Legends to supplemental figures

Supplemental Figure 1. 3D time-lapse recording of PML bodies in (A) a U2OS cell and (B) a W8 MEF, both expressing EYFP-PML. Both cells were not treated with MMS.

Supplemental Figure 2. The amount of SUMOylated PML decreases during MMS treatment and returns after recovery from treatment.

Supplemental Figure 3. Double labeling of exogenous TRF1 and telomeric DNA in (A) a nontreated U2OS cell and (B) an MMS treated U2OS cell. Telomeric DNA was hybridized with a telomere specific PNA probe (red) and exogenous TRF1 was labeled using a anti-GFP antibody (green). Nuclei were counterstained with DAPI (blue).

Supplemental Figure 4. Localization of Sp100 (green) and TRF2 (red) in a U2OS cell without (A) and with (B) MMS treatment as shown by immunofluorescence. Note that the Sp100 localization before and after treatment is similar to that observed for PML (see also figure 5). Although the cell shown in (A) does not show a colocalization of Sp100 with TRF2, a colocalization of the two is observed in U2OS cells that show in addition to regular PML bodies ALT-associated PML bodies.

Supplemental Figure 5. The DNA damage markers γH2AX and 53BP1 are mostly not present at telomeric DNA at which PML bodies form. (A-C) Representative immunofluorescence images of U2OS cells expressing DsRed-TRF1 and stained with antibodies directed against PML (green) and γH2AX (cyan). (A) U2OS cell without treatment, (B) treated for 2 hours with MMS and (C) recovered from MMS treatment. Arrows indicate sites of newly formed PML bodies that colocalize with telomeric DNA but not with DNA damage foci. (D, E) Representative immunofluorescence images of U2OS cells stained with antibodies specific for 53BP1 (green) and TRF2 (red). (D) U2OS cell treated with MMS for 2 hours and (E) U2OS cell 2 hours after recovery from MMS treatment. Nuclei were counterstained with DAPI (blue).

Supplemental Figure 6. PML bodies form in NB4 cells when treated with arsenic trioxide. (A) PML does not preferentially localize at telomeric DNA in untreated NB4 cells. NB4 cells were fixed, hybridized with a telomere-specific PNA probe and stained with anti-PML antibody. PML is present in a diffuse and punctate pattern (yellow) which does not significantly overlap with the staining pattern of telomeric DNA (red). (B) Arsenic trioxide-treated NB4 cells show PML bodies that localize at telomeric DNA. NB4 cells were treated with arsenic trioxide for 8 hours, fixed, hybridized with a telomere-specific PNA probe and stained with anti-PML antibody. Nuclear DNA was counter stained with DAPI.

Supplemental Figure 7. SUMOylation-deficient PML does not localize at telomeric DNA in U2OS cells that recover form MMS treatment. U2OS cells were cotransfected with DsRed-TRF1 and EYFP-tagged SUMOylation-deficient PML, treated with MMS for 1.5 hours and allowed to recover in fresh medium. During the recovery phase, cells were fixed and analyzed by fluorescence microscopy. The image shows the formation of PML aggregates (yellow), which do not colocalize with telomeric DNA (red).

