Title

3D genomic capture of regulatory immuno-genetic profiles in COVID-19 patients for prognosis of severe COVID disease outcome

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Abstract

Human infection with the SARS-CoV-2 virus leads to coronavirus disease (COVID-19). A striking characteristic of COVID-19 infection in humans is the highly variable host response and the diverse clinical outcomes, ranging from clinically asymptomatic to severe immune reactions leading to hospitalization and death. Here we used a 3D genomic approach to analyse blood samples at the time of COVID diagnosis, from a global cohort of 80 COVID-19 patients, with different degrees of clinical disease outcomes. Using 3D whole genome *EpiSwitch*[®] arrays to generate over 1 million data points per patient, we identified a distinct and measurable set of differences in genomic organization at immune-related loci that demonstrated prognostic power at baseline to stratify patients with mild forms of illness and those with severe forms that required hospitalization and intensive care unit (ICU) support. Further analysis revealed both well established and new COVID-related dysregulated pathways and loci, including innate and adaptive immunity; ACE2; olfactory, $G\beta\gamma$, Ca^{2+} and nitric oxide (NO) signalling; prostaglandin E2 (PGE2), the acute inflammatory cytokine CCL3, and the T-cell derived chemotactic cytokine CCL5. We identified potential therapeutic agents for mitigation of severe disease outcome, with several already being tested independently, including mTOR inhibitors (rapamycin and tacrolimus) and general immunosuppressants (dexamethasone and hydrocortisone). Machine learning algorithms based on established *EpiSwitch*[®] methodology further identified a subset of 3D genomic changes that could be used as prognostic molecular biomarker leads for the development of a COVID-19 disease severity test.

Background

As of January 2021, an estimated 85 million people have been infected with the SARS-CoV-2 virus worldwide [1]. Infection with the virus has been shown to induce a wide range of clinical symptoms; some individuals experience asymptomatic or mild disease courses that do not require hospitalization while others develop severe coronavirus disease (COVID-19) triggered by a strong systemic immune response that can lead to acute respiratory failure, thromboembolic phenomena, microvascular disease, viral sepsis and sometimes death [2]. Another early feature associated with more severe disease is significant hypoxemia that commonly occurs in the absence of other systemic symptoms [3]. Several clinical features for disease severity risk following SARS-CoV-2 infection have been identified through epidemiological studies. These include advanced age, male gender, obesity, diabetes, hypertension, the existence of underlying medical conditions such as neurologic disability particularly stroke and renal disease, and immunocompromised status [4]. However, a subgroup of 'healthy' patients without these risk factors nevertheless can develop significant disease leading to increased morbidity and mortality. Identifying the cellular and molecular factors responsible for the heterogeneous disease presentation in COVID-19 is critical for understanding individual disease risk and designing appropriate therapeutic interventions.

Recent studies have identified a central role for the immune system in mediating disease severity following SARS-CoV-2 infection. Individuals with severe disease have higher levels of circulating inflammatory mediators

including interleukin (IL)-2, IL-6, IL-7, IL-10, tumour necrosis factor (TNF), granulocyte colony-stimulating factor (G-CSF), monocyte chemoattractant protein-1 (MCP1), and macrophage inflammatory protein 1 alpha (MIP1 α) after SARS-CoV-2 infection compared to individuals with mild disease [5, 6]. IL6, in particular, became the focus of attention as a potential disease severity biomarker, as well as a candidate for therapeutic intervention, due to its central role in mediating cytokine storms and the availability of existing targeted therapeutic drugs against it [7, 8]. However, subsequent studies called into question the prominence of IL6 and cytokine storms in SARS-Cov2 infections and several clinical trials of drugs targeting IL6 showed inconsistent results and no clinical benefit, especially for the moderately ill patients [9–13].. However, reports on IL6 treatment with tocilizumab for a specific subgroup of critically ill patients in the first two days of ICU admission demonstrated reduced risk of mortality [14]. While elevated levels of pro-inflammatory cytokines are observed in COVID-19, the exact mechanisms of immune dysfunction leading to severe symptoms remain unclear. As individuals exposed to COVID-19 infections develop a range of acute or long-term systemic inflammatory responses, the ability to identify prognostic profiles for severe clinical cases where patients develop hyperinflammation, do not respond to the standard of care, and progress to needing ICU support is of immediate clinical utility.

The 3D configuration of the genome plays a crucial role in gene regulation [15–17]. The 3D genome acts as the regulatory interface and integration point for multiple inputs: genetic variants and genetic risks, epigenetic modifications, metabolic signals and transcriptional events, influencing cellular phenotype and ultimately clinical outcomes [15, 18, 19]. *EpiSwitch*[®] is a chromosome

conformation capture (3C) methodology [20, 21] that has been translated to practice for the discovery of blood-based 3D genomic biomarkers and for patient stratification. To date, 3D genomic biomarkers based on *EpiSwitch*[®] technology have been used to successfully stratify melanoma patients, prognostically stratify patients with fast and slow progressing motor neurone disease, stratify patients with symptomatic and pre-symptomatic Huntington's disease, diagnose patients with thyroid cancer and various stages of prostate cancer, prognostically stratify patients for treatment in diffuse large B cell lymphoma, and predictively stratify patients with non-small cell lung cancer for response to the PD-L1 immuno-checkpoint inhibitor, avelumab [22–30].

Here we used the *EpiSwitch*[®] Explorer array platform for whole genome profiling of COVID patients at the time of confirmed infection to identify 3D genomic profiles, also known as chromosome conformation signatures (CCS), associated with the development of severe clinical outcomes, requiring ventilation and admission to intensive care units (ICU). After analysing 1.1 million annotated sites across the whole genome for each patient, we found significant and reproducible systemic differences in the 3D genomic profiles of patients presenting different levels of COVID-19 disease severity (mild and severe forms of the disease). To predict disease severity in patients, a subset of prognostic markers at immune-related loci displaying alternative 3D genomic conformations were used to develop a molecular classifier. The markers consistently present at baseline of diagnosis and associated with mild or severe outcomes revealed a regulatory network of biological signalling pathways dysregulated at the level of 3D genome architecture. Analysis of the protein

products of the immune-related genomic loci from the predictive marker panel identified a set of existing therapeutic agents that may have clinical utility in managing COVID-19 symptoms. The 3D genomic profile in COVID-19 patients identified through the use of *EpiSwitch*[®] Explorer arrays demonstrated prognostic power and could be further refined using the established *EpiSwitch*[®] methodology to develop a PCR-based test for prognosis of severe outcomes in COVID-19 [22–30].

Materials and Methods

Patient characteristics

Clinical PBMC and whole blood samples from consented patients were obtained from academic collaborators and commercial sources. A total of 80 patients from 4 sample cohorts were used in this study, comprising a multinational set of COVID-19 cases: asymptomatic, mild hospitalized and severe (ICU support) - from the United Kingdom, the United States, and Peru. All samples were collected at the time of diagnosis of COVID infection with PCR test. Patients were then observed over the period of up to several weeks for clinical manifestations of COVID disease. The age of the patients ranges from 24 to 95, with median at 70 years (**Table 1**).

Table 1: Patient information used in all the cohorts.

Cohort	Gender	Age	Race	Ethnicity	Country Collected	COVID Severity
1	Male	80	Other	Any other ethnic group	United Kingdom	Mild
1	Female	85	White	White/Any Other White Background	United Kingdom	Mild
1	Male	75	White	White British	United Kingdom	Mild
1	Male	36	Asian	Asian / Any Other Asian Background	United Kingdom	Severe
1	Male	70	White	White British	United Kingdom	Severe
1	Male	87	White	White - British	United Kingdom	Mild
1	Female	89	White	White/Any Other White Background	United Kingdom	Mild
1	Female	95	White	White/Any Other White Background	United Kingdom	Mild

1	Male	49	White	White/Any Other White Background	United Kingdom	Severe
1	Female	83	Other	Any other ethnic group	United Kingdom	Severe
1	Male	73	White	White/Any Other White Background	United Kingdom	Severe
2	Male	62	White	Non-Hispanic	United States	Asymptomatic
2	Male	55	White	Non-Hispanic	United States	Mild/Moderate
2	Female	62	Black	Non-Hispanic	United States	Asymptomatic
2	Male	63	White	Non-Hispanic	United States	Mild/Moderate
2	Male	71	White	Non-Hispanic	United States	Mild/Moderate
2	Female	24	Black	Non-Hispanic	United States	Mild/Moderate
2	Female	67	Black	Non-Hispanic	United States	Mild/Moderate
2	Male	65	White	Non-Hispanic	United States	Mild/Moderate
2	Female	57	Black	Non-Hispanic	United States	Mild/Moderate
2	Female	30	Black	Unknown	United States	Mild/Moderate
2	Male	71	White	Non-Hispanic	United States	Asymptomatic
2	Male	59	White	Non-Hispanic	United States	Mild/Moderate
3	Male	67	White	Non-Hispanic	United States	Severe
3	Female	62	White	Non-Hispanic	United States	Mild
3	Male	87	White	Non-Hispanic	United States	Mild
3	Female	82	White	Non-Hispanic	United States	Severe
3	Female	47	White	Hispanic	United States	Severe
3	Female	47	White	Non-Hispanic	United States	Severe
3	Female	27	White	Non-Hispanic	United States	Asymptomatic
3	Female	Unk ^a	White	Non-Hispanic	United States	Asymptomatic
3	Female	52	White	Non-Hispanic	United States	Asymptomatic
3	Female	42	White	Non-Hispanic	United States	Mild - Not hospitalised
3	Male	72	White	Non-Hispanic	United States	Mild - Not hospitalised
3	Male	67	White	Non-Hispanic	United States	Mild
3	Female	Unk	Black	Non-Hispanic	United States	Severe
3	Female	Unk	Unk	Unk	United States	Mild - Not hospitalised
3	Female	38	White	Non-Hispanic	United States	Asymptomatic
4	Male	84	N/A	Hispanic	Peru	ICU
4	Male	56	N/A	Hispanic	Peru	Hospitalised
4	Female	82	N/A	Hispanic	Peru	Hospitalised
4	Male	88	N/A	Hispanic	Peru	Hospitalised
4	Male	78	N/A	Hispanic	Peru	Hospitalised
4	Male	79	N/A	Hispanic	Peru	Hospitalised
4	Male	70	N/A	Hispanic	Peru	ICU
4	Male	64	N/A	Hispanic	Peru	ICU
4	Male	72	N/A	Hispanic	Peru	ICU
4	Male	70	N/A	Hispanic	Peru	Hospitalised
4	Female	67	N/A	Hispanic	Peru	ICU
4	Female	57	N/A	Hispanic	Peru	Hospitalised
4	Female	84	N/A	Hispanic	Peru	ICU

