

## Supplemental information

### Epigenetic Reprogramming Reverses the Relapse Specific Gene Expression Signature and Restores Chemosensitivity in Childhood B-Lymphoblastic Leukemia

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#### Supplemental Figure legends

##### **Figure S1. Vorinostat is negatively connected with the ALL relapse/diagnosis signature.**

The bar view is constructed from horizontal lines, each representing an individual treatment-control pair (instance). Each of the 12 vorinostat instances in the “bar view” is colored in black, Colors applied on remaining instances reflect the sign of their scores (green, positive; gray, null; red, negative). Similarly 182 instances for trichostatin A are represented in next panel.

##### **Figure S2. In vitro cell viability assays of vorinostat and decitabine individually in combination with chemotherapy**

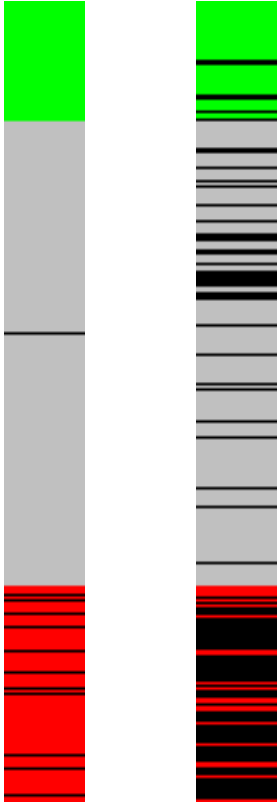
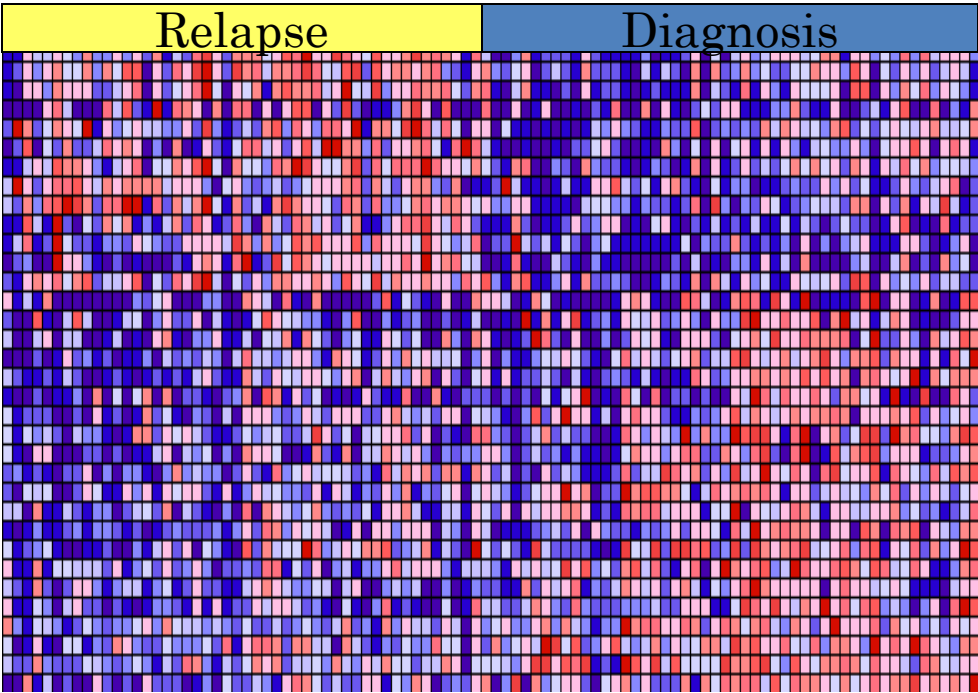
(A) Reh and (B) RS4: 11 cell lines were incubated with vorinostat and various chemotherapeutic agents were added after a 24 or 48h intervals; etoposide (24 h), prednisolone (24 h), doxorubicin (24 h) and cytarabine (48 h). (C) and (D) shows the effect of decitabine pretreatment for 24 h followed by chemotherapeutic agents in Reh and RS4:11 cell lines respectively.

#### Supplemental Table Legends

**Table S1: List of relapse specific genes analyzed and the effect of vorinostat treatment**

**Table S2: Primers used in various assays**

Figure S1.



Vorinostat

Trichostatin A

Figure S2

A.

