Low baseline pulmonary levels of cytotoxic lymphocytes as a predisposing risk factor for severe COVID-19

Pascal H.G. Duijf [1, 2, 3 *]

[1] Institute of Health and Biomedical Innovation, Queensland University of Technology (QUT), Faculty of Health, School of Biomedical Sciences, Brisbane QLD, Australia.

[2] Centre for Data Science, Queensland University of Technology (QUT), Brisbane QLD, Australia.

[3] University of Queensland Diamantina Institute, The University of Queensland, Translational Research Institute, Brisbane QLD, Australia.

[*] Correspondence: Pascal H.G. Duijf, email: pascal.duijf@qut.edu.au

Abstract

COVID-19 is caused by the coronavirus SARS-CoV-2 and currently has detrimental human health, community and economic impacts around the world. It is unclear why some SARS-CoV-2-positive individuals remain asymptomatic, while others develop severe symptoms. Baseline pulmonary levels of anti-viral leukocytes, already residing in the lung prior to infection, may orchestrate an effective early immune response and prevent severe symptoms. Using "*in silico* flow cytometry", we deconvoluted the levels of all seven types of anti-viral leukocytes in 1,927 human lung tissues. Baseline levels of CD8+ T cells, resting NK cells and activated NK cells, as well as cytokines that recruit these, are significantly lower in lung tissues with high expression of the SARS-CoV-2 entry receptor ACE2. We observe this in univariate analyses, in multivariate analyses, and in two independent datasets. Relevantly, ACE2 mRNA and protein levels very strongly correlate in human cells and tissues. Above findings also largely apply to the SARS-CoV-2 entry protease TMPRSS2. Both SARS-CoV-2-infected lung cells and COVID-19 lung tissues show upregulation of CD8+ T cell- and NK cell-recruiting cytokines. Moreover, tissue-resident CD8+ T cells and inflammatory NK cells are significantly more abundant in bronchoalveolar lavages from mildly affected COVID-19 patients, compared to severe cases. This suggests that these lymphocytes are important for preventing severe symptoms. Elevated ACE2 expression increases sensitivity to coronavirus infection. Thus, our results suggest that some individuals may be exceedingly susceptible to develop severe COVID-19 due to concomitant high pre-existing ACE2 and TMPRSS expression and low baseline cytotoxic lymphocyte levels in the lung.

Keywords: COVID-19, SARS-CoV-2, ACE2, TMPRSS2, T cells, NK cells

Introduction

Coronaviruses are viruses belonging to the family of *Coronaviridae* (1). They are large, single-stranded RNA viruses that often originate from bats and commonly infect mammals. While the majority of coronavirus infections cause mild symptoms, some can cause severe symptoms, such as pneumonia, respiratory failure and sepsis, which may lead to death (2, 3).

Coronavirus zoonosis constitutes a serious health risk for humans. Indeed, in recent history, transmissions of three types of coronaviruses to humans have led to varying numbers of deaths. The outbreak of the Severe Acute Respiratory Syndrome (SARS) epidemic, which is caused by the SARS coronavirus (SARS-CoV), originated in Guangdong, China in 2002 and led to nearly 800 deaths (4). The Middle East Respiratory Syndrome coronavirus (MERS-CoV) outbreak, which emerged in Saudi Arabia in 2012, similarly caused about 800 deaths, although with over 8,000 cases, nearly four times as many cases were reported (4). Finally, Coronavirus Disease 19 (COVID-19), caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), is currently causing a pandemic. On 1 May 2020, the World Health Organization reported over 3 million confirmed cases and over 220,000 patients to have succumbed to COVID-19 around the world (5). However, the factual number of deaths is probably considerably higher (6). In addition, this figure is still soaring, on 1 May 2020 at a rate exceeding 6,400 deaths per day (5).

To infect target cells, coronaviruses use their spike (S) glycoprotein to bind to receptor molecules on the host cell membrane. Angiotensin-converting enzyme 2 (ACE2) has been identified as the main SARS-CoV-2 entry receptor on human cells (7, 8), while the serine protease TMPRSS2, or potentially cathepsin B and L, are used for S-protein priming to facilitate host cell entry (7). SARS-CoV-2 S-protein has a 10 to 20-fold higher affinity to human ACE2 than SARS-CoV S-protein (9). Moreover, ACE2 expression proportionally increases the susceptibility to S protein-mediated coronavirus infection (10-12). Hence, increased expression of ACE2 is thought to increase susceptibility to COVID-19 (13-15).

Epithelial cells of the respiratory tract, including the lung, are primary SARS-CoV-2 target cells (16-18). These cells can sense viral infection via pattern recognition receptors (PRRs). PRRs, including Toll-like receptors and NOD-like receptors, recognize pathogen-associated molecular patterns (PAMPs) (19). Upon PRR activation, a range of pro-inflammatory cytokines and chemokines are produced and released in order to activate the host's immune system. Interferons (IFNs), in particular type I and type III IFN, are among the principal cytokines to recruit immune cells (19, 20).

Six types of leukocytes have been implicated in detecting and responding to viral infections in the lung, a major site of SARS-CoV-2 infection, which also presents with severe COVID-19 symptoms. The cytotoxic activities of CD8+ T cells and NK cells can facilitate early control of viral infections by clearing infected cells and avoiding additional viral dissemination (21, 22). Dendritic cells specialize in sensing infections, including by viruses, and inducing an immune response (23). CD4+ T cells contribute to viral clearance by promoting production of cytokines and interactions between CD8+ T cells and dendritic cells (24). M1 macrophages interact with pulmonary epithelial cells to fight viral infections in the lung (25). Finally, neutrophils may contribute to clearance of viral infections through phagocytosis of virions and viral particles. However, their precise role is uncertain (26).

SARS-CoV-2 is considerably more efficient in infection, replication and production of infectious virus particles in human lung tissue than SARS-CoV (17). Strikingly, despite this, SARS-CoV-2 initially does not significantly induce type I, II or III IFNs in infected human lung cells and tissue (17, 27). When this does occur, it may in fact promote further SARS-CoV-2 infection, as IFNs directly upregulate expression of the SARS-CoV-2 receptor ACE2 (28). These observations suggest that baseline levels of leukocytes, which already reside in the lung prior to infection, may be important in mounting a rapid immune response against SARS-CoV-2 infection and prevent severe COVID-19 symptoms. As stated above, ACE2 expression level may be a predictor of increased susceptibility to COVID-19 (10-15)}. Thus, we here investigated the relationship between ACE2 and TMPRSS2 expression and the levels of seven leukocyte types implicated in anti-viral immune response in human lung tissue.

Results

We used bulk RNAseq gene expression data from the 578 human lung tissues present in the Genotype-Tissue Expression (GTEx) database (29, 30), because this is the largest publicly available dataset with clinical information. Using an established "*in silico* flow cytometry" pipeline (31), we estimated the levels of CD8+ T cells, resting and activated NK cells, M1 macrophages, dendritic cells, CD4+ T cells and neutrophils in these tissues **(Fig. S1a-c**, **Table S1)**. We compared these to ACE2 expression levels in these lung tissues. This revealed that ACE2 expression is negatively correlated with the levels of CD8+ T cells, resting and activated NK cells and M1 macrophages ($p < 8\times10^{-6}$, Pearson correlations) **(Fig. 1a-c)**. However, there are no statistically significant correlations between ACE2 expression and the levels of CD4+ T cells, dendritic cells and neutrophils ($p > 0.05$) **(Fig. S2a-d)**. Thus, the levels of a majority of leukocytes involved in anti-viral immune responses are significantly lower in lung tissues with high ACE2 expression levels.

It is possible that some of above observations are linked to phenotypic characteristics, such as sex, age, body mass index (BMI), race or smoking status. To test the robustness of our findings, we applied multivariable regression analysis that includes these five covariates **(Table S2)**, as well as the levels of the seven above leukocyte types or states. This showed that only 4 of the 12 variables significantly contribute to predicting ACE2 expression levels, specifically the levels of CD8+ T cells, resting NK cells, activated NK cells and M1 macrophages **(Fig. 1a-c, Table S3)**. Notably, none of the five added phenotypic covariates showed statistically significant contributions. Consistently, we found limited statistically significant correlations between these variables and ACE2 expression in univariate analyses, irrespective of whether they were analyzed as continuous data or binned into discrete ordinal categories **(Fig. S4a-j)**. Thus, the levels of four types of leukocytes that respond to viral infection are low in lung tissue with high ACE2 expression levels independently of phenotypic covariates.

Next, we tested whether above observations could be validated in an independent cohort of individuals. For this, we used the, to our knowledge, largest publicly available lung tissue dataset. The Laval University, University of British-Columbia, Groningen University (LUG) dataset includes microarray gene expression data of 1,349 human lung tissues. Following determination of ACE2

expression levels and estimation of the levels of CD8+ T cells, resting NK cells, activated NK cells and M1 macrophages **(Fig. S1a-c**, **Table S1)**, we found that three of the four also negatively correlated with ACE2 expression in this independent dataset ($p<2\times10^{-8}$) **(Fig. 1d-f)**. With a correlation coefficient of R=0.096, only M1 macrophages did not correlate with ACE2 expression in this dataset **(Fig. S3)**. Thus, our observations indicate that the baseline levels of three types of cytotoxic lymphocytes, specifically CD8+ T cells, resting NK cells and activated NK cells, are robustly and consistently low in lung tissue with high expression of the SARS-CoV-2 receptor ACE2.

To more rigorously assess our observations, we employed a range of additional analyses. Although highly statistically significant (all p values <4.1x10^{-6}, **Fig. 1**), the absolute Pearson R values between baseline levels of ACE2 and the three lymphocyte types were seemingly low, as they ranged between 0.2 and 0.3 **(Fig 1a-c)**. To test how strong these are in relative terms, we calculated the Pearson R and p values of 1000 randomly sampled other genes. This revealed that the ACE2 R and p values were significantly lower than expected by chance (all one-sample t test $p<2.2\text{x}10^{-16}$; **Fig 2a,b**). In addition, these R and p values ranked in the top 0.4 to 11 percentiles of strongest and most significant correlations for each of the three leukocyte types **(Fig 2a,b)**. Thus, the seemingly low correlations between ACE2 mRNA and cytotoxic lymphocyte levels in the lung are not only highly statistically significant, but also strong in relative terms.

Further, we tested how well ACE2 mRNA and protein levels correlate. Using the mRNA and protein levels in 52 cell lines, we find that ACE2 mRNA levels strongly correlate with ACE2 protein levels in human cells (Pearson R=0.8155, $p=1.8\text{x}10^{-13}$; **Fig. 2c**). In fact, ACE2 ranks in the top 1.8 percentile of over 12,000 genes with the strongest mRNA-protein level correlations ($p<2.2\text{x}10^{-16}$; **Fig 2d**). Using two ACE2-specific antibodies, immunochemistry on 40 human tissues also shows a strong ACE2 mRNA-protein correlation (p=0.0011, Kruskal-Wallis test; **Fig. 2e**) and this is additionally validated by a meta-analysis that we conducted using nine published studies (Pearson R=0.7130 , p=0.0013; **Fig. 2f**, **Table S4**). Therefore, we conclude that ACE2 mRNA and protein levels very strongly correlate, both in human cells and in human tissues.

Above, we found that baseline ACE2 levels in the lung negatively correlate with CD8+ T cells and resting and activated NK cells in multivariate analyses and in an independent dataset **(Fig. 1a-f)**. Several cytokines, including CCL2-5, CXCL9, CXCL10, CXCL16 and IL18, are known to chemotactically attract CD8+ T cells and NK cells (32-37). Consistently, we find that the baseline levels of these chemokines in human lung tissue typically significantly correlate with the baseline levels of CD8+ T cells and resting and activated NK cells (**Fig. 2g, Fig. S5**). Additionally, as expected given our above results, we find significant negative correlations between the levels of ACE2 and the levels of six of these eight cytokines in the lung **(Fig. 2h)**. These findings lend further support to our previous observations, suggesting that high levels of said cytokines in the lung establish a favorable milieu for cytotoxic lymphocytes, which correlates with low ACE2 levels.

Next, we assessed the direct consequences of SARS-CoV-2 infection. *In vitro* SARS-CoV-2 infection of human lung cells invariably leads to upregulation of all eight abovementioned CD8+ T cell- and NK cell-attracting cytokines, with six of these increases showing statistical significance **(Fig. 2i)**. Similarly, compared to control lung tissues, all eight cytokines are upregulated in lung tissues from COVID-19 patients, with five showing statistical significance **(Fig. 2j)**. Moreover, the levels of CD8+ T cells and NK cells are higher in bronchoalveolar lavages of mildly affected COVID-19 patients than in severe cases, with CD8+ T cells and a subset of NK cells, inflammatory NK cells, showing a statistically significant higher level **(Fig. 2k)**. These findings are corroborated in a different cohort of patients, additionally showing a highly significant increase in a tissue-resident signature score for CD8+ T cells **(Fig. 2k)**. Thus, together, these observations suggest that SARS-CoV-2 infection of lung cells stimulates CD8+ T cell- and NK cell-attracting cytokines and that these cytotoxic lymphocytes are important for preventing severe symptoms of COVID-19.

Finally, we tested whether the levels of the SARS-CoV-2 host cell protease TMPRSS2 shows similar correlations with the levels of CD8+ T cells and NK cells in the lung. In univariate analyses, baseline TMPRSS2 levels in the lung show significant negative correlations with these lymphocyte levels, although in multivariate analyses, these are only statistically significant for CD8+ T cells and

activated NK cells **(Fig. 3a-c)**. The corresponding *R* and *p* values are typically also significantly lower than expected by chance **(Fig. 3d,e)**. Furthermore, TMPRSS2 mRNA and protein levels strongly correlate (R=0.8048, $p<2.2x10^{-16}$, **Fig. 3f**) and TMPRSS2 is in the top 2.5 percentile of genes that show the strongest mRNA-protein correlation ($p<2.2x10^{-16}$, **Fig. 3g**). Additionally, TMPRSS2 expression tends to correlate negatively with CD8+ T cell- and NK cell-attracting cytokines **(Fig. 3h)**. Therefore, albeit typically to a lesser extent, baseline TMPRSS2 expression levels in the lung negatively correlate with the levels of CD8+ T cells and NK cells in a manner similar to ACE2.

Taken together, our observations suggest that a subgroup of individuals may be exceedingly susceptible to developing severe COVID-19 due to concomitant high pre-existing ACE2 and TMPRSS2 expression and low baseline levels of CD8+ T cells and NK cells in the lung **(Fig. 3i)**.

Discussion

We investigated the baseline expression levels of the SARS-CoV-2 host cell entry receptor ACE2 and the host cell entry protease TMPRSS2 and the baseline levels of all seven types of anti-viral leukocytes in 1,927 human lung tissue samples. Although SARS-CoV-2 cellular tropism is broad (16-18), we focused on lung tissue. In addition to epithelial cells elsewhere in the respiratory tract, alveolar epithelial cells are thought to be a primary SARS-CoV-2 entry point (16, 28). Consistently, the SARS-CoV-2 receptor ACE2 is expressed in these cells at the mRNA and protein levels (28, 38-40). Moreover, in severely affected COVID-19 patients, the lungs are among the few organs that present with the most life-threatening symptoms. "Cytokine storm"-induced acute respiratory distress syndrome (ARDS), widespread alveolar damage, pneumonia, and progressive respiratory failure have been observed (41, 42). These indications frequently require admission to intensive care units (ICUs) and mechanical ventilation and may ultimately be fatal.

Early after infection, rapid activation of the innate immune system is of paramount importance for the clearance of virus infections. Infected cells typically do so through release of pro-inflammatory cytokines and chemokines, in particular type I and III interferons (19, 20). Notably, however, several

studies have highlighted multiple complexities related specifically to SARS-CoV2 and innate immune system activation at early stages. First, unlike SARS-CoV, SARS-CoV-2-infected lung tissue initially fails to induce a range of immune cell-recruiting molecules, including several interferons (17, 27), suggesting that leukocytes are ineffectively recruited to infected lung shortly after infection. Second, the host cell entry receptor ACE2 has been identified as an interferon target gene (28). Thus, even when interferons are upregulated in order to recruit immune cells, concomitant upregulation of ACE2 expression may in fact exacerbate SARS-CoV-2 infection (28).

These findings suggest that the levels of immune cells that already reside in the lung prior to infection are more critical for dampening SARS-CoV-2 infection at early stages than they are for fighting infections of other viruses. Cytotoxic lymphocytes, including CD8+ T cells and NK cells, are key early responders to virus infections and these are the cells whose baseline levels we here identify as significantly reduced in lung tissue with elevated ACE2 and TMPRSS2 expression. That these immune cells are important in preventing severe COVID-19 is supported by the fact that their levels are significantly higher in bronchoalveolar lavages from mild patients than from severe patients. Therefore, our results suggest that individuals with increased baseline susceptibility to SARS-CoV-2 infection in the lungs may also be less well equipped from the outset to mount a rapid anti-viral cellular immune response **(Fig. 3i)**.

Several observations indicate that these cytotoxic lymphocytes are critically important for effective control of SARS-CoV-2 infection. Recent studies showed that CD8+ T cells in peripheral blood are considerably reduced and functionally exhausted in COVID-19 patients, in particular in elderly patients and in severely affected patients that require ICU admission (43-45). Reduced CD8+ T cell counts also predict poor COVID-19 patient survival (43). Additionally, CD8+ T cell- and NK cell-attracting cytokines are upregulated in SARS-CoV-2-infected human lung cells and in lung tissues from COVID-19 patients and the levels of CD8+ T cells and NK cells are higher in bronchoalveolar lavages of mildly affected COVID-19 patients than in severe cases (46, 47).

We found that the five phenotypic parameters, sex, age, BMI, race and smoking history did not statistically significantly contribute to variation in ACE2 expression in human lung tissue, neither in univariate nor in multivariate analyses. This is consistent with some studies but inconsistent with others (42, 48-50). These paradoxical observations may be partially explained by varying gender, age and race distributions within each study cohort.

Further research will be required to elucidate the precise mechanisms of SARS-CoV-2-induced activation of the innate immune system early after infection. However, the link that we identified between high baseline ACE2 and TMPRSS2 expression and reduced cytotoxic lymphocyte levels in human lung tissue prior SARS-CoV-2 infection is striking. It suggests that increased susceptibility to SARS-CoV-2 infection in the lungs may be accompanied by a poorer ability to mount a rapid innate immune response at early stages. This may predict long-term outcome of individuals infected with COVID-19, given that the levels of CD8+ T cell and NK cell are significantly higher in bronchoalveolar lavages of mild cases compared to severe patients (46, 47). Finally, it may contribute to the substantial variation in COVID-19 clinical presentation, ranging from asymptomatic to severe respiratory and other symptoms.

Methods

Discovery dataset and processing

Gene expression data and corresponding phenotype data from human lung tissues (n=578) were obtained from the Genotype-Tissue Expression (GTEx) Portal (https://gtexportal.org), managed by the National Institutes of Health (NIH). Gene expression data were publicly available. Access to phenotype data required authorization. The GTEx protocol was previously described (29, 30). Briefly, total RNA was extracted from tissue. Following mRNA isolation, cDNA synthesis and library preparation, samples were subjected to HiSeq2000 or HiSeq2500 Illumina TrueSeq RNA sequencing. Gene expression levels were obtained using RNA-SeQC v1.1.9 (51) and expressed in transcripts per million (TPM). Reported expression levels were $\log_2$-transformed, unless otherwise indicated.

Validation dataset and processing

For validation purposes, the Laval University, University of British-Columbia, Groningen University (LUG) lung tissue dataset (n=1,349) was used. This dataset was accessed via Gene Expression Omnibus (GEO; https://www.ncbi.nlm.nih.gov/geo), accession number GSE23546, and was previously described (52). Briefly, total RNA from human lung tissue samples was isolated, quantified, quality-checked and used to generate cDNA, which was amplified and hybridized to Affymetrix gene expression arrays. Arrays were scanned and probe-level gene expression values were normalized using robust multichip average (RMA). These normalized values were obtained from GEO. To collapse probe-level expression data to single expression levels per gene, for each gene the probe with the highest median absolute deviation (MAD) was used. The MAD for each probe p was calculated using equation (1).

$$\mathrm{MAD}(p) = M(|p_i - M(p)|) \quad (1)$$

Herein, M is the median, p_i denotes probe p's expression level in sample i, and $M(p)$ represents the median signal of probe p.

***In silico* cytometry**

The levels of seven types of leukocytes involved in anti-viral cellular immune response, specifically CD8+ T cells, resting NK cells, activated NK cells, M1 macrophages, CD4+ T cells, dendritic cells and neutrophils, were estimated in the discovery and validation lung tissue samples using a previously described approach (31). Specifically, the following workflow was used. First, only non-log-transformed expression values were used. Thus, where required, expression values for all samples in the discovery and validation datasets were reverse-$\log_2$-transformed using equation 2.

$$c = 2^{c_l} - 1 \quad (2)$$

Herein, c denotes the calculated non-$\log_2$-transformed expression counts and c_l denotes the previously reported $\log_2$-transformed expression counts. Next, to compensate for potential technical differences between signatures and bulk sample gene expression values due to inter-platform variation, bulk-mode batch correction was applied. To ensure robustness, deconvolution was statistically analyzed using 100

permutations. Pearson correlation coefficients *R*, root mean squared errors (RMSA) and *p* values are reported on a per-sample level in **Fig. S1a-c** and **Table S1**.

