Quantitative Proteomics Analysis Reveals Novel Targets of miR-21 in Zebrafish Embryos

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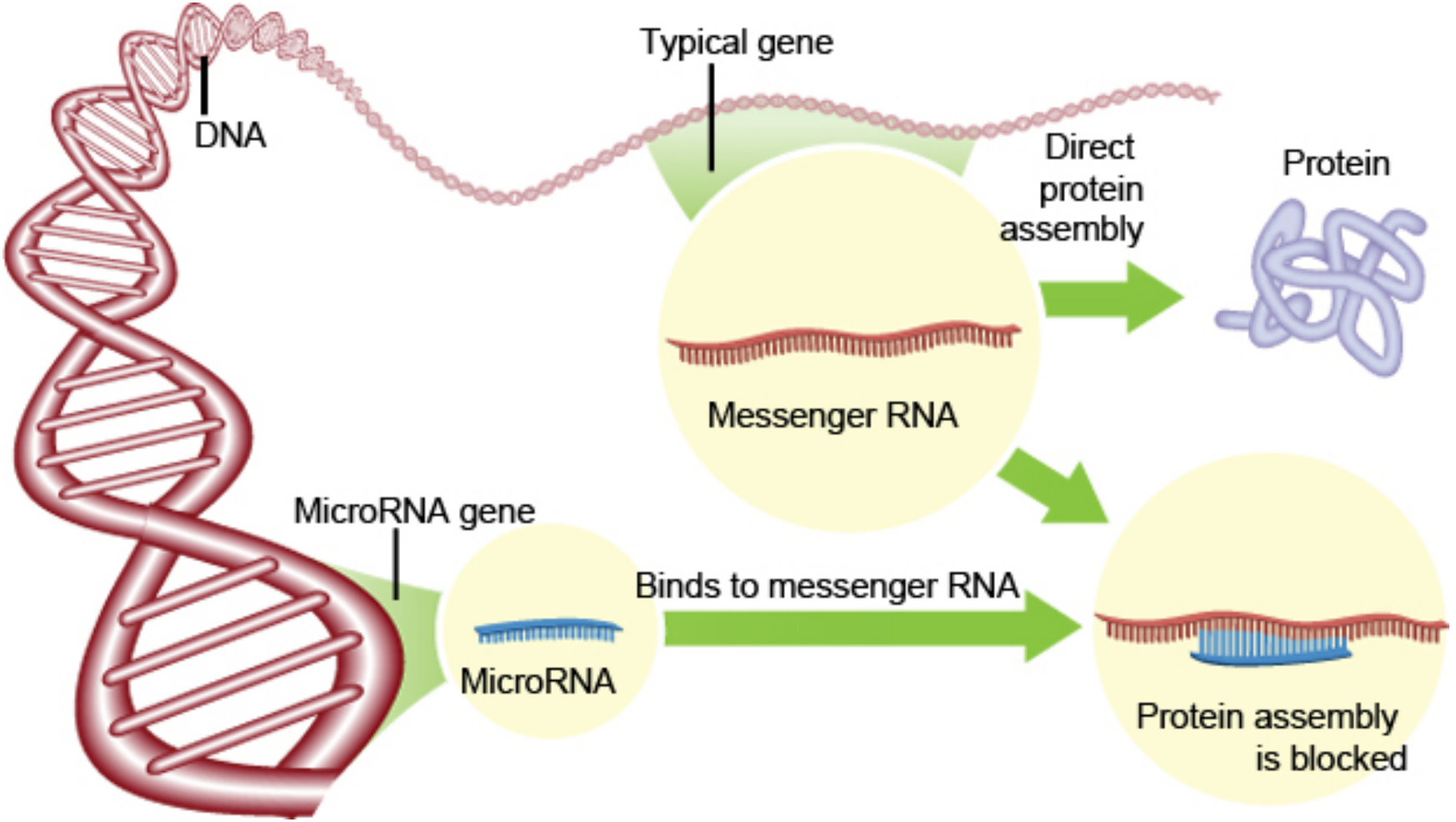
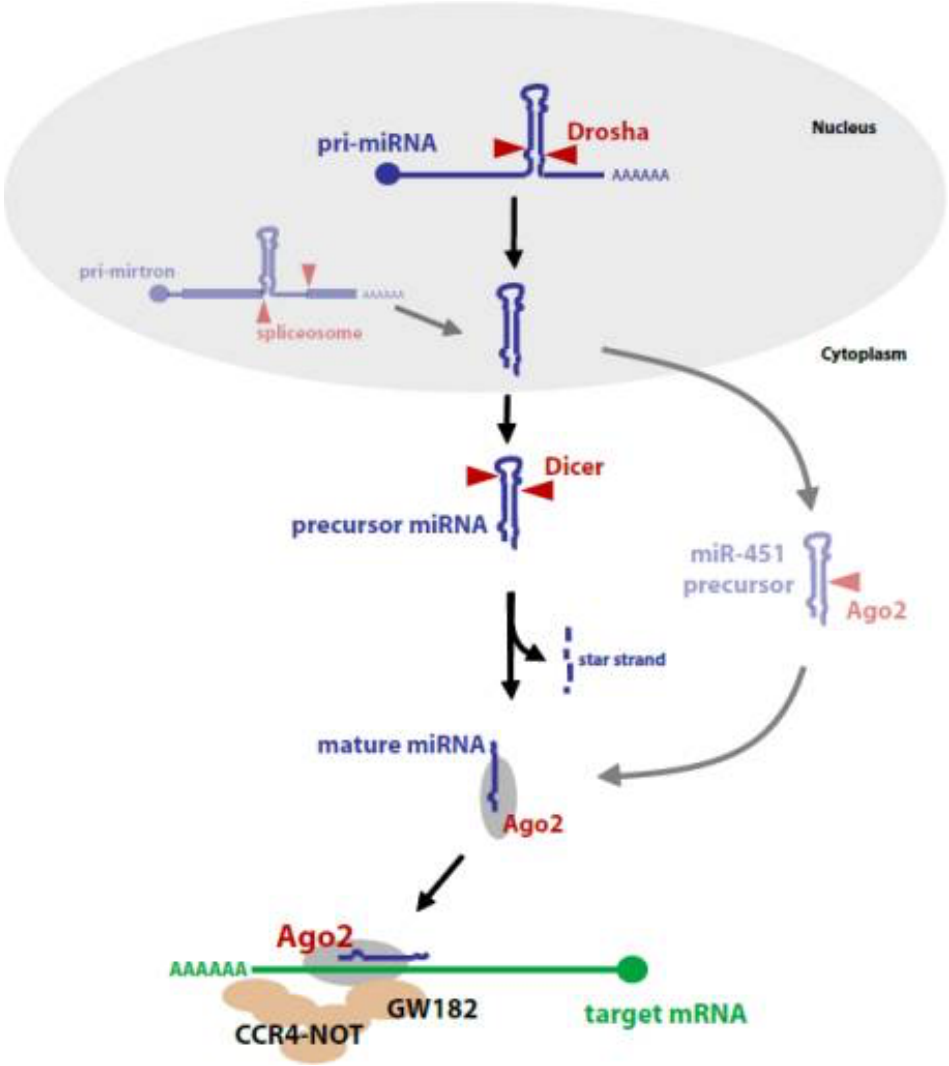
Quantitative Proteomics Analysis Reveals Novel Targets of miR-21 in Zebrafish Embryos. Sci Rep. 2017 Jun 22;7(1):4022. doi: 10.1038/s41598-017-04166-x.

