SUPPLEMENTARY METHODS

Human primary samples and cell lines

Non-tumoral fresh human tonsils were obtained with the approval of the Institutional Review Boards in accordance with the Declaration of Helsinki. Tissues were either fixed and embedded in paraffin, or submitted to magnetic-based separation of IgD^+, $CD27^+$ and $CD71^+$ cells (Miltenyi Biotec). The purity of the isolated cells was higher than 90% as measured by FACS. The human GC-like DLBCL cell line OCI-Ly1 was grown in medium containing 90% Iscove's medium (Cellgro), 10% FBS (Gemini Bio-Products) and 1% penicillin/streptomycin (Gibco).

Immunohistochemistry and Immunofluorescence

Slides were deparaffinized and antigen retrieved in 1mM EDTA pH 8.0 using steaming for 30 min. Indirect IHC was performed and developed by DAB color substrates (Vector Laboratories) as previously described[10]. Primary antibodies against CD3, CD79α, CD68, CD21, IgD, FOXP1, peanut agglutinin (PNA), and BCL6 are listed in Table S3. Slides were viewed with a light microscope (AxioSkop2; Carl Zeiss Microimaging, Inc.) using 10X/0.50, 20X/0.50, 40X/0.90 or 100X/1.30 Plan Neofluar objective lenses. Images were taken using a color camera (AxioCam, Carl Zeiss) and processed using Axiovision software (Carl Zeiss).

Western blot

Cells were lysed in RIPA buffer. Equal amounts of total protein were separated by SDS-PAGE and transferred to a nitrocellulose membrane (Amersham Biosciences). Primary antibodies against FOXP1, BCL6 and actin are listed in Table S3. The signal from HRP-conjugated secondary antibodies was developed using Lumi-Light PLUS Western Blotting Substrate (Roche).

Array-based chromatin immunoprecipitation (ChIP-on-chip)

A total of 50 x 10^6 OCI-Ly1 cells were cross-linked with 1% formaldehyde. After sonication, immunoprecipitations were performed using 10μg Rabbit polyclonal antibody against FOXP1 (Abcam ab16645) or normal rabbit IgG antibody (Sigma–Aldrich). After validation of the enrichment by Q-ChIP, FOXP1 ChIP products and their respective input genomic fragments were amplified by ligation-mediated PCR. The products were cohybridized with the respective input samples to NimbleGen promoter arrays representing 1.5 kb of promoter sequence from 24,275 genes with 10 oligonucleotides each (human genome v. 35, May 2004) according to the manufacturer's protocol (NimbleGen Systems). Three replicates were performed (GEO Series GSE44243).

Computational analyses

Following our previously described strategy[30,31], we assayed moving averages of log-ratio of any 3 neighboring probes, calculated a background control as the random permutation of the log ratios of all probes, and determined a maximum value for each gene promoter. The cut-off for each array was established as higher than 99th percentile of the 24175 log-ratio values generated from random permutation probes. A locus with maximum moving average above cut-offs in two replicates was considered a potential binding site. Since this high stringency overlapping approach produces higher false negative rates, we also computed the correlations among peaks between the replicates as a way to rescue promoters that did not pass the cut-off in one replicate. High correlation between the signals of promoter demonstrates that the probes in each promoter follow a similar pattern, indicating the presence of an underestimated peak. Therefore, we calculated the Pearson correlation coefficient of the probes signal of the promoter between replicates, and promoters with a correlation higher that 0.8 were rescued and

included in our final set of FOXP1 targets. Ingenuity Pathway Analysis (IPA) determined the most significantly enriched biological functions and/or related diseases (www.ingenuity.com). De novo motif analysis was performed using the FIRE motif discovery program[32]. The over-representation of FIRE-discovered motifs in different gene sets was assessed using the hypergeometric distribution. Only motif matches with CompareACE scores > 0.8 were reported (1.0 being the maximum score achievable). To investigate the expression levels in B cells of FOXP1 target genes we analyzed a large cDNA microarray-based gene expression dataset, which we published previously[33] (GEO Series GSE25639). Normal primary samples (Figure 2 and GSE25639[33]) were hybridized to Affymetrix Human Genome U133 Plus 2.0 Array, and DLBCL samples (Figure S2 and www.broad.mit.edu/cancer/pub/dlbcl[42]) were hybridized to Affymetrix HT_HG-U133A/B Arrays, which contain 9 and 8 probes to detect FOXP1 transcripts, respectively. All probes contribute to determine overall FOXP1 expression in our analyses. R and Bioconductor were used for preprocessing and statistical analysis (www.r-project.org), and the Genesis software (Institute for Genomics and Bioinformatics, Graz University of Technology) was used to visualize and cluster FOXP1 target genes expression data.

Real time PCR analyses

Total RNA was extracted from harvested cells using TRIzol (Invitrogen) as described in manufacturer´s protocol. RNA was converted into cDNA using random hexanucleotides and M-MLV reverse transcriptase (Promega). Quantitative RT-PCR (QRT-PCR) was performed with SYBR green (Applied Biosystems) in an ABI 7900 HT real-time thermal cycler (Applied Biosystems). cDNAs were tested in replicates, and data was normalized to the *GAPDH* housekeeping gene, and presented either as $2^{-\Delta Ct}$ or as relative to control ($2^{-\Delta\Delta Ct}$). GLTs expression analyses were normalized to Igβ levels and represented relative to those of

unstimulated WT cells. For quantitative ChIP (Q-ChIP), DNA fragments enriched by ChIP as described before were quantified in the same ABI 7900 HT real-time thermal cycler. Input DNA was used for Ct normalization. IgG isotype control and the *PRR20A* gene, which is not a FOXP1 target, were used as negative controls. All primers used in these experiments are listed in Table S3.

siRNA knockdown experiments

Targeting of the 3'UTR region of *hFOXP1* (Dharmacon) with two different siRNAs: siRNA1 (5'-CAACTTAGCCAGCGCAATA-3') or siRNA2 (5'-GCCAAGGCCTTCTGACAATT-3'), was able to knock-down FOXP1 effectively 48 hours after siRNA transfection as measured by QRT-PCR. After transfections, expression profiles were normalized to untreated cells and differences were represented as fold-changes relative to the scramble control.

Mice

Transgenic mice over-expressing the human *FOXP1* cDNA (NM_032682.5) under the control of Eμ enhancer and SR-alpha mouse promoter were developed through pronuclear DNA injection technology in the C57BL/6 mouse strain (www.genoway.com). The pEμSR vector was kindly provided by Dr. Jerry Adams (WEHI, Melbourne, Australia). Mice were characterized for transgene integration by PCR and Southern blot analysis (see primers in Table S3), crossed onto the C57BL/6 genetic background and housed in specific pathogen-free animal facility. All experiments were conducted with protocols approved by the Ethical Committee of Animal Experimentation of the University of Navarra.