Univariate statistical analyses

Log_2-transformed expression levels of ACE2 in lung tissue samples were compared to the estimated levels of seven leukocyte types or states. Pearson correlation analyses were performed to determine Pearson correlation coefficients *R* and *p* values. *P* values were adjusted at a false discovery rate of 0.05 to yield *q* values, as previously described (53). Straight lines represent the minimized sum of squares of deviations of the data points with 95% confidence intervals shown. Continuous phenotypic covariates were analyzed in the same way and, additionally, as discrete ordinal categories after binning. Discrete and binned phenotype data were statistically evaluated using Mann-Whitney *U* tests. All analyses were performed in the *R* computing environment (*R* Project for Statistical Computing, Vienna, Austria).

Multivariate regression analyses

Multivariate analyses were performed using standard ordinary least squares regression, summarized in equation 3.

$$Y = \beta_0 + \sum_{k=1}^{n} (\beta_k X_k) + \varepsilon \quad (3)$$

Herein, β_0 denotes the intercept, while β_k represents the slope of each variable X_k in a model with n variables and ε denotes the random error component. These analyses was performed using *R*.

Messenger RNA and protein levels in human cells

Available ACE2 and TMPRSS2 mRNA and corresponding protein levels in 52 and 124 human cell lines, respectively, were obtained from references (54, 55). The correlations between mRNA and protein levels were analyzed by linear regression analysis using Pearson correlations. Pearson coefficients *R* for mRNA-protein level correlations were also determined for 12,015 other genes. To test whether the ACE2 and TMPRSS2 coefficients and *p* values were statistically significantly lower than for other genes, one-sample *t* tests were used.

Messenger RNA and protein levels in human tissues

Two types of analyses were performed to compare mRNA and protein levels in human tissues. First, mRNA expression levels from 40 human tissues were obtained from the Human Protein Atlas (https://www.proteinatlas.org). These represented the consensus normalized mRNA expression levels from three sources, specifically, Human Protein Atlas in-house RNAseq data, RNAseq data from the Genotype-Tissue Expression (GTEx) project and CAGE data from FANTOM5 project. Corresponding ordinal human tissue protein expression levels ('not detected', 'low', 'high') were also obtained from the Human Protein Atlas. These were based on immunohistochemical staining of the tissues using DAB (3,3'-diaminobenzidine)-labeled antibodies (HPA000288, CAB026174), followed by knowledge-based annotation, as described on said website. A Kruskal-Wallis test was performed to assess whether mRNA and protein levels significantly correlated. Second, a meta-analysis was performed. For this, mRNA and protein expression levels were obtained from nine different sources. Tissue mRNA levels were obtained from six sources, as determined by Northern blot (56), quantitative RT-PCR (57), microarray hybridization (58), RNA sequencing (59) and cap analysis of gene expression (60). Tissue protein levels were obtained from three sources, as determined by mass spectrometry (61) and immunohistochemistry (39, 62). For each source and tissue, mRNA and protein expression levels were scored as 'not detected', 'low', 'intermediate' or 'high' and these received scores of 0-3, respectively. For each tissue, final mRNA and protein scores were calculated by averaging and scores from the respective six and three sources **(Table S4)**. Strength and significance level of the correlation between the final scores were determined by linear regression analysis using Pearson correlation.

Cytokine levels in SARS-CoV-2-infected lung cells

SARS-CoV-2-induced fold increases in the expression levels of eight cytotoxic lymphocyte-attracting cytokines, CCL2-5, CXCL9, CXCL10, CXCL16 and IL18, were determined from reference (27). These represent fold increase in expression in Calu-3 lung cells, 24 hours after infection with SARS-CoV-2 at a multiplicity of infection of 2, compared to uninfected Calu-3 cells.

Cytokine levels in control and COVID-19 lung tissues

Baseline levels of above eight cytokines in lung tissues were obtained from the GTEX project, as described above, and compared to the baseline levels of ACE2, TMPRSS2, CD8+ T cells, resting and activated NK cells in these tissues, estimated as described above. Their levels were compared using Spearman's rank correlations (*R* and *p* values). For comparison of cytokine levels in post-mortem COVID-19 lung tissues (n=2) to those in healthy, uninfected lung tissues (n=2), fold increases were determined following RNAseq analyses and previously reported (27).

Lymphocyte levels in COVID-19 bronchoalveolar lavages

The levels of CD8+ T cells, NK cells and inflammatory NK cells in bronchoalveolar lavages from mild (n=2) and severe (n=22) COVID-19 patients were reported elsewhere and determined using single-cell RNAseq (46). Statistical significance levels were assessed using Mann-Whitney *U* tests. The levels of T cells and NK cells, as well as CD8+ T cell tissue-resident signature score, in bronchoalveolar lavages from moderate (n=3) and severe/critical (n=5) COVID-19 patients were reported in another study (47).

Acknowledgements

I thank Dr. Marianna Datseris, M.D. for critically reading the manuscript. This work was supported by the School of Biomedical Sciences, Queensland University of Technology. This study used publicly available and controlled data from the Genotype-Tissue Expression (GTEx) Project. GTEx was supported by the Common Fund of the Office of the Director of the National Institutes of Health (commonfund.nih.gov/GTEx). Additional funds were provided by the NCI, NHGRI, NHLBI, NIDA, NIMH, and NINDS. Donors were enrolled at Biospecimen Source Sites funded by NCI\Leidos Biomedical Research, Inc. subcontracts to the National Disease Research Interchange (10XS170), Roswell Park Cancer Institute (10XS171), and Science Care, Inc. (X10S172). The Laboratory, Data Analysis, and Coordinating Center (LDACC) was funded through a contract (HHSN268201000029C) to the The Broad Institute, Inc. Biorepository operations were funded through a Leidos Biomedical Research, Inc. subcontract to Van Andel Research Institute (10ST1035). Additional data repository and project management were provided by Leidos Biomedical Research, Inc. (HHSN261200800001E). The

Brain Bank was supported supplements to University of Miami grant DA006227. Statistical Methods development grants were made to the University of Geneva (MH090941 & MH101814), the University of Chicago (MH090951, MH090937, MH101825, & MH101820), the University of North Carolina - Chapel Hill (MH090936), North Carolina State University (MH101819), Harvard University (MH090948), Stanford University (MH101782), Washington University (MH101810), and to the University of Pennsylvania (MH101822). The datasets used for the analyses described in this study were obtained from dbGaP at http://www.ncbi.nlm.nih.gov/gap through dbGaP accession number phs000424.v8.p2.

References

1. Coronaviridae Study Group of the International Committee on Taxonomy of V. 2020. The species Severe acute respiratory syndrome-related coronavirus: classifying 2019-nCoV and naming it SARS-CoV-2. Nat Microbiol 5:536-544. https://doi.org/10.1038/s41564-020-0695-z.
2. Liu Y, Yan LM, Wan L, Xiang TX, Le A, Liu JM, Peiris M, Poon LLM, Zhang W. 2020. Viral dynamics in mild and severe cases of COVID-19. Lancet Infect Dis https://doi.org/10.1016/S1473-3099(20)30232-2.
3. Gattinoni L, Chiumello D, Caironi P, Busana M, Romitti F, Brazzi L, Camporota L. 2020. COVID-19 pneumonia: different respiratory treatments for different phenotypes? Intensive Care Med https://doi.org/10.1007/s00134-020-06033-2.
4. Xie M and Chen Q. 2020. Insight into 2019 novel coronavirus - An updated interim review and lessons from SARS-CoV and MERS-CoV. Int J Infect Dis 94:119-124. https://doi.org/10.1016/j.ijid.2020.03.071.
5. 2020. Coronavirus disease 2019 (COVID-19), Situation Report 102. World Health Organization
6. Li R, Pei S, Chen B, Song Y, Zhang T, Yang W, Shaman J. 2020. Substantial undocumented infection facilitates the rapid dissemination of novel coronavirus (SARS-CoV-2). Science 368:489-493. https://doi.org/10.1126/science.abb3221.
7. Hoffmann M, Kleine-Weber H, Schroeder S, Kruger N, Herrler T, Erichsen S, Schiergens TS, Herrler G, Wu NH, Nitsche A, Muller MA, Drosten C, Pohlmann S. 2020. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell 181:271-280 e8. https://doi.org/10.1016/j.cell.2020.02.052.
8. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W, Si HR, Zhu Y, Li B, Huang CL, Chen HD, Chen J, Luo Y, Guo H, Jiang RD, Liu MQ, Chen Y, Shen XR, Wang X, Zheng XS, Zhao K, Chen QJ, Deng F, Liu LL, Yan B, Zhan FX, Wang YY, Xiao GF, Shi ZL. 2020. A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 579:270-273. https://doi.org/10.1038/s41586-020-2012-7.
9. Wrapp D, Wang N, Corbett KS, Goldsmith JA, Hsieh CL, Abiona O, Graham BS, McLellan JS. 2020. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science 367:1260-1263. https://doi.org/10.1126/science.abb2507.
10. Hofmann H, Geier M, Marzi A, Krumbiegel M, Peipp M, Fey GH, Gramberg T, Pohlmann S. 2004. Susceptibility to SARS coronavirus S protein-driven infection correlates with expression of

angiotensin converting enzyme 2 and infection can be blocked by soluble receptor. Biochem Biophys Res Commun 319:1216-21. https://doi.org/10.1016/j.bbrc.2004.05.114.

11. Mossel EC, Huang C, Narayanan K, Makino S, Tesh RB, Peters CJ. 2005. Exogenous ACE2 expression allows refractory cell lines to support severe acute respiratory syndrome coronavirus replication. J Virol 79:3846-50. https://doi.org/10.1128/JVI.79.6.3846-3850.2005.
12. Li W, Moore MJ, Vasilieva N, Sui J, Wong SK, Berne MA, Somasundaran M, Sullivan JL, Luzuriaga K, Greenough TC, Choe H, Farzan M. 2003. Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature 426:450-4. https://doi.org/10.1038/nature02145.
13. Fang L, Karakiulakis G, Roth M. 2020. Are patients with hypertension and diabetes mellitus at increased risk for COVID-19 infection? Lancet Respir Med 8:e21. https://doi.org/10.1016/S2213-2600(20)30116-8.
14. Guo J, Huang Z, Lin L, Lv J. 2020. Coronavirus Disease 2019 (COVID-19) and Cardiovascular Disease: A Viewpoint on the Potential Influence of Angiotensin-Converting Enzyme Inhibitors/Angiotensin Receptor Blockers on Onset and Severity of Severe Acute Respiratory Syndrome Coronavirus 2 Infection. J Am Heart Assoc 9:e016219. https://doi.org/10.1161/JAHA.120.016219.
15. Zhuang MW, Cheng Y, Zhang J, Jiang XM, Wang L, Deng J, Wang PH. 2020. Increasing host cellular receptor-angiotensin-converting enzyme 2 expression by coronavirus may facilitate 2019-nCoV (or SARS-CoV-2) infection. J Med Virol https://doi.org/10.1002/jmv.26139.
16. Sungnak W, Huang N, Becavin C, Berg M, Queen R, Litvinukova M, Talavera-Lopez C, Maatz H, Reichart D, Sampaziotis F, Worlock KB, Yoshida M, Barnes JL, Network HCALB. 2020. SARS-CoV-2 entry factors are highly expressed in nasal epithelial cells together with innate immune genes. Nat Med https://doi.org/10.1038/s41591-020-0868-6.
17. Chu H, Chan JF, Wang Y, Yuen TT, Chai Y, Hou Y, Shuai H, Yang D, Hu B, Huang X, Zhang X, Cai JP, Zhou J, Yuan S, Kok KH, To KK, Chan IH, Zhang AJ, Sit KY, Au WK, Yuen KY. 2020. Comparative replication and immune activation profiles of SARS-CoV-2 and SARS-CoV in human lungs: an ex vivo study with implications for the pathogenesis of COVID-19. Clin Infect Dis https://doi.org/10.1093/cid/ciaa410.
18. Chu H, Chan JFW, Yuen TTT, Shuai H, Yuan S, Wang Y, Hu B, Yip CCY, Tsang JOL, Huang X, Chai Y, Yang D, Hou Y, Chik KKH, Zhang X, Fung AYF, Tsoi HY, Cai JP, Chan WM, Ip JD, Chu AWH, Zhou J, Lung DC, Kok KH, To KKW, Tsang OTY, Chan KH, Yuen KY. 2020. Comparative tropism, replication kinetics, and cell damage profiling of SARS-CoV-2 and SARS-CoV with implications for clinical manifestations, transmissibility, and laboratory studies of COVID-19: an observational study. Lancet Microbe April 21, 2020:1-10. https://doi.org/10.1016/S2666-5247(20)30004-5.
19. Newton AH, Cardani A, Braciale TJ. 2016. The host immune response in respiratory virus infection: balancing virus clearance and immunopathology. Semin Immunopathol 38:471-82. https://doi.org/10.1007/s00281-016-0558-0.
20. Crouse J, Kalinke U, Oxenius A. 2015. Regulation of antiviral T cell responses by type I interferons. Nat Rev Immunol 15:231-42. https://doi.org/10.1038/nri3806.
21. Schmidt ME and Varga SM. 2018. The CD8 T Cell Response to Respiratory Virus Infections. Front Immunol 9:678. https://doi.org/10.3389/fimmu.2018.00678.
22. Hammer Q, Ruckert T, Romagnani C. 2018. Natural killer cell specificity for viral infections. Nat Immunol 19:800-808. https://doi.org/10.1038/s41590-018-0163-6.
23. Cook PC and MacDonald AS. 2016. Dendritic cells in lung immunopathology. Semin Immunopathol 38:449-60. https://doi.org/10.1007/s00281-016-0571-3.
24. Sant AJ and McMichael A. 2012. Revealing the role of CD4(+) T cells in viral immunity. J Exp Med 209:1391-5. https://doi.org/10.1084/jem.20121517.
25. Harper RW. 2017. Partners in Crime: Epithelial Priming of Macrophages during Viral Infection. Am J Respir Cell Mol Biol 57:145-146. https://doi.org/10.1165/rcmb.2017-0153ED.

26. Galani IE and Andreakos E. 2015. Neutrophils in viral infections: Current concepts and caveats. J Leukoc Biol 98:557-64. https://doi.org/10.1189/jlb.4VMR1114-555R.
27. Blanco-Melo D, Nilsson-Payant BE, Liu WC, Uhl S, Hoagland D, Moller R, Jordan TX, Oishi K, Panis M, Sachs D, Wang TT, Schwartz RE, Lim JK, Albrecht RA, tenOever BR. 2020. Imbalanced Host Response to SARS-CoV-2 Drives Development of COVID-19. Cell 181:1036-1045 e9. https://doi.org/10.1016/j.cell.2020.04.026.
28. Ziegler CGK, Allon SJ, Nyquist SK, Mbano IM, Miao VN, Tzouanas CN, Cao Y, Yousif AS, Bals J, Hauser BM, Feldman J, Muus C, Wadsworth MH, 2nd, Kazer SW, Hughes TK, Doran B, Gatter GJ, Vukovic M, Taliaferro F, Mead BE, Guo Z, Wang JP, Gras D, Plaisant M, Ansari M, Angelidis I, Adler H, Sucre JMS, Taylor CJ, Lin B, Waghray A, Mitsialis V, Dwyer DF, Buchheit KM, Boyce JA, Barrett NA, Laidlaw TM, Carroll SL, Colonna L, Tkachev V, Peterson CW, Yu A, Zheng HB, Gideon HP, Winchell CG, Lin PL, Bingle CD, Snapper SB, Kropski JA, et al. 2020. SARS-CoV-2 Receptor ACE2 Is an Interferon-Stimulated Gene in Human Airway Epithelial Cells and Is Detected in Specific Cell Subsets across Tissues. Cell 181:1016-1035 e19. https://doi.org/10.1016/j.cell.2020.04.035.
29. Consortium GT. 2013. The Genotype-Tissue Expression (GTEx) project. Nat Genet 45:580-5. https://doi.org/10.1038/ng.2653.
30. Consortium GT, Laboratory DA, Coordinating Center -Analysis Working G, Statistical Methods groups-Analysis Working G, Enhancing Gg, Fund NIHC, Nih/Nci, Nih/Nhgri, Nih/Nimh, Nih/Nida, Biospecimen Collection Source Site N, Biospecimen Collection Source Site R, Biospecimen Core Resource V, Brain Bank Repository-University of Miami Brain Endowment B, Leidos Biomedical-Project M, Study E, Genome Browser Data I, Visualization EBI, Genome Browser Data I, Visualization-Ucsc Genomics Institute UoCSC, Lead a, Laboratory DA, Coordinating C, management NIHp, Biospecimen c, Pathology, e QTLmwg, Battle A, Brown CD, Engelhardt BE, Montgomery SB. 2017. Genetic effects on gene expression across human tissues. Nature 550:204-213. https://doi.org/10.1038/nature24277.
31. Newman AM, Liu CL, Green MR, Gentles AJ, Feng W, Xu Y, Hoang CD, Diehn M, Alizadeh AA. 2015. Robust enumeration of cell subsets from tissue expression profiles. Nat Methods 12:453-7. https://doi.org/10.1038/nmeth.3337.
32. Brown CE, Vishwanath RP, Aguilar B, Starr R, Najbauer J, Aboody KS, Jensen MC. 2007. Tumor-derived chemokine MCP-1/CCL2 is sufficient for mediating tumor tropism of adoptively transferred T cells. J Immunol 179:3332-41. https://doi.org/10.4049/jimmunol.179.5.3332.
33. Serody JS, Burkett SE, Panoskaltsis-Mortari A, Ng-Cashin J, McMahon E, Matsushima GK, Lira SA, Cook DN, Blazar BR. 2000. T-lymphocyte production of macrophage inflammatory protein-1alpha is critical to the recruitment of CD8(+) T cells to the liver, lung, and spleen during graft-versus-host disease. Blood 96:2973-80.
34. Zumwalt TJ, Arnold M, Goel A, Boland CR. 2015. Active secretion of CXCL10 and CCL5 from colorectal cancer microenvironments associates with GranzymeB+ CD8+ T-cell infiltration. Oncotarget 6:2981-91. https://doi.org/10.18632/oncotarget.3205.
35. Spranger S, Dai D, Horton B, Gajewski TF. 2017. Tumor-Residing Batf3 Dendritic Cells Are Required for Effector T Cell Trafficking and Adoptive T Cell Therapy. Cancer Cell 31:711-723 e4. https://doi.org/10.1016/j.ccell.2017.04.003.
36. Wendel M, Galani IE, Suri-Payer E, Cerwenka A. 2008. Natural killer cell accumulation in tumors is dependent on IFN-gamma and CXCR3 ligands. Cancer Res 68:8437-45. https://doi.org/10.1158/0008-5472.CAN-08-1440.
37. Fan Z, Yu P, Wang Y, Wang Y, Fu ML, Liu W, Sun Y, Fu YX. 2006. NK-cell activation by LIGHT triggers tumor-specific CD8+ T-cell immunity to reject established tumors. Blood 107:1342-51. https://doi.org/10.1182/blood-2005-08-3485.
38. Bertram S, Heurich A, Lavender H, Gierer S, Danisch S, Perin P, Lucas JM, Nelson PS, Pohlmann S, Soilleux EJ. 2012. Influenza and SARS-coronavirus activating proteases TMPRSS2 and HAT are

expressed at multiple sites in human respiratory and gastrointestinal tracts. PLoS One 7:e35876. https://doi.org/10.1371/journal.pone.0035876.

