Leibniz Institute on Aging –
Fritz Lipmann Institute

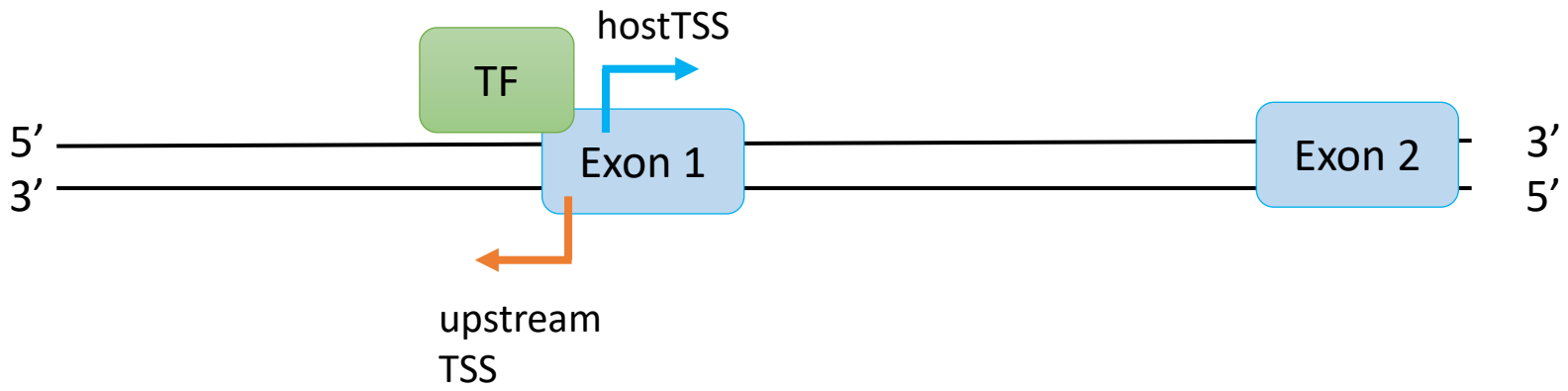
# Convergent promoters: cooperation, collision or collision leading to cooperation?

Elina Weichens, Kanstantsin Siniuk

12.02.2025

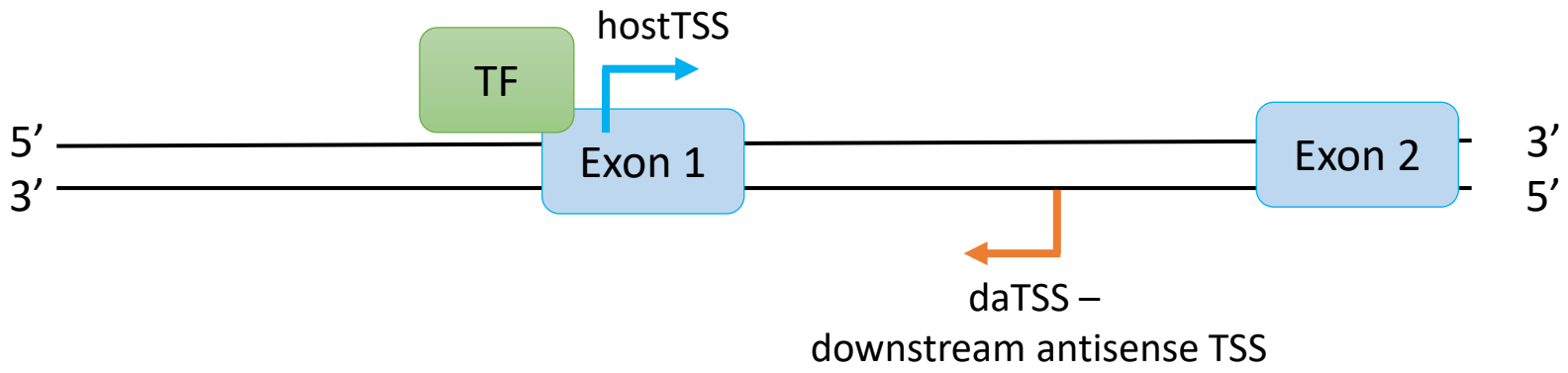
Leibniz Association

# Classic view of promoter structure




TSS: transcription start site
TF: transcription factor

# Alternative view of promoter structure

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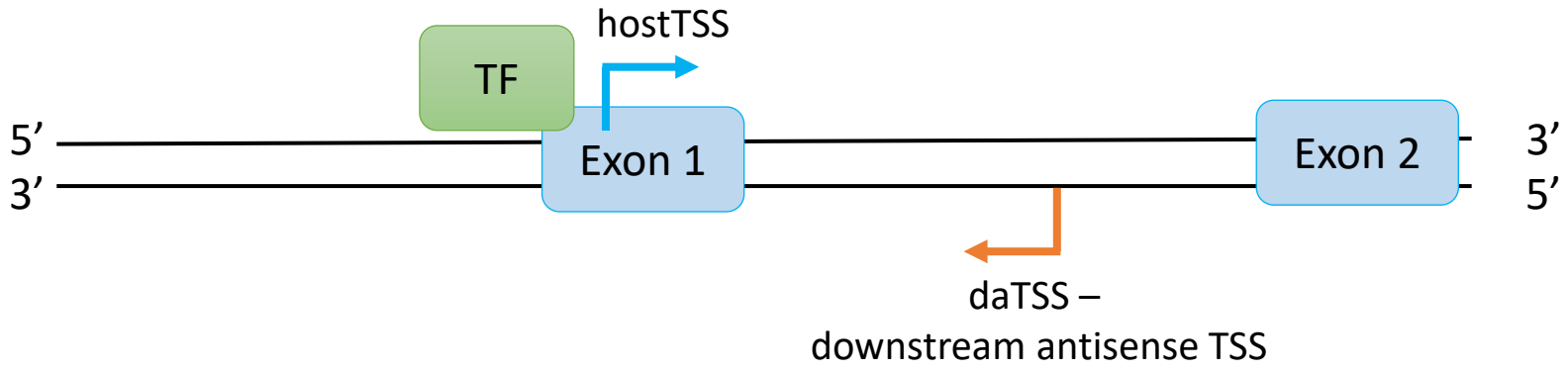

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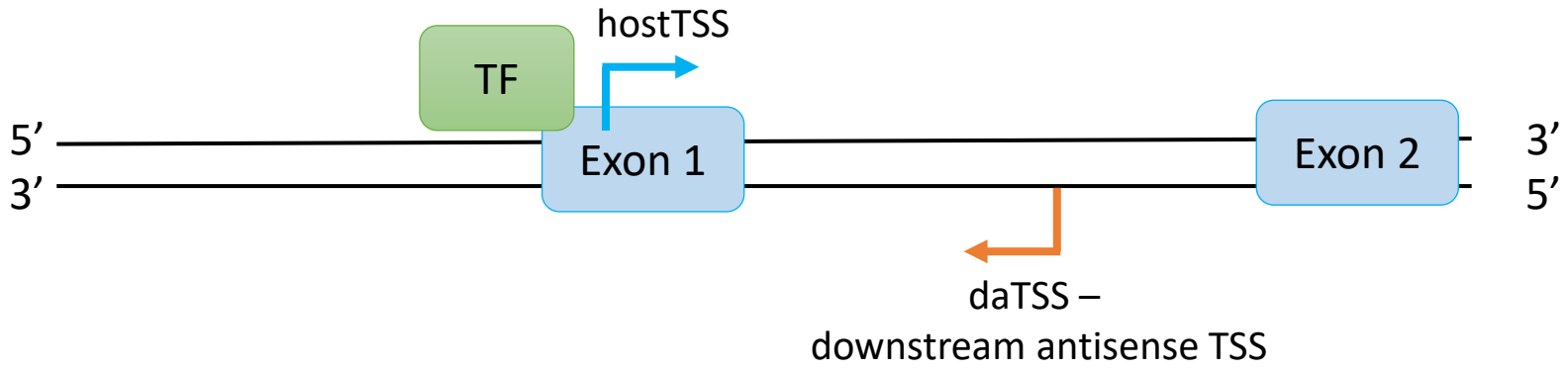


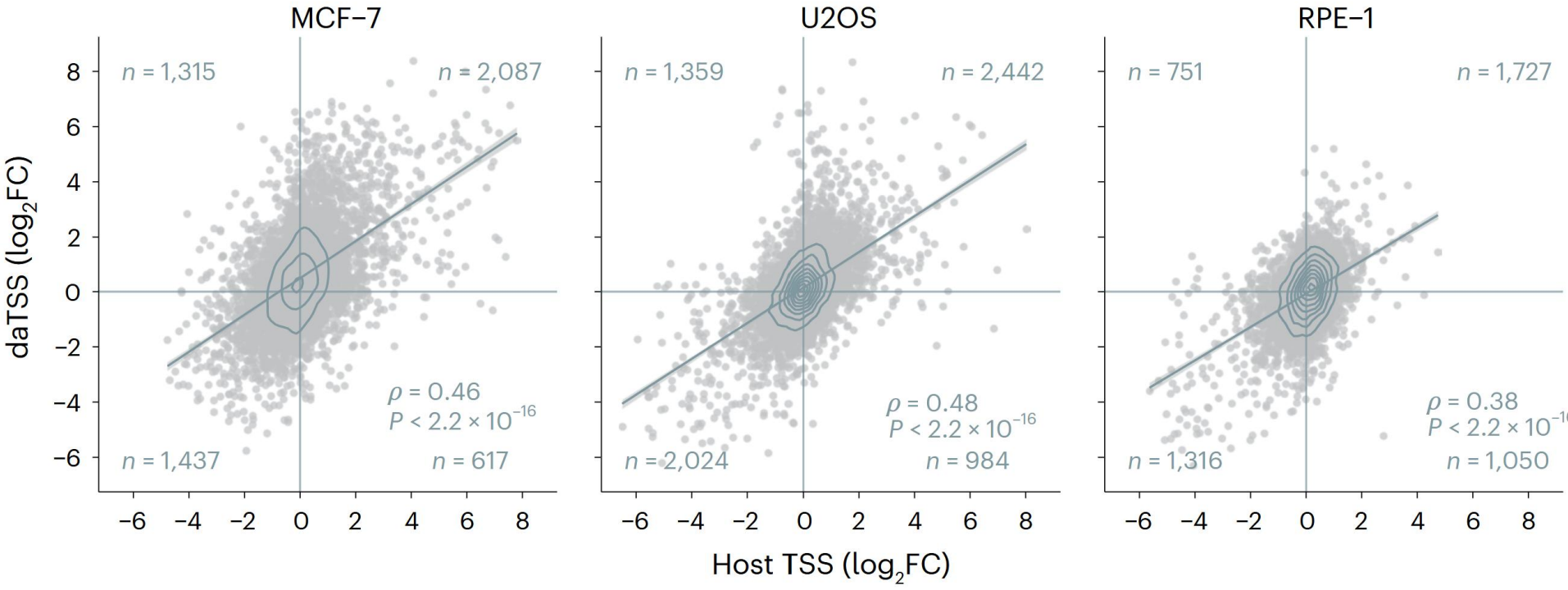

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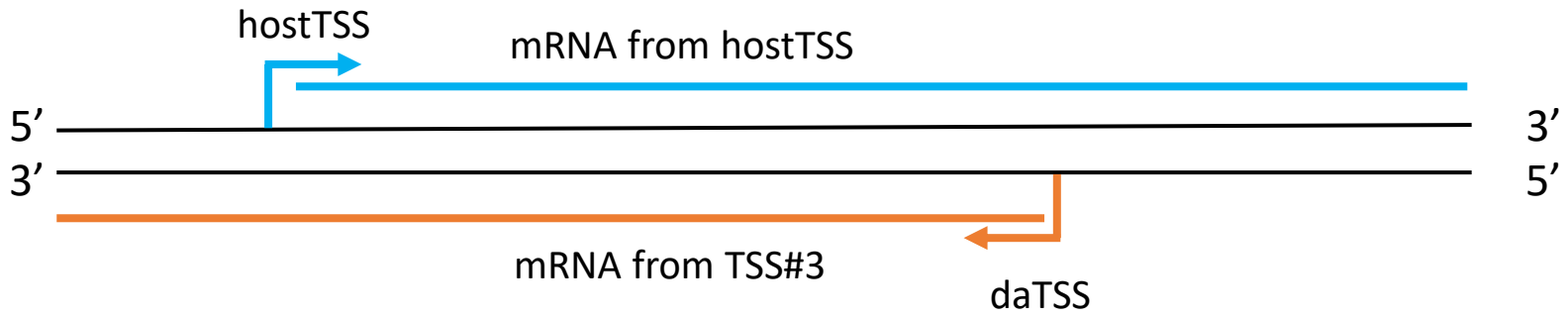

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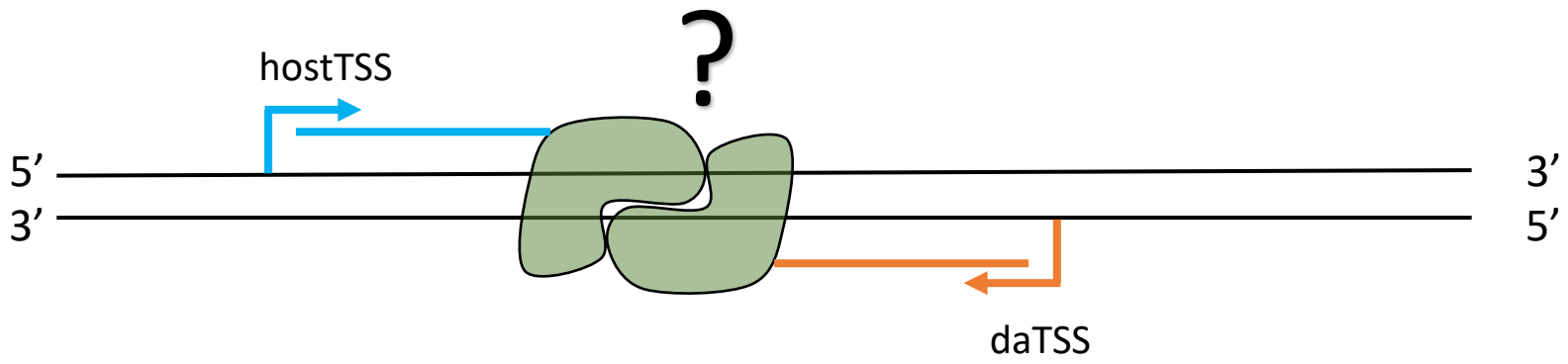

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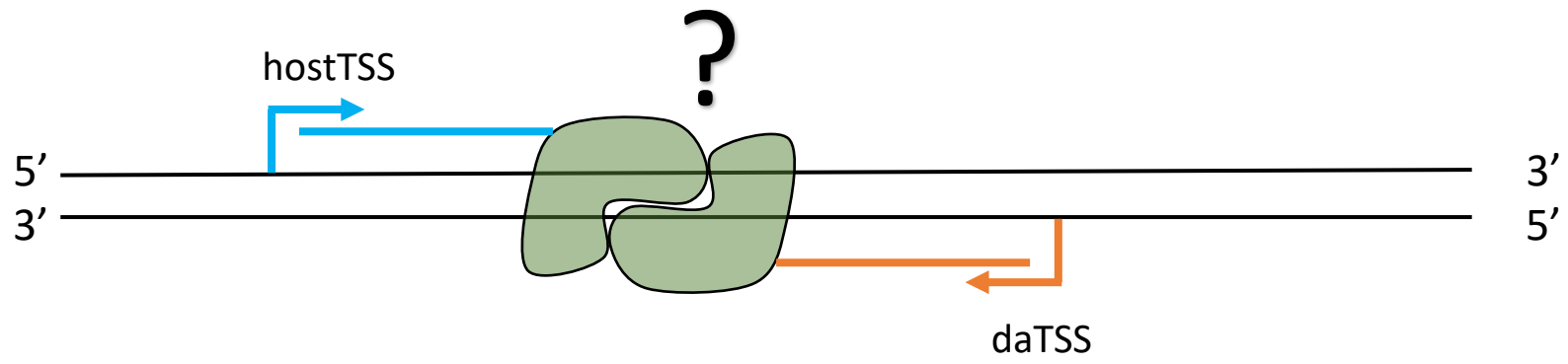
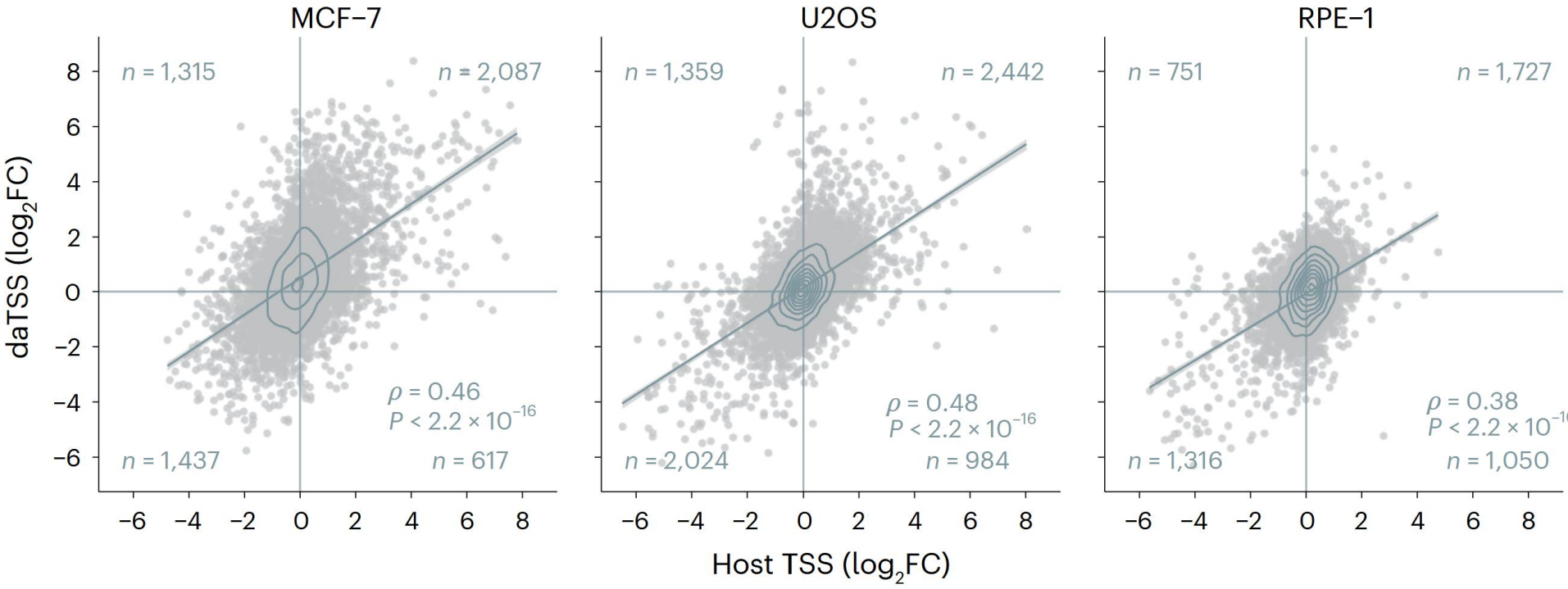
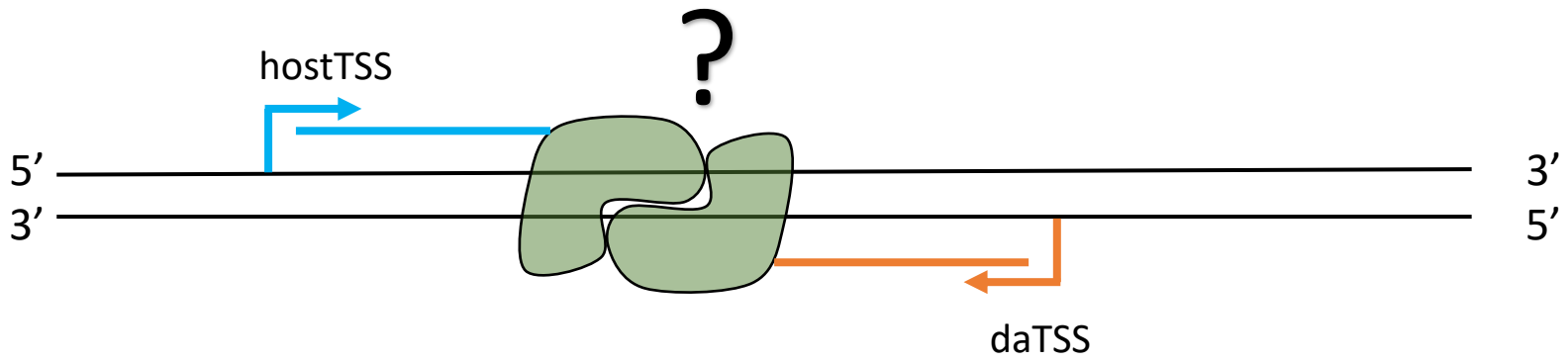
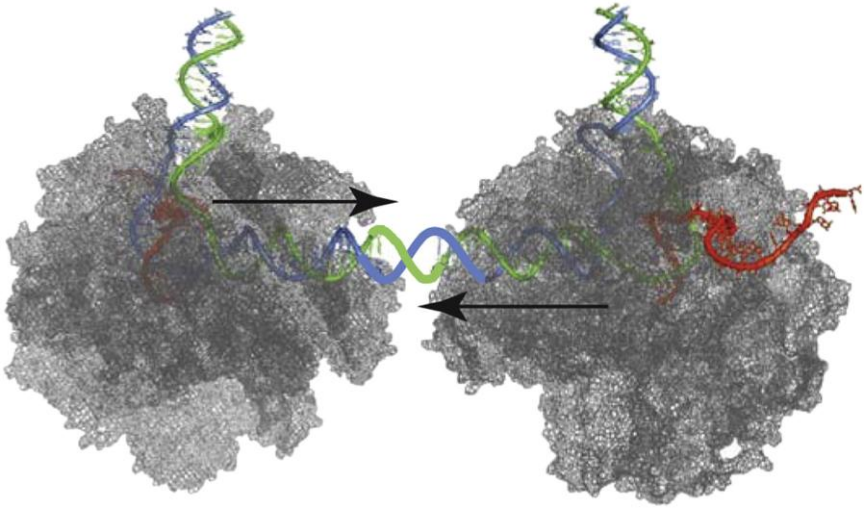


