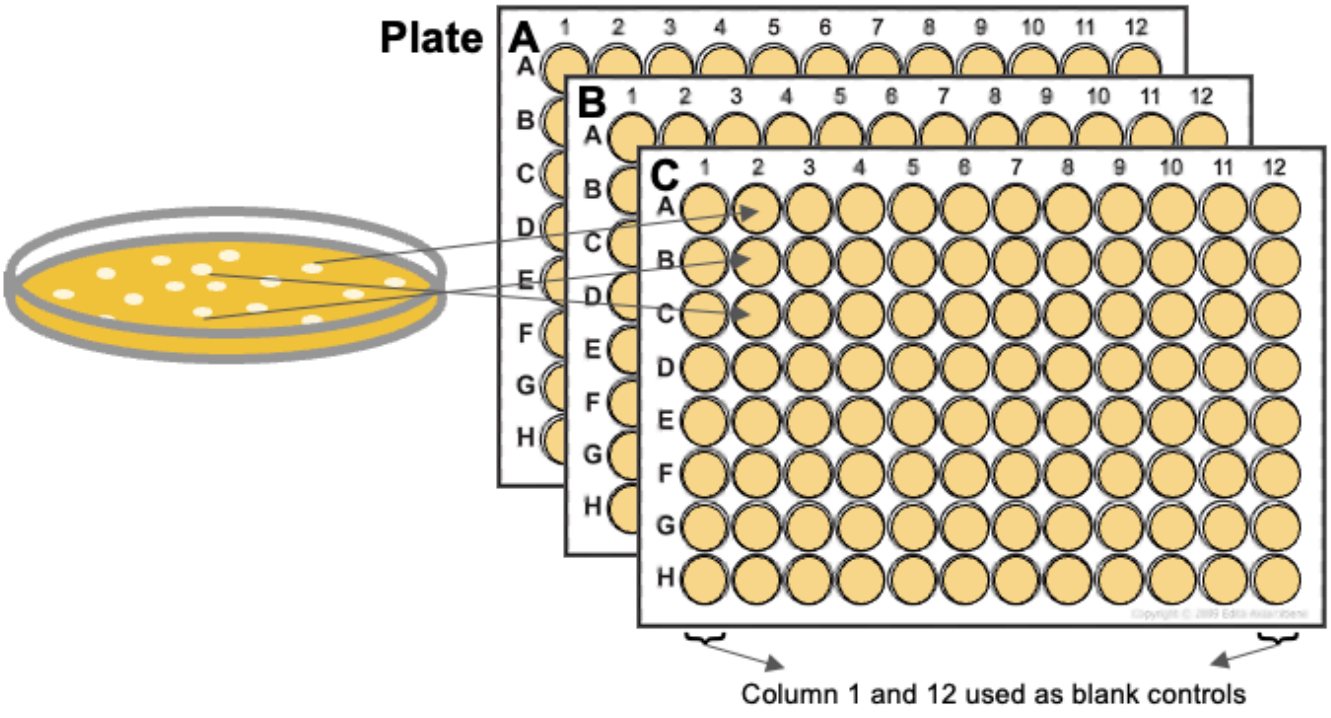
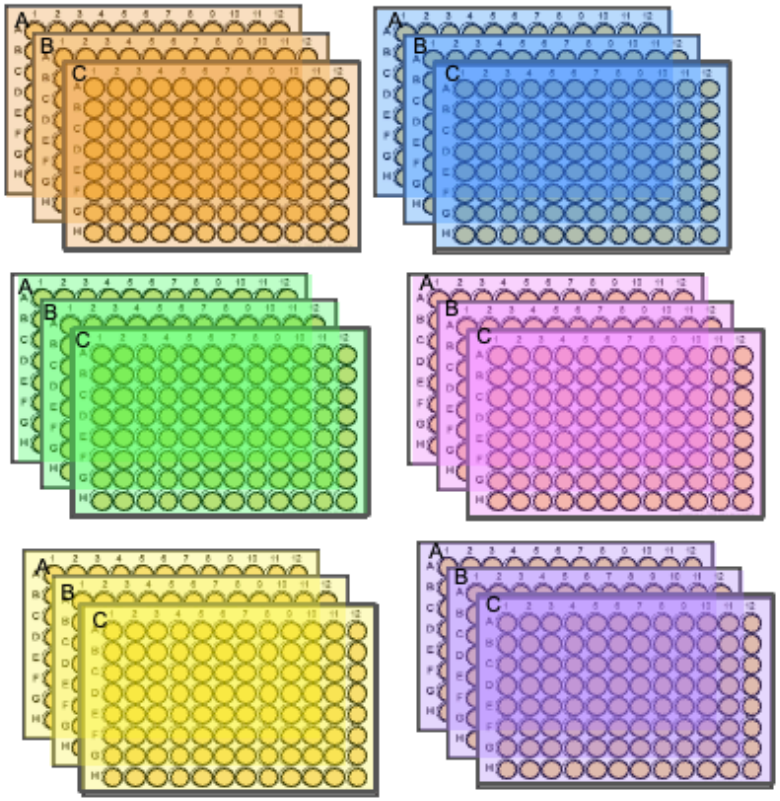
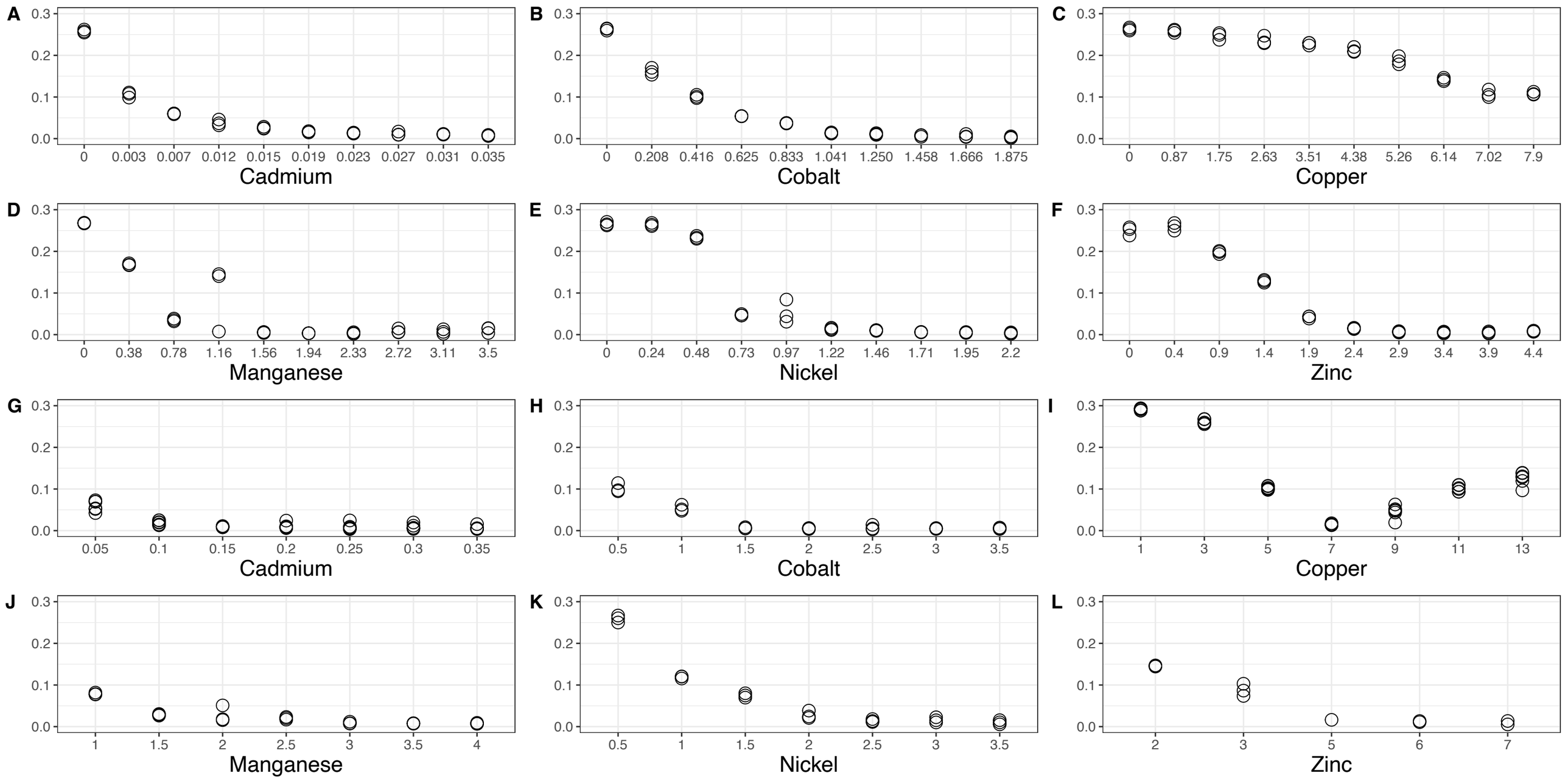
The ancestral strain W303 background is grown up in liquid media and plated on petri dishes to sample 240 single colonies.

240 single-colony ancestral populations in 3 (A, B, C) 96-well plates (180 per plate).
Yeast was grown up and then diluted to the same OD to have equivalent starting population sizes.

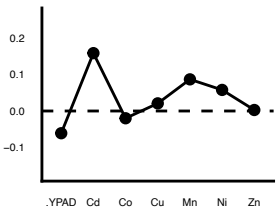


A, B, and C plates with 240 normalized populations are used to inoculate metal media. Yeast was left to grow for two weeks. When growth was observed in a well, the well was then sampled after 24 hours.

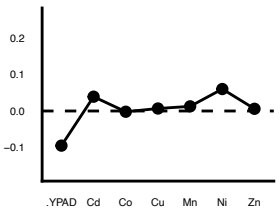



A
0.3
0.2
0.1
0.0
0
0.003
0.007
0.012
0.015
0.019
0.023
0.027
0.031
0.035
Cadmium
B
0
0.208
0.416
0.625
0.833
1.041
1.250
1.458
1.666
1.875
Cobalt
C
0
0.87
1.75
2.63
3.51
4.38
5.26
6.14
7.02
7.9
Copper
D
0
0.38
0.78
1.16
1.56
1.94
2.33
2.72
3.11
3.5
Manganese
E
0
0.24
0.48
0.73
0.97
1.22
1.46
1.71
1.95
2.2
Nickel
F
0
0.4
0.9
1.4
1.9
2.4
2.9
3.4
3.9
4.4
Zinc
G
0.05
0.1
0.15
0.2
0.25
0.3
0.35
Cadmium
H
0.5
1
1.5
2
2.5
3
3.5
Cobalt
I
1
3
5
7
9
11
13
Copper
J
1
1.5
2
2.5
3
3.5
4
Manganese
K
0.5
1
1.5
2
2.5
3
3.5
Nickel
L
2
3
5
6
7
Zinc

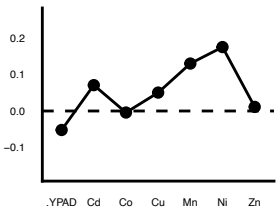
cdbm_21



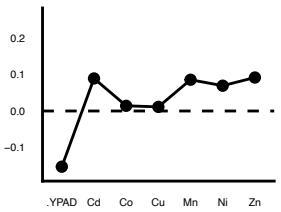
cdbm_30



cdbm_42



cobm_1



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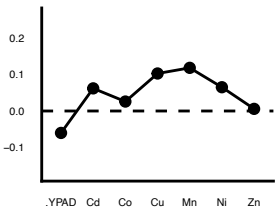
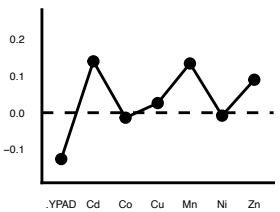


cdbm_22



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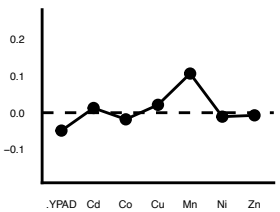

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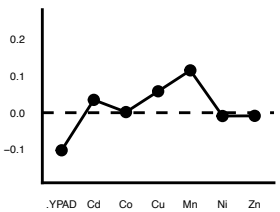

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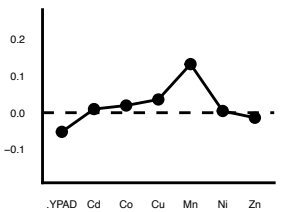

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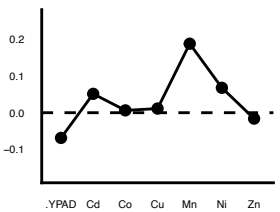
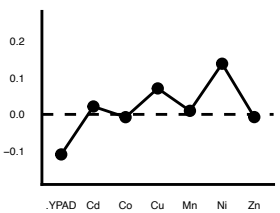
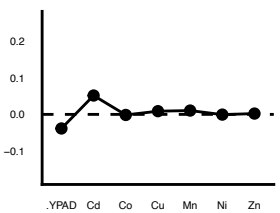


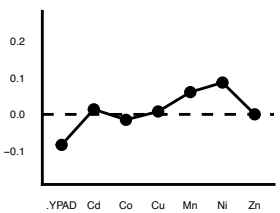
cdbm_23



cdbm_32



cdbm_44



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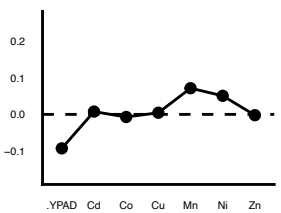

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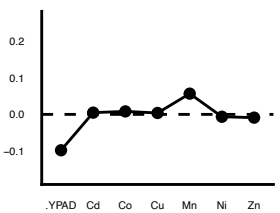

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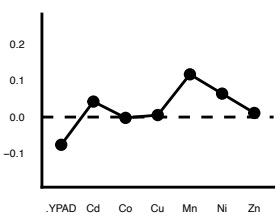

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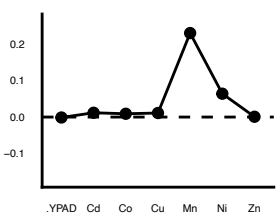

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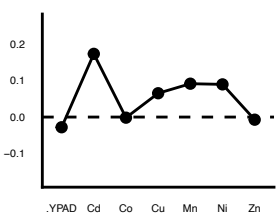

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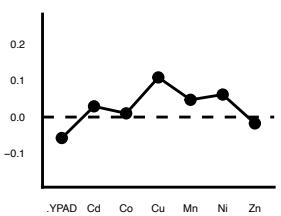

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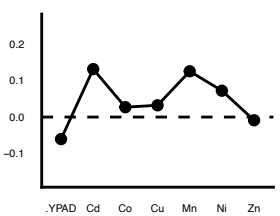

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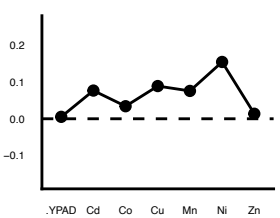

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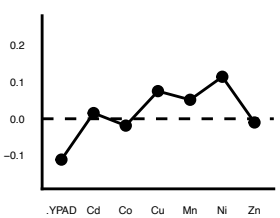

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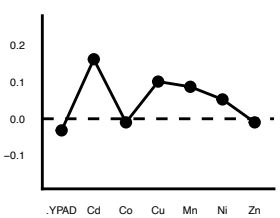

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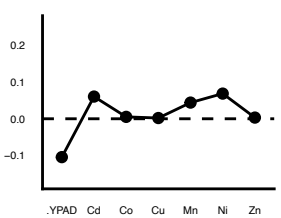

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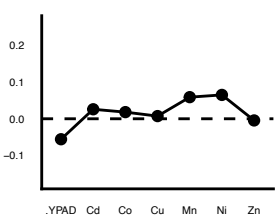

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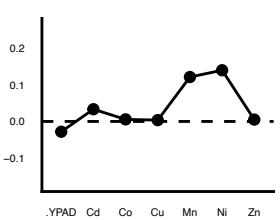

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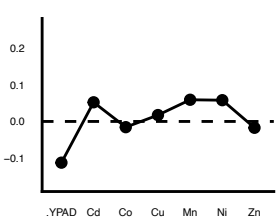
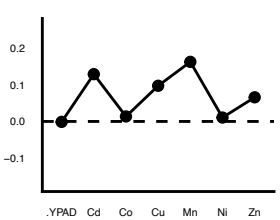


cdbm_47



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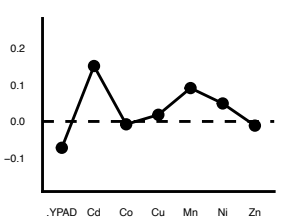

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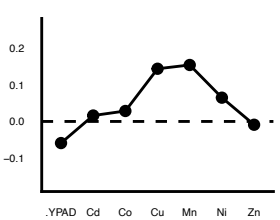

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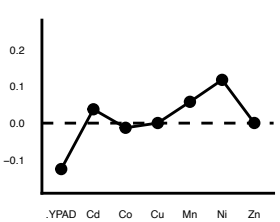

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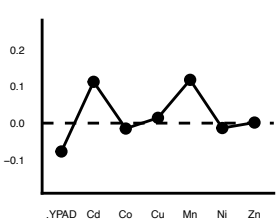

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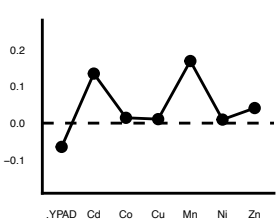

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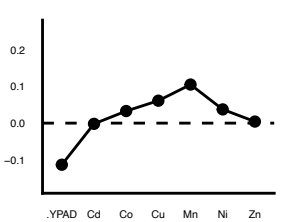

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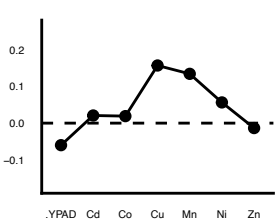

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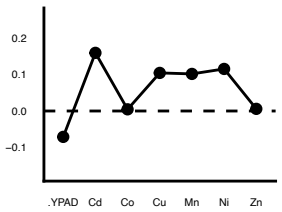

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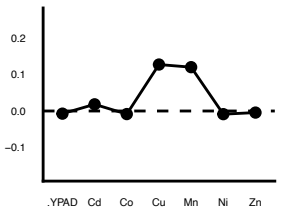

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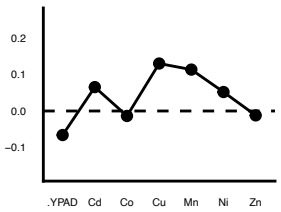

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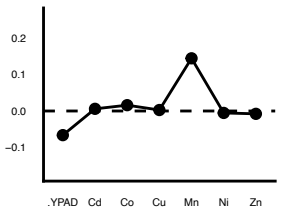

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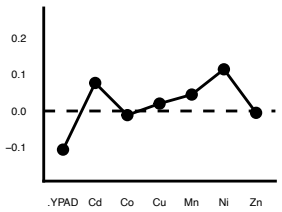

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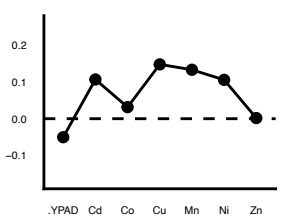

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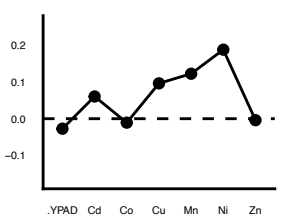

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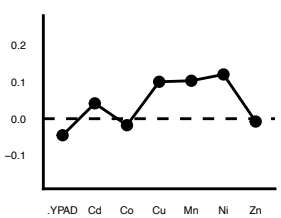

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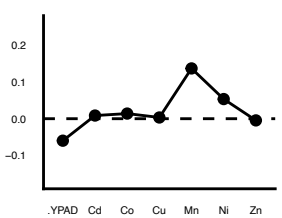

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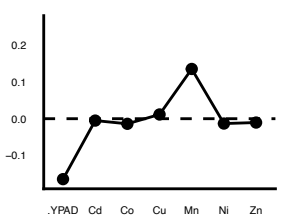

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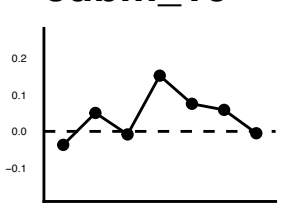

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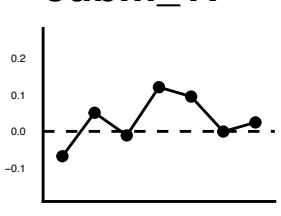
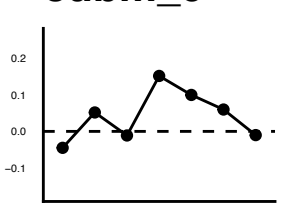


cubm_9



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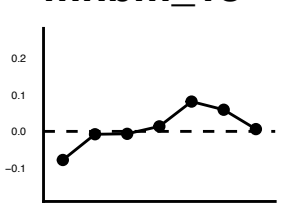

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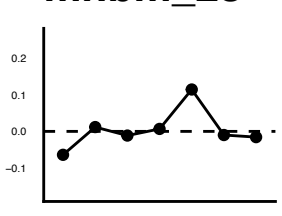

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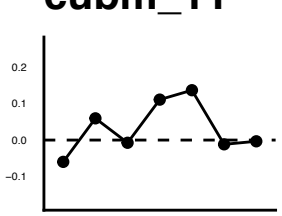

