhibitor, and PD98059, an ERK1/2 inhibitor. Isoprenaline treatment also led to ERK1/2 phosphorylation. In addition, knockdown of β-arrestin2 by siRNA specifically suppressed cytokine release when a high concentration of isoprenaline (1 mM) was used.

Conclusion: Our results suggest that activation of the β 2 -adrenoceptor in 16HBE14o- cells stimulated the PKA/Src/ERK1/2 and/or β-arrestin2 signaling pathways, leading to IL-6 release. Therefore, our data reveal that β 2 -adrenoceptor signaling plays a role in the immune regulation of human airway epithelia.

Full Text

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Figures

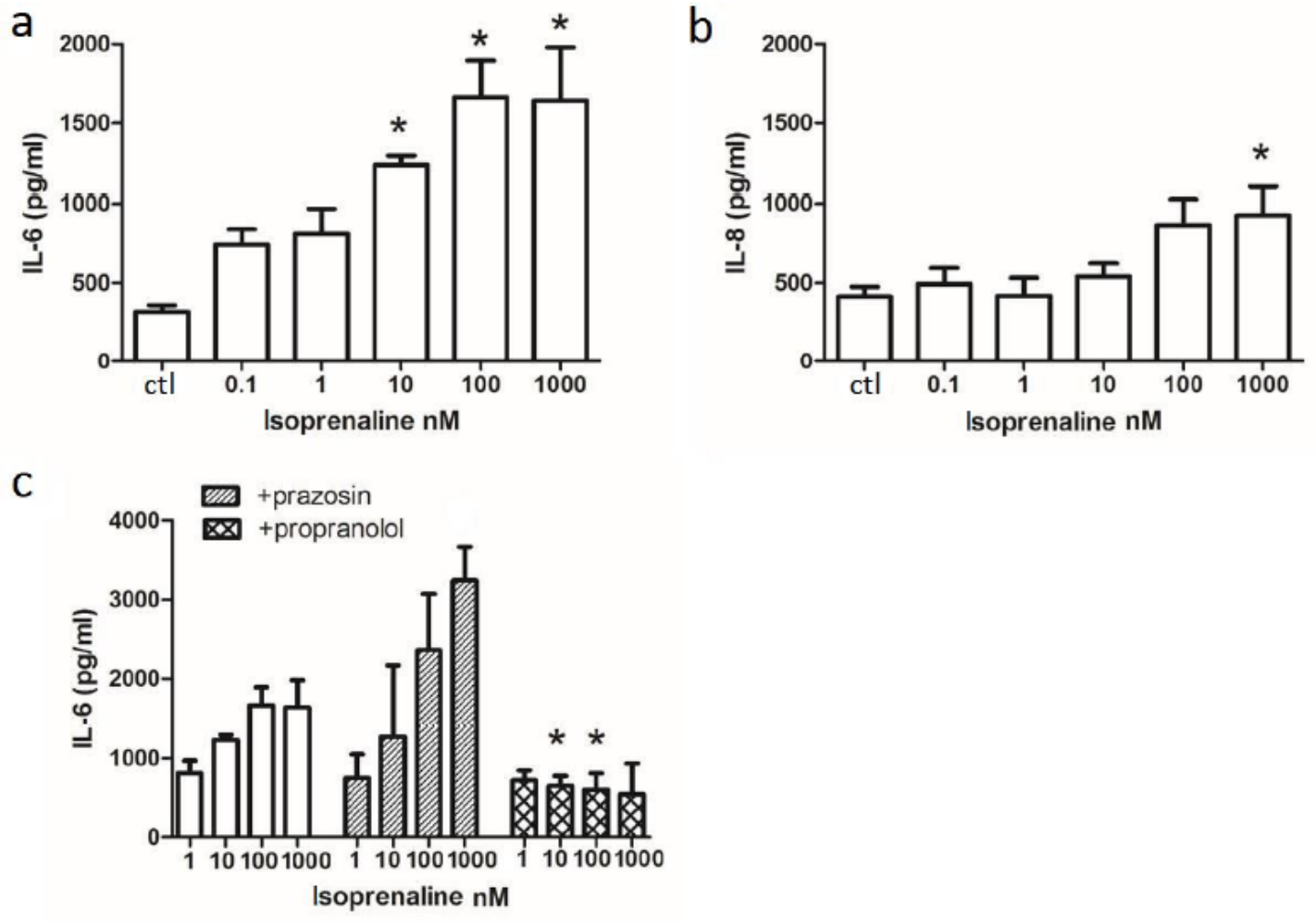

Figure 1

Isoprenaline induces IL-6 and IL-8 release. a – b. 16HBE14o- cells were treated with different concentrations of isoprenaline for 6 hrs. IL-6 (a) and IL-8 (b) release were quantified by ELISA. Each column represents the mean ± S.E. (n=5-7; *p < 0.05 compared with control (ctl) group; one-way ANOVA with Dunnett's post hoc test). c. Cells were pretreated with prazosin (1 μM) or propranolol (10 μM) for 2 hrs, and then the cells were stimulated with isoprenaline in the presence of the inhibitors prior to quantification of IL-6 secretion by ELISA. Each column represents the mean ± S.E. (n=4-6; *p < 0.05 compared with the same concentration of isoprenaline in the control group without inhibitor; Student's t-test).

