Regulation of normal B-cell differentiation and malignant B-cell survival by OCT2

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The requirement for the B-cell transcription factor OCT2 (octamer-binding protein 2, encoded by *Pou2f2*) in germinal center B cells has proved controversial. Here, we report that germinal center B cells are formed normally after depletion of OCT2 in a conditional knockout mouse, but their proliferation is reduced and in vivo differentiation to antibody-secreting plasma cells is blocked. This finding led us to examine the role of OCT2 in germinal center-derived lymphomas. shRNA knockdown showed that almost all diffuse large B-cell lymphoma (DLBCL) cell lines are addicted to the expression of OCT2 and its coactivator OCA-B. Genome-wide chromatin immunoprecipitation (ChIP) analysis and gene-expression profiling revealed the broad transcriptional program regulated by OCT2 that includes the expression of STAT3, IL-10, ELL2, XBP1, MYC, TERT, and ADA. Importantly, genetic alteration of OCT2 is not a requirement for cellular addiction in DLBCL. However, we detected amplifications of the *POU2F2* locus in DLBCL tumor biopsies and a recurrent mutation of threonine 223 in the DNA-binding domain of OCT2. This neomorphic mutation subtly alters the DNA-binding preference of OCT2, leading to the transactivation of noncanonical target genes including *HIF1a* and *FCRL3*. Finally, by introducing mutations designed to disrupt the OCT2–OCA-B interface, we reveal a requirement for this protein–protein interface that ultimately might be exploited therapeutically. Our findings, combined with the predominantly B-cell–restricted expression of OCT2 and the absence of a systemic phenotype in our knockout mice, suggest that an OCT2-targeted therapeutic strategy would be efficacious in both major subtypes of DLBCL while avoiding systemic toxicity.

cancer biology | lymphoma | germinal center

Octamer-binding protein 2 (OCT2), a B-cell–restricted transcription factor encoded by the gene *POU2F2*, binds to an octamer DNA motif 5′-ATGCAAAT-3′ (1). It belongs to the POU domain family of transcription factors that uses both a POU homeodomain and a POU-specific domain to bind DNA (2–4). DNA binding induces a conformational change in the POU domain that permits recruitment of the coactivator OCA-B (also known as "BOB-1" or "OBF1," encoded by *POU2AF1*), which stabilizes the complex and further enhances transcriptional activation (5–10).

OCT2 and OCA-B are largely restricted in expression to the B-cell lineage (1, 7–10). Although OCT2 initially was thought to direct the B-cell–restricted expression of Ig genes by binding to octamer motifs in their promoters, OCT2-knockout cell lines subsequently were shown to transcribe Ig genes normally (11). Because mice with germline deletion of *Pou2f2* die shortly after birth from an undetermined cause (12), fetal liver and bone marrow chimeras have been used to investigate the function of OCT2-deficient B cells. Such mice have reduced B1 and marginal zone B cells, and B-cell proliferation and Ig secretion are reduced when the cells are stimulated in vitro (12, 13). The role of OCT2 in antigen-dependent germinal center responses is controversial, with one study finding a defect in the germinal center response to NP-OVA immunization (14) and another reporting normal germinal center formation after influenza challenge (15). OCA-B–deficient mice have normal B-cell development but are unable to mount a germinal center response (16–18). Thus, current evidence suggests that OCT2 and OCA-B have important functions in the later stages of B-cell differentiation, but the precise role, if any, for OCT2 in the germinal center reaction is unclear.

Germinal centers form when a mature B cell encounters antigen in the context of CD4 T-cell help and are characterized by intense B-cell proliferation and hypermutation of Ig genes (19). B cells with improved affinity for the immunizing antigen as a result of Ig hypermutation are selected and eventually differentiate into either memory B cells or long-lived plasma cells. Diffuse large B-cell lymphoma (DLBCL), the most common type of non-Hodgkin lymphoma, is derived from B cells that have transited the germinal center (19). The germinal center B-cell–like (GCB) subtype of DLBCL retains expression of germinal center B-cell–restricted genes, whereas the activated B-cell–like (ABC) DLBCL subtype appears to be derived from postgerminal center plasmablastic cells (20). Both OCT2 and OCA-B are highly expressed in normal germinal center B cells and in almost all cases of DLBCL (21, 22). A role for OCA-B in DLBCL was proposed based on the identification of a DLBCL-specific super-enhancer near the OCA-B promoter, but this study did not investigate whether OCA-B acts by binding to OCT2 or to the related and ubiquitously expressed POU domain factor octamer-binding

Significance

Diffuse large B-cell lymphoma (DLBCL) is the most common form of non-Hodgkin lymphoma and is incurable in roughly 30% of cases. Here we demonstrate the addiction of both major subtypes of DLBCL to the expression of the transcription factor OCT2 (octamer-binding protein 2) and its co-activator OCA-B. We clarify the role of OCT2 in normal germinal center biology and identify the genes and pathways that it regulates in malignant B cells. Our findings suggest that pharmacological agents designed to target OCT2 itself or the OCT2–OCA-B interface would be an effective and nontoxic therapeutic strategy in DLBCL.

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Data deposition: The sequence reported in this paper has been deposited in the Gene Expression Omnibus (GEO) database, www.ncbi.nlm.nih.gov/geo.

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protein 1 (OCT1) (23). One study of follicular lymphoma described apparent loss-of-function mutations in *POU2F2*, leading to the suggestion that OCT2 may prevent the formation of lymphomas (24). In the present study, we set out to clarify the role of OCT2 in normal germinal center reactions and to determine whether OCT2 acts to promote or restrain the development of germinal center-derived lymphomas.

Results

Abnormal Germinal Center Function and Plasma Cell Differentiation in OCT2-Deficient Mice. We generated conditional *Pou2f2*-knockout mice from targeted ES cells created by the European Conditional Mouse Mutagenesis Consortium. Lox P sites placed on either side of exons 8–11 ensured deletion of the entire POU domain (Fig. S1*A*). *Pou2f2*$^{fl/fl}$ mice were crossed first to FLPE recombinase mice (25) to excise the neomycin cassette and then to ERT2-Cre mice in which the Cre recombinase is tamoxifen inducible (26). We confirmed correct gene targeting by Southern blotting (Fig. S1 *B–D*) and observed efficient Cre-mediated deletion in germinal center B cells and in splenic B cells (Fig. S1 *E* and *F*). Cre-mediated deletion was associated with the persistence of a *Pou2f2* transcript and the production of an unstable protein that lacked exons 8–11 (Fig. S1 *F* and *G*). Homozygous deletion of *Pou2f2* was not associated with any sign of ill health or altered behavior in mice observed for more than 2 mo after deletion. Heterozygous floxed (*Pou2f2*$^{fl/wt}$), Cre-expressing mice were used as controls.

