

# Webinar

## Understanding the function of miRNA *in vivo*

**Taconic**  
Smart Solutions To Improve Human Health

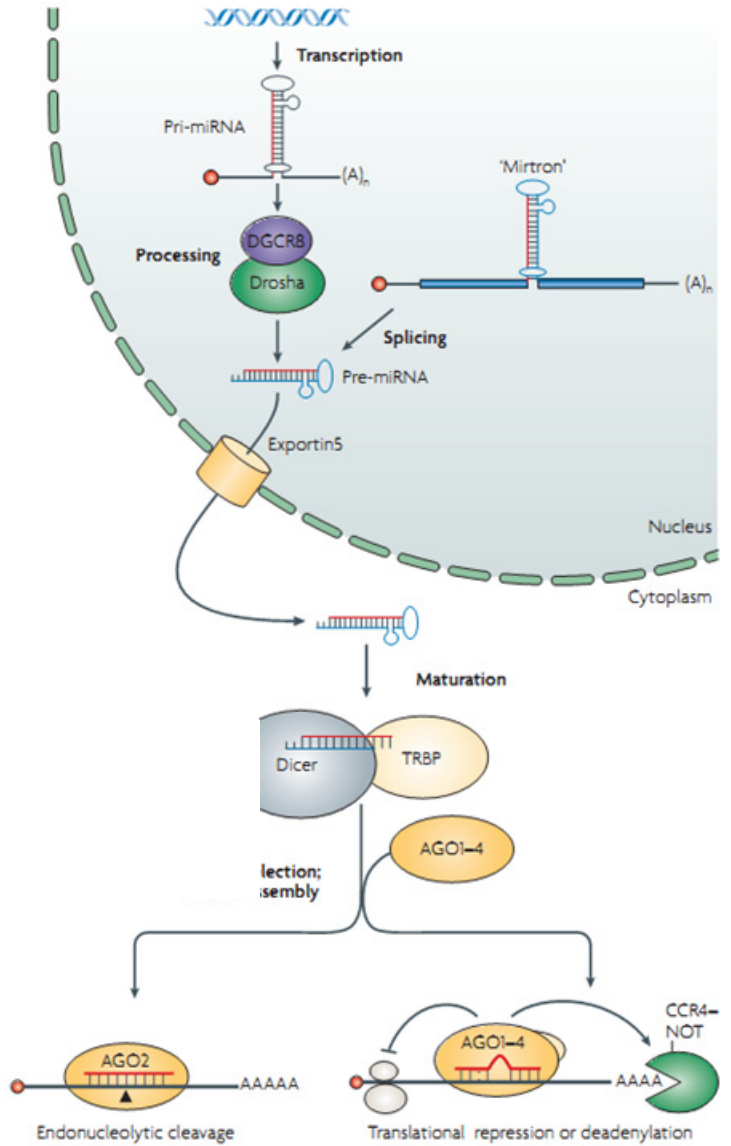


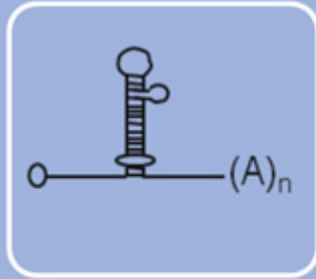
27 April 2011, Jost Seibler, Head of R&D

# Understanding the function of miRNAs *in vivo*



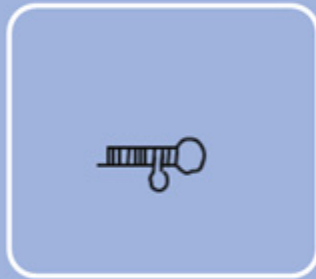
- Knockout mice: > 25 reports
- Over-expression in mice: > 5 reports
- Deletion/mutation of target sequences of miRNAs: ?





## Pri-miRNA

- Knockout of single miRNAs or cluster
- Overexpression of single miRNA or cluster



## Pre-miRNA

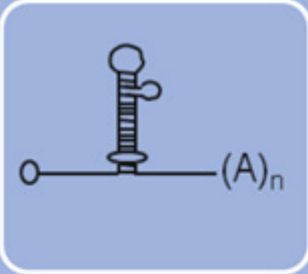
- Overexpression
- (Knockout)



## Mature miRNA

- Overexpression

# Modifications of miRNA expression



## Pri-miRNA

- Knockout of single miRNAs or cluster
- Overexpression of single miRNA or cluster



