AFF3-DNA methylation interplay in maintaining the mono-allelic expression pattern of *XIST* in terminally differentiated cells

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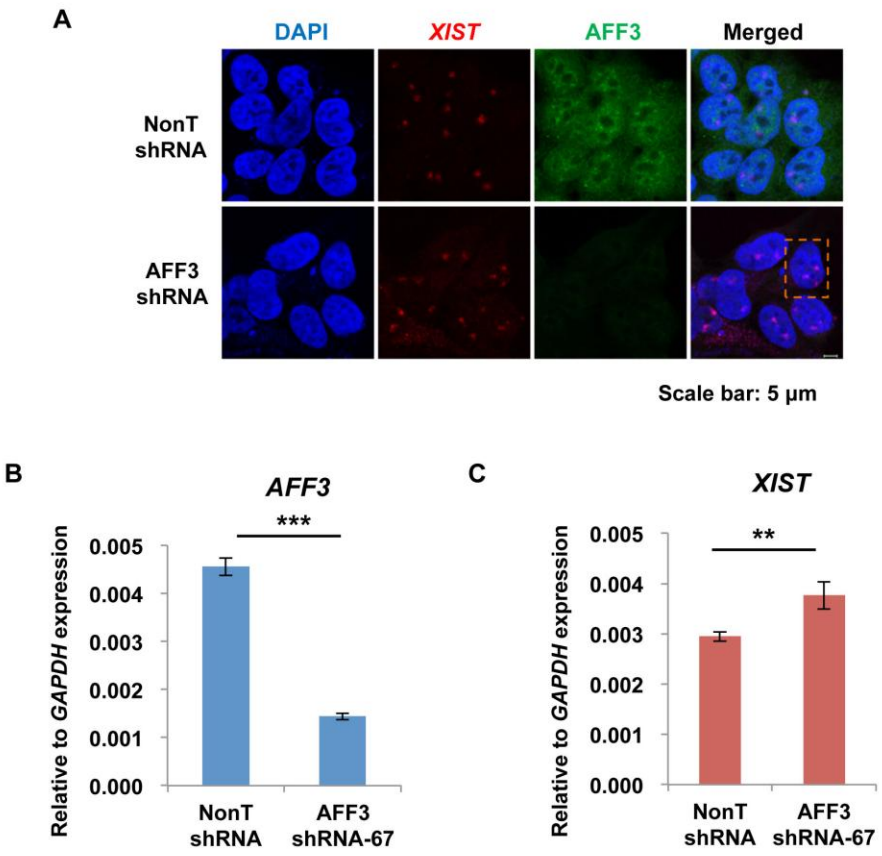
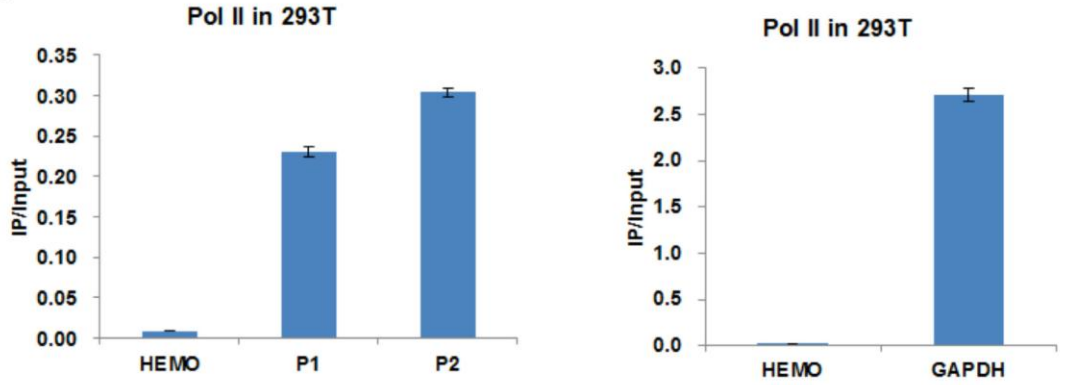
Figure S1



