PML protein organizes heterochromatin domains where it regulates histone H3.3 loading by ATRX/DAXX

Supplemental Information

List of Supplemental tables (in a single Excel file)

Supplemental Table 1. Genomic positioning of PADs and genes found in PADs

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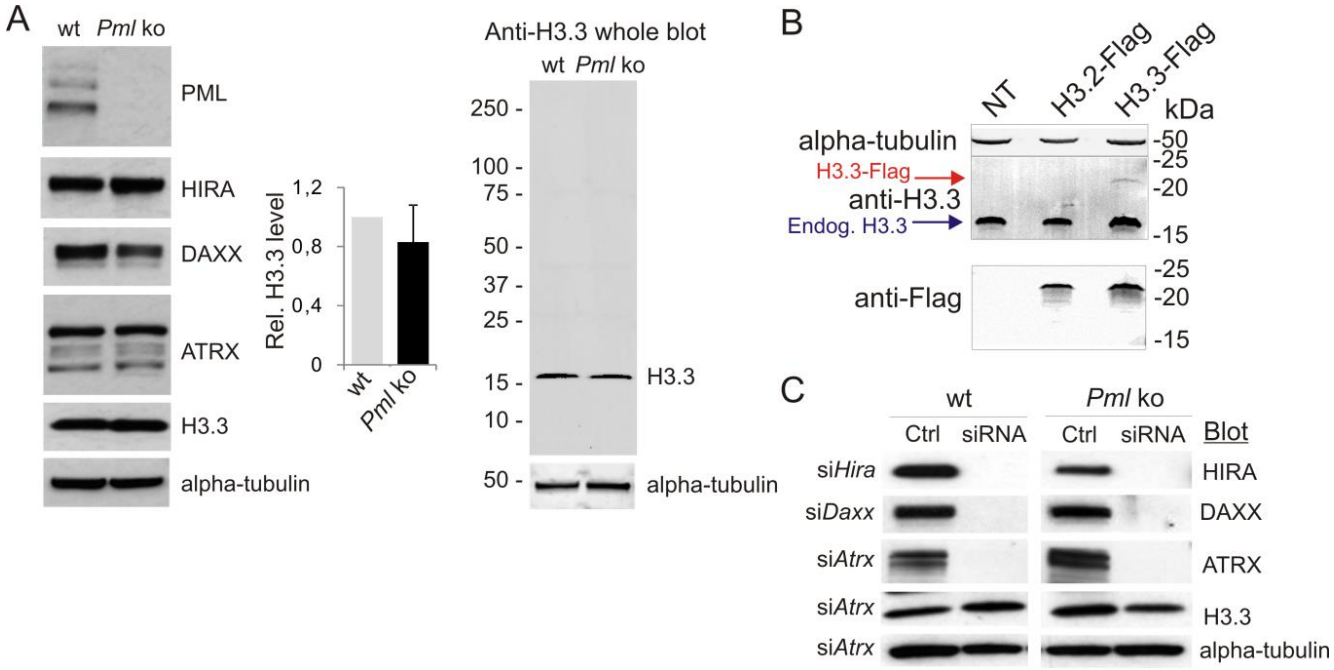
