

Genetic predisposition to chronic lymphocytic leukemia is mediated by a *BMF* super-enhancer polymorphism

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SUMMARY

Chronic lymphocytic leukemia (CLL) is an adult B-cell malignancy. Genome-wide association studies show that variation at 15q15.1 influences CLL risk. We deciphered the causal variant at 15q15.1 and the mechanism by which it influences tumorigenesis. We imputed all possible genotypes across the locus and then mapped highly associated SNPs to areas of chromatin accessibility, evolutionary conservation and transcription factor binding. SNP rs539846 C>A, the most highly associated variant ($P = 1.42 \times 10^{-13}$, odds ratio = 1.35), localizes to a super-enhancer defined by extensive histone H3 lysine 27 acetylation in intron 3 of *BMF* (B-cell lymphoma 2 (BCL2) modifying factor). The rs539846-A risk allele alters a conserved RELA binding motif, disrupts RELA binding and is associated with decreased *BMF* expression in CLL. These findings are consistent with rs539846 influencing CLL susceptibility through differential RELA binding, with direct modulation of *BMF* expression impacting on anti-apoptotic BCL2, a hallmark of oncogenic dependency in CLL.

INTRODUCTION

Although genome-wide association studies (GWAS) have frequently identified statistically significant associations within non-coding regions of the genome, the underlying causal variant has been elucidated in only a few instances. GWAS of chronic lymphocytic leukemia (CLL) have identified 31 risk loci, with the signal annotating *BMF* (B-cell lymphoma 2 modifying factor) at 15q15.1, being highly robust (Berndt et al., 2016; Berndt et al., 2013; Crowther-Swanepoel et al., 2010; Di Bernardo et al., 2008; Slager et al., 2011; Slager et al., 2012; Speedy et al., 2014).

Elevated expression of the anti-apoptotic protein B-cell lymphoma 2 (*BCL2*) is a hallmark of CLL, driving the accumulation of mature leukemic lymphocytes (Hanada et al., 1993). *BMF*, a BH3-only pro-apoptotic member of the *BCL2* protein family, neutralizes the anti-apoptotic activity of *BCL2* through direct interaction (Puthalakath et al., 2001). Here, we sought to identify the causal polymorphism(s) driving the 15q15.1 association with CLL susceptibility as a basis for understanding *BCL2* addiction mechanisms in CLL.

RESULTS

Fine-mapping of the 15q15.1 CLL risk locus

A previous GWAS reported an association between rs8024033 at 15q15.1 and CLL risk (Berndt et al., 2013). To refine the association signal, we performed fine-mapping of the 15q15.1 CLL risk locus by imputation of our European GWAS to 1000 Genomes Project (Abecasis et al., 2012) and UK10K (UK 10K Consortium, 2015) reference panels. By this approach we identified four risk SNPs with minor allele frequency > 0.01 and association $P < 5.0 \times 10^{-7}$ (**Figure 1A, Table S1**). The lead SNP, rs539846 (odds ratio = 1.35, $P = 1.42 \times 10^{-13}$), maps to the third intron of *BMF* and is in high linkage disequilibrium (LD, $r^2 = 0.91$) with the published SNP, rs8024033. We verified the fidelity of imputed rs539846 genotypes by Sanger sequencing in a subset of 176 CLL cases, demonstrating >95% concordance.

To rule out the existence of multiple statistical signals at the *BMF* locus, we repeated association testing conditional on rs539846 genotypes, observing no significant variants (most significant variant: rs181168015, $P = 1.52 \times 10^{-4}$; **Figure S1**). We also found no rare non-synonymous variants in *BMF* in the germline exomes of 141 CLL cases (enriched for genetic susceptibility by virtue of family history, **Supplemental Information**). Collectively these results are consistent with a single underlying variant at the 15q15.1 locus.

Definition of rs539846 as a plausible CLL risk SNP

To further prioritize candidate CLL risk variants we examined the regulatory potential of SNPs in LD ($r^2 > 0.2$) with rs539846, based upon epigenetic data from lymphoblastoid cell lines (LCLs, (Encode Project Consortium, 2012)) and primary CLL cells. These data showed that rs539846 resides within an active enhancer region inferred by DNaseI sensitivity, H3K4me1 and H3K27ac histone modifications in both cell types (**Figure 1B, Table S1**). Moreover, analysis of histone H3K27ac data from lymphoid cells of both B-cell and T-cell lineages defines a B-cell specific 15q15.1 super-enhancer, that spans approximately 80kb, encompassing the CLL risk locus (Hnisz et al., 2013), whilst Hi-C data from LCLs (Rao et al., 2014) show that this putative super-enhancer element overlaps a chromatin contact domain (**Figure 2A**).

