DAXX-dependent supply of soluble (H3.3-H4) dimers to PML bodies pending deposition into chromatin

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Supplemental information

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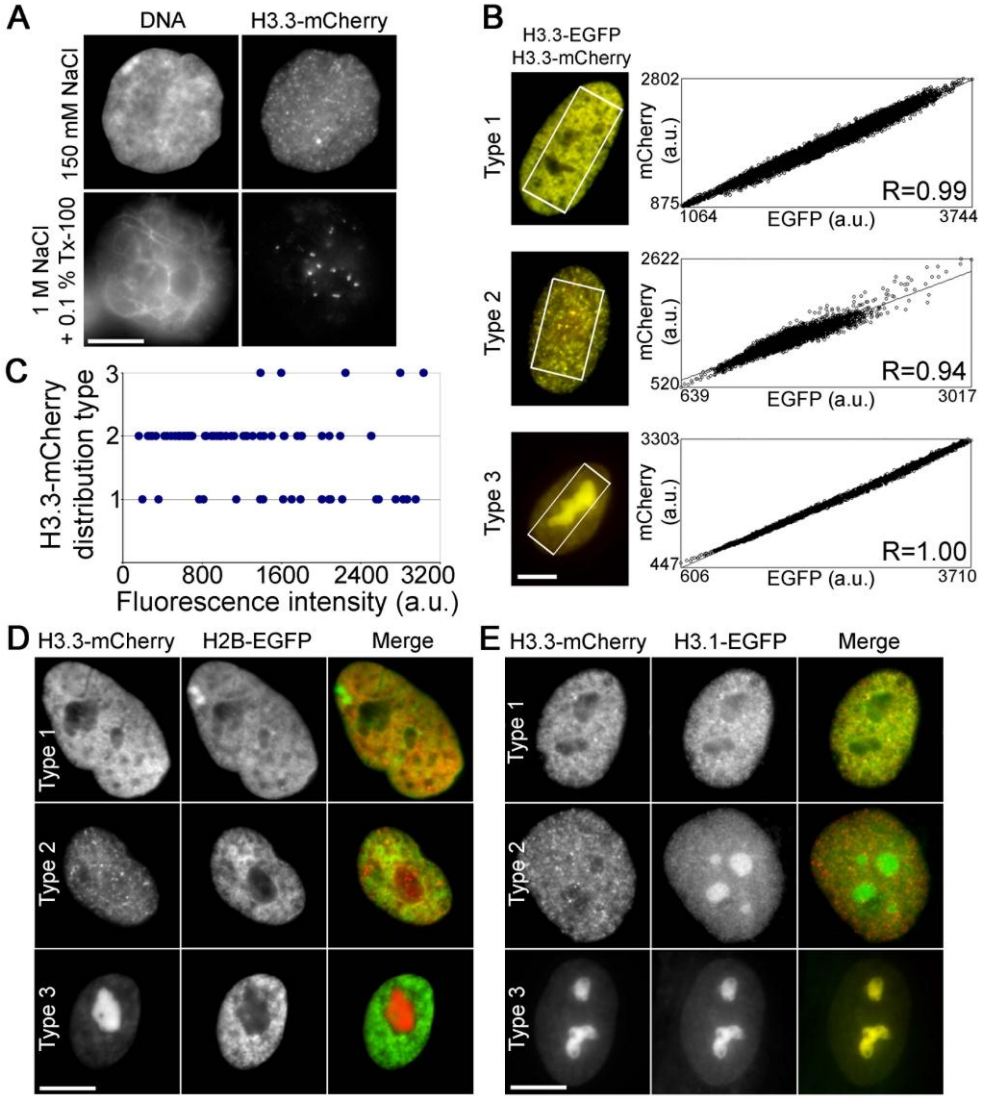
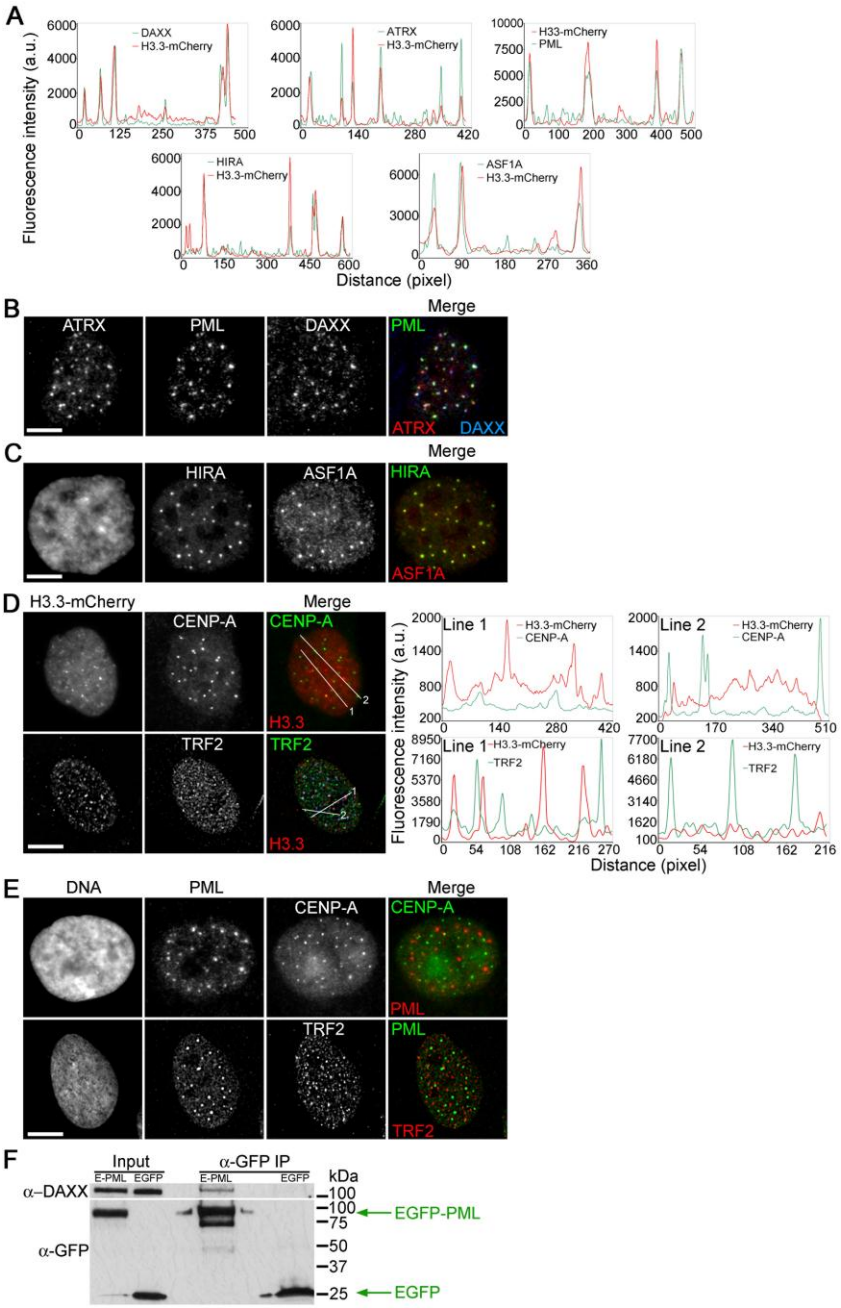
Supplemental Movie

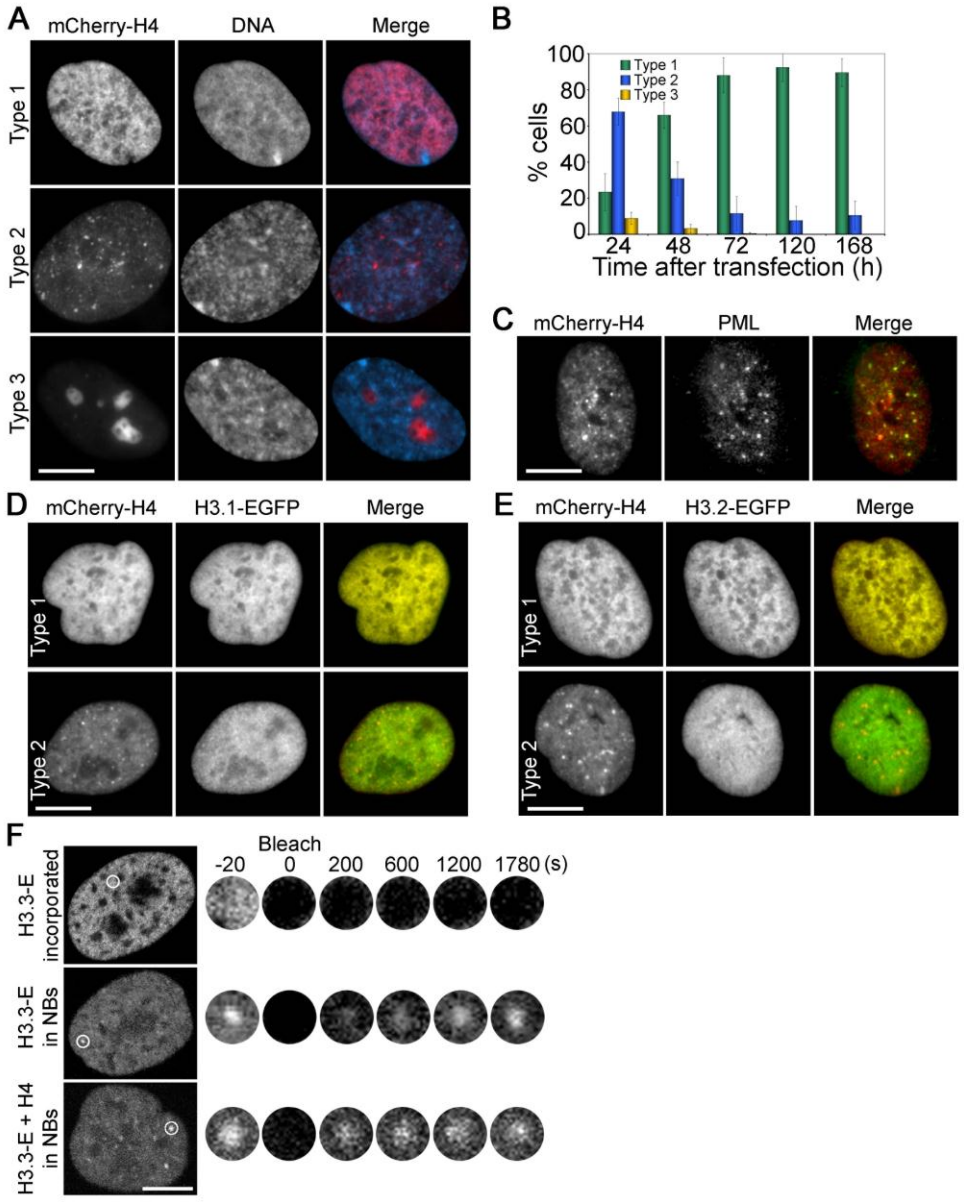
Supplemental Movie 1. Time-lapse video imaging of H3.3-mC transiently expressed in MSCs. Video recording was started 24 h after transfection and pictures taken every 20 min for 37 h.



