Supplemental Material and Methods

Animal material

Eμ-TCL1 mice on the C57BL/6J background were provided by Dr. Marco Herling (University of Cologne, Cologne, Germany). All animal experiments were performed upon approval and according to local regulations. Malignant B-cells were isolated using magnetic microbeads (Miltenyi biotec) for depletion of CD90.2$^+$ cells and subsequent enrichment of CD5$^+$ cells according to the manufacturer's protocols. Purity of the cells was always >90% as determined by multi-color flow cytometry. The murine stroma cell line M2-10B4 was purchase from ATCC (Manassas, VA).

Cell culture

Human and murine co-culture

The human stroma cell line HS-5 or primary mesenchymal stroma cells (pMSC) were seeded at a density of 5 x 10^4 /ml in RPMI-1640 (Sigma-Aldrich) supplemented with 2 mM L-glutamine (Sigma-Aldrich), 10 % FCS (c.c.pro) and 40 U/ml Penicillin-Streptomycin (ThermoFisher Scientific) henceforth called R10$^+$ per well in a 24-well plate (Greiner bio-one). Cells were maintained at 37°C, 5% CO_2 for 24h. Subsequent, medium was exchanged to fresh R10$^+$ and CLL PBMCs (>75% B-CLL frequency for flow cytometry, >90% B-CLL frequency for gene expression analysis and ChIP) were seeded at a density of 2 x 10^6/ml (HS-5:CLL ratio = 1:40). Cultures were incubated for 1-6 days as detailed at 37°C, 5% CO_2. For indicated experiments CLL cells were re-purified after co-culture using anti-CD90 magnetic microbeads (Miltenyi biotec) for stroma cell depletion according to the manufacturer's protocol.

Transwell experiments were similarly conducted with the stromal cells in the lower compartments separated from the CLL PBMCs in the upper chamber in volumes recommended by the manufacturer (Greiner bio-one). Cultures of CLL PBMCs with conditioned medium from HS-5 were set up in a similar way adding either 1 ml fresh R10$^+$ to the CLL cell suspension or 1 ml of HS-5 conditioned medium. HS-5 conditioned medium was prepared by incubating 2 x 10^6 HS-5 cells per T75 flask in 20 ml R10$^+$ for 4 days. After a centrifugation step the cell-free medium was stored at -20°C.

For selected analyses HS-5 cells were cultured in absence and presence of 5 µM Lenalidomide for 24h. Then, stroma cells were harvested, washed and seeded as described above.

The murine stroma cell line M2-10B4 was seeded at a density of 5 x 10^4 /ml in $R10^+$ in a 96-well flat-bottom plate (Greiner bio-one) and cultured for 24h at 37°C, 5% CO_2. Medium was exchanged to fresh $R10^+$ and freshly isolated CLL cells from the spleen of a Eµ-TCL1 mouse were added at a density of 5 x 10^5 /ml (M2-10B4:CLL ratio = 1:10). Cultures were incubated for 3 days at 37°C, 5% CO_2.

For the treatment of CLL samples with Notch ligands recombinant human Jagged1, Jagged2, DLL1 and DLL4 (R&D Systems) were coated for 4h at 4°C in a 48-well flat-bottom plate at a concentration of 10 µg/ml. Afterwards, CLL PBMCs were cultured for 1-6 days at a density of 10^6 cells/ml.

All inhibitors utilized in the cell culture experiments are listed in the Supplemental Table 3.

T-cell activation culture

CLL PBMCs were analyzed for T-cell frequency by flow cytometry prior to the seeding. PBMCs and magnetic-bead isolated T-cells (CD3 microbeads, human; Miltenyi biotec) were seeded at a density of 10^6 /ml in $R10^+$. T-cells were stimulated using activation/expansion beads coated with anti-CD2/CD3/CD28 (Miltenyi biotec) at a ratio of 1:4. Samples were treated with anti-human CD274 (Thermo Fisher Scientific, clone MIH1) and the corresponding isotype as a control (mouse IgG1, κ; Thermo Fisher Scientific) at a concentration of 10 µg/ml. Samples were cultured for 72h at 37°C, 5% CO_2.

CLL/T-cell co-culture

Human T-cells were isolated from CLL PBMCs using anti-CD3 magnetic beads (Miltenyi biotec) according to the manufacturer's instructions. The T-cell-depleted fraction was cultured for 3 days in absence/presence of HS-5 cells as described above. T-cells were maintained for the same time at a density of 10^6 /ml in $R10^+$ at 37°C, 5% CO_2. CLL cells were re-purified from the stroma layer by anti-CD19 magnetic microbeads (Miltenyi biotec) according to the manufacturer's instructions. T-cells and CLL cells were seeded at a ratio of 1:1 in an autologous setup. Cultures were stimulated with anti-CD2/CD3/CD28 coated activation/expansion beads (Miltenyi biotec) at a bead:T-cell ratio of 1:8. In selected experiments samples were treated with anti-human CD274 (Thermo Fisher Scientific,

clone MIH1) and the corresponding isotype as a control (mouse IgG1, κ; Thermo Fisher Scientific) at a concentration of 10 µg/ml. Cultures were maintained for 48h at 37°C, 5% CO_2.

Murine CLL/M2-10B4 co-cultures were setup as described above. Murine T-cells were isolated from the lymph nodes of the same donor mouse using the Pan T Cell Isolation Kit II, mouse (Miltenyi biotec) as outlined by the manufacturer. CLL cells were re-purified from the stroma layer by CD90 depletion (CD90.2 microbeads, Miltenyi biotec). T-cells and CLL cells were seeded at a ratio of 2:1 and stimulated with anti-CD3/CD28 coated activation/expansion beads (Miltenyi biotec) at a bead:T-cell ratio of 1:8. Cultures were maintained for 48h at 37°C, 5% CO_2.

Flow Cytometry and Sorting

Cells were stained according to the manufacturer's recommendations using fluorochrome-coupled antibodies (Supplemental Table 2). For *ex vivo* analyses CLL and healthy donor (HD) PBMCs were used directly after density gradient isolation. Cells from the *in vitro* cultures were harvested in FACS tubes after the indicated incubation time. All samples were collected by centrifugation at 300x *g* for 5 min at 4°C. After washing with PBS, samples were stained with Zombie Aqua™ Fixable Viability Kit (Biolegend) for dead-cell discrimination according to manufacturer's instructions at a final dilution of 1:500. Samples were blocked with FcR Blocking Reagent, human (Miltenyi biotec) for 10 min at 4°C. Fluorochrome-coupled antibodies against surface proteins were directly added to the samples at recommended dilutions and incubated for 20 min at 4°C. Cells were either analyzed directly after washing (surface stainings) or further stained for intracellular proteins (intracellular stainings) using the Leucoperm™ fixation/permeabilization system (Biorad) according to the product manual.

