TET1 plays an essential oncogenic role in MLL-rearranged leukemia
**TET1 plays an essential oncogenic role in MLL-rearranged leukemia**

Hao Huang,†, Xi Jiang,*†, Zejuan Li,*†, Yuanyuan Li,*†, Chun-Xiao Song,† Chunjiang He,§, Miaosun Sun,§, Ping Chen,¶, Sandeep Gurbuxani,**, Jiapeng Wang,§, Gia-Ming Hong,§, Abdel G. Elkahlonu,§, Stephen Arnovitz,§, Jinhua Wang,§, Keith Szulwach,§, Li Lin*, Craig Street*, Mark Wunderlich*, Meelad Dawlaty†, Mary Beth Neilly†, Rudolf Jaenisch†, Feng-Chun Yang,§, James C. Mullu§,º, Peng Jin†, Paul P. Liu†, Janet D. Rowley,a,1 Mingjiang Xu,§, Chuan He§, and Jianjun Chen†,2

†Department of Medicine, ‡Department of Chemistry and Institute for Biophysical Dynamics, ¶Committee on Genetics, Genomics and Systems Biology, and †Department of Pathology, University of Chicago, Chicago, IL 60637; **Department of Pediatrics, Indiana University School of Medicine, Indianapolis, IN 46202; ¶Genetics and Molecular Biology Branch, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD 20892; ‡Department of Human Genetics, Emory University School of Medicine, Atlanta, GA 30322; †Division of Experimental Hematology and Cancer Biology, Cincinnati Children’s Hospital Medical Center, University of Cincinnati College of Medicine, Cincinnati, OH 45229; and AWhitehead Institute and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02142

Contributed by Janet D. Rowley, June 5, 2013 (sent for review May 17, 2013)

The ten-eleven translocation 1 (TET1) gene is the founding member of the TET family of enzymes (TET1/2/3) that convert 5-methylcytosine to 5-hydroxymethylcytosine. Although TET1 was first identified as a fusion partner of the mixed lineage leukemia (MLL) gene in acute myeloid leukemia carrying t(10;11), its definitive role in leukemia is unclear. In contrast to the frequent down-regulation (or loss-of-function mutations) and critical tumor-suppressor roles of the three TET genes observed in various types of cancers, here we show that TET1 is a direct target of MLL-fusion proteins and is significantly up-regulated in MLL-rearranged leukemia, leading to a global increase of 5-hydroxymethylcytosine level. Furthermore, our both in vitro and in vivo functional studies demonstrate that TET1 plays an indispensable oncogenic role in the development of MLL-rearranged leukemia, through coordination with MLL-fusion proteins in regulating their critical cotargets, including homeobox A9 (Hoxa9)/myeloid ecotropic viral integration 1 (Meis1)/pre-B-cell leukemia homeobox 3 (Pbx3) genes. Collectively, our data delineate an MLL-fusion/Tet1/Hoxa9/Meis1/Pbx3 signaling axis in MLL-rearranged leukemia and highlight TET1 as a potential therapeutic target in treating this presently therapy-resistant disease.

R

ecently, the ten-eleven translocation (Tet) proteins (including Tet1/2/3) have been shown to be able to convert 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC), resulting in active or passive DNA demethylation (1–4). In contrast to homologous mutation of Tet3 that results in neontal lethality (5), mice carrying either Tet1+/- or Tet2+/- largely appear to develop normally (6–10). Although high abundance of Tet1 and Tet2 has been observed in mouse embryonic stem cells (mESCs) and Tet proteins have been implicated in hydroxymethylation and epigenetic regulation of stem cells (11–16), evidence is emerging that Tet1 and Tet2 are likely not required for the pluripotency and maintenance of mESCs (6, 8–11, 15). **TET1** is the founding member of the family, first identified as a fusion partner of the mixed lineage leukemia (MLL) gene in patients with acute myeloid leukemia (AML) carrying t(10;11) (q22;q23) (17, 18), but its definitive function in leukemia is unknown. Loss-of-function mutations of Tet2 (but not TET1 or TET3) have been frequently observed in myeloid cancers, in which TET2 functions as a critical tumor suppressor (7–9, 19–21). Notably, substantial down-regulation of all three TET genes has been reported in various types of solid tumors (3, 22–25), and **TET1** has been shown to be an essential tumor suppressor in prostate and breast cancers (26, 27). Thus, one may expect that all three TET genes are tumor suppressors in various cancers.

