

读书报告

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A Regulatory Role for MicroRNA 33* in Controlling Lipid Metabolism Gene Expression

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miR-33* miR-33a miR-33b miR-33a-5p miR-33a-3p

miRNA*, 从哪里来?

Although both strands of the duplex are necessarily produced in equal amounts by transcription, their accumulation is **asymmetric** at the steady state. By convention, the most abundant duplex strand is defined as the mature miRNA strand, whereas the **less abundant** strand is known as the “passenger strand,” or miRNA star strand (herein referred to as miRNA*).

however, it is thought that strand selection is dictated by the relative **thermodynamic stability** of each duplex end .

为什么要研究miRNA*?

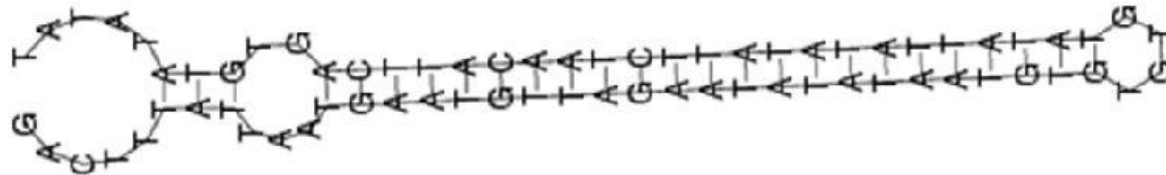
The strand with unstable base pairs at the 5' end typically **evades degradation** and is incorporated into Argonaute (Ago) complexes, where it **mediates posttranscriptional gene silencing** .

While the functional role of miR-33 has been highly investigated, the role of its passenger strand, miR-33*, has not been addressed.

怎样来研究miRNA-33*?

cell: Human hepatic (Huh7), human monocytic (THP1)

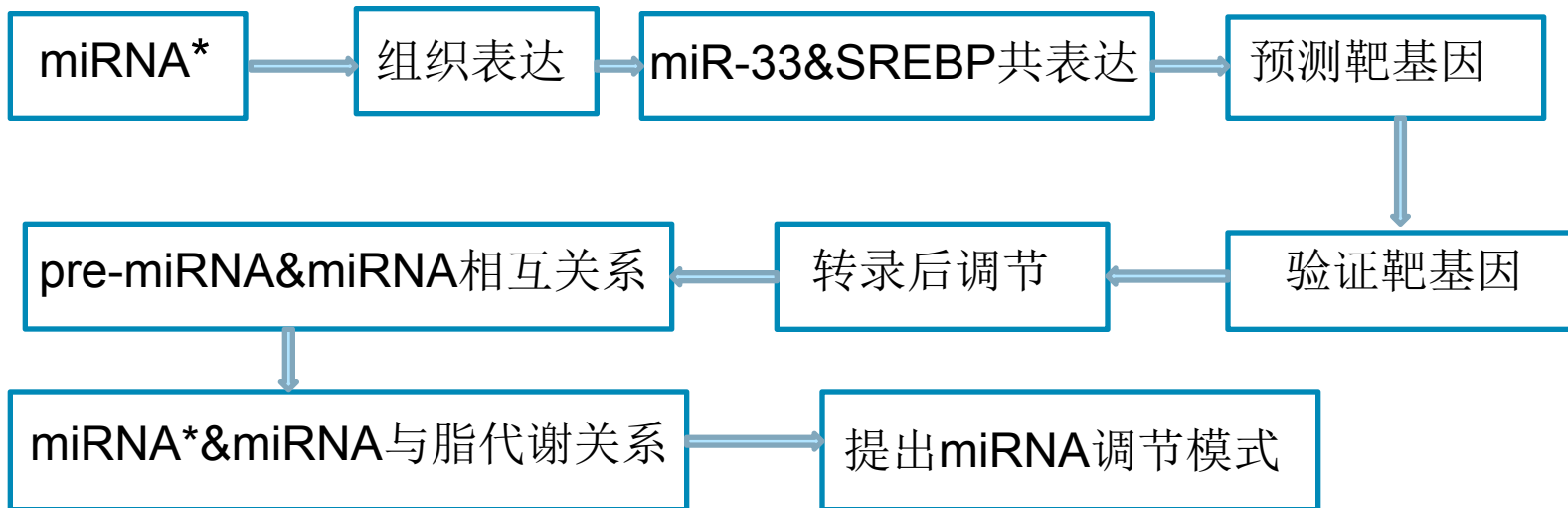
miRNA: miRNA mimic (miR-33a, miR-33b, miR-33a*, or miR-33b*) miRNA inhibitor (Inh-miR-33a, InhmiR-33a*, Inh-miR-33b, or Inh-miR-33b*) nontargeting control mimic (CM) inhibitor negative-control sequence (CI)



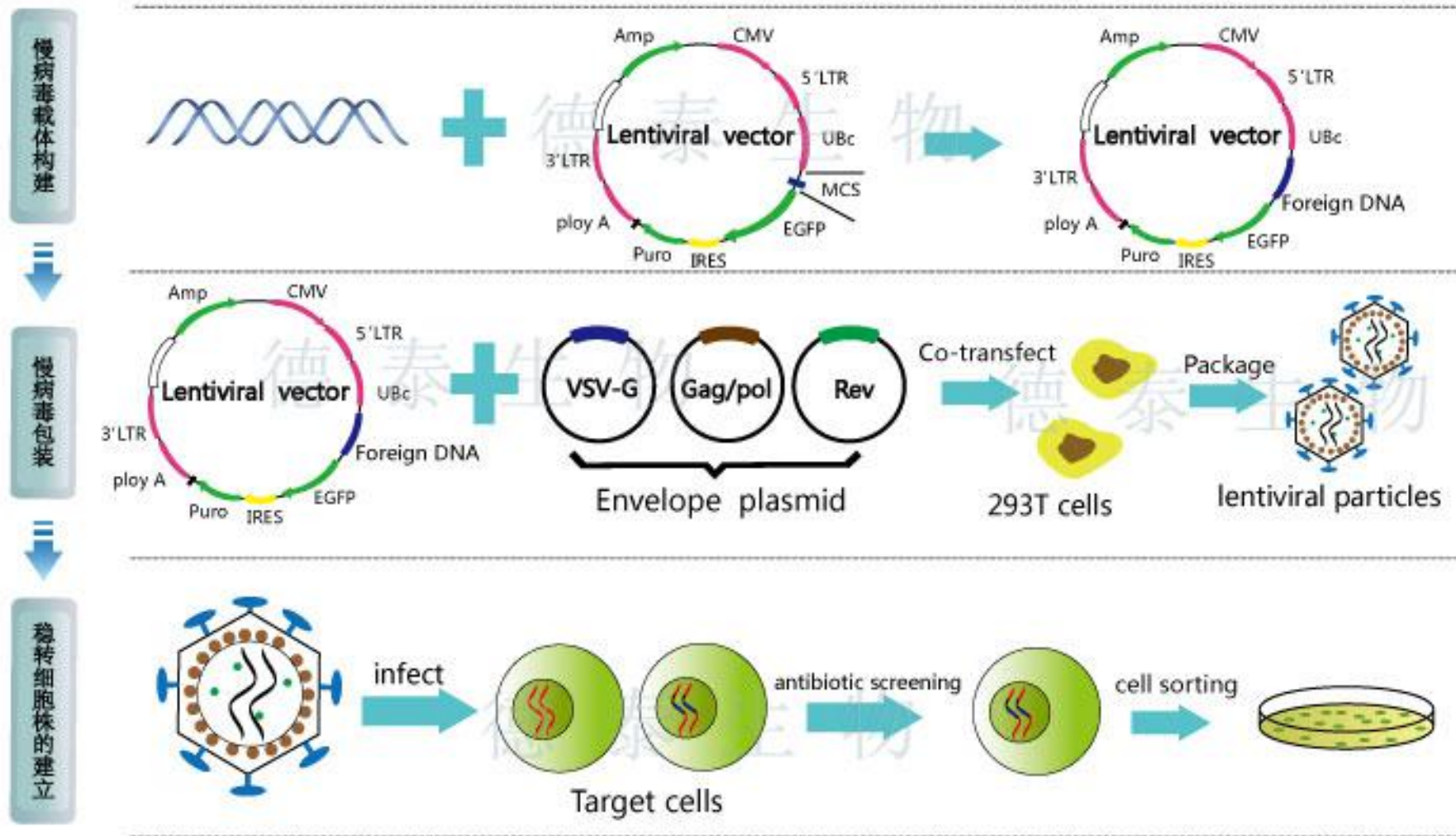
pre-miRNA

怎样来研究miRNA-33*?

