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Title	Dissecting the role of SMN multimerization in its dissociation from the Cajal body using harmine as a tool compound
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Supplementary figure 1

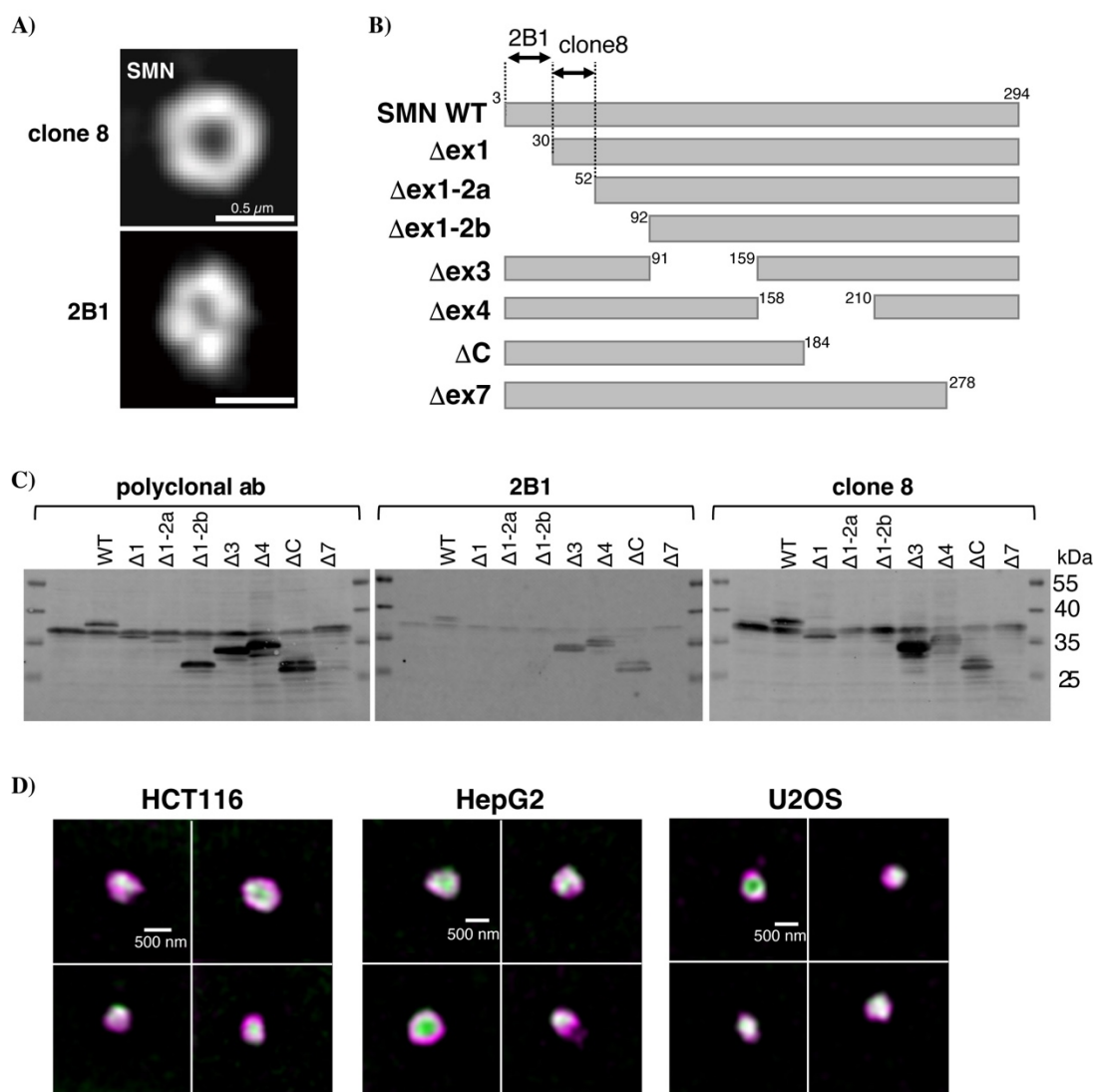


Fig. S1.

(A) CB/Gems were visualized using two anti-SMN antibody clones and images were captured by super-resolution microscopy. (B) (C) The epitopes of each SMN antibody were determined by detecting SMN deletion mutants in Western blotting. (B) Schematic representation of the protein regions contained in each SMN deletion mutant. (C) 2B1 and clone8 recognize different epitopes that likely located in exon 2b and exon 2a, respectively. (D) SIM images of CB/Gems stained with anti-SMN (green) and anti-Coilin (magenta) antibodies in HCT116, HepG2 and U2OS cells as indicated above each image.

