SUPPLEMENTARY APPENDIX

Silenced B-cell receptor response to autoantigen in a poor-prognostic subset of chronic lymphocytic leukemia

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Bergh et al. Supplementary Tables and Figures

Supplementary Table 1. Antibodies and reagents

Ab reactivity	Conjugate	Species/Clone	Company	Method used
CD5	PerCpcy5.5	Mouse/UCHT2	BioLegend	FC[3]
CD5	PE	Mouse/UCHT2	BD Biosciences	FC
CD19	PE-cy7	Mouse/HIB19	BioLegend	FC
CD19	PeCy5	Mouse/HIB19	BioLegend	FC
BrdU	FITC	Mouse/B44	BD Biosciences	FC
IgM	RPE	Mouse/M15/8	ABD Serotec	FC
CD36	PE	Mouse/CB38	BD Biosciences	FC
CD36	APC/Cy7	Mouse/336213	BioLegend	FC
SR-PSOX (CXCL16)[1]	-	Rat/256213	R&D systems	FC
SR-B1[2]	-	Rabbit/Polyclonal	LifeSpan BioSciences	FC
CD25	APC	Mouse/BC96	BD Biosciences	FC
CD86	PE	Mouse/IT2.2	BD Biosciences	FC
Ig kappa light chain	-	Mouse/G20-193	BD Biosciences	FC
IgD	-	Mouse	DAKO	FC
IgM $F(ab')_2$	-	Goat/Polyclonal	Southern Biotechnology	Stimuli
IgM	HRP	Rabbit/Polyclonal	DAKO	ELISA
IgM	ALP	Goat/Polyclonal	Sigma-Aldrich	Luminescence ELISA
Phospho-p44/42 MAPK (ERK1/2)(Thr202/Tyr204)	-	Rabbit	Cell Signaling Technology	WB[4]
P44/42 MAPK (ERK1/2)	-	Rabbit	Cell Signaling Technology	WB
Rabbit Ig	HRP	Goat/Polyclonal	DAKO	WB
Other reagents				
CpG oligodeoxynucleotide (ODN 10104, stimulatory CpG type B, human specific)	-		Coley Pharmaceutical Group and InVivogen	Stimuli
nLDL	Biotin		Gift from Sohvi Hörkkö	Stimuli/FC
MDA-LDL	Biotin		Gift from Sohvi Hörkkö	Stimuli/FC/IF[5]
MDA-BSA	Biotin		Gift from Sohvi Hörkkö	FC/IF
AnnexinV	Pacific blue or FITC		BioLegend/BD Biosciences	FC
Streptavidin	Alexa488		Molecular Probes	IF/FC
Fluo-4 AM	-		Invitrogen	FC

[1] SR-PSOX, Scavenger receptor for PhosphatidylSerine and Oxidized lipoproteins, [2] SR-B1, Scavenger receptor class B member 1, [3] FC, Flow cytometer, [4] WB, Western blot, [5] IF, Immunofluorescence

Supplementary Figure 1

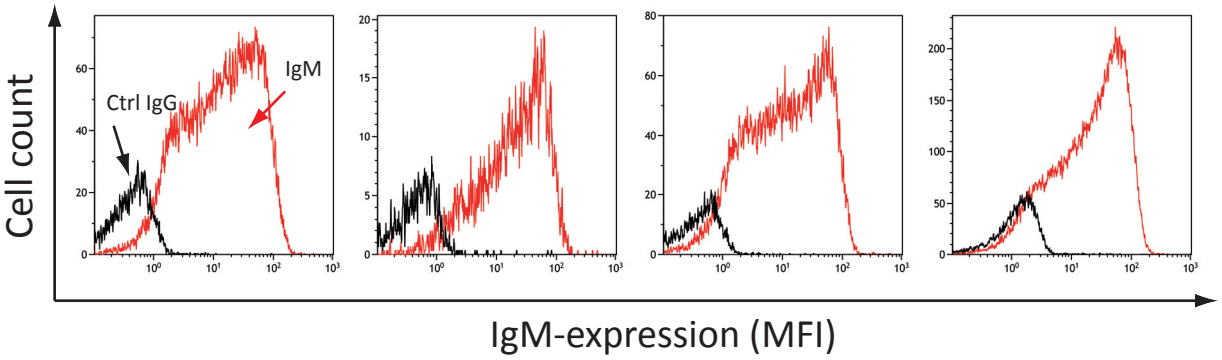

Figure 1. Surface expression of IgM in normal B-cells. IgM surface expression was analyzed in $CD19^{+}$ B-cells from four healthy individuals. (Mean MFI = 17; range 12-25).