Glucose uptake was analyzed by the use of 6-NBDG (6-(N-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl)amino)-6-Deoxyglucose; ThermoFisher Scientific). Cells were washed in PBS and glucose-free medium by centrifugation (300 x *g*, 5 min, 4°C) and resuspended in glucose-free medium containing 0.3 mM 6-NBDG. Samples were incubated for 15 min at 37°C, 5% CO_2 and subsequently recorded after two washing steps.

Absolute cell count /µl was determined using 123count eBeads (ThermoFisher Scientific).

All samples were recorded on a BD FACS Canto II after appropriate fluorescence compensation. Obtained data was analyzed using FlowJo V9.5 or V10 (FlowJo LLC).

Fluorescence-activated cell sorting (FACS) was performed at the Core Unit Cell Sorting of the University Erlangen-Nuremberg using fluorochrome-conjugated antibodies (Supplemental Table 2) according manufacturer's recommendations.

Quantitative real-time PCR

Samples were lysed after culture and RNA was prepared using the innuPREP RNA Mini Kit 2.0 (analytik jena) as recommended by the manufacturer. Synthesis of cDNA was performed using the SuperScript™ II Reverse Transcriptase (ThermoFisher Scientific) together with random decamers (ThermoFisher Scientific), dNTP-Set 1 (Carl Roth), and RNaseOUT™ Recombinant Ribonuclease Inhibitor (ThermoFisher Scientific) according to the protocol provided to the SuperScript™ II Reverse Transcriptase.

Quantitative real-time PCR reactions were set up using the Luna® Universal qPCR Master Mix (New England Biolabs) and QuantiTect Primer Assays (Qiagen, see Supplemental Table 4) in accordance to the manufacturer's recommendations. PCR reactions were run on a Rotor-Gene Q machine (Qiagen) and relative gene expression levels were quantified using the 2-standard method.

For analysis of micro-RNAs (miRs) total RNA was extracted using the miRNeasy Micro Kit (Qiagen) with on-column DNAse digestion (RNase-free DNase Set, Qiagen) according to the manufacturer's protocol. Relative expression was assessed using the miScript II RT Kit (Qiagen) with the recommended protocol for mature miRs and according miScript Primer Assays (Qiagen, see Supplemental Table 4) together with the miScript SYBR® Green PCR Kit (Qiagen) using miR16 and miR484 for normalization.

Gene set enrichment analysis

Gene Set Enrichment Analysis was performed using the public dataset GSE21029[1] on the GSEA software provided by the Broad Institute (MIT, CA, USA) with gene sets available by the Molecular Signatures Database v6.2[2].

Chromatin immuno-precipitation (ChIP)

For chromatin immune-precipitations (ChIP) both primary CLL PBMCs (B-CLL cell frequency >90%) as well as CLL cell lines (Mec-1, Eheb) were used. ChIPs were performed using the ChIP-IT® Express Enzymatic Kit (Active motif) according to the manufacturer's protocol. Precipitation was conducted with a c-Myc (D3N8F) Rabbit mAb (Cell Signaling Technology) and the corresponding normal rabbit IgG as control (Cell Signaling Technology). Precipitated samples were analyzed by use of quantitative real-time PCR for DNA fragments spanning regions around the PD-L1 promoter using 5 different primer pairs as indicated in Supplemental Table 5.

Supplemental Information

Supplemental Table 1: Patient's characteristics

Case	Sex	Age [Yrs]	Case	Sex	Age [Yrs]	Case	Sex	Age [Yrs]
1	M	60	**77**	F	71	**155**	M	70
5	F	79	**80**	M	59	**160**	F	51
6	M	71	**81**	M	79	**162**	F	68
9	M	73	**85**	M	72	**166**	M	79
10	M	80	**86**	M	71	**169**	F	71
11	F	77	**90**	F	86	**170**	M	53
15	M	75	**91**	M	83	**172**	M	34
18	M	52	**92**	M	59	**174**	M	65
22	M	67	**94**	M	52	**178**	M	67
23	M	55	**99**	F	78	**179**	M	84
30	M	91	**103**	M	67	**183**	F	74
32	F	81	**104**	F	53	**188**	M	40
34	F	73	**111**	F	91	**192**	F	87
37	M	52	**113**	M	76	**193**	M	62
38	M	68	**115**	F	81	**198**	F	82
40	M	82	**118**	M	75	**199**	M	59
41	F	71	**119**	F	63	**200**	F	65
42	M	64	**121**	M	40	**202**	M	74
43	M	68	**131**	F	76	**203**	F	82
49	M	82	**135**	M	43	**208**	F	76
50	M	72	**136**	M	56	**209**	M	75
51	M	75	**137**	F	80	matched-pair donors:		
57	M	65	**138**	F	76	**WOT0173**	M	80
61	F	74	**142**	F	78	**WET0405**	M	81
63	F	71	**143**	M	72	**AA02P/597-FA**	M	54
65	F	76	**144**	M	77	**AA03P/593-PCM**	M	52
66	F	68	**147**	F	81	**AA04P/370-CECM**	M	63
67	M	77	**149**	M	83	**AA07P/611-FA**	M	67
72	F	55	**151**	F	49	**AA09P/629-RE**	F	84
74	F	48	**153**	M	65	**77-137/138**	M	49
75	M	68	**154**	M	68	**121-230/231**	M	80