Supplementary figure 2

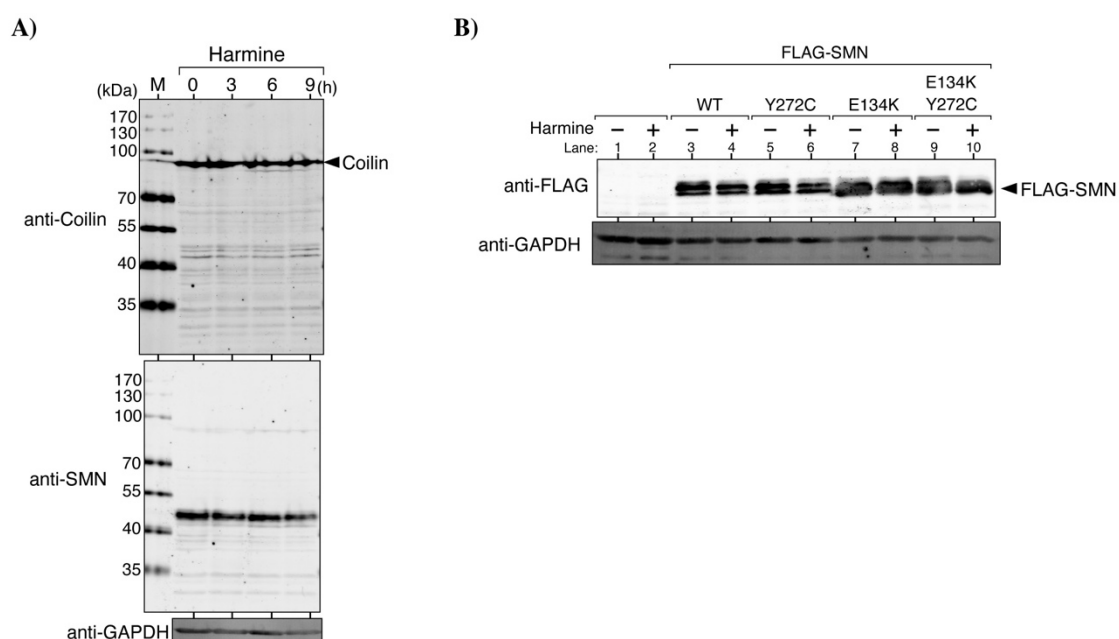


Fig. S2.

Harmine treatment did not have any effects on the protein expression of SMN and Coilin. (A) The expression of endogenous Coilin and SMN after harmine exposure was detected by anti-Coilin (top panel) and anti-SMN (middle panel) antibodies. No changes were observed for both Coilin and SMN instead of their significant localization changes. GAPDH was used as a loading control (lower panel). (B) Expression levels of SMN mutants were not altered in the presence of harmine. SMN was detected as multiple bands for all samples in this condition, but the SMN E134K and E134K/Y272C mutants showed lower bands than others, which is consistent with the previous report (Wang and Dreyfuss, 2001).