# Yes, RNA polymerases collide into each other and terminate transcription*



* According to some papers

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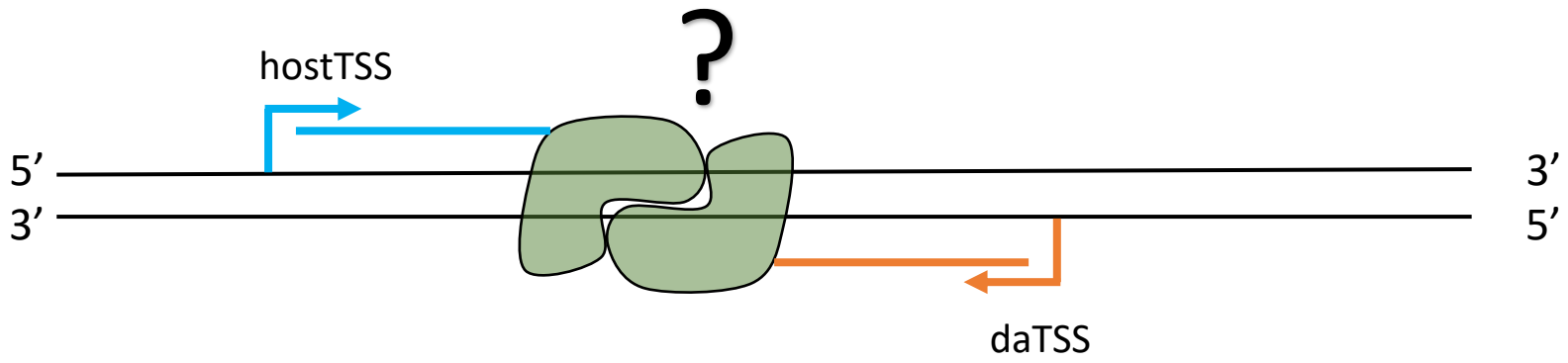


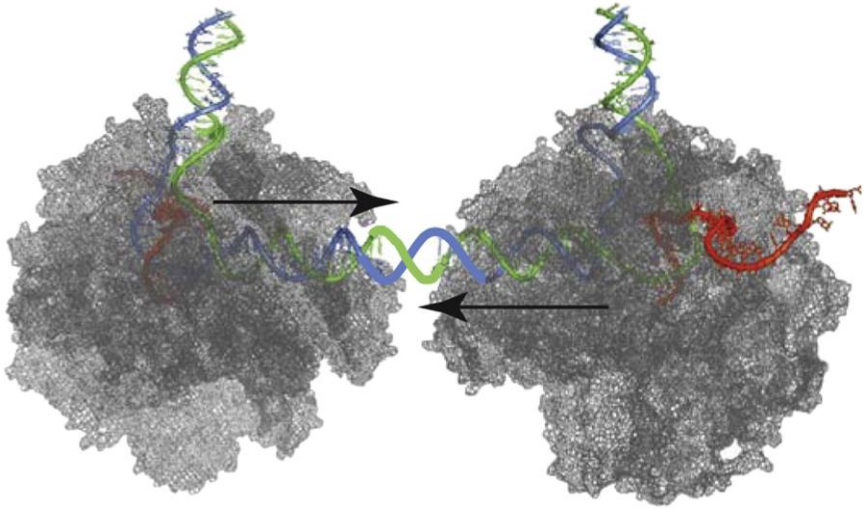

Molecular Cell
Article

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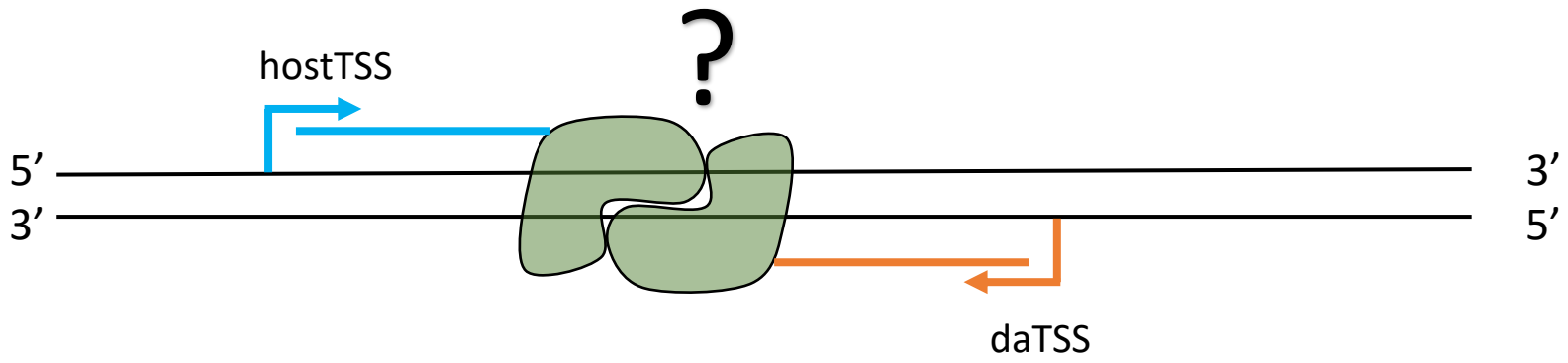


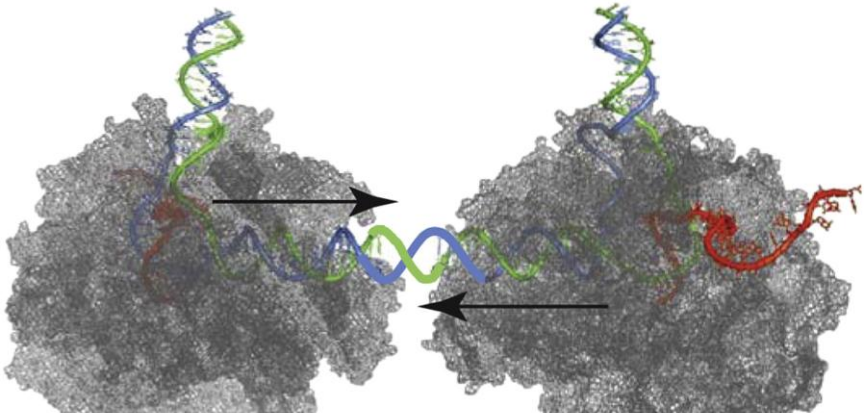

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Elizabeth M. Prescott and Nick J. Proudfoot*

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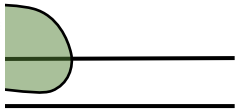
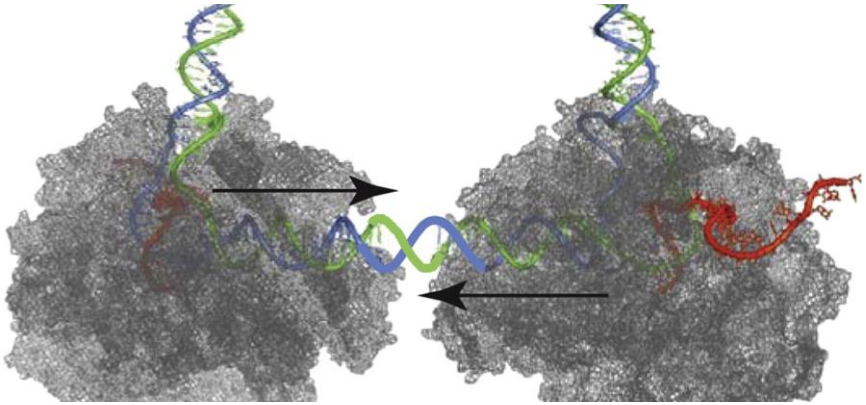
5416–5425 Nucleic Acids Research, 2006, Vol. 34, No. 19
doi:10.1093/nar/gkl668
Published online 29 September 2006




## Collision events between RNA polymerases in convergent transcription studied by atomic force microscopy

Neal Crampton[1,2], William A. Bonass[1], Jennifer Kirkham[1], Claudio Rivetti[3] and Neil H. Thomson[1,2,*]



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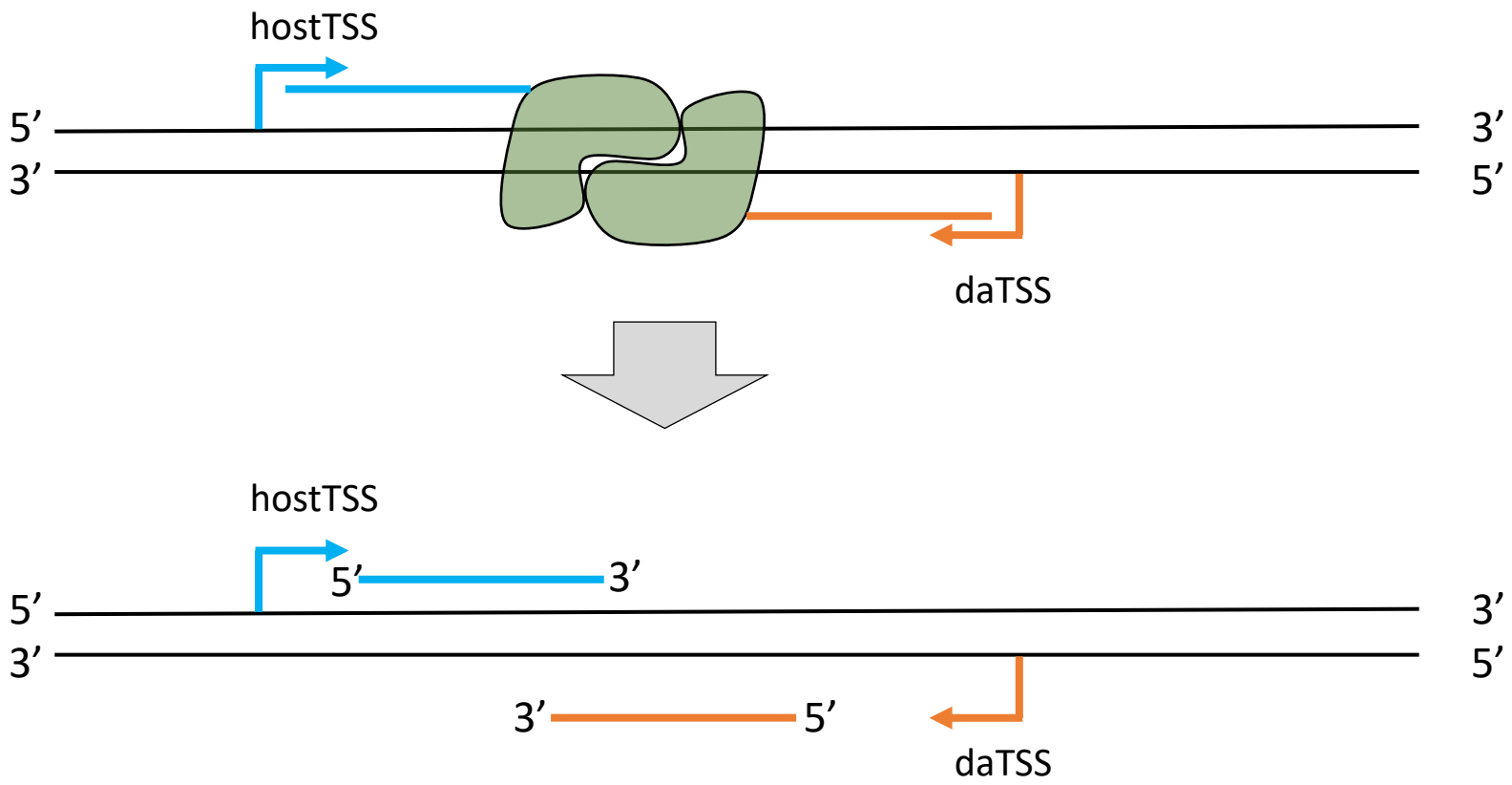
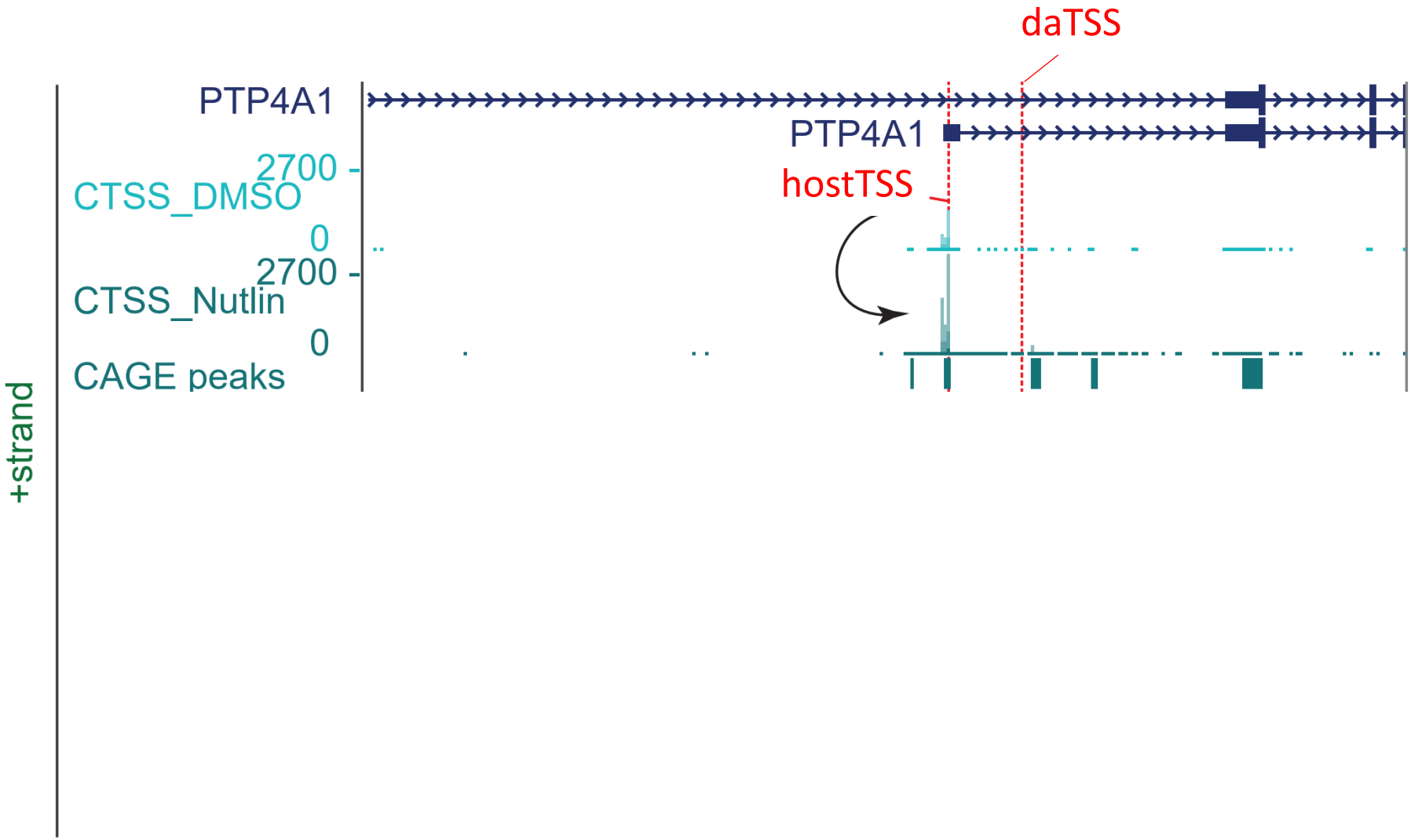