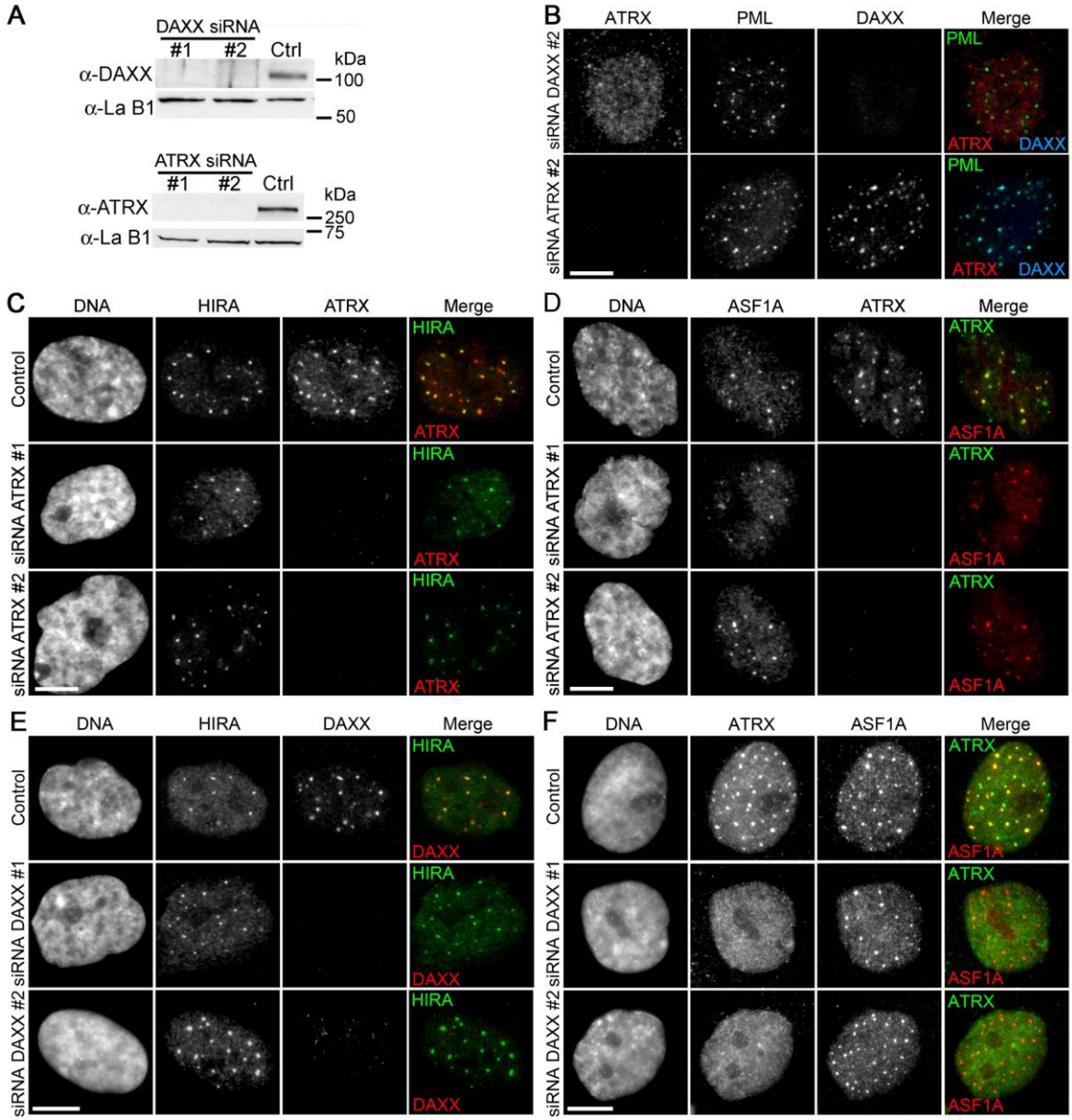
Supplemental Figure 1. Intranuclear localization of epitope-tagged H3.3 and canonical histones in MSCs. (A) H3.3-mC nuclear bodies are resistant to in situ extraction with Triton X-100 and high salt:Cells were extracted on coverslips with PBS (150 mM NaCl; control) or with 0.1% Triton-X100 and 1 M NaCl for 20 min in PBS before fixation. Note the persistence of H3.3-mC NBs after extraction. (B) Correlation analysis of fluorescence overlap between co-expressed H3.3-mC and H3.3-EGFP, for type 1, 2 and 3 distribution patterns. Scatter plots show for the cell on the left (boxed area) the correlation between green pixel intensity (x-coordinate) and red pixel intensity (y-coordinate); R, correlation coefficient. (C) Distribution pattern of tagged H3.3 is not dependent on H3.3-mC expression level: distribution of indicated H3.3-mC patterns as a function of expression level (fluorescence intensity; a.u., arbitrary units) 48 h after transfection. (D) Intranuclear localization of H3.3-mC and H2B-EGFP 24 h after co-transfection. (E) Intranuclear localization of H3.3-mC and H3.1-EGFP 24 h after co-transfection. Scale bars, 10 µm.



