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Stress decreases host viral resistance and increases Covid susceptibility in embryonic stem cells.

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Abstract

Stress-induced changes in viral receptor and susceptibility gene expression were measured in embryonic stem cells (ESC) and differentiated progeny. Rex1 promoter-Red Fluorescence Protein reporter ESC were tested by RNAseq after 72hr exposures to control hyperosmotic sorbitol under stemness culture (NS) to quantify stress-forced differentiation (SFD) transcriptomic programs. Control ESC cultured with stemness factor removal produced normal differentiation (ND). Bulk RNAseq transcriptomic analysis showed significant upregulation of two genes involved in Covid-19 cell uptake, Vimentin (VIM) and Transmembrane Serine Protease 2 (TMPRSS2). SFD increased the hepatitis A virus receptor (Havcr1) and the transplacental Herpes simplex 1 (HSV1) virus receptor (Pvrl1) compared with ESC undergoing ND. Several other coronavirus receptors, Glutamyl Aminopeptidase (ENPEP) and Dipeptidyl Peptidase 4 (DPP4) were upregulated significantly in SFD>ND. Although stressed ESC are more susceptible to infection due to increased expression of viral receptors and decreased resistance, the necessary Covid-19 receptor, angiotensin converting enzyme (ACE)2, was not expressed in our experiments. TMPRSS2, ENPEP, and DPP4 mediate Coronavirus uptake, but are also markers of extra-embryonic endoderm (XEN), which arise from ESC undergoing ND or SFD. Mouse and human ESCs differentiated to XEN increase TMPRSS2 and other Covid-19 uptake-mediating gene expression, but only some lines express ACE2. Covid-19 susceptibility appears to be genotype-specific and not ubiquitous. Of the 30 gene ontology (GO) groups for viral susceptibility, 15 underwent significant stress-forced changes. Of these, 4 GO groups mediated negative viral regulation and most genes in these increase in ND and decrease with SFD, thus suggesting that stressed increases ESC viral susceptibility.

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79 **Introduction**

80
81 The Covid19/2 pandemic has caused trillions of dollars of economic disruption. World-wide, Covid19
82 infects hundreds of millions and has killed over 450,000 Americans in its first year ([https://covid.cdc.gov/covid-
83 data-tracker/#cases_casesper100klast7days](https://covid.cdc.gov/covid-data-tracker/#cases_casesper100klast7days)). Key elements of a successful Covid19 medical responses are
84 testing, tracking, reducing symptoms, vaccination, and risk analysis of viral effects dependent on susceptibility.
85 An important element of risk analysis is whether environmental toxicants, urban hormonal stresses and nutrition
86 affect susceptibility. Stresses have been linked in a dose-dependent manner to susceptibility through infection
87 under circumstances where host responses and symptoms play a lesser role. ¹

88 Covid19 is particularly alarming as the newest 3 coronaviruses since 2003 are the most severe:
89 SARS1/Covid1, MERS and the current Covid19/Covid2/SARS2. Coronaviruses have unique dangerous patho-
90 genic features, a large genome of 30mb, 3 open reading frames producing 29 proteins, genomic proof reading
91 capability that maintains pathogenic genomes during replication, and yet the ability to swap genetic sequences
92 and change susceptibility and interactome efficiency² during coinfection of host cells³. CoV2 has evolved
93 changes that make it more clinically dangerous than previous coronaviruses. For example, it can infect upper
94 respiratory throat and bronchi and create flu like symptoms or infect lungs and create pneumonia and death. Of
95 the 7 total and 3 severe human coronaviruses (Cov1, Cov2, MERS), only Cov2 infects both the upper respiratory
96 tract and lungs.

97 It is not surprising that host variation in susceptibility is a lesser focus, but it is still important to
98 understand whether environmental stimuli change host susceptibility including virus uptake and interactome var-
99 iation. This is especially dangerous in utero where early first trimester, placental syncytiotrophoblasts coexpress
100 TMPRSS2 and ACE2^{4,5}, which are both required for cellular uptake and potential transplacental transfer of virus.
101 Interestingly, it has been reported that human syncytiotrophoblasts (outer placental cells facing maternal circu-
102 lation) and viruses do not co-express TMPRSS2 and ACE2⁶ suggesting that early embryos/fetuses and their
103 stem cells are more exposed to Covid19 than stem cells in later fetuses. In addition, the other decidual tissue,
104 extraembryonic endoderm, is also thought to co-express TMRPSS2 and ACE2, where co-expression is neces-
105 sary for Covid19 virion uptake^{4,7}. Thus, the two membranes between the embryo/fetus and maternal virus, may
106 both take up the virus and expose embryonic stem cells and/or derivative differentiated lineages. If stress in-
107 creased Covid19 susceptibility genes and decreased viral resistance genes, lytic cell death in the early embryo

108 could be loss before detection of placental hormones and would not be recorded as a miscarriage. Occult losses
109 or the stress-induced epigenetic memory of survival of early ESC lineage cells could lead to lineage imbalance
110 or epigenetic error affecting the life of the placenta, yolk sac and neonate.

111 ***From the host cell perspective***, a small number of genes is needed for cellular binding and uptake of the Cov2
112 virion. Throughout the host cells are protein interactomes, which can be targeting to enable drug repurposing,
113 but also yield markers identifying susceptible cells^{2,8-10}. Key markers for susceptibility are a small set of genes
114 binding receptors and fusogenic proteinases that clip the receptor and enable membrane fusion; cell surface and
115 receptors - ACE2 human β Cov1/2), Anpep and Anpep (β Cov2 co-receptor candidate)¹¹, DPP4 (MERS β Cov),
116 and fusogenic serine proteases; TMPRSS2 (β Cov1/2), Cathepsins b/l (Ctsb, Ctsl), Furin (β Cov1/2), Vim¹².

117 There is a general knowledge of viral susceptibility genes, but also specific reports that ESC have antiviral
118 responses and several groups of genes are necessary for these responses; histone chaperones, sumoylation
119 factors, and chromatin modifiers which prevent proviral transcriptional activity^{13,14}. It is important to test whether
120 known ESC viral resistance genes change expression during normal or stressed stemness or early differentia-
121 tion. Gene ontology groups are a means to test host viral interactions, especially negative regulation or resistance
122 by host, in larger interacting gene sets¹⁵.

123 We report here that nearly all these markers increase with normal ESC differentiation and increase further still
124 with stress, and that viral resistance (i.e., negative regulatory) gene GO Groups increase with normal differenti-
125 ation but decrease with stress. Taken together these transcriptomic changes, if accompanied by proteomic
126 changes, would lead to greater viral infection in earliest cells in the ESC lineage, probably XEN lineage cells,
127 which are essential for embryonic survival by the start of gastrulation, two days after the embryo implants into
128 the uterus¹⁶.

132 **Materials and Methods**

134 **Materials**

135 Germline competent mESC-D3 cells were purchased from ATCC (Manassas, VA). DMEM medium was obtained
136 from HyClone (Logan, UT). Gibco™ glutamax and sodium pyruvate supplement solutions were from Life Tech-
137 nologies (Grand Island, NY). ESC-qualified EmbryoMax fetal bovine serum, 0.1% gelatin solution and ESGRO™
138 Mouse LIF medium supplement were from EMD Millipore (Billerica, MA). Rex1 promoter reporter that enables
139 expression of red fluorescent protein mApple were from Allele Biotechnology (San Diego, CA) as described
140 previously^{17,18}. MEM non-essential amino acid solution, sorbitol, 2-mercaptoethanol and other chemicals were
141 from Sigma (St. Louis, MO). QIAzol Lysis reagent for mRNA isolation was from QiaGen (Ann Arbor, MI)

142 **Embryonic stem cell culture and RNA preparation**

143 Rex1-RFP embryonic stem cells were cultured as described previously^{17,18}. Rex1-RFP ESCs were opti-
144 mized at passage for exponential growth during the stimulus period, which began 18hr after passage at 24%
145 confluence. Germline competent mESC-D3 cells (ATCC, Manassas, VA) from Doetschman et al¹⁹ were cultured
146 in the absence of feeder cells in DMEM (Gibco, Grand Island, NY) supplemented with 15% mESC-screened
147 fetal bovine serum (HyClone, Logan, UT), 2mM L-glutamine, 1mM sodium pyruvate, 1 mM nonessential amino
148 acids, 0.1 mM 2-mercaptoethanol (Sigma, St. Louis, MO), and 1000 U/mL murine leukemia inhibitory factor (LIF;
149 Millipore, Temecula, CA) on 0.1% gelatin-coated dishes at 37°C in humidified air with 5% CO₂²⁰. mESCs were
150 cultured overnight after passaging before stimulation with sorbitol. Osmolality of ESC media with and without
151 added 200-300mM sorbitol was determined previously²¹. ESC were washed at Tfinal and lysed using Trizol.