## Pre-miRNA

- Overexpression



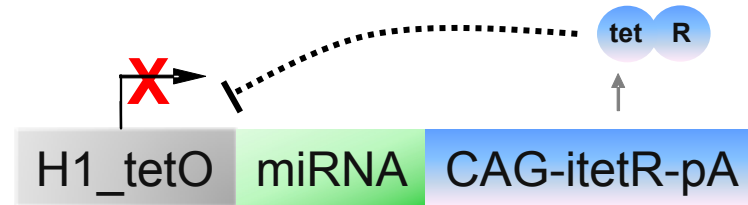
## Mature miRNA

- Overexpression

# Inducible miRNA expression

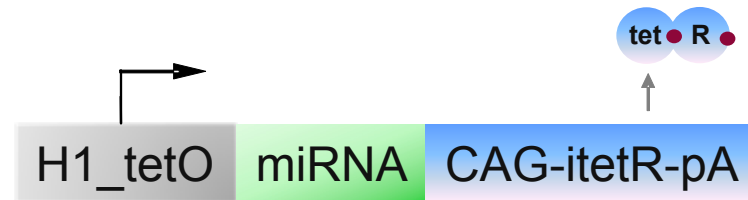


No expression of miRNA in the absence of doxycycline



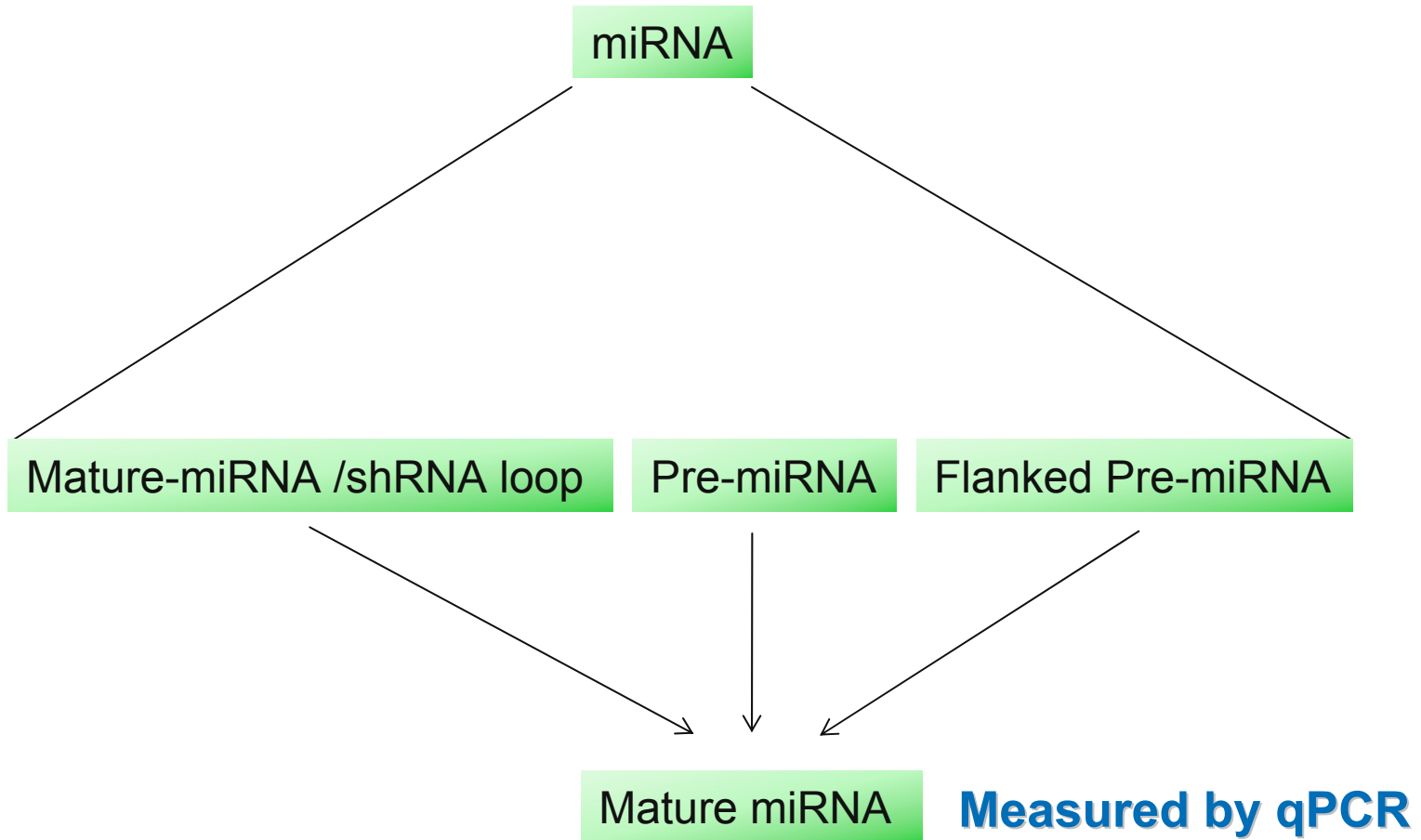
*doxycycline*