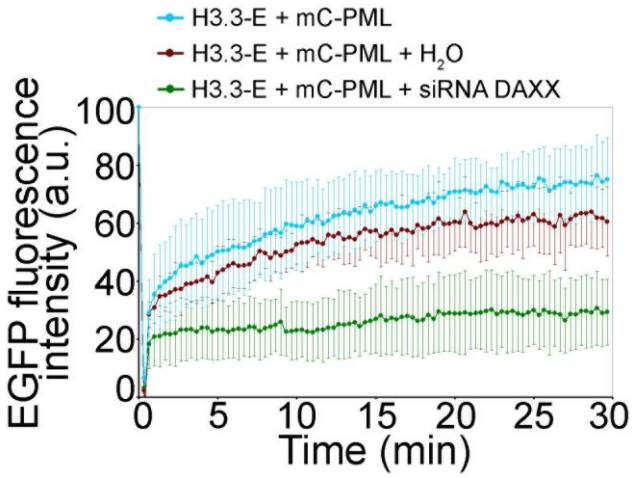
Supplemental Figure 2. Quantitative analysis of co-localization of H3.3-Cherry with H3.3 chaperones and centromeric and telomeric landmarks. (A) Fluorescence intensities of immunolabeled DAXX, ATRX, PML, HIRA and ASF1A (green lines) together with H3.3-mC (red lines), along regions delineated in Figure 2A. (B) Immunolocalization of ATRX, PML and DAXX in non-transfected cells. (C) Immunolocalization of HIRA and ASF1A in non-transfected cells (left panel, DNA stained with DAPI). (D) Intranuclear immunolocalization of CENPA and TRF2 in cells expressing H3.3-mC (24 h after transfection). White lines 1 and 2 (Merge) delineate regions of fluorescence quantified on the right. (E) Immunolocalization of PML together with CENPA or TRF2. Note the lack of co-localization. Images in lower panels are deconvoluted. Scale bars, 7 µm. (F) Immunoblot analysis of DAXX detection in GFP-Trap® pull-downs of EGFP-PML, in cells expressed EGFP-PML (E-PML) or EGFP alone (EGFP).



