Genome-wide expression analysis reveals six contravened targets of EZH2 associated with breast cancer patient survival

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S. N0.	**Histologic type**	**Grade**	**Metastatic status**
1	Invasive ductal carcinoma	2	lymph node metastatic
2	Papillary carcinoma	1	
3	Invasive ductal carcinoma	2	lymph node metastatic
4	Invasive ductal carcinoma	3	lymph node metastatic
5	Invasive ductal carcinoma	3	
6	Invasive ductal carcinoma	2	
7	Invasive ductal carcinoma	2	
8	Invasive ductal carcinoma	1	
9	NORMAL AREA		
10	Invasive ductal carcinoma	2	
11	Invasive ductal carcinoma	2	lymph node metastatic
12	NORMAL AREA		
13	NORMAL AREA		
14	Invasive ductal carcinoma	2	lymph node metastatic
15	Invasive ductal carcinoma	3	lymph node metastatic

Table S1 A. **Tissue microarray patient sample details.** Eleven Invasive ductal carcinoma patient samples along with one sample from papillary carcinoma patient were included in the the study. To compare the expression of EZH2 in cancerous as well as non-cancerous breast tissue, three normal breast tissues were taken into consideration.

			Adjacent NORMAL	Tumor	
S. No.	**Sample ID**	**Grade**			
1	1364-16	2		X	
2	1388-16	3			
3	3731/16	3			
4	B-1479/16	3			No amp in PMEPA1
5	B-1513-16	1			No amp in PMEPA1
6	H-394/16	1			No amp in PMEPA1
7	400/16	1	X		
8	B-257-17	2	X	X	
9	B-336-17	3			
10	B-399-17	3	X		
11	482-17	1			
12	B-898-17	0			
13	H-125/17	2			
14	H-127/17	3			
15	H-138/17	1			

Table S1 B. **Patient sample details used for Transcript analysis.** Fifteen Histologically similar breast cancer patient samples of different grades were analyzed for the mRNA expression of EZH2 and its targets to validate the relevance of the online dataset. 'X' denotes no expression shown. Three of the samples showed no PMEPA1 expression.

Antibody/Reagent/Kit	Catalog no.	Company
α-tubulin antibody	T5168	Sigma Aldrich, USA
anti-EZH2 antibody anti-SUMF1 antibody	D2C9-5246 PAC879Hu01	Cell Signaling Technology Cloud Clone Corp.
anti-Rabbit IgG (whole molecule)–Peroxidase antibody produced in goat secondary antibody	A0545	Sigma Aldrich, USA
anti-mouse HRP conjugated secondary antibody	A3682-1ML	Sigma Aldrich, USA
4, 5-dimethylthiazol-2yl)-2, 5-diphenyl tetrazolium bromide (MTT)	2102227.1	MP Biomedical, USA
SYBER Green	RT-SY2X-03+NRWOU	Eurogentech
SuperScript® First-Strand Synthesis System for RT-PCR	11904018	Invitrogen, Carlsbad, CA
Dnase I Kit	AMPD1-1KT	Sigma Aldrich, USA
INTERFERin Polyplus-Transfection reagent	409-10	Polyplus-transfection® SA
PVDF membrane	88518, 0.45µm,	Thermo scientific
Skimmed milk	RM1254-500G	Himedia
ECL-HRP for X-ray Film Kit	K-12045-D50	
Trizol	T9424	Sigma Aldrich, USA

Table S2. Table provides the details of reagents used in the study. All reagents used in the study were obtained from authentic companies.

Pooled EZH2si Duplexes (Eurogentech)
5’-GGG-AAA-GUG-UAU-GAU-AAA-U55-3’
5’-AUU-UAU-CAU-ACA-CUU-UCC-C55-3’
5’-CAC-AAG-UCA-UCC-CAU-UAA-A55-3’
5’-UUU-AAU-GGG-AUG-ACU-UGU-G55-3’
5’-GGA-UGG-UAC-UUU-CAU-UGA-A55-3’
5’-UUC-AAU-GAA-AGU-ACC-AUC-C55-3’

