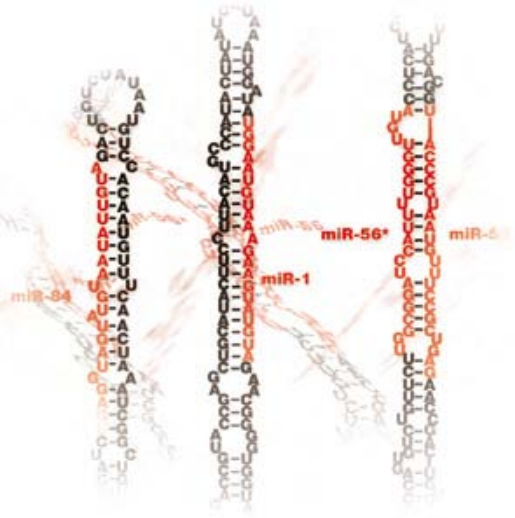


miR-33 as a Therapeutical Target for the Metabolic Syndrome

RNA Oligonucleotides, NIH
Bethesda, Dec 15-16, 2011

Carlos Fernández-Hernando Ph.D

DISCLOSURE: C F-H HAS PATENTS ON THE USE OF MIR-33 INHIBITORS

Metabolic Syndrome

Metabolic syndrome is a combination of medical disorders that,when occuring together, increase the risk of developing cardiovascular disease and diabetes

- ☐ Low levels of High-density lipoproteins (HDL)
- ☐ High levels of plasma triglycerides
- ☐ Insulin Resistance

miR-33

Central Dogma of Molecular Genetics

Genes to messenger to proteins

(*Francis Crick, 1957*)

Replication

DNA → RNA → Protein

transcription translation

miRNAs

Small non-coding RNA

(Victor Ambros, 1993)

(Gary Ruvkun, 1993)

C. elegans

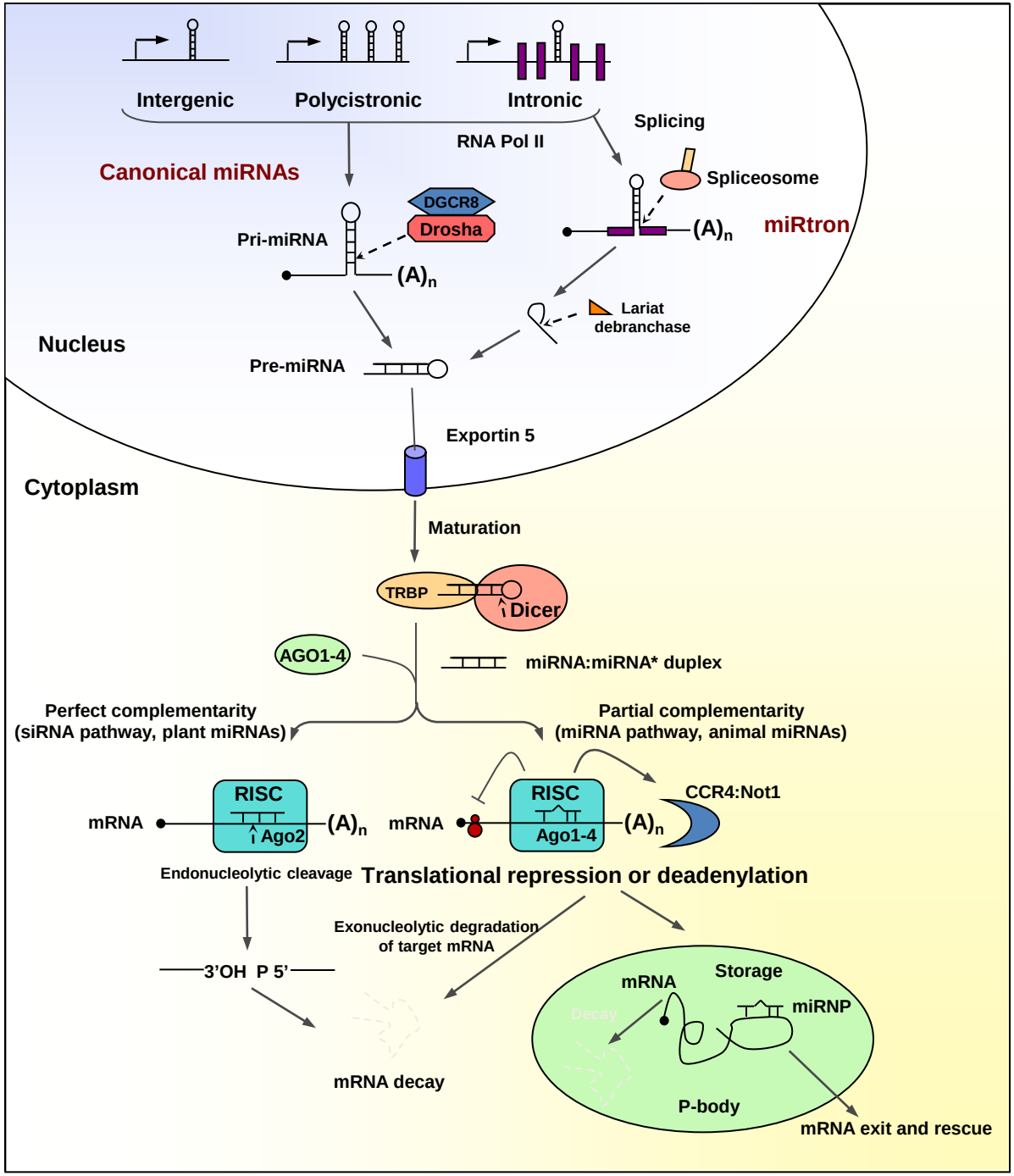
lin-4

let-7 *(conserved across animal phylogeny)*

(Gary Ruvkun, 2000)

The human genome may encode over 1000 miRNAs, which may target about 60% of mammalian genes

