Functional assessment of individual miRNA binding sites: time to go *in vivo*

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June 27, 2016

microRNA target prediction

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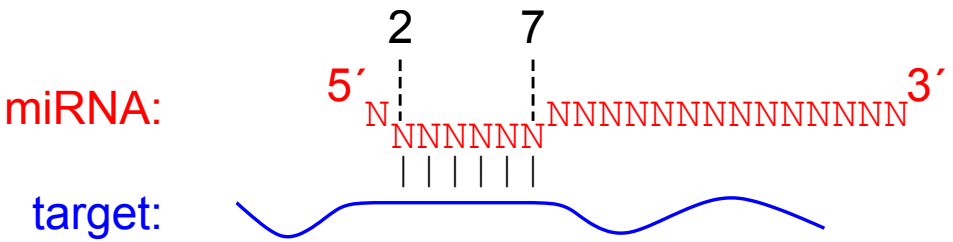

$\longrightarrow$ the "seed"

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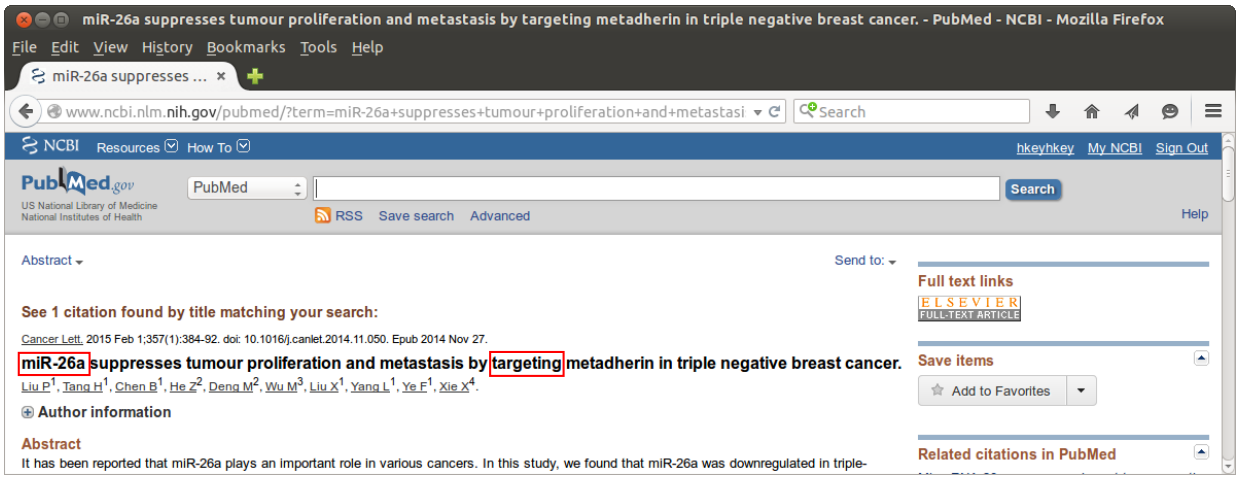

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miRNA-mediated repression is very modest (usually $<$ 2-fold): lower than well tolerated fluctuations in gene expression (*e.g.*, haplosufficiency). Why have these sites been conserved if they are not functional?

Comparative genomics yield false positives

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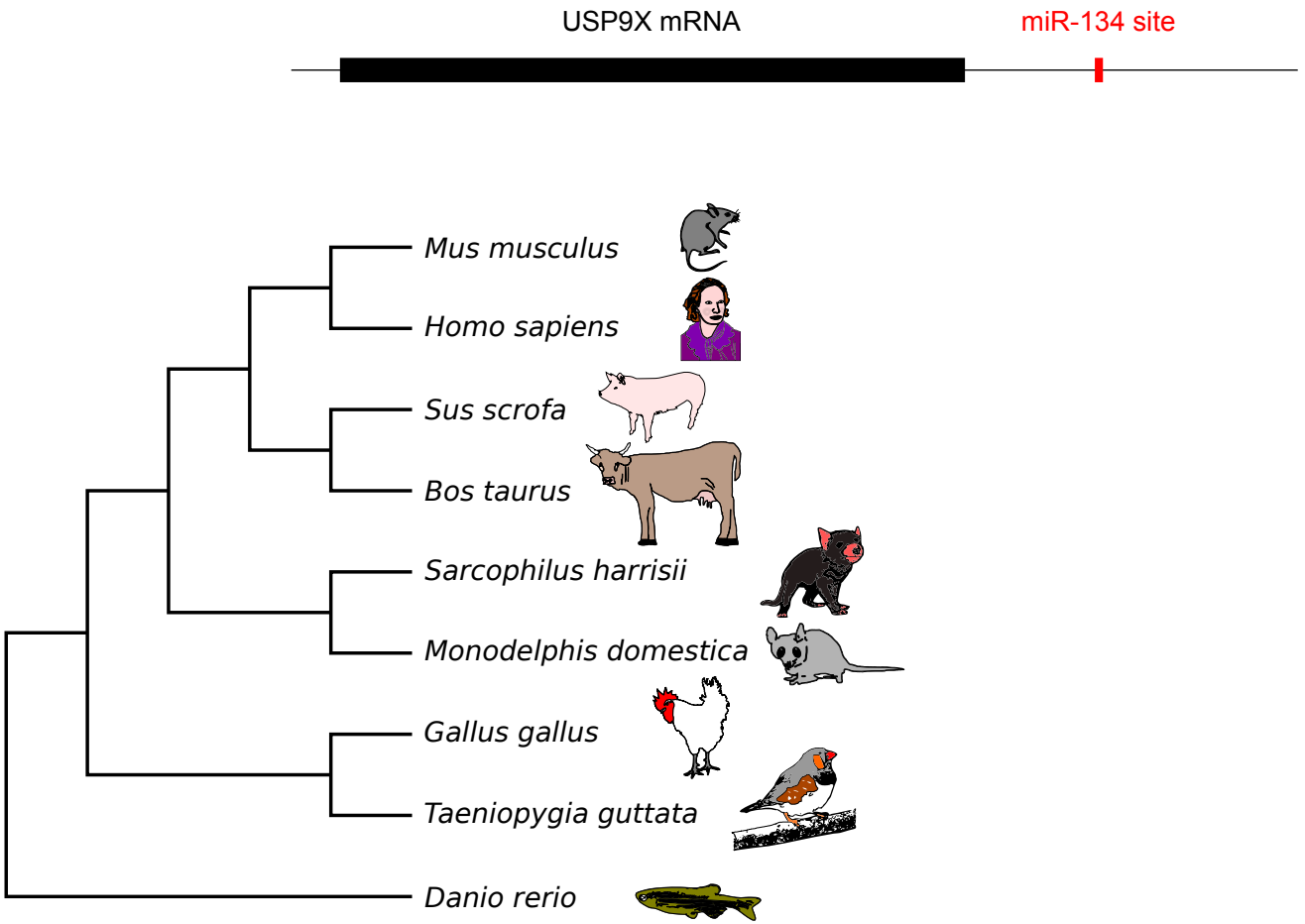

