JCB

Supplemental material

Ogawa and Imamoto, https://doi.org/10.1083/jcb.201712042

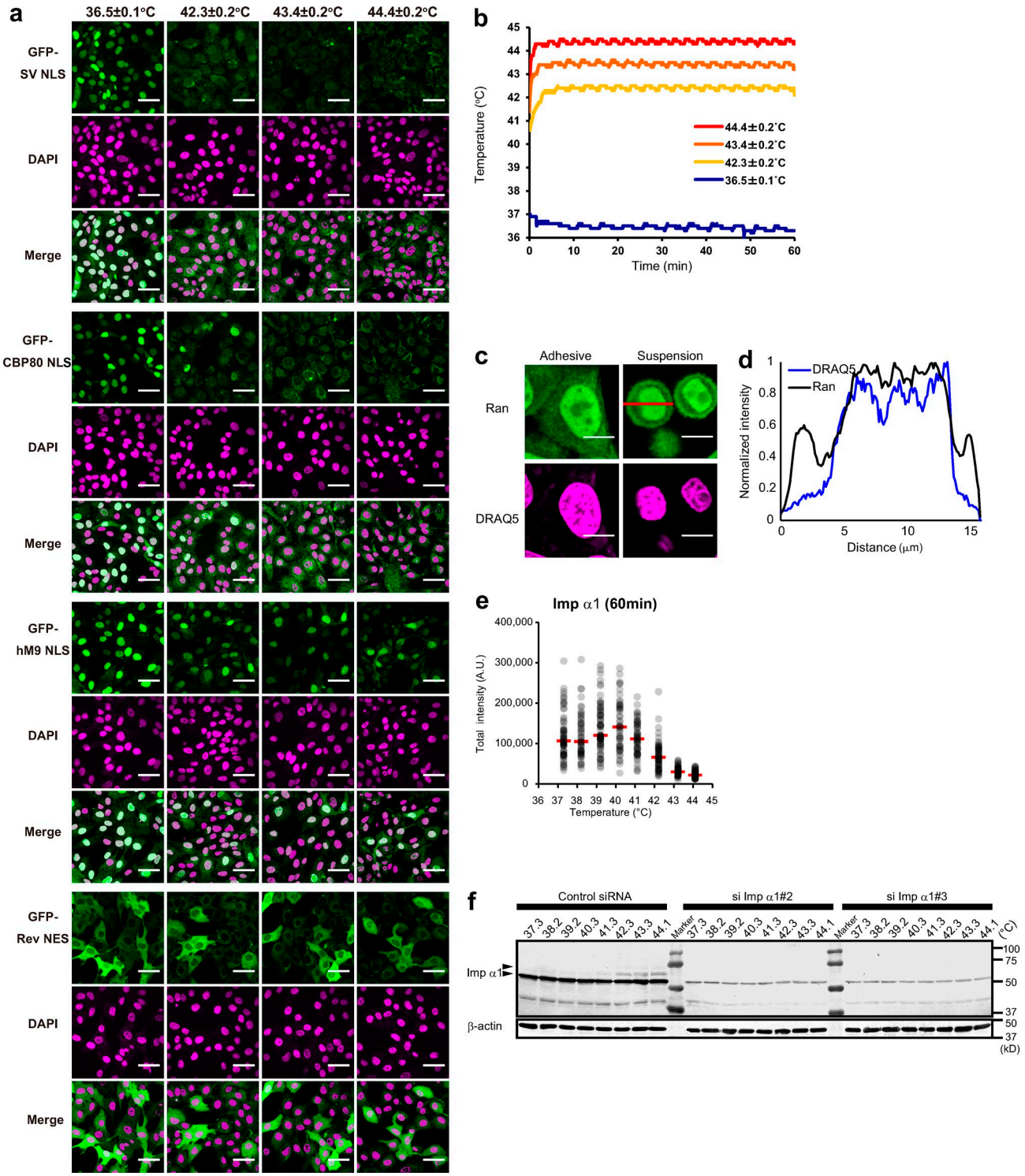

Figure S1. **Influences of temperature rise to nuclear transport efficiencies and transport factors; related to Figs. 2 and 3. (a)** hTERT-RPE1 cells stably expressing GFP-fused SV NLS, CBP80 NLS, hM9 NLS, or Rev NES were grown in 24-well glass-bottomed plates. After 24 h, the cells were exposed to various temperatures for 1 h using a conventional water bath. Then, the cells were fixed and stained with DAPI. Bars, 50 µm. **(b)** The temperature stability of the assay system using a water bath was verified using a precisely calibrated thermologger with the sensor set directly below the 24-well plate. After the temperature stabilized, the 24-well plate was set. The temperature was plotted every second over 60 min. **(c and d)** Adherent- and suspension-cultured HeLa S3 cells were stained with anti-Ran antibody and DRAQ5. Line profile of Ran distribution in a suspension-cultured cell (red line in d) is shown. Blue and black lines indicate Ran and DRAQ5 signals, respectively. Unlike under adhesion conditions, in suspension-cultured cells, Ran was partially localized near the plasma membrane independent of environmental temperatures. **(e)** After 60 min incubation at indicated temperatures, the cells were stained with anti–Imp α1 antibody. Total intensities of individual cells were