4	Male	49	N/A	Hispanic	Peru	Hospitalised
4	Female	83	N/A	Hispanic	Peru	ICU
4	Male	88	N/A	Hispanic	Peru	ICU
4	Female	66	N/A	Hispanic	Peru	ICU
4	Female	77	N/A	Hispanic	Peru	ICU
4	Male	89	N/A	Hispanic	Peru	ICU
4	Male	70	N/A	Hispanic	Peru	Hospitalised
4	Male	78	N/A	Hispanic	Peru	ICU
4	Female	77	N/A	Hispanic	Peru	Hospitalised
4	Female	83	N/A	Hispanic	Peru	ICU
4	Male	69	N/A	Hispanic	Peru	Hospitalised
4	Female	63	N/A	Hispanic	Peru	ICU
4	Female	82	N/A	Hispanic	Peru	ICU
4	Male	60	N/A	Hispanic	Peru	Hospitalised
4	Male	78	N/A	Hispanic	Peru	Hospitalised
4	Male	75	N/A	Hispanic	Peru	ICU
4	Male	92	N/A	Hispanic	Peru	ICU
4	Female	64	N/A	Hispanic	Peru	Hospitalised
4	Male	75	N/A	Hispanic	Peru	Hospitalised
4	Male	64	N/A	Hispanic	Peru	ICU
4	Female	79	N/A	Hispanic	Peru	ICU
4	Male	78	N/A	Hispanic	Peru	Hospitalised
4	Female	77	N/A	Hispanic	Peru	ICU
4	Male	86	N/A	Hispanic	Peru	Hospitalised
4	Female	77	N/A	Hispanic	Peru	ICU
4	Male	68	N/A	Hispanic	Peru	ICU
4	Male	63	N/A	Hispanic	Peru	Hospitalised
4	Male	68	N/A	Hispanic	Peru	ICU
4	Male	71	N/A	Hispanic	Peru	ICU

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168 ^a *Unk: Unknown.*

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170 *Array design*

171 Custom microarrays were designed using the *EpiSwitch*[®] pattern recognition
172 algorithm, which operates on Bayesian-modelling and provides a probabilistic
173 score that a region is involved in long-range chromatin interactions. It was used
174 to annotate the GRCh38 human genome assembly across ~1.1 million sites
175 with the potential to form long-range chromosome conformations [22–30]. The

most probable interactions were identified and filtered on probabilistic score and proximity to protein, long non-coding RNA, or microRNA coding sequences. Predicted interactions were limited to *EpiSwitch*[®] sites greater than 10 kb and less than 300 kb apart. Repeat masking and sequence analysis was used to ensure unique marker sequences for each interaction. The *EpiSwitch*[®] Explorer array (Agilent Technologies, Product Code X-HS-AC-02), containing 60-mer oligonucleotide probes was designed to interrogate potential 3D genomic interactions. In total, 964,631 experimental probes and 2,500 control probes were added to a 1 x 1 M CGH microarray slide design. The experimental probes were placed on the design in singlicate with the controls in groups of 250. The control probes consisted of six different *EpiSwitch*[®] interactions that are generated during the extraction processes and used for monitoring library quality. A further four external inline control probe designs were added to detect non-human (*Arabidopsis thaliana*) spike in DNA added during the sample labelling protocol to provide a standard curve and control for labelling. The external spike DNA consists of 400 bp ssDNA fragments from genomic regions of *A. thaliana*. Array-based comparisons were performed described previously, with the modification of only one sample being hybridised to each array slide in the Cy3 channel [22–30].

Preparation of genomic templates

Chromatin with intact chromosome conformations from each blood sample was extracted using the *EpiSwitch*[®] Explorer Array Kit following the manufacturer's instructions (Oxford BioDynamics Plc) [22–30]. The *EpiSwitch*[®] Explorer arrays were performed as published previously, with the modification of only one

sample being hybridised to each array slide in the Cy3 channel. *EpiSwitch*[®] Explorer arrays, based on Agilent SureSelect array platform, allow for the highly reproducible, non-biased interrogation of ~1.1 million anchor sites for 3D genomic interactions (964,631 experimental probes and 2500 control probes).

Statistical analysis

The four COVID cohorts were normalised by background correction and quantile normalisation, using the *EpiSwitch*[®] R analytic package, which is built on the Limma and dplyr libraries. The four datasets were then combined into one sample set containing 80 samples. Data was corrected for batch effects using ComBat R script. Parametric (Limma R library, Linear Regression) and non-parametric (*EpiSwitch*[®] RankProd R library) statistical methods were performed to identify 3D genomic changes that demonstrated a difference in abundance between the Mild and Severe COVID-19 classes. Asymptomatic patients (10 samples) were excluded from this analysis. The resulting data from both procedures were further filtered based on adjusted p-value (FDR correction) and abundance scores (AS). Only 3D genomic markers with adjusted p-value ≤ 0.05 and AS $-1.1 \leq$ or ≥ 1.1 were selected. Both filtered lists from Limma and RankProd analysis were compared and the intersection of the two lists was selected for further processing.

Genome mapping and linear discriminant analysis

1000 3D genomic markers from the statistically filtered list with the greatest and lowest abundance scores were selected for genome mapping. Mapping was carried out using Bedtools *closest* function for the 3 closest protein coding loci

(Gencode v33). The resulting list of ‘Severe’ and ‘Mild’ 3D genomic markers were further annotated for relatedness to immunological processes using the ‘immune process’ annotation from Gene Ontology and gene lists for immune aging and trained immunity [31–33]. Significant 3D genomic markers with associated protein coding loci involved in immune processes were then ordered by adjusted p-value (adj.P.Val), then abundance score. The top 100 3D genomic markers from this combined filter were then utilized for linear discriminant analysis (LDA) using the MASS library and visualized using the ggplot2 package in R.

Machine learning

Two approaches were selected for model building, linear discriminant analysis (LDA) and eXtreme Gradient Boosting (XGBoost). Both procedures were performed using R, with caret, XGBoost, and SHAPforxgboost libraries for the XGBoost model and MASS library for LDA model.

Biological network and drug target analysis

Network analysis for functional/biological relevance of the 3D genomic markers was performed using the Hallmark Gene Sets and BioCarta and Reactome Canonical Pathway gene sets from the Molecular Signatures Database (MSigDB) [34]. Protein interaction networks were generated using the Search Tool for the Retrieval of Interacting proteins (STRING) database [35]. Candidate drugs were identified using the GeneAnalytics platform (geneanalytics.genecards.org) [36].

Results

Array-based profiling of COVID-19 patient cohorts

We used whole-genome *EpiSwitch*[®] Explorer arrays to screen peripheral blood mononuclear cells (PBMC) samples collected at the time of confirmed COVID-19 infection from 38 patients in three independent cohorts from the US and the UK. Interestingly, all three cohorts showed separation by principal component analysis (PCA) for Mild or Severe (requiring ICU admission) outcomes without pre-selection or reduction of the array markers (**Figure 1**), suggesting that 3D genomic profiles associated with different clinical outcomes existed and could be distinguished.

Figure 1. PCA plot of three independent COVID-19 cohorts from the UK and USA for Mild and Severe (ICU) disease outcomes. *The PCA plot of COVID-19 patients that exhibited mild disease outcomes (blue circles) and severe disease outcomes requiring ICU admission (orange squares) is based on whole genome profiling of all 964 thousand CCS markers screened, without any marker reduction.*

Identification of the top prognostic 3D genomic markers for Severe & Mild COVID-19 disease outcomes

To evaluate the biological relevance of the observed separation of Mild and Severe COVID-19 outcomes by 3D genomic markers, we focused on the top 750 markers prognostically associated with each of the two outcomes. The statistical testing employed in this study to determine statistically significant 3D genomic markers benefits from using both parametric testing (Limma) and non-parametric testing (*EpiSwitch*[®] RankProd), both procedures that correct for multiple testing by using False Discovery Rate (FDR) corrections. The RankProd approach also has a resampling step to control for random rank importance, adding another layer of statistical stringency in marker selection when testing a large number of possibilities. The top markers selected were filtered based on an adjusted (FDR) P value ≤ 0.05 , and high abundance scores (AS), $-1.1 \leq$ or ≥ 1.1 . These criteria were applied to the parametric and non-parametric statistical sets, producing the top 750 intersecting markers. Similar approaches and thresholds for FDR cut-offs were utilized in previously published biomarker development studies using *EpiSwitch*[®] [22–30].

We next evaluated the top 750 3D genomic markers, identified from 964,631 whole genome screened cis-interactions, according to the functional roles of the genes encoded across their genomic locations using Hallmark Gene Sets as well as BioCarta and Reactome canonical pathway analysis (**Figure 2**). The list of affected pathways and corresponding genetic loci with individual 3D genomic changes is provided in **Supplementary Tables 1a, b**. When evaluating the biological function of the genes within the genomic regions identified as being dysregulated between patients who developed mild or severe outcomes in COVID-19, a number of biological pathways with known associations to COVID-19 were identified including; the olfactory signalling pathway ACE2, innate and adaptive immune systems, IL6 and JAK-STAT signalling, calcium signalling, (NO) nitric oxide signalling, coagulation, complement, interferon gamma response, TGF beta signalling, TNF alpha signalling, and apoptosis [37–42]. In addition, the MSP-RON systemic inflammatory response was identified as a potentially novel signalling pathway dysregulated in COVID-19.

Figure 2. Mapping of top 3D genomic markers to biological pathways.
Gene set analysis of the top 750 3D genomic markers separating Mild and Severe (ICU) COVID-19 outcomes using Hallmark (A), BioCarta (B), and Reactome (C) gene and canonical pathway lists. Thickness of edges indicates

the number of mapping dysregulated genes and color indicates statistical rank
*(orange - high rank; blue - lower rank). See also **Supplementary Tables 1a, b.***

Interestingly, SARS-CoV-2 has been reported to drive hyperactivation of CD4+ T-cells and immune paralysis (due to loss of FOXP3 negative feedback) to promote pathogenesis of disease [43]. CD25+ (IL2 alpha receptor) FOXP3- are considered hyperactive T-cells and fail to differentiate into regulatory T-cells (Tregs) and produce Furin to promote viral entry into lung epithelial cells [43]. CD28 (see Fig.2C, Reactome, ICU) may function with IL2 to mediate the feedbacks necessary to repress a potentially overstimulated immune response in COVID19. With CD25 being the IL2 alpha receptor, IL-2 acts as a potent growth factors for CD25-expressing activated T cells (see Fig.2A Hallmark, ICU). The prevalence of both IL2 and CD25 indicates that a positive feedback loop for T-cell activation is established in severe COVID-19 leading to the production of multiple effector cytokines. This may be because of a reduction of FOXP3-mediated negative regulation to allow functional Tregs to be produced. The CD25+ T-cells in severe patients are likely to die partly by cytokine deprivation or become hyperactivated in severe disease – i.e. FOXP3 negative cells may become ex-Tregs or hyperactivated T-cells (leading to T cell paralysis). These abnormally activated T-cells produce Furin which activates the Spike pro-protein cleavage and promotes viral entry into cells.

Regarding the immune checkpoints, IL2 expression activates FOXP3 and prolonged activation results in the expression of immune checkpoints such as CTLA-4 and FOXP3, which represses transcription of effector cytokines,

suppression of T-cell responses and resolution of inflammation (i.e. in normal cells). In severe COVID-19, hyperactivated macrophages may present antigens to CD4+ T-cells which are activated and differentiate into CD25+, IL10R+ early activated T-cells which produced IL10 rather than IL2 and there is no Foxp3 expression to start the negative feedback. IL10 further enhances the activation of CD25+ T-cells which express immune checkpoints, multiple cytokines and Furin. Multifaceted Th differentiation leads to unfocused T-cell responses and paralyses the T-cell system.