152 **RNA isolation, cDNA library prep, and RNAseq**

153 RNA expression analysis was done by the Wayne State University Applied Genomics Technology Center with
154 the following method. An aliquot of the RNA was assessed by microfluidics using the ScreenTape for the Agilent
155 2200 TapeStation. The electrophoretogram, RNA Integrity Number (RIN), and the ratio of the 28S:18S RNA
156 bands were optimized for overall quality of the RNA as done previously. RNA-seq was used to determine ex-
157 pression profiles. Lexogen's QuantSeq 3'mRNA-seq Library Prep Kit (FWD for Illumina) will be utilized for build-
158 ing RNA-seq libraries from 0.1-200 ng of total RNA in 5 µl of nuclease-free ultrapure water. Libraries will be
159 quantified on the Qubit and Agilent 2200 TapeStation using the DNA High Sensitivity Screen tape. The barcoded

160 libraries were multiplexed at equimolar concentrations and sequenced on an Illumina NovaSeq 6000. Data were
161 demultiplexed using Illumina's CASAVA 1.8.2 software. After quality was assessed ²², and reads were aligned
162 to the mouse genome (Build mm9) ²³ and tabulated for each gene region ²⁴. Differential gene expression analysis
163 was used to compare transcriptome changes between conditions ²⁵. Significantly altered genes (log fold change
164 ≥ 2 ; FDR ≤ 0.05) were used to identify affected pathways ²⁶.

165 **Statistics**

166 Data from at least three independent biological experiments were analyzed using Microsoft Excel and presented
167 as means \pm SEM. Statistical analysis was done by student *t*-test. Geometric means analysis was used in the
168 fluorescent flow cytometry study; the brightness of the fluorescent labeled cells ranges from low-to-high over a
169 logarithmic range of fluorescence axis. This feature of fluorescent flow cytometry fits this criterion of geometric
170 mean quantification, where higher reliability and repeatability were achieved than arithmetic mean quantifica-
171 tion²⁷.

176 Results

177 The experimental protocol used a 72hr stimulus of ESC to normal stemness (NS) with stemness and
178 proliferation-maintain Leukemia inhibitory factor (LIF), normal differentiation by LIF removal (ND), and three lev-
179 els of hyperosmotic stress as a positive control of stress-forced differentiation (SFD) used in previous studies
180 (**Figure 1**^{18,21,28}). The 72hr stimulus was previously established for use with Rex1-RFP^{17,18} and Pdgfra-GFP²⁸
181 transgenic ESCs as it enables some differentiation and a 2-3fold change in stemness and 1st differentiated line-
182 age, respectively. Dose of 200, 250 and 300mM sorbitol creates reduced growth, near zero growth, and negative
183 growth, respectively. But, at highest dose initial death occurs only in the first 12hr and by 72hr there is approxi-
184 mately 5% death (data not shown,²¹), enabling assay of robust levels of stress responses where highest stimulus-
185 response is induced. Comparisons are first used to test for significant and high fold change (FC) differences
186 between ND from NS and then how SFD differs from ND. The final comparison is between SFD and NS to test
187 how stress induces changes from the starting state of NS or naïve pluripotency equivalent to the source of ESC
188 in the inner cells mass of the preimplantation embryo within 4.5 days of fertilization²⁹⁻³².

189 In **Figure 2A**, the majority of XEN 1st differentiated lineage genes from two previous reports^{33,34} increased
190 in cultured ESC here with ND (green box) with LIF removal but also increased more during exposure to 300mM
191 sorbitol mediated SFD (red box) despite LIF. All other lineages reverse directions between increase in ND/NS
192 and decrease in SFD/ND. Originating 0th lineage/Naïve pluripotency decreased with ND/2nd lineage (to primarily
193 formative pluripotency data not shown³⁵) and increased with SFD. Also, primed pluripotency/ 3rd lineage in-
194 creased with ND and decreased with SFD. In **Figure 2B**, most genes in 4 different viral GO group processes
195 increase during ND compared with NS and decrease during SFD compared with ND. In **Figure 2C**, the majority
196 of Covid19 or Covid1/MERS receptor genes increase with SFD compared with ND and ND more than NS in a
197 manner like 1st lineage XEN increase in **Figure 2A**. Most single viral susceptibility genes increase with ND and
198 SFD whether this is significant. Since TMPRSS2 is specific to endoderm and increases with ND and SFD are
199 XEN, this suggests that stress may increase viral susceptibility by transdifferentiating ESC to XEN despite LIF
200 presence. **Figure 2D** shows the three sequential stages of embryogenesis, before and after implantation, and
201 the lineage number assignments in temporal sequence as naïve pluripotency restricts to formative and then
202 primed pluripotency at the start of gastrulation.

In **Figure 3** basal counts for coronavirus susceptibility genes in ESC culture under NS/naïve conditions tend to be at low levels, as for most transplacental viruses, suggesting low susceptibility in stem cells under naïve pluripotent culture, but a possible expression in subpopulations of cells in heterogeneous culture. Only one gene, Vim is expressed above 10counts in the Coronavirus receptor/susceptibility gene group, whereas 3 genes, Pvr1, Pvr and Hn1 are expressed above 10counts in transplacental viruses. After LIF removal for 72hr as ESCs begin the transit from Naïve pluripotency (NS) to Formative pluripotency (ND) and other subpopulations arise, most viral susceptibility genes upregulate (10/15) but no gene expression FC are significant. However, comparing ND to SFD highest dose at 300mM sorbitol stress 8/15 genes have significant FC by Pvalue and/or FDR 10/15 genes upregulate. All 3 of the host coronavirus susceptibility genes that are significant in SFD FC are upregulated (Tmprss2, VIM, DPP4) and 2/5 of the other genes, mostly transplacental virus susceptibility genes are upregulated (Havcr1, Pvr1).

We analyzed total counts of mRNA for 20 viral receptors [³⁶ Chapter on Biology of Viruses and Viral Diseases [James D. Chappell](#) and [Terence S. Dermody](#)]³⁶⁻³⁹ in ESCs cultured under 1) NS conditions and then FC changes from NS to 2) ND or 3) 300mM sorbitol highest SFD to elucidate viral susceptibility under these three conditions. **Supplemental table 1.** Eight of twenty viruses had > 10 mRNA counts/cell during NS culture. In rank order these were the GNB2L1 > Rpsa > Ldlr > Pvr12 > Cul5 > Icam1 > vim > Pvr1 genes which are receptors for these viruses, respectively; influenza A > Sindbis > Rhino minor sero group > herpes 2 > encephalomyocarditis > Rhino major sero group > Covid2 > Herpes 1. The highest upregulated genes/viral receptors with ND were CR2/Epstein Barr and Anpep/several mammalian corona viruses. The highest downregulated genes/viral receptors with ND were CD4/HIV and Tnfrsf23/Pseudorabies. But the FC for all up- and down-regulated genes were not significant (pValue or FDR). The highest upregulated genes/viral receptors for 300mM sorbitol SFD were Havcr1/hepatitis A, Tnfrsf23/Pseudorabies, Pvr1/Herpes 1, and Vim/Covid2. Havcr1, Pvr1, and Vim were all significant for pValue and FDR. The highest downregulated genes/viral receptors for 300mM sorbitol SFD were CR2/Epstein-Barr and Cd4/HIV, but none of these were significant.

Pdgfra-GFP ESC intermediate and bright subpopulations mark 1st differentiated lineage XEN cells, assayed by flow cytometry increased with increasing sorbitol above 300 to 400mM sorbitol, despite LIF presence (**Figure 4A**). Only minor increases in intermediate bright Pdgfra+ cells at 200-250mM sorbitol. Interestingly, 300mM sorbitol despite LIF produced almost equal increases in Pdgfra+ intermediate and bright cells as retinoic

231 acid at 1uM with LIF removal an established inducer of XEN cells^{28,40} . Since retinoic acid at this level is a normal
232 morphogen for XEN cells⁴⁰, this suggests that 300mM overrides LIF and forces differentiation of a subpopulation
233 of ESC.