39. Hamming I, Timens W, Bulthuis ML, Lely AT, Navis G, van Goor H. 2004. Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. J Pathol 203:631-7. https://doi.org/10.1002/path.1570.
40. Zou X, Chen K, Zou J, Han P, Hao J, Han Z. 2020. Single-cell RNA-seq data analysis on the receptor ACE2 expression reveals the potential risk of different human organs vulnerable to 2019-nCoV infection. Front Med https://doi.org/10.1007/s11684-020-0754-0.
41. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, Zhang L, Fan G, Xu J, Gu X, Cheng Z, Yu T, Xia J, Wei Y, Wu W, Xie X, Yin W, Li H, Liu M, Xiao Y, Gao H, Guo L, Xie J, Wang G, Jiang R, Gao Z, Jin Q, Wang J, Cao B. 2020. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet 395:497-506. https://doi.org/10.1016/S0140-6736(20)30183-5.
42. Chan JF, Yuan S, Kok KH, To KK, Chu H, Yang J, Xing F, Liu J, Yip CC, Poon RW, Tsoi HW, Lo SK, Chan KH, Poon VK, Chan WM, Ip JD, Cai JP, Cheng VC, Chen H, Hui CK, Yuen KY. 2020. A familial cluster of pneumonia associated with the 2019 novel coronavirus indicating person-to-person transmission: a study of a family cluster. Lancet 395:514-523. https://doi.org/10.1016/S0140-6736(20)30154-9.
43. Diao B, Wang C, Tan Y, Chen X, Liu Y, Ning L, Chen L, Li M, Liu Y, Wang G, Yuan Z, Feng Z, Zhang Y, Wu Y, Chen Y. 2020. Reduction and Functional Exhaustion of T Cells in Patients With Coronavirus Disease 2019 (COVID-19). Front Immunol 11:827. https://doi.org/10.3389/fimmu.2020.00827.
44. Zheng M, Gao Y, Wang G, Song G, Liu S, Sun D, Xu Y, Tian Z. 2020. Functional exhaustion of antiviral lymphocytes in COVID-19 patients. Cell Mol Immunol https://doi.org/10.1038/s41423-020-0402-2.
45. Liu J, Li S, Liu J, Liang B, Wang X, Wang H, Li W, Tong Q, Yi J, Zhao L, Xiong L, Guo C, Tian J, Luo J, Yao J, Pang R, Shen H, Peng C, Liu T, Zhang Q, Wu J, Xu L, Lu S, Wang B, Weng Z, Han C, Zhu H, Zhou R, Zhou H, Chen X, Ye P, Zhu B, Wang L, Zhou W, He S, He Y, Jie S, Wei P, Zhang J, Lu Y, Wang W, Zhang L, Li L, Zhou F, Wang J, Dittmer U, Lu M, Hu Y, Yang D, et al. 2020. Longitudinal characteristics of lymphocyte responses and cytokine profiles in the peripheral blood of SARS-CoV-2 infected patients. EBioMedicine 55:102763. https://doi.org/10.1016/j.ebiom.2020.102763.
46. Wauters E, Van Mol P, Garg AD, Jansen S, Van Herck Y, Vanderbeke L, Bassez A, Boeckx B, Malengier-Devlies B, Timmerman A, Van Brussel T, Van Buyten T, Schepers R, Heylen E, Dauwe D, Dooms C, Gunst J, Hermans G, Meersseman P, Testelmans D, Yserbyt J, Matthys P, Tejpar S, CONTAGIOUS collaborators, Neyts J, Wauters J, Qian J, Lambrechts D. 2020. Discriminating mild from critical COVID-19 by innate and adaptive immune single-cell profiling of bronchoalveolar lavages. bioRxiv Preprint:10 July 2020. https://doi.org/10.1101/2020.07.09.196519.
47. Liao M, Liu Y, Yuan J, Wen Y, Xu G, Zhao J, Cheng L, Li J, Wang X, Wang F, Liu L, Amit I, Zhang S, Zhang Z. 2020. Single-cell landscape of bronchoalveolar immune cells in patients with COVID-19. Nat Med 26:842-844. https://doi.org/10.1038/s41591-020-0901-9.
48. Lloyd-Sherlock P, Ebrahim S, Geffen L, McKee M. 2020. Bearing the brunt of covid-19: older people in low and middle income countries. BMJ 368:m1052. https://doi.org/10.1136/bmj.m1052.
49. Yang X, Yu Y, Xu J, Shu H, Xia J, Liu H, Wu Y, Zhang L, Yu Z, Fang M, Yu T, Wang Y, Pan S, Zou X, Yuan S, Shang Y. 2020. Clinical course and outcomes of critically ill patients with SARS-CoV-2 pneumonia in Wuhan, China: a single-centered, retrospective, observational study. Lancet Respir Med https://doi.org/10.1016/S2213-2600(20)30079-5.
50. Smith JC, Sausville EL, Girish V, Yuan ML, Vasudevan A, John KM, Sheltzer JM. 2020. Cigarette Smoke Exposure and Inflammatory Signaling Increase the Expression of the SARS-CoV-2 Receptor ACE2 in the Respiratory Tract. Dev Cell 53:514-529 e3. https://doi.org/10.1016/j.devcel.2020.05.012.

51. DeLuca DS, Levin JZ, Sivachenko A, Fennell T, Nazaire MD, Williams C, Reich M, Winckler W, Getz G. 2012. RNA-SeQC: RNA-seq metrics for quality control and process optimization. Bioinformatics 28:1530-2. https://doi.org/10.1093/bioinformatics/bts196.
52. Obeidat M, Hao K, Bosse Y, Nickle DC, Nie Y, Postma DS, Laviolette M, Sandford AJ, Daley DD, Hogg JC, Elliott WM, Fishbane N, Timens W, Hysi PG, Kaprio J, Wilson JF, Hui J, Rawal R, Schulz H, Stubbe B, Hayward C, Polasek O, Jarvelin MR, Zhao JH, Jarvis D, Kahonen M, Franceschini N, North KE, Loth DW, Brusselle GG, Smith AV, Gudnason V, Bartz TM, Wilk JB, O'Connor GT, Cassano PA, Tang W, Wain LV, Soler Artigas M, Gharib SA, Strachan DP, Sin DD, Tobin MD, London SJ, Hall IP, Pare PD. 2015. Molecular mechanisms underlying variations in lung function: a systems genetics analysis. Lancet Respir Med 3:782-95. https://doi.org/10.1016/S2213-2600(15)00380-X.
53. Benjamini Y and Hochberg Y. 1995. Controlling the False Discovery Rate: A practical and powerful approach to multiple testing. J R Stat Soc Ser B-Stat Methodol 57:289-300.
54. Ghandi M, Huang FW, Jane-Valbuena J, Kryukov GV, Lo CC, McDonald ER, 3rd, Barretina J, Gelfand ET, Bielski CM, Li H, Hu K, Andreev-Drakhlin AY, Kim J, Hess JM, Haas BJ, Aguet F, Weir BA, Rothberg MV, Paolella BR, Lawrence MS, Akbani R, Lu Y, Tiv HL, Gokhale PC, de Weck A, Mansour AA, Oh C, Shih J, Hadi K, Rosen Y, Bistline J, Venkatesan K, Reddy A, Sonkin D, Liu M, Lehar J, Korn JM, Porter DA, Jones MD, Golji J, Caponigro G, Taylor JE, Dunning CM, Creech AL, Warren AC, McFarland JM, Zamanighomi M, Kauffmann A, Stransky N, et al. 2019. Next-generation characterization of the Cancer Cell Line Encyclopedia. Nature 569:503-508. https://doi.org/10.1038/s41586-019-1186-3.
55. Nusinow DP, Szpyt J, Ghandi M, Rose CM, McDonald ER, 3rd, Kalocsay M, Jane-Valbuena J, Gelfand E, Schweppe DK, Jedrychowski M, Golji J, Porter DA, Rejtar T, Wang YK, Kryukov GV, Stegmeier F, Erickson BK, Garraway LA, Sellers WR, Gygi SP. 2020. Quantitative Proteomics of the Cancer Cell Line Encyclopedia. Cell 180:387-402 e16. https://doi.org/10.1016/j.cell.2019.12.023.
56. Tipnis SR, Hooper NM, Hyde R, Karran E, Christie G, Turner AJ. 2000. A human homolog of angiotensin-converting enzyme. Cloning and functional expression as a captopril-insensitive carboxypeptidase. J Biol Chem 275:33238-43. https://doi.org/10.1074/jbc.M002615200.
57. Harmer D, Gilbert M, Borman R, Clark KL. 2002. Quantitative mRNA expression profiling of ACE 2, a novel homologue of angiotensin converting enzyme. FEBS Lett 532:107-10. https://doi.org/10.1016/s0014-5793(02)03640-2.
58. Wu C, Jin X, Tsueng G, Afrasiabi C, Su AI. 2016. BioGPS: building your own mash-up of gene annotations and expression profiles. Nucleic Acids Res 44:D313-6. https://doi.org/10.1093/nar/gkv1104.
59. Uhlen M, Zhang C, Lee S, Sjostedt E, Fagerberg L, Bidkhori G, Benfeitas R, Arif M, Liu Z, Edfors F, Sanli K, von Feilitzen K, Oksvold P, Lundberg E, Hober S, Nilsson P, Mattsson J, Schwenk JM, Brunnstrom H, Glimelius B, Sjoblom T, Edqvist PH, Djureinovic D, Micke P, Lindskog C, Mardinoglu A, Ponten F. 2017. A pathology atlas of the human cancer transcriptome. Science 357:https://doi.org/10.1126/science.aan2507.
60. Consortium F, the RP, Clst, Forrest AR, Kawaji H, Rehli M, Baillie JK, de Hoon MJ, Haberle V, Lassmann T, Kulakovskiy IV, Lizio M, Itoh M, Andersson R, Mungall CJ, Meehan TF, Schmeier S, Bertin N, Jorgensen M, Dimont E, Arner E, Schmidl C, Schaefer U, Medvedeva YA, Plessy C, Vitezic M, Severin J, Semple C, Ishizu Y, Young RS, Francescatto M, Alam I, Albanese D, Altschuler GM, Arakawa T, Archer JA, Arner P, Babina M, Rennie S, Balwierz PJ, Beckhouse AG, Pradhan-Bhatt S, Blake JA, Blumenthal A, Bodega B, Bonetti A, Briggs J, Brombacher F, Burroughs AM, et al. 2014. A promoter-level mammalian expression atlas. Nature 507:462-70. https://doi.org/10.1038/nature13182.
61. Kim MS, Pinto SM, Getnet D, Nirujogi RS, Manda SS, Chaerkady R, Madugundu AK, Kelkar DS, Isserlin R, Jain S, Thomas JK, Muthusamy B, Leal-Rojas P, Kumar P, Sahasrabuddhe NA, Balakrishnan L, Advani J, George B, Renuse S, Selvan LD, Patil AH, Nanjappa V, Radhakrishnan A, Prasad S, Subbannayya T, Raju R, Kumar M, Sreenivasamurthy SK, Marimuthu A, Sathe GJ,

Chavan S, Datta KK, Subbannayya Y, Sahu A, Yelamanchi SD, Jayaram S, Rajagopalan P, Sharma J, Murthy KR, Syed N, Goel R, Khan AA, Ahmad S, Dey G, Mudgal K, Chatterjee A, Huang TC, Zhong J, Wu X, et al. 2014. A draft map of the human proteome. Nature 509:575-81. https://doi.org/10.1038/nature13302.

62. Thul PJ, Akesson L, Wiking M, Mahdessian D, Geladaki A, Ait Blal H, Alm T, Asplund A, Bjork L, Breckels LM, Backstrom A, Danielsson F, Fagerberg L, Fall J, Gatto L, Gnann C, Hober S, Hjelmare M, Johansson F, Lee S, Lindskog C, Mulder J, Mulvey CM, Nilsson P, Oksvold P, Rockberg J, Schutten R, Schwenk JM, Sivertsson A, Sjostedt E, Skogs M, Stadler C, Sullivan DP, Tegel H, Winsnes C, Zhang C, Zwahlen M, Mardinoglu A, Ponten F, von Feilitzen K, Lilley KS, Uhlen M, Lundberg E. 2017. A subcellular map of the human proteome. Science 356:https://doi.org/10.1126/science.aal3321.

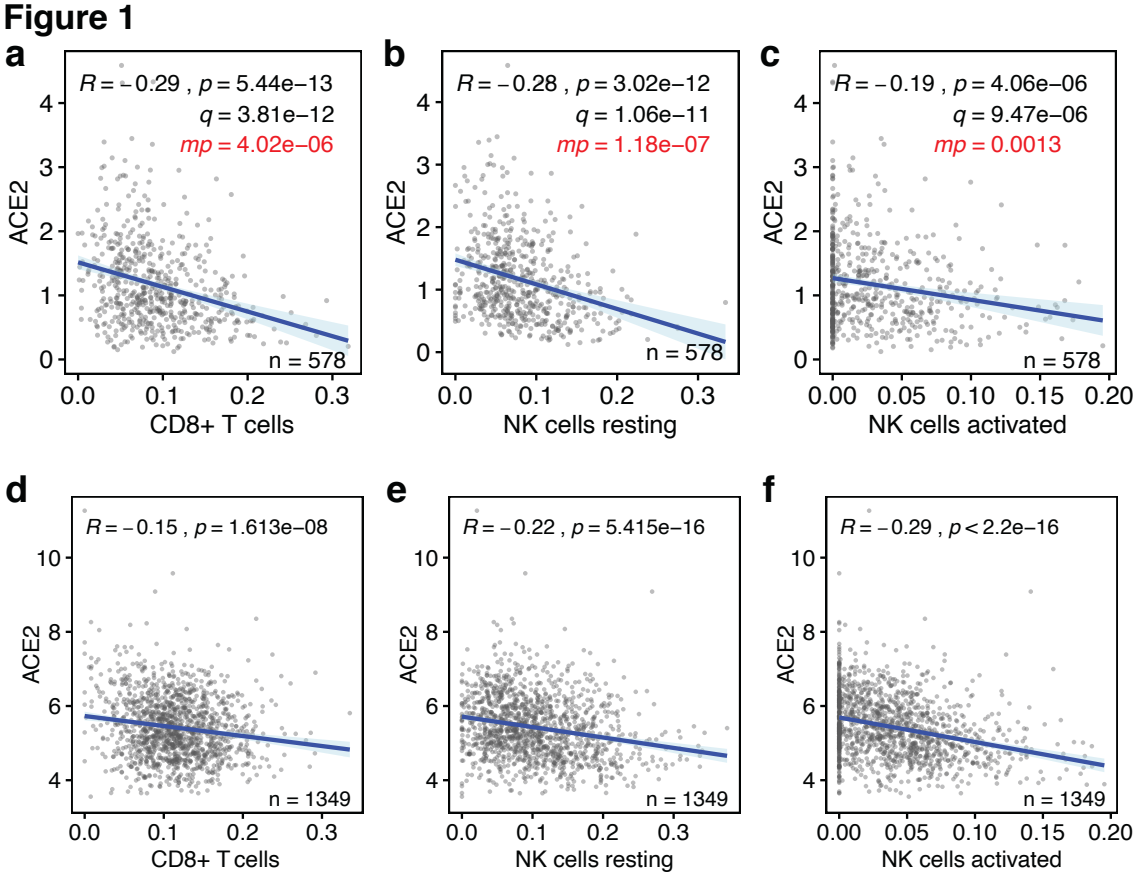


Figure 1. Baseline levels of cytotoxic lymphocytes inversely correlate with ACE2 expression in the lung.

(a-c) The correlations between the baseline levels of CD8+ T cells, resting NK cells and activated NK cells (x-axes) and the those of the SARS-CoV-2 host cell receptor ACE2 (y-axes) in human lung tissue are shown. Data are from the GTEx dataset (n=578). **(d-f)** The same correlations are shown for lung tissues in the LUG dataset (n=1,349). Regression lines and 95% confidence intervals are shown. *R* and *p* values: Pearson correlations; *q* values: Benjamini-Hochberg-adjusted *p* values using a false discovery rate of 0.05; *mp* values: multivariate *p* values (see Table S3).

Figure 2

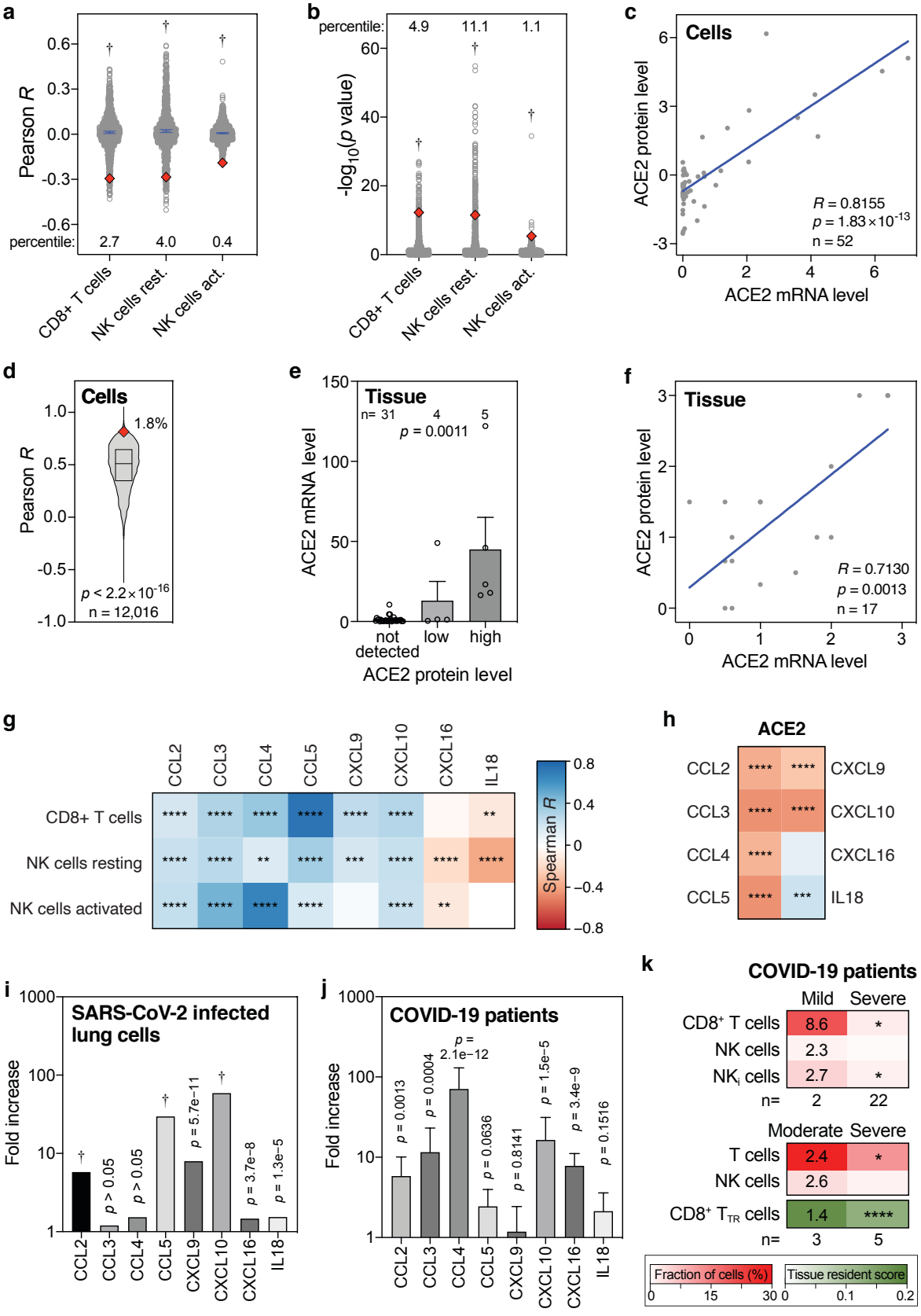


Figure 2. Levels of ACE2 mRNA, ACE2 protein, CD8+ T cells, NK cells and cytokines in lung cells, lung tissues and COVID-19 patient samples.

(a, b) Pearson *R* and -$\log_{10}$(*p* values) of correlations between 1000 randomly sampled genes and the levels of indicated lymphocytes in lung tissues were determined and plotted. ACE2 Pearson *R* and *p* values are shown as red diamonds. Blue lines indicate means with 95% confidence intervals. Percentiles for ACE2 with respect to the 1000 random *R* and *p* values are shown. Data are from the GTEx dataset (n=578). *P* values: one-sample *t* tests. **(c)** Correlations between ACE2 mRNA and protein levels in 52 cell lines. *R* and *p* values: Pearson correlations. **(d)** Pearson correlation *R* values between mRNA and protein levels of 12,016 genes are compared to the ACE2 *R* coefficient (red diamond). The box represents the median and interquartile range. The ACE2 *R* percentile is also shown. *P* value: one-sample *t* test. **(e)** Bar graph showing the correlation between ACE2 mRNA and protein levels in human tissues. Means with standard errors of the means are shown. Samples are from the Human Protein Atlas. *P* value: Kruskal-Wallis test. **(f)** Meta-analysis scatter plot showing the correlation between ACE2 mRNA and protein levels in 17 human tissues. Data are from 9 different studies, as detailed in Table S4. *P* value: Pearson correlation. **(g, h)** Heatmaps showing Spearman correlations between the levels of ACE2 or indicated cytotoxic lymphocytes and eight cytokines that recruit these cells in human lung tissues from the GTEx dataset (n=578). Colors of tiles represent Spearman *R*, as per scale bar on the right. Spearman significance levels are shown by asterisks. See also Fig. S5. **(i, j)** Fold increase in expression levels of indicated cytokines in Calu-3 lung cells 24 hours after SARS-CoV-2 infection compared to uninfected Calu-3 cells (i), and in post-mortem COVID-19 lung tissues (n=2) to those in healthy, uninfected lung tissues (n=2) (j). **(k)** Comparison of indicated fractions of lymphocyte levels in bronchoalveolar lavages of mild/moderate and severe COVID-19 patients, as determined in two separate studies (46, 47). The second also determined a tissue resident (TR) score for CD8+ T cells. Numbers in the mild/moderate column on the left show fold increase compared to the respective severe cases on the right. Asterisks in the severe column on the right represent statistical significance levels, as determined my Mann-Whitney *U* tests (top panel), or *t* tests (middle and bottom panels) comparing mild/moderate to severe cases. *P* value abbreviations: *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$; ****, $p<0.0001$; †, $p<2.2\text{x}10^{-16}$.

