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Transcription factor Oct1 protects against hematopoietic stress and promotes acute myeloid leukemia

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A better understanding of the development and progression of acute myelogenous leukemia (AML) is necessary to improve patient outcome. Here we define roles for the transcription factor Oct1/Pou2f1 in AML and normal hematopoiesis. Inappropriate reactivation of the *CDX2* gene is widely observed in leukemia patients and in leukemia mouse models. We show that Oct1 associates with the *CDX2* promoter in both normal and AML primary patient samples, but recruits the histone demethylase Jmjd1a/Kdm3a to remove the repressive H3K9me2 mark only in malignant specimens. The CpG DNA immediately adjacent to the Oct1 binding site within the *CDX2* promoter exhibits variable DNA methylation in healthy control blood and bone marrow samples, but complete demethylation in AML samples. In MLL-AF9-driven mouse models, partial loss of Oct1 protects from myeloid leukemia. Complete Oct1 loss completely suppresses leukemia but results in lethality from bone marrow failure. Loss of Oct1 in normal hematopoietic transplants results in superficially normal long-term reconstitution; however, animals become acutely sensitive to 5-fluorouracil, indicating that Oct1 is dispensable for normal hematopoiesis but protects blood progenitor cells against external chemotoxic stress. These findings elucidate a novel and important role for Oct1 in AML.

Acute myeloid leukemia (AML) is the most common form of adult acute leukemia and is associated with high mortality rates. In the United States there are more than 10,000 AML deaths each year, with a survival rate of ~50% in adults <65 and ~20% in adults >65 [1,2]. Despite great advances in our understanding of the molecular basis of the disease and improvements for Flt3-mutant AML [3–5], the standard of treatment for most forms of AML remains a cyclophosphamide/doxorubicin-based chemotherapeutic regimen [6]. New targets and therapies are therefore needed that may require different approaches to the study of AML. Historically, the genetic alterations in AML have been the major area studied and the main approach used in seeking to understand and to treat the disease. Genomic investigations have cataloged multiple genetic lesions associated with AML [7], including translocations that generate abnormal fusion proteins such as MLL-AF9 and MLL-ENL in approximately 7% of adult AML cases/10% of acute lymphocytic leukemia (ALL) cases, and oncogenic mutations in key genes such as FLT3 and N-Ras in approximately 30% and 10% of AML patients, respectively.

In addition to genetic lesions, studies have identified unmutated factors in leukemia that contribute to the malignant phenotype. These factors may or may not be epigenitically up-regulated, and can be important therapeutic targets. An example of an unmutated factor whose expression is unaltered in most leukemia samples is

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BRD4. BRD4 is important for leukemia maintenance, and can be targeted by JQ1 and related compounds [8]. A number of other genes and their products are commonly mis- or overexpressed in AML, for example, *Hoxa9* [9], *Meis1* [10], *Pou2af1*/*Ocab*/*Bob1* [11], and *Cdx2* [12–15]. The relatively high incidence of these changes makes them targets in a larger fraction of AML patients compared with genetic alterations, and the epigenetic nature of the misexpression suggests that these alterations could be more readily reversible.

Cdx2 encodes a developmentally essential homeodomain transcription factor critical for trophectoderm specification in the early mouse embryo [16]. Later in development, *Cdx2* plays a prominent role in the gastrointestinal tract [17–19] and in primitive hematopoiesis [20]. In adults, *Cdx2* expression is confined mostly to the gastrointestinal tract, where it has a tumor-suppressive role [18,21]. Human *CDX2* expression is not observed in normal blood cells, but inappropriate *CDX2* expression is observed in most cases of adult and pediatric AML and ALL (including those with canonical translocations, complex and normal karyotypes) and in murine leukemia models [12–15]. Forced Cdx2 expression is sufficient to initiate leukemia in mice [13,22]. *CDX2* is rarely involved in translocations associated with AML, though the translocation did not appear to be the transforming event [22]. The molecular pathways leading to abnormal *CDX2* de-repression and misexpression in leukemic cells are not well understood, though it is known that CDX2 promotes self-renewal through the expression of downstream targets such as *Hox* genes and *Meis1* [12,13,23].

The transcription factor Oct1/Pou2f1 is known to regulate *Cdx2* in colon cancer, pancreatic cells, intestinal endocrine cells, and trophoblast stem cells [24–27]. Oct1 is related to the Oct4 pluripotency transcription factor. Oct1 is widely expressed and promotes resistance to genotoxic and oxidative stress, glycolytic metabolism. and malignant transformation [28–31] (for review, see [32–34]). Oct1 amplification and/or Oct1 overexpression correlate with tumor aggressiveness in esophageal, gastric, prostate, lung, head and neck, cervical, and colorectal cancer [35–44]. Oct1 also regulates stem cell and immune memory phenotypes [45,46] and promotes thymic lymphoma in mouse models [28]. Oct1 constitutively occupies binding sites in some target genes [47–49], while binding to others after exposure to exogenous stresses such as hydrogen peroxide [50].

Oct1 can both positively and negatively regulate target gene expression. In a repressive modality, the chromatin-modifying complex NuRD associates with Oct1 to repress target genes, whereas in a positive modality, the histone lysine demethylase Jmjd1a/Kdm3a is recruited. NuRD is a complex of proteins containing nucleosome remodeling and histone deacetylase activity associated primarily with transcriptional repression [51–53]. Jmjd1a (also known as KDM3A and Jhdm2a) is a histone lysine demethylase with specificity toward mono- and dimethyl H3K9 [54]. Switching from negative to positive modalities occurs in response to MAPK signals [24] or expression of another Oct1 cofactor, OCA-B/Bob.1 [46]. OCA-B is not strongly expressed in myeloid cells, but OCA-B misexpression occurs in ~30% of primary AML tumor samples, where it is a negative prognostic factor [11].

Here we report that Oct1 is constitutively bound to *CDX2* in both normal and malignant human primary samples and that its cofactors change in the malignant condition in a manner that correlates with *CDX2* misexpression. Oct1 associates with NuRD in normal bone marrow samples but switches cofactors to Jmjd1a in AML samples. The association of Jmjd1a at the *CDX2* promoter correlates with loss of the repressive mark it removes, H3K9me2, and with a complete lack of DNA methylation near a conserved CpG dinucleotide adjacent to the Oct1 binding site. Suppression of Oct1 protects animals from MLL-AF9-driven leukemia, though complete loss results in hematopoietic failure in the presence of this fusion oncoprotein. Similar bone marrow failure occurs in animals when Oct1 is deleted from normally engrafted bone marrow and the mice are treated with 5-fluorouracil (5-FU), suggesting that loss of Oct1 sensitizes hematopoietic stem cells to proliferative stress from either the presence of oncoproteins or exposure to chemotoxic stress. Cumulatively, the results indicate that Oct1 is an important regulator of leukemogenicity and hematopoietic stress.

Methods

Blood and bone marrow samples

Surplus peripheral blood and bone marrow aspirates from AML patients were originally submitted for clinical flow cytometric evaluation at ARUP Laboratories (Salt Lake City, UT). Approval to utilize de-identified leftover patient samples was obtained from the University of Utah Institutional Review Board (IRB No. 7275). All experiments were performed in accordance with this approved protocol. Normal controls were collected from individuals in whom no malignancy or clonality was detected by flow cytometry. Normal bone marrow samples were composed predominantly of myeloid cells and all demonstrated approximately normal (~1%) myoblasts.

Quantitative real-time polymerase chain reaction

RNA was isolated using TRIzol (Invitrogen), and cDNA was synthesized using the Maxima Kit (Invitrogen) and random hexamers (Invitrogen). Sequences for quantification of human *CDX2* were taken from Shakya et al. [24].

Chromatin immunoprecipitation

Oct1, Jmjd1a, H3K9me2, and NuRD/Mta2 chromatin immunoprecipitation (ChIP) was performed as described previously, using

a method that calculates enrichment relative to input DNA and relative to isotype control antibody and an intergenic sequence [24,55]. Antibodies used were as follows: Oct1—Bethyl A1301-716A and A301-717A (used 1:1); Jmjd1a—Abcam ab91252; Mta2—Abcam ab50209; H3K9me2—Abcam ab8898. The human *Cdx2* primer pair was described previously [24].

