

Supplementary Materials

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Supplementary Methods

Production of recombinant proteins

Recombinant ApoE2, ApoE3, and ApoE4 were produced in early-passage Expi293 transfected with plasmids pCMV4-ApoE2/E3/E4 according to the manufacturer's protocol (ThermoFisher). pCMV4-ApoE3 Addgene plasmid # 87086 was a gift from Bradley Hyman. pCMV-ApoE2 and ApoE4 were obtained by mutagenesis. After three days, supernatants were collected by centrifugation at 2000xg for 10 min, 0.45 µm-filtrated and concentrated using Amicon Ultra-4 Centrifugal Filter Device (Millipore). Finally, supernatants were sterile filtered using 0.22 µm filter. The purity and yields of ApoE proteins were assayed by Coomassie blue staining (Invitrogen) against BSA control and by immunoblotting with ApoE antibody (AB947, Millipore) as described below. Average ApoE yields upon collection were 50 µg/ml, and 500-1000 µg/ml after further processing. Control supernatants were produced by transfecting Expi293 cells with an empty plasmid, processed identically and used in all experiments as a control condition.

Immunoblotting of recombinant ApoE and CLL cell lysates

CLL B cells were centrifuged at 1000 xg 10 min at room temperature and resuspended in RIPA buffer (ThermoFisher). Cells were incubated for 5 min on ice, centrifuged at 10,000 xg for 10 min at 4°C and supernatants were collected. 10 µg of total protein lysate, or 1-5 µg of concentrated recombinant ApoE were separated on 4-12% polyacrylamide Bold™ precast gel in MOPS SDS running buffer (ThermoFisher). Separated proteins were transferred to a PVDF membrane by using iBlot 2 dry blotting system (ThermoFisher) by applying an electric field of 20 V for 1 min, 23 V for 3 min and 25 V for 2 min. The following antibodies were used for immunoblotting: rabbit anti-SLC31A1/CTR1 antibody (EPR7936, abcam), goat anti-ApoE (AB947, Millipore), mouse anti β-actin antibody (MAB1501, Sigma), anti-mouse HRP (Jackson ImmunoResearch), anti-goat HRP (Jackson ImmunoResearch) and anti-rabbit HRP (Jackson ImmunoResearch).

Flow cytometry assay of CLL cell activation

B cell activation was tested by flow cytometry staining of CD86 (clone B7-2 eBioscience), CD58 (clone TS2/9 Biolegend) and HLA-DR (clone L243 Biolegend) 72 h post-activation.

CLL cell culturing for RNA sequencing analysis

7x10⁶ CLL B cells (purity 93-99%) seeded with 2.1x10⁶ hCD40L-expressing 3T3 feeder cells were cultured with α -IgM/IL-21/IL-4 as described above. Where indicated, unstimulated B cells were used as a control. Cells in Figure 2B were treated with 70 μ g/ml ApoE or control Expi293-derived supernatants. 96 hours later (Figure 1F) or 72 hours later (Figure 2B) later cells were stained with ZombieAqua and CD19 antibody (clone HIB19, Biolegend). Viable CD19⁺ CLL cells were sorted on BD FACS Aria, and total RNA extracted. RNA-seq libraries were prepared and sequenced as described in Supplementary Materials.

RNA sequencing and raw data processing

Immediately after sorting, RNA from B cells was extracted by using the NucleoSpin RNA extraction kit (Machery-Nagel). Extracted total RNA was quantitatively and qualitatively evaluated using NanoDrop 1000 (Thermo Fisher Scientific) and Agilent Bioanalyzer 2100 (Agilent), respectively. RNA-seq libraries were prepared from 1 μ g of total RNA, using the TruSeq® Stranded mRNA Library Prep (Illumina) according to the manufacturer's protocol. The resulting libraries were quality checked for the insert size using a DNA 1000 Chip on Bioanalyzer 2100 (Agilent) and quantified using Qubit™ dsDNA BR Assay Kit on Qubit 2.0 Fluorometer (Thermo Fisher Scientific). Equimolarly pooled libraries were sequenced on NovaSeq6000 Sequencing System (Illumina, Inc) using 2x150 bp paired-end mode. Real-time image analysis and base calling were performed directly on the Novaseq instrument using the recommended sequencing control software. Illumina BCL2FASTQ v2.20 software (<https://emea.support.illumina.com/downloads/bcl2fastq-conversion-software-v2-20.html>) was used for de-multiplexing and production of FASTQ sequence files. FASTQ sequence files were subsequently quality checked with FASTQC software (<http://www.bioinformatics.bbsrc.ac.uk/projects/fastqc>). Subsequently, sequences with a low-quality score Q<20, or those including only adaptor dimers, were discarded from the analysis. The resulting set of selected reads were aligned onto the complete human genome using STAR version 2.7.3 (Dobin and Gingeras, 2015) using hg38 Genome Assembly and Gencode.v35 as gene definition. The resulting Mapped reads were used as input for feature Counts function of Rsubread packages (<https://bioconductor.org/packages/release/bioc/html/Rsubread.html>).

RNAseq data analysis

ComBat-seq was used to estimate batch effect¹. Differentially expressed genes were filtered using the DESeq2 R package (Deseq2 version: 1.38.3; R version: 4.2.2)². Raw read counts were used as input to generate the log2 fold changes, *p* values and adjusted *p* values applying default parameters. Shrinkage of effect size was performed using the lfcShrink function (apeglm)³. Differences in gene expression were considered significant for adjusted *p* values ≤ 0.01 and log2 fold change ≥ 1 or ≤ -1 . Over-representation analysis (ORA) was performed in g:Profiler against GO biological process⁴, with term size set to contain up to 350 genes. Heatmaps of differentially expressed genes involved in cellular response to copper ions, B cell receptor pathway, WNT pathway, PI3K-AKT and mTOR pathways were plotted using pheatmap R package.

Public RNAseq datasets used

TPM values of ApoE receptor expression in blood-derived unstimulated CLL cells (reported in Figure 1F) were extracted from the published CLL dataset EGAD00001004046⁵. Normalized expression levels of *ApoE* (reported in Figure S13) were extracted from pre-processed single cell RNA-seq data of the human bone marrow (marker centroids contained in .xls table mmc8 of the manuscript)⁶.

Comparative analysis of CLL and RS transcriptomes

RNAseq expression data in primary CLL samples were extracted from the following datasets: GSE92626, GSE66117, GSE176141, GSE119103, EGAD00001000258^{5,7–11}. RNA from primary RS samples were obtained from formalin-fixed paraffin-embedded lymph node/bone marrow biopsies and processed with Stranded Total RNA Prep Ligation with Ribo-Zero Plus (Illumina), following manufacturer's protocol. Libraries were subjected to a 75 cycles paired-end sequencing with a NextSeq 550 sequencer (Illumina). Reads were aligned to a reference genome (UCSC hg19) and transcripts quantified with Salmon v1.4.0 to obtain transcript per million (TPM) normalized counts, using the RNA-Seq Alignment tool (Illumina). The differential expression analysis (DEA) was conducted using DESeq2 v1.30.1 to identify the set of differentially expressed genes (DEG) between RS and CLL samples. RS data from primary samples are part of a manuscript in preparation (Vaisitti T et al.).

Mesenchymal stromal cell cultures, ApoE immunoblotting and immunofluorescence (related to Figure S13)

CLL bone marrow material was processed as described¹². Following antibodies were used for immunoblotting: goat anti-ApoE (AB947, Millipore), mouse anti β -actin antibody (MAB1501, Sigma). For immunofluorescence microscopy, cells were fixed in 80 mM PIPES, 2 mM MgCl₂, 5 mM EGTA and 4% PFA for 20 min on ice and permeabilized in 0.1% Triton X-100, 1% BSA in PBS for 15 min at RT. Cells were incubated with rabbit anti-ApoE (E7X2A) (49285, Cell Signalling) and anti-smooth muscle actin followed by goat anti-rabbit IgG Alexa Fluor 488 and anti-mouse IgG Alexa Fluor 555 (ThermoFisher Scientific). Images were acquired at the Opera Phenix automated confocal system (PerkinElmer) using 63X water objective.

