Supplemental Information

Novel sub-cellular localizations and intra-molecular interactions may define new functions of Mixed Lineage Leukemia protein. Amit Mahendra Karole, Swathi Chodisetty, Aamir Ali, Nidhi Kumari and Shweta Tyagi.

Supplemental Methods

Immunoblotting

U2OS whole cell or nuclear extract (100ug) were subjected to the SDS-PAGE gel electrophoresis and proteins were transferred to the PVDF membrane. Membranes was blocked with 4% skimmed milk for 1hr, followed by incubation with primary antibodies against MLL_N (1:100, Bethyl A300-086A), MLL_C (1:100, Bethyl A300-374A), or β- Actin (1:5000, Sigma A5441) as indicated. These blots were further incubated with HRP conjugated secondary antibodies anti-mouse IgG (Sigma-Aldrich-A9044) and anti-rabbit IgG (Sigma-Aldrich-A0545). The signal was detected using Amersham ECL Prime Western Blotting Detection Reagent (RPN2232).

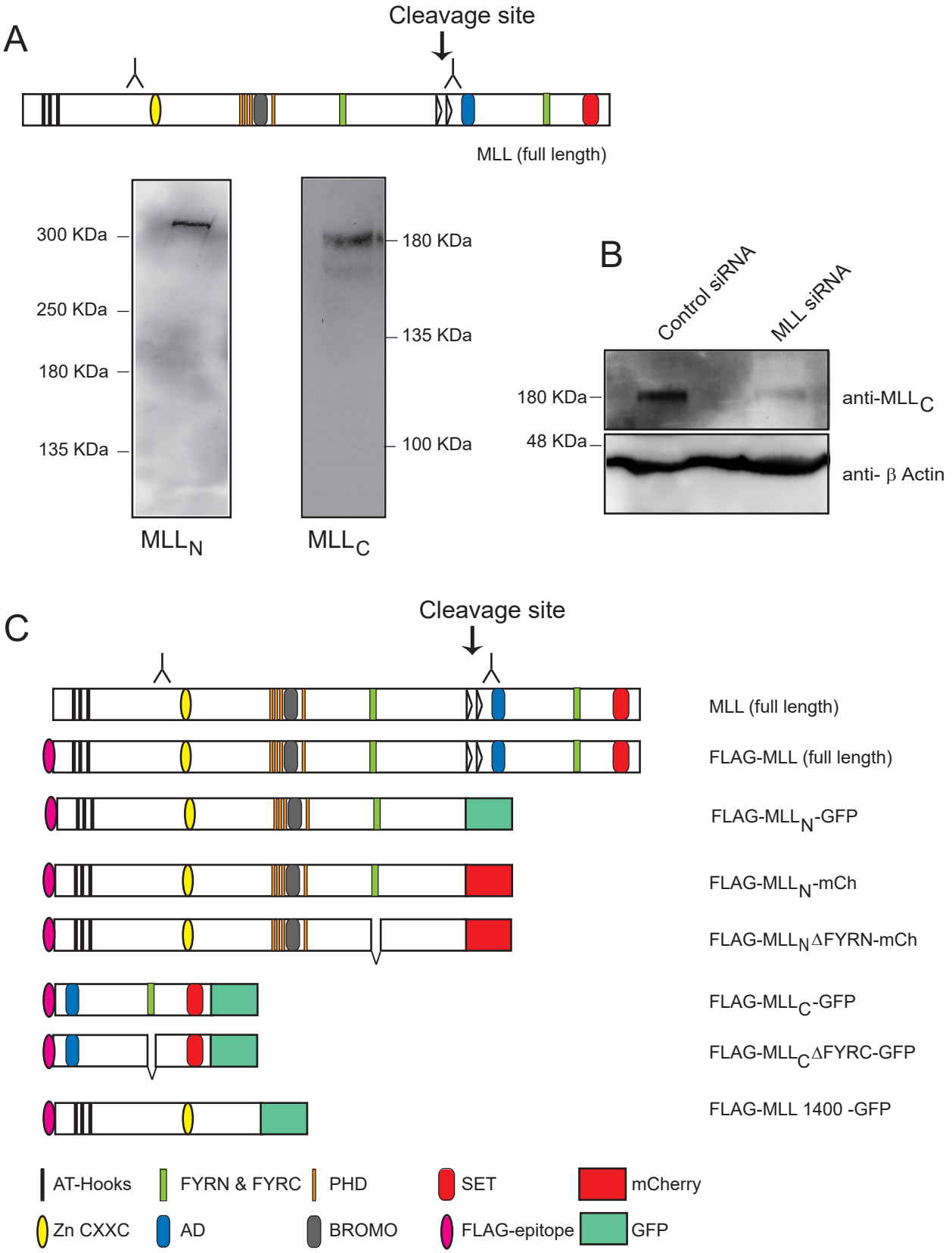
Supplemental Figure Legends

Supplemental Figure 1: **Schematic of MLL constructs used in this study**.

