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Figure S1

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3 **Supplementary Information**

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23 **Supplementary Material and methods**

24 **Cells and cell culture conditions.** The source and culture conditions of Huh7.5.1 cells have

25 been described[1]. HeLa cells were purchased from ATCC and cultured in Dulbecco´s

26 modified Eagle medium (Gibco® DMEM GlutaMAX™, ThermoFisher Scientific) containing 1%

27 sodium pyruvate as described for Huh7.5.1 cells[1].

Viruses and infectivity assays. Cell culture-derived recombinant cell culture-derived hepatitis C virus (HCVcc) Jc1 (genotype 2a/2a chimera), H77R2a (genotype 1a/2a chimera engineered for *Renilla* luciferase expression), Con1R2a (genotype 1b/2b chimera engineered for *Renilla* luciferase expression), and JcR2a (genotype 2a/2a chimera engineered for *Renilla* luciferase expression) were generated in Huh7.5.1 cells as described[1, 2, 3, 4]. HCVcc infectivity was determined by calculating the 50% tissue culture infectious dose (TCID50) using anti-NS5A antibody as described[5, 6] or by assessing luciferase activity. HCVcc were used at 10^5-10^6 TCID50/mL throughout the study. HCV RNA was purified using a QIAmp viral RNA minikit (Qiagen) and analyzed by one-step RT-qPCR using a Sensi Fast NO ROX kit (Bioline) according to the manufacturer's instructions. Standard curves were performed using 10-fold dilution series of HCV RNA.

Purification of HCVcc particles using sucrose cushion or iodixanol density gradient. HCVcc (JcR2a) were concentrated 10-fold using a Vivaspin column (GE Healthcare). For sucrose cushion purification, HCVcc were purified by overlaying 3.5 mL of culture media on 1.5 mL of 20% sucrose, and by ultracentrifuging samples for 4h at 40,000 rpm on a SW-55 rotor (Beckman Coulter). Purified HCVcc were resuspended in 30 µL of PBS for analysis via immunocapture and electron microscopy. Density distributions of infectious HCVcc were determined by overlaying 0.5 mL culture media on a 5 mL, 4%-40% iodixanol step gradient, and ultracentrifuging samples for 16h at 40,000 rpm on a SW-55 rotor (Beckman Coulter): 625 µl fractions were carefully harvested from the top of each tube, and density was determined by weighing. Infectivity of each fraction was quantified by TCID50 using anti-NS5A antibody as described[5, 6], while HCV RNA of fractions was purified and analyzed as described above. ApoB and ApoE concentrations of fractions were determined by enzyme-linked immunosorbent assay (Human Apolipoprotein B or E ELISA[PRO] kit, Mabtech) undiluted or in a 1:50 dilution, respectively, according to the manufacturer's instructions (Mabtech).

1   **miRNA mimics and siRNAs.** Non-targeting control miRNA, miR-501-3p mimic, miR-619-3p

2   mimic, antagomiR-122, antagomiR-501-3p, non-targeting control antagomiR, non-targeting

3   control siRNA, siRNAs targeting *OGT, CD81* or *apoliporotein E* (*ApoE*) and a library of 28

4   custom ON-TARGETplus smart pool siRNAs were purchased from Dharmacon (GE

5   Healthcare).

6

7   **miRNA expression analysis.** Total RNA (100 ng) was purified from control or HCV-infected

8   Huh7.5.1 cells using Tri reagent® (Thermo Scientific) and Direct-zol™ RNA purification kit

9   (Zymo Research). Total RNA was first polyadenylated and reverse transcribed using a

10 miScript II RT system (Qiagen) according to the manufacturer's instructions. The obtained

11 cDNA was subjected to RT-qPCR using miScript SYBR Green kit (Qiagen). Primers were the

12 mature miRNA sequence for the forward primer (Thermo Scientific) and the universal miScript

13 primer (Qiagen) for the reverse primer. Data were analyzed by the ΔΔCt method using small

14 nucleolar RNA, C/D box 61 (SNORD61) as an endogenous reference and the non-infected

15 samples as a calibrator[7].

16

17 **Antibodies**. Rabbit anti-OGT antibodies DM-17 and AL24 were purchased from Sigma or

18 kindly provided by Dr. G. W. Hart and Dr. S. Hardivillé (Johns Hopkins University School of

19 Medicine, Baltimore, MD)[8], respectively. Mouse anti-β-actin antibody was purchased from

20 Abcam and mouse, rabbit or sheep HRP-conjugated secondary antibodies (A9044, A0545

21 and A3415, respectively) were purchased from Sigma. Sheep anti-NS5A serum for

22 determination of TCID50 was a kind gift from M. Harris[9]. Human anti-E2 (AR3A) antibody[10]

23 for electron microscopy analysis was kindly provided by Mansun Law (SCRIPPS, California,

24 USA).

25

26 **Western blotting.** OGT and actin protein expression in human cells was assessed by

27 Western blot as described[8] with some modifications. Briefly, cells were lysed in lysis buffer

28 no. 6 (R&D Systems) according to the manufacturer's instructions. Equal amounts of protein

1   (40 μg) were size-separated through a Mini PROTEAN® TGX Stain-Free™ gel electrophoresis

2   (Bio-Rad) and transferred to PVDF membranes (Bio-Rad). Immunoblots were performed

3   using rabbit anti-OGT (1:2000) and mouse anti-β-actin (1:1000) antibodies[8, 11]. Antigen-

4   antibody complexes were detected by incubating the membrane with the appropriate HRP-

5   conjugated secondary antibodies (1:5000; 1:10,000) and imaged by enhanced

6   chemiluminescence with a ChemiDoc MP imager (Bio-Rad). Quantification of protein

7   expression was performed using ImageLab™ 5.2.1 software (BioRad). For analysis of OGT

8   and GAPDH expression in liver tissue from HCV transgenic (FL-N/35) or wild-type mice[12],

9   crude protein extracts were prepared by homogenization of frozen mouse livers (50–100 μg)

10 in tissue lysis buffer from the Ambion PARIS RNA (Thermo Scientific) and protein isolation kit,

11 supplemented with protease inhibitors (cOmpleteTM EDTA-free protease inhibitor mixture,

12 Sigma-Aldrich) and phosphatase inhibitors (PhosSTOPTM, Sigma-Aldrich), using a tissue

13 homogenizer (MP Fast Prep24, MP Biomedicals, Santa Ana, CA) and MP Lysing Matrix A

14 tubes. Proteins were quantified using the BCA assay (Thermo Fisher Scientific). Western

15 blotting was performed as described above.

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17 **Immunocapture and electron microscopy analysis of viral particles.** Sucrose-cushion

18 purified or iodixanol gradient fractionated HCVcc (JcR2a) produced in cells transfected with a

19 non-targeting siRNA control or a pool of siRNA against OGT were transferred via anti-E2

20 antibody AR3A on electron microscopy (EM) grids through immunocapture (IC) as

21 described[13]. Particles were stained with uranyl acetate dihydrate and observed in a JEOL

22 1230 electron microscope. Series of electron micrographs were acquired at random from IC

23 EM grids. The images were then analyzed with Image-J software, to determine the particle

24 size distribution.

25

26 **Gene expression analysis in patient-derived liver tissue**. For OGT expression analysis in

27 patient's samples, raw data were retrieved from the Gene Expression Omnibus (GSE84346)

28 and re-analyzed by quality-trimming (cutadapt) and mapping (HISAT2) to human genome

assembly hg19. Reads mapping to Gencode v.19 genes were counted using htseq-count and

normalized applying DESeq2. Activity and fibrosis scores as well as viral load were taken from

the supplemental data published in[14]. To analyze OGT expression in liver tissue of chronic

hepatitis B or C patients, FPKM values and clinical data were retrieved from The Cancer

Genome   Atlas   (TCGA,   https://www.cancer.gov/about-

nci/organization/ccg/research/structural-genomics/tcga). This data set includes samples from

39 HCV-infected patients, 83 hepatitis B virus (HBV)-infected, 80 patients with alcoholic liver

disease (ALD) and 13 patients with non-alcoholic fatty liver disease (NAFLD).

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Supplementary figure legends

Figure S1. (A) Effect of miR-501-3p inhibition on HCV infectivity. Huh7.5.1 cells were transfected with control antagomiR (Ctrl), antagomiR-122 as loss-of-function control to perturb HCV replication and antagomiR-501-3p, prior to infection with HCVcc (JcR2a) according to the two-step protocol depicted in Fig. 1A. After 48h, supernatants were transferred onto naive Huh7.5.1 cells. After 72h, Renilla Luciferase activity of infected Huh7.5.1 cells was determined. Data are expressed as mean percentage as compared to Ctrl ± s.d. Results are from four independent experiments in quadruplicate. The dashed line indicates values from vehicle-treated cells set at 100%. Statistics**: ***, *p*-value < 0.05, Mann-Whitney test. (B) miR-501-3p expression upon HCV infection. Huh7.5.1 cells were infected with HCVcc (JcR2a).

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1   After 72h, RNA was purified and miR-501-3p expression analyzed by RT-qPCR. Percentage

2   of miR-501-3p expression relative to uninfected Huh7.5.1 cells (Ctrl). Results are presented

3   as mean ± s.d. from three independent experiments in duplicate. The dashed line indicates

4   values from uninfected Huh7.5.1 cells set at 100%. Statistics: *, *p*-value < 0.05, Mann-Whitney

5   test.

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7   **Supplementary Table 1. A genome-wide miRNA mimic screen identifies cellular**

8   **miRNAs modulating HCV infection.** Log2(FC), lfdr and effect on HCV infection in part 1 and

9   part 2 of the screen are shown for the individual miRNAs of the miRNA mimic library. In red:

10   proviral effect, in blue: antiviral effect. FC: fold change, lfdr: local false discovery rate