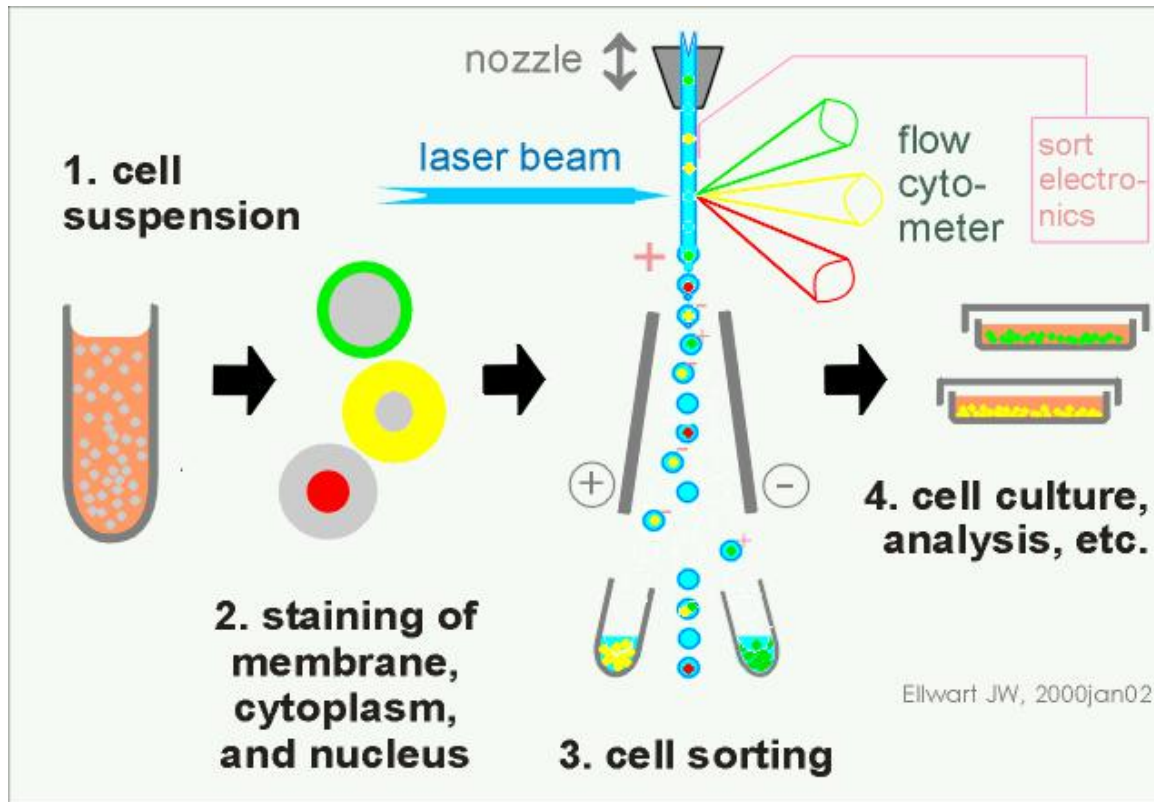
Technical roadmap



怎样来研究miRNA-33*?



怎样来研究miRNA-33*?



工作原理：让荧光染色的细胞在稳定的液流推动装置作用下通过直径为50-100um的小孔并排列成单行，每个细胞依次而且恒速通过激光束的照射区，细胞受激光照射后产生散射光和荧光。通过检测散射光可知细胞的体积，检测荧光可知细胞DNA或RNA的含量。根据所规定的参量可把指定的细胞亚群从整个群体中分选出来，以便进一步的研究分析。

怎样来研究miRNA-33*?

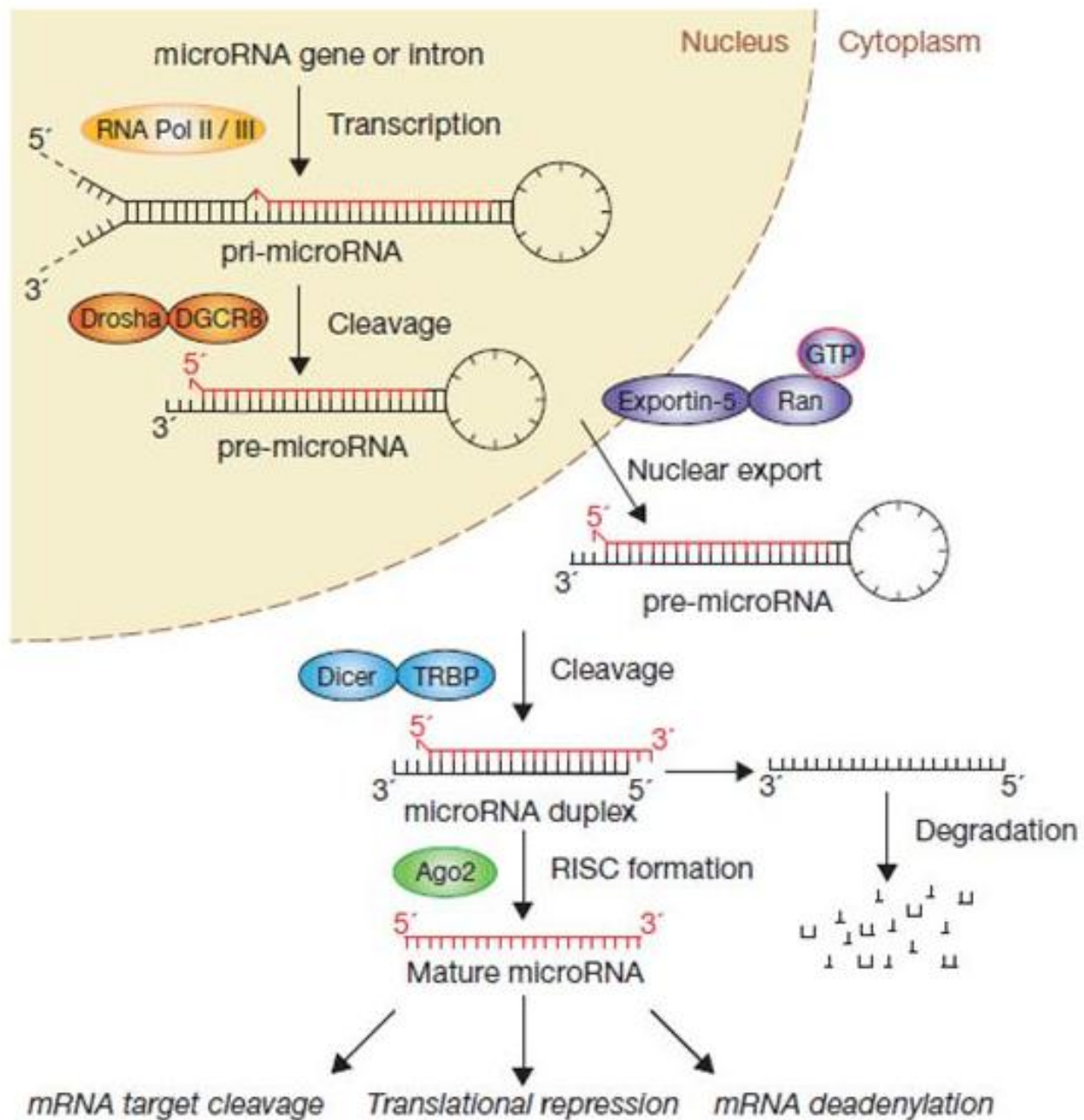
Ago2-IP

通过用合成的miRNA转染细胞，孵育后裂解细胞，用特异的抗AGO2抗体对RISC进行免疫共沉淀，对获得的mRNA进行表达分析。

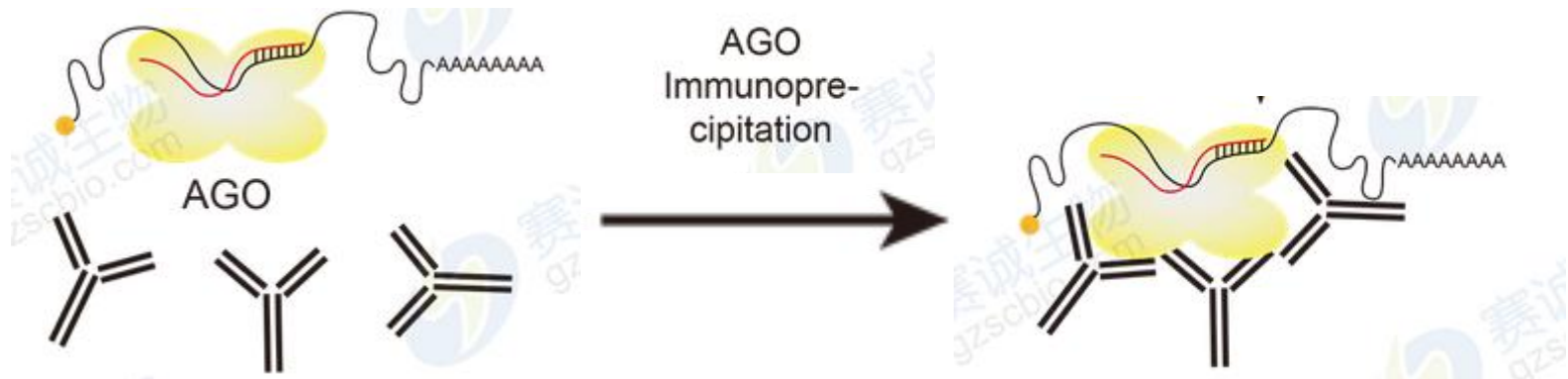
AGO蛋白是RNA诱导沉默复合体（RNA-induced silencing complex, RISC）的核心催化元件，能够与小RNA结合并且在小RNA的指导下结合靶基因，进一步剪切或抑制靶基因的表达。

在细胞中，成熟的miRNA分子可与Argonaute(Ago)蛋白家族形成RNA沉默复合体(RISC)，降解靶mRNA或者抑制转录。在人类中普遍存在4种Ago蛋白（Ago1-Ago4），在Ago家族蛋白中表达最多的是Ago2，它具有Slicer活性，可切割其靶RNA，在microRNA调控路径中具有重要意义。针对这种Ago蛋白，利用抗体，经RISC免疫沉淀，即***microRNA与靶mRNA共沉淀***，由此可回收microRNA。

