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**Identification of uniquely expressed transcription factors in highly purified B-cell lymphoma samples**

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Keywords:	B-cell lymphomas, Cell sorting, Transcription factors, Gene expression



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Transcription factors defining subtypes of B-cell lymphomas

## Identification of uniquely expressed transcription factors in highly purified B-cell lymphoma samples

Running title: **Transcription factors defining subtypes of B-cell lymphomas**

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**ABSTRACT**

Transcription factors (TFs) are critical for B-cell differentiation, affecting gene expression both by repression and transcriptional activation. Still, this information is not used for classification of B-cell lymphomas (BCLs). Traditionally, BCLs are diagnosed based on a phenotypic resemblance to normal B-cells; assessed by immunohistochemistry or flow cytometry, by using a handful of phenotypic markers. In the last decade diagnostic and prognostic evaluation has been facilitated by global gene expression profiling (GEP), providing a new powerful means for the classification, prediction of survival and response to treatment of lymphomas. However, most GEP studies have typically been performed on whole tissue samples, containing varying degrees of tumor cell content, which results in uncertainties in data analysis. In this study global GEP analyses were performed on highly purified, flow-cytometry sorted tumor-cells from eight subgroups of BCLs. This enabled identification of TFs that can be uniquely associated to the tumor cells of chronic lymphocytic leukemia (CLL), diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL), hairy cell leukemia (HCL) and mantle cell lymphoma (MCL). The identified transcription factors influence both the global and specific gene expression of the BCLs and have possible implications for diagnosis and treatment.

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## INTRODUCTION

B-cell lymphomas (BCLs) constitute a diverse set of tumors, both morphologically and clinically, that are mainly classified in relation to normal B-cell differentiation[1]. However, it is now clear that some lymphoid malignancies, for example hairy cell leukemia (HCL), cannot easily be traced to a specific normal B-cell counterpart or differentiation stage, why much more detailed molecular analysis is called for [2]. Gene expression profiling has lately proven to be a powerful tool in the understanding of BCL biology and it has, for instance been used to demonstrate how diffuse large B-cell lymphoma (DLBCL) can be subgrouped based on unique gene expression profiles [3], which also can be associated to prognosis [4]. In both DLBCL and mantle cell lymphoma (MCL), prognostic signatures have been defined by the gene expression of the lymphoma cells [3, 5]. This is in contrast to FL where the aggressiveness of the disease is mainly determined by the cellular microenvironment [6-8], composed of both immunocytes and stromal cells. Thus, for most lymphoma entities the utilization of pure cell populations would provide an unmet need to identify specific genes related to each subtype of tumor cells and to trace their cellular origin.

Similar to immunophenotyping and gene expression profiling, it has recently been shown that patterns of microRNA expression define different stages of B-cell differentiation and also that these differences can be used to classify BCLs [9]. In contrast to most microRNAs, which mainly work through repression of genes, transcription factors (TFs) regulates gene expression both by repression and transcriptional activation of genes. Several of these genes, such as PAX5 [10], BCL6 [11], IRF-4 [12], BLIMP-1[13] and XBP1 [14], are critical in B-cell differentiation. However, the TF SOX11, which is not involved in normal B-cell differentiation, has recently

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2  
3 been identified as a strong diagnostic marker for MCL [15] highlighting the importance of  
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5 analyzing not only B-cell-associated markers in the assessment of lymphoma gene regulation.  
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8 Since TFs are important players in normal B-cell differentiation but also in disease  
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10 progression [12] we focused our analysis specifically on the regulation of those, to potentially  
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12 identify new functional, diagnostic and therapeutic targets. In the present study, highly purified,  
13  
14 flow-cytometry sorted, B-cells from eight different BCL entities (follicular lymphoma (FL),  
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16 transformed DLBCL (DLBCL-tr), DLBCL with a germinal center (GC) phenotype (DLBCL-  
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18 GC), DLBCL with a non-GC phenotype (DLBCL-non-GC), MCL, chronic lymphocytic  
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20 lymphoma (CLL), leukemic version of MCL (L-MCL), HCL and four subpoulations derived  
21  
22 from non-malignant tonsils (naïve B-cells (N), centrocytes (CC), centroblasts (CB), memory B-  
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24 cells (M)) were analyzed, using global gene expression analysis. By using purified tumor cells we  
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26 were able to identify unique TFs for the different diagnostic B-cell lymphoma subgroups. These  
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28 TFs are not only potential new molecular targets, but partly also influence the difference in global  
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30 and specific gene expression observed between B-cell lymphomas.  
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## MATERIAL AND METHODS

### Collection and isolation of tumor cells

The study was conducted according to the Helsinki declaration and with approval of the Uppsala University Ethics Committee (approval 01-399). All specimens in this study were diagnosed according to the World Health Organization (WHO) criteria [16] including; FL, n = 12; DLBCL-tr, n = 6; DLBCL-GC, n = 7; DLBCL-non-GC, n = 10; MCL, n=12; CLL, n=15; L-MCL, n=6 and HCL, n=10. Of note, some samples (FL, n = 12; DLBCL-tr, n = 6) have been analyzed previously in a study where the transformation from FL to DLBCL-tr was examined [17]. Freshly frozen tumor cell suspensions were collected from the biobank at the Department of Pathology, Uppsala University Hospital (Uppsala, Sweden) (FL, DLBCL, MCL, CLL, HCL) and from the biobank at Rigshospitalet (Copenhagen, Denmark) (L-MCL). The subgrouping of DLBCL into GC and non-GC subgroups was based on immunohistochemistry for CD10, BCL6 and IRF-4 according to previous publications [18, 19]. After diagnosis, the cells were sorted, using a FACS Aria (Becton Dickinson, Franklin Lakes, New Jersey, USA), based on their expression of CD19, kappa ( $\kappa$ ) light chain or lambda ( $\lambda$ ) light chain and lack of CD3, using antibodies towards these antigens, CD19-PB (Dako, Glostrup, Denmark),  $\kappa$ -APC (Becton Dickinson),  $\lambda$ -PE (Dako) and CD3-FITC (Becton Dickinson). This enabled the identification of a BCL population based on identical light-chain clonality. The fraction of tumor cells in the samples prior to sorting varied (see Results section) but all samples were generally purified to >97% as assessed by FACS analysis. The isolated cells were then lysed in Trizol (Invitrogen Corporation, California, USA), as previously described [20]. The patient material details are shown in Table I.

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### Collection and isolation of non-malignant tonsil B-cells

The non-malignant B-cell populations (N, CC, CB, M) were derived from three fresh pediatric tonsils specimens obtained from Lund University Hospital (Lund, Sweden) and purified as previously described [20]. The cells were stained in a stepwise procedure: 1) mouse anti-human-IgM (Becton Dickinson), 2) goat anti-mouse-PE-Texas-Red (Invitrogen), 3) goat anti-human-IgG-biotin (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA, USA), goat anti-human-IgA-biotin (Invitrogen), 4) Streptavidin-APC (Becton Dickinson), mouse anti-human-CD27-FITC (Becton Dickinson), rabbit anti-human-IgD-PE (Dako), mouse anti-human-CD38-PerCPCy5.5 (Becton Dickinson), mouse anti-human-CD5-PECy7 (Becton Dickinson) and mouse anti-human-CD19-APCCy7 (Becton Dickinson) and sorted, using a FACSVantage Diva (Becton Dickinson) to a purity of >95%. Notably, plasmablasts and plasmacells were excluded from the analysis by separating cells with very high CD38 expression (plasmablast and plasmacells) from cells with high CD38 expression (CC, CB). The freshly isolated cells were all lysed in Trizol (Invitrogen). Details of which markers that were used to separate the different B-cell populations are shown in Table II.