NanoString expression of Kdm3a, Mta2, Pou2f1

Expression of *KDM3A*, *MTA2* and *POU2F1* in frozen primary human samples was measured using NanoString and a custom gene array built on the leukemia gene expression panel. RNA was prepared from blood cells using an RNeasy Micro kit (Qiagen) and an RNA clean and concentrator kit (Zymo, Irvine, CA). Analysis and normalization of the raw data were conducted with nSolver Analysis Software v4.0 (NanoString Technologies, Seattle, WA), with negative control subtraction and positive control normalization, and normalization with housekeeping genes on the leukemia panel.

Bisulfite sequencing

Bisulfite DNA modification and analysis were performed as published [24]. Primers for bisulfite-converted DNA were *CDX2* promoter forward, 5′ ATGATAGATATTAATGGTTGGAGA, and *CDX2* promoter reverse, 5′ ACTCCTATCTCCAAACTCAAAT.

Mice

All mice used in the study were on the pure C57BL6/J background. The *Oct1* (*Pou2f1*) conditional allele, along with the genotyping protocol, was described previously [46]. Mx1-Cre mice [56] were obtained from the Jackson Laboratory (Bar Harbor, ME).

Virus production

The pMSCV-puro-MLL-AF9 plasmid was obtained from the laboratory of Dr. Robert G. Roeder. The 293T cells were transfected with 5 μg of plasmid DNA together with 5 μg of pCL-Eco packaging plasmid using polyethyleneimine (PEI)-mediated transfection. Viral supernatants were harvested 48 and 72 hours posttransfection, passed through a 0.45-μm filter, and sequentially transduced into bone marrow cells as described below.

Transplantation and leukemia generation

Bone marrow transduction of MLL-AF9 and the subsequent transplant into lethally irradiated mice were performed as described previously [57]. Briefly, 5 days prior to bone marrow harvest, 6- to 9-week-old mice were injected with 200 mg/kg 5-FU (Sigma). Bone marrow was cultured in RPMI medium (Sigma) supplemented with 20% fetal bovine serum (Sigma), 100 U/mL penicillin (Thermo Fisher Scientific), 100 μg/mL streptomycin (Thermo Fisher Scientific), 2 mmol/L L-glutamine (Thermo Fisher Scientific), 20 ng/mL mouse interleukin-3 (mIL-3; Fitzgerald, North Acton, MA), 50 ng/mL mIL-6 (Fitzgerald), and 50 ng/mL mouse stem cell factor (mSCF; Fitzgerald) for 4 days. Viral transduction was performed at 3 and 4 days by spin infection at 1500*g* for 1.5 hours at 37°C in the presence of 4 μg/mL polybrene (Sigma). Transduced bone marrow cells were injected into lethally irradiated (two split doses of 450 rad, 1 hour apart) by retro-orbital (RO) injection. After transplant, sulfamethoxazole/trimethoprim (STI Pharma LLC, Langhorne PA) was provided to mice in the drinking water for 3 weeks. Mice were monitored daily for any signs of sickness or disease. Complete blood counts were conducted weekly and at endpoint using a Drew Scientific Hemavet 950FS with manufacturer mouse settings. Mice were euthanized when the WBC/RBC ratio fell <100 or when the mice appeared moribund. Spleens were taken from euthanized mice to confirm splenomegaly.

pIpC administration

Poly(deoxyinosinic-deoxycytidylic) acid (pIpC, Sigma P-4929) was administered at 12.5 μg/g intraperitoneally, four injections spaced 48 hours apart, for the Oct1 conditional allele experiments.

Peripheral blood smears

Two microliters of blood was placed on a microscope slide, smeared, methanol fixed, and stained using Wright staining according to the manufacturer's instructions. De-identified peripheral blood smears were imaged and evaluated at 500 × by a hematopathologist.

Results

Oct1 cofactor switching and DNA demethylation are associated with abnormal Cdx2 expression in primary human AML

Using real-time reverse transcription quantitative polymerase chain reaction (RT-qPCR), we observed elevated *CDX2* expression in malignant high-blast-count human AML patient samples (Figure 1A), as previously reported [13]. *CDX2* expression also correlates with increased histone H4 acetylation at the *CDX2* promoter in ChIP assays, as expected (not shown). The *CDX2* upstream promoter region contains a conserved canonical Oct1-binding "octamer" sequence and proximal CpG motif 51 bp upstream of the transcription start site (Figure 1B). We performed ChIP at this region using Oct1 antibodies in primary human samples. There was considerable sample-to-sample variance among samples from different patients with different disease etiologies, blast counts, and so on. Nevertheless, we found that Oct1 binding to the *CDX2* promoter could be detected in both tumor and healthy control samples (Figure 1C). Oct1 recruits two chromatin-modifying activities, NuRD/Mta2 and Jmjd1a, in a mutually exclusive fashion either to negatively regulate or to positively regulate gene expression [24]. Jmjd1a (also known as KDM3A) is a histone lysine demethylase with specificity toward mono- and dimethyl H3K9 [54]. We therefore also performed ChIP for Mta2, Jmjd1a, and H3K9me2 at the Oct1 binding site in the *CDX2* promoter. In contrast to Oct1, NuRD components were only enriched in the normal samples, while Jmjd1a was enriched in AML samples (Figure 1D,E). Consistent with the activity

