Supplementary Information

Supplementary Table1. Methylation across well-characterized, imprinted germline ASMs
Excel File

Supplementary Table 2. List of 2468 NORED regions
Excel File

Supplementary Table 3. List of 2487 MethylMosaic bimodal regions
Excel File

Supplementary Table 4. Gene set enrichment for 2468 NORED regions
Excel File

Supplementary Table 5. Gene set enrichment for 2487 MethylMosaic bimodal regions
Excel File

Supplementary Table 6. Gene set enrichment for 207 bimodal NORED regions
Excel File

Supplementary Table 7. Gene set enrichment for 2261 NORED exclusive regions
Excel File

Supplementary Table 8. Gene set enrichment for 2335 MethylMosaic bimodal exclusive regions
Excel File

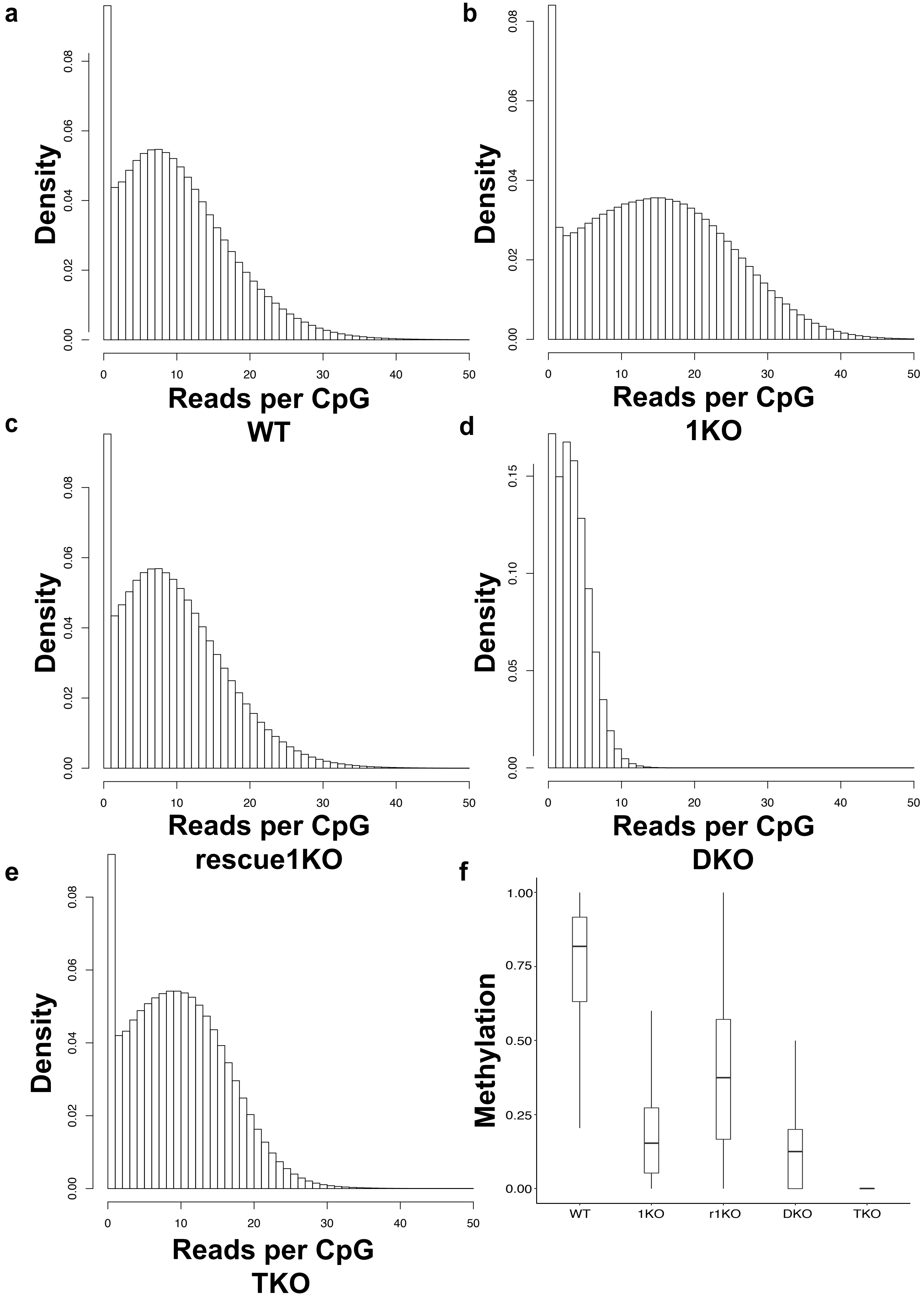
Supplementary Figure 1. Coverage and methylation level for WT, 1KO, r1KO, DKO, and TKO ESCs. (a - e) Coverage per CpG site in WT (a), 1KO (b), r1KO (c), DKO (d), and TKO (e) ESCs. (f) Distribution of CpG methylation for CpG sites with minimum coverage of five in ESCs. Global average methylation for all covered CpG sites in WT, 1KO, r1KO, DKO, and TKO was 0.727, 0.176, 0.369, 0.157, 0.006, respectively.