Seven days after immunization with sheep red blood cells, the numbers of splenic B cells, T cells, and germinal center B cells were normal, with no difference in the proportion of germinal center light zone (centrocyte) and dark zone (centroblast) cells (Fig. 1*A* and Fig. S2 *A* and *B*). Similarly, when immunized with the thymus-dependent immunogen NP-KLH, knockout mice had normal numbers of germinal center B cells (Fig. S2*B*). However, the function of the germinal center B cells in knockout animals was not normal, in that their cell-cycle progression was reduced at 14 d after immunization with NP-KLH, as assessed by the incorporation of 5-ethynyl-2′-deoxyuridine (EdU) (Fig. 1*B* and Fig. S2*C*). Furthermore, serum antigen-specific Ig levels were reduced in knockout animals after immunization with NP-KLH (Fig. 1*C*). By day 28, NP-specific IgM, total NP-specific IgG1, and high-affinity NP-specific IgG1 levels were reduced by 5.5-, 10.6-, and 61-fold, respectively. Moreover, NP-specific antibody-secreting cells were almost absent in both spleen and bone marrow when measured by ELISpot assays 28 d after immunization (Fig. 1*D* and Fig. S2*D*). In contrast, normal numbers of memory B cells (Gr1$^-$, CD11d$^-$, IgM$^-$, IgD$^-$, CD138$^-$, NP$^+$, IgG1$^+$, CD38$^+$) were present 28 d after immunization (Fig. S2*E*) and were able to expand normally when mice were rechallenged with NP-KLH 30 d after primary immunization (Fig. 1*E* and Fig. S2*F*). However, OCT2-deficient mice were unable to produce Ig in response to secondary immunization (Fig. S2*G*). Thus, although OCT2-deficient mice were able to initiate germinal center formation normally and produce normal numbers of memory B cells, the proliferation of B cells within the germinal center was reduced, and the production of antibody-secreting plasma cells was blocked.

DLBCL Cell Lines Are Addicted to the Expression of both OCT2 and OCA-B. Because of the germinal center phenotype in *Pou2F2*-knockout mice, we examined the dependence of human lymphoma cell lines on OCT2 by RNAi. shRNA-mediated knockdown of OCT2 expression was toxic for most DLBCL cell lines tested but not for T-cell, Hodgkin, or mantle cell lymphoma lines (Fig. S3*A*). In a larger panel of cell lines, OCT2 was confirmed to be essential in both the ABC and GCB DLBCL lines but not in control T-cell lines (Fig. 2*A*). A similar spectrum of toxicity was observed using a second, independent OCT2 shRNA (Fig. S3*B*), and the toxicity of both shRNAs could be reversed by ectopic expression of OCT2 (Fig. S3*C*). OCT2-depleted cells showed both increased apoptotic cell death, as measured by cleavage of caspase 3 and PARP, and reduced cell-cycle progression, with an increase in G1-phase cells (Fig. S3 *D* and *E*). Similarly, knockdown of OCA-B was toxic for all ABC and GCB DLBCL cell lines tested but not for T-cell lines (Fig. 2*B*), demonstrating the essential role of OCT2 and OCA-B in both common subtypes of DLBCL.

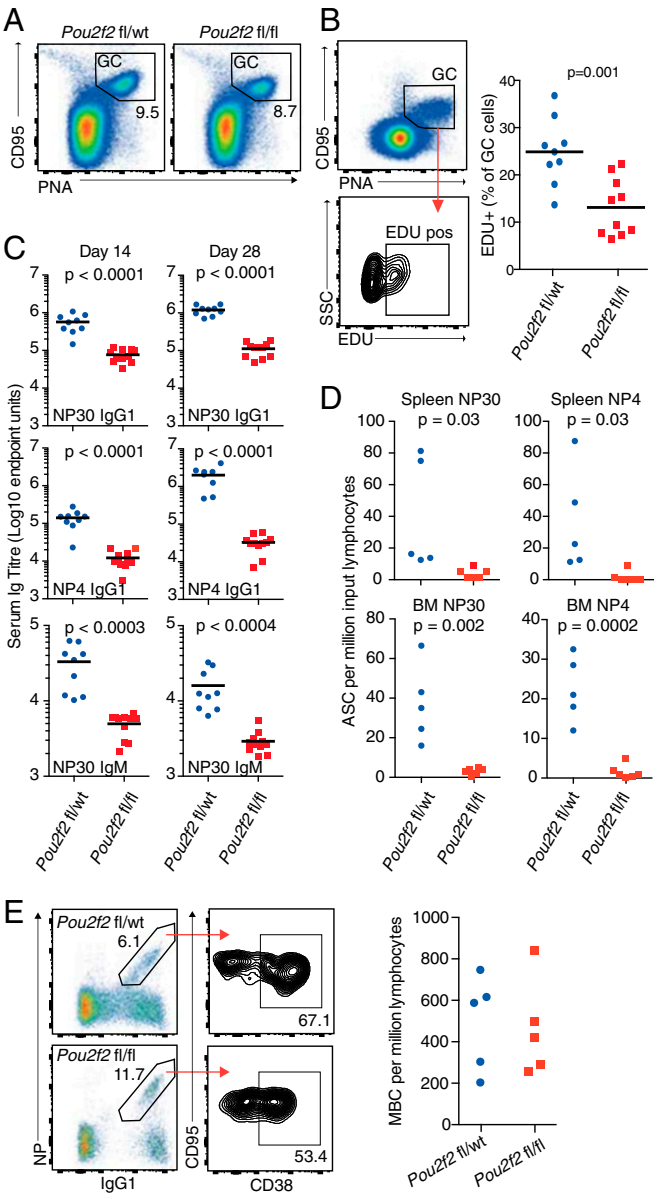

Fig. 1. Defective germinal center function in *Pou2f2*-knockout mice. (*A*) FACS plots gated on B220$^+$ splenic B cells showing germinal center B cells in mice 7 d after immunization with sheep red blood cells. (*B*) Germinal center B-cell turnover measured by pulsed EdU incorporation in mice 14 d after immunoprecipitation with NP-KLH. (*C*) NP-specific serum IgG1 and IgM Ig responses at day 14 and day 28 after immunoprecipitation with NP-KLH. Total and high-affinity Ig measured with NP30- and NP4-conjugated BSA is indicated along with the measured isotype. (*D*) Antigen-specific antibody-secreting cells (ASC) quantified by ELISpot on spleen or bone marrow (BM) 28 d after immunization with NP30 (total)- or NP4 (high-affinity)–conjugated BSA. (*E*) Representative FACS plots of NP-specific memory B cells from mice 7 d after secondary immunization. FACS plots shown are gated on B220$^+$, Dump$^-$(Gr1$^-$CD11d$^-$IgM$^-$IgD$^-$CD138$^-$). Cells with a memory B-cell (MBC) phenotype (B220$^+$, Dump$^-$,CD38$^+$) are enumerated as cells per million lymphocytes.