What is microRNA (miRNA)?



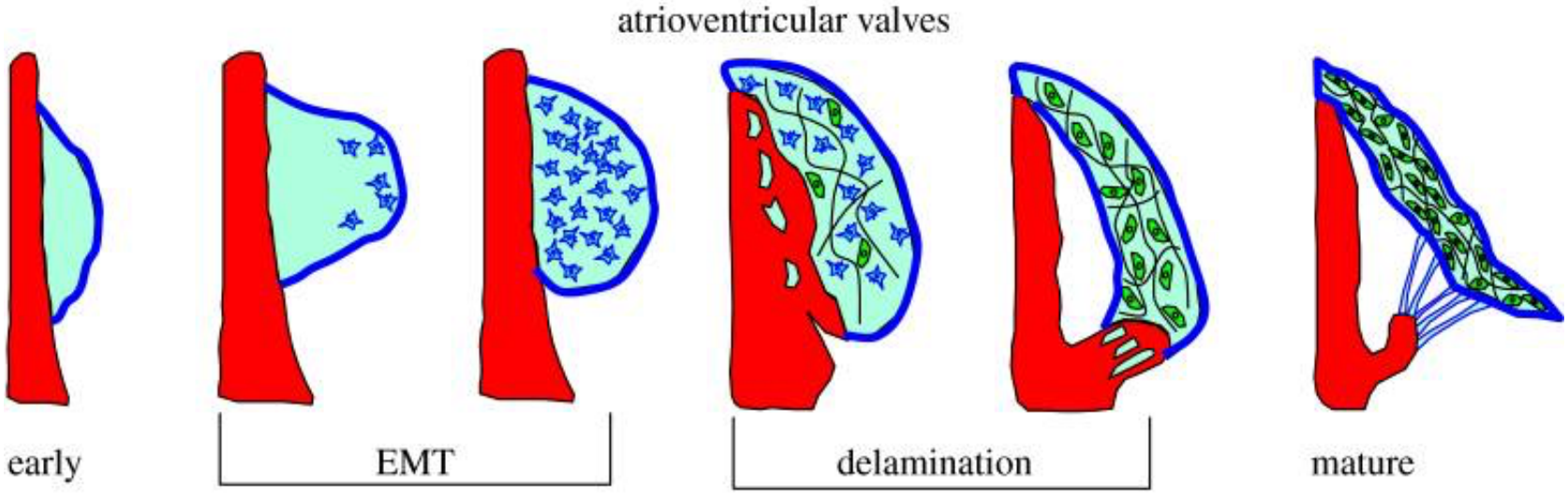
miRNAs cause suppression or degradation of target mRNAs

How are miRNAs created?



>60% of human protein coding genes can be regulated by miRNAs

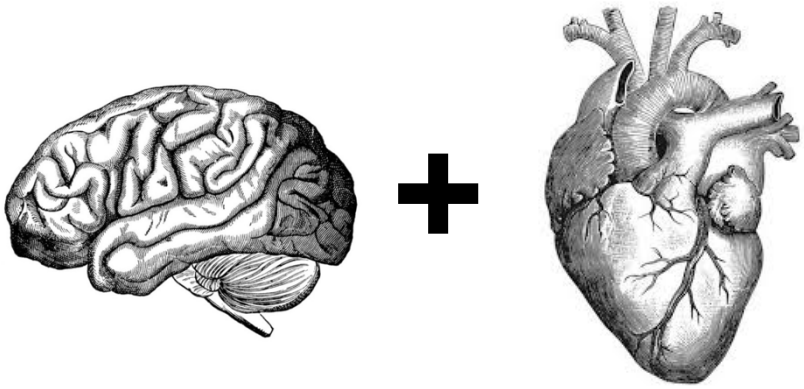
What is cardiac valvulogenesis & miR-21?



miR-21 is critical for heart valve formation and development

What is known about miR-21?

Highly expressed



Gene targets in human cancer cells

PDCD4

PTEN

TPM1

SPRY2

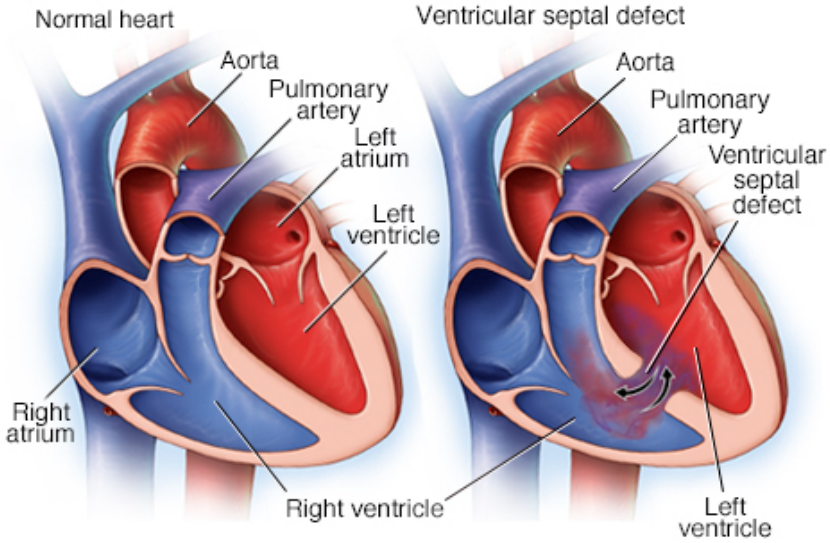
Global gene targets are unknown

Why is this all relevant to humans?

