SUPPLEMENTARY DATA

Identification of microRNA-27a as a key regulator of cholesterol homeostasis

by

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Supplementary Figures

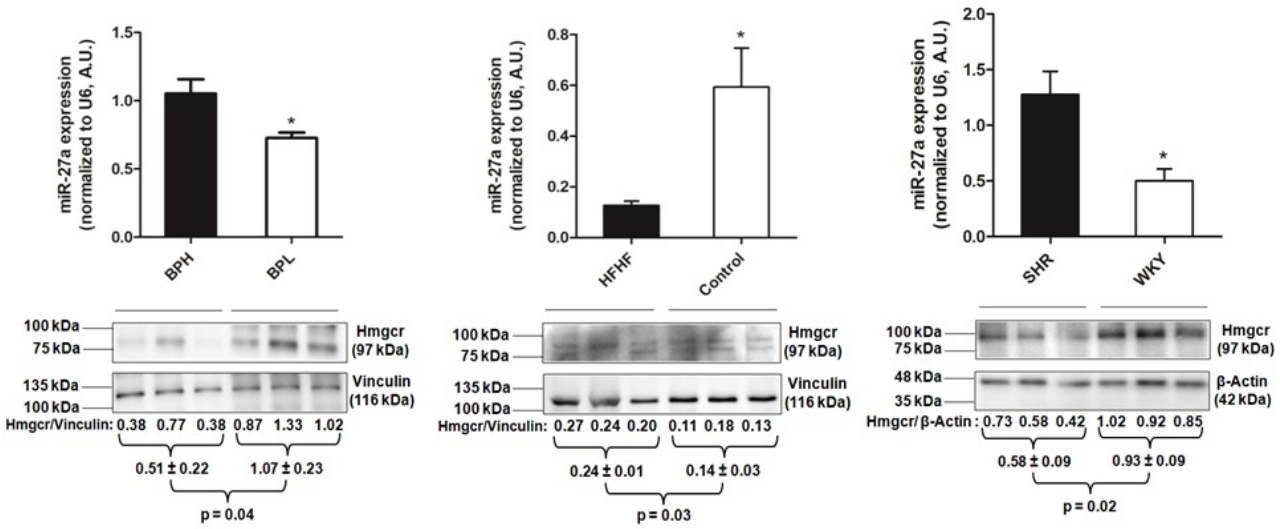

Fig.S1: Inverse correlation between Hmgcr levels and miR-27a expression in BPH vs. BPL, HFHF vs, Control and SHR vs. WKY liver tissues. Liver tissue samples from male BPH/ BPL mice at the age of 5 −7 weeks were procured from the Jackson Laboratory (Bar Harbor, ME) following institutional norms. Likewise, male SHR/WKY liver tissue samples at the age of 4-6weeks were procured from the Division of Pharmacology, CDRI (Lucknow, India) while liver tissues from male Sprague-Dawley (SD) rats fed with high fat and fructose diet (HFHF) and normal chow-diet fed rats at the age of 32 weeks were obtained from National Institute of Nutrition (Hyderabad, India). miRNAs and total protein were isolated from the liver tissues of the aforementioned animals and were probed for Hmgcr and miR-27a levels.

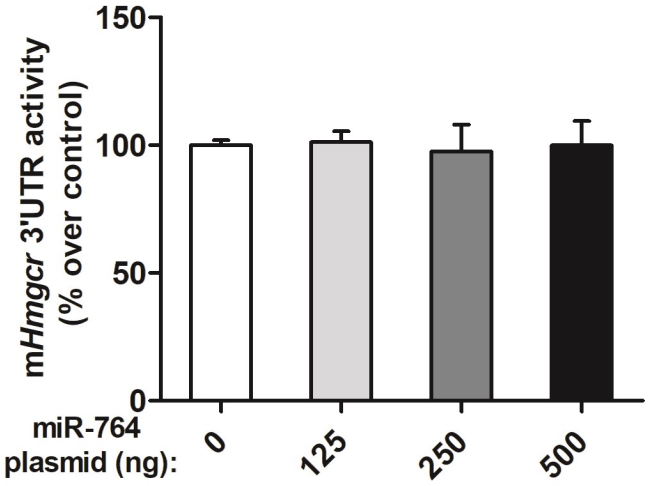

Fig.S2: Effect of miR-764 over-expression on mHmgcr-3'UTR. The mHmgcr-3'UTR reporter construct was co-transfected with increasing doses of miR-764 expression plasmid in AML12 cells and luciferase activity was assayed. Values were normalized to total protein and are mean ± SE of triplicate values. No statistical significance was observed by one-way ANOVA with Newman-Keuls multiple comparison test.