miRNA expression upon induction with doxycycline

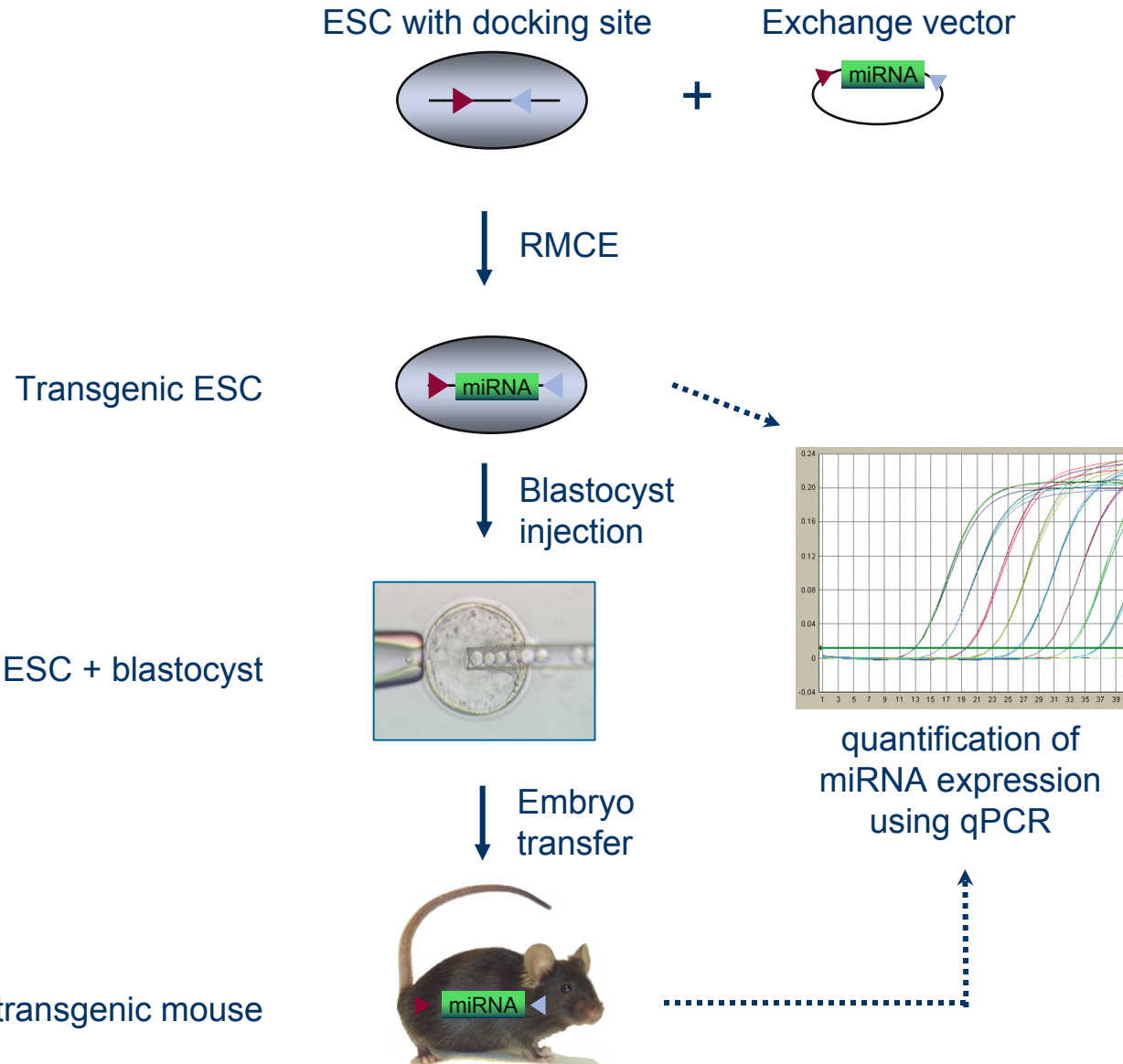


miRNA expression upon induction

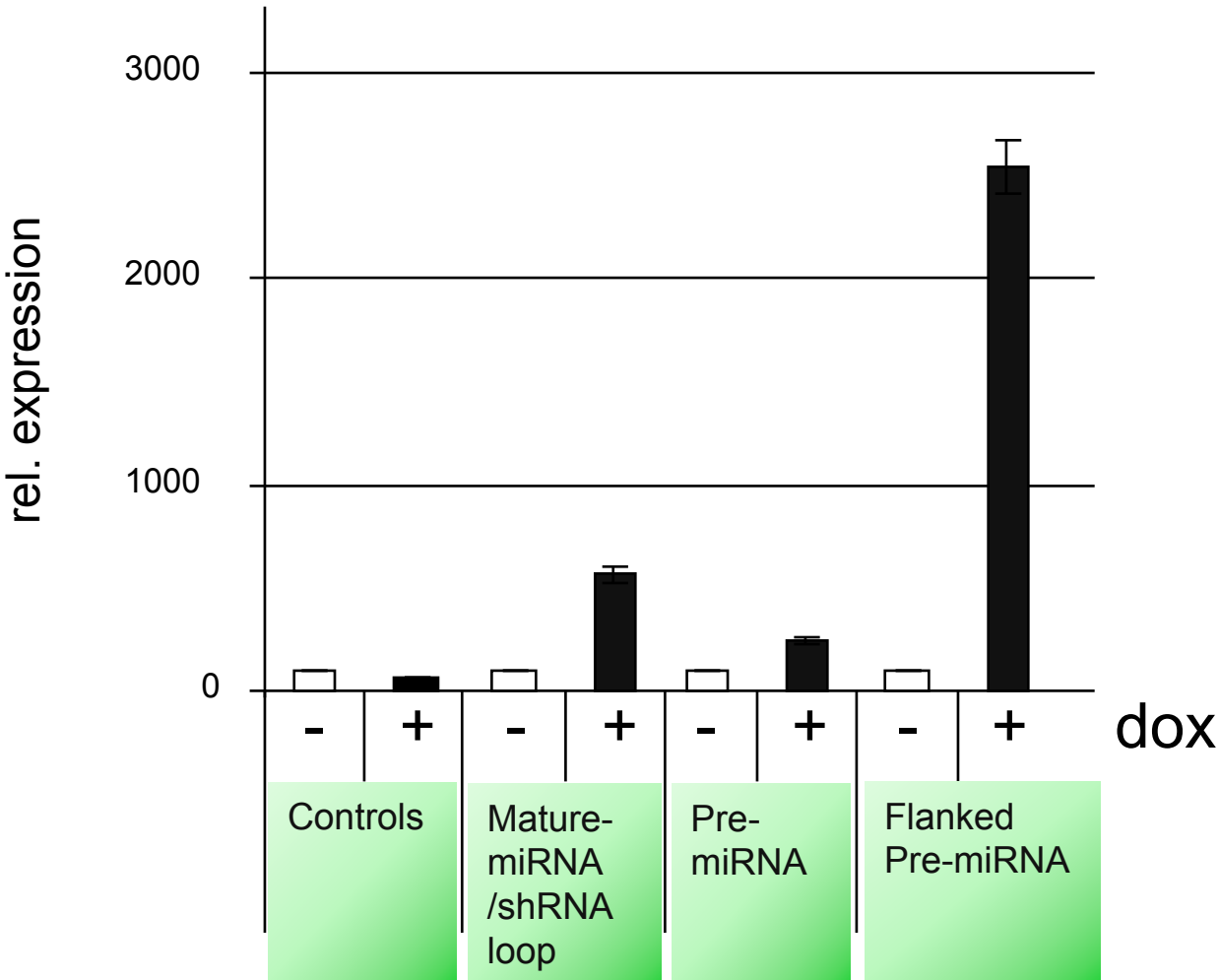




# miRNA expression in ESC and mice

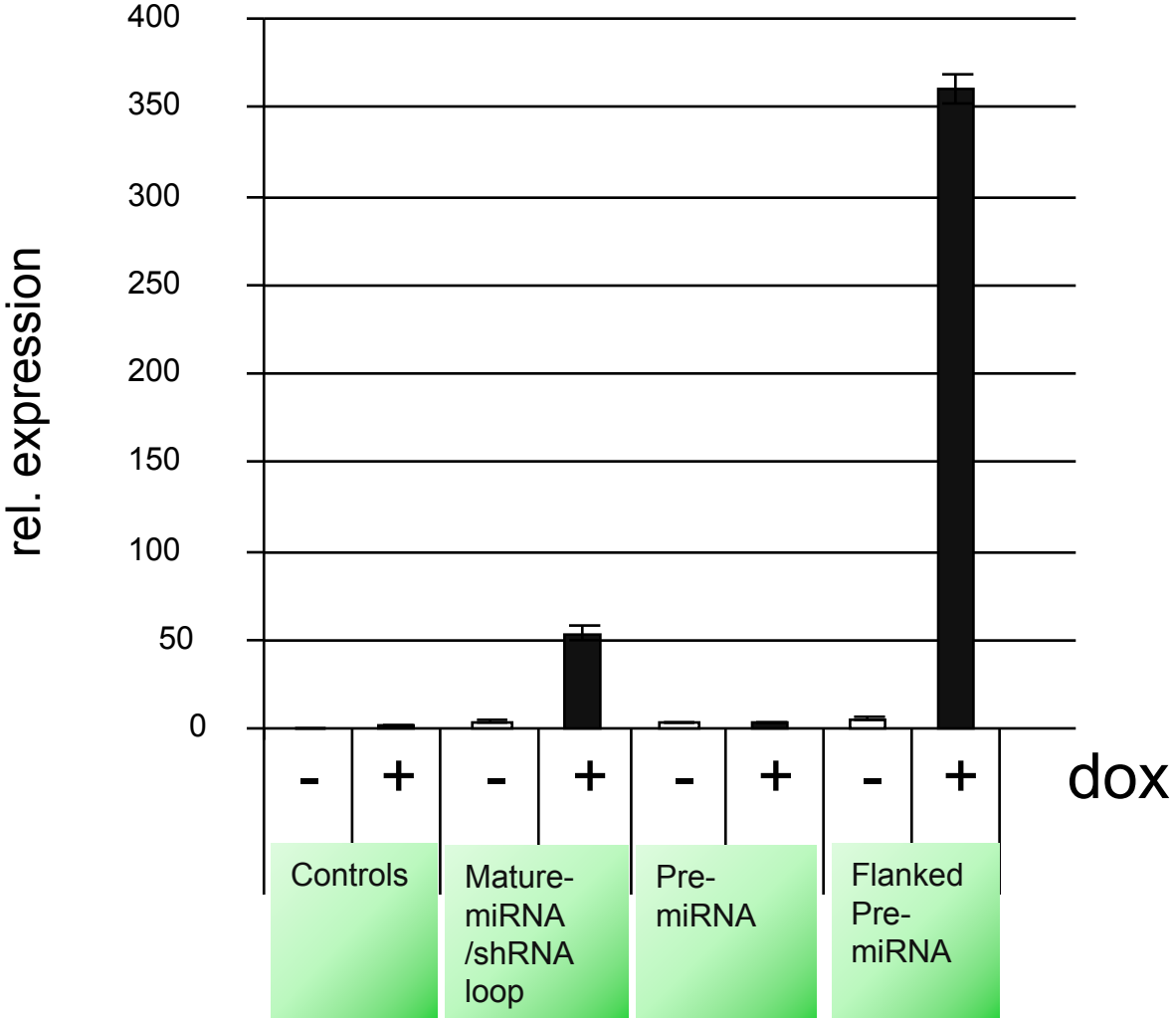


# Example #1: miR-138 in ES cells





# Example #2 