Supplementary Table S1a. Characteristics of the patients investigated at the Cancer Sciences Unit, Southampton (CSU)

Sample ID	Cohort	Gender	Rai Stage	IGHV use	IGHV homology (%)	sIgM levels (MFI)	sIgM Ca^{2+} (%)	sIgD levels (MFI)	sIgD Ca^{2+} (%)	ZAP70 flow	CD38 flow
280	Initial and expanded	Male	II	3-48	99.0	50	88	11	44	≥30%	<30%
281	Initial and expanded	Male	II	3-23	92.0	11	6	4	6	<30%	<30%
470	Initial and expanded	Male	I	3-7	95.1	10	12	6	14	<30%	<30%
473	Initial and expanded	Male	0	4-31	100	106	50	58	76	≥30%	≥30%
475	Initial and expanded	Male	I	1-3	92.0	53	2	150	71	≥30%	<30%
482	Initial and expanded	Male	I	4-30-4	95.9	19	2	41	47	<30%	≥30%
493	Initial and expanded	Female	I	4-34	96.5	30	5	71	29	<30%	<30%
501	Initial and expanded	Female	II	3-9	100	77	51	69	72	≥30%	≥30%
504	Initial and expanded	Male	II	1-2	100	402	47	128	65	<30%	≥30%
543	Initial and expanded	Female	I	3-23	94.8	6	3	2	1	<30%	<30%
585	Initial and expanded	Male	II	2-5	100	175	8	65	22	<30%	≥30%
604	Initial and expanded	Male	II	3-30	94.1	94	44	58	66	<30%	<30%
635	Initial and expanded	Female	II	3-21	98.9	67	82	58	85	<30%	<30%
668	Initial and expanded	Male	II	3-30	100	71	54	124	85	≥30%	<30%
684	Initial and expanded	Male	I	3-15	91.8	29	7	32	64	<30%	<30%
695	Initial and expanded	Male	I	3-21	99.6	80	82	105	86	nd	<30%
156	Expanded	Male	II	5-51	97.6	25	24	43	60	<30%	<30%
348	Expanded	Male	I	3-15	94.9	66	4	47	69	<30%	<30%
449	Expanded	Female	I	3-11	93.1	34	44	52	24	≥30%	≥30%
457	Expanded	Female	I	3-30	91.3	41	5	18	5	<30%	<30%
489	Expanded	Male	I	4-31	91.0	26	4	111	74	<30%	<30%
498	Expanded	Female	I	3-48	91.7	16	3	126	23	<30%	<30%
570	Expanded	Male	I	4-34	95.1	13	3	27	37	<30%	<30%
577	Expanded	Female	II	1-46	96.9	22	31	35	55	<30%	<30%
618	Expanded	Female	II	3-11	100	145	68	60	71	≥30%	≥30%
653	Expanded	Female	II	3-7	92.4	28	3	15	3	<30%	<30%
669	Expanded	Male	II	1-2	100	36	52	39	72	≥30%	<30%

nd, not determined

Supplementary Table S1b. Characteristics of the patients investigated at the German Cancer Research Center (DKFZ)

Sample ID	Gender	IGHV use	IGHV homology (%)	ZAP70 flow
CLL20	Female	3-7	94.2	nd
CLL21	Male	1-69	100	≥30%
CLL23	Male	1-3	97.5	≥30%
CLL25	Male	4-61	88.4	<30%
CLL30	Male	1-8	96.9	<30%
CLL32	Male	3-23	91.8	<30%
CLL33	Female	3-30	87.6	nd
CLL34	Male	1-69	100	≥30%
CLL35	Male	3-15	90.0	<30%
CLL36	Male	1-46	100	≥30%
CLL38	Female	3-30	91.0	<30%
CLL40	Female	3-21	95.1	<30%
CLL42	Male	3-48	92.8	<30%
CLL43	Male	3-30	97.4	<30%
CLL44	Female	3-74	91.1	<30%
CLL45	Male	3-15	88.2	<30%
CLL46	Female	4-59	98.9	<30%
CLL47	Male	1-46	100	nd
CLL48	Male	3-23	95.0	<30%
CLL49	Male	3-20	90.6	<30%
CLL50	Male	3-9	90.7	<30%
CLL51	Female	1-46	100	≥30%
CLL52	Male	3-15	91.0	≥30%
CLL53	Male	1-69	97.2	nd
CLL54	Female	1-3	100	≥30%
CLL55	Male	3-39	94.2	<30%
CLL58	Male	3-23	87.8	<30%
CLL59	Male	3-48	87.9	<30%
CLL60	Male	1-69	100	≥30%
CLL64	Male	3-23	93.6	≥30%
CLL65	Female	3-23	84.4	≥30%
CLL66	Male	5-51	100	≥30%
CLL67	Male	3-23	93.6	<30%
CLL68	Male	4-34	89.6	<30%
CLL69	Female	3-23	89.6	<30%
CLL72	Male	1-69	100	≥30%