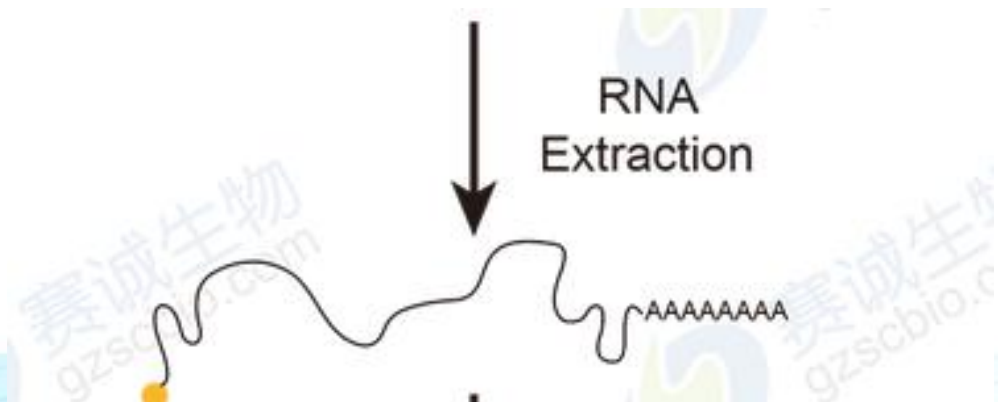
<http://www.bb100.com/experiment/16-338802-1.html>



miRNA生产过程的“线性化”经典途径

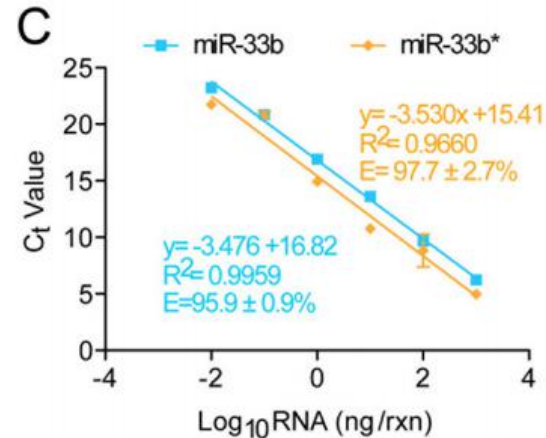
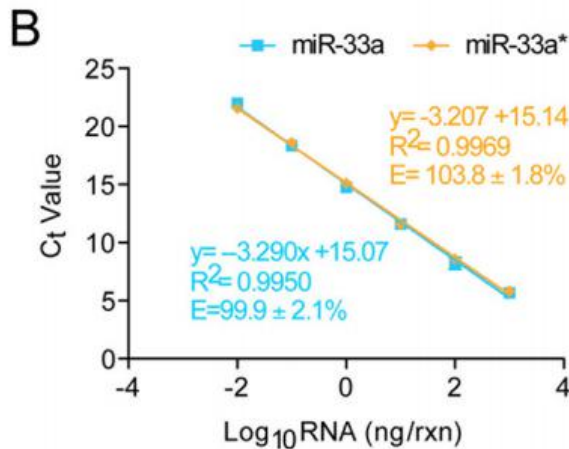
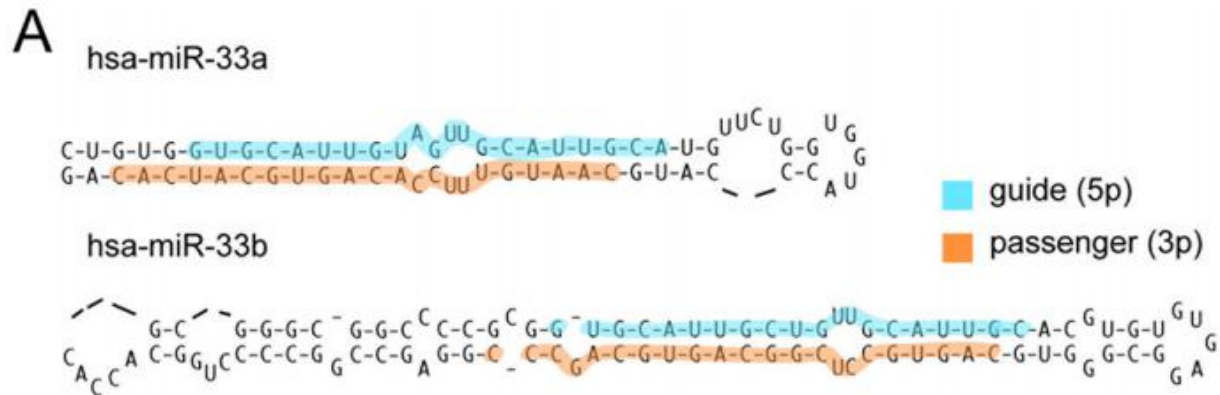


Ago-IP

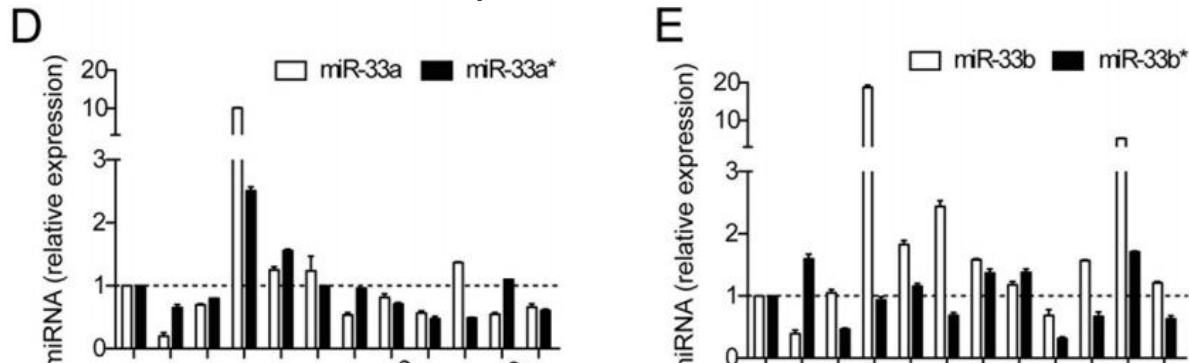


RESULTS

1. miR-33* is evolutionarily conserved and accumulates to steady state levels in human, mouse, and nonhuman primate tissues.



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Altogether, these results demonstrate that we were able to determine specifically the expression of mature miR-33a and -a* and miR-33b and -b* and that these miRNAs are expressed in most human tissues.

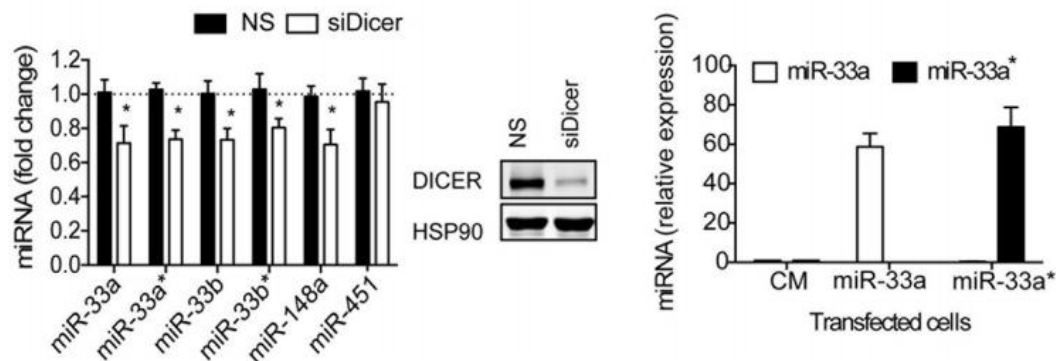


FIG 1 miR-33a and -a* and miR-33b and -b* are differentially expressed in human tissues.

2. miR-33 duplex arms were detectable in (Huh7) and were synchronously expressed with their host genes, Srebp-1 and Srebp-2.