Supplementary Figure 2

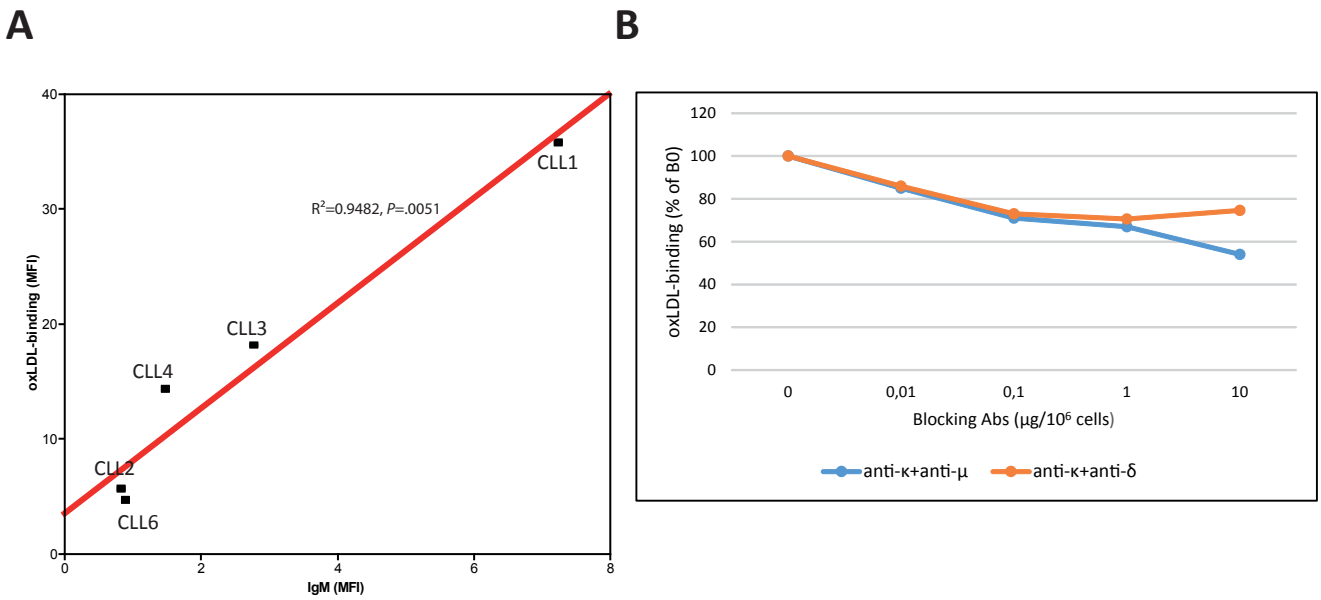

Figure 2. Cell surface receptor analysis. (A) The level of oxLDL-binding correlated with the level of sIgM-expression (R2 = 0.948, P = .005, n = 5) **(B)** oxLDL-binding was blocked in a dose-dependent manner by anti-IgM (anti-µ + anti-κ) or anti-IgD (anti-δ + anti-κ) Abs.