in ES cells





## ARTICLES

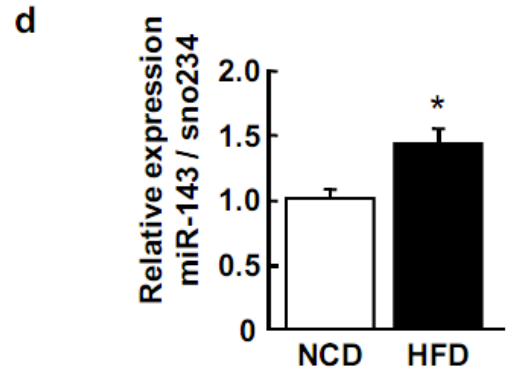
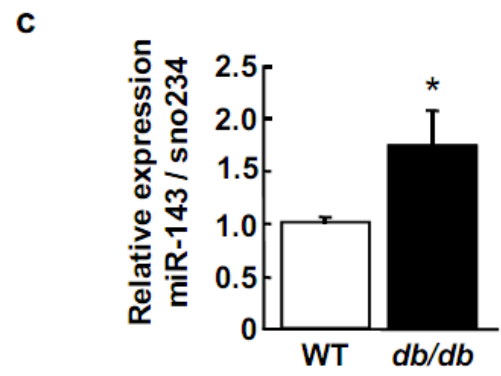
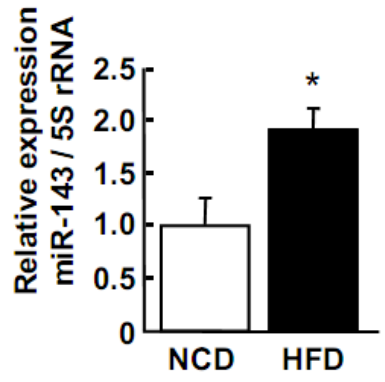
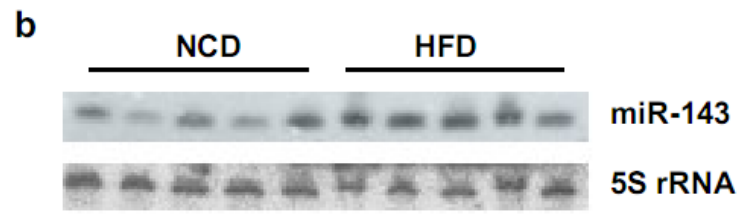
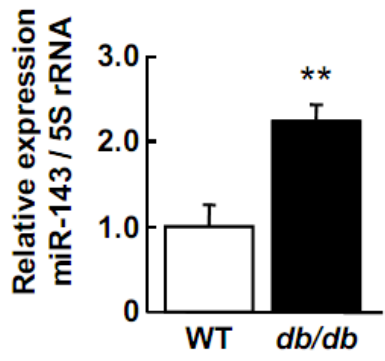
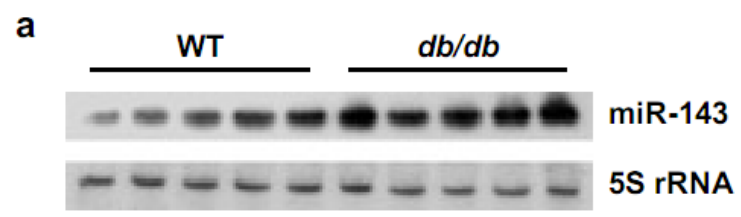
nature  
cell biology