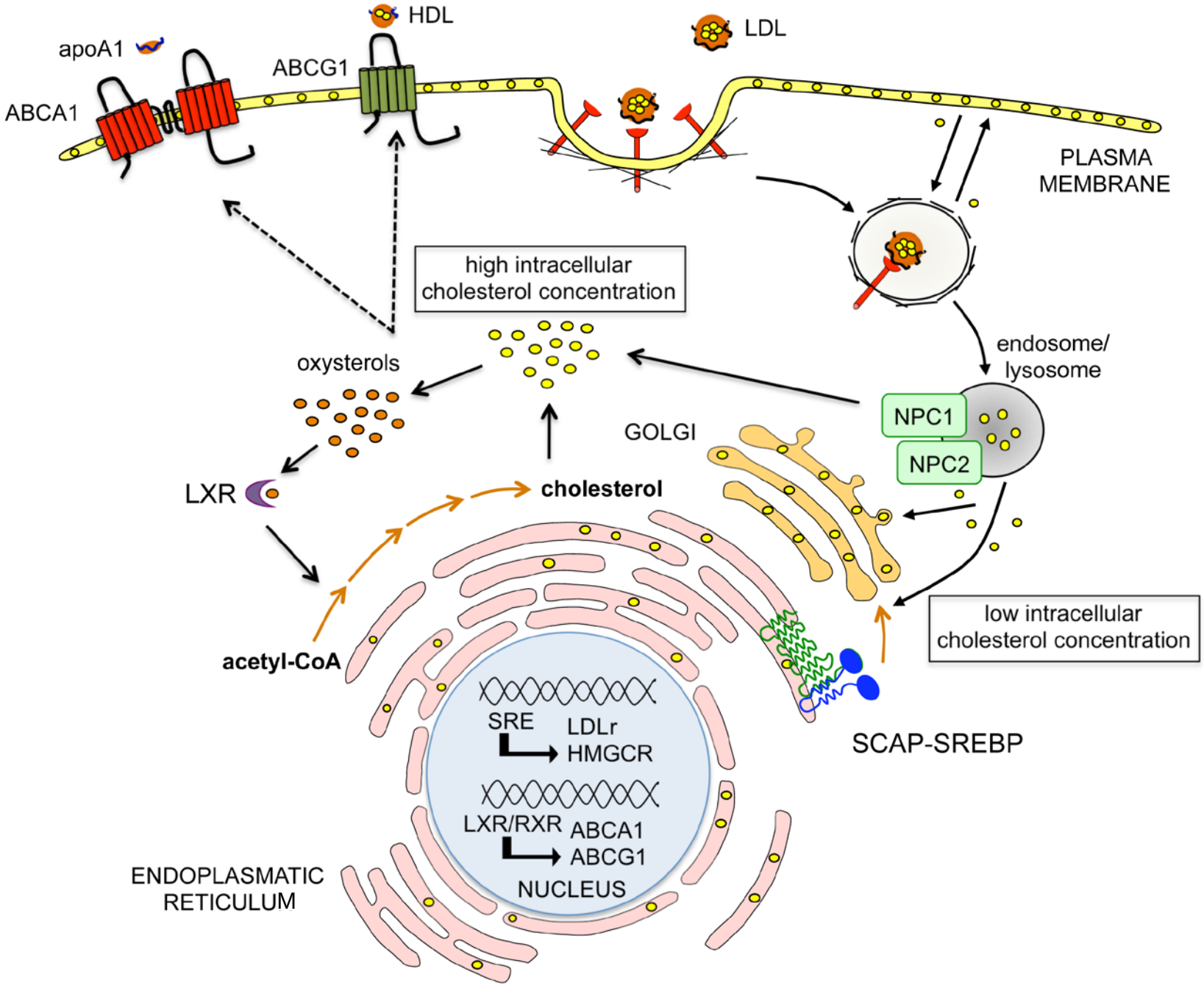
microRNAs: Biogenesis



In mammalian cells, miRNAs are initially transcribed as parts of longer molecules (pri-miRNAs of >1000 nt), are processed in the nucleus into shorter (70-100 nt) hairpin pre-miRNAs by Drosha, and are then transported to the cytoplasm, via an Exportin 5-dependent mechanism, where they are digested by a second, doubled-stranded specific ribonuclease called Dicer to produce a double-stranded miRNA.

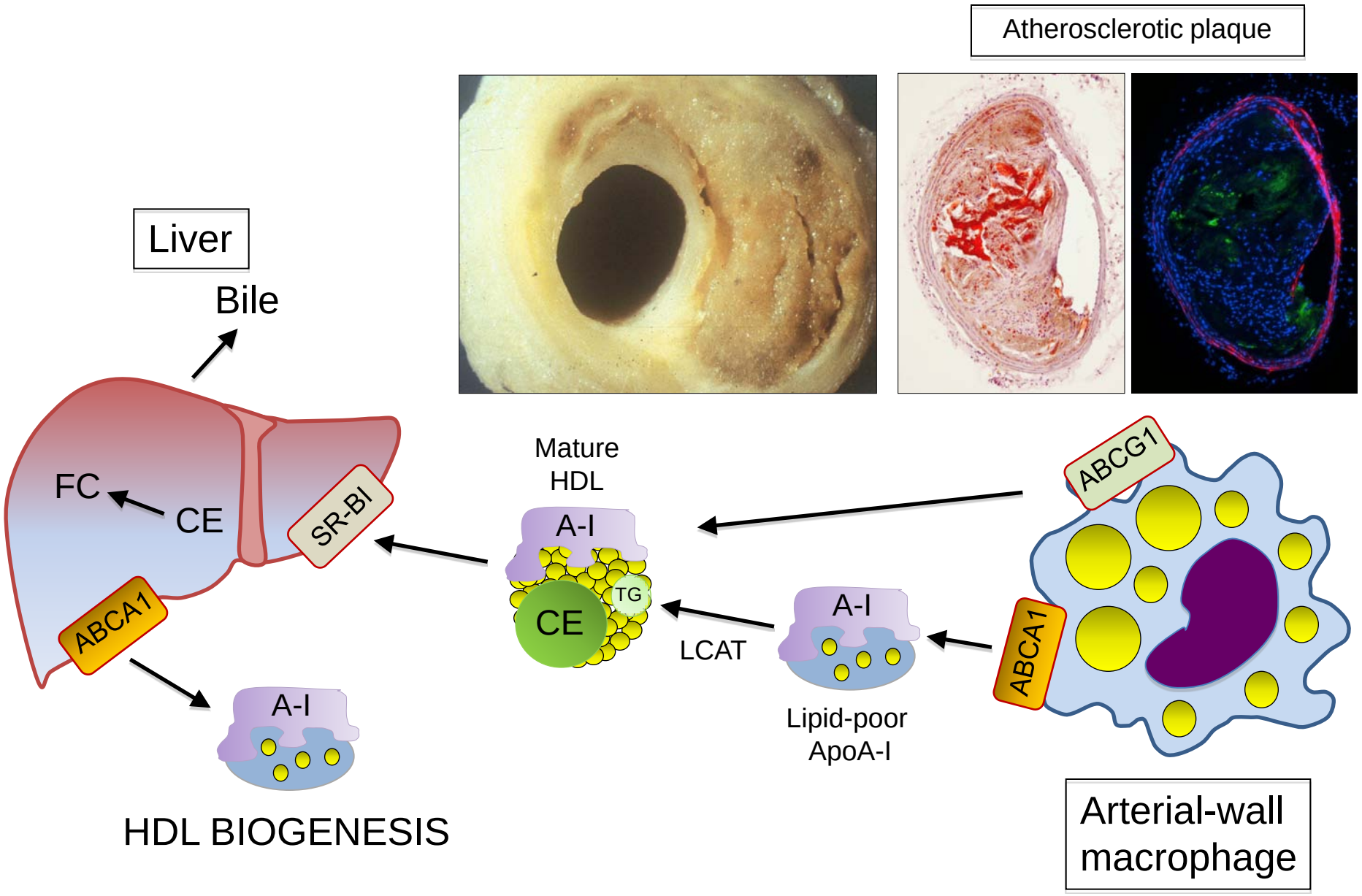
The miRNA-duplex are incorporated into a ribonucleoprotein complex (RISC) complex that mediates the downregulation of target gene activity by translational inhibition or target mRNA degradation, resulting in reduced levels of the corresponding protein or transcript.

Suárez Y and Sessa WC. ***Circ Res****. 2009*



Moore KJ, Rayner KJ, Suárez Y and Fernández-Hernando C. ***Trends Endocrinol Met.*** *2010*

