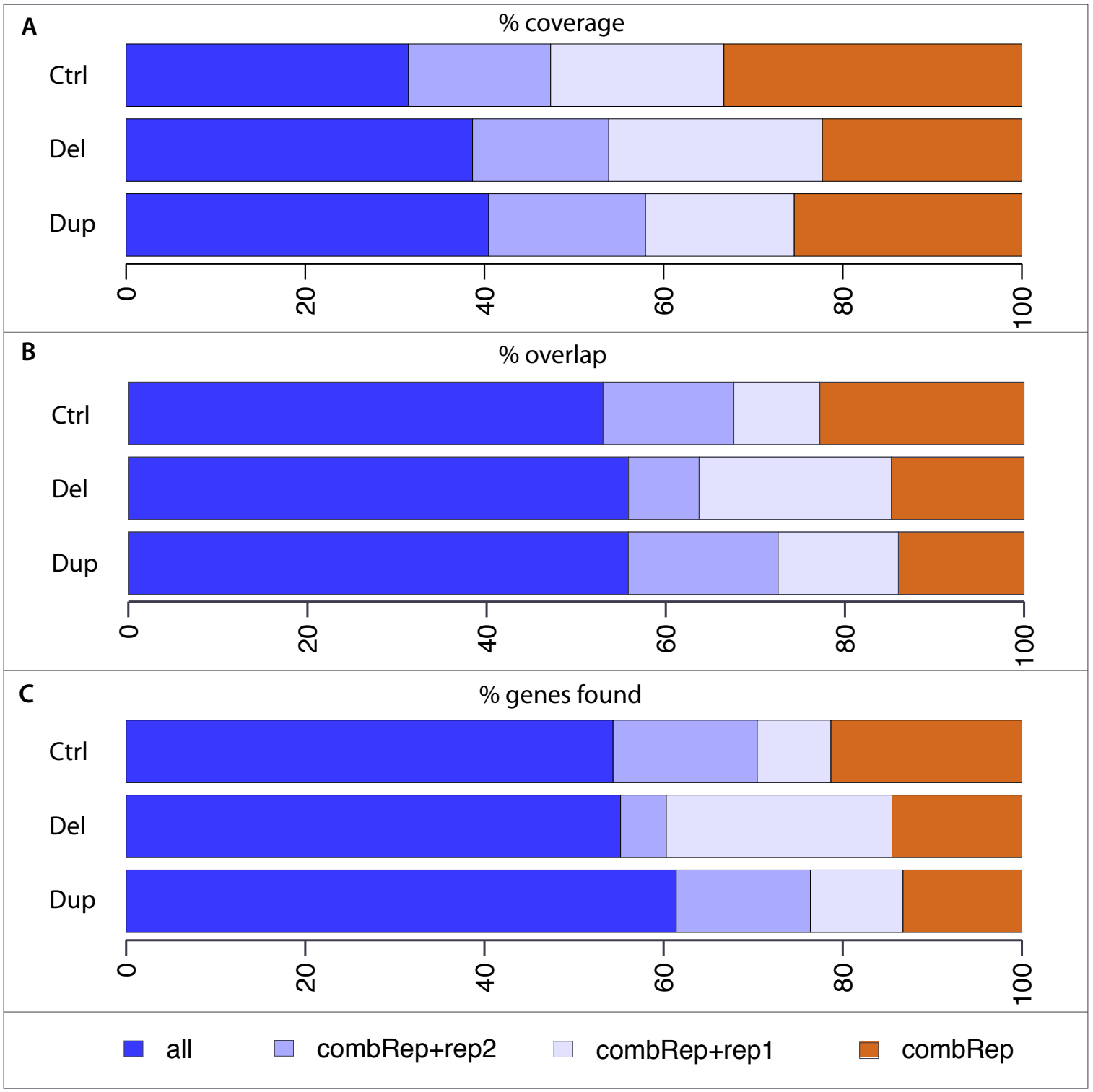
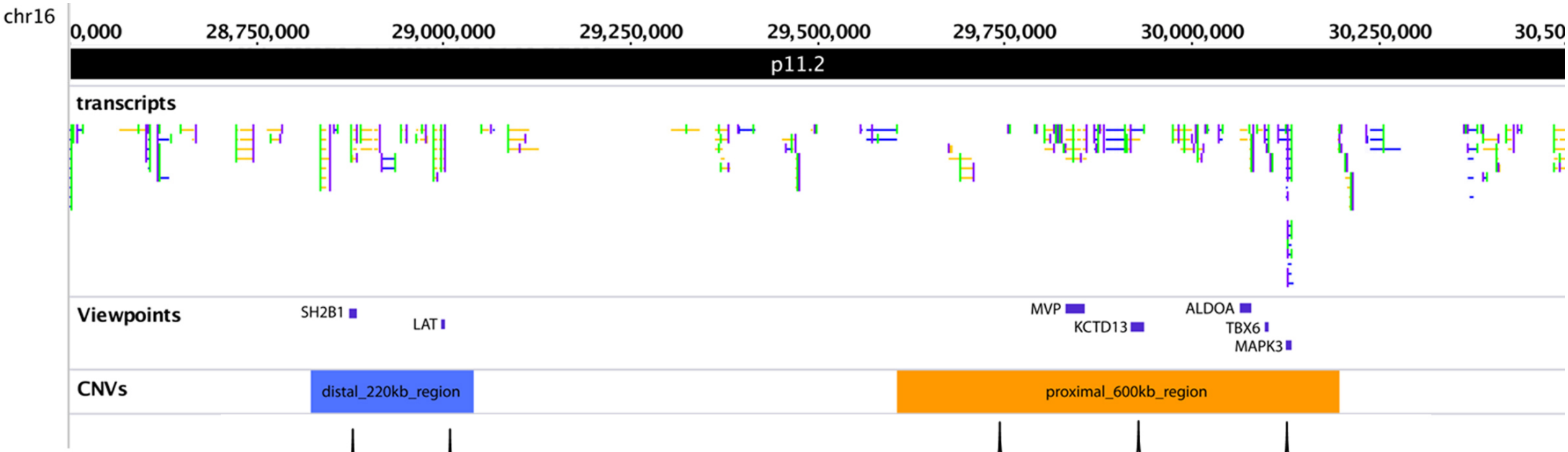

A
% coverage
Ctrl
Del
Dup
0
20
40
60
80
100
B
% overlap
Ctrl
Del
Dup
0
20
40
60
80
100
C
% genes found
Ctrl
Del
Dup
0
20
40
60
80
100
all
combRep+rep2
combRep+rep1
combRep

Figure S1: Comparison of 4C-seq replicates
BRICKS obtained after averaging replicates (combRep) are compared to BRICKS found in each replicate separately (rep1 and rep2) according to **(A)** percentage of the total length of combRep BRICKS also included in the intersection of rep1 and rep2 BRICKS, in only one of the replicates or in none; **(B)** percentage of the combRep BRICKS that overlap with the intersection of rep1 and rep2 BRICKS, with only one of the replicates or with neither; and **(C)** percentage of genes found within combRep BRICKS that are also found in the intersection of rep1 and rep2 BRICKS, in only one of the replicates or in none.



SH2B1
Common variants associated with **BMI, serum leptin and body fat** in GWAS

LAT
Adaptor molecule participating in **AKT activation**

MVP
Epistatic effect on **zebrafish head size with KCTD13**

ALDOA
Dosage sensitive gene affecting **brain morphology** in zebrafish

MAPK3
Epistatic effect on **zebrafish head size with KCTD13**

KCTD13
Dosage sensitive driver of **micro/macrocephaly phenotypes in zebrafish**

TBX6
Candidate gene for the **vertebral malformations**

Figure S2: Selection of 4C viewpoints
Function and mapping position of the genes selected as viewpoints for 4C-seq analysis.