Immunizations

Series of 2- to 8-month-old mice were immunized intraperitoneally with 0.5 x 10^9 sheep red blood cells (SRBCs) (Innovative research). Generally, SRBC immunizations were performed on day 0 and day 7, and animals were sacrificed at day 14. For FACS analysis of GC B cell frequencies, animals were immunized with SRBCs only once at day 0 and then sacrificed at day 14. To study T-dependent immune responses, 2-month-old mice were immunized intraperitoneally with 100 μg of $NP_{(36)}$-CGG (NP-Chicken Gamma Globulin) (Biosearch Technologies) on day 0 and were re-challenged with the same antigen 4 weeks later. At the indicated times after immunization, mice were bled or sacrificed.

Flow cytometry and cell sorting

Murine bone marrow cells, peripheral blood cells, splenocytes and thymocytes were analyzed by flow cytometry using fluorochrome-conjugated antibodies for the following surface proteins: CD4, CD8, CD21, CD23, CD25, CD45 (B220), CD95/Fas, CD117(cKit), GL-7, IgD, IgM, IgG1 (BD or Biolegend), IgG3 (Southern Biotech) and PNA (Vector Laboratories). PI was used for the exclusion of dead cells. Data were acquired on FACSCalibur flow cytometer (BD) and analyzed using FlowJo software. Sorting of murine $CD19^+$ and $Gr1^+$ splenic cells, or thymic CD3+ cells was performed through magnetic sorting (MiltenyiBiotec). FACSAria IIu sorter (BD) was used to sort ~5x10^5 murine GC splenic B cells ($B220^+Fas^+GL7^{hi}$) and perform transcriptional analysis.

CSR analyses

Blood was collected and serum separated at the indicated times for ELISA. 96-well plates were coated with $NP_{(36)}$-CGG at 4ºC overnight. Anti-NP Ig levels were detected with goat anti-mouse IgG1-HRP (1:4000), anti-mouse-IgG3-HRP (1:2000) and anti-mouse IgM-HRP

(1:4000) (Southern Biotech) and visualized with ABTS substrate (2,2′-Azinobis(3-ethylbenzthiazoline Sulfonic Acid) Diamonium salt; Thermo scientific). For *ex vivo* CSR, murine splenic $CD19^+$ B cells were magnetically-sorted (Miltenyi AutoMACS) and cultured in complete RPMI media supplemented with LPS 50μg/ml (for switching to IgG3) or LPS + IL-4 (50ng/ml) (for switching to IgG1). After 72 h of stimulation, IgM, IgG1 and IgG3 surface expression markers were measured by flow cytometry as previously described[34]. CSR in WT B cells was set to 100%. GLTs were analyzed after 48 h of stimulation by RT-PCR on RNA extracted with TRIzol (Invitrogen), using primers previously described[35].

SHM analysis

Total RNA was extracted with TRIzol (Invitrogen) from splenic B cells that were isolated after T cell depletion from NP-immunized mice. High-fidelity RT-PCR and nested PCR, Zero Blunt Topo cloning (Invitrogen) and sequencing of the V_H186.2 region were performed as previously described[36].

SUPPLEMENTARY TABLE S1. List of FOXP1 target genes in the OCI-Ly1 cell line

FOXP1 target genes	Rescued promoters	BCL6 target genes [Ci et al.[30]]	Cellular context for BCL6 targeting [Ci et al.[30]]	potential FOXP1 function (primary cells) [Fig. 2]	potential FOXP1 function (DLBCL) [Fig. S2]	Description
AASDHPPT	-	YES	DLBCL	na	complex	Aminoadipate-semialdehyde dehydrogenase-phosphopantetheinyl transferase
ADAM29	-	NO	-	activation	na	ADAM metallopeptidase domain 29
ADAMTSL4	r	NO	-	repression	activation	Thrombospondin repeat-containing protein 1
ADRM1	r	NO	-	complex	complex	Adhesion regulating molecule 1
AIDA	r	YES	both	na	na	Axin interactor, dorsalization associated
AKT1S1	-	YES	both	complex	na	AKT1 substrate 1 (proline-rich)
ALDH16A1	r	NO	-	activation	complex	Aldehyde dehydrogenase 16 family, member A1
ALDH9A1	r	YES	DLBCL	activation	complex	aldehyde dehydrogenase 9 family, member A1
ALG10	-	NO	-	complex	na	Asparagine-linked glycosylation 10 homolog (yeast, alpha-1,2-glucosyltransferase)
AMY2B	r	NO	-	activation	activation	Amylase, alpha 2B (pancreatic)
APBB1IP	-	NO	-	complex	activation	Amyloid beta (A4) precursor protein-binding, family B, member 1 interacting protein
ATF5	r	YES	DLBCL	repression	complex	Activating transcription factor 5
ATF6	-	YES	both	activation	activation	Activating transcription factor 6
ATP5O	r	NO	-	repression	complex	ATP synthase, H+ transporting, mitochondrial F1 complex, O subunit
BAT4	-	NO	-	activation	activation	HLA-B associated transcript 4
BCLAF1	-	NO	-	activation	complex	BCL2-associated transcription factor 1
BIK	-	NO	-	repression	complex	BCL2-interacting killer (apoptosis-inducing)
BMS1P1	r	NO	-	na	na	BMS1 pseudogene 1
BMS1P5	r	NO	-	activation	na	BMS1 pseudogene 5
BTG1	-	NO	-	activation	complex	B cell translocation gene 1, anti-proliferative
BUB3	r	YES	both	repression	complex	BUB3 budding uninhibited by benzimidazoles 3 homolog (yeast)
C10ORF140	r	YES	DLBCL	activation	na	Chromosome 10 open reading frame 40

