The PML-associated protein DEK regulates the balance of H3.3 loading on chromatin and is important for telomere integrity

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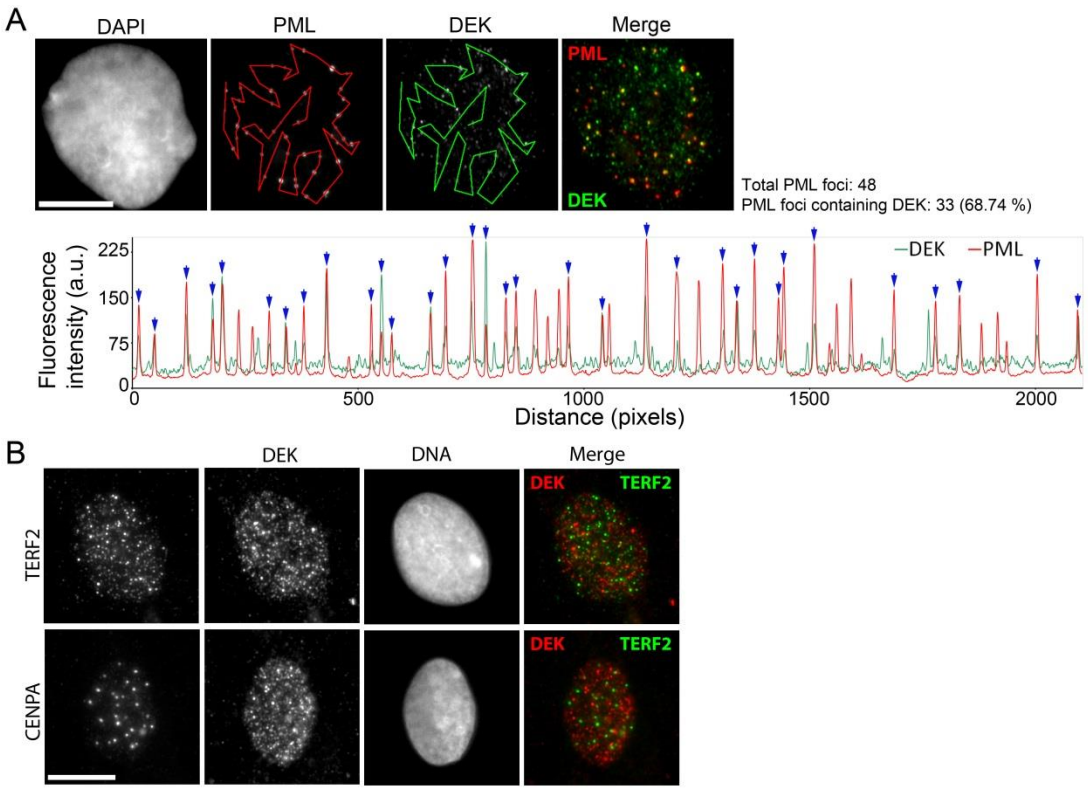
