Supplemental data for

In silico and *in vitro* identification of microRNAs that regulate HNF4A expression.

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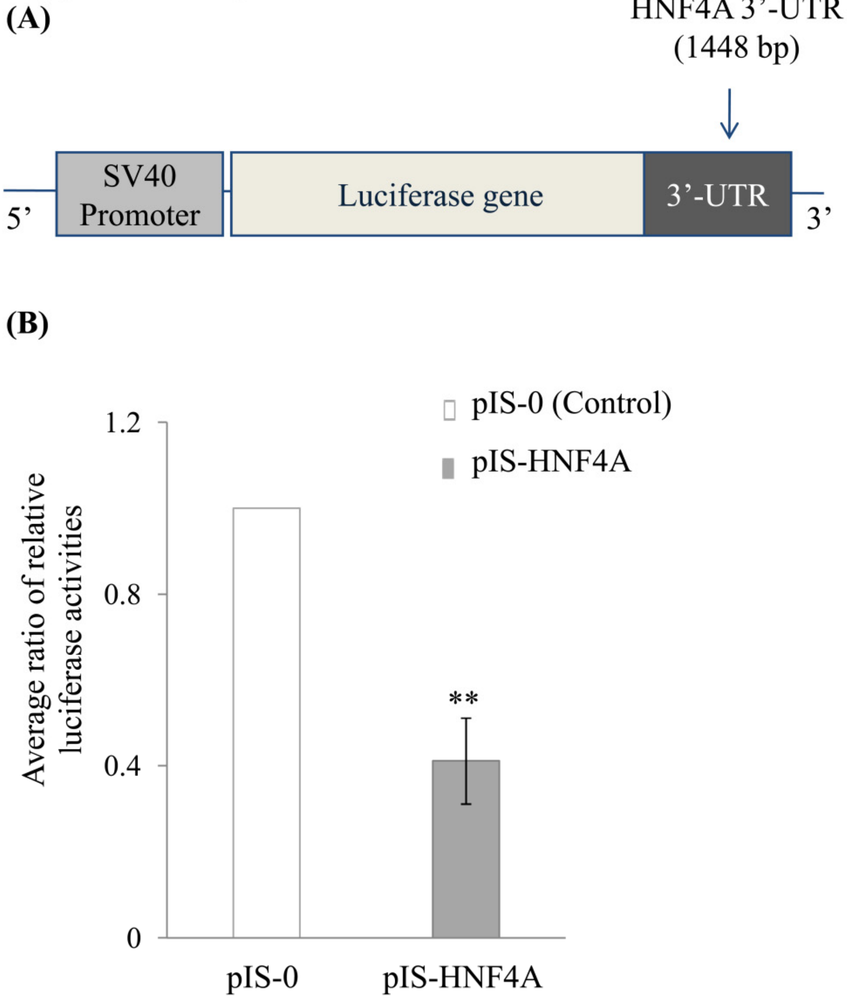
Submitted to Drug Metabolism and Disposition

Supplemental Figures:

Supplemental Figure 1: Regulatory elements in the 3'-UTR of HNF4A. (A) Schematic representation of the pIS-HNF4A vector. (B) Transfection of HeLa cell line with 200ng of pIS-0 (control) or pIS-HNF4A plasmids. *Renilla* luciferase plasmid was used as an internal control. Dual luciferase assays were performed 24 hr after transfection. The transfections were performed in triplicate on three different days. Data are expressed as the pIS-HNF4A luciferase activity corrected for *Renilla* luciferase and normalized to pIS-0 within each experiment (mean ± SEM; n=3 independent experiments). ** indicates $p < .001$.