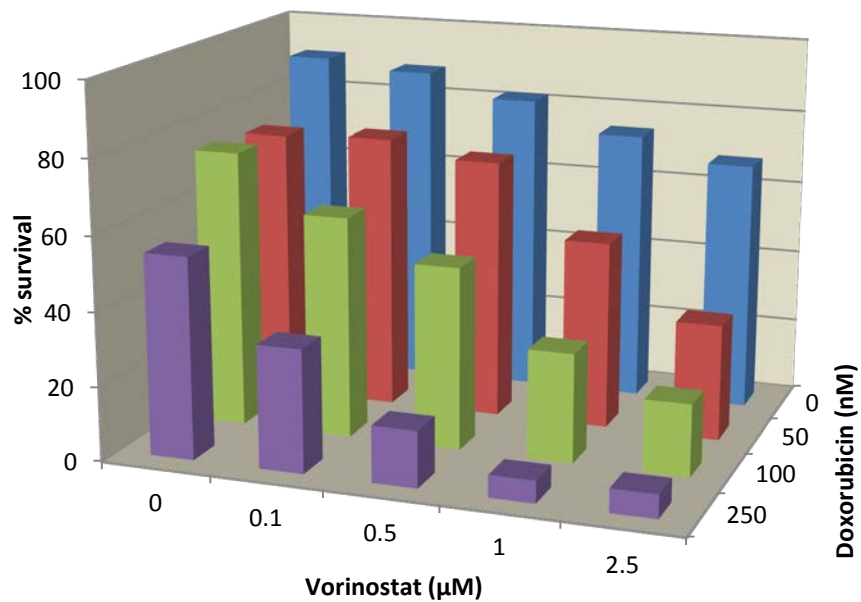
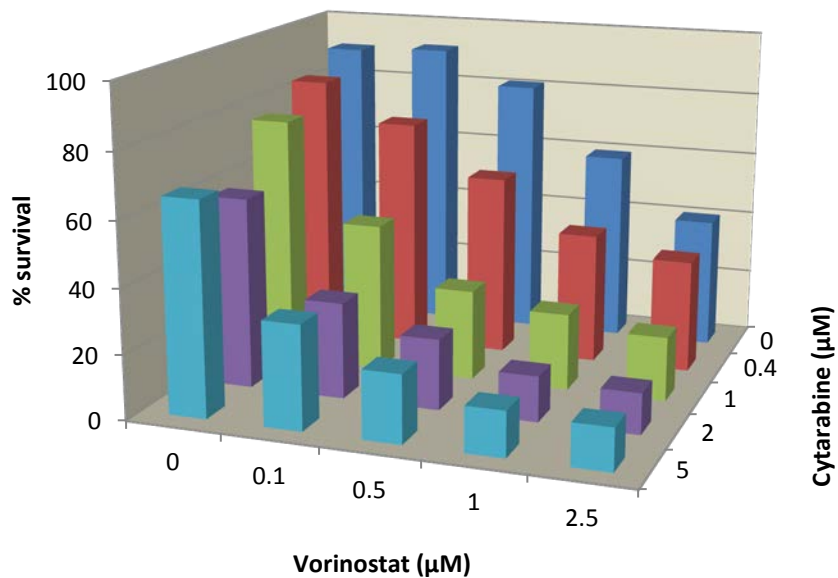
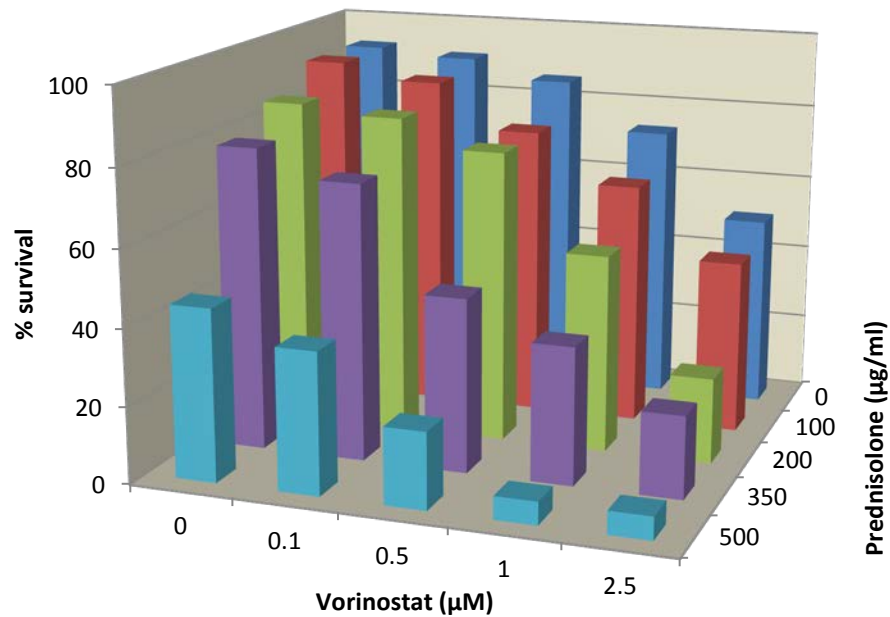
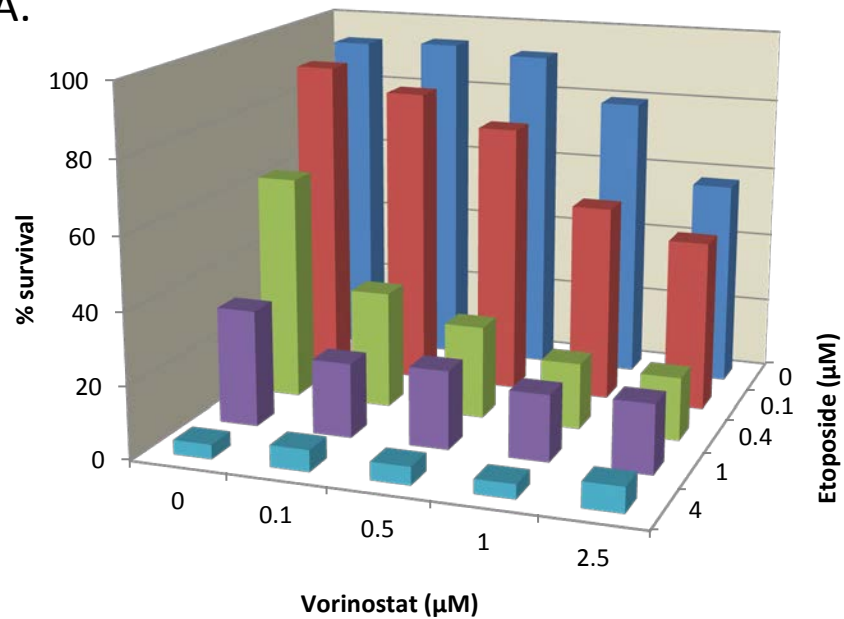


Figure S2

B.