### Isolation of mRNA and sample preparation

Briefly, total RNA was isolated from sorted cells (malignant and non-malignant) lysed in Trizol (Invitrogen), and the RNA quality was controlled. The RNA was then subjected to *in vitro* transcription, according to the Two-cycle Eukaryotic Targeting Labeling assay protocol (Affymetrix, Inc., Santa Clara, CA, USA). The labeled cRNA was hybridized to the Human Genome U133 Plus 2.0 arrays containing ~ 50,000 transcripts (Affymetrix). This complete procedure is described in detail previously [17, 20]. All arrays included in the study passed the

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2  
3 initial quality control, including comparison of scaling factors, background, noise and GAPDH  
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5 expression with threshold values defined by Affymetrix.  
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## 8 9 **Gene expression analyses**

10 For each probe set, the expression level is given as a signal value in the GeneChip Operating  
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12 Software (GCOS) 1.4 (Affymetrix). The signals on each array were scaled to an average target  
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14 value of 100 (MAS5). These normalized signal values were imported into Gene Spring GX 10  
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16 (Agilent) and logarithmic transformed.  
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21 **Identification of present transcripts.** All transcripts on the array (referred to as “Array total  
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23 transcripts”) were filtered to have a present call (as defined by the algorithm in GCOS 1.4) in all  
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25 three samples in at least one of the four non-malignant B-cell populations N, CC, CB and M. This  
26  
27 resulted in 23019 transcripts, from now on referred to as “B-cell present transcripts”. Furthermore  
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29 the “Array total transcripts” were filtered to be present in at least two of 78 BCL samples  
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31 resulting in 39555 transcripts referred to as “BCL present transcripts”. These lists of transcripts  
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33 were further analyzed as described below.  
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38 **Identification of transcription factors.** In addition the ENSEMBL database (2008-05-30)  
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40 was filtered for all genes identifications (ID) annotated as “transcription factor activity” resulting  
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42 in 1859 genes. These genes corresponded to 2597 transcripts available on Affymetrix Human  
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44 Genome Plus 2.0 arrays since some of the TF genes were represented by more than one  
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46 transcript. To this list we added three Affymetrix transcripts encoding Sox11 resulting in a total  
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48 of 2600 transcripts encoding TFs (referred to as “Array total TF transcripts”). These transcripts  
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50 were further filtered to be present in at least two of the 78 BCL samples and 1886 transcripts  
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52 hereafter referred to as “BCL present TFs transcripts”, passed this filter and were further  
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54 analyzed. To characterize the genes corresponding to the identified transcripts, annotation in  
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3 Affymetrix were used. An overview of the different analyzes performed in this study is shown in  
4  
5 Figure 1.  
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8 **ANOVA.** To identify transcripts with a unique expression profile in the different BCL  
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10 entities, the “BCL present transcripts” as well as the “BCL present TF transcripts” were analyzed  
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12 using one-way ANOVA test (F-test) followed by Tukey’s Honestly Significant Difference (HSD)  
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14 post hoc test, with the false discovery rate (FDR) controlled by the Benjamini and Hochberg  
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16 method [21] ( $q < 0.05$ ). Intersections of transcripts significantly separating one group from all  
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18 other groups were identified. However, for some entities it was not possible to find transcripts  
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20 that differed significantly thus these entities (DLBCL and MCL subgroups, respectively) were  
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22 grouped together. The analysis was performed in Gene Spring (Agilent).  
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27 **Unsupervised subgroup identification.** Variance filtration was applied on the “B-cell  
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29 present transcript” to explore unsupervised clustering of different entities of BCLs. The filtration  
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31 process was terminated as soon as one group, defined as having all or all but one of the nearest  
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33 neighbors within the same group, was formed and the group was removed. Filtration was then  
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35 performed again for the remaining samples. Since variance filtration depends on the samples  
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37 present in the analysis, all “B-cell present transcripts” were used for all filtrations. This procedure  
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39 was iterated until no more groups could be separated. The analysis was performed using Qlucore  
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41 Omics Explorer 2.0 (Qlucore AB, Lund, Sweden).  
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46 **Interaction analysis.** To analyze for interactions between identified TFs and potential down-  
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48 stream genes, the lists of uniquely expressed TFs and genes associated with each biological  
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50 group, as determined by unsupervised clustering, were imported to the Ingenuity Pathway  
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52 Analysis tool (Ingenuity® Systems, Mountain view, CA, USA [www.ingenuity.com](http://www.ingenuity.com)) and a  
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network analysis performed. Interactions defined by IPA as expression, transcription and activation were further investigated in the literature to identify specific interactions.

**Supervised identification of differentially expressed transcripts.** “B-cell present transcripts” and “Array total TF transcripts” were filtered to distinguish the three non-malignant B-cell groups (N, GC B-cells (including CB and CC) and M), using multi group comparison (F-test), controlling for false discovery rate by the Benjamini and Hochberg method [21] ( $p < 0.003$ ,  $q < 0.01$  respectively  $p < 0.003$ ,  $q < 0.05$ ) The resulting lists, “B-cell F-test transcripts” and “B-cell F-test TF transcripts” contained 5886 and 479 transcripts, respectively. To validate these analyses, permutation tests, where the subgroup-labels were randomly assigned, was performed repetitively and resulted in insignificant number of genes confirming the statistic significance of the above identified transcripts. The analysis was performed using Qlucore Omics Explorer 2.0 (Qlucore AB, Lund, Sweden).

**Correlation analysis.** To enable comparison between samples in the non-malignant group and samples in the malignant group, all samples were normalized to the mean using the elimination factor algorithm in Qlucore Omics Explorer 2.0 (Qlucore AB).

Two different methods were used to analyze the non-malignant B-cells and the BCL entities ( $n=90$ ) by supervised analyses; (i) The different populations were visualized by a PCA and each sample was associated to its nearest neighbor (based on Euclidian distance) using both “B-cell F-test transcripts” and “B-cell F-test TF transcripts”, (ii) The Pearson correlation between each entity of malignant and non-malignant B-cells was calculated based on the “B-cell F-test transcripts”.

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## RESULTS

### Analysis of purified B-cell lymphomas

The proportion of tumor cells in each complex BCL sample varied; CLL (69% to 97%), DLBCL-GC (20% to 80%), DLBCL-nonGC (20% to 90%), DLBCL-tr (60% to 80%), FL (35% to 80%), HCL (61% to 97%), L-MCL (63% to 93%), MCL (59% to 97%), clearly reflecting the need for enrichment. By using flow-cytometry based cell-sorting, the clonal B-cell population was increased from a heterogeneous 20-97% to a homogenous >97%, allowing a quality assured interpretation of gene expression data relevant for the tumor cells. This procedure dramatically improved the analytical resolution in this study and enabled identification of gene expression differences in tumor cells.

### B-cell lymphoma entities express unique sets of transcription factors.

Since TFs orchestrate a large part of all cellular processes and functions, we hypothesized that these factors can likely be used to distinguish a unique biology associated with each BCL entity. Consequently, we sought to specifically identify TFs that can be uniquely linked to different BCLs. We generated a list of 2600 transcripts corresponding to known TFs based on annotations from the ENSEMBL database, which we designated “Array total TF transcripts”. To identify TFs with a unique expression profile in each of the BCL entities, an F-test followed by Tukey HSD post hoc test ( $q = 0.05$ ) was performed and transcripts significantly deregulated between CLL, DLBCL (including DLBCL-GC, DLBCL-nonGC and DLBCL-tr samples), FL, HCL and MCL (including MCL and L-MCL samples) could be identified.

The number of unique TF transcripts, and corresponding characterized genes, varied between entities, such as FL ( $n = 10$ , corresponding to 7 genes), DLBCL ( $n = 11$ , corresponding to 10

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genes), MCL (n = 19, corresponding to 10 genes), CLL (n = 33, corresponding to 21 genes) and HCL (n = 169, corresponding to 82 genes) (Table III and Figure 2a- e). This analysis revealed that HCL displays the largest number of distinct transcripts, clearly distinguishing it from the other BCL entities. MCL could not be separated from L-MCL, nor could the different DLBCL subgroups be distinguished based on TF expression profiles.

The relative mRNA expression of all unique TF transcripts in BCLs and non-malignant B-cell populations are shown in Figure 2. Of note, most of the transcripts, identified to be deregulated in BCLs, were not shown to be differentially regulated during normal B-cell differentiation. Among these TFs (Figure 2, Table III and Table SI), tumor suppressor genes or TFs regulating tumor suppressors were identified for all of the entities including expression of *FOXO1* [22] in FL cells, *PA2G4* [23] in DLBCL cells, *SOX11* [24] in MCL cells and *PRDM2* [25] in CLL. Furthermore, *SOX4* and *EGRI*, known, depending on tumor type, to act both as tumor suppressors [26-29] and oncogenes [30, 31] showed lower expression in CLL cells. Finally, HCL cells had a high expression of *SOX4*, and also a low expression of *FOXPI* [32]. These results suggest a functional role for the TFs related to the malignant transformation. Of note, *IRF-4*, expressed in high levels in DLBCL-nonGC [33] was for the first time demonstrated to be active in MCL. Furthermore, HCL expressed a high number (n = 169) of unique TF transcripts, where *MAF* [34] may direct HCL towards a myeloid cell differentiation [35, 36].