Since causal SNPs that drive GWAS associations may function by altering transcription factor binding, we examined whether 15q15.1 candidate risk SNPs disrupt predicted JASPAR motifs. This

revealed that rs539846 alters a highly conserved base within a putative RELA-binding motif (GGGACTTT[C/A]C, phastCons score = 1.00, Genomic Evolutionary Rate Profiling (GERP) score = 4.81) (**Figure 1B**). ENCODE transcription factor chromatin immunoprecipitation sequencing (ChIP-seq) in LCLs confirmed the presence of RELA binding across this site (**Figure 1B**) in cells homozygous for the rs539846-C allele (non-risk allele, preserving the RELA motif). Within the 15q15.1 chromatin contact domain, 4C-seq in the MEC1 CLL cell line showed a high frequency of three dimensional contacts between the viewpoint (adjacent to rs539846) and the distal end of the predicted super-enhancer, with both points overlapping regions of RELA and CTCF binding (**Figure 2B**).

No other candidate CLL risk variant at the 15q15.1 locus showed the unique combination of evolutionary conservation, active enhancer localization and disruption of a transcription factor binding motif, thus re-affirming that rs539846 is the single best causal SNP candidate.

rs539846 alters RELA-mediated enhancer activity

We next performed luciferase reporter assays to determine the effect of rs539846 on enhancer activity. MEC1 cells transfected with constructs containing the risk A allele demonstrated a significant reduction in normalized luminescence compared to the C allele ($P = 0.015$, **Figure 3A/B**), indicating that the intact RELA motif is required for enhancer activity. We assayed protein-DNA interactions for rs539846-C and A alleles using electrophoretic mobility shift assays (EMSA). The C allele formed stronger protein-DNA complexes compared with the A allele (**Figure 3C**) and in an EMSA super-shift assay RELA was preferentially recruited to the C allele (**Figure 3C**).

rs539846 alters RELA-mediated regulation of *BMF*

To determine whether *BMF* is a target of RELA-mediated regulation, we first queried the International Cancer Genome Consortium (ICGC) dataset, revealing a correlation between *RELA* and *BMF* expression in CLL (**Table S2A**; $P = 0.004$). In order to establish a direct relationship between *RELA* and *BMF* expression we performed siRNA experiments in MEC1 cells where knockdown of *RELA* was accompanied by a significant reduction in *BMF* mRNA (**Figure 4A**, $P = 0.02$, **Figure S2**). We also investigated whether rs539846 genotype was associated with *BMF* transcript levels in 426 primary CLL cases. We observed a significant dose relationship between the rs539846-A risk allele and reduced *BMF* mRNA ($P = 0.0003$; **Figure 4B**). No association was seen between rs539846 genotype and levels of other genes within 1Mb of the SNP (false discovery

rate <0.05 , **Table S2B**). To investigate whether rs539846 genotype might influence splicing of *BMF* we examined RNA-seq data from 30 CLL cases, finding no evidence of aberrant splicing. We also found no differences in the splicing levels of known *BMF* exons between rs539846 risk allele and non-risk allele homozygotes (**Figure S3**).

Impact of rs539846 on prognosis and survival in CLL patients

CLL can be classified on the basis of several prognostic factors including immunoglobulin heavy-chain variable (*IGHV*) mutation status, expression levels of CD38, ZAP70 and CLLU1 as well as somatic genomic abnormalities (trisomy 12, 13q14 deletion, 6q21 deletion, 11q23 deletion, 17p13 deletion, *NOTCH1* mutation and *SF3B1* mutation). We found no association between rs539846 genotype and these features in a subset of UK-GWAS and ICGC study cases (**Table S3**). There was also no association between rs539846 and overall patient outcome (**Table S4A**) and we noted that *BMF* transcript levels were not associated with patient survival (**Table S4B**).

DISCUSSION

Collectively, our data demonstrate that the underlying molecular mechanism for the 15q15.1 CLL risk locus is mediated through rs539846, which resides within a transcriptional enhancer and disrupts a conserved RELA transcription factor binding site. Our data are compatible with the rs539846-A allele conferring increased CLL risk through reduced RELA-mediated expression of the pro-apoptotic BCL2-family gene, *BMF*. Furthermore, epigenetic and chromosome conformation capture data are consistent with rs539846 localizing within a chromatin contact domain, overlapping a B-cell super-enhancer (Hnisz et al., 2013). This interval, anchored by divergent CTCF binding sites, forms a 'loop domain' (Rao et al., 2014) which is expected to bring two regions of RELA binding, separated by a linear distance of around 65Kb, into physical contact close to the promoter of *BMF*.

RELA (also known as p65) is a sub-unit of the NF-kappa-B protein (NFKB) complex. This transcription factor complex regulates expression of genes involved in biological processes such as proliferation, survival and inflammation. NFKB signaling is constitutively active in CLL (Furman et al., 2000) whilst high levels of the pro-survival gene *BCL2*, an established NFKB target, are a hallmark of the disease (Scarfo and Ghia, 2013).