Figure 3

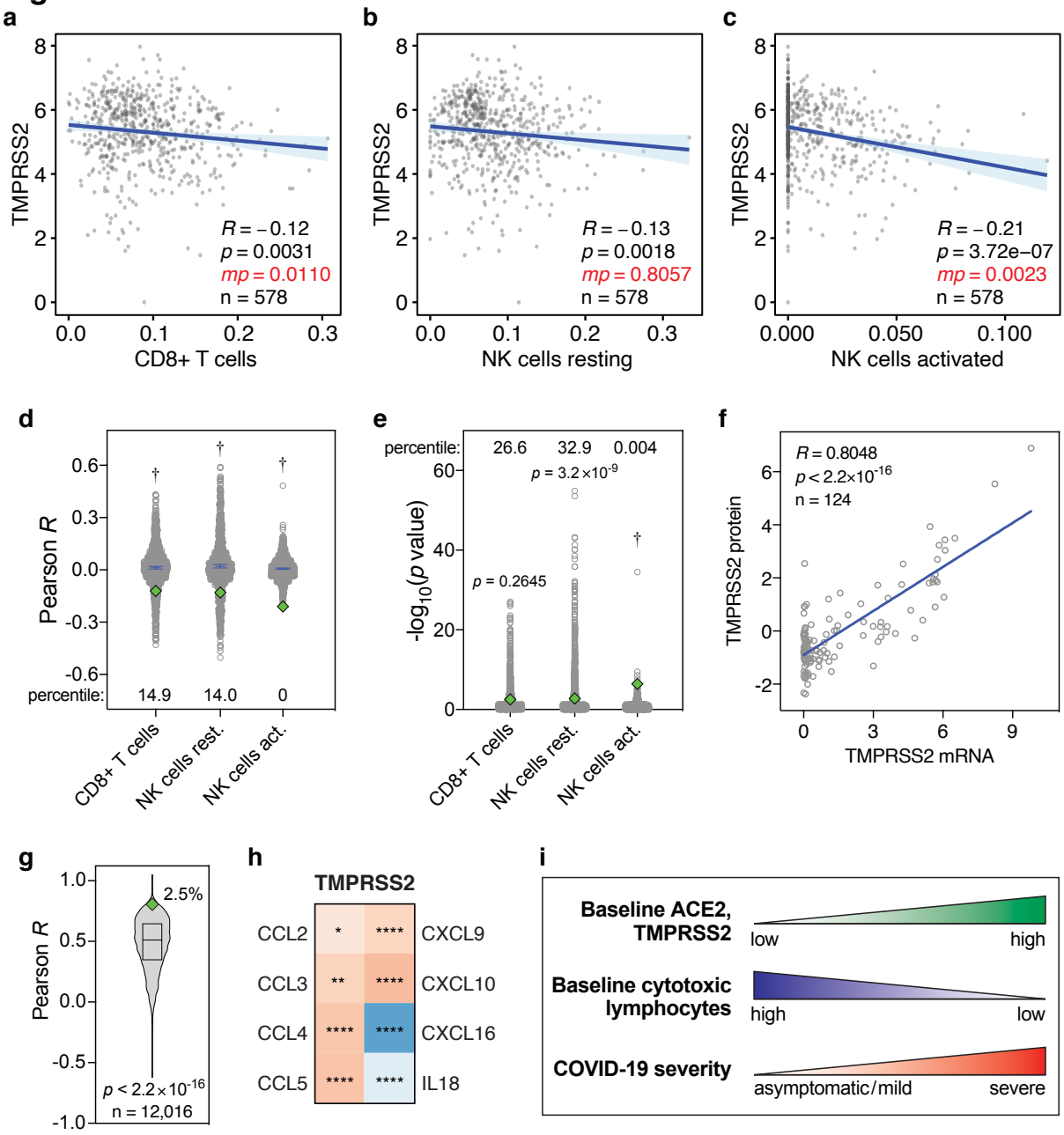


Figure 3. Levels of TMPRSS2 mRNA, TMPRSS2 protein and cytokines in lung cells and tissues. **(a-c)** Pearson correlations between baseline levels of indicated lymphocytes and TMPRSS2 in human lung tissue, as Figure 1a-c. Data are from the GTEx dataset (n=578). **(d, e)** Pearson *R* and -log$_{10}$(*p* values) of correlations between 1000 randomly sampled genes and the levels of indicated lymphocytes in lung tissues, as in Figure 2a,b. TMPRSS2 Pearson *R* and *p* values are shown as green diamonds. **(f)** Correlations between TMPRSS2 mRNA and protein levels in 124 cell lines. *R* and *p* values: Pearson correlations. **(g)** Pearson correlation *R* values between mRNA and protein levels of 12,016 genes are compared to the TMPRSS2 *R* coefficient (green diamond). The box represents the median and interquartile range. The TMPRSS2 *R* percentile is also shown. *P* value: one-sample *t* test. **(h)** Heatmap showing Spearman correlations between the levels of TMPRSS2 and cytokines in human lung tissues from the GTEx dataset (n=578), as in Figure 2h. **(i)** Individuals with high baseline levels of ACE2 and TMPRSS2 show low baseline tissue-resident levels of cytotoxic lymphocytes in the lung. We propose that this may jointly predispose these individuals to development of severe COVID-19.

Supplementary Information

Low baseline pulmonary levels of cytotoxic lymphocytes as a predisposing risk factor for severe COVID-19

Pascal H.G. Duijf

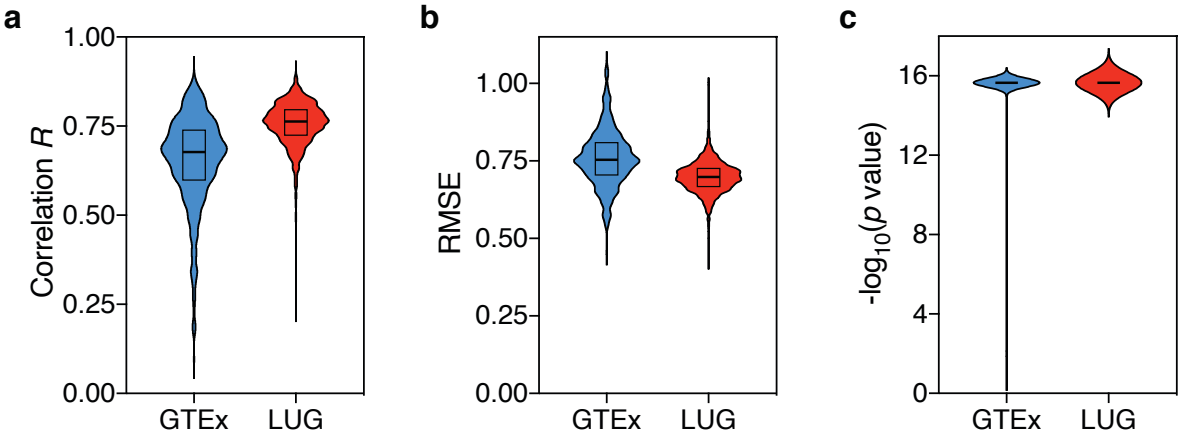


Figure S1. *In silico* cytometry statistics.
Per-sample statistics of *in silico* cytometry on human lung tissues are shown for the GTEx (n=578) and LUG (n=1,349) datasets. **(a)** Pearson correlation coefficients *R*. **(b)** Root mean square errors (RMSE). **(c)** *p* values. *P* values < 2.2×10^{-16} were processed as equal to 2.2×10^{-16}. Source data are provided in Table S1.

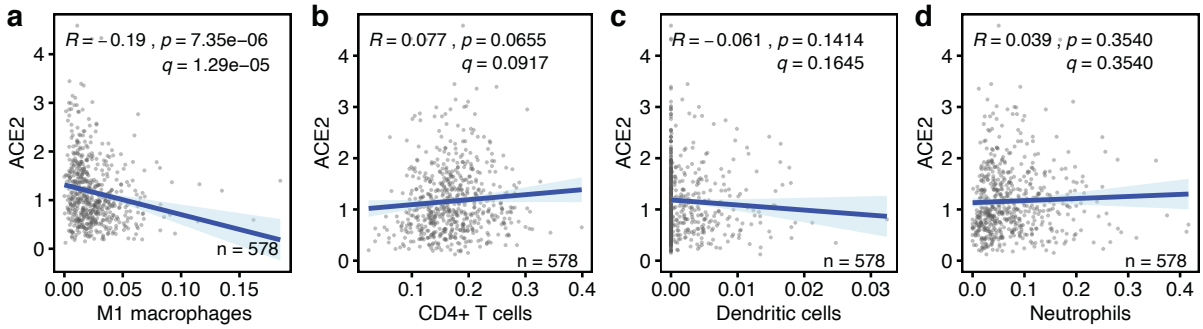
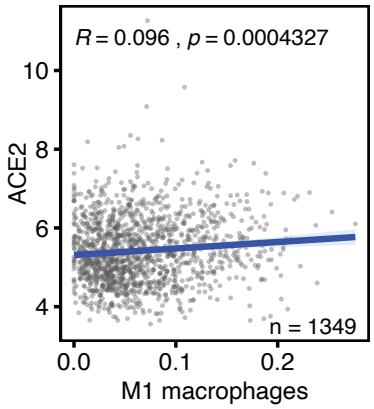


Figure S2. Correlations between baseline anti-viral leukocyte levels and ACE2 expression levels in human lung tissues.
(a-d) The correlations between the baseline levels of indicated immune cell types (x-axes) and the expression level of the SARS-CoV-2 host cell receptor ACE2 (y-axis) in human lung tissue are shown. Data are from the GTEx dataset (n=578). Regression lines and 95% confidence intervals are shown. *R* and *p* values: Pearson correlations; *q* values: Benjamini-Hochberg-adjusted *p* values using a false discovery rate of 0.05. See also Figure 1.

Figure S3. Correlation between baseline M1 macrophage levels and ACE2 expression level in human lung tissue.
The correlation between these variables is shown as described in Figure S2. Data are from the LUG dataset (n=1,349).



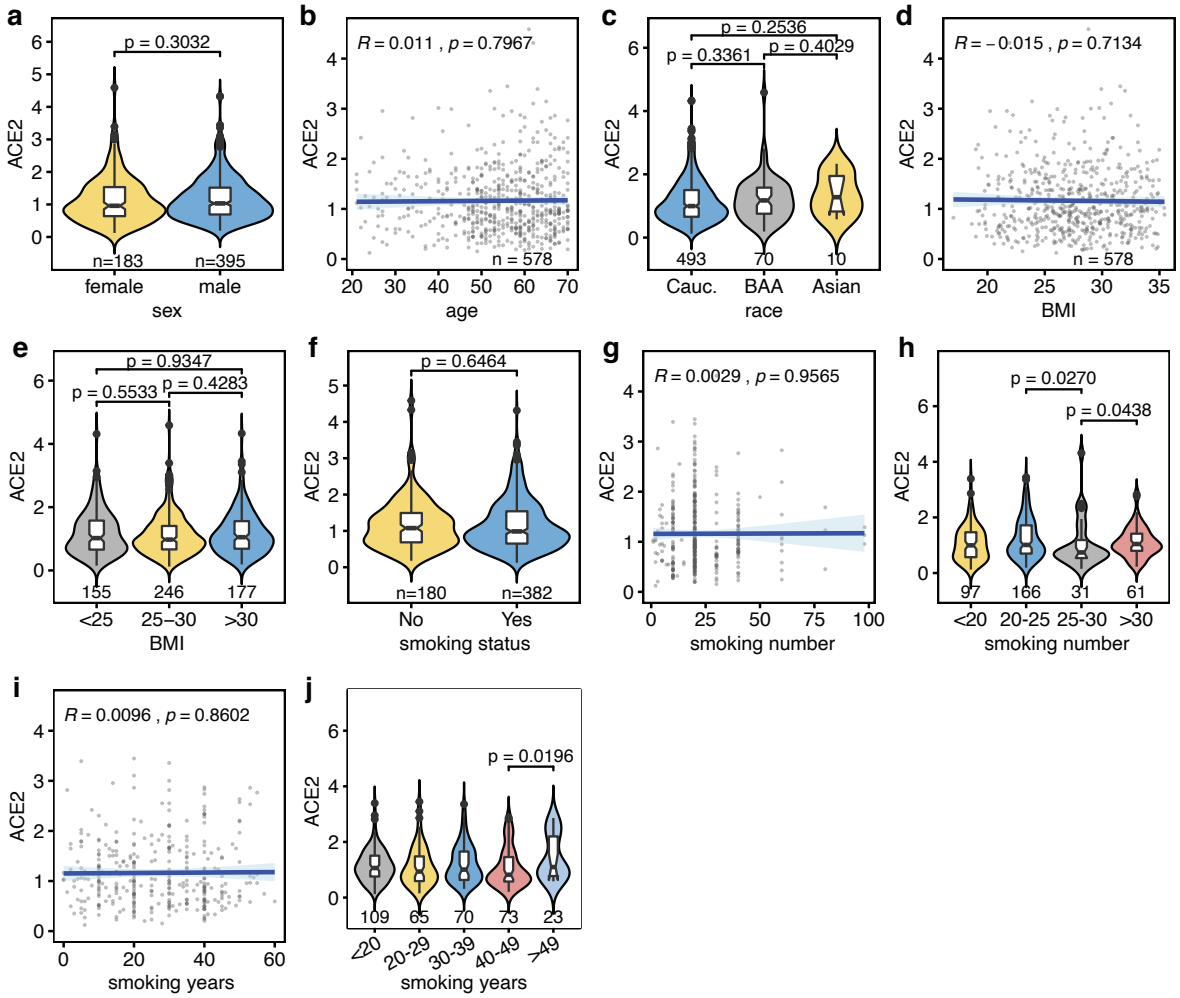


Figure S4. Univariate analyses of phenotypic covariates included in multivariate analyses.
Univariate analyses of five covariates that were included in multivariate analyses of ACE2 and TMPRSS2 expression in human lung tissue using the GTEx dataset (Tables S2, S3). **(a)** Sex. **(b)** Age, **(c)** Race. **(d, e)** Body mass index. **(f-j)** Smoking behavior, referring to smoking status (smoker/non-smoker) (f), number of units smoked during the smoke period (g, h) and number of years smoked (i, j). *P* values in categorical analyses: Mann-Whitney *U* tests. *R* and *p* values in continuous analyses: Pearson correlations. *P* values in panels h and j are only shown if $p<0.05$. Sample numbers (n) are shown on the x-axes.

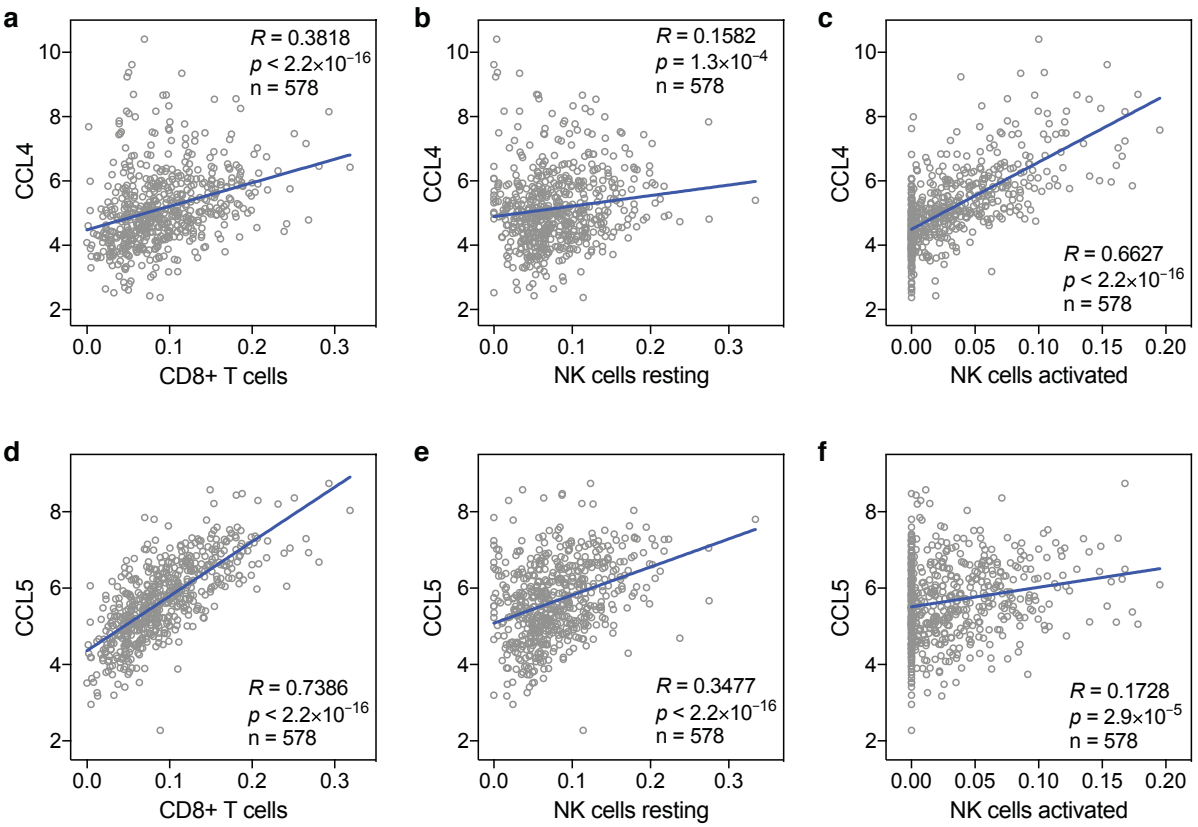


Figure S5. Correlations between baseline levels of cytotoxic lymphocytes and CCL4 and CCL5 in human lung tissue.

The correlations between baseline levels of the chemokines CCL4, CCL5 and CD8+ T cells, resting and activated NK cells in human lung tissue are shown. Data are from the GTEx dataset (n=578). *R* and *p* values: Spearman correlations. See also Figure 2g.

Table S1. Source data of *in silico* cytometry statistics

For each sample in the GTEx (n=578) and LUG (n=1,349) datasets, Pearson correlation coefficient *R*, root mean square error (RMSE) and -log10(*p* value) are shown. These are the source data of Figure S1.

