

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

Erwan Delbarre, Kristina Ivanauskiene, Thomas Küntziger, and Philippe Collas

### **Supplemental information**

#### **Supplemental Figures**

**Supplemental Figure 1.** Intranuclear localization of epitope-tagged H3.3 and canonical histones in MSCs.

**Supplemental Figure 2.** Quantitative analysis of co-localization of H3.3-Cherry with H3.3 chaperones and centromeric and telomeric landmarks.

**Supplemental Figure 3.** Characterization of intranuclear localization of mCherry-H4.

**Supplemental Figure 4.** DAXX down-regulation delocalizes ATRX from PML bodies but does not affect HIRA or ASF1A.

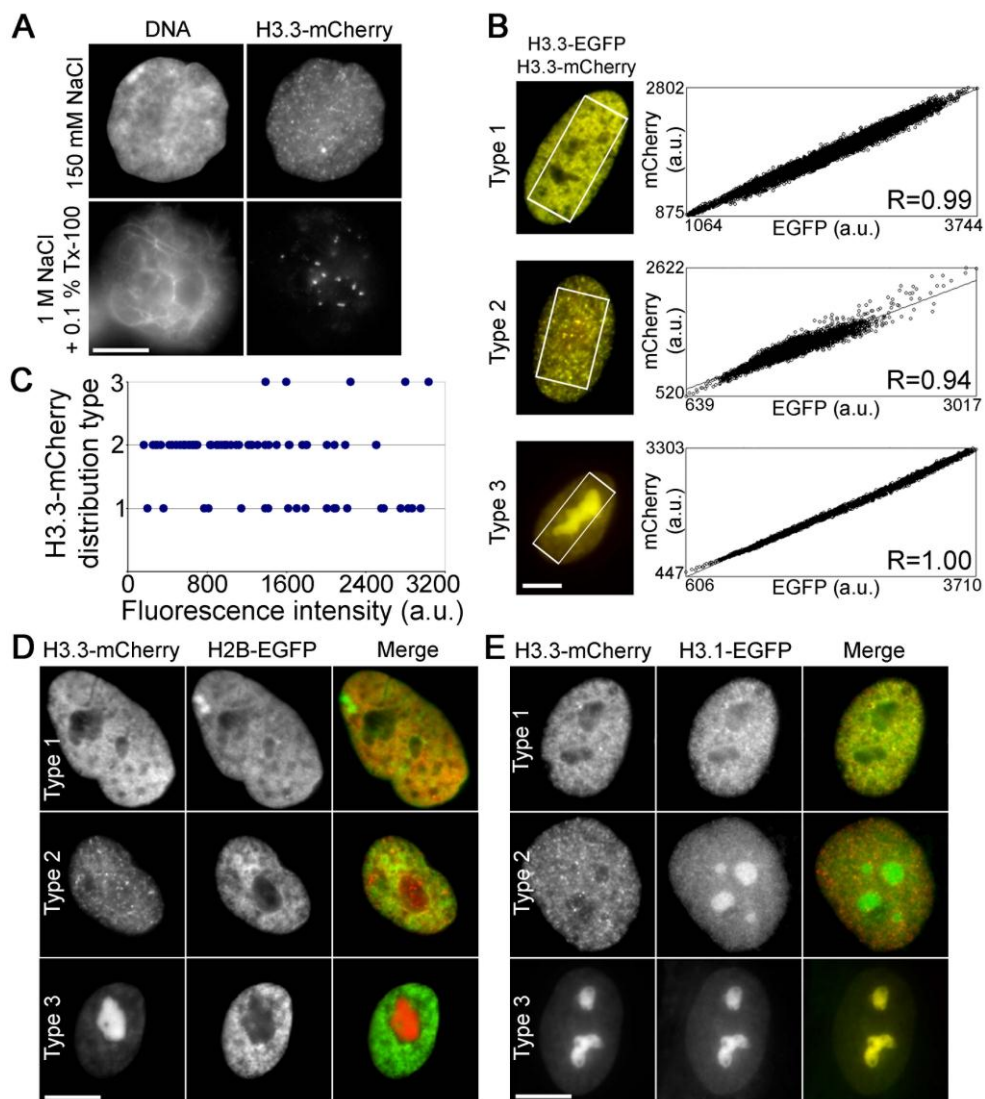
**Supplemental Figure 5.** FRAP analysis of H3.3-EGFP localized at PML bodies marked by mCherry-PML.