Supplementary figure 3

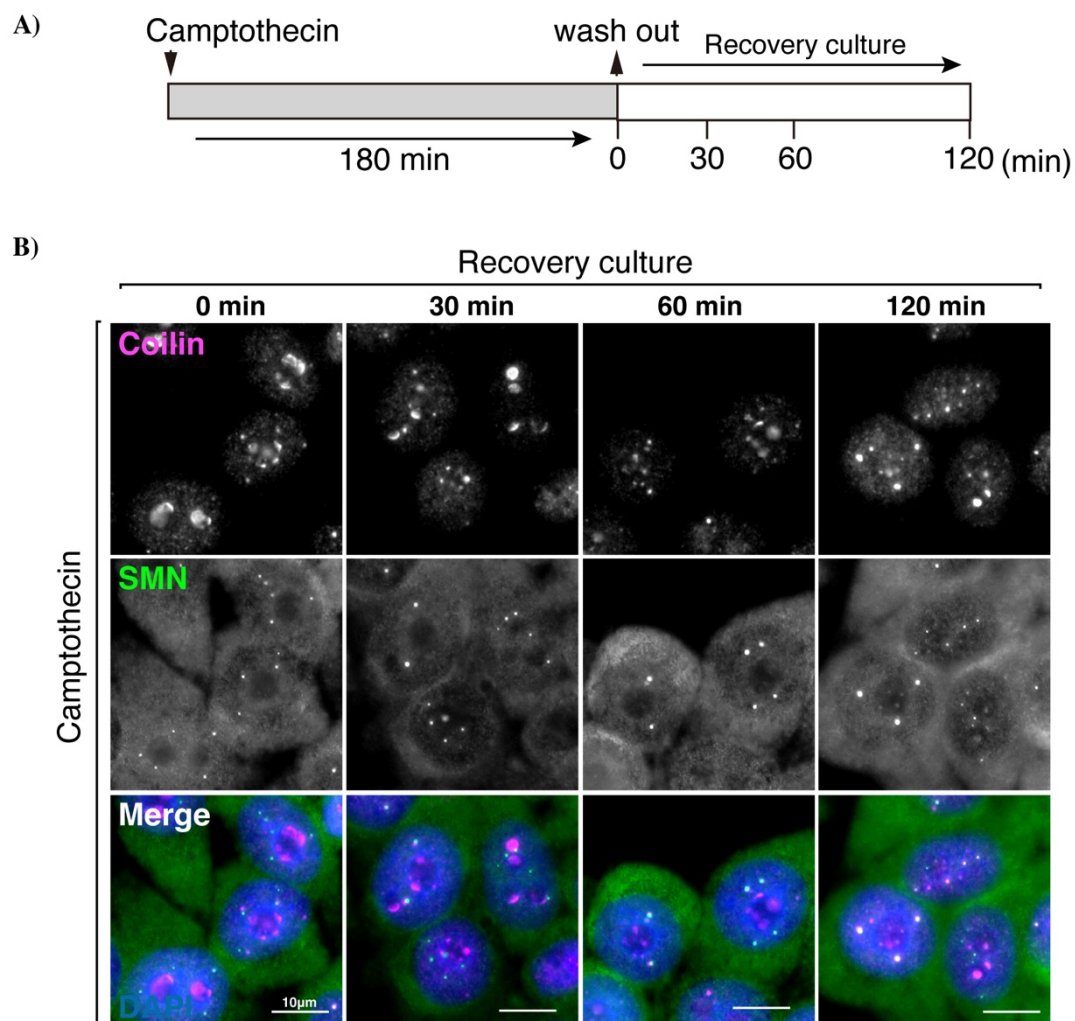


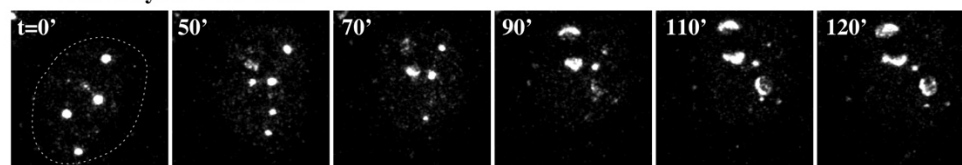
Fig. S3.

Coilin rapidly reassembles Cajal bodies after washout of camptothecin similar to harmine. (A) Schematic time course of the release experiment. (B) Coilin and SMN staining at indicated times. The effect of camptothecin is slightly stronger than that of harmine, so that Coilin is still present around PNC 60min after washout compared to harmine.

