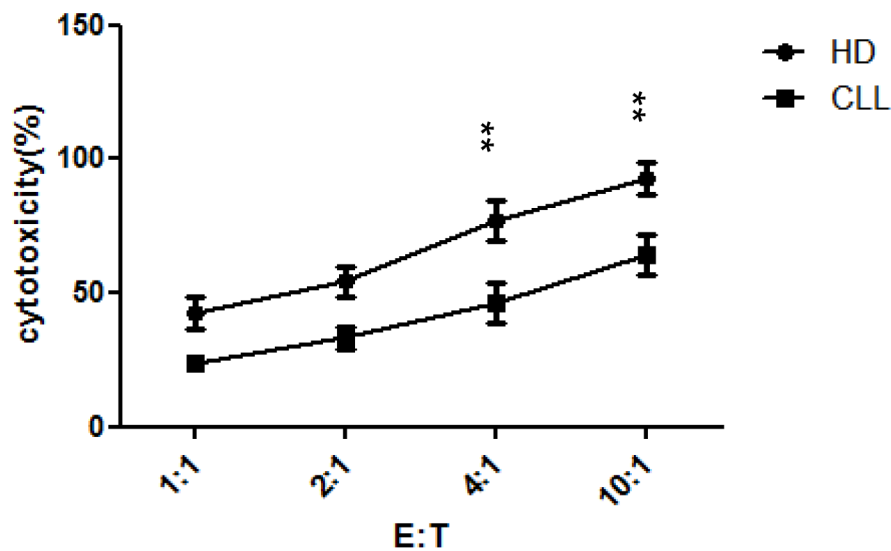
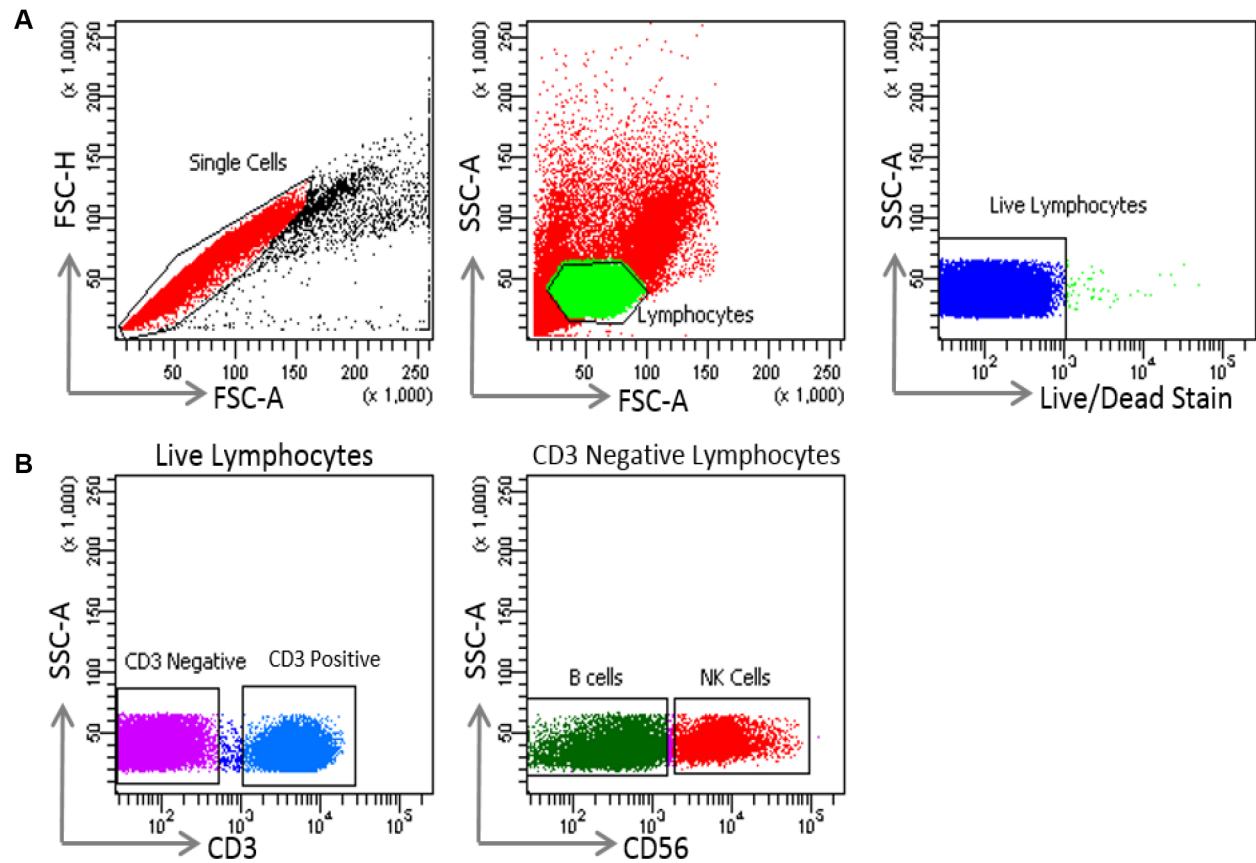