**Supplemental Figure 6.** Down-regulation or dismantlement of PML disperses H3.3 chaperones.

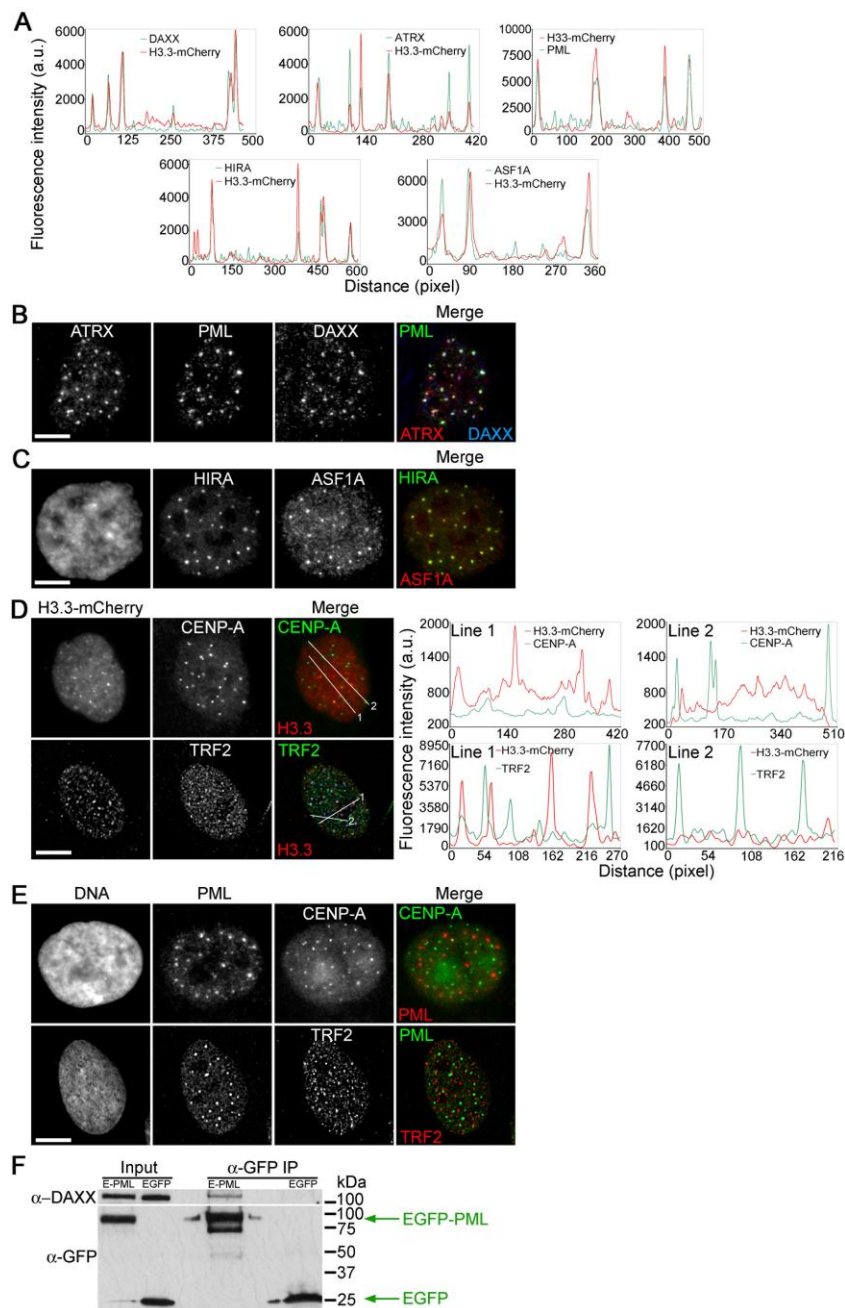
**Supplemental Figure 7.** Targeting of H3.3(H113A)-EGFP to PML bodies is mediated by DAXX.

