# Promoters revisited

Elina Wiechens – 16.02.2023

Traditional view of transcription initiation
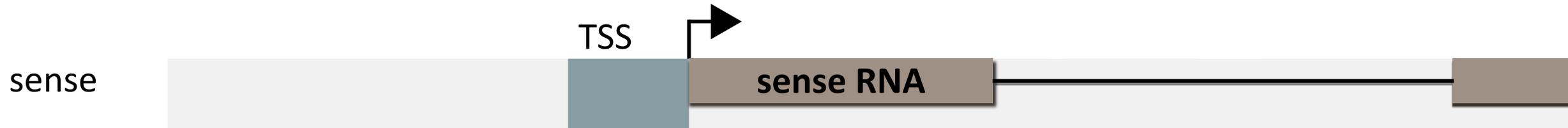
Divergent transcription in promoter regions initiates multiple transcripts

TSS#2

sense

sense RNA

upstream antisense RNA

antisense

TSS#1
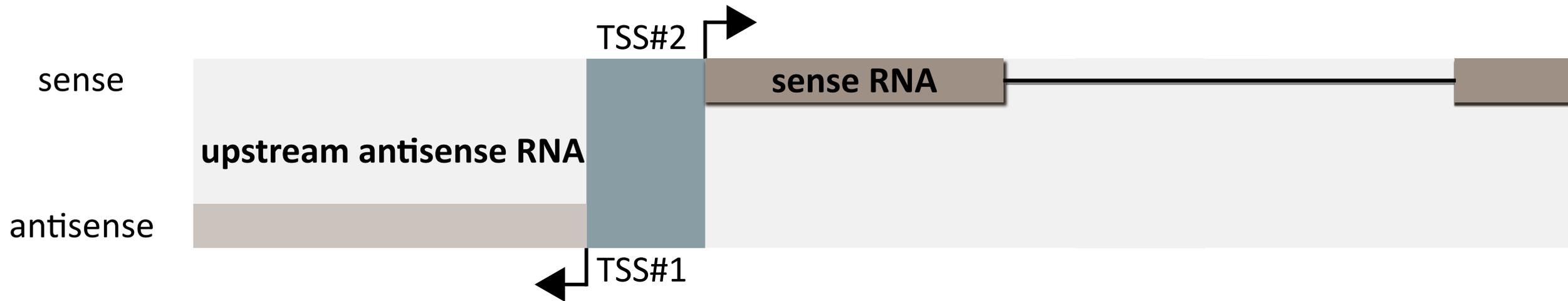
Native Elongating Transcript Sequencing Reveals Human Transcriptional Activity at Nucleotide Resolution

Cell

Andreas Mayer,[1,4] Julia di Iulio,[1,4] Seth Maleri,[1] Umut Eser,[1] Jeff Vierstra,[2] Alex Reynolds,[2] Richard Sandstrom,[2] John A. Stamatoyannopoulos,[2,3] and L. Stirling Churchman[1,*]

Mayer *et al.* (2015, Cell)

Downstream Antisense Transcription Predicts Genomic Features That Define the Specific Chromatin Environment at Mammalian Promoters

PLOS | GENETICS

Christopher A. Lavender[1,2], Kimberly R. Cannady[1], Jackson A. Hoffman[1], Kevin W. Trotter[1], Daniel A. Gilchrist[1¤], Brian D. Bennett[2], Adam B. Burkholder[2], Craig J. Burd[3,4], David C. Fargo[2*], Trevor K. Archer[1*]

Lavender *et al.* (2016, PLoS Genetics)
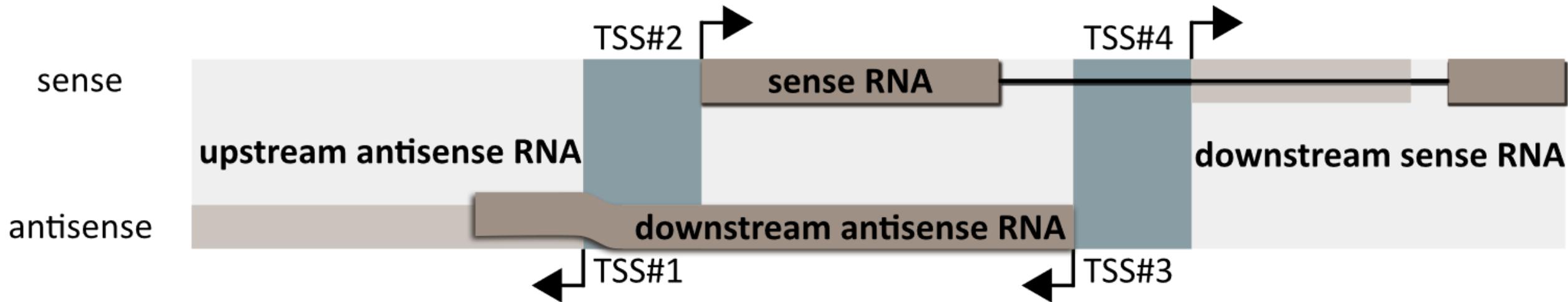
Principles for RNA metabolism and alternative transcription initiation within closely spaced promoters

nature genetics

Yun Chen[1,2], Athma A. Pai[3], Jan Herudek[4], Michal Lubas[2,4], Nicola Meola[4], Aino I. Järvelin[5,8], Robin Andersson[1], Vicent Pelechano[5,8], Lars M. Steinmetz[5,6,7], Torben Heick Jensen[4], and Albin Sandelin[1,2]

Chen *et al.* (2017, Nature Genetics)

# Antisense transcription-dependent chromatin signature modulates sense transcript dynamics

Thomas Brown[†], Françoise S Howe[†], Struan C Murray[†], Meredith Wouters, Philipp Lorenz, Emily Seward, Scott Rata, Andrew Angel[*] & Jane Mellor[**]

molecular systems biology

Brown *et al.* (2018, Molecular Systems Biology)

Dynamic experimental setup

Figure by Katjana Schwab
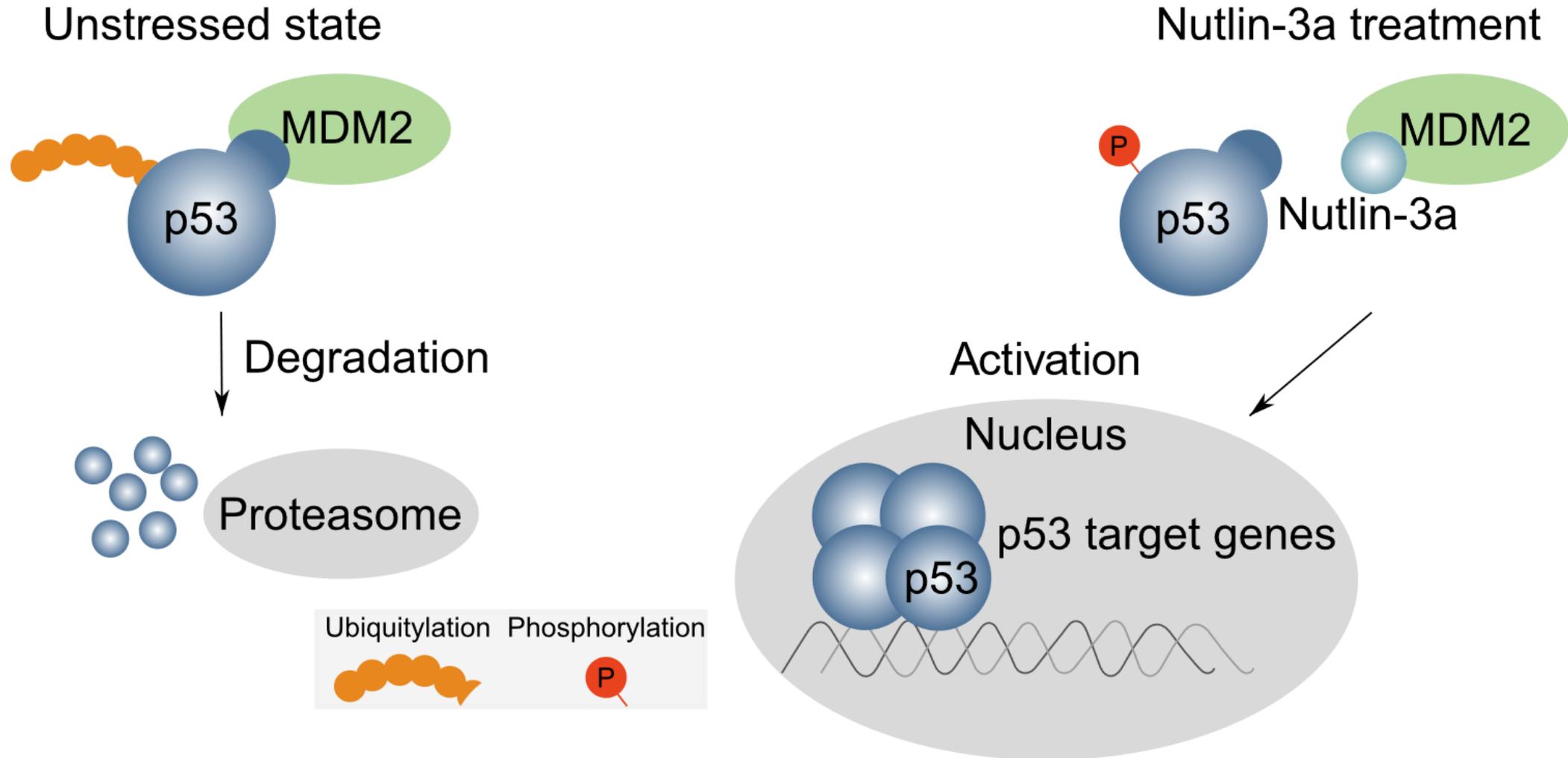
# Identification of closely spaced promoter

Identifying closely spaced promoters in
Cap analysis gene expression (CAGE) – Sequencing data