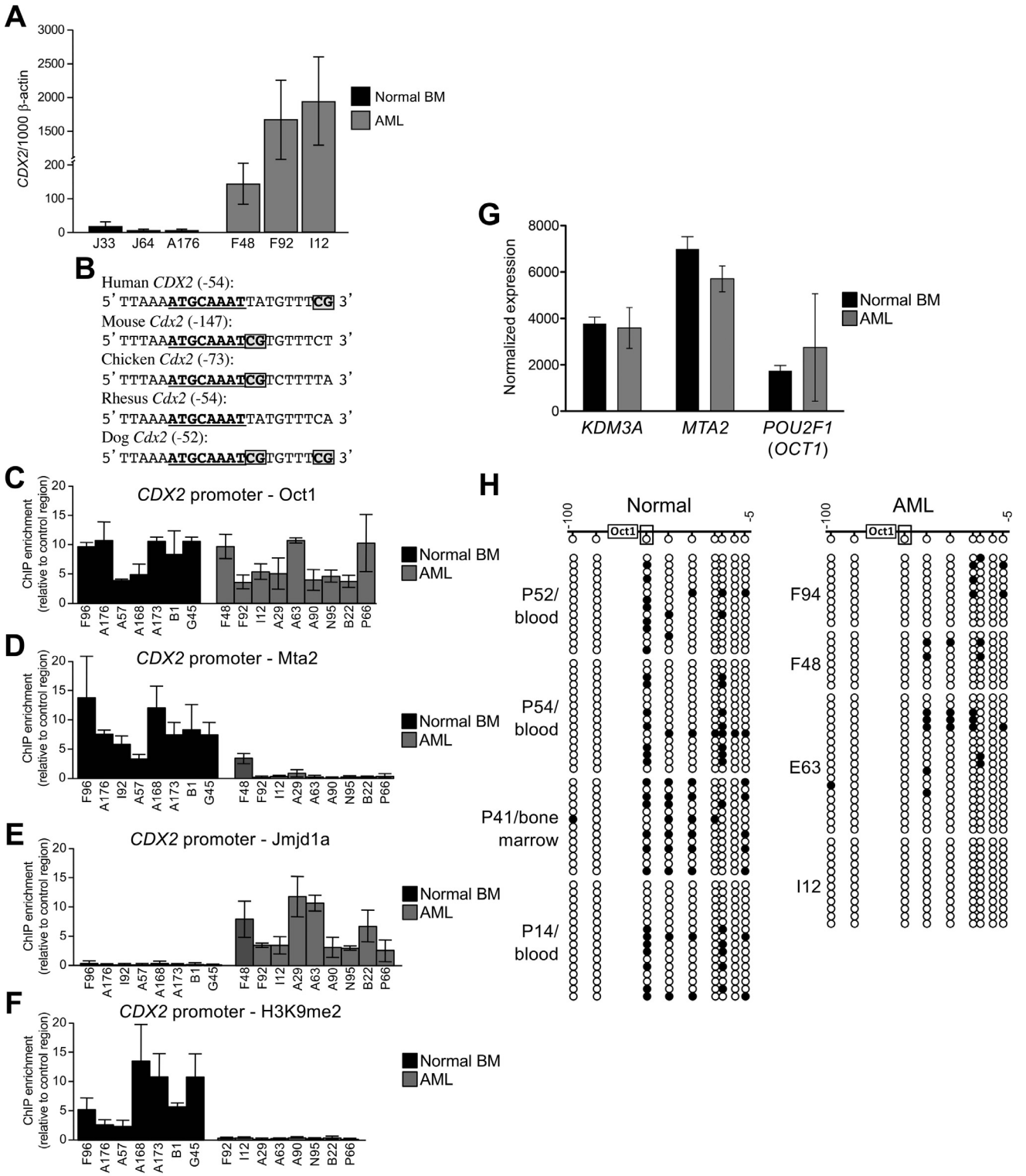

Figure 1. Oct1 coactivator switching and local demethylation at *CDX2* in human AML. (**A**) *CDX2* qRT-PCR in normal and leukemic patient bone marrow samples. Expression was normalized using *β*-actin. Averages of triplicate experiments are shown ±standard deviations. (**B**) Species comparison of the *CDX2* promoter region. Oct protein binding sites are underlined. CG sites are boxed. (**C**) Oct1 ChIP enrichment at the *CDX2* promoter using human AML isolates and normal bone marrow controls. Enrichment is shown relative to isotype control antibodies and an intergenic region. Average ChIP enrichment is shown ±standard deviation. (**D**) ChIP enrichment using Mta2 (NuRD) antibodies at the same region. (**E**) ChIP enrichment using Jmjd1a antibodies. (**F**) H3K9me2 antibodies. (**G**) Expression of *JMJD1A* (*KDM3A*), *MTA2,* and *OCT1* (*POU2F1*) genes was measured in primary human AML and normal samples using NanoString. n = 5 for AML samples, including samples B22 and A29 from (**A**–**F**), and n = 3 for normal bone marrow samples. (**H**) Methylation status of CpGs adjacent to the Oct1 binding site in the *CDX2* upstream promoter region was assessed using bisulfite sequencing in normal blood and bone marrow controls (*left*) or in primary AML samples (*right*). *Dark circles* indicate the presence of DNA methylation in a particular sequencing read (reads denoted by rows).

of Jmjd1a, strong H3K9me2 enrichment was identified near the *CDX2* Oct1 binding site in normal but not AML specimens (Figure 1F). These findings indicate that Oct1 recruits different cofactors depending on cellular context, switching from NuRD components to Jmjd1a in AML, which corresponds to higher *CDX2* expression and reduced H3K9me2 enrichment. Expression of *JMJD1A* (*KDM3A*), *MTA2*, and *OCT1* (*POU2F1*) was broadly similar comparing normal and malignant samples (Figure 1G), indicating that the differences observed in ChIP were due to differential recruitment rather than differential expression. The regulation of Oct1 at the level of coactivator switching is consistent with TCGA data indicating no survival differences in patients with high or low levels of Oct1 (Supplementary Figure E1, online only, available at www.exphem.org).

At the *Il2* gene locus in mouse T cells, Oct1 associates with Jmjd1a, which mediates local H3K9me2 demethylation and promotes DNA hypomethylation [24]. DNA methylation has been studied at *CDX2* in the context of ALL and AML [15]; however, only the body of the gene and proximal promoter region were analyzed. Moreover, methylation at these sites was averaged. The upstream distal promoter containing the octamer motif that binds Oct1 has therefore not been studied. Bisulfite sequencing analysis of the Oct1 binding region revealed a strikingly complete absence of CpG methylation in all AML samples at this position (Figure 1H). In contrast, healthy human peripheral blood and bone marrow controls exhibited substantial DNA methylation in the same region. As a control, we also analyzed the body of the *CDX2* gene downstream of the promoter. Consistent with prior findings [15], we observed little difference between these same AML and control samples (Supplementary Figure E2, online only, available at www.exphem.org). Thus, the epigenetic state of the *CDX2* promoter, specifically sites surrounding the Oct1 site, is unique to the transformed state.

Conditional Oct1 deletion protects against leukemia

To determine the role of Oct1 in a mouse model of high-risk AML, we utilized retroviral MLL-AF9 transduction of primary murine hematopoietic progenitors [58,59] and the transplantation of these cells into lethally irradiated recipient mice. In this model, signs of leukemia such as splenomegaly, lymphocytosis, and presence of AML blasts in the peripheral blood can be monitored in recipient mice, which typically develop an oligoclonal AML (Figure 2A) and succumb within about 10 weeks [57]. As Oct1 homozygous germline deletion is embryonic lethal [27,60], we utilized an *Oct1* conditional allele (previously described in [46]) onto Mx1-Cre [56] to generate $Oct1^{+/fl}$;Mx1-Cre and $Oct1^{fl/\Delta}$;Mx1-Cre donor bone marrow for MLL-AF9 transduction and subsequent transplant into C57BL/6J recipient mice. Although there are potential caveats to using Mx1-Cre [61], we found that *Oct1* is present before pIpC is administered and that *Oct1* is effectively deleted in the blood of transplanted mice when pIpC is administered 5 weeks posttransplant (Figure 2B).

We observed significantly increased survival with the loss of even a single *Oct1* allele, either by germline deletion ($Oct1^{fl/\Delta}$;Mx1-Cre PBS treated: $p = 0.0198$, hazard ratio [HR] = 3.279) or pIpC-induced deletion ($Oct1^{+/fl}$;Mx1-Cre pIpC treated: $p = 0.0211$, HR = 3.062) (Figure 2C). Loss of both Oct1 alleles, using $Oct1^{fl/\Delta}$;Mx1-Cre mice administered pIpC, resulted in even longer survival (Figure 2C; $p = 0.0017$, HR=4.472). Ultimately however, these mice became anemic and were euthanized. The lethality associated with total Oct1 loss was not due to leukemia as WBC counts were not elevated and leukemic blasts were not observed in the peripheral blood of these mice (Figure 2D, E) or bone marrow (not shown), and mice did not exhibit splenomegaly (not shown). Rather, lethality was associated with hematopoietic failure without leukemia. RBC and WBC counts in these animals dropped precipitously in all endpoint mice compared with normal and leukemic controls (Figure 2F, G). These data suggest that loss of Oct1 protects mice against MLL-AF9-driven AML; however, mice also suffer from hematopoietic failure with the loss of Oct1 in the presence of MLL-AF9.

Conditional Oct1 deletion in nonleukemic mice results in stable hematopoietic engraftment but hypersensitivity to 5-FU treatment

To determine if acute Oct1 deletion impairs normal hematopoiesis in the non-leukemic setting, we transplanted bone marrow from mice with the Oct1 conditional allele into lethally irradiated recipients as illustrated in Figure 2 (but without addition of the MLL-AF9 oncoprotein) and used pIpC to delete one or both alleles of Oct1 in the adult bone marrow. $Oct1^{+/fl}$;Mx1-Cre and $Oct1^{fl/\Delta}$;Mx1-Cre mice were injected intraperitoneally with pIpC to delete Oct1 at 5 weeks (Figure 3A) and 12 weeks (Supplementary Figure E3, online only, available at www.exphem.org) posttransplant. After pIpC injection, mice with and without Oct1 deletion appeared normal and healthy by examination and by blood counts (not shown). At 25 weeks, all mice were injected intraperitoneally with 5-FU, which depletes rapidly dividing intermediate progenitor cells, leading to mobilization of the stem cell pool [62]. $Oct1^{fl/\Delta}$;Mx1-Cre mice injected with pIpC died within 2 weeks of 5-FU treatment. The decreased blood counts, particularly red blood cell count (Figure 3D) and decrease in peripheral blood cellularity (Figure 3C, D) are all consistent with hematopoietic failure. Put together, our data suggest that Oct1 is dispensable for normal hematopoiesis, but is necessary for the hematopoietic proliferative stress response caused by an oncogene or 5-FU treatment.