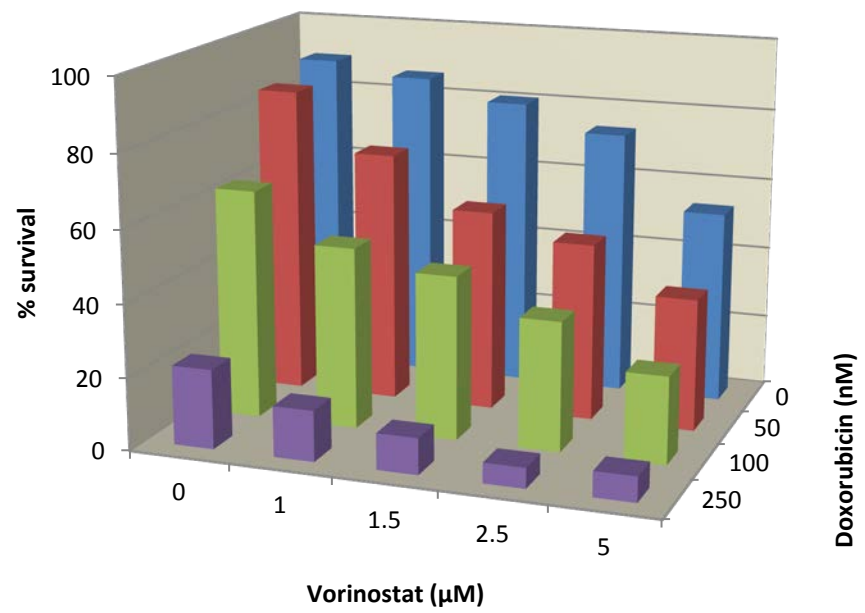
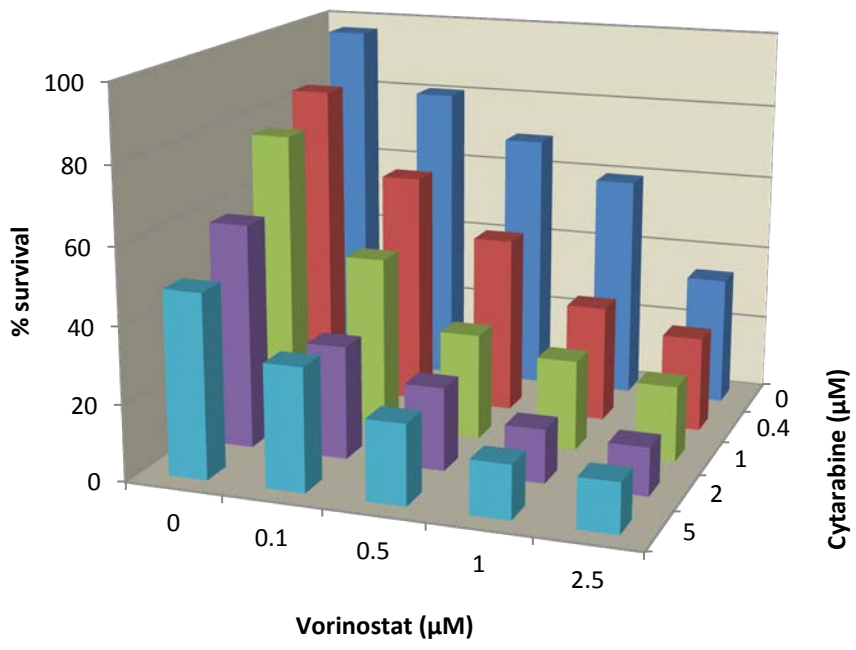
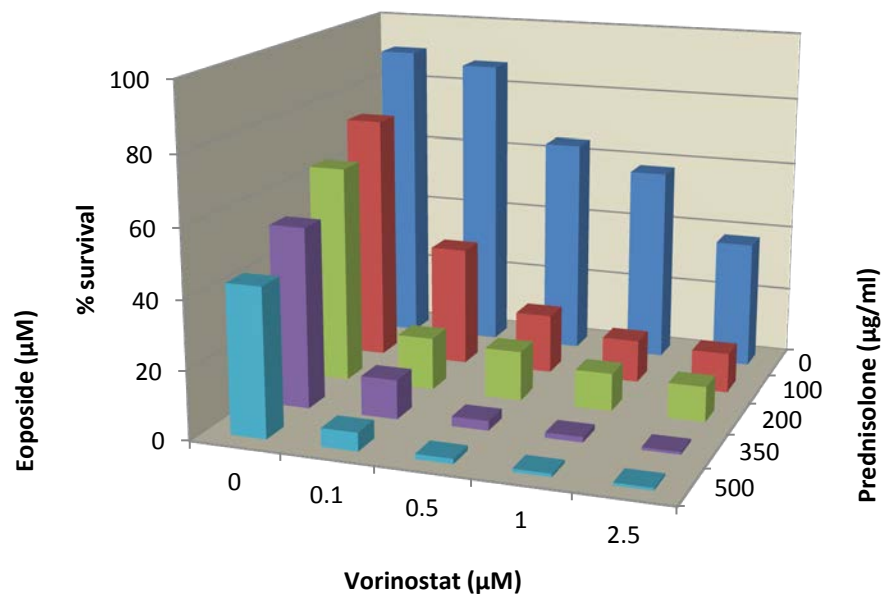
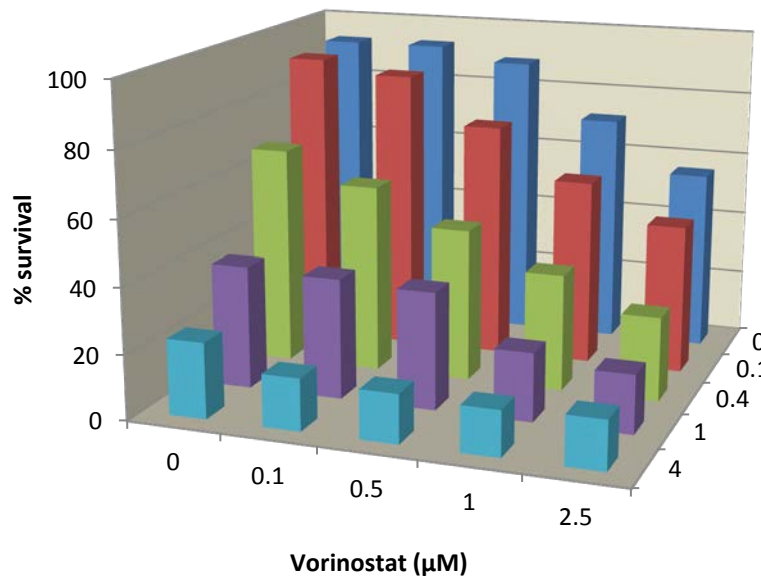
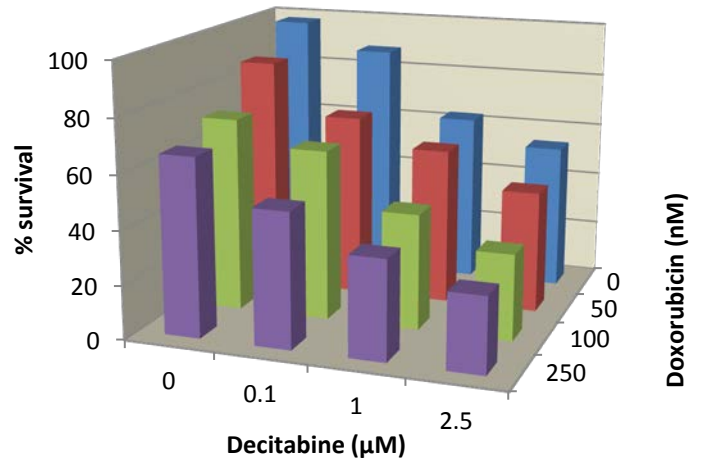
