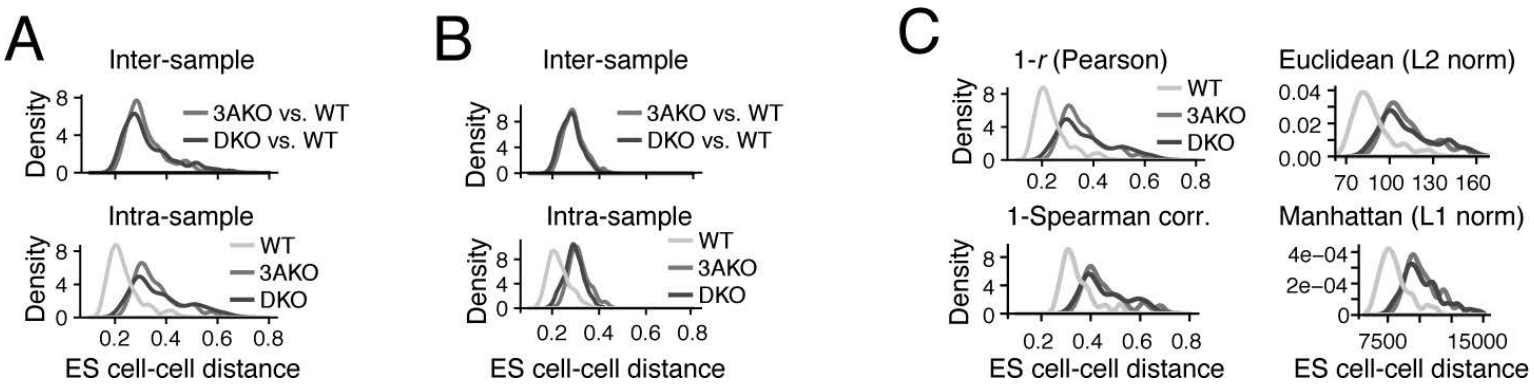

Figure S1 supporting Figure 1: Increased cellular variation in *DNMT3A* and *DNMT3A/3B* knockout ES cells

A. Inter-sample (top) and intra-sample (bottom) density distribution of pairwise cell-cell distances (1-Pearson correlation coefficient) for in silico sorted undifferentiated WT (*n* = 162), 3AKO (*n* = 74), and DKO cells (*n* = 74).

B. Inter-sample (top) and intra-sample (bottom) density distribution of pairwise cell-cell distances (1-Pearson correlation coefficient) for only the highest quality cells (number of genes detected > 7,000) for wildtype (*n* = 149), 3AKO (*n* = 56), and DKO (*n* = 58) ES cells.

C. Intra-sample density distribution of pairwise cell-cell distances in *in silico* sorted undifferentiated WT (*n* = 162), 3AKO (*n* = 74), and DKO cells (*n* = 74) using four different distances: 1- Pearson correlation coefficient (**top left**), 1- Spearman rank correlation (**bottom left**), Euclidean L2 norm (**top right**) and Manhattan L1 norm (**bottom right**).

