

Supplementary Information

Histone methylase MLL4 plays critical roles in cell cycle progression and cell viability and its knockdown suppresses growth of c xenografted tumor *in vivo*

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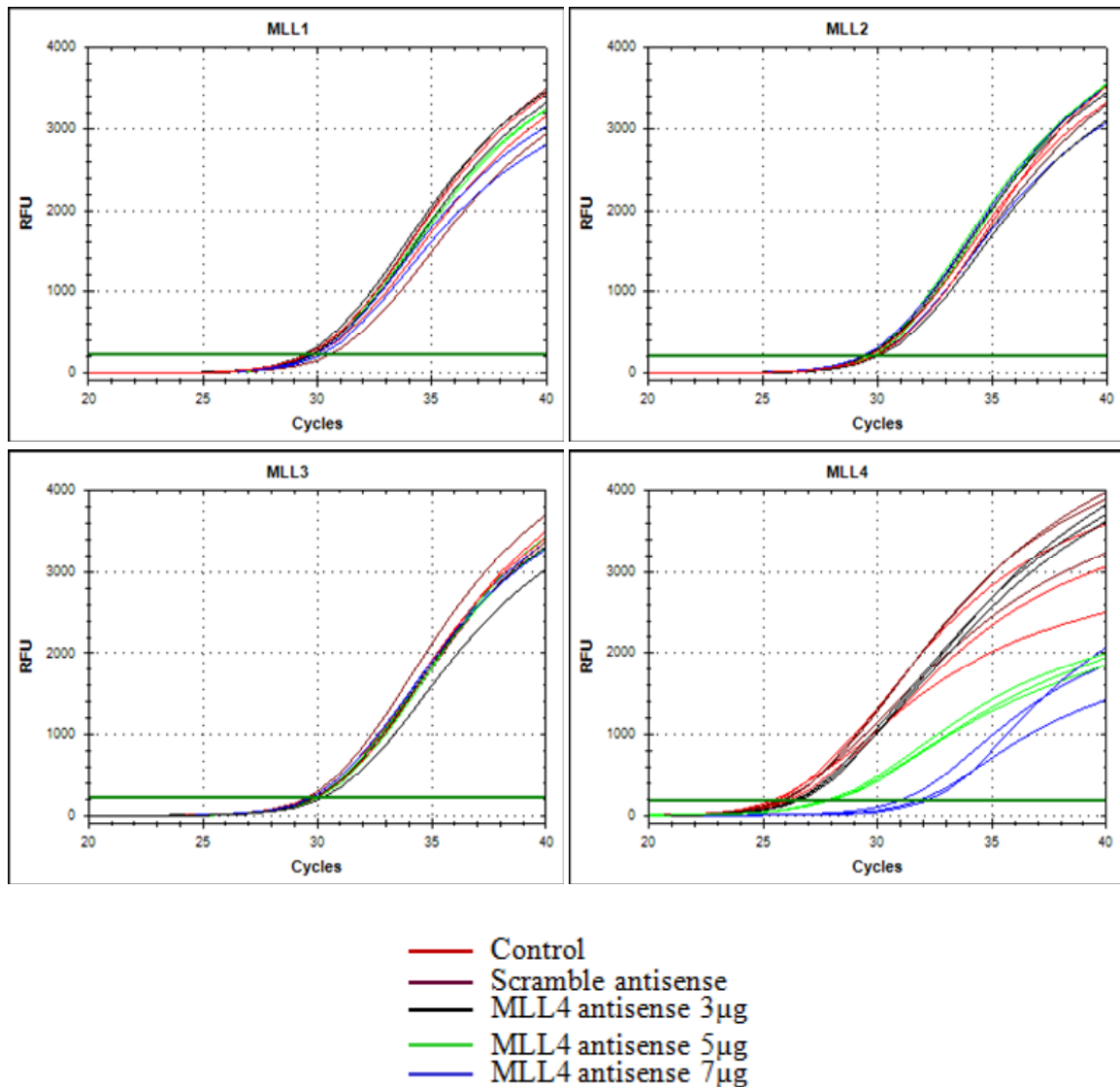


Figure S1. Real-time PCR analysis on the expression of MLL1, MLL2, MLL3 and MLL4 upon MLL4-knockdown. SW480 cells were transfected with varying concentrations of MLL4 antisense for 48 h. RNA was analyzed by real-time PCR using primers specific to MLL1, MLL2, MLL3, MLL4 and GAPDH (loading control). Relative numbers of amplicons at different PCR cycles are plotted. Each reaction is done with at least three replicates each times and repeated for two times.