Reverse Cholesterol Transport

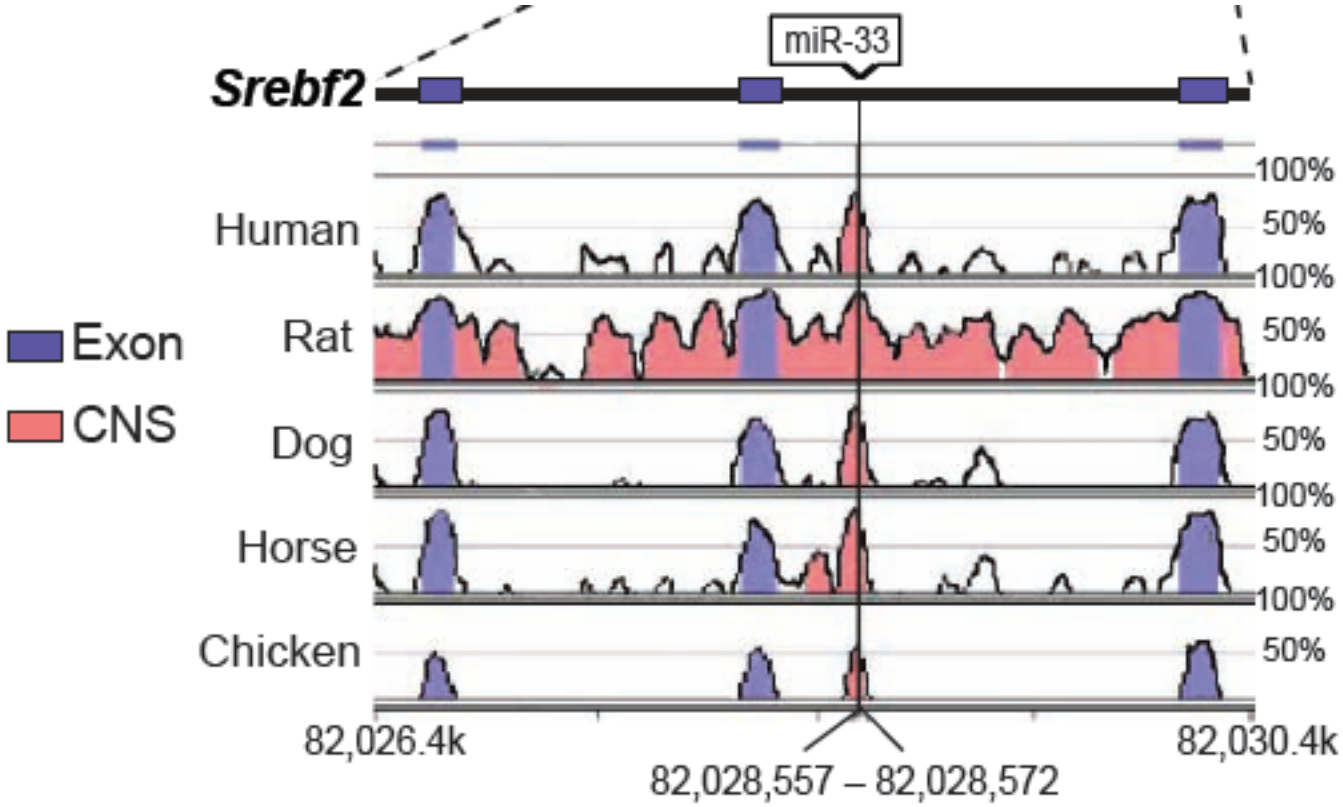


Science *2010 Jun 18;328(5985): 1570-3*

REPORTS

MiR-33 Contributes to the Regulation of Cholesterol Homeostasis

Katey J. Rayner,[1,2]* Yajaira Suárez,[1]* Alberto Dávalos,[1] Saj Parathath,[1] Michael L. Fitzgerald,[2] Norimasa Tamehiro,[2] Edward A. Fisher,[1] Kathryn J. Moore,[1,2]†‡ Carlos Fernández-Hernando [1]†‡



Putative miR-33 Gene Targets

- ABCA1 (3 potential binding sites)

- Reverse transport of cholesterol (cholesterol efflux)
- HDL biogenesis

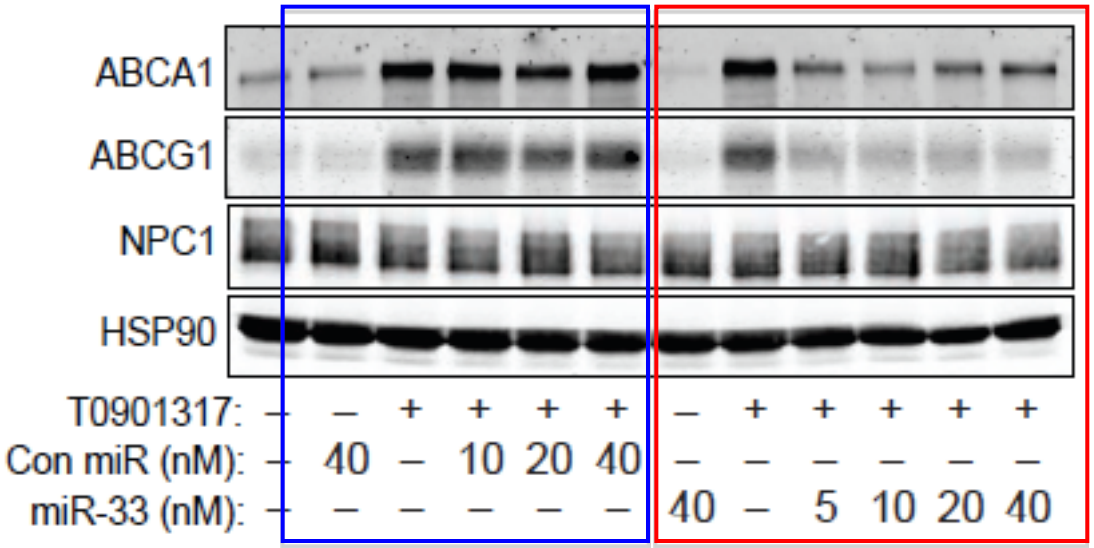
- ABCG1 (2 potential binding sites, only in rodents)

- Reverse transport of cholesterol (cholesterol efflux)

- NPC1 (2 potential binding sites)

- Intracellular cholesterol trafficking

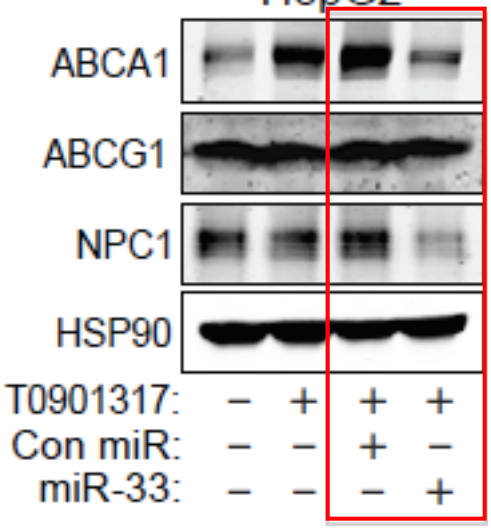
miR-33 reduces expression of ABCA1 and ABCG1 in mouse Mø, hepatocytes and endothelial cells