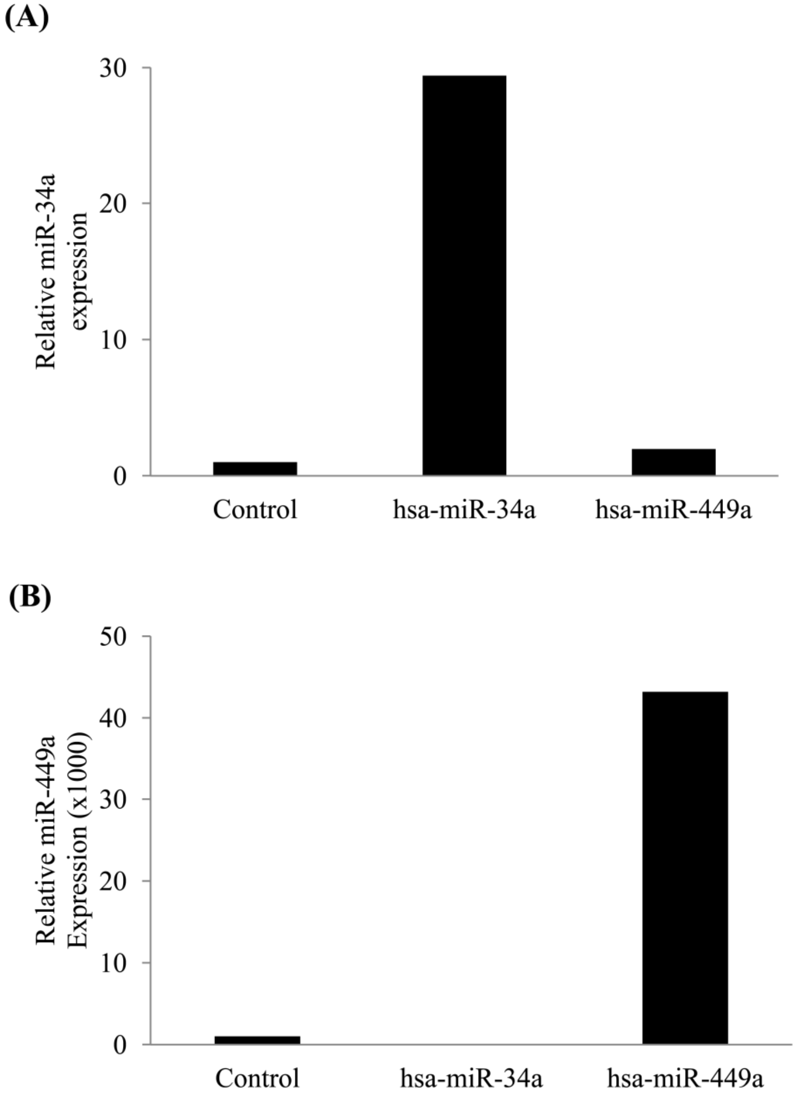
Supplemental Figure 1



Supplemental Figure 2: miRNA expression in cells transfected with miRNAs.

HepG2 cells were transfected with cel-miR-67 (control), hsa-miR-34a, or hsa-miR-449a. MiRNA expression (A) hsa-miR-34a and (B) hsa-miR-449a were measured by TaqMan assays. Quantities are expressed relative to the cel-miR-67 transfected cells.

Supplemental Figure 2

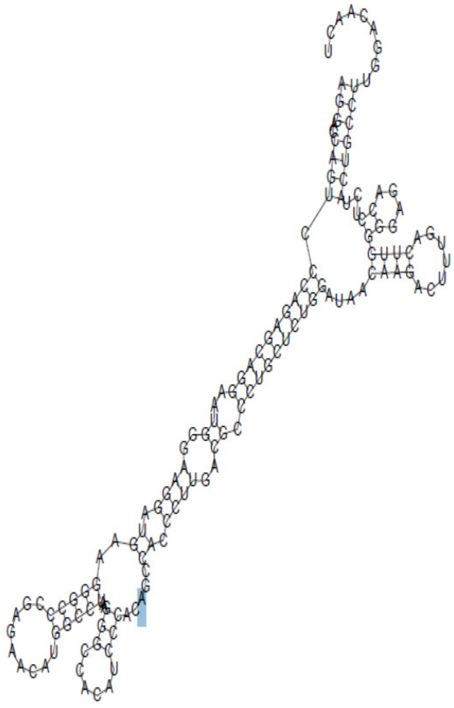


Supplemental Figure 3: **Secondary structure prediction.** Secondary structures of wildtype and variant HNF4A mRNAs were predicted using RNAFold (Gruber et al., 2008). A 70 bp sequence flanking the nucleotide on either side of the SNP (rs11574744) was used to assess the minimum folding energy and secondary structure. The wildtype and variant nucleotides are highlighted.