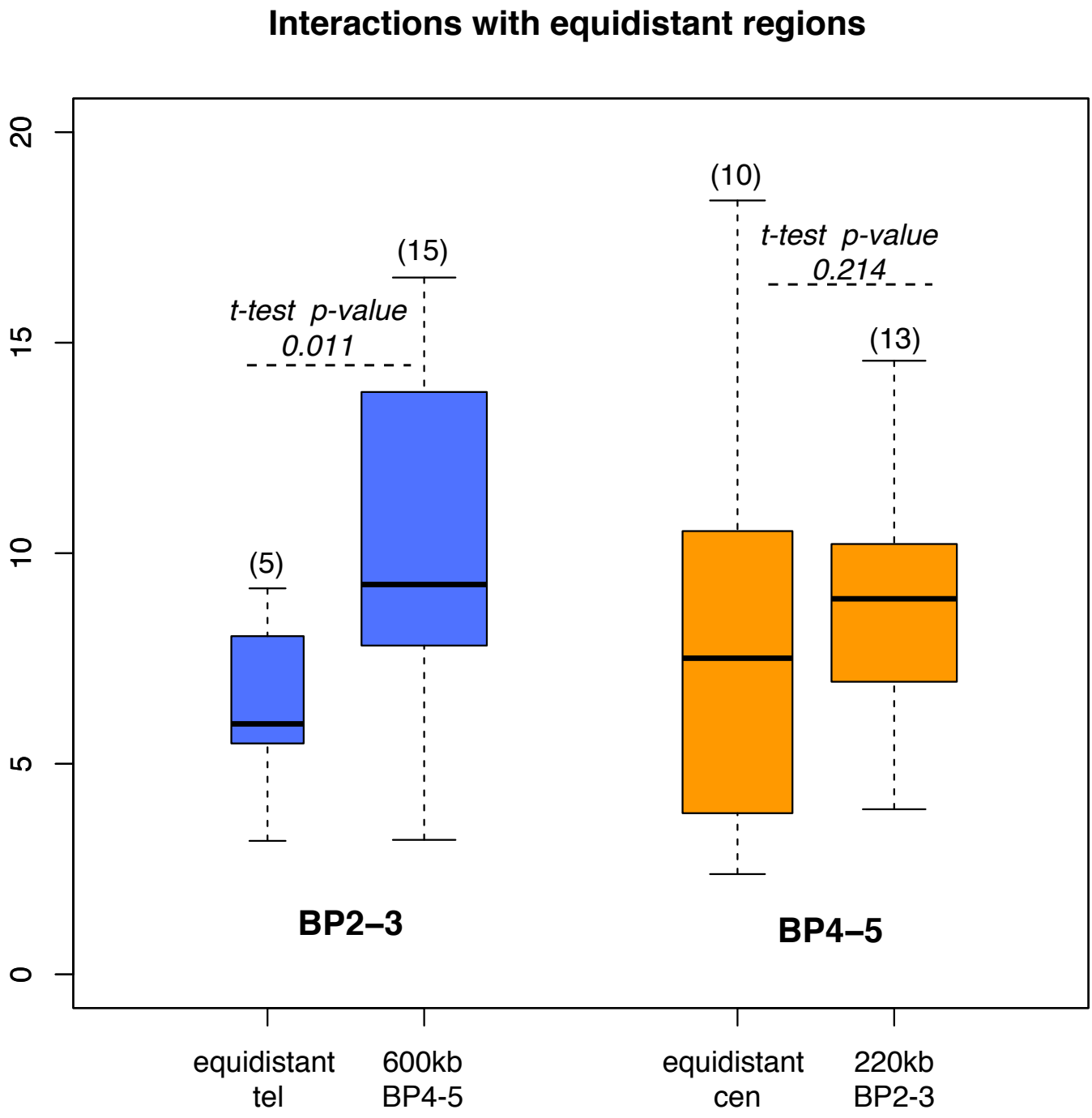
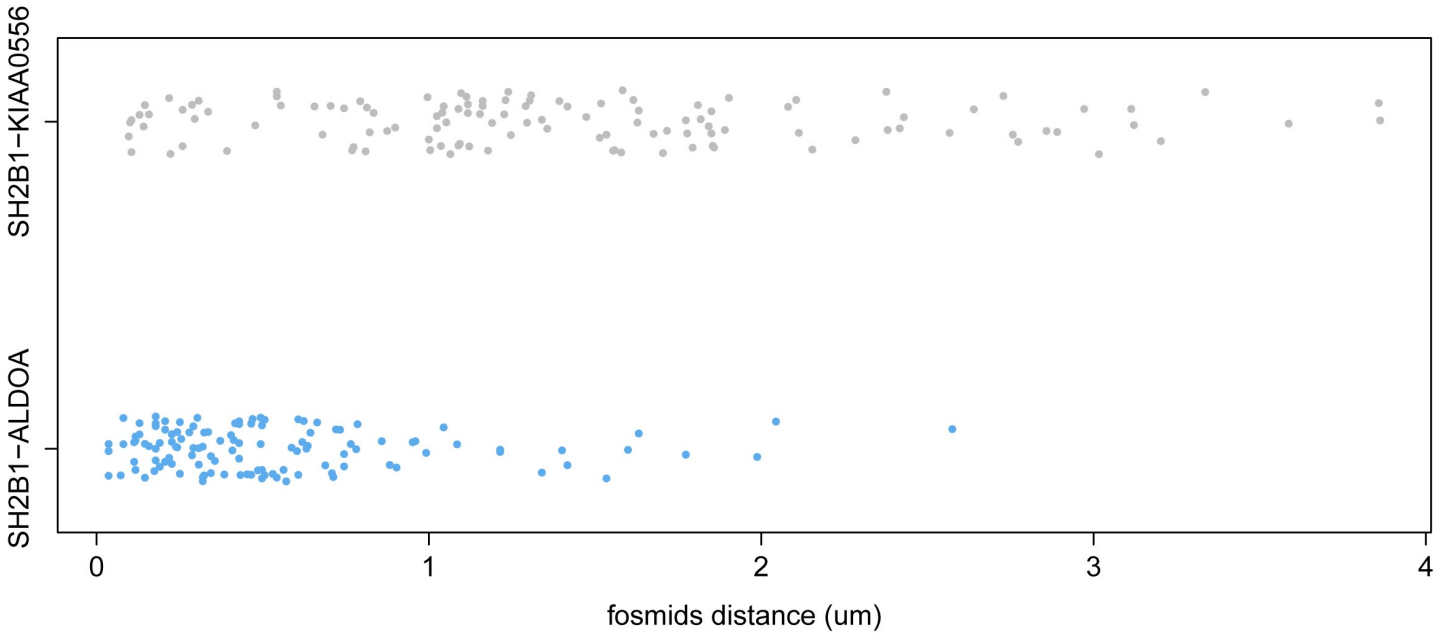
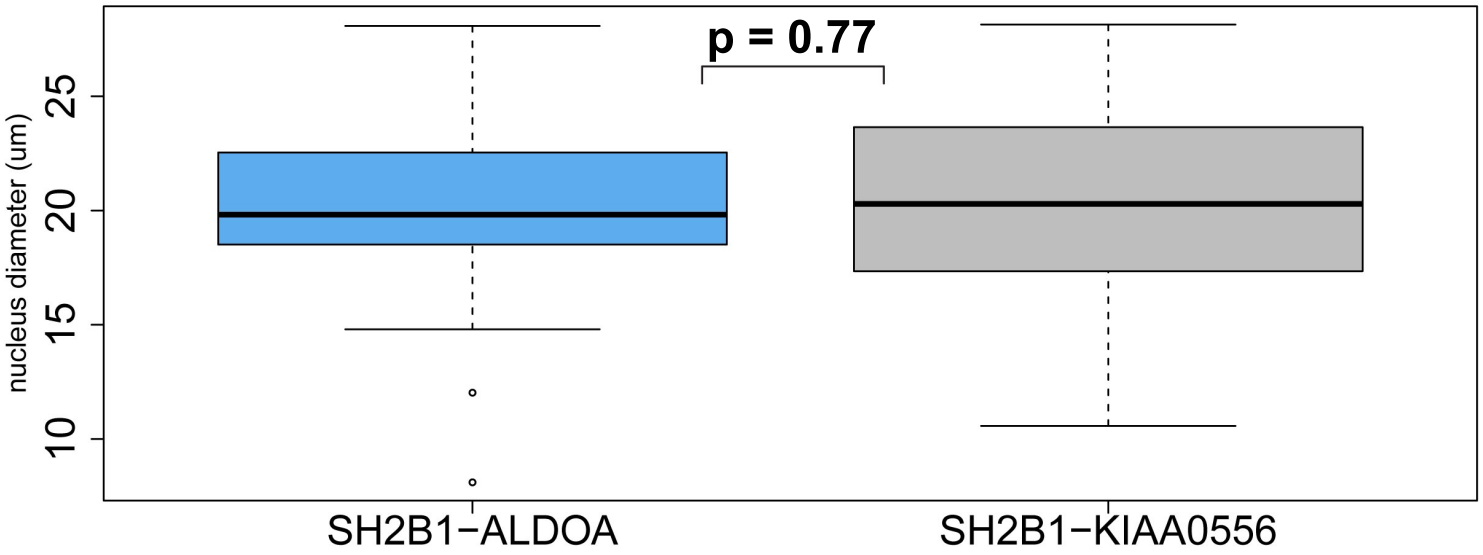