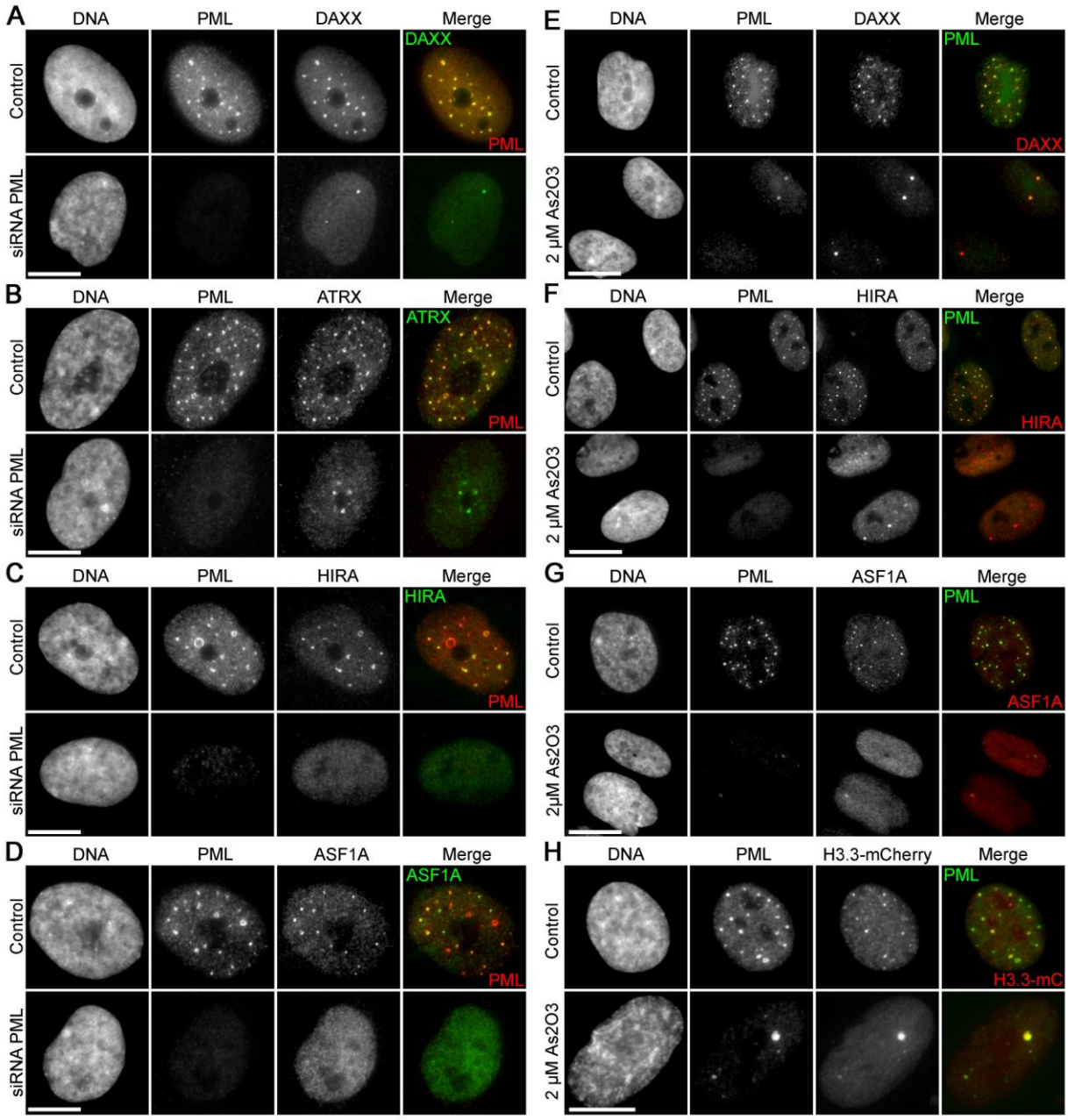
Supplemental Figure 3. Characterization of intranuclear localization of mCherry-H4. (A) Intranuclear localization of mC-H4 24 h after transfection. (B) Time-course distribution of mC-H4 patterns shown in (A) (mean ± SD of 3 experiments; >120 cells per experiment). (C) Co-localization of mC-H4 and immunolabeled PML 24 h after mC-H4 transfection. (D) Intranuclear localization of mC-H4 and H3.1-EGFP 24 h after co-transfection. (E) Intranuclear localization of mC-H4 and H3.2-EGFP 24 h after co-transfection. (F) Time-lapse images of H3.3-EGFP FRAP analysis under indicated conditions (left), 20 s before bleach, during bleaching (0) and during fluorescence recovery, in the bleached area circled on the left images. One representative cell for each condition is shown. Scale bars, 10 µm.



