Expanded View Figures

Figure EV1. *HOTAIR* overexpression has marginal phenotypic and transcriptomic consequences on MDA-MB-231.

A Proliferation assays in two different cell culture conditions. Cells were counted with an automatic cell counter at the time indicated on the plot (individual experiments and mean, $n = 2$).

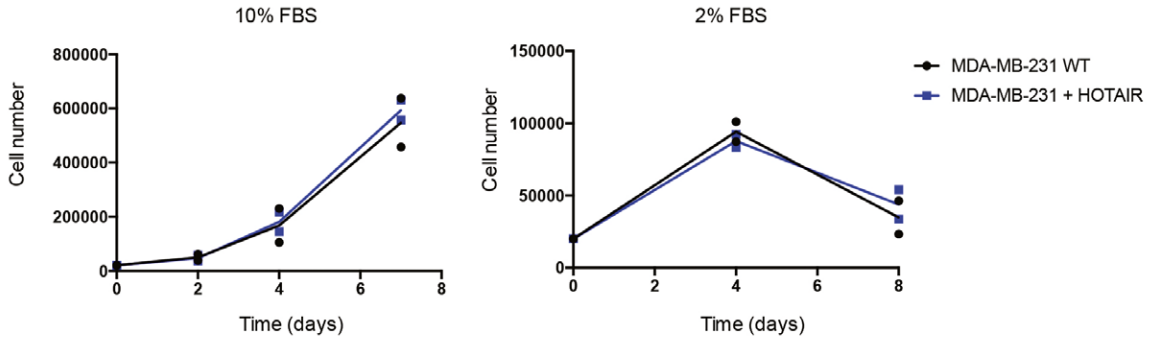
B Pearson correlation matrix between each single-sequenced sample. Value varies between 1 and 0.85.

C Number of differentially expressed genes depending on cutoff parameter.

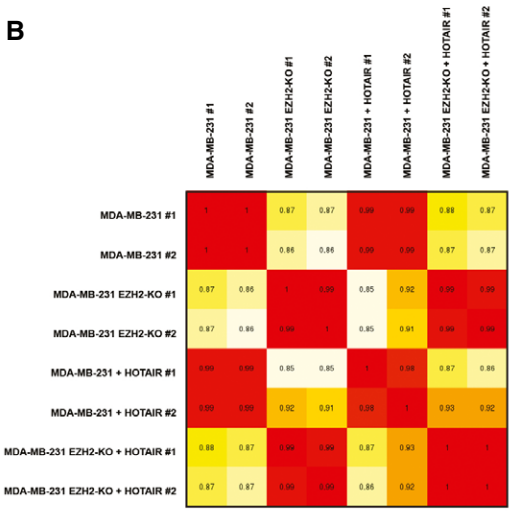
D Volcano plots representing gene expression change upon overexpression of *HOTAIR* in MDA-MB-231 EZH2++– and focusing on genes either previously reported to gain H3K27me3 upon overexpression of *HOTAIR* (left panel) or to be located with 100 kb of a binding sites for *HOTAIR* (right panel) (*y*-axis: $\log_{10}$ *P*-value, *x*-axis: $\log_2$ fold change). Red dots represent genes whose expression changes by more than twofold with a *P*-value < 0.05.

Source data are available online for this figure.

A



B



C

P value <	0.05	0.05	0.01	0.01
LogFC >	1	2	1	2
WT vs WT + HOTAIR	32	20	9	8
KO EZH2 vs KO EZH2 + HOTAIR	20	13	0	0
WT vs KO EZH2	1272	465	1209	463