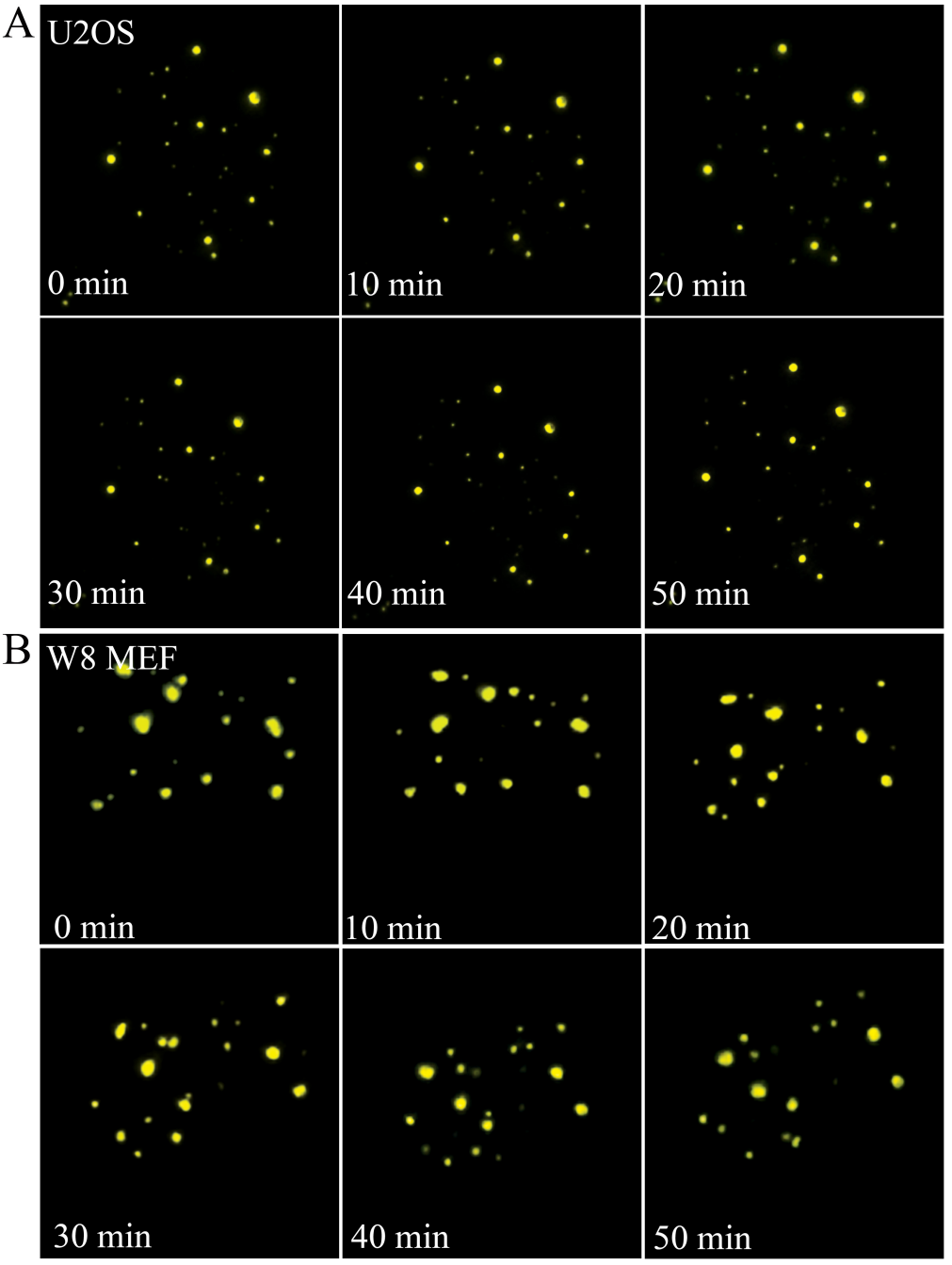
Supplemental movies

Supplemental Movie 1. MMS treatment of U2OS cells expressing EYFP-PML elicits PML nuclear body disassembly. Several fusion events of PML bodies can be seen before disassembly.

Supplemental Movie 2. U2OS cell expressing EYFP-PML and DsRed-TRF1. During recovery from MMS-treatment, PML bodies form on telomeric DNA.

Supplemental Movie 3. W8 MEF expressing EYFP-PML and DsRed-TRF1. Several *de novo* formation events of PML bodies on telomeric DNA can be seen.

