SUPPLEMENTARY MATERIAL

A genome-wide association study of IgM antibody against phosphorylcholine: shared genetics and phenotypic relation to chronic lymphocytic leukemia

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Figure S1. Individual genome-wide association studies of IgM anti-PC in the discovery phase

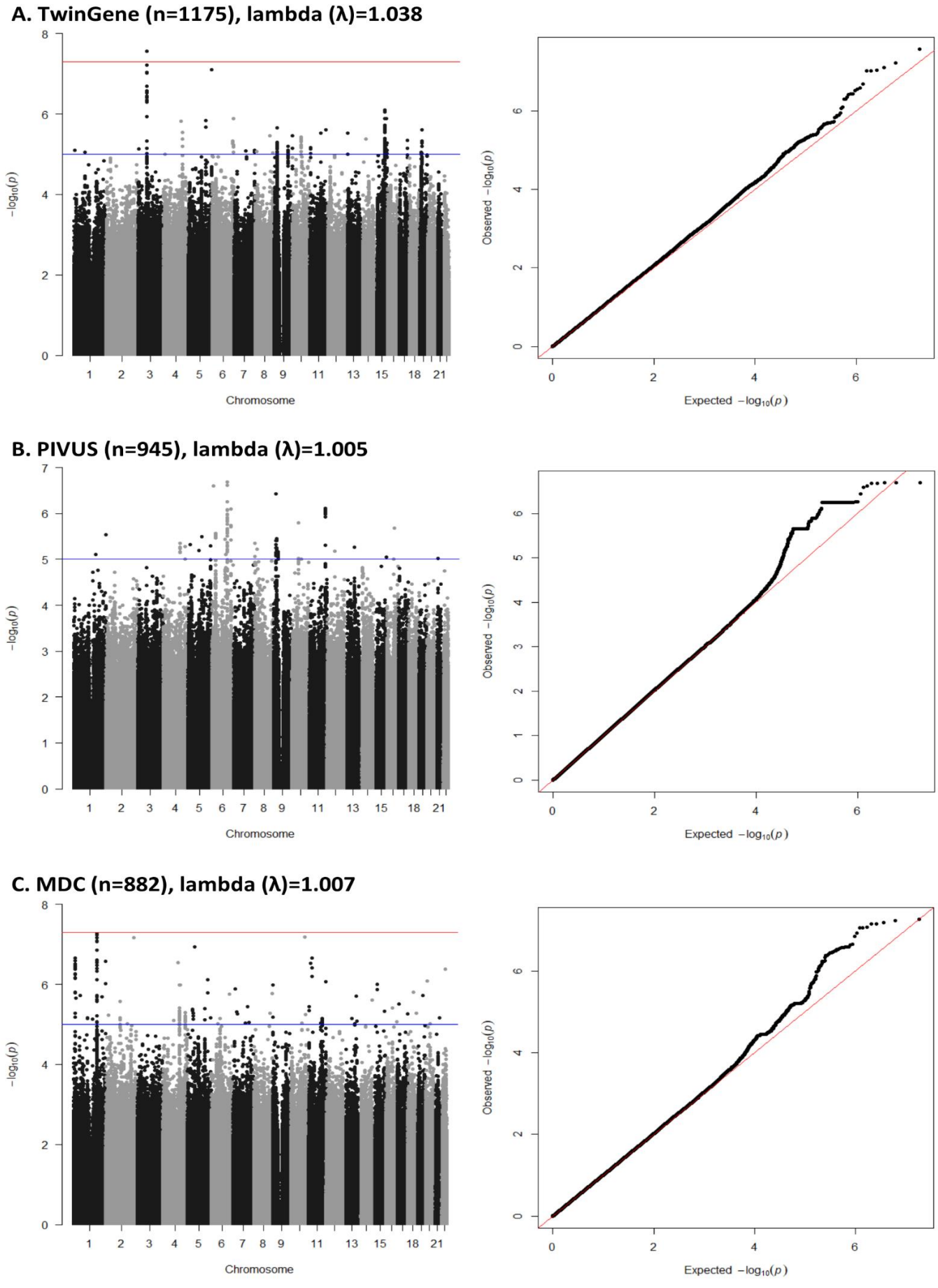


Figure S2. Marks of regulation in the LD block of 11q24.1 from UCSC Genome Browser

