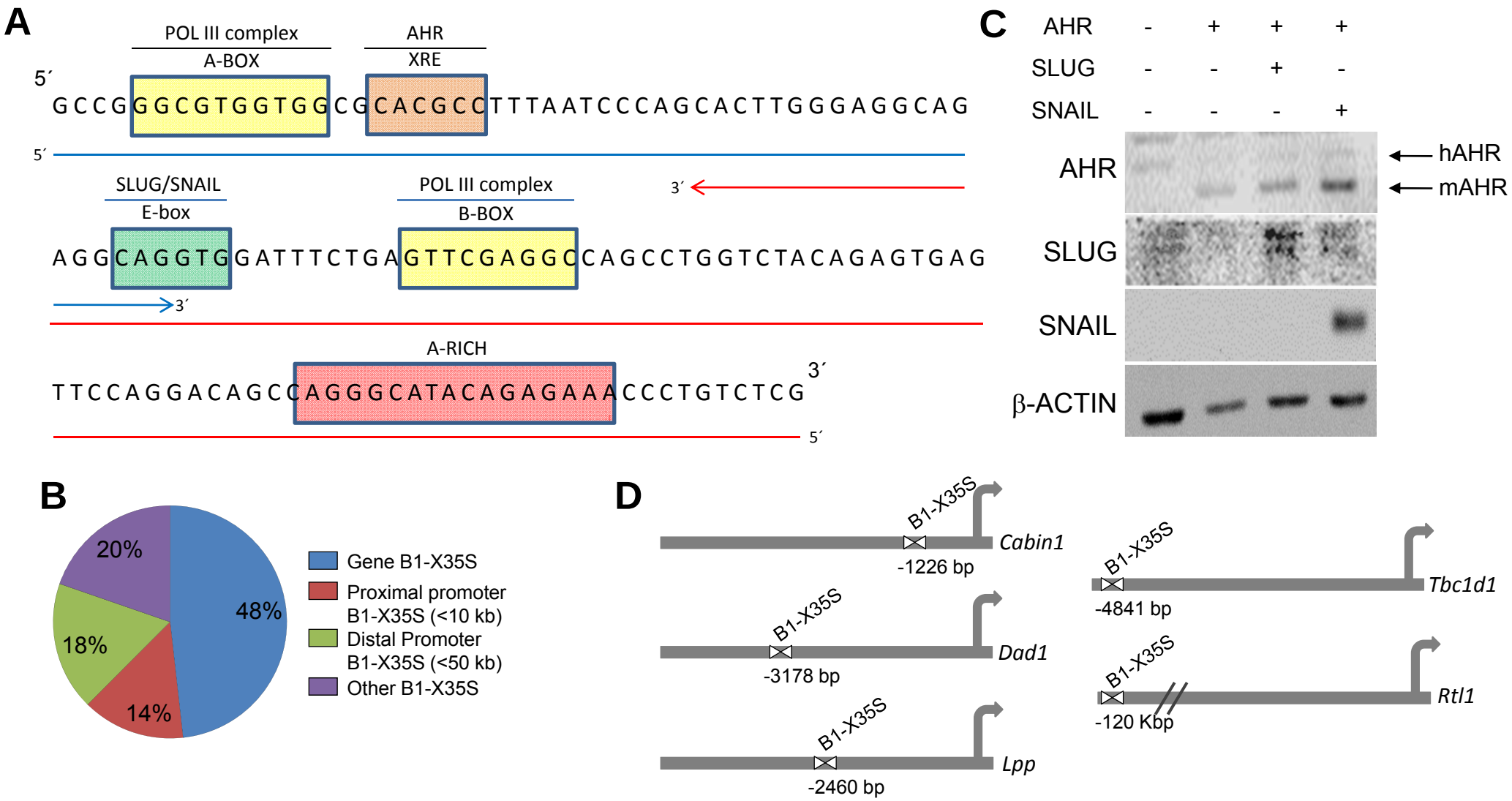


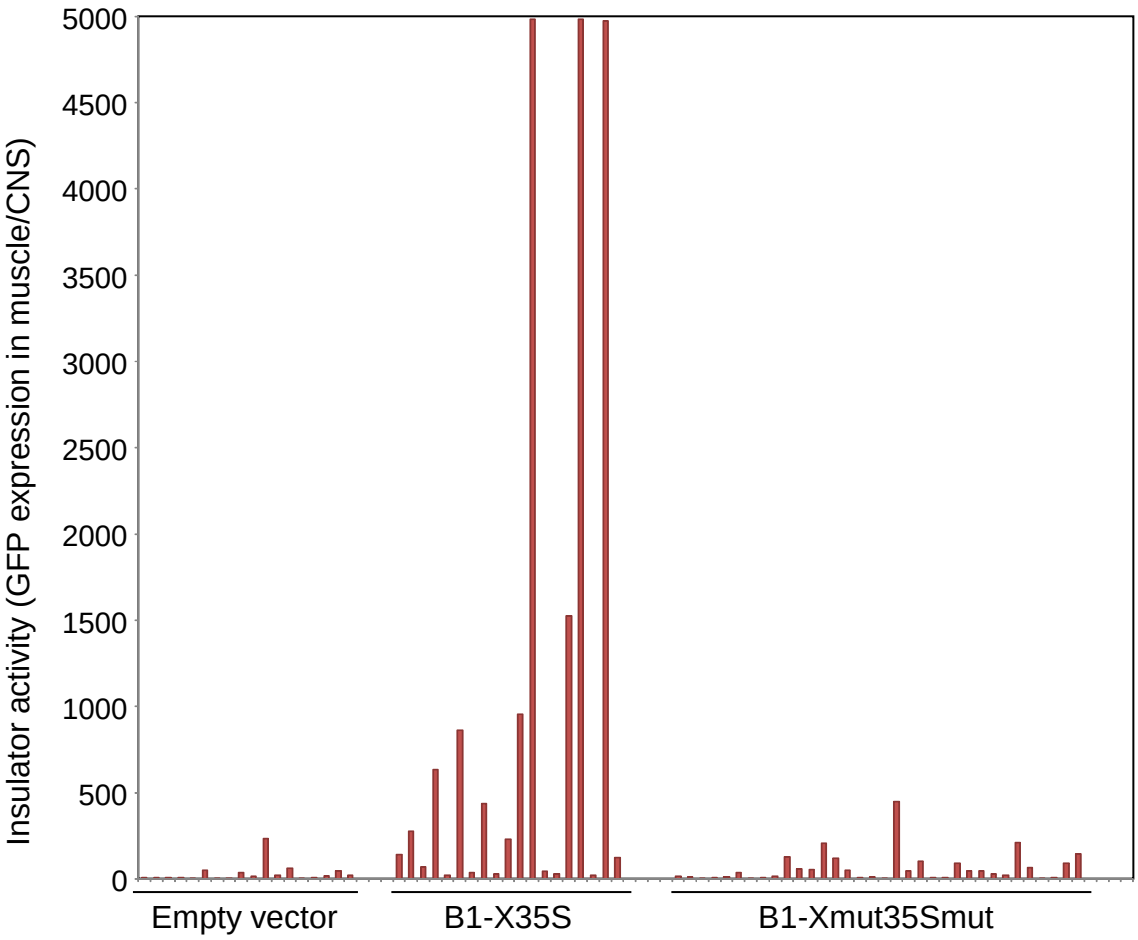
Supplementary Figure 1. Consensus sequences for DNA binding proteins in the B1-X35S retrotransposon and its prevalence in several genomic locations.

(A) Elements for the dioxin binding AhR (XRE), the epithelial-to-mesenquimal regulators SLUG and SNAIL (E-box) and for RNA polymerase III complex (A and B boxes) are indicated. The long blue and red arrows indicate the sequence of the forward and reverse primers used to amplify by PCR the full length B1-X35S retrotransposon (see Table SII).

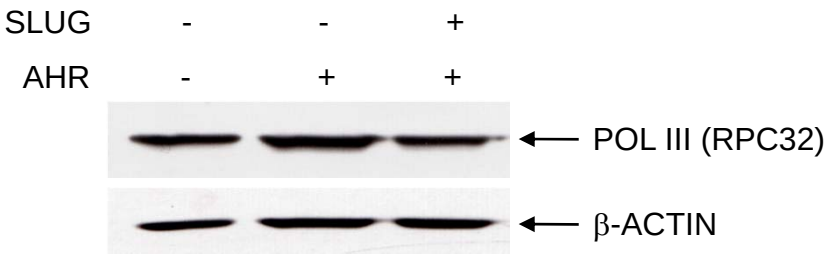
(B) B1-X35S localization was determined in the proximal promoter (red), distal promoter (green), non-promoter gene regions (blue) and any other location excluding all of the above (purple).

