Supplemental Materials and Methods

Antibodies: Anti-SRF (cat# Sc-335) and anti-Igf1R (sc-712) (Santa Cruz Biotech), and anti-ADAM-10 (14-6211) were from e-Bioscience, anti-Ku70 (cat# MS-329-P) (Labvision), p-MAPK (#4370) and MAPK (#9102), pIgf1Rβ (#3024) and Igf1Rβ (#3027) (Cell signaling technology) and anti-GAPDH (cat# mAB 374) (Chemicon International)

Primers

Primers used to amplify miR-122 gene from mouse genomic DNA

miR122-F:	TCTTCCTGGAATTCAAGCCTTT
miR122-R:	AGTGGGCCTAGTGCTGGAAA

The following primers were used for RT-PCR

Human ADAM10

RT-F:	CAA CCT ACG AAT GAA GAG GGA CAC
RT-R:	CCA CCA CGA GTC TGG ATG AAT C

Human SRF

RT-F:	AGC ATA GGG GGG AGG GTC TTT TAC
RT-R:	AGG GTT AGA GGT TAG GGG GTT CTC

Igf1R-F:	TCTCTCCTCTCTGCTTCATAACGG
Igf1R-R:	TCCAAAGACAGGTCAGTCGGTC

ADAM17-F:	CTGAACAACGACACCTGCTGC
ADAM17-R:	GCAGAAAGGGATGCATTTCCCA

The following primers were used to amplify 3'-UTRs.

SRF-3'UTR-F:	TTTCACCCCATCCCAGATAGC
SRF-3'UTR-de1miR122-F:	CACCTCCCATTTCTGTTG
SRF-3'UTR-R:	CCCCCATCCCTTCACCTAATC
ADAM10-3'UTR-F:	GCCCATTCAGCAACCCCAG
ADAM10-3'UTR-R:	ACTTGTGCCCGTAGCAGCC
ADAM10-3'UTR-d122-F:	CATCTCCAAACTAAACCCTCAC
ADAM10-3'UTR-R:	ACTTGTGCCCGTAGCAGCC
IGF1R-3'UTR-F:	TCTCTCCTCTCTGCTTCATAACGG
IGF1R-3'UTR-R:	TCCAAAGACAGGTCAGTCGGTC
IGF1R-3'UTR-del miR22-F:	GGGAACTGGACACAATAGGTC
IGF1R-3'UTR del miR-122 R:	GCTTGAATCCATTGACTGC

Plasmid constructs: Mouse miR-122 gene was amplified from 129SvJ genomic DNA and cloned into TA vector. It was retrieved with BamH I and cloned into the same site of p-Rev-tet-off (for HepG2), pBabe-puro (for SK-Hep-1) and pMSCV (for Hep3B). Stable cell lines were generated by infection with retroviral particles obtained in phoenix cells after transfection with these parental vectors or recombinant plasmids. SRF and IgF1R expression vectors were obtained from Addgene.

Cell invasion assay: BiocoatTM tumor invasion system (BD bioscience) was used to evaluate the invasion ability for the miR122 overexpressed cell lines following the product manual except that the incubation time was extended to 72 hours because of the slower rate of invasion ability of HepG2 cells. The invaded cells were stained with Hema 3 (Fisher Biotech) and analyzed by SigmaScan Pro 5 (SPSS) program. All analysis is under the same procedure using digitalized image. All cells were identified and overlaid with a separate digital layer. The total cell number

was estimated by measuring the total pixels within the separate digital layer. The criteria used in identifying and overlaying cells were identical for the same cell line.

Table 1. Relative miR-122 Expression in FFP HCC and matching liver tissues. RNA isolated from formalin-fixed paraffin embedded tissues were subjected to real-time RT-PCR analysis with Taqman probes and primers specific for miR-122 and RNU6B respectively. miR-122 level was normalized to RNU6B. The data are mean of triplicate assays.

