Structural and dynamical analysis of integrated human/SARS-CoV-2 metabolic models present novel treatment strategies against COVID-19

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Structural and dynamical analysis of integrated human/SARS-CoV-2 metabolic models present novel treatment strategies against Covid-19

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The coronavirus disease 2019 (COVID-19) pandemic caused by the new coronavirus (SARS-CoV-2) is currently responsible for over 500 thousand deaths in 216 countries across the world and is affecting over 10 million people. The absence of FDA approved drugs against the new SARS-CoV-2 virus has highlighted an urgent need to design new drugs. We developed an integrated model of the human cell and the SARS-CoV-2 virus to provide insight into the pathogenetic mechanism of the virus and to support current therapeutic strategies. We show the biochemical reactions required for the growth and general maintenance of the human cell, first of all, in its healthy state. We then demonstrate how the entry of the SARS-CoV-2 virus into the human cell causes biochemical and structural changes, leading to a change of cell functions or cell death. We have completed a comparative analysis of our model and other previously generated cell type models and highlight 48 pathways and over 800 reactions hijacked by the virus for its replication and survival. We designed a new tool which predicts 15 unique reactions as drug targets from our models (the integrated human macrophage, human airway epithelial cells and the SARS-CoV-2 virus) and provide a platform for future studies on viral entry inhibition and drug optimisation strategies.

INTRODUCTION

SARS-COV-2, the causative agent of the COVID-19 disease, belongs to a group of viruses commonly known as β-coronavirus. This class of viruses is responsible for mild-to-fatal respiratory tract infections in animals and birds. Whilst the common cold is more commonly associated with the mild forms of the disease, the previous MERS and SARS-2002 infections and the current COVID-19 disease belong to the group of fatal diseases. The genome of the virus responsible for the ongoing COVID-19 disease, SARS-CoV-2, has ~80% sequence identity to SARS-CoV and 96% identical at the whole-genome level to a bat coronavirus (Zhou *et al*., 2020). The SARS-CoV-2 virus affects the lower respiratory tract cells and the upper cells in the pharyngeal region (Huang *et al*., 2020; Chen *et al*., 2020); and the range of viral infections ranges from asymptomatic, mild, moderate and severe cases. Previous studies in China show that 86% of cases of infection and the contagiousness of the virus were undocumented before travel restrictions were imposed (Li *et al*., 2020). Therefore, there are still many unknown factors regarding the stages of infection and transmissibility patterns of the virus. Studies in France demonstrate the transmission potential of asymptomatic persons and suggest varying dynamics of transmission in children (Danis *et al*., 2020). The human

angiotensin-converting enzyme 2 (human-ACE-2 protein) has been identified as the cell receptor for both the SARS-2002 virus and the SARS-CoV-2 virus. The ACE-2 enzyme, which has a primary function of controlling blood pressure, is usually found in the epithelial cells of the heart, lungs, kidneys and intestine (Hamming *et al*., 2014; Donoghue *et al*., 2000).

The mechanism of replication of the SARS-CoV-2 virus in the human cell involves an initial binding and attachment of the spike (S) glycoprotein to the ACE2 receptor of its host. During endocytosis, the genetic material of the virus is injected into the host cell, where it loses its protective envelope. The virus, now ready for replication, is released into the nucleus of the human cell (Fig 1). Subsequent assembly and maturation of viral proteins lead to cell death and a proliferation of the virus within the human body.

The lack of FDA approved drugs against Covid-19, coupled with the difficulties encountered globally in containing the virus, prompted the WHO to declare the outbreak a pandemic in March 2020. The has led to intensified efforts around the world to fight this disease. Previous studies in drug target identification against viral diseases such as Zika, Chikungunya and Dengue by Aller *et al*., 2018 introduced a system of integrating the host's macrophage and viral metabolic networks to predict a set of host reactions which, when constrained, can inhibit viral production. A recent study by Renz *et al*., 2020 demonstrates a similar approach and predicts drug targets against the SARS-CoV2 virus. Targets of known antiviral drugs predicted from both studies demonstrate the applicability of the integrated human/virus metabolic modelling in drug target identification.

We have built on these approaches by developing an integrated epithelial cell / SARS-CoV-2 virus metabolic model and employed a combination of structural and dynamical analyses to assess the model and make predictions. We have designed and developed a new tool (*findCPcli*) to carry out such analyses and to predict drug targets. We have also performed a comparative analysis of our model and another previously generated cell-type models.

RESULTS

Comparative analysis of integrated models of infected human epithelial cell and the macrophage cell with the SARS-CoV-2 virus

We constructed an integrated genome-scale metabolic model (GEM) of the human airway epithelial cell (Wang *et al.,* 2017), with the SARS-CoV-2 virus using the methods described in Aller *et al*., 2018 and Renz *et al*., 2020. The new GEM (iBBEC4660) was refined by using the human metabolic networks in the HumanCyc database (Romero *et al*., 2004). We performed a comparative analysis of the essential and unique reactions needed for the viability of the virus in the epithelial cell/ SARS-CoV-2 integrated model and the GEM constructed by Renz *et al.,* 2020. Our results show how the virus heightens its virulence mechanisms by modifying

the host's defences within different cell compartments. Consequently, we suggest treatment regimens based on different stages of viral infection and replication.

Host dependent metabolic pathways

We initially demonstrated the biochemical requirements for the growth and maintenance of the human airway epithelial and macrophage cells and used the integrated models to show the essential host reactions needed for the survival and viability of the SARS-CoV-2 virus within the host's cell compartments. We have validated our models by mapping the experimentally characterised human/SARS-CoV-2 virus protein-protein interaction data from Gordon *et al*., 2020 on the *in-silico* virus-integrated human macrophage and epithelial cells. We identified 48 metabolic pathways from 334 metabolic pathways in the human metabolic network, including the biosynthesis and degradation pathways of amino acids, fatty acids, carbohydrates, amines, cofactors as well as core components of the central mRNA metabolism (Fig 2).

The 48 metabolic pathways that were mapped to the protein-protein interaction network produced by Gordon *et al*., 2020 are referred to as PPi-Pathway Intersection nodes in this manuscript (Fig 3). These include cysteine, methionine and selenocysteine amino acid biosynthetic pathways, C20 prostanoid hormone biosynthetic pathways, Vitamin D3 and Vitamin K epoxide cycle. The degradation pathways identified include the lysine, tryptophan, methionine, fatty acid degradation, ceramide and sphingolipid recycling pathways and phospholipases degradation; amine and heme degradation (Fig 3).