Transcript 1
**Transcript 2**

RNA-Seq reads

RNA-Seq coverage

CAGE-Seq reads

CAGE coverage

Example: MDM4

8
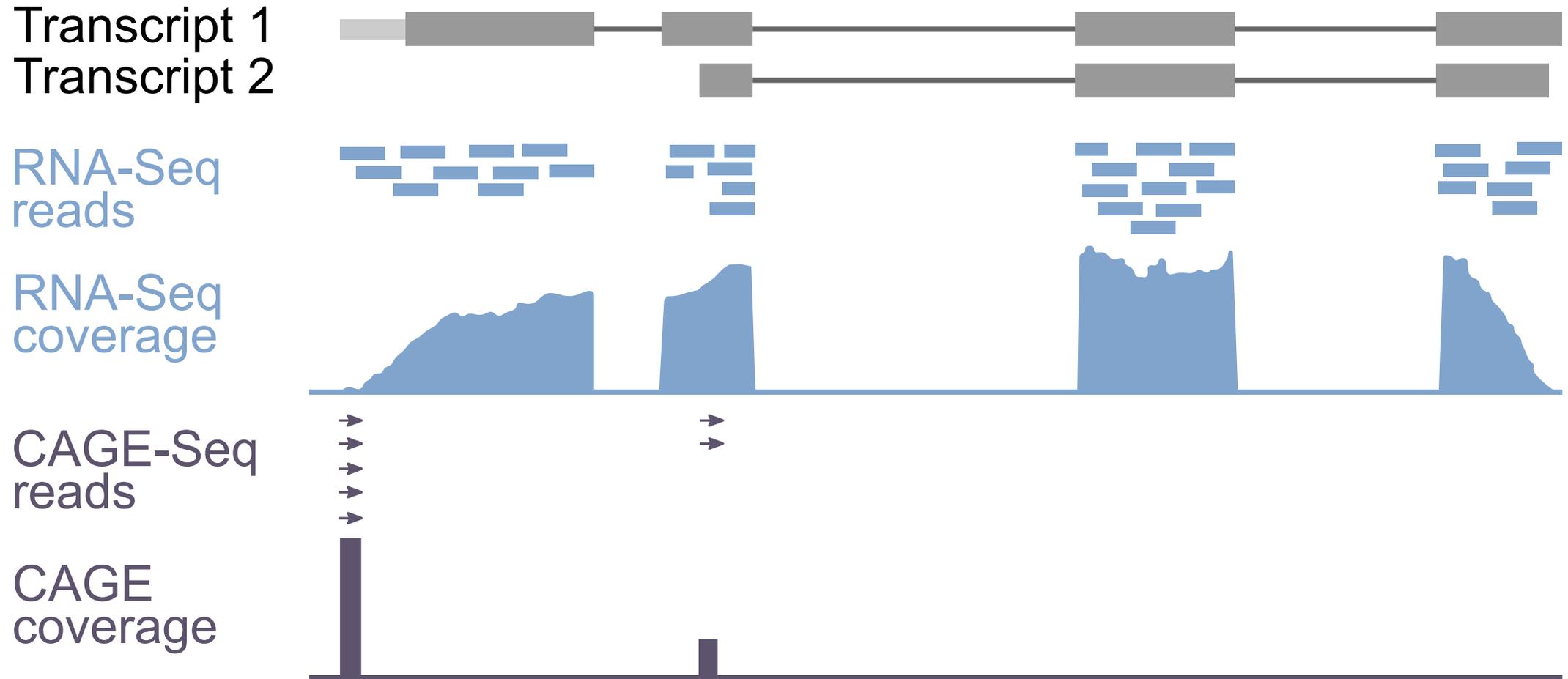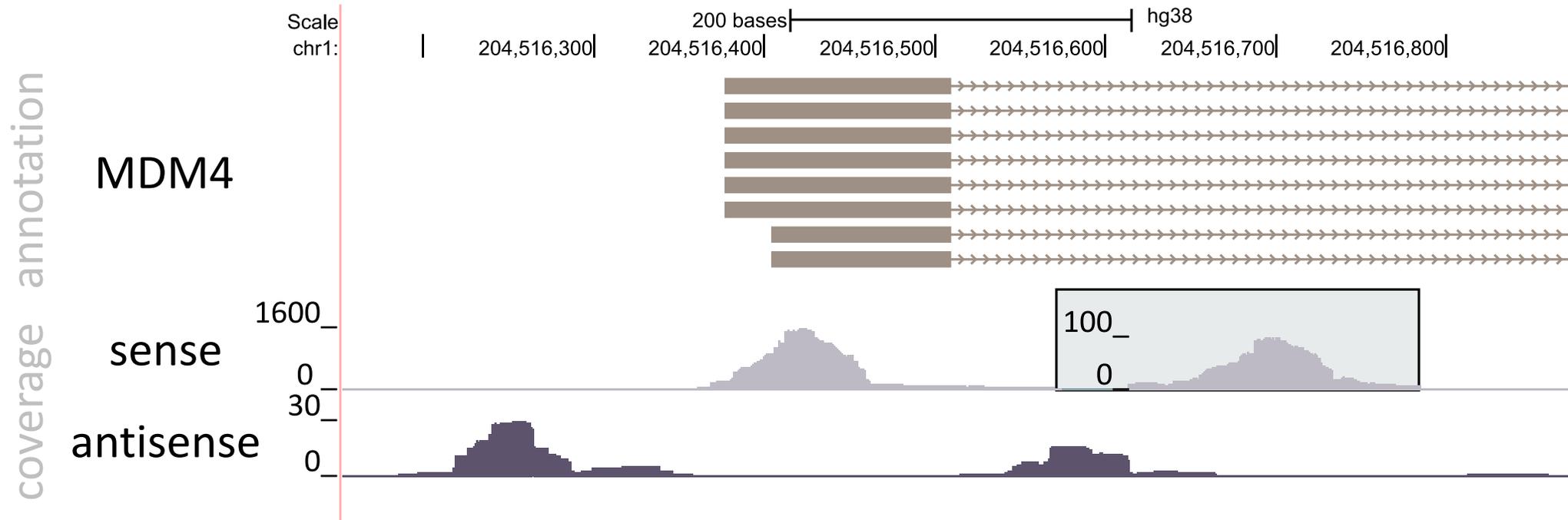
Example: MDM4

TSS#1: upstream antisense RNA
TSS#2: sense RNA
TSS#3: downstream antisense RNA
TSS#4: downstream sense RNA
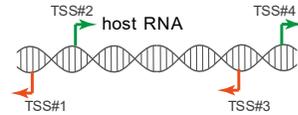
annotation

MDM4

Scale
chr1:

200 bases   hg38

204,516,300   204,516,400   204,516,500   204,516,600   204,516,700   204,516,800

peaks

sense

TSS#2   TSS#4

antisense

TSS#1   TSS#3

84 553 CAGE-Seq peaks

bidirectional PeakPair ≤ 400 bp

9 575 bidirectional PeakPairs

Closely spaced promoter ≤ 2 500 bp

1 049 closely spaced promoter

9

Closely spaced promoter in the epigenome

Constructed by Martin Fischer

Closely spaced promoter in the epigenome

TSS/Promoter mark

H3K4me3

G4 quadruplex

R-loops
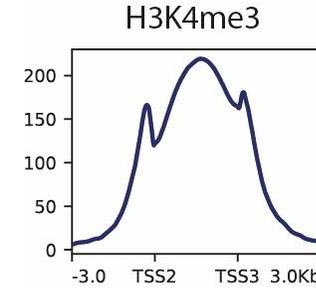
H3K4me3
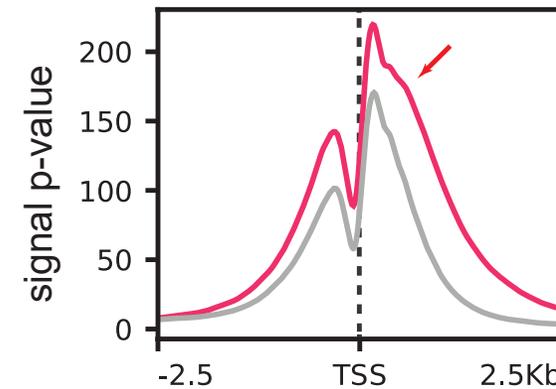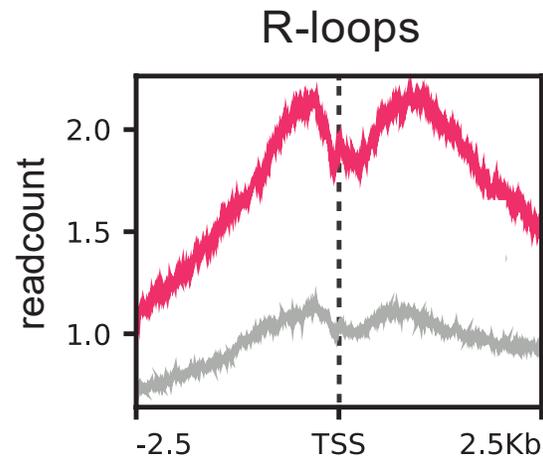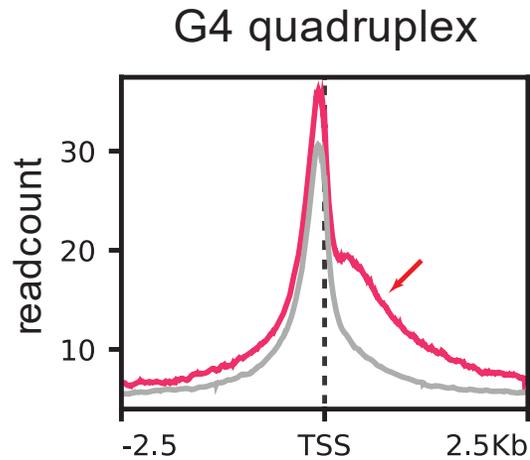
— Closely spaced promoter
— Non Closely spaced promoter

