microRNA pseudo-targets

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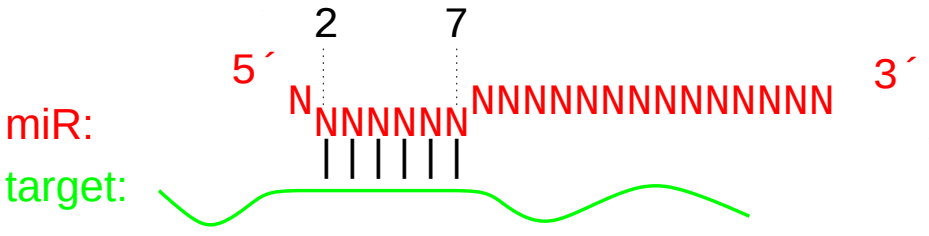
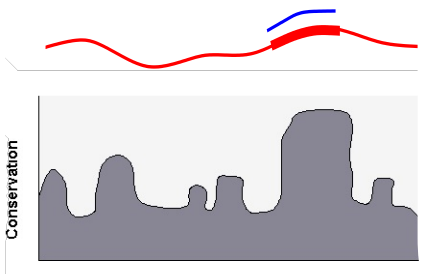
microRNA target identification



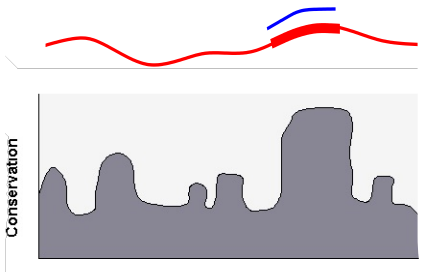
$\longrightarrow$ the “seed”

Identification of miRNA targets



Most computational programs for target prediction: search seed matches in 3´ UTRs, select the ones that were conserved in evolution.

Identification of miRNA targets



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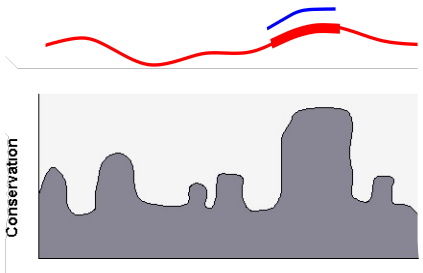
Such short matches are very frequent (60 % of human coding genes seem to be targeted: Friedman *et al.*, 2009).

Identification of miRNA targets



Most computational programs for target prediction: search seed matches in 3´ UTRs, select the ones that were conserved in evolution.

Such short matches are very frequent (60 % of human coding genes seem to be targeted: Friedman *et al.*, 2009).

$\Longrightarrow$ miRNAs are implicated in every physiological process in animals.

Paradox

miRNA-mediated repression is very modest (usually $<$ 2-fold) (Baek *et al.*, 2008, Selbach *et al.*, 2008), but biological processes are robust: they can tolerate considerable fluctuations in parameters (such as genetic variation) and still generate invariant phenotypic outputs.

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$\Longrightarrow$ how miRNAs accomplish any significant regulation (a small change in gene expression triggers a physiological effect)?

An alternative hypothesis

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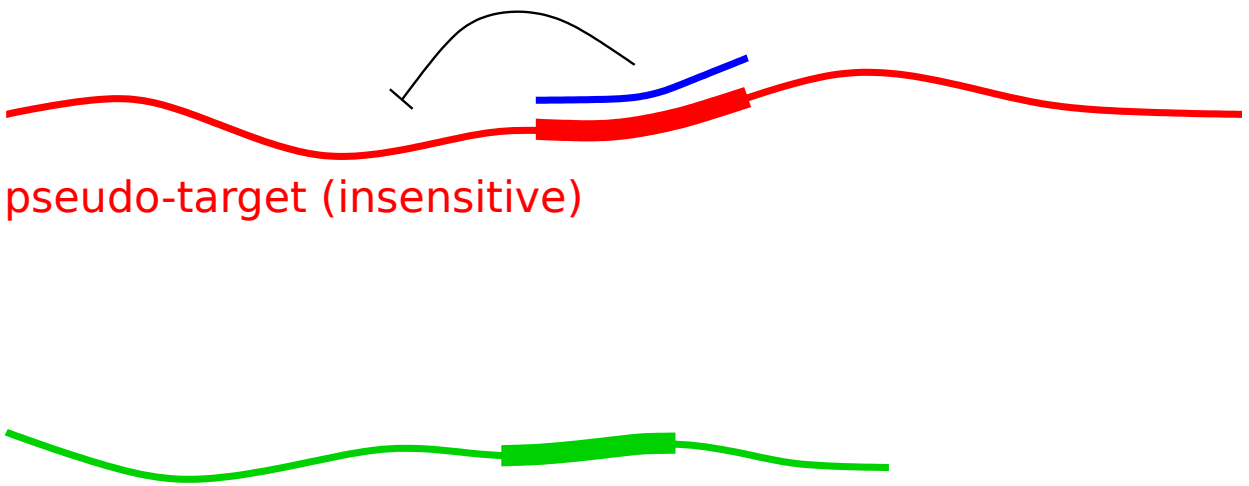
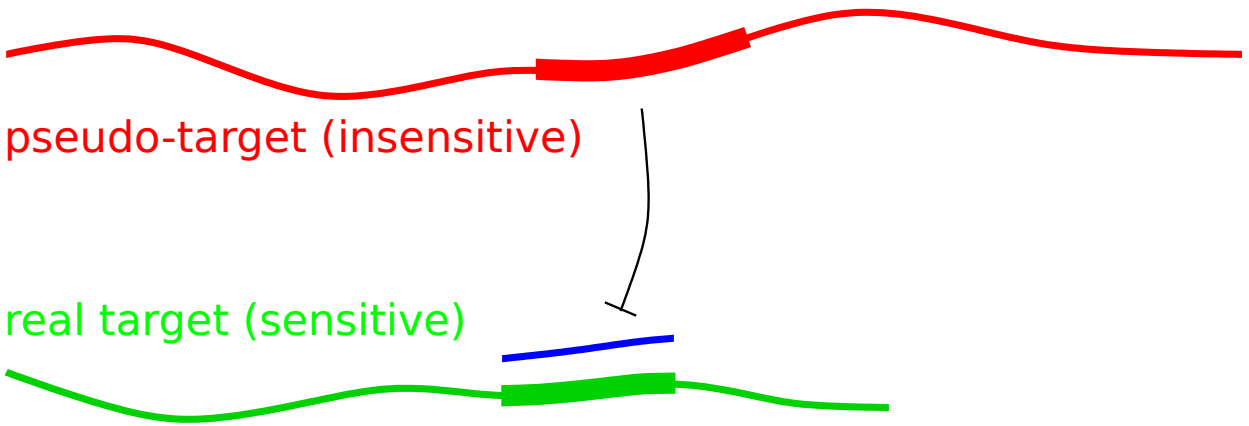
- Most computationally predicted targets are not *functionally* targeted (not repressed enough).
- Their phylogenetic conservation means that these binding sites have a function.