Supplemental Tables

Supplemental Table S1. CLL patient characteristics (n=52 donors).

ID	CLL	Rai Binet stage	Age	Sex	IGHV status	CD38	Cytogenetics	WBC/uL	RNAseq
CLL03	1	I/A	86	F	MUT	Neg	Del 13	69000	yes
CLL05	2	0/A	64	M	U-MUT	ND	Neg	21000	
CLL07	3	II/A	63	M	MUT	Neg	Del 13	27000	
CLL8	4	0/A	79	F	MUT	Neg	Neg	22000	
CLL10	5	0/A	82	M	MUT	Pos	Del 13	28000	
CLL11	6	0/A	79	F	MUT	Neg	Neg	21000	
CLL13	7	0/A	66	F	MUT	Neg	Neg	43000	
CLL15	8	0/A	60	M	U-MUT	Neg	Neg	16300	
CLL16	9	I/A	55	F	U-MUT	Neg	Neg	78000	
CLL17	10	0/A	88	F	U-MUT	Neg	Neg	107000	
CLL18	11	IV/C	70	F	MUT	Pos	Neg	12000	
CLL19	12	0/A	54	M	MUT	Neg	Neg	20000	
CLL20	13	I/A	69	M	U-MUT	Pos	Tris 12	20000	
CLL21	14	I/A	83	M	MUT	Neg	Del 13	22000	
CLL22	15	I/A	86	M	U-MUT	ND	ND	14000	
CLL23	16	0/A	75	F	MUT	ND	Neg	100000	
CLL24	17	0/A	59	M	MUT	Neg	Neg	7800	
PS105	18	I/B	78	M	MUT	Neg	Del 13	29000	
CLL25	19	III/B	83	F	U-MUT	Neg	Del13	120700	
CLL26	20	III/C	84	M	U-MUT	ND	Tris 12	30700	
CLL27	21	0/A	69	M	U-MUT	ND	ND	7100	
CLL28	22	I/A	64	F	U-MUT	ND	Del 13	59200	
CLL29	23	II/A	66	M	MUT	Neg	Neg	13840	
CLL30	24	II/A	64	M	U-MUT	Neg	Del13	39990	
CLL31	25	0/A	82	F	MUT	Neg	Neg	26840	
CLL32	26	0/A	56	F	MUT	Pos	Del13	10130	
CLL33	27	I/A	67	M	MUT	ND	Neg	20700	
CLL34	28	0/A	72	M	MUT	ND	Del13	41600	
CLL35	29	0/A	76	M	MUT	ND	Neg	25600	
CLL36	30	0/A	76	M	MUT	Neg	Neg	123000	yes
CLL39	31	0/A	ND	M	ND	ND	ND	12100	yes

CLL40	32	I/A	74	F	MUT	Pos	Del13	7800	yes
CLL41	33	II/B	60	M	U-MUT	Neg	ND	55000	yes
CLL42	34	0/A	72	M	MUT	ND	Del13	4300	yes
CLL45	35	0/A	54	M	ND	Pos	ND	4300	
CLL46	36	0/A	82	F	MUT	Neg	Del13	16550	
CLL47	37	0/A	77	F	MUT	Pos	Tris 12	58300	
CLL48	38	0/A	90	M	ND	Neg	ND	11840	
CLL49	39	I/A	83	F	ND	NA	ND	8880	
CLL50	40	0/A	57	M	MUT	Neg	Del13	87200	yes
CLL52	41	0/A	67	M	MUT	Neg	Neg	10930	
CLL55	42	I/A	89	F	MUT	Neg	Del13	76000	yes
CLL57	43	0/A	71	M	MUT	Neg	Del13	46000	
CLL62	44	0/A	72	M	ND	ND	ND	11000	
CLL63	45	0/A	67	F	ND	Neg	ND	12000	
CLL64	46	0/A	83	M	MUT	Neg	ND	33000	
CLL65	47	0/A	69	M	MUT	Neg	ND	17100	
CLL66	48	II/B	75	M	U-MUT	Pos	Neg	23000	
CLL67	49	0/A	72	M	MUT	Neg	ND	15000	yes
CLL68	50	0/A	59	F	MUT	Neg	Neg	15000	
CLL69	51	0/A	55	F	ND	Neg	ND	15000	
CLL70	52	I/A	84	F	MUT	Neg	Neg	65000	

Supplemental Table S2 (.xls file). Corresponding to Figure 2. DESeq2 and gene enrichment analysis results for RNA sequencing data on CLL cells activated in co-cultures with 3T3-CD40L cells, cytokines and indicated stimuli (recombinant ApoE3 or control condition) for 72h. Tab1 of the table reports all RNAseq-identified transcripts ranked according to the fold-change difference of expression in ApoE-treated CLL cells vs control CLL cells. Tab 2 of the table consequently reports RNAseq-identified DEG transcripts having fold change ≥ 1 or ≤ -1 , and $p_{adj} < 0.01$. Tab 3 of the Table contains gProfiler gene enrichment analysis of DEGs from the Tab 2.

Supplemental Table S3 (.xls file). Corresponding to Figure 5. DESeq2 and gene enrichment analysis results for RNA sequencing data on CLL cells with low and high WBCs activated in co-cultures with 3T3-CD40L cells, cytokines and indicated stimuli treated with ApoE for 72h. Tab1 of the table reports all RNAseq-identified transcripts ranked according to the fold-change difference of expression in B cells with aggressive and indolent phenotype. Tab 2 of the table consequently reports RNAseq-identified DEG transcripts having fold change ≥ 0.6 or ≤ -0.6 , and $p_{adj} < 0.05$. Tab 3 of the Table contains gProfiler gene enrichment analysis of DEGs from the Tab 2.

Supplemental Table S4. Richter syndrome patient-derived xenografts used in the study.

Sc: sub-cutaneous injection; ip: intra-peritoneum injection.

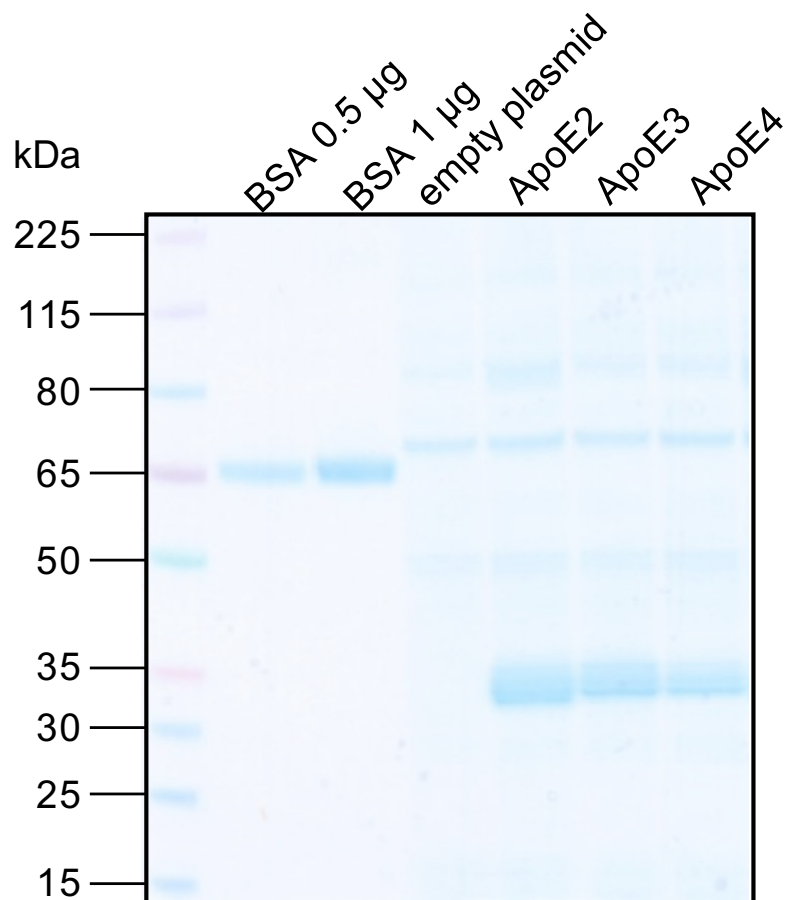
Patient code / date of xenograft isolation	Xenograft passage / inoculum	Genetic abberations/mutations	Reference
RS1316	13, ip	Trisomy 12, mutations	Vaisitti et al. ¹³
RS1316	14, ip	in <i>BTK</i> , <i>KRAS</i> , <i>MED12</i> , and <i>NOTCH2</i>	
RS9737	15, sc	Biallelic inactivation of	Vaisitti et al. ¹³
RS9737	14, sc	<i>CDKN2A</i> and <i>TP53</i> , monoallelic deletion of 11q23 (<i>ATM</i>), mutations of <i>BTK</i> , <i>KRAS</i> , <i>EGR2</i> , and <i>NOTCH1</i>	
IP867/17	10, sc	Amplification of <i>MYC</i> , <i>TP53</i> deletion	Vaisitti et al. ¹⁴