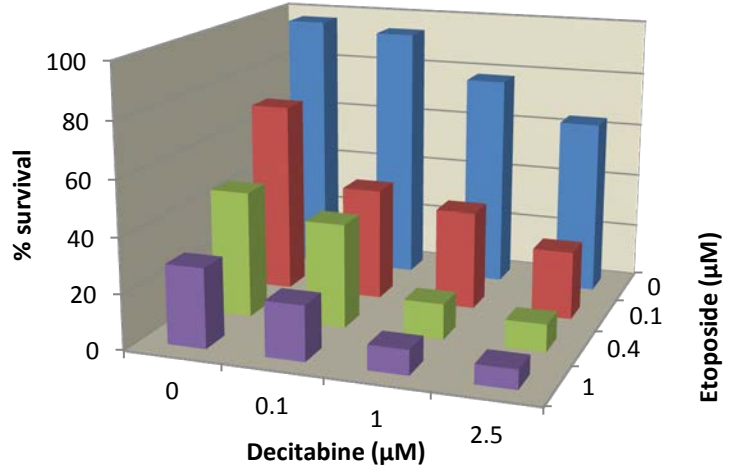
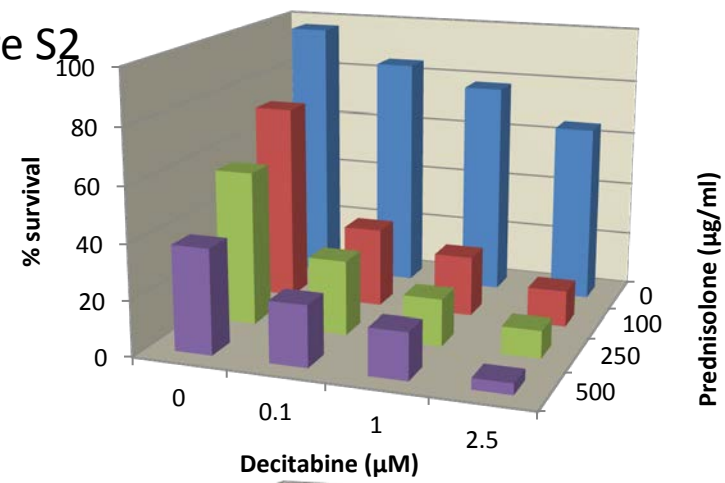
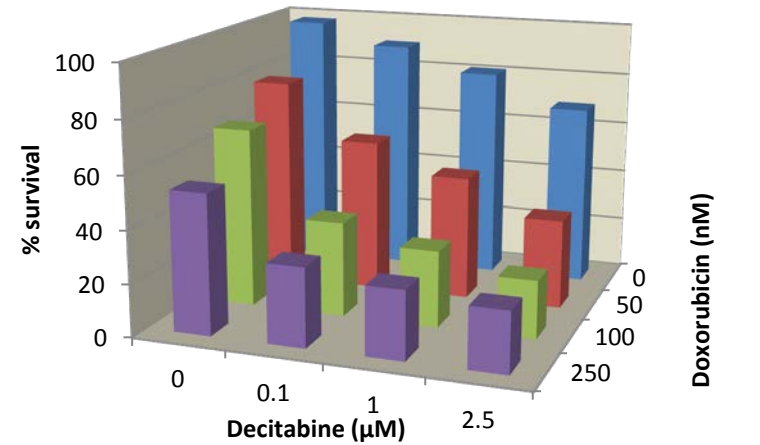
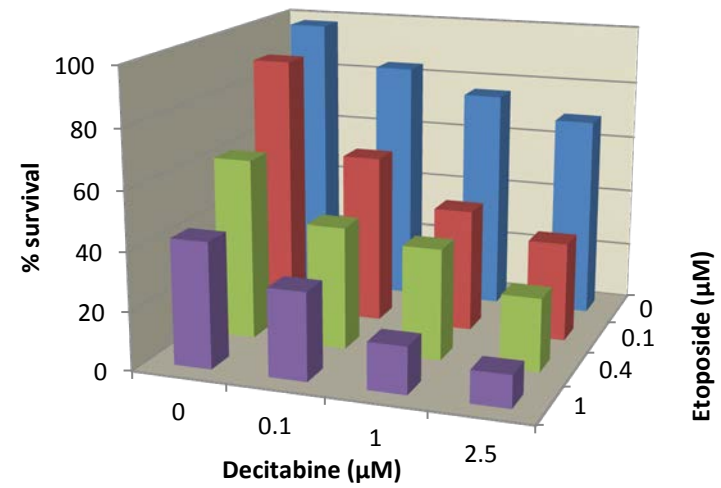
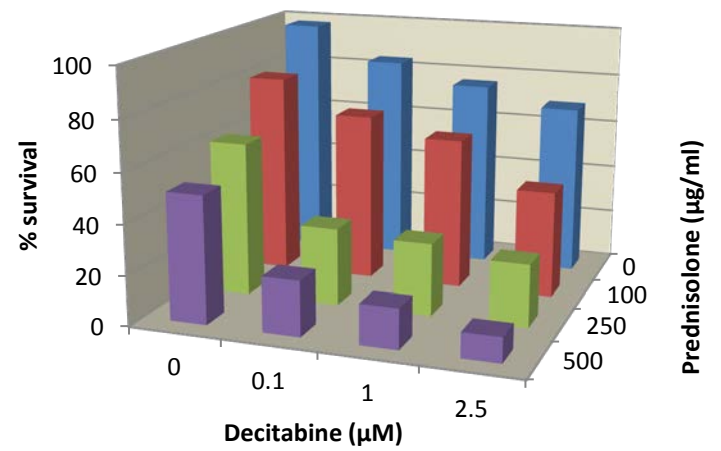