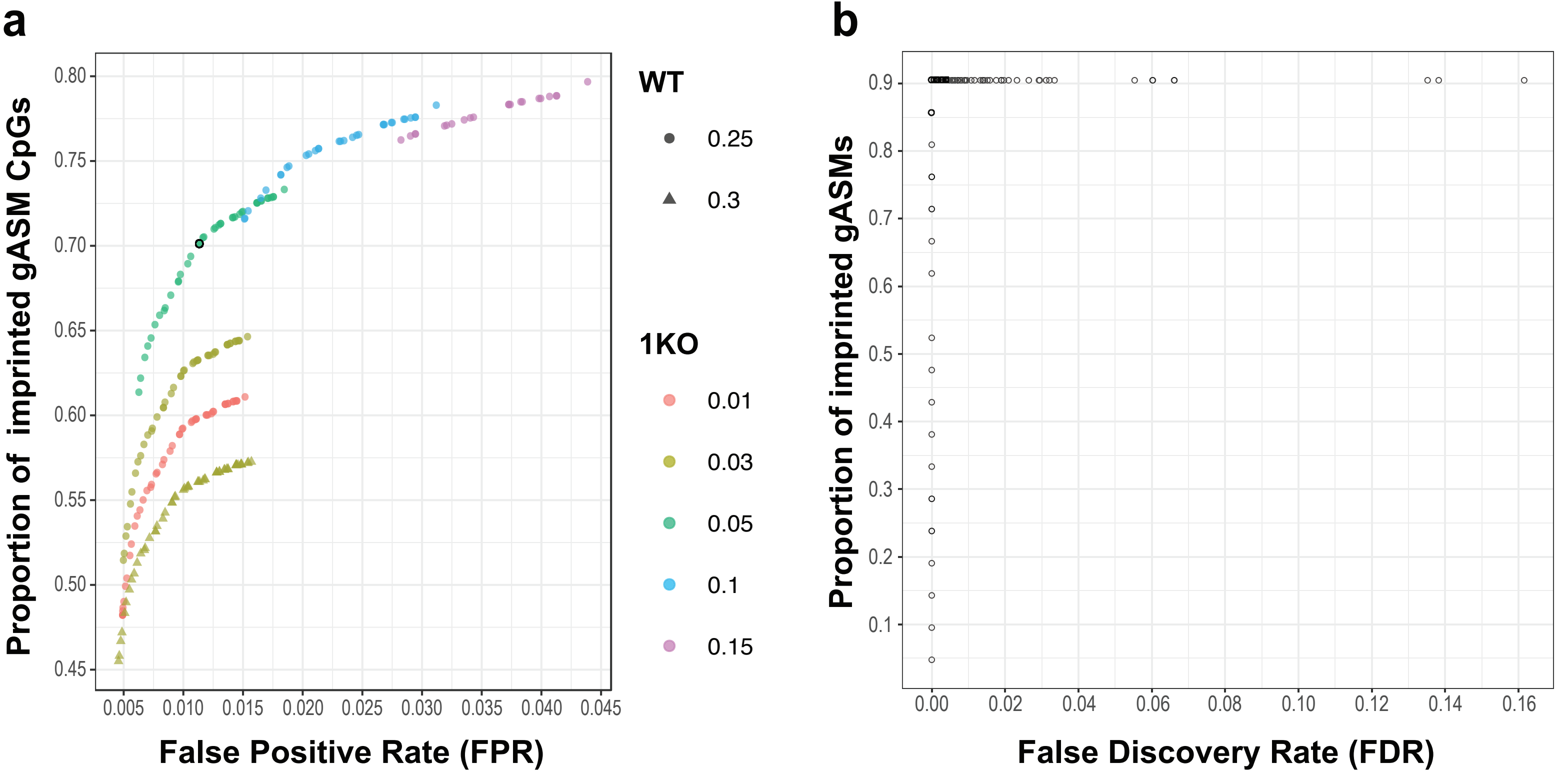
Supplementary Figure 2. NORED method evaluation. (a) ROC-like curve for individual CpG sites that meet three NORED criteria at various cutoffs for WT, 1KO, and r1KO. Cutoff for WT parameter is a circle for 0.25 and a triangle for 0.30. Cutoff for 1KO parameter is indicated by color. Each circle/triangle represents a tested r1KO value for combinations of WT and 1KO. For r1KO values are between WT and 1KO cutoffs at increments of 0.005. Circle with black outline indicates values for three parameters to select individual CpG sites to combine into regions (r1KO = 0.125). (b) Proportion of gASMs identified compared to region-level FDR

