

Supplementary Information

For Breast Cancer Research and Treatment

Histone methyltransferases regulate the transcriptional expression of ER α and the proliferation of tamoxifen-resistant breast cancer cells

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Supplementary Table 1

Gene	Accession number		Nucleotide sequence	Species	Purpose	Reference
ER α	NM_000125	Sense	5'-CACATGAGTAACAAAGGCATGG-3'	Human		1
	NM_001122742	Antisense	5'-ATGAAGTAGAGCCCGCAGTG-3'			
MLL1	NM_001197104	Sense	5'-GAAGTGGTTCCTGAGAATGG-3'	Human		-
		Antisense	5'-CACAGTCGAGAGATCATTAG-3'			
MLL2	NM_014727	Sense	5'-TGATCGAGAAAAGTGCAAGAG-3'	Human		-
		Antisense	5'-CTGGTGGTAACGGAACCTATAG-3'			
MLL3	NM_170606	Sense	5'-AGTCTTCAGGAGGGTCTATG-3'	Human		-
		Antisense	5'-CACAGGGAAGAGTGTCTTAG-3'			
MLL4	NM_003482	Sense	5'-CTCTGGATGGGATTGATGCT-3'	Human		-
		Antisense	5'-CGTGGCTCTCCTGTTCTTC-3'			
SET1A	NM_014712	Sense	5'-CATCGAATACGTGGGTCAGA-3'	Human		2
		Antisense	5'-AATGCCCTCCTGCACGTA-3'			
SET1B	NM_001353345	Sense	5'-GGGATCTGGACAAAAACAA-3'	Human	qRT-PCR primer	2
		Antisense	5'-CCCACGTAGAACTCATCGATCT-3'			
PR	NM_001202474	Sense	5'-ACAGGACCCCTCCGACGAAAA-3'	Human		-
		Antisense	5'-AGCTGTCTCCAACCTTGCACC-3'			
TFF1	NM_003225	Sense	5'-ACCATGGAGAACAAGGTGAT-3'	Human		-
		Antisense	5'-AAATTCACACTCTCTCTG-3'			
CCND1	NM_053056	Sense	5'-GGATGCTGGAGGTCTGCGA-3'	Human		-
		Antisense	5'-AGAGGCCACGAACATGCAAG-3'			
c-Myc	NM_002467	Sense	5'-AAAGGCCCCCAAGGTAGTTA-3'	Human		
		Antisense	5'-GCACAAGAGTTCCGTAGCTG-3'			
GREB1	NM_01466	Sense	5'-GTGGTAGCCGAGTGGACAAT-3'	Human		
		Antisense	5'-ATTTGTTTCCAGCCCTCCTT-3'			
β -actin	NM_001101	Sense	5'-CGTGGGCCGCCCTAGGCACCA-3'	Human		-
		Antisense	5'-TTGGCTTAGGGTTCAGGGGGG-3'			
18s rRNA	NR_145820	Sense	5'-TGCATGGCCGTTCTTAGTTG-3'	Human		-
		Antisense	5'-AGTTAGCATGCCAGAGTCTCGTT-3'			
ChIP 1	NM_001122742	Sense	5'-CTACCGACTCAGAACGGATTT-3'	Human	ChIP primer	-
		Antisense	5'-CTCAGAGACTGTCTTCTTATGCT-3'			
ChIP 2	NM_000125	Sense	5'-AGGAGCTGGCGGAGGGCGTTTCG-3'	Human		-
		Antisense	5'-AGCGCATGTCCCGCCGACACGC-3'			
MLL1	NM_001197104	Sense	5'-AAGAAGUCAGAGUGCGAAGUC-3'	Human		-
MLL2	NM_014727	Sense	5'-GAAGGGCAUCGGGUGCUAUAU-3'	Human		-
MLL3	NM_170606	Sense	5'-GUGAUGAGGUGCGACAGAU-3'	Human		-
MLL3 #2	NM_170606	Sense	5'-GAGUGUUAGUGAGCCAUGA-3'	Human	siRNA	-
MLL4	NM_003482	Sense	5'-GCAGUUUGUGCACUCCAAG-3'	Human		-
SET1A	NM_014712	Sense	5'-GGAAAGAGCCAUCGGAUUUU-3'	Human		-
SET1A #2	NM_014712	Sense	5'-GUCAGAGAACAGCUACCAA-3'	Human		-
SET1B	NM_001353345	Sense	5'-CAAGCUUGUUAUCAUGUAAAU-3'	Human		-