American Heart Association®

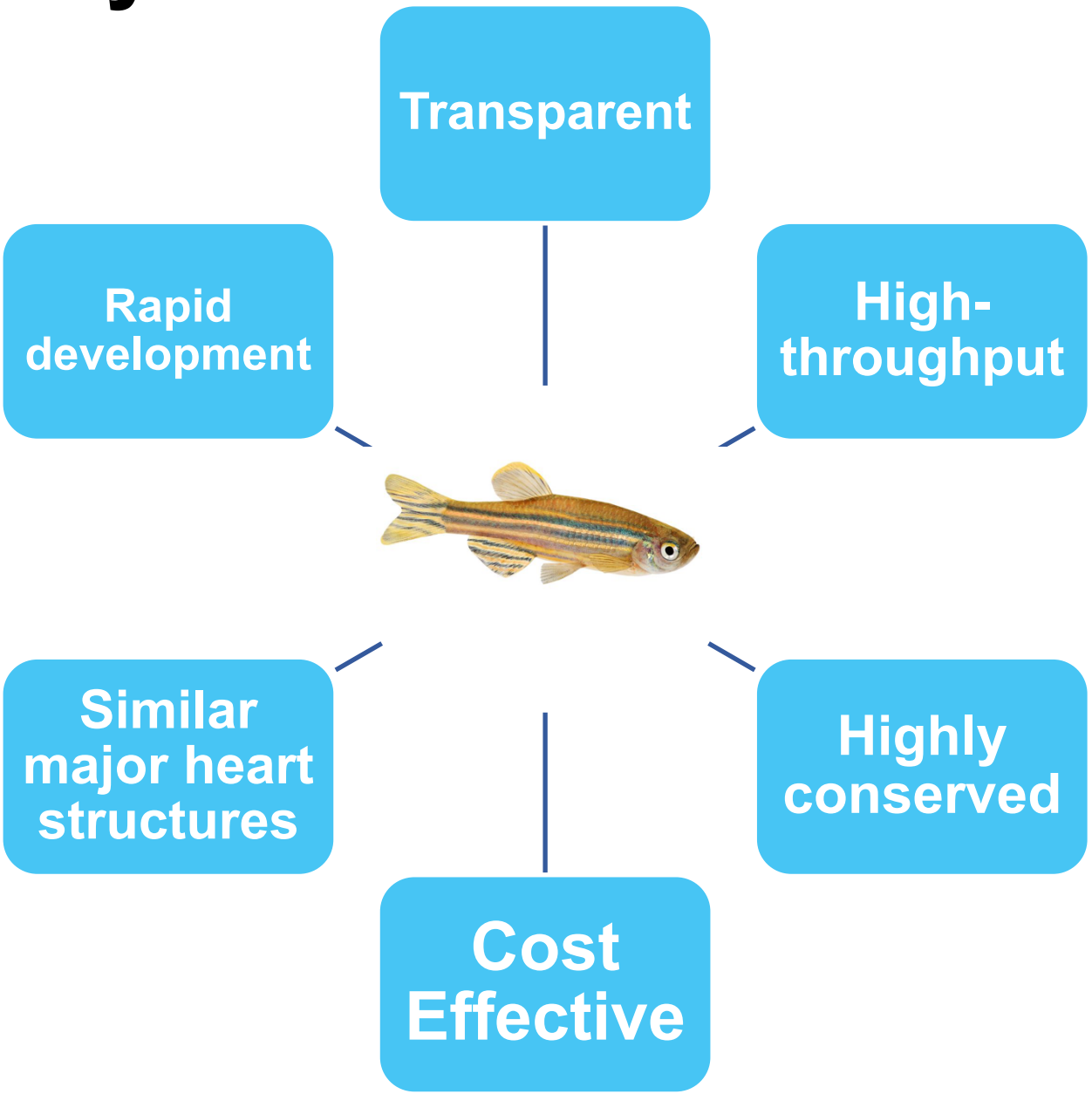
Congenital Defects - A simplified glossary

- Healthy Heart Function
- Aortic Valve Stenosis (AVS)
- Atrial Septal Defect (ASD)
- Coarctation of the Aorta (CoA)
- Complete Atrioventricular Canal defect (CAVC)
- d-Transposition of the great arteries
- Ebstein's Anomaly
- l-transposition of the great arteries
- Patent Ductus Arteriosis (PDA)
- Pulmonary Valve Stenosis
- Single Ventricle Defects
- Tetralogy of Fallot
- Total Anomalous Pulmonary Venous Connection (TAPVC)
- Truncus Arteriosus
- Ventricular Septal Defect (VSD)



Congenital heart diseases are the most common human birth defect

Why were zebrafish used?



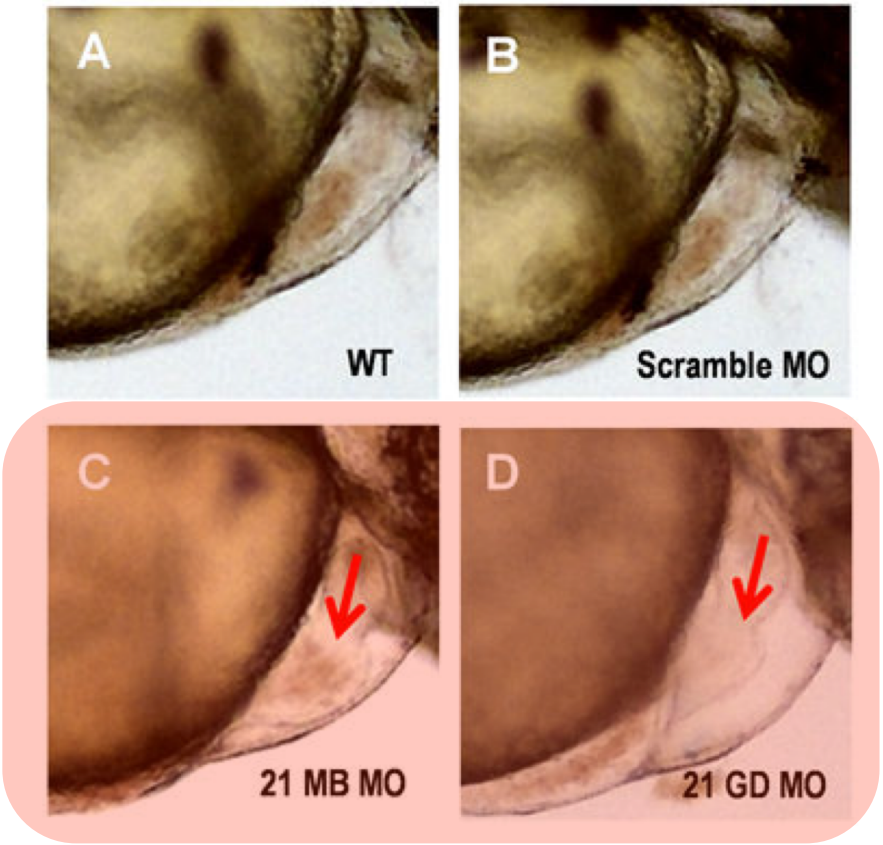
How was TMT proteomics used?