Figure S2

C.



D.



**Table S1: List of relapse specific genes analyzed and the effect of vorinostat treatment**

<b>Genes*</b>	<b>Up or down regulated in relapse signature compared to diagnosis</b>	<b>Pt 1</b>	<b>Pt 2</b>	<b>Pt 3</b>	<b>Reh</b>	<b>RS4:11</b>	<b>MV4:11</b>
BIRC5	Upregulated	Y	Y	Y	Y	Y	Y
PSAP	Upregulated	N	N	N	N	N	N
AP2S1	Upregulated	N	Y	N	N	Y	N
PTBP1	Upregulated	Y	Y	Y	N	Y	Y
FOXM1	Upregulated	Y	Y	Y	Y	Y	Y
GTSE1	Upregulated	N	Y	Y	N	N	Y
TAF13	Upregulated	Y	N	N	N	N	N
HMGA1	Upregulated	N	Y	Y	N	N	N
MCM7	Upregulated	Y	Y	Y	Y	Y	N
KIF2C	Upregulated	N	Y	Y	N	N	Y
GTSE1	Upregulated	N	Y	N	Y	N	N
AP2S1	Upregulated	N	N	N	N	Y	N
TYMS	Upregulated	Y	Y	Y	N	Y	Y
FANCD2	Upregulated	Y	Y	Y	N	Y	Y
KIAA0101	Upregulated	N	Y	Y	Y	Y	N
PTBP1	Upregulated	Y	Y	Y	N	Y	Y
MRPS18B	Upregulated	N	N	N	Y	Y	Y
ORC6L	Upregulated	N	Y	Y	N	N	N
PRR11	Upregulated	N	N	N	Y	N	N
ATP1A1	Upregulated	N	Y	Y	N	N	N
CTTN	Upregulated	Y	Y	NA	NA	N	N
BGN	Upregulated	NA	NA	NA	NA	NA	NA
SLC9A3R1	Upregulated	N	N	N	N	N	N
TK1	Upregulated	N	Y	N	N	Y	N
GAA	Upregulated	N	N	N	Y	Y	N
SLC37A4	Upregulated	N	N	N	N	Y	N
AMOTL2	Upregulated	NA	N	NA	NA	NA	NA
AKR1C1	Upregulated	N	Y	N	Y	N	N
ESPL1	Upregulated	N	Y	N	N	N	N