(A) Immunoblot of MLL, detected with anti-MLL_N and MLL_C antibodies. Line diagram shows the full-length MLL with its domain architecture and antibody epitope position is indicated on top. Lower panel shows the immunoblot of MLL probed with antibodies against N-terminal subunit of MLL, anti-MLL_N (the epitope spans 720-780 amino acids), and against C terminal MLL, anti-MLLC (the epitope spans 2725-2775 amino acids).

(B) Analysis of MLL knockdown using MLL siRNA by immunoblotting. The U2OS cells were transfected with control and MLL siRNA #1 (against the ORF[36]). The blot was probed with anti-MLL_C and anti-β-Actin antibody. (**A-B**) Molecular weight markers are indicated.

(C) Line diagram showing MLL full-length domain position and the various MLL mutants/constructs used in the study. As shown, MLL comprises of AT-Hooks: DNA binding domain; FYRN & FYRC: heterodimerization domains ; PHD: plant homeo domain, Zn CXXC domain- Zinc finger domain, BROMO: Bromodomain ;AD: transactivation domain, and Su(var)3-9, Enhancer-of-zeste, Trithorax (SET) domain.GFP and mCherry fusions are shown as indicated. Recombinant proteins have FLAG-epitope tag at the N-terminal. mCh, mCherry.