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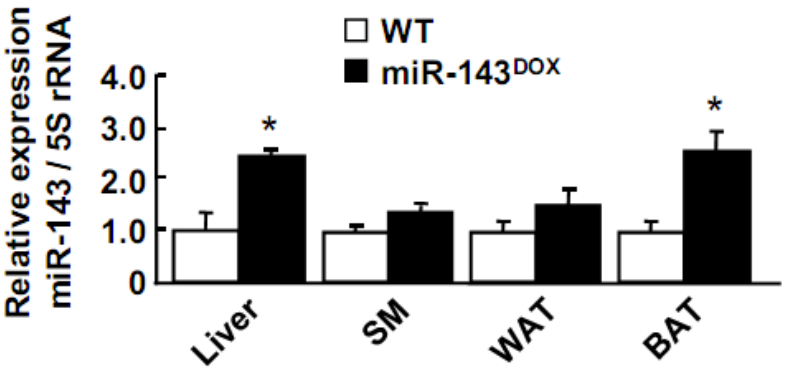
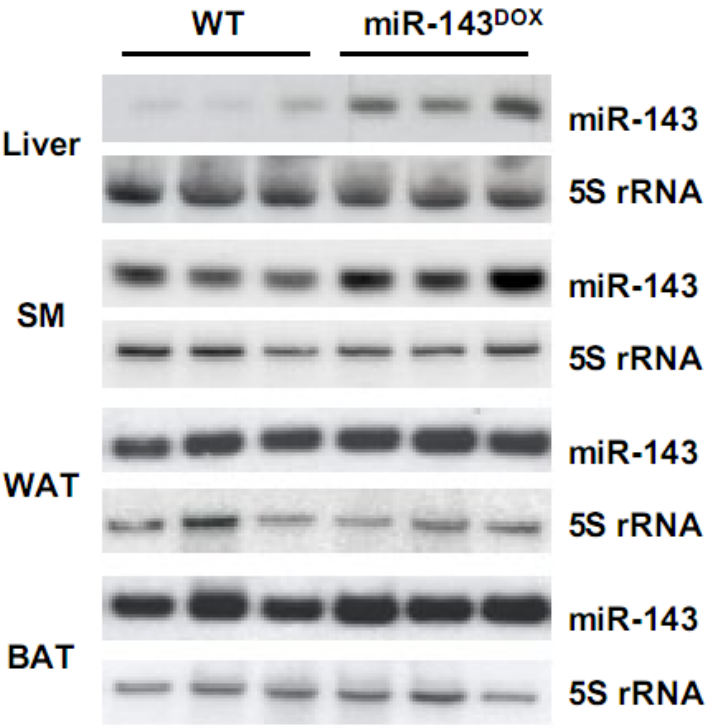
# Obesity-induced overexpression of miRNA-143 inhibits insulin-stimulated AKT activation and impairs glucose metabolism

Sabine D. Jordan<sup>1</sup>, Markus Krüger<sup>2</sup>, Diana M. Willmes<sup>1</sup>, Nora Redemann<sup>1</sup>, F. Thomas Wunderlich<sup>1</sup>, Hella S. Brönneke<sup>3</sup>, Carsten Merkwirth<sup>4</sup>, Hamid Kashkar<sup>5</sup>, Vesa M. Olkkonen<sup>6</sup>, Thomas Böttger<sup>2</sup>, Thomas Braun<sup>2</sup>, Jost Seibler<sup>7</sup> and Jens C. Brüning<sup>1,8</sup>

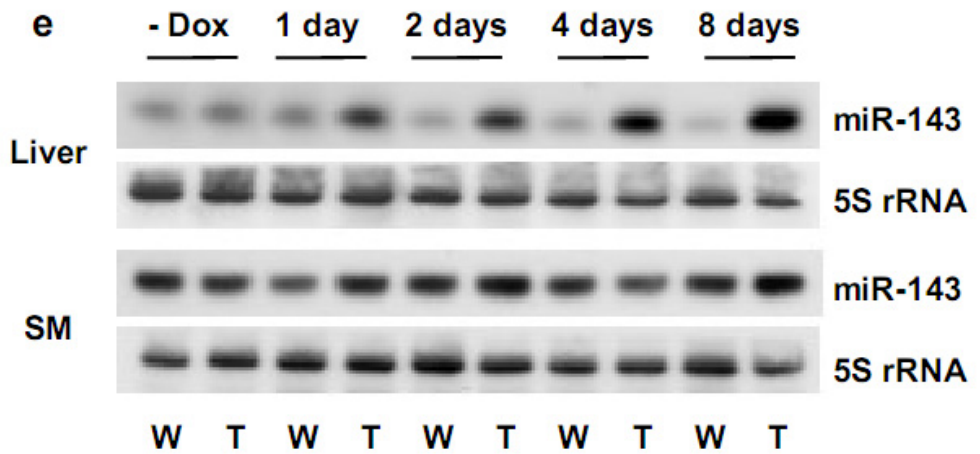
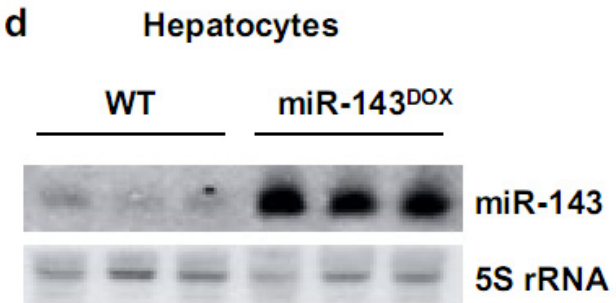
# miR-143 over expressed in liver of obesity models