Supplemental Table S5. Comparative expression analysis of genes functionally implicated in protection against ferroptosis between RS and CLL patients. List of ferroptosis resistance genes was composed based on the literature^{15–18}. Statistical values were derived from DESeq2 analysis on 14 RS and 269 CLL transcriptomes. Genes are listed according to levels of expression in CLL cells.

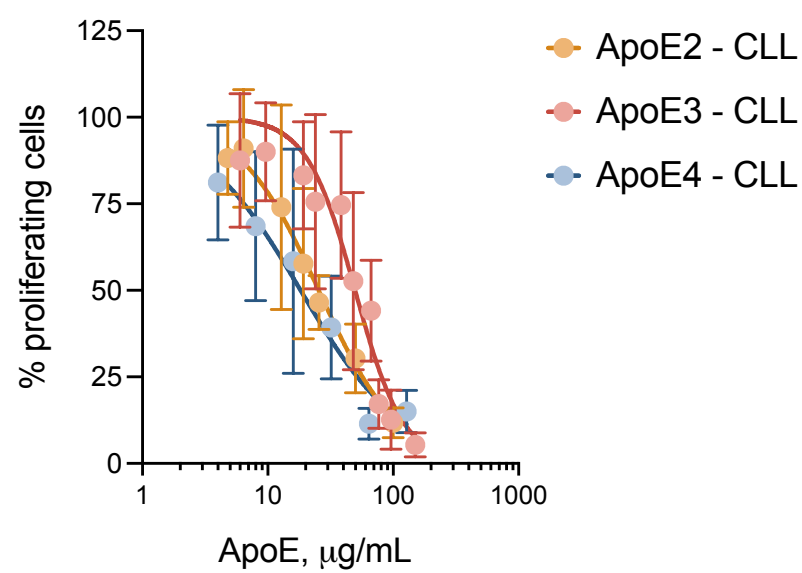
Symbol	Log ₂ F C	adj. p-val	Median RS	Average Expr. RS	St.Dev. RS	Median CLL	Average Expr. CLL	St.Dev. CLL
<i>CD44</i>	1.84	1.68E-04	138.35	368.54	868.64	147.00	186.86	148.70
<i>GPX4</i>	-1.42	4.30E-03	39.29	44.34	24.72	107.76	117.99	58.32
<i>GSS</i>	1.61	7.39E-16	35.37	38.48	10.19	15.36	15.73	7.57
<i>HMGCR</i>	1.61	1.08E-06	30.68	37.89	15.71	14.59	16.72	9.86
<i>AIFM2</i>	1.18	1.75E-06	6.04	6.76	2.33	4.52	4.40	2.33
<i>GCLM</i>	2.2	5.01E-07	9.06	9.82	3.16	2.19	2.34	1.29
<i>SLC7A11</i>	3.54	5.39E-05	1.84	2.28	1.13	0.27	0.40	0.27
<i>AKR1C3</i>	2.99	3.65E-03	0.82	3.84	10.00	0.24	0.86	1.78

Supplemental Figures

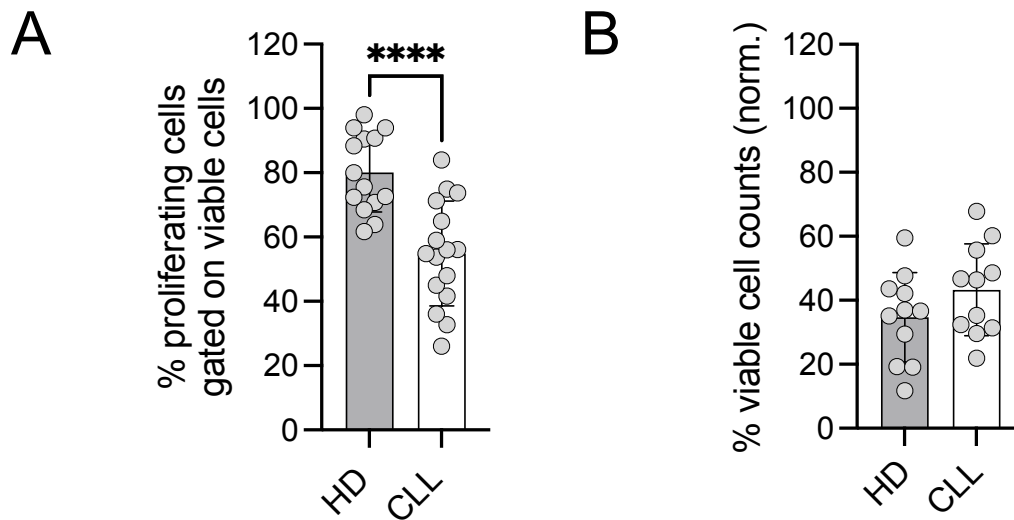
Supplemental Figure S1. SDS-PAGE analysis of Expi293-produced recombinant ApoE2, ApoE3 and ApoE4. Expi293 were transfected with either empty control plasmid or pCMV4 plasmids encoding ApoE isoforms. Supernatants of transfected Expi293 cells were concentrated 10-fold by using 30K membranes (eliminating proteins <30 kDa weight) and sterile-filtered.



Supplemental Figure S2. ApoE isoforms inhibit CLL cell proliferation. Proliferation of viable B cells from CLL patients (n=6-10) cultured in the presence of 3T3-hCD40L feeder cells, α -IgM stimulatory antibodies and IL4/IL21 and treated with increasing concentration of ApoE isoforms for 5-6 days. Graphs report mean values $\pm$ SD.

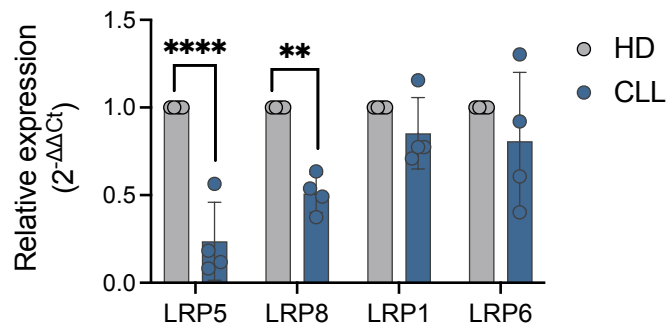


Supplemental Figure S3. ApoE affects proliferation of healthy B cells. (A) Proliferation of viable cells and (B) total counts of viable cells isolated from healthy donors (HD, n=11) or CLL patients (n=11) cultured in the presence of 3T3-hCD40L feeder cells, α -IgM/IL4/IL21 and treated with 50 μ g/mL ApoE3 for 5-6 days. Values were normalized to controls treated with supernatants from Expi293 cells transfected with an empty vector. Graphs report mean values $\pm$ SD. ****P < 0.0001 (Mann-Whitney test).