### **Unsupervised clustering of B-cell lymphomas and comparison to diagnostic subgroups.**

To verify that the diagnostic subgroups are biologically distinct when analyzing purified tumor cells, an unsupervised clustering was performed. Our results demonstrated that the majority of samples clearly associated with their diagnostic subgroup (Figure 3). Remarkably, we observed

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3 11233 transcripts that clearly separated group A, including all 10 HCL samples (Figure 3a), and  
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5 as in the TF-analysis, HCL demonstrated a distinct transcriptional profile. After excluding group  
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7 A, a new filtration resulted in 253 transcripts that separated the data set into two distinct groups  
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9 (Figure 3b). Group B1 included all 15 CLL samples, but also 1/12 FL and 1/6 L-MCL sample  
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11 and group B2 included all 12 MCL samples and 5/6 L-MCL samples the remaining samples  
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13 constitute 11/12 FL samples, all 7 DLBCL-GC samples, all 10 DLBCL-nonGC samples, and all 6  
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15 DLBCL-tr samples that could not be further separated. The unsupervised analysis showed that  
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17 HCL, CLL, MCL and DLBCL/FL are recognized as biological groups and that DLBCL and FL  
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19 cannot be separated.  
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24 The unsupervised clustering indicates that differences in expression of TFs, influence the  
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26 global transcription profile, since the number of uniquely expressed TFs correlates to the total  
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28 number of genes used to separate each B cell lymphoma subgroup. This association is further  
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30 supported by the finding that genes down-stream of the unique TFs were identified in the same  
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32 BCL subgroup. These associations were based on an analysis where unique TFs were compared  
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34 with genes associated with the specific subtypes in the unsupervised analysis, as assessed by  
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36 identifying relations using IPA (Ingenuity® Systems). For example, in HCL (i) *PPAR $\gamma$*  are  
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38 involved in the regulation of *CAR2* [37] and *KLF4* [38], (ii) *SIX3* is shown to increase the  
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40 expression of *CCND1* [39] and (iii) *TBX21* is known to increase the expression of *CXCR3* [40].  
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42 In CLL genes verified to affect *CCND1* are *LEF1*, showing a high expression, and *E2F3*,  
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44 showing a low expression [41, 42]. However, more than 11 unique TF transcripts, were needed to  
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46 affect the global gene expression as demonstrated by the inability to separate FL and DLBCL.  
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### **Supervised clustering using B-cell associated transcripts**

FL and DLBCL are clinically identified as different diseases [16], despite this, the unsupervised clustering could not separate them. Therefore we explored the separation of all BCL entities based on B-cell associated genes, which forms the basis of the current diagnostic strategy. These BCL entities were analyzed using 5886 transcripts (“B-cell F-test transcripts”) significantly separating the B-cell populations (N, GC B-cells and M) ( $q < 0.01$ ).

To ensure the biological relevance when analyzing the relation between different malignant and non-malignant B-cell populations, two different statistical methods were used, (i) PCA in combination with nearest neighbor (Figure 4) and (ii) Pearson correlation analysis (Figure 5). Notable, in BCLs, the gene expression of the identified “B-cell F-test transcripts” are within the same range as normal B-cells, as illustrated by PCA plots (Figure 4a-d). This is of interest, since it was expected that BCLs would display a more extreme expression of genes involved in for example proliferation. In addition, memory B-cells and naïve B-cells are known to have different functions, however, the strong correlation between them suggest them to be transcriptionally similar (Figure 4 and Figure 5).

By using the “B-cell F-test transcripts”, we were able to separate the three different subgroups of DLBCL, as well as FLs (Figure 4 and Figure 5). Although, pure lymphoma cells were analyzed and, thus the intratumoral heterogeneity was significantly reduced, a profound heterogeneity in the different DLBCL-tumor specimens was still observed. This heterogeneity is likely due to translocations and random mutations in genes other than immunoglobulin genes [43]. However, the DLBCL-tr and DLBCL-GC showed more similar gene expression compared to DLBCL-nonGC and correlated closely (Figure 4a-4b and Figure 5a-5b). Notably, DLBCL-tr and DLBCL-GC evolve through different mechanisms but some DLBCL-GC may have passed

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3 through an undiagnosed primary FL and may thus be misdiagnosed. These two subgroups also  
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5 demonstrate a closer relation to GC B-cells, their suggested precursor cell, than to the resting B-  
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7 cells (M and N), as previously reported [44, 52].  
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10 CLL and HCL, as well as L-MCL and MCL show a closer association to the resting B-cell  
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12 populations (M and N), especially to memory B-cells, than to the proliferating GC B-cells  
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14 (Figure 4a). However, separation of L-MCL and MCL were difficult also using these B-cell  
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16 associated genes (Figure 4a, Figure 4d), underlining the clinical paradigm that these entities  
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18 should be considered as a single disease [16]. In addition, we demonstrated that CLL could not be  
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20 separated based on IGHV mutational status. This result is in agreement with previous studies  
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22 identifying only a limited number of genes to be differentially regulated between IGHV mutated  
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24 and un-mutated CLL [45, 46].  
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**DISCUSSION**

As a B-cell undergoes malignant transformation, distinct phenotypic characteristics, also associated with normal B-cell differentiation, are often conserved [47]. Consequently, the classification of BCLs has historically been associated with the cell of origin [16]. Therefore, previous studies have analyzed lymphomas according to origin to understand the functional and biological context of each specific lymphoma entity [3, 48-52]. However, it is clear that many differences related to the diverse clinical outcome among different B-cell lymphomas cannot be related to B-cell associated features but must be explained by specific features related to each unique lymphoma subgroup.

Historically, most microarray studies have assessed the transcriptome from whole tissue samples, comprising various tumor cells, non-malignant infiltrating cells and stromal cells. In the case of FL it has been shown that the overall survival is to a great extent determined by the interplay between tumor cells and the surrounding bystander cells [6-8]. In the case of MCL and DLBCL, on the other hand, the clinical outcome is mainly determined by the tumor cell alone [3, 5]. Thus for most analyses, as when analyzing BCLs for tumor cell-associated antigens and when correlating BCL cells to their normal counterparts, it is of major importance that the mRNA analyzed are from homogenous cell populations. In the present study, we analyzed a set of eight flow-cytometry sorted populations of BCLs (CLL, DLBCL-GC, DLBCL-tr, DLBCL-nonGC, FL, HCL, L-MCL and MCL) and four populations of non-malignant tonsil B-cells from different pre-defined stages of differentiation (N, CB, CC, M). Thus, as homogenous cell populations were used, results can be related to difference in cellular gene expression in contrast to variation in tumor tissue content.