nd, not determined

Supplementary Table S2. IL10 primers.

Primer name	Position (hg19)	Forward primer sequence	Reverse primer sequence
IL10-3	chr1:206945122-206945472	TTTAGATTAAGTTATGGGTTTGGTGA	CCCCACTATAAACATCCAATTTAAC
IL10-5	chr1:206945783-206946243	TTTTTTTTGTAAGTTTGTTTTGTGG	AAAAAACCCAATTATTTCTCAATCC
IL10-6	chr1:206946101-206946518	AGTTGATTTTTTGGGGAGAATAGTT	CCCCAAACACAATTAAAATAAAAAA
IL10-7	chr1:206946317-206946783	TTTTAAGTAAAAATGAGGGGGTGG	AAACTCCAACCACAAAAACTTACAA
IL10-8	chr1:206945446-206945766	ATTGTTTTGTTGTTTGGTTTTTTTG	CTCACCAAACCCATAACTTAATCTAAA
IL10-9	chr1:206943889-206944369	TTAGTTTTATTTGGAGGAGGTGATG	TTCCTCTACTTAAAAACCTTAACTCCC
IL10-10	chr1:206944376-206944731	GTTAAAGGAGTTTTTGTTGGAGGAT	TCAAACAAAACTTAACAACCCAAAT
IL10-11	chr1:206942758-206943094	AGTTTGGGTTGAATTTTTAGGTGTA	AAAAACTCAATAAACCCCATTCTTC
IL10-12	chr1:206942448-206942849	AGTTTGTTGGGAATTTAGGTTGAAT	ATATTCTCCCACCAACACACTAAAC
IL10-13	chr1:206939689-206940029	TAAATGTGAGATTGGTTTTTGATTG	CTCAAAATAAAAAACTTCAAACCCC

Supplementary Table S3. DNA oligonucleotides used for luciferase reporter and proximity ligation assays.

Luciferase reporter assay:

Name	Forward primer sequence	Reverse primer sequence
CLL-VMR2 (IL10 intron 3)	GGCCCTTCTTAGAGCTTTGC	CTCACTCACCTTTGGCTCCT
STAT3 site mutagenesis	AATGACTCACAAACAACATTCAAGCTGTTTG	CAAACAGCTTGAATGTTGTTTGTGAGTCATT

Luciferase pathway reporter oligo:

STAT3 reporter	GGTTCCCGTAAATGCATCAGGTTCCCGTAAATGCATCAGGTTCCCGTAAATGCATCAGGTTCCCGTAAATGCATCAGTTCCCGTAAATGCATCAGGTTCCCGTAAATGCATCAGGTTCCCGTAAATGCATCAGGTTCCCGTAAATGCATC

Proximity ligation assay:

STAT3 consensus motif	**CTACGTTCCTGGAATACT**CATCGCCCTTGGACTACGACTGACGAACCGCTTTGCCTGACTGATCGCTAAATCTGG
CLL-VMR2 (IL10 intron 3)	**CTACGAATAACAGGAAAGCTACT**CATCGCCCTTGGACTACGACTGACGAACCGCTTTGCCTGACTGATCGCTAAATCTGG

For mutagenesis primers the mutated bases are underlined. For proximity ligation assay bold sequences indicate annealed double-stranded binding motifs.