NK cell function is markedly impaired in patients with chronic lymphocytic leukaemia but is preserved in patients with small lymphocytic lymphoma

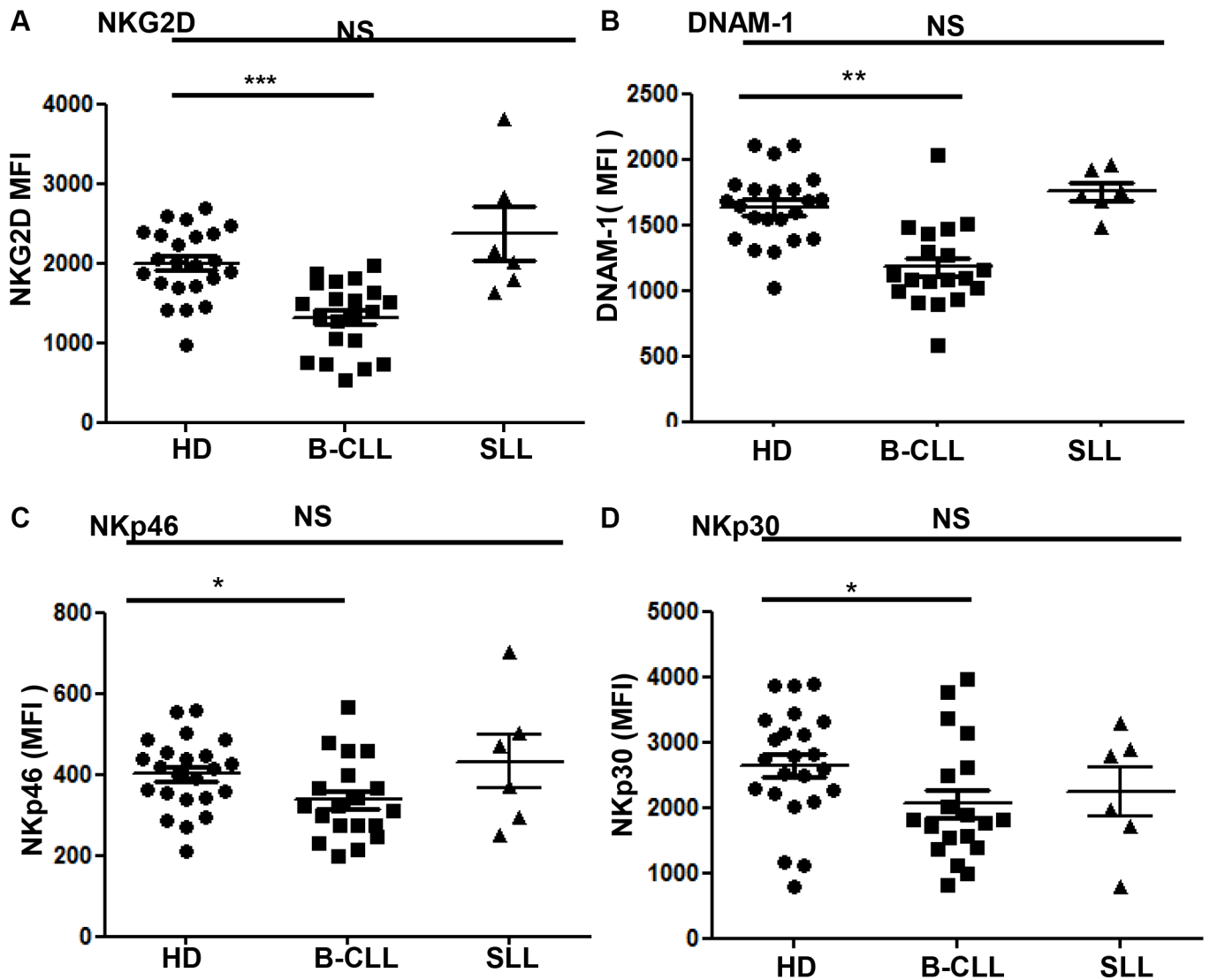
Supplementary Materials



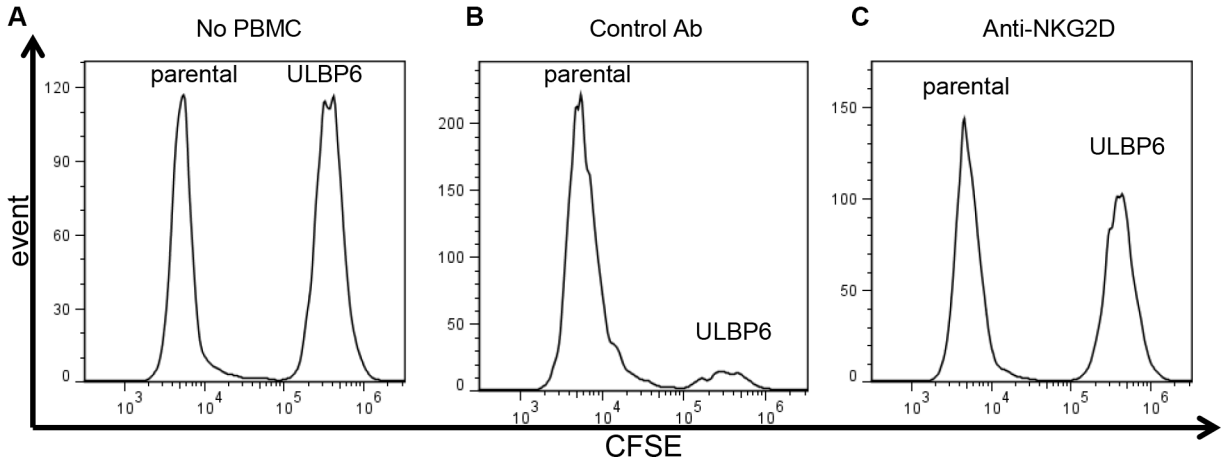
Supplementary Figure S1: The cytotoxicity to K562 cells is reduced by NK cells from patients with B-CLL. NK cells were enriched and the cytotoxicity to K562 cells were measured using DELFIA® EuTDA Cytotoxicity Reagents (AD0116 PerkinElmer Inc). Briefly, K562 cells were first loaded with an acetoxymethyl ester of BATDA before they were co-cultured with enriched NK cells from HD and B-CLL patients at a different effector/target(E/T) ratio for 4 hrs. TDA release after target cell lysis was detected by addition of Europium solution and measurement of the fluorescence signal. Specific cytotoxicity was calculated using the formula: % Specific release = (Experimental release (counts) – Spontaneous release (counts))/(Maximum release (counts) – Spontaneous release (counts)) × 100. Data are shown as