TMT identified a global profile of miR-21 regulated proteins

Why use proteomics instead of genomics?

What were the overall objectives of this study?

Fig. 1: What was the phenotypic effect of miR-21 knockout?



miR-21 knockout resulted in abnormal loss of atrioventricular valve constriction

Fig. 2A: How were miR-21 regulated proteins identified?

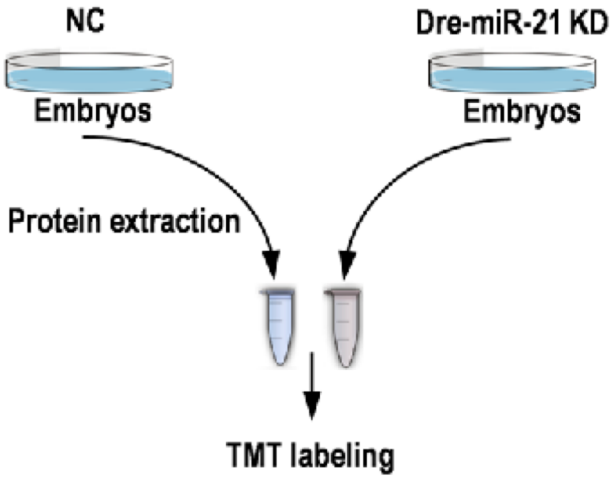
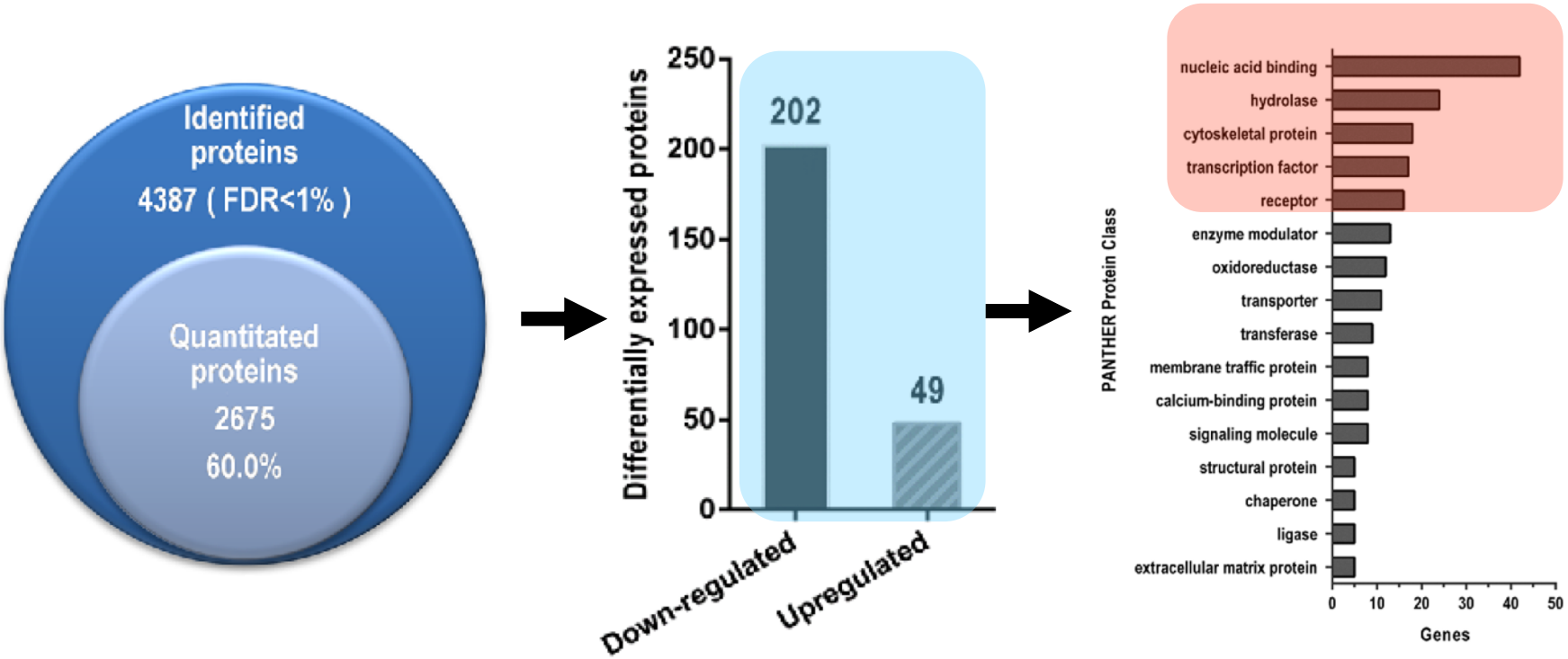
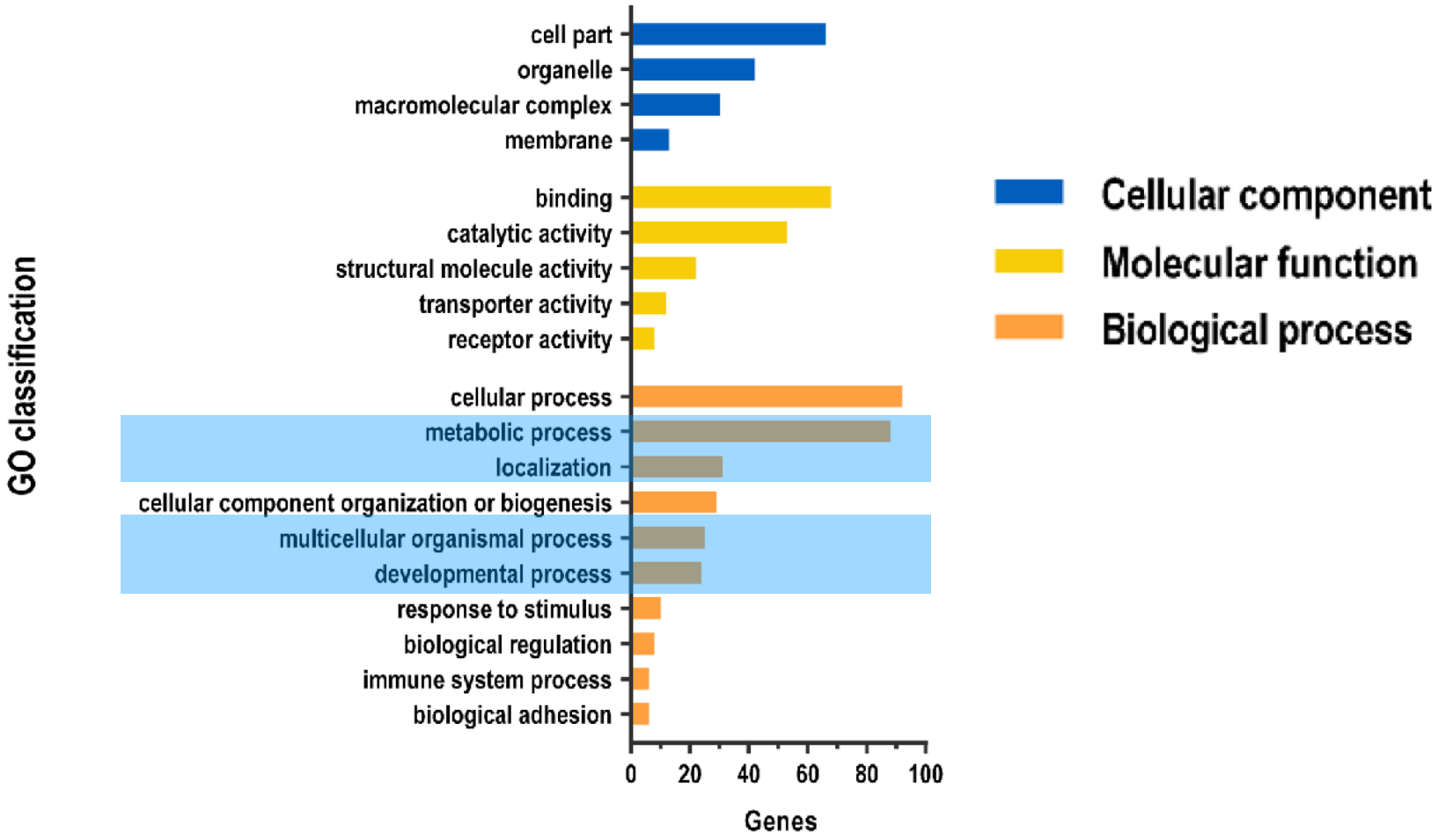