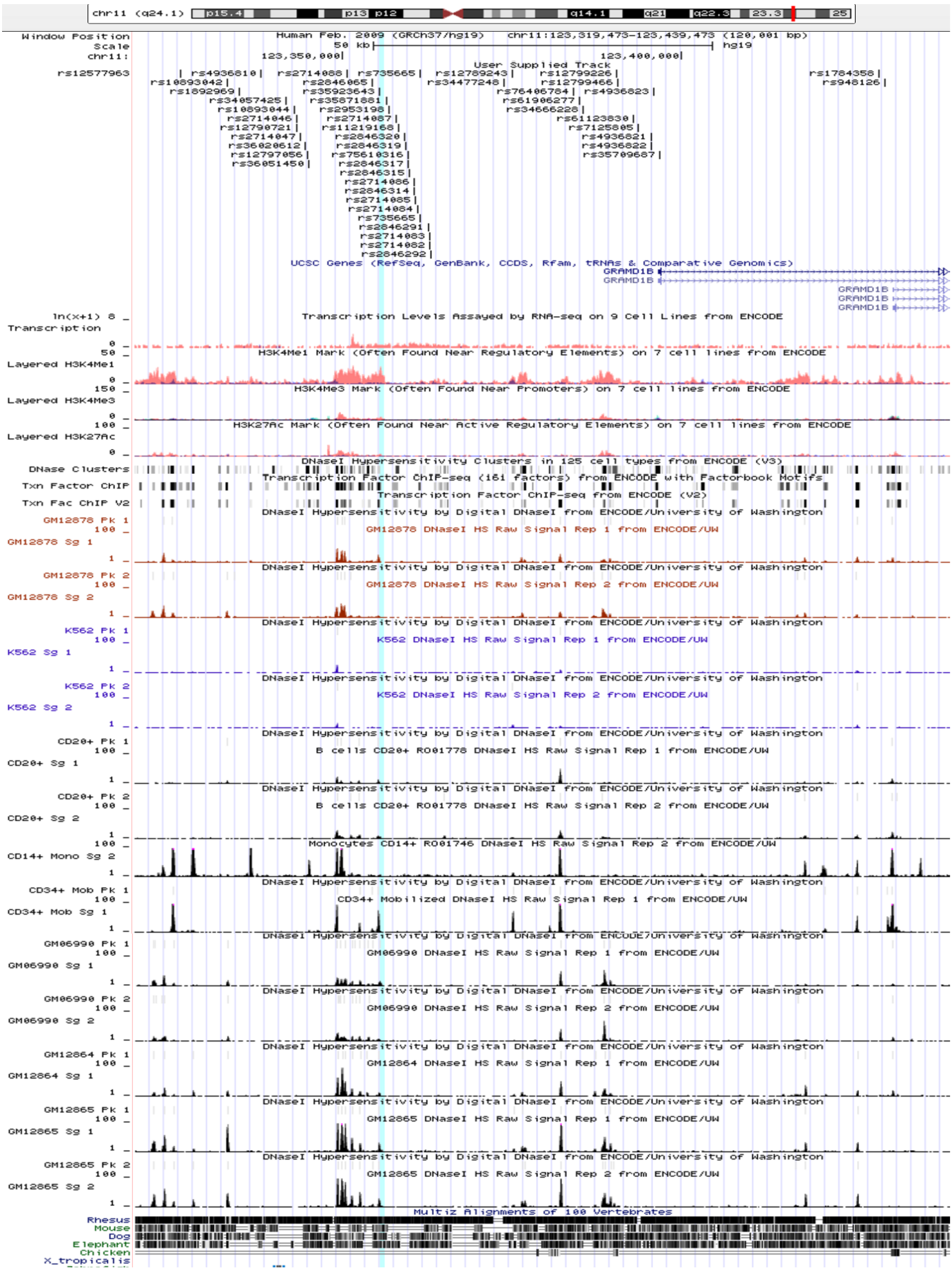


Figure S3. Functional prediction of rs35923643 in leukemia- or immune- cells

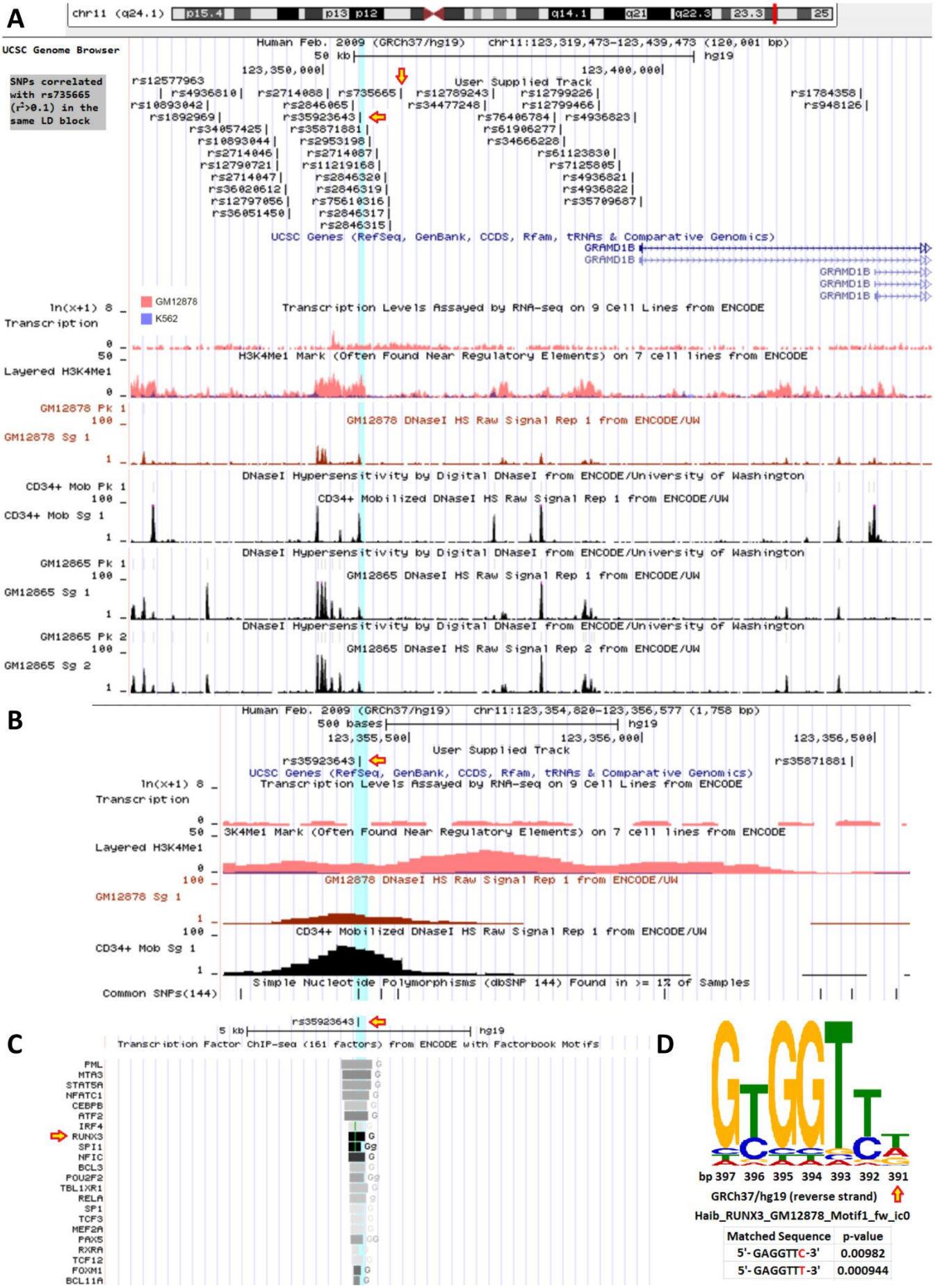
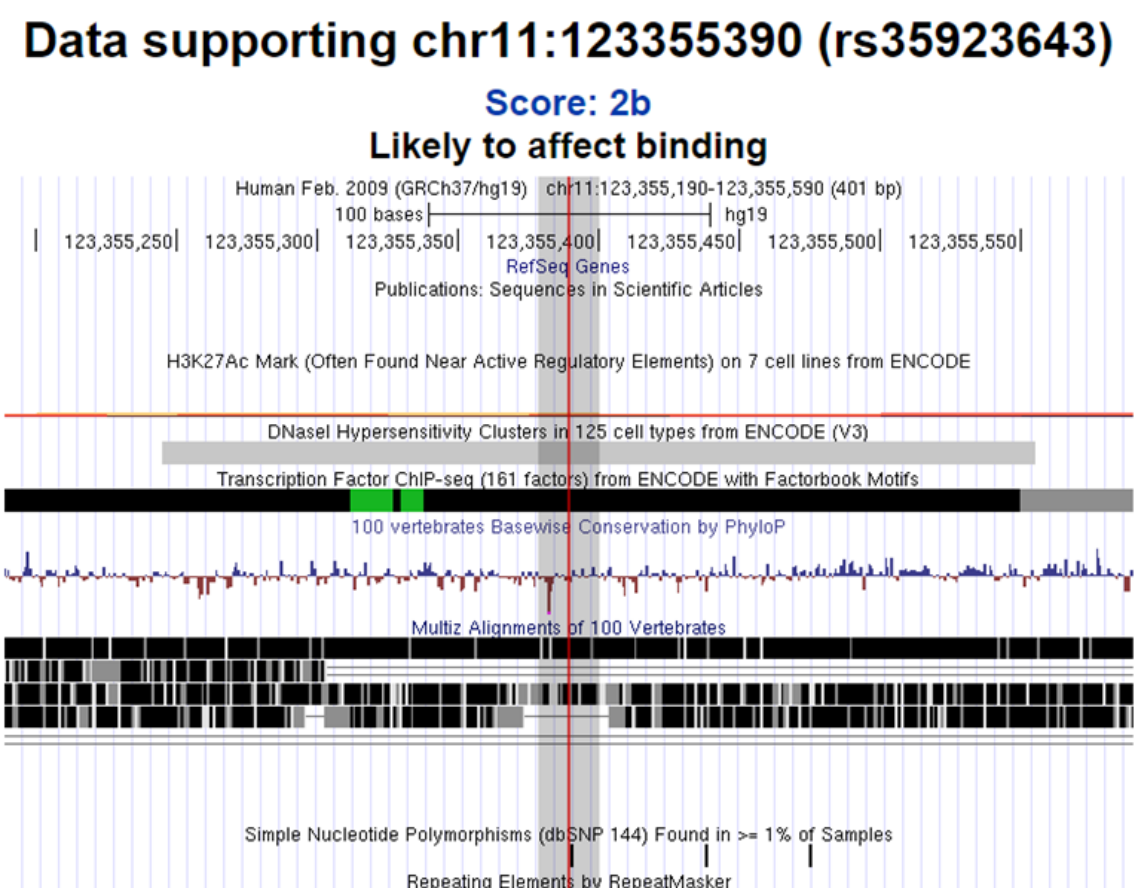


