



## Early View

Original Research Article

# Beneficial effects of the AT<sub>2</sub> receptor agonist buloxibutid (C21) against acute alveolar epithelial cell inflammation during anti-viral responses

Franco Conforti, Joseph Bell, Robert Ridley, Lareb Dean, Matthew Loxham, James Parkin, Aiman Alzetani, Mark G Jones, Carl-Johan Dalsgaard, Johan Raud, Donna E Davies

Please cite this article as: Conforti F, Bell J, Ridley R, *et al.* Beneficial effects of the AT<sub>2</sub> receptor agonist buloxibutid (C21) against acute alveolar epithelial cell inflammation during anti-viral responses. *ERJ Open Res* 2026; in press (<https://doi.org/10.1183/23120541.00249-2025>).

This manuscript has recently been accepted for publication in the *ERJ Open Research*. It is published here in its accepted form prior to copyediting and typesetting by our production team. After these production processes are complete and the authors have approved the resulting proofs, the article will move to the latest issue of the ERJOR online.

Copyright ©The authors 2026. This version is distributed under the terms of the Creative Commons Attribution Non-Commercial Licence 4.0. For commercial reproduction rights and permissions contact [permissions@ersnet.org](mailto:permissions@ersnet.org)

# Beneficial effects of the AT<sub>2</sub> receptor agonist buloxibutid (C21) against acute alveolar epithelial cell inflammation during anti-viral responses

*Authors: Franco Conforti<sup>1,2</sup>, Joseph Bell<sup>1,2</sup>, Robert Ridley<sup>1,2</sup>, Lareb Dean<sup>1,2</sup>, Matthew Loxham<sup>1,2</sup>, James Parkin<sup>1,2</sup>, Aiman Alzetani<sup>2,3</sup>, Mark G Jones<sup>1,2</sup>, Carl-Johan Dalsgaard<sup>4</sup>, Johan Raud<sup>4</sup> and Donna E Davies<sup>1,2</sup>.*

## Affiliations:

<sup>1</sup>Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, Southampton SO16 6YD, UK.

<sup>2</sup>NIHR Southampton Biomedical Research Centre, University Hospital Southampton, Southampton SO16 6YD, UK;

<sup>3</sup>University Hospital Southampton, Southampton SO16 6YD, UK;

<sup>4</sup>Vicore Pharma AB, Stockholm, Sweden.

Correspondence: Franco Conforti, Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, Southampton SO16 6YD, UK. E-mail: fc3g11@soton.ac.uk.

## Take-home message:

- The potent AT<sub>2</sub> receptor agonist, buloxibutid, reduces the proinflammatory pathogenetic response in a model of viral infection in primary human ATII cells.
- Crucially, buloxibutid treatment does not alter the protective alveolar antiviral responses.

Conflict of interest: JR is an employee of Vicore Pharma AB and holds shares and share options in the company. C-JD is a former employee of Vicore Pharma AB and holds shares and share options in the company. FC, JB, RR, LD, ML, JP, AA, MGJ and DD have nothing to declare.

## ABSTRACT

*Aim:* Activation of the angiotensin II type 2 receptor (AT<sub>2</sub> receptor; encoded by *AGTR2*) has been shown to be beneficial during tissue injury and repair. Therefore, we aimed to investigate the expression of AT<sub>2</sub> receptor in human alveolar type II (ATII) cells, a cell population responsible of lung repair and regeneration and the effect of the AT<sub>2</sub> receptor agonist buloxibutid (also known as C21), in an *in vitro* model of viral infection using primary ATII cells.

*Methods:* We described the expression of AT<sub>2</sub> receptor mRNA using publicly available lung single-cell RNA sequencing datasets. We evaluated the effects of buloxibutid on ATII cell biology at baseline and in response to treatment with double stranded RNA (polyinosinic:polycytidylic acid, a pathogen associated molecular pattern) using MTS cytotoxicity assay, transcriptomic analysis and ELISA.

*Results:* We found that buloxibutid was well tolerated by ATII cells under all conditions tested. RNA sequencing demonstrated that ATII cells responded to polyinosinic:polycytidylic acid with induction of a characteristic antiviral innate immune response. Gene set enrichment analysis revealed that buloxibutid caused a significant suppression of polyinosinic:polycytidylic acid-induced pro-inflammatory

responses whereas it was without effect on the expression of antiviral genes.

*Conclusions:* Our findings suggest that buloxibutid may have therapeutic potential for treatment of respiratory viral pneumonias by limiting excessive pro-inflammatory responses that have the potential to lead to a cytokine storm, while maintaining a protective antiviral response.

## INTRODUCTION

The renin-angiotensin system (RAS) is an important hormonal system that plays a crucial role not only in regulating blood pressure and fluid balance in the body but also in the pathophysiology of several diseases affecting the kidney, neuronal system, cardiocirculatory system and lung. The Angiotensin II (Ang-II) hormone is the principal mediator of the RAS acting via the angiotensin II type 1 receptor (AT<sub>1</sub> receptor), which is abundantly expressed in different tissues, and the angiotensin II type 2 receptor (AT<sub>2</sub> receptor), the expression of which has been shown to increase during tissue injury. Pharmacological activation of the AT<sub>2</sub> receptor has shown significant therapeutic benefit, as well as an ability to modulate immune and stem cell responses in order to promote tissue repair and regeneration (Fatima, Patel and Hussain 2021).

Circulating angiotensin I (Ang-I) is cleaved to generate angiotensin II (Ang-II) through the activity of angiotensin converting enzyme (ACE). Ang-II acts on AT<sub>1</sub> receptor or AT<sub>2</sub> receptor with opposing effects: AT<sub>1</sub> receptor promotes inflammation, fibrosis, alveolar-endothelial damage and increases microvascular permeability, while AT<sub>2</sub> receptor reduces inflammation, promotes alveolar-endothelial survival together with barrier integrity, and can reduce oedema and lung fibrosis (Wang et al. 2019). ACE is abundantly expressed in the

pulmonary capillary network and so, in some pathological conditions, Ang-II can cause excessive activation of AT<sub>1</sub> receptor to cause increased alveolar-capillary permeability, alveolar oedema, hypoxemia, sepsis and possibly death (Wang et al. 2019, Patel et al. 2020).

Since the availability of the non-peptide AT<sub>2</sub> receptor agonist buloxibutid, the anti-inflammatory effect of AT<sub>2</sub> receptor activation has been validated in different models of tissue injury and disease including brain injury, atherosclerosis, arthritis and kidney injury (Gao et al. 2022, Jabber, Mohammed and Hadi 2023, Sampson et al. 2016, Ismael and Ishrat 2022, Steckelings et al. 2022). In murine models of acute and chronic cigarette smoke-induced chronic obstructive pulmonary disease, activation of the AT<sub>2</sub> receptor by buloxibutid has been reported to exhibit anti-inflammatory effects resulting in the restoration of the lung function (Mei et al. 2020). However, little is known about the effects of buloxibutid in injury/repair of the human lung when homeostasis is perturbed by a viral infection. In cases of viral pneumonia, alveolar epithelial cells and vascular endothelial cells are damaged leading to the abnormal accumulation of fluid in the lung alveoli. This is accompanied by release of inflammatory cytokines from both immune and non-immune cells. If the inflammatory response is excessive, acute lung injury (ALI) or even acute respiratory distress syndrome (ARDS) can develop (Gao et al. 2020). The recent COVID-19 pandemic has

increased awareness of the potential deadly consequences of severe viral pneumonia caused by viruses like SARS-CoV-2, respiratory syncytial virus (RSV) as well as influenza and has highlighted the need for better control of virus-induced inflammation.

Considering the critical role of RAS homeostasis in lung injury and repair, we postulated that the AT<sub>2</sub> receptor agonist buloxibutid would control inflammatory responses in primary human ATII cells exposed to polyinosinic:polycytidylic acid (poly I:C), a synthetic double-stranded RNA which was used to mimic a viral infection.

## **RESULTS**

**Buloxibutid treatment did not show any significant cytotoxicity on primary human ATII cells.**

Buloxibutid is a potent agonist of AT<sub>2</sub> receptor and its activation has been reported to be beneficial in different lung conditions where ATII cells play a vital role in lung homeostasis (Mei et al. 2020, Menk et al. 2018, Sumners et al. 2019, Steckelings and Sumners 2020). Data mining of online published single cell-sequencing datasets (Habermann et al. 2020, Sikkema et al. 2023) of normal and pathological lung tissue identified that AT<sub>2</sub> receptor is mainly expressed in ATII cells (Fig.1A and Supp.

Fig.1) thus identifying these cells as a likely therapeutic target. In our dose response experiments, exposure of primary human ATII cells to buloxibutid in absence or presence of poly I:C did not affect cell morphology or cell density (Supp. Fig.2). Similarly, using an MTS cytotoxicity assay, we found no significant harmful effects of buloxibutid on primary human ATII cells in presence or absence of poly I:C (Fig.1B). Moreover, buloxibutid treatment did not affect AT<sub>2</sub> receptor gene (*AGTR2*) expression in ATII cells (Fig.1C). Our findings strongly suggest that buloxibutid is well tolerated by ATII cells and does not negatively regulate AT<sub>2</sub> receptor gene expression.

### **Buloxibutid treatment inhibits inflammatory cytokine release in response to poly I:C without modulating antiviral genes.**

Polyinosinic:polycytidylic acid (poly I:C) is a synthetic analogue of double-stranded (ds)RNA that mimics viral infection by activating the RNA viral sensors, Toll-like receptor 3 (TLR3) and RNA helicases (Harris et al. 2013). Therefore, for initial experiments, we performed a dose-response study by treating ATII cells with different concentrations of buloxibutid in absence or presence of poly I:C. Consistent with the anti-inflammatory role of AT<sub>2</sub> receptor, we found that a small, non-significant suppressive effect of Buloxibutid on polyI:C-induced IL-6 gene

expression ( $p=0.99, 0.73, 0.27$  for 1000 nM buloxibutid vs control, 10nM, and 100 nM buloxibutid, respectively) was accompanied by a significant decrease in IL-6 protein release (Fig.2A). In contrast, the ability of double stranded RNA to induce *MX1* and *RSAD2*, interferon-inducible genes that inhibit the replication of a variety of viruses including HIV and influenza (Schoggins and Rice 2011), was not affected by buloxibutid treatment (Fig.2B). Our findings suggest a distinct anti-inflammatory role for buloxibutid during viral infection while maintaining antiviral gene expression.