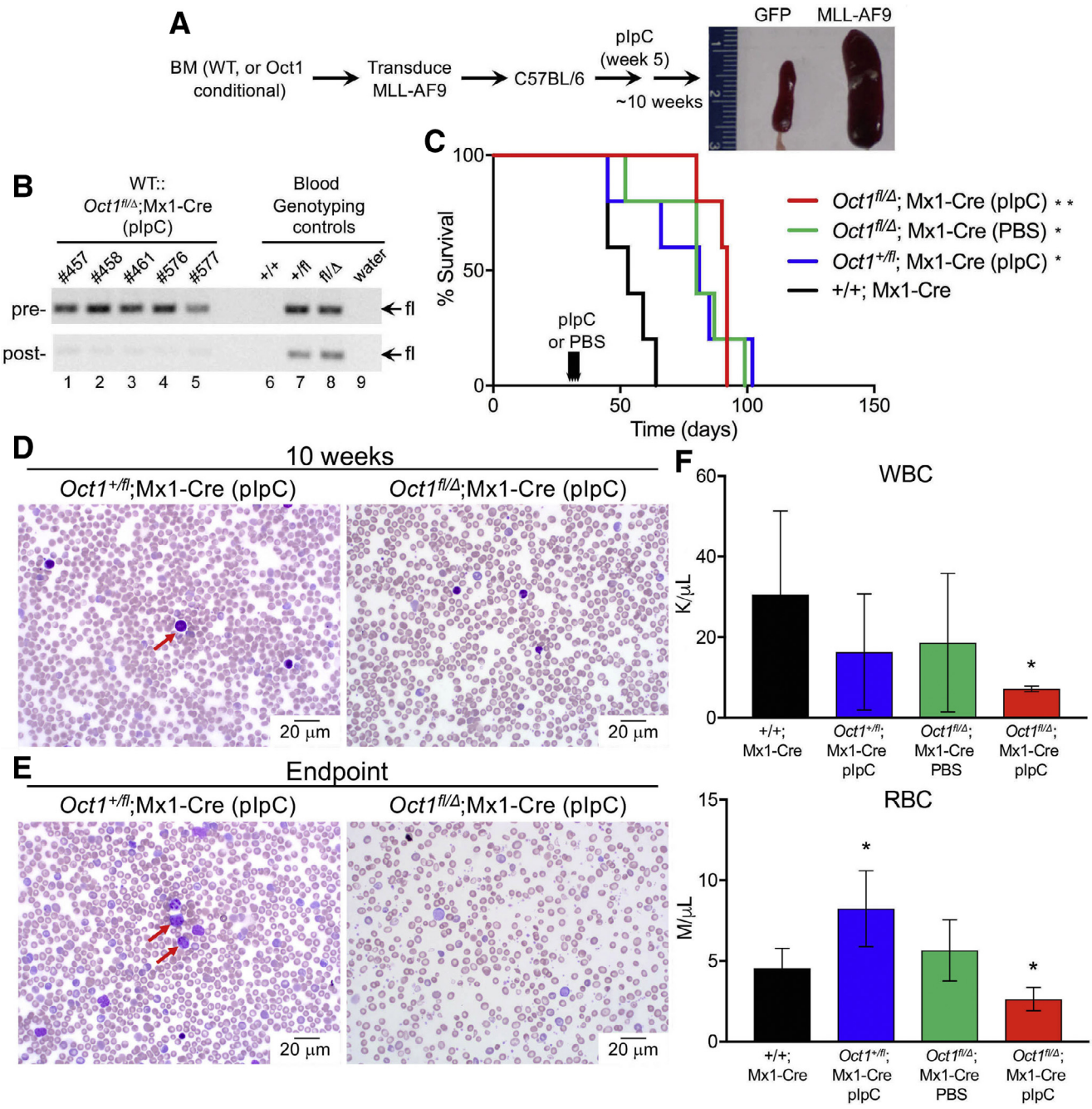

Figure 2. Oct1 conditional deletion protects mice from leukemia. (**A**) Experimental design outlining bone marrow transplant protocol and a representative picture of the splenomegaly induced in this AML model. (**B**) *Oct1* conditional flox allele deletion measured by semiquantitative PCR with genomic DNA from peripheral blood pre- and post-pIpC injection. (**C**) Kaplan–Meier plot revealing survival (%) of mice in time (days) posttransplant, n = 5 per group. Significance was determined by using the log-rank (Mantel–Cox) test and hazard ratio (log rank) test to compare against +/+;Mx1-Cre survival. Oct1$^{fl/\Delta}$;Mx1-Cre (pIpC): $p = 0.0017$, HR = 4.472; *Oct1*$^{fl\Delta/}$;Mx1-Cre (PBS): $p = 0.0198$, HR = 3.279; *Oct1*$^{+/fl}$;Mx1-Cre (pIpC): $p = 0.0211$, HR=3.062. (**D**) Peripheral blood smears, Wright stained, made at 10 weeks or the endpoint (**E**) of the experiment. (**F**) RBC and WBC counts at experimental endpoint. Compared with +/+;Mx1-Cre counts made using Student's *t* test: WBC Oct1$^{fl/\Delta}$;Mx1-Cre (pIpC), $p = 0.0359$; RBC *Oct1*$^{+/fl}$;Mx1-Cre (pIpC), $p = 0.0144$; Oct1$^{fl/\Delta}$;Mx1-Cre (pIpC), $p = 0.017$ (other conditions not significant).

Discussion

Here we report that the transcription factor Oct1, a known promoter of stem cell phenotypes and malignant transformation [28,45], is essential to sustain AML in an MLL-AF9-driven murine model. By combining this mouse AML model system with a conditional Oct1 knockout allele [46], we found that loss of even a single Oct1 allele significantly delayed leukemogenesis.

