

Supplemental Methods:

Cell culture: HEK293 (ATCC) and retroviral packaging Plat-E¹ cells were maintained in DMEM supplemented with 10% fetal bovine serum (FBS), penicillin, and streptomycin (Invitrogen). MEF lines were generated by immortalizing fibroblasts isolated from E14 embryos with the SV40 T/t antigen. MEFs were maintained in the same basic medium supplemented with non-essential amino acids, glutamine, and gentamicin (Invitrogen). Murine hematopoietic cells were grown in RPMI 1640 medium supplemented with 5 ng/ml murine recombinant IL-3 (PeproTech), 10% FBS, glutamine, penicillin and streptomycin. Human U937 (ATCC), P31/Fujioka², K562 (ATCC), and THP-1 (ATCC) cells were grown in the same medium without IL-3.

Transfection/Infection of cell lines: HEK293 and Plat-E cells were transfected by the calcium phosphate method. MEFs were infected by co-culture with filtered Plat-E supernatant in the presence of Polybrene (2 µg/ml). Murine fetal liver lines were infected by spinoculation. Transfection/infection efficiencies were verified by GFP percentage by flow cytometry (Accuri C6).

Immunostaining and microscopy: Transfected HEK293 cells or infected MEFs were grown on coverslips in 6-well plates. Cells were washed with phosphate-buffered saline (PBS), fixed with 3.7% paraformaldehyde (20 min), permeabilized with 0.1% Triton X-100 in PBS (10 min), and blocked with 10% FBS (1 h). Cells were incubated with anti-Flag M2 (Sigma) or anti-DOT1L (Santa Cruz) for 1 hr at room temperature (RT). For IF of hematopoietic cells grown in suspension, cells were allowed to settle onto Poly-D-Lysine-coated coverslips overnight. Cells were fixed in 3.7% paraformaldehyde, permeabilized with 0.3% Triton X-100, washed, and blocked with PBS containing 12% normal donkey serum, BSA, and Tween-20 for 1 hr. Because of the lower expression of the fusion protein in these hematopoietic cells, CALM-AF10 could not be detected using the anti-FLAG antibody, and a polyclonal anti-CALM antibody (Sigma) was used. This antibody also detects endogenous murine Calm, and therefore we performed these experiments in *Calm*^{NULL} CALM-AF10-expressing cells. All cells were incubated with AlexaFluor568 or 488-conjugated secondary antibodies (Invitrogen) for 40

min at RT. After further washing, cells were stained with DAPI and mounted onto slides using Fluoromount-G (Southern Biotech). Samples were examined at RT using a Zeiss LSM 510 confocal on an Axio Observer microscope using a Zeiss Plan-Apochromat 63x/1.4 or a 100x/1.4 oil objective. Pinholes were set to 1 airy unit for each channel, line averaging of 8, 1024 x 1024 image format, and a 2X optical zoom for the 100x objective. Images of cell and colony morphology were obtained with the Leica Microsystems DFC425 attached to a Leica DMLB at RT.

Western blotting: Western blots were performed according to standard protocols. Primary antibodies included: FLAG M2 (Sigma), CALM (Sigma), Actin (Sigma), Histone H3 (Cell Signaling), and di-methylated H3K79 (Cell Signaling). Fluorescently conjugated secondary antibodies were incubated for 1 hr at RT, and blots were developed using the Odyssey Infra-red imaging system (Li-Cor Biosciences). To quantitate levels of di-me-H3K79, the integrated intensities of the bands were measured by the Odyssey imaging system and normalized to Actin or total Histone H3.

Real Time RT-PCR of *Hoxa* genes and RT-PCR of *CALM-AF10*: Total RNA was isolated from MEFs or leukemic cells using the RNeasy Mini kit (Qiagen). Total RNA was reverse transcribed using the Superscript II kit (Invitrogen). Quantitative PCR amplification was performed using the iQ Sybr Mix (Bio-Rad) with the iQ5 Optical System (Bio-Rad). Expression levels were normalized to the levels of the endogenous housekeeping genes *β 2M* and *GAPDH* by the comparative threshold (CT) method. All primers are available upon request.

