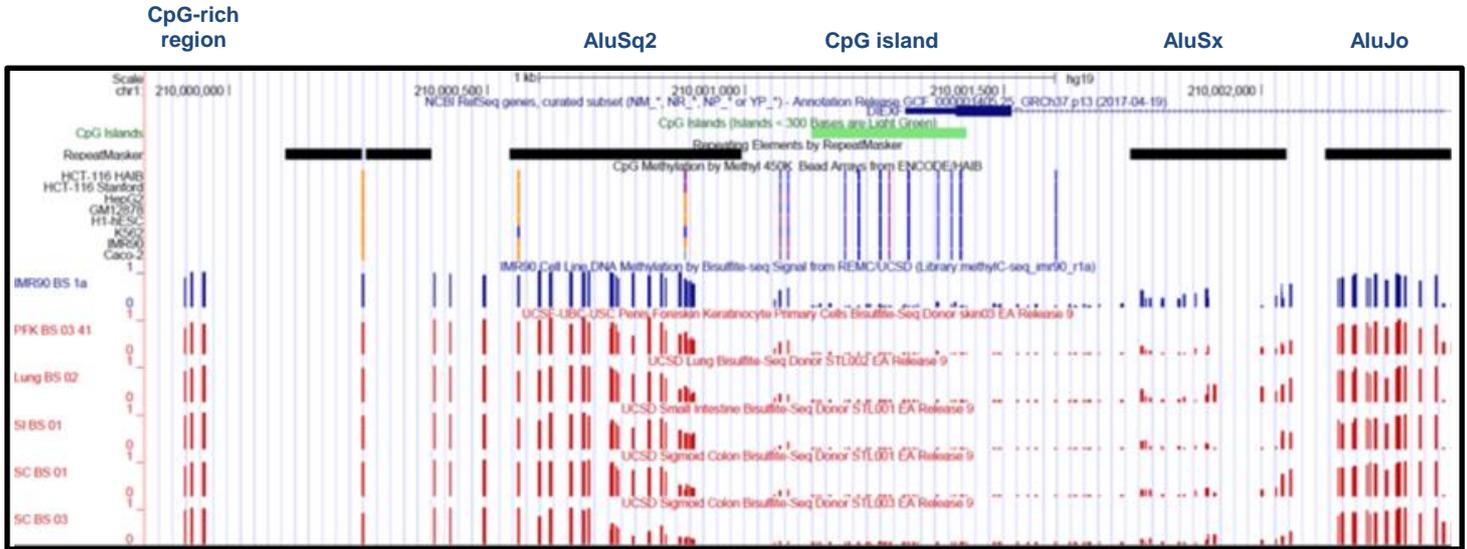


SUPPLEMENTAL FIGURES

**Tissue and cancer specific expression of DIEXF is
epigenetically mediated by an Alu repeat**

Figure S1



- orange = methylated (score >= 600)
- purple = partially methylated (200 < score < 600)
- bright blue = unmethylated (0 < score <= 200)
- black = NA (score = 0)

Figure S1. DNA methylation profile of the *DIEXF* gene promoter region. Annotated CpG islands and repeat elements are indicated using the Repeat Masker (hg 19, updated 2009-04-24). (A) DNA methylation data have been obtained from ENCODE (generated using the Infinium Human Methylation 450K bead array platform from Illumina) and the Roadmap Epigenomics (generated using whole genome bisulfite sequencing).

