An *In Silico* Analysis of Potential SARS-CoV-2 Interactions with Proteins Involved in Placental Functions

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Abstract:

COVID-19 is a rapidly evolving medical emergency that has drawn global attention, unprecedented in any disease of its kind in recent times. The magnitude of the health crisis emerging from this pandemic has overwhelmed health care workers worldwide and called in for extraordinary measures to contain this virus. A simple Pubmed query on "COVID-19" returned with 12214 articles (as on May 17[th,] 2020), published just within a few months. A detailed survey revealed around 250 clinical reports, 8 clinical trials, 9 meta-analyses, and 906 reviews that were published during this time span. Combining the strings "COVID-19 and Pregnancy" yielded a total of 132 reports while querying "COVID-19 and Placenta" returned with just 11 articles Even taking into considerations that few materials are in the PrePrint Server, we still have a gross under-representation of studies addressing the effect of this disease on pregnancy outcome and maternal & child health. An essential aspect of a successful pregnancy is proper placentation, where transiently invasive placental trophoblast cells invade the maternal endometrium to establish a functional feto-maternal communication. Based on the elegant study by David. E. Gordon, et al. published in Nature (April 30, 2020), which identified 332 human host proteins interacting with SARS-nCoV2 using an affinity-based purification, we interrogated several gene expression data sets available at NCBI-GEO related to trophoblast invasion and differentiation. Both of these processes are indispensable for placentation and fetal survival. Our analysis showed several overlaps with the interactome proteins implying that SARS-CoV-2 infection can affect several proteins, which are crucial for trophoblasts function. GeneMANIA and STRING based functional analysis further revealed that several of that SARS-CoV-2 interacting trophoblast proteins as a hub for the protein-protein interaction network. Our study thus elucidates the possible effect of SARS-CoV-2 infection on placenta formation and pregnancy outcome.

Keywords: SARS-CoV-2, COVID-19, Pregnancy, Trophoblasts, Interactome, Placenta.

Background:

COVID-19, the viral respiratory disease caused by Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2) was first reported in Wuhan, Hubei province, China, in December 2019. (1,2) As on May 17, 2020, there had been 4,760,420 cases and 314,138 deaths attributed to COVID-19, the over-all mortality being 6.59%.(3) Even though individuals with co-morbidities like hypertension, diabetes, cardiovascular disease, chronic respiratory disease and cancer, and individuals aged more than 60 years are at a higher risk for severe disease and death,(4) a large number of the affected individuals, indeed more than 50% of COVID-19 patients, belong to the reproductive age group; (5) Close to 40% of the affected individuals are females.(6) Piecing all these information together, a large number of females in the reproductive age group have been already infected with SARS-Cov2, or are at potential risk of infection, given the R0 of about 2.28 of COVID-19.(7)

A characterization of pregnant females who were hospitalized for delivery during the COVID-19 outbreak showed that 15.4% of these women were positive for SARS-CoV-2, and among these, 87.9% were asymptomatic. Though the studies are limited, there is no evidence to suggest that the previous severe corona viral epidemics – Severe Acute Respiratory Syndrome (SARS) and Middle East Respiratory Syndrome (MERS) – were associated with increased disease susceptibility in pregnant females.(8) Though the currently limited data does not suggest increased COVID-19 severity in pregnant women compared to the general population, (9) since the physiological and mechanical adaptations in pregnancy are associated with increased susceptibility to infections especially those involving the cardiorespiratory system,(10) the absence of evidence at the early stage of this disease does not preclude this possibility of risk to both mother and the child.

The major consensus at this stage of COVID-19 is that there is no vertical transmission of SARS-CoV-2, like the predecessor coronaviruses causing SARS and MERS, (8,11–13) though there was one recent report on a probable case of vertical transmission.(14) SARS-CoV-2 infection in the third trimester of pregnancy was found to be associated with an increased risk of preterm delivery and intrauterine fetal distress and the requirement for Caesarean sections.(15) At present, there is limited data on the effect of SARS-CoV-2 infection during the first trimester of pregnancy on the pregnancy outcome. However, during SARS of 2003, pregnant females who presented with the disease during the first trimester had an adverse outcome, with 57% of pregnancies ending in spontaneous miscarriage, and 4 out of 5 patients who presented with the disease progressing into preterm delivery. (16) The reasons behind this adverse outcome in pregnancy in females infected with SARS-CoV remains enigmatic.

The placenta is a transient organ that mediates not only the mechanical function of attachment of the developing fetus to the uterus but also plays an indispensable role in facilitating gaseous exchange, transport of nutrients as well as the removal of waste product throughout the pregnancy.(17) Trophoblast cells derived from the outer trophectoderm layer of the developing blastocyst are the major cell types responsible for the formation of the placenta and the extraembryonic membranes, besides providing endocrine support, these cells have stem cell like properties capable of differentiating along several lineages each with a specialized function. Trophoblast cells differentiate into two layers – the mononuclear

cytotrophoblasts (CTB) and multinucleated syncytiotrophoblasts (STB) (Figure 1). The former cells form the core of the primary, secondary and tertiary villi, while the syncytiotrophoblasts form the covering. The cytotrophoblasts function as undifferentiated progenitors for the overlaying syncytiotrophoblasts. The cytotrophoblast cells penetrate the syncytial layer to get in direct contact with the endometrium, where it attaches to the decidua via anchoring villi as part of trophoblast cell columns aided by the release of anchoring protein trophouteronectin.(18)

In addition to the exchange of gas and nutrients, the placenta is also a site for the bi-directional transfer of metabolites, small molecules as well as pathogens and viruses.(19) Though trophoblast cells of the placenta assist in the protective role against pathogens that may cross the maternal-fetal barrier, vertical transmission of pathogens like toxoplasma and listeria as well as viruses (Zika virus, rubella, cytomegalovirus, etc.) do take place in varied proportions. Most of these infections pose severe fetal complications and congenital abnormalities, leading to miscarriage as well as fetal death. In our present study, we plan to explore the harmful effects of SARS-CoV-2 on the placenta and the trophoblast cells in pregnant females with COVID-19 disease.

Since the placenta executes and orchestrates fetal growth-related pathways, placental dysfunction has deleterious effects on the outcome of pregnancy. The first step in addressing the question of whether an adverse pregnancy outcome can occur in SARS-CoV-2 infection is to study the interaction between SARS-CoV-2 and the proteins that are associated with important placental functions like invasion and differentiation. In this study, we use an *in-silico* based approach, employing the SARS-CoV-2 human interactome, differential expression analysis of genes associated with critical placental functions to address the possible consequences of COVID-19 on pregnancy outcome.

Figure 1: This study aims to decipher the potential interactions between the proteins of SARS-CoV-2 and various proteins that are important in the key functions of placenta.

Materials and Methods:

SARS-CoV-2 Human Interactome:
Gordon et al. identified 332 proteins that can interact with 26 of the 29 SARS-CoV-2 proteins, using an affinity-purification (following cloning of viral proteins and their expression in HEK293T cells) and mass spectrometry approach.(20–22) These proteins are listed online (and in Supplementary Table 1)

Identification of Proteins Involved in Crucial Functions of Placenta:
Datasets from NCBI-GEO involving studies on placenta were analyzed for differential gene expression using GEO-2R. Briefly, GEO-2R uses two R-packages from the Bioconductor project – GEOquery, which parses the data to submit it to limma R – to make comparisons between the input samples. Limma stands for 'linear models for microarray analysis'; it is a statistical tool for identification of the differentially expressed genes in the input samples. The datasets used and the samples for differential expression are summarised in Table 1. The datasets were chosen to identify genes that are upregulated during various physiological processes and states involving the placenta – including invasion of trophoblasts, trophoblast differentiation, and disease conditions associated with pregnancy.

Table 1: Summary of Datasets Used to Study Placental Functions and Pathology

Dataset	Condition	Figure No.
GSE9984	Term placenta vs First trimester placenta	Figure 3
GSE28551	Term placenta vs First trimester placenta	Figure 3
GSE9773	Villous vs Extravillous trophoblasts	Figure 6
GSE130339	Day 7 (differentiated) vs day 0 (undifferentiated) trophoblasts	Figure 9
GSE20510	JEG3 vs HTR-8/SVneo cell lines	Figure 15
GSE20510	JEG3 vs SGHPL5 cell lines	Figure 15
GSE66273	Pre-eclampsia vs Control placenta	Figure 19
GSE48424	Pre-eclampsia vs Control placenta	Figure 19

The significant differentially expressed genes were identified by a cut-off adjusted p-value of 0.05. The adjusted p-values were employed to minimize the false discovery rate. In this study, Benjamini & Hochberg false discovery rate method was used to calculate the adjusted p-values. From the significant differentially expressed genes, those with a $\log_2$ fold change of ≥ 1 and ≤ -1 were considered significant upregulated and downregulated, respectively.

Genes/proteins involved in critical placental functions or that are differentially expressed in placental pathology were also obtained by text mining, and literature search of studies involving such analysis – the genes that are upregulated in cytotrophoblasts, syncytiotrophoblasts and migratory trophoblasts were obtained from a study by West et al., (23) and the differentially expressed genes in gestational diabetes mellitus from a study by Radaelli et al.(24)

Identification of Proteins Involved in Placental Functions that can Bind to SARS-CoV-2:

For each dataset, the significant upregulated and downregulated proteins that were identified as compared to the list of proteins interacting with SARS-CoV-2. The lists were compared using Venny 2.1.0 (25) to identify the overlapping proteins. The functions of the proteins that were recurring across multiple study scenarios were further analyzed using text mining and network analysis using GeneMANIA(26) and/or STRING(27) to evaluate their interactome and possible biological function.

A flow chart summarising the complete approach in the discovery of the proteins involved in placental functions that can bind to SARS-CoV-2 is given in Figure 2.
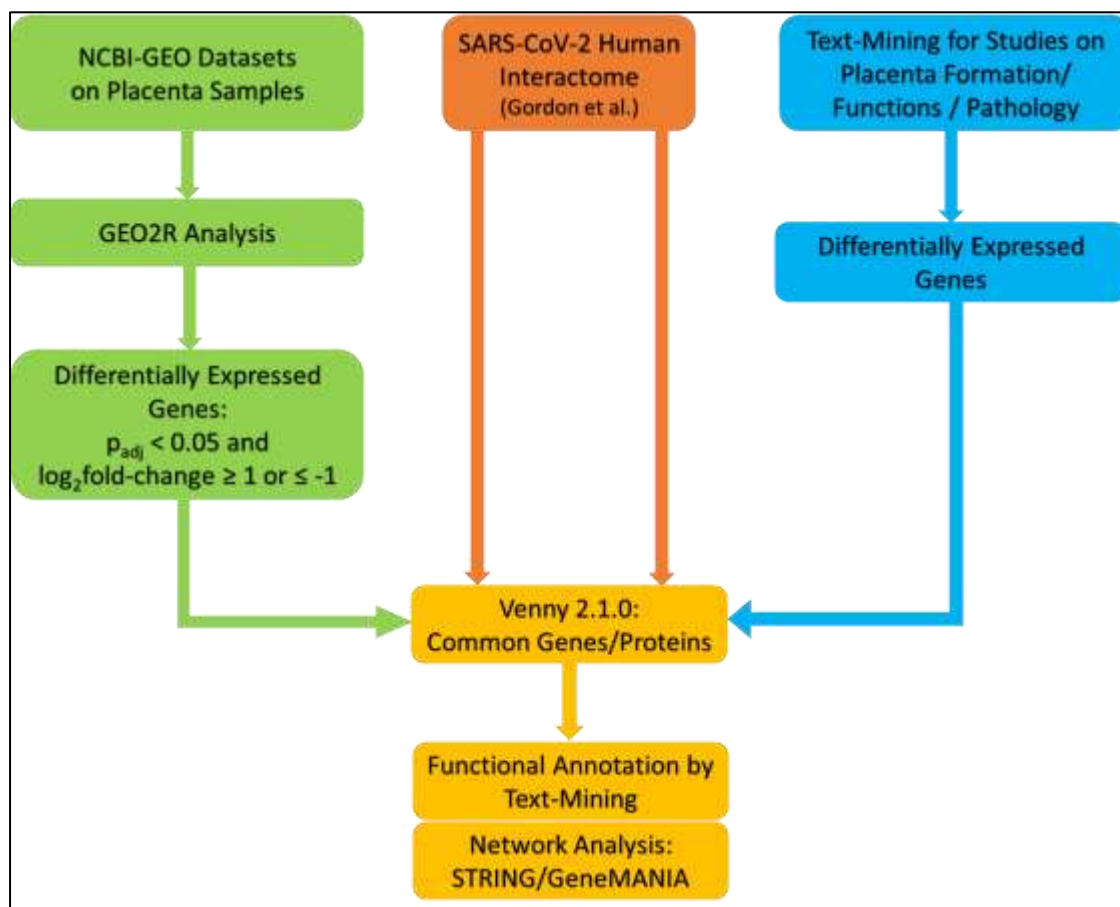
Figure 2: Summary of Workflow

Identification of Common Elements in SARS-CoV-2 Human Interactome and Zika Virus Human Interactome:

Since Zika virus infection in pregnancy has been proven to be associated with vertical transmission of the virus, and disruption of the placental physiology with alterations like abnormal blood flow and aberrant synthesis of biological mediators,(28) we also studied the possible overlap between the human proteins that interact with Zika virus and the SARS-CoV-2 human interactome. For this, a list of human proteins from SK-N-BE2 neuroblastoma cells that interact with Zika viral proteins were obtained from a study by Scaturro et al.(29) These were compared with SARS-CoV-2 human interactome. The lists were compared using Venny

2.1.0 and the relevance of the overlapping proteins in placental functions were studied using text-mining and network analysis.