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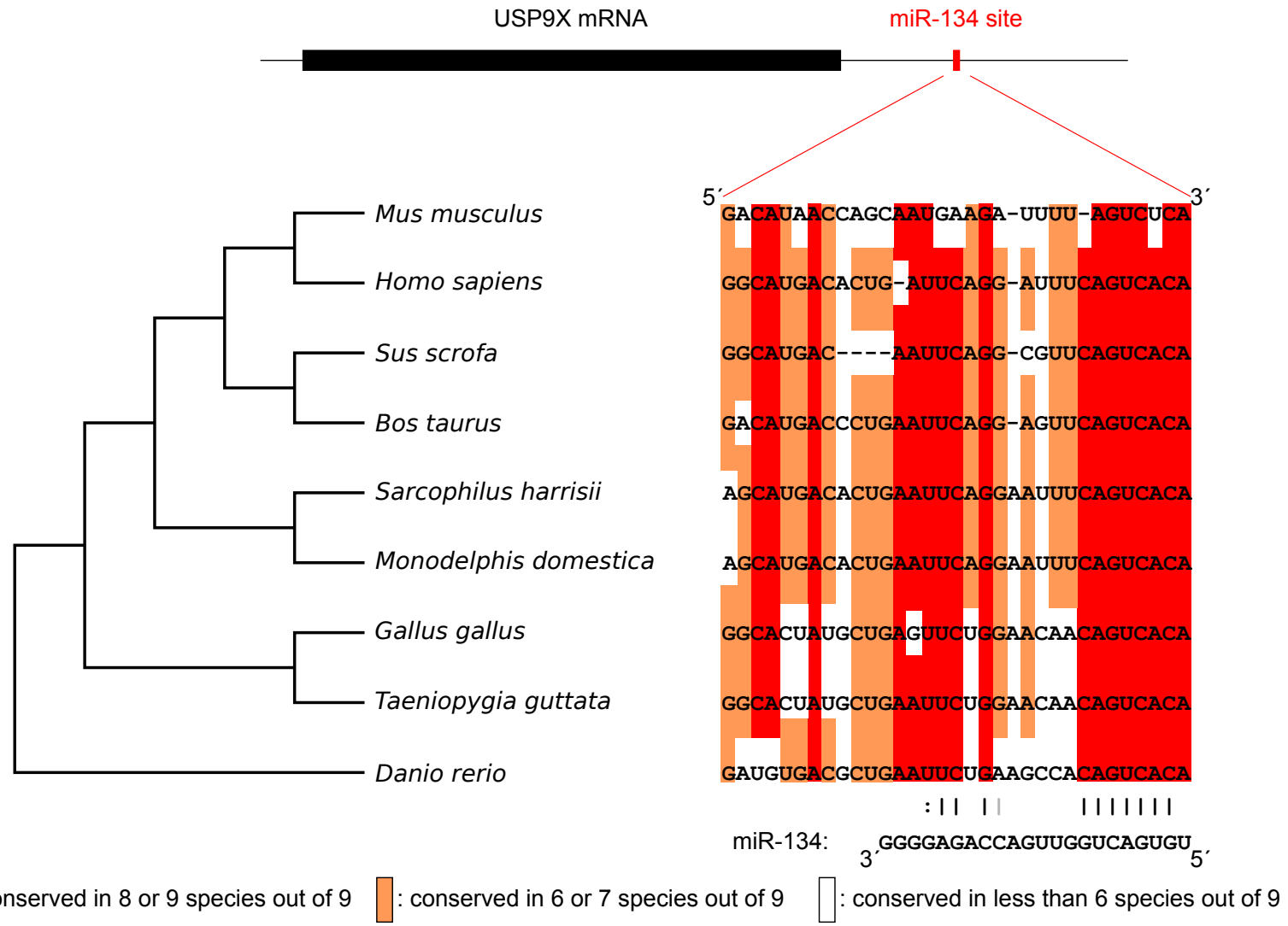