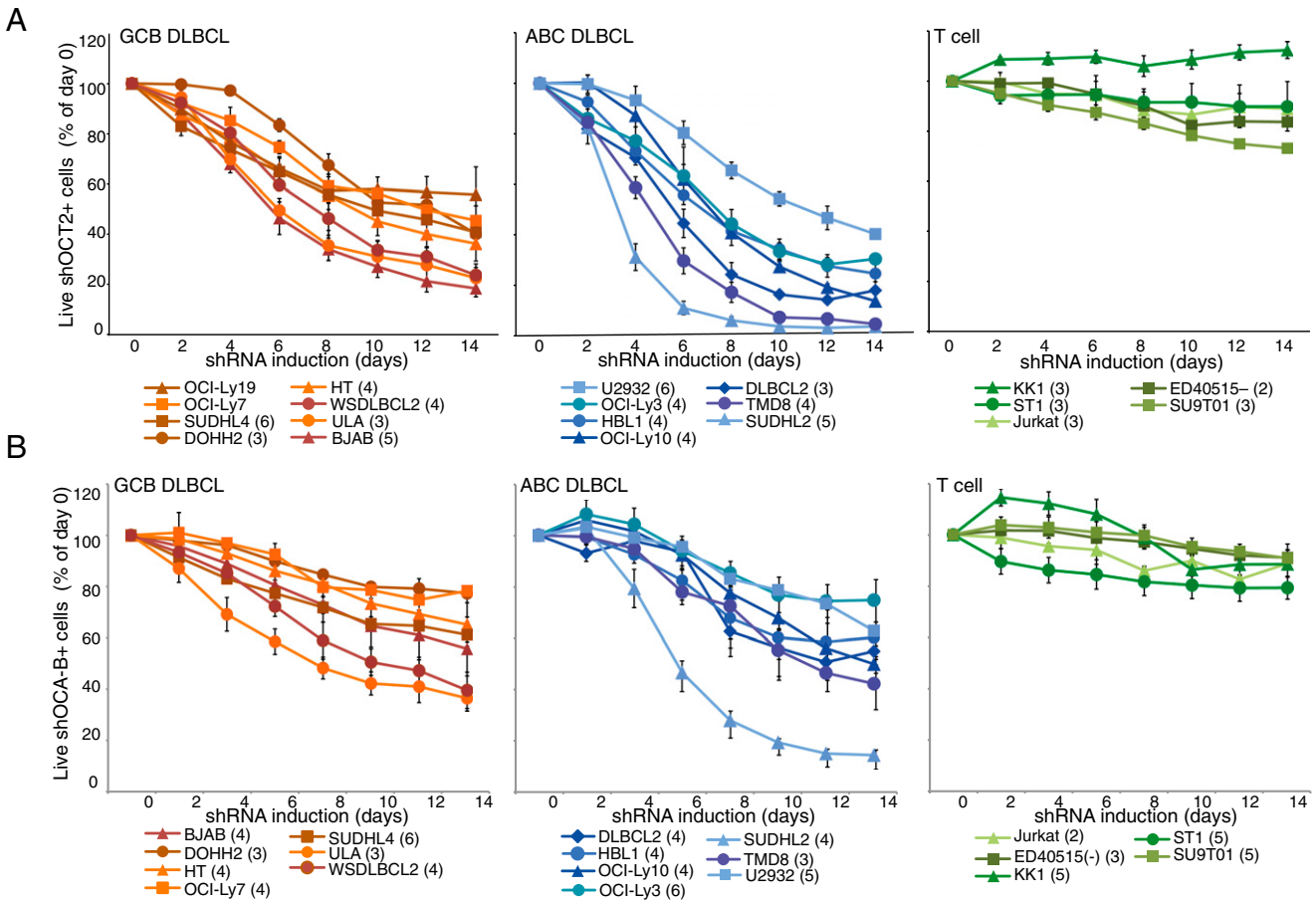

Fig. 2. DLBCL cell lines are addicted to the expression of OCT2. Viability of DLBCL lines transduced with shRNAs targeting OCT2 (shOCT2 #1) (*A*) or OCA-B (*B*). Shown are the percentages of live shRNA-expressing cells over time relative to the day 0 value. Error bars represent the SEM of replicate experiments; the number of replicates is shown in parenthesis.

OCT2 and OCA-B Bind an Overlapping Repertoire of Genes. To identify gene targets of OCT2 and OCA-B, we performed genome-wide chromatin immunoprecipitation (ChIP) analysis (ChIP sequencing, ChIP-Seq) in the HBL-1 ABC DLBCL line. Both OCT2 and OCA-B bound a large number of genomic sites, with 29,208 and 28,376 binding peaks, respectively. These were enriched in promoter-proximal regions: 38% of OCT2 peaks and 34% of OCA-B peaks were located within a window from 15 kb upstream to 2 kb downstream of a transcriptional start site (TSS) (Fig. 3*A*). Analysis of promoter peaks revealed OCT2 and OCA-B interaction with an overlapping repertoire of protein-coding genes (Fig. 3*B*). OCT2 peaks frequently coincided with OCA-B peaks, with their intersection increasing as a function of peak size. Among the 1,000 largest OCA-B peaks, almost all coincided with OCT2 binding. By contrast, OCT2 was able to bind DNA as an isolated factor, with 35% of OCT2-binding regions lacking OCA-B interaction. Using the DNA motif discovery program MEME (27), we found the most strongly enriched motif within both OCT2 and OCA-B peak regions was the octamer 5′-ATGCAAAT-3′ (Fig. 3*D*). A slightly altered motif, 5′-ATGCATAT-3′, was enriched at sites binding OCT2 in isolation and may allow a partially overlapping palindromic motif similar to the MORE sequence identified for OCT1 binding that precludes OCA-B binding (28). No octamer-related sequence was identified within OCA-B peaks lacking OCT2 binding. The octamer motif was found with increasing frequency as a function of OCA-B peak size, being present in 80% of the largest OCA-B peaks. In contrast, no octamer motif was present in 50% of the largest OCT2 peaks, suggesting that OCT2 may be recruited to DNA through an association with other, as yet unidentified, DNA-binding factors (Fig. 3*E*). Fig. 3*F* shows representative OCT2 and OCA-B binding profiles.