Supplementary Figures

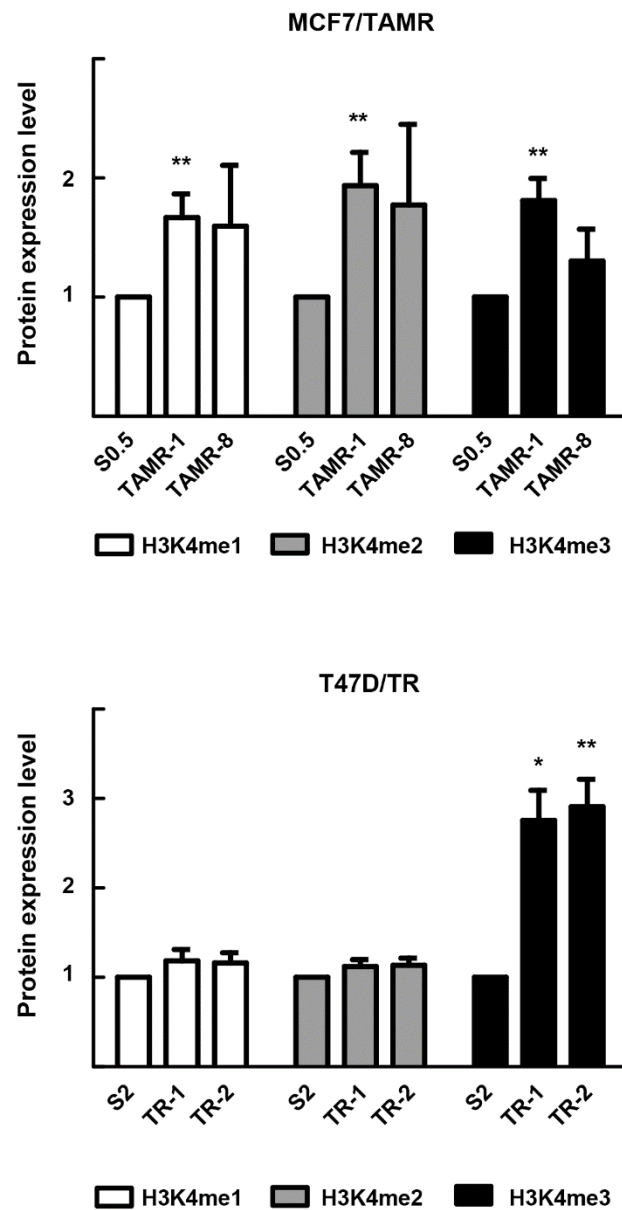


Figure 1. Histone H3K4 methylation status is increased in tamoxifen-resistant breast cancer cells. Band intensities of each protein in Figure 1A were quantified and normalized to that of Histone H3. Data presented as mean $\pm$ SEM (n=11 for MCF7 and n=4 for T47D). *, P<0.05 and **, P<0.01.