Our results identify host dependency factors required for the SARS-CoV-2 virus infection, replication, survival and viability within different cell compartments and provide insight into novel treatment strategies.

Essential reactions for the host and viral metabolism

The Flux Balance Analysis (FBA) algorithm (Orth *et al*., 2010) was used to compute both the maximum growth rate of the cell in the absence of virus and the maximum growth rate of the virus in the cell (host optimum and virus optimum conditions). We identified 52 essential reactions in the macrophage (iAB-AMØ-1410) model and 10 reactions in the epithelial cell model (iBBEC4660) essential for the virus to propagate (Tables 1 in S1 Table and 2 in S2 Table). It was also demonstrated that: a) the maximum growth rate of the macrophage cell in the absence of virus was 0.0269 h^{-1} (Table 1 in S1 Table); and 0.012 for the human airway epithelial cell (Table 2 in S2 Table); b) the maximum growth rate of the virus in the macrophage cell was 0.0144 h^{-1} and 0.0181 in the human airway epithelial cell. These numerical results mean that 0.0144 h^{-1} is the theoretical maximum of the growth rate of the virus in the human macrophage cell. If this flux is assigned to the viral growth reaction, then Flux Variability

Analysis (FVA) (Orth *et al*., 2010) can be used to calculate the ranges of fluxes allowed for the remaining reactions in the cell while the virus is being replicated at its optimum condition. The execution of FVA under such conditions produced a zero growth of the host cell, i.e. both the lower and upper flux bounds of the reaction indicate that the growth is zero. This means that if the virus is replicating at its maximum rate then the cell cannot reproduce.

Bottleneck reactions and the prioritization of potential drug targets

The bottleneck reactions identified by the *findCPcli* tool are unique reactions in a metabolic network required for the growth and survival of the organism and, like chokepoint reactions, are potential drug targets (Yeh *et al.,* 2004; Oarga *et al.,* 2020). Whilst the classical chokepoint reactions identify unique reactions from a stoichiometric model, we improve on this approach by using the structural and dynamical information of the integrated Human/SARS-CoV-2 metabolic model within the airway epithelial cell and the macrophage cell to predict potential drug targets against the SARS-CoV-2 virus.

We initially identified 1595 bottleneck reactions required for the virus' maintenance and replication in the *human macrophage* cell; these include pathways in lipid metabolism, coenzyme transport and metabolism, energy production and conversion, amino acid and nucleotide transport and metabolism (Table 1 in S1 Table). In the human airway epithelial cell, 1819 bottleneck reactions were initially identified; these include the biosynthesis and degradation pathways of amino acids, fatty acids, carbohydrates, amines, cofactors as well as some components of the central mRNA metabolism (Table 2 in S2 Table).

To validate/account for the results, and because each bottleneck reaction should be balanced by at least one other reaction that produces or consumes that metabolite, we have excluded reactions in the model with dead-end metabolites. The bottleneck reactions are further prioritised by interrogating the dynamical information in the model using the flux variability analysis, which determines if a reaction is reversible. The bottleneck reactions are potential drug targets as they are indispensable for the maintenance and replication of the virus within the host. In order to rank the potential drug targets identified, we prioritised enzymes for unique reactions that occur at the nodes of intersection between the bottleneck and essential reactions and the experimental results from the human/virus protein-protein interaction network (Gordon *et al*., 2020) (Fig 3). We refer to these as PPi-Pathway intersection nodes.

The PPi-Pathway intersection (PPi) nodes identified are present in biosynthesis pathways such as the cysteine and S-adenosyl-L-methionine biosynthetic pathways. In both pathways, the enzyme S-adenosylmethionine synthase (Mat2b), catalyses the phosphorylation reaction of methionine to S-adenosyl-L-methionine. During infection, the viral protein Nsp9 is seen to react with MAT2B (Gordon *et al*., 2020) (Fig 4a/b). Another viral protein, Nsp8 also interacts

with the enzyme O-phosphoseryl-tRNA(Sec) selenium transferase (SEPSECS), which catalyses the last step of the L-selenocysteine biosynthesis pathway (Fig 5).

PPi nodes also occur in a network of various fatty acid and stearate biosynthetic pathways with Nsp2 interacting with the very long-chain acyl-CoA synthetase (SLC27A2) (Fig 6a). In other fatty acid biosynthetic pathways, γ-linolenate biosynthesis, Nsp7 interacts with ACSL3 (Fig 6a)The viral protein, Nsp2, also interacts with POR in other pathways including vitamin D3 biosynthesis, L-tryptophan degradation, ceramide and sphingolipid recycling (Fig 6b).

In carbohydrate metabolism, a PPi node is identified at the glycan & oligosaccharide biosynthetic pathways, and specifically where two mannose residues are added in α(1→2) linkages to the nascent oligosaccharide and catalysed by the enzyme ALG11. The viral protein Nsp4 interacts with ALG11 during the infection of the SARS-CoV-2 virus (Fig 7). Other viral proteins, Nsp7 (Fig 8) reacts with ACSL3 and ORF8a interacts with HS2ST1 (Fig 8b), a key enzyme involved in the heparan sulfate biosynthesis pathway. The first enzyme of the N-linked oligosaccharide processing pathway, mannosyl-oligosaccharide α-1,2-glucosidase (MOGS), also interacts with Nsp7 and ORF8a (Fig 8c).

PPi nodes specific to the human macrophage cell include the O-phosphoseryl-tRNA(Sec) selenium transferase in the L-selenocysteine biosynthetic pathway, which interacts with the viral protein Nsp8. The alkylglycerone-phosphate synthase/Nps7 PPi node, which is present in the Phospholipid/Plasmalogen biosynthetic pathway is also specific to the macrophage cell. Alternatively, PP-pathway intersection nodes common to both human airway epithelial cell and the macrophage cell are the MAT2B/Nsp9 intersection pathways present in the cysteine metabolism and L-methionine degradation. We did not identify PPi nodes specific to the human epithelial cell.

Discussion

Metabolic pathway perturbations in the human cell due to the Covid-19 disease are a reflection of the viral entry and infection of SARS-CoV-2 and of the immune regulation changes in the human body. We have used *in silico* system models to study the interactions of SARS-CoV-2 in the host and propose new treatment management regimens. We have built on studies using the human alveolar macrophage model iAB-AMØ-1410 (Bordbar *et al.,* 2010) as host cells and SARS-CoV-2 (Renz *et al*., 2020); influenza (Aller *et al*. 2017), and tuberculosis as pathogens. Previous studies have also demonstrated the role of the angiotensin converting enzyme 2 (ACE2) as the receptor for both the SARS-CoV and the SARS-CoV-2. The ACE2 cells are expressed in the human airway epithelial cells (Yan *et al*., 2020; Jia *et al*., 2005). In this study, we have constructed an integrated human epithelial cell and the SARS-CoV-2 virus to provide insight into the infection patterns of the virus in the human body.