Supplemental Table 2: Antibodies for Flow Cytometry

Antibody	Fluorochrome	Clone	Manufacturer	Order No.
human				
CD3	PE/Cy7	SK7	Biolegend	344816
CD3	Pacific Blue	UCHT1	Biolegend	300431
CD4	FITC	OKT4	Biolegend	317408
CD5	FITC	UCHT2	Biolegend	300606
CD5	APC	UCHT2	Biolegend	300612
CD14	PerCP/Cy5.5	HCD 14	Biolegend	325622
CD16	BV421	3G8	Biolegend	302038
CD19	PE/Cy7	HIB19	Biolegend	302216
CD19	APC/Cy7	SJ25C1	Biolegend	363010
CD25	FITC	M-A251	Biolegend	356106
CD25	APC	M-A251	Biolegend	356110
CD45	APC	HI30	Biolegend	304037
CD45	PerCP/Cy5.5	2D1	Biolegend	368504
CD69	PerCP/Cy5.5	FN50	Biolegend	310926
CD137	PE/Cy7	4B4-1	Biolegend	309818
CD137	APC/Cy7	4B4-1	Biolegend	309830
CD184 (CXCR4)	PE	12G5	Biolegend	306505
CD274	PE	29E.2A3	Biolegend	329706
CD274	APC	29E.2A3	Biolegend	329708
EZH2	AlexaFluor 647	11/EZH2	BD	563491
Glut-1	APC	202915	R&D Systems	FAB1418A
IFNγ	APC/Cy7	4S.B3	Biolegend	502530
Ki67	PerCP/Cy5.5	Ki-67	Biolegend	350520
Myc	FITC	9E10	abcam	ab117599
Myc	AlexaFluor 488	9E10	Biorad	MCA2200A488
TNFα	APC	MAb11	Biolegend	502912
mouse				
CD3	eFluor450	17A2	Thermo Fisher	48-0032-82
CD5	FITC	53-7.3	Biolegend	100605
CD19	PE/Cy7	6D5	Biolegend	115519
CD25	FITC	3C7	Biolegend	101907
CD69	APC/Cy7	H1.2F3	Biolegend	104525
CD137	APC	17B5	Biolegend	106110
CD274	PE	MIH7	Biolegend	155404
EZH2	BV421	11/EZH2	BD	562963
Myc	AlexaFluor647	9E10	NovusBio	NB600-302AF647

Supplemental Table 3: Inhibitors

Compound	Manufacturer	Order No.	Concentration in culture
10058-F4	Selleckchem	S7153	50 µM
BMS-708163	Selleckchem	S1262	8 µM
3-Deazaneplanocin A (DZNeP)	Selleckchem	S7120	10 µM
EPZ011989	Selleckchem	S7805	50 µM
Lenalidomide	Sigma-Aldrich	SML2283	5 µM
Pomalidomide	Selleckchem	S1567	5 µM
Thalidomide	Selleckchem	S1193	5 µM

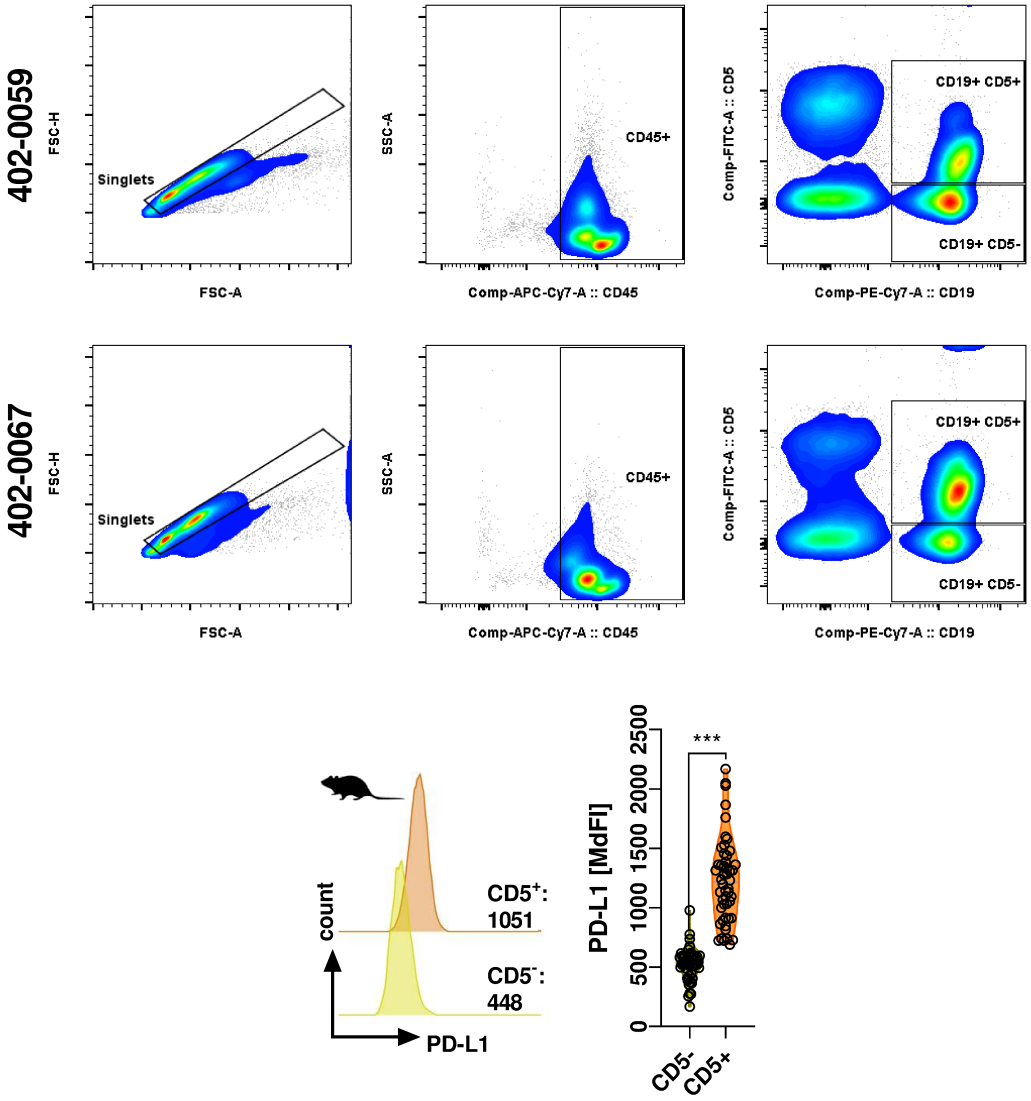
Supplemental Table 4: Primer for quantitative real-time PCR

Gene	Quantitect Primer Assay	Name	NCBI Ref. Seq.
ACTB	QT01680476	Actin, beta	NM_001101, XM_006715764
CCNB1	QT00006615	Cyclin B1	NM_031966
CD274	QT00082775	Programmed death ligand 1	NM_014143, XM_006716759
DLL1	QT00057631	Delta Like Canonical Notch Ligand 1	NM_005618, XM_005266934, XM_006725014
DLL3	QT00021791	Delta Like Canonical Notch Ligand 3	NM_016941, NM_203486
EZH2	QT00054614	Enhancer of zeste homolog 2	NM_004456, NM_001203249, NM_001203247, NM_001203248, NM_152998
HK2	QT00013209	Hexokinase 2	NM_000189
HES1	QT00039648	Hes Family BHLH Transcription Factor 1	NM_005524
JAG1	QT00031948	Jagged 1	NM_000214
LDHA	QT00001687	lactate dehydrogenase A	NM_001135239, NM_001165416, NM_001165415, NM_005566
MYC	QT00035406	MYC Proto-Oncogene	NM_002467
miR16	MS00008813	hsa-miR-16-2-3p	MIMAT0004518
miR26a	MS00029239	hsa-miR-26a-5p	MIMAT0000082
miR101	MS00008372	hsa-miR-101-3p	MIMAT0000099