miRNA ID	Mature Sanger ID	Library ID	Mature Sequence	Part 1			Part 2		
				Log2(FC)	lfdr	Effect on HCV	Log2(FC)	lfdr	Effect on HCV
hsa-let-7a	MIMAT0000062	C-300473-05	UGAGGUAGUAGGUUGUAUAGUU	-1.701719359696617	3.9917e-05	TRUE	-3.28400225976063	0.0018396	TRUE
hsa-let-7b	MIMAT0000063	C-300476-05	UGAGGUAGUAGGUUGUGUGGUU	-1.746840911917792	8.3715e-05	TRUE	-3.61453853652595	0.009387	TRUE
hsa-let-7d	MIMAT0000065	C-300478-07	AGAGGUAGUAGGUUGCAUAGUU	-1.530759329763348	0.00023985	TRUE	-2.74632167025162	0.057617	TRUE
hsa-let-7e	MIMAT0000066	C-300479-05	UGAGGUAGGAGGUUGUAUAGUU	-1.30271875995011	0.0014577	FALSE	-1.90993819018051	0.081661	TRUE
hsa-let-7f	MIMAT0000067	C-300480-05	UGAGGUAGUAGAUUGUAUAGUU	-2.221784356196614	6.7884e-06	TRUE	-3.76872186786763	0.014453	TRUE
hsa-let-7g	MIMAT0000414	C-300583-05	UGAGGUAGUAGUUUGUACAGUU	-1.912695266519093	6.7884e-06	TRUE	-2.718787559599202	0.057617	TRUE
hsa-miR-101	MIMAT0000099	C-300518-07	UACAGUACUGUGAUAACUGAA	-0.886376348041933	0.0030028	FALSE	-1.9796810124946	0.11887	TRUE
hsa-miR-103-as	MIMAT0007402	C-301453-00	UCAUAGCCCUGUACAAUGCUGCU	-1.11208100417218	0.0074787	FALSE	-2.91287954813545	0.057617	TRUE
hsa-miR-106a*	MIMAT0004517	C-301159-01	CUGCAAUGUAAGCACUUCUUAC	-1.32520114049511	3.9917e-05	TRUE	-2.3773966141336	0.11887	TRUE
hsa-miR-1178	MIMAT0005823	C-301319-00	UUGCUCACUGUUCUUCCCUAG	-1.225567286667959	0.00061109	FALSE	-2.3126959903038	0.08542	TRUE
hsa-miR-1178-5p	MIMAT0022940	C-301922-00	CAGGGUCAGCUGAGCAUG	-1.16672245017752	0.00026644	TRUE	-0.780775920183507	0.72813	FALSE
hsa-miR-1185	MIMAT0005798	C-301317-00	AGAGGAAACCCUUUGUAUGUU	-1.2315368528283	8.3715e-05	TRUE	-0.721316088104541	0.76975	FALSE
hsa-miR-1200	MIMAT0005863	C-301326-00	CUCCUGAGCCAUUCUGAGCCUC	-1.248666404694154	0.00054279	FALSE	-2.09711908610931	0.08542	TRUE
hsa-miR-1205	MIMAT0005869	C-301331-00	UCUGCAGGGUUUGCUUUGAG	-0.801511472736558	0.0053924	FALSE	-3.77846481724539	0.0018396	TRUE
hsa-miR-1207-3p	MIMAT0005872	C-301334-00	UCAGCUGGCCCUCAUUUC	-1.10040811819955	0.00013963	TRUE	-1.69117584901668	0.26604	FALSE
hsa-miR-122	MIMAT0000421	C-300591-05	UGGAGUGUGACAAUGGUGUUUG	-0.70369537171691	0.040577	FALSE	-3.13425305602985	0.028781	TRUE
hsa-miR-122*	MIMAT0004590	C-301046-01	AACGCCAUUAUCACACUAAAUA	-0.557131436052541	0.037103	FALSE	-1.83404561163316	0.11887	TRUE
hsa-miR-1226	MIMAT0005577	C-301284-01	UCACCAGCCCUGUGUUCCCUAG	-0.741744822716559	0.0099219	FALSE	-1.89688153334356	0.11887	TRUE
hsa-miR-1228*	MIMAT0005582	C-301287-01	GUGGGCGGGGGCAGGUGUGUG	-0.947568053691086	0.0049772	FALSE	-2.29154418859234	0.11887	TRUE
hsa-miR-1237-5p	MIMAT0022946	C-302618-00	CGGGGGCGGGGCCGAAGCGCG	-0.956259568660452	0.021994	FALSE	-2.34546898601693	0.057617	TRUE
hsa-miR-1244	MIMAT0005896	C-301865-00	AAGUAGUUGGUUUGUAUGAGAUGGGUU	-1.17945761818024	6.1693e-05	TRUE	-0.989861012597079	0.5811	FALSE
hsa-miR-1245b-3p	MIMAT0019951	C-302405-00	UCAGAUGAUCUAAAGGCCUAUA	-0.414846884881959	0.32544	FALSE	-2.27819747964432	0.081661	TRUE
hsa-miR-1255b-2-3p	MIMAT0022725	C-301882-00	AACCACUUUCUUUGUCUCAUCCA	-0.427268737714942	0.061384	FALSE	-2.58892190360625	0.023579	TRUE
hsa-miR-1258	MIMAT0005909	C-301384-00	AGUUAGGAUUAGGUCGUGGAA	-1.57210875214914	1.5894e-05	TRUE	-1.63979276673705	0.36109	FALSE
hsa-miR-125b-2*	MIMAT0004603	C-301061-01	UCACAAGUCAGGCUCUUGGGAC	-0.858902068537019	0.0052694	FALSE	-1.87406855538924	0.11887	TRUE
hsa-miR-1260b	MIMAT0015041	C-301716-00	AUCCCACCACUGCCACCAU	-0.476317862532308	0.10991	FALSE	2.46099391534969	0.057617	TRUE
hsa-miR-1270	MIMAT0005924	C-301867-00	CUGGAGAUAUGGAAGAGCUGUGU	-1.23553336541613	3.9917e-05	TRUE	-2.24160380684469	0.11887	TRUE
hsa-miR-1277	MIMAT0005933	C-301411-00	UACGUAGAUAUAUAUGUAUUU	-0.745198002045239	0.0074787	FALSE	-3.58615793633136	0.014453	TRUE
hsa-miR-1278	MIMAT0005936	C-301417-00	UAGUACUGUGCAUAUCAUCUAU	-0.494528345361641	0.089234	FALSE	1.60846287059147	0.12226	TRUE
hsa-miR-1283	MIMAT0005799	C-301315-00	UCUACAAAGGAAAGCGCUUUC	-0.538161112092319	0.061384	FALSE	-2.67520055202511	0.08542	TRUE
hsa-miR-1284	MIMAT0005941	C-301423-00	UCUAUACAGACCCUGGCUUUUC	-0.412436737654723	0.099257	FALSE	-2.28002191497783	0.11527	TRUE
hsa-miR-1287	MIMAT0005878	C-301341-00	UGCUGGAUCAGUGGUUCGAGUC	-1.20683701032783	0.00023985	TRUE	-1.98393331688063	0.08542	TRUE
hsa-miR-1288	MIMAT0005942	C-301424-00	UGGACUGCCCUGAUCUGGAGA	-0.704889827052788	0.0021076	FALSE	-2.89790974514386	0.057617	TRUE
hsa-miR-129-3p	MIMAT0004605	C-301063-01	AAGCCCUUACCCCAAAAAGCAU	-0.999602920325209	0.0021076	FALSE	-1.88681635888787	0.11887	TRUE
hsa-miR-1291	MIMAT0005881	C-301345-00	UGGCCCUGACUGAAGACCAGCAGU	-1.21284220826454	0.00061109	FALSE	-2.02374608043906	0.11887	TRUE
hsa-miR-1293	MIMAT0005883	C-301347-00	UGGGUGGUCUGGAGAUUUGUGC	-1.39687224389743	0.00026844	TRUE	-2.31295737423138	0.057617	TRUE
hsa-miR-1294	MIMAT0005884	C-301348-00	UGUGAGGUUGGCAUUGUUGUCU	-1.44051810095446	8.3715e-05	TRUE	-1.95652126826796	0.057617	TRUE
hsa-miR-1295	MIMAT0005885	C-301349-00	UUAGGCCGCAGAUCUGGGUGA	-1.2640855905457	0.00087976	FALSE	-2.34227893604624	0.11887	TRUE
hsa-miR-1295b-5p	MIMAT0022593	C-302563-00	CACCCAGACUGCGGCCCUAAU	-0.454374224389677	0.17522	FALSE	-2.73444344828677	0.023579	TRUE
hsa-miR-1298	MIMAT0005800	C-301318-00	UUCAUUCGGCUGUCCAGAUGUA	-0.26688230187125	0.32544	FALSE	-2.44553337713544	0.11887	TRUE
hsa-miR-1302	MIMAT0005890	C-301869-00	UUGGGACAUACUUAUGCUAAU	-1.35138412635794	3.6716e-05	TRUE	-2.60359528922327	0.057617	TRUE
hsa-miR-1302	MIMAT0005890	C-301354-00	UUUGGGACAUACUUAUGCUAAA	-1.11168868347783	0.00013793	TRUE	-1.29913904583381	0.51048	FALSE
hsa-miR-1307	MIMAT0005951	C-301434-00	ACUCGGCGUGGCGUCGGUCGUG	-1.12000717587297	0.00054279	FALSE	-2.10295176202445	0.057617	TRUE
hsa-miR-1307-5p	MIMAT0022727	C-301875-00	UCGACCGGACCUCGACCGGCU	-0.803481003211417	0.0030028	FALSE	-1.76491161834359	0.11887	TRUE
hsa-miR-1322	MIMAT0005963	C-301436-00	GAUGAUGCGCUGAUGCUG	-1.23817643374983	6.1693e-05	TRUE	-1.29920754317295	0.46329	FALSE
hsa-miR-1323	MIMAT0005795	C-301311-00	UCAAAACUGAGGGGCAUUUUCU	-0.756897047236657	0.096607	FALSE	-2.43084213259459	0.11887	TRUE
hsa-miR-135b*	MIMAT0004698	C-301200-01	AUGUAGGGCUAAAAGCCAUGGG	-1.49530939618559	0.0018747	FALSE	-2.98188718020305	0.050122	TRUE
hsa-miR-139-3p	MIMAT0004552	C-301036-03	UGGAGACGCGGCCCUGUUGGAGU	-1.02065377094153	0.00079918	FALSE	-2.77458369079406	0.019699	TRUE
hsa-miR-140-3p	MIMAT0004597	C-301055-01	UACCACAGGGUAGAACCACGG	-0.172675601522775	1	FALSE	2.56894688372309	0.11887	TRUE
hsa-miR-142-3p	MIMAT0000434	C-300610-03	UGUAGUGUUUCCUACUUUAUGGA	-1.00474764620935	0.00023985	TRUE	-1.7577126946261	0.22566	FALSE
hsa-miR-146a	MIMAT0000449	C-300630-03	UGAGAACUGAAUUCCAUGGGUU	0.279820319960640	0.17522	FALSE	1.66479155066337	0.11887	TRUE
hsa-miR-150*	MIMAT0004610	C-301067-01	CUGGUACAGGCCUGGGGGACAG	-1.46501526099695	0.00023985	TRUE	-1.19620229448666	0.40677	FALSE
hsa-miR-151b	MIMAT0010214	C-301973-00	UCGAGGAGCUCACAGUCU	-1.03668648961171	3.9917e-05	TRUE	-2.8985532020104	0.057617	TRUE
hsa-miR-182	MIMAT0000259	C-300557-07	UUUGGCAAUGGUAGAACUCACACU	-0.581360116993454	0.03164	FALSE	-1.81305061189634	0.11887	TRUE
hsa-miR-184	MIMAT0000454	C-300635-03	UGGACGGAGAACUGAUAAGGGU	-1.31700777618768	3.9917e-05	TRUE	-1.52851322717993	0.36109	FALSE
hsa-miR-185	MIMAT0000455	C-300636-07	UGGAGAGAAAGGCAGUUCCUGA	-1.59673044023595	0.00013963	TRUE	-1.74454670174015	0.1633	FALSE
hsa-miR-18b*	MIMAT0004751	C-301187-01	UGCCCUAAAUGCCCCUUCUGGC	-0.825568919117593	0.0027607	FALSE	-2.30862554668178	0.023579	TRUE
hsa-miR-1909	MIMAT0007883	C-301456-00	CGCAGGGGCCGGGUGCUCACCG	-0.511863313449074	0.17522	FALSE	2.49877552994666	0.11887	TRUE
hsa-miR-1915*	MIMAT0007891	C-301466-00	ACCUUGCCUUGCUGCCCGGGCC	-1.71935071114424	8.3715e-05	TRUE	-1.13532112712663	0.46329	FALSE
hsa-miR-196b*	MIMAT0009201	C-301305-00	UCGACAGCACGACACGCCUUC	-0.949306838412525	0.00054279	FALSE	-3.1358874675771	0.023579	TRUE
hsa-miR-19b	MIMAT0000074	C-300489-03	UGUGCAAAUCCAUGCAAAACUGA	-1.01461439258753	0.00013793	TRUE	-2.75192926226938	0.014453	TRUE
hsa-miR-19b-1*	MIMAT0004491	C-301021-01	AGUUUUGCAGGUUUGCAUCCAGC	-1.68388148726454	0.0030028	FALSE	-2.85695043305243	0.014453	TRUE
hsa-miR-19b-2*	MIMAT0004492	C-301139-01	AGUUUUGCAGGUUGCAUUGA	-0.594068837054943	0.037103	FALSE	-2.06967686748099	0.11887	TRUE
hsa-miR-200b*	MIMAT0004571	C-301144-01	CAUCUUACUGGGCAGCAUUGGA	-1.05721574165849	0.0074787	FALSE	-4.59657299807562	0.0018396	TRUE
hsa-miR-203b-5p	MIMAT0019813	C-302277-00	UAGUGGUCCUAAACAUUUCACA	-1.0335612225008	0.00016067	TRUE	-2.13526669981558	0.08542	TRUE
hsa-miR-208a	MIMAT0000241	C-300537-03	AUAAGACGAGCAAAAAGCUUGU	-0.823665097293502	0.0030028	FALSE	-1.54639877799123	0.11887	TRUE
hsa-miR-21	MIMAT0000076	C-300492-03	UAGCUUAUCAGACUGAUGUUGA	-0.768228630205262	0.096607	FALSE	3.29607415881261	0.057617	TRUE
hsa-miR-211-3p	MIMAT0022694	C-301905-00	GCAGGGACGCAAAGGGGUGC	-0.812107428438748	0.0027607	FALSE	-2.21028883028492	0.08542	TRUE
hsa-miR-2114	MIMAT0011156	C-301489-00	UAGUCCCUUCCUUGAAGCGGUC	-0.958796902783202	0.00016326	TRUE	-1.5768286317563	0.26604	FALSE
hsa-miR-2114*	MIMAT0011157	C-301490-00	CGAGCCUCAAGCAAGGGACUU	-1.02419077915881	0.00013793	TRUE	-2.57866858794879	0.057617	TRUE
hsa-miR-2117	MIMAT0011162	C-301496-00	UGUUCUCUUUGCCAAGGACAG	-0.806177347742896	0.00054279	FALSE	-2.55943646917884	0.057617	TRUE
hsa-miR-216a-3p	MIMAT0022844	C-301886-00	UCACAGUGGUCUCUGGGAUUAU	-1.47806207137632	8.3715e-05	TRUE	-1.62257411616963	0.1633	FALSE
hsa-miR-22	MIMAT0000077	C-300493-03	AAGCUGCCAGUUGAAGAACUGU	-1.14812626998085	0.00054279	FALSE	-2.8254651928315	0.009387	TRUE
hsa-miR-220b	MI0005529	C-301218-01	CCACCACCGUAAGCUGCACAU	-1.10856445415353	0.12653	FALSE	-2.50799687656707	0.11887	TRUE
hsa-miR-221*	MIMAT0004568	C-301163-01	ACCUGGCAUACAAUGUAGAUUU	-1.0647285020049	0.00016067	TRUE	0.493351763855846	0.86349	FALSE
hsa-miR-223*	MIMAT0004570	C-301197-01	CGUGUAUUUGACAAGCUGAGUU	-0.596047980424722	0.0074787	FALSE	-2.64001675119474	0.057617	TRUE
hsa-miR-2276	MIMAT0011775	C-301481-00	UCUGCAAGUGUCAGAGGCGAGG	-1.23563628890583	3.9917e-05	TRUE	-0.719842576977581	0.76975	FALSE
hsa-miR-2277-3p	MIMAT0011777	C-301482-00	UGACAGCGCCCUGCCUGGCUC	-1.22725149342576	3.9917e-05	TRUE	-2.2780178572373	0.11887	TRUE
hsa-miR-23a*	MIMAT0004496	C-301025-01	GGGGUUCCUGGGGAUGGGAUUU	-0.773715976814457	0.0018747	FALSE	-2.22574058493535	0.11887	TRUE
hsa-miR-25*	MIMAT0004498	C-301183-01	AGGCGGAGACUUGGGCAAUUG	-1.14410824968417	0.0018747	FALSE	-2.13159960818718	0.057617	TRUE
hsa-miR-2681-3p	MIMAT0013516	C-301978-00	UAUCAUGGAGUUGGUAAAGCAC	-0.841542410888718	0.00023985	TRUE	-0.564927523090931	0.86349	FALSE
hsa-miR-2681-5p	MIMAT0013515	C-301977-00	GUUUUACCACCUCCAGGAGACU	-1.20496402288836	3.6716e-05	TRUE	-2.40988448605506	0.057617	TRUE
hsa-miR-2682-3p	MIMAT0013518	C-301980-00	CGCCUCUUCAGCGCUGCUUUCC	-0.908772237664288	0.00013793	TRUE	-1.43184476418853	0.29187	FALSE
hsa-miR-2682-5p	MIMAT0013517	C-301979-00	CAGGCAGUACGUAUUGACAGUGCGC	-1.24830345350196	1.5894e-05	TRUE	-2.80779774369284	0.014453	TRUE
hsa-miR-26b	MIMAT0000083	C-300501-07	UUCAAGUAAUUCAGGAUAGGU	-0.405072630482104	0.12653	FALSE	-2.50877510131769	0.081661	TRUE
hsa-miR-27a	MIMAT0000084	C-300502-03	UUCACAGUGGCUAAGUUCCGC	-1.32790971119818	3.9917e-05	TRUE	-1.95676458087438	0.1633	FALSE
hsa-miR-27a*	MIMAT0004501	C-301028-01	AGGGCUUAGCUGCUUGUGAGCA	-0.887009503478049	0.0052694	FALSE	-3.44308089121935	0.028781	TRUE
hsa-miR-27b*	MIMAT0004588	C-301154-01	AGAGCUUAGCUGAUUGGUGAAC	-1.00848877755825	0.0014577	FALSE	-2.34115081043794	0.050122	TRUE
hsa-miR-28-5p	MIMAT0000085	C-300503-05	AAGGAGCUCACAGUCUAUUGAG	-0.358344656239635	0.17522	FALSE	-1.5918060993224	0.11887	TRUE
hsa-miR-2861	MIMAT0013802	C-301642-00	GGGGCCUGGCGGUGGGCGG	-0.615484847088555	0.0046172	FALSE	-2.44784331747309	0.081661	TRUE
hsa-miR-2964a-3p	MIMAT0019748	C-302218-00	AGAAUUGCGUUUGGACAACAGU	-1.13977573653624	6.1693e-05	TRUE	-0.558827938583115	0.76975	FALSE
hsa-miR-298	MIMAT0004901	C-301212-01	AGCAGAAGCAGGGAGGUUCUCCCA	-0.456122353506126	0.54004	FALSE	-2.83068032162142	0.11887	TRUE
hsa-miR-299-5p	MIMAT0002890	C-300854-03	UGGUUUACCGUCCCACUACAU	-1.25864926851518	0.00023985	TRUE	-0.317867194423722	1	FALSE
hsa-miR-29a*	MIMAT0004503	C-301178-01	ACUGAUUUCUUUUGGUGUUCAG	-1.35357089379682	0.0021076	FALSE	-2.75349311494331	0.057617	TRUE
hsa-miR-29b1-1*	MIMAT0004514	C-301150-01	GCUGGUUUCAUAUGGUGGUUUAGA	-0.970652939446982	0.015067	FALSE	-2.38342736203068	0.11887	TRUE
hsa-miR-301b	MIMAT0004958	C-301252-01	CAGUGCAAUGAUAUUGUCAAAGC	-0.837318501823261	0.0014577	FALSE	-2.28547978225747	0.11887	TRUE
hsa-miR-302b*	MIMAT0000714	C-300668-07	ACUUUAACAUGGAAGUGCUUUC	-0.550910910384927	0.037103	FALSE	-1.76045353694163	0.12226	TRUE
hsa-miR-302f	MIMAT0005932	C-301410-00	UAAUUGCUUCCAUGUUU	-1.5960005623826	1.5894e-05	TRUE	-3.17802223827817	0.014453	TRUE
hsa-miR-30b	MIMAT0000420	C-300590-03	UGUAAACAUCCUACACUCAGCU	-0.759206041294187	0.0018747	FALSE	-2.5817157170778	0.057617	TRUE
hsa-miR-30c-1*	MIMAT0004674	C-301199-01	CUGGGAGAGGGUUGUUUACUCC	-1.437877868685483	8.3715e-05	TRUE	0.291968781990601	1	FALSE
hsa-miR-30c-2*	MIMAT0004550	C-301034-01	CUGGGAGAAGGCUGUUUACUCU	-1.18964958597662	8.3715e-05	TRUE	-0.203715081689554	1	FALSE
hsa-miR-30d	MIMAT0000245	C-300543-03	UGUAAACAUCCCCGACUGGAAG	-0.469794284968542	0.061384	FALSE	-2.24325814749711	0.08542	TRUE
hsa-miR-3115	MIMAT0014977	C-301644-00	AUAUGGGUUUUACUAGUUGGU	-0.595634685516391	0.0052694	FALSE	-2.22531374934429	0.11887	TRUE
hsa-miR-3116	MIMAT0014978	C-301645-00	UGCCUGGAACAAUGAUGGAGAUUU	-1.61818516940984	6.7884e-06	TRUE	-1.27627977004857	0.51048	FALSE
hsa-miR-3117	MIMAT0014979	C-301647-00	AUAGGACUCAUAUAGUGCCAG	-1.079446926933698	6.1693e-05	TRUE	0.583708702116637	1	FALSE
hsa-miR-3119	MIMAT0014981	C-301651-00	UGGCUUUUAACUUUGAUGGC	-0.77136494055742	0.00087976	TRUE	-2.20041353445194	0.11887	TRUE
hsa-miR-3121	MIMAT0014983	C-301654-00	UAAAUAGAGUAGGCAAAGGACA	-0.550473953423974	0.0074787	FALSE	-2.06892978205979	0.11887	TRUE
hsa-miR-3124	MIMAT0014986	C-301657-00	UUCGCGGGCGAAGGCAAAGUC	-1.13549064733399	3.9917e-05	TRUE	-0.910461837996433	0.5811	FALSE
hsa-miR-3126-5p	MIMAT0014989	C-301661-00	UGAGGGACGAAUGCCAGAAGCA	-1.43373948465677	1.5894e-05	TRUE	-0.744651597941774	0.76975	FALSE
hsa-miR-3127	MIMAT0014990	C-301662-00	AUCAGGGCUUGUGGAAUGGGAAG	-0.992180086498039	0.00013793	TRUE	-1.20167440176899	0.51048	FALSE
hsa-miR-3130-3p	MIMAT0014994	C-301665-00	GCUGCACCGGAGACUGGGUAA	-0.645251817092445	0.0021076	FALSE	-3.33421355039417	0.023579	TRUE
hsa-miR-3130-5p	MIMAT0014995	C-301666-00	UACCCAGUCUCCGGUGCAGCC	-1.12058237373742	6.1693e-05	TRUE	-2.44166606880781	0.11887	TRUE
hsa-miR-3131	MIMAT0014996	C-301669-00	UCGAGGACUGGUGGAAGGGCCUU	-1.104753338687	6.1693e-05	TRUE	-0.776623344642163	0.76864	FALSE
hsa-miR-3132	MIMAT0014997	C-301670-00	UGGGUAGAGAAGGAGCUCAGAGGA	-1.06496839888313	8.3715e-05	TRUE	-2.09592053963675	0.11887	TRUE
hsa-miR-3136	MIMAT0015003	C-301676-00	CUGACUGAAUAGGUAGGGUCAUU	-0.837973854149906	0.00054279	FALSE	-2.76623087444917	0.057617	TRUE
hsa-miR-3137	MIMAT0015005	C-301678-00	UCUGUAGCCUGGGUCGAUGAUG	-1.05179643968079	8.3715e-05	TRUE	-1.1088570611196	0.51579	FALSE
hsa-miR-3138	MIMAT0015006	C-301679-00	UGUGGACAGUGAGUGGGAGGGGAGU	-0.924469604318995	0.00013793	TRUE	-1.78790357269758	0.22566	FALSE
hsa-miR-3139	MIMAT0015007	C-301680-00	UAGGAGCUCAACAGUGCCUGGU	-1.37716815069908	1.5894e-05	TRUE	-1.31106163706907	0.46329	FALSE
hsa-miR-3140	MIMAT0015008	C-301681-00	AGCUUUUGGGAAUUCAGGUAU	-1.58079760272847	6.7884e-06	TRUE	-3.18144016432131	0.014453	TRUE
hsa-miR-3142	MIMAT0015011	C-301684-00	AAGGCCUUUCUGAACCUUCAGA	-0.977053358967313	0.00013963	TRUE	-1.89446739540295	0.1633	FALSE
hsa-miR-3144-5p	MIMAT0015014	C-301687-00	AGGGGACCAAAGAGAUAUAUAG	-1.13947775501217	3.9917e-05	TRUE	0.414321660104908	1	FALSE
hsa-miR-3147	MIMAT0015019	C-301692-00	GGUUGGGGCAGUGAGGAGGGUGUGA	-0.942186557775687	0.00023985	TRUE	-0.157149408887514	1	FALSE
hsa-miR-3148	MIMAT0015021	C-301694-00	UGGAAAAAACGGGUAGGUUU	-1.14553360648851	3.9917e-05	TRUE	-1.08300314433575	0.51579	FALSE
hsa-miR-3150	MIMAT0015023	C-301696-00	CUGGGGAGAUCCUCGAGGUUGG	-1.4167699283789	1.5894e-05	TRUE	-0.986527775853387	0.5811	FALSE
hsa-miR-3150b-5p	MIMAT0019226	C-301961-00	CAACCUCGAGGAUCUCCCAGC	-1.04471163915449	3.9917e-05	TRUE	-2.12053729635218	0.08542	TRUE
hsa-miR-3151	MIMAT0015024	C-301697-00	GGUGGGGCAAUGGGAUCAGGU	-1.02537680303558	0.00013793	TRUE	-1.56671822128811	0.26604	FALSE
hsa-miR-3152	MIMAT0015025	C-301698-00	UGUGUUAGAAUAGGUGGCACUUUA	-1.13601969432149	6.1693e-05	TRUE	-2.17593163296179	0.11887	TRUE
hsa-miR-3157	MIMAT0015031	C-301704-00	UUCAGCCAGGCUAGUGCAGUCU	-1.81838789899633	0.00023985	TRUE	-0.798984655509925	0.76975	FALSE
hsa-miR-3180	MIMAT0018163	C-301583-03	UGGGGCGGAGCUUCCGGAGGCC	-1.376334135637947	0.00013793	TRUE	-1.97473785577519	0.050122	TRUE
hsa-miR-3186-5p	MIMAT0015067	C-301749-00	CAGGCGUCUGUCUCACGUGGCUU	-0.536494994531891	0.061384	FALSE	-2.19613374331388	0.11887	TRUE
hsa-miR-3190-5p	MIMAT0015073	C-301756-02	UGUGGCCAGCUAGUCCUCCCA	-1.568982136910	3.9917e-05	TRUE	-3.02592555730969	0.014453	TRUE
hsa-miR-3191-5p	MIMAT0022732	C-302750-00	CUCUCUGGCCGUCUACCUUCCA	-0.896644736818671	0.01741	FALSE	-2.93191050011036	0.057617	TRUE
hsa-miR-32*	MIMAT0004505	C-301181-01	CAAUUUAGUGUGUGUGAUAUUU	-0.561723514860645	0.37761	FALSE	-2.47247129599692	0.11887	TRUE

