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Tris(1,3-dichloro-2-propyl) phosphate Induces Genome-Wide Hypomethylation Within Early Zebrafish Embryos

38 Pages
28 Figures

Supporting Information Figure Legends

Figure S1. Representative selected-ion chromatograms (SICs) for monitoring the *m/z* 242→126 (A) and *m/z* 247→126 (B) transitions for the $[M+H]^+$ ions of the unlabeled 5-mdC and $[^{13}C_5]$-5-mdC, respectively, in the nucleoside mixture of genomic DNA extracted from zebrafish embryos. Shown in the insets are the MS^2 spectra for the unlabeled and labeled 5-mdC.

Figure S2. Representative selected-ion chromatograms (SICs) for monitoring the *m/z* 268→152→135 (A) and *m/z* 273→157→139 (B) transitions for the $[M+H]^+$ ions of the unlabeled dG and $[^{15}N_5]$-dG, respectively, in the nucleoside mixture of genomic DNA extracted from zebrafish embryos. Shown in the insets are the MS^3 spectra for the unlabeled and labeled dG.

Figure S3. Calibration curves for the quantification of 5-mdC (A) and dG (B).

Figure S4. Position-specific signal-to-noise ratios for cytosine methylation within a CpG context on **chromosome 1 (chr1)**. Following initiation of 250 μM 5-azaC or 2 μM TDCIPP exposure at 0.75 hpf, genomic DNA was extracted from 2-hpf embryos and, following bisulfite conversion, whole-genome bisulfite sequencing (WGBS) was conducted on Illumina's HiSeq 2500 platform. All SNRs are relative to vehicle controls and reflect variation among two replicates per control group and three replicates per treatment group. SNRs>0 and SNRs<0 represent hypermethylation and hypomethylation, respectively, relative to vehicle controls.

Figure S5. Position-specific signal-to-noise ratios for cytosine methylation within a CpG context on **chromosome 2 (chr2)**. Following initiation of 250 μM 5-azaC or 2 μM TDCIPP exposure at

0.75 hpf, genomic DNA was extracted from 2-hpf embryos and, following bisulfite conversion, whole-genome bisulfite sequencing (WGBS) was conducted on Illumina's HiSeq 2500 platform. All SNRs are relative to vehicle controls and reflect variation among two replicates per control group and three replicates per treatment group. SNRs>0 and SNRs<0 represent hypermethylation and hypomethylation, respectively, relative to vehicle controls.

Figure S6. Position-specific signal-to-noise ratios for cytosine methylation within a CpG context on **chromosome 3 (chr3)**. Following initiation of 250 μM 5-azaC or 2 μM TDCIPP exposure at 0.75 hpf, genomic DNA was extracted from 2-hpf embryos and, following bisulfite conversion, whole-genome bisulfite sequencing (WGBS) was conducted on Illumina's HiSeq 2500 platform. All SNRs are relative to vehicle controls and reflect variation among two replicates per control group and three replicates per treatment group. SNRs>0 and SNRs<0 represent hypermethylation and hypomethylation, respectively, relative to vehicle controls.

Figure S7. Position-specific signal-to-noise ratios for cytosine methylation within a CpG context on **chromosome 4 (chr4)**. Following initiation of 250 μM 5-azaC or 2 μM TDCIPP exposure at 0.75 hpf, genomic DNA was extracted from 2-hpf embryos and, following bisulfite conversion, whole-genome bisulfite sequencing (WGBS) was conducted on Illumina's HiSeq 2500 platform. All SNRs are relative to vehicle controls and reflect variation among two replicates per control group and three replicates per treatment group. SNRs>0 and SNRs<0 represent hypermethylation and hypomethylation, respectively, relative to vehicle controls.

Figure S8. Position-specific signal-to-noise ratios for cytosine methylation within a CpG context on **chromosome 5 (chr5)**. Following initiation of 250 μM 5-azaC or 2 μM TDCIPP exposure at 0.75 hpf, genomic DNA was extracted from 2-hpf embryos and, following bisulfite conversion, whole-genome bisulfite sequencing (WGBS) was conducted on Illumina's HiSeq 2500 platform. All SNRs are relative to vehicle controls and reflect variation among two replicates per control group and three replicates per treatment group. SNRs>0 and SNRs<0 represent hypermethylation and hypomethylation, respectively, relative to vehicle controls.

Figure S9. Position-specific signal-to-noise ratios for cytosine methylation within a CpG context on **chromosome 6 (chr6)**. Following initiation of 250 μM 5-azaC or 2 μM TDCIPP exposure at 0.75 hpf, genomic DNA was extracted from 2-hpf embryos and, following bisulfite conversion, whole-genome bisulfite sequencing (WGBS) was conducted on Illumina's HiSeq 2500 platform. All SNRs are relative to vehicle controls and reflect variation among two replicates per control group and three replicates per treatment group. SNRs>0 and SNRs<0 represent hypermethylation and hypomethylation, respectively, relative to vehicle controls.

Figure S10. Position-specific signal-to-noise ratios for cytosine methylation within a CpG context on **chromosome 7 (chr7)**. Following initiation of 250 μM 5-azaC or 2 μM TDCIPP exposure at 0.75 hpf, genomic DNA was extracted from 2-hpf embryos and, following bisulfite conversion, whole-genome bisulfite sequencing (WGBS) was conducted on Illumina's HiSeq 2500 platform. All SNRs are relative to vehicle controls and reflect variation among two replicates per control group and three replicates per treatment group. SNRs>0 and SNRs<0 represent hypermethylation and hypomethylation, respectively, relative to vehicle controls.

Figure S11. Position-specific signal-to-noise ratios for cytosine methylation within a CpG context on **chromosome 8 (chr8)**. Following initiation of 250 μM 5-azaC or 2 μM TDCIPP exposure at 0.75 hpf, genomic DNA was extracted from 2-hpf embryos and, following bisulfite conversion, whole-genome bisulfite sequencing (WGBS) was conducted on Illumina's HiSeq 2500 platform. All SNRs are relative to vehicle controls and reflect variation among two replicates per control group and three replicates per treatment group. SNRs>0 and SNRs<0 represent hypermethylation and hypomethylation, respectively, relative to vehicle controls.

Figure S12. Position-specific signal-to-noise ratios for cytosine methylation within a CpG context on **chromosome 9 (chr9)**. Following initiation of 250 μM 5-azaC or 2 μM TDCIPP exposure at 0.75 hpf, genomic DNA was extracted from 2-hpf embryos and, following bisulfite conversion, whole-genome bisulfite sequencing (WGBS) was conducted on Illumina's HiSeq 2500 platform. All SNRs are relative to vehicle controls and reflect variation among two replicates per control group and three replicates per treatment group. SNRs>0 and SNRs<0 represent hypermethylation and hypomethylation, respectively, relative to vehicle controls.

Figure S13. Position-specific signal-to-noise ratios for cytosine methylation within a CpG context on **chromosome 10 (chr10)**. Following initiation of 250 μM 5-azaC or 2 μM TDCIPP exposure at 0.75 hpf, genomic DNA was extracted from 2-hpf embryos and, following bisulfite conversion, whole-genome bisulfite sequencing (WGBS) was conducted on Illumina's HiSeq 2500 platform. All SNRs are relative to vehicle controls and reflect variation among two replicates per control

group and three replicates per treatment group. SNRs>0 and SNRs<0 represent hypermethylation and hypomethylation, respectively, relative to vehicle controls.

Figure S14. Position-specific signal-to-noise ratios for cytosine methylation within a CpG context on **chromosome 11 (chr11)**. Following initiation of 250 μM 5-azaC or 2 μM TDCIPP exposure at 0.75 hpf, genomic DNA was extracted from 2-hpf embryos and, following bisulfite conversion, whole-genome bisulfite sequencing (WGBS) was conducted on Illumina's HiSeq 2500 platform. All SNRs are relative to vehicle controls and reflect variation among two replicates per control group and three replicates per treatment group. SNRs>0 and SNRs<0 represent hypermethylation and hypomethylation, respectively, relative to vehicle controls.

Figure S15. Position-specific signal-to-noise ratios for cytosine methylation within a CpG context on **chromosome 12 (chr12)**. Following initiation of 250 μM 5-azaC or 2 μM TDCIPP exposure at 0.75 hpf, genomic DNA was extracted from 2-hpf embryos and, following bisulfite conversion, whole-genome bisulfite sequencing (WGBS) was conducted on Illumina's HiSeq 2500 platform. All SNRs are relative to vehicle controls and reflect variation among two replicates per control group and three replicates per treatment group. SNRs>0 and SNRs<0 represent hypermethylation and hypomethylation, respectively, relative to vehicle controls.

Figure S16. Position-specific signal-to-noise ratios for cytosine methylation within a CpG context on **chromosome 13 (chr13)**. Following initiation of 250 μM 5-azaC or 2 μM TDCIPP exposure at 0.75 hpf, genomic DNA was extracted from 2-hpf embryos and, following bisulfite conversion, whole-genome bisulfite sequencing (WGBS) was conducted on Illumina's HiSeq 2500 platform.

All SNRs are relative to vehicle controls and reflect variation among two replicates per control group and three replicates per treatment group. SNRs>0 and SNRs<0 represent hypermethylation and hypomethylation, respectively, relative to vehicle controls.

Figure S17. Position-specific signal-to-noise ratios for cytosine methylation within a CpG context on **chromosome 14 (chr14)**. Following initiation of 250 μM 5-azaC or 2 μM TDCIPP exposure at 0.75 hpf, genomic DNA was extracted from 2-hpf embryos and, following bisulfite conversion, whole-genome bisulfite sequencing (WGBS) was conducted on Illumina's HiSeq 2500 platform. All SNRs are relative to vehicle controls and reflect variation among two replicates per control group and three replicates per treatment group. SNRs>0 and SNRs<0 represent hypermethylation and hypomethylation, respectively, relative to vehicle controls.

Figure S18. Position-specific signal-to-noise ratios for cytosine methylation within a CpG context on **chromosome 15 (chr15)**. Following initiation of 250 μM 5-azaC or 2 μM TDCIPP exposure at 0.75 hpf, genomic DNA was extracted from 2-hpf embryos and, following bisulfite conversion, whole-genome bisulfite sequencing (WGBS) was conducted on Illumina's HiSeq 2500 platform. All SNRs are relative to vehicle controls and reflect variation among two replicates per control group and three replicates per treatment group. SNRs>0 and SNRs<0 represent hypermethylation and hypomethylation, respectively, relative to vehicle controls.