Interactions with equidistant regions
0
5
10
15
20
(5)
(15)
t-test p-value
0.011
BP2–3
(10)
(13)
t-test p-value
0.214
BP4–5
equidistant
tel
600kb
BP4-5
equidistant
cen
220kb
BP2-3

Figure S3: The 220kb BP2-3 viewpoints display preferential interactions with the BP4-BP5 region compared with the telomeric equidistant region
Significances of BRICKS identified with viewpoints mapping within the 220kb BP2-BP3 region and mapping within the 600kb BP4-BP5 interval (right blue boxplot) compared with those in the equidistant, same lengths and telomeric region (left blue boxplot). Significances of BRICKS identified with viewpoints mapping within the 600kb BP4-BP5 region and mapping within the 220kb BP2-BP3 interval (right orange boxplot) compared with those in the equidistant, same lengths and centromeric region (left orange boxplot). The number of BRICKs is indicated in brackets and p-values of a one-sided t-test (alternative "less") are reported above each comparison.


SH2B1−KIAA0556
SH2B1−ALDOA
0
1
2
3
4
fosmids distance (um)


p = 0.77
nucleus diameter (um)
10
15
20
25
SH2B1−ALDOA
SH2B1−KIAA0556

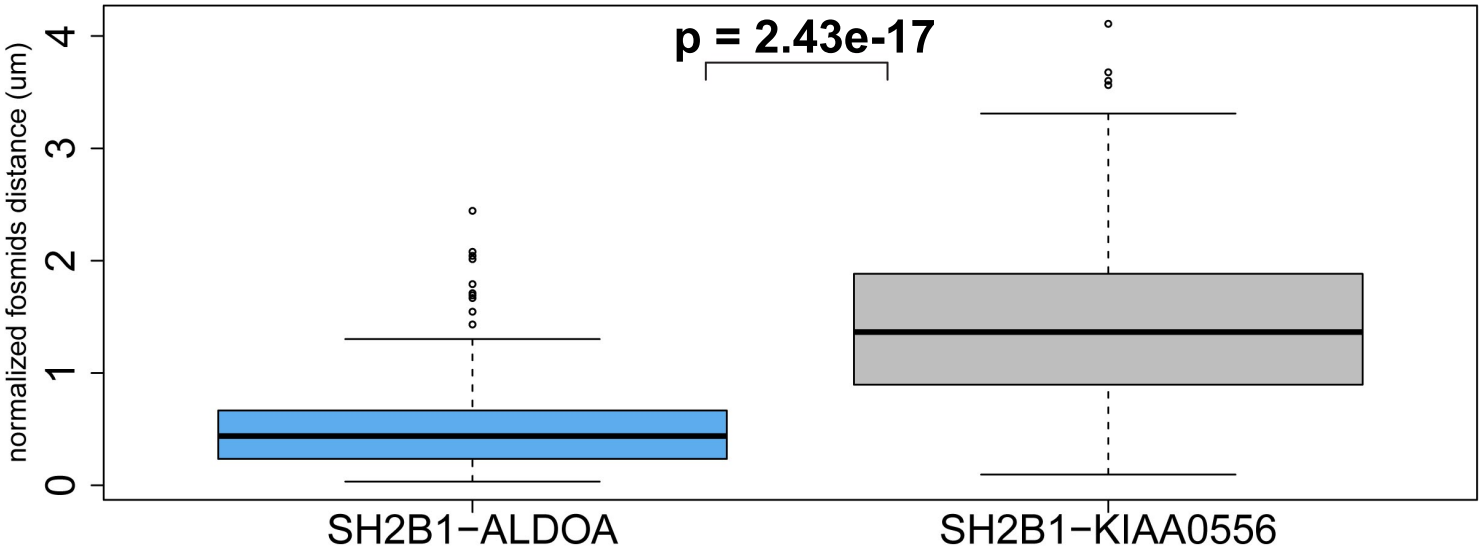

p = 2.43e-17
normalized fosmids distance (um)
0
1
2
3
4
SH2B1−ALDOA
SH2B1−KIAA0556

Figure S4: FISH shows co-localization of the 220kb and 600kb intervals
Fluorescence *in situ* hybridization experiments show co-localization of *SH2B1* that maps to the 220 kb interval with *ALDOA* that map to the 600kb interval but not with the equidistant *KIAA0556* locus. **(A)** Distribution of interphase nuclei foci distances *SH2B1-ALDOA* (light blue) and *SH2B1-KIAA0556* (grey)(n=120 for each experiment). **(B)** Distribution of the measured nucleus diameters in the *SH2B1-ALDOA* (light blue) and *SH2B1-KIAA0556* experiments (grey)(n=60 nuclei). **(C)** Distribution of interphase nuclei foci distances *SH2B1-ALDOA* (light blue) and *SH2B1-KIAA0556* (grey) normalized by the nucleus diameter (n=120 for each experiment).