Transcription interference?
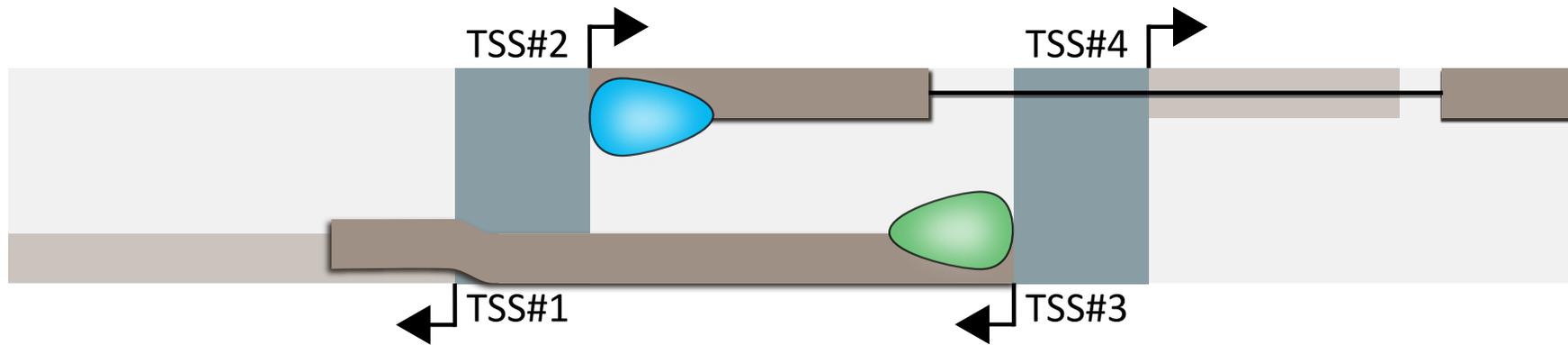
Promoter destruction
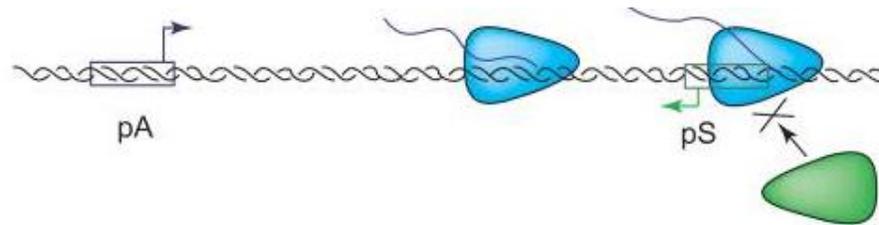
Collision
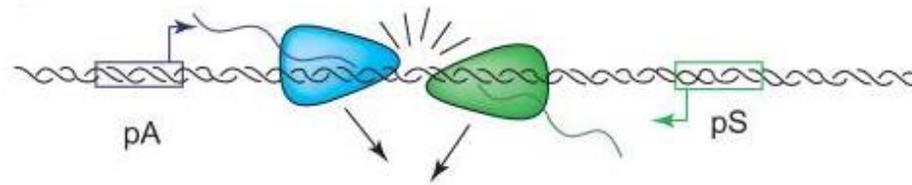
Roadblock
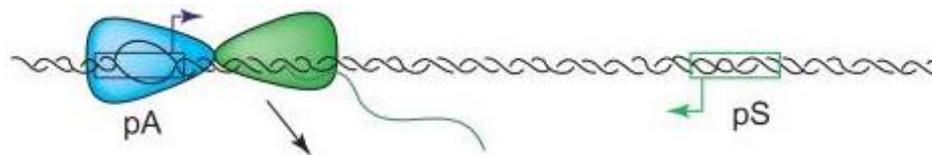
# Change in TSS expression in all bidirectional PeakPairs

n=1636

n=3375

$R = 0.49$

n=2859

n=1705

TSS#2(Log$_2$FC)*

TSS#1(Log$_2$FC)*
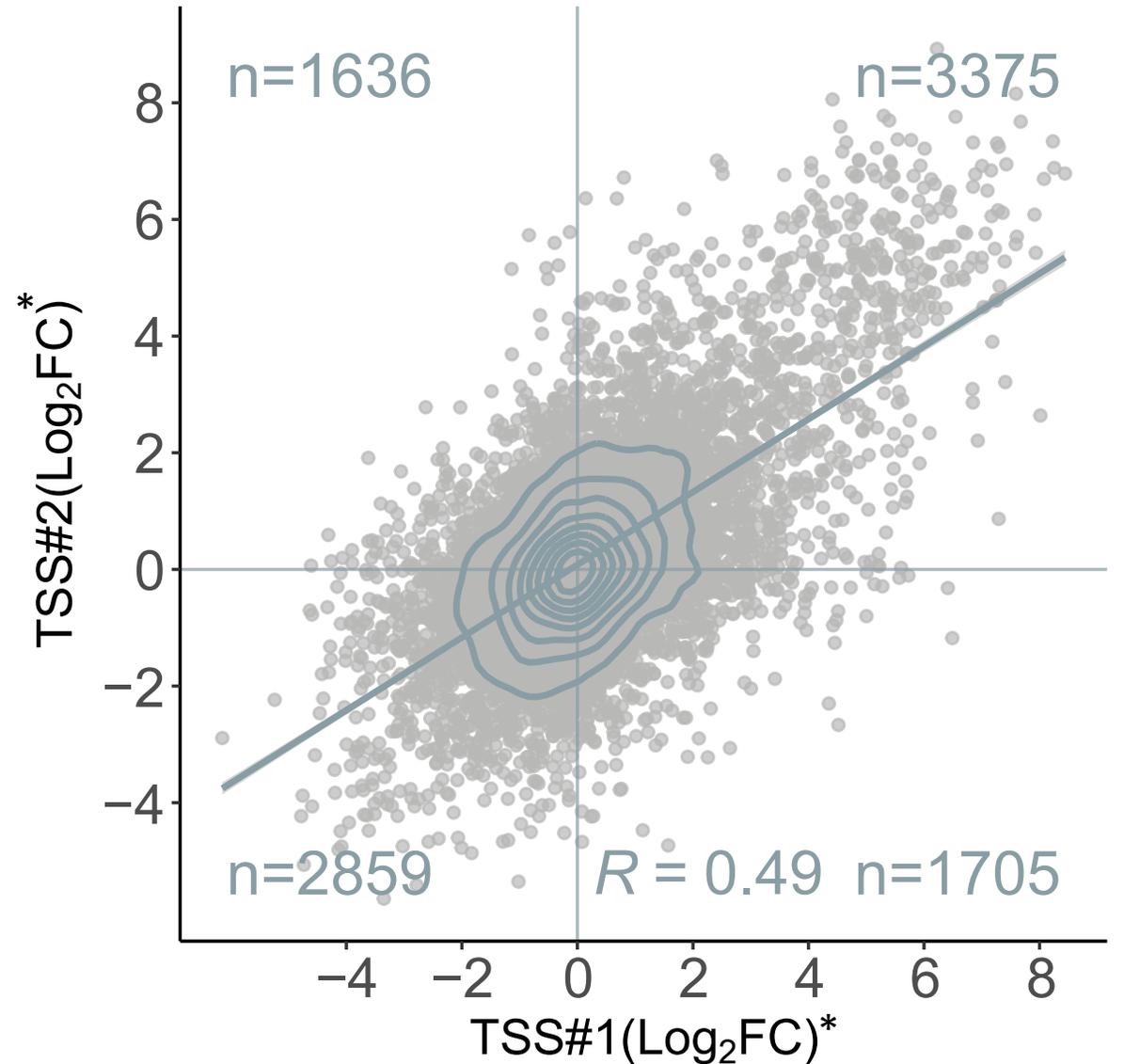
*Log$_2$(TSS expression in Nutlin/ TSS expression in DMSO)