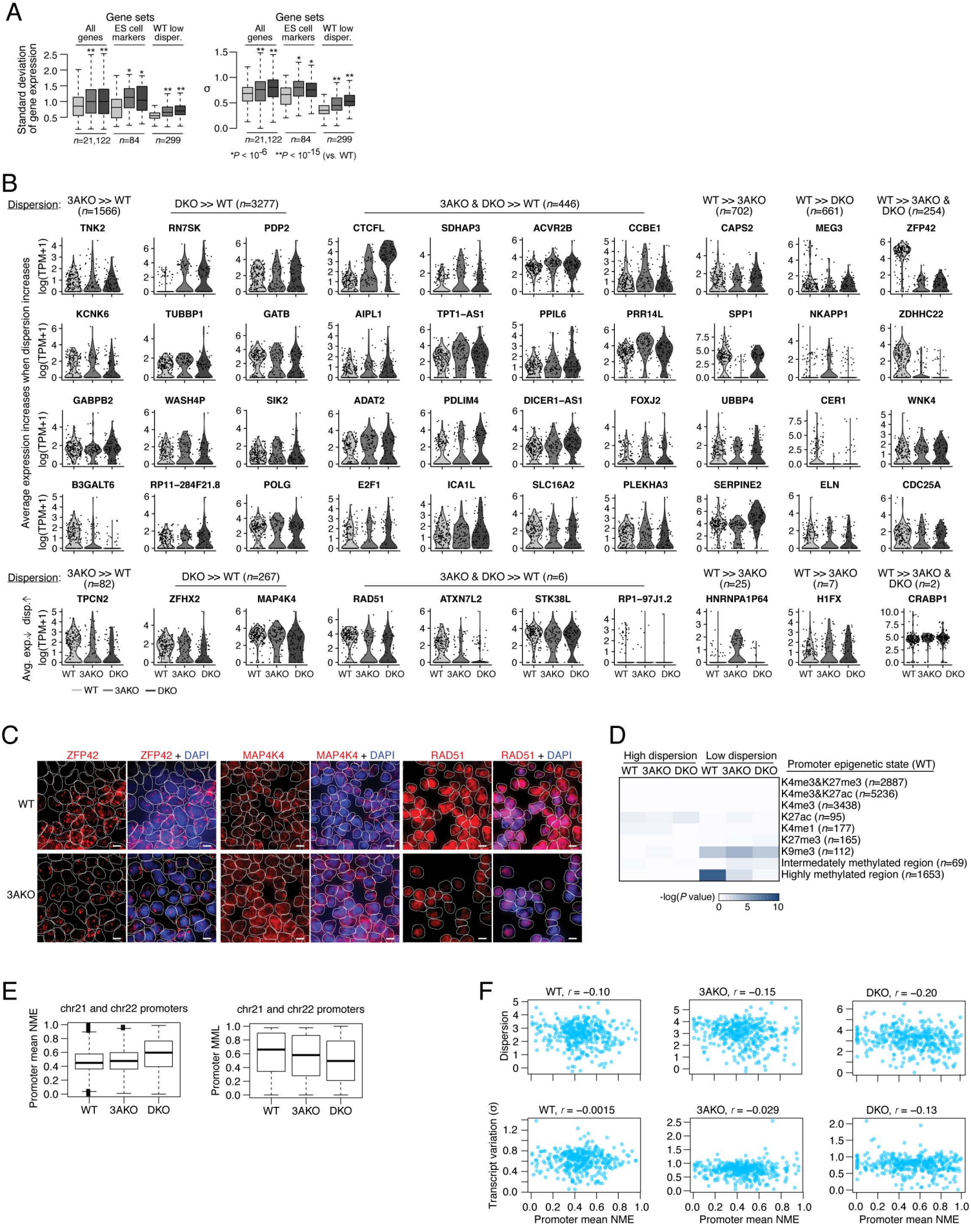

Figure S2 supporting Figure 2: Relationship between DNA methylation level, mean methylation entropy and transcript variation in *DNMT3A* and *DNMT3A/3B* knockouts

A. Box plots of gene expression standard deviation computed across all cells (**left**) and only among cells with detectable gene expression (σ, **right**) for gene sets composing of all genes, ES cell markers, and WT low dispersion genes for WT, 3AKO, and DKO ES cells. Boxes display the interquartile range while the bold line shows the median and whiskers extend to the most extreme data point that is no more than 1.5 times the interquartile range.
B. Violin plots of *log* gene expression level, *log*(TPM+1), for 50 selected genes that have a difference in dispersion greater than 1.5 between two samples, where the samples being compared are annotated using column headers on the top and the overall number of genes present in each category is shown in parentheses. The change in average expression relative to dispersion is annotated along rows on the left. The majority of genes (>90%) that increase in dispersion also increase in average expression. TPM = transcripts per million fragments mapped.
C. Representative images of RNA FISH experiment showing staining for DAPI (blue) and red fluorescent probes targeting ZFP42 (left), MAP4K4 (middle) and RAD51 (right) in WT (top) and 3AKO (bottom) ES cells. Cell segmentation is shown using white outlines. White bar in bottom right corner of each panel indicates a distance of 10 microns.
D. Genomic enrichment analysis for high (left) and low (right) transcript dispersion genes in WT, 3AKO, and DKO sorted ES cells overlapped with the promoter epigenetic state of matching WT ES cells (Gifford et al., 2013, Tsankov et al., 2015b). We observe a high enrichment of highly methylated promoter regions at low dispersion WT genes but this enrichment decreases for low dispersion 3AKO and DKO genes.
E. Boxplot of the promoter mean normalized methylation entropy (NME; left) and mean methylation level (MML; right) measured for WT, 3AKO, and DKO ES cell WGBS data for all chromosome 21 and 22 promoters using the approach in (Jenkinson et al., 2017). Boxes display the interquartile range while the bold line shows the median and whiskers extend to the most extreme data point that is no more than 1.5 times the interquartile range.
F. Correlation scatter plots of transcriptional variation measured in terms of dispersion (top) and standard deviation (σ) of detectable transcripts (bottom) versus promoter mean normalized methylation entropy for all WT (left), 3AKO (middle) and DKO (right) promoters on chromosomes 21 and 22.