C19ORF56	r	NO	-	repression	na	Chromosome 19 open reading frame 56
C1ORF58	r	YES	both	repression	na	Chromosome 1 open reading frame 58
C22ORF26	r	NO	-	activation	na	Chromosome 22 open reading frame 26
C2ORF24	r	YES	both	complex	na	Chromosome 2 open reading frame 24
C3ORF38	r	YES	both	repression	na	Chromosome 3 open reading frame 38
C4ORF3	r	NO	-	repression	na	Chromosome 4 open reading frame 3
C5ORF33	r	YES	DLBCL	repression	na	Chromosome 5 open reading frame 33
C5ORF44	r	NO	-	activation	na	Chromosome 5 open reading frame 44
C7ORF10	r	YES	both	na	na	Chromosome 7 open reading frame 10
C7ORF11	r	YES	both	repression	na	Chromosome 7 open reading frame 11
C7ORF30	-	NO	-	repression	na	Chromosome 7 open reading frame 30
C8ORF41	r	YES	DLBCL	repression	na	Chromosome 8 open reading frame 41
C9ORF100	r	YES	DLBCL	repression	na	Chromosome 9 open reading frame 100
CALM2	-	NO	-	repression	na	Calmodulin 2 (phosphorylase kinase, delta)
CBLL1	-	YES	both	repression	complex	Cbl proto-oncogene, E3 ubiquitin protein ligase-*like 1*
CBX3	-	YES	both	repression	complex	Chromobox homolog 3 (HP1 gamma homolog, Drosophila)
CD37	r	YES	DLBCL	activation	complex	Cell differentiation antigen 37
CD55	r	YES	both	activation	complex	CD55 molecule, decay accelerating factor for complement (Cromer blood group)
CDC42EP5	r	NO	-	repression	activation	CDC42 effector protein (Rho GTPase binding) 5
CEP68	r	NO	-	activation	complex	Centrosomal protein 68kDa
CFLAR	r	NO	-	activation	complex	CASP8 and FADD-like apoptosis regulator
CHMP2B	r	YES	both	activation	complex	Chromatin modifying protein 2B
CHTF8	r	NO	-	activation	na	CTF8, chromosome transmission fidelity factor 8 homolog (S. cerevisiae)
CIRH1A	r	YES	both	complex	activation	Cirrhosis, autosomal recessive 1A (cirhin)
CITED2	-	YES	both	repression	complex	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2
CKAP2	-	YES	DLBCL	repression	complex	Cytoskeleton associated protein 2
COPG	-	NO	-	repression	complex	Coatomer protein complex,

						subunit gamma
COX7C	-	YES	DLBCL	repression	complex	Cytochrome c oxidase subunit VIIc
CRADD	-	YES	DLBCL	activation	complex	CASP2 and RIPK1 domain containing adaptor with death domain
CRIPT	r	YES	both	repression	activation	Cysteine-rich PDZ-binding protein
CSNK2B	-	NO	-	na	complex	Casein kinase 2, beta polypeptide
CTDSP2	r	NO	-	repression	complex	CTD (carboxy-terminal domain, RNA polymerase II, polypeptide A) small phosphatase 2
CXORF42	r	NO	-	na	na	Chromosome X open reading frame 42
CXXC4	-	NO	-	activation	na	CXXC finger 4
DAZAP2	-	YES	DLBCL	activation	complex	DAZ associated protein 2
DBF4	r	NO	-	repression	complex	DBF4 homolog (S. cerevisiae)
DCAKD	r	NO	-	repression	na	Dephospho-CoA kinase domain containing
DHTKD1	-	NO	-	repression	complex	Dehydrogenase E1 and transketolase domain containing 1
DHX40	-	NO	-	repression	complex	DEAH (Asp-Glu-Ala-His) box polypeptide 40
DHX8	-	NO	-	repression	na	DEAH (Asp-Glu-Ala-His) box polypeptide 8
DICER1	r	YES	DLBCL	complex	complex	Dicer1, Dcr-1 homolog (Drosophila)
DLEU1	-	NO	-	na	na	Deleted in lymphocytic leukemia 1 (non-protein coding)
DLEU2	r	NO	-	na	na	Deleted in lymphocytic leukemia 2 (non-protein coding)
DLGAP1	r	NO	-	activation	activation	Discs, large (Drosophila) homolog-associated protein 1
DUSP10	-	NO	-	repression	complex	Dual specificity phosphatase 10
DUSP11	-	YES	DLBCL	repression	complex	Dual specificity phosphatase 11 (RNA/RNP complex 1-interacting)
ECT2	-	NO	-	repression	complex	Epithelial cell transforming sequence 2 oncogene
EFCAB2	r	NO	-	repression	activation	EF-hand calcium binding domain 2
EIF1	r	YES	DLBCL	activation	complex	Eukaryotic translation initiation factor 1
EIF4ENIF1	-	YES	both	repression	complex	Eukaryotic translation initiation factor 4E nuclear import factor 1
ELOVL6	r	YES	DLBCL	complex	complex	ELOVL family member 6, elongation of long chain fatty acids (FEN1/Elo2, SUR4/Elo3-like, yeast)

ERCC5	r	YES	GC B-cell	activation	complex	Excision repair cross-complementing rodent repair deficiency, complementation group 5
ERH	r	YES	DLBCL	repression	activation	Enhancer of rudimentary homolog (Drosophila)
EXO1	-	NO	-	repression	complex	Exonuclease 1
FAM119B	r	NO	-	activation	complex	Family with sequence similarity 119, member B
FAM134A	r	YES	both	complex	complex	Family with sequence similarity 134, member A
FAM60A	r	YES	both	na	complex	Family with sequence similarity 60, member A
FCHO1	-	NO	-	repression	na	FCH domain only 1
FCHO2	r	YES	DLBCL	complex	activation	FCH domain only 2
FLJ13224	r	YES	both	na	na	Hypothetical protein FLJ13224
FLJ35776	r	NO	-	na	activation	Hypothetical protein FLJ35776
FLJ40330	r	NO	-	na	na	Hypothetical FLJ40330
FUZ	r	YES	both	activation	na	Fuzzy homolog (Drosophila)
GIT1	r	YES	DLBCL	complex	complex	G protein-coupled receptor kinase interactor 1
GORAB	r	NO	-	repression	na	Golgin, RAB6-interacting
GORASP1	r	NO	-	complex	complex	Golgi reassembly stacking protein 1, 65kDa
GPATCH3	r	NO	-	activation	complex	G patch domain containing 3
GPR108	r	YES	DLBCL	complex	activation	G protein-coupled receptor 108
GTF3C2	-	YES	DLBCL	activation	complex	General transcription factor IIIC, polypeptide 2, beta 110kDa
GTPBP1	-	NO	-	complex	complex	GTP binding protein 1
HBP1	-	YES	GC B-cell	activation	complex	HMG-box transcription factor 1
HDAC9	-	YES	both	repression	complex	Histone deacetylase 9
HEXIM1	r	YES	both	activation	complex	Hexamethylene bis-acetamide inducible 1
HIST1H1E	r	NO	-	repression	na	Histone cluster 1, H1e
HIST1H2AJ	r	NO	-	na	na	Histone cluster 1, H2aj
HIST1H2BB	-	YES	DLBCL	repression	na	Histone cluster 1, H2aj
HIST1H2BD	r	YES	both	activation	complex	Histone cluster 1, H2bd
HIST1H2BM	-	YES	both	repression	na	Histone cluster 1, H2bm
HIST2H2AA3	r	YES	both	na	complex	Histone cluster 2, H2aa3
HIST2H2AA4	r	YES	both	na	na	Histone cluster 2, H2aa4
HIST2H2AB	-	YES	both	na	na	Histone cluster 2, H2ab
HIST2H3A	r	YES	both	na	na	Histone cluster 2, H3a
HIST2H3C	r	YES	both	na	na	Histone cluster 2, H3c
HNRNPA2B1	r	YES	both	complex	activation	Histone cluster 2, H2b1
HOXA5	r	NO	-	repression	complex	Homeobox A5