**RNA-sequencing show a significant effect of buloxibutid treatment on inflammatory pathways without affecting antiviral responses.**

In order to further evaluate the anti-inflammatory role of buloxibutid in the context of a viral infection, we performed RNA-sequencing on A7II cells treated without and with buloxibutid in presence or absence of poly I:C. Bioinformatic analysis showed that gene expression profiles were largely dependent on presence or absence of poly I:C (Fig.3A), where the top differentially expressed genes were identified as being involved in cellular response to virus (Fig.3B).

Virus infection activates specific proinflammatory pathways that include the interferon system, JAK-STAT and NF- $\kappa$ B which in turn promote the expression of cytokines, chemokines and antiviral factors in order to preserve tissue homeostasis and block viral spreading (Nan, Wu and Zhang 2017, Pfeffer 2011). In order to understand how changes in the expression of individual genes might reflect broader changes within the cell, we performed gene set enrichment analysis, which highlights pathways enriched in differentially expressed genes in our RNA-seq results. This showed that, in the presence of polyI:C, buloxibutid significantly downregulated a range of inflammation-associated pathways (Fig.4A). Further interrogation of these pathways individually showed that the effect of buloxibutid was seen across multiple genes within each pathway, and cells were clearly defined by the presence or absence of buloxibutid (Fig.4B and Supp. Table2). Of note, some of the pro-inflammatory factors and chemokines down-regulated by buloxibutid (Fig.5A and Supp. Fig.3) are those that have been implicated as mediators of the cytokine storm, a hyperinflammatory state that is associated with severe uncontrolled respiratory viral infection in some individuals (Murdaca et al. 2021, Kirsch-Volders and Fenech 2021). In contrast key antiviral transcripts were not affected by buloxibutid treatment (Fig.5B). These findings demonstrate the potential of buloxibutid to act exclusively as an anti-inflammatory agent with

no effect on the antiviral cell response during a respiratory viral infection.

## **Discussion and conclusions**

Our work provides evidence of an anti-inflammatory effect of the AT<sub>2</sub> receptor agonist buloxibutid in our model of viral infection in primary human ATII cells. Buloxibutid was able to significantly attenuate inflammation pathways including markers of acute inflammation that are typical of the deleterious cytokine storm. Most importantly, the advantageous anti-inflammatory effect of buloxibutid did not alter the crucial alveolar antiviral responses that are required to control viral replication and spread and so limit further progression of virus-induced lung injury.

The global impact in human history of respiratory diseases like Spanish flu, SARS, MERS, influenza A H1N1 2009 and COVID-19 highlight the crucial role of proinflammatory pathogenetic mechanisms in the respiratory system known as “cytokine storm”. During a respiratory viral infection, a normal immune response to tackle virus spreading can progress in an uncontrolled immune response characterised by an hyperinflammatory status typical of the so called cytokine storm (Murdaca et al. 2021, Kirsch-Volders and Fenech 2021). As respiratory viruses develop various strategies to evade the anti-

viral immune response the rapidly growing infection triggers the uncontrolled production of typical mediators of the cytokine storm including cytokines such as IL-6, IL-8, IL-1 $\beta$  and GM-CSF, and chemokines such as CCL2, CCL-5, IP-10 and CCL3. This is due an exaggerated activation and expansion of immune cells leading to hypersecretion of deleterious amounts of proinflammatory cytokines resulting in acute lung damage, systemic inflammation, multiorgan dysfunction and possibly death (Murdaca et al. 2021, Kirsch-Volders and Fenech 2021). During the COVID19 pandemic, many people died due to the cytokine storm induced by SARS-CoV2 due to the limited availability of effective anti-inflammatory therapy for COVID-19. Corticosteroids such as dexamethasone were widely used after platform trials demonstrated a significant decrease in mortality among patients who were receiving either invasive mechanical ventilation or oxygen alone at randomization but not among those receiving no respiratory support (Group et al. 2021). While corticosteroids have a potent anti-inflammatory effect, they also suppress the antiviral response (Bahsoun et al. 2023). In fact recent studies have indicated increased mortality and delayed viral clearance when either high dose corticosteroids or early initiation of treatment was administered (Cron 2022, Bahsoun et al. 2023). In contrast to corticosteroids, buloxibutid is able to reduce inflammation while retaining the protective antiviral response. Moreover, buloxibutid has shown efficacy in COVID-

19 caused by the original Wild-Type SARS-CoV-2 variant that replicates primarily in ATII cells (Tornling et al. 2021, Szekely et al. 2021). In this study, treatment of hospitalised patients with moderately severe COVID-19 with buloxibutid for 7 days on top of standard of care (including glucocorticoids and remdesivir in the majority of the subjects) reduced the requirement for oxygen supplementation.

In view of the critical role of proinflammatory pathogenetic mechanisms involved in severe lung viral infections, there is a strong need to identify the most suitable immunomodulatory strategy that does not blunt the antiviral response. Our results suggest that buloxibutid could be a potential approach to dampen the pathogenetic cytokine storm while leaving the antiviral mechanism unaltered in order to suppress residual virus spread and limit further tissue damage. Most importantly, by acting upstream of the cytokine storm network, buloxibutid could help to prevent damage to alveolar epithelium and of the microvasculature, modulating the exaggerated infiltration of inflammatory cells and promoting epithelial repair (Fig.6). While this study provides important insights into the cellular responses of alveolar epithelial cells during viral mimic stimulation, certain limitations should be acknowledged. First, all experiments were conducted in vitro, which, while allowing for controlled mechanistic investigations, may not fully capture the complex interactions present in the in vivo lung environment, including cell

crosstalk, vascular influences, and effects of the tissue microenvironment. Second, although the use of primary cells is a strength of this study, the relatively small number of experimental replicates (n) and donors is a limitation. Third, although ATII cells are a key component of the distal lung and play a central role in respiratory viral infections, we did not assess responses in airway epithelial cells, which represent the initial site of viral entry and are crucial in early response to viral infection. Lastly, we used polyI:C as a surrogate for viral infection. While polyI:C is a well-established tool to study innate immune activation, it does not fully replicate the complexities of live viral infection, including viral replication and shedding. Future studies incorporating live virus models would be valuable to confirm the relevance of our findings.

## **METHODS**

### **Primary alveolar type II (ATII) isolation**

Human lung experiments were approved by the Southampton and South-West Hampshire and the Mid and South Buckinghamshire Local Research Ethics Committees, and all subjects gave written informed consent.

Primary ATII culture were established from macroscopically normal regions of surgically resected lung parenchyma tissue in

accord with the method described by Witherden et al. ,2001(Witherden and Tetley 2001). Briefly, the lung tissue was perfused with 0.9% sodium chloride solution and infused with 0.25% Trypsin solution (Sigma-Aldrich, Poole, UK) at 37 °C for 45 min. After trypsin digestion, the tissues were finely cut in the presence of newborn calf serum (NCS) (Life Technologies Limited, Paisley, UK) and DNase 250 µg/ml (Sigma-Aldrich, Poole, UK), then cells were filtered by sequential passage through a 400-µm metal mesh and 40- µm nylon filter. The cells were re-suspended in DCCM-1 medium (Biological Industries Ltd, Kibbutz Beit-Haemek, Israel) supplemented with 1% penicillin, 1% streptomycin, and 1% L-glutamine, and incubated at 37 °C in a humidified incubator for 2 h in tissue culture flasks to allow differential adherence and removal of contaminating cells. The ATII cells were re-suspended in fresh DCCM-1 supplemented with 10% NCS, 1% penicillin, 1% streptomycin and 1% L-glutamine (all supplements from Life Technologies Limited, Paisley, UK) and plated on collagen 1 (PureCol 5005-b, Advanced BioMatrix Inc, California, USA) coated 96-well plates at 60% density; after 48h purity was tested by staining for alkaline phosphatase.

### **Cell treatment and cytotoxicity assay**

Buloxibutid was dissolved in dimethylsulphoxide (DMSO) (Sigma-Aldrich, Gillingham, UK) at a final concentration of 0.1% and 0.1% DMSO was used as vehicle CTRL. Human primary ATII cells at passage 1 were cultured at a cell density of 70-80% on collagen 1 (PureCol 5005-b, Advanced BioMatrix Inc, California, USA) coated 96well plate and treated for 24h without or with buloxibutid in presence or absence of poly I:C HMW (InvivoGen Europe, Toulouse, France). After 24h treatment, any cytotoxic effect has been evaluated using an MTS assay *CellTiter 96® AQueous One Solution Cell Proliferation Assay* from Promega UK Ltd. in accord with the manufacture instruction. A vehicle control (DMSO) was run in each experiment.

### **Reverse transcription and quantitative PCR (RTqPCR)**

Human primary ATII cells were cultured at a cell density of 70-80% on collagen 1 coated 96 well plate and treated for 24h without or with buloxibutid in presence or absence of poly I:C (InvivoGen Europe, Toulouse, France). After the 24h treatment, RNA was isolated using Monarch® Total RNA Miniprep Kit (New England Biolabs Limited, Herts, UK). RNA was reversed to cDNA using precision nanoScript2 Reverse Transcription Kit from Primer Design, Southampton, UK or High-Capacity cDNA Reverse Transcription Kit from Life Technologies Limited, Paisley, UK.

Probe-based qPCR primers sets were obtained from Primer Design, Southampton, UK (*MX1 catalog no. DD-hu-600-MX1; RSAD2 catalog no. DD-hu-600-RSAD2; UBC/GAPDH catalog no. HK-PP-hu-d-UBC/GAPDH*) and SYBR green qPCR primers sets were obtained from Life Technologies Limited, Paisley, UK (*IL6: FW-CTGGCAACAATGAGTCTACCTT and RV-GCCACAGCGAGGTTGAAGAT; AGTR2: FW-ACTCACCTCTTCAGAACGAATTG and RV-CCATCTTTGGAAGGTTTCAGGTTG*). Changes in mRNA expression using the following primers were analysed by qPCR and normalized to the housekeeping genes ubiquitin C and glyceraldehyde-3-phosphate dehydrogenase (*UBC and GAPDH*). Data were analysed using the  $\Delta\Delta CT$  method.