Sample	Liver	HCC	S.D. (Liver)	S.D. (HCC)	P value
#1	1.052244727	0.006430906	0.090714595	0.001016835	0.002453844
#2	1.041157564	0.193816554	0.132133931	0.025091983	0.007360203
#3	0.932472669	0.100516694	0.161059539	0.015884224	0.014719997
#4	0.958143847	0.218197898	0.218184974	0.014069144	0.031239664
#5	1.069003027	2.060121406	0.123443423	0.096127627	0.003886913
#6	1.175778703	0.342413425	0.309418432	0.022984168	0.042675704
#8	1.031575375	1.237187643	0.058160652	0.109741936	0.042251899
#9	1.048975668	0.018579916	0.126185004	0.00187797	0.004833455
#10	1.048245391	0.809460515	0.083624914	0.066571139	0.034997728
#11	1.022390653	0.58728845	0.069052767	0.039421165	0.01068081
#12	1.000144141	0.431388641	0.020796289	0.052025093	0.002129725
#13	0.996436131	0.2692586	0.056179486	0.023883976	0.00106103
#14	1.00505971	0.012941851	0.120902198	0.001308101	0.004821062
#15	1.233633161	0.617207228	0.833461668	0.041546944	0.031956387
#16	1.010744813	0.182661973	0.184939605	0.016394124	0.018546489

Table 2. Relative expression of ADAM-10 in primary human HCC and matching liver tissues. RNA isolated from snap-frozen tissues was subjected to real-time RT-PCR analysis with Taqman probes and primers specific for miR-122 and RNU6B respectively. miR-122 level was normalized to RNU6B. The data are mean of triplicate assays.

Liver	HCC		Liver	HCC
36.70010739	94.20373654	Mean	41.75731779	76.76302609
68.96117159	144.7793847	SD	22.18149301	59.01279562
56.79580146	48.42608203	Median	41.28976721	56.01387688
23.06626292	15.11293939	Q1	23.06626292	37.73188785
21.82201442	26.68047376	Q3	62.15128779	94.20373654
41.28976721	37.73188785	Min	0.357920822	15.11293939
45.18313218	156.25	Max	72.89320246	209.0511804
21.37292385	26.86605114	25th per	23.06626292	37.73188785
28.79432065	56.01387688	50th per	18.22350429	18.28198903
0.357920822	41.00455795	75th per	20.86152058	38.18985966
63.45721847	62.58358419	Min	22.7083421	22.61894846
72.89320246	79.21558436	Max	10.74191467	114.8474439
62.15128779	209.0511804			

Table 3. Relative expression of miR-122 in primary human HCC and matching liver tissues. RNA isolated from snap-frozen tissues was subjected to real-time RT-PCR analysis with Taqman probes and primers specific for miR-122 and RNU6B respectively. miR-122 level was normalized to RNU6B. The data are mean of triplicate assays.

Tumor (miR-122/RNU6B)	Liver (miR-122/RNU6B)	S.D.(Tumor)	S.D.(Liver)	P value
1.990123498	5.370199912	0.282685	0.317147	0.000161
1.319507911	2.121260136	0.153658	0.120849	0.000327
4.572897978	3.298719659	0.371862	0.432962	0.018042
0.057474607	1.017294395	0.009471	0.055351	7.75E-06
0.872436237	2.920821991	0.121616	0.332491	0.000557
2.305154051	5.782327083	0.234514	0.895089	0.002875
0.775725755	4.131735489	0.018914	0.76353	0.0016
0.788817419	0.464585953	0.094318	0.008482	0.004052
5.551041531	0.343982706	0.943687	0.041431	0.000672
0.159600334	1.765714072	0.013328	0.481167	0.004452

Supplemental Figure Legends

Fig. S1. **Ectopic expression of miR-122 inhibited anchorage independent growth of SK-Hep-1 cells.** Identical number ($1X10^3$) of cells was allowed to grow on soft agar plates for two weeks. Clones were stained with crystal violet, photographed with a digital camera.