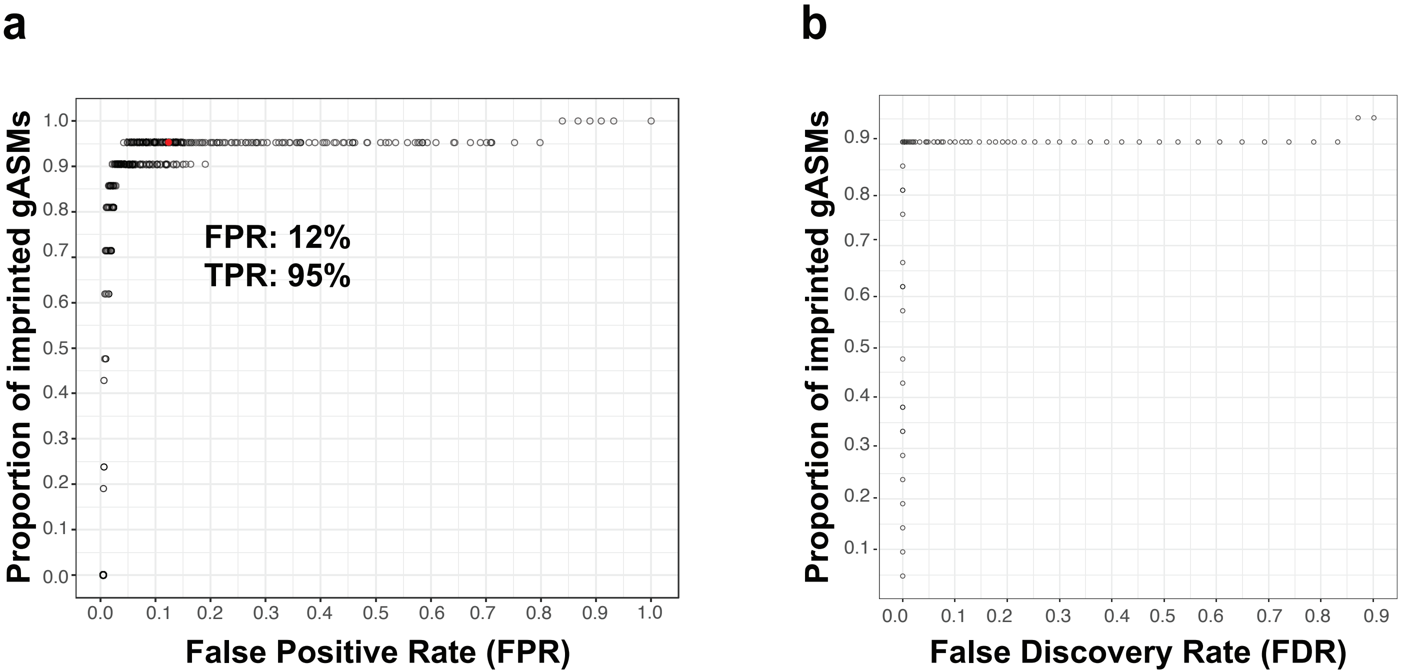
Supplementary Figure 3. MethylMosaic method evaluation. (a) ROC-like curve for CpG-centered windows at various cutoffs for hypermethylation and hypomethylation indices (see Methods) Red point indicates bounds for indices (between 0.2 to 0.75) to select CpG-centered windows to combine into regions. (b) Proportion of gASMs identified at region-level FDR.