### **ELISA and multiplex electrochemiluminescence immunoassay analysis**

Human Interleukin 6 (IL6) and Monocyte chemoattractant protein-1 (MCP1) were evaluated in culture media using a DuoSet ELISA (R&D, Abingdon, UK) in accordance with manufacturer's instructions. Human Interleukin 15 (IL15), Human Interleukin 1-beta (IL1 $\beta$ ), Macrophage Inflammatory Protein 1 Alpha (MIP1a) and tumor necrosis factor alpha (TNF $\alpha$ ) were evaluated in culture media using a multiplex electrochemiluminescence immunoassay (U-PLEX MSD, Rockville, Maryland, USA). Each sample was evaluated in

duplicates and the mean value used for subsequent analyses.  $p < 0.05$  was accepted as statistically significant. \*  $p < 0.05$ .

### **RNA sequencing and Bioinformatic Analyses**

Cells were harvested 24h after treatment and RNA was isolated using Monarch<sup>®</sup> Total RNA Miniprep Kit (New England Biolabs). RNA sequencing was performed by Novogene (UK) using an Illumina Novaseq 6000 sequencer after passing the quality control checks. RNA samples were enriched for mRNA using polyA enrichment using poly-T-oligo magnetic beads. After fragmentation, cDNA was synthesised using random hexamer primers. ~150bp paired end read sequencing was performed on an Illumina Novaseq 6000 sequencer. Kallisto running in paired-end mode was used to pseudoalign raw fastq data with human reference transcriptome hg38 (from human reference genome GRCh38), using RefSeq's transcripts to generate raw counts data. Raw counts were imported into R using the tximport package. Differential expression analysis was performed using edgeR, with Benjamini-Hochberg multiple test correction. Normalised counts per million reads (CPM) were calculated using EdgeR. Gene set enrichment analysis was performed using NCBI's GSEA software (<https://www.gsea-msigdb.org/gsea/index.jsp>), run in preranked mode, with genes ranked by  $-\log_{10}$  (adjusted P-value). Heatmaps were produced using pheatmap. Graphs were produced using ggplot2.

## **Statistics**

All the experiments were performed using 4 different donors (3 male and 1 female) . 'n' indicates the number of repeated experiments with different donors. ELISA and qPCR results are expressed as means of  $\pm$  SD. Differences between groups were assessed using Kolmogorov-Smirnov test, Mann-Whitney test, oneway-ANOVA or 2ways-ANOVA test with Dunnett's multiple comparisons test. All data were analysed using Prism (GraphPad, CA, USA).  $p < 0.05$  was accepted as statistically significant. \*  $p < 0.05$  and  $p\text{-value} < 0.01$  \*\*.

## **References**

- Bahsoun, A., Y. Fakhri, R. Zareef, F. Bitar & M. Arabi (2023) Corticosteroids in COVID-19: pros and cons. *Front Med (Lausanne)*, 10, 1202504.
- Cron, R. Q. (2022) No perfect therapy for the imperfect COVID-19 cytokine storm. *Lancet Rheumatol*, 4, e308-e310.
- Fatima, N., S. N. Patel & T. Hussain (2021) Angiotensin II Type 2 Receptor: A Target for Protection Against Hypertension, Metabolic Dysfunction, and Organ Remodeling. *Hypertension*, 77, 1845-1856.
- Gao, W., L. Shen, D. D. Long, T. T. Pan, D. Wang, X. Q. Chai & S. S. Hu (2022) Angiotensin II type 2 receptor pharmacological agonist, C21, reduces the inflammation and pain hypersensitivity in mice with joint inflammatory pain. *Int Immunopharmacol*, 110, 108921.
- Gao, Y. L., Y. Du, C. Zhang, C. Cheng, H. Y. Yang, Y. F. Jin, G. C. Duan & S. Y. Chen (2020) Role of Renin-Angiotensin System in Acute Lung Injury Caused by Viral Infection. *Infect Drug Resist*, 13, 3715-3725.
- Group, R. C., P. Horby, W. S. Lim, J. R. Emberson, M. Mafham, J. L. Bell, L. Linsell, N. Staplin, C. Brightling, A. Ustianowski, E. Elmahi, B. Prudon, C. Green, T. Felton, D. Chadwick, K.

- Rege, C. Fegan, L. C. Chappell, S. N. Faust, T. Jaki, K. Jeffery, A. Montgomery, K. Rowan, E. Juszczak, J. K. Baillie, R. Haynes & M. J. Landray (2021) Dexamethasone in Hospitalized Patients with Covid-19. *N Engl J Med*, 384, 693-704.
- Habermann, A. C., A. J. Gutierrez, L. T. Bui, S. L. Yahn, N. I. Winters, C. L. Calvi, L. Peter, M. I. Chung, C. J. Taylor, C. Jetter, L. Raju, J. Roberson, G. Ding, L. Wood, J. M. S. Sucre, B. W. Richmond, A. P. Serezani, W. J. McDonnell, S. B. Mallal, M. J. Bacchetta, J. E. Loyd, C. M. Shaver, L. B. Ware, R. Bremner, R. Walia, T. S. Blackwell, N. E. Banovich & J. A. Kropski (2020) Single-cell RNA sequencing reveals profibrotic roles of distinct epithelial and mesenchymal lineages in pulmonary fibrosis. *Sci Adv*, 6, eaba1972.
- Harris, P., S. Sridhar, R. Peng, J. E. Phillips, R. G. Cohn, L. Burns, J. Woods, M. Ramanujam, M. Loubeau, G. Tyagi, J. Allard, M. Burczynski, P. Ravindran, D. Cheng, H. Bitter, J. S. Fine, C. M. Bauer & C. S. Stevenson (2013) Double-stranded RNA induces molecular and inflammatory signatures that are directly relevant to COPD. *Mucosal Immunol*, 6, 474-84.
- Ismael, S. & T. Ishrat (2022) Compound 21, a Direct AT2R Agonist, Induces IL-10 and Inhibits Inflammation in Mice Following Traumatic Brain Injury. *Neuromolecular Med*, 24, 274-278.
- Jabber, H., B. Mohammed & N. R. Hadi (2023) Investigating the renoprotective effect of C21 in male mice with sepsis via modulation of p-AKT/PI3K expression. *J Med Life*, 16, 203-209.
- Kirsch-Volders, M. & M. Fenech (2021) Inflammatory cytokine storms severity may be fueled by interactions of micronuclei and RNA viruses such as COVID-19 virus SARS-CoV-2. A hypothesis. *Mutation Research-Reviews in Mutation Research*, 788.
- Mei, D., W. S. D. Tan, W. Liao, C. K. M. Heng & W. S. F. Wong (2020) Activation of angiotensin II type-2 receptor protects against cigarette smoke-induced COPD. *Pharmacol Res*, 161, 105223.
- Menk, M., J. A. Graw, C. von Haefen, H. Steinkraus, B. Lachmann, C. D. Spies & D. Schwaiberger (2018) Angiotensin II type 2 receptor agonist Compound 21 attenuates pulmonary inflammation in a model of acute lung injury. *J Inflamm Res*, 11, 169-178.
- Murdaca, G., F. Paladin, A. Tonacci, S. Isola, A. Allegra & S. Gangemi (2021) The Potential Role of Cytokine Storm Pathway in the Clinical Course of Viral Respiratory Pandemic. *Biomedicines*, 9.

- Nan, Y., C. Wu & Y. J. Zhang (2017) Interplay between Janus Kinase/Signal Transducer and Activator of Transcription Signaling Activated by Type I Interferons and Viral Antagonism. *Front Immunol*, 8, 1758.
- Patel, S. N., N. Fatima, R. Ali & T. Hussain (2020) Emerging Role of Angiotensin AT2 Receptor in Anti-Inflammation: An Update. *Curr Pharm Des*, 26, 492-500.
- Pfeffer, L. M. (2011) The role of nuclear factor  $\kappa$ B in the interferon response. *J Interferon Cytokine Res*, 31, 553-9.
- Sampson, A. K., J. C. Irvine, W. A. Shihata, D. Dragoljevic, N. Lumsden, O. Huet, T. Barnes, T. Unger, U. M. Steckelings, G. L. Jennings, R. E. Widdop & J. P. Chin-Dusting (2016) Compound 21, a selective agonist of angiotensin AT2 receptors, prevents endothelial inflammation and leukocyte adhesion in vitro and in vivo. *Br J Pharmacol*, 173, 729-40.
- Schoggins, J. W. & C. M. Rice (2011) Interferon-stimulated genes and their antiviral effector functions. *Curr Opin Virol*, 1, 519-25.
- Sikkema, L., C. Ramirez-Suastegui, D. C. Strobl, T. E. Gillett, L. Zappia, E. Madisson, N. S. Markov, L. E. Zaragosi, Y. Ji, M. Ansari, M. J. Arguel, L. Apperloo, M. Banchemo, C. Becavin, M. Berg, E. Chichelnitskiy, M. I. Chung, A. Collin, A. C. A. Gay, J. Goteschniering, B. Hooshiar Kashani, K. Inecik, M. Jain, T. S. Kapellos, T. M. Kole, S. Leroy, C. H. Mayr, A. J. Oliver, M. von Papen, L. Peter, C. J. Taylor, T. Walzthoeni, C. Xu, L. T. Bui, C. De Donno, L. Dony, A. Faiz, M. Guo, A. J. Gutierrez, L. Heumos, N. Huang, I. L. Ibarra, N. D. Jackson, P. Kadur Lakshminarasimha Murthy, M. Lotfollahi, T. Tabib, C. Talavera-Lopez, K. J. Travaglini, A. Wilbrey-Clark, K. B. Worlock, M. Yoshida, C. Lung Biological Network, M. van den Berge, Y. Bosse, T. J. Desai, O. Eickelberg, N. Kaminski, M. A. Krasnow, R. Lafyatis, M. Z. Nikolic, J. E. Powell, J. Rajagopal, M. Rojas, O. Rozenblatt-Rosen, M. A. Seibold, D. Sheppard, D. P. Shepherd, D. D. Sin, W. Timens, A. M. Tskov, J. Whitsett, Y. Xu, N. E. Banovich, P. Barbry, T. E. Duong, C. S. Falk, K. B. Meyer, J. A. Kropski, D. Pe'er, H. B. Schiller, P. R. Tata, J. L. Schultze, S. A. Teichmann, A. V. Misharin, M. C. Nawijn, M. D. Luecken & F. J. Theis (2023) An integrated cell atlas of the lung in health and disease. *Nat Med*, 29, 1563-1577.
- Steckelings, U. M. & C. Sumners (2020) Correcting the imbalanced protective RAS in COVID-19 with angiotensin AT2-receptor agonists. *Clin Sci (Lond)*, 134, 2987-3006.

