Supplementary information

1. Stock solutions

Reagents and stock solutions used in our experiments were as follows. Chemicals were purchased from FUJIFILM Wako Pure Chemical Corp (Osaka, Japan), unless stated otherwise.

1) Dulbecco's modified Eagle's medium nutrient mixture F-12 Ham at 4°C.
2) Fetal calf serum (GE Healthcare HyClone, Pittsburgh, PA) at -80°C.
3) Penicillin-streptomycin (100×) solution at 4°C.
4) Phosphate buffered saline (PBS): 137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, and 1.8 mM KH_2PO_4 at room temperature.
5) Bovine serum albumin (BSA; Takara Bio, Kusatsu, Shiga, Japan) at 4°C.
6) 10 mg/mL 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindo-carbocyanine perchlorate [DiI or DiIC18(3); Takara Bio Inc.] in DMSO at -20°C.
7) 10 mg/mL 3,3'-dioctadecyloxacarbo-cyanine perchlorate [DiO or $DiOC_{18}(3)$; Takara Bio Inc.] in DMSO at -20°C.
8) 1 mg/mL reversine (Funakoshi, Tokyo, Japan) in DMSO at -20°C.
9) 1 mg/mL pactilaxel in DMSO at -20°C.
10) 10 mg/mL Hoechst 33342 (Dojindo, Kumamoto, Japan) in H_2O at -20°C.
11) 4% paraformaldehyde in PBS at -20°C.
12) 100 mg/mL (10%) digitonin in DMSO at -20°C.

2. Antibodies

1) Anti-RanBP2 antibody was obtained from Dr. Nishitani, University of Hyogo, Japan [8].
2) Secondary antibodies were purchased from Medical and Biological Laboratories (Nagoya, Japan).

3. Supplementary procedures

3-1. Culture conditions and drug treatments

Cells were obtained from the RIKEN cell bank (Tsukuba, Japan) or American Type Culture Collection (Manassas, Virginia, USA). HeLa human cervical cancer cells, HuH-7 human hepatocarcinoma cells, and C2C12 mouse myoblastic cells were maintained in Dulbecco's modified Eagle's medium/Ham's F-12 nutrient mixture (D-MEM) with 10% fetal calf serum and antibiotics at 37 °C in a 5% CO_2 incubator using culture dishes

obtained from Violamo (AS ONE, Osaka, Japan). For pharmacological induction of micronuclei (MN), we used the following conditions: 5×10^5 cells on coverslips in 12-well plates were cultured in the presence of 1 μg/mL paclitaxel for 16 h, followed by incubation for 2 h with 200 ng/mL reversine [13, 14].

3-2. DiI/DiO staining, indirect immunofluorescence analyses, and image processing

Cells were grown on coverslips in D-MEM containing 50 μg/mL DiI/DiO at 37°C. When the dye was applied to the cells for ~10 min, substantial fluorescent signals were detected throughout the cytoplasm, including the plasma membrane. However, when DiI/DiO incubation was extended to 30–60 min, a significant amount of fluorescent signals negatively contrasted a large ovoid-shaped structure in most cells, which might represent Hoechst 33342-positive nuclei. If DiI/DiO incubation was continued for longer than 60 min, cells appeared to undergo apoptosis, because many membrane blebs emerged on the cellular cortex. When indirect immunofluorescence was not performed, the cells should be fixed after 30–60-min of DiI/DiO incubation using PBS containing 4% paraformaldehyde, followed by Hoechst 33342 staining (see below). If indirect immunofluorescence was carried out, the cells should be fixed using PBS containing 4% paraformaldehyde for 20 min at room temperature. After fixation, 100 μg/mL (0.01%) digitonin in PBS was applied for 5 min, followed by blocking with 1% bovine serum albumin in PBS for 15 min at room temperature. The cells were labeled with primary (anti-RanBP2 antibody) and secondary antibodies accordingly [14]. During the secondary antibody incubation, samples were costained with 10 μg/mL Hoechst 33342 at room temperature to visualize DNA. Samples were observed under FV1200 laser scanning confocal microscope (Olympus, Tokyo, Japan) equipped with Fluoview software. For each image, at least six slices from the z-axis were captured using 1 micron as the step size. ImageJ was used for image processing.