Peritoneal macrophages

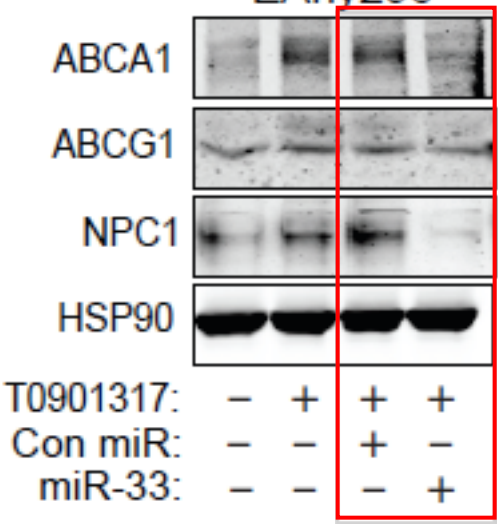
Hepatocyte

HepG2

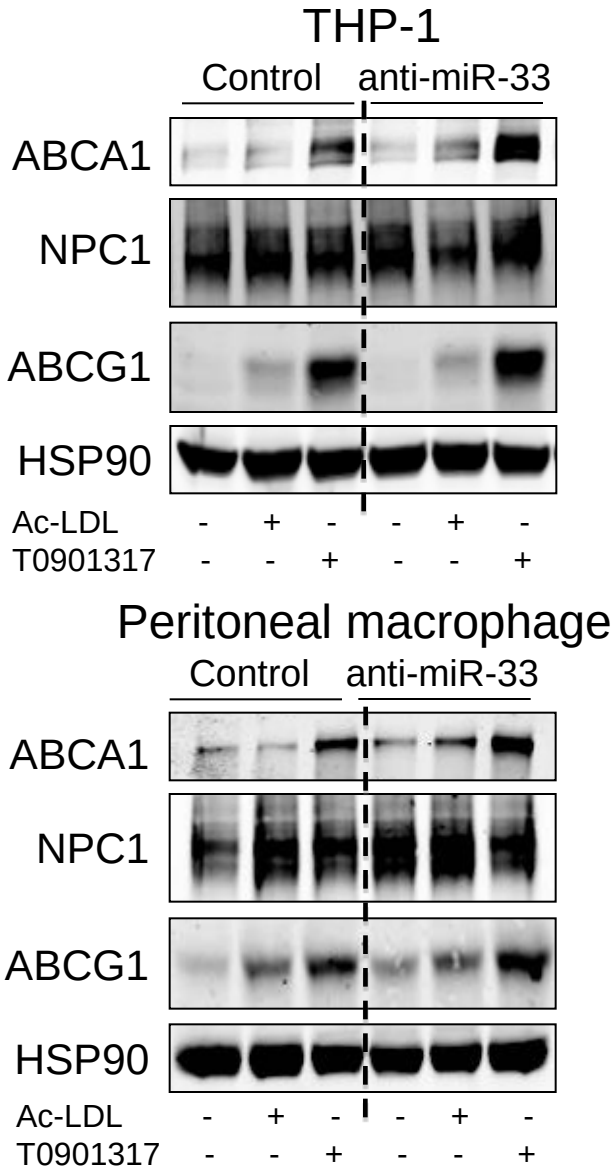


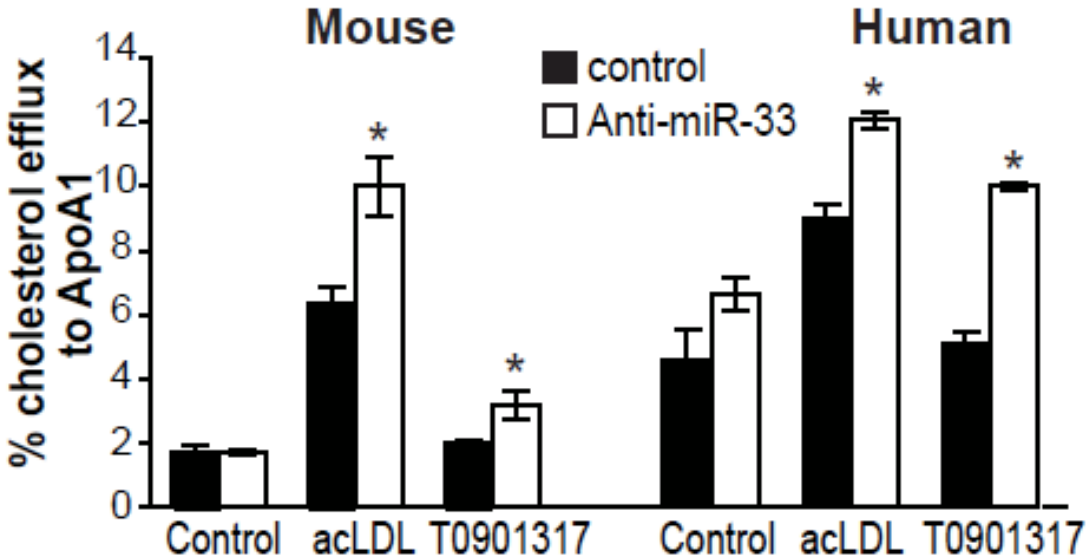
Endothelial Cell

EAhy296



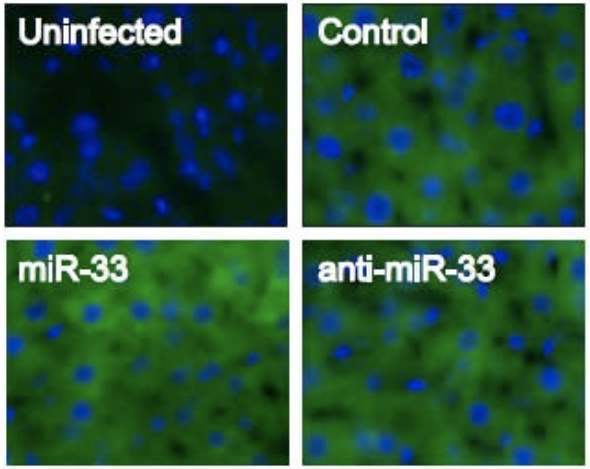
Antagonism of miR-33 increases ABCA1 and cholesterol efflux

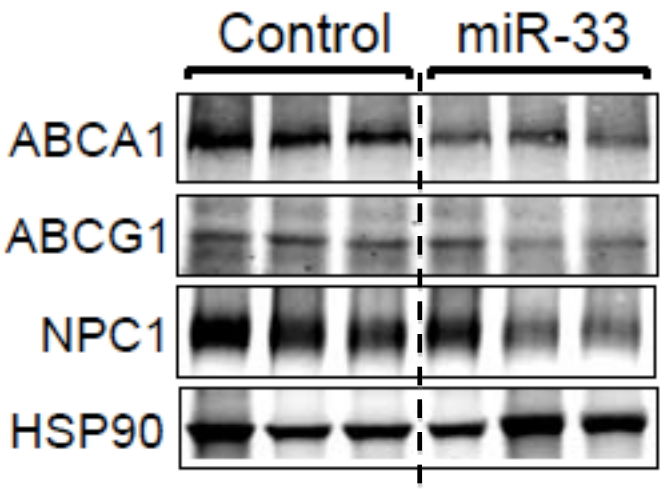


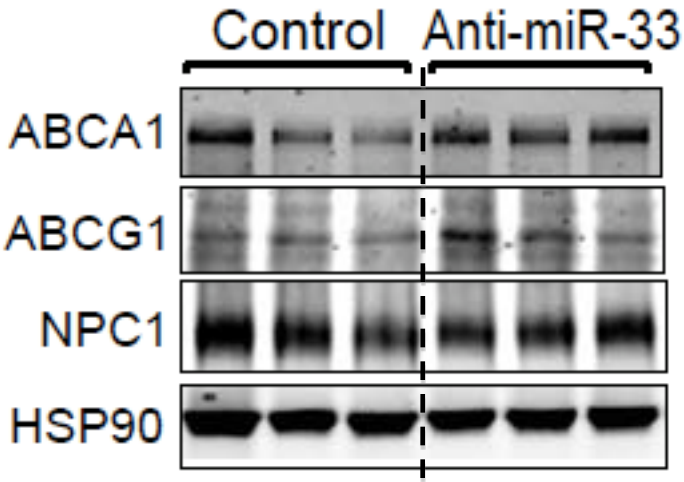


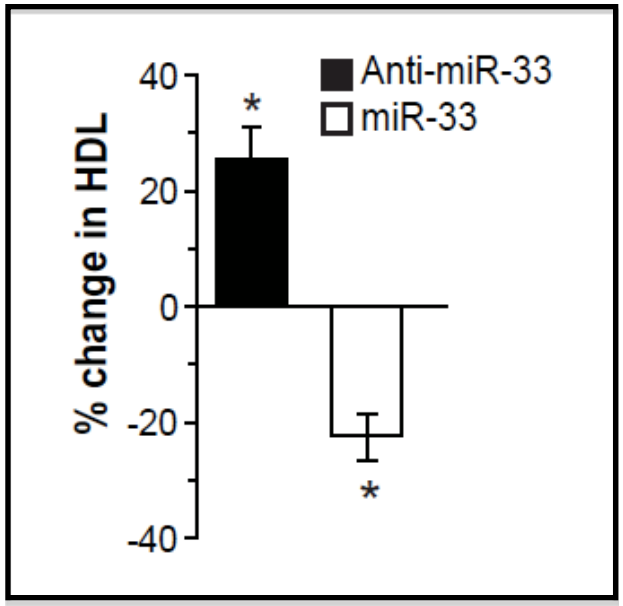
Rayner KJ* and Suárez Y* *et al.* ***Science*** *2010.*

In vivo delivery of miR-33 or anti-miR33 regulates hepatic ABCA1 expression and plasma HDL levels