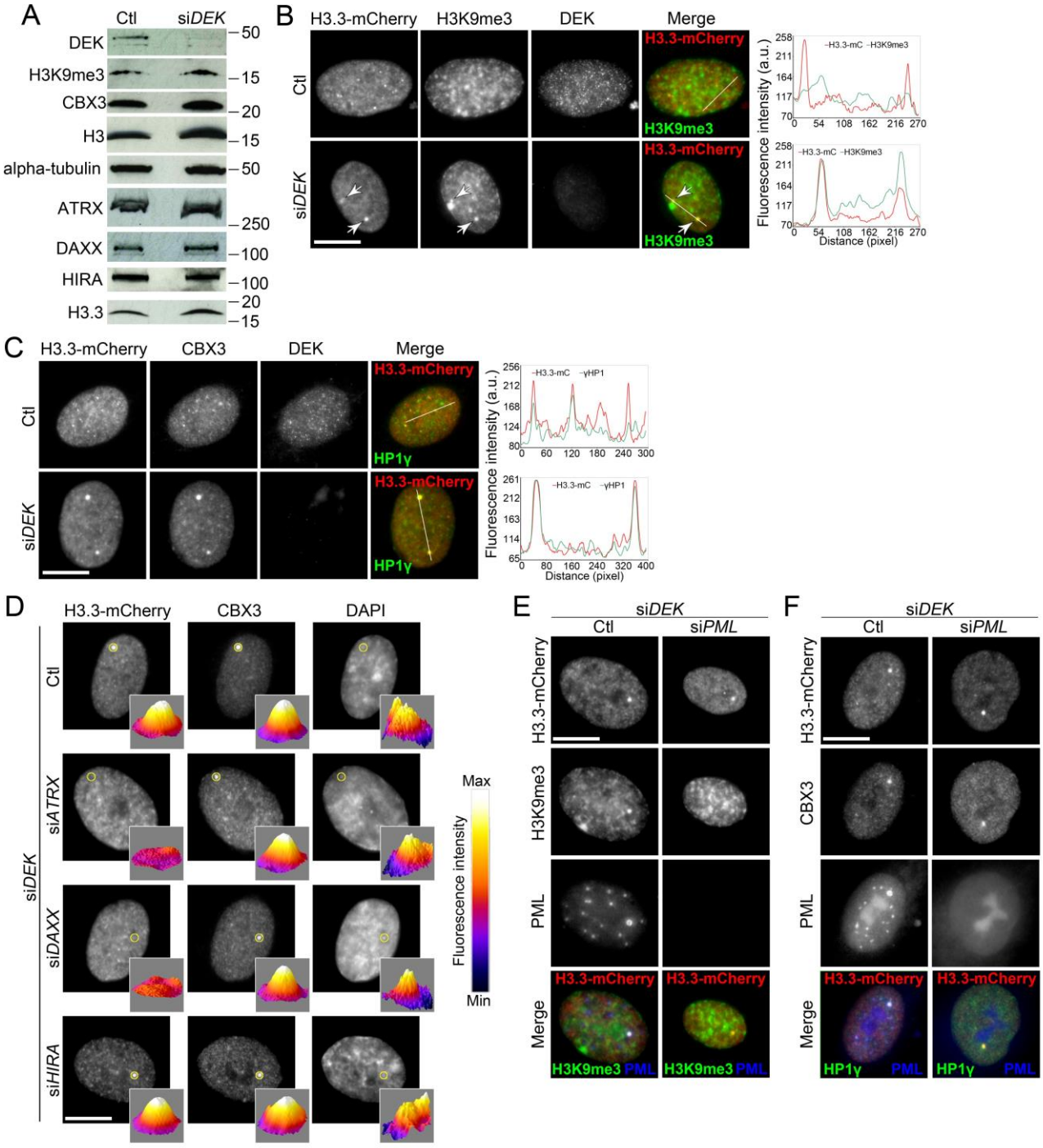
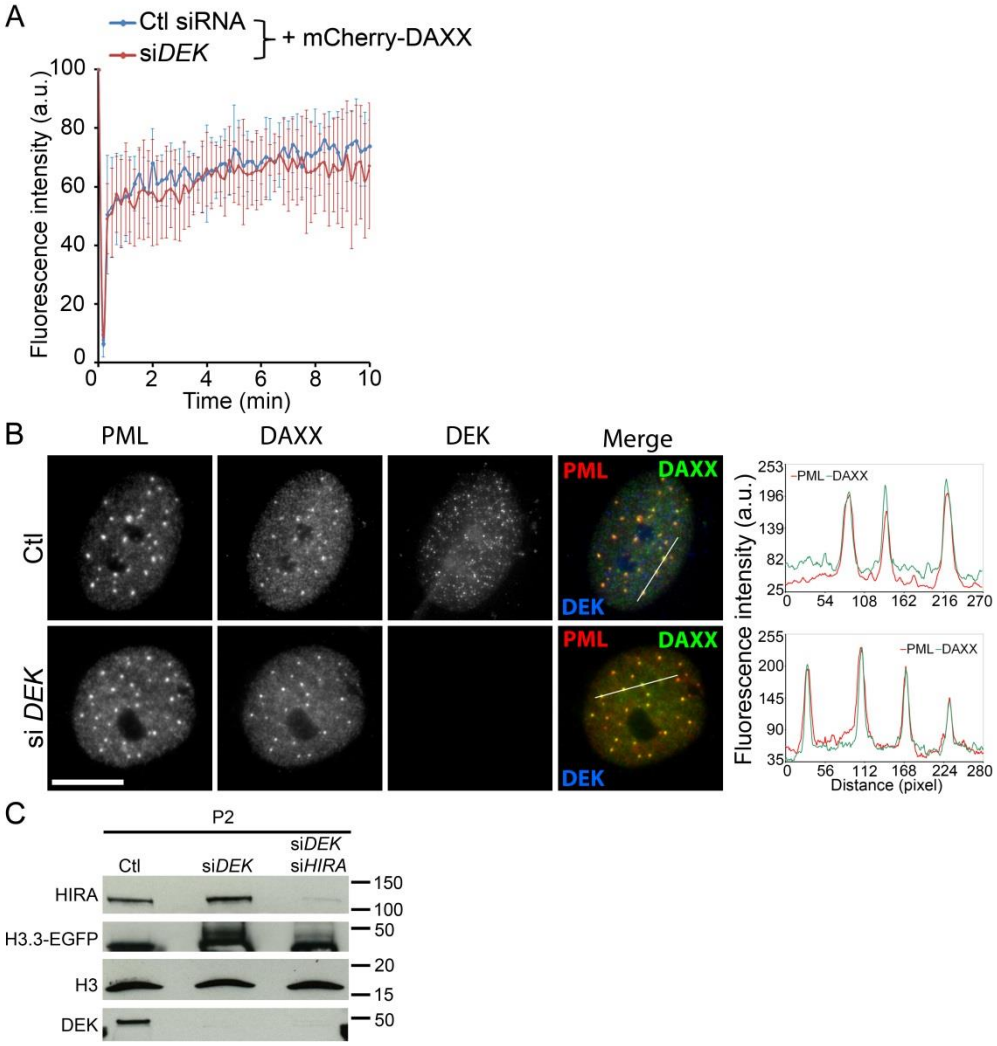