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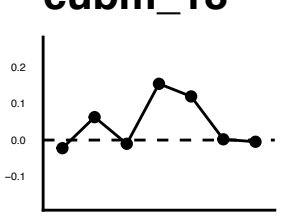
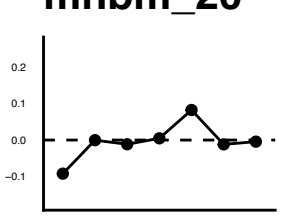
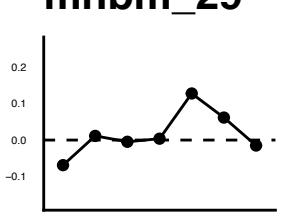


mnbm_12

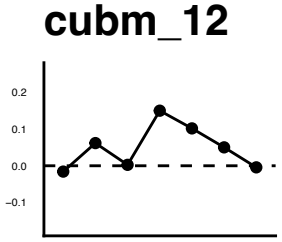
mnbm_20



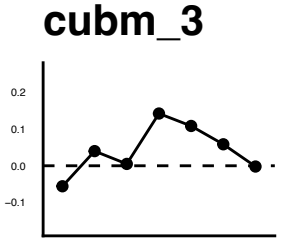
mnbm_29



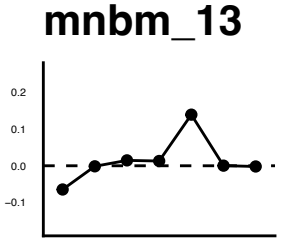
cubm_12



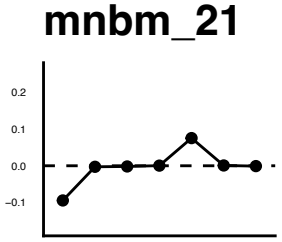
cubm_3



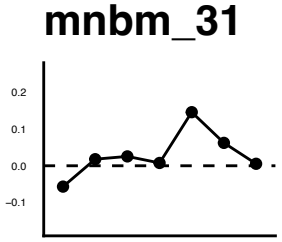
mnbm_13



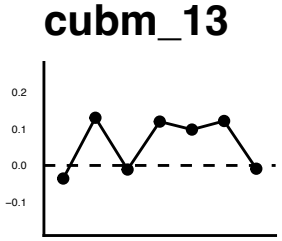
mnbm_21



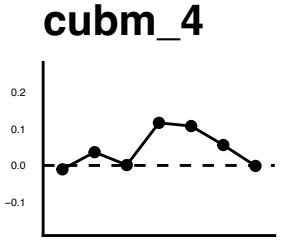
mnbm_31



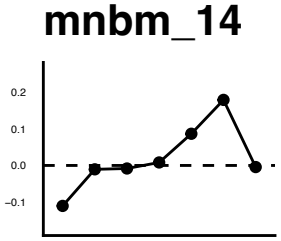
cubm_13



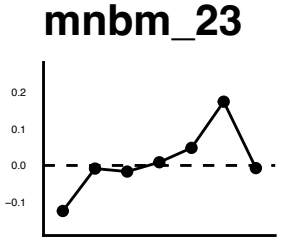
cubm_4



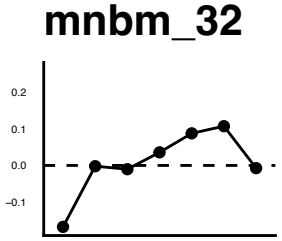
mnbm_14



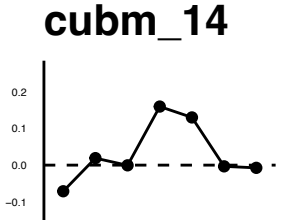
mnbm_23



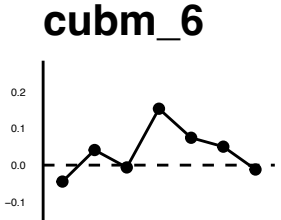
mnbm_32



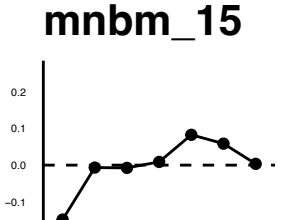
cubm_14



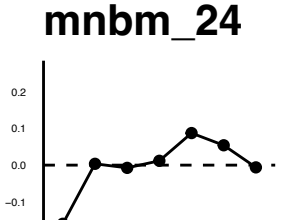
cubm_6



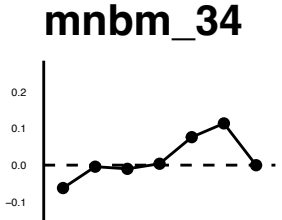
mnbm_15

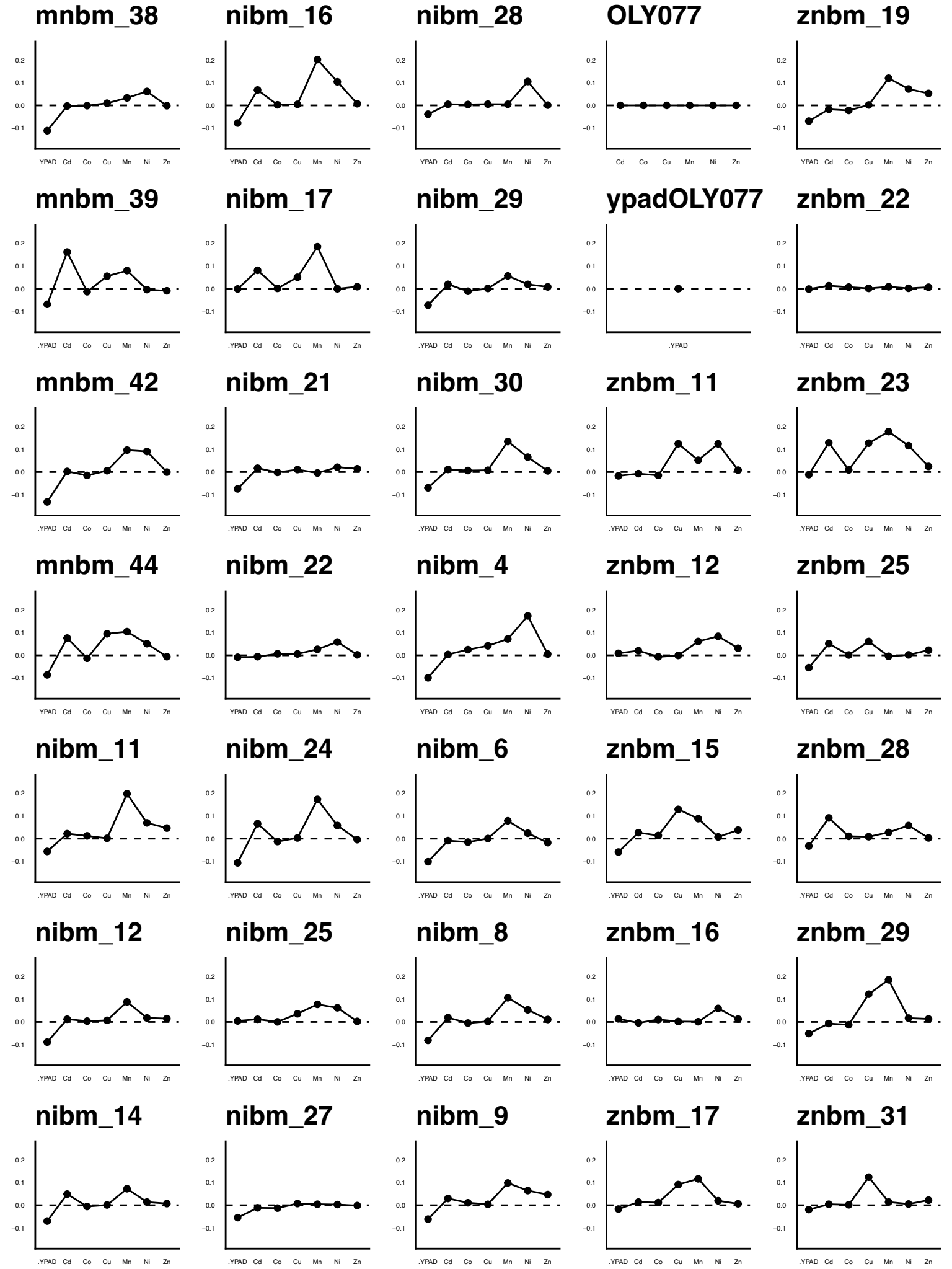


mnbm_24

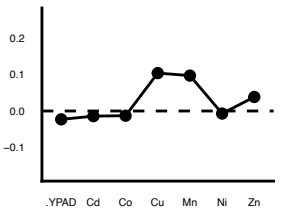


mnbm_34

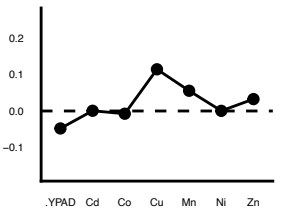



mnbm_38
nibm_16
nibm_28
OLY077
znbm_19
mnbm_39
nibm_17
nibm_29
ypadOLY077
znbm_22
mnbm_42
nibm_21
nibm_30
znbm_11
znbm_23
mnbm_44
nibm_22
nibm_4
znbm_12
znbm_25
nibm_11
nibm_24
nibm_6
znbm_15
znbm_28
nibm_12
nibm_25
nibm_8
znbm_16
znbm_29
nibm_14
nibm_27
nibm_9
znbm_17
znbm_31
0.2
0.1
0.0
−0.1
.YPAD
Cd
Co
Cu
Mn
Ni
Zn

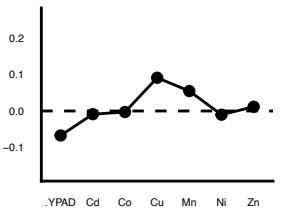
znbm_32



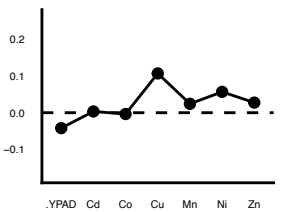
znbm_43



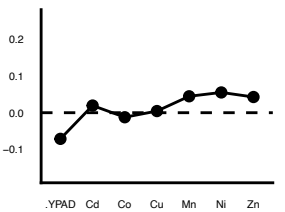
znbm_7



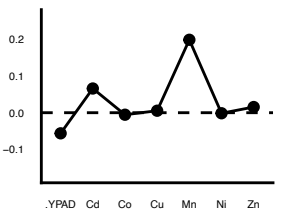
znbm_34



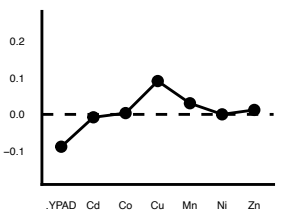
znbm_44



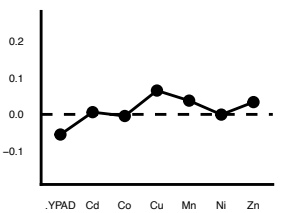
znbm_8



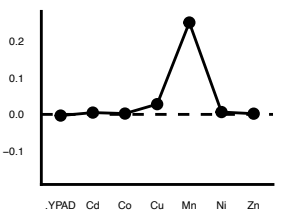
znbm_37



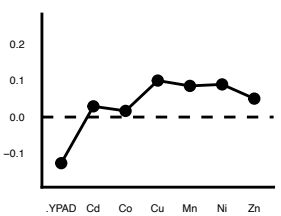
znbm_45



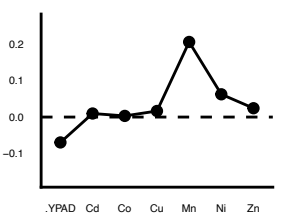
znbm_9



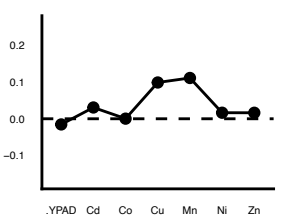
znbm_38



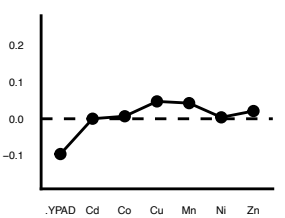
znbm_46



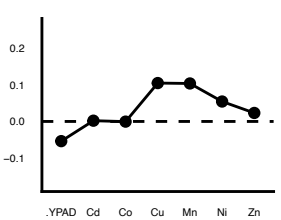
znbm_39



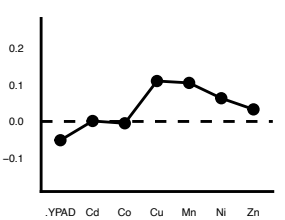
znbm_47



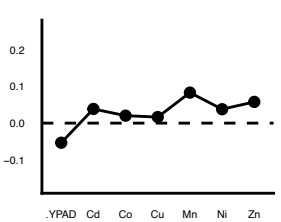
znbm_41



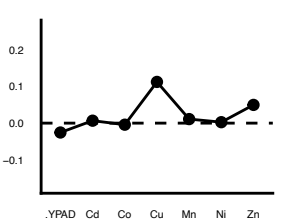
znbm_5

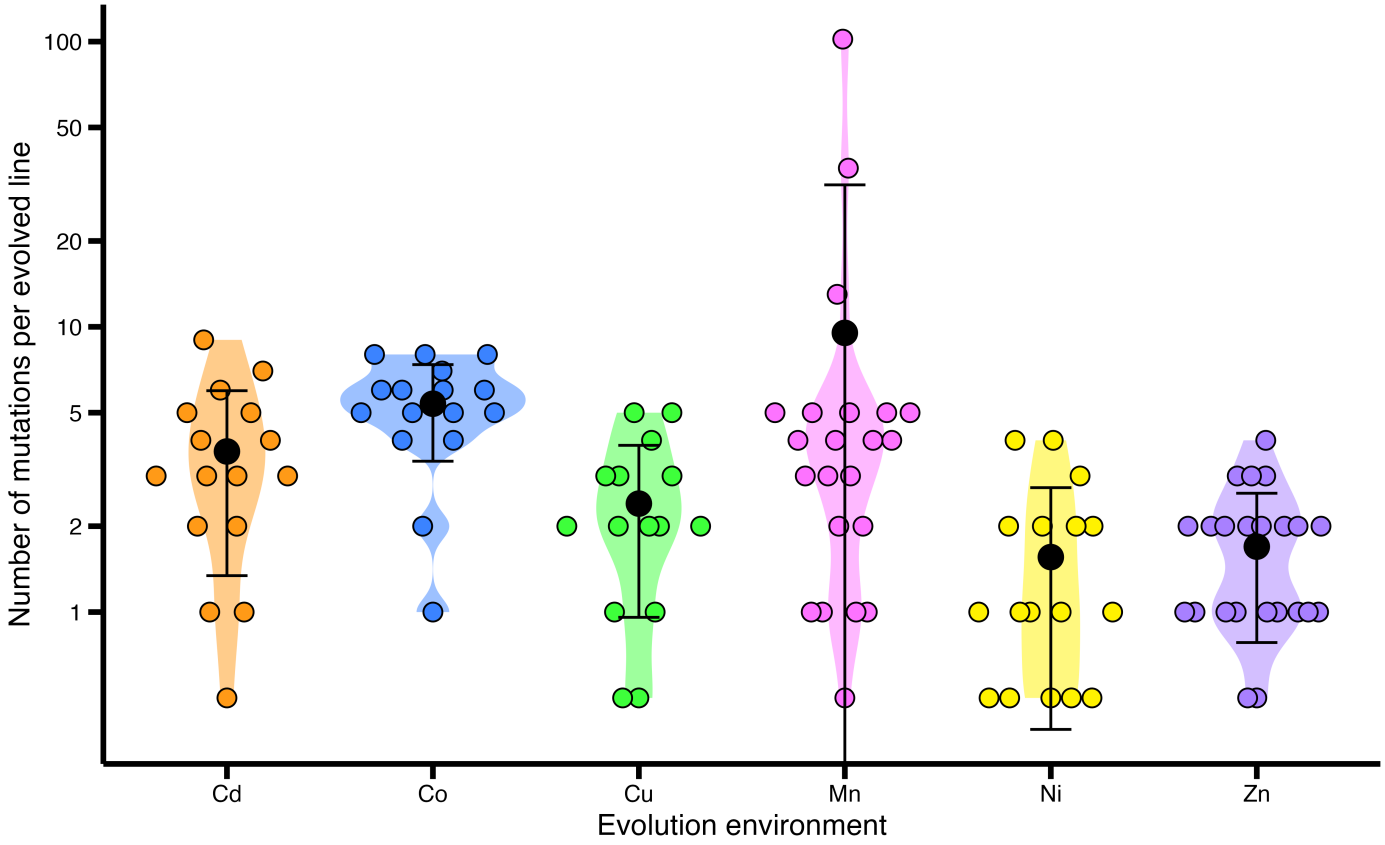


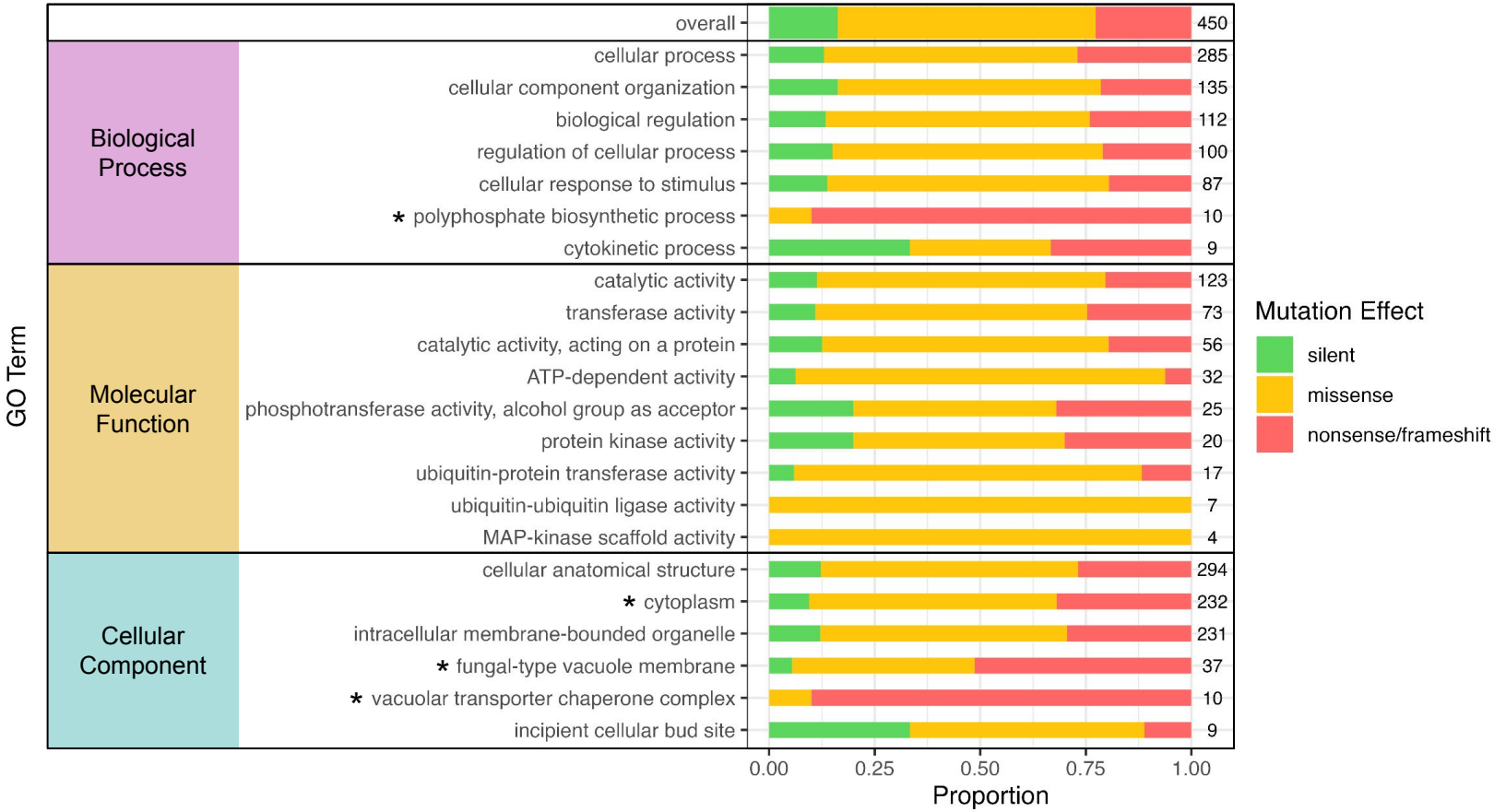
znbm_42

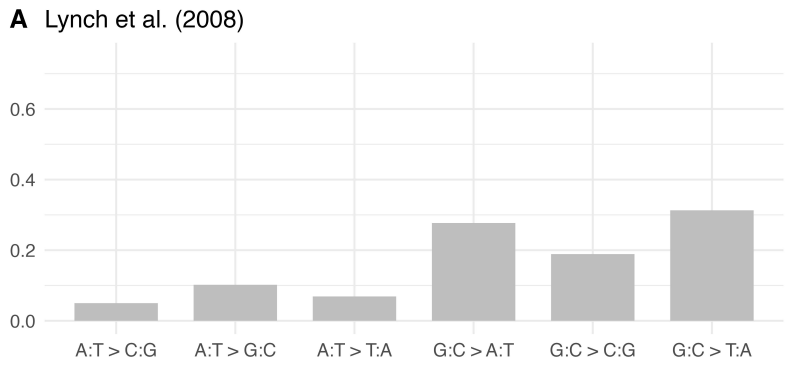


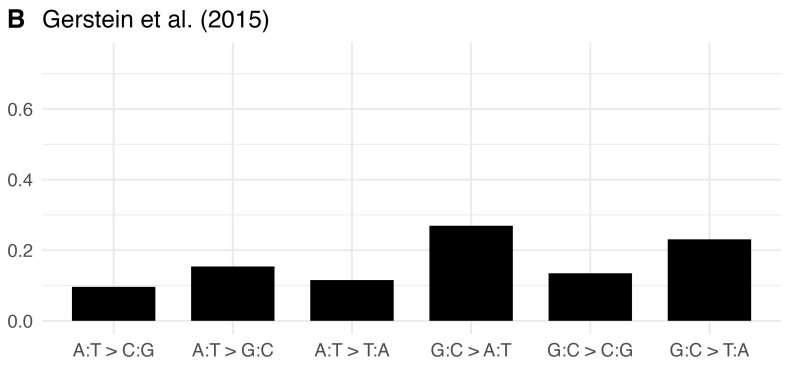
znbm_6

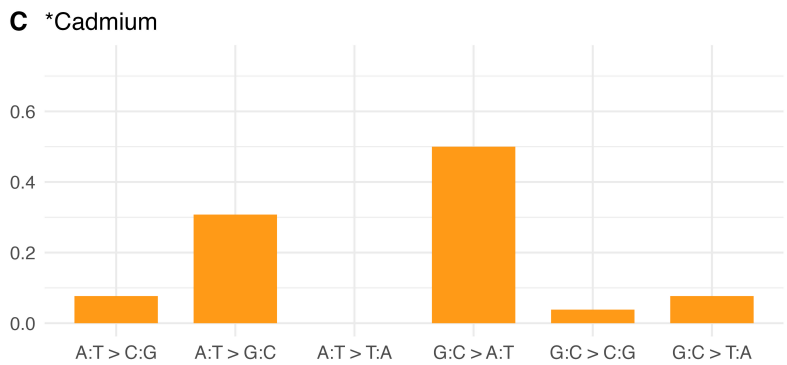


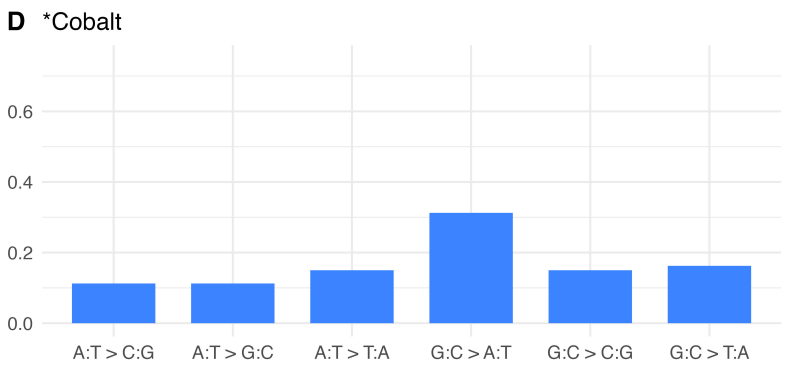

Number of mutations per evolved line
100
50
20
10
5
2
1
Cd
Co
Cu
Mn
Ni
Zn
Evolution environment