hsa-miR-323-5p	MIMAT0004696	C-301085-01	AGGUGGUCCGUGGCGCGUUCGC	-0.997451491983143	0.0049772	FALSE	-2.55198466781697	0.014453	TRUE
hsa-miR-323b-5p	MIMAT0001630	C-301724-00	AGGUUGUCCGUGGUGAGUUCGCA	-0.870040751918234	0.00054279	FALSE	-3.51049237466915	0.014453	TRUE
hsa-miR-324-3p	MIMAT0000762	C-300705-05	ACUGCCCCAGGUGCUGGGC	-0.930125097721032	0.0021076	FALSE	-2.18712046531723	0.081661	TRUE
hsa-miR-328	MIMAT0000752	C-300695-03	CUGGCCCUCUCUGCCCUUCCGU	-1.1639709975505	8.3715e-05	TRUE	-1.57395137899233	0.26604	FALSE
hsa-miR-339-3p	MIMAT0004702	C-301185-01	UGACGCCCUCGACGACGAGCCG	-0.424124048504712	0.061384	FALSE	-2.06515171637643	0.08542	TRUE
hsa-miR-342-5p	MIMAT0004694	C-301083-01	AGGGGUGCUAUCUGUGAUUGA	-1.66163157467999	0.00016326	TRUE	-2.01939161434937	0.12226	TRUE
hsa-miR-345-3p	MIMAT0022698	C-301887-00	GCCCUGAACGAGGGGCUCUGGAG	-0.839605975652715	0.0021076	FALSE	-2.80466918793858	0.023579	TRUE
hsa-miR-346	MIMAT0000773	C-300712-03	UGUCUGCCCGCAUGCCUGCCUCU	-1.22592404544756	0.00023985	TRUE	-1.35203588028221	0.20114	FALSE
hsa-miR-34a*	MIMAT0004557	C-301145-01	CAAUCAGCAAGUAUACUGCCCU	-1.27197605437233	6.1693e-05	TRUE	-0.378051811558674	1	FALSE
hsa-miR-3529-5p	MIMAT0019828	C-302292-00	AGGUAGACUGGGAUUUGUUGUU	-0.674576387471472	0.034257	FALSE	-1.99747275957492	0.11887	TRUE
hsa-miR-3612	MIMAT0017989	C-301505-00	AGGAGGCAUCUUGAGAAAUGGA	-1.26286494709099	0.00026644	TRUE	-1.44238772362423	0.51048	FALSE
hsa-miR-3616-3p	MIMAT0017996	C-301512-00	CGAGGGCAUUUCAUGAUGCAGGC	-1.10327166715049	0.00054279	FALSE	-1.69108153949499	0.11887	TRUE
hsa-miR-3619-3p	MIMAT0019219	C-301952-00	GGGACCAUCCUGCCUGCUGUGG	-0.361591800679582	0.037103	FALSE	-2.1201206119135	0.11887	TRUE
hsa-miR-3620-5p	MIMAT0022967	C-302619-00	GUGGGCUGGGCUGGGCUGGGCC	-0.963210303847923	0.01741	FALSE	-2.95077111022971	0.014453	TRUE
hsa-miR-3622b-5p	MIMAT0018005	C-301522-00	AGGCAUGGGAGGUCAGGUGA	-1.03124833680421	0.00087976	FALSE	-1.86140566900932	0.08542	TRUE
hsa-miR-365	MIMAT0000710	C-300666-03	UAAUGCCCCUAAAAAUCCUUAU	-1.07713706038737	0.03164	FALSE	-2.565586989697	0.12226	TRUE
hsa-miR-3654	MIMAT0018074	C-301532-00	GACUGGACAAGCUGAGGAA	-0.394926641879625	0.096607	FALSE	-1.99274206927806	0.057617	TRUE
hsa-miR-365b-5p	MIMAT0022833	C-301900-00	AGGGACUUUCAGGGGCACUGG	-1.17934943470377	0.00026644	TRUE	-1.48034463468101	0.22566	FALSE
hsa-miR-3666	MIMAT0018088	C-301546-00	CAGUGCAAGUGUAGAUGCCGA	-0.8322031427779	0.0027607	FALSE	-2.76031104596189	0.009387	TRUE
hsa-miR-3667-3p	MIMAT0018090	C-301547-00	ACCUUCCUCUCCAUGGGUUGUCU	-1.53737109621222	6.1693e-05	TRUE	-0.189890808664278	1	FALSE
hsa-miR-3674	MIMAT0018097	C-301555-00	AUUGUAGAACCUAAGAUUGGCC	-0.686422173208126	0.0099219	FALSE	-1.66427885423157	0.08542	TRUE
hsa-miR-3675-5p	MIMAT0018098	C-301557-00	UAUGGGGCUUCUGUAGAGAUUUC	-0.704414688397948	0.0074787	FALSE	-1.47755963621301	0.11887	TRUE
hsa-miR-3676-5p	MIMAT0022734	C-301954-00	AGGAGAUCCUGGGUU	-0.876412168433536	0.00016326	TRUE	-2.81631813362903	0.028781	TRUE
hsa-miR-3680	MIMAT0018106	C-301565-00	GACUCACUCACAGGAUUGUGCA	-0.710055072199479	0.0074787	FALSE	-1.48818626629073	0.11887	TRUE
hsa-miR-3680*	MIMAT0018107	C-301564-00	UUUUGCAUGACCCUGGGAGUAGG	-0.68553099516054	0.0099219	FALSE	-1.8440688012842	0.057617	TRUE
hsa-miR-3681	MIMAT0018108	C-301566-00	UAGUGGAUGAUGCACUCUGUGC	-1.41416859913926	0.00013793	TRUE	-1.60964747472672	0.1633	FALSE
hsa-miR-3681*	MIMAT0018109	C-301567-00	ACACAGUGCUUCAUCCACUACU	-1.39472879333958	0.00013793	TRUE	-1.15667754722324	0.26604	FALSE
hsa-miR-3682	MIMAT0018110	C-301568-00	UGAUGAUACAGGUGGAGGUAG	-1.46673360047121	8.3715e-05	TRUE	-0.643125216570606	0.72813	FALSE
hsa-miR-3682-5p	MIMAT0019222	C-301956-00	CUACUUCUACCUGUGUUAUCAU	-1.23125674549655	1.5894e-05	TRUE	-2.81908923583428	0.023579	TRUE
hsa-miR-3684	MIMAT0018112	C-301570-00	UUAGACCUAGUACACGUCCU	-0.580463595828849	0.021994	FALSE	-1.45573759159026	0.11887	TRUE
hsa-miR-3688	MIMAT0018116	C-301574-00	UAUGGAAAGACUUUGCCACUCU	-0.832441806479729	0.0027607	FALSE	-2.02033498247755	0.057617	TRUE
hsa-miR-3688-5p	MIMAT0019223	C-301957-00	AGUGGCAAAGUCUUUCCAUACU	-0.588424467468018	0.0030028	FALSE	-2.09619814608759	0.11887	TRUE
hsa-miR-3689b*	MIMAT0018181	C-301587-00	CUGGGAGGUGUGAUAUUGUGGU	-1.45567876271802	9.6971e-05	TRUE	-1.732466728882077	0.081661	TRUE
hsa-miR-3691	MIMAT0018120	C-301578-00	AGUGGAUGAUGGAGACUCGGUAC	-1.18761474653793	0.00054279	TRUE	-1.75547134579776	0.057617	TRUE
hsa-miR-3691-3p	MIMAT0019224	C-301958-00	ACCAAGUCUGCGUCAUCCUCUC	-0.810629756562153	0.00026644	TRUE	-1.96328717500158	0.11887	TRUE
hsa-miR-374a	MIMAT0000727	C-300681-05	UUAUAAUACAACCUGAUAAGUG	-0.718874319759212	0.0021076	FALSE	-2.60836689817691	0.081661	TRUE
hsa-miR-374c	MIMAT0018443	C-301630-00	AUAAUACAACCUGCUAAGUGCU	-0.337432743025632	0.32544	FALSE	-1.98175619738517	0.11887	TRUE
hsa-miR-374c-3p	MIMAT0022735	C-301969-00	CACUUAGCAGGUUGUAUUAUAU	-0.646260597978354	0.0018747	FALSE	-2.63477891317834	0.028781	TRUE
hsa-miR-376c	MIMAT0000720	C-300674-05	AACAUAGAGGAAAUUCCACGU	-0.624380737173221	0.0053924	FALSE	-2.68639877338995	0.057617	TRUE
hsa-miR-378b	MIMAT0014999	C-301672-00	ACUGGACUUGGAGGCAGAA	-0.950272981825821	0.00016326	TRUE	-0.901403427172712	0.72813	FALSE
hsa-miR-378g	MIMAT0018937	C-302000-00	ACUGGGCUUGGAGUCAGAAG	-0.836483927856313	0.00023985	TRUE	-1.88595985918819	0.11887	TRUE
hsa-miR-380*	MIMAT0000734	C-300688-03	UGGUUGACCAUAGAACAUGCGC	-1.15838836623363	0.0018747	FALSE	-2.13916483631805	0.12226	TRUE
hsa-miR-381-5p	MIMAT0022862	C-301920-00	AGCGAGGUUGCCCUUUGUAUAU	-0.569567310635211	0.021994	FALSE	-2.14982823754668	0.08542	TRUE
hsa-miR-383	MIMAT0000738	C-300692-03	AGAUCAGAAGGUGAUUGUGGCU	-0.925079491666264	0.0052694	FALSE	-2.8317437192294	0.050122	TRUE
hsa-miR-3907	MIMAT0018179	C-301585-00	AGGUGCUCCAGGCUGGCUCACA	-1.38465900005315	0.00013793	TRUE	-0.257773760992354	1	FALSE
hsa-miR-3910	MIMAT0018184	C-301590-00	AAAGGCAUAAAACCAAGACA	-0.501095287980396	0.037103	FALSE	-1.43931285152292	0.11887	TRUE
hsa-miR-3913-3p	MIMAT0019225	C-301959-00	AGACAUCAAGAUCAGGUCCCAAA	-2.00645940017379	2.9753e-06	TRUE	-3.02362886477847	0.014453	TRUE
hsa-miR-3918	MIMAT0018192	C-301600-00	ACAGGGCCGCAGAUGGAGACU	-0.975422481909615	0.015067	FALSE	-2.442731722350024	0.081661	TRUE
hsa-miR-3922-5p	MIMAT0019227	C-301962-00	UCAAGGCCAGAGGUCCCAAGCA	-1.00561107859174	6.1693e-05	TRUE	-1.71505157929257	0.1633	FALSE
hsa-miR-3935	MIMAT0018350	C-301618-00	UGUAGAUACGAGCACCAGCCAC	-0.43578505271152	0.17522	FALSE	-2.47251139472301	0.057617	TRUE
hsa-miR-3972	MIMAT0019357	C-302158-00	CUGCCAGCCCGUUCCAGGAC	-1.16965458786124	0.00023985	TRUE	-1.70649157359084	0.40677	FALSE
hsa-miR-3975	MIMAT0019360	C-302161-00	UGAGGCUAAUGCACUACUUCAC	-1.37543164339877	8.3715e-05	TRUE	-1.26480301471027	0.51579	FALSE
hsa-miR-425*	MIMAT0001343	C-300718-07	AUCGGGAAUGUCGUGUCCGCCC	-0.937175749542432	0.00054279	TRUE	-3.15498966014918	0.028781	TRUE
hsa-miR-4253	MIMAT0016882	C-301816-00	AGGGCAUGUCCAGGGGGU	-1.35070000422013	0.00016326	TRUE	-2.478920178845828	0.11887	TRUE
hsa-miR-4290	MIMAT0016921	C-301856-00	UGCCCUCCUUUCUUCCCUC	-1.2579851622971	0.00026644	TRUE	-0.780428141336904	0.86349	FALSE
hsa-miR-4306	MIMAT0016858	C-301792-00	UGGAGGAAAGGCAGUA	-1.10160128889539	0.0046172	FALSE	-2.08580333486915	0.11887	TRUE
hsa-miR-4312	MIMAT0016864	C-301798-00	GGCCUUGUUCCUGUCCCCA	-0.866321092589685	0.0030028	FALSE	-2.71345507691741	0.11887	TRUE
hsa-miR-4314	MIMAT0016868	C-301802-00	CUCUGGGAAAUGGGACAG	-1.5364651282067	8.3715e-05	TRUE	-1.29173007577176	0.5811	FALSE
hsa-miR-4417	MIMAT0018929	C-301991-00	GGUGGGCUUCCCGGAGGG	-0.611560680456615	0.0021076	FALSE	-2.09593331520836	0.11887	TRUE
hsa-miR-4418	MIMAT0018930	C-301992-00	CACUGCAGGACUCAGCAG	-0.946166316606651	0.00013793	TRUE	-1.12537501301133	0.46329	FALSE
hsa-miR-4419a	MIMAT0018931	C-301993-00	UGAGGGAGGACAGUCA	-1.57297371821019	6.7884e-06	TRUE	-1.73343971226014	0.1633	FALSE
hsa-miR-4420	MIMAT0018933	C-301995-00	GUCACUGAUGUCUGUAGCUGAG	-0.924563865566564	0.00013963	TRUE	-0.533919074584704	0.86349	FALSE
hsa-miR-4422	MIMAT0018935	C-301997-00	AAAAGCAUCAGGAAGUCCCA	-0.890570506779186	0.00023985	TRUE	0.609244483333412	0.86349	FALSE
hsa-miR-4423-3p	MIMAT0018936	C-301999-00	AUAGGCACCAAAAAGCAACAA	-0.908201173118296	0.00013793	TRUE	-0.922983857808818	0.5811	FALSE
hsa-miR-4423-5p	MIMAT0019232	C-301998-00	AGUUGCCUUUUUGUUCCCAUGC	-0.973577427508824	8.3715e-05	TRUE	-1.75215013385816	0.1633	FALSE
hsa-miR-4425	MIMAT0018940	C-302003-00	UGUUGGGAUUCAGCAGGACCAU	-2.09487999782656	6.0932e-07	TRUE	-4.02209575968718	0.0018396	TRUE
hsa-miR-4426	MIMAT0018941	C-302004-00	GAAGAUGGACGUAUCUU	-1.51937100953274	6.7884e-06	TRUE	-1.79705743821345	0.12226	TRUE
hsa-miR-4427	MIMAT0018942	C-302005-00	UCUGAAUAGAGUCUGAAGAGU	-0.853522749406319	0.00023985	TRUE	-1.87772807758284	0.1633	FALSE
hsa-miR-4428	MIMAT0018943	C-302006-00	CAAGGAGACGGGAACAUGGAGC	-1.00869386291556	6.1693e-05	TRUE	-2.00933698314586	0.12226	TRUE
hsa-miR-4430	MIMAT0018945	C-302008-00	AGGCUGGAGUGAGCGGAG	-0.738546756609574	0.00054279	FALSE	-2.96189732903016	0.014453	TRUE
hsa-miR-4431	MIMAT0018947	C-302010-00	GCGACUCUGAAAACUAAGAGU	-0.605956299781477	0.0021076	FALSE	-1.84934532554394	0.11887	TRUE
hsa-miR-4432	MIMAT0018948	C-302011-00	AAAGACUCUGCAAGAUGCCU	-1.01744360848873	6.1693e-05	TRUE	-3.10500591742549	0.023579	TRUE
hsa-miR-4433-3p	MIMAT0018949	C-302012-00	ACAGGAGUGGGGGUGGGACA	-0.842019427210466	0.00023985	TRUE	-1.23537194391469	0.40677	FALSE
hsa-miR-4433-5p	MIMAT0020956	C-302013-00	CGUCCCACCCCCCACUCCUGU	-1.14337035396948	3.6715e-05	TRUE	-1.97664293733303	0.11887	TRUE
hsa-miR-4434	MIMAT0018950	C-302014-00	AGGAGAAGUAAAGUAGAA	-0.883254828802553	0.00013963	TRUE	-1.1657175196267	0.46329	FALSE
hsa-miR-4435	MIMAT0018951	C-302015-00	AUGGCCAGAGCUCACACAGAGG	-0.883497505941514	0.00023985	TRUE	-0.615152700112339	0.76975	FALSE
hsa-miR-4436a	MIMAT0018952	C-302016-00	GCAGGACAGGCAGAAGUGGAU	-1.04036408328386	3.9917e-05	TRUE	-1.592082960587719	0.22566	FALSE
hsa-miR-4437	MIMAT0018953	C-302018-00	UGGGCUCAGGGUACAAAGGUU	-1.08972765547642	3.9917e-05	TRUE	-1.15841014023857	0.51048	FALSE
hsa-miR-4438	MIMAT0018956	C-302021-00	CACAGGCUUAGAAAAGACAGU	-1.17220321847248	3.6715e-05	TRUE	-1.92817351938145	0.11887	TRUE
hsa-miR-4440	MIMAT0018958	C-302023-00	UGUCGUGGGGCUUUGCUUCGUUG	-1.11752167173499	3.9917e-05	TRUE	-2.33357733512522	0.08542	TRUE
hsa-miR-4441	MIMAT0018959	C-302024-00	ACAGGGAGGAGAUUGUA	-1.2984552451679	1.5894e-05	TRUE	-0.431268961949208	0.86349	FALSE
hsa-miR-4442	MIMAT0018960	C-302025-00	GCCGGACAAGAGGGAGG	-0.97262245143818	0.00023985	TRUE	-1.15721358729306	0.46329	FALSE
hsa-miR-4443	MIMAT0018961	C-302026-00	UUGGAGGCGUGGGGUUUU	-1.75283027769511	3.4785e-06	TRUE	-2.08359341608655	0.11887	TRUE
hsa-miR-4445-5p	MIMAT0018963	C-301976-00	AGAUUGUUUCUUUGUCCUGCA	-1.26374145331415	1.5894e-05	TRUE	-4.01626597303824	0.0018396	TRUE
hsa-miR-4447	MIMAT0018966	C-302029-00	GGUGGGGCUGUUGUUU	-0.841752979683909	0.00023985	TRUE	-2.88111133299715	0.028781	TRUE
hsa-miR-4449	MIMAT0018968	C-302031-00	CGUCCCGGGCCUGCCGCGAGGCA	-0.853462598263724	0.00023985	TRUE	-1.80522351536343	0.1633	FALSE
hsa-miR-4450	MIMAT0018971	C-302034-00	UGGGGAUUUGGAGAAGUGGUGA	-1.06492940641686	4.5608e-05	TRUE	-2.95948317827462	0.014453	TRUE
hsa-miR-4451	MIMAT0018973	C-302037-00	UGGUAGAGCUGAGGACA	-1.13976267989773	3.6715e-05	TRUE	-0.526465448512174	0.86349	FALSE
hsa-miR-4452	MIMAT0018974	C-302038-00	UUGAAUUCUUGGCCUUAAGUGAU	-0.813274313439393	0.00026644	TRUE	-0.166636659928165	1	FALSE
hsa-miR-4453	MIMAT0018975	C-302039-00	GAGCUUGGUCUGUAGCGGU	-1.18208500406966	3.9917e-05	TRUE	-2.62562177516204	0.050122	TRUE
hsa-miR-448	MIMAT0001532	C-300721-05	UUGCAUAUGUAGGAUGUCCCAU	-1.39737830033137	0.00054279	FALSE	-2.29949072457764	0.11887	TRUE
hsa-miR-449c	MIMAT0010251	C-301488-00	UAGGCAGUGUAUUGCUAGCGGCUGU	-0.977360548104279	0.00013963	TRUE	-1.65073035658006	0.26604	FALSE
hsa-miR-4507	MIMAT0019044	C-302113-00	CUGGGUGGCGUGGGCUGGG	-1.08254852754089	0.015067	FALSE	-2.63018823024416	0.11887	TRUE
hsa-miR-450a-3p	MIMAT0022700	C-301907-00	AUUGGGGACAUUUUGCAUUCAU	-1.3171609377353	0.00013793	TRUE	-1.14626230328136	0.46329	FALSE
hsa-miR-451	MIMAT0001631	C-300734-05	AAACCGUUACCAUUACUGAGUU	-1.43368456108961	8.3715e-05	TRUE	-0.268670734386333	1	FALSE
hsa-miR-452	MIMAT0001635	C-300735-07	AACUGUUUGCAGAGGAAACUGA	-0.355271404072044	0.12563	FALSE	-2.45598627080639	0.050122	TRUE
hsa-miR-4525	MIMAT0019064	C-302136-00	GGGGGGAUGUGCAUGCUGGUU	-1.29162472134399	0.00013793	TRUE	-1.82359755370572	0.26604	FALSE
hsa-miR-4526	MIMAT0019065	C-302137-00	GCUGACAGCAGGGCUGGCCGU	-0.819545705994525	0.0021076	FALSE	-3.08101976925394	0.057617	TRUE
hsa-miR-4527	MIMAT0019066	C-302138-00	UGGCUCGCAAAGAGAUGACUGU	-1.48851753125946	3.9917e-05	TRUE	-1.1928606551261	0.5811	FALSE
hsa-miR-4533	MIMAT0019072	C-302145-00	UGGAAGGAGGUGACGGGCUUGU	-1.18624537355721	0.00016326	TRUE	-0.593158084514742	0.86349	FALSE
hsa-miR-4536-3p	MIMAT0020959	C-301930-00	UCGGUCAUAUAUCUACCACAU	-1.07232143191926	0.00054279	FALSE	-3.00264840143944	0.014453	TRUE
hsa-miR-4536-5p	MIMAT0019078	C-301929-00	UGUGGUAGAUAUAUAGCGAU	-1.31712298567513	0.00013963	TRUE	-1.61271864964865	0.20114	FALSE
hsa-miR-4538	MIMAT0019081	C-302154-00	GAGCUUGGAUGAGCUGGCCUGA	-1.22689177781842	0.00013963	TRUE	-1.39982249594569	0.5811	FALSE
hsa-miR-4539	MIMAT0019082	C-302155-00	GCUGAACUGGGCUGAGCUGGGC	-0.662063245722664	0.0074787	FALSE	-2.55330760069272	0.11887	TRUE
hsa-miR-4632-5p	MIMAT0022977	C-302499-00	GAGGGCAGCGUGGGUGUGGCGGA	-0.597712246032446	0.015067	FALSE	-2.79546040686595	0.057617	TRUE
hsa-miR-4642	MIMAT0019702	C-302175-00	AUGGCAUGCCCCUGGUGUGC	-1.24506307782347	0.00013963	TRUE	-1.87231348016783	0.26604	FALSE
hsa-miR-4645-3p	MIMAT0019706	C-302179-00	AGACAGUAGUUCUUGCCUGGUU	-1.12025038846698	0.00026644	TRUE	-1.08522858184346	0.5811	FALSE
hsa-miR-4650-3p	MIMAT0019714	C-302184-00	AGGUAGAAUGAGGCCUGACAU	-1.52761158686618	3.9917e-05	TRUE	-2.47248261479947	0.11887	TRUE
hsa-miR-4651	MIMAT0019715	C-302187-00	CGGGGUGGGUGAGGCGGGC	-1.12142324138952	0.00023985	TRUE	-1.06587157649805	0.5811	FALSE
hsa-miR-4652-5p	MIMAT0019716	C-302188-00	AGGGGACUGGUAAUAAGAACA	-1.12850660881293	0.00023985	TRUE	-0.668591975163071	0.86349	FALSE
hsa-miR-4655-5p	MIMAT0019721	C-302194-00	CACCGGGGAUGGCAGAGGGUCG	-1.21402640640325	0.00016326	TRUE	-1.71020871463468	0.40677	FALSE
hsa-miR-466	MIMAT0015002	C-301675-00	AUACACAUACACGCAACACACAU	-1.57268812194273	1.5894e-05	TRUE	-1.36589716672211	0.46329	FALSE
hsa-miR-4664-3p	MIMAT0019738	C-302210-00	CUUCCGGCUCGUGAGCCCCGU	-1.12808833805518	8.3715e-05	TRUE	-1.31446201824497	0.26604	FALSE
hsa-miR-4664-5p	MIMAT0019737	C-302209-00	UGGGGUGCCCACUCCGCAAGUU	-1.35541980608348	3.6715e-05	TRUE	-0.623887404733994	0.76864	FALSE
hsa-miR-4665-5p	MIMAT0019739	C-302211-00	CUGGGGGACGCGUGAGCGCGAGC	-1.14650290801351	6.1693e-05	TRUE	-2.55003959556736	0.014453	TRUE
hsa-miR-4666b	MIMAT0022485	C-302595-00	UUGCAUGUCAGAUUGUAAUUCCC	-1.25134263280118	0.0053924	FALSE	-1.96334534104703	0.11887	TRUE
hsa-miR-4668-5p	MIMAT0019745	C-302216-00	AGGGAAAAAAAAAAGAAUUGUC	-1.06833102929774	0.00013793	TRUE	-1.46510395349218	0.1633	FALSE
hsa-miR-4672	MIMAT0019754	C-302224-00	UUACACAGCUGGACAGAGGCA	-1.69132941190395	1.5655e-05	TRUE	-1.829706183938803	0.11887	TRUE
hsa-miR-4674	MIMAT0019756	C-302226-00	CUGGGCUCGGGACGCGCGGCU	-1.30671632029371	3.9917e-05	TRUE	-1.87257075861882	0.08542	TRUE
hsa-miR-4675	MIMAT0019757	C-302227-00	GGGGCGUGUGAUUGGACAGCAGG	-0.49750400822998	0.015067	FALSE	-1.657784791860002	0.11887	TRUE
hsa-miR-4677-5p	MIMAT0019760	C-302231-00	UUGUUCUUUGGUCCCUUCCUU	-1.01207284849144	0.00013963	TRUE	-2.02433296093914	0.1633	TRUE
hsa-miR-4678	MIMAT0019762	C-302232-00	AAGGUAAUUGUUCAGACUUAUGA	-0.678062145099685	0.0021076	FALSE	-1.8265679594388	0.11887	TRUE
hsa-miR-4682	MIMAT0019767	C-302236-00	UCUGAGUCCUGGGAUCGGGUCU	-0.95136903371094	0.00023985	TRUE	-0.279156377102257	1	FALSE
hsa-miR-4683	MIMAT0019768	C-302237-00	UGGAGAUCCAGUGCUGCGCGGAU	-1.03845589118353	0.00013793	TRUE	-2.39905380190628	0.023579	TRUE
hsa-miR-4684-3p	MIMAT0019770	C-302239-00	UGUUGCAAGUGGUGGAGCGU	-0.997924294092217	0.00016326	TRUE	-1.70225287410888	0.1633	TRUE
hsa-miR-4687-3p	MIMAT0019775	C-302242-00	UGGCUGUUGGAGGGGGCAGGC	-1.08006757648135	9.6971e-05	TRUE	-2.49518358442824	0.023579	TRUE
hsa-miR-4688	MIMAT0019777	C-302244-00	UAGGGGCAGCAGAGGACCUGG	-0.933026739975212	0.00023985	TRUE	-2.48777268680185	0.023579	TRUE
hsa-miR-4689	MIMAT0019778	C-302245-00	UUGAGGAGACAUGGUGGGGGC	-1.02151466268037	0.00023985	TRUE	-1.54092925086873	0.1633	FALSE
hsa-miR-4692	MIMAT0019783	C-302250-00	UCAGGCAGUGGAUGCCUUAGAU	-0.988783938058404	0.00016326	TRUE	1.14868084400672	0.40677	FALSE
hsa-miR-4693-3p	MIMAT0019785	C-302252-00	UGAGAGUGGAAUUCACAGUAUUU	-1.44962435716591	3.6715e-05	TRUE	-1.73885084830262	0.11887	TRUE
hsa-miR-4694-3p	MIMAT0019787	C-302253-00	CAAAUGCAGGAUAACACCU	-0.938557148822229	0.00023985	TRUE	-1.50626462839686	0.1633	FALSE
hsa-miR-4694-5p	MIMAT0019786	C-302254-00	AGGUGUUAUCCUAUCCAUUUGC	-0.952993076462483	0.00023985	TRUE	-2.88415743782109	0.014453	TRUE
hsa-miR-4695-3p	MIMAT0019789	C-302458-00	UGACUCACCGCUGCCCUCCUUC	-1.73712530730471	3.6715e-05	TRUE	-2.61115417981241	0.11887	TRUE
hsa-miR-4696	MIMAT0019790	C-302255-00	UGCAAGACGGAUACUGUCAUCU	-1.05967205505661	0.00013793	TRUE	-3.02679657583108	0.009387	TRUE
hsa-miR-4697-5p	MIMAT0019791	C-302257-00	AGGGGGCGCAGUCACUGACGUG	-1.49838261147195	1.5894e-05	TRUE	-1.93121149945073	0.08542	TRUE

