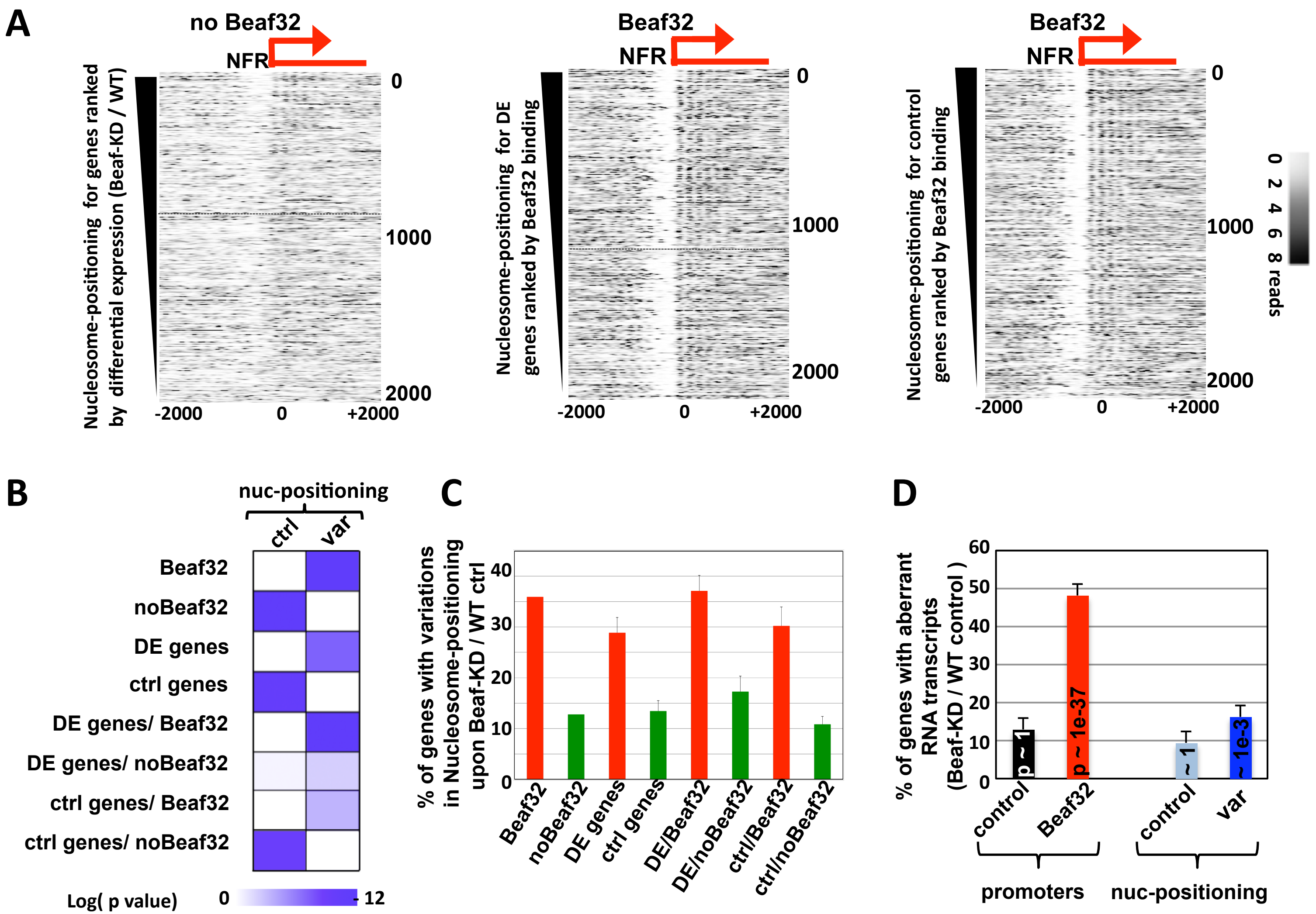
A
no Beaf32
NFR
Nucleosome-positioning for genes ranked by differential expression (Beaf-KD / WT)
0
1000
2000
-2000
0
+2000
Beaf32
NFR
Nucleosome-positioning for DE genes ranked by Beaf32 binding
0
1000
2000
-2000
0
+2000
Beaf32
NFR
Nucleosome-positioning for control genes ranked by Beaf32 binding
0
1000
2000
-2000
0
+2000
0 2 4 6 8 reads
B
nuc-positioning
ctrl
var
Beaf32
noBeaf32
DE genes
ctrl genes
DE genes/ Beaf32
DE genes/ noBeaf32
ctrl genes/ Beaf32
ctrl genes/ noBeaf32
Log( p value)
0
12
C
% of genes with variations in Nucleosome-positioning upon Beaf-KD / WT ctrl
0
10
20
30
40
Beaf32
noBeaf32
DE genes
ctrl genes
DE/Beaf32
DE/noBeaf32
ctrl/Beaf32
ctrl/noBeaf32
D
% of genes with aberrant RNA transcripts (Beaf-KD / WT control )
0
10
20
30
40
50
60
p ~ 1
p ~ 1e-37
~ 1
~ 1e-3
control
Beaf32
control
var
promoters
nuc-positioning

**Figure S2. Nucleosome positioning defects upon insulator protein depletion**

**A.** Heat map of nucleosome-positioning as measured by MNase-Seq (see Methods) for DE genes whose promoters were not bound by Beaf32 (left panel), bound by Beaf32 (middle panel) or for control genes whose promoters were bound by Beaf32 (right panel). Nucleosomes were aligned relative to TSS (x-axis; position 0) of genes. The dotted lines mark the lower limit for DE genes. NFR, Nucleosome Free Regions (see also Figure 1B).

**B.** Heat map representing the intersection analysis between the list of genes with similar levels of nucleosome positioning or changes in nucleosome-positioning defects ('ctrl' and 'var, respectively; as found upon Beaf32-KD as compared to control cells; see Methods) and genes whose promoters were bound by Beaf32 ('Beaf32') or not ('no Beaf32') and/or genes that were differentially expressed ('DE genes') or not (control) genes. Each intersection was tested using fisher exact test. See panel C for the percentages of genes corresponding to each intersection.

**C.** Histogram showing the relative enrichment of genes where most significant changes in nucleosome-positioning were detected between MNase-Seq read counts in Beaf32-KD compared to WT cells as in panel B. The error bars represent the standard deviation obtained by analyzing reads from 3 independent experiments in Beaf32-KD compared to WT.

**D.** Detection of aberrant RNASeq reads in Beaf32-KD compared to WT cells. Histogram showing the relative enrichment of genes showing 'aberrant' RNASeq reads (outside defined exons; see Methods) in Beaf32-KD compared to WT cells, as a function of: left, the binding in promoters of Beaf32 (red) or not (control, black); right: variations in nucleosome-positioning ('Beaf32-KD / WT', group of genes harboring most significant changes in nucleosome positioning; see Figure 1C) or not ('control'). The error bars represent the standard deviation obtained by analyzing reads from 4 independent RNASeq experiments in Beaf32-KD compared to WT.