Figure S2

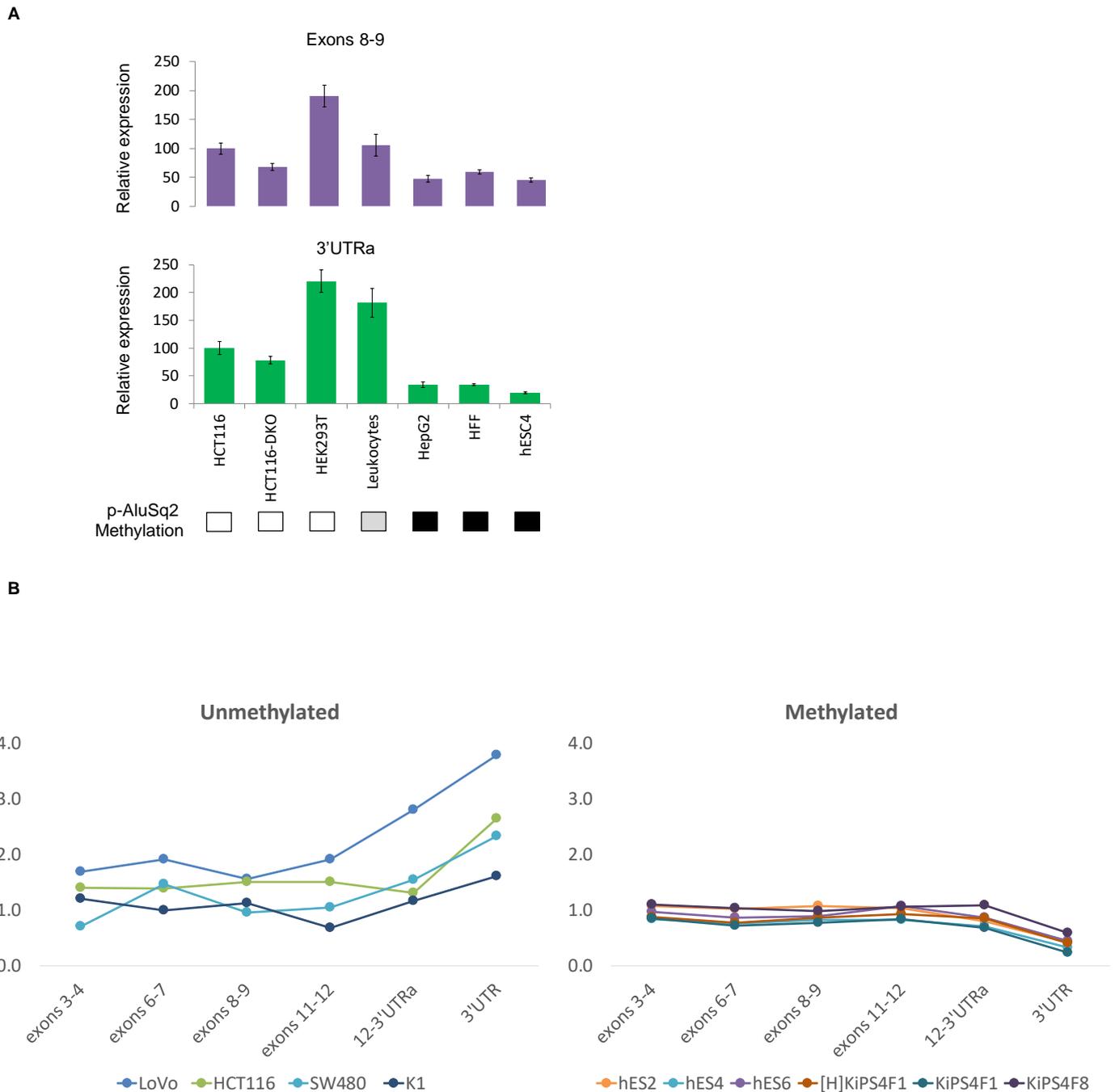


Figure S2. *DIEXF* gene transcriptional characterization. (A) Relative expression of *DIEXF* analyzed by RT-qPCR in different cell lines using 2 different primer pairs (Exons 8-9 covering different *DIEXF* transcripts and 12-3'UTRa specific of the long 8.5 kb transcript). The methylation status of p-AluSq2 for each sample is indicated in a box next to its name using a grey scale (black: full methylation; grey: partially methylated; white: no methylation; see Figure 1 for more details). Expression levels were normalized using 2 reference genes (*PPIA* and *PSMC4*) and represented relative to HCT116 cells. (B) Expression profiles of different *DIEXF* exons analyzed by RT-qPCR in different cell lines with the p-AluSq2 unmethylated or methylated. Expression levels were normalized using 2 reference genes (*PPIA* and *PSMC4*). Data were normalized by the mean of all samples for each amplified region.