D

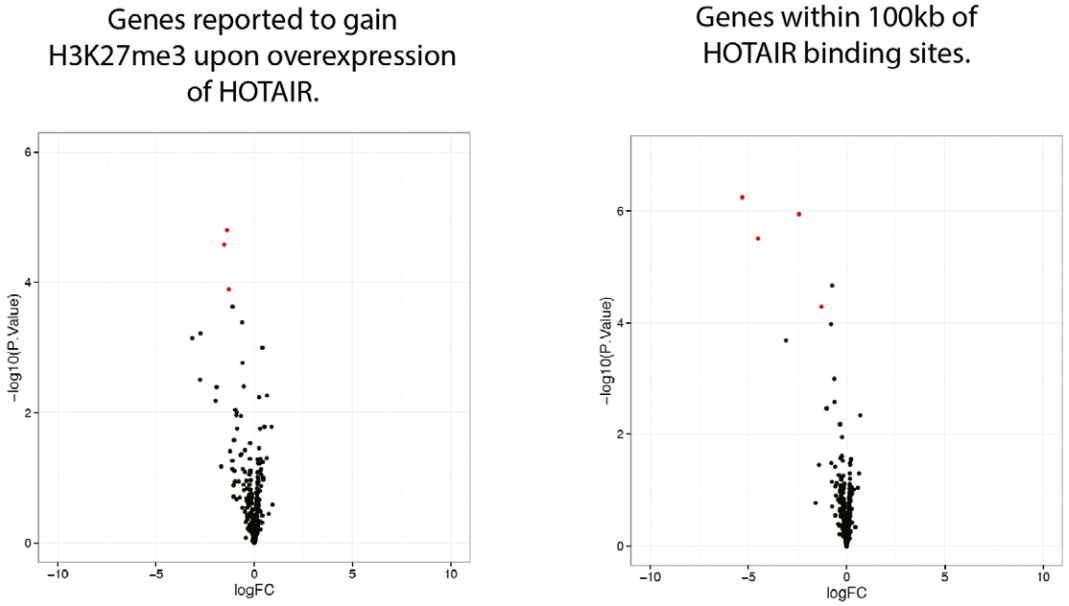


Figure EV1.

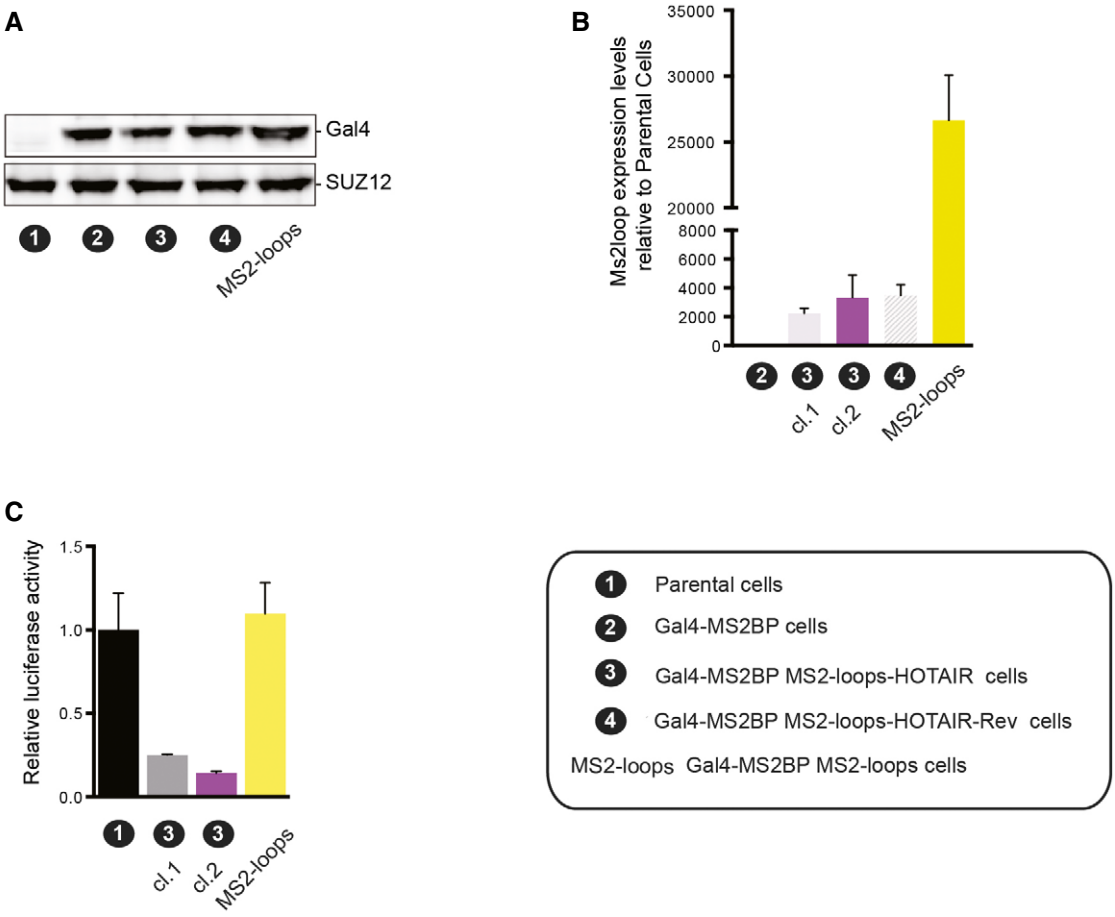


Figure EV2. Expression of Gal4-MS2CP fusion proteins and luciferase activity in clones overexpressing *HOTAIR*.