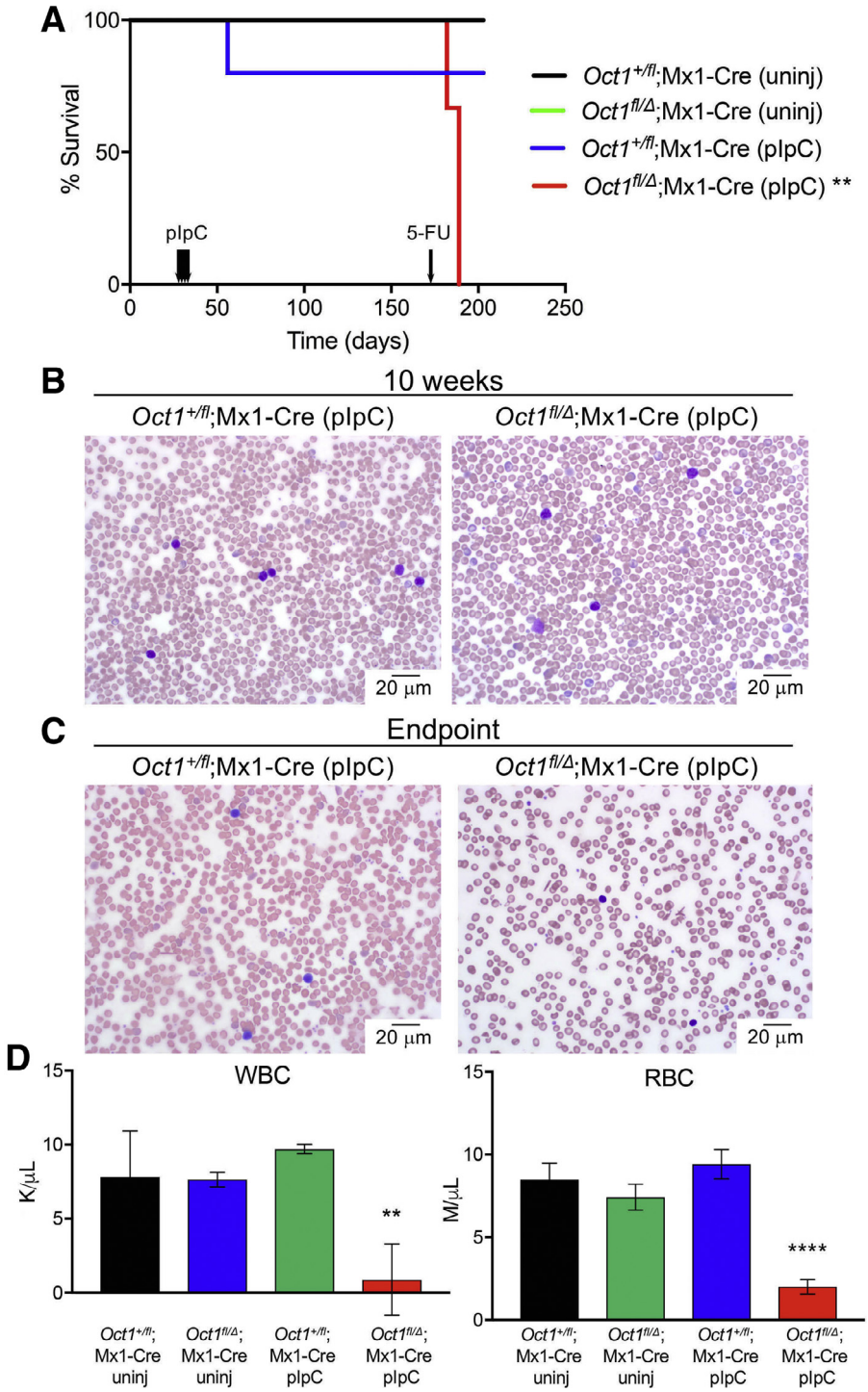

Figure 3. Oct1 is dispensable for homeostatic hematopoiesis but protects mice from hematopoietic stress. (**A**) Kaplan–Meier plot revealing survival (%) over time (days) posttransplant. Significance was determined using the log-rank (Mantel–Cox) test to compare each condition with *Oct1*$^{+/fl}$;Mx1-Cre uninjected. *Oct1*$^{+/fl}$;Mx1-Cre uninjected, n = 4; *Oct1*$^{fl/\Delta}$;Mx1-Cre uninjected, n = 3, $p > 0.99$; *Oct1*$^{+/fl}$;Mx1-Cre pIpC injected, n = 5, $p > 0.99$; *Oct1*$^{fl/\Delta}$;Mx1-Cre pIpC injected, n = 6, $p = 0.0051$. (**B**) Peripheral blood smears, Wright stained, made at 10 weeks or endpoint (**C**) of the experiment. (**D**) RBC and WBC counts at experimental endpoint. Compared with *Oct1*$^{+/fl}$;Mx1-Cre uninjected counts using Student's *t* test: WBC Oct1$^{fl/\Delta}$;Mx1-Cre (pIpC), $p = 0.0041$; RBC Oct1$^{fl/\Delta}$;Mx1-Cre (pIpC), $p < 0.0001$.

Interestingly, conditional deletion of both Oct1 alleles completely protected mice from leukemia, but also resulted in bone marrow failure, evidenced by pancytopenia and lethality. This phenotype was not observed in nontransformed, transplanted hematopoietic progenitors. The underlying mechanism of how Oct1

deletion protects from leukemia, be it grow arrest, cell death, or differentiation, or some combination of these, remains to be determined.

Malignant transformation caused by deregulation of oncogenes and tumor suppressors can result in increased cellular stress, for example, oxidative, metabolic or DNA damage-associated [63]. Cancer cells must adapt to these stresses, resulting in changes that can make cancer cells dependent on activities that are not transforming themselves but are nevertheless needed to promote the cancer phenotype. Because these activities provide less vital functions in nontumor cells, these pathways may be targetable in a therapeutic context. Oct1 is associated with stress resistance, and its loss confers hypersensitivity to oxidative and genotoxic stress [29]. Because Oct1 deletion in leukemia was protective but caused bone marrow failure, likely as a result of oncogenic stress, we wanted to further investigate the role of Oct1 in hematopoietic stress. To test Oct1's role in hematopoietic stress resistance in adult animals, we treated mice that had been engrafted with Oct1 conditional bone marrow and subsequently treated with pIpC to delete Oct1 with 5-FU, a cytotoxic DNA base analog that in a wild-type context kills proliferating cells in the bone marrow. Mice with no Oct1 died within 2 weeks of 5-FU injection from bone marrow failure. This result is consistent with prior observations in the hematopoietic system with Oct1, specifically that Oct1-deficient cells fail to compete with wild-type cells in competitive fetal liver transplants and fail in secondary fetal liver transplants [45]. Other transcription factors including Bmi1 and HLF exhibit similar stress-dependent phenotypes [64–66].

Using human specimens, we found that Oct1 associated with the *CDX2* gene promoter changes from cofactors that are repressive to activating cofactors in normal progenitors versus AML, respectively. Cdx2 is critical for development of the early embryo [67] and in endoderm, where it acts as a tumor suppressor in gut epithelial cells [18]. In the blood system, Cdx2 is important for fetal hematopoiesis [20], but is not normally expressed in adult progenitors or differentiated cells. *CDX2* is only rarely mutated or translocated in leukemia, but is almost universally reactivated and highly expressed in AML and ALL and in murine leukemia models, regardless of karyotype [12–15,22,23]. In leukemic cells, Cdx2 activates *Meis1* and *Hoxa9*, genes essential for leukemia progression [12,13,23]. The pathways and mechanisms that inappropriately activate *Cdx2* have remained obscure. ChIP-qPCR reveals that Oct1 is bound to the *CDX2* promoter in human cells, and that Oct1 occupancy at the *CDX2* promoter does not change in AML. Instead, the cofactors known to associate with Oct1 change in the malignant versus normal states. In normal cells the negative cofactor NuRD/Mta2 associates with the Oct1-bound region, while in malignant cells the activating cofactor Jmjd1a instead localizes with Oct1. Furthermore, Jmjd1a association corresponds to reduced H3K9me2 enrichment at the *CDX2* promoter.

The precise upstream mechanisms that control Oct1 cofactor switching have not been determined but could be due to MAP kinase signaling or expression of the Oct1 cofactor OCA-B, as we have reported that either can drive Jmjd1a association with Oct1 [24,46]. MAPK activity is frequently augmented in leukemia because of, for example, Flt3 internal tandem duplication or N-Ras mutation. ERK, for example, is active in 50%–80% of AML samples [68,69] and in >30% of ALL [70]. Oct1 cofactor switching is known to be regulated by MAPK signals, specifically MEK-ERK signaling [24,36,71]. In primary $CD4^+$ T cells, MAP kinase signaling causes Oct1 bound at the interleukin-2 promoter to switch from a repressive to an activating mode characterized by Jmjd1a association [24]. A study focused on melanoma and leukemia identified Oct1 at the center of a pathway linking mutant BRAF and oncogenic MAPK signaling to lipid metabolism, through the direct Oct1 target *Hmgcl* [71]. Interestingly, in the gut where Cdx2 is tumor suppressive, the *Cdx2* gene is downmodulated by MEK-ERK signaling [21,24]. Similarly, OCA-B expression is associated with higher AML relapse rates and poor prognosis [11]. Also untested is the role of the Oct1 paralog protein Oct2 in this process. Oct2 binds the same DNA sequences as Oct1 [72], and also associates with OCA-B [73]. Higher Oct2 expression is also associated with poor prognosis in AML [11].

Although CpG methylation is often analyzed on a region-, locus-, or genome-wide scale, methylation changes at one or a small number of CpG methylation sites can strongly influence gene expression [24,74–78]. We and others previously reported findings of local DNA methylation control by Oct1 [24,79]. These changes can be linked to changes in Oct1 cofactor status [24], most likely because de novo DNA methyltransferases are known to dock with nucleosomes containing H3K9me2 to methylate nearby CpGs [77,80,81]. We therefore studied DNA methylation in the region surrounding the Oct1 binding site upstream of the human *CDX2* promoter. Prior work revealed few differences in DNA methylation at the downstream *CDX2* core promoter and transcription initiation region [15]. Consistent with the switch in Oct1 cofactors, we found that the CpG dinucleotide near the Oct1 binding site, Just upstream of the *CDX2* core promoter, is completely demethylated specifically in malignant cells. DNA methyltransferases associate with complexes that recognize H3K9 methylation in nucleosomes [80,82], providing a potential mechanistic link between association of the Jmjd1a cofactor and the observed local DNA

demethylation. Oct1 binding sites are associated with dynamic changes in local DNA methylation during hematopoiesis [83]. These findings support the hypothesis that Oct1 becomes activated posttranslationally to regulate *CDX2*, and potentially other target genes, as part of a malignant program in AML.

The *POU2F1* (*OCT1*) locus is located at position 1q24.2 in humans. Chromosomal gain in this region is associated with both solid and hematological malignancy. Chromosome 1q amplification is observed in AML generation, during clonal evolution, and in cases of Fanconi-associated AML [84–90]. These cases are rare, however, and elevated *OCT1* (*POU2F1*) message levels do not broadly correlate with worse outcome in AML. The finding that changes in Oct1 activity, through altered cofactor associations but not mutation or elevated expression, drive a malignant leukemic program is consistent with the observation that Oct1 target sites, including *CDX2*, are highly enriched in the promoters of significantly upregulated genes in lung and breast adenocarcinoma, leukemia, and myeloid leukemia stem cells, without concomitant increases in Oct1 mRNA levels [38,91–94]. In some of these cases Oct1 sites are more enriched than are sites for other transcription factors. For example, an embryonic stem cell gene expression signature was identified in myeloid leukemia stem cells associated with MLL-transformed leukemia-initiating cells, although this signature lacked Oct4 [91]. We speculate that Oct1 association with cofactors that enhance transcriptional activation potential may fulfill this role.