Figure S19. Position-specific signal-to-noise ratios for cytosine methylation within a CpG context on **chromosome 16 (chr16)**. Following initiation of 250 μM 5-azaC or 2 μM TDCIPP exposure at 0.75 hpf, genomic DNA was extracted from 2-hpf embryos and, following bisulfite conversion,

whole-genome bisulfite sequencing (WGBS) was conducted on Illumina's HiSeq 2500 platform. All SNRs are relative to vehicle controls and reflect variation among two replicates per control group and three replicates per treatment group. SNRs>0 and SNRs<0 represent hypermethylation and hypomethylation, respectively, relative to vehicle controls.

Figure S20. Position-specific signal-to-noise ratios for cytosine methylation within a CpG context on **chromosome 17 (chr17)**. Following initiation of 250 μM 5-azaC or 2 μM TDCIPP exposure at 0.75 hpf, genomic DNA was extracted from 2-hpf embryos and, following bisulfite conversion, whole-genome bisulfite sequencing (WGBS) was conducted on Illumina's HiSeq 2500 platform. All SNRs are relative to vehicle controls and reflect variation among two replicates per control group and three replicates per treatment group. SNRs>0 and SNRs<0 represent hypermethylation and hypomethylation, respectively, relative to vehicle controls.

Figure S21. Position-specific signal-to-noise ratios for cytosine methylation within a CpG context on **chromosome 18 (chr18)**. Following initiation of 250 μM 5-azaC or 2 μM TDCIPP exposure at 0.75 hpf, genomic DNA was extracted from 2-hpf embryos and, following bisulfite conversion, whole-genome bisulfite sequencing (WGBS) was conducted on Illumina's HiSeq 2500 platform. All SNRs are relative to vehicle controls and reflect variation among two replicates per control group and three replicates per treatment group. SNRs>0 and SNRs<0 represent hypermethylation and hypomethylation, respectively, relative to vehicle controls.

Figure S22. Position-specific signal-to-noise ratios for cytosine methylation within a CpG context on **chromosome 19 (chr19)**. Following initiation of 250 μM 5-azaC or 2 μM TDCIPP exposure

at 0.75 hpf, genomic DNA was extracted from 2-hpf embryos and, following bisulfite conversion, whole-genome bisulfite sequencing (WGBS) was conducted on Illumina's HiSeq 2500 platform. All SNRs are relative to vehicle controls and reflect variation among two replicates per control group and three replicates per treatment group. SNRs>0 and SNRs<0 represent hypermethylation and hypomethylation, respectively, relative to vehicle controls.

Figure S23. Position-specific signal-to-noise ratios for cytosine methylation within a CpG context on **chromosome 20 (chr20)**. Following initiation of 250 μM 5-azaC or 2 μM TDCIPP exposure at 0.75 hpf, genomic DNA was extracted from 2-hpf embryos and, following bisulfite conversion, whole-genome bisulfite sequencing (WGBS) was conducted on Illumina's HiSeq 2500 platform. All SNRs are relative to vehicle controls and reflect variation among two replicates per control group and three replicates per treatment group. SNRs>0 and SNRs<0 represent hypermethylation and hypomethylation, respectively, relative to vehicle controls.

Figure S24. Position-specific signal-to-noise ratios for cytosine methylation within a CpG context on **chromosome 21 (chr21)**. Following initiation of 250 μM 5-azaC or 2 μM TDCIPP exposure at 0.75 hpf, genomic DNA was extracted from 2-hpf embryos and, following bisulfite conversion, whole-genome bisulfite sequencing (WGBS) was conducted on Illumina's HiSeq 2500 platform. All SNRs are relative to vehicle controls and reflect variation among two replicates per control group and three replicates per treatment group. SNRs>0 and SNRs<0 represent hypermethylation and hypomethylation, respectively, relative to vehicle controls.

Figure S25. Position-specific signal-to-noise ratios for cytosine methylation within a CpG context on **chromosome 22 (chr22)**. Following initiation of 250 μM 5-azaC or 2 μM TDCIPP exposure at 0.75 hpf, genomic DNA was extracted from 2-hpf embryos and, following bisulfite conversion, whole-genome bisulfite sequencing (WGBS) was conducted on Illumina's HiSeq 2500 platform. All SNRs are relative to vehicle controls and reflect variation among two replicates per control group and three replicates per treatment group. SNRs>0 and SNRs<0 represent hypermethylation and hypomethylation, respectively, relative to vehicle controls.

Figure S26. Position-specific signal-to-noise ratios for cytosine methylation within a CpG context on **chromosome 23 (chr23)**. Following initiation of 250 μM 5-azaC or 2 μM TDCIPP exposure at 0.75 hpf, genomic DNA was extracted from 2-hpf embryos and, following bisulfite conversion, whole-genome bisulfite sequencing (WGBS) was conducted on Illumina's HiSeq 2500 platform. All SNRs are relative to vehicle controls and reflect variation among two replicates per control group and three replicates per treatment group. SNRs>0 and SNRs<0 represent hypermethylation and hypomethylation, respectively, relative to vehicle controls.

Figure S27. Position-specific signal-to-noise ratios for cytosine methylation within a CpG context on **chromosome 24 (chr24)**. Following initiation of 250 μM 5-azaC or 2 μM TDCIPP exposure at 0.75 hpf, genomic DNA was extracted from 2-hpf embryos and, following bisulfite conversion, whole-genome bisulfite sequencing (WGBS) was conducted on Illumina's HiSeq 2500 platform. All SNRs are relative to vehicle controls and reflect variation among two replicates per control group and three replicates per treatment group. SNRs>0 and SNRs<0 represent hypermethylation and hypomethylation, respectively, relative to vehicle controls.

Figure S28. Position-specific signal-to-noise ratios for cytosine methylation within a CpG context on **chromosome 25 (chr25)**. Following initiation of 250 μM 5-azaC or 2 μM TDCIPP exposure at 0.75 hpf, genomic DNA was extracted from 2-hpf embryos and, following bisulfite conversion, whole-genome bisulfite sequencing (WGBS) was conducted on Illumina's HiSeq 2500 platform. All SNRs are relative to vehicle controls and reflect variation among two replicates per control group and three replicates per treatment group. SNRs>0 and SNRs<0 represent hypermethylation and hypomethylation, respectively, relative to vehicle controls.

Figure S1.

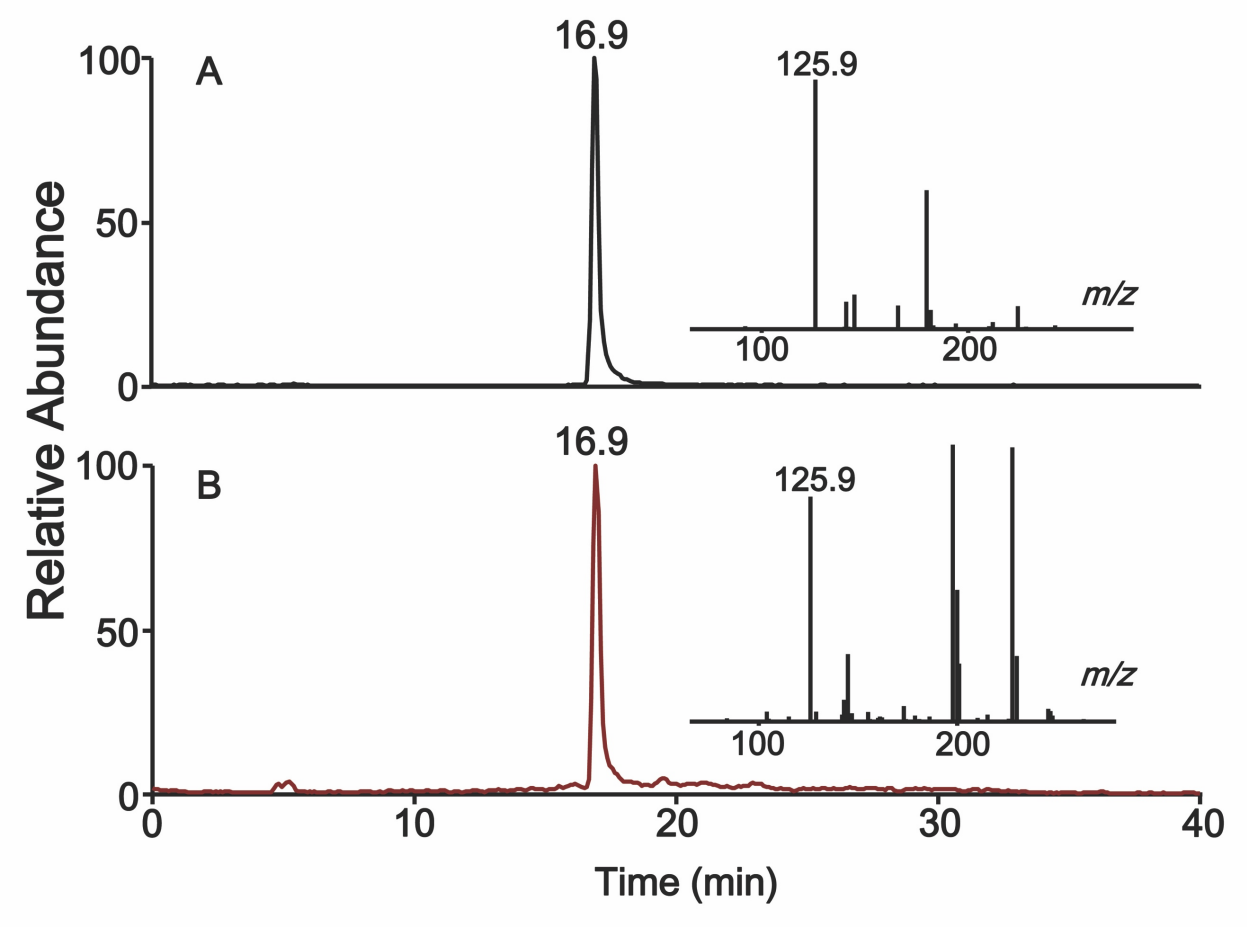

A
B
16.9
125.9
m/z
100
200
Relative Abundance
100
50
0
0
10
20
30
40
Time (min)

Figure S2.