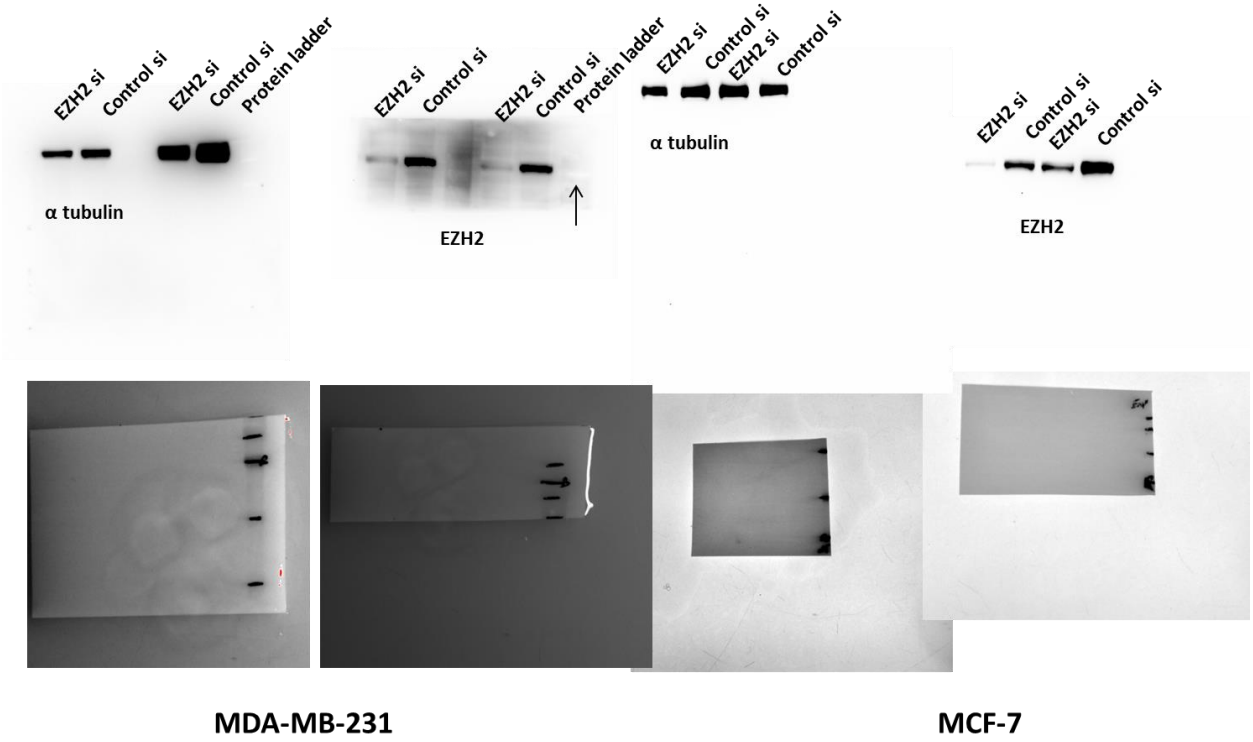
Table S3: siRNA Duplexes. Specific siRNA sequences targeting EZH2 Provided by Eurogentec was used for efficient EZH2 knockdown.

Sl. No.	qRT-Primer Name	Primer Sequence 5' to 3'
1	GPNMB RT F	GAACCTTCAGCCTTAACC
2	GPNMB RT R	CTACAATTGTGATGGTGGC
3	PMEPA1 RT F	TGGAGTTTGTTCAGATC
4	PMEPA1 RT R	TGGGATTCCGTTGC
5	COL5A1 RT F	CTGCTCCCATGCCT
6	COL5A1 RT R	CCCCTCCAAGTCATC
7	VGLL4 RT F	GCCCACACTGACCC
8	VGLL4 RT R	ACCCAGAGCTTTGGC
9	POMT2 RT F	TCCAGCATGTTGACAGGTATCCT
10	POMT2 RT R	CATAAGCCAGAGGGTGGAAGAG
11	SUMF1 RT F	CCATCCCTGCTGGAGTA
12	SUMF1 RT R	GGTCTTCACTTGCTCACTC
13	5'Ex1Ezh2	AGAAGGGACCAGTTTGTTGG
14	3'Ex1Ezh2	TTCATCAGCTCGTCTGAACC
15	GAPDH RT FW	AAGATCATCAGCAATGCCTC
16	GAPDH RT RV	CTCTTCCTCTTGTGCTCTTG

Table S4: Real Time primer Sequence. Quantitative Real time assay using specific primers was performed to study the expression of EZH2 and its identified targets. Primers against GAPDH were taken for normalization.