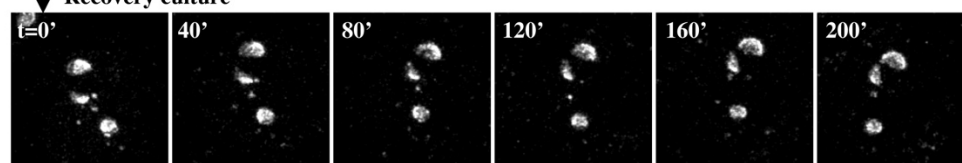
Supplementary figure 4

A) HeLa / Coilin-mRuby2

Actinomycin D treatment

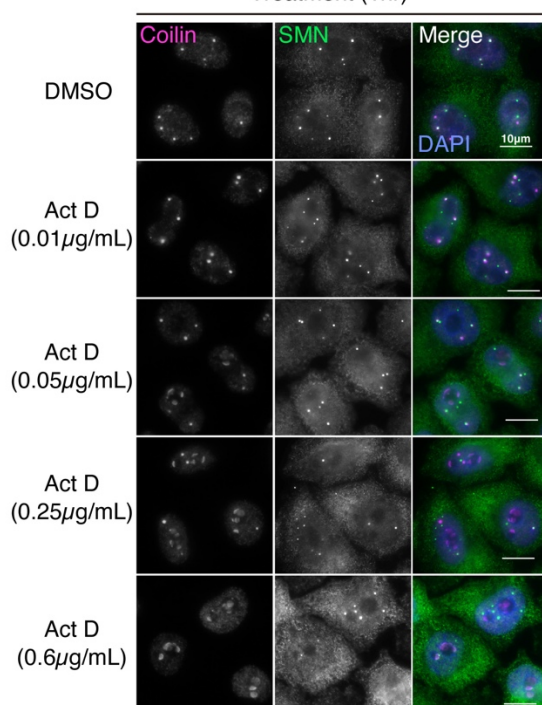


Recovery culture



B)

Treatment (1hr)



C)

Recovery (4hr)

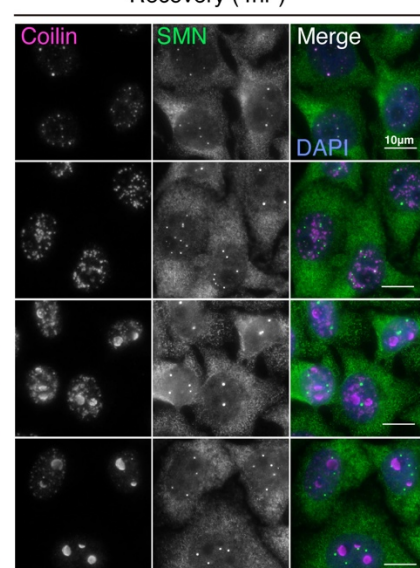


Fig. S4.

(A) Frames at the indicated time from Supplementary Movie 3 and 4 of a nucleus were presented as snapshots as shown in Fig. 5(F); the dashed line indicates the nuclear boundary. mRuby2-fusion Coilin was expressed in HeLa cells and the cells were scanned every 2 minutes for 6.5 hrs (2.5 hrs for actinomycin D treatment at a concentration of 0.6 $\mu\text{g/mL}$ and 4 hrs for recovery). (B) The relocation of Coilin to the perinucleolar components (PNC) depends on the concentration of Actinomycin D. At low concentrations, one hour of treatment is not enough to induce relocation to the PNC. However, after four hours of recovery culture, the number of puncta stained with Coilin increases. At high concentrations, the relocation occurs rapidly and is irreversible.