Chromatin Immunoprecipitation (ChIP): Modification-specific histone ChIP assays were performed as described previously.³ Immunoprecipitation was performed with 1 μ g of anti-H3K79me2 or anti-H3K4me3 antibodies (Abcam) incubated overnight at 4°C. Salmon sperm-conjugated protein G sepharose beads (35 μ L; Millipore) were then added and rocked for an additional 3 hr at 4°C. Following RNase A and proteinase K treatment, DNA was purified with a PCR purification kit (Qiagen) and amplified by real time PCR. Amplification values are normalized to input and are presented as fold enrichment over negative control (empty vector or *Hoxa9/Meis1*). Primer

sequences used to amplify promoter regions of the *Hoxa* genes upstream of the TSS are available upon request.

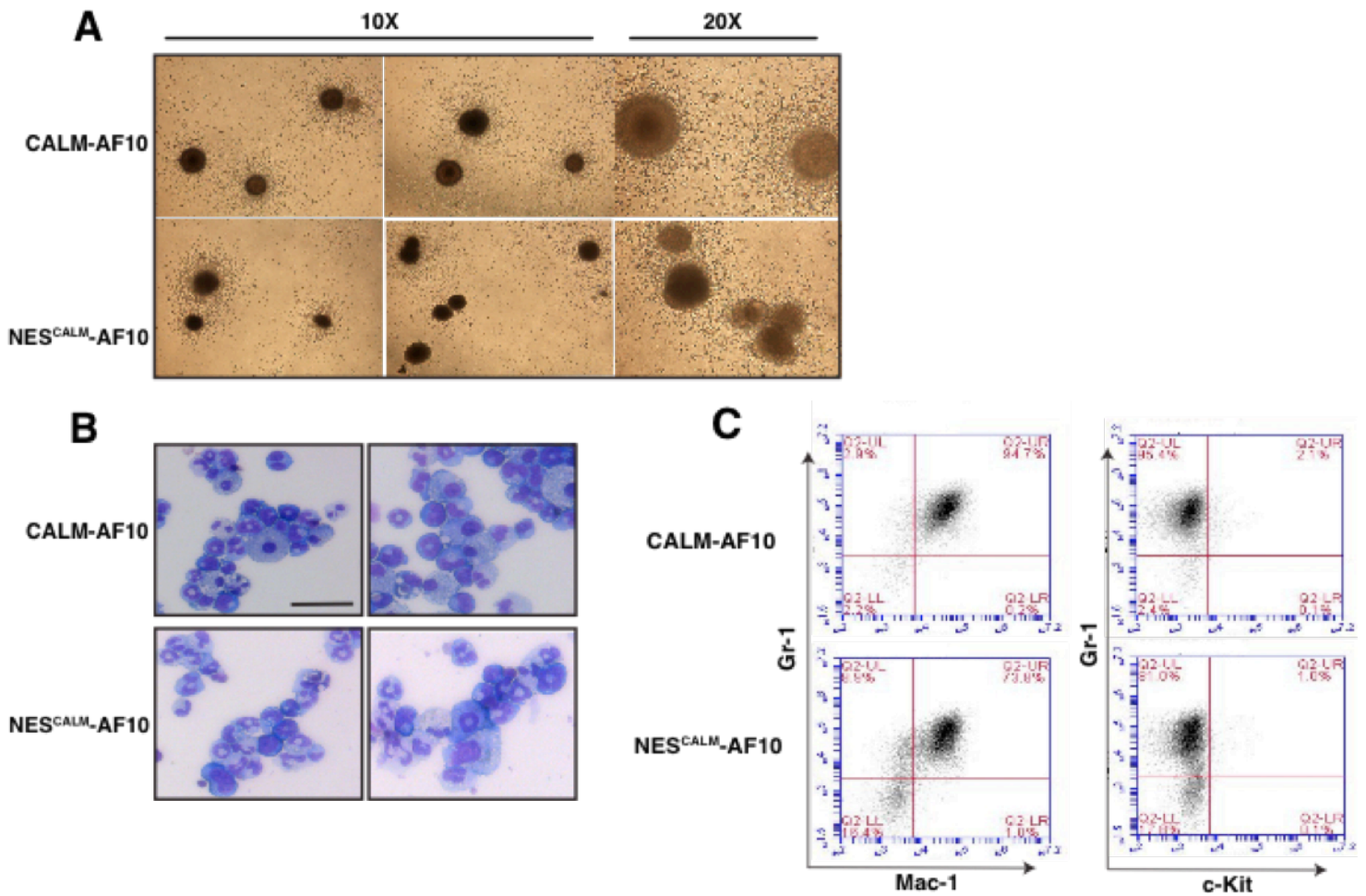
Inhibition of CRM1-dependent nuclear export: Prior to IF analysis, HEK293 and murine leukemia cells were treated with 10 nM or 0.1 nM LMB for 1 h or 12 hr, respectively. MEFs were treated with 1 nM LMB for approximately 24 hr before ChIP analysis. Human leukemic cell lines (U937, P31/Fujioka, K562, and THP-1) were grown in the presence of varying concentrations (0, 0.1, 0.5, and 1 nM) of LMB (Sigma) for 17 and 42 hours. Cell viability was analyzed by flow cytometry, and total cell number was recorded as a percent of untreated.