Our *in silico* comparative analyses of the SARS-CoV-2 viral infection between two different conditions (infected human macrophage and airway epithelial cells) show the requirements of viability of the virus between these two conditions. Our results compliment previous efforts to propose drug targets and repurposing strategies including the SARS-CoV-2-Human Protein-Protein Interaction Map (Gordon *et al*., 2020) and studies from Joseph Steward, 2020 which identified host dependency factors facilitating virus infection. We also provide additional resource to the Covid-19 disease map (Ostaszewsli *et al*., 2020). In addition, we have designed a new algorithm (Oarga *et al*., 2020) and used the dynamic information of the human/virus models to predict new treatment regimens. Here we demonstrate the flux changes from lipid metabolism, cofactor biosynthetic pathways, redox balance and immune regulation indicative of pathogenic reactions arising from the Covid-19 viral infections.

Inhibition of viral entry and replication

The components of the plasma membrane, such as cholesterol and sphingolipid-rich lipid, are involved in virus penetration, entry, replication and infection (Wang *et al*., 2008; Abu-Farha *et al*., 2020). In this study, we demonstrate current therapeutic strategies that interfere with different stages of the viral cycle by targeting lipid metabolism as well as proposing new treatment strategies.

The dynamical changes of flux metabolism in our *in-silico* virus optimal models show significant increase in viral infection. Four reactions involved in the biosynthesis of fatty acids with predicted non-zero fluxes in the host model exhibit an average increase of 190% in their maximum fluxes in the viral model (the maximum increase is 298%). The average increase of 32 reactions in lipid metabolism with non-zero fluxes in the host model is 277% (the maximum increase is 498%). With respect to sphingolipid metabolism, 14 out of 15 reactions with non-zero fluxes in the host model exhibit an average increase of 228% (the maximum increase is 298%) and similar increases in phospholipases and palmitic acid biosynthesis (Fig 6). We show an average increase of 190% in cholesterol and fatty acid metabolism during viral infection and demonstrate the essentiality of these pathways to SARS-CoV-2 virus. Previous studies have shown that cholesterol and fatty acids are main components of the viral membranes and needed for viral replication (Heaton and Randall, 2011); therefore, drugs inhibiting these pathways such as AM580, Statins, Fibrate (Fiévet *et al*., 2009) will be essential for both early and late stages of the Covid-19 disease.

Sphingolipids are composed of both hydrophobic and hydrophilic units and play a large role in the endocytic or exocytic viral entry processes into the cell (Dimitrov *et al*., 2004). The pH-dependent endocytic process is further enhanced by the presence of clathrin, a protein present in the plasma membrane, Golgi apparatus and in the cytoplasm, whilst the exocytic

route involves viral crossing through the plasma membrane at neutral pH. Our results show a 3-fold increase in sphingolipid metabolism during viral infection (Fig 6); we hypothesise that drugs inhibiting sphingolipid metabolism and/or the endocytosis process will inhibit infection of SARs-CoV-2 virus. Indeed, a sphingosine kinase-2 (SphK2) inhibitor, Opaganib, which has proved beneficial in the treatment of Covid-19 is currently in phase 2/3 clinical trials in the US, and now in UK and Italy. Previous studies also demonstrate that chloroquine and hydroxychloroquine elevate the pH of endosomes in the cells and directly inhibits endocytosis and the exocytic process (Munro *et al*., 1997, Devaux *et al*., 2020). More recent studies have also shown that artemisinin-inhibited endocytosis (Uzzun *et al*., 2020, Hoppe *et al*., 2004). A recent study by Abu-Farha described other lipid modifying drugs including LJ-001, Arbidol, Methyl-B-cyclodextrin (Mazzon and Marsh 2019; de Wilde *et al*., 2014; Blaising *et al*., 2013. We highlight critical reactions as drug targets for lipid metabolism for the SARS-CoV-2 virus (table 2) and in our PPi-pathway intersection nodes (Fig 3).

Our lists of bottleneck, essential reactions and PPi-pathway intersection nodes also include critical points in the biosynthesis of phospholipids. We show that these reactions are essential for viral infection and replication (Fig 3) and propose that targeting the phospholipase enzyme or the interacting Nsp2 protein could inhibit viral replication. Our results support previous studies from Muller *et al*., 2018, that targeting the phospholipase enzyme could inhibit the early stage of Covid-19 disease.

Redox homeostasis and antioxidant therapy

Redox homeostasis refers to the ability of the cell to maintain its balance amidst infections and other unstable cellular environmental factors. Delgardo-Roche and Mesta, 2020 have described oxidative stress as a key player in Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) Infection with cytokine production. Foyer and Noctor 2005 have previously shown that antioxidants, such as glutathione and ascorbate, are important metabolites for the cellular redox state. Our studies have identified key target enzymes involved in the metabolism of glutathione and ascorbic acid as bottleneck and essential reactions; including glutathione synthase, glutathione peroxidase and ascorbic acid oxidase (Table 2). We also demonstrate an increase in flux of these enzymatic reactions on infection of the virus. In a recent study, Horowitz *et al*., 2020 previously demonstrated how the use of high dose oral and/or IV glutathione on severe outcomes of SARS-CoV-2 virus led to favourable treatment outcomes. Other studies have shown that steroids such as dexamethasone and Methylpredisone to treat severe cases of Covid-19. Due to possible side effects of steroid treatment, we propose the use glutathione as therapy for severe cases of Covid-19 in the aged population and other severe cases with cytokine storm syndrome.