(C) Western blot detection of AHR, SLUG and SNAIL in human HEK 293 cells under basal conditions and after transient transfection with the corresponding expression plasmids. Note that the transfected high affinity AHRb1 mouse protein has a lower molecular weight (~90 kDa) than its human endogenous homolog (~106 kDa).

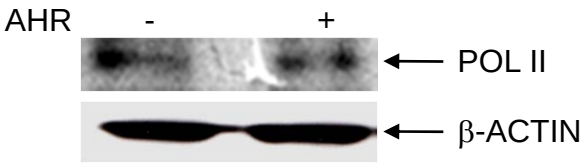
(D) Location of the B1-X35S element in the *Cabin1, Dad1, Lpp, Tbc1d1 and Rtl1* gene promoters. Nucleotide positions are refered to the TSS.



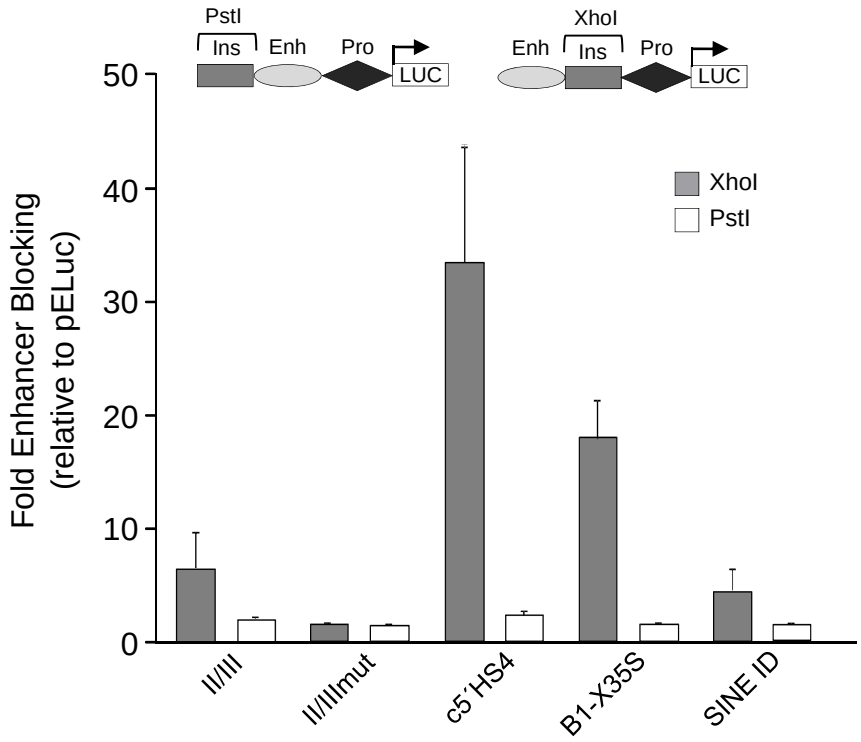
Supplementary Figure 2. Analysis of insulator activity in individual specimens of zebrafish transfected with the indicated constructs. Insulator activity was measured as the ratio of GFP signal driven by a muscle promoter with respect to that modulated by a CNS enhancer. At least 19 animals were quantified for B1-X35S and 34 for B1-Xmut35Smut. Eighteen additional zebra fish specimens were analyzed as negative controls (empty vector without retrotransposon). Higher values indicate lower expression in CNS and thus stronger insulation from the Z48 enhancer. The experiment was repeated twice.



