DOT1L complex regulates transcriptional initiation in human erythroleukemic cells

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DOT1L, the only H3K79 methyltransferase in human cells and a homolog of the yeast Dot1, normally forms a complex with AF10, AF17, and ENL or AF9, is dysregulated in most cases of mixed-lineage leukemia (MLLr), and has been believed to regulate transcriptional elongation on the basis of its colocalization with RNA polymerase II (Pol II), the sharing of subunits (AF9 and ENL) between the DOT1L and super elongation complexes, and the distribution of H3K79 methylation on both promoters and transcribed regions of active genes. Here we show that DOT1L depletion in erythroleukemic cells reduces its global occupancy without affecting the traveling ratio or the elongation rate (assessed by 4sUDRB-seq) of Pol II, suggesting that DOT1L does not play a major role in elongation in these cells. In contrast, analyses of transcription initiation factor binding reveal that DOT1L and ENL depletions each result in reduced TATA binding protein (TBP) occupancies on thousands of genes. More importantly, DOT1L and ENL depletions concomitantly reduce TBP and Pol II occupancies on a significant fraction of direct (DOT1L-bound) target genes, indicating a role for the DOT1L complex in transcription initiation. Mechanistically, proteomic and biochemical studies suggest that the DOT1L complex may regulate transcriptional initiation by facilitating the recruitment or stabilization of transcription factor IID, likely in a monoubiquitinated H2B (H2Bub1)-enhanced manner. Additional studies show that DOT1L enhances H2Bub1 levels by limiting recruitment of the Spt-Ada-Gcn5-acetyltransferase (SAGA) complex. These results advance our understanding of roles of the DOT1L complex in transcriptional regulation and have important implications for MLLr leukemias.

DOT1L complex | transcriptional initiation | TFIID | SAGA | H2B monoubiquitination

C u - u u u u u (TF) u 50% (1). A u u u RNA (P I, II, III), u P II u u - u u u u ,u u - u . T u u

u SET1/MLL u u H3K4 u (15, 16). I u , u H3K4 (H3K4 3) u TAF3 u u TFIID (17). MLL u (MLL-FP), u *MLL1* 5′ 1 60 u , u u MLL , u u u DOT1L u u (18).

DOT1L, H3K79 u D 1, AF10, AF17, AF9 ENL, u u SEC (19, 20); u (DOT), (21). A DOT1L , DOT1L SIRT1- (22), AF10 u H3K79 (23), AF9 ENL H3 u DOT1L (24, 25), u AF17 u . M , AF9 ENL, AF10 AF17 u MLL1 MLL (14). T DOT1L u P II, u u SEC, H3K79 - u (20). M , H3K79 D 1/DOT1L u u u H2BK120 (H2B 1) (26). O , D 1 H2B 1 (27), B 1- u u , u DOT-1.1 u H2B 1 *Caenorhabditis elegans* (28). P DOT1L H2B 1 u .

T u DOT1L, u MLL , u u u . W u u DOT1L DOT1L, u TFIID -MLL MLL , DOT1L u H2B u u SAGA .

Results

DOT1L Promotes the Chromatin Association of Pol II in Human Cells.

W u u , HEL K562, DOT1L u -MLL DOT1L u (29) u - u . P - P II - P II 5 (-5) 2 (-2) C- (CTD), CTD u u u . T DOT1L , HEL - RNA u (u) - (u P II (*SI Appendix*, *Supplementary Methods*). DOT1L (KD) u CTD -2 - P II, u (*SI Appendix*, F . S1*A*). C u u -2 P II *c-MYC* *CTNNB1* - DOT1L u DOT1L KD (*SI Appendix*, F . S1 *B* *C*). I , u -5 P II , u DOT1L (*SI Appendix*, F . S1 *D* *E*).