Supplemental Movie 4. PML-/- MEF expressing EYFP-PML and DsReTRF1. Within the circles, *de novo* formed PML bodies are shown that dissociate from telomeric DNA.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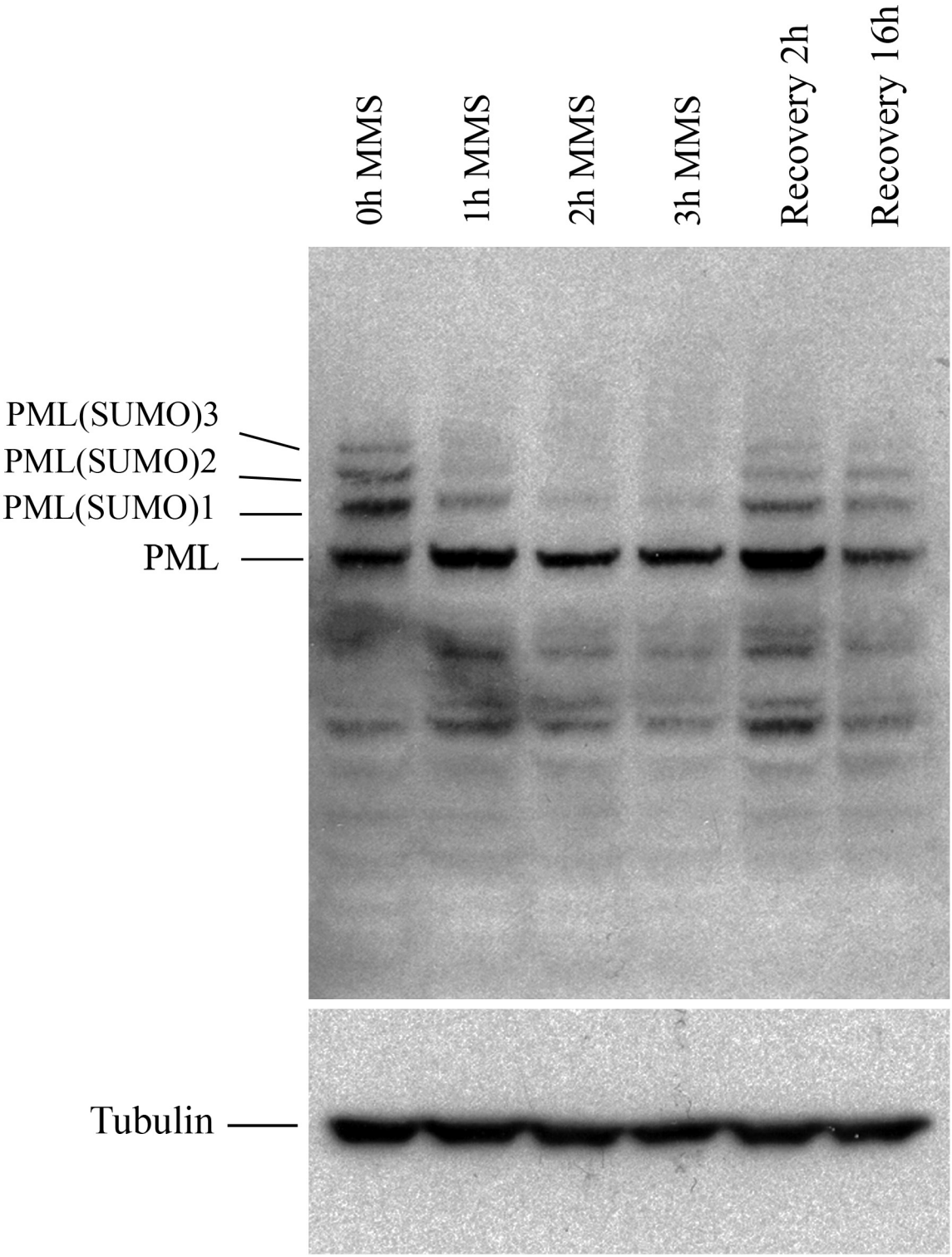
