Supplementary data

Epigenetic Repression of *microRNA-129-2* Leads to Overexpression of *SOX4* Oncogene in Endometrial Cancer

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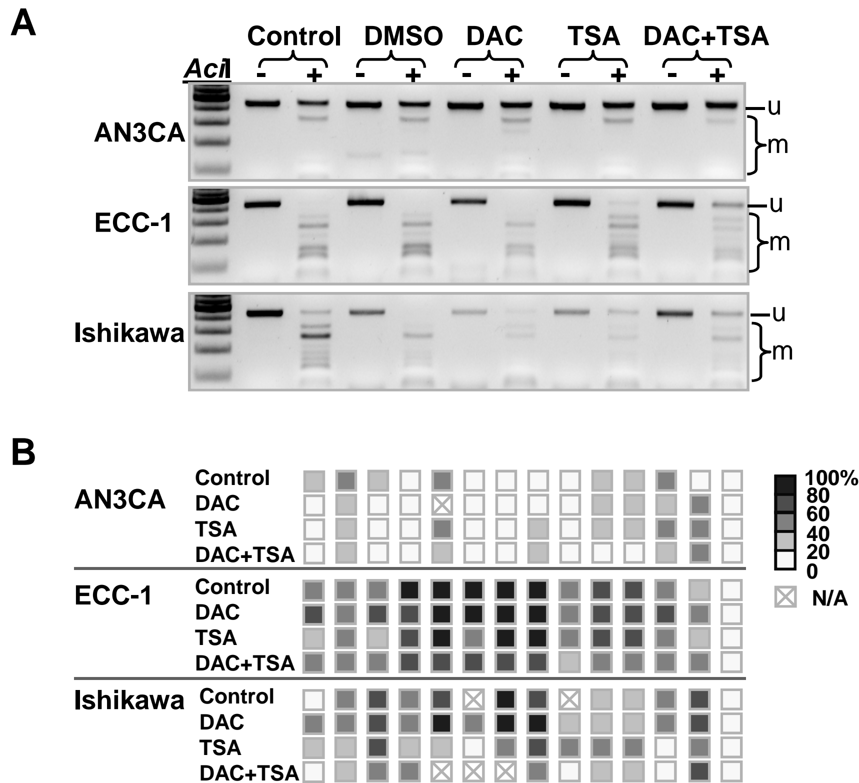

Figure S1. DNA de-methylation analysis of the *miR-129-2* CpG sites in endometrial cancer cells. ***A,*** COBRA analysis in cancer cells treated with DAC, TSA, vehicle (DMSO) or without any treatment (control). u, unmethylated band; m, methylated bands; +, *AciI* restriction enzyme added; -, without *AciI*. ***B,*** quantitative DNA methylation analysis using MassARRAY in cells exposed to agents as in Fig S2*A*. Each row represents a sample and each column represents an individual CpG site or a combination of CpG sites. Color-coding reflected the degree of methyltion with black being 100% and white being 0%; N/A, not analyzable.