S. No.	Oligo Name	Oligo Sequence 5' to 3'
1	CoL5A1 site 1 (F)	TGTGAAGTGTTGTCTATCTGAG
2	CoL5A1 site 1 (R)	CTAATGACGAAGGTGGTTTG
3	CoL5A1 site 2 (F)	GGGCTCAAGTAATCTGTCTG
4	CoL5A1 site 2 (R)	ATGGTGAAACCCTGTCTGTA
5	CoL5A1 site 3 (F)	TATTTTTGTAGAGGTGGGGTCT
6	CoL5A1 site 3 (R)	GGTAGGAGGATTGCTTGAGT
7	CoL5A1 site 4 (F)	CTTCTATGGGCTGTGGACAA
8	CoL5A1 site 4 (R)	GCAGCCATATCTCTAGGACC
9	CoL5A1 site 5 (F)	TGAGGGAACTATGTGAGTGAG
10	CoL5A1 site 5 (R)	TGGGGAGGACTCTCGT
11	CoL5A1 site 6 (F)	TCAAGGAGAGGATGGATGT
12	CoL5A1 site 6 (R)	AGGCAGACAGATGGACAC
13	GPNMB site 2 (F)	CCATAGTCTGTTGAATACCC
14	GPNMB site 2 (R)	TCTTGTAGCATTTGTTCTGG
15	GPNMB site 1 (F)	TTCCTTTTTGTTGGAGTCAG
16	GPNMB site 1 (R)	ATAAGTCAGGTGTGGTGTTGC
17	PEMAP1 site 1 (F)	ATTGGGCTTTACCTTTCTCT
18	PEMAP1 site 1 (R)	GGTTCACATCACAGGACTCT
19	PEMAP1 site 2 (F)	TGTGGTCAGAGAGAGTGCTT
20	PEMAP1 site 2 (R)	TAATAGTGGGGGACTTCAATAC
21	PEMAP1 site 3 (F)	AATAGTGCCCAGGTTGAGAC
22	PEMAP1 site 3 (R)	AGGTAGAGAAGAGGGTCAGG
23	PEMAP1 site 4 (F)	GAAGTTGGTGTGGGTCATTT
24	PEMAP1 site 4 (R)	GCTGTGTGTCCTCCATTTAG
25	POMT2 site 1 (F)	GTTCTCTACCTCCTCCCAGT
26	POMT2 site 1 (R)	ACAATAAGCAGGCATGAGTT
27	POMT2 site 2 (F)	CTAGGCAACAGAGCAAGACT
28	POMT2 site 2 (R)	CGAGATCATACCAATGGACT
29	POMT2 site 3 (F)	GAGGGAAATTAGGCGATATT
30	POMT2 site 3 (R)	TACCCTTTTGTGTGTGTGTG
31	POMT2 site 4 (F)	AGCTAAGATCACGCTACTGC
32	POMT2 site 4 (R)	TAGGTTCATCCATGTTGTCA
33	POMT2 site 5 (F)	CAGCTACTAAGGAGGCTGAG
34	POMT2 site 5 (R)	GGAGAGATTGAGCATTTCTG
35	SUMF1 site 1 (F)	TCAGGCAGCAAGTTTCTACT
36	SUMF1 site 1 (F)	GCTCTTTTACCTCACCTCCT
37	SUMF1 site 2 (F)	ATAAATTCCAGGACCCAAAG
38	SUMF1 site 2 (F)	CAGGGATGTGTGACTAAGG
39	VGLL4 site 1 (F)	GGAAGAAGAGGAAAAAGAGG
40	VGLL4 site 1 (R)	CCTCCCCAATCTAGTTCTTT
41	VGLL4 site 2 (F)	TCGGAGTGAAGAGAAGAAAA
42	VGLL4 site 2 (R)	TCATACTTCTAAGGGGCAAA

Table S5: Primer sequences for CHIP-qPCR: Primers specific to binding sites of EZH2 on its identified target genes



Blot images for western blot assay

Figure S1. Uncropped image of western blot done for knockdown studies displayed in Fig. 3A(i) and 3B(ii)

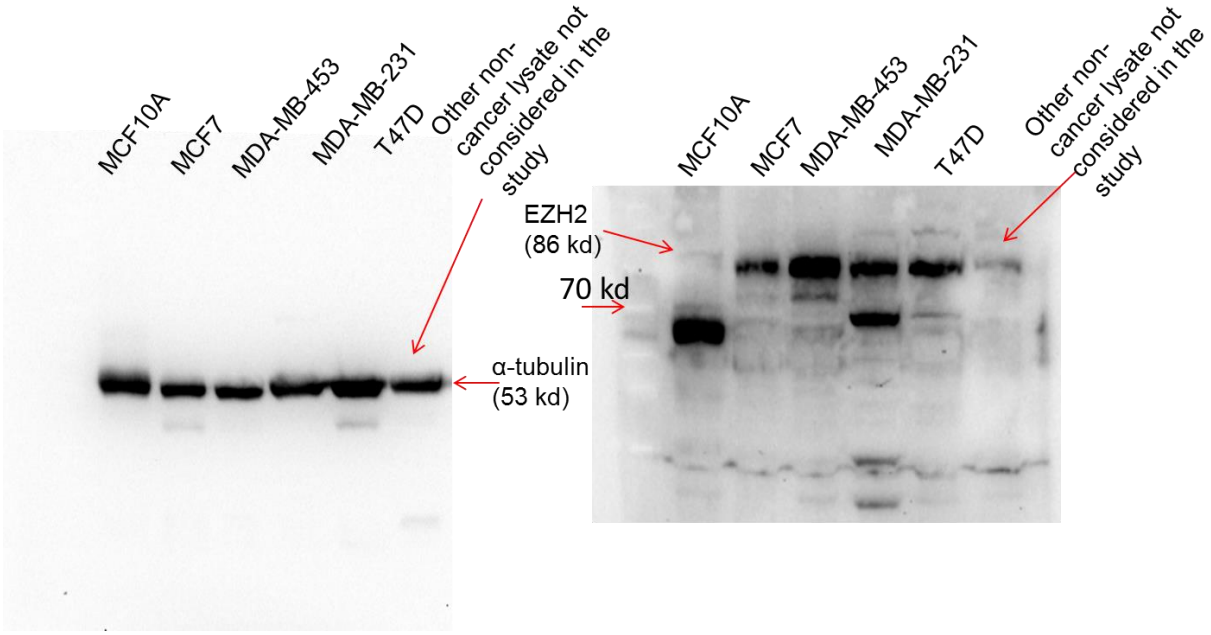
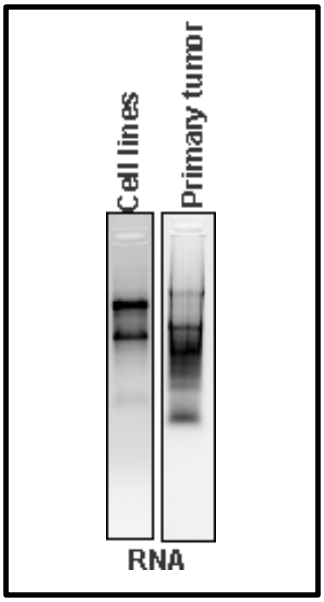
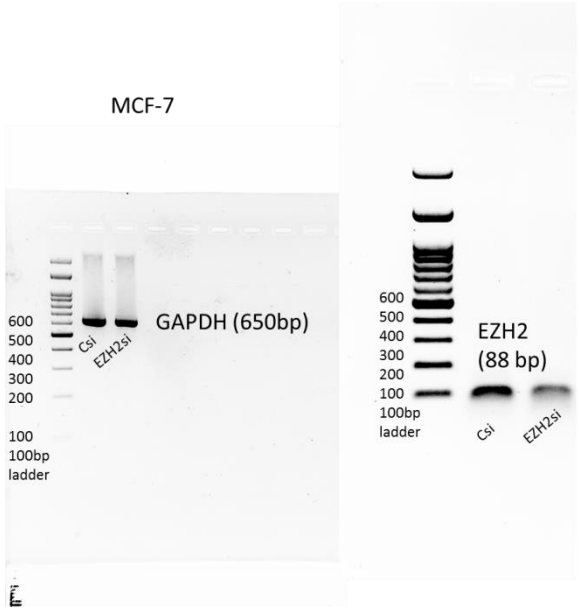