Here we provide direct evidence that *BMF* is transcriptionally regulated by RELA, in-keeping with the somewhat counter-intuitive observation that levels of pro-apoptotic BMF are high in CLL (Mackus et al., 2005). In the normal response to cellular stress, BMF interacts with BCL2 at the mitochondrial surface and neutralizes its anti-apoptotic properties (Puthalakath et al., 2001). In CLL, it is hypothesized that although cells maintain some ability to induce pro-apoptotic BH3-only proteins like BMF in response to oncogenic stress, apoptosis ultimately fails due to over-expression of pro-survival proteins.

Our data suggest that in individuals carrying the rs539846 risk allele, *BMF* transcript levels are reduced and thus the apoptotic response may be attenuated further. Indeed, previous studies in the myeloma cell line, U266 have reported that siRNA-mediated knockdown of *BMF* is associated with a decrease in apoptosis following treatment with arsenic trioxide (Morales et al., 2008) whilst mice lacking *Bmf* develop a B-cell lymphadenopathy caused by a resistance of B-cells to apoptosis (Labi et al., 2008).

We did not observe an association between the SNP and prognostic markers or patient survival in CLL. This is consistent with differential expression of *BMF* being important in the early phases of CLL rather than disease progression *per se*. We do however acknowledge that our analysis had <50% power to demonstrate a 10% difference in patient outcome and to robustly determine the relationship between *BMF* expression and patient outcome requires much larger patient cohorts.

Finally, this study underlines the importance of BH3-only proteins such as *BMF* in CLL development. Recently, a number of BH3-mimetics have been developed as potential therapies for lymphoid malignancies (Billard, 2013). These molecules are designed to mimic endogenous BH3-only proteins and bind to pro-survival members of the BCL2 family, facilitating the induction of apoptosis. One example, ABT-199 (Venetoclax), selectively targets BCL2 and in recent clinical trials involving relapsed or refractory CLL patients gave an overall response rate of 79% (Roberts et al., 2016). Our findings thus further demonstrate the utility of association studies to define clinically relevant oncogenic pathways.

EXPERIMENTAL PROCEDURES

Ethics

Ethical approval for this study was obtained from the UK Multi-Research Ethics Committee (MREC 99/1/082).

Fine-mapping of the 15q15.1 locus

We made use of data from two published CLL GWAS. Briefly, (1) UK-CLL-1 (Di Bernardo et al., 2008), a scan of 517 cases using Illumina HumanCNV370-Duo BeadChips with Hap1.2M-Duo Custom array data on 2,698 individuals from the Wellcome Trust Case Control Consortium 2 (WTCCC2) 1958 Birth cohort as controls (2) UK-CLL-2 (Speedy et al., 2014), 1,403 cases using the Illumina Omni Express BeadChips with Hap1.2M-Duo Custom array data on 2,501 individuals from the UK Blood Service Control Group serving as controls. Individuals with low call rate (<90%), extremely high or low heterozygosity ($P < 1.0 \times 10^{-4}$) and those evaluated to be of non-European ancestry (using HapMap version 2 populations as a reference) were excluded.

GWAS data were imputed using 1000 Genomes Project phase 1 integrated release 3, March 2012 (Abecasis et al., 2012) and UK10K 2014 release (UK 10K Consortium, 2015) as reference in conjunction with IMPUTE2 v2.1.1 software (Howie et al., 2009). Genotypes were aligned to the positive strand in both imputation and genotyping. Poorly imputed SNPs defined by an information measure, $I_s < 0.80$, were excluded. The association between each SNP and CLL risk was assessed by Cochran-Armitage trend test. To look for independent effects, conditional logistic regression analysis was performed. SNP rs539846 was included as a covariate and association statistics for SNPs within the interval chr15:40379030-40532514 were recalculated (region spans all SNPs in LD $r^2 > 0.2$ with rs539846). To validate imputed rs539846 genotypes, we performed Sanger sequencing in 176 CLL GWAS cases. Primers are listed in **Table S5**.

Epigenetic annotation

To explore the epigenetic profile of the interval, we examined LCL chromatin state segmentation, DNase-seq, histone modification and transcription factor ChIP-seq data from the ENCODE project (Encode Project Consortium, 2012; Ernst and Kellis, 2012). In addition, ChIP-seq (H3K4me3, H3K4me1, and H3K27ac) and DNase-seq data generated using standard protocols within the Blueprint Consortium from cells of a CLL patient with mutated *IGHV* (>90% tumour cell content)

were also examined (Puente et al., 2015). Detailed protocols are available from the Blueprint Consortium (<http://www.blueprint-epigenome.eu>). We also used HaploReg (Ward and Kellis, 2012) to examine whether rs539846 or proxy SNPs ($r^2 > 0.2$ in 1000 Genomes EUR reference panel) annotate transcription factor binding sites or enhancer elements. We assessed sequence conservation using GERP (Cooper et al., 2005) and PhastCons (Siepel et al., 2005). We searched for overlap with annotated 'super-enhancer' regions in lymphoid cell types from B-cell (CD19⁺ and CD20⁺) and T-cell (CD4⁺ naïve and memory; CD8⁺ naïve and memory) lineages (Hnisz et al., 2013).