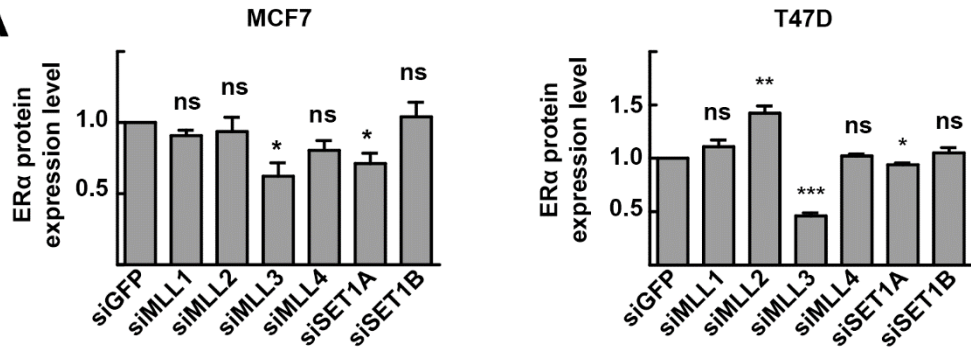
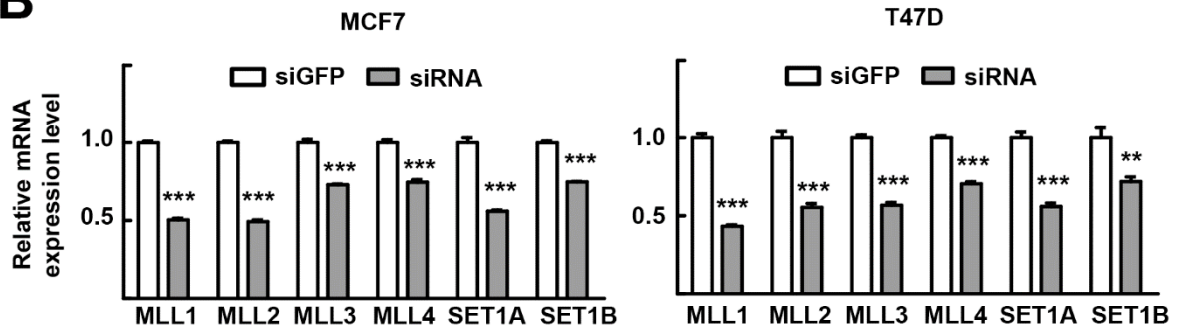
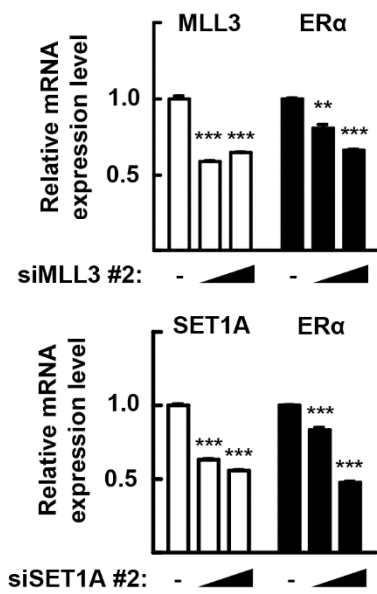
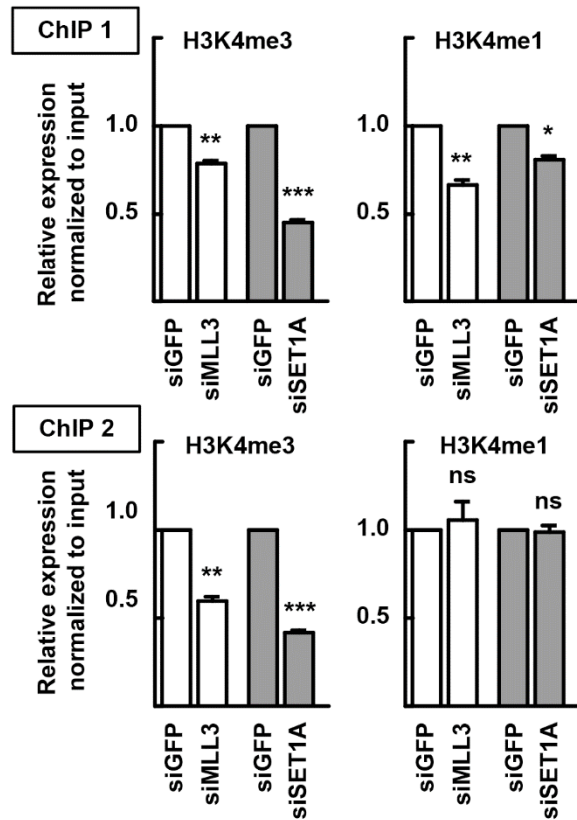
A**B****C****D**