Figure S4. Evidence for affecting binding from RegulomeDB



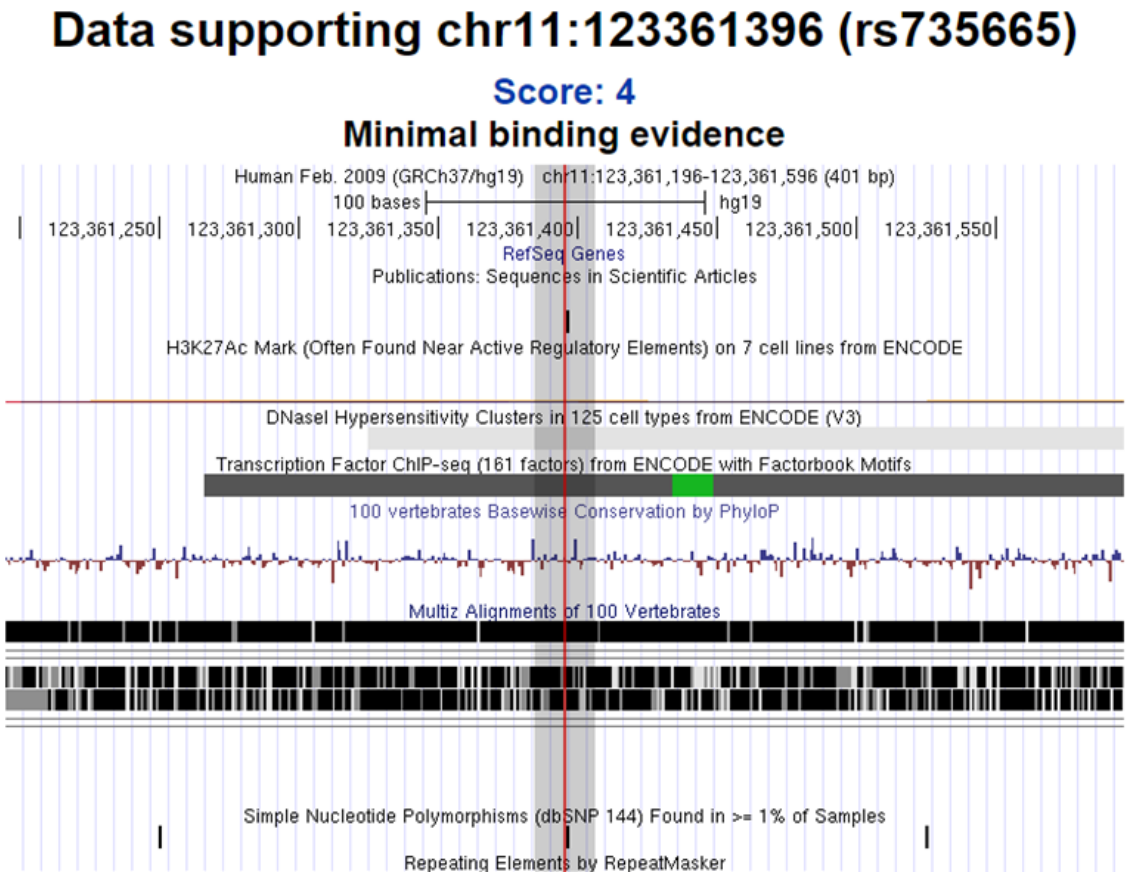


Figure S5. IgM anti-PC values before and after the first CLL diagnosis

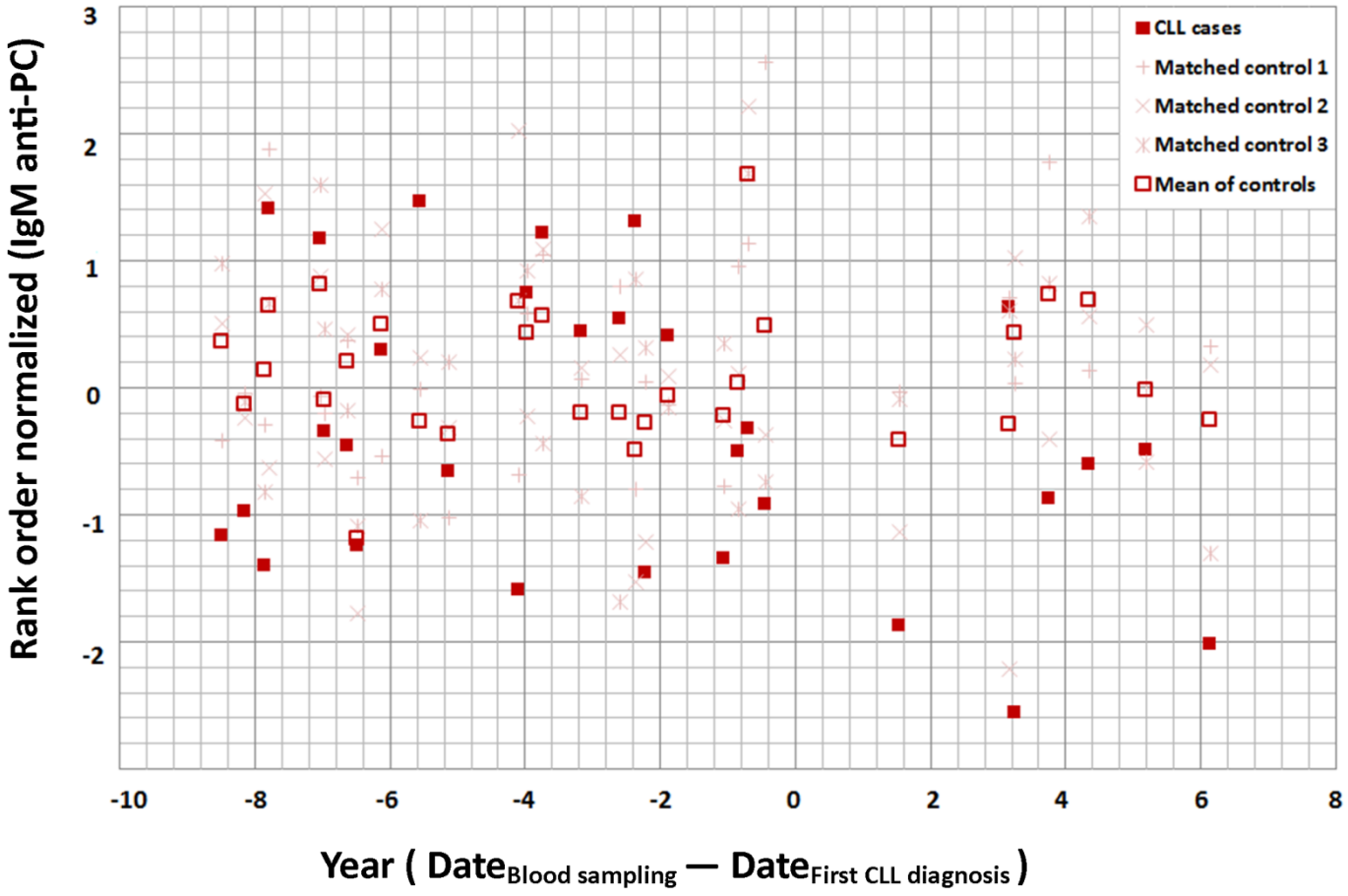