Supplementary Figure 3

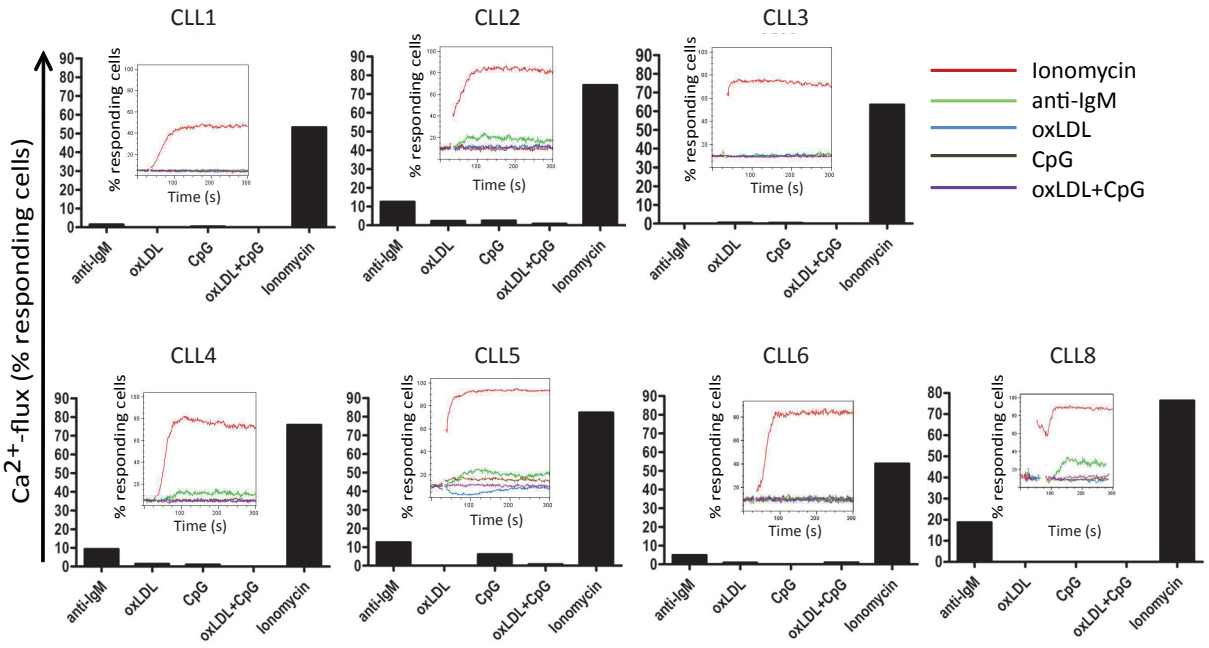

Figure 3. oxLDL ligation does not induce Ca^{2+} - flux. Subset #1 lymphocytes from 7 CLL cases were analyzed for Ca^{2+} -release. Cells were loaded with the Ca^{2+} sensitive dye Fluo-4 AM and analyzed by flow cytometry before and after addition of anti-IgM $F(ab')_2$ (20 µg/mL), oxLDL (50 µg/mL) and/or CpG (10 µg/mL). The calcium ionophore ionomycin was used as positive control. The diagrams show percent responding cells of total viable cells. The insert in each diagram show flow images for Ca^{2+} -flux responses.

Supplementary Figure 4

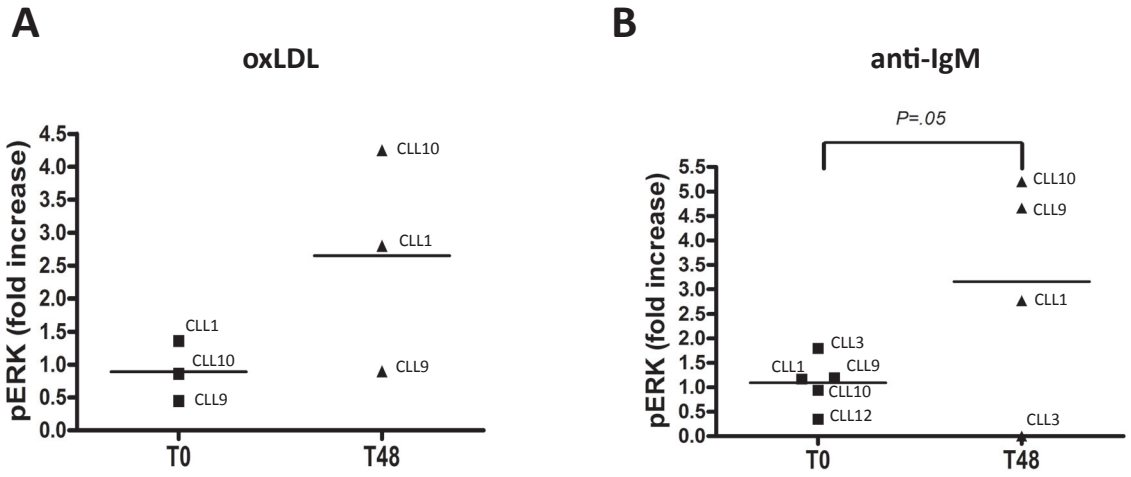

Figure 4. Constitutive phosphorylation of ERK1/2 at basal level. Cells were cultured for 48 hours and ERK1/2 phosphorylation was analyzed by immunoblotting immediately (T0) and after 48 hours (T48). Cells were stimulated at both time points with 50 µg/mL oxLDL or 10 µg/mL anti-IgM $F(ab')_2$ for 5 minutes. The diagram show data on pERK/total ERK fold-increase. (**A**) after oxLDL exposure; (**B**) after anti-IgM exposure

