

Supplementary material 1

Table 1. Relative quantification of drug induced gene re-expression in the independent biological replicas (indicated by numbers 1 and 2). The qPCR assays were performed in a StepOne Real-time PCR System (Applied Biosystems) and the results were calculated using the $\Delta\Delta C_t$ -method normalized to glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) expression levels. For each gene and cell line, the results are expressed as fold change relative to the respective reference (untreated controls).

RELATIVE EXPRESSION LEVELS (FOLD CHANGE)

CELL LINES GENES and treatments	184A1	184B5	BT-20	BT-474	BT-483	BT-549	Hs 578T	MCF 10A	MCF7	MDA-MB- 134-IV	MDA- MB-231	MDA- MB-361	MDA- MB-415	MDA- MB-436	MDA- MB-453	MDA- MB-468	SK-BR-3	T-47D	ZR-75-30	ZR-75-1
	SEMAB3B																			
Reference (untreated cell line)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
5-Aza-dC 1	2.61	0.88	0.98	3.63	3.08	1.08	1.33	0.73	7.43	1.69	0.34	3.50	2.14	1.22	5.82	1.68	3.92	1.35	0.43	10.39
5-Aza-dC 2	3.06	1.03	1.18	5.64	2.44	1.18	1.62	0.77	3.45	1.47	0.34	3.93	2.60	2.29	7.72	1.78	2.24	1.33	0.54	10.14
TSA 1	0.46	1.08	0.15	1.43	1.11	1.25	1.34	0.66	0.92	1.12	0.47	1.11	0.68	1.20	2.19	0.70	20.91	1.06	0.44	1.45
TSA 2	0.68	1.29	0.15	1.65	1.08	0.93	1.47	0.66	1.13	0.76	0.43	1.13	0.71	1.27	1.95	0.83	46.53	1.10	0.14	1.38
5-Aza-dC+TSA 1	1.39	1.18	1.65	3.22	2.91	1.28	1.79	0.71	3.82	0.86	0.66	4.85	1.83	2.35	6.88	2.50	3.61	1.79	0.90	5.13
5-Aza-dC+TSA 2	1.38	1.07	1.23	3.13	2.85	1.46	1.49	0.64	3.32	1.31	0.72	5.63	2.11	1.74	5.33	2.16	2.04	1.74	0.65	5.46
HYAL3																				
Reference (untreated cell line)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
5-Aza-dC 1	1.09	2.54	1.15	1.25	1.15	2.17	1.52	1.87	1.13	0.88	3.68	2.02	1.65	1.70	1.96	1.87	1.61	1.36	0.72	4.38
5-Aza-dC 2	1.21	2.51	1.23	1.78	1.24	1.96	1.54	1.55	1.01	1.14	4.40	2.84	1.78	2.27	1.90	2.10	1.17	1.24	0.71	3.21
TSA 1	0.95	1.78	0.32	1.41	0.99	1.30	1.17	1.34	0.67	1.49	1.54	1.83	0.69	1.23	1.03	0.81	2.65	0.91	1.63	1.06
TSA 2	0.93	0.86	0.84	1.04	0.90	1.09	1.24	1.15	0.75	0.77	1.10	1.58	0.69	1.08	1.15	0.96	1.14	0.89	1.15	0.73
5-Aza-dC+TSA 1	1.17	2.68	1.04	5.04	1.05	2.51	2.07	2.83	0.94	0.86	3.35	2.78	1.41	1.83	4.26	1.91	1.55	1.11	1.07	1.98
5-Aza-dC+TSA 2	0.90	2.53	1.20	3.67	0.93	2.30	1.99	2.11	1.06	0.99	3.13	3.26	1.37	1.99	1.32	1.82	0.98	1.01	1.27	1.12
HYAL1																				
Reference (untreated cell line)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
5-Aza-dC 1	124.26	63.79	7.01	5.44	25.65	55.66	5.57	84.38	30.69	12.33	7.77	21.49	2.37	17.27	89.89	5.62	11.24	8.47	3.58	32.07
5-Aza-dC 2	127.88	61.85	7.91	5.29	23.73	54.26	5.71	98.07	31.01	7.50	6.88	25.44	2.36	14.43	49.05	8.16	3.27	8.63	3.35	27.76
TSA 1	1.23	0.92	1.73	3.94	0.63	1.61	1.19	0.44	2.05	1.59	5.11	0.96	0.53	0.58	1.17	2.42	10.65	1.52	1.65	0.62
TSA 2	1.21	1.21	1.47	2.85	1.42	1.46	0.89	0.77	1.66	1.83	1.10	0.63	0.53	0.63	0.97	4.15	1.00	1.46	2.89	0.43
5-Aza-dC+TSA 1	126.63	58.89	8.10	58.57	58.45	43.26	7.67	106.79	41.77	8.89	6.67	22.75	1.32	26.94	142.25	6.93	13.80	9.01	4.22	20.37
5-Aza-dC+TSA 2	68.82	44.35	8.28	29.82	46.30	39.88	6.79	106.01	36.88	15.84	6.75	34.29	1.96	25.75	44.03	12.34	2.65	7.02	3.72	15.81
HYAL2																				
Reference (untreated cell line)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
5-Aza-dC 1	1.21	1.37	1.18	1.19	1.19	1.41	1.29	2.29	1.83	1.31	1.35	1.03	1.24	0.51	1.99	1.05	1.71	1.48	0.61	1.58
5-Aza-dC 2	1.28	1.33	1.25	1.11	1.27	1.36	1.33	1.99	1.74	1.24	1.50	1.17	1.37	0.70	1.68	0.98	1.10	1.45	0.66	1.15
TSA 1	0.98	1.03	1.84	1.07	0.97	1.27	1.29	1.24	1.01	1.34	1.04	0.89	0.72	0.79	1.16	1.22	1.78	1.33	0.69	0.71
TSA 2	0.68	1.00	1.35	1.09	0.80	1.23	1.22	1.08	0.95	1.02	0.82	0.91	0.71	0.72	1.20	1.00	1.56	1.17	0.71	0.56