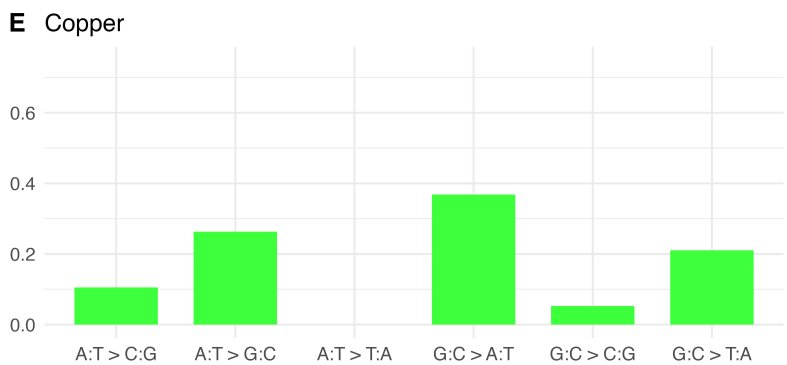

GO Term
Biological Process
Molecular Function
Cellular Component
overall
cellular process
cellular component organization
biological regulation
regulation of cellular process
cellular response to stimulus
* polyphosphate biosynthetic process
cytokinetic process
catalytic activity
transferase activity
catalytic activity, acting on a protein
ATP-dependent activity
phosphotransferase activity, alcohol group as acceptor
protein kinase activity
ubiquitin-protein transferase activity
ubiquitin-ubiquitin ligase activity
MAP-kinase scaffold activity
cellular anatomical structure
* cytoplasm
intracellular membrane-bounded organelle
* fungal-type vacuole membrane
* vacuolar transporter chaperone complex
incipient cellular bud site
450
285
135
112
100
87
10
9
123
73
56
32
25
20
17
7
4
294
232
231
37
10
9
0.00
0.25
0.50
0.75
1.00
Proportion
Mutation Effect
silent
missense
nonsense/frameshift

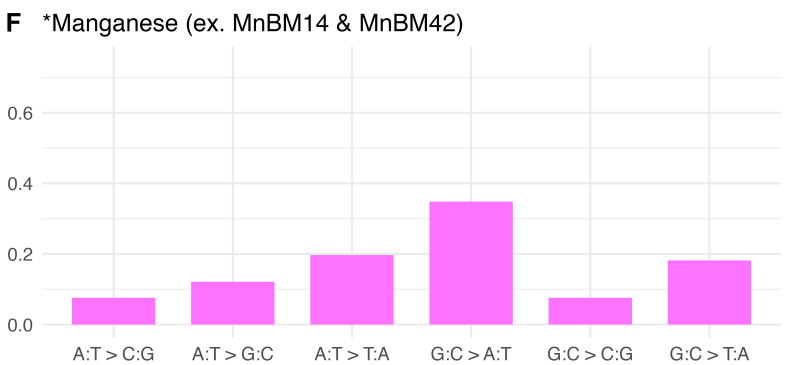

A Lynch et al. (2008)
0.6
0.4
0.2
0.0
A:T > C:G
A:T > G:C
A:T > T:A
G:C > A:T
G:C > C:G
G:C > T:A

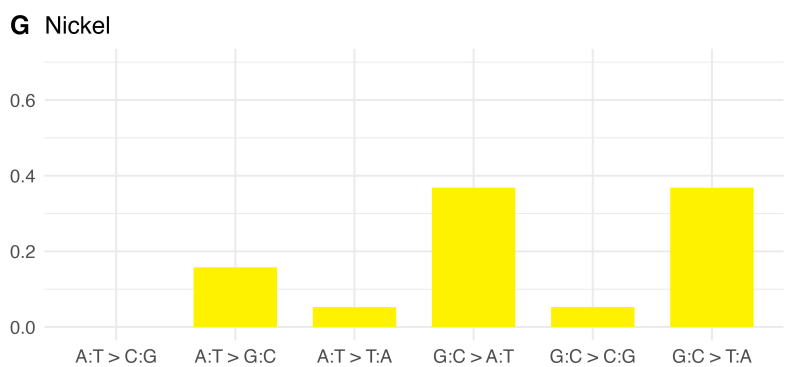

B Gerstein et al. (2015)
0.6
0.4
0.2
0.0
A:T > C:G
A:T > G:C
A:T > T:A
G:C > A:T
G:C > C:G
G:C > T:A

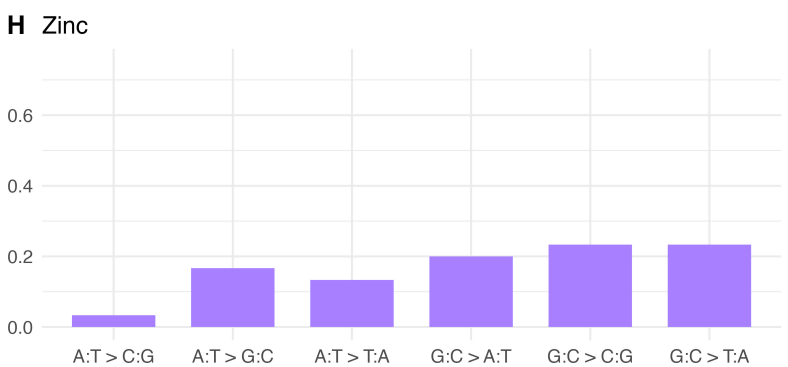

C *Cadmium
0.6
0.4
0.2
0.0
A:T > C:G
A:T > G:C
A:T > T:A
G:C > A:T
G:C > C:G
G:C > T:A

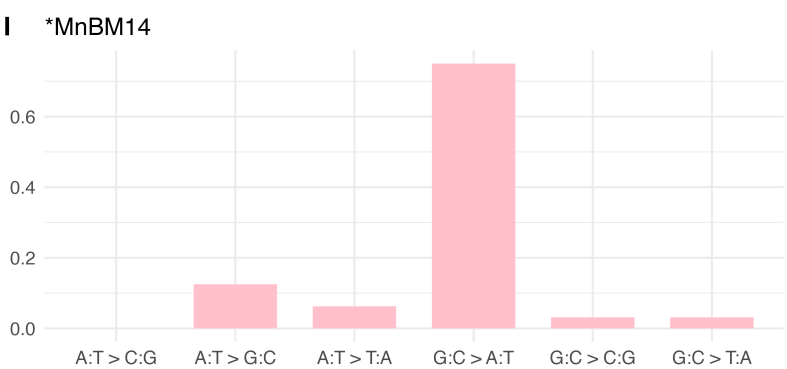

D *Cobalt
0.6
0.4
0.2
0.0
A:T > C:G
A:T > G:C
A:T > T:A
G:C > A:T
G:C > C:G
G:C > T:A

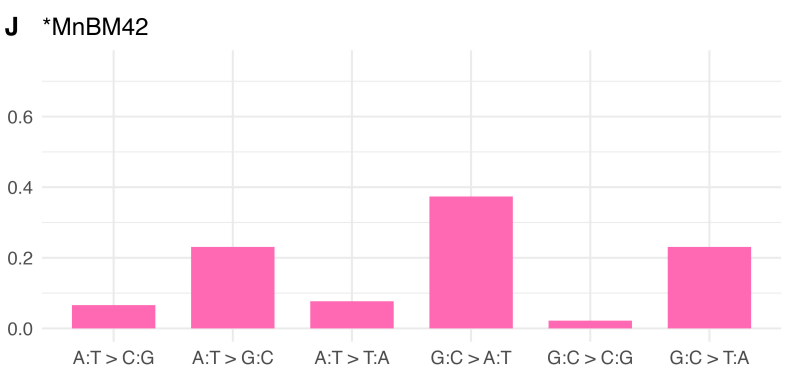

E Copper
0.6
0.4
0.2
0.0
A:T > C:G
A:T > G:C
A:T > T:A
G:C > A:T
G:C > C:G
G:C > T:A

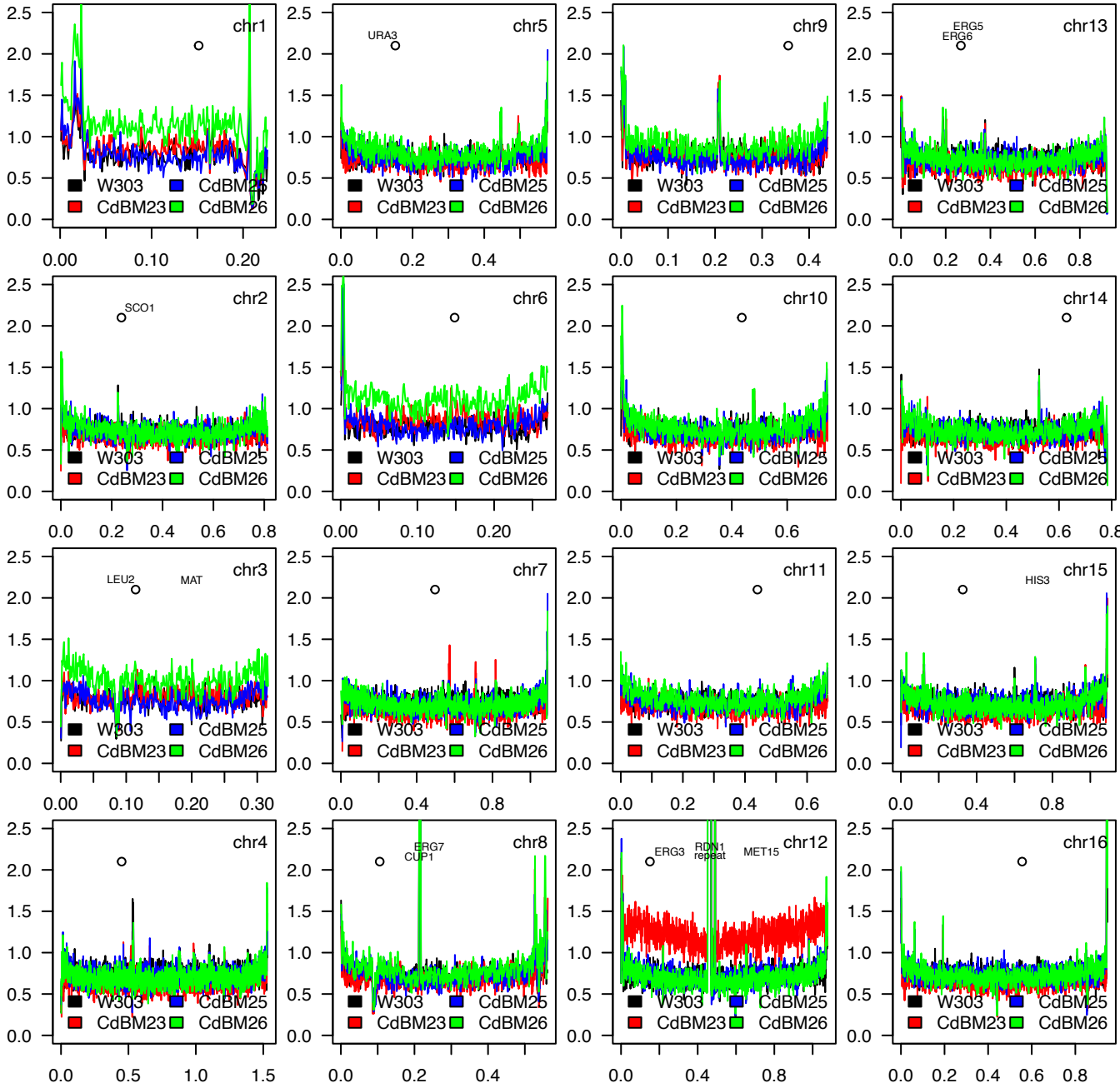

F *Manganese (ex. MnBM14 & MnBM42)
0.6
0.4
0.2
0.0
A:T > C:G
A:T > G:C
A:T > T:A
G:C > A:T
G:C > C:G
G:C > T:A

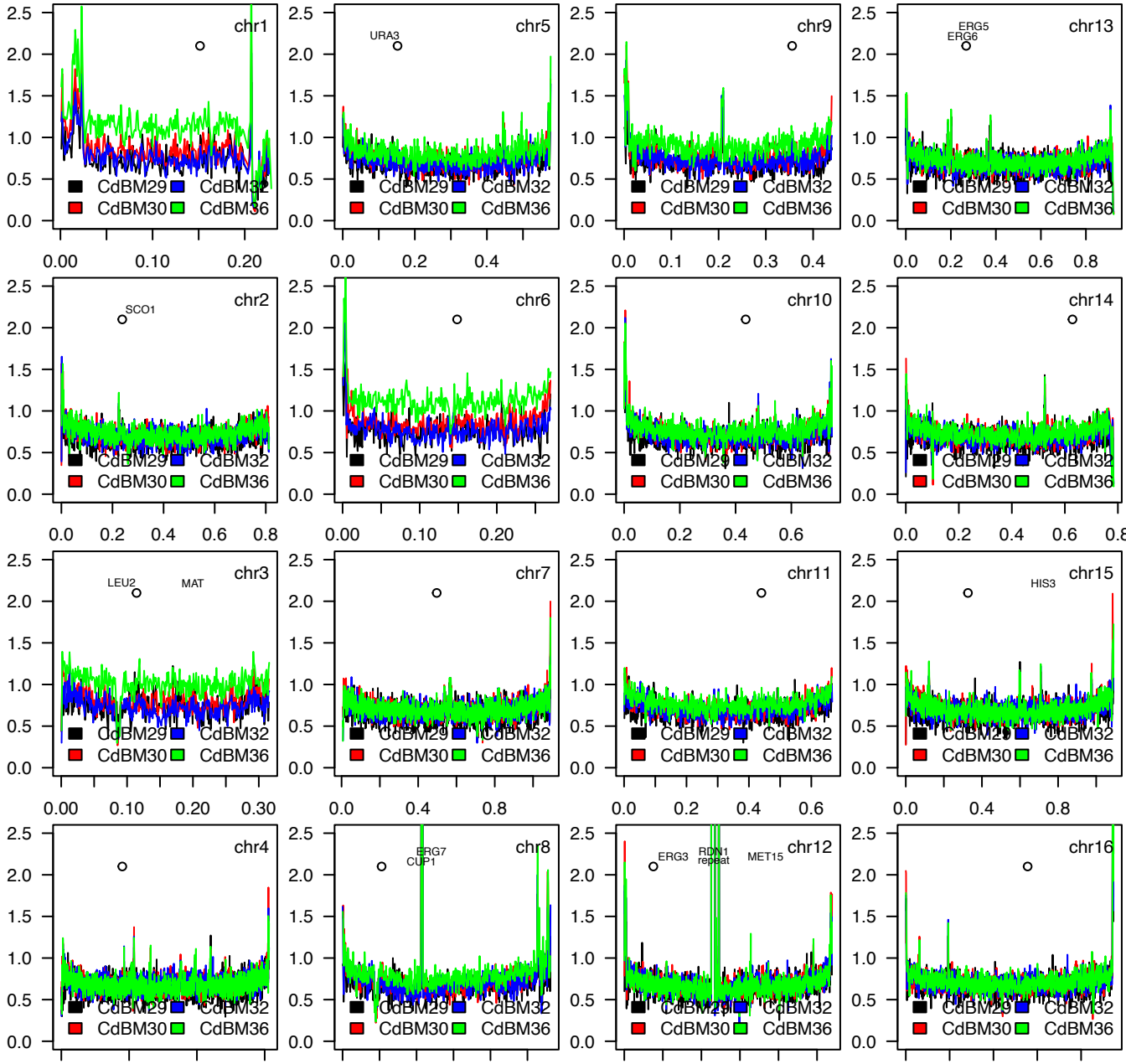

G Nickel
0.6
0.4
0.2
0.0
A:T > C:G
A:T > G:C
A:T > T:A
G:C > A:T
G:C > C:G
G:C > T:A


H Zinc
0.6
0.4
0.2
0.0
A:T > C:G
A:T > G:C
A:T > T:A
G:C > A:T
G:C > C:G
G:C > T:A


I *MnBM14
0.6
0.4
0.2
0.0
A:T > C:G
A:T > G:C
A:T > T:A
G:C > A:T
G:C > C:G
G:C > T:A


J *MnBM42
0.6
0.4
0.2
0.0
A:T > C:G
A:T > G:C
A:T > T:A
G:C > A:T
G:C > C:G
G:C > T:A


chr1
chr5
URA3
chr9
chr13
ERG5
ERG6
chr2
SCO1
chr6
chr10
chr14
chr3
LEU2
MAT
chr7
chr11
chr15
HIS3
chr4
chr8
ERG7
CUP1
chr12
ERG3
RDN1
repeat
MET15
chr16
W303
CdBM25
CdBM23
CdBM26