4. Supplementary Figure and figure legend

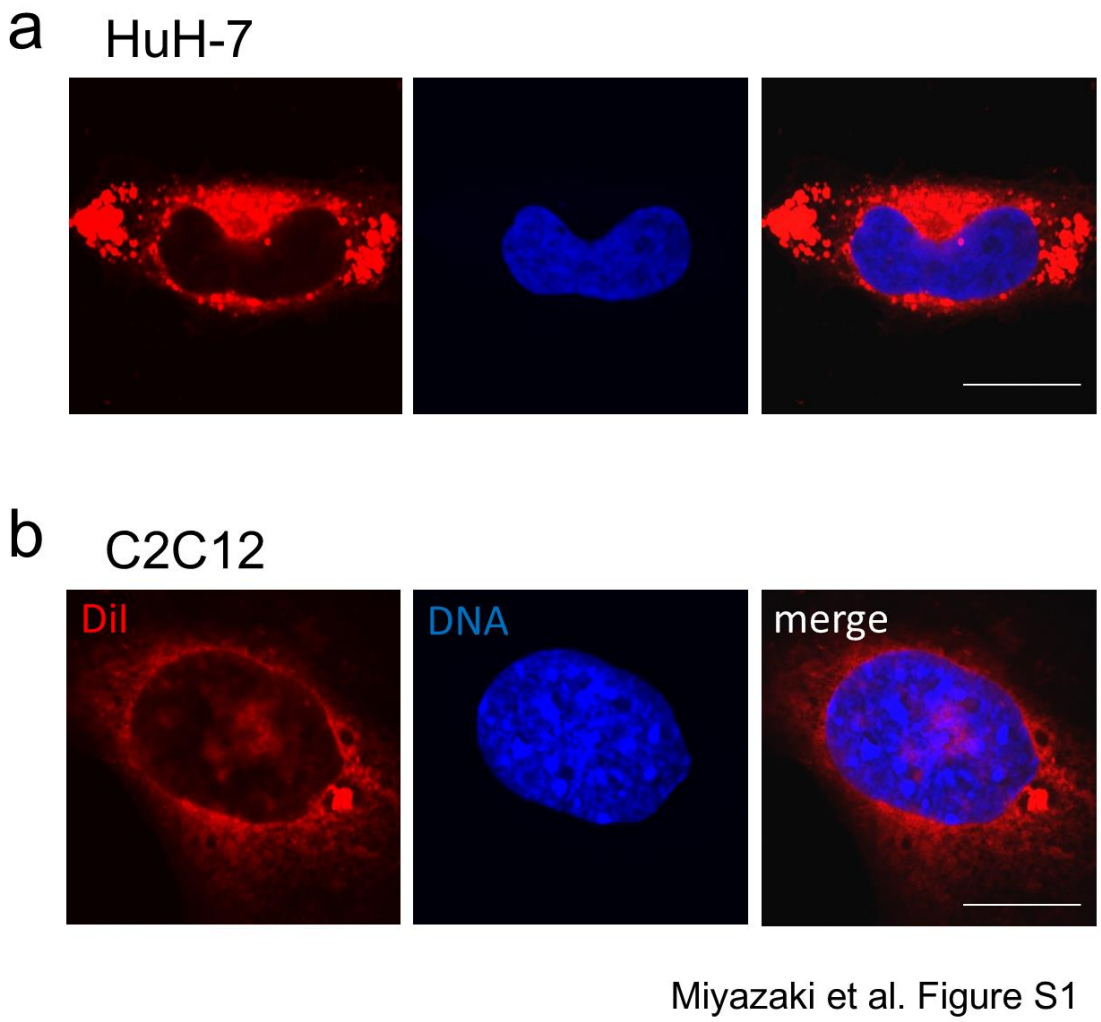

Figure S1. Application of the DiI staining procedure to other cell lines. (a) HuH-7 human hepatocyte cells were incubated with DiI, followed by fixation using paraformaldehyde. The nuclear area was visualized by DNA staining using Hoechst 33342. Fluorescence images were taken by an Olympus XI71 microscope imaging system. (b) C2C12 mouse myocyte cells were incubated with DiI, followed by fixation using paraformaldehyde. The nuclear area was outlined by DNA staining using Hoechst 33342. In a and b, all fluorescence images were taken by an FV1200 laser scanning confocal microscope imaging system. Bar: 10 μm.