HS2ST1	-	YES	both	repression	complex	Heparan sulfate 2-O-sulfotransferase 1
HTATSF1	r	YES	DLBCL	repression	complex	HIV-1 Tat specific factor 1
IL4I1	r	YES	DLBCL	repression	complex	Interleukin 4 induced 1
ING3	r	NO	-	repression	complex	Inhibitor of growth family, member 3
KBTBD3	r	YES	DLBCL	activation	activation	Kelch repeat and BTB (POZ) domain containing 3
KDM5C	r	NO	-	complex	na	Lysine (K)-specific demethylase 5C
KIAA0430	r	YES	both	complex	complex	KIAA0430
KIAA0586	r	YES	both	repression	complex	KIAA0586
KIAA0913	r	NO	-	complex	complex	KIAA0913
KLF12	-	NO	-	repression	complex	Kruppel-like factor 12
KLRC2	r	YES	DLBCL	na	na	Killer cell lectin-like receptor subfamily C, member 2
LENG9	-	YES	both	activation	na	Leukocyte receptor cluster (LRC) member 9
LOC100133469	r	NO	-	na	na	Hypothetical LOC100133469
LOC150381	r	NO	-	na	na	Hypothetical LOC150381
LOC285074	r	YES	DLBCL	na	activation	Hypothetical LOC285074
LOC648740	r	NO	-	na	na	Hypothetical LOC648740
LRMP	-	NO	-	repression	complex	Lymphoid-restricted membrane protein
LRP10	r	YES	both	complex	complex	Low density lipoprotein receptor-related protein 10
LTA4H	-	YES	both	activation	complex	Leukotriene A4 hydrolase
MALAT1	r	YES	GC B-cell	na	activation	Metastasis associated lung adenocarcinoma transcript 1 (non-protein coding)
MAML3	-	YES	GC B-cell	repression	complex	Mastermind-like 3
MBD6	-	YES	both	complex	complex	Methyl-CpG binding domain protein 6
MBOAT7	r	NO	-	activation	na	Membrane bound O-acyltransferase domain containing 7
MDM2	r	NO	-	repression	complex	Mdm2, transformed 3T3 cell double minute 2, p53 binding protein (mouse)
MEPCE	r	YES	DLBCL	complex	complex	Methylphosphate capping enzyme
METTL2A	r	NO	-	na	activation	Methyltransferase like 2A
MIAT	r	NO	-	na	na	Myocardial infarction associated transcript (non-protein coding)
MIR484	r	NO	-	na	na	MicroRNA 484
MLL	-	YES	both	repression	complex	Myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila)
MNAT1	-	YES	DLBCL	repression	na	Menage a trois homolog 1, cyclin H assembly factor (Xenopus laevis)
MPZL2	r	NO	-	complex	activation	Myelin protein zero-like 2

MPZL3	r	NO	-	na	activation	Myelin protein zero-like 3
MRPS18B	r	YES	both	na	complex	Mitochondrial ribosomal protein S18B
MSH2	-	YES	DLBCL	repression	complex	MutS homolog 2, colon cancer, nonpolyposis type 1 (E. coli)
MSL2	r	NO	-	activation	na	Male-specific lethal 2-like 1 (Drosophila)
MTA3	r	NO	-	repression	complex	Metastasis associated 1 family, member 3
MTMR14	r	NO	-	repression	complex	Myotubularin related protein 14
MYST3	-	NO	-	activation	complex	MYST histone acetyltransferase (monocytic leukemia) 3
NAA25	r	NO	-	na	na	N(alpha)-acetyltransferase 25, NatB auxiliary subunit
NCF1B	r	NO	-	na	na	Neutrophil cytosolic factor 1B pseudogene
NDE1	r	YES	both	complex	complex	NudE nuclear distribution gene E homolog 1 (A. nidulans)
NEIL1	r	YES	both	repression	complex	Nei endonuclease VIII-like 1 (E. coli)
NEK9	r	YES	DLBCL	complex	complex	NIMA (never in mitosis gene a)- related kinase 9
NFATC4	-	YES	both	complex	activation	Nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 4
NMI	r	YES	DLBCL	complex	complex	N-myc (and STAT) interactor
NMT1	r	NO	-	complex	complex	N-myristoyltransferase 1
NPC1	r	YES	both	repression	complex	Niemann-Pick disease, type
NPIPL3	r	NO	-	na	na	Nuclear pore complex interacting protein-like 3
NSL1	r	NO	-	activation	complex	NSL1, MIND kinetochore complex component, homolog (S. cerevisiae)
NUP37	-	NO	-	repression	complex	Nucleoporin 37kDa
NUP62	r	YES	DLBCL	repression	complex	**N**ucleoporin 62kDa
OGT	-	YES	DLBCL	activation	complex	O-linked N-acetylglucosamine (GlcNAc) transferase (UDP-N-acetylglucosamine:polypeptide-N-acetylglucosaminyl transferase)
OSR2	r	NO	-	repression	na	Odd-skipped related 2 (Drosophila)
OTUD5	r	YES	DLBCL	complex	activation	OTU domain containing 5
PAPD4	-	YES	DLBCL	repression	activation	PAP associated domain containing 4
PAX5	r	YES	DLBCL	complex	na	Paired box 5
PHIP	r	NO	-	complex	complex	Pleckstrin homology domain interacting protein
PIGF	-	YES	both	complex	complex	Phosphatidylinositol glycan anchor biosynthesis, class F
PIH1D1	r	NO	-	repression	complex	PIH1 domain containing 1