- Steckelings, U. M., R. E. Widdop, E. D. Sturrock, L. Lubbe, T. Hussain, E. Kaschina, T. Unger, A. Hallberg, R. M. Carey & C. Sumners (2022) The Angiotensin AT<sub>2</sub> Receptor: From a Binding Site to a Novel Therapeutic Target. *Pharmacol Rev*, 74, 1051-1135.
- Sumners, C., A. A. Peluso, A. H. Haugaard, J. B. Bertelsen & U. M. Steckelings (2019) Anti-fibrotic mechanisms of angiotensin AT. *Acta Physiol (Oxf)*, 227, e13280.
- Wang, D., X. Q. Chai, C. G. Magnussen, G. R. Zosky, S. H. Shu, X. Wei & S. S. Hu (2019) Renin-angiotensin-system, a potential pharmacological candidate, in acute respiratory distress syndrome during mechanical ventilation. *Pulm Pharmacol Ther*, 58, 101833.
- Witherden, I. R. & T. D. Tetley (2001) Isolation and Culture of Human Alveolar Type II Pneumocytes. *Methods Mol Med*, 56, 137-46.

## **Figure legends**

**Fig.1:** AT<sub>2</sub> receptor gene (AGTR2) expression in lung cells and buloxibutid treatment (10–1000nM) of primary ATII cells in absence or presence of poly I:C. A) Banovich/Kropski dataset from the IPF atlas averaged per subject and braked by disease confirm the high expression of AT<sub>2</sub> receptor in ATII cells in both control donors and ILD patients. B) MTS cell viability assay showed no significant effect of buloxibutid treatment on ATII cell viability (n=4/5). # – significance vs. equivalent buloxibutid-treated, poly-IC free culture. C) AT<sub>2</sub> receptor gene expression is not modulated by buloxibutid compound treatment in absence or presence of poly I:C (n=3/4). Values are expressed as means of ± SD, of n=3-5 independent experiments, each performed using cells from a different donor. Samples were generated by pooling lysates from 3 wells per condition keeping donors

separate and analysed in technical duplicate. Analysis: 2ways-ANOVA (p-value <0.01<sup>##</sup> and <0.0001<sup>####</sup> ).

**Fig.2:** Expression of the inflammatory marker, IL6, and of anti-viral genes during buloxibutid treatment (10-1000nM) in absence or presence of poly I:C. A) Buloxibutid treatment shows a small, non-significant suppressive effect in IL6 gene expression induced by poly I:C, confirmed by a significant decrease in IL-6 protein release, measured by ELISA (bottom panel, Mann-Whitney p-value < 0.05<sup>#</sup>; oneway-ANOVA p-value < 0.05<sup>\*</sup>). B) Buloxibutid treatment does not affect induction of the anti-viral genes, MX1 or RSAD2, by poly I:C. Values are expressed as means of  $\pm$  SD, of n=3-4 independent experiments, each performed using cells from a different donor. Samples were generated by pooling lysates or supernatants from 3 wells per condition keeping donors separate and analysed in technical duplicate. Analysis: 2ways-ANOVA (p-value < 0.05<sup>\*</sup>).

**Fig.3:** RNA sequencing of primary ATII cells treated with buloxibutid 1000nM in absence or presence of poly I:C. A) Heat map of all differentially expressed genes show a strong clustering mainly between conditions without or with poly I:C in absence or presence of buloxibutid 1000nM. B) Heatmap of GO\_RESPONSE\_TO\_VIRUS genes show top differentially expressed genes clustering between conditions without or with

poly I:C. n=4-5 independent experiments, each performed using cells from a different donor. Samples were generated by pooling lysates from 3 wells per condition keeping donors separate.

**Fig.4:** Gene set enrichment analysis (GSEA) of RNA sequencing datasets. A) GSEA analysis shows that mainly inflammation related pathways are significantly downregulated by buloxibutid 1000nM in presence of poly I:C compared to poly I:C alone (p-value < 0.05). B) Heat maps showing downregulation of the top 3 pathways of GSEA analysis by buloxibutid 1000nM in presence of poly I:C compared to poly I:C alone. n=5 independent experiments, each performed using cells from a different donor. Samples were generated by pooling lysates from 3 wells per condition keeping donors separate.

**Fig.5:** Mediators of the cytokine storm and antiviral transcripts during buloxibutid treatment. A) The Heatmap generated from RNA-sequencing shows that transcripts of the mediators of the cytokine storm are increased by poly I:C and down regulated by buloxibutid 1000nM treatment in presence of poly I:C. B) Antiviral genes induced by poly I:C treatment mimicked viral infection are not affected by buloxibutid compound 1000nM treatment. n=5 independent experiments, each performed using cells from a different donor. Samples were generated by pooling lysates from 3 wells per condition keeping donors separate.

**Fig.6:** Graphical representation of role of buloxibutid in viral infection and inflammation. buloxibutid treatment during virus infection may help to reduce the cytokine storm by acting mainly on the alveolar cell mitigating the immune cell response.

### **Supplementary legends**

**Supp. Fig.1:** AGTR2 transcript is highly expressed in ATII cells in the lung. A) Krasnow human lung cell atlas from single-cell RNA sequencing cell compartment. B-C) AGTR2 is mainly expressed in the epithelial compartment and strongly correlates with SFTPC expression that is a specific marker of ATII cells.

**Supp. Fig.2:** Buloxibutid (10-1000nM) treatment of primary ATII cells in absence or presence of poly I:C. shows no effects on ATII cell morphology and density.

**Supp. Fig.3:** ATII cell protein release of the mediators of the cytokine storm is significantly down regulated by buloxibutid 1000nM treatment in presence of poly I:C treatment. Values are expressed as means of  $\pm$  SD. Kolmogorov-Smirnov test (p-value < 0.05 \* and p-value < 0.01 \*\* ).

**Supp. Table 1:** Gene table of GO\_RESPONSE\_TO\_VIRUS top differentially expressed genes

**Supp. Table 2:** Data tables of GSEA hallmark interferon gamma response, hallmark TNFA signaling via NFkB and hallmark inflammatory response.