Change in TSS expression in bidirectional PeakPairs
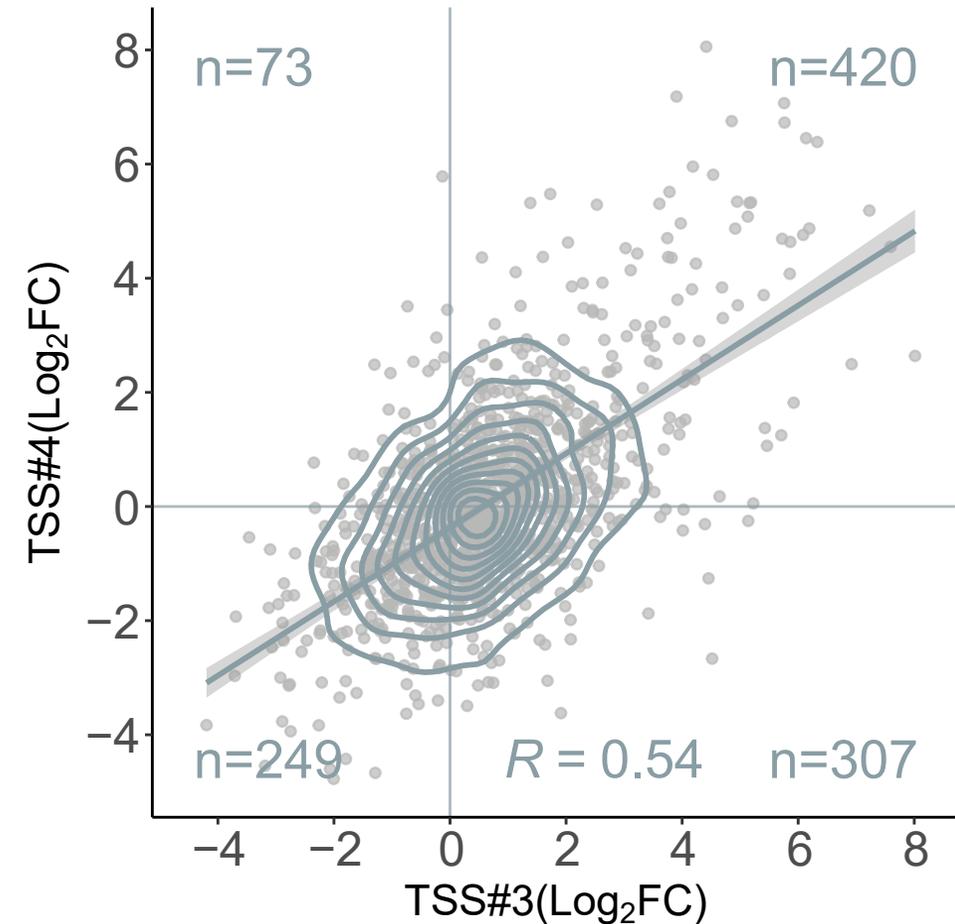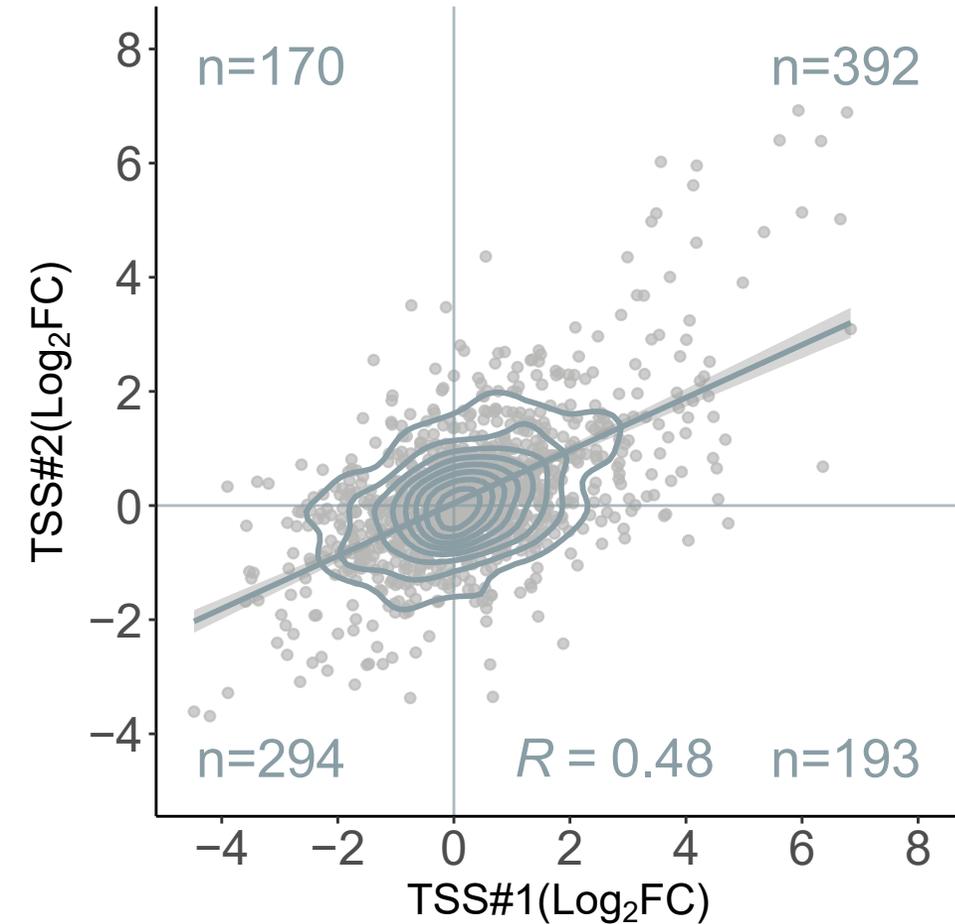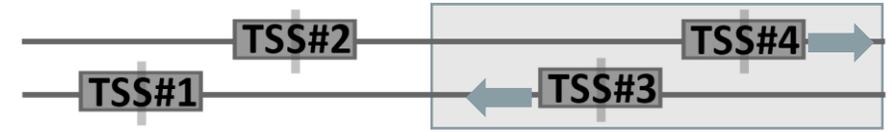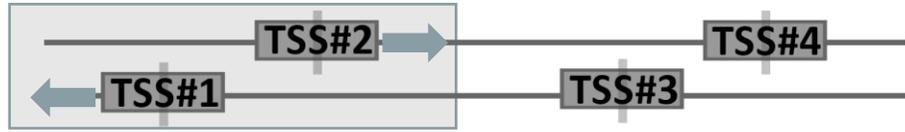
Change in TSS expression in bidirectional PeakPairs
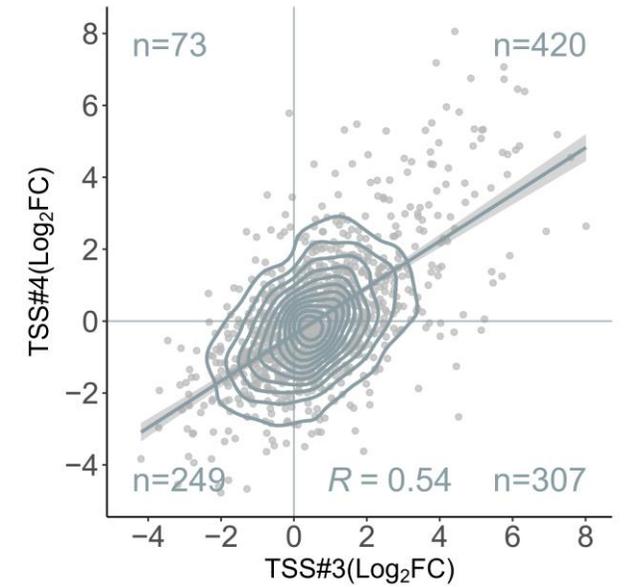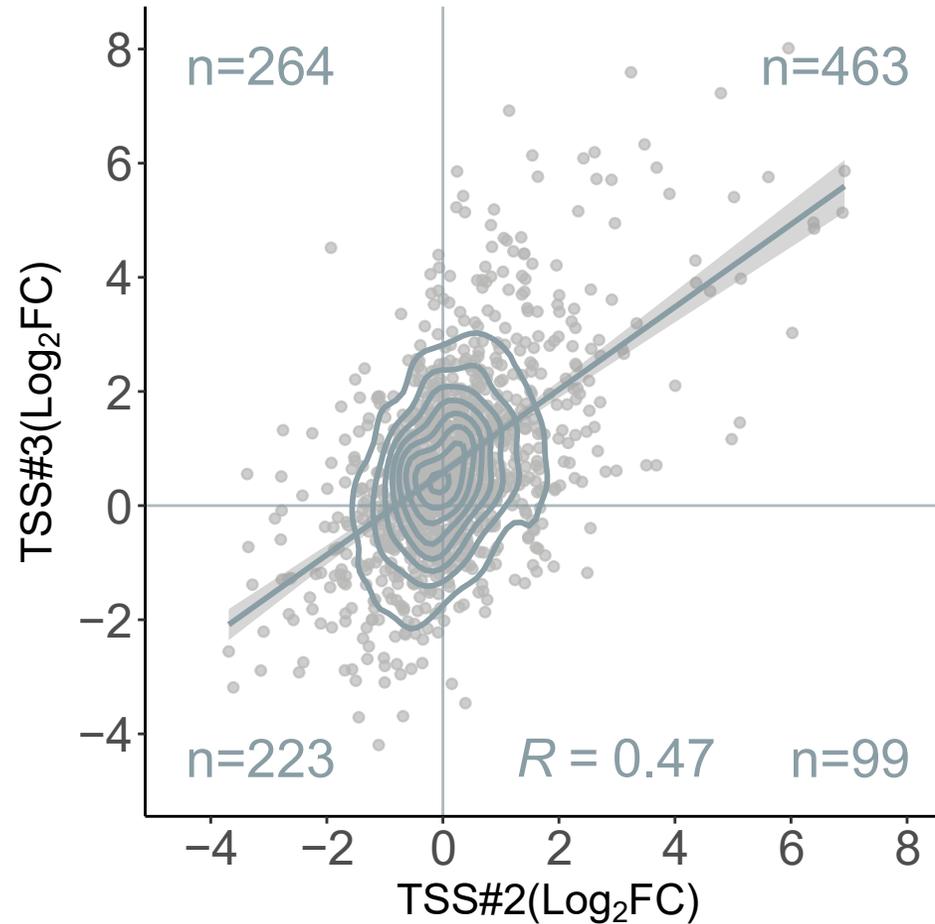