PIK3CG	-	YES	GC B-cell	repression	complex	Phosphoinositide-3-kinase, catalytic, gamma polypeptide
PIK3IP1	r	YES	both	activation	complex	Phosphoinositide-3-kinase interacting protein 1
PIM1	r	YES	both	repression	complex	Pim-1 oncogene
PKIB	r	YES	GC B-cell	repression	activation	Protein kinase (cAMP-dependent, catalytic) inhibitor beta
PLEKHA8	r	YES	both	repression	activation	Pleckstrin homology domain containing, family A (phosphoinositide binding specific) member 8
POU2AF1	-	NO	-	repression	complex	POU class 2 associating factor 1
PPOX	-	NO	-	repression	complex	Protoporphyrinogen oxidase
PPP1R10	r	YES	both	activation	complex	Protein phosphatase 1, regulatory subunit 10
PRDM1	r	NO	-	activation	activation	PR domain containing 1, with ZNF domain
PRDM15	-	YES	DLBCL	repression	complex	PR domain containing 15
PRDM2	r	NO	-	complex	complex	PR domain containing 2, with ZNF domain
PRDX5	-	YES	GC B-cell	repression	activation	Peroxiredoxin 5
PROX1	-	YES	GC B-cell	activation	activation	Prospero homeobox 1
PSIP1	-	YES	both	repression	complex	PC4 and SFRS1 interacting protein 1
PUS1	r	NO	-	activation	complex	Pseudouridylate synthase 1
PWWP2A	r	NO	-	repression	activation	PWWP domain containing 2A
RALGPS2	r	YES	DLBCL	activation	complex	Ral GEF with PH domain and SH3 binding motif 2
RASGEF1B	r	YES	both	complex	na	RasGEF domain family, member 1B
RB1	r	NO	-	repression	complex	Retinoblastoma 1 (including osteosarcoma)
RBM22	-	NO	-	repression	activation	RNA binding motif protein 22
RBM34	r	YES	both	activation	complex	RNA binding motif protein 34
RFC1	r	NO	-	repression	complex	Replication factor C (activator 1) 1, 145kDa
RFTN1	r	YES	GC B-cell	repression	complex	Raftlin, lipid raft linker 1
RIF1	-	NO	-	complex	activation	RAP1 interacting factor homolog (yeast)
RMND5A	r	YES	DLBCL	repression	complex	Required for meiotic nuclear division 5 homolog A (S. cerevisiae)
RNASEK	r	YES	both	activation	na	Ribonuclease, RNase K
RNF11	r	YES	both	activation	complex	Ring finger protein 11
RPF1	r	NO	-	na	na	Ribosome production factor 1 homolog (S. cerevisiae)
RPL4	r	YES	DLBCL	repression	complex	Ribosomal protein L4
RPS14	-	NO	-	activation	complex	Ribosomal protein S14
RPS24	r	NO	-	activation	activation	Ribosomal protein S24
RPS27L	-	NO	-	repression	complex	Ribosomal protein S27-like
RRM2B	r	YES	both	repression	activation	Ribonucleotide reductase

						M2 B (TP53 inducible)
RRP15	r	NO	-	activation	complex	Ribosomal RNA processing 15 homolog (S. cerevisiae)
RSBN1L	r	YES	DLBCL	activation	activation	Round spermatid basic protein 1-like
RSRC1	r	NO	-	repression	complex	Arginine/serine-rich coiled-coil 1
RXRB	r	NO	-	na	complex	Retinoid X receptor, beta
SAP30L	r	YES	both	complex	complex	SAP30-like
SATB1	-	YES	both	activation	complex	SATB homeobox 1
SC5DL	-	YES	both	repression	complex	Sterol-C5-desaturase (ERG3 delta-5-desaturase homolog, S. cerevisiae)-like
SECISBP2	r	NO	-	activation	activation	SCY1-like 1 binding protein 1
SEP15	r	YES	both	na	na	15 kDa selenoprotein
SERINC1	r	YES	GC B-cell	activation	complex	Serine incorporator 1
SETD7	r	YES	both	repression	activation	SET domain containing (lysine methyltransferase) 7
SH3BP5L	r	NO	-	complex	activation	SH3-domain GRB2-like endophilin B1
SH3GLB1	r	YES	DLBCL	activation	complex	SH3-domain GRB2-like endophilin B1
SLC25A40	r	NO	-	complex	activation	Solute carrier family 25, member 40
SLC39A7	-	NO	-	na	complex	Solute carrier family 39 (zinc transporter), member 7
SLC39A9	r	YES	DLBCL	complex	activation	Solute carrier family 39 (zinc transporter), member 9
SLC4A1AP	-	YES	both	repression	activation	Solute carrier family 4 (anion exchanger), member 1, adaptor protein
SMAD2	r	NO	-	repression	complex	SMAD family member 2
SPAST	r	YES	DLBCL	activation	complex	Spastin
SRP54	r	NO	-	repression	complex	Signal recognition particle 54kDa
STAT6	-	YES	GC B-cell	complex	complex	Signal transducer and activator of transcription 6, interleukin-4 induced
STK11IP	-	YES	both	repression	na	Serine/threonine kinase 11 interacting protein
STK35	-	YES	DLBCL	complex	activation	Serine/threonine kinase 35
SUPT7L	r	YES	both	repression	complex	Suppressor of Ty 7 (S. cerevisiae)-like
TAGLN2	r	NO	-	activation	complex	Transgelin 2
TATDN3	r	NO	-	activation	activation	TatD DNase domain containing 3
TBC1D15	-	YES	GC B-cell	activation	complex	TBC1 domain family, member 15
TBC1D17	r	YES	both	activation	na	TBC1 domain family, member 17
TBL1XR1	r	YES	both	complex	activation	Transducin (beta)-like 1 X-linked receptor 1
TCF7L2	-	NO	-	complex	complex	Transcription factor 7-like 2 (T-cell specific, HMG-box)
TEAD2	r	NO	-	activation	na	TEA domain family member 2

TESK2	r	NO	-	complex	na	Testis-specific kinase 2
TIMM9	-	YES	both	complex	complex	Translocase of inner mitochondrial membrane 9 homolog (yeast)
TK2	-	YES	DLBCL	complex	complex	Thymidine kinase 2, mitochondrial
TLR1	-	YES	both	activation	complex	Toll-like receptor 1
TMEM126A	r	NO	-	repression	activation	Transmembrane protein 126
TMEM140	r	YES	both	activation	complex	Transmembrane protein 140
TMEM167A	r	NO	-	repression	na	Transmembrane protein 167A
TMEM167B	r	YES	DLBCL	activation	na	Transmembrane protein 167B
TMX4	r	NO	-	activation	na	Thioredoxin-related transmembrane protein 4
TOP2B	r	YES	both	repression	complex	Topoisomerase (DNA) II beta 180kDa
TP53	r	YES	both	repression	complex	Tumor protein p53
TPP2	-	YES	both	complex	complex	Tripeptidyl peptidase II
TRDMT1	r	NO	-	complex	complex	tRNA aspartic acid methyltransferase 1
TRIB1	r	NO	-	activation	complex	Tribbles homolog 1 (Drosophila)
TRIM23	r	YES	both	activation	complex	Tripartite motif containing 23
TRMT112	r	NO	-	repression	na	tRNA methyltransferase 11-2 homolog (S. cerevisiae)
TSEN34	r	NO	-	activation	complex	tRNA splicing endonuclease 34 homolog (S. cerevisiae)
TSFM	-	NO	-	repression	complex	Ts translation elongation factor, mitochondrial
TTC21A	r	NO	-	complex	na	Tetratricopeptide repeat domain 21A
TXNDC15	r	NO	-	repression	na	Thioredoxin domain containing 15
TXNIP	-	YES	both	activation	complex	Thioredoxin interacting protein
UBE2B	r	YES	DLBCL	repression	complex	Ubiquitin-conjugating enzyme E2B
UBE2S	-	YES	DLBCL	na	complex	Ubiquitin-conjugating enzyme E2S
UBE3C	r	NO	-	repression	complex	Ubiquitin protein ligase E3C
UBL7	r	YES	DLBCL	repression	activation	Ubiquitin-like 7 (bone marrow stromal cell-derived)
UBXN8	r	NO	-	activation	na	UBX domain protein 8
USP21	r	NO	-	activation	na	Ubiquitin specific peptidase 21
VCPIP1	r	YES	both	repression	na	Valosin containing protein (p97)/p47 complex interacting protein 1
VNN2	-	NO	-	repression	complex	Vanin 2
VPREB3	-	NO	-	repression	complex	Pre-B lymphocyte gene 3
VPS4B	-	YES	both	activation	complex	Vacuolar protein sorting 4

