

SUPPLEMENTARY FIGURES

Figure S1. Validation of BEAF-32 depletion and its impact on chromatin organization and gene expression.

A. Western blot of nuclear extracts prepared from BEAF-32 depleted cells ('Beaf-KD') compared to control cells depleted of the *luciferase* gene (Luc-KD) used as an exogenous control gene (see Materials and Methods)[17], after incubation with anti-BEAF-32 (upper blot) or anti-Actin antibodies (lower blot) next to a protein Molecular Weight marker (MW).

B. Gene expression analysis by RT-qPCR in BEAF-32-depleted and control luciferase-depleted cells, using primers specific for *beaf-32* genes normalized to control genes (see Materials and Methods).

C. Hi-C contact maps for luciferase-depleted (Luc-KD, left) and BEAF-32-depleted (Beaf-KD, right) conditions at three resolutions (10 kb, 25 kb, and 50 kb). TADs were detected as described (black triangles; see Materials and Methods). The averaged insulation scores are also shown below the 2D plots (blue tracks).

D. Boxplot showing the distribution of insulation scores for the TADs altered upon Beaf-KD compared to Luc-KD ("altered TADs") as compared to unchanged TADs ("conserved TADs").

E. Proportion of up-regulated (red) and down-regulated (blue) genes found to be associated with altered- or conserved- TADs. The difference was tested using a paired Wilcoxon test (p-values of 0.979 and 0.42 for up- or down-regulated genes, respectively).

F. Boxplot showing the distribution of insulation scores in Beaf-KD as compared to Luc-KD control cells, for the BEAF-32 sites not associated with a TAD border (p-value of 5×10^{-8} ; as tested using a paired Wilcoxon test).

Figure S2. Analysis of leakage scores and insulation changes at BEAF-32 binding sites.

A. Boxplot showing the distribution of leakage scores (insulator permissiveness), calculated by quantifying LRCs across BEAF-32 sites, in Beaf-KD relative to control Luc-KD cells (see Materials and Methods). Based on these scores, genomic regions were ranked into quantiles ranging from the highest increase in leakage (top 5%, Q1) to the lowest leakage (lowest 5%, Q20). The top 5% group (Q1) displayed significantly higher leakage scores than groups 4–20 (paired Wilcoxon test).

B. Same as in panel A, except that leakage scores were compared between Q1 (top 5%) and Q20 (lowest 5%). Note that leakage scores were significantly higher in Q1 than in Q20 (paired Wilcoxon test, $p\text{-value} < 2.2 \times 10^{-16}$).

C. Boxplot comparing insulation scores (see Materials and Methods) in Beaf-KD (red) and Luc-KD (blue) for genomic regions exhibiting the highest leakage (top 5%; paired Wilcoxon test, $p\text{-value} = 3 \times 10^{-107}$).

D. Comparison of leakage scores between Beaf-KD (red) and Luc-KD (blue) conditions. Leakage scores were calculated between Beaf-KD and Luc-KD after normalizing the interaction levels across insulators (upper-left region of the aggregation matrix) by the signal in the control region located in the lower-left region of the aggregation matrix (see Materials and Methods). The difference was assessed using a paired Wilcoxon test ($p\text{-value} = 0.00196$).

Figure S3. Assessing permissiveness at insulators versus enhancers.

A. 2D plots showing an example of leakages observed across BEAF-32 binding sites. Luc-KD shows a lower leakage signal compared to Beaf-KD (compare upper and middle 2D plot). The lower 2D plot displays Beaf-KD/Luc-KD interaction values.

B. Distance plot comparing the relative distances of permissive BEAF-32 binding sites (associated with the top 5% of leakage scores), to the borders of TADs that were altered or conserved upon Beaf-KD ($p\text{-value} = 0.418$, as tested using a paired Wilcoxon test).

C. ROC analysis testing the ability of the method, quantifying leakages across sites, to distinguish enhancers (sites associated with H3K27ac and H3K4me1 marks; see Materials and Methods) from insulator-associated factors (dCTCF, CP190, Su(Hw), Zw5 and BEAF-32) in group Top5% / Q1 (AUC = 99.5%). Note that the distinction is also possible for less permissive regions as shown for group lowest 5% / Q20 (AUC = 94.76%), as expected (see text).

Figure S4. Detection of enhancer–promoter long-range contacts by HiCaggR, as a function of leakage.