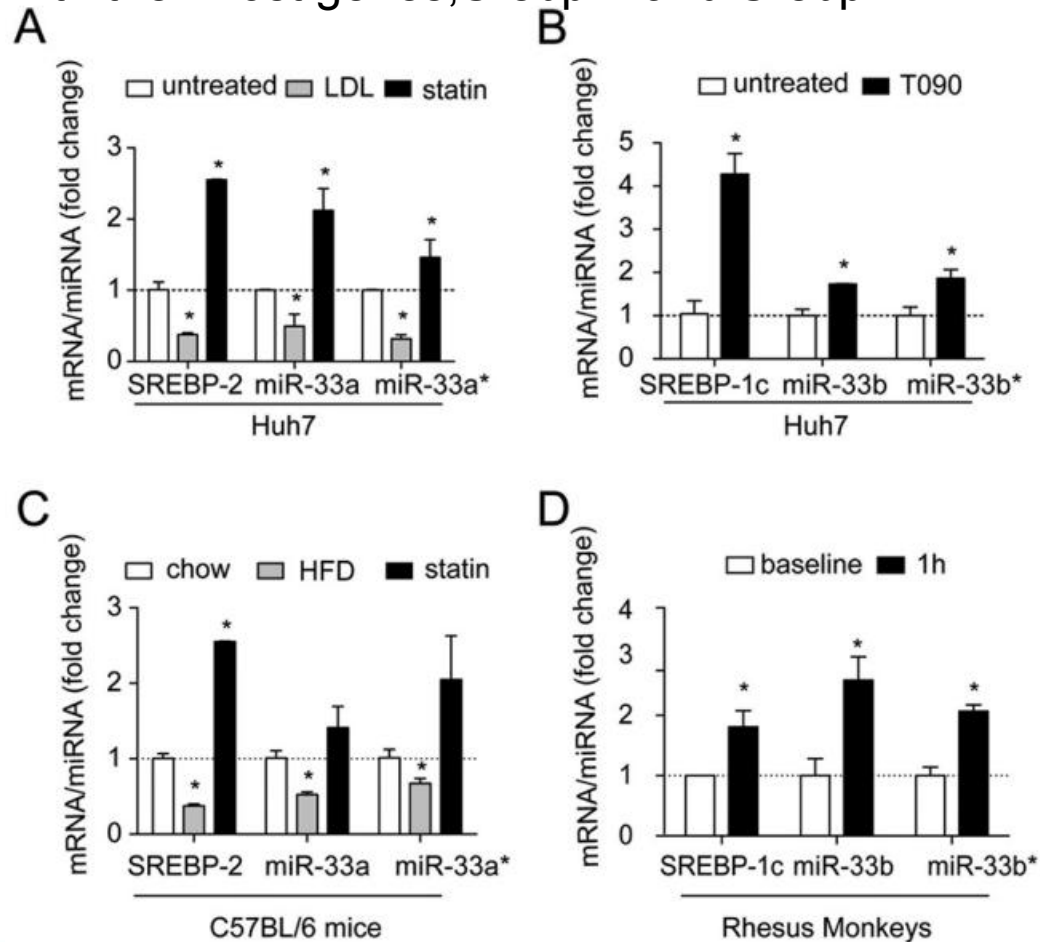


FIG 2 miR-33a and -a* and miR-33b and -b* are **coexpressed** with their host genes, Srebp-2 and Srebp-1.

3. miR-33 and miR-33* are predicted to regulate a ***similar number of target genes***.

TABLE 1 Predicted lipid metabolism target genes for miR-33a*

Gene product	Target gene predicted by algorithm ^a		Conserved in mice
	miRanda	miRWalk	
ABCA1	X		Yes
NPC1	X		Yes
CROT	X		No
CPT1a	X	X	Yes
AMPK α	X	X	Yes
IRS2	X		Yes
SRC1	X	X	No
SRC3		X	Yes
RIP140	X	X	Yes
NFYC		X	Yes

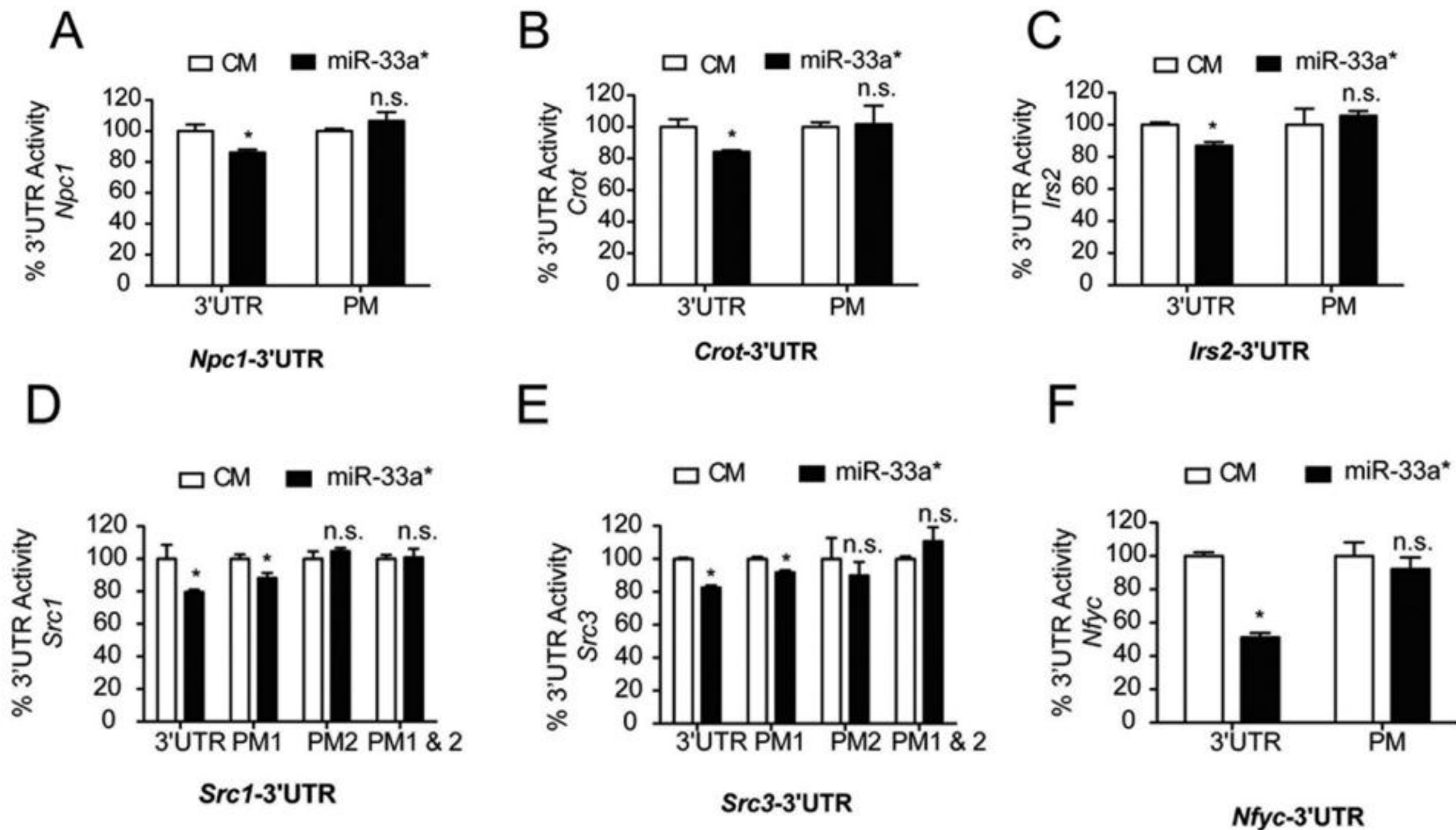
^a X, predicted binding site present.

TABLE 2 Predicted lipid metabolism target genes for miR-33b*

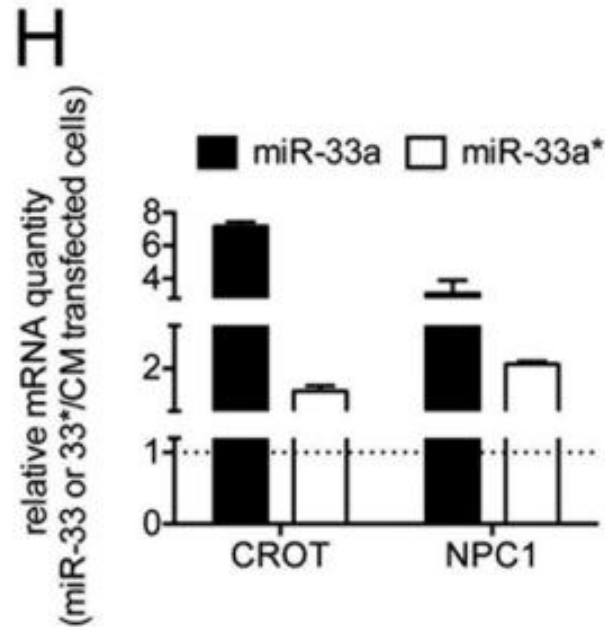
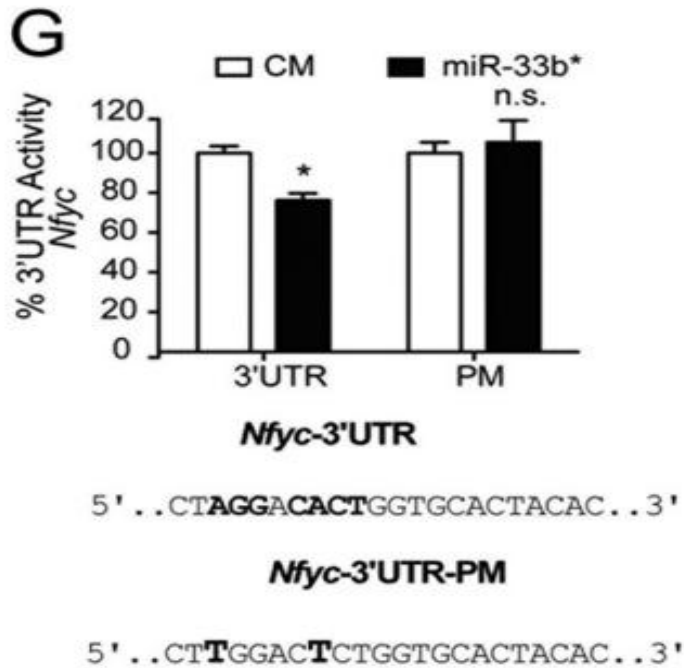
Gene product	Target gene predicted by algorithm ^a		Conserved in mice
	miRanda	miRWalk	
ABCA1	X	X	No
CS	X		No
SRC3	X		No
RIP140	X		No
NFYC	X		No

^a X, predicted binding site present.

4.miR-33* species can repress targets via perfect and near-perfect seed matches.