T DOT1L u P II HEL , C IP- u (C IP-) -2 P II (*SI Appendix*, *Supplementary Methods*). N , DOT1L KD u , DOT1L P II u (F . 1 *A*–*C*). T DOT1L KD HEL , RNA- u (RNA-) (*SI Appendix*, *Supplementary Methods*). A u 820 - u 552 u u DOT1L KD (*SI Appendix*, F . S2). T DOT1L P II -MLL u u , P II C IP- DOT1L u (KO) K562 CRISPR/C 9 u (F . 1*D* *SI Appendix*, *Supplementary Methods*). T u P II DOT1L KO u u DOT1L P II u (F . 1 *E* *F*). I DOT1L K562 , RNA- 1,105 - u 1,551 u - u DOT1L KO (*SI Appendix*, F . S3 *A* *B*). T DOT1L, C IP- K562 11,040 8,619 DOT1L- u , - (FDR) < 0.01 (*SI Appendix*, F . S3*C*). I u (30), u DOT1L u K562 (*SI Appendix*, F . S3*C*). S u , DOT1L C IP- RNA- , 894 , 452 , - u 442 , u u (*SI Appendix*, F . S3 *D* *E*).

T P II u DOT1L u K562 u u u u : u I, DOT1L- u RNA u DOT1L (DOT1L); u II, DOT1L- u u RNA u DOT1L ; u III, DOT1L- u u RNA u DOT1L ; u IV, DOT1L- u u RNA u DOT1L (F . 1*G*). W u I DOT1L u II IV DOT1L . A u F .1*G*, u 1) u I u II, - u P II u TSS , u - (5%) P II u TSS ; 2) u III, (15%) u P II u TSS u u I II , u , - u P II u TSS P II u TSS ; 3) u IV, -u P II u , (8%) u P II u TSS u III (F . 1*G*). T , u , DOT1L- u , P II u DOT1L. A , u u , u RNA u I u II DOT1L . P ( ) u : 1) u (., TFIID) u DOT1L, 2) u RNA (u) (31).

T DOT1L u P II - DOT1L- u u - DOT1L P II. T , DOT1L P II C IP- K562 , u (*SI Appendix*, F . S3*F*). T P II - RNA (32), DOT1L P II DOT1L - RNA . I ,u - u DOT1L C IP- RNA- (*SI Appendix*, F . S3*G*).

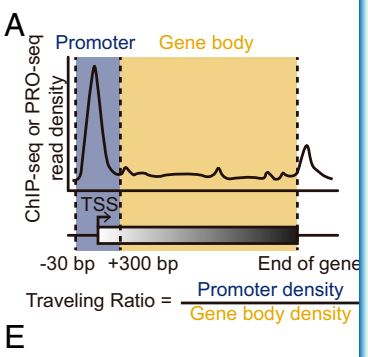
H u DOT1L P II -MLL , DOT1L u P II MLL . F u - , THP1 MOLM-13 (MLL) DOT1L (SGC0946) u DOT1L (33). A H3K79 2 C IP- *SI Appendix*, F . S4 *A B*, DOT1L u H3K79 2 THP1 , 19,743 14,896 DMSO- SGC0946- , . B - DOT1L DOT1L - u P II (*HOXA9 MEIS1*) DOT1L MLL-AF9 (*SI Appendix*, *Supplementary Methods* F . S4 *C–H*). T u u DOT1L u P II -MLL u (HEL K562), u MLL u . H , (34), u SGC0946 - DOT1L P II H3K79 DOT1L u TFIID.

DOT1L May Not Play a Major Role in Transcriptional Elongation in Human Erythroleukemic Cells. T u u u . DOT1L u u ; DOT1L u u - u , u - (35). T DOT1L u u , (TR) P II (36) P II C IP- HEL K562 , (F . 2*A* *SI Appendix*, *Supplementary Methods*). DOT1L KD (HEL) KO (K562) TR P II (F . 2 *B C*), u (35).

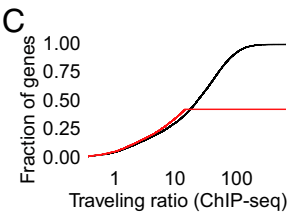
T , u u - u (PRO-) DOT1L KD HEL u P II u (*SI Appendix*, *Supplementary Methods*). N , DOT1L KD u P II u TR P II (F . 2 *D–F*), u u DOT1L u ! u . T u DOT1L u ,

(4 UDRB-) - DRB - RNA u u (4 U) (37) (*SI Appendix, Supplementary Methods*). V 1,243 - u 1,734 u u (*SI Appendix*, F . S5), 518 - u 311 u - u DOT1L (F . 2 *G H*), OT1L u P II (F . 2 *I–M* u u DOT1L ! u .