Figure S6. Global allele frequency of SNP rs735665 in the 1000 Genomes Project Phase 3 from Ensembl (GRCh37/hg19)

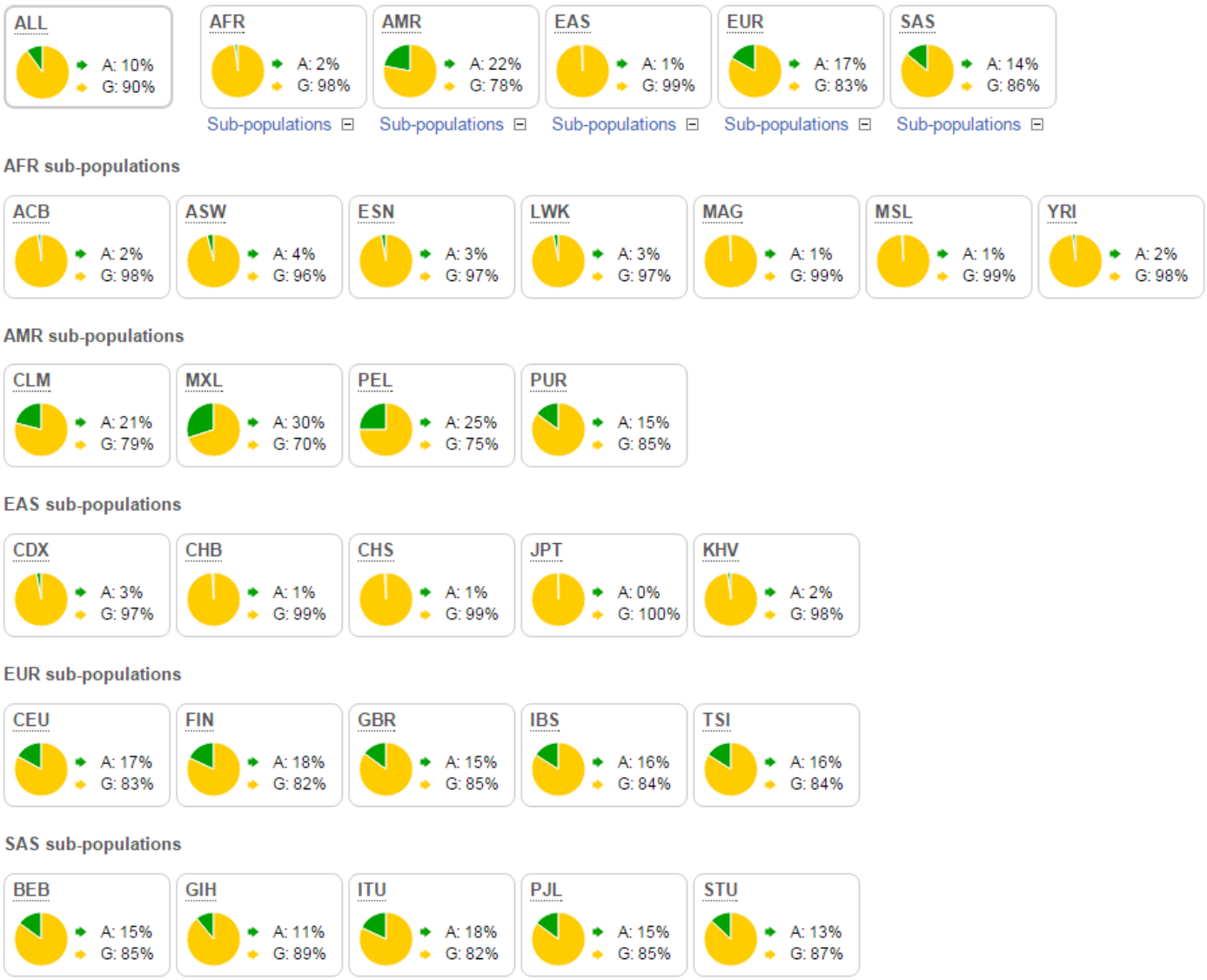
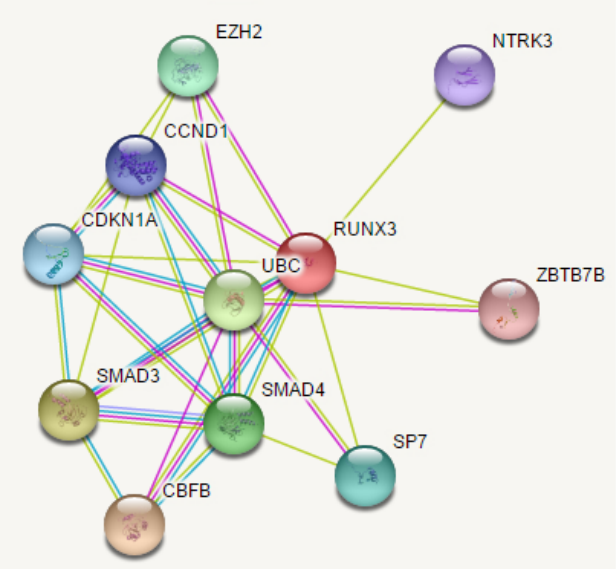