Supplemental Figure 1. DEK associates with PML NBs but not with telomeres or centromeres in human MSCs. (A) Example of co-localization of DEK with endogenous PML NBs along the traced line. A total of 40 cells were analyzed. DEK co-localizes with 60.2% (±18) of PML NBs. (B) Immunofluorescence localization of DEK, TERF2 (telomere marker) and CENPA (centromere marker). Bars, 10 µm.

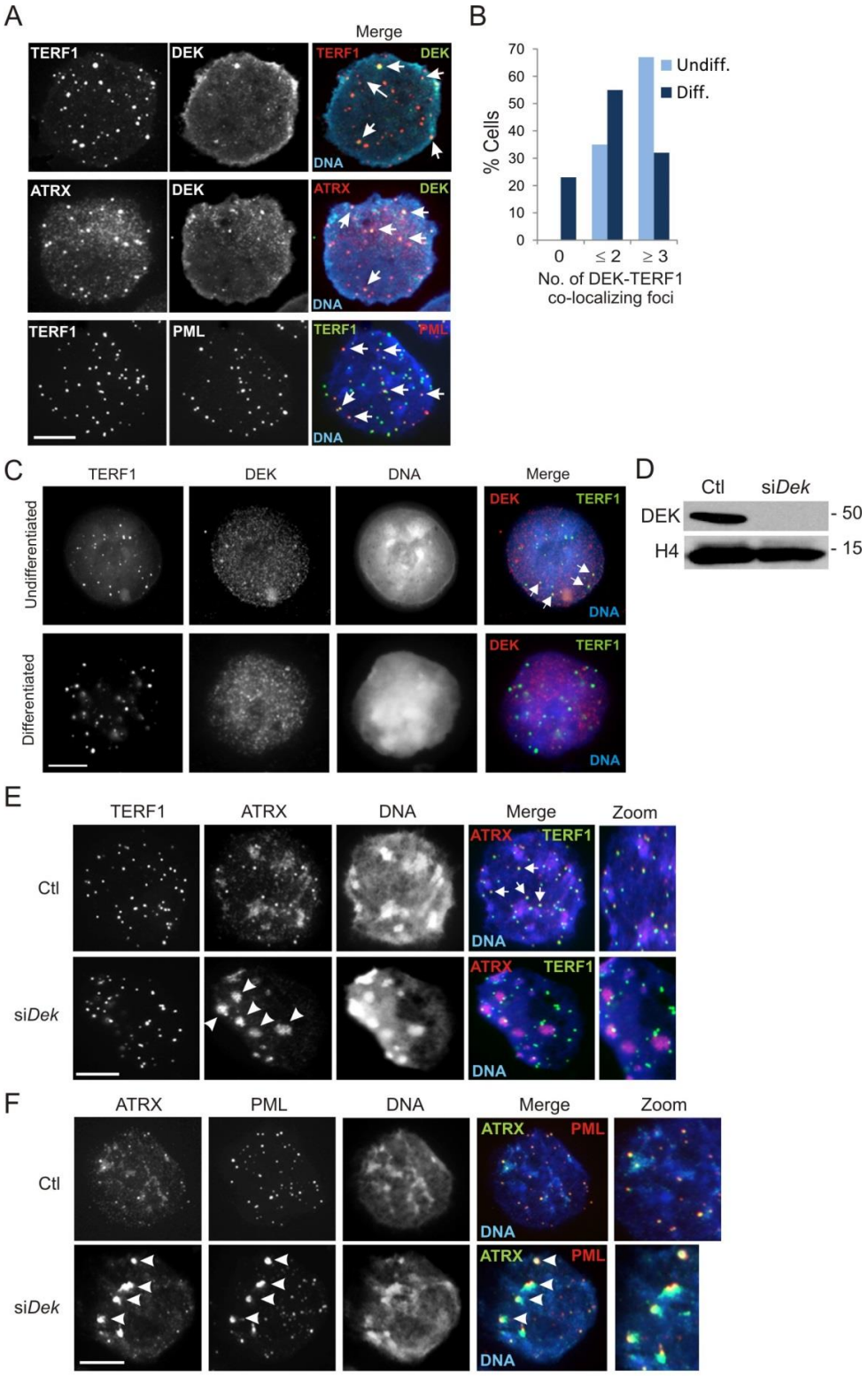


Supplemental Figure 2. DEK depletion in MSCs results in heterochromatin foci enriched in H3K9me3 and CBX3, and in DAXX/ATRX-dependent enrichment of H3.3 at these sites. (A) Western blot analysis of indicated proteins after DEK knock-down by siRNA; Ctl, control siRNA. H3 and alpha-tubulin were used as loading controls. (B) Localization of H3.3-mCherry, H3K9me3 and DEK in control and DEK-depleted cells, 48 h after transfection of H3.3-mC. DEK was knocked-down for 4 days by siRNA, at which time H3.3-mC was transfected concomitantly with a

second round of DEK knock-down. Note the large H3.3-mC foci coinciding with H3K9me3 masses after DEK depletion (arrows). Line graph representations of co-localizations are shown. (C) Localization of H3.3-mC, CBX3 and DEK in control and DEK-depleted cells, 48 h after transfection of H3.3-mC. (D) Three-dimensional representation of H3.3-mC enrichment at CBX3-containing DAPI-dense foci in DEK-depleted cells. Cells were also treated with siRNAs to *ATRX*, *DAXX* or *HIRA*, or with a control siRNA (Ctl). Circles delineate the quantified areas. Knock-down of ATRX or DAXX, but not HIRA, reduces H3.3-mC enrichment at these sites. The gray level scale (arbitrary units) is identical within the marks examined. (E) Localization of H3.3-mCherry, H3K9me3 and PML in DEK-depleted cells also depleted of PML. Note H3.3-mC enrichment at H3K9me3 masses even after PML depletion (arrow). (F) Cells as in (E) were labeled with antibodies to CBX3. Bars, 10 µm.