DOT1L Appears to Regulate Transcriptional Initiation in Human Erythroleukemic Cells. O TFIID/ TBP, TFIIA, TFIIB P II u u . T u P II u TSS DOT1L- K562 (F . 1 *A E 2D*), DOT1L DOT1L u T , C IP- TBP, TFIIA, TFIIB DOT1L KO K562 .



B



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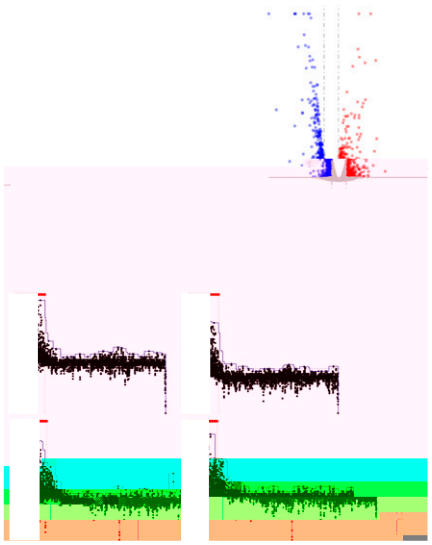
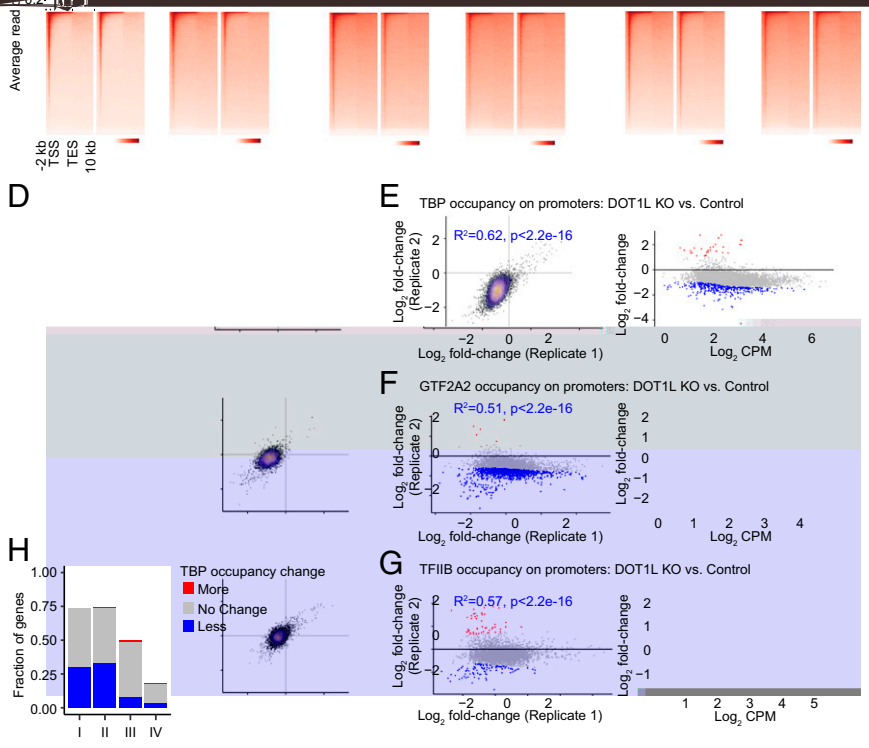


(F . 3 *A–G* *SI Appendix, Supplementary Methods*), u
DOT1L
. T
DOT1L TFIID
TFIIA, C IP-
. W u P II (*SI*
Appendix, F . S3*F*), TFIID TFIIA
DOT1L (*SI Appendix*, F . S6
A *B*).
T DOT1L TFIID
TFIIA DOT1L
u u
(F .1*G*). W u DOT1L
u TFIID TSS
30% u I u II
TFIID -
TSS (1%). I -
u III IV, (10%
) u TFIID
TSS u u I II
(F . 3*H*). T u DOT1L
TFIID U u
TFIIA
(*SI Appendix*, F . S6*C*).
T u I II
TFIID TFIIA DOT1L ,
u . W

u DOT1L TFIID TFIIA -
DOT1L- u
RNA DOT1L , u I
u DOT1L
TFIID TFIIA (*SI Appendix*, F .
S6 *D* *E*). C P II
RNA (32),
TFIID P II
DOT1L- u u
DOT1L u I u II
DOT1L- TFIID
TFIID- P II u
u I DOT1L
.