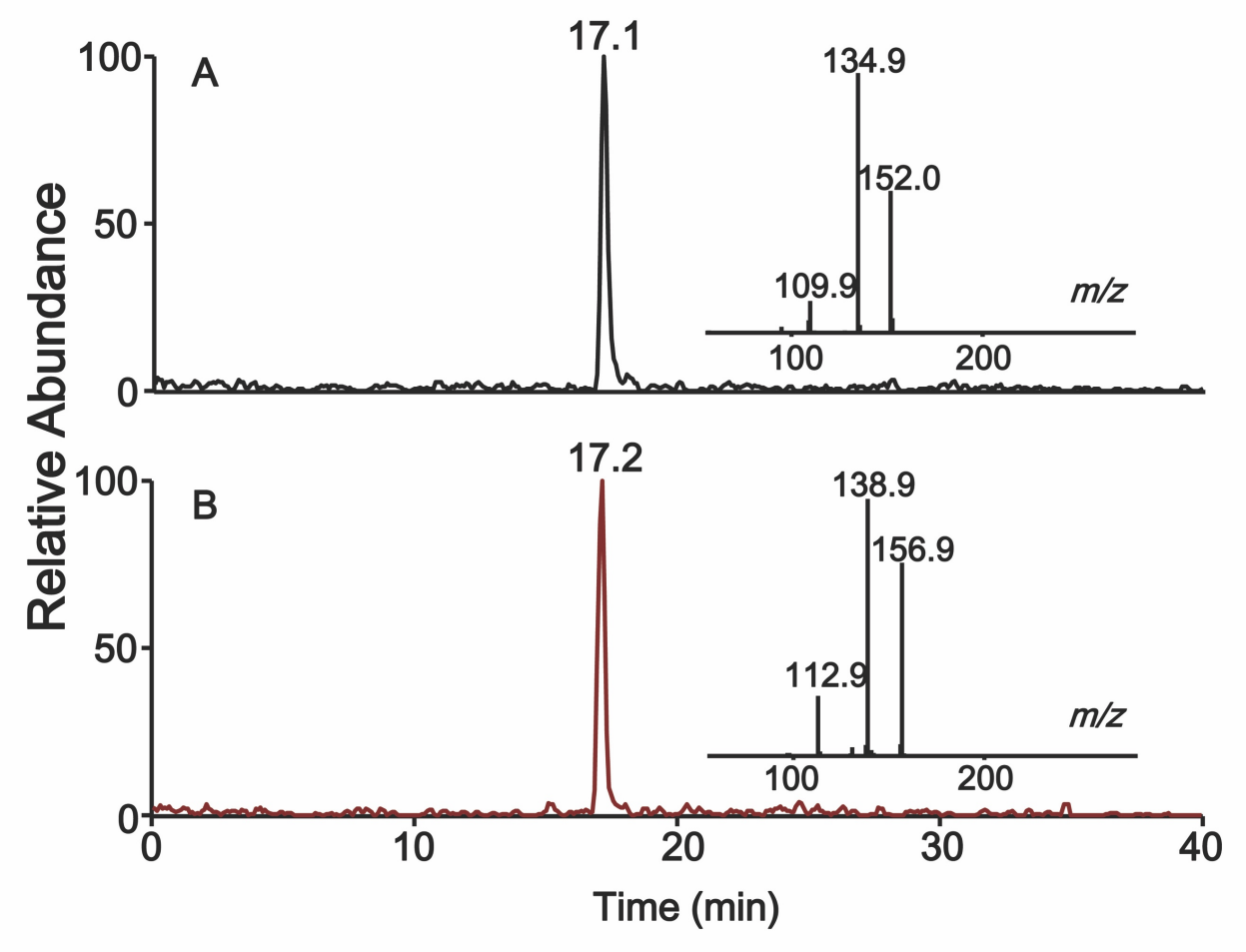

A
17.1
134.9
152.0
109.9
m/z
100
200
B
17.2
138.9
156.9
112.9
m/z
100
200
Relative Abundance
100
50
0
0
10
20
30
40
Time (min)

Figure S3.

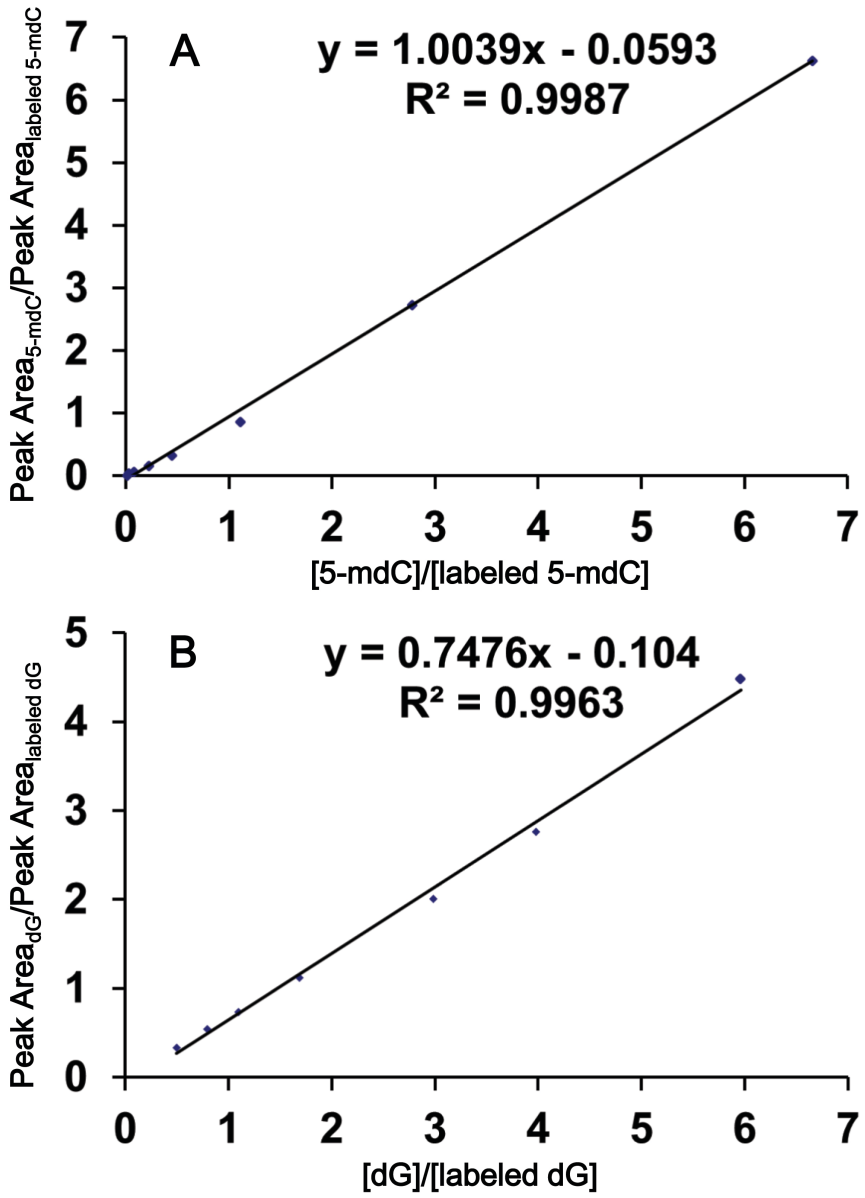

A
y = 1.0039x - 0.0593
R² = 0.9987
Peak Area5-mdC/Peak Arealabeled 5-mdC
[5-mdC]/[labeled 5-mdC]
B
y = 0.7476x - 0.104
R² = 0.9963
Peak AreadG/Peak Arealabeled dG
[dG]/[labeled dG]

Figure S4.

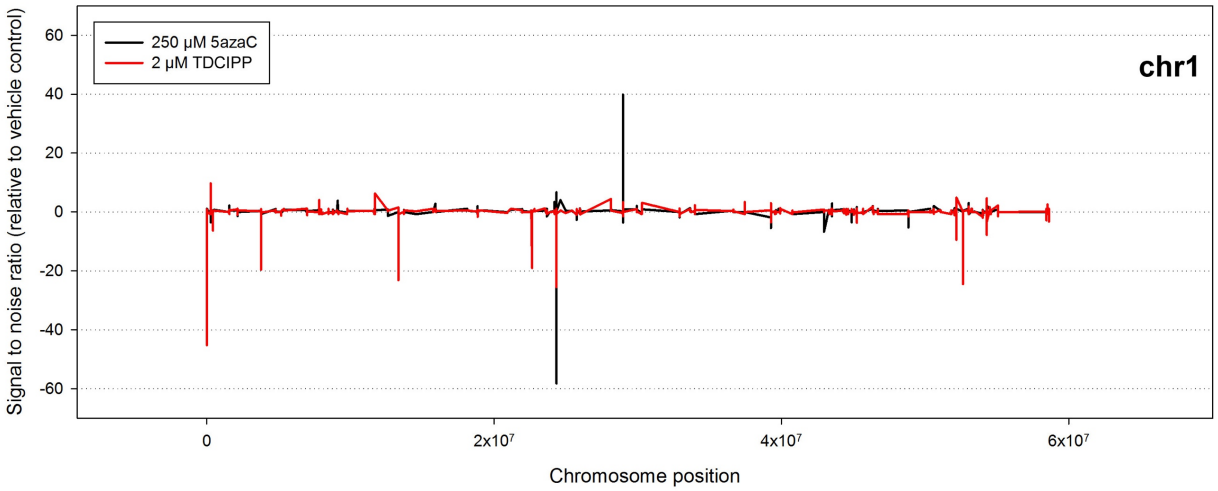

250 µM 5azaC
2 µM TDCIPP
chr1
Signal to noise ratio (relative to vehicle control)
60
40
20
0
-20
-40
-60
0
2x10⁷
4x10⁷
6x10⁷
Chromosome position

Figure S5.

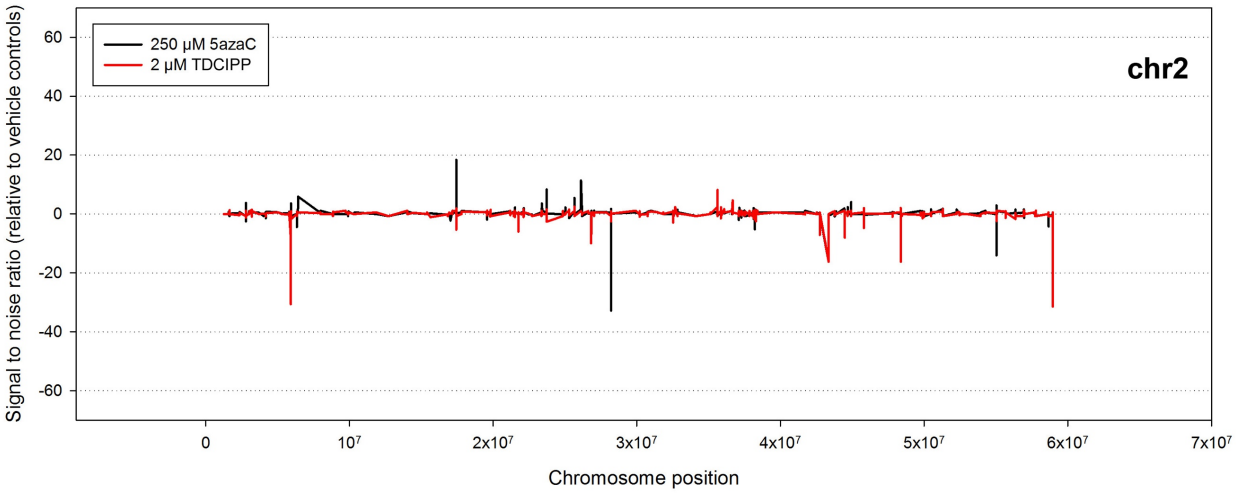

250 µM 5azaC
2 µM TDCIPP
chr2
Signal to noise ratio (relative to vehicle controls)
60
40
20
0
-20
-40
-60
0
10^7
2x10^7
3x10^7
4x10^7
5x10^7
6x10^7
7x10^7
Chromosome position

Figure S6.

