# Effects of SARS-CoV-2 Spike S1 Subunit on the Interplay between HBV- and HCC-Related Molecular Processes in Human Liver: Biological Significance and Management in COVID-19 Patients.

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**SUPPLEMENTS**

**Table 1S -** Comparison between human proteins interacting to S1, as found in [X] and [X]

| ACE2 | ACE2 |
|---|---|
| AGER | AGTR1 |
| **DPP4** | AKT2 |
| **ESR1** | APOE |
| KIF18A | ASGR1 |
| PRC1 | AVPR1B |
| RRAGC | C1QB |
| S100A8 | C1QC |
| SFN | CD46 |
| TLN1 | CFH |
| **TLR4** | CFP |
| TMPRSS2 | CLEC4M |
|  | COP1 |
|  | CR2 |
|  | **DPP4** |
|  | **ESR1** |
|  | F10 |
|  | FLT1 |
|  | IL12RB1 |
|  | ITGB6 |
|  | LYPLA2 |
|  | MBL2 |
|  | NID1 |
|  | SDC1 |
|  | SDC2 |
|  | SNCA |
|  | **TLR4** |

In bold the 4 proteins in common.

$f(x) = 358.1\ x^{-1.3}$

$R^2 = 0.4911$
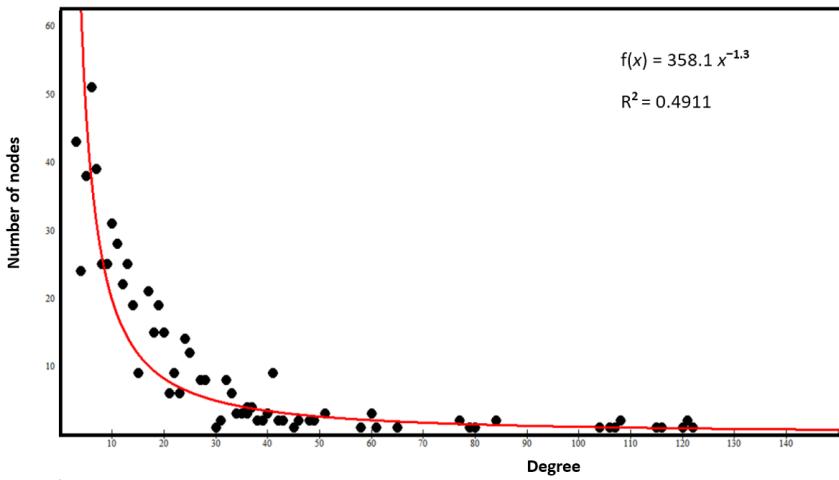
**Figure 1S** – Distribution graph of interactome-12. The distribution conforms to the power law with an R2 value of 0.4911. The value is still acceptable although at the low limits of reliability. This implies heterogeneities in the distribution, which makes the results difficult to explain. However, it is not a surprising result because the distribution reflects the overall structural and functional behavior of the entire interactome where proteins with different roles from each other and subjected to many functional stress by viral proteins in complex and metabolically differentiated cellular environments.

**Table 2S – Proteins involved in Hepatitis B and HCC according to KEGG analysis of the interactome-12**

1) **hsa05161 Hepatitis B,     90/158; strength: 1.22; signal:6.91; FDR: $2.37 \times 10^{-67}$.**
   CREB3L3, MAPK13, MAPK12, MAPK1, TAB1, SOS2, NFKBIA, PIK3R2, NFKB1, MAPK14, TICAM1, KRAS, TLR2, CREBBP, PIK3R3, MAP2K2, MAPK3, EP300, PRKCG, PIK3CA, ATF2, CDK2, RB1, TP53, CREB3L4, ARAF, NFATC3, MAP2K1, FOS, BID, CREB3L2, TBK1, SMAD3, MAPK11, ATF4, CREB3, MAP2K3, CREB5, TAB2, MAP3K7, NRAS, IRAK1, CHUK, JUN, YWHAB, SRC, TLR4, ELK1, PIK3CD, JAK2, AKT2, GRB2, TIRAP, TRAF3, BAD,  STAT3, MAPK8, NFATC2, PTK2B, IRF7, MAP2K7, MAP3K1, RELA, SOS1, CREB1, NFATC1, MAPK9, RAF1, MAVS, HRAS,  PRKCA, MAP2K4, TICAM2, BRAF, PIK3R1, IKBKB, JAK3, TRAF6, AKT1, MAP2K6, IRF3, IRAK4, CCNA2, CREB3L1, IKBKG, PRKCB, MYD88, JAK1, AKT3, PIK3CB.

2) **hsa05225 Hepatocellular carcinoma, 54/161; strength: 0.99; signal: 3.69; FDR: $1.38 \times 10^{-31}$.**
   GADD45B, MAPK1, SOS2, PIK3R2, RPS6KB1, CCND1, PLCG1, GADD45G, KRAS, PIK3R3, MAP2K2, GAB1, MAPK3, PRKCG, PIK3CA, STAT3, SHC2, RB1, TP53, EGFR, ARAF, MAP2K1, RPS6KB2, MET, SHC4, SMAD3, MTO, NRAS, GADD45A, PTEN, SHC3, BCL2L1, ELK1, PIK3CD, CSNK1A1L, AKT2, GRB2, BAD, SOS1, SHC1, RAF1, HRAS, PRKCA, CDKN2A, BRAF, PIK3R1, AKT1, ACTG1, PLCG2, ACTB, PRKCB, IGF1R, AKT3, PIK3CB.