Statistical Analysis: Data are presented as mean plus or minus SEM (n = 3 or more). Statistical analysis was performed by one-way ANOVA followed by Dunnett's post-test when three or more groups of data were compared. Statistical analyses of cell viability experiments were performed by two-way ANOVA followed by Bonferroni's Multiple Comparison Test. Values were considered statistically significant when *P* values were less than .05. All analyses were done using PRISM software (GraphPad Software, Inc.).

References for Supplemental Methods:

1. Morita S, Kojima T, Kitamura T. Plat-E: an efficient and stable system for transient packaging of retroviruses. *Gene Ther.* 2000;7(12):1063-1066.
2. Narita M, Shimizu K, Hayashi Y, et al. Consistent detection of CALM-AF10 chimaeric transcripts in haematological malignancies with t(10;11)(p13;q14) and identification of novel transcripts. *Br J Haematol.* 1999;105(4):928-937.
3. Okada Y, Feng Q, Lin Y, et al. hDOT1L links histone methylation to leukemogenesis. *Cell.* 2005;121(2):167-178.

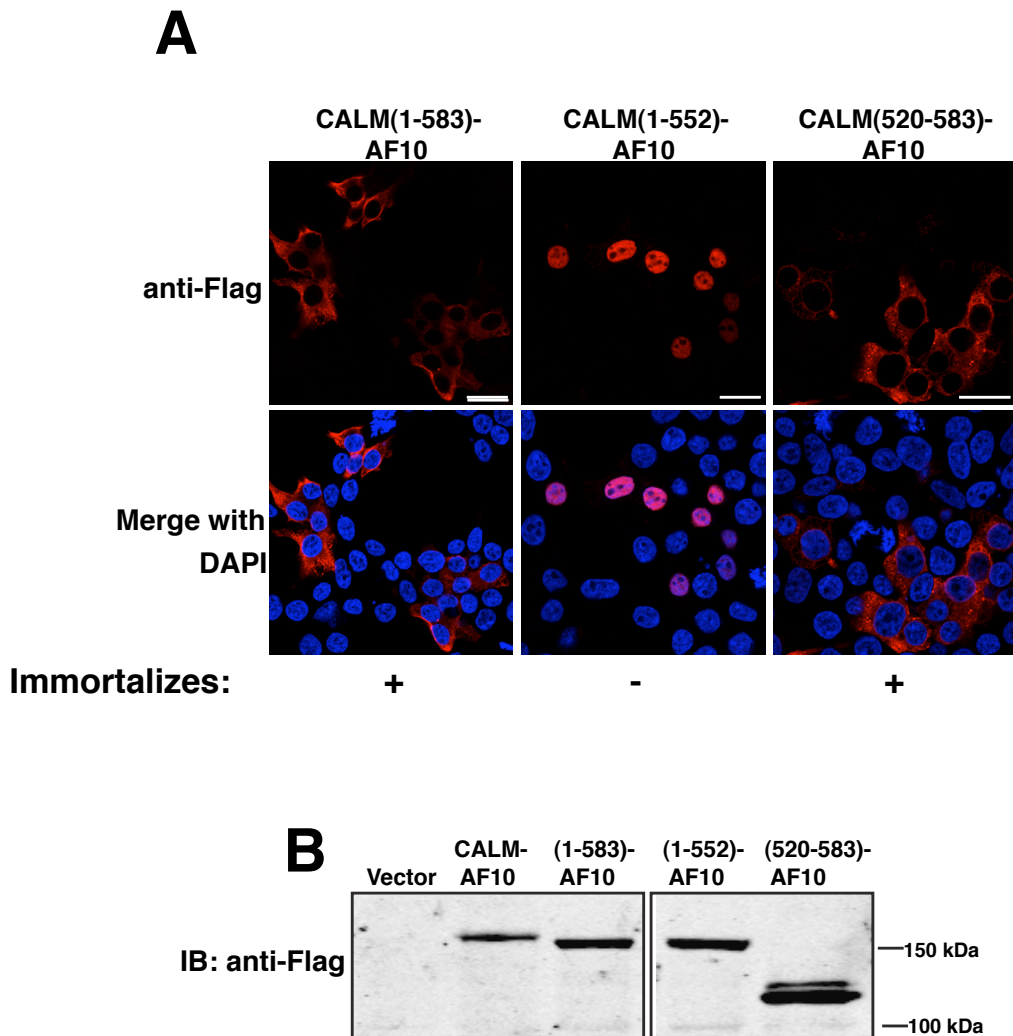
Supplemental Figure 1



Supplemental Figure 1: The morphology and immunophenotypes of CALM-AF10 and NES^{CALM}-AF10 *in vitro* methylcellulose colonies are indicative of myeloid transformation. (A)