OCT2 and OCA-B Regulate a Broad Program of B-Cell Gene Expression. To identify genes whose expression depends on OCT2 and OCA-B, we performed gene-expression profiling after shRNA-mediated knockdown of these factors in three ABC and two GCB DLBCL lines. For each cell line we generated a list of genes whose expression decreased significantly following knockdown of OCT2 and/or OCA-B and examined these lists for overlap with gene-expression signatures generated from normal and malignant immune cells (Table S1) (29). Signatures enriched in three or more cell lines are shown in Table S1 and include signatures associated with B-cell transcription factors (IRF4, NF-κB, STAT3, TCF3), oncogenic signaling pathways (MYD88, JAK), and B-cell differentiation. We generated an "OCT2_Common_UP" signature comprising genes that changed in expression in two or more cell lines (Fig. S4). The genes in this signature with OCT2 peaks in their promoter regions were defined as OCT2 direct target genes. OCT2 direct target genes that belong to functionally related signatures (Table S1) are summarized in Fig. 4*A*; those that have overlapping OCT2 and OCA-B ChIP-Seq peaks are shown in red.

Of particular interest was the regulation of genes that have established roles in germinal center biology, plasma cell differentiation, or lymphomagenesis, including *CD22*, *EBF1*, *ELL2*, *XBP1*, *MYC*, *TERT*, *ADA*, *IL-10*, and *STAT3*. For most of the enriched signatures that reflect the action of transcription factors

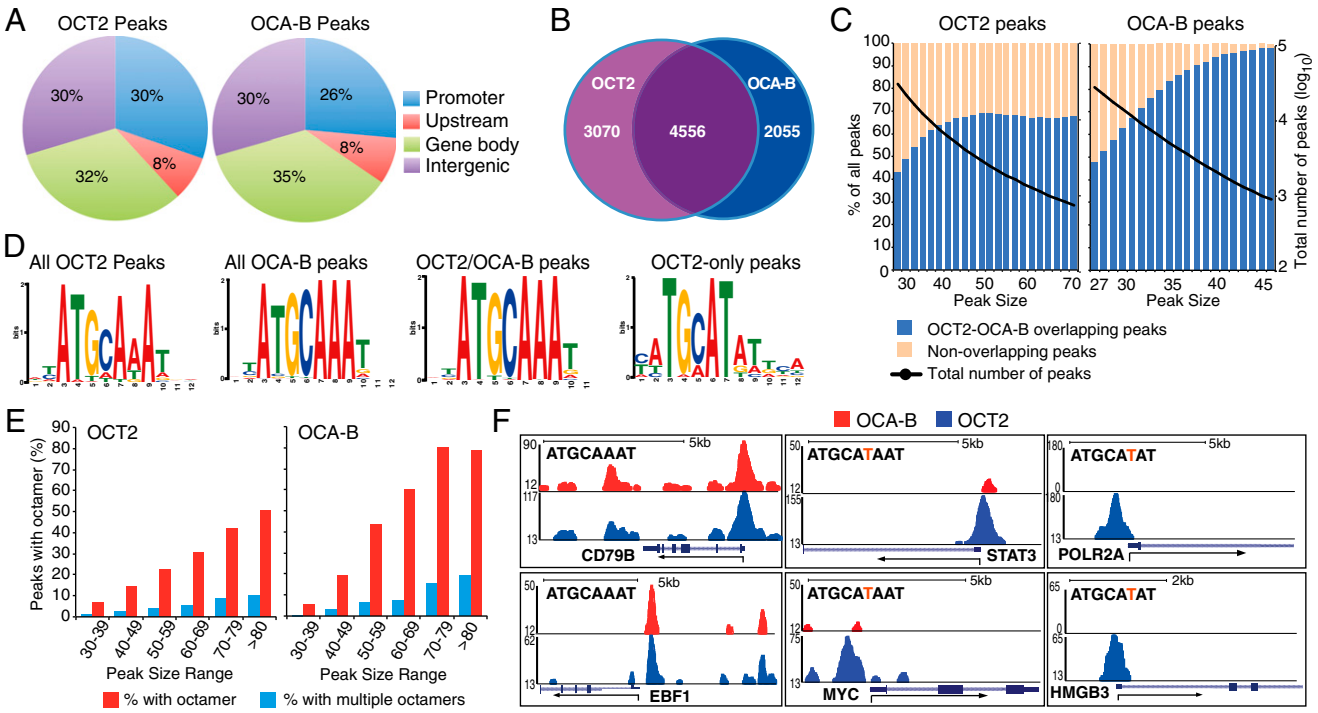

Fig. 3. OCT2 and OCA-B bind an overlapping repertoire of genomic loci. (*A*) Classification of OCT2- and OCA-B–binding peaks as promoter (TSS ± 2 kb), upstream (TSS −15 kb to −2 Kb), gene body, or intergenic regions. (*B*) Overlap of genes with an OCT2 or OCA-B peak within a gene window (TSS −15 kb through gene body). (*C*, *Left*) Proportion of OCT2-binding peaks that overlap with OCA-B peaks as a function of OCT2 peak size. (*Right*) Proportion of OCA-B peaks overlapping with an OCT2 peak as a function of OCA-B peak size. The total number of peaks (black line) is overlaid. (*D*) Motif discovery using MEME. The most enriched motifs are shown based upon the top 1,000 peaks from each class of peak as indicated. (*E*) Percentage of OCT2 or OCA-B peaks that contain the octamer motif (ATGCA[A/T]AT) as a function of peak size. (*F*) Examples of OCT2-binding peaks (blue) with large (*Left*), small (*Center*), or no (*Right*) overlapping of OCA-B peaks (red peaks). The best octamer sequence is indicated. The *y* axis shows ChIP-Seq read counts.

or signaling regulators, OCT2 did not influence the expression of the regulatory factor itself but rather influenced the expression of its downstream target genes. An exception is the regulation of JAK/STAT3 signatures (Fig. S5 *A* and *B*), which appeared to be caused by decreased expression of IL-10 and STAT3 in the absence of OCT2, as confirmed by quantitative PCR (qPCR), ELISA, and immunoblot analysis (Fig. 4 *B*–*D*). Direct binding of OCT2 to the *STAT3* promoter was confirmed by ChIP (Fig. S5*C*). We observed a significant correlation between OCT2 and STAT3 mRNA levels in both ABC and GCB DLBCL tumors profiled by RNA sequencing (RNA-Seq) (Fig. 4*E*) (30). A previous study suggested that OCT2 expression is itself controlled by IL-10–JAK–STAT3 signaling (31). We confirmed this regulation by analysis of mRNA from HBL-1 ABC DLBCL cells treated with JAK kinase inhibitors (ruxolitinib, AZD1480) or stimulated with IL-10 (Fig. S5*D*), suggesting the existence of a positive feedback loop. Other than OCT2 itself, no single gene (*STAT3*, *MYC*, *MYB*, *ADA*, and *BCL6*) could reverse the toxicity of OCT2 depletion in DLBCL lines when expressed ectopically (Fig. S5*E*); this finding is consistent with the concept that OCT2 regulates a network of many genes rather than a single dominant downstream target.