ENL May Regulate Transcriptional Initiation in Erythroleukemic Cells. T ENL AF9 u u u
DOT1L SEC
u -
YEATS H3 u (24, 25).
I u u SEC, ENL AF9
P II u
u (25). Q DOT1L
u u
AF9 ENL DOT1L u
(24, 25) ENL AF9 u
DOT1L .

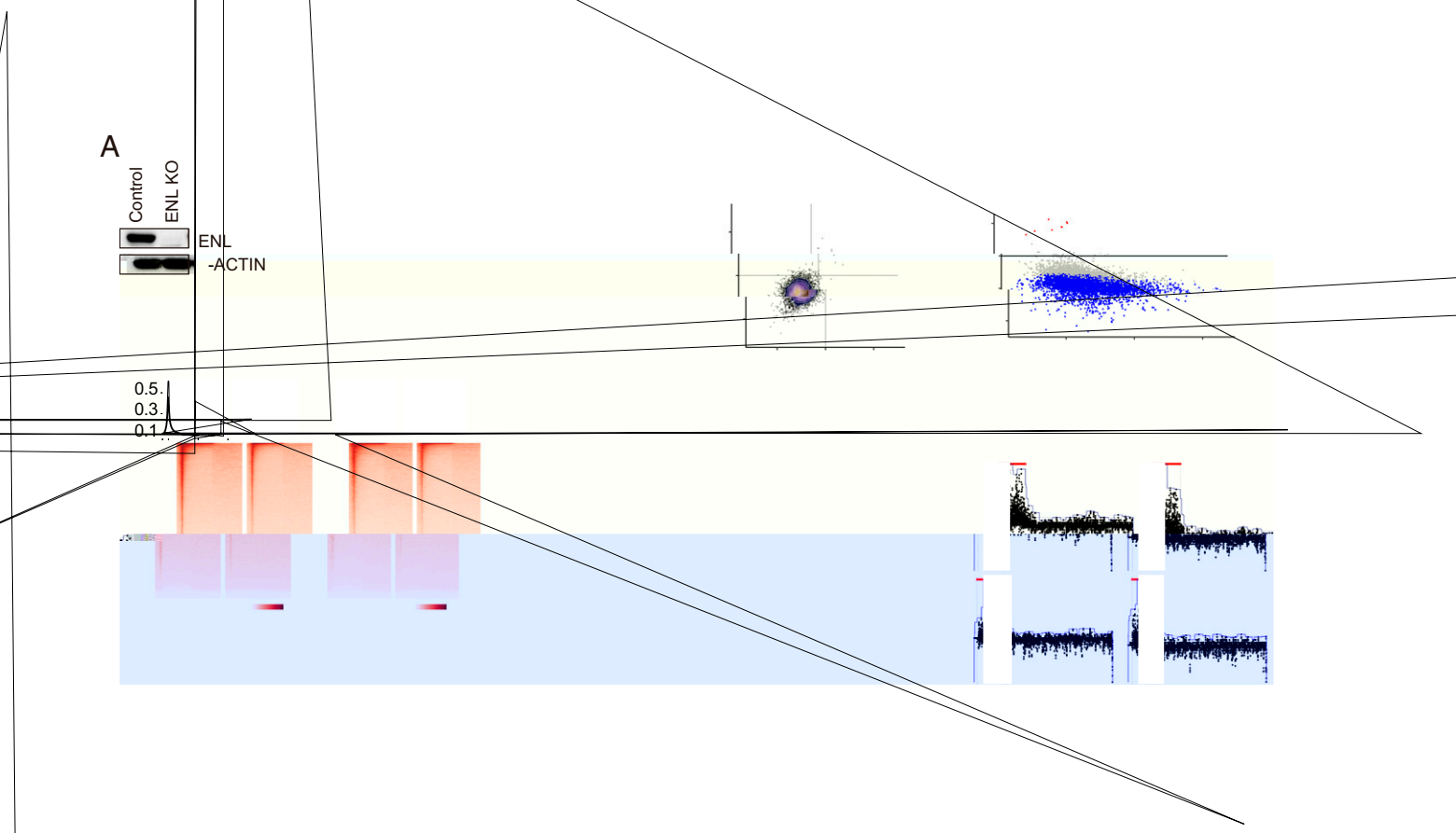


T , C IP- TBP ENL KO K562 CRISPR/C 9 (F . 4*A*). T ENL u TBP (F . 4 *B, D, E*), u TBP *MYC* DOT1L (F . 4*C*), u TFIID/TBP , u .

S ENL DOT1L SEC (12), u ENL u - u DOT1L. ENL u DOT1L TBP DOT1L- u (u I II) DOT1L (u I). A F . 4*F*, u : 1) 37% DOT1L- u 36% DOT1L - u TBP u ENL DOT1L ; 2) 14% DOT1L- u 17% DOT1L u TBP - u DOT1L , u u ENL ; 3) 27% DOT1L u 20% DOT1L u TBP u ENL , u u DOT1L ; 4) 21% DOT1L u 28% DOT1L TBP u ENL DOT1L ; 5) TBP u ENL DOT1L . T DOT1L ENL TBP DOT1L- u , , DOT1L ENL u u DOT1L , - u u u u SEC u u.

T ENL u u K562 4 UDRB- ENL KO . W u ENL KO u P II (F . 4 *G–K*), u AF9 ENL SEC. N , u ENL u - K562 , u ENL TBP u DOT1L , u DOT1L.