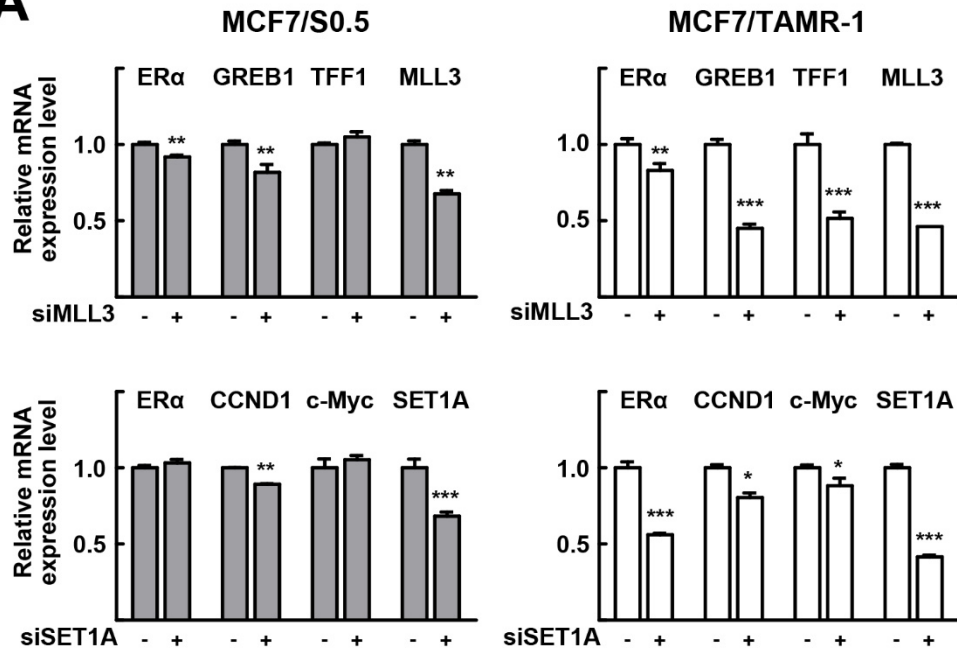
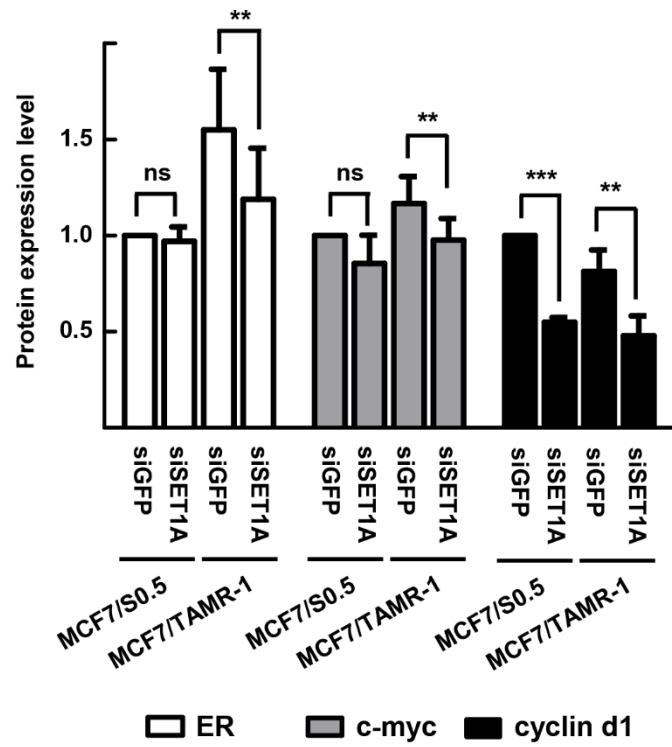
Figure 2. MLL3 and SET1A regulate ER α gene expression

(A) Band intensities of each protein in Figure 2A were quantified and normalized to that of α -tubulin. Data presented as mean $\pm$ SEM (n=4). *, P< 0.05, **, P<0.01 and ***, P< 0.001.

(B) MCF7 and T47D cells were transfected with indicated siRNA for 48 h. Total RNA obtained from the MCF7 cells were subjected to qRT-PCR analysis. Each siRNA specific for MLL1, MLL2, MLL3, MLL4, SET1A, and SET1B downregulates the expression of each gene with 40~50% efficiency. Data presented as mean $\pm$ SEM (n=3). ***, P< 0.001.

(C) MCF7 cells were transfected with indicated siRNA for 48 h. Total RNA obtained from the MCF7 cells were subjected to qRT-PCR analysis. Data presented as mean $\pm$ SEM (n=3). **, P<0.01 and ***, P< 0.001.