chr1
chr5
URA3
chr9
chr13
ERG5
ERG6
chr2
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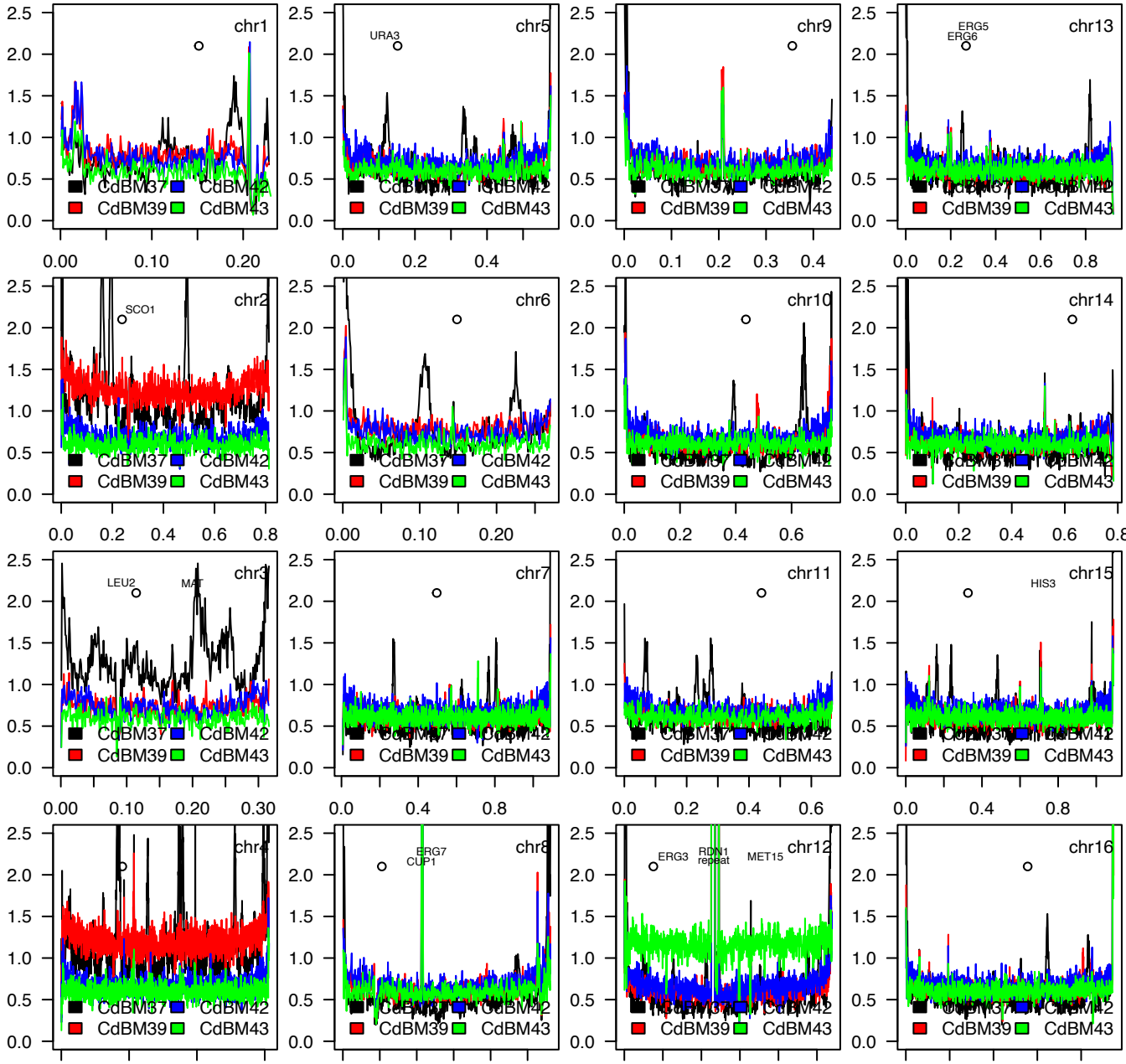
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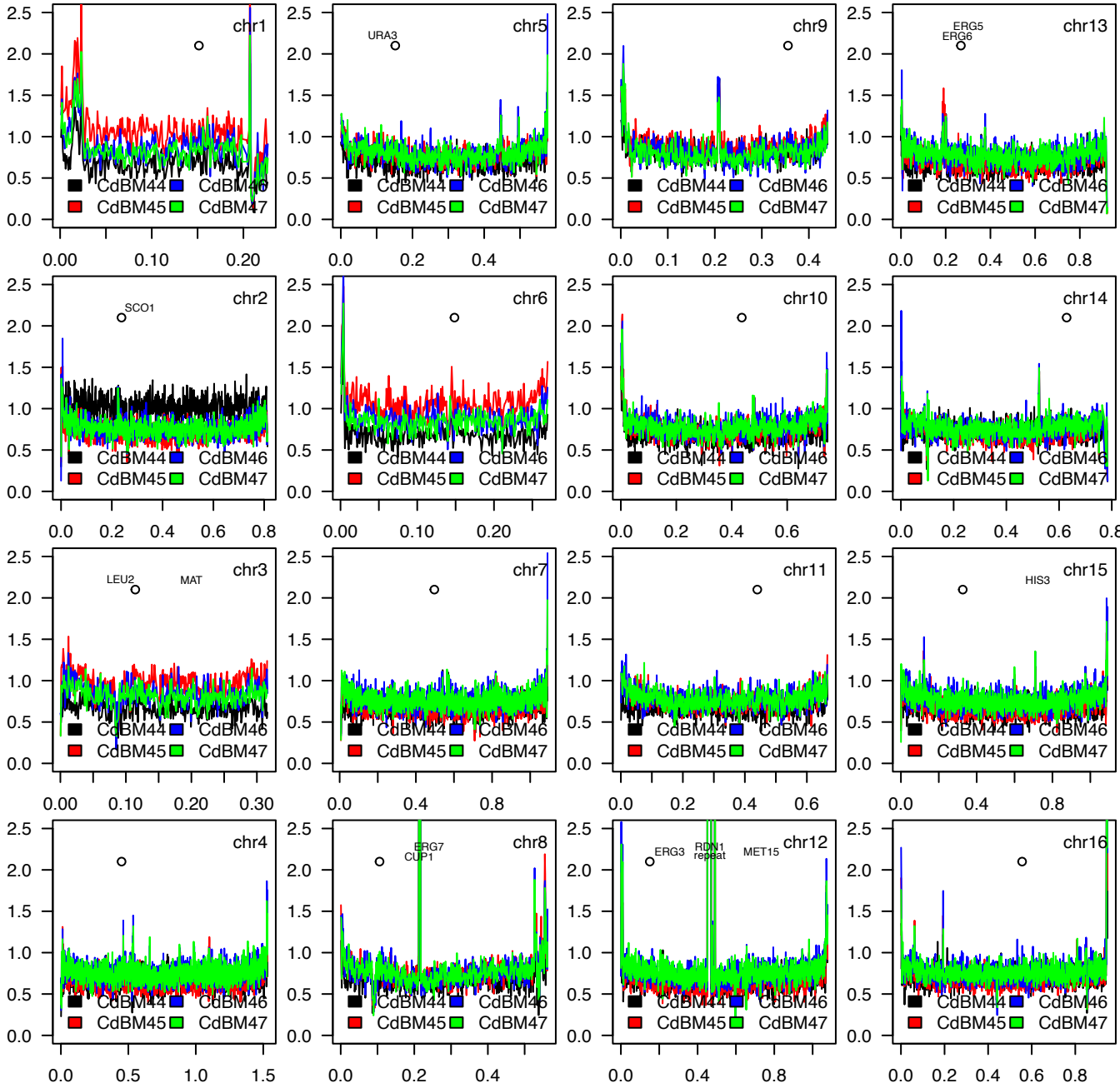
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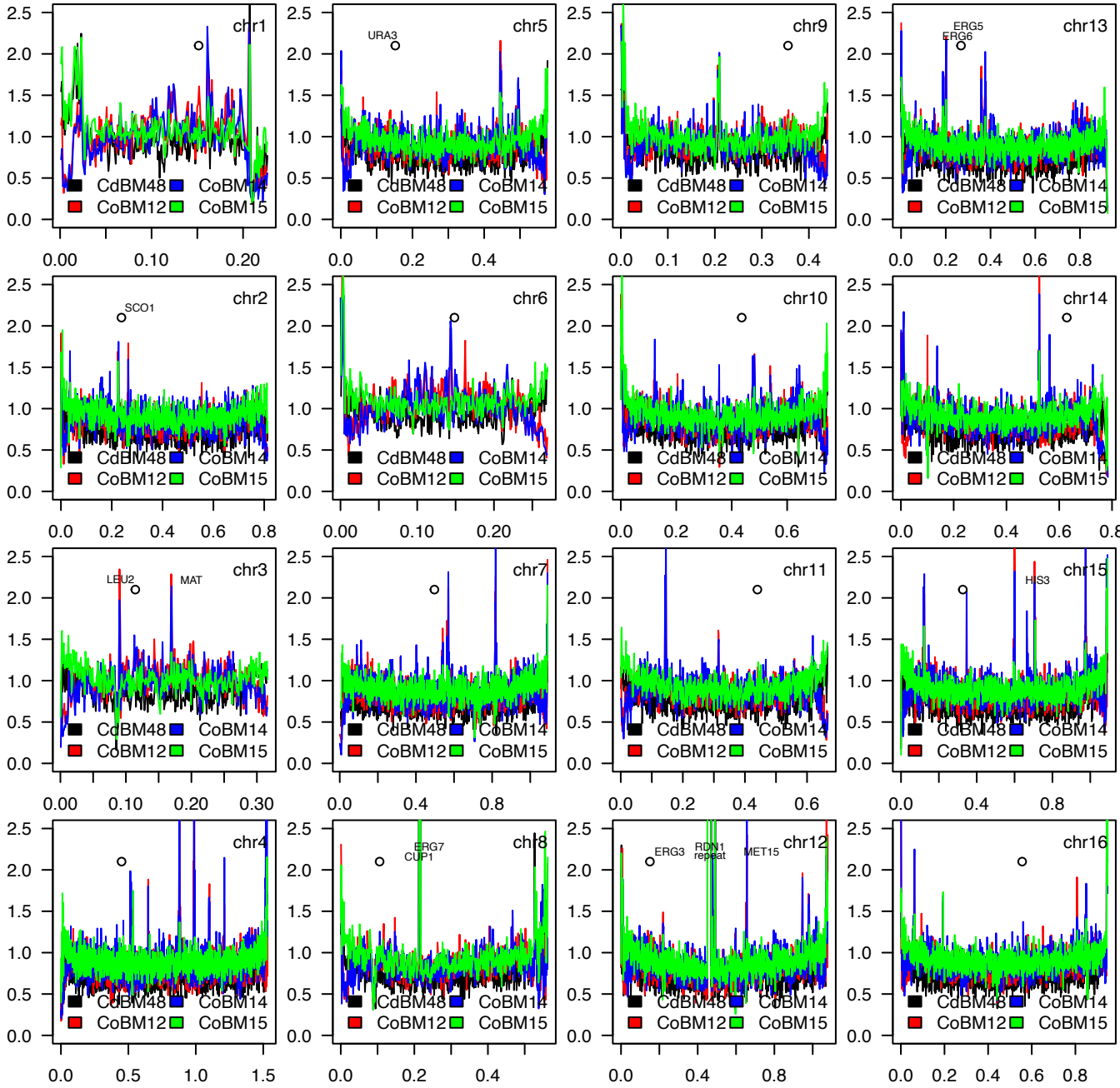
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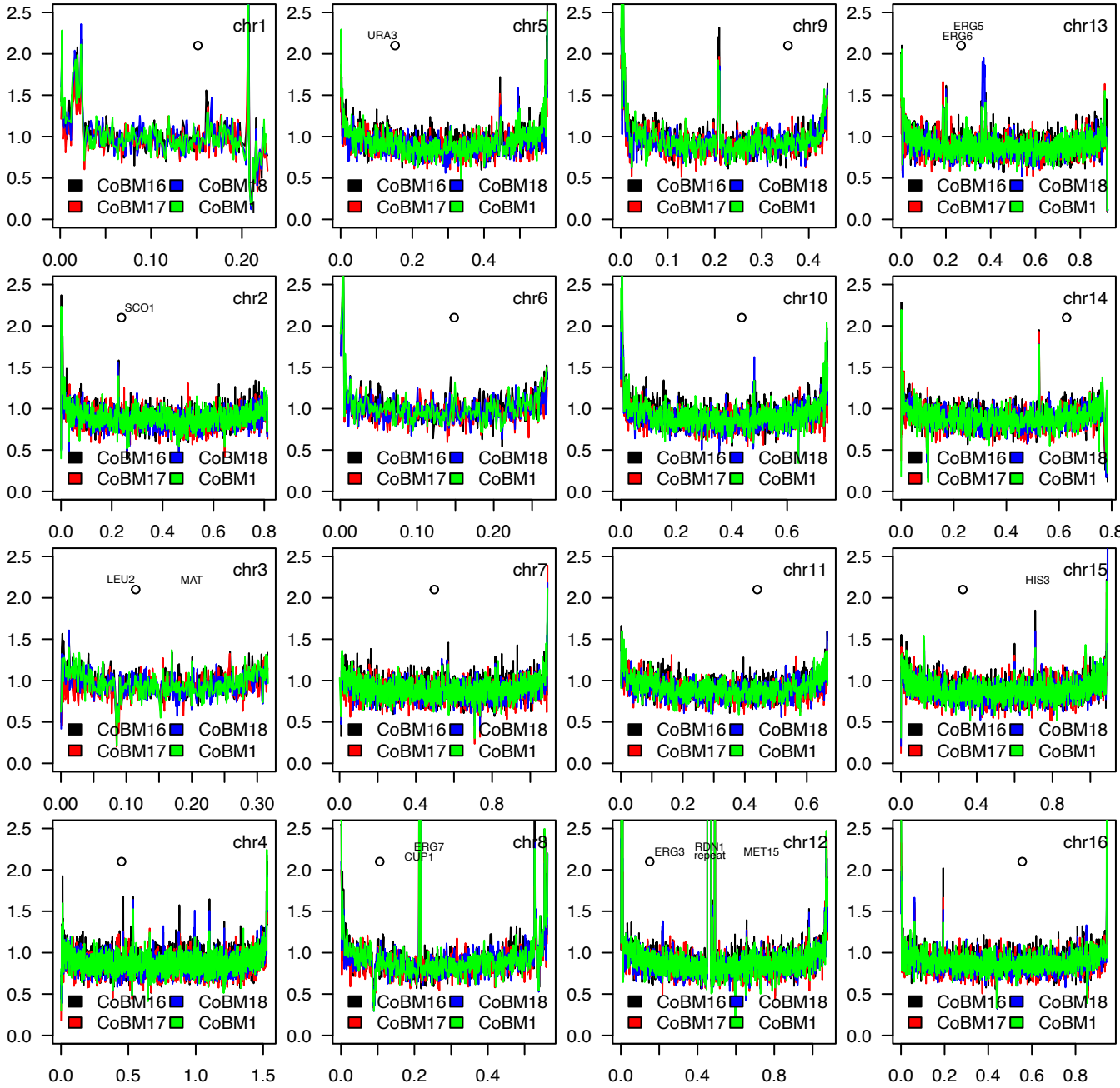
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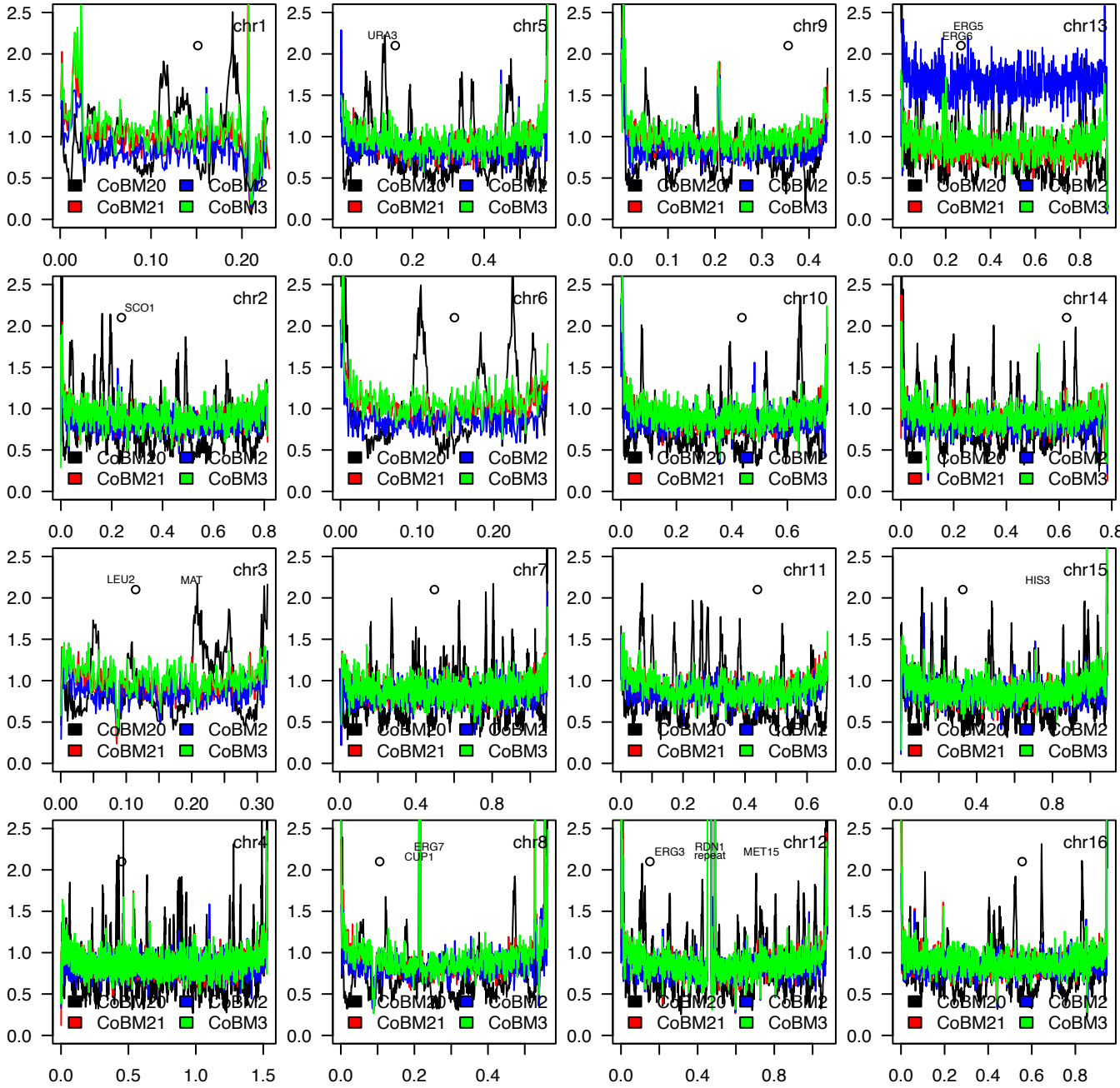
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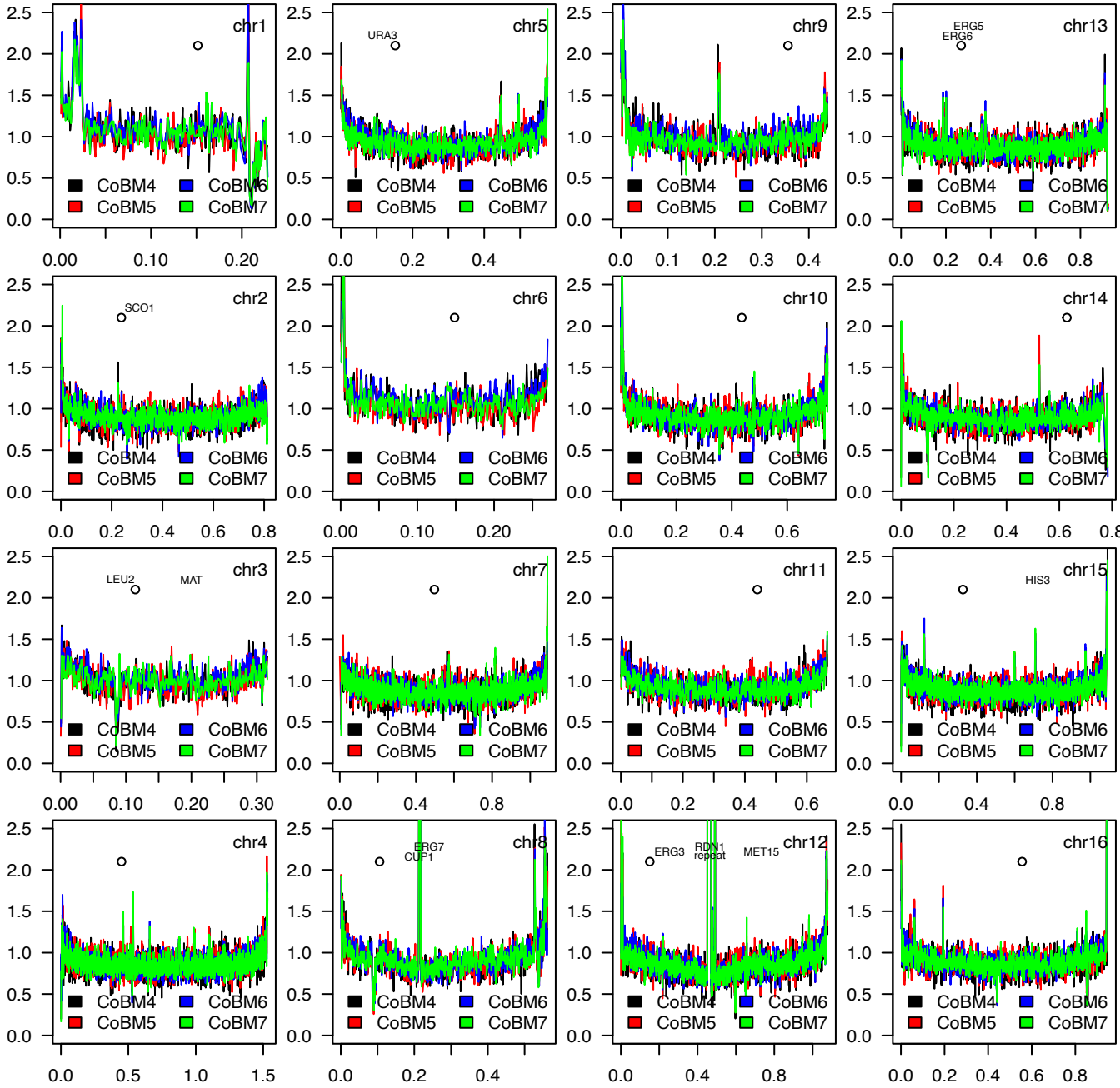
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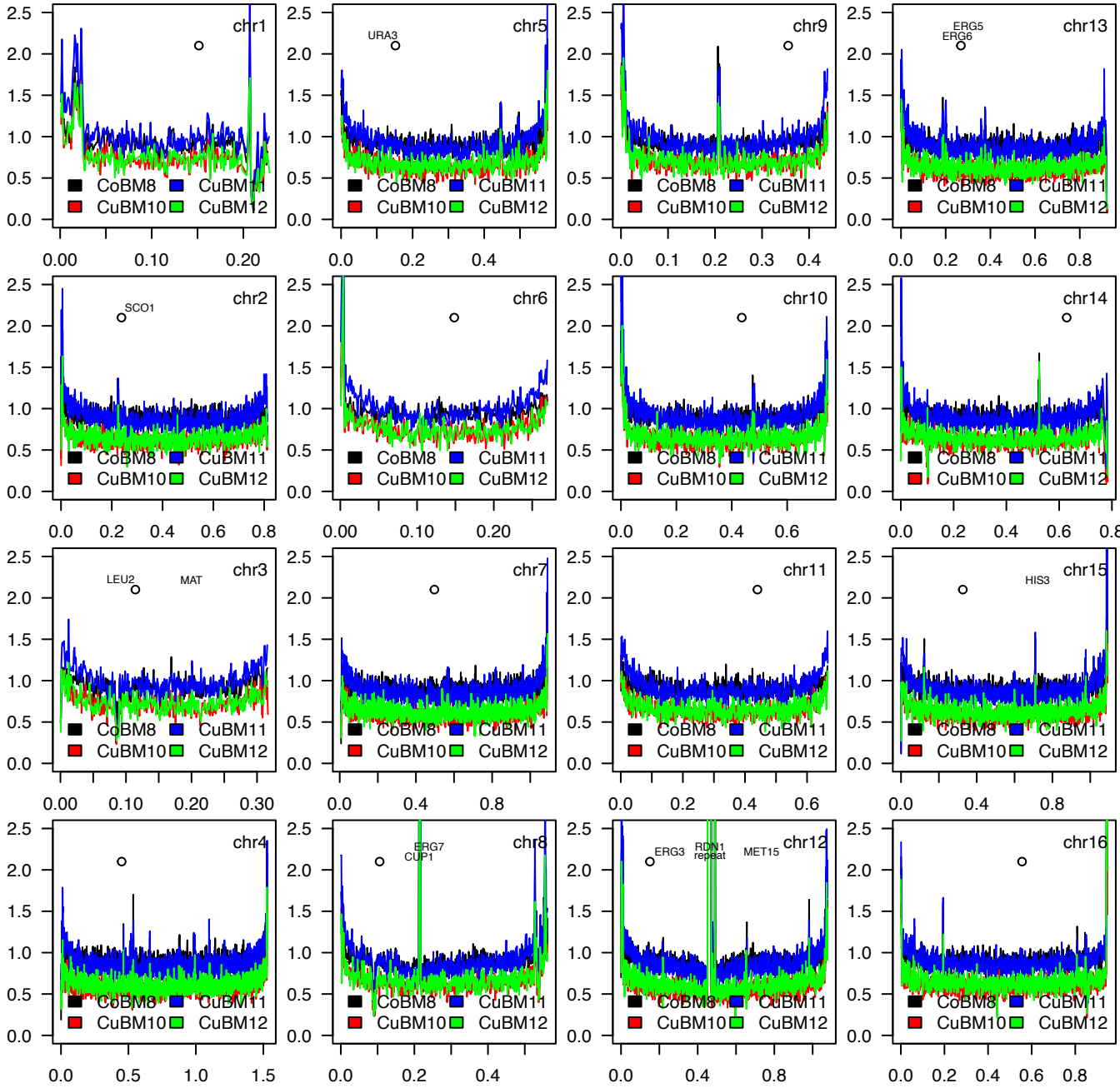
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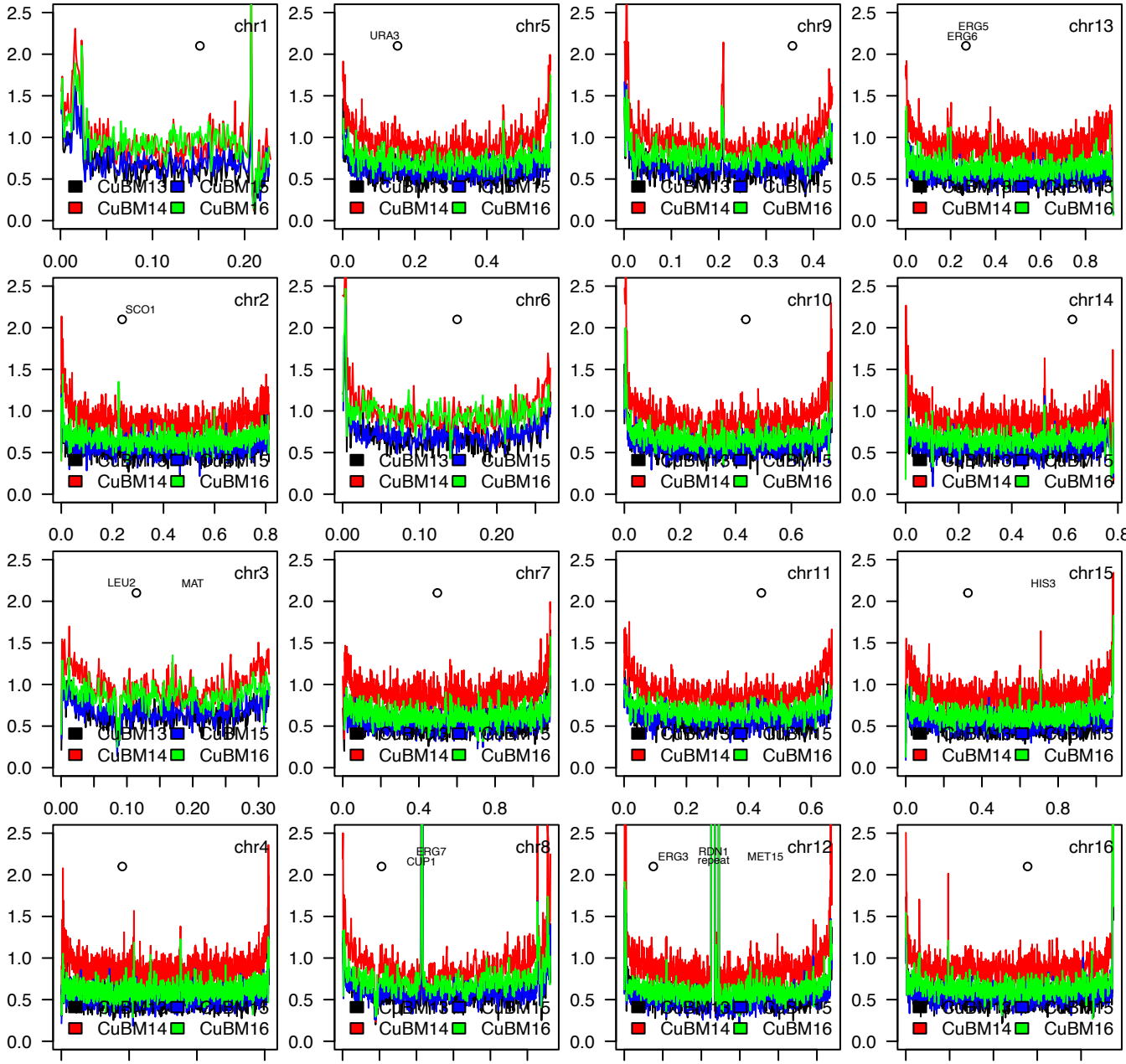
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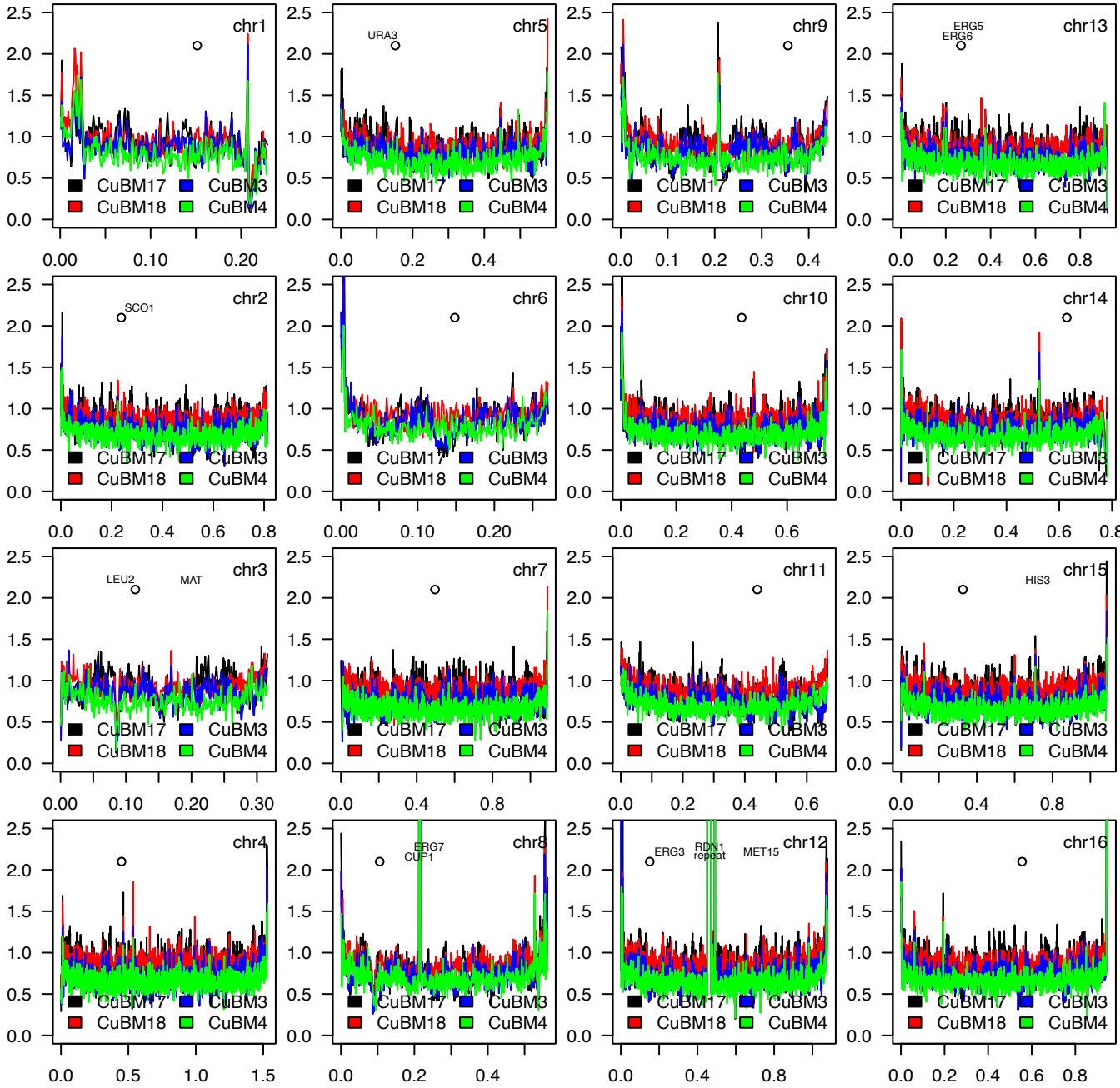
