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Supplemental Data

Symmetrical Dose-Dependent DNA-Methylation Profiles in Children with Deletion or Duplication of 7q11.23

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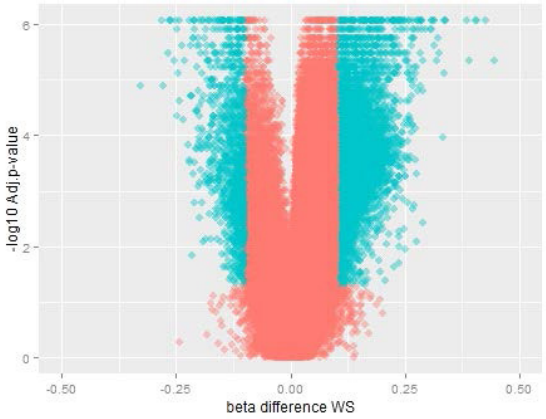
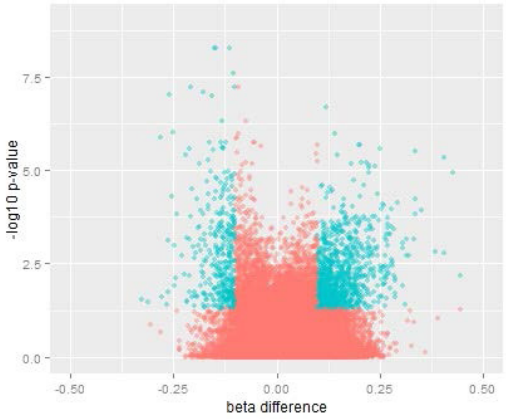
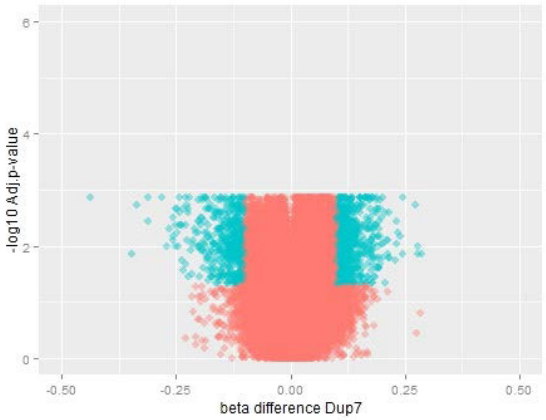
A


-log10 Adj.p-value
beta difference WS

B


-log10 p-value
beta difference

C


-log10 Adj.p-value
beta difference Dup7

D

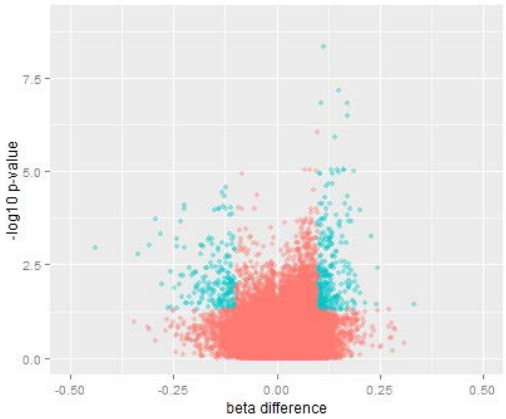
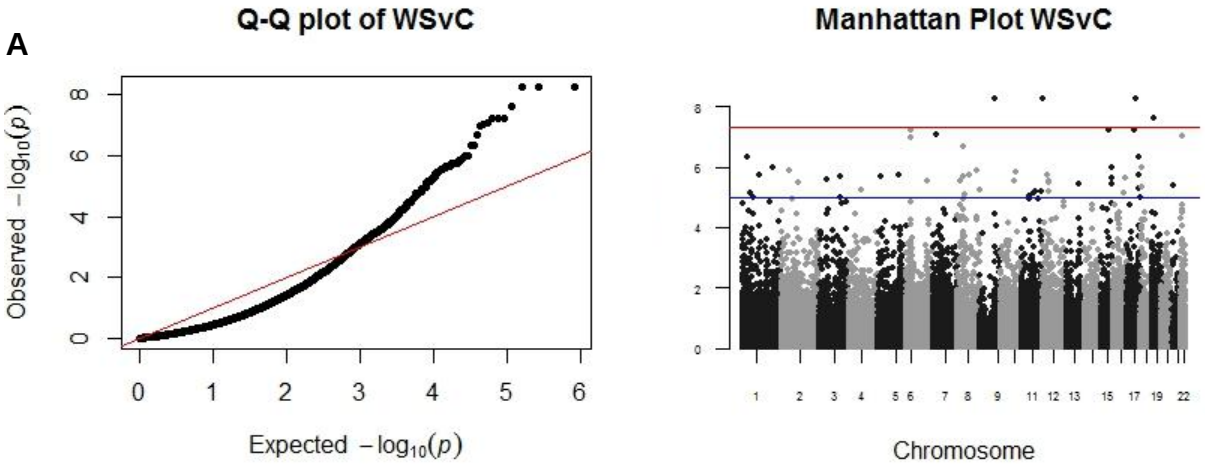
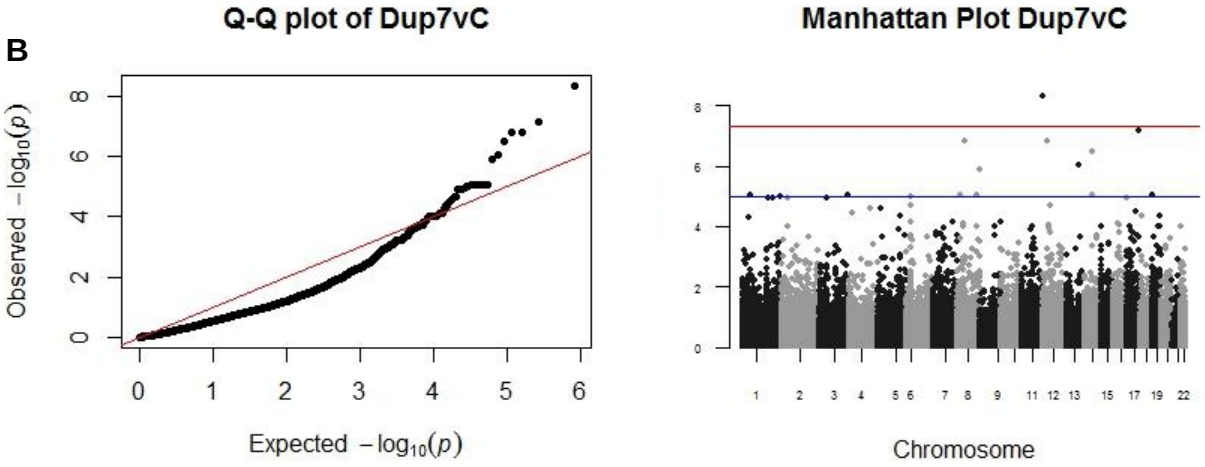