# Example of convergent promoters



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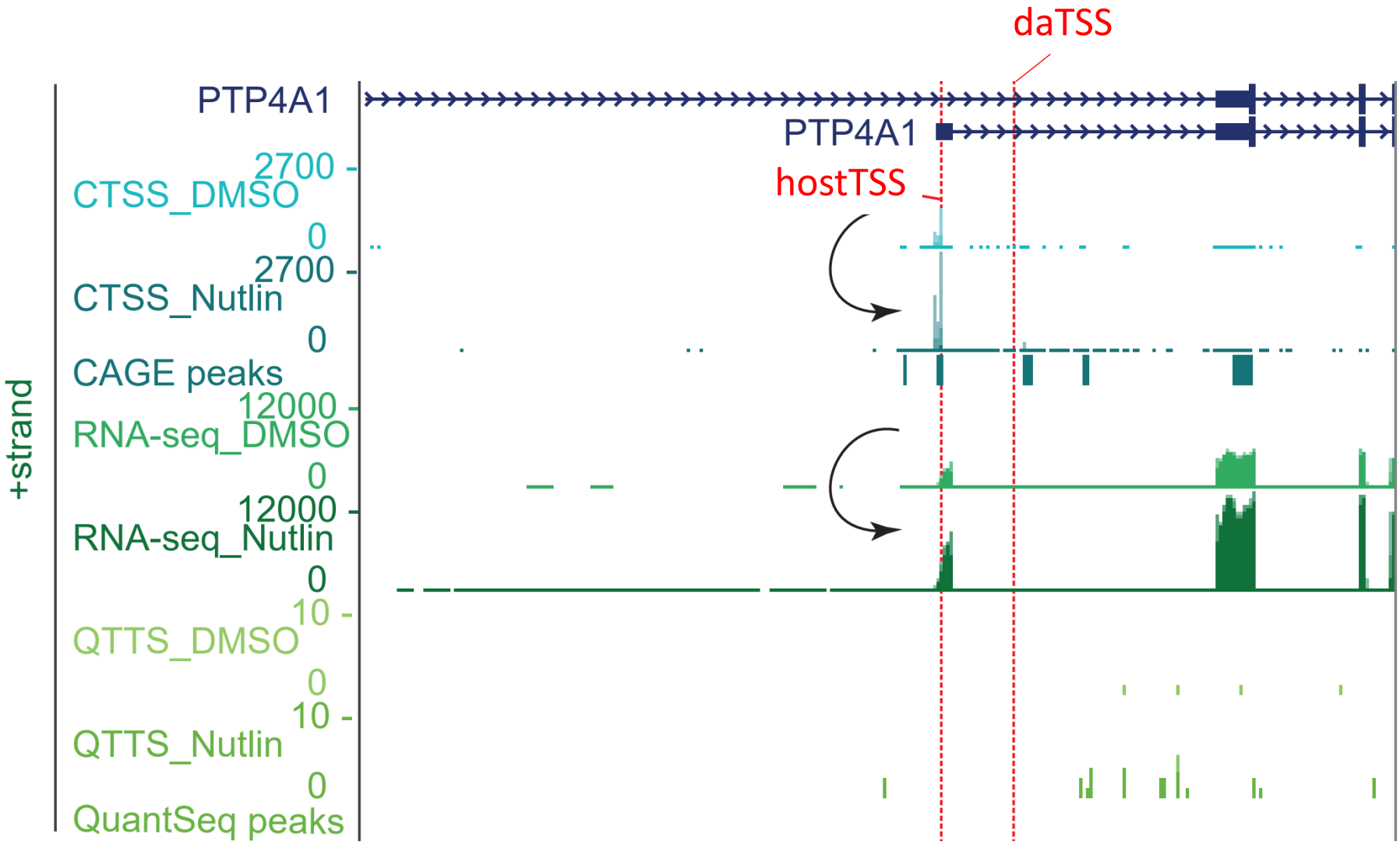

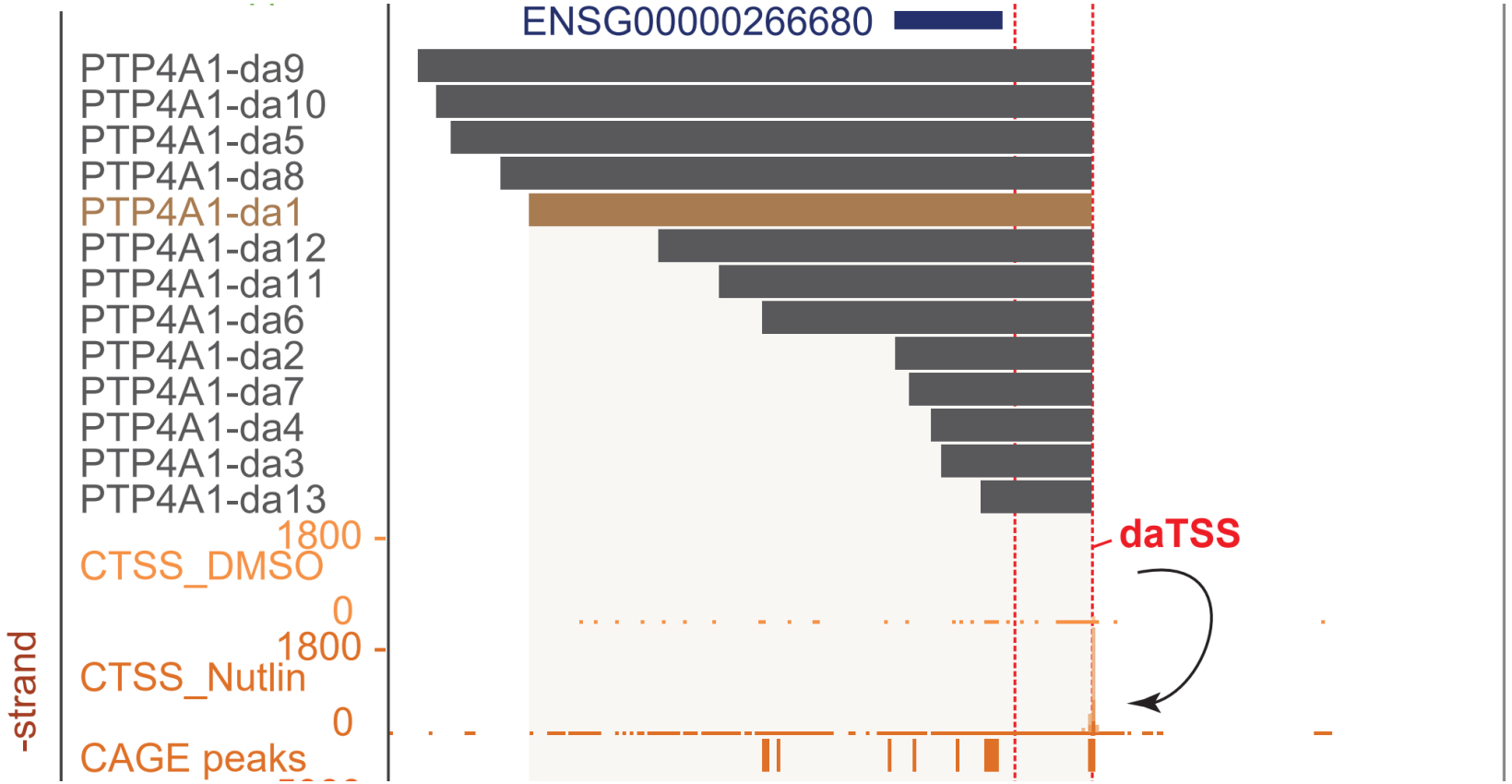
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PTP4A1-da5
PTP4A1-da8
PTP4A1-da1
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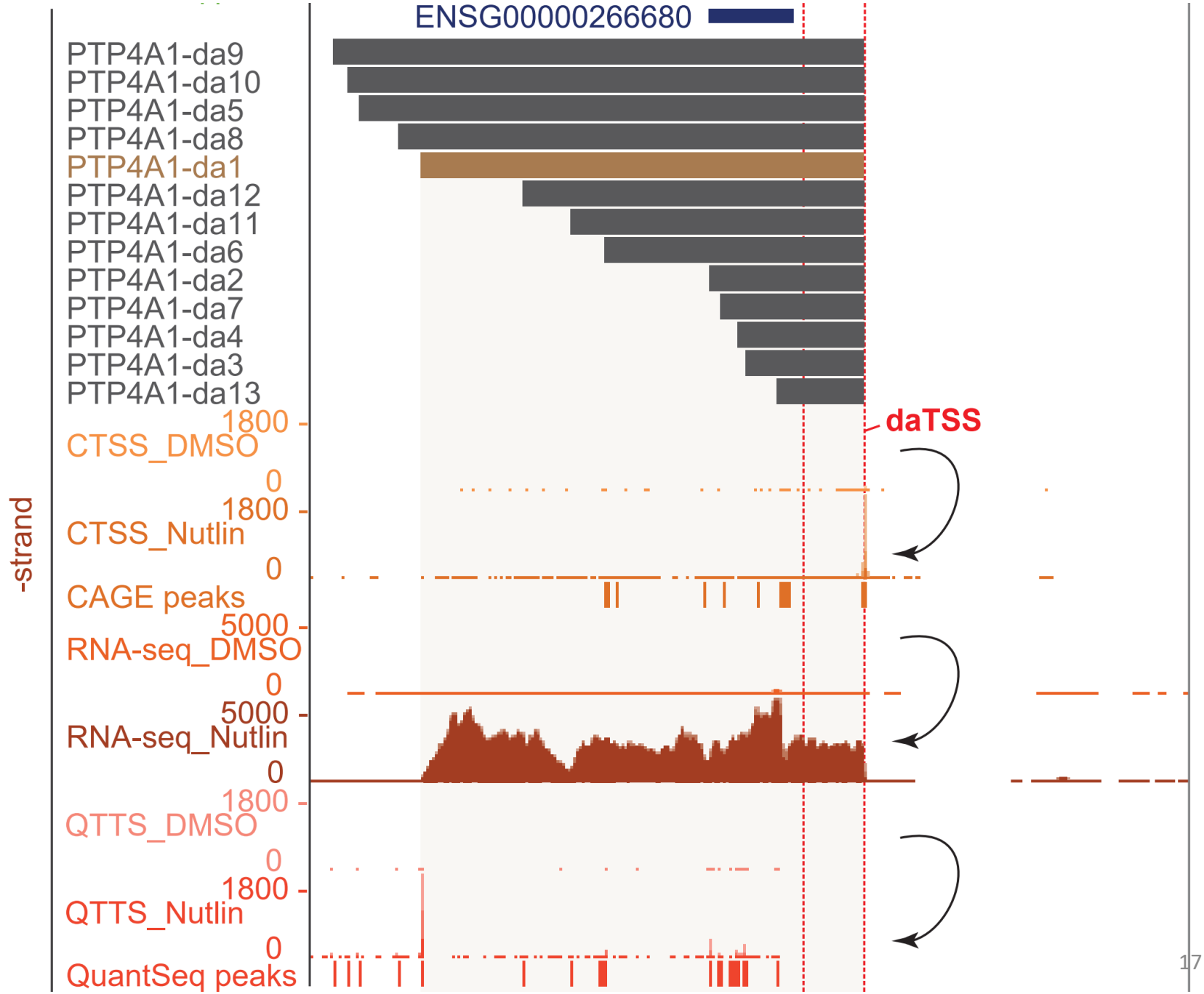
PTP4A1-da11
PTP4A1-da6
PTP4A1-da2
PTP4A1-da7
PTP4A1-da4
PTP4A1-da3
PTP4A1-da13
1800
CTSS_DMSO
0
1800
CTSS_Nutlin
0
CAGE peaks
-strand
daTSS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PTP4A1-da6
PTP4A1-da2
PTP4A1-da7
PTP4A1-da4
PTP4A1-da3
PTP4A1-da13
daTSS
1800
CTSS_DMSO
0
1800
CTSS_Nutlin
0
CAGE peaks
5000
RNA-seq_DMSO
0
5000
RNA-seq_Nutlin
0
1800
QTTS_DMSO
0
1800
QTTS_Nutlin
0
QuantSeq peaks
-strand

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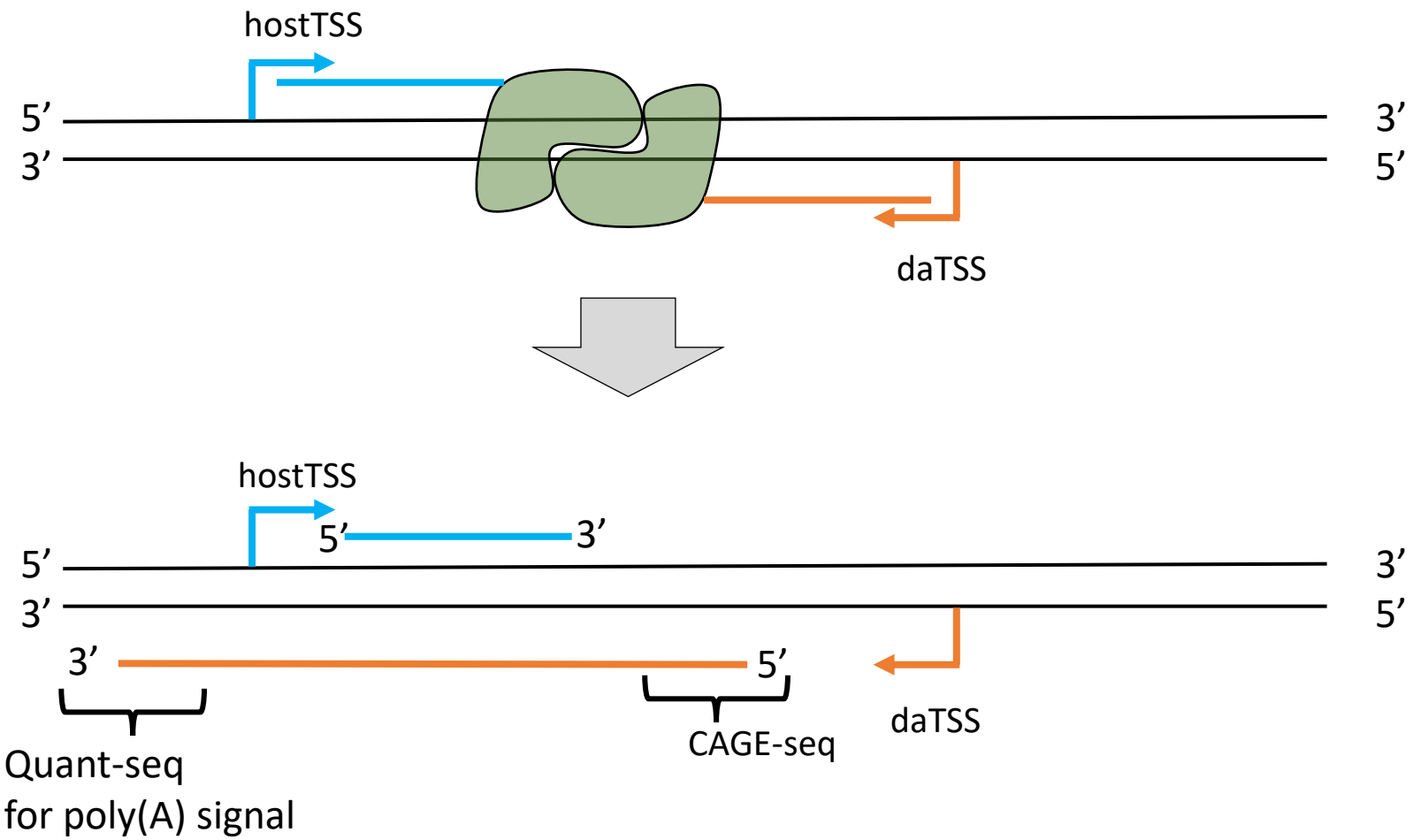

