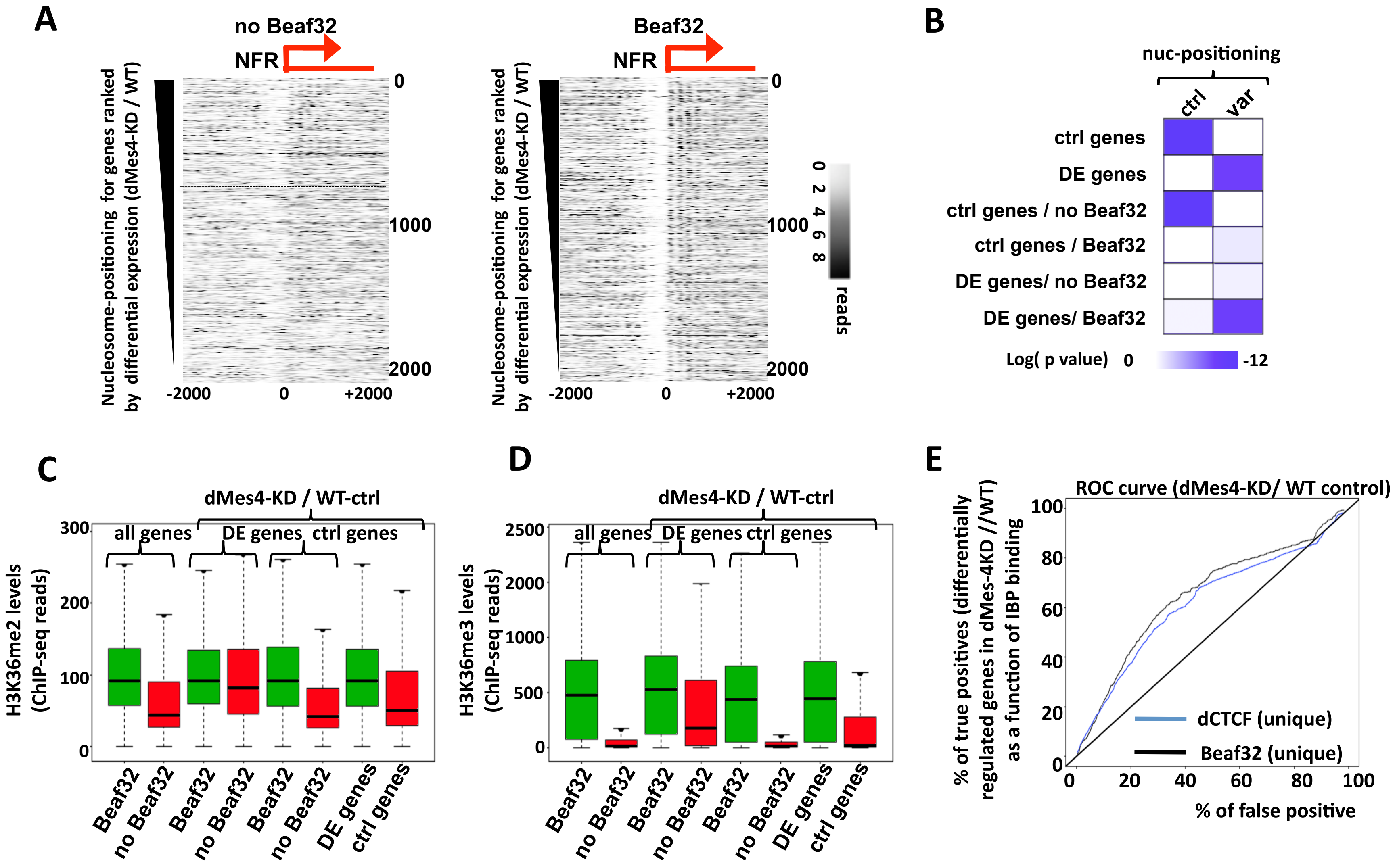
A
no Beaf32
NFR
Beaf32
NFR
Nucleosome-positioning for genes ranked by differential expression (dMes4-KD / WT)
0
1000
2000
-2000
0
+2000
0 2 4 6 8
reads
B
nuc-positioning
ctrl
var
ctrl genes
DE genes
ctrl genes / no Beaf32
ctrl genes / Beaf32
DE genes/ no Beaf32
DE genes/ Beaf32
Log( p value) 0
-12
C
dMes4-KD / WT-ctrl
all genes
DE genes
ctrl genes
H3K36me2 levels (ChIP-seq reads)
300
200
100
0
Beaf32
no Beaf32
Beaf32
no Beaf32
Beaf32
no Beaf32
DE genes
ctrl genes
D
dMes4-KD / WT-ctrl
all genes
DE genes
ctrl genes
H3K36me3 levels (ChIP-seq reads)
2500
2000
1000
500
0
Beaf32
no Beaf32
Beaf32
no Beaf32
Beaf32
no Beaf32
DE genes
ctrl genes
E
ROC curve (dMes4-KD/ WT control)
% of true positives (differentially regulated genes in dMes-4KD //WT) as a function of IBP binding
100
80
60
40
20
0
0
20
40
60
80
100
% of false positive
dCTCF (unique)
Beaf32 (unique)

**Figure S5. Genome-wide correlations between genes regulated by dMes-4 and nucleosome positioning or H3K36me2/me3 levels**

**A.** Heat map of nucleosome-positioning as measured by MNase-Seq in wild-type cells (see Methods) after ranking of genes whose promoters were bound by Beaf32 (right panel) or not (left panel) by their differential expression upon dMes-4KD as compared to control cells. Nucleosomes were aligned relative to the TSS (x-axis; position 0) of genes. The dotted lines mark the lower limit for DE genes. NFR, Nucleosome Free Regions (see Figure E2 for a similar analysis of DE genes upon Beaf32-KD).

**B.** Heat map representing the intersection analysis between the list of genes with most significant nucleosome-positioning defects upon Beaf32-KD as compared to control cells ('var'; see Figure E2B) or with similar levels of nucleosome positioning ('ctrl') and genes whose promoters were bound by Beaf32 ('Beaf32') or not ('no Beaf32'). Each intersection was tested using fisher exact test. See panel C-D for the levels of H3K36me2 and H3K36me3 corresponding to each intersection.

**C.** Box plot showing the levels of H3K36me2 (in ChIP-Seq reads; see Methods) for each intersection shown in panel B. Reads were counted within windows corresponding to gene bodies (from +500 to the end of each gene) in wild-type cells and for all genes or specifically for the genes differentially expressed upon dMes4-KD as compared to control genes ('DE genes') or for control genes with not change in expression in dMes4-KD.

**D.** Same as panel C except that H3K36me3 levels are indicated (in ChIP-Seq reads; see Methods). Reads were counted within windows corresponding to gene bodies (from +500 to the end of each gene) in wild-type cells and for all genes or specifically for the genes differentially expressed upon dMes4-KD as compared to control genes ('DE genes') or for control genes with not change in expression in dMes4-KD.

**E.** Impact of dMes-4 as a function of Beaf32 and dCTCF binding. Receiver operating characteristic (ROC) curve analysis (see Methods) of DE genes in dMes-4KD compared to WT cells allowing to test the influence of dMes-4 on differential expression for genes flanking a unique dCTCF binding site (< 500bp from TSS; blue curve) or a Beaf32 binding site (< 500bp from TSS; black curve). Y-axis, % of true positives (differentially expressed genes); X-axis, % of false positives (constant expression), depending on the calculated threshold for differential expression (see also Figure 3C for a combinatorial analysis).