Hi-C and definition of a topological domain at the 15q15.1 locus

We made use of publicly available Hi-C data on GM12878 cells (Rao et al., 2014), based on combined replicates digested using *Mbol*, analyzed using the balanced Knight-Ruiz normalization method (Knight and Ruiz, 2013) with a uniform resolution of 5Kb. Contact domains were defined with the Arrowhead algorithm (Rao et al., 2014).

Cell culture

MEC1 (human CLL) cells were grown in Iscove's Modified Dulbecco's Medium (Life Technologies) supplemented with 10% fetal calf serum.

4C-Sequencing

4C-seq libraries were prepared as described (van de Werken et al., 2012), using 10 million MEC1 cells cross-linked with 2% formaldehyde. Using 4C primer design software (<http://mnlab.uchicago.edu/4Cpd/>) we identified a viewpoint adjacent to SNP rs539846. Primary and secondary restriction enzymes were *DpnII* and *HindIII* (cut sites, chr15:40,397,659-40,397,662bp and chr15:40,396,692-40,396,697bp, respectively). Primers are listed in **Table S5**. Libraries were sequenced on an Illumina MiSeq, to obtain 150bp single-end reads. Reads were mapped to the human genome using Bowtie (version 2.1.0) and filtered for PHRED score <30. Implementing standard procedures, unique 4C-seq reads were allocated to blind and non-blind fragments. Profiles for the two classes of fragments were obtained at 100bp resolution and an average profile for a 5 Kb running window was computed. For data visualization we used Vispig (Scales et al., 2014) and incorporated processed ChIP-seq data from the ENCODE Project (Encode Project Consortium, 2012).

Plasmid construction and luciferase assays

Allele-specific fragments of a 591bp region spanning rs539846 were amplified from human genomic DNA using primers detailed in **Table S5**, cloned into the PCR8/GW/TOPO vector and then transferred into pGL3 *luc2* promoter vector using Gateway technology (Life Technologies). Reporter constructs were introduced into MEC1 cells by nucleofection, using program X-01 on the Amaxa Nucleofector I (Amaxa Biosystems). Typically, 5×10^6 cells were resuspended in 100 μ l Cell Line Nucleofector Solution V and mixed with 3 μ g reporter plasmid DNA and 60ng of internal control plasmid (pRL-SV40). Transiently transfected cells were grown for 24 hours before assaying with the Dual-Luciferase Reporter Assay System (Promega) and the Fluoroskan Ascent FL plate reader (Labsystems). Relative luciferase activity was calculated as the ratio of luminescence from the experimental reporter to that of the control reporter. Each transfection experiment was repeated three times and statistical significance was calculated using the Student's *t*-test.

Electrophoretic mobility-shift assay

Nuclear protein was extracted from MEC1 cells using NE-PER nuclear and cytoplasmic extraction kits (Thermo Fisher Scientific). Infrared dye DY-682 labelled (Eurofins Genomics) and unlabeled (Life Technologies) complementary oligonucleotides flanking rs539846 (5'-GAGGGGACTTT[C/A]CCTCCCCAAC-3' and 5'-GTTTGGGAGG[G/T]AAAGTCCCCTC-3') were annealed to generate double-stranded EMSA probes. Each 20 μ l binding reaction contained 50fmol labelled target DNA, 1X binding buffer (10mM Tris, 50mM KCl, 1mM DTT; pH 7.5), 1 μ g poly (dI.dC) (Sigma-Aldrich), 2.5mM DTT, and 10 μ g nuclear protein extract. The reaction mix was incubated in the dark for 30 minutes at room temperature. Competition assays were performed by adding 200-fold molar excess of unlabeled probes to the binding reaction. 'Supershift' EMSAs were conducted by adding 2 μ g RELA antibody (Santa Cruz Biotechnology) to the binding reaction and incubating for 15 minutes prior to the addition of labelled probe. Post incubation, 2 μ l of 10X Orange loading dye (LI-COR Biosciences) was added to the reaction mix and the DNA-protein complexes were resolved by electrophoresis on a 6% DNA retardation gel (Life Technologies) in 0.5X Tris-borate-EDTA (TBE) at 4°C. Gels were imaged using the Odyssey[®] Fc Infrared Imaging System (LI-COR Biosciences).