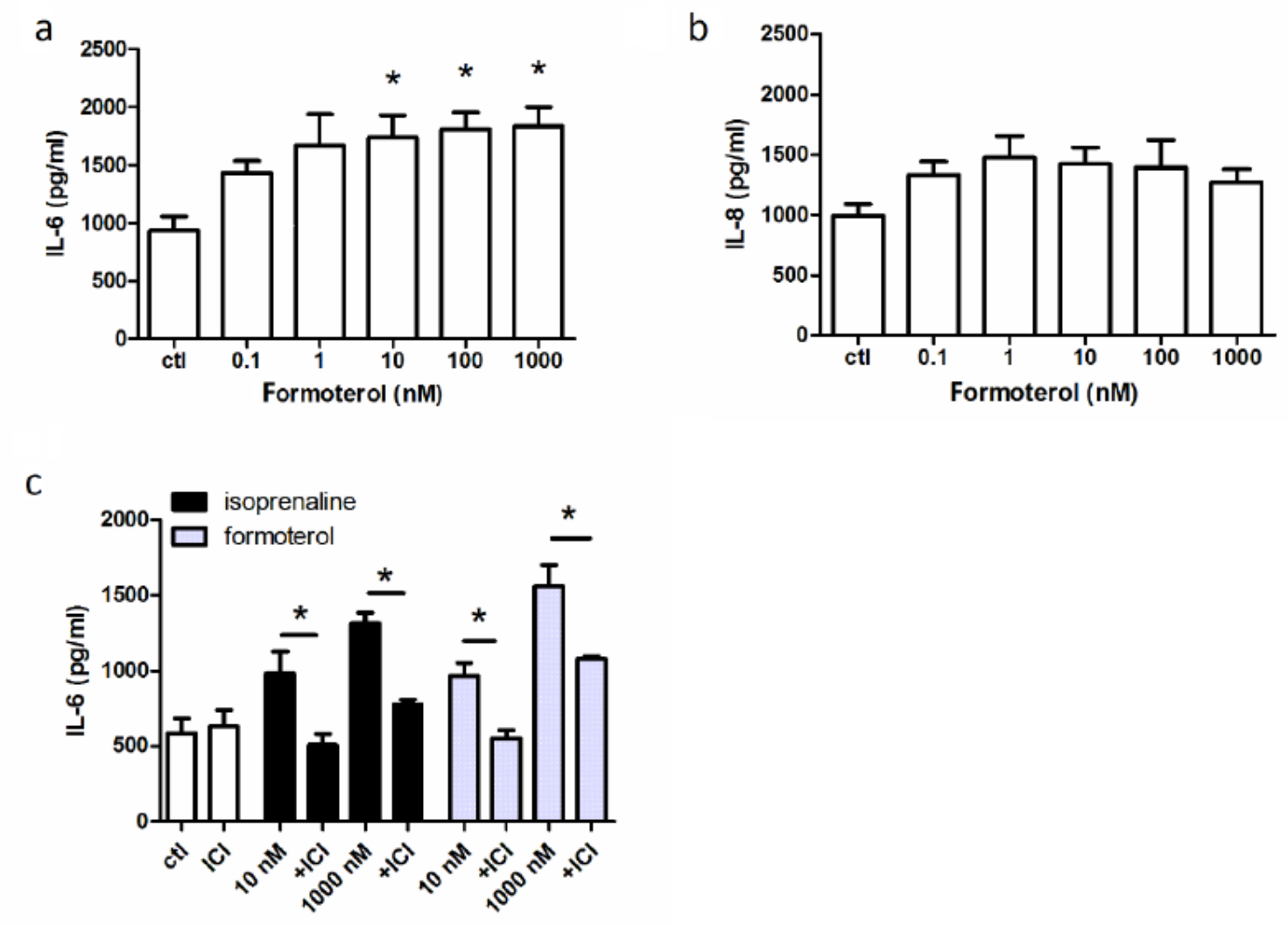

Figure 2

Isoprenaline-induced cytokine release is dependent on the β2-adrenoceptor. a – b. 16HBE14o- cells were treated with different concentrations of formoterol for 6 hrs and then IL-6 (a) and IL-8 (b) release were quantified by ELISA. Each column represents the mean ± S.E. (n=4-5; *p < 0.05 compared with control (ctl) group; one-way ANOVA with Dunnett's post hoc test). c. Cells were pretreated with ICI 118551 (10 μM; ICI) for 2 hrs before stimulation of the cells with isoprenaline or formoterol for 6 hrs. Each column represents the mean ± S.E. (n=3-5; *p < 0.05 compared with the same concentration of isoprenaline or formoterol without inhibitor; Student's t-test).