Results:

SARS-CoV-2 Interacting Proteins that are Upregulated in First Trimester and Term Placentas
Analysis of two different datasets, GSE9984 and GSE28551, were performed to identify the SARS-CoV-2 interacting proteins that are upregulated in first-trimester placenta and term placenta. The data sets revealed that 3 proteins (GSE9984) and 14 proteins (GSE28551), upregulated in first-trimester placenta interacting with SARS-CoV-2 in the two queried data sets (Figures 3a and 3b). We further observed 21 proteins (GSE9984) and 19 proteins (GSE28551) that were upregulated in term placenta in these two data sets (Figures 3a and 3b) that could interact with SARS-CoV-2. We then identified the candidate proteins in the first trimester (3 and 14) and term placenta (21 and 19) that were common in the two data sets. Our results yielded LOX as a common candidate in the term placenta and SCARB1 and PRKAR2B in the first trimester that could potentially interact with CoV2 (Figure 3c).
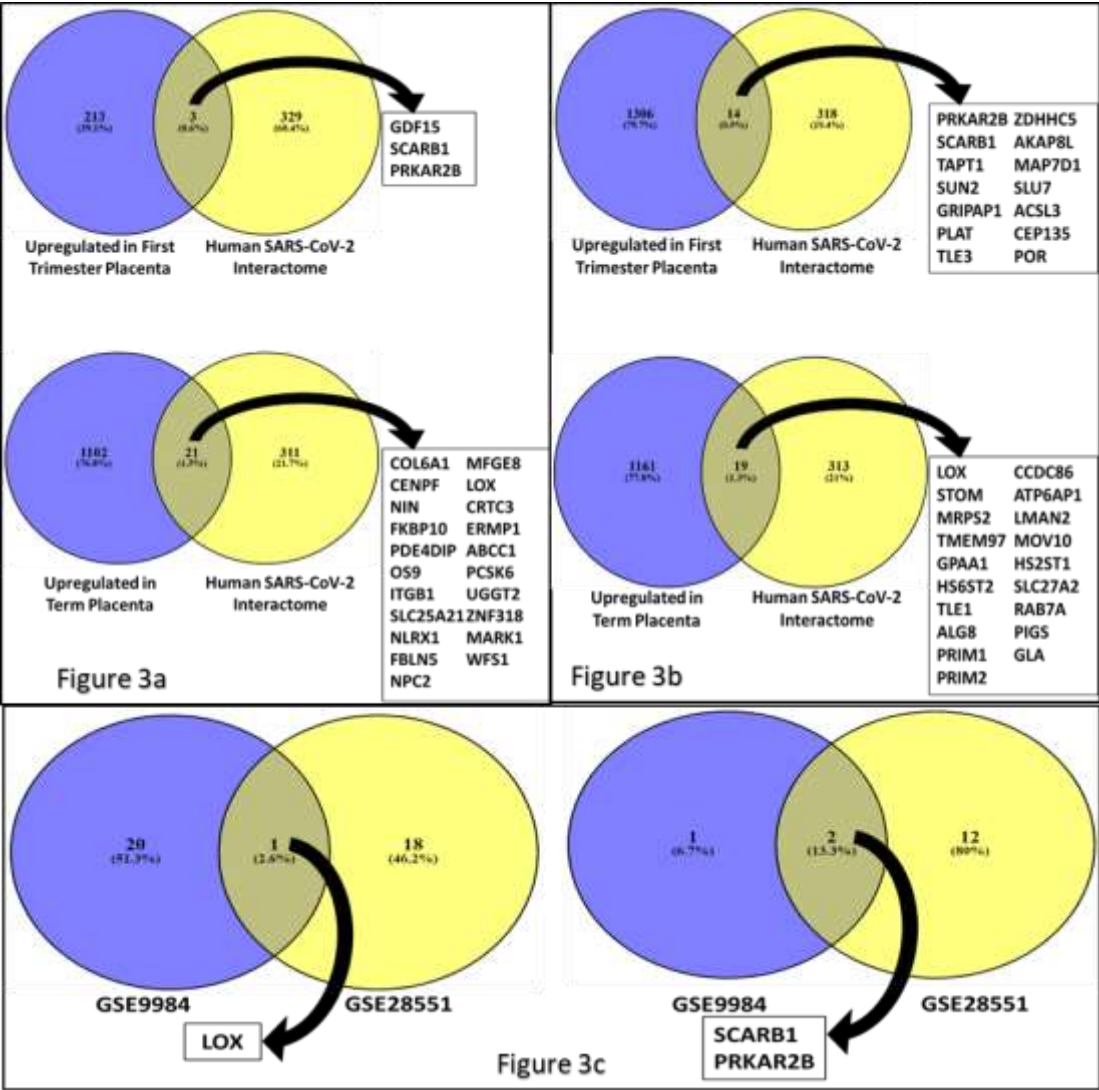

Figure 3: SARS-CoV-2 Interacting Proteins that are Upregulated in First Trimester Placenta and Term Placenta. Figures 3a and 3b show data from GSE9984 and GSE28551, respectively.

Figure 3c shows the overlapping proteins in term placenta (left) and first trimester placenta (right) from GSE9984 and GSE28551, that interact with SARS-CoV-2. The expansions of all the protein names and the SARS-CoV-2 proteins interacting with them are given in Supplementary Table 2.

LOX (lysyl oxidase) upregulated in term placenta can interact with SARS-CoV-2 orf8. Since the downregulation of LOX has been implicated in impaired trophoblast migration and invasion (30), it will be interesting to further validate its interaction with SARS-CoV2 orf8 in an experimental system and study the outcome of this interaction in relation to trophoblast functions. The interaction network of LOX is shown in Figure 4.

SCARB1 and PRKAR2B were the two common first trimester proteins from both the datasets. Scavenger receptor class B type 1 or SCARB1 functions as the receptor for HDL and is important in HDL uptake by trophoblasts (31), and along with CD81, it forms the entry receptor for hepatitis C,(32) which is an RNA virus-like SARS-CoV2. It interacts with SARS-CoV2 nsp7. PRKAR2B or cAMP-dependent protein kinase type II-beta regulatory subunit interacts with SARS-CoV2 nsp13.

Figure 4: LOX interaction network

SARS-CoV-2 Interacting Proteins that are Upregulated in Villous and Extravillous Trophoblasts

Differentiation of trophoblast stem cells along villous and extravillous lineages is a critical early decision in the formation of the placenta and establishing effective feto-maternal communication (Figure 5). Mononuclear cytotrophoblasts (CTBs) forming placental villi through branching morphogenesis, give rise to mature tertiary villi forming villous cytotrophoblast (vCTB). Villous syncytiotrophoblast (vSTB) formed upon the fusion of this vCTB, forms the outer layers of the floating villi bathing in maternal blood that is drained into the intervillous space towards the chorionic plate coming from the basal plate. The vSTB also produces pregnancy steroid hormones and participates in exchange for oxygen and transport of nutrients,(33) a feat critical for supporting pregnancy. At sites where the villi interact with the decidua, forming trophoblast cell columns, a migratory lineage of trophoblast, called extravillous trophoblast (EVT) arises.(34) EVT can be found in various anatomical locations: in

the chorionic plate, smooth chorion, the cell islands and columns, the basal plate, the placental septa, and in walls and lumina of uteroplacental vessels.(35) EVT is a complex heterogeneous population in itself, manifesting proliferative or invasive or both the characteristics.(35) Broadly, EVT can leave the placenta core and invade the maternal endometrium, interdigitating through the extracellular spaces of the endometrium and the myometrium. Few subpopulations of EVT arises, within the maternal uterine compartment – interstitial CTBs (iCTBs), colonizing the decidual stroma, and endovascular CTBs (evCTBs), eroding and remodeling the maternal spiral arteries securing an uninterrupted blood flow towards the placenta. CTB lineage commitments and genesis of trophoblast subpopulations are indispensable for pregnancy, as evidenced in gestational trophoblastic orders arising due to defective trophoblast induced alterations at the feto-maternal interface.(36,37)
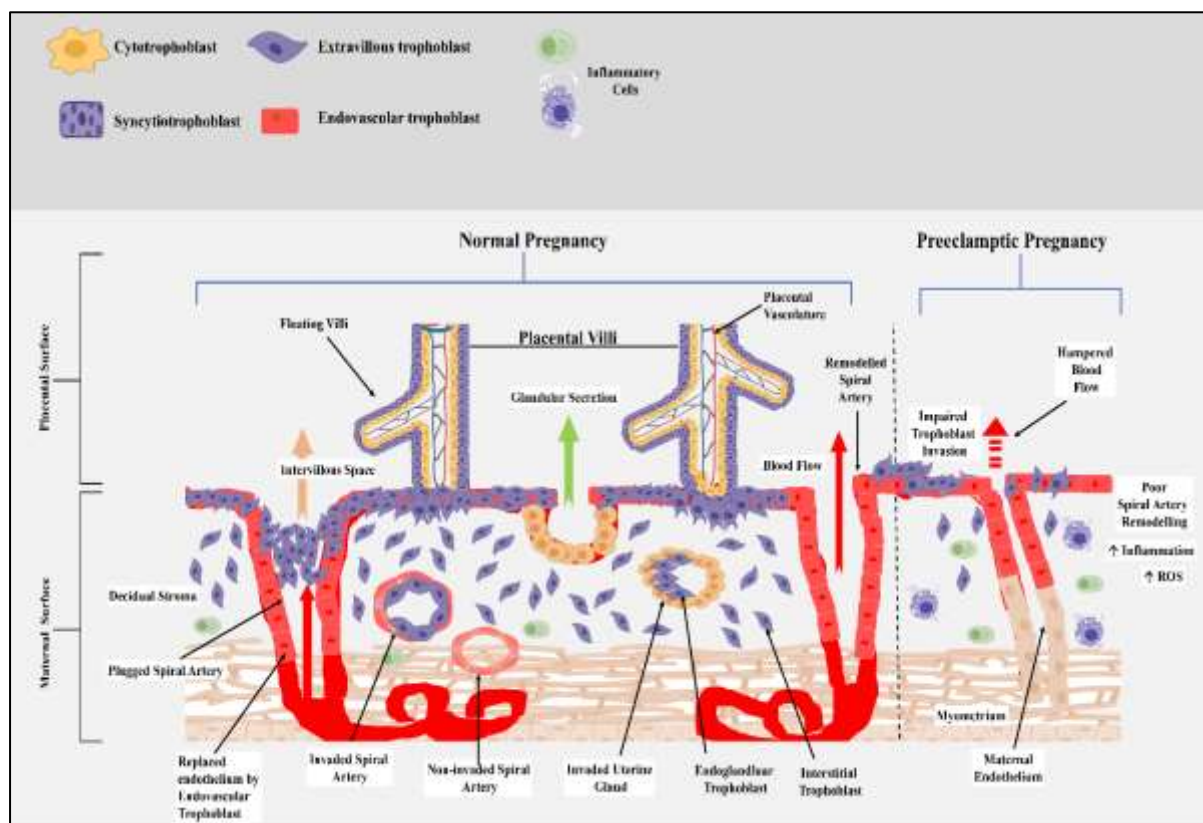
Figure 5: Trophoblast cells in Placenta interacting with maternal endometrium in Normal Pregnancy and in Preeclampsia

Considering the importance of both villous and extravillous lineages of trophoblast in placentation and fetal development(38) we investigated if SARS-CoV-2 interacting proteins are crucial during this differentiation program. We analyzed the villous and extravillous data set (GSE9773) (Figure 6). As mentioned above, the EVT populations are highly motile and exhibit a migratory phenotype, which enables them to leave the villous core and enter the maternal endometrium. As seen in the intersecting Venn, GSE9773 identified 5 common entries between villous CTB and SARS-CoV-2 interactome. COL6A1 (collagen type VI α1 chain), identified as one target, is reported to play a critical role in cell migration (39) and cancer metastasis.(40)
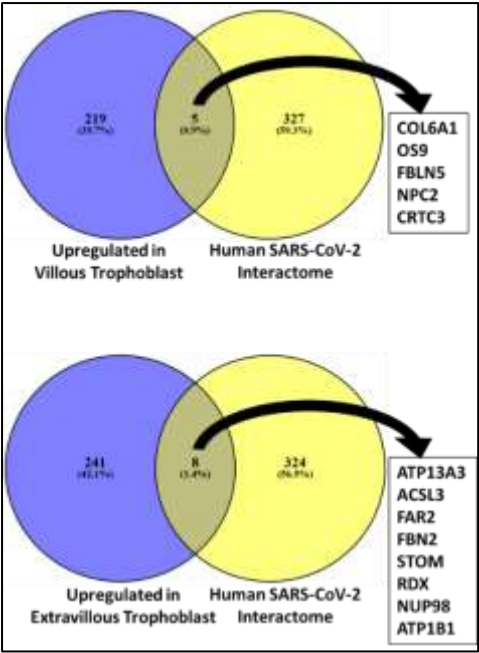
Figure 6: SARS-CoV-2 Interacting Proteins that are Upregulated in Villous and Extravillous Trophoblasts. The data was obtained by analysing GSE9984. The expansions of all the gene names are given in Supplementary Table 2.