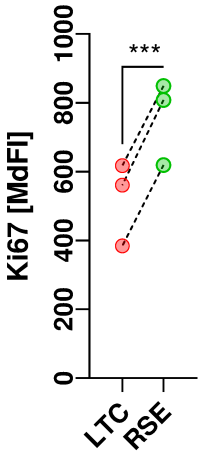
Supplemental Table 5: *CD274* primer for ChIP

#	fw sequence	rev sequence	product length
1	TGGACTGACATGTTTCACTTTCT	CAAGGCAGCAAATCCAGTTT	148 bp
2	TGATGCTAGGCTGGAGGTCTG	TGATGCTAGGCTGGAGGTCTG	121 bp
3	TGATCAGCAACACTATTTGGGA	TGTTAGGTATCAGATTGTTGCCA	142 bp
4	ACAGGGCAGGTTCTACTAGGT	TGCCCACAGCCACATAAACT	191 bp
5	CGCAAATCACTGAGCAGCAA	GCAGTGTTCAGGGTCTACCT	113 bp

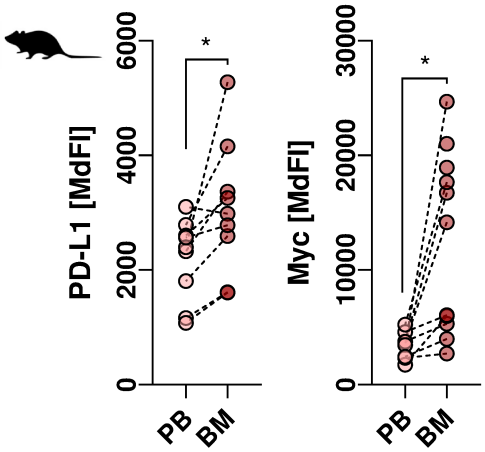
Supplemental Figures



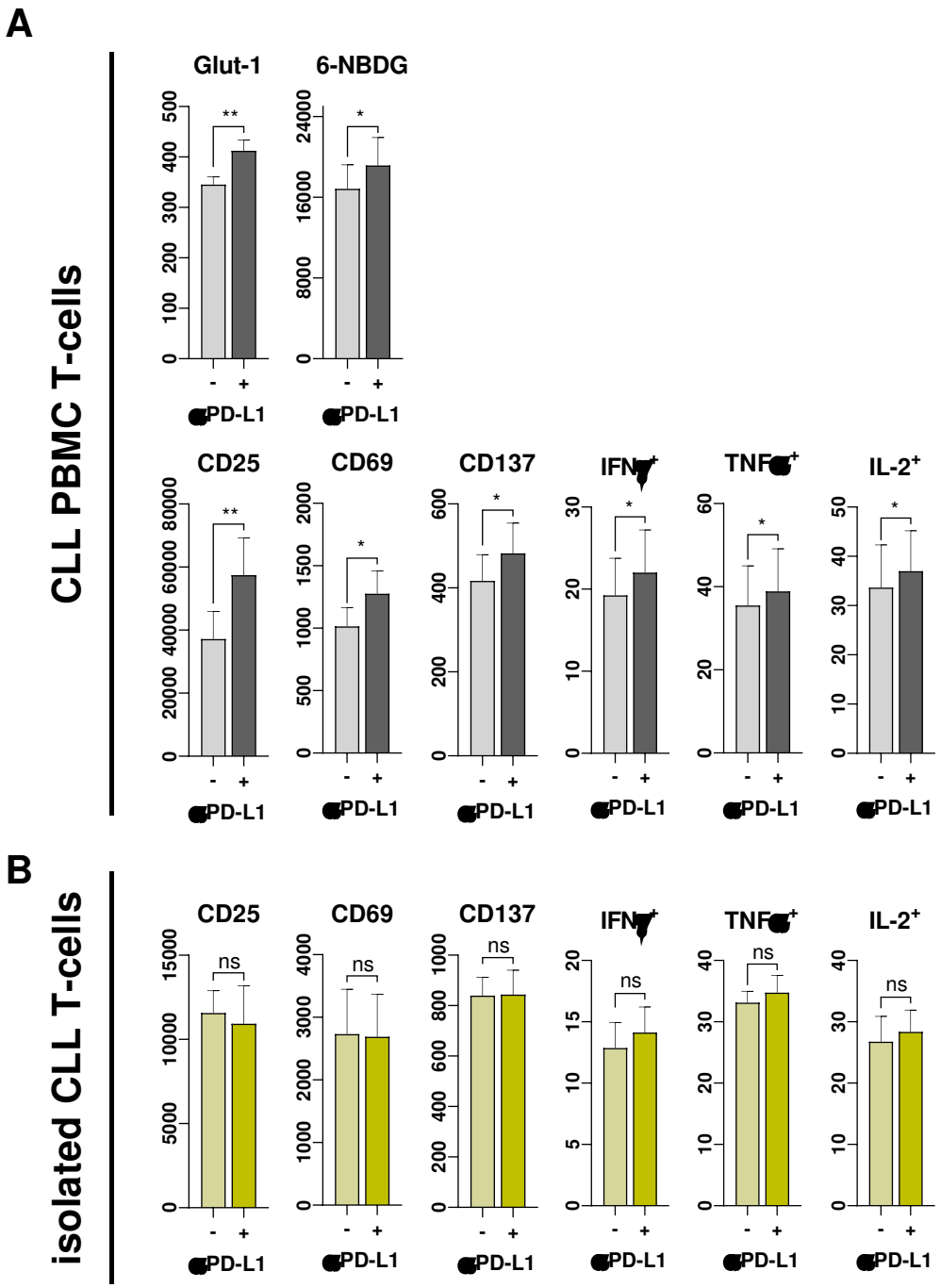
Supplemental Figure 1: Top: Gating strategy for malignant $CD5^+$ and non-malignant $CD5^-$ $CD19^+$ lymphocytes in peripheral blood from two different Eμ-TCL1 mice (402-0059, 402-0067). Bottom: PD-L1 expression was analyzed *ex vivo* on the surface of non-malignant ($CD5^-$) and malignant ($CD5^+$) $CD19^+$ lymphocytes from the blood of 5 month old Eμ-TCL1 mice by flow cytometry. The left histogram shows one representative example (numbers indicating median fluorescence intensity [MdFI]) and the right graph summarizes the values from 50 animals. *** p<0.001