Gene expression and splicing analysis

We used Spearman's rank correlation to assess the relationship between *BMF* and *RELA* transcript levels in the ICGC dataset (Puente et al., 2015). Expression quantitative trait locus analyses were performed for all genes in the 1Mb region around rs539846, using Affymetrix Human Genome Array U219 data on 426 CLL patients (Puente et al., 2015). Four cases with 15q15.1 copy number

losses were excluded. Genotypes were determined by imputation as described and were confirmed from whole genome-sequencing (WGS) data in 145 samples, with >99% concordance. The association between SNP genotype and expression was evaluated by linear regression controlling for false discovery using 'matrixEQTL' (Shabalín, 2012) implemented in R (version 3.2.0). To assess the impact of rs539846 on splicing we used RNA-seq data from CLL tumors (Puente et al., 2015), counting individual k-mers supporting each of the possible splicing events. Sample genotype for SNP rs539846 was determined from WGS data and differences between k-mer counts for rs539846-CC and -AA homozygotes were evaluated using a Student's *t*-test.

siRNA knockdown

siRNA targeting *RELA* and a control siRNA (**Table S5**) were obtained from Eurofins Genomics. MEC1 cells were transfected with 100nM siRNA using nucleofection as described. Total RNA was extracted 24 hours post-transfection using the RNeasy Plus Mini Kit (Qiagen). cDNA was produced using SuperScript® II Reverse Transcriptase (Life Technologies). Knockdown efficiency was measured by qPCR and western blot using standard protocols. *RELA* antibody was used with GAPDH antibody (FL-335; sc-25778, HRP) (Santa Cruz Biotechnology) as the loading control. Transcript levels of *RELA* and *BMF* were quantified using SYBR Green PCR mastermix (Life Technologies) and normalized to *GAPDH*. The experiment was repeated three times. Primer sequences are detailed in **Table S5**.

Association between rs539846 genotype and clinical variables

Logistic regression was used to test the association between rs539846 genotype and prognostic factors. Trisomy 12, 13q14 deletion, 6q21 deletion, 11q23 deletion, 17p13 deletion, CD38 expression, ZAP70 expression, CLLU1 expression, *NOTCH1* mutation and *SF3B1* mutation status were determined in a subset of UK-CLL-1 patients, who were participants in the LRF CLL4 Trial (Catovsky et al., 2007), as previously described (Gonzalez et al., 2013; Oscier et al., 2010; Oscier et al., 2013). *IGHV* mutation status was determined as per BIOMED-2 protocols (van Dongen et al., 2003) in a subset of patients from UK-CLL-1, UK-CLL-2 and the ICGC CLL project (Puente et al., 2015). In accordance with published criteria (van Krieken et al., 2007), we classified sequences with germline homology of $\geq 98\%$ as unmutated and those with homology $< 98\%$ as mutated. Survival analysis was performed using data from a subset of UK-CLL-1 and ICGC CLL project cases. Analysis was carried out using the log-rank test (using time from diagnosis to death or censoring at the end of follow-up).

ACCESSION CODES

Data used in this manuscript are deposited in the European Genome-Phenome Archive under accession numbers EGAD00010000875 and EGAS00000000092.

AUTHOR CONTRIBUTIONS

RH designed the study, drafted the manuscript with contributions from all other authors; RK, GS, HS and JS performed the experiments; PL, HS, RK, GS and GM performed the bioinformatics analysis; DC and JA performed sample recruitment; RH obtained financial support; in Spain, SB, JMS, DMG, IS, XSP and JGA performed data analysis; CLO and EC supervised the data analysis.

ACKNOWLEDGEMENTS

Bloodwise provided principal funding (LRF05001, LRF06002 and LRF13044). We also acknowledge support from Cancer Research UK (C1298/A8362 supported by the Bobby Moore Fund) and the Arbib Charitable Fund. GS is in receipt of a PhD studentship from The Institute of Cancer Research funded by Cancer Research UK. We also acknowledge support from The Spanish Ministry of Science and Innovation (MICINN) through the Instituto de Salud Carlos III (ISCIII) International Cancer Genome Consortium for Chronic Lymphocytic Leukemia (ICGC-CLL Genome Project). This study uses data generated by the Wellcome Trust Case-Control Consortium 1 and 2. A full list of the investigators who contributed to generation of the data is available at <http://www.wtccc.org.uk/>. We are grateful to all investigators and patients for their participation. We also thank the clinicians and other staff who contributed to the blood sample and data collection for this study.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