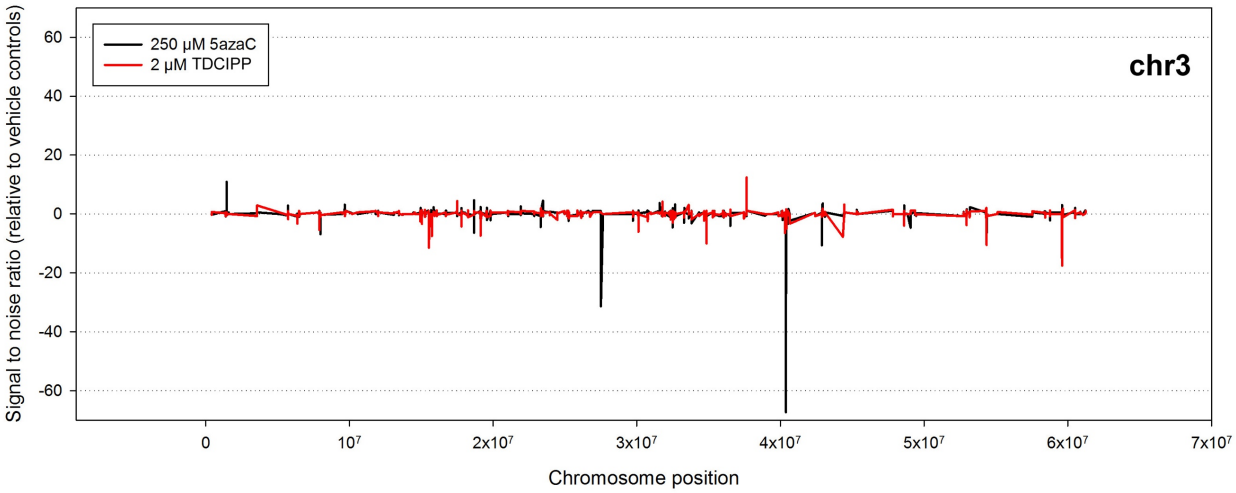

250 µM 5azaC
2 µM TDCIPP
chr3
Signal to noise ratio (relative to vehicle controls)
60
40
20
0
-20
-40
-60
0
10^7
2x10^7
3x10^7
4x10^7
5x10^7
6x10^7
7x10^7
Chromosome position

Figure S7.

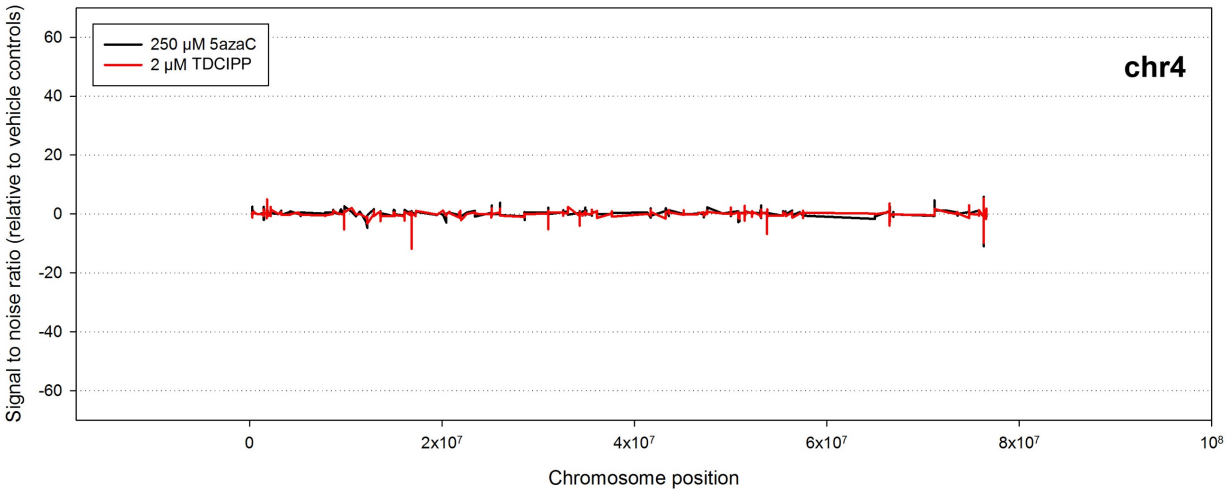

250 µM 5azaC
2 µM TDCIPP
chr4
Signal to noise ratio (relative to vehicle controls)
60
40
20
0
-20
-40
-60
0
2x10^7
4x10^7
6x10^7
8x10^7
10^8
Chromosome position

Figure S8.

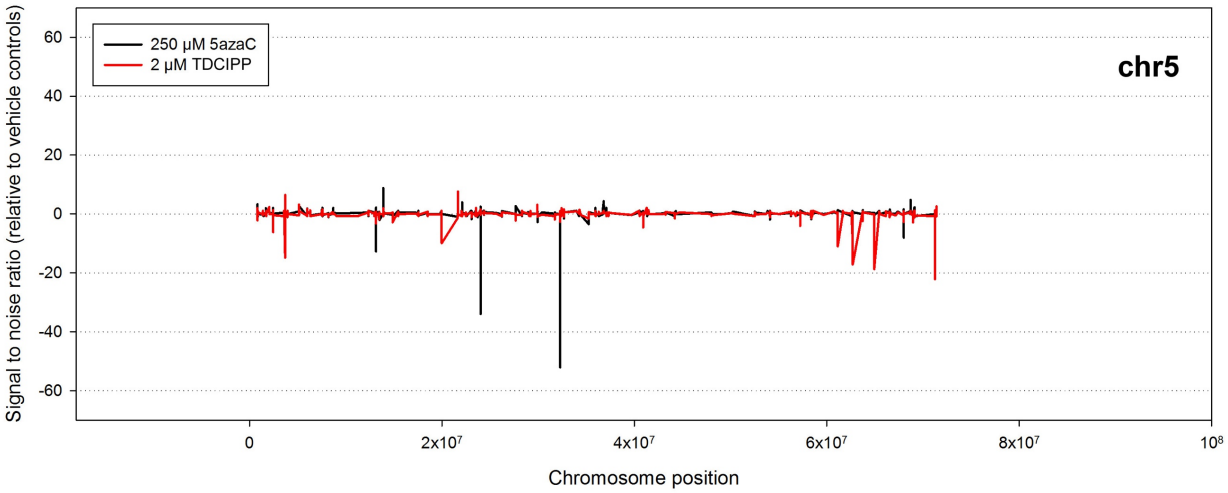

250 µM 5azaC
2 µM TDCIPP
chr5
Signal to noise ratio (relative to vehicle controls)
60
40
20
0
-20
-40
-60
0
2x10⁷
4x10⁷
6x10⁷
8x10⁷
10⁸
Chromosome position

Figure S9.

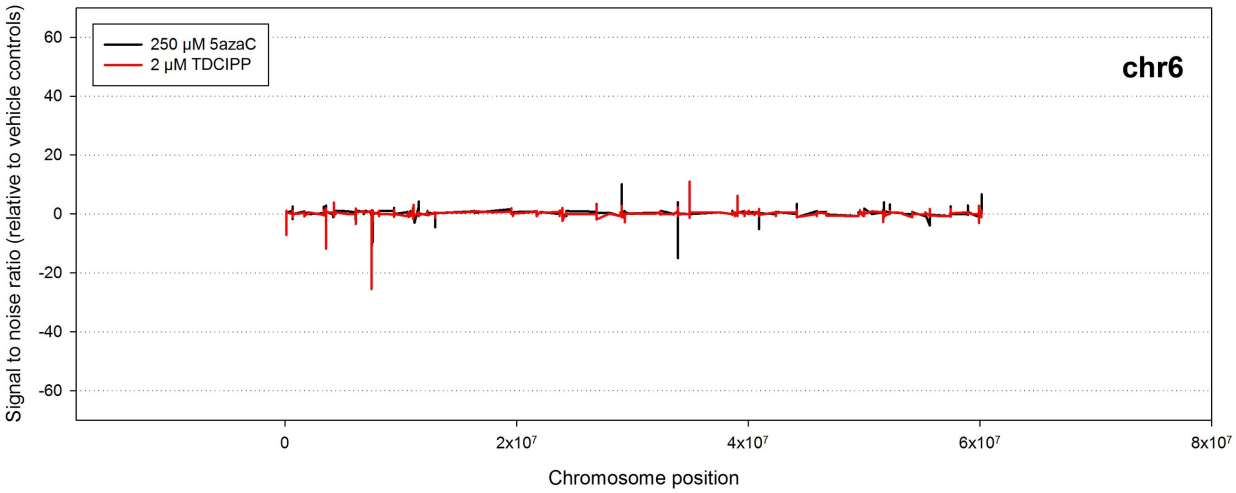

250 µM 5azaC
2 µM TDCIPP
chr6
Signal to noise ratio (relative to vehicle controls)
60
40
20
0
-20
-40
-60
0
2x10^7
4x10^7
6x10^7
8x10^7
Chromosome position

Figure S10.