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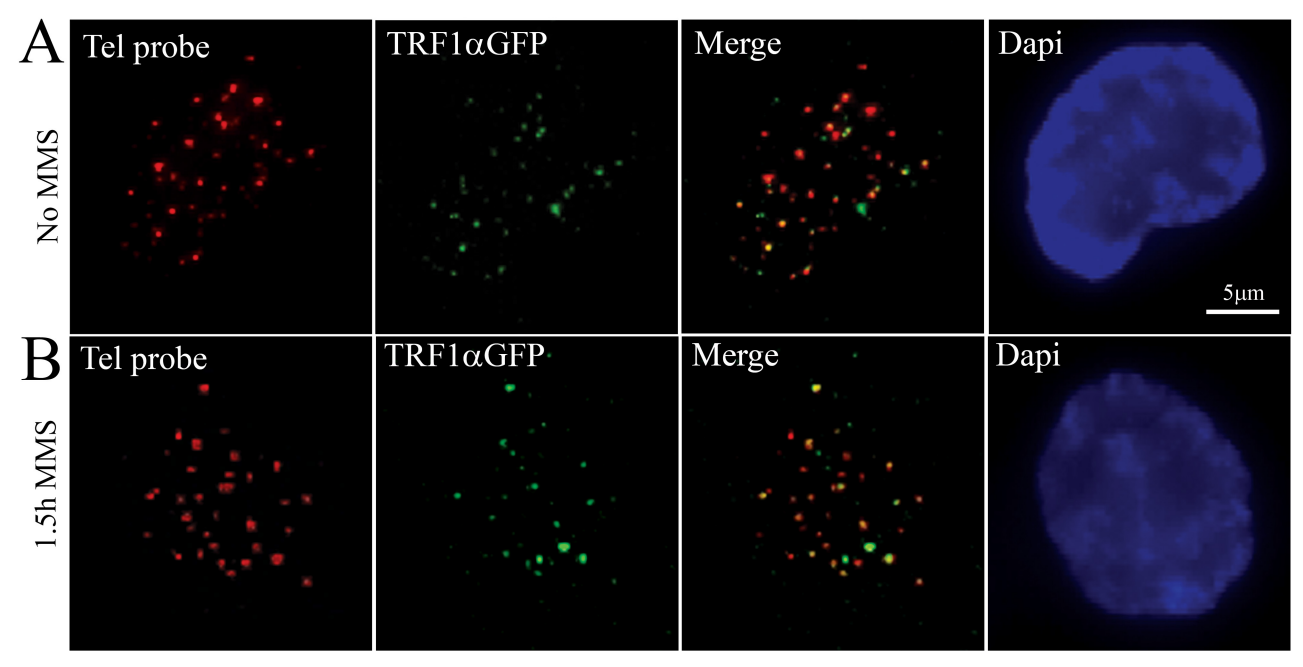
1h MMS
2h MMS
3h MMS
Recovery 2h
Recovery 16h
PML(SUMO)3
PML(SUMO)2
PML(SUMO)1
PML
Tubulin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Merge
Dapi
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B