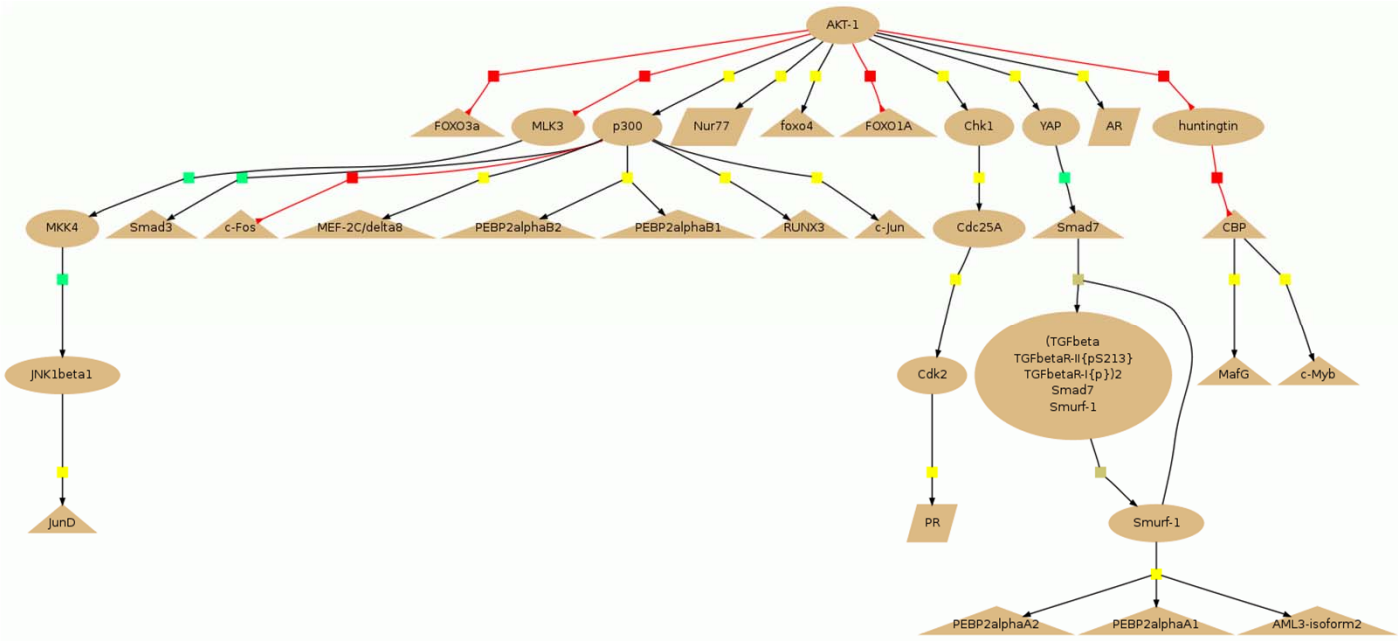
Supplementary Figure 3. Western blot detection of RNA POL III complex subunit RPC32 in Hepa I cells under basal conditions and after transient transfection with expression plasmids for AHR and SLUG. Protein levels of β-ACTIN were used as loading control.