mean values of the specific cytotoxicity from 3 HD and 3 patients with B-CLL. Error bars represent standard errors and significance was determined using two-way ANOVA, $p < 0.01$ (**).



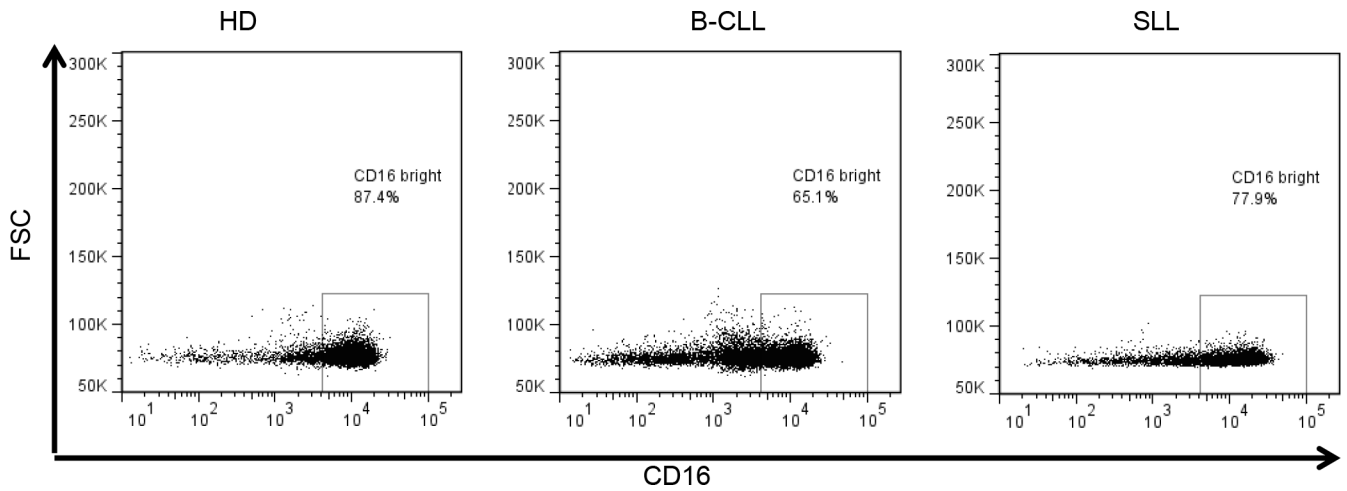
Supplementary Figure S2: Flow cytometry gating strategy. Single cells were firstly identified from whole PBMCs. Gated live lymphocytes were then used to identify NK cell populations as CD3 negative, CD56 positive cells.



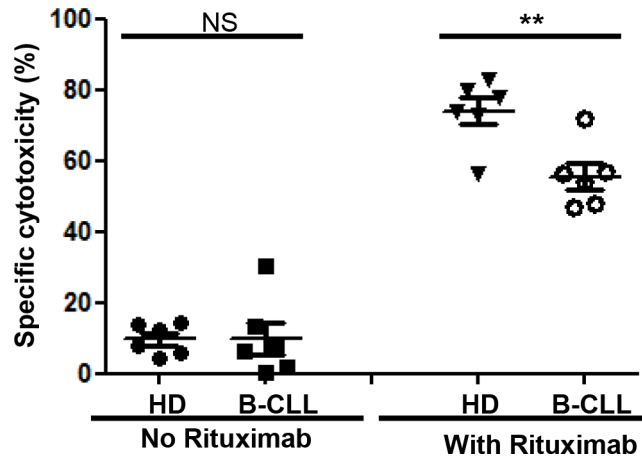
Supplementary Figure S3: NKG2D, DNAM-1, NKp46 and NKp30 receptors on NK cells are downregulated in patients with B-CLL but not patients with SLL. The NKG2D (A), DNAM-1 (B), NKp46 (C) and NKp30 (D) surface expression on NK populations from HD, patients with B-CLL and patients with SLL were studied using flow cytometry. Data shown are mean values for mean fluorescence intensity(MFI) of surface staining. Error bars represent standard errors and significance was determined using Mann-Whitney testing, $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***).