Rayner KJ* and Suárez Y* *et al.* ***Science*** *2010.*

THERAPEUTIC INHIBITION OF miR-33 WITH ANTI-miR-33

1. Rayner KJ* and Suarez Y* *et al*. *Science* 2010. **Lentivirus**

2. Najafi-Shoushtari SH *et al*. *Science* 2010. **LNA oligonucleotides**

3. Marquart TJ *et al*. *PNAS* 2010. **Adenovirus and LNA oligonucleotides**

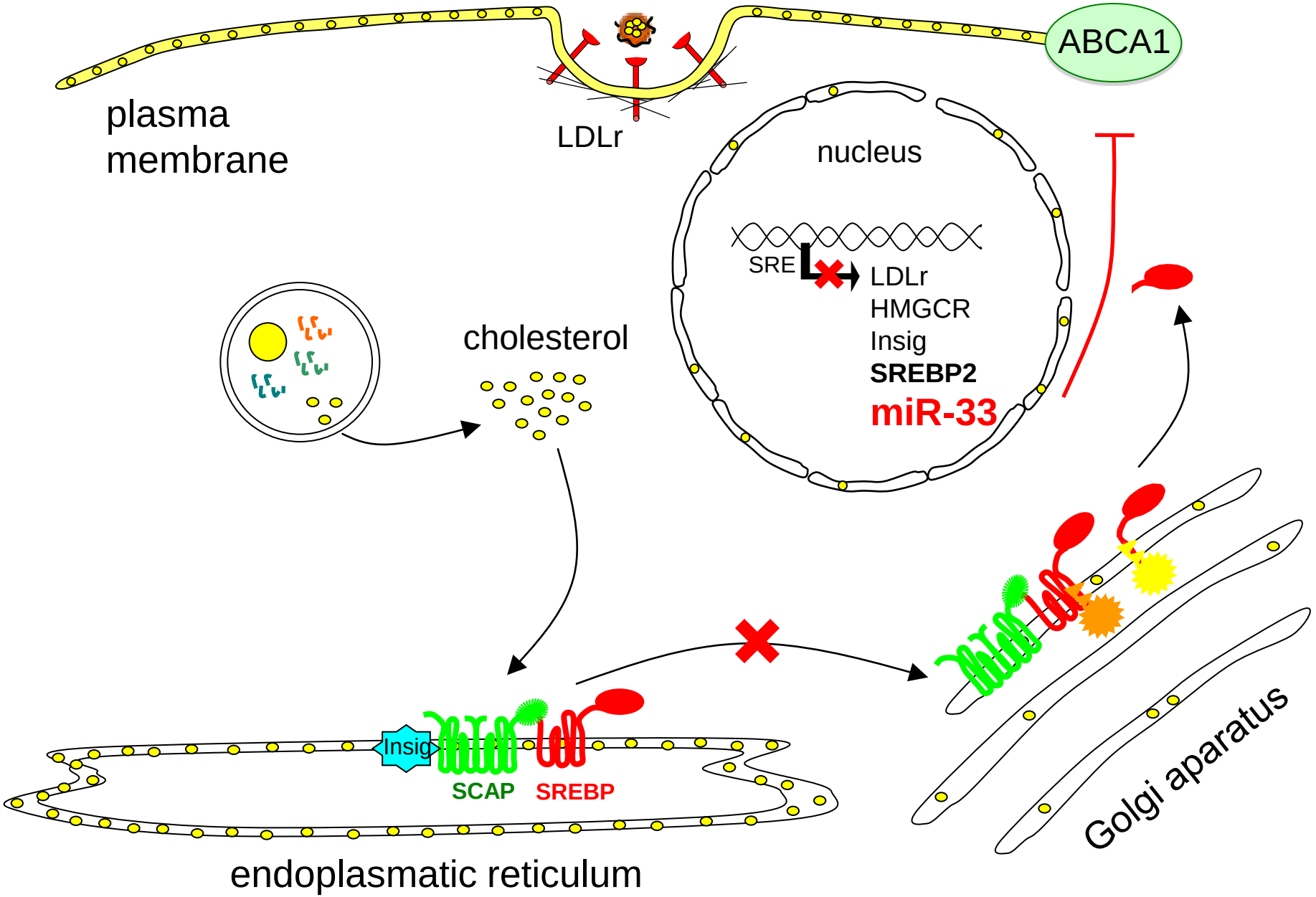
4. Horie T *et al*. *PNAS* 2010. **Knockout mice**

5. Rayner K *et al*. *JCI* 2011. **2´F/MOE-modified oligonucleotides**

6. Dávalos A* and Goedeke L* *et al*. *PNAS* 2011. **Transgenic fly.**

7. Rayner K *et al*. *Nature* 2011 *(20 Oct, 2011*). **Non-human primates**

Cellular cholesterol trafficking and compartmentalization



hsa-miR-33a versus *hsa-miR-33b*

hsa-miR-33a: 5´-G**UGCAUUG**U**A**GU**UGCAUUG**CA-3

Seed sequence

hsa-miR-33b: 5´-G**UGCAUUG**C**U**GU**UGCAUUG**CA-3

Seed sequence

Srebp-2: cholesterol metabolism (MVA-pathway, LDLr)

Srebp-1:

- *Srebp 1a*: cholesterol metabolism (MVA-pathway, LDLr)
- *Srebp 1c*: fatty acid metababolism (FAS, SCD,…)

Srebp/miR-33 conserved in ***Drosophila melanogaster***

-Do not synthesize sterols

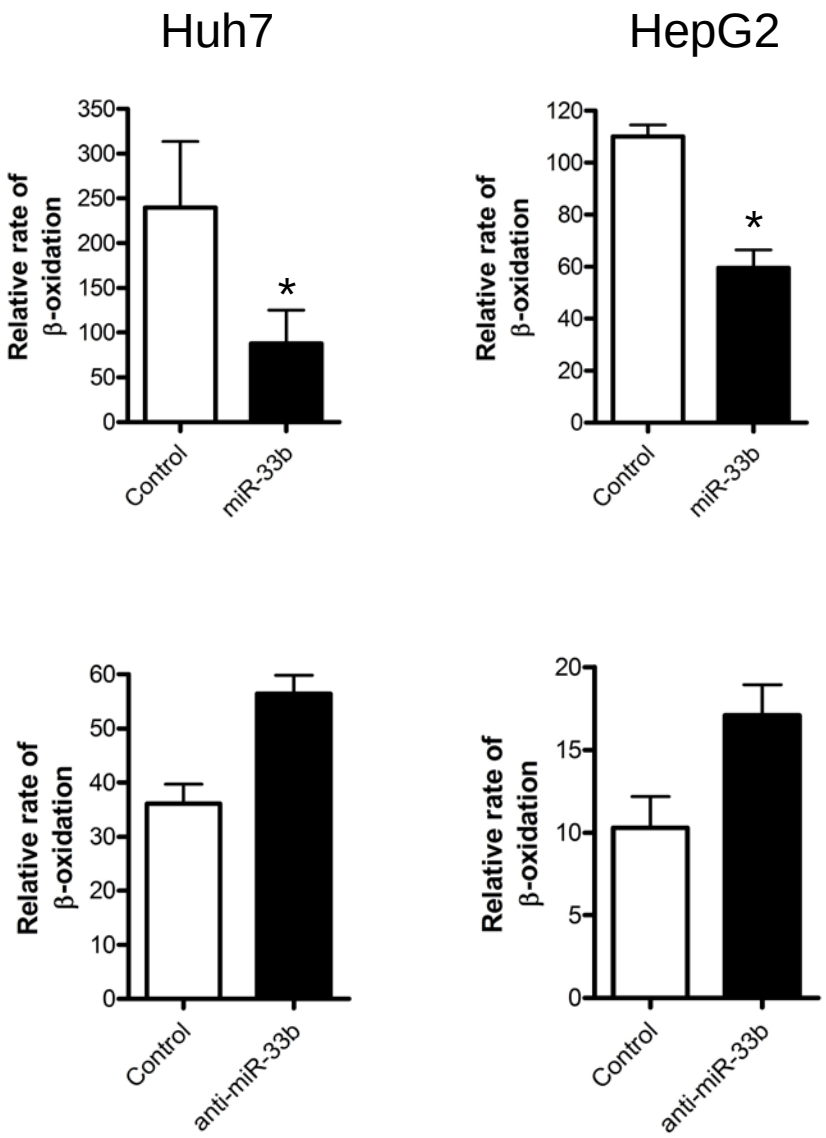
- Fruit fly genome does not contain ABCA1

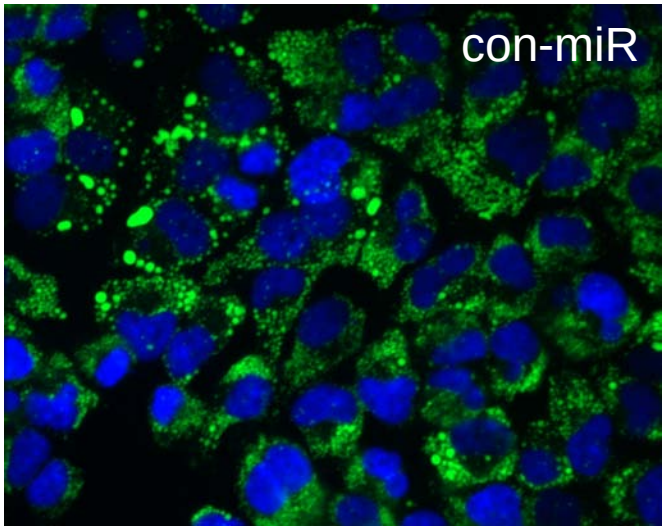
Putative miR-33 Gene Targets (Fatty acid oxidation genes)