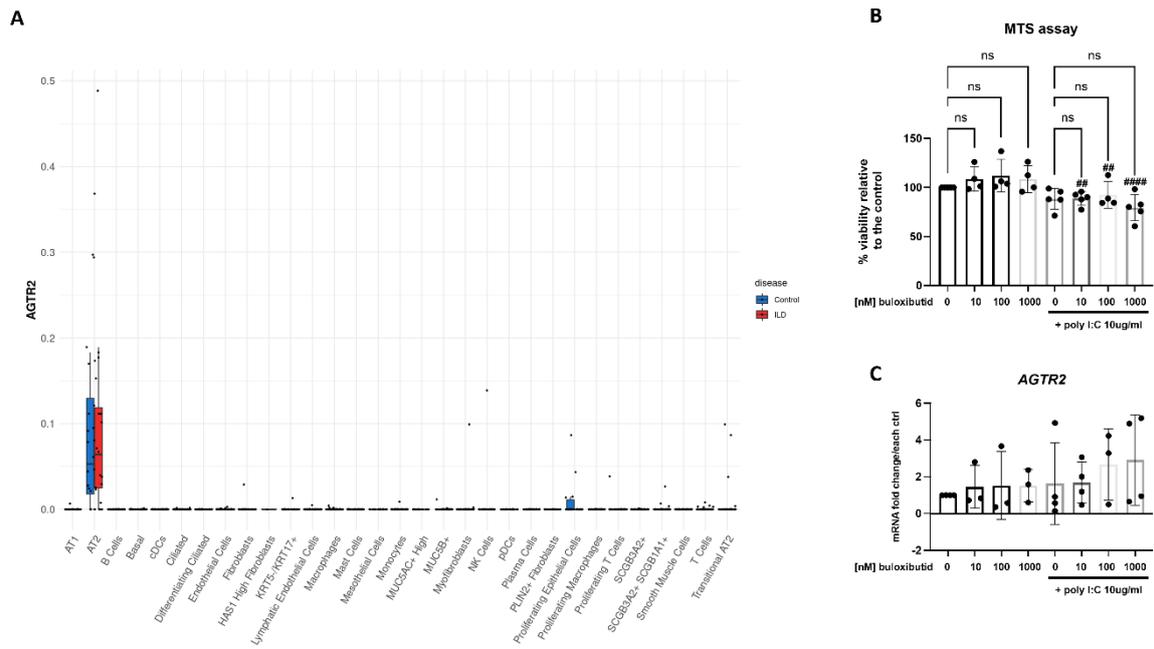


Figure 1

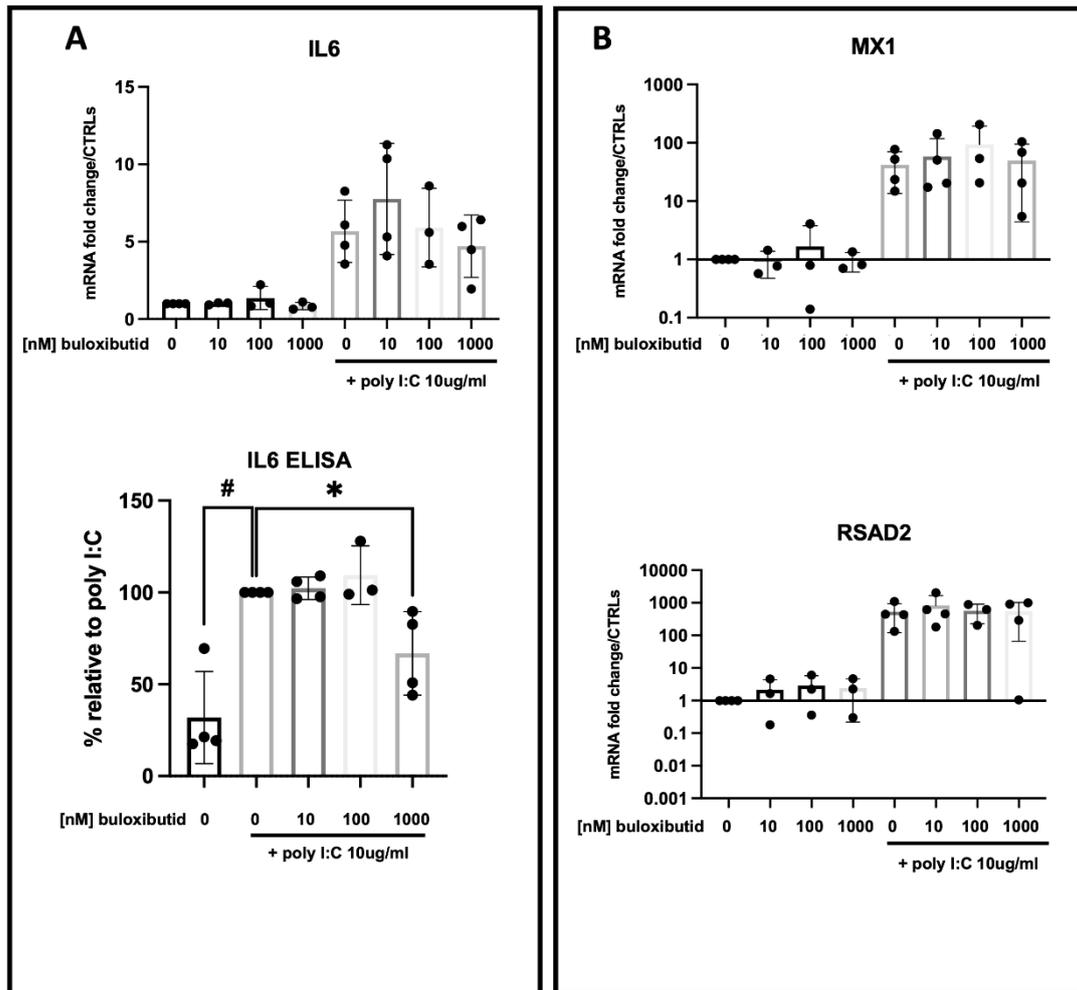


Figure 2



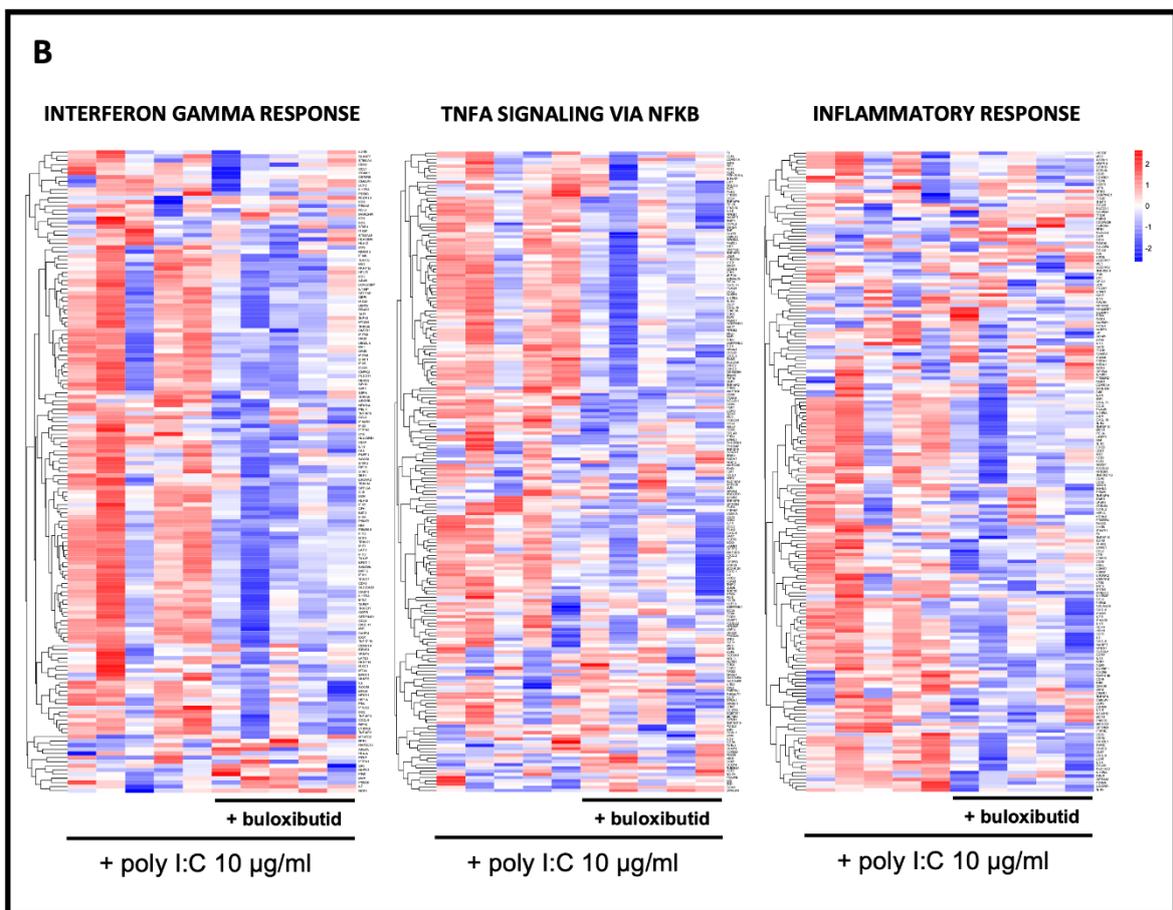
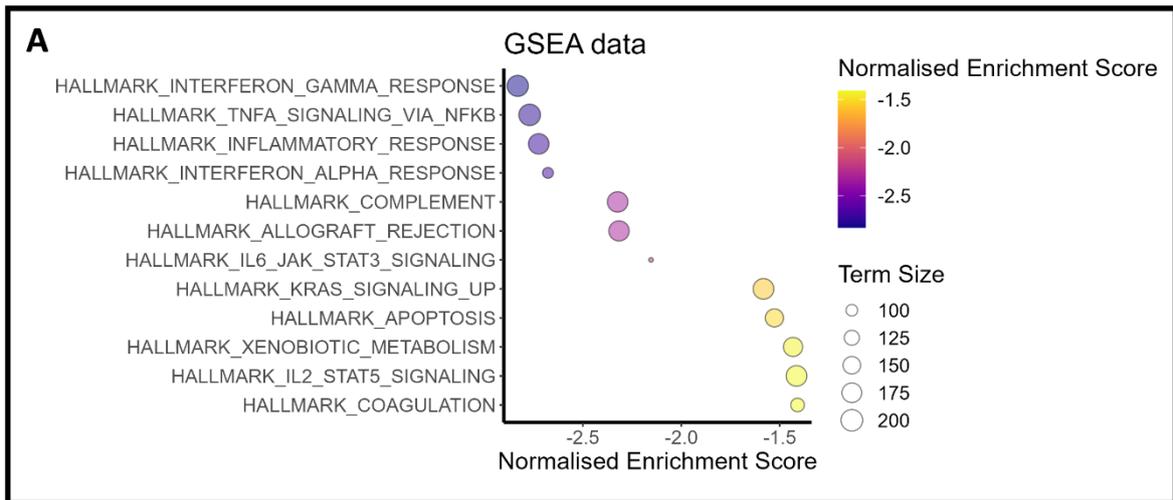