Supplementary Figure S4: The cytotoxicity to CHO-ULBP6 is NKG2D dependent. PBMC-based assay of specific cytotoxic responses to CHO cells expressing ULBP6 in a representative healthy donor, in the presence (C) or absence (B) of blocking anti-NKG2D antibody.



Supplementary Figure S5: Gating strategy for CD16^{bright}. NK cells identified from PBMCs using CD56 and CD3. Then CD16 bright population was gated according to CD16 staining pattern on the NK cell populations.



Supplementary Figure S6: Rituximab induced NK cytotoxicity assay using B cells from healthy donor as target. B cells from HD were enriched and incubated with NK cells from HD or patients with B-CLL, with or without Rituximab, for 16 hours. The specific lysis was calculated according to the relative cell counting using flow cytometry. Data shown are mean values of the specific cytotoxicity by NK cells, error bars represent standard errors and significance was determined using Mann-Whitney testing, $P < 0.01$ (**).

Phosphprotein(38)

| Gene symbol | description | CLL(Fold changes) | SLL(Fold changes |
|-------------|---|---------------------|-------------------|
| ADAM9 | metallopeptidase domain 9 | -1.624341 | -1.515804 |
| CD69 | CD69 molecule | -1.556424 | -1.670129 |
| ARHGAP31 | Cdc42 GTPase-activating protein | -4.258728 | -5.377674 |
| FOSL2 | FOS-like antigen 2 | -1.640036 | -1.59406 |
| GRASP | GRP1 (general receptor for phosphoinositides 1)-associated scaffold protein | -2.272311 | -1.874172 |
| NECAP1 | NECAP endocytosis associated 1 | 1.554797 | 1.67798 |
| SAR1A | SAR1 homolog A | 1.517245 | 1.619355 |
| SKIL | SKI-like oncogene | -2.27814 | -1.785319 |
| WWC2 | WW and C2 domain containing 2 | -1.949762 | -1.703699 |
| ANKRD28 | ankyrin repeat domain 28 | 2.301504 | 1.614136 |
| AUTS2 | autism susceptibility candidate 2 | -2.210972 | -2.062404 |
| BAZ1A | bromodomain adjacent to zinc finger domain, 1A | -1.821478 | -2.050731 |
| CREB1 | cAMP responsive element binding protein 1 | -1.839904 | -1.770372 |
| CREM | cAMP responsive element modulator | -1.665931 | -1.858836 |
| CASP8 | caspase 8, apoptosis-related cysteine peptidase | 1.525664 | 1.622755 |
| C1orf21 | chromosome 1 open reading frame 21 | -1.801542 | -1.661947 |
| C16orf54 | chromosome 16 open reading frame 54 | 2.001255 | 1.589108 |
| C5orf25 | chromosome 5 open reading frame 25 | -1.652645 | -1.661897 |
| DUSP16 | dual specificity phosphatase 16 | -1.827226 | -1.699478 |
| FAM177A1 | family with sequence similarity 177, member A1 | -1.951131 | -1.912894 |
| GLG1 | golgi apparatus protein 1 | -1.943558 | -1.555048 |
| HSF2 | heat shock transcription factor 2 | 1.661108 | 1.811061 |
| HIST1H3A | histone cluster 1 | -1.931873 | 1.70527 |
| HIST1H3B | histone cluster 1 | -1.693491 | 2.056228 |
| IGF1R | insulin-like growth factor 1 receptor | -1.685208 | -1.68879 |
| ITGA6 | integrin, alpha 6 | -3.467409 | -1.786681 |
| IRF2BP2 | interferon regulatory factor 2 binding protein 2 | -1.605661 | -1.750973 |
| MED14 | mediator complex subunit 14 | 1.569984 | -1.511179 |
| MYADM | myeloid-associated differentiation marker | 2.117809 | 1.849359 |
| NR1D1 | nuclear receptor subfamily 1, group D, member 1 | 1.617725 | 1.775971 |
| PLN | phospholamban | 1.812395 | 2.523499 |
| PHLDB2 | pleckstrin homology-like domain, family B, member 2; | -3.350928 | -2.411696 |
| KCTD10 | potassium channel tetramerisation domain containing 10 | -1.814611 | -1.826904 |
| SUDS3 | suppressor of defective silencing 3 homolog | 1.703513 | 1.630459 |
| TXNIP | thioredoxin interacting protein | 1.613654 | 2.011565 |
| VAV1 | vav 1 guanine nucleotide exchange factor | -1.69213 | -1.917351 |
| ZFP36 | zinc finger protein 36 | -1.717131 | 1.558329 |
| ZMYND11 | zinc finger, MYND domain containing 11 | -1.56747 | -1.578275 |