#### **Supplemental Movie**

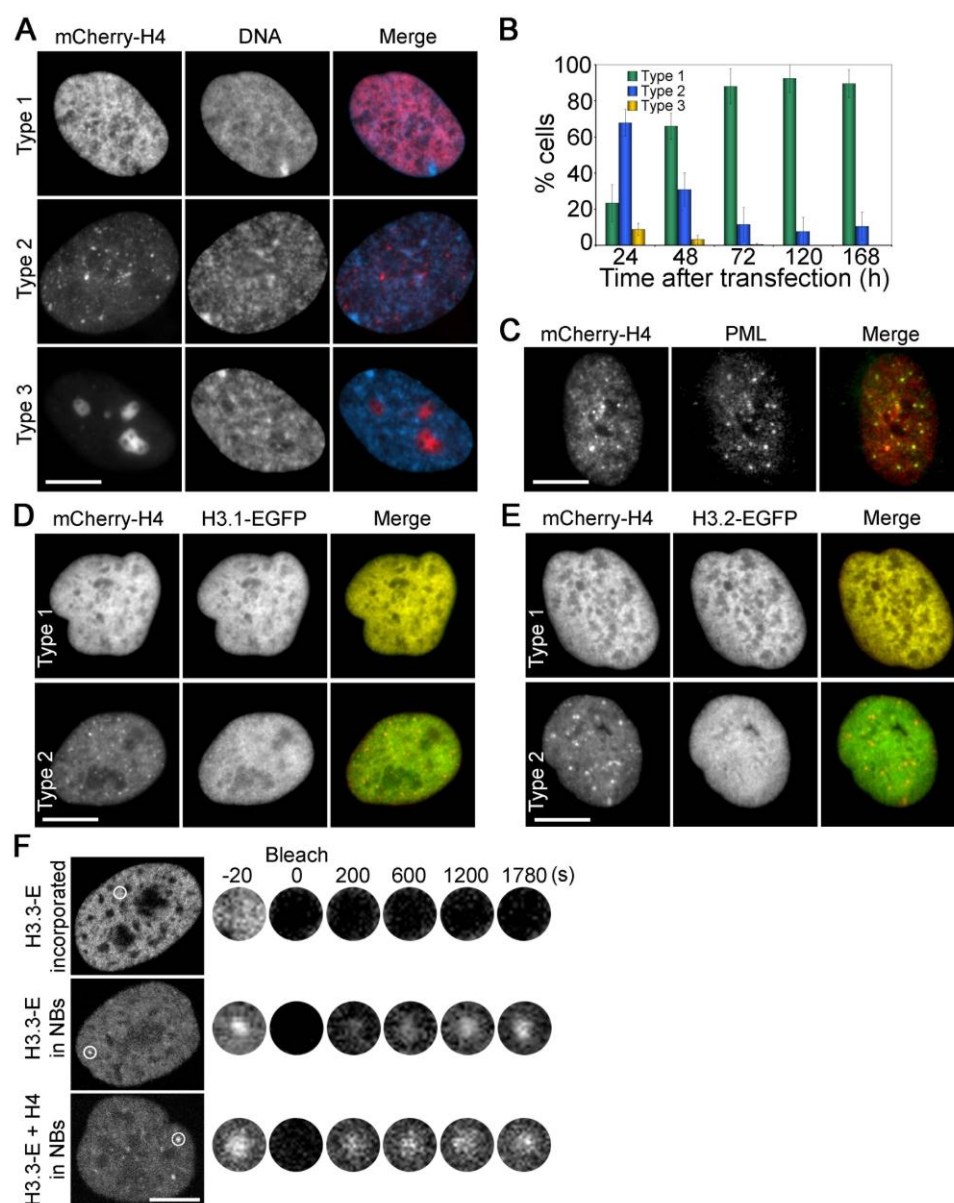
**Supplemental Movie 1.** Time-lapse video imaging of H3.3-mC transiently expressed in MSCs. Video recording was started 24 h after transfection and pictures taken every 20 min for 37 h.