Figure 4

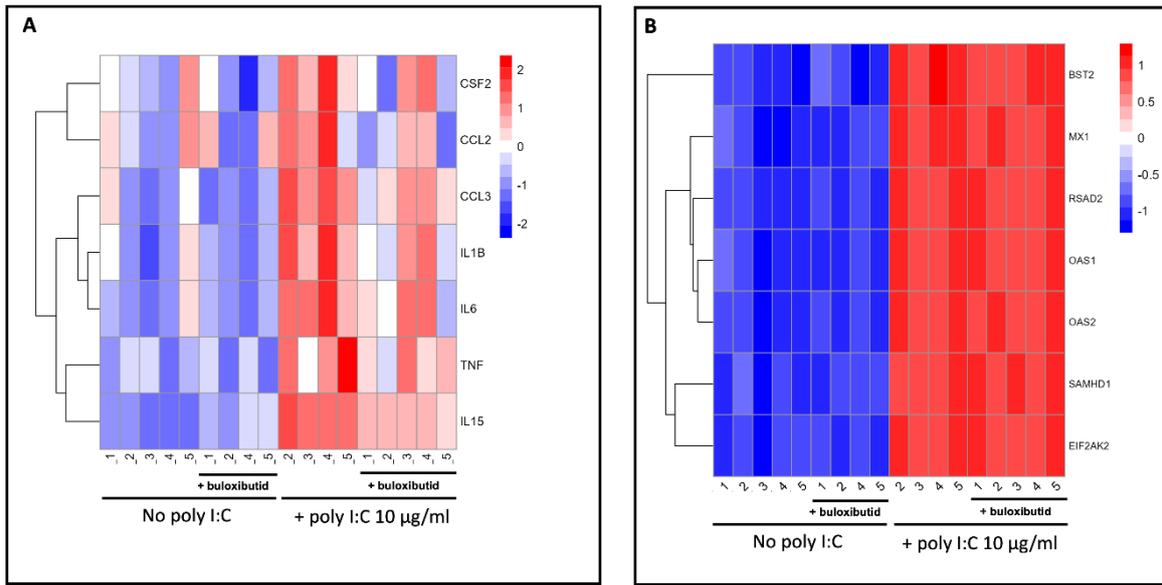


Figure 5

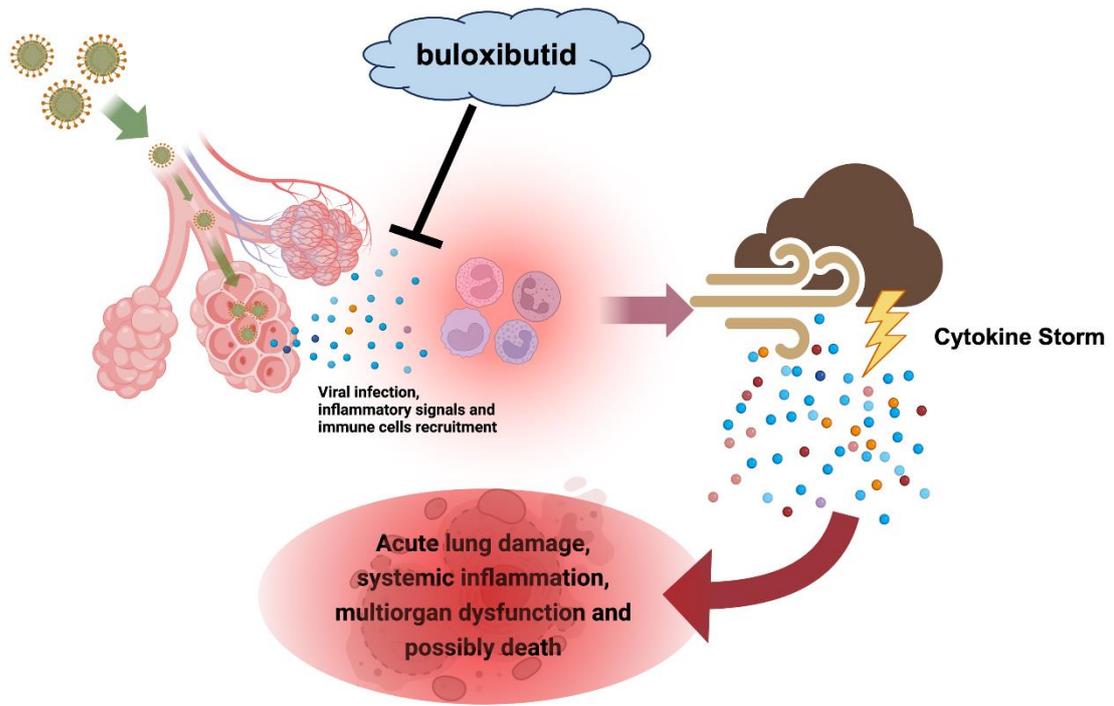
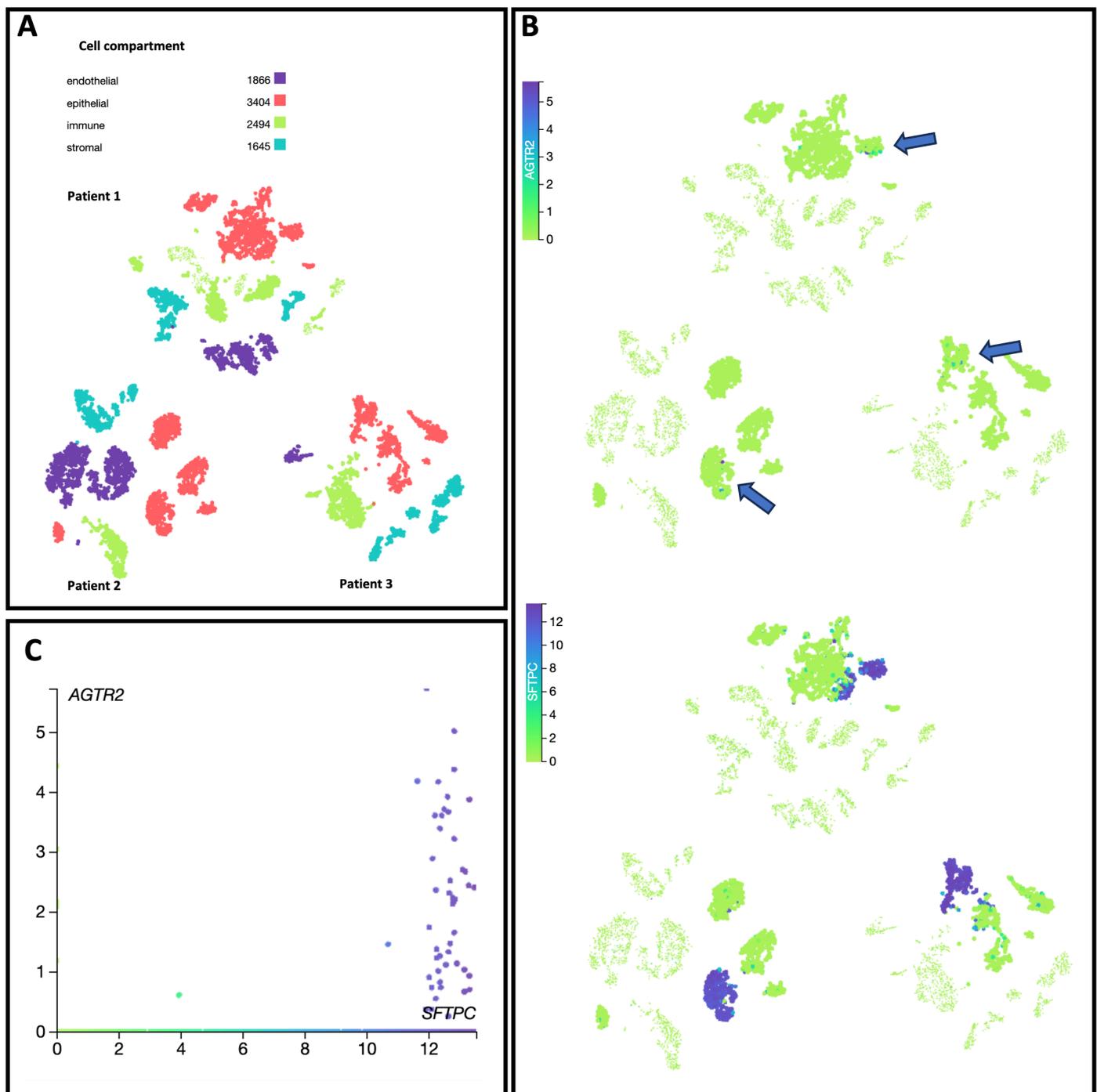
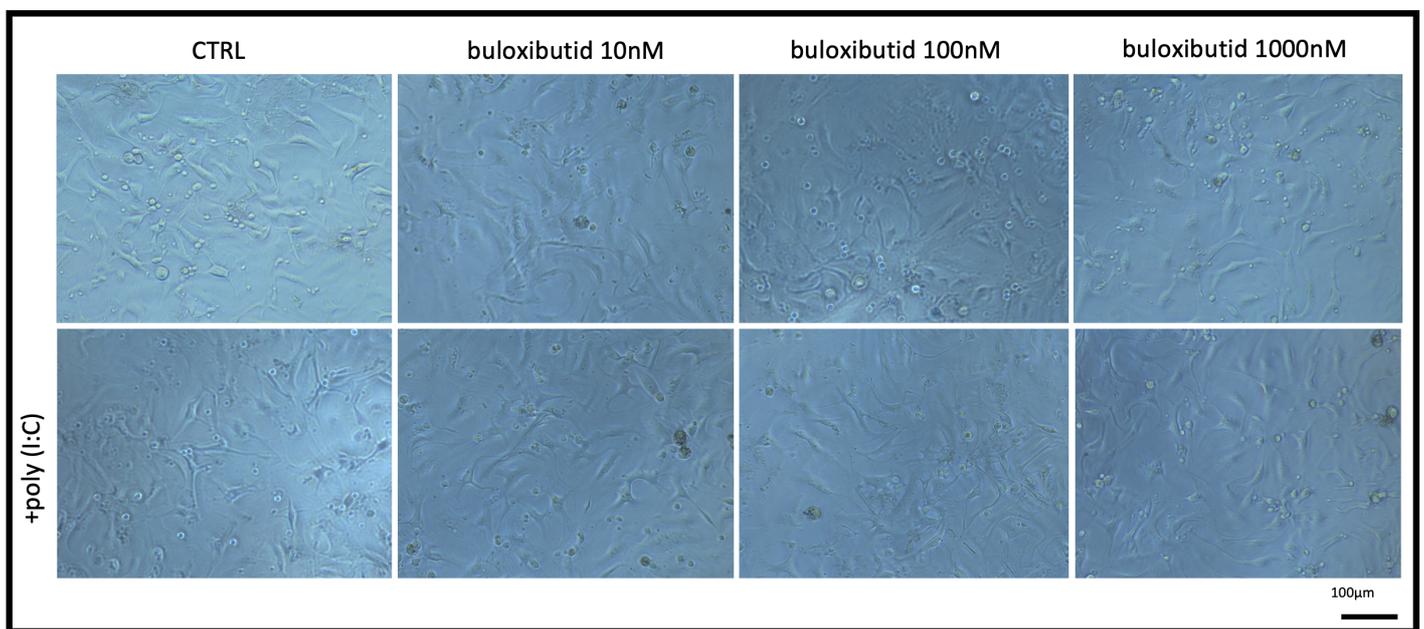


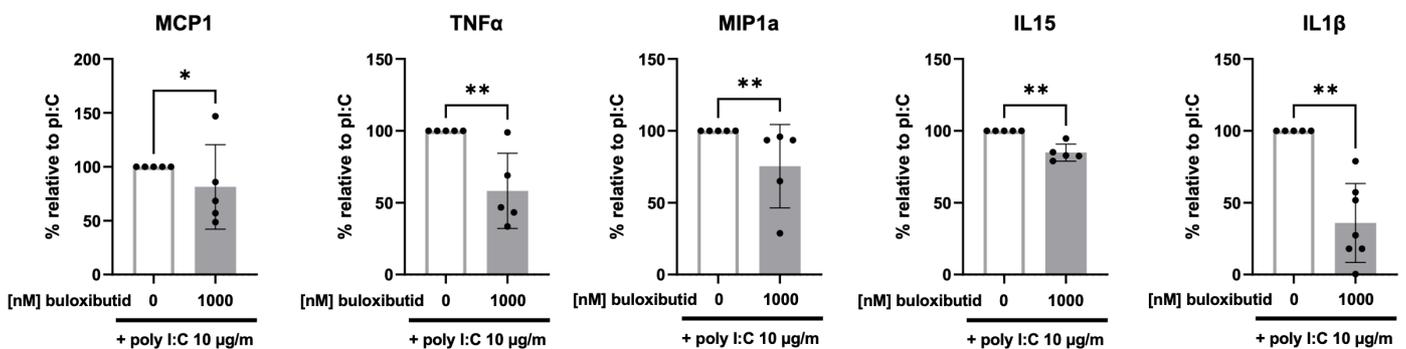
Figure 6



**Supp. Fig.1:** AGTR2 transcript is highly expressed in ATII cells in the lung. A) Krasnow human lung cell atlas from single-cell RNA sequencing cell compartment. B-C) AGTR2 is mainly expressed in the epithelial compartment and strongly correlates with SFTPC expression that is a specific marker of ATII cells.