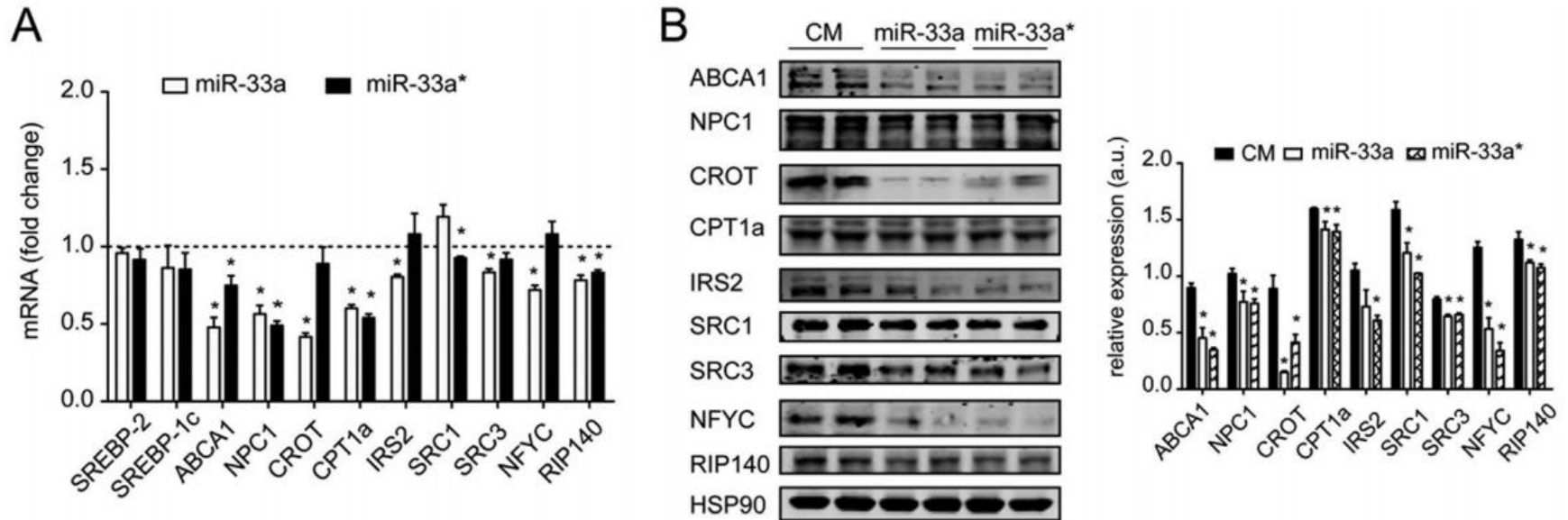


PM: point mutations



To further determine the direct effect of miR-33a and -a* on some of their common target genes, we performed Ago2 immunoprecipitation in Huh7 cells transfected with miR-33a and miR-33a* and assessed the expression of CROT and NPC1 mRNA. Interestingly, the expression of CROT and NPC1 was increased in cells transfected with miR-33a and miR-33a* compared with that of cells that overexpressed a negative-control mimic (CM), suggesting that both miRNAs interact directly with CROT and NPC1 in the RISC.

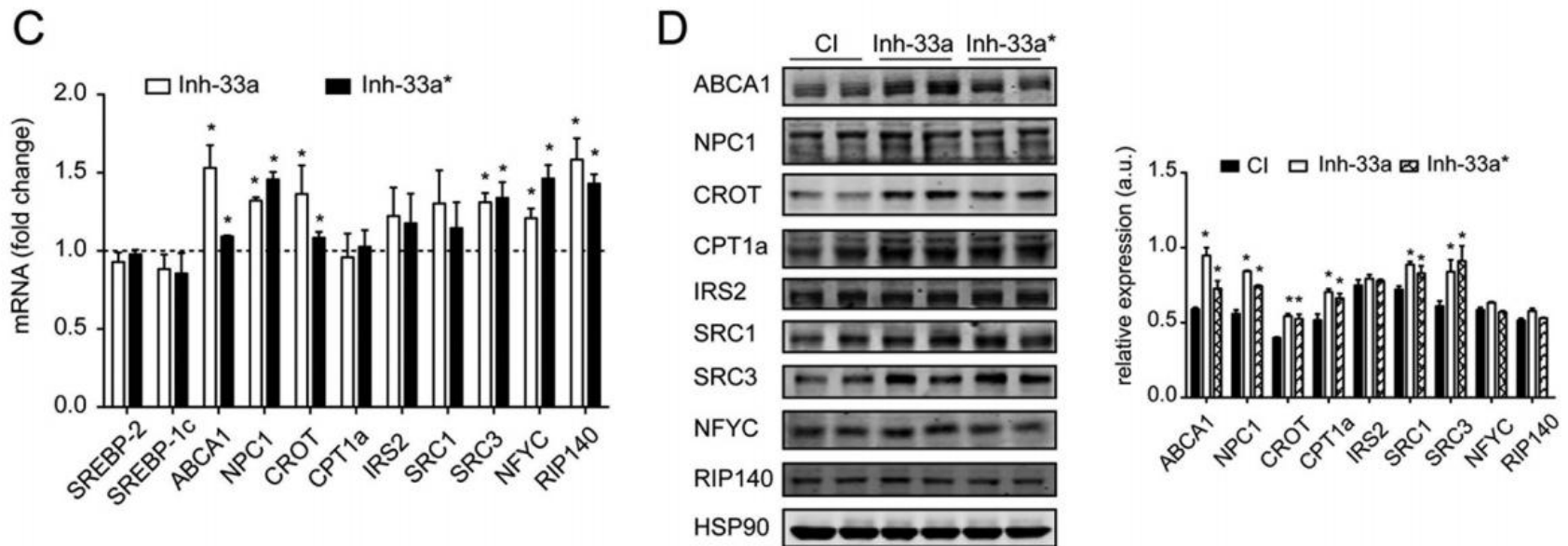
5. miR-33a or miR-33a* regulates gene expression in *Huh7 cells*.



CM: a control mimic

HSP90: heat shock protein 90

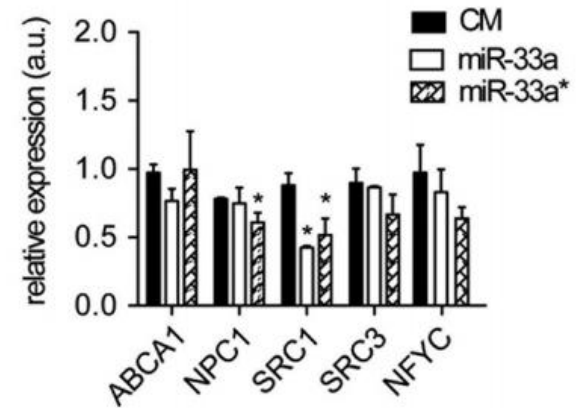
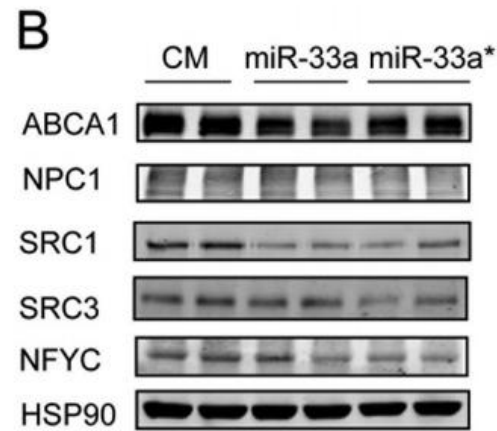
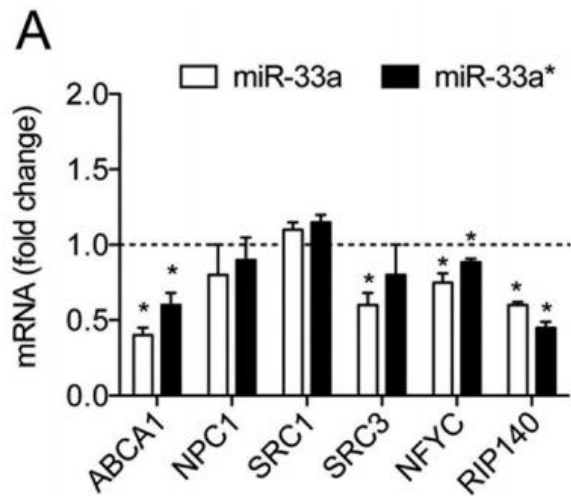
5.miR-33a* regulates gene expression in *Huh7 cells*



CI: a control inhibitor

FIG 4 Posttranscriptional regulation of ABCA1, NPC1, CROT, CPT1a, IRS2, SRC1, SRC3, NFYC, and RIP140 by miR-33a and miR-33a* in Huh7 cells.

6. miR-33a or miR-33a* regulates gene expression in *THP1 cells*.



6.miR-33a or miR-33a* regulates gene expression in *THP1 cells*.