Figure S3

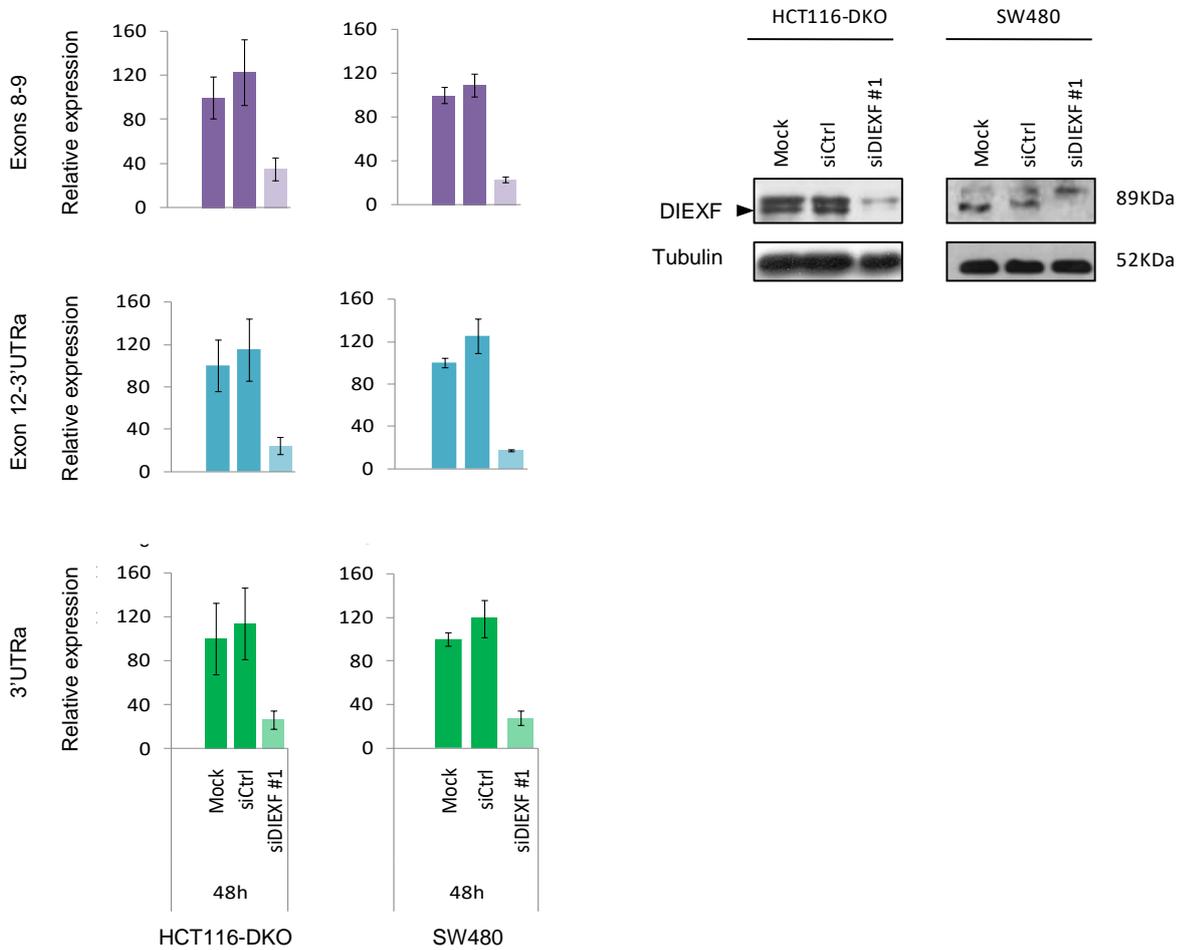


Figure S3. Silencing of *DIEXF* in HCT116-DKO and SW480 by small interfering RNA. A siRNA located in exon 6 common to all transcripts (siDIEXF#1) was used. As controls we used the same cell line plus Lipofectamine 2000 (Mock) and transfected with a non-targeting control siRNA control (siCtrl). (A) mRNA expression levels of *DIEXF* gene measured by RT-qPCR using different sets of primers at 48h after transfection of the siRNA. Expression levels were normalized using 2 reference genes (PPIA and PSMC4) and expressed relative to Mock. (B) Western Blot showing the *DIEXF* protein levels at 48h post-transfection. The band that corresponds to *DIEXF* protein is indicated with an arrow.