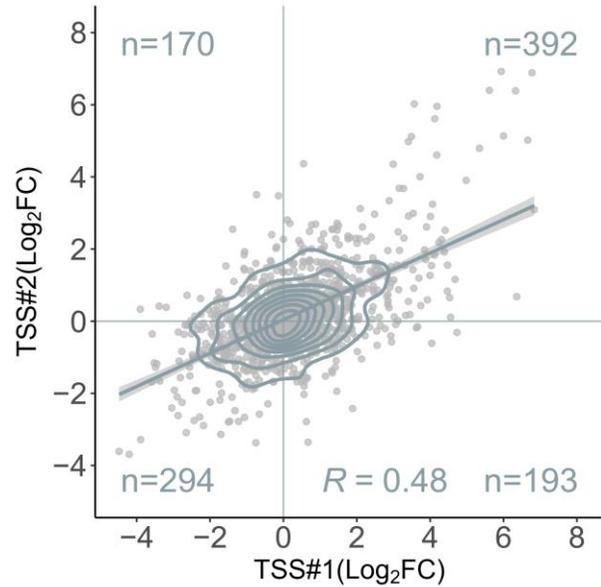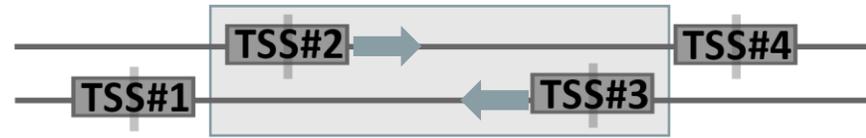
Cooperativity of closely spaced promoter:
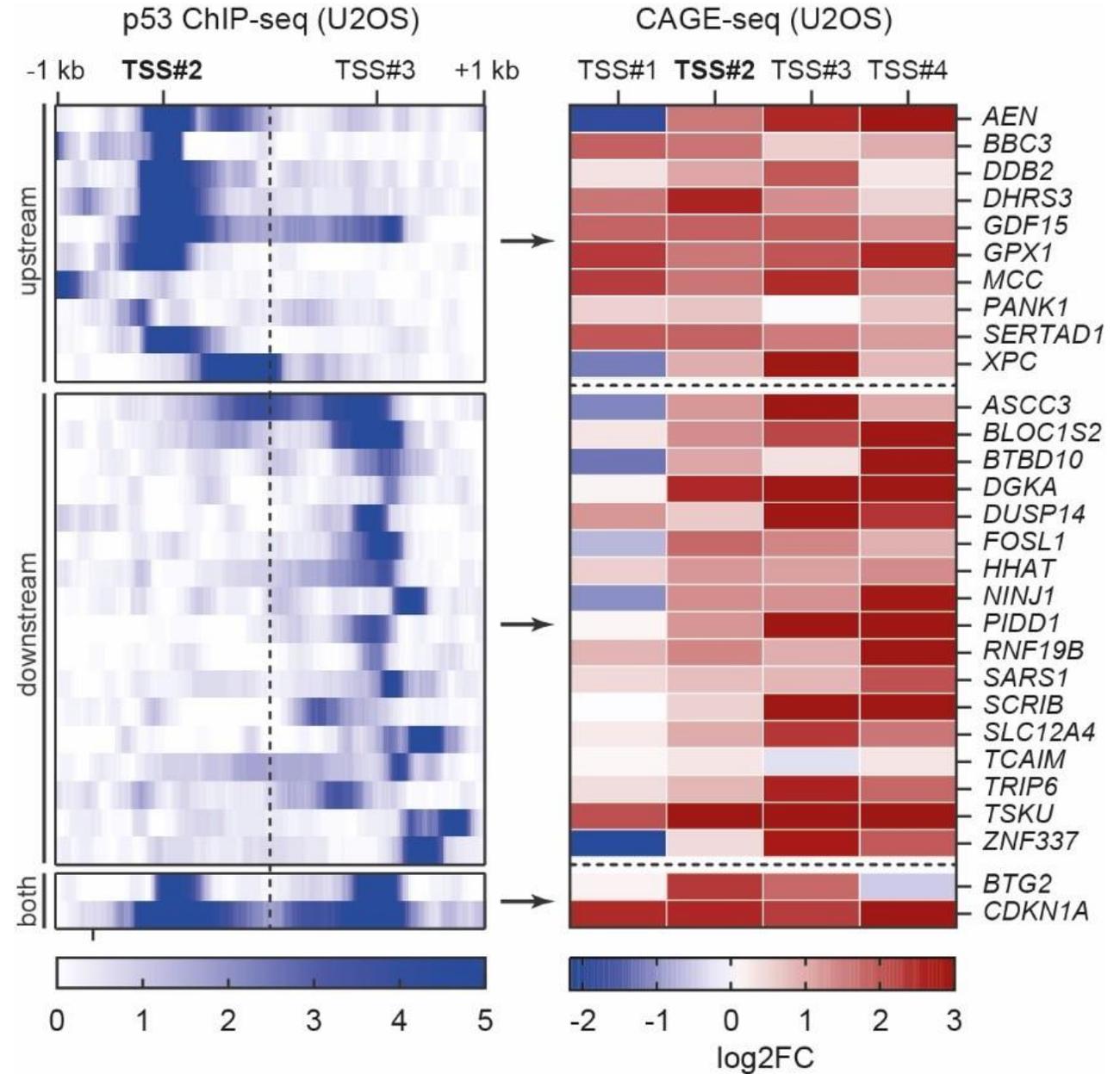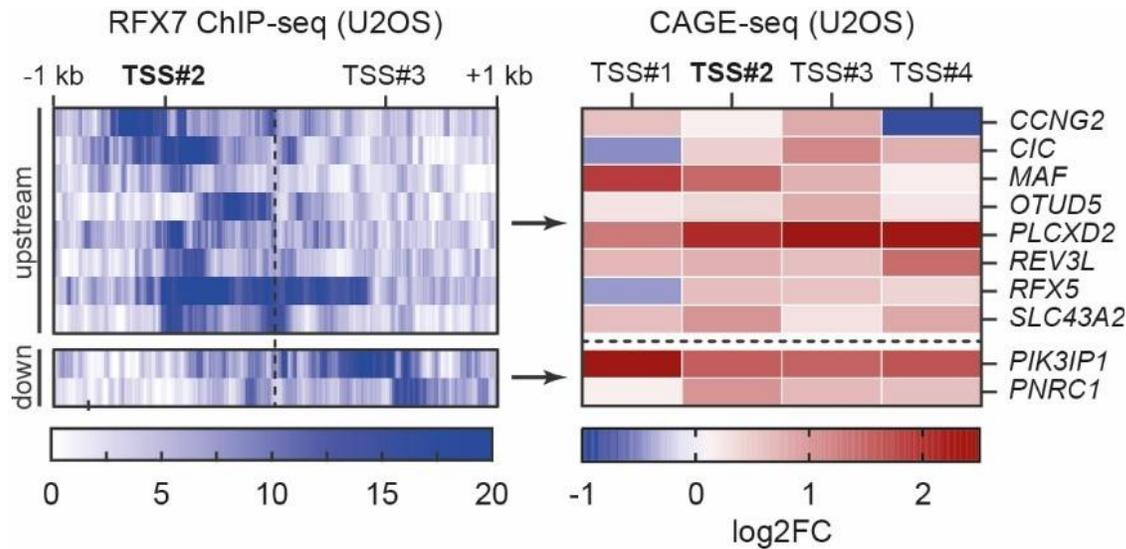
Cooperativity of closely spaced promoter:
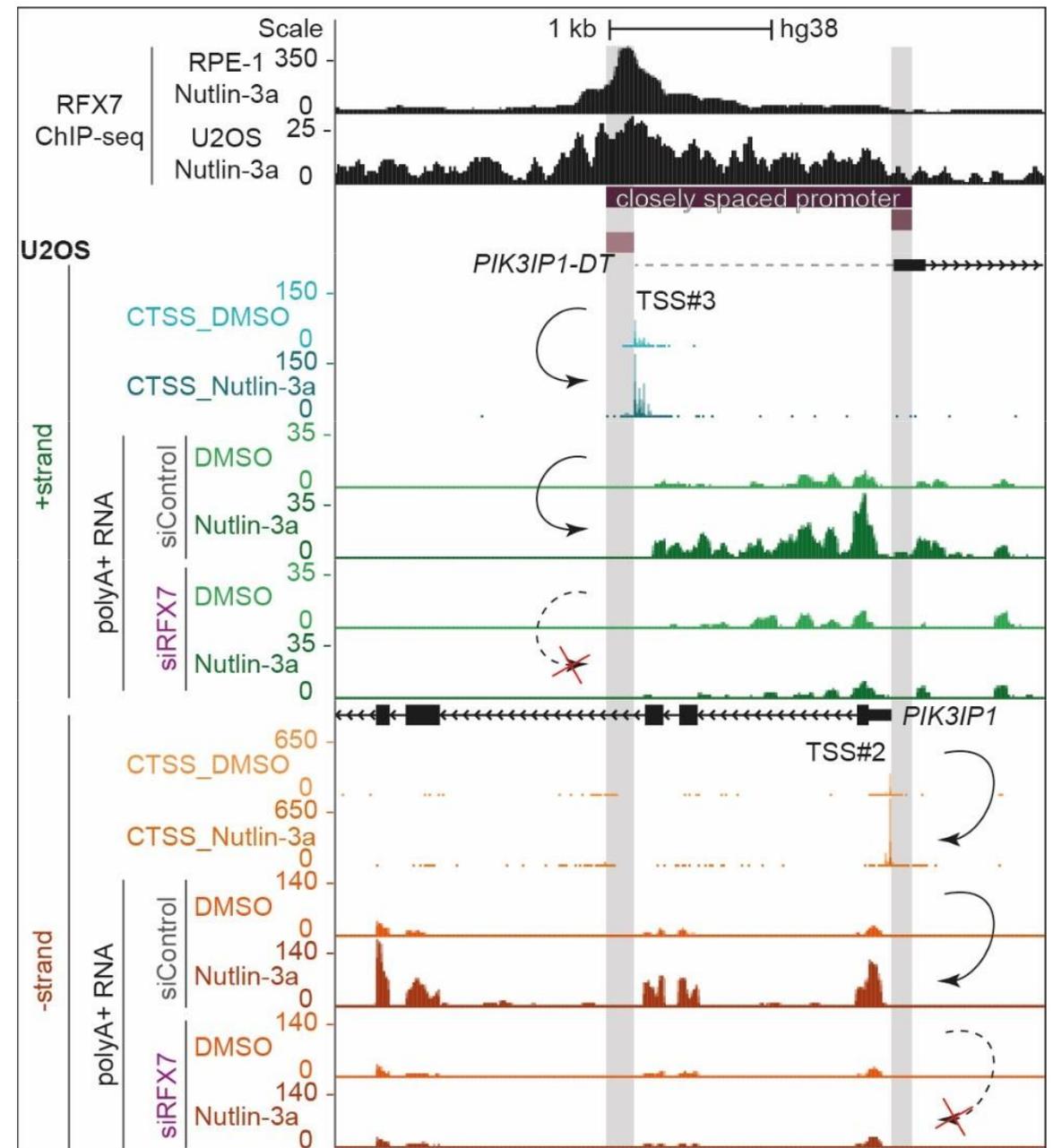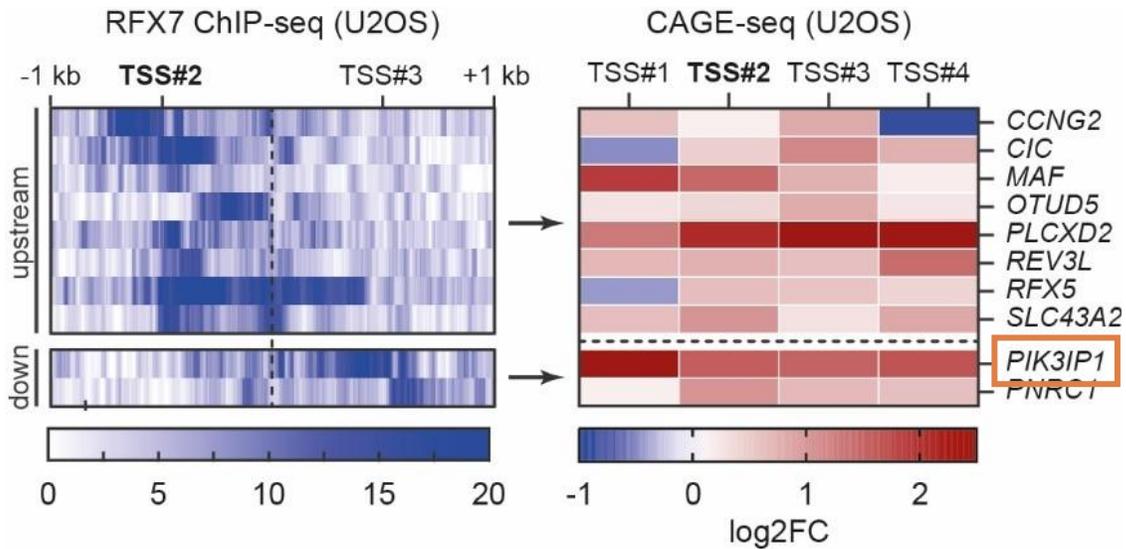
Summary