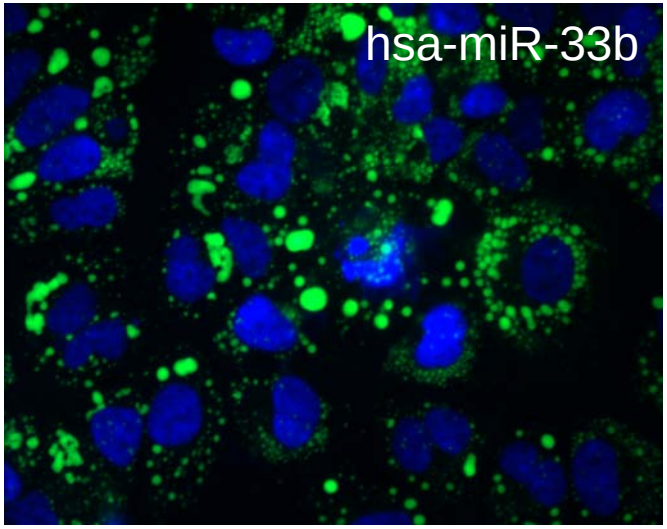
- CPT1a (Carnitine palmitoyl transferase 1A)
- CROT (Carnitine O-octaniltransferase)
- HADHB (Hydroxyacyl-CoA-dehydrogenase)
- AMPK (AMP Kinase subunit-α)