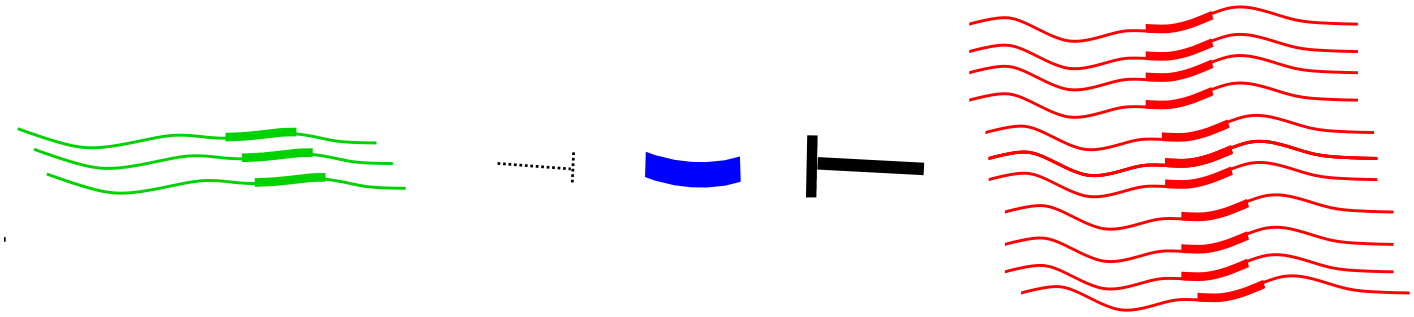


An alternative hypothesis

- Most computationally predicted targets are not *functionally* targeted (not repressed enough).
- Their phylogenetic conservation means that these binding sites have a function.
- That function could be to repress the miRNA by titrating it.



Discriminative prediction 1



According to the new hypothesis:

miRNA binding sites should be better conserved in abundantly expressed pseudo-targets

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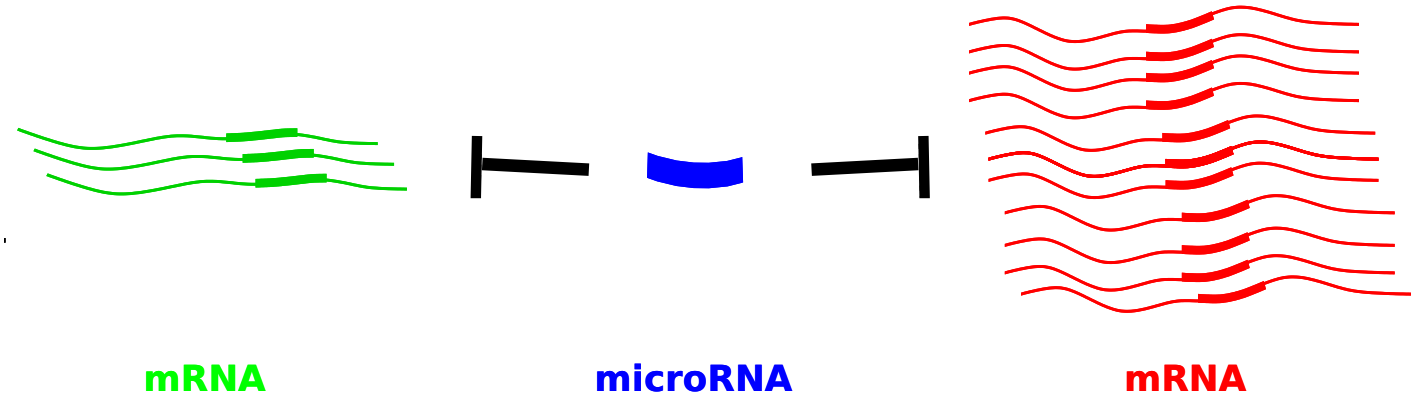

According to the new hypothesis:

miRNA binding sites should be better conserved in abundantly expressed pseudo-targets

According to the current theory:

miRNA binding sites conservation is not expected to correlate with gene expression

Are seed match conservation and mRNA abundance correlated?

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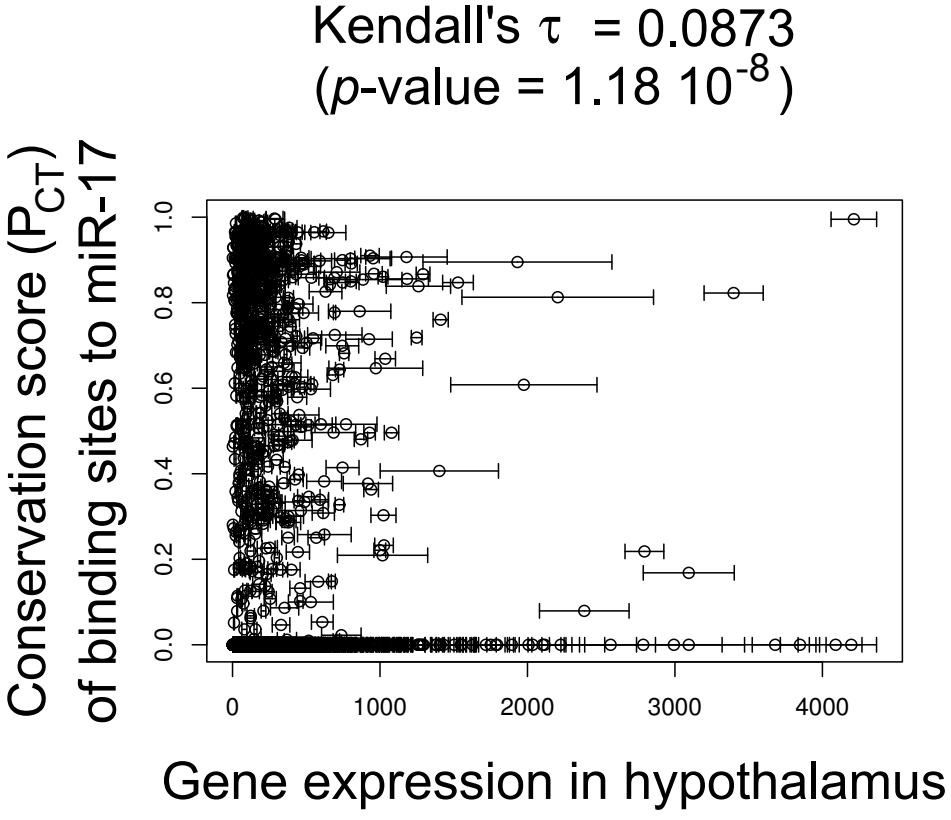
miRNA binding site conservation: measured by TargetScan's "P_{CT}" score (Friedman *et al.*, 2009).

Quantification of mRNA abundance: extracted from published microarray experiments (Mackiewicz *et al.*, 2007, Thorrez *et al.*, 2008).

Are seed match conservation and mRNA abundance correlated?