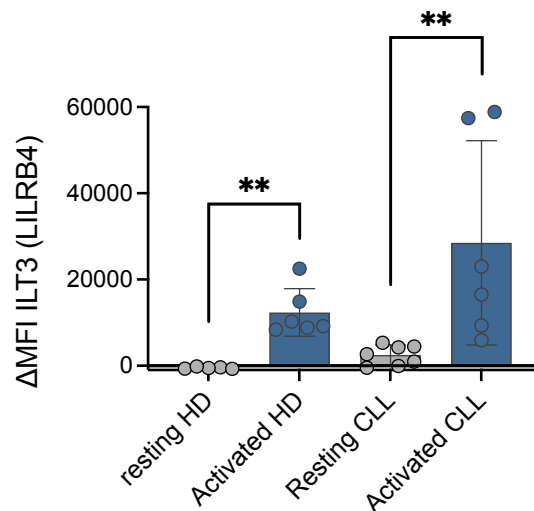


Supplemental Figure S4. Expression of ApoE receptors in B cells from healthy donors and CLL patients. (A) B cells from healthy donors (n=4 donors) and CLL patients (n=4 donors) were stimulated in the presence of 3T3-hCD40L feeder cells and α -IgM/IL4/IL21 for 4 days. Expression of ApoE receptors was evaluated by qPCR using the following primers: LRP5_fwd GGACACCAACATGATCGAGTCG, LRP5_rev CGCTCAATGCTGTGCAGATTCC, LRP8_fwd GAAGCGGAACTATTCACGCCTC, LRP8_rev ATGAGGACCTCCTGCTCTTTTCG, LRP1_fwd CAACGGCATCTCAGTGGACTAC, LRP1_rev TGTTGCTGGACAGAACCACCTC, LRP6_fwd CAGTTGGAGTGGTGCTGAAAGG, LRP6_rev CCATCCAAAGCAGCCCGTTCAA. Relative expression was normalized to ACTB and was expressed as $2^{-\Delta\Delta C_t}$. Ct, threshold cycles. (B) Quantification of ILT3 expression evaluated by flow cytometric analysis in B cells from healthy donors (n=5 donors) and CLL patients (n=6 donors) stimulated as in panel A. PE/Cy7-conjugated anti-human CD85k/ILT3 clone ZM4.1 was used for staining of human ILT3. Graphs report mean values $\pm$ SD. ****P < 0.0001, **P < 0.01 (Mann-Whitney test).

A

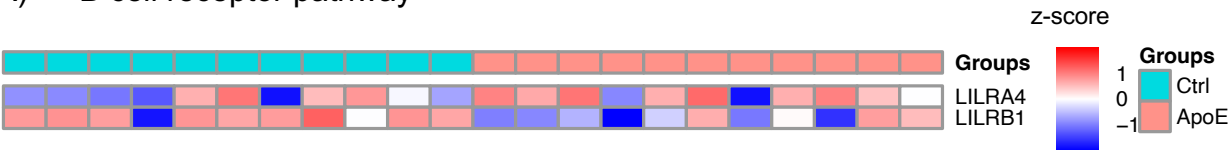


B

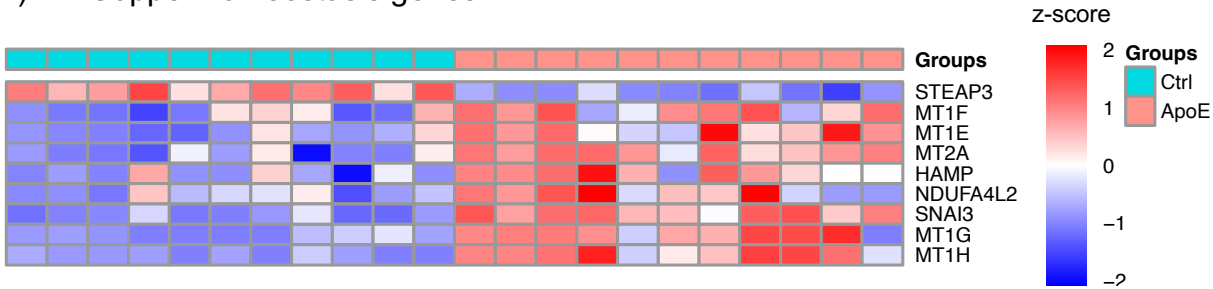


Supplemental Figure S5. ApoE3 influence on gene expression in pathways with a key role in CLL pathogenesis. CLL cells cultured with control supernatant of Expi293 cells (Ctrl) or 70 µg/ml of recombinant ApoE3 (ApoE). Genes belonging to indicated pathways have statistically different expression levels between ApoE-treated and ctrl-treated CLL cells. Thresholds used for filtering: fold change ≥ 1 or ≤ -1 ; $p_{adj} < 0.05$; base mean > 20 . Statistical comparison was done using DESeq2. Heatmaps report variance stabilized expression value (vsd) calculated using DESeq2 and scaled in the row direction using pheatmap R package.