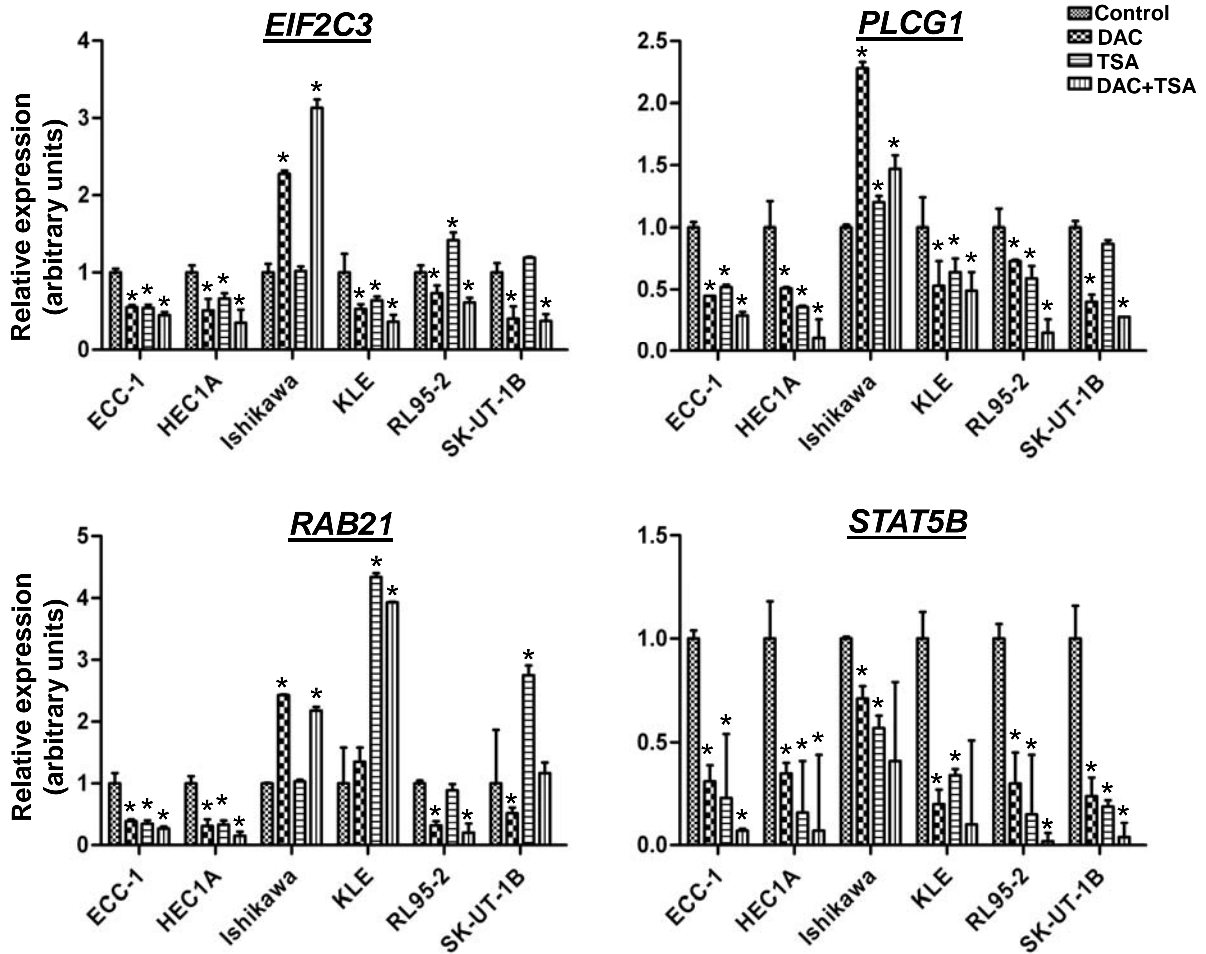

Figure S2. DNA demethylation and hyperacetylation treatments reduced gene expression of *miR-129-2* targets. Total RNAs from cancer cells treated with DAC and/or TSA in relation to untreated cell line were subjected to RT-qPCR analysis. *GAPDH* was used as an internal control. *Bar*, mean from triplicates; *Error bar*, SD; *, $P < 0.05$.