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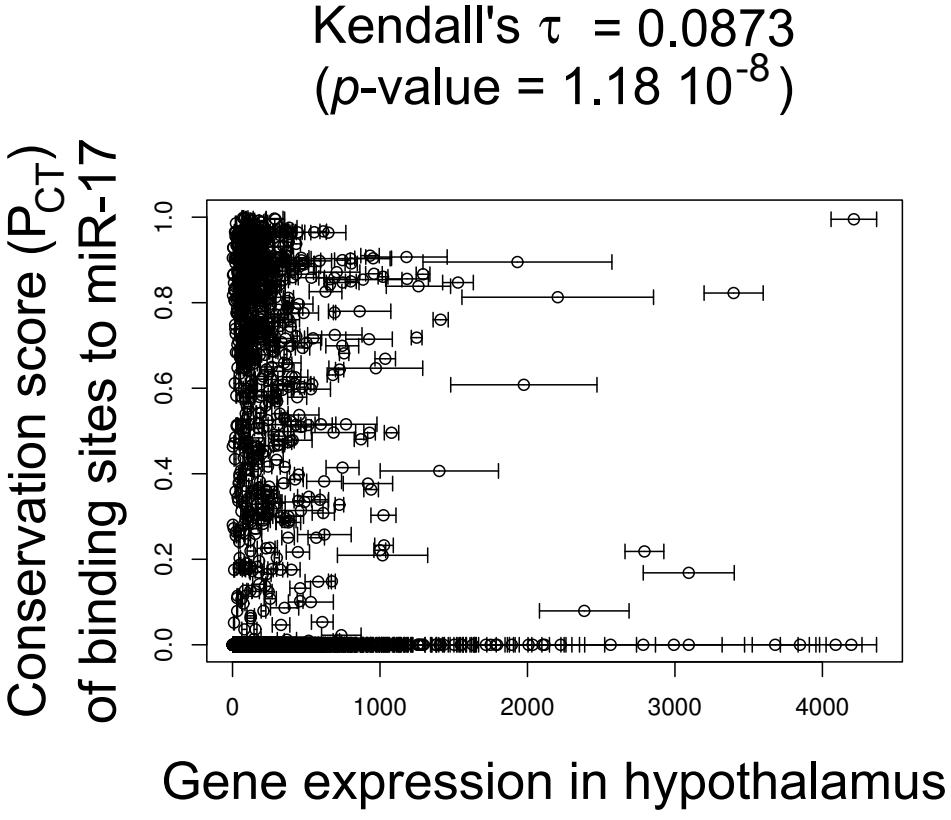

abundantly expressed miR-17 targets tend to bear highly conserved binding sites

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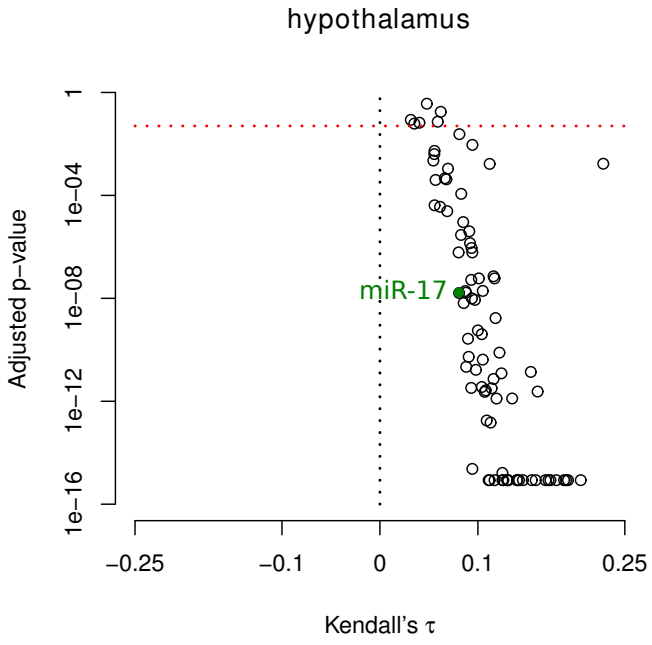

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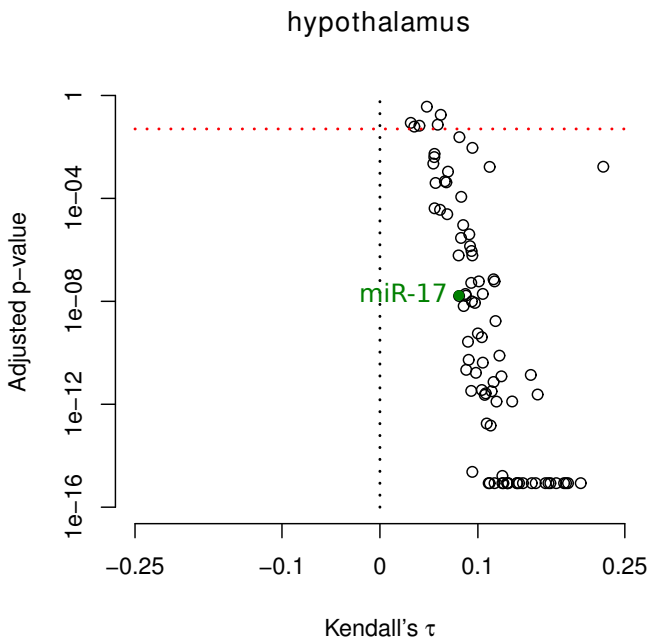

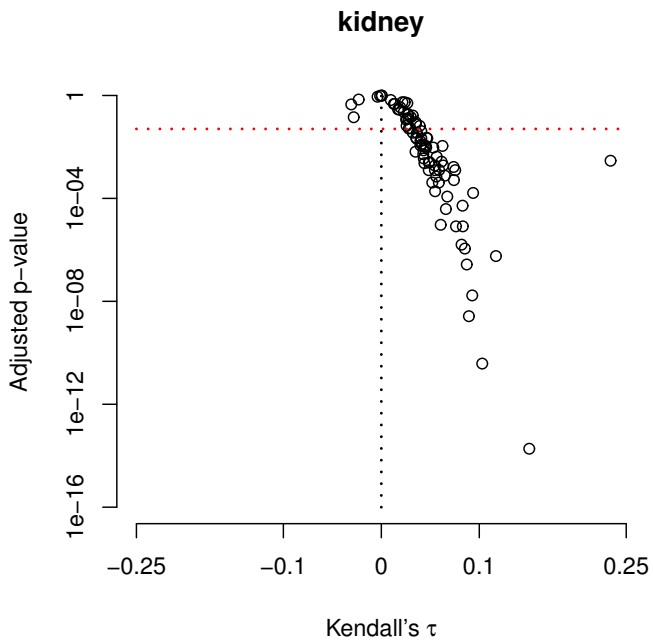

+34 other mouse tissues, same results: ▸ Supplementary data

+33 fly tissues, same results: ▸ Supplementary data

Discriminative prediction 2

According to the current theory:

miRNA targets are tightly regulated (inter-individual fluctuation should not exceed miRNA-guided repression).

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According to the new hypothesis:

Pseudo-target expression levels can fluctuate between individuals in a natural population (phenotype is robust).

Natural variability vs. miRNA-guided repression

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Baek *et al.*, 2008: quantification of miR-223-mediated repression in mouse neutrophils.

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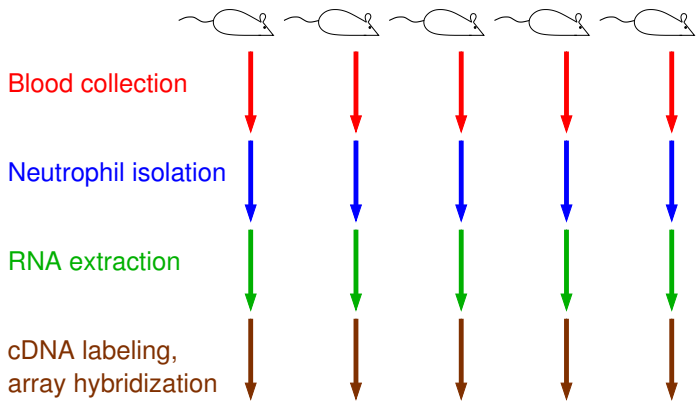