DOT1L Can Recruit TFIID via Physical Interactions. M ,



DOT1L SAGA -IP (F . 6*A* *B*). T -IP u u DOT1L, u SAGA H L (F . 6*C* *SI Appendix, Supplementary Methods*). W u SAGA u u DOT1L ,u u DOT1L (F . 6*D*). T DOT1L u SAGA H2B 1 , C IP- PCAF, u u SAGA u H2B 1 DOT1L KO K562 . N , DOT1L PCAF,u DOT1L u SAGA u (F . 6 *E* *G*). T DOT1L H2B 1,u (27), DOT1L H2B 1 u (F . 6 *F* *G*). S u PCAF C IP- , , u, CUT&T PCAF DOT1L KO, u (F . 6 *H* *I* *SI Appendix, Supplementary Methods*). T u u , - H2B 1 DOT1L (26),u DOT1L H2B 1 u u SAGA u . T u SAGA DOT1L, u u (F . 1*G* 3*H*). W u u I II, (7%) SAGA u (4.3% 1.4%,) u III IV (*SI Appendix*, F . S9*A*), u DOT1L SAGA - u DOT1L- u . T u DOT1L u SAGA, C IP- . W u u P II, TFIID TFIIA (*SI Appendix*, F . S3*F* S6 *A* *B*), SAGA DOT1L (*SI Appendix*, F . S9*B*),u DOT1L

