Supplemental material

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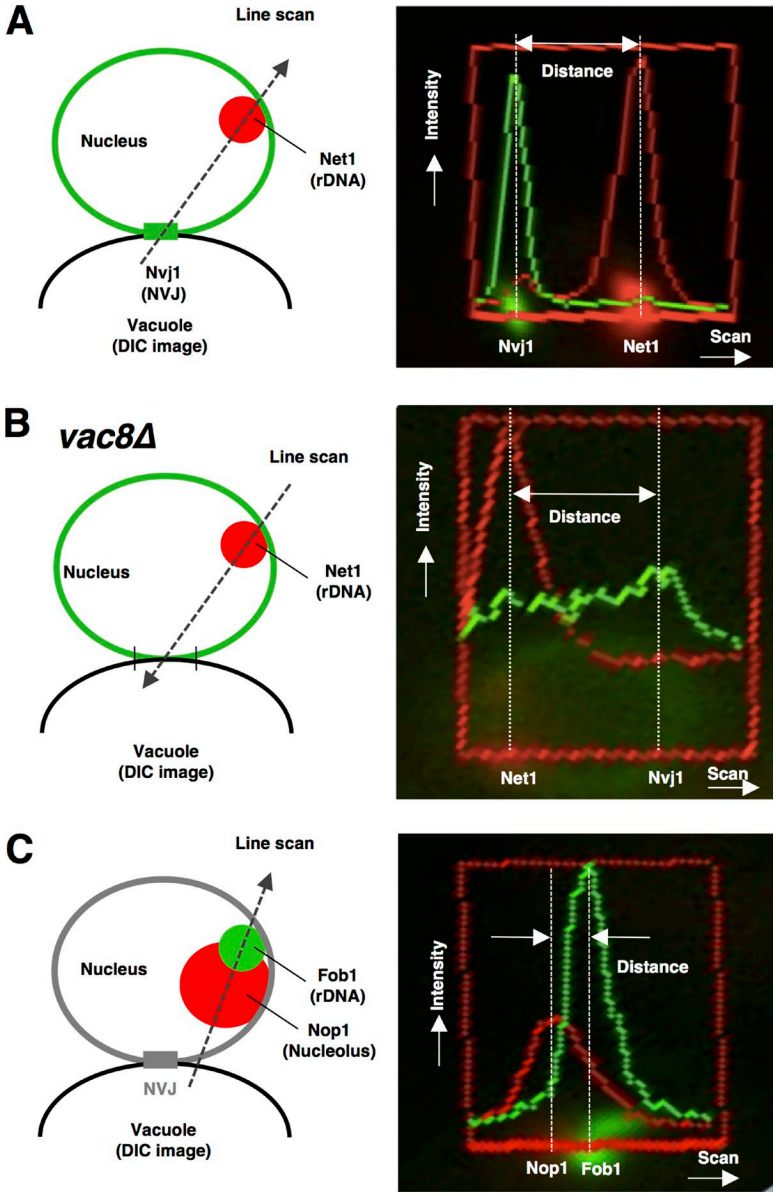

Figure S1. **Measurements of distances between rDNA, nucleolar proteins, and the NVJ. (A)** Illustration for measurement of the distance between rDNA (monitored using Net1-RFP) and the NVJ (monitored using Nvj1-GFP) in cells (left). An example for measurement of the distance using a captured cell image (right). See Materials and methods for details. **(B)** Measurement of distance between rDNA (monitored using Net1-RFP) and the NVJ (monitored using Nvj1-GFP) in *vac8Δ* cells. Nvj1 did not accumulate in the case of this mutant. See Materials and methods for details. **(C)** Measurement between nucleolar proteins (monitored using RFP-Nop1) and rDNA (monitored using Fob1-GFP) in cells. See Materials and methods for details.