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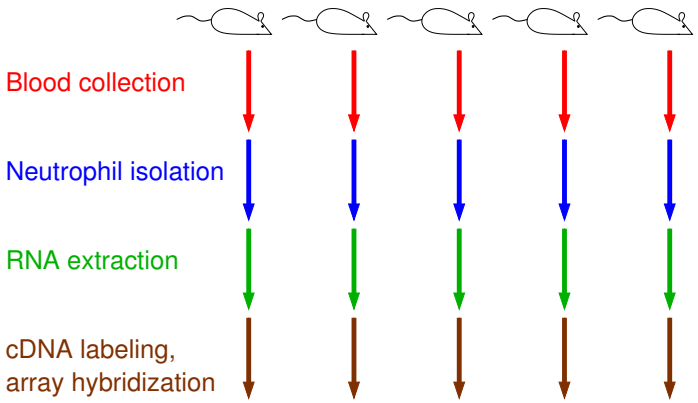
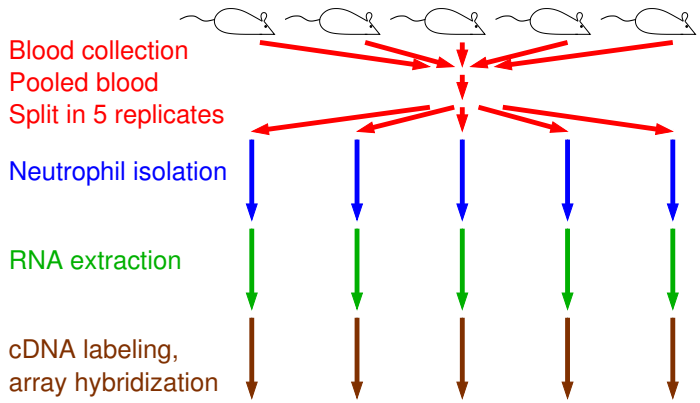
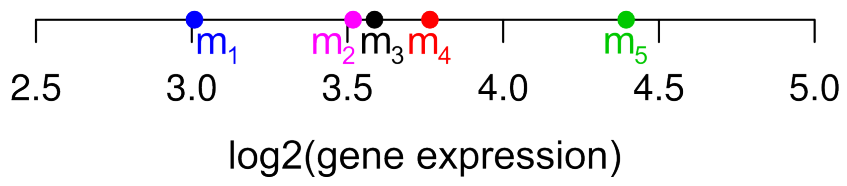




Natural variability vs. miRNA-guided repression

Gene *Styx*



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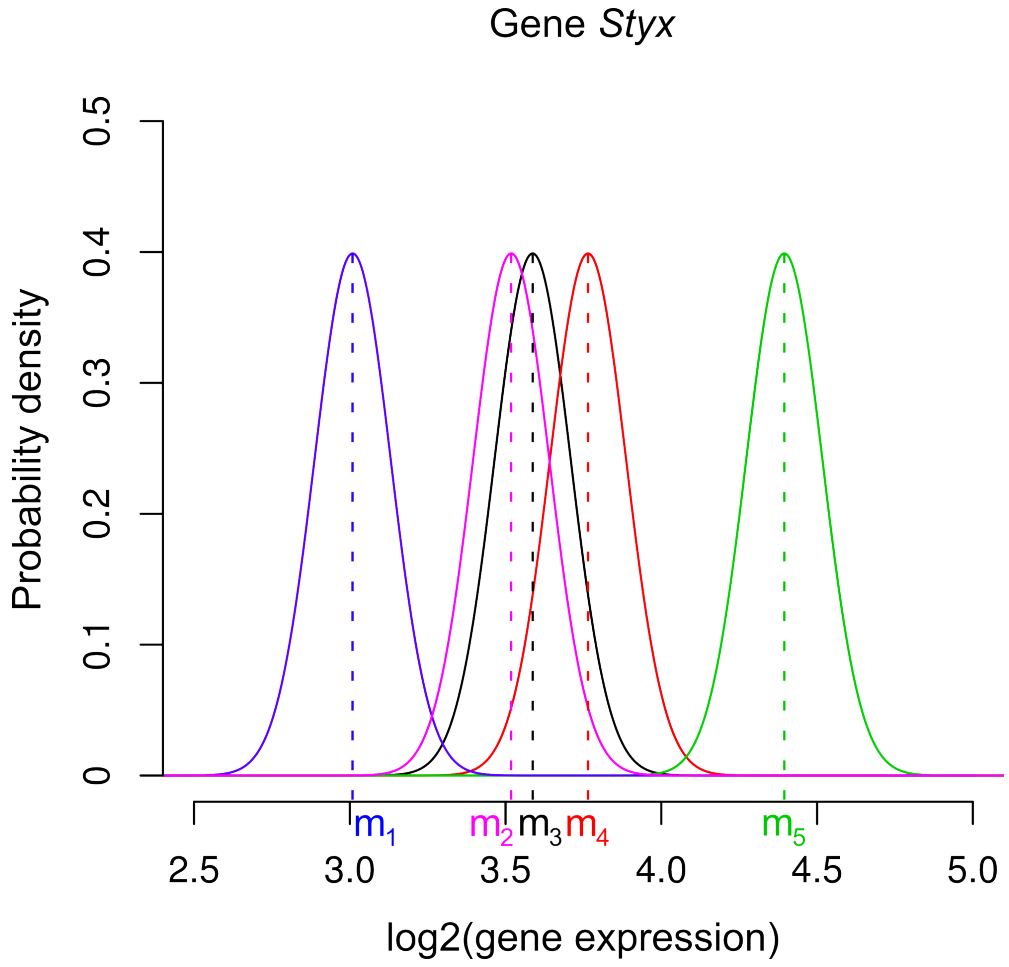

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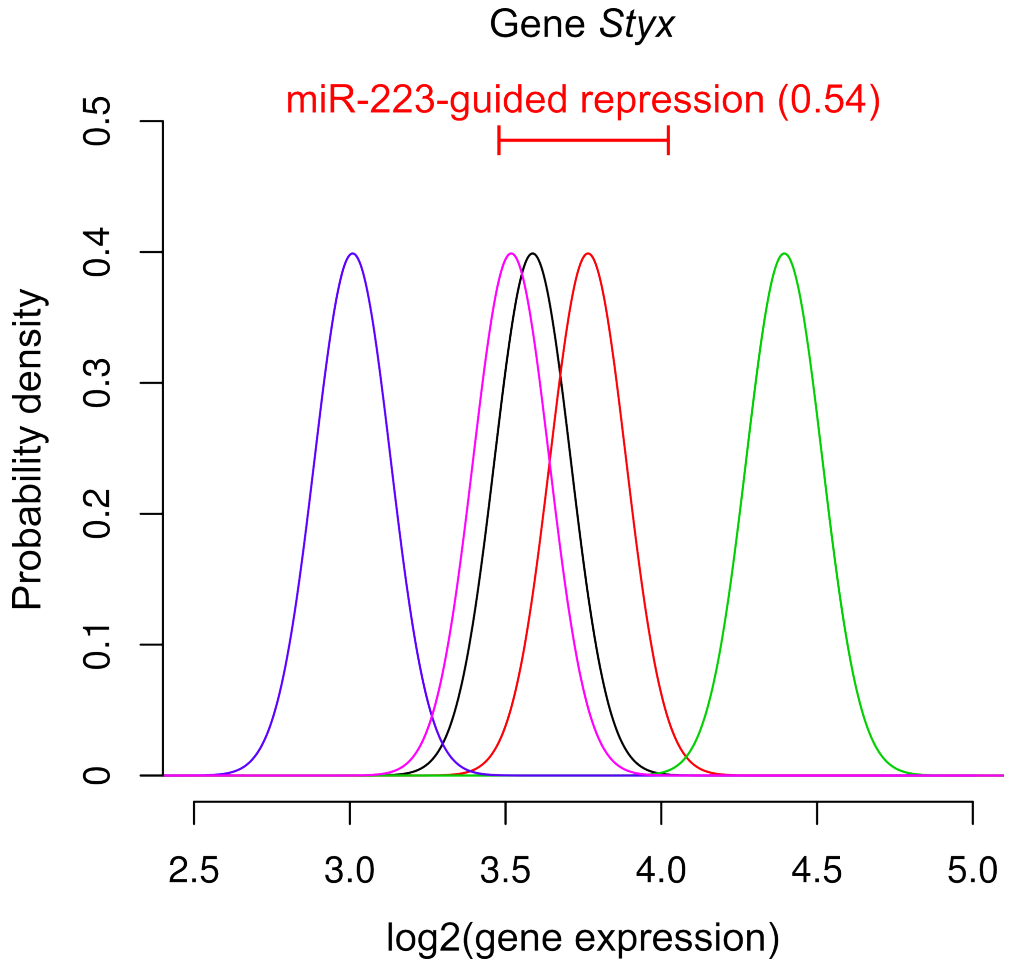