Figure S2. Uncropped image of immunoblot for EZH2 expression in different breast cancer cell lines displayed in Fig. 1B(i).

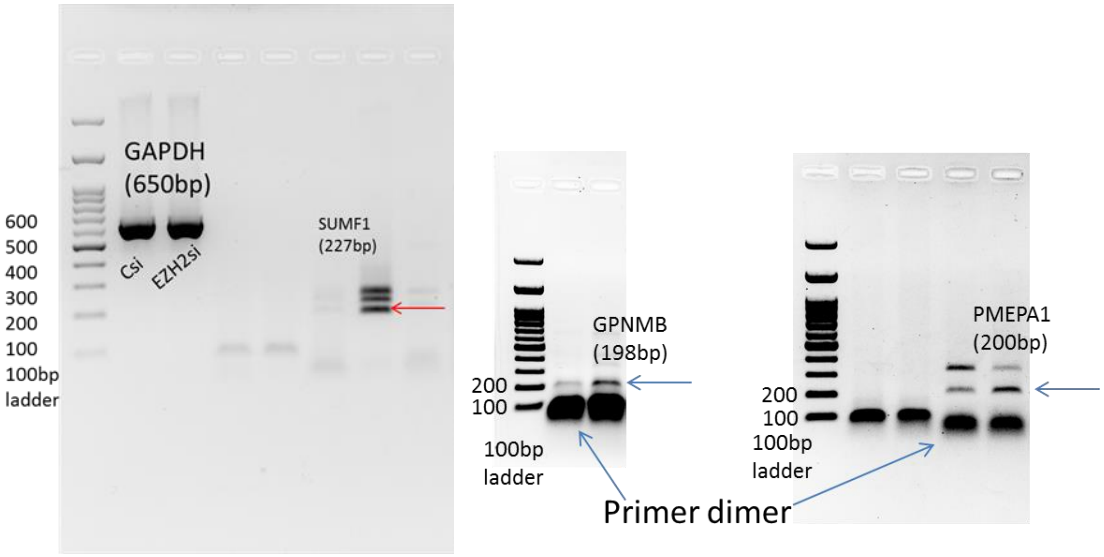
Quality of RNA used in the study



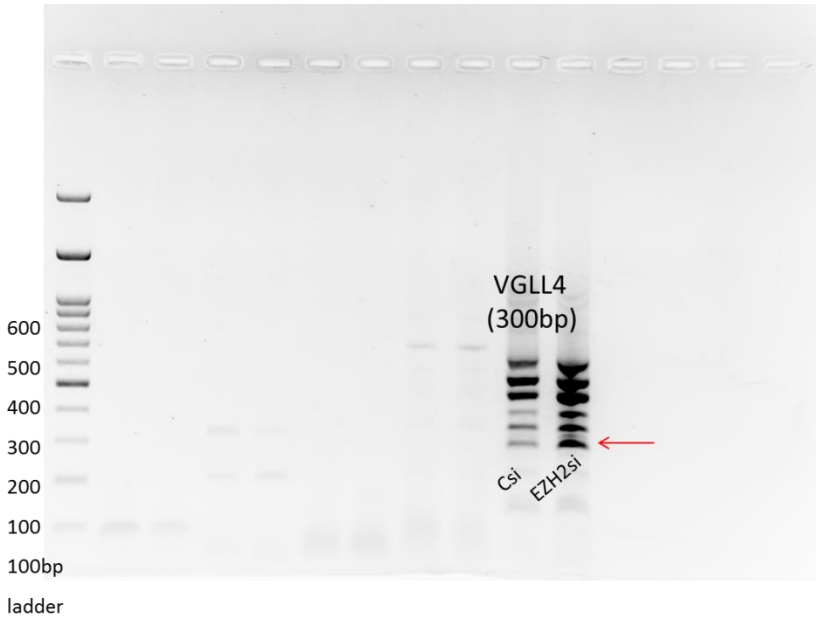
(i)