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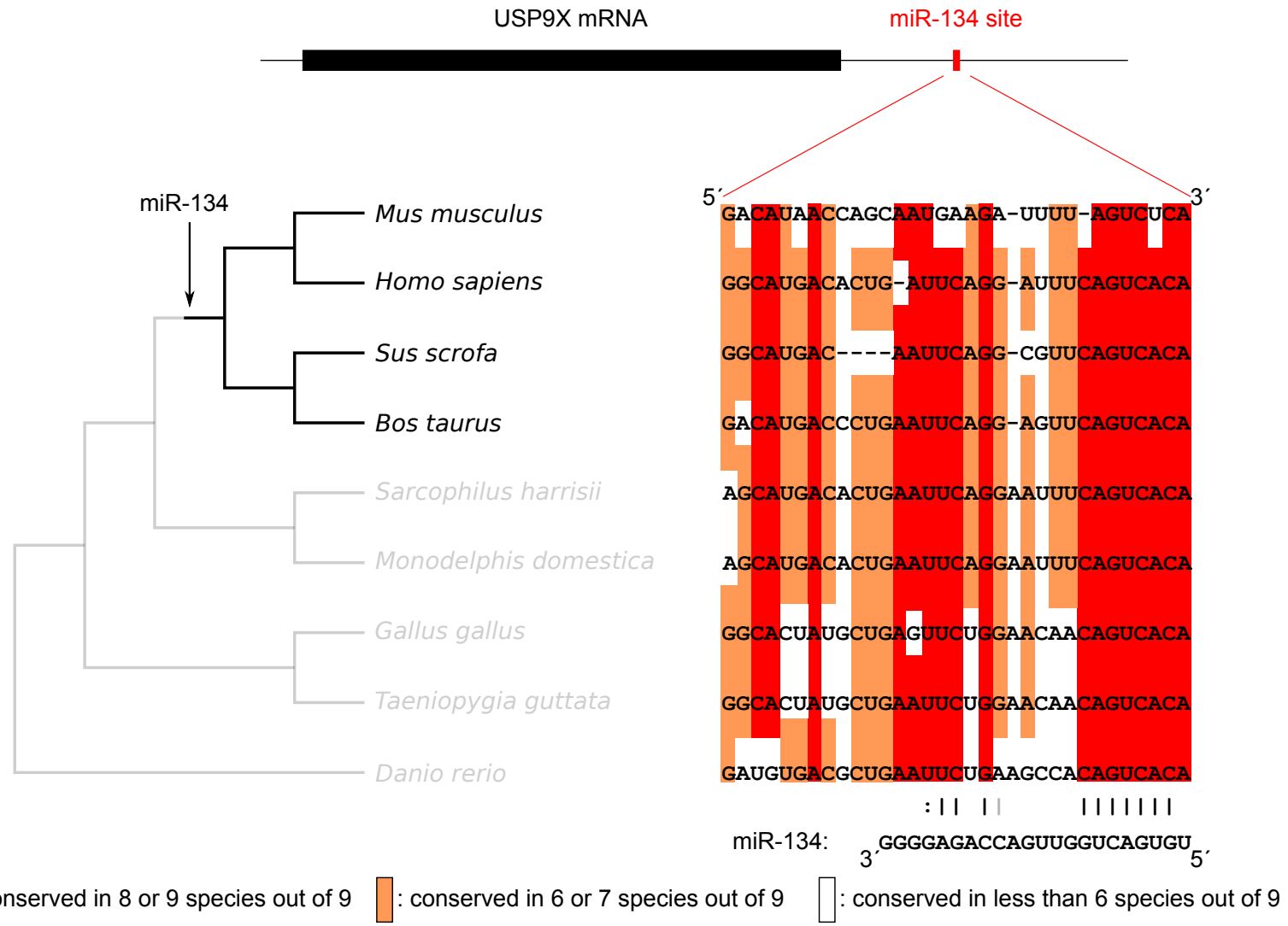

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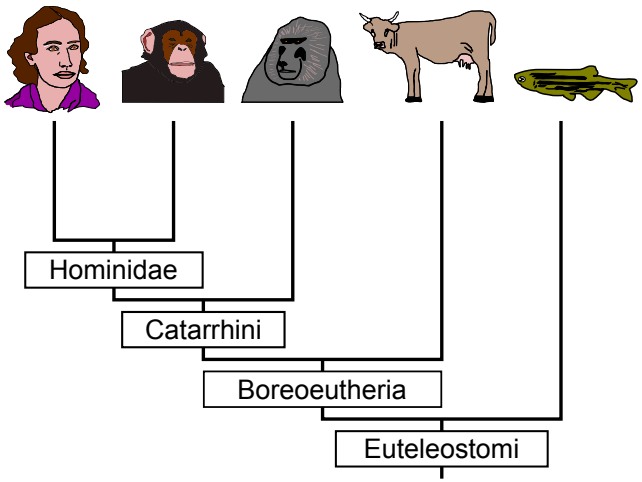