3) **30 genes in common between HBV and HCC.**
   AKT1, AKT2, AKT3, ARAF, BAD, BRAF, ELK1, GRB2, HRAS, KRAS, MAP2K1, MAP2K2, MAPK1, MAPK3, NRAS, PIK3CA, PIK3CB, PIK3CD, PIK3R1, PIK3R2, PIK3R3, PRKCA, PRKCB, PRKCG, RAF1, RB1, SMAD3, SOS1, SOS2, TP53.
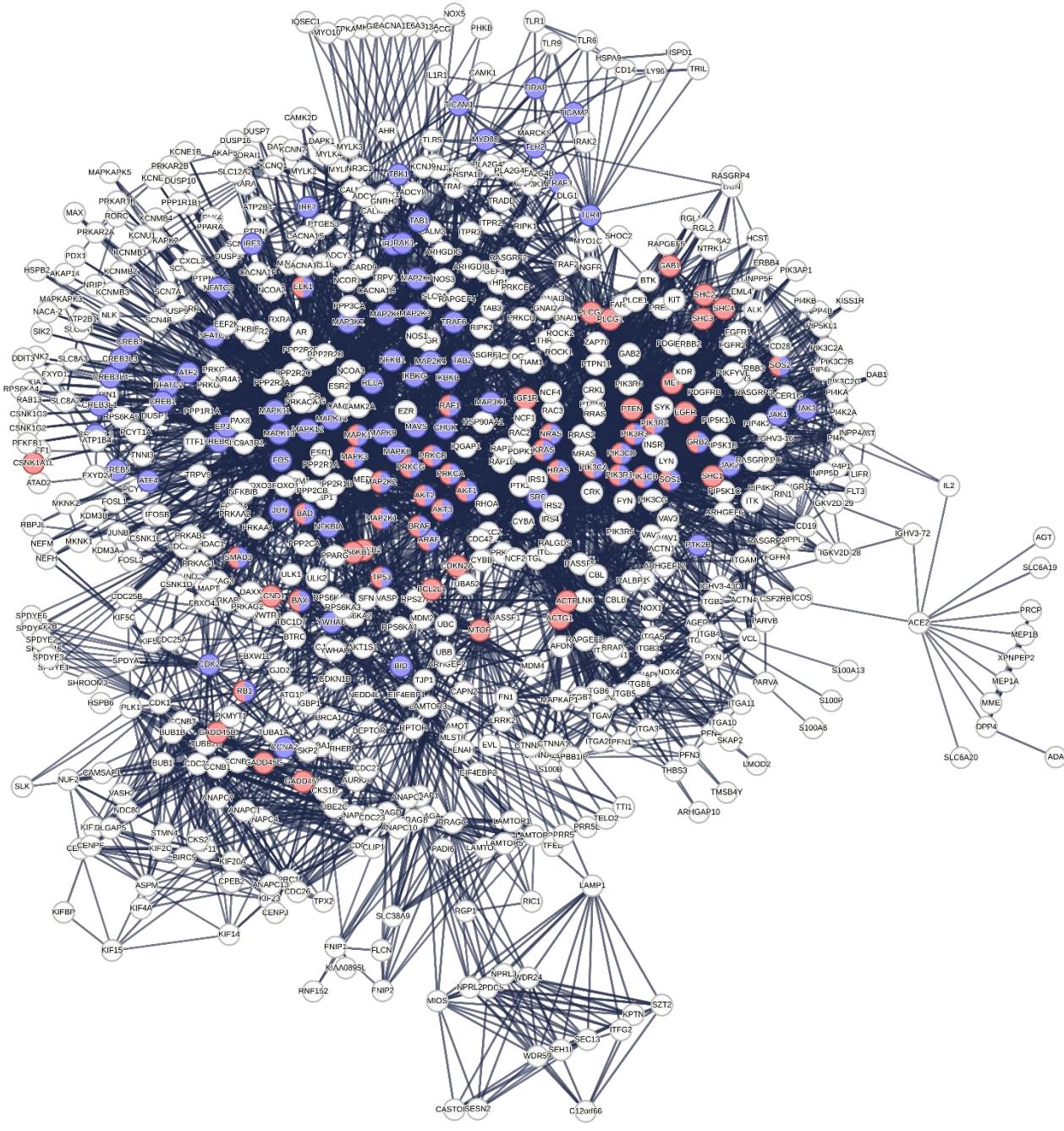
**Figure 2S** – Distribution of HBV and HCC genes in Interactome-12. The figure shows in blue the genes involved in hepatitis B, in red those of hepatocellular carcinoma and in red/blue the 30 genes in common. As can be seen these genes massively involve the central core.
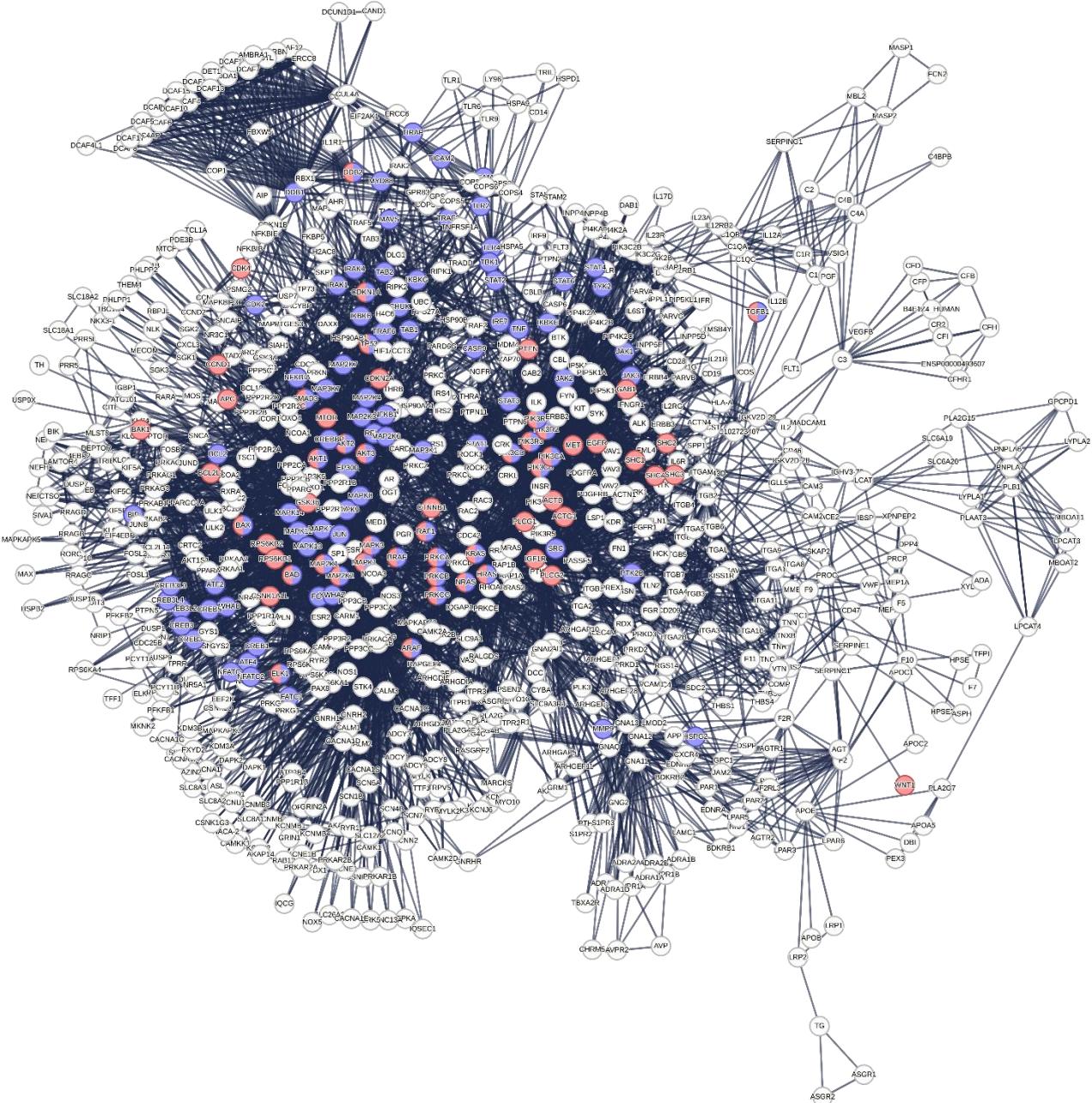
**Figure 3S** – Distribution of HBV and HCC genes in Interactome-814 (as named in [11] for its 814 nodes). Number of nodes: 814; number of edges: 6451; mean node degree: 15.9; mean local clustering coefficient: 0.547; expected number of edges: 1498; PPI enrichment p-value: < 1.0e-16. This interactome was modeled as reported in [11]. The presence of KEGG terms for HBV and HCC has been detected but never studied previously [11]. Nodes are colored to highlight the general representation of the genes involved. Blue nodes refer to hsa05161 Hepatitis B; 101 of 158 1.19 6.89 1.01e-71 and red ones to hsa05225 Hepatocellular carcinoma; 56 of 161 0.93 3.33 2.02e-29. Common genes (HCC/HBV) are in red/blue.

**Table 3S – Proteins involved in Hepatitis B and HCC according to KEGG analysis of the interactome-814**

1) **hsa05161 Hepatitis B; 101 of 158 1.19 6.89 1.01e-71**

CREB3L3, MAPK13, MAPK12, MAPK1, TAB1, NFKBIA, TGFB1, PIK3R2, NFKB1, MAPK14, TICAM1, KRAS, DDB2, TLR2, CREBBP, PIK3R3, MAP2K2, MAPK3, EP300, PRKCG, PIK3CA, ATF2, STAT3, CDK2, TP53, CREB3L4, ARAF, BAX, STAT6, NFATC3, DDB1, MAP2K1, FOS, STAT2, BID, CREB3L2, TBK1, CASP9, SMAD3, MAPK11, ATF4, CREB3, MAP2K3, CREB5, STAT4, STAT1, TAB2, MAP3K7, NRAS, IRAK1, CHUK, JUN, MMP9, YWHAB, SRC, TLR4, HSPG2, ELK1, PIK3CD, JAK2, AKT2, TIRAP, TRAF3, BAD, MAPK8, YWHAZ, NFATC2, PTK2B, IRF7, MAP2K7, BCL2, MAP3K1, RELA, CDKN1A, CREB1, NFATC1, MAPK9, TNF, RAF1, MAVS, HRAS, PRKCA, MAP2K4, TICAM2, BRAF, PIK3R1, IKBKB, TYK2, JAK3, TRAF6, AKT1, IKBKE, MAP2K6, IRAK4, CREB3L1, IKBKG, PRKCB, MYD88, JAK1, AKT3, PIK3CB

2) **hsa05225 Hepatocellular carcinoma; 56 of 161 0.93 3.33 2.02e-29**

MAPK1, TGFB1, PIK3R2, RPS6KB1, CCND1, PLCG1, KRAS, DDB2, APC, CDK4, PIK3R3, MAP2K2, GAB1, MAPK3, PRKCG, PIK3CA, SHC2, TP53, EGFR, ARAF, BAX, WNT1, MAP2K1, RPS6KB2, MET, GSK3B, SHC4, SMAD3, MTOR, NRAS, PTEN, BAK1, SHC3, BCL2L1, ELK1, PIK3CD, CSNK1A1L, AKT2, BAD, CDKN1A, SHC1, RAF1, HRAS, PRKCA, CDKN2A, BRAF, PIK3R1, AKT1, ACTG1, PLCG2, ACTB, CTNNB1, PRKCB, IGF1R, AKT3, PIK3CB

3) **29 genes in common between HBV and HCC.**

AKT1, AKT2, BAD, HRAS, PIK3CD, TP53, PIK3CA, PIK3CB, MAPK3, RAF1, SMAD3, TGFB1, PIK3R1, PIK3R2, PIK3R3, CDKN1A, PRKCG, PRKCA, PRKCB, DDB2, KRAS, NRAS, MAP2K1, MAP2K2, BAX, BRAF, ARAF, MAPK1, ELK1.

**Table 4S Comparison of enriched terms of the two interactomes.**

The terms being compared are: Biological Process (GO), Molecular Function (GO), Cellular Component (GO), and KEGG Pathways.
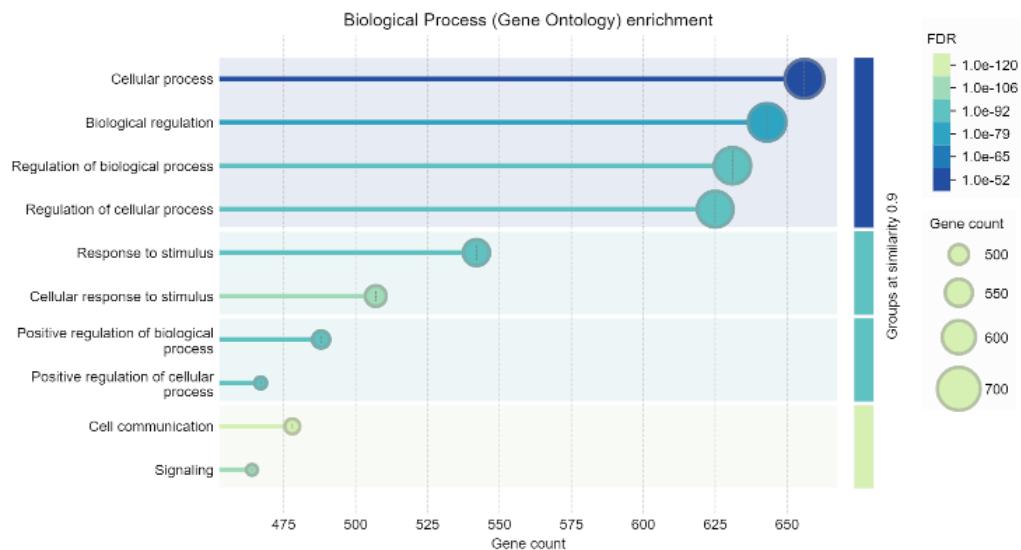


Interactome-12
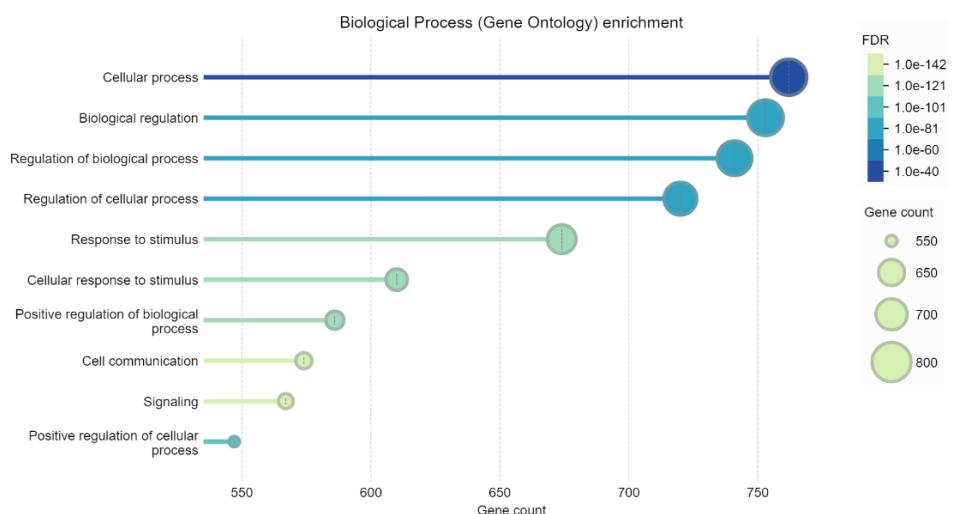


Interactome-814
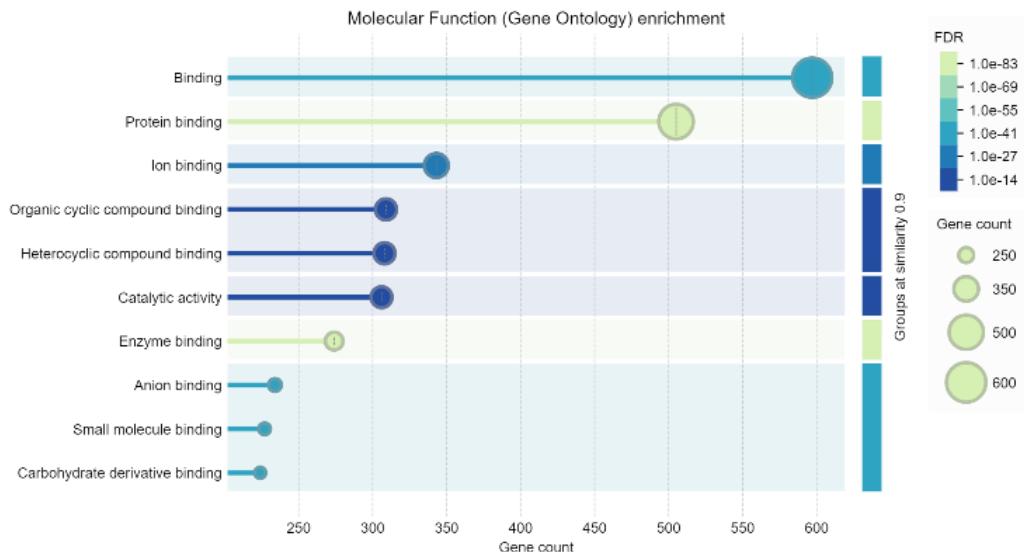
Interactome-12



Interactome-814

Cellular Component (Gene Ontology) enrichment

Interactome-12



Cellular Component (Gene Ontology) enrichment
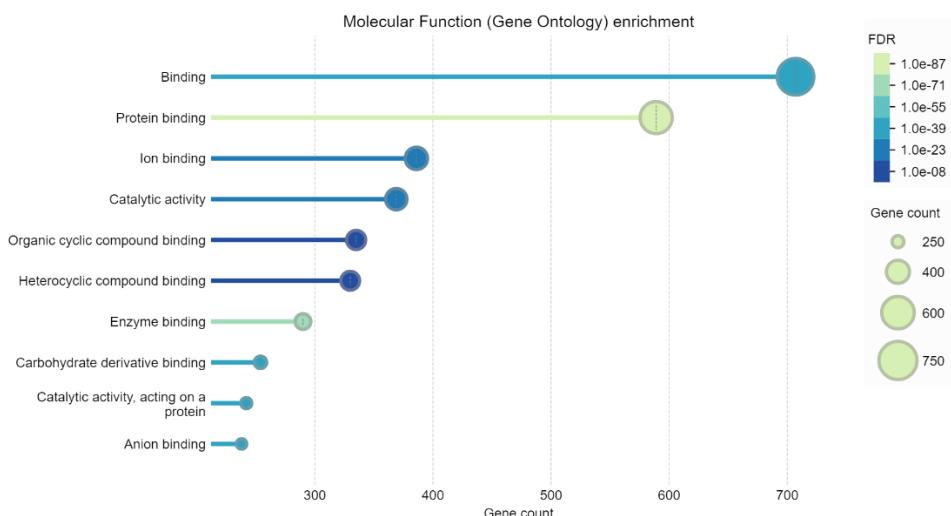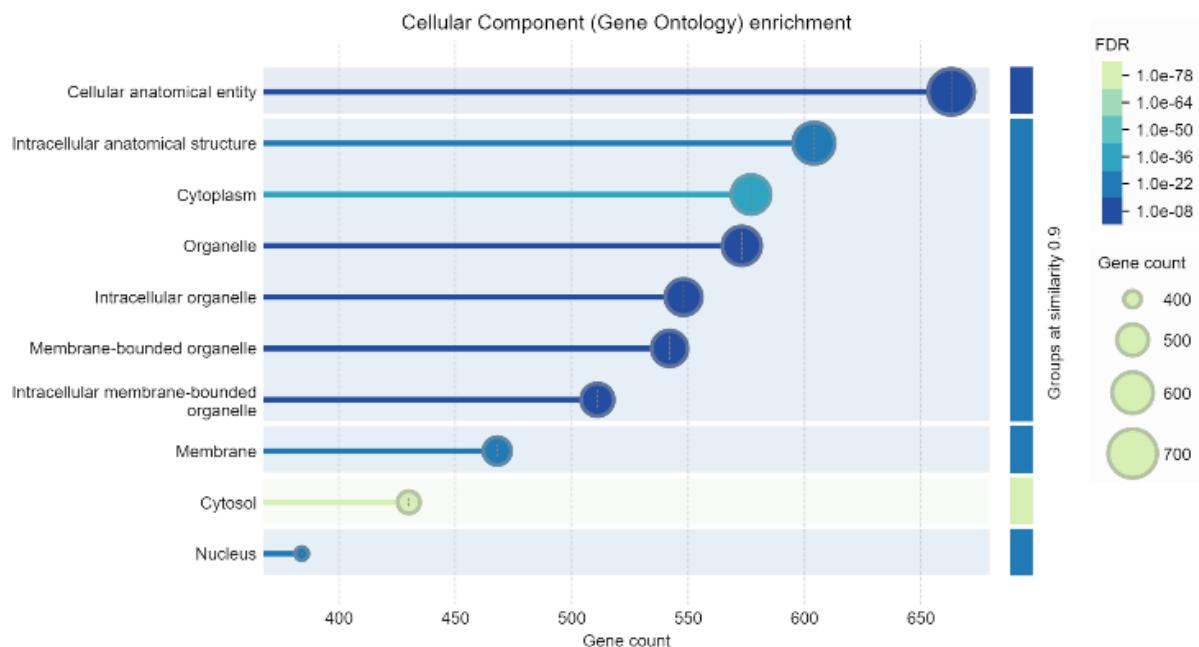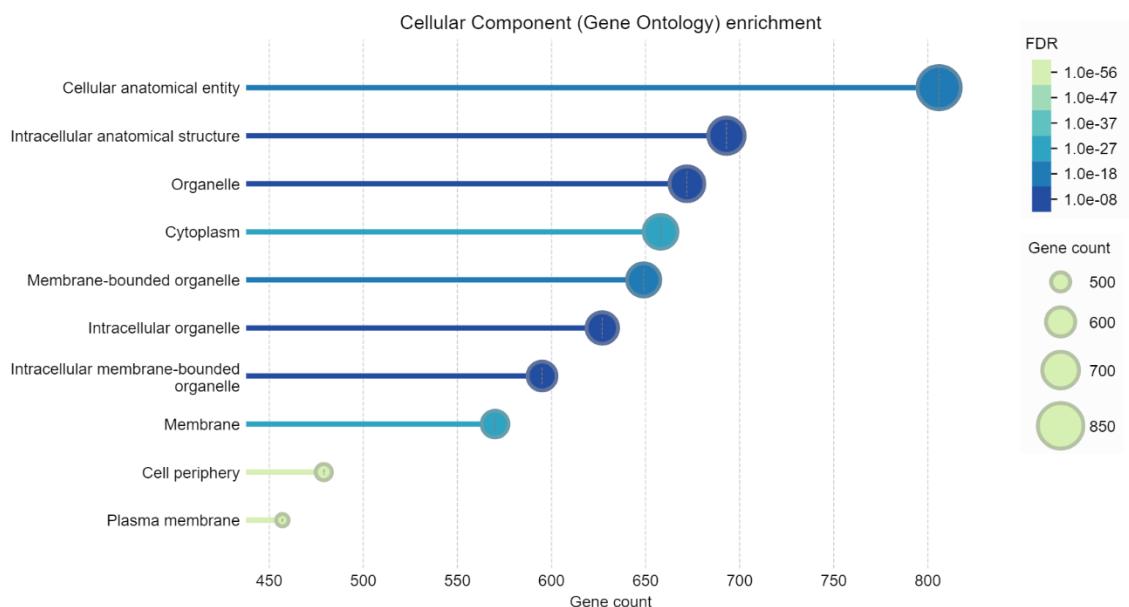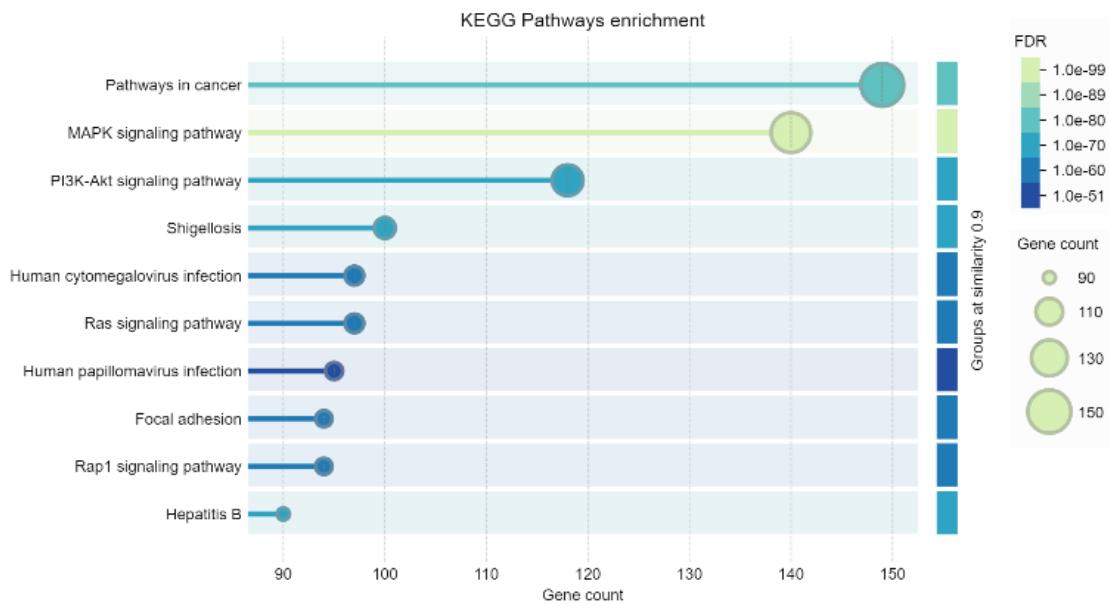
Interactome-814
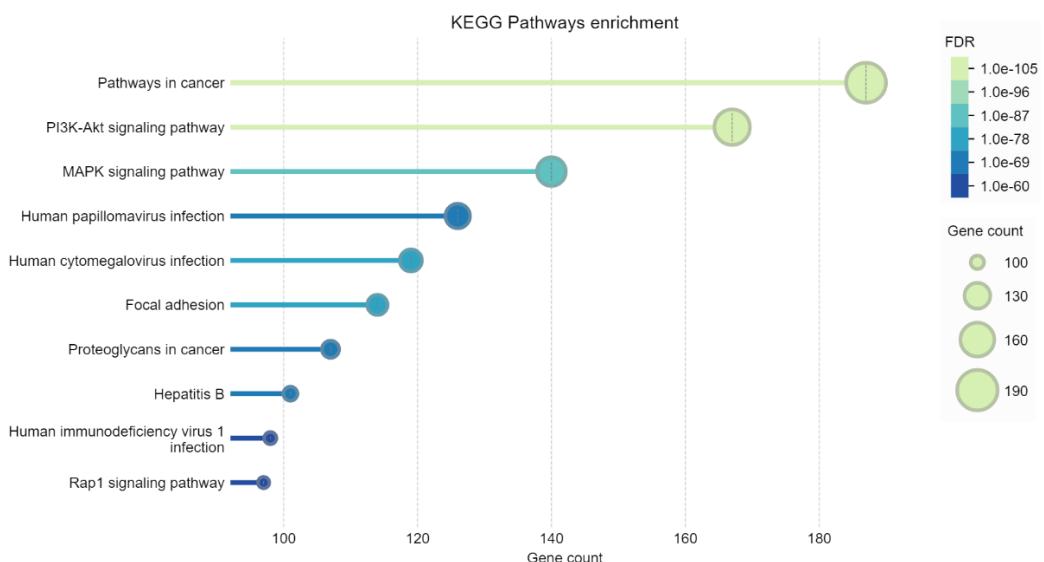
Interactome-12



Interactome-814

**Parameters used**: Similarity: > 0.9; Gene count; Nr. of terms shown: 10. The comparisons were calculated by STRING on a semantic basis, grouping the functionally similar terms, and on the number of genes involved. The number of genes is a more direct physical measure of the complexity of a biological phenomenon. The analysis of interactomes, through the similarity parameter, uses Semantic Web technologies to detect explicit and implicit functional redundancies, thus improving the understanding of protein interactions [135]. By contributing to the mapping of protein interactions, the review allows us to understand, from a systems perspective, host-pathogen interactions [136]. KEGG analysis shows that cancer as well as HBV pathways are present in both interactomes with a high number of genes. The high similarity value has compacted all genes that in the various terms play functions related to cancer or HBV.
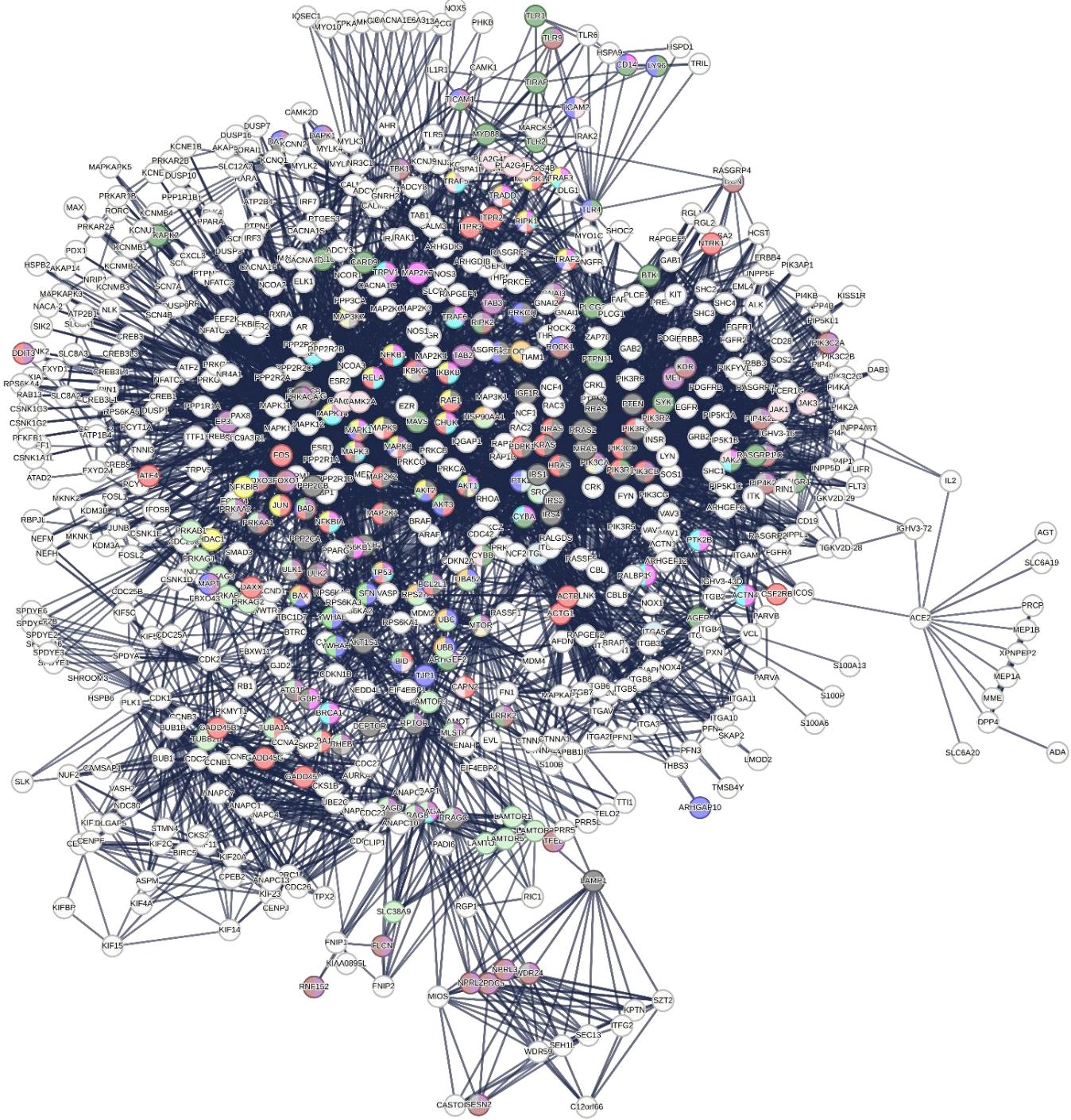
**Figure 4S** – Distribution of cell deaths among nodes of the Interactome-12 according to Table 5S. The figure shows that many genes, especially those in the central core, are involved in different patterns of cell death (multi-colored nodes) that are characteristic of both HBV and HCC. This makes it very difficult to find explanations about the fate of the two diseases.

**Table 5S** - Distribution of cell deaths among nodes of the Interactome-12

| Biological Process (Gene Ontology) | | | | | |
|---|---|---|---|---|---|
| GO-term | description | count in network | strength | signal | false discovery rate |
| GO:0010506 | Regulation of autophagy | 56 of 346 | 0.67 | 1.9 | 4.41e-18 |
| GO:0010508 | Positive regulation of autophagy | 34 of 146 | 0.83 | 2.09 | 7.07e-15 |
| GO:0034612 | Response to tumor necrosis factor | 31 of 199 | 0.66 | 1.35 | 1.06e-09 |
| GO:1903557 | Positive regulation of tumor necrosis factor superfamily cytokine pro… | 28 of 105 | 0.89 | 2.08 | 1.71e-13 |
| GO:0071356 | Cellular response to tumor necrosis factor | 27 of 175 | 0.65 | 1.23 | 2.05e-08 |
| GO:0016241 | Regulation of macroautophagy | 23 of 159 | 0.62 | 1.04 | 8.46e-07 |
| GO:2000811 | Negative regulation of anoikis | 7 of 19 | 1.03 | 0.87 | 0.00023 |
| GO:0043276 | Anoikis | 6 of 12 | 1.16 | 0.89 | 0.00024 |

| KEGG Pathways | | | | | |
|---|---|---|---|---|---|
| pathway | description | count in network | strength | signal | false discovery rate |
| hsa04140 | Autophagy - animal | 61 of 131 | 1.13 | 5.04 | 4.71e-42 |
| hsa04210 | Apoptosis | 53 of 131 | 1.07 | 4.24 | 2.92e-34 |
| hsa04217 | Necroptosis | 24 of 147 | 0.68 | 1.32 | 5.09e-09 |

| Reactome Pathways | | | | | |
|---|---|---|---|---|---|
| pathway | description | count in network | strength | signal | false discovery rate |
| HSA-109581 | Apoptosis | 35 of 175 | 0.77 | 1.89 | 3.82e-14 |
| HSA-1632852 | Macroautophagy | 32 of 133 | 0.85 | 2.11 | 7.71e-15 |
| HSA-109606 | Intrinsic Pathway for Apoptosis | 14 of 52 | 0.89 | 1.36 | 1.63e-07 |
| HSA-5218859 | Regulated Necrosis | 11 of 58 | 0.74 | 0.86 | 7.04e-05 |

| WikiPathways | | | | | |
|---|---|---|---|---|---|
| pathway | description | count in network | strength | signal | false discovery rate |
| WP2036 | TNF-related weak inducer of apoptosis (TWEAK) signaling pathway | 22 of 42 | 1.18 | 2.98 | 3.23e-16 |
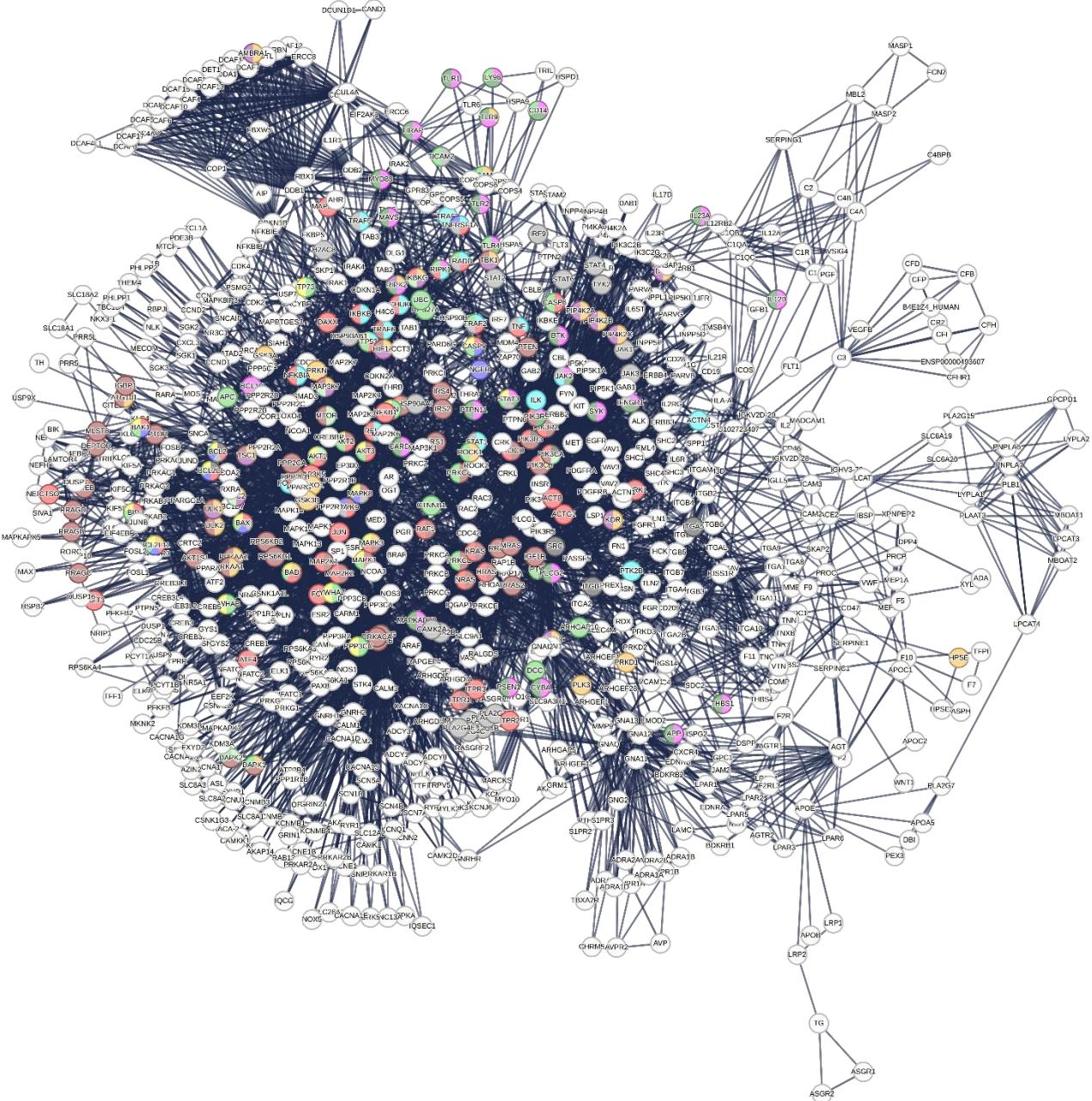
**Figure 5S** – Distribution of cell deaths among nodes of the Interactome-814 according to Table 6S. Again, the figure shows that many genes, especially those in the central core, are involved in different modes of cell death (multi-colored nodes) that are characteristic of both HBV and HCC.

**Table 6S** - Distribution of cell deaths among nodes of the Interactome-814

**Biological Process (Gene Ontology)**

| GO-term | description | count in network | strength | signal | false discovery rate | |
|---|---|---|---|---|---|---|
| GO:0010508 | Positive regulation of autophagy | 35 of 146 | 0.76 | 1.85 | 1.95e-13 | 🟠 |
| GO:1903557 | Positive regulation of tumor necrosis factor superfamily cytokine prod… | 29 of 105 | 0.83 | 1.88 | 1.84e-12 | 🟣 |
| GO:0032760 | Positive regulation of tumor necrosis factor production | 28 of 101 | 0.83 | 1.85 | 4.46e-12 | 🟢 |
| GO:0033209 | Tumor necrosis factor-mediated signaling pathway | 20 of 56 | 0.94 | 1.8 | 2.65e-10 | 🔵 |
| GO:0016239 | Positive regulation of macroautophagy | 17 of 74 | 0.75 | 1.11 | 1.51e-06 | 🟣 |
| GO:2000811 | Negative regulation of anoikis | 8 of 19 | 1.01 | 0.95 | 7.99e-05 | ⚫ |
| GO:0043276 | Anoikis | 7 of 12 | 1.15 | 1.02 | 6.08e-05 | 🟢 |

**KEGG Pathways**

| pathway | description | count in network | strength | signal | false discovery rate | |
|---|---|---|---|---|---|---|
| hsa04140 | Autophagy - animal | 65 of 131 | 1.08 | 4.73 | 2.71e-41 | 🔴 |
| hsa04210 | Apoptosis | 55 of 131 | 1.01 | 3.86 | 3.15e-32 | 🔴 |
| hsa04217 | Necroptosis | 36 of 147 | 0.77 | 2.01 | 2.87e-15 | ⚪ |
| hsa04215 | Apoptosis - multiple species | 11 of 30 | 0.95 | 1.3 | 7.19e-07 | 🔵 |

**Reactome Pathways**

| pathway | description | count in network | strength | signal | false discovery rate | |
|---|---|---|---|---|---|---|
| HSA-109581 | Apoptosis | 42 of 175 | 0.76 | 2.06 | 2.30e-16 | 🟢 |
| HSA-109606 | Intrinsic Pathway for Apoptosis | 21 of 52 | 0.99 | 2.09 | 6.39e-12 | 🟡 |

**Table 7S** – The 220 genes that control liver cell death during covid.

ACTB ACTG1 ACTN4 ADA AGER AGT AKT1 AKT1S1 AKT2 AKT3 ARHGAP10 ARHGEF2 ATF2 ATF4 ATG101 BAD BAX BCL10 BCL2L1 BID BRAF BTK CAMK2A CAMK2D CAPN2 CARD9 CD14 CDC25A CDC25 B CDC25C CDKN1B CDKN2A CHUK CREB3 CREB3L1 CRKL CSF2RB CTNNA1 CYBA CYBB DAPK1 DAPK2 DAXX DCN DDIT3 DEPDC5 DEPTOR ERBB3 ERBB4 FLCN FNIP1 FOS FOXO1 FOXO3 GADD45A GADD45B GADD45G GNAI3 HRAS HSP90AA1 HSPD1 IFNGR1 IGBP1 IGF1R IKBKB IKBKG IL2 ILK IRF3 IRF7 IRS1 IRS2 IRS4 ITGA4 ITGA5 ITGAM ITGAV ITGB1 ITPR2 ITPR3 JAK2 JAK3 JUN KDR KRAS KRAS LAMP1 LAMTOR1 LAMTOR2 LAMTOR3 LAMTOR4 LAMTOR5 2 LRRK2 LY96 LYN MAP2K1 MAP2K2 MAP2K4 MAP2K7 MAP3K14 MAP3K5 MAP3K7 MAPK1 MAPK3 MAPK8 MAPK9 MAPKAPK2 MAPT MAVS MK NK2 MLST8 MRAS MTOR MYD88 NFKB1 NFKBIA NGFR NPRL2 NPRL3 NR3C1 NRAS NTRK1 PAX8 PDPK1 PDX1 PIK3C2A PIK3CA PIK3CB PIK3CD PIK3CG PIK3R1 PIK3R2 PIK3R3 PIP4K2A PIP4K2B PIP4K2C PLCG1 PLCG2 PPARA PPARG PPP2CA CB PRKAA1 PRKAA2 PRKAB1 PRKAB2 PRKACA PRKACB PRKACG PRKAG1 PRKAG2 PRKAG3 PRKCA PRKCD PRKCI PRKCQ PTEN PTK2 PTK2B PTPN11 RAF1 R APGEF2 RASGRP1 RB1 RELA RGL2 RHEB RHOA RIPK1 RIPK2 RNF152 ROCK1 RORC RPS27A RPS6KB1 RPS6KB2 RPTOR RRAGA RRAGB RRAGC RRAGD RRAS RRAS2 RRAS2 RYR2 SESN2 SFN SLC38A9 SMAD3 SP1 SRC SYK TBK1 TFEB TICAM1 TICAM2 TIRAP TJ P1 TLR1 TLR2 TLR4 TLR9 TP53 TRADD TRAF2 TRAF3 TRAF5 TRAF6 TUBA1A TUBA1B TUBB2B UBA52 UBB UBC ULK1 ULK2 WDR24 YWHAB YWHAH