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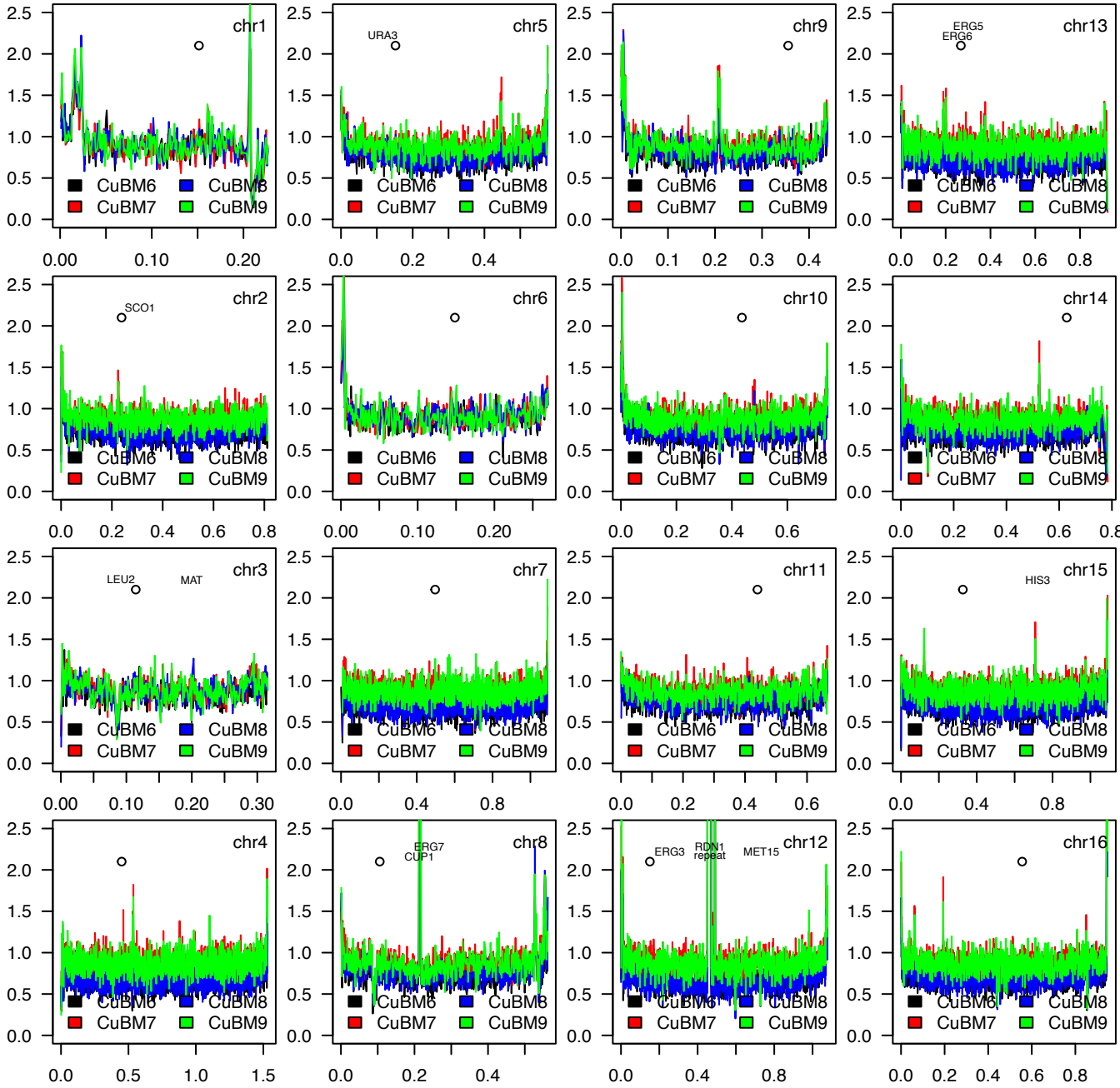
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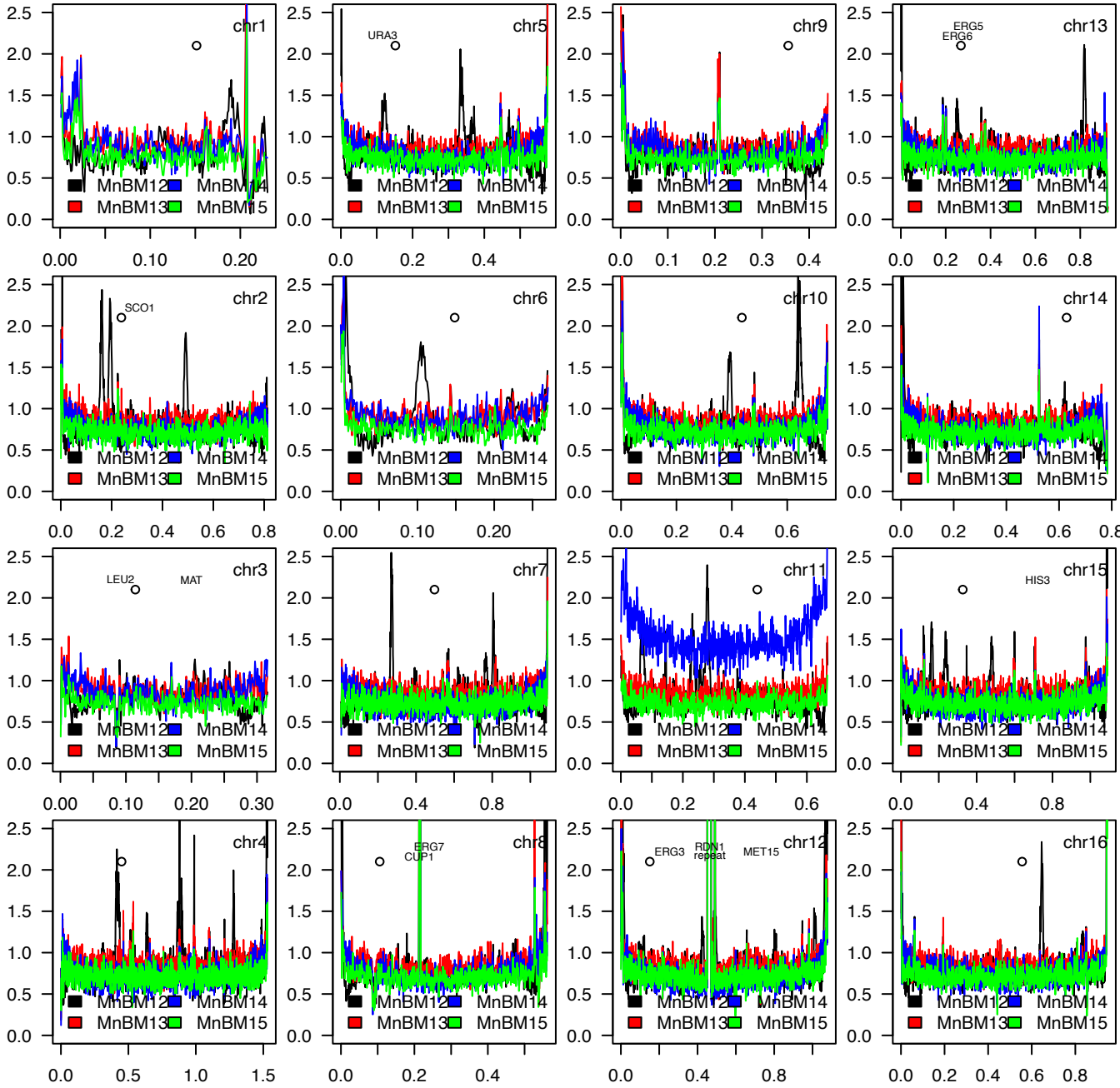
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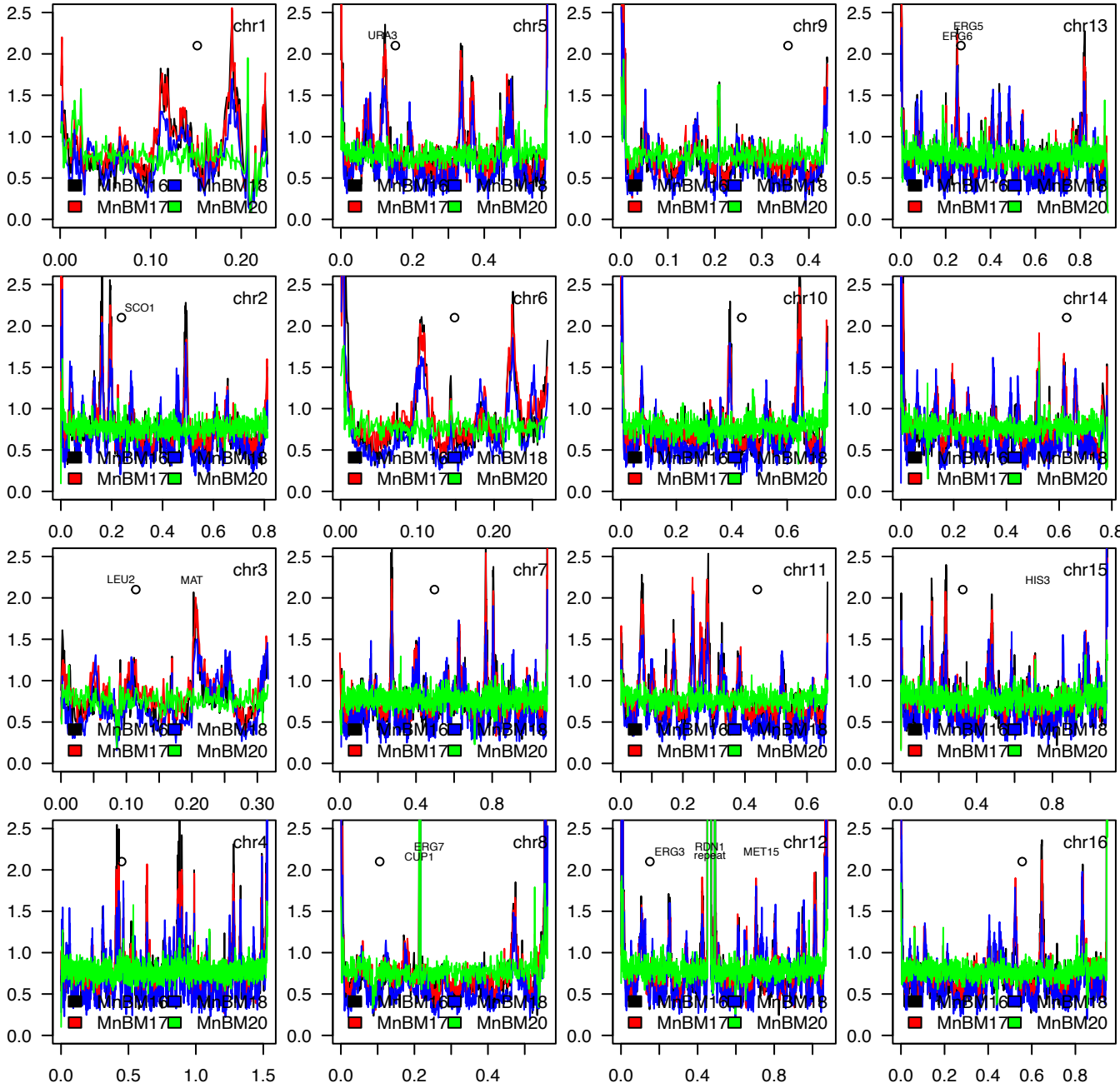
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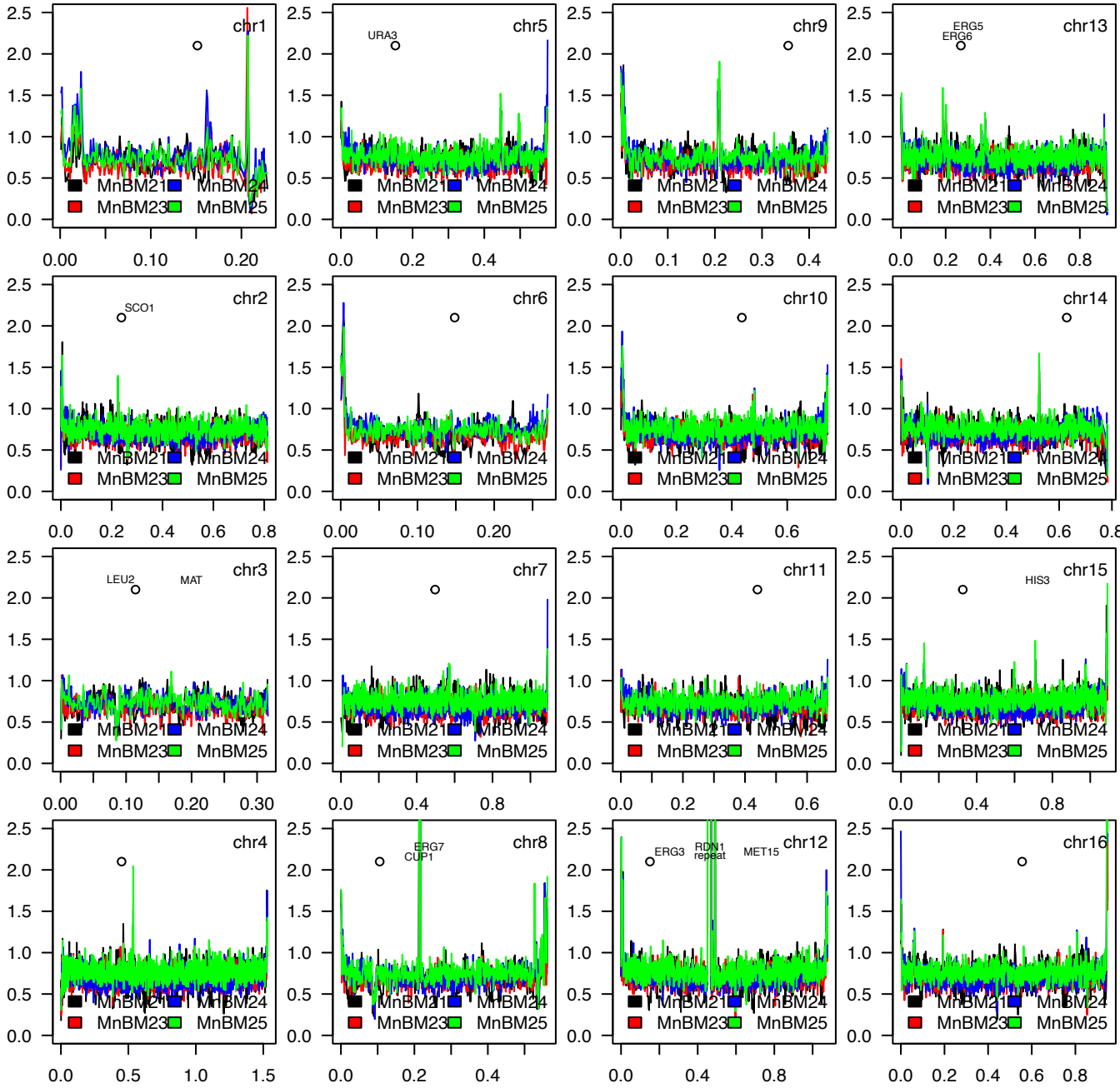
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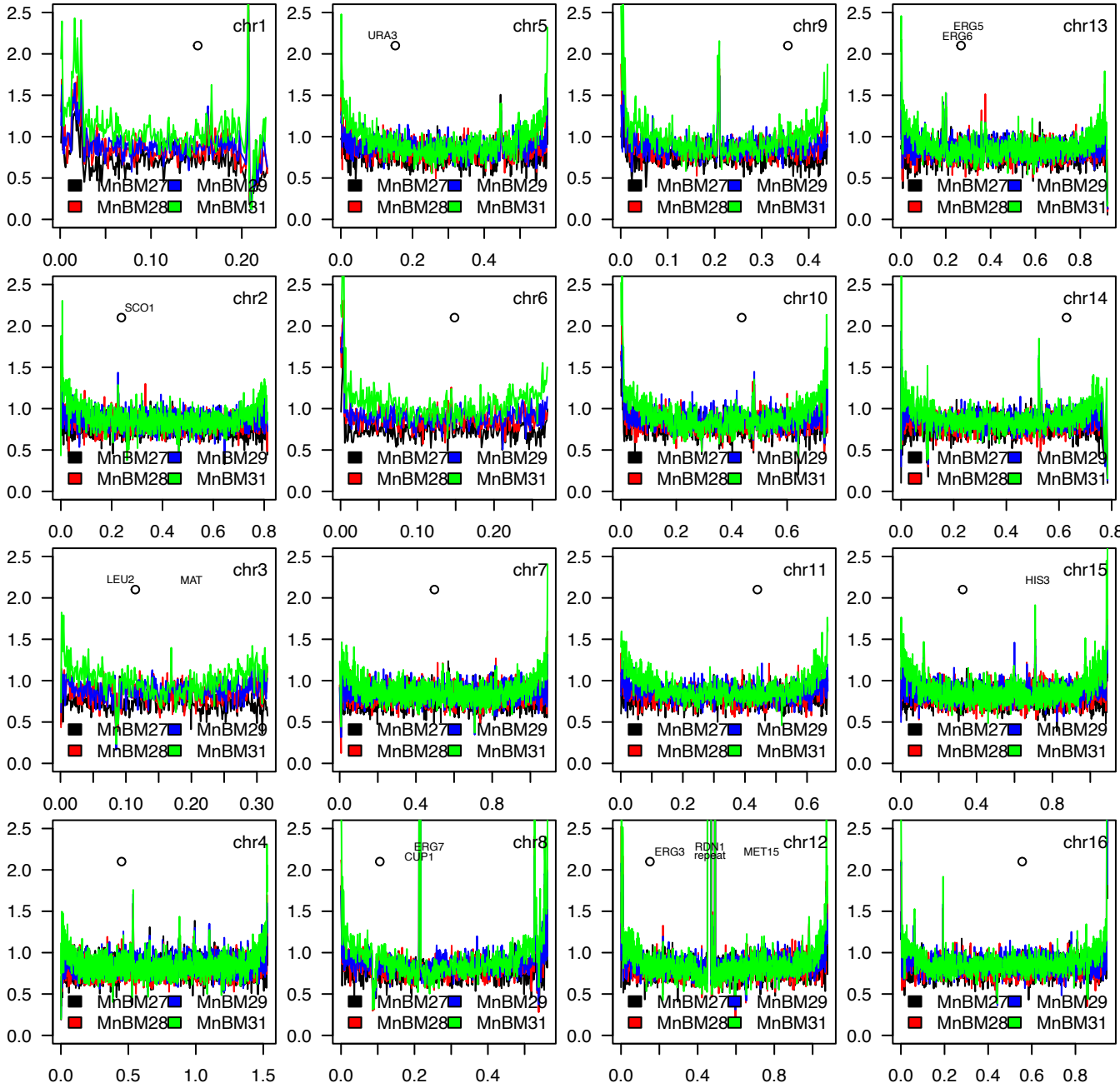
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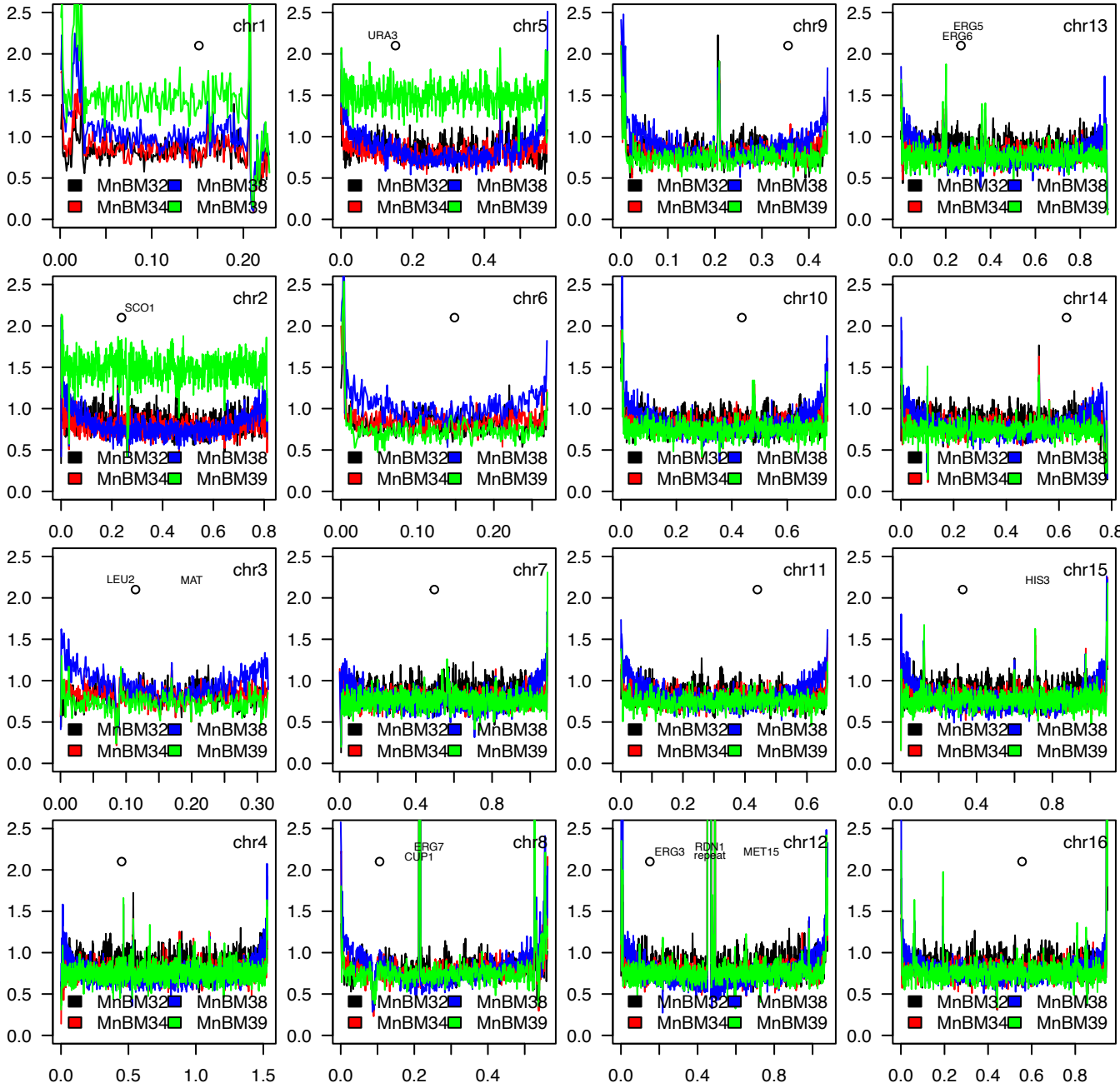
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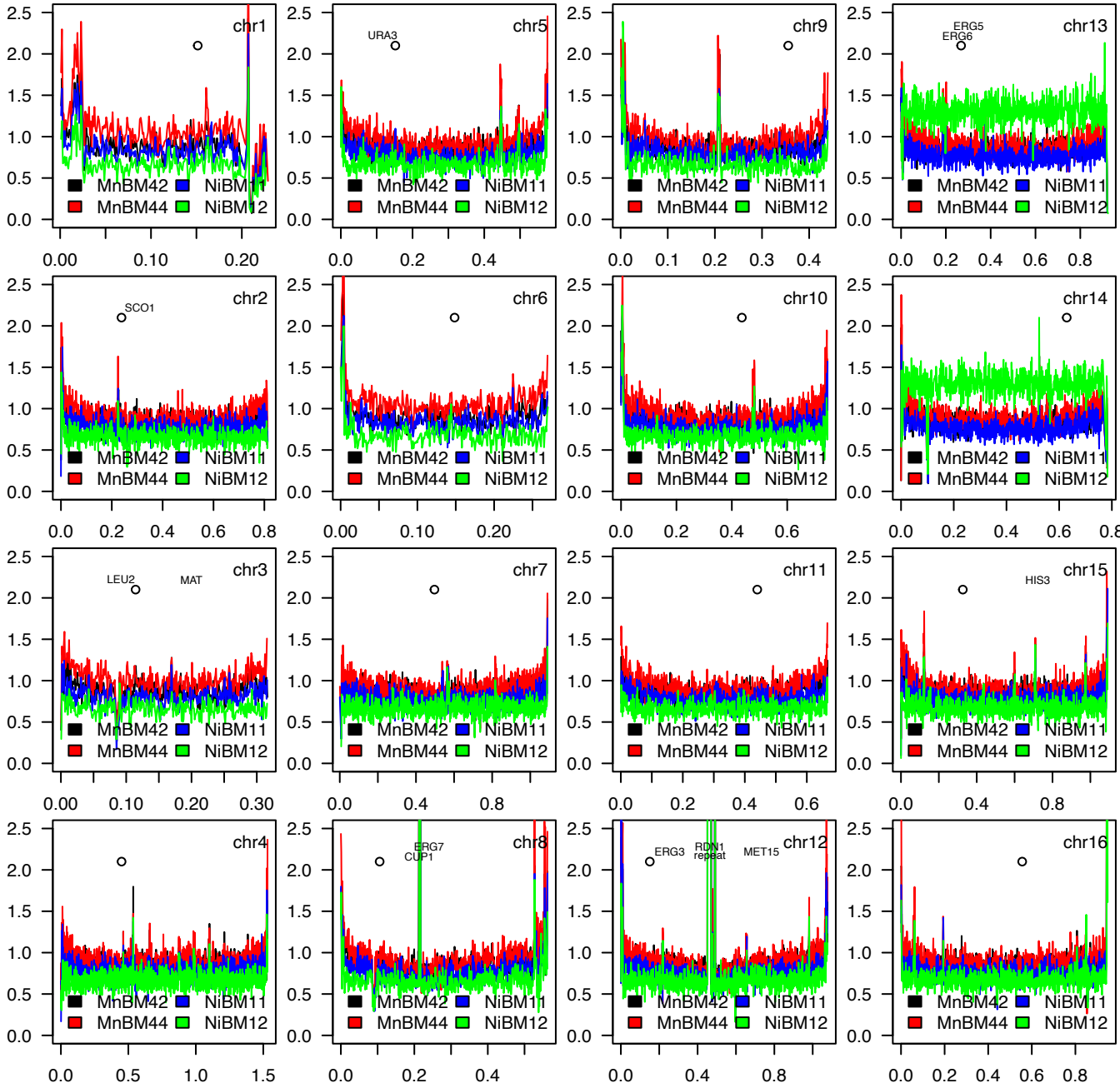
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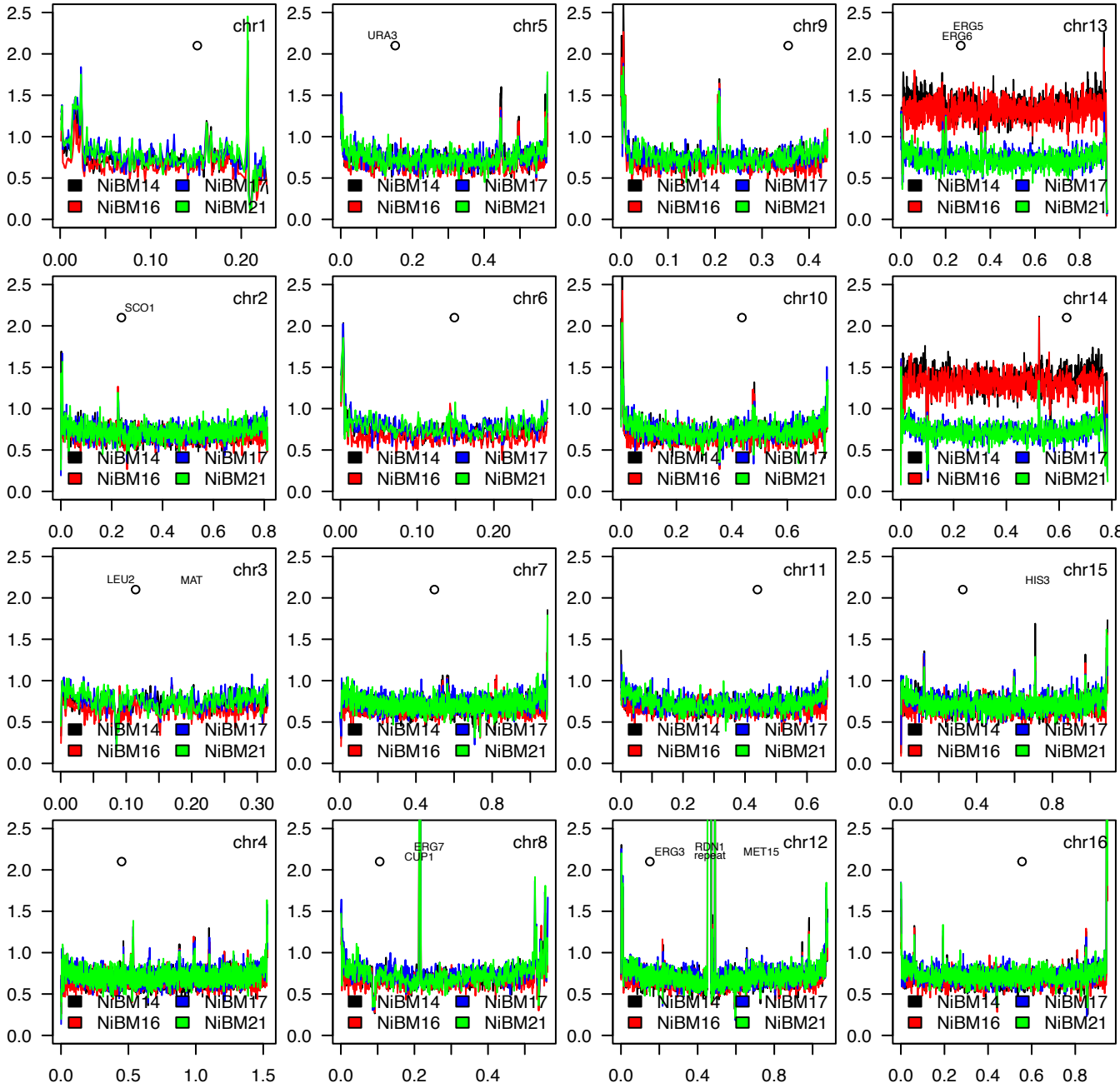
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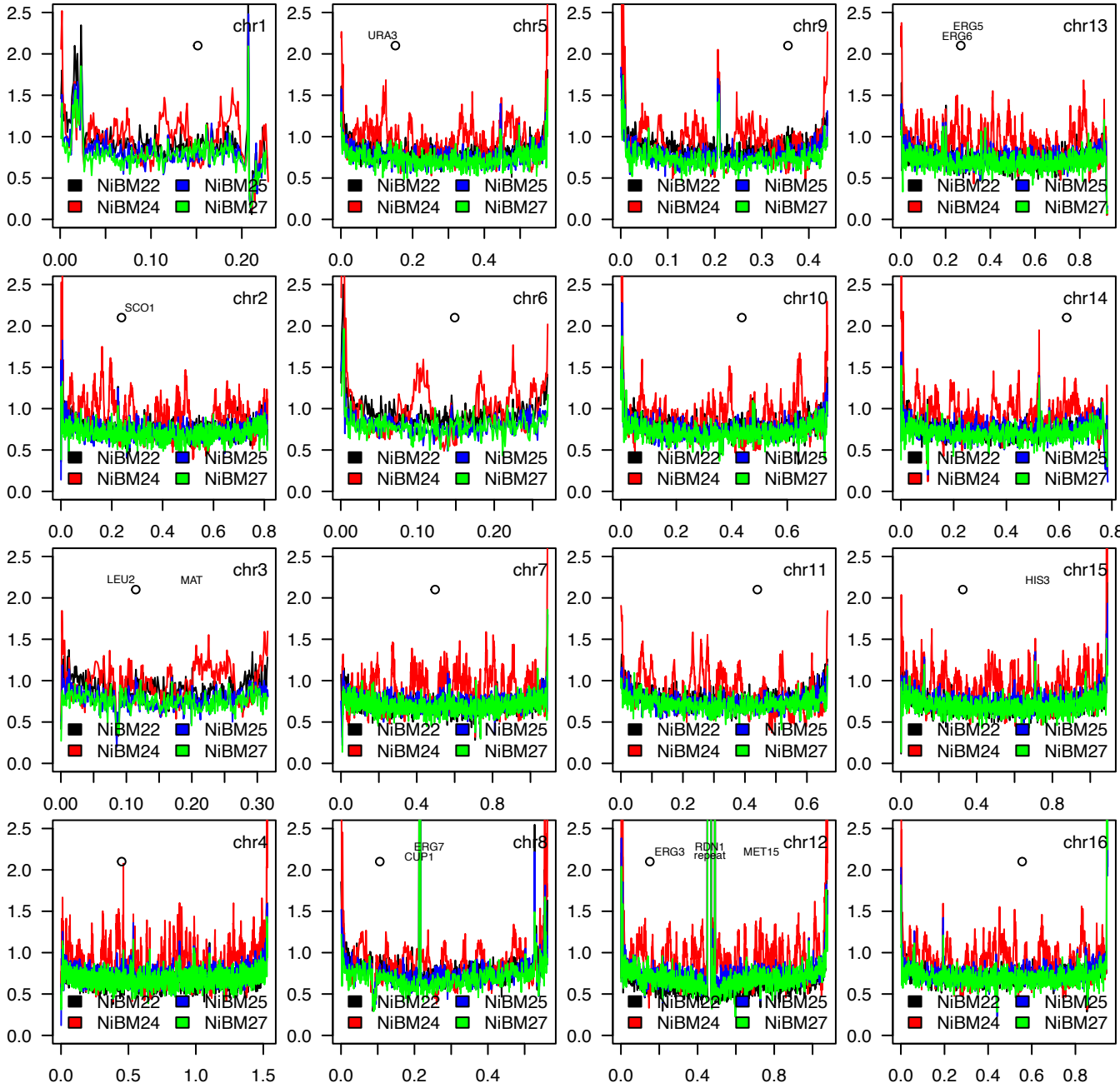