234 Stress increases Covid19 susceptibility genes that are markers of 1st lineage XEN cells and their deriva-
235 tive extraembryonic endoderm lineages (**Figure 2, 3**) and induces 1st lineage as shown here and previously^{21,28,41}
236 . We thus surveyed mouse and human ESC and derivative XEN lineage differentiation at NCBI GEO profiles for
237 expression of covid19 susceptibility genes TMPRSS2 and ACE2 and the XEN determining factor at the time of
238 lineage allocation in the blastocyst, Gata6, which is essential in XEN lineage allocation in the blastocyst^{42,43} . All
239 three genes were expressed at low or nil levels in undifferentiated embryonic stem cells but increase in some or
240 all lineages of mouse or human XEN cells (**Figure 4A, B**). There was apparent heterogeneity in XEN differenti-
241 ation and covid19 expression in different genetic backgrounds or due to other elements of quality of cell lines.

242 Of 15 significant changing host viral GO groups, 14 reverse most of their genes' expression from ND/ND
243 to SFD 300mM/ND with 13 increasing with ND and decreasing with SFD and 1 decreasing with ND and increas-
244 ing with SFD (**Figure 5A**). One GO group increased with ND and SFD. Rank of significance was higher during
245 SFD>ND for all 15 GO groups. Significance was rare for ND/NS and only 4/15 groups were significant changed
246 but for SFD/ND all 15 groups changed significantly.

247 There are approximately 300 host viral interaction GO groups amongst approximately 45,000 groups (
248 <https://www.ebi.ac.uk/QuickGO/searchterms/viral>, [https://en.wikipedia.org/wiki/Gene_ontol-
249 ogy#Terms and ontology](https://en.wikipedia.org/wiki/Gene_ontology#Terms_and_ontology)). In ESC, there were 92 genes in the 15 significant host viral response GO groups
250 which are listed at the top of **Supplemental Table 2**. At the bottom of the table, are two auxiliary tables that
251 show how the 92 genes are shared amongst all 15 groups and amongst the 4 negative regulatory groups. In the
252 four negative regulatory groups the most common expressed genes in 3/4 groups are - jun, tetherin (aka Bst)2,
253 and Interferon-induced transmembrane protein 1 (Ifitm1), and 4/4 groups are - 2'-5'-oligoadenylate synthetase
254 1 (Oas1b), Tripartite motif-containing 28 (Trim28), and Interferon-stimulated gene 15 (Isg15). Most of these
255 have antiviral activities and some have known function in pluripotent cells. For all 15 groups the most commonly
256 expressed gene are; in 11/15 groups; oas1b and Isg15, in 9/15 groups; TRIM28, and in 7/15 groups; Krüppel-
257 associated box zinc finger protein (Zfp)809, Ifitm1, Nucleoporin 93 (Nup93), and nucleotide-binding oligomeriza-
258 tion domain, leucine rich repeat containing X1 (NlrX1).

259 At the other end of the expression spectrum are 22 genes expressed in only one GO group. Of these 22, 9 are
260 of great interest because there is high counts and high and significant fold change in 2-3 of 3 possible compari-
261 sons; ND/ND or SFD/ND or SFD/NS. These 9 are Tmprss2, Proenkephalin (Penk), Basic leucine zipper tran-
262 scription factor, ATF-like (Batf3), Myeloid differentiation primary response 88 (Myd88), Mitogen-activated protein
263 kinase 11 (MAPK11, aka p38MAPK β), heat shock protein beta-1 (Hspb1, aka HSP27), THO complex subunit 6
264 homolog (Thoc6), alpha-actin-2 (Acta2), and Autophagy Related 16 Like 2 (Atg16l2).

265 The highest dose at 300mM is a “demonstration dose” with highest fold change and significance, but so
266 harsh in decreasing cell number during 72hr exposure, that this would be too morbid a dose to consider as
267 affecting normal development. We thus assayed the lowest dose at 200mM sorbitol for which of the significant
268 doses at 300mM were also affected significantly and found that only 5/15 host viral response GO groups were
269 affected. Of the GO groups significantly changed at 200mM 4/5 are in the top 5 most significant. Interestingly, of
270 the 15 GO groups for host viral genes that had insignificance and lack of large change in ranking between
271 unstress and stress groups at 300mM sorbitol (**Figure 5B**), the only directional regulatory were for 3 positive GO
272 groups.

273 To confirm even expression of ESC cultured with 5 stimuli, 2 unstressed NS and ND (naïve and formative
274 pluripotency, respectively), and 3 stressed (200mM, 250mM, and 300mM sorbitol) we tested for even expression
275 with non-significant FC between stimuli and with low FC below 1.4. This was done by assaying the top twenty
276 expressed genes at 300mM determined here and by using previous reported loading control⁴⁴⁻⁴⁶ (**Figure 6A, B**).
277 For the top 20 highest expressed genes here, 13 are expressed at high stoichiometry in ribosomes, related to
278 RNA loading controls in past studies of stress forced expression changes in mouse ESC^{17,18,21,28}, but one of
279 these had high and significant FC between NS and SFD (**Figure 6A**). Of the 7 other highest expressed non-
280 ribosomal genes, only Tuba1b was also disqualified for high FC and significance. For more traditional loading
281 controls, only Hprt was disqualified as a loading control for FC>1.4 and significance, but several other genes
282 had FC>1.4 without significance (Gapdh, PGK1, B2M, Ppia, Hmbs) (**Figure 6B**). Altogether, the data from ribo-
283 somal, other highest expressed, and traditional loading controls suggest that total mRNA counts, and genes
284 expressed per cell are well supported.

Discussion

We report here that studies of single host viral susceptibility genes and several GO groups of host viral response and interaction genes change with stress that increases viral Covid19 susceptibility specifically as well increasing susceptibility to as all viruses in general.

The culture model for ESC exposure used here replicated previous 72hr exposures used to test for stress effects on Rex1-RFP 0th lineage NS or naïve pluripotency loss¹⁸ and Pdgfra-GFP 1st differentiated lineage XEN gain²⁸ and the sorbitol doses of 200, 250 and 300mM yield reduce but positive growth, near nil growth, and negative growth through initial apoptosis and chronic adaptation, respectively.

Most coronavirus susceptibility genes increase when NS differentiates to ND without stress by LIF removal and increase more when SFD at 300mM expression is compared with ND. In addition to increase to susceptibility due to single gene increases, the majority of all 4 negative regulatory GO groups increase with NS to ND, putatively suppressing viral processes. But most genes reverse and decrease expression with SFD compared with ND, suggesting that viral susceptibility due to stress-forced loss of resistance would exacerbate the coronavirus effects after entrance into the host cell. Taken together, stressed ESC express increased Covid receptors and decreased negatively regulatory GO groups genes, which may be pathogenically synergistic. But does this occur in the stressed ESC themselves or in stress-forced differentiation to 1st lineage.

Several lines of evidence suggest that XEN cells differentiated from ESCs may be the primary candidate cell for Covid19 infection. Several Covid19 genes are known to be expressed by XEN cells: TMPRSS2⁴⁷ and DPP4 are expressed mostly by XEN visceral lineage^{48,49}, ENPEP is expressed by mostly XEN⁵⁰, and VIM is expressed mostly by XEN parietal endoderm⁵¹. Thus, the induction of coronavirus is associated with the induction of XEN lineages. These covid susceptibility genes are expressed mostly at exceptionally low levels in the NS/Naïve pluripotency starting state but increase with ND and more with SFD compared to NS. This suggests that low levels of these genes are expressed in naïve ESCs during NS culture but an induction of a small subpopulation of 1st lineage endoderm.

Flow cytometry was performed to test for stimulation induced increase from NS naïve pluripotency to ND formative pluripotency and to test for 1st subpopulation size increase in XEN. There was a large increase to an intermediate bright Pdgfra-GFP subpopulation of ~20% and smaller bright subpopulation of ~1% with 300mM sorbitol despite LIF and this was equivalent to the normal 1st lineage XEN inducer retinoic acid with LIF removal

316 ^{40,49}. But a key deficit of induction of covid19 susceptibility by stress was the lack of ACE2 in mouse ESC tested
317 here in NS ND or SFD 300mM sorbitol. Both mouse and human ESC lines produce ACE2 and TMPRSS2 genes
318 necessarily co-expressed for covid19 uptake after induction of XEN by cultural manipulation of mouse ESC⁵² or
319 transgenic overexpression of genes in human ESC, sox7 or sox17⁵³. But not all mouse or human ESC lines
320 expressed high levels of ACE2 suggesting genetic heterogeneity or some other deficit in inducing a full suscep-
321 tibility interactome.