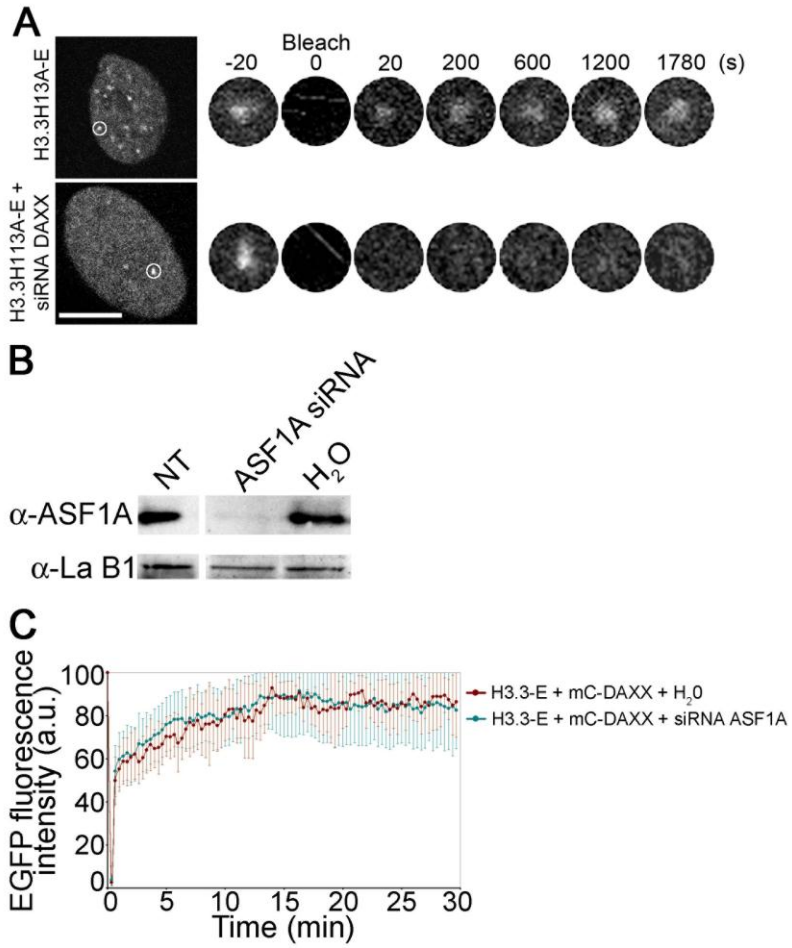
Supplemental Figure 4. DAXX down-regulation delocalizes ATRX from PML bodies but does not affect HIRA or ASF1A. (A) Immunoblot analysis of DAXX and ATRX down-regulation using two different siRNA oligonucleotides. Lamin B1 is shown as loading control. (B) Immunolocalization of ATRX, PML and DAXX after down-regulation of DAXX (top; siRNA #2) or ATRX (bottom; siRNA #2). Data with siRNAs #1 are shown in Figure 4B. (C, D) ATRX down-regulation using two different siRNA oligonucleotides maintains localization of HIRA (C) or ASF1A (D) at NBs. HIRA, ATRX and ASF1A were immunolabeled. (E) DAXX down-regulation using two different siRNA oligonucleotides maintains HIRA localization at NBs. HIRA and DAXX were immunolabeled. (F) DAXX down-regulation with two different siRNA oligonucleotides (left) maintains ASF1A at NBs despite de-localization of ATRX. ATRX and ASF1A were immunolabeled. Scale bars, 10 μm.



Supplemental Figure 5. FRAP analysis of H3-3-EGFP localized at PML bodies marked by mCherry-PML. FRAP analysis of H3.3-EGFP expressed at PML bodies detected by expression of mC-PML, with or without siRNA-mediated DAXX down-regulation (mean ± SD of 6-12 cells).



Supplemental Figure 6. Down-regulation or dismantlement of PML disperses H3.3 chaperones. (A-D) siRNA-mediated down-regulation of PML disperses DAXX, ATRX, HIRA and ASF1A. PML and chaperones were detected by immunofluorescence. (E-H) Dismantlement of PML bodies with 2 µM AS_2O_3 also causes dispersion of H3.3 chaperones. Note in a few instances where PML bodies are only partially dismantled and remain detected, DAXX, ASF1A and H3.3-mC also co-localize with PML. Scale bars, 10µm.



Supplemental Figure 7. Targeting of H3.3(H113A)-EGFP to PML bodies is mediated by DAXX. (A) Time-lapse images of H3.3(H113A)-EGFP FRAP analysis without or with siRNA-mediated DAXX down-regulation, 20 s before bleach, upon bleaching (0) and during fluorescence recovery, in the area circled on left images. Note the fluorescence recovery of H3.3(H113A)-EGFP both in foci and in the surrounding soluble pool (top row), whereas only the soluble fraction recovers after DAXX down-regulation (bottom row). (B) Western blot analysis of ASF1A knock-down by siRNA in non-transfected cells (NT) and cells expressing H3.3-EGFP (right two lanes). H_2O, sham-depleted cells. (C) FRAP analysis of H3.3-EGFP in cells expressing mC-DAXX, with or without siRNA-mediated ASF1A knock-down (mean ± SD of 9-16 cells).