Another target, FBLN5 (fibulin-5) is a member of the fibulin family that alters cell adhesive and invasive properties and is expressed in human villous cytotrophoblasts as reported by Winship, et al.(41) Derived from decidua and EVT; it regulates EVT invasion and placentation in women. Gauster, et al.(42) based on cell culture experiments with the villous trophoblast-derived placental fusogenic cell line BeWo, showed that fibulin-5 expression was downregulated during functional differentiation and intercellular fusion. BeWo under hypoxia showed a reduced tendency to fuse, along with an increase in FBLN5 expression. Moore, et al.(43) localized major microfibrillar networks in amnion. Other targets NPC2 (along with NPC1) are required for egress of lysosomal cholesterol, by which cholesterol is removed from the late endocytic pathway.(44) Placenta has been an active steroidogenic tissue; this pathway is therefore of paramount importance, which can be perturbed upon interaction with SARS-CoV-2. We then identified the overlapping proteins between EVT and SARS-CoV-2 interactome, which yielded eight interacting members (GSE9984). FAR2 is abundantly expressed in placenta and trophoblast (Figure 7), but currently, its function is unknown.
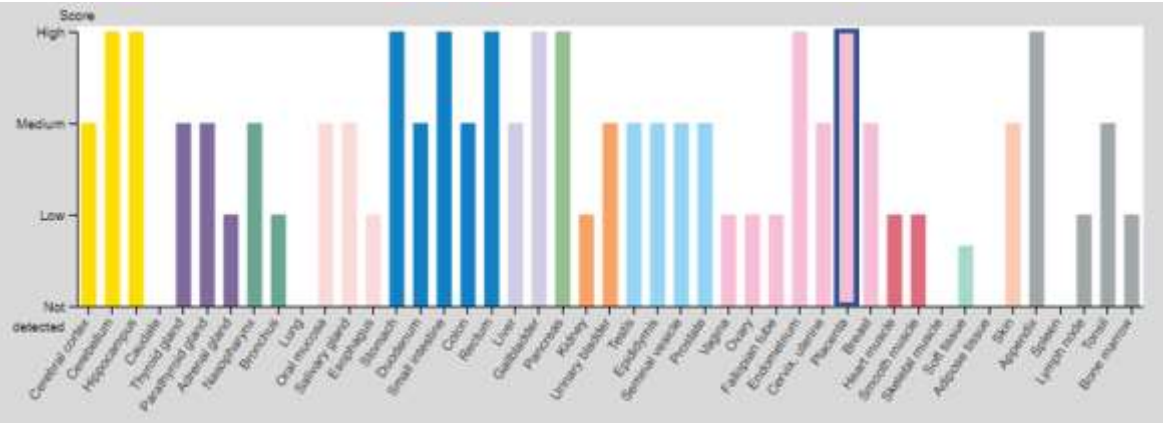
Figure 7: FAR2 Protein Expression in Placenta [Courtesy: Human Protein Atlas(45)]

We next looked into the role of STOM (Stomatin) gene as one of the candidates in our analysis.
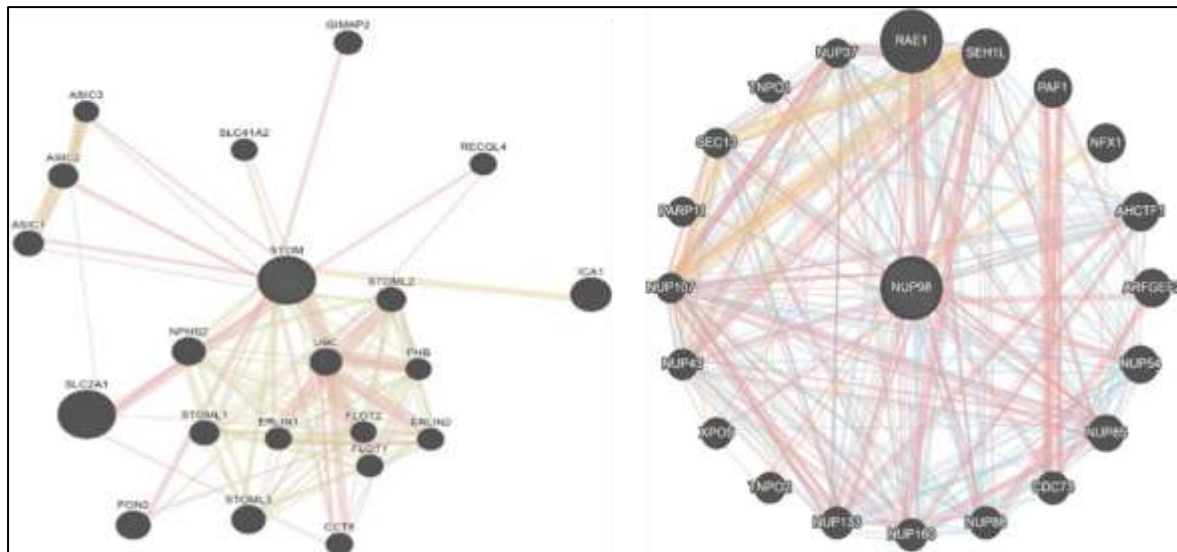
Figure 8: GENEMANIA network showing STOM and NUP98 as hub of a complex interactome. Several cell cycle genes associated with NUP98.

Chen, et al.(46) found that STOM can induce trophoblast fusion and hence could play a role in STB formation. Similarly, NUP98 plays a role in the nuclear pore complex (NPC) assembly and /or maintenance. NUP98, along with NUP96, are involved in bi-directional transport across the NPC. NUP98 seems critical for embryonic stem cell development, as was associated with pregnancy loss.(47) The interaction networks of STOM and NUP98 are shown in Figure 8. Therefore analyzing the genes highly expressed in villous and extravillous trophoblasts, we conclude that several key proteins related to trophoblast fusion, invasion, and genome maintenance seem to be part of SARS-CoV-2 interactome.

SARS-CoV-2 Interacting Proteins that are Upregulated during Villous Trophoblast Differentiation

Our observation was also reinforced when we analyzed the next data gene set (GSE130339). This gene data set associated with early and latter day 7 trophoblast differentiation (Figure 9). STOM and NUP98 were also found to be enriched here. Of particular interest is FBN2. A recent report published by Yu Y et al.(48) mentions that FBN encodes asprosin, a glucogenic hormone, following furin cleavage of the C-terminus of profibrillin. They identified a peptide hormone, placensin encoded by FBN2 in trophoblasts of human placenta. Placensin secretion by immortalized human trophoblastic HTR-8/SVneo cells is accompanied by an increase in matrix metalloproteinase-9 (MMP9) expression, thereby promoting cell invasion.

FBN2 also seems to interact with GCM1 (Figure 10), a critical factor for trophoblast differentiation. GCM1 is a master regulator of trophoblast differentiation and seems to be associated with several HDAC complex and is possible that this alters the epigenetic landscape in the cell, an event needed prior to differentiation and lineage commitment.
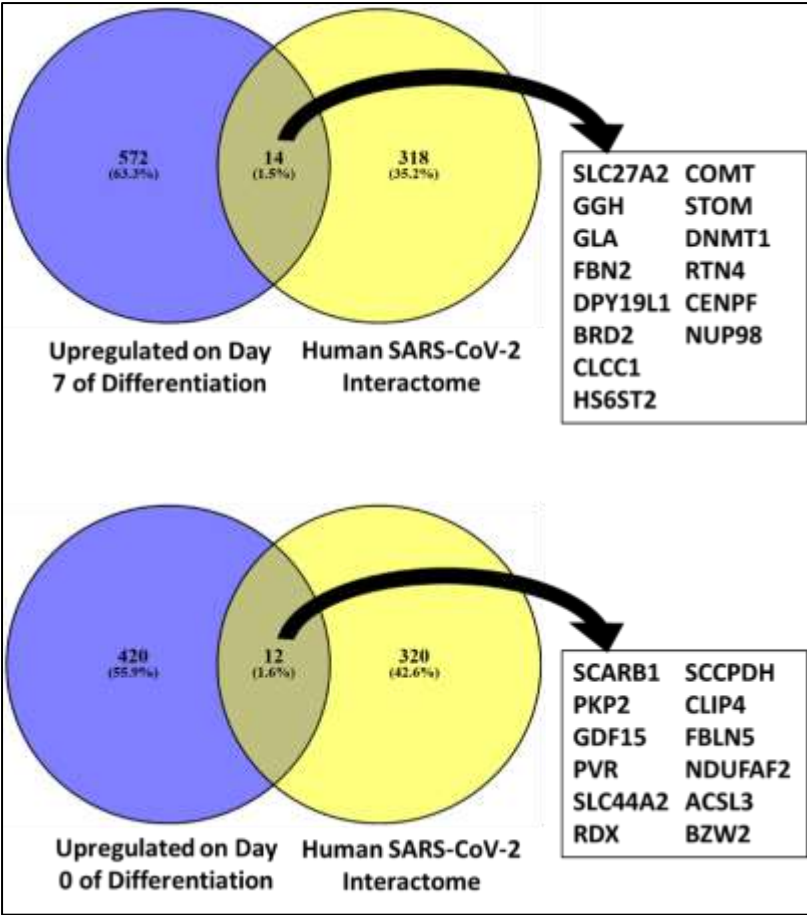
Figure 9: SARS-CoV-2 Interacting Proteins that are Upregulated on Day 0 and Day 7 of Villous Trophoblast Differentiation. The data was obtained by analyzing GSE130339. The expansions of all the gene names are given in Supplementary Table 2.
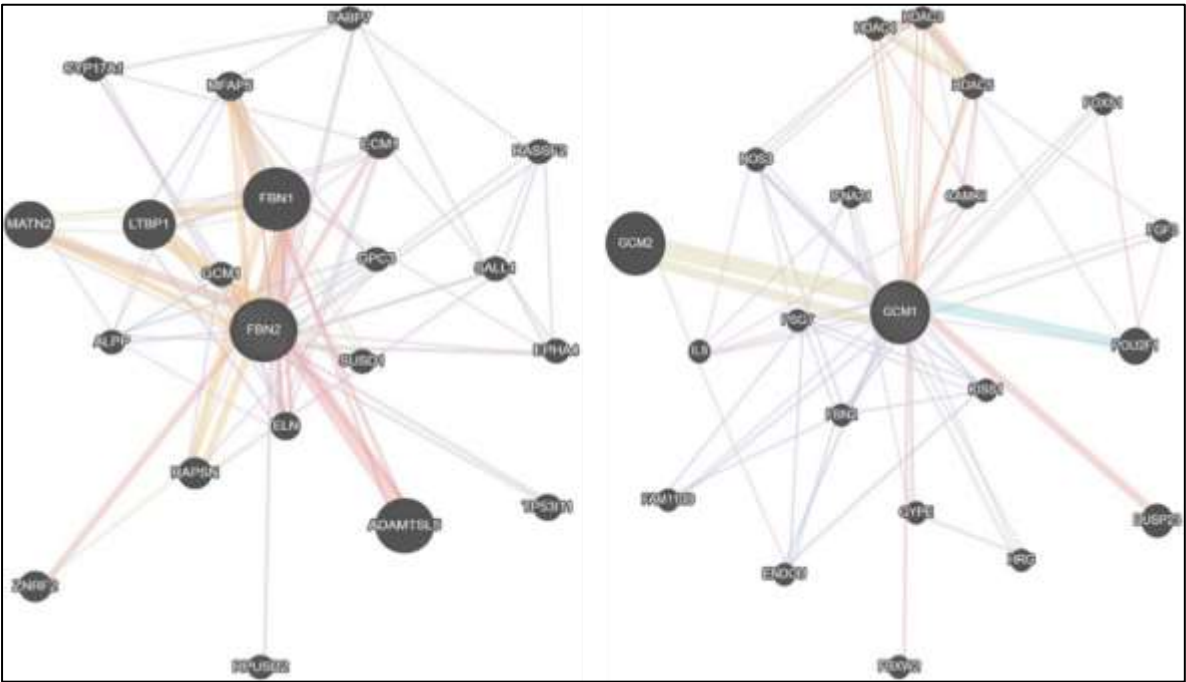

Figure 10: GENEMANIA network showing FBN and GCM1 interactome

Of interest is GDF15 present in Day 0 differentiation. Recently Turco, et al.(49), using placental organoids derived from early placental villi, found that these cultures organize into villous-like structures and secretes placental-specific peptides and hormones, including human chorionic gonadotropin (hCG), growth differentiation factor 15 (GDF15). This gene encodes a secreted ligand of TGF superfamily, which binds to various TGF-beta receptors leading to recruitment and activation of SMAD family transcription factors that regulate gene expression. GDF15 is involved in trophoblast development where it controls apoptosis and differentiation.(50) GDF15 interactome showed p53 and ERBB2 as close neighbors (Figure 11).
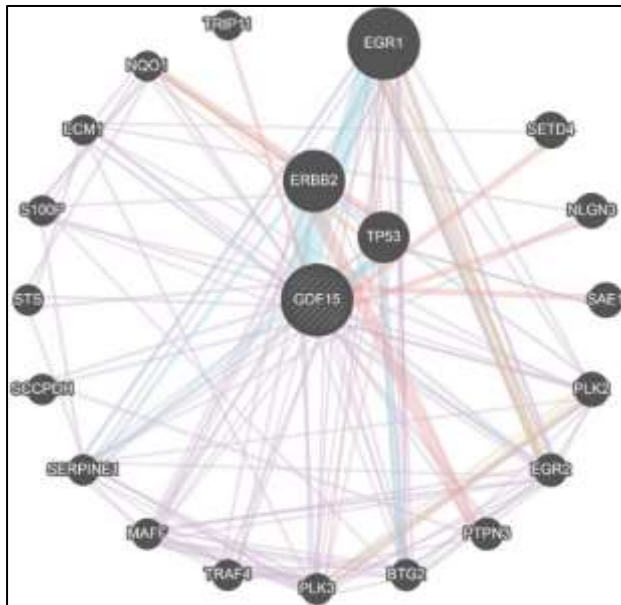
Figure 11: GENEMANIA network showing GDF1 interactome

SARS-CoV-2 Interacting Proteins that are Upregulated in Cytotrophoblasts, Syncytiotrophoblasts and Migrating Trophoblasts

We did the data mining for SARS-CoV-2 interactome with genes highly expressed in cytotrophoblasts, syncytiotrophoblasts, and migrating trophoblasts (Figure 12). A significant number of genes upregulated in CTB were found to overlap with SARS-CoV-2 interactome (approx. 94, also see supplementary table 3), implying that SARS-CoV-2 can strongly influence CTB stem cells. We observed about ten overlapping proteins related to STB formation.