A Western blot analysis with Gal4 antibody on nuclear extracts from parental cell line (1) MS2BP, (2) MS2BP *MS2-HOTAIR* (3) or MS2BP *MS2-HOTAIR-Rev* (4) and MS2 loop cell lines. SUZ12 was used as a loading control.

B qRT–PCR to detect MS2-hybrid RNAs in the cell lines indicated on the *x*-axis. *Y*-axis represents MS2 loop RNA levels normalized to actin and calculated over the parental cells (mean ± SD, $n = 2$).

C Relative luciferase activity in the cell lines indicated on the *x*-axis. Values represent the relative luciferase activity normalized to the amount of protein (mean ± SD, $n = 3$).

Data information: Correspondence between numbers and model cell lines is indicated at the bottom right.
Source data are available online for this figure.

Figure EV3. Chromatin regulation upon recruitment of *HOTAIR*.

A ChIP experiments with H3K27me3 and primers located along the luciferase reporter (left) or control regions (MYT1 and ACT) (right) in the cell lines indicated on the *x*-axis of each graph (individual experiments and mean, $n = 2$). A H3K27me3 antibody of different origin was used in this assay as compared to Fig 3.

B ChIP experiments with H3K9me2 (top), H3K27ac (middle), or RNA polymerase II (bottom) in the cell lines indicated on the *x*-axis of each graph. Primers used are indicated in the color legend (individual experiments and mean, $n = 2$).

C Analysis of DNA methylation. Schematic representation of the portion of the reporter construct analyzed for DNA methylation is displayed on top. It comprises the Gal4-binding sites (5× UAS), the TK minimal promoter and part of the luciferase gene. DNA methylation analysis by bisulfite cloning is shown below in three different cell lines as indicated on the right. Black circles indicate methylated CpGs, and white circles indicate unmethylated CpGs. The percentage of methylated CpG is indicated below each condition.

Data information: Correspondence between numbers and model cell lines is indicated at the bottom right.
Source data are available online for this figure.