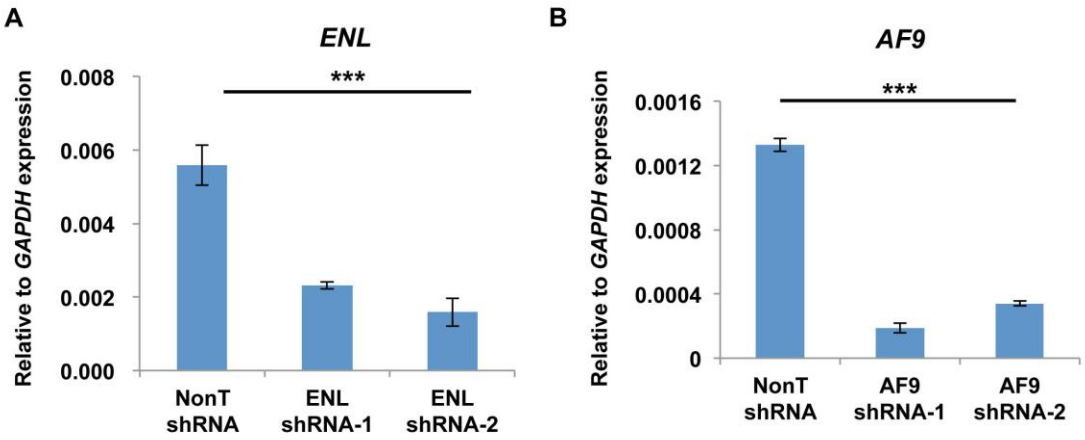
Supplementary Figure S1. Regulation of *XIST* gene expression by AFF3. (A) *XIST* RNA FISH (red) combined with AFF3 immunostaining (green) in control and AFF3 knockdown HEK293T cells. The orange box highlights HEK293T cells containing three *XIST* RNA clouds after AFF3 knockdown. Scale bar represents 5 μm. (B and C) Knockdown of AFF3 using an independent shRNA leads to up-regulation of XIST RNA in IMR-90 cells. (B) RT-qPCR showing the efficiency of AFF3 knockdown mediated by an independent shRNA in IMR-90 cells. (C) RT-qPCR showing an increase of XIST RNA level by treating with the independent AFF3 shRNA in IMR-90 cells. (B and C) The expression of *AFF3* and *XIST* was normalized to the expression of *GAPDH*. Results shown are technical replicates from representative biological replicates. Error bars represent standard deviations. Significant differences are marked with an asterisk (t-test, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

Figure S2



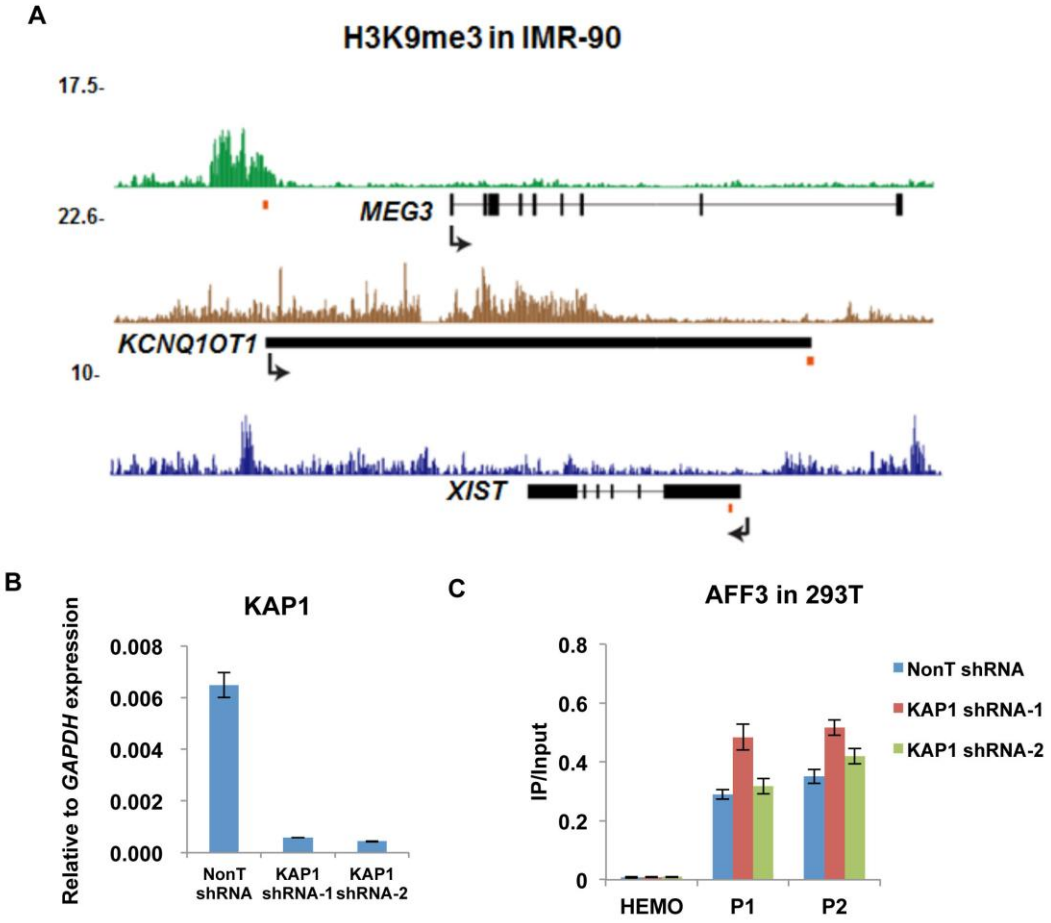
Supplementary Figure S2. Pol II ChIP-qPCR in HEK293T at the *XIST* DMR and the *GAPDH* promoter. The *HEMO* served as a negative control. Error bars represent standard deviations.

