THE JOURNAL OF CELL BIOLOGY

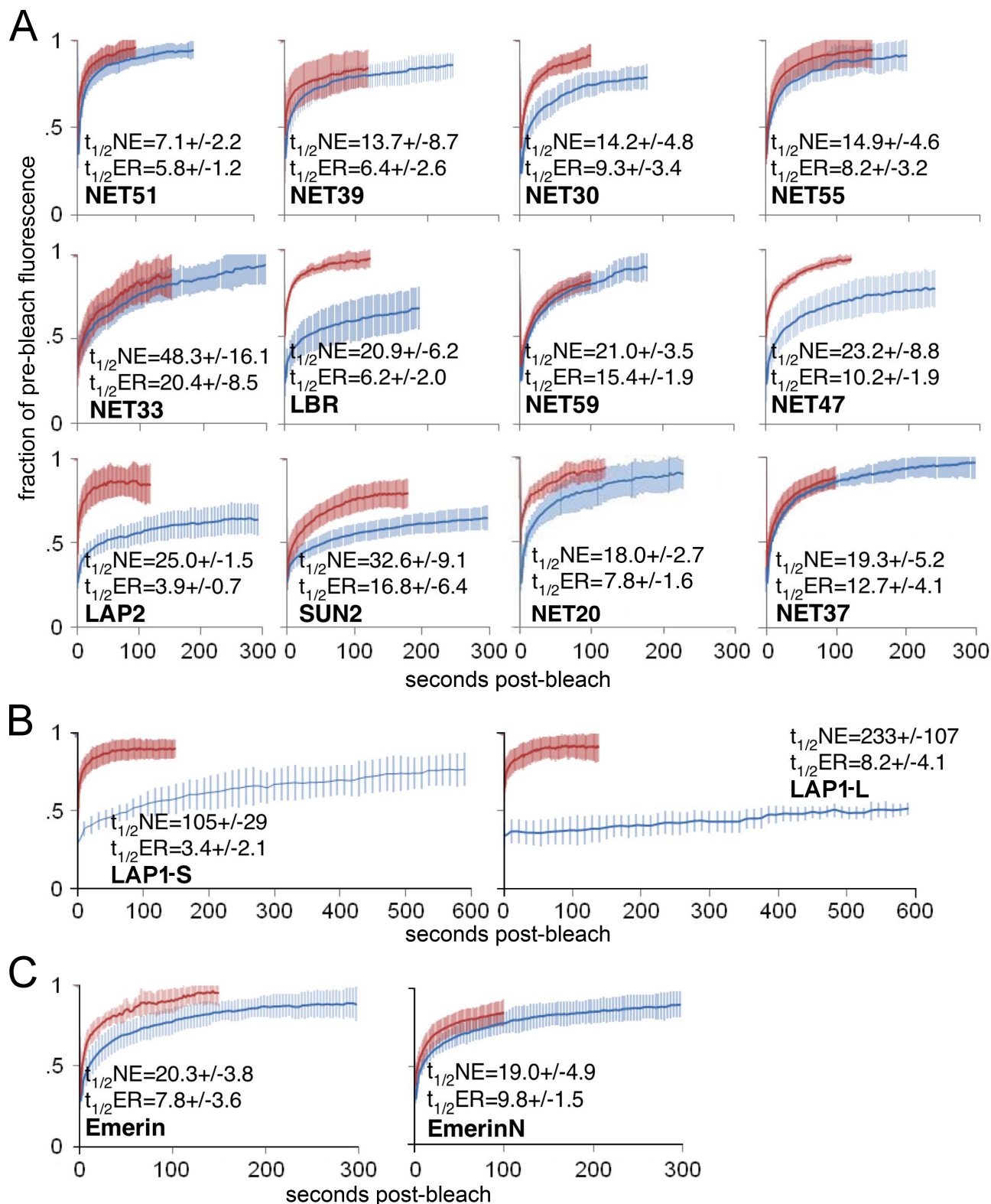**Figure S1. NE and ER FRAP for all NETs.** Graphs of FRAP curves used to generate $t_{1/2}$s for both NE (blue) and ER (red). A mean of at least eight experiments is shown for each NET, with error bars indicating SD. (A) NETs in 300-s experiments. (B) LAP1 recovery was very slow, so experiments were performed for 600 s. (C) Emerin was tested as both a C-terminal GFP fusion (left, Emerin), as was the case for NETs throughout this paper, and as an N-terminal GFP fusion (EmerinN). No notable differences were observed.