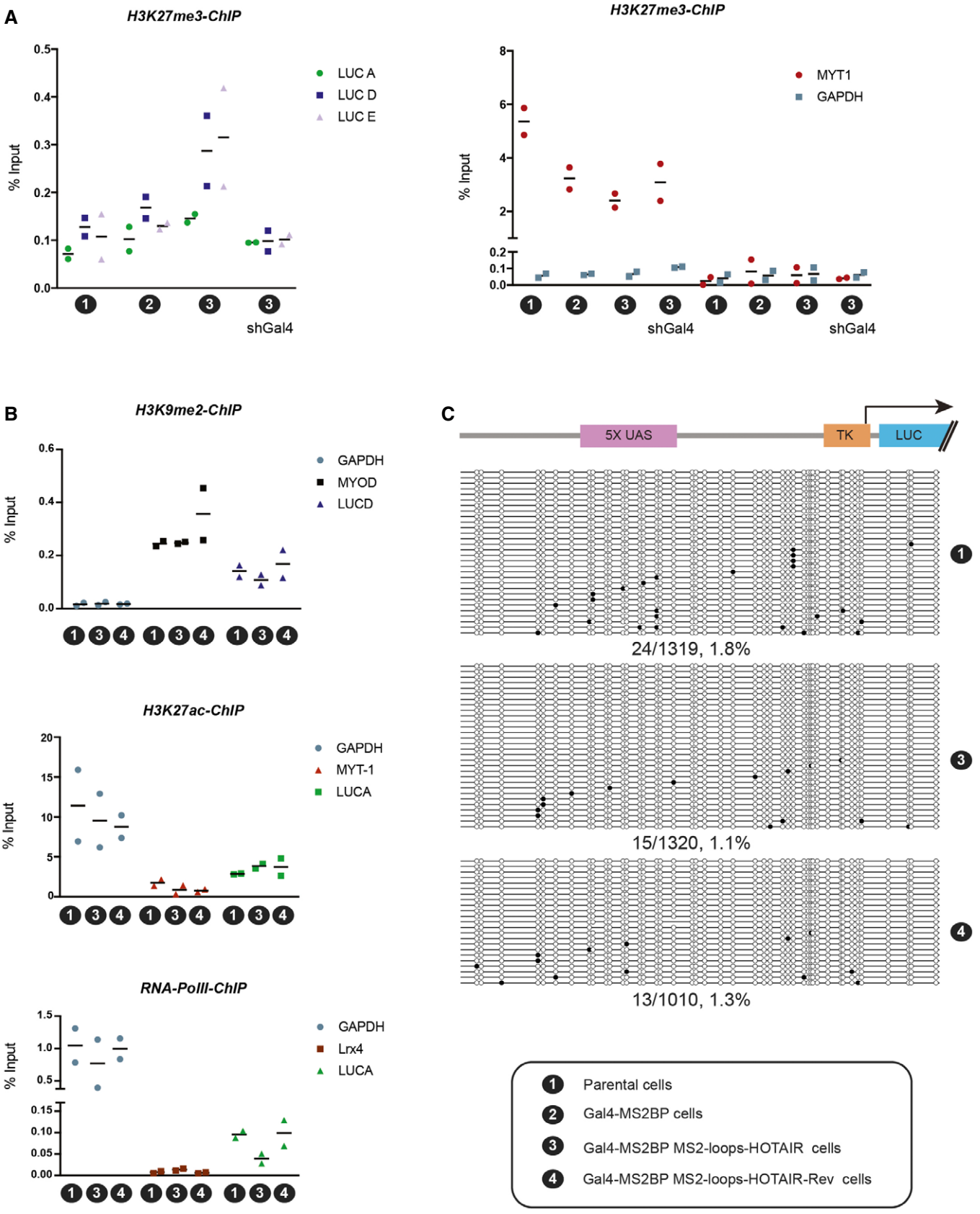


Figure EV3.

Figure EV4. PRC2 and *HOTAIR in vitro* production.

A Scheme for PRC2 production in Sf9 insect cells and purification over MiniQ column (left panel). Coomassie of eluted fractions. EZH2, SUZ12, EED-FLAG and RbAp48 proteins are indicated (right panel).

B Agarose gel showing 2.2-kb full-length *in vitro* transcribed *HOTAIR* and *HOTAIR-Rev* RNAs.

C Left: Scheme representing the interacting partners (chromatin and RNA) analyzed by sucrose gradient sedimentation on the right. Right: Native chromatin was incubated with or without biotinylated *HOTAIR/HOTAIR-Rev* RNAs, and chromatin conformation was analyzed by density gradient centrifugation.

D Left: Scheme representing the interacting partners (chromatin, RNA and PRC2) analyzed by sucrose gradient on the right. Right: PRC2 and native chromatin were incubated with or without biotinylated *HOTAIR/HOTAIR-Rev* RNAs and their profile analyzed by density gradient centrifugation.

Source data are available online for this figure.