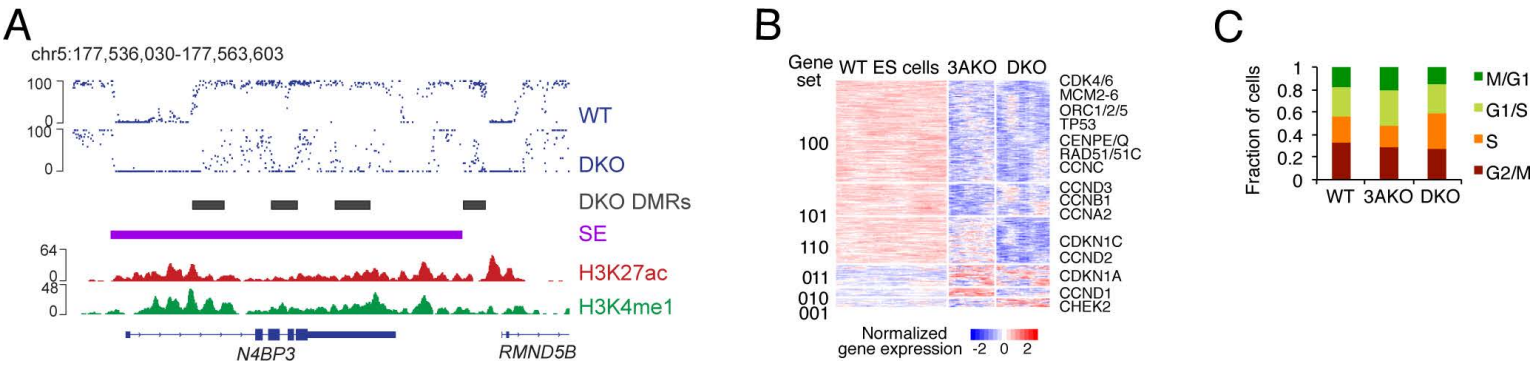

Figure S3 supporting Figure 3: Widespread transcriptional repression and changes in cell cycle gene expression in *DNMT3A* and *DNMT3A/B* knockout ES cells

A. Browser tracks display methylation levels for WT and DKO cells over a 28kb region on chromosome 5. Grey bars highlight DKO-specific differentially methylated regions (DMRs; difference > 0.6, $P < 0.01$). An ES cell super-enhancer (Hnisz et al., 2013) is highlighted in purple with ENCODE ChIP-seq data for H3K27ac and H3K4me1 in H1 ES cells displayed below. CpGs located within the super-enhancer region lose substantial methylation upon loss of DNMT3A and 3B.

B. Differentially expressed cell cycle annotated genes (right; rows) for sorted population of WT, 3AKO, and DKO ES cells (columns) ordered by progress in the cell cycle. Gene sets (left) are defined in Fig. 2A.