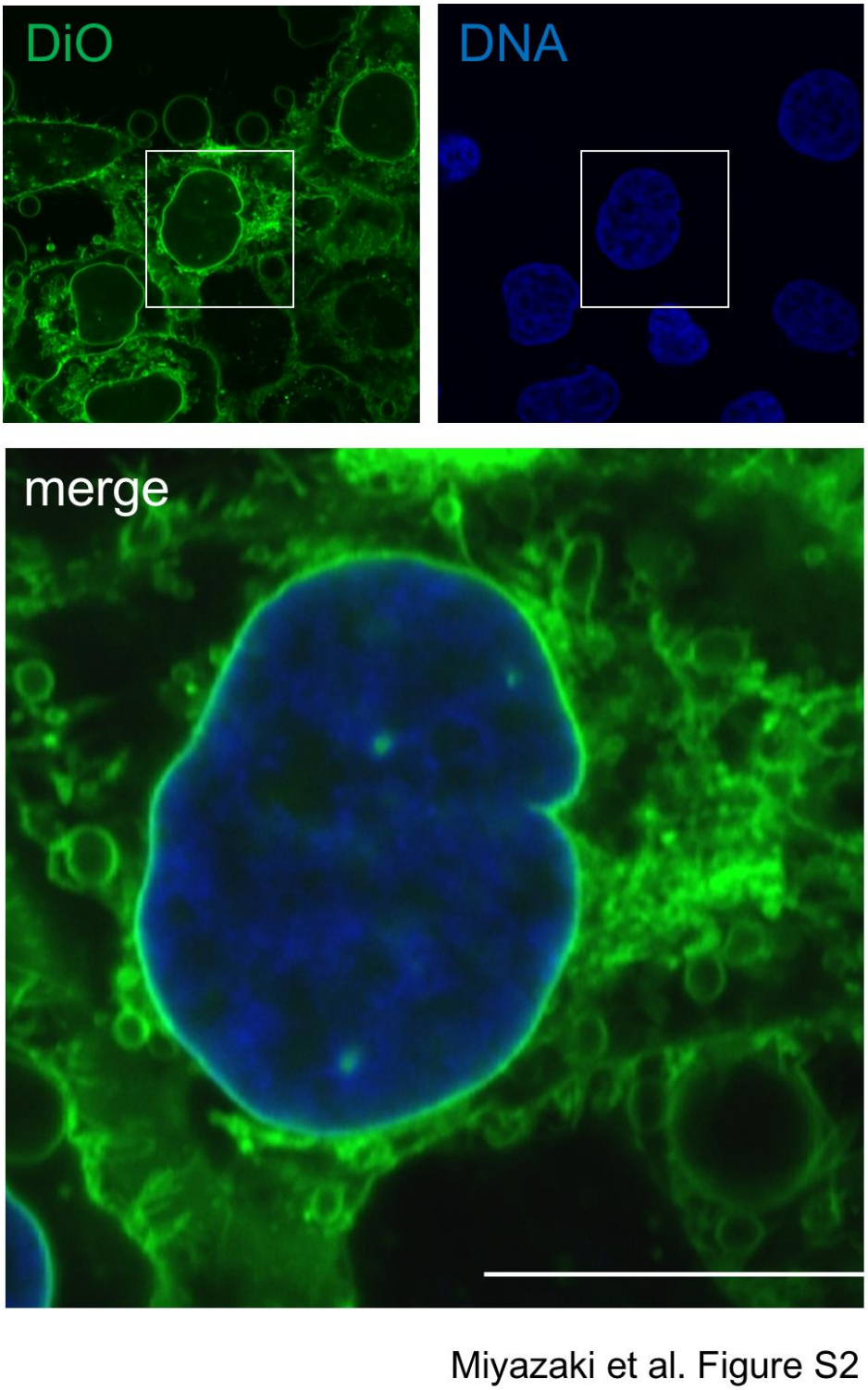

Figure S2. Fluorescence visualization of the nuclear boundary using the lipophilic dye DiO. DiO and Hoechst 33342-DNA staining patterns are represented at the upper-left and upper-right, respectively. A merged image is shown at the bottom. Bar: 10 μm.