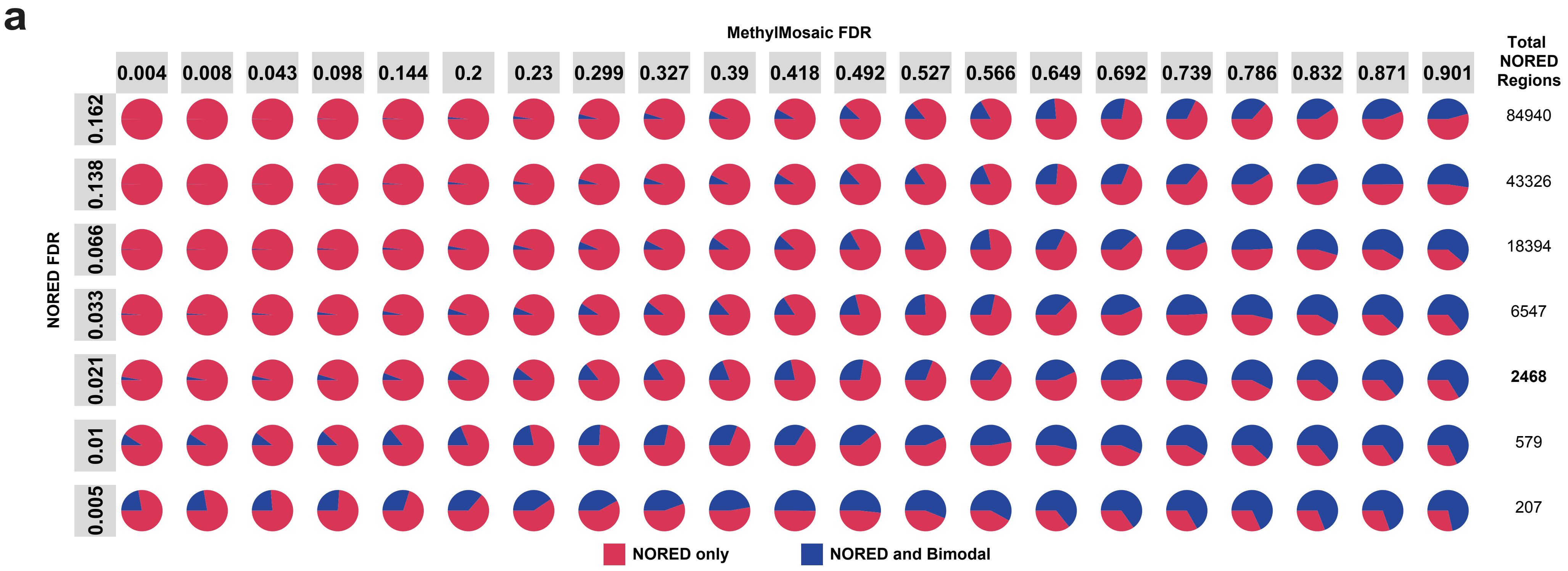
Supplementary Figure 4. Overlap of NORED and MethylMosaic demonstrates that two methods are independent, but complementary. (a) Proportion of NORED regions that are also bimodal in MethylMosaic at various FDRs for each method. Total number of NORED regions at each FDR cutoff is listed to the right. (b) Proportion of MethylMosaic regions that are also NORED at various FDRs for each method. Total number of MethylMosaic regions at each FDR cutoff is listed below.