C. Fraction of cells in M/G1, G1/S, S, and G2/M phase for *in silico* sorted WT, 3AKO, and DKO ES cell populations.

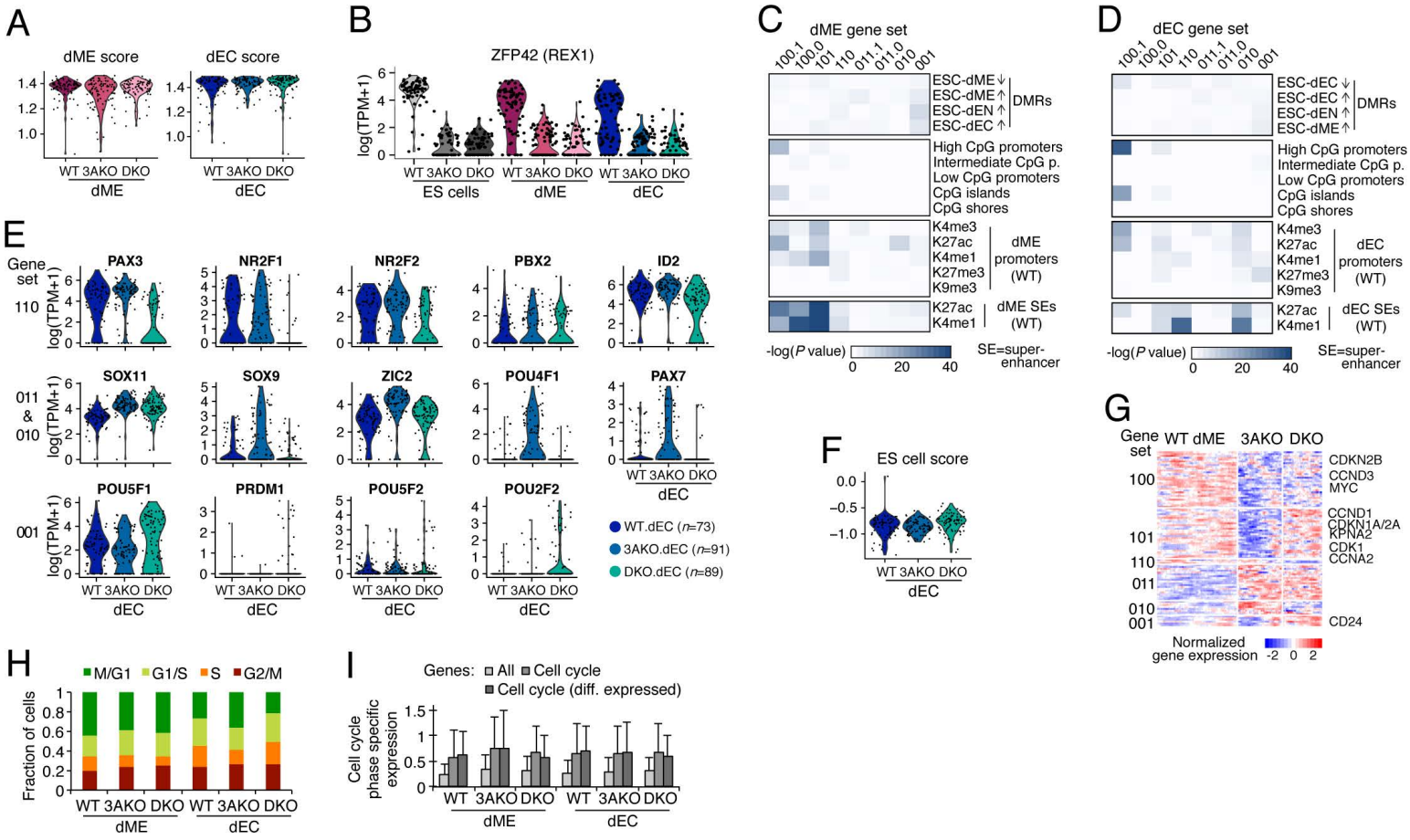

Figure S4 supporting Figure 4: Transcriptional misregulation in *DNMT3A/B* knockout cells following mesoderm differentiation

A. Violin plot of mesoderm (left) and ectoderm (right) scores for WT, 3AKO, and DKO cells following 5 days of differentiation towards mesoderm and ectoderm, respectively. Each dot represents a cell.

B. Distribution of ZFP42 expression for *in silico* sorted WT, 3AKO, and DKO ES (left), mesoderm (middle), and ectoderm (right) cells.

C. Genomic enrichment analysis for gene sets (columns) defined in Figure 4F against DNA methylation, CpG density features, and chromatin data collected in matching WT dME cells (Gifford et al., 2013, Tsankov et al., 2015b). DMR = differentially methylated region; K = lysine histone 3; me3 = tri-methylation; ac = acetylation; me1 = mono-methylation.

D. Genomic enrichment analysis for gene sets (columns) defined in Figure 4G against DNA methylation, CpG density features, and chromatin data collected in matching WT dEC cells.