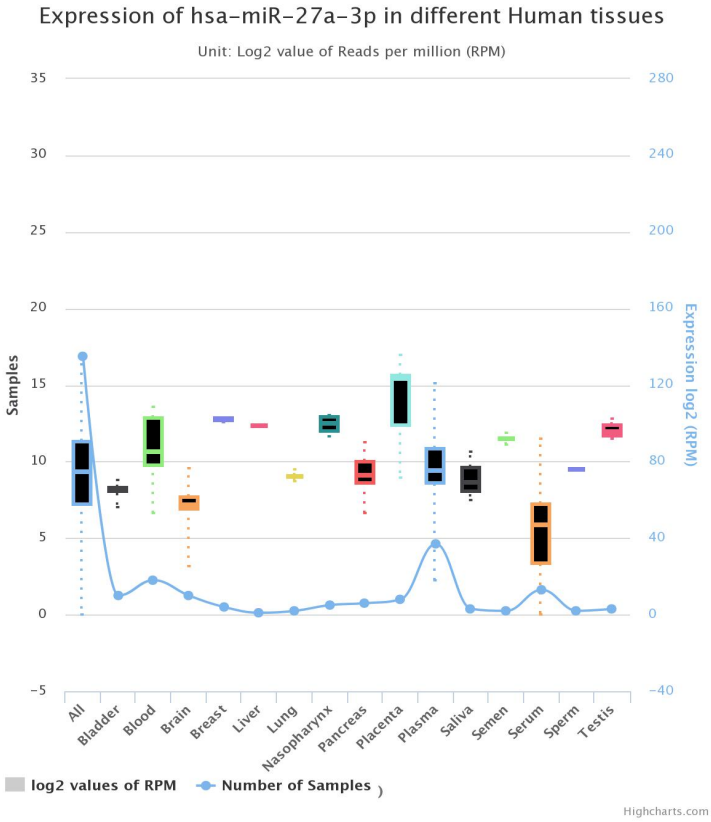

Fig.S3: Expression profile of hsa-miR-27a-3p in different human tissues as retrieved from miRmine (http://guanlab.ccmb.med.umich.edu/mirmine/)

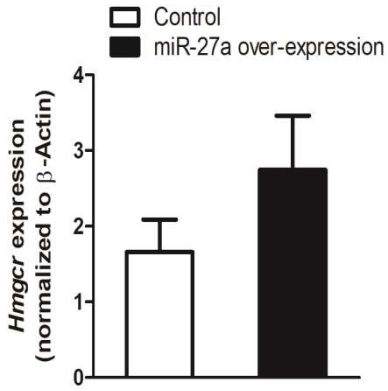

Fig.S4: Effect of miR-27a over-expression on Hmgcr mRNA levels. The relative expression of *Hmgcr* upon over-expression of miR- 27a (LNA27a) in AML12 cells was determined by qPCR using gene-specific primers. *Hmgcr* expression was normalized to β-actin mRNA in the same sample. No statistical significance was observed by Student's *t*-test (unpaired, 2-tailed).