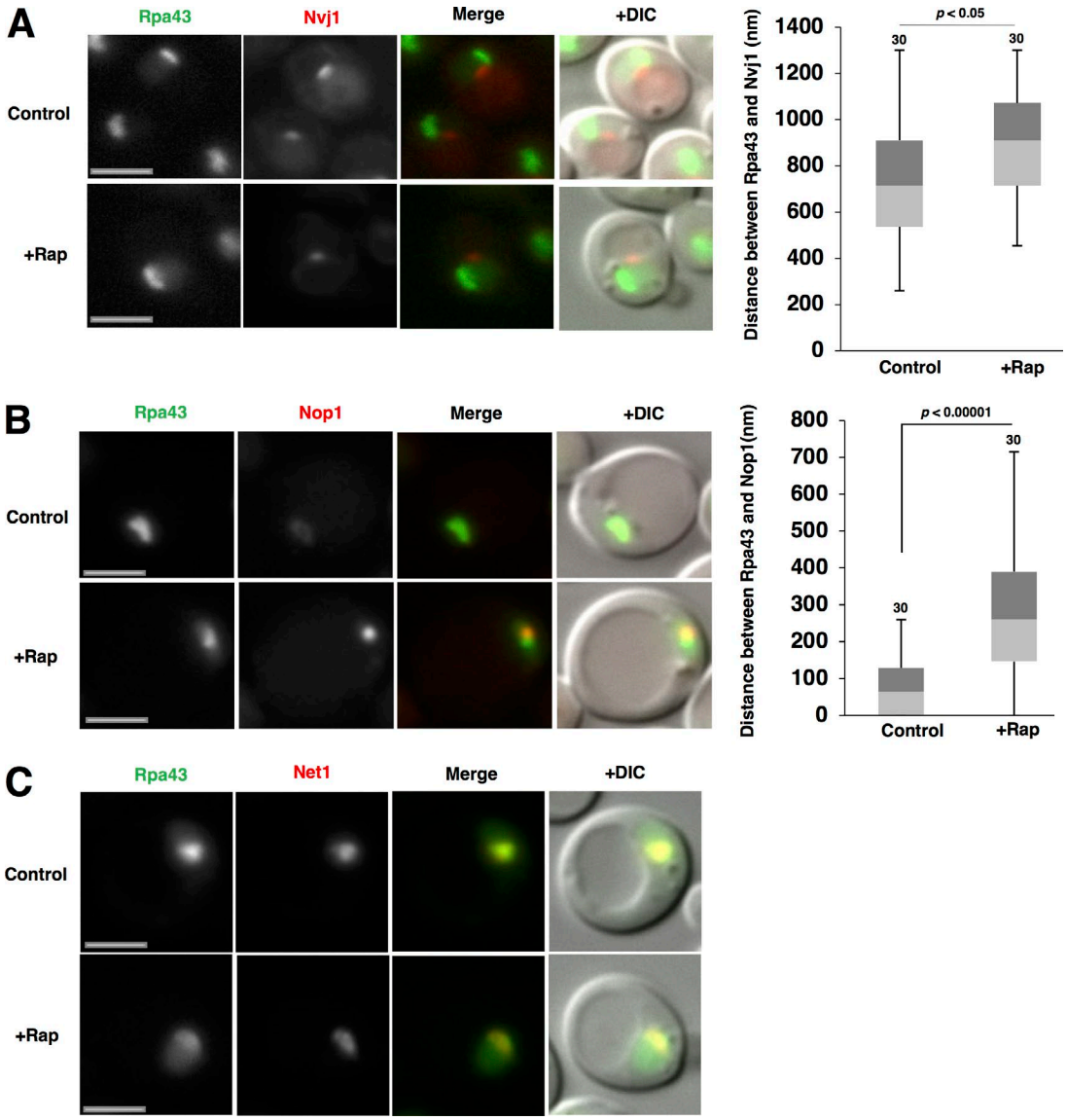

Figure S2. **Rpa43 moves far from the NVJ and dissociates from nucleolar proteins after TORC1 inactivation. (A)** Cells of strain SCU5408 (*RPA43-GFP NVJ1-mRuby2*) were treated with rapamycin for 1 h. The distance between the Rpa43-GFP and Nvj1-mRuby2 peaks is shown in the box plot. **(B)** Cells of strain SCU366 (*RPA43-GFP*) harboring pSCU618 (pRFP-NOP1) plasmid were treated with rapamycin for 1 h. The distance between the Rpa43-GFP and RFP-Nop1 peaks is shown in the box plot. **(C)** Cells of strain SCU5410 (*RPA43-GFP NET1-mRuby2*) were treated with rapamycin for 1 h. Bars, 2.5 µm.