-log10 p-value
beta difference

Figure S1: Volcano plots pre- and post-adjustment for cell type heterogeneity. (**A**) Methylation data for the WS cohort pre-adjustment for cell type. Blue data points depict probes that are both significant ($P \leq 0.05$) and have a delta beta of ≥ 10% relative to TD controls. Pre-adjustment resulted in 87391 significant probes, of which 7466 had a delta beta of ≥ 10% (6260 hypermethylated and 1206 hypomethylated relative to TD). (**B**) Adjusting for cell type heterogeneity within the WS cohort resulted in 5154 significant probes, of which 1413 had a delta beta of ≥ 10% relative to TD controls (1032 hypermethylated and 381 hypomethylated). (**C**) Pre-adjustment within the Dup7 cohort relative to TD controls resulted in 35789 significant probes, of which 1333 had a delta beta of ≥ 10% (779 hypermethylated and 554 hypomethylated relative to TD). (**D**) Adjusting for cell type heterogeneity within the Dup7 cohort resulted in 3126 significant probes relative to TD controls, of which 508 had a delta beta ≥ 10% (306 hypermethylated, 202 hypomethylated).

A


Q-Q plot of WSvC
Observed $-\log_{10}(p)$
Expected $-\log_{10}(p)$
Manhattan Plot WSvC
Chromosome

B


Q-Q plot of Dup7vC
Observed $-\log_{10}(p)$
Expected $-\log_{10}(p)$
Manhattan Plot Dup7vC
Chromosome

C

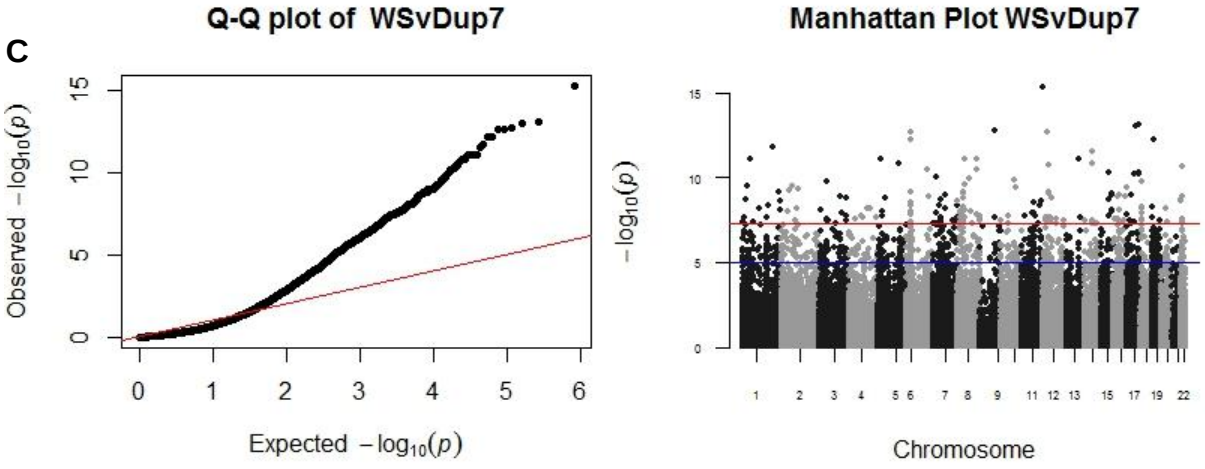

Q-Q plot of WSvDup7
Observed $-\log_{10}(p)$
Expected $-\log_{10}(p)$
Manhattan Plot WSvDup7
$-\log_{10}(p)$
Chromosome

Figure S2: Quantile-quantile (QQ) and Manhattan plots of methylation data. QQ-plots depict significance values after adjusting for cell type heterogeneity. Red lines show the threshold for genome-wide significance and blue lines the threshold for a suggestive association. QQ-plot and Manhattan plot of (**A**) WS methylation data relative to TD controls, (**B**) Dup7 methylation data relative to TD controls and (**C**) WS methylation data relative to Dup7.