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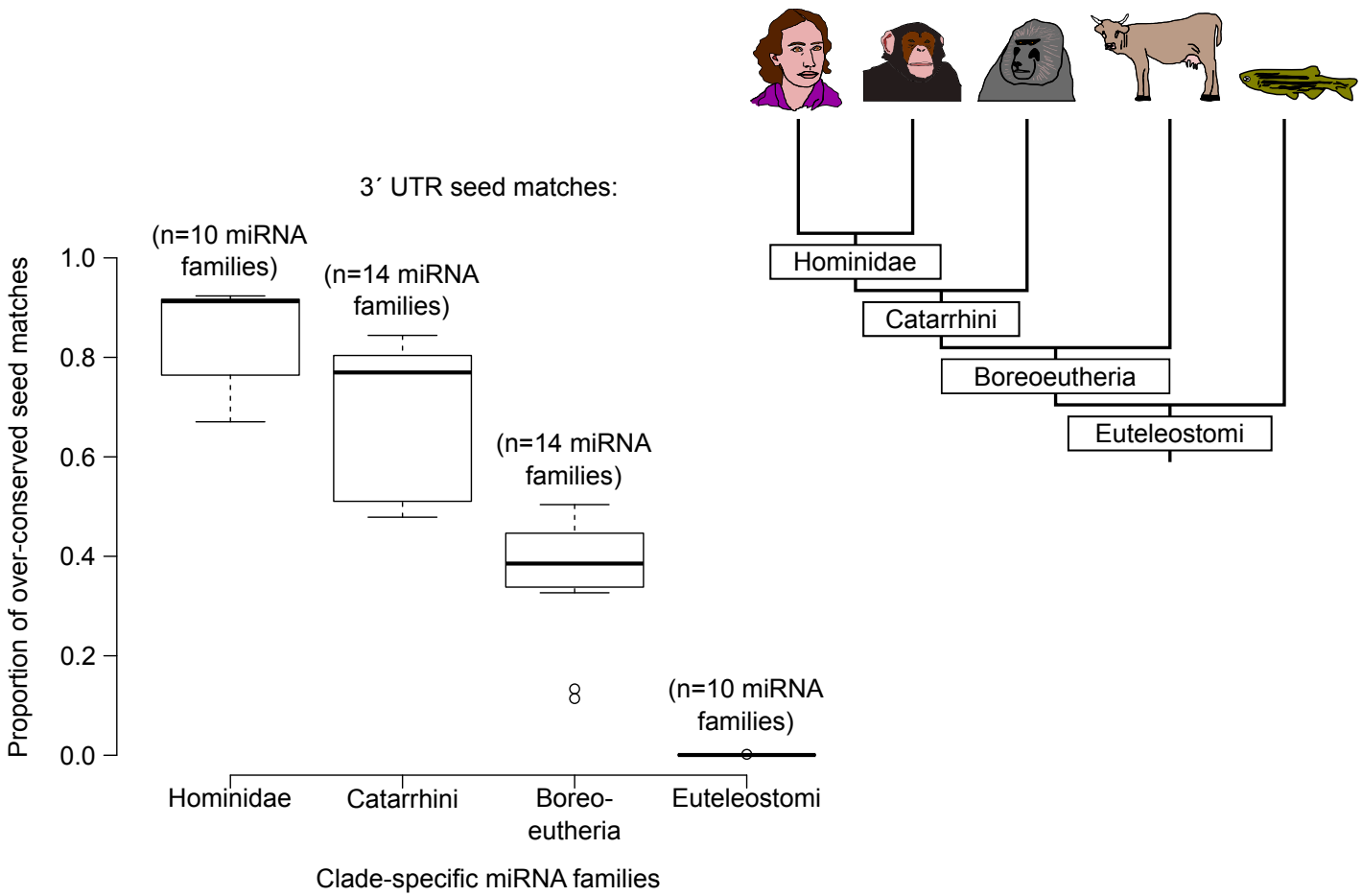

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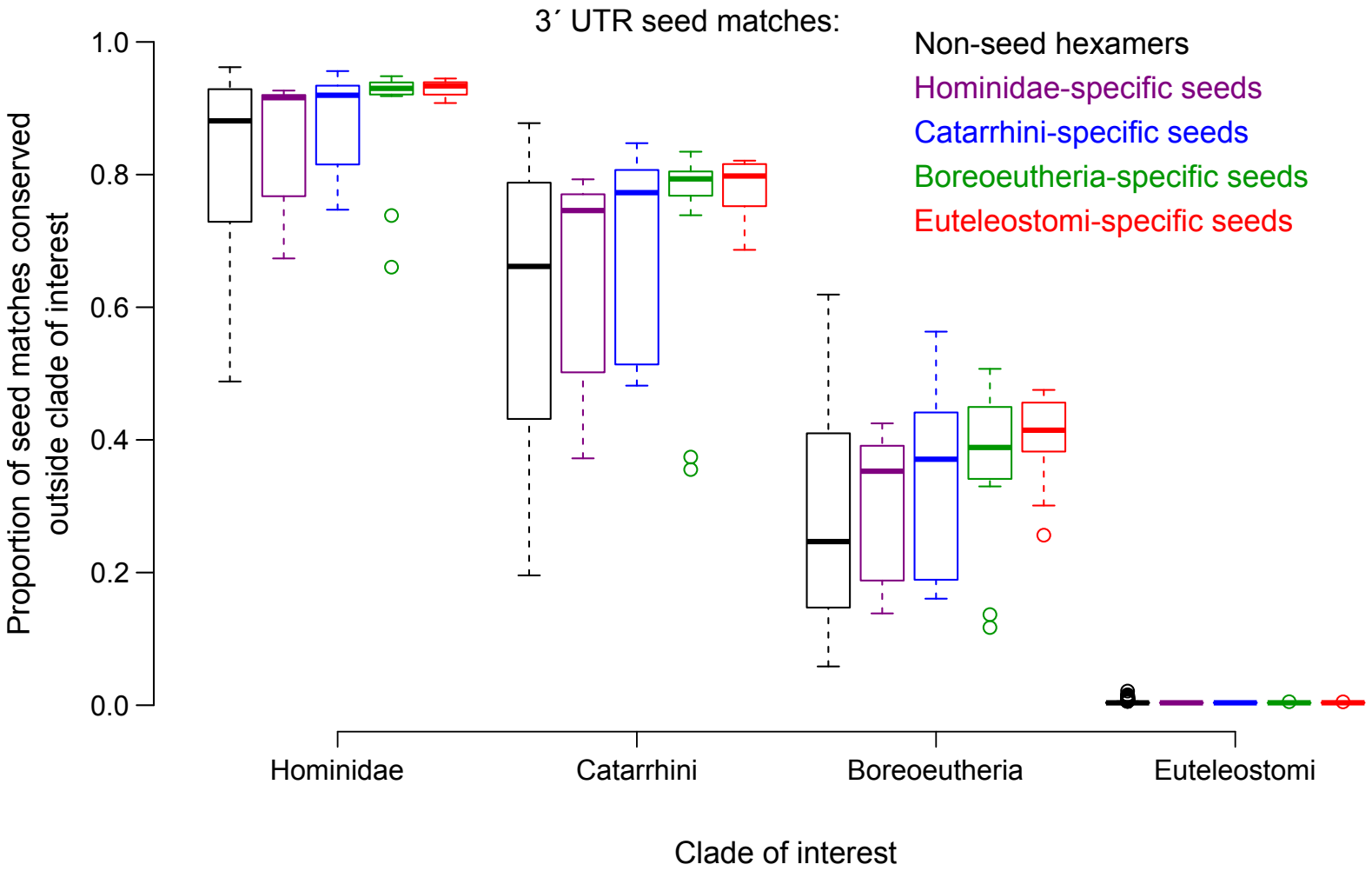