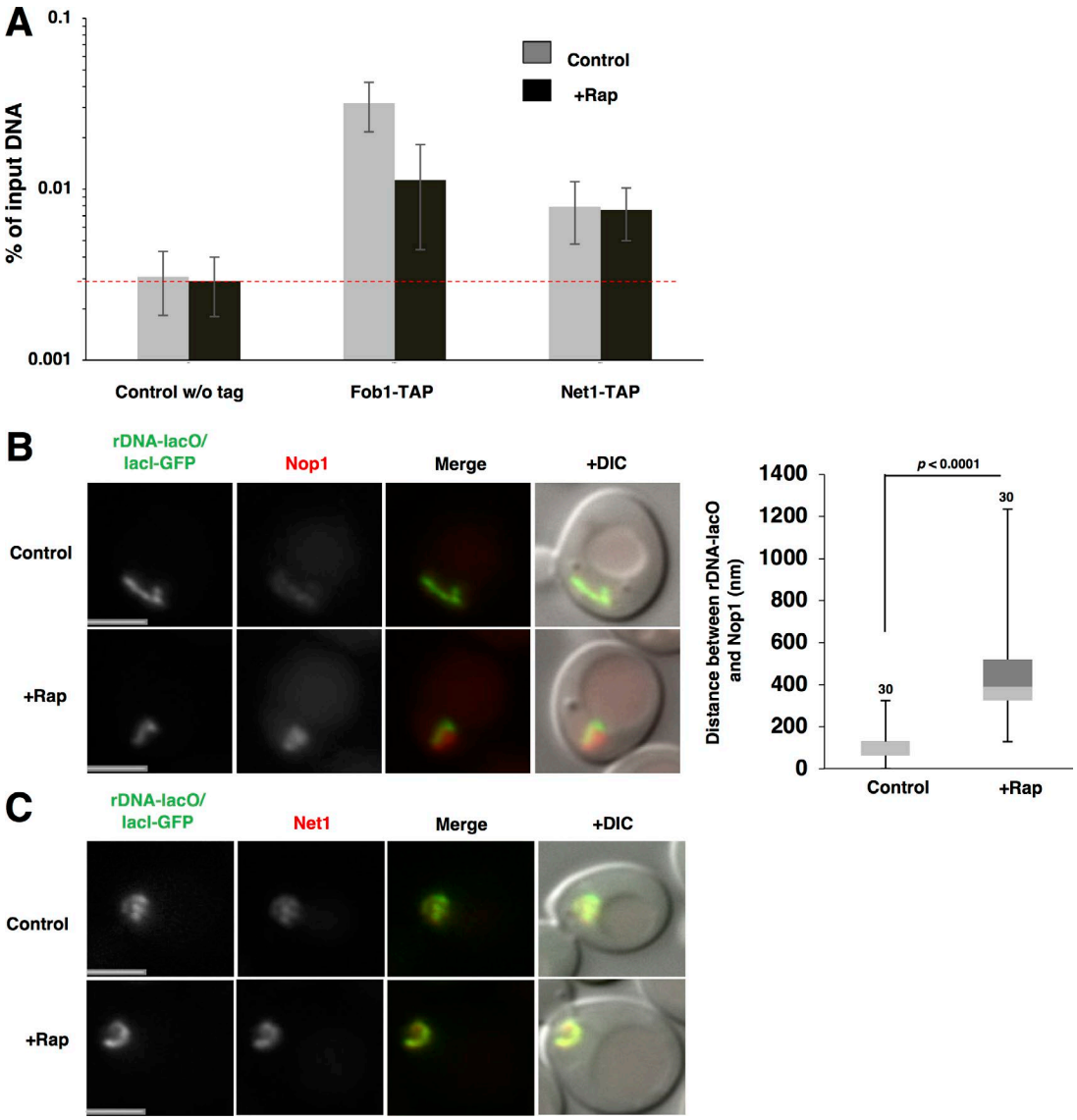

Figure S3. **Profiling of several rDNA markers after TORC1 inactivation. (A)** Exponentially growing cells of strains SCU5328 (*FOB1-TAP*) and SCU2610 (*NET1-TAP*) were treated with rapamycin for 1 h. Cell extracts were subjected to ChIP assay to quantify binding of Fob1 and Net1 with rDNA. DNA content in each immunoprecipitation sample relative to that of its input sample is shown as mean ± SD. WT strain without TAP tag (BY4741) was used as a control. **(B)** Cells of strain SCU5313 (*rDNA-lacO lacI-GFP*) harboring plasmid pSCU618 (pRFP-NOP1) were treated with rapamycin for 1 h. The distance between the lacI-GFP and RFP-NOP1 peaks is shown in the box plot. Numbers above the bars are sample sizes. P-values were calculated using two-tailed Mann–Whitney *U* test. **(C)** Cells of strain SCU5412 (*rDNA-lacO lacI-GFP NET1-mRuby2*) were treated with rapamycin for 1 h. Bars, 2.5 µm.