The **MLL** gene, located at human chromosome 11 band q23 (11q23), is frequently involved in chromosome translocations occurring in ~10% of total leukemia, including ~80% of infant acute leukemia, usually associated with poor prognosis (28–30). The critical feature of MLL rearrangements is the generation of a chimeric transcript consisting of 5’ MLL and 3’ sequences of a partner gene [80% involving ALL 1-fused gene from chromosome 9 (AF9), AF6, AF10, elongation factor RNA polymerase II (ELL), or eleven-nineteen leukemia (ENL) in AML] (29, 30). Although TET1 was first identified as a fusion partner of the MLL gene in AML carrying t(10;11)(q22;q23), this fact does not give any clear clue about its pathological role in leukemia because fusion of MLL to even an artificial inducible dimerization domain caused activation of its transforming potential (31–33).

In the present study, through a large-scale, genome-wide gene expression profiling of 100 AML patient samples and nine normal bone marrow (BM) control samples, we show that **TET1** is aberrantly overexpressed in MLL-rearranged AML. We then performed a series of in vitro and in vivo functional and mechanistic studies. Our studies indicate that **TET1** is a direct target gene of MLL-fusion proteins and thus is aberrantly overexpressed in MLL-rearranged leukemia, in which it plays a critical oncogenic role through cooperating MLL-fusion proteins in regulating a set of important oncogenic cotargets including homeobox A9 (Hoxa9), myeloid ecotropic viral integration 1 (Meis1), and pre-B-cell leukemia homeobox 3 (Pbx3).

**Results**

**TET1** is aberrantly overexpressed in **MLL**-rearranged AML. We performed a large-scale, global gene expression profiling assay of 100 human AMLs with common chromosomal translocations (12 with MLL rearrangements and 88 without; see Table S1) and nine normal BM control [three each for CD34+ hematopoietic stem/progenitor cell, CD33+ myeloid progenitor cell, and mononuclear cell (MNC)] samples by use of microarrays. We found that **TET1** was expressed at a significantly higher level.


Data deposition: The microarray data reported in this paper have been deposited in the Gene Expression Omnibus (GEO) database, www.ncbi.nlm.nih.gov/geo (accession nos. GSE34184 and GSE30285).

1H.H., X.J., Z.L., and Y.L. contributed equally to this work.

2To whom correspondence may be addressed. E-mail: rowley@bsd.uchicago.edu or jchen@medicine.bsd.uchicago.edu or jchen@bsd.uchicago.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1310656110/-/DCSupplemental.
in MLL-rearranged AML than in normal controls \((P = 0.01)\) (Fig. 1D); in contrast, neither TET2 (Fig. 1B) nor TET3 (Fig. 1C) is significantly dysregulated in MLL-rearranged AML relative to normal controls. Our cytometry analysis (Fig. S1) and previous studies \((34, 35)\) showed that the MLL-rearranged leukemic cells usually are CD33+/CD34−. Thus, normal CD33+ cells would be better controls than normal CD34+ cells for MLL-rearranged leukemic cells. If compared with normal CD33+ cells, TET1 was significantly up-regulated \((P = 0.01)\) whereas both TET2 \((P = 0.01)\) and TET3 \((P = 0.05)\) were significantly down-regulated in MLL-rearranged AML samples (Figs. 1A–C). As leukemic MNC cells were used for all human patient samples in the microarray assay, we also compared the expression levels of TET genes between MLL-rearranged patient samples (i.e., leukemic MNC cells) and normal MNC cell samples; we found that only TET1 is significantly up-regulated \((P = 0.04)\) in MLL-rearranged AML whereas TET2 and TET3 did not show a significant change. The overexpression of TET1 in human MLL-rearranged AML cell samples compared with normal CD33+ or MNC cells was confirmed by quantitative PCR (qPCR) assay of additional samples (Fig. 1D).

Among normal hematopoietic cells, compared with CD34+ hematopoietic stem/progenitor cells, committed CD33+ myeloid progenitor cells (i.e., relatively more mature cells) exhibit significantly lower TET1 expression but a higher level of TET2 or TET3 expression (Figs. 1A–C). MNC cells, on the other hand, are a mixed population containing both primitive progenitors and committed cells, in which the TET genes are expressed at a level relatively similar to that seen in CD34+ cells, but significantly higher (TET1) or lower (TET2/3) than that seen in CD33+ cells (Figs. 1A–C). Thus, our data suggest that TET1 is likely down-regulated whereas TET2 and TET3 are up-regulated during normal hematopoiesis.