Figure S4

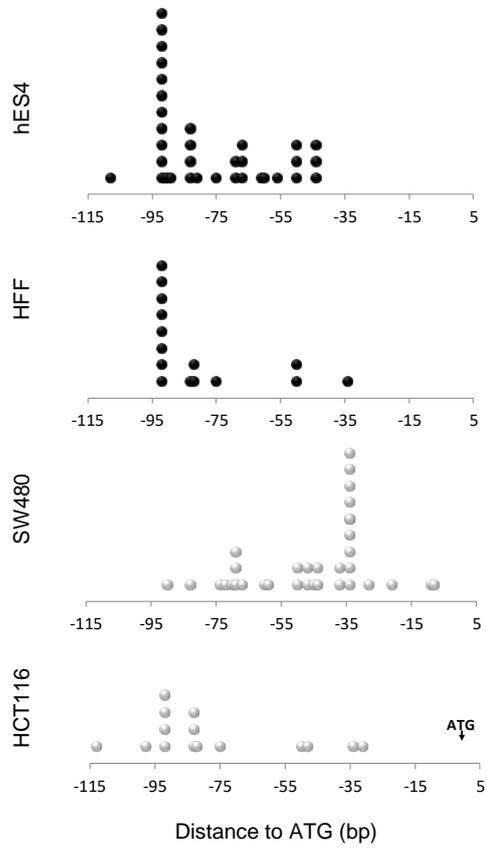


Figure S4. Transcriptional regulation of *DIEXF*. Location of Transcription Start Sites identified by 5'RACE in two cell lines with the p-AluSq2 fully methylated (hES4 and HFF) and two cell lines harboring low or null methylation of the p-AluSq2 (SW480 and HCT116). Positions are relative to the start codon (ATG, position 1). Each circle corresponds to a clone.

Figure S5

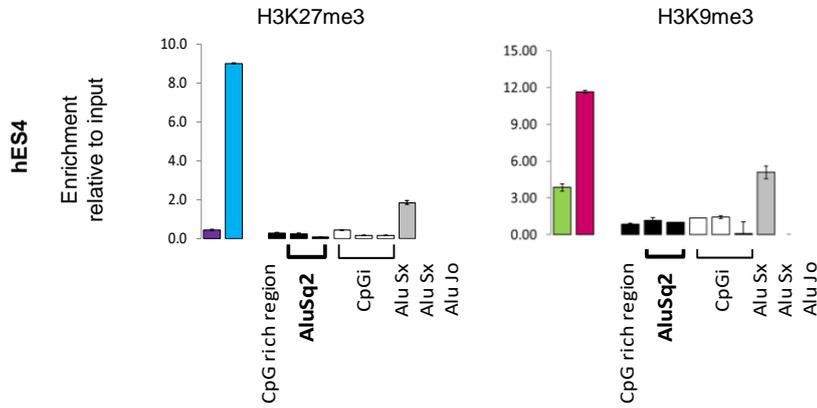


Figure S5. Enrichment in histone modifications associated with inactive chromatin along the *DIEXF* promoter region. ChIP assays were performed with antibodies against H3K27me3 and H3K9me3. Different genomic elements within *DIEXF* promoter region (see Figure 1C) were analyzed by qPCR in hES4. In the cell lines HFF, SW480, LoVo, HCT116-DKO, HCT116 we did not obtain any enrichment of inactive marks. CPLX2 (purple bar) and DRD1 (blue bar) were used as negative and positive control, respectively, for H3K27me3, while GAPDH (green bar) and 16CEN (red bar) were used as negative and positive control, respectively, for H3K9me3. Results are reported as enrichment of immunoprecipitated DNA relative to the input. The DNA methylation levels of each region are depicted using a grey scale (black: full methylation; grey: partially methylated; white; no methylation; see Figure 1 for more details).