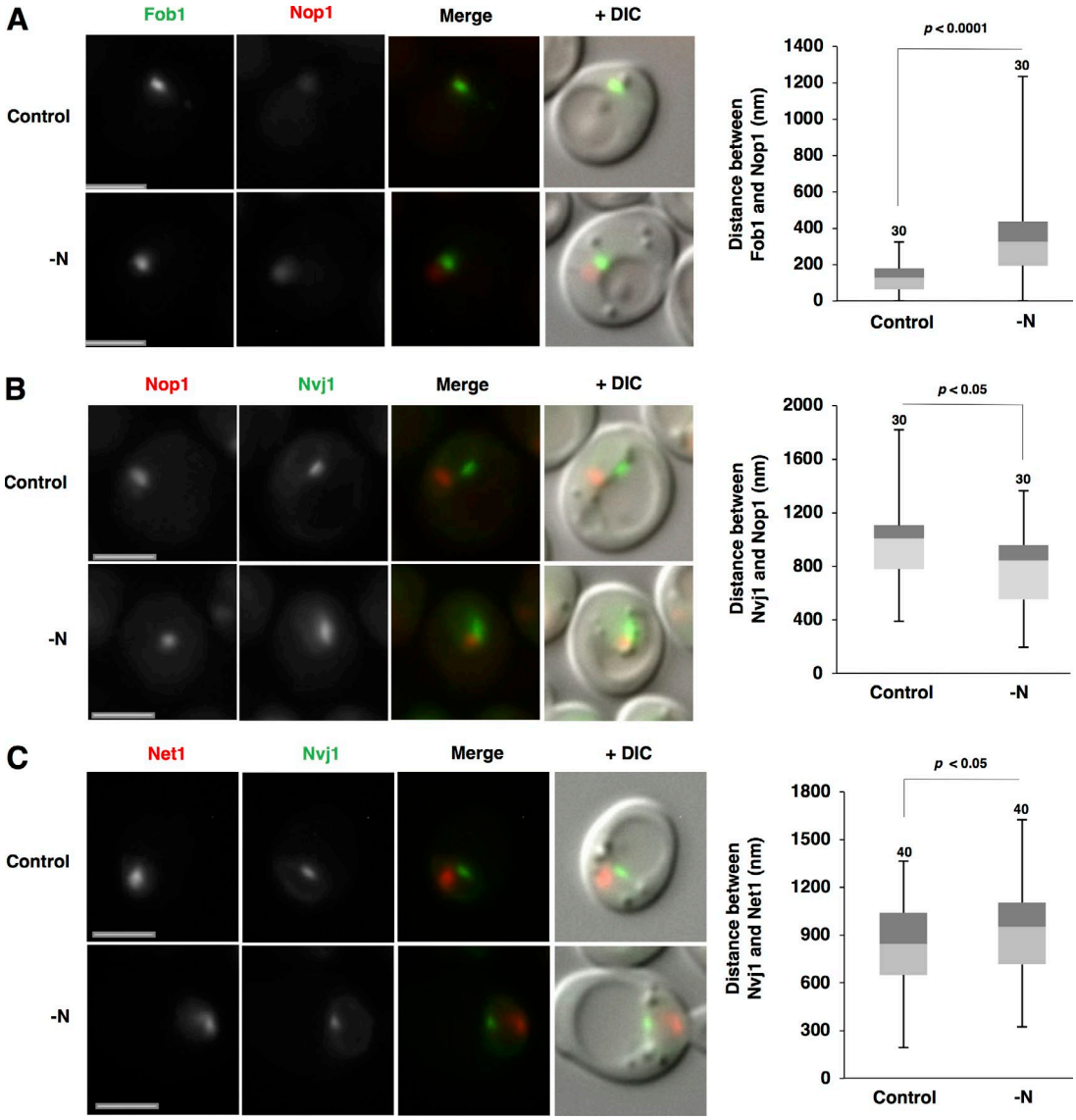

Figure S4. **Nitrogen starvation promotes intranuclear repositioning of nucleolar proteins and rDNA. (A)** Cells of strain SCU359 (*FOB1-GFP*) harboring plasmid pSCU618 (pRFP-NOP1) were transferred to nitrogen-deficient media for 1 h. The distance between the Fob1-GFP and RFP-Nop1 peaks is shown in the box plot. **(B)** Cells of strain SCU3287 (*NVJ1-GFP*) harboring plasmid pSCU618 (pRFP-NOP1) were transferred to nitrogen-deficient media for 1 h. The distance between the Nvj1-GFP and RFP-Nop1 peaks is shown in the box plot. **(C)** Cells of strain SCU4433 (*NVJ1-GFP NET1-mRuby2*) were transferred to nitrogen-deficient media for 1 h. The distance between the Nvj1-GFP and Net1-RFP peaks is shown in the box plot. Bars, 2.5 µm.