Dávalos A* and Goedeke L* *et al*. ***PNAS***. *2011*

hsa-miR-33b reduce fatty acid oxidation

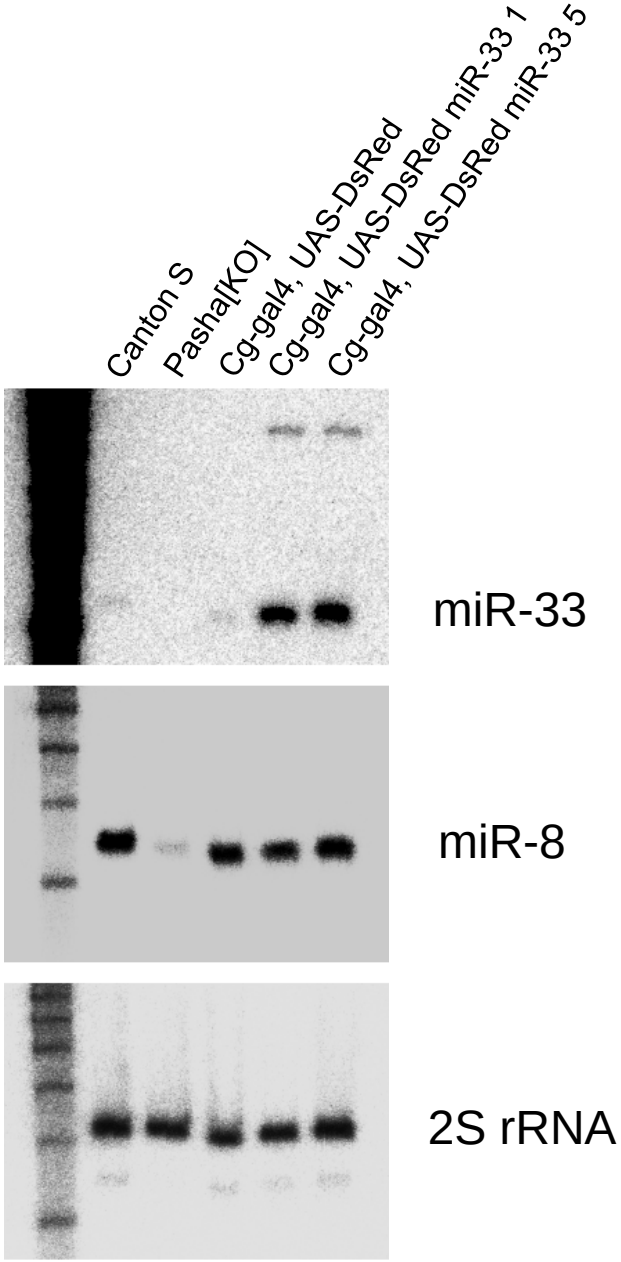


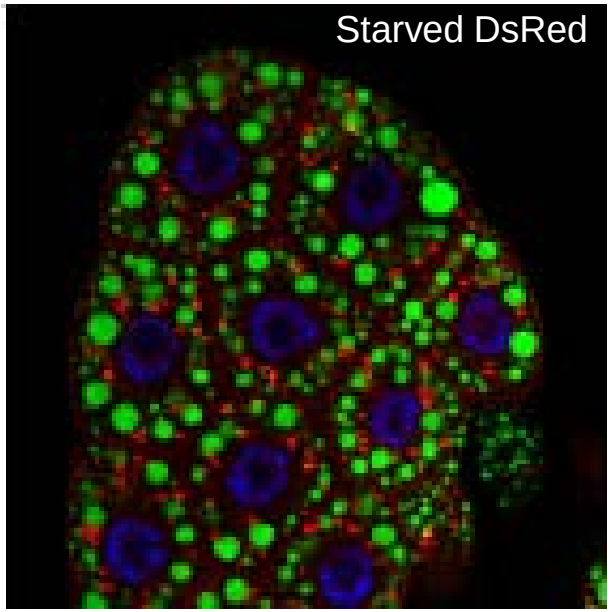


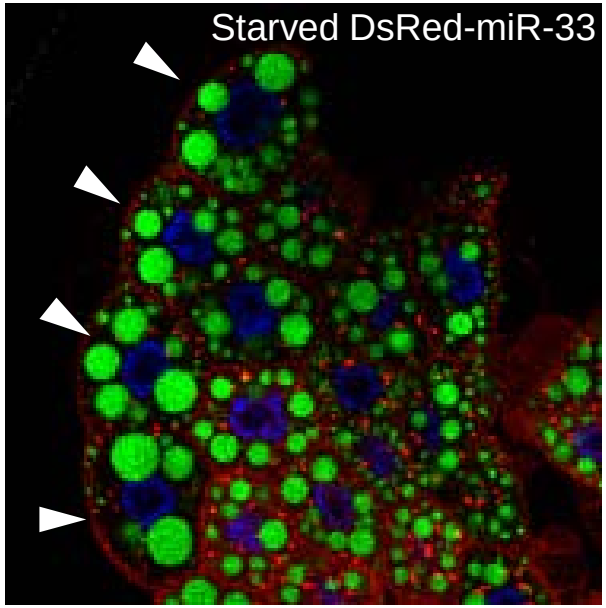


Dávalos A* and Goedeke L* *et al*. ***PNAS***. *2011*

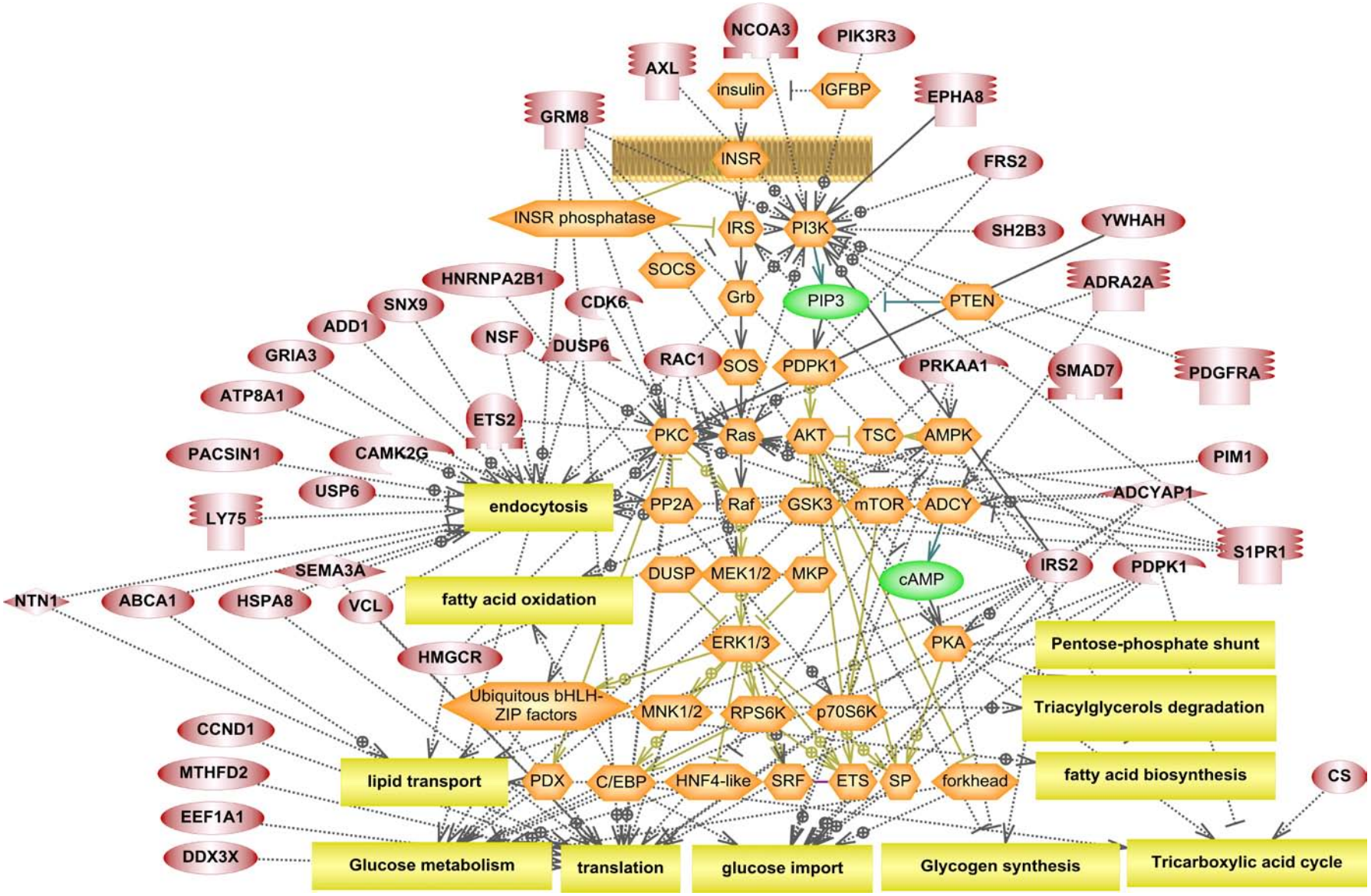
miR-33 reduce fatty acid oxidation in vivo







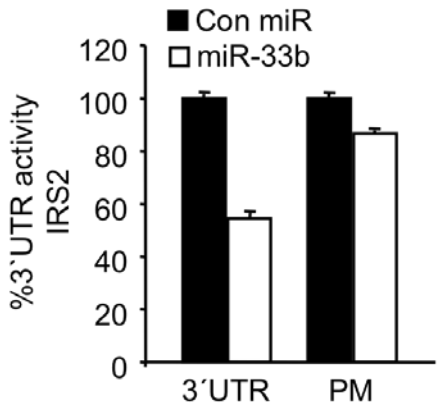
hsa-miR-33b regulates insulin signaling

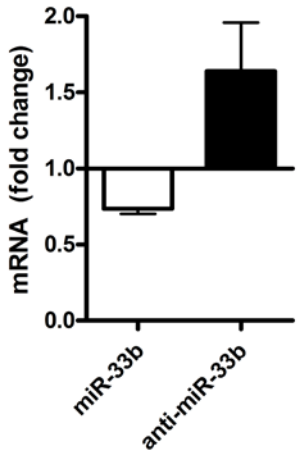


hsa-miR-33b regulates IRS2 expression

IRS2-3'UTR

Hsa	5'…G**CAATGCA**AAAGTCTTCCTGCTTCC…3'
Ptr	5'…G**CAATGCA**AAAGTCTTCCTGCTTCC…3'
Mml	5'…G**CAATGCA**AAAGTCTTCCTGCTTCC…3'
Mmu	5'…G**CAATGCA**GCTGTCTTCCTGCTTCC…3'
Rno	5'…G**CAATGCA**GCTGTCTTCCTGCTTCC…3'



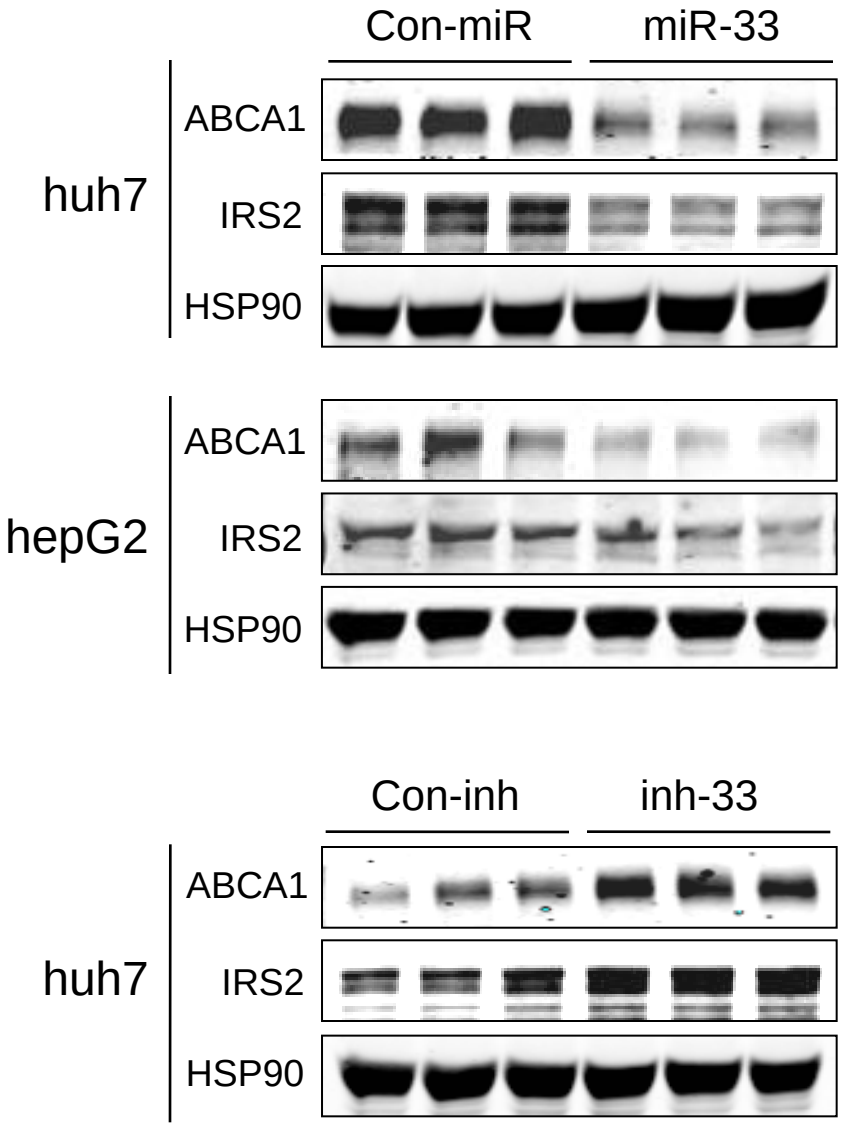


IRS2-3'UTR

5'…GCAATGCAAAAGTCTTCCTGCTTCC…3'