Supplemental Figure 3

Wildtype
MFE: -47.60 kcal/mol

Mutant
MFE: -47.60 kcal/mol



Supplemental Tables:

Supplemental Table 1: MiRNAs predicted to target the 3'-UTR of *HNF4A*.

RefSeq Gene ID [a]	**3'-UTR length (bp)**	**Bioinformatic Programs** [b, c]					**Total no. of unique miRNAs** [d]	**Overlap** [e]
		miRBase Targets	**miRanda**	**Target Scan**	**RNA22**	**PITA**		
NM_000457	1724	11	12	112	251	63	350	99

Footnotes: [a] RefSeq Gene id is taken from UCSC Genome browser; [b] PicTar predictions are not included in the table as HNF4A does not appear to be a part of the program's database; [c] Versions of the bioinformatic programs: miRBase Targets using miRBase release 11.0, miRanda and TargetScan version 4.2 uses miRBase release 10.0, and for RNA22, we used miRBase release 10.0, PITA uses miRBase release 9.0; [d] Total number of unique miRNAs predicted by at least one program; [e] Total number of miRNAs predicted to target *HNF4A* by at least 2 programs.

Supplementary Table 2: List of miRNAs predicted by different bioinformatic algorithms to target the 3'-UTR of HNF4A. Note: versions of the bioinformatic programs: for miRBase Targets we used miRBase release 11.0, miRanda and TargetScan version 4.2 using miRBase release 10.0, and for RNA22, we used miRBase release 10.0, PITA uses miRBase release 9.0. PicTar predictions are not included in the table as HNF4A does not appear to be a part of the program's database.