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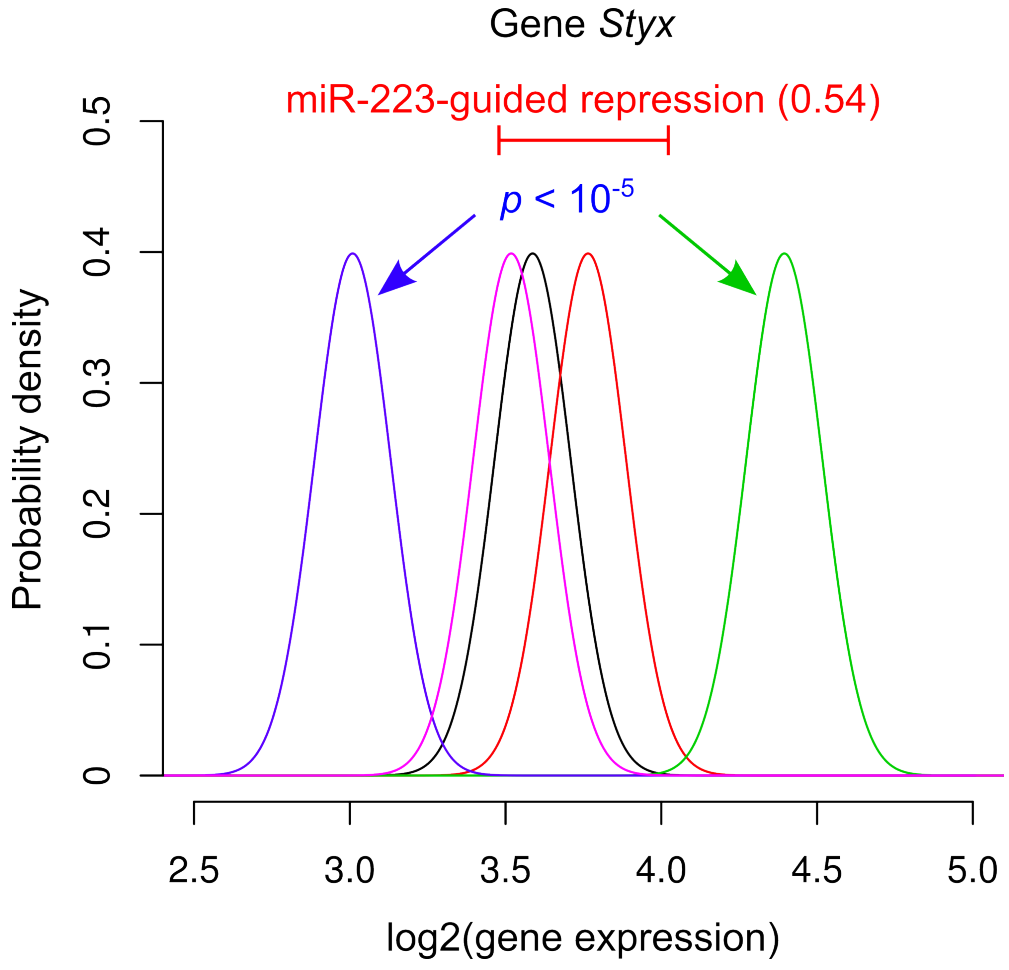

p: probability that the difference between two individual mice is smaller than miRNA-guided repression

Natural variability vs. miRNA-guided repression

For 168 predicted targets out of 189:
inter-individual fluctuations across 5 wild-type mice exceeds miRNA-mediated regulation (p-value < 0.05).

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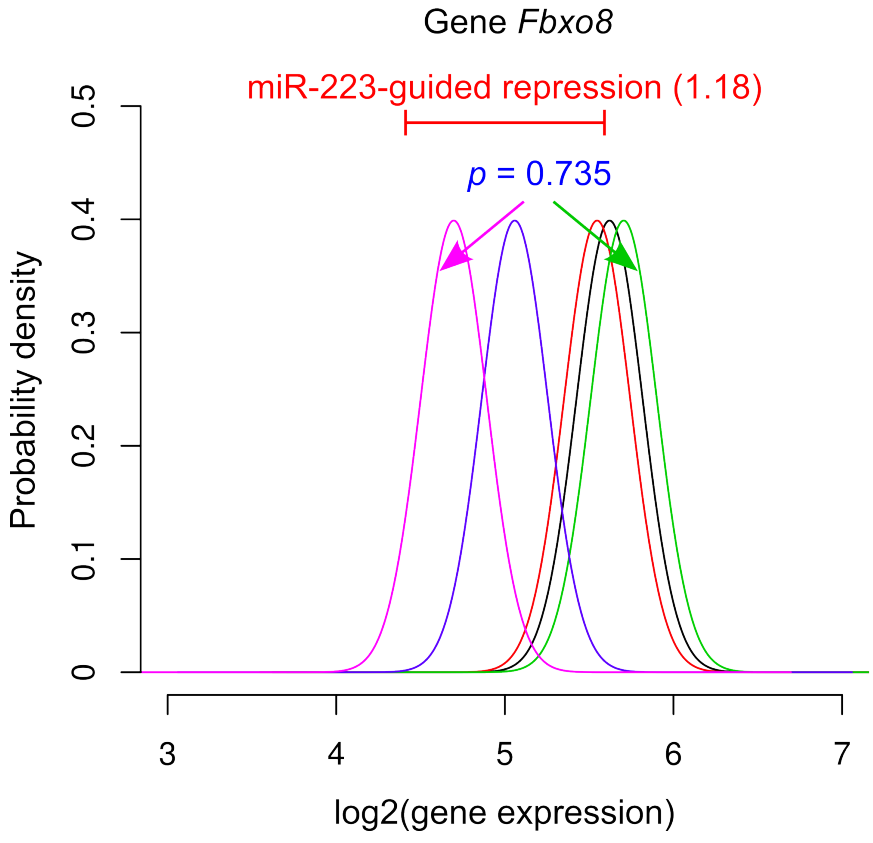
the remaining 21 targets:



Conclusion: revisiting miRNA target definition

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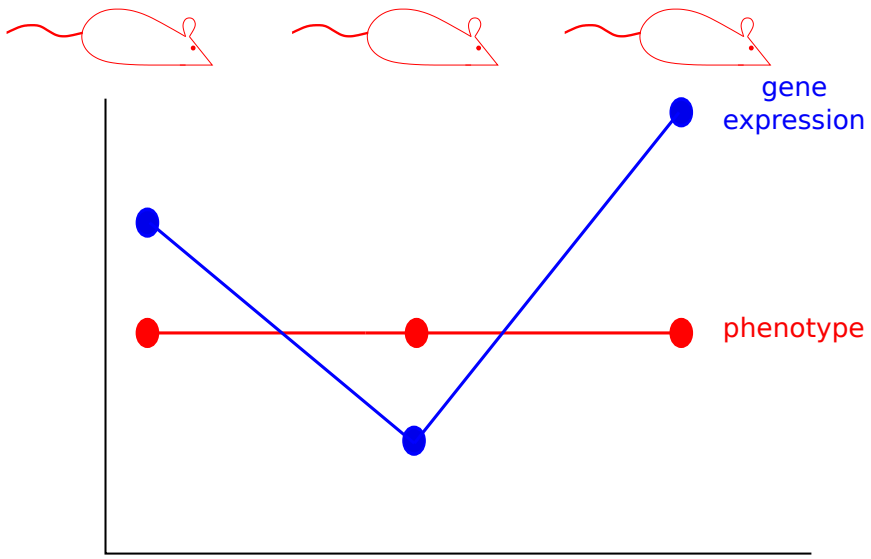
Every measurable change in gene expression does not translate into a macroscopic, evolutionarily selectable phenotype.