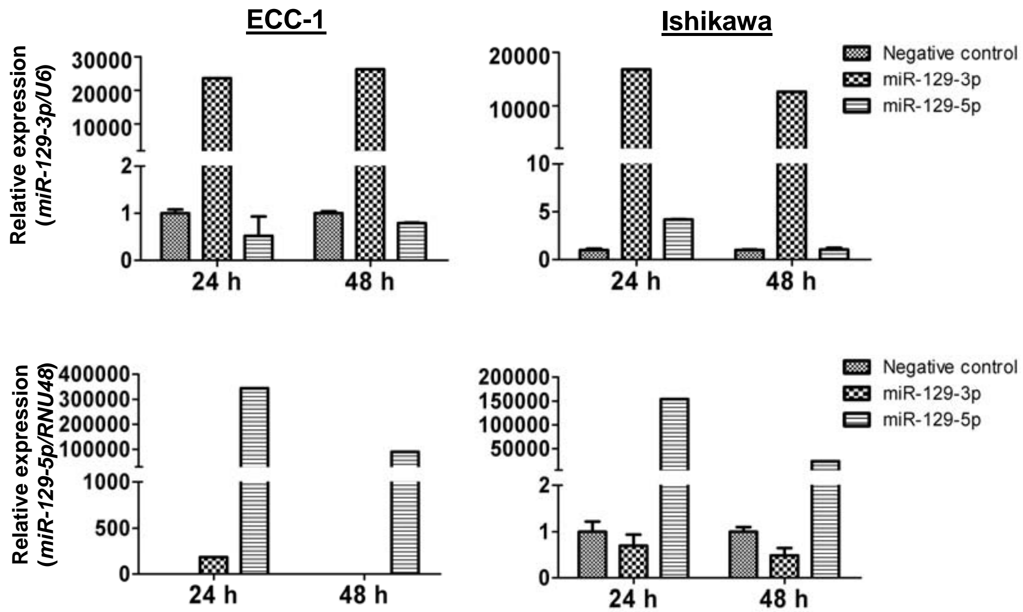

Figure S3. Relative *miR-129-2* expression in ECC-1 and Ishikawa cells after transient transfection with miRNAs or a negative control pre-miR precursor RNA oligonucleotide, respectively, for 24 or 48 h. *U6* or *RNU48* served as internal control. *Error bars*, SD from triplicates.