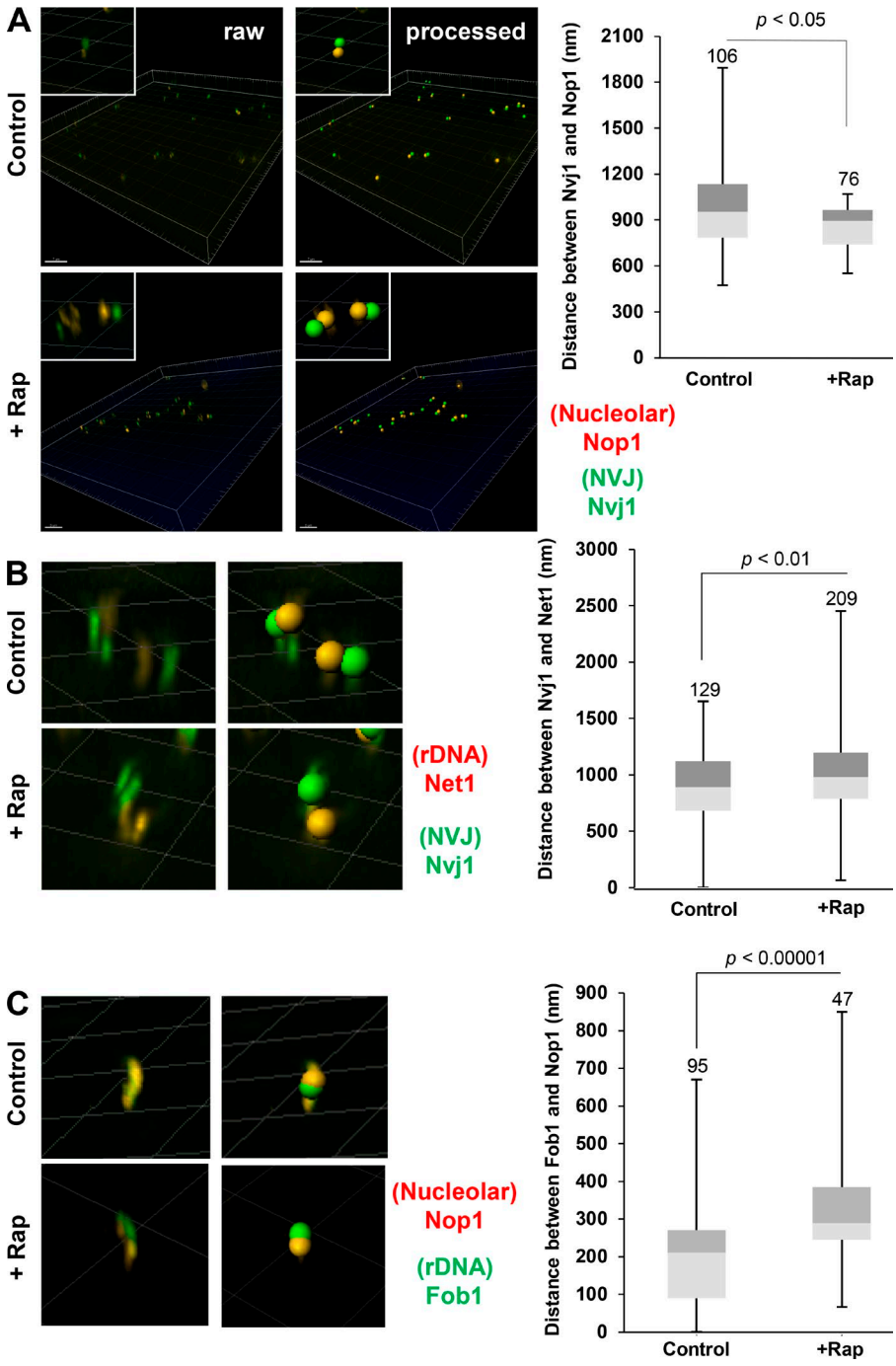

Figure S5. **3D analysis of the relocation of nucleolar proteins to the NVJ and rDNA escape from the NVJ after TORC1 inactivation. (A)** Exponentially growing cells of strains SCU3287 (*NVJ1-GFP*) harboring plasmid pSCU618 (pRFP-NOP1) were treated with rapamycin for 1 h. Cell images with GFP and RFP signals were captured using a DeltaVision microscopy imaging system. Bars: (control cells) 7 µm; (rapamycin-treated cells) 5 µm. Inset: Enlarged signal images. The distance between the Nvj1-GFP and RFP-Nop1 peaks, measured using Imaris software, is shown in the box plot. Numbers above the bars are sample sizes. P-values were calculated using a two-tailed Mann–Whitney *U* test. **(B)** Exponentially growing cells of strain SCU4433 (*NVJ1-GFP NET1-mRuby2*) were treated with rapamycin for 1 h. Cell images with GFP and RFP signals were captured using the DeltaVision microscopy imaging system. Bars, 5 µm. The distance between the Nvj1-GFP and Net1-RFP peaks, measured using Imaris software, is shown in the box plot. **(C)** Exponentially growing cells of strains SCU359 (*FOB1-GFP*) harboring plasmid pSCU618 (pRFP-NOP1) were treated with rapamycin for 1 h. Cell images with GFP and RFP signals were captured using a DeltaVision microscopy imaging system. Bars, 5 µm. The distance between the Fob1-GFP and RFP-Nop1 peaks, measured using Imaris software, is shown in the box plot.