plotted. The red bars show the median of >50 cells at eight temperature conditions (total n = 462). All plots in each graph were calculated from one simultaneous experiment. **(f)** 48 h after transfection of either negative control, Imp α1 #2, or Imp α1 #3 siRNA, the cells were incubated at various temperatures for 60 min. Then, endogenous Imp α1 were detected using WB.

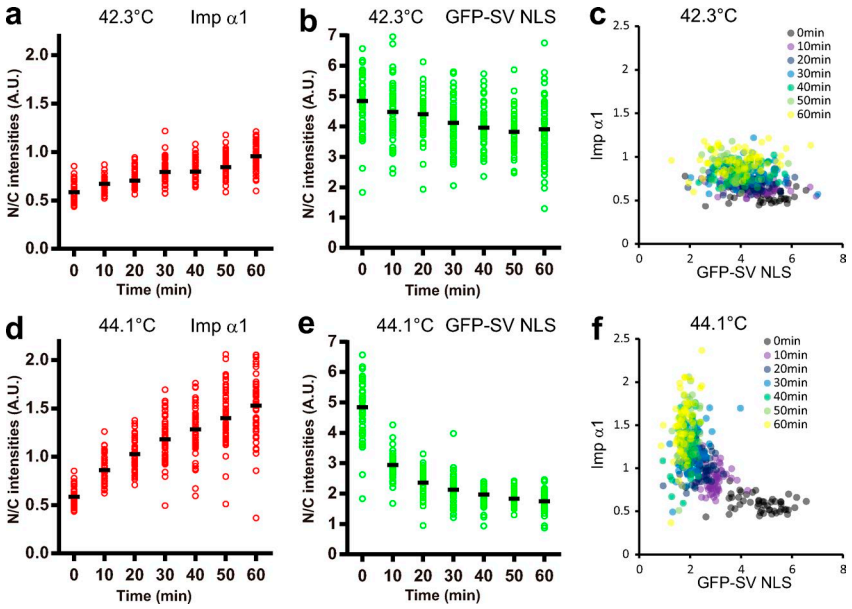

Figure S2. **Relationships between nuclear accumulations of Imp α1 and the inhibitions of GFP-SV NLS at 42.3 and 44.1°C; related to Fig. 4 (b–d). (a, b, d, and e)** After the incubation at either 42.3 **(a and b)** or 44.1°C **(d and e)**, the GFP-SV NLS–expressing stable HeLa S3 cell lines were fixed at various time points. Then, cells were stained with anti–Imp α1 antibody and DRAQ5 and imaged. N/C intensities of endogenous Imp α1 (red) and GFP-SV NLS (green) versus time (min) were plotted. Black bars show the median at each temperature. Black bars show the median of >50 cells at seven time points (total n = 403 [a and b] and 409 [d and e]). All plots in each graph were calculated from one simultaneous experiment. **(c and f)** Scatter plots of the N/C intensities of GFP-SV NLS versus endogenous Imp α1 at 42.3°C (c) or 44.1°C (f). On the x and y axes, N/C intensities of GFP-SV NLS and Imp α1 are shown, respectively. Over 50 cells were plotted at each time point (total n = 403 [c] and 409 [f]).