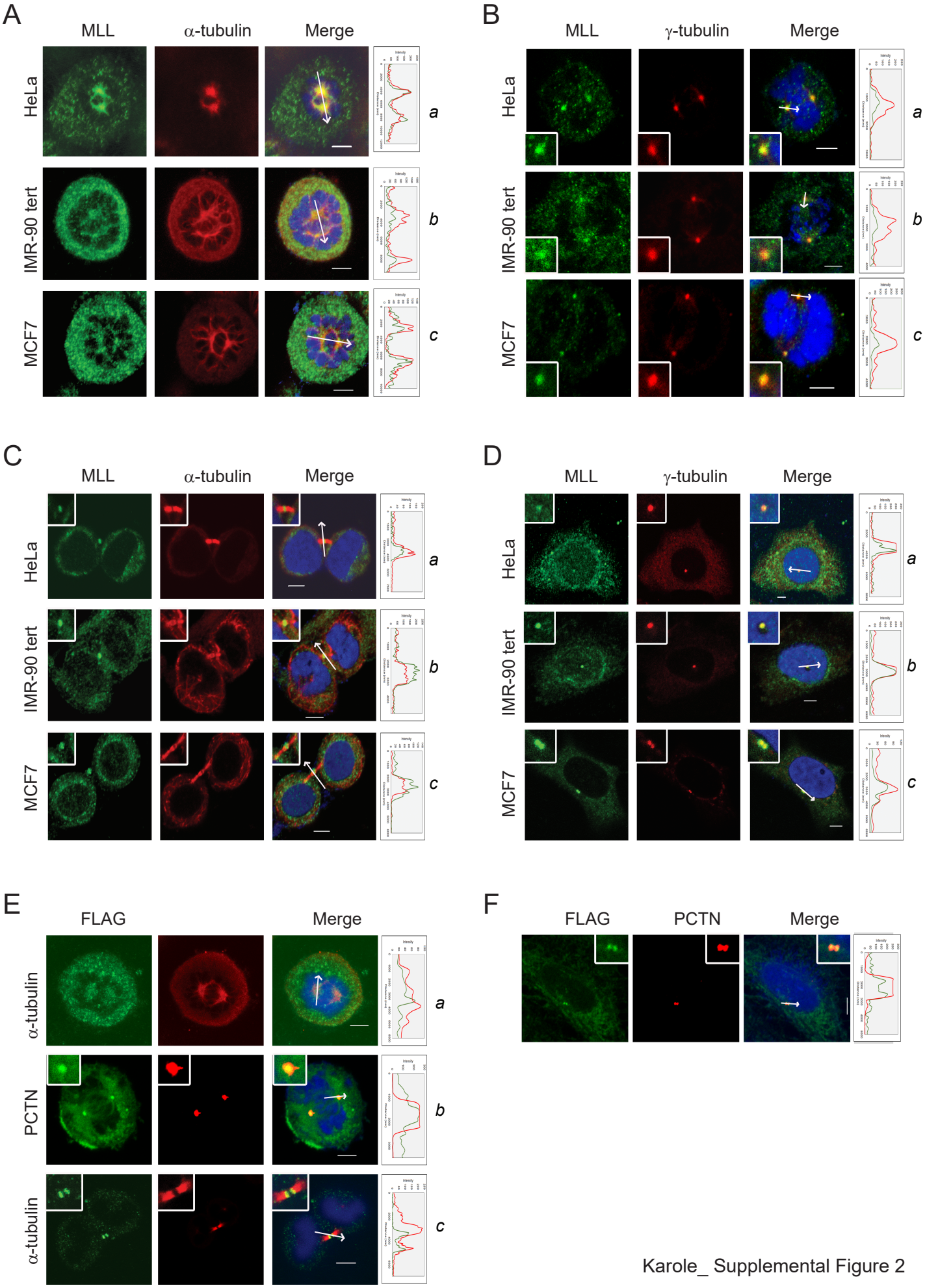
Karole_Supplemental Figure 1

Supplemental Figure 2: **MLL localization in multiple cell lines.**

(A-C) Immunofluorescence staining (IFS) of endogenous MLL_C in HeLa (panel a), IMR90-tert (panel b) and MCF7 (panel c) cells in mitosis. The cells were co-stained with anti-MLL_C (green) and anti-α-tubulin (red) to mark spindle **(A)** or midbody**(C)** or anti-γ-tubulin (red) to mark centrosome **(B).**

(**D**) IFS of HeLa (panel a), IMR90-tert (panel b) and MCF7 (panel c) in interphase. Cells were stained with endogenous anti-MLL_C (green) and anti-γ-tubulin (red) to mark centrosome.

(E, F) IFS staining of U2OS cells stably expressing the MLL full-length FLAG-epitope tag stained with anti-FLAG antibody (green). **(E)** Cells in mitosis were co-stained with anti-α-tubulin (red) to mark spindle (panel a) or midbody (panel c) or anti-pericentrin (red) to mark centrosome (panel b). **(F)** Interphase cells were stained with anti-pericentrin (red) to mark the centrosome. **(A-F)** DNA was stained with 4, 6-diamidino-2-phenylindole (DAPI, blue). Co-localization profile shown on left, in plot-profile analysis indicated with white arrow, shows the MLL (green line) with respective marker γ-tubulin or α-tubulin or pericentrin (red line). Scale bar, 5μm.See inset for zoomed-in images.



Karole_ Supplemental Figure 2

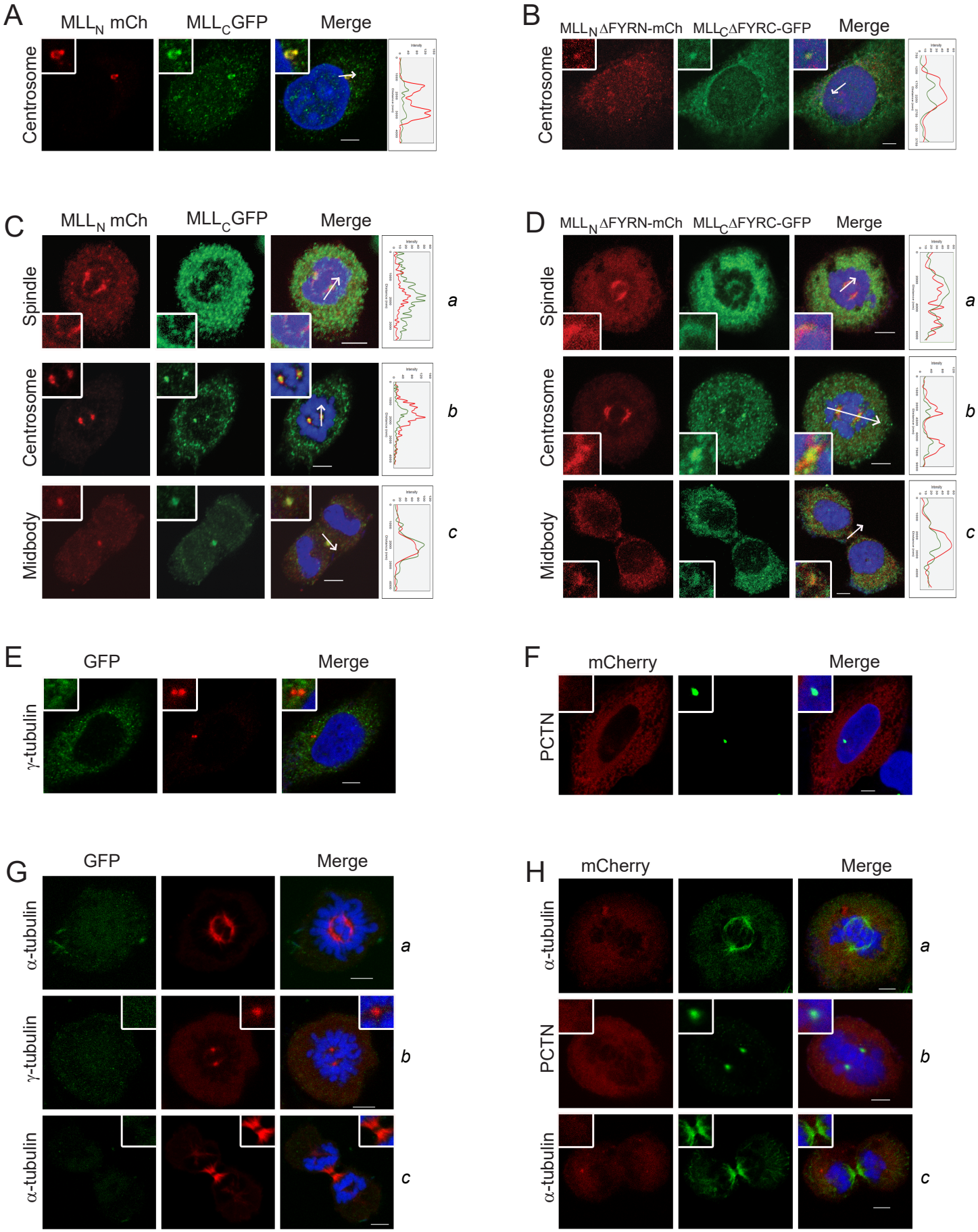
Supplemental Figure 3: **Ectopic MLL_N and MLL_C localizes to centrosome, spindle and midbody independent of each other**.

