**Figure S1. Systems biology approaches are used to study the interactions between hepatocytes and HCV.** (A)Diagram describing how HCV particles are assembled from *iHepatocytes2322* resources. The black and gray arrows represent *iHepatocytes2322* and *iHCV* reactions, respectively. AAs, amino acids; DAG, diacylglycerolipids; NNs, DNA and RNA nucleotides; LPA, lysophosphatidic acid; NS5B, HCV RNA polymerase; PA, phosphatidate; TAG, triglycerolipid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; and SM, sphingomyelin. (B) Diagram describing the antimetabolite algorithm for a metabolite *m1*. The FBA maximizes the HCV assembly reaction where the flux values (*v1 … v6*) for all reactions (*R1...R6*) involving *m1* are constrained to zero. (C) Diagram describing the Reporter Metabolite algorithm for metabolite *m1*. The *Z* score was calculated from the adjusted *P*-value (*q1 … q6*) for each gene associated with reactions *R1... R6*. (D) Diagram describing the Reporter Subnetwork algorithm. The reaction graph is extracted from the GEM, where the nodes are reactions and the edges describe two nodes that share at least one metabolite. The reactions *R1...R6* are fully connected because they share the metabolite *m1*. The *Zscore (Z1 … Z8)* was calculated for each reaction from its *q*-value for genes associated with these reactions. The Reporter Subnetwork analysis identifies highly scored subnetworks in a connective manner (*R1*, *R6* and *R7*) using a simulated annealing algorithm.



**Figure S2 The acyl-carnitine metabolites are changed significantly in dysplastic nodule and early HCC.** The color represents the negative logarithm P value of changed metabolites.