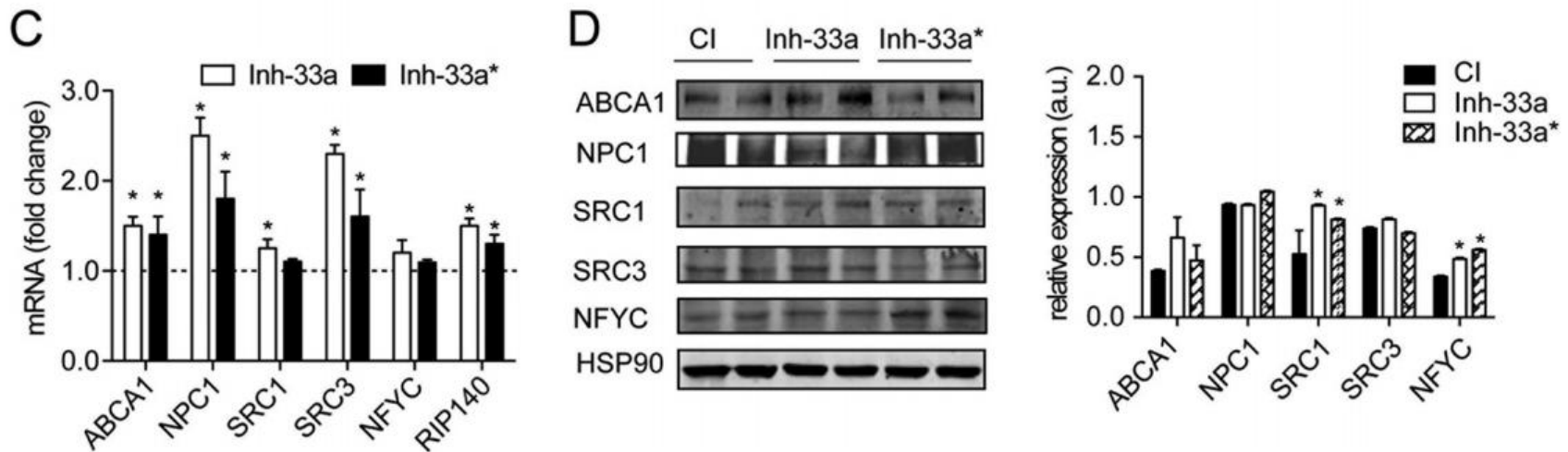
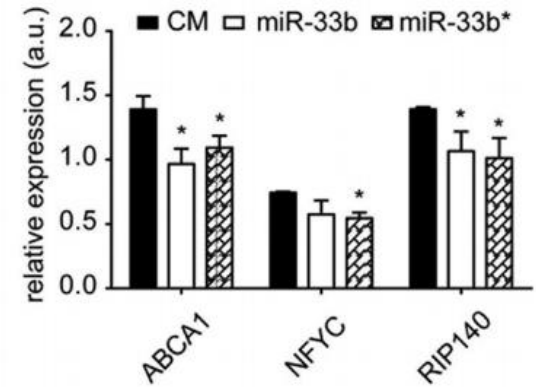
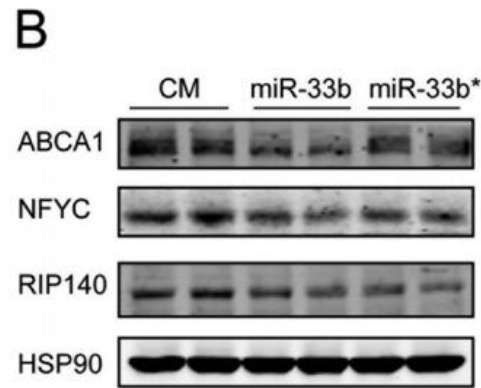
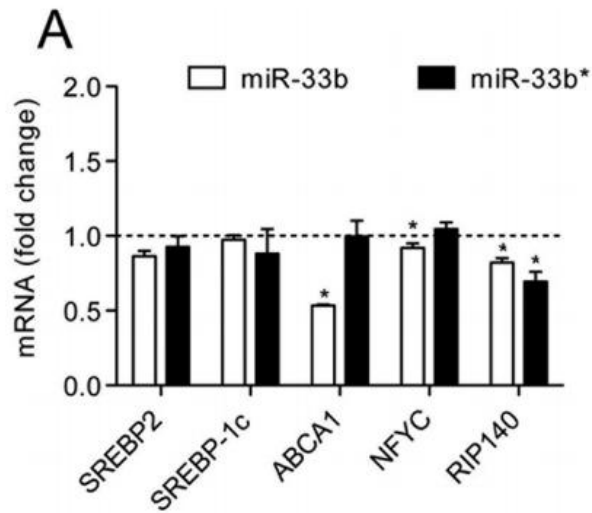


FIG 5 Posttranscriptional regulation of ABCA1, NPC1, SRC1, SRC3, NFYC, and RIP140 by miR-33a and miR-33a* in THP1 cells.

7. miR-33b or miR-33b* regulates gene expression in Huh7 cells.



7. miR-33b or miR-33b* regulates gene expression in Huh7 cells.

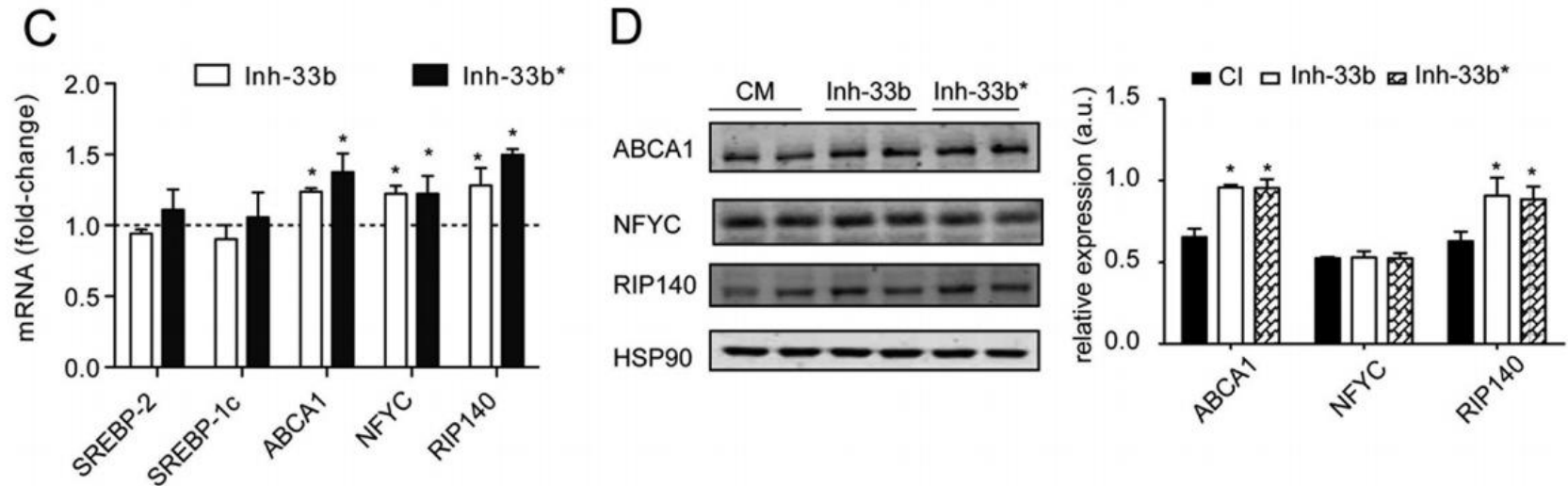
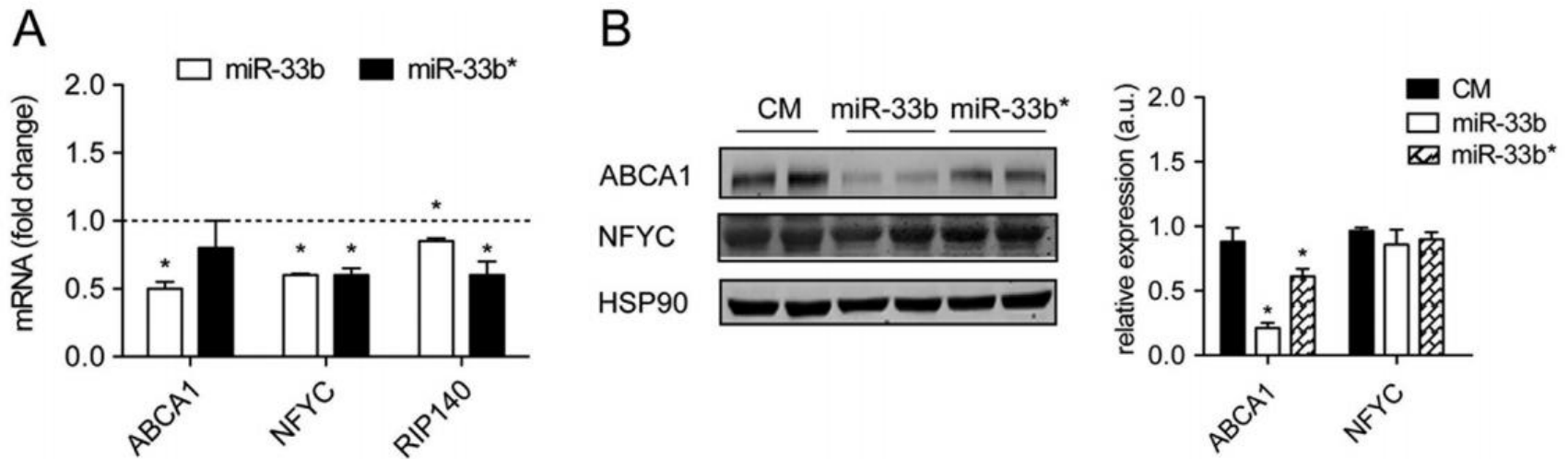


FIG 6 Posttranscriptional regulation of ABCA1, NFYC, and RIP140 by miR-33b and miR-33b* in Huh7 cells.

8. miR-33b or miR-33b* regulates gene expression in THP1 cells.



8.miR-33b or miR-33b* regulates gene expression in THP1 cells.