**Supp. Fig.2:** Buloxibutid (10-1000nM) treatment of primary ATII cells in absence or presence of poly I:C. shows no effects on ATII cell morphology and density.



**Supp. Fig.3:** ATII cell protein release of the mediators of the cytokine storm is significantly down regulated by buloxibutid 1000nM treatment in presence of poly I:C treatment. Values are expressed as means of  $\pm$  SD. Kolmogorov-Smirnov test (p-value < 0.05 \* and p-value < 0.01 \*\* ).

## GO\_RESPONSE\_TO\_VIRUS

GENE SYMBOL	NCBI GENE ID	name
SRC	6714	SRC proto-oncogene, non-receptor tyrosine kinase
CHRM2	1129	cholinergic receptor muscarinic 2
CXCL12	6387	C-X-C motif chemokine ligand 12
CYP1A1	1543	cytochrome P450 family 1 subfamily A member 1
DDX3X	1654	DEAD-box helicase 3 X-linked
IVNS1ABP	10625	influenza virus NS1A binding protein
HYAL3	6677	sperm adhesion molecule 1
HYAL2	8692	hyaluronidase 2
SMPD1	6609	sphingomyelin phosphodiesterase 1
URI1	8725	URI1 prefoldin like chaperone
LAMTOR5	10542	late endosomal/lysosomal adaptor, MAPK and MTOR activator 5
BANF1	8815	barrier to autointegration nuclear assembly factor 1
HNRNPUL1	11100	heterogeneous nuclear ribonucleoprotein U like 1
ACTA2	59	actin alpha 2, smooth muscle
CFL1	6944	vacuolar protein sorting 72 homolog
HYAL1	6677	sperm adhesion molecule 1
CCT5	22948	chaperonin containing TCP1 subunit 5
STMN1	3925	stathmin 1
ODC1	89874	solute carrier family 25 member 21
ENO1	2023	enolase 1
PRKRA	8575	protein activator of interferon induced protein kinase EIF2AK2
TPT1	7178	tumor protein, translationally-controlled 1
RPS15A	6210	ribosomal protein S15a
EEF1G	1937	eukaryotic translation elongation factor 1 gamma
AP1S1	1174	adaptor related protein complex 1 subunit sigma 1
DDX1	1653	DEAD-box helicase 1
CLU	1191	clusterin
IFNAR1	3454	interferon alpha and beta receptor subunit 1
NPC2	10577	NPC intracellular cholesterol transporter 2
MAVS	57506	mitochondrial antiviral signaling protein
ITGB6	3694	integrin subunit beta 6
DCLK1	9201	doublecortin like kinase 1
XPR1	9213	xenotropic and polytropic retrovirus receptor 1
TLR8	51311	toll like receptor 8
FGR	2268	FGR proto-oncogene, Src family tyrosine kinase
CHUK	1147	component of inhibitor of nuclear factor kappa B kinase complex
CXCR4	7852	C-X-C motif chemokine receptor 4
DDX21	54606	DEAD-box helicase 56
BCL3	602	BCL3 transcription coactivator
IRAK3	11213	interleukin 1 receptor associated kinase 3
CCL22	6367	C-C motif chemokine ligand 22
DHX58	79132	DEH-box helicase 58
IL17RA	23765	interleukin 17 receptor A
CDK6	1021	cyclin dependent kinase 6
GTF2F1	2962	general transcription factor IIF subunit 1
PSMA2	5683	proteasome 20S subunit alpha 2
TBX21	30009	T-box transcription factor 21
CCL11	6356	C-C motif chemokine ligand 11
TNF	7124	tumor necrosis factor
GATA3	2625	GATA binding protein 3
CCL4	6351	C-C motif chemokine ligand 4
TNFSF4	7292	TNF superfamily member 4
TBK1	29110	TANK binding kinase 1
IKKB	3551	inhibitor of nuclear factor kappa B kinase subunit beta
DUOX2	50506	dual oxidase 2
IL12A	3592	interleukin 12A
DHX36	170506	DEAH-box helicase 36
BST2	684	bone marrow stromal cell antigen 2
TRIM22	10346	tripartite motif containing 22
TLR3	7098	toll like receptor 3
MYD88	4615	MYD88 innate immune signal transduction adaptor
CCL5	6352	C-C motif chemokine ligand 5
ISG20	3669	interferon stimulated exonuclease gene 20
CXCL10	3627	C-X-C motif chemokine ligand 10
ZC3HAV1	56829	zinc finger CCCH-type containing, antiviral 1
MST1R	4486	macrophage stimulating 1 receptor
IFITM2	10581	interferon induced transmembrane protein 2
IFITM1	8519	interferon induced transmembrane protein 1
IFITM3	10410	interferon induced transmembrane protein 3
EIF2AK2	5610	eukaryotic translation initiation factor 2 alpha kinase 2
OAS3	4940	2'-5'-oligoadenylate synthetase 3
NMI	4641	myosin IC
IFIT3	3437	interferon induced protein with tetratricopeptide repeats 3
IFIT2	3433	interferon induced protein with tetratricopeptide repeats 2
IFIT1	3434	interferon induced protein with tetratricopeptide repeats 1
ADAR	103	adenosine deaminase RNA specific
MX2	4600	MX dynamin like GTPase 2
MX1	4599	MX dynamin like GTPase 1
RSAD2	91543	radical S-adenosyl methionine domain containing 2
OAS1	4938	2'-5'-oligoadenylate synthetase 1
OSA2	57492	AT-rich interaction domain 1B
IFI44	10561	interferon induced protein 44
DDX58	23586	RNA sensor RIG-I
IFIH1	64135	IFIH1 interferon induced with helicase C domain 1
IRF7	3665	interferon regulatory factor 7
DDX60	55601	DEXD/H-box helicase 60
ISG15	9636	ISG15 ubiquitin like modifier
SAP30BP	29115	SAP30 binding protein
CCL8	6355	C-C motif chemokine ligand 8
LILRB1	10859	leukocyte immunoglobulin like receptor B1
HMGA2	8091	high mobility group AT-hook 2
EXT1	2131	exostosin glycosyltransferase 1
FOSL1	8061	FOS like 1, AP-1 transcription factor subunit
IFIT1B	439996	interferon induced protein with tetratricopeptide repeats 1B
CREBZF	58487	CREB/ATF bZIP transcription factor
BCL2L1	598	BCL2 like 1
ITGB8	3696	integrin subunit beta 8
IKBK	8517	inhibitor of nuclear factor kappa B kinase regulatory subunit gamma
AUP1	550	AUP1 lipid droplet regulating VLDL assembly factor
IFNAR2	3455	interferon alpha and beta receptor subunit 2
BATF3	55509	basic leucine zipper ATF-like transcription factor 3
IFNGR2	3460	interferon gamma receptor 2
IFNGR1	3459	interferon gamma receptor 1
PIM2	11040	Pim-2 proto-oncogene, serine/threonine kinase
YJU2B	81576	YJU2 splicing factor homolog B