Supplementary Figure 5

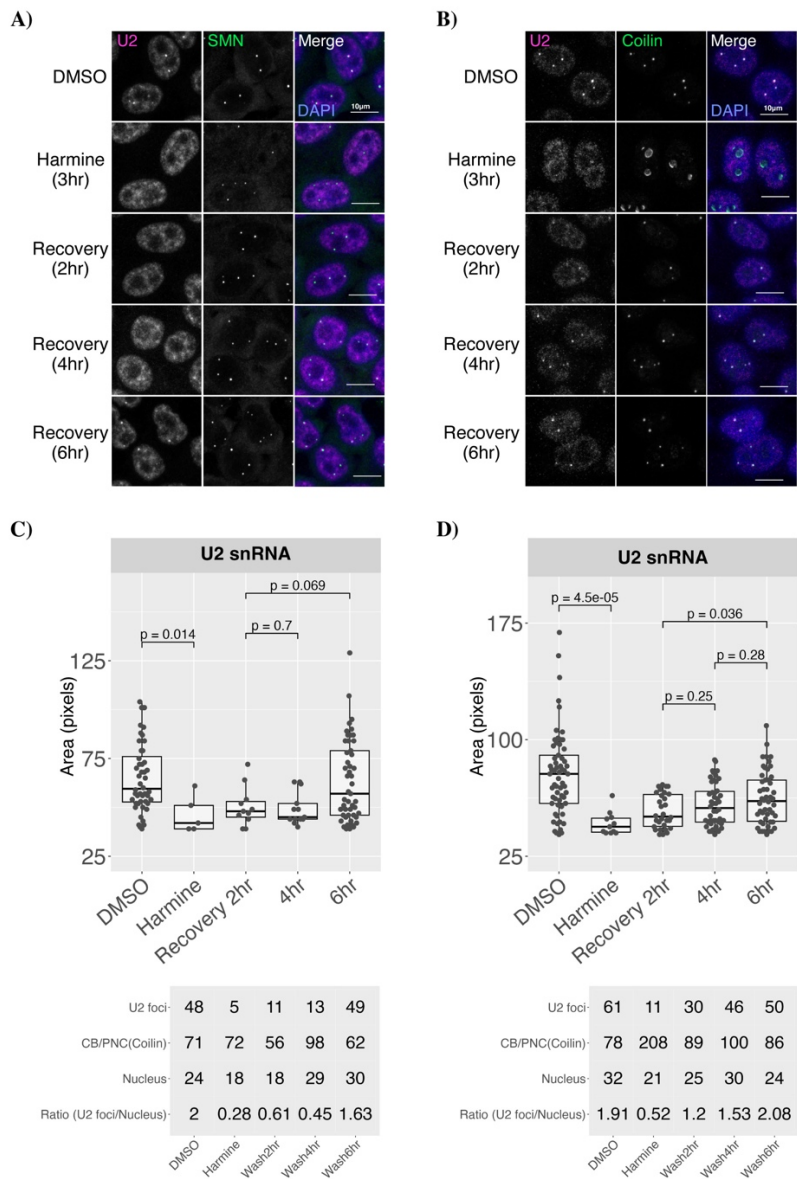


Fig. S5.

Recovery of U2 snRNA in the reassembled Cajal bodies was observed by confocal microscopy. U2 snRNA was detected by co-staining with the SMN antibody (A) and the Coilin antibody (B). The combination of secondary antibodies, which varied depending on the animal species of the primary antibody, caused differences in the detection sensitivity of U2. (C) U2 snRNA was detected in Cajal bodies labeled with the Coilin antibody after 120 minutes of recovery culture, with the number (presented below the plots) and size of U2 foci increasing over time. 1 pixel = 0.0064 μm^2 . The numbers of nuclei used for calculation and the numbers of detected dots are presented below the plots. The experiments were independently repeated twice.