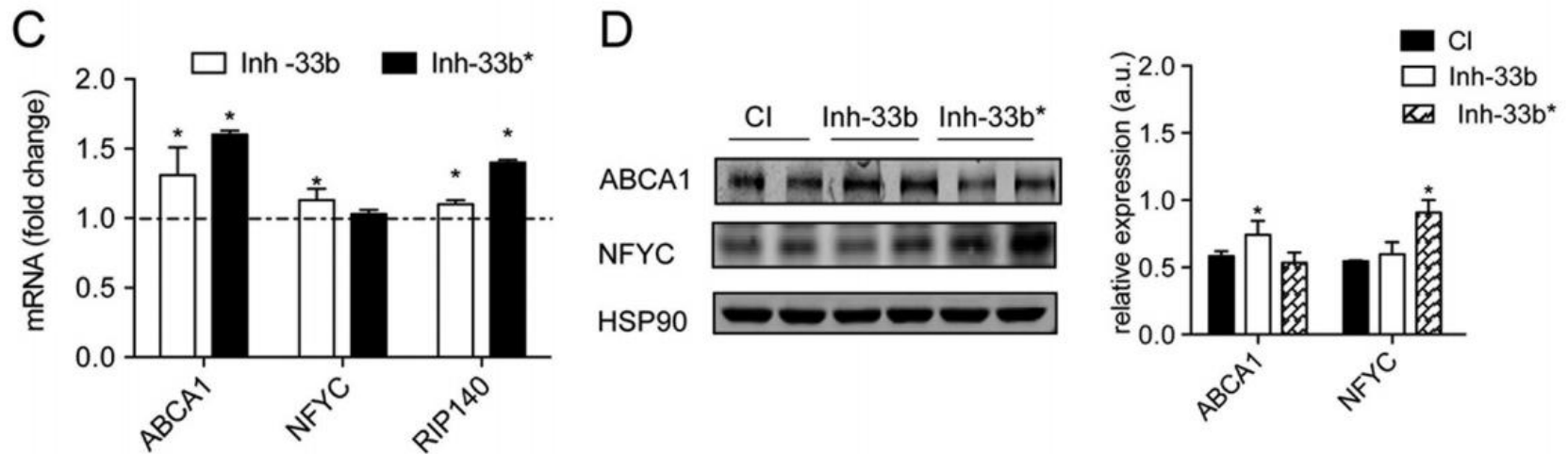
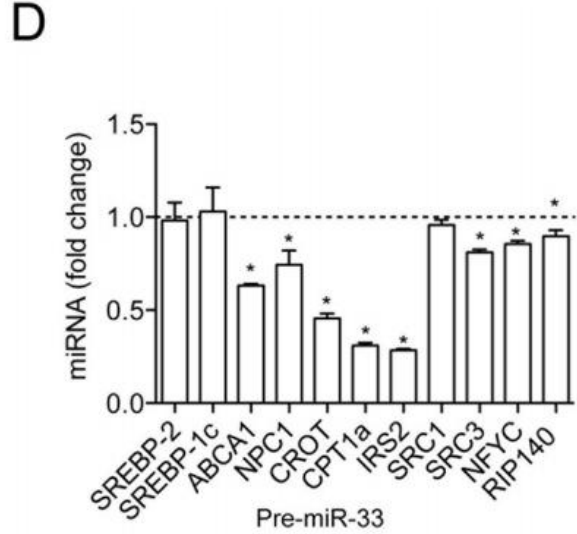
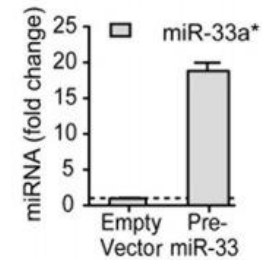
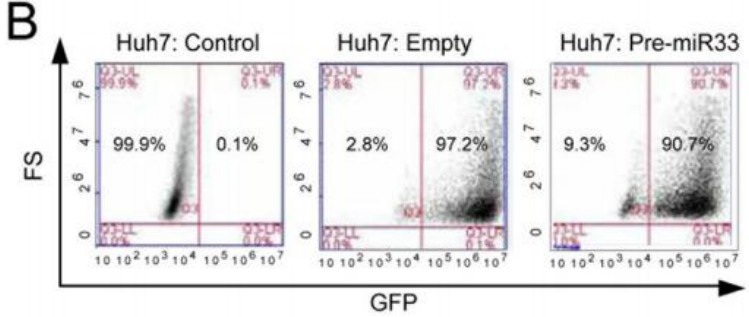
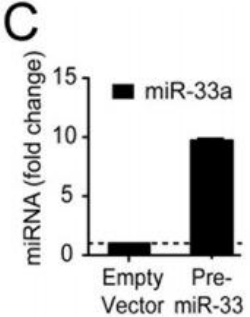
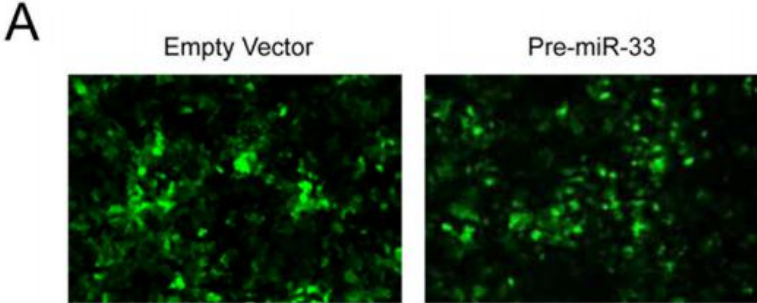


FIG 7 Posttranscriptional regulation of ABCA1, NFYC, and RIP140 by miR-33b and miR-33b* in THP1 cells.

9. miR-33 and miR-33* differentially regulate target gene expression.



9.miR-33 and miR-33* *differentially* regulate target gene expression.

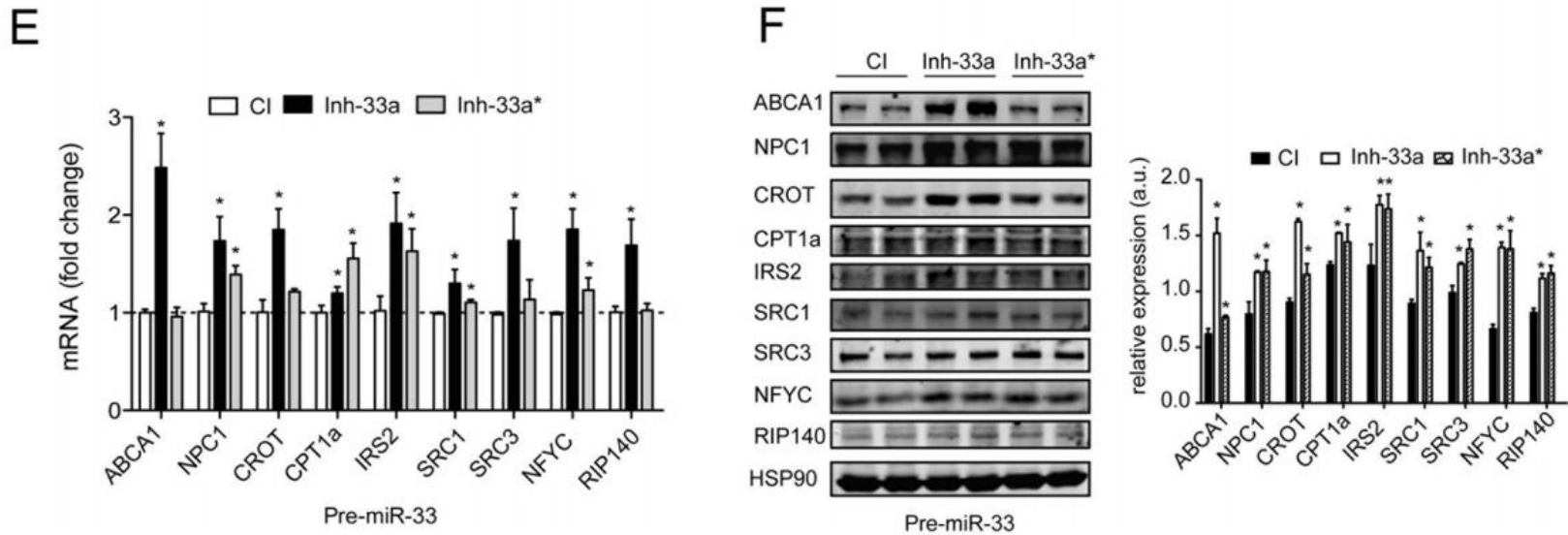
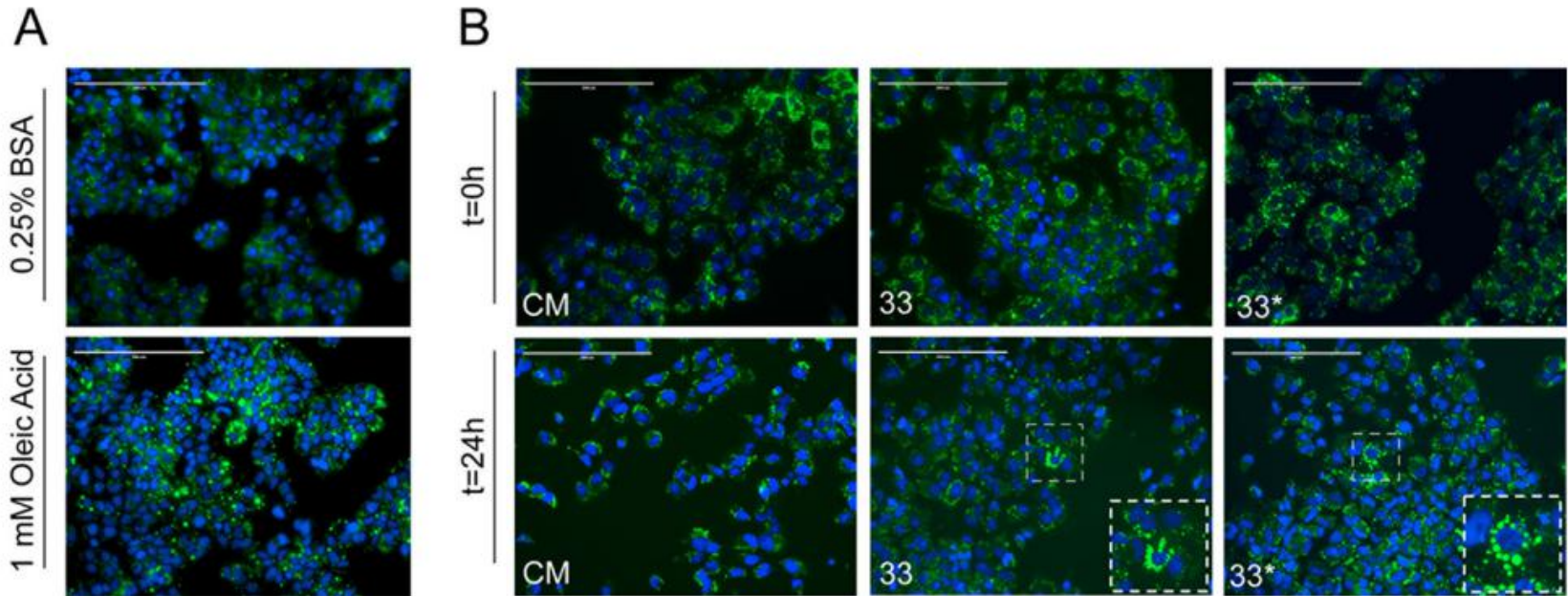