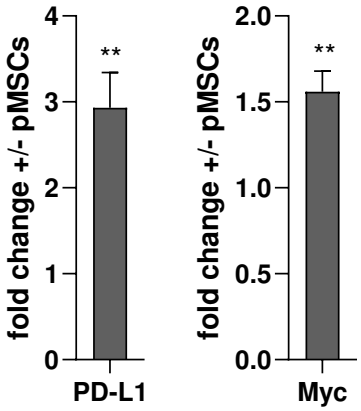
Supplemental Figure 2: Proliferation as analyzed by Ki67 staining in flow cytometry in LTCs and RSEs (n=3) corresponding to Figure 1B. *** p<0.001



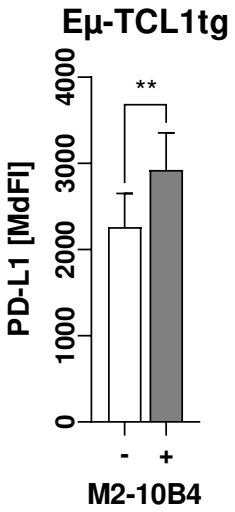
Supplemental Figure 3: Surface expression of PD-L1 (left, n=9) and intracellular levels of c-Myc (right, n=11) were analyzed by flow cytometry in $CD19^+$ $CD5^+$ CLL cells in matched pairs of PB and BM of Eμ-TCL1 mice. * p<0.05



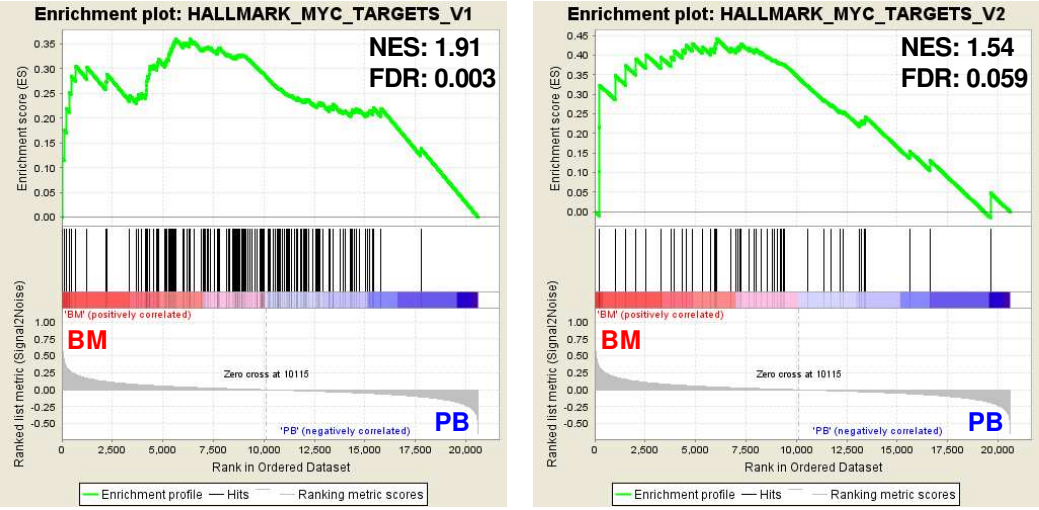
Supplemental Figure 4: CLL PBMCs (A, grey) or isolated CLL T-cells (B, yellow) were stimulated with anti-CD2/CD3/CD28 coated activation expansion beads (T-cell:bead ratio = 1:2) for 72 h in absence (-, light grey/yellow) or presence (+, dark grey/yellow) of 10 µg/ml of anti-PD-L1 antibody. The corresponding IgG isotype was used as control (-, light grey/yellow). Afterwards, T-cells were exemplary analyzed for their metabolic competence and activation by means of flow cytometry staining for surface level of Glut-1 (n=6) and glucose uptake (n=6, utilizing the fluorescent glucose analog 6-NBDG), as well as CD25 (grey n=9, yellow n=5), CD69 (n=6), CD137 (n=5), IFNγ (n=5), TNFα (n=4), and IL-2 (grey n=4, yellow n=6). Error bars represent the standard error mean. * p<0.05, ** p<0.01, ns not significant



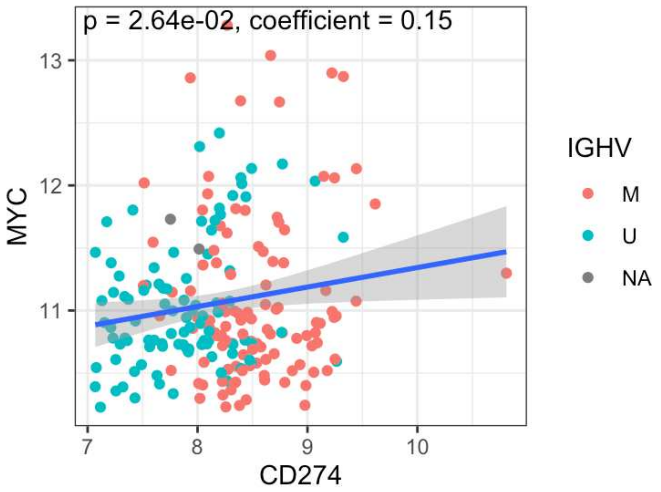
Supplemental Figure 5: CLL PBMCs were cultured in absence/presence of pMSCs for 3 days. Protein levels of surface PD-L1 (left, n=6) and intracellular Myc (right, n=6) were analyzed by flow cytometry. Values are depicted as the fold change between presence (+) and absence (-) of stroma.



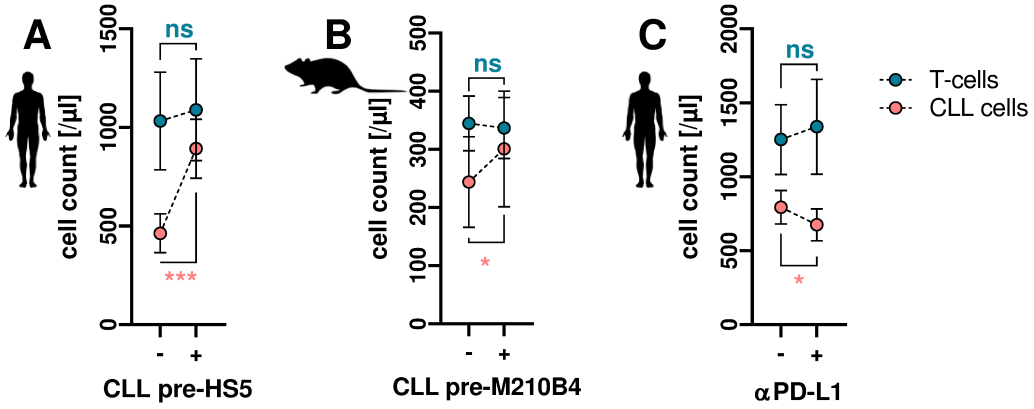
Supplemental Figure 6: Murine CLL cells isolated from the spleen of Eμ-TCL1 mice were cultured in absence (-, white) or presence (+, grey) of the murine BM stroma cell line M2-10B4 for 72h. Surface levels of PD-L1 on $CD19^+$ $CD5^+$ lymphocytes were determined by flow cytometry (n=5). Error bars represent the standard error mean. * p<0.05, ** p<0.01, *** p<0.001



