Supplemental Material to:

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ANGPT2 promoter methylation is strongly associated with gene expression and prognosis in chronic lymphocytic leukemia

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Supplementary Table 1. Time to first treatment (TTFT) and overall survival (OS) of 88 CLL patients in relation to classical prognostic factors

	TTFT (median months)	N° treated cases	P-value	OS (median months)	N° dead cases	P-value
Clinical stage						
Binet A	117	15/40	<0.001	177	15/50	0.003
Binet B-C	2	14/19		72	15/26	
IGHV mutational status						
Mutated (<98%)	117	10/35	<0.001	177	14/46	<0.001
Unmutated (≥98%)	18	20/27		61	22/40	
CD38						
CD38 negative (<30%)	97	16/39	0.010	121	21/54	0.011
CD38 positive (≥30%)	5	9/12		57	14/21	
FISH stratification						
Low risk	97	13/35	<0.001	177	16/49	<0.001
Intermediate/high risk	3	14/16		61	15/23	

Legend: Low FISH risk = no abnormalities or del(13q); Intermediate/high FISH risk = del(11q), del(17p) or trisomy 12.

Abbreviations: FISH = fluorescence in situ hybridization; IGHV = variable region of immunoglobulin heavy chain genes.

Supplementary Table 2. Levels of ANGPT2 expression and CpG methylation compared between PBMCs and sorted B cell samples

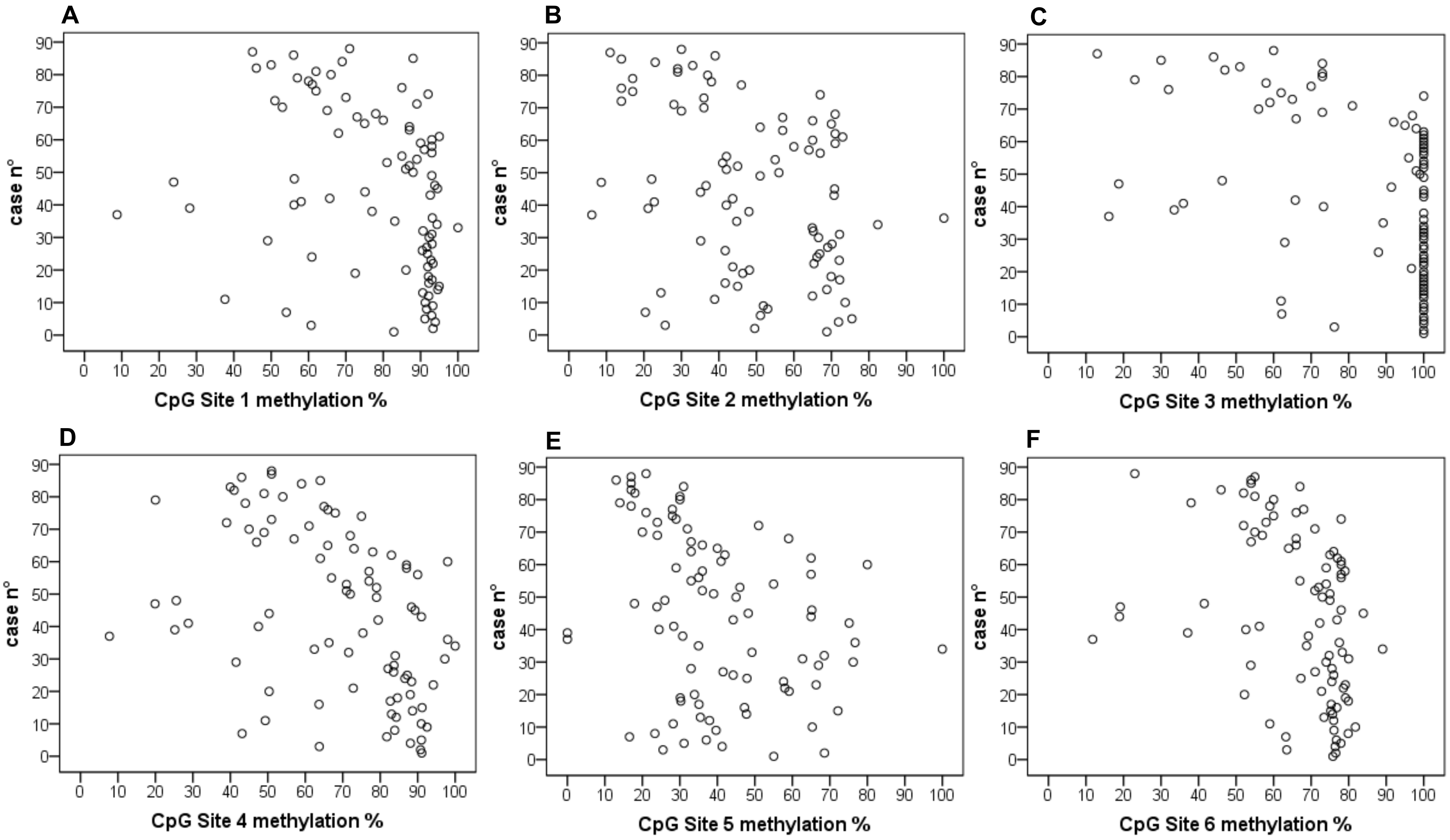
Case number	Sample type	IGHV mutational status	Relative ANGPT2 mRNA expression to B2M	Meth % CpG Site 1	Meth % CpG Site 2	Meth % CpG Site 3	Meth % CpG Site 4	Meth % CpG Site 5	Meth % CpG Site 6	Meth % average all CpG sites
CLL 1	PBMCs	UM	$8,75x10^{-5}$	56	22	46	26	18	42	35
CLL 2	PBMCs	UM	$2,75x10^{-5}$	83	45	89	66	35	69	65
CLL 3	PBMCs	M	0	93	72	100	83	35	75	76
CLL 4	PBMCs	UM	$6,12x10^{-4}$	58	23	36	29	28	56	38
CLL 5	PBMCs	M	$7,43x10^{-4}$	56	42	73	47	24	53	49
CLL 6	PBMCs	UM	$6,85x10^{-5}$	77	48	100	75	31	69	67
CLL 7	PBMCs	M	$2,93x10^{-6}$	91	75	100	91	31	78	78
CLL 8	PBMCs	M	$8,91x10^{-7}$	93	72	100	84	63	80	82
CLL 1	sorted		$3,30x10^{-4}$	53	29	45	32	35	32	38
CLL 2	sorted		$1,12x10^{-4}$	72	34	100	62	65	26	60
CLL 3	sorted		0	100	64	100	100	78	64	84
CLL 4	sorted		$1,90x10^{-3}$	47	11	25	39	28	26	29
CLL 5	sorted		$2,01x10^{-3}$	41	26	100	48	44	32	49
CLL 6	sorted		$6,40x10^{-5}$	64	37	100	97	54	57	68
CLL 7	sorted		0	100	60	100	100	67	69	83
CLL 8	sorted		0	100	65	100	100	72	69	84
HD 1	sorted		0	100	58	100	100	100	64	87
HD 2	sorted		$2,22x10^{-6}$	100	60	100	100	100	60	87