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3 TFs have not been a central focus of drug development, but recent advances suggest them to  
4 be worthwhile targets for cancer therapy [12, 53, 54]. In this study TFs, not necessarily linked to  
5 B-cell differentiation but to distinct biological features of each BCL subgroup and potentially  
6 responsible for the malignant transformation were identified. Most of these identified TFs did not  
7 show any differential regulation in normal B-cell differentiation, probably due to the fact that  
8 genes separating non-malignant B-cells in many cases are shared between more than one B-cell  
9 lymphoma subtype. Furthermore, TFs with direct or indirect tumor suppressor function were  
10 found in each BCL subgroup as well as other individual genes, explaining functional features  
11 previously reported to be associated with the specific subgroup. Amongst others, HCL, shown to  
12 be a distinct group in all analyses performed, has, for example previously been shown to occupy  
13 phagocytic capacity [55, 56]. This is reflected in the distinct gene expression profile observed for  
14 HCL cells as compared to other BCLs and non-malignant B-cells using both a focused list of TFs  
15 and unselected genes.  
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34 For several of the HCL and CLL-associated TFs, downstream targets are found among the  
35 unselected genes identified in the unsupervised clustering. Thus, not only is the number of  
36 transcripts separating different lymphoma subgroups related to the number of unique TFs but also  
37 specific examples of down-stream genes are identified. However, for DLBCLs and FLs where  $\leq$   
38 11 transcripts coding for TFs could be identified, the unsupervised clustering was unable to  
39 distinguish these subpopulations. The inability to subgroup DLBCL and FL indicated that there  
40 are a certain number of TF genes that are needed to affect the global gene expression to that  
41 extent that unsupervised clustering can successfully identify distinct groups. Despite this, it is  
42 clear that FL and DLBCL have both morphological and clinical differences although they are  
43 both related to the GC B-cell stage. However, using genes associated with normal B-cell  
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3 differentiation we were able to separate also FL and the different molecular groups of DLBCL,  
4 including DLBCL-tr, DLBCL-non GC and DLBCL-GC from each other. This suggests that these  
5 BCLs differ in genes important for normal B-cells differentiation, as previously seen for DLBCL  
6 [3, 57, 58].  
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12 FL, like DLBCL, is known to originate from GC B-cells [59, 60]. but gene expression  
13 profiles of FL were surprisingly dissimilar to GC B-cells in our study. This might be explained  
14 by the t(14;18) translocation in FL leading to an over expression of BCL2 [61], which regulates  
15 other pathways and thereby changing the expression profile. Lymphomas with this translocation  
16 tend to have an indolent growing pattern [16, 62], likely to result in a distinct gene expression  
17 profile.  
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27 Our correlation analysis showed that HCL and CLL samples were, in agreement with the  
28 unsupervised clustering and the high number of uniquely expressed TFs, distinct and  
29 homogenous populations having a transcriptional profile enabling separation in a different  
30 dimension, compared to the non-malignant B-cells (Figure 4a, c and Figure 5a). In addition, in  
31 agreement with the unsupervised clustering, leukemic and nodal MCL are clinically regarded as  
32 one disease, therefore a higher Pearson correlation coefficient between these subgroups was  
33 expected. Potentially, this somewhat low correlation could be explained by the heterogeneity  
34 observed within the L-MCL samples in this study (Figure 4d). Nevertheless, specific genomic  
35 changes associated with leukemic dissemination and shorter survival times have been observed  
36 previously [63, 64]. The TF *IRF-4* known to separate DLBCL-nonGC from DLBCL-GC [57] and  
37 to be expressed in a variety of lymphomas of mature B cells (CLL, MM, DLBCL-nonGC) [57,  
38 65, 66] here show an high mRNA expression in MCL. However previous studies have not been  
39 able to confirm the IRF-4 protein expression in MCL [67]. The functional role of *IRF-4* in  
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3 lymphomas has become evident lately although yet not fully elucidated [12]. Studies show an  
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5 association between polymorphism in the *IRF-4* locus and development of CLL [68] as well as  
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7 the involvement of the gene in multiple myeloma [69]. The functional effect of *IRF-4* in MCL  
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9 have to be further assessed in future studies.  
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12 We showed that HCLs have a gene expression profile distinct from all other BCLs. This  
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14 gene profile could not easily be assigned to any of the non-malignant B-cell populations but were  
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16 most closely related to memory B-cells (Figure 4a). This is in agreements with previous studies  
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18 where similarities to both memory B-cells [34] and splenic marginal zone B-cells [70-73] has  
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20 been observed. The cause of the distinct gene expression profile of HCL cells could be either due  
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22 to an onset of a unique signaling pathways compared to the other BCLs which is indicated by the  
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24 high expression of *MAF* suggested to change the expression profile towards a myeloid  
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26 differentiation [35, 36], or a distinct cell of origin, potentially a splenic B-cell, which remains to  
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28 be determined.  
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34 In summary, we have identified TFs that uniquely identify eight different B-cell lymphoma  
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36 subtypes, using highly purified BCLs which enabled the specific analysis of the gene expression  
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38 of tumor cells. The identified TFs partly also explain the downstream global and specific gene  
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40 expression of individual subgroups of BCL. Interestingly, these TFs may be novel functional,  
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42 diagnostic, prognostic or therapeutic targets.  
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3 **Abbreviations**

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5 BCL: B-cell lymphoma; CB: centroblasts; CC: centrocytes; CLL: chronic lymphocytic leukemia;  
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8 DLBCL: diffuse large B-cell lymphomas; DLBCL-GC: DLBCL with a germinal center (GC)  
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10 phenotype, DLBCL-nonGC: DLBCL with a nonGC phenotype; FDC: False discovery rate; FL:  
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12 follicular lymphoma; GCOS: GeneChip Operating Software, HCL: hairy cell leukemia, L-MCL;  
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14 leukemic version of mantle cell lymphoma; MCL: mantle cell lymphoma; M: memory B-cell, N:  
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16 naïve B-cell; PCA: principal component analysis; PCC: Pearson correlation coefficient; TF:  
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18 transcription factor.  
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28  
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30  
31 USA for his work filtering ENSEMBLE for transcription factors.  
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37 **Authorship Contributions**

38  
39 UA Contributed to the design of the study, was responsible for the analysis of the  
40  
41 microarray data, performed statistical analysis and wrote the manuscript.  
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44 PE Contributed to the design of the study and final decision on statistical analysis of  
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46 gene expression data and revision of the manuscript.  
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49 CP Contributed to the design of the study, discussions on statistical analysis of gene  
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51 expression data and revision of the manuscript.  
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54 CMH Responsible for preparation and flow cytometry sorting of non-malignant B-cells  
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56 and revision of the manuscript.  
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- 1 U. Andreasson *et al.* Transcription factors defining subtypes of B-cell lymphomas  
2  
3 MJ Contributed to the design of the study and the revision of the manuscript.  
4  
5 NA Responsible for evaluation of material and clinical data for material (L-MCL) used  
6  
7 for gene expression analysis.  
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9  
10 MB Provided clinical data, identified and collected samples used for gene expression  
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12 analysis and revision of the manuscript.  
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15 CS Responsible for diagnosis and selection of material used for gene expression  
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17 analysis.  
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20 RR Responsible for collection of samples used for gene expression analysis and  
21  
22 revision of the manuscript.  
23  
24  
25 C.A.K B Contributed to the design of the study and revision of the manuscript.  
26  
27 SE Responsible for the design of the study, interpretation of data and writing of the  
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34 All authors approved of the final manuscript.  
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### 39 **Disclosure of Conflicts of Interest**

40 The authors declare no conflict of interest  
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## FIGURE LEGENDS

### **Figure 1. Schematic figure of the different analyses performed.**

Four major analyses of the collected gene expression data were performed. In the figure the designated name and number of filtered transcripts are presented, as well as the characteristics of the different analyses performed. Red lines indicate analyses based on transcripts encoding transcription factors (as defined in ENSEMBL – see Material and Methods).

### **Figure 2. Heat map representing sets of unique transcription factors for each B-cell lymphoma subtype.**

The relative expression of each transcription factor, filtered to have unique profiles in different B-cell lymphoma entities, is visualized in malignant entities (n=5, including 78 samples) as well as in the non-malignant populations (n=3, including 12 samples). The corresponding gene names are shown in Supplementary material (Table SI). Red color indicates up regulation, green color indicates down regulation, whereas black color indicates no change. Notable these heat maps can only be used for qualitative assessment of the expression of each gene over the samples.

### **Figure 3. Subgrouping of B-cell lymphomas through variance filtration.**

PCA plots are used to visualize the results from the unsupervised variance filtration used to group different BCL specimens. The two nearest neighbors for each sample is also visualized in the plots. Transcripts are filtered for high variance across samples, and when a group of samples have one or less nearest-neighbors outside the group it is defined as a distinct group and removed from the analysis. a) Already at 11233 transcripts, group A, containing 10/10 HCL samples, was formed. The visualization was made for 7849 transcripts to improve resolution in the picture,

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with the variance in the three major principal components being; 1 = 15%, 2 = 11% and 3 = 7%.

b) The remaining samples from a) was further filtered and 253 transcripts were used to separate the next two groups, with the variance in the three major principal components being; 1 = 25%, 2 = 10% and 3 = 7%. Group B1 included all 15 CLL samples, but also 1/12 FL and 1/6 L-MCL sample and group B2 included all 12 MCL samples and 5/6 L-MCL samples. The remaining samples in b) could not be further separated and consisted of all 23 DLBCL samples and 11/12 FL.

**Figure 4. PCA representation of B-cell lymphoma entities and non-malignant B-cells based on B-cell associated transcripts.**

PCA plots were used to visualize the relation between different lymphoma entities and non-malignant B-cell populations. The nearest neighbor for each sample is also visualized in the plots. The analysis is based on 5886 transcripts (B-cell F-test transcript) filtered by multi group comparison ( $p = 0.003$ ,  $q = 0.01$ ) to maximize the separation of the non-malignant B-cell populations centroblasts and centrocytes (GC-B-cells,  $n=6$ ), naïve B-cells (N,  $n=3$ ) and memory B-cells (M,  $n = 3$ ). a) The mean values for all populations are visualized in the PCA plot, with the variance in the three major principal components being; 1 = 47%, 2 = 14% and 3 = 12%. b) All samples in the three non-malignant B-cell populations and all FL, DLBCL-GC, DLBCL-nonGC and DLBCL-tr samples are visualized, with the variance in the three major principal components being; 1 = 31%, 2 = 9% and 3 = 7%. c) All samples in the three non-malignant B-cell populations and all CLL and HCL samples are visualized, with the variance in the three major principal components being; 1 = 26%, 2 = 14% and 3 = 11%, d) All samples in the three non-malignant B-cell populations and all L-MCL and MCL samples are visualized with the variance in the three major principal components being; 1 = 34%, 2 = 11% and 3 = 11%.