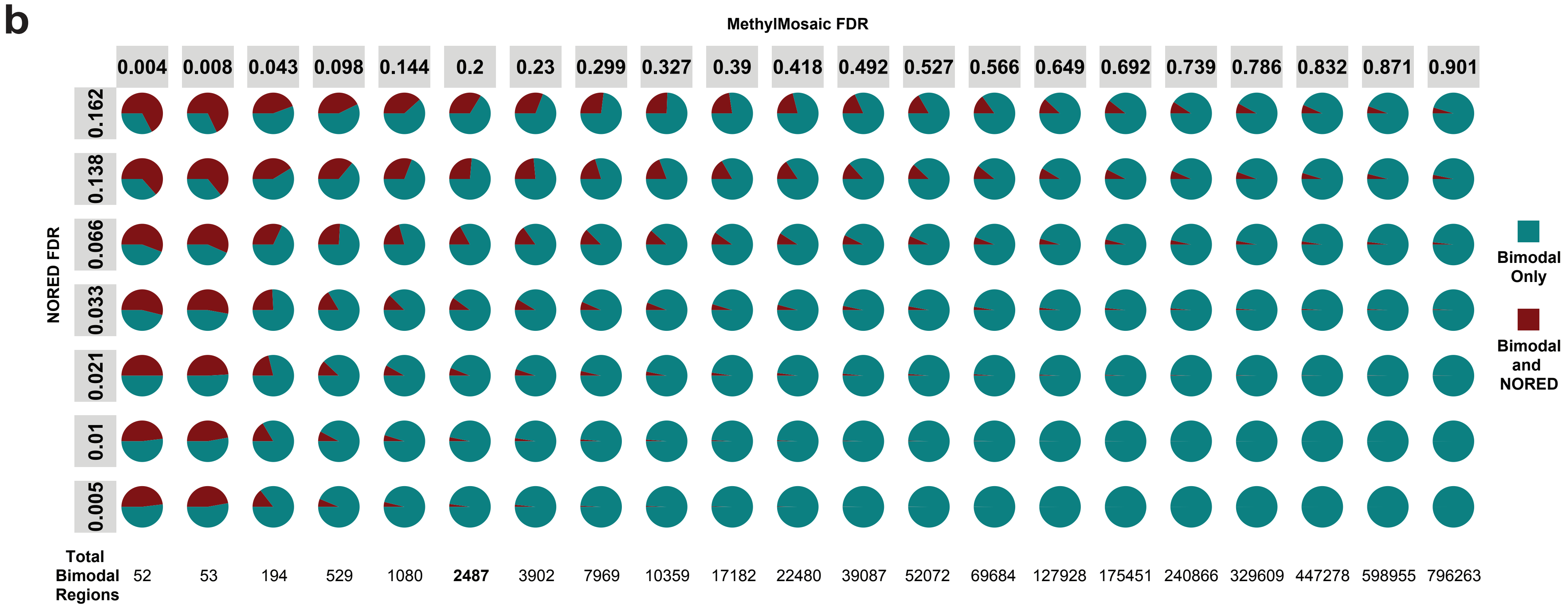
Supplementary Figure 5. Genomic sequencing confirms that WT ESCs are homozygous at *Hcn2/Polrmt* locus.