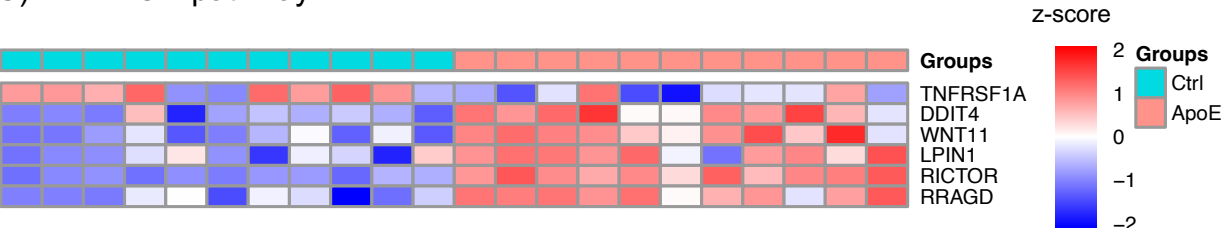
(A) B cell receptor pathway



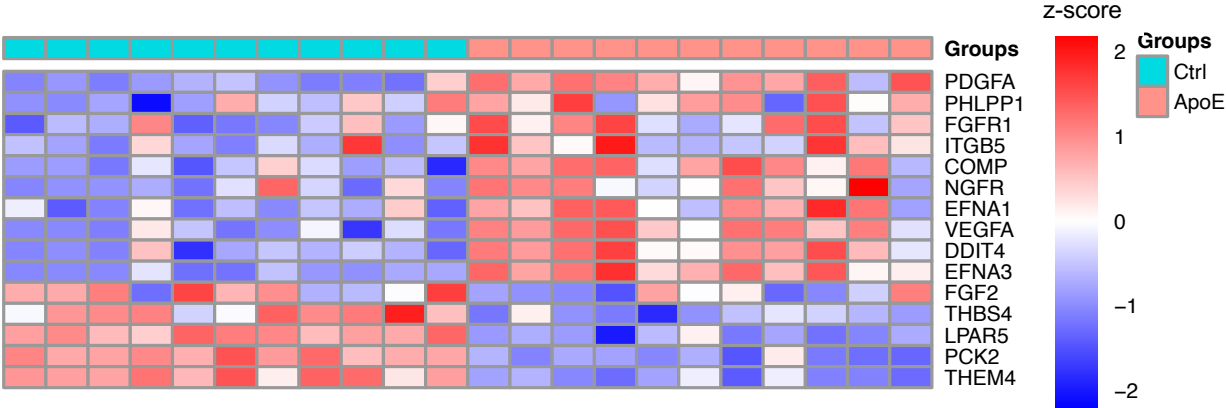
(B) Copper homeostasis genes



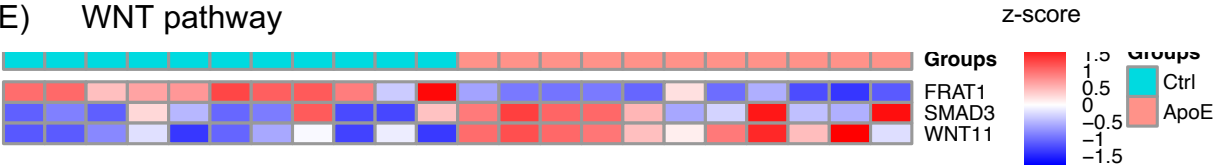
(C) mTOR pathway



(D) PI3K-Akt pathway

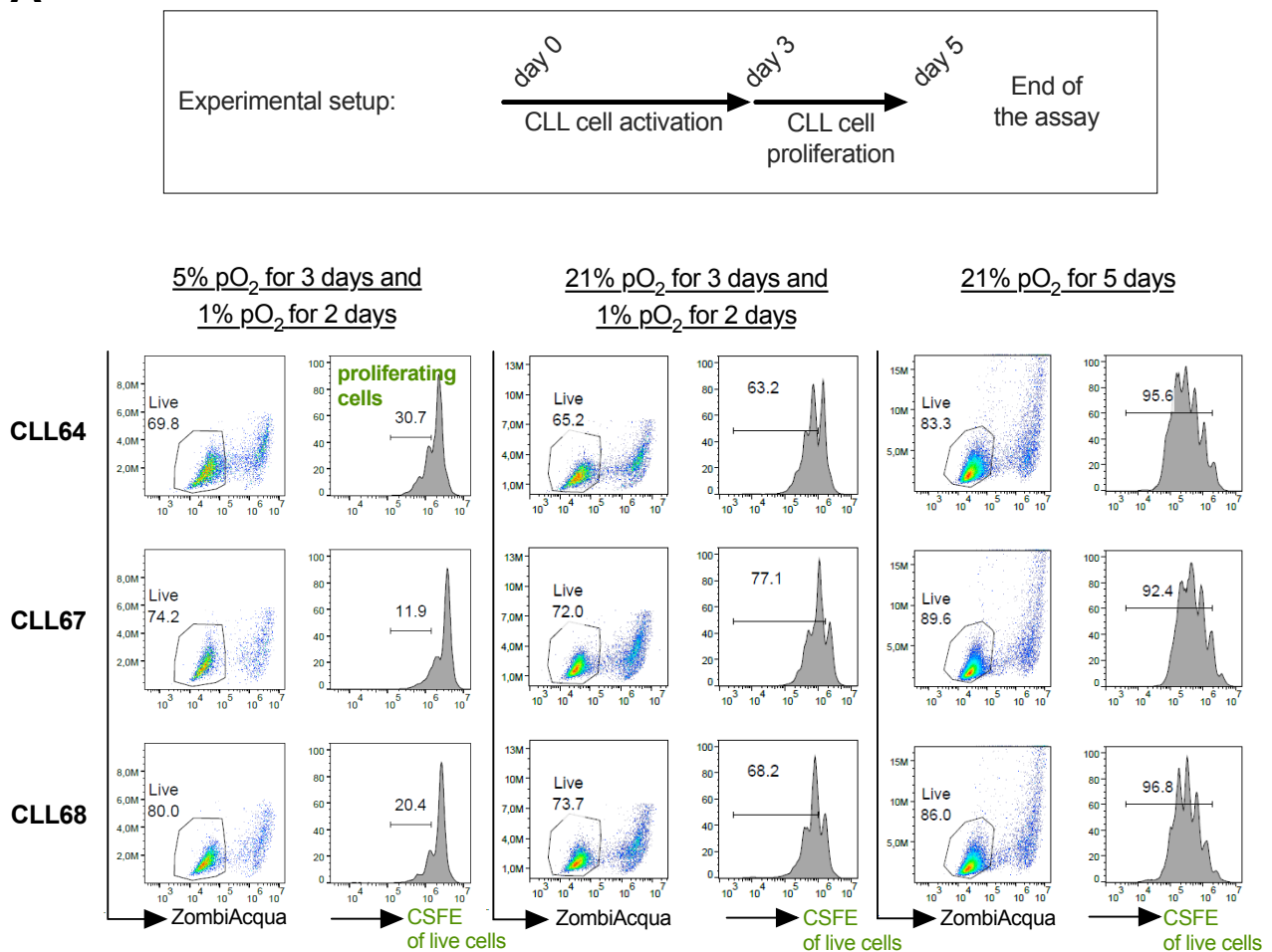


(E) WNT pathway



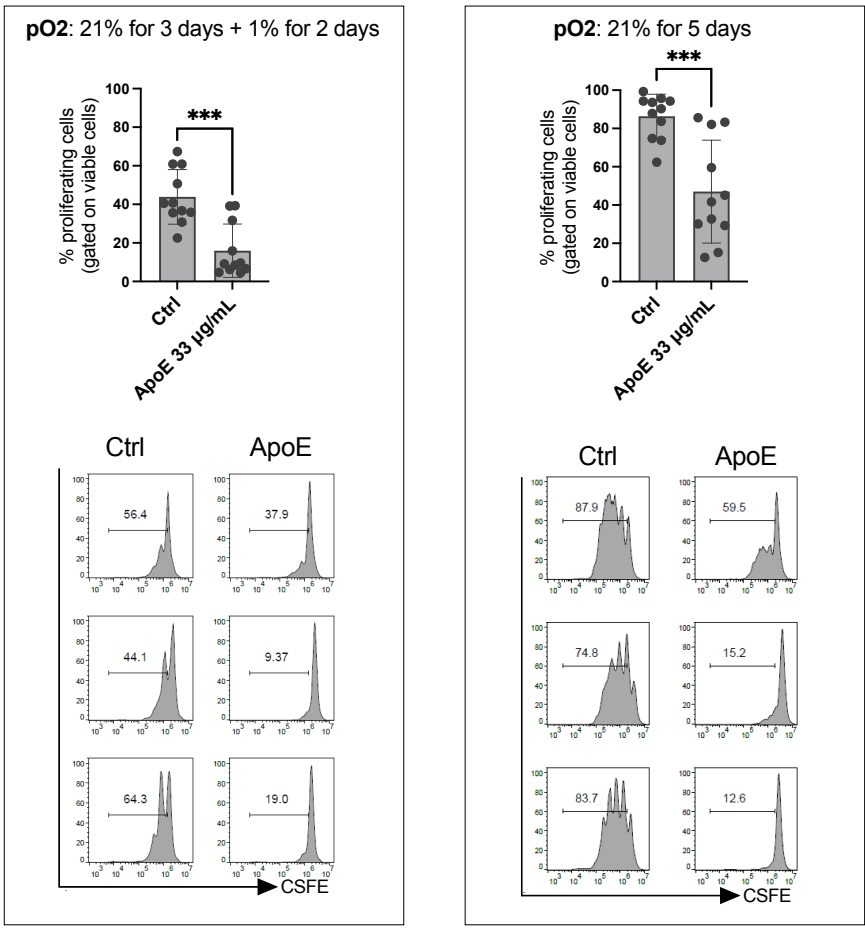
Supplemental Figure S6. ApoE-mediated inhibition of CLL proliferation occurs in hypoxic conditions. (A) Optimization of culturing conditions for CLL cell proliferation assay in hypoxia. CLL cells fail to proliferate when cultured for 5 days at pO₂ 1% in the presence of 3T3-hCD40L feeder cells, α -IgM/IL4/IL21 and treated with 50 μ g/mL ApoE3 (data not shown). Hence, a hybrid normo-hypoxic schedule was set up, as indicated in the experimental setup scheme. Once put in culture, CLL cells require 3 days for full activation before they start to proliferate. We reasoned that might require to be exposed to higher pO₂ during this phase. We cultured CLL cells at pO₂ 5% or 21% for 3 days, before moving them to pO₂ 1% for 2 more days. CLL cell viability was restored with the 5%-1% combination, however their proliferation was still strongly inhibited (left plot at the bottom of the panel), in comparison to the fully normoxic condition (right plot). Instead, the 21%-1% combination resulted in a detectable CLL cell proliferation (central plot).