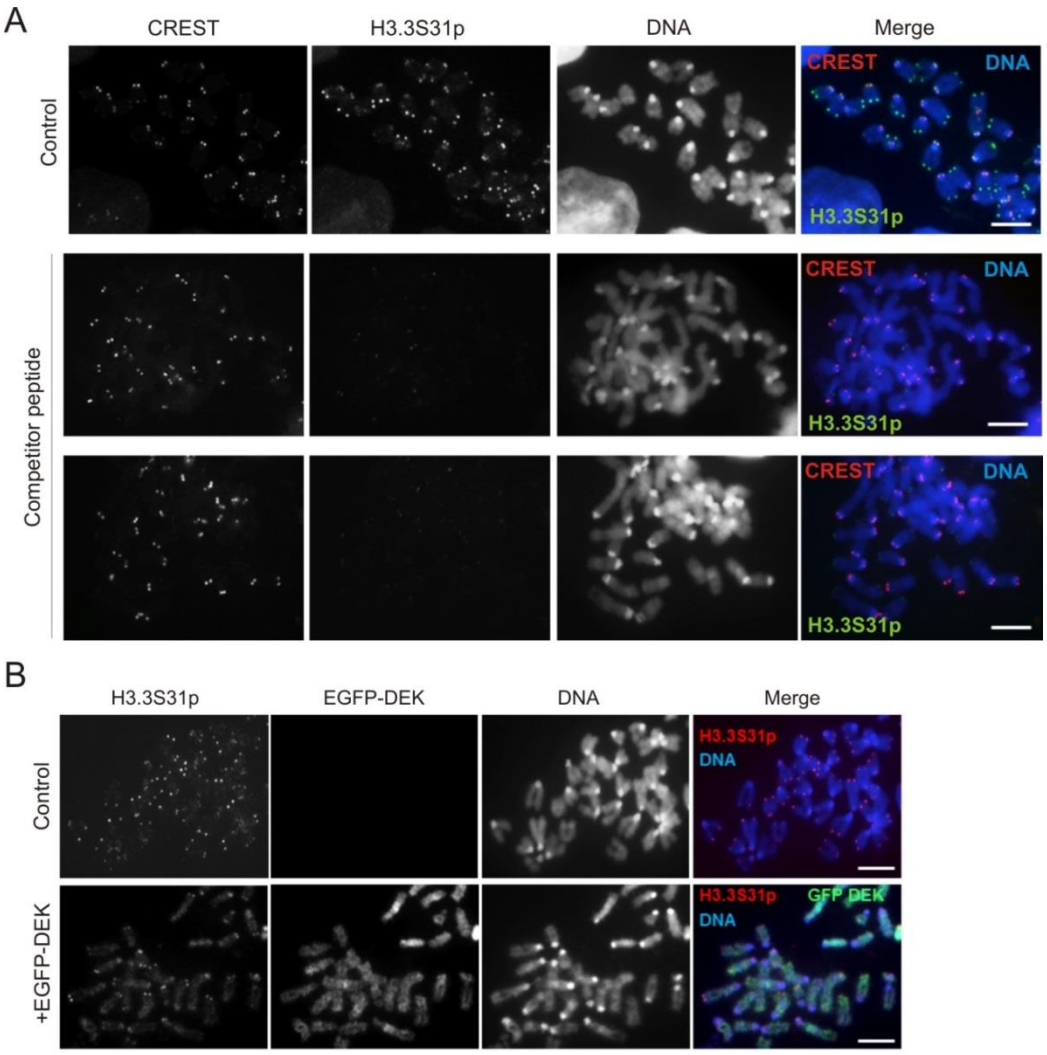


Supplemental Figure 3. The DAXX-dependent pathway of H3.3 targeting to PML NBs is intact in DEK-depleted cells. (A) FRAP analysis of H3.3-EGFP at PML NBs after overexpression of mCherry-DAXX, in control and DEK-depleted cells. (B) DEK depletion maintains DAXX at PML NBs: immunofluorescence localization of PML, DAXX and DEK in control and DEK-depleted cells. Bar, 10 µm. (C) Immunoblot of the MNase-insoluble (P2) fraction resulting from cell fractionation (see Fig. 3C for immunoblots of the other fractions).