miRbase Targets	miRanda	TargetScan	RNA22	PITA	Overlap between two or more algorithms
hsa-miR-143	hsa-miR-29b	hsa-miR-1	hsa-let-7a	hsa-miR-132	hsa-miR-125a
hsa-miR-18a	hsa-miR-133a	hsa-miR-125a	hsa-let-7b	hsa-miR-134	hsa-miR-130b
hsa-miR-18b	hsa-miR-133b	hsa-miR-125b	hsa-let-7c	hsa-miR-198	hsa-miR-132
hsa-miR-197	hsa-miR-198	hsa-miR-130a	hsa-let-7d	hsa-miR-200a*	hsa-miR-133a
hsa-miR-340	hsa-miR-214	hsa-miR-130b	hsa-let-7e	hsa-miR-203	hsa-miR-133b
hsa-miR-34b	hsa-miR-24	hsa-miR-133a	hsa-let-7g	hsa-miR-205	hsa-miR-134
hsa-miR-382	hsa-miR-296	hsa-miR-133b	hsa-let-7i	hsa-miR-212	hsa-miR-143
hsa-miR-512-5p	hsa-miR-302c	hsa-miR-143	hsa-miR-103	hsa-miR-214	hsa-miR-150
hsa-miR-554	hsa-miR-326	hsa-miR-146a	hsa-miR-107	hsa-miR-220	hsa-miR-154
hsa-miR-613	hsa-miR-337	hsa-miR-146b	hsa-miR-10a	hsa-miR-224	hsa-miR-17-3p
hsa-miR-766	hsa-miR-378	hsa-miR-147	hsa-miR-122a	hsa-miR-28	hsa-miR-18a
	hsa-miR-412	hsa-miR-148a	hsa-miR-125a	hsa-miR-296	hsa-miR-18b
		hsa-miR-148b	hsa-miR-126*	hsa-miR-299-5p	hsa-miR-194
		hsa-miR-150	hsa-miR-127	hsa-miR-302b*	hsa-miR-195
		hsa-miR-151	hsa-miR-128a	hsa-miR-302c*	hsa-miR-197
		hsa-miR-152	hsa-miR-128b	hsa-miR-337	hsa-miR-198
		hsa-miR-154	hsa-miR-129	hsa-miR-340	hsa-miR-200a*
		hsa-miR-15a	hsa-miR-130b	hsa-miR-342	hsa-miR-205
		hsa-miR-15b	hsa-miR-132	hsa-miR-34b	hsa-miR-206
		hsa-miR-16	hsa-miR-133a	hsa-miR-378	hsa-miR-21
		hsa-miR-17-3p	hsa-miR-133b	hsa-miR-382	hsa-miR-212
		hsa-miR-185	hsa-miR-134	hsa-miR-384	hsa-miR-214
		hsa-miR-18a	hsa-miR-135a	hsa-miR-411	hsa-miR-220
		hsa-miR-18b	hsa-miR-135b	hsa-miR-412	hsa-miR-24
		hsa-miR-194	hsa-miR-136	hsa-miR-452*	hsa-miR-28
		hsa-miR-195	hsa-miR-140	hsa-miR-453	hsa-miR-296
		hsa-miR-197	hsa-miR-143	hsa-miR-485-3p	hsa-miR-299-5p
		hsa-miR-205	hsa-miR-144	hsa-miR-491	hsa-miR-29b
		hsa-miR-206	hsa-miR-145	hsa-miR-493-3p	hsa-miR-29c
		hsa-miR-208	hsa-miR-149	hsa-miR-503	hsa-miR-302c
		hsa-miR-21	hsa-miR-150	hsa-miR-511	hsa-miR-302c*
		hsa-miR-214	hsa-miR-154	hsa-miR-512-5p	hsa-miR-326
		hsa-miR-24	hsa-miR-17-3p	hsa-miR-513	hsa-miR-328
		hsa-miR-26a	hsa-miR-181b	hsa-miR-516-3p	hsa-miR-337
		hsa-miR-26b	hsa-miR-181d	hsa-miR-518c*	hsa-miR-338
		hsa-miR-299-5p	hsa-miR-182	hsa-miR-532	hsa-miR-340
		hsa-miR-29a	hsa-miR-182*	hsa-miR-542-5p	hsa-miR-342
		hsa-miR-29b	hsa-miR-184	hsa-miR-545	hsa-miR-346
		hsa-miR-29c	hsa-miR-187	hsa-miR-558	hsa-miR-34a
		hsa-miR-301	hsa-miR-188	hsa-miR-585	hsa-miR-34b
		hsa-miR-326	hsa-miR-189	hsa-miR-590	hsa-miR-34c
		hsa-miR-328	hsa-miR-18a*	hsa-miR-593	hsa-miR-361
		hsa-miR-337	hsa-miR-191*	hsa-miR-597	hsa-miR-370
		hsa-miR-338	hsa-miR-193b	hsa-miR-608	hsa-miR-378
		hsa-miR-346	hsa-miR-194	hsa-miR-613	hsa-miR-382
		hsa-miR-34a	hsa-miR-195	hsa-miR-615	hsa-miR-384
		hsa-miR-34b	hsa-miR-196a	hsa-miR-617	hsa-miR-412
		hsa-miR-34c	hsa-miR-196b	hsa-miR-624	hsa-miR-449
		hsa-miR-361	hsa-miR-197	hsa-miR-629	hsa-miR-449b
		hsa-miR-370	hsa-miR-198	hsa-miR-636	hsa-miR-453
		hsa-miR-378	hsa-miR-199a	hsa-miR-637	hsa-miR-454-3p
		hsa-miR-382	hsa-miR-199a*	hsa-miR-640	hsa-miR-484
		hsa-miR-383	hsa-miR-199b	hsa-miR-642	hsa-miR-485-3p
		hsa-miR-384	hsa-miR-200a*	hsa-miR-650	hsa-miR-485-5p
		hsa-miR-412	hsa-miR-202	hsa-miR-658	hsa-miR-491
		hsa-miR-421	hsa-miR-202*	hsa-miR-659	hsa-miR-493-3p
		hsa-miR-421-3p	hsa-miR-204	hsa-miR-661	hsa-miR-497
		hsa-miR-424	hsa-miR-205	hsa-miR-671	hsa-miR-503
		hsa-miR-433-5p	hsa-miR-206	hsa-miR-765	hsa-miR-511
		hsa-miR-449	hsa-miR-20a	hsa-miR-766	hsa-miR-512-5p
		hsa-miR-449b	hsa-miR-21	hsa-miR-767-5p	hsa-miR-513