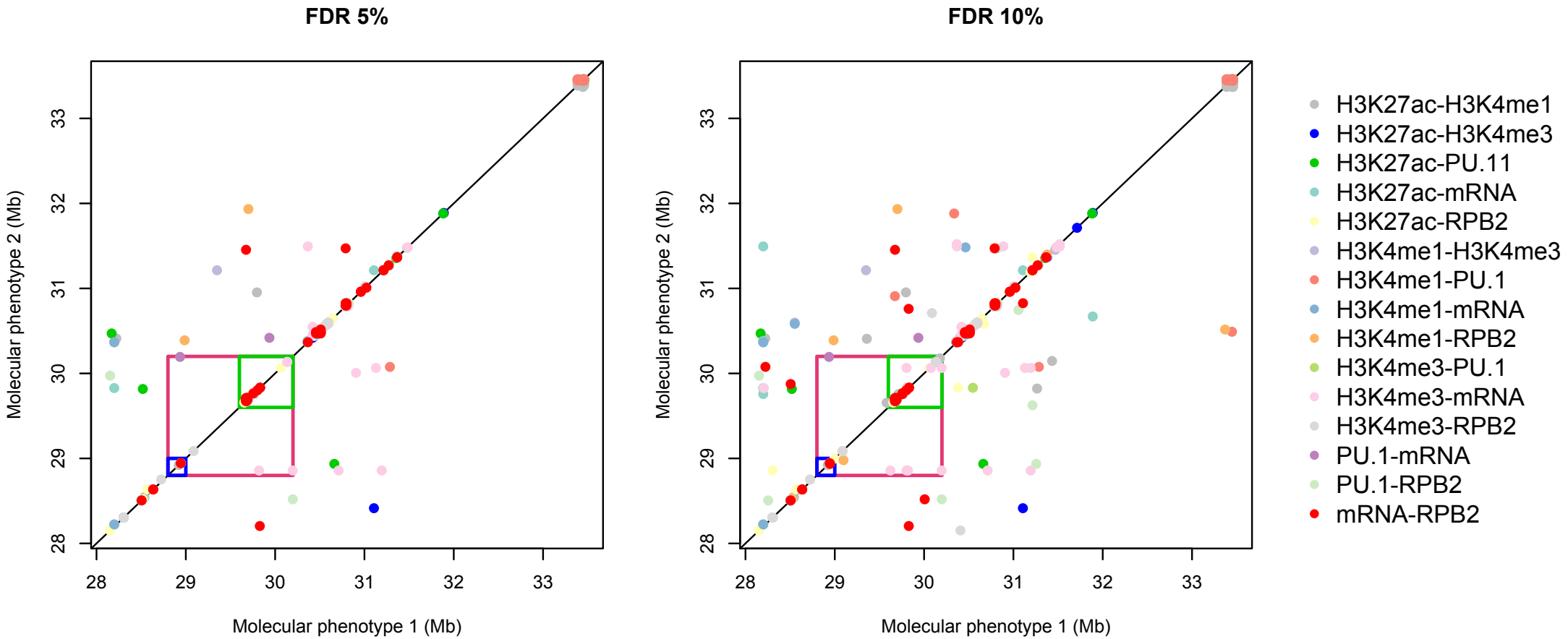

FDR 5%
FDR 10%
Molecular phenotype 2 (Mb)
Molecular phenotype 1 (Mb)
28
29
30
31
32
33
H3K27ac-H3K4me1
H3K27ac-H3K4me3
H3K27ac-PU.11
H3K27ac-mRNA
H3K27ac-RPB2
H3K4me1-H3K4me3
H3K4me1-PU.1
H3K4me1-mRNA
H3K4me1-RPB2
H3K4me3-PU.1
H3K4me3-mRNA
H3K4me3-RPB2
PU.1-mRNA
PU.1-RPB2
mRNA-RPB2

Figure S5: Associations between molecular phenotypes within the 16p11.2 cytoband

Pairwise molecular associations within the 16p11.2 region (28.1-34.6 Mb) between H3K4me1, H3K4me3, H3K27ac, PU.1 and RPB2 binding and mRNA levels in LCLs of 47 unrelated individuals at 5% (left panel) and 10% FDR (right panel). The positions of the 220kb BP2-BP3 and 600kb BP4-BP5 intervals are delimited by blue and green rectangles, respectively. Associations that support the 4C observed loopings between the 16p11.2 600kb BP4-BP5 and BP2-BP3 intervals are enclosed in the red rectangle. The nature of the molecular phenotypes participating in the associations is indicated on the right.

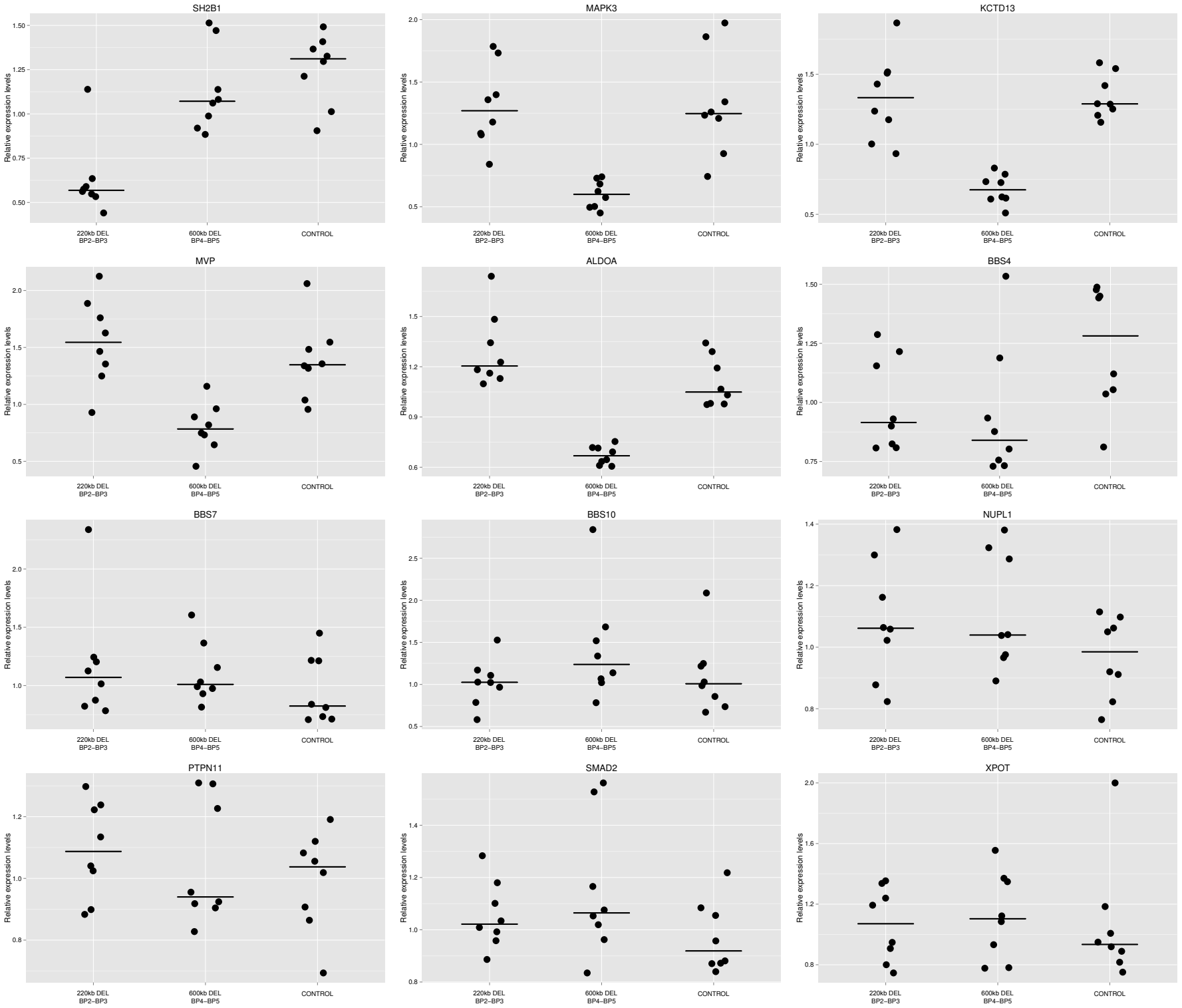

SH2B1
MAPK3
KCTD13
MVP
ALDOA
BBS4
BBS7
BBS10
NUPL1
PTPN11
SMAD2
XPOT
Relative expression levels
220kb DEL
BP2–BP3
600kb DEL
BP4–BP5
CONTROL