Immune regulation

The SARS-CoV-2 virus is able to proliferate unhindered in infected cells, due to the lack of immunity in humans (Felsenstein *et al*., 2020). The result is cell death, a release of viral particles to the extracellular environment and a general hyperactivity of the immune system in some patients with severe Covid-19 disease and subsequent, lung inflammation and cytokine syndrome. Immunocompromised patients or those with underlying symptoms such as diabetes, hypertension and transplantation are most affected (Zhong *et al*., 2020). Whilst clinical trials are ongoing worldwide with various antivirals and immune modulating treatments, there is currently limited knowledge on the host dependency factors responsible for the individual outcomes of the disease. Our results provide insight into the immune evasion strategies of SARS-CoV2; we demonstrate changes in the flux metabolism of vitamin D and tryptophan metabolism during viral infection. Vitamin D is important for bone growth and turnover and a low vitamin D status is associated with an increased susceptibility to upper respiratory tract infections (Mitchell F, 2020). Previous studies have shown that a supplementation of vitamin D prevents acute respiratory tract infections (Martineau *et al*., 2016). Our results highlight vitamin D as an essential reaction in the PPi-pathway intersection nodes and we show the viral protein Nsp2 interaction with key enzymes in the vitamins D and C metabolism pathways (Fig 3 and 6). SARS-CoV-2 viral infection causes metabolic perturbations of vitamin D metabolism in the host resulting to disruptions in cellular homeostasis. We propose support therapy management strategies where vitamin D supplements are provided to all Covid-19 patients. Our results also show that tryptophan, melatonin and prostaglandins, important compounds for immunity and homeostasis (Platten *et al*., 2019; Gitto *et al*., 2010), are affected by the infection of SARS-CoV-2 virus and we provide insight into the viral mechanism of action within the human body.

In summary, we have provided a platform for drug target prediction against Covid-19, and for future studies on viral entry inhibition, antioxidant therapy and immune regulation.

METHODS

We manually curated the human airway epithelial cell initially constructed by Wang *et al.,* 2017 with gene expression datasets of the human airway epithelial cell (Deprez *et al*., 2020; Braga *et al*., 2020) and the humancyc database (Romero *et al*., 2014) to produce a new GEM, (iBBEC4660); i for *in-silico*, BB for the first author's name, EC for airway epithelial cell and 4660 for the number of open reading frames. In order to assess and predict the performance of the models, we made use of Flux Balance Analysis (FBA) and Flux Variability Analysis (FVA) (Orth *et al*., 2010). FBA is a computational method that can be applied efficiently to genome scale models to estimate the fluxes of reactions at steady state. It is based on the solution of a linear programming problem that maximizes an objective function of interest subject to a set of constraints on the fluxes of the reactions. The linear programming problem associated with FBA can be expressed as:

$$\max c \cdot v$$
$$s.t.\ S \cdot v = 0$$
$$L \leq v \leq U$$

where *v* is the vector of fluxes, *c* represents the objective, *S* is the stoichiometry matrix, and *L* and *U* are lower and upper bounds on the fluxes. Thus, $c \cdot v$ is the objective function, which usually refers to the growth rate of the organism, and $S \cdot v = 0$ represents the balance of fluxes at steady state.

FVA is also based on the solution of linear programming problems, and its main use is the computation of ranges of fluxes that are compatible with given flux constraints. For instance, if the growth rate predicted by FBA is μ_{max}, then the range of fluxes of a given reaction *i* that are compatible with such growth rate can be obtained by minimizing and maximizing the following programming problem:

$$\max v_i$$
$$s.t.\ S \cdot v = 0$$
$$L \leq v \leq U$$
$$v_{growth} = \mu_{max}$$

where v_{growth} is the flux of the reaction associated with growth. FBA and FVA were applied on the metabolic network of the host, both with and without reaction modelling the production of the virus, by using the Python toolbox COBRApy (Ebrahim *et al*., 2013).

Bottleneck reactions, like chokepoint reactions, are required for the reaction synthesis and the removal of these reactions will cause an accumulation or depletion of the metabolites; they represent potential drug targets. The tool *findCPcli* is a command line application intended for the computation of bottleneck reactions on genome scale models. The source code of the tool is available at github.com*/findCP/findCPcli.* This tool is distributed as a *Python* package and requires an installation of a *Python* language interpreter with a version *3*.5 or higher and the application can be installed with the *pip* package management tool.

The pathway maps were created with the pathway collage software (Paley *et al*., 2016) and the model was deposited in the BioModels database (Chelliah *et al*., 2015) and assigned the identifier “MODEL2007210001”.

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AUTHOR CONTRIBUTIONS

Conceived and designed the study: BP. Bannerman, RA. Floto. Acquisition and analysis of data: BP. Bannerman, J Julvez, A. Oarga. Data Interpretation: BP. Bannerman, RA. Floto, J Julvez, TL. Blundell, P. Moreno. Wrote the paper: B. P. Bannerman, J Julvez, A. Oarga, RA Floto. Revised the paper: B. P. Bannerman, J Julvez, A. Oarga, P. Moreno, TLBlundell, RAFloto