**TET1 Is a Direct Target Gene of MLL and Particularly MLL-Fusion Proteins.** It is well known that MLL-fusion proteins bind to the promoters of a group of critical target genes, such as HOX9 and MEIS1, and promote their expression through recruiting DOT1 like (DOT1L)-mediated methylation of histone H3 lysine 79 \((36-38)\). To elucidate the mechanism underlying the up-regulation of TET1 in MLL-rearranged leukemia, we performed chromatin immunoprecipitation (ChIP) assays. As shown in Fig. 2A, MLL (see MLL-C binding) and particularly MLL-fusion proteins (see the portion of MLL-N binding exceeding that of MLL-C) are significantly enriched at the CpG area (sites 2 and 3), but not the distal upstream site (site 1), of TET1 in human MONOMAC-6 cells (an MLL-AF9 leukemia line), associated with a significant enrichment of histone H3 lysine \((K)\) 79 \((H3K79)\) dimethylation \((H3K79me2)\) to the sites. A similar pattern was observed in twofold MLL-rearranged AML cell lines including THP-1 \((9,11)\) and K562 \((4,11)\) cells. In contrast, no such enrichment was observed in K562, a negative control cell line with no MLL rearrangements (Fig. 2A). Notably, there is no significant difference between MLL-N binding and MLL-C binding in K562, indicating that the affinity of the antibody against MLL N-terminal is similar to that of the antibody against MLL C-terminal. Thus, the substantially enhanced enrichment of MLL-N binding compared with MLL-C binding in MONOMAC-6 cells is not due to the affinity difference of the antibodies; instead, the enhancement is due to the fact that the antibody against MLL N-terminal can bind to both the wild-type and MLL-rearranged alleles whereas the antibody against MLL C-terminal can only bind to the single wild-type allele in MONOMAC-6 cells. Consistent with the direct binding of MLL-fusion proteins to the promoter region of TET1 observed in MLL-rearranged leukemic cells (Fig. 2A), forced expression of MLL fusion proteins could significantly up-regulate Tet1 endogenous expression in both mouse (Fig. 2B) and human (Fig. 2C) hematopoietic progenitor cells. Conversely, Tet1 expression was significantly \((P < 0.01)\) down-regulated in MLL-ENL-estrogen receptor inducible (ERtm) mouse myeloid cells carrying tamoxifen-inducible MLL-ENL \((39, 40)\) when expression of MLL-ENL was depleted after withdrawal of 4-Hydroxy-tamoxifen (4-OHT). The opposite was true for Fas, a control gene that is
down-regulated in MLL-rearranged leukemia (41) (Fig. 2D). Thus, our data suggest that TET1 is a direct target gene of MLL and, particularly, MLL-fusion proteins.

**Tet1 Plays an Essential Oncogenic Role in MLL-Rearranged Leukemia.** To assess the functional importance of Tet1 expression in MLL-rearranged leukemias, we conducted both loss- and gain-of-function studies. First, we synthesized three Tet1 small hairpin RNAs (shRNAs), including shTet1-a (i.e., the mTet1-shRNA-A used in ref. 14), shTet1-b (i.e., the mTet1-shRNA-5 used in ref. 15), and shTet1-a+b (i.e., a combination to achieve a stronger knock-down effect), along with a scrambled shRNA (as a negative control for Tet1 shRNAs). We then cloned each shRNA into a retroviral vector, namely pGFP-V-RS (Origene). Meanwhile, we synthesized a Flag-tagged mouse Tet1 (amino acids 1367–2039) (12) that has been shown to be able to exhibit a comparable regulatory function as full-length Tet1 (4, 12) and cloned it into an murine stem cell virus puromycin (MSCVpuro) vector. In colony-forming/replating assays, we showed that depletion of endogenous Tet1 expression by shTet1-a, shTet1-b, and particularly shTet1-a+b, significantly (P < 0.05, t test) inhibited MLL-AF9-mediated immortalization of mouse BM progenitor cells (Fig. 3A) whereas forced expression of Tet1 led to the opposite effect (Fig. 3B). The degree of the above inhibition or enhancement effect on cell immortalization was parallel with the magnitude of the decrease or increase of Tet1 expression (Fig. 3 C and D) and that of 5mC level (Fig. 3E), indicating that Tet1 functions in a dosage-dependent manner.