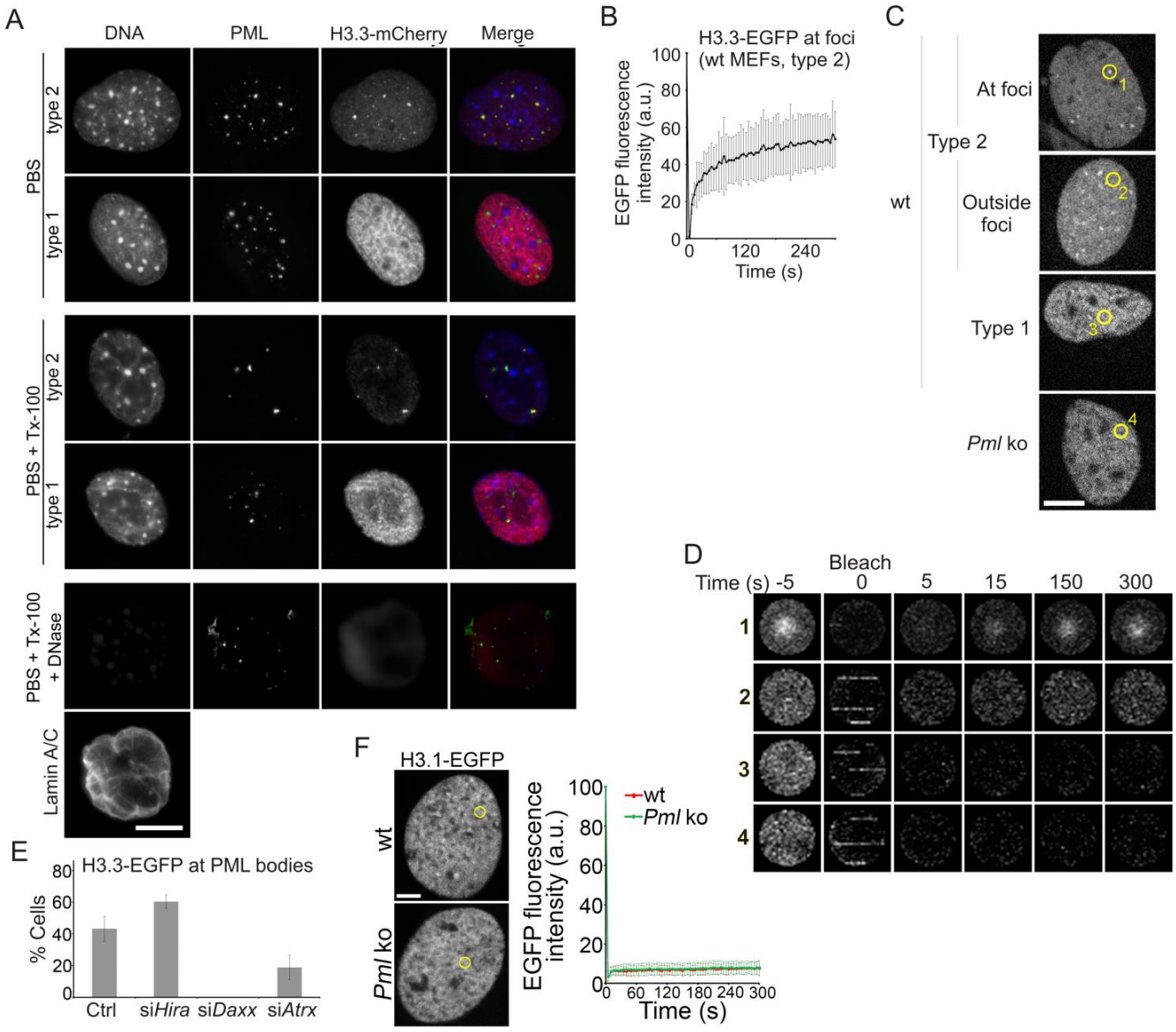
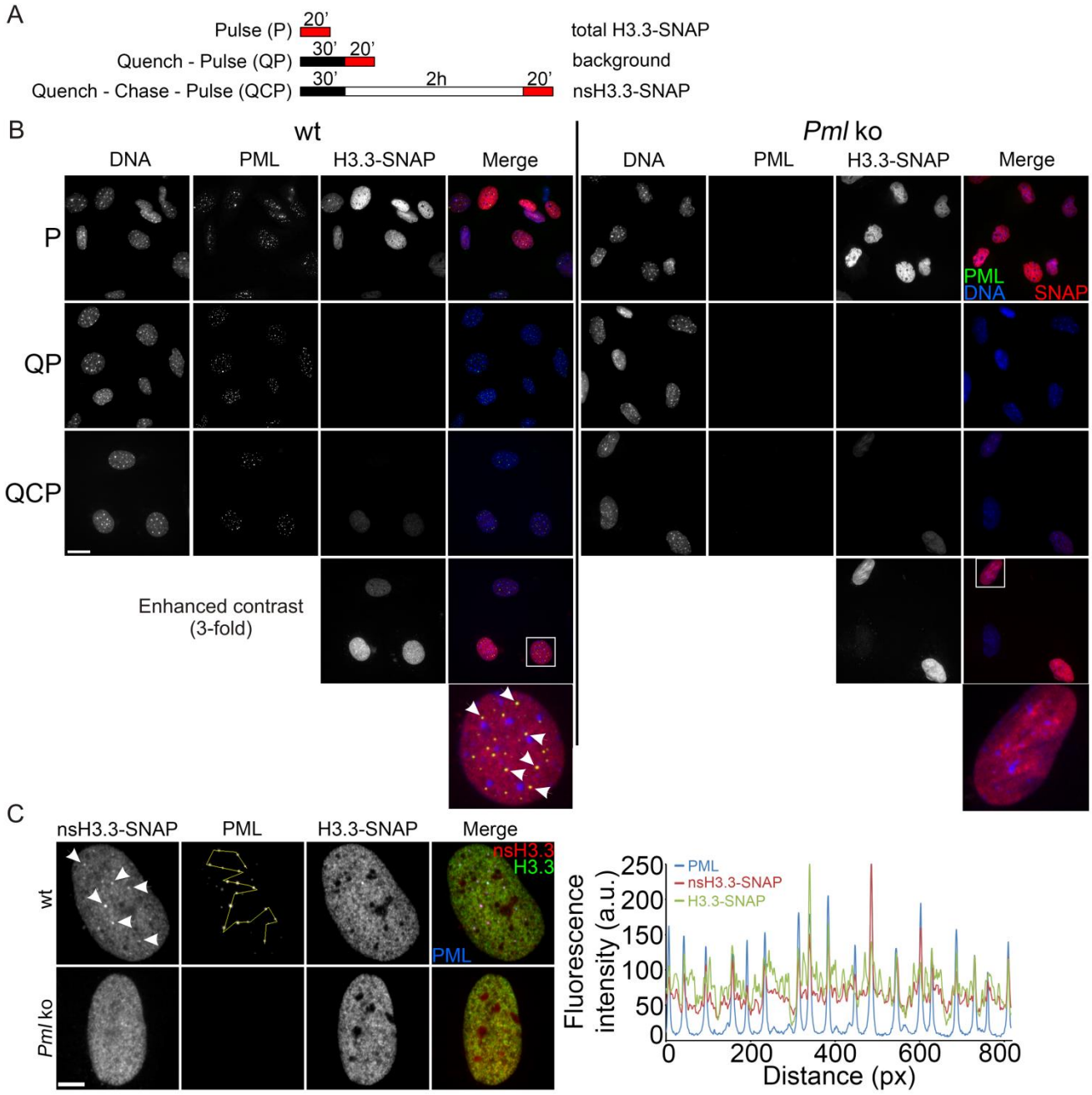
Supplemental Table 4 ChIP-qPCR primers and genomic location of amplicons



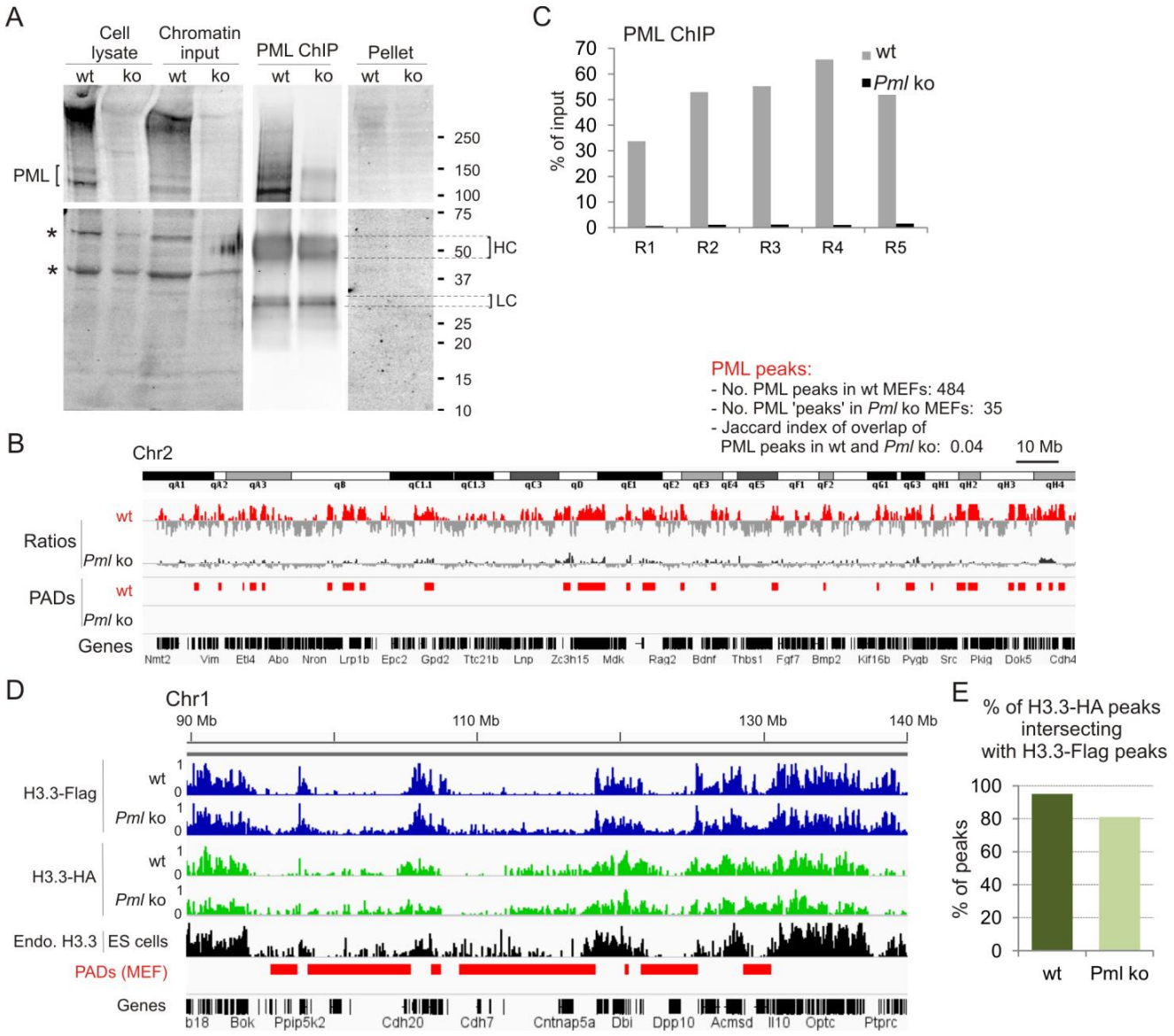
Supplemental Figure 1. Characterization of wt and *Pml* ko MEFs. (A) Western blot of HIRA, DAXX and ATRX in wt and *Pml* ko MEFs. Proportion of H3.3 in *Pml* ko relative to wt MEFs, and related to alpha-tubulin as internal reference, determined by Western blotting (mean ± s.d; 3 experiments). Right, whole-gel Western blot of endogenous H3.3. (B) Western blot analysis of anti-H3.3 antibody specificity using MEFs that were non-transfected (NT) or that expressed H3.2-Flag or H3.3-Flag. The H3.3 antibody specifically detects H3.3-Flag (red arrow) while the Flag antibody detects H3.2-Flag and H3.3-Flag (lower blot). The H3.3 antibody also detects endogenous H3.3 (blue arrow). (C) Western blot assessment of knockdowns of HIRA, DAXX and ATRX with siRNAs. This blot is representative of all blots done to verify complete knock-downs in all relevant experiments.