Fig. 2B & 2C: What proteins does miR-21 regulate?



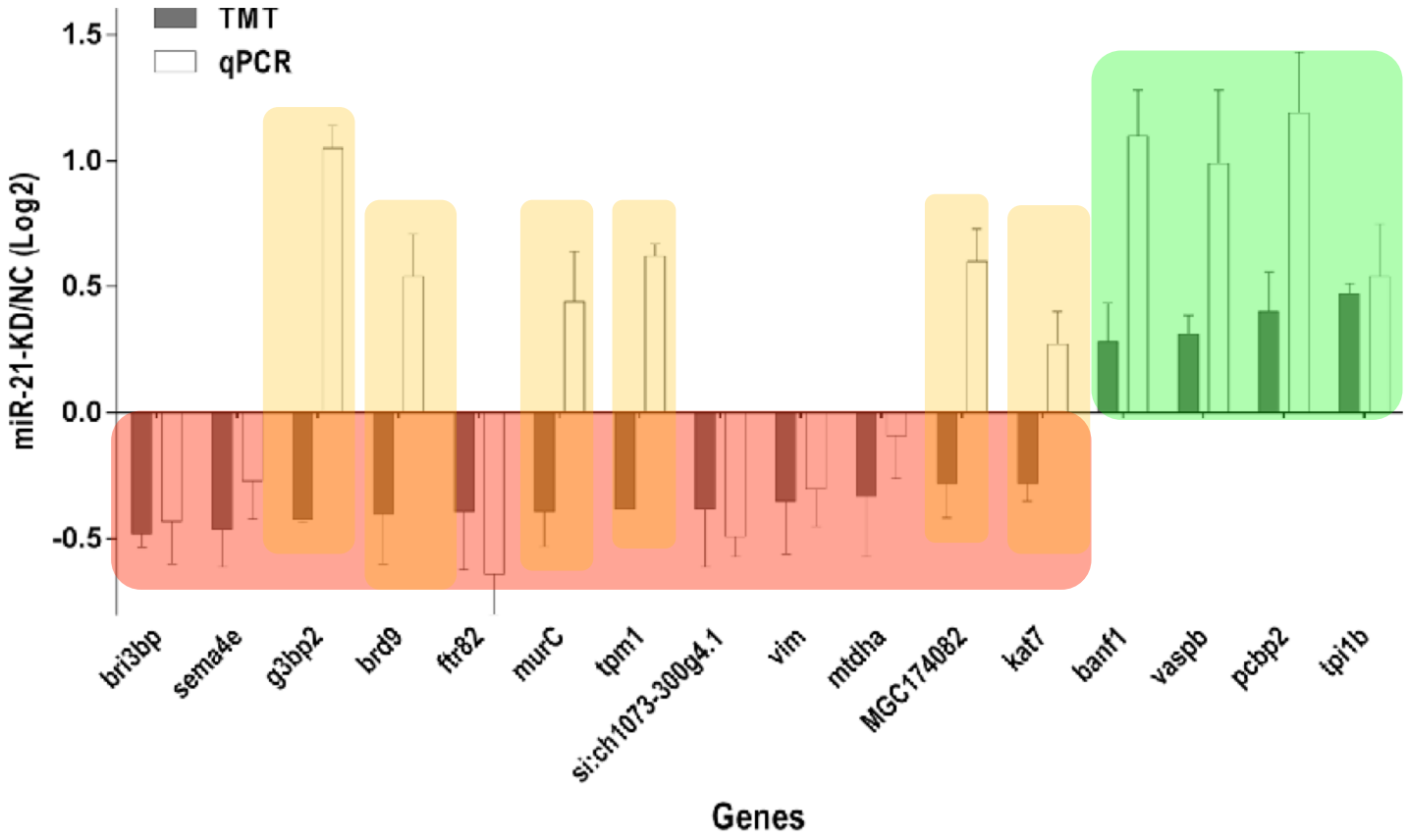
251 proteins of various functions were dysregulated after miR-21 knockout