Supplementary figure 6

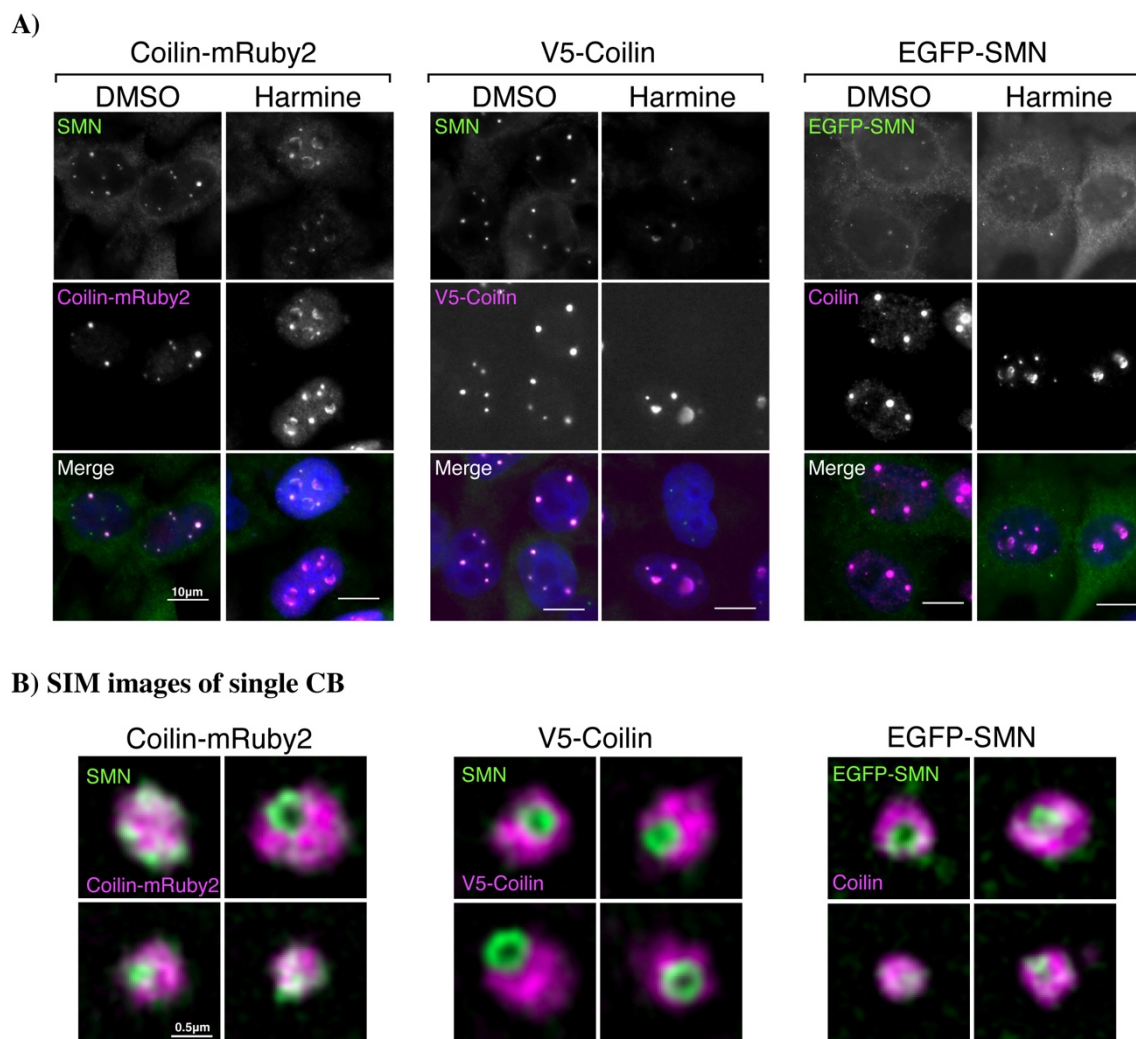


Fig. S6.

Overexpression of either SMN or Coilin causes forced recruitment of SMN to the PNC after harmine exposure (50 μ M, 4hrs). (A) The cells expressing Coilin-mRuby2 or V5- tagged Coilin were stained with anti-SMN antibody to detect the localization of endogenous SMN. The cells expressing EGFP-SMN were stained with anti-coilin antibody to detect the localization of endogenous coilin. SMN was overlapped with Coilin both in the absence or presence of harmine. After harmine exposure, SMN was also observed at PNC in addition to Cajal bodies. (B) Images of Cajal bodies obtained by super-resolution microscopy showed that no separation of SMN and Coilin puncta occurs in the cell transfected with either Coilin-mRuby2 or EGFP-SMN, whereas V5- tagged Coilin did not cause such structural disruption.