Closely spaced promoters...

- are surprisingly prevalent in the genome

- possess a distinct chromatin structure

- elicit convergent transcription without the need for spatial separation

- overcome transcription interference

- cooperate to enable co-regulation by transcription factors, such as p53
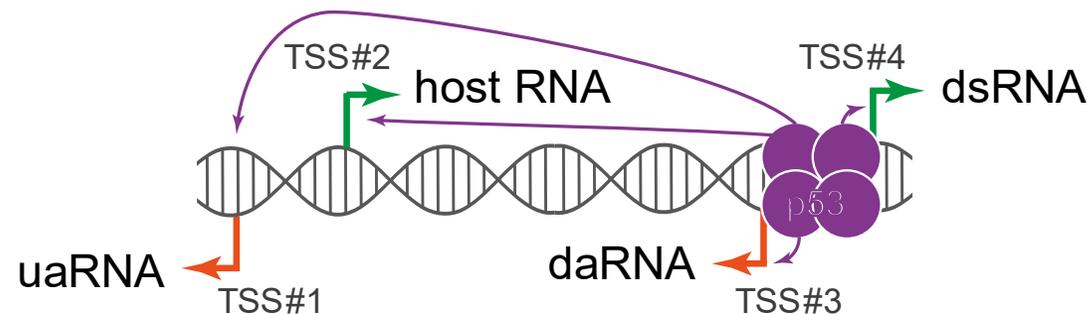
Special thanks goes to
Steve Hoffman,
Martin Fischer,
Alena van Bömmel,
Konstantin Riege, and
everyone else at the RG Hoffmann

Thank you for
your attention!

We are hiring!