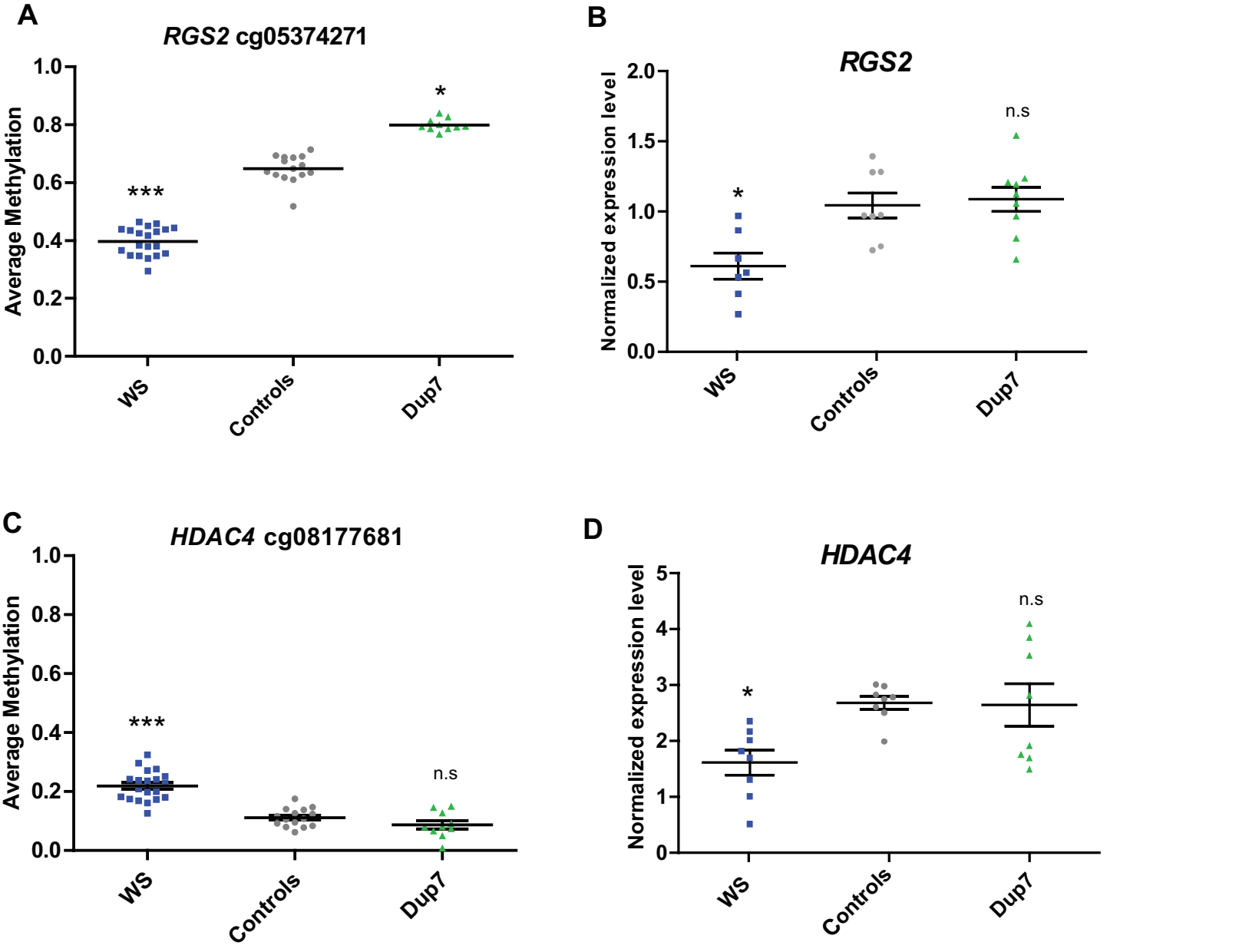

A
RGS2 cg05374271
Average Methylation
1.0
0.8
0.6
0.4
0.2
0.0
***
*
WS
Controls
Dup7
B
RGS2
Normalized expression level
2.0
1.5
1.0
0.5
0.0
*
n.s
WS
Controls
Dup7
C
HDAC4 cg08177681
Average Methylation
1.0
0.8
0.6
0.4
0.2
0.0
***
n.s
WS
Controls
Dup7
D
HDAC4
Normalized expression level
5
4
3
2
1
0
*
n.s
WS
Controls
Dup7

Figure S3: Differential methylation alters expression of *RGS2* and *HDAC4*. (**A**) Average methylation level at *RGS2* cg05374271 showed a significant effect of genotype ($P < 0.001$, Kruskal-Wallis) with levels of 39.7% in the WS group, 64.9% in the TD group and 79.9% in the Dup7 group, resulting in a WS DM of -25.2% (adj. $P = 9.96E-07$) and Dup7 DM of 15% (adj. $P = 1.1E-05$) (**B**) Expression analysis of *RGS2* across the WS, TD and Dup7 cohorts revealed a significant decrease in expression within the WS group only ($P < 0.01$, Kruskal-Wallis). (**C**) Average methylation level at *HDAC4* cg08177681 showed a significant effect of genotype ($P < 0.0001$, Kruskal-Wallis) with levels of 21.9% in the WS group, 11.1% in the TD group and 8.7% in the Dup7 group, resulting in a WS DM of 10.8% and a non-significant change in Dup7 methylation. (**D**) Expression analysis of *HDAC4* across WS, TD controls and Dup7 identified a significant decrease in expression within the WS group ($P < 0.01$, Kruskal-Wallis). * $P < 0.05$, *** $P < 0.001$ using Dunn's post-hoc analysis.

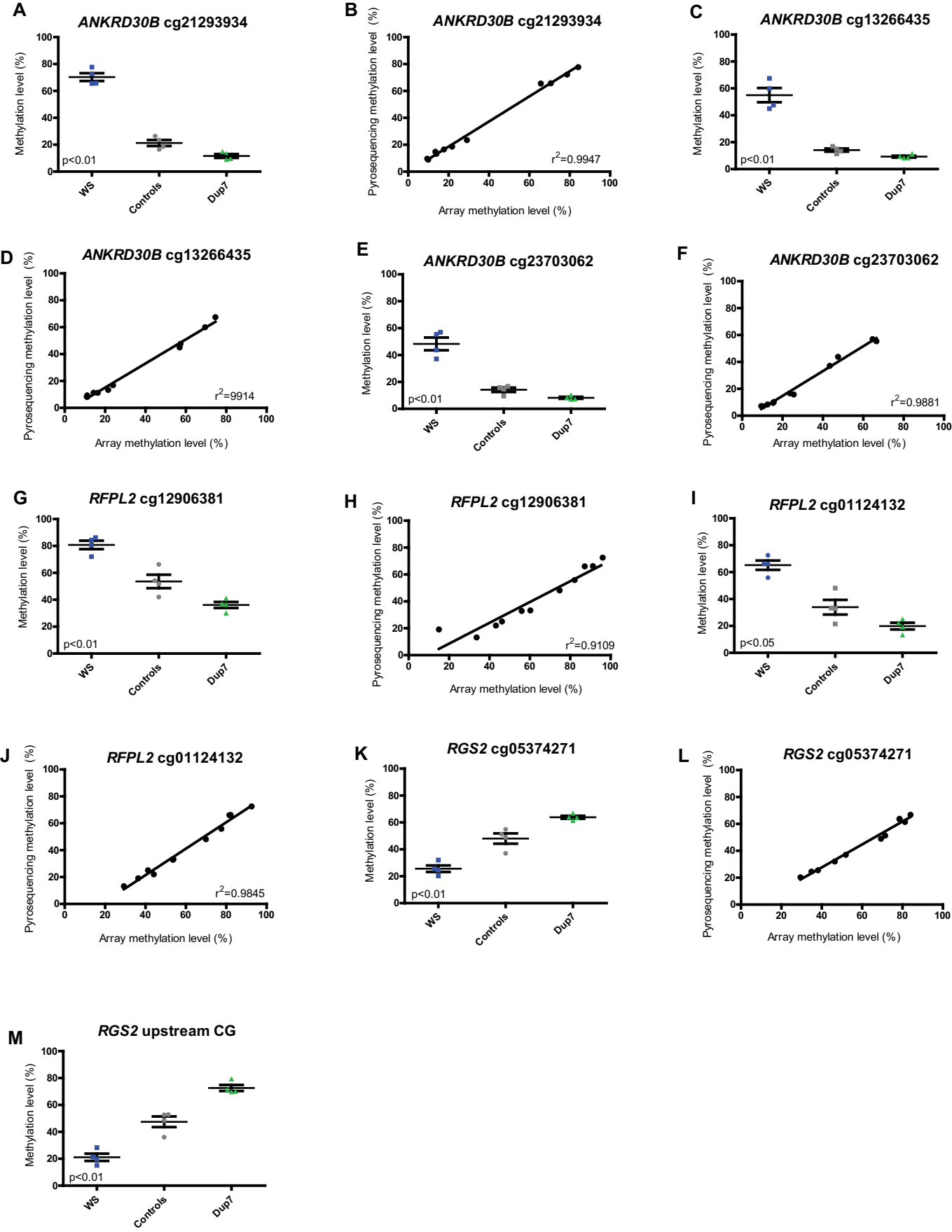
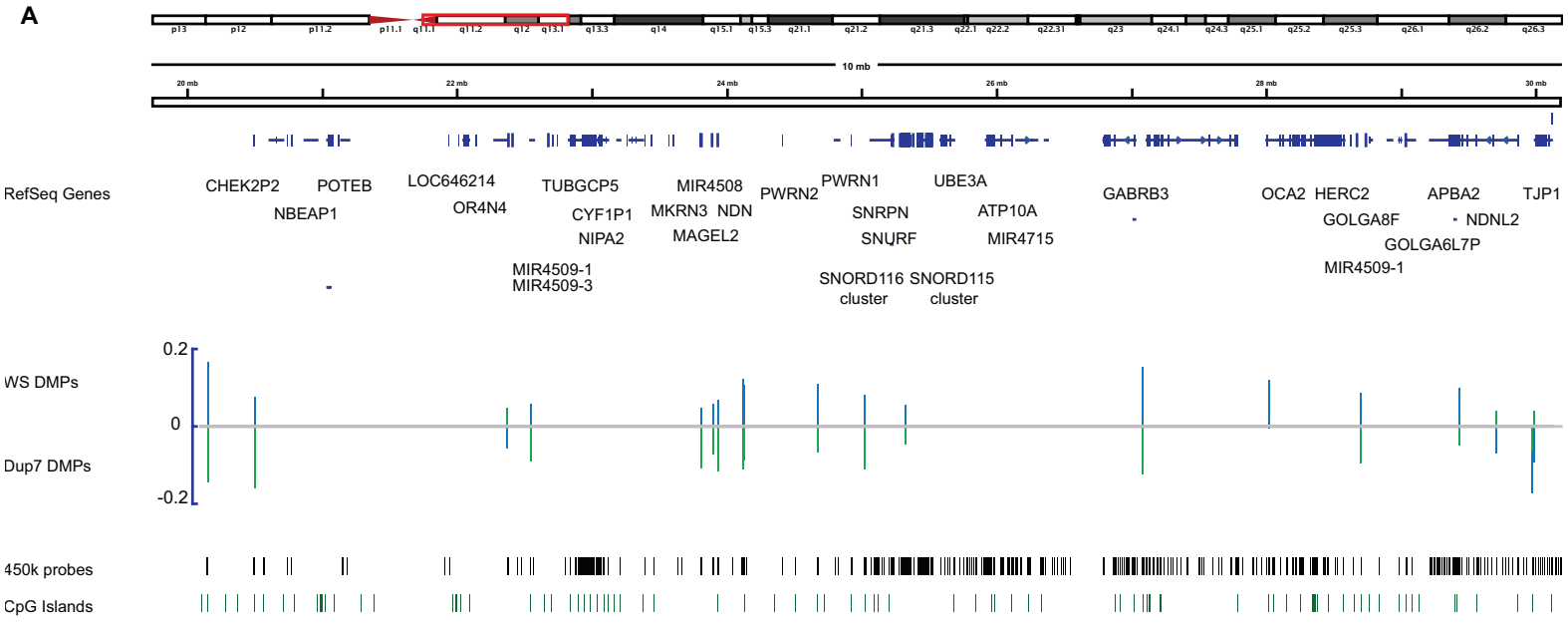
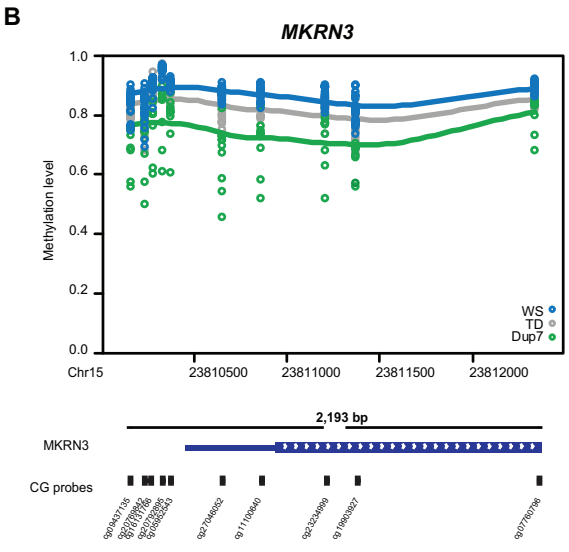