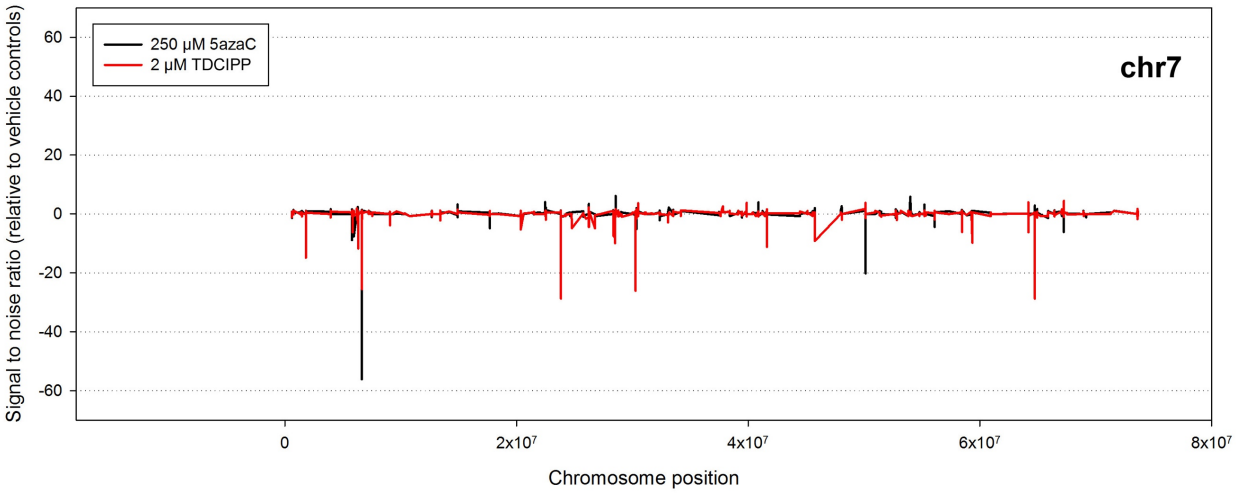

250 µM 5azaC
2 µM TDCIPP
chr7
Signal to noise ratio (relative to vehicle controls)
60
40
20
0
-20
-40
-60
0
2x10^7
4x10^7
6x10^7
8x10^7
Chromosome position

Figure S11.

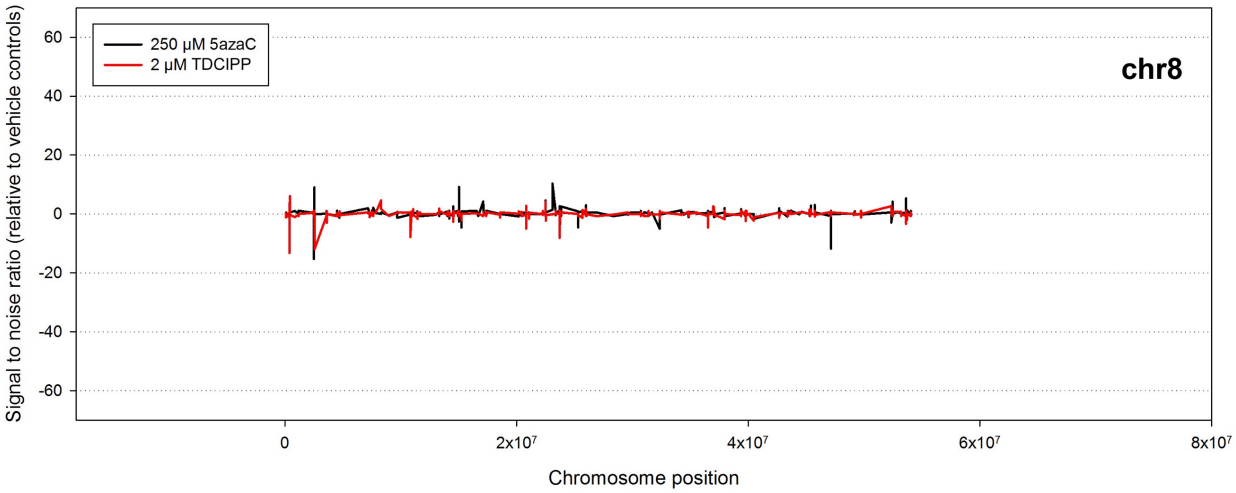

250 µM 5azaC
2 µM TDCIPP
chr8
Signal to noise ratio (relative to vehicle controls)
60
40
20
0
-20
-40
-60
0
2x10^7
4x10^7
6x10^7
8x10^7
Chromosome position

Figure S12.

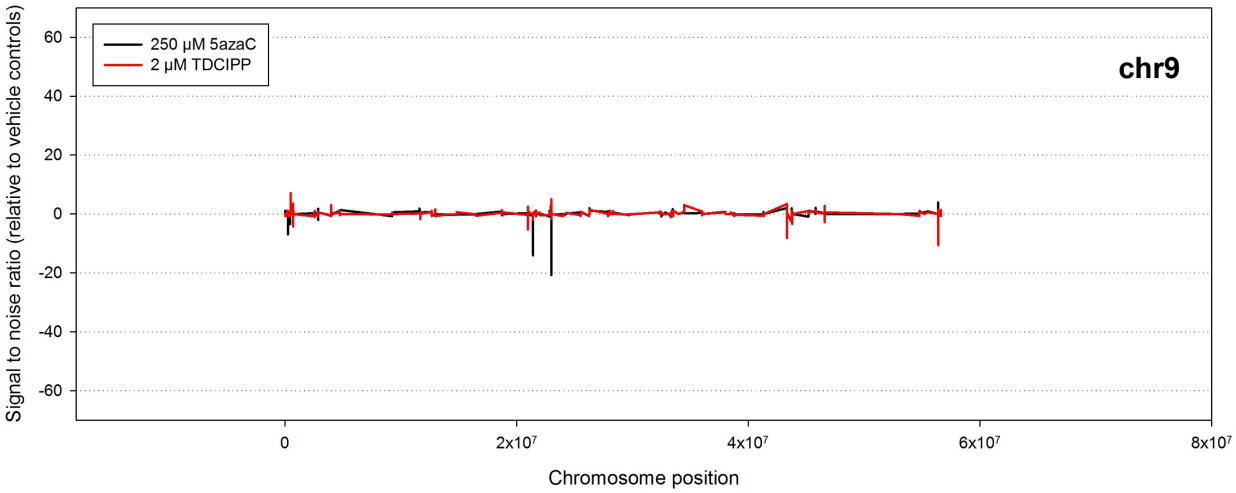

250 µM 5azaC
2 µM TDCIPP
chr9
Signal to noise ratio (relative to vehicle controls)
60
40
20
0
-20
-40
-60
0
2x10^7
4x10^7
6x10^7
8x10^7
Chromosome position

Figure S13.

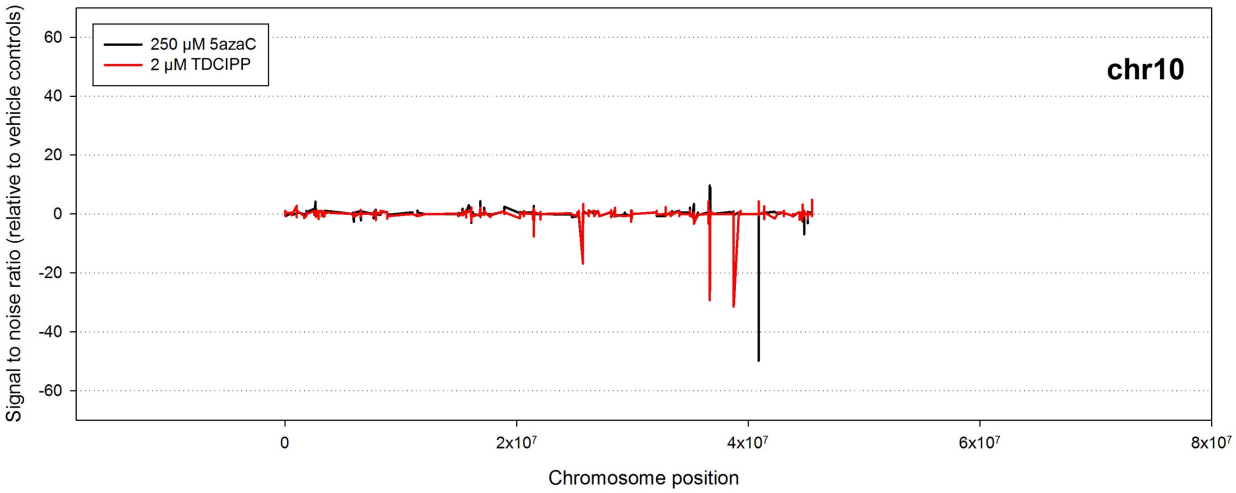

250 µM 5azaC
2 µM TDCIPP
chr10
Signal to noise ratio (relative to vehicle controls)
60
40
20
0
-20
-40
-60
0
2x10^7
4x10^7
6x10^7
8x10^7
Chromosome position

Figure S14.

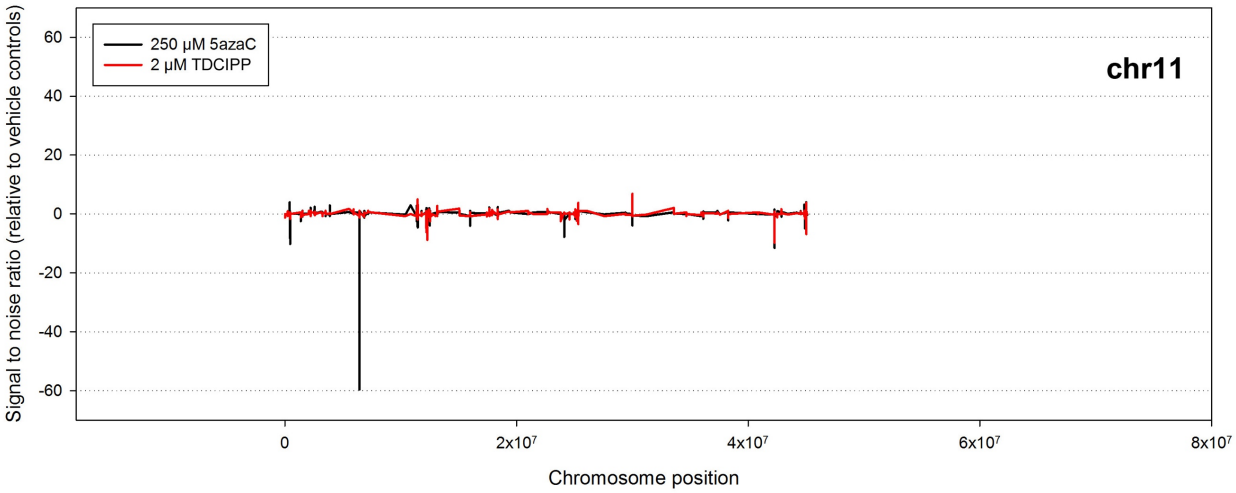
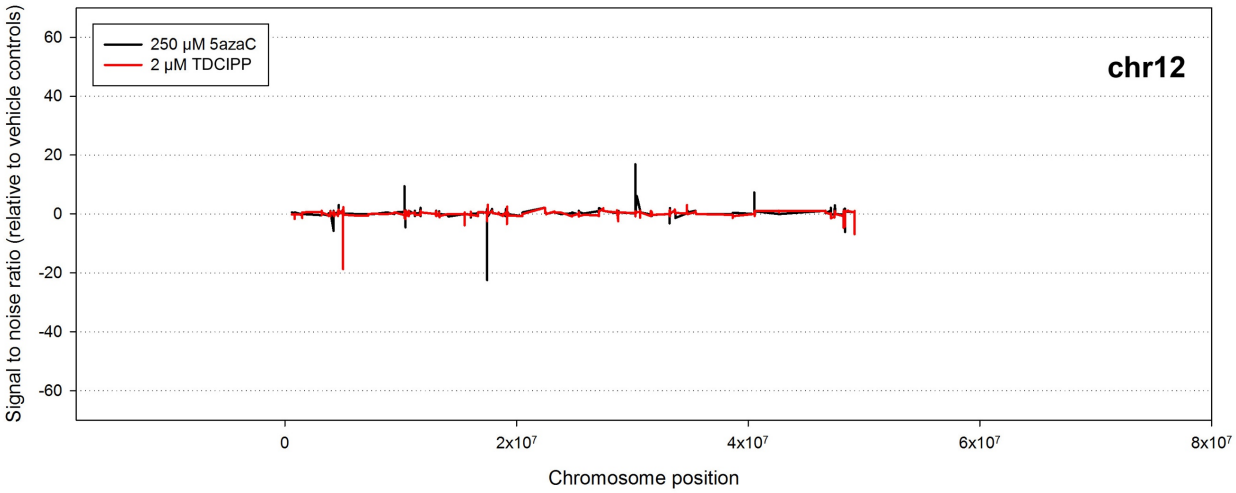