Intracellular signalling cascade(11)

| Gene symbol | description | CLL(Fold changes) | SLL(Fold changes) |
|-------------|---|---------------------|---------------------|
| ADAM9 | metallopeptidase domain 9 | -1.624341 | -1.515804 |
| ARHGAP31 | Cdc42 GTPase-activating protein | -4.258728 | -5.377674 |
| SAR1A | SAR1 homolog A | 1.517245 | 1.619355 |
| CREM | cAMP responsive element modulator | -1.665931 | -1.858836 |
| DUSP16 | dual specificity phosphatase 16 | -1.827226 | -1.699478 |
| GADD45B | growth arrest and DNA-damage-inducible, beta | -2.248157 | -1.937782 |
| IGF1R | insulin-like growth factor 1 receptor | -1.685208 | -1.68879 |
| MED14 | mediator complex subunit 14 | -1.569984 | -1.511179 |
| PHLDB2 | pleckstrin homology-like domain, family B, member 2 | -3.350928 | -2.411696 |
| VAV1 | vav 1 guanine nucleotide exchange factor | -1.69213 | -1.917351 |
| ZFP36 | zinc finger protein 36, C3H type | -1.717131 | 1.558329 |

Apoptosis(9)

| Gene symbol | description | CLL(Fold changes) | SLL(Fold changes) |
|-------------|---|---------------------|---------------------|
| ADAM9 | metallopeptidase domain 9 | -1.624341 | -1.515804 |
| NLRP3 | NLR family, pyrin domain containing 3 | -1.844568 | -1.658023 |
| CREB1 | cAMP responsive element binding protein 1 | -1.839904 | -1.770372 |
| CASP8 | caspase 8, apoptosis-related cysteine peptidase | 1.525664 | 1.622755 |
| CUL4A | cullin 4A | -2.061744 | -1.691255 |
| IGF1R | insulin-like growth factor 1 receptor | -1.685208 | -1.68879 |
| SERPINB9 | serpin peptidase inhibitor, clade B | 1.738767 | 1.936593 |
| TXNIP | thioredoxin interacting protein | 1.613654 | 2.011565 |
| VAV1 | vav 1 guanine nucleotide exchange factor | -1.69213 | -1.917351 |

Transcription regulation(15)

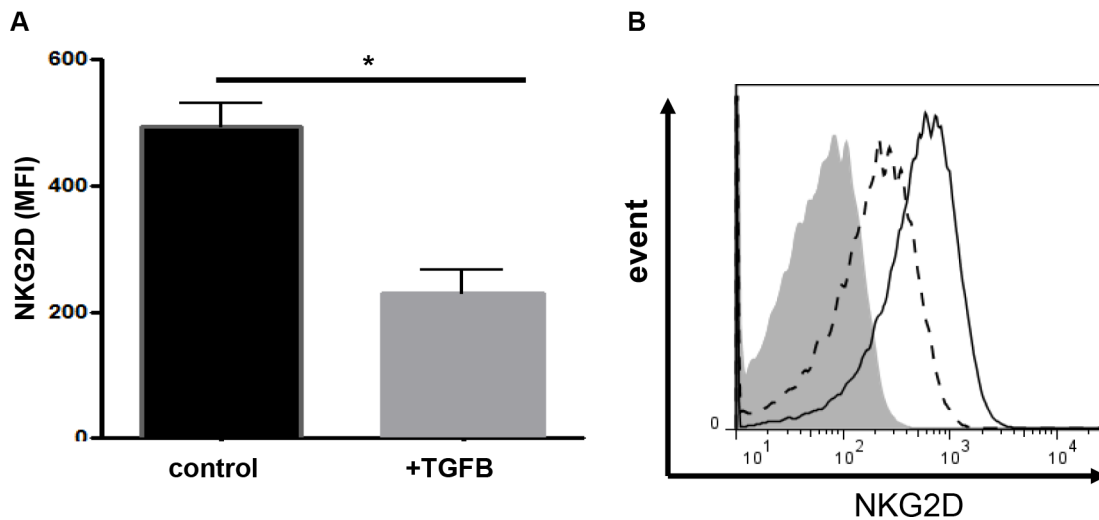
| Gene symbol | description | CLL(Fold changes) | SLL(Fold changes) |
|-------------|--|---------------------|---------------------|
| SKIL | SKI-like oncogene | -2.27814 | -1.785319 |
| AHR | aryl hydrocarbon receptor | 2.199513 | 2.401935 |
| BRMS1L | breast cancer metastasis-suppressor 1-like | -1.580083 | 1.658639 |
| BAZ1A | bromodomain adjacent to zinc finger domain, 1A | -1.821478 | -2.050731 |
| CREB1 | cAMP responsive element binding protein 1 | -1.839904 | -1.770372 |
| CREM | cAMP responsive element modulator | -1.665931 | -1.858836 |
| HSF2 | heat shock transcription factor 2 | 1.661108 | 1.811061 |
| IRF2BP2 | interferon regulatory factor 2 binding protein 2 | -1.605661 | -1.750973 |
| JMJD6 | jumonji domain containing 6 | -2.100287 | -1.62571 |
| MED14 | mediator complex subunit 14 | -1.569984 | -1.511179 |
| NR1D1 | nuclear receptor subfamily 1, group D, member 1 | 1.617725 | 1.775971 |
| SUDS3 | suppressor of defective silencing 3 homolog | 1.703513 | 1.630459 |
| TXNIP | thioredoxin interacting protein | 1.613654 | 2.011565 |
| ZNF710 | zinc finger protein 710 | -1.652275 | -2.033264 |
| ZMYND11 | zinc finger, MYND domain containing 11 | -1.56747 | -1.578275 |