Genes*	Up or down regulated in relapse signature compared to diagnosis	Pt 1	Pt 2	Pt 3	Reh	RS4:11	MV4:11
RELN	Upregulated	N	Y	N	NA	N	NA
HIST1H4A	Upregulated	N	N	N	N	N	N
ST7	Upregulated	NA	Y	Y	NA	N	Y
AP2S1	Upregulated	N	N	N	N	Y	N
NEF-sp, LOC81691	Upregulated	N	Y	N	N	N	N
DMC1	Upregulated	N	Y	N	Y	NA	N
septin 8	Upregulated	N	N	N	N	N	N
GART	Upregulated	Y	Y	N	NA	N	N
GRN	Upregulated	N	N	N	N	N	N
AKR1C2	Upregulated	Y	N	N	NA	N	N
TMEM97	Upregulated	Y	Y	N	N	Y	N
C10orf56	Upregulated	N	N	N	N	N	N
C10orf56	Upregulated	N	N	N	N	N	N
MSTP9	Upregulated	N	N	N	NA	Y	Y
OBSL1	Upregulated	NA	NA	Y	NA	NA	N
MLC1	Upregulated	N	Y	N	NA	N	N
RECQL4	Upregulated	N	Y	Y	N	Y	N
LAPTM4B	Upregulated	Y	Y	NA	NA	NA	NA
PLK3	Upregulated	N	N	Y	N	N	N
TUBA3D	Upregulated	N	Y	Y	N	N	N
PNPO	Upregulated	N	N	N	N	N	N
CENPM	Upregulated	N	Y	N	N	Y	N
CENTA2	Upregulated	Y	Y	Y	Y	N	N
PRMT7	Upregulated	Y	N	Y	N	N	N
POLQ	Upregulated	Y	Y	N	Y	Y	Y
MEIG1	Upregulated	N	N	N	N	N	N
ESPL1	Upregulated	N	Y	N	N	N	N
CD9	Downregulated	N	Y	N	Y	N	Y
C3orf37	Downregulated	Y	N	N	Y	Y	Y

<b>Genes*</b>	<b>Up or down regulated in relapse signature compared to diagnosis</b>	<b>Pt 1</b>	<b>Pt 2</b>	<b>Pt 3</b>	<b>Reh</b>	<b>RS4:11</b>	<b>MV4:11</b>
NR3C1	Downregulated	N	N	N	Y	Y	Y
SETX	Downregulated	N	N	N	Y	Y	N
GABBR1	Downregulated	Y	Y	Y	Y	N	Y
IFIT5	Downregulated	Y	N	N	Y	N	Y
IFIT5	Downregulated	Y	N	N	Y	N	NA
KLF7	Downregulated	N	Y	N	Y	Y	Y
DOPEY2	Downregulated	N	N	Y	Y	Y	N
BPG181B	Downregulated	Y	Y	Y	Y	Y	N
IQCB1	Downregulated	N	N	N	Y	Y	Y
HRK	Downregulated	Y	Y	N	Y	NA	Y
ZNF238	Downregulated	NA	NA	NA	Y	NA	Y
NCOA3	Downregulated	Y	Y	N	Y	Y	N
MED21	Downregulated	N	N	N	N	N	N
MED21	Downregulated	N	Y	N	N	N	N
KIAA0922	Downregulated	N	N	Y	Y	Y	N
ITSN2	Downregulated	N	N	N	Y	Y	N
SSBP2	Downregulated	Y	N	N	N	N	Y
ZMIZ1	Downregulated	N	N	N	Y	Y	Y
KBTBD2	Downregulated	N	N	N	N	Y	N
ARID5B	Downregulated	Y	N	N	Y	Y	N
ZCCHC11	Downregulated	N	N	N	Y	Y	N
FEM1C	Downregulated	N	N	N	Y	Y	N
TDRD7	Downregulated	Y	Y	N	Y	Y	Y
RABEP1	Downregulated	Y	Y	Y	Y	N	N
YTHDC1	Downregulated	N	Y	N	Y	N	NA
MACF1	Downregulated	N	N	N	Y	Y	N
CHD7	Downregulated	Y	Y	N	Y	N	N
ATF7IP	Downregulated	Y	N	N	N	N	N
GRAMD1C	Downregulated	Y	Y	Y	Y	Y	Y