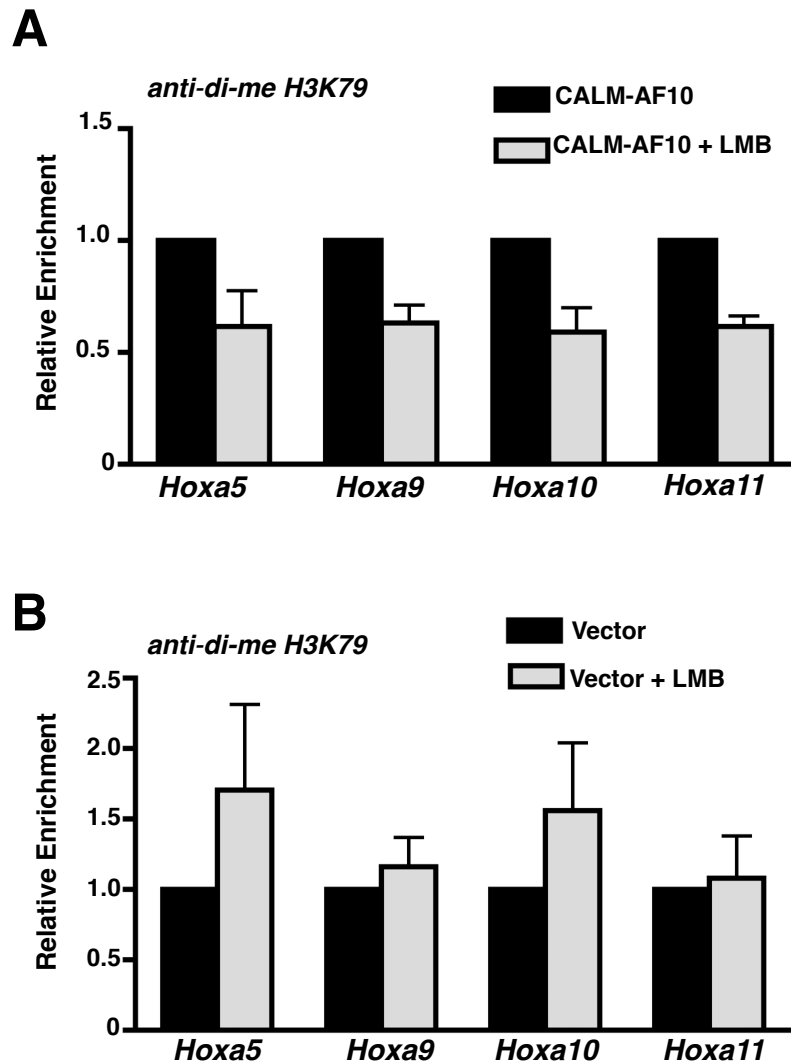
Colony morphology of murine bone marrow cells retrovirally transduced with CALM-AF10 (upper panels) or NES^{CALM}-or 20X magnifications. (B) Cells from tertiary colonies were pooled, and 300,000 cells were cytopun onto coverslips to assess cellular morphology. Two representative pictures of stained CALM-AF10 (upper) or NES^{CALM}-AF10 (lower) immortalized cells are shown. Scale bar represents 40 μ m for all panels. (C) Flow cytometric analyses of cells from CALM-AF10 (upper) or NES^{CALM}-AF10 (lower) colonies. Cells were co-stained for Gr-1 and Mac-1 (left) or Gr-1 and c-Kit (right).