miRbase Targets	miRanda	TargetScan	RNA22	PITA	Overlap between two or more algorithms
		hsa-miR-454-3p	hsa-miR-210	hsa-miR-768-5p	hsa-miR-515-3p
		hsa-miR-484	hsa-miR-211	hsa-miR-801	hsa-miR-518c*
		hsa-miR-485-5p	hsa-miR-212		hsa-miR-520g
		hsa-miR-490	hsa-miR-214		hsa-miR-520h
		hsa-miR-493-3p	hsa-miR-217		hsa-miR-525
		hsa-miR-497	hsa-miR-218		hsa-miR-526a
		hsa-miR-499	hsa-miR-22		hsa-miR-526c
		hsa-miR-504	hsa-miR-220		hsa-miR-532
		hsa-miR-505	hsa-miR-221		hsa-miR-545
		hsa-miR-512-5p	hsa-miR-222		hsa-miR-554
		hsa-miR-513	hsa-miR-24		hsa-miR-575
		hsa-miR-515-3p	hsa-miR-27a		hsa-miR-590
		hsa-miR-519e	hsa-miR-27b		hsa-miR-593
		hsa-miR-520f	hsa-miR-28		hsa-miR-597
		hsa-miR-520g	hsa-miR-29b		hsa-miR-608
		hsa-miR-520h	hsa-miR-29c		hsa-miR-613
		hsa-miR-525	hsa-miR-302a		hsa-miR-615
		hsa-miR-526a	hsa-miR-302b		hsa-miR-624
		hsa-miR-526c	hsa-miR-302c		hsa-miR-629
		hsa-miR-532	hsa-miR-302c*		hsa-miR-631
		hsa-miR-539	hsa-miR-302d		hsa-miR-636
		hsa-miR-542-3p	hsa-miR-30a-3p		hsa-miR-637
		hsa-miR-545	hsa-miR-30e-3p		hsa-miR-640
		hsa-miR-548c	hsa-miR-30e-5p		hsa-miR-642
		hsa-miR-551a	hsa-miR-31		hsa-miR-650
		hsa-miR-551b	hsa-miR-324-3p		hsa-miR-658
		hsa-miR-575	hsa-miR-324-5p		hsa-miR-659
		hsa-miR-579	hsa-miR-325		hsa-miR-661
		hsa-miR-590	hsa-miR-326		hsa-miR-663
		hsa-miR-613	hsa-miR-328		hsa-miR-668
		hsa-miR-616	hsa-miR-331		hsa-miR-671
		hsa-miR-620	hsa-miR-337		hsa-miR-765
		hsa-miR-625	hsa-miR-338		hsa-miR-766
		hsa-miR-631	hsa-miR-339		hsa-miR-767-5p
		hsa-miR-633	hsa-miR-340		hsa-miR-768-5p
		hsa-miR-634	hsa-miR-342		hsa-miR-769-5p
		hsa-miR-637	hsa-miR-345		hsa-miR-801
		hsa-miR-640	hsa-miR-346		hsa-miR-9
		hsa-miR-641	hsa-miR-34a		
		hsa-miR-642	hsa-miR-34b		
		hsa-miR-650	hsa-miR-34c		
		hsa-miR-660	hsa-miR-361		
		hsa-miR-661	hsa-miR-362		
		hsa-miR-663	hsa-miR-370		
		hsa-miR-668	hsa-miR-373		
		hsa-miR-671	hsa-miR-375		
		hsa-miR-765	hsa-miR-378		
		hsa-miR-766	hsa-miR-382		
		hsa-miR-768-5p	hsa-miR-412		
		hsa-miR-769-5p	hsa-miR-422a		
		hsa-miR-9	hsa-miR-422b		
			hsa-miR-423		
			hsa-miR-425-3p		
			hsa-miR-431		
			hsa-miR-432		
			hsa-miR-432*		
			hsa-miR-433		
			hsa-miR-448		
			hsa-miR-449		
			hsa-miR-449b		
			hsa-miR-450		