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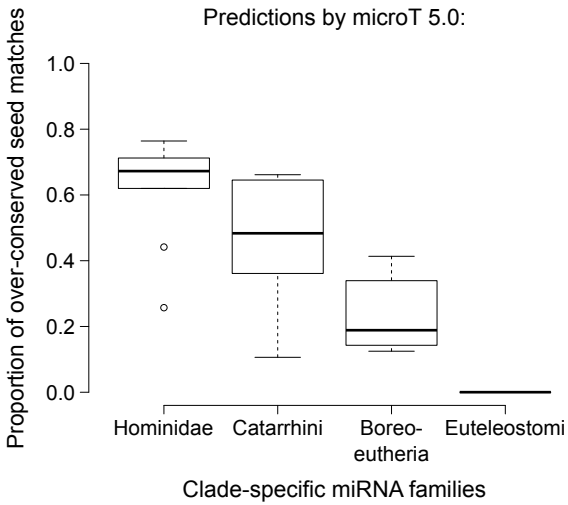

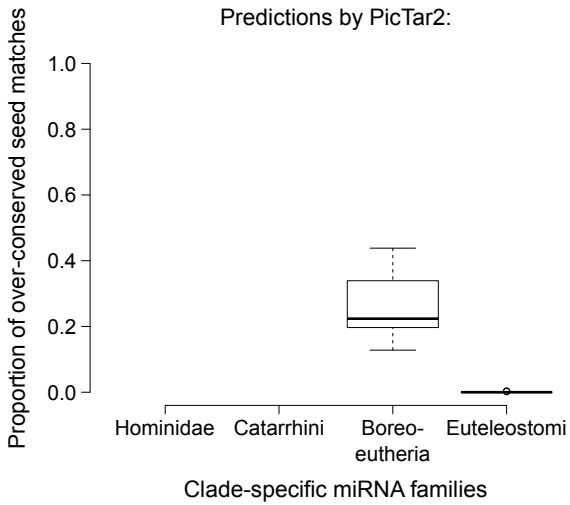

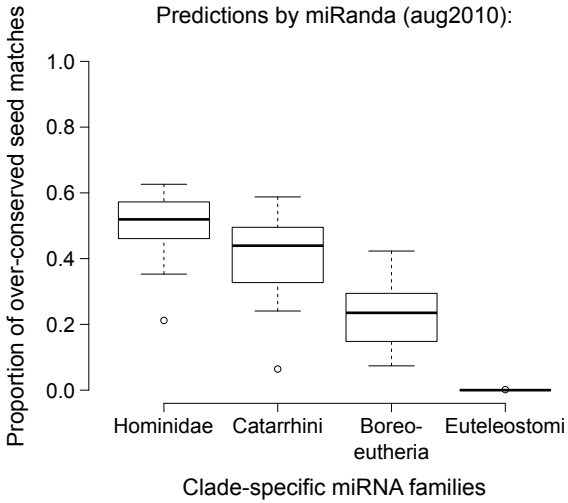

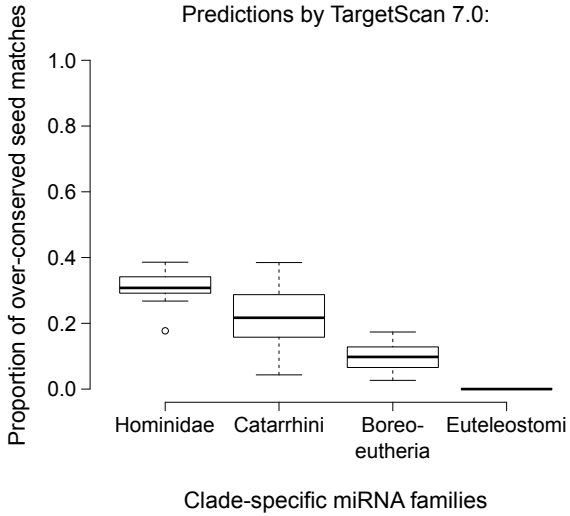

Conclusion

Many predicted miRNA binding sites are conserved in a miRNA-independent fashion.

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Eliminate over-conserved sites from predictions.

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Eliminate over-conserved sites from predictions.

Assess macroscopic *in vivo* phenotypes rather than changes in protein abundance.

Acknowledgements

Anna Sergeeva:

Laura Martinez:

Blaise Li:

Natalia Pinzón:

Isabelle Busseau:

Delphine Mazé:

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Supplementary data

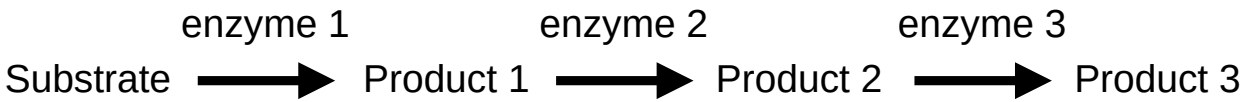
Introduction:

- Robustness of biological pathways
- Issues with published pseudo-targets

Conclusion:

- Pseudo-targets for other regulators?
- Propagation of gene expression perturbation

Biological robustness



Reported examples of pseudo-targets

A proposed pseudo-target: PTENP1, that de-repressed PTEN (Poliseno *et al.*, 2010).

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But PTENP1 mRNA is $\approx$ 100 times less abundant than the PTEN mRNA (Ebert and Sharp, 2010).