REFERENCES

- Abecasis, G.R., Auton, A., Brooks, L.D., DePristo, M.A., Durbin, R.M., Handsaker, R.E., Kang, H.M., Marth, G.T., and McVean, G.A. (2012). An integrated map of genetic variation from 1,092 human genomes. *Nature* 491, 56-65.
- Berndt, S.I., Camp, N.J., Skibola, C.F., Vijai, J., Wang, Z., Gu, J., Nieters, A., Kelly, R.S., Smedby, K.E., Monnereau, A., *et al.* (2016). Meta-analysis of genome-wide association studies discovers multiple loci for chronic lymphocytic leukemia. *Nature communications* 7, 10933.
- Berndt, S.I., Skibola, C.F., Joseph, V., Camp, N.J., Nieters, A., Wang, Z., Cozen, W., Monnereau, A., Wang, S.S., Kelly, R.S., *et al.* (2013). Genome-wide association study identifies multiple risk loci for chronic lymphocytic leukemia. *Nature genetics* 45, 868-876.
- Billard, C. (2013). BH3 mimetics: status of the field and new developments. *Mol Cancer Ther* 12, 1691-1700.
- Catovsky, D., Richards, S., Matutes, E., Oscier, D., Dyer, M.J., Bezares, R.F., Pettitt, A.R., Hamblin, T., Milligan, D.W., Child, J.A., *et al.* (2007). Assessment of fludarabine plus cyclophosphamide for patients with chronic lymphocytic leukaemia (the LRF CLL4 Trial): a randomised controlled trial. *Lancet* 370, 230-239.
- Cooper, G.M., Stone, E.A., Asimenos, G., NISC Comparative Sequencing Program, Green, E.D., Batzoglou, S., and Sidow, A. (2005). Distribution and intensity of constraint in mammalian genomic sequence. *Genome Research* 15, 901-913.
- Crowther-Swanepoel, D., Broderick, P., Di Bernardo, M.C., Dobbins, S.E., Torres, M., Mansouri, M., Ruiz-Ponte, C., Enjuanes, A., Rosenquist, R., Carracedo, A., *et al.* (2010). Common variants at 2q37.3, 8q24.21, 15q21.3 and 16q24.1 influence chronic lymphocytic leukemia risk. *Nature genetics* 42, 132-136.
- Di Bernardo, M.C., Crowther-Swanepoel, D., Broderick, P., Webb, E., Sellick, G., Wild, R., Sullivan, K., Vijayakrishnan, J., Wang, Y., Pittman, A.M., *et al.* (2008). A genome-wide association study identifies six susceptibility loci for chronic lymphocytic leukemia. *Nature genetics* 40, 1204-1210.
- Encode Project Consortium (2012). An integrated encyclopedia of DNA elements in the human genome. *Nature* 489, 57-74.
- Ernst, J., and Kellis, M. (2012). ChromHMM: automating chromatin state discovery and characterization. *Nature methods* 9, 215-216.

Furman, R.R., Asgary, Z., Mascarenhas, J.O., Liou, H.-C., and Schattner, E.J. (2000). Modulation of NF- κ B Activity and Apoptosis in Chronic Lymphocytic Leukemia B Cells. *The Journal of Immunology* *164*, 2200-2206.

Gonzalez, D., Else, M., Wren, D., Usai, M., Buhl, A.M., Parker, A., Oscier, D., Morgan, G., and Catovsky, D. (2013). CLLU1 expression has prognostic value in chronic lymphocytic leukemia after first-line therapy in younger patients and in those with mutated IGHV genes. *Haematologica* *98*, 274-278.

Hanada, M., Delia, D., Aiello, A., Stadtmauer, E., and Reed, J.C. (1993). bcl-2 gene hypomethylation and high-level expression in B-cell chronic lymphocytic leukemia. *Blood* *82*, 1820-1828.

Hnisz, D., Abraham, B.J., Lee, T.I., Lau, A., Saint-Andre, V., Sigova, A.A., Hoke, H.A., and Young, R.A. (2013). Super-enhancers in the control of cell identity and disease. *Cell* *155*, 934-947.

Howie, B.N., Donnelly, P., and Marchini, J. (2009). A Flexible and Accurate Genotype Imputation Method for the Next Generation of Genome-Wide Association Studies. *PLoS Genetics* *5*, e1000529.

Knight, P.A., and Ruiz, D. (2013). A fast algorithm for matrix balancing. *IMA Journal of Numerical Analysis* *33*, 1029-1047.

Labi, V., Erlacher, M., Kiessling, S., Manzl, C., Frenzel, A., O'Reilly, L., Strasser, A., and Villunger, A. (2008). Loss of the BH3-only protein Bmf impairs B cell homeostasis and accelerates γ irradiation-induced thymic lymphoma development. *The Journal of Experimental Medicine* *205*, 641-655.

Mackus, W.J.M., Kater, A.P., Grummels, A., Evers, L.M., Hooijbrink, B., Kramer, M.H.H., Castro, J.E., Kipps, T.J., van Lier, R.A.W., van Oers, M.H.J., *et al.* (2005). Chronic lymphocytic leukemia cells display p53-dependent drug-induced Puma upregulation. *Leukemia* *19*, 427-434.