322 Stress increases significance of 15 viral host response GO groups and an increase in rank of significance
323 from ND to SFD. Highest increases in rank are over 10fold from 64% to 5.9%, but the most interesting are two
324 that increase significantly from NS to ND and decrease significantly from SFD compared with ND. The least
325 change is 47.2% to 32.8% (viral genome replication). but this is also a reversal from majority gene expression
326 increase to majority decrease. Only one significant GO group does not undergo a reversal and it increases from
327 98.3 to 32.8% ranking from ND to SFD (regulation of defense of host to virus). Two GO groups are not listed in
328 any pairwise comparison and are unranked but are highly significant, with 20.0% and 23.6%, for SFD 300mM
329 sorbitol compared to ND.

330 Six of the most shared genes are expressed in 3 or 4 of the four negative regulatory GO groups mediating
331 viral resistance and 5/6 of these genes have been reported to mediate viral resistance. Individual genes ex-
332 pressed in 3 of 4 of the negative regulatory host viral response GO groups are of great interest. Jun, Bst2 and
333 Ifitm1 are expressed in three of the four groups. Jun is a stress transcription factor implicated in the stress
334 response of ESC, TSC and early embryos. ⁵⁴ Bst2, also known as tetherin is a protein associated with plasma-
335 lemma lipid rafts and has been reported to inhibit retrovirus by preventing viral particles' diffusion into infected
336 cells. ⁵⁵ Ifitm1/3 is Interferon-induced transmembrane protein 1. IFITM proteins are antiviral restriction factors for
337 influenza A virus replication, probably at the early step of life cycle such as cellular entry and membrane fusion.

338 ⁵⁶

339 Individual genes expressed in 4 of 4 of the negative regulatory host viral response GO groups are also
340 of great interest. Oas1b or 2'-5'-oligoadenylate synthetase 1 is an enzyme that is important in the innate immune
341 response to viral infection ⁵⁷. Isg15 or Interferon-stimulated gene 15 is a secreted cytokine and has intracellular
342 function like ubiquitin, ISG15 is covalently linked by a C-terminal motif on newly-synthesized proteins, a process

343 called ISGylation mediating both activation and inhibition of antiviral immunity ⁵⁸ although viral resistance is me-
344 diated in mice but not humans ⁵⁹. Trim28 is in the tripartite motif (TRIM) protein family, which is a group of E3
345 ligases implicated in the regulation of a variety of cellular functions including innate immunity to virus which may
346 occur by viral protein degradation in proteasomes and TRIM28 may block transition from latent to lytic cycle ⁶⁰.
347 TRIM28 also has a role in maintaining pluripotency through epigenetic regulation ⁶¹ and is necessary to maintain
348 epigenetic stability and imprinting during zygotic genome activation in mouse 2-cell stage embryos ⁶². Although
349 the phenotype is not the same as the ones studied here, the power of TRIM28 is reported in its role as the
350 hierarchical epigenetic regulator of the complex polygenic, polyphenic cause of obesity, which TRIM28, depend-
351 ing on its wild type or haplo-insufficient activity, flips into an obese or non-obese state in isogenic animals that is
352 dependent on the expression levels of a handful of imprinted genes ⁶³. TRIM28 is important in preventing provirus
353 and endogenous retrovirus replication in ESC to protect germline lineage cells from insertional mutagenesis ¹³.
354 Interestingly, although most viral resistance genes decrease with stress, TRIM28 increases significantly with
355 stress by both Pvalue and FDR (**Supplemental Figure 2**). Covid-19 is not a retrovirus as it does not rely on
356 reverse transcriptase and a DNA intermediate for replication. On the other hand some Cov2 sequence has been
357 detected in host cells, suggesting that the RNA genome is reverse transcribed into DNA at low frequency and
358 small parts integrate into the genome ⁶⁴. Taken together viral resistance is decreased by stress, and for some
359 genes the amount of viral resistance function with stress is less than basal NS state as well as the ND state. It
360 will be important to establish whether these genes affect viral resistance to coronaviruses as reported for their
361 viral resistance function for other viruses and whether they are expressed and mediate function in XEN lineage
362 cells. The specific mechanisms of viral resistance in ESC and the subpopulations of cells that are resistant or
363 susceptible due to these mechanisms, need to be tested. It will be important to test whether Covid-19 can infect
364 ESC and/or XEN cells and whether insertional mutagenesis can affect the germ line of ESC genomes.

365 Four genes common to 3-4/4 negatively regulatory GO groups are also highly shared amongst all 15 host
366 viral GO groups, but Bst2 and jun are not as highly shared. Genes highly shared amongst all 15 groups are: in
367 11/15 groups; oas1b and lsg15, in 9/15 groups; TRIM28, and in 7/15 groups; Krüppel-associated box zinc finger
368 protein (Zfp809), Ifitm1, Nucleoporin 93 (Nup93), and nucleotide-binding oligomerization domain, leucine rich
369 repeat containing X1 (NlrX1). New genes common to all 15 but not most shared in 4 negative regulatory are
370 ZFP809, NlrX1, and Nup93. ZFP809 can mediate epigenetic anti-retroviral effects through histone modification

371 more than DNA methylation after sequence recognition of foreign DNA⁶⁵. Endogenous retroviruses are silenced
372 primarily by DNA methylation in somatic tissue but by H3 methylation (H3K9me3) in embryos and ESC⁶⁶⁻⁶⁸.
373 Nlr1 found exclusively in the mitochondrial membrane, increases viral susceptibility by sequestering outer mi-
374 tochondrial membrane proteins (MAVS) from another antiviral protein and thereby prevents mitochondrial anti-
375 viral immunity by preventing TBK1 to IRF3 to IFN β transcription⁶⁹. Nup93 is a nucleoporin that mediates mito-
376 chondrial activated TBK1 import to activate IRF3 and IFNB as part of innate immunity to retroviruses that Nlr1
377 blocks⁷⁰.

378 High stress at 300mM leads to high caspase 3 associated apoptosis in the first 12hr of stimulus but
379 healthy cells with less than or about 5% death at 72hr, whereas cell growth at 250mM is static and at 200mM
380 significantly more cells accumulated at 72hr with a growth rate of about 75% at NS at 0% sorbitol²¹. At 200mM
381 sorbitol only a third (5/15) of the GO groups significantly changed compared with all (15/15) groups at 300mM
382 sorbitol. As expected, four fifths (4/5) of the significantly changed GO groups are in the top 5 highest significant
383 changed groups at 300mM sorbitol and are changed in the same direction at 200mM. Thus, the quality and
384 direction of the response at 200mM is similar that at 300mM sorbitol but is less significant and of lower ranking.
385 Loading controls of several categories suggested that the conclusions for counts per cell for different genes,
386 number of genes expressed, and GO groups genes were solid interpretations. It is likely that in vivo highest
387 stress may come from exposures to multiple stressors that synergize to create stress like 300mM sorbitol or
388 higher.

389 The greatest loss of human life occurs soon after fertilization, as the early embryo begins exponential
390 growth and implants into the uterus to access maternal nutrition^{54,71,72}. Just before implantation, embryonic stem
391 cells and placental trophoblast stem cells arise in the embryo and have the capability of forming all 200+ cell
392 types in adult humans and 10 lineages of the placenta, respectively. Viruses can harm the placenta or cross the
393 placenta and infect the embryo or fetus: either of these events can have long term health risks for the fetus and
394 offspring⁷³. The first key problem here is that stress induces several coronavirus receptors including TMPRSS2,
395 Dpp4, Anpep and Enpep, and stress induces ACE2 in some XEN lineages. The second problem is that host
396 resistance genes that are expressed in normal stemness and increase with normal unstressed differentiation,
397 decrease with stress; Oas1b, Isg15, Jun, Ifitm1, Bst2, Tspan7, Fam111a, and Eif2ak2. Taken together these
398 data suggest that stress may lead to viral entry into first lineage XEN cells. This could lead to embryo death due

399 to lack of sufficient XEN function ¹⁶. But, although Covid-19 does not require reverse transcriptase and a repli-
400 cative DNA intermediate that might insert into the host genome at high rates, there have been reports of Covid-
401 19 gene sequences in host genomic DNA ⁶⁴. These could be passed along to offspring and become transgen-
402 erational as 1st lineage XEN cells have been shown to revert into totipotent 2-cell embryo like cells⁷⁴ characteristic
403 of the early post-fertilization embryo.