Sample n	GTEx R	GTEx RMSE	GTEx - log10 pval	LUG R	LUG RMSE	LUG - log10 pval
1	0.76787006	0.64982766	15.6575773	0.802959	0.649639	15.65758
2	0.33960379	0.95534778	15.6575773	0.766379	0.707377	15.65758
3	0.69311497	0.73304977	15.6575773	0.787641	0.676389	15.65758
4	0.7996746	0.60995383	15.6575773	0.681417	0.76724	15.65758
5	0.81489669	0.5873594	15.6575773	0.742665	0.717019	15.65758
6	0.7328384	0.69463011	15.6575773	0.801268	0.654958	15.65758
7	0.55167376	0.83420724	15.6575773	0.728905	0.715461	15.65758
8	0.6898905	0.73547426	15.6575773	0.779413	0.679019	15.65758
9	0.74337414	0.7268448	15.6575773	0.773965	0.690504	15.65758
10	0.48677652	0.87413605	15.6575773	0.777735	0.69054	15.65758
11	0.55088695	0.83618046	15.6575773	0.789392	0.682538	15.65758
12	0.72198448	0.72751001	15.6575773	0.747533	0.711919	15.65758
13	0.44847706	0.89376979	15.6575773	0.842775	0.586167	15.65758
14	0.70299836	0.72682982	15.6575773	0.761953	0.700298	15.65758
15	0.743309	0.70328228	15.6575773	0.753612	0.708944	15.65758
16	0.62707528	0.79388999	15.6575773	0.804003	0.657556	15.65758
17	0.65149292	0.77493294	15.6575773	0.810269	0.653416	15.65758
18	0.71063774	0.74524749	15.6575773	0.771608	0.701303	15.65758
19	0.61600528	0.8063179	15.6575773	0.797468	0.669538	15.65758
20	0.53761731	0.84298471	15.6575773	0.757637	0.707872	15.65758
21	0.61042154	0.79213496	15.6575773	0.706046	0.744027	15.65758
22	0.76173265	0.68612869	15.6575773	0.757324	0.704732	15.65758
23	0.5923411	0.81590966	15.6575773	0.788137	0.684214	15.65758
24	0.57111551	0.82945874	15.6575773	0.758922	0.67449	15.65758
25	0.70245483	0.74771223	15.6575773	0.800876	0.675024	15.65758
26	0.71931585	0.70114944	15.6575773	0.748056	0.717971	15.65758
27	0.67884846	0.74083394	15.6575773	0.739715	0.723634	15.65758
28	0.7007555	0.72235034	15.6575773	0.780562	0.674622	15.65758
29	0.72923843	0.71907274	15.6575773	0.782376	0.682771	15.65758
30	0.55756861	0.82973695	15.6575773	0.726186	0.73166	15.65758
31	0.62325326	0.79139104	15.6575773	0.813847	0.649338	15.65758
32	0.63886768	0.77930092	15.6575773	0.805055	0.660688	15.65758
33	0.44888773	0.89528101	15.6575773	0.736178	0.715388	15.65758
34	0.68212751	0.74938995	15.6575773	0.811061	0.663174	15.65758
35	0.73205242	0.68283704	15.6575773	0.68782	0.758606	15.65758
36	0.68262106	0.75168703	15.6575773	0.819304	0.652341	15.65758
37	0.48008816	0.88349271	15.6575773	0.762902	0.701996	15.65758
38	0.45440411	0.89026941	15.6575773	0.685527	0.761058	15.65758
39	0.60434542	0.79776549	15.6575773	0.768102	0.690433	15.65758
40	0.82054193	0.57404279	15.6575773	0.835847	0.617019	15.65758
41	0.69813151	0.75070076	15.6575773	0.835447	0.633442	15.65758
42	0.62125354	0.78878221	15.6575773	0.774305	0.690438	15.65758
43	0.75157392	0.68798327	15.6575773	0.821192	0.634717	15.65758
44	0.38777408	0.92960941	15.6575773	0.736161	0.721822	15.65758
45	0.82524164	0.63701517	15.6575773	0.774914	0.694771	15.65758
46	0.51701022	0.86026052	15.6575773	0.817019	0.65777	15.65758
47	0.56007914	0.83677468	15.6575773	0.8374	0.597228	15.65758
48	0.71308175	0.70717759	15.6575773	0.763472	0.70808	15.65758
49	0.62706859	0.79677808	15.6575773	0.723793	0.732254	15.65758
50	0.71172223	0.74130879	15.6575773	0.840075	0.586469	15.65758
51	0.77950068	0.64522993	15.6575773	0.780776	0.690774	15.65758
52	0.68360264	0.74101263	15.6575773	0.781202	0.687471	15.65758
53	0.84172362	0.62704355	15.6575773	0.784475	0.654503	15.65758
54	0.31660643	0.95729498	15.6575773	0.796829	0.669602	15.65758
55	0.68785982	0.75314287	15.6575773	0.700059	0.745584	15.65758
56	0.70464449	0.74925695	15.6575773	0.795957	0.679328	15.65758
57	0.70970752	0.74479217	15.6575773	0.823785	0.631725	15.65758
58	0.62238904	0.79602679	15.6575773	0.793232	0.683047	15.65758
59	0.52698581	0.84930394	15.6575773	0.819992	0.646026	15.65758
60	0.73123587	0.70101211	15.6575773	0.749767	0.699034	15.65758
61	0.7674915	0.70041919	15.6575773	0.727959	0.726717	15.65758
62	0.62789934	0.78044364	15.6575773	0.770688	0.703886	15.65758
63	0.60410025	0.80136883	15.6575773	0.737926	0.714894	15.65758
64	0.57227658	0.82550032	15.6575773	0.749034	0.706154	15.65758
65	0.76991092	0.65403992	15.6575773	0.746872	0.711794	15.65758
66	0.68588497	0.73930282	15.6575773	0.742272	0.717509	15.65758
67	0.60545218	0.80540731	15.6575773	0.693004	0.74553	15.65758
68	0.72715468	0.70593705	15.6575773	0.725727	0.725521	15.65758
69	0.82942443	0.63454869	15.6575773	0.805247	0.660876	15.65758
70	0.71425946	0.7176295	15.6575773	0.825733	0.620069	15.65758
71	0.77765493	0.66701741	15.6575773	0.766407	0.69845	15.65758
72	0.70485216	0.71288925	15.6575773	0.738098	0.727688	15.65758
73	0.70049108	0.72082562	15.6575773	0.761357	0.701782	15.65758
74	0.49294211	0.87118578	15.6575773	0.772676	0.688762	15.65758
75	0.74102721	0.69347067	15.6575773	0.738963	0.717142	15.65758
76	0.68292334	0.74742974	15.6575773	0.820638	0.654015	15.65758
77	0.68202831	0.74271381	15.6575773	0.804679	0.677565	15.65758
78	0.66058818	0.77004578	15.6575773	0.814325	0.667653	15.65758
79	0.61258367	0.7911726	15.6575773	0.715896	0.727023	15.65758
80	0.66021942	0.76245576	15.6575773	0.757175	0.711845	15.65758
81	0.73545094	0.70171276	15.6575773	0.745553	0.71137	15.65758
82	0.76539688	0.70190687	15.6575773	0.812	0.653151	15.65758
83	0.52249671	0.85192189	15.6575773	0.747185	0.716424	15.65758
84	0.50301948	0.86526106	15.6575773	0.803403	0.669913	15.65758
85	0.51410756	0.85722208	15.6575773	0.787927	0.672406	15.65758
86	0.54248051	0.84295889	15.6575773	0.672214	0.76654	15.65758
87	0.54325456	0.83888963	15.6575773	0.705861	0.743058	15.65758
88	0.67174415	0.7604468	15.6575773	0.704747	0.743665	15.65758
89	0.19835402	1.04850401	2	0.804903	0.650558	15.65758
90	0.80546361	0.6228925	15.6575773	0.774205	0.670835	15.65758
91	0.77078186	0.66191298	15.6575773	0.733251	0.716882	15.65758
92	0.61667107	0.80159867	15.6575773	0.756188	0.711612	15.65758
93	0.6965581	0.72055837	15.6575773	0.799211	0.669125	15.65758
94	0.72414493	0.69095633	15.6575773	0.775547	0.686414	15.65758
95	0.56751768	0.82297723	15.6575773	0.771394	0.687734	15.65758
96	0.68384218	0.7419826	15.6575773	0.772536	0.694775	15.65758
97	0.55867087	0.83016121	15.6575773	0.739445	0.719632	15.65758
98	0.59189641	0.80927822	15.6575773	0.731612	0.725989	15.65758
99	0.66689221	0.74551067	15.6575773	0.762586	0.705917	15.65758
100	0.76408857	0.68936908	15.6575773	0.755818	0.682473	15.65758
101	0.78195749	0.65704796	15.6575773	0.781971	0.6349	15.65758
102	0.62157124	0.79616277	15.6575773	0.782796	0.679205	15.65758
103	0.69262931	0.73158418	15.6575773	0.711814	0.741561	15.65758
104	0.35999342	0.93722591	15.6575773	0.830347	0.667358	15.65758
105	0.57105776	0.82676047	15.6575773	0.742403	0.721198	15.65758
106	0.61157077	0.79751651	15.6575773	0.8256	0.632411	15.65758
107	0.62836027	0.78725474	15.6575773	0.723734	0.72605	15.65758
108	0.63730953	0.77172128	15.6575773	0.676773	0.758942	15.65758
109	0.55034452	0.8364782	15.6575773	0.788154	0.685592	15.65758
110	0.58071617	0.81963676	15.6575773	0.841142	0.578848	15.65758
111	0.64288144	0.76919284	15.6575773	0.74721	0.712274	15.65758
112	0.76962275	0.6741102	15.6575773	0.779528	0.681984	15.65758
113	0.74316718	0.6962846	15.6575773	0.815183	0.62824	15.65758
114	0.74932054	0.7188616	15.6575773	0.7221	0.740452	15.65758
115	0.70768548	0.7150471	15.6575773	0.74192	0.71447	15.65758
116	0.64318529	0.77146204	15.6575773	0.747699	0.711697	15.65758
117	0.50027396	0.86654572	15.6575773	0.76586	0.700596	15.65758
118	0.65288856	0.7635483	15.6575773	0.797885	0.666194	15.65758
119	0.5870206	0.81213873	15.6575773	0.798512	0.671222	15.65758
120	0.69896254	0.7171962	15.6575773	0.698032	0.744293	15.65758
121	0.77664185	0.6616216	15.6575773	0.760781	0.701755	15.65758
122	0.67996473	0.73349422	15.6575773	0.795132	0.666717	15.65758
123	0.37997051	0.92877873	15.6575773	0.757785	0.703435	15.65758
124	0.72629742	0.69520622	15.6575773	0.784242	0.679389	15.65758
125	0.63413228	0.77743163	15.6575773	0.82885	0.658733	15.65758
126	0.58253376	0.81290462	15.6575773	0.812851	0.663289	15.65758
127	0.4026485	0.9152936	15.6575773	0.773322	0.690661	15.65758
128	0.5887239	0.81019846	15.6575773	0.864425	0.539901	15.65758
129	0.60223864	0.80525946	15.6575773	0.801334	0.664347	15.65758
130	0.67338731	0.73945285	15.6575773	0.826393	0.650289	15.65758
131	0.6775215	0.74103379	15.6575773	0.768024	0.690069	15.65758
132	0.81529304	0.62630844	15.6575773	0.76203	0.706059	15.65758
133	0.80319014	0.65161174	15.6575773	0.774558	0.703944	15.65758
134	0.67283271	0.76493473	15.6575773	0.809132	0.65769	15.65758
135	0.60445736	0.7972835	15.6575773	0.799303	0.665812	15.65758
136	0.67770028	0.7593337	15.6575773	0.810735	0.658945	15.65758
137	0.73847589	0.72632252	15.6575773	0.739253	0.720143	15.65758
138	0.74601418	0.72300831	15.6575773	0.662395	0.771096	15.65758
139	0.70236846	0.73427486	15.6575773	0.758509	0.702472	15.65758
140	0.81865779	0.57914457	15.6575773	0.678185	0.757806	15.65758
141	0.74338605	0.72207604	15.6575773	0.42344	0.908166	15.65758
142	0.84727784	0.64446399	15.6575773	0.81911	0.64399	15.65758
143	0.81034984	0.66762845	15.6575773	0.669108	0.769303	15.65758
144	0.87262691	0.56850327	15.6575773	0.816395	0.660872	15.65758
145	0.73770041	0.71204382	15.6575773	0.793646	0.684506	15.65758
146	0.72007362	0.72191013	15.6575773	0.761751	0.693291	15.65758
147	0.35159383	0.95375304	15.6575773	0.735312	0.718459	15.65758
148	0.68133293	0.74550045	15.6575773	0.860572	0.577393	15.65758
149	0.64469881	0.78311107	15.6575773	0.738542	0.728356	15.65758
150	0.76699006	0.65770387	15.6575773	0.736961	0.715982	15.65758
151	0.65261002	0.77708712	15.6575773	0.852436	0.606723	15.65758
152	0.69092728	0.72802414	15.6575773	0.815421	0.643371	15.65758
153	0.66815765	0.75516493	15.6575773	0.852101	0.600422	15.65758
154	0.72338	0.72113486	15.6575773	0.823008	0.647346	15.65758
155	0.61294783	0.80524366	15.6575773	0.81933	0.649554	15.65758
156	0.68029826	0.76974972	15.6575773	0.824095	0.637462	15.65758
157	0.6574042	0.77689403	15.6575773	0.793561	0.684478	15.65758
158	0.66517798	0.75270397	15.6575773	0.779435	0.670839	15.65758
159	0.60275426	0.79751146	15.6575773	0.761359	0.703594	15.65758
160	0.64954584	0.77630178	15.6575773	0.768137	0.696951	15.65758
161	0.33783197	0.9496664	15.6575773	0.826736	0.647421	15.65758
162	0.64145073	0.77269795	15.6575773	0.736982	0.71602	15.65758
163	0.57302665	0.82115977	15.6575773	0.762553	0.681568	15.65758
164	0.66410007	0.76511238	15.6575773	0.711566	0.737221	15.65758
165	0.69744735	0.72988507	15.6575773	0.74463	0.720925	15.65758
166	0.84479041	0.63148691	15.6575773	0.844184	0.609722	15.65758
167	0.65019929	0.75998999	15.6575773	0.737026	0.722095	15.65758
168	0.61418142	0.81586006	15.6575773	0.782789	0.677807	15.65758
169	0.24425278	0.99608952	15.6575773	0.724606	0.723865	15.65758
170	0.79420928	0.68015397	15.6575773	0.820238	0.638723	15.65758
171	0.69498389	0.72872033	15.6575773	0.677837	0.760919	15.65758
172	0.54730348	0.8376161	15.6575773	0.757639	0.709778	15.65758
173	0.59771908	0.80182968	15.6575773	0.752393	0.699098	15.65758
174	0.79089542	0.61525168	15.6575773	0.772723	0.691779	15.65758
175	0.73268118	0.71735237	15.6575773	0.789165	0.662873	15.65758
176	0.64544132	0.77395979	15.6575773	0.790608	0.678914	15.65758
177	0.72990485	0.73814333	15.6575773	0.755187	0.712886	15.65758
178	0.69403073	0.75451906	15.6575773	0.859342	0.61968	15.65758
179	0.71895773	0.70207397	15.6575773	0.787019	0.674599	15.65758
180	0.84347043	0.61851737	15.6575773	0.740603	0.718527	15.65758
181	0.70251848	0.75091921	15.6575773	0.77875	0.650124	15.65758
182	0.72978884	0.72399499	15.6575773	0.806435	0.669966	15.65758
183	0.59919488	0.81391231	15.6575773	0.658888	0.772346	15.65758
184	0.66052791	0.75134944	15.6575773	0.69686	0.749192	15.65758
185	0.65948202	0.77740456	15.6575773	0.753158	0.706553	15.65758
186	0.54895017	0.84003207	15.6575773	0.733403	0.714169	15.65758
187	0.76845936	0.66732278	15.6575773	0.774229	0.685554	15.65758
188	0.60680086	0.80270009	15.6575773	0.715689	0.736403	15.65758
189	0.54034402	0.84167912	15.6575773	0.818287	0.660603	15.65758
190	0.69362566	0.74127999	15.6575773	0.74269	0.723009	15.65758
191	0.34119349	0.95065248	15.6575773	0.838666	0.614078	15.65758
192	0.76556764	0.65038664	15.6575773	0.814702	0.660265	15.65758