Acknowledgements

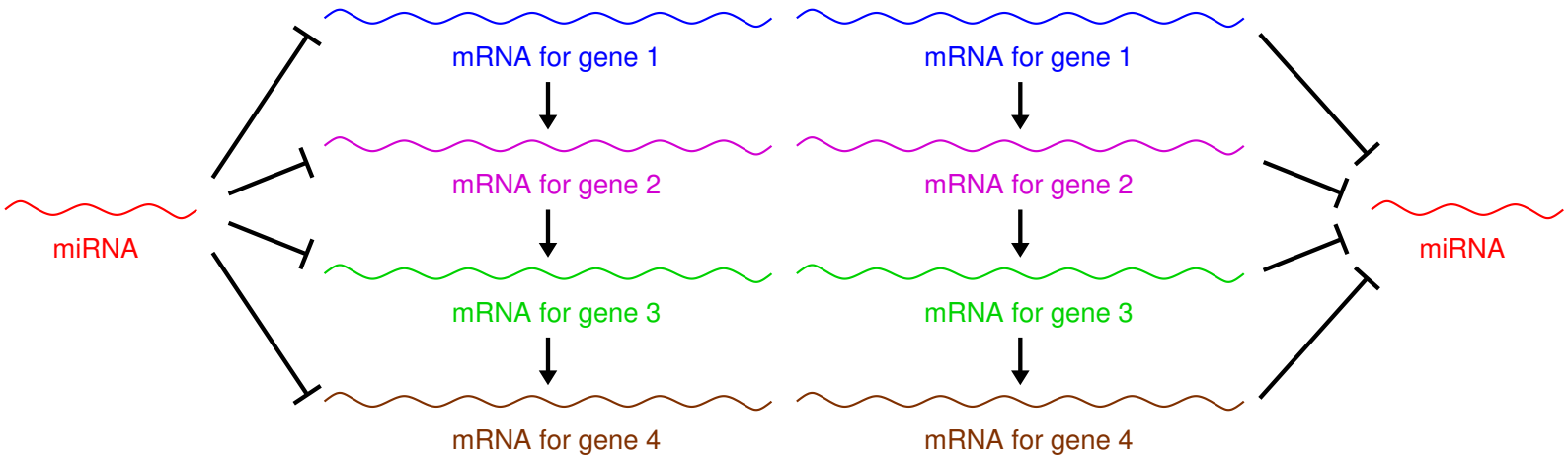
Hervé Seitz,
Anna Sergeeva,
and Laura Martinez

Jessy Presumey and Florence Apparailly (INM, Montpellier, France)

Supplementary data

- Alternative interpretations of properties of predicted miRNA targets.
- Conservative approximations in the assessment of abundance/conservation correlation
- Positive correlation between gene expression and conservation of miRNA binding sites
- Dose-sensitivity and seed match conservation

Alternative interpretations of properties of predicted miRNA targets.



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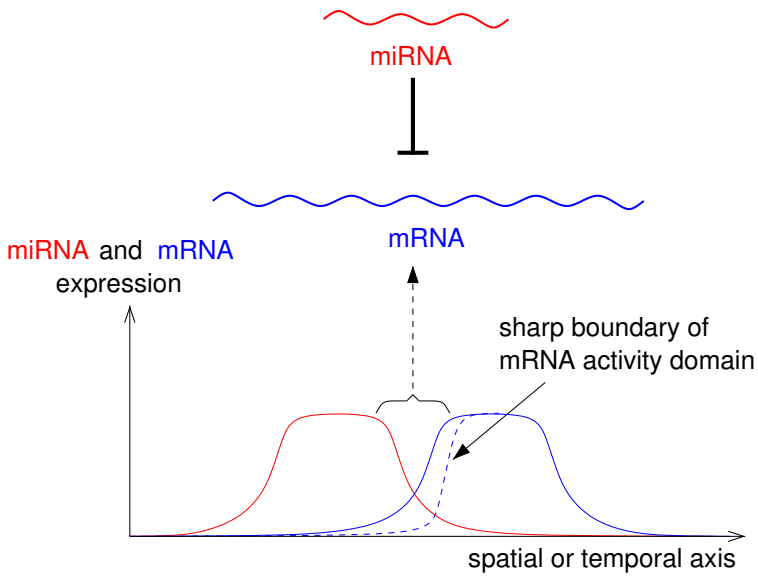
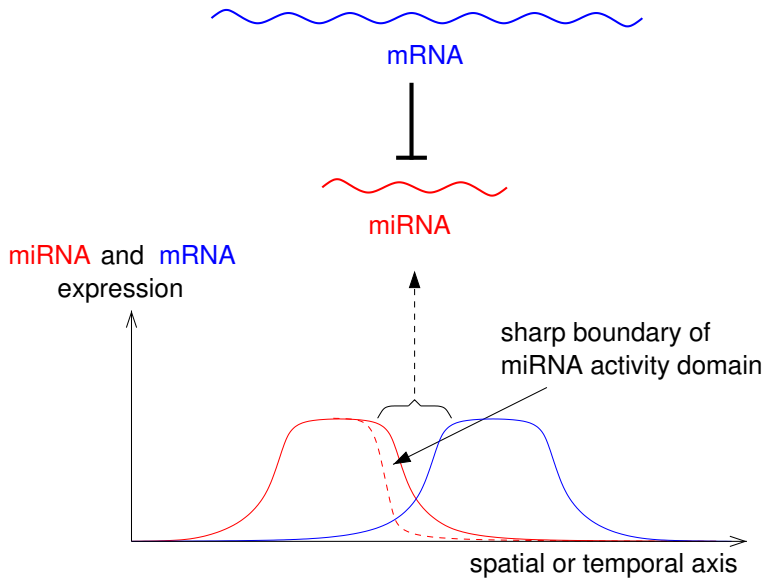
Alternative interpretations of properties of predicted miRNA targets.