Together, our data reveal a role for Oct1 in resistance to chemotoxic stress and support of AML, and identify the known AML oncogene, *CDX2,* as an Oct1 target in AML cells.

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Conflict of interest disclosure

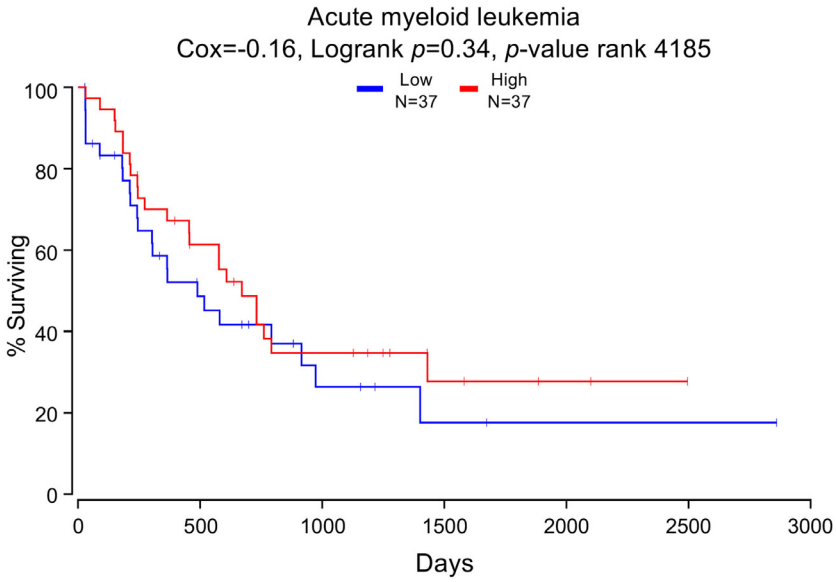
The authors declare they have no competing financial interests regarding this work.

References

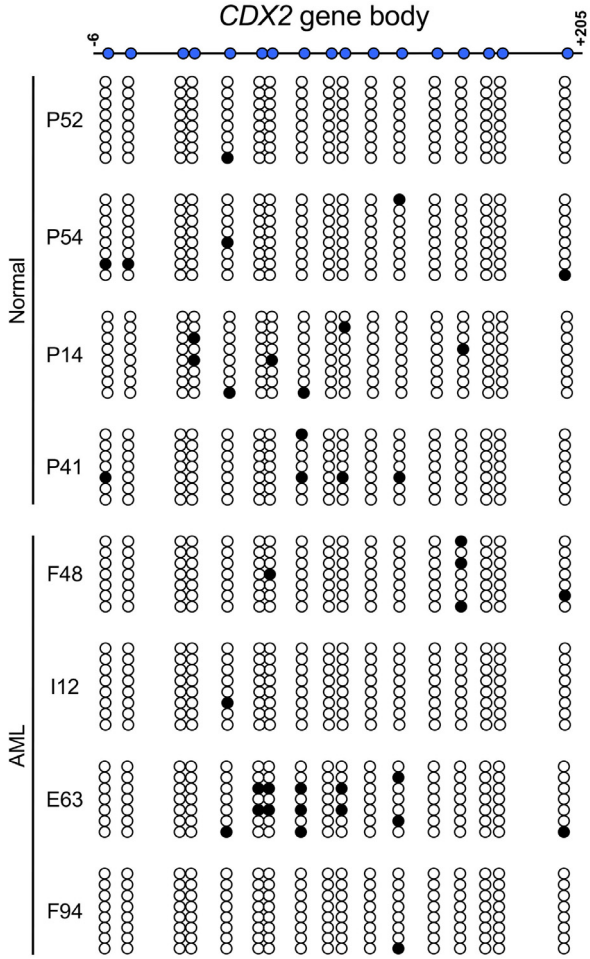
1. De Kouchkovsky I, Abdul-Hay M. Acute myeloid leukemia: a comprehensive review and 2016 update. Blood Cancer J. 2016;6:e441.
2. Wouters BJ, Delwel R. Epigenetics and approaches to targeted epigenetic therapy in acute myeloid leukemia. Blood. 2016;127:42–52.
3. Lancet JE. Is the overall survival for older adults with AML finally improving? Best Pract Res Clin Haematol. 2018;31:387–390.
4. Talati C, Sweet K. Recently approved therapies in acute myeloid leukemia: a complex treatment landscape. Leuk Res. 2018;73:58–66.
5. Stone RM. What FLT3 inhibitor holds the greatest promise? Best Pract Res Clin Haematol. 2018;31:401–404.
6. Dombret H, Gardin C. An update of current treatments for adult acute myeloid leukemia. Blood. 2016;127:53–61.
7. Graubert TA, Mardis ER. Genomics of acute myeloid leukemia. Cancer J. 2011;17:487–491.
8. Zuber J, Shi J, Wang E, et al. RNAi screen identifies Brd4 as a therapeutic target in acute myeloid leukaemia. Nature. 2011;478:524–528.
9. Golub TR, Slonim DK, Tamayo P, et al. Molecular classification of cancer: class discovery and class prediction by gene expression monitoring. Science. 1999;286:531–537.
10. Kumar AR, Li Q, Hudson WA, et al. A role for MEIS1 in MLL-fusion gene leukemia. Blood. 2009;113:1756–1758.
11. Advani AS, Lim K, Gibson S, et al. OCT-2 expression and OCT-2/BOB.1 co-expression predict prognosis in patients with newly diagnosed acute myeloid leukemia. Leuk Lymphoma. 2010;51:606–612.
12. Rawat VP, Thoene S, Naidu VM, et al. Overexpression of CDX2 perturbs HOX gene expression in murine progenitors depending on its N-terminal domain and is closely correlated with deregulated HOX gene expression in human acute myeloid leukemia. Blood. 2008;111:309–319.
13. Scholl C, Bansal D, Dohner K, et al. The homeobox gene CDX2 is aberrantly expressed in most cases of acute myeloid leukemia and promotes leukemogenesis. J Clin Invest. 2007;117:1037–1048.
14. Riedt T, Ebinger M, Salih HR, et al. Aberrant expression of the homeobox gene CDX2 in pediatric acute lymphoblastic leukemia. Blood. 2009;113:4049–4051.
15. Thoene S, Rawat VP, Heilmeier B, et al. The homeobox gene CDX2 is aberrantly expressed and associated with an inferior prognosis in patients with acute lymphoblastic leukemia. Leukemia. 2009;23:649–655.
16. Niwa H, Toyooka Y, Shimosato D, et al. Interaction between Oct3/4 and Cdx2 determines trophectoderm differentiation. Cell. 2005;123:917–929.
17. Suh E, Traber PG. An intestine-specific homeobox gene regulates proliferation and differentiation. Mol Cell Biol. 1996;16:619–625.
18. Guo RJ, Suh ER, Lynch JP. The role of Cdx proteins in intestinal development and cancer. Cancer Biol Ther. 2004;3:593–601.
19. Lu CW, Yabuuchi A, Chen L, Viswanathan S, Kim K, Daley GQ. Ras-MAPK signaling promotes trophectoderm formation from embryonic stem cells and mouse embryos. Nat Genet. 2008;40:921–926.
20. Wang Y, Yabuuchi A, McKinney-Freeman S, et al. Cdx gene deficiency compromises embryonic hematopoiesis in the mouse. Proc Natl Acad Sci USA. 2008;105:7756–7761.
21. Krueger F, Madeja Z, Hemberger M, McMahon M, Cook SJ, Gaunt SJ. Down-regulation of Cdx2 in colorectal carcinoma cells by the Raf-MEK-ERK 1/2 pathway. Cell Signal. 2009;21:1846–1856.
22. Rawat VP, Cusan M, Deshpande A, et al. Ectopic expression of the homeobox gene Cdx2 is the transforming event in a mouse model of t(12;13)(p13;q12) acute myeloid leukemia. Proc Natl Acad Sci USA. 2004;101:817–822.