**Early HCC**

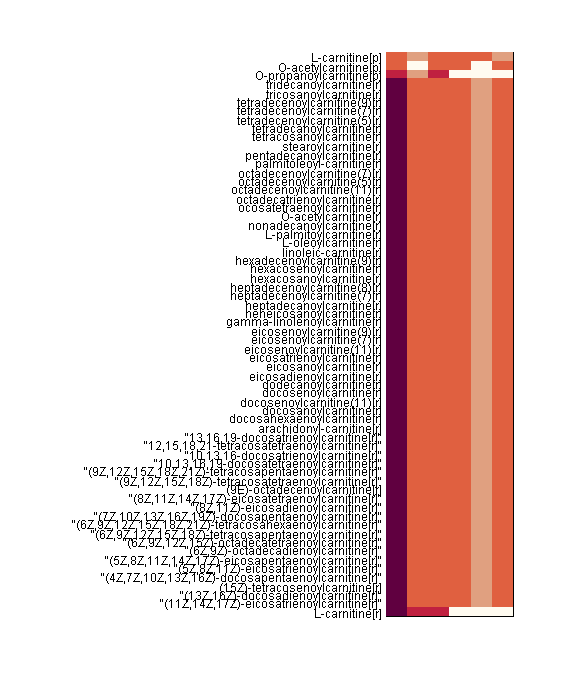
Up

Down

**Dysplastic**

Up

Down



**Reporter Metabolite**



**-log P value**

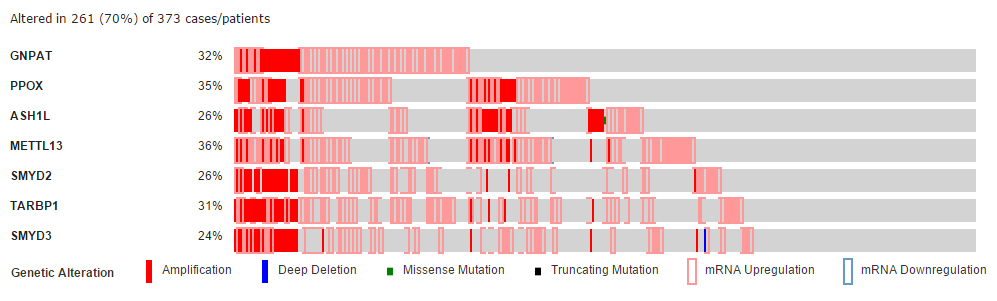
**+5**

**0**

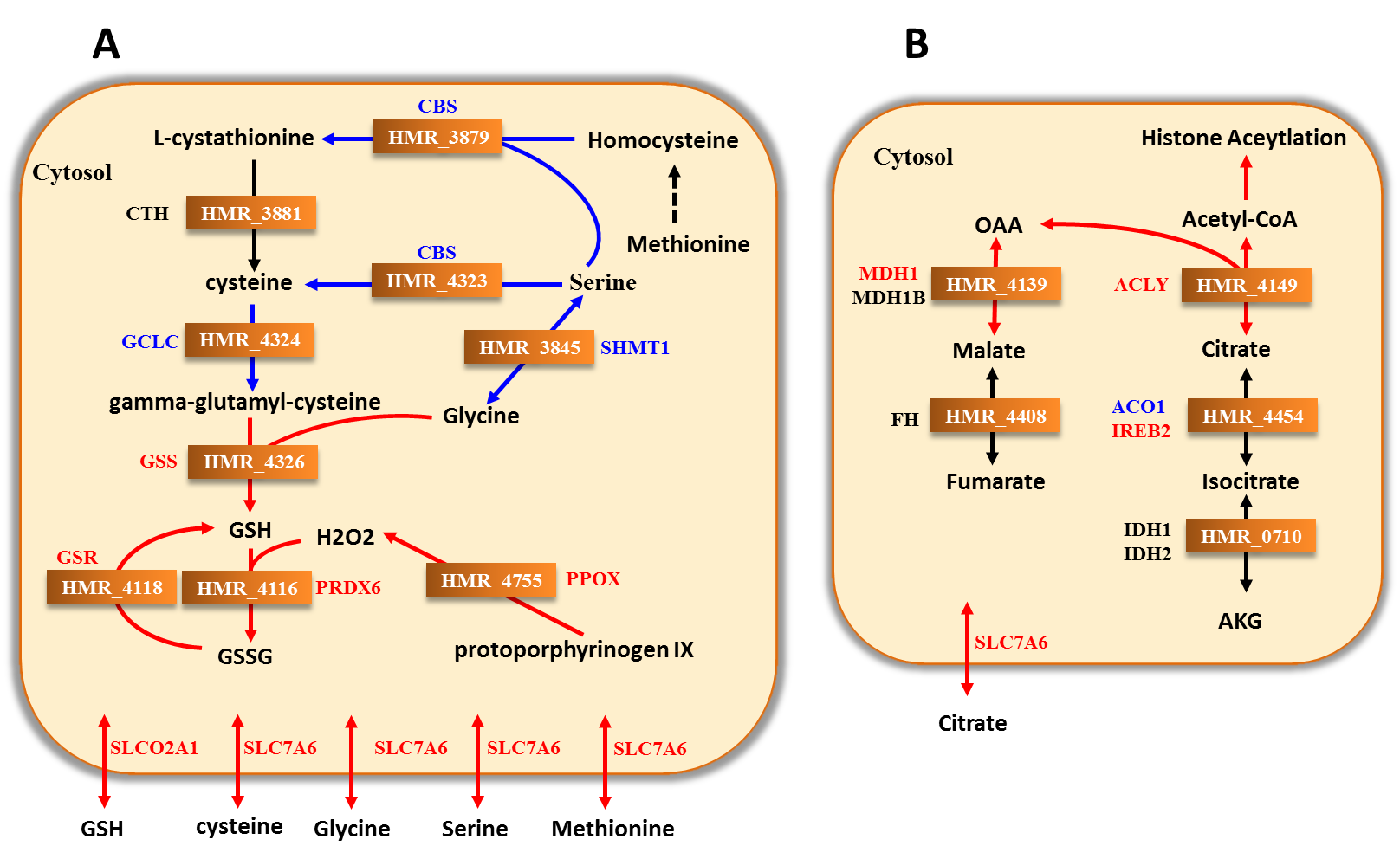
**Figure S3. DNA methylation probes for the *BBOX1* and *BCAT1* genes with delta beta values = 0.1 (adjusted *P*-value < 0.05).** Red and green circles are the average beta values for probes across all of the cancer and normal TCGA samples, respectively. Blue and gray rectangles indicate that the probe is in the promoter region and gene body region, respectively. The bold black line in the rectangle indicates that this probe is a CpG island.

**D:\Users\Desktop\Rplot03.tiff**

**Figure S4. Oncoprint for the *ASH1L*, *METTL13*, *SMYD2*, *TARBP1,* *SMYD3*, *GNPAT* and *PPOX* genes obtained from cBioPortal (**[**1**](#_ENREF_1)**) (see the link** <http://www.cbioportal.org/index.do?cancer_study_list=lihc_tcga&cancer_study_id=lihc_tcga&genetic_profile_ids_PROFILE_MUTATION_EXTENDED=lihc_tcga_mutations&genetic_profile_ids_PROFILE_COPY_NUMBER_ALTERATION=lihc_tcga_gistic&genetic_profile_ids_PROFILE_MRNA_EXPRESSION=lihc_tcga_rna_seq_v2_mrna_median_Zscores&Z_SCORE_THRESHOLD=2.0&data_priority=0&case_set_id=lihc_tcga_log2CNA&case_ids=&patient_case_select=sample&gene_set_choice=user-defined-list&gene_list=GNPAT%0D%0APPOX%0D%0AASH1L%0D%0AMETTL13%0D%0ASMYD2%0D%0ATARBP1%0D%0ASMYD3%0D%0A%0D%0A%0D%0A%0D%0A%0D%0A%0D%0A%0D%0A%0D%0A&clinical_param_selection=null&tab_index=tab_visualize&Action=Submit>**)**