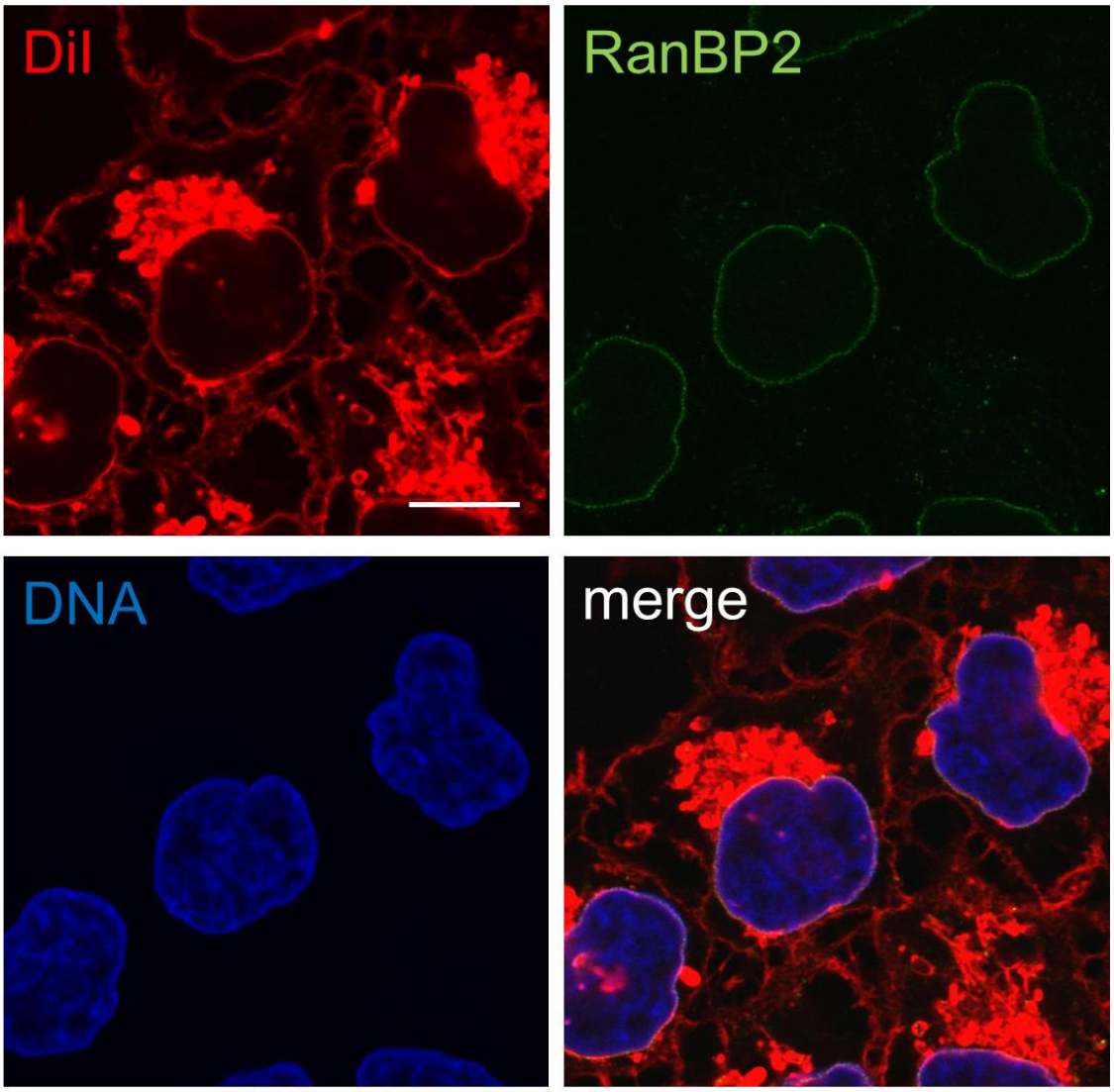

Figure S3. The DiI signals detected around the nuclear boundary appeared to be merge with the RanBP2 antibody signals, which represent the NE associated nuclear pore complex. After DiI staining (upper-left), indirect immunofluorescence analysis was performed using an anti-RanBP2 nuclear pore protein antibody (upper-right). Nuclear DNA was visualized with Hoechst 33342 (lower-left). Merged image is shown at the lower-right. All fluorescence images were taken by an FV1200 laser scanning confocal microscope imaging system. Bar: 10 μm.