Of special interest is RBX1, a ring box 1 protein which is found to be abundantly expressed in placenta (Figure 13). Cullin 3 (CUL3), is a scaffold protein that assembles into a large number of ubiquitin ligase complexes, similar to Skp1-Cullin 1-F-box protein complex. CUL3 associates with Bric-a-brac-Tramtrack-Broad (BTB) complex and Rbx1 to form a BTB-CUL3-Rbx1 (BCR) ubiquitin ligase complex. CUL3 was found to be highly expressed in the invasive EVTs of human placenta villi from normal pregnant women, and the expression of CUL3 in the less invasive EVTs from PE patients was significantly lower.(51) CUL3 also promoted the invasion and migration of human trophoblast cells in the human EVT cell line HTR8/SVneo as well as in placental explants. These results show that RbX1 interaction with SARS-CoV-2 proteins is crucial for EVT invasion.
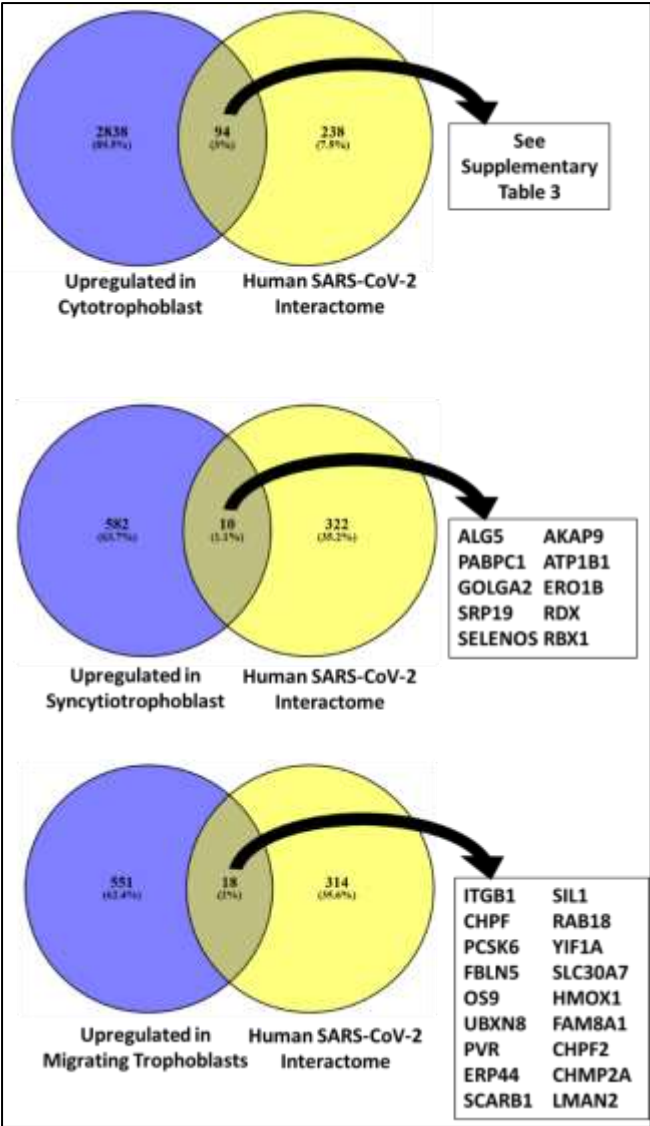
Figure 12: SARS-CoV-2 Interacting Proteins that are Upregulated in Cytotrophoblasts, Syncytiotrophoblasts, and Migrating Trophoblasts. The expansions of all the gene names are given in Supplementary Table 2.
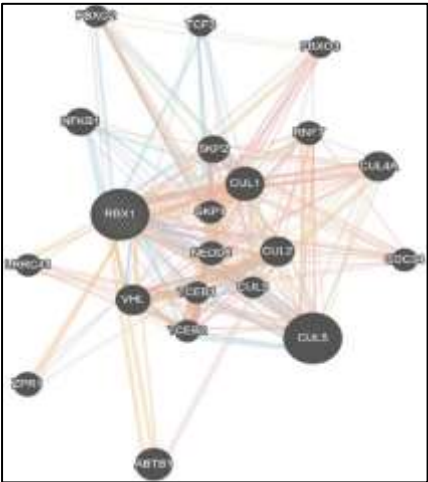

Figure 13: RBX1 CUL3 complex predominantly expressed in invasive EVT

Further, we found 18 genes related to migratory trophoblast overlapped with SARS-CoV-2 interactome. Of particular interest is HMOX-1, which produces a signaling molecule, carbon monoxide (CO). Bilban, et al.(34) reported that modulation of HMOX-1 expression in loss-of-function as well as gain-of-function cell models (BeWo and HTR8/SVneo, respectively) demonstrated a reciprocal relationship of HMOX-1 expression with trophoblast migration. Also, HMOX-1 expression led to an increase in peroxisome proliferator-activated receptor (PPAR) gamma. The HMOX-1 interactome is depicted in Figure 14.
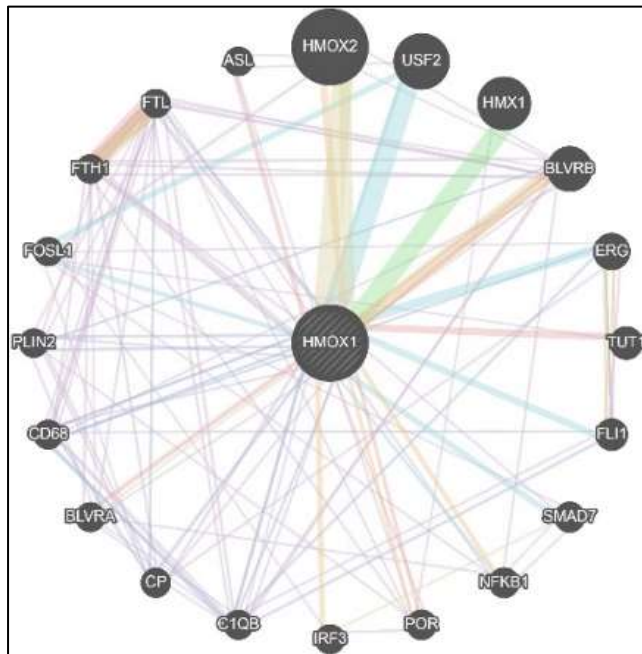
Figure 14: HMOX-1 interactome. Several of the interacting partners play an important role in trophoblast invasion.

SARS-CoV-2 Interacting Proteins that are Upregulated in Placental Cell Lines

One of the most challenging aspects of placenta research is the availability of fresh tissue samples. Further, with the development of newer and safer methodologies for termination of pregnancy using medications, obtaining first-trimester tissue samples is even more difficult. This has resorted to the placenta researchers using cell lines based approaches to address their biological question. There are currently seven (BeWo, JEG3, SGHPL5, JAR, HTR-SVNeo, ACH1P-3P, Spa-26) widely used trophoblasts derived cell lines available, that serve the purpose. These cell lines are more resilient compared to the trophoblast obtained from primary cultures having an extended life span and more comfortable to grow and propagate to address trophoblast function. JEG3, JAR, BeWo, ACH1P-3P are all choriocarcinoma derived, while SGHPL5, HTR, Spa-26, Swan71, TCL-1, RST1, RSVT-2 are all immortalized. Much of our knowledge about the trophoblast system was obtained using these cell lines and immortalized cells as a model system. We next explored that to what extent SARS-CoV-2 interacting proteins overlap with those derived from the cell lines (Figure 15). This will enable us to know if these systems can also be used to study SARS-CoV-2 infection in proxy of using placental trophoblast cells, which are somewhat difficult to obtain in adequate amounts. Our analysis interrogated gene set data from HTR/SVneo, SGHPL5, and JEG3 cell lines, three of the most popular trophoblast cell lines using the dataset GSE20510.
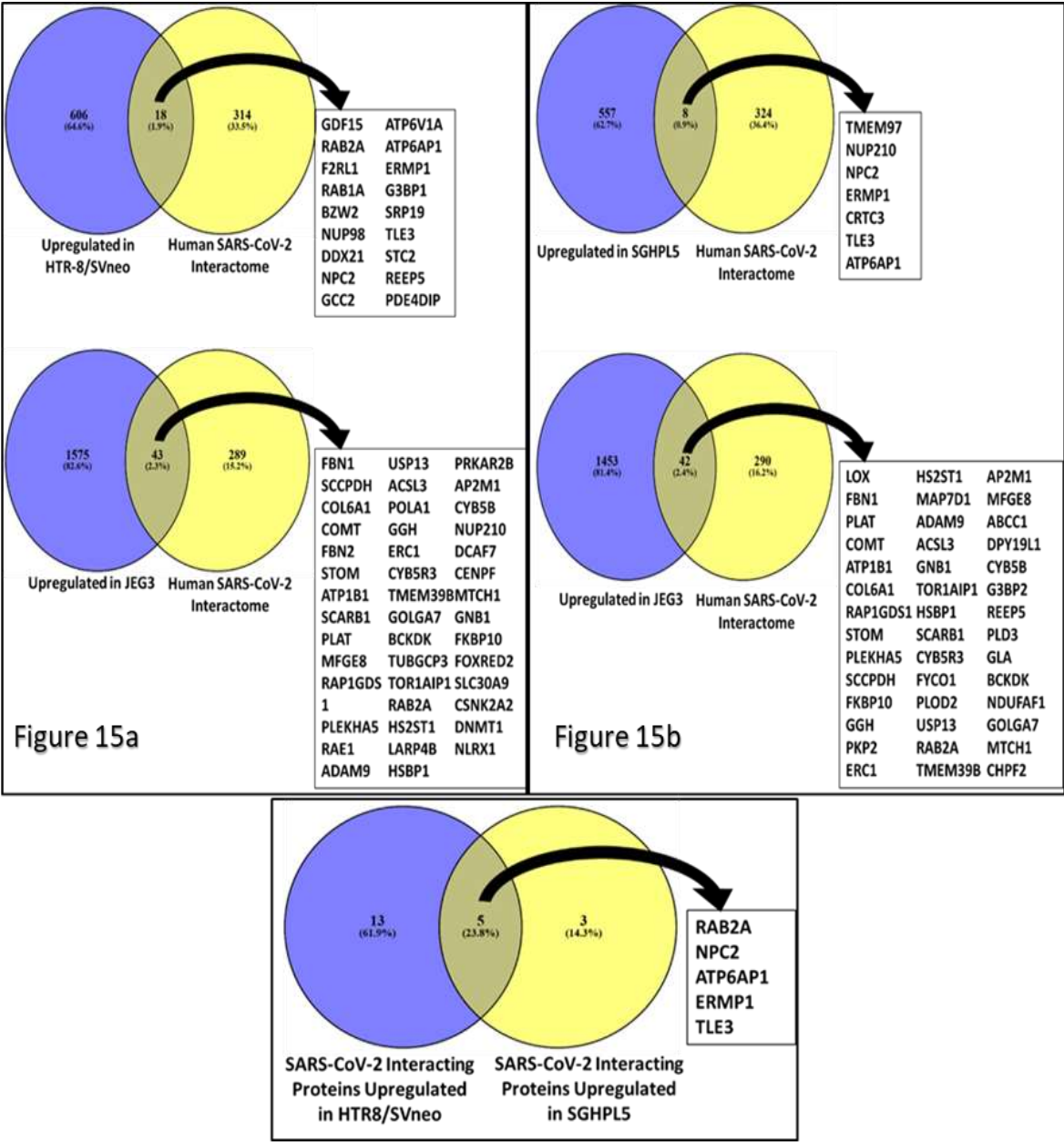
Figure 15a

Figure 15b

Figure 15c

Figure 15: SARS-CoV-2 Interacting Proteins that are Upregulated in Different Placental Cell Lines. Figure 15a and 15b show genes upregulated in HTR-8/SVneo and SGHPL5 cells, respectively, in comparison with JEG3 cells. Figure 15c shows the overlapping proteins upregulated in HTR-8/SVneo and SGHPL5 that interact with SARS-CoV-2. The dataset used was GSE20510. The expansions of all the gene names are given in Supplementary Table 2.

Our results show 18 and 43 overlapping proteins between HTR/SVneo and JEG3 cell lines, respectively, and 8 and 42 overlapping proteins between SGHPL-5 and JEG3 cell lines, respectively. 5 proteins overlapped between the representative early placental cell lines – SGHPL5 and JEG3. We next looked for the overlaps between the genes upregulated in the early placental cell lines – HTR8/SVneo and SGHPL5 – and early placenta and the human SARS-CoV2 interactome, using datasets GSE20510 and GSE28551. The result showed the commanility of transducin-like enhancer protein 3 (TLE3) (Figure 16).
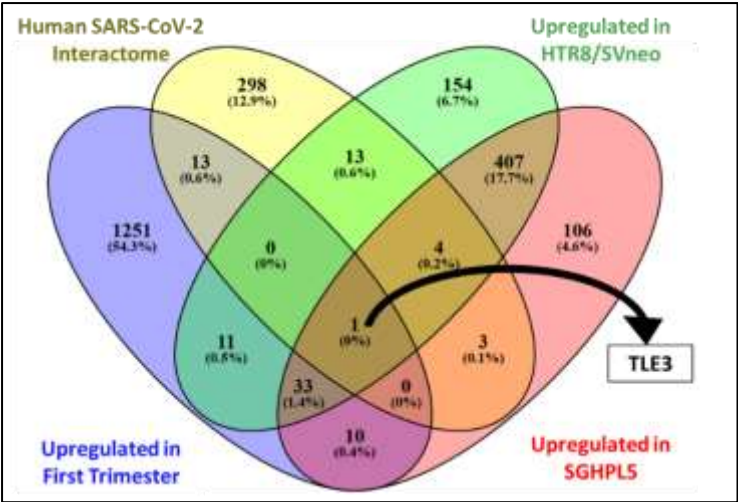
Figure 16: Combined analysis of datasets GSE20510 and GSE28551 involving genes that are upregulated in first trimester placenta and surrogates used in in vitro studies for first-trimester placenta – HTR8/SVneo and SGHPL5 cell lines, and the human SARS-CoV2 interactome.