Morales, A.A., Gutman, D., Lee, K.P., and Boise, L.H. (2008). BH3-only proteins Noxa, Bmf, and Bim are necessary for arsenic trioxide-induced cell death in myeloma. *Blood* *111*, 5152-5162.

Oscier, D., Wade, R., Davis, Z., Morilla, A., Best, G., Richards, S., Else, M., Matutes, E., Catovsky, D., and Chronic Lymphocytic Leukaemia Working Group, U.K.N.C.R.I. (2010). Prognostic factors identified three risk groups in the LRF CLL4 trial, independent of treatment allocation. *Haematologica* *95*, 1705-1712.

Oscier, D.G., Rose-Zerilli, M.J., Winkelmann, N., Gonzalez de Castro, D., Gomez, B., Forster, J., Parker, H., Parker, A., Gardiner, A., Collins, A., *et al.* (2013). The clinical significance of NOTCH1 and SF3B1 mutations in the UK LRF CLL4 trial. *Blood* *121*, 468-475.

Puente, X.S., Bea, S., Valdes-Mas, R., Villamor, N., Gutierrez-Abril, J., Martin-Subero, J.I., Munar, M., Rubio-Perez, C., Jares, P., Aymerich, M., *et al.* (2015). Non-coding recurrent mutations in chronic lymphocytic leukaemia. *Nature advance online publication*.

Puthalakath, H., Villunger, A., O'Reilly, L.A., Beaumont, J.G., Coultas, L., Cheney, R.E., Huang, D.C.S., and Strasser, A. (2001). Bmf: A Proapoptotic BH3-Only Protein Regulated by Interaction with the Myosin V Actin Motor Complex, Activated by Anoikis. *Science* 293, 1829-1832.

Rao, S.S., Huntley, M.H., Durand, N.C., Stamenova, E.K., Bochkov, I.D., Robinson, J.T., Sanborn, A.L., Machol, I., Omer, A.D., Lander, E.S., *et al.* (2014). A 3D map of the human genome at kilobase resolution reveals principles of chromatin looping. *Cell* 159, 1665-1680.

Roberts, A.W., Davids, M.S., Pagel, J.M., Kahl, B.S., Puvvada, S.D., Gerecitano, J.F., Kipps, T.J., Anderson, M.A., Brown, J.R., Gressick, L., *et al.* (2016). Targeting BCL2 with Venetoclax in Relapsed Chronic Lymphocytic Leukemia. *N Engl J Med* 374, 311-322.

Scales, M., Jager, R., Migliorini, G., Houlston, R.S., and Henrion, M.Y. (2014). visPIG--a web tool for producing multi-region, multi-track, multi-scale plots of genetic data. *PLoS One* 9, e107497.

Scarfo, L., and Ghia, P. (2013). Reprogramming cell death: BCL2 family inhibition in hematological malignancies. *Immunology letters* 155, 36-39.

Shabalin, A.A. (2012). Matrix eQTL: ultra fast eQTL analysis via large matrix operations. *Bioinformatics* 28, 1353-1358.

Siepel, A., Bejerano, G., Pedersen, J.S., Hinrichs, A.S., Hou, M., Rosenbloom, K., Clawson, H., Spieth, J., Hillier, L.W., Richards, S., *et al.* (2005). Evolutionarily conserved elements in vertebrate, insect, worm, and yeast genomes. *Genome Research* 15, 1034-1050.

Slager, S.L., Rabe, K.G., Achenbach, S.J., Vachon, C.M., Goldin, L.R., Strom, S.S., Lanasa, M.C., Spector, L.G., Rassenti, L.Z., Leis, J.F., *et al.* (2011). Genome-wide association study identifies a novel susceptibility locus at 6p21.3 among familial CLL. *Blood* 117, 1911-1916.

Slager, S.L., Skibola, C.F., Di Bernardo, M.C., Conde, L., Broderick, P., McDonnell, S.K., Goldin, L.R., Croft, N., Holroyd, A., Harris, S., *et al.* (2012). Common variation at 6p21.31 (BAK1) influences the risk of chronic lymphocytic leukemia. *Blood* 120, 843-846.

Speedy, H.E., Di Bernardo, M.C., Sava, G.P., Dyer, M.J., Holroyd, A., Wang, Y., Sunter, N.J., Mansouri, L., Juliusson, G., Smedby, K.E., *et al.* (2014). A genome-wide association study identifies multiple susceptibility loci for chronic lymphocytic leukemia. *Nature genetics* 46, 56-60.