						homolog B (S. cerevisiae)
WARS2	-	YES	both	repression	activation	Tryptophanyl tRNA synthetase 2, mitochondrial
WASF2	-	YES	DLBCL	complex	activation	WAS protein family, member 2
WDR48	r	NO	-	activation	complex	WD repeat domain 48
WDR83	r	NO	-	na	na	WD repeat domain 8
WRAP53	r	NO	-	repression	na	WD repeat containing, antisense to TP53
XPO7	r	NO	-	repression	complex	exportin 7
XRCC4	-	NO	-	repression	complex	X-ray repair complementing defective repair in Chinese hamster cells 4
YIPF4	r	YES	both	repression	complex	Yip1 domain family, member 4
ZBTB5	-	NO	-	complex	complex	Zinc finger, CCHC domain containing 5
ZCCHC7	-	NO	-	repression	activation	Zinc finger, CCHC domain containing 7
ZCWPW1	-	YES	DLBCL	activation	na	Zinc finger, CW type with PWWP domain 1
ZFP106	r	YES	both	repression	complex	Zinc finger protein 106 homolog (mouse)
ZFP36L1	-	YES	both	activation	complex	Zinc finger protein 36, C3H type-like 1
ZNF250	r	NO	-	activation	na	Zinc finger protein 250
ZNF585B	-	NO	-	na	na	Zinc finger protein 585B
ZNF687	r	YES	both	repression	na	Zinc finger protein 687
ZSCAN18	r	NO	-	activation	complex	Zinc finger and SCAN domain containing 18
ZWILCH	r	YES	DLBCL	repression	activation	Zwilch, kinetochore associated, homolog (Drosophila)

r: computationally rescued following the criteria specified in methods; both: bound to the corresponding promoter in both GC B-cells and DLBCL cells; na: non-available expression data from naïve-GC-memory cells (Fig. 2).

SUPPLEMENTARY TABLE S2. Selected significant BioFunctions identified by IPA analysis of FOXP1 target genes in the OCI-Ly1 cell line

Overall IPA analysis

Category	Functions Annotation	p-Value	Key Targets	# Molecules
Gene Expression	expression of RNA	9.30E-07	ATF5, ATF6, BCLAF1, BTG1, CBX3, CFLAR, CIRH1A, CITED2, CKAP2, CTDSP2, DICER1, ECT2, EIF1, GTF3C2, HBP1, HDAC9, HEXIM1, HIST2H3C (includes others), HNRNPA2B1, HOXA5, HTATSF1, KAT6A, KDM5C, KLF12, MAML3, MDM2, MLL, MNAT1, MSH2, NFATC4, NMI, NUP62, OGT, OSR2, PAX5, PHIP, POU2AF1, PRDM1, PRDM2, PROX1, PSIP1, PUS1, RB1, RFC1, RNASEK, RNF11, RPS14, RPS24, RPS27L, RXRB, SATB1, SMAD2, STAT6, TBL1XR1, TCF7L2, TEAD2, TP53, TXNIP, USP21, ZFP36L1	60
Cancer	diffuse B-cell lymphoma	2.16E-06	BTG1, CFLAR, MDM2, PAX5, PIM1, PRDM1, PRDM2, TP53	8
DNA Replication, Recombination, and Repair	checkpoint control	3.87E-06	BUB3, DBF4, EXO1, MDM2, MSH2, PIM1, RB1, TP53, ZWILCH	9
Cancer	Lympho-hematopoietic cancer	1.04E-05	BCLAF1, BTG1, CD55, CFLAR, DICER1, DLEU1, DLEU2, EXO1, GIT1, HDAC9, KAT6A, MDM2, MLL, MSH2, PAX5, PIM1, PRDM1, PRDM2, PSIP1, RB1, RPL4, RPS14, RRM2B, RXRB, SATB1, SRP54, STAT6, TOP2B, TP53, TRIB1, XRCC4	31
Hematological System Development and Function	differentiation of leukocytes	2.84E-04	BIK, CITED2, DICER1, HDAC9, HOXA5, KAT6A, MLL, MPZL2, MSH2, OGT, PAX5, PIK3CG, POU2AF1, PRDM1, RB1, RFTN1, SATB1, SMAD2, STAT6, TLR1, TP53, XRCC4	22
Infectious Disease	Viral Infection	3.71E-04	AMY2B, APBB1IP, BCLAF1, CBLL1, CD55, CEP68, CHMP2B, COPG1, CSNK2B, DAZAP2, DICER1, DLGAP1, ERCC5, FCHO2, GORASP1, HIST1H2BD, HIST2H2AA3/HIST2H2AA4, HTATSF1, KAT6A, KLRC2, LTA4H, MDM2, MSH2, NDE1, NEK9, NMT1, NUP62, OGT, PRDM2, PSIP1, RPS14, SMAD2, SPAST, STAT6, TAGLN2, TOP2B, TP53, TXNIP, UBE2B, UBE3C, VNN2, VPS4B, WASF2, WDR83	44
Cellular Development	differentiation of blood cells	4.85E-04	BIK, CITED2, DICER1, HDAC9, HOXA5, KAT6A, MLL, MPZL2, MSH2, OGT, PAX5, PIK3CG, POU2AF1, PRDM1, RB1, RFTN1, RPS14, SATB1, SMAD2, STAT6, TLR1, TP53, WASF2, XRCC4	24
Humoral Immune Response	class switching	7.77E-04	CD37, EXO1, MSH2, PRDM1, STAT6, XRCC4	6

IPA analysis on the FOXP1/BCL6 overlapping target genes

Category	Functions Annotation	p-Value	Key Targets	# Molecules
Gene Expression	activation of DNA endogenous promoter	1.05E-06	ATF5, ATF6, CITED2, CKAP2, DICER1, GTF3C2, HDAC9, HEXIM1, HIST2H3C (includes others), HTATSF1, MAML3, MLL, MNAT1, NFATC4, NMI, PAX5, PROX1, PSIP1, SATB1, STAT6, TBL1XR1, TP53, TXNIP	23
Cell Cycle	aneuploidy	3.53E-05	BUB3, PIM1, TP53, TPP2, UBE2B	5