404 Key data suggest that maternal stress hormones sensitize the early post-implantation embryos and its
405 ESC and TSC lineages to bisphenol A, leading to higher rates of miscarriage than either stressor alone⁷⁵. Both
406 types of stem cells in the implanting embryos respond to maternal stress hormones (adrenaline, cortisol) by
407 growing more slowly ⁵⁴ and other stressors such as benzopyrene of bisphenyl A slow growth and exacerbate
408 stress hormone caused embryo loss in vivo ⁷⁶. Viral infectivity of 5 viruses was shown to be proportional to the
409 amount of psychological stress and independent of other immune factors such as white blood cell counts ¹,
410 suggesting a focus stress-induced changes on viral receptors as a means to susceptibility. The data presented
411 here support the hypothesis that stress can increase viral susceptibility by increasing receptor expression, but
412 also suggest that stress can decrease host viral resistance, compounding Cov2 pathogenic potential in the early
413 post-fertilization embryo around the time of embryo implantation into the uterus. Direct testing whether ESC and
414 their early differentiated progeny can be infected is needed, and whether this leads to lineage imbalance or
415 genetic or epigenetic changes. It is also important to test whether environmental stressors, not just control hy-
416 perosmotic stress can change susceptibility of the ESC lineage.

417 418 419 420 **Acknowledgements**

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The authors have no conflicts of interest.

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Consent to participate (include appropriate statements)

No human subjects were used in the studies reported here and thus consent to participate is not applicable.

Consent for publication (include appropriate statements)

All authors have consented to submission of this manuscript

Availability of data and material (data transparency)

All data are available for communication or inspection

Code availability (software application or custom code)

No codes were created for these studies, all software and analysis were from software in the public domain or freely accessible.

Authors' contributions

Design of the work; data acquisition, data analysis, data interpretation

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Figure 1. Paradigm: 72hr exposure of embryonic stem cells (**ESC**) – grown in culture as stem cells – will identify stress levels that override growth and stemness and create teratogenic and epigenetic changes that persist and affect health. **Terminology:** we compare three states of ESC - normal stemness (**NS**) normal differentiation (**ND**) and the override; stress-forced differentiation (**SFD**). SFD is given at three doses that create normal growth to negative growth at this highest dose of positive control hyperosmotic. +control stress is hyperosmotic sorbitol at three doses.

Figure 2 Functional developmental gene groups (**A**) and GO group gene expression (**B**) are affected during normal differentiation without stress and stress-forced override of stemness at 300mM sorbitol, and single Viral susceptibility gene expression (**C**) as described in Figure 1. At the left are gene or gene group names. On the right, are fold changes in green (increase) or red (decrease) between ND from NS and on the far right SFD at 300mM Sorbitol compared to ND. (**A**) On the right total genes/group are black and decreasing and increasing genes are in red and green, respectively. The 8 developmental marker genesets arising in Embryos from E3.5-6.5 in vivo and in ESC in vitro from previous cited studies (in parentheses); **1**)-⁷⁷ 3 core "0" or initiating naïve pluripotency (NS) genes downstream of LIF receptor: Stat3, Klf4, Tbx3 with 5 other naïve genes Tcf3, Nanog, Essrb, Pou5F1, and an additional 2 Gjb5, Scep1 ⁷⁸ **2**) ten 2nd lineage formative pluripotency genes: Dusp6, Trip6, Lin28b ⁷⁸, Otx2, Zic2⁷⁹ CD47, Sox11, Egr1, Dnmt3b^{29,30,80,81}, 3) Epha4a 3-three 3rd lineage "primed" pluripotency Sema6a, Car14, Jakmip2^{30,77} 4) 21 1st lineage early XEN genes; Amn, Cd63, Ctsl, Fbp2, Gpx3, Lama1, Gsn, Lpar3, Lrpap1, Man2c1, P4ha2, PGK1, Col4a1/2, Podxl, S100a10, Serpinh1, Slc2a3, Sparc, Srgn, Tfp1 ³⁴ 5) 20 1st lineage late XEN genes; Amn, Cd63, Ctsl, Clu, Ctsh, Lama1, EPAS1, Fst, Gata4/6, Lamb1, Lrpap1, P4ha2, Ph4b, Col4a1/2, Pga5, Serpinh1, Sparc, Upp1 ³⁴) 6) 15 1st lineage XEN genes AFP, Gata4, Sox17, Lamb1, Lama1, Aqp8, Vim, Plac1, Rhox6/9, Emp2, TROMA/krt8, Col4a1/2, Amn³³), for lineages 0/1/2/3, and 7) Eighteen Aerobic glycolysis and Warburg genes; Slc2a1/Glut1, HK1, Pfkfb3, PGI-GPI, PFK1, Aldoa/c, Tpi1, Gapdh, PGK1, PGAM1, ENO1, PKM2, LDHA/B, PDH1, PDK1, SLc16a3-MCT4³⁵ Starting "0" ESC lineage (E3.5-

692 4.5) is Naïve pluripotent stem cells or NS from **figure 1**, 1st lineage from NS pluripotency is extraembryonic
693 endoderm/XEN and later lineages are restrictions of Naïve stemness: 2nd lineage formative pluripotency (E5.5)
694 and 3rd lineage primed pluripotency. **(B)** The majority of genes in 4 viral negative regulatory groups increase from
695 NS to ND, blocking viral pathogenesis, but gene expression in most genes in these groups reverses with SFD
696 300mM sorbitol compared with ND. **(C)** 6 coronavirus and 3 known transplacental susceptibility genes were
697 studied, and upregulation or downregulation are indicated by fold change and significance by Pvalue and FDR.
698 **(D)** A diagram of pre-implantation blastocyst at E4.5 (4.5 days after fertilization) and post-implantation embryo
699 at E5.5 and near the start of gastrulation at E6.25. In the terminology of this figure, the 0th lineage is ICM/ESC at
700 E4.5 which is naïve pluripotency/NS, 1st lineage is XEN arising before implantation, 2nd lineage is ND/formative
701 pluripotency and 3rd lineage is primed pluripotency just before the start of gastrulation.

702
703 **Figure 3** Stress changes expression for 6 host ESC genes for Coronavirus susceptibility, 8 genes for known
704 transplacentally-transmitted virus susceptibility and 1 additional virus of highly significant changed susceptibility
705 for virus uptake. These are single genes analyzed from bulk RNAseq for counts X \pm SD for ESC cultured under
706 NS for 72hr, and then FC (green increase and red decrease) for ND/NS and for SFD highest dose at 300mM
707 sorbitol/ND and then SFD highest dose/NS. Blue indicates significant FC between one culture treatment to an-
708 other. On the far- right Green highlights indicate Coronavirus and yellow indicates trans-placentally-transmitted
709 virus.

710
711 **Figure 4.** First differentiated lineage XEN cells increase with 300mM sorbitol stress as much as positive control
712 XEN morphogen retinoic acid, and first lineage mouse and human ESC differentiated to first lineage by culture
713 conditions of Sox17 transgenic overexpression, cause induction of ACE2 and Tmprss2 Covid19 susceptibility
714 genes. **(A)** Pdgfra-GFP ESC were exposed to 72hr LIF+ (NS), LIF- (ND), 1uM retinoic acid a normal morphogen
715 for 1st lineage XEN induction from ESC or 200-400mM sorbitol in the presence of LIF to test for a stress dose
716 that causes similar % of bright and intermediate bright 1st lineage Pdgfra+ cells as assayed by flow cytometry²⁸
717 , **(B)** Three mouse ESC lines were culture under conditions to maintain stemness (far right) or XEN differentiation
718 (3 histogram bars on left) and tested for ACE2, Tmprss2 Covid19 susceptibility genes or Gata6 XEN determining
719 transcription factor, from NCBI GEO database; GSM40110/22/23/24⁵². **(C)** Two human ESC lines were culture

720 under conditions to maintain stemness (2 histogram bars on far right) or two human ESC lines transdifferentiated
721 to XEN differentiation using first lineage driver Sox17 (2 histogram bars on left) and tested for ACE2, Tmprss2
722 Covid19 susceptibility genes or Gata6 XEN determining transcription factor, from NCBI GEO database;
723 GSM2722918/19/20/21⁵³
724