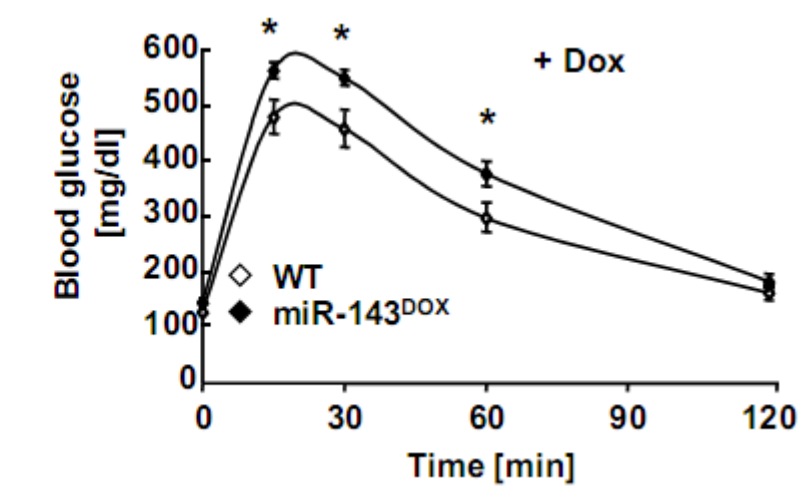
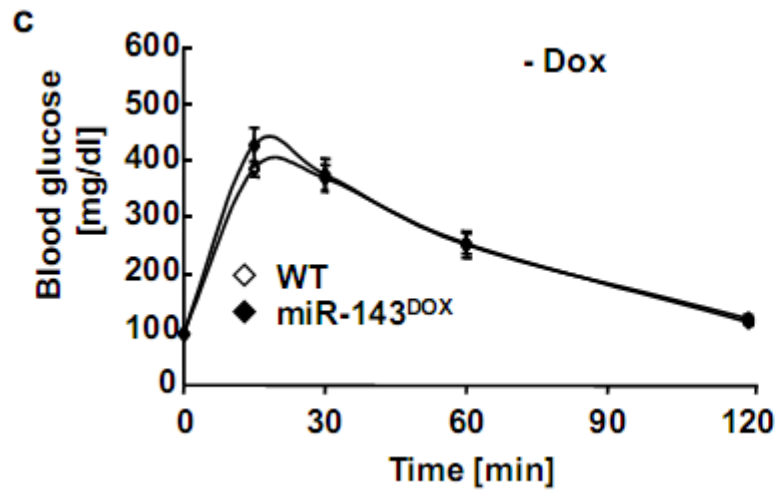
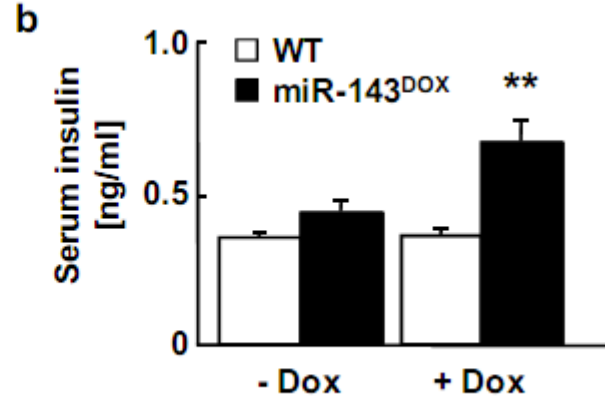
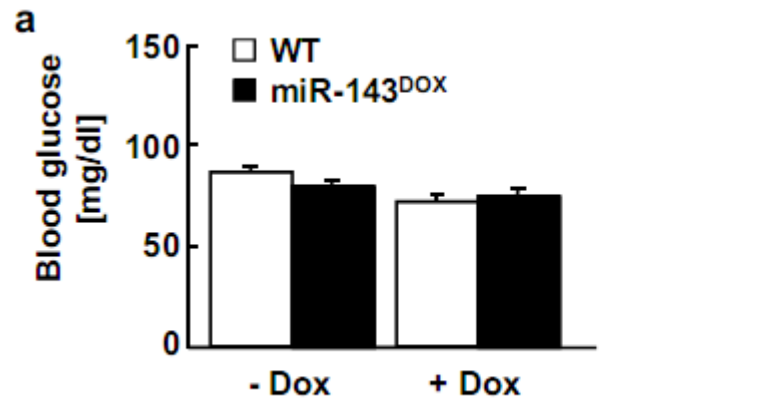
# miR-143 over expressed in transgenic mice



# miR-143 over expressed in liver

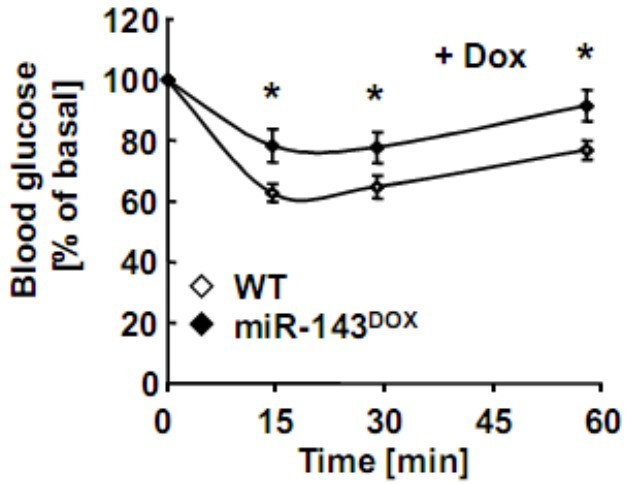
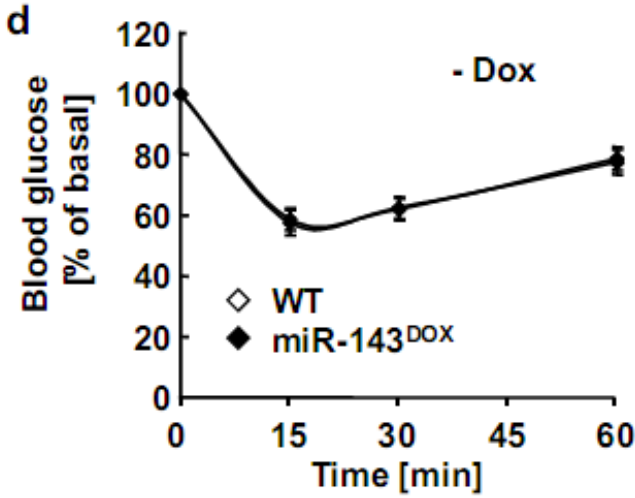


