





SUPPLEMENTARY FIGURE 3







_	AF-1	DBD		AF-2/LBD	
ER a	A/B	С	D	E	F

B

Α



Coomassie Blue

*



C WB Input IP IP 0.5% CTRL Dnmt3b ERα



SUPPLEMENTARY FIGURE 6



Supplementary data

Supplementary Figure Legends:

Supplementary Figure 1 Degradation of ERα abolishes the repression of *CYP1A1* expression and the decrease in AhR binding at the *CYP1A1* promoter in presence of E2.

CYP1A1 (A) and *CYP1B1* (B) mRNA levels were quantified in MCF7 cells grown in estrogen free media and treated with DMSO, TCDD, TCDD+E2, ICI, TCDD+ICI, and TCDD+E2+ICI for 24h. ChIPs of AhR (C) and ERa (D) were performed in MCF7 cells treated with ICI 24h prior addition of TCDD for 90 min.

Supplementary Figure 2 AhR binding at the *CYP1B1* **promoter is not affected in H2A.Z-depleted cells or in the presence of E2.**

(A) Schematic representation of the *CYP1B1* promoter. The position of the amplicons A, B, C and D used in the qPCR analyses are illustrated. (B) ChIP of AhR was performed in MCF7 cells infected with shCT or shH2A.Z constructs for 5 days and, then treated or not with 10 nM TCDD during 90 min. ChIPs of ER α (C), AhR (D), RNA polymerase II (E) were performed in MCF7 cells grown in estrogen-free medium for 3 days, then treated with DMSO, 10 nM TCDD or 10 nM TCDD+100 nM E2 for 90 min.

Supplementary Figure 3 Verification of the efficiency of DNMT knockdowns.

Western blot were performed on protein extracts from MCF7 cells infected with constructs against DNMT1 (A), DNMT3A (B) or DNMT3B (C). Actin was used as loading control.

Supplementary Figure 4 DNMT3B can directly interact with ERα.

³⁵S-labeled, *in vitro* translated DNMT3B, was subjected to GST pull-down assays using either GST or GST-ER α protein domains as depicted (A). ³⁵S-labeled, in vitro translated ER α , was subjected to GST pull-down assays using either GST or GST-DNMT3B protein fragments as depicted (B). Input represents 10% of the *in vitro* translated material used in each assay. Western blot of ER α on immunoprecipitated DNMT3B from MCF7 cell extract (C). Input represents 0.5% of the protein extract used in the immunoprecipitation assay.

Supplementary Figure 5 Levels of DNA methylation in different *CYP1A1* promoter regions.

The cartoon represents *CYP1A1* promoter first 1.5 kb from the transcription start site (TSS) and containing five XREs. We splitted this region into five regions and analyzed them by bisulfite sequencing. Bisulfite sequencing was performed in MCF7 cells in full media.

Supplementary Figure 6 shRNA constructs directed to Dnmt enzymes are specific for each individual enzyme.

Immunoblotting experiments using Dnmt1, 3A, and 3B antibodies have been performed to verify that our respective shRNA constructs are specific for their cognate target protein within the Dnmt family.

Supplementary Tables

Sh CT/pLVTHM	GTCACGATAAGACAATGATAT
Sh CT/pLKO.1-puro	Non-Mammalian shRNA (shc002, Sigma)
Sh Dnmt1-1	GCCCAATGAGACTGACATCAA
Sh Dnmt1-2	GCCGAATACATTCTGATGGAT
Sh Dnmt1-3	GAGGTTCGCTTATCAACTAA
Sh Dnmt3a-1	CCAGATGTTCTTCGCTAATAA
Sh Dnmt3a-2	CCCAAGGTCAAGGAGATTATT
Sh Dnmt3b-1	CCTGTCATTGTTTGATGGCAT
Sh Dnmt3b-2	CCATGCAACGATCTCTCAAAT
Sh Dnmt3b-3	GCCCGTGATAGCATCAAAGAA
Sh H2A.Z-4	ATACTCTAACAGCTGTCCA