250 µM 5azaC
2 µM TDCIPP
chr11
Signal to noise ratio (relative to vehicle controls)
60
40
20
0
-20
-40
-60
0
2x10^7
4x10^7
6x10^7
8x10^7
Chromosome position

Figure S15.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2 µM TDCIPP
chr12
Signal to noise ratio (relative to vehicle controls)
60
40
20
0
-20
-40
-60
0
2x10^7
4x10^7
6x10^7
8x10^7
Chromosome position

Figure S16.

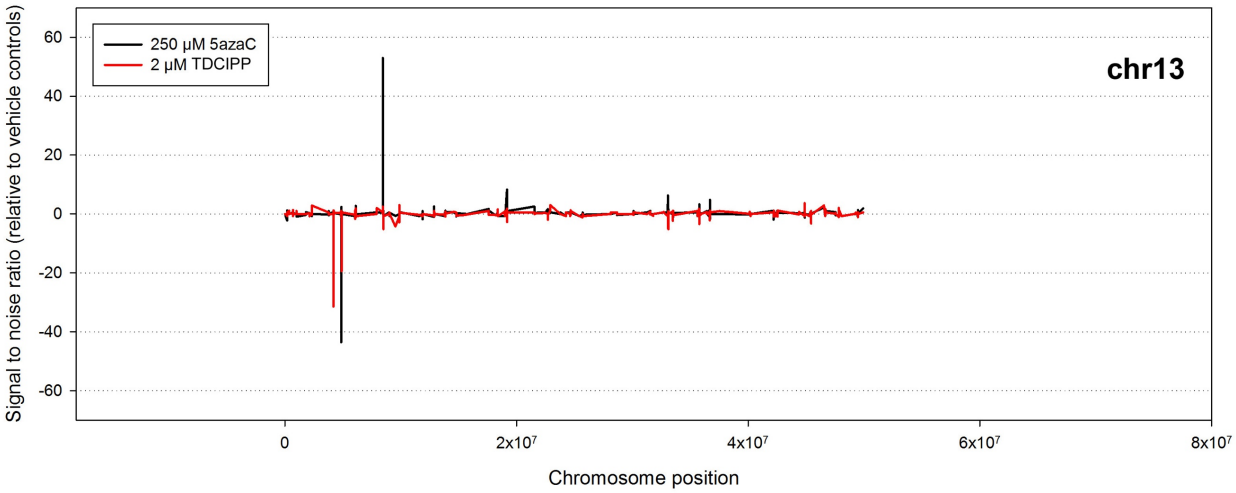

250 µM 5azaC
2 µM TDCIPP
chr13
Signal to noise ratio (relative to vehicle controls)
60
40
20
0
-20
-40
-60
0
2x10⁷
4x10⁷
6x10⁷
8x10⁷
Chromosome position

Figure S17.

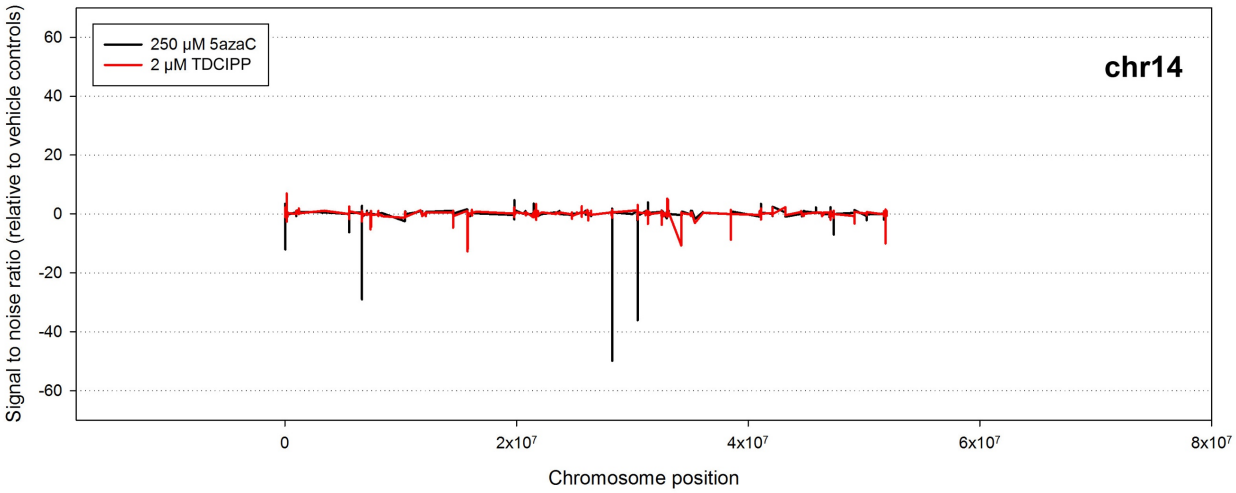

250 µM 5azaC
2 µM TDCIPP
chr14
Signal to noise ratio (relative to vehicle controls)
60
40
20
0
-20
-40
-60
0
2x10^7
4x10^7
6x10^7
8x10^7
Chromosome position

Figure S18.

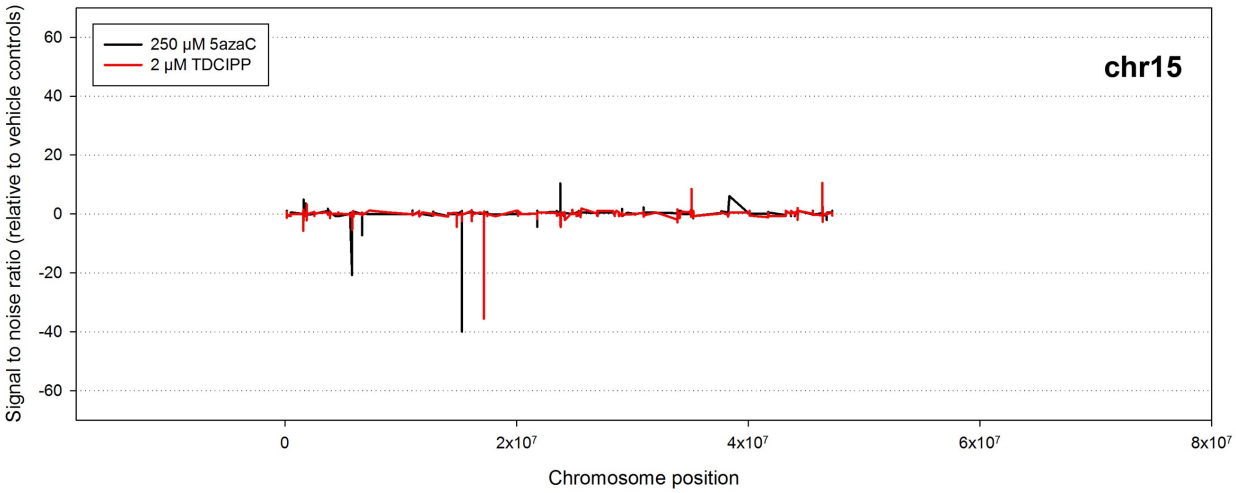

250 µM 5azaC
2 µM TDCIPP
chr15
Signal to noise ratio (relative to vehicle controls)
60
40
20
0
-20
-40
-60
0
2x10^7
4x10^7
6x10^7
8x10^7
Chromosome position

Figure S19.

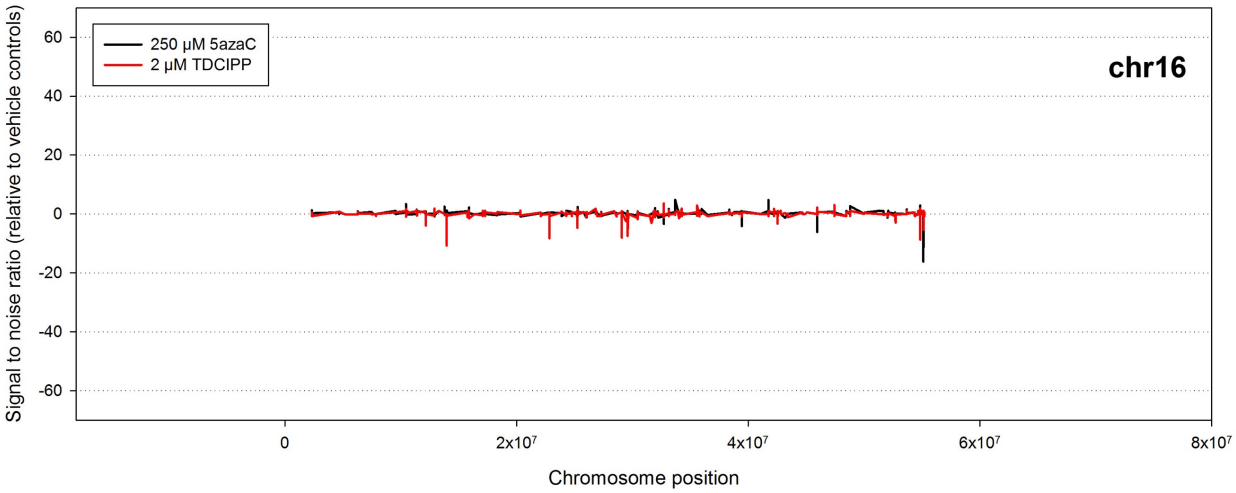

250 µM 5azaC
2 µM TDCIPP
chr16
Signal to noise ratio (relative to vehicle controls)
60
40
20
0
-20
-40
-60
0
2x10^7
4x10^7
6x10^7
8x10^7
Chromosome position

Figure S20.