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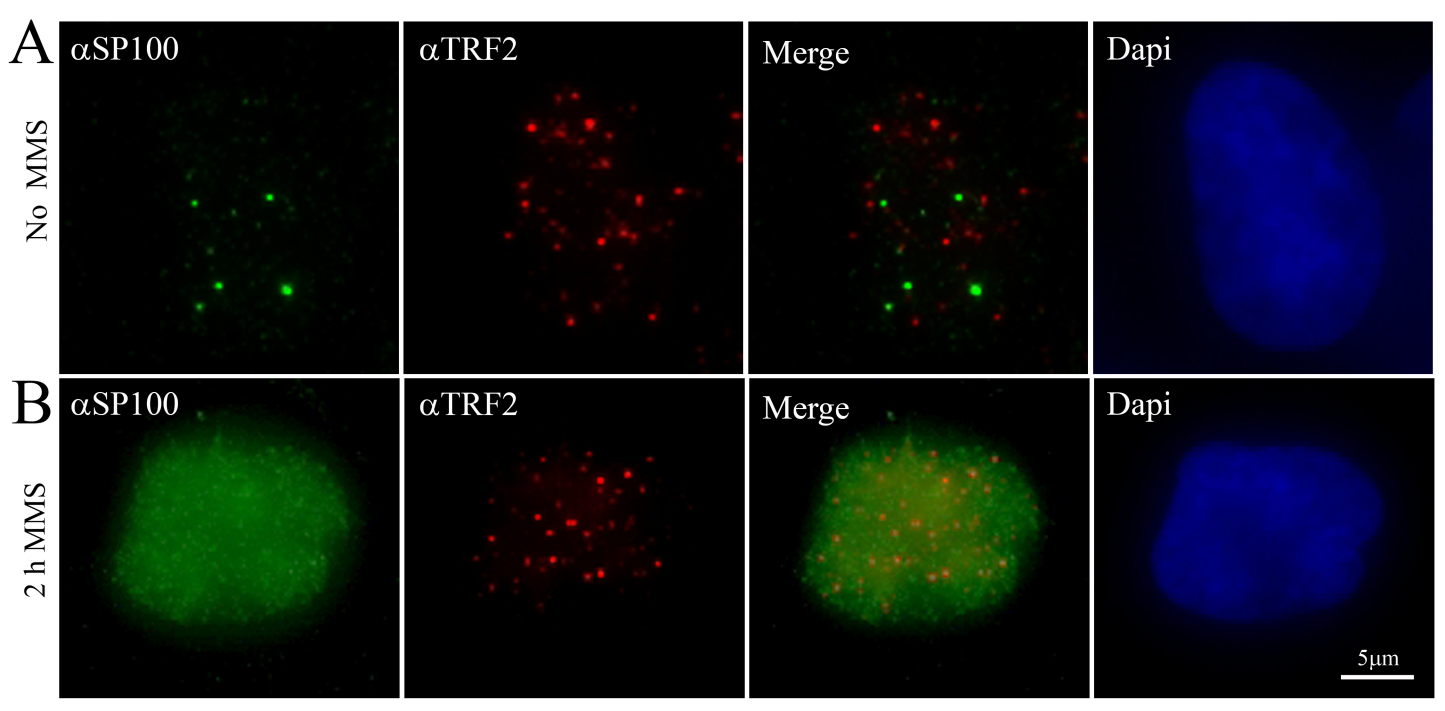
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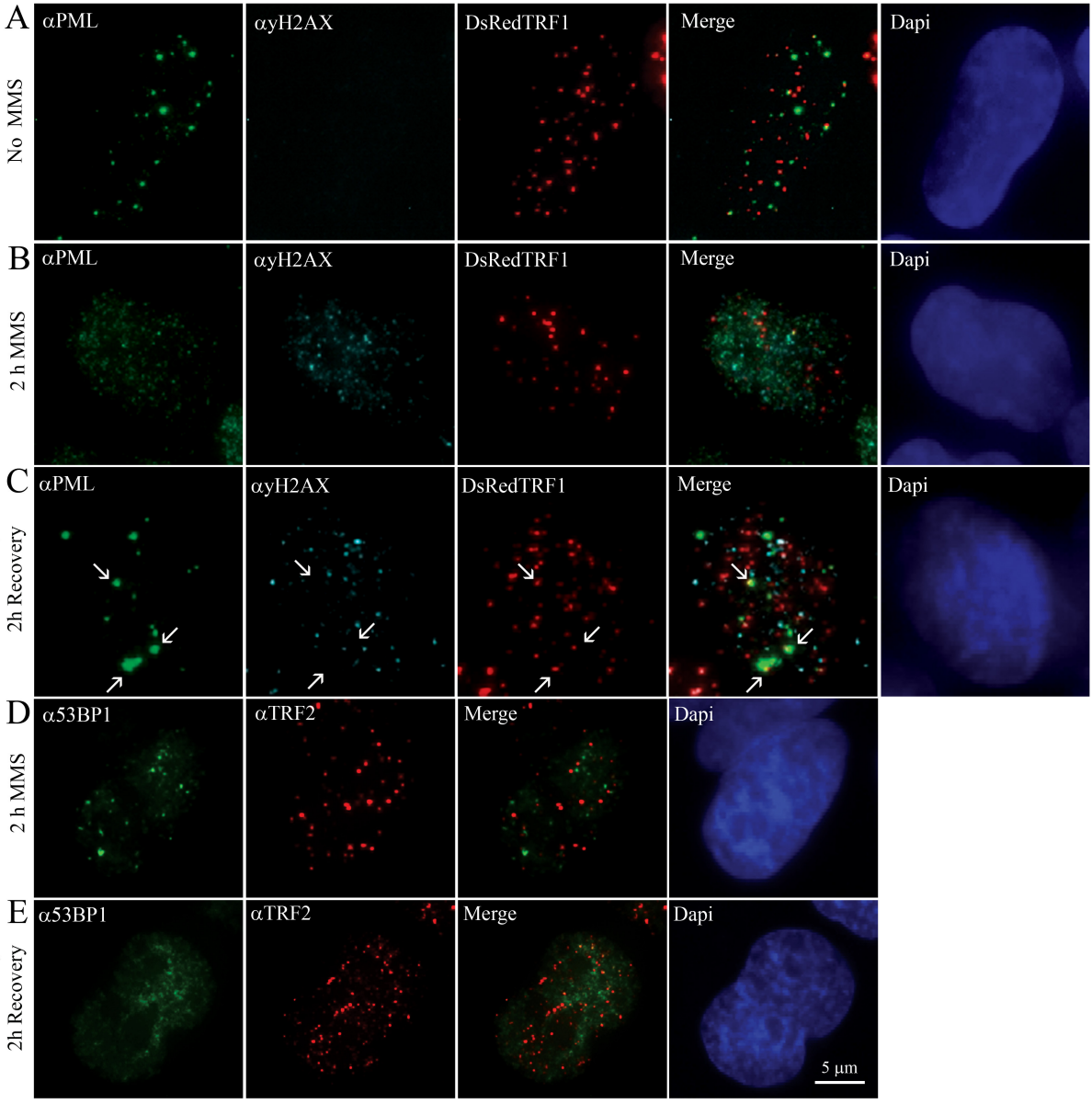
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Merge
Dapi
C
1.5h MMS,
2h Recovery
αPML