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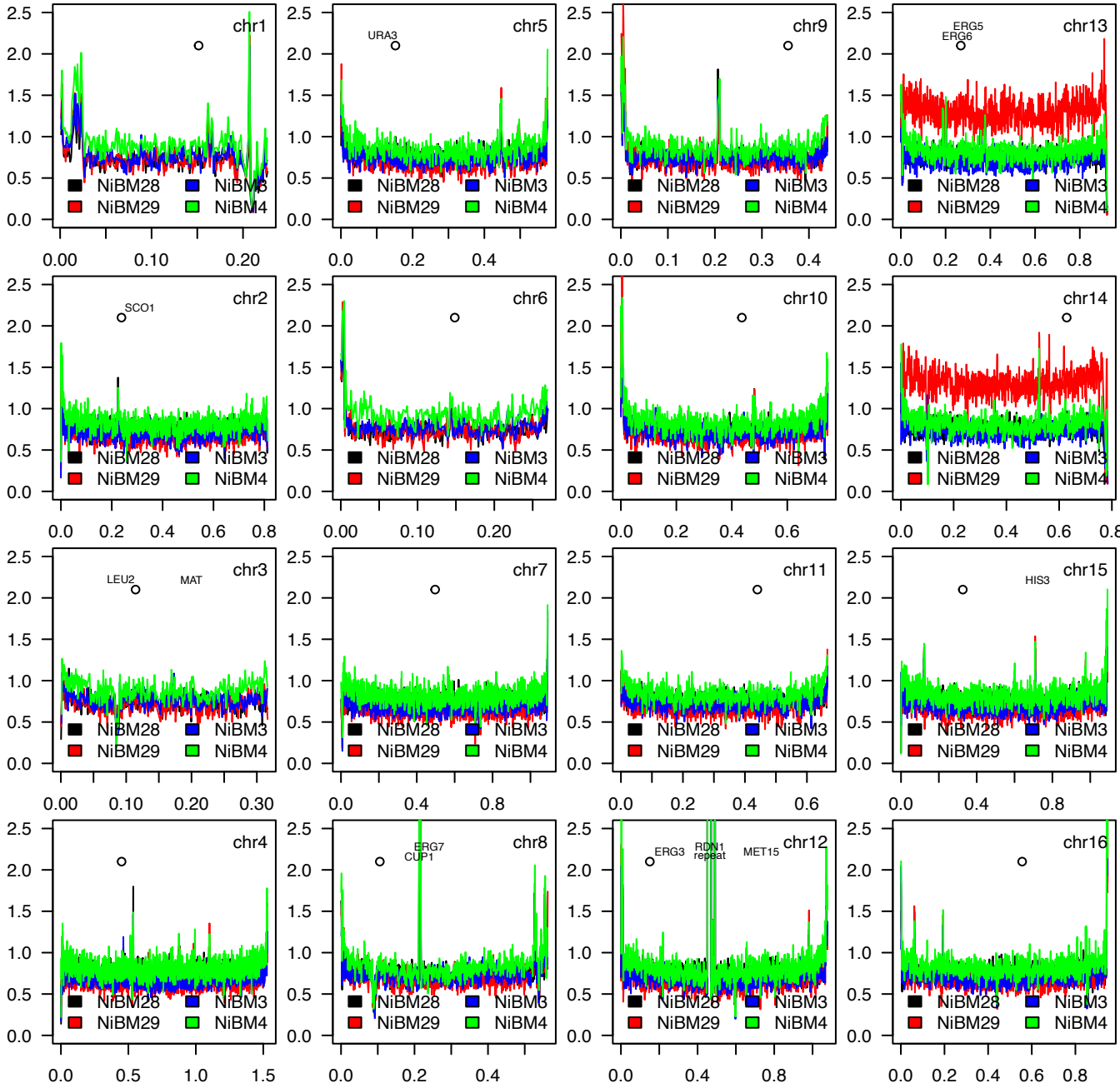
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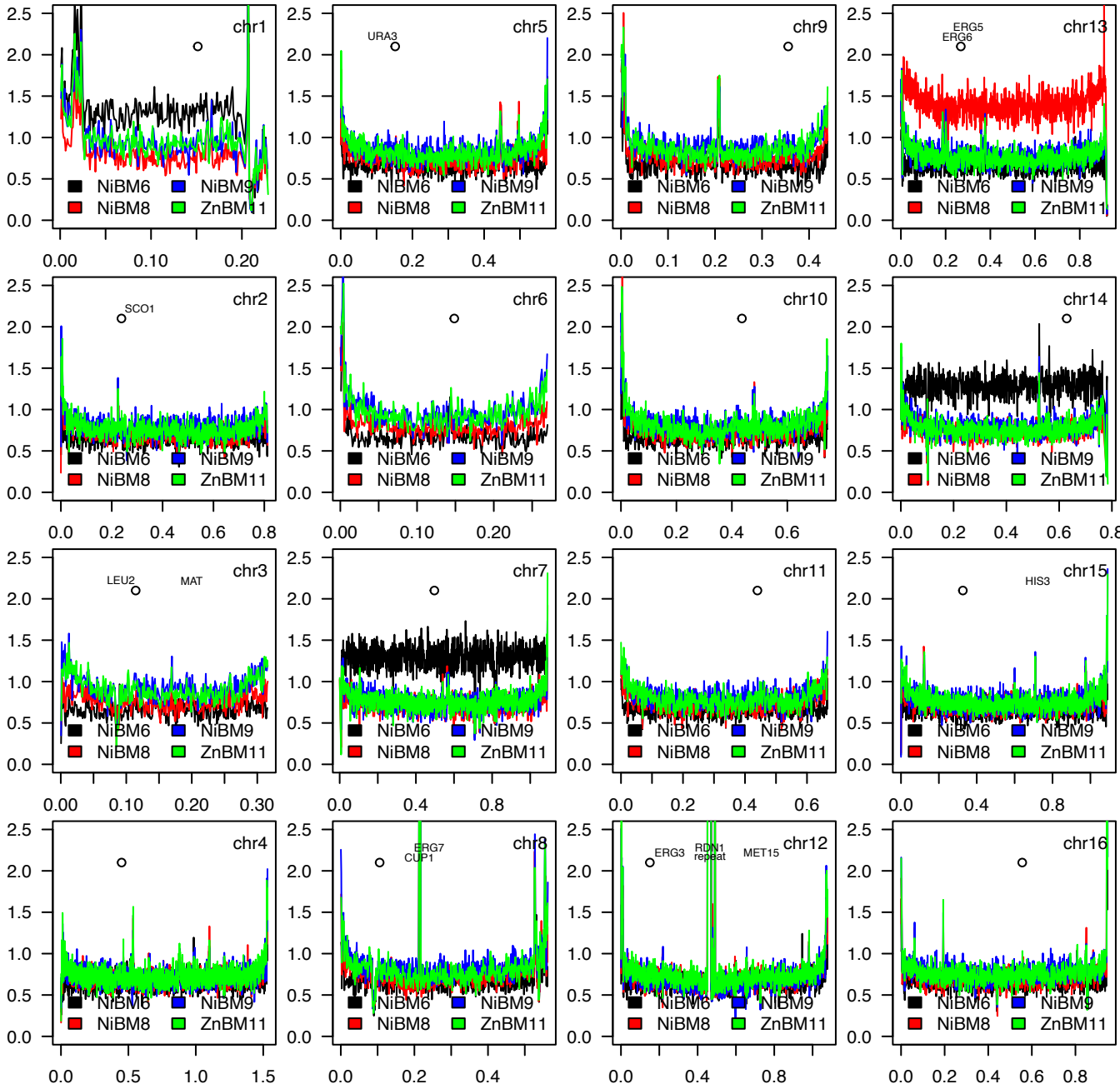
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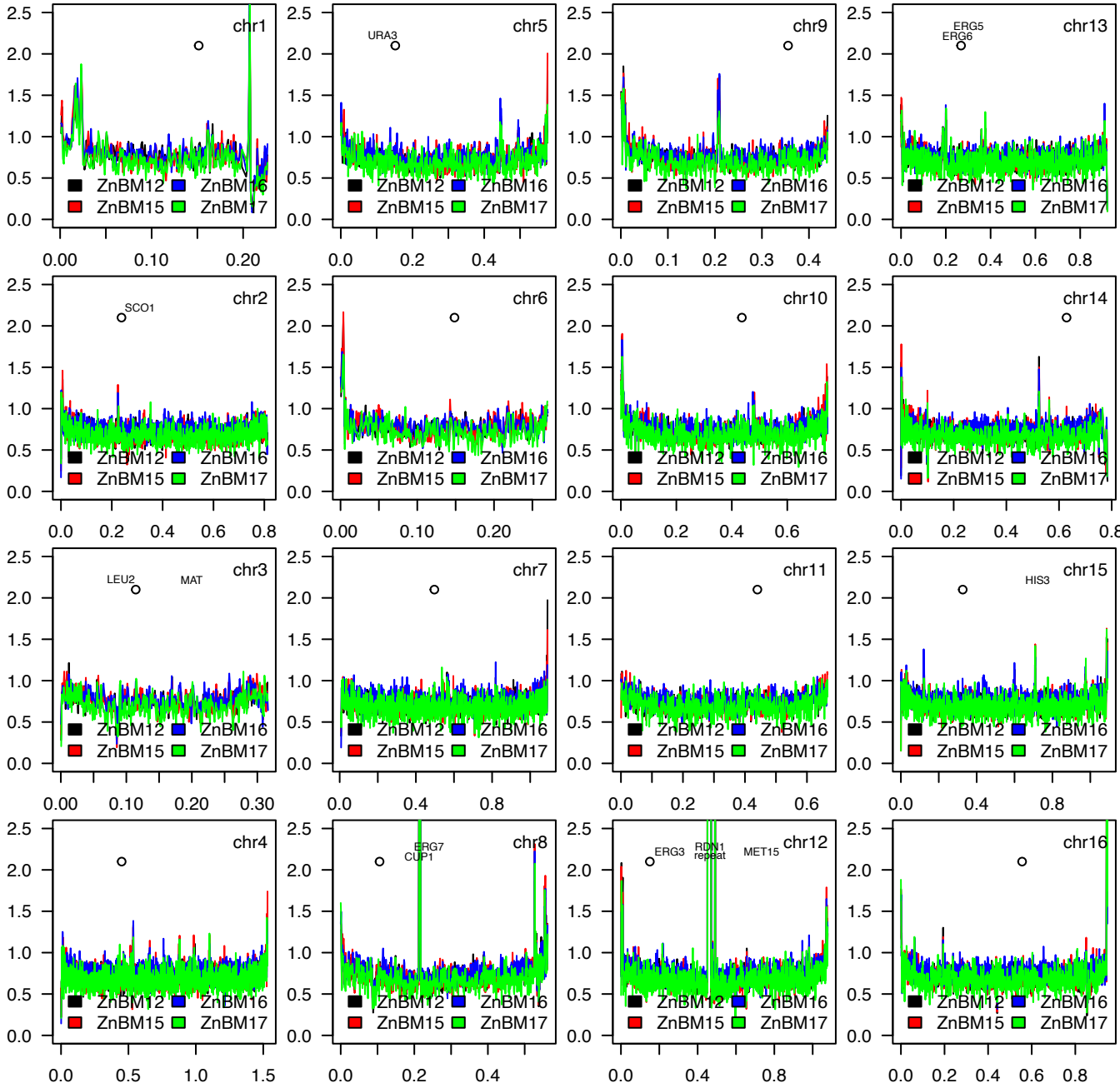
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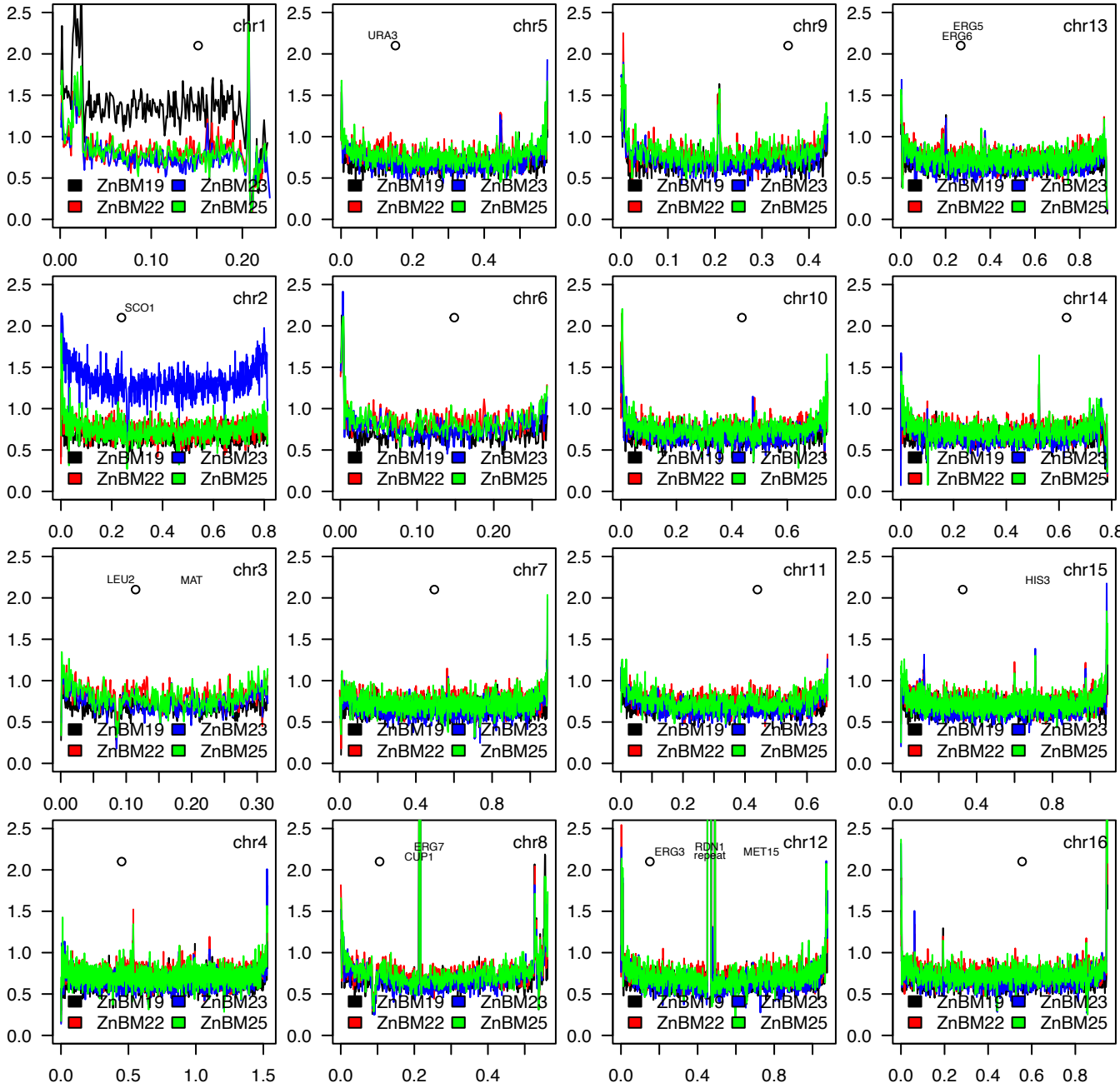
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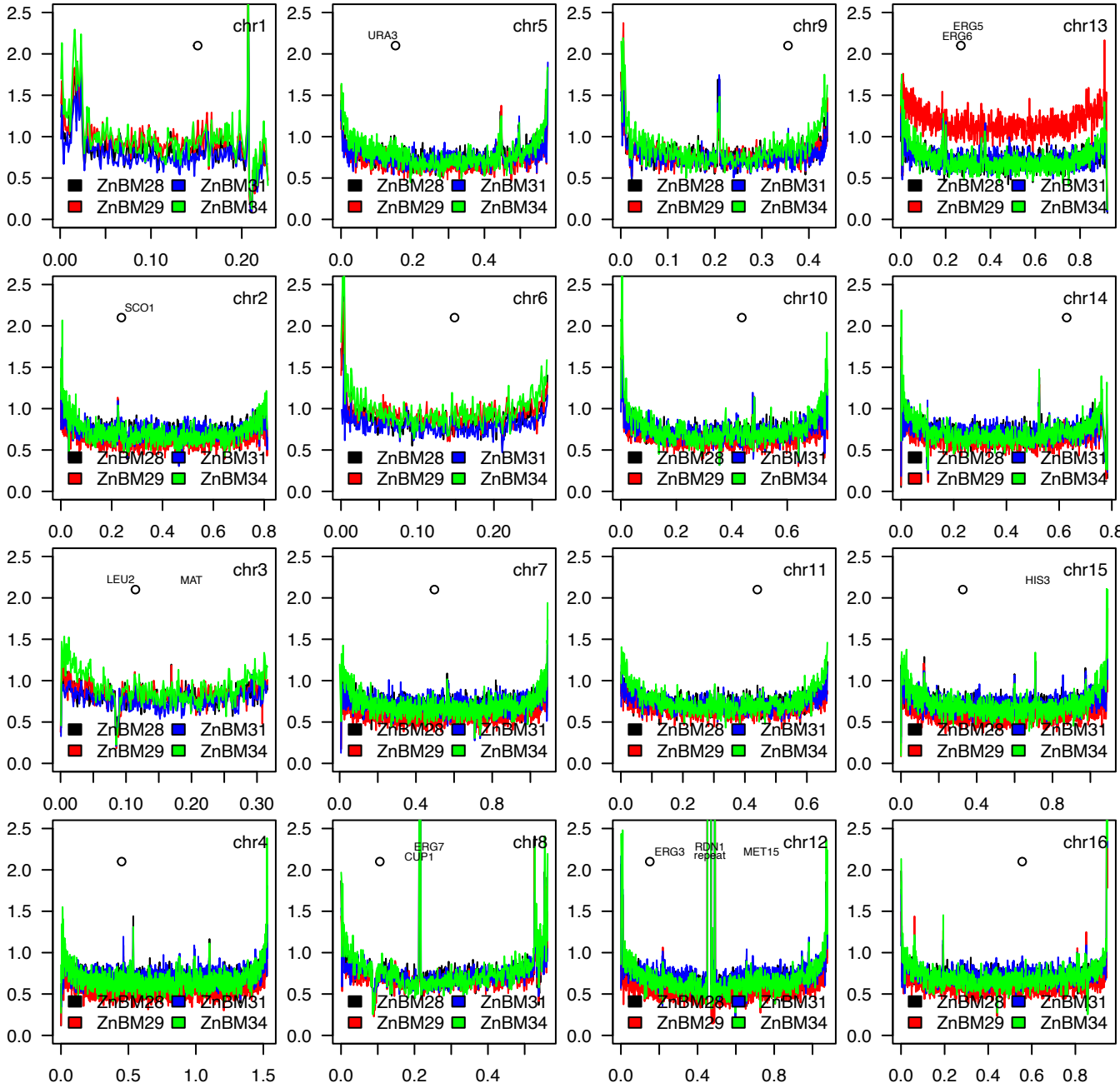
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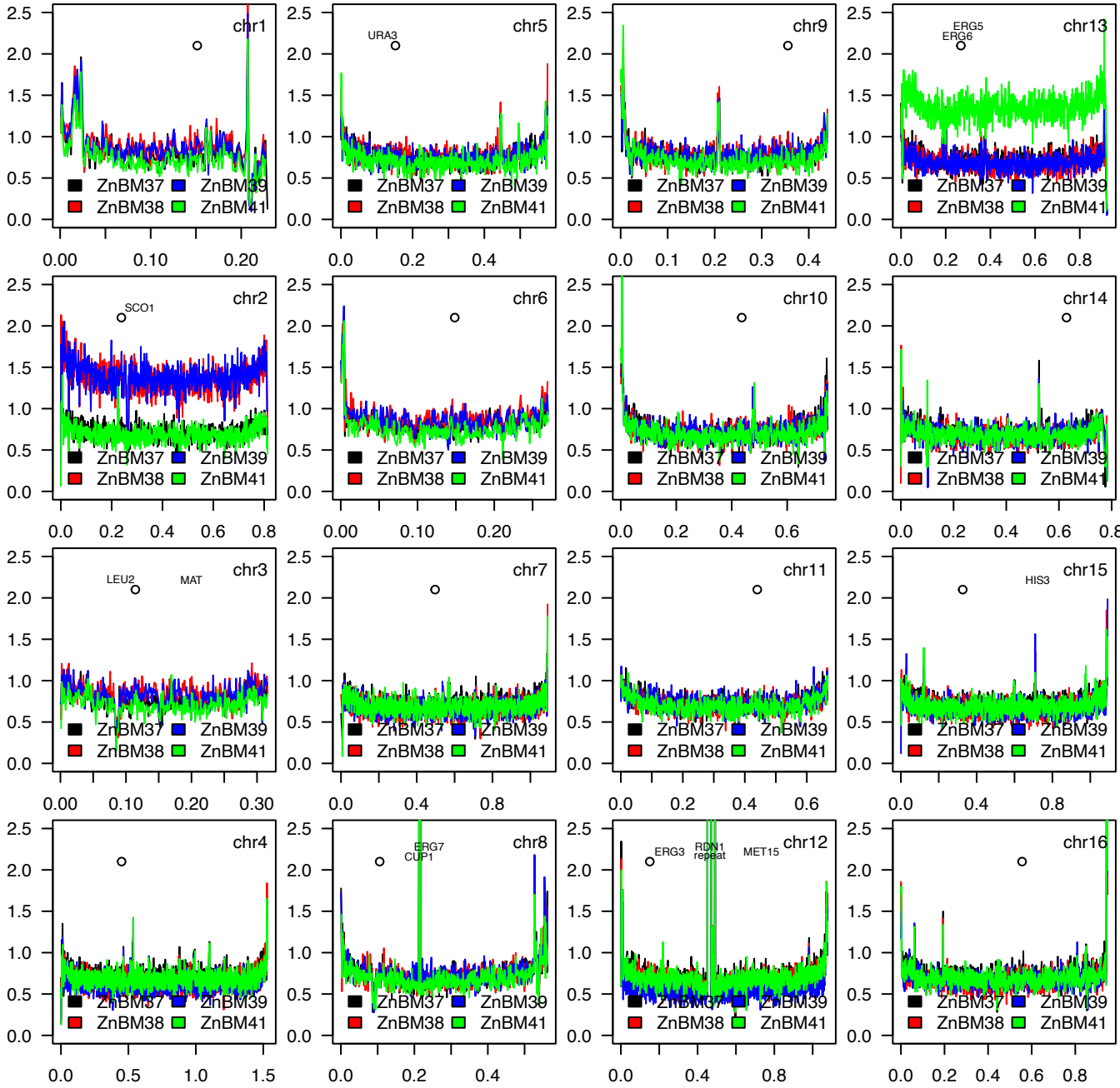
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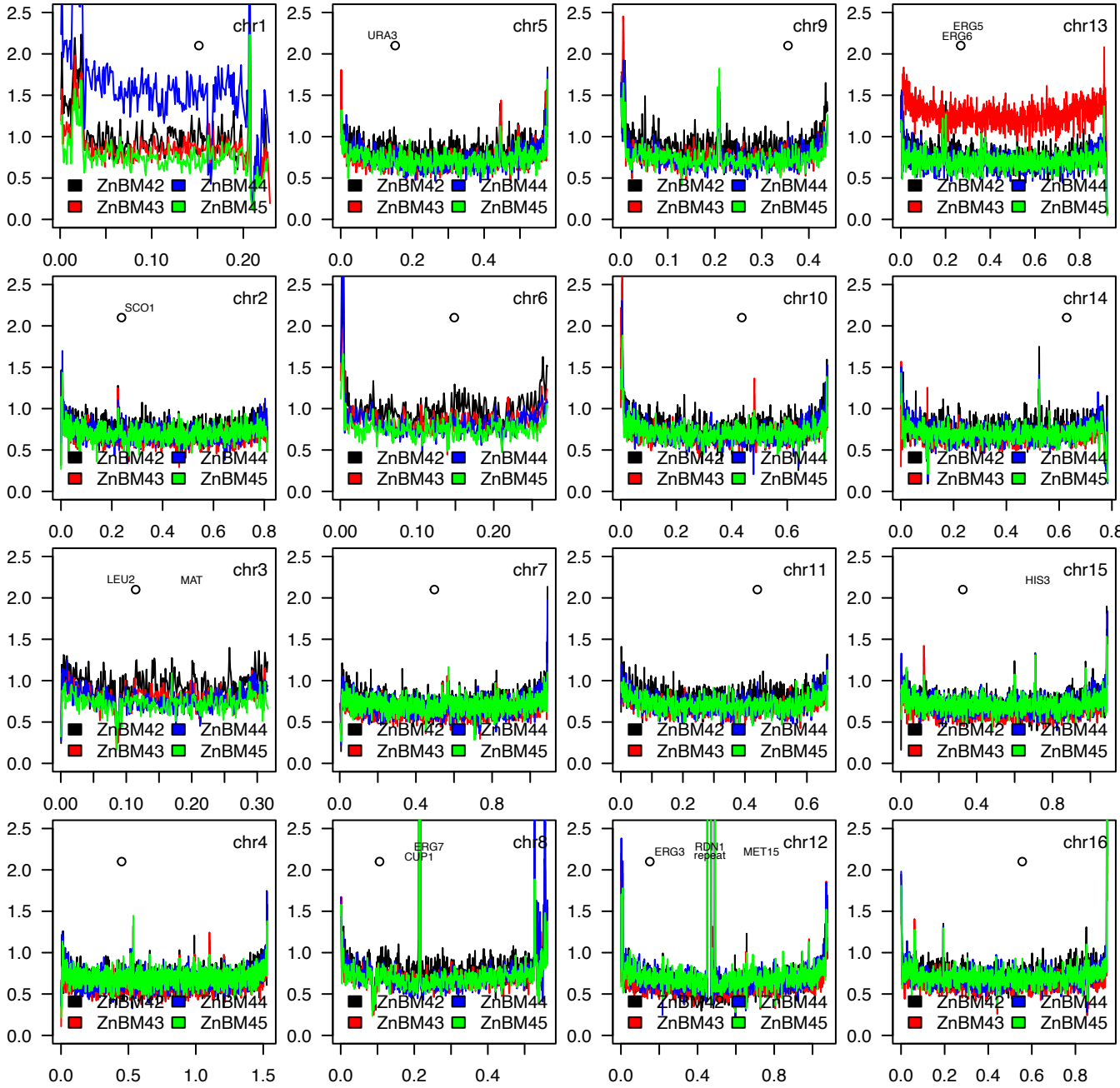
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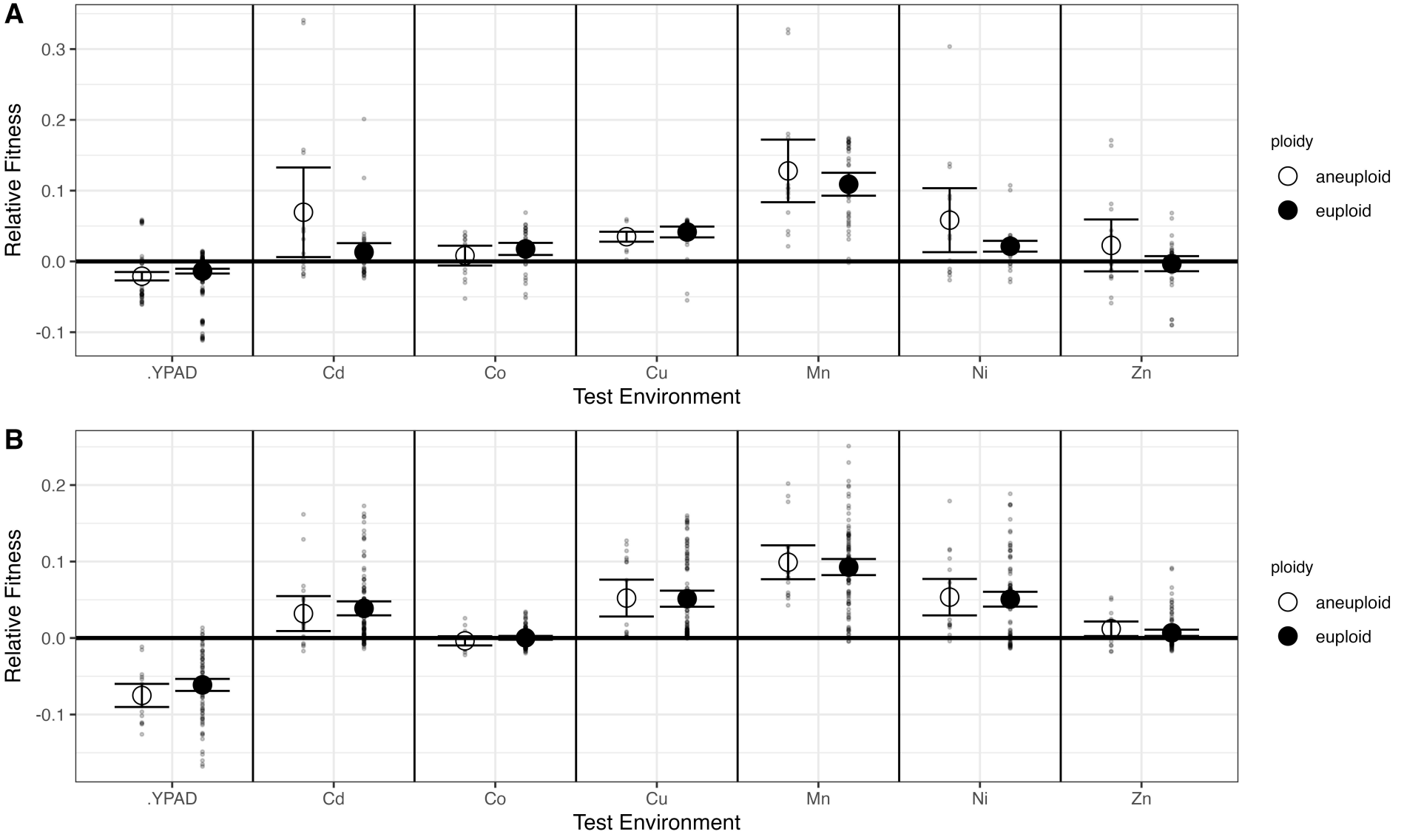
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A