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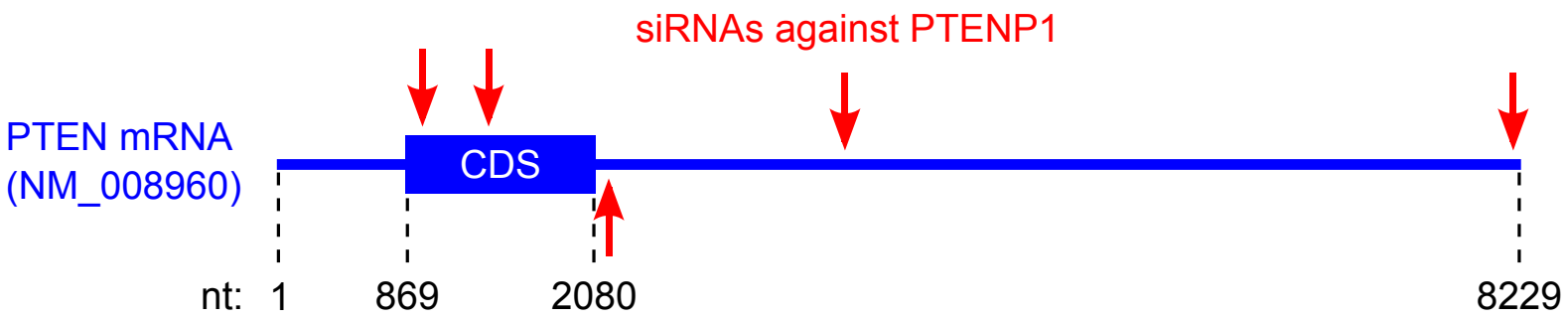

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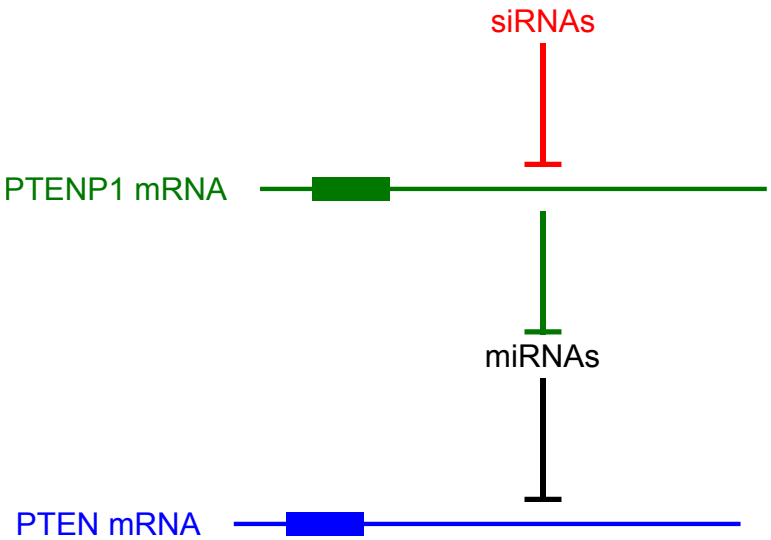
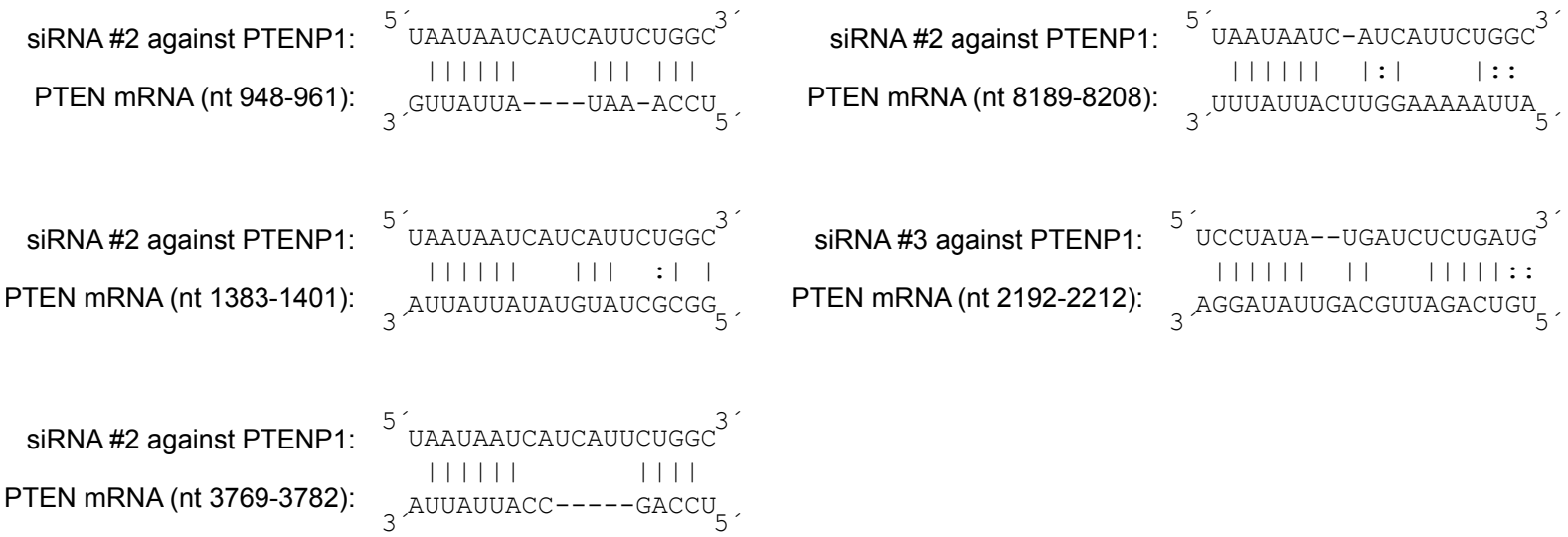
Proposed by Poliseno *et al* (2010):



More probably:

Reported examples of pseudo-targets



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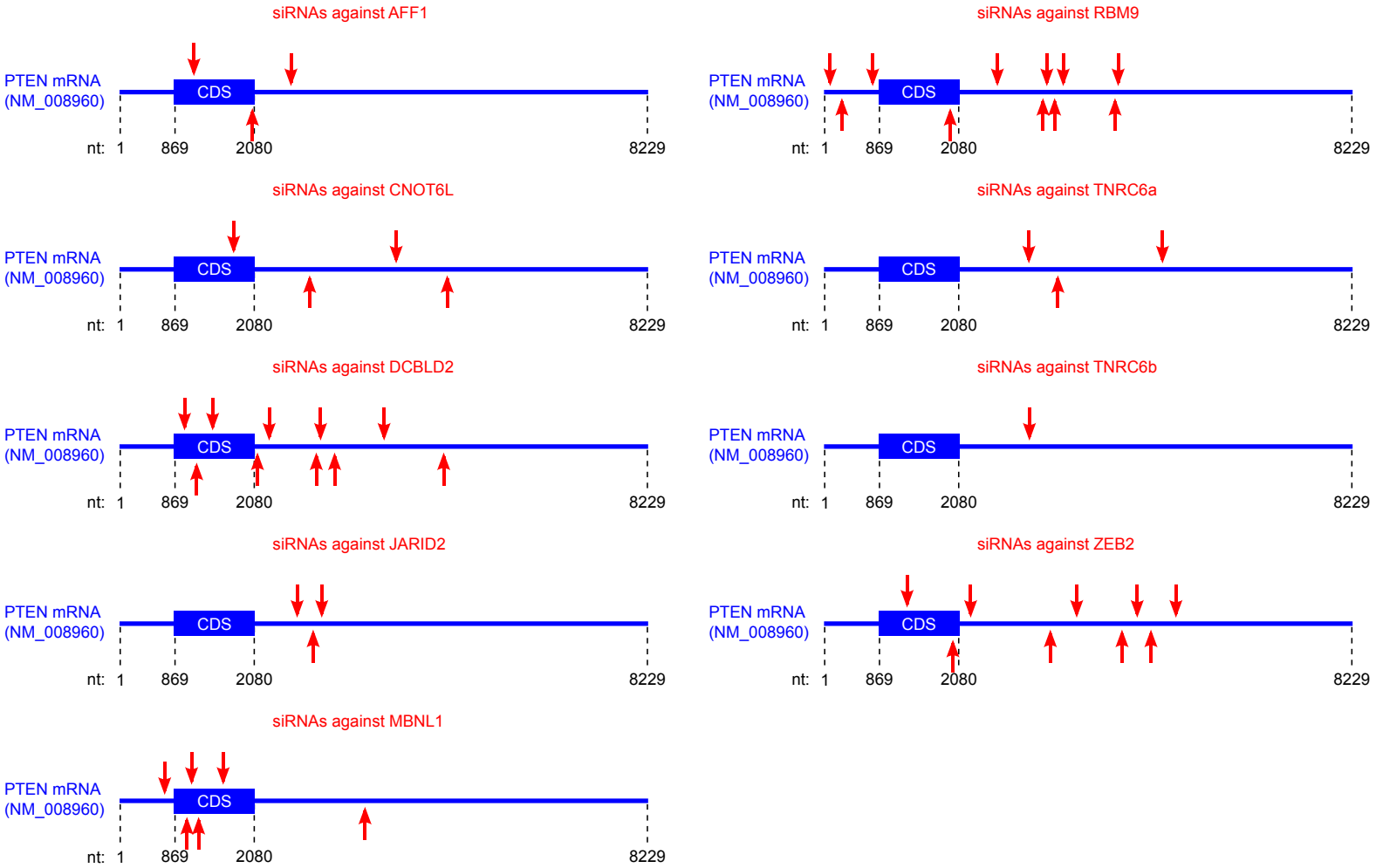

Pseudo-targets for other regulators?

- Transcription factors: experimentally identified binding sites are poorly conserved among vertebrates (Odom *et al.*, 2007 and Schmidt *et al.*, 2010).