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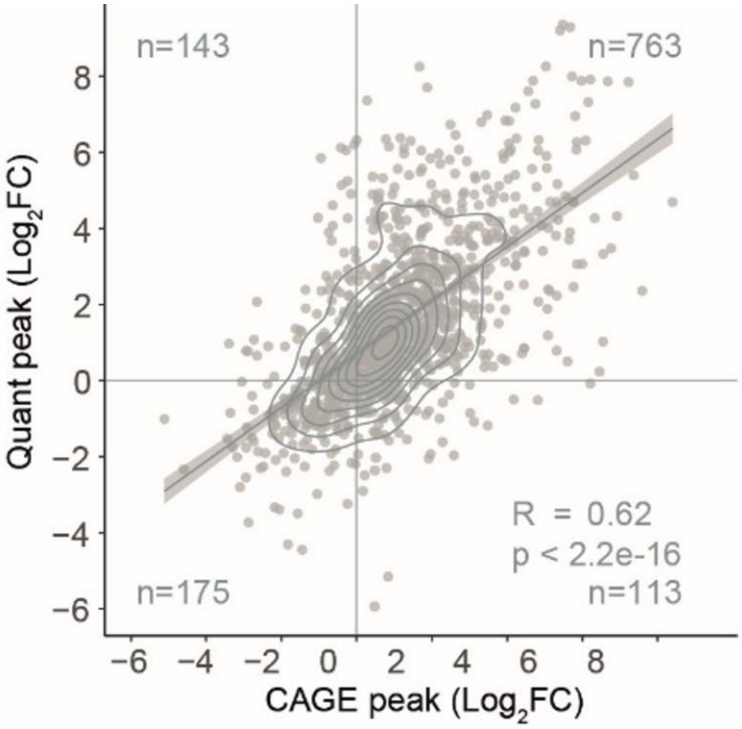
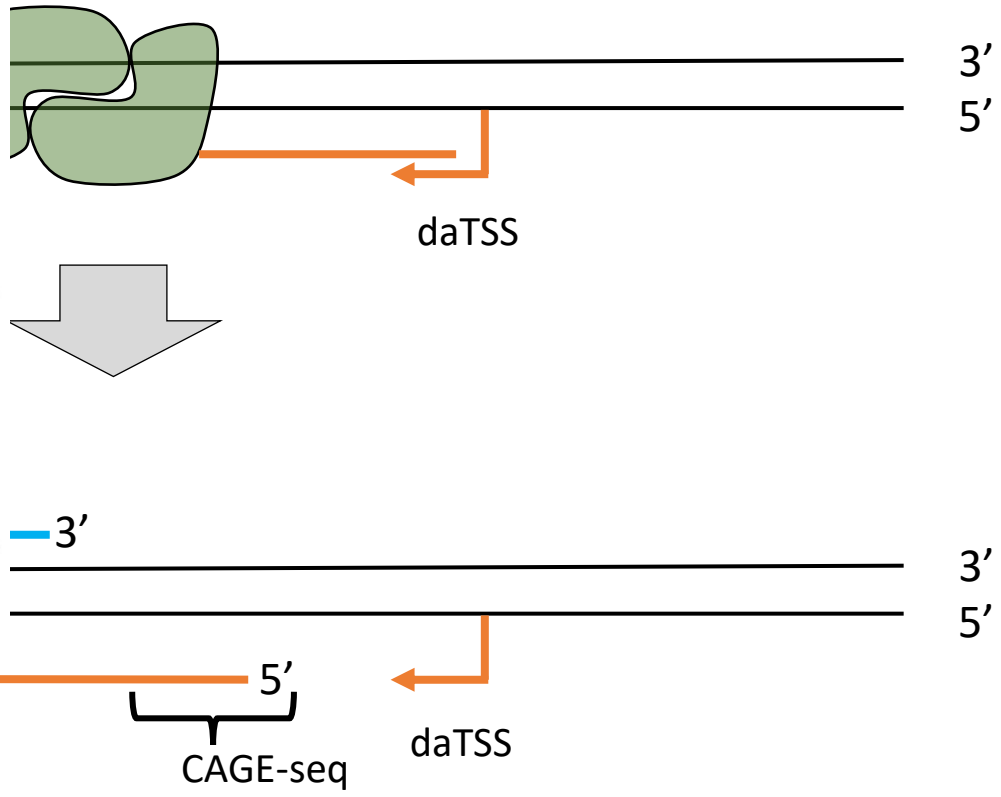
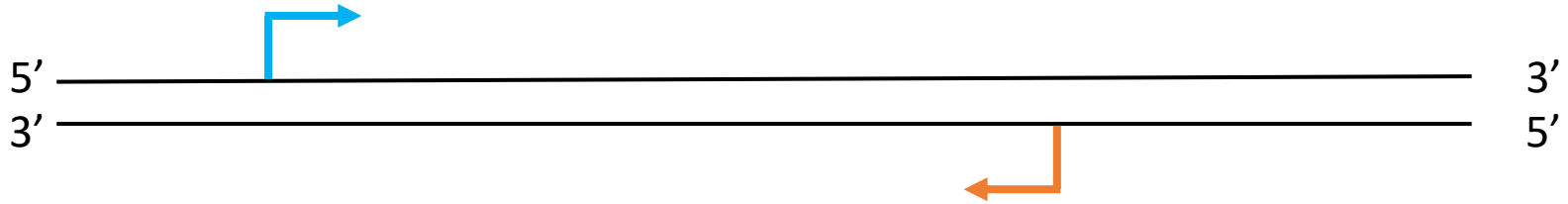
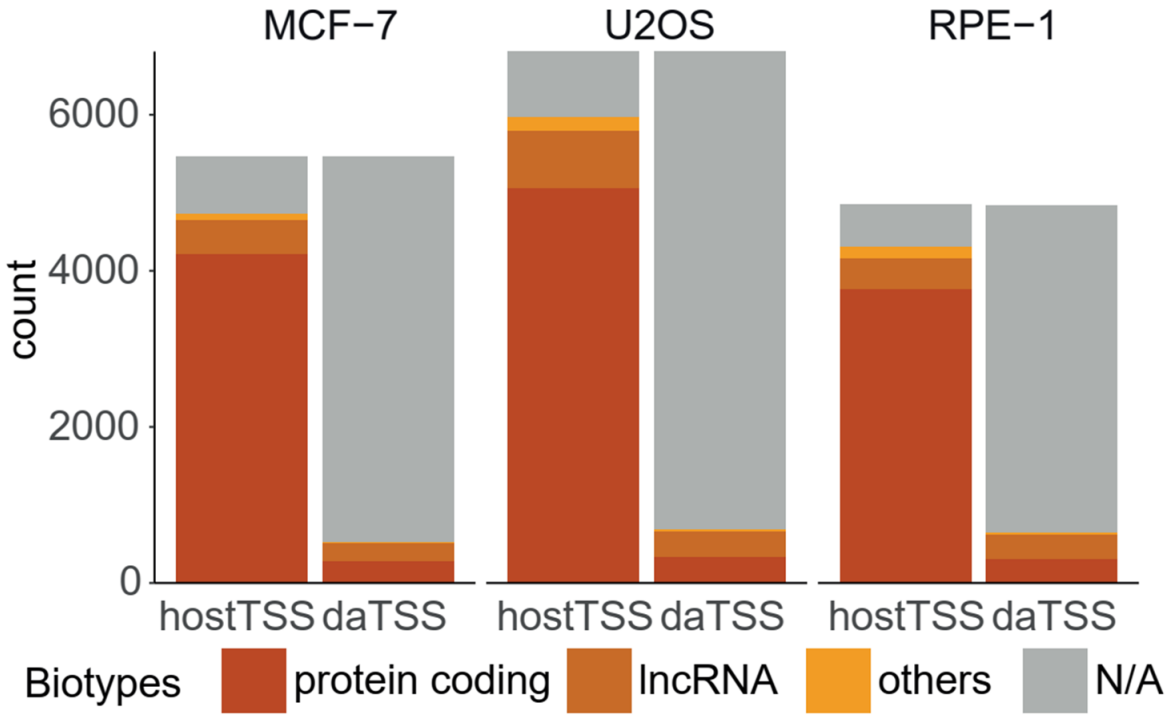


# How common are downstream antisense RNAs at TSS#3?



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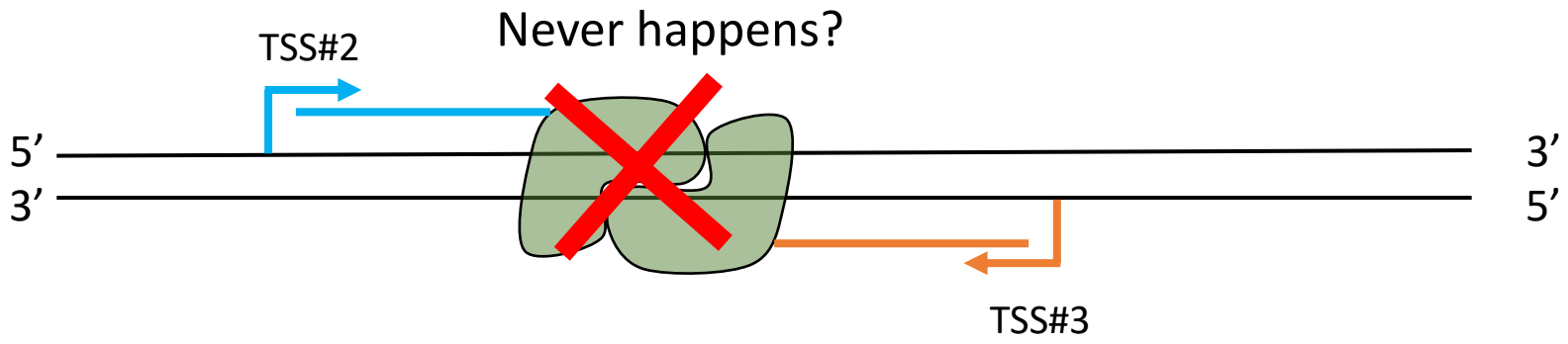
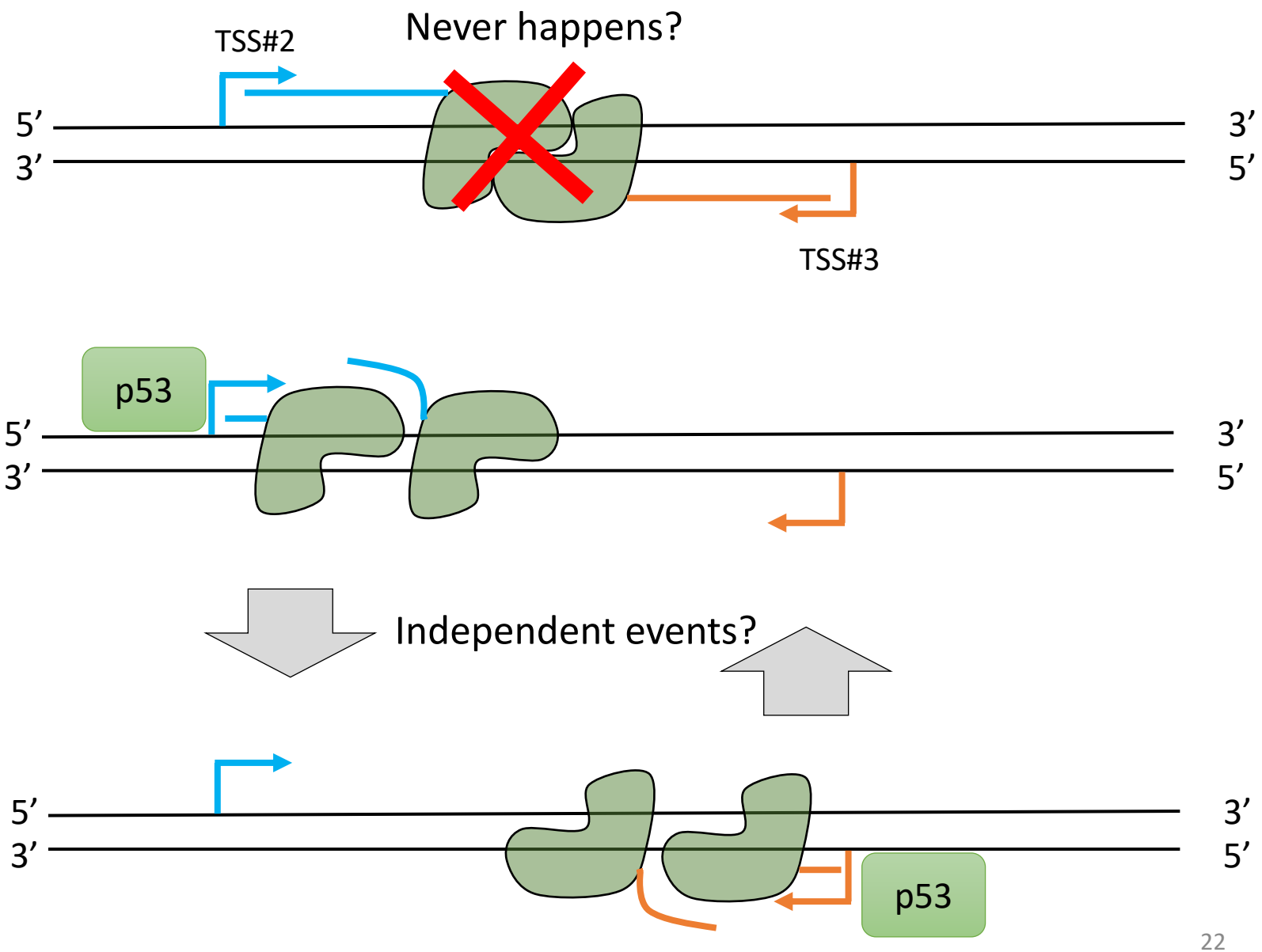
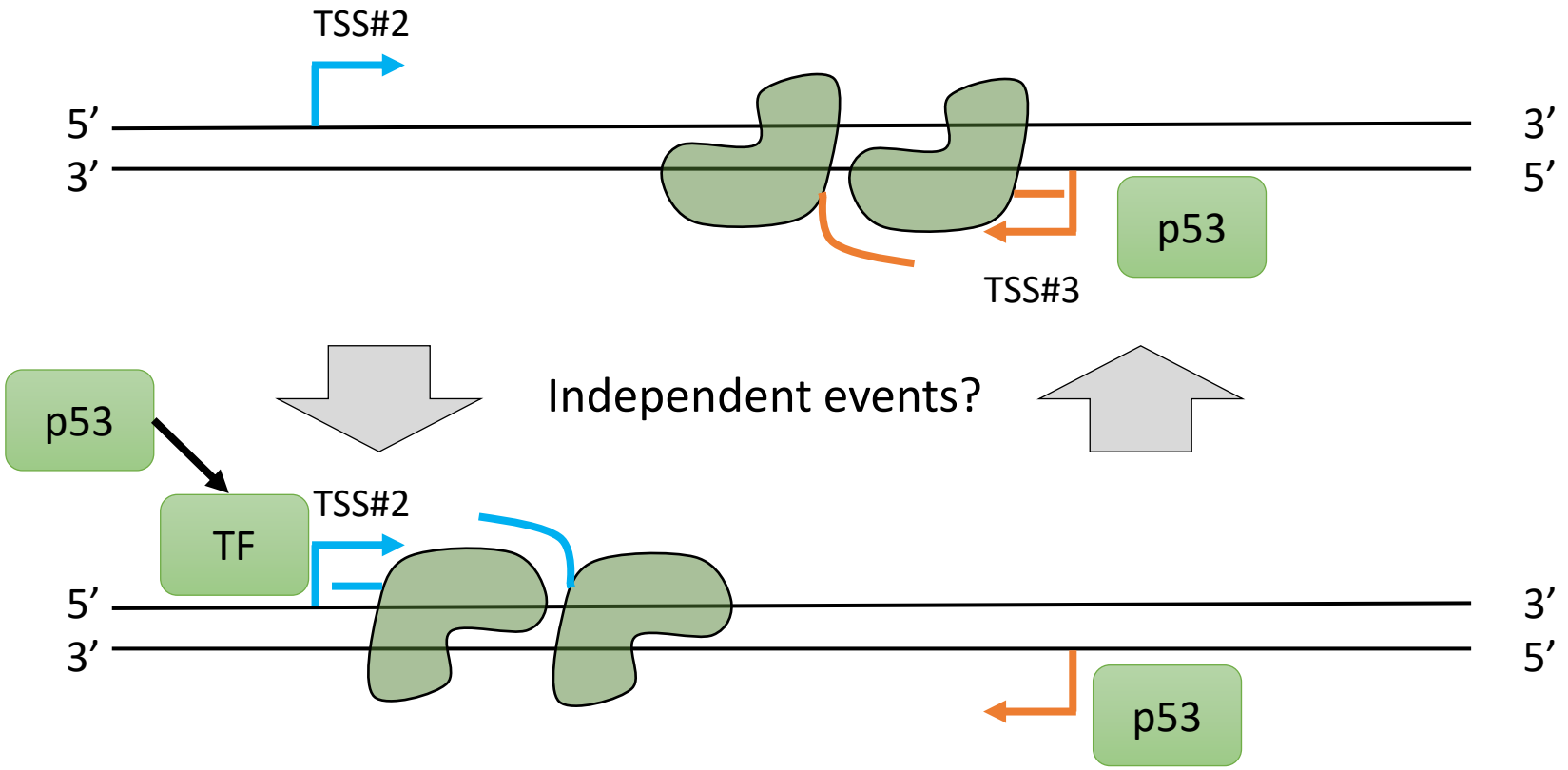


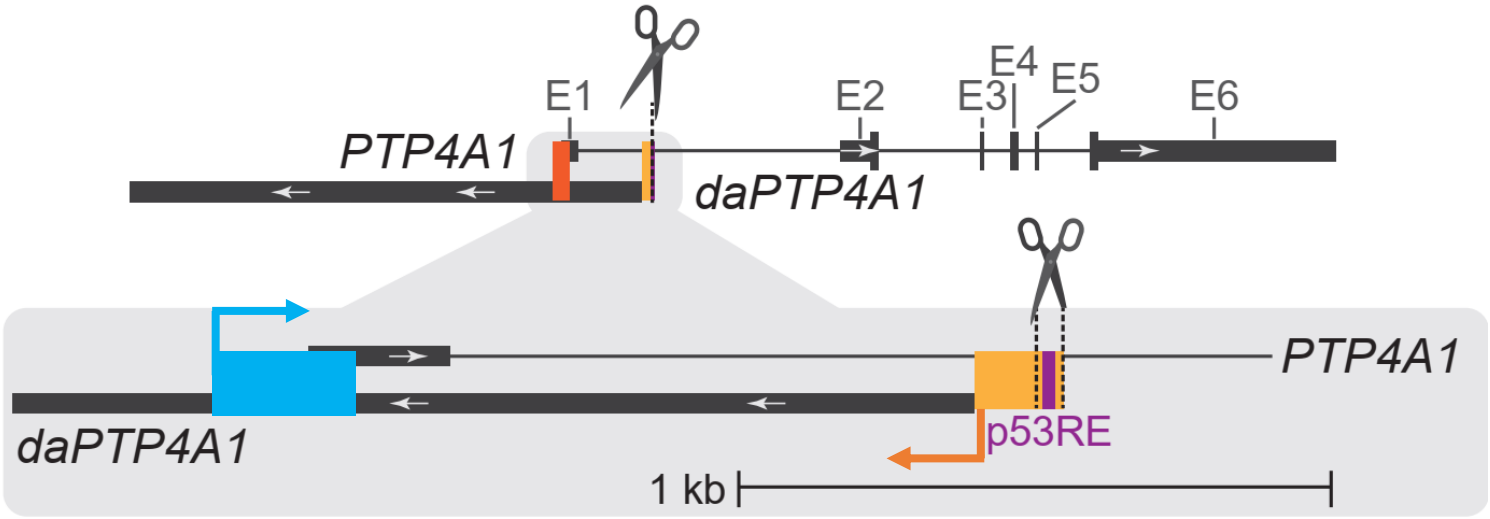
# Independent regulation of convergent promoters?