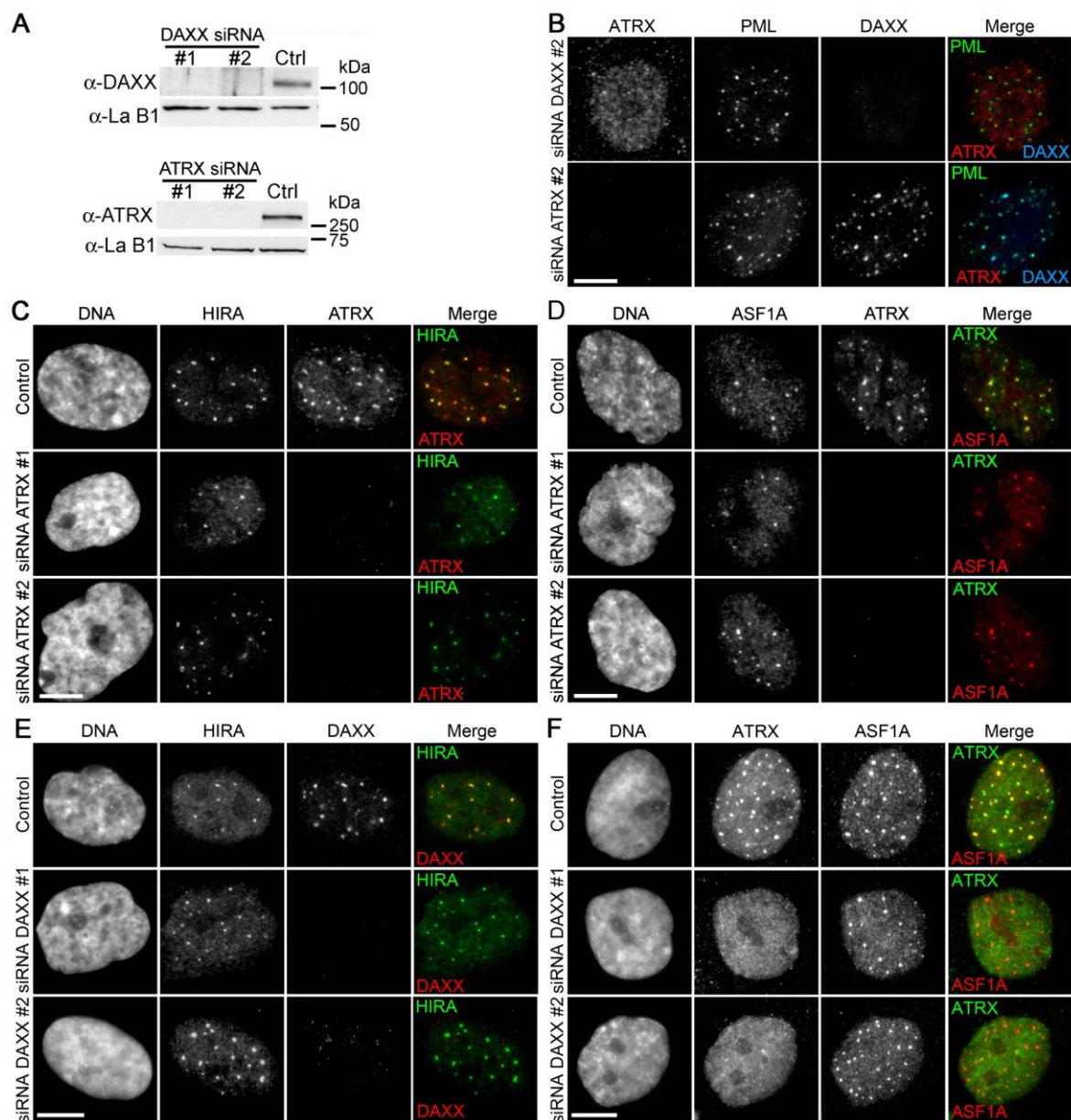
**Supplemental Figure 1.** Intranuclear localization of epitope-tagged H3.3 and canonical histones in MSCs. (A) H3.3-mC nuclear bodies are resistant to in situ extraction with Triton X-100 and high salt: Cells were extracted on coverslips with PBS (150 mM NaCl; control) or with 0.1% Triton-X100 and 1 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  $\mu$ m.