Chromosome	W303	Cd.37	Cd.39	Cd.44	Cd.45	Co.2	Mn.14	Mn.39	Ni.6	Ni.8	Ni.12	Ni.14	Ni.16	Ni.29	Zn.19	Zn.23	Zn.29	Zn.38	Zn.39	Zn.41	Zn.43	Zn.44	Zn.47
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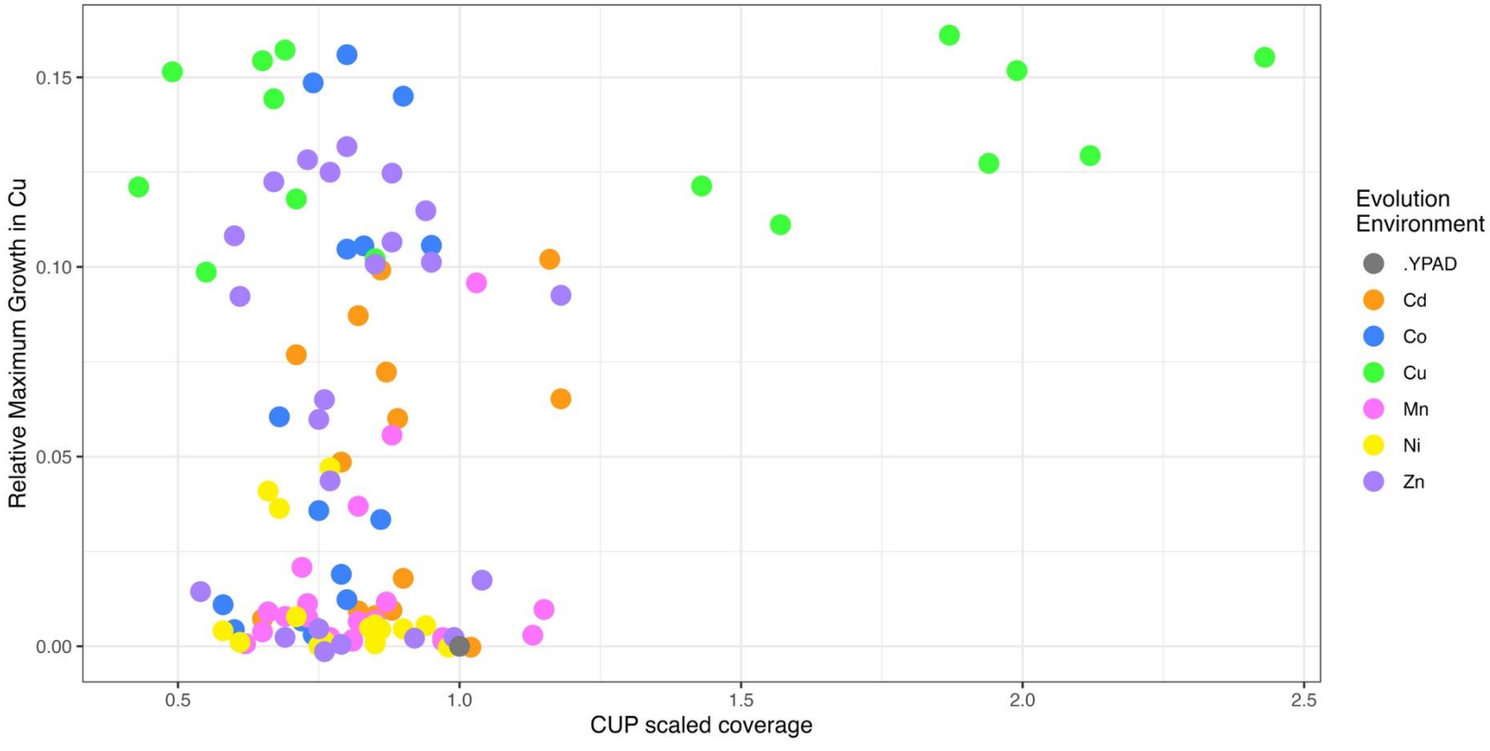
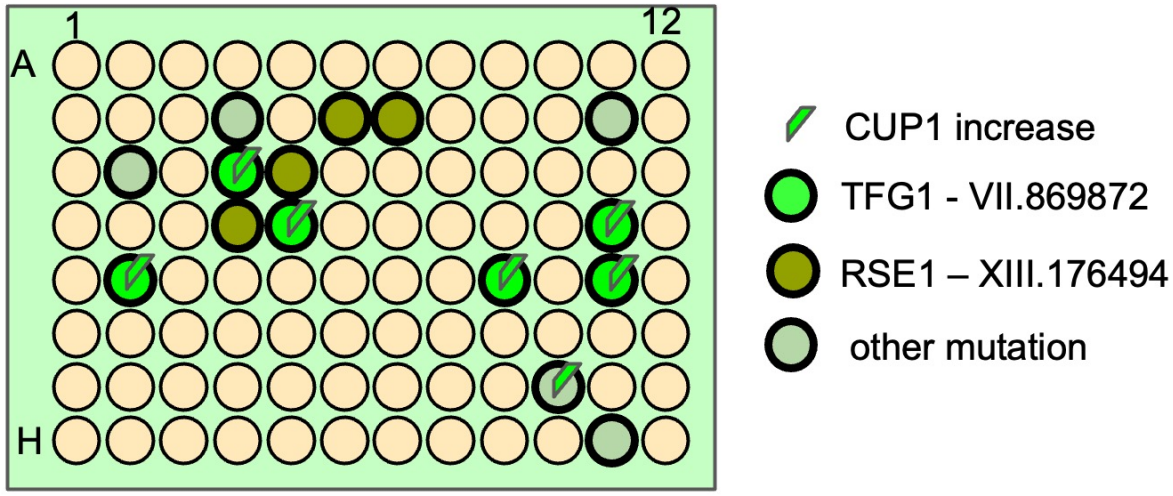
II	1	1.7	1.7	1.6	1.0	1.0	1.0	1.8	0.9	1.0	0.9	0.9	0.9	0.9	1.0	2.0	1.0	1.9	2.0	1.0	1.0	1.0	0.4
III	1	1.7	1.0	1.0	1.3	0.9	1.1	0.8	0.9	1.0	0.9	0.9	0.9	0.9	0.9	1.0	1.3	1.1	1.1	0.9	1.1	1.1	0.4
IV	1	1.6	1.6	1.0	0.9	1.0	0.9	0.9	0.9	1.0	1.0	0.9	0.9	0.9	1.0	1.0	0.9	0.9	0.9	0.9	0.9	1.0	0.4
V	1	0.9	0.9	1.0	1.1	1.0	1.0	1.7	0.9	1.0	0.9	0.9	0.9	1.0	0.9	1.0	1.0	1.0	1.0	0.9	1.0	1.0	0.8
VI	1	1.1	1.0	1.0	1.4	0.9	1.1	0.8	0.9	1.0	0.9	0.9	0.9	1.0	0.9	1.1	1.4	1.2	1.1	1.0	1.2	1.1	0.6
VII	1	0.8	0.9	1.0	1.0	1.0	1.0	0.9	1.8	1.0	0.9	0.9	0.9	0.9	1.0	1.0	0.9	0.9	0.9	1.0	0.9	1.0	0.8
VIII	1	0.8	0.9	1.0	1.1	1.0	1.0	0.9	0.9	1.0	0.9	0.9	0.9	0.9	1.0	1.0	1.0	1.0	1.0	0.9	1.0	1.0	0.8
IX	1	0.8	0.9	1.2	1.2	0.9	1.0	0.9	0.9	1.0	0.9	1.0	0.9	1.0	0.9	1.0	1.2	1.1	1.1	1.0	1.1	1.1	0.8
X	1	0.8	0.9	1.0	1.0	1.0	1.0	0.9	0.9	1.0	0.9	1.0	0.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.6
XI	1	0.8	0.9	1.0	1.1	1.0	2.0	0.9	0.9	1.0	0.9	0.9	0.9	0.9	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.8
XII	1	0.8	0.8	0.7	0.9	0.8	0.8	0.9	0.7	0.7	0.7	0.7	0.7	0.7	1.1	0.7	0.8	0.8	0.8	0.9	0.7	0.8	1.0
XIII	1	0.8	0.9	1.0	1.0	1.9	1.0	0.9	0.9	1.9	1.8	1.8	1.8	1.8	1.0	1.0	1.8	1.0	1.0	1.9	1.9	1.0	1.6
XIV	1	0.8	0.9	1.1	1.0	1.0	1.0	0.9	1.8	1.0	1.8	1.8	1.8	1.8	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.8
XV	1	0.8	0.8	1.0	1.0	1.0	1.0	0.9	0.9	1.0	1.0	0.9	0.9	0.9	1.0	1.0	0.9	0.9	0.9	1.0	0.9	1.0	0.8
XVI	1	0.8	0.9	1.1	1.0	1.0	1.0	0.9	0.9	1.0	1.0	0.9	0.9	0.9	1.0	1.0	0.9	0.9	1.0	1.0	1.0	1.0	0.8