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**Figure 5. Pearson correlation coefficients between different entities of malignant and non-malignant B-cells.**

a) Pearson correlation coefficient (PCC) are calculated using the mean value for the 5886 transcripts (B-cell F-test transcripts) filtered by F-test ( $p = 0.003$ ,  $q = 0.01$ ) to maximize the separation of the non-malignant B-cell populations ( $n = 3$ ). PCC are calculated for all malignant and non-malignant B-cell entities. b) The groups with the highest PCC values are listed.

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Transcription factors defining subtypes of B-cell lymphomas

**REFERENCES**

1. LeBien TW, Tedder TF. B lymphocytes: how they develop and function. *Blood* 2008;112:1570-1580.
2. Jaffe ES, Harris NL, Stein H, Isaacson PG. Classification of lymphoid neoplasms: the microscope as a tool for disease discovery. *Blood* 2008;112:4384-4399.
3. Alizadeh AA, Eisen MB, Davis RE, Ma C, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. *Nature* 2000;403:503-511.
4. Rosenwald A, Wright G, Chan WC, Connors JM, et al. The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. *N Engl J Med* 2002;346:1937-1947.
5. Rosenwald A, Wright G, Wiestner A, Chan WC, et al. The proliferation gene expression signature is a quantitative integrator of oncogenic events that predicts survival in mantle cell lymphoma. *Cancer Cell* 2003d;3:185-197.
6. Dave SS, Wright G, Tan B, Rosenwald A, et al. Prediction of survival in follicular lymphoma based on molecular features of tumor-infiltrating immune cells. *N Engl J Med* 2004;351:2159-2169.
7. de Jong D. Molecular pathogenesis of follicular lymphoma: a cross talk of genetic and immunologic factors. *J Clin Oncol* 2005;23:6358-6363.
8. Glas AM, Knoops L, Delahaye L, Kersten MJ, et al. Gene-expression and immunohistochemical study of specific T-cell subsets and accessory cell types in the transformation and prognosis of follicular lymphoma. *J Clin Oncol* 2007;25:390-398.
9. Zhang J, Jima DD, Jacobs C, Fischer R, et al. Patterns of microRNA expression characterize stages of human B-cell differentiation. *Blood* 2009;113:4586-4594.
10. Nutt SL, Heavey B, Rolink AG, Busslinger M. Commitment to the B-lymphoid lineage depends on the transcription factor Pax5. *Nature* 1999;401:556-562.
11. Chang CC, Ye BH, Chaganti RS, Dalla-Favera R. BCL-6, a POZ/zinc-finger protein, is a sequence-specific transcriptional repressor. *Proc Natl Acad Sci U S A* 1996;93:6947-6952.
12. Shaffer AL, Emre NC, Romesser PB, Staudt LM. IRF4: Immunity. Malignancy! Therapy? *Clin Cancer Res* 2009;15:2954-2961.
13. Turner CA, Jr., Mack DH, Davis MM. Blimp-1, a novel zinc finger-containing protein that can drive the maturation of B lymphocytes into immunoglobulin-secreting cells. *Cell* 1994;77:297-306.
14. Shaffer AL, Shapiro-Shelef M, Iwakoshi NN, Lee AH, et al. XBP1, downstream of Blimp-1, expands the secretory apparatus and other organelles, and increases protein synthesis in plasma cell differentiation. *Immunity* 2004;21:81-93.
15. Ek S, Dictor M, Jerkeman M, Jirström K, et al. Nuclear expression of the non B-cell lineage Sox11 transcription factor identifies mantle cell lymphoma. *Blood* 2008;111:800-805.
16. Swerdlow SH, Campo, E., Harris, N. L. et al. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues Lyon, France: International Agency for research on Cancer; 2008.

U. Andreasson *et al.*

Transcription factors defining subtypes of B-cell lymphomas

17. Andreasson U, Dictor M, Jerkeman M, Berglund M, et al. Identification of molecular targets associated with transformed diffuse large B cell Lymphoma using highly purified tumour cells. *American Journal of Hematology* 2009;84.
18. Berglund M, Thunberg U, Amini RM, Book M, et al. Evaluation of immunophenotype in diffuse large B-cell lymphoma and its impact on prognosis. *Mod Pathol* 2005;18:1113-1120.
19. Hans CP, Weisenburger DD, Vose JM, Hock LM, et al. A significant diffuse component predicts for inferior survival in grade 3 follicular lymphoma, but cytologic subtypes do not predict survival. *Blood* 2003;101:2363-2367.
20. Andreasson U, Ek S, Merz H, Rosenquist R, et al. B cell lymphomas express CX3CR1 a non-B cell lineage adhesion molecule. *Cancer Lett* 2008;259:138-145.
21. Benjamini Y, Hochberg, Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal Statistical Society Series B (Statistical Methodology)* 1995:289-300.
22. Bouchard C, Lee S, Paulus-Hock V, Loddenkemper C, et al. FoxO transcription factors suppress Myc-driven lymphomagenesis via direct activation of Arf. *Genes Dev* 2007;21:2775-2787.
23. Liu Z, Oh SM, Okada M, Liu X, et al. Human BRE1 is an E3 ubiquitin ligase for Ebp1 tumor suppressor. *Mol Biol Cell* 2009;20:757-768.
24. Gustavsson E, Sernbo, S., Andersson, E., Brennan, D.J., Dictor, M., Jerkeman, M., Borrebaeck, C.A.K., Ek, S. . Sox11 functions as a tumor suppressor under epigenetic control in mantle cell lymphomas. *Lund: Immunotechnology*; 2009.
25. Canote R, Du Y, Carling T, Tian F, et al. The tumor suppressor gene RIZ in cancer gene therapy (review). *Oncol Rep* 2002;9:57-60.
26. Baron V, Adamson ED, Calogero A, Ragona G, et al. The transcription factor Egr1 is a direct regulator of multiple tumor suppressors including TGFbeta1, PTEN, p53, and fibronectin. *Cancer Gene Ther* 2006;13:115-124.
27. Gibbs JD, Liebermann DA, Hoffman B. Egr-1 abrogates the E2F-1 block in terminal myeloid differentiation and suppresses leukemia. *Oncogene* 2008;27:98-106.
28. Gibbs JD, Liebermann DA, Hoffman B. Leukemia suppressor function of Egr-1 is dependent on transforming oncogene. *Leukemia* 2008;22:1909-1916.
29. Pan X, Zhao J, Zhang WN, Li HY, et al. Induction of SOX4 by DNA damage is critical for p53 stabilization and function. *Proc Natl Acad Sci U S A* 2009;106:3788-3793.
30. Pramoonjago P, Baras AS, Moskaluk CA. Knockdown of Sox4 expression by RNAi induces apoptosis in ACC3 cells. *Oncogene* 2006;25:5626-5639.
31. Zheng C, Ren Z, Wang H, Zhang W, et al. E2F1 Induces tumor cell survival via nuclear factor-kappaB-dependent induction of EGR1 transcription in prostate cancer cells. *Cancer Res* 2009;69:2324-2331.
32. Rayoo M, Yan M, Takano EA, Investigators K, et al. Expression of the forkhead box transcription factor FOXP1 is associated with estrogen receptor alpha, estrogen receptor beta and improved survival in familial breast cancers. *J Clin Pathol* 2009.
33. Lam LT, Davis RE, Pierce J, Hepperle M, et al. Small molecule inhibitors of I kappa B kinase are selectively toxic for subgroups of diffuse large B-cell lymphoma defined by gene expression profiling. *Clin Cancer Res* 2005;11:28-40.