Fig. 3: What is the GO of miR-21 regulated proteins?



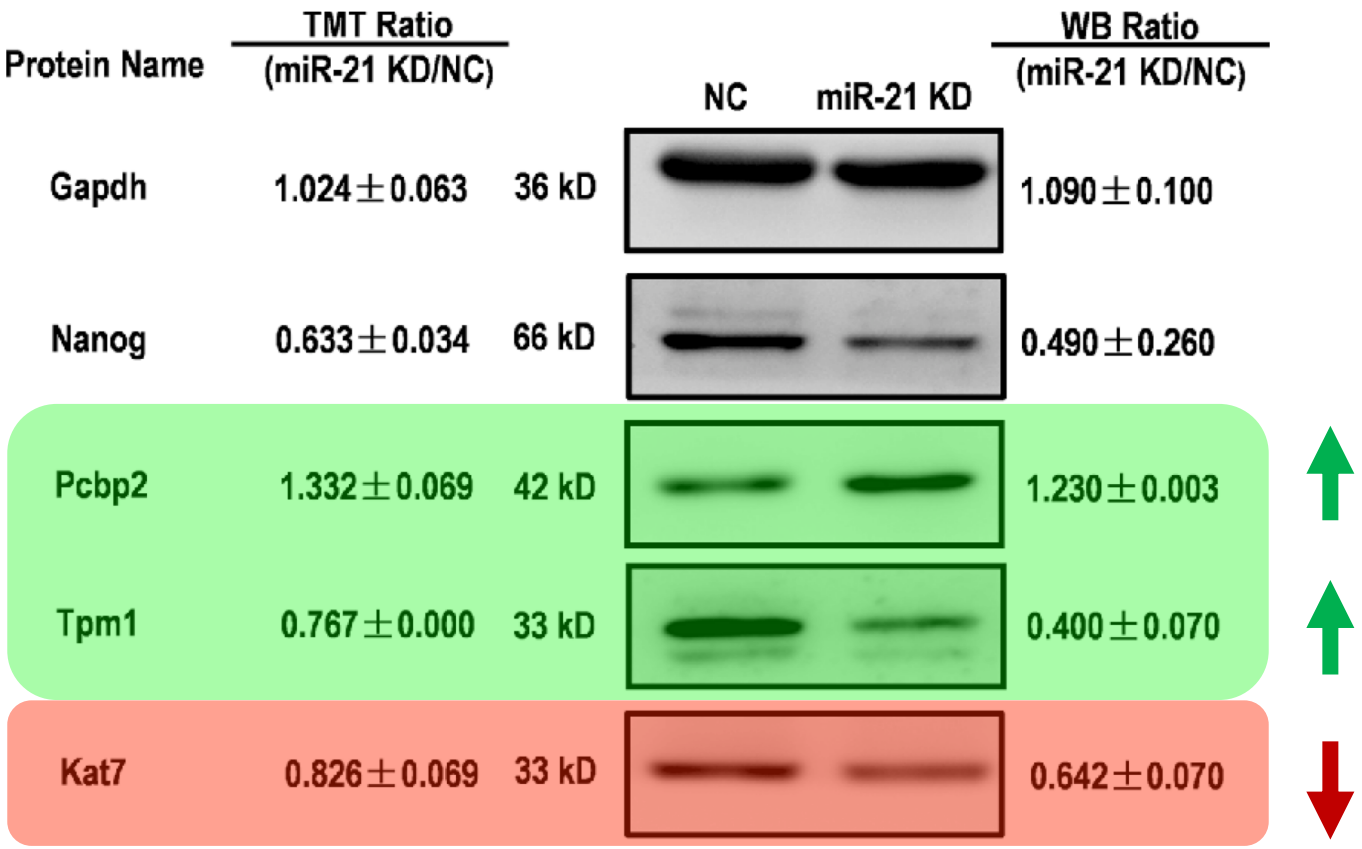
miR-21 regulates diverse functions across widespread biological processes

Fig. 4: What is the mRNA to protein ratio of the identified DEPs?



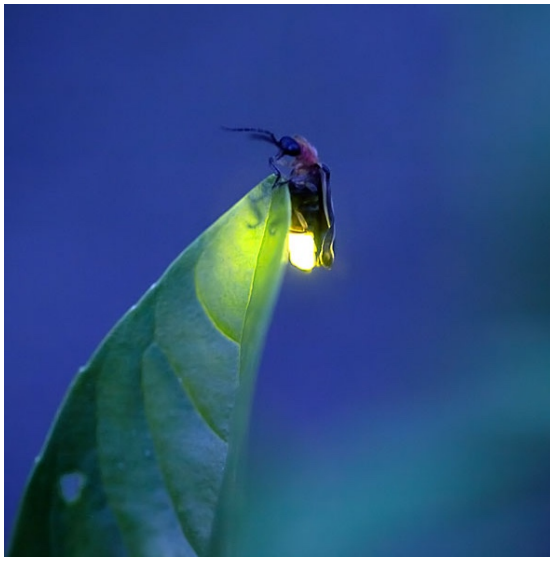
49 genes upregulated and 202 genes downregulated after knockout