In addition, we transfected TET1 small interfering RNA (siRNA) oligos into human MLL-rearranged leukemic cells. Knockdown of TET1 by siRNAs resulted in a significant (P < 0.05; log-rank test) increase in apoptosis (Fig. S2A) and decrease in cell viability (Fig. S2B) of human MONOMAC-6 and THP-1 cells, both carrying the t(9,11) aberration. The effects of siTET1 could be reversed by cotransfected mouse Tet1, suggesting that the observed effects of siTET1 are not due to off-targeting. A similar pattern was observed in analysis of the effects on cell growth/proliferation (Fig. S2C). The changes in TET1 expression were associated with 5mC level changes (Fig. S2D).

Furthermore, we also conducted in vivo mouse BM transplantation (BMT) assays. Knocking down expression of Tet1 by shTet1-a, shTet1-b, and particularly shTet1-a+b significantly (P < 0.005; log-rank test) delayed MLL-AF9-mediated leukemogenesis in recipient mice (Fig. 3F). All of the leukemic mice died from AML (Fig. S3), and, notably, depletion of Tet1 expression significantly reduced spleen size and decreased white blood cell counts (Table S2). Depletion of Tet1 expression by shTet1-a, shTet1-b, and particularly shTet1-a+b substantially reduced the proportion of immature blast cells in both BM and peripheral blood (PB), associated with a reduction of leukemia cell infiltration and disruption of organ architecture in spleen and liver (Fig. 3G), as well as a decrease of 5mC in spleen (Fig. 3H).

**Critical Cotargets of TET1 and MLL Fusions.** HOXA9, MEIS1, and PBX3 are the three most well-studied critical oncogenic targets of MLL fusions (36, 39, 42–48). Interestingly, previous genome-wide ChIP-seq or ChiP-chip assays of Tet1 in mESCs suggest that Hoxa9, Meis1, or Pbx3 are potential direct target genes of Tet1 in mESCs (14–16) (Fig. S4). To determine whether these three MLL-fusion targets are also direct targets of TET1 in MLL-rearranged leukemic cells, we conducted conventional ChiP assays using MONOMAC-6 cells as a model. We found that TET1, similar to MLL fusion proteins, could bind to promoter regions of the three genes, associated with significantly increased levels of H3K79me2 (Figs. 4 A–C). We next assessed the effect of TET1 on their expression. We found that knockdown of TET1 expression by siRNA oligos and ectopic expression of mouse Tet1 in MONOMAC-6 cells resulted in a significant down-regulation (Fig. 4D) and up-regulation (Fig. 4E) of the three genes, respectively. Similar effects of knockdown of Tet1 expression by shRNA constructs on expression of the three targets in mouse BM progenitor cells transduced with MLL-AF9 were also observed in vitro (Fig. 4F) and in vivo (Fig. 4G). We next showed that forced expression of HOXA9, MEIS1, or PBX3 can largely reverse the effects of TET1 depletion by siTET1 on cell apoptosis, viability, and growth/proliferation of both MONOMAC-6 and KOCL-48 (the latter carrying MLL-AF4) cells (Fig. 4 H and I).
Validation in a Tet1 Knockout Model. After completion of the above studies, we have very recently obtained a Tet1−/− mouse strain (6). As shown in Fig. 5A, the expression levels of all three validated target genes including Hoxa9, Meis1, and Pbx3 are significantly down-regulated in Tet1−/− BM progenitor (lineage-negative (Lin−)) cells compared with their wild-type counterpart.

The Tet1 knockout results in a significant inhibition on cell transduction mediated by MLL-AF9 (Fig. 5B), similar to that caused by shRNA-Tet1-a+b (Fig. 3A). Such inhibition can be largely reversed by forced expression of Hoxa9 (Fig. 5B). As expected, the expression levels of all three target genes are significantly down-regulated in MLL-AF9-transduced Tet1−/− colony cells compared with MLL-AF9-transduced wild-type colony cells (Fig. 5C). As Hoxa9 has been shown to be able to regulate expression of Meis1 and Pbx3 (49–51), it is not surprising that forced expression of Hoxa9 can largely restore the overlapexpression of those genes in MLL-AF9–transduced Tet1−/− cells (Fig. 5C). More importantly, knockout of Tet1 significantly inhibited MLL-AF9–mediated leukemogenesis (Fig. 5D and E), in a manner similar to that caused by shRNA-Tet1-a+b (Fig. 3F and G). As expected, leukemia BM cells from MLL-AF9/Tet1−/− (i.e., Tet1-KO MA9) mice exhibited a significant decrease of 5mC than those from MLL-AF9/Tet1-wild-type (i.e., Tet1-WT MA9) mice (Fig. S5).