TLE3 is a transcriptional co-repressor. In murine models, TLE3 known out was associated with abnormal placental development with a severe defect in the vasculature and intrauterine death. TLE3 has also involved in Notch-3 mediated signaling. (52) The interaction network of TLE3 using STRING shows its association with various proteins involved in the transcriptional regulation like Histone deacetylase 1 (HDAC1), CREB-binding protein (CREBBP), C-terminal-binding protein 1 (CTBP1) and Forkhead box protein A1 (FOXA1) (Figure 16a). GENEmania of TLE3 interactome showed strong associations with COPB1 and COPZ1. These are components of coat protein I (COPI) complex which is involved in intracellular protein trafficking. They are also involved in the binding of SARS-CoV (cause of SARS of 2003) spike protein (S protein) to the membrane protein (M-protein) , a process required for viral entry into the endoplasmic reticulum (ER)-Golgi intermediate compartment (ERGIC), budding and viral shedding.(53)
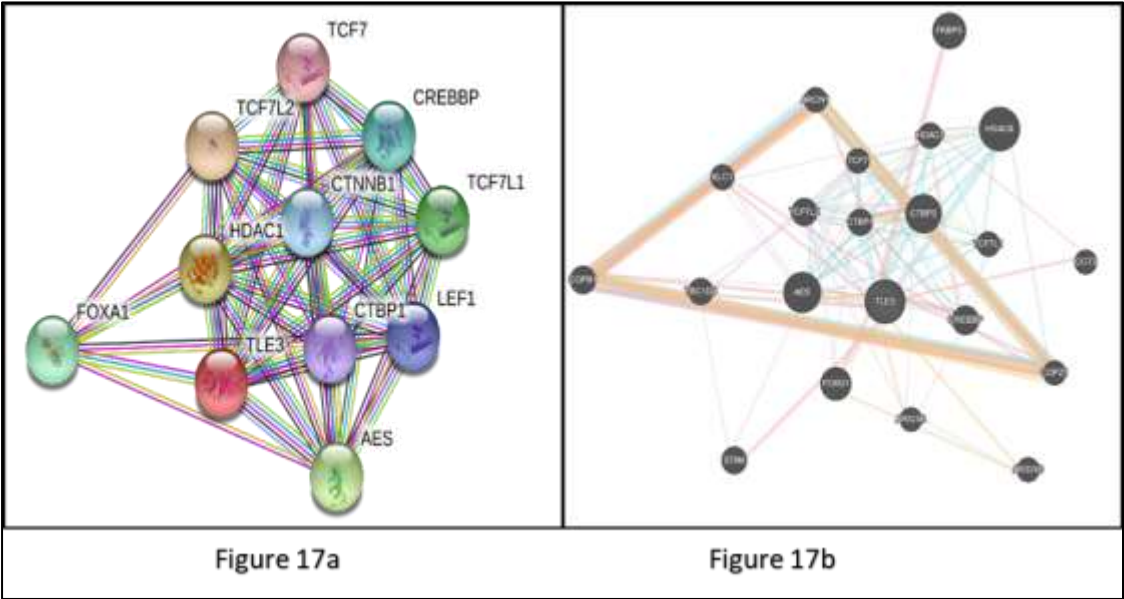

Figure 17: STRING (Figure 17a) and GeneMANIA (Figure 17b) analysis showing TLE3 interacting proteins

We further explored on ARCNI, as one of the potential TLE3 interacting partners as evident from GeneMANIA (Figures 17b and 18). Results show ARCN1 as a hub, interacting with a large family of COP proteins (COPA, COPB1, COPB2, COPG1, COPG2, COPE, COPZ1) as well as Golgi components (GOLPH3, GOLPH3L). As discussed above, COP plays an important role in virus entry, assembly, and transmission, thereby indicating that these trophoblast cell lines may suffice as a suitable model to study the viral infection cycle.
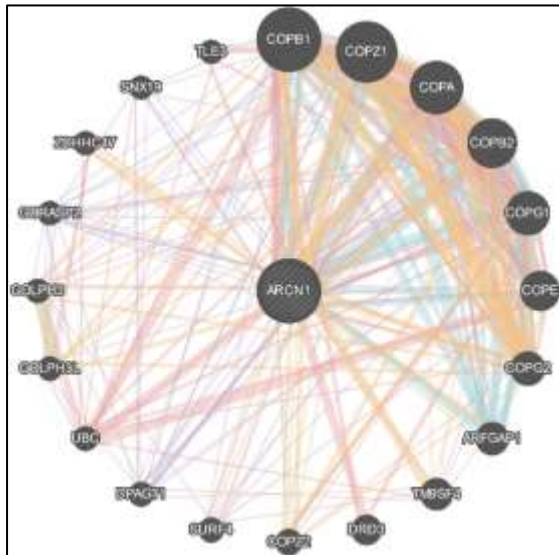

Fig 18. ARCNI interactome showing association with COP complexes

SARS-CoV-2 Interacting Proteins that are Altered in Pre-eclampsia and Gestational Diabetes Mellitus

So far, we saw that a significant number of genes associated with trophoblast invasion, differentiation, and migration that could potentially interact with SARS-CoV-2. This prompted us to speculate the status of engagement between SARS-CoV-2 and females with preeclampsia (PE) (Figure 5). PE is a common event affecting 10-12% of pregnant females, usually begins after 20 weeks of pregnancy in a woman whose blood pressure had been normal. It can lead to serious, even fatal complications. ACOG defines gestational hypertension as "hypertension without proteinuria, or severe features develop after 20 weeks of gestation, and blood pressure levels return to normal in the postpartum period". Further, there may be no typical symptoms. High blood pressure and protein in the urine are key features that still might be absent. There may also be swelling in the legs and water retention, but this can be hard to distinguish from a normal pregnancy. Hypertension during pregnancy has been classified into four categories: (i) Preeclampsia/Eclampsia, (ii) Chronic hypertension: This involves hypertension (>140 mm Hg systolic BP or >90 mm Hg diastolic BP) that predates pregnancy, (iii) Chronic hypertension with superimposed preeclampsia and (iv) Gestational hypertension where we have BP elevation in the absence of proteinuria or systemic findings which was formerly known as Pregnancy Induced Hypertension(PIH).

The datasets for PE included GSE66273, where gestational age for PE was 31.0 (30.9–34.0) weeks, and controls were 31.2 (29.3–33.2) weeks, and GSE48424, where gestational age for PE was 34 (31-35) weeks and controls, was (33-37) weeks. Our analysis of the dataset of genes

overexpressed in preeclampsia using GSE66273 revealed MFGE8 (milk fat globule-epidermal growth factor-factor 8) as one of the targets that was also upregulated in PE (Figures 19a and 20).

Figure 19: SARS-CoV-2 Interacting Proteins that are Altered in Pre-eclampsia (Figures 19 a,b, and c) and Gestational Diabetes Mellitus (Figure 19d). The expansions of all the gene names are given in Supplementary Table 2.

Figure 20: Protein Interaction Network of MFGE8.

MFGE8 protein is highly expressed in human chorionic villi at all trimesters of gestation and in murine implantation sites.(54) MFGE8 performs an important role in physiological conditions during menstrual endometrium remodeling and implantation, and dysfunctions of

its expression may be associated with endometrial pathological conditions. Further, Schmitz, et al.(55) investigated that MFGE8 and its receptor integrin αvβ3 play an important role in the attachment of trophoblast cells to the endometrial epithelium. Yu, et al.(56) reported MFGE8 as a master regulator for TGFbeta1 in orchestrating EMT, an event that seems to be dysfunctional in PE. Of particular interest is that MMP2 (72 kDa MMP) as one of the interacting partners for MFGE8 as identified in the gene interaction model (Figure 18). MMP2 has been strongly associated with the onset of PE.(57,58) Therefore; we can conclude that SARS-CoV-2 interaction with MFGE8 could have potentially serious consequences in the onset of PE.

We next investigated the genes down-regulated in PE overlapping with SARS-CoV-2 interactome and identified PLAT as one member (Figure 19b). PLAT (Plasminogen Activator Tissue Type) interaction network comprises (Figure 21) of several serine proteases and their inhibitors - SERPINE1 (also called PAI-1, Plasminogen Activator Inhibitor), PLAU (Plasminogen Urokinase Activator), PLG (Plasminogen). One of the PLAT interacting partners, LRP1 (LDL receptor Related Protein 1) is reported to be poorly expressed in preeclamptic group placental in SGA (Small for Gestational Age) delivering mothers (59) which explains the atherosclerotic phenotype of preeclamptic placentas. Several of the members of this interaction network (SERPINE1, LRP1, PDGFD, JunD) seems to be associated with preeclampsia and PIH(60,61)

Figure 21: Protein Interaction Network of PLAT
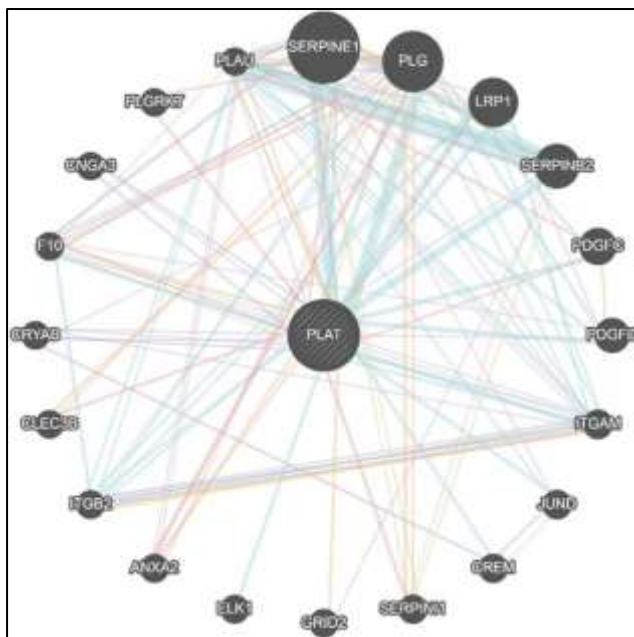
Analysis of GSE48424 (Figure 17c) showed one of the overlapping proteins upregulated in PE and human SARS-CoV-2 as F2LR1. F2LR1 or coagulation factor II (thrombin) receptor-like 1, also known as Protease-activated receptor 2. PAR2 expression was found to be absent in normal placental endothelial cells but overexpressed in placental endothelial cells of pre-eclampsia patients. Since this is an extracellular receptor, it will be intriguing to know whether pre-eclampsia placental will be at risk for SARS-CoV-2 binding compared to normal pregnancies.(62)

The list of genes with altered expression in gestational diabetes mellitus (GDM) was obtained from a study by Radaelli, et al.(24) The average gestational age in GDM was 38.5 ± 0.5 weeks and in controls was 38.9 ± 0.4 weeks. Among the overlapping genes with SARS-CoV-2 (Figure 19d) interactome was FBN2 or Fibulin 2, which has already been mentioned previously as upregulated during extra-villous trophoblast differentiation. Another protein in the common Venn is poliovirus receptor (PVR) or CD155, which was found to interact with actin and integrin alpha-V, which are known factors affecting cell motility and migration; thus CD155 is involved in tumor cell invasion and migration, a shared property of tumor cells and trophoblast cells.(63) The STRING analysis also showed the confirmed this finding (Figure 22).
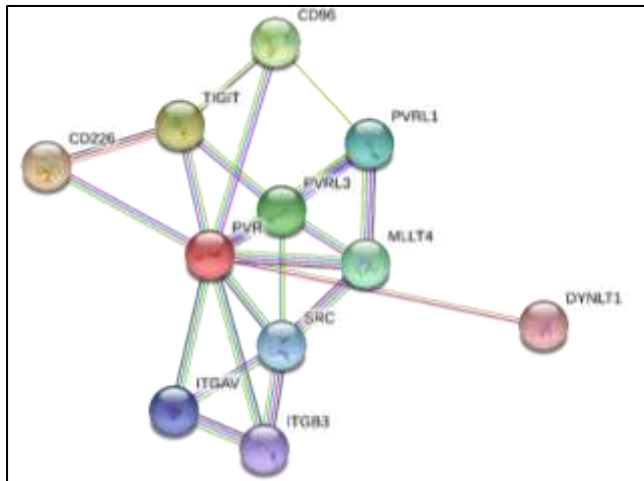

Figure 22: Protein Interaction Network of PVR (CD155)

SARS-CoV-2 Interacting Proteins that are also Involved in Binding to Zika Virus Proteins

Based on the fact that infection with Zika virus causes fetal abnormality, we explored the overlaps between Zika virus and SARS-CoV-2 interactomes to address if there are any common intersecting pathways and, therefore, could predict the possible outcome of SARS-CoV-2 infection on the fetus. Zika virus is transmitted primarily by *Aedes aegypti* mosquitoes**.** Though most people with Zika virus infection do not manifest symptoms, during pregnancy, Zika viral infection is reported to cause fetal abnormality in the newborn. Babies were born with microcephaly and other congenital malformations.(64,65) Infection with Zika virus is also associated with other complications of pregnancy, including preterm birth and miscarriage. Zika infection causes microcephaly in the developing fetus is confirmed in the large European cohort study, the EUROCAT (the European Surveillance of Congenital Anomalies) as well in a Brazilian study.(66)

Of the 406 proteins that bind to Zika virus, according to Scaturro et al. (29), 20 were also found to interact with SARS-CoV-2. Among these 20, 10 proteins were highly expressed in cytotrophoblasts, 1 in syncytiotrophoblasts, and 2 in migratory trophoblasts (Figure 23). Thus, 13 human proteins highly expressed in the placenta, which are potentially involved in the pathogenesis of Zika virus infection, are also possible targets of SARS-CoV-2.