Figure S7. Functional partners with RUNX3 predicted from STRING database (version 10)



runt-related transcription factor 3; CBF binds to the core site, 5'-PYGPYGGT-3', of a number of enhancers and promoters, including murine leukemia virus, polyomavirus enhancer, T-cell receptor enhancers, lck, IL-3 and GM-CSF promoters

Identifier: ENSP00000343477, RUNX3
Organism: Homo sapiens

- show protein sequence
- homologs among STRING organisms
- Pathways, Functions, Resources (GeneCards)

Nodes:

Network nodes represent proteins

splice isoforms or post-translational modifications are collapsed, i.e. each node represents all the proteins produced by a single, protein-coding gene locus.

Node Size

small nodes: protein of unknown 3D structure

large nodes: some 3D structure is known or predicted

Node Color

colored nodes: query proteins and first shell of interactors

white nodes: second shell of interactors

Edges:

Edges represent protein-protein associations

associations are meant to be specific and meaningful, i.e. proteins jointly contribute to a shared function; this does not necessarily mean they are physically binding each other.

Known Interactions

from curated databases

experimentally determined

Predicted Interactions

gene neighborhood

gene fusions

gene co-occurrence

Others

textmining

co-expression

protein homology

Your Input:

		Neighborhood	Gene Fusion	Cooccurence	Coexpression	Experiments	Databases	Textmining	[Homology]	Score
RUNX3	*runt-related transcription factor 3; CBF binds to the core site, 5'-PYGPYGGT-3', of a number of enhancers and promoters, including murine leukemia virus, polyomavirus enhancer, T-cell receptor enhancers, lck, IL-3 and GM-CSF promoters (429 aa)*									

Predicted Functional Partners:

CBFB	*core-binding factor, beta subunit; CBF binds to the core site, 5'-PYGPYGGT-3', of a number of enhancers and promoters, includ...*					●	●	●		0.996
SMAD3	*SMAD family member 3 (425 aa)*					●	●	●		0.996
UBC	*ubiquitin C (685 aa)*					●				0.994
SMAD4	*SMAD family member 4; Common SMAD (co-SMAD) is the coactivator and mediator of signal transduction by TGF-beta (trans...*						●	●		0.987
EZH2	*enhancer of zeste homolog 2 (Drosophila) (751 aa)*					●		●		0.951
SP7	*Sp7 transcription factor; Transcriptional activator essential for osteoblast differentiation. Binds to SP1 and EKLF consensus s...*							●		0.947
CDKN1A	*cyclin-dependent kinase inhibitor 1A (p21, Cip1); May be the important intermediate by which p53/TP53 mediates its role as a...*							●		0.944
CCND1	*cyclin D1; Regulatory component of the cyclin D1-CDK4 (DC) complex that phosphorylates and inhibits members of the retino...*					●		●		0.939
NTRK3	*neurotrophic tyrosine kinase, receptor, type 3; Receptor for neurotrophin-3 (NT-3). This is a tyrosine- protein kinase receptor. K...*							●		0.933
ZBTB7B	*zinc finger and BTB domain containing 7B; Transcription regulator that acts as a key regulator of lineage commitment of imm...*							●		0.928

Figure S8. Potential functional pathways RUNX3 involved from STRING database

Network Stats

number of nodes: 11
number of edges: 29
average node degree: 5.27
clustering coefficient: 0.842

expected number of edges: 19
PPI enrichment p-value: 0.0195
your network has significantly more interactions than expected (what does that mean?)

Functional enrichments in your network

Note: some enrichments may be expected here (why?)

Biological Process (GO)

pathway ID	*pathway description*	*count in gene set*	*false discovery rate*
GO:0010604	positive regulation of macromolecule metabolic process	10	6e-05
GO:2000738	positive regulation of stem cell differentiation	4	6e-05
GO:0045786	negative regulation of cell cycle	6	0.000151
GO:0045930	negative regulation of mitotic cell cycle	5	0.000187
GO:0001932	regulation of protein phosphorylation	7	0.000273

(more ...)