hsa-miR-4699-5p	MIMAT0019794	C-302259-00	AGAAGAUUGCAGAGUAAGUUCC	-0.321333385517737	0.096607	FALSE	-1.70896571432969	0.11887	TRUE
hsa-miR-4700-5p	MIMAT0019796	C-302262-00	UCUGGGGAUGAGGACAGUGUGU	-0.63878129737828	0.0046172	FALSE	-2.49301512707352	0.023579	TRUE
hsa-miR-4701-5p	MIMAT0019798	C-302460-00	UUGGCCACCACACCUACCCCUU	-1.254075921715140	0.00016326	TRUE	-2.10511844533172	0.1633	FALSE
hsa-miR-4706	MIMAT0019806	C-302269-00	AGCGGGGAGGAAGUGGGCGCUGCUU	-0.640574202002897	0.0030028	FALSE	-1.80378336355061	0.11887	TRUE
hsa-miR-4707-3p	MIMAT0019808	C-302270-00	AGCCCGCCCCAGCCGAGGCUU	-0.833524657548794	0.00079918	FALSE	-2.32407172887442	0.057617	TRUE
hsa-miR-4708-3p	MIMAT0019810	C-302273-00	AGCAAGGCGGCAUCUCUCUGAU	-0.969695371193823	0.00023985	TRUE	-0.525873276306624	0.86349	FALSE
hsa-miR-4708-5p	MIMAT0019809	C-302272-00	AGAGAUGCCGCCUUGCUCCUU	-0.822684020528361	0.00079918	FALSE	-2.10220660879154	0.08542	TRUE
hsa-miR-4709-3p	MIMAT0019812	C-302275-00	UUGAAGAGGAGGUGCUCUGUAGC	-0.664327400587703	0.0027607	FALSE	-1.68675985049984	0.11887	TRUE
hsa-miR-4709-5p	MIMAT0019811	C-302274-00	ACAACAGUGACUUGCUCUCCAA	-1.089698011660567	9.6971e-05	TRUE	-0.80626334250579	0.5811	FALSE
hsa-miR-4711-5p	MIMAT0019816	C-302279-00	UGCAUCAGGCCAGAAGACAUGAG	-0.502094584622719	0.015067	FALSE	-2.22945919207469	0.057617	TRUE
hsa-miR-4712-3p	MIMAT0019819	C-302282-00	AAUGAGAGACCUGUACUGUAU	-1.26003058356888	3.9917e-05	TRUE	-2.52822910278788	0.028781	TRUE
hsa-miR-4713-5p	MIMAT0019820	C-302283-00	UUCUCCCACUACCAGGCUCCCA	-1.41056492413791	3.6715e-05	TRUE	0.152837140204165	1	FALSE
hsa-miR-4714-5p	MIMAT0019822	C-302286-00	AACUCUGACCCCUUAGGUUGAU	-0.971641326370144	0.00023985	TRUE	-1.9820676345786	0.08542	TRUE
hsa-miR-4715-3p	MIMAT0019825	C-302288-00	GUGCCACCUUAACUGCAGCCAAU	-0.831711497711227	0.0014577	FALSE	-1.64639738941666	0.11887	TRUE
hsa-miR-4720-3p	MIMAT0019834	C-302297-00	UGCUUAAGUUGUACCAAGUAU	-0.267039361511682	0.37761	FALSE	-2.41942301448004	0.057617	TRUE
hsa-miR-4726-3p	MIMAT0019846	C-302309-00	ACCCAGGUUCCCUCUGGCCGCA	-1.05457626001572	0.0046172	FALSE	-1.96757030993053	0.11887	TRUE
hsa-miR-4726-5p	MIMAT0019845	C-302310-00	AGGGCCAGAGGAGCCUGGAGUGG	-0.548731137329315	0.061384	FALSE	-2.20367296738285	0.11887	TRUE
hsa-miR-4730	MIMAT0019852	C-302314-00	CUGGCGGAGCCCAUUCCAUGCCA	-1.14323623131763	0.0021076	FALSE	-3.22072847594229	0.014453	TRUE
hsa-miR-4731-3p	MIMAT0019854	C-302316-00	CACACAAGUGGCCCCCAACACU	-0.715286408900071	0.021994	FALSE	-2.11972721193743	0.11887	TRUE
hsa-miR-4733-5p	MIMAT0019857	C-302319-00	AAUCCCAAGCUAGACCCGGGU	-0.918486094585803	0.0074787	FALSE	-2.80327741332156	0.023579	TRUE
hsa-miR-4740-3p	MIMAT0019870	C-302329-00	GCCCGAGAGGAUCCGUCCCUGC	-0.824911201381423	0.015067	FALSE	-3.46216635615516	0.014453	TRUE
hsa-miR-4743-5p	MIMAT0019874	C-302462-00	UGGCCGGAUGGGACAGGAGGCAU	-1.02760222969445	0.00061109	FALSE	-2.22597648616421	0.11887	TRUE
hsa-miR-4745-3p	MIMAT0019879	C-302464-00	UGGCCCGGCGACGUCUCACGGUC	-1.23742515486855	0.00016326	TRUE	-2.78131955234324	0.08542	TRUE
hsa-miR-4747-3p	MIMAT0019883	C-302339-00	AAGGCCCGGGCUUUCCUCCCAG	-0.465231590374108	0.12653	FALSE	-2.90430878110698	0.023579	TRUE
hsa-miR-4747-5p	MIMAT0019882	C-302340-00	AGGGAAGGAGGCUUGGUCUUAG	-0.431504547805173	0.17522	FALSE	-1.91877072137694	0.12226	TRUE
hsa-miR-4748	MIMAT0019884	C-302341-00	GAGGUUUGGGGAGGAUUUGCU	-0.555957276676544	0.061384	FALSE	-2.01827086334852	0.11887	TRUE
hsa-miR-4750-3p	MIMAT0022979	C-302491-00	CCUGACCCACCCCCUCCCGCAG	-0.714754040609532	0.0074787	FALSE	-2.39203721905178	0.11887	TRUE
hsa-miR-4755-5p	MIMAT0019895	C-302349-00	UUUCCCUCAGAGCCUGGCUUU	-0.539113669488979	0.061384	FALSE	-2.80807175968132	0.057617	TRUE
hsa-miR-4760-5p	MIMAT0019906	C-302359-00	UUUAGAUUGAACAUGAAGUUAG	-0.823743933447296	0.015067	FALSE	-2.78675399181231	0.028781	TRUE
hsa-miR-4768-5p	MIMAT0019920	C-302372-00	AUUCUCUCGGAUCCCAUGGAU	-0.784576616178355	0.061384	FALSE	-2.06931455868509	0.11887	TRUE
hsa-miR-4774-3p	MIMAT0019930	C-302383-00	AUUGCCUAACAUGUGCCAGAA	-0.980156502965659	0.03164	FALSE	-1.92072766628618	0.12226	TRUE
hsa-miR-4778-3p	MIMAT0019937	C-302393-00	UCUUCUUCCUUUUGCAGAGUUGA	-0.468380465892242	0.17522	FALSE	-2.12161516444605	0.11887	TRUE
hsa-miR-4778-5p	MIMAT0019936	C-302392-00	AAUUCUGUAAAGGAAGAAGAGG	-0.108428651324849	1	FALSE	2.20881646867654	0.08542	TRUE
hsa-miR-4782-3p	MIMAT0019945	C-302398-00	UGAUUGUCUUCAUAUCUAGAAC	-0.415227659905038	0.32544	FALSE	-1.91949188729733	0.11887	TRUE
hsa-miR-4792	MIMAT0019964	C-302418-00	CGGUGAGCGCUCGCUGGC	-0.681007711978918	0.096607	FALSE	-2.3586952181417	0.057617	TRUE
hsa-miR-4799-5p	MIMAT0019976	C-302431-00	AUCUAAAUGCAGCAUGCCAGCU	-0.362222805879368	0.32544	FALSE	-1.88523388837028	0.11887	TRUE
hsa-miR-4800-5p	MIMAT0019978	C-302435-00	AGUGGACCGAGGAAGGAAGGA	-1.32275981315377	0.0074787	FALSE	-2.73500556024618	0.028781	TRUE
hsa-miR-4804-3p	MIMAT0019985	C-302441-00	UGCUUAACCUUGCCCUCGAAA	-0.336610566496367	0.37761	FALSE	-2.24438580605872	0.11887	TRUE
hsa-miR-486-3p	MIMAT0004762	C-301211-01	CGGGGCAGCUCAGUACAGGAU	-0.915928947716196	0.0027607	FALSE	-1.90219636227184	0.11887	TRUE
hsa-miR-487a	MIMAT0002178	C-300747-03	AAUCAUACAGGGACACCUGGU	-0.747164545262124	0.015067	FALSE	-2.42832566173827	0.040079	TRUE
hsa-miR-488	MIMAT0004763	C-301189-01	UUGAAAGGCUAUUUCUUGGUC	-0.908290335869122	0.01741	FALSE	-2.18110128296813	0.11887	TRUE
hsa-miR-491-3p	MIMAT0004765	C-301091-01	CUUAUGCAAGAUUCCCUUCUAC	-1.62232250510605	0.00023985	TRUE	-1.07582371390113	0.46329	FALSE
hsa-miR-492	MIMAT0002812	C-300757-05	AGGACCUGCGGGACAAGAUUCUU	-0.468584149908068	0.061384	FALSE	-2.72511166766836	0.014453	TRUE
hsa-miR-494	MIMAT0002816	C-300761-05	UGAAACAUACACGGGAAACCUC	-0.970219284972552	0.0021076	FALSE	-3.24114885056762	0.014453	TRUE
hsa-miR-499b-3p	MIMAT0019898	C-302351-00	AACAUCACUGCAAGUCUUAACA	-0.450448811655319	0.10991	FALSE	-2.12877293491975	0.11887	TRUE
hsa-miR-5001-3p	MIMAT0021022	C-302470-00	UUCUGCCUCUGUCCCAGGUCCUU	-1.334286639345599	0.00013793	TRUE	-1.54637406738355	0.40677	FALSE
hsa-miR-5003-5p	MIMAT0021025	C-302474-00	UCACAACAACCUUGCAGGCUGA	-1.32073825957867	0.00013793	TRUE	-1.18738346839705	0.51579	FALSE
hsa-miR-5004-5p	MIMAT0021027	C-302477-00	UGAGGACAGGGCAAAUUCACGA	-1.00354080968358	0.00079918	FALSE	-3.14572597959306	0.057617	TRUE
hsa-miR-5006-5p	MIMAT0021033	C-302479-00	UUGCCAGGGCAGGAGGUGGAA	-1.29090326952223	0.00013963	TRUE	-1.67173993218432	0.40677	FALSE
hsa-miR-501-3p	MIMAT0004774	C-301167-01	AAUGCACCCGGGCAAGGAUUCU	-0.0731774010060508	1	FALSE	1.96439959148781	0.057617	TRUE
hsa-miR-5087	MIMAT0021079	C-301942-00	GGGUUUGUAGCUUUGCUGGCAUG	-0.359939924285983	0.10991	FALSE	-1.78188598696404	0.11887	TRUE
hsa-miR-509-5p	MIMAT0004779	C-301166-01	UACUGCAGACAGUGGCAAUCA	-1.74945283553728	0.00013793	TRUE	-2.47315672999895	0.081661	TRUE
hsa-miR-5092	MIMAT0021084	C-302456-00	AAUCCACGCUGAGCUGGGUCAUC	-1.18967239061038	0.00023985	TRUE	-1.511756387148	0.46329	FALSE
hsa-miR-513a-5p	MIMAT0002877	C-300844-07	UUCACAGGGAGGUGUCAU	-1.05997050839063	0.0074787	FALSE	-2.37670537648159	0.11887	TRUE
hsa-miR-513c-3p	MIMAT0022728	C-301908-00	UAAAUUUCACCUUUCUUGAGAAGA	-0.803025559359546	0.0046172	FALSE	-2.73190794276724	0.014453	TRUE
hsa-miR-514	MIMAT0002883	C-300851-07	AUUGACACUUCUGUGAGUAGA	-0.348236527420602	0.12653	FALSE	-1.56224654603507	0.11887	TRUE
hsa-miR-516a-5p	MIMAT0004770	C-301104-01	UUCUCGAGGAAAGAAGCACUUUC	-0.681407959396855	0.0046172	FALSE	-2.18673664105892	0.11887	TRUE
hsa-miR-517a	MIMAT0002852	C-300811-05	AUCGUGCAUCCCUUUAGAGUGU	-1.09820612826732	0.0018747	FALSE	-2.32535259864992	0.08542	TRUE
hsa-miR-517b-3p	MIMAT0002857	C-300817-06	AUCGUGCAUCCCUUUAGAGUGU	-1.68014170163934	3.9917e-05	TRUE	-1.45577777456063	0.26604	FALSE
hsa-miR-517c	MIMAT0002866	C-300832-03	AUCGUGCAUCCUUUUAGAGUGU	-1.39967029576568	0.00054279	FALSE	-3.92792989935189	0.009387	TRUE
hsa-miR-518a-5p	MIMAT0005457	C-301099-01	CUGCAAAGGGAAGCCCUUUC	-1.69701165144962	0.00013963	TRUE	-3.84851299481868	0.014453	TRUE
hsa-miR-518b	MIMAT0002844	C-300798-03	CAAAGCGCUCCCCUUUAGAGGU	-1.15354841149699	0.00087976	FALSE	-2.22045683232246	0.08542	TRUE
hsa-miR-518c*	MIMAT0002847	C-300804-03	UCUCUGGAGGGAAGCACUUUCUG	-1.15870376927669	0.0014577	FALSE	-3.20841244988613	0.014453	TRUE
hsa-miR-518d-5p	MIMAT0005456	C-301100-01	CUCUAGAGGGAAGCACUUUCUG	-0.509493211764305	0.54004	FALSE	-2.82765759572384	0.081661	TRUE
hsa-miR-5192	MIMAT0021123	C-302496-00	AGGAGAGUGGAUUCCAGGUGGU	-0.929717635688366	0.0014577	FALSE	-4.01918995190962	0.014453	TRUE
hsa-miR-5196-5p	MIMAT0021128	C-301981-00	AGGGAAGGGGACGAGGGUUGGG	-1.16761124143466	3.6715e-05	TRUE	-2.56840698714633	0.040079	TRUE
hsa-miR-520a-3p	MIMAT0002834	C-300788-03	AAAGUGCUUCCCUUUGGACUGU	0.0374069999290813	1	FALSE	2.98674734617433	0.08542	TRUE
hsa-miR-520c-3p	MIMAT0002846	C-300803-05	AAAGUGCUUCCUUUUAGAGGGU	-0.199389168688778	0.37761	FALSE	-1.76230191681356	0.11887	TRUE
hsa-miR-520h	MIMAT0002867	C-300833-03	ACAAAGUGCUUCCCUUUAGAGU	-0.694676795373559	0.015067	FALSE	-2.26960151079303	0.08542	TRUE
hsa-miR-532-5p	MIMAT0002888	C-300867-01	CAUGCCUUGAGUGUAGGACCGU	-0.687783861572295	0.021994	FALSE	-3.38518185188287	0.014453	TRUE
hsa-miR-539-3p	MIMAT0022705	C-301909-00	AUCAUACAAGGACAAUUCUUU	-0.712513192949551	0.0014577	FALSE	-1.7777539713116	0.12226	TRUE
hsa-miR-544b	MIMAT0015004	C-301677-00	ACCUGAGGUUGUGCAUUUCUAA	-1.14820342901038	3.9917e-05	TRUE	-0.449549813604399	0.91039	FALSE
hsa-miR-548ab	MIMAT0018928	C-301990-00	AAAAGUAAUUGUGGAUUUUGU	-0.602579302030159	0.0021076	FALSE	-2.46432616915756	0.057617	TRUE
hsa-miR-548ac	MIMAT0018938	C-302001-00	CAAAAACCGGCAAUUACUUUUG	-0.979633570213558	6.1693e-05	TRUE	-2.0217602235335	0.11887	TRUE
hsa-miR-548ae	MIMAT0018864	C-302019-00	CAAAAACUGCAAUUACUUUA	-0.606375099488669	0.0030028	FALSE	-2.77725501966519	0.057617	TRUE
hsa-miR-548ah-3p	MIMAT0020957	C-302036-00	CAAAAACUGCAGUUACUUUUGC	-0.668405768437197	0.0014577	FALSE	-2.22611084098903	0.081661	TRUE
hsa-miR-548at-5p	MIMAT0022277	C-302544-00	AAAAGUUAUUGCGGUUUUGCU	-0.0453519642557928	1	FALSE	-1.8924808826669	0.11887	TRUE
hsa-miR-548au-3p	MIMAT0022922	C-302561-00	UGGCAGUUACUUUUGCCACCAG	-1.52595082150064	0.0021076	FALSE	-3.17672563043804	0.014453	TRUE
hsa-miR-548ax	MIMAT0022474	C-302582-00	AGAAGUAAUUGCGGUUUUGCCA	-0.564257743083232	0.10991	FALSE	-2.34784280477006	0.057617	TRUE
hsa-miR-548ay-5p	MIMAT0025452	C-302660-00	AAAAGUAAUUGUGGUUUUUGC	-0.653127591660778	0.021994	FALSE	-3.23819702467989	0.014453	TRUE
hsa-miR-548h-3p	MIMAT0022723	C-301888-00	CAAAAACCGCAAUUACUUUUGCA	-1.06047561824622	0.00054279	FALSE	-2.88715465104665	0.014453	TRUE
hsa-miR-548l	MIMAT0005889	C-301353-00	AAAAGUAAUUGCGGGUUUUGUC	-0.59227174574884	0.061384	FALSE	-2.37472439626248	0.11887	TRUE
hsa-miR-548s	MIMAT0014987	C-301658-00	AUGGCCAAAACUGCAGUUAUUUU	-0.871664637647317	0.00054279	FALSE	-2.25194852231406	0.11887	TRUE
hsa-miR-549	MIMAT0003333	C-300991-01	UGACAACUAUGGAUGAGCUCU	-0.71851748320711	0.01741	FALSE	-2.02535291589875	0.12226	TRUE
hsa-miR-550b-2-5p	MIMAT0022737	C-301971-00	AUGUGCCUGAGGGAGUAAGACA	-0.509912734915377	0.0074787	FALSE	-1.840206886668008	0.12226	TRUE
hsa-miR-5580-5p	MIMAT0022273	C-302541-00	UGCUGGCUCAUUUCAUAUGUGU	-0.250725616752794	0.54004	FALSE	-2.09116428366529	0.08542	TRUE
hsa-miR-5583-3p	MIMAT0022282	C-302549-00	GAAUAUGGGUAUAUUAGUUUGG	-0.580364168150873	0.10991	FALSE	-2.828220915146	0.014453	TRUE
hsa-miR-5583-5p	MIMAT0022281	C-302548-00	AAACUAAUAUACCAUAUUUCUG	-0.741198390433839	0.050037	FALSE	-2.77350973293048	0.014453	TRUE
hsa-miR-568	MIMAT0003232	C-300886-01	AUGUAUAAAUGUAUACACAC	-0.546512240064326	0.050037	FALSE	-2.16900241945777	0.11887	TRUE
hsa-miR-5681a	MIMAT0022469	C-302576-00	AGAAAGGGGUGGCAAUACCUCUU	-0.554451480617061	0.10991	FALSE	-2.10313927081946	0.11887	TRUE
hsa-miR-5681b	MIMAT0022480	C-302589-00	AGGUAUUGCCACCCUUUCUAGU	-0.990407574357977	0.015067	FALSE	-2.24966625727449	0.11887	TRUE
hsa-miR-5685	MIMAT0022475	C-302583-00	ACAGCCCAGCAGUUAUCAGGGG	-1.10866666817995	0.0099219	FALSE	-1.81151890217456	0.11887	TRUE
hsa-miR-5686	MIMAT0022477	C-302586-00	UAUCGUAUCGUAUUGUAUUGU	-0.618603205233964	0.096607	FALSE	-3.26787707054355	0.014453	TRUE
hsa-miR-5687	MIMAT0022478	C-302587-00	UUAGAACGUUUUAGGGAUCAAU	-0.4136100982672	0.25618	FALSE	-1.96140285293105	0.11887	TRUE
hsa-miR-5688	MIMAT0022479	C-302588-00	UAACAAACACCUGUAAAACAGC	-0.496708860788786	0.17522	FALSE	-2.54610797665859	0.028781	TRUE
hsa-miR-5692a	MIMAT0022484	C-302593-00	CAAAUAAUACCACAGUGGGUGU	-0.71714659454192	0.061384	FALSE	-2.47331051365896	0.057617	TRUE
hsa-miR-5692c	MIMAT0022476	C-302584-00	AAUAAAUACACAGUAGGGUGUAC	-0.930027776637268	0.021994	FALSE	-2.41733401849822	0.057617	TRUE
hsa-miR-5697	MIMAT0022490	C-302600-00	UCAAGUAGUUUCAUGAUAAAGG	-0.509179181511475	0.17522	FALSE	-1.87679966081257	0.11887	TRUE
hsa-miR-5701	MIMAT0022494	C-302604-00	UUAUUGUCACGGUCUGAUU	-0.713050083304961	0.061384	FALSE	-2.19998791399188	0.081661	TRUE
hsa-miR-5702	MIMAT0022495	C-302605-00	UGAGUCAGCAACAUAUCCCAUG	-0.634695457873147	0.089234	FALSE	-2.91461280705682	0.014453	TRUE
hsa-miR-571	MIMAT0003236	C-300890-01	UGAGUUGGCCAUCUGAGUGAG	-1.04012448788184	0.00016326	TRUE	-1.06802200266461	0.5811	FALSE
hsa-miR-574-3p	MIMAT0003239	C-300893-03	CACGCUCAUGCACACACCCACA	-1.4521414321912	0.0018747	FALSE	-2.74091004364483	0.057617	TRUE
hsa-miR-578	MIMAT0003243	C-300897-01	CUUCUUGUGCUCUAGGAUUGU	-0.525376007672939	0.038627	FALSE	-2.14162436977285	0.081661	TRUE
hsa-miR-579	MIMAT0003244	C-300898-03	UUCAUUUGGUAUAAACCGCGAUU	-1.2654167775587	0.0030028	FALSE	-3.8111485398612	0.014453	TRUE
hsa-miR-582-5p	MIMAT0003247	C-300901-01	UUACAGUUGUUCAACCAGUUACU	-1.2010641323251	0.00070025	FALSE	-2.60372535467359	0.040079	TRUE
hsa-miR-586	MIMAT0003252	C-300906-01	UAUGCAUUGUAUUUUUAGGUCC	-0.445099473295375	0.10991	FALSE	-3.97498411008623	0.014453	TRUE
hsa-miR-593*	MIMAT0003261	C-300917-01	AGGCACCAGCCAGGCAUUGCUCAGC	-1.46368716545378	0.00023985	TRUE	-1.69498877920483	0.1633	FALSE
hsa-miR-606	MIMAT0003274	C-300931-01	AAACUACUGAAAAUCAAAGAU	-0.637631782545375	0.32544	FALSE	-2.73649791406254	0.11887	TRUE
hsa-miR-607	MIMAT0003275	C-300932-01	GUUCAAAUCCAGAUCUAUAAC	-0.096398469841977	1	FALSE	2.3242183673772	0.11887	TRUE
hsa-miR-6071	MIMAT0023696	C-302625-00	UUCUGCUGCCCGCCAAGGC	-0.528822533996229	0.12653	FALSE	-2.1934583463063	0.11887	TRUE
hsa-miR-6075	MIMAT0023700	C-302629-00	ACGGCCCAGGCGGCAUUGGUG	-0.592038077604373	0.037103	FALSE	-2.63925181780449	0.057617	TRUE
hsa-miR-608	MIMAT0003276	C-300933-01	AGGGGUGGUGUUGGGACAGCUCCGU	-1.414763754940469	9.6971e-05	TRUE	-1.32647981772413	0.22566	FALSE
hsa-miR-6080	MIMAT0023705	C-302634-00	UCUAGUGCGGGCGUUCCCG	-0.377302781648075	0.17522	FALSE	-3.12142545628473	0.023579	TRUE
hsa-miR-6081	MIMAT0023706	C-302635-00	AGGAGCAGUGCCGGCCCAAGGCGCC	-1.07161737179969	0.0018747	FALSE	-2.06272320366808	0.11887	TRUE
hsa-miR-6086	MIMAT0023711	C-302640-00	GGAGGUUGGGAAGGGCAGAG	-0.556948350994301	0.040903	FALSE	-2.86501870739423	0.028781	TRUE
hsa-miR-6133	MIMAT0024615	C-302655-00	UGAGGGAGGGAUGGGGGUA	-1.00026187079406	0.0021076	FALSE	-3.11682515905473	0.014453	TRUE
hsa-miR-617	MIMAT0003286	C-300943-01	AGACAUCCCAUUUGAAGGUGGC	-1.41203659250412	0.00023985	TRUE	-0.966712399788375	0.51579	FALSE
hsa-miR-619	MIMAT0003288	C-300945-01	GACCUGGACAUGUUUGUGCCCAGU	0.0866202298806936	1	FALSE	2.48494644624597	0.014453	TRUE
hsa-miR-620	MIMAT0003289	C-300946-01	AUGGAGAUAGAUAUAUGAAAU	-0.746644845920626	0.096607	FALSE	-3.35303260797096	0.057617	TRUE
hsa-miR-621	MIMAT0003290	C-300947-01	GGCUAGCAACAGCGCUUACCU	-0.524135944687041	0.49143	FALSE	-3.56178952288145	0.023579	TRUE
hsa-miR-624*	MIMAT0003293	C-300950-01	UAGUACCAGUACCUUGUGUUCA	-1.18323865292578	8.3715e-05	TRUE	-2.08818623024311	0.11887	TRUE
hsa-miR-627	MIMAT0003296	C-300953-01	GUGAGUCUCUAAGAAAAGAGGA	-1.05366486573687	0.00016326	TRUE	-1.71256975426944	0.22566	FALSE
hsa-miR-631	MIMAT0003300	C-300957-01	AGACCUGGCCCAGACCUCAGC	-2.01925344204044	3.9917e-05	TRUE	-1.07267199301577	0.51048	FALSE
hsa-miR-634	MIMAT0003304	C-300961-01	AACCAGCACCCCAACUUUGGAC	-1.301238506046349	0.00016067	TRUE	-0.64623077279342	0.76864	FALSE
hsa-miR-638	MIMAT0003308	C-300965-01	AGGGAUCGCGGGCGGGUGGCGGCCU	-0.639193662554625	0.03164	FALSE	-1.99612853656179	0.12226	TRUE
hsa-miR-642a-3p	MIMAT0020924	C-301902-00	AGACACAUUUGGAGAGGGAACC	-0.669360409389209	0.0099219	FALSE	-2.67786191538987	0.023579	TRUE
hsa-miR-642b-5p	MIMAT0022736	C-301970-00	GGUUCCCUCUCCAAAUGUGUCU	-0.982372985036737	6.1693e-05	TRUE	-2.91448575097456	0.023579	TRUE
hsa-miR-644	MIMAT0003314	C-300971-01	AGUGUGGCUUUCUUAGAGC	-1.1328764954543	0.0014577	FALSE	-2.28201156273226	0.12226	TRUE
hsa-miR-646	MIMAT0003316	C-300973-01	AAGCAGCUGCCUCUGAGGC	-1.373355174868112	3.9917e-05	TRUE	-1.88378259141162	0.1633	FALSE
hsa-miR-647	MIMAT0003317	C-300974-01	GUGGCUGCACACCUUCCUCUU	-0.662202123721489	0.015067	FALSE	-2.34112755480514	0.057617	TRUE
hsa-miR-6499-3p	MIMAT0025451	C-302659-00	AGCAGUGUUGUUUGUGCCCACA	-0.789311054725994	0.0099219	FALSE	-2.42776308682332	0.08542	TRUE
hsa-miR-650	MIMAT0003320	C-300977-01	AGGAGGCAGCGCUCUCAGGAC	-1.32232002725072	0.00054279	FALSE	-2.38532145199764	0.11887	TRUE
hsa-miR-6503-3p	MIMAT0025463	C-302671-00	GGGACUAGGAUGCAGACCUCC	-0.701857748301807	0.01741	FALSE	-2.26797239631951	0.08542	TRUE
hsa-miR-6503-5p	MIMAT0025462	C-302670-00	AGGACUGCAUUCAAAUCCCCAGA	-1.288292791561107	0.00061109	FALSE	-4.16301032924927	0.009387	TRUE