Again, forced expression of Hoxa9 could partially reverse the delay of leukemogenesis caused by Tet1 knockout (Fig. 5D and E), further indicating that Hoxa9 is an important target of Tet1.

Taken together, the results from the Tet1−/− model are consistent with those that we observed from the Tet1-shRNA models, highlighting the important oncogenic role of Tet1 in the pathogenesis of MLL-rearranged leukemia.

Discussion

In contrast to the frequent down-regulation of the $TET$ genes in various types of solid tumors (3, 22–27), we found that Tet1 is significantly up-regulated in MLL-rearranged leukemia, in which this gene was first identified. Our data indicate that Tet1 is a direct target gene of MLL-fusion proteins, and MLL fusions bind to the promoter region of Tet1 and promote its expression directly in both human and mouse hematopoietic stem/progenitor cells, cumulating in a global increase of 5mC. More importantly, distinct from the tumour-suppressor roles of both Tet1 and Tet2 reported in various types of cancers (7–9, 24–27), here we provide compelling evidence to show that Tet1 plays an essential oncogenic role in MLL-rearranged leukemia. Such a finding has two layers of significance: (i) it highlights the critical influence of cell/tissue context on the function of a given gene in tumorigenesis, such as Tet1, which functions as a tumor-suppressor gene in solid tumors, but as an oncogene in leukemogenesis mediated by MLL fusions; and (ii) despite their similar catalytic activities in oxidation of 5mC, Tet1 likely plays a distinct pathological role in leukemogenesis compared with Tet2.
Remarkably, through analysis of potential direct targets of Tet1 detected from mESCs (14–16), we found that a set of critical target genes of MLL-fusion proteins such as HOXA9, Meis1, and Pbx3, are also likely targeted directly by Tet1 in mESCs. Through conventional ChIP-qPCR assays, we confirmed that these three genes are genuine cotarget genes of MLL-fusion proteins and TET1 in MLL-rearranged leukemic cells. As expected, their expression levels are significantly down-regulated when Tet1 expression is depleted in hematopoietic cells, particularly in those transduced with MLL fusions.

It is probably not a coincidence that TET1 was discovered as a partner gene of MLL in a translocation in AML (17, 18). MLL has around 100 different partners, and the functions of some of them have been identified (30, 52). MLL itself is a large multifunctional protein (homolog of Drosophila trithorax) with many functions related to chromatin structure; many of MLL’s partners are members of chromatin-modifying complexes including ENL, AF9, AF4, and AF10, which interact with DOT1L, and ELL, AF4/FMR2 family member 1 (AFF1), and AFF4, which interact with positive transcription elongation factor b (P-TEFB) (37, 53–56). Thus, a fusion protein consisting of MLL and one of the members of the complexes would be much more effective in promoting regulation of critical targets and enhancing cell proliferation than the two proteins produced independently in the cell and meeting at a critical location by chance. It is important, in the future, to systematically investigate how TET1 cooperates with MLL fusions in regulating their cotargets. It is possible that they have critical but transient interactions, or they belong to two distinct functional complexes that exert synergistic functions in regulating transcription of cotargets.

The effects of the aforementioned cotargets of TET1 and MLL fusions on cell viability/growth and apoptosis of MLL-rearranged leukemia cells have been well demonstrated (42–44, 48, 50). Thus, it is not a surprise that knockdown of TET1 induced apoptosis and inhibited cell viability/growth of MLL-rearranged leukemic cells. We showed that forced expression of HOXA9 could only partially rescue the inhibitory effects of Tet1 knockout on MLL-AF9-induced leukemogenesis in vivo (Fig. 5 D and E), which might be owing to the possibility that Tet1 also regulates some other important target genes that are not in the HOXA9/Meis1/Pbx3 signaling axis in MLL-rearranged leukemia. *P < 0.05; **P < 0.01, t test.