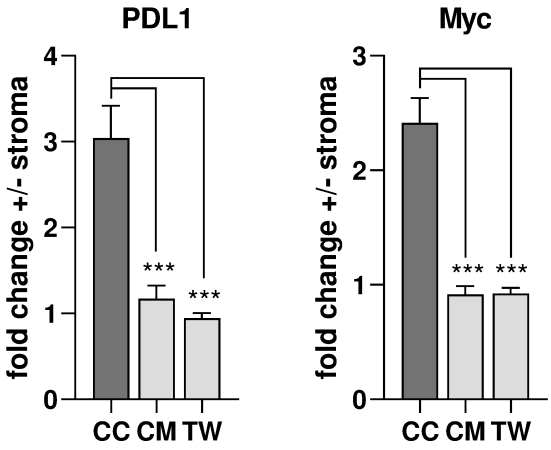
Supplemental Figure 7: Gene expression data from a public dataset (GSE21029) including samples from CLL BM and PB (n=26) were subjected to gene set enrichment analysis. Genes of the Hallmark_Myc_Targets_V1 and HALLMARK_MYC_TARGETS_V2 are depicted with the normalized enrichment score (NES) and the false discovery rate (FDR).



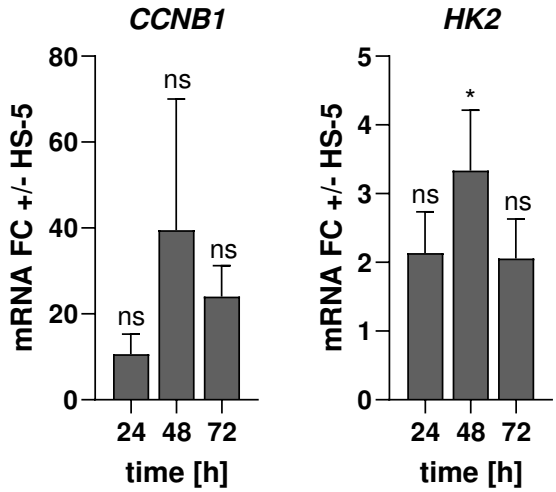
Supplemental Figure 8: Human CLL cells were analyzed for the pairwise correlation of *CD274* and *MYC* expression (shown as log2 RNAseq counts) depicting *IGHV*-mutated (M, red, n=110) and unmutated (U, green, n=94) CLL cases. CLL PBMCs were processed and sequenced as previously described[3]. NA: *IGHV* data not available



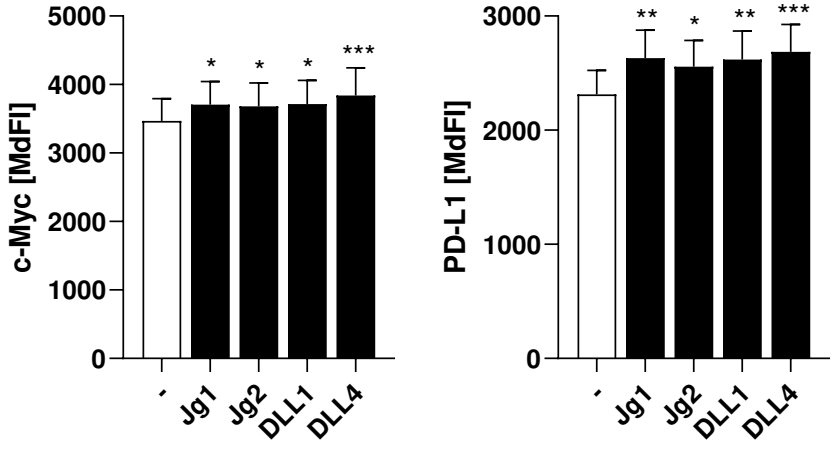
Supplemental Figure 9 (corresponding to Figure 2): Human (A, n=9) and murine (B, n=4) T-cells were co-cultured with CLL cells either untreated (-) or pre-educated by bone marrow stromal contact (+; HS-5 or M2-10B4 respectively). Samples were analyzed for CLL and T-cell count /µl by flow cytometry. In selected experiments (C, n=5) T-cells co-cultured with stroma-educated CLL-cells were additionally treated with a PD-L1 blocking antibody and corresponding isotype control as described in materials and methods. * p<0.05, *** p<0.001, ns not significant



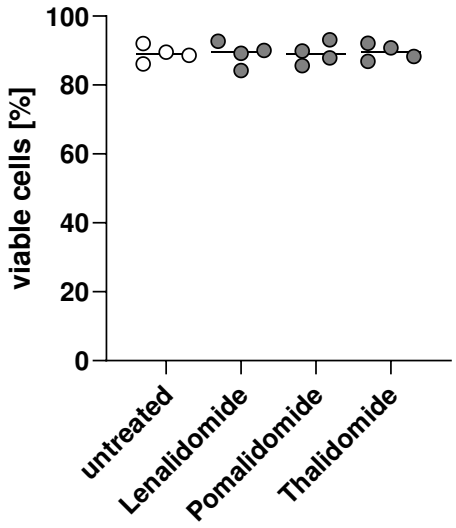
Supplemental Figure 10: CLL PBMCs were cultured for 3 days either alone, in presence of HS-5 cells (CC), with conditioned medium from HS-5 cells (CM) or together with HS-5 cells separated by a transwell (TW). Protein levels of surface PD-L1 (n=3-9) and intracellular Myc (n=3-9) were analyzed by flow cytometry and depicted as fold change of presence (+) and absence (-) of stroma. Error bars depict the standard error mean. ***p<0.001