# Impaired glucose tolerance by miR-143

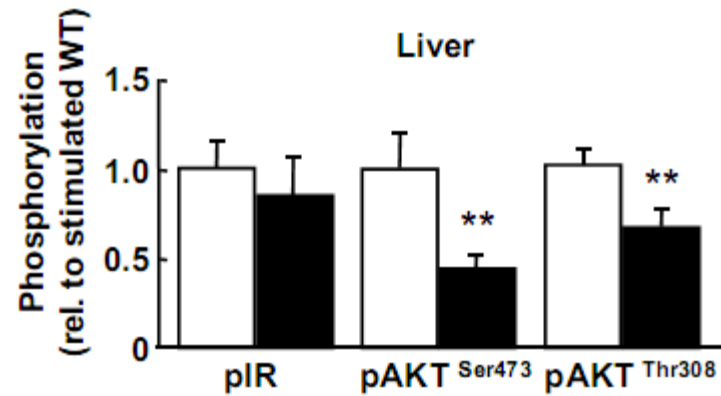
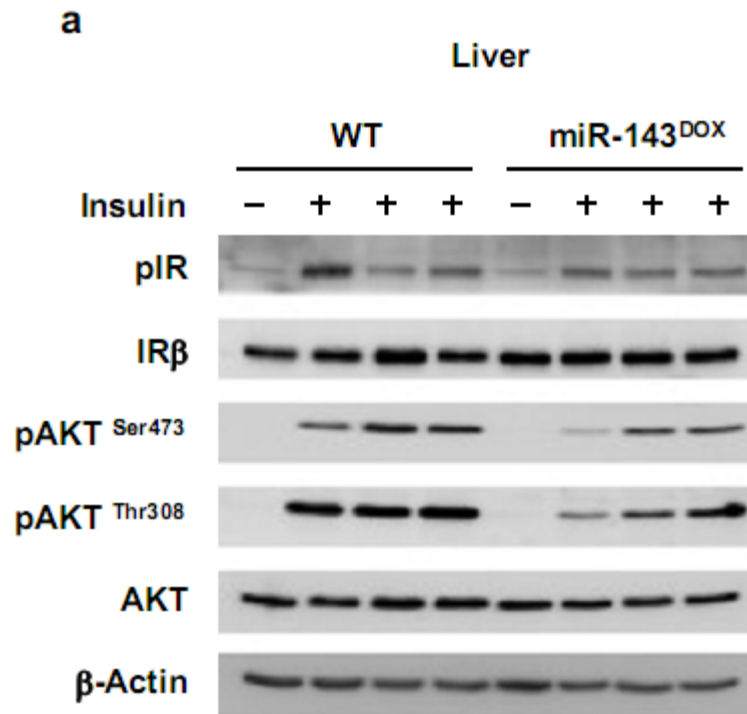




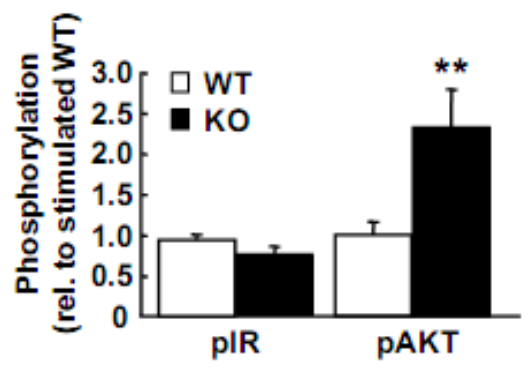
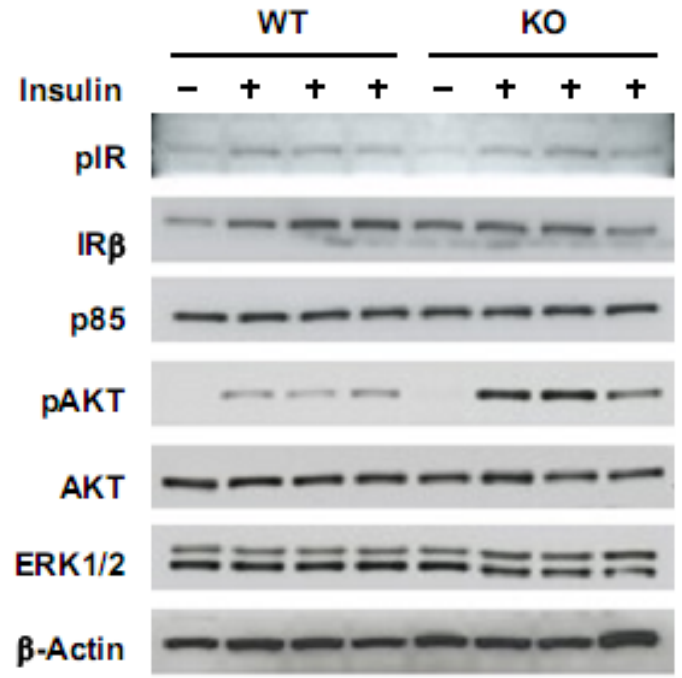
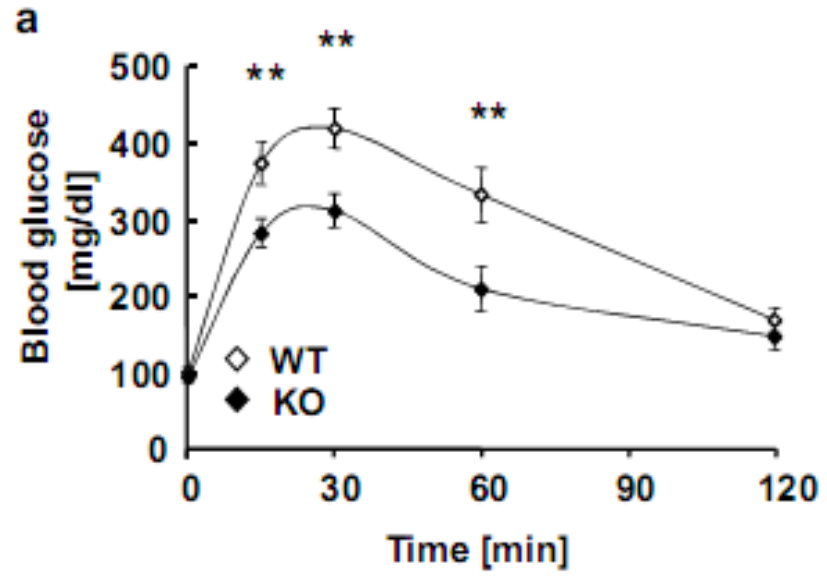
# Impaired insulin tolerance by miR-143