The genes and signatures most negatively correlated with OCT2 and OCA-B were enriched for those reflecting the action of type I IFN. Indeed, knockdown of OCT2 led to enhanced transcription from an IFN response element luciferase reporter (Fig. S5*F*). Furthermore, overexpression of OCT2 substantially reversed the expression of genes from the IFN-3 gene-expression signature, which includes genes that are induced by IFN (Fig. S5 *G* and *H*). Thus, it is conceivable that the toxicity of OCT2 depletion may be mediated in part by the induction of a type I IFN response, which is deleterious to DLBCL cells (32).

To identify genes regulated by OCT2 in normal mouse B cells, we profiled gene expression in purified splenic B cells from wild-type or *Pou2f2*$^{fl/fl}$ mice in which *Pou2f2* deletion was induced ex vivo by tamoxifen. Cells were analyzed after 48 h of tamoxifen treatment, at which time almost complete depletion of OCT2 protein was observed by immunoblot. Analysis of genes with lower expression in the knockout B cells revealed enrichment of multiple gene ontology (GO) terms related to Toll-like receptor signaling, B-cell proliferation, and B-cell activation (Table S1). Individual genes of particular interest included *Lmo2*, *Cd22*, *Il10, Bach2*, *Spib*, and *Stat3*, which either were identified as OCT2 targets in DLBCL cells or previously were suggested to be OCT2 targets. We used qRT-PCR to validate *Stat3*, *Ada*, *Ell2* (each of which also was an OCT2 target in DLBCL) as OCT2 targets in sorted germinal center B cells from immunized mice (Fig. 4*F*). Taken together, these studies demonstrate that OCT2 controls a broad program of genes and pathways critical for normal B-cell function in both malignant human B cells and normal mouse B cells.

Recurrent Genetic Aberrations Targeting OCT2 in DLBCL. Analysis of previously published array comparative genomic hybridization (aCGH) data from DLBCL identified five samples with amplifications encompassing the *POU2F2* locus, each of which had increased OCT2 mRNA levels (Fig. 5*A*) (33). In three cases, the amplicon was less than 1 MB in size, and in the GCB DLBCL line BJAB the amplicon included only one other gene, *ZNF574*, of unknown function, suggesting that these amplifications were selected to increase OCT2 expression. An additional six cases had amplifications of the *POU2AF1* locus, encoding OCA-B, with overexpression of OCA-B mRNA (Fig. 5*A*). The minimal common amplified region included only six other genes, none of which is expressed in B cells, suggesting that OCA-B was the

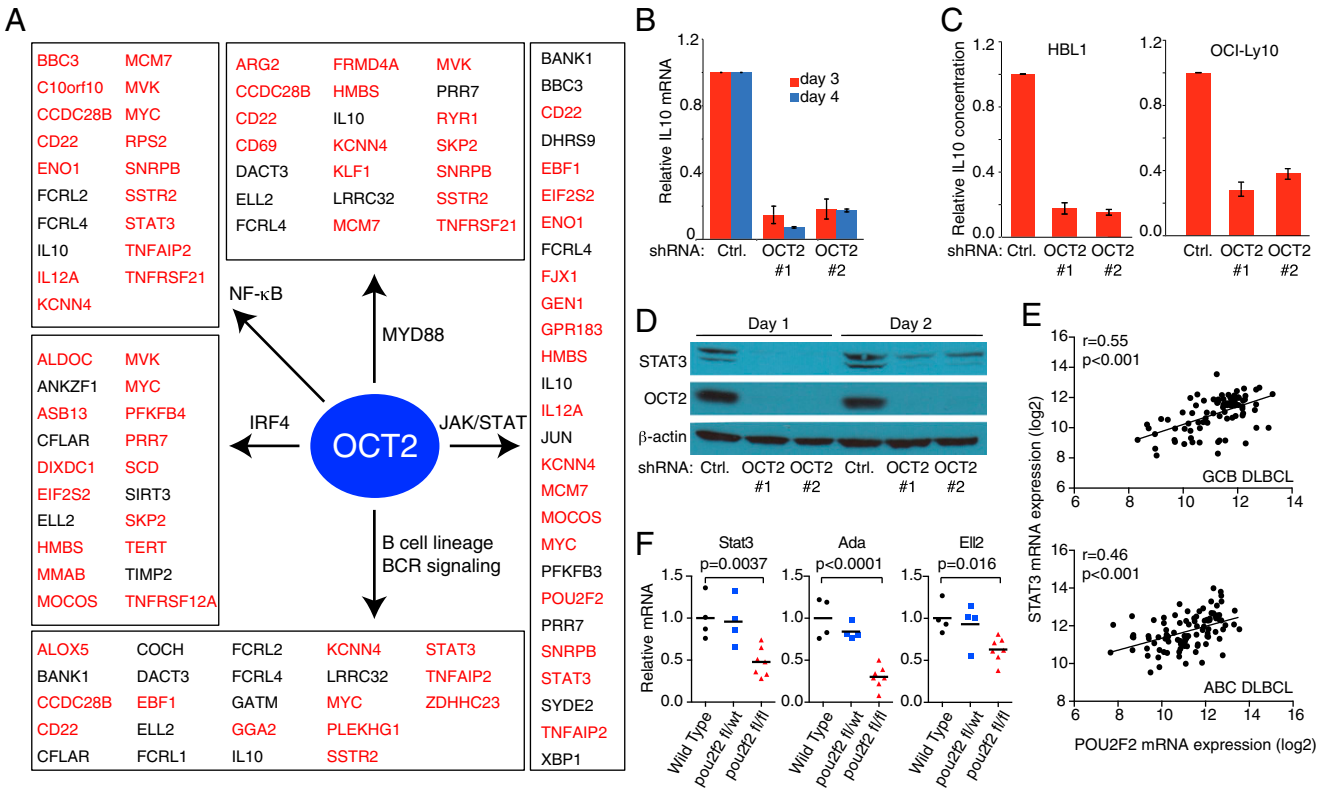

Fig. 4. OCT2 and OCA-B regulate a broad program of B-cell gene expression. (*A*) Direct OCT2 target genes identified by ChIP-Seq and by reduced expression following OCT2 knockdown. Genes are grouped by overlap with functionally related gene-expression signatures. Genes with OCT2 and OCA-B overlapping ChIP-Seq peaks within the gene window are in red. OCT2 isolated peaks within the gene window are in black. Bold type indicates the peak is within 2 kb of the TSS. (*B*) IL-10 mRNA expression quantified by qRT-PCR following OCT2 shRNA knockdown relative to B2M in HBL-1 cells. (*C*) IL-10 measured by ELISA in cell supernatants following OCT2 shRNA knockdown in HBL-1 and Ly10 cells. (*D*) Expression of total STAT3 following OCT2 shRNA knockdown in TMD8. (*E*) Correlation of POU2F2 and STAT3 mRNA expression by digital gene expression in GCB (*Upper*) and ABC (*Lower*) DLBCL biopsy specimens. (*F*) Confirmation of selected changes in mRNA expression by qRT-PCR analysis of RNA from sorted germinal center B cells from mice 7 d after i.p. immunization with sheep red blood cells.

focus of these genetic events. These recurrent amplifications of the genes encoding OCT2 and OCA-B suggest an oncogenic role for these factors in DLBCL.

