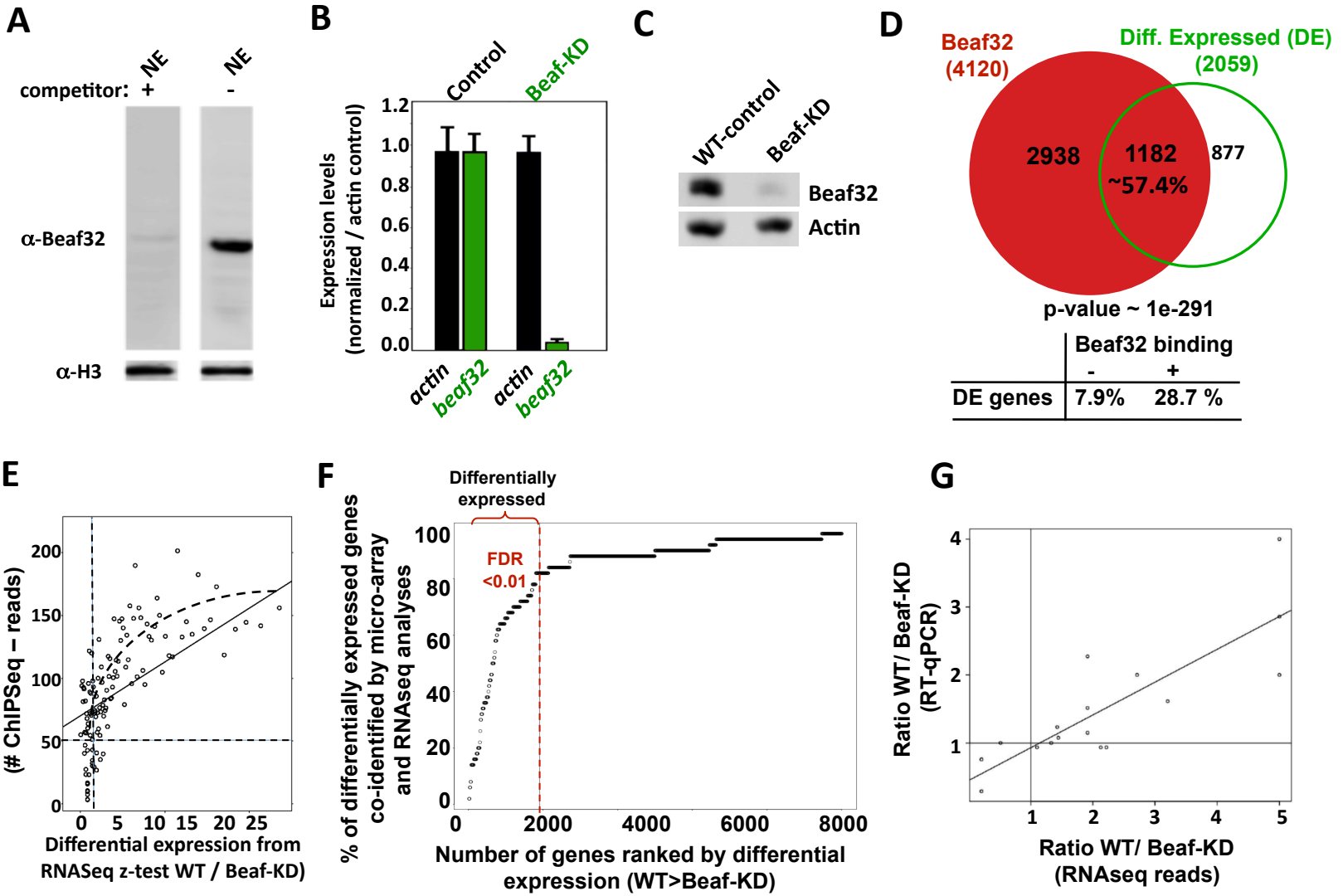
A
NE
NE
competitor: +
-
α-Beaf32
α-H3
B
Control
Beaf-KD
Expression levels
(normalized / actin control)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
actin
beaf32
actin
beaf32
C
WT-control
Beaf-KD
Beaf32
Actin
D
Beaf32
(4120)
Diff. Expressed (DE)
(2059)
2938
1182
~57.4%
877
p-value ~ 1e-291
Beaf32 binding
-
+
DE genes
7.9%
28.7 %
E
Beaf32 binding
(# ChIPSeq – reads)
200
150
100
50
0
0
5
10
15
20
25
Differential expression from
RNASeq z-test WT / Beaf-KD)
F
Differentially
expressed
FDR
<0.01
% of differentially expressed genes
co-identified by micro-array
and RNAseq analyses
100
80
60
40
20
0
0
2000
4000
6000
8000
Number of genes ranked by differential
expression (WT>Beaf-KD)
G
Ratio WT/ Beaf-KD
(RT-qPCR)
4
3
2
1
1
2
3
4
5
Ratio WT/ Beaf-KD
(RNAseq reads)