Molecular Function (GO)

pathway ID	*pathway description*	*count in gene set*	*false discovery rate*
GO:0005072	transforming growth factor beta receptor, cytoplasmic mediator activity	2	0.0121
GO:0044212	transcription regulatory region DNA binding	5	0.0121
GO:0043565	sequence-specific DNA binding	5	0.0274
GO:0070412	R-SMAD binding	2	0.0274
GO:0016538	cyclin-dependent protein serine/threonine kinase regulator activity	2	0.0323

Cellular Component (GO)

pathway ID	*pathway description*	*count in gene set*	*false discovery rate*
GO:0071141	SMAD protein complex	2	0.00258
GO:0000790	nuclear chromatin	4	0.00452
GO:0000228	nuclear chromosome	4	0.00902
GO:0000307	cyclin-dependent protein kinase holoenzyme complex	2	0.00902
GO:0031981	nuclear lumen	8	0.00902

KEGG Pathways

pathway ID	*pathway description*	*count in gene set*	*false discovery rate*
05220	Chronic myeloid leukemia	3	0.00171
04068	FoxO signaling pathway	3	0.00304
04110	Cell cycle	3	0.00304
05161	Hepatitis B	3	0.00304
05206	MicroRNAs in cancer	3	0.00304

(more ...)

PFAM Protein Domains

pathway ID	*pathway description*	*count in gene set*	*false discovery rate*
PF03166	MH2 domain	2	0.00471
PF03165	MH1 domain	2	0.00589

INTERPRO Protein Domains and Features

pathway ID	*pathway description*	*count in gene set*	*false discovery rate*
IPR001132	SMAD domain, Dwarfin-type	2	0.00336
IPR013019	MAD homology, MH1	2	0.00336
IPR013790	Dwarfin	2	0.00336
IPR003619	MAD homology 1, Dwarfin-type	2	0.00503
IPR017855	SMAD domain-like	2	0.00503

(more ...)

Statistical background

For the above enrichment analysis, the following statistical background is assumed: Whole Genome ▼ UPDATE

Table S1. Genome-wide significant SNPs in the discovery GWAS meta-analysis of IgM anti-PC

SNP	CHR	POS	A1	A2	FREQ_ A1	INFO	Beta	SE	I^2(%)	Phet	P-value
rs35871881	**11**	**123356451**	**G**	**A**			**0.174**	**0.031**	**61.2**	**0.08**	**1.95e-08**
TwinGene			G	A	0.250	0.986	0.158	0.049			0.001
PIVUS			G	A	0.241	0.970	0.263	0.053			7.72e-07
MDC			G	A	0.215	0.969	0.086	0.059			0.145
rs12798553	**11**	**123353693**	**A**	**G**			**0.174**	**0.031**	**61.0**	**0.08**	**1.98e-08**
TwinGene			A	G	0.248	0.980	0.160	0.049			0.001
PIVUS			A	G	0.240	0.969	0.263	0.053			8.58e-07
MDC			A	G	0.215	0.967	0.085	0.059			0.151
rs35923643	**11**	**123355391**	**G**	**A**			**0.177**	**0.032**	**53.9**	**0.11**	**2.51e-08**
TwinGene			G	A	0.230	0.999	0.153	0.050			0.002
PIVUS			G	A	0.225	0.980	0.263	0.054			1.18e-06
MDC			G	A	0.188	0.987	0.101	0.061			0.097
rs141373354	**1**	**68736970**	**A**	**G**			**0.578**	**0.104**	**0.0**	**0.71**	**2.60e-08**
TwinGene			A	G	0.980	0.910	0.625	0.159			9.22e-05
PIVUS			A	G	0.986	0.901	0.658	0.203			0.001
MDC			A	G	0.980	0.913	0.457	0.181			0.011
rs116361873	**1**	**68728886**	**G**	**A**			**0.576**	**0.104**	**0.0**	**0.68**	**2.99e-08**
TwinGene			G	A	0.981	0.918	0.626	0.159			8.83e-05
PIVUS			G	A	0.986	0.910	0.660	0.202			0.001
MDC			G	A	0.981	0.935	0.446	0.181			0.014
rs735665	**11**	**123361397**	**A**	**G**			**0.174**	**0.031**	**54.3**	**0.11**	**3.12e-08**
TwinGene			A	G	0.232	NA	0.145	0.050			0.004
PIVUS			A	G	0.229	NA	0.261	0.053			1.05e-06
MDC			A	G	0.188	NA	0.103	0.060			0.089
rs36020612	**11**	**123344435**	**T**	**C**			**0.182**	**0.033**	**61.1**	**0.08**	**3.73e-08**
TwinGene			T	C	0.222	0.963	0.161	0.052			0.002
PIVUS			T	C	0.218	0.909	0.279	0.057			9.47e-07
MDC			T	C	0.186	0.900	0.090	0.064			0.156
rs36051450	**11**	**123344959**	**C**	**T**			**0.179**	**0.033**	**60.6**	**0.08**	**4.26e-08**
TwinGene			C	T	0.224	0.962	0.158	0.052			0.002
PIVUS			C	T	0.221	0.932	0.274	0.056			1.00e-06
MDC			C	T	0.187	0.924	0.089	0.063			0.154

CHR: chromosome number; POS: position of the SNP in human genome (GRCh37/hg19); A1: effect allele; A2: non-effect allele; FREQ_A1: frequency of effect allele; INFO: imputation quality info; Beta: effect size per standard deviation of rank order normalized IgM anti-PC per allele; SE: standard error; I^2: the percentage of variation across studies that is due to heterogeneity rather than chance; Phet: P-value for heterogeneity; NA: not available, because SNP was directly genotyped; PIVUS: Prospective Investigation of the Vasculature in Uppsala Seniors; MDC: Malmö Diet and Cancer study.