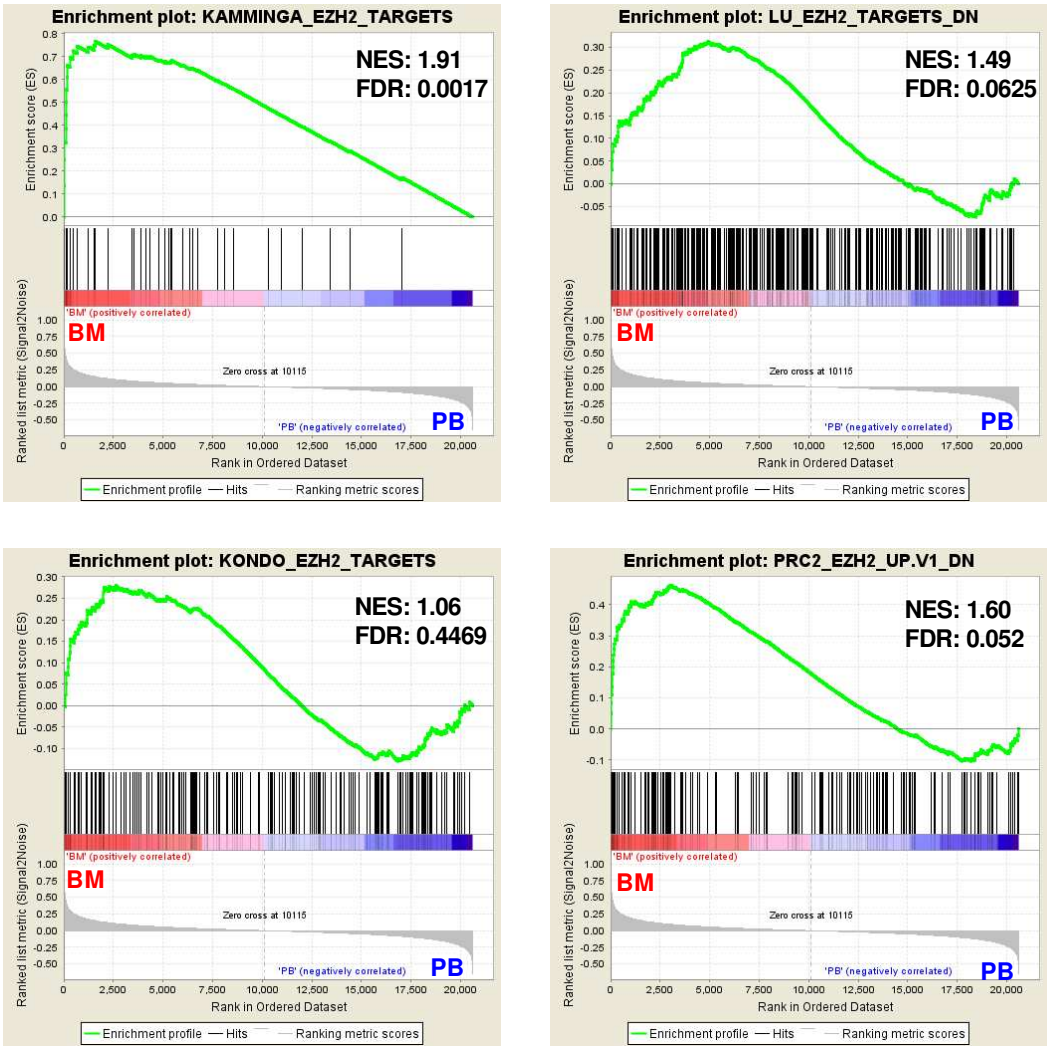
Supplemental Figure 11: Total RNA was extracted from CLL cells cultured in absence/presence of HS-5 stroma cells after indicated times and quantified for *CCNB1* (n=3) and *HK2* (n=6-9) gene expression by real-time PCR. Relative gene expression values are depicted as the fold change (FC) +/- stroma. Error bars represent the standard error mean. * p<0.05, ns: not significant



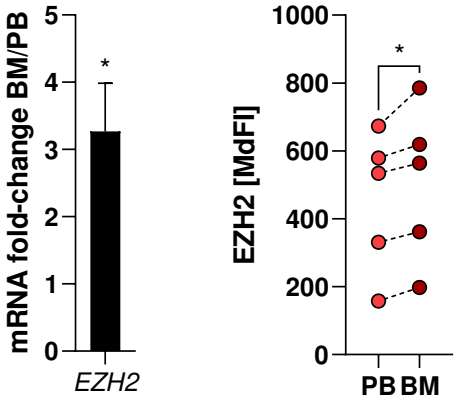
Supplemental Figure 12: Induction of c-Myc and PD-L1 by recombinant human Notch ligands. CLL-patient derived PBMCs were treated for 1-6 days with respective recombinant Notch ligands coated to a 48-well flat-bottom plate at a concentration of 10 µg/ml. Intracellular levels of c-Myc and surface expression of PD-L1 were determined by means of flow cytometry (n=6). Error bars represent the standard error mean. * p<0.05, ** p<0.01, *** p<0.001



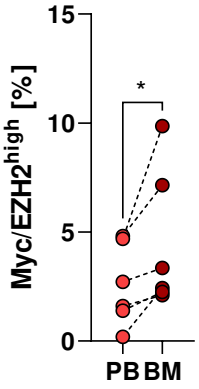
Supplemental Figure 13: HS-5 cells were culture for 24h in absence (untreated) or presence of 5 µM IMiDs (Lenalidomide, Pomalidomide, Thalidomide). Afterwards, the frequency of viable cells amongst all was assessed by flow cytometry using Zombie Aqua™ Fixable Viability Kit (Biolegend).



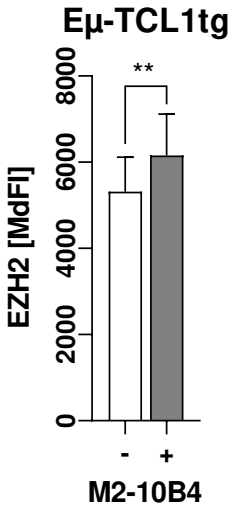
Supplemental Figure 14: Gene expression data from a public dataset (GSE21029[1]) including samples from CLL BM and PB (n=26) were subjected to gene set enrichment analysis[2]. Genes of the gene sets KAMMINGA-EZH2_TARGETS[4], LU_EZH2_TARGETS_DN[5], KONDO_EZH2_TARGETS[6], and PRC2_EZH2_UP.V1_DN[7] from the MSigDB[8] are depicted with the corresponding normalized enrichment score (NES) and the false discovery rate (FDR).