Fig. S2. **Ectopic expression of miR-122 inhibited clonogenic survival of Hep3B cells.** A. Northern blot analysis of Hep3B cells expressing miR-122 or transfected with the vector was performed. B. Clonogenic survival of Hep3B cells. Identical number ($1X10^3$) of single cells (vector transfected or miR-122 expressing) was plated in 60 mm dish. The number of colonies formed were stained and counted after two weeks. The assay was performed in triplicate.

Fig. S3. **Expression of miR-122 arrested HepG2 cells, impeded their ability to invade through the basement membrane.** A. Northern blot analysis of HepG2 cells expressing miR-122 or control RNA was performed as described in Fig.1. B. Growth of HepG2 cells (5000/well of 96 well plate) expressing miR-122 was inhibited compared to those transfected with control RNA as measured by MTT assay. Each sample was assayed in quadruplicate. C. HepG2-tet-off cells ($2x10^5$) were subjected to invasion assay using Matrigel coated chambers and cells invaded after 72 hr were stained with Hema 3, photographed and counted.

Fig. S4. **Depletion of miR-122 promoted tumorigenic properties of Huh-7 cells.** A. Northern blot analysis of Huh-7 cells and mouse liver RNA (15 μg) was performed as described in Fig. 2. B. MTT assay to measure cell growth. C. $^3H_1$-thymidine incorporation assay as described in Fig 2. D. Cell migration assay. Cells ($1x10^5$) in serum free medium were seeded in the top chamber of a trans-well plate and allowed to migrate to the bottom chamber containing complete medium. Cells that migrated to the bottom chamber after 24 hr were stained and counted. E. Clonogenic survival assay. Identical number of cells (500 in 60 mm dish) was plated in triplicate and the colonies formed after 10 days were stained and counted. The colonies formed in cells transfected with control RNA was taken as 100%.

Fig. S5. **Real-time RT-PCR analysis of ADAM-10 and ADAM-17 in HCC cells.** A. Real-time RT-PCR analysis of ADAM-10 in HCC cells B. RT-PCR analysis of ADAM-17 in HCC cells. RNA from HCC cells expressing miR-122 or vector (HepG2, Hep3B and SK-Hep-1) or depeleted of miR-122 (Huh-7) were used for these assays. The data was normalized to 18S rRNA.

Fig. S6. SRF is the target of miR-122. A. miR-122 complementary site in 3'-UTR of SRF. B. Firefly luciferase driven by 3'-UTR of SRF is inhibited by miR-122. Hep3B cells were co-transfected with pIS0-3'-UTR-SRF or pIS0-3'-UTR-SRF-Δ-miR-122 and 50 nM miR-122 mimetic or control RNA with pRL-TK as an internal control. RLU-1 and RLU-2 activities were measured after 48 hr and RLU1/RLU2 values were plotted.

Fig.S7. **Expression of Igf1R, a target of miR-122, inversely correlated to miR-122 level.** A. miR-122 cognate site on Igf1R 3'-UTR. B. Luciferase activities in Hep3B cells transfected with miR-122 or control RNA along with pIS0-3'-UTR-Igf1R or pIS0-3'-UTR-Igf1R-Δ-miR-122. C. Expression of Igf1R in HCC and matching liver tissues. D. Pearson correlation of expression of miR-122 and Igf1R in human tissues.

Fig. S8. **Ectopic expression of SRF or Igf1R partially reversed growth inhibitory properties of miR-122 in HepG2 cells.** Cells transfected with the vector (Control) or stably expressing miR-122 were

transfected with expression vectors for SRF, Igf1R or respective empty vectors. After 48 hrs cells were trypsinized and subjected to MTT assay (A, B), migration (C) and clonogenic survival assays.

Fig. S9. **Ectopic miR-122 inhibited motility of HDMEC cells in scratch and wound healing assay.** A fine scratch made in ~80% confluent cells and migration of cells was monitored microscopically to see the movement of the cells which can close the gap.

Fig. S10. **Survival of miR-122 expressing Hep3B cells was significantly reduced after sorafenib treatment.** Cells were seeded on 96 well plates and were treated with different concentrations of sorafenib dissolved in DMSO. Cell survival was measured 24 hr later using MTT assay.