A. Barplot showing the number of significant enhancer–promoter contacts as identified using HiCaggR, as a function of leakages in the Luc-KD control condition (see Materials and Methods).

B. Detection of the most significant enhancer–promoter long-range contacts as a function of the ranking of genomic regions by insulator permissiveness (from top 5% to lowest 5%). The plots show the E-P contacts that were specifically detected in the control condition (Luc-KD; left plot) or in the BEAF-32 depleted condition (Beaf-KD, right). Significant interactions highlighted in red were detected through HiCaggR (see Materials and Methods).

C. Barplots showing the total number of enhancer-promoter pairs in control (Luc-KD, left; in blue) and BEAF-32 depleted cells (Beaf-KD, right; in red). Note that most E-P pairs were not found to interact significantly through HiCaggR (as represented by grey dots in panel B).

D. Boxplot comparing the distribution of distances between enhancers and promoters found in genomic regions with most (top 5%) or least (lowest 5 %) leakage in control (Luc-KD; in blue) or BEAF-32 depleted condition (Beaf-KD; in red). The significance of the differences in distances was tested using a paired Wilcoxon test (p-value = 0.41 and 0.18, for the top 5% and the lowest 5%, respectively).

E. Matrix testing the enrichment of down-regulated genes depending on leakage (Beaf-KD compared to Luc-KD). Heatmap of p-values (left) and heatmap of odds ratios (right), as obtained using a Fisher's exact test.

F. Cumulative plot comparing the relative distances of up-regulated genes as detected upon Beaf-KD, with the borders of TADs that were either altered or conserved upon Beaf-KD compared to Luc-KD condition (p-value = 0.82, as tested using a paired Wilcoxon test).

Figure S5. Single-cell integration and gene expression correlations in leakage-associated regions.

A. UMAP 1 and 2 of multiomic data (see Materials and Methods) highlighting the quantification by scRNA-seq of *beaf-32* (purple) in Luc-KD (left plot) or Beaf-KD (right plot). Note that no cluster was found to be specifically enriched in either condition.

B. Matrix testing the relative enrichment (shown as odds ratio) of down-regulated genes as obtained from multiomics data (scRNA-seq) depending on the leakage ranking (Beaf-KD compared to Luc-KD). Enrichment was tested using a Fisher exact test.

C. Distribution of gene expression variance as obtained from scRNA-seq data for all genes (Log ratio in Beaf-KD compared to Luc-KD; p-value of 0.949, as obtained using a paired Wilcoxon test).

D. Heatmap showing the Spearman correlation coefficients calculated from scRNA-seq data for up-regulated genes in Beaf-KD within the top 5% of regions displaying the highest leakage (log Beaf-KD/Luc-KD ratio).

E. Barplot showing the enrichment of down-regulated genes across the 4 clusters identified and displayed in the correlation heatmap. Only cluster 3 shows a significant enrichment (Fisher's exact test, p-value = 2.8×10^{-2}).

F. Gene Ontology analysis for the four gene-clusters identified by hierarchical clustering for up-regulated genes (left panel). For comparison, the GOs associated with genes flanking a BEAF-32 binding site are also indicated (right panel).

Table S1: List of novel enhancer-promoter contacts.

The table lists novel enhancer-promoter interactions detected by Hi-C specifically upon BEAF-32 depletion, but not in control cells, within loci exhibiting the highest leakage (top 5%) across a BEAF-32 site.