The nucleocapsid (N) protein of the SARS-Cov-1 virus is sumoylated (see Fig.2C Reactome, ICU) and binds to hUbc9, a ubiquitin conjugating enzyme of the sumoylation system [44]. SARS-CoV-2 N protein is likely to be sumoylated at several sites included K62. This pathway is a potential target for treatments as SUMOylation is required for homo-oligomerisation and self-association of the N protein required for the formation of viral RNP and nucleocapsid assembly.

PD-L1 expression in severe COVID-19 patients (see Fig.2C Reactome, ICU) is likely to be linked to immunosuppressive phenotypes in innate immune cells and to support lymphopenia through apoptosis of lymphocytes [45]. It is possible that PD-1 signalling is not able to control hyperactivated T cells and resolution of hyperinflammatory stage. It remains to be investigated if PD-L1 expression on lung epithelia may also regulate PD-1 expressing T-cells (as shown for influenza and Rous sarcoma viruses [46, 47]).

Oxidative phosphorylation (see Fig. 2A Hallmark, ICU) may prove to be the link between the metabolic state of cells in people with predisposing conditions (T1D, heart attacks, obesity, use of steroids, etc), and dysregulation of the homeostasis of CD25+ T-cells and Fox3p expressing Tregs. Activation of Tregs is impaired in Type1 diabetics, but is also reduced in severe COVID patients [48, 49]. FOXP3 expression is reduced in CD25+ CD4+ T-cells in patients who have had heart attacks [50]. Leptin released from adipocytes also prevents CD25+CD4+ T-cell proliferation [51]. T-cell activation is dependent on glycolysis and oxidative phosphorylation while Treg differentiation is more dependent on oxidative phosphorylation and inhibited by glycolysis [52, 53]. This could be because of the hypoxic lung in severe COVID which leads to higher levels of glycolysis – hence reduced Treg differentiation – via HIF-1alpha activation (which mediates glycolysis and so promotes degradation of FoxP3 proteins (reduced feedback loop blocking Treg differentiation). Type 1 interferons (see Fig.2A Hallmark, Mild) and downstream pathways are suppressed in severe patients (i.e. lower levels of IFT1,2,3 and IF1TM1), with lower levels of TNF ligands TRAIL, LIGHT and surface proteins SLAMF1, KLRB1, all of which have roles in viral infections [43, 49].

The profound hypoxia associated with more severe disease may well result from viral damage to hypoxic pulmonary vasoconstriction, which is a protective mechanism that diverts blood flow towards the healthier regions of the lung where oxygen uptake can still occur [54]. The regulation of blood flow within the lung is dependent on both Ca²⁺ signalling and NO. The mechanisms associated with acute hypoxia signalling are not understood, but an interesting link has

been made in the carotid body between this mechanism and the olfactory receptor Olfr78 [55]. In fact, *EpiSwitch* array analysis identified 3 statistically significant CCS markers at the Olfr78/OR51E2 locus. A number of other features relating to hypoxia in SARS-CoV-2 infection may be caused by viral infection of carotid body type 1 cells [56].

Olfactory signalling (Reactome ICU) and NO (Biocarta ICU) pathways are linked, as NO acts as a neurotransmitter involved in neural olfactory processes in the central nervous system. and also inhibits viral replication [57, 58] With the loss of smell both sustentacular cells and basal cells appear to be affected, and both express ACE2 (the receptor for the SARS-CoV-2 virus) and TMPRSS2 (a serine protease controlling viral entry into cells) [59].

Linear Discriminant Analysis across COVID-19 patient cohorts

The unbiased whole genome array screening on three independent cohorts of COVID patients, coupled with the pathway analyses on the top 750 markers, strongly supported immune related genomic loci and pathways associated with different clinical outcomes of COVID-19. To refine this further, we added a fourth blood cohort of hospitalized COVID-19 patients from Lima, Peru which at the time of collection had one of the highest COVID-19 fatality rates in the world (3.5%). Of the 42 hospitalized patients in this cohort, 18 remained on the ward with mild disease and 26 progressed to ICU support. Thus, when combined with the 38 patients in the first 3 cohorts, a total of 80 patients who were screened by the whole genome array were used, providing 77.3 million data points from patients clinically assessed as Asymptomatic (7), Mild (40) and

Severe (35). With the focus on prognosis of severe (ICU) outcomes, we reduced our analysis to the top 100 immuno-genetic components of the 3D genomic markers (see **Materials and Methods**) statistically associated with Severe (ICU) outcome in clinical annotations (**Table 2**). Next, this data was subject to Linear Discriminant Analysis (LDA). By LDA, the top 100 Severe (ICU) markers were able to demonstrate statistically significant difference for patients with different clinical outcomes - asymptomatic, mild and severe (ICU) (**Figure 3**).

Figure 3. Linear Discriminant Analysis (LDA) for COVID-19 disease severity. The top 100 immune-related 3D genomic markers associated with Severe (ICU) clinical outcomes were used to characterize 80 COVID-19 patients with different disease severity levels ranging from asymptomatic (Asymptomatic, blue circles), Mild presentations of disease (green circles) and

428 *this with Severe presentations (red circles). See also Table 2 a,b. (Y axis -*
429 *patient categories; X axis - Linear Discriminant Coordinate 1 (LD1).*
430
431 **Table 2a: Top 100 3D genomic markers associated with Severe (ICU)**
432 **clinical outcomes.**

Marker ^a	Closest Genes ^b	RPs ^c	FC ^d	pfp ^e	P.value ^f
Hg38_1_112153186_112272476_FF	CTTNBP2NL,KCND3,TXNP3,WNT2B	6632.327399	1.432700804	0.005113164	0.005113164
Hg38_1_112153186_112262846_FR	CTTNBP2NL,KCND3,TXNP3,WNT2B	6615.221985	1.432377731	0.00216811	0.002123472
Hg38_22_25299532_25482069_FR	AL022324.1,AL022324.2,AL022332.2,CRY BB2P1,IGLL3P,IGLVIVOR22-1,LRP5L	28171.7163	1.261392393	0.00019327	8.52662E-05
Hg38_12_8956668_9052223_RF	HNRNPA1P34,KLRG1,VDAC2P2 AC130289.1,AC130289.2,LGALS9,LGALS	32830.14986	1.248199493	0.000191193	8.21396E-05
Hg38_17_27705009_27778065_RF	9DP,NOS2 AC005697.1,AC130289.1,AC130289.2,LG	31710.89437	1.248110436	0.000190318	4.07352E-05
Hg38_17_27705009_27865712_RF	ALS9DP,NOS2 KLK15,KLK2,KLK3,KLK4,KLK5,KLK6,KLK	32302.86692	1.244388651	0.000190318	6.08132E-05
Hg38_19_50840717_50961422_FR	P1,PPIAP59 AL512288.2,EYA3,PPP1R8,RPA2,SMPDL	41082.56724	1.239284032	0.000190318	2.48894E-05
Hg38_1_27836402_28029845_FR	3B,THEMIS2,XKR8	34252.74921	1.238018648	0.000190318	6.60302E-05
Hg38_10_101411215_101490136_RF	BTRC,DPCD,POLL	33830.59298	1.237745489	0.000232634	0.000129254
Hg38_18_42444490_42488772_RR	AC087683.1,PIK3C3,RIT2,RNA5SP454	36961.50644	1.230563488	0.000270694	0.000185233
Hg38_1_99639845_99714401_FF	AGL,FRRS1,HMGB3P10,PALMD	39107.43986	1.228138328	0.000226279	0.000123122
Hg38_19_34393710_34499949_FR	AC092073.1,GPI,PDCD2L,UBA2,WTIP AC083822.1,AC092933.1,AC117490.1,FB	38265.62504	1.227970267	0.000250933	0.000155726
Hg38_3_196356745_196597689_FF	XO45,RNF168,RPS29P3,SMCO1,UBXN7, WDR53	38295.8272	1.227284759	0.000194259	8.66473E-05
Hg38_1_99670351_99714401_FF	AGL,FRRS1,HMGB3P10,PALMD	38478.96973	1.227283877	0.000236458	0.00014024
Hg38_11_9685855_9716901_RF	AC011979.1,AC011979.2,SWAP70 AC005697.1,AC130289.1,AC130289.2,LG	39606.72308	1.226172597	0.000190318	1.7589E-05
Hg38_17_27705009_27879507_RF	ALS9DP,LYRM9,NOS2	39888.90639	1.223195987	0.000190318	6.32774E-05
Hg38_5_76572659_76680168_RF	F2R,F2RL2,IQGAP2	42674.08273	1.220877422	0.000190318	2.56474E-05
Hg38_6_132711125_132761455_FF	VNN1,VNN2,VNN3	41891.67247	1.220844426	0.000197577	9.24207E-05
Hg38_4_154222023_154421645_FR	AC110753.1,AC110775.1,DCHS2,FGB	41672.67989	1.220594126	0.000295776	0.000219223
Hg38_15_98883743_99043890_FF	IGF1R,PGPEP1L,SYNM	42502.98791	1.219932467	0.000217426	0.000113829
Hg38_22_25482040_25619499_RF	CRYBB2P1,GRK3,LRP5L,YES1P1 AL603764.1,AL603764.2,C10orf120,DMBT	45257.16951	1.219326086	0.000190318	3.14883E-05
Hg38_10_122685605_122705532_FF	1	47360.38753	1.218546484	0.000225914	0.000122259
Hg38_1_99538093_99618679_FR	AGL,AL365220.1,HMGB3P10,PALMD AC015688.1,AC015688.2,AC015688.3,AC	45455.75237	1.217793578	0.000190585	7.27088E-05
Hg38_17_27602913_27705038_FR	015688.4,AC015688.5,AC130289.1,AC130 289.2,CPDP1,ITM2BP1,KSR1,LGALS9,N	45673.11179	1.214995239	0.000190318	2.61731E-05
Hg38_18_42413426_42488772_RR	OS2P1	40889.09224	1.214925448	0.000445143	0.000381491
Hg38_3_184240659_184480470_FR	AC087683.1,PIK3C3,RIT2,RNA5SP454 ALG3,CAMK2N2,CHRD,CLCN2,ECE2,EE	44523.82545	1.21420253	0.000221256	0.000117135
Hg38_11_9685855_9839717_RF	F1AKMT4,EIF2B5,EIF4G1,FAM131A,POL R2H,PSMD2,THPO,VWA5B2	44637.16946	1.213208971	0.000232634	0.000129317
Hg38_3_184390826_184480470_FR	AC011979.1,SBF2,SWAP70	46450.621	1.212777042	0.000190318	6.55571E-05
Hg38_17_27609056_27705038_RR	AC128714.1,CHRD,EIF2B5,THPO AC015688.1,AC015688.2,AC015688.3,AC	43991.22324	1.212729895	0.000191193	8.04439E-05
Hg38_7_29911761_30016644_FR	015688.4,AC015688.5,AC130289.1,AC130 289.2,CPDP1,ITM2BP1,KSR1,LGALS9,N	41863.49514	1.212672171	0.000471617	0.000410584
	OS2P1				
	FKBP14,SCRN1,WIPF3				