hsa-miR-6504-3p	MIMAT0025465	C-302672-00	CAUUACAGCACAGCCAUUCU	-0.219268298279075	0.49143	FALSE	-2.3682663259571	0.08542	TRUE
hsa-miR-6505-3p	MIMAT0025467	C-302675-00	UGACUUCUACCUCUUCCAAAG	-1.41016990839787	0.00054279	FALSE	-2.92045878806631	0.040079	TRUE
hsa-miR-6507-5p	MIMAT0025470	C-302679-00	GAAGAAUAGGAGGGACUUUGU	-0.637513482741369	0.03164	FALSE	-2.64696299700191	0.057617	TRUE
hsa-miR-6514-3p	MIMAT0025485	C-302693-00	CUGCCUGUUCUUCCACUCCAG	-1.57253367763337	0.00023985	TRUE	-0.857463656453242	0.72813	FALSE
hsa-miR-652-5p	MIMAT0022709	C-301928-00	CAACCCUAGGAGAGGGUGCCAUUCA	-0.590578760858739	0.01741	FALSE	-2.04113601671663	0.11887	TRUE
hsa-miR-654-5p	MIMAT0003330	C-300988-01	UGGUGGGCCGCAGAACAUGUGC	-1.241020883078823	0.00054279	FALSE	-4.37321715649902	0.0018396	TRUE
hsa-miR-659	MIMAT0003337	C-300994-01	CUUGGUUCAGGGAGGGUCCCCA	-0.865338332757254	0.0053924	FALSE	-2.2970254587848	0.12226	TRUE
hsa-miR-660-3p	MIMAT0022711	C-301897-00	ACCUCCUGUGUGCAUGGAUUA	-0.914364872105563	0.0018747	FALSE	-2.7989676696545	0.028781	TRUE
hsa-miR-661	MIMAT0003324	C-300981-01	UGCCUGGGUCUCUGGCCUGCGCGU	-0.966061272920722	0.0046172	FALSE	-2.04789510522588	0.11887	TRUE
hsa-miR-664b-3p	MIMAT0022272	C-302539-00	UUCAUUUGCCUCCCAGCCUACA	-0.63588937093853	0.061384	FALSE	-2.38779084523873	0.057617	TRUE
hsa-miR-671-5p	MIMAT0003880	C-301000-03	AGGAAGCCCUGGAGGGGCUGGAG	-1.01268622344544	0.00023985	TRUE	-0.995050180707598	0.5811	FALSE
hsa-miR-6721-5p	MIMAT0025852	C-302707-00	UGGGCAGGGGCUUAUUGUAGGAG	-1.0522716562067	0.0021076	FALSE	-2.86092601806193	0.028781	TRUE
hsa-miR-7	MIMAT0000252	C-300546-07	UGGAAGACUAGUGAUUUUGUUGU	-0.833080606802588	0.0014577	FALSE	-2.34382127982937	0.11887	TRUE
hsa-miR-718	MIMAT0012735	C-301486-00	CUUCCGCCCCGCCGGGCGUCG	-0.712476270533885	0.0014577	FALSE	-2.51689917044679	0.08542	TRUE
hsa-miR-744	MIMAT0004945	C-301242-01	UGCGGGGCUAGGGCUAACAGCA	-1.31292964878657	0.0027607	FALSE	-2.17130853543699	0.057617	TRUE
hsa-miR-744*	MIMAT0004946	C-301241-01	CUGUUGCCACUAACCUCAACCU	-0.909425861300587	3.9917e-05	TRUE	-2.41920375944258	0.11887	TRUE
hsa-miR-764	MIMAT0010367	C-301640-00	GCAGGUGCUCACUUGUCCUCCU	-1.1596052122796	6.1693e-05	TRUE	-0.963991799367289	0.5811	FALSE
hsa-miR-767-3p	MIMAT0003883	C-301003-01	UCUGCUCAUACCCAUGGUUUCU	-0.875815222995269	0.0021076	FALSE	-2.87852305265755	0.009387	TRUE
hsa-miR-801	MI0005202	C-301014-01	GAUUGCUCUGCGUGCGGAAUCGAC	-1.02838938735747	0.00023985	TRUE	0.128066636696993	1	FALSE
hsa-miR-873-3p	MIMAT0022717	C-301921-00	GGAGACUGAUGAGUUCCCGGGA	-0.45500701580676	0.050037	FALSE	-2.17559760678154	0.08542	TRUE
hsa-miR-888*	MIMAT0004917	C-301227-01	GACUGACACCUCUUUGGGUGAA	-0.798719536763374	0.015067	FALSE	-1.83668949541677	0.11887	TRUE
hsa-miR-940	MIMAT0004983	C-301269-01	AAGGCAGGGCCCCCGCUCCCC	-1.31504996360232	0.00054279	FALSE	-2.52010733193133	0.11887	TRUE
hsa-miR-96	MIMAT0000095	C-300514-07	UUUGGCACUAGCACAUUUUUGCU	-0.788957233607577	0.0074787	FALSE	-2.19234338350236	0.11527	TRUE
hsa-miR-99a	MIMAT0000097	C-300516-03	AACCCGUAGAUCCGAUCUUGUG	-0.986802247020601	0.0030028	FALSE	-2.42723567464877	0.11887	TRUE