5-Aza-dC+TSA 1	1.38	1.24	1.38	1.28	1.33	1.26	1.77	2.22	1.37	0.51	1.67	0.91	0.84	0.62	2.50	1.13	1.49	1.80	0.69	1.22
5-Aza-dC+TSA 2	0.71	1.26	1.98	1.10	1.49	1.40	1.92	2.20	1.53	0.83	1.65	0.67	1.05	0.49	1.37	1.03	1.77	1.66	0.58	1.65
TUSC2																				
Reference (untreated cell line)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
5-Aza-dC 1	1.14	2.09	1.06	1.67	1.35	1.55	1.78	3.86	1.43	1.44	2.01	1.32	1.87	1.60	1.91	1.40	2.16	1.83	0.70	2.75
5-Aza-dC 2	0.98	2.62	0.92	2.28	1.18	1.44	1.71	3.56	1.67	1.49	3.92	1.47	2.09	2.36	1.65	1.84	2.32	1.82	0.98	2.88
TSA 1	0.94	1.07	0.00	1.62	1.18	1.15	2.26	0.77	1.14	1.36	1.22	0.91	0.91	0.84	1.23	0.15	1.74	1.12	2.85	2.24
TSA 2	0.59	1.01	0.00	1.45	0.91	0.90	2.33	1.07	0.94	1.13	0.67	0.85	0.96	1.25	1.21	0.76	1.26	1.27	2.51	2.33
5-Aza-dC+TSA 1	1.48	2.61	0.97	2.12	1.06	1.36	4.32	3.78	1.04	0.51	3.14	2.08	1.89	2.91	1.54	2.75	1.92	2.18	0.92	2.91
5-Aza-dC+TSA 2	0.43	2.37	1.15	1.69	1.10	1.30	3.77	3.78	1.04	0.77	1.24	1.68	1.62	2.23	0.62	0.70	2.27	2.09	0.72	2.24
RASSF1																				
Reference (untreated cell line)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
5-Aza-dC 1	0.92	1.35	1.97	1.17	1.29	0.93	2.10	2.82	3.08	1.11	2.80	1.52	0.81	3.13	1.99	2.22	2.43	2.50	0.55	1.92
5-Aza-dC 2	0.86	1.35	2.22	1.84	1.24	0.93	2.09	2.72	1.93	1.23	3.18	3.01	0.97	4.00	2.86	5.54	2.34	2.45	0.54	1.56
TSA 1	0.86	1.09	1.15	1.34	0.90	1.56	1.61	1.58	0.99	1.29	1.80	1.24	0.67	0.84	1.13	1.17	2.46	1.55	0.38	0.85
TSA 2	0.61	1.29	1.38	0.95	0.72	1.03	1.36	1.24	0.89	0.79	1.21	0.99	0.67	0.83	1.03	1.02	1.56	1.30	0.43	0.88
5-Aza-dC+TSA 1	0.99	1.77	1.38	0.87	1.02	0.89	3.27	3.09	1.30	0.56	4.33	1.24	0.59	7.46	2.22	6.77	3.68	1.67	0.78	1.14
5-Aza-dC+TSA 2	0.34	1.69	1.86	0.77	0.93	0.80	3.23	2.80	1.32	0.58	2.50	2.62	0.79	5.29	2.22	4.20	1.90	1.25	0.69	1.61
ZMYND10																				
Reference (untreated cell line)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
5-Aza-dC 1	0.96	1.11	1.54	4.55	1.13	0.92	1.51	1.01	0.99	0.43	1.66	2.51	1.59	5.23	1.27	0.74	3.37	1.28	0.76	1.88
5-Aza-dC 2	1.03	1.70	1.61	7.64	1.28	0.93	1.53	0.90	0.97	0.16	1.32	2.34	1.77	7.66	1.29	0.82	3.02	1.27	0.80	2.12
TSA 1	0.62	0.23	1.46	12.36	0.91	0.98	1.43	1.14	0.76	0.58	2.29	1.53	0.92	0.72	1.33	1.26	5.40	1.20	4.73	1.57
TSA 2	0.55	0.29	1.63	24.73	0.79	1.06	1.62	0.97	0.64	1.00	1.86	1.59	0.91	1.26	1.27	1.30	1.45	1.22	5.29	1.43
5-Aza-dC+TSA 1	0.86	1.88	1.13	56.26	0.67	0.53	2.07	1.29	0.57	0.59	2.11	2.05	1.17	8.91	1.17	2.50	3.79	0.71	2.01	2.69
5-Aza-dC+TSA 2	0.54	1.27	1.10	47.86	0.50	0.59	1.95	1.29	0.62	0.76	2.08	2.57	0.78	6.00	0.89	2.02	1.64	1.00	1.02	2.30
NPRL2																				
Reference (untreated cell line)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
5-Aza-dC 1	0.87	1.20	1.13	1.57	0.98	0.85	1.57	1.21	2.31	1.12	1.39	1.01	1.28	2.14	1.66	1.22	33.01	1.03	0.83	2.63
5-Aza-dC 2	0.86	1.14	1.06	1.91	0.92	0.93	1.34	1.72	1.68	0.95	1.38	1.21	1.41	2.62	1.55	1.34	1.78	1.16	1.26	2.70
TSA 1	0.96	1.25	1.29	1.18	0.80	1.18	1.24	1.12	1.10	1.23	0.97	0.97	0.83	1.14	1.29	1.15	4.30	0.96	2.89	2.52
TSA 2	0.58	0.95	1.06	1.10	0.78	1.02	0.89	1.05	0.85	0.99	1.04	0.87	0.82	1.34	1.43	1.19	1.46	1.16	2.45	2.25
5-Aza-dC+TSA 1	0.90	1.41	1.99	2.23	0.76	1.01	1.22	1.56	1.36	0.99	1.08	2.01	1.36	2.84	1.82	1.82	3.13	1.49	0.95	2.05
5-Aza-dC+TSA 2	0.82	1.43	1.99	1.81	0.77	0.89	1.23	1.14	1.47	1.03	1.13	1.48	1.45	2.76	1.34	1.77	1.70	1.55	1.00	1.50
TMEM115																				