Table S2. Replication results for the eight genome-wide significant SNPs in PRACSIS

SNP	CHR	POS	A1	A2	FREQ_ A1	INFO	Beta	SE	P-value
rs35871881	11	123356451	G	A	0.230	0.953	0.184	0.067	0.0064
rs12798553	11	123353693	A	G	0.229	0.946	0.185	0.068	0.0064
rs35923643	11	123355391	G	A	0.205	0.975	0.247	0.069	0.0004
rs141373354	1	68736970	A	G	0.982	0.883	0.305	0.219	0.1644
rs116361873	1	68728886	G	A	0.982	0.904	0.306	0.219	0.1640
rs735665	11	123361397	A	G	0.206	0.993	0.244	0.068	0.0004
rs36020612	11	123344435	T	C	0.199	0.933	0.250	0.072	0.0005
rs36051450	11	123344959	C	T	0.201	0.947	0.250	0.071	0.0004

CHR: chromosome number; POS: position of the SNP in human genome (GRCh37/hg19); A1: effect allele; A2: non-effect allele; FREQ_A1: frequency of effect allele; INFO: imputation quality info; Beta: effect size per standard deviation of rank order normalized IgM anti-PC per allele; SE: standard error.

Table S3. Meta-analysis for the successfully replicated SNPs (sorted by association P-value)

SNP (Position)	Study	Info	Beta	SE	P-value	Phet
rs35923643 G/A	Meta		0.189	0.029	4.34×10^{-11}	0.159
(Chr11: 123355391)	*TwinGene*	0.999	0.153	0.050	0.002	
	PIVUS	0.980	0.263	0.054	1.18×10^{-6}	
	MDC	0.987	0.101	0.061	0.097	
	PRACSIS	0.975	0.247	0.069	0.0004	
rs735665 A/G	Meta		0.186	0.028	5.03×10^{-11}	0.150
(Chr11: 123361397)	*TwinGene*	NA	0.145	0.050	0.004	
	PIVUS	NA	0.261	0.053	1.05×10^{-6}	
	MDC	NA	0.103	0.060	0.089	
	PRACSIS	0.993	0.244	0.068	0.0004	
rs36020612 T/C	Meta		0.193	0.030	1.06×10^{-10}	0.118
(Chr11: 123344435)	*TwinGene*	0.963	0.161	0.052	0.002	
	PIVUS	0.909	0.279	0.057	9.47×10^{-7}	
	MDC	0.900	0.090	0.064	0.156	
	PRACSIS	0.933	0.250	0.072	0.0005	
rs36051450 C/T	Meta		0.191	0.030	1.09×10^{-10}	0.116
(Chr11: 123344959)	*TwinGene*	0.962	0.158	0.052	0.002	
	PIVUS	0.932	0.274	0.056	1.00×10^{-6}	
	MDC	0.924	0.089	0.063	0.154	
	PRACSIS	0.947	0.250	0.071	0.0004	
rs12798553 A/G	Meta		0.176	0.028	3.17×10^{-10}	0.158
(Chr11: 123353693)	*TwinGene*	0.980	0.160	0.049	0.001	
	PIVUS	0.969	0.263	0.053	8.58×10^{-7}	
	MDC	0.967	0.085	0.059	0.151	
	PRACSIS	0.946	0.185	0.068	0.0064	
rs35871881 G/A	Meta		0.176	0.028	3.25×10^{-10}	0.160
(Chr11: 123356451)	*TwinGene*	0.986	0.158	0.049	0.001	
	PIVUS	0.970	0.263	0.053	7.72×10^{-7}	
	MDC	0.969	0.086	0.059	0.145	
	PRACSIS	0.953	0.184	0.067	0.0064	

SNP is presented with effect allele/alternative allele (chromosome number and position in human genome GRCh37/ hg19). Info: imputation quality; NA: not available, because SNP is directly genotyped; Beta: effect size per standard deviation of rank order normalized IgM anti-PC per allele; SE: standard error; Phet: P-value for heterogeneity; The two SNPs (rs141373354 and rs116361873) on chromosome 1 are not successfully replicated in PRACSIS, association P-values are 0.1644 and 0.1640, respectively.

Table S4. Levels of IgM anti-PC among genotypes of rs735665 and rs35923643

		Discovery phase			Replication
		TwinGene	*PIVUS*	*MDC*	*PRACSIS*
Genotypes of rs735665	**N_{GG}**	697	568	556	407
	N_{GA}	411	322	254	212
	N_{AA}	67	55	33	27
Raw values of IgM anti-PC	**GG**	40.3 (23.2-67.2)	39.5 (24.8-68.1)	46.0 (28.6-75.1)	31.9 (19.7-56.3)
	GA	48.7 (23.8-83.2)	45.8 (28.9-75.6)	51.1 (32.8-75.5)	39.7 (24.1-71.0)
	AA	40.6 (21.1-78.6)	52.0 (34.6-110.9)	58.6 (34.4-78.1)	47.1 (28.9-59.7)
Normalized values of IgM anti-PC in GWAS	**GG**	-0.07±0.99	-0.11±0.98	-0.05±0.99	-0.11±1.01
	GA	0.14±1.03	0.13±0.99	0.05±1.00	0.18±0.98
	AA	0.01±1.08	0.43±1.09	0.22±0.86	0.23±0.69
Genotypes of rs35923643	**N_{AA}**	705	564	583	409
	N_{AG}	403	312	266	210
	N_{GG}	67	53	33	27
Raw values of IgM anti-PC	**AA**	40.0 (23.1-66.8)	39.4 (24.8-68.1)	47.2 (28.9-75.5)	31.9 (19.6-56.2)
	AG	50.7 (24.0-83.9)	46.8 (29.1-76.3)	51.7 (32.8-77.4)	39.7 (24.1-71.0)
	GG	40.6 (21.1-78.6)	49.8 (34.6-107.9)	58.6 (34.4-78.1)	47.1 (28.9-59.7)
Normalized values of IgM anti-PC in GWAS	**AA**	-0.08±0.99	-0.11±0.98	-0.04±1.00	-0.11±1.01
	AG	0.16±1.03	0.14±0.99	0.05±1.01	0.18±0.98
	GG	0.01±1.08	0.41±1.10	0.22±0.86	0.23±0.69

PIVUS: Prospective Investigation of the Vasculature in Uppsala Seniors; MDC: Malmö Diet and Cancer study; PRACSIS: Prognosis and Risk in Acute Coronary Syndromes in Sweden.