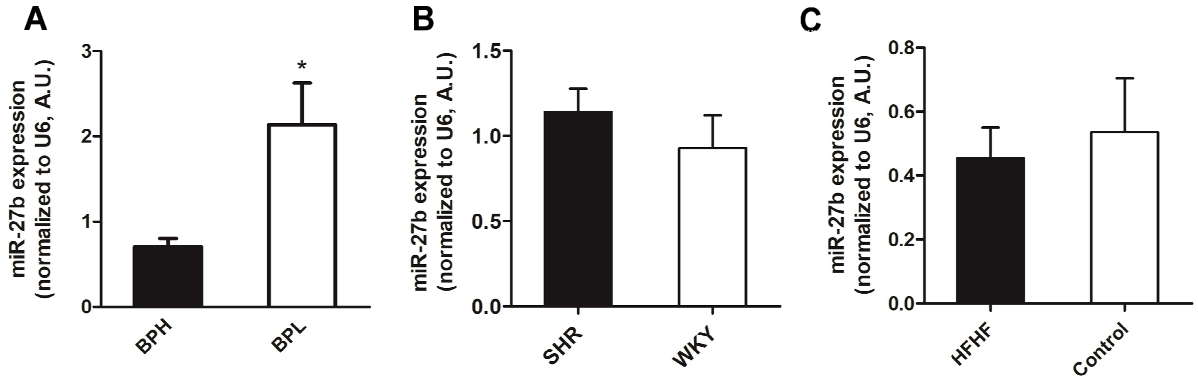

Fig.S5: Expression analysis of miR-27b in liver tissues of BPH/BPL, SHR/WKY and HFHF/Control animals. miRNAs were isolated from the liver tissues of (A) BPH vs. BPL, (B) SHR vs. WKY and (C) Sprague-Dawley (SD) rats fed with high fat-fructose diet (HFHF) vs. normal chow-diet fed rats (Control) were probed for miR-27b levels by qPCR. Statistical significance was determined by Student's *t*-test (unpaired, 2-tailed).

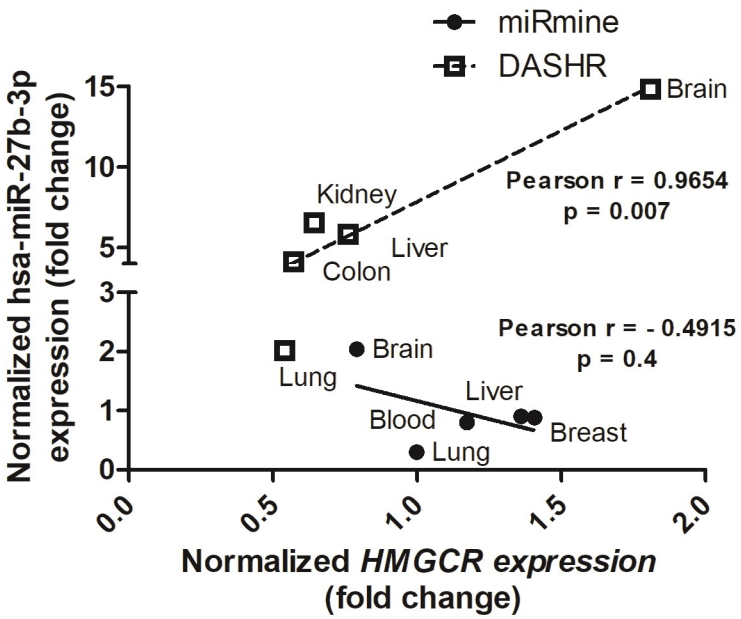

Fig.S6: Correlation analysis between *HMGCR* and hsa-miR-27b-3p expression in different tissues. Endogenous *HMGCR* expression profiles in different human tissues was obtained from the GTEx portal while hsa-miR-27b-3p expression data was retrieved from miRmine and DASHR respectively as detailed in the materials and methods. The Pearson r and p values for each database are shown.