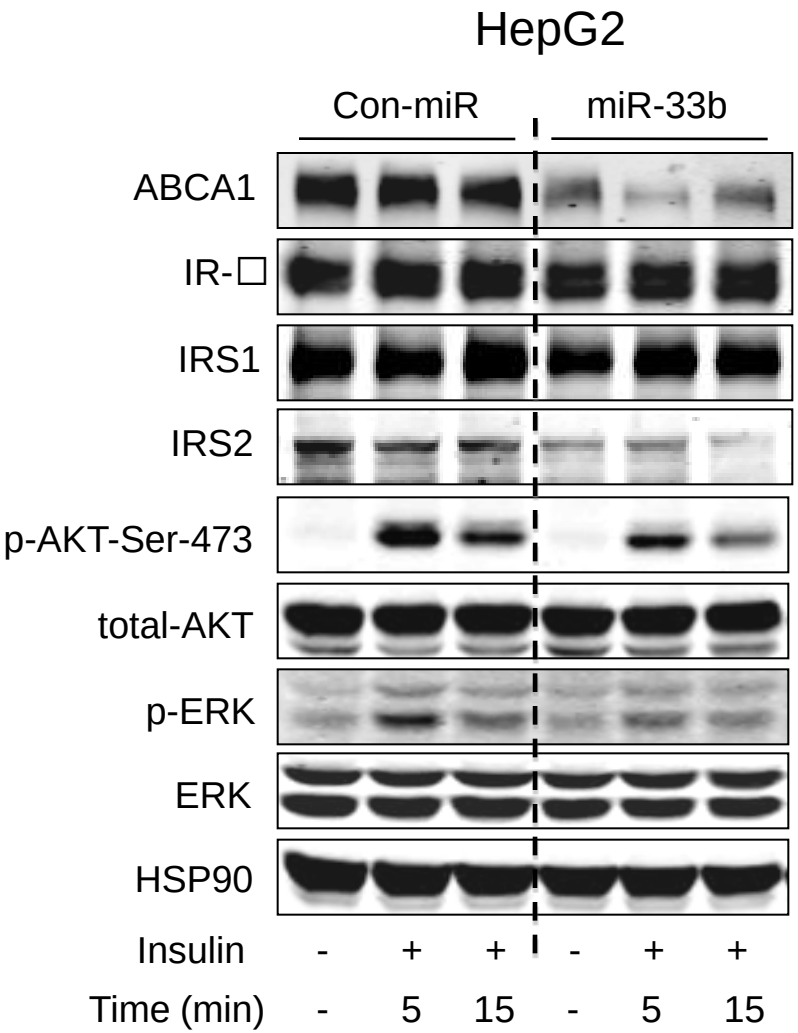
IRS2-3'UTR-PM

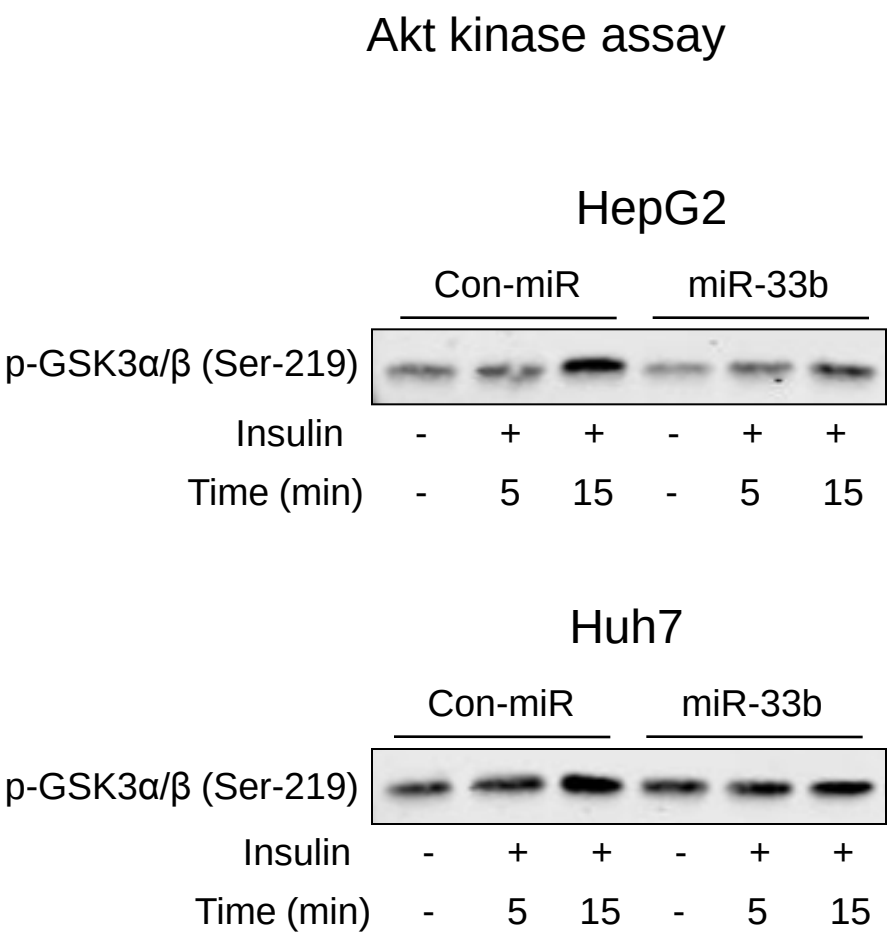
5'…GC**TT**TGCAAAAGTCTTCCTGCTTCC…3'



Dávalos A* and Goedeke L* *et al.* ***PNAS****. 2011*

hsa-miR-33b regulates insulin signaling





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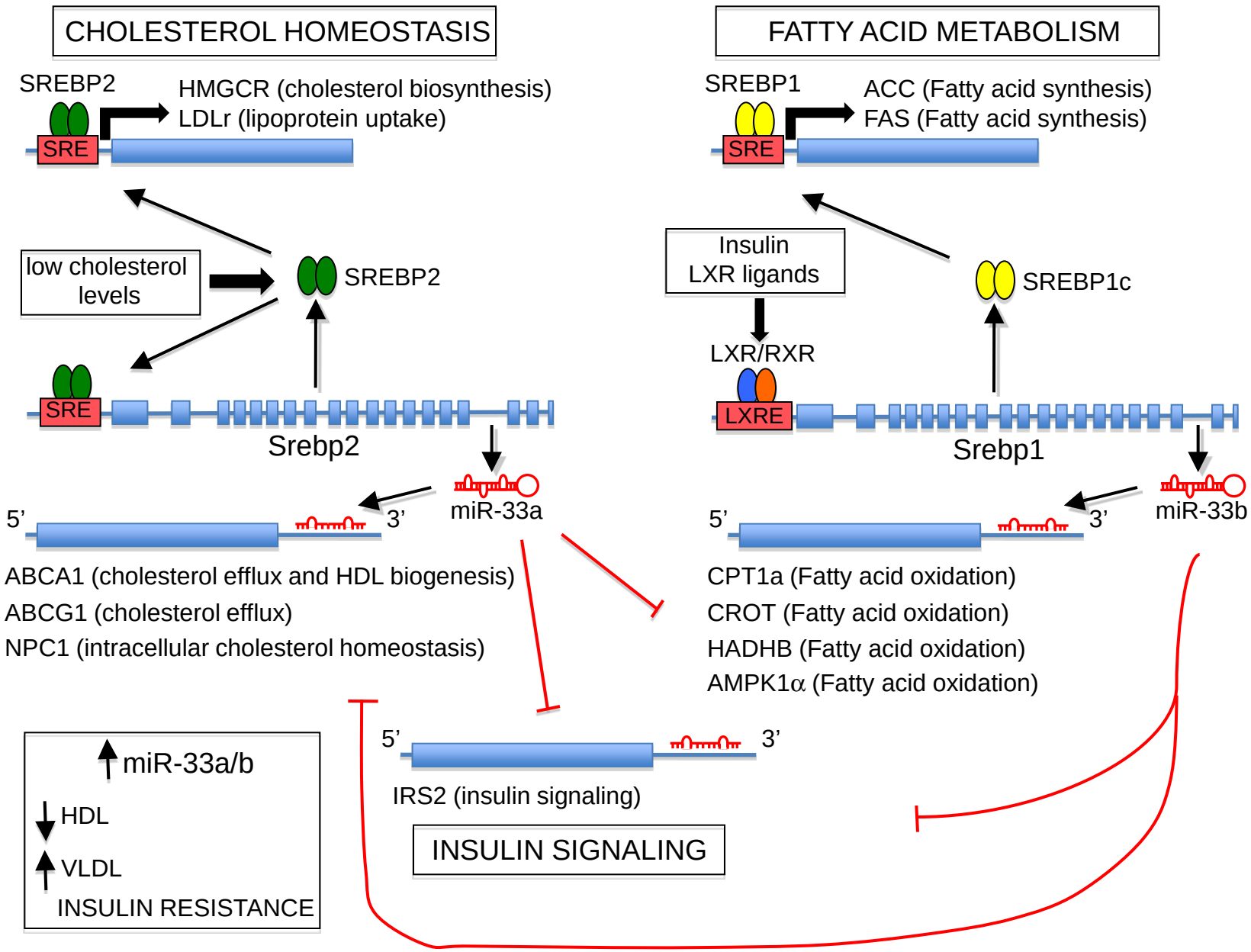

Dávalos A* and Goedeke L* *et al.* ***PNAS***. *2011*

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