193	0.72599161	0.74095472	15.6575773	0.7571	0.715478	15.65758
194	0.66043078	0.75269024	15.6575773	0.75468	0.709712	15.65758
195	0.56001772	0.83338094	15.6575773	0.716521	0.732976	15.65758
196	0.81838675	0.60658809	15.6575773	0.821864	0.636654	15.65758
197	0.77302519	0.63590636	15.6575773	0.647836	0.784919	15.65758
198	0.54390926	0.84098442	15.6575773	0.747368	0.708886	15.65758
199	0.67961129	0.75558107	15.6575773	0.763616	0.689703	15.65758
200	0.60649215	0.7960565	15.6575773	0.812353	0.6419	15.65758
201	0.48456997	0.87433601	15.6575773	0.79388	0.651875	15.65758
202	0.81596176	0.64460334	15.6575773	0.761849	0.690674	15.65758
203	0.74966186	0.66777995	15.6575773	0.781606	0.663368	15.65758
204	0.75464334	0.65831696	15.6575773	0.821076	0.660814	15.65758
205	0.74968041	0.72670082	15.6575773	0.796044	0.666209	15.65758
206	0.81399703	0.58351606	15.6575773	0.791838	0.680987	15.65758
207	0.68506628	0.75996461	15.6575773	0.789881	0.689701	15.65758
208	0.6360702	0.78975549	15.6575773	0.809869	0.647442	15.65758
209	0.58242464	0.81682636	15.6575773	0.724377	0.731891	15.65758
210	0.68684771	0.75712965	15.6575773	0.804232	0.670361	15.65758
211	0.75432658	0.70731847	15.6575773	0.772383	0.700347	15.65758
212	0.74763336	0.70352207	15.6575773	0.79732	0.668725	15.65758
213	0.7345029	0.69096848	15.6575773	0.820374	0.643138	15.65758
214	0.38555341	0.92473624	15.6575773	0.786387	0.671845	15.65758
215	0.66480243	0.77317655	15.6575773	0.722954	0.725167	15.65758
216	0.53238055	0.8501063	15.6575773	0.661335	0.76966	15.65758
217	0.49614753	0.87117367	15.6575773	0.747917	0.713147	15.65758
218	0.62848809	0.78155805	15.6575773	0.754777	0.713618	15.65758
219	0.34756065	0.9461676	15.6575773	0.812003	0.672584	15.65758
220	0.71911963	0.70564645	15.6575773	0.752694	0.706264	15.65758
221	0.59834917	0.81128019	15.6575773	0.790308	0.685963	15.65758
222	0.47498616	0.87943216	15.6575773	0.836905	0.637796	15.65758
223	0.60689839	0.79634132	15.6575773	0.799052	0.669879	15.65758
224	0.50019681	0.86724342	15.6575773	0.793398	0.663917	15.65758
225	0.62729209	0.79058412	15.6575773	0.781364	0.688733	15.65758
226	0.46225126	0.88865426	15.6575773	0.780071	0.690821	15.65758
227	0.67231135	0.76832341	15.6575773	0.787926	0.675839	15.65758
228	0.64748332	0.76768051	15.6575773	0.82181	0.631713	15.65758
229	0.62251406	0.78420097	15.6575773	0.843974	0.604237	15.65758
230	0.8183653	0.67757687	15.6575773	0.731429	0.728452	15.65758
231	0.75270747	0.71496551	15.6575773	0.763006	0.705997	15.65758
232	0.6068207	0.79963664	15.6575773	0.74788	0.711903	15.65758
233	0.58879924	0.8080764	15.6575773	0.750129	0.707874	15.65758
234	0.19851404	1.02630196	2	0.796418	0.649542	15.65758
235	0.71390061	0.74582221	15.6575773	0.719688	0.729439	15.65758
236	0.76283788	0.68148595	15.6575773	0.76701	0.698827	15.65758
237	0.60732441	0.79707316	15.6575773	0.557045	0.836521	15.65758
238	0.54137454	0.84031461	15.6575773	0.786279	0.675397	15.65758
239	0.57237844	0.82061417	15.6575773	0.772489	0.694027	15.65758
240	0.89292037	0.45411744	15.6575773	0.791821	0.645896	15.65758
241	0.65583111	0.75916878	15.6575773	0.725282	0.728549	15.65758
242	0.774149	0.70954979	15.6575773	0.835758	0.615321	15.65758
243	0.67806547	0.76133731	15.6575773	0.749561	0.716408	15.65758
244	0.49364517	0.86885487	15.6575773	0.667615	0.773075	15.65758
245	0.536933	0.84322847	15.6575773	0.765581	0.683119	15.65758
246	0.70872418	0.7067122	15.6575773	0.734532	0.72122	15.65758
247	0.77589014	0.67296294	15.6575773	0.674216	0.766718	15.65758
248	0.80072619	0.61393943	15.6575773	0.803479	0.666537	15.65758
249	0.65988256	0.77526385	15.6575773	0.787501	0.678435	15.65758
250	0.68068467	0.74091501	15.6575773	0.76172	0.703398	15.65758
251	0.73244551	0.70928926	15.6575773	0.795979	0.678793	15.65758
252	0.75354693	0.67422913	15.6575773	0.776025	0.688462	15.65758
253	0.63254677	0.79478404	15.6575773	0.798442	0.680254	15.65758
254	0.6153526	0.79113624	15.6575773	0.738534	0.71802	15.65758
255	0.56034604	0.82753621	15.6575773	0.787161	0.676819	15.65758
256	0.77369824	0.65969904	15.6575773	0.744086	0.717224	15.65758
257	0.85411372	0.56176758	15.6575773	0.786093	0.665727	15.65758
258	0.57687458	0.81685974	15.6575773	0.801868	0.644505	15.65758
259	0.69854543	0.73007923	15.6575773	0.735325	0.72027	15.65758
260	0.26945451	0.97889818	15.6575773	0.820929	0.652595	15.65758
261	0.84318789	0.58198357	15.6575773	0.742597	0.714365	15.65758
262	0.69618896	0.75954336	15.6575773	0.81396	0.632136	15.65758
263	0.44164111	0.89711877	15.6575773	0.825774	0.638587	15.65758
264	0.63749278	0.78479043	15.6575773	0.705999	0.744879	15.65758
265	0.86949088	0.57450335	15.6575773	0.817806	0.658609	15.65758
266	0.8281639	0.5636942	15.6575773	0.768556	0.680677	15.65758
267	0.66002965	0.75860091	15.6575773	0.849624	0.608813	15.65758
268	0.75685307	0.71151029	15.6575773	0.737617	0.723191	15.65758
269	0.57332907	0.82288263	15.6575773	0.710483	0.723503	15.65758
270	0.84539899	0.59464806	15.6575773	0.784225	0.68118	15.65758
271	0.76104507	0.65077789	15.6575773	0.728	0.728922	15.65758
272	0.6640011	0.76063509	15.6575773	0.740465	0.716417	15.65758
273	0.66142851	0.75573128	15.6575773	0.792563	0.679757	15.65758
274	0.09561775	1.06392067	0.92081875	0.804311	0.656906	15.65758
275	0.59691428	0.81195173	15.6575773	0.764963	0.695617	15.65758
276	0.66214351	0.77044357	15.6575773	0.796255	0.672442	15.65758
277	0.77865783	0.65298519	15.6575773	0.827325	0.657303	15.65758
278	0.54618294	0.84078972	15.6575773	0.662821	0.768781	15.65758
279	0.6933973	0.73413878	15.6575773	0.762835	0.700561	15.65758
280	0.68076046	0.7351417	15.6575773	0.751751	0.695009	15.65758
281	0.64173496	0.7808481	15.6575773	0.738474	0.711002	15.65758
282	0.62576569	0.80110413	15.6575773	0.816107	0.653323	15.65758
283	0.7198404	0.69761934	15.6575773	0.775223	0.682029	15.65758
284	0.55056818	0.83413069	15.6575773	0.836076	0.628981	15.65758
285	0.70713266	0.71949422	15.6575773	0.751681	0.701574	15.65758
286	0.42489912	0.90477606	15.6575773	0.783174	0.692962	15.65758
287	0.731716	0.71285487	15.6575773	0.768599	0.690812	15.65758
288	0.81458549	0.62653519	15.6575773	0.741483	0.706972	15.65758
289	0.66883619	0.74993427	15.6575773	0.779969	0.693146	15.65758
290	0.59842802	0.80157266	15.6575773	0.68451	0.759285	15.65758
291	0.65410643	0.77468509	15.6575773	0.818814	0.645443	15.65758
292	0.71982128	0.70302377	15.6575773	0.748352	0.713861	15.65758
293	0.84054467	0.58340764	15.6575773	0.772643	0.689538	15.65758
294	0.65200613	0.78014444	15.6575773	0.682554	0.758181	15.65758
295	0.43740953	0.8993488	15.6575773	0.764508	0.673325	15.65758
296	0.69769203	0.74067558	15.6575773	0.73447	0.722677	15.65758
297	0.62942763	0.79526233	15.6575773	0.740265	0.722765	15.65758
298	0.34273059	0.95188053	15.6575773	0.757274	0.714485	15.65758
299	0.86048091	0.54256793	15.6575773	0.841756	0.592278	15.65758
300	0.61614358	0.79170506	15.6575773	0.800507	0.654171	15.65758
301	0.67335919	0.75445336	15.6575773	0.770732	0.701699	15.65758
302	0.35345743	0.94275013	15.6575773	0.768785	0.698409	15.65758
303	0.71042911	0.74494089	15.6575773	0.767138	0.701982	15.65758
304	0.8477898	0.53334989	15.6575773	0.864484	0.58774	15.65758
305	0.67903314	0.73579422	15.6575773	0.731853	0.725057	15.65758
306	0.63507411	0.79044464	15.6575773	0.737426	0.715749	15.65758
307	0.64005978	0.78176292	15.6575773	0.77896	0.686587	15.65758
308	0.66719365	0.75715937	15.6575773	0.671694	0.759826	15.65758
309	0.68957512	0.75765528	15.6575773	0.753502	0.716508	15.65758
310	0.48493149	0.88565917	15.6575773	0.756836	0.700051	15.65758
311	0.39856453	0.92223475	15.6575773	0.758049	0.707652	15.65758
312	0.78137492	0.66483924	15.6575773	0.790423	0.68897	15.65758
313	0.78942621	0.64359881	15.6575773	0.814714	0.667971	15.65758
314	0.76627041	0.66710032	15.6575773	0.824011	0.654317	15.65758
315	0.825058	0.58586794	15.6575773	0.811953	0.63457	15.65758
316	0.69565437	0.72924025	15.6575773	0.736519	0.721321	15.65758
317	0.61540866	0.79928767	15.6575773	0.817248	0.628069	15.65758
318	0.67771209	0.75088031	15.6575773	0.70467	0.746934	15.65758
319	0.52363313	0.85124364	15.6575773	0.75058	0.71488	15.65758
320	0.87085539	0.54369011	15.6575773	0.673283	0.761646	15.65758
321	0.81630204	0.61709377	15.6575773	0.816021	0.658796	15.65758
322	0.64594725	0.7857347	15.6575773	0.745291	0.710347	15.65758
323	0.44286124	0.89777843	15.6575773	0.762003	0.70251	15.65758
324	0.56375425	0.82688696	15.6575773	0.743416	0.704938	15.65758
325	0.66011937	0.77486623	15.6575773	0.730243	0.715486	15.65758
326	0.64587618	0.7778332	15.6575773	0.76491	0.691397	15.65758
327	0.81350517	0.63650257	15.6575773	0.83337	0.653997	15.65758
328	0.67892547	0.74199618	15.6575773	0.7595	0.706742	15.65758
329	0.67768412	0.75595947	15.6575773	0.783379	0.683794	15.65758
330	0.57931722	0.82302225	15.6575773	0.823863	0.638998	15.65758
331	0.8277393	0.60774943	15.6575773	0.727557	0.728405	15.65758
332	0.63872241	0.78657921	15.6575773	0.85217	0.641538	15.65758
333	0.79485073	0.61411864	15.6575773	0.783887	0.686954	15.65758
334	0.67857081	0.75136256	15.6575773	0.822722	0.645722	15.65758
335	0.63648825	0.78721863	15.6575773	0.742859	0.718157	15.65758
336	0.81332719	0.61821189	15.6575773	0.733941	0.722593	15.65758
337	0.57055958	0.82872707	15.6575773	0.825853	0.627373	15.65758
338	0.63713995	0.78837872	15.6575773	0.758108	0.704373	15.65758
339	0.68086742	0.75679822	15.6575773	0.71921	0.721512	15.65758
340	0.72934189	0.72734226	15.6575773	0.757114	0.710346	15.65758
341	0.83192681	0.64921726	15.6575773	0.75686	0.717091	15.65758
342	0.78246491	0.63642252	15.6575773	0.829994	0.634412	15.65758
343	0.60465147	0.80669847	15.6575773	0.79388	0.683538	15.65758
344	0.53641883	0.84358496	15.6575773	0.780861	0.675445	15.65758
345	0.68972396	0.74980173	15.6575773	0.731879	0.718151	15.65758
346	0.75841901	0.68770193	15.6575773	0.77734	0.681967	15.65758
347	0.80725538	0.64476958	15.6575773	0.752977	0.706004	15.65758
348	0.5775612	0.86913574	15.6575773	0.79143	0.662081	15.65758
349	0.69500163	0.75067582	15.6575773	0.722789	0.727697	15.65758
350	0.66147917	0.7569634	15.6575773	0.829693	0.633217	15.65758
351	0.61676661	0.79240063	15.6575773	0.781361	0.685954	15.65758
352	0.82856033	0.60122032	15.6575773	0.654186	0.7776	15.65758
353	0.70617192	0.72990498	15.6575773	0.712173	0.734328	15.65758
354	0.73457706	0.68664734	15.6575773	0.824215	0.647241	15.65758
355	0.31359627	0.9629867	15.6575773	0.769599	0.669602	15.65758
356	0.45186184	0.89129695	15.6575773	0.805541	0.659896	15.65758
357	0.59299302	0.81308118	15.6575773	0.79006	0.69253	15.65758
358	0.53800577	0.84725377	15.6575773	0.736027	0.732259	15.65758
359	0.76431043	0.70604349	15.6575773	0.714312	0.735142	15.65758
360	0.17025679	1.03808476	2	0.761171	0.70684	15.65758
361	0.68547218	0.75350995	15.6575773	0.688967	0.757115	15.65758
362	0.66379789	0.75864982	15.6575773	0.827252	0.637705	15.65758
363	0.73369752	0.71775636	15.6575773	0.818534	0.667334	15.65758
364	0.64338608	0.77553813	15.6575773	0.835611	0.609172	15.65758
365	0.69103689	0.7409776	15.6575773	0.776392	0.696328	15.65758
366	0.64556511	0.76519122	15.6575773	0.766746	0.691208	15.65758
367	0.69142432	0.75020049	15.6575773	0.824394	0.627898	15.65758
368	0.71035602	0.70416429	15.6575773	0.74401	0.696828	15.65758
369	0.57393374	0.82158152	15.6575773	0.80995	0.653886	15.65758
370	0.7680132	0.64222566	15.6575773	0.822591	0.612494	15.65758
371	0.51849301	0.85746835	15.6575773	0.728594	0.726031	15.65758
372	0.74486079	0.68363681	15.6575773	0.768782	0.709896	15.65758
373	0.71912939	0.72351411	15.6575773	0.7362	0.723434	15.65758
374	0.66855253	0.75030359	15.6575773	0.802292	0.64371	15.65758
375	0.71500557	0.7087689	15.6575773	0.728628	0.710208	15.65758
376	0.57674964	0.82619145	15.6575773	0.759353	0.693681	15.65758
377	0.75240956	0.69466386	15.6575773	0.763747	0.704828	15.65758
378	0.73022512	0.73899983	15.6575773	0.772411	0.695916	15.65758
379	0.71941842	0.7101646	15.6575773	0.73949	0.724477	15.65758
380	0.67653551	0.747337	15.6575773	0.795691	0.664514	15.65758
381	0.80205415	0.63943573	15.6575773	0.729668	0.723158	15.65758
382	0.67812514	0.74188468	15.6575773	0.789552	0.671616	15.65758
383	0.70053945	0.73368547	15.6575773	0.81552	0.665196	15.65758
384	0.57307317	0.81988861	15.6575773	0.788056	0.68082	15.65758
385	0.66552644	0.76345452	15.6575773	0.775621	0.694257	15.65758
386	0.65402125	0.76145584	15.6575773	0.798758	0.641243	15.65758
387	0.6513516	0.76530441	15.6575773	0.72543	0.73445	15.65758
388	0.31307892	0.95940365	15.6575773	0.786517	0.681791	15.65758
389	0.61140943	0.79425297	15.6575773	0.755092	0.696874	15.65758
390	0.73779991	0.67695028	15.6575773	0.740136	0.722908	15.65758
391	0.26437683	0.98097019	15.6575773	0.78094	0.683336	15.65758
392	0.49064682	0.8706901	15.6575773	0.823799	0.640414	15.65758
393	0.53421455	0.84814431	15.6575773	0.786727	0.677893	15.65758
394	0.76957242	0.64990945	15.6575773	0.80014	0.675833	15.65758
395	0.76838601	0.69168466	15.6575773	0.855299	0.613982	15.65758
396	0.74465165	0.72117884	15.6575773	0.731772	0.717993	15.65758
397	0.82607666	0.56872887	15.6575773	0.760158	0.703722	15.65758
398	0.54116533	0.84420808	15.6575773	0.823615	0.648303	15.65758

399	0.75380773	0.69201404	15.6575773	0.754296	0.710553	15.65758
400	0.70056472	0.73641256	15.6575773	0.828156	0.62062	15.65758
401	0.30317843	0.97100511	15.6575773	0.744932	0.720282	15.65758
402	0.42704837	0.90400651	15.6575773	0.760689	0.697853	15.65758
403	0.73250295	0.68569706	15.6575773	0.743454	0.716956	15.65758
404	0.63998157	0.77148253	15.6575773	0.797208	0.674443	15.65758
405	0.18982386	1.02679717	2	0.803261	0.64979	15.65758
406	0.83707936	0.55007538	15.6575773	0.767848	0.693432	15.65758
407	0.74339517	0.67975992	15.6575773	0.833875	0.634358	15.65758
408	0.74793287	0.67889893	15.6575773	0.820188	0.623863	15.65758
409	0.70750911	0.71679886	15.6575773	0.824295	0.654846	15.65758
410	0.77867297	0.67583158	15.6575773	0.700145	0.749118	15.65758
411	0.69289092	0.7567346	15.6575773	0.787082	0.681237	15.65758
412	0.83317722	0.56288769	15.6575773	0.789655	0.679567	15.65758
413	0.70542758	0.74607318	15.6575773	0.818843	0.651949	15.65758
414	0.60418303	0.81035751	15.6575773	0.788503	0.683418	15.65758
415	0.77263732	0.68624395	15.6575773	0.749337	0.713422	15.65758
416	0.77999501	0.64430522	15.6575773	0.857768	0.596827	15.65758
417	0.65819066	0.76349394	15.6575773	0.721292	0.738618	15.65758
418	0.84534771	0.61412027	15.6575773	0.742444	0.719647	15.65758
419	0.72107122	0.74360552	15.6575773	0.756215	0.697051	15.65758
420	0.69186243	0.76327487	15.6575773	0.823636	0.661281	15.65758
421	0.67704186	0.7557326	15.6575773	0.824067	0.644177	15.65758
422	0.61801931	0.79213051	15.6575773	0.838228	0.606988	15.65758
423	0.39774019	0.92016505	15.6575773	0.826148	0.66437	15.65758
424	0.66725127	0.76451426	15.6575773	0.764849	0.688124	15.65758
425	0.86750801	0.54605993	15.6575773	0.750045	0.692775	15.65758
426	0.65224792	0.76255366	15.6575773	0.71395	0.738439	15.65758
427	0.70968908	0.70488503	15.6575773	0.812222	0.649952	15.65758
428	0.8144281	0.61899063	15.6575773	0.794581	0.670675	15.65758
429	0.55162367	0.8352793	15.6575773	0.711564	0.739971	15.65758
430	0.81451033	0.65313459	15.6575773	0.774304	0.678878	15.65758
431	0.64322032	0.76959099	15.6575773	0.815513	0.651896	15.65758
432	0.76139424	0.6525384	15.6575773	0.699841	0.747594	15.65758
433	0.70921862	0.72550022	15.6575773	0.811825	0.637925	15.65758
434	0.57747588	0.82639924	15.6575773	0.759339	0.701199	15.65758
435	0.71591986	0.72409077	15.6575773	0.72731	0.729214	15.65758
436	0.47779155	0.880142	15.6575773	0.834849	0.618359	15.65758
437	0.49645427	0.86737501	15.6575773	0.791193	0.687659	15.65758
438	0.57743264	0.82305894	15.6575773	0.640255	0.78536	15.65758
439	0.72408992	0.73562376	15.6575773	0.747989	0.71532	15.65758
440	0.65438578	0.76223506	15.6575773	0.775596	0.682084	15.65758
441	0.77561601	0.673137	15.6575773	0.664528	0.77254	15.65758
442	0.65915061	0.75561091	15.6575773	0.744109	0.702744	15.65758
443	0.64163782	0.78081529	15.6575773	0.823738	0.662318	15.65758
444	0.63293546	0.78185073	15.6575773	0.728052	0.732315	15.65758
445	0.47748474	0.87907839	15.6575773	0.704574	0.745085	15.65758
446	0.79135335	0.64989083	15.6575773	0.723887	0.731429	15.65758
447	0.78444278	0.66513336	15.6575773	0.794666	0.671867	15.65758
448	0.66124032	0.75088453	15.6575773	0.836126	0.613719	15.65758
449	0.84725654	0.57327395	15.6575773	0.755826	0.700347	15.65758
450	0.6443371	0.78954395	15.6575773	0.75978	0.715384	15.65758
451	0.64475131	0.76588908	15.6575773	0.770362	0.669441	15.65758
452	0.74418936	0.67489822	15.6575773	0.855937	0.585868	15.65758
453	0.72247041	0.72688859	15.6575773	0.762756	0.700346	15.65758
454	0.51252129	0.86008798	15.6575773	0.743388	0.722329	15.65758
455	0.22879971	1.04332886	15.6575773	0.758161	0.713607	15.65758
456	0.49451615	0.86879374	15.6575773	0.784977	0.667649	15.65758
457	0.61730463	0.79382221	15.6575773	0.809956	0.658432	15.65758
458	0.72501316	0.74525514	15.6575773	0.803999	0.652058	15.65758
459	0.69454045	0.74772783	15.6575773	0.788514	0.685062	15.65758
460	0.63909089	0.77040152	15.6575773	0.765208	0.706371	15.65758
461	0.66181163	0.75262712	15.6575773	0.787266	0.667723	15.65758
462	0.55251219	0.83822132	15.6575773	0.829424	0.64137	15.65758
463	0.80513944	0.68464999	15.6575773	0.744724	0.711264	15.65758
464	0.70250859	0.73723424	15.6575773	0.768015	0.701385	15.65758
465	0.59414622	0.80920074	15.6575773	0.848811	0.622095	15.65758
466	0.61966274	0.78486906	15.6575773	0.796293	0.662787	15.65758
467	0.52473404	0.85249113	15.6575773	0.808454	0.671259	15.65758
468	0.60496308	0.80896415	15.6575773	0.742548	0.721366	15.65758
469	0.61949589	0.79070222	15.6575773	0.811742	0.669353	15.65758
470	0.69907747	0.73108434	15.6575773	0.73915	0.723346	15.65758
471	0.74682731	0.72107425	15.6575773	0.754893	0.717161	15.65758
472	0.68695287	0.72899135	15.6575773	0.782761	0.691515	15.65758
473	0.70027547	0.74764463	15.6575773	0.76757	0.691957	15.65758
474	0.67665174	0.74817816	15.6575773	0.78047	0.692034	15.65758
475	0.73865186	0.68108967	15.6575773	0.859199	0.559392	15.65758
476	0.52289529	0.85180319	15.6575773	0.668443	0.768252	15.65758
477	0.33180736	0.95022672	15.6575773	0.716968	0.732784	15.65758
478	0.59534322	0.81261156	15.6575773	0.815329	0.652696	15.65758
479	0.702301	0.72743886	15.6575773	0.803292	0.672427	15.65758
480	0.76350165	0.70133905	15.6575773	0.790684	0.678166	15.65758
481	0.77611628	0.7048954	15.6575773	0.80878	0.667171	15.65758
482	0.71592386	0.69943891	15.6575773	0.797176	0.647981	15.65758
483	0.7804117	0.67984787	15.6575773	0.75695	0.702238	15.65758
484	0.85593272	0.61143133	15.6575773	0.770794	0.690627	15.65758
485	0.74228279	0.7278532	15.6575773	0.828818	0.628046	15.65758
486	0.60588402	0.80759028	15.6575773	0.763802	0.697486	15.65758
487	0.71823562	0.70427794	15.6575773	0.801204	0.669957	15.65758
488	0.6636924	0.77504323	15.6575773	0.759127	0.700359	15.65758
489	0.69713735	0.73683862	15.6575773	0.759708	0.706338	15.65758
490	0.67121011	0.77080842	15.6575773	0.710417	0.741314	15.65758
491	0.78699172	0.64169254	15.6575773	0.797238	0.676997	15.65758
492	0.60100456	0.81047883	15.6575773	0.761262	0.706335	15.65758
493	0.81389203	0.64419667	15.6575773	0.764715	0.697881	15.65758
494	0.65987459	0.77273028	15.6575773	0.770325	0.700429	15.65758
495	0.699245	0.74799964	15.6575773	0.851678	0.599122	15.65758
496	0.79823767	0.65839407	15.6575773	0.760197	0.694588	15.65758
497	0.29238768	0.97692468	15.6575773	0.72815	0.720757	15.65758
498	0.44636892	0.89441426	15.6575773	0.756012	0.713311	15.65758
499	0.73933841	0.70345159	15.6575773	0.797833	0.682087	15.65758
500	0.58807227	0.81453685	15.6575773	0.714215	0.734489	15.65758
501	0.77527752	0.71043088	15.6575773	0.710306	0.745301	15.65758