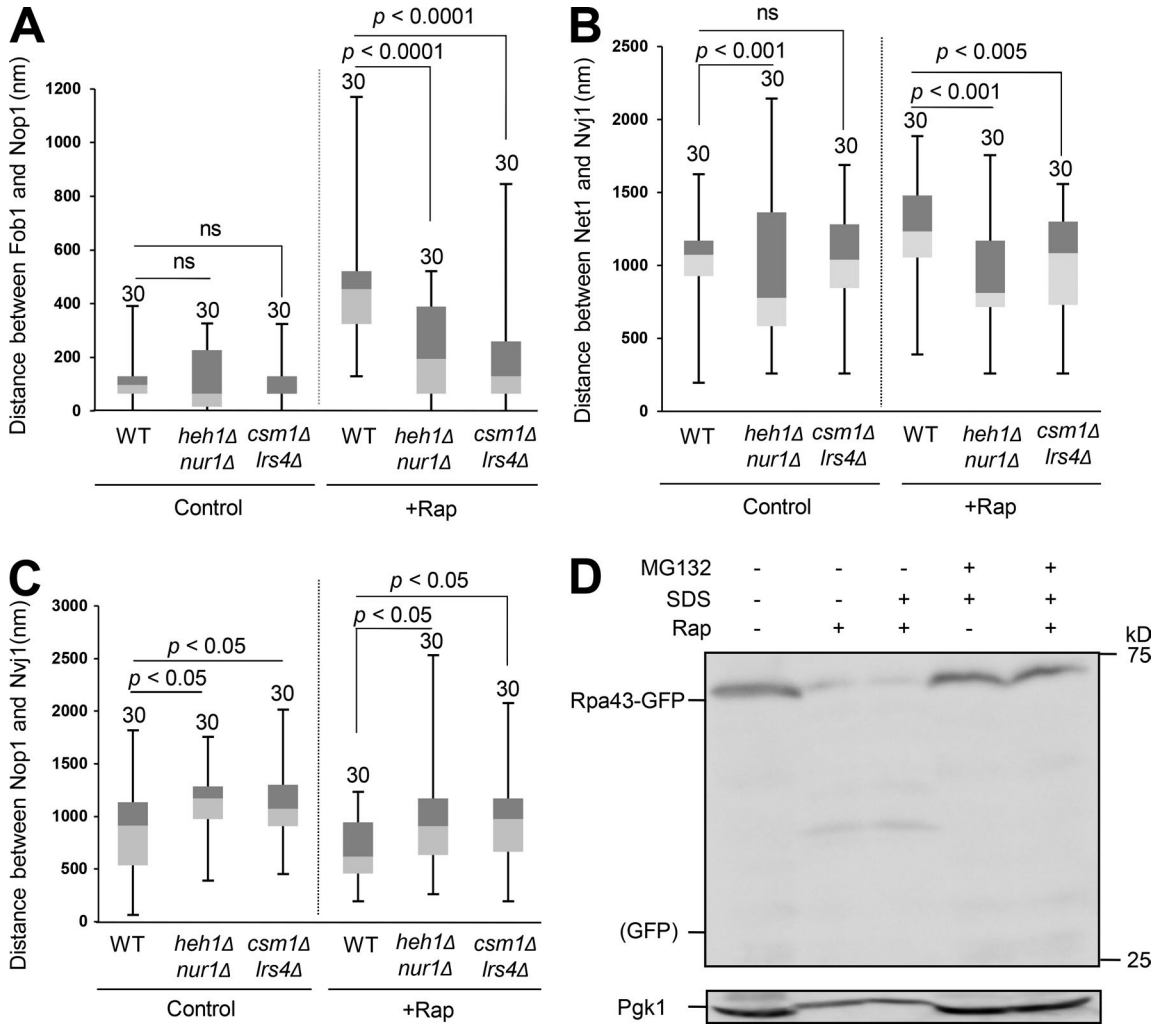

Figure S6. **The CLIP–cohibin axis is required for the relocation of rDNA and nucleolar proteins after TORC1 inactivation. (A)** Cells of strains SCU4823 (*FOB1-GFP*), SCU5370 (*heh1Δ nur1Δ FOB1-GFP*), and SCU5386 (*csm1Δ lrs4Δ FOB1-GFP*) harboring plasmid pSCU618 (pRFP-NOP1) were treated with rapamycin for 1 h. The distance between the Fob1-GFP and RFP-Nop1 peaks is shown in the box plot. **(B)** Cells of strains SCU5029 (*NVJ1-GFP NET1-mRuby2*), SCU5378 (*heh1Δ nur1Δ NVJ1-GFP NET1-mRuby2*), and SCU5392 (*csm1Δ lrs4Δ NVJ1-GFP NET1-mRuby2*) were treated with rapamycin for 1 h. The distance between the Nvj1-GFP and Net1-RFP peaks is shown in the box plot. **(C)** Cells of strains SCU3411 (*NVJ1-GFP*), SCU5374 (*heh1Δ nur1Δ NVJ1-GFP*), and SCU5388 (*csm1Δ lrs4Δ NVJ1-GFP*) harboring plasmid pSCU618 (pRFP-NOP1) were treated with rapamycin for 1 h. The distance between the Nvj1-GFP and RFP-Nop1 peaks is shown in the box plot. **(D)** Cells of strains SCU5404 (*pdr5Δ RPA43-GFP*) were treated with 200 ng/ml rapamycin, 50 µg/ml MG132, and/or 0.003% SDS for 3 h. Whole cell extracts were subjected to Western blotting. Pgk1 was detected as the loading control using an anti-Pgk1 antibody.