Figure S6

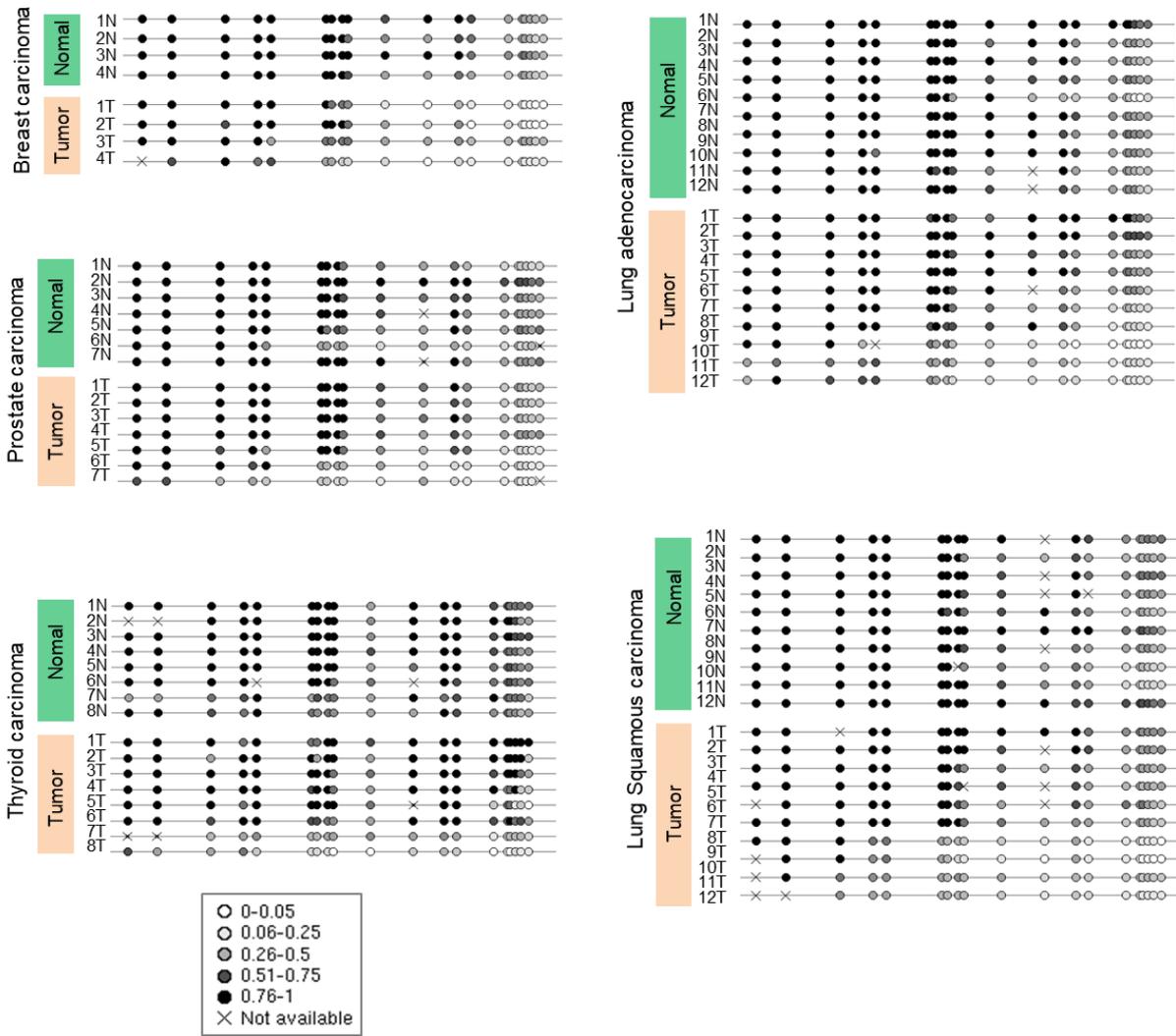


Figure S6. AluSq2 DNA methylation status and *DIEXF* expression in different cancer types. Analysis was performed by bisulfite sequencing in normal-tumor pairs of patients with breast, prostate, thyroid, lung adenocarcinoma and lung squamous carcinoma. Each circle represents a CpG and the DNA methylation level is represented using a grey scale: black is fully methylated and white fully unmethylated.