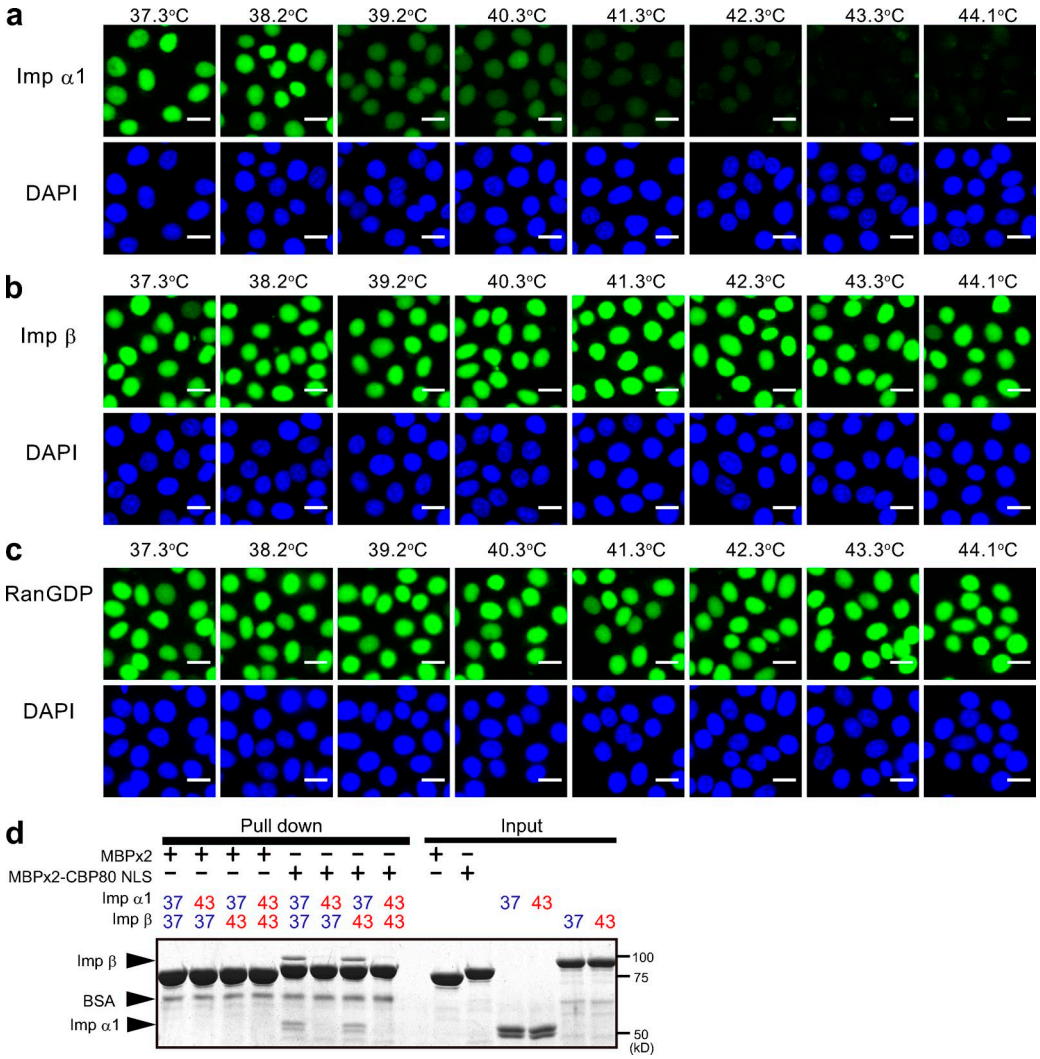

Figure S3. **Representative images from Fig. 5 (c–e) and thermosensitivity of bindings between Imp α1 and CBP80 NLS. (a–c)** Imp α1 (a), Imp β (b), or RanGDP (c) was preincubated for 1 h at indicated various temperatures. The remaining two transport factors were preincubated at 37.3°C for 1 h. Then, the import assays of GST-SV NLS–GFP were performed. Bars, 20 µm. **(d)** Imp α1 and Imp β were independently preincubated at either 37.3 or 43.3°C for 60 min. Then, these proteins and either MBPx2 or MBPx2–CBP80 NLS were incubated with amylose beads. Proteins bound to beads were analyzed by Coomassie brilliant blue staining.