Supplementary Table S4. CpG-induced cytokine secretion in the supernatant of CLL cells

CLL ID	IGHV status	IL-10	IL-6	GM-CSF	IL-5	IFN-gamma	TNF-alpha	IL-2	IL-4	IL-8	IL-1b
482	M	2998.5	86.1	0.0	0.0	0.0	0.0	0.0	0.0	180.3	0.0
684	M	1161.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	6.0	0.0
475	M	64946.4	303.5	0.0	0.0	0.0	236.4	0.0	0.0	139.5	0.0
604	M	2677.1	0.0	0.0	0.0	0.0	35.3	0.0	0.0	0.0	9.6
281	M	1101.7	7.8	4.4	0.2	0.0	3333.6	10.8	4.1	295.1	0.0
493	M	1539.9	111.0	5.3	0.4	0.0	1112.3	6.2	4.9	1034.3	0.0
470	M	8813.9	818.0	11.7	1.1	0.0	232.8	0.0	6.5	79.6	0.0
543	M	10702.5	94.2	7.3	0.6	0.0	567.0	24.9	3.5	36.1	18.8
501	U	490.5	185.0	0.0	0.0	0.0	554.3	0.0	0.0	0.0	42.5
695	U	2253.7	222.9	0.0	0.0	0.0	1095.5	1.2	0.0	173.6	75.6
280	U	1237.1	0.0	0.0	0.0	0.0	1193.1	2.1	0.0	84.1	111.5
668	U	871.7	396.9	0.0	0.0	0.0	11.6	0.0	0.0	7.0	59.8
585	U	20369.7	6.4	7.0	0.8	0.0	21.9	0.0	2.0	18.3	0.0
473	U	4380.9	117.5	7.1	0.4	0.0	106.1	28.4	3.5	219.9	30.1
635	U	232.5	23.1	0.0	0.0	0.0	149.4	0.0	0.0	131.2	0.0
504	U	467.0	68.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Supplementary Table S5. Intracellular production of IL-10 following CpG+PIB stimulation in CLL

Sample ID	Initial cohort	IL-10$^+$ cells (%)
280	x	3.5
281	x	9.7
470	x	20.9
473	x	10.4
475	x	30.9
482	x	27.5
493	x	21.3
501	x	2.8
504	x	9.1
543	x	9.2
585	x	10
604	x	19.5
635	x	2.6
668	x	3
684	x	19
695	x	1.8
156		7.2
348		12.8
449		19.6
457		4.8
489		12.4
498		10.4
570		15
577		11.7
618		1.3
653		34.4
669		1.1

Values indicate percentage of IL-10$^+$ cells of all CD19$^+$/CD5$^+$ CLL cells following stimulation by CpG+PIB. Stimulation conditions prior to determination of IL10$^+$ CLL cells are described in the text. X indicates patients from the intital cohort investigated for correlative analysis with IL-10 cytokine secretion.

Supplementary Table S6. Basal and induced *IL10* transcript levels following CpG stimulation in CLL

Sample ID	Basal *IL10* transcript levels*	Induced *IL10* transcript levels
CSU cohort		
280	0.00208	0.119
281	0.00918	0.085
470	0.00774	0.249
473	0.00067	0.167
475	0.24846	1.134
482	0.17294	0.647
493	0.06601	0.222
501	0.00015	0.031
543	0.00396	0.215
585	0.02174	0.264
604	0.06747	0.846
635	0.00108	0.014
668	0.00402	0.15
684	0.07845	0.419
695	0.00173	0.159
DFKZ cohort		
CLL20	0.012	nd
CLL21	0.011	nd
CLL23	0.041	nd
CLL25	0.024	nd
CLL30	0.461	nd
CLL32	0.157	nd
CLL33	0.546	nd
CLL34	0.002	nd
CLL35	0.011	nd
CLL36	0.000	nd
CLL38	0.037	nd
CLL40	0.045	nd
CLL42	0.577	nd
CLL43	0.029	nd
CLL44	0.468	nd
CLL45	0.013	nd
CLL46	0.009	nd
CLL47	0.007	nd
CLL48	0.115	nd
CLL49	0.080	nd
CLL50	0.010	nd
CLL51	0.000	nd
CLL52	0.403	nd
CLL53	0.046	nd
CLL54	0.000	nd
CLL55	0.046	nd
CLL58	0.322	nd
CLL59	0.177	nd
CLL60	0.005	nd
CLL64	0.003	nd
CLL65	0.139	nd
CLL66	0.006	nd
CLL67	0.003	nd
CLL68	0.061	nd
CLL69	0.182	nd
CLL72	0.000	nd

Values indicate relative *IL10* transcript levels in CLL cells (>99% purity) cultured for 24 hours without (basal) or with CpG stimulation (induced) *in vitro* . Stimulation conditions prior to measurement of the relative *IL10* transcript levels are described in the text.