A
ANKRD30B cg21293934
Methylation level (%)
p<0.01
WS
Controls
Dup7
B
ANKRD30B cg21293934
Pyrosequencing methylation level (%)
r2=0.9947
Array methylation level (%)
C
ANKRD30B cg13266435
Methylation level (%)
p<0.01
WS
Controls
Dup7
D
ANKRD30B cg13266435
Pyrosequencing methylation level (%)
r2=9914
Array methylation level (%)
E
ANKRD30B cg23703062
Methylation level (%)
p<0.01
WS
Controls
Dup7
F
ANKRD30B cg23703062
Pyrosequencing methylation level (%)
r2=0.9881
Array methylation level (%)
G
RFPL2 cg12906381
Methylation level (%)
p<0.01
WS
Controls
Dup7
H
RFPL2 cg12906381
Pyrosequencing methylation level (%)
r2=0.9109
Array methylation level (%)
I
RFPL2 cg01124132
Methylation level (%)
p<0.05
WS
Controls
Dup7
J
RFPL2 cg01124132
Pyrosequencing methylation level (%)
r2=0.9845
Array methylation level (%)
K
RGS2 cg05374271
Methylation level (%)
p<0.01
WS
Controls
Dup7
L
RGS2 cg05374271
Pyrosequencing methylation level (%)
Array methylation level (%)
M
RGS2 upstream CG
Methylation level (%)
p<0.01
WS
Controls
Dup7

Figure S4: Pyrosequencing validation of DM probes in WS, Dup7 and TD controls confirms dose-dependent changes in DNA methylation. Methylation level of 4 WS, 4 TD and 4 Dup7 samples were analyzed using secondary pyrosequencing assays and the methylation level at each CpG is shown. Significance testing between the three groups was performed using the Kruskal-Wallis test. (**A**) Average methylation levels across *ANKRD30B* cg21293934 were 70.3% in the WS group, 21.28% in the TD group and 11.65% in the Dup7 group, corresponding to a WS DM of 49.02% and Dup7 DM of -9.63%. (**B**) Comparison of pyrosequencing and array methylation levels resulted in a significant correlation of R^2=0.9947 (P < 0.0001) using linear regression. (**C**) Average methylation levels across *ANKRD30B* cg13266435 were 55% in the WS group, 14.11% in the TD group and 9.26% in the Dup7 group, resulting in a WS DM of 40.9% and Dup7 DM of -4.85%. (**D**) Comparative analysis of *ANKRD30B* cg13266435 showed a significant correlation of R^2=0.9825 (P < 0.0001). (**E**) Average methylation levels across *ANKRD30B* cg23703062 were 48.33% in the WS group,14.17% in the TD group and 8.22% in the Dup7 group, resulting in a WS DM of 34.16% and Dup7 DM of -5.95%. (**F**) Comparative analysis of *ANKRD30B* cg23703062 showed a significant correlation of R^2=0.9881 (P < 0.0001). (**G**) Average methylation levels across *RFPL2* cg12906381 were 80.8% in the WS group, 53.62% in the TD group and 36.1% in the Dup7 group, resulting in a WS DM of 27.18% and Dup7 DM of -17.52%. (**H**) Comparative analysis of *RFPL2* cg12906381 showed a significant correlation of R^2=0.9919 (P < 0.0001). (**I**) Average methylation levels across *RFPL2* cg01124132 were 65.17% in the WS group, 33.95% in the TD group and 19.87% in the Dup7 group, resulting in WS DM of 31.22% and Dup7 DM of -14.08%. (**J**) Comparative analysis of *RFPL2* cg01124132 revealed a

significant correlation of R^2=0.9845 (*P* < 0.0001). (**K**) Average methylation levels across *RGS2* cg05374271 were 25.63% in the WS group, 48.05% in the TD group and 63.87% in the Dup7 group, resulting in a WS DM of -22.42% and Dup7 DM of 15.82%. (**L**) Comparative analysis of *RGS2* cg05374271 showed a significant correlation of R^2=0.9825 (*P* < 0.0001). (**M**) A CpG upstream of cg05374271 in RGS2 was captured in the same pyrosequencing assay, however it was not captured on the array. Average methylation levels from pyrosequencing across cg05374271 were 21.09% in the WS group, 47.48% in the TD group and 72.65% in the Dup7 group, resulting in a WS DM of -26.39% and Dup7 DM of 25.17%. Significance testing for average methylation changes was performed using the Kruskal-Wallis test.