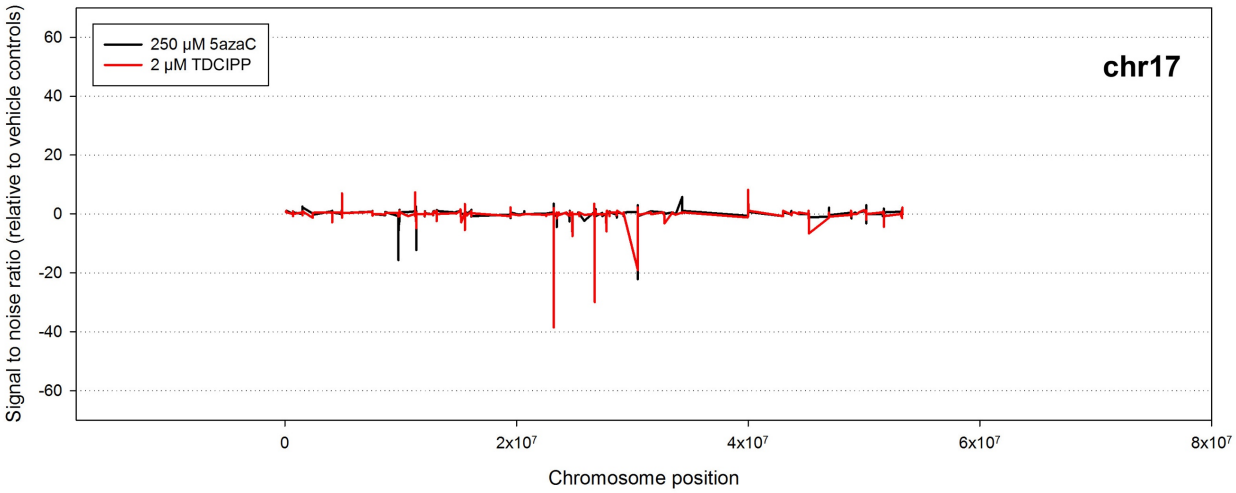

250 µM 5azaC
2 µM TDCIPP
chr17
Signal to noise ratio (relative to vehicle controls)
60
40
20
0
-20
-40
-60
0
2x10^7
4x10^7
6x10^7
8x10^7
Chromosome position

Figure S21.

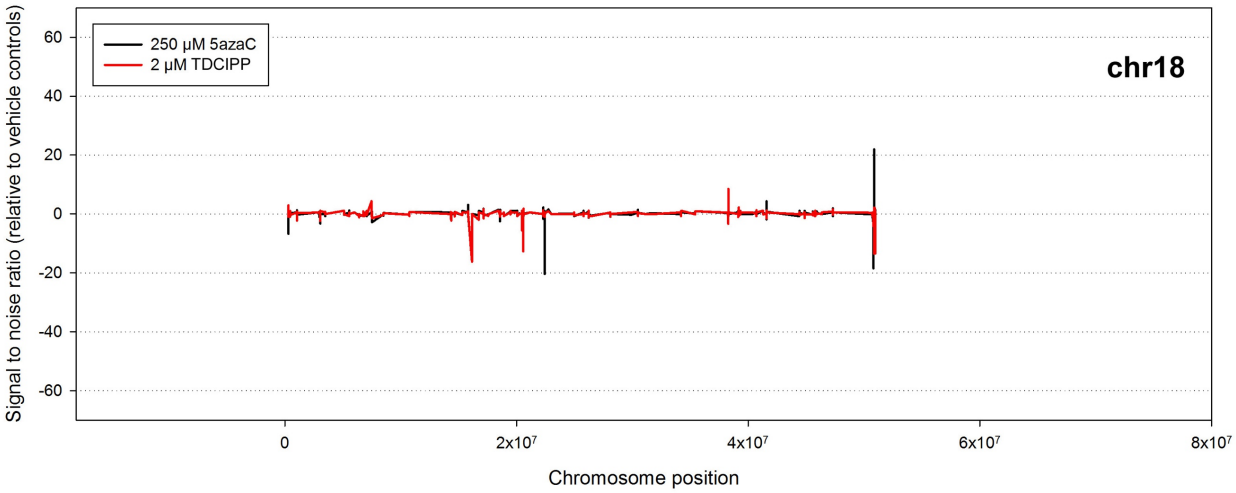

250 µM 5azaC
2 µM TDCIPP
chr18
Signal to noise ratio (relative to vehicle controls)
60
40
20
0
-20
-40
-60
0
2x10^7
4x10^7
6x10^7
8x10^7
Chromosome position

Figure S22.

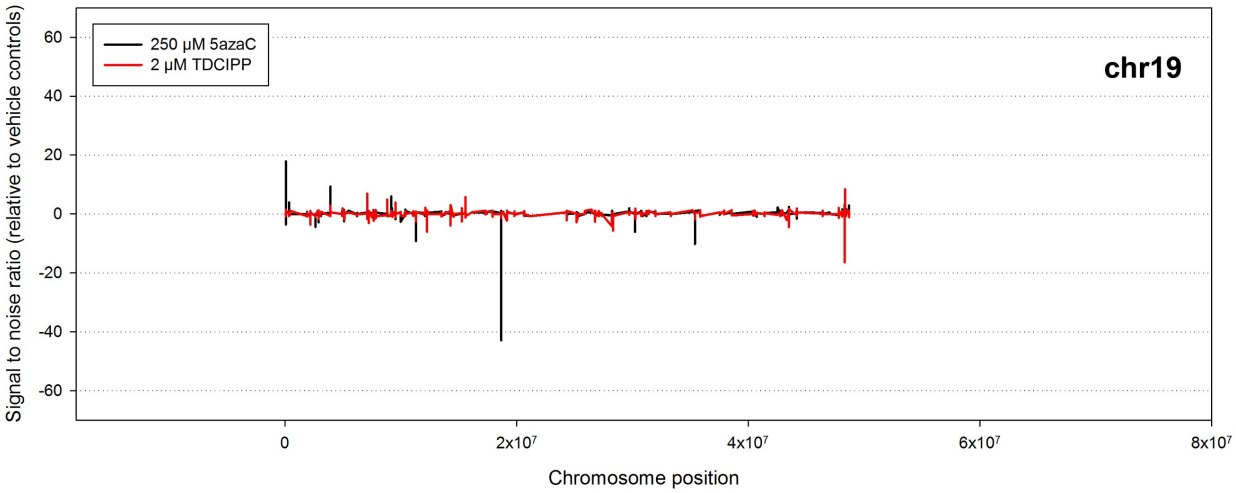

250 µM 5azaC
2 µM TDCIPP
chr19
Signal to noise ratio (relative to vehicle controls)
60
40
20
0
-20
-40
-60
0
2x10⁷
4x10⁷
6x10⁷
8x10⁷
Chromosome position

Figure S23.

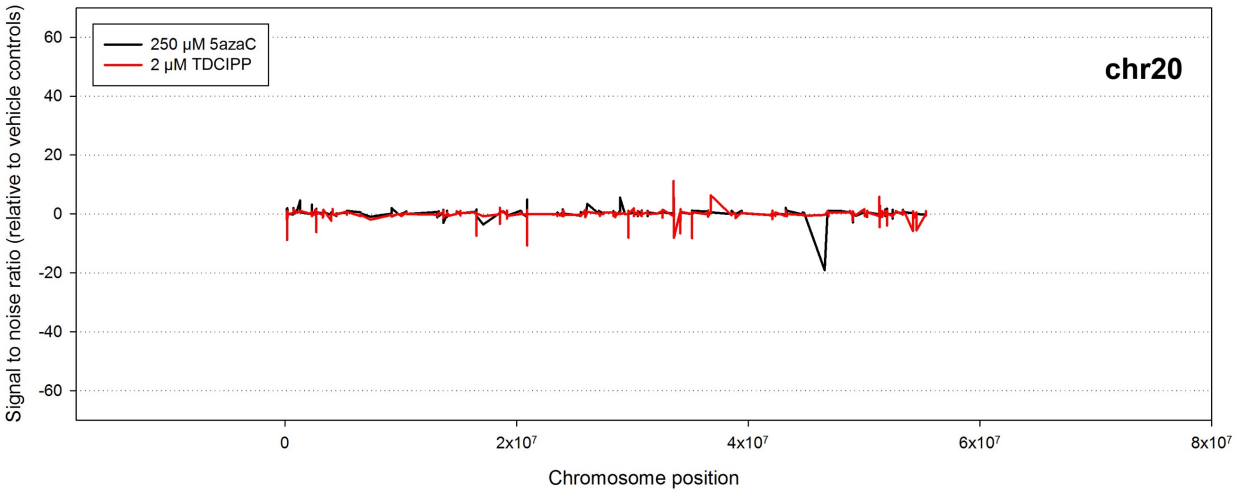

250 µM 5azaC
2 µM TDCIPP
chr20
Signal to noise ratio (relative to vehicle controls)
60
40
20
0
-20
-40
-60
0
2x10^7
4x10^7
6x10^7
8x10^7
Chromosome position

Figure S24.