Supplementary figure 7

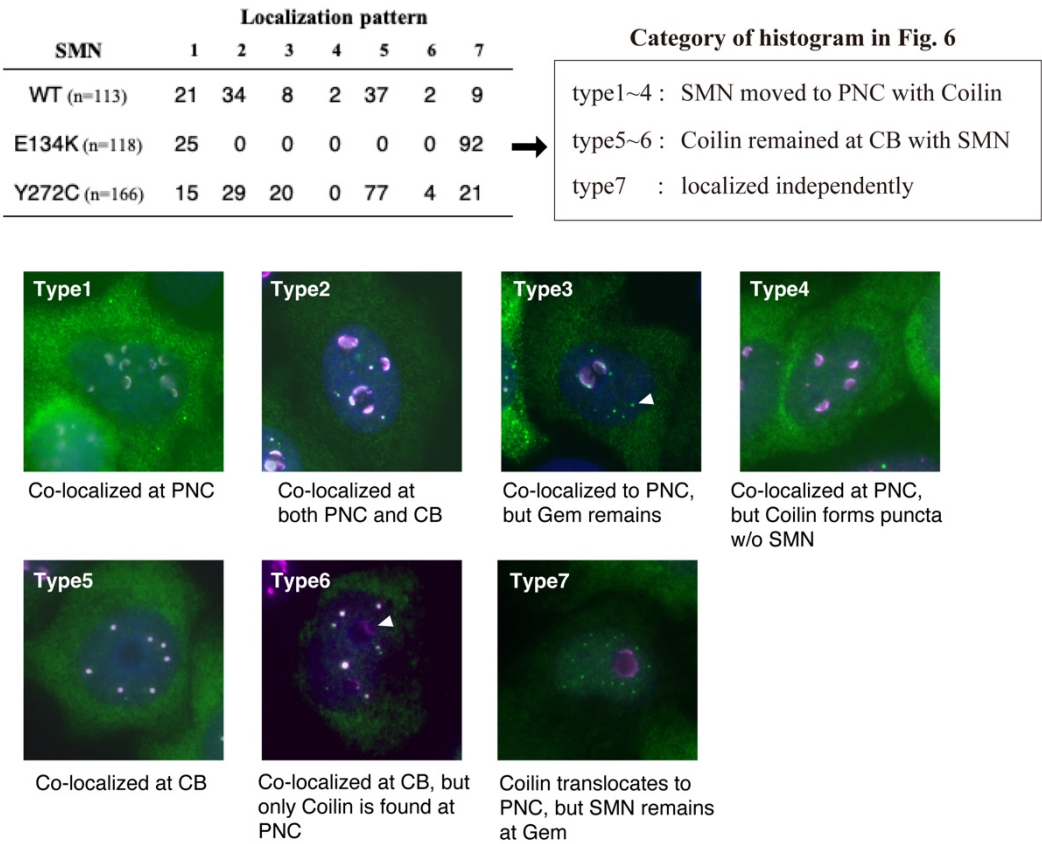


Fig. S7.

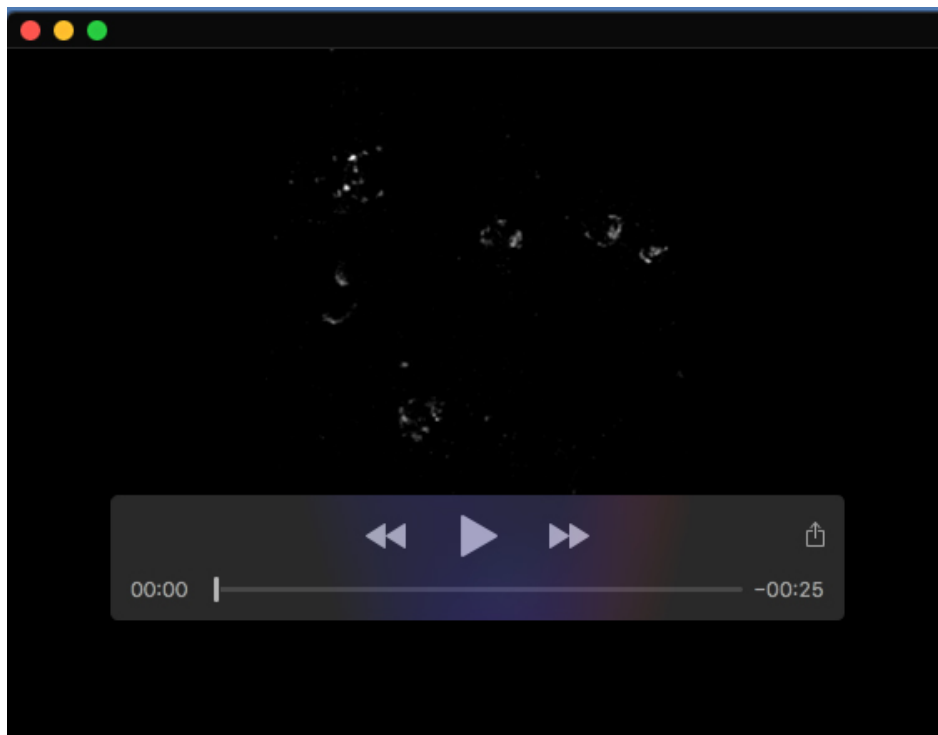
Classification by co-localization pattern of overexpressed SMN and Coilin after harmine treatment. The colocalization pattern was classified as Types 1-7 according to the lower panel. Table shows the count of cells with colocalization patterns of Type1-7. Bar graph in Fig. 6D were created with Type1~4 as SMN moved to PNC with Coilin, Type5~6 as Coilin remained at CB with SMN and Type7 as localized independently.