Table S1. ShRNA targeting sequences

Table S2. Primers used for RT-qPCR

36B4 RT-FWD	5'-CGACCTGGAAGTCCAACTAC-3'
36B4-RT-REV	5'-ATCTGCTGCATCTGCTTG-3'

CYP1A1-RT-FWD	5'- TGAACCCCAGGGTACAGAGA-3'
CYP1A1-RT-REV	5'- GGCCTCCATATAGGGCAGAT-3'
CYP1B1-RT-FWD	5'-AACGTACCGGCCACTATCAC-3'
CYP1B1-RT-REV	5'- CCACGACCTGATCCAATTCT-3'

 Table S3. Antibodies used for ChIP experiments		
AhR	(H-211) sc-5579, Santa Cruz Biotechnology	
Dnmt3b	ab2851, Abcam	
ERα	(HC-20) sc-543, Santa Cruz Biotechnology	
H2A.Z	Raised against an C-terminal H2A.Z peptide (CSLIGKKGQQKT)	
H3	b1791, Abcam	
RNA pol II	(8WG16) MMS-126R, Covance	

Table S4. Primers used for qPCR analysis of ChIPs experiments

CVP1A1-A-FWD	$5'_{-}$ CTTCGTGTCGTGCCACAG_3'
CVD1A1 A DEV	5' - ACCOUNTERCOUNTERCOUNTER 2'
CIPIAI-A-KEV	5 - AUUUICIAUUICIUCUIUIU-5
CYP1A1-B-FWD	5'- CAGCACTAAGGCGATCCTAGA-3'
CYP1A1-B-REV	5'- GATTGAAGGATCGGAATGGA-3'
CYP1A1-C-FWD	5'- CGTACAAGCCCGCCTATAAA-3'
CYP1A1-C-REV	5'- CTGGGATCACAAGGATCAGG-3'
CYP1A1-D-FWD	5'- CATGTCGGCCACGGAGTTTCTTC-3'
CYP1A1-D-REV	5'- ACAGTGCCAGGTGCGGGTTCTTTC-3'
CYP1B1-A-FWD	5'- AAGCTGTGCCATAACCCAAG-3'
CYP1B1-A-REV	5'- TAATTTGCGTGCAGACAAGC-3'
CYP1B1-B-FWD	5'-ATGACTGGAGCCGACTTTCC-3'
CYP1B1-B-REV	5'-GGCGAACTTTATCGGGTTG-3'
CYP1B1-C-FWD	5'-GACCCCCAGTCTCAATCTCA-3'
CYP1B1-C-REV	5'-AGTCTCTTGGCGTCGTCAGT-3'
CYP1B1-D-FWD	5'-TGCTTCATTTGTATGTCAAAGC-3'
CYP1B1-D-REV	5'-GGCTAAGTTCTGGGACATGAA-3'

Table S5. Antibodies used for Western blot experiments

Actin	A2066, Sigma
Dnmt1	(H-300) sc-20701, Santa Cruz Biotechnology
Dnmt3a	(H-295) sc-20703, Santa Cruz Biotechnology
Dnmt3b	(H-230) sc-20704, Santa Cruz Biotechnology
ERα	(HC-20) sc-543, Santa Cruz Biotechnology
H2A.Z	ab4174, Abcam
Н3	06-755, Upstate

Table S6. Percent of inp	ut for antibodies u	sed in ChIP ex	periments
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Antibodies	CYP1A1 promoter(%Input)	CYP1B1 promoter(%Input)
AhR	0.004-0.1	0.01-1
Dnmt3b	0.004-0.02	0.005-0.025

ER	0.005-0,1	0.02-0.3
H2A.Z	0.04-1	0.05-1
НЗ	0.5-1	0.5-1
RNA polII	0.001-0.4	0.08-0.8