others(47)

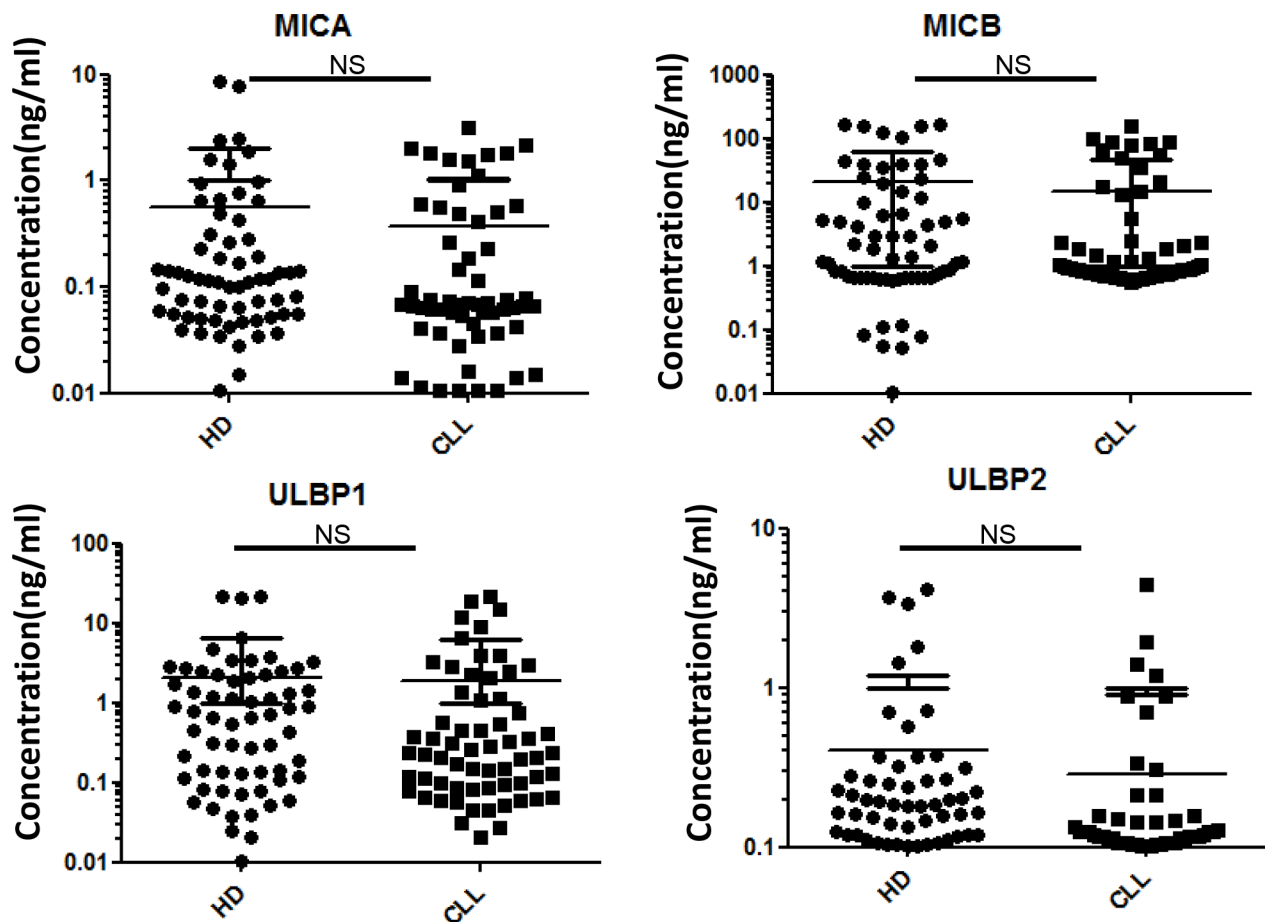
| Gene symbol | description | CLL(Fold changes) | SLL(Fold changes) |
|----------------------|--|---------------------|---------------------|
| ALOX5AP | Arachidonate 5-Lipoxygenase-Activating Protein | -1.735243 | -1.831961 |
| ANKH | Inorganic Pyrophosphate Transport Regulator | -2.077653 | -1.712332 |
| ARSD-AS1 | ARSD Antisense RNA 1 | -2.785523 | -1.63 |
| C10orf31 | CELF2 antisense RNA 1 | 2.423804 | 1.928385 |
| DAAM1 | Dishevelled Associated Activator Of Morphogenesis | -1.75864 | -1.645267 |
| DNAJB7 | DnaJ (Hsp40) Homolog, Subfamily B, Member 7 | 1.682713 | 1.575715 |
| DTHD1 | Death Domain Containing 1 | 1.710254 | 1.663715 |
| FAM105A | Family With Sequence Similarity 105, Member A | -1.853797 | -1.564916 |
| FAM105B | Family with sequence similarity 105, member B | -2.468972 | -2.330079 |
| GPR174 | Probable G-protein coupled receptor 174 | 2.164354 | 1.810403 |
| HSD17B12 | Estradiol 17-beta-dehydrogenase 12 | -1.690102 | -1.513342 |
| IER2 | immediate early response 2 | -1.56747 | -1.684103 |
| IGHD2-21 | immunoglobulin heavy diversity 2-21 | -1.79005 | 2.531513 |
| JAG1 | jagged 1 protein | -1.750184 | -2.061796 |
| LOC100131096 | TNRC6C antisense RNA 1 | 1.60214 | 1.547565 |
| LOC100506647 | uncharacterized LOC100506647 | 2.056228 | 1.536875 |
| LOC202181 | SUMO-interacting motifs containing 1 pseudogene | -1.566997 | -1.675314 |
| METRNL | meteorin, glial cell differentiation regulator-like | -1.564501 | -1.652475 |
| MGC57346 | ADP-ribosylation factor pseudogene | 1.660398 | 2.175902 |
| MIR1200 | microRNA 1200 | -3.606001 | -1.582421 |
| MIR4441 | microRNA 4441 | -2.639016 | 2.479415 |
| MIR643 | microRNA 643 | 1.881437 | 2.653125 |
| MRPL10 | mitochondrial ribosomal protein L10 | 1.50759 | 1.700504 |