A
RefSeq Genes
CHEK2P2
POTEB
NBEAP1
LOC646214
OR4N4
TUBGCP5
CYF1P1
NIPA2
MIR4509-1
MIR4509-3
MIR4508
MKRN3
NDN
MAGEL2
PWRN2
PWRN1
SNRPN
SNURF
SNORD116
cluster
SNORD115
cluster
UBE3A
ATP10A
MIR4715
GABRB3
OCA2
HERC2
GOLGA8F
APBA2
NDNL2
GOLGA6L7P
MIR4509-1
TJP1
WS DMPs
Dup7 DMPs
0.2
0
-0.2
450k probes
CpG Islands


B
MKRN3
Methylation level
WS
TD
Dup7
Chr15
23810500
23811000
23811500
23812000
2,193 bp
MKRN3
CG probes

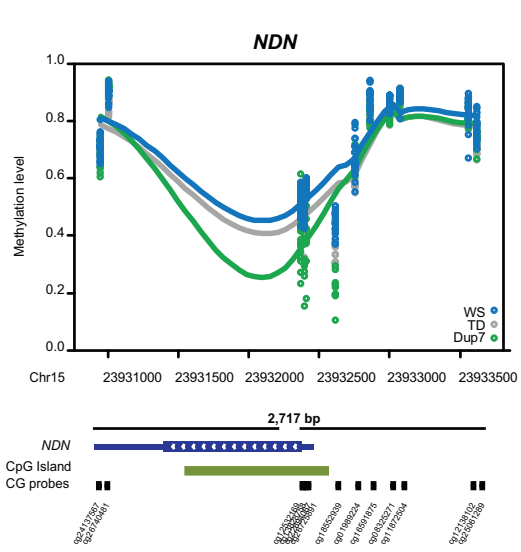
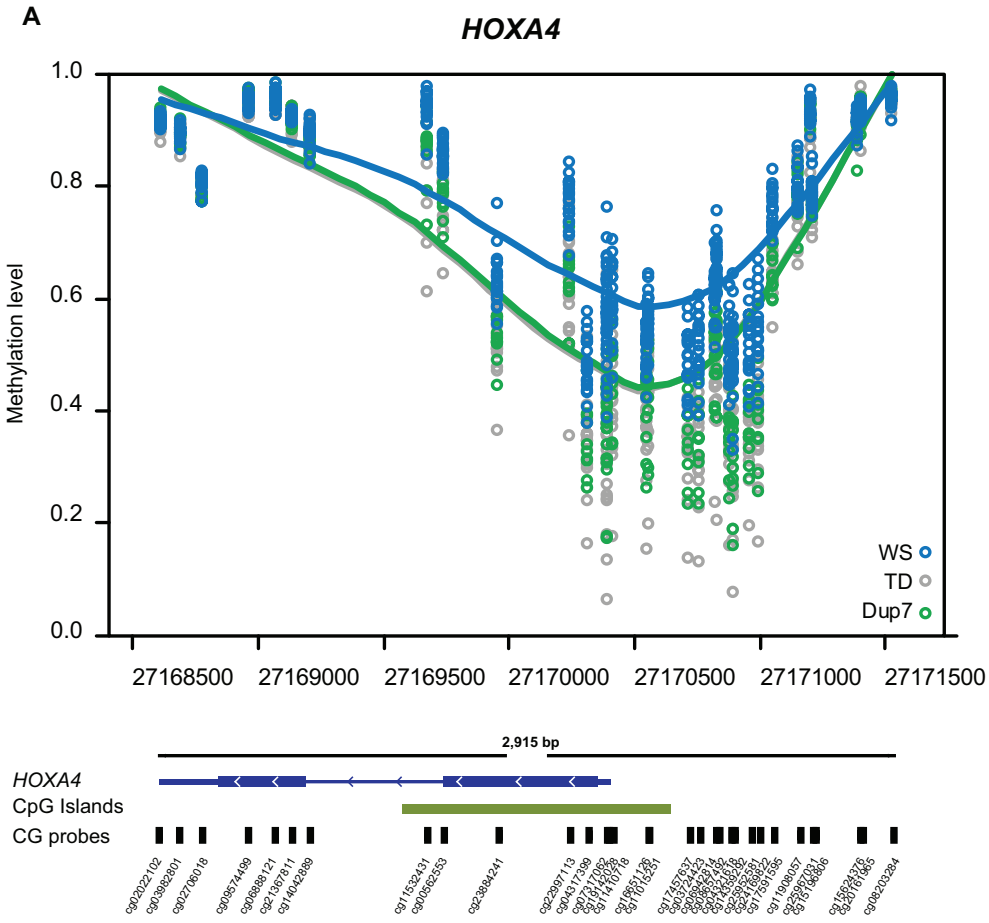

C
NDN
Methylation level
WS
TD
Dup7
Chr15
23931000
23931500
23932000
23932500
23933000
23933500
2,717 bp
NDN
CpG Island
CG probes

Figure S5: Differential methylation identified across *MKRN3* and *NDN,* within the imprinted Prader-Willi and Angelman syndrome region at 15q11-13. (**A**) Average DNA methylation levels in WS (blue) and Dup7 (green) spanning the imprinted Prader-Willi and Angelman syndrome region. Significantly DM probes with a change in methylation of >10% are plotted from the symmetrically opposite WS v Dup7 dataset (**B**) A DMR representing significant hypomethylation of 10 probes within *MKRN3* was identified in children with Dup7 (mean DMR *P* = 4.94e-07, chr15:23810163-23812334). Children with WS showed a non-significant gain of methylation. (**C**) A DMR representing significant hypomethylation of 5 CG probes within *NDN* was found in children with Dup7 (mean DMR *P* = 9.86e-04, chr15:23932370-23933620). A non-significant increase in methylation was detected for children with WS.