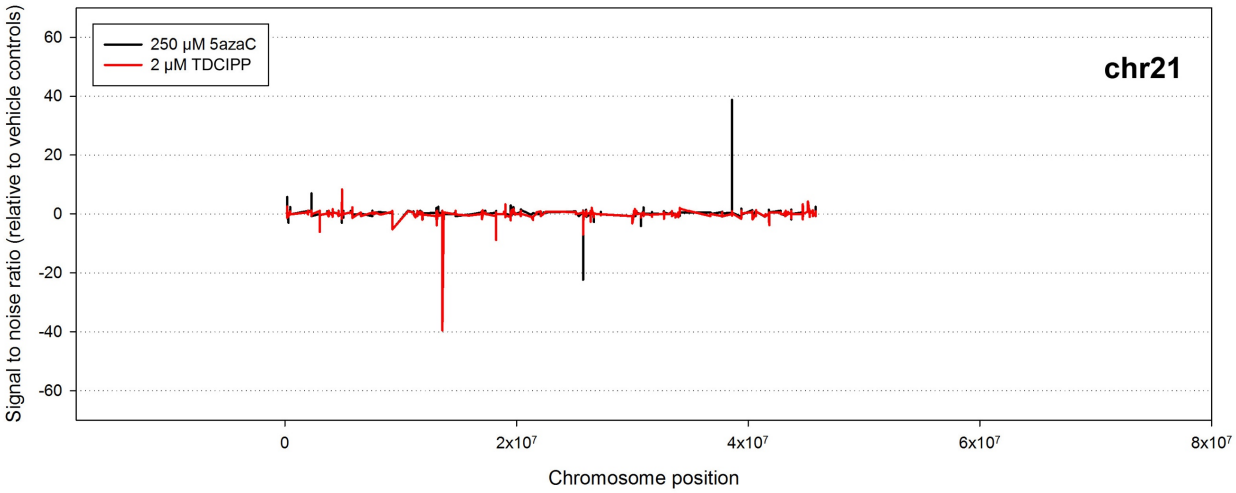

250 µM 5azaC
2 µM TDCIPP
chr21
Signal to noise ratio (relative to vehicle controls)
60
40
20
0
-20
-40
-60
0
2x10^7
4x10^7
6x10^7
8x10^7
Chromosome position

Figure S25.

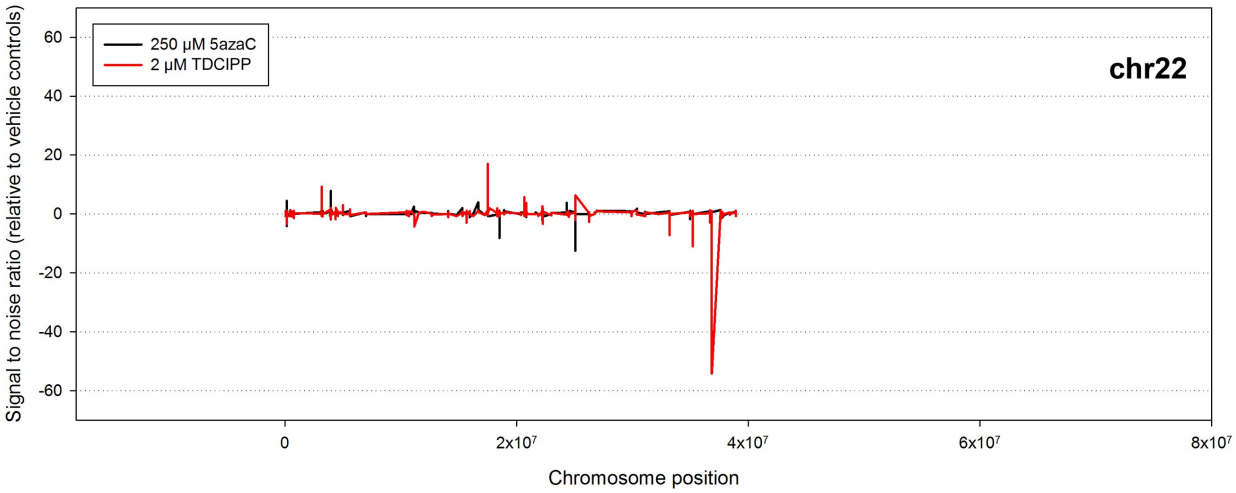

250 µM 5azaC
2 µM TDCIPP
chr22
Signal to noise ratio (relative to vehicle controls)
60
40
20
0
-20
-40
-60
0
2x10^7
4x10^7
6x10^7
8x10^7
Chromosome position

Figure S26.

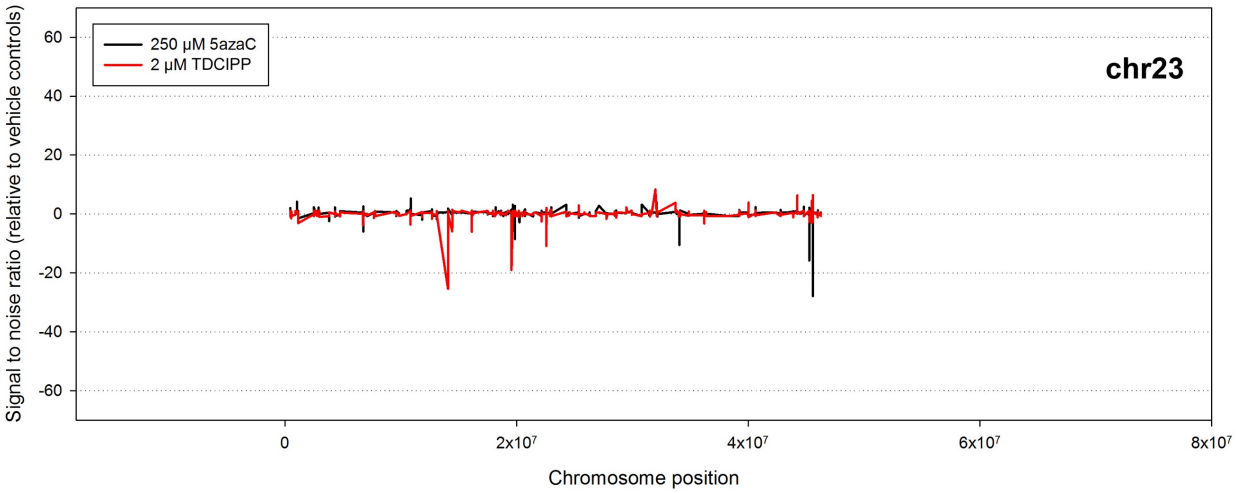

250 µM 5azaC
2 µM TDCIPP
chr23
Signal to noise ratio (relative to vehicle controls)
60
40
20
0
-20
-40
-60
0
2x10^7
4x10^7
6x10^7
8x10^7
Chromosome position

Figure S27.

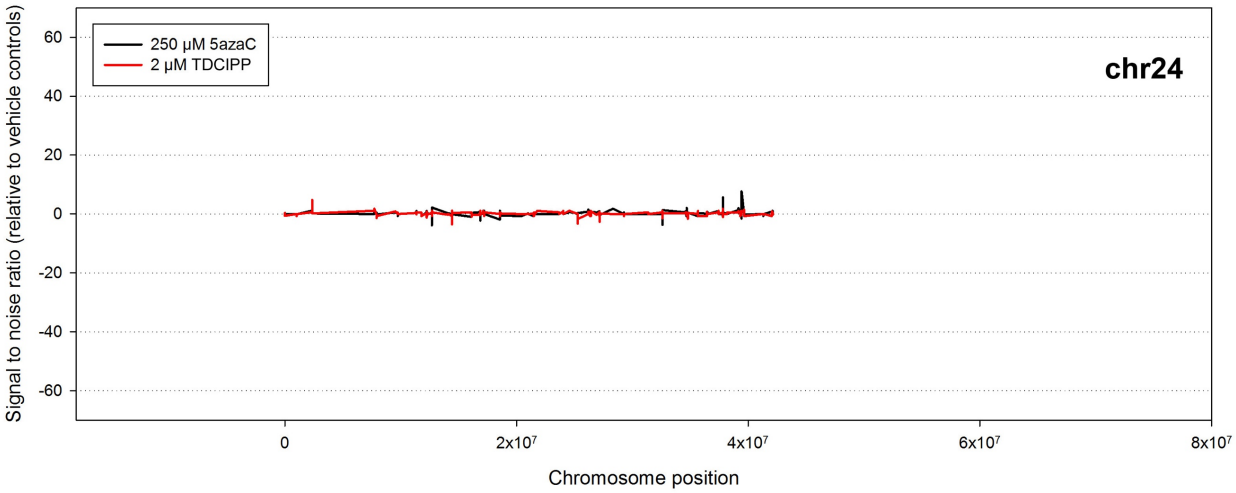
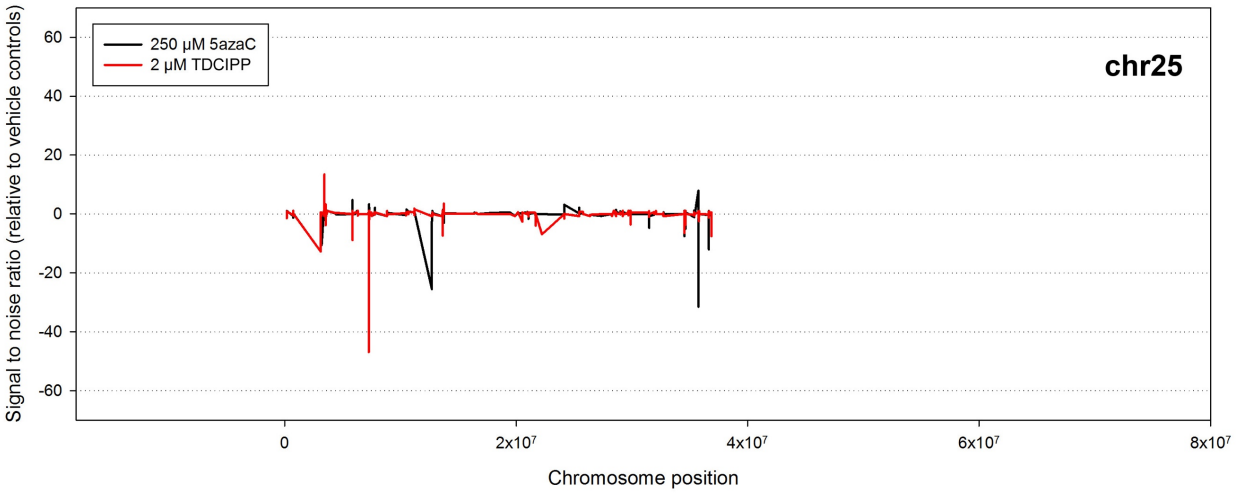

250 µM 5azaC
2 µM TDCIPP
chr24
Signal to noise ratio (relative to vehicle controls)
60
40
20
0
-20
-40
-60
0
2x10^7
4x10^7
6x10^7
8x10^7
Chromosome position

Figure S28.


250 µM 5azaC
2 µM TDCIPP
chr25
Signal to noise ratio (relative to vehicle controls)
60
40
20
0
-20
-40
-60
0
2x10^7
4x10^7
6x10^7
8x10^7
Chromosome position