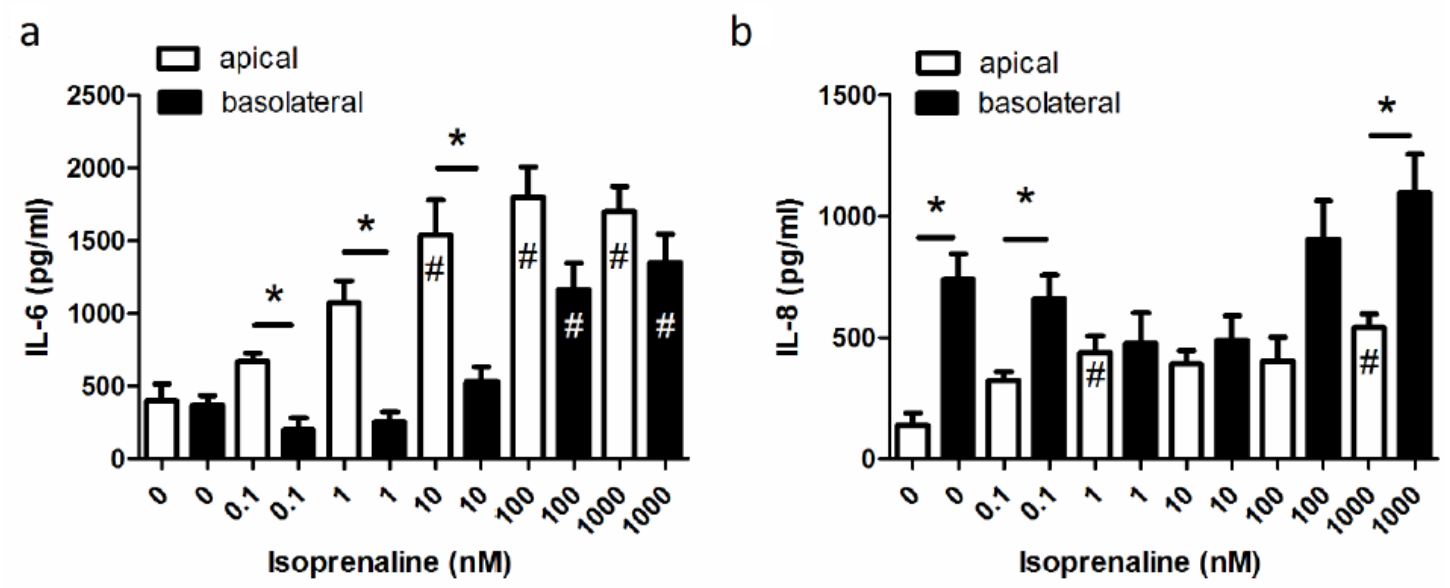

Figure 3

Polarized secretion of IL-6 and IL-8 is induced by isoprenaline. a – b. Different concentrations of isoprenaline were added to the apical side of the 16HBE14o- epithelia grown on Transwell-COL membrane for 6 hrs. Both apical and basolateral samples were collected to quantify the secretion of IL-6 (a) and IL-8 (b) by ELISA. Each column represents the mean ± S.E. n=4. #p < 0.05 compared with control group; one-way ANOVA with Dunnett's post hoc test. *p < 0.05 compared between apical and basolateral secretion of cytokines, as calculated by the Student's t-test.