# Indirect influence of p53 on hostTSS and daTSS?



# Knockout of p53 responsive element (p53RE) to check causality



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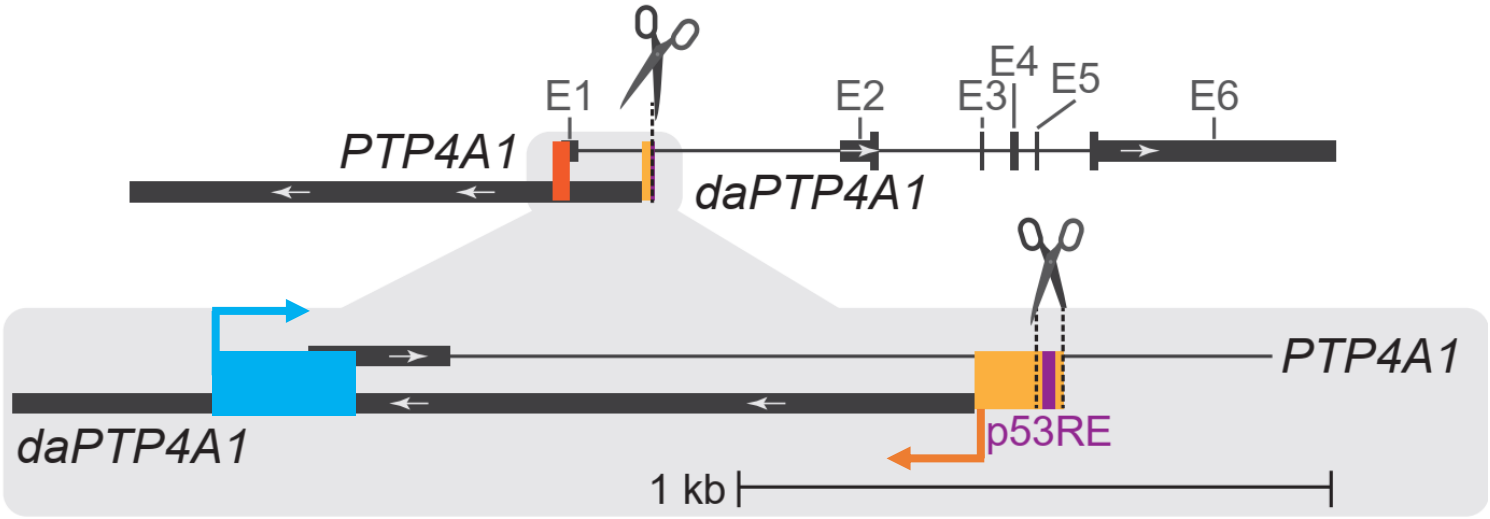


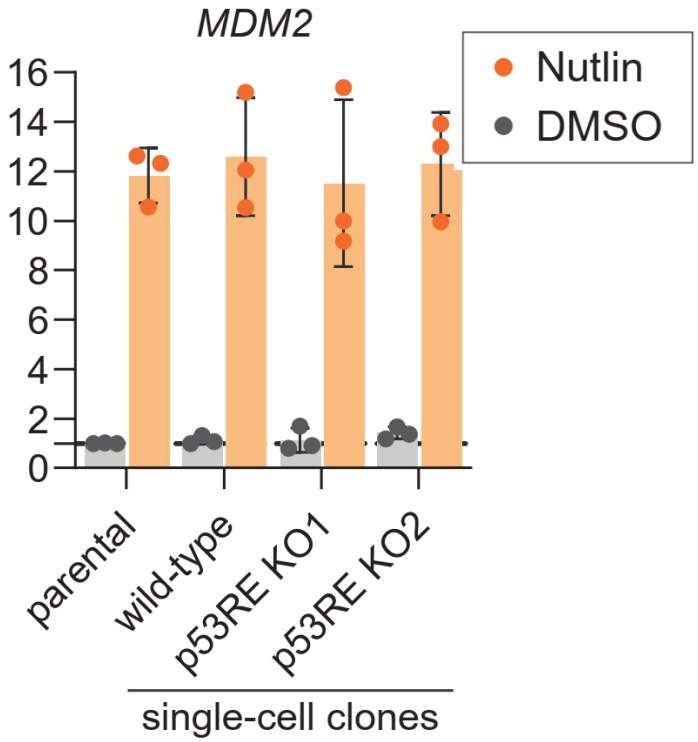

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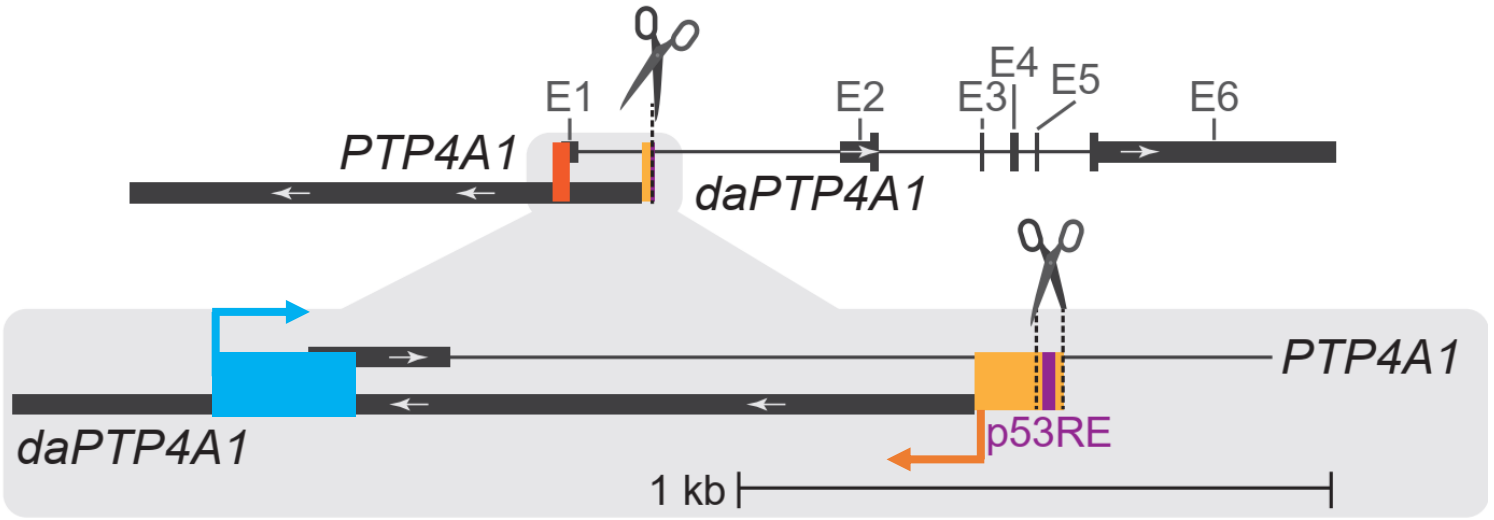


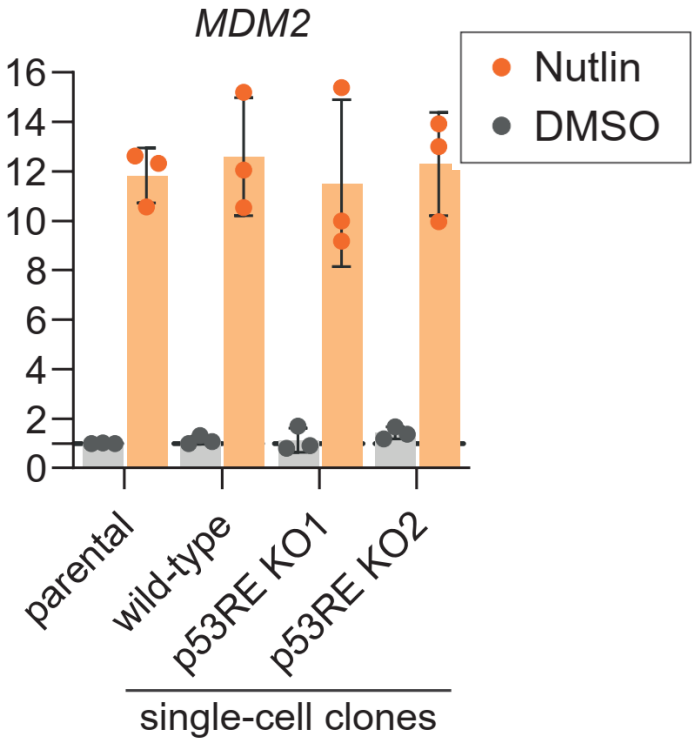


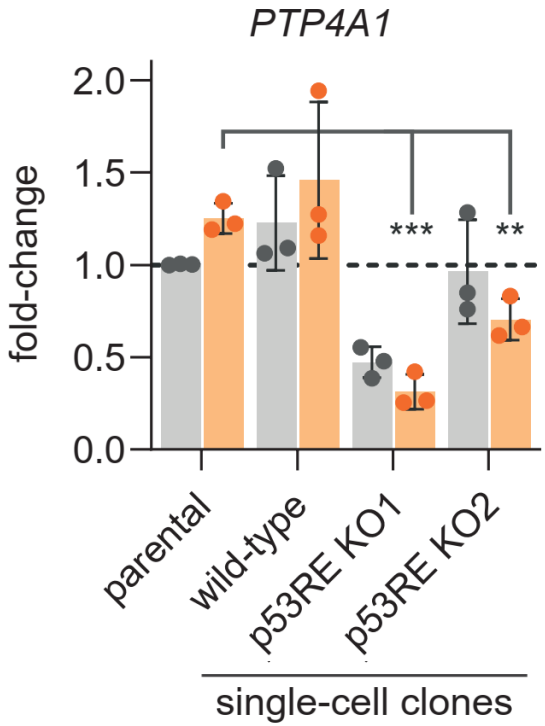

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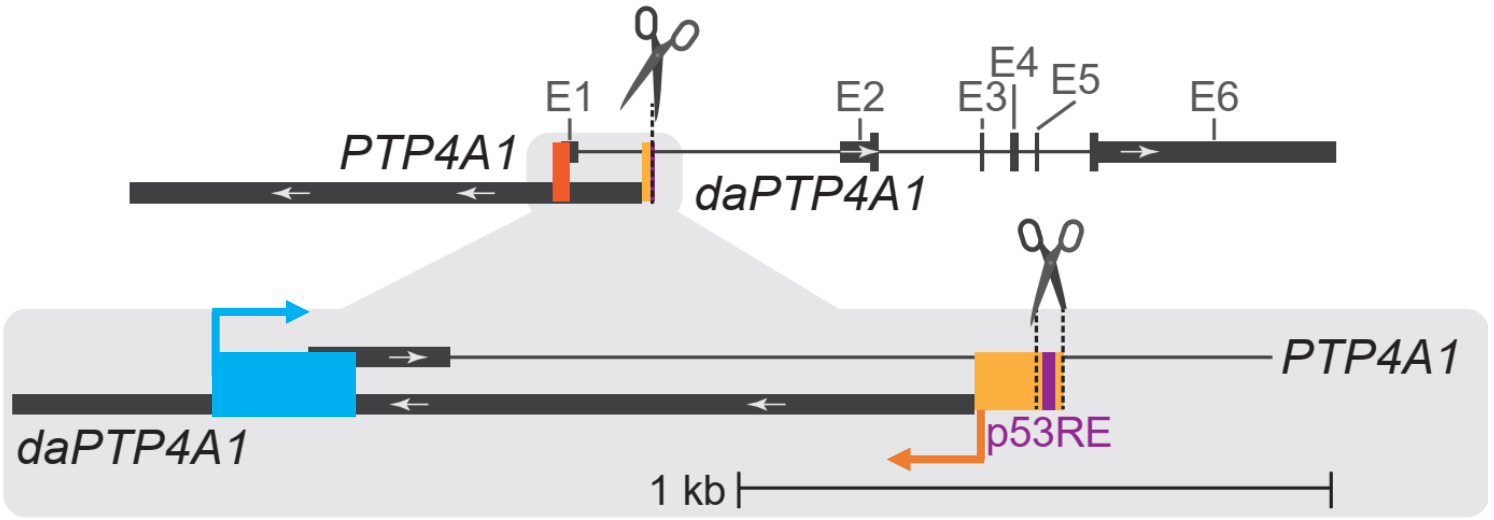


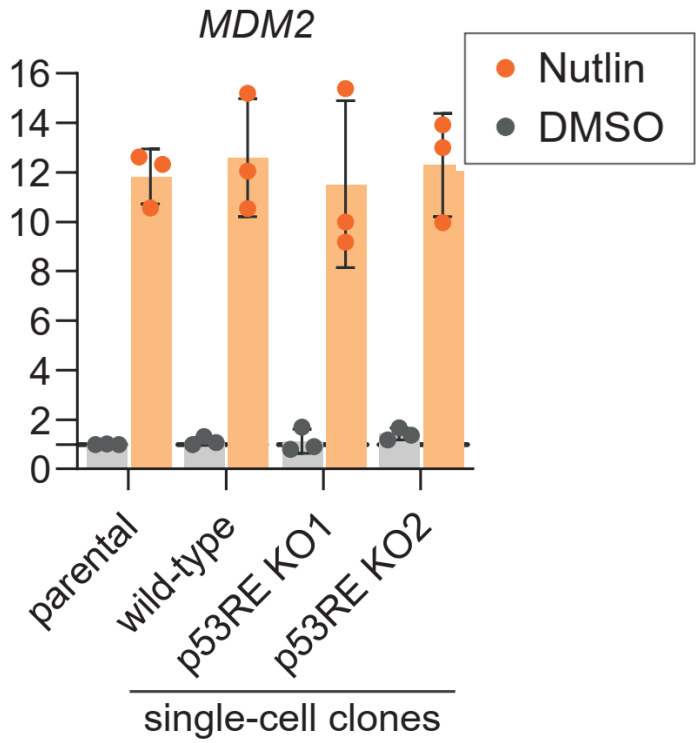


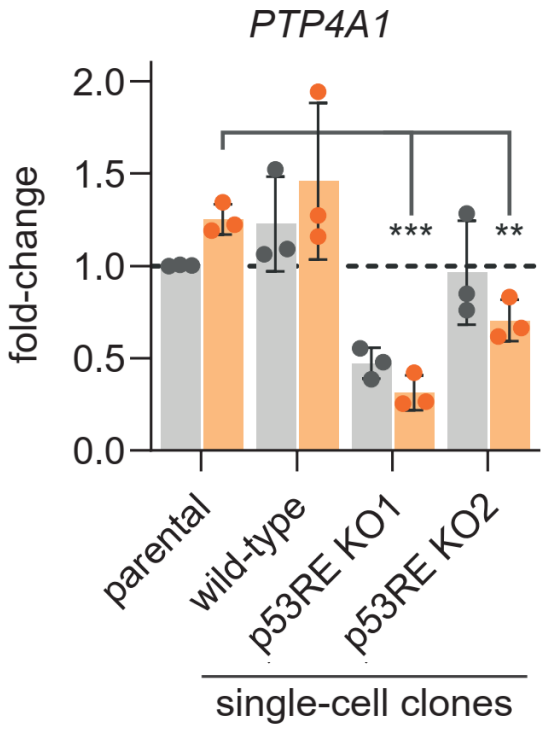


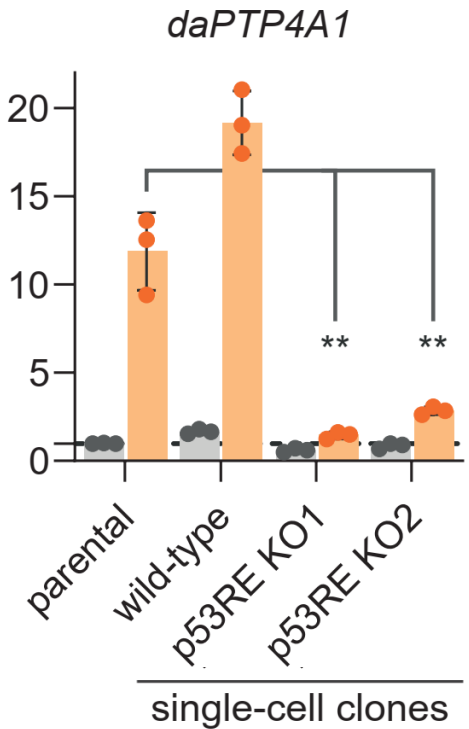

# Major take-home points

- ¼ of active TSSs in human cell lines leads to overlapping convergent transcription
- Expression of hostTSS and daTSS are positively correlated and causally linked.

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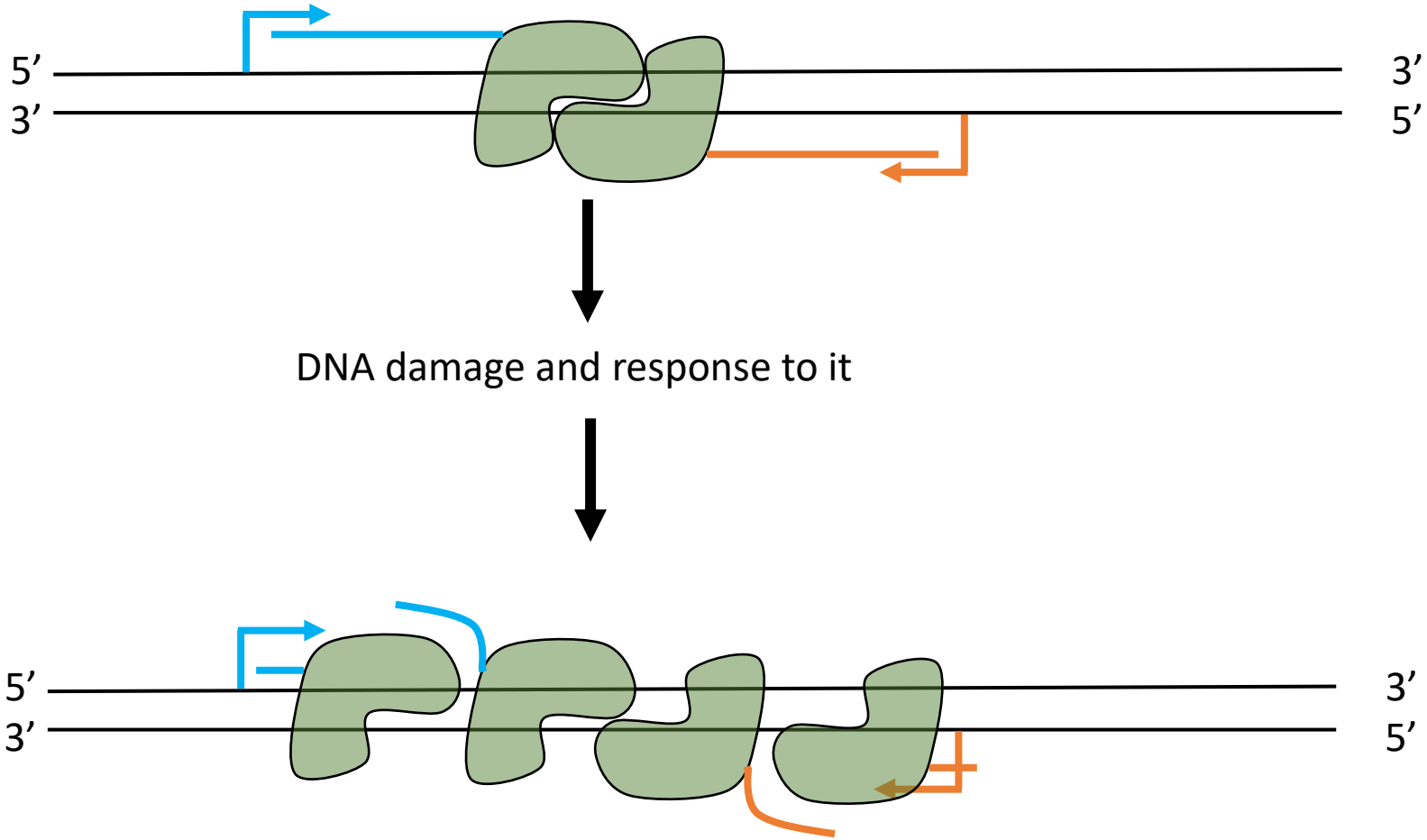
… but what is the mechanism behind this link?