Cellular Assembly and Organization	formation of mitotic spindle	3.53E-05	CKAP2, DICER1, NDE1, NEK9, TP53	5
Cell Cycle	checkpoint control	3.31E-04	BUB3, MSH2, PIM1, TP53, ZWILCH	5
Infectious Disease	Viral Infection	4.56E-04	CBLL1, CD55, CHMP2B, DAZAP2, DICER1, ERCC5, FCHO2, HIST1H2BD, HIST2H2AA3/HIST2H2AA4, HTATSF1, KLRC2, LTA4H, MSH2, NDE1, NEK9, NUP62, OGT, PSIP1, SPAST, STAT6, TOP2B, TP53, TXNIP, UBE2B, VPS4B, WASF2	26
Hematological Disease	hematologic cancer	1.18E-03	CD55, GIT1, HDAC9, MLL, MSH2, PAX5, PSIP1, RRM2B, SATB1, STAT6, TOP2B, TP53	12
Hematopoiesis	differentiation of leukocytes	1.20E-03	CITED2, DICER1, HDAC9, MLL, MSH2, OGT, PAX5, PIK3CG, RFTN1, SATB1, STAT6, TLR1, TP53	13
Cancer	Cancer	1.79E-03	AIDA, AKT1S1, ATF5, CBX3, CD55, CIRH1A, DICER1, EIF1, ELOVL6, ERCC5, GIT1, HBP1, HDAC9, HIST1H2BD, HIST2H2AA3/HIST2H2AA4, LRP10, MALAT1, MBD6, MLL, MSH2, NDE1, NEIL1, NEK9, NPC1, NUP62, PAX5, PIK3CG, PIK3IP1, PIM1, PLEKHA8, PRDX5, PROX1, PSIP1, RPL4, RRM2B, SATB1, STAT6, STK35, TOP2B, TP53, TRIM23, TXNIP, UBE2B, UBE2S, WARS2, YIPF4	46
Cellular Growth and Proliferation	proliferation of cells	3.39E-03	AKT1S1, ATF5, ATF6, BUB3, CBLL1, CD37, CD55, CITED2, CRADD, DICER1, EIF1, GIT1, HBP1, HEXIM1, HNRNPA2B1, IL4I1, MAML3, MLL, MNAT1, NDE1, NEIL1, NFATC4, NUP62, OGT, PAX5, PIGF, PIK3CG, PIK3IP1, PIM1, PROX1, RRM2B, SATB1, SETD7, SPAST, STAT6, TP53, TPP2, TXNIP, UBE2B, WASF2, ZFP36L1	41
Cell Cycle	cell cycle progression	3.68E-03	BUB3, CKAP2, DICER1, HBP1, MLL, MSH2, NDE1, NEK9, PIM1, PROX1, SETD7, STAT6, TOP2B, TP53, TPP2, UBE2S, VCPIP1	17
Cell Death and Survival	apoptosis	3.69E-03	AKT1S1, ATF5, ATF6, CD55, CITED2, CKAP2, CRADD, DICER1, ERCC5, HEXIM1, MLL, MNAT1, MSH2, NFATC4, NPC1, NUP62, OGT, PAX5, PIK3CG, PIK3IP1, PIM1, PRDX5, RRM2B, SATB1, SEP15, SH3GLB1, STAT6, TLR1, TP53, TPP2, TXNIP, UBE2B, ZFP36L1	33
DNA Replication, Recombination, and Repair	repair of DNA	7.19E-03	ERCC5, MSH2, NEIL1, RRM2B, TP53, UBE2B	6
Cellular Function and Maintenance	autophagy	1.22E-02	ATF6, DICER1, NPC1, SH3GLB1, TLR1, TP53	6

SUPPLEMENTARY TABLE S3. List of primers, siRNAs and antibodies

Q-ChIP

	FORWARD	*REVERSE*
DICER	GAATTCGACTGCCTCCATTG	AGCTAAGCTCTCCGGGAAAC
PRR20A	CTCCAGCAGTCGGCTTTC	GGAGGTGTCCACAGGTTCAC
PAX5	CCTAGGGGGAAGAGCCTAGAG	GATTGTGGCGAAATCTGCTC
PIM1	TCCCACCCTCGTTTTAGATG	GAGGAAATCACGAAGCAAATG
POU2AF1	CTCAACTGGGAGGAAACACC	AGGGGAGGGGCTACTGTG
PRDM1	CGAAGAGTACAAGAGCGATGG	ACAAGGCTGGGTGAATTCTG

QRT-PCR

	FORWARD	*REVERSE*
BCL6	AGCCACAAGACCGTCCATAC	CGAGTGTGGGTTTTCAGGTT
DICER	GCTGAAACTGCAACTGACC	TCTTTCATAAAGCCCACTTCTG
PRDM1	CTACCCTTATCCCGGAGAGC	GCTCGGTTGCTTTAGACTGC
hGAPDH	ACTTTGTCAAGCTCATTTCC	CACAGGGTACTTTATTGATG
mGAPDH	ACTTTGTCAAGCTCATTTCC	TGCAGCGAACTTTATTGATG
hFOXP1 (Fig. 1)	CAGATATTGCGCAGAACCAAG	AAGCAAACATTCGTGTGAACC
hFOXP1 (Fig. 3)	GGCAACTTAGCCAGCGCAATA	GCATAGGAGATCTGCCTGGA
mFOXP1	AGGCTGTGAGGCGGTTTGT	CATTGAGCTGTGCTTCTATCG
PAX5	ATGTTTGCCTGGGAGATCAG	GGGTGGCTGCTGTACTTTTG
PIM1	GCTCGGTCTACTCAGGCATC	AGTGCCATTAGGCAGCTCTC
POU2AF1	CTATGCCTCTCCGCCACTC	CCACGGGAAATAGGTGAGG
γ1 GLT	TCGAGAAGCCTGAGGAATGT	ATAGACAGATGGGGGTGTCG
γ3 GLT	AGAGTCAGCCTCAAGGAGATGAT	CAGGGACCAAGGGATAGACAG
Igβ	GCTCAGAGACAGAGCAGTGACC	AGATCTGGGAACAAGGGCTTC

Genotyping PCR for the presence of the human FOXP1 transgene

	FORWARD	*REVERSE*
hFOXP1	AACAGCAACGAGAGTGACAGCAGTCC	AGCCACCACCTTCTGATAGGCAGC

Southern blot

	FORWARD	*REVERSE*
hFOXP1	GACTTTTGCAGGCTCCACCAGACC	AGCAAGCAGGGTCAGGCAAAGC

siRNA knockdown

	siRNA1	*siRNA2*
hFOXP1	CAACTTAGCCAGCGCAATA	GCCAAGGCCTTCTGACAATT

Antibodies

	REFERENCE	*USE*
h/m actin	Calbiochem	WB (1:5000)