Based on a report of OCT2 POU domain mutations in follicular lymphoma (24), we sequenced the exons encoding the POU domain in 207 cases of DLBCL. Twelve nonsynonymous, heterozygous mutations were observed, with a mutational hot spot targeting threonine 223 (T223) in seven cases, substituting either alanine or serine (Table S2). T223 is located in the POU-specific domain and inserts into the DNA major groove, making contact with G_3 of the octamer motif (Fig. 5*B*). Compared with wild-type OCT2, ectopic expression of OCT2 T223A was much less effective in rescuing DLBCL cells from the toxicity of OCT2 knockdown (Fig. 5*B* and Fig. S6*A*). To test whether the T223A mutant isoform alters OCT2 DNA binding, we designed "Biotag" constructs that express either wild-type or T223A OCT2 fused to a peptide sequence that can be biotinylated by the bacterial enzyme BirA. We engineered the DLBCL cell line BJAB to express BirA, transduced the cells with the OCT2 Biotag constructs, and performed ChIP-Seq using streptavidin to purify chromatin bound to these OCT2 isoforms (Fig. S6*B*). The majority of regions bound by wild-type OCT2 also were bound by OCT2 T223A, indicating that T223A is not a complete loss-of-function mutant (Fig. 5 *D* and *G*). Roughly 10% of the T223A binding peaks (n = 2,975) had reduced or no binding by wild-type OCT2. MEME analysis of these peaks revealed strong enrichment for an altered octamer motif, 5′-ATACAAAT-3′, which has an alanine in the position of the motif that is contacted by T223 (Fig. 5 *E*, *F*, and *H*). To identify genes regulated by OCT2 T223A, we profiled gene expression in lymphoma lines in which we ectopically expressed either wild-type or T223A OCT2 following knockdown of endogenous OCT2 expression. Although cells expressing OCT2 T223A had reduced expression of previously identified OCT2 target genes and signatures (Table S3), the expression levels of a number of genes and signatures were increased by this mutant. This gene set was enriched for genes with OCT2 T223A binding peaks, such as *FCRL2*, *FCRL3*, and *HIF1A* (Table S3). Accordingly, signatures of HIF1α-dependent target genes were enriched in HBL-1 cells expressing T223A (Table S3), and increased cell-surface expression of FCRL2 and FCRL3 was confirmed by flow cytometry (Fig. S6*C*).

Four of the DLBCL biopsies with OCT2 T223 mutation were included in a previous RNA-Seq study (30), allowing us to search for genes that were expressed differentially in these cases versus others with wild-type OCT2. Integrative analysis revealed 10 genes that were more highly expressed in OCT2 T223A$^+$ biopsies and also were bound preferentially and up-regulated by OCT2 T223A in vitro. Among these was *HIF1A*, and accordingly, multiple signatures of HIF1α target genes were enriched in OCT2 T223A$^+$ cases (Table S3). Also included was *FCRL3*, which was bound and regulated by OCT2 T223A in our cell line models. Taken together, these findings suggest that OCT2 T223A is an altered-function mutant that subtly shifts the repertoire of OCT2-regulated genes.

The OCT2–OCA-B Interface as a Potential Target for Therapeutic Development. Because both OCT2 and OCA-B are essential in DLBCL lines, we investigated whether the interface between