(ii)



(iii)



(iv)

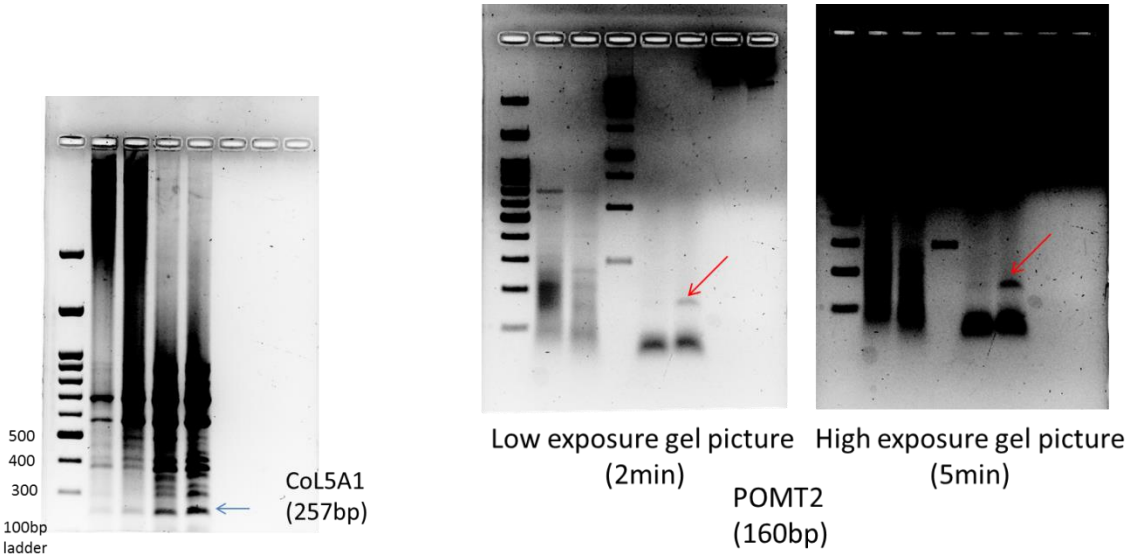
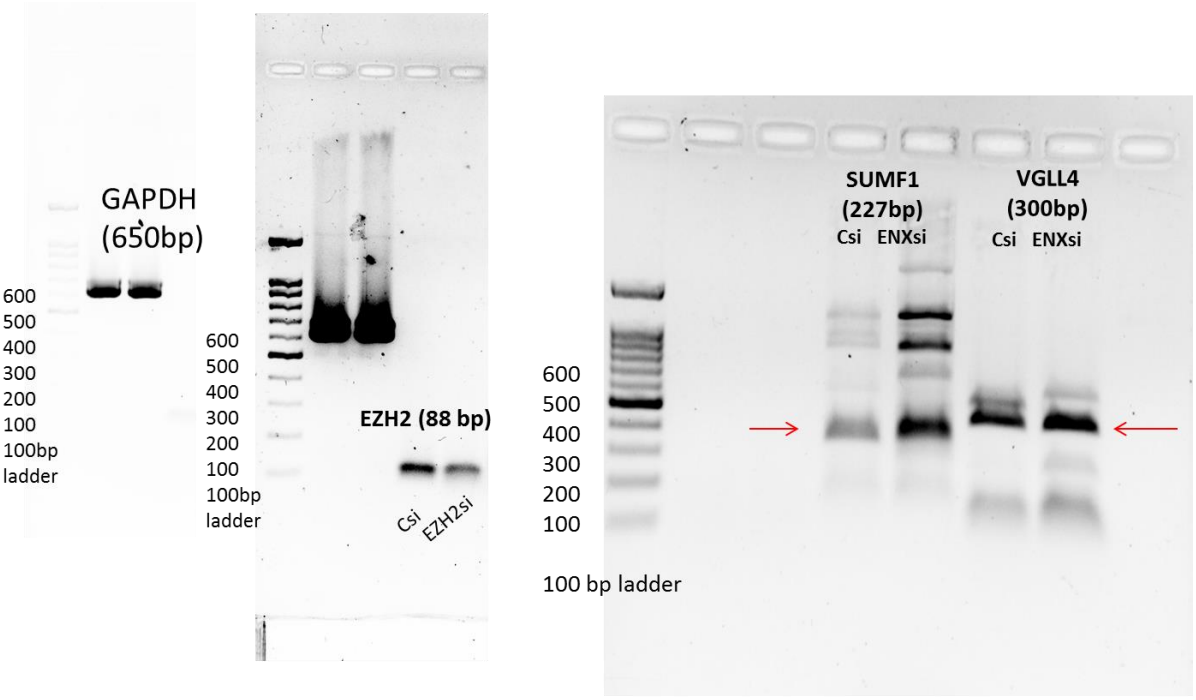