Fig. 5. Effects of Tet1-knockout and the signaling-pathway model. (A) Expression changes of three target genes in Tet1 knockout BM progenitor (i.e., Lin−) cells relative to wild-type controls. (B and C) Effect of Tet1 knockout on MA9-mediated cell transformation and the corresponding expression changes of the targets. (D) Kaplan-Meier survival analysis of the BM transplantation recipient mice. The median survival of Tet1-WT_MA9 (MSCVneo-MA9+MSCVpuro cotransduced into wild-type mouse BM progenitor cells; n = 8), Tet1-KO_MA9 (MSCVneo-MA9+MSCVpuro cotransduced into Tet1−/− mouse BM progenitor cells; n = 9), and Tet1-KO_MA9+HOXA9 (MSCVneo-MA9+MSCVpuro-HOXA9 cotransduced into Tet1−/− mouse BM progenitor cells; n = 5) is 66, >150, and 85 d, respectively, Tet1-WT_MA9 vs. Tet1-KO_MA9, P < 0.00001; Tet1-WT_MA9 vs. Tet1-KO_MA9+HOXA9, P = 0.018; Tet1-KO_MA9 vs. Tet1-KO_MA9+HOXA9, P = 0.019; log-rank test. (E) Wright-Giemsa-stained BM cell cytosin and PB smear, and hematoxylin/eosin (H&E)-stained spleen and liver paraffin sections of transplantation recipient mice are shown. (Scales bars: 10 μm in BM/PB and 100 μm in spleen/liver.) (F) The model of the MLL-fusion/Tet1/Hoxa9/Meis1/Pbx3 signaling axis in MLL-rearranged leukemia. *P < 0.05; **P < 0.01, t test.

Materials and Methods
Microarray Profiling of 109 Human Samples. The samples were analyzed by use of Affymetrix GeneChip Human Exon 1.0 ST arrays (Affymetrix). The data have been deposited in the Gene expression Omnibus (GEO) repository with the accession numbers GSE34184 and GSE30285.
Chromatin Immunoprecipitation and In Vivo or In Vivo Functional Studies. Those were performed as described previously (41, 47, 48, 50) with some modifications. See Table S3 for primer sequences for the chromatin immunoprecipitation (ChIP) assay.

**ShmC Labeling Reaction, Dot-Blot, and LC-MS/MS Assays.** These assays were conducted as described previously (57–59) with some modifications.

**Supplemental Information.** A detailed description of all of the materials and methods used appears in SI Materials and Methods.

**ACKNOWLEDGMENTS.** We thank Drs. Robert K. Slany, Gregory Hannon, Scott Hammond, Lin He, Scott Armstrong, and Michael Thirman for providing cell lines or plasmids. This work was supported in part by National Institute of Health Grants CA127277 (to J.C.), NS079625 and HD673162 (to P.J.), HG006827 (to Chuan He), HL112294 (to M.X.), and CA118319 (to J.C.M.), the Intramural Program of the National Human Genome Research Institute (A.G.E. and P.P.L.), the American Cancer Society Research Scholar grant (J.C.), the Emory Genetics Discovery Fund (P.J.), a Winship Cancer Institute Kennedy Seed Grant and a Winship Multi-Investigator Pilot Grant (to J.C.), the G. Harold and Leila Y. Mathers Charitable Foundation (J.C.), Gabrielle’s Angel Foundation (J.C., Z.L., H.H., X.J., and G.-M.H.), a Leukemia and Lymphoma Society (LLS) Translational Research grant (to J.D.R. and J.C.), an LLS Special Fellowship (to Z.L.), and the University of Chicago Committee on Cancer Biology Fellowship Program (J.X.). M.S. was supported by Department of Defense Predoctoral Traineeship Award W81XWH-10-1-0396. J.C.M. is an LLS Scholar.

31. Martin ME, et al. (2003) Dimerization of MLL fusion proteins immortalizes hematopoietic cell lines or plasmids. This work was supported in part by National Institute of Health Grants CA127277 (to J.C.), NS079625 and HD673162 (to P.J.), HG006827 (to Chuan He), HL112294 (to M.X.), and CA118319 (to J.C.M.), the Intramural Program of the National Human Genome Research Institute (A.G.E. and P.P.L.), the American Cancer Society Research Scholar grant (J.C.), the Emory Genetics Discovery Fund (P.J.), a Winship Cancer Institute Kennedy Seed Grant and a Winship Multi-Investigator Pilot Grant (to J.C.), the G. Harold and Leila Y. Mathers Charitable Foundation (J.C.), Gabrielle’s Angel Foundation (J.C., Z.L., H.H., X.J., and G.-M.H.), a Leukemia and Lymphoma Society (LLS) Translational Research grant (to J.D.R. and J.C.), an LLS Special Fellowship (to Z.L.), and the University of Chicago Committee on Cancer Biology Fellowship Program (J.X.). M.S. was supported by Department of Defense Predoctoral Traineeship Award W81XWH-10-1-0396. J.C.M. is an LLS Scholar.