23. Wang Z, Iwasaki M, Ficara F, et al. GSK-3 promotes conditional association of CREB and its coactivators with MEIS1 to facilitate HOX-mediated transcription and oncogenesis. Cancer Cell. 2010;17:597–608.
24. Shakya A, Kang J, Chumley J, Williams MA, Tantin D. Oct1 is a switchable, bipotential stabilizer of repressed and inducible transcriptional states. J Biol Chem. 2011;286:450–459.
25. Jin T, Li H. Pou homeodomain protein OCT1 is implicated in the expression of the caudal-related homeobox gene Cdx-2. J Biol Chem. 2001;276:14752–14758.
26. Wang P, Wang Q, Sun J, et al. POU homeodomain protein Oct-1 functions as a sensor for cyclic AMP. J Biol Chem. 2009;284: 26456–26465.
27. Sebastiano V, Dalvai M, Gentile L, et al. Oct1 regulates trophoblast development during early mouse embryogenesis. Development. 2010;137:3551–3560.
28. Shakya A, Cooksey R, Cox JE, Wang V, McClain DA, Tantin D. Oct1 loss of function induces a coordinate metabolic shift that opposes tumorigenicity. Nat Cell Biol. 2009;11:320–327.
29. Tantin D, Schild-Poulter C, Wang V, Hache RJ, Sharp PA. The octamer binding transcription factor Oct-1 is a stress sensor. Cancer Res. 2005;65:10750–10758.
30. Bellance N, Pabst L, Allen G, Rossignol R, Nagrath D. Oncosecretomics coupled to bioenergetics identifies alpha-amino adipic acid, isoleucine and GABA as potential biomarkers of cancer: Differential expression of c-Myc, Oct1 and KLF4 coordinates metabolic changes. Biochim Biophys Acta. 2012;1817:2060–2071.
31. Vázquez-Arreguín K, Maddox J, Kang J, Park D, Cano RR, Factor RE, Ludwig T, Tantin D. BRCA1 through its E3 ligase activity regulates the transcription factor Oct1 and carbohydrate metabolism. Mol Cancer Res. 2018;16:439–452.
32. Tantin D. Oct transcription factors in development and stem cells: insights and mechanisms. Development. 2013;140:2857–2866.
33. Zhao FQ. Octamer-binding transcription factors: genomics and functions. Front Biosci (Landmark Edition). 2013;18:1051–1071.
34. Vazquez-Arreguin K, Tantin D. The Oct1 transcription factor and epithelial malignancies: old protein learns new tricks. Biochim Biophys Acta. 2016;1859:792–804.
35. Hwang-Verslues WW, Chang PH, Jeng YM, et al. Loss of corepressor PER2 under hypoxia up-regulates OCT1-mediated EMT gene expression and enhances tumor malignancy. Proc Natl Acad Sci USA. 2013;110:12331–12336.
36. Qian J, Kong X, Deng N, et al. OCT1 is a determinant of synbindin-related ERK signalling with independent prognostic significance in gastric cancer. Gut. 2014;64:37–48.
37. Almeida R, Almeida J, Shoshkes M, et al. OCT-1 is overexpressed in intestinal metaplasia and intestinal gastric carcinomas and binds to, but does not transactivate, CDX2 in gastric cells. J Pathol. 2005;207:396–401.
38. Reymann S, Borlak J. Transcription profiling of lung adenocarcinomas of c-myc-transgenic mice: identification of the c-myc regulatory gene network. BMC Syst Biol. 2008;2:46.
39. Wang Z, Zhu S, Shen M, et al. STAT3 is involved in esophageal carcinogenesis through regulation of Oct-1. Carcinogenesis. 2013;34:678–688.
40. Xiao S, Liao S, Zhou Y, Jiang B, Li Y, Xue M. High expression of octamer transcription factor 1 in cervical cancer. Oncol Lett. 2014;7:1889–1894.
41. Xu SH, Huang JZ, Xu ML, et al. ACK1 promotes gastric cancer epithelial–mesenchymal transition and metastasis through AKT-POU2F1-ECD signalling. J Pathol. 2015;236:175–185.
42. Obinata D, Takayama K, Urano T, et al. Oct1 regulates cell growth of LNCaP cells and is a prognostic factor for prostate cancer. Int J Cancer. 2012;130:1021–1028.
43. Li Y, Dong M, Kong F, Zhou J. Octamer transcription factor 1 mediates epithelial–mesenchymal transition in colorectal cancer. Tumour Biol. 2015;36:9941–9946.
44. Sharpe DJ, Orr KS, Moran M, et al. POU2F1 activity regulates HOXD10 and HOXD11 promoting a proliferative and invasive phenotype in head and neck cancer. Oncotarget. 2014;5:8803–8815.
45. Maddox J, Shakya A, South S, et al. Transcription factor Oct1 is a somatic and cancer stem cell determinant. PLoS Genet. 2012; 8:e1003048.
46. Shakya A, Goren A, Shalek A, et al. Oct1 and OCA-B are selectively required for CD4 memory T cell function. J Exp Med. 2015;212:2115–2131.
47. Singh H, Sen R, Baltimore D, Sharp PA. A nuclear factor that binds to a conserved sequence motif in transcriptional control elements of immunoglobulin genes. Nature. 1986;319:154–158.
48. Sive HL, Roeder RG. Interaction of a common factor with conserved promoter and enhancer sequences in histone H2B, immunoglobulin, and U2 small nuclear RNA (snRNA) genes. Proc Natl Acad Sci USA. 1986;83:6382–6386.
49. Verrijzer CP, Alkema MJ, van Weperen WW, Van Leeuwen HC, Strating MJ, van der Vliet PC. The DNA binding specificity of the bipartite POU domain and its subdomains. EMBO J. 1992; 11:4993–5003.
50. Kang J, Gemberling M, Nakamura M, et al. A general mechanism for transcription regulation by Oct1 and Oct4 in response to genotoxic and oxidative stress. Genes Dev. 2009;23:208–222.
51. Zhang Y, Ng HH, Erdjument-Bromage H, Tempst P, Bird A, Reinberg D. Analysis of the NuRD subunits reveals a histone deacetylase core complex and a connection with DNA methylation. Genes Development. 1999;13:1924–1935.
52. Xue Y, Wong J, Moreno GT, Young MK, Cote J, Wang W. NURD, a novel complex with both ATP-dependent chromatin-remodeling and histone deacetylase activities. Mol Cell. 1998;2: 851–861.
53. Tong JK, Hassig CA, Schnitzler GR, Kingston RE, Schreiber SL. Chromatin deacetylation by an ATP-dependent nucleosome remodelling complex. Nature. 1998;395:917–921.
54. Yamane K, Toumazou C, Tsukada Y, et al. JHDM2A, a JmjC-containing H3K9 demethylase, facilitates transcription activation by androgen receptor. Cell. 2006;125:483–495.
55. Tantin D, Voth WP, Shakya A. Efficient chromatin immunoprecipitation using limiting amounts of biomass. J Vis Exp. 2013;75:e50064.
56. Kuhn R, Schwenk F, Aguet M, Rajewsky K. Inducible gene targeting in mice. Science. 1995;269:1427–1429.
57. Stubbs MC, Kim YM, Krivtsov AV, et al. MLL-AF9 and FLT3 cooperation in acute myelogenous leukemia: development of a model for rapid therapeutic assessment. Leukemia. 2008;22:66–77.
58. Schoch C, Schnittger S, Klaus M, Kern W, Hiddemann W, Haferlach T. AML with 11q23/MLL abnormalities as defined by the WHO classification: incidence, partner chromosomes, FAB subtype, age distribution, and prognostic impact in an unselected series of 1897 cytogenetically analyzed AML cases. Blood. 2003;102:2395–2402.
59. Krivtsov AV, Twomey D, Feng Z, et al. Transformation from committed progenitor to leukaemia stem cell initiated by MLL-AF9. Nature. 2006;442:818–822.
60. Wang VE, Schmidt T, Chen J, Sharp PA, Tantin D. Embryonic lethality, decreased erythropoiesis, and defective octamer-dependent promoter activation in Oct-1-deficient mice. Mol Cell Biol. 2004;24:1022–1032.
61. Velasco-Hernandez T, Sawen P, Bryder D, Cammenga J. Potential pitfalls of the Mx1-Cre system: implications for experimental modeling of normal and malignant hematopoiesis. Stem Cell Rep. 2016;7:11–18.