Figure S3. Uncropped images of agarose gel pictures for expression of (i) GAPDH, EZH2 (ii)SUMF1, GPNMB, PMEPA1 (iii) VGLL4 (iv) CoL5A1, POMT2 upon EZH2 silencing in MCF-7 breast cancer cells displayed in Figure 3E.

(i)


GAPDH
(650bp)
600
500
400
300
200
100
100bp
ladder
600
500
400
300
200
100
100bp
ladder
EZH2 (88 bp)
Csi
EZH2si
SUMF1
(227bp)
Csi ENXsi
VGLL4
(300bp)
Csi ENXsi
600
500
400
300
200
100
100 bp ladder

(ii)

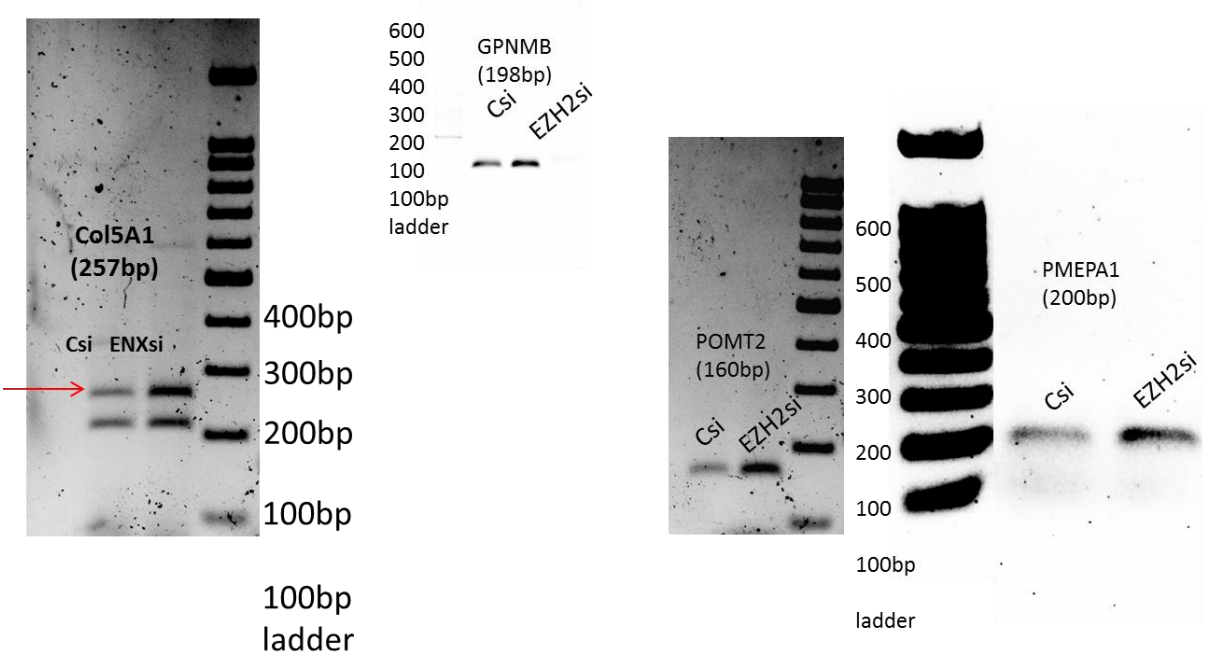

Col5A1
(257bp)
Csi ENXsi
400bp
300bp
200bp
100bp
100bp
ladder
600
500
400
300
200
100
100bp
ladder
GPNMB
(198bp)
Csi
EZH2si
POMT2
(160bp)
Csi
EZH2si
600
500
400
300
200
100
100bp
ladder
PMEPA1
(200bp)
Csi
EZH2si

Figure S4. Uncropped images of agarose gel pictures for expression of (i) GAPDH, EZH2, SUMF1, VGLL4 (ii) CoL5A1, GPNMB, POMT2, PMEPA1 upon EZH2 silencing in MDA-MB-231 breast cancer cells displayed in Figure 3F.