Figure S3



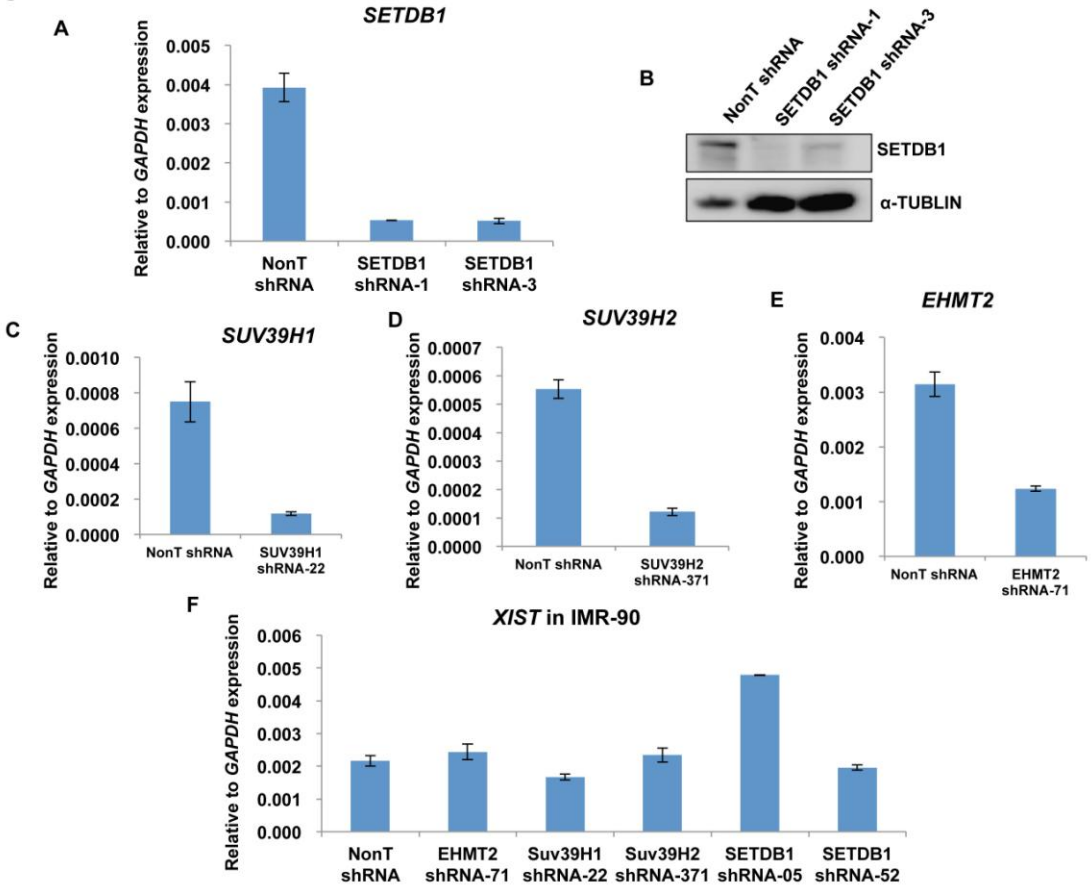
Supplementary Figure S3. Knockdown efficiency of ENL and AF9. (A and B) RT-qPCR showing the efficiency of shRNA-mediated ENL (A) and AF9 (B) knockdown in IMR-90 cells. The expression of *ENL* and *AF9* was normalized to the expression of *GAPDH*. Results shown are technical replicates from representative biological replicates. Error bars represent standard deviations. Significant differences are marked with an asterisk (t-test, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$).