U. Andreasson *et al.*

Transcription factors defining subtypes of B-cell lymphomas

34. Basso K, Liso A, Tiacci E, Benedetti R, et al. Gene expression profiling of hairy cell leukemia reveals a phenotype related to memory B cells with altered expression of chemokine and adhesion receptors. *J Exp Med* 2004;199:59-68.
35. Hedge SP, Kumar A, Kurschner C, Shapiro LH. c-Maf interacts with c-Myb to regulate transcription of an early myeloid gene during differentiation. *Mol Cell Biol* 1998;18:2729-2737.
36. Hegde SP, Zhao J, Ashmun RA, Shapiro LH. c-Maf induces monocytic differentiation and apoptosis in bipotent myeloid progenitors. *Blood* 1999;94:1578-1589.
37. Yu S, Matsusue K, Kashireddy P, Cao WQ, et al. Adipocyte-specific gene expression and adipogenic steatosis in the mouse liver due to peroxisome proliferator-activated receptor gamma1 (PPARgamma1) overexpression. *J Biol Chem* 2003;278:498-505.
38. Drori S, Girnun GD, Tou L, Szwaya JD, et al. Hic-5 regulates an epithelial program mediated by PPARgamma. *Genes Dev* 2005;19:362-375.
39. Goudreau G, Petrou P, Reneker LW, Graw J, et al. Mutually regulated expression of Pax6 and Six3 and its implications for the Pax6 haploinsufficient lens phenotype. *Proc Natl Acad Sci U S A* 2002;99:8719-8724.
40. Sundrud MS, Grill SM, Ni D, Nagata K, et al. Genetic reprogramming of primary human T cells reveals functional plasticity in Th cell differentiation. *J Immunol* 2003;171:3542-3549.
41. D'Amico M, Hulit J, Amanatullah DF, Zafonte BT, et al. The integrin-linked kinase regulates the cyclin D1 gene through glycogen synthase kinase 3beta and cAMP-responsive element-binding protein-dependent pathways. *J Biol Chem* 2000;275:32649-32657.
42. Ohtani K, DeGregori J, Nevins JR. Regulation of the cyclin E gene by transcription factor E2F1. *Proc Natl Acad Sci U S A* 1995;92:12146-12150.
43. Chaganti RS, Nanjangud G, Schmidt H, Teruya-Feldstein J. Recurring chromosomal abnormalities in non-Hodgkin's lymphoma: biologic and clinical significance. *Semin Hematol* 2000;37:396-411.
44. Davies AJ, Rosenwald A, Wright G, Lee A, et al. Transformation of follicular lymphoma to diffuse large B-cell lymphoma proceeds by distinct oncogenic mechanisms. *Br J Haematol* 2007;136:286-293.
45. Klein U, Tu Y, Stolovitzky GA, Mattioli M, et al. Gene expression profiling of B cell chronic lymphocytic leukemia reveals a homogeneous phenotype related to memory B cells. *J Exp Med* 2001;194:1625-1638.
46. Rosenwald A, Alizadeh AA, Widhopf G, Simon R, et al. Relation of gene expression phenotype to immunoglobulin mutation genotype in B cell chronic lymphocytic leukemia. *J Exp Med* 2001;194:1639-1647.
47. Shaffer AL, Rosenwald A, Staudt LM. Lymphoid malignancies: the dark side of B-cell differentiation. *Nat Rev Immunol* 2002;2:920-932.
48. Ek S, Högerkorp CM, Dictor M, Ehinger M, et al. Mantle cell lymphomas express a distinct genetic signature affecting lymphocyte trafficking and growth regulation as compared with subpopulations of normal human B cells. *Cancer Res* 2002;62:4398-4405.
49. Ellmark P, Högerkorp CM, Ek S, Belov L, et al. Phenotypic protein profiling of different B cell sub-populations using antibody CD-microarrays. *Cancer Lett* 2008;265:98-106.
50. Pascual V, Liu YJ, Banchereau J. Normal human B cell sub-populations and their malignant counterparts. *Baillieres Clin Haematol* 1997;10:525-538.

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51. Rosenwald A. DNA microarrays in lymphoid malignancies. *Oncology (Williston Park)* 2003a;17:1743-1748; discussion 1750, 1755, 1758-1749 passim.
52. Rosenwald A, Staudt LM. Gene expression profiling of diffuse large B-cell lymphoma. *Leuk Lymphoma* 2003b;44 Suppl 3:S41-47.
53. Dunleavy K, Pittaluga S, Czuczman MS, Dave SS, et al. Differential efficacy of bortezomib plus chemotherapy within molecular subtypes of diffuse large B-cell lymphoma. *Blood* 2009;113:6069-6076.
54. Libermann TA, Zerbini LF. Targeting transcription factors for cancer gene therapy. *Curr Gene Ther* 2006;6:17-33.
55. Jansen J, Meijer CJ, van der Valk P, de Bruyn WC, et al. Phagocytic potential of hairy cells. *Scand J Haematol* 1979;23:69-79.
56. Rosner MC, Golomb HM. Phagocytic capacity of hairy cells from seventeen patients. *Virchows Arch B Cell Pathol Incl Mol Pathol* 1982;40:327-337.
57. Hans CP, Weisenburger DD, Greiner TC, Gascoyne RD, et al. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. *Blood* 2004;103:275-282.
58. Wright G, Tan B, Rosenwald A, Hurt EH, et al. A gene expression-based method to diagnose clinically distinct subgroups of diffuse large B cell lymphoma. *Proc Natl Acad Sci U S A* 2003;100:9991-9996.
59. Kuppers R, Klein U, Hansmann ML, Rajewsky K. Cellular origin of human B-cell lymphomas. *N Engl J Med* 1999;341:1520-1529.
60. Ott G, Rosenwald A. Molecular pathogenesis of follicular lymphoma. *Haematologica* 2008;93:1773-1776.
61. Godon A, Moreau A, Talmant P, Baranger-Papot L, et al. Is t(14;18)(q32;q21) a constant finding in follicular lymphoma? An interphase FISH study on 63 patients. *Leukemia* 2003;17:255-259.
62. Harris NL, Jaffe ES, Stein H, Banks PM, et al. A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. *Blood* 1994;84:1361-1392.
63. Martinez-Climent JA, Vizcarra E, Sanchez D, Blesa D, et al. Loss of a novel tumor suppressor gene locus at chromosome 8p is associated with leukemic mantle cell lymphoma. *Blood* 2001;98:3479-3482.
64. Vizcarra E, Martinez-Climent JA, Benet I, Marugan I, et al. Identification of two subgroups of mantle cell leukemia with distinct clinical and biological features. *Hematol J* 2001;2:234-241.
65. Iida S, Rao PH, Butler M, Corradini P, et al. Deregulation of MUM1/IRF4 by chromosomal translocation in multiple myeloma. *Nat Genet* 1997;17:226-230.
66. Ito M, Iida S, Inagaki H, Tsuboi K, et al. MUM1/IRF4 expression is an unfavorable prognostic factor in B-cell chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL). *Jpn J Cancer Res* 2002;93:685-694.
67. Tsuboi K, Iida S, Inagaki H, Kato M, et al. MUM1/IRF4 expression as a frequent event in mature lymphoid malignancies. *Leukemia* 2000;14:449-456.
68. Di Bernardo MC, Crowther-Swanepoel D, Broderick P, Webb E, et al. A genome-wide association study identifies six susceptibility loci for chronic lymphocytic leukemia. *Nat Genet* 2008;40:1204-1210.

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- 4 69. Shaffer AL, Emre NC, Lamy L, Ngo VN, et al. IRF4 addiction in multiple myeloma. *Nature* 2008;454:226-231.
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- 6 70. Tierens A, Delabie J, Malecka A, Wang J, et al. Splenic marginal zone lymphoma with villous lymphocytes shows on-going immunoglobulin gene mutations. *Am J Pathol* 2003;162:681-689.
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- 9 71. Walsh SH, Rosenquist R. Immunoglobulin gene analysis of mature B-cell malignancies: reconsideration of cellular origin and potential antigen involvement in pathogenesis. *Med Oncol* 2005;22:327-341.
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- 12 72. Vanhentenrijk V, De Wolf-Peeters C, Wlodarska I. Comparative expressed sequence hybridization studies of hairy cell leukemia show uniform expression profile and imprint of spleen signature. *Blood* 2004;104:250-255.
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- 15 73. Vanhentenrijk V, Tierens A, Wlodarska I, Verhoef G, et al. V(H) gene analysis of hairy cell leukemia reveals a homogeneous mutation status and suggests its marginal zone B-cell origin. *Leukemia* 2004;18:1729-1732.
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**Table I. Clinical information of the B-cell lymphoma samples**

Samples	Grade <sup>1</sup>	Sex (F/M)	Stage <sup>2</sup> I-II	Stage <sup>2</sup> III-IV	IGVH mutational status "mutated"	IGVH mutational status "unmutated"
CLL (n=15)	NA <sup>3</sup>	4/11	NA <sup>3</sup>	NA <sup>3</sup>	7	8
DLBCL-GC (n=7)	NA <sup>3</sup>	5/2	2	4	7	0
DLBCL-nonGC (n=10)	NA <sup>3</sup>	5/5	4	4	10	0
DLBCL-tr (n=6)	NA <sup>3</sup>	2/4	0	5	6	0
FL (n=12)	I-II	6/6	2	6	NA <sup>3</sup>	NA <sup>3</sup>
HCL (n=10)	NA <sup>3</sup>	4/6	NA <sup>3</sup>	NA <sup>3</sup>	7	3
L-MCL (n=6)	NA <sup>3</sup>	6/0	0	6	NA <sup>3</sup>	NA <sup>3</sup>
MCL (n= 12)	NA <sup>3</sup>	4/8	1	10	2	10

<sup>1</sup> Grade at diagnosis, when available<sup>2</sup> Stage, when available<sup>3</sup> NA = not available

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**Table II. Sorting parameters for each non-malignant B-cell population**