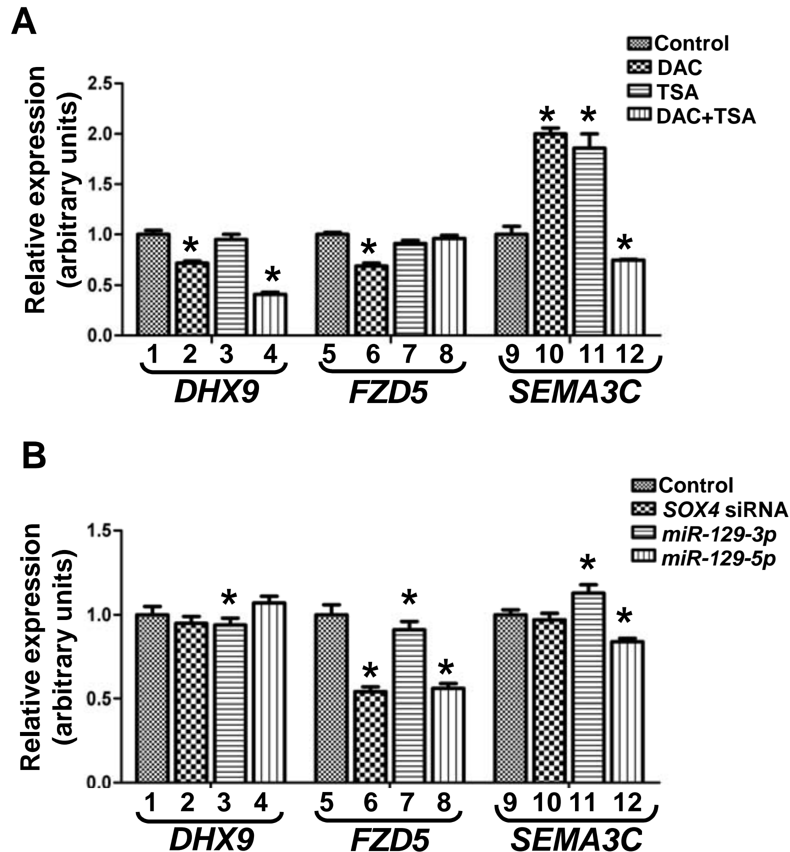

Figure S4. Expression analysis of *SOX4* target genes after (***A***) DAC and/or TSA treatments or (***B***) *SOX4* siRNA, *miR-129-3p or miR-129-5p* transfection in ECC-1 cells. Total RNAs from cancer cells in relation to untreated cells were subjected to RT-qPCR analysis. *GAPDH* was used as an internal control. *Bar*, mean from triplicates; *Error bar*, SD. . *, $P < 0.05$. ***A***. partial repression of three SOX4-regulated genes was observed, likely attributed to the re-expression of the *miR-129-2* locus, an upstream regulator of SOX4. As *miR-129-2* was epigenetically silenced in endometrial cancer cells, its expression was partially reactivated in these cells after the DAC and/or TSA treatment. This reactivation might down-regulate SOX4, which abolished its control on the expression of the three target genes. The pharmacologic mechanisms of these epigenetic treatments, however, can be complicated. As a result, other factors may be activated in a feedback loop, resulting in up-regulation of *SEMA3C* in cells treated with DAC or TSA (lanes 10 and 11, respectively). ***B***. suppression of SOX4-targeted genes by ectopically expressed *miR-129-3p* and *-5p* and transiently transfected with *SOX4* siRNA. The expression of these target genes was negatively regulated by the *miR-129-2* locus, but positively controlled by *SOX4*. One exception is the *DHX9* gene likely due to suboptimal treatment conditions in these cells. Alternatively, other "positive" factors might continuously regulate the expression of this gene.