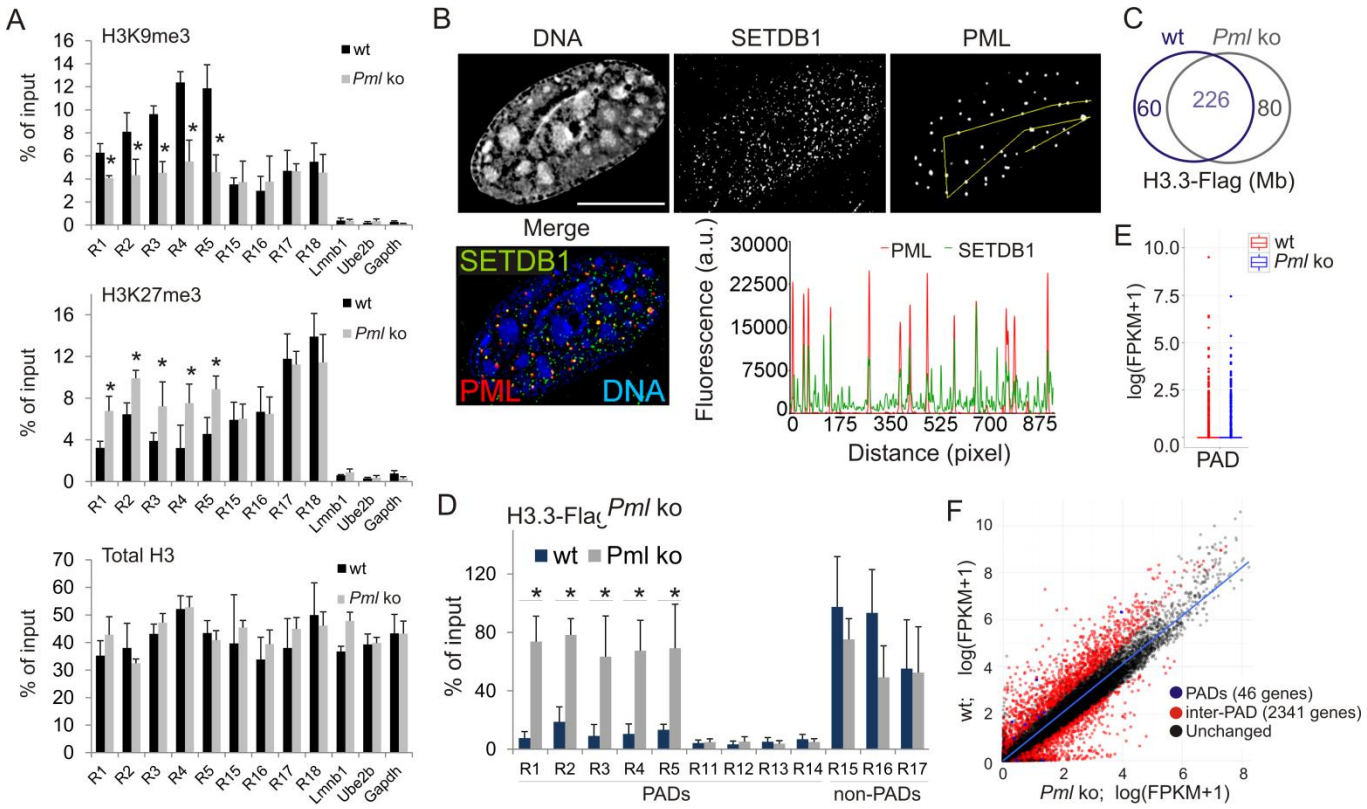
Supplemental Figure 2. Epitope-tagged H3.3 mobility and extractability in wt and *Pml* ko MEFs. (A) Epitope-tagged H3.3 at PML bodies is detergent-insoluble but DNase-sensitive. Detection of PML and H3.3-mCherry in wt MEFs treated with PBS, PBS + 0.1% Triton-X100, or PBS + 0.1% Triton-X100 + 1 mg/ml DNase I, before fixation and immunostaining. DNA was stained with DAPI. The nucleus in the lower panels was delineated by lamin A/C immunostaining. Bar, 10 µm. (B) FRAP analysis of H3.3-EGFP at foci (PML bodies) in 'type 2' wt MEFs (mean ± s.d., 21 cells). (C) Representative photobleached areas (yellow circles) in FRAP experiments. Numbering: 1, H3.3-EGFP at foci in type 2 cells; 2, H3.3-EGFPoutside foci in type 2 cells; 3, H3.3-EGFP in chromatin in type 1 cells; 4, H3.3 in chromatin in *Pml* ko cells. Bar, 10 µm. (D) Representative bleached areas in the four conditions shown in (C), imaged during fluorescence recovery. (E) Percentage of H3.3-EGFP at PML bodies in wt MEFs; mean ± s.d of 260 cells in 2 replicates. (F) FRAP analysis of H3.1-EGFP in indicated bleached areas in chromatin (yellow circles; mean ± s.d., 9-11 cells).