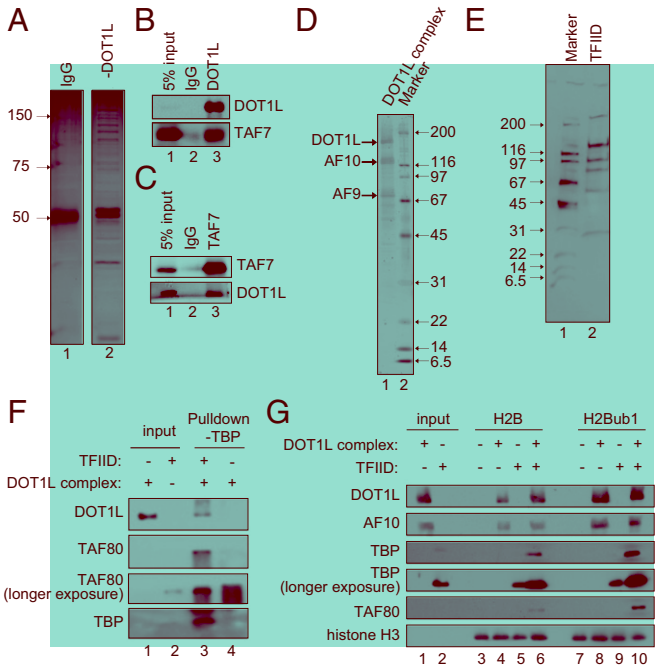


Fig. 5. DOT1L interacts with transcriptional initiation factors. (*A*) Silver staining of proteins immunoprecipitated by rabbit IgG (lane 1) and a DOT1L antibody (lane 2) and separated on an SDS/PAGE gel. (*B*) Western blot analyses of TFIID subunit TAF7 coimmunoprecipitated with DOT1L. (*C*) Western blot analyses of DOT1L coimmunoprecipitated with TAF7. (*D* and *E*) Silver staining of purified DOT1L complex (*D*) and TFIID (*E*) separated on SDS-PAGE gels. (*F*) Western blot analyses of co-IP assays with TFIID as a bait and DOT1L complex as prey. Lanes 1 and 2 contained 10% of input samples. A longer exposure is also shown for TAF80 to better reveal the TFIID input. (*G*) Immobilized template assays of the recruitment of TFIID by DOT1L complex on chromatin templates assembled with unmodified H2B versus H2Bub1. Lanes 1 and 2 contained 10% and 5% inputs for DOT1L complex and TFIID, respectively, with a longer TBP exposure also shown to better reveal the input TFIID.