# Collision as enhancer of transcription?



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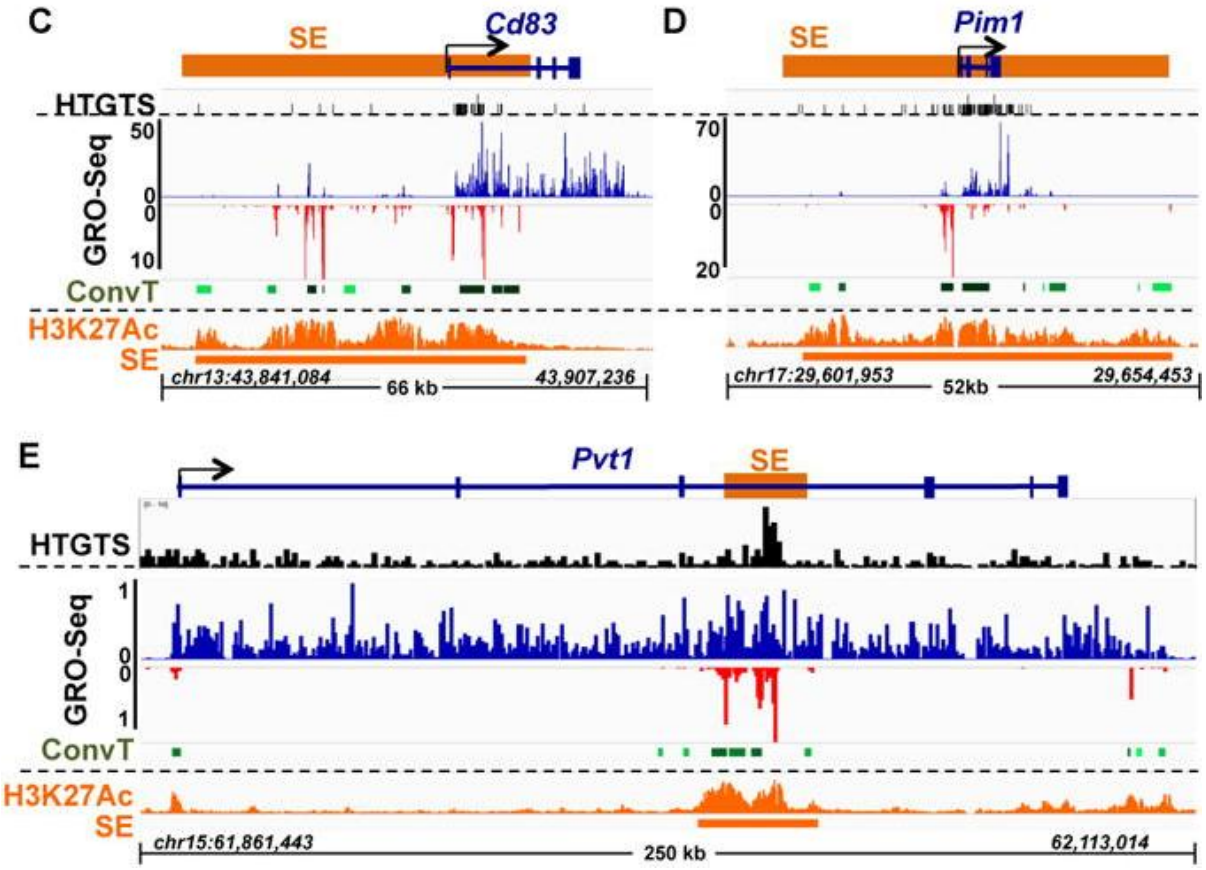
Published in final edited form as:
*Cell*. 2014 December 18; 159(7): 1538–1548. doi:10.1016/j.cell.2014.11.014.

## Convergent Transcription At Intragenic Super-Enhancers Targets AID-initiated Genomic Instability



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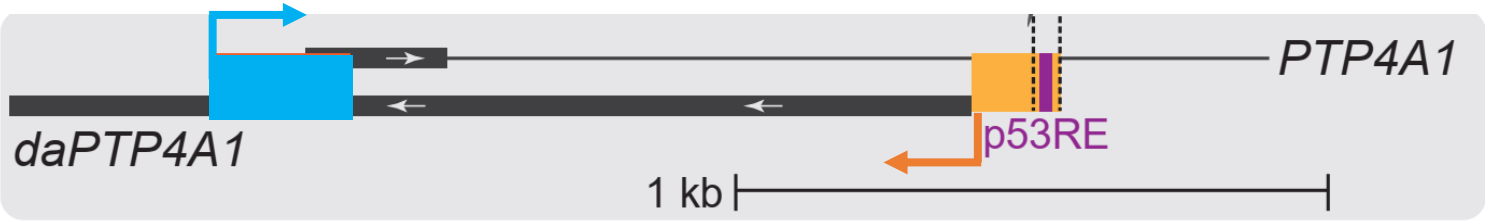
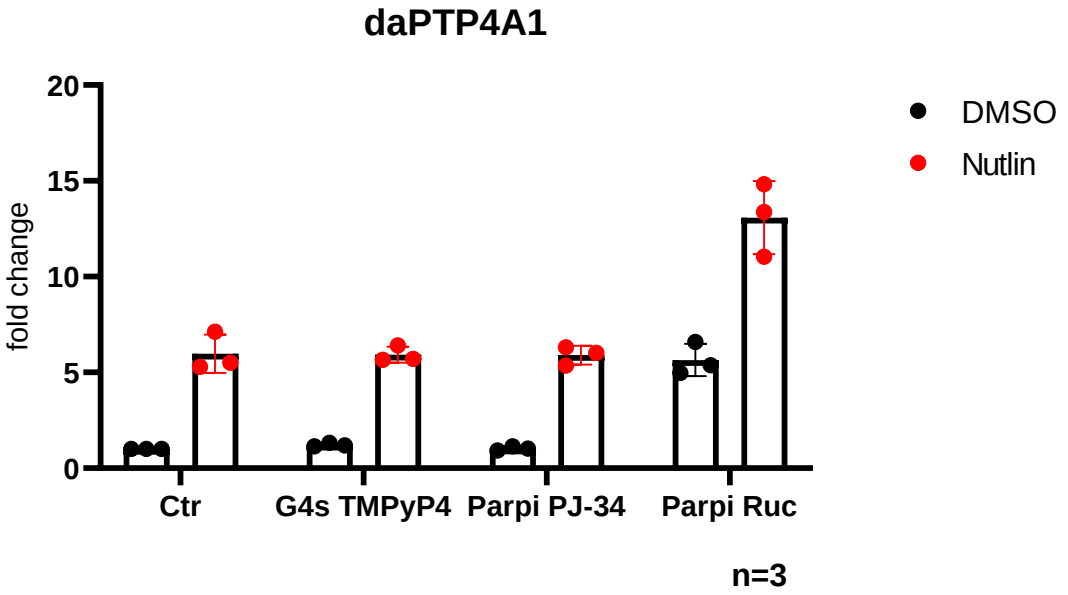
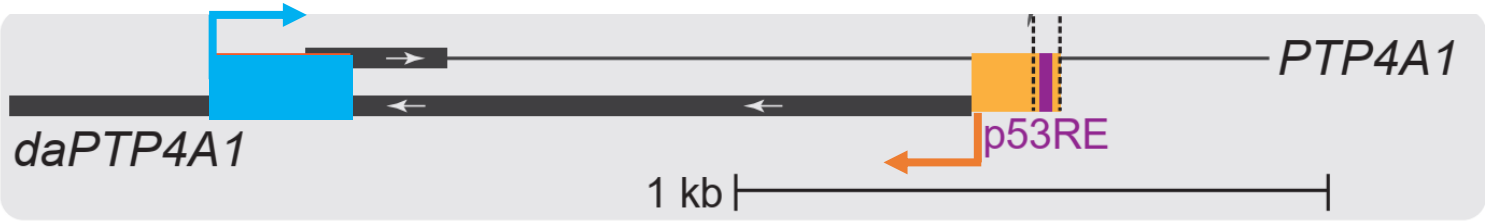
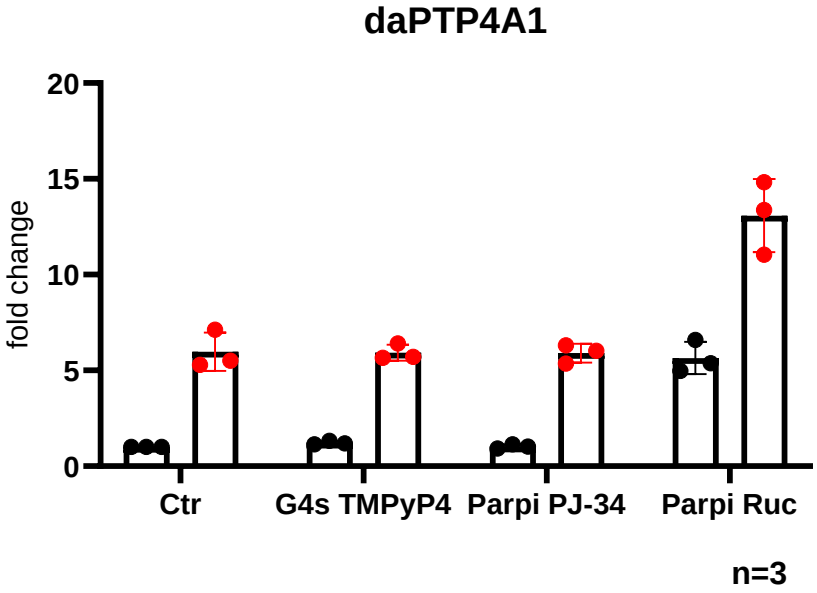
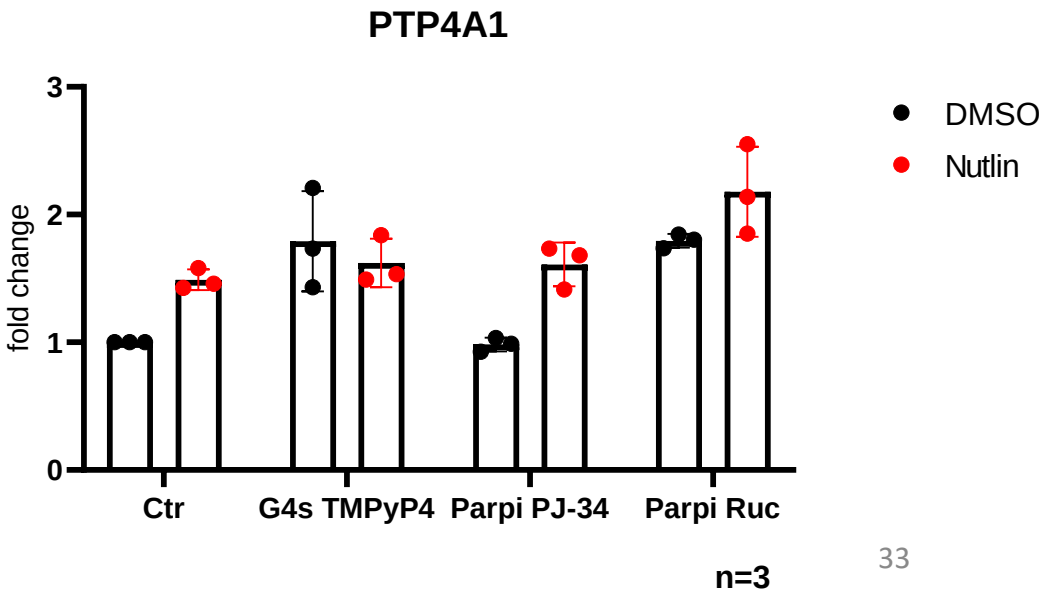


# Parp1 seems to mediate cooperativity



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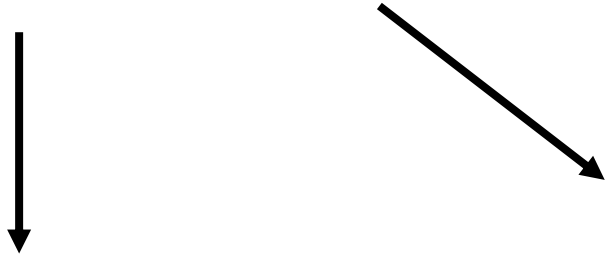
Parp1 doesn't exist in yeast



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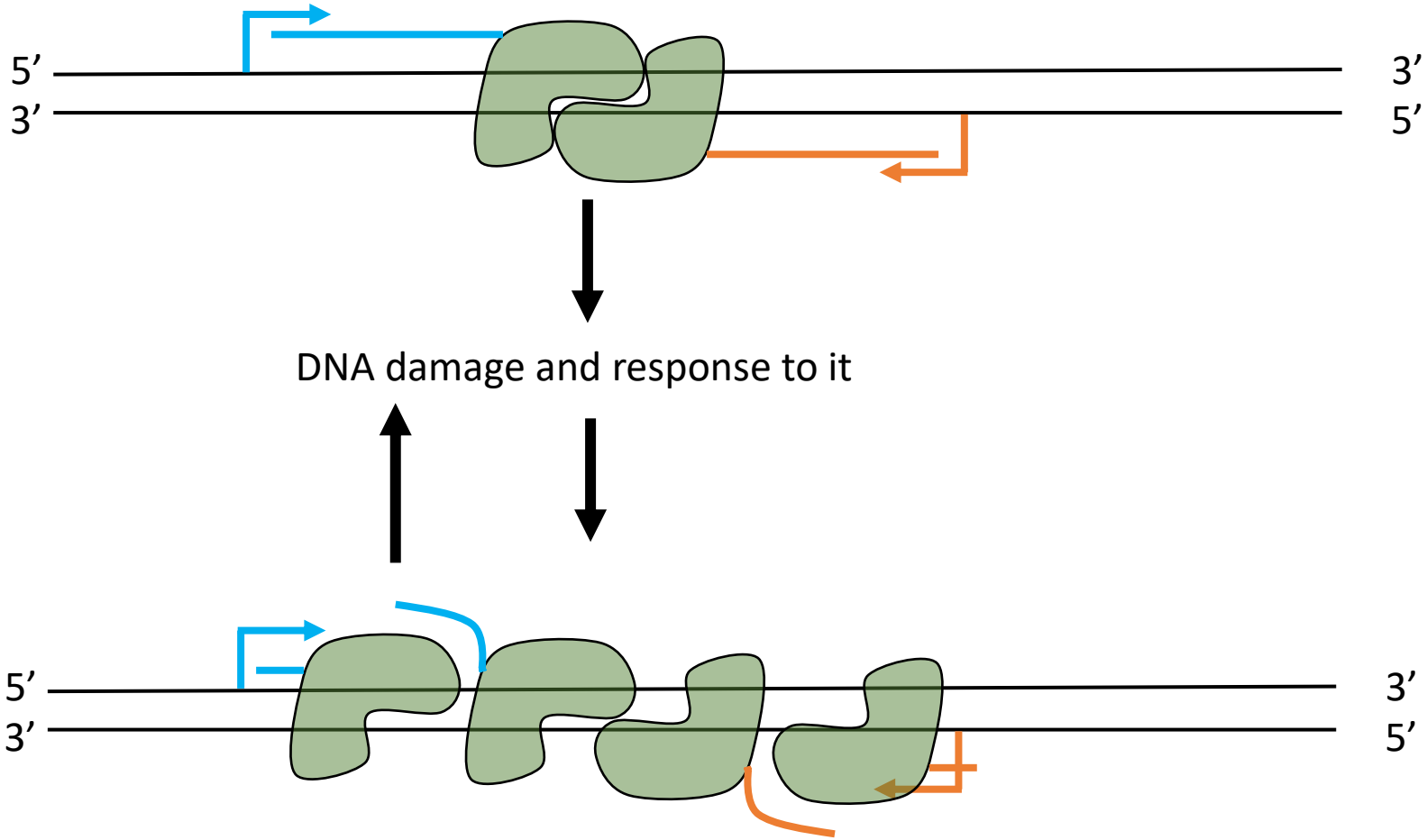
# Transcription as driver of mutagenesis



# Battle for DNA damage?

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# Acknowledgements

Thanks:
Steve Hoffmann
Martin Fischer
Elina Weichens
Tushar Patel
**Konstantin Riege**
Robert Schwarz
Katjana Schwab
Silke Förste

Sequencing Facility

Thank you for your attention!

nature genetics



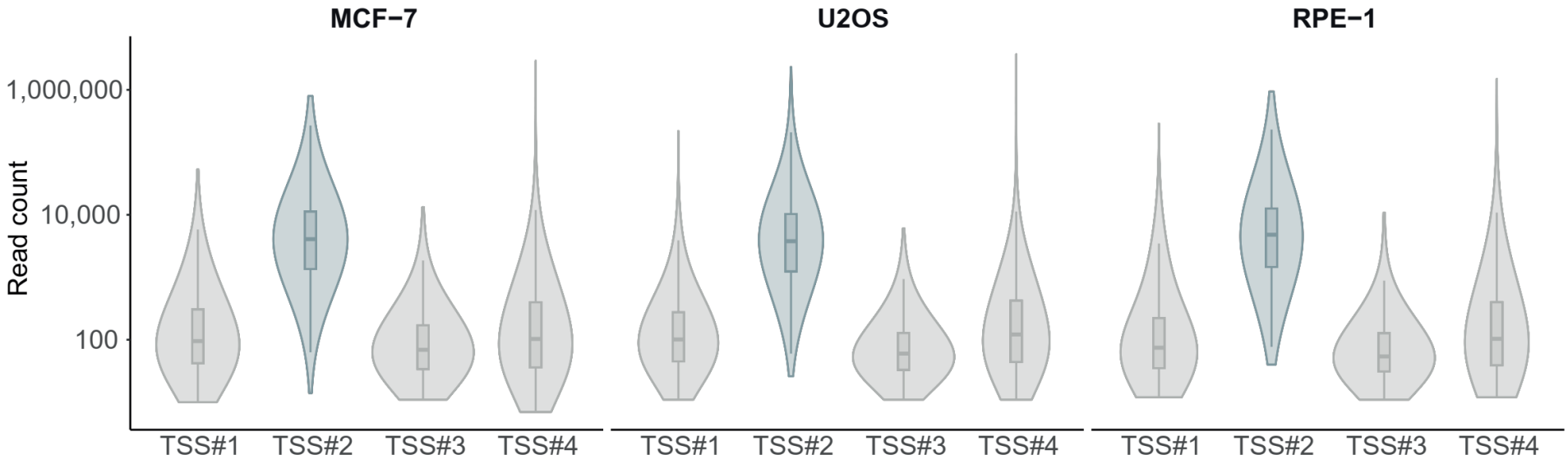
Article | Open access | Published: 06 January 2025