Supplemental Figure 2



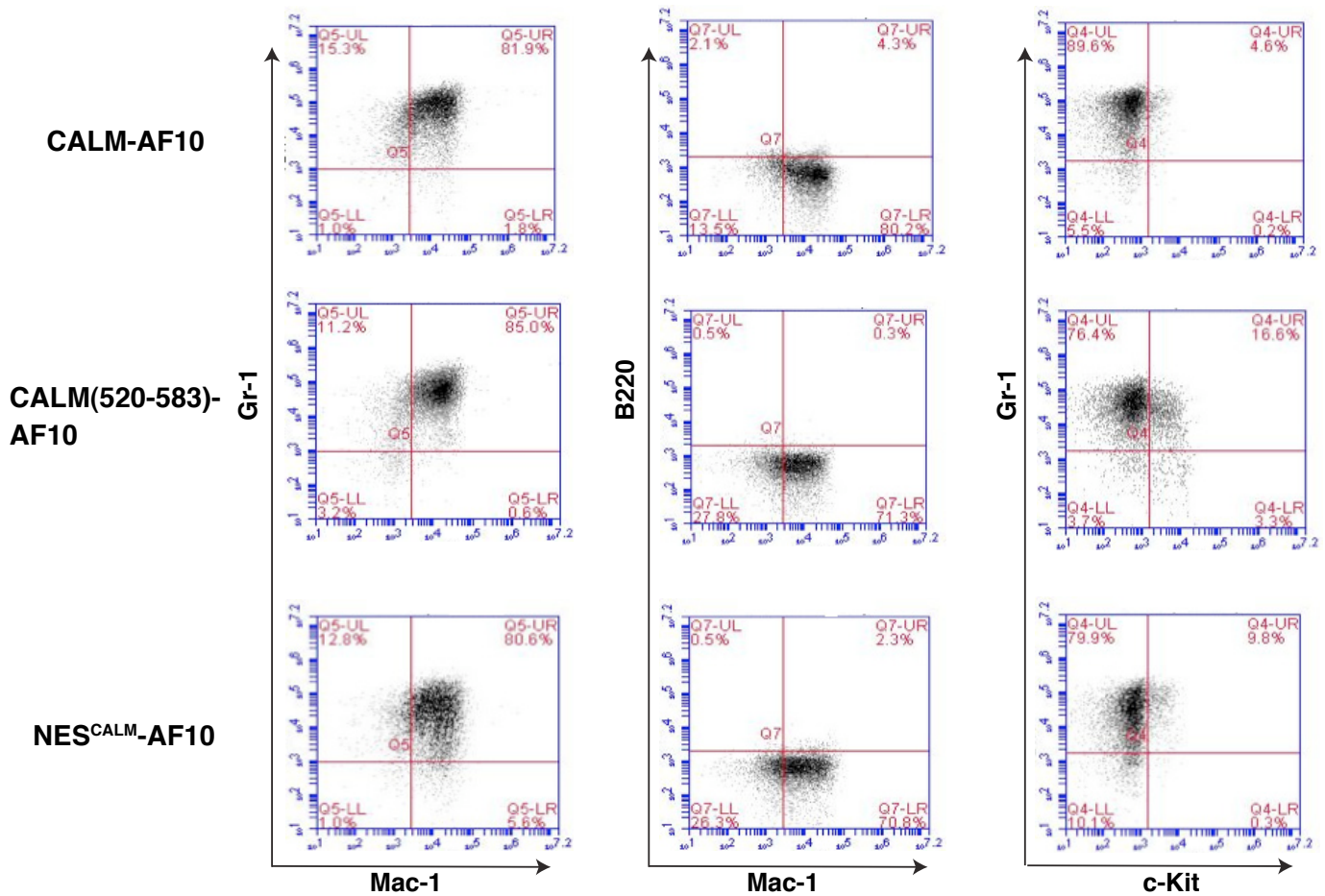
Supplemental Figure 2: Transforming ability of CALM-AF10 truncation mutants correlates with cytoplasmic localization. (A) Confocal IF analysis of HEK293 cells transiently transfected with CALM-AF10 truncation mutants. Cell nuclei were stained with DAPI (blue). Scale bars represent 20 μ m. The transforming ability of each of the mutants is shown below each panel. **(B)** Western blot of HEK293 cells transiently transfected with CALM-AF10 truncation mutants and blotted with anti-Flag antibodies.

Supplemental Figure 3



Supplemental Figure 3: Leptomycin B treatment lowers local H3K79 di-methylation at the *Hoxa* locus in CALM-AF10 cells. CHIP analysis of H3K79 di-methylation in the promoter regions of the *Hoxa* genes was performed in (A) CALM-AF10- (B) or Vector-expressing cells untreated (black bars) or treated with 1 nM LMB for 24 hr (grey bars). Results are shown as percent of input normalized to the untreated conditions (mean \pm SEM compiled from 3 independent experiments). A statistically significant reduction (Student's t-test; $P < 0.05$) in di-methyl H3K79 ChIP was observed at *Hoxa9* and *Hoxa11* in LMB-treated CALM-AF10 cells.

Supplemental Figure 4



Supplemental Figure 4: Primary leukemias generated by CALM-AF10, CALM(520-583)-AF10 and NES^{CALM}-AF10 have similar immunophenotypes. Flow cytometric analyses of cells from CALM-AF10 (upper row), CALM(520-583)-AF10 (middle row) or NES^{CALM}-AF10 (bottom row) leukemic bone marrow cells. Cells were co-stained for Gr-1 and Mac-1 (left column), B220 and Mac-1 (middle column) or Gr-1 and c-Kit (right column). For each construct, data are from one leukemic mouse and are representative of at least n=3 mice.