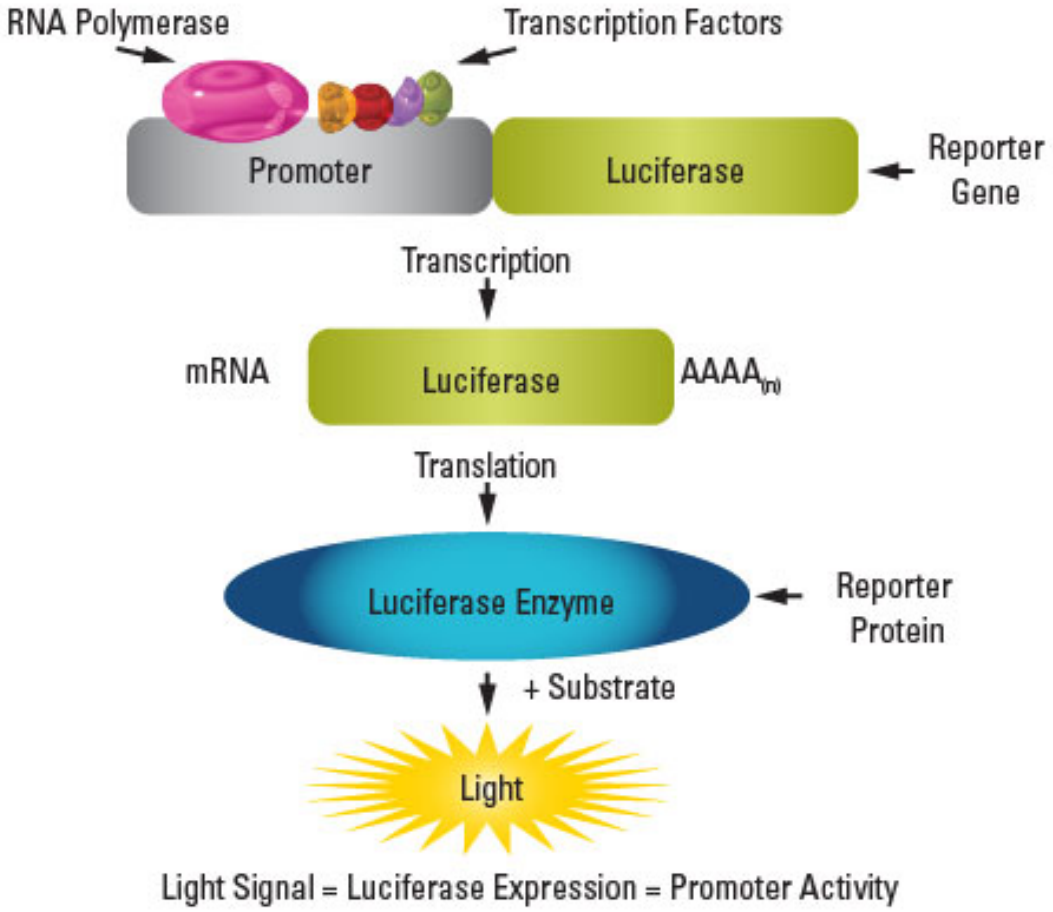
Fig. 5: Was the MS data accurate?



Western blot data confirmed upregulated and downregulated proteins

What is a luciferase reporter assay?