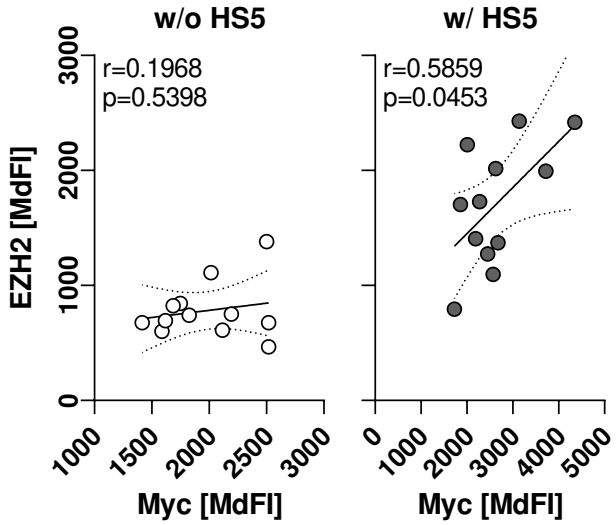
Supplemental Figure 15: Total RNA was prepared from CD19^{+} lymphocytes isolated from matched-pairs of human CLL PB and BM. Relative gene expression of *EZH2* was quantified by real-time PCR and is depicted as the fold-change between BM and PB (left, n=7). Intracellular levels of EZH2 were analyzed by flow cytometry in CD19^{+} lymphocytes in the same matched-pairs of PB and BM showing the median fluorescence intensity (MdFI). Error bars represent the standard error mean. * p<0.05



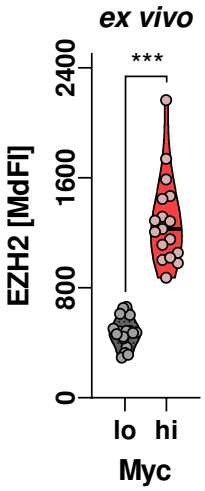
Supplemental Figure 16: The Myc/EZH2^{high} subpopulation was analyzed by flow cytometry in CD19^{+} CLL cells *ex vivo* in peripheral blood (PB) and bone marrow (BM) derived patient samples (n=6). * p<0.05



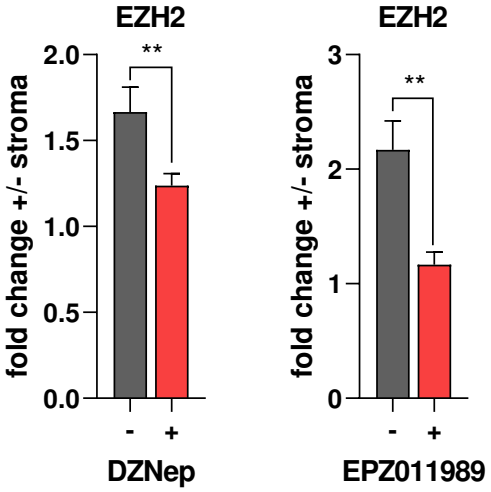
Supplemental Figure 17: Murine CLL cells isolated from the spleen of Eμ-TCL1 mice were cultured in absence (-, white) or presence (+, grey) of the murine BM stroma cell line M2-10B4 for 72h. Intracellular levels of EZH2 in $CD19^{+}$ $CD5^{+}$ lymphocytes were determined by flow cytometry (n=4). Error bars represent the standard error mean. * p<0.05, ** p<0.01, *** p<0.001



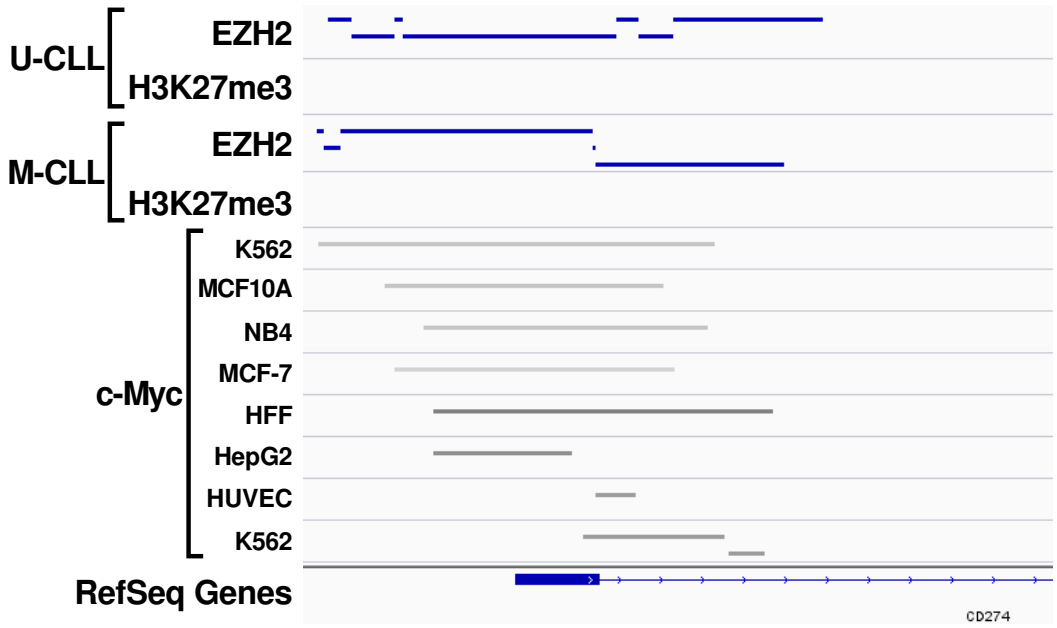
Supplemental Figure 18: Protein levels of EZH2 and Myc as determined by flow cytometry (based on the median fluorescence intensity [MdFI]) were subjected to a Pearson correlation analysis of CLL cells cultured in absence (w/o, white circles, n=12) or presence (w/, grey circles, n=12) HS-5 stroma cells for 3 days.



Supplemental Figure 19: CLL PBMCs were analyzed *ex vivo* for their Myc content in $CD19^{+}$ lymphocytes by flow cytometry. Samples were gated on the 15%-lowest (black) and 15%-highest (red) Myc expressing cells (as shown in Fig.2E). Within these gates, intracellular levels of EZH2 were semi-quantified based on the median fluorescence intensity (MdFI) by flow cytometry (n=17). *** p<0.001



Supplemental Figure 20: CLL PBMCs were cultured on HS-5 BM stroma for 3 days in absence (grey) or presence (red) of the EZH2 inhibitors DZNep (10 µM) and EPZ011989 (50 µM). Protein levels of intracellular EZH2 were analyzed by flow cytometry and are depicted as the fold change +/- stroma. Error bars show the standard error mean. ** p<0.01



Supplemental Figure 21: Chromatin immunoprecipitation (ChIP) data from a public dataset (GSE115772[9]) as well as the ENCODE database were analyzed in the integrative genome viewer (IGV) for occupancy of EZH2, tri-methylated lysine 27 at histone 3 (H3K27me3) and c-Myc at the promoter region of *CD274* (RefSeq Genes). Data is shown for *IGHV* unmutated CLL (U-CLL), *IGHV* mutated CLL (M-CLL), as well as different cancer cell lines (K562, MCF10A, NB4, MCF-7, HFF, HepG2, HUVEC).

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