725 **Figure 5** Fifteen Viral GO groups were significant for stress induced fold changes in host ESC host-viral genome
726 interactome genome rank of significance when comparing ND culture to NS culture but mostly SFD culture com-
727 pared with NS or ND culture and 15 other viral host interactome GO Groups were not significant. **(A)** 15 host
728 viral GO groups for virus interaction had increasing significance at 300mM SFD compared with ND from top to
729 bottom. Bottom two rows show total GO groups and significant GO groups for a comparison of culture ESC
730 treatments.
731

732 **Figure 6.** Loading controls from highest copy number (A) and from previously published reviews (B). For ESD
733 we typically use GAPDH, ACTB, and 18S loading controls which report high copy number RNA from ribosomes.
734 Red shows decreasing expression from NS, green shows increasing expression from NS, pink shows ribosome-
735 associated genes with names indicated in footnotes at the bottom, blue shows significance, orange shows order
736 of ranking by highest expression in ESC at 300mM sorbitol and dark green is expression of the same genes as
737 NS or 0mM sorbitol culture.
738

739 **Supplemental figure 1.** More comprehensive list of stress effects on viral receptor gene expression includes
740 Viruses listed by WHO as highest priority ([https://www.who.int/activities/prioritizing-diseases-for-research-and-](https://www.who.int/activities/prioritizing-diseases-for-research-and-development-in-emergency-contexts)
741 [development-in-emergency-contexts](https://www.who.int/activities/prioritizing-diseases-for-research-and-development-in-emergency-contexts)), List of Wiki virus list (<https://en.wikipedia.org/wiki/Virus>).
742

743 **Supplemental figure 2.** (A) Comprehensive analysis of all genes expressed in all 15 significant host viral
744 interaction GO groups. Includes X \pm -SD for Counts during NS ESC culture, ND/NS FC ratio of mean counts,
745 SFD/ND FC ratio of mean counts, and SFD/NS FC ratio of mean counts.

746 (B) Ninety-one genes expressed in the 15 groups were analyzed here for how
747 many GO groups each gene is listed in.

748
749
750
751
752
753

(C) Twenty-one genes expressed uniquely in one GO group.

(D) Ten genes important in provirus silencing and suppression of endogenous retrovirus transposition ¹³.

Supplemental figure 3. Low ranking host viral GO groups which were not significant when comparing 300mM sorbitol SFD to ND. (associated with Figure 6A)

Figures

Embryonic stem cell – Rex1-RFP, Pdgfra-GFP

High throughput screen (HTS)

Passage → T_{zero} → T_{final}

3 SFD doses

bulk RNAseq
Flow cytometry

0	200	250	300	Sorbitol (mM)
Exponential	Good	Static	Negative	Proliferation

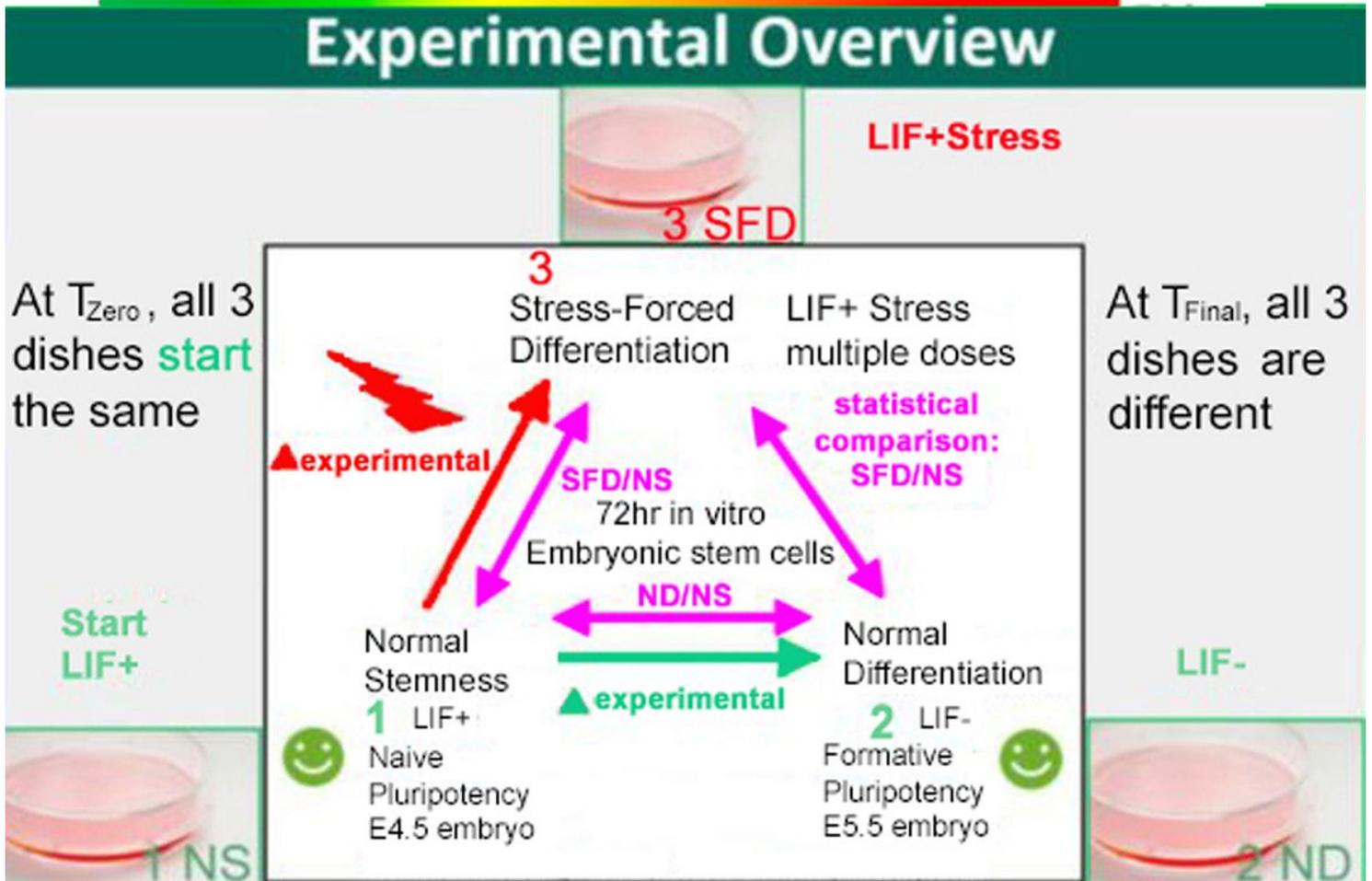


Figure 1

Please see the manuscript file to view the figure caption.