Table S5. Polygenic risk score analyses between immunoglobulins and CLL risk

Base — Target	P_T	N_{SNPs}	OR	SE	r^2	P-value
IgA — CLL	5.0e-15	2	1.06	0.17	0.00%	0.75
	5.0e-10	10	0.89	0.11	0.01%	0.29
	5.0e-09	22	0.87	0.09	0.02%	0.12
	5.0e-08*	**31**	**0.88**	**0.08**	**0.02%**	**0.11**
	5.0e-07	41	0.93	0.07	0.00%	0.31
	1.0e-06	45	0.91	0.07	0.02%	0.20
IgG — CLL	5.0e-15	5	0.69	0.10	0.12%	0.007
	5.0e-10	10	0.68	0.10	0.14%	0.0003
	5.0e-09	12	0.67	0.10	0.15%	0.0001
	5.0e-08	17	0.73	0.09	0.12%	0.0004
	5.0e-07*	**20**	**0.68**	**0.09**	**0.18%**	**3.9e-06**
	1.0e-06	22	0.67	0.09	0.20%	1.2e-05
IgM — CLL	5.0e-15	5	0.84	0.16	0.01%	0.27
	5.0e-10	18	0.70	0.09	0.14%	0.0001
	5.0e-09	33	0.72	0.07	0.19%	5.3e-06
	5.0e-08*	**43**	**0.69**	**0.07**	**0.28%**	**4.2e-08**
	5.0e-07	59	0.79	0.06	0.14%	0.0001
	1.0e-06	66	0.75	0.06	0.23%	8.4e-07
IgM anti-PC — CLL	**5.0e-08***	**1**	**17.55**	**0.36**	**0.59%**	**1.2e-15**
	5.0e-07*	**1**	**17.55**	**0.36**	**0.59%**	**1.2e-15**
	1.0e-06*	**1**	**17.55**	**0.36**	**0.59%**	**1.2e-15**
	5.0e-06	6	1.87	0.11	0.31%	7.3e-09
	5.0e-05	39	1.11	0.04	0.05%	0.02
	5.0e-04	386	1.00	0.02	0.00%	0.87
	0.005	3237	1.00	0.01	0.00%	0.90
	0.05	22405	1.02	0.003	0.24%	3.1e-07
	0.5	137132	1.01	0.002	0.10%	0.001

Standardized polygenic risk score (PRS) of the base phenotype was used to predict the target phenotype. P_T: P-value threshold of the association between single nucleotide polymorphisms (SNPs) and the base phenotype, * means the best P_T that defined as the threshold corresponding to the largest explained variance; N_{SNPs}: number of independent SNPs included in the best P_T quantile; OR: odds ratio per standard deviation; SE: standard error; r^2: Nagelkerke r^2, the proportion of target variation explained by PRS of SNPs in the best P_T quantile.

Table S6. Signal for each transcription factor and matched sequence from FIMO

TF	Signal	Motif	Strand	Start	End	P-value	Q-value	Matched Sequence
ATF2	487	M1	-	389	399	0.00986	1	CTGAGGTTTTG
BCL11A	426	×						
BCL3	202	×						
CEBPB	264	×						
FOXM1	549	×						
IRF4	160	M2	+	381	395	0.00665	1	CCAACCCCCAAAACC
		M3	+	384	398	0.00369	1	ACCCCCAAAACCTCA
MEF2A	144	×						
MTA3	496	×						
NFATC1	331	×						
NFIC	732	M4	-	386	391	0.00862	0.528	TTGGGG
PAX5	388	M5	-	378	397	0.00555	0.499	GAGGTTTTGGGGGTTGGAGG
PML	319	×						
POU2F2	327	×						
RELA	228	×						
RUNX3	1000	M6	-	391	397	0.000944	0.216	GAGGTTT
		M7	+	391	400	0.00029	0.208	AAACCTCAGA
		M7	+	382	391	0.00409	0.602	CAACCCCCAA
		M8	+	384	399	0.000894	0.301	ACCCCCAAAACCTCAG
		M8	+	383	398	0.00279	0.335	AACCCCCAAAACCTCA
		M8	+	376	391	0.00289	0.335	CCCCTCCAACCCCCAA
		M9	+	382	399	4.5e-05	0.0607	CAACCCCCAGAACCTCAG
		M9	+	382	399	1.86e-05	0.0251	CAACCCCCAAAACCTCAG
		M9	+	391	408	0.00673	0.479	AAACCTCAGAAATTGGCT
		M10	+	391	400	0.000414	0.229	AAACCTCAGA
		M10	+	383	392	0.000972	0.341	AACCCCCAAA
		M10	+	382	391	0.00156	0.369	CAACCCCCAA
RXRA	131	M11	-	382	396	0.0012	0.423	AGGTTTTGGGGGTTG
		M11	-	381	395	0.00358	0.834	GGTTTTGGGGGTTGG
		M12	-	389	405	0.00907	0.699	CAATTTCTGAGGTTTTG
SP1	156	×						
SPI1	1000	M13	+	314	328	8.52e-06	0.0117	ATGAGGAACAGAGAG
		M14	+	314	324	0.000166	0.226	ATGAGGAACAG
STAT5A	357	M15	-	375	390	0.00373	0.367	TGGGGGTTGGAGGGGG
		M15	-	385	400	0.00495	0.367	TCTGAGGTTTTGGGGG
		M15	-	387	402	0.00503	0.367	TTTCTGAGGTTTTGGG
		M15	-	377	392	0.00982	0.431	TTTGGGGGTTGGAGGG
TBL1XR1	220	×						
TCF12	154	×						
TCF3	131	×						

The position of rs35923643 (at the 391bp of submitted sequence) is labeled in red. M1: Haib_ATF2_H1-hESC_ Motif1 _fw_ic0; M2: IRF4_full_TCAAGG20NCG_AD_NCGAAACCGAAACYN_fw_ic0; M3: Irf4_fw_ic0; M4: MA0161_NFIC_fw _ic0; M5: MA0014_Pax5_fw_ic0; M6: Haib_RUNX3_GM12878_Motif1_fw_ic0; M7: RUNX3_ DBD_TACGGA30NGGC_AI_NAACCGCAAN_fw_ic0; M8: RUNX3_DBD_TACGGA30NGGC_AI_NA ACCGC AAACCRCAN_fw_ic0; M9: RUNX3_DBD_TACGGA30NGGC_AI_WAACCRCAAWAACCRCAN_fw_ ic0; M10: RUNX3_full_TCTCCC20NGA_AE_NAACCRCAAN_fw_ic0; M11: MA0074_RXRA_VDR_fw_ic0; M12: MA0115_NR1H2_RXRA_fw_ic0; M13: Haib_SPI1_GM12878_Motif1_fw_ic0; M14: Haib_SPI1_K562_Motif1_ fw_ic0; M15: Haib_STAT5A _GM12878_Motif1_fw_ic0.