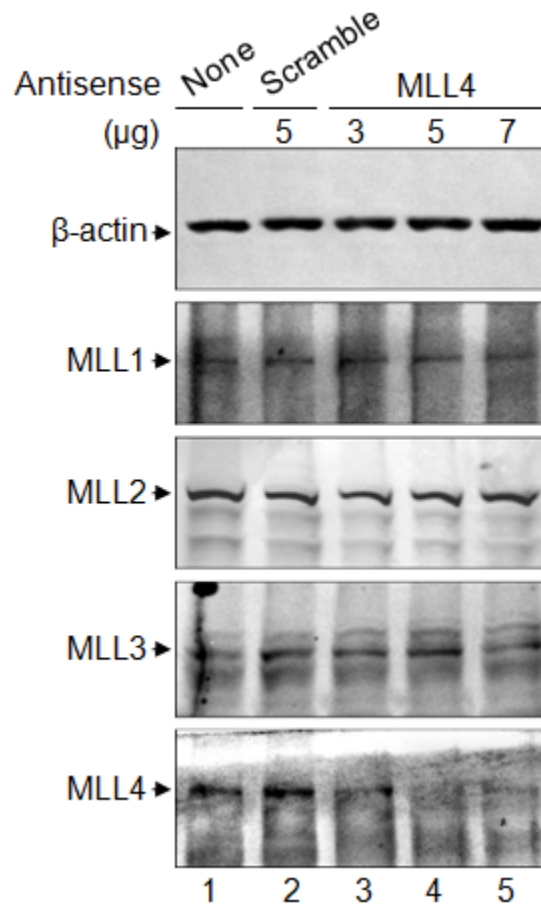


Figure S2. Effects of MLL4-specific antisense treatment on expression of MLL1, MLL2, MLL3 and MLL4 in colon cancer cell. SW480 cells were transfected with varying concentrations of MLL4 antisense for 48 h. The protein extract was analyzed by Western blot using antibodies specific to MLL1, MLL2, MLL3, MLL4 and β -actin (loading control).

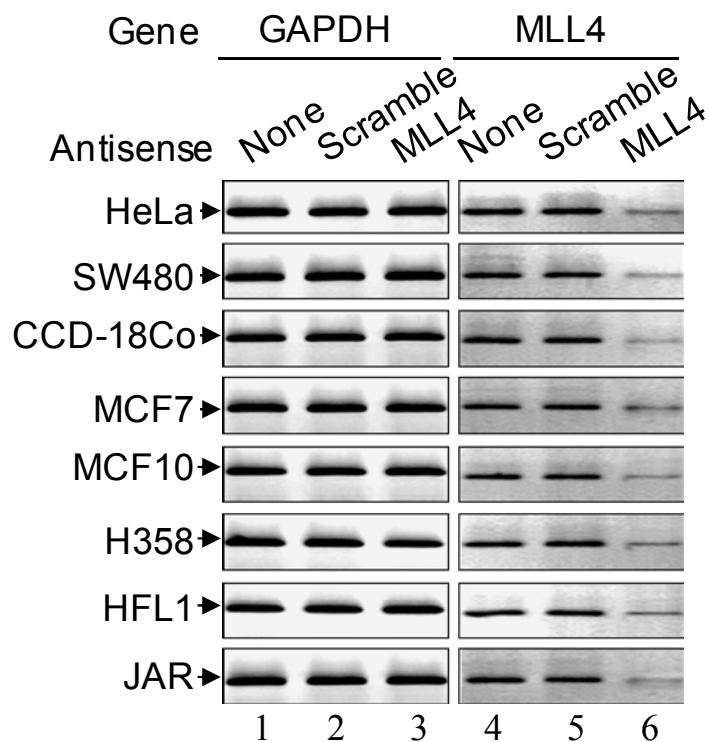


Figure S3. Knockdown of MLL4 in different malignant and non malignant cell lines using MLL4 specific antisense. Human cervical cancer (HeLa), colorectal adenocarcinoma (SW480), nonmalignant colon fibroblast (CCD-18Co), human adenocarcinoma mammary (MCF7), non malignant mammary gland fibrocystic (MCF10), human bronchioalveolar carcinoma (H358), nonmalignant lung fibroblast (HFL1) and human choriocarcinoma placenta (JAR) cells were grown upto 60% confluency prior to treatment with MLL4 phosphorothioate antisense. A scramble antisense with no homology with MLL4 was used as control. The RNA extract of the cells were subjected to RT-PCR using primers specific to MLL4. GAPDH was used as control.

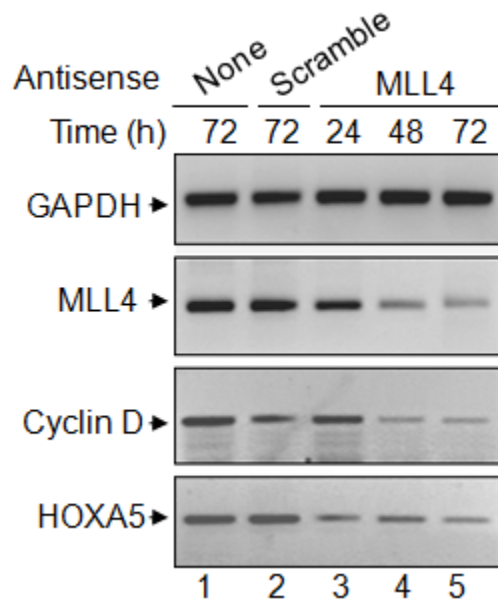


Figure S4. Temporal studies on the effects of MLL4-knockdown on the expression of MLL4 target genes such as Cyclin D and HOXA5. SW480 cells were transfected with MLL4 antisense for varying time points (24h, 48h and 72h). The RNA was extracted and subjected to PCR analysis using primers specific to MLL4, cyclin D and HOXA5. GAPDH was used as control.