Table S1. Antibody list

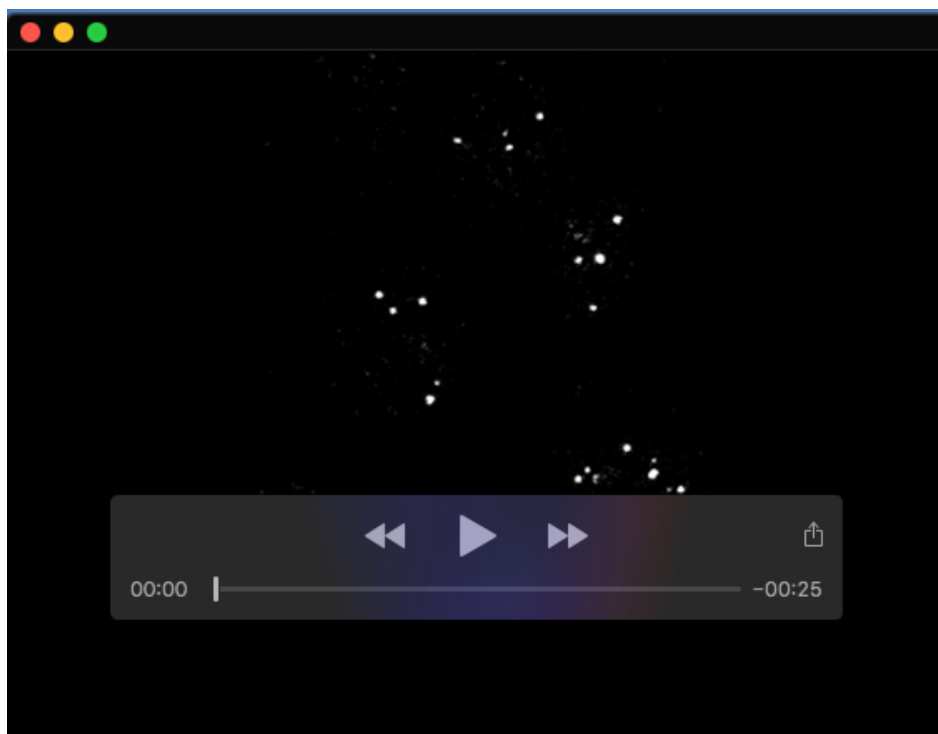
<i>Target name</i>	<i>Catalog code</i>	<i>Supplier</i>	<i>Species</i>
anti-Coilin	0967-1-AP	Proteintech	rabbit polyclonal
anti-Coilin	c-300	SantaCruz	rabbit polyclonal
anti-SMN (clone 8/SMN)	610646	BD Biosciences	mouse monoclonal
anti-SMN (2B1)	NB100-1936SS	Novus	mouse monoclonal
anti-SMN2	22329-1-AP	Proteintech	rabbit polyclonal
anti-Fibrillarin	38F3	Abcam	mouse monoclonal
anti-Nopp140 (E-7)	sc-374033	SantaCruz	mouse monoclonal
anti-Gemin2 (2E17)	ab6084	Abcam	mouse monoclonal
anti-GAPDH	10494-1-AP	Proteintech	rabbit polyclonal
anti-DYKDDDK (FLAG tag)	012-22384	Wako	mouse monoclonal
anti-V5	13202	cell signaling	rabbit monoclonal
anti-DIG	11333062910	Roche	mouse monoclonal
anti-FITC	A889	Invitrogen	rabbit polyclonal
anti-RbIgG-Cy2	ab6940	Abcam	secondary antibody
anti-MsIgG-Cy3	AP124C	Sigma	secondary antibody
anti-RbIgG-Cy3	AP132C	Sigma	secondary antibody
anti-MsIgG-Alexa488	A11001	Invitrogen	secondary antibody
anti-MsIgG-Cy2	715-225-150	Jackson	secondary antibody



Movie 1. Time-lapse imaging after harmine exposure by observing Coilin-mRuby2 transiently expressed in HeLa cells. Images were acquired every 2 min for 3 hrs after harmine exposure. After 90 min of harmine treatment Coilin rapidly disappears from Cajal bodies and accumulates in PNC.



Movie 2. Time-lapse imaging of Coilin-mRuby2 during Cajal body reformation that was transiently expressed in HeLa cells. Images were acquired every 2 min for 3 hrs after washout of harmine. After 30 min of recovery culture, Coilin reforms Cajal bodies simultaneously with the disappearance from PNC.



Movie 3. Time-lapse imaging of Coilin-mRuby2 in HeLa cells. Images were acquired every 2 min for 2.5 hrs after exposure to actinomycin D. After 60 min of actinomycin D treatment Coilin rapidly disappears from Cajal bodies and accumulates in the PNC.



Movie 4. Time-lapse imaging of Coilin-mRuby2 transiently expressed in HeLa cells. Images were acquired every 2 min for 4 hrs after washout of actinomycin D. During this time period, Coilin never leaves the PNC.