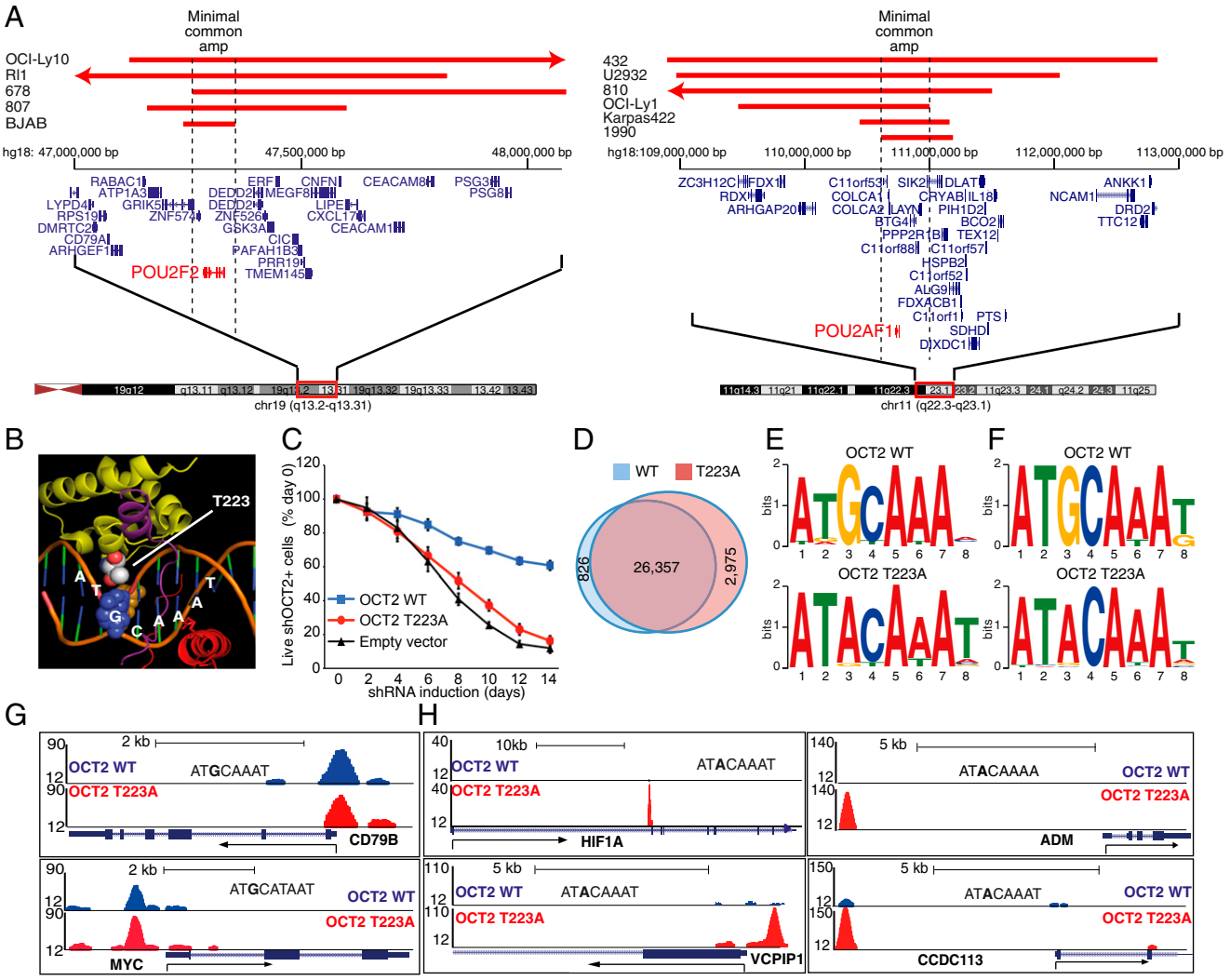

Fig. 5. Recurrent genetic alteration of POU2F2 in DLBCL. (*A*) Amplification of the *POU2F2* and *POU2AF1* loci in DLBCL biopsies and cell lines analyzed by aCGH. Red lines indicate amplified regions. (*B*) Crystal structure of the OCT1 POU domain interacting with the octamer sequence and OCA-B. Yellow helices represent POU-specific domains, the red helix is a POU homeodomain, and the purple helix is OCA-B. The elemental structure of T223 and the guanine at position three of the octamer motif are shown. (*C*) Wild-type OCT2 but not OCT2 T223A is able to rescue Ly10 cells depleted of OCT2 by shRNA. (*D*) Overlap between genes with a wild-type OCT2 or T223A BioChIP peak in a gene window (TSS −15 kb through gene body). (*E*) Motif discovery using Weeder based on the top 1,000 OCT2 BioChIP peaks. (*Upper*) Wild-type OCT2. (*Lower*) OCT2. (*F*) Motif discovery using MEME based on the top 1,000 OCT2 BioChIP peaks. (*Upper*) Wild-type OCT2. (*Lower*) OCT2 T223A. (*G*) Examples of genes with similar binding between wild-type and mutant OCT2. Best octamer motifs are shown. (*H*) Examples of genes with preferential binding by OCT2 T223A. The best octamer motif identified within the 200-nt peak window is indicated.

these two proteins might be a potential therapeutic target. The POU domain of OCT2 is highly homologous to that of OCT1, allowing us to use the crystal structure of the ternary complex of OCT1, OCA-B, and the octamer motif along with mutational studies of OCT1 to predict which amino acids of OCT2 are critical for its interaction with OCA-B (5, 6). We created OCT2 and OCA-B mutant isoforms that were predicted to disrupt the association of these proteins by introducing single-alanine substitutions of residues in the OCT2 POU-specific domain (L184 and E185; Pubmed accession NP_001234923) and the POU homeodomain (K335, I339), as well as a proline substitution of L32 of OCA-B (Fig. 6*A*). Although the toxicity of OCT2 or OCA-B knockdown in the ABC DLBCL line OCI-Ly10 was reversed by ectopic expression of the respective wild-type isoforms, the mutant OCT2 and OCA-B isoforms had little, if any, activity (Fig. 6 *B* and *C* and Fig. S6 *D*–*G*). Thus, the residues that facilitate the interaction of OCT2 and OCA-B are essential for the survival of DLBCL cells.

Discussion

Our genetic and functional analyses have clarified the role of OCT2 in normal and malignant B cells. Previous studies of Rag2-deficient mice reconstituted with OCT2-knockout bone marrow have yielded conflicting evidence regarding the role of OCT2 in the germinal center reaction (14, 15). By conditional deletion of *Pou2f2*, we observed normal formation of germinal centers in the absence of OCT2, but the cell-cycle progression of the B cells in these germinal centers was impaired. Although the development of memory B cells proceeded normally following immunization of OCT2-deficient mice, these mice were strikingly unable to form antibody-secreting plasma cells. Hence, our genetic analysis revealed that OCT2 influences the dynamic behavior of germinal center B cells in vivo by promoting their proliferation and plasmacytic differentiation.

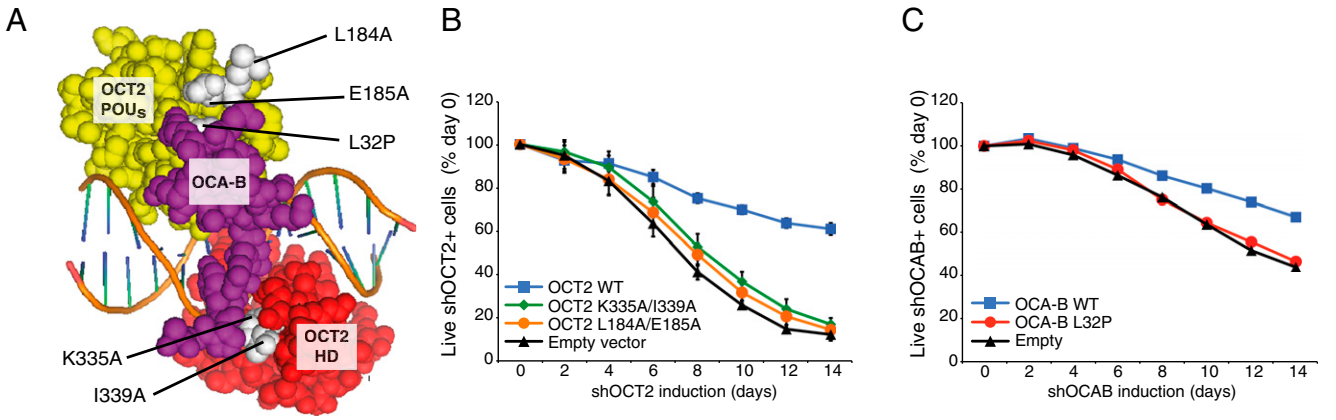

Fig. 6. The OCT2–OCA-B interface as a potential target for drug therapy. (*A*) Crystal structure showing the OCT1 POU domain (yellow) in complex with OCA-B (purple) and the octamer motif. Residues mutated to abolish the OCT2–OCA-B interaction are shown in white. (*B*) Rescue experiment in Ly10 cells transduced with an OCT2 shRNA and either wild-type or POU domain mutant OCT2 constructs, as indicated. (*C*) Rescue experiment in Ly10 cells transduced with OCA-B shRNA and either wild-type or mutant (L32P) OCA-B.