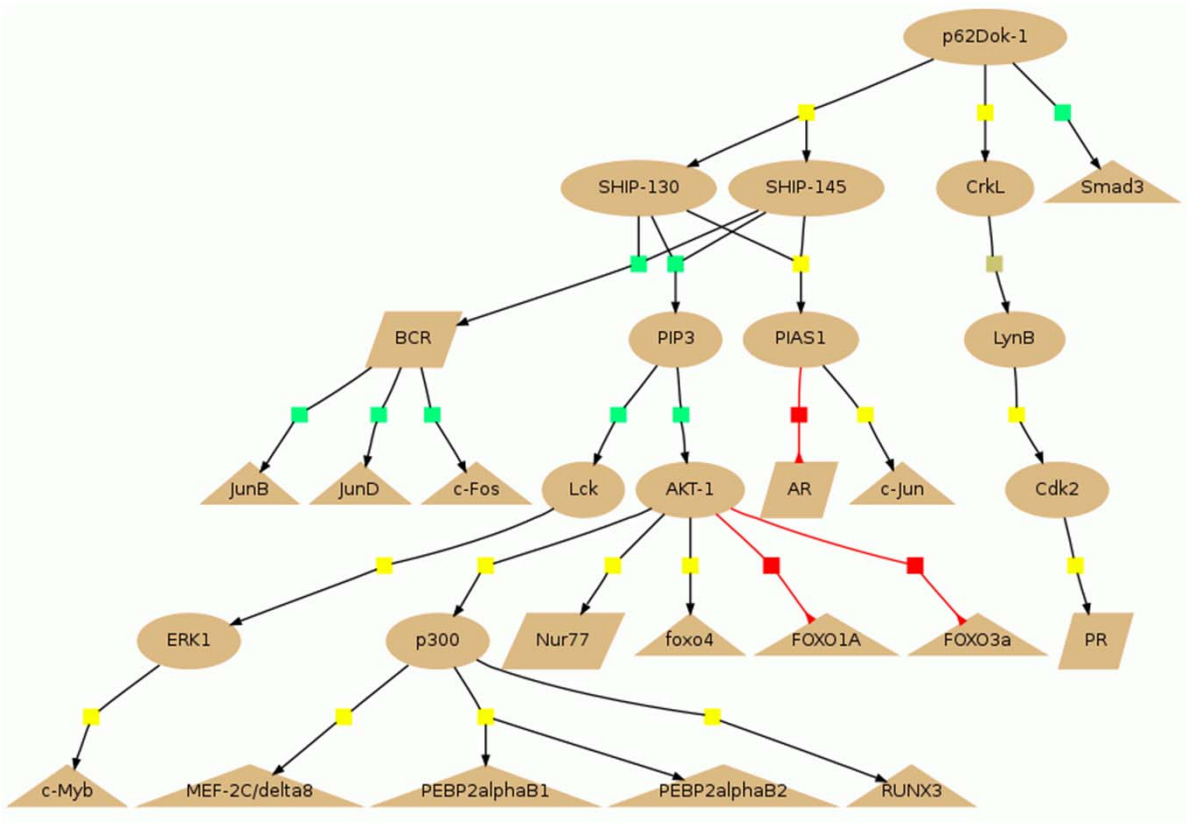


Supplementary Figure 4. Western blot detection of RNA POL II in Hepa I cells under basal conditions and after transient transfection with expression plasmids for AHR and SLUG. Protein levels of β-ACTIN were used as loading control.