Hg38_8_5125372_5302538_FF	AC007718.1,AC019176.2,AC019176.3,AN GPT2	46856.24945	1.212339076	0.000190585	7.8309E-05
Hg38_1_109341941_109359750_RR	MYBPHL,PSMA5,SORT1	48297.45591	1.211313728	0.000190318	2.73916E-05
Hg38_8_5120560_5302538_FF	AC007718.1,AC019176.2,AC019176.3,AN GPT2	43978.91795	1.210971319	0.000270694	0.000188689
Hg38_11_9663012_9685884_FR	AC011979.1,AC011979.2,SWAP70	44994.92012	1.210935714	0.000203616	0.000100216
Hg38_2_223395100_223450604_FF	AP1S3,HIGD1AP4,KCNE4,SCG2	36942.13688	1.210848175	0.001762385	0.001715734
Hg38_22_34722668_34750147_RR	AL021877.1,ISX,LINC01399,TOM1	35013.59465	1.210759976	0.003185687	0.003148208
Hg38_22_34696714_34750147_RR	AL021877.1,ISX,LINC01399,TOM1	42265.79077	1.210755417	0.000460206	0.000399296
Hg38_11_9577172_9685884_FR	AC011979.1,AC011979.2,RPL23AP65,SW AP70,WEE1	44448.90982	1.210288615	0.000266947	0.000170375
Hg38_3_196470838_196597689_FF	AC092933.1,FBXO45,RNF168,RPS29P3, SMCO1,WDR53	46109.70739	1.209957577	0.000236458	0.000138509
Hg38_3_10166865_10293602_RF	AC022384.1,GHRL,IRAK2,SEC13,TATDN 2	46779.70177	1.209213182	0.000235024	0.000134793
Hg38_16_20286574_20445752_FR	ACSM2A,ACSM5,GP2,PDILT,UMOD AC120036.1,AC120036.2,AC120036.3,AT P6V1G1P2,IGLV8OR8-	54241.62999	1.209175245	0.000190318	2.47322E-05
Hg38_8_47130821_47283106_FR	1,NDUFA5P12,PRKDC,RPL10AP2,SPIDR	48044.37065	1.20891203	0.000203616	0.00010061
Hg38_6_46139224_46175482_FF	ACTG1P9,ENPP4,ENPP5	46958.78429	1.208676033	0.000190318	5.85964E-05
Hg38_1_241874934_242040769_FF	BECN2,CFL1P4,EXO1,MAP1LC3C,RPL23 AP20	47180.45513	1.208555322	0.000190318	6.62673E-05
Hg38_5_76400224_76572688_RR	AC112173.1,IQGAP2,SNRPCP2	45337.39374	1.207074623	0.000235024	0.000133546
Hg38_11_59849051_59881154_FF	CBLIF,OOSP3,TCN1	47867.7306	1.204466691	0.000270694	0.000186333
Hg38_2_68144388_68359483_FR	AC017083.3,CNRIP1,PLEK,PNO1,PPP3R 1,WDR92	51605.70057	1.204265587	0.000198471	9.45656E-05
Hg38_2_223307535_223450604_FF	AP1S3,HIGD1AP4,KCNE4,SCG2	49372.04609	1.203832699	0.000377016	0.000308266
Hg38_3_3002170_3126031_FF	CNTN4,IL5RA,TRNT1	48427.09125	1.20361951	0.000203616	9.86734E-05
Hg38_8_47246986_47283106_FR	AC120036.1,IGLV8OR8-1,PRKDC,SPIDR C19orf85,FIZ1,ISOC2,NAT14,SBK2,SBK3, SHISA7,SSC5D,ZNF524,ZNF579,ZNF628, ZNF865	47376.87168	1.203047691	0.000323677	0.000248469
Hg38_19_55428739_55609271_RR		47009.69299	1.202678261	0.000386009	0.000316755
Hg38_19_50907983_50961422_RR	KLK4,KLK5,KLK6,PPIAP59	51087.9601	1.202676692	0.000190318	3.69637E-05
Hg38_6_13822113_14075828_RF	CD83,MCUR1,MRPL35P1,RNF182	51551.70406	1.202099372	0.000190318	3.79683E-05
Hg38_2_215174891_215344486_FF	ABCA12,ATIC,FN1	48345.93731	1.201468439	0.000309338	0.000232913
Hg38_9_83699493_83789570_FF	GKAP1,KIF27,UBQLN1	48416.43058	1.201216095	0.000398896	0.000329676
Hg38_5_76572659_76725304_RF	F2R,F2RL2,IQGAP2	52489.5729	1.201051522	0.000190585	7.80929E-05
Hg38_1_27971171_28029845_FR	AL512288.2,EYA3,SMPDL3B,XKR8	51485.31893	1.200030214	0.000205013	0.00010311
Hg38_2_227820025_227868257_RR	CCL20,DAW1,TDGF1P2	46004.21086	1.198654468	0.000208559	0.000105506
Hg38_12_25206967_25256704_FR	CASC1,ETFRF1,KRAS	52498.54024	1.198534797	0.000190318	5.24934E-05
Hg38_7_66305772_66398671_FF	CRCP,GTf2IP9,SKP1P1,TPST1 HERC4,MYPN,POU5F1P5,RPS3AP38,SIR T1	54136.9225	1.19847735	0.000424345	0.000356949
Hg38_10_67960384_68133275_FR		54837.31692	1.198308347	0.000190318	5.88163E-05
Hg38_11_78974906_79152599_RF	COPS8P3,GAB2,NARS2,TENM4	41989.93216	1.1980759	0.003159364	0.003112902
Hg38_16_20348274_20445752_RR	ACSM2A,ACSM5,PDILT,UMOD CABS1,CSN3,MUC7,OPRPN,SMR3A,SM R3B	55380.96235	1.198072413	0.000190318	2.38385E-05
Hg38_4_70255223_70476582_FF		52402.38034	1.197988929	0.000331089	0.000256107
Hg38_12_45019370_45087145_FF	AC008127.2,ANO6,DBX2,RACGAP1P	51383.90016	1.197718744	0.000436269	0.000370828
Hg38_16_12334388_12375827_FF	CPPED1,RPS23P6,SNX29	52699.22445	1.197394377	0.000267757	0.000174042
Hg38_17_39781054_39819919_RR	AC090844.1,GRB7,IKZF3,KRT8P34	55019.10601	1.19677388	0.000190585	7.78741E-05
Hg38_3_157568738_157614358_FF	PTX3,SLC66A1L,VEPH1	53401.93153	1.196698463	0.000236458	0.000138568
Hg38_20_32238035_32290178_FF	KIF3B,PLAGL2,POFUT1	48801.16551	1.196661797	0.00035878	0.000285969
Hg38_5_139331499_139356679_FF	MATR3,PAIP2,SLC23A1	53797.60168	1.196248355	0.000270694	0.000187911
Hg38_6_13822113_14016923_RF	CD83,MCUR1,MRPL35P1,RNF182	58355.99643	1.195984218	0.000190318	1.12567E-05
Hg38_5_55883135_56069519_FR	AC016596.3,IL31RA,IL6ST AMIGO1,ATXN7L2,CYB561D1,GNAI3,GN AT2,GPR61,PSMA5,SORT1,SYPL2	53914.7156	1.195682592	0.000190318	6.19733E-05
Hg38_1_109359721_109614645_RF		54950.53026	1.194986266	0.000190318	2.4109E-05

Hg38_19_50732810_50961422_RR	AC010325.1,AC010325.3,ACP4,C19orf48,GPR32,GPR32P1,KLK1,KLK15,KLK2,KLK3,KLK4,KLK5,KLK6,KLKP1,LINC01869,PP1AP59	56016.13008	1.194608978	0.000190318	4.85841E-05
Hg38_8_55805339_55947043_FF	LYN,TGS1,TMEM68	55621.8783	1.194497412	0.000190318	4.73235E-05
Hg38_X_70955507_71218087_FF	AL590764.2,CXorf65,FOXO4,GJB1,IL2RG,MED12,NLGN3,NUTF2P7,RPS23P8,SNX12,SOC5P4	56885.39132	1.194295196	0.000190318	4.47041E-05
Hg38_5_76572659_76639964_RF	AC112173.1,F2RL2,IQGAP2	58308.47106	1.193449221	0.000190318	2.06378E-05
Hg38_20_4769899_4890448_FF	AL133396.1,RASSF2,SLC23A2	59927.43482	1.1931546	0.000190318	3.53671E-05
Hg38_3_105278353_105431101_RF	AC091804.1,ALCAM,CBLB	56327.14698	1.19304865	0.000241232	0.000148287
Hg38_19_42642160_42697488_RR	CEACAM8,CEACAMP1,CEACAMP5,RPS10P28	55006.12013	1.192289525	0.000211794	0.000109015
Hg38_5_76402807_76572688_FR	AC112173.1,IQGAP2,SNRPCP2	53090.77575	1.19220398	0.000269965	0.0001777
Hg38_2_223212101_223450604_FF	HIGD1AP4,KCNE4,SCG2	58337.7325	1.192155643	0.000203616	9.92034E-05
Hg38_19_38902738_39089179_FR	AC011455.2,ACP7,CCER2,FBXO17,FBXO27,MRPS12,NFKBIB,SARS2,SIRT2	57992.17317	1.191812951	0.000190318	3.70888E-05
Hg38_1_17050232_17198111_FF	PADI1,PADI2,SDHB	57847.82668	1.191782438	0.000190318	3.95164E-05
Hg38_5_76423309_76572688_FR	AC112173.1,F2RL2,IQGAP2	58745.04935	1.191324001	0.000190318	5.82141E-05
Hg38_20_34795172_34933972_FF	ACSS2,GGT7,GSS,HMGB3P1,NCOA6	60708.3298	1.190714179	0.000190318	2.03084E-05
Hg38_12_8124954_8343628_FF	AC092111.3,AC092745.2,AC092745.3,ALG1L10P,CLEC4A,DEFB109F,ENPP7P5,FAM86FP,FAM90A1,GCSHP4,POU5F1P3,ZNF705A	60019.25444	1.190702538	0.000190318	5.73893E-05
Hg38_17_1289899_1411148_FR	CRK,TRARG1,YWHA	53984.44619	1.190499917	0.000367955	0.000294364
Hg38_1_109359721_109391474_RF	MYBPHL,PSMA5,SORT1	56576.1692	1.190008843	0.000190585	7.12243E-05
Hg38_8_30150657_30174586_FR	DCTN6,LEPROTL1,MBOAT4	55692.9615	1.189894605	0.000267757	0.000173585
Hg38_12_8705596_8956697_RR	A2ML1,KLRG1,M6PR,PHC1,RIMKL,RPSP51	58603.68516	1.189574098	0.000241232	0.00014759
Hg38_4_146421997_146576279_RR	POU4F2,SLC10A7,TTC29	49274.49382	1.189341371	0.001503703	0.001455054
Hg38_20_31418842_31617274_RR	DEFB122,DEFB123,DEFB124,HM13,ID1,MCTS2P,REM1	59083.38395	1.189054381	0.000197577	9.1207E-05
Hg38_5_76384752_76572688_FR	AC112173.1,IQGAP2,SNRPCP2	54851.10909	1.188958993	0.000349576	0.000274341
Hg38_1_150622137_150760974_FF	CTSS,ENSA,GOLPH3L,HORMAD1	64127.37577	1.188853014	0.000190318	2.07508E-05
Hg38_8_47283077_47496228_RF	AC024451.1,AC024451.2,PRKDC,SPIDR	57314.15933	1.188487932	0.000296454	0.000221421
Hg38_8_47257078_47283106_FR	AC120036.1,IGLV8OR8-1,PRKDC,SPIDR	50064.97609	1.18834688	0.000953713	0.000903222
Hg38_20_46123165_46316628_FF	CD40,CDH22,RPL13P2	59459.05109	1.187993343	0.0002561	0.000160439
Hg38_11_59842025_59881154_FF	CBLIF,OOSP3,TCN1	53784.52324	1.187956606	0.000386774	0.00031852
Hg38_17_75816290_75954012_FF	AC087289.3,ACOX1,FBF1,MRPL38,TRIM47,TRIM65,UNC13D,UNK,WBP2	60099.77655	1.187782434	0.000281289	0.000199385