αyH2AX
DsRedTRF1
Merge
Dapi
D
2 h MMS
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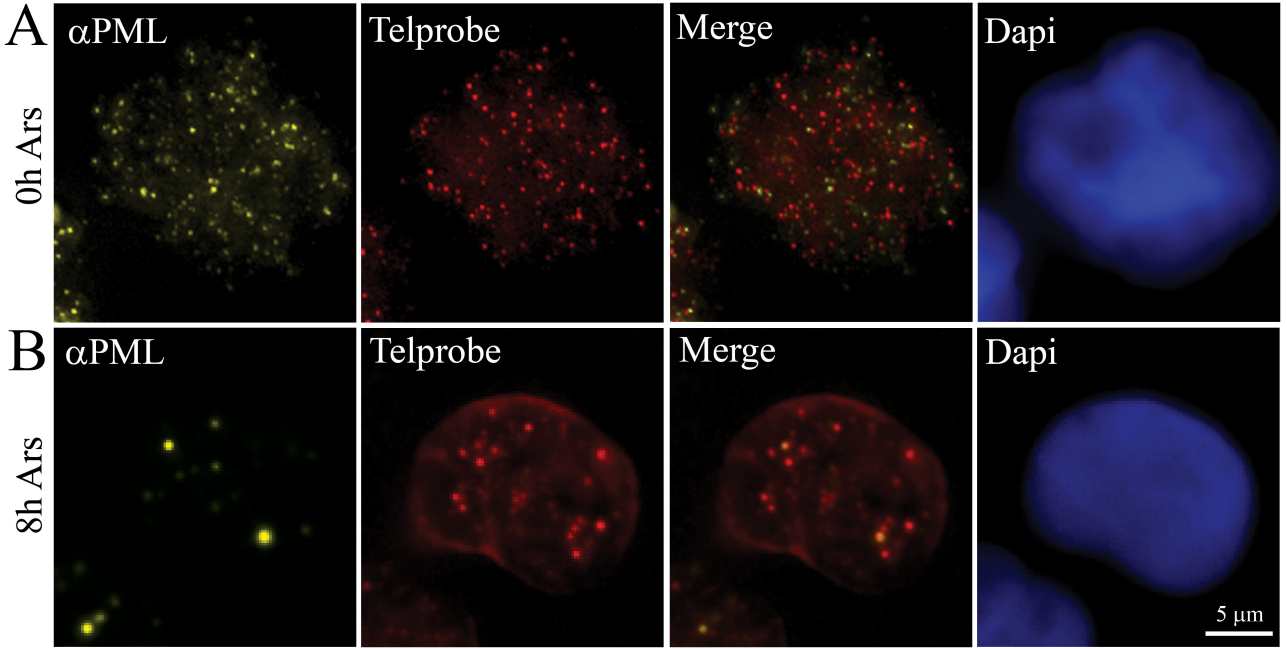
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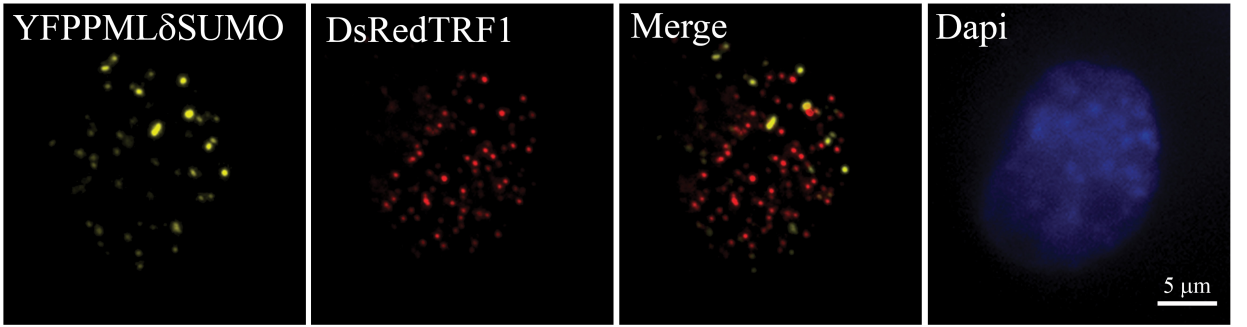
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