Reference (untreated cell line)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
5-Aza-dC 1	1.04	1.26	1.07	1.23	1.15	1.00	1.12	1.17	12.12	1.21	1.40	1.66	1.63	1.03	1.54	2.05	1.18	2.12	0.58	2.25
5-Aza-dC 2	1.08	1.29	1.08	1.46	1.18	1.03	1.12	1.17	7.41	1.15	2.67	1.80	1.42	1.65	2.30	2.11	0.93	1.82	0.46	2.08
TSA 1	1.00	1.34	0.80	1.92	0.98	1.16	1.06	0.81	36.00	1.04	1.16	1.28	0.94	0.84	1.15	1.10	1.90	1.62	0.77	2.13
TSA 2	0.97	1.11	0.85	1.66	0.98	1.09	0.87	1.04	6.63	1.17	1.16	1.04	0.93	0.99	1.25	1.10	0.78	1.63	0.57	1.84
5-Aza-dC+TSA 1	1.10	1.56	1.23	2.37	1.21	1.22	1.08	1.24	5.43	1.00	1.39	1.85	1.65	1.73	1.89	2.51	2.23	3.22	0.54	2.84
5-Aza-dC+TSA 2	0.96	1.44	1.31	2.08	1.18	1.13	1.11	1.28	2.41	1.22	1.40	2.47	2.05	1.48	1.83	2.86	1.09	3.40	0.51	2.97
CACNA2D2																				
Reference (untreated cell line)	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
5-Aza-dC 1	6.13	5.06	2.43	1.17	0.67	8.07	2.23	30.70	0.88	0.50	1.99	1.05	0.78	1.65	2.17	1.33	9.84	3.24	0.56	1.64
5-Aza-dC 2	11.06	7.47	2.34	1.67	0.55	8.41	3.09	17.14	1.42	0.83	2.51	1.19	0.68	3.28	2.98	1.59	3.89	3.67	0.54	1.80
TSA 1	0.84	1.64	0.46	1.72	0.94	2.36	1.82	1.13	0.84	0.85	3.03	0.90	0.55	1.16	1.15	1.20	10.67	1.44	0.79	1.95
TSA 2	0.70	0.95	0.69	1.82	0.97	1.36	1.62	0.73	0.73	0.61	3.56	1.08	0.49	1.11	0.97	1.09	1.50	1.88	0.73	2.47
5-Aza-dC+TSA 1	7.84	13.09	2.44	2.36	0.42	10.71	2.64	52.35	0.92	0.70	15.56	1.97	0.82	3.76	1.53	10.48	13.96	4.69	0.65	1.56
5-Aza-dC+TSA 2	10.99	21.29	1.81	2.59	0.48	10.45	4.23	56.50	1.29	0.48	9.58	1.78	0.70	3.84	2.33	5.97	4.15	4.44	0.52	1.10