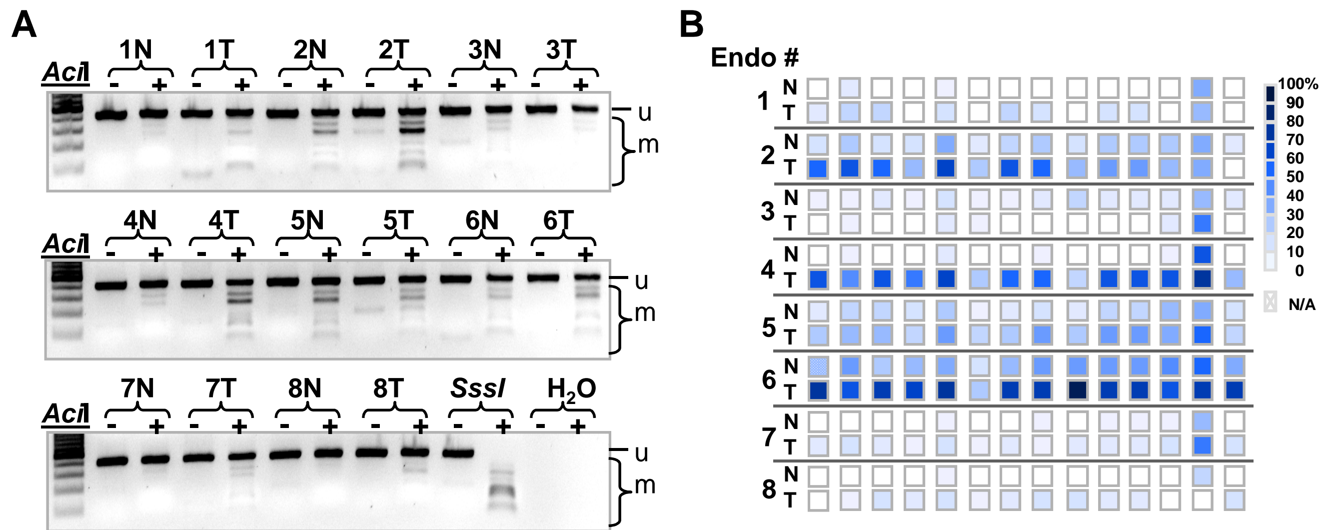

Figure S5. Methylation analysis of the *miR-129-2* CpG island in primary endometrial tumors and adjacent normal endometrium. ***A,*** COBRA analyzed 8 pairs of endometrial tissues. The number indicats the identical patient. N, adjacent normal specimen; T, endometrial cancer tissue; *SssI*, positive control; H_2O, negative control; *AciI*, restriction enzyme cutting on CCGC sites; +, *AciI* added, -, without *AciI*. ***B,*** quantitative DNA methylation analysis using MassARRAY in 8 paired samples of endometrial tissues as Fig. S4*A*. Measured methylation levels of the samples were corrected using a standard curve. Color-coding reflects the degree of methyltion with dark blue being 100% and white being 0%; N/A, not analyzable.

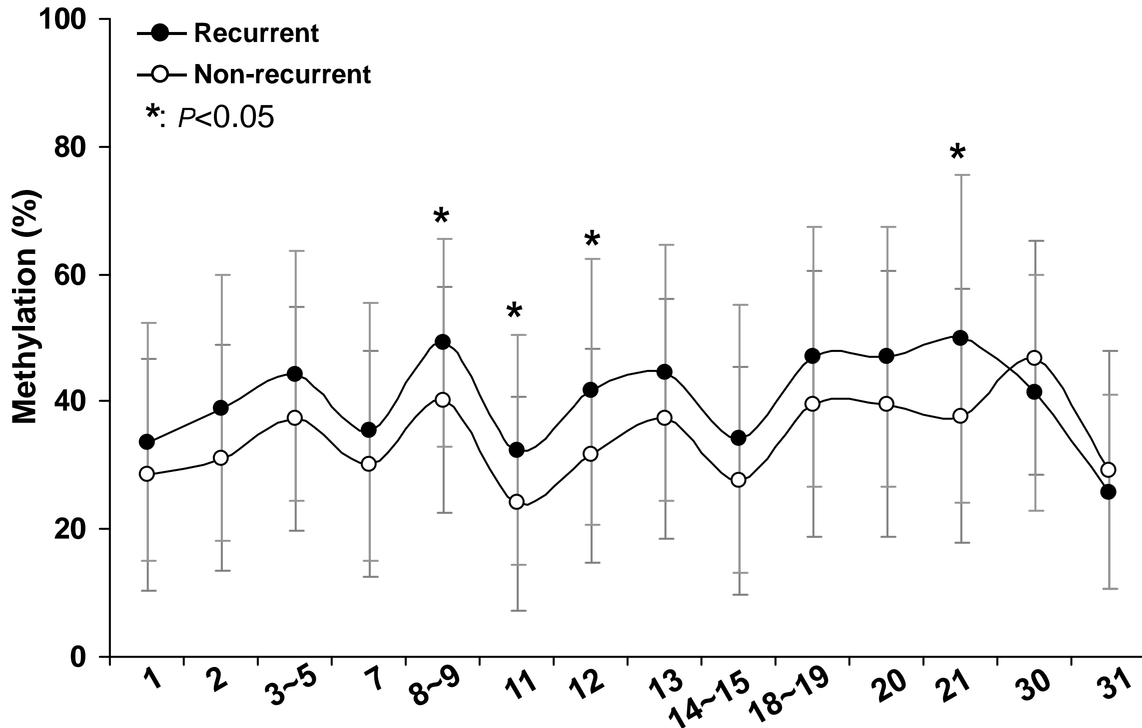

Figure S6. Statistical analysis of methylation on each CpG unit of *miR-129-2* between recurrent and non-recurrent groups. Normal pool was used as threshold, if a recurrent or non-recurrent sample had methylation level less than normal pool level at that site, then this sample was removed from analysis. The data was analyzed by Wilcoxon test. * indicates as a statistical significance between two groups.