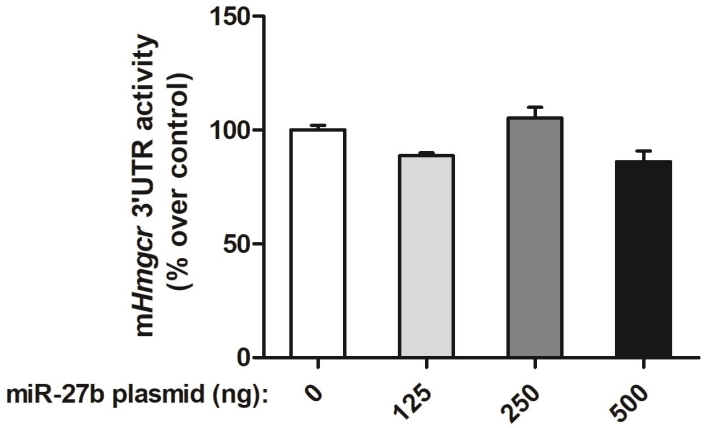

Fig.S7: Effect of miR-27b over-expression on mHmgcr-3'UTR. The mHmgcr-3'UTR reporter construct was co-transfected with increasing doses of miR-764 expression plasmid in AML12 cells and luciferase activity was assayed. Values were normalized to total protein and are mean ± SE of triplicate values. No statistical significance was observed by one-way ANOVA with Newman-Keuls multiple comparison test.

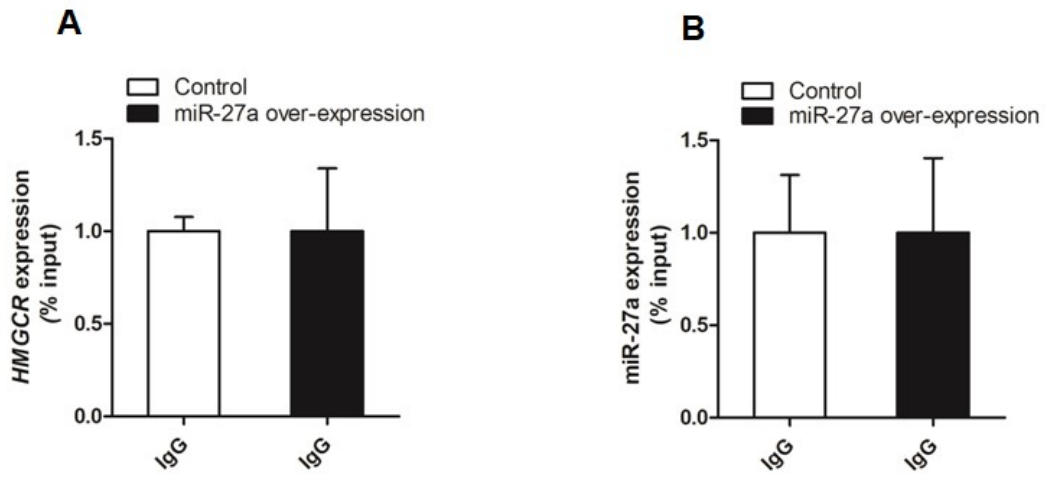

Fig.S8: Ribonucleoprotein Ago2 precipitation analysis in HuH-7 cells over-expressing miR-27a. HuH-7 cells were transfected with miR-27a expression plasmid. HuH-7 cells transfected with pcDNA3.1 were used as control. After 30-36 hours of transfection, miRNA-27-RISC complexes were immunoprecipitated with Ago2/ pre-immune anti-mouse IgG antibody and *HMGCR*, miR-27a levels were measured by qPCR using gene specific primers. The (A) *HMGCR* and (B) miR-27a expression was normalized to the corresponding input in the RNA fraction immunoprecipitated with pre-immune anti-mouse IgG antibody; expressed as % input and is mean ± SE for quadruplets. No statistical significance was observed by Student's *t*-test (unpaired, 2-tailed).