(5-Aza-dC) – treatment with 1 μ M of 5-Aza-2'-deoxycytidine (Sigma) for 96 hours ; (TSA) – treatment with 150 ng/mL of trichostatin A (Sigma) for 24 hours; (5-Aza-dC + TSA) – combined treatment with 1 μ M of 5-Aza-dC for 96 hours followed by an additional period of 24 hours with 150 ng/mL of TSA.

Supplementary Material

Table 2. Primers sequences used for High Resolution Melting (HRM), Chromatin Immunoprecipitation (ChIP) and Copy Number Analysis.

GENES	FORWARD (5'-3')	REVERSE (5'-3')
High Resolution Melting (HRM) Analysis		
<i>CACNA2D2</i>	AGGAGAGGTCGAGTTATCGTT	CCATATTTCCATTCAAAATACGA
<i>HYAL1</i>	TTAGTGTTGATAGCGTGGG	TTTATAAAAAATCACCCCACC
<i>RASSF1*</i>	GGGTTTTATAGTTTTTGTATTTAGGTT	AACTCAATAAACTCAAACCTCCCC
<i>TMEM115</i>	TGGTTTTTTGGGATTTGTAGTT	AATCAAACCTAATCAACTCC
<i>TUSC2</i>	TTTGTGGGATTTGGGATTT	ACTACCATAACCTACCCCAAC
<i>NPRL2</i>	TATGTTTGAATTGGTATTAAT	CCCTCCCAACTAACTCTACT
<i>ZMYND10</i>	GAAAGCGTTGGGAATTTAAATATT	CCCGCCCCTAAAAATAACTC
Chromatin Immunoprecipitation (ChIP) and Copy Number Analysis		
<i>RASSF1**</i>	TGGGGTGTGAGGAGGGGACGA	AGAGCCGCGCAATGGA
<i>TMEM115</i>	CCAGACCACGGAGGTTCTGT	CTCTAGCCCCCACACTTGCT
<i>TUSC2</i>	TAGGCCCTGAAGTGCAGGTT	CTCCCGGTCTTTTGGGTGTA
<i>NPRL2</i>	GGCGCAAAGCCATGTAAGAC	CCGCAGCAGGGAACTACAAT
<i>ZMYND10</i>	GGGACGCGAGAAATAGAGGAA	TCTGTCCTCAGACGCCAAGTC
<i>GAPDH**</i>	TACTAGCGGTTTTACGGGCG	TCGAACAGGAGGAGCAGAGAGCGA

* Di GIOIA S, BIANCHI P, DESTRO A, et al. Quantitative evaluation of RASSF1A methylation in the non-lesional, regenerative and neoplastic liver. BMC Cancer. 2006; 6:89-101.

** KAWAMOTO K, OKINO ST, PLACE RF, et al. Epigenetic modifications of RASSF1A gene through chromatin remodeling in prostate cancer. Clin Cancer Res 2007; 13:2541-8.