Population	Designation	CD19	CD5	IgD	IgM	CD38	CD27	IgA/IgG
Naïve B-cells	N	+	-	+	+	-	-	n.d. <sup>1</sup>
Centroblast	CB	+	-	-	-	+	n.d. <sup>1</sup>	-
Centrocyte	CC	+	-	-	-	+	n.d. <sup>1</sup>	+
Memory B-cells	M	+	-	-	-	-	+	+

<sup>1</sup> n.d - not determined

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**Table III. Unique characterized TF genes for each B-cell lymphoma group<sup>1</sup>.**

<b>Gene symbol<sup>2</sup></b>	<b>Gene symbol</b>
<b><u>FL</u></b>	<b><u>CLL</u></b>
ZNF496*	EBF1*
TSHZ1*	LASS4*
CCRN4L*	EGR*1
HOPX	TRERF1*
STAT5B	FOS*
FOXO1	ZNF9*3
TSC22D4	E2F3*
	SOX4*
<b><u>DLBCL</u></b>	BHLHB3*
EP300*	LOC641518
MLL*	PHTF1
TSC22D2*	LEF1
KLF2	C21orf41
PHF1	RXRA
ZNF274	THRA
CREBBP	SMAD4
ZNF238	YY1
PTTG1	ARNT
PA2G4	PRDM2
	PBX3
	HIRA
<b><u>HCL<sup>3</sup></u></b>	
TCF4*	
TAF4B*	<b><u>MCL/L-MCL</u></b>
ETS1*	NR3C1*
MYBL2*	SEMA4A*
TCF7L2	TCF25
MAF	MEF2C
GAS7	MAFB
MYF6	SALL2
SIX3	GTF2IRD1
HOPX	IRF4
PRDM16	SOX8
TSHZ3	SOX11
ETV5	
TBX15	
SOX5	
ASCL2	
SOX4	
PPARG	

<sup>1</sup> This table is an assortment of all TF transcripts present in Supplementary material (Table S1)

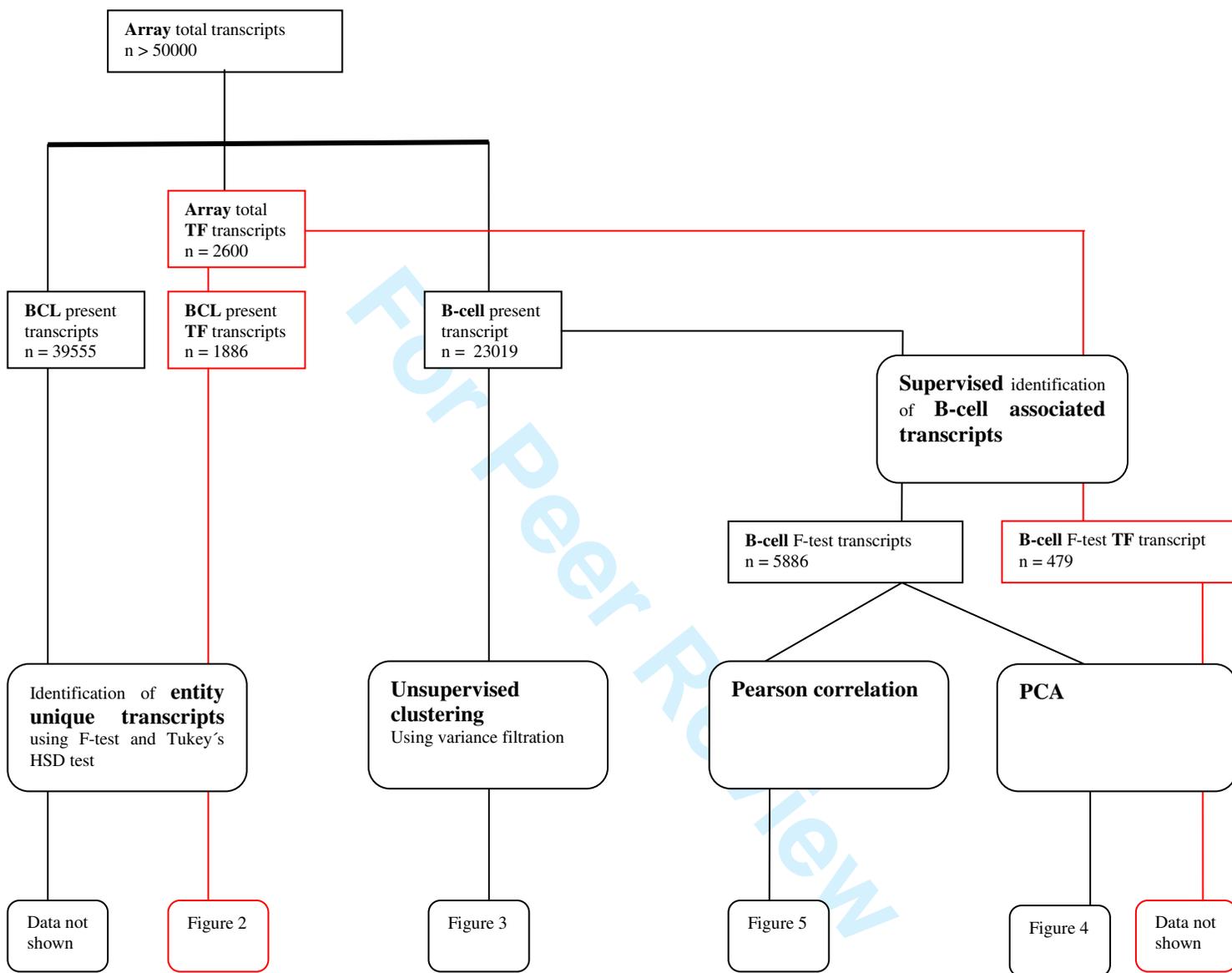
<sup>2</sup> \* equals low expression of the transcript in the specified group.

<sup>3</sup> The characterized genes corresponding to the 50 transcripts (of total 169) with best q-value.