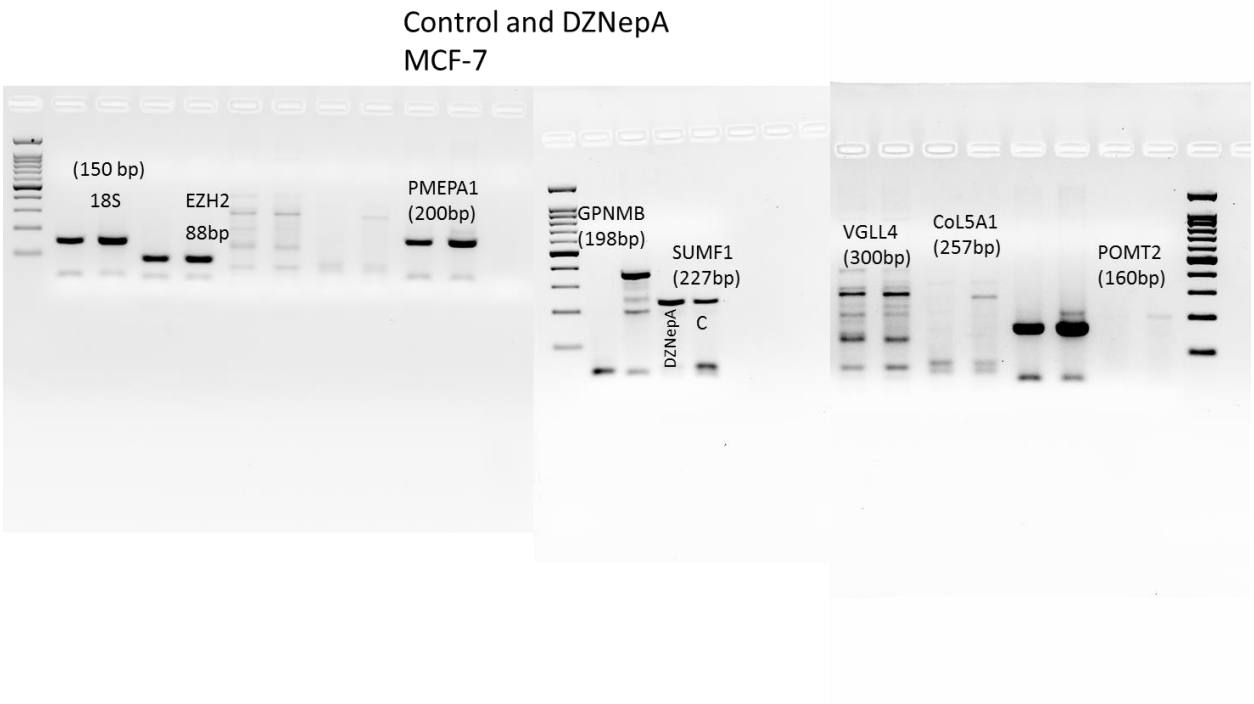


Figure S5. Uncropped images of agarose gel pictures for expression of 18S, EZH2, PMEPA1, GPNMB, SUMF1, VGLL4, CoL5A1 and POMT2 upon DZNepA treatment in MCF-7 breast cancer cells displayed in Figure 10A.