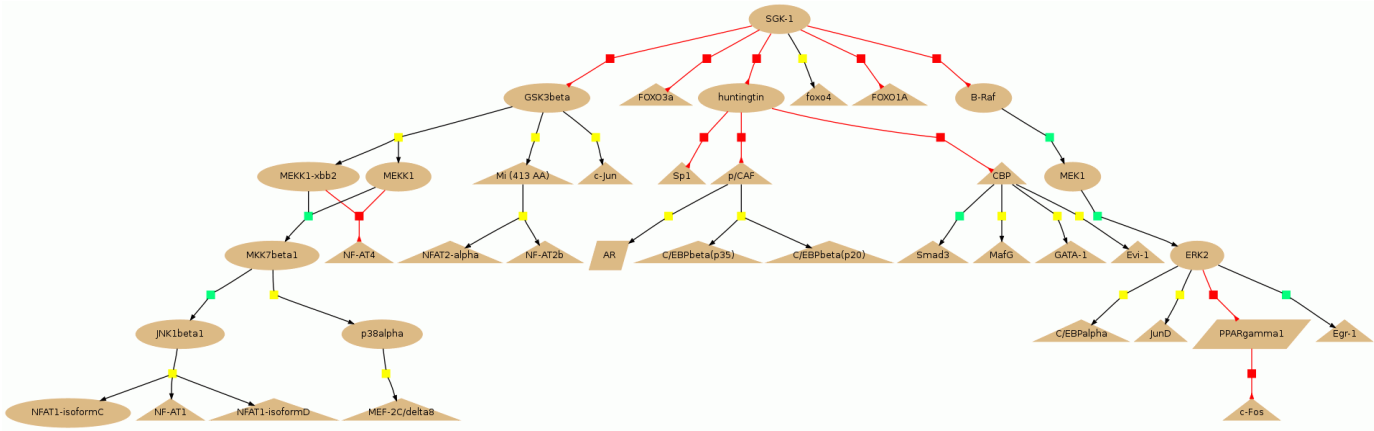


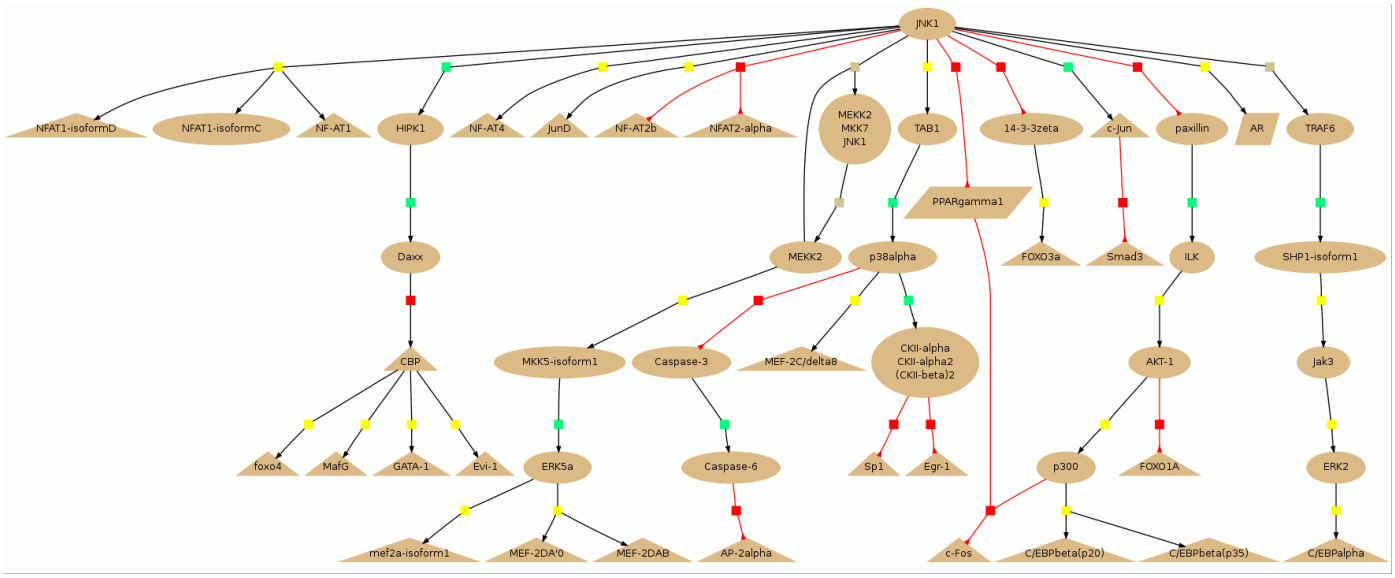
Supplementary Figure 5. Enhancer blocking assay to analyze the insulator activity of a SINE ID element. The SINE ID was cloned flanking the enhancer and promoter (PstI construct) or between both regulatory sequences (XhoI construct) and its ability to regulate luciferase activity measured in transfected MEK 293 cells. The SINE ID has a much lower insulator activity than B1-X35S. The experiment was repeated twice.