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Figure 1

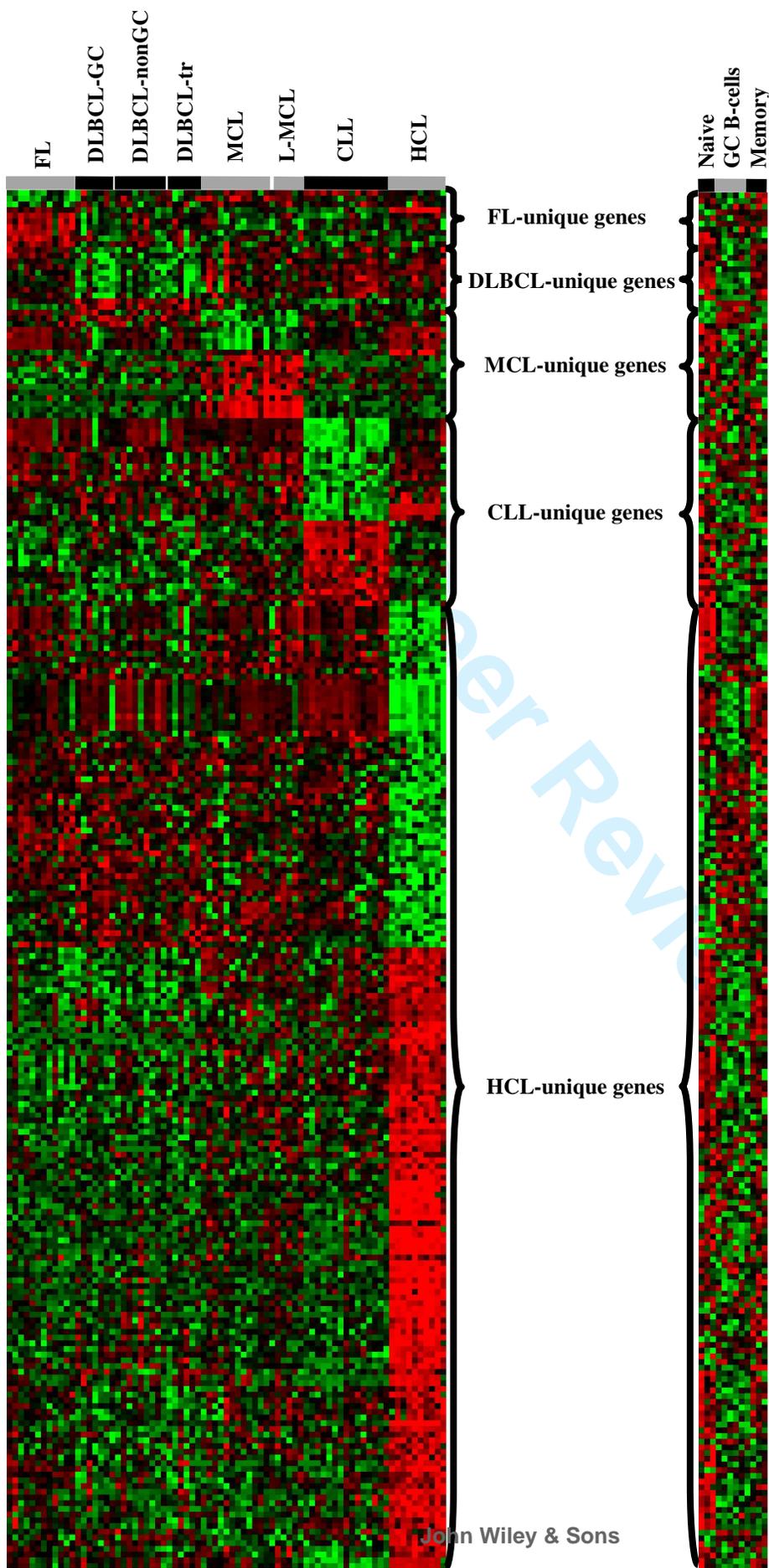


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Figure 2

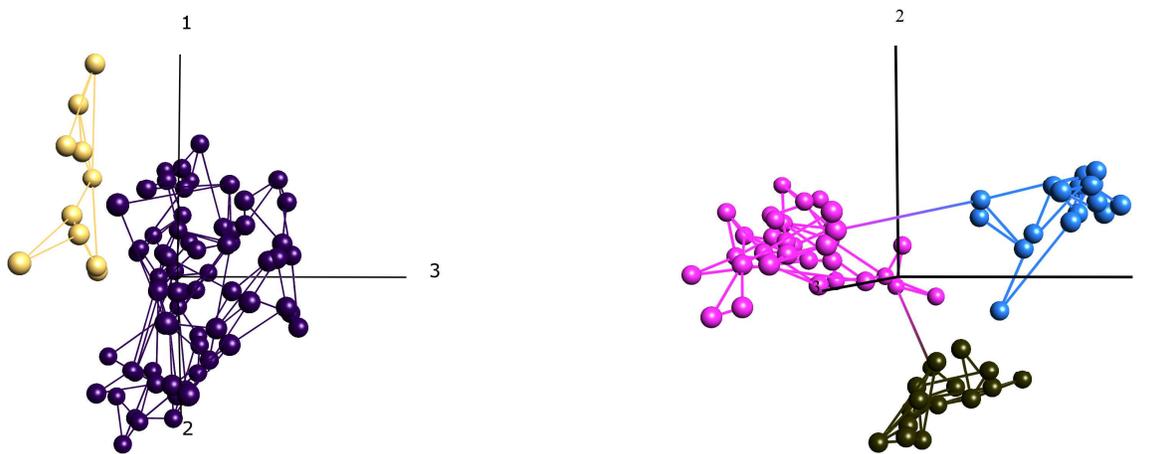
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Figure 3



a)

b)



Group A (10 HCL samples)



Remaining samples a)



Group B1 (15 CLL, 1 FL and 1 L-MCL samples)



Group B2 (12 MCL and 5 L-MCL samples)

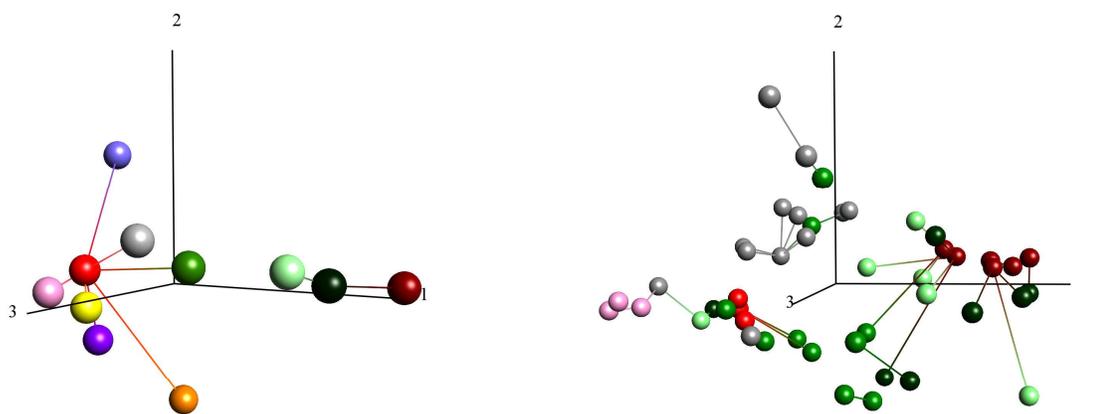


Remaining samples b)

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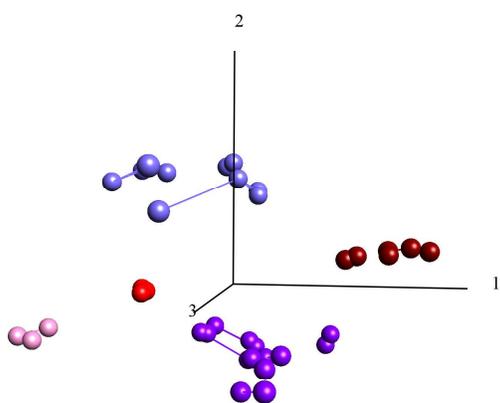
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Figure 4

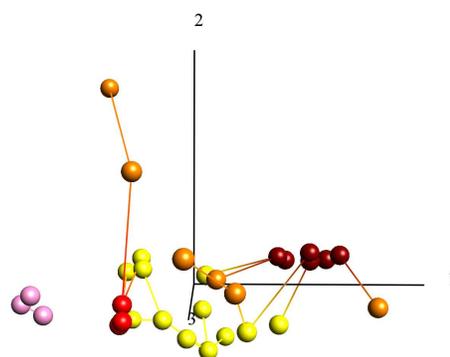


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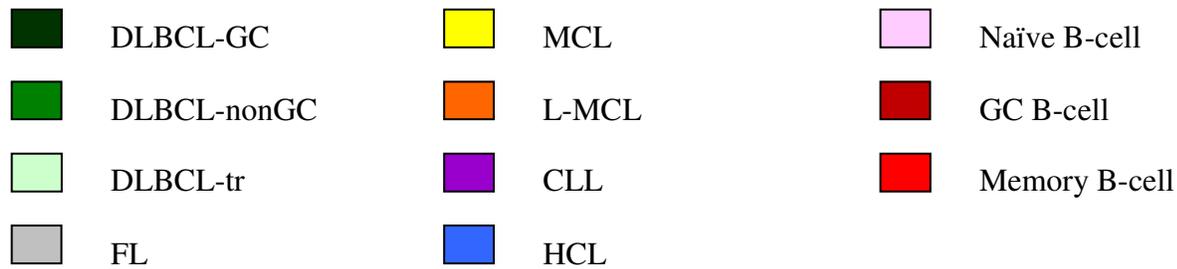
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Figure 5a

	DLBCL-nonGC	DLBCL-tr	FL	MCL	L-MCL	CLL	HCL	GC B	Memory	Naïve
DLBCL-GC	0.47	0.69	0.10	-0.39	-0.37	-0.57	-0.56	0.77	-0.72	-0.70
DLBCL-nonGC		0.37	0.16	-0.25	-0.44	-0.36	-0.44	0.32	-0.30	-0.29
DLBCL-tr			0.29	-0.45	-0.41	-0.55	-0.54	0.66	-0.60	-0.61
FL				-0.31	-0.44	-0.25	-0.22	0.13	-0.02	-0.18
MCL					0.36	0.12	-0.02	-0.45	0.34	0.47
L-MCL						0.19	0.00	-0.26	0.15	0.30
CLL							0.12	-0.44	0.40	0.41
HCL								-0.45	0.48	0.36
GC B									-0.89	-0.94
Memory										0.69



Figure 5b

<i>Population 1</i>	<i>Population 2</i>	<i>PCC</i>
DLBCL-GC	GC B-cells	0.77
DLBCL-GC	DLBCL-tr	0.69
Memory B-cells	Naïve B-cells	0.69
DLBCL-tr	GC B-cells	0.66
HCL	Memory B-cells	0.48
DLBCL-GC	DLBCL-nonGC	0.47
MCL	Naïve B-cells	0.47
CLL	Naïve B-cells	0.41
CLL	Memory B-cells	0.40
DLBCL-nonGC	DLBCL-tr	0.37
MCL	L-MCL	0.36
HCL	Naïve B-cells	0.36
MCL	Memory B-cells	0.34