Legend
Antiviral effect
Proviral effect

Note: Hits were selected using the following Local False Discovery Rate (lfdr) thresholds: 0.00027 in screen part 1 and 0.1226 in screen part 2. Positive log2 fold change values indicate a proviral effect of the miRNA on the HCV life cycle, while negative log2 fold change values indicate an antiviral effect.

1 **A functional microRNA screen uncovers O-linked N-acetylglucosamine transferase as**

2 **a host factor modulating hepatitis C virus morphogenesis and infectivity**

3

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2

1   **Abstract**

2

3   **Objective:** Infection of human hepatocytes by the hepatitis C virus (HCV) is a multistep

4   process involving both viral and host factors. microRNAs (miRNAs) are small non-coding

5   RNAs that post-transcriptionally regulate gene expression. Given that miRNAs were

6   indicated to regulate between 30% and 75% of all human genes, we aimed to investigate the

7   functional and regulatory role of miRNAs for the HCV life cycle.

8   **Design:** To systematically reveal human miRNAs affecting the HCV life cycle, we performed

9   a two-step functional high-throughput miRNA mimic screen in Huh7.5.1 cells infected with

10   recombinant cell culture-derived HCV. miRNA targeting was then assessed using a

11   combination of computational and functional approaches.

12   **Results:** We uncovered miR-501-3p and miR-619-3p as novel modulators of HCV

13   assembly/release. We discovered that these miRNAs regulate O-linked N-acetylglucosamine

14   (O-GlcNAc) transferase (OGT) protein expression and identified OGT and O-GlcNAcylation

15   as regulators of HCV morphogenesis and infectivity. Furthermore, increased OGT

16   expression in patient-derived liver tissue was associated with HCV-induced liver disease and

17   cancer.

18   **Conclusion:** miR-501-3p and miR-619-3p and their target OGT are previously undiscovered

19   regulatory host factors for HCV assembly and infectivity. In addition to its effect on HCV

20   morphogenesis,   OGT   may   play   a   role   in   HCV-induced   liver   disease   and

21   hepatocarcinogenesis.

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3

| | **Significance of this study** |

What is already known about this subject?

- To establish chronic infection, the hepatitis C virus (HCV) hijacks cellular factors including microRNAs (miRNAs), known to post-transcriptionally regulate gene expression.

- miRNAs may positively or negatively modulate HCV infection either by directly targeting the viral genome or indirectly by regulating virus-associated cellular pathways[1, 2].

What are the new findings?

- A functional miRNA mimic screen uncovered miR-501-3p and miR-619-3p to enhance late steps of HCV infection.

- miR-501-3p regulates the expression of O-linked N-acetylglucosamine transferase (OGT) at the protein level.

- Silencing of OGT expression or inhibition of O-linked N-acetylglucosaminylation (O-GlcNAcylation) leads to an increase in the infectivity and size of HCV particles.

- OGT expression increases in patient-derived liver tissue during liver disease progression and cancer.

How might it impact on clinical practice in the foreseeable future?

- As upregulation of OGT and increased O-GlcNAcylation of proteins have been associated with various forms of cancer, OGT may play a dual role in HCV morphogenesis as well as pathogenesis of HCV-induced liver disease and carcinogenesis.

4

1   **Introduction**

2   Chronic hepatitis C is a major cause of chronic liver disease and hepatocellular carcinoma

3   (HCC). Since the approval of pan-genotypic direct-acting antivirals (DAAs), it is considered a

4   curable disease in more than 90% of treated patients. Nonetheless, an estimated 71 million

5   individuals are still infected by the hepatitis C virus (HCV) and several challenges remain;

6   viral cure reduces but does not eliminate the HCC risk in patients with advanced fibrosis[3],

7   the majority of infected patients has limited access to therapy and DAA failure/viral

8   resistance has been reported in a subset of patients[4, 5]. To overcome these limitations,

9   approaches to target host factors involved in HCV infection and pathogenesis are

10 developed[6, 7]. Interestingly, defined host factors that contribute to the establishment of

11 chronic HCV infection and represent potential antiviral targets, e.g. epidermal growth factor

12 receptor[8], also play a role in liver disease pathogenesis and represent candidate targets for

13 treatment of advanced liver disease and HCC prevention[9]. Thus, uncovering host factors

14 usurped by HCV not only contributes to a better understanding of virus-host interactions

15 underlying the HCV life cycle but also to the identification of potential targets for treatment of

16 liver disease and prevention of HCC.

17   The establishment of various models to study HCV infection has shed light on the

18 molecular mechanisms that govern the HCV life cycle, which can be subdivided into early

19 steps, including viral entry, translation and replication as well as late steps, including

20 assembly and release of new virions. Each step of the HCV replication cycle relies on

21 specific virus-host interactions that involve host proteins and microRNAs (miRNAs)[7], small

22 non-coding RNAs that regulate gene expression at the post-transcriptional level. One miRNA

23 can target numerous messenger RNAs (mRNAs) by base-pairing with a complementary site

24 that is typically located within the 3′ untranslated region (3′UTR) of the mRNA. Accumulating

25 evidence indicates that miRNAs participate to HCV replication by exerting pro- or antiviral

26 effects. The breakthrough discovery of the direct targeting of HCV by miR-122, the most

27 abundant miRNA in the liver, revealed the crucial role of this miRNA for HCV

28 translation/replication that contributes to progression to chronic HCV infection[1, 10]. miR-

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1   122 antisense oligonucleotides <u>were subsequently</u> developed as host-targeting antivirals[11,

2   12]. Other miRNAs <u>can</u> indirectly target HCV by regulating host factors that participate in

3   antiviral responses and immune surveillance[2, 13, 14]. Since up to 60% of all human

4   protein-coding genes were reported to be under miRNA-mediated regulation and miRNAs

5   are involved in basically every biological process, we hypothesized that miRNAs provide a

6   tool for loss-of-function approaches to uncover novel HCV host factors. We performed

7   genome-wide high-throughput modulation of the human miRNome and analyzed the<u>ir</u> impact

8   on HCV infection by combining computational and functional approaches.

9

10   **Material and methods**

11   **Cells, cell culture conditions, viruses, virus purification, infectivity assays, miRNAs,**

12   <u>**antagomiRs,**</u> **siRNAs, antibodies, immunoblot, immunocapture, electron microscopy**

13   **analysis of viral particles** <u>**and gene expression analysis in liver tissue**</u> are described in

14   the Supplementary information.

15

16   **Functional miRNA/siRNA screens.** Huh7.5.1 cells were transfected with the miRIDIAN

17   human miRNA mimic library (mIRBase 19) <u>comprising</u> more than 2000 mature miRNAs or 28

18   ON-TARGETplus smart pool siRNAs (20 nM, Dharmacon) using Interferin HTS (Polyplus) in

19   a 96-well format[8]. After 48h, a viability test (Presto Blue, Thermo Scientific) was performed

20   prior to a two-step infection assay[15, 16, 17]. During part <u>1</u> of the protocol, 50 µL of HCV cell

21   culture-derived particles (HCVcc, JcR2a) were incubated with cells during 4h. The inoculum

22   was removed and cells were incubated with 150 µl of medium for 48h. In part 2, supernatants

23   from part 1 cells were transferred onto naïve Huh7.5.1 cells and part 1 cells were lysed to

24   determine luciferase activity[17, 18]. After 72h, part 2 cells were lysed to determine luciferase

25   activity[17]. siCD81 (20 nM), antagomiR-122 (100 nM) and siApoE (20 nM) were used as

26   positive controls[17]. A non-targeting siRNA with no sequence complementarity to any

27   human gene or homology to any human miRNA was used as negative control.

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6

Inhibitor treatment. Four hours following HCV RNA electroporation[8], Huh7.5.1 cells were incubated with vehicle or inhibitors of OGT (peracetylated 5-thio-N-acetylglucosamine (Ac$_4$5S-GlcNAc)[19]) or OGA (Thiamet G (Sigma))[20]. After 96h, supernatants were transferred onto naïve Huh7.5.1 cells for 72h prior to determination of luciferase activity while electroporated cells were lysed to determine luciferase activity.

Gene expression analyses. Total RNA was purified[17] and transcribed into cDNA using Maxima reverse transcriptase (Thermo Scientific). *GAPDH* and *OGT* mRNA was detected by real time qPCR using iTaq™ Universal Probes Supermix (Bio-Rad) and TaqMan Gene Expression Assay (Thermo Scientific). Relative *OGT/GAPDH* gene expression was calculated by the ΔΔCt method[21].

Dual luciferase reporter gene assay. The human *OGT* 3'UTR sequence was retrieved from NCBI (NM_181672.2) and Ensembl genome browser (ENST00000373719.3). A fragment of the *OGT* 3′UTR (positions 3380-3837, NM_181672.2) (Thermo Fisher Scientific GENEART) was cloned between the *Not*I and *Xho*I sites downstream of a *Renilla* luciferase cassette in a psiCHECK2 plasmid (Promega). A mutated version of this construct (9-bp substitution in the predicted miR-501-3p target site) was generated as described[22]. The functionality of the *OGT* 3′UTR was assessed as described[23]. The miRIDIAN mimic negative control 1 was used as control. *Renilla* and *firefly* luciferase activity was assessed 48h after transfection into HeLa cells using Dual-Luciferase Reporter assay (Promega).

Bioinformatic and statistical analysis. Data analysis and statistical treatment for the miRNA mimic screen were performed in R (www.r-project.org). Cell measurement data used in further analysis were cell viability and luciferase activity. In total 26 sets of plates (performed in triplicate) were tested. The presence of multiple wells with negative and positive controls on each plate allowed stepwise normalization intra- and inter-plate. First, intra-plate zonal bias was examined and a model of median effects across the entire screen

7

determined using the median-polish algorithm[24] and all plates corrected accordingly. Then the dataset was examined for outlier plates, i.e. plates where all individual measurements correlate very poorly with the other remaining replicates. Three and 9 plates were excluded for part 1 and part 2 of the screen, respectively, based on poor median correlation (r < 0.7) so that the remaining plates correlation improved substantially (> 40%). Next, the plates were normalized inter replicates using the particularly robust quantile-quantile approach[25]. Finally, the data were tested using a moderated t-test (empirical Bayes shrinkage, R-package limma[26]) for the null-hypothesis of no change of a given miRNA compared to the negative control. The resulting *p*-values for independent testing of each miRNA where corrected for the multiple testing situation and expressed as local false discovery rate (lfdr, R-package fdrtool[27]). The testing was performed independently for part 1 and 2 of the screen and candidate miRNAs selected for each part. For data from part 1, a lfdr threshold of 0.00027 was used. Data from part 2 were subject to increase inherent stochastic noise and for this reason the minimum acceptable relative risk of false positives was increased to 0.1226 (i.e. maximum 15% risk for each of the retained hits).

Other datasets were analyzed using the two-tailed Mann-Whitney test, Wilcoxon test, Spearman correlation or the two-tailed unpaired t-test for data with normal distribution as assessed by D'Agostino and Pearson omnibus and Shapiro-Wilk normality tests (GraphPad Prism v.6 package).

Results

Genome-wide identification of human miRNAs affecting the HCV life cycle. We performed a genome-wide screen in human hepatoma Huh7.5.1 cells using a genomic miRNA mimics library and a two-step infection assay[17] with a luciferase reporter virus (JcR2a), which allowed us to functionally assess the role of miRNAs during the early steps (part 1 - viral entry/translation/replication) and the late steps (part 2 - viral assembly/release/infectivity) of the HCV life cycle (Fig. 1A). Silencing of *CD81* and *ApoE*,

8

two essential host factors required for HCV entry or assembly, respectively, was performed

in parallel using small interfering RNA (siRNA) as controls. Silencing of *CD81* resulted in a

reduction of HCV infection in part 1 and consequently in part 2 of the screen since reduced

viral entry in the first part of the assay leads to a reduced production of viral particles (Fig.

1B)[17]. Silencing of *ApoE* resulted in a marked inhibition of HCV infection only in part 2 of

the assay, consistent with the role of ApoE in HCV assembly (Fig. 1B)[17]. The screen

identified 427 miRNAs (corresponding to about 16% of the library) that significantly

modulated HCV infection (lfdr < threshold, Supplementary Table 1 and Fig. 1C): 186 miRNAs

affected HCV infection in part 1, 309 miRNAs affected HCV infection in part 2, including 68

hits in part 1 and part 2. The limited number of part 1 and 2 hits may be due to the fact that a

single miRNA may modulate the expression of several proteins, which may have different

roles in the viral life cycle. Most hits were observed to dampen HCV infection independently

of any significant alteration of cell viability (data not shown). The 186 miRNAs modulating the

early steps of HCV infection all decreased viral infection. Among the 309 miRNAs that had

an impact in part 2, 11 miRNAs increased HCV infection by at least 3-fold while 298 miRNAs

inhibited HCV infection by at least 2.7-fold. Hits from the screen included the let-7 family[2,

28], miR-27a[29] and miR-29 family[30] that were already shown to inhibit HCV infection, as

well as miR-21[31] and miR-146a-5p[17] that were shown to stimulate HCV infection thus

supporting the relevance of our findings. Collectively, our screen identified a set of miRNAs

whose overexpression overall impairs HCV infection by affecting viral

entry/translation/replication and/or virion assembly/egress/infectivity.

miR-619-3p, miR-501-3p and OGT play a role in late steps of the HCV life cycle. We

focused our analysis on miRNAs that modulate late steps of the HCV life cycle, as the

molecular mechanisms of HCV assembly/release remain only partially understood. Our

screen identified 241 miRNAs that modulated late steps without affecting early steps of

infection: 11 miRNAs increased HCV infection while 230 miRNAs decreased HCV infection.

Among the miRNAs that increased HCV infection, miR-140-3p, miR-501-3p, miR-619-3p and

9

miR-4778-5p have not yet been associated with HCV. Since they enhanced HCV infection in part 2 without affecting part 1, these miRNAs may target host genes that control virus assembly/egress/infectivity. We first confirmed the effect of these miRNAs in independent experiments using the same protocol as for the screen. Overexpression of miR-619-3p or miR-501-3p consistently led to an increase in the infection of progeny virions (Fig. 1D) while infection was decreased with progeny virions from antagomiR-transfected cells (Supplementary Figure S1A). miR-619-3p or miR-501-3p were thus selected for further investigation. To study the molecular mechanisms by which these miRNAs affect HCV infection, we generated a list of predicted miRNA targets using DIANA, TargetScan Human v6.2 and miRDB databases, and selected candidate targets based on their expression in our Huh7.5.1 cells as assessed by microarray (data not shown). Ingenuity Pathway Analysis enabled us to refine the gene list by selecting 28 genes involved in the following functional networks or pathways that contribute to the HCV life cycle[32, 33, 34]: lipid metabolism and cholesterol biosynthesis, protein maturation and processing at the endoplasmic reticulum (ER), components of the endosomal sorting complex, adipocyte biogenesis, cellular morphology and cell inflammation (Table 1).

To assess whether knock-down of these 28 candidate targets affects virus production, we performed a siRNA-based screen using siRNA pools exhibiting strong silencing without cytotoxicity (Fig. 2). Silencing of *CD81* and antagomiR-122 served as controls for part 1; knock-down of *ApoE* served as control for part 2 (Fig. 2). Hits were defined as genes whose knock-down modulated HCV infection in at least one part of the screen with high significance (Fig. 2, p-value $\leq$ 0.0001, Mann-Whitney U-test). HCV entry/translation/replication was significantly modulated by silencing of *PPP3CA, CEBPA, MID1, WDFY3, DCX* and *SLC35D1*. HCV assembly/egress/infectivity was significantly modulated by knock-down of *PPP3CA, CSDE1, GAN, USP37, CEBPA, MID1, WDFY3, DCX, MAPK9, SLC35D1, DCC, RNF144A, PPP2R2C* and *OGT*. Strikingly, only the silencing of *OGT* was associated with an enhancement of HCV assembly/release/infectivity (p-value = 0.0002), while that of the other hits was associated with reduced HCV infection (Fig. 2).