B



Distribution on the deep-well plate of copper mutations

Plate A



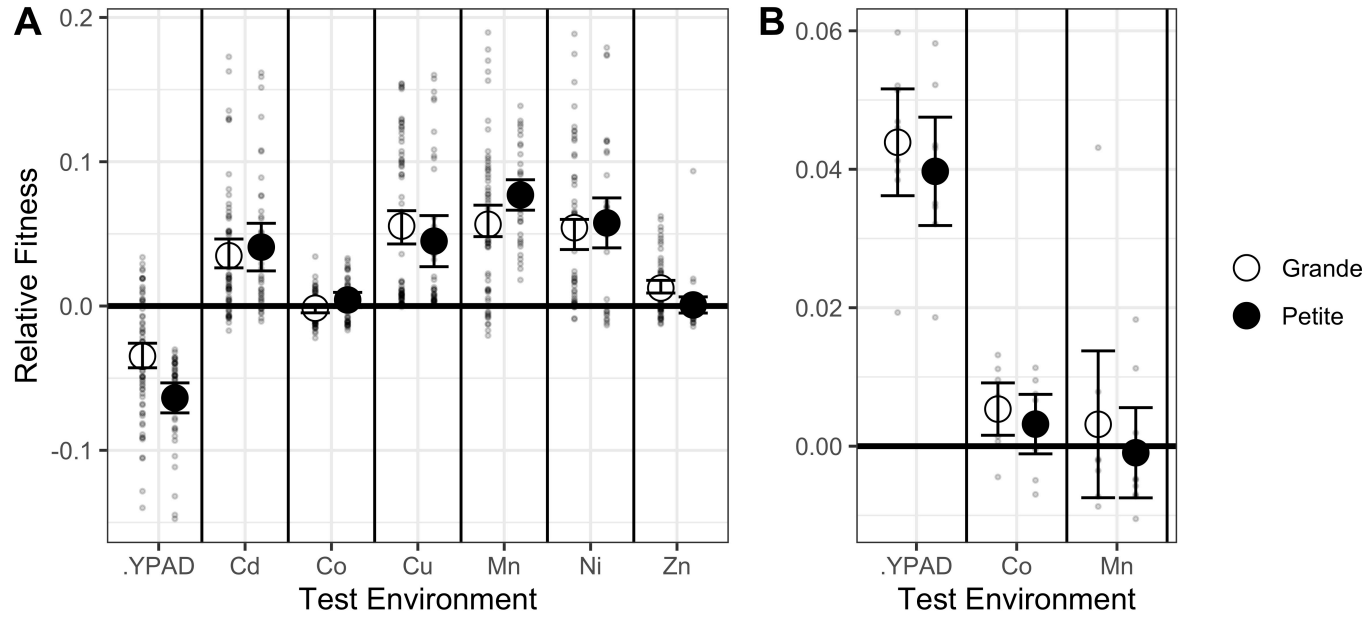
CUP1 increase

TFG1 - VII.869872

RSE1 – XIII.176494

other mutation

CuBM10	4d	RSE1, NGG1
CuBM11	5d	TFG1, NGG1
CuBM12	6b	RSE1, NGG1
CuBM13	7b	RSE1, NGG1
CuBM14	9e	TFG1, BUL1, FIG4, PYK2, NGG1
CuBM15	10g	COQ1
CuBM17	11d	TFG1, NGG1
CuBM18	11e	ABP1, ROG1, TFG1, PRP2, NGG1
CuBM3	11h	PMA1
CuBM4	5c	RSC1, NGG1, RSE1
CuBM6	2c	DNF1, ATG2
CuBM7	2e	BLM10, TFG1, NGG1
CuBM9	4c	MMS4, TFG1, KSP1, NGG1


A
B
Relative Fitness
0.2
0.1
0.0
-0.1
.YPAD
Cd
Co
Cu
Mn
Ni
Zn
Test Environment
0.06
0.04
0.02
0.00
.YPAD
Co
Mn
Test Environment
Grande
Petite


C
15S rRNA
COX1
ATP8
ATP6
COB
OLI1
VAR1
21S rRNA
COX2
COX3
RPM1
Mitochondrial coverage
7500
5000
2500
0
0
25000
50000
75000
cu_3
mn_13
mn_14
mn_17
mn_20
mn_23
mn_25
mn_27
mn_28
mn_32
mn_34
mn_38
mn_39
mn_42
mn_44
ni_24
ni_4
W303