# Gene regulation by convergent promoters

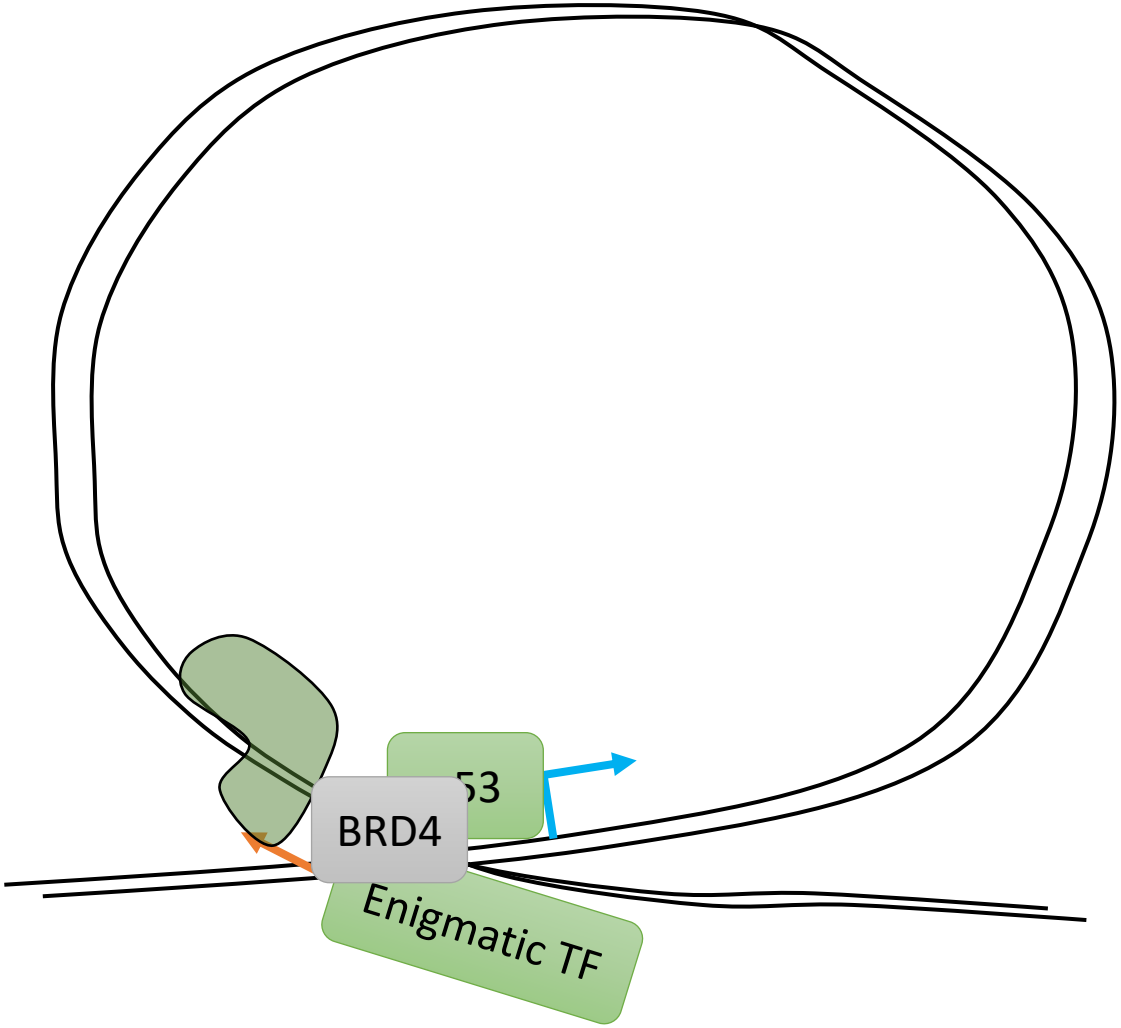
Elina Wiechens, Flavia Vigliotti, Kanstantsin Siniuk, Robert Schwarz, Katjana Schwab, Konstantin Riege, Alena van Bömmel, Ivonne Görlich, Martin Bens, Arne Sahm, Marco Groth, Morgan A. Sammons, Alexander Loewer, Steve Hoffmann ✉ & Martin Fischer ✉

*Nature Genetics* **57**, 206–217 (2025) | Cite this article

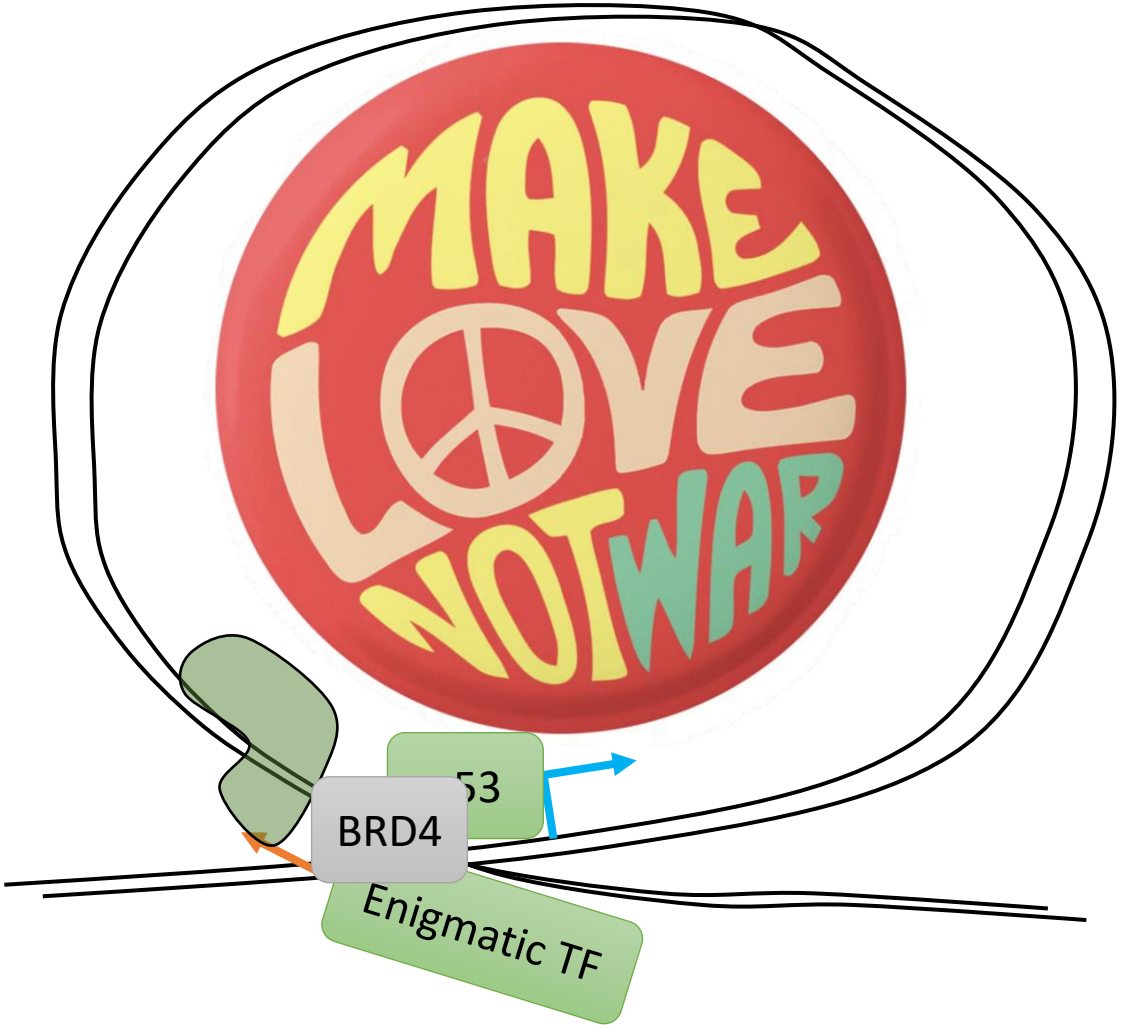
# What are expression levels of transcripts from TSS 1, 2, 3, 4?