Figure S1. Genome-wide analyses highlight a role of Beaf32 in gene expression

A. Western blotting analysis of nuclear extracts using anti-Beaf32 antibodies in the presence (+) or absence (-) of peptide competitor ('competitor'), as indicated. Lower blot: Anti-histone H3 antibodies were used for loading control ('anti-H3').

B. Graph representing the results from 3 independent RT-qPCR analyses to measure the mRNA levels of Beaf32 (green) and actin (black) in Beaf32 knock-down ('Beaf32-KD') or wild-type ('Control') cells. The error bar corresponds to the standard deviation of the measure. The y axis shows the relative fold change in expression normalized to *actin* control.

C. Western blotting analysis of nuclear extracts prepared from Beaf32-KD or control cells using anti-Beaf32 or anti-actin control antibodies for loading control.

D. Venn diagram showing the intersection analysis between differentially expressed (DE) genes whose expression is affected in Beaf32-KD cells compared to WT cells (2,059 genes) as measured in two independent DGE-Seq experiments (see Methods) and genes that are flanked (< 500bp from TSSs) by one of the 4,120 Beaf32 binding site (> 25 ChIPSeq reads; see Methods). The indicated p-value was obtained by fisher exact test. Also indicated are the percentages of DE genes depending on whether their promoters are bound by Beaf32 or not. Note that approximately 91% (3,749) of the 4,120 Beaf32 sites correspond to 'direct' Beaf32 binding sites (Liang et al., 2014) that harbor the CGATA motifs and/or that localize close to a TSS.

E. Plot of the correlation between differential expression between WT and Beaf32-KD cells and Beaf32 binding. All drosophila genes were scored according to their differential expression levels as measured by DGE-Seq (x-axis; see Methods) after ranking of genes according to the levels of Beaf32 binding to their promoters as measured by ChIP-Seq (y-axis; see Methods). The dotted lines represent the thresholds for Beaf32 binding (horizontal line) and for DE genes (vertical line).

F. Correlation between RNA-Seq and microarray analysis or RT-qPCR and for differentially expressed genes. Graph representing the percentage (%) of differentially regulated genes identified through DGE-Seq (2,059 genes) upon Beaf32-depletion ('Beaf32-KD') and most differentially expressed genes identified by microarray analyses (see Methods). The red bar marks the FDR<0.01 for the DGE-Seq including 85% of most differentially expressed genes identified by microarray analyses (p-value ~1e-28).

G. Graph showing the linear regression line for gene expression in WT- compared to Beaf32-KD-cells obtained by both DGE-Seq and by RT-qPCR (Pearson's correlation coefficient: 0.83; p-value~ 3.2e-5) for 18 genes tested using the absolute quantification component of pyQPCR (http://pyqpcr.sourceforge.net/)(see Methods). Each point corresponds to a gene whose expression was measured in triplicates as a ratio of expression between WT/Beaf32-KD cells, either for DGE-Seq reads or for the number of copies as measured by RT-qPCR. On the x-axis is represented the ratio by DGE-seq and on the y-axis the ratio by RT-qPCR.