# Preliminary data: Single-molecule FISH of nascent RNA
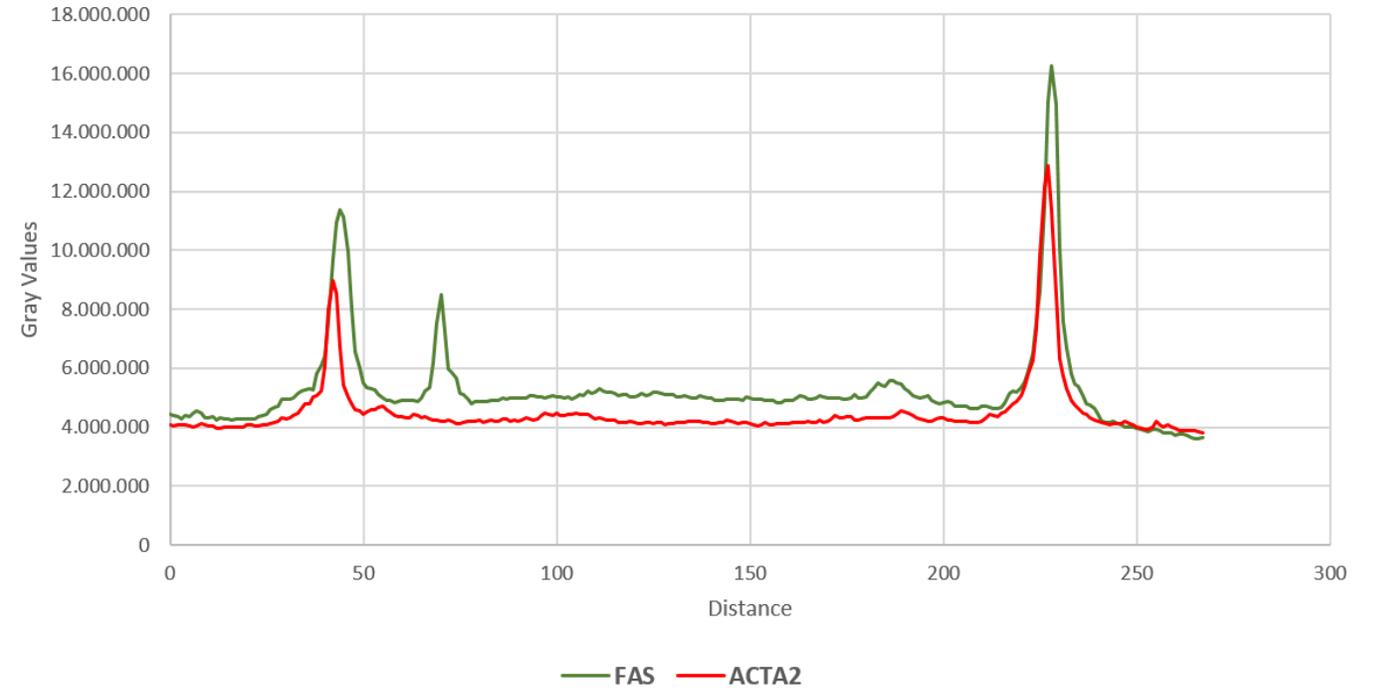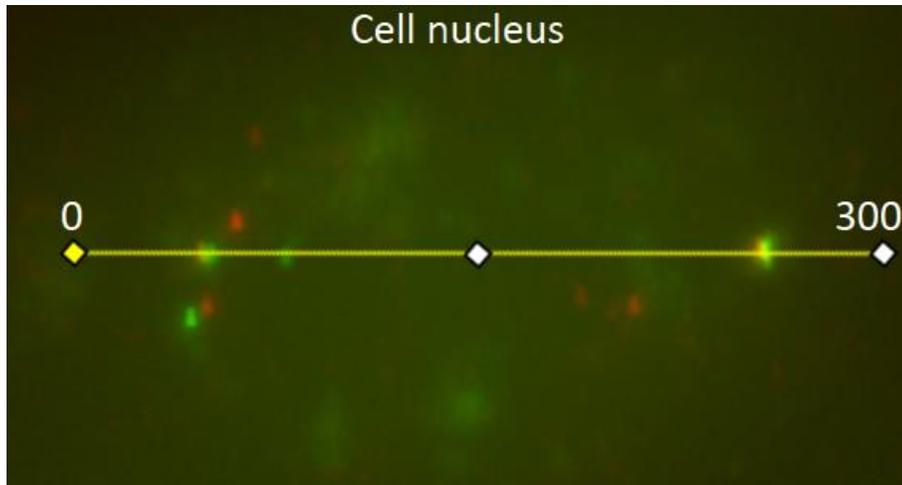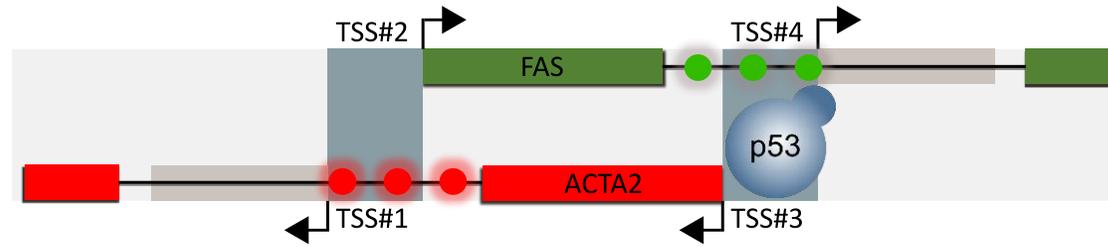
# Identifying bidirectional peak pairs and closely spaced promoters on MCF-7 CAGE-Seq data
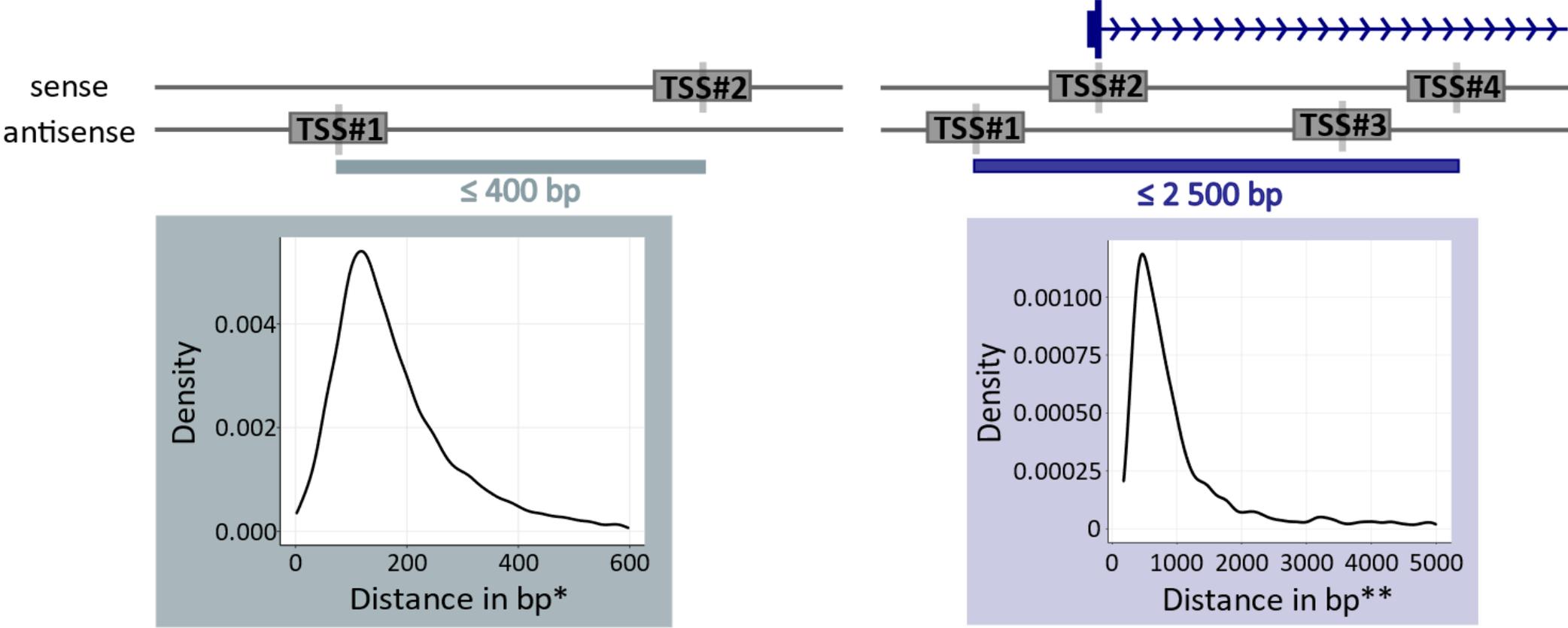
Identified closely spaced promoter
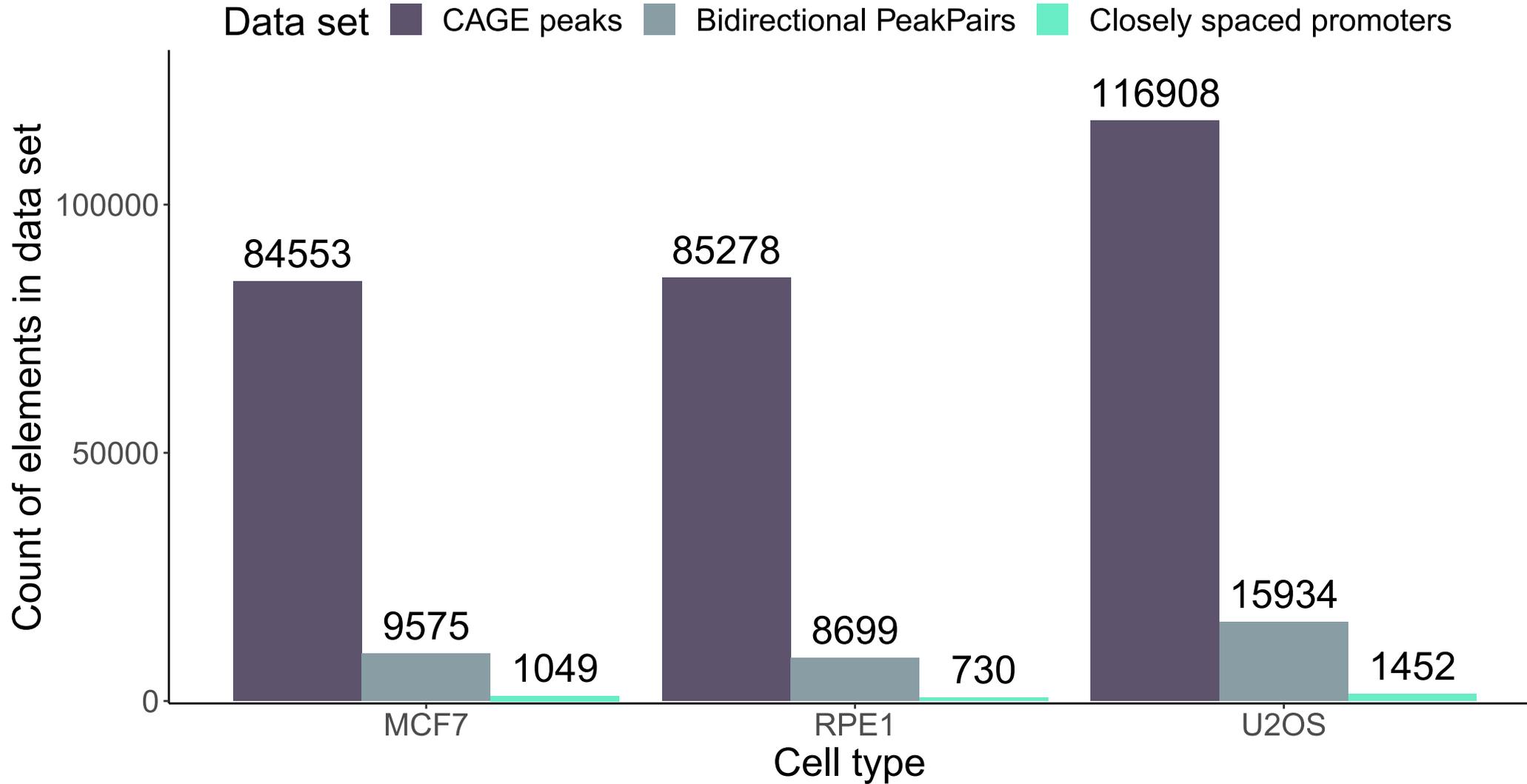
# TSS expression in convergent transcription