Figure S4



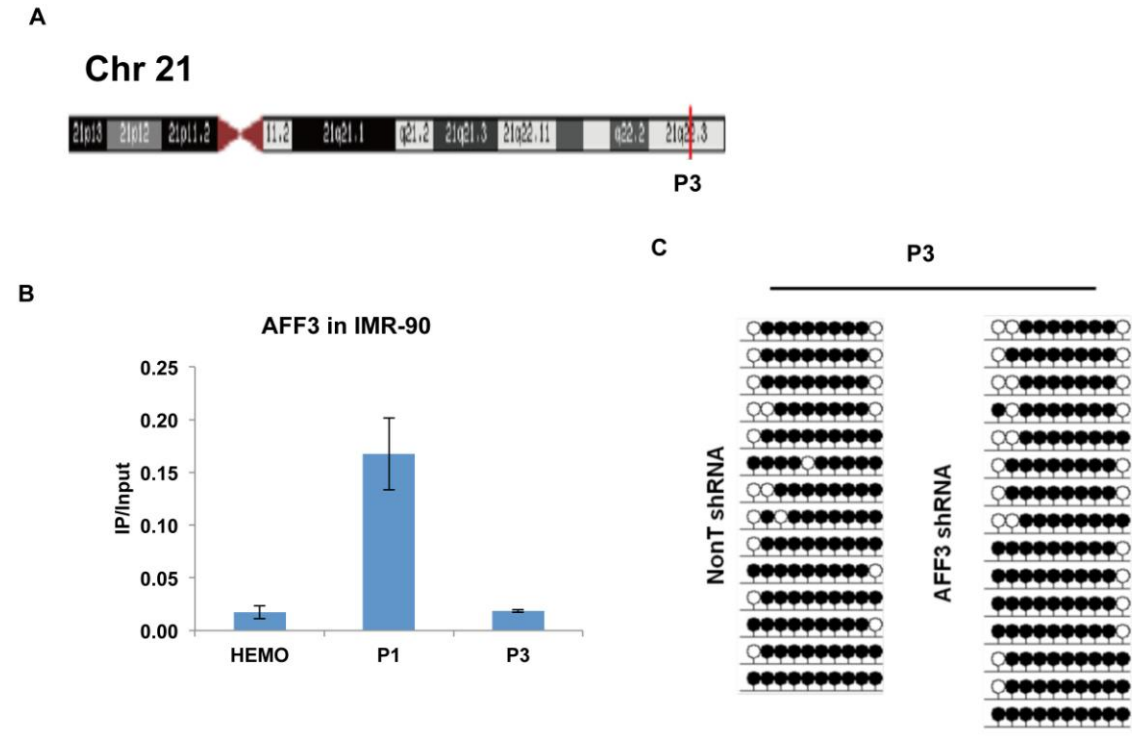
Supplementary Figure S4. The recruitment of AFF3 to the *XIST* DMR is KAP1 independent. (A)Track examples of H3K9me3 occupancies at imprinted genes in IMR-90 cells. (B) RT-qPCR showing the efficiency of shRNA-mediated KAP1 knockdown in IMR-90 cells. The expression of *KAP1* was normalized to the expression of *GAPDH*. Results shown are technical replicates from representative biological replicates. Error bars represent the standard deviation. (C) ChIP-qPCR showing that the occupancy of AFF3 at the *XIST* DMR remains unchanged after KAP1 knockdown in HEK293T cells. The *HEMO* gene serves as a negative control for ChIP-qPCR. Error bars represent the standard deviation.

Figure S5



Supplementary Figure S5. Depletion of the H3K9 methytransferases does not seem to have great effects on *XIST* expression. (A) RT-qPCR showing the efficiency of shRNA-mediated SETDB1 knockdown in IMR-90 cells. (B) Knockdown of SETDB1 by lentiviral mediated shRNA in IMR-90 cells. SETDB1 protein levels were measured by western blotting. α-TUBLIN was used as a loading control. (C-F) RT-qPCR showing the efficiency of shRNA-mediated SUV39H1 (C), SUV39H2 (D) and EHMT2 (E) knockdown in IMR-90 cells. (F) RT-qPCR showing that XIST RNA level remains unchanged after EHMT2, SUV39H1, SUV39H2 and SETDB1 knockdown in IMR-90 cells. (A, C-F) The expression of the genes tested was normalized to the expression of *GAPDH*. Results shown are technical replicates from representative biological replicates. Error bars represent the standard deviation.

Figure S6



Supplementary Figure S6. AFF3 does not affect the methylation status of the AFF3 unbound region. (A) Schematic illustration of the location of the AFF3 unbound region (P3) located on chromosome 21. (B) ChIP-qPCR shows that AFF3 is recruited to the *XIST* DMR (P1) but not the P3 region on chromosome 21 in IMR-90 cells. The *HEMO* served as a negative control. Error bars represent standard deviations. (C) Bisulfite sequencing analysis of the P3 region in control and AFF3 knockdown IMR-90 cells. Methylated and unmethylated cytosines are designated by filled and unfilled circles, respectively. Each line indicates a unique DNA clone.