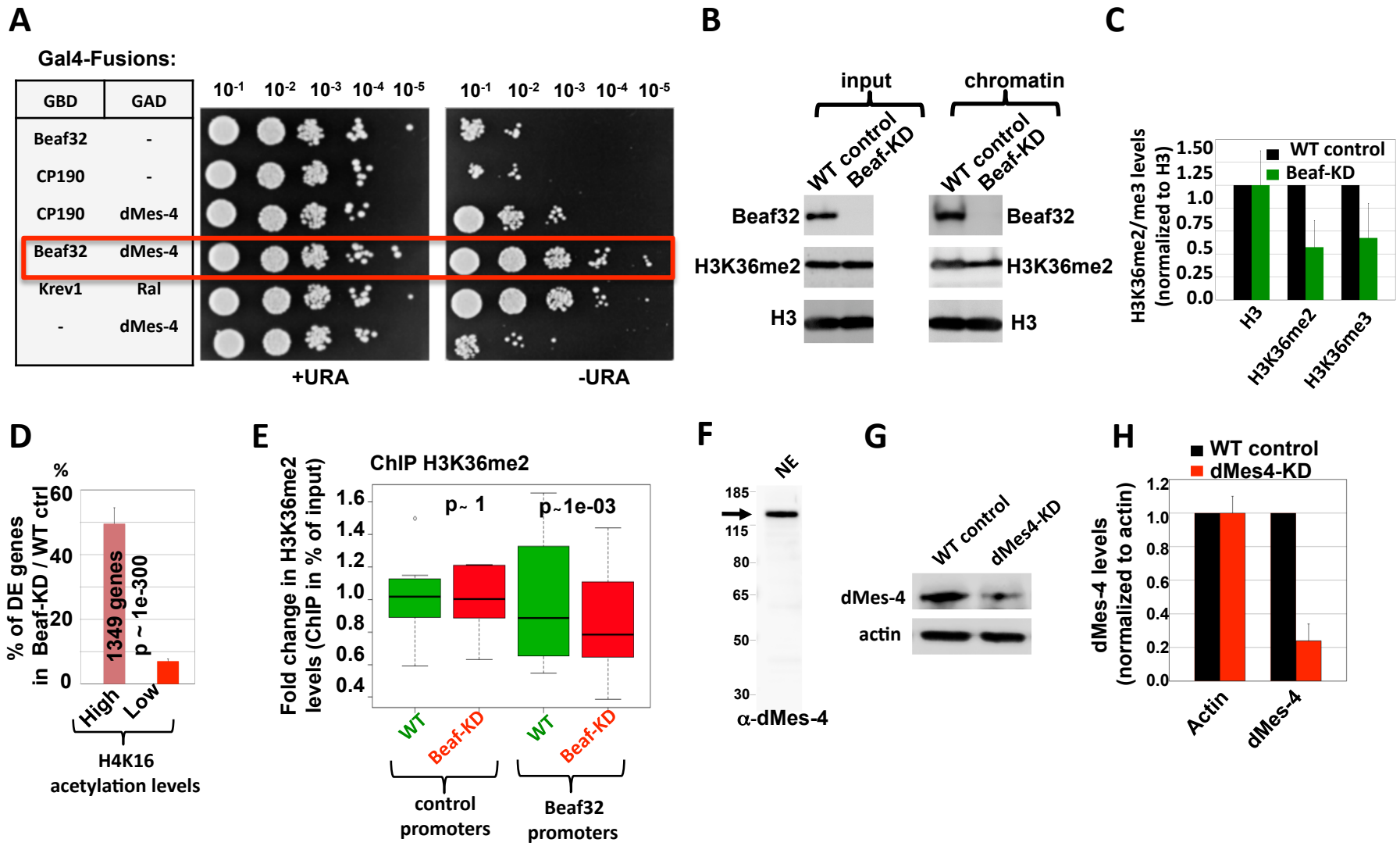
A
Gal4-Fusions:
GBD
GAD
Beaf32
-
CP190
-
CP190
dMes-4
Beaf32
dMes-4
Krev1
Ral
-
dMes-4
10-1 10-2 10-3 10-4 10-5
10-1 10-2 10-3 10-4 10-5
+URA
-URA
B
input
chromatin
WT control
Beaf-KD
WT control
Beaf-KD
Beaf32
H3K36me2
H3
Beaf32
H3K36me2
H3
C
H3K36me2/me3 levels (normalized to H3)
1.50
1.25
1.0
0.75
0.5
0.25
0.0
WT control
Beaf-KD
H3
H3K36me2
H3K36me3
D
% of DE genes in Beaf-KD / WT ctrl
%
60
40
20
0
1349 genes
p ~ 1e-300
High
Low
H4K16 acetylation levels
E
ChIP H3K36me2
Fold change in H3K36me2 levels (ChIP in % of input)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
p~ 1
p~1e-03
WT
Beaf-KD
WT
Beaf-KD
control promoters
Beaf32 promoters
F
NE
185
115
80
65
50
30
α-dMes-4
G
WT control
dMes4-KD
dMes-4
actin
H
WT control
dMes4-KD
dMes-4 levels (normalized to actin)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Actin
dMes-4

Figure S3. Characterization of dMes-4 and insulator protein depletions

A. Beaf32 directly interacts with dMes-4. Data obtained by yeast 2 hybrid using the indicated various combinations of bait and target constructs expressing fusion protein Beaf32, dMes-4 and CP190 proteins compared to positive controls (Krev1-Ral) in cells replicated on nonselective (+Ura) or selective (-Ura) plates (see Methods).

B. Western blotting analysis of chromatin fractions purified through sucrose cushions ('chromatin'; see Methods) prepared from Beaf32-KD or control cells or of the corresponding nuclear extracts ('input'), using anti-Beaf32, anti-H3K36me2 antibodies or total anti-histone H3 for loading control.

C. Quantification of H3K36me2/3 levels in Beaf32-KD or control cells as compared to loading control from the western blotting analysis in Figure 2E and in panel B (see Methods).

D. Histogram showing the percentage of DE genes as determined by expression analysis in Beaf32-KD compared to WT cells as a function of the levels of histone acetylation (H4K16ac; High or Low, light red and red bar, respectively) within the corresponding promoter regions (-500 to 0 bp from TSS).

E. Box plot showing the results of ChIP performed in Beaf32-KD (red boxes) compared to WT-control (mock-depleted) cells (green boxes) in percent of input (y-axis) with anti- H3K36me2 antibodies or IgG control, for promoters harboring a Beaf32 site or not as indicated. ChIP data were analyzed by qPCR analyses in triplicates and for three independent measures normalized to three control loci (see Methods).

F. Western blotting analysis of nuclear extracts using anti-dMes-4 antibodies. The arrow indicates the unique band detected corresponding to the molecular weight of dMes-4 (1423 AA).

G. Western blotting analysis of nuclear extracts prepared from dMes-4-KD or control cells using anti-dMes-4 or anti-actin control antibodies for loading control. The efficiency of dMes-4-KD was confirmed by RTqPCR analysis (see Figure 4A).

H. Quantification of dMes-4 depletion as compared to loading control from the western blotting analysis in panel F (see Methods).