Supplementary Figure 5

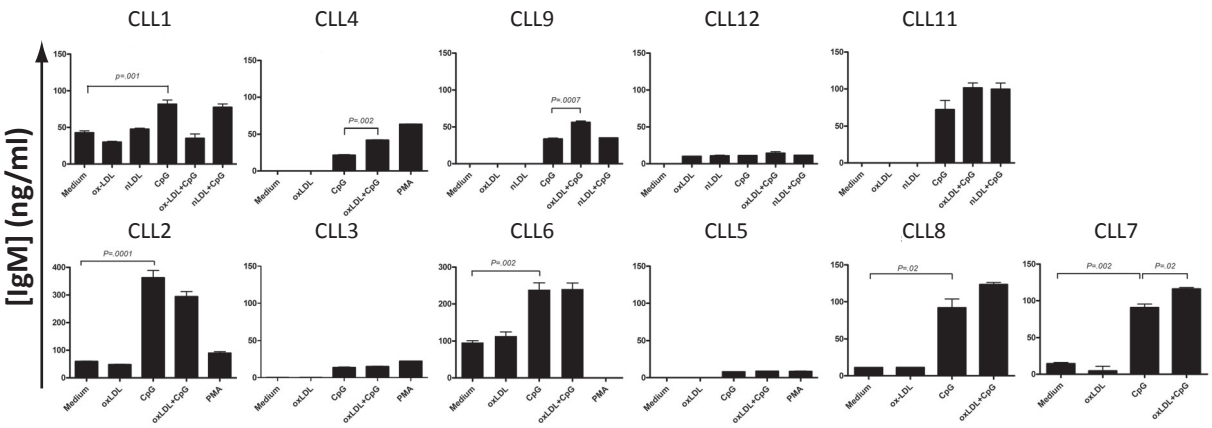

Figure 5. IgM release from CLL subset #1 cells upon BcR and TLR stimulation. PBMC (45-98% $CD5^{+}/CD19^{+}$) from 11 subset #1 patients were cultured in the presence or absence of nLDL (25 µg/mL), oxLDL (25 µg/mL), anti-IgM $F(ab')_2$ (10 µg/mL), PMA (50 ng/mL) and/or CpG (5 µg/mL) for 72 hours. Culture supernatants were collected and analyzed for IgM content in ELISA. Data are expressed as mean ± SEM.