* Basal *IL10* transcript levels were determined using different assays for the CSU and the DKFZ cohort and the results are not comparable between cohorts

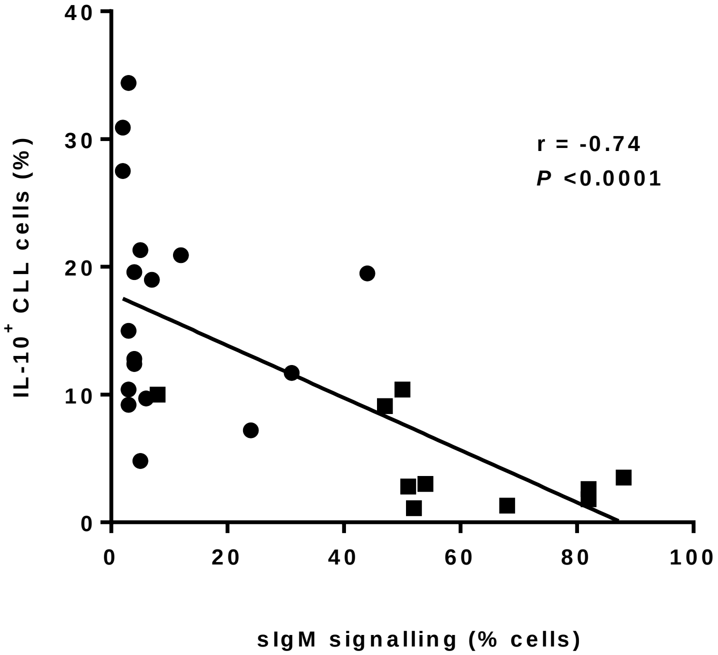
nd, *IL10* transcript levels were not determined following CpG stimulation in the DKFZ cohort

Supplementary Figure legends

Supplementary Figure S1. Production of IL-10 by CLL correlates with sIgM signaling capacity. PBMCs from CLL patients at CSU (n=27) were cultured at 2 x 10^7/ml and stimulated with CpG (7.5µg/ml). Following 24 hours stimulation, PMA, ionomycin and brefeldin A was added for 5 hours before IL-10 production by $CD19^+CD5^+$ cells was detected by flow cytometry. Correlation between IL-10 production and sIgM signaling capacity. Circles represent M-CLL, Squares represent U-CLL. Linear regression and Spearman correlation are shown.

Supplementary Figure S2. DNA methylation analysis and the relative positions of known *IL10* enhancer elements. (A) Schematic diagram of the *IL10* locus showing H3K27ac in GM12878 cells, inter-species sequence conservation, the positions of CLL-VMRs 1&2 and previously characterized enhancers, as well as the positions of 450K probes for the -12 kb upstream enhancer, promoter and CLL-VMR1. DNA methylation levels determined by 450K arrays in 122 CLL cases at DKFZ separated by IGHV mutation status. The *IL10* upstream enhancer (cg12073436), promoter (cg10978799), and CLL-VMR1 (cg17067005) are shown (M-CLL, n=64; U-CLL, n=58; statistical evaluation performed by t-test). (B) DNA methylation of the IL10 promoter and CLL-VMRs 1&2 in IGHV CLL subgroups as determined by MassARRAY (statistical evaluation performed by t-test). (C) DNA methylation, as determined by MassARRAY, of three regions of the *IL10* gene locus compared with unstimulated *IL10* expression in 36 CLL cases (DFKZ cohort). *IL10* expression was analyzed by qPCR relative to HPRT transcripts.

Supplementary figure S1



Supplementary figure S2