A

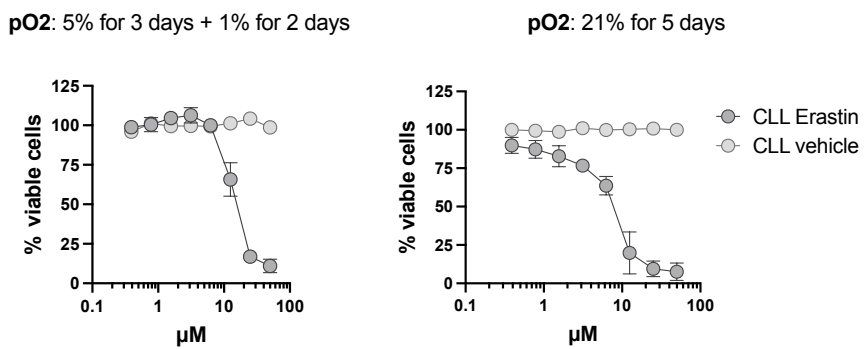


(B) Proliferation of CLL cells (n=11 donors) stimulated with 3T3-hCD40L/ α -IgM/IL4/IL21, treated with 50 μ g/mL ApoE3 or control supernatant and cultured at pO2 21% for 3 days and pO2 1% for 2 more days. CLL cells cultured in normoxia for 5 days are shown for comparison. Representative flow cytometry profiles of CFSE dilution as a readout of cellular proliferation are shown for both conditions. (C) Viability of stimulated CLL B cells treated with increasing concentrations of erastin-2 or vehicle (DMSO) and cultured for 5 days at pO2 5% for 3 days and pO2 1% for 2 more days or in normoxia (n=3 donors). Graphs report mean values $\pm$ SD. ***P < 0.001 (Mann-Whitney test).

B

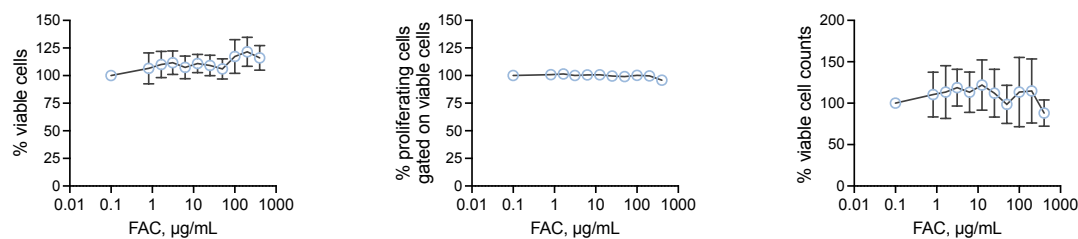


C

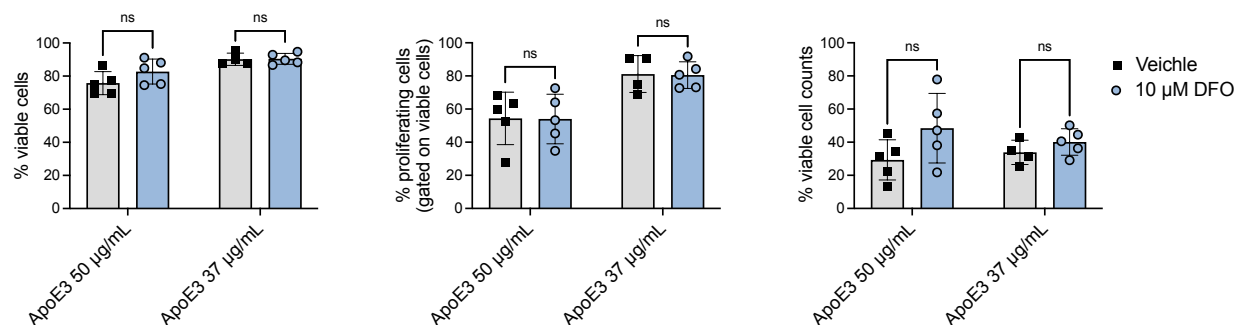


Supplemental Figure S7. Modulation of iron levels appears not have direct impact on ferroptosis or ApoE toxicity in proliferating CLL cells. (A) Cell viability, proliferation of viable cells and total counts of viable CLL cells (n=5 donors) treated with various concentrations of ferric ammonium citrate (FAC) for 5 days. Ferric ammonium citrate is estimated to have at least 15% of total ferric content, hence the highest used concentration (400 $\mu\text{g/mL}$) corresponds to at least 1 mM Fe^{3+} . Values are normalized to the untreated control. (B) Cell viability, proliferation of viable cells and total counts of viable CLL cells (n=5 donors) treated with 50 and 37 $\mu\text{g/mL}$ ApoE for 5 days in presence of 10 μM deferoxamine (DFO) or vehicle. Values were expressed as percentage calculated on the control group treated with control supernatant from Expi293 cells transfected with an empty vector. Data were analyzed by Mann-Whitney test. Ns, not significant.