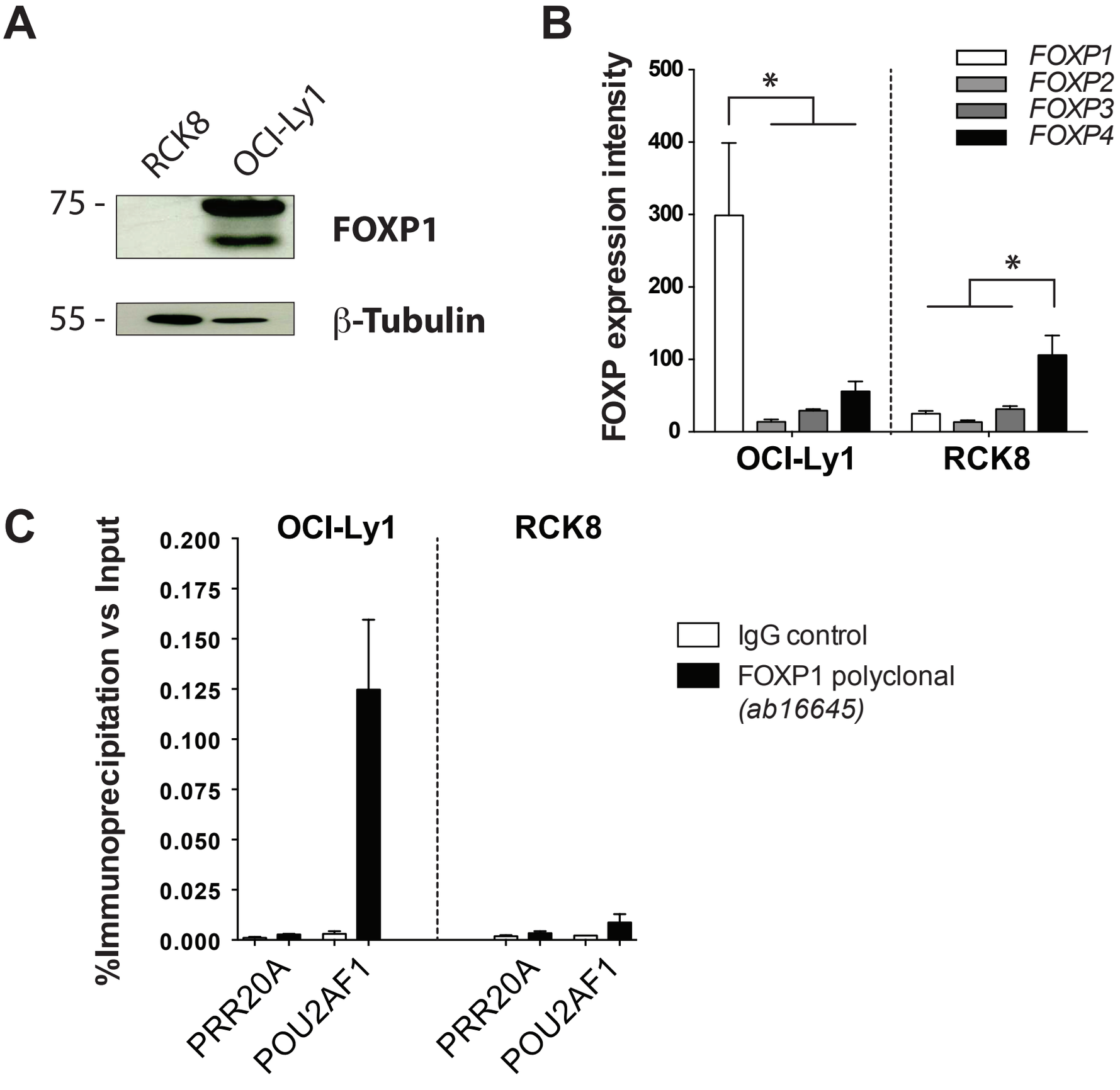
h BCL6	Dako	WB (1:500), IHC (1:10), IF (1:10)
h CD3	Dako	IHC (1:100)
h CD79α	Dako	IHC (1:40)
h CD21	Dako	IHC (1:50)
m CD21	Abcam	IHC (1:50)
h CD68	Dako	IHC (1:50)
h IgD	Dako	IHC (1:50)
h/m FOXP1	Abcam (polyclonal ab16645; Lot: GR55101-1)	WB (1:5000), IHC (1:1000), IF (1:200)
PNA	Vector Laboratories	IHC (1:1000)
h/m β-tubulin	Sigma	WB (1:3000)

SUPPLEMENTARY FIGURE LEGENDS

Supplementary Figure S1. FOXP1 expression in the OCI-Ly1 cell line. (A) Western blotting with anti-FOXP1 polyclonal antibody in a panel of GC-like DLBCL cell lines shows the predominance of the longer isoform in the OCI-Ly1 cells, while the RCK8 cells exhibit very low levels of FOXP1 expression. (B) Bar graph representing DNA microarray data (average signal of the probes ± SEM), depicting the expression intensity in OCI-Ly1 and RCK8 cells of the four FOXP family members 1-2-3-4 (GEO series GSE42203). Consistent with previous protein analysis, significant high-levels of *FOXP1* mRNA expression are demonstrated only in the OCI-Ly1 cell line, and not in RCK8 cells. (C) Q-ChIP analyses with anti-FOXP1 polyclonal antibody confirm FOXP1 occupancy at POU2AF1 target gene and not at PRR20A negative control in OCI-Ly1 FOXP1-positive cells. The absence of cross reactivity with other expressed FOXP family members is confirmed in RCK8 FOXP1-negative cells. Data are displayed as average fold enrichment relative to the input in replicated experiments.

Supplementary Figure S2. Expression of FOXP1 target genes in human DLBCL samples. (A) Graphical heat map representation of the transcript abundance of FOXP1 target genes identified by ChIP-on-chip in the DLBCL cell line OCI-Ly1. The data are derived from previously published expression arrays[42], consisting of tumor specimens from DLBCL patients classified either as GC-, ABC- or Type3-subtype. Red and green indicate high and low abundance, respectively. After sorting the samples (columns) based on FOXP1 expression levels (in a row at the bottom), around 75% of the targets exhibited a complex interrelationship with FOXP1, while 25% of targets clustered in a group that exhibited a positive association with FOXP1 expression in all DLBCL subtypes. (B) Expression centroid views evaluating the variability of gene expression inside each cluster. The bottom cluster of genes positively associates with increasing levels of FOXP1 in all DLBCL subtypes.

Supplementary Figure S1



Supplementary Figure S2