502	0.68434553	0.74872971	15.6575773	0.709174	0.745781	15.65758
503	0.83047442	0.65821388	15.6575773	0.872401	0.59495	15.65758
504	0.6976008	0.72715378	15.6575773	0.756366	0.70927	15.65758
505	0.45165395	0.89565841	15.6575773	0.795147	0.685827	15.65758
506	0.79364162	0.68147898	15.6575773	0.821229	0.585546	15.65758
507	0.72811617	0.69173956	15.6575773	0.735752	0.722935	15.65758
508	0.3902897	0.92240038	15.6575773	0.735647	0.712962	15.65758
509	0.67516731	0.76343468	15.6575773	0.731546	0.696748	15.65758
510	0.75947913	0.70850583	15.6575773	0.647702	0.782054	15.65758
511	0.68924142	0.75321705	15.6575773	0.786892	0.686214	15.65758
512	0.6297176	0.785607	15.6575773	0.719129	0.72712	15.65758
513	0.72722106	0.72063911	15.6575773	0.695228	0.754684	15.65758
514	0.68145715	0.74036507	15.6575773	0.74899	0.716309	15.65758
515	0.51438259	0.85866594	15.6575773	0.796234	0.651528	15.65758
516	0.43424789	0.9026355	15.6575773	0.795587	0.655116	15.65758
517	0.71897431	0.7125138	15.6575773	0.754364	0.699159	15.65758
518	0.71162204	0.73098453	15.6575773	0.805436	0.654452	15.65758
519	0.67510939	0.74560581	15.6575773	0.716585	0.739683	15.65758
520	0.55766011	0.83863379	15.6575773	0.882547	0.567297	15.65758
521	0.59129059	0.80753152	15.6575773	0.856421	0.604516	15.65758
522	0.74657949	0.66745387	15.6575773	0.828337	0.626874	15.65758
523	0.7467179	0.6935483	15.6575773	0.678775	0.763916	15.65758
524	0.5065352	0.86178731	15.6575773	0.847128	0.638282	15.65758
525	0.78742553	0.69741061	15.6575773	0.733981	0.721383	15.65758
526	0.66099005	0.75171893	15.6575773	0.737659	0.722559	15.65758
527	0.56290276	0.83293325	15.6575773	0.772807	0.712841	15.65758
528	0.611358	0.8006772	15.6575773	0.683897	0.747429	15.65758
529	0.74761565	0.708675	15.6575773	0.63868	0.789178	15.65758
530	0.65065973	0.78088125	15.6575773	0.834756	0.639608	15.65758
531	0.73842592	0.73296533	15.6575773	0.74666	0.707866	15.65758
532	0.63274813	0.79526962	15.6575773	0.712008	0.733241	15.65758
533	0.47611626	0.87863921	15.6575773	0.760208	0.706667	15.65758
534	0.6269829	0.78209819	15.6575773	0.790969	0.682922	15.65758
535	0.60912433	0.80380809	15.6575773	0.73324	0.729745	15.65758
536	0.74499571	0.70205367	15.6575773	0.832417	0.653584	15.65758
537	0.27125903	0.99280928	15.6575773	0.719435	0.731626	15.65758
538	0.61933423	0.79427226	15.6575773	0.758461	0.694801	15.65758
539	0.59298449	0.81821617	15.6575773	0.723226	0.724692	15.65758
540	0.69632199	0.74780338	15.6575773	0.759046	0.701491	15.65758
541	0.8432606	0.57369159	15.6575773	0.802775	0.672218	15.65758
542	0.67268601	0.7613017	15.6575773	0.743187	0.721746	15.65758
543	0.33926101	0.94618681	15.6575773	0.8015	0.671635	15.65758
544	0.68111123	0.76874461	15.6575773	0.701943	0.74554	15.65758
545	0.67881553	0.74609584	15.6575773	0.74564	0.721689	15.65758
546	0.54046712	0.84097136	15.6575773	0.828306	0.638664	15.65758
547	0.74375653	0.7035178	15.6575773	0.760761	0.709859	15.65758
548	0.62636949	0.78886823	15.6575773	0.741058	0.720378	15.65758
549	0.61438182	0.80033816	15.6575773	0.805449	0.600432	15.65758
550	0.67019108	0.75087283	15.6575773	0.721638	0.732072	15.65758
551	0.7569947	0.71983827	15.6575773	0.647006	0.784101	15.65758
552	0.58326934	0.81337943	15.6575773	0.738943	0.71594	15.65758
553	0.68913881	0.74352379	15.6575773	0.752531	0.662123	15.65758
554	0.78771451	0.68555313	15.6575773	0.855221	0.539384	15.65758
555	0.69969	0.75047493	15.6575773	0.780485	0.695053	15.65758
556	0.76775235	0.64835877	15.6575773	0.780038	0.673303	15.65758
557	0.69798146	0.72025493	15.6575773	0.848988	0.593368	15.65758
558	0.66441686	0.7613705	15.6575773	0.831556	0.561812	15.65758
559	0.74960655	0.68920277	15.6575773	0.708012	0.747469	15.65758
560	0.65651583	0.76636283	15.6575773	0.76714	0.701602	15.65758
561	0.76831415	0.69572604	15.6575773	0.793772	0.672402	15.65758
562	0.60516233	0.80489271	15.6575773	0.764574	0.64737	15.65758
563	0.73098764	0.72198431	15.6575773	0.81134	0.654706	15.65758
564	0.7850624	0.64925151	15.6575773	0.858014	0.629467	15.65758
565	0.714201	0.70662617	15.6575773	0.857895	0.617429	15.65758
566	0.1812916	1.01924653	2	0.765667	0.668773	15.65758
567	0.17227393	1.03528054	2	0.741609	0.725371	15.65758
568	0.79890394	0.66154572	15.6575773	0.718383	0.724958	15.65758
569	0.76437784	0.6876607	15.6575773	0.776485	0.699374	15.65758
570	0.40525942	0.91632942	15.6575773	0.785652	0.674369	15.65758
571	0.62821331	0.790657	15.6575773	0.79425	0.673281	15.65758
572	0.54054335	0.84392697	15.6575773	0.748109	0.7239	15.65758
573	0.76135566	0.71534245	15.6575773	0.764618	0.701386	15.65758
574	0.68841508	0.74224981	15.6575773	0.700809	0.750011	15.65758
575	0.5650588	0.82783287	15.6575773	0.873118	0.513347	15.65758
576	0.61925186	0.78776	15.6575773	0.778786	0.645726	15.65758
577	0.75747844	0.66199282	15.6575773	0.736532	0.728779	15.65758
578	0.52336018	0.85151132	15.6575773	0.747596	0.714281	15.65758
579				0.829477	0.607059	15.65758
580				0.833785	0.659942	15.65758
581				0.797244	0.663176	15.65758
582				0.807191	0.666704	15.65758
583				0.654101	0.769864	15.65758
584				0.787908	0.686874	15.65758
585				0.831786	0.653351	15.65758
586				0.792458	0.677405	15.65758
587				0.799621	0.676005	15.65758
588				0.804854	0.659336	15.65758
589				0.7692	0.68541	15.65758
590				0.819506	0.645388	15.65758
591				0.739765	0.716171	15.65758
592				0.703424	0.728333	15.65758
593				0.880527	0.599636	15.65758
594				0.74548	0.717302	15.65758
595				0.802857	0.672605	15.65758
596				0.809635	0.592931	15.65758
597				0.836957	0.646949	15.65758
598				0.839056	0.6416	15.65758
599				0.81157	0.680155	15.65758
600				0.800692	0.655562	15.65758
601				0.780288	0.698029	15.65758
602				0.755929	0.689811	15.65758
603				0.81974	0.663588	15.65758
604				0.723193	0.731441	15.65758

605	0.836401	0.628687	15.65758
606	0.911251	0.419262	15.65758
607	0.818817	0.6423	15.65758
608	0.815959	0.587867	15.65758
609	0.775377	0.701169	15.65758
610	0.810637	0.676703	15.65758
611	0.783418	0.686293	15.65758
612	0.827488	0.651619	15.65758
613	0.830243	0.633429	15.65758
614	0.76299	0.706539	15.65758
615	0.777326	0.683108	15.65758
616	0.792992	0.688547	15.65758
617	0.791698	0.667083	15.65758
618	0.746328	0.709129	15.65758
619	0.824635	0.653013	15.65758
620	0.780386	0.695657	15.65758
621	0.890327	0.548828	15.65758
622	0.765789	0.695786	15.65758
623	0.793323	0.67593	15.65758
624	0.799253	0.685113	15.65758
625	0.707269	0.713864	15.65758
626	0.815809	0.659695	15.65758
627	0.756838	0.708618	15.65758
628	0.758913	0.712994	15.65758
629	0.806806	0.670664	15.65758
630	0.725551	0.702166	15.65758
631	0.789219	0.677406	15.65758
632	0.798177	0.671401	15.65758
633	0.771027	0.67813	15.65758
634	0.809229	0.666031	15.65758
635	0.752316	0.7117	15.65758
636	0.745579	0.711261	15.65758
637	0.745375	0.717513	15.65758
638	0.828201	0.622256	15.65758
639	0.819101	0.644291	15.65758
640	0.783208	0.690717	15.65758
641	0.639238	0.786195	15.65758
642	0.728835	0.728735	15.65758
643	0.5941	0.810263	15.65758
644	0.743587	0.716656	15.65758
645	0.819951	0.659432	15.65758
646	0.71859	0.736019	15.65758
647	0.817048	0.659215	15.65758
648	0.814462	0.666707	15.65758
649	0.737011	0.71207	15.65758
650	0.751921	0.716585	15.65758
651	0.771256	0.690252	15.65758
652	0.779024	0.688401	15.65758
653	0.784727	0.669316	15.65758
654	0.763146	0.688507	15.65758
655	0.761684	0.700649	15.65758
656	0.744078	0.718388	15.65758
657	0.839238	0.631888	15.65758
658	0.712365	0.70875	15.65758
659	0.781958	0.696883	15.65758
660	0.764964	0.708114	15.65758
661	0.781655	0.694766	15.65758
662	0.88273	0.513199	15.65758
663	0.708519	0.743325	15.65758
664	0.78099	0.685178	15.65758
665	0.705255	0.723169	15.65758
666	0.78502	0.685646	15.65758
667	0.696065	0.734747	15.65758
668	0.832355	0.645889	15.65758
669	0.687384	0.756173	15.65758
670	0.778759	0.689232	15.65758
671	0.739144	0.727058	15.65758
672	0.766936	0.700403	15.65758
673	0.831052	0.656697	15.65758
674	0.76816	0.703657	15.65758
675	0.80481	0.656479	15.65758
676	0.830248	0.662945	15.65758
677	0.799644	0.675043	15.65758
678	0.755421	0.682801	15.65758
679	0.807969	0.663481	15.65758
680	0.778601	0.703357	15.65758
681	0.678404	0.759408	15.65758
682	0.844275	0.562663	15.65758
683	0.771829	0.692486	15.65758
684	0.827057	0.658848	15.65758
685	0.8889	0.520816	15.65758
686	0.779557	0.685926	15.65758
687	0.826954	0.656704	15.65758
688	0.769125	0.699903	15.65758
689	0.881961	0.496594	15.65758
690	0.710025	0.745151	15.65758
691	0.815741	0.637419	15.65758
692	0.864562	0.58562	15.65758
693	0.731239	0.725962	15.65758
694	0.725731	0.733802	15.65758
695	0.809398	0.650864	15.65758
696	0.844813	0.629186	15.65758
697	0.772659	0.696847	15.65758
698	0.785803	0.684959	15.65758
699	0.875638	0.559683	15.65758
700	0.745452	0.708584	15.65758
701	0.629989	0.794424	15.65758
702	0.814794	0.658299	15.65758
703	0.755485	0.715518	15.65758
704	0.831513	0.634615	15.65758
705	0.852114	0.583726	15.65758
706	0.849181	0.608123	15.65758
707	0.766739	0.672542	15.65758
708	0.687113	0.751077	15.65758
709	0.775649	0.668987	15.65758
710	0.73186	0.728451	15.65758
711	0.794779	0.668587	15.65758
712	0.746058	0.714136	15.65758
713	0.783579	0.684422	15.65758
714	0.793903	0.623058	15.65758
715	0.797154	0.664462	15.65758
716	0.829359	0.654034	15.65758
717	0.794461	0.67782	15.65758
718	0.746753	0.713377	15.65758
719	0.764302	0.709912	15.65758
720	0.821783	0.669849	15.65758
721	0.600036	0.803218	15.65758
722	0.773492	0.697938	15.65758
723	0.748952	0.708535	15.65758
724	0.802202	0.669293	15.65758
725	0.872055	0.608634	15.65758
726	0.821294	0.652607	15.65758
727	0.726352	0.722836	15.65758
728	0.659818	0.765166	15.65758
729	0.782503	0.684321	15.65758
730	0.688529	0.744634	15.65758
731	0.773486	0.705232	15.65758
732	0.691429	0.749325	15.65758
733	0.71483	0.729752	15.65758
734	0.78104	0.684301	15.65758
735	0.75907	0.690635	15.65758
736	0.610114	0.800979	15.65758
737	0.756082	0.695542	15.65758
738	0.76771	0.686997	15.65758
739	0.804666	0.665904	15.65758
740	0.664061	0.753398	15.65758
741	0.810006	0.663316	15.65758
742	0.749778	0.721999	15.65758
743	0.810336	0.665186	15.65758
744	0.712186	0.735793	15.65758
745	0.783865	0.679325	15.65758
746	0.796486	0.682023	15.65758
747	0.796407	0.671123	15.65758
748	0.804208	0.675282	15.65758
749	0.800447	0.654541	15.65758
750	0.767746	0.693829	15.65758
751	0.732253	0.728506	15.65758
752	0.751563	0.699475	15.65758
753	0.81538	0.675123	15.65758
754	0.823838	0.657871	15.65758
755	0.758944	0.710429	15.65758
756	0.695817	0.739976	15.65758
757	0.826663	0.628033	15.65758
758	0.853517	0.595221	15.65758
759	0.797715	0.681695	15.65758
760	0.785589	0.691973	15.65758
761	0.795076	0.679921	15.65758
762	0.768809	0.719103	15.65758
763	0.84491	0.646007	15.65758
764	0.773846	0.684647	15.65758
765	0.84135	0.614592	15.65758
766	0.808888	0.672955	15.65758
767	0.782672	0.682618	15.65758
768	0.7018	0.747612	15.65758
769	0.820353	0.624411	15.65758
770	0.763721	0.692248	15.65758
771	0.770611	0.680024	15.65758
772	0.725137	0.731628	15.65758
773	0.739857	0.718038	15.65758
774	0.855136	0.583444	15.65758
775	0.752022	0.712958	15.65758
776	0.736903	0.721651	15.65758
777	0.793606	0.622588	15.65758
778	0.822497	0.644963	15.65758
779	0.825582	0.619915	15.65758
780	0.772236	0.692406	15.65758
781	0.791914	0.653098	15.65758
782	0.808445	0.672087	15.65758
783	0.793581	0.687456	15.65758
784	0.679075	0.760631	15.65758
785	0.775771	0.674573	15.65758
786	0.855273	0.603479	15.65758
787	0.766861	0.699522	15.65758
788	0.833812	0.63632	15.65758
789	0.671904	0.767981	15.65758
790	0.627788	0.780373	15.65758
791	0.691161	0.754252	15.65758
792	0.800067	0.633382	15.65758
793	0.681481	0.747214	15.65758
794	0.896852	0.506732	15.65758
795	0.826931	0.646884	15.65758
796	0.81027	0.685558	15.65758
797	0.833139	0.601492	15.65758
798	0.866313	0.595174	15.65758
799	0.761802	0.70396	15.65758
800	0.708014	0.73629	15.65758
801	0.777637	0.693333	15.65758
802	0.820679	0.64775	15.65758
803	0.783761	0.667992	15.65758
804	0.745293	0.71143	15.65758
805	0.722151	0.743919	15.65758
806	0.761253	0.710745	15.65758
807	0.73444	0.683018	15.65758
808	0.768755	0.704782	15.65758
809	0.746894	0.717701	15.65758
810	0.707796	0.743212	15.65758

811	0.691524	0.75594	15.65758
812	0.78693	0.666508	15.65758
813	0.745628	0.698497	15.65758
814	0.74929	0.713002	15.65758
815	0.768514	0.698464	15.65758
816	0.842632	0.584736	15.65758
817	0.7051	0.730224	15.65758
818	0.76721	0.699879	15.65758
819	0.787893	0.671389	15.65758
820	0.780374	0.682974	15.65758
821	0.785363	0.692493	15.65758
822	0.859342	0.549345	15.65758
823	0.767217	0.704088	15.65758
824	0.739796	0.720221	15.65758
825	0.728577	0.733938	15.65758
826	0.733453	0.726341	15.65758
827	0.732387	0.72961	15.65758
828	0.815105	0.630482	15.65758
829	0.839441	0.65636	15.65758
830	0.834698	0.650292	15.65758
831	0.771269	0.704258	15.65758
832	0.789611	0.686827	15.65758
833	0.735719	0.72393	15.65758
834	0.814591	0.616849	15.65758
835	0.790419	0.689815	15.65758
836	0.78473	0.682878	15.65758
837	0.813936	0.656387	15.65758
838	0.829929	0.611358	15.65758
839	0.772189	0.694661	15.65758
840	0.707238	0.720096	15.65758
841	0.685376	0.754889	15.65758
842	0.769271	0.691431	15.65758
843	0.744316	0.714659	15.65758
844	0.880236	0.542133	15.65758
845	0.760082	0.702195	15.65758
846	0.763591	0.702477	15.65758
847	0.809324	0.672497	15.65758
848	0.822625	0.612721	15.65758
849	0.766008	0.712739	15.65758
850	0.785518	0.69913	15.65758
851	0.675955	0.765904	15.65758
852	0.800859	0.602692	15.65758
853	0.858401	0.561668	15.65758
854	0.769692	0.692131	15.65758
855	0.816124	0.659429	15.65758
856	0.771042	0.692388	15.65758
857	0.771223	0.686221	15.65758
858	0.670844	0.76087	15.65758
859	0.737992	0.727044	15.65758
860	0.672762	0.762582	15.65758
861	0.887975	0.483904	15.65758
862	0.73406	0.723293	15.65758
863	0.816208	0.655008	15.65758
864	0.841274	0.657643	15.65758
865	0.799198	0.687463	15.65758
866	0.835788	0.629502	15.65758
867	0.777863	0.689189	15.65758
868	0.695276	0.752255	15.65758
869	0.712226	0.736885	15.65758
870	0.773715	0.697803	15.65758
871	0.79545	0.687114	15.65758
872	0.765891	0.688616	15.65758
873	0.795922	0.683263	15.65758
874	0.769179	0.682906	15.65758
875	0.760249	0.70093	15.65758
876	0.819652	0.651266	15.65758
877	0.70846	0.709759	15.65758
878	0.803284	0.649877	15.65758
879	0.80757	0.666417	15.65758
880	0.775111	0.697829	15.65758
881	0.763048	0.704407	15.65758
882	0.768287	0.701009	15.65758
883	0.767751	0.694695	15.65758
884	0.822764	0.634876	15.65758
885	0.850516	0.640262	15.65758
886	0.591747	0.816696	15.65758
887	0.73863	0.725974	15.65758
888	0.748382	0.720216	15.65758
889	0.804458	0.656342	15.65758
890	0.769842	0.692604	15.65758
891	0.778384	0.691461	15.65758
892	0.728208	0.728898	15.65758
893	0.81855	0.672021	15.65758
894	0.704846	0.746881	15.65758
895	0.72821	0.730615	15.65758
896	0.77736	0.658292	15.65758
897	0.701517	0.744183	15.65758
898	0.784194	0.643621	15.65758
899	0.802241	0.66083	15.65758
900	0.825347	0.655826	15.65758
901	0.783879	0.671634	15.65758
902	0.812159	0.651685	15.65758
903	0.787115	0.68212	15.65758
904	0.765787	0.695202	15.65758
905	0.789955	0.691247	15.65758
906	0.743388	0.691064	15.65758
907	0.657245	0.76763	15.65758
908	0.379121	0.93263	15.65758
909	0.778152	0.68659	15.65758
910	0.708266	0.741077	15.65758
911	0.741949	0.703251	15.65758
912	0.732645	0.709869	15.65758
913	0.733462	0.719083	15.65758
914	0.716453	0.742462	15.65758
915	0.654978	0.773194	15.65758
916	0.625046	0.795415	15.65758
917	0.714263	0.730403	15.65758
918	0.770429	0.693096	15.65758
919	0.749573	0.693034	15.65758
920	0.772131	0.695857	15.65758
921	0.766798	0.691671	15.65758
922	0.756766	0.698513	15.65758
923	0.640414	0.779451	15.65758
924	0.750113	0.706342	15.65758
925	0.719802	0.728077	15.65758
926	0.777063	0.679308	15.65758
927	0.663746	0.766772	15.65758
928	0.719106	0.712363	15.65758
929	0.751487	0.692049	15.65758
930	0.790472	0.674228	15.65758
931	0.605095	0.802587	15.65758
932	0.755214	0.715396	15.65758
933	0.697897	0.74966	15.65758
934	0.785667	0.692032	15.65758
935	0.739355	0.719973	15.65758
936	0.741586	0.708798	15.65758
937	0.771365	0.688013	15.65758
938	0.740556	0.698675	15.65758
939	0.696002	0.751329	15.65758
940	0.806964	0.656185	15.65758
941	0.681813	0.755193	15.65758
942	0.668765	0.764835	15.65758
943	0.677381	0.757851	15.65758
944	0.751058	0.663902	15.65758
945	0.732774	0.689789	15.65758
946	0.67583	0.757583	15.65758
947	0.771558	0.680492	15.65758
948	0.697133	0.727558	15.65758
949	0.810621	0.659512	15.65758
950	0.784512	0.665114	15.65758
951	0.840039	0.615174	15.65758
952	0.694421	0.744816	15.65758
953	0.712999	0.7328	15.65758
954	0.838536	0.616874	15.65758
955	0.65724	0.771597	15.65758
956	0.744268	0.710347	15.65758
957	0.729235	0.72432	15.65758
958	0.70442	0.729031	15.65758
959	0.775332	0.695943	15.65758
960	0.630596	0.789718	15.65758
961	0.701197	0.723258	15.65758
962	0.806464	0.633677	15.65758
963	0.703745	0.725434	15.65758
964	0.582657	0.822075	15.65758
965	0.700899	0.717601	15.65758
966	0.696693	0.742772	15.65758
967	0.720849	0.714114	15.65758
968	0.716702	0.729774	15.65758
969	0.756091	0.676041	15.65758
970	0.761053	0.694696	15.65758
971	0.825623	0.619598	15.65758
972	0.760872	0.685556	15.65758
973	0.767353	0.701338	15.65758
974	0.751818	0.716972	15.65758
975	0.672787	0.758048	15.65758
976	0.70122	0.735332	15.65758
977	0.548242	0.841881	15.65758
978	0.653347	0.778939	15.65758
979	0.657986	0.758886	15.65758
980	0.693729	0.739627	15.65758
981	0.75958	0.68512	15.65758
982	0.787659	0.682513	15.65758
983	0.783072	0.673346	15.65758
984	0.852235	0.565936	15.65758
985	0.756683	0.704229	15.65758
986	0.776682	0.70108	15.65758
987	0.744442	0.717364	15.65758
988	0.705559	0.729741	15.65758
989	0.691645	0.735575	15.65758
990	0.766992	0.682072	15.65758
991	0.723486	0.723949	15.65758
992	0.624424	0.793921	15.65758
993	0.224089	1.000564	15.65758
994	0.742459	0.701235	15.65758
995	0.669515	0.762791	15.65758
996	0.800951	0.650713	15.65758
997	0.688341	0.743621	15.65758
998	0.680654	0.742041	15.65758
999	0.750087	0.70924	15.65758
1000	0.781958	0.684516	15.65758
1001	0.837911	0.624383	15.65758
1002	0.771139	0.686127	15.65758
1003	0.735688	0.722035	15.65758
1004	0.747783	0.686443	15.65758
1005	0.727815	0.690349	15.65758
1006	0.762345	0.700186	15.65758
1007	0.718939	0.72389	15.65758
1008	0.746073	0.719766	15.65758
1009	0.705781	0.738709	15.65758
1010	0.793995	0.65613	15.65758
1011	0.723311	0.723929	15.65758
1012	0.629387	0.786832	15.65758
1013	0.77112	0.669028	15.65758
1014	0.77787	0.66907	15.65758
1015	0.669405	0.76766	15.65758
1016	0.705858	0.742072	15.65758