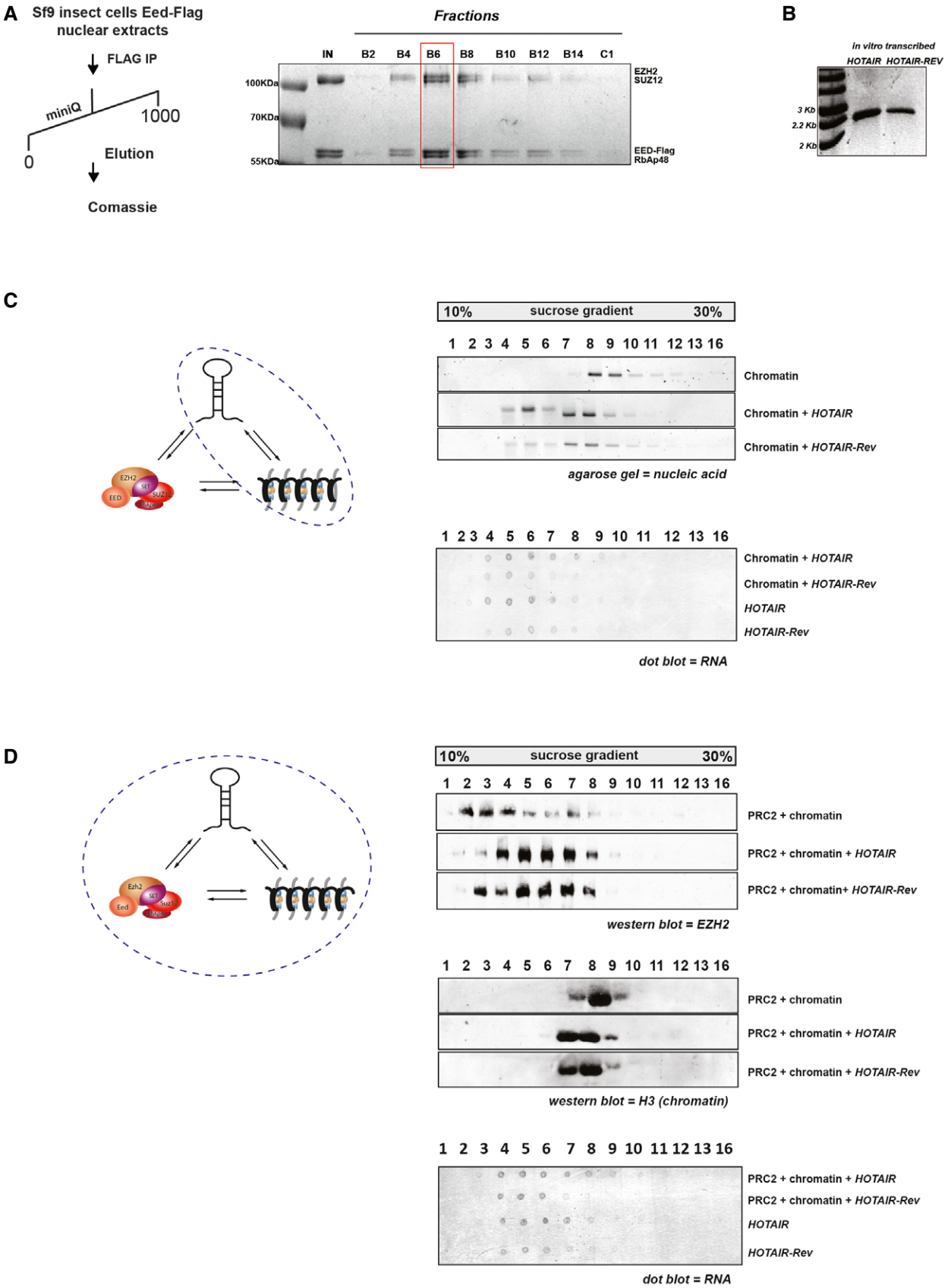


Figure EV4.

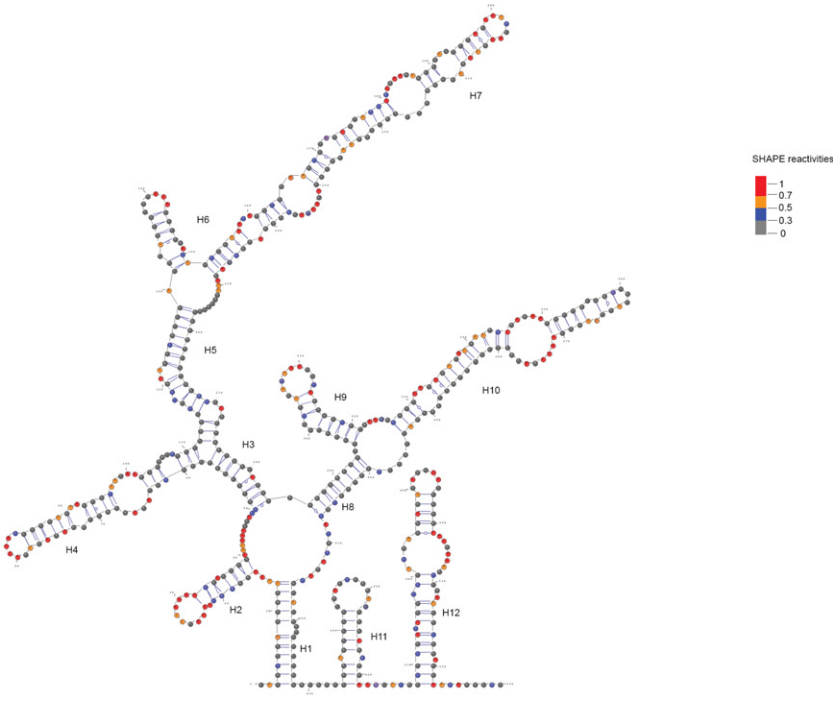


Figure EV5. *HOTAIR* domain 1 secondary structure.

Secondary structure model for the first 530 bp of *HOTAIR* RNA obtained from the RNAstructure software using the 1M7 reactivity map obtained in this study and displayed with predictive secondary structure. *HOTAIR* D1 domain as mapped with 1M7 SHAPE probing from our experiment is shown. SHAPE reactivity is depicted by colored nucleotides. Highly reactive nucleotides are displayed in red and orange, and low reactive nucleotides are displayed in black or blue according to the values reported in the legend.

Source data are available online for this figure.