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434 **Table 2b: Top 100 3D genomic markers associated with Mild clinical**
435 **outcomes.**

Marker ^a	Closest Genes ^b	RPs ^c	FC ^d	pfp ^e	P.value ^f
Hg38_12_57455972_57739450_FR	AC022506.1,AC025165.6,AGAP2,ARHGAP9,ARHGAP25,B4GALNT1,DCTN2,DDIT3,DTX3,GLI1,INHBE,KIF5A,MARS1,MBD6,OS9,PIP4K2C,RPL13AP23,SLC26A10,TSPAN31	20695.53	-1.27396	0.005801	0.005728
Hg38_12_8219312_8342000_RR	AC092745.2,AC092745.3,ALG1L10P,CLEC4A,ENPP7P5,FAM86FP,FAM90A1	22476.39	-1.25793	0.003679	0.003275
Hg38_12_57455972_57601784_FR	AC022506.1,ARHGAP9,DCTN2,DDIT3,GLI1,INHBE,KIF5A,MARS1,MBD6,PIP4K2C	26019.29	-1.25154	0.003591	0.003182
Hg38_3_194098667_194165164_FF	CPN2,DPPA2P3,HES1	36563.85	-1.21996	0.001529	0.00092
Hg38_12_57455972_57741912_FF	AC022506.1,AC025165.6,AGAP2,ARHGAP9,ARHGAP25,B4GALNT1,DCTN2,DDIT3,DTX3,GLI1,INHBE	39468.24	-1.20992	0.002057	0.001478

	,KIF5A,MARS1,MBD6,OS9,PIP4K2C,RPL13AP23,S LC26A10,TSPAN31				
Hg38_3_194044422_194098696_RF	CPN2,DPPA2P3,HES1	41488.62	-1.20427	0.001906	0.00132
Hg38_19_55711884_55778461_RF	NLRP9,RFPL4A,RFPL4AL1,RFPL4AP1	47528.2	-1.20352	0.000549	8.55E-05
Hg38_3_194098667_194197530_FF	CPN2,DPPA2P3,HES1	41354.35	-1.20266	0.002534	0.002063
Hg38_1_121342022_121413896_FF	AC243994.1,FCGR1B,MTIF2P1,SRGAP2C	53004.09	-1.19708	0.001529	0.000923
Hg38_22_24151422_24244908_FR	CABIN1,GGT5,GGTLC4P,SUSD2	39791.51	-1.19575	0.005043	0.004913
Hg38_20_48471131_48503400_RF	ARFGEF2,PREX1,SNAP23P1 AC104581.1,AC104581.2,ALOX15B,CNTROB,GUC Y2D,KCNAB3,TRAPPC1	46296.68	-1.1859	0.00225	0.001671
Hg38_17_7929730_8058198_RF	AL359837.1,BTG2,CHI3L1,CHIT1,FMOD,NPM1P40	64657.79	-1.18343	0.001365	0.00076
Hg38_1_203182882_203368482_FF	ADORA1,CHI3L1,MYBPH,MYOG,PPFIA4	59766.31	-1.18113	0.000484	4.59E-05
Hg38_1_203071963_203182911_RF	ATP5MGL,CYB5R3,POLDIP3,RRP7BP	57174.39	-1.1803	0.000693	0.000208
Hg38_22_42580439_42682841_FR	ACTBP8,AL121835.1,CNR1	53123.91	-1.17891	0.001088	0.000478
Hg38_6_88165454_88196986_RF	AC104581.2,CNTROB,KCNAB3,TRAPPC1 AC008749.1,AC010525.2,EPN1,NLRP9,RFPL4A,R FPL4AL1,RFPL4AP1	63654.73	-1.1786	0.002297	0.001782
Hg38_17_7929730_7946563_RF	CHI3L1,CHIT1,MYBPH	52010.98	-1.1774	0.00137	0.000769
Hg38_19_55674840_55778461_RF	AC018695.1,AC018695.5,COX4I1,IRF8	60382.51	-1.17524	0.000692	0.000188
Hg38_1_203182882_203229090_FF	AC018695.1,AC018695.5,COX4I1,IRF8	61234.27	-1.1745	0.000692	0.000204
Hg38_16_85802243_86052828_RR	AC079250.1,KCNK12,MSH2	60406.66	-1.17448	0.000484	3.64E-05
Hg38_16_85802243_85995118_RR	EPCAM,KCNK12,MSH2	58149.21	-1.17432	0.000581	0.000142
Hg38_2_47530515_47601968_FF	BTG2,CHI3L1,CHIT1,FMOD,NPM1P40	57045.55	-1.17382	0.002057	0.001468
Hg38_2_47354007_47530544_RF	MYT1L,PXDN,TPO	57499.77	-1.1738	0.002172	0.001595
Hg38_1_203182882_203350382_FR	AC008897.1,CERT1,COL4A3BP,HMGCR	65021.95	-1.17231	0.000549	0.000115
Hg38_2_1633516_1675005_RF	CPN2,DPPA2P3,HES1	68553.53	-1.17152	0.001906	0.001327
Hg38_5_75336769_75362900_FR	ARFGEF2,PREX1,RNA5SP486 AC116353.4,CD14,DND1,HARS1,HARS2,IK,NDUF A2,TMCO6,WDR55,ZMAT2	59160.03	-1.17129	0.001304	0.000704
Hg38_3_194098667_194189209_FR	CBLIF,MRPL16,OOSP1,OOSP3,OOSP4A,OOSP4B ,SRD5A3P1,STX3,TCN1 OR52N1,OR52N4,OR52N5,OR52P1P,OR52U1P,O R56B1,OR56B2P,TRIM22,TRIM5	56698.07	-1.17075	0.002297	0.001765
Hg38_20_48373935_48503400_RF	AL512329.1,FOXC1,FOXQ1,FOXQ2 AC008749.1,AC010525.2,EPN1,NLRP9,RFPL4A,R FPL4AL1,RFPL4AP1	55522.2	-1.16948	0.002305	0.001809
Hg38_5_140633077_140734458_RR	DCTN6,LEPROTL1,MBOAT4 AP000721.1,AP006333.1,COX8A,MACROD1,MARK 2,NAA40,OTUB1,RCOR2 CD14,DND1,HARS1,HARS2,IK,NDUFA2,TMCO6,W DR55,ZMAT2	54888.94	-1.16939	0.00442	0.004159
Hg38_11_59792210_59978171_RR	AC008897.1,CERT1,COL4A3BP,HMGCR	74005.01	-1.16876	0.000484	3.28E-05
Hg38_11_5691214_5795431_FF	AL359837.1,BTG2,CHI3L1,CHIT1,FMOD,NPM1P40	88583.08	-1.16839	0.002316	0.001828
Hg38_6_1238871_1325089_RR	GALC,GPR65,SHLD2P2	82994.49	-1.16813	0.000922	0.000314
Hg38_19_55694909_55778461_RF	AKAP17A,ASMT,P2RY8	64771.68	-1.16793	0.000635	0.000169
Hg38_8_30132538_30177089_RR	AC023932.1,AQP4,ARIH2P1,CDH2 AL445528.1,AL445528.2,AL590560.2,CRP,CRPP1, DUSP23,FCRL6,SLAMF8,VSIG8 AL445528.1,AL590560.2,CRP,DUSP23,FCRL6,SLA MF8,VSIG8	74556.43	-1.16791	0.005043	0.004888
Hg38_11_63909129_64174761_FF	AC092111.3,CLEC4A,DEFB109F,FAM90A1,GCSH P4,POU5F1P3,ZNF705A AC092111.3,CLEC4A,DEFB109F,FAM90A1,GCSH P4,NECAP1,POU5F1P3,ZNF705A	63195.39	-1.16722	0.000922	0.000315
Hg38_5_140633077_140703814_RR	ALG1L10P,CLEC4A,ENPP7P5,FAM86FP,FAM90A1	58926.92	-1.16618	0.003577	0.003151
Hg38_5_75336769_75421308_FR	ALG1L10P,CLEC4A,ENPP7P5,FAM86FP,FAM90A1	64569.86	-1.16603	0.001304	0.000699
Hg38_1_203182882_203382014_FR	ALG1L10P,CLEC4A,ENPP7P5,FAM86FP,FAM90A1	65019.22	-1.16536	0.001039	0.000437
Hg38_14_87771425_87820850_FF	AC092745.2,AC092745.3,ALG1L10P,CLEC4A,ENP P7P5,FAM86FP,FAM90A1,RPS3AP43	52190.13	-1.16416	0.003577	0.003154
Hg38_X_1815904_1830910_RR		91544.04	-1.16364	0.001257	0.000635
Hg38_18_28150491_28322553_RR		72068.78	-1.162	0.000549	0.000109
Hg38_1_159614100_159866607_RF		86219.89	-1.14393	0.000549	0.000114
Hg38_1_159708296_159866607_RF		104796.2	-1.13452	0.00044	2.23E-05
Hg38_12_8124954_8219341_FR		17037.23	-1.29189	0.004735	0.004479
Hg38_12_8089989_8219341_FR		18859.98	-1.27661	0.005043	0.004915
Hg38_12_8219312_8280587_RR		22456.55	-1.25801	0.004368	0.004085
Hg38_12_8219312_8285130_RF		24421.3	-1.24525	0.004314	0.004004
Hg38_12_8219312_8360807_RR		24631.85	-1.24298	0.006047	0.006047