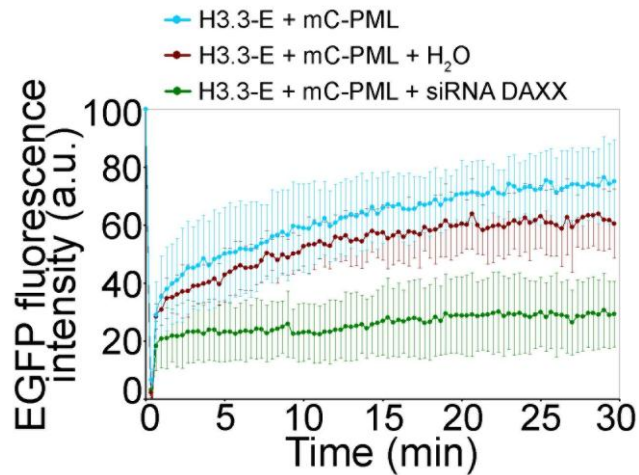
**Supplemental Figure 2.** Quantitative analysis of co-localization of H3.3-Cherry with H3.3 chaperones and centromeric and telomeric landmarks. (A) Fluorescence intensities of immunolabeled DAXX, ATRX, PML, HIRA and ASF1A (green lines) together with H3.3-mC (red lines), along regions delineated in Figure 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  $\mu$ m. (F) Immunoblot analysis of DAXX detection in GFP-Trap<sup>®</sup> pull-downs of EGFP-PML, in cells expressed EGFP-PML (E-PML) or EGFP alone (EGFP).



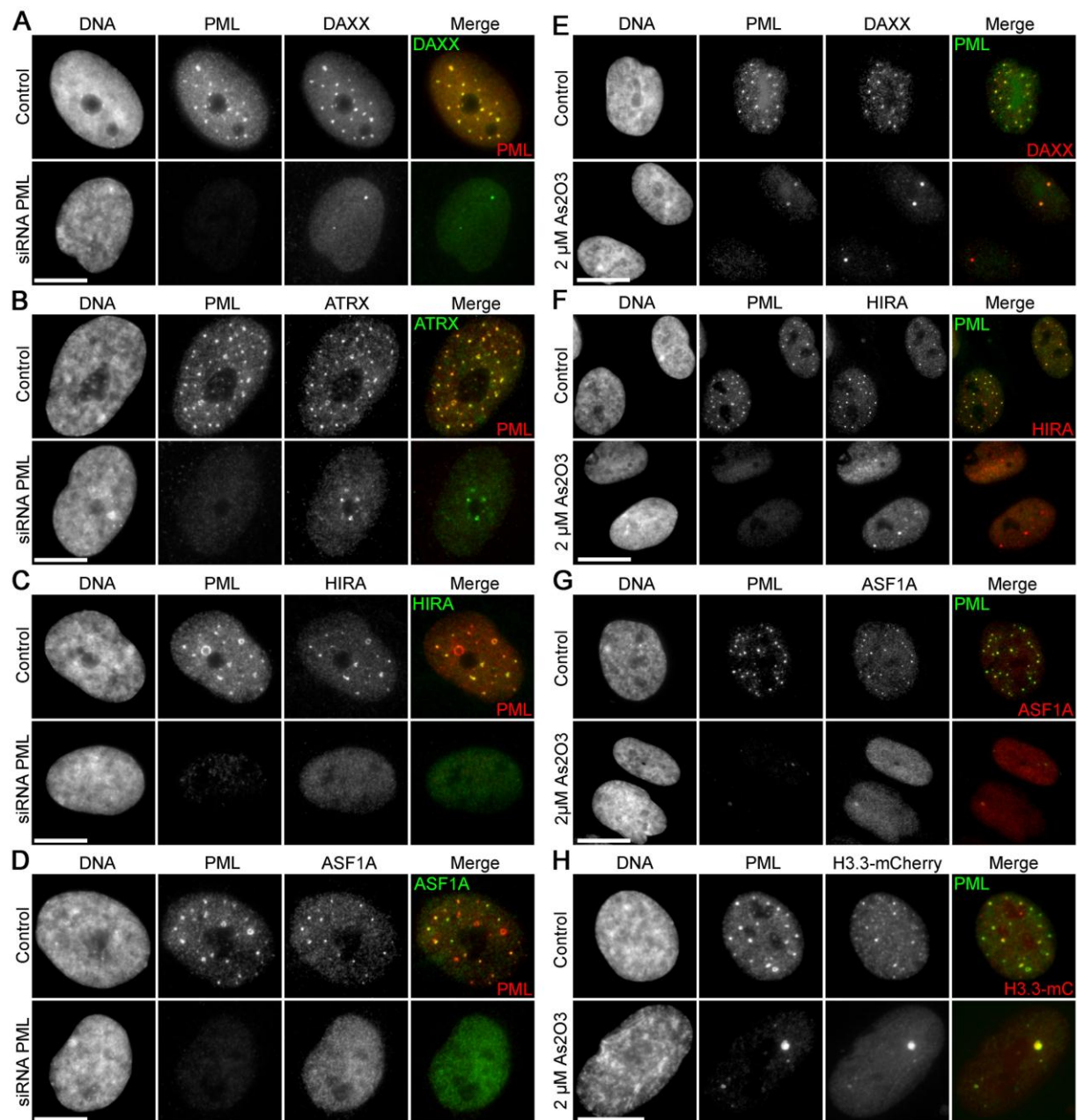
**Supplemental Figure 3.** Characterization of intranuclear localization of mCherry-H4. (A) Intranuclear localization of mC-H4 24 h after transfection. (B) Time-course distribution of mC-H4 patterns shown in (A) (mean  $\pm$  SD of 3 experiments; >120 cells per experiment). (C) Co-localization of mC-H4 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  $\mu$ m.