Figure S6: Ciliopathy gene expression levels are modified in cells of both 16p11.2 600kb BP4-BP5 and 16p11.2 BP2-BP3 deletion carriers. RNA levels of genes involved in primary cilium function and related pathways are modified in both 600kb BP4-BP5 and 220kb BP2-BP3 deletion patients' cells. Relative expression levels measured by quantitative PCR of BBS4, BBS7, BBS10, PTPN11, SMAD2, XPOT, NUP58 (a.k.a NUPL1), as well as 16p11.2 imbalanced interval genes ALDOA, KCTD13, MAPK3, MVP and SH2B1 in LCLs of eight unrelated carriers of the 220kb BP2-BP3 deletion, eight unrelated carriers of the 600kb BP4-BP5 deletion and eight unrelated age- and sex-matched control individuals (CONTROL). Note the significant diminution of the hemizygote gene SH2B1, but not of the neighboring normal-copy ALDOA, KCTD13, MVP and MAPK3 in 220kb deletion carriers and the reciprocal diminutions of the hemizygote ALDOA, KCTD13, MVP and MAPK3 but not of the neighboring normal-copy SH2B1 in 600 kb deletion carriers. The bar indicates the median.

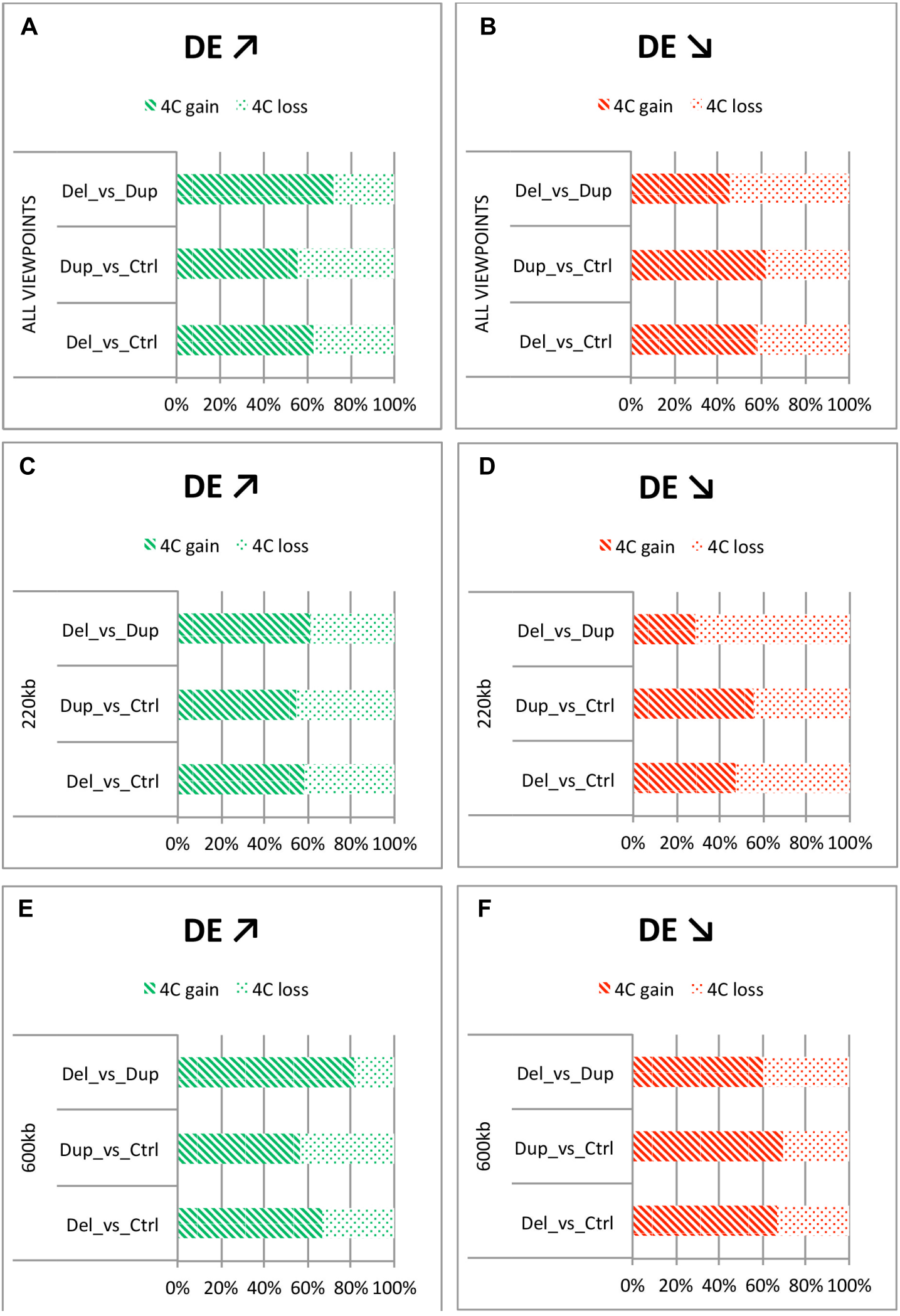
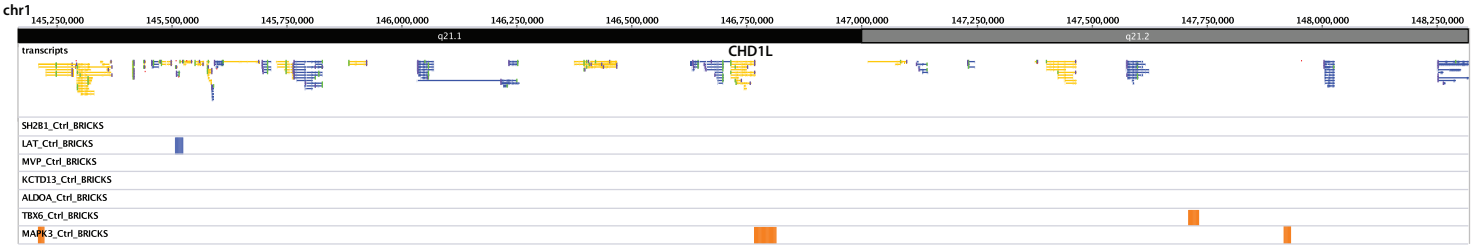

A
DE ↗
4C gain
4C loss
ALL VIEWPOINTS
Del_vs_Dup
Dup_vs_Ctrl
Del_vs_Ctrl
0% 20% 40% 60% 80% 100%
B
DE ↘
4C gain
4C loss
ALL VIEWPOINTS
Del_vs_Dup
Dup_vs_Ctrl
Del_vs_Ctrl
0% 20% 40% 60% 80% 100%
C
DE ↗
4C gain
4C loss
220kb
Del_vs_Dup
Dup_vs_Ctrl
Del_vs_Ctrl
0% 20% 40% 60% 80% 100%
D
DE ↘
4C gain
4C loss
220kb
Del_vs_Dup
Dup_vs_Ctrl
Del_vs_Ctrl
0% 20% 40% 60% 80% 100%
E
DE ↗
4C gain
4C loss
600kb
Del_vs_Dup
Dup_vs_Ctrl
Del_vs_Ctrl
0% 20% 40% 60% 80% 100%
F
DE ↘
4C gain
4C loss
600kb
Del_vs_Dup
Dup_vs_Ctrl
Del_vs_Ctrl
0% 20% 40% 60% 80% 100%

Figure S7 : 4C contacts perturbation and gene differential expression
Distribution of gain / loss of contacts for up- and down-regulated genes for each comparison: Deletion (Del) vs. Duplication (Dup), Dup vs. Control (Ctrl) and Del vs. Ctrl. Panels (A) and (B) show up-/down-regulated genes with altered BRICKS found in all viewpoints, panels (C) and (D) in the 220kb viewpoints only, and panels (D) and (F) in the 600kb viewpoints only.