Figure S1

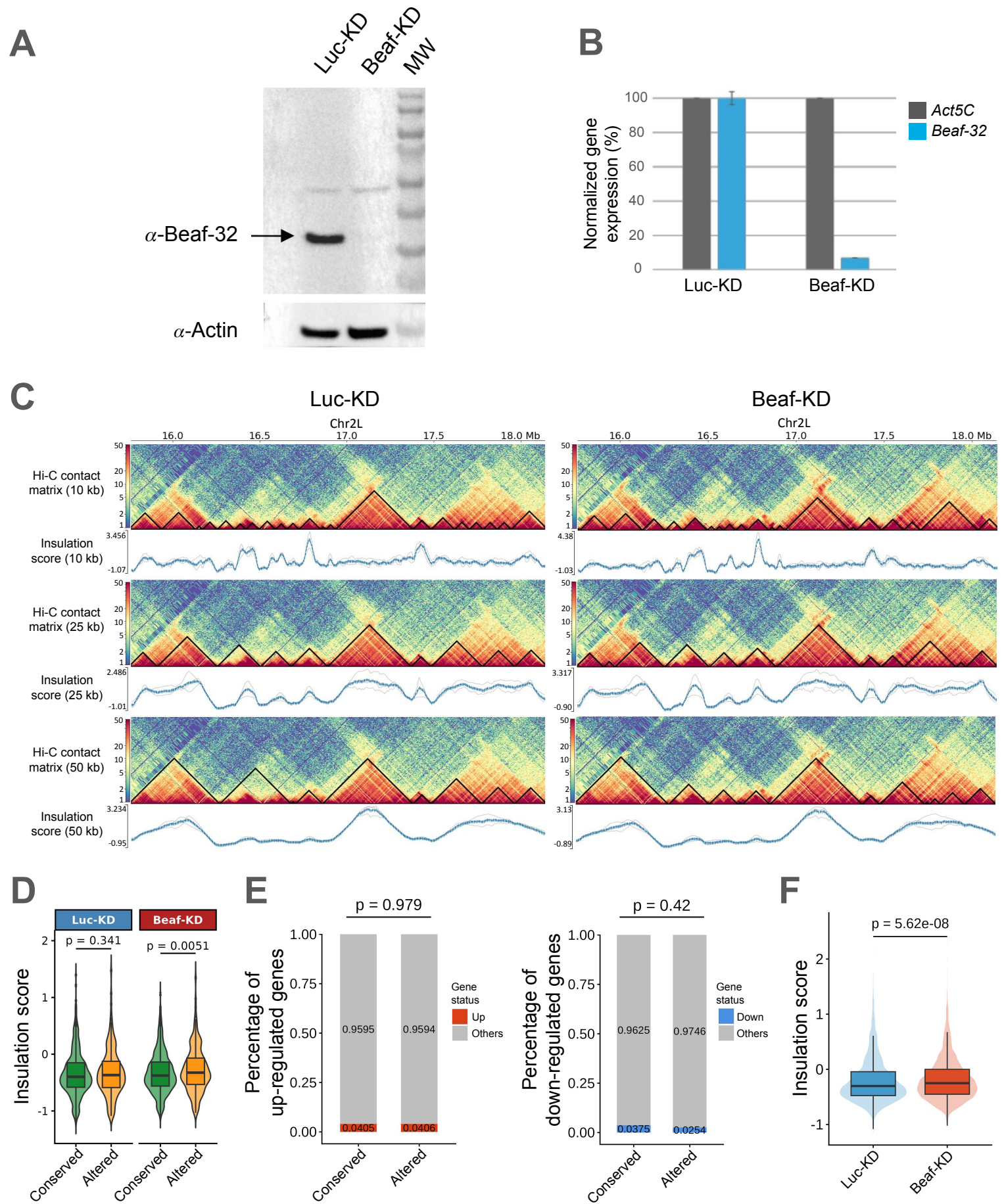


Figure S2

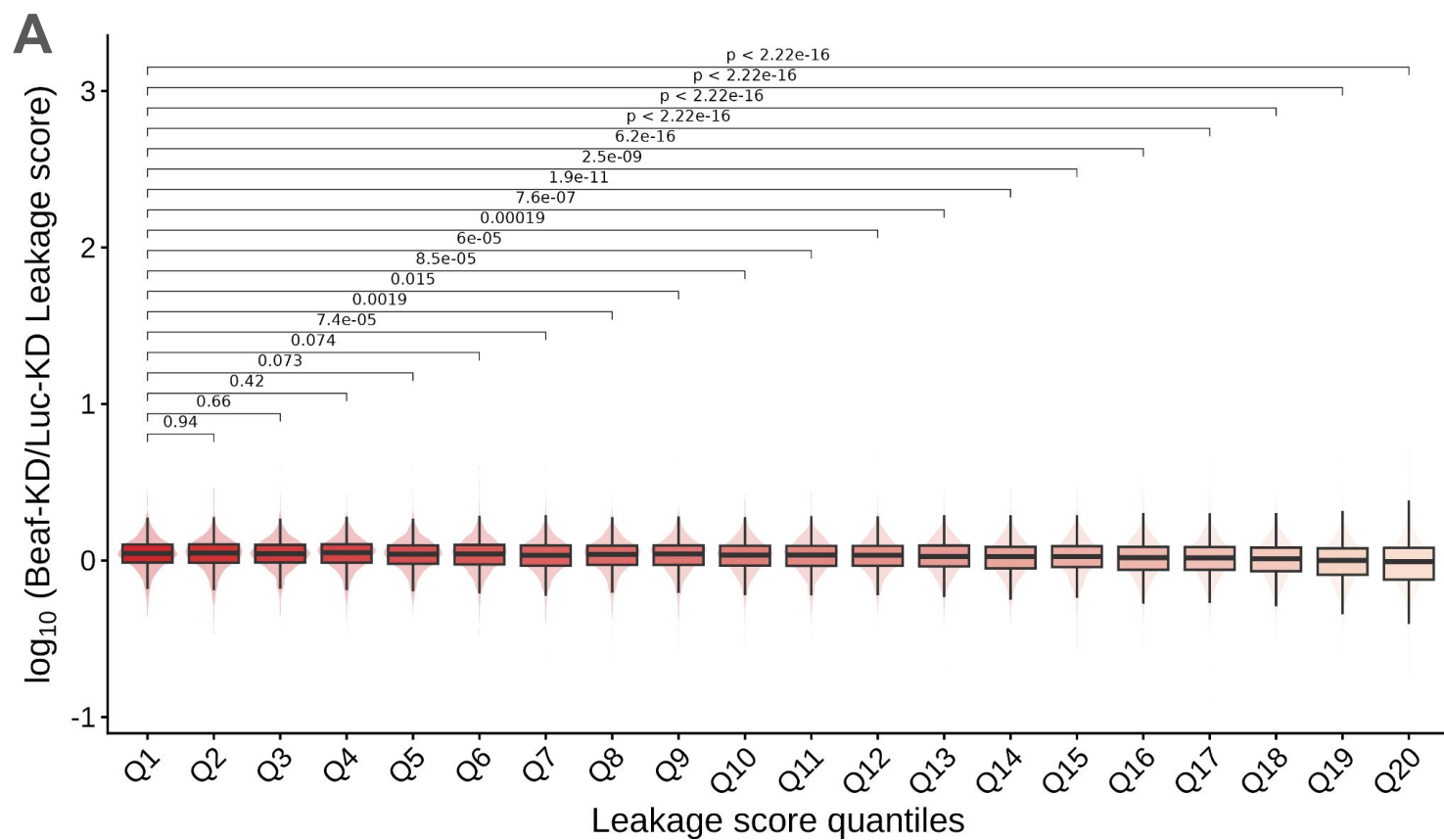