miRbase Targets	miRanda	TargetScan	RNA22	PITA	Overlap between two or more algorithms
			hsa-miR-452		
			hsa-miR-453		
			hsa-miR-454-3p		
			hsa-miR-455		
			hsa-miR-483		
			hsa-miR-484		
			hsa-miR-485-3p		
			hsa-miR-485-5p		
			hsa-miR-486		
			hsa-miR-491		
			hsa-miR-493-3p		
			hsa-miR-497		
			hsa-miR-501		
			hsa-miR-502		
			hsa-miR-503		
			hsa-miR-506		
			hsa-miR-508		
			hsa-miR-509		
			hsa-miR-510		
			hsa-miR-511		
			hsa-miR-512-5p		
			hsa-miR-515-3p		
			hsa-miR-515-5p		
			hsa-miR-516-5p		
			hsa-miR-517*		
			hsa-miR-517a		
			hsa-miR-517b		
			hsa-miR-518a		
			hsa-miR-518c*		
			hsa-miR-518e		
			hsa-miR-518f*		
			hsa-miR-519a		
			hsa-miR-519d		
			hsa-miR-519e*		
			hsa-miR-520a*		
			hsa-miR-520b		
			hsa-miR-520g		
			hsa-miR-520h		
			hsa-miR-524		
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			hsa-miR-526a		
			hsa-miR-526b		
			hsa-miR-526b*		
			hsa-miR-526c		
			hsa-miR-532		
			hsa-miR-545		
			hsa-miR-550		
			hsa-miR-554		
			hsa-miR-557		
			hsa-miR-564		
			hsa-miR-565		
			hsa-miR-566		
			hsa-miR-571		
			hsa-miR-572		
			hsa-miR-573		
			hsa-mIr-574		
			hsa-miR-575		
			hsa-miR-578		
			hsa-miR-580		
			hsa-miR-581		

miRbase Targets	miRanda	TargetScan	RNA22	PITA	Overlap between two or more algorithms
			hsa-miR-584		
			hsa-miR-586		
			hsa-miR-587		
			hsa-miR-589		
			hsa-miR-591		
			hsa-miR-593		
			hsa-miR-594		
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			hsa-miR-602		
			hsa-miR-608		
			hsa-miR-609		
			hsa-miR-611		
			hsa-miR-612		
			hsa-miR-613		
			hsa-miR-614		
			hsa-miR-615		
			hsa-miR-619		
			hsa-miR-622		
			hsa-miR-623		
			hsa-miR-624		
			hsa-miR-626		
			hsa-miR-628		
			hsa-miR-629		
			hsa-miR-631		
			hsa-miR-632		
			hsa-miR-636		
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			hsa-miR-671		
			hsa-miR-7		
			hsa-miR-758		
			hsa-miR-765		
			hsa-miR-766		
			hsa-miR-767-3p		
			hsa-miR-767-5p		
			hsa-miR-768-5p		
			hsa-miR-769-3p		

miRbase Targets	miRanda	TargetScan	RNA22	PITA	Overlap between two or more algorithms
			hsa-miR-769-5p		
			hsa-miR-770-5p		
			hsa-miR-801		
			hsa-miR-9		
			hsa-miR-92		
			hsa-miR-92b		
			hsa-miR-96		

Supplemental Table 3: ***HNF4A*** **3'-UTR SNPs predicted to destroy or create a miRNA target site.**

dbSNP rs #	**Minor allele frequency (%)**	**Seed sequence [Wildtype/ Derived allele]**	**Old target destroyed**	**New target created**
rs11574744	0-4.0 [a]	AC[T/A]GCCA	-34a, -34c, -449a, -449b	
rs11574745	0-2.5	A[C/T]CTTCA	-493-3p	
rs6130615	11-61[a]	TAATG[C/T]G		-323
rs6103734	0 [b]	GTCA[G/A]GA	-378	
rs6103735	0 [b]	TTAA[G/A]GA		-302b*

Footnote: [a] Seattle SNPs was used in addition to dbSNP to identify the minor allele frequency (MAF); [b] The MAF appears to be 0% in over 200 individuals that have been genotyped for these two SNPs.