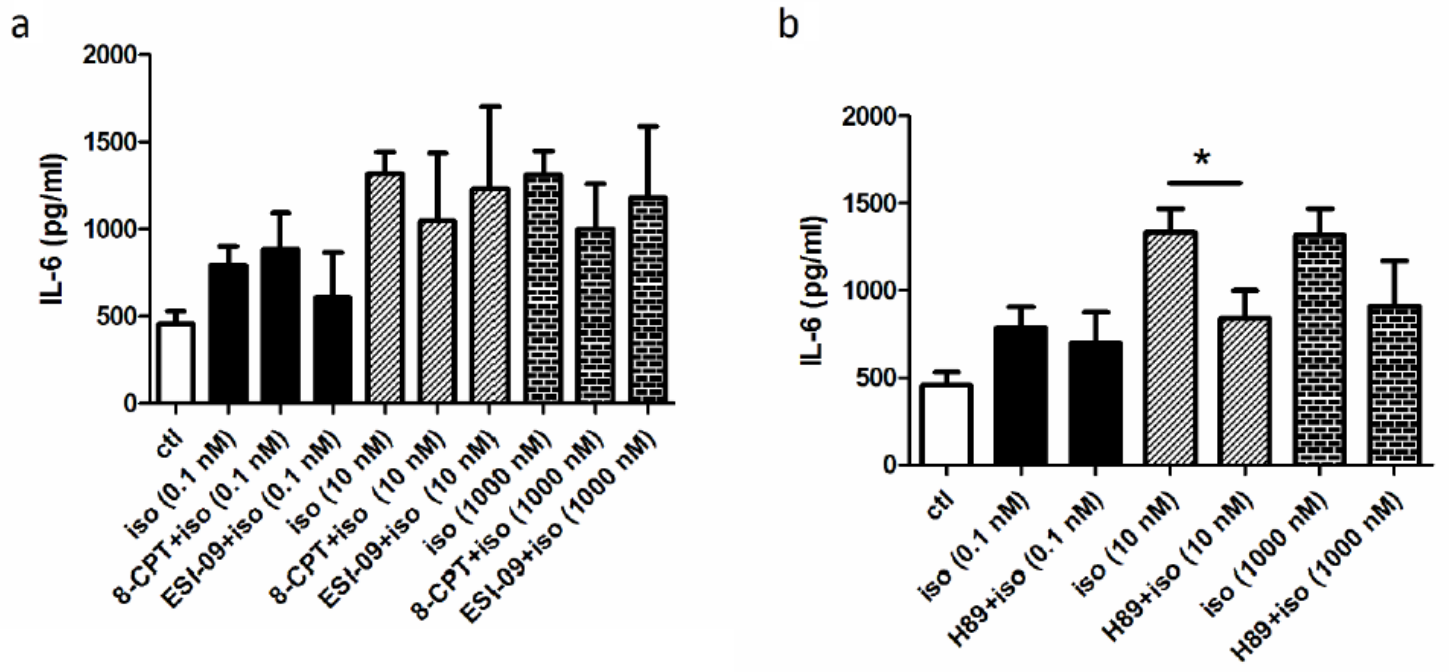

Figure 4

cAMP-dependent signaling pathways are involved in isoprenaline-mediated IL-6 release. a. Cells were pretreated with EPAC activator 8-CPT (5 μM) or EPAC inhibitor ESI-09 (5 μM) for 2 hrs before addition of isoprenaline (iso) for 6 hrs. IL- 6 was quantified by ELISA. b. The effect of PKA inhibitor H89 (10 μM) on isoprenaline-induced IL-6 was examined. Each column represents the mean ± S.E. n=3-5; *p < 0.05, as calculated by the Student's t-test.