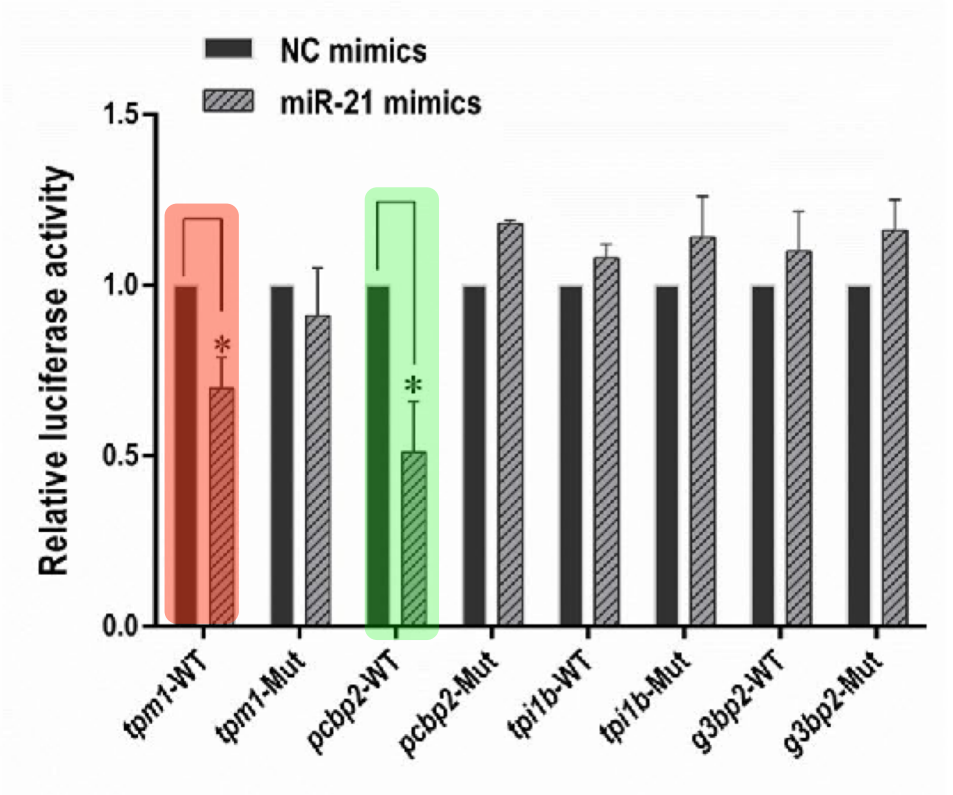




Luciferase activity directly correlates with amount of target protein

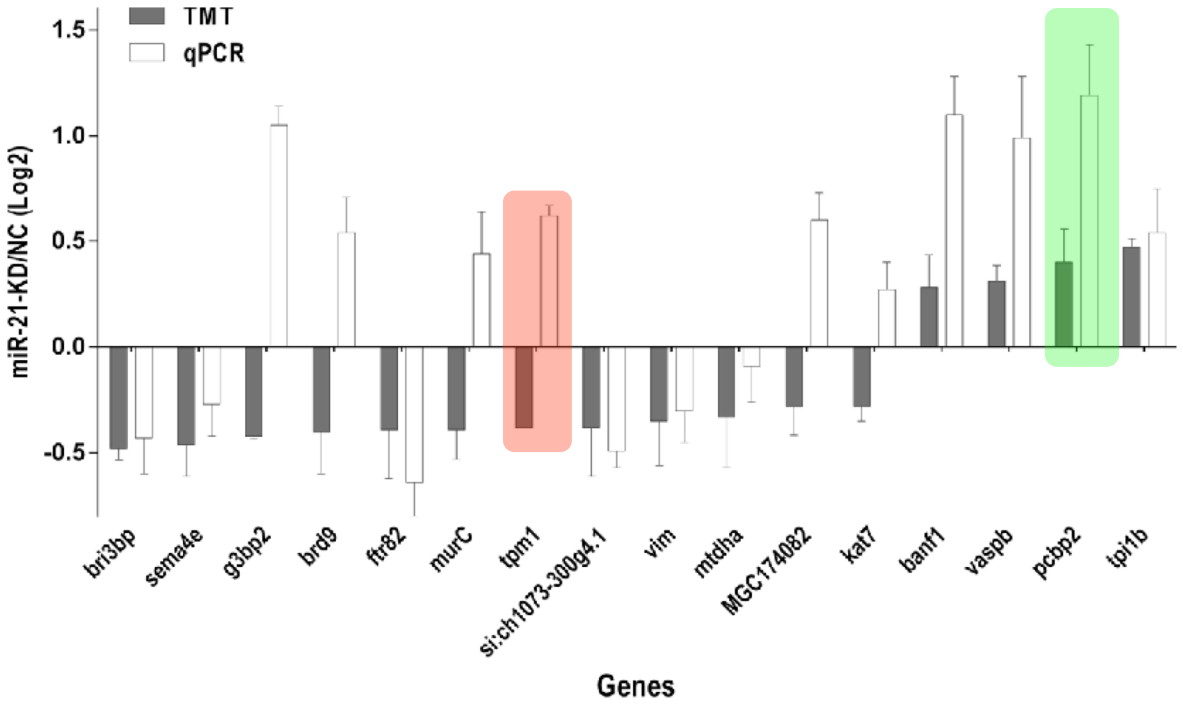
Fig. 6A & 6B:

```
3′cgguuGUGGUCAGAC-UAUUCGAu 5′  dre-miR-21
       | ||  ||   :||||||
5′atttcCTCCCATCTGTGTAAGCTc 3′  tpm1-WT
5′atttcCTCCCATCTGTGTCCUATc 3′  tpm1-Mut

3′cggUUGUGGUCAGACUA-UUCGAu 5′  dre-miR-21
     |:| :|:||||||| |||||
5′cggAGCTTCGGTCTGATCAAGCTc 3′  pcbp2-WT
5′cggAGCTTCGGTCTGATCCCUATc 3′  pcbp2-Mut

3′cggUUGUGGUCAGACUAUUCGau 5′   dre-miR-21
     |||::| | || ||||||
5′agaAACGTCTG-CTCATAAGCcc 3′   tpi1b-WT
5′agaAACGTCTG-CTCAGCCUAcc 3′   tpi1b-Mut

3′cggUUGUGGUCAGACUAUUCGAU 5′   dre-miR-21
     ||:| |   :| ||||||||
5′ctgAATAGCTCATTAATAAGCTA 3′   g3bp2-WT
5′ctgAATAGCTCATTAAGCCUATA 3′   g3bp2-Mut
```



Tpm1 **and** ***pcbp2*** **are direct, novel targets of miR-21**

What are *tpm1* and *pcbp2?*



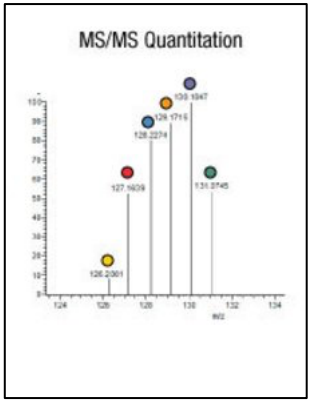
Tpm proteins

Modulate muscle contractions

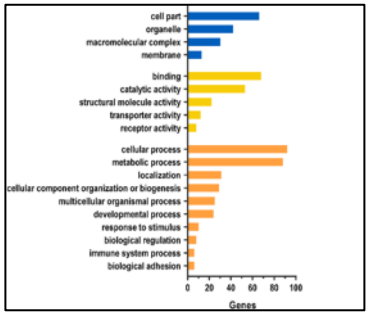
PCBP proteins

Interact with ss-poly (C) regions

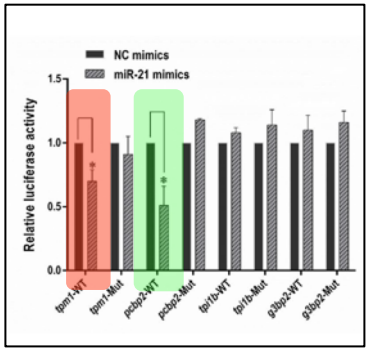
Summary



TMT mass spectrometry is a way to identify and quantify proteins

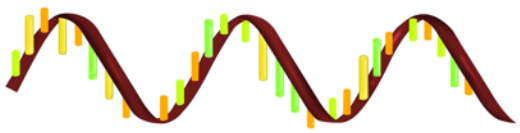


miR-21 has many targets and is involved in heart valve development

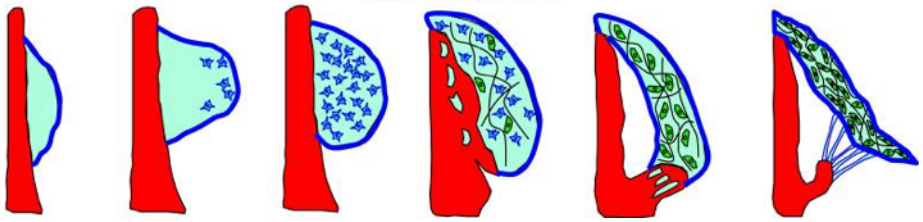


Tpm1 **and** ***pcbp2*** **are novel targets of miR-21**

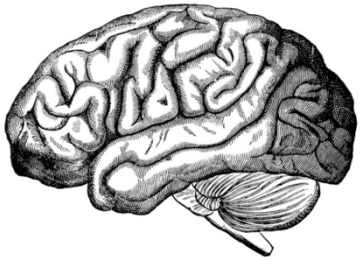
Future Directions



New mRNA targets?



Regulatory pathways affected in valvulogenesis?



Role of miR-21 in brain tissue?

Questions?

References

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Images

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