# ONLINE SUPPLEMENT

## MATERIALS AND METHODS

### Monocyte and neutrophil isolation

Peripheral blood was collected from volunteers and neutrophils and monocytes were isolated using a modified standard protocol. Briefly, 40mL of blood was mixed with 10mL acid citrate dextrose (ACD). Ten millilitres of phosphate buffered saline (PBS) (Gibco, Carlsbad, CA, USA) and 6mL of 10% dextran (MP Biomedicals, Santa Ana, CA, USA) were added and sedimentation was allowed to occur at room temperature for 20 minutes. The upper layer was removed, overlayed on Ficoll Paque-PLUS (GE Healthcare, Little Chalfont, UK) and centrifuged at 490x*g* for 10 minutes. The buffy coat containing monocytes was isolated and incubated with CD14 magnetic beads (Miltenyi Biotec, Bergisch, Germany) for 15 minutes at 4°C. The cell pellet containing neutrophils was resuspended in sterile water for 30 seconds to lyse remaining red blood cells before osmolarity was re-established with equal parts of 2x PBS. Cells were then incubated for 30 minutes at 4°C with CD16 magnetic beads (Miltenyi Biotec). Magnetically labelled cells ($CD14^+$ monocytes and $CD16^+$ neutrophils) were run separately through magnetic columns as per the manufacturer's instructions to positively select out each cell type. Typical purity for each cell population was 99% or greater assessed by microscopy.

### Confirmation of neutralising activity of anti-TGF-β1 antibodies

Primary airway smooth muscle (ASM) cells were seeded in 12 well plates at $6.4x10^{-4}$ cells per well in 5% FBS DMEM (Gibco) for 24 hours. Cells were then quiesced for 24 hours in 0.1% BSA (Sigma Aldrich) DMEM before stimulation with TGF-β1 (0.3ng/mL) (R&D Systems) in the presence or absence of anti-TGF-β1 antibodies (1.5µg/mL) (R&D Systems) for 24 hours at 37°C with 5% $CO_2$. After 24 hours, cell-free supernatant was collected and stored at -80°C for analysis.

**Detection of $PGE_2$**

$PGE_2$ was detected in supernatants from previous experiments where monocytes and neutrophils were treated with indomethacin ($10^{-6}$M). A $PGE_2$ ELISA kit (Cayman Chemical, Ann Arbor, MI, USA) was used as per the manufacturer's instructions. Detection limit was 7.8pg/mL.

**Quantitative PCR (qPCR)**

IL-6, CXCL8, IFN-α, IFN-β, IL-28, IL-29A, TNF-α and VEGF mRNA expression was detected using qPCR. Total cellular RNA was isolated using the NucleoSpin® RNA kit (Machery Nagel, Düren, Germany) according to the manufacturer's instructions. 80ng of total RNA was converted to cDNA using MMLV-RV reaction kits (Invitrogen, Carlsbad, CA, USA). qPCR was carried out on cDNA using the StepOneTM Real-Time PCR system (Applied Biosystems, Carlsbad, CA, USA) using Taqman Gene Expression Assays (Applied Biosystems). Assays were carried out in triplicate using a multiplexed reaction mixture containing the primer and probes for the target genes (IL-6, CXCL8, IFN-α, IFN-β, IL-28, IL-29A, TNF-α, VEGF) and for ubiquitously expressed ribosomal RNA (18S rRNA) as a housekeeping gene. Relative expression was normalised to 18S rRNA expression and quantification performed using the 2ΔΔCT method.