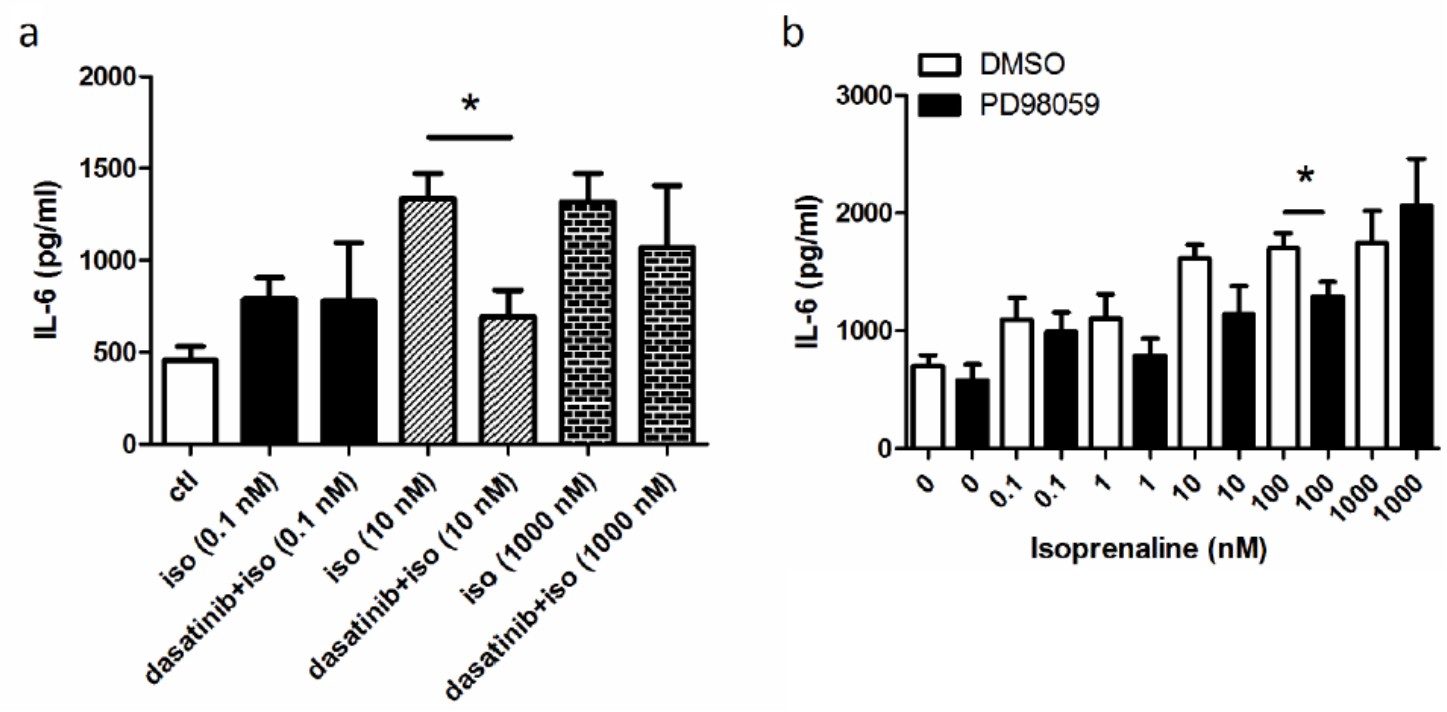

Figure 5

Src and ERK1/2 are involved in isoprenaline-induced IL-6 secretion. a – b. Cells were treated with dasatinib (10 μM) or PD98059 (10 μM) for 2 hrs before isoprenaline (iso) treatment at different concentrations for 6 hrs. The effect of dasatinib (a) and PD98059 (b) on IL-6 release was examined. Each column represents the mean ± S.E. n=4-6; *p < 0.05, as calculated by the Student's t-test.