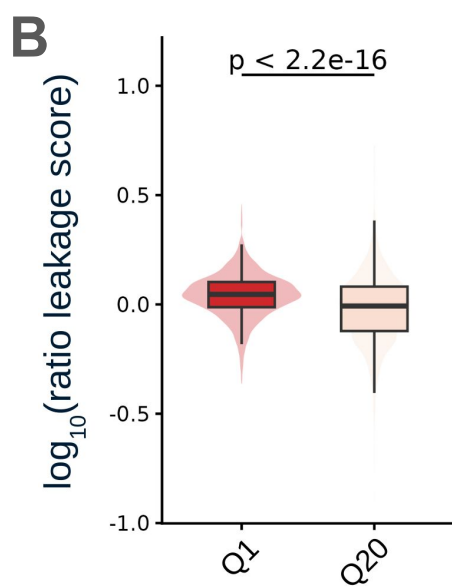
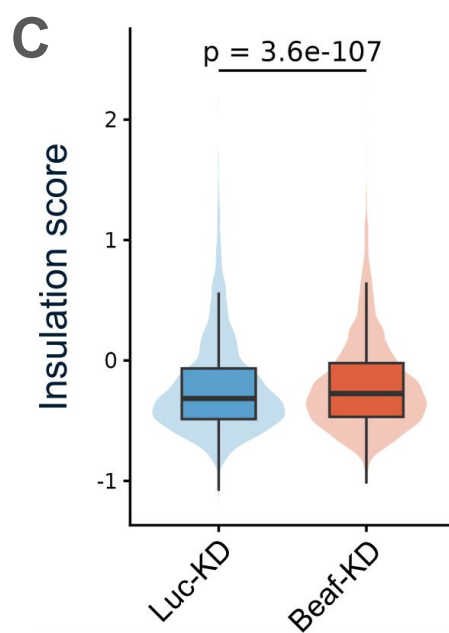
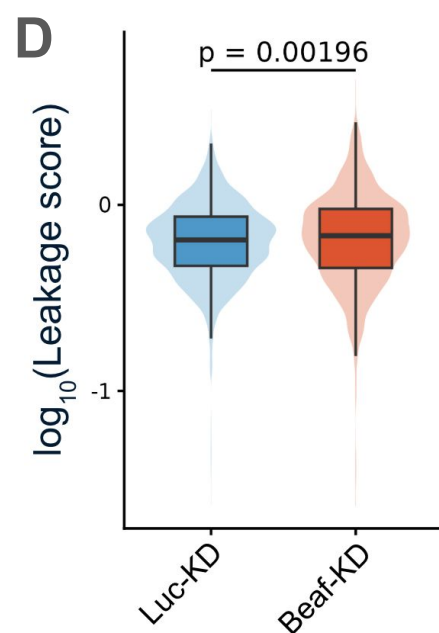