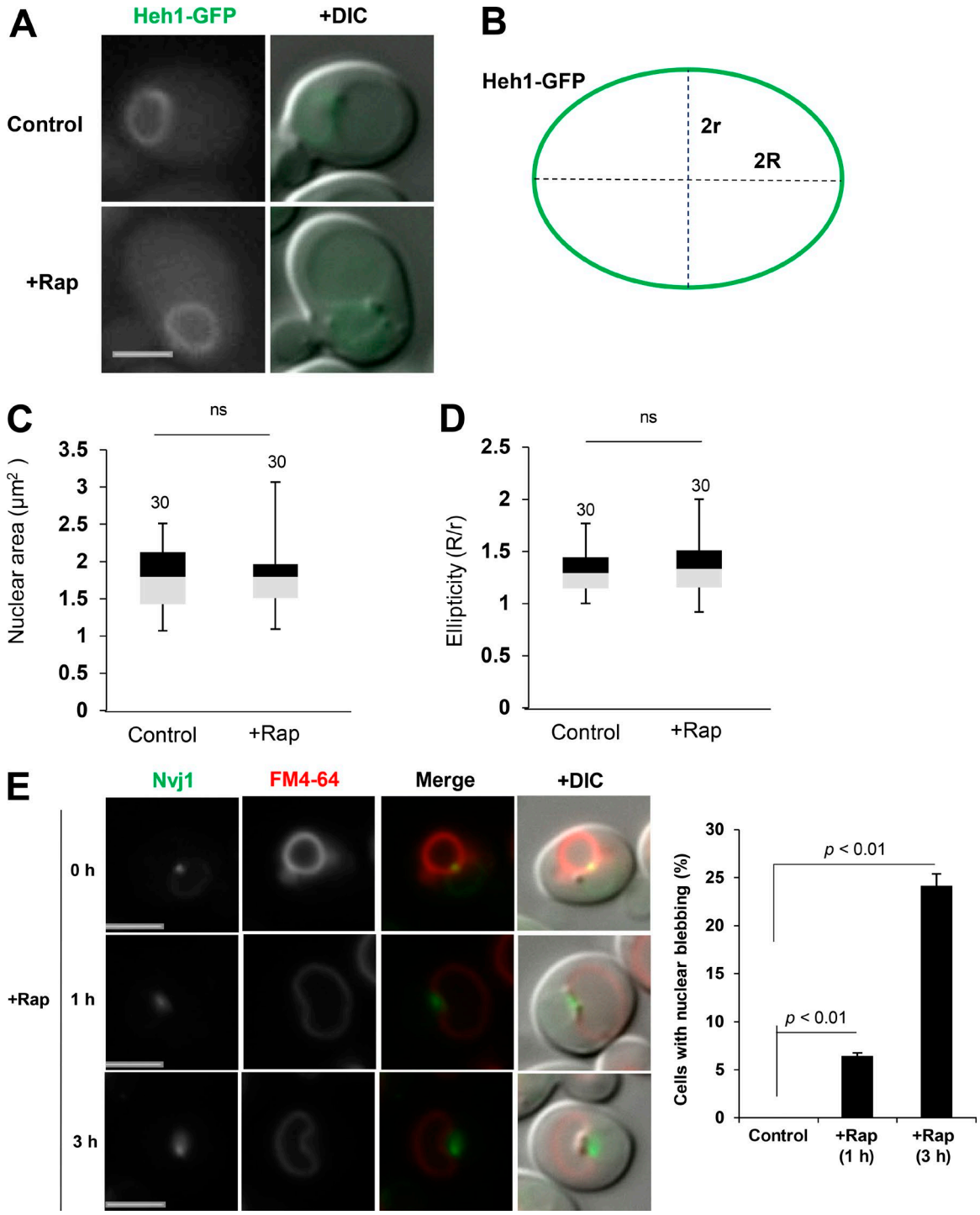

Figure S7. **Assessment of nuclear shape and size after TORC1 inactivation and nuclear invagination into the vacuole during micronucleophagy. (A)** Exponentially growing cells of strain SCU4205 (HEH1-GFP) were treated with rapamycin for 1 h. Cell images with GFP signals were captured using a fluorescence microscope. Bars, 2.5 µm. **(B)** Nuclear shape and size were monitored using Heh1-GFP localized on the nuclear membrane. We fitted nuclei to ellipses and measured the long axis (2R) and the short axis (2r) of them. **(C)** Nuclear areas (µm^2) are shown in the box plot. Numbers above the bars are sample sizes. P-values were calculated using a two-tailed Mann–Whitney *U* test. ns, not significant. **(D)** Ellipticity (R/r) of the nucleus is shown in the box plot. Numbers above the bars are sample sizes. P-values were calculated using a two-tailed Mann–Whitney *U* test. ns, not significant. **(E)** Cells of strain SCU3287 (*NVJ1-GFP*) prestained with 1 µl/ml FM4-64 (a vacuolar membrane dye) for 2 h were treated with 200 ng/ml rapamycin for the indicated times. Cells with nuclear invagination were counted in each experiments (>200 cells) and mean value (±SD) obtained from three independent experiments are shown as a percentage.

Provided online are two tables in Excel. Table S1 shows strains used in this study. Table S2 shows plasmids used in this study.