A
HOXA4
Methylation level
1.0
0.8
0.6
0.4
0.2
0.0
WS
TD
Dup7
27168500
27169000
27169500
27170000
27170500
27171000
27171500
2,915 bp
HOXA4
CpG Islands
CG probes

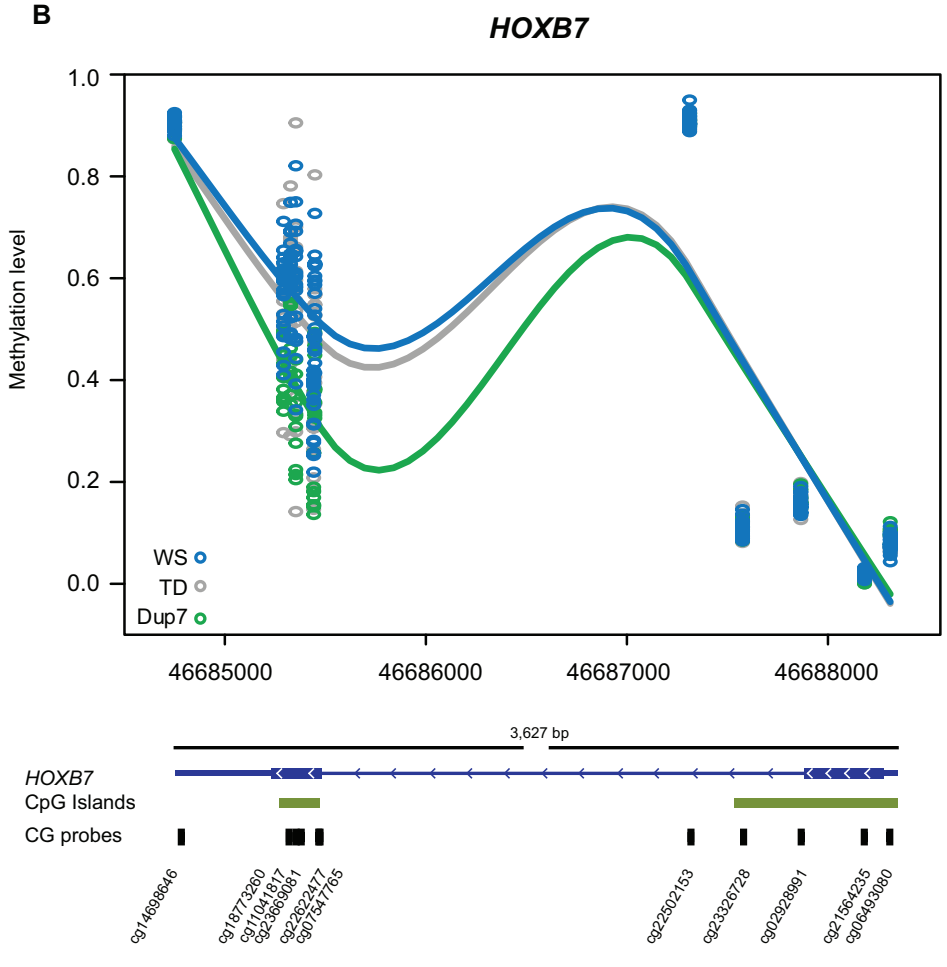

B
HOXB7
Methylation level
1.0
0.8
0.6
0.4
0.2
0.0
WS
TD
Dup7
46685000
46686000
46687000
46688000
3,627 bp
HOXB7
CpG Islands
CG probes

Figure S6: Cohort specific DMRs identified across *HOXA4* and *HOXB7*. Scatter plots fitted with Loess curves show DNA methylation changes across children with WS (blue), TD children (grey) and children with Dup7 (green). (**A**) Hypermethylation of *HOXA4* identified in the WS cohort exclusively. Of the 32 probes spanning *HOXA4*, 17 probes showed a gain of methylation of ≥10% (mean DMR *P* = 1.15e-03, chr7:27,168,962-27,171,528). DM probes are highlighted in the line plot, showing the selective gain of methylation across the promoter region of *HOXA4* in the WS cohort only. (**B**) Hypomethylation of *HOXB7* was identified specifically within the Dup7 cohort. 3 probes across this region were significantly DM by at least 10% relative to TD controls (mean DMR *P* = 1.13e-07, chr17:46,684,750-46,685,448).