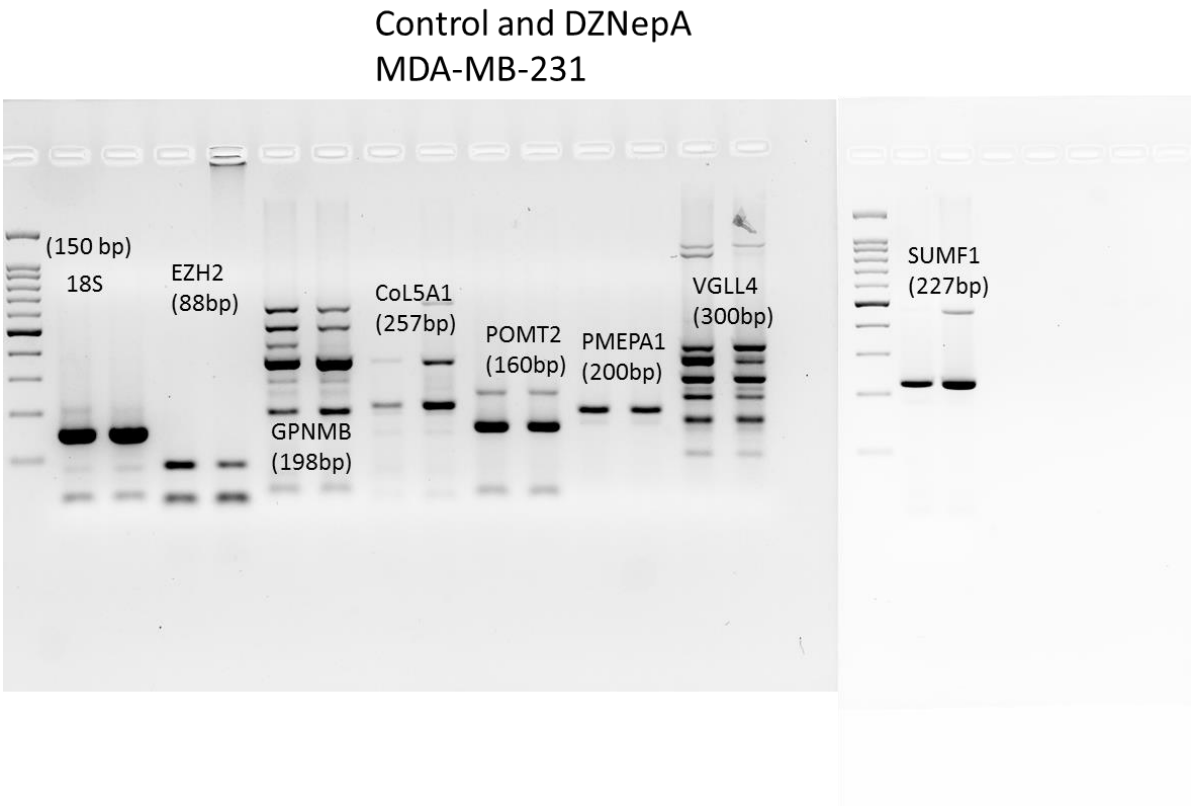


Figure S6. Uncropped images of agarose gel pictures for expression of 18S, EZH2, PMEPA1, GPNMB, SUMF1, VGLL4, CoL5A1 and POMT2 upon DZNepA treatment in MDA-MB-231 breast cancer cells displayed in Figure 10B.

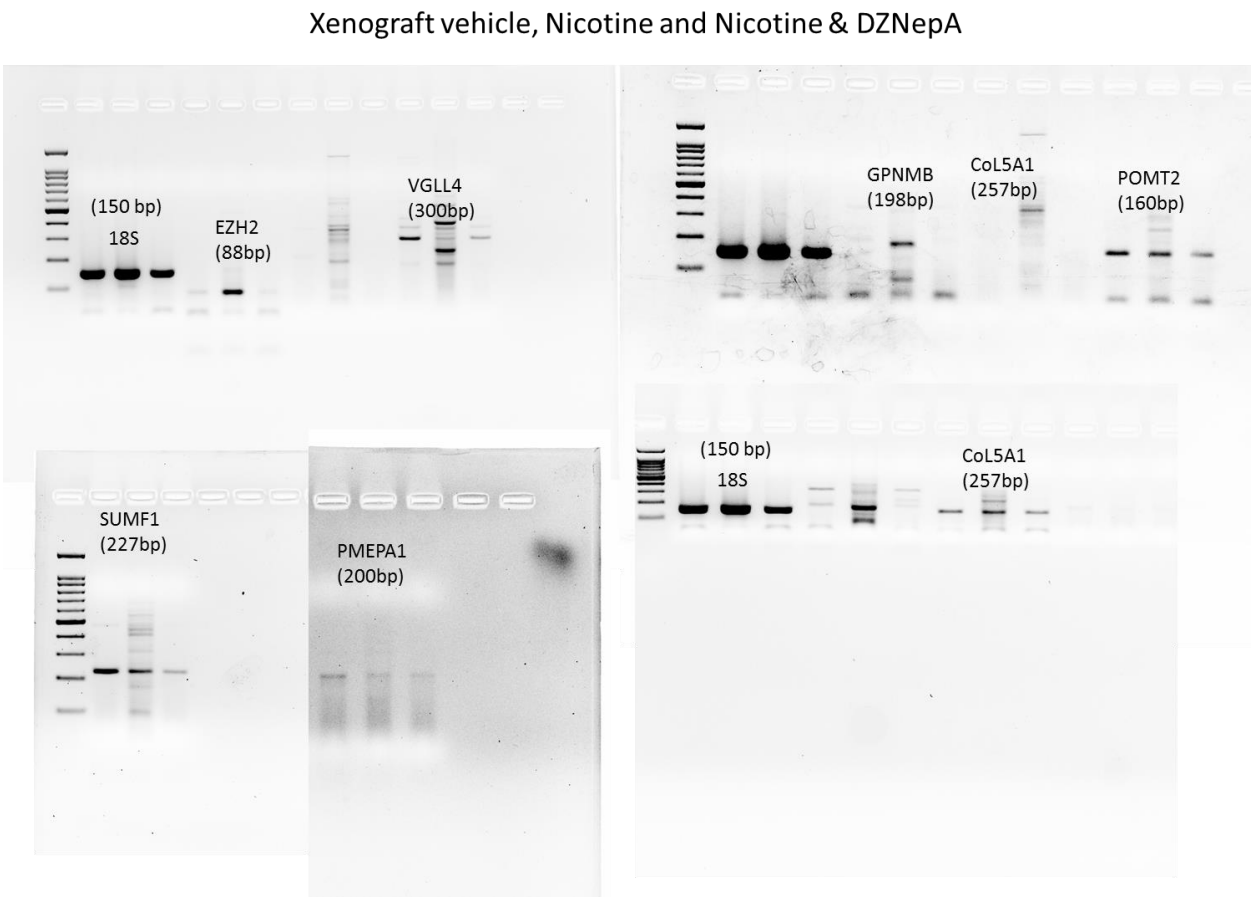


Figure S7. Uncropped images of agarose gel pictures for expression of 18S, EZH2, PMEPA1, GPNMB, SUMF1, VGLL4, CoL5A1 and POMT2 in xenograft extracted from vehicle, nicotine and nicotine& DZNepA treatment mice displayed in Figure 10C.