10

1   These results indicate that the down-regulation of *OGT* phenocopies the effect of miR-501-

2   3p and miR-619-3p on HCV infection (Fig. 2) <u>and</u> suggest OGT as a novel player <u>in</u> the HCV

3   life cycle.

4

5   **miR-501-3p <u>post-transcriptionally</u> regulates OGT expression.** To study whether <u>miR-501-</u>

6   <u>3p and miR-619-3p target</u> OGT, we analyzed OGT RNA and protein levels in Huh7.5.1 cells

7   following overexpression of miR-501-3p or miR-619-3p. While neither miRNA had an impact

8   on OGT RNA levels (Fig. 3A), up-regulation of miR-501-3p significantly decreased OGT

9   protein expression by ~65% (Fig. 3B, *p*-value $\leq$ 0.05, t-test). miR-619-3p also decreased

10 OGT expression but less robustly than miR-501-3p (Fig. 3B), prompting us to focus our

11 investigation on miR-501-3p. To assess whether OGT is a functional target of miR-501-3p<u>,</u>

12 <u>we</u> subcloned a fragment of the *OGT* mRNA 3′UTR that harbors the predicted miR-501-3p

13 target site in the *Renilla* luciferase expression cassette (RLuc) of a dual luciferase reporter

14 construct. Co-transfection of miR-501-3p mimic with the wild-type 3'UTR reporter (RLuc wt

15 *OGT* 3'UTR) significantly decreased luciferase activity as compared to the empty vector (Fig.

16 3C, p-value < 0.05, t-test). In contrast, the repression of luciferase expression was lost when

17 the reporter with mutated miR-501-3p binding site (RLuc mt *OGT* 3'UTR) was used (Fig. 3C).

18 <u>These</u> data are consistent in indicating that miR-501-3p mediates post-transcriptional

19 regulation of OGT.

20

21 **O-GlcNAcylation modulates HCVcc infectivity.** To investigate whether OGT modulates

22 HCV assembly and/or infectivity, we determined infectious virus titer (TCID50) and HCV RNA

23 levels to calculate the specific infectivity of HCVcc particles generated in OGT-silenced

24 Huh7.5.1 cells. Interestingly, OGT-silencing led to a significant increase in the TCID50 and

25 the specific infectivity of HCVcc (Fig. 4A, *p*-value $\leq$ 0.05, Mann-Whitney test). <u>Noteworthy</u>,

26 the effect of OGT on HCVcc infectivity was genotype-independent as demonstrated by

27 increased infectivity of HCVcc bearing the envelope glycoproteins of genotypes 1a, 1b and

28 2a upon OGT-silencing (Fig. 4B). We next sought to investigate how OGT could modulate

11

HCVcc infectivity. OGT is the only enzyme that catalyzes the addition of N-acetylglucosamine (O-GlcNAc) to serine and threonine residues of proteins. Moreover, OGT has a scaffold function and promotes binding of proteins in multiprotein complexes[35]. To assess whether the enzymatic activity of OGT modulates HCVcc infectivity, we used pharmacological inhibitors of OGT ($Ac_4$5S-GlcNAc) or O-GlcNAcase (OGA) (Thiamet G), the OGT counterpart that removes O-GlcNAc (Fig. 4C). $Ac_4$5S-GlcNAc led to a significant enhancement of HCVcc infectivity in a dose-dependent manner, while the opposite effect was observed with Thiamet G (Fig. 4D, *p*-value $\leq$ 0.05, Mann-Whitney test). Collectively, these results demonstrate that O-GlcNAcylation modulates HCVcc infectivity.

OGT-silencing affects HCVcc biophysical properties and size distribution. To further assess how OGT may impact HCVcc morphogenesis, we analyzed the structural and biophysical properties of HCVcc produced in siCtrl- and siOGT-transfected Huh7.5.1 cells following iodixanol gradient ultracentrifugation. Silencing of OGT led to the production of more infectious HCVcc with higher density (Fig. 5A-B) as well as higher ApoE concentrations (Fig. 5C) suggesting that OGT/O-GlcNAcylation affects the biophysical properties of HCVcc. No change in apoB concentrations were observed between HCVcc produced from siCtrl- or siOGT-transfected cells (Fig. 5D), in line with the model that HCV lipoviroparticles contain several exchangeable ApoE molecules and one non-exchangeable apoB[36]. We also visualized HCVcc by electron microscopy (EM) following anti-E2 antibody immunocapture[36] to assess whether OGT-silencing had an impact on HCVcc size. Particle size distribution was assessed from a series of randomly acquired electron micrographs. A shift towards bigger sizes was observed for sucrose-cushion purified HCVcc generated in OGT-silenced Huh7.5.1 cells as compared to control HCVcc (Fig. 6A-B). This shift was also observed in different fractions of iodixanol gradient-separated HCVcc (Fig. 6C-F) in line with the higher infectivity and ApoE concentrations of HCVcc generated in OGT-silenced Huh7.5.1 cells (Fig. 5A-C). These data suggest that OGT-silencing affects the lipidation of HCVcc.

12

OGT expression increases in liver disease. Since silencing of OGT promotes HCV infectivity, we assessed whether HCV infection in turn had an effect on miR-501-3p and OGT expression. In Huh7.5.1 cells, HCV infection lead to small but significant increase of miR-501-3p and decrease of OGT levels (Fig. 7A-B and Supplementary Fig. 1B; *p*-value < 0.05, Mann-Whitney test), which may promote viral infection given the pro- and antiviral roles of miR-501-3p and O-GlcNAcylation, respectively (Fig. 1C-D and 4D). In contrast, no significant difference of OGT expression was observed between the livers of HCV transgenic and wild-type mice[37] (data not shown) suggesting that HCV proteins do not directly modulate OGT expression. In liver tissue from HCV-infected patients, HCV RNA levels were not correlated with OGT expression (Fig. 7C, Spearman correlation: 0.06004019, p-value = 0.7661) suggesting that in patients there is likely no direct effect of HCV on OGT expression.

O-GlcNAcylation has been associated with a variety of cancers, including HCC recurrence linked to increased O-GlcNAcylation after liver transplantation[38]. We therefore investigated OGT expression in chronic liver disease and HCC. While there was a trend for increased OGT expression in liver tissue from HCV-infected patients with fibrosis and inflammation (Fig. 7D-E), OGT levels were markedly and significantly elevated in the tumor liver tissue of patients chronically infected with HCV or hepatitis B virus and patients with alcoholic liver disease or non-alcoholic fatty liver disease as compared to non-tumor tissue (Fig. 7F, *p*-value < 0.05, Wilcoxon test). These data suggest that OGT expression increases in HCC in an etiology-independent manner. Collectively, these results suggest that OGT expression is likely increased in HCV-induced liver disease and cancer through inflammation and fibrosis rather than by HCV itself.

Discussion

By focusing on miRNAs affecting late steps of the viral life cycle, we uncovered that i) miR-501-3p regulates the expression of OGT; ii) silencing of OGT expression or inhibition of its enzymatic activity increases the infectivity of HCV particles; and iii) OGT knock-down leads

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to the release of bigger HCV particles. Our data suggest that O-GlcNAcylation affects HCV morphogenesis and infectivity.

While we were characterizing the role of OGT/O-GlcNAcylation for HCV morphogenesis, Li and colleagues published their functional genomics study of HCV-miRNA interactions[2]. By conducting genome wide miRNA mimic and hairpin inhibitor screens, they identified a set of miRNAs exhibiting a pro- or antiviral effect on HCV. Characterization of the underlying molecular processes showed that miR-25, let-7 and miR-130 families restrict viral infection by decreasing the expression of cellular HCV co-factors[2]. Despite similarities in the cell type and HCV infection models used here and by Li and colleagues, our screen only displays a small overlap with their study (9% common miRNA hits). This is not surprising given the small overlap between previous siRNA screens to uncover HCV host factors[8, 15] and is likely due i) to the different sizes of miRNA mimic libraries as the library used here was more than 2-times larger than the one used by Li and co-workers, and ii) to the markedly distinct pipelines for hit selection that were used in the two studies. Nonetheless, both screens were consistent in confirming the proviral role of miR-146a-5p in promoting HCV assembly/egress that we previously reported[17] and the global multistep inhibitory effects of the let-7 family on HCV infection[28], further corroborating the involvement of these miRNAs in fine-tuning the HCV life cycle. Both studies also consistently indicated that miR-518a-5p, miR-517-3p, miR-185 and members of the miR-302 family inhibit early steps of HCV infection, while miR-586, miR-620 and members of the miR-200 family inhibit late steps of viral infection. Since none of these miRNAs except miR-185 has been previously associated with HCV infection[39], it might be interesting to further characterize the involvement of these miRNAs in HCV-host interactions. Interestingly, an overall proviral effect of miR-501-3p was also observed by Li and colleagues[2], however the mechanism of action was not studied. By characterizing the role of miR-501-3p in the HCV life cycle, we uncovered OGT as a miR-501-3p target in liver-derived cells and showed for the first time a link between O-GlcNAcylation and HCV infection. These results indicate that genome-wide miRNA functional

14

1　screens represent a powerful strategy to dissect the role of miRNAs in pathogen-host

2　interactions.

3　　While N-glycosylation of HCV envelope glycoproteins plays an important role for

4　escape from virus-neutralizing antibodies[40], so far no functional association between HCV

5　and O-glycosylation has been reported. In contrast to N-linked glycosylation that consists of

6　the attachment of a glycan to a nitrogen of an asparagine residue of proteins in the ER/Golgi

7　prior to their trafficking to the plasma membrane and/or their secretion, the glycosylation of

8　serine and threonine residues with O-GlcNAc is a post-translational modification (PTM) of

9　intracellular proteins that are localized in the nucleus, cytoplasm or mitochondria. The O-

10　glycosylation/deglycosylation of proteins is catalyzed by a single pair of nucleo-cytoplasmic

11　enzymes, OGT/OGA. O-GlcNAcylation is complementary to protein

12　phosphorylation/dephosphorylation, another more broadly known abundant protein PTM that

13　involves numerous kinases/phosphatases. OGT/OGA are often found in protein complexes

14　that also include kinases/phosphatases and a protein can be either O-GlcNAcylated or

15　phosphorylated on a same residue to fine-tune cellular signaling[41]. O-GlcNAcylation and

16　phosphorylation on the same or neighboring serine or threonine residue is known as yin yang

17　site[42].

18　　O-GlcNAcylation plays a major role in the regulation of metabolic pathways in the

19　liver, including insulin signaling, bile acid metabolism and lipogenesis[35]. The large number

20　of OGT/OGA substrates and cellular pathways regulated by O-GlcNAcylation hampers a

21　detailed characterization of the role of these proteins in HCV infection. Since i) HCV

22　assembly takes place at ER-derived membranes, ii) OGT/OGA are not known to localize in

23　the ER lumen, and iii) O-GlcNAcylation of extracellular proteins containing EGF-like domains

24　is catalyzed by EGF domain-specific OGT (EOGT) in the ER lumen in an OGT-independent

25　manner[43]), OGT/OGA most likely modulate HCV infection by post-translationally modifying

26　one or several cellular factors required for HCV morphogenesis rather than by affecting viral

27　proteins, although HCV glycoproteins contain putative O-GlcNAcylation sites as determined

28　using OGlcNAcScan, OGTsite and YingOYang1.2 bioinformatics tools (data not shown).

15

1   Regarding HCV host factors that may be regulated by OGT/OGA, O-GlcNAcylation

2   sites have been predicted in human CLDN1[44] and OCLN at serine sites that can also be

3   phosphorylated and this has been suggested to potentially play a role for HCV entry[45].

4   However, in our experimental setting we did not observe a significant effect of OGT-silencing

5   on the early steps of HCV infection, suggesting that O-GlcNAcylation of CLDN1 and/or

6   OCLN likely does not play a major role in HCV infection. Other host factors important for the

7   HCV life cycle are well-known O-GlcNAcylated proteins, as for example various nuclear pore

8   complex proteins (Nups) including Nup98, Nup153 and Nup155 that are involved in HCV

9   replication and assembly and/or may be associated with viral particles[46, 47, 48]. However,

10   since depletion of Nups was reported to alter HCV replication and/or assembly but to have

11   no impact on the specific infectivity of HCV particles[46] in contrast to the depletion of OGT

12   as shown here, it is unlikely that a modulation of Nup O-GlcNAcylation accounts for the

13   effects of OGT-silencing and/or OGT/OGA inhibitors on HCVcc infectivity observed in our

14   study. This is in line with our observation that OGT knock-down had no effect on Dengue

15   virus (DENV) replication and infectivity (unpublished observations KH, MZ and Evelyne

16   Schaffer, IBMC, Strasbourg), although Nup98 had been suggested to potentially play a role

17   for DENV infection[46]. These data suggest that OGT does not broadly modulate the

18   infectivity of viruses of the *Flaviviridae* family.

19   However, OGT and/or O-GlcNAcylation have been reported to play a role in the

20   infection with other viruses[49, 50, 51]. Interestingly, while OGT expression modulates the

21   levels of human papillomavirus 16 (HPV16) oncoproteins E6 and E7[52], E6 in turn can up-

22   regulate OGT to increase O-GlcNAcylation and the oncogene activities of HPV[53],

23   suggesting that OGT/O-GlcNAcylation could play a role in virus-induced cancer. In cell

24   culture, HCV infection appeared to be associated with a minor decrease in OGT expression

25   in line with an antiviral role of O-GlcNAcylation. In contrast, an increased OGT expression

26   was observed in HCC tissues of HCV-infected patients. Since OGT has been suggested to

27   activate oncogenic signaling pathways in non-alcoholic steatohepatitis-related HCC[54] and

28   O-GlcNAcylation has been associated with HCC recurrence linked to increased O-

16

GlcNAcylation after liver transplantation[38], these data suggest that in addition to their effect on the HCV life cycle, OGT/O-GlcNAcylation may also play a role in HCV-induced hepatocarcinogenesis.

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22

1 **Figure legends**

2 **Figure 1. High-throughput screen identifies human miRNAs that regulate the HCV life**

3 **cycle.** (A) Schematic outline of the miRNA mimic screen strategy. Huh7.5.1 cells were

4 transfected with miRNA mimics or controls prior to infection with *Renilla* luciferase HCVcc

5 (JcR2a) two days later (part 1). Cell supernatants of part 1 were used to inoculate naïve

6 Huh7.5.1 cells (part 2). Cells from part 1 and part 2 were lysed at the end of each infection

7 step (2 and 3 days post infection, respectively) to determine luciferase activity. (B)

8 Modulation of HCV entry and replication (part 1) and/or assembly and infectivity (part 2) upon

9 transfection of control non-targeting siRNA (siCtrl, negative control), siCD81 (inhibiting viral

10 entry) or siApoE (inhibiting viral assembly). By inhibiting HCV entry, siCD81 impacts part 1

11 as well as part 2. In contrast, by specifically impairing late steps of HCV replication cycle,

12 siApoE inhibits HCV infection only in part 2. The box plots show the sample lower quartile

13 (25th percentile; bottom of the box), the median (50th percentile; horizontal line in box) and

14 the upper quartile (75th percentile; top of the box) of relative light units (RLU) in each lysate.

15 The whiskers indicate s.d. Data are from three independent experiments. (C) Effects of

16 miRNA overexpression on each part of the HCV life cycle. Data were tested using a

17 moderated t-test (empirical Bayes shrinkage, R-package limma[26]) for the null-hypothesis of

18 no change of a given miRNA compared to the negative control. The resulting p-values for

19 independent testing of each miRNA where corrected for the multiple testing situation and

20 expressed as local false discovery rate (lfdr, R-package fdrtool[27]). miRNAs having a

21 significant effect on either part 1 or 2 of the screen are below the thresholds indicated by

22 dashed lines (lfdr < 0.00027 or 0.1226, respectively). miRNAs that were previously reported

23 to impact on HCV infection as well as miR-140-3p, miR-501-3p, miR-619-3p and miR-4778-

24 5p are highlighted in blue (Log2(FC) < 0) or red (Log2(FC) > 0). Data are from three

25 independent experiments. (D) Effect of miR-140-3p, miR-501-3p, miR-619-3p and miR-4778-

26 5p on the HCV life cycle. Huh7.5.1 cells were transfected with siCtrl (Ctrl), miR-140-3p, miR-

27 501-3p, miR-619-3p or miR-4778-5p and infection experiments were carried out as described

28 in A. HCV infection was determined as luciferase activity. Results represent mean

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2
3   1   percentage ± s.d. from three independent experiments in triplicate. The dashed line indicates
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5   2   values from control-transfected cells set at 100%. Statistics**: *,** *p*-value ≤ 0.05, Mann-Whitney
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7   3   test.
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10   4
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12   5   **Figure 2. OGT is a novel host cell factor involved in the late steps of the HCV life cycle.**
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14   6   Huh7.5.1 cells were transfected with a set of siRNAs against 28 predicted targets of miR-
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16   7   501-3p and/or miR-619-3p, and infected with HCVcc JcR2A according to the two-step
17
18   8   protocol depicted in Fig. 1A. siCD81, antagomiR-122 and siApoE were used as loss-of-
19
20   9   function controls to perturb HCV entry, translation/replication and assembly, respectively.
21
22   10   miR-501-3p and miR-619-3p, which were ineffective in part 1 of the screen but enhanced
23
24   11   HCV infection in part 2, were transfected in parallel. HCV infection was quantified as fold
25
26   12   change of luciferase activity with respect to negative control (siCtrl). Results for different
27
28   13   replicates are shown as individual points. For each gene, median fold change of luciferase
29
30   14   activity ± s.d. is shown as black horizontal lines. The dashed line indicates a fold change of
31
32   15   1. Data are from three independent experiments in triplicate. Results for miR-501-3p, miR-
33
34   16   619-3p and siOGT that increase HCV infection in part 2 are depicted in red. Results for
35
36   17   siRNA targeting *PPP3CA, CEBPA, MID1, WDFY3, DCX, SLC35D1, CSDE1, GAN, USP37,*
37
38   18   *MAPK9, DCC, RNF144A,* or *PPP2R2C* that significantly modulated HCV infection in part 1
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40   19   and/or part 2 but did not phenocopy the effect of miR-501-3p and miR-619-3p are depicted in
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42   20   blue.
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44   21
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47   22   **Figure 3. miR-501-3p mediates post-transcriptional regulation of OGT by decreasing**
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49   23   **its expression at the protein level.** Huh7.5.1 cells were transfected with siCtrl (Ctrl), a pool
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51   24   of siRNA against *OGT*, miR-501-3p or miR-619-3p. After 96h, RNA and proteins were
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53   25   purified, and OGT expression analyzed by RT-qPCR and Western blot. (A) Percentage of
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55   26   *OGT* mRNA expression in miRNA-transfected cells as compared to negative control. Results
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57   27   are presented as mean ± s.d. and are from three independent experiments in triplicate. The
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59   28   dashed line indicates values from control-transfected cells set at 100%. Statistics: *, *p*-value
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< 0.05, t-test (B) OGT protein expression. Left: percentage of OGT protein expression in siRNA- or miRNA-transfected cells as assessed by quantification of Western blots. OGT levels were normalized to actin levels using ImageLab™ 5.2.1 software (BioRad). Results are presented as mean ± s.d. and are from three independent experiments. The dashed line indicates values from control-transfected cells set at 100%. Statistics: *, *p*-value < 0.05, t-test. Right: representative Western blot analysis. (C) Analysis of miRNA targeting of *OGT* expression by dual luciferase reporter assay. Left: HeLa cells were co-transfected with a miR-501-3p mimic and a dual luciferase reporter plasmid containing either wild type miR-501-3p (RLuc wt *OGT* 3'UTR) or mutated miR-501-3p binding site (RLuc mt *OGT* 3'UTR) to modulate RLuc expression. Co-transfection of the miR-501-3p mimic and empty RLuc vector was used as control. Data are expressed as mean percentage of *Renilla* luciferase activity ± s.d. normalized to *firefly* luciferase, and relative to co-transfection of the vectors with non-targeting miRNA (miR-Ctrl). Results are from three independent experiments in triplicate. The dashed line indicates values from control-transfected cells set at 100%. Statistics: *, *p*-value < 0.05, t-test. Right: Schematic representation of the used constructs.