Hg38_12_8167748_8219341_FR	AC092111.3,CLEC4A,DEFB109F,FAM90A1,ZNF705A	26120.5	-1.24257	0.003834	0.003468
Hg38_12_8219312_8328104_RR	AC092745.2,ALG1L10P,CLEC4A,ENPP7P5,FAM86FP,FAM90A1	26483.92	-1.23755	0.004736	0.004516
Hg38_12_8219312_8255981_RR	ALG1L10P,CLEC4A,ENPP7P5,FAM86FP,FAM90A1	26177.67	-1.23732	0.005043	0.004853
Hg38_X_1815904_1862623_RR	AKAP17A,ASMT,P2RY8	75133.7	-1.18512	0.001736	0.001164
Hg38_3_196597660_196703198_FR	CEP19,NRROS,PIGX	74115.71	-1.18402	0.003518	0.003043
Hg38_5_75336769_75484207_FF	AC008897.1,CERT1,COL4A3BP,HMGCR	57905.16	-1.17777	0.001039	0.000435
Hg38_2_161741288_161924901_FF	DPP4,RPEP5,SLC4A10	62589.08	-1.17446	0.000549	0.000113
Hg38_6_123596_213963_RF	AL035696.2,CICP18,DUSP22,OR4F1P	75804.3	-1.16879	0.00044	2.02E-05
Hg38_1_27649540_27806062_RF	AL020997.1,AL031729.1,CHMP1AP1,FAM76A,IFI6,RPEP3,STX12	66756.33	-1.16674	0.001014	0.000364
Hg38_X_133717418_133849606_FR	AL009174.1,GPC3,RPSAP63	73268.77	-1.16659	0.00096	0.00034
Hg38_3_55467639_55543310_RF	CACNA2D3,ERC2,WNT5A	72517.35	-1.16579	0.000549	9.19E-05
Hg38_4_145150602_145254511_FF	ABCE1,OTUD4,RPS23P4	68632.72	-1.16196	0.00189	0.001292
Hg38_19_55633865_55778461_RF	AC008749.1,AC010525.2,CCDC106,EPN1,NLRP9,RFPL4A,RFPL4AL1,RFPL4AP1,U2AF2,ZNF580,ZNF581	75169.92	-1.16176	0.000549	0.000102
Hg38_5_75336769_75398049_FR	AC008897.1,CERT1,COL4A3BP,HMGCR	68717.29	-1.16169	0.001257	0.000625
Hg38_12_57455972_57582436_FF	AC022506.1,ARHGAP9,DCTN2,DDIT3,GLI1,INHBE,KIF5A,MARS1,MBD6	56735.6	-1.16122	0.006007	0.005982
Hg38_17_7811364_7929759_RR	AC104581.2,CHD3,CYB5D1,DNAH2,KCNAB3,KDM6B,NAA38,RNF227,TMEM88,TRAPPC1	64335.59	-1.16112	0.00096	0.000339
Hg38_12_54362865_54554769_FR	GPR84,GTSF1,ITGA5,NCKAP1L,PDE1B,ZNF385A	77978	-1.16096	0.000508	5.4E-05
Hg38_19_55568483_55778461_FF	AC008749.1,AC010525.2,CCDC106,EPN1,FIZ1,NLRP9,RFPL4A,RFPL4AL1,RFPL4AP1,U2AF2,ZNF524,ZNF579,ZNF580,ZNF581,ZNF784,ZNF865	68297.2	-1.16038	0.001257	0.000628
Hg38_11_71586126_71832443_FR	ALG1L9P,AP000867.3,AP000867.4,DEFB108B,ENPP7P8,FAM86C1,KRTAP5-11,OR7E4P,OR7E87P,RPS3AP41,SNRPCP14,UNC93B6,ZNF705E	77867.09	-1.15966	0.000484	4.7E-05
Hg38_6_88196957_88306519_FF	ACTBP8,AL121835.1,CNR1	66691.6	-1.15954	0.001088	0.000501
Hg38_6_29574420_29588525_FR	GABBR1,OR2H2,OR2H5P,RPL13AP,TMEM183AP1,UBD	70449.91	-1.15914	0.001688	0.001104
Hg38_5_75336769_75441390_FR	AC008897.1,CERT1,COL4A3BP,HMGCR	64955.58	-1.15804	0.004074	0.003713
Hg38_1_6450605_6528778_RR	ESPN,NOL9,PLEKHG5,TNFRSF25	71701.08	-1.15795	0.005924	0.005874
Hg38_1_203182882_203270779_FF	CHI3L1,CHIT1,NPM1P40	73183.64	-1.15762	0.001039	0.000428
Hg38_5_100904230_100940286_FR	AC113385.2,FAM174A,SLCO4C1,ST8SIA4	71609.33	-1.15744	0.001661	0.001079
Hg38_13_52856865_53051264_RR	OLFM4,PCDH8,PPIAP26	71949.73	-1.15673	0.000549	0.000103
Hg38_13_52856865_52963923_RR	OLFM4,PCDH8,PPIAP26	68520.61	-1.15546	0.001039	0.000399
Hg38_3_105200051_105366544_FR	AC091804.1,ALCAM,CBLB	66104.49	-1.15531	0.001039	0.000419
Hg38_11_71706658_71832443_FR	ALG1L9P,DEFB108B,ENPP7P8,FAM86C1,ZNF705E	64412.6	-1.1553	0.001523	0.000897
Hg38_5_53482719_53612929_RR	AC108114.1,FST,NDUFS4	77444.92	-1.15507	0.000484	4.2E-05
Hg38_1_20185384_20244465_FR	PLA2G2C,PLA2G2F,UBXN10	62651.67	-1.15497	0.002295	0.001733
Hg38_7_44346976_44471838_RF	AC004453.1,CAMK2B,NUDCD3	66580.98	-1.15452	0.001386	0.00079
Hg38_2_47530515_47620210_FF	AC079250.1,KCNK12,MSH2	72271.33	-1.15428	0.001661	0.001067
Hg38_16_85802243_85911983_RR	AC018695.1,AC018695.5,COX4I1,IRF8	74094.78	-1.15413	0.00061	0.000157
Hg38_16_85802243_86036166_RR	AC018695.1,AC018695.5,COX4I1,IRF8	77409.19	-1.15377	0.000484	3.05E-05
Hg38_8_143703139_143989720_RR	CCDC166,EPPK1,FAM83H,GRINA,IQANK1,MAPK15,NRBP2,PARP10,PLEC,PUF60,SCRIB,ZNF707	73024.5	-1.15362	0.002297	0.001793
Hg38_19_38110011_38361547_RF	AC011479.2,C19orf33,CATSPERG,DPF1,KCNK6,PP1R14A,SIPA1L3,SPINT2,YIF1B	72414.85	-1.15338	0.000521	6.69E-05
Hg38_15_53422678_53572577_FR	AC084759.1,ONECUT1,UNC13C,WDR72	74543.22	-1.15279	0.00044	2.21E-05
Hg38_16_85802243s3_85867042_RR	AC018695.1,AC018695.5,COX4I1,IRF8	76315.72	-1.1527	0.000549	0.000104
Hg38_13_52856865_52977269_RR	OLFM4,PCDH8,PPIAP26	85912.11	-1.15263	0.000212	2.74E-06
Hg38_19_11345773_11410838_FR	CCDC159,EPOR,PLPPR2,RAB3D,RGL3,SWSAP1,TMEM205	65883.23	-1.15262	0.001555	0.000949
Hg38_6_88064646_88196986_RF	AKIRIN2,AL121835.1,CNR1,SPACA1	76118.53	-1.15215	0.001039	0.000419

Hg38_14_87632985_87771454_RF	GALC,GPR65,SHLD2P2	60903.03	-1.15187	0.004368	0.004091
Hg38_6_31492267_31623703_RF	AIF1,AL663061.2,ATP6V1G2,ATP6V1G2-DDX39B,DDX39B,LST1,LTA,LTB,MCCD1,MICB,NC R3,NFKBIL1,PPIAP9,PRRC2A,RPL15P4,TNF,UQC RHP1	64391.76	-1.15116	0.001795	0.001211
Hg38_1_203182882_203394694_FF	AL359837.1,BTG2,CHI3L1,CHIT1,FMOD,NPM1P40 ABHD16A,AL662899.3,AL662899.4,APOM,BAG6,C 6orf47,CSNK2B,GPANK1,LY6G5B,LY6G5C,LY6G6 D,LY6G6E,LY6G6F,LY6G6F-LY6G6D,PRRC2A	84256.99	-1.15101	0.000521	7.13E-05
Hg38_6_31623674_31710726_FR	AC006967.1,MNX1,NOM1,UBE3C	76086.54	-1.15062	0.000549	0.000107
Hg38_7_156960825_157154336_RR	AC079250.1,KCNK12,MSH2	67505.74	-1.15058	0.004267	0.003925
Hg38_2_47530515_47563878_FR	BTG2,CHI3L1,CHIT1,NPM1P40	82371.48	-1.14946	0.001014	0.000376
Hg38_1_203182882_203323342_FF	AL049861.1,LMO4,RNA5SP52	79022.09	-1.1492	0.001257	0.000642
Hg38_1_87788997_87826750_RF	PXDN,SNTG2,TPO	86647	-1.14909	0.001184	0.000569
Hg38_2_1263663_1424767_RF	GPR139,GPRC5B,IQCK	71442.26	-1.14902	0.001319	0.000724
Hg38_16_19858844_20038485_FR		75834.09	-1.14887	0.003207	0.002747

436

437 ^a Marker: the marker's ID

438 ^b Closest Gene: the genes around the genomic loci of the marker

439 ^c RPs: The Rank Product statistics evaluated per each marker

440 ^d FC: Fold changes of average expressions (ICU/Mild)

441 ^e pfp: Estimated percentage of false positive predictions (pfp), both considering
442 upregulated and downregulated markers.

443 ^f P.value: estimated pvalues for each marker

444

445 **Biological network analysis and therapeutic implications.** Ranking top 100
446 immune-related 3D genomic markers associated with severe (ICU) outcome by
447 adjusted p-value, then by abundance, we found the top 20 markers to be (**Table**
448 **2**) to be at genetic loci involved in macrophage-stimulating protein (MSP)-RON
449 signalling (KLK5, NOS2, KLK3), G-Beta Gamma (Gβγ) Signalling (WNT2B,
450 NOS2, VEGFC) and pathways related to regulation of nitric oxide. The top 20
451 3D genomic markers associated with Mild clinical outcomes in COVID-19
452 (**Table 2**) are at genomic loci involved in the regulation of RAC1 activation

(PREX1, ARHGAP9), MHC II antigen presentation (KIF5A, DCTN2) and MHC 1 mediated antigen processing and presentation (FCGR1B, DCTN2, KIF5A). Interestingly, RAC1 signalling negatively regulates T cell migration via TCR signalling and inhibiting RAC1 restores T cell migration suggesting that essential mechanisms for T cell control are lacking in patients with Severe clinical presentations of COVID-19 [60].

When we mapped the genomic location of the top 100 3D genomic markers associated with Severe (ICU) clinical outcomes, we found broad genomic distribution with a notable high density at regions on chromosomes 5, 17, 20 and 22 (**Figure 4**).

Figure 4. Genome wide mapping of 3D genomic loci associated with severe COVID-19 outcomes. Genomic locations and distribution of the top 100 3D genomic markers for Severe (ICU) clinical outcome. Individual human chromosomes are shown on the y-axis (chr1-chr22 along with the X and Y sex chromosomes). The heatmap shows the number of markers within a 0.3Mb genomic window with green representing a low density of markers and red

472 *indication a high density of markers. Sites with a high density of markers are*
 473 *seen on chromosomes 5, 17, 20 and 22.*

474

475 Analysis of the top 3D genomic markers associated with Severe (ICU) COVID-
 476 19 outcomes using the Search Tool for Retrieval of Interacting Genes
 477 (STRING) database, we found a protein-protein interaction network with hubs
 478 on TNF, IL6, VEGFA, INS, TLR6, STAT1, MAPK1 and MAPK3
 479 (Supplementary Figure 1 and Supplementary Table 2).