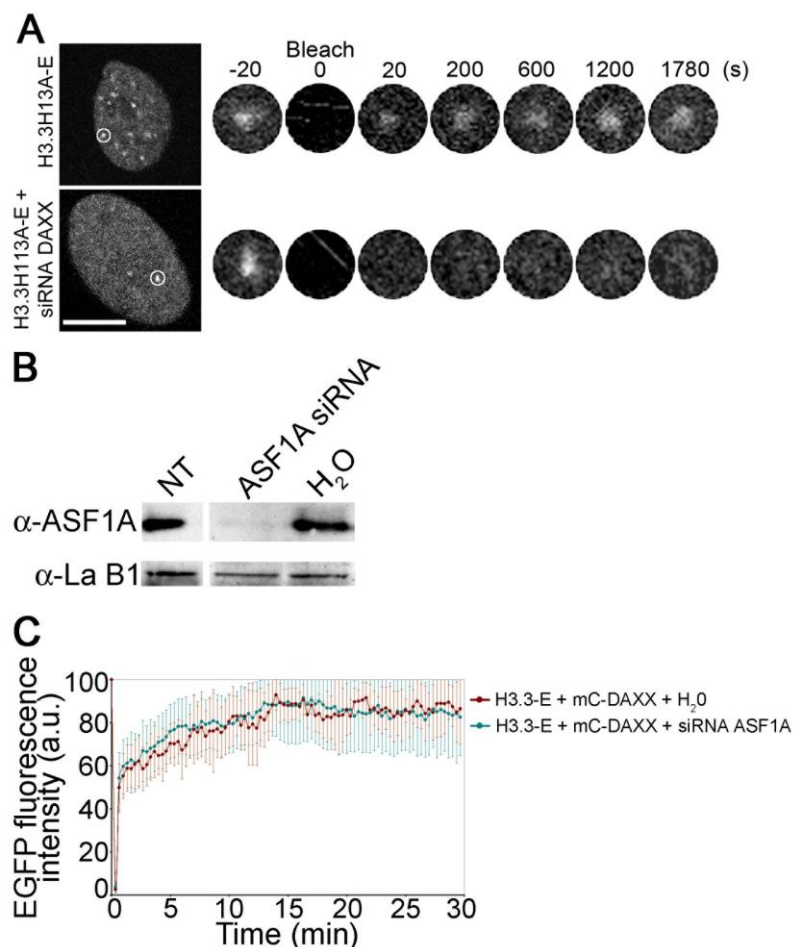
**Supplemental Figure 4.** DAXX down-regulation delocalizes ATRX from PML bodies but does not affect HIRA or ASF1A. (A) Immunoblot analysis of DAXX and ATRX down-regulation using two different siRNA oligonucleotides. Lamin B1 is shown as loading control. (B) Immunolocalization of ATRX, PML and DAXX after 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  $\pm$  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  $\mu$ M As<sub>2</sub>O<sub>3</sub> 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, 10 $\mu$ m.



**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<sub>2</sub>O, sham-depleted cells. (C) FRAP analysis of H3.3-EGFP in cells expressing mC-DAXX, with or without siRNA-mediated ASF1A knock-down (mean  $\pm$  SD of 9-16 cells).