| PLIN2 | perilipin 2 | 2.049041 | 1.630855 |
| PPP1R14BP3 | protein phosphatase 1, regulatory (inhibitor) subunit 14B pseudogene 3 | -1.662206 | -1.798865 |
| RN5S196 | RNA, 5S ribosomal pseudogene 196 | -1.670176 | 1.866066 |
| RN5S212 | RNA, 5S ribosomal pseudogene 212 | 1.984273 | 2.98566 |
| RN5S229 | RNA, 5S ribosomal pseudogene 229 | 1.632762 | 1.700264 |
| RN5S268 | RNA, 5S ribosomal pseudogene 268 | 2.769351 | 6.03132 |
| RN5S368 | RNA, 5S ribosomal pseudogene 368 | 1.518613 | 1.788121 |
| RN5S371 | RNA, 5S ribosomal pseudogene 3671 | -3.451426 | -3.753289 |
| RN5S394 | RNA, 5S ribosomal pseudogene 394 | 2.098807 | 2.010691 |
| RNU5E-1 | RNA, U5E small nuclear 1 | -1.591073 | 1.853176 |
| RNU7-45P | RNA, U7 small nuclear 45 pseudogene | 1.91246 | 1.653218 |
| RNY4P7 | RNA, Ro-associated Y4 pseudogene 7 | 1.52938 | 2.475524 |
| RSF1-IT2 | RSF1 intronic transcript 2 | 1.759194 | 1.59446 |
| SIK3-IT1 | SIK3 intronic transcript 1 | 1.517751 | 1.682244 |
| SLC25A30 | solute carrier family 25, member 30 | 1.958755 | 2.179846 |
| SNORA12 | small nucleolar RNA, H/ACA box 12 | 1.511508 | 2.532863 |
| SNORD62B // SNORD62A | small nucleolar RNA, C/D box 62A /B | 1.526259 | 1.526259 |
| SOAT1 | sterol O-acyltransferase 1 | 1.584228 | 1.822816 |
| TCP11L2 | t-complex 11, testis-specific-like 2 | -1.855092 | -2.161073 |
| TMEM14E | transmembrane protein 14E | 2.496681 | 2.715892 |
| UPF3AP1 | UPF3A pseudogene 1 | -1.530705 | -1.503339 |
| VTRNA1-3 | vault RNA 1-3 | 2.281941 | 4.980698 |
| WWC2-AS1 | WWC2 antisense RNA 1 | -1.814426 | -1.718231 |
| ZBTB20-AS2 | ZBTB20 antisense RNA 2 | -2.234574 | 1.635804 |

Supplementary Figure S7: The genes that have been significantly modified in both patients with B-CLL or SLL.