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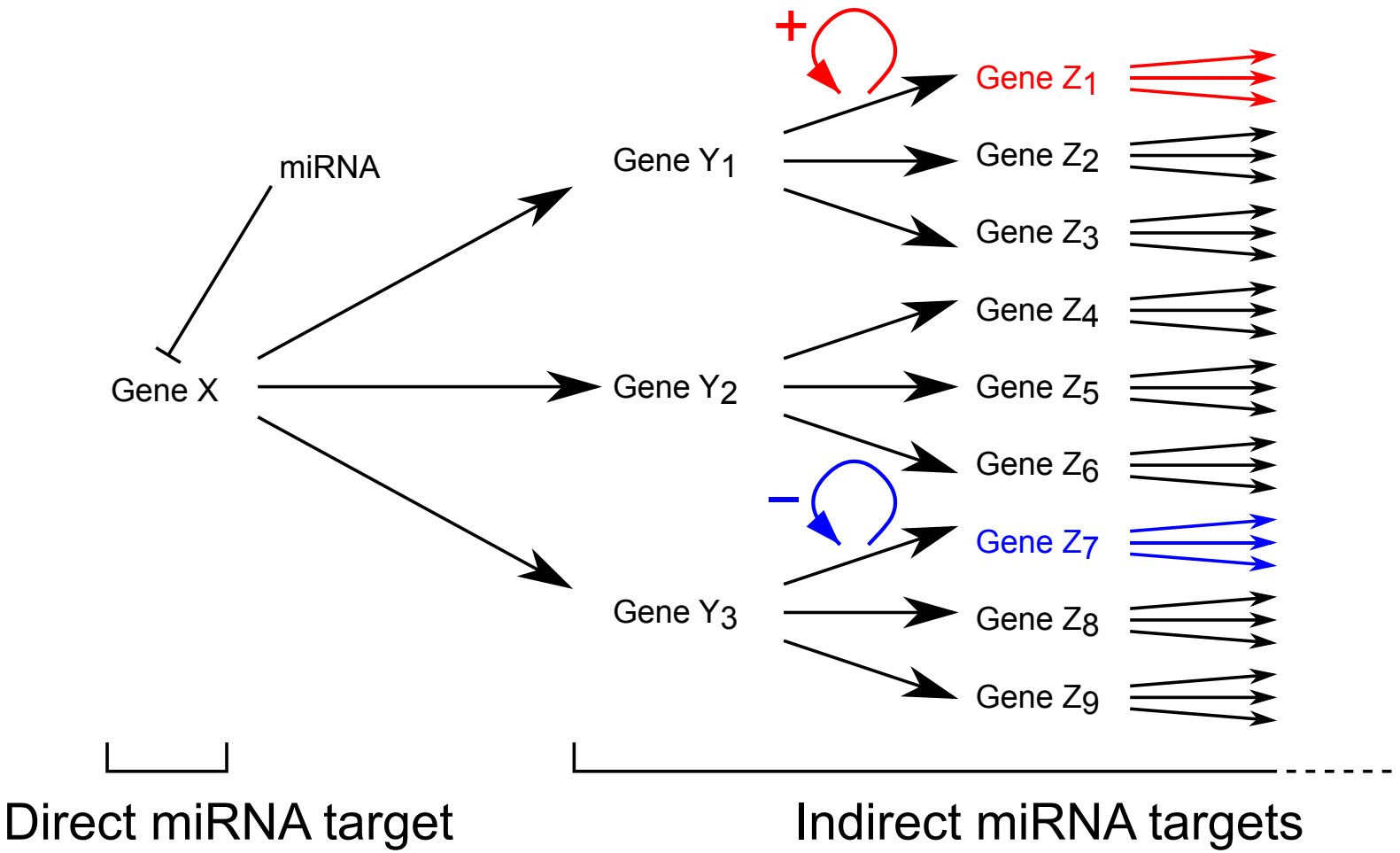
Real molecular events, which are neutral in evolutionary terms?

◂ Return

Propagation of gene expression perturbation



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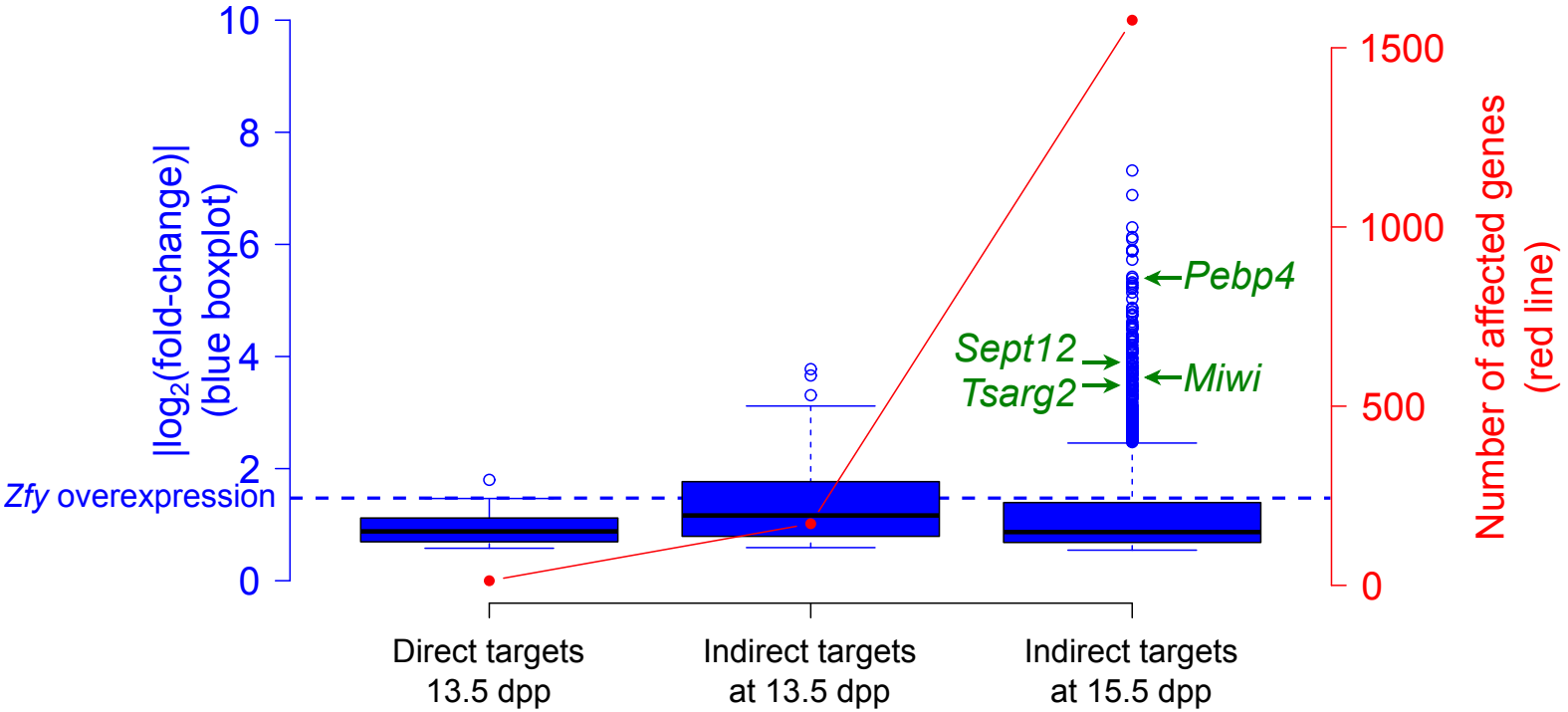

(in collaboration with H. Royo and J. Turner, MRC, London)