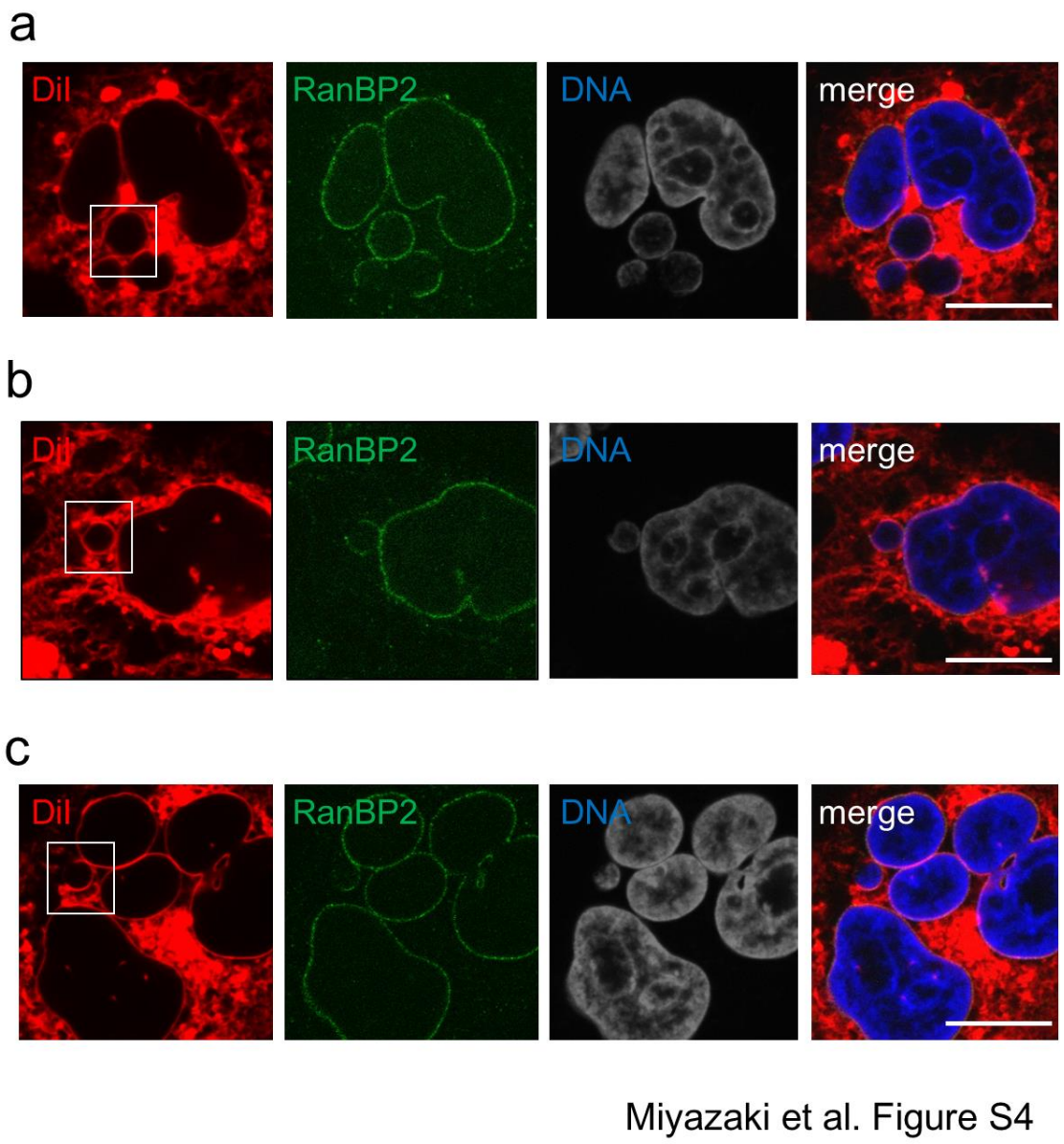

Figure S4. After MN were induced pharmacologically in HeLa cells, the nuclear boundary was visualized by the DiI and antibody costaining method. White boxed areas indicate images of micronucleus with seemingly intact nuclear boundary, which showed continuous DiI and RanBP2 signals (a and see also Figure 2a), micronucleus with the nuclear boundary region exhibiting continuous DiI signals, but deficient for RanBP2 signals (b and see also Figure 2b), and micronucleus with the nuclear boundary region deficient for both DiI and RanBP2 signals (c and see also Figure 2c), respectively. Bar: 10 μm.