(D) Band intensities of each DNA gel band in Figure 3C were quantified using ImageJ and normalized to that of input. Data represent three readings of each band as mean $\pm$ SEM (n=3). *, P< 0.05, **, P<0.01 and ***, P< 0.001.

A**B**

C

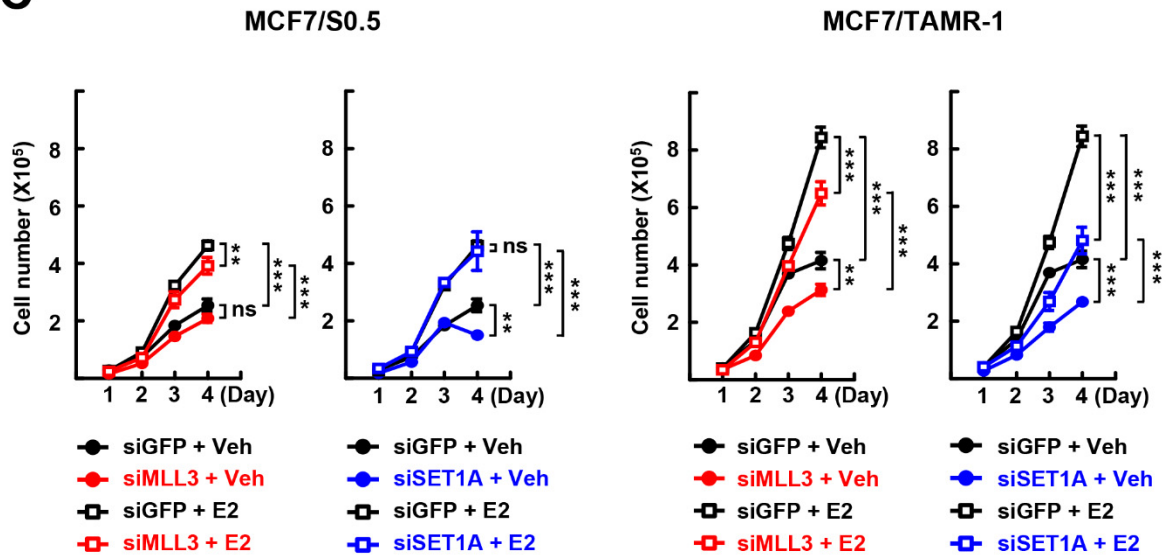


Figure 3. ER α target gene expression and cell growth inhibition was more obvious in the tamoxifen-resistant cells compared with their parent cells

(A) The MCF7/S0.5 and MCF7/TAMR-1 cells were grown in 2% charcoal-stripped FBS. The cells were transfected with siMLL3 or siSET1A for 48 h and 100nM E2 was treated for 24 h. Total RNA obtained from the MCF7/S0.5 and MCF7/TAMR-1 cells were subjected to qRT-PCR analysis. Data presented as mean $\pm$ SEM (n=3). *, P< 0.05, **, P< 0.01 and ***, P< 0.001.

(B) Band intensities of each protein in Figure 3B were quantified and normalized to that of α -tubulin. Data presented as mean $\pm$ SEM (n=6). *, P< 0.05, **, P<0.01 and ***, P< 0.001.

(C) The MCF7/S0.5 and MCF7/TAMR-1 cells were grown in 2% charcoal-stripped FBS. The cells were transfected with siMLL3 or siSET1A and 10nM E2 or vehicle was treated. The number of viable cells were counted using a hemocytometer. Cell numbers were presented as the mean $\pm$ SEM from duplicate plates. One of two independent experiments with similar results are presented. **, P< 0.01 and ***, P< 0.001.

Supplementary Reference

1. Treeck O, Lattrich C, Springwald A et al (2010) Estrogen receptor beta exerts growth-inhibitory effects on human mammary epithelial cells. *Breast Cancer Res Treat* 120(3):557-65. doi: 10.1007/s10549-009-0413-2.
2. Castellini L, Moon EJ, Razorenova OV et al (2017) KDM4B/JMJD2B is a p53 target gene that modulates the amplitude of p53 response after DNA damage. *Nucleic Acids Res* 20;45(7):3674-3692. doi: 10.1093/nar/gkw1281.