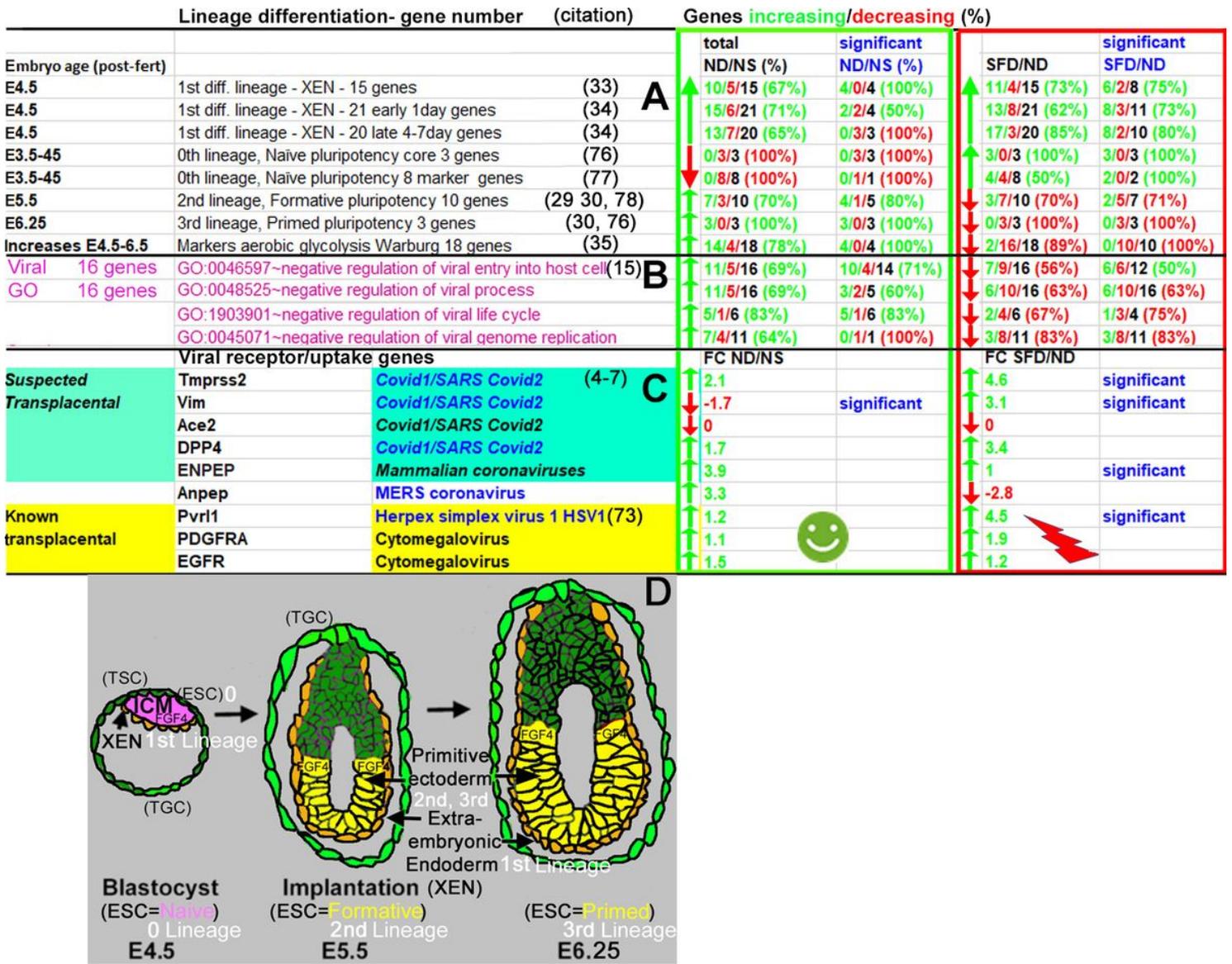


Figure 2

Please see the manuscript file to view the figure caption.

Naïve pluripotency		Formative pluripotency		Increasing		Decreasing		Significant		Stress-forced differentiation		Stress-forced differentiation		Coronavirus	
Normal stemness		Normal differentiation		Viral		Viral		Viral		Viral		Viral		Fetal Exposure	
Viral NS		Viral ND vs NS		Viral ND vs NS		Viral ND vs NS		Viral ND vs NS		Viral ND vs NS		Viral ND vs NS		Known transplacental viruses	
Receptor Counts		Receptor		Receptor		Receptor		Receptor		Receptor		Receptor		VIRUS receptor	
Gene	X	SD	Gene	Fold Change	PValue	FDR	Gene	Fold Change	PValue	FDR	Gene	Fold Change	PValue	FDR	
Tmprss2	1	1	Tmprss2	2.087582525	0.27992	1	Tmprss2	4.65914894	0.00172	0.03671	Tmprss2	9.726357906	4.92E-05	3.65E-03	Covid1/SARS Covid2
Vim	41	18.2	Vim	-1.69560349	0.06753	1	Vim	3.109789272	6.74E-05	3.29E-03	Vim	1.834030944	0.02559	0.23976	Covid1/SARS Covid2
Ace2	0	0	Ace2	-1	1	1	Ace2	-1	1	1	Ace2	-1	1	1	Covid1/SARS Covid2
DPP4	2	1	Dpp4	1.682067259	0.41625	1	Dpp4	3.419998887	0.02527	0.22634	Dpp4	5.752668152	0.00301	0.06203	Covid1/SARS Covid2
Anpep	1	0.5	Anpep	3.263007828	0.22242	1	Anpep	-2.8464626	0.28006	0.73567	Anpep	1.146337854	0.90224	1	MERS
ENPEP	0	0	Enpep	3.861329928	0.25606	1	Enpep	1.020853863	0.98636	1	Enpep	3.941853573	0.25093	0.73924	Mammalian coronaviruses
Havcr1	0	0	Havcr1	-1	1	1	Havcr1	55.40502405	7.01E-05	3.39E-03	Havcr1	55.40502405	5.90E-05	4.21E-03	Hepatitis A
Pvrl1	10	4.4	Pvrl1	1.169125638	0.6763	1	Pvrl1	4.478391584	1.11E-05	8.27E-04	Pvrl1	5.235802419	1.94E-06	3.07E-04	Herpes simplex virus 1 HSV1
PDGFRA	1.7	2.1	Pdgfra	1.071379406	0.92879	1	Pdgfra	1.871586912	0.41504	0.87954	Pdgfra	2.005179675	0.37055	0.83849	Cytomegalovirus
EGFR	6	3	Egfr	1.517187199	0.23639	1	Egfr	1.121841471	0.72322	1	Egfr	1.702043519	0.12498	0.55402	Cytomegalovirus
Hn1	291	176.7	Hn1	-1.04872437	0.80021	1	Hn1	-1.8339746	0.0016	0.0349	Hn1	-1.92333386	0.00066	0.0231	Mumps virus
PVR	19.1	8.2	PVR	1.177230264	0.55541	1	Pvr	-1.92810155	0.02641	0.2319	Pvr	-1.63782873	0.09962	0.50068	Poliovirus
Mog	0	0	Mog	-1	1	1	Mog	10.18897667	0.06515	0.39363	Mog	10.18897667	0.06251	0.39157	Rubella virus
pvr14	1.7	0.6	Pvr14	2.605888289	0.15236	1	Pvr14	-5.68496653	0.02217	0.20947	Pvr14	-2.18158489	0.35218	0.81653	Measles virus
St3gal4	8	2.6	St3gal4	-1.51460822	0.31822	1	St3gal4	1.293484023	0.5414	1	St3gal4	-1.1709524	0.69327	1	Zika virus

Figure 3

Please see the manuscript file to view the figure caption.