Of particular interest are the MRPS (Mitochondrial Ribosomal Proteins)(Figures 24 and 25). These family of proteins plays a crucial role during trophoblast syncytialization process.(67) The human mitochondrial ribosomal protein (MRP) gene family comprises of 30 genes which code for mitochondrial ribosomal small subunit and 50 genes for the large subunit. Poidatz, et al.(67)

found that MRPS participates in ERRγ (Estrogen related receptor- gamma) mediated fusion of trophoblast cell to form a syncytium along with the release of hCG hormone.
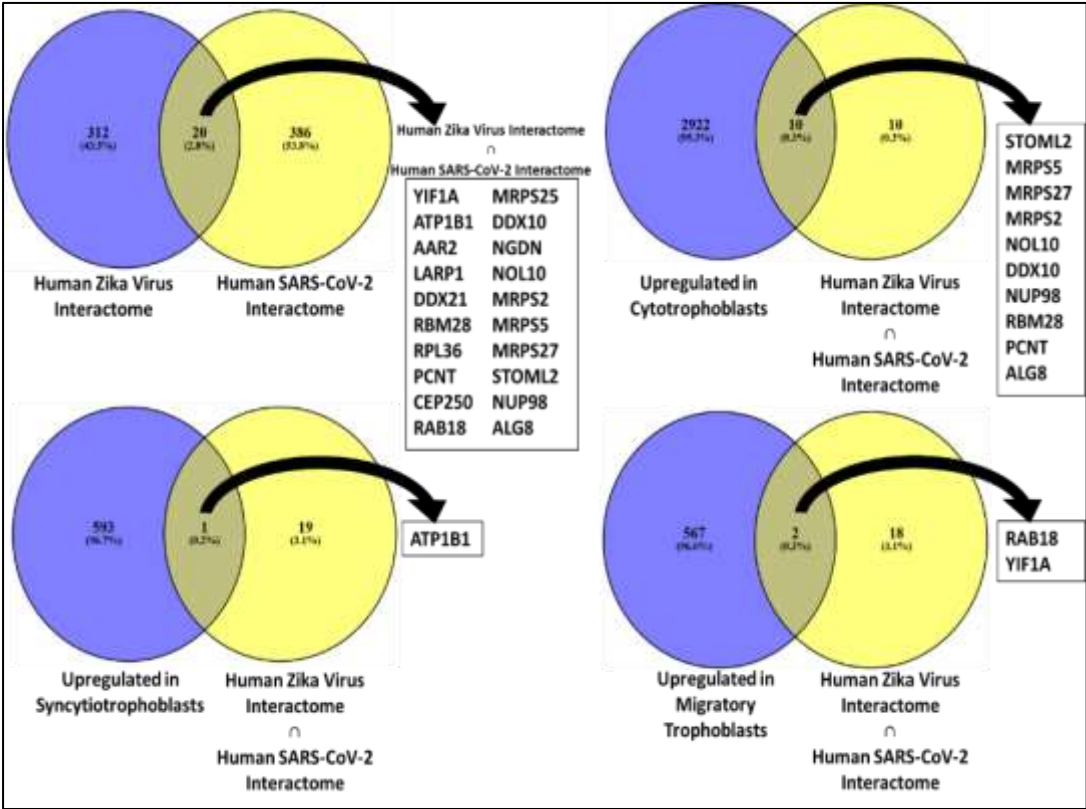

Figure 23: Left Upper Panel Shows the Overlap Between SARS-CoV-2 Interactome and Zika Virus Interactome in Humans. 13 of These Proteins were found to be highly expressed in placenta. The expansions of all the gene names are given in Supplementary Table 2.
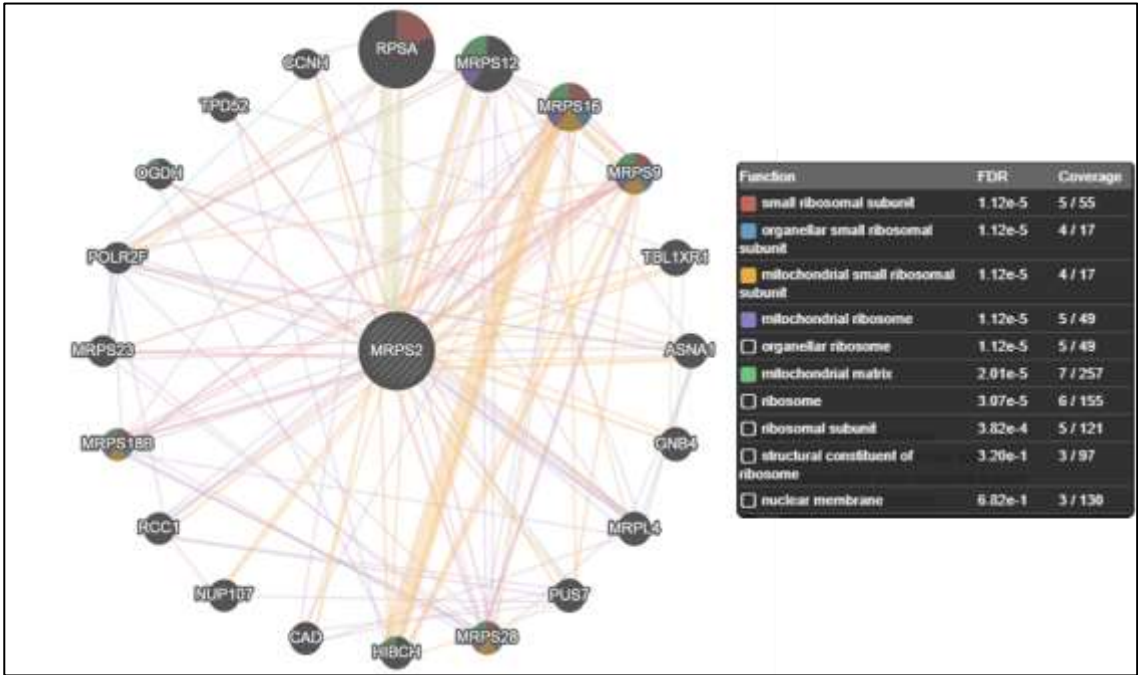

Figure 24: Mitochondrial Ribosomal Protein (MRPS) families showing ALL of their interacting partners
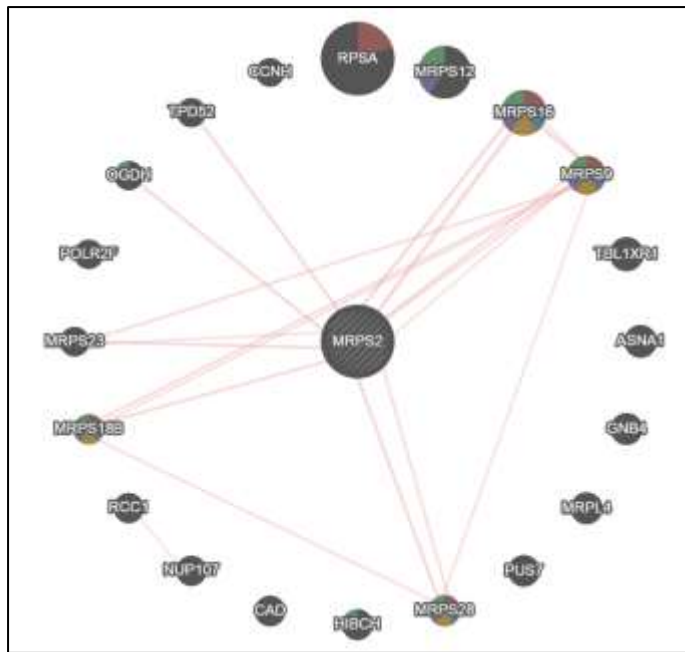
Figure 25: MRSP members physically interacting with each other as a protein complex

Discussion:

We investigated, *in silico*, the effect of SARS-CoV2 infection in pregnant females. Using a large number of publicly available gene expression data sets from NCBI-GEO related to trophoblast invasion, differentiation, and migration and query against the 332 human interactome as reported by Gordon, et al.(20–22) we identified several overlaps between them. Several of the key genes associated with trophoblast invasion, villous vs. extravillous differentiation, as well as migratory behavior of these cells seem to be a part of this interactome. Our analysis also identified many novel, unreported candidate proteins that could be targeted during these infections. One of the candidate genes that has come up in our analysis is LOX (Lysyl Oxidase), which seems to be differentially expressed between early and term placenta. LOX seems to be interacting with several lysyl oxidases like proteins (LOXL1, L2, L3, L4), collagens (COL31A), tubulin (TUB), and Apoptotic protease activating factor 1 (APAF1).

In our analysis, we also explored the differentially expressed proteomes from trophoblast cell lines to see their extent of overlaps. The logic of using the cell lines (JEG3, BeWo, SGHPL5, HTR SVneo) is based on the observation that several investigators use these cell lines as a proxy for primary trophoblasts. We found a significant number of overlaps with CoV2, indicating that these cells can be used as a model to study viral infection. Another prominent overlap that was found amongst various early placental tissue and its surrogates and SARS-CoV2 interactome was the transcriptional co-repressor TLE3, the functioning of which is vital in the formation of placental vasculature. TLE3 was also found to form networks with components of COPI vesicles, which were found to be vital in the entry of the SARS-CoV-2 to ERGIC, and viral shedding. COPI complexes help to form the vesicle coat that plays a role in traffic contents between Golgi stacks and cargoes between the Golgi apparatus and the endoplasmic reticulum.(68) This also implies that inhibition of COP1 complex using specific pharmacological agents can prevent the virus infection cycle, at least as observed with the influenza virus.(68)

Since the SARS-CoV-2 interactome reported by Gordon, et al.(20–22) does not include cell surface proteins, we also manually sifted through the list of genes upregulated in various processes and states associated with placenta to check for the entry receptors – ACE2 and TMPRSS2 – of SARS-CoV2.(69) TMPRSS2 is up-regulated in term placenta (GSE28551) while ACE2 is upregulated in extravillous trophoblasts (GSE9773), further making the interaction between SARS-CoV2 and placental tissue probable.

Not only did we analyzed the trophoblast gene expression under normal physiological state and the interaction with the SARS-CoV-2, but we also explored them under pathological states like preclampsia and GDM, two of the most common associated pathological states in pregnancy. We found a few interesting overlapping members, both in PE and GDM. PLAT and MFGE8 in PE is of particular interest as it seems to be associated with serine proteases and their regulators, many of which were reported to be associated with matrix remodeling, invasion, and vasculogenesis. Simialrly, analysis of SARS-CoV-2 and Zika virus interactome showed numerous overlaps, with a number of proteins belonging to the Mitochondrial Ribosomal Protein family, which is important in syncytium formation.

One limitation of the current analysis is that the human proteins interacting with SARS-CoV-2 obtained after cloning the viral proteins in HEK293T cells (which are human embryonic kidney cells) may not be exactly similar to the interactome of SARS-CoV-2 in placental cells. However, to the best of our knowledge, there are no reports available on the latter, and at this stage, the former study is unique in that manner. Further, we investigated the abundance of the HEK interacting proteome in placenta and found that a majority of these proteins are also represented in the placental trophoblast cells indicating that we could potentially see these similar interactions conserved there.

We attempted KEGG and GO pathway analysis with DAVID 6.8 (70) for functional annotation of the proteins interacting with SARS-CoV2, that are overlapping with those involved in key roles in placental biology. However, the analysis did not suggest any significant enrichment of specific biological processes or pathways. This could be due to the already restricted number of proteins of the SARS-CoV-2 interactome, which is further narrowed down by a screen for their role in placental function.

There are no clinical reports on the outcome of COVID-19 during the first trimester of pregnancy when the trophoblast invasion and differentiation are very critical. Further, the effect of COVID-19 on pregnancies associated with co-morbidities like preeclampsia and gestational diabetes mellitus is not known. The lack of this information should be factored in before making any rigid conclusions from our study, which is solely based on *in silico* analysis.

Conclusion:
Our findings suggest that SARS-CoV-2 might have a profound influence on the placentation process through their effect on the trophoblast cells. Given the fact that several of the viral proteins physically interact with host proteins that are critical components of trophoblast invasion, migration, proliferation, and differentiation processes, we speculate that SARS-CoV-2 infection can have detrimental consequences on the pregnancy outcome. We, however, want to add a note of caution that our conclusion is entirely based upon *in silico* data analysis

and therefore lack experimental validation. Given the robustness of the process and the individual variability, the final results may differ. It will be interesting to analyze more clinical results to substantiate our prediction. We performed a text mining-based Pubmed search to identify the clinical consequences of COVID-19 on pregnancy. Baergen and Heller (71) observed that amongst 50% of COVID-19 positive pregnant females, there was evidence of fetal vascular malperfusion or fetal vascular thrombosis, a possibility well predicted in our current study given the fact that SARS-CoV-2 interaction with EVT can alter trophoblast mediated alteration of maternal vasculature, leading to poor perfusion and hypoxia.(72) Stefanovic (73) reported that the rate of Cesarean deliveries among COVID-19 women is unacceptably high and that while significant attention is devoted to the mother, the fetus also needs to be treated as a patient and deserves even more attention. Though clinical studies are limited, following our findings, it becomes, even more, a priority to take into account the possible consequences of COVID-19 on pregnancy outcomes and prepare health care professionals to adopt effective management to avoid an adverse health crisis affecting both mother and the child.