(A, B) IFS of U2OS cells co-expressing MLL_N-mCherry and MLL_C-GFP **(A)** or MLL_N-ΔFYRN mCherry and MLL_CΔ FYRC GFP **(B)**. The interphase cells were stained with anti-mCherry (red) and anti-GFP (green) antibodies.

(C, D) IFS of U2OS cells stably co-expressing MLL_N-mCherry and MLL_C-GFP **(C)** or MLLN-ΔFYRN mCherry and MLL_C Δ FYRC GFP**(D)**. The mitotic cells were stained with anti-mCherry (red) and anti-GFP (green) antibodies to check for the localization of ectopically expresses proteins at spindle (panel a), centrosome (panel b) and midbody (panel c). **(A-D)** Co-localization pattern depicted on left indicates the extent of localization shown for recombinant MLL_C (green line) with recombinant MLL_N (red). The area of interest are highlighted with white arrow.

(E, F) IFS of U2OS interphase cells stably expressing pcDNA GFP or mCherry vector control. The GFP (**E**) or mCherry (**F**) expressing cells in interphase were stained with anti-γ-tubulin (red) **(E)** or anti-pericentrin (green) **(F)** antibodies.

(G, H) U2OS cells stably expressing pcDNA GFP or mCherry vector control were imaged for different stages of mitosis. **(G)** The cell expressing GFP were stained with α-tubulin (red) to mark spindle (panel a) or midbody (panel c) and with anti-γ-tubulin (red) to mark centrosome (panel b). **(H)**The cells in mitosis expressing mCherry were stained with anti-α-tubulin (green) to mark spindle (panel a) or midbody (panel c), and with anti-pericentrin (green) to mark centrosome (panel b). **(A-H)**DNA was stained with DAPI (blue). Scale bar, 5μm. See inset for zoomed-in images.

