Title: Long-acting $\beta_2$-agonists increase fluticasone propionate-induced mitogen-activated protein kinase phosphatase 1 (MKP-1) in airway smooth muscle cells

Dr. Melanie Manetsch MManetsch@ccia.unsw.edu.au ¹, Mr. Md. Mostafizur Rahman mrah7373@uni.sydney.edu.au ¹, Mr. Brijeshkumar Patell bpat5142@uni.sydney.edu.au ¹, Ms. Emma Ramsay emma.ramsay@student.unsw.edu.au ¹, Ms. Nowshin Rumzhum nrum3025@uni.sydney.edu.au ¹, Dr. Hatem Alkhouri hatem.alkhouri@sydney.edu.au ¹, Ms. Qi Ge qi.ge@sydney.edu.au ² and Prof. Alaina Ammit alaina.ammit@sydney.edu.au ¹. ¹ Respiratory Research Group, Faculty of Pharmacy, University of Sydney, Sydney, NSW, Australia, 2006 and ² Woolcock Institute of Medical Research, University of Sydney, Sydney, NSW, Australia.

Body: Mitogen-activated protein kinase phosphatase 1 (MKP-1) represses MAPK-driven signalling and plays an important anti-inflammatory role in asthma and airway remodelling. Although MKP-1 is corticosteroid-responsive and increased by cAMP-mediated signalling, the upregulation of this critical anti-inflammatory protein by long-acting $\beta_2$-agonists and clinically-used corticosteroids has been incompletely examined to date. To address this, we investigated MKP-1 gene expression and protein upregulation induced by two long-acting $\beta_2$-agonists (salmeterol and formoterol), alone or in combination with the corticosteroid fluticasone propionate (abbreviated as fluticasone) in primary human airway smooth muscle (ASM) cells in vitro. $\beta_2$-agonists increased MKP-1 protein in a rapid but transient manner, while fluticasone induced sustained upregulation. Together, $\beta_2$-agonists increased fluticasone-induced MKP-1 and modulated ASM synthetic function (measured by interleukin 6 (IL-6) and interleukin 8 (IL-8) secretion). As IL-6 expression (like MKP-1) is cAMP/adenylate cyclase-mediated, the long-acting $\beta_2$-agonist formoterol increased IL-6 mRNA expression and secretion. Nevertheless, when added in combination with fluticasone, $\beta_2$-agonists significantly repressed IL-6 secretion induced by tumour necrosis factor α (TNFα). Conversely, as IL-8 is not cAMP-responsive, $\beta_2$-agonists significantly inhibited TNFα-induced IL-8 in combination with fluticasone, where fluticasone alone was without repressive effect. In summary, long-acting $\beta_2$-agonists increase fluticasone-induced MKP-1 in ASM cells and repress synthetic function of this immunomodulatory airway cell type.