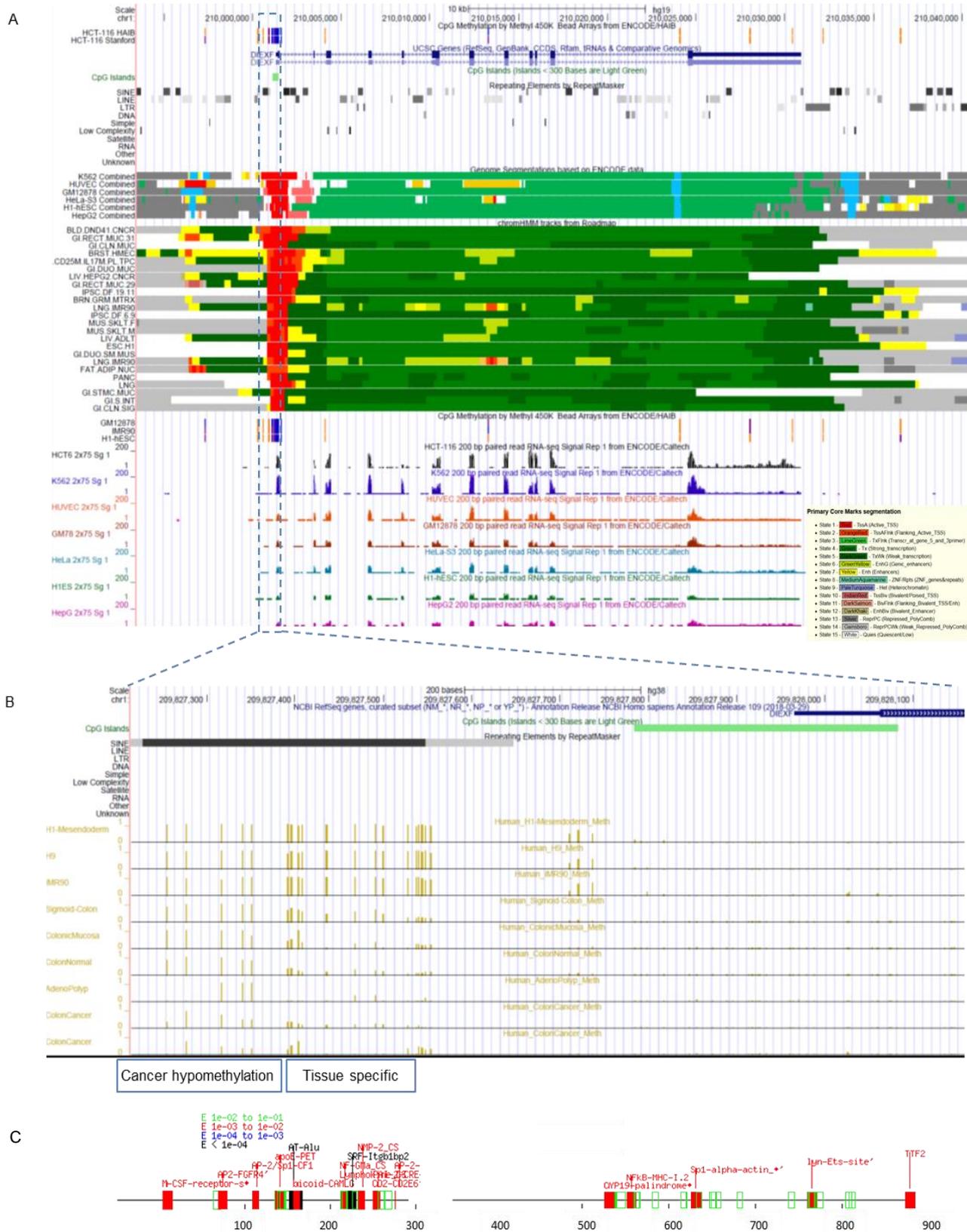


Figure S7. (A) Epigenomic and transcriptomic profile of *DIEXF* gene and promoter region in different tissue types. (B) Zoom in of the promoter region including the AluSq2 element and DNA methylation profile determined by WGBS in human embryonic stem cells, IMR90 fibroblasts, normal colon mucosa and colon cancers. The differentially methylated regions in tissue types and cancer are indicated below. (C) Location of transcription factor binding sites predicted using the Tfsitescan tool along the region displayed in B.