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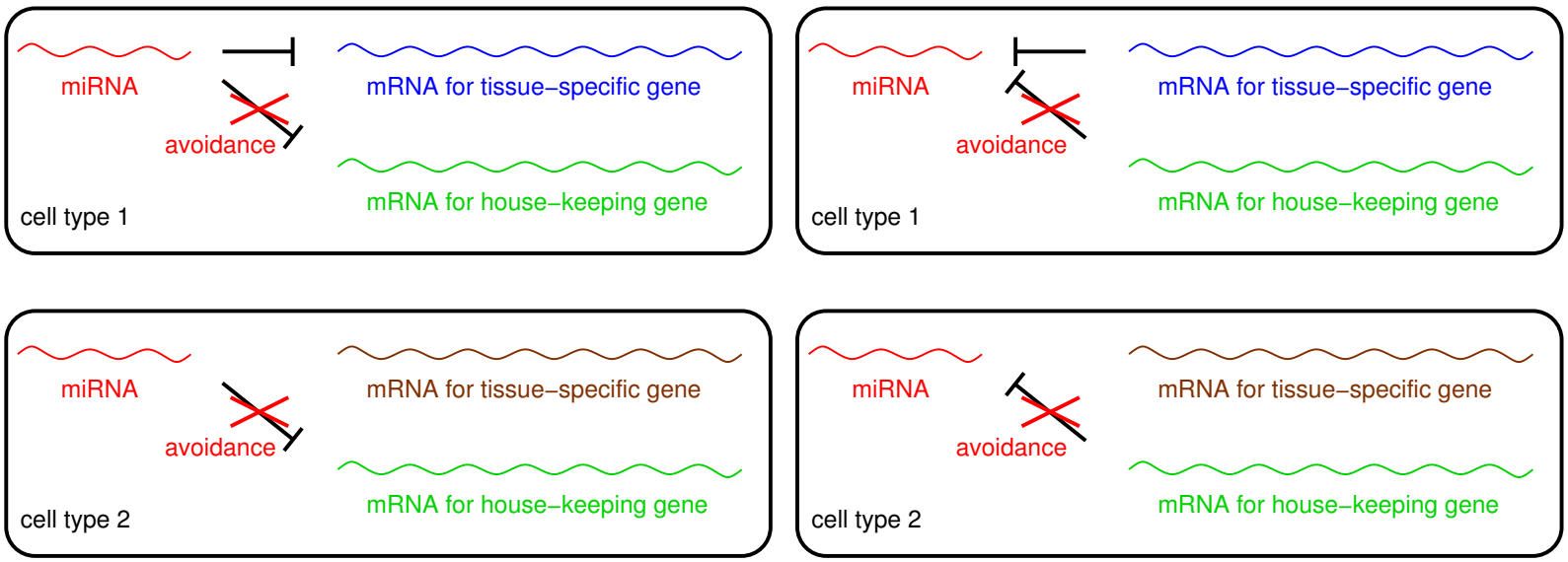

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mRNA abundance and seed match conservation

- Most predicted targets are expected to be pseudo-targets (conservative approximation: we will consider every predicted target).
- miRNA binding site should correlate with that mRNA's abundance in the cells where miRNA titration is beneficial (conservative approximation: we will consider whole tissues and organs).
- Poorly abundant mRNAs (without a real titration effect on the miRNA) should not exhibit such correlation (conservative approximation: we will consider every mRNA).

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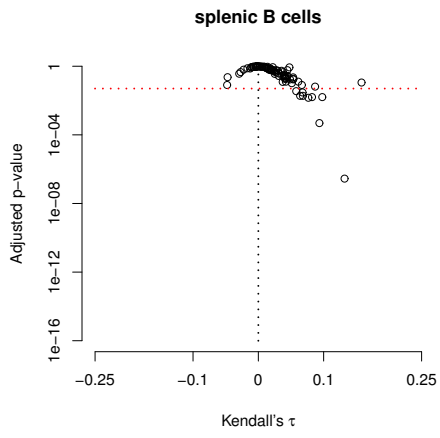
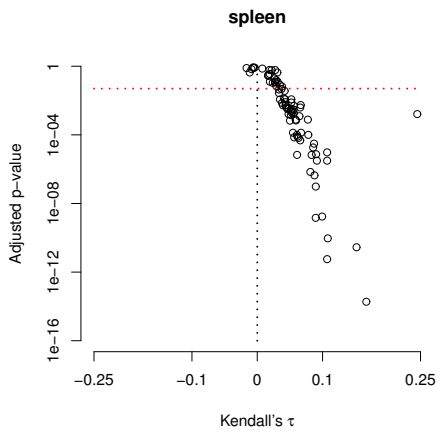
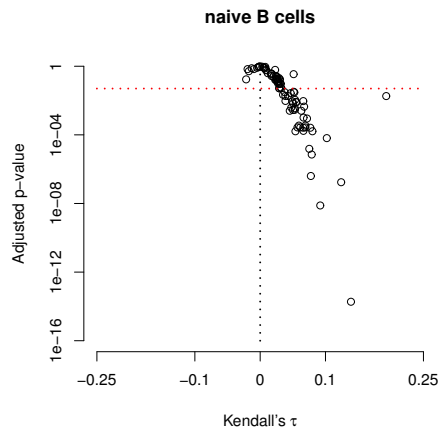
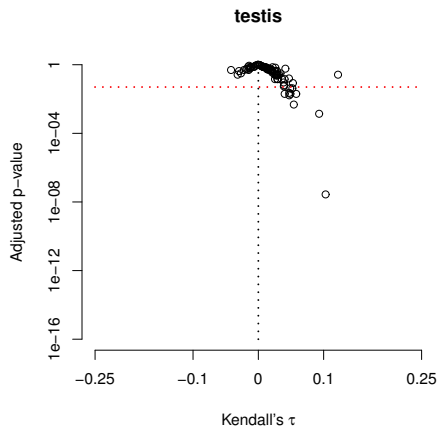
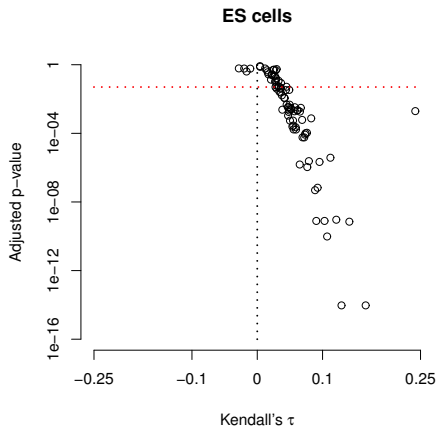
mRNA abundance and seed match conservation