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Figures

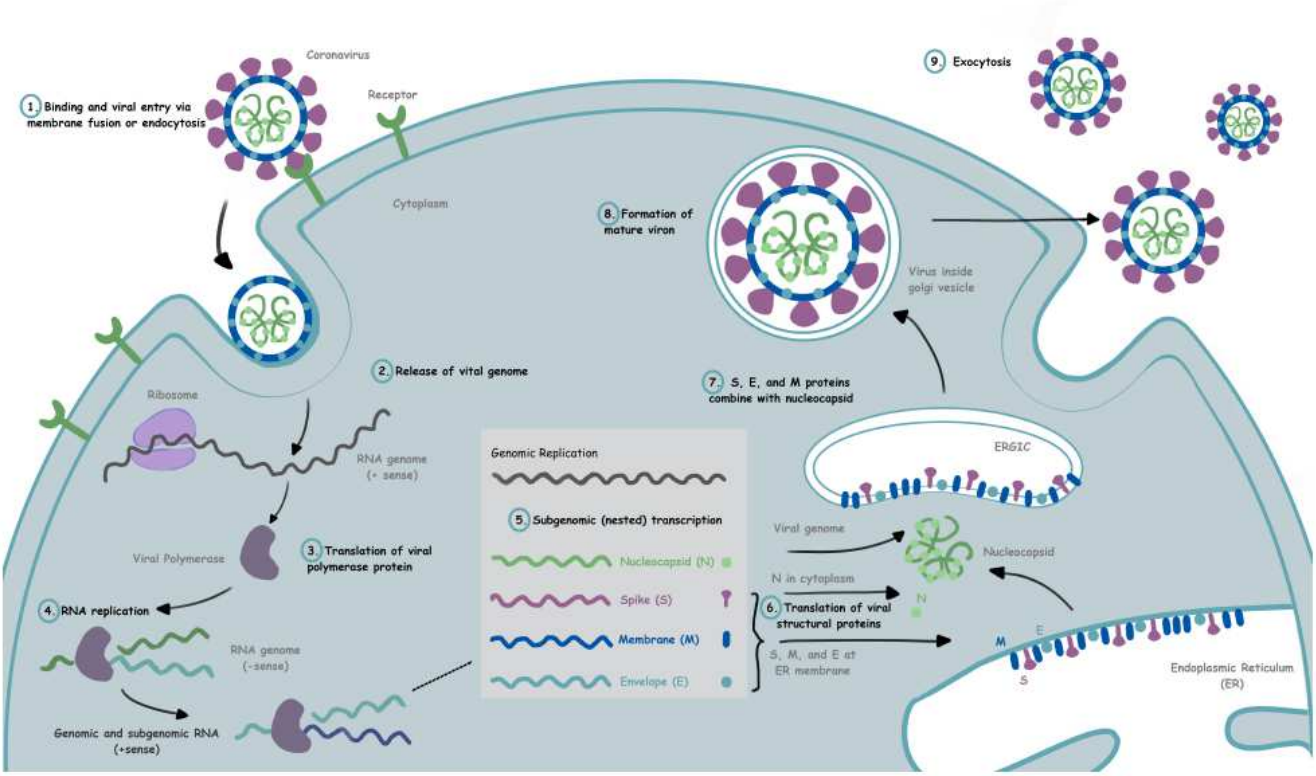

Figure 1

The mechanism of replication of the SARS-CoV-2 virus in the human cell

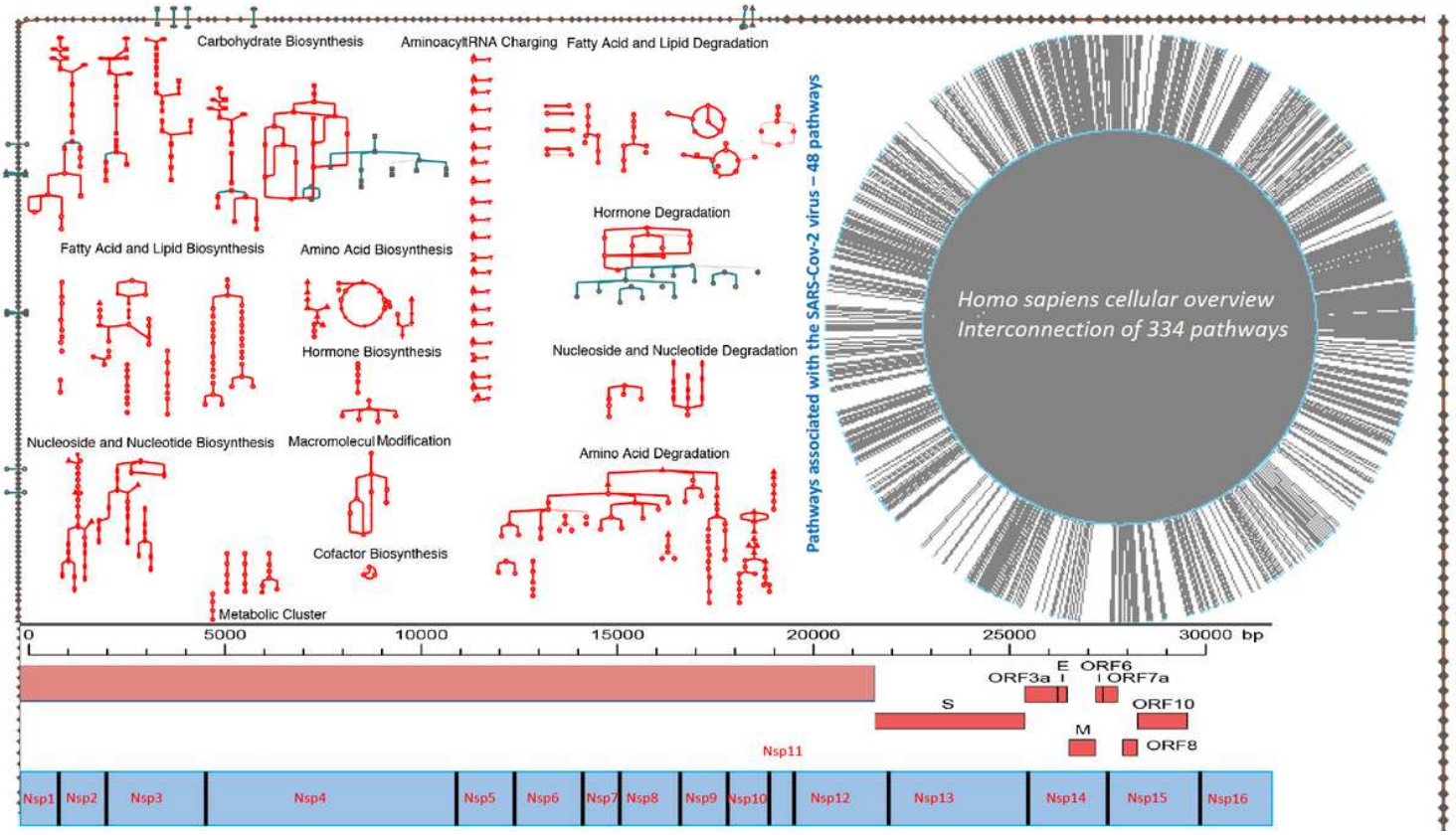

Figure 2

SARS-CoV-2 viral genome & host dependent metabolic pathways

sno	Pathway	Subpathway	Main Protease2	Nsp2	Nsp4	Nsp7	Nsp8	Nsp9	ORF3a	ORF8a
1	**Biosynthesis**	Amino acids	x	x	x	x	L-selenocysteine biosynthesis [SEPSECS]	Cys, Met [MAT2B] / Selenom	x	x
3	Biosynthesis	Fatty acids	x	Fatty acids, long fatty acids & stearate biosynthesis [SLC27A2, POR]		Fatty acids, long fatty acids & stearate biosynthesis ACSL3, Phospholipid/Plasmalogen bio [AGPS]		x	x	x
4	Biosynthesis	Carbohydrate biosynthesis	x	x	Glycan & oligosaccharide biosynthesis [ALG11]	Glycan [HS2ST1, MOGS]		x	Glycan [ALG5], Oligosaccharide [ALG5]	Glycan [CHPF, HS6ST2], Oligosaccharide, glycosaminoglycan [CHPF, HS6ST2]
5	Biosynthesis							x	x	x
6	Biosynthesis	Cofactors	Vitamin K epoxide cycle GGCX	Vitamin D3 Biosynthesis [POR]		x		single carbon carrier metabolism [MAT2B]	x	x
7	Biosynthesis	Hormones	x	[POR]	x	C20 prostanoid biosynthesis [PTGES2]		x	x	x
8	Biosynthesis	tRNA charging	TARS2	x	x	x		x	x	x
9	**Degradation**	Amino acids	L-Lysine degradation [AASS]	L-tryptophan degradation [POR]	x			L-methionine degradation [MAT2B]	x	x
11	Degradation	Fatty acids	x	Ceramide and sphingolipid recycling [SLC27A2, POR]	x	Sphingolipid recycling and fatty acid degradation [ACSL3]		x	x	Phospholipases [PLD3]
12	Degradation			Fatty acid degradation [SLC27A2	x			x	x	x
13	Degradation	Amine degradation			x	COMT		x	x	x
14	Degradation	Cofactors	x		x				Heme [HMOX1]	x
15	Degradation	Hormones	x	Hormone [melatonin]degradation [POR]	x	Adrenalin, L-dopa degradation [COMT]			x	x
16	**Central mRNA**	Transcription	AKAP8L, SLC30A9	x	x	NAT14	NSD2 / Histone-lysine N	GTF2F2, NUP62, ZNF503		x