**SUPPLEMENTARY FIGURES**

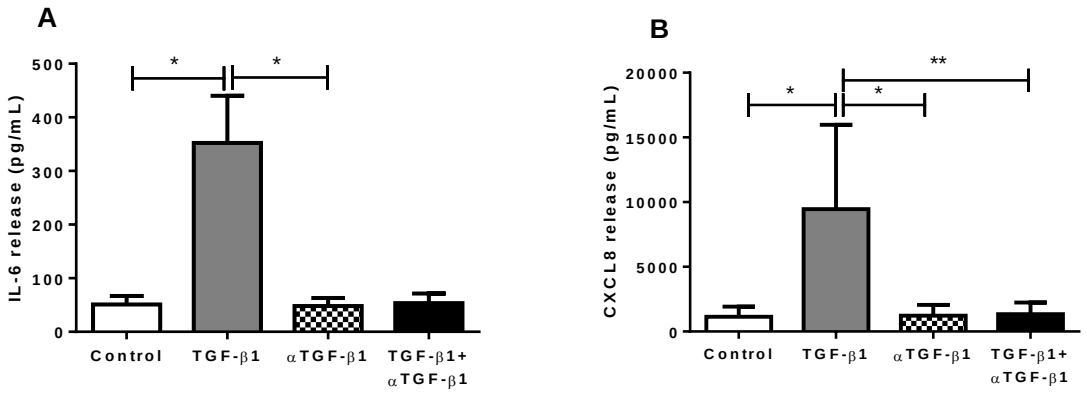


**Figure S1. Anti-TGF-β1 antibodies neutralise recombinant TGF-β1 to prevent cytokine release from primary airway smooth muscle cells.** (A) IL-6 and (B) CXCL8 release from TGF-β1 (0.3ng/mL) stimulated primary airway smooth muscle cells in the presence or absence of neutralising anti-TGF-β1 antibodies (1.5µg/mL) after 24h (n=6). Data are presented as mean±SEM. *p<0.05, **p<0.01.

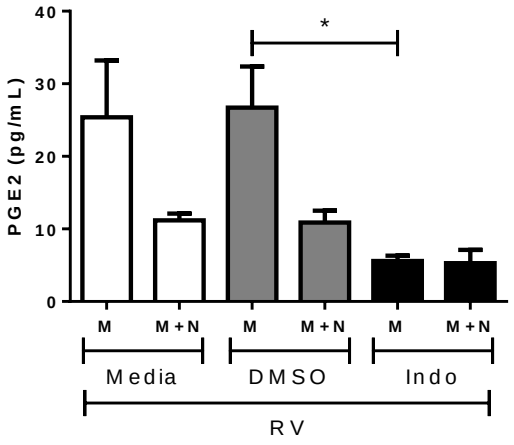


**Figure S2. Indomethacin prevents the generation of $PGE_2$ from monocytes stimulated with RV16.** $PGE_2$ release from monocytes and monocytes co-cultured with neutrophils in media alone, DMSO vehicle control and indomethacin ($10^{-6}$M) and stimulated with RV16 (MOI1) after 24h (n=4). Data are presented as mean±SEM. *p<0.05.

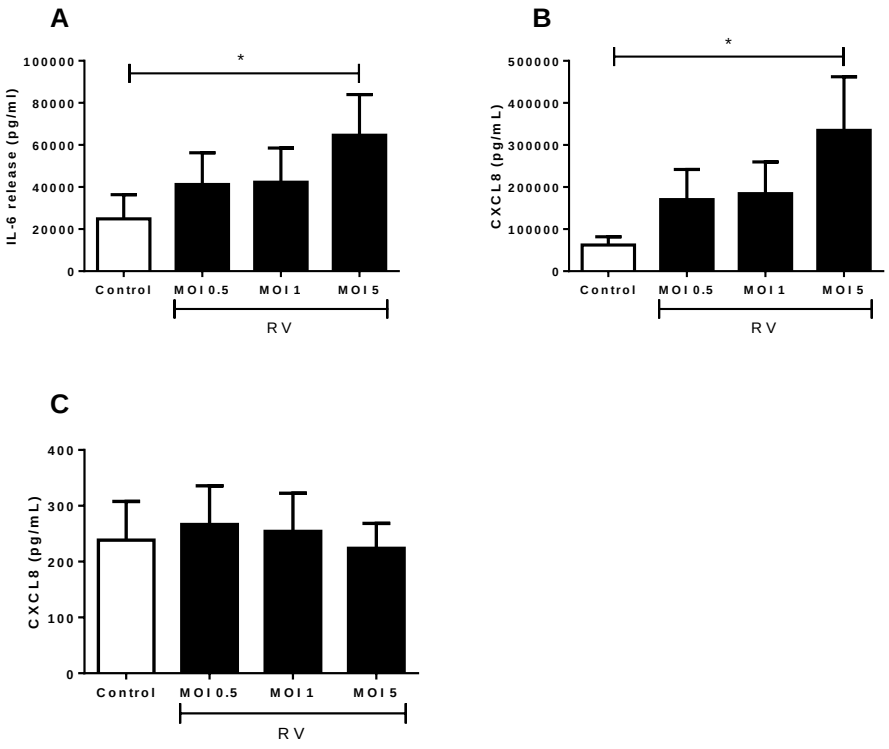


**Figure S3. Concentration dependant effects of RV16 on monocytes and neutrophils.** (A) IL-6 and (B&C) CXCL8 release from monocytes (A&B) and neutrophils (C) stimulated with RV16 (MOI0.5-5) after 24h (n=3-4). Data are presented as mean±SEM. *$p<0.05$.