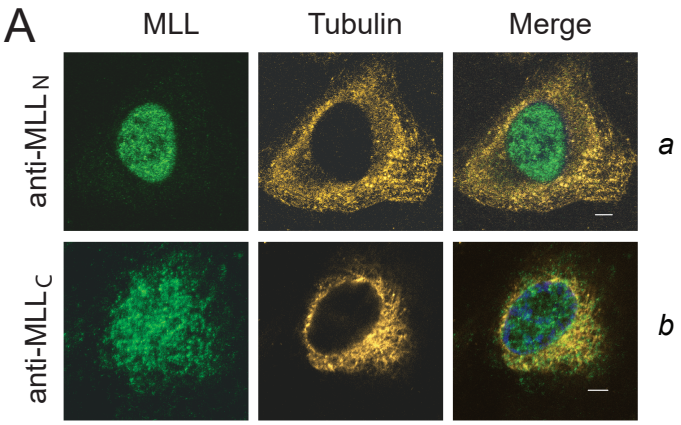


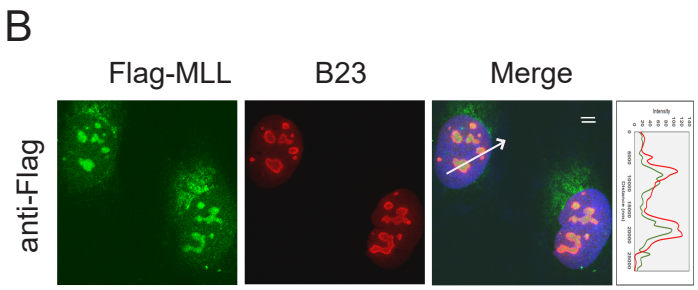
Karole_ Supplemental Figure 3

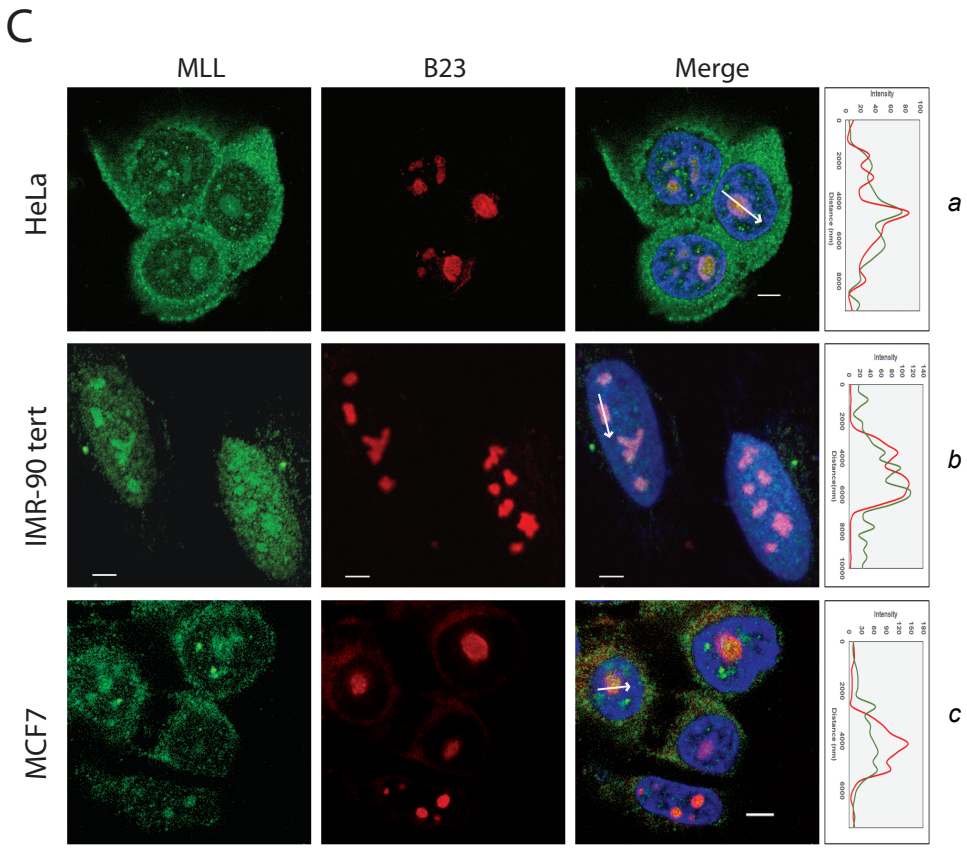
Supplemental Figure 4: Nucleolar localization of MLL in multiple cell lines.

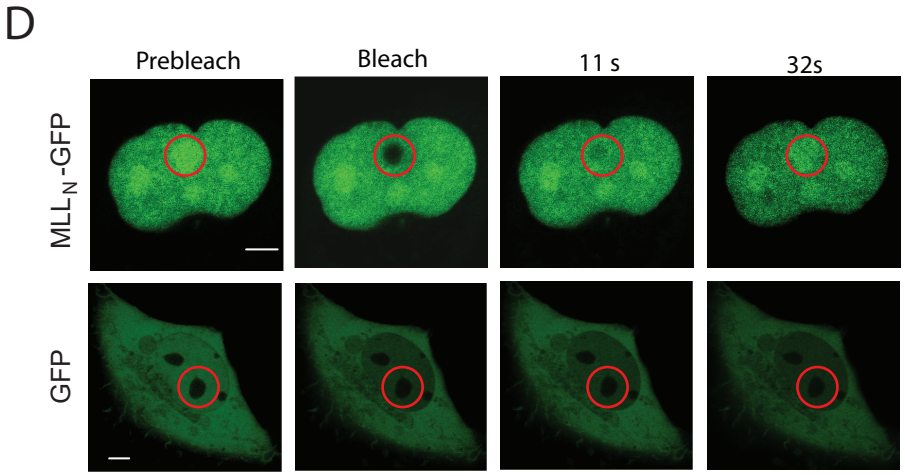
(A) IFS of endogenous MLL_C in HeLa (panel a), IMR90-tert (panel b) and MCF7 (panel c) cells in interphase. The cells were stained with anti-MLL_C (green) and anti-B23 (red) antibodies. Nucleoli were marked with B23 and DNA was stained with DAPI (blue). The colocalization profile shown on left in the form of plot-profile of the selected area indicated by white arrow. Scale bar, 5µm.