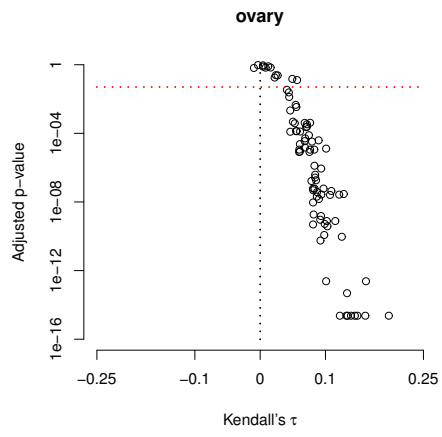












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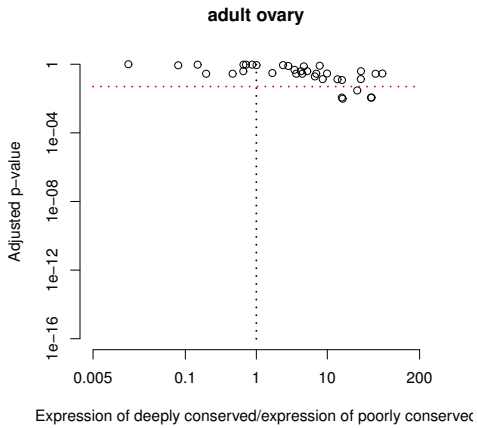
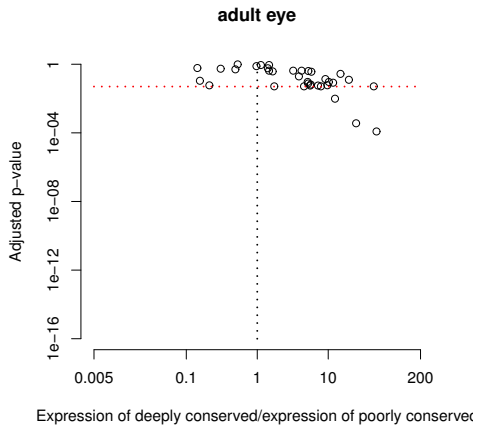
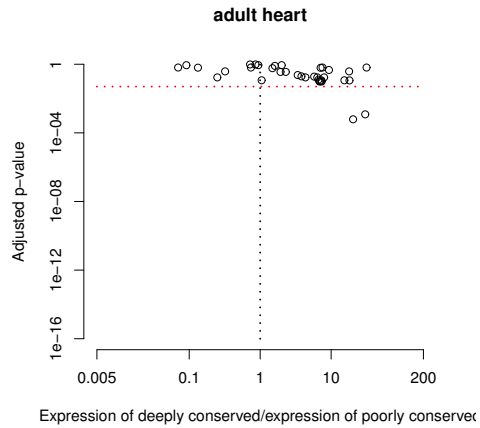
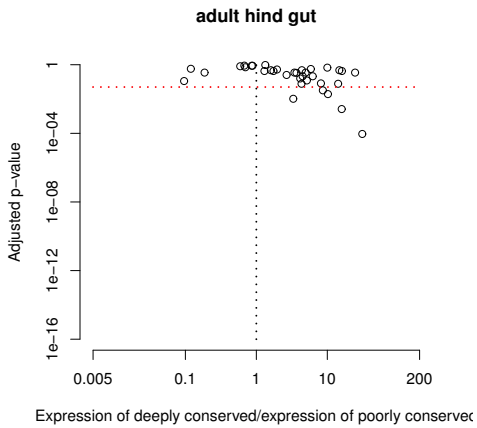
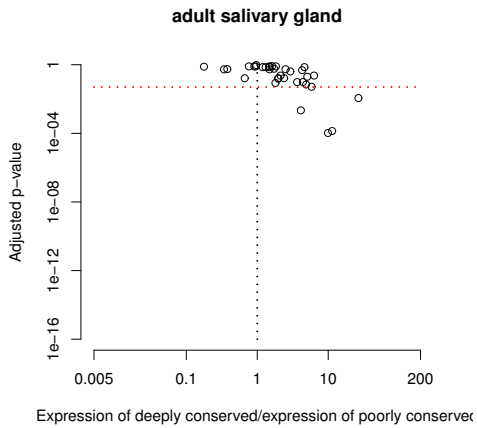
mRNA abundance and seed match conservation

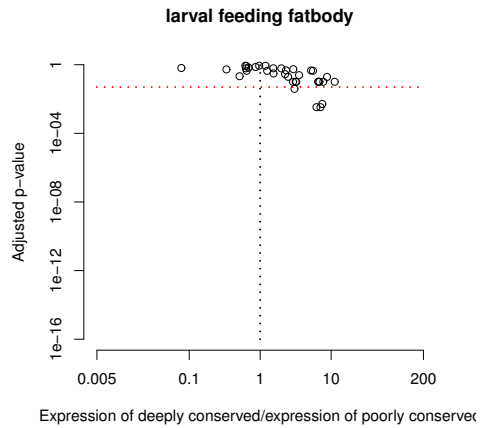












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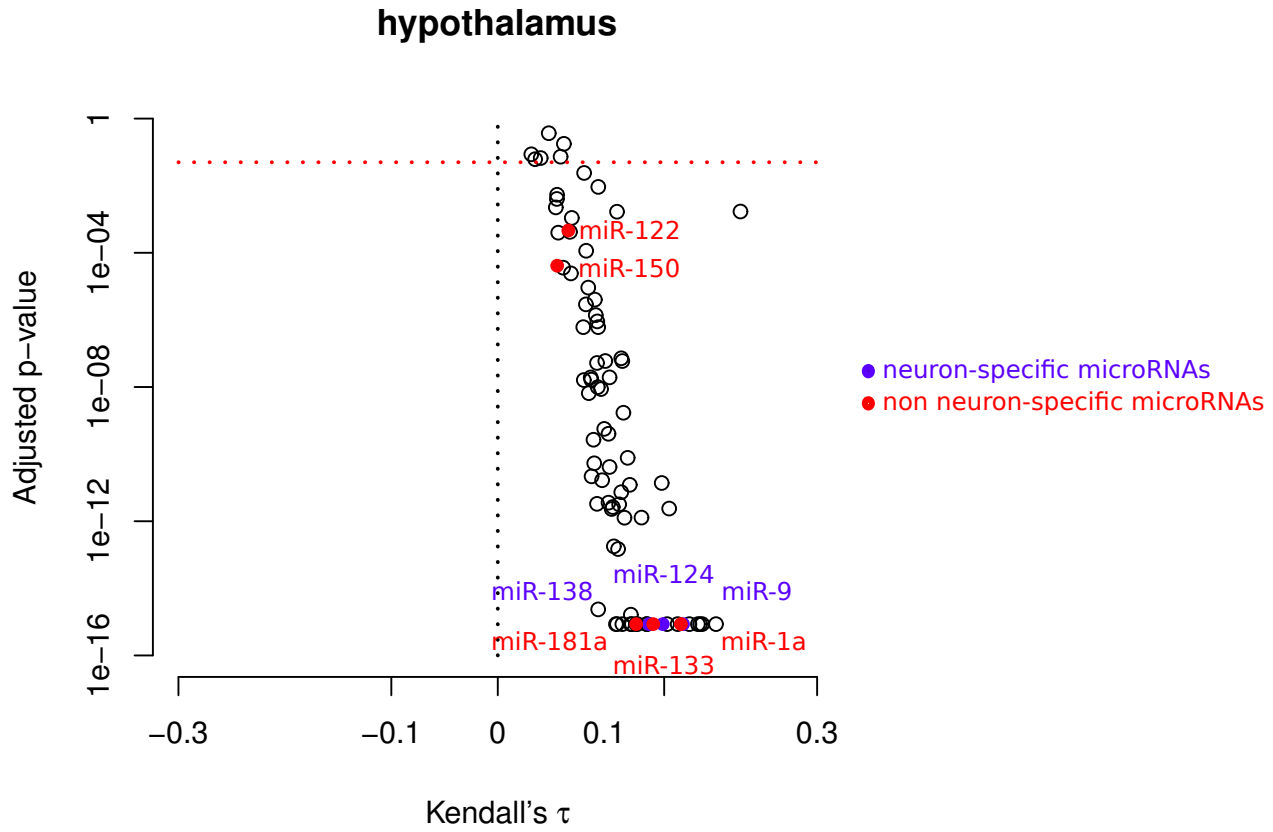
mRNA abundance ans seed match conservation



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Dose-sensitivity and seed match conservation

Assessment of dose sensitivity	Expected by pseudo-target theory	Observed
Heuristic predicting haplo-insufficiency in humans (Huang et al., 2010)	Positive correlation between haplo-insufficiency probability and site conservation	T=+0.190 ($p < 2.2\ 10^{-16}$)
Size of gene family	Negative correlation between family size and site conservation	worm: T=-0.057 (p =8.7 10^{-13}) mouse: T=+0.013 (p = 0.017)
	Genes with deeply-conserved sites belong to smaller gene families	fly: poorly-conserved: 2.7genes/family deeply-conserved: 1.8 genes/family (p =1.6 10^{-5})

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