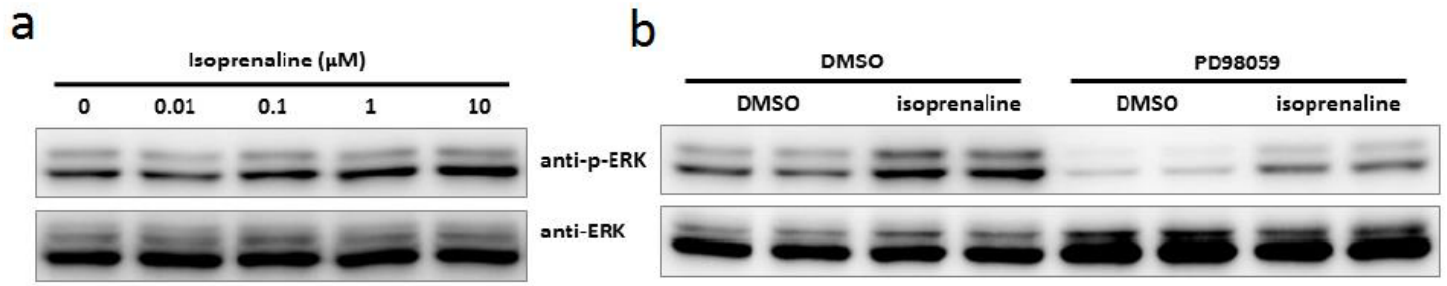

Figure 6

Effect of isoprenaline on ERK1/2 phosphorylation. a. 16HBE14o- cells were stimulated with different concentrations of isoprenaline for 5 min. b. Cells were treated with DMSO or PD98059 (10 μM) for 15 min

followed by isoprenaline (10 μM) stimulation for 5 min. Representative images of western blots are shown. n=3.

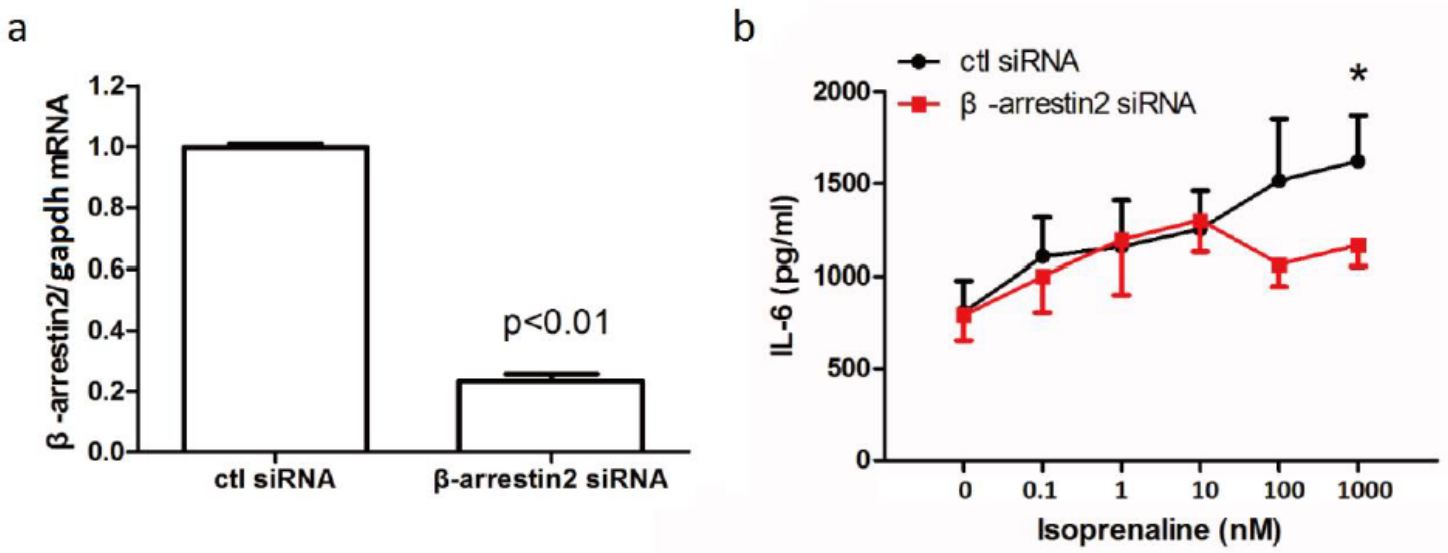

Figure 7

β-arrestin2 mediates isoprenaline-induced IL-6 release. a. The efficiency of β-arrestin2 knockdown (KD) was verified by real-time PCR (n=3). The expression of β-arrestin2 mRNA was normalized by the level of GAPDH mRNA. b. The effect of β-arrestin2 KD on isoprenaline-induced IL-6 release was examined. Each data point represents the mean ± S.E. n=5; *p < 0.05 compared with the same concentration of isoprenaline between the control (ctl siRNA) and KD groups (β-702 arrestin2 siRNA), as calculated by the Student's t-test.