Abbreviations: CLL = chronic lymphocytic leukemia; HD = healthy donor; PBMCs = peripheral blood mononuclear cells; sorted = sorted B cells; IGHV = immunoglobulin heavy chain variable genes; UM = unmutated; M = mutated; Meth % = methylation percentage.

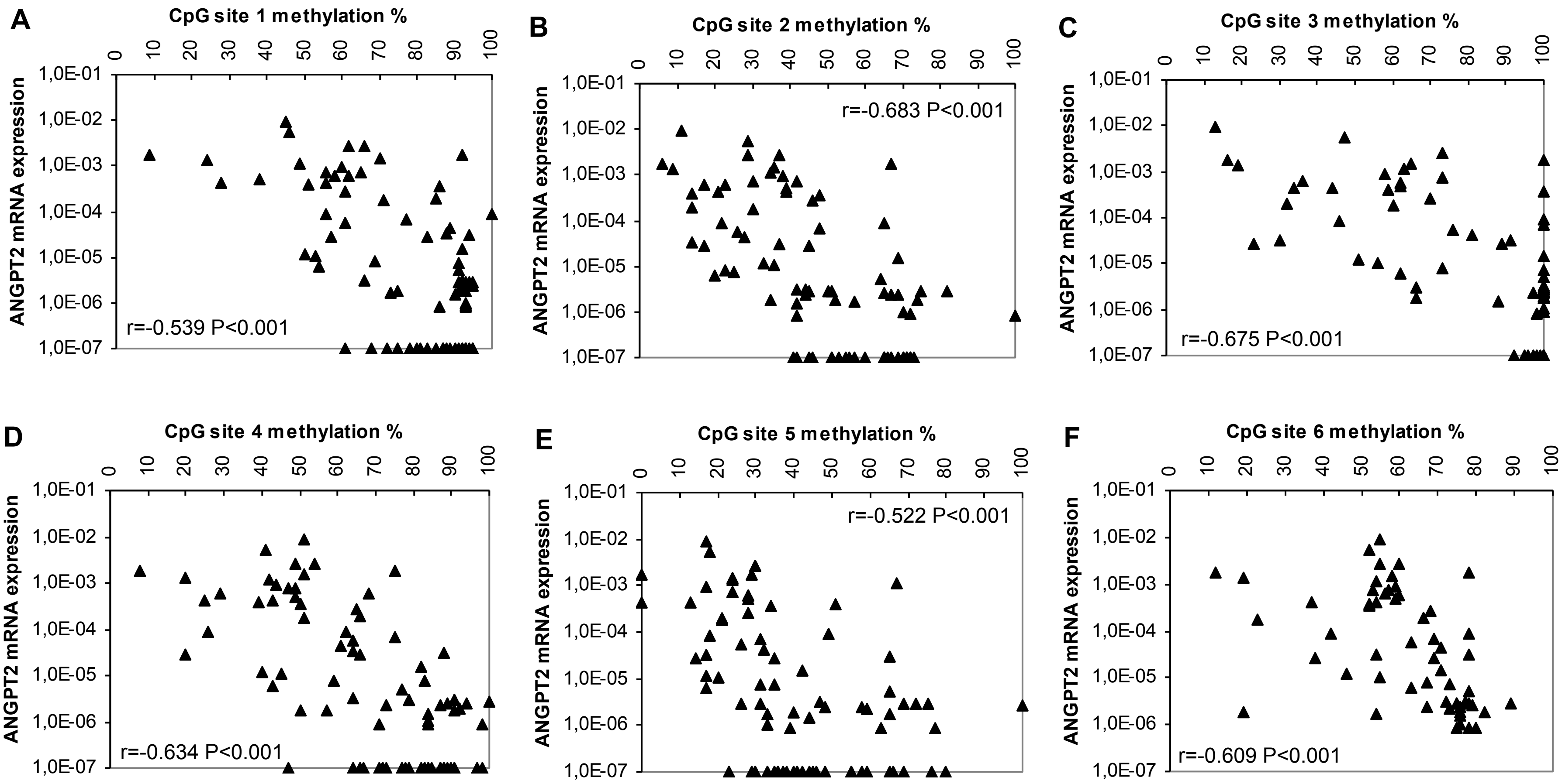
Supplementary Table 3. *ANGPT2* CpG methylation percentages and apoptotic cell rates in MEC1 and RAMOS cell lines after 72 hours of DAC/TSA treatment in a representative experiment

	Treatment	Meth % CpG 1	Meth % CpG 2	Meth % CpG 3	Meth % CpG 4	Meth % CpG 5	Meth % CpG 6	Meth % average all CpG sites	Apoptotic cell rate (%)
MEC1	control	100	53	100	100	100	60	86	4.1
MEC1	5 μM DAC	50	29	100	57	100	27	61	2.5
MEC1	10 μM DAC	40	33	100	68	46	35	54	9.8
MEC1	10 μM DAC + TSA	19	26	95	28	33	19	37	35.3
RAMOS	control	100	63	100	100	85	78	88	10.1
RAMOS	5 μM DAC	45	42	100	49	58	33	55	3.9
RAMOS	10 μM DAC	39	37	100	44	32	27	47	1.4
RAMOS	10 μM DAC + TSA	15	22	86	25	30	16	32	37.9

Abbreviations: DAC = 5-aza-2’-deoxycytidine; TSA = Trichostatin A; Meth % = methylation percentage.



Supplementary Figure 1. The scatter plots show the distributions of methylation percentages (%) for each CpG site among 88 CLL cases. CpG site 1 is represented in panel A, CpG site 2 in panel B, CpG site 3 in panel C, CpG site 4 in panel D, CpG site 5 in panel E and CpG site in panel F.



Supplementary Figure 2. The scatter plots show the inverse correlations between the relative *ANGPT2* mRNA expression to B2M (in log10 scale) and the methylation percentage (%) for each CpG site in 88 CLL patients (all Spearman tests). $1x10^{-7}$ value coincides with the max sensibility of the RQ-PCR method for ANGPT2 mRNA expression and here is used instead of zero. CpG site 1 is shown in panel A, CpG site 2 in panel B, CpG site 3 in panel C, CpG site 4 in panel D, CpG site 5 in panel E and CpG site 6 in panel F.