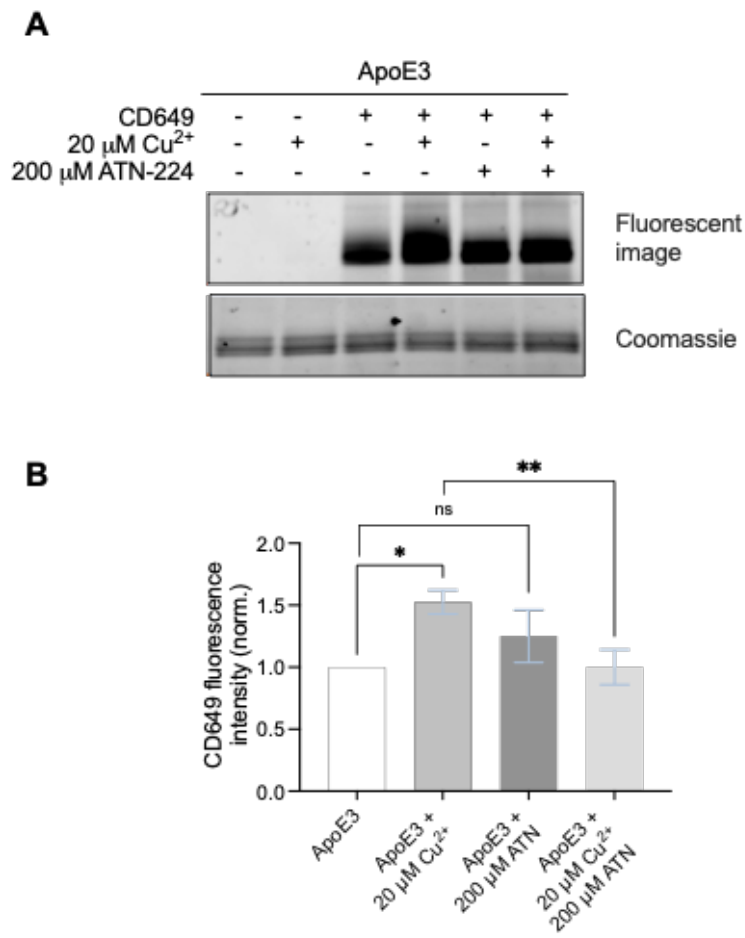
A



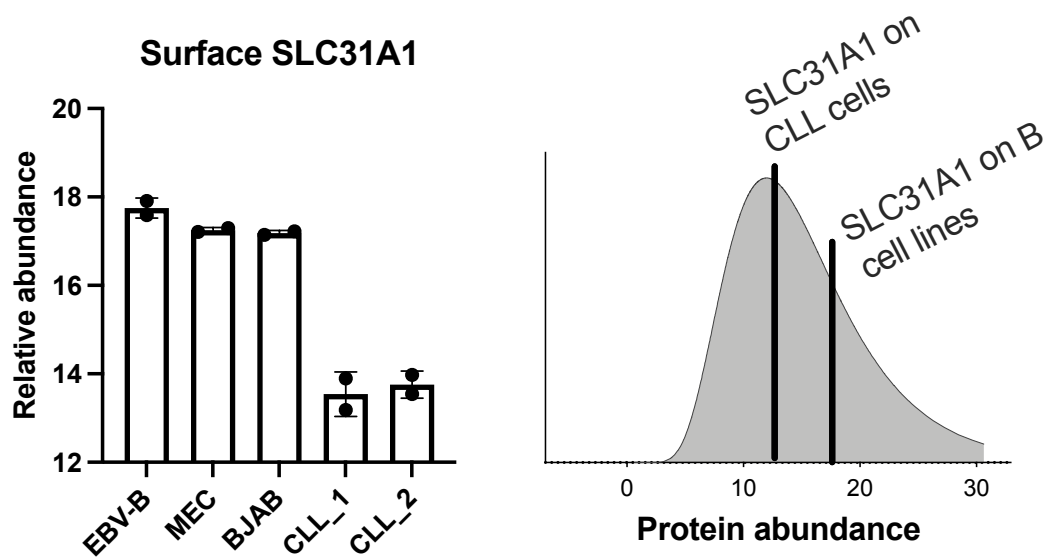
B



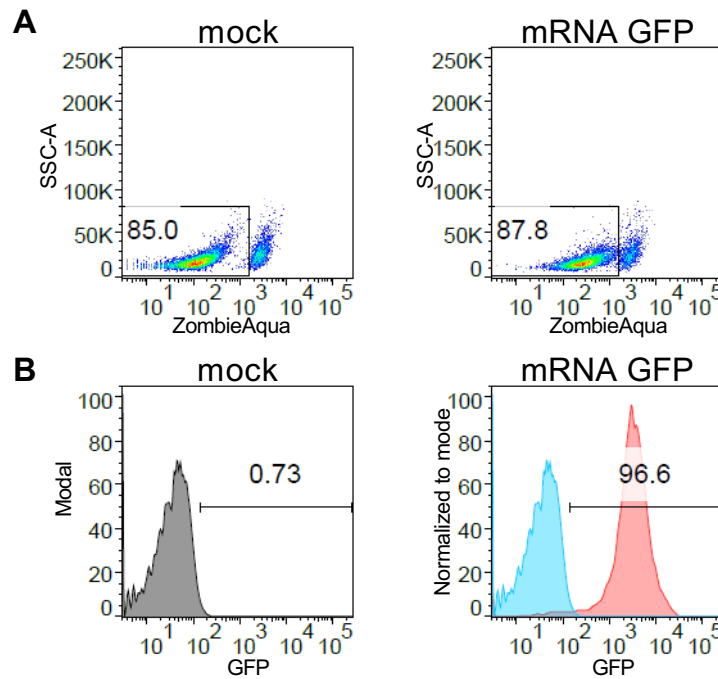
Supplemental Figure S8. Validation of CD649 signal readout on recombinant ApoE3 using the copper chelator ATN-224. (A) SDS-PAGE analysis of recombinant ApoE3 pre-incubated with CD649 at the indicated conditions. 2 μg of recombinant ApoE3 was resuspended in 50 μL PBS, in the presence or absence of 20 μM CuSO_4 , for 5 min at RT. 20 μM of CuSO_4 were pre-incubated with 200 μM of the copper chelator ATN-224 for 5 min at room temperature. 2 μM of CD649 probe were added for 2 hours at room temperature. Samples were resuspended in SDS-PAGE loading buffer, boiled 5 min at 90°C and loaded in a 4-12% polyacrylamide Bolt precast gels in MOPS SDS buffer (ThermoFisher). (B) Quantification of CD649 fluorescence intensity (n=3-4 samples). Data were analyzed by Mann-Whitney test. * $P < 0.05$, ** $P < 0.01$.



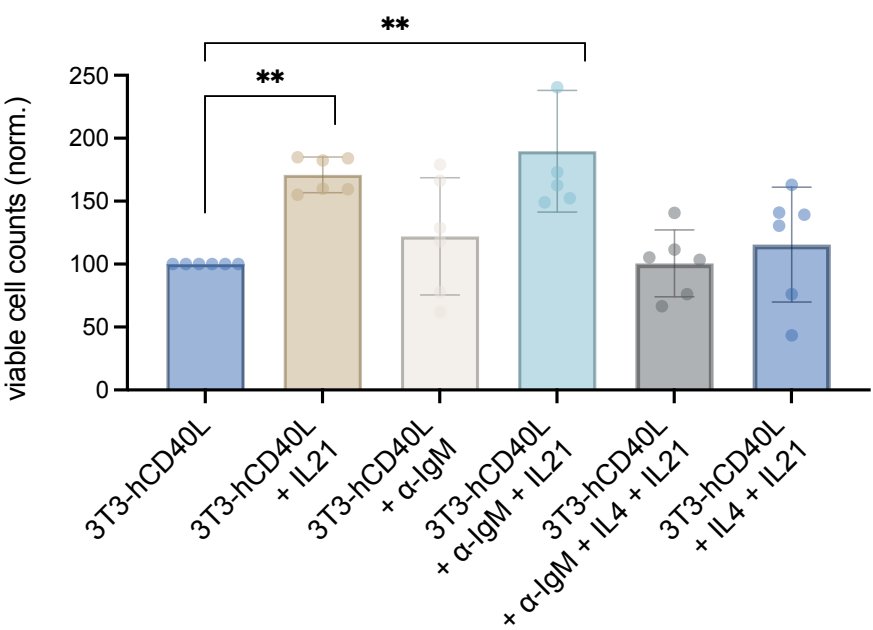
Supplemental Figure S9. SLC31A1 expression in primary CLL cells. Surface proteomics analysis¹⁹ of B cell lines and primary B cells revealed that B cells express only one copper importer, SLC31A1, while being negative for SLC31A2. Plot reports label free quantification values of surface expression of SCL31A1, relative to overall protein abundance detected across samples (derived from Supplementary Table S1 of the reference 19).



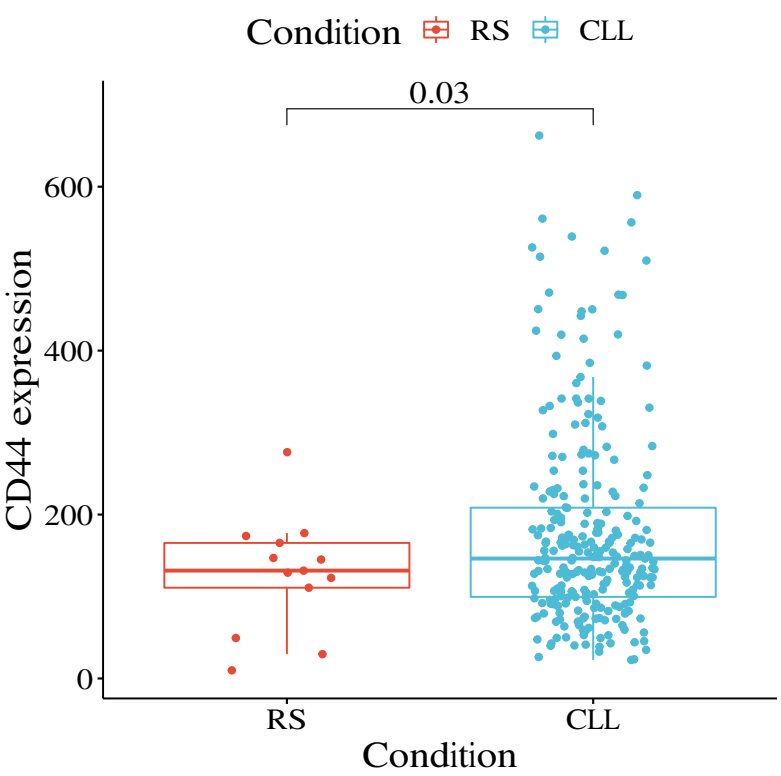
Supplemental Figure S10. Application of an optimized electroporation method preserving cell viability and allowing highly efficient overexpression of exogenous genes in primary CLL cells. Depicted are representative data generated using *GFP* mRNA for electroporation following optimized protocol²⁰. Cell viability (A) and transfection efficiency (B) evaluated 24h post-electroporation in CLL cells either mock-electroporated or electroporated with GFP mRNA.