FIG 8 Both arms of the miR-33 duplex contribute to the posttranscriptional regulation of target gene expression.

10.miR-33* inhibits cellular fatty acid oxidation.



stained with Bodipy (green) and DAPI (blue).
green:脂滴 blue: 细胞核

10.miR-33* inhibits cellular fatty acid oxidation.

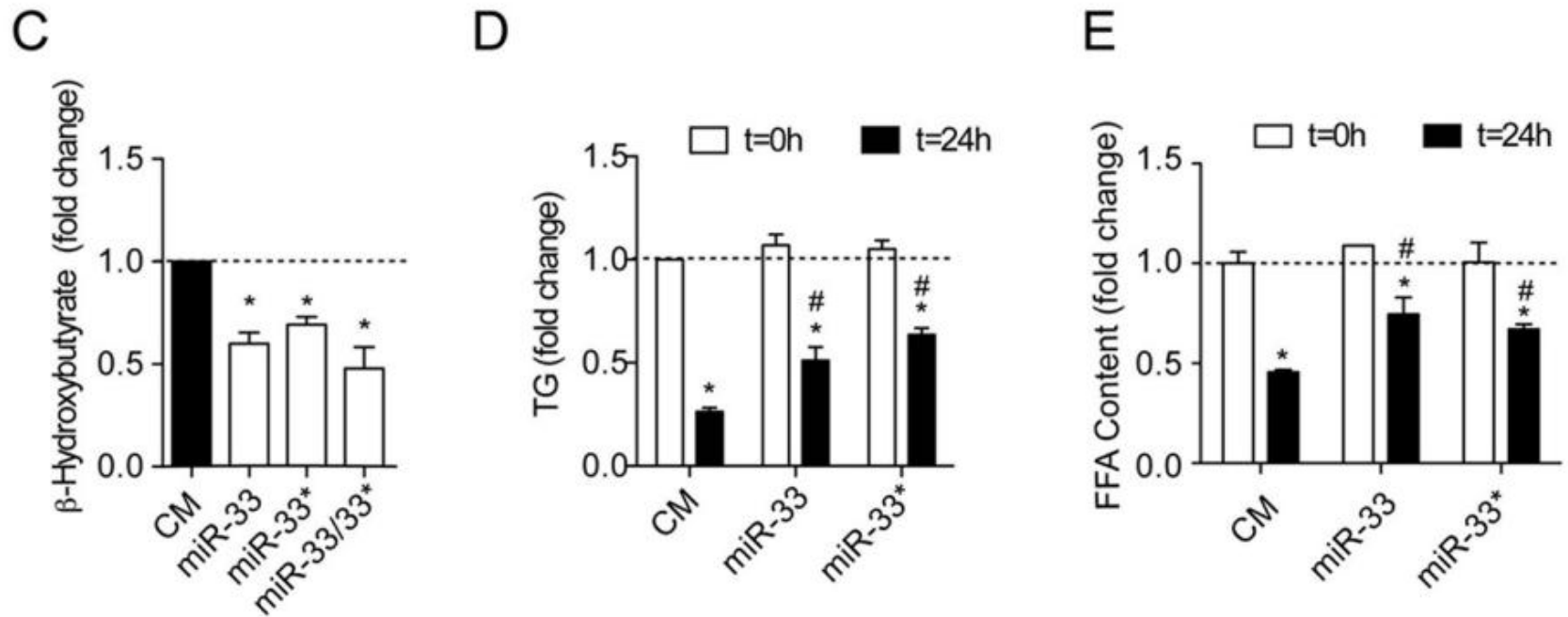


FIG 9 miR-33* cooperates with miR-33 to regulate fatty acid oxidation in human hepatic cells.

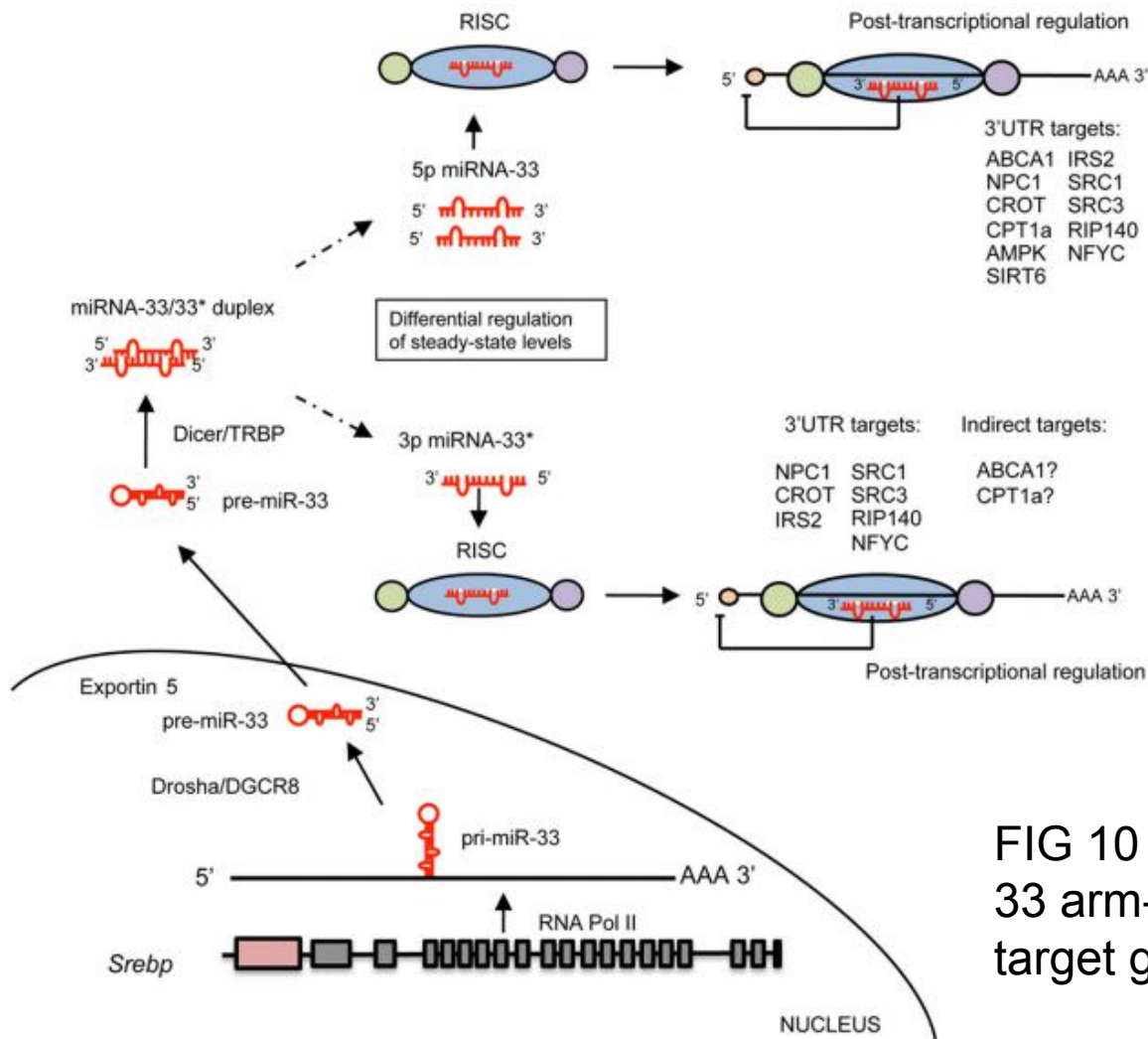


FIG 10 Proposed model of miR-33 arm-specific processing and target gene regulation.

谢谢大家

敬请批评指正