Figure 3

PPi-pathway intersection nodes

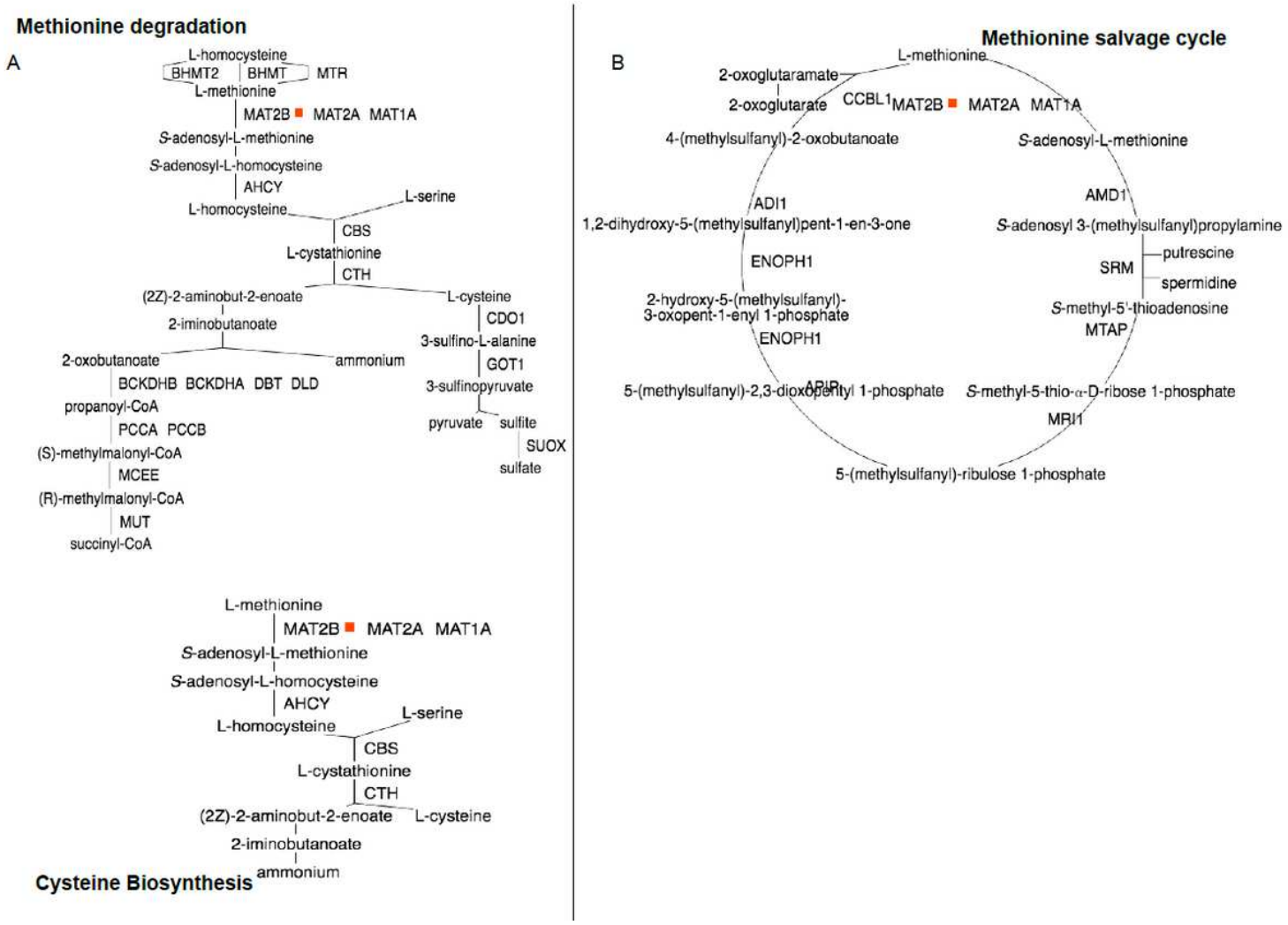

Figure 4

PPi-Pathway intersection node - Nsp9

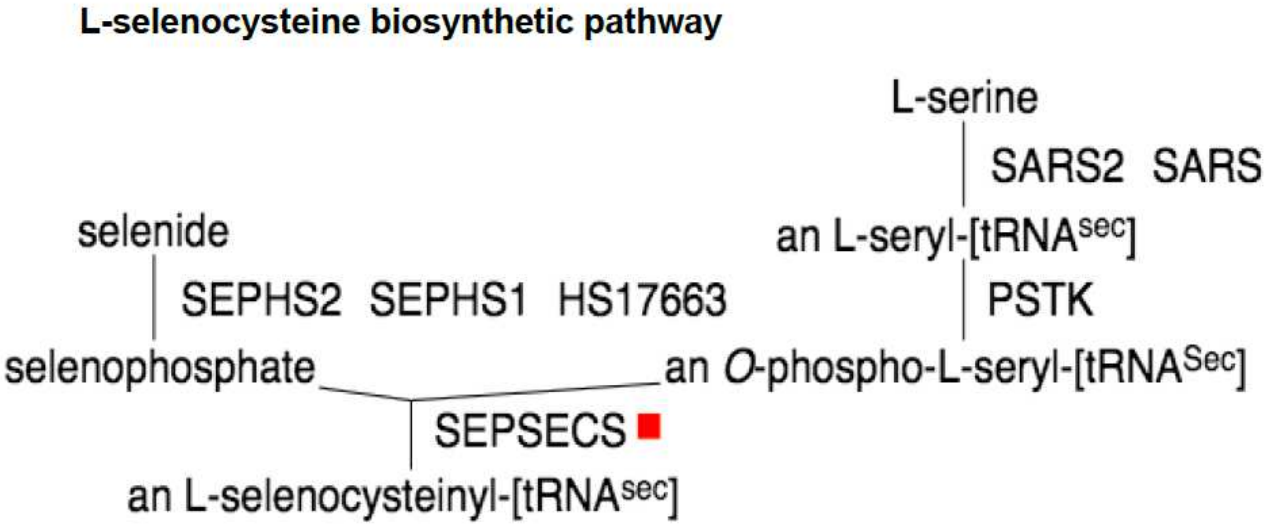

Figure 5

PPi-Pathway intersection node - Nsp8