!


chr1
145,250,000
145,500,000
145,750,000
146,000,000
146,250,000
146,500,000
146,750,000
147,000,000
147,250,000
147,500,000
147,750,000
148,000,000
148,250,000
q21.1
q21.2
transcripts
CHD1L
SH2B1_Ctrl_BRICKS
LAT_Ctrl_BRICKS
MVP_Ctrl_BRICKS
KCTD13_Ctrl_BRICKS
ALDOA_Ctrl_BRICKS
TBX6_Ctrl_BRICKS
MAPK3_Ctrl_BRICKS

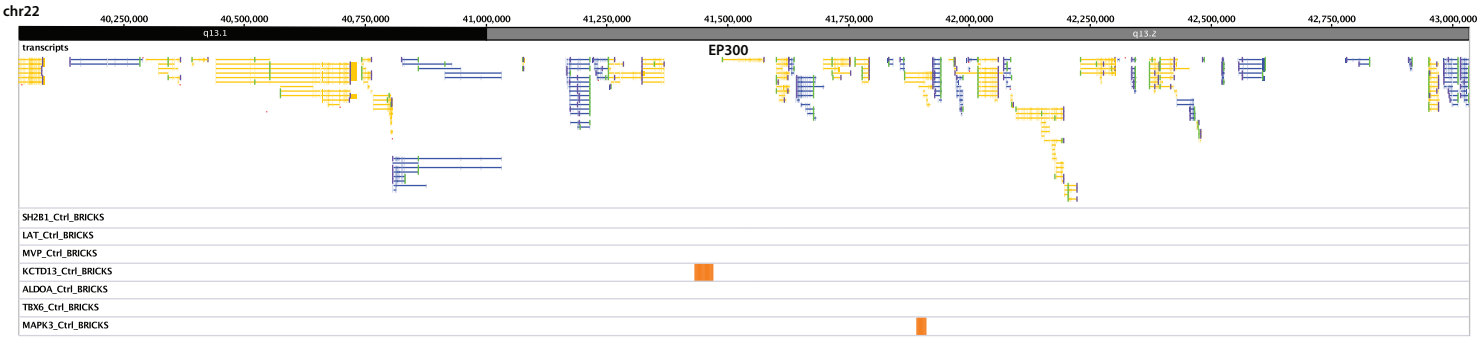
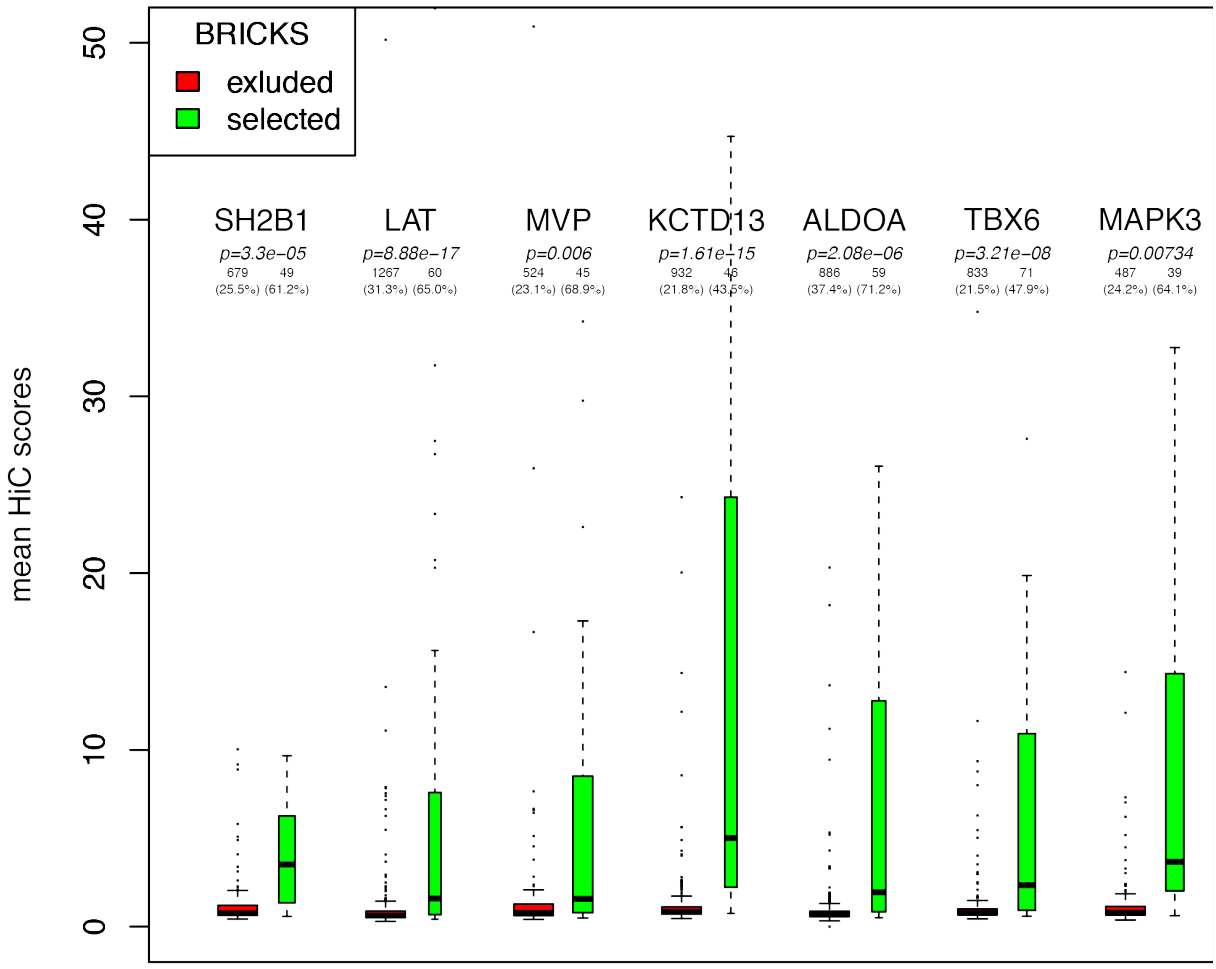

chr22
40,250,000
40,500,000
40,750,000
41,000,000
41,250,000
41,500,000
41,750,000
42,000,000
42,250,000
42,500,000
42,750,000
43,000,000
q13.1
q13.2
transcripts
EP300
SH2B1_Ctrl_BRICKS
LAT_Ctrl_BRICKS
MVP_Ctrl_BRICKS
KCTD13_Ctrl_BRICKS
ALDOA_Ctrl_BRICKS
TBX6_Ctrl_BRICKS
MAPK3_Ctrl_BRICKS

Figure S8: Chromatin-interacting regions
Examples of regions (BRICKS) interacting with 16p11.2 viewpoints showing some of the interacting genes, i.e. CHD1L (A) and EP300 (B). Other examples are shown in Figure 4A.

!