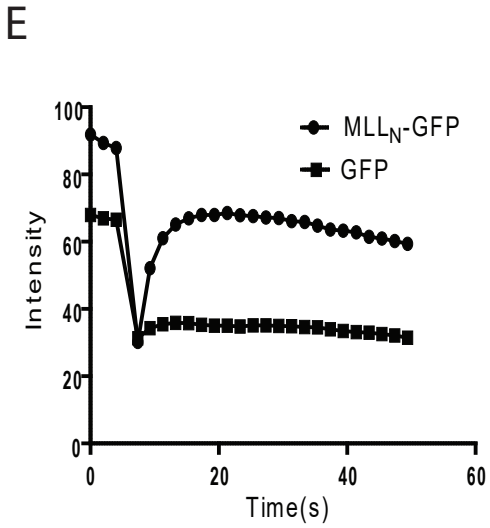
(B-C) Fluorescence recovery after photo bleaching (FRAP) experiment was performed on U2OS cells expressing MLL_N-GFP and pcDNA GFP–mCherry vector control. **(B)** Representative images show stages at pre-bleach, bleach, 11seconds and 32 seconds in recovery respectively. **(C)** FRAP curves of MLL_N-GFP and pcDNA GFP–mCherry vector control in interphase cells. Graph shows relative intensity vs time interval. Four experiments were done and results from one representative experiment are shown here.











Karole_Supplemental Figure 4

Supplemental Figure 5: Localization of MLL fusion proteins.

(A) Nucleolar Localization Sequence (NoLS) analysis of MLL1400. MLL 1400 amino acid sequence was analyzed for nucleolar localization sequence with the help of Nucleolar Localization Sequence Detector (NOD) (Scott et al., 2010) by using Jpred secondary structure prediction method (http://www.compbio.dundee.ac.uk/jpred). The results were plotted in terms of NoLS predictions per residue.

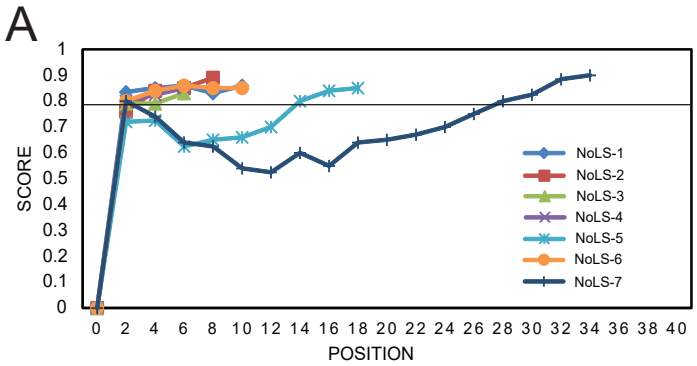
(B) Localization MLL and MLL fusion proteins after Actinomycin D treatment. Cells expressing MLL_N-GFP, MLL-AF4-GFP and MLL-AF9-GFP were treated with Actinomycin D (Sigma-Aldrich A1410) at 0.08 μg/ml. for 1 hr and subjected to IFS. Subsequently, cells were fixed and co-stained with anti-GFP (green) and anti-UBF (red) antibodies. UBF was used to mark nucleoli.