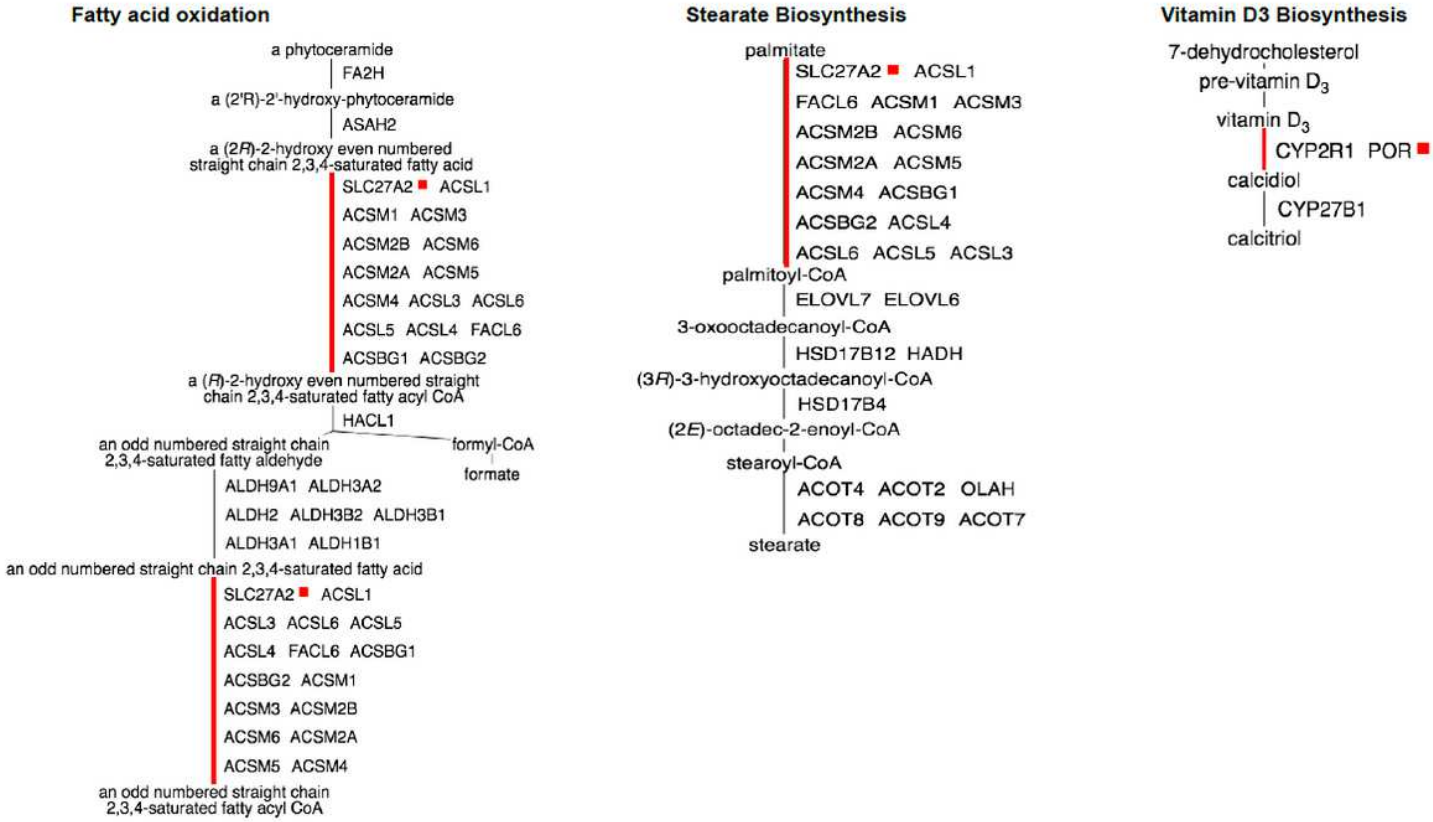

Figure 6

PPi-Pathway intersection node - Nsp2 as denoted by the red square blocks. The red lines indicate an increase in flux of the highlighted reactions when the virus hijacks the system