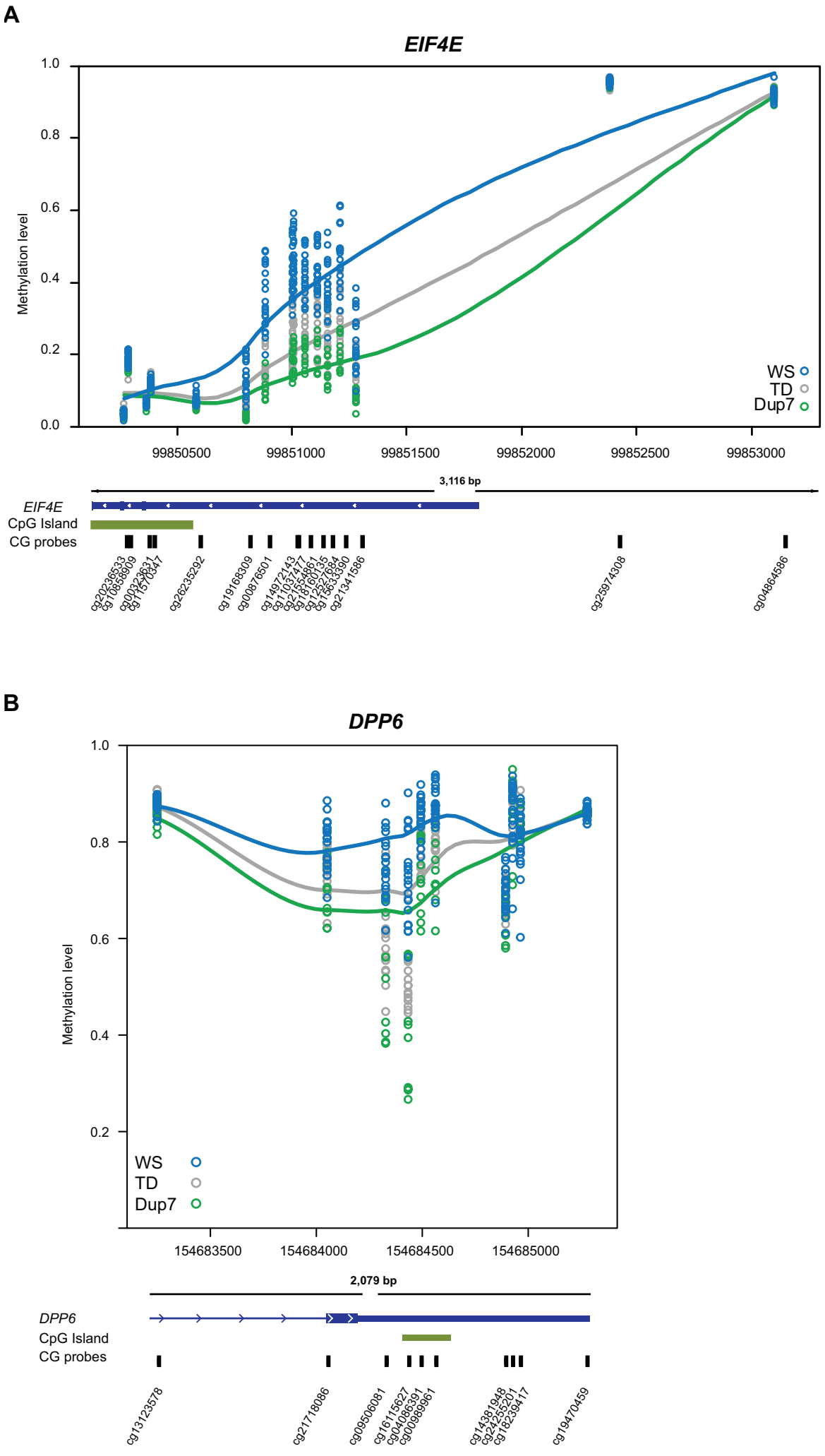

A
EIF4E
Methylation level
1.0
0.8
0.6
0.4
0.2
0.0
99850500
99851000
99851500
99852000
99852500
99853000
WS
TD
Dup7
3,116 bp
EIF4E
CpG Island
CG probes
cg20236533
cg10858809
cg00223631
cg11570047
cg26235292
cg19168309
cg00876501
cg14972143
cg11027477
cg21554861
cg18160135
cg12527684
cg15633590
cg21341586
cg25974308
cg04864586
B
DPP6
Methylation level
1.0
0.8
0.6
0.4
0.2
154683500
154684000
154684500
154685000
WS
TD
Dup7
2,079 bp
DPP6
CpG Island
CG probes
cg13123578
cg21718086
cg09506081
cg16115627
cg04086391
cg00989961
cg14381948
cg22435201
cg18239417
cg19470459

Figure S7: Dose-dependent DNA methylation identified across *EIF4E* and *DPP6.*

(**A**) A DMR representing dose-dependent methylation changes identified across the promoter region of *EIF4E*. Of the 24 probes that span *EIF4E,* 15 probes that span the region of differential methylation are shown in the line graph. 8 probes show a DM of ≥10% (mean DMR *P* = 8.6e-04, chr4:99,849,709-99,851,281). The Dup7 group showed a non-significant trend of hypomethylation relative to TD. (**B**) A DMR representing dose-dependent methylation changes identified in *DPP6.* Of 8 DM probes, 3 probes showed DM of >10% in the WS group relative to the Dup7 group (cg09506081, cg16115627, cg04086391; mean DMR *P* = 4.54e-03, chr7:154,683,249-154,684,963).

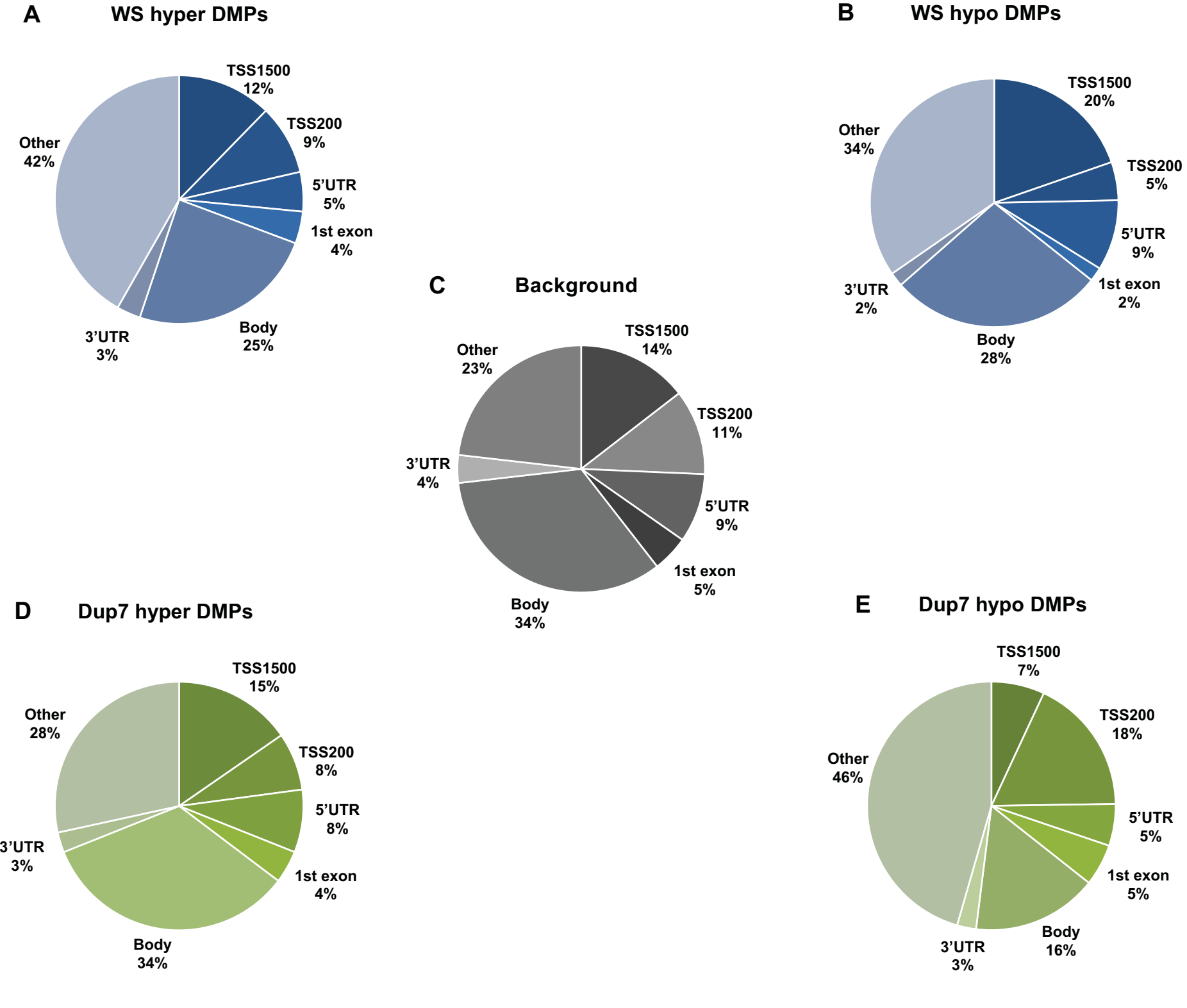

A
WS hyper DMPs
TSS1500
12%
TSS200
9%
5'UTR
5%
1st exon
4%
Body
25%
3'UTR
3%
Other
42%
B
WS hypo DMPs
TSS1500
20%
TSS200
5%
5'UTR
9%
1st exon
2%
Body
28%
3'UTR
2%
Other
34%
C
Background
TSS1500
14%
TSS200
11%
5'UTR
9%
1st exon
5%
Body
34%
3'UTR
4%
Other
23%
D
Dup7 hyper DMPs
TSS1500
15%
TSS200
8%
5'UTR
8%
1st exon
4%
Body
34%
3'UTR
3%
Other
28%
E
Dup7 hypo DMPs
TSS1500
7%
TSS200
18%
5'UTR
5%
1st exon
5%
Body
16%
3'UTR
3%
Other
46%

Figure S8: Relative position of significantly differentially methylated CGs (beta ≥ 10%). Position of DM probes using annotations from UCSC RefGene Group of (**A**) WS hypermethylated DM probes, (**B**) WS hypomethylated DM probes, (**C**) background probes on the array calculated as all probes retained after filtering out SNPs and cross-reactive probes, (**D**) Dup7 hypermethylated DM probes and (**E**) Dup7 hypomethylated probes. Positions outside UCSC Refgene Group annotations are listed as ‘other’ and include distal and intergenic regions.