Figure S3

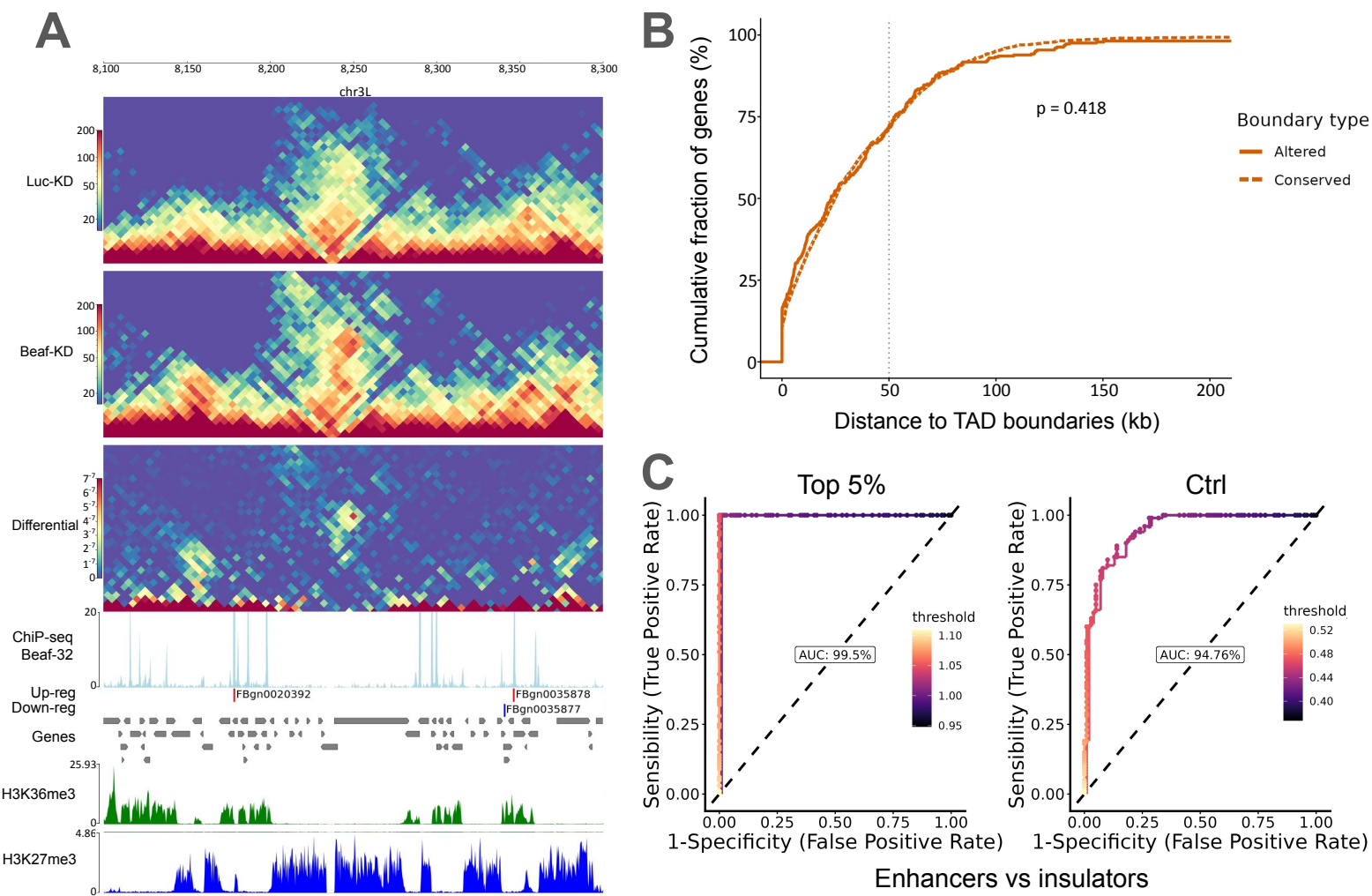