A


HiC in BRICKS
BRICKS
exluded
selected
mean HiC scores
0
10
20
30
40
50
SH2B1
p=3.3e−05
679 49
(25.5%) (61.2%)
LAT
p=8.88e−17
1267 60
(31.3%) (65.0%)
MVP
p=0.006
524 45
(23.1%) (68.9%)
KCTD13
p=1.61e−15
932 46
(21.8%) (43.5%)
ALDOA
p=2.08e−06
886 59
(37.4%) (71.2%)
TBX6
p=3.21e−08
833 71
(21.5%) (47.9%)
MAPK3
p=0.00734
487 39
(24.2%) (64.1%)

B

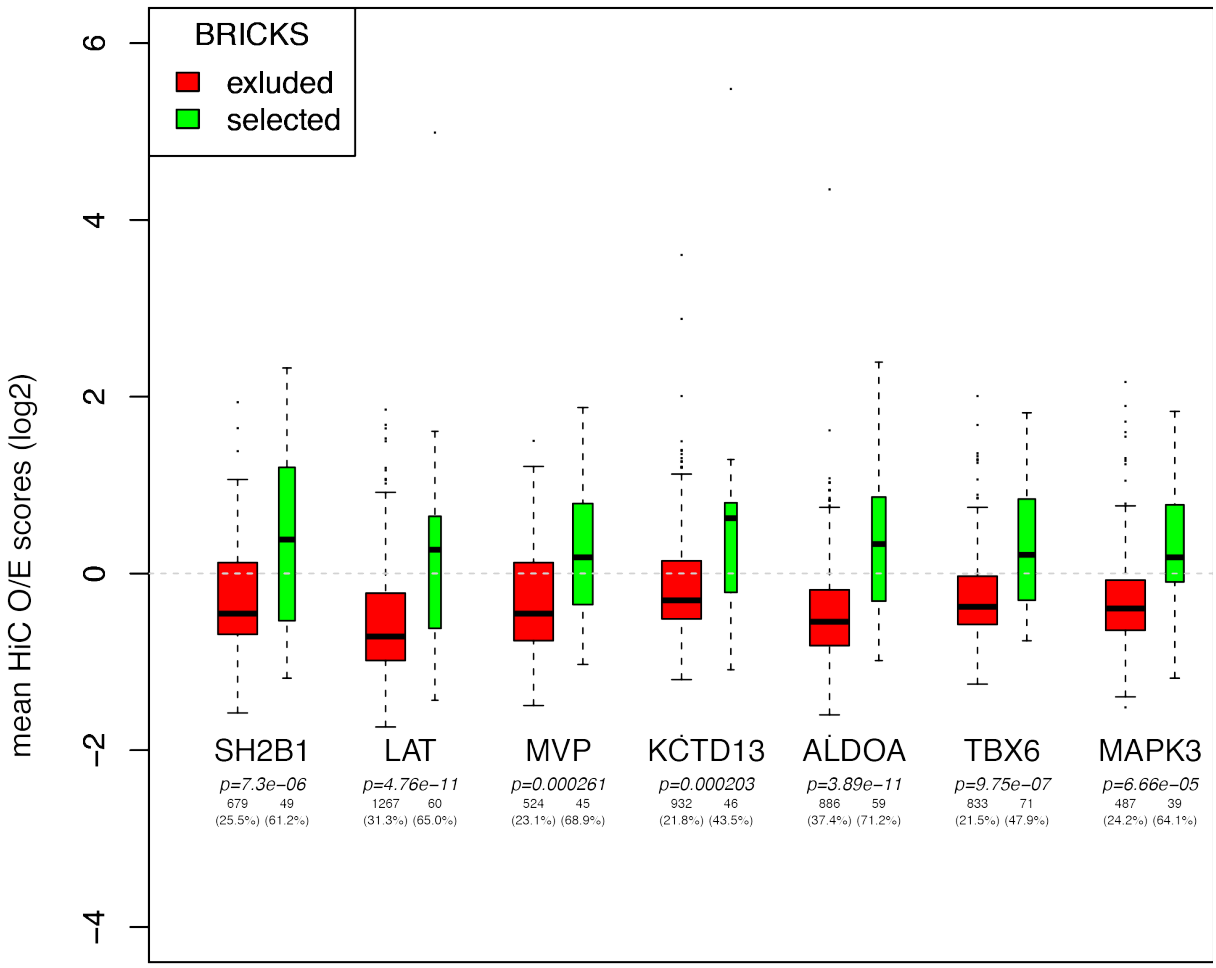

HiC in BRICKS
O/E scores
BRICKS
exluded
selected
mean HiC O/E scores (log2)
−4
−2
0
2
4
6
SH2B1
p=7.3e−06
679 49
(25.5%) (61.2%)
LAT
p=4.76e−11
1267 60
(31.3%) (65.0%)
MVP
p=0.000261
524 45
(23.1%) (68.9%)
KCTD13
p=0.000203
932 46
(21.8%) (43.5%)
ALDOA
p=3.89e−11
886 59
(37.4%) (71.2%)
TBX6
p=9.75e−07
833 71
(21.5%) (47.9%)
MAPK3
p=6.66e−05
487 39
(24.2%) (64.1%)

Figure S9: Distribution of Hi-C scores in selected versus non-selected BRICKS

Virtual 4C-seq tracks were generated for each viewpoint from the GM12878 Hi-C results of reference 70 (5kb resolution) by extracting the Hi-C vectors from the KR normalized observed (top panel) and observed/expected matrices (bottom panel). BRICKS found with each viewpoint were quantified by the mean Hi-C signals. The p-values of two-sided t-tests are reported for each comparison, together with the number of Hi-C bins and the % of non-NA bins.

!