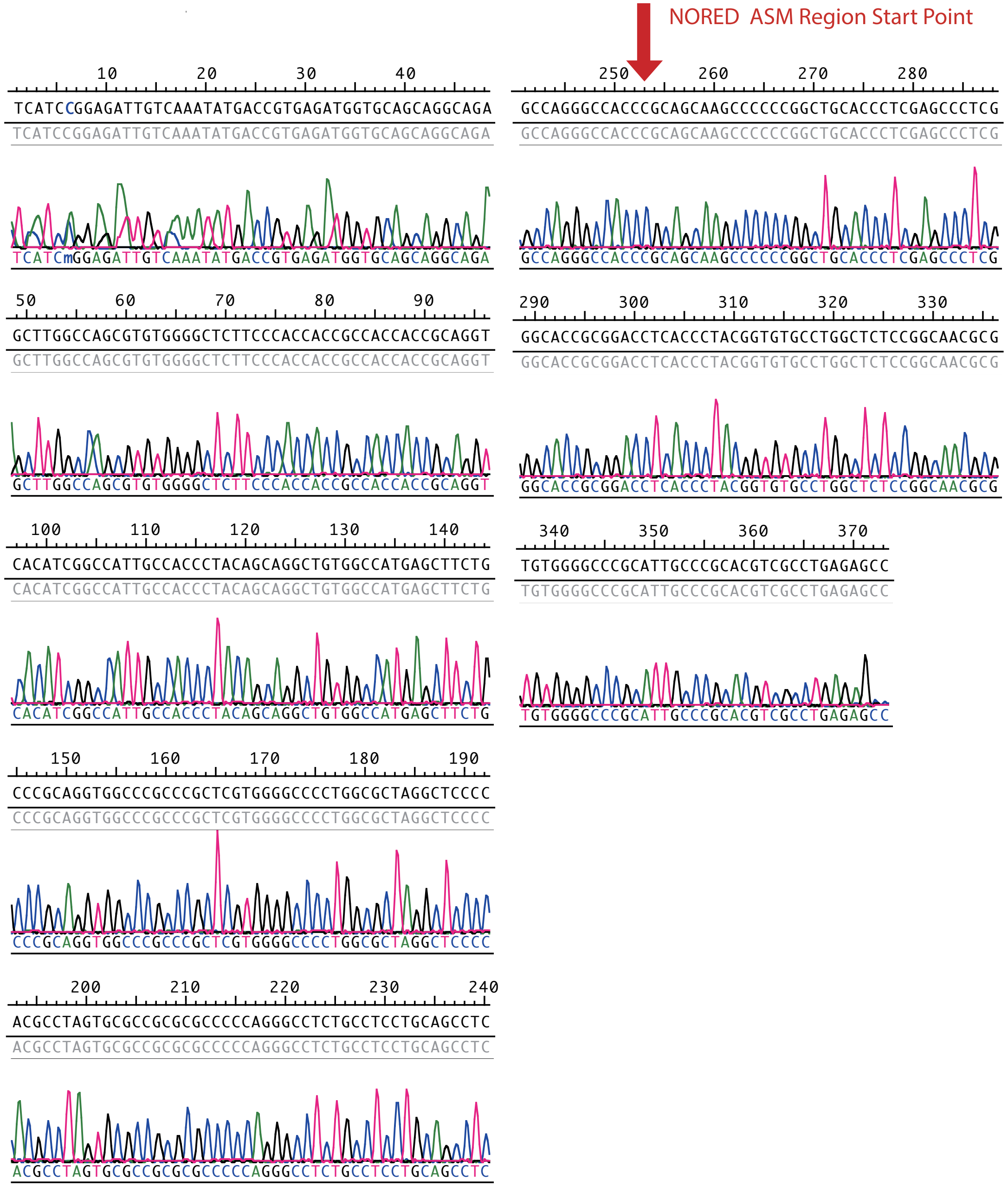

a
Density
0.00
0.02
0.04
0.06
0.08
0
10
20
30
40
50
Reads per CpG
WT
b
Density
0.00
0.02
0.04
0.06
0.08
0
10
20
30
40
50
Reads per CpG
1KO
c
Density
0.00
0.02
0.04
0.06
0.08
0
10
20
30
40
50
Reads per CpG
rescue1KO
d
Density
0.00
0.05
0.10
0.15
0
10
20
30
40
50
Reads per CpG
DKO
e
Density
0.00
0.02
0.04
0.06
0.08
0
10
20
30
40
50
Reads per CpG
TKO
f
Methylation
0.00
0.25
0.50
0.75
1.00
WT
1KO
r1KO
DKO
TKO