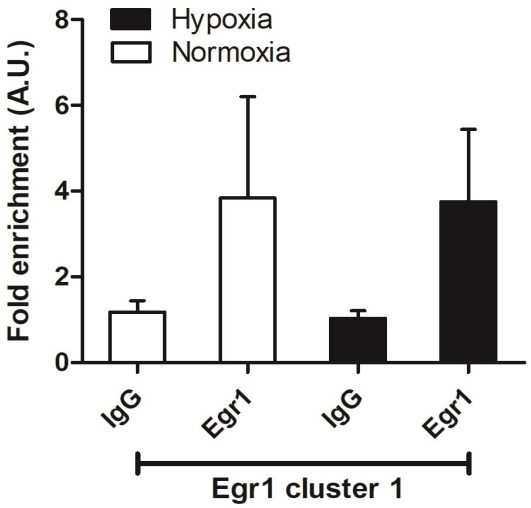

Fig.S9: Effect of hypoxic stress on binding of Egr1 with miR-27a promoter domain. ChIP assay was carried out with chromatin isolated from AML12 cells treated with/without exposure to hypoxia. qPCR was performed with DNA purified from respective cocktails using one primer pair viz. P1 amplifying 184 bp DNA segments (Egr1 cluster 1) in the proximal miR-27a promoter domain. No statistical significance was observed by one-way ANOVA with Newman-Keuls multiple comparison test.

Supplementary Tables

Supplementary Table 1

Primers used for qPCR

Name of the primer	Sequence (5' to 3')
miR-27a-SL-primer	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGA TACGACGCGGAAC
miR-27b-SL-primer	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGA TACGACGCAGAAC
U6-SL-primer	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGA TACGACTATGGAAC
mmu-miR-27-FP	ACACT TTCACAGTGGCTAA
U6-FP	CTGCGCAAGGATGACACGCA
universal RP	GTGCAGGGTCCGAGGT
m*Hmgcr*-FP	GAGAATGCAGAGAAAGGTG
m*Hmgcr*-RP	GGCGAATAGACACACCAC
mβ-Actin-FP	CTTCTTTGCAGCTCCTTCGTT
mβ-Actin-RP	TTCTGACCCATTCCCACCA
r*Hmgcr*-FP	ACCTGCTGCCATAAACTGGAT
r*Hmgcr*-RP	ACCACCTTGGCTGGAATGAC
rβ-Actin-FP	GCTGTGCTATGTTGCCCTAG
rβ-Actin-RP	CGCTCATTGCCGATAGTG
hHmgcr-FP	TCGGTGGCCTCTAGTGAGAT
hHmgcr-RP	TGTCCCCACTATGACTTCCC
m*Mvk*-FP	GGTGTGGTCGGAACTTCCC
m*Mvk*-RP	CCTTGAGCGGGTTGGAGAC
m*SS*-FP	ATGGAGTTCGTCAAGTGTCTAGG
m*SS*-RP	CGTGCCGTATGTCCCCATC

Abbreviations: FP-forward primer, RP-Reverse primer, SL-stem loop

Supplementary Table 2

***In silico* tools and databases employed in the study**

Tool/database	Link	Reference
Rat Genome Database	http://rgd.mcw.edu/rgdweb/search/qtls.html?100	(1)
VISTA	http://genome.lbl.gov/vista/mvista/submit.shtml	(2)
miRWalk	http://zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk2/	(3)
miRanda	http://www.microrna.org/	(4)
TargetScan	http://www.targetscan.org/vert_72/	(5)
PITA	https://genie.weizmann.ac.il/pubs/mir07/mir07_prediction.html	(6)
RNA22	https://cm.jefferson.edu/rna22/Interactive/	(7)
RNAhybrid	https://bibiserv2.cebitec.uni-bielefeld.de/rnahybrid	(8)
GTEx portal	https://www.gtexportal.org/home/	(9)
miRmine	http://guanlab.ccmb.med.umich.edu/mirmine/	(10)
DASHR	http://www.lisanwanglab.org/DASHR/smdb.php	(11)
Primer 3	http://primer3.ut.ee/	(12)
LASAGNA	http://biogrid-lasagna.engr.uconn.edu/lasagna_search/	(13)
JASPAR	http://jaspar.genereg.net/	(14)

Supplementary Table 3

Expression of genes involved in cholesterol biosynthesis upon transfection of 60 nM of locked nucleic acid inhibitor of 27a (LNA27a) or control oligos in AML12 cells as determined by qPCR

Gene	Fold-change (LNA 27a /control)	p-value (Student's *t*-test)
Hmgcr	3.84	0.01
Mvk	3.05	0.04
SS	12.02	0.02
Ldlr	7.57	0.04

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