Supplementary Figure 6

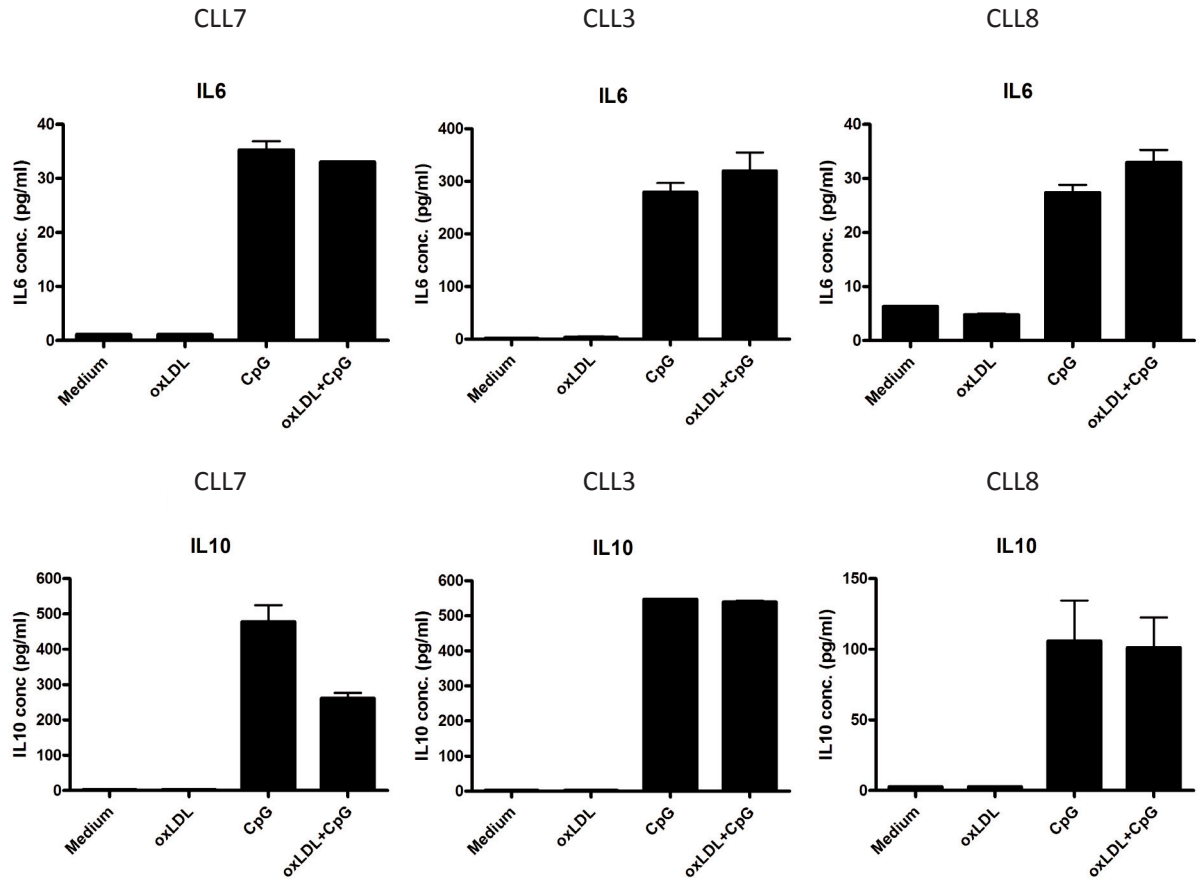

Figure 6. Cytokine release from CLL subset #1 cells upon BcR and TLR stimulation. CLL cells from three subset #1 CLL patients were cultured in the presence or absence of oxLDL (25 µg/mL), nLDL (25 µg/mL), anti-IgM F(ab´)$_2$ (10 µg/mL), and/or CpG (5 µg/mL) for 72 hours. Culture supernatants were collected and analyzed for 10 different cytokines in Luminex-assay. Data for IL6 and IL10 are presented and the concentrations are expressed as mean ±SEM.

Supplementary Figure 7

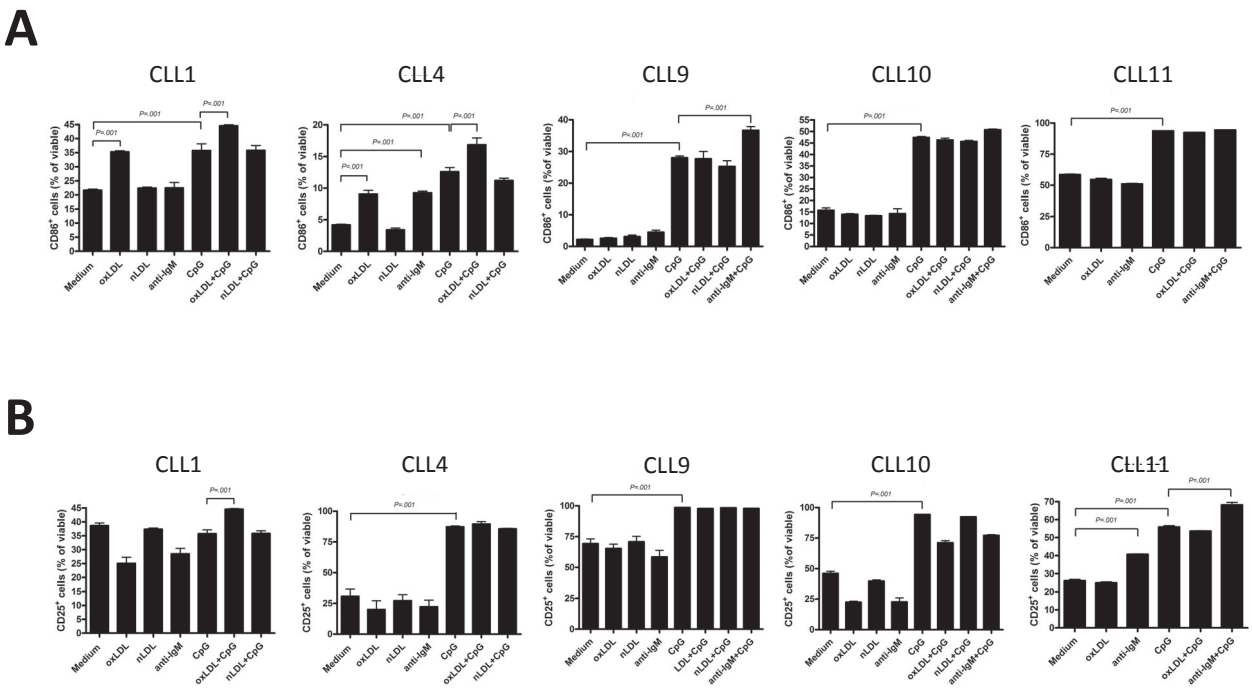

Figure 7. Surface expression of CD25 and CD86 after BcR and TLR stimulation. CLL subset #1 cells from five patients were cultured in the presence or absence of oxLDL (25 µg/mL), nLDL (25 µg/mL), anti-IgM F(ab´)$_2$ (10 µg/mL), and/or CpG (5 µg/mL) for 24 hours. Surface expression of CD86 (**A**) and CD25 (**B**) was measured with flow cytometry. Data are expressed as mean ±SEM.