INTERFERON GAMMA RESPONSE

TNFA SIGNALING VIA NFKB

INFLAMMATORY RESPONSE

NAME	SYMBOL	RANK IN GENE LIST	RANK METRIC SCORE	RUNNING ES	CORE ENRICHMENT
row_0	DHOSL	5	2.81493563	0.02203946No	
row_1	FFR1	540	1.07126583	0.02064844No	
row_2	FCGR2A	182	0.9823405	-0.01132126No	
row_3	NCFB3	143	0.86683156	-0.03004776No	
row_4	NCOI1	82	0.53778798	0.01027074No	
row_5	RARGF6	2708	0.50971491	-0.10665644No	
row_6	ANKK4	2728	0.50682457	-0.10367515No	
row_7	PMN1	2728	0.46588158	-0.14602551No	
row_8	IL1	3726	0.37651031	-0.15628786No	
row_9	KLKI1	721	0.25561052	0.01587977No	
row_10	METTL7B	4868	0.24742386	-0.21970939No	
row_11	UPL1	4917	0.24219707	-0.22061577No	
row_12	PPP1R3	1534	0.13288854	0.13031807No	
row_13	IFI1	6715	0.10975349	-0.32488544No	
row_14	MVP	6748	0.10778154	-0.32928148No	
row_15	MARCKS1	6748	0.07828208	-0.32928148No	
row_16	MHF2D	7376	0.06998869	-0.36180280No	
row_17	GZMA	7657	0.05387748	-0.3793124No	
row_18	STGAL5	9188	0.02164167	-0.51291536No	
row_19	PSMB2	9646	-0.05151873	-0.48482074No	
row_20	GBP6	9770	-0.05489209	-0.50162979No	
row_21	ITGB7	9884	-0.06633193	-0.51790529No	
row_22	CDKN1A	9922	-0.06745199	-0.50982404No	
row_23	PSMB8	10249	-0.08739078	-0.52802472No	
row_24	PTEN	10498	-0.10348467	-0.54179565No	
row_25	SR	10519	-0.10506405	-0.54224726No	
row_26	IRF9	10539	-0.10683339	-0.54249944No	
row_27	SAMD11	10539	-0.11833602	-0.55190178No	
row_28	PLA2G4A	10770	-0.12511533	-0.5543355No	
row_29	IRF8	10787	-0.12232574	-0.55413101No	
row_30	MXI1	10857	-0.12721519	-0.55717913No	
row_31	RNF123	10962	-0.13550528	-0.56252511No	
row_32	VAMP5	11128	-0.14708467	-0.57584180No	
row_33	LATS2	11217	-0.15148494	-0.57482176No	
row_34	OAS3	11536	-0.17602569	-0.59232434No	
row_35	SCCTM1	11575	-0.17495488	-0.59232434No	
row_36	IFIA2K2	11881	-0.18950233	-0.6060328No	
row_37	BST2	11881	-0.20393126	-0.60784344No	
row_38	OAS2	12022	-0.21026102	-0.61293204No	
row_39	PMF4B	12082	-0.22171939	-0.61578796No	
row_40	E5F83	12166	-0.22812814	-0.61809329No	
row_41	IFI1	12251	-0.23025311	-0.61809329No	
row_42	IFI4L	12251	-0.23653829	-0.61896342No	
row_43	IFI4L	12251	-0.23477712	-0.61789576No	
row_44	L18BP1	12251	-0.23803759	-0.61789576No	
row_45	IFI252	12301	-0.23870106	-0.61743600No	
row_46	PTC2	12497	-0.25470754	-0.62715066No	
row_47	AP3B4	12497	-0.25032318	-0.62577436No	
row_48	ARID5B	12667	-0.26931428	-0.63291862No	
row_49	CMTR1	12696	-0.27122091	-0.63241266No	
row_50	EPST1	12788	-0.28207179	-0.63553323No	
row_51	MXI2	12830	-0.28663786	-0.63701741No	
row_52	KAT2	12903	-0.29399878	-0.63729237No	
row_53	IFI202	13204	-0.29282902	-0.63652746No	
row_54	RSAD2	13305	-0.30449304	-0.63871044No	
row_55	MTA2	13324	-0.31838316	-0.64272427No	
row_56	HLA-A	13324	-0.32882862	-0.64466125No	
row_57	HLA-G	13324	-0.33078903	-0.64518151No	
row_58	CSF2RB	13324	-0.33011623	-0.64518151No	
row_59	IRF5	13345	-0.33406383	-0.64549047No	
row_60	RBC1L1	13351	-0.34159673	-0.64307344No	
row_61	IFI443	13351	-0.34488388	-0.64307344No	
row_62	C3ORF	13351	-0.34599846	-0.64192455No	
row_63	SEPRIN1	13429	-0.34905111	-0.63925565No	
row_64	ISG20	13429	-0.34905111	-0.63925565No	
row_65	UBASH3B	13429	-0.34905111	-0.63925565No	
row_66	H1A-DOA1	13470	-0.35289872	-0.63523144No	
row_67	H1A-B	13470	-0.35289872	-0.63523144No	
row_68	STAT1	13500	-0.35791287	-0.62911254No	
row_69	CXCL11	13501	-0.35813036	-0.62626855No	
row_70	HELA	13527	-0.36153138	-0.62487765No	
row_71	TRIM25	13536	-0.36275807	-0.62246794No	
row_72	CH2L2	13557	-0.36517583	-0.62075055No	
row_73	HEX1	13557	-0.36617698	-0.62075055No	
row_74	XXID1	13584	-0.36871957	-0.61639111No	
row_75	IFIA2	13702	-0.37820813	-0.61841127No	
row_76	IFIA2	13702	-0.38037028	-0.61620743No	
row_77	IFIA4	13719	-0.38455063	-0.61511127No	
row_78	STROSA4	13903	-0.41121219	-0.62792193No	
row_79	PMP	14012	-0.41842427	-0.62179173No	
row_80	ZBP1	14088	-0.42748594	-0.62239169No	
row_81	CIF	14185	-0.43128991	-0.62388053No	
row_82	IFI443	14185	-0.43452352	-0.62388053No	
row_83	DOXG	14238	-0.44772529	-0.62140999No	
row_84	TRIM14	14238	-0.45423552	-0.62491127No	
row_85	TRIM26	14238	-0.46772888	-0.62054451No	
row_86	LGALS3BP	14441	-0.49207282	-0.64284252No	
row_87	IRF2	14441	-0.49207282	-0.64284252No	
row_88	CMK1R1	14453	-0.49386444	-0.61820765No	
row_89	VAMP8	14505	-0.50174466	-0.61728076No	
row_90	CD14	14505	-0.50202223	-0.61728076No	
row_91	HERC6	14540	-0.50738506	-0.61116581No	
row_92	STAT1	14542	-0.50800834	-0.60719067No	
row_93	IFI443	14542	-0.50800834	-0.60719067No	
row_94	IFI2	14557	-0.50944123	-0.59987655No	
row_95	SLAMF7	14559	-0.51649484	-0.59771575No	
row_96	CC15	14606	-0.51649484	-0.59771575No	
row_97	IL15RA	14606	-0.51764875	-0.59802781No	
row_98	C3ORF	14606	-0.52033184	-0.58802199No	
row_99	BANK1	14606	-0.52077063	-0.58844276No	
row_100	LY86	14714	-0.53794783	-0.58388066No	
row_101	H1A-DM2A	14734	-0.54083862	-0.58418516No	
row_102	STAT1	14734	-0.54083862	-0.58418516No	
row_103	NOX3	14911	-0.57118022	-0.58195155No	
row_104	ZNF41	14935	-0.57419306	-0.57973199No	
row_105	CMR2	14966	-0.58162026	-0.57547417No	
row_106	PTPN6	14999	-0.58760112	-0.57245959No	
row_107	SOX2	15019	-0.59172528	-0.56560125No	
row_108	IGS15	15020	-0.59333502	-0.56400444No	
row_109	ITP1	15036	-0.59423503	-0.56088104No	
row_110	NLRK5	15036	-0.59526103	-0.56088104No	
row_111	TRAF3	15048	-0.60295242	-0.55418212No	
row_112	IFI1	15201	-0.60513074	-0.55023222No	
row_113	LCF3	15245	-0.61355163	-0.54782176No	
row_114	PLSCR1	15281	-0.64200993	-0.55089171No	
row_115	IL13RA1	15281	-0.64402713	-0.54728786No	
row_116	IFI202	15287	-0.65009716	-0.54284252No	
row_117	AUTS2	15425	-0.67382261	-0.54551515No	
row_118	CFH	15478	-0.68689702	-0.54113799No	
row_119	STAT1	15478	-0.68844652	-0.53971964No	
row_120	PSMB10	15489	-0.68844652	-0.53971964No	
row_121	PMP11	15508	-0.69370311	-0.52627575No	
row_122	TRAF3	15508	-0.69370311	-0.52627575No	
row_123	CASP1	15544	-0.70123356	-0.51719599No	
row_124	IFI15	15569	-0.70619186	-0.51300474No	
row_125	PAGE1	15629	-0.71821131	-0.51091766No	
row_126	ITM3	15629	-0.72551405	-0.50488775No	
row_127	PSMA3	15638	-0.72482681	-0.49706704No	
row_128	TAF1	15661	-0.72949026	-0.49524776No	
row_129	VCAAM1	15664	-0.73065176	-0.48933065No	
row_130	SPFN	15674	-0.73462051	-0.48428676No	
row_131	ADAM	15674	-0.73790179	-0.47815871No	
row_132	DDX58	15687	-0.73742301	-0.47320476No	
row_133	CD69	15708	-0.74397653	-0.46879476No	
row_134	MYD88	15748	-0.75456594	-0.46449727No	
row_135	POE4B	15769	-0.75949561	-0.45985877No	
row_136	DDI1	15769	-0.76044809	-0.45985877No	
row_137	IFI1	15813	-0.77455855	-0.45061288No	
row_138	IRF7	15813	-0.77791849	-0.44463163No	
row_139	IFI5	15828	-0.78266868	-0.44320317No	
row_140	SAMD11	15878	-0.79624759	-0.43511036No	
row_141	BATF2	15885	-0.79871875	-0.42712222No	
row_142	WARS1	15885	-0.80214346	-0.42270702No	
row_143	H1A-DRB1	15899	-0.80594846	-0.41706142No	
row_144	ICAM1	15899	-0.81071911	-0.41155575No	
row_145	IFIA	15899	-0.81339139	-0.40521807No	
row_146	CD274	15904	-0.83776028	-0.40721533No	
row_147	TNFAIP3	16001	-0.84908511	-0.39662794No	
row_148	CC17	16001	-0.84778924	-0.39560476No	
row_149	B2M	16024	-0.85075534	-0.38438633No	
row_150	IFI1	16024	-0.87102057	-0.38101707No	
row_151	IFI41	16024	-0.90079222	-0.37848476No	
row_152	GRB4	16178	-0.90881121	-0.37202744No	
row_153	CD3	16197	-0.93107164	-0.36578099No	
row_154	TOX18	16218	-0.93691018	-0.36352363No	
row_155	PSME2	16245	-0.93861018	-0.36352363No	
row_156	CASP8	16245	-0.94067513	-0.36222572No	
row_157	LOP18	16245	-0.95253283	-0.33996667No	
row_158	RP22	16293	-0.96371913	-0.33385805No	
row_159	SP110	16293	-0.97093159	-0.33081544No	
row_160	IFI1	16378	-1.00273708	-0.32278244No	
row_161	NFKB1A	16418	-1.00807806	-0.31483379No	
row_162	CITA	16418	-1.02007913	-0.30812808No	
row_163	IFI1	16418	-1.02485873	-0.30055533No	
row_164	NFKB1	16449	-1.04041527	-0.29242545No	
row_165	TNFSF10	16449	-1.04791318	-0.28649549No	
row_166	TNXP1	16474	-1.05666294	-0.27898186No	
row_167	LY86	16474	-1.05777603	-0.27898186No	
row_168	NM1	16528	-1.08234668	-0.26584161No	
row_169	IFNA2	16581	-1.10622603	-0.25972126No	
row_170	TRIM21	16572	-1.10656513	-0.24998409No	
row_171	RNF31	16572	-1.10957176	-0.24129897No	
row_172	IFI1	16588	-1.11784901	-0.23307266No	
row_173	BTG1	16610	-1.13761038	-0.22528075No	
row_174	CASP7	16624	-1.14603887	-0.21677026No	
row_175	IFI1	16624	-1.18515588	-0.20998199No	
row_176	SOCS1	16701	-1.21018992	-0.20728183No	
row_177	NAMPT	16746	-1.24276512	-0.19515176No	
row_178	TNFAIP6	16746	-1.26355851	-0.18623455No	
row_179	OGFR	16821	-1.35274056	-0.17866561No	
row_180	CASP4				