1017	0.680278	0.753871	15.65758
1018	0.753652	0.662044	15.65758
1019	0.820061	0.647687	15.65758
1020	0.72079	0.723806	15.65758
1021	0.776586	0.688046	15.65758
1022	0.687348	0.754433	15.65758
1023	0.690358	0.739867	15.65758
1024	0.783328	0.661857	15.65758
1025	0.506323	0.861537	15.65758
1026	0.67595	0.754128	15.65758
1027	0.757751	0.710844	15.65758
1028	0.75452	0.709793	15.65758
1029	0.757314	0.696604	15.65758
1030	0.815508	0.612907	15.65758
1031	0.71877	0.729066	15.65758
1032	0.743899	0.715105	15.65758
1033	0.790251	0.658635	15.65758
1034	0.732677	0.729678	15.65758
1035	0.35503	0.964937	15.65758
1036	0.709782	0.76099	15.65758
1037	0.531547	0.847224	15.65758
1038	0.732107	0.723587	15.65758
1039	0.744643	0.674368	15.65758
1040	0.627713	0.793194	15.65758
1041	0.756276	0.688384	15.65758
1042	0.653043	0.780371	15.65758
1043	0.560027	0.833088	15.65758
1044	0.711066	0.725508	15.65758
1045	0.756527	0.710687	15.65758
1046	0.818695	0.634769	15.65758
1047	0.695607	0.735414	15.65758
1048	0.714009	0.72878	15.65758
1049	0.712248	0.741944	15.65758
1050	0.775953	0.672263	15.65758
1051	0.744133	0.703175	15.65758
1052	0.772121	0.684686	15.65758
1053	0.713474	0.728495	15.65758
1054	0.771891	0.685924	15.65758
1055	0.809628	0.654636	15.65758
1056	0.760103	0.692118	15.65758
1057	0.78302	0.678274	15.65758
1058	0.745836	0.714558	15.65758
1059	0.768741	0.697168	15.65758
1060	0.704826	0.749205	15.65758
1061	0.775161	0.683603	15.65758
1062	0.732497	0.702145	15.65758
1063	0.794772	0.653777	15.65758
1064	0.704785	0.741416	15.65758
1065	0.610918	0.803309	15.65758
1066	0.73975	0.703612	15.65758
1067	0.578734	0.819566	15.65758
1068	0.755852	0.712753	15.65758
1069	0.757024	0.697733	15.65758
1070	0.790732	0.687048	15.65758
1071	0.63718	0.77523	15.65758
1072	0.672559	0.764661	15.65758
1073	0.551601	0.837156	15.65758
1074	0.838523	0.610499	15.65758
1075	0.78146	0.649677	15.65758
1076	0.757695	0.682712	15.65758
1077	0.765066	0.691627	15.65758
1078	0.798581	0.656941	15.65758
1079	0.66402	0.761724	15.65758
1080	0.720704	0.730526	15.65758
1081	0.660943	0.768078	15.65758
1082	0.819114	0.637775	15.65758
1083	0.74674	0.695437	15.65758
1084	0.709397	0.741257	15.65758
1085	0.711678	0.738512	15.65758
1086	0.688208	0.753062	15.65758
1087	0.793762	0.656493	15.65758
1088	0.708538	0.730728	15.65758
1089	0.801037	0.669775	15.65758
1090	0.641376	0.776633	15.65758
1091	0.736028	0.720062	15.65758
1092	0.674931	0.768255	15.65758
1093	0.776621	0.659614	15.65758
1094	0.767389	0.680831	15.65758
1095	0.790682	0.632699	15.65758
1096	0.841569	0.63514	15.65758
1097	0.743903	0.704644	15.65758
1098	0.805389	0.618343	15.65758
1099	0.696449	0.748044	15.65758
1100	0.719927	0.715891	15.65758
1101	0.69704	0.73399	15.65758
1102	0.651679	0.771676	15.65758
1103	0.755278	0.670244	15.65758
1104	0.720316	0.722533	15.65758
1105	0.737407	0.719694	15.65758
1106	0.662154	0.766654	15.65758
1107	0.830692	0.631771	15.65758
1108	0.791845	0.666042	15.65758
1109	0.649861	0.777237	15.65758
1110	0.686171	0.760505	15.65758
1111	0.816981	0.643317	15.65758
1112	0.764978	0.679219	15.65758
1113	0.614046	0.795592	15.65758
1114	0.639398	0.78214	15.65758
1115	0.704238	0.733455	15.65758
1116	0.755563	0.695235	15.65758
1117	0.785704	0.692678	15.65758
1118	0.758935	0.708937	15.65758
1119	0.781297	0.676508	15.65758

1120	0.71271	0.741053	15.65758
1121	0.590683	0.814181	15.65758
1122	0.717742	0.722819	15.65758
1123	0.692886	0.739174	15.65758
1124	0.630878	0.791091	15.65758
1125	0.735303	0.720045	15.65758
1126	0.631029	0.780588	15.65758
1127	0.722456	0.724475	15.65758
1128	0.531931	0.848575	15.65758
1129	0.70148	0.746213	15.65758
1130	0.763469	0.685513	15.65758
1131	0.639119	0.773494	15.65758
1132	0.772997	0.687042	15.65758
1133	0.828557	0.623948	15.65758
1134	0.695512	0.755683	15.65758
1135	0.742055	0.690035	15.65758
1136	0.6752	0.765561	15.65758
1137	0.813241	0.638919	15.65758
1138	0.800103	0.673278	15.65758
1139	0.778431	0.663085	15.65758
1140	0.665075	0.758563	15.65758
1141	0.239762	0.998118	15.65758
1142	0.66556	0.757436	15.65758
1143	0.700001	0.735559	15.65758
1144	0.678126	0.762227	15.65758
1145	0.747102	0.705114	15.65758
1146	0.803634	0.660102	15.65758
1147	0.783473	0.669712	15.65758
1148	0.747155	0.708985	15.65758
1149	0.725198	0.702334	15.65758
1150	0.685829	0.75145	15.65758
1151	0.502274	0.864317	15.65758
1152	0.782728	0.672239	15.65758
1153	0.72986	0.727101	15.65758
1154	0.773939	0.697421	15.65758
1155	0.687333	0.743767	15.65758
1156	0.707543	0.731405	15.65758
1157	0.845588	0.611515	15.65758
1158	0.710151	0.732487	15.65758
1159	0.641018	0.772645	15.65758
1160	0.661364	0.776176	15.65758
1161	0.808727	0.653302	15.65758
1162	0.766915	0.69704	15.65758
1163	0.686071	0.753808	15.65758
1164	0.599673	0.805603	15.65758
1165	0.794448	0.629683	15.65758
1166	0.802811	0.644151	15.65758
1167	0.651216	0.772093	15.65758
1168	0.795941	0.674203	15.65758
1169	0.72548	0.711849	15.65758
1170	0.739106	0.712871	15.65758
1171	0.730707	0.71683	15.65758
1172	0.698956	0.743106	15.65758
1173	0.809202	0.661838	15.65758
1174	0.821564	0.578432	15.65758
1175	0.632834	0.784984	15.65758
1176	0.577107	0.821497	15.65758
1177	0.707338	0.741701	15.65758
1178	0.715981	0.736117	15.65758
1179	0.764327	0.675078	15.65758
1180	0.807443	0.655474	15.65758
1181	0.681317	0.739627	15.65758
1182	0.73531	0.704112	15.65758
1183	0.66009	0.755459	15.65758
1184	0.740376	0.714956	15.65758
1185	0.776033	0.672729	15.65758
1186	0.740137	0.69061	15.65758
1187	0.63157	0.787747	15.65758
1188	0.754081	0.697833	15.65758
1189	0.74844	0.685375	15.65758
1190	0.727209	0.735502	15.65758
1191	0.656472	0.77443	15.65758
1192	0.796227	0.658925	15.65758
1193	0.772166	0.672837	15.65758
1194	0.715671	0.736194	15.65758
1195	0.52654	0.849773	15.65758
1196	0.732145	0.726056	15.65758
1197	0.705633	0.734931	15.65758
1198	0.768547	0.702023	15.65758
1199	0.71499	0.738296	15.65758
1200	0.764234	0.694914	15.65758
1201	0.673909	0.756292	15.65758
1202	0.682181	0.744902	15.65758
1203	0.774757	0.684742	15.65758
1204	0.680445	0.757292	15.65758
1205	0.69218	0.741157	15.65758
1206	0.76709	0.67628	15.65758
1207	0.736914	0.707589	15.65758
1208	0.708526	0.729553	15.65758
1209	0.708515	0.728573	15.65758
1210	0.740751	0.717606	15.65758
1211	0.623889	0.794416	15.65758
1212	0.807316	0.656808	15.65758
1213	0.762856	0.706352	15.65758
1214	0.712044	0.736508	15.65758
1215	0.588046	0.817042	15.65758
1216	0.755743	0.686084	15.65758
1217	0.757567	0.666973	15.65758
1218	0.278226	0.974871	15.65758
1219	0.725258	0.723067	15.65758
1220	0.70005	0.736196	15.65758
1221	0.58577	0.814576	15.65758
1222	0.723187	0.720456	15.65758

1223	0.714422	0.730269	15.65758
1224	0.577502	0.822202	15.65758
1225	0.693312	0.751261	15.65758
1226	0.791282	0.685651	15.65758
1227	0.564387	0.829423	15.65758
1228	0.747661	0.704331	15.65758
1229	0.720655	0.713909	15.65758
1230	0.662961	0.769072	15.65758
1231	0.72137	0.721816	15.65758
1232	0.686161	0.748134	15.65758
1233	0.809834	0.661821	15.65758
1234	0.76412	0.698151	15.65758
1235	0.689511	0.733263	15.65758
1236	0.792592	0.657457	15.65758
1237	0.766558	0.712442	15.65758
1238	0.761543	0.706918	15.65758
1239	0.550016	0.837194	15.65758
1240	0.730906	0.726227	15.65758
1241	0.793114	0.681477	15.65758
1242	0.784768	0.672592	15.65758
1243	0.747936	0.716881	15.65758
1244	0.725548	0.721379	15.65758
1245	0.629535	0.794369	15.65758
1246	0.839963	0.619946	15.65758
1247	0.630581	0.786691	15.65758
1248	0.802012	0.65231	15.65758
1249	0.704737	0.732707	15.65758
1250	0.684682	0.75004	15.65758
1251	0.751803	0.681922	15.65758
1252	0.727949	0.725403	15.65758
1253	0.695998	0.751598	15.65758
1254	0.484333	0.874303	15.65758
1255	0.698134	0.743205	15.65758
1256	0.714551	0.727829	15.65758
1257	0.66066	0.782367	15.65758
1258	0.785997	0.641922	15.65758
1259	0.784753	0.691787	15.65758
1260	0.779707	0.685682	15.65758
1261	0.825568	0.627843	15.65758
1262	0.72802	0.711189	15.65758
1263	0.75862	0.696339	15.65758
1264	0.747344	0.700294	15.65758
1265	0.694342	0.746211	15.65758
1266	0.860389	0.599314	15.65758
1267	0.759755	0.703841	15.65758
1268	0.642002	0.777851	15.65758
1269	0.776608	0.633207	15.65758
1270	0.744324	0.704364	15.65758
1271	0.741291	0.712387	15.65758
1272	0.744685	0.694415	15.65758
1273	0.702378	0.745417	15.65758
1274	0.779596	0.6634	15.65758
1275	0.756676	0.701942	15.65758
1276	0.677364	0.771027	15.65758
1277	0.714177	0.733026	15.65758
1278	0.722679	0.730909	15.65758
1279	0.702159	0.743194	15.65758
1280	0.74375	0.702985	15.65758
1281	0.812572	0.642327	15.65758
1282	0.720098	0.735015	15.65758
1283	0.749565	0.69603	15.65758
1284	0.75384	0.709555	15.65758
1285	0.779544	0.686506	15.65758
1286	0.585673	0.817444	15.65758
1287	0.62267	0.793432	15.65758
1288	0.75075	0.679543	15.65758
1289	0.769074	0.714154	15.65758
1290	0.672913	0.755533	15.65758
1291	0.791189	0.677605	15.65758
1292	0.687371	0.748525	15.65758
1293	0.728507	0.727564	15.65758
1294	0.712912	0.744501	15.65758
1295	0.770852	0.664634	15.65758
1296	0.722733	0.720011	15.65758
1297	0.779218	0.666609	15.65758
1298	0.647898	0.772769	15.65758
1299	0.796565	0.6778	15.65758
1300	0.777649	0.685544	15.65758
1301	0.697163	0.750899	15.65758
1302	0.723201	0.736601	15.65758
1303	0.663783	0.771957	15.65758
1304	0.79649	0.632939	15.65758
1305	0.716883	0.734674	15.65758
1306	0.826048	0.635109	15.65758
1307	0.81099	0.665067	15.65758
1308	0.721699	0.720644	15.65758
1309	0.779726	0.691522	15.65758
1310	0.707097	0.750393	15.65758
1311	0.768244	0.65827	15.65758
1312	0.693818	0.742331	15.65758
1313	0.654357	0.774034	15.65758
1314	0.731014	0.727412	15.65758
1315	0.820342	0.606148	15.65758
1316	0.719218	0.72763	15.65758
1317	0.792234	0.649942	15.65758
1318	0.607372	0.801541	15.65758
1319	0.767538	0.694009	15.65758
1320	0.672924	0.771797	15.65758
1321	0.700652	0.744471	15.65758
1322	0.704809	0.723656	15.65758
1323	0.814738	0.629158	15.65758
1324	0.790582	0.628781	15.65758
1325	0.718023	0.713256	15.65758
1326	0.783174	0.692256	15.65758
1327	0.675715	0.755211	15.65758
1328	0.659093	0.769057	15.65758
1329	0.668308	0.758882	15.65758
1330	0.669046	0.752132	15.65758
1331	0.744776	0.714642	15.65758
1332	0.670585	0.764935	15.65758
1333	0.68995	0.740489	15.65758
1334	0.607847	0.796431	15.65758
1335	0.754045	0.70841	15.65758
1336	0.611958	0.805104	15.65758
1337	0.746985	0.705477	15.65758
1338	0.753129	0.69097	15.65758
1339	0.825727	0.661501	15.65758
1340	0.697593	0.745985	15.65758
1341	0.621878	0.792016	15.65758
1342	0.760842	0.704345	15.65758
1343	0.771142	0.677164	15.65758
1344	0.775522	0.678941	15.65758
1345	0.488443	0.872785	15.65758
1346	0.687731	0.754729	15.65758
1347	0.72357	0.736731	15.65758
1348	0.756148	0.677644	15.65758
1349	0.727772	0.709451	15.65758

Table S2. Phenotypic characteristics of GTEx lung tissue donors

Feature		Number of individuals (%)	Median (range)
Sex			
	Female	183 (31.7)	
	Male	395 (68.3)	
Age			
	<40	73 (12.6)	56 (21 - 70)
	40-49	93 (16.1)	
	50-59	200 (34.6)	
	60-69	190 (32.9)	
	>69	22 (3.8)	
Body mass index			
	<25	155 (26.8)	27.7 (17.0 - 35.4)
	25-30	246 (42.6)	
	>30	177 (30.6)	
Race			
	Caucasian	493 (85.9)	
	Black/African American	70 (12.2)	
	Asian	10 (1.7)	
	American Indian/Alaska Native	1 (0.2)	
Smoking status			
	Non-smoker	180 (32.0)	
	Smoker	382 (68.0)	

Table S3. Multivariate analysis of ACE2 expression in human lung tissue

Variable	β value	S.E.	*t* value	*p* value	
CD8+ T cells	-2.719	0.584	-4.656	**4.02e-06**	****
NK cells resting	-3.128	0.583	-5.366	**1.18e-07**	****
NK cells activated	-2.281	0.704	-3.241	**0.0013**	**
M1 macrophages	-4.963	1.339	-3.707	**0.0002**	***
CD4+ T cells	0.453	0.513	0.882	0.3781	
Dendritic cells	-12.793	6.688	-1.913	0.0563	
Neutrophils	-0.398	0.469	-0.848	0.3971	
Sex = male (vs. female)	0.065	0.056	1.16	0.2466	
Age	0.004	0.002	1.799	0.0726	
BMI	-0.004	0.006	-0.556	0.5787	
Race = Caucasian (vs. non-Caucasian)	-0.096	0.073	-1.313	0.1899	
Smoking status = yes (vs. no)	-0.025	0.058	-0.433	0.6650	

Abbreviations: S.E., Standard Error.

Table S4. Meta-analysis of ACE2 mRNA and protein levels in 17 human tissues

Source data of Fig. 2f.

Source data type	Source data method	Adipose tissue	Airway epithelium	Brain	Cardiac muscle	Colorectal tissue	Endometrium	Gall bladder	Kidney	Liver	Lung	Oral mucosa/esophagus	Skin	Small intestine	Smooth muscle	Spleen	Stomach	Testis	Ref.
mRNA	NB	0	N	N	3	2	0	N	3	0	0	N	0	2	0	N	N	3	[1]
mRNA	qRT-PCR	1	1	0	2	2	1	2	2	1	1	1	0	3	1	0	1	3	[2]
mRNA	MA	2	2	2	2	2	1	N	3	2	2	N	1	3	2	N	N	3	[3]
mRNA	RNAseq	2	N	2	2	1	1	3	3	0	0	0	1	3	2	N	0	3	[4]
mRNA	RNAseq	N	N	N	N	N	N	N	N	N	N	1	N	N	N	N	N	N	[5]
mRNA	CAGE	0	N	0	1	2	0	1	1	0	0	0	N	3	0	0	N	2	[6]
Protein	MS	N	N	0	1	0	N	1	3	1	0	0	N	N	N	N	N	3	[7]
Protein	IHC	3	1	1	N	1	N	N	3	1	3	2	3	3	3	3	0	N	[8]
Protein	IHC	0	0	0	1	2	0	3	3	0	0	0	0	3	0	0	0	3	[9]
Scores (averages)																			
mRNA score		1.0	1.5	1.0	2.0	1.8	0.6	2.0	2.4	0.6	0.6	0.5	0.5	2.8	1.0	0.0	0.5	2.8	
Protein score		1.5	0.5	0.3	1.0	1.0	0.0	2.0	3.0	0.7	1.0	0.7	1.5	3.0	1.5	1.5	0.0	3.0	

Abbreviations: CAGE, cap analysis of gene expression; IHC, immunohistochemistry; MA, microarray; MS, mass spectrometry; N, not determined; NB Northern blot.

Supplementary References

1. Tipnis, S.R., et al., *A human homolog of angiotensin-converting enzyme. Cloning and functional expression as a captopril-insensitive carboxypeptidase.* J Biol Chem, 2000. **275**(43): p. 33238-43.
2. Harmer, D., et al., *Quantitative mRNA expression profiling of ACE 2, a novel homologue of angiotensin converting enzyme.* FEBS Lett, 2002. **532**(1-2): p. 107-10.
3. Wu, C., et al., *BioGPS: building your own mash-up of gene annotations and expression profiles.* Nucleic Acids Res, 2016. **44**(D1): p. D313-6.
4. Uhlen, M., et al., *A pathology atlas of the human cancer transcriptome.* Science, 2017. **357**(6352).
5. GTEx Consortium, *Genetic effects on gene expression across human tissues.* Nature, 2017. **550**(7675): p. 204-213.
6. FANTOM Consortium, RIKEN PMI, CLST (DGT), *A promoter-level mammalian expression atlas.* Nature, 2014. **507**(7493): p. 462-70.
7. Kim, M.S., et al., *A draft map of the human proteome.* Nature, 2014. **509**(7502): p. 575-81.
8. Hamming, I., et al., *Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis.* J Pathol, 2004. **203**(2): p. 631-7.
9. Thul, P.J., et al., *A subcellular map of the human proteome.* Science, 2017. **356**(6340).