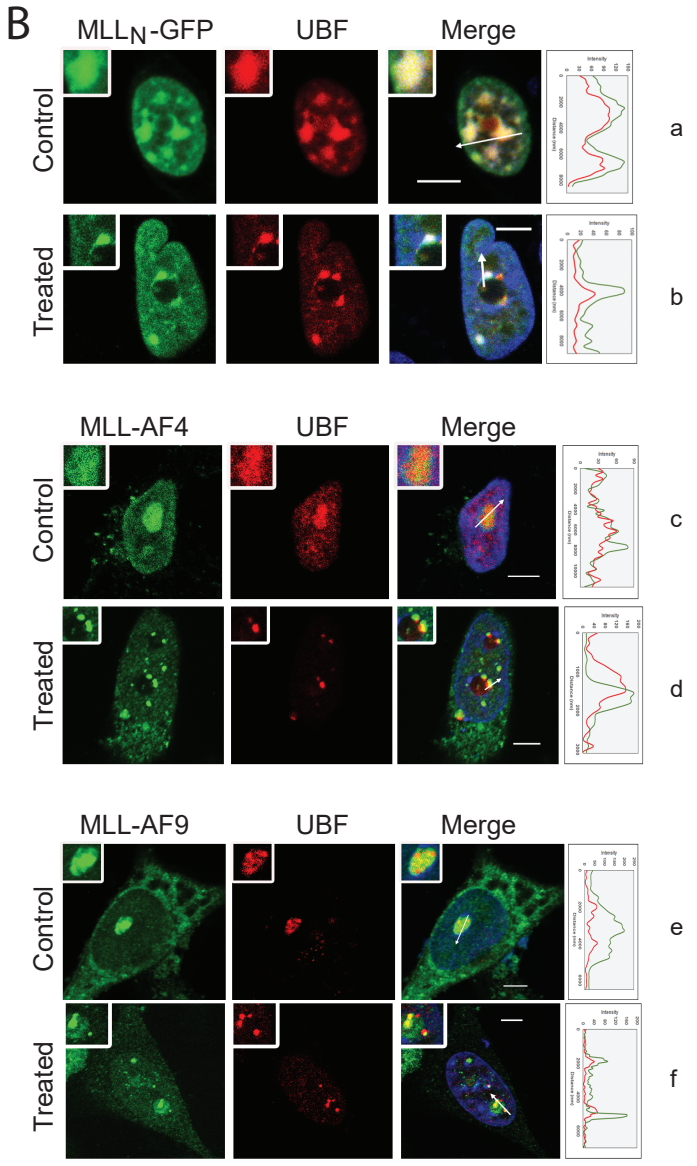
(C) Cells expressing MLL-AF4-GFP and MLL-AF9-GFP were fixed and stained with anti-GFP (green, panel a, b) and anti-MLL_C (red, panel a, b) to detect endogenous MLL protein. The localization of both signals were analyzed at nucleolar region.

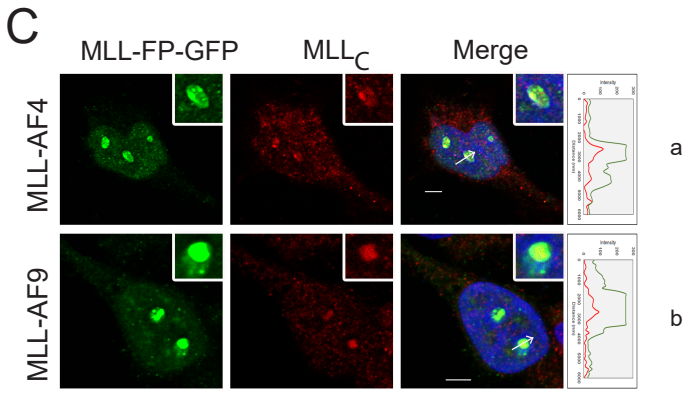
(D) Localization of the MLL fusion proteins and endogenous MLL protein is shown at centrosome. The cells were co-stained with anti-GFP (green panel a, b) and anti-MLL_C antibodies (red, panel a,b).

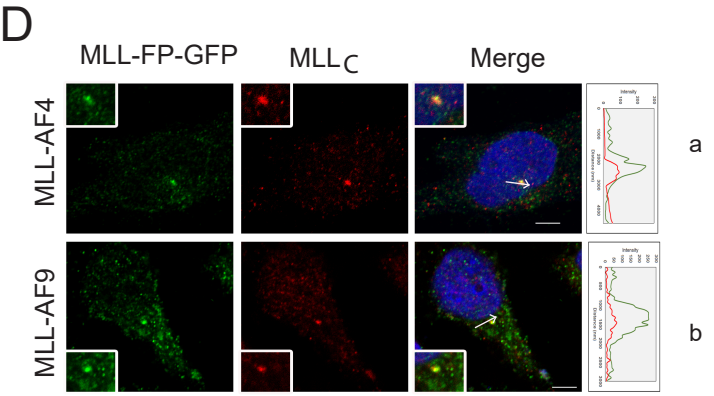
(E) Localization of MLL fusion proteins and MLL_C were analyzed at mitotic spindle (panel a, b), centrosome (panel c, d) and midbody (panel e, f) respectively. The cells expressing MLL-AF4-GFP and MLL-AF9-GFP were fixed and stained with anti-GFP (green) and anti -MLL_C antibody (red). The fixations have been described in Materials and Methods, except panel f where cells were fixed for 2 mins with methanol.