480

481

482

483 **Supplemental Figure 1. STRING Network associated with Severe (ICU)**
 484 **clinical outcomes in COVID-19.** *The proteins encoded by genes in the top 3D*
 485 *genomic markers associated with severe clinical outcomes in COVID-19 show*
 486 *a network with hubs at inflammatory mediators (TNF, IL6, VEGFA), immune-*
 487 *related receptors and signalling mediators (TLR4, STAT1, MAPK1) and the*
 488 *pleiotropic transcription factor MYC. See also Supplementary Table 2.*

489

490 Next, we used the network of genes under 3D genomic control for susceptibility
491 to severe COVID-19 outcome to evaluate the existing drugs with known gene
492 targets. We were trying to evaluate them as potential therapeutic tools for
493 mitigation of severe disease outcomes. Using GeneAnalytics we identified 25
494 drug candidates with potential utility for treating COVID-19 disease (**Table 3**).
495 Interestingly, the analysis based on 3D genomic profiling of severe COVID-19
496 patients identified at the second highest score Dexamethasone, which has
497 been now reported as beneficial in reducing mortality among severe patients
498 [61].

499

500 **Table 3: Potential Therapeutic Agent for Mitigation of Severe Disease**
501 **Outcomes.**

Score	Name	Number of Genes	Number of Matched Genes	Matched Genes (Symbols)
66.218012	Rapamycin	311	22	FLT3, FLT1, RPS6KB1, TSC1, CD28, STAT1, PIK3R1, IL18, MYC, NF1, PRKCA, RPS6, PRKCB, INS, STK11, VEGFA, RAF1, PDPK1, PIK3CB, IL7, MAPK3, MAPK1
58.655334	Dexamethasone	717	27	CXCL2, RPS6KB1, IKBKB, CD28, IL6, STAT1, PIK3R1, BIRC3, H4-16, IL18, MYC, FCER2, IL6ST, VEGFC, CCL3, INS, STK11, VEGFA, RAF1, TNF, IL7, CLEC7A, TLR4, KLK3, CD83, MAPK3, MAPK1
49.415037	Pge2	418	20	CXCL2, IKBKB, IL6, CCR7, STAT1, IL18, FCER2, IL6ST, PRKCA, VEGFC, CCL3, PRKCB, VEGFA, RAF1, TNF, IL7, TLR4, CD83, MAPK3, MAPK1
44.896712	Imatinib	172	14	FLT3, FLT1, CCR7, STAT1, PIK3R1, MYC, PRKCA, RPS6, VEGFA, GAB2, RAF1, PDPK1, LYN, MAPK1
39.965545	Retinoic Acid	935	24	FLT3, RPS6KB1, IL6, STAT1, H4-16, IL18, MYC, NF1, FCER2, IL6ST, PRKCA, DPP4, VEGFC, RPS6, CCL3, PRKCB, MUC7, RAF1, TNF, IL7, LYN, TLR4, MAPK3, MAPK1
39.312624	RANTES	230	14	CXCL2, IKBKB, IL6, CCR7, STAT1, IL18, MYD88, DPP4, CCL3, LTB, TNF, IL7, TLR4, MAPK3
31.205938	Curcumin	180	11	IKBKB, IL6, STAT1, BIRC3, IL18, MYC, VEGFA, RAF1, TNF, MAPK3, MAPK1
30.087237	Calcitriol	194	11	IL6, STAT1, MYC, FCER2, PRKCA, PRKCB, TNF, PIK3CB, TLR4, KLK3, CD83
29.870899	Sorafenib	71	8	FLT3, FLT1, BIRC3, NF1, VEGFA, RAF1, TNF, MAPK1
29.858701	Tacrolimus	197	11	RPS6KB1, CD28, CCR7, IL18, MYC, CCL3, INS, RAF1, TNF, IL7, TLR4, FLT1, RPS6KB1, IKBKB, BIRC3, MYC, PRKCA, PRKCB, VEGFA, RAF1, TLR4, KLK3, MAPK3, MAPK1
29.848169	Paclitaxel	314	13	FLT3, IKBKB, STAT1, PIK3R1, BIRC3, H4-16, MYC, PRKCA, CCL3, PIK3CB, KLK3, MAPK3
29.627724	Etoposide	256	12	FLT1, RPS6KB1, LEF1, STAT1, BIRC3, IL18, MYC, PRKCA, PRKCB, VEGFA, RAF1, LYN, TLR4, MAPK3, MAPK1
28.416533	Cisplatin	489	15	
27.863827	Immunosuppressive Agents	85	8	FLT3, TSC1, CD28, MYC, NF1, DPP4, CD22, TNF
27.418446	Doxorubicin	362	13	FLT3, FLT1, STAT1, BIRC3, IL18, MYC, PRKCA, DPP4, VEGFA, RAF1, TNF, IL7, KLK3
27.020127	Calcium	2384	31	CXCL2, FLT1, RPS6KB1, CD28, GRB7, CCR7, PIK3R1, BIRC3, IL18, NF1, FCER2, IL6ST, PRKCA, CD27, LGALS9, DPP4, VEGFC, RPS6, CCL3,

				DMBT1, PRKCB, STK11, REG1A, PDPK1, PIK3CB, IL7, LYN, BLNK, TLR4, MAPK3, MAPK1
26.202619	CCL3	63	7	CXCL2, IL6, CCR7, IL18, CCL3, TNF, TLR4
25.456246	Trastuzumab	68	7	PIK3R1, H4-16, VEGFC, CCL3, VEGFA, RAF1, MAPK1
25.012201	Everolimus	41	6	RPS6KB1, TSC1, PIK3R1, MYC, NF1, VEGFA
24.336196	Immunologic Factors	117	8	FLT3, TSC1, CD28, MYC, NF1, DPP4, CD22, TNF
23.525819	Sorafenib Tosylate	9	4	FLT1, VEGFC, VEGFA, RAF1
22.936924	Rosiglitazone	133	8	RPS6KB1, IL6, STAT1, IL18, DPP4, INS, TNF, MAPK1
22.849199	IL 10	53	6	IL6, IL18, CCL3, TNF, IL7, TLR4
22.776058	Imiquimod	27	5	IL6, STAT1, IL18, TNF, TLR4
22.457678	Hydrocortisone	139	8	FLT1, IL6, FCER2, CCL3, INS, TNF, IL7, KLK3

502

503 ^a Score: The binomial distribution is used to test the null hypothesis that the
504 input genes are not over-represented within any SuperPath. GO term or
505 compound. The presented score for each match is a transformation (-log2) of
506 the resulting p-value, where higher scores indicate better matches. Results with
507 p-values lower than 10⁻⁵⁰ are assigned the maximum score.

508 ^b Name: The compound Name.

509 ^c Number of Genes: Total number of Genes.

510 ^d Number of Matched Genes: The number of genes in the entity that match the
511 input genes, Matched Genes (Symbols): The symbols of the matched genes.

512

513 **Generation and testing of a blood-based 3D genomic predictive classifier** 514 **for COVID-19 disease severity**

515 Based on the established *EpiSwitch*[®] methodology for the discovery,
516 evaluation, feature reduction and validation of 3D genome biomarkers in the
517 format of a clinical assay [22–30], we wanted to evaluate the top 3D genomic
518 marker leads identified here for further evidence of their prognostic stratification
519 power before it could be developed into PCR-based *EpiSwitch*[®] test. The top
520 50 3D genomic markers associated with Severe (ICU) disease outcome were
521 selected to build a classifier using machine learning algorithms at the level of
522 *EpiSwitch*[®] array readouts. Markers associated with Severe (ICU) outcomes
523 were selected over those associated with Mild clinical outcomes due to the tight

524 clustering of the patient samples seen in the LDA analysis. Of the 80 patient
525 samples used in this study, 38 samples (UK and USA) were peripheral blood
526 mononuclear cells (PBMCs) and 42 (Peru) were whole blood collections. As
527 *EpiSwitch*[®] clinical assays are developed on standard whole blood input [22–
528 30], we used the 42 whole blood samples for classifier development and testing.
529 Within these samples, 24 were Severe (ICU) patients and 18 were hospitalized
530 patients with a Mild disease course. The 42 samples were randomly divided
531 into a Training set of 30 samples and Test set of 12 samples. The use of both
532 LDA and XGBoost algorithms to build classifier models was done to reduce the
533 potential of data overfitting by either model. In addition to using multiple
534 machine learning approaches to build classifying models, 10-fold cross
535 validation was utilized to ensure robust and rigorous training models were built
536 especially for XGBoost, this was carried out using the caret package in R. Both
537 models were built using the top 50 3D genomic markers associated with Severe
538 (ICU) outcomes. The LDA model built using the 30-patient Training set showed
539 good separation by clinical outcome classes in both the Training and Test sets
540 **(Figure 5A).**

Figure 5. 3D genomic markers can be used to classify COVID-19 disease outcomes. LDA plot of the Training (A) and Training and Test (B) sets of COVID-19 patients using the top 50 3D genomic markers associated with Severe (ICU) clinical outcomes. The markers showed separation of Mild

547 (circles) and Severe (triangles) outcomes in Training Set (A), and across all 42
 548 patients in training (triangle) and testing (circle) sets (B). Miscalled samples - 2
 549 milds and 1 severe, are marked. Y axis - patient categories; X axis - Linear
 550 Discriminant Coordinate 1 (LD1).

551

552 As shown in Fig. 5, the LDA model miss-called 2 Mild patients as Severe and
 553 1 Severe patient as Mild (**Figure 5B**). The overall confusion matrix for the
 554 overall Training and Test sample cohort of 42 patients is shown in **Figure 6**.

555

A

	Predicted Mild	Predicted Severe
Actual Mild	16	2
Actual Severe	1	23

B

Statistic	Value	95% CI
Sensitivity	94.12%	71.31-99.85%
Specificity	92.00%	73.97-99.02%
Positive Likelihood Ratio	11.76	3.10-44.69
Negative Likelihood Ratio	0.06	0.01-0.43
Disease prevalence (*)	40.48%	25.63-56.72%
Positive Predictive Value (*)	88.89%	67.80-96.81%
Negative Predictive Value (*)	95.83%	77.39-99.36%
Accuracy (*)	92.86%	80.52-98.50%

556

557 **Figure 6. Confusion matrix and summary statistics for the LDA classifier.**

558 (A) LDA Classifier performance on the combined Training and Tests sets of 42
 559 Mild and Severe (ICU) COVID-19 patients. (B) LDA classifier performance
 560 values for main test validation criteria and 95% confidence intervals (CI).

561

562 This LDA model result mirrors the classification of the Test samples using the
563 model built on XGBoost (**Supplementary Figure 2**). Overall, both LDA and
564 XGBoost models were able obtain a positive predictive value (PPV) of >83%
565 for identifying ICU-severe patients with a balanced accuracy of >76%.
566

A

	Predicted Mild	Predicted Severe
Actual Mild	4	2
Actual Severe	1	5

B

Statistic	Value
Sensitivity	71.43%
Specificity	80.00%
Disease prevalence (*)	58.33%
Positive Predictive Value (*)	83.33%
Negative Predictive Value (*)	66.67%
Accuracy (*)	75.7%

567
568 **Supplemental Figure 2. Confusion matrix and summary statistics for the**
569 **XGBoost classifier.** (A) XGBoost classifier performance on the Test set of 12
570 Mild and Severe (ICU) COVID-19 patients. (B) XGBoost classifier performance
571 values for main test validation criteria and 95% confidence intervals (CI).

572

573 Discussion

574 COVID infections lead to highly heterogeneous courses of COVID disease,
575 from asymptomatic to mild to severe at considerable risk of fatality. With all of
576 its genetic diversity and evolution, numerous mutations and genetic variants,
577 including recent UK (B.1.1.7) and South African (B.1.1.354) clades, the SARS-
578 CoV-2 virus clearly shows strong variations in infectivity. However, there is little
579 evidence for the virus to being responsible for heterogeneity of the disease [62].