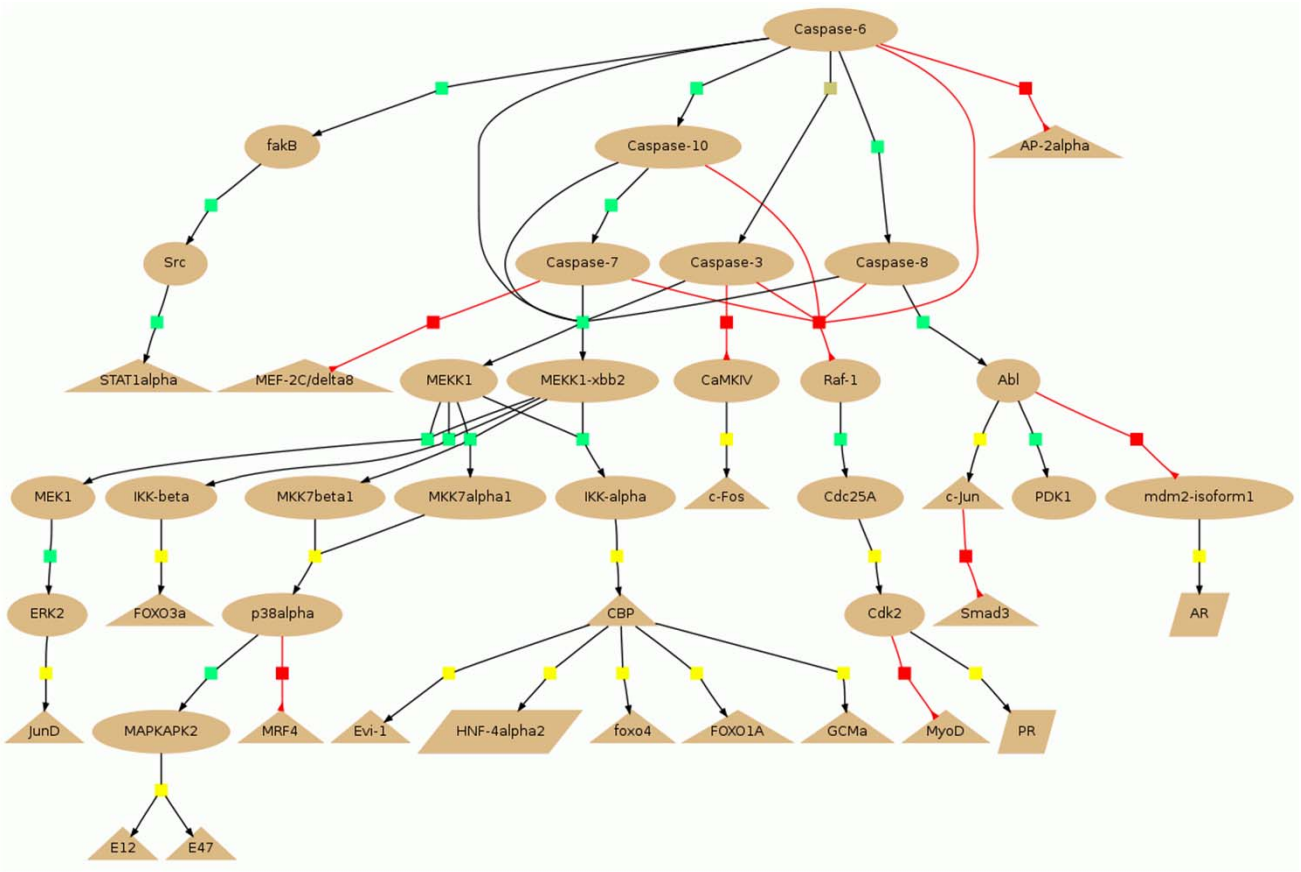


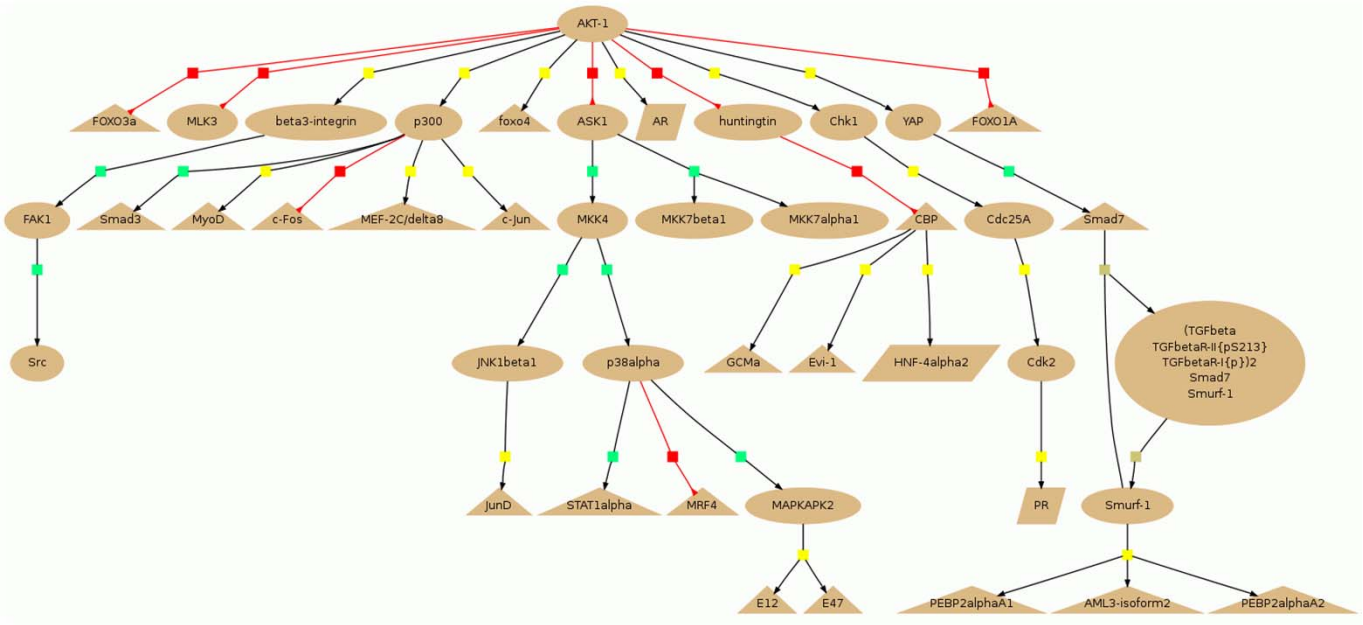
Supplementary Figure 6. There were 2555 genes having a B1-X35S element in their promoters (-10 kbp to +1) and Explain identified two significant networks: AKT1 (false discovery rate FDR=0.01) and p62DOK1 (FDR=0.026). AKT1 is known as a thymoma viral proto-oncogene with serine-threonine kinase activity that acts in G protein-coupled receptor (GPCR) pathways regulating cell proliferation, apoptosis and angiogenesis. In humans, AKT1 is aberrantly expressed in ovarian neoplasms, gliomas, and various other cancers. p62DOK1 (docking protein 1) binds ABL and RASGAP and it is involved in cell adhesion to the extracellular matrix, migration and growth. Human p62DOK1 is associated with chronic lymphocytic leukemia. Explain program (BioBase; http://biobase-international.com/pages/index.php?id=286).