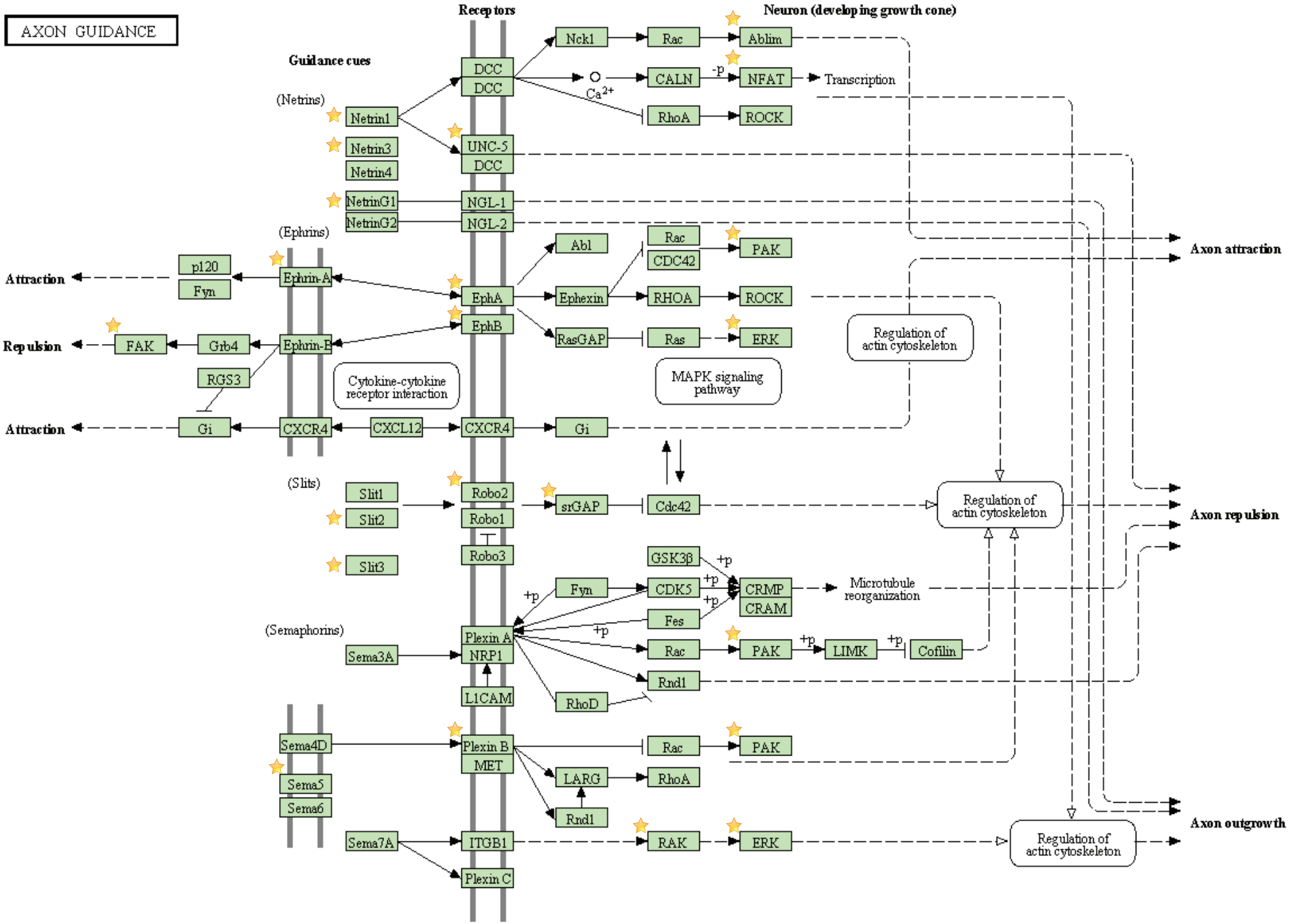


Figure S10: KEGG pathway with modified chromatin interactions
The 1193 genes that show modified chromatin interaction in cells of 16p11.2 600kb rearrangement carriers are enriched in members of the hsa04360 “axon guidance” KEGG pathway. The genes with modified chromatin interactions are marked with yellow stars.

!

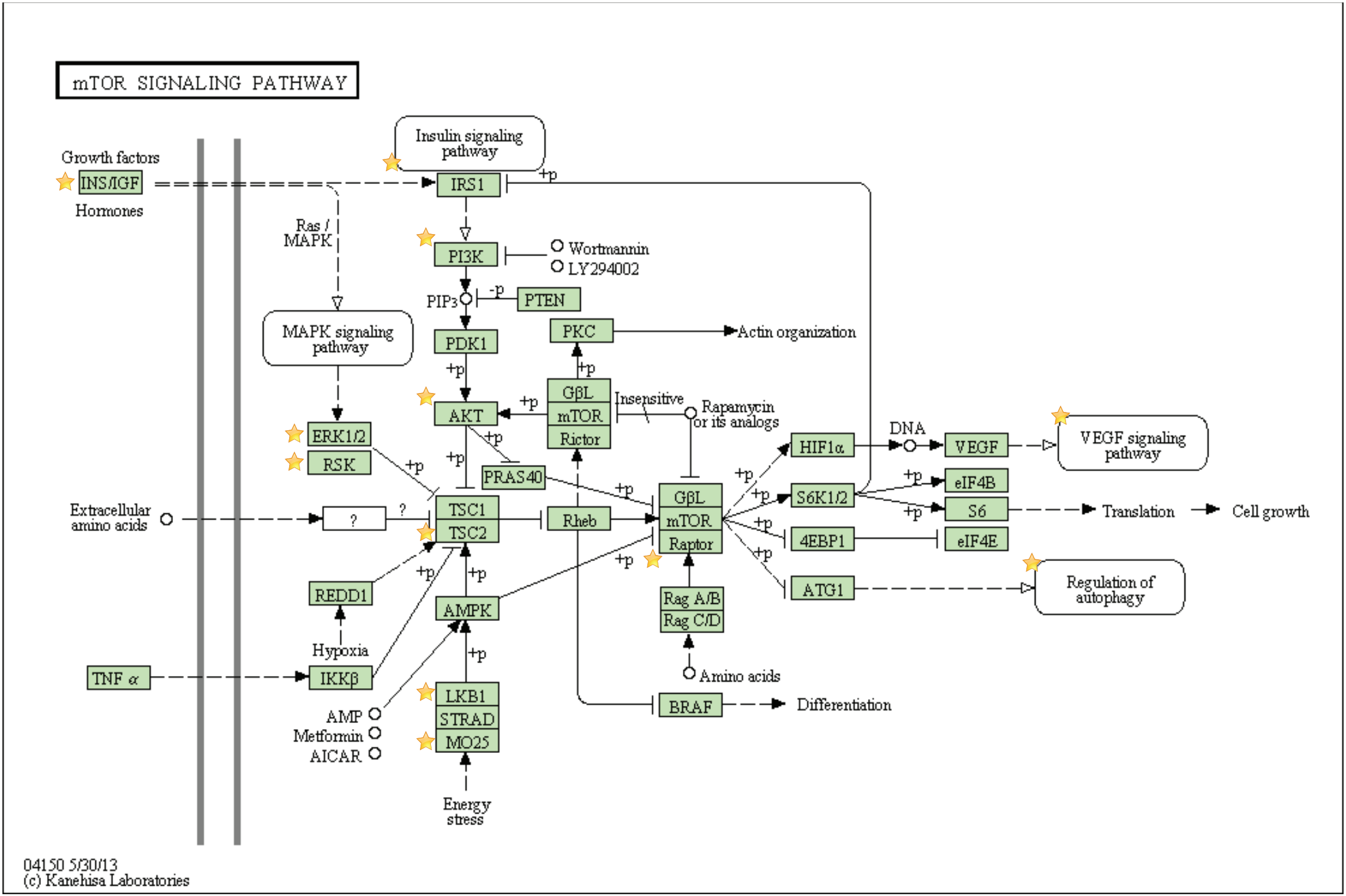

mTOR SIGNALING PATHWAY
Growth factors
INS/IGF
Hormones
Insulin signaling pathway
IRS1
+p
Ras / MAPK
PI3K
Wortmannin
LY294002
PIP3
-p
PTEN
MAPK signaling pathway
PDK1
PKC
Actin organization
+p
+p
GβL
mTOR
Rictor
Insensitive
Rapamycin or its analogs
AKT
+p
+p
ERK1/2
RSK
+p
+p
PRAS40
+p
HIF1α
DNA
VEGF
VEGF signaling pathway
+p
+p
eIF4B
S6K1/2
+p
S6
Translation
Cell growth
Extracellular amino acids
?
?
TSC1
TSC2
Rheb
GβL
mTOR
Raptor
+p
+p
4EBP1
eIF4E
+p
+p
+p
ATG1
Regulation of autophagy
REDD1
+p
+p
AMPK
Rag A/B
Rag C/D
Hypoxia
+p
Amino acids
TNF α
IKKβ
LKB1
STRAD
MO25
AMP
Metformin
AICAR
BRAF
Differentiation
Energy stress
04150 5/30/13
(c) Kanehisa Laboratories

Figure S11: KEGG pathway with modified chromatin interactions
The 1193 genes that show modified chromatin interaction in cells of 16p11.2 600kb rearrangement carriers are enriched in members of the hsa04150 “mTOR signaling” KEGG pathway. The genes with modified chromatin interactions are marked with yellow stars.

!