Environmental factors, and largely the host itself, appears to be the major factor in defining disease severity and outcomes. As part of underlying mechanisms of heterogeneity, multiple studies point at the emerging evidence of differently pre-programed host innate immune cells in mild and severe disease courses, with strong differences in cellular responses occurring from the very early stages of infection [62].

Here, for the first time, we used an established 3D genomics approach to identify molecular biomarkers, known as chromosome conformation signatures (CCS), to capture the systemic differences in severe COVID-19 disease outcomes at the earliest stages of infection. The 3D genome acts as an integrator of multiple molecular inputs; from genetic risks, epigenetic modifications, transcriptional events and metabolic signalling and serves as a bridge between the stable conditional 3D genome and clinical outcomes [63, 64]. Preceding in the cascade of regulatory events the relevant and complex changes in gene expression, alterations in 3D genome structure represent a novel class of molecular readouts with the potential to forecast cellular and physiological phenotypes [63]. The *EpiSwitch* 3D genomic arrays used here have been reduced to a simple liquid biopsy testing platform with a demonstrated ability to provide diagnostic, prognostic, and predictive patient stratifications in a wide range of therapeutic areas [22–30], including COVID-19.

We used whole 3D genome arrays to analyse molecular profiles across 1.1 million sites per patient from 80 COVID-infected patients from around the world.

605 In agreement with earlier observations, the regulatory 3D genome contained a
606 stable network of 3D genomic alterations tightly associated with distinct clinical
607 outcomes. The 3D genomic changes identified here provide further insight into
608 functional networks of genome regulation after SARS-CoV-2 infection, as many
609 were associated with genomic loci implicated in immune regulation. By
610 biological network analysis, we identified several immune-related pathways that
611 showed differential regulation in severe forms of COVID-19, including the
612 (MSP)-RON pathway, Gβγ signalling, and nitric oxide (NO) signalling. MSP-
613 RON signalling mediates systemic inflammatory responses and abnormalities
614 in MSP-RON signalling can lead to onset of autoimmune disease [65]. Perhaps
615 a more central mediator of the inflammatory response guiding the difference
616 between mild and severe forms of COVID-19 identified by our study is Gβγ,
617 which directly activates PI3Kγ and is involved in the recruitment of neutrophils
618 to sites of inflammation. Inhibition of PI3Kγ significantly reduces inflammation
619 and PI3Kγ is the common signalling effector for many different chemokine and
620 receptors involved in promoting inflammation [66, 67]. Last, pro-inflammatory-
621 macrophages upregulate inducible nitric oxide synthase (NOS2) and produce
622 high steady state NO concentrations. NO is also responsible for modulating
623 virtually all steps of innate and adaptive immunity [68, 69]. However, NO can
624 also cause oxidative stress, which is especially damaging to the host due to
625 increased tissue damage, a commonly seen pulmonary event seen in severe
626 COVID-19 [2, 70, 71]. The cytokines IFN-γ and TNF-α, the latter of which was
627 identified as a mediator of the severe clinical outcomes in COVID-19 in this
628 study, are strong inducers of NOS2 [72]. Involvement of NO pathway may also
629 explain higher risk in patients with microvascular pathologies: hypertension,

diabetes, smoking, stroke, chronic kidney disease and age. It would be interesting to assess to what extent the respiratory failure in COVID patients is associated with hypoxia due to vascular mechanisms.

Our work has clinical diagnostic implications. Several cytokines and chemokines including TNF, MIP1 α , and IL6 have been observed to be elevated using transcriptomics in patients with severe forms of COVID-19 [73, 74]. However, when measured at the protein level, these molecular factors aren't elevated until after the onset of severe symptoms and are therefore unlikely to be causal in disease onset but may contribute to worsening of the severe disease. The 3D genomic changes on the other hand, represent one of the earliest steps in dynamic cellular phenotypic transitions and do not rely on longitudinal sampling during infection in order to predict disease severity outcome. We also identified some potentially novel therapeutic strategies for managing COVID-19. Interestingly, several of the drugs identified here as potential therapeutic tools have been tested independently in clinical trials for COVID-19, including mTOR inhibitors (rapamycin and tacrolimus) and general immunosuppressants (dexamethasone and hydrocortisone) [75–78]. In addition, we identified a potentially novel therapeutic pathway for COVID-19 that did not have evidence of being explored at the time of this writing based on a search of the ClinicalTrials.gov database. The signalling lipid prostaglandin E2 (PGE2), the cell signalling mediator calcium, the acute inflammatory phase cytokine CCL3 (also known as MIP1 α) and the T-cell derived chemotactic cytokine CCL5 (also known as RANTES) were identified as potential pathways of therapeutic interest and intersect on a common

signalling cascade. PGE2 exerts its cellular effects through binding to one of four cell membrane receptors (EP1-4) [79]. Binding to the EP1 or EP3 receptors increases intracellular calcium, while binding to EP2 and EP4 receptors triggers cyclic AMP mediated signalling events [79]. While PGE2 can act as a potent anti-inflammatory ligand, inhibiting the production of CCL3 in dendritic cells *in vivo* and the production of CCL5 mRNA and protein expression in LPS-activated macrophages *in vitro*, it can also be proinflammatory in certain lung conditions such as COPD, lung cancer, and several viral infections [80–82]. Elevated levels of PGE2 have been observed in SARS-CoV-2 infected patients and increased PGE2 has been postulated to correlate with enhanced COVID-19 severity in males [83, 84]. Although initial efforts at reducing PGE2 synthesis in COVID-19 through the use of non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin and ibuprofen have been controversial [85], our results suggest that prostaglandin signalling in immune cells may play an important role in mediating disease severity and should be evaluated as both a therapeutic intervention point as well as a consideration in clinical management of COVID-19 (e.g. patients with a history of NSAID use) [82].

Our findings also point to some logical next steps for advancing a clinically useful test for predicting COVID-19 severity. The 3D genomic markers identified by the bespoke *EpiSwitch*[®] Expoler array platform showed strong predictive power for COVID-19 disease outcome. Following previously published examples, these markers (or a subset of them) could be translated into a MIQE-compliant [22–30] qPCR-detectable format, reduced in number by machine learning on expanded patient cohorts, and used to develop a

molecular classifier model with a minimal signature for validation on an independent testing cohort [22–30]. Interestingly, in our array study we found stratification of samples using either PBMC or whole blood collections highly consistent. The samples were collected at the time of COVID diagnosis, which took place from very early pre-symptomatic to advanced clinical manifestation stages of the disease. This suggests that the systemic 3D immune-genetic profiling, captured by *EpiSwitch*[®] technology, represents consistent features of regulatory state, including epigenetic status, prognostically associated with severe clinical outcome and represented in whole blood and PBMC samples.

With advance of vaccination programs around the world, the latest estimates from real-world broad immunization campaign in Israel demonstrate that for the age group >60 (89.9% received first dose and 80% received both doses of BNT162b2 BioNTech/Pfizer vaccine) hospitalization has dropped by 36% and severe cases by 29% [86, 87]. The latest Emergency Use Authorization of the single-shot Johnson & Johnson COVID-19 vaccine (JNJ-78436725) is based on the phase 3 ENSEMBLE trial results with overall 66% effectiveness in preventing moderate and severe disease, including 72% in US, 57% in Latin America, and 57% effectiveness in South Africa, tested against variant B.1.351 [88]. Together, this strongly suggests that in the immediate future, even in the context of active immunization, prognosis of COVID severity stratification would remain a valuable risk-mitigation tool for a significant part of the population.

The objective of the current study was to interrogate whether the clinical outcomes of severe versus mild profiles could be distinguished prognostically

through the use of 3D genomics as a biomarker modality. As a secondary goal, we sought to use 3D genomics to better understand regulatory networks involved in the development of COVID-19 illness to aid in development or repurposed use of known therapeutic agents to control and mitigate severe outcomes. Open access to the data obtained here and direct access to *EpiSwitch*[®] Explorer array kits used in this study (**Materials and Methods**) should provide the research community with tools to further refine COVID-19 disease profiles and help better understand disease risk profiling towards the broader goal of promoting improved and informed clinical decision making.

Conclusions

While the collective understanding of the biology behind SARS-CoV-2 infection leading to development of COVID-19 has been a focus of recent research efforts, the molecular factors that underly differences in COVID-19 disease presentation/severity are largely unknown and largely dictated by the host. There is a high need for high content technologies that could be reduced to practice for assessment of cellular, systemic host profiles to identify potential biomarkers and underlying molecular network mechanisms addressing outcome prediction. Taking an array-based *EpiSwitch*[®] approach, we identified blood-based differences in 3D immuno-genetic molecular profiles of patients with mild forms of COVID-19 compared to those with more severe clinical outcomes. Our results suggest that 3D immuno-genetic profiles in multi-cohort study could be used as a biomarker modality for COVID specific disease classification and outcome prediction.

In line with established *EpiSwitch*[®] methodology [22–30], further feature reduction in PCR format for the initial findings and biomarker leads described here, use of an extended patient cohort and an independent cohort validation will serve to further validate the biological relevance of the 3D genomic changes and could help to establish the minimal set of prognostic markers that could robustly stratify patients. Ultimately, as a next step, a reduced set of markers in *EpiSwitch*[®] MIQE-compliant qPCR format could be developed and used as a clinical prognostic assay to help patients understand their likely risk of developing severe disease outcomes if infected with the SARS-CoV-2 virus. Such a test could assist in informed clinical decisions on patient care in cases of a confirmed SARS-CoV-2 infection. It could also act as a prognostic measure, providing risk evaluation through systemic profiling prior to exposure to SARS-CoV-2 and assist in informed decisions on personal lifestyle, social, and workforce activities.

Declarations

C.K., A.W., F.S., M.S., J.W. E.H. and A.A. are full-time employees at Oxford BioDynamics plc and have no other competing financial interests.

Author Contributions

EH, AA conceived the study, MS, EH and AR assisted with *EpiSwitch*[®] array design. WM, AB and ZL assisted with design of experiments. MS and AA planned and reviewed experiments. CK, AW, FCS and EH analysed the data. RP, AD, PB, BE performed experiments. RV, AB, DP, PAR, JM helped with

interpretation of the data and writing of the manuscript. WW, CK, EH, and AA.
wrote and reviewed the manuscript.

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Competing interests

The authors declare that they have no competing interests.

Consent for publication

Written informed consent for publication was obtained from all authors.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

All patients signed informed consent forms prior to providing blood samples. All ethical guidelines were followed.

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1025

A

B

C

A

	Predicted Mild	Predicted Severe
Actual Mild	16	2
Actual Severe	1	23

B

Statistic	Value	95% CI
Sensitivity	94.12%	71.31-99.89%
Specificity	97.80%	73.97-99.85%
Positive Likelihood Ratio	17.46	1.10-84.69
Negative Likelihood Ratio	0.16	0.01-1.43
Disease prevalence (*)	49.48%	25.63-56.77%
Positive Predictive Value (*)	88.89%	67.80-96.81%
Negative Predictive Value (*)	95.83%	77.39-99.36%
Accuracy (*)	97.86%	88.52-99.57%