Supplemental Figure 3. Neo-synthesized H3.3-SNAP is targeted to PML bodies in wt MEFs. (A) Detection of neo-synthesized H3.3 using a SNAP tag (nsH3.3-SNAP). nsH3.3-SNAP is assessed after quenching the SNAP tag followed by a 2 h chase and a final pulse with a fluorescent SNAP ligand (QCP). Total H3.3-SNAP is visualized after a single pulse (P) and background associated with unbound SNAP ligand is assessed after quenching and immediately pulsing (QP). (B) Visualization of H3.3-SNAP under each condition in wt and *Pml* ko MEFs. Bar, 20 µm. nsH3.3-SNAP is detected at foci coinciding with immunolabeled PML bodies in wt MEFs in QCP condition (bottom panels, enlarged nucleus, arrows; left). (C) Detection of nsH3.3-SNAP at PML bodies, and quantification of fluorescence along the trace (graph). Bar, 5 µm.



Supplemental Figure 4. PML antibody validation for ChIP and genomic enrichment of epitope-tag H3.3. (A) Western blot of PML ChIPs from wt and *Pml* ko MEFs; pellet, insoluble fraction left after collection of input chromatin used for ChIP. *Unspecific bands. HC, Ig heavy chains and LC, light chains. (B) Browser view of PML (ChIP/input ratios; scale -0.5/+0.5) and PML peaks (PADs) in wt and *Pml* ko MEFs. Overlap information between peaks of PML in wt and *Pml* ko MEFs is provided. (C) ChIP-qPCR of PML in PAD sites (R1-R5) in wt and *Pml* ko MEFs. See Supplemental Table 4 for position of amplicons. (D) Browser views of H3.3-Flag and H3.3-HA in wt and *Pml* ko MEFs, and of endogenous H3.3 ChIPed from mouse ES cells (Elsässer et al. 2015, Nature 522, 240-244). ES cell data were from GEO GSE59189, tracks GSM1429905 (ChIP) and GSM1429901 (input). (E) Percent of H3.3-Flag peaks intersecting with H3.3-HA peaks in wt and *Pml* ko MEFs.



Supplemental Figure 5. Chromatin modifications in PADs. (A) ChIP-qPCR analysis of H3K9me3, H3K27me3 and total H3 in indicated regions in wt and *Pml* ko MEFs; *P < 0.05; Fisher's exact test; mean ± s.d., 3 experiments. (B) Immunofluorescence co-detection of SETDB1 and PML in wt MEFs, and measurement of pixel overlap in a representative nucleus, assessing co-localization of PML and SETDB1 (graph). DNA was stained with DAPI. Bar, 10 µm. (C) Venn diagram of H3.3-Flag peak overlap in wt and *Pml* ko MEFs. (D) ChIP-qPCR of H3.3-Flag in wt and *Pml* ko MEFs in indicated regions; mean ± s.d., *P < 0.05 compared to wt, Fisher's exact test. See Supplementary Table 4 for position of amplicons. (E) Gene expression levels in PADs in wt and *Pml* ko MEFs, determined in a triplicate RNA-seq experiment. (F) Scatter plot of gene expression levels in wt vs. *Pml* ko MEFs. Differentially expressed genes in PADs (blue) and between PADs (inter-PADs; red) are highlighted (> 2-fold, α < 0.05; triplicate RNA-seq data).