Conflicts of Interest: NONE

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Supplementary Information:

Supplementary Table 1: Human Proteins Interacting with SARS-CoV-2

AP3B1	DDX21	TLE3	TIMM10B	MRPS25	CLCC1	SMOC1
BRD4	RBM28	TLE5	TIMM9	DDX10	VPS11	PLEKHF2
BRD2	RPL36	GOLGA3	HDAC2	LARP7	SUN2	FBXL12
CWC27	GOLGA7	GOLGA2	GPX1	MEPCE	ALG5	UGGT2
ZC3H18	ZDHHC5	GOLGB1	TRMT1	NGDN	STOML2	CHPF2
SLC44A2	POLA1	GRIPAP1	ATP5MG	EXOSC8	NUP98	ADAMTS1
PMPCB	PRIM1	CEP350	ATP6AP1	NARS2	RAE1	HYOU1
YIF1A	PRIM2	PDE4DIP	SIGMAR1	NOL10	MTCH1	FKBP7
ATP1B1	POLA2	CEP135	ATP13A3	CCDC86	HEATR3	ADAM9
ACADM	COLGALT1	CEP68	AGPS	SEPSECS	MDN1	FKBP10
ETFA	PKP2	CNTRL	CYB5B	EXOSC5	PLOD2	SLC9A3R1
STOM	AP2A2	ERC1	ACSL3	EXOSC3	TOR1A	CHMP2A
GGCX	GFER	GCC2	CYB5R3	AATF	STC2	CSDE1
ATP6V1A	ERGIC1	CLIP4	RALA	HECTD1	PLAT	TOMM70
PSMD8	AP2M1	NIN	COMT	MRPS2	ITGB1	MARK3
REEP5	GRPEL1	CEP112	RAB5C	MRPS5	CISD3	MARK2
PMPCA	TBCA	MIPOL1	RAB7A	EXOSC2	COL6A1	DPH5
ANO6	SBNO1	USP13	RAB8A	MRPS27	PVR	DCTPP1
PITRM1	BCKDK	GCC1	RAB2A	GTF2F2	DNMT1	MARK1
SLC30A9	AKAP8	JAKMIP1	RAB10	FBN1	LOX	PTBP2
FASTKD5	MYCBP2	CDK5RAP2	RAB14	FBN2	PCSK6	BAG5
SLC30A7	SLU7	AKAP9	RHOA	NUP214	INHBE	UBXN8
TUBGCP3	RIPK1	GORASP1	RAB1A	NUP62	NPC2	GPAA1
COQ8B	UBAP2L	FYCO1	GNB1	DCAF7	MFGE8	WFS1
SAAL1	TYSND1	C1orf50	GNG5	EIF4H	OS9	ABCC1
REEP6	PDZD11	CEP250	LMAN2	NUP54	NPTX1	F2RL1
INTS4	PRRC2B	TBK1	MOGS	MIB1	POGLUT2	SCAP
SLC25A21	UBAP2	HOOK1	TOR1AIP1	SPART	POGLUT3	DPY19L1
TUBGCP2	ZNF318	NINL	MTARC1	NEK9	ERO1B	TMEM97
TARS2	CRTC3	GLA	QSOX2	ZNF503	PLD3	SLC30A6
RTN4	USP54	IMPDH2	HS2ST1	NUP88	FOXRED2	TAPT1
FAM8A1	ZC3H7A	SIRT5	NDUFAF2	NUP58	CHPF	ERMP1
AASS	LARP4B	NUTF2	SCCPDH	MAT2B	PUSL1	NLRX1
AKAP8L	RBM41	ARF6	SCARB1	FBLN5	EMC1	RETREG3
AAR2	TCF12	RNF41	NAT14	PPT1	GGH	PIGO
BZW2	PPIL3	SLC27A2	DCAKD	CUL2	ERLEC1	FAR2
RRP9	PLEKHA5	EIF4E2	FAM162A	MAP7D1	IL17RA	ECSIT
PABPC1	TBKBP1	POR	DNAJC19	THTPA	NGLY1	ALG8
CSNK2A2	CIT	RAP1GDS1	SELENOS	ZYG11B	HS6ST2	TMEM39B
CSNK2B	HSBP1	WASHC4	PTGES2	TIMM8B	SDF2	GHITM
G3BP1	PCNT	FKBP15	RAB18	RBX1	NEU1	ACAD9
PABPC4	CEP43	GIGYF2	MPHOSPH10	ELOC	GDF15	NDFIP2
LARP1	PRKAR2A	IDE	SRP72	ELOB	TM2D3	BCS1L
FAM98A	PRKACA	TIMM10	ATE1	HMOX1	ERP44	NDUFAF1

SNIP1	PRKAR2B	ALG11	NSD2	TRIM59	EDEM3	TMED5
UPF1	RDX	NUP210	SRP19	ARL6IP6	SIL1	NDUFB9
MOV10	CENPF	TIMM29	SRP54	VPS39	POFUT1	PIGS
G3BP2	TLE1	DNAJC11				

Supplementary Table 2: Expansion of Abbreviated Protein Names Listed in Various Figures and the SARS-CoV-2 Proteins Interacting with These Proteins

Protein Symbol	Protein name	SARS-CoV-2 Protein
AAR2	AAR2 splicing factor homolog(AAR2)	SARS-CoV2 M
AATF	Apoptosis antagonizing transcription factor(AATF)	SARS-CoV2 nsp8
ABCC1	ATP binding cassette subfamily C member 1(ABCC1)	SARS-CoV2 orf9c
ACAD9	Acyl-coa dehydrogenase family member 9(ACAD9)	SARS-CoV2 orf9c
ACSL3	Acyl-coa synthetase long-chain family member 3(ACSL3)	SARS-CoV2 nsp7
ADAM9	ADAM metallopeptidase domain 9(ADAM9)	SARS-CoV2 orf8
AGPS	Alkylglycerone phosphate synthase(AGPS)	SARS-CoV2 nsp7
AKAP8	A-kinase anchoring protein 8(AKAP8)	SARS-CoV2 nsp12
AKAP8L	A-kinase anchoring protein 8 like(AKAP8L)	SARS-CoV2 M
AKAP9	A-kinase anchoring protein 9(AKAP9)	SARS-CoV2 nsp13
ALG5	ALG5, dolichyl-phosphate beta-glucosyltransferase(ALG5)	SARS-CoV2 orf3a
ALG8	ALG8, alpha-1,3-glucosyltransferase(ALG8)	SARS-CoV2 orf9c
ANO6	Anoctamin 6(ANO6)	SARS-CoV2 M
AP2M1	Adaptor related protein complex 2 mu 1 subunit(AP2M1)	SARS-CoV2 nsp10
ARL6IP6	ADP ribosylation factor like gtpase 6 interacting protein 6(ARL6IP6)	SARS-CoV2 orf3a
ATP13A3	Atpase 13A3(ATP13A3)	SARS-CoV2 nsp6
ATP1B1	Atpase Na+/K+ transporting subunit beta 1(ATP1B1)	SARS-CoV2 M
ATP6AP1	Atpase H+ transporting accessory protein 1(ATP6AP1)	SARS-CoV2 nsp6
ATP6V1A	Atpase H+ transporting V1 subunit A(ATP6V1A)	SARS-CoV2 M
BCKDK	Branched chain ketoacid dehydrogenase kinase(BCKDK)	SARS-CoV2 nsp12
BCS1L	BCS1 homolog, ubiquinol-cytochrome c reductase complex chaperone(BCS1L)	SARS-CoV2 orf9c
BRD2	Bromodomain containing 2(BRD2)	SARS-CoV2 E
BZW2	Basic leucine zipper and W2 domains 2(BZW2)	SARS-CoV2 M
CCDC86	Coiled-coil domain containing 86(CCDC86)	SARS-CoV2 nsp8
CENPF	Centromere protein F(CENPF)	SARS-CoV2 nsp13
CEP135	Centrosomal protein 135(CEP135)	SARS-CoV2 nsp13
CEP250	Centrosomal protein 250(CEP250)	SARS-CoV2 nsp13
CHMP2A	Charged multivesicular body protein 2A(CHMP2A)	SARS-CoV2 orf9b
CHPF	Chondroitin polymerizing factor(CHPF)	SARS-CoV2 orf8
CHPF2	Chondroitin polymerizing factor 2(CHPF2)	SARS-CoV2 orf8
CLCC1	Chloride channel CLIC like 1(CLCC1)	SARS-CoV2 orf3a
CLIP4	CAP-Gly domain containing linker protein family member 4(CLIP4)	SARS-CoV2 nsp13
COL6A1	Collagen type VI alpha 1 chain(COL6A1)	SARS-CoV2 orf8

COMT	Catechol-O-methyltransferase(COMT)	SARS-CoV2 nsp7
CRTC3	CREB regulated transcription coactivator 3(CRTC3)	SARS-CoV2 nsp12
CSDE1	Cold shock domain containing E1(CSDE1)	SARS-CoV2 orf9b
CSNK2A2	Casein kinase 2 alpha 2(CSNK2A2)	SARS-CoV2 N
CYB5B	Cytochrome b5 type B(CYB5B)	SARS-CoV2 nsp7
CYB5R3	Cytochrome b5 reductase 3(CYB5R3)	SARS-CoV2 nsp7
DCAF7	DDB1 and CUL4 associated factor 7(DCAF7)	SARS-CoV2 nsp9
DCAKD	Dephospho-coa kinase domain containing(DCAKD)	SARS-CoV2 nsp7
DCTPP1	Dctp pyrophosphatase 1(DCTPP1)	SARS-CoV2 orf9b
DDX10	DEAD-box helicase 10(DDX10)	SARS-CoV2 nsp8
DDX21	Dexd-box helicase 21(DDX21)	SARS-CoV2 N
DNAJC11	Dnaj heat shock protein family (Hsp40) member C11(DNAJC11)	SARS-CoV2 nsp4
DNMT1	DNA methyltransferase 1(DNMT1)	SARS-CoV2 orf8
DPH5	Diphthamide biosynthesis 5(DPH5)	SARS-CoV2 orf9b
DPY19L1	Dpy-19 like 1(DPY19L1)	SARS-CoV2 orf9c
ECSIT	ECSIT signalling integrator(ECSIT)	SARS-CoV2 orf9c
EMC1	ER membrane protein complex subunit 1(EMC1)	SARS-CoV2 orf8
ERC1	ELKS/RAB6-interacting/CAST family member 1(ERC1)	SARS-CoV2 nsp13
ERMP1	Endoplasmic reticulum metallopeptidase 1(ERMP1)	SARS-CoV2 orf9c
ERO1B	Endoplasmic reticulum oxidoreductase 1 beta(ERO1B)	SARS-CoV2 orf8
ERP44	Endoplasmic reticulum protein 44(ERP44)	SARS-CoV2 orf8
EXOSC2	Exosome component 2(EXOSC2)	SARS-CoV2 nsp8
EXOSC3	Exosome component 3(EXOSC3)	SARS-CoV2 nsp8
EXOSC5	Exosome component 5(EXOSC5)	SARS-CoV2 nsp8
F2RL1	F2R like trypsin receptor 1(F2RL1)	SARS-CoV2 orf9c
FAM8A1	Family with sequence similarity 8 member A1(FAM8A1)	SARS-CoV2 M
FAR2	Fatty acyl-coa reductase 2(FAR2)	SARS-CoV2 orf9c
FASTKD5	FAST kinase domains 5(FASTKD5)	SARS-CoV2 M
FBLN5	Fibulin 5(FBLN5)	SARS-CoV2 nsp9
FBN1	Fibrillin 1(FBN1)	SARS-CoV2 nsp9
FBN2	Fibrillin 2(FBN2)	SARS-CoV2 nsp9
FKBP10	FK506 binding protein 10(FKBP10)	SARS-CoV2 orf8
FOXRED2	FAD dependent oxidoreductase domain containing 2(FOXRED2)	SARS-CoV2 orf8
FYCO1	FYVE and coiled-coil domain containing 1(FYCO1)	SARS-CoV2 nsp13
G3BP1	G3BP stress granule assembly factor 1(G3BP1)	SARS-CoV2 N
G3BP2	G3BP stress granule assembly factor 2(G3BP2)	SARS-CoV2 N
GCC2	GRIP and coiled-coil domain containing 2(GCC2)	SARS-CoV2 nsp13
GDF15	Growth differentiation factor 15(GDF15)	SARS-CoV2 orf8
GGH	Gamma-glutamyl hydrolase(GGH)	SARS-CoV2 orf8
GHITM	Growth hormone inducible transmembrane protein(GHITM)	SARS-CoV2 orf9c
GLA	Galactosidase alpha(GLA)	SARS-CoV2 nsp14
GNB1	G protein subunit beta 1(GNB1)	SARS-CoV2 nsp7
GOLGA2	Golgin A2(GOLGA2)	SARS-CoV2 nsp13