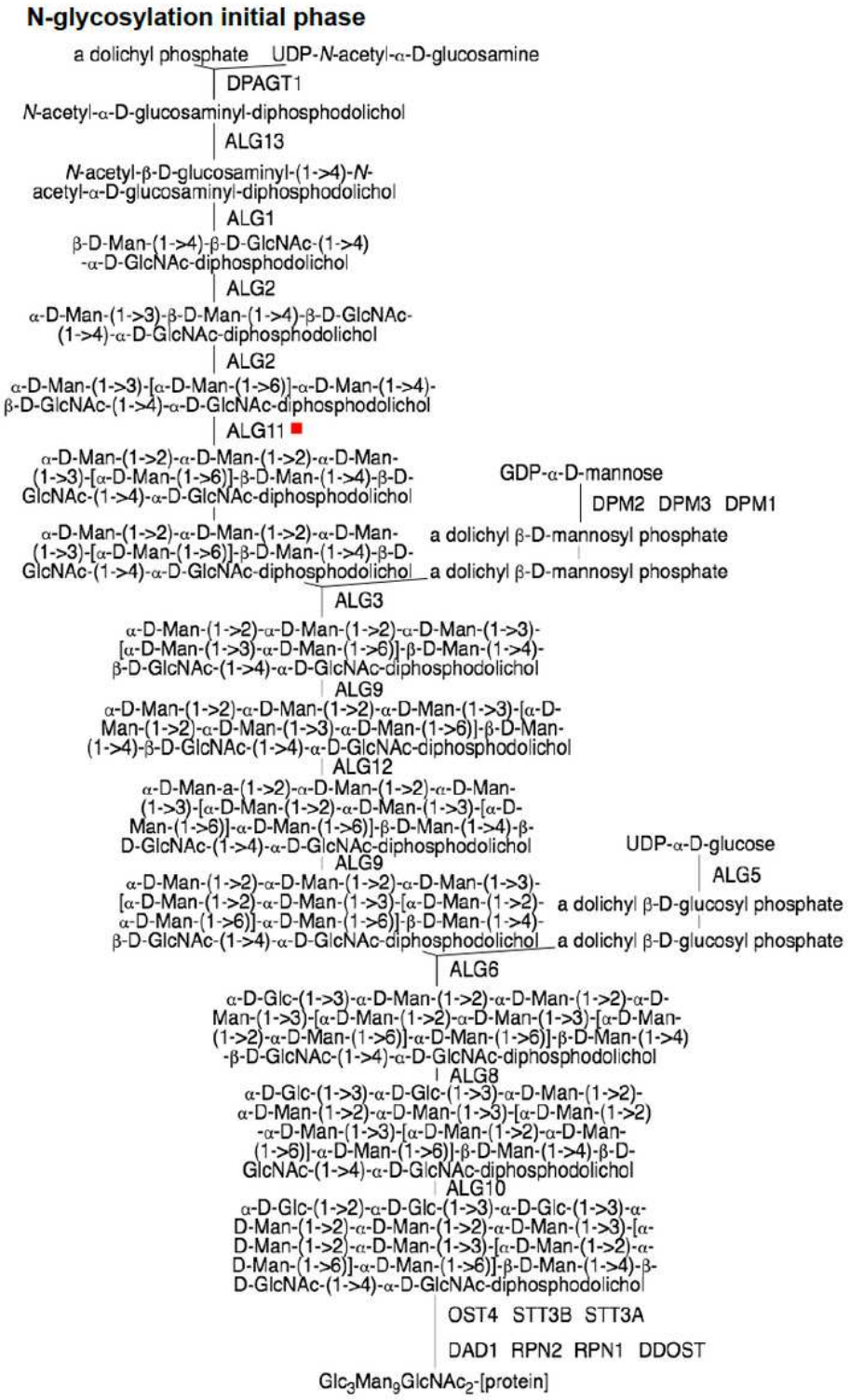

Figure 7

PPi-Pathway intersection node - Nsp4

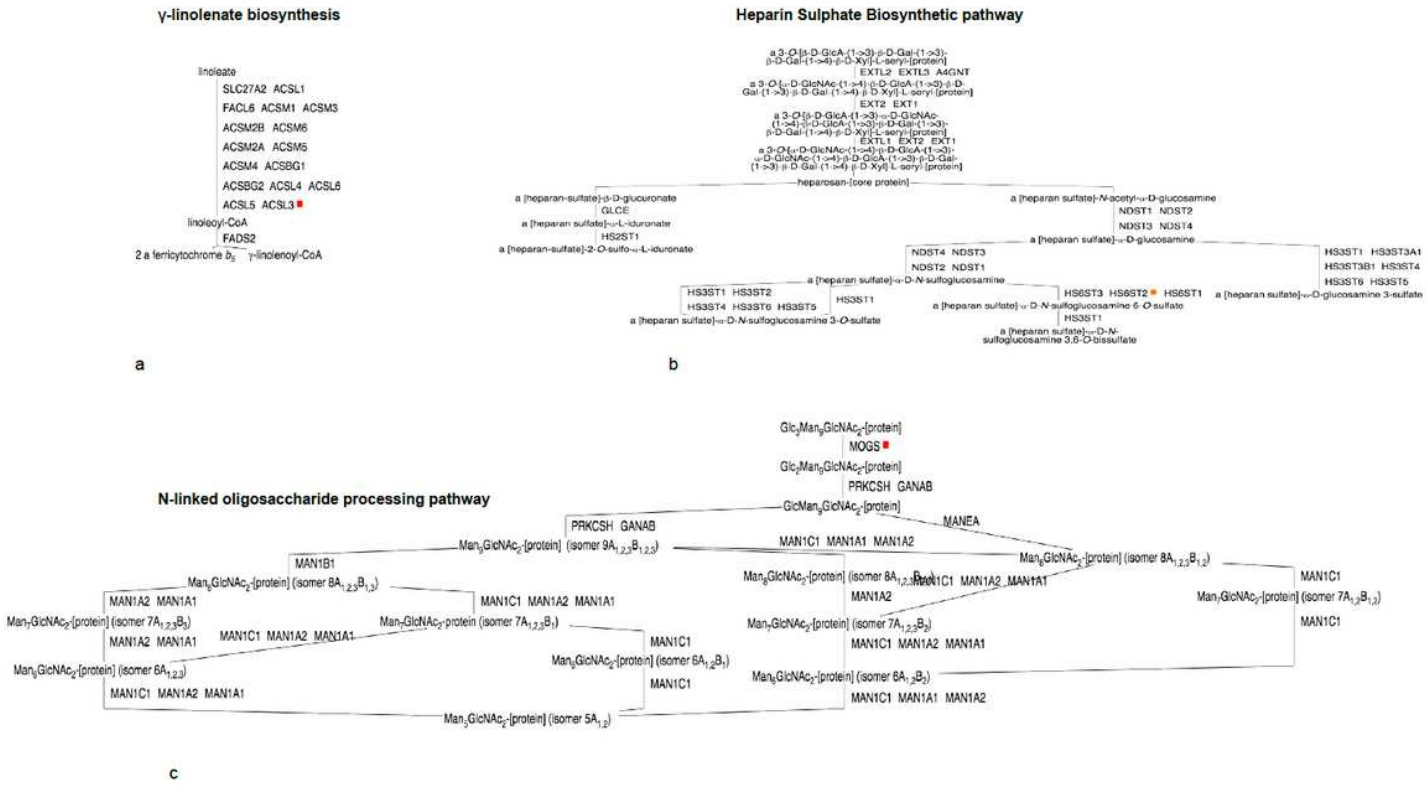

Figure 8

a. PPi-Pathway intersection node - Nsp7 b. PPi-Pathway intersection node - Orf8a c. PPi-Pathway intersection node - Nsp7 and Orf8a

Supplementary Files

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- Supplementary.zip