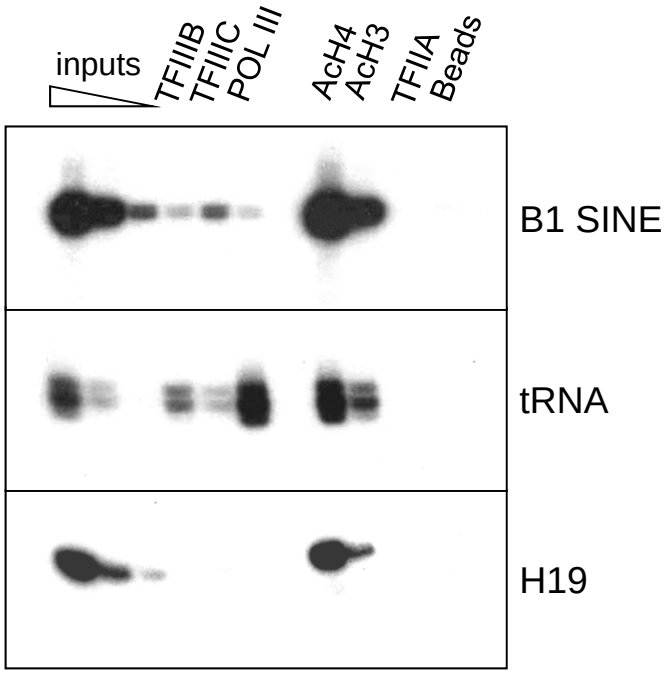


Supplementary Figure 7. In 4997 genes the B1-X35S element was located within the transcriptional unit, mainly in introns but also in 5'UTR and 3'UTRs. Explain found two main transcriptional networks: SGK-1 (FDR=0.008) and JNK-1 (FDR=0.046) (Supplementary Fig. 6). SGK-1 (serum-glucocorticoid regulated kinase 1) is a protein serine-threonine kinase with anti-apoptotic activity that acts in neurite development. Human SGK1 is associated with hyper-insulinism, type 2 diabetes mellitus, pulmonary fibrosis, and several neoplasms. JNK-1 (c-Jun NH2 terminal protein kinase 1) is a mitogen activated protein kinase that regulates protein phosphorylation, JAK-STAT cascade, apoptosis, caspase activity and angiogenesis. Explain program (BioBase; http://biobase-international.com/pages/index.php?id=286).





Supplementary Figure 8. A total of 5325 genes were identified between -10 kbp and -50 kbp encompassing transcriptional networks for CASPASE-6 (FDR=0) and, again, AKT-1 (FDR=0.012), although using other intermediate signaling molecules. Explain program (BioBase; http://biobase-international.com/pages/index.php?id=286).



Supplementary Figure 9. Comparison by ChIP of crosslinking of endogenous TFIIIB, TFIIIC and POL III at B1 SINEs and $tRNA^{Leu}$ genes. The Pol II-dependent H19 locus is included as a control. Positive control antibodies recognize acetylated histones H3 (AcH3) and H4 (AcH4), whilst antibody against TFIIA and beads without antibody provide negative controls.