Genes*	Up or down regulated in relapse signature compared to diagnosis	Pt 1	Pt 2	Pt 3	Reh	RS4:11	MV4:11
ZNF586	Downregulated	N	N	N	N	N	N
SMEK2	Downregulated	N	Y	Y	Y	Y	Y
CCDC88A	Downregulated	N	Y	N	N	Y	Y
RNFT1	Downregulated	N	N	N	Y	Y	NA
FLJ11286	Downregulated	Y	N	N	N	N	Y
POLR2J4	Downregulated	N	N	Y	Y	Y	Y
AFF1	Downregulated	Y	Y	N	Y	N	N
GLS	Downregulated	N	N	N	Y	Y	N
ZEB2	Downregulated	Y	N	N	NA	Y	N
TOPORS	Downregulated	Y	N	Y	Y	Y	N
NFYA	Downregulated	N	N	Y	N	N	N
C7orf23	Downregulated	Y	N	N	Y	N	N
NF1	Downregulated	NA	NA	NA	NA	NA	NA
S1PR1	Downregulated	Y	Y	N	NA	NA	NA
CRIP1	Downregulated	N	Y	N	Y	N	Y
BCL10	Downregulated	N	Y	N	N	N	N
CKMT2	Downregulated	N	NA	NA	NA	NA	NA
KMO	Downregulated	N	N	N	NA	NA	NA
HERC3	Downregulated	N	Y	N	NA	NA	NA
SPOCK3	Downregulated	NA	NA	NA	NA	NA	NA
C6orf32	Downregulated	Y	Y	N	NA	NA	NA
GAB1	Downregulated	Y	N	N	NA	Y	NA
RFX3	Downregulated	NA	NA	NA	NA	NA	NA
CDR1	Downregulated	NA	NA	NA	NA	NA	NA
DR1	Downregulated	N	Y	N	N	N	N
TSC1	Downregulated	N	Y	N	N	N	N
FUSIP1	Downregulated	N	N	N	N	N	N
BLZF1	Downregulated	NA	NA	NA	NA	NA	NA
KMO	Downregulated	N	N	N	NA	NA	NA

<b>Genes*</b>	<b>Up or down regulated in relapse signature compared to diagnosis</b>	<b>Pt 1</b>	<b>Pt 2</b>	<b>Pt 3</b>	<b>Reh</b>	<b>RS4:11</b>	<b>MV4:11</b>
RPS27A	Downregulated	N	N	N	Y	N	N
ITPR1	Downregulated	Y	Y	N	N	Y	N
GRINL1A	Downregulated	N	N	N	N	N	N
TUG1	Downregulated	N	N	Y	Y	N	Y
TTC9	Downregulated	NA	N	NA	NA	N	NA
KLHL9	Downregulated	N	Y	NA	NA	N	NA
LBA1	Downregulated	N	N	N	NA	NA	NA
TMCC1	Downregulated	Y	Y	N	N	N	N
FUSIP1	Downregulated	N	N	N	N	N	N
CENTD1	Downregulated	Y	Y	N	NA	NA	NA
DIS3	Downregulated	N	N	N	Y	Y	N
SPTBN1	Downregulated	NA	NA	NA	NA	NA	NA
CCDC88C	Downregulated	Y	Y	Y	Y	Y	Y
SPTBN1	Downregulated	NA	NA	NA	NA	NA	NA
ZNF460	Downregulated	NA	NA	N	NA	NA	NA
SLC25A30	Downregulated	NA	NA	NA	NA	NA	Y
ITPR1	Downregulated	Y	Y	N	N	Y	N
WBP11	Downregulated	N	N	N	N	N	N
AFTPH	Downregulated	N	Y	N	Y	N	N
RBM7	Downregulated	Y	N	N	N	N	N
INTS6	Downregulated	N	N	N	Y	Y	N
ARID1A	Downregulated	N	N	N	N	N	N
CSGALNACT1	Downregulated	Y	N	N	NA	NA	Y
SRBD1	Downregulated	N	Y	N	NA	N	NA
ZBTB10	Downregulated	Y	N	N	Y	Y	N
B3GNT2	Downregulated	Y	Y	N	N	Y	N
VCP1P1	Downregulated	Y	N	N	N	N	N
CHD7	Downregulated	NA	NA	NA	NA	NA	NA
FCRL2	Downregulated	NA	NA	NA	NA	NA	NA
TECTA	Downregulated	NA	NA	NA	NA	NA	NA

<b>Genes*</b>	<b>Up or down regulated in relapse signature compared to diagnosis</b>	<b>Pt 1</b>	<b>Pt 2</b>	<b>Pt 3</b>	<b>Reh</b>	<b>RS4:11</b>	<b>MV4:11</b>
EGLN1	Downregulated	N	N	N	Y	N	N
RAB9A	Downregulated	Y	Y	N	N	Y	N
Transcribed locus	Downregulated	NA	NA	NA	NA	NA	NA
ALOX5	Downregulated	NA	N	Y	NA	NA	NA
CDS2	Downregulated	Y	N	N	N	N	N
SFRS10	Downregulated	N	N	N	N	Y	N
ABCD4	Downregulated	NA	N	N	NA	NA	NA
FRMD4B	Downregulated	Y	N	N	Y	Y	NA