# Outlook: spatial cooperation can be one of explanations

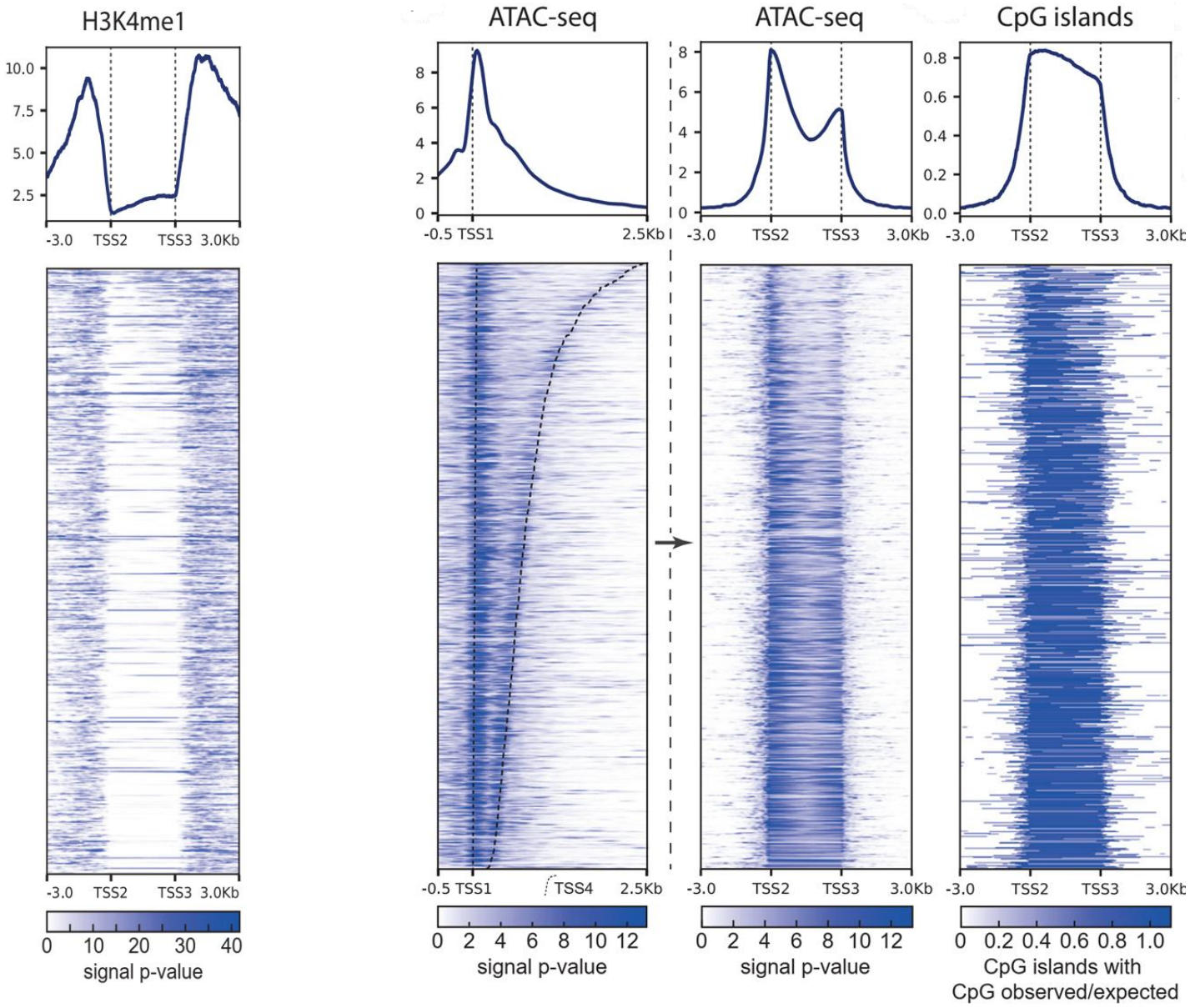


# Outlook: spatial cooperation can be one of explanations

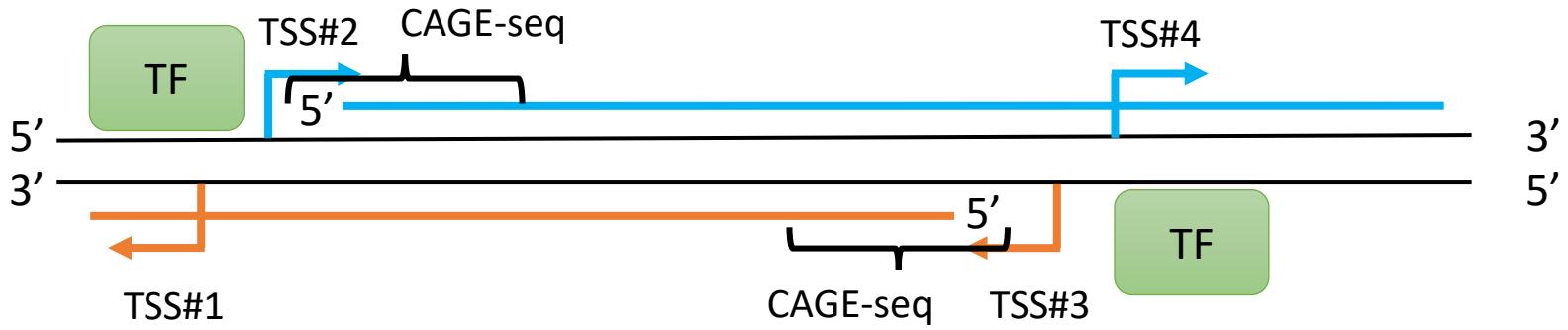


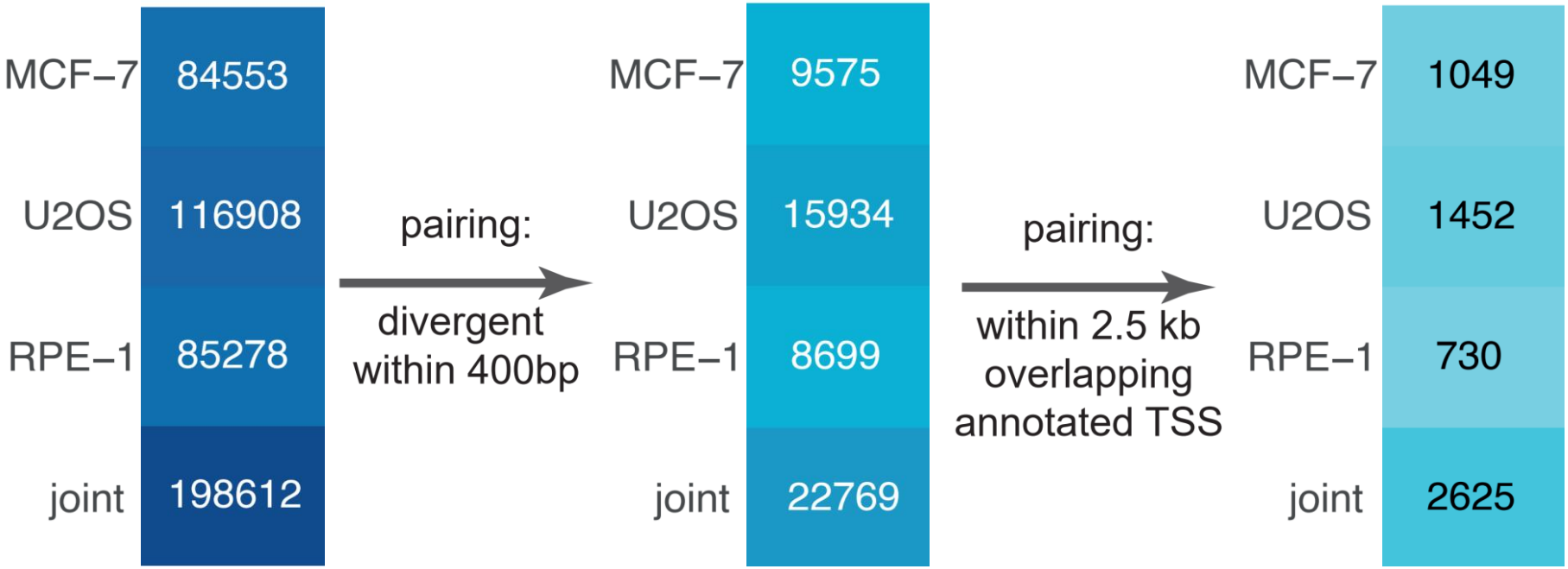
# Supplementary slides

# How do you know that TSS#3 is not enhancer/eRNA?

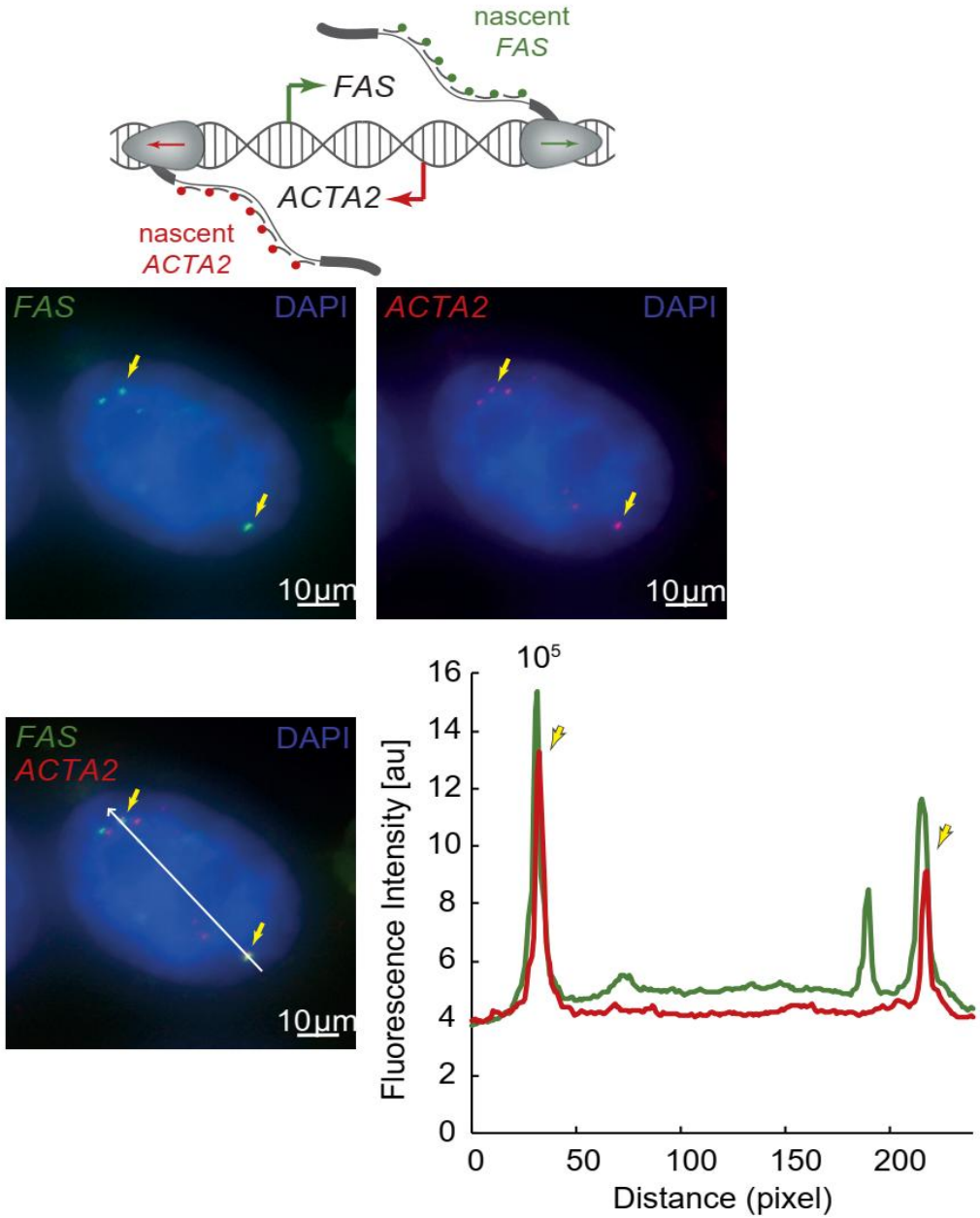


# Mystery of convergent promoters

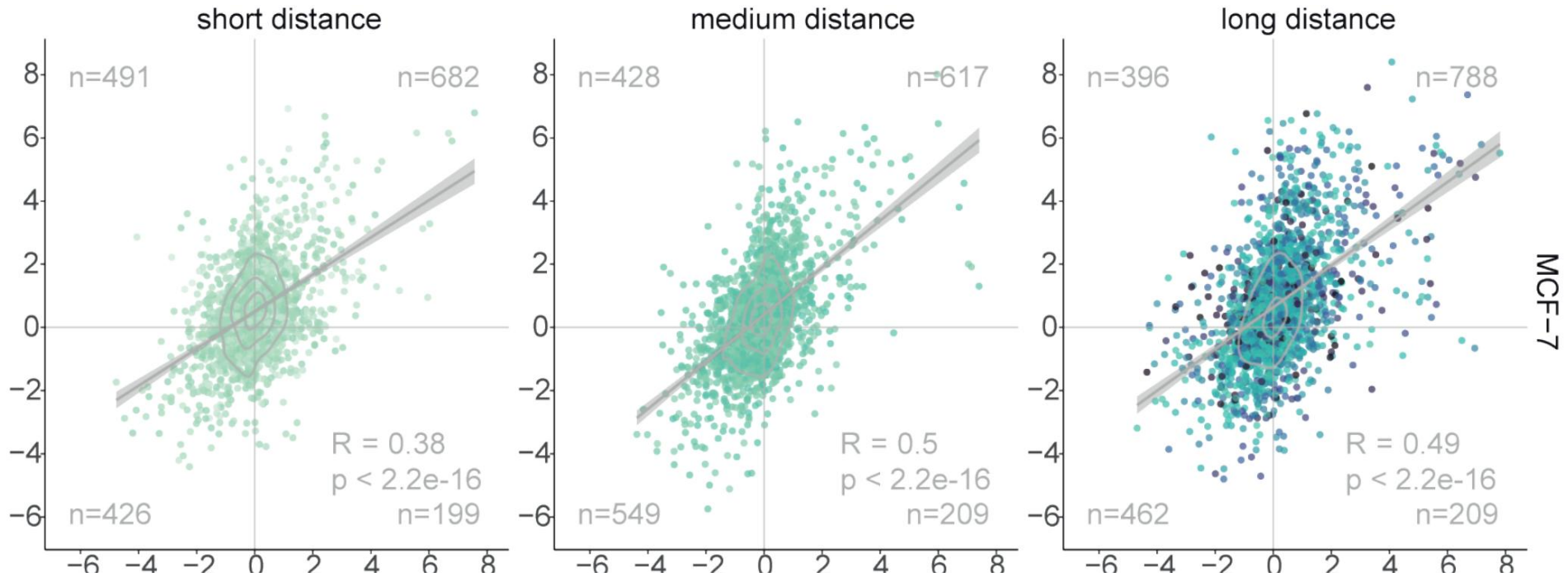


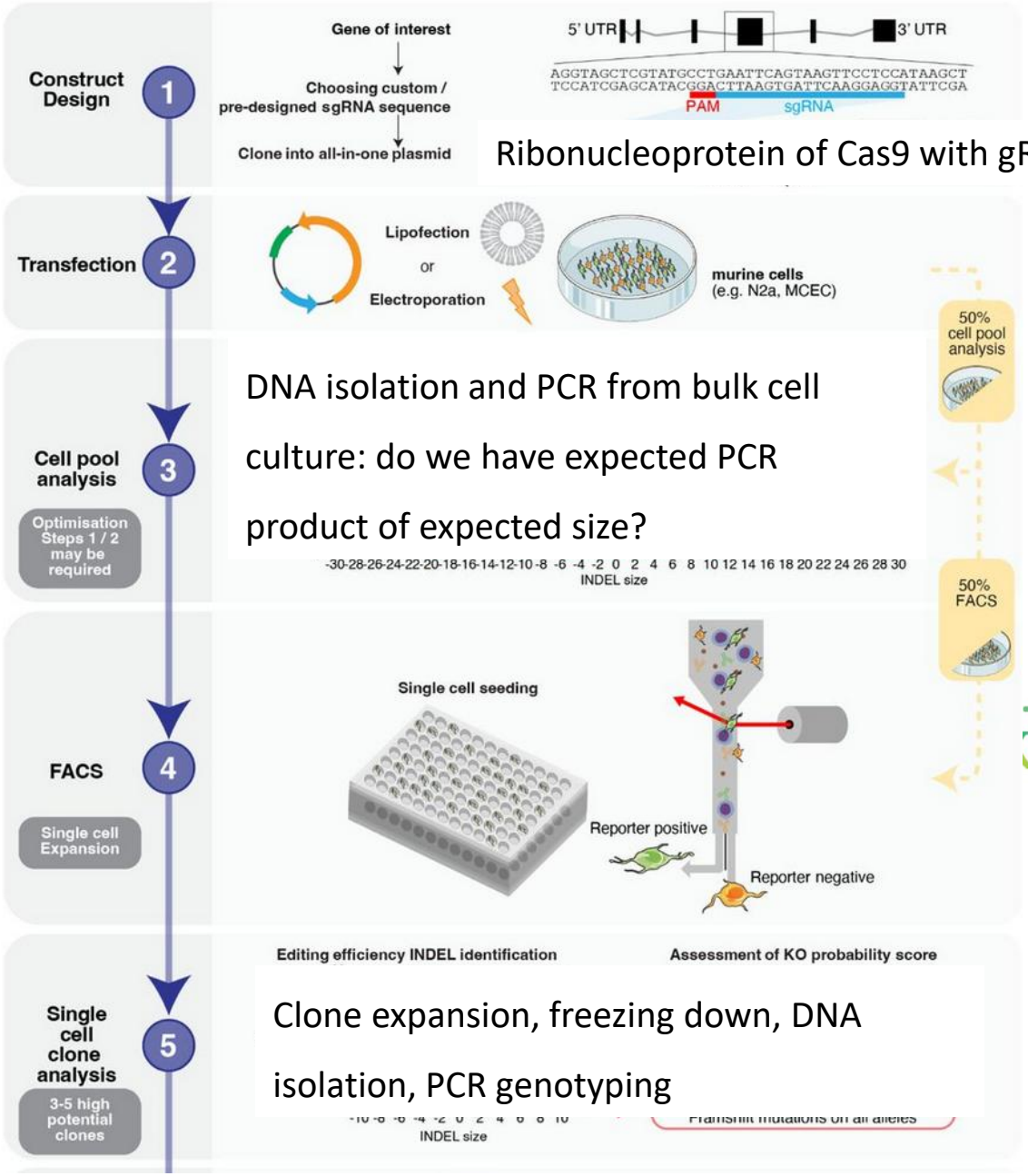
# Maybe TSS 3 and TSS 4 are expressed from different alleles?



# Long distance promotes cooperation between TSS #2 and #3



# CRISPR: workflow with cells

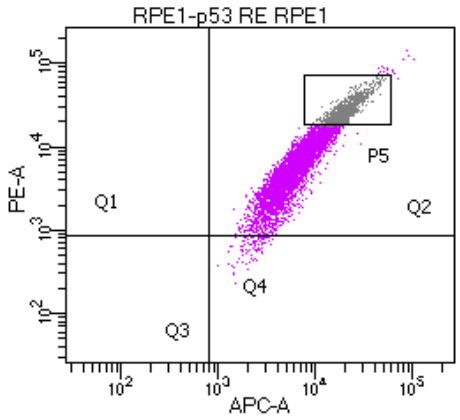


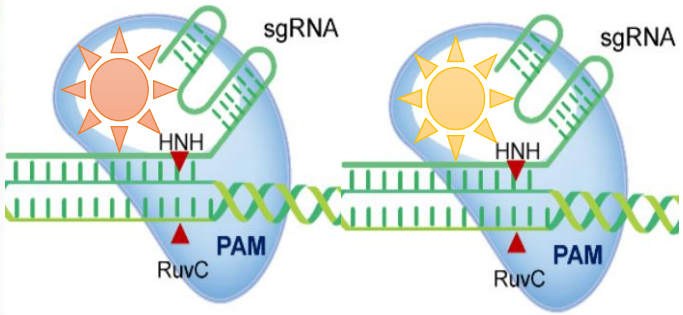

Ribonucleoprotein of Cas9 with gRNA

DNA isolation and PCR from bulk cell culture: do we have expected PCR product of expected size?

Clone expansion, freezing down, DNA isolation, PCR genotyping

Selection of cells with high signals from both fluorophores




*Modified from H.Tobias et al. 2023*

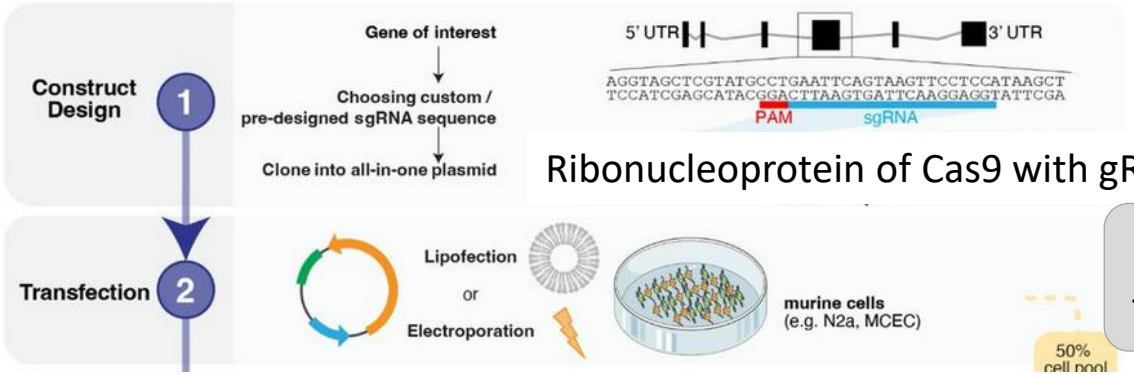
# What can go wrong? Risk management – risks and bottlenecks


Selection of inefficient gRNA, selection of wrong cell line

- Elina and Konstantin checked RNA-seq data for SNPs in targeted regions.
- Selection using IDT gRNA tool.
- Exclusion MCF7 cells due to genomic instability (in top 5 out of 60 cell lines).

*Modified from H.Tobias et al. 2023*

# What can go wrong? Risk management – risks and bottlenecks

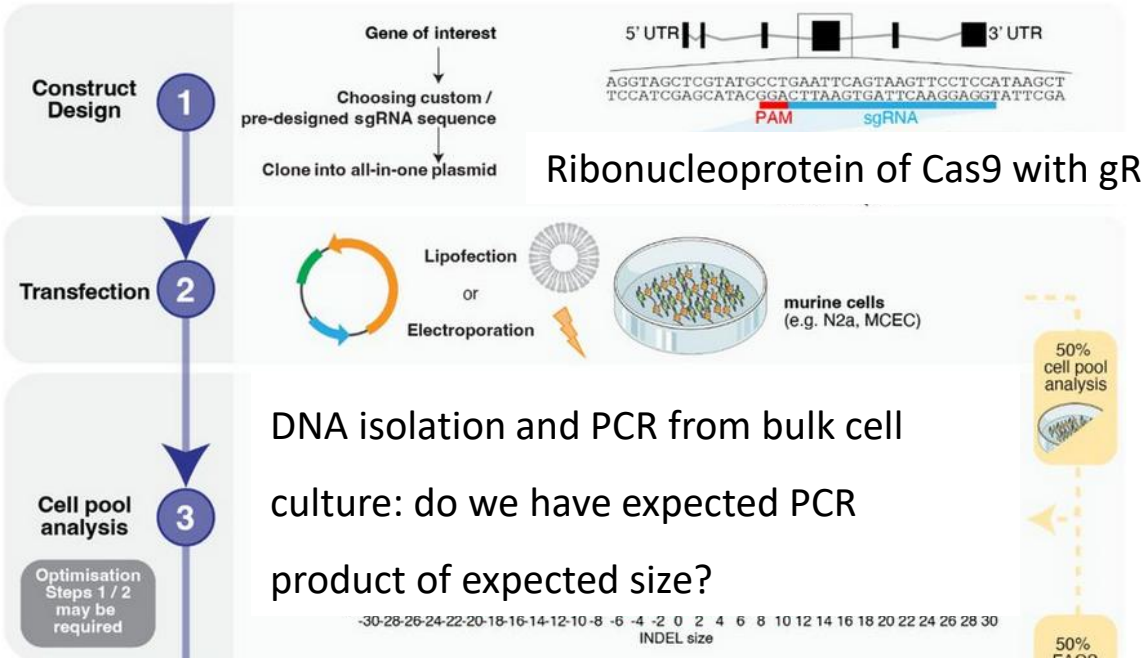



Ribonucleoprotein of Cas9 with gRNA

Optimization of Lipofection, first trial – all U2OS cells dead.

*Modified from H.Tobias et al. 2023*

# CRISPR: workflow with cells



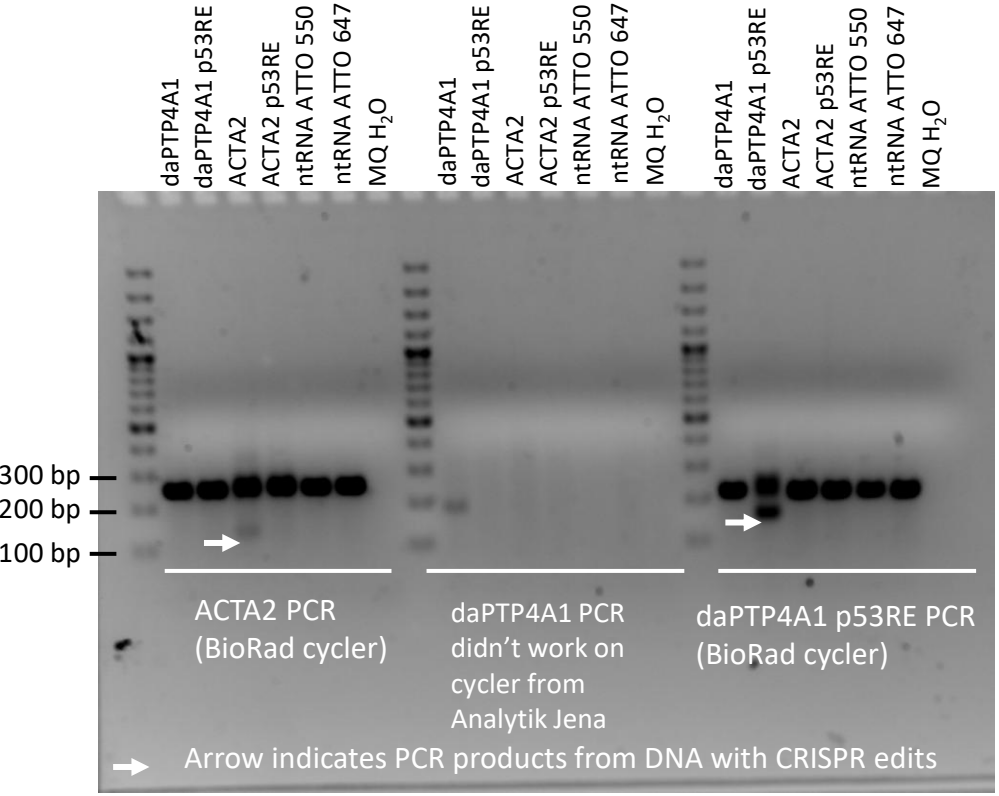

Ribonucleoprotein of Cas9 with gRNA

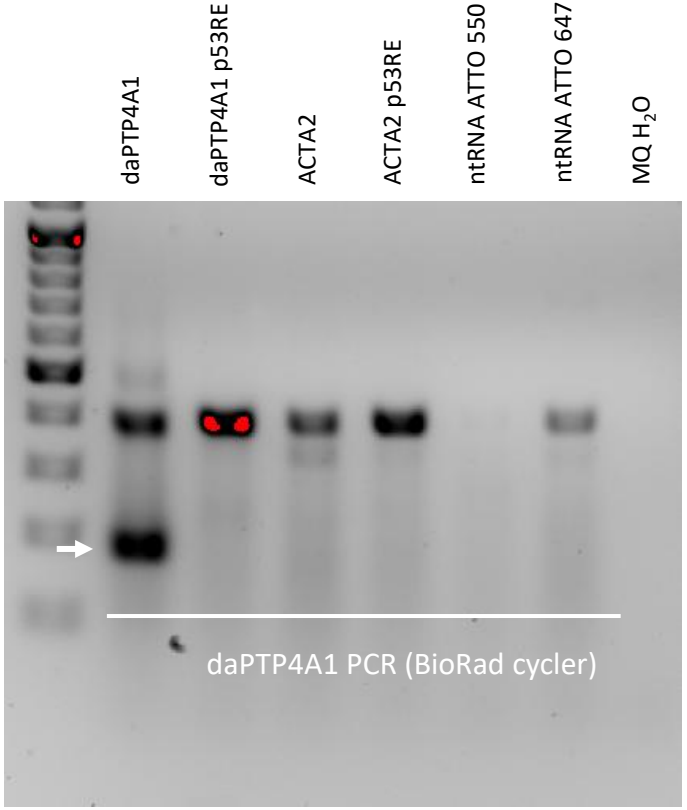
DNA isolation and PCR from bulk cell culture: do we have expected PCR product of expected size?



*Modified from H.Tobias et al. 2023*

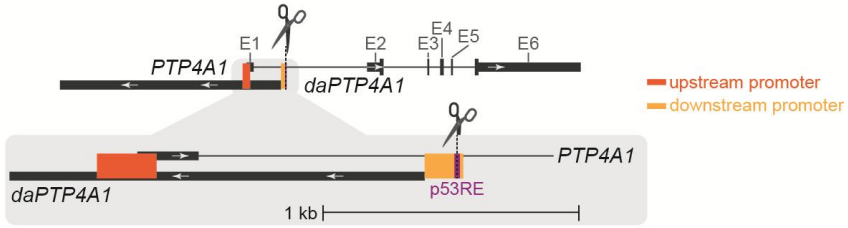
# CRISPR: does gRNA work at all? Genotyping of cell pools

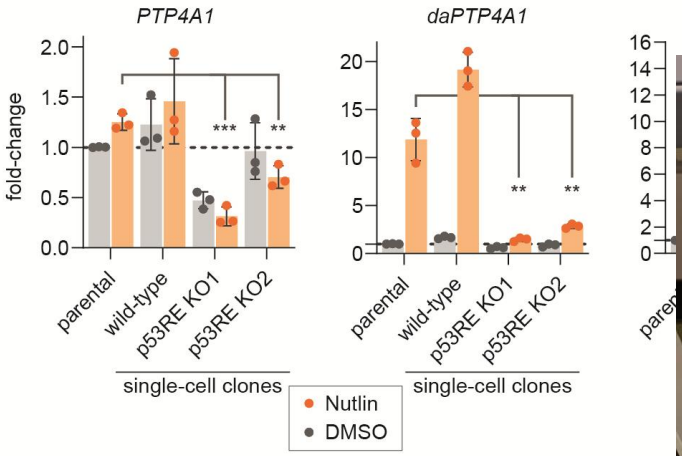



**Aim:**
**To delete daPTP4A1 TSS (one gene targeting)**
**To delete p53RE next to daPTP4A1 TSS (second).**
**Do that in RPE1 and U2OS cell lines.**

# Knockout of p53RE reduces PTP4A1 and daPTP4A1 expression




**Maybe outsource?**