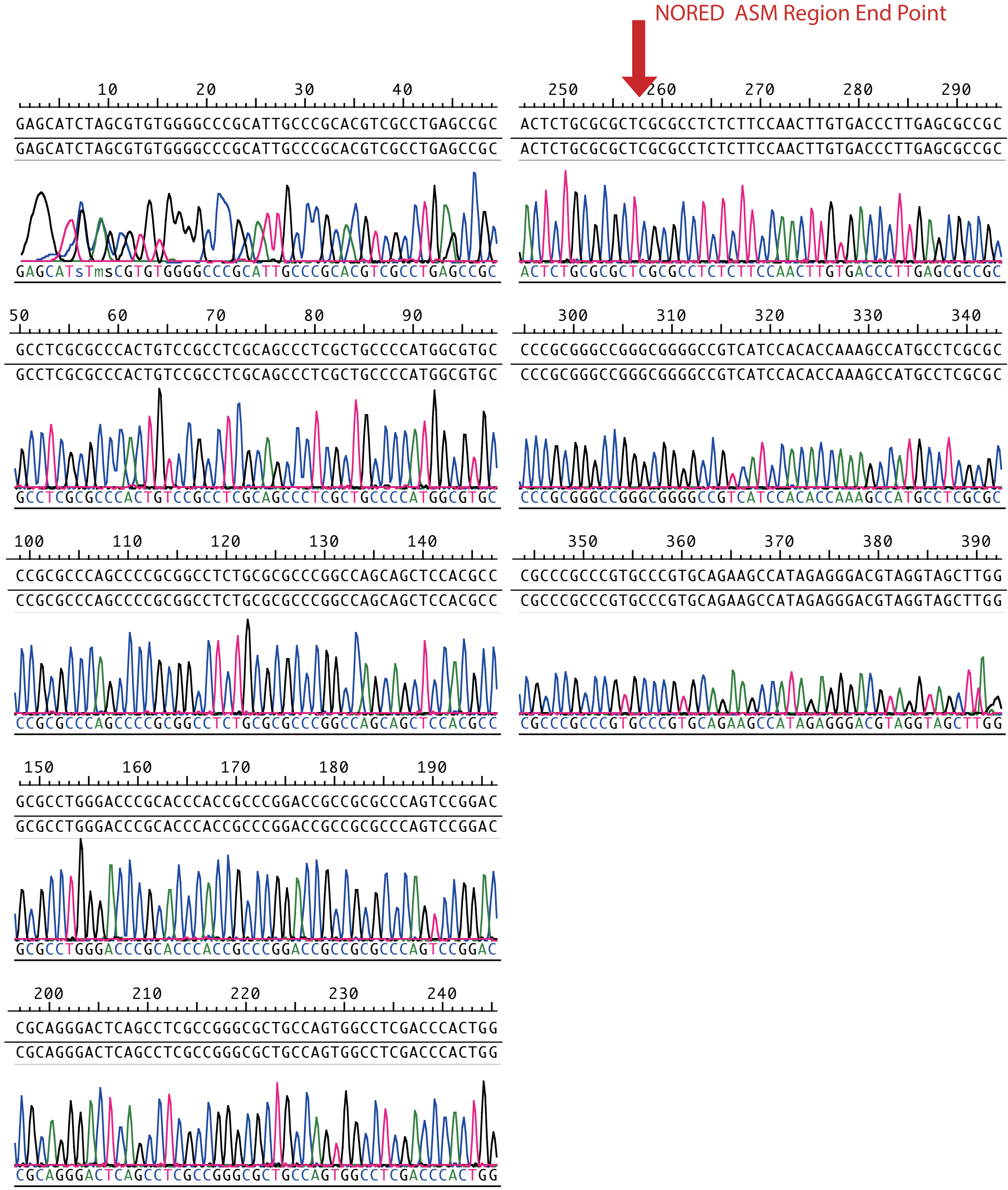

a
Proportion of imprinted gASM CpGs
0.80
0.75
0.70
0.65
0.60
0.55
0.50
0.45
0.005
0.010
0.015
0.020
0.025
0.030
0.035
0.040
0.045
False Positive Rate (FPR)
WT
0.25
0.3
1KO
0.01
0.03
0.05
0.1
0.15
b
Proportion of imprinted gASMs
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.00
0.02
0.04
0.06
0.08
0.10
0.12
0.14
0.16
False Discovery Rate (FDR)


a
b
Proportion of imprinted gASMs
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
FPR: 12%
TPR: 95%
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
False Positive Rate (FPR)
Proportion of imprinted gASMs
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
False Discovery Rate (FDR)


a
MethylMosaic FDR
0.004 0.008 0.043 0.098 0.144 0.2 0.23 0.299 0.327 0.39 0.418 0.492 0.527 0.566 0.649 0.692 0.739 0.786 0.832 0.871 0.901
Total NORED Regions
NORED FDR
0.162 84940
0.138 43326
0.066 18394
0.033 6547
0.021 **2468**
0.01 579
0.005 207
NORED only
NORED and Bimodal


b
MethylMosaic FDR
0.004 0.008 0.043 0.098 0.144 0.2 0.23 0.299 0.327 0.39 0.418 0.492 0.527 0.566 0.649 0.692 0.739 0.786 0.832 0.871 0.901