E. Violin plots of *log*(TPM+1) gene expression for key developmental and oncogenic TFs misregulated in dEC 3AKO and/or DKO mutants. TFs displayed were either downregulated in DKO (top row; gene set 110), upregulated in 3AKO (middle row; gene sets 011 & 010), or upregulated in DKO (bottom row; gene set 001).

F. Violin plot of ES cell scores for WT, 3AKO, and DKO cells following 5 days of differentiation towards ectoderm. DKO dEC sample has a higher median and standard deviation in ES cell scores.
G. Differentially expressed cell cycle annotated genes (right; rows) for sorted population of WT, 3AKO, and DKO dME cells (columns) ordered by progress in the cell cycle. Gene sets (left) are defined in panel Figure 4F.
H. Fraction of cells in M/G1, G1/S, S, and G2/M phase for sorted WT, 3AKO, and DKO dME (left) and dEC (right) cell populations.
I. Distribution of cell cycle phase specific expression for sorted WT, 3AKO, and DKO dME (left) and dEC (right) cells considering all genes, cell cycle annotated genes, and differentially expressed cell cycle annotated genes. Error bars indicate one standard deviation.

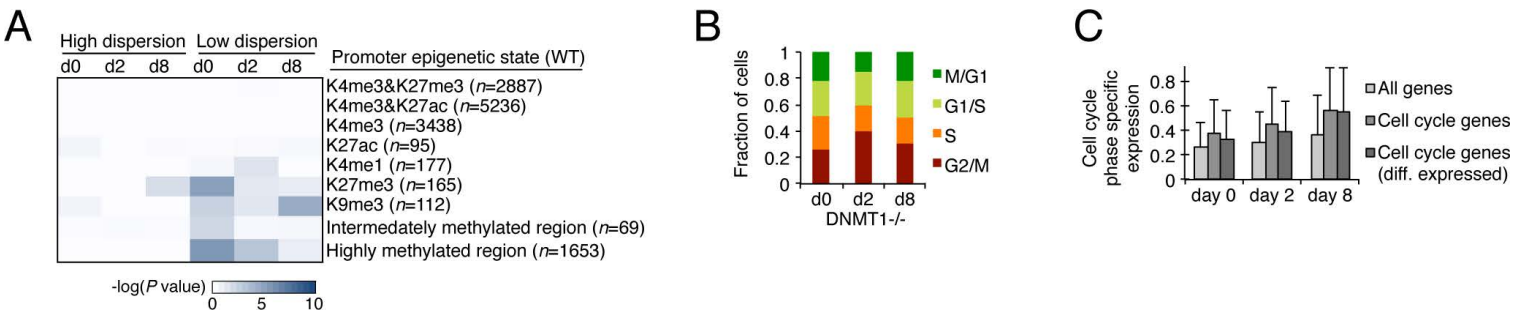

Figure S5 supporting Figure 5: Loss of *DNMT1* triggers increased transcript variation and differentiation

A. Genomic enrichment analysis for high (left) and low (right) transcript dispersion genes at day 0, 2, and 8 sorted ES cells following DOX treatment overlapped with the promoter epigenetic state of WT HUES64 ES cells (Gifford et al., 2013, Tsankov et al., 2015b). We observe a high enrichment of highly methylated promoter regions at day 0 low dispersion genes but this enrichment gradually decreases for low dispersion day 2 and day 8 genes while the enrichment at H3K9me3 promoters remains.

B. Fraction of cells in M/G1, G1/S, S, and G2/M phase for *in silico* sorted ES cells at day 0, 2, and 8.

C. Distribution of cell cycle phase specific expression for day 0, 2, and 8 sorted ES cells considering all genes, known cell cycle associated genes, and known, differentially expressed cell cycle annotated genes. Error bars indicate one standard deviation.

Table S1: Differentially expressed genes in wildtype (WT), *DNMT3A-/-* (3AKO) and *DNMT3A/B-/-* (DKO) ES cells

Three-way differentially expressed genes (rows in spreadsheet "Markers") for sorted population of WT, 3AKO, and DKO ES cells, displayed in **Fig. 2A**. Genes are separated into 6 clusters (100, 101, 110, 011, 010, and 001), where 1 or 0 indicates high or low expression for the respective condition (order: WT, 3AKO, DKO). Spreadsheets "100" to "001" contain functional enrichment analysis for genes in each cluster from spreadsheet "Markers" against the REACTOME database.

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