In contrast to the rather selective germinal center defects resulting from OCT2 deficiency, we observed that all human DLBCL lines tested are strongly addicted to the expression of OCT2. DLBCL tumors arise from normal B cells that have traversed the germinal center and either retain the germinal center phenotype, in the case of the GCB subtype, or undergo partial plasmacytic differentiation, in the case of the ABC subtype. The defect in proliferation in OCT2-deficient germinal center B cells may contribute to the toxicity of OCT2 knockdown in DLBCL lines. However, depletion of OCT2 in DLBCL not only blocks proliferation but also induces cell death, suggesting that OCT2 functions in a somewhat different or augmented fashion in malignant versus normal B cells. A role for OCA-B in DLBCL was revealed previously by the discovery of a DLBCL-specific superenhancer in the *POU2AF1* locus (23), but that study did not address whether OCA-B mediates its effects in DLBCL through OCT1 or OCT2. We cannot exclude a contribution from OCT1, but our data suggest that OCT2 is the key factor cooperating with OCA-B. This view is supported by our genetic analysis of DLBCL tumors, which revealed recurrent amplification of the *POU2F2* locus. Likewise, in DLBCL tumors we observed recurrent amplification of the *POU2AF1* locus but not the *POU2F1* locus, which encodes OCT1. However, amplification of *POU2F2* and *POU2AF1* were detected in only ~3% (5/172) of DLBCL tumors tested; by itself, this result might have led to the conclusion that OCT2 and OCA-B contribute only infrequently to DLBCL pathogenesis. Given the universal addiction of DLBCL lines to OCT2 and OCA-B, our study highlights the power of functional genomic methodologies to uncover essential cancer mechanisms.

By combining ChIP-Seq with gene-expression profiling, we identified multiple OCT2 target genes that play important roles in B-cell function and differentiation, including *ELL2*, *XBP1*, *MYC*, *TERT*, *ADA*, *IL-10*, and *STAT3*. Gene-expression signature analysis revealed that many of the enriched signatures (MYD88, NF-κB, STAT3, and IRF4) are involved in key regulatory pathways in DLBCL. Although OCT2 did not regulate *IRF4* or *PRDM1* directly, the direct regulation of *STAT3* and its activation by autocrine production of IL-10 were particularly intriguing, given the known role of *STAT3* in the transactivation of *PRDM1* and the initiation of plasma cell differentiation. STAT3 appears to play a particularly important role in the differentiation of germinal center B cells to plasma cells (34), an effect that may depend upon synergism with CD40 signaling (35). There are similarities between the phenotypes of OCT2 conditional knockout mice and IL-21 receptor knockout mice, in which germinal centers and memory B cells are formed but germinal center proliferation and plasma cell differentiation are reduced (36). These similarities are intriguing, given that IL-21 receptor signaling activates STAT3. The OCT2 target ELL2 is required for the alternative splicing needed to produce soluble Ig. Together with XBP1 (also an OCT2 target), ELL2 regulates the unfolded protein response during plasma cell differentiation. *Ell2* knockouts show decreased plasma cell numbers as well as reduced expression of OCA-B, suggesting a feed-forward loop during plasma cell differentiation (37). The OCT2 target gene *ADA* encodes adenosine deaminase, which prevents apoptosis during B-cell activation and is required for the formation of germinal centers (38). Thus, the identified OCT2 transcriptional program is consistent with the growth and survival of normal and malignant germinal center B cells and also with the control of postgerminal center plasma cell differentiation by OCT2.

The recurrent *POU2F2* T223A mutation targets a residue in the POU-specific domain that directly contacts the octamer motif within the DNA major groove. A previous analysis posited that this mutation was loss of function (24), but this analysis conflicts with other evidence that OCT2 performs an oncogenic function in DLBCL and with the fact that DLBCL tumors do not acquire nonsense or frame-shift mutations in *POU2F2*, nor do they delete this gene frequently. Instead, our ChIP-Seq and gene-expression data suggest that OCT2 T223A is a neomorphic mutation that is associated with some reduction in transcriptional programs regulated by wild-type OCT2 (Table S3) but also with a gain in expression of noncanonical target genes that have an altered octamer motif in which the base contacted by T223 is changed from guanine to adenine. In DLBCL tumors, the T223A mutation is invariably heterozygous, suggesting a role for the remaining wild-type OCT2 allele. This observation may explain why cells engineered to express only OCT2 T223A were not as fit as those with wild-type OCT2. Alternatively, DLBCL tumors with OCT2 T223A may acquire additional genetic or epigenetic changes that permit them to tolerate its altered regulatory repertoire. In this regard, an intriguing hypothesis is that the OCT2 T223A isoform may promote lymphomagenesis by blocking plasmacytic differentiation, which OCT2 normally promotes. Indeed, genetic events that block full plasmacytic differentiation are recurrent in ABC DLBCL, including multiple lesions that inactivate Blimp1 (reviewed in ref. 39). Hence, DLBCL tumors may acquire T223A and lose one wild-type OCT2 allele, partially blocking plasmacytic differentiation. In addition, one of the noncanonical targets of OCT2 T223A is *FCRL3*, which encodes a transmembrane protein with both ITAM and ITIM domains that enhances TLR9-mediated B-cell proliferation and survival while inhibiting plasma cell differentiation (40). Future analysis of T223A knockin mice would be helpful in testing the hypothesis that OCT2 T223A blocks postgerminal center plasmacytic differentiation.

The present study identifies OCT2 as an attractive therapeutic target in DLBCL. Knockdown of OCT2 was toxic to models of both ABC and GCB DLBCL, suggesting that agents targeting OCT2 would transcend the many regulatory differences between these subtypes (39). Recent clinical trials have focused on oncogenic pathways in ABC DLBCL (41), but methods of targeting GCB DLBCL are needed also. The B-cell–restricted expression of OCT2 and OCA-B suggests that inhibiting these proteins in human patients would not be associated with generalized toxicity. Consistent with this notion, the induced deletion of OCT2 in adult mice was associated with no outward signs of ill health. Finally, our work suggests that agents targeting the OCT2–OCA-B interface might be one possible strategy. Alternatively, new methods to target proteins for destruction using small molecules (42, 43) might be aimed at OCT2 for the therapy of DLBCL.

Materials and Methods

Pou2f2-targeted JM8A3.N1 ES cells were purchased from the European Conditional Mouse Mutagenesis (EUCOMM) Consortium (clone ID HEPD0690_4_H11), International Knockout Mouse Consortium (IKMC) Project 82506 [allele name Pou2f2_tm1a(EUCOMM) Hmgu], and Mouse Genome Informatics (MGI) (allele ID MGI:101897). All animal experiments were carried out in accordance with the National Cancer Institute Animal Care and Use Committee guidelines and approval. A detailed description of all methods is available in *SI Materials and Methods*.

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