H2B 1 SAGA

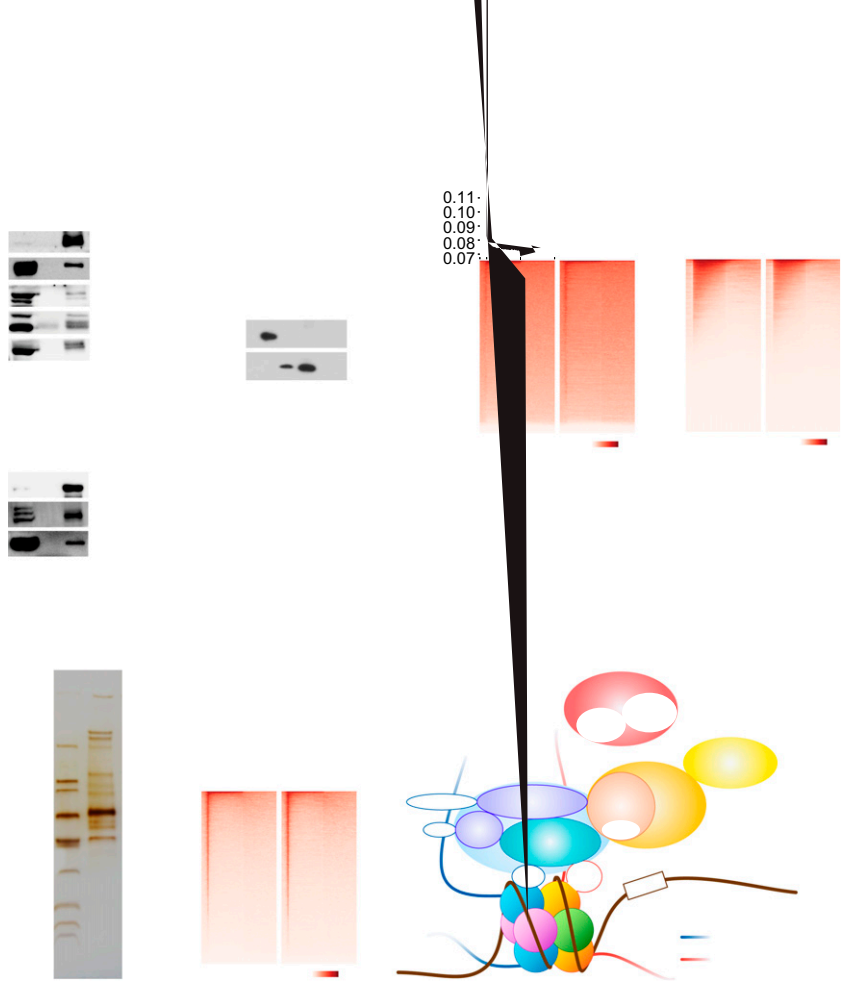
Discussion

DOT1L AF10, AF17, ENL/AF9, MLL . R DOT1L ENL . I DOT1L DOT1L TFIID H2B 1 SAGA . A DOT1L - F . 6*J*.

An Apparent Role for the DOT1L Complex in Transcriptional Initiation in Erythroleukemic Cells. T DOT1L P II, ENL AF9 (P-TEF ELL1), H3K79 2 Q TR (C IP- PRO-) (4 UDRB-) P II DOT1L ! . T

ES DOT1L SEC (35). I DOT1L (TFIIA TFIID) DOT1L ENL, TFIID DOT1L TFIID DOT1L DOT1L TFIID H3K79 . Q AF9/ ENL TFIID TAF5 TAF6 (38) TFIID–DOT1L DOT1L TFIID TFIID TFIID (TBP, TAF) (39). R DOT1L ( ) - : 1) ENL/AF9 YEATS H3 (24, 25), 2) AF10 H3K27 (23), 3) DOT1L ( ) (21, 40). I M DOT1L (F . 5*A* *SI Appendix*, F . S7*A*) DOT1L . T DOT1L

The Binding of DOT1L to H2Bub1 Prevents Its Deubiquitination by the SAGA Complex. H3K79 D 1/DOT1L H2B 1 (26). R D 1 H2B H2B 1 U 8 SAGA, B 1/R 6 P 1 (26), B 1 (27). I *C. elegans* DOT-1.1 ZFP-1 (DOT1L AF10) P II H2B 1, (28). Q D 1 H2B 1, DOT1L . T SAGA H2B 1 DOT1L, H2B 1 DOT1L (26), DOT1L H2B 1 SAGA. O DOT1L H2B 1 SAGA DOT1L, (20, 35), SIRT1 H3K79 (22), (30), . I DOT1L H2B 1 (DOT1L), H3 (AF9/ENL), TFIID ( ), SAGA DUB H2B 1 (41, 42). A DOT1L SAGA SAGA HAT . H SAGA, HAT DUB (5, 43),



MLL FPs Could Potentially Promote Transcriptional Initiation through the DOT1L Complex. MLL-FP u MLL u , , *HOXA9* *MESI1*, , (44, 45). C ! u MLL1 u u SEC DOT1L - , SEC DOT1L , MLL-FP Q DOT1L , MLL u (AF10, AF17, ENL AF9) u u , u u MLL-FP , u (MLL-FP) DOT1L , u . A u DOT1L u DOT1L , u MLL1 N u C G (u CXXC) (46) PAF1C (47, 48), u u u u - C , u u DOT1L , u u , u MLL-FP MLL u .

Materials and Methods

Cells and Cell Culture. Human cells HEL, THP1, and MOLM-13 were cultured in RPMI-1640 + 10% FBS + 2% penicillin/streptomycin + 2 mM L-glutamine + 55 μM β-mercaptoethanol. Human cells K562 were cultured in 90% DMEM + 10% FBS + 2% penicillin/streptomycin + 2 mM L-glutamine.

See *SI Appendix* for additional details and descriptions of RNAi, CRISPR-Cas9 genome editing, ChIP-seq, RNA-seq, PRO-seq, 4sUDRB-seq, ATAC-seq, CUT&-Tag, co-IP, immobilized template assay, and mass spectrometry analyses.

Data Availability. Next-generation sequencing data have been deposited in the Gene Expression Omnibus (GEO) database, https://www.ncbi.nlm.nih.gov/geo (accession no. GSE161367). The mass spectrometry proteomics data have been deposited to the ProteomeXchange Consortium via the PRIDE (49) partner repository (dataset identifier PXD024874). All other study data are included in the article and *SI Appendix*.

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