Figure 4. Silencing of *OGT* affects HCV morphogenesis and infectivity. (A) Analysis of HCV infectivity. Huh7.5.1 cells were transfected with siCtrl, a pool of siRNA against *OGT* or ApoE as a loss-of-function control to perturb HCV assembly, prior to infection with HCVcc (Jc1) two days later (entry and replication). Mock-transfected cells were used as control (Ctrl). After another 48h, intra- and extracellular HCVcc particles were used to infect naïve Huh7.5.1 cells (assembly and infectivity). Virus supernatants of Huh7.5.1 cells were assayed by (left) endpoint dilution assay (TCID50). Intra- and extracellular HCV RNA was purified and analyzed by RT-qPCR to calculate (right) the specific infectivity (TCID50/RNA). Data are expressed as mean percentage as compared to control ± s.d. Results are from four independent experiments in triplicate. The dashed line indicates values from control-transfected cells set at 100%. Statistics**: ***, *p*-value < 0.05, Mann-Whitney test. (B) Genotype-independent effect of OGT on HCV infection. Huh7.5.1 cells were transfected with siCtrl or

25

1 siOGT prior to infection with HCVcc JcR2a (genotype 2a), H77R2a (genotype 1a) or

2 Con1R2a (genotype 1b). Experiments were carried out and analyzed as described in A. Data

3 are expressed as mean percentage of Renilla luciferase activity as compared to control ± s.d.

4 Results are from three independent experiments in quadruplicate. The dashed line indicates

5 values from control-transfected cells set at 100%. Statistics**: ***, *p*-value < 0.05, Mann-Whitney

6 test. (C) Activity of OGT/OGA inhibitors on O-GlcNAcylation. The activity of $Ac_4$5S-GlcNAc

7 (OGT inhibitor) or Thiamet G (OGA inhibitor) on O-GlcNAcylation of proteins in Huh7.5.1

8 cells was demonstrated by Western blot as described in Supplementary Methods. (D) Effect

9 of O-GlcNAcylation on HCV infectivity. Huh7.5.1 cells were electroporated with HCVcc

10 (JcR2a), prior to treatment with increasing concentrations of $Ac_4$5S-GlcNAc (OGT inhibitor,

11 left) or Thiamet G (OGA inhibitor, right) 4h later. After 96h, supernatants were transferred

12 onto naïve Huh7.5.1 cells and electroporated cells were lysed to determine luciferase

13 activity. Luciferase activity in infected Huh7.5.1 cells was assessed 72h later. Data are

14 expressed as mean percentage as compared to control ± s.d. Results are from three

15 independent experiments in quadruplicate. The dashed line indicates values from vehicle-

16 treated cells set at 100%. Statistics**: ***, *p*-value < 0.05, Mann-Whitney test.

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18 **Figure 5. Silencing of *OGT* modulates HCVcc biophysical properties.** (A) Separation of

19 HCVcc by iodixanol density gradient ultracentrifugation. HCVcc were produced in non-

20 targeting siRNA control- or siOGT-transfected Huh7.5.1 cells. After overlaying HCVcc

21 (JcR2A) on a 4%-40% iodixanol step gradient and ultracentrifugation for 16h, fractions of

22 HCV particles were used to infect naïve Huh7.5.1 cells in order to determine TCID50. HCV

23 RNA of each fraction was purified and analyzed by RT-qPCR. Data are expressed as mean ±

24 s.d. from three independent experiments. (B) Specific infectivity (TCID50/RNA) was

25 calculated and the density was determined by weighting each fraction. Specific infectivity of

26 each fraction is expressed as fold change as compared to the total infectivity of the control.

27 Data are expressed as mean ± s.d. from three independent experiments. (C-D) ApoE and

28 ApoB concentrations in the individual fractions were determined by ELISA. The dashed lines

26

indicate limits of quantification of the assays. Data are expressed as mean ± s.d. from three independent experiments.

Figure 6. Silencing of *OGT* increases the size of HCVcc. (A) Representative pictures of HCV particles generated in Huh7.5.1 cells transfected with non-targeting siRNA (siCtrl) or siOGT. (B-F) Comparative analysis of particle size distribution for immunocapture (IC) from HCV particles produced in Huh7.5.1 cells transfected with siCtrl or siOGT prior to infection with HCVcc (JcR2a) following sucrose-cushion purification (B) or iodixanol gradient fractionation (C-F) of HCVcc. HCVcc were transferred via anti-E2 antibody AR3A on electron microscopy (EM) grids through IC. Particle size distribution was assessed from a series of randomly acquired electron micrographs with Image-J software (NIH). Results from one of three (A-B) or two (C-F) independent experiments are shown. Black lines: size distribution of immunocaptured HCVcc produced in siCtrl-transfected cells. Grey lines: size distribution of immunocaptured HCVcc produced in siOGT-transfected cells.

Figure 7. *OGT* expression increases in HCC. (A-B) Huh7.5.1 cells were infected with HCV (JcR2a). After 72h, RNA and proteins were purified, and *OGT* expression analyzed by RT-qPCR and Western blot. (A) Percentage of *OGT* mRNA expression relative to uninfected Huh7.5.1 cells (Ctrl). Results are presented as mean ± s.d. from three independent experiments in duplicate. The dashed line indicates values from uninfected Huh7.5.1 cells set at 100%. Statistics: *, *p*-value < 0.05, Mann-Whitney test. (B) OGT protein expression. Left: percentage of OGT protein expression relative to uninfected Huh7.5.1 cells (Ctrl) following quantification of Western blots as described in Supplementary Methods. Results are presented as mean ± s.d. from three independent experiments. The dashed line indicates values from uninfected Huh7.5.1 cells set at 100%. Statistics: *, *p*-value < 0.05, Mann-Whitney test. Right: representative Western blot analysis of OGT and actin. (C) *OGT* expression and viral load in liver tissue from 22 HCV-infected patients and 6 patients not infected with HCV described in[55]. Spearman correlation: rho = 0.06004019, p-value = 0.77.

27

1 (D-E) OGT expression in liver tissue from 22 HCV-infected patients and 6 patients not

2 infected with HCV according to fibrosis (D) or activity (E) scores described in[55]. Wilcoxon

3 test: F1 vs F0 p-value = 0,38; F2 vs F0 p-value = 0,18; F3 vs F0 p-value = 0,43; F4 vs F0 p-

4 value = 0,17; A1 vs A0 p-value = 0,28; A2 vs A0 p-value = 0,23; A3 vs A0 p-value = 0,09. (F)

5 *OGT* expression in tumor (HCC) and non-tumor (Ctrl) liver tissue from 39 HCV-infected

6 patients, 83 HBV-infected, 80 patients with alcoholic liver disease (ALD) and 13 patients with

7 non-alcoholic liver disease (NAFLD) as described in Supplementary Methods. *, *p*-value <

8 0.05, Wilcoxon test.

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28

1 **Table 1. Computational analysis of miR-501-3p and miR-619-3p targets and pathway**

2 **enrichment.**

miRNA ID	Target gene symbol	_Pathway_ or network
miR-501-3p	_MEF2A_; _PPP3CA_; _PPP3CC_	_Calcium signaling_
	HMGCS1	_Cholesterol biosynthesis_
	AFF4; _CHMP1B_; _CUX1_; _DCLK1_; _LMX1A_; _PTBP2_; _RBMS1_; _RC3H1_; _SCN2A_; _SEC63_; _ZFHX4_	Inflammatory response, dermatological diseases and conditions, inflammatory disease
	CDK6; _CSDE1_; _GLI2_; _HOXD10_; _LSM5_; _MEF2A_; _MYCN_; _OGT_; _PPP2R2C_; _PPP2R5E_; _SEMA3C_; _TFDP2_	Cellular development, nervous system development and function; organ morphology
	CIT; _COL10A1_; _FNBP1L_; _GAN_; _HERC1_; _KPNA4_; _NONO_; _SHPRH_; _STRN_; _TARDBP_; _UBE2H_; _USP37_	Cell death and survival; cellular compromise; free radical scavenging
	ATXN1; _CBLL1_; _CEBPA_; _DCC_; _PEX5L_; _RCC2_; _RNF144A_; _ZC3H12C_	Cell morphology, cellular assembly and organization; cellular function and maintenance
miR-619-3p	_RUNX1T1_; _SMAD3_	_Adipocyte biogenesis_
	FOXG1; _GPBP1_; _MID1_; _MKL2_; _MSI1_; _PCBP2_; _WDFY3_	Cell cycle; organismal injury and abnormalities; cancer
	ACVR2B; _DCX_; _ESRRG_; _MAPK9_; _OGT_; _PCBP1_; _PDE3B_; _SMAD3_; _SMARCC1_; _TGFB3_; _PAPOLA_	Carbohydrate metabolism, energy production; small molecule biochemistry
	RUNX1T1; _SHANK2_; _SLC35D1_	Gene expression, lipid metabolism, small molecule biochemistry

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3   **Supplementary Information**
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23   **Supplementary Material and methods**

24   **Cells and cell culture conditions.** The source and culture conditions of Huh7.5.1 cells have

25   been described[1]. HeLa cells were purchased from ATCC and cultured in Dulbecco´s

26   modified Eagle medium (Gibco® DMEM GlutaMAX™, ThermoFisher Scientific) containing 1%

27   sodium pyruvate as described for Huh7.5.1 cells[1].

Viruses and infectivity assays. Cell culture-derived recombinant cell culture-derived hepatitis C virus (HCVcc) Jc1 (genotype 2a/2a chimera), H77R2a (genotype 1a/2a chimera engineered for *Renilla* luciferase expression), Con1R2a (genotype 1b/2b chimera engineered for *Renilla* luciferase expression), and JcR2a (genotype 2a/2a chimera engineered for *Renilla* luciferase expression) were generated in Huh7.5.1 cells as described[1, 2, 3, 4]. HCVcc infectivity was determined by calculating the 50% tissue culture infectious dose (TCID50) using anti-NS5A antibody as described[5, 6] or by assessing luciferase activity. HCVcc were used at 10^5-10^6 TCID50/mL throughout the study. HCV RNA was purified using a QIAmp viral RNA minikit (Qiagen) and analyzed by one-step RT-qPCR using a Sensi Fast NO ROX kit (Bioline) according to the manufacturer's instructions. Standard curves were performed using 10-fold dilution series of HCV RNA.

Purification of HCVcc particles using sucrose cushion or iodixanol density gradient. HCVcc (JcR2a) were concentrated 10-fold using a Vivaspin column (GE Healthcare). For sucrose cushion purification, HCVcc were purified by overlaying 3.5 mL of culture media on 1.5 mL of 20% sucrose, and by ultracentrifuging samples for 4h at 40,000 rpm on a SW-55 rotor (Beckman Coulter). Purified HCVcc were resuspended in 30 µL of PBS for analysis via immunocapture and electron microscopy. Density distributions of infectious HCVcc were determined by overlaying 0.5 mL culture media on a 5 mL, 4%-40% iodixanol step gradient, and ultracentrifuging samples for 16h at 40,000 rpm on a SW-55 rotor (Beckman Coulter): 625 µl fractions were carefully harvested from the top of each tube, and density was determined by weighing. Infectivity of each fraction was quantified by TCID50 using anti-NS5A antibody as described[5, 6], while HCV RNA of fractions was purified and analyzed as described above. ApoB and ApoE concentrations of fractions were determined by enzyme-linked immunosorbent assay (Human Apolipoprotein B or E ELISA[PRO] kit, Mabtech) undiluted or in a 1:50 dilution, respectively, according to the manufacturer's instructions (Mabtech).

1   **miRNA mimics and siRNAs.** Non-targeting control miRNA, miR-501-3p mimic, miR-619-3p

2   mimic, antagomiR-122, antagomiR-501-3p, non-targeting control antagomiR, non-targeting

3   control siRNA, siRNAs targeting *OGT, CD81* or *apoliporotein E* (*ApoE*) and a library of 28

4   custom ON-TARGETplus smart pool siRNAs were purchased from Dharmacon (GE

5   Healthcare).

6

7   **miRNA expression analysis.** Total RNA (100 ng) was purified from control or HCV-infected

8   Huh7.5.1 cells using Tri reagent® (Thermo Scientific) and Direct-zol™ RNA purification kit

9   (Zymo Research). Total RNA was first polyadenylated and reverse transcribed using a

10   miScript II RT system (Qiagen) according to the manufacturer's instructions. The obtained

11   cDNA was subjected to RT-qPCR using miScript SYBR Green kit (Qiagen). Primers were the

12   mature miRNA sequence for the forward primer (Thermo Scientific) and the universal miScript

13   primer (Qiagen) for the reverse primer. Data were analyzed by the ΔΔCt method using small

14   nucleolar RNA, C/D box 61 (SNORD61) as an endogenous reference and the non-infected

15   samples as a calibrator[7].

16

17   **Antibodies**. Rabbit anti-OGT antibodies DM-17 and AL24 were purchased from Sigma or

18   kindly provided by Dr. G. W. Hart and Dr. S. Hardivillé (Johns Hopkins University School of

19   Medicine, Baltimore, MD)[8], respectively. Mouse anti-β-actin antibody was purchased from

20   Abcam and mouse, rabbit or sheep HRP-conjugated secondary antibodies (A9044, A0545

21   and A3415, respectively) were purchased from Sigma. Sheep anti-NS5A serum for

22   determination of TCID50 was a kind gift from M. Harris[9]. Human anti-E2 (AR3A) antibody[10]

23   for electron microscopy analysis was kindly provided by Mansun Law (SCRIPPS, California,

24   USA).

25

26   **Western blotting.** OGT and actin protein expression in human cells was assessed by

27   Western blot as described[8] with some modifications. Briefly, cells were lysed in lysis buffer

28   no. 6 (R&D Systems) according to the manufacturer's instructions. Equal amounts of protein

1   (40 µg) were size-separated through a Mini PROTEAN® TGX Stain-Free™ gel electrophoresis

2   (Bio-Rad) and transferred to PVDF membranes (Bio-Rad). Immunoblots were performed

3   using rabbit anti-OGT (1:2000) and mouse anti-β-actin (1:1000) antibodies[8, 11]. Antigen-

4   antibody complexes were detected by incubating the membrane with the appropriate HRP-

5   conjugated secondary antibodies (1:5000; 1:10,000) and imaged by enhanced

6   chemiluminescence with a ChemiDoc MP imager (Bio-Rad). Quantification of protein

7   expression was performed using ImageLab™ 5.2.1 software (BioRad). For analysis of OGT

8   and GAPDH expression in liver tissue from HCV transgenic (FL-N/35) or wild-type mice[12],

9   crude protein extracts were prepared by homogenization of frozen mouse livers (50–100 µg)

10   in tissue lysis buffer from the Ambion PARIS RNA (Thermo Scientific) and protein isolation kit,

11   supplemented with protease inhibitors (cOmpleteTM EDTA-free protease inhibitor mixture,

12   Sigma-Aldrich) and phosphatase inhibitors (PhosSTOPTM, Sigma-Aldrich), using a tissue

13   homogenizer (MP Fast Prep24, MP Biomedicals, Santa Ana, CA) and MP Lysing Matrix A

14   tubes. Proteins were quantified using the BCA assay (Thermo Fisher Scientific). Western

15   blotting was performed as described above.

16

17   **Immunocapture and electron microscopy analysis of viral particles.** Sucrose-cushion

18   purified or iodixanol gradient fractionated HCVcc (JcR2a) produced in cells transfected with a

19   non-targeting siRNA control or a pool of siRNA against OGT were transferred via anti-E2

20   antibody AR3A on electron microscopy (EM) grids through immunocapture (IC) as

21   described[13]. Particles were stained with uranyl acetate dihydrate and observed in a JEOL

22   1230 electron microscope. Series of electron micrographs were acquired at random from IC

23   EM grids. The images were then analyzed with Image-J software, to determine the particle

24   size distribution.

25

26   **Gene expression analysis in patient-derived liver tissue**. For OGT expression analysis in

27   patient's samples, raw data were retrieved from the Gene Expression Omnibus (GSE84346)

28   and re-analyzed by quality-trimming (cutadapt) and mapping (HISAT2) to human genome

assembly hg19. Reads mapping to Gencode v.19 genes were counted using htseq-count and normalized applying DESeq2. Activity and fibrosis scores as well as viral load were taken from the supplemental data published in[14]. To analyze OGT expression in liver tissue of chronic hepatitis B or C patients, FPKM values and clinical data were retrieved from The Cancer Genome Atlas (TCGA, https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga). This data set includes samples from 39 HCV-infected patients, 83 hepatitis B virus (HBV)-infected, 80 patients with alcoholic liver disease (ALD) and 13 patients with non-alcoholic fatty liver disease (NAFLD).

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Supplementary figure legends

Figure S1. (A) Effect of miR-501-3p inhibition on HCV infectivity. Huh7.5.1 cells were transfected with control antagomiR (Ctrl), antagomiR-122 as loss-of-function control to perturb HCV replication and antagomiR-501-3p, prior to infection with HCVcc (JcR2a) according to the two-step protocol depicted in Fig. 1A. After 48h, supernatants were transferred onto naive Huh7.5.1 cells. After 72h, Renilla Luciferase activity of infected Huh7.5.1 cells was determined. Data are expressed as mean percentage as compared to Ctrl ± s.d. Results are from four independent experiments in quadruplicate. The dashed line indicates values from vehicle-treated cells set at 100%. Statistics**:** *, p-value < 0.05, Mann-Whitney test. (B) miR-501-3p expression upon HCV infection. Huh7.5.1 cells were infected with HCVcc (JcR2a).

1   After 72h, RNA was purified and miR-501-3p expression analyzed by RT-qPCR. Percentage

2   of miR-501-3p expression relative to uninfected Huh7.5.1 cells (Ctrl). Results are presented

3   as mean ± s.d. from three independent experiments in duplicate. The dashed line indicates

4   values from uninfected Huh7.5.1 cells set at 100%. Statistics: *, *p*-value < 0.05, Mann-Whitney

5   test.

6

7   **Supplementary Table 1. A genome-wide miRNA mimic screen identifies cellular**

8   **miRNAs modulating HCV infection.** Log2(FC), lfdr and effect on HCV infection in part 1 and

9   part 2 of the screen are shown for the individual miRNAs of the miRNA mimic library. In red:

10   proviral effect, in blue: antiviral effect. FC: fold change, lfdr: local false discovery rate