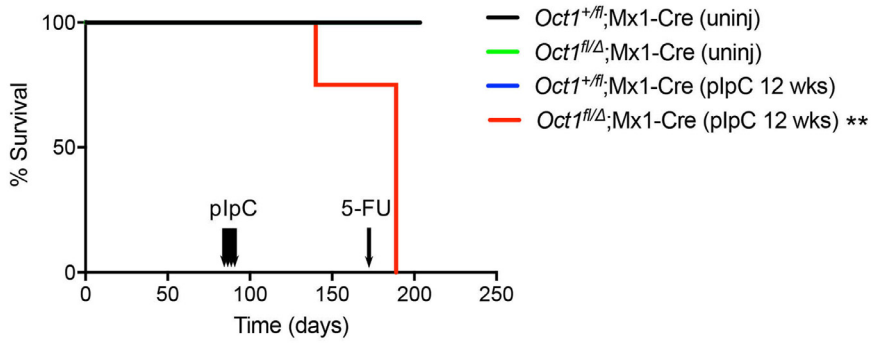
62. Lerner C, Harrison DE. 5-Fluorouracil spares hemopoietic stem cells responsible for long-term repopulation. Exp Hematol. 1990;18:114–118.
63. Luo J, Solimini NL, Elledge SJ. Principles of cancer therapy: oncogene and non-oncogene addiction. Cell. 2009;136:823–837.
64. Lessard J, Sauvageau G. Bmi-1 determines the proliferative capacity of normal and leukaemic stem cells. Nature. 2003;423:255–260.
65. Komorowska K, Doyle A, Wahlestedt M, et al. Hepatic leukemia factor maintains quiescence of hematopoietic stem cells and protects the stem cell pool during regeneration. Cell Rep. 2017;21:3514–3523.
66. Wahlestedt M, Ladopoulos V, Hidalgo I, et al. Critical modulation of hematopoietic lineage fate by hepatic leukemia factor. Cell Rep. 2017;21:2251–2263.
67. Strumpf D, Mao CA, Yamanaka Y, et al. Cdx2 is required for correct cell fate specification and differentiation of trophectoderm in the mouse blastocyst. Development. 2005;132:2093–2102.
68. Ricciardi MR, McQueen T, Chism D, et al. Quantitative single cell determination of ERK phosphorylation and regulation in relapsed and refractory primary acute myeloid leukemia. Leukemia. 2005;19:1543–1549.
69. Zebisch A, Staber PB, Delavar A, et al. Two transforming C-RAF germ-line mutations identified in patients with therapy-related acute myeloid leukemia. Cancer Res. 2006;66:3401–3408.
70. Gregorj C, Ricciardi MR, Petrucci MT, et al. ERK1/2 phosphorylation is an independent predictor of complete remission in newly diagnosed adult acute lymphoblastic leukemia. Blood. 2007;109:5473–5476.
71. Kang HB, Fan J, Lin R, et al. Metabolic rewiring by oncogenic BRAF V600E links ketogenesis pathway to BRAF-MEK1 signaling. Mol Cell. 2015;59:345–358.
72. Staudt LM, Clerc RG, Singh H, LeBowitz JH, Sharp PA, Baltimore D. Cloning of a lymphoid-specific cDNA encoding a protein binding the regulatory octamer DNA motif. Science. 1988;241:577–580.
73. Luo Y, Fujii H, Gerster T, Roeder RG. A novel B cell-derived coactivator potentiates the activation of immunoglobulin promoters by octamer-binding transcription factors. Cell. 1992;71:231–241.
74. Seguin-Estevez Q, De Palma R, Krawczyk M, et al. The transcription factor RFX protects MHC class II genes against epigenetic silencing by DNA methylation. J Immunol. 2009;183:2545–2553.
75. Mancini DN, Rodenhiser DI, Ainsworth PJ, et al. CpG methylation within the 5′ regulatory region of the BRCA1 gene is tumor specific and includes a putative CREB binding site. Oncogene. 1998;16:1161–1169.
76. Bruniquel D, Schwartz RH. Selective, stable demethylation of the interleukin-2 gene enhances transcription by an active process. Nat Immunol. 2003;4:235–240.
77. El Gazzar M, Yoza BK, Chen X, Hu J, Hawkins GA, McCall CE. G9a and HP1 couple histone and DNA methylation to TNFalpha transcription silencing during endotoxin tolerance. J Biol Chem. 2008;283:32198–32208.
78. Kim ST, Fields PE, Flavell RA. Demethylation of a specific hypersensitive site in the Th2 locus control region. Proc Natl Acad Sci USA. 2007;104:17052–17057.
79. Hori N, Nakano H, Takeuchi T, et al. A dyad oct-binding sequence functions as a maintenance sequence for the unmethylated state within the H19/Igf2-imprinted control region. J Biol Chem. 2002;277:27960–27967.
80. Lehnertz B, Ueda Y, Derijck AA, et al. Suv39h-mediated histone H3 lysine 9 methylation directs DNA methylation to major satellite repeats at pericentric heterochromatin. Curr Biol. 2003;13:1192–1200.
81. Feldman N, Gerson A, Fang J, et al. G9a-mediated irreversible epigenetic inactivation of Oct-3/4 during early embryogenesis. Nat Cell Biol. 2006;8:188–194.
82. Rose NR, Klose RJ. Understanding the relationship between DNA methylation and histone lysine methylation. Biochim Biophys acta. 2014;1839:1362–1372.
83. Hodges E, Molaro A, Dos Santos CO, et al. Directional DNA methylation changes and complex intermediate states accompany lineage specificity in the adult hematopoietic compartment. Mol Cell. 2011;44:17–28.
84. Johansson B, Brondum-Nielsen K, Billstrom R, Schiodt I, Mitelman F. Translocations between the long arms of chromosomes 1 and 5 in hematologic malignancies are strongly associated with neoplasms of the myeloid lineages. Cancer Genet Cytogenet. 1997;99:97–101.
85. de Souza Fernandez T, Ornellas MH, Otero de Carvalho L, Tabak D, Abdelhay E. Chromosomal alterations associated with evolution from myelodysplastic syndrome to acute myeloid leukemia. Leuk Res. 2000;24:839–848.
86. Johansson B, Moorman AV, Haas OA, et al. Hematologic malignancies with t(4;11)(q21;q23)—A cytogenetic, morphologic, immunophenotypic and clinical study of 183 cases. European 11q23 Workshop participants. Leukemia. 1998;12:779–787.
87. Panagopoulos I, Teixeira MR, Micci F, et al. Acute myeloid leukemia with inv(8)(p11q13). Leuk Lymphoma. 2000;39:651–656.
88. Beach DF, Barnoski BL, Aviv H, et al. Duplication of chromosome 1 dup(1)(q21q32) as the sole cytogenetic abnormality in a patient previously treated for AML. Cancer Genet. 2012;205:665–668.
89. Rochowski A, Olson SB, Alonzo TA, Gerbing RB, Lange BJ, Alter BP. Patients with Fanconi anemia and AML have different cytogenetic clones than de novo cases of AML. Pediatr Blood Cancer. 2012;59:922–924.
90. Mehta PA, Harris RE, Davies SM, et al. Numerical chromosomal changes and risk of development of myelodysplastic syndrome—Acute myeloid leukemia in patients with Fanconi anemia. Cancer Genet Cytogenet. 2010;203:180–186.
91. Somervaille TC, Matheny CJ, Spencer GJ, et al. Hierarchical maintenance of MLL myeloid leukemia stem cells employs a transcriptional program shared with embryonic rather than adult stem cells. Cell Stem Cell. 2009;4:129–140.
92. Mattison J, Kool J, Uren AG, et al. Novel candidate cancer genes identified by a large-scale cross-species comparative oncogenomics approach. Cancer Res. 2010;70:883–895.
93. Li L, Li M, Sun C, et al. Altered hematopoietic cell gene expression precedes development of therapy-related myelodysplasia/acute myeloid leukemia and identifies patients at risk. Cancer Cell. 2011;20:591–605.
94. Ben-Porath I, Thomson MW, Carey VJ, et al. An embryonic stem cell-like gene expression signature in poorly differentiated aggressive human tumors. Nat Genet. 2008;40:499–507.



Supplemental Figure E1. Kaplan-Meier survival curves were downloaded and modified using OncoLnc (Anaya et al., OncoLnc: linking TCGA survival data to mRNAs, miRNAs, and IncRNAs. PeerJ Computer Science 2:e67) and gene symbol *POU2F1*. Survival in days of patients in which their AML cell's *OCT1* (*POU2F1*) mRNA expression fell in the bottom (''Low'') or top (''High'') quartiles of the group as a whole is shown.



Supplemental Figure E2. *Cdx2* proximal promoter and 5′ region DNA methylation status in human leukemia and normal control samples. Position is shown relative to the transcription initiation site.



Supplemental Figure E3. Oct1 is dispensable for homeostatic hematopoiesis but protects mice from hematopoietic stress. Similar to Figure 3A except that plpC was administered at 12 weeks instead of at week 5.