\* Few genes are represented as multiple probes

Y- Yes, Expression reversed after vorinostat exposure

N- No, Expression not changed after vorinostat exposure

NA- Not analyzed, Genes with expression intensity across the chip < 16

Table S2: Primers used in various assays

### Primers used for qRT-PCR

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B2M	Forward	5'-ATGTGTCTGGGTTTCATCCATCC-3'
	Reverse	5'-AGTCACATGGTTCACACGGCA-3'
BIRC5	Forward	5'-CATCTCTACATTCAAGAACTGG-3'
	Reverse	5'-GGTTAATTCTTCAAACCTGCTTC-3'
FOXO1	Forward	5'-GGAGGAAATGCCACACTTAGCG-3'
	Reverse	5'-TAGGACTTCTGGGTCTTGGGGTG-3'
HRK	Forward	5'-GTTGGTGAAAACCCTGTGT-3'
	Reverse	5'-TAAATAGCATTGGGGTGTCT-3'

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### Primers from Qiagen

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COL6A2	QT00067039
CSMD1	QT00043169
FANCD2	QT00034370
GATA5	QT01673777
TYMS	QT00052423
WT1	QT00059003
CDKN2A	QT00089964
GATA4	QT00031997
HOXA9	QT01002365
NR3C1	QT00020608
PTPRO	QT00037695
APC	QT02407671

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## Primers used for chromatin immunoprecipitation assays

Genes	Location		Sequence
NR3C1	1500 bp upstream	Forward	5'-CCAGATATGCTCCATTTCACTTC-3'
		Reverse	5'-CTTTTGTGGTAGGAAGCTCAGA-3'
NR3C1	TSS	Forward	5'-TTGAGTAGAGGCGAATCACTTTC-3'
		Reverse	5'-GTTTCTATTCCTTCCCCACTCAT-3'
HRK	1500 bp upstream	Forward	5'-TGTGGTATCCTGCACAGAGG-3'
		Reverse	5'-AATGCACCAGCATCTCACTG-3'
BIRC5	2000 bp upstream	Forward	5'-CTTCAACTTACAAACGGTTCAGG-3'
		Reverse	5'-CTCCTGTTTTCTTTCTCTCTCC-3'
BIRC5	5000 bp upstream	Forward	5'-AACTTATGGGGTTGCTCTCTTTC-3'
		Reverse	5'-TTTAATAGAGACGGGGTTTCACC-3'
FOXM1	1500 bp upstream	Forward	5'-GGCATCAGATCTCACACCAA-3'
		Reverse	5'-CACCGTCTCCAAAACAAACA-3'
FOXM1	TSS	Forward	5'-CTGTTCAAAATGCCCAAGT-3'
		Reverse	5'-TGCTGTGATGATGCTGTGAA-3'

## Primers used for MSP

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GATA4 <sup>1</sup>	Unmethylated Forward	5'-TTTGTATAGTTTTGTAGTTTGTGTTTAGT-3'
	Unmethylated Reverse	5'-CCCAACTCACAACCTCAAATCCCCA-3'
	Methylated Forward	5'-GTATAGTTTCGTAGTTTGCCTTAGC-3'
	Methylated Reverse	5'-AACTCGCGACTCGAATCCCCG-3'
APC <sup>1</sup>	Unmethylated Forward	5'-GTGTTTTATTGTGGAGTGTGGGT-3'
	Unmethylated Reverse	5'-CCAATCAACAACTCCCAACAA-3'
	Methylated Forward	5'-TATTGCGGAGTGC GGTC-3'
	Methylated Reverse	5'-TCGACGAACTCCCGACGA-3'
HOXA9 <sup>2</sup>	Unmethylated Forward	5'-GTATGGTTAATGGGGGTGTGGGTTT-3'
	Unmethylated Reverse	5'-CCATACCCAACACCTAACCCACCCAACCCA-3'
	Methylated Forward	5'-GGTTAATGGGGCGCGGGCGTC-3'
	Methylated Reverse	5'-AACGCCTAACCCGCCGACCCG-3'
NR3C1 <sup>2</sup>	Unmethylated Forward	5'-TTGGTTTTGTTTGTGTTTAGGT-3'
	Unmethylated Reverse	5'-CCATCCCAATCCCACTACTTCAAC-3'
	Methylated Forward	5'-TCGGTTTCGTTTCGTTTAGGTTC-3'
	Methylated Reverse	5'-CGTCCCGATCCCACTACTTCGAC-3'
CDKN2A <sup>3</sup>	Unmethylated Forward	5'-TTATTAGAGGGTGGGGTGGATTGT-3'
	Unmethylated Reverse	5'-CAACCCCAAACCACAACCATAA-3'
	Methylated Forward	5'-TTATTAGAGGGTGGGGCGGATCGC-3'
	Methylated Reverse	5'-GACCCCGAACCGGACCGTAA-3'
PTPRO <sup>4</sup>	Unmethylated Forward	5'-ATGTTTTTGGAGGATTTGGGT-3'
	Unmethylated Reverse	5'-ATACCCCATCACTACACAAACA-3'
	Methylated Forward	5'-CGTTTTTGGAGGATTTGGGC-3'
	Methylated Reverse	5'-AAAACACGACTACGCTAACG-3'

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## References

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