GOLGA7	Golgin A7(GOLGA7)	SARS-CoV2 Spike
GPAA1	Glycosylphosphatidylinositol anchor attachment 1(GPAA1)	SARS-CoV2 orf9c
GRIPAP1	GRIP1 associated protein 1(GRIPAP1)	SARS-CoV2 nsp13
HDAC2	Histone deacetylase 2(HDAC2)	SARS-CoV2 nsp5
HMOX1	Heme oxygenase 1(HMOX1)	SARS-CoV2 orf3a
HOOK1	Hook microtubule tethering protein 1(HOOK1)	SARS-CoV2 nsp13
HS2ST1	Heparan sulfate 2-O-sulfotransferase 1(HS2ST1)	SARS-CoV2 nsp7
HS6ST2	Heparan sulfate 6-O-sulfotransferase 2(HS6ST2)	SARS-CoV2 orf8
HSBP1	Heat shock factor binding protein 1(HSBP1)	SARS-CoV2 nsp13
IDE	Insulin degrading enzyme(IDE)	SARS-CoV2 nsp4
IMPDH2	Inosine monophosphate dehydrogenase 2(IMPDH2)	SARS-CoV2 nsp14
INTS4	Integrator complex subunit 4(INTS4)	SARS-CoV2 M
ITGB1	Integrin subunit beta 1(ITGB1)	SARS-CoV2 orf8
LARP1	La ribonucleoprotein domain family member 1(LARP1)	SARS-CoV2 N
LARP4B	La ribonucleoprotein domain family member 4B(LARP4B)	SARS-CoV2 nsp12
LMAN2	Lectin, mannose binding 2(LMAN2)	SARS-CoV2 nsp7
LOX	Lysyl oxidase(LOX)	SARS-CoV2 orf8
MAP7D1	MAP7 domain containing 1(MAP7D1)	SARS-CoV2 orf10
MARK1	Microtubule affinity regulating kinase 1(MARK1)	SARS-CoV2 orf9b
MARK2	Microtubule affinity regulating kinase 2(MARK2)	SARS-CoV2 orf9b
MARK3	Microtubule affinity regulating kinase 3(MARK3)	SARS-CoV2 orf9b
MAT2B	Methionine adenosyltransferase 2B(MAT2B)	SARS-CoV2 nsp9
MDN1	Midasin AAA atpase 1(MDN1)	SARS-CoV2 orf7a
MFGE8	Milk fat globule-EGF factor 8 protein(MFGE8)	SARS-CoV2 orf8
MOGS	Mannosyl-oligosaccharide glucosidase(MOGS)	SARS-CoV2 nsp7
MOV10	Mov10 RISC complex RNA helicase(MOV10)	SARS-CoV2 N
MRPS2	Mitochondrial ribosomal protein S2(MRPS2)	SARS-CoV2 nsp8
MRPS25	Mitochondrial ribosomal protein S25(MRPS25)	SARS-CoV2 nsp8
MRPS27	Mitochondrial ribosomal protein S27(MRPS27)	SARS-CoV2 nsp8
MRPS5	Mitochondrial ribosomal protein S5(MRPS5)	SARS-CoV2 nsp8
MTCH1	Mitochondrial carrier 1(MTCH1)	SARS-CoV2 orf6
NARS2	Asparaginyl-trna synthetase 2, mitochondrial (putative)(NARS2)	SARS-CoV2 nsp8
NDUFAF1	NADH:ubiquinone oxidoreductase complex assembly factor 1(NDUFAF1)	SARS-CoV2 orf9c
NDUFAF2	NADH:ubiquinone oxidoreductase complex assembly factor 2(NDUFAF2)	SARS-CoV2 nsp7
NGDN	Neuroguidin(NGDN)	SARS-CoV2 nsp8
NIN	Ninein(NIN)	SARS-CoV2 nsp13
NLRX1	NLR family member X1(NLRX1)	SARS-CoV2 orf9c
NOL10	Nucleolar protein 10(NOL10)	SARS-CoV2 nsp8
NPC2	NPC intracellular cholesterol transporter 2(NPC2)	SARS-CoV2 nsp4
NUP210	Nucleoporin 210(NUP210)	SARS-CoV2 nsp9
NUP54	Nucleoporin 54(NUP54)	SARS-CoV2 nsp9
NUP58	Nucleoporin 58(NUP58)	SARS-CoV2 nsp9

NUP62	Nucleoporin 62(NUP62)	SARS-CoV2 nsp9
NUP88	Nucleoporin 88(NUP88)	SARS-CoV2 nsp15
NUP98	Nucleoporin 98(NUP98)	SARS-CoV2 orf8
NUTF2	Nuclear transport factor 2(NUTF2)	SARS-CoV2 N
OS9	OS9, endoplasmic reticulum lectin(OS9)	SARS-CoV2 N
PABPC1	Poly(A) binding protein cytoplasmic 1(PABPC1)	SARS-CoV2 nsp13
PCNT	Pericentrin(PCNT)	SARS-CoV2 orf8
PCSK6	Proprotein convertase subtilisin/kexin type 6(PCSK6)	SARS-CoV2 nsp13
PDE4DIP	Phosphodiesterase 4D interacting protein(PDE4DIP)	SARS-CoV2 orf9c
PIGO	Phosphatidylinositol glycan anchor biosynthesis class O(PIGO)	SARS-CoV2 orf9c
PIGS	Phosphatidylinositol glycan anchor biosynthesis class S(PIGS)	SARS-CoV2 M
PITRM1	Pitrilysin metallopeptidase 1(PITRM1)	SARS-CoV2 nsp1
PKP2	Plakophilin 2(PKP2)	SARS-CoV2 orf8
PLAT	Plasminogen activator, tissue type(PLAT)	SARS-CoV2 orf8
PLD3	Phospholipase D family member 3(PLD3)	SARS-CoV2 nsp12
PLEKHA5	Pleckstrin homology domain containing A5(PLEKHA5)	SARS-CoV2 orf8
PLOD2	Procollagen-lysine,2-oxoglutarate 5-dioxygenase 2(PLOD2)	SARS-CoV2 M
PMPCA	Peptidase, mitochondrial processing alpha subunit(PMPCA)	SARS-CoV2 M
PMPCB	Peptidase, mitochondrial processing beta subunit(PMPCB)	SARS-CoV2 orf8
POFUT1	Protein O-fucosyltransferase 1(POFUT1)	SARS-CoV2 nsp1
POLA1	DNA polymerase alpha 1, catalytic subunit(POLA1)	SARS-CoV2 nsp1
POLA2	DNA polymerase alpha 2, accessory subunit(POLA2)	SARS-CoV2 nsp2
POR	Cytochrome p450 oxidoreductase(POR)	SARS-CoV2 nsp1
PRIM1	Primase (DNA) subunit 1(PRIM1)	SARS-CoV2 nsp1
PRIM2	Primase (DNA) subunit 2(PRIM2)	SARS-CoV2 nsp13
PRKAR2B	Protein kinase camp-dependent type II regulatory subunit beta(PRKAR2B)	SARS-CoV2 nsp7
PTGES2	Prostaglandin E synthase 2(PTGES2)	SARS-CoV2 orf8
PUSL1	Pseudouridylate synthase-like 1(PUSL1)	SARS-CoV2 orf8
PVR	Poliovirus receptor(PVR)	SARS-CoV2 nsp7
RAB10	RAB10, member RAS oncogene family(RAB10)	SARS-CoV2 nsp7
RAB18	RAB18, member RAS oncogene family(RAB18)	SARS-CoV2 nsp7
RAB1A	RAB1A, member RAS oncogene family(RAB1A)	SARS-CoV2 nsp7
RAB2A	RAB2A, member RAS oncogene family(RAB2A)	SARS-CoV2 nsp7
RAB5C	RAB5C, member RAS oncogene family(RAB5C)	SARS-CoV2 nsp7
RAB7A	RAB7A, member RAS oncogene family(RAB7A)	SARS-CoV2 nsp7
RAB8A	RAB8A, member RAS oncogene family(RAB8A)	SARS-CoV2 orf6
RAE1	Ribonucleic acid export 1(RAE1)	SARS-CoV2 nsp2
RAP1GDS1	Rap1 gtpase-GDP dissociation stimulator 1(RAP1GDS1)	SARS-CoV2 N
RBM28	RNA binding motif protein 28(RBM28)	SARS-CoV2 orf10
RBX1	Ring-box 1(RBX1)	SARS-CoV2 nsp13

RDX	Radixin(RDX)	SARS-CoV2 M
REEP5	Receptor accessory protein 5(REEP5)	SARS-CoV2 N
RPL36	Ribosomal protein L36(RPL36)	SARS-CoV2 N
RRP9	Ribosomal RNA processing 9, small subunit (SSU) processome component, homolog (yeast)(RRP9)	SARS-CoV2 M
RTN4	Reticulon 4(RTN4)	SARS-CoV2 M
SAAL1	Serum amyloid A like 1(SAAL1)	SARS-CoV2 nsp12
SBNO1	Strawberry notch homolog 1(SBNO1)	SARS-CoV2 nsp7
SCARB1	Scavenger receptor class B member 1(SCARB1)	SARS-CoV2 nsp7
SCCPDH	Saccharopine dehydrogenase (putative)(SCCPDH)	SARS-CoV2 nsp7
SELENOS	Selenoprotein S(SELENOS)	SARS-CoV2 nsp6
SIGMAR1	Sigma non-opioid intracellular receptor 1(SIGMAR1)	SARS-CoV2 orf8
SIL1	SIL1 nucleotide exchange factor(SIL1)	SARS-CoV2 M
SLC25A21	Solute carrier family 25 member 21(SLC25A21)	SARS-CoV2 nsp2
SLC27A2	Solute carrier family 27 member 2(SLC27A2)	SARS-CoV2 M
SLC30A7	Solute carrier family 30 member 7(SLC30A7)	SARS-CoV2 M
SLC30A9	Solute carrier family 30 member 9(SLC30A9)	SARS-CoV2 E
SLC44A2	Solute carrier family 44 member 2(SLC44A2)	SARS-CoV2 orf9b
SLC9A3R1	SLC9A3 regulator 1(SLC9A3R1)	SARS-CoV2 nsp12
SLU7	SLU7 homolog, splicing factor(SLU7)	SARS-CoV2 nsp8
SRP19	Signal recognition particle 19(SRP19)	SARS-CoV2 orf8
STC2	Stanniocalcin 2(STC2)	SARS-CoV2 M
STOM	Stomatin(STOM)	SARS-CoV2 orf3b
STOML2	Stomatin like 2(STOML2)	SARS-CoV2 orf3a
SUN2	Sad1 and UNC84 domain containing 2(SUN2)	SARS-CoV2 orf9c
TAPT1	Transmembrane anterior posterior transformation 1(TAPT1)	SARS-CoV2 M
TARS2	Threonyl-trna synthetase 2, mitochondrial (putative)(TARS2)	SARS-CoV2 nsp13
TLE1	Transducin like enhancer of split 1(TLE1)	SARS-CoV2 nsp13
TLE3	Transducin like enhancer of split 3(TLE3)	SARS-CoV2 orf9c
TMEM39B	Transmembrane protein 39B(TMEM39B)	SARS-CoV2 orf9c
TMEM97	Transmembrane protein 97(TMEM97)	SARS-CoV2 orf9b
TOMM70	Translocase of outer mitochondrial membrane 70(TOMM70)	SARS-CoV2 nsp7
TOR1AIP1	Torsin 1A interacting protein 1(TOR1AIP1)	SARS-CoV2 nsp5_C145A
TRMT1	Trna methyltransferase 1(TRMT1)	SARS-CoV2 M
TUBGCP3	Tubulin gamma complex associated protein 3(TUBGCP3)	SARS-CoV2 nsp12
UBAP2	Ubiquitin associated protein 2(UBAP2)	SARS-CoV2 nsp12
UBAP2L	Ubiquitin associated protein 2 like(UBAP2L)	SARS-CoV2 orf9c
UBXN8	UBX domain protein 8(UBXN8)	SARS-CoV2 orf8
UGGT2	UDP-glucose glycoprotein glucosyltransferase 2(UGGT2)	SARS-CoV2 nsp13
USP13	Ubiquitin specific peptidase 13 (isopeptidase T-3)(USP13)	SARS-CoV2 orf9c
WFS1	Wolframin ER transmembrane glycoprotein(WFS1)	SARS-CoV2 M

YIF1A	Yip1 interacting factor homolog A, membrane trafficking protein(YIF1A)	SARS-CoV2 Spike
ZDHHC5	Zinc finger DHHC-type containing 5(ZDHHC5)	SARS-CoV2 nsp12
ZNF318	Zinc finger protein 318(ZNF318)	SARS-CoV2 M

Supplementary Table 3: Proteins Upregulated in Cytotrophoblasts That Interact with SARS-CoV-2

FASTKD5	G3BP1	CYB5B	NUP88	POLA2	HDAC2
STOML2	TMEM97	IMPDH2	ARL6IP6	AATF	MTCH1
NUP54	EXOSC3	GHITM	RRP9	MAT2B	NUTF2
UBAP2L	EXOSC2	MRPS5	NARS2	PMPCA	DNAJC11
SAAL1	DCTPP1	NUP58	PTGES2	TUBGCP3	MRPS27
MRPS2	RAB10	TOR1AIP1	GNB1	PMPCB	ACAD9
CCDC86	AGPS	SIGMAR1	TARS2	INTS4	NUP62
EMC1	NOL10	CSDE1	DDX10	BZW2	ECSIT
SBNO1	DCAKD	IDE	BRD2	SLC9A3R1	SLC30A9
BCKDK	BCS1L	NUP98	SLC44A2	POR	GPAA1
CLCC1	PKP2	DNMT1	RBM28	PIGO	CENPF
TRMT1	UBAP2	TOMM70	MOV10	RAB5C	PRIM1
PCNT	ALG8	MOGS	ATP6V1A	ZDHHC5	MARK2
MARK3	POFUT1	RAB8A	SUN2	TLE1	ANO6
EXOSC5	AKAP8	DPH5	RAE1	AKAP8L	
PIGS	PUSL1	MDN1	PITRM1	ERMP1	