UK 10K Consortium (2015). The UK10K project identifies rare variants in health and disease. *Nature* 526, 82-90.

van de Werken, H.J.G., de Vree, P.J.P., Splinter, E., Holwerda, S.J.B., Klous, P., de Wit, E., and de Laat, W. (2012). Chapter Four - 4C Technology: Protocols and Data Analysis. In *Methods in enzymology*, W. Carl, and C.D. Allis, eds. (Academic Press), pp. 89-112.

van Dongen, J.J., Langerak, A.W., Bruggemann, M., Evans, P.A., Hummel, M., Lavender, F.L., Delabesse, E., Davi, F., Schuurink, E., Garcia-Sanz, R., *et al.* (2003). Design and standardization of PCR primers and protocols for detection of clonal immunoglobulin and T-cell receptor gene recombinations in suspect lymphoproliferations: report of the BIOMED-2 Concerted Action BMH4-CT98-3936. *Leukemia* 17, 2257-2317.

van Krieken, J.H., Langerak, A.W., Macintyre, E.A., Kneba, M., Hodges, E., Sanz, R.G., Morgan, G.J., Parreira, A., Molina, T.J., Cabecadas, J., *et al.* (2007). Improved reliability of lymphoma diagnostics via PCR-based clonality testing: report of the BIOMED-2 Concerted Action BHM4-CT98-3936. *Leukemia* 21, 201-206.

Ward, L.D., and Kellis, M. (2012). HaploReg: a resource for exploring chromatin states, conservation, and regulatory motif alterations within sets of genetically linked variants. *Nucleic acids research* 40, D930-934.

FIGURE LEGENDS

Figure 1. Genetic mapping and epigenetic landscape at the 15q15.1 locus.

(A) SNAP plot of the 15q15.1 chronic lymphocytic leukemia susceptibility locus. Genotyped (triangles) and imputed (dots) SNPs are shown based on their megabase chromosomal position on the x-axis and $-\log_{10} P$ -value on the y-axis. Color intensity of each symbol reflects the extent of linkage disequilibrium with rs539846 (white $r^2=0$ to dark red $r^2=1$). Recombination rates, estimated using HapMap samples of European ancestry, are shown by a light blue line. Physical positions are based on NCBI build 37 of the human genome. Also shown are relative gene positions and chromatin state segmentation (ChromHMM) for GM12878 derived from ENCODE project data.

(B) ChIP-seq data for H3K4Me1, H3K4Me3, H3K27Ac histone modifications and DNaseI hypersensitivity (HS) are illustrated for GM12878 from ENCODE (brown) and for CLL cells from the Blueprint Project (blue). ChIP-seq data for RELA in GM12878 is also shown. Data are shown relative to the genomic arrangement of *BMF* and were plotted in the UCSC Genome Browser. Also illustrated is the position weighted matrix for RELA and the motif sequence (underlined) altered by rs539846 (red box).

Figure 2. Contact profile of the *BMF* 4C viewpoint in combination with ChIP-seq data.

(A) Heatmap representing chromatin interactions in GM12878 cells at 40.32-40.52Mb on chromosome 15. Chromatin contact domains called by the Arrowhead algorithm are marked by white lines (Rao et al., 2014). The contact domain and CD19⁺ B-cell super-enhancer (Hnisz et al., 2013) encompassing rs539846 are labelled in pink and yellow, respectively.

(B) 4C-seq analyses in MEC1 CLL cells indicate the formation of a loop domain between rs539846 and *cis*-regulatory elements. The 4C viewpoint (green box) lies adjacent to rs539846 (dotted line). A 10kb masked region (grey box) is also marked. ENCODE ChIP-seq data from GM12878 cells show the correspondence between loop formation and CTCF and RELA transcription factor occupancy. Canonical transcripts and chromosome 15 position are also shown.

Figure 3. rs539846 affects RELA-bound enhancer activity

(A) Allele-specific constructs containing a 591bp putative regulatory sequence flanking rs539846 were cloned into the pGL3-promoter luciferase reporter vector.

(B) The ratio of luminescence from the experimental pGL3-rs539846 constructs to the Renilla internal control, pRL-SV40, was normalized to the empty pGL3-SV40 vector. Data shown are mean \pm standard error from 3 independent experiments performed in triplicate. Difference in expression was assessed by the Student's *t* test. The rs539846-A risk allele had significantly decreased enhancer activity over the protective allele.

(C) EMSA showing differential binding of MEC1 nuclear protein to the rs539846-C allele (protective) and the rs539846-A allele (risk). Binding of double-stranded A-allele and C-allele probes to MEC1 nuclear extract show a marked reduction of DNA-protein binding associated with the A-allele.

Figure 4. RELA expression and rs539846 genotype are correlated with *BMF* transcript levels

(A) siRNA knockdown of RELA reduces transcript levels of *BMF*. Data shown are mean \pm standard error for three independent replicates relative to GAPDH reference mRNA, normalized to control siRNA. *P*- values were determined with a two-tailed *t*-test.

(B) The rs539846 CLL risk allele (A) was associated with lower *BMF* mRNA levels in 462 patient samples. Boxplot indicates the median (horizontal line), first to third quartiles (box) and 1.5 times the interquartile range (whiskers) of *BMF* expression.