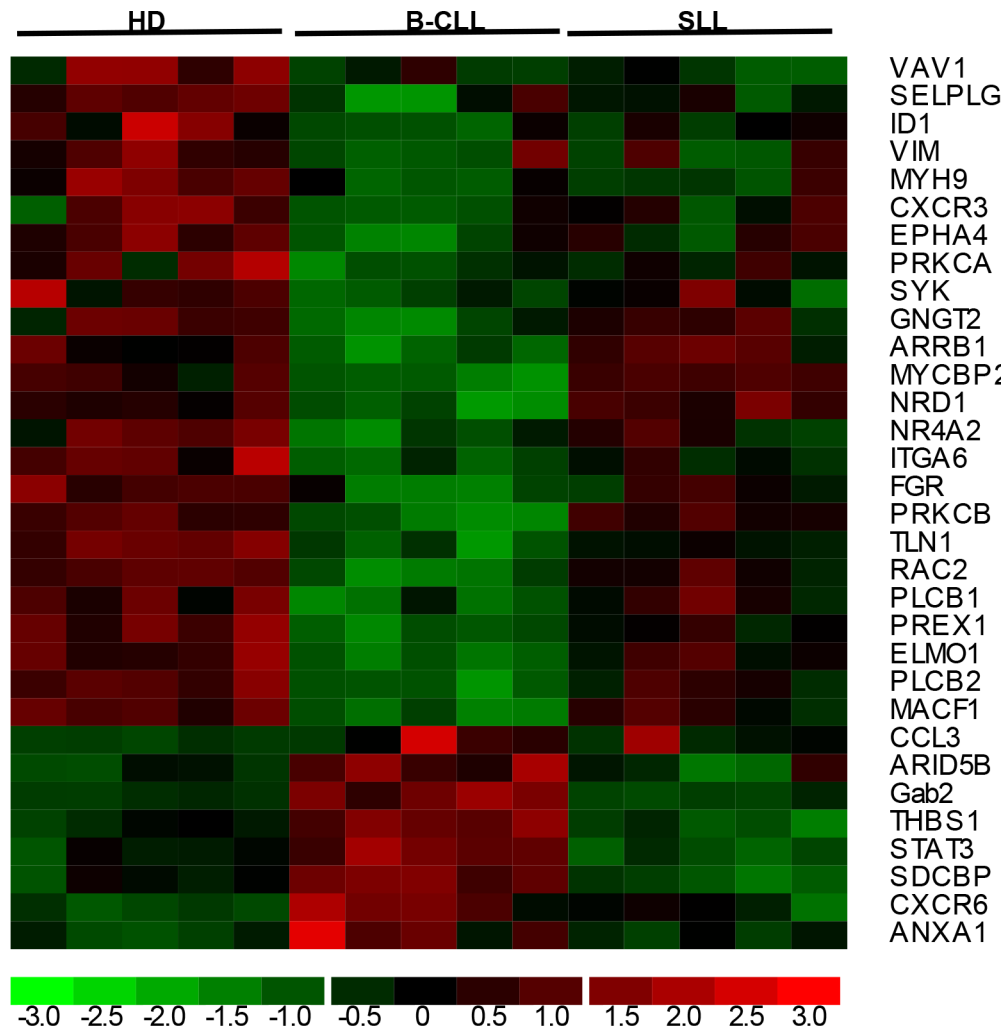
A list of the 93 genes that are upregulated or downregulated on microarray analysis of NK cells taken from patients with B-CLL or SLL patients compared with HD. This list includes 46 genes which are downregulated in both B-CLL and SLL patients, 36 genes which are upregulated in both B-CLL patients and SLL patients and 11 genes that are differentially regulated in B-CLL and SLL patients. They have been classified using DAVID Gene Functional Classification Tool (<https://david.ncifcrf.gov/home.jsp>). 38 genes have been identified to be phosphoproteins; 11 genes have been identified to be involved in an intracellular signaling cascade; 9 genes have been identified to be related to regulation of apoptosis and 15 genes have been identified to be related to transcription regulation.



Supplementary Figure S8 :TGFβ can downregulate the NKG2D expression of NK cells. (A) NK cells were incubated with TGFβ-1 for 48hrs and surface expression of NKG2D was assessed using flow cytometry. Data shown are mean values of MFI of NKG2D staining from 5 separate donors , error bars represent standard errors and significance was determined using Mann-Whitney tests, $P < 0.01(**)$. (B) An example histogram plot of NKG2D expression, solid black line represents no TGFβ-1 treatment, dashed black line represents with TGFβ-1 treatment and grey-filled histograms represent isotype control staining.



Supplementary Figure S9: The concentration of soluble NKG2D ligands in the serum of patients with B-CLL are not upregulated compared with HD. The serum from 64 healthy donors and 64 patients with B-CLL were tested for concentration of four ligands of NKG2D: MICA, MICB, ULBP1 and ULBP2. ELISA kits were obtained from R&D systems: DY1300, DY1599, DY1380 and DY1298.



Supplementary Figure S10: The relative intensity profiles of the migration related genes. The relative intensity profiles of 32 genes involved in cell migration, cell motion and chemokine signalling pathway that were significantly modulated in B-CLL patients group compared with HD. Each column represents one sample, while each row represents relative hybridization intensities of an individual gene. The colour on the heatmap indicates the magnitude of the relative expression of genes across the samples, with brighter red indicating higher expression and brighter green indicating lower expression.