NORED FDR
0.162
0.138
0.066
0.033
0.021
0.01
0.005
Bimodal Only
Bimodal and NORED
Total Bimodal Regions
52 53 194 529 1080 **2487** 3902 7969 10359 17182 22480 39087 52072 69684 127928 175451 240866 329609 447278 598955 796263


NORED ASM Region Start Point
1 TCATCCGGAGATTGTCAAATATGACCGTGAGATGGTGCAGCAGGCAGA
50 GCTTGGCCAGCGTGTGGGGCTCTTCCCACCACCGCCACCACCGCAGGT
100 CACATCGGCCATTGCCACCCTACAGCAGGCTGTGGCCATGAGCTTCTG
150 CCCGCAGGTGGCCCGCCCGCTCGTGGGGCCCCTGGCGCTAGGCTCCCC
200 ACGCCTAGTGCGCCGCGCGCCCCCAGGGCCTCTGCCTCCTGCAGCCTC
250 GCCAGGGCCACCCGCAGCAAGCCCCCCGGCTGCACCCTCGAGCCCTCG
290 GGCACCGCGGACCTCACCCTACGGTGTGCCTGGCTCTCCGGCAACGCG
340 TGTGGGGCCCGCATTGCCCGCACGTCGCCTGAGAGCC


NORED ASM Region End Point
1 GAGCATCTAGCGTGTGGGGCCCGCATTGCCCGCACGTCGCCTGAGCCGC
50 GCCTCGCGCCCACTGTCCGCCTCGCAGCCCTCGCTGCCCCATGGCGTGC
100 CCGCGCCCAGCCCCGCGGCCTCTGCGCGCCCGGCAGCAGCTCCACGCC
150 GCGCCTGGGACCCGCACCCACCGCCCGGACCGCCGCGCCCAGTCCGGAC
200 CGCAGGGACTCAGCCTCGCCGGGCGCTGCCAGTGGCCTCGACCCACTGG
250 ACTCTGCGCGCTCGCGCCTCTCTTCCAACTTGTGACCCTTGAGCGCCGC
300 CCCGCGGGCCGGGCGGGGCCGTCATCCACACCAAAGCCATGCCTCGCGC
350 CGCCCGCCCGTGCCCGTGCAGAAGCCATAGAGGGACGTAGGTAGCTTGG