Figure S1. Phosphorylation status of Pol II CTD on PCs in parental HCT116 cells
Parental HCT116 cells were fixed and stained with antibodies against Pol II CTD (Alexa Fluor 488; green) and S2P, T4P, S5P, or S7P (Cy5; magenta). All images are MIP images of Z-stacks at 0.30 μm intervals to cover the entire nucleus (29–62 stacks). Scale bar, 5 μm.
Parental HCT116 and KI cells were fixed and stained with antibodies against coilin, SMN, TCAB1, fibrillarin, and NPAT, and Cy5-labeled secondary antibody (magenta). PCs were detected with EGFP–RPB1 in KI cells (A) or using Alexa Fluor 488-labeled anti-CTD antibodies in parental HCT116 cells (B) (green). Confocal images of the entire nucleus were acquired at 0.30 μm Z-intervals (29–62 stacks). MIP images are shown. Yellow lines indicate nuclear peripheries. Scale bars, 5 μm.
Figure S3. Coilin–SNAP was more representative to the endogenous coilin than SNAP–coilin

Cells expressing SNAP-tagged coilin were established using coilin-KO cells that were generated from KI cells. Both N- and C-terminally SNAP-tagged versions (SNAP–coilin and coilin–SNAP, respectively) were examined. (A) Western blotting. Whole-cell lysates prepared from parental HCT116 (P), KI, coilin-KO expressing SNAP–coilin (clone 1; SC), and coilin-KO expressing coilin–SNAP (clone 1; CS) were separated on an SDS-polyacrylamide gel and were probed with antibodies against coilin and ACTB. (B) and (C) Immunofluorescence. (B) Pol II (green) was detected using anti-CTD antibody (parental HCT116) or EGFP–RPB1 (KI, CS, and SC), and coilin (magenta) was detected using the specific antibody. (C) Cells were stained with antibodies against coilin (CBs; green) and NPAT (HLBs; magenta). Nuclear DNA was counterstained with Hoechst 33342 (blue). Nuclear areas are indicated by yellow lines. (D) Association rates between two heterotopic NBs in parental HCT116, KI, CS, and SC cells. “PCs with CBs” and “HLBs with CBs” indicate the percentages of PCs and HLBs, respectively, that were co-localized with, adjacent to, or non-associated with CBs (top). “CBs with PCs” and “CBs with HLBs” indicate the percentage of CBs that were co-localized with, adjacent to, or non-associated with PCs and HLBs, respectively (bottom). Scale bars, 10 μm.
**Figure S4. Association of NBs in living cells**

The entire nuclear images of those in Fig. 4C with contrast enhancement. The first row represents the contrast-enhanced images of that in Fig. 4. Time-lapse images of EGFP–RPB1 (green), NPAT–HaloTag (JF646; magenta), and coilin–SNAP (TMR; cyan) in coilin-KO cells expressing both NPAT–HaloTag and coilin–SNAP. MIP images of 13 Z-stacks at 0.75 μm intervals are shown. The magnified views of foci indicated by yellow arrowheads are shown in Fig. 4C. Yellow lines indicate nuclear peripheries. Scale bar, 5 μm.
Figure S5. Phosphorylation status of Pol II CTD on PCs did not depend on CBs or HLBs

(A) Coilin-KO cells were fixed and stained with Pol II CTD antibodies (magenta). PCs detected using EGFP–RPB1 (green) harbored S5P and S7P, as in coilin-positive KI cells. MIP images of Z-stacks at 0.30 μm intervals to cover the entire nucleus (29–62 stacks) are shown. (B–D) S5P levels in PCs with or without association with CBs or HLBs. (B) Immunofluorescence: Parental HCT116 cells were stained with antibodies against S5P (green) and coilin (magenta). Nuclear DNA was counterstained using Hoechst 33342 (blue). Arrowheads and asterisks indicate S5P foci (PCs) with and without a CB, respectively. An MIP image of 47 Z-stacks at 0.30 μm intervals is shown. (C) S5P levels in S5P foci with and without CBs. Intensity ratio of CB-associating PCs to CB-free PCs was expressed as “average intensity of a S5P focus with a CB in a cell” divided by “average intensity of a S5P focus without a CB in the same cell” (n = 100 cells). (D) S5P levels in S5P foci with and without HLBs. Intensity ratio of HLB-associating PCs to HLB-free PCs was expressed as “average intensity of a S5P focus with an HLB in a cell” divided by “average intensity of a S5P focus without an HLB in the same cell” (n = 100 cells). Scale bars, 5 μm.
Figure S6. PCs in coilin-KO cells

(A) Coilin-KO cells that stably expressed mCherry–PCNA were established. Confocal images of EGFP–RPB1 (green) and mCherry–PCNA (magenta) in the coilin-KO cells were acquired using time-lapse microscopy. MIP images of 15 Z-stacks at 0.75 μm intervals are shown. PCs nucleated in early S phase in coilin-KO cells, as observed in KI cells.

(B) Coilin-KO cells were fixed and stained with antibodies against coilin, NPAT, SMN, TCAB1, and fibrillarin, and Cy5-labeled secondary antibodies (magenta). PCs were detected using EGFP–RPB1 (green). Confocal images of the entire nucleus were acquired at 0.30 μm Z-intervals (29–62 stacks). MIP images are shown. Yellow lines indicate nuclear peripheries. Scale bars, 5 μm.
Figure S7. Effects of coilin overexpression on associations between NBs

(A) and (B) Immunofluorescence. KI cells overexpressing coilin–SNAP (KI CS OE) and KI cells were fixed and stained with SNAP–Cell 430 (cyan), antibodies against coilin (CB; yellow) and either of NPAT (HLB; magenta; A) or SMN (magenta; B). PCs were detected by EGFP–RPB1 (green). MIP images of confocal sections that cover the entire nucleus (0.3 μm Z-intervals; 27–31 stacks) are shown. Nuclear areas are indicated by yellow lines.

(C) Association rates between two heterotopic NBs in KI (n = 100 cells) and coilin–SNAP overexpressing cells (KI CS OE, n = 35 cells). The graphs of KI cells are reproduction of those in Fig. S3D for a ease of comparison. See Fig. 3D legend for graph description. Scale bars, 5 μm.
Figure S8. Effects of PC depletion by triptolide treatment on CB and HLB association in parental HCT116 cells

Parental HCT116 cells were treated with 5 μM triptolide or DMSO (vehicle) for 2 h, fixed, and stained with antibodies against Pol II CTD (PC; green), coilin (CB; magenta), and NPAT (HLB; cyan). Nuclear DNA was counterstained with Hoechst 33342 (blue). (A) MIP images of confocal sections. Confocal images of the entire nucleus were acquired at 0.30 μm Z-intervals (41–50 stacks). Nuclear areas are indicated by yellow lines. (B) Association rates between CBs and HLBs in the absence or presence of triptolide. “CBs with HLBs” indicates the percentage of CBs that are co-localized with, adjacent to, or non-associated with HLBs (n = 30 cells). “HLBs with CBs” indicates the percentage of HLBs that are co-localized with, adjacent to, or non-associated with CBs (n = 30 cells). Scale bar, 5 μm.