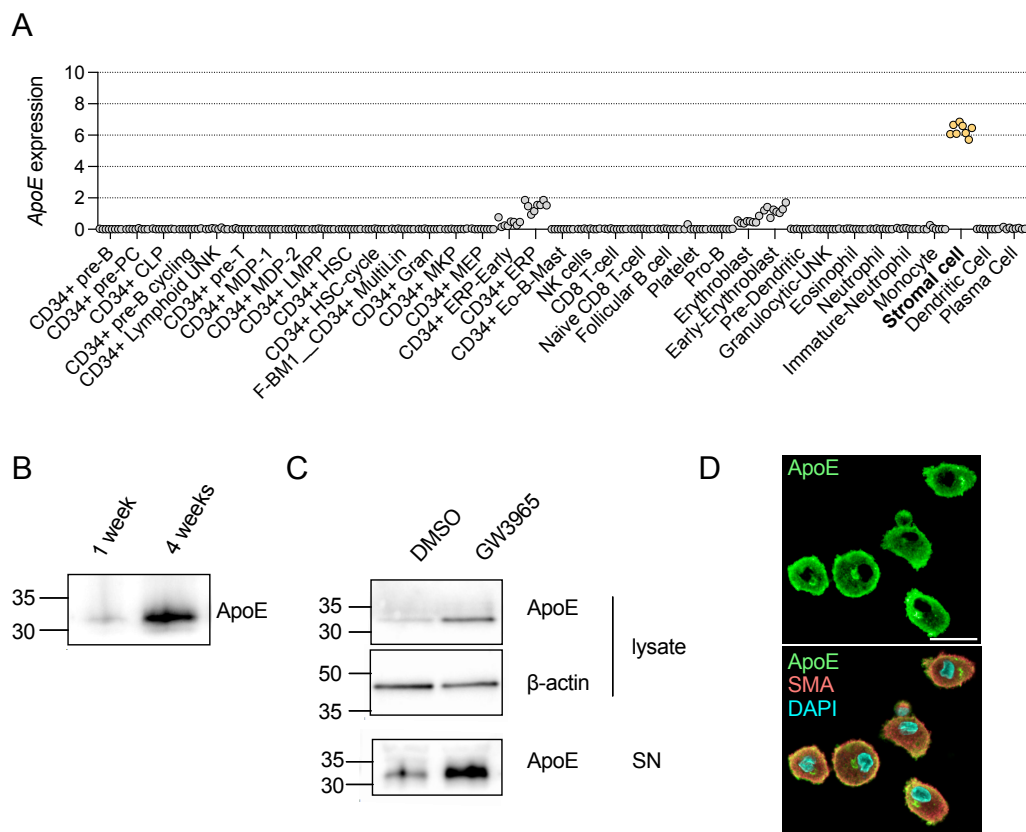
Supplemental Figure S11. Optimization of culturing conditions for patient-derived xenograft cells derived from Richter Syndrome patients' cells. Total viable cell numbers were compared between cell cultures set up with CD40L-expressing 3T3 feeder cells and indicated stimuli (n=3 donors). Graphs report mean values $\pm$ SD. **P < 0.01 (Mann-Whitney test).



Supplemental Figure S12. Repetition of statistical analysis evaluating differences in *CD44* expression between RS and CLL cells. RNAseq counts of *CD44* in a cohort of RS patients (n=13; one outlier with TPM=3491.747 was removed) versus CLL cohort (n=269 donors). TPM, transcripts per million. *P* value was calculated using the student's t-test.

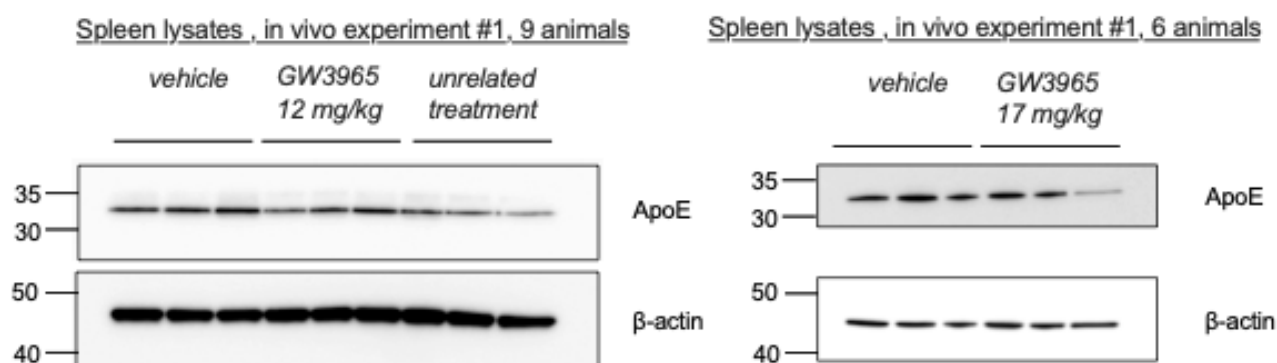


Supplemental Figure S13. ApoE is produced in the CLL bone marrow. Experimental details for stromal cell culturing, immunoblotting and immunofluorescence are indicated in Supplementary Methods. (A) Single-cell RNAseq analysis of bone marrow lymphocyte populations from 8 healthy donors⁶ suggests that ApoE could be produced by mesenchymal marrow stromal cells. (B) Temporal analysis secretion of ApoE from mesenchymal stromal cells derived from the CLL bone marrow biopsy. Stromal cell cultures were established from the CLL bone marrow biopsy. Indicated weeks are counted starting from cell culture establishment as described¹². (C) Production of ApoE from stromal cells 72h after their stimulation with DMSO or 3 μ M GW3965, a small molecule agonist of the liver X receptors (LXRs)²¹. Intracellular ApoE levels were evaluated in cellular lysates; secreted ApoE was evaluated in cell culture supernatant. (D) Immunofluorescence of stromal cells stained for ApoE, smooth muscle actin (SMA) and nuclei (DAPI). Scale bar, 20 μ m.

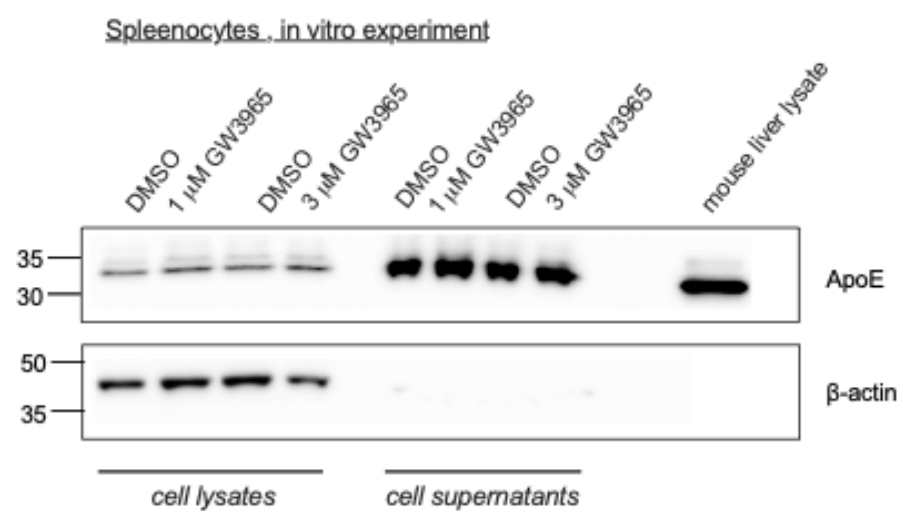


Supplemental Figure S14. LXR agonist GW3965 does not upregulate ApoE expression in C57BL/6 splenocytes. (A) ApoE expression in the spleen of C57BL/6 mice (n=15). Two independent experiments were performed. Experiment #1: Animals (n=3 per group) were either untreated, treated with 12 mg/kg LXR agonist GW3965 or unrelated drug (daily i.p. injections for 26 days). Experiment #2: Animals (n=3 per group) were either untreated or treated with LXR agonist GW3965 (17 mg/kg i.p., daily treatment for 34 days). GW3965 literature reports treatment concentrations in the 5-20 mg/kg range²²⁻²⁴. 10 ug of proteins of total organ lysates were loaded equally for all samples. Samples were blotted using anti-mouse ApoE mAb (Cell Signaling clone E7X2A, cat.n. 28542) and anti-mouse beta actin (MAB1501, Sigma). (B) ApoE expression in *in vitro* cultured C57B/L6 splenocytes. Cells were cultured with 1-3 μ M GW3965 or vehicle (DMSO), as indicated. 10 ug of proteins of cell supernatants or cell lysates were loaded equally for all samples.

A



B



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