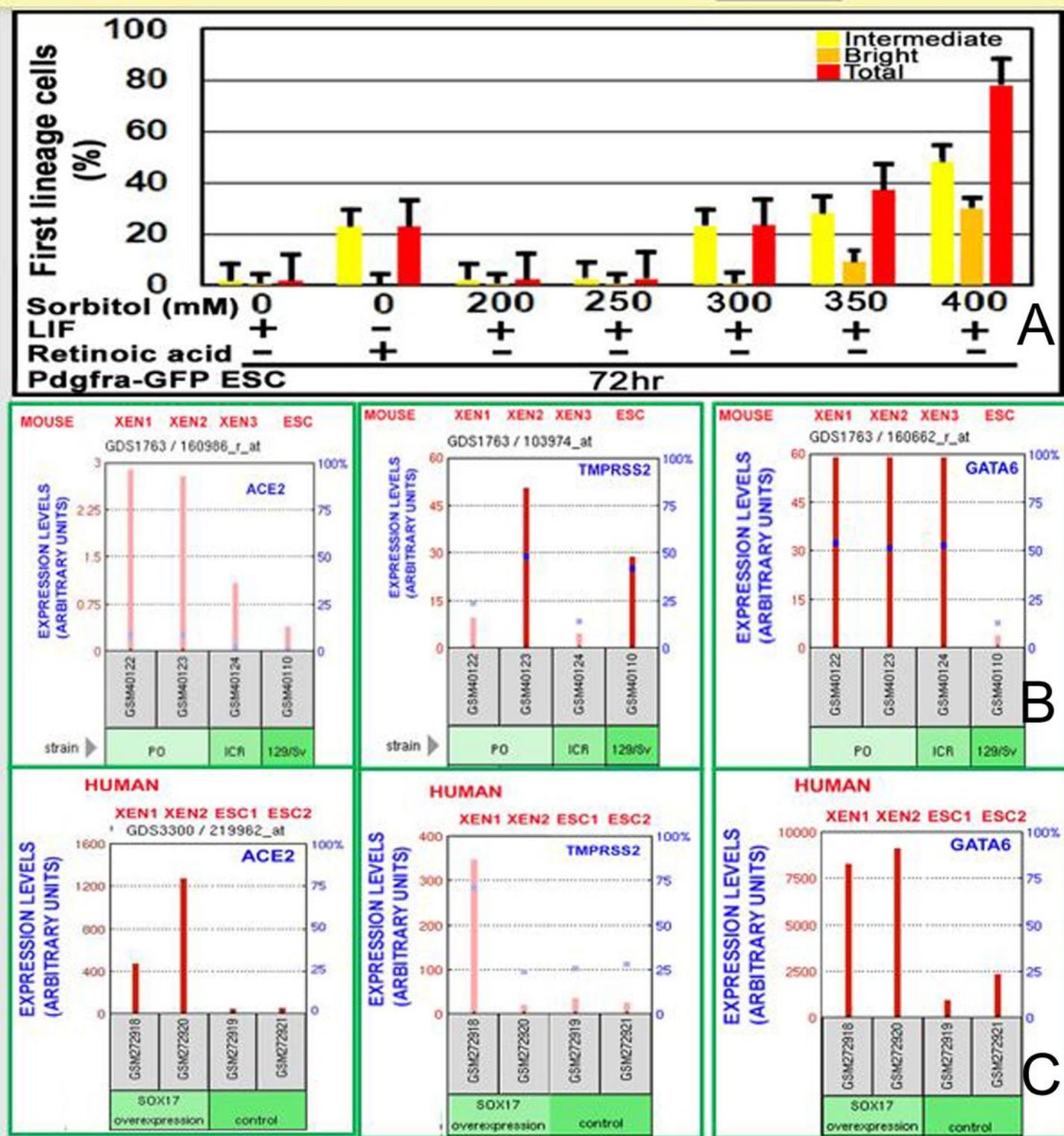


Figure 4

Please see the manuscript file to view the figure caption.

GO Group: change in expression of genes, significance rank of group, % rank, and significance

Gene expression change (mostly↑)	Gene expression change (mostly↓)							Significant Order of Significance Rank % of Significance Rank	GO Term	
	ND/ NS	SFD200/ Rank%	SFD250/ NS	SFD300/ NS	SFD200/ ND	SFD250/ ND	SFD300/ ND			Rank%
↑	1705	64.0%	818	2066	976	882	1955	229	5.9%	GO:0050792-regulation of viral process
↑	1027	38.6%	550	1490	1506	896	1944	271	7.0%	GO:0016032-viral process
↑	524	19.7%	1920	2458	503	1801	1947	272	7.0%	GO:0048525-negative regulation of viral process
↑	422	15.9%	591	2331	2434	397	1951	361	9.3%	GO:0009615-response to virus
↑	992	37.3%	789	2653	3006	709	1965	548	14.1%	GO:0051607-defense response to virus
↑	1506	56.6%	2622	2904	712	2490	1960	664	17.1%	GO:1903901-negative regulation of viral life cycle
↑	1013	38.1%	Not listed	3331	1032	1551	1762	746	19.2%	GO:0045071-negative regulation of viral genome replication
Rank#	2382	89.8%	704	2254	2537	806	2549	771	19.9%	GO:0050688-regulation of defense response to virus
↓	Not listed	Not listed	Not listed	Not listed	Not listed	Not listed	Not listed	779	20.0%	GO:0045091-regulation of single stranded viral RNA replication via double stranded DNA ntermediate
↑	Not listed	Not listed	Not listed	Not listed	Not listed	Not listed	Not listed	917	23.6%	GO:0039692-single stranded viral RNA replication via double stranded DNA ntermediate
↑	2275	85.5%	2713	3078	1514	2723	1924	985	25.3%	GO:1903900-regulation of viral life cycle
↑	1453	54.4%	Not listed	3188	1539	1649	2417	1190	30.8%	GO:0045069-regulation of viral genome replication
↑	2617	98.3%	990	2446	2960	1426	3352	1211	30.8%	GO:0050691-regulation of defense response to virus by host
↑	1257	47.2%	Not listed	3118	1594	1910	2767	1272	32.8%	GO:0019079-viral genome replication
↑	Not listed	Not listed	2189	961	24.7%	Not listed	Not listed	2604	67.1%	GO:0046597-negative regulation of viral entry into host cell
Total GO groups										
Significant	701	1146	1220	1069	916	1211	1289			
All	2662	2909	3772	3883	3048	3626	3878			

Figure 5

Please see the manuscript file to view the figure caption.

LIF-/+	TOP 20 highest counts	SFD/ND	NS	ORDER OF RANKING	A
	Fold Change	PValue	FDR	Naïve Pluripotency	
				Ctrl Ave	Sorb 300 Ave
Eif2s2	-1.17	0.46	0.94	21307.33	16723.66
Rps14	1.018	0.94	1	13802.66	13475.33
Hsp90ab1	-1.28	0.27	0.74	18920.66	13423.33
Actb	1.078	0.72	1	11941.33	12056.66
Gnb2l1	-1.06	0.77	1	8837.66	8178.66
Rps12	1.21	0.29	0.74	6849.66	8131.33
Rps20	-1.3	0.14	0.58	11778.66	7536.33
Rpl23a	1.01	0.95	1	7381.66	7371.33
Rps21	1.37	0.13	0.56	5350	7338.66
Rplp2	1.50	0.03	0.26	5135.66	7301
Rpl8	-1.25	0.29	0.74	9315.66	6570.66
Fus	1.12	0.68	1	5790.33	5653
Prdx1	1.07	0.70	1	4900	5302
Rps3	1.22	0.31	0.76	4341.33	5260.33
Serbp1	1.06	0.75	1	5261.33	5256
Hsp90aa1	-1.49	0.08	0.46	8484.33	5154.33
Rplp1	1.33	0.26	0.73	3593.33	4931
Sod1	-1.45	0.08	0.47	7761.33	4912.66
Rps5	-1.28	0.22	0.72	6850.6	4894.33
Tuba1b	2.17	0.00	0.02	2359	4853

EIF2S2 is an initiating protein elongation factor that binds tRNA to 40S ribosomal subunits
RPS are 40S structural ribosomal proteins
RPLP are 60S structural acidic proteins
RPL are 60S ribosomal structural proteins
GNB2L1/RACK1 is a ribosomal 40S subunit binding protein
Serbp1 interacts with GNB2L1/RACK1 and the ribosomal 40S subunit

Gene	Normal stemness			Normal differentiation ND/NS			Stress-forced differentiation SFD/ND				Stress-forced differentiation SFD/NS			
	X	SD	gene	Fold Change	PValue	FDR	gene	Fold Change	PValue	FDR	gene	Fold Change	PValue	FDR
ACTB	11941.3	7805.87	Actb	-1.26	0.26	1	reversal Actb	1.36	0.13	0.57	Actb	1.07	0.72	1
GAPDH	130.7	177	Gapdh	2.24	0.12	1	reversal Gapdh	-1.60	0.36	0.81	Gapdh	1.40	0.51	0.99
Pgk1	19.7	22.9	Pgk1	1.10	0.87	1	reversal Pgk1	-2.00	0.29	0.73	Pgk1	-1.80	0.36	0.83
Ppia	61.7	76.8	Ppia	-1.24	0.70	1	reversal Ppia	1.71	0.34	0.80	Ppia	1.37	0.57	1
RPLP0	2352.67	1389.73	Rplp0	1.15	0.48	1	reversal Rplp0	-1.19	0.38	0.84	Rplp0	-1.03	0.86	1
B2M	17.66	7.37	B2m	1.23	0.56	1	reversal B2m	-2.01	0.06	0.38	B2m	-1.63	0.19	0.67
YWHAZ	878.66	554.58	Ywhaz	-1.19	0.38	1	reversal Ywhaz	-1.10	0.63	1	Ywhaz	-1.31	0.17	0.64
Sdha	258	98.7	Sdha	1.03	0.85	1	reversal Sdha	-1.12	0.53	1	Sdha	-1.08	0.65	1
TFRC	319.66	106.64	Tfrc	1.07	0.74	1	reversal Tfrc	-1.14	0.54	1	Tfrc	-1.06	0.78	1
GUSB	15	7.810	Gusb	1.06	0.82	1	in kind Gusb	1.19	0.54	1	Gusb	1.27	0.40	0.87
HMBS	28	12.76	Hmbs	1.37	0.24	1	in kind Hmbs	1.04	0.88	1	Hmbs	1.43	0.18	0.66
HPRT	15.66	1.52	Hprt	-1.63	0.24	1	in kind Hprt	-2.29	0.08	0.43	Hprt	-3.74	0.00	0.07
TBP	16	1.73	Tbp	1.20	0.58	1	in kind Tbp	1.13	0.70	1	Tbp	1.37	0.35	0.82

Figure 6

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