Figure S4

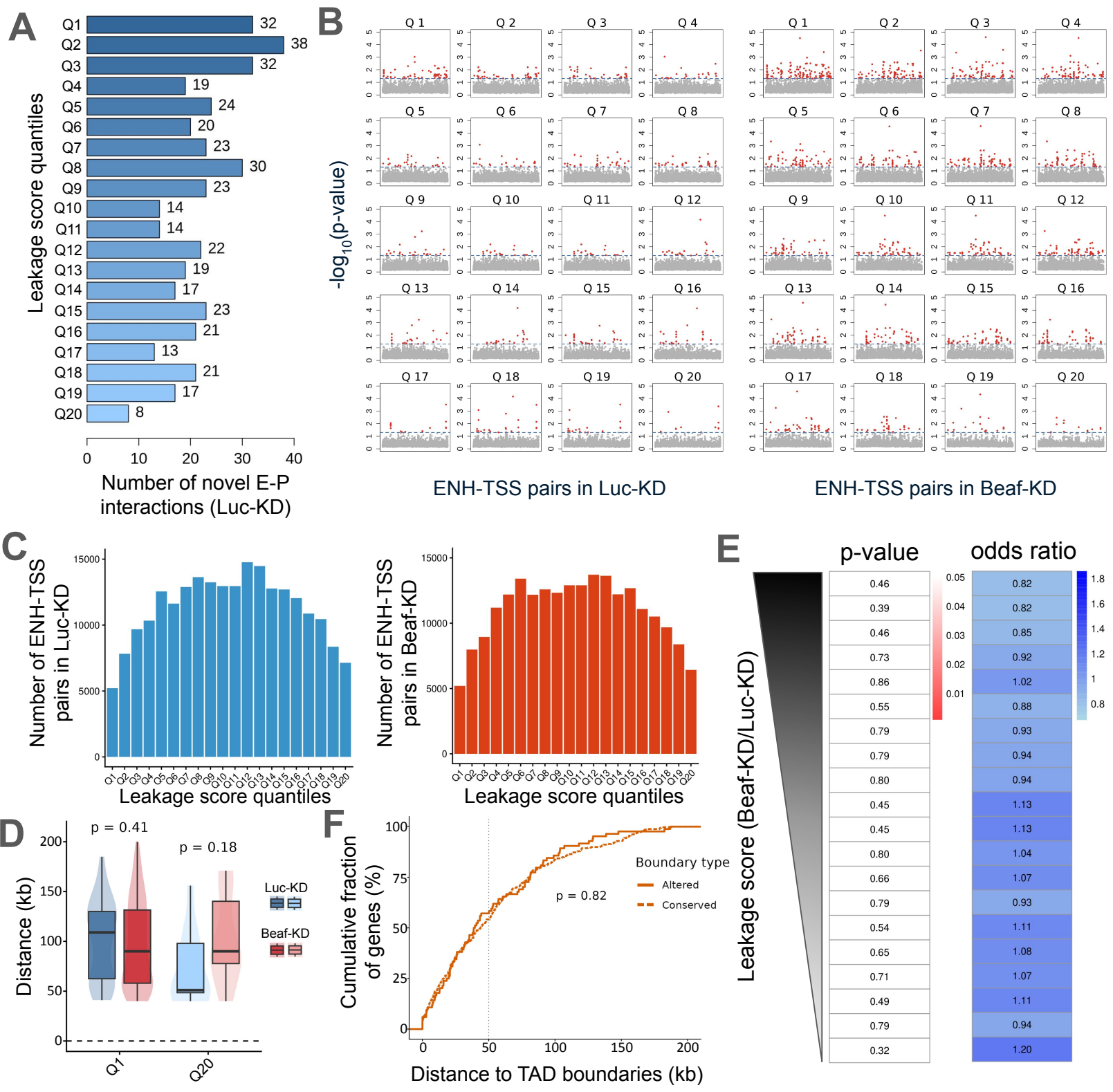


Figure S5