A
B
C
D
Z Scores

Figure S9: Enrichment analysis of DM probes using ENCODE data reveals significant enrichment in H3K9me3 and CTCF that spans many different cell types. Top 50 most significant enrichments from (**A**) WS hypermethylated probes (**B**) WS hypomethylated probes (**C**) Dup7 hypermethylated probes and (**D**) Dup7 hypomethylated probes. Random intersections are scaled by mean and standard deviation. The blue line represents the Z-score associated with a Bonferroni adjusted q = 0.05

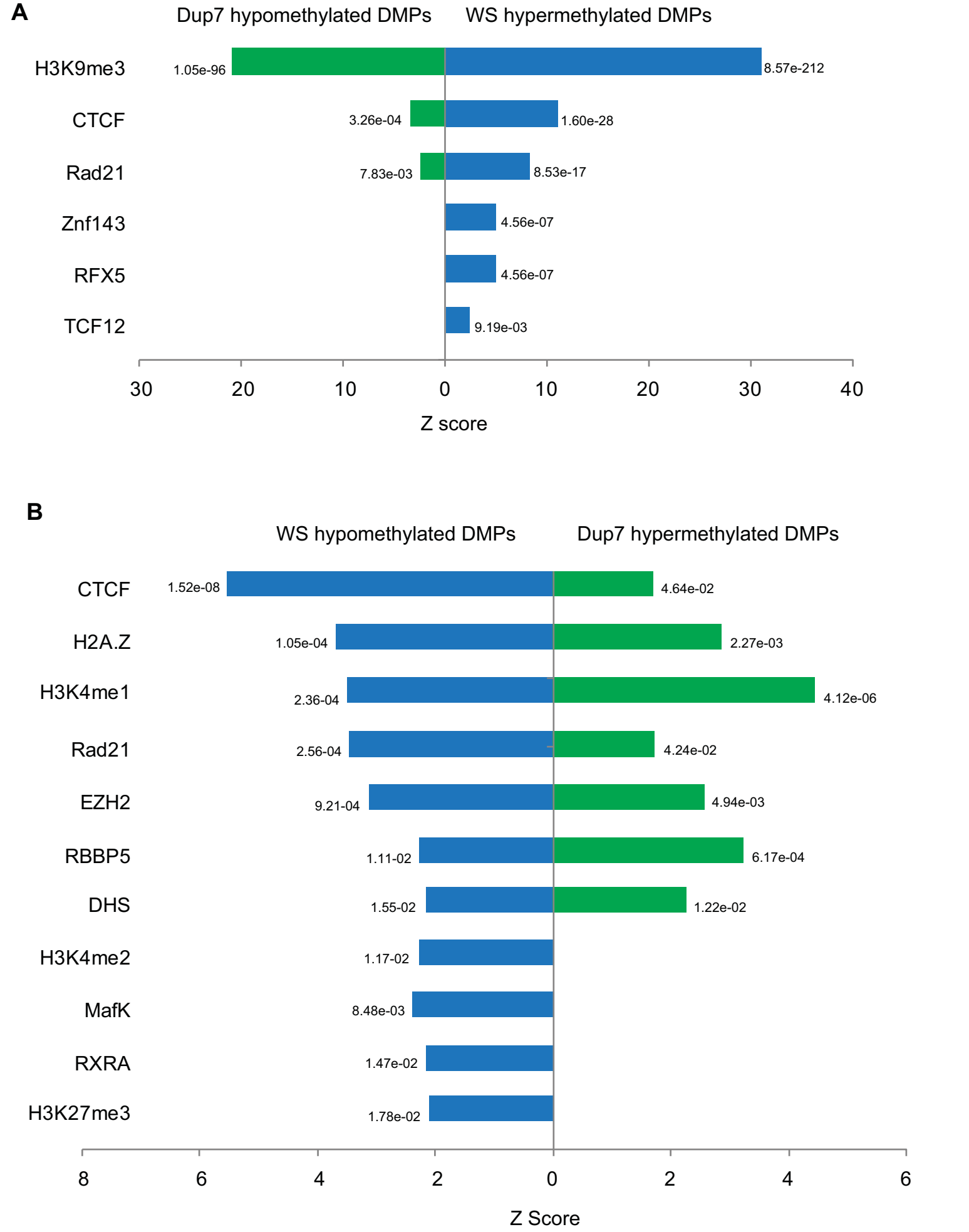

A
Dup7 hypomethylated DMPs
WS hypermethylated DMPs
H3K9me3
CTCF
Rad21
Znf143
RFX5
TCF12
1.05e-96
8.57e-212
3.26e-04
1.60e-28
7.83e-03
8.53e-17
4.56e-07
4.56e-07
9.19e-03
30
20
10
0
10
20
30
40
Z score
B
WS hypomethylated DMPs
Dup7 hypermethylated DMPs
CTCF
H2A.Z
H3K4me1
Rad21
EZH2
RBBP5
DHS
H3K4me2
MafK
RXRA
H3K27me3
1.52e-08
4.64e-02
1.05e-04
2.27e-03
2.36-04
4.12e-06
2.56-04
4.24e-02
9.21-04
4.94e-03
1.11-02
6.17e-04
1.55-02
1.22e-02
1.17-02
8.48e-03
1.47e-02
1.78e-02
8
6
4
2
0
2
4
6
Z Score

Figure S10: Enrichment analysis using ENCODE data identifies significant and dose-dependent enrichment in histone marks and transcription factor binding sites in H1-hESCs. (**A**) Significant enrichment in H3K9me3, CTCF and Rad21binding sites were identified in WS hypermethylated (blue) and Dup7 hypomethyated (green) probes, using ENCODE H1-hESCS data. Additional enrichment in binding sites for developmentally important transcription factors ZNF143, RFX5 and TCF12 were identified within the WS hypermethylated group (blue). (**B**) Significant enrichment for several transcriptional regulator binding sites and histone marks were identified in WS hypomethylated (blue) and Dup7 hypermethylated (green) probes, using ENCODE H1-hESCS data.

Table S1. Demographic information for participants at time of blood draw

Group	Mean age (years) ± SD	Range (years)	Sex	Ethnicity
WS	6.1±1.58	2.8-8.5	14 F 6 M	19 participants were of European ancestry. One participant's mother was of Asian ancestry
Dup7	7.8±2.44	4.4-10.7	5 F 5 M	Eight participants were of European ancestry. Two participants' fathers were of mixed European and Native American ancestry
TD controls	5.5±1.43	2.4-7.4	12 F 3 M	All 15 participants were of European ancestry.