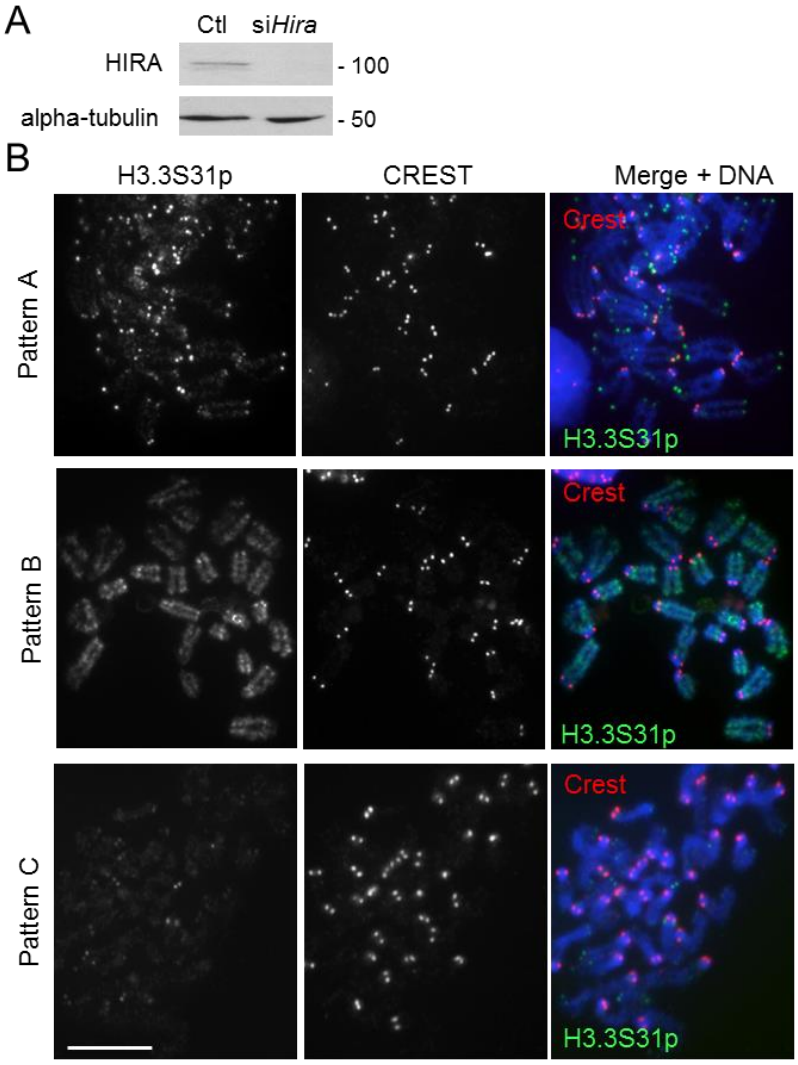


Supplemental Figure 4. Loss of DEK in ESCs leads to dissociation of PML and ATRX from telomeres. (A) Immunofluorescence localization of TERF1, DEK, ATRX and PML in mouse ESCs. Arrows point to examples of co-localizations. (B) Percentage of undifferentiated and differentiated

ESCs with 0, ≤ 2 or ≥ 3 telomeres co-localizing with DEK. (C) Immunofluorescence localization of TERF1 and DEK in undifferentiated and differentiated ESCs. Differentiation was induced by removal of leukemia inhibitory factor and addition of retinoic acid. (D) Western blot assessment of DEK knock-down by siRNA. Histone H4 was used as loading control. (E) Immunolocalization of TERF1 and ATRX in control and DEK-depleted cells. Arrows in Ctl cells point to co-localization of TERF1 and ATRX; arrowheads in si*Dek* panels point to ATRX enrichment in chromocenters. (F) Immunolocalization of ATRX and PML in control and DEK-depleted cells. Arrowheads point to enhanced PML and ATRX labeling in chromocenters. Bars, 5 μm.



Supplemental Figure 5. H3.3S31p detection in a peptide competition assay and after overexpression of EGFP-DEK. (A) Specificity of anti-H3.3S31p antibodies used in this study, assessed by peptide competition in immunofluorescence analysis of ESC metaphase spreads using a peptide containing phosphorylated S31 of H3.3. CREST was labeled to mark centromeres. Results show that the antibody is specific. (B) Overexpression of EGFP-DEK does not elicit a significant increase in H3.3S31p on mitotic chromosomes. Bars, 5 μm.



Supplemental Figure 6. DEK depletion in ESCs reduces H3.3 loading on telomeres and leads to HIRA-dependent relocation of H3.3 on chromosome arms. (A) Western blot assessment of HIRA knock-down by siRNA in ESCs. (B) Representation of H3.3S31p immunostaining patterns (Patterns A-C) on mitotic chromosome spreads. CREST is labeled as centromere marker. Bar, 5 μm.