# miR-143 inhibits insulin-stimulated signaling at AKT phosphorylation



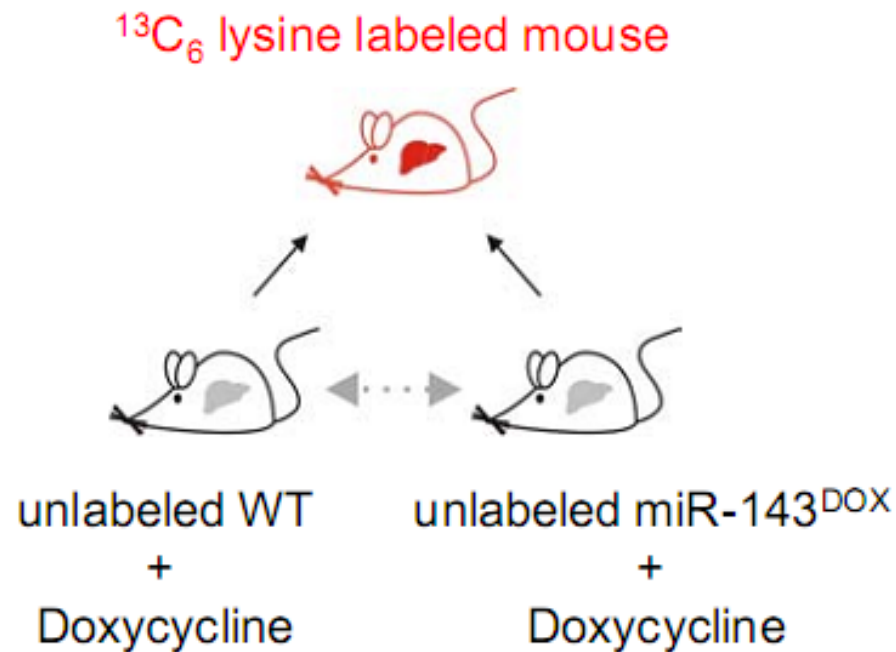
# KO of miR-143 has opposite effects



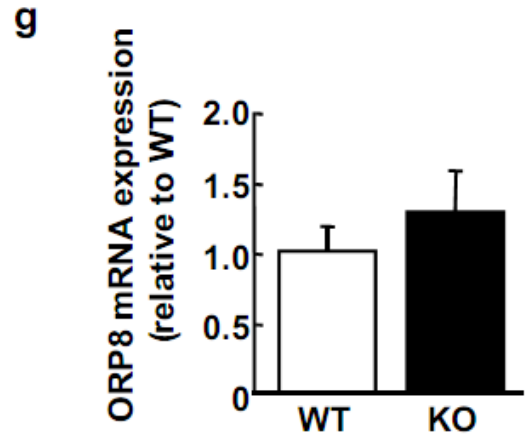
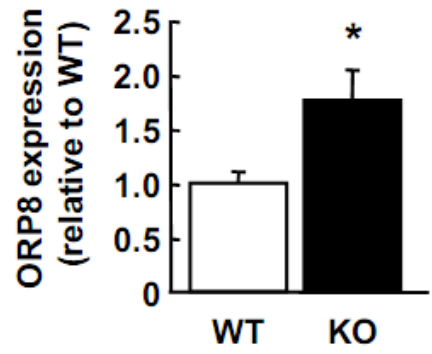
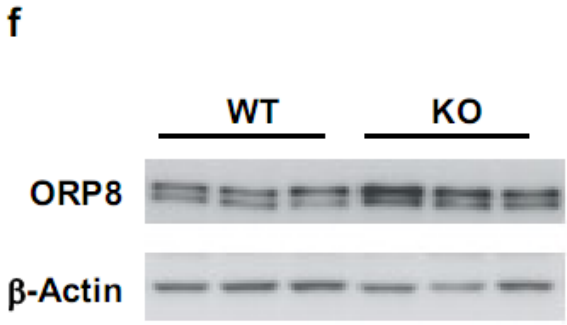
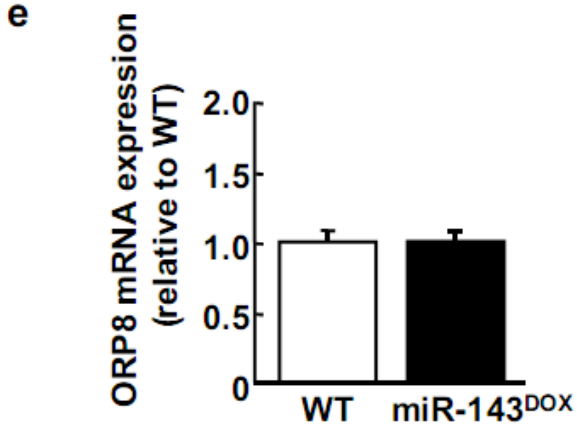
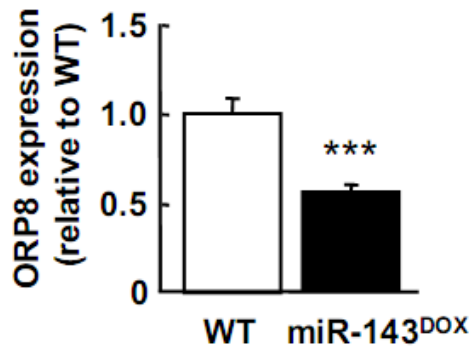
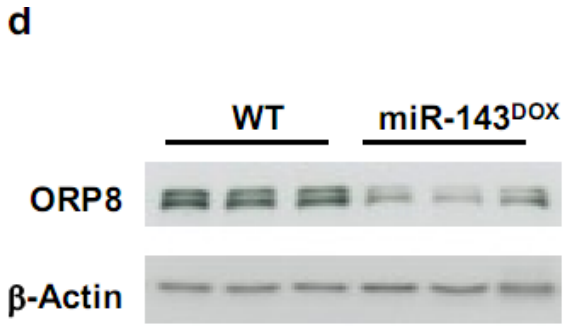
# In vivo SILAC to find miR-143 targets



Combination of inducible miR-143 overexpression with quantitative proteomics:



# Orp8 as target of miR-143





- miR-143 up regulation leads to insulin resistance
- miR-143 inhibits insulin-stimulated AKT activation
- KO of miR-143/145 cluster are protected from obesity-associated insulin resistance
- ORP8 seems to be the relevant target in liver





- Over-expression and knockout technologies allowing in depth understanding of miRNA function *in vivo*
- Modifications of the mouse genome
  - Constitutive, Tissue Specific or Inducible Knockout
  - Constitutive, Tissue Specific or Inducible Overexpression
- Next: Expression of two miRNAs in parallel
  - One transcript
  - Two independent H1-miRNA cassettes