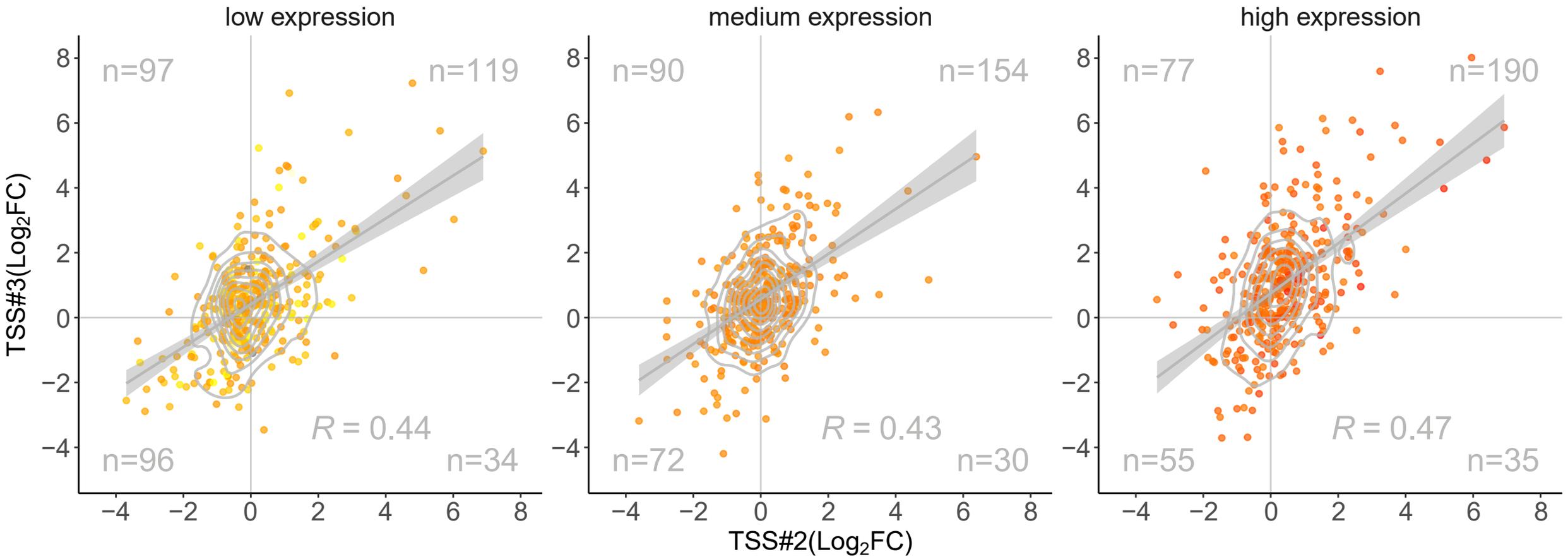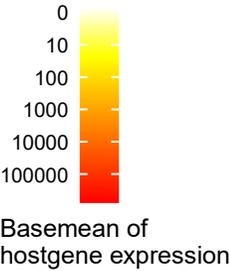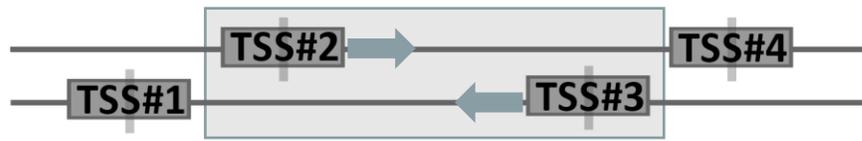
TSS distance in convergent transcription
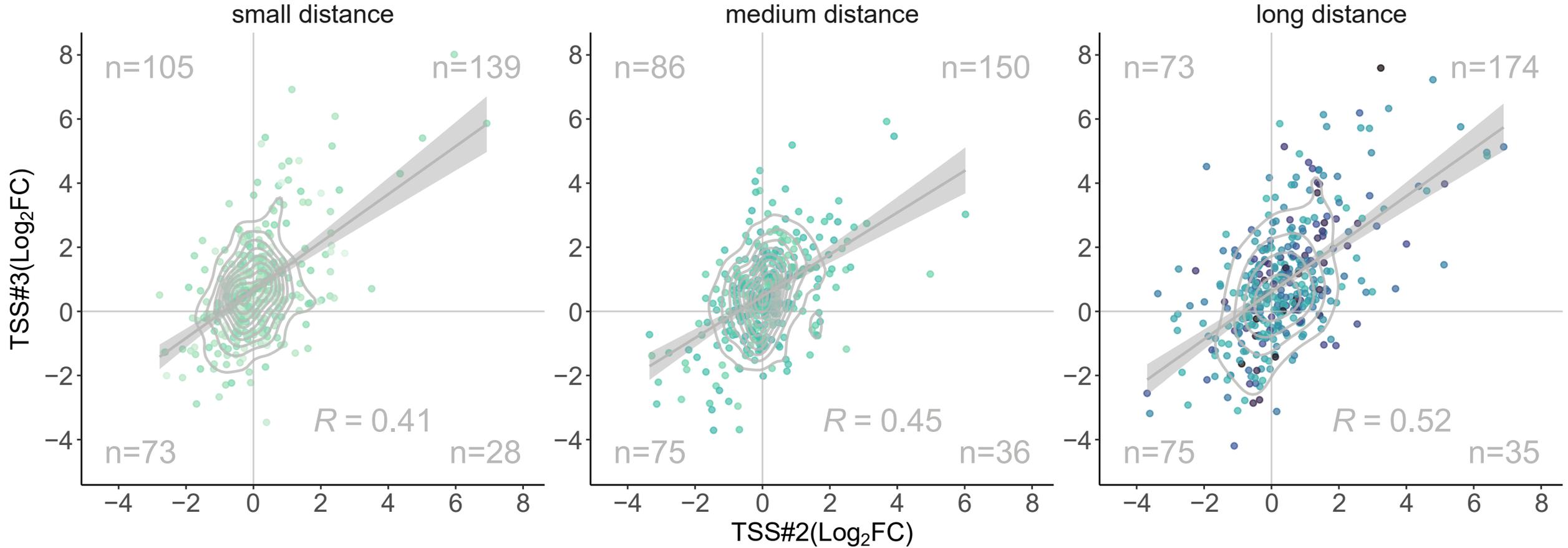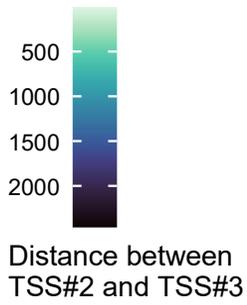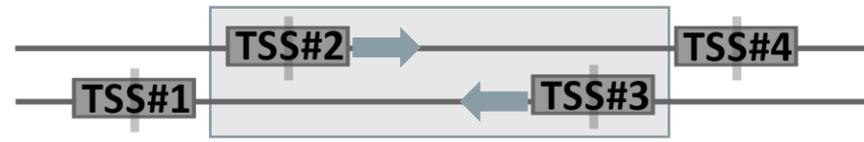
# Host-gene expression and TSS expression in MCF-7
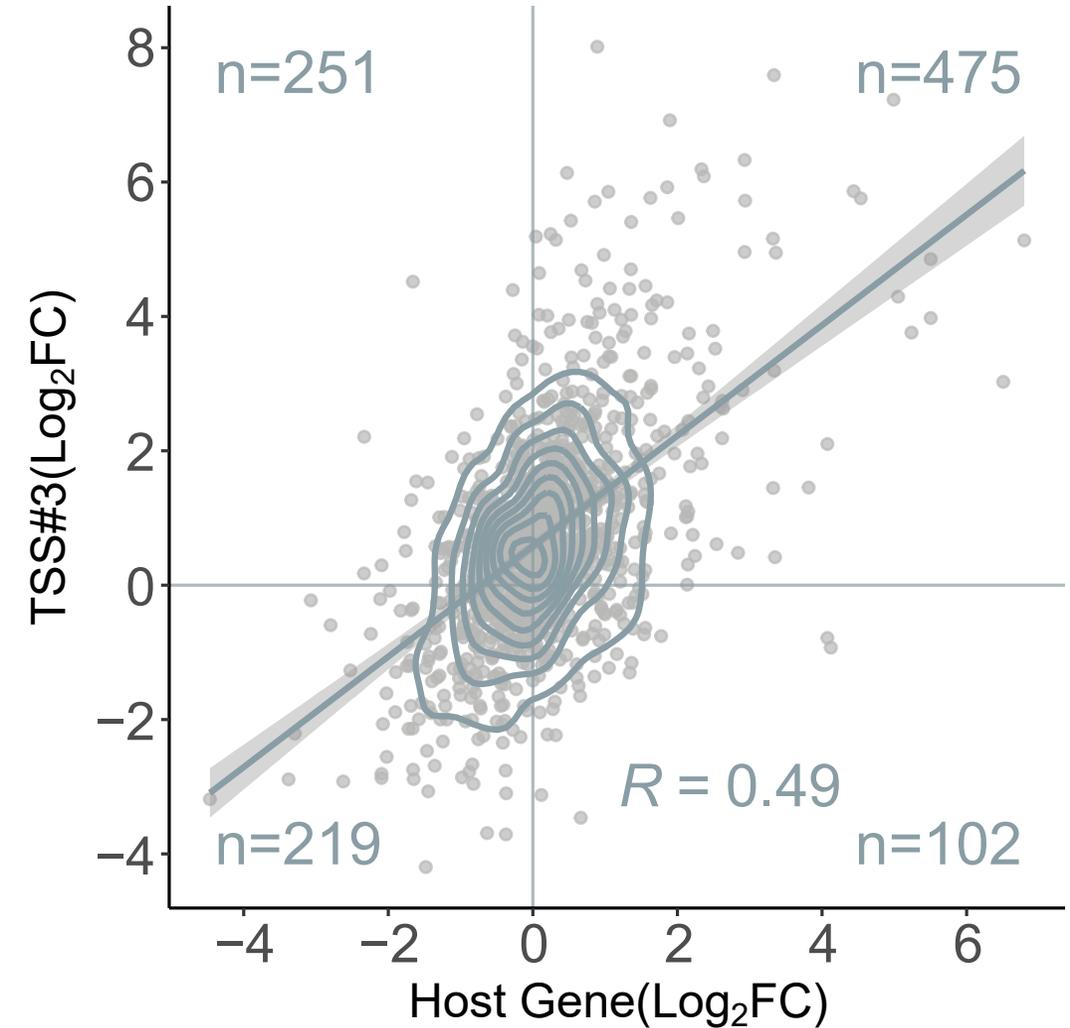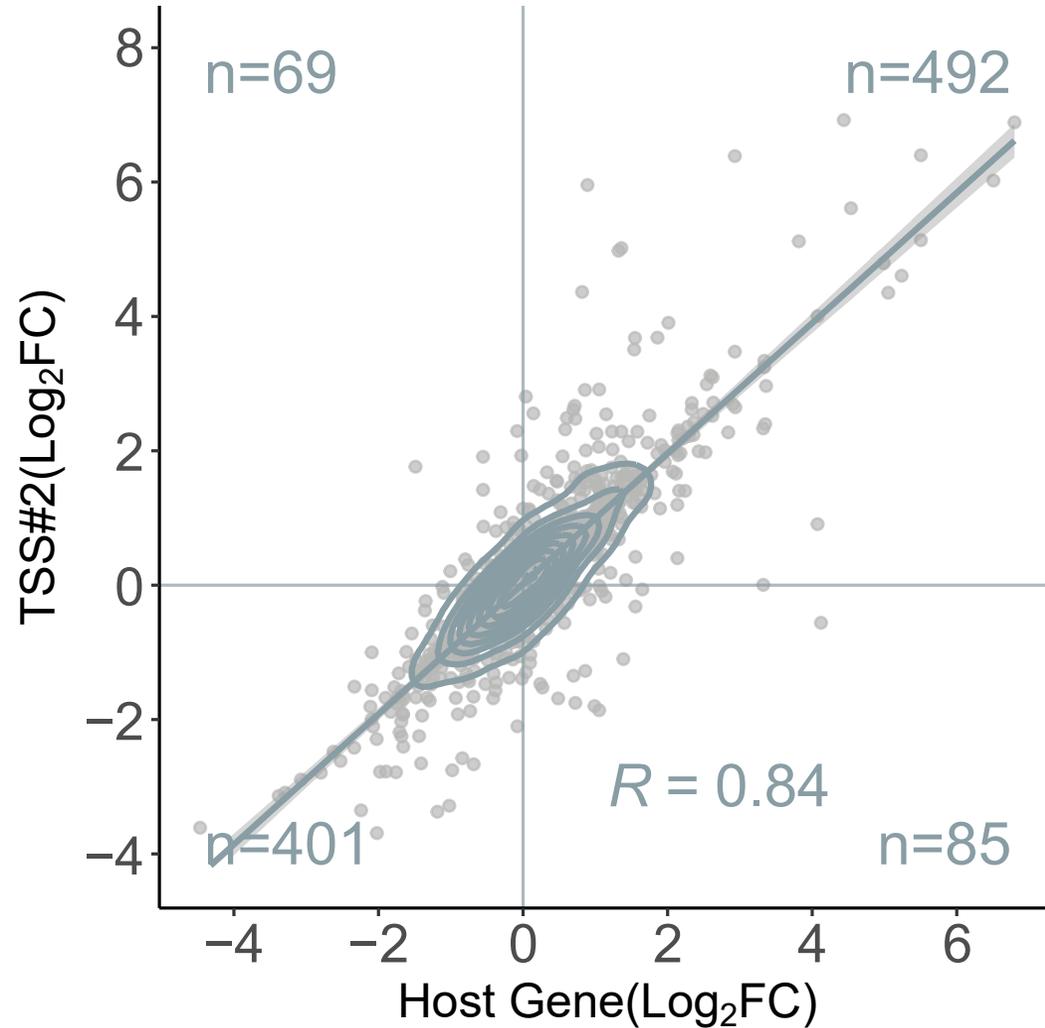
Read count of TSSs
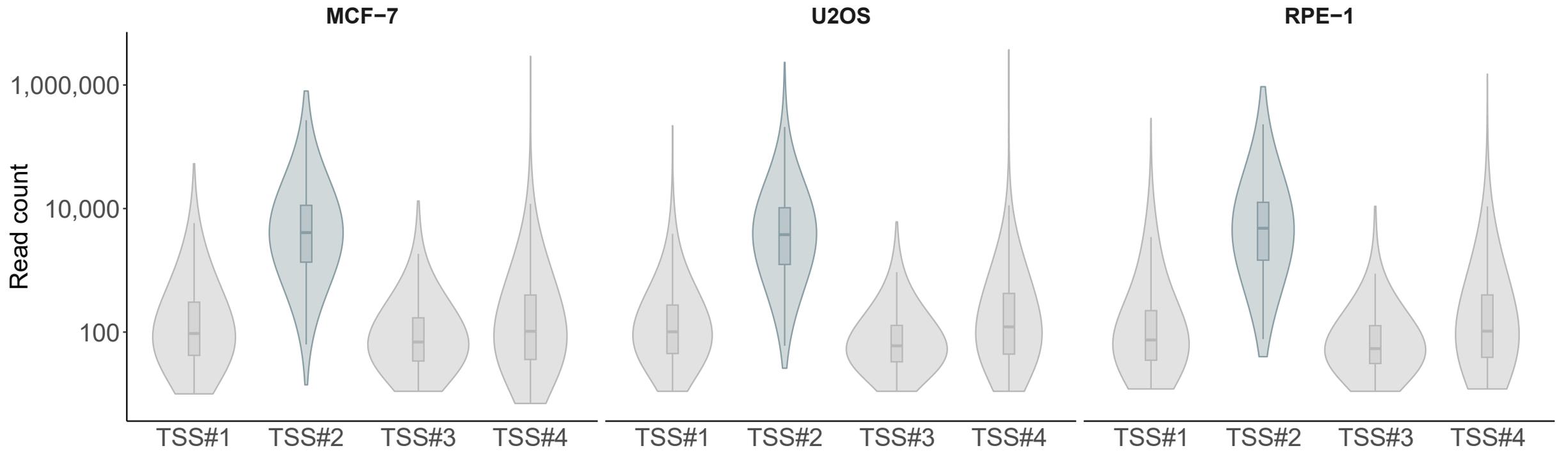
Biotypes of annotated features