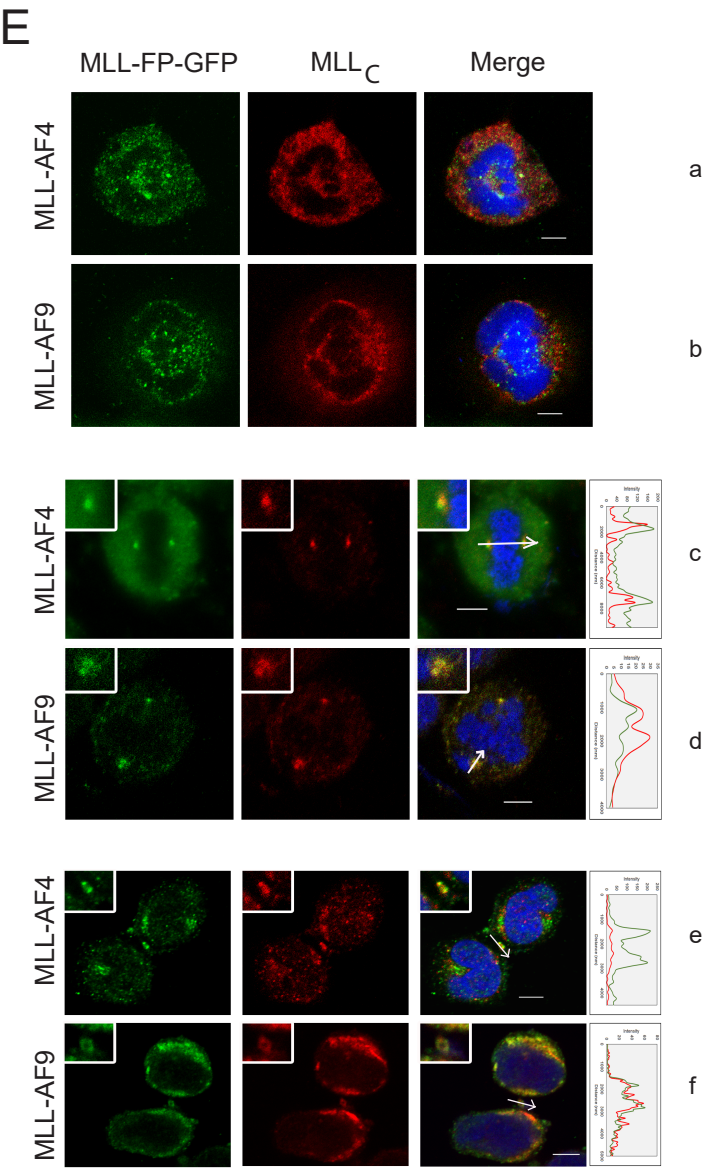
(F) IFS of leukemic cell line THP-1 (with MLL-AF9 fusion) stained with anti-MLL_C antibody. THP-1 cells were fixed and co-stained with anti-MLL_C (green) and anti-UBF (red) or Centrin1 (red) antibodies. The anti-MLL_C antibody detects only wild type MLL_C but not MLL-AF9 fusion protein (see Figure S1A). UBF and Centrin1 were used to mark nucleoli and centrosome respectively. (**B**-**F**) DNA was stained with DAPI (blue). The plot profile analysis (on left) of areas indicated (white arrow) depicts co-localization profile of GFP tagged MLL fusion proteins (green line) with MLL_C (red line; **B**, **D**, **E**) or different organelle markers (red; **C**) as indicated. The co-localization profile of MLL_C (green line) with respective markers (red line) is shown in **F**. Scale bar, 5μm. See inset for zoomed-in images.

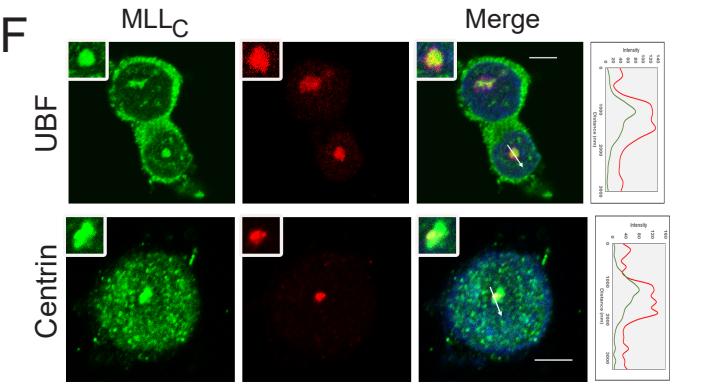












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