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**Figure S5. Oxidative stress reactions and the role of SLC7A6 in transporting amino acids and citrate to the cell are integrated with gene expression in early HCC.** (A) Oxidative stress reactions were reported in the Reporter Subnetwork analysis of the early HCC stage. **(B)** Biosynthesis of TCA metabolites from extracellular citrate using *SLC7A6*.Blue, red and black arrows indicate that the gene(s) associated with this reaction are downregulated, upregulated and unregulated (adjusted *P*-value > 0.05), respectively. The reaction is also unregulated if it involves both upregulated and downregulated genes.



# Supplementary Tables

Table S1 Lipid composition of HCV particles obtained from Scholtes et al ([2](#_ENREF_2))

|  |  |
| --- | --- |
|  | in vitro LVP |
| RNA | 1 |
| apoB/RNA | 106.31 |
| Triacylglycerol. (TAG)/RNA | 104.97x106.31 |
| Phospholipids (PL)/RNA | 84.23x 106.31 |
| (TCho)/RNA | 65.52x106.31 |
| PL composition | 28.7 phosphatidylethanolamine + 61.4 phosphatidylcholine +  0 phosphatidylserine +  3.4 Phosphatidylinositol +  6.4 sphingomyelin.=> 100 PL |

Table S2 Antimetabolites that inhibit HCV assembly and validations from the literature.

|  |  |  |
| --- | --- | --- |
| Host-targets | Summary | Ref |
| Acyl-CoA | Liefhebber et al downregulated the acyl-CoA synthetase long-chain family member 1 gene to reduce HCV assembly. | ([3](#_ENREF_3)) |
| Acyl-glycerol |
| cholesterol esters |
| (R)-5-diphosphomevalonate | Jin Ye reviewed the role of mevalonate pathway in HCV assembly. | ([4](#_ENREF_4)) |
| (R)-5-phosphomevalonate |
| (R)-mevalonate |
| Presqualene-PP | Saito et al downregulated the squalene synthase gene to reduce HCV assembly. | ([5](#_ENREF_5)) |
| Squalene |
| Squalene 2,3-oxide | Owens et al suggested that both of oxidosqualene cyclase and lanosterol demethylase can be used as antiviral targets for HCV assembly. | ([6](#_ENREF_6)) |
| lanosterol |
| dimethylallyl-PP | Ye et al and Kapadia and Chisari reported the role of geranylgeranylation in HCV replication. | ([7](#_ENREF_7), [8](#_ENREF_8)) |
| farnesyl-PP |
| geranyl-PP |
| isopentenyl-pPP |
| cholesterol | Takano et al and Rodgers et al downregulated the *DHCR24* and *DHCR7* genes to reduce HCV assembly. | ([9](#_ENREF_9), [10](#_ENREF_10)) |
| inositol | Bishé et al reviewed the role of phosphoinositides in the HCV assembly. | ([11](#_ENREF_11)) |
| 1D-myo-inositol-3-phosphate |
| 3-dehydrosphinganine | Sakamoto et al downregulated the serine palmitoyltransferase gene to reduce HCV assembly. | ([12](#_ENREF_12)) |
| sphinganine |
| sn-glycerol-3-phosphate | Sn-glycerol-3-phosphate may prevent lipid biosynthesis through Kennedy pathway. |  |
| 1,2-diacylglycerol-LD-TAG pool (DAG) | Herker et al downregulated the *DGAT1* gene to reduce HCV assembly. | ([13](#_ENREF_13)) |

Table S3 Metabolites that were removed from the model before running the Reporter Subnetwork algorithm.

|  |
| --- |
| Metabolite Name |
| H2O |
| CO2 |
| O2 |
| H+ |
| HCO3- |
| Na+ |
| CoA |
| Pi |
| Ppi |
| AMP |
| ADP |
| ATP |
| NAD+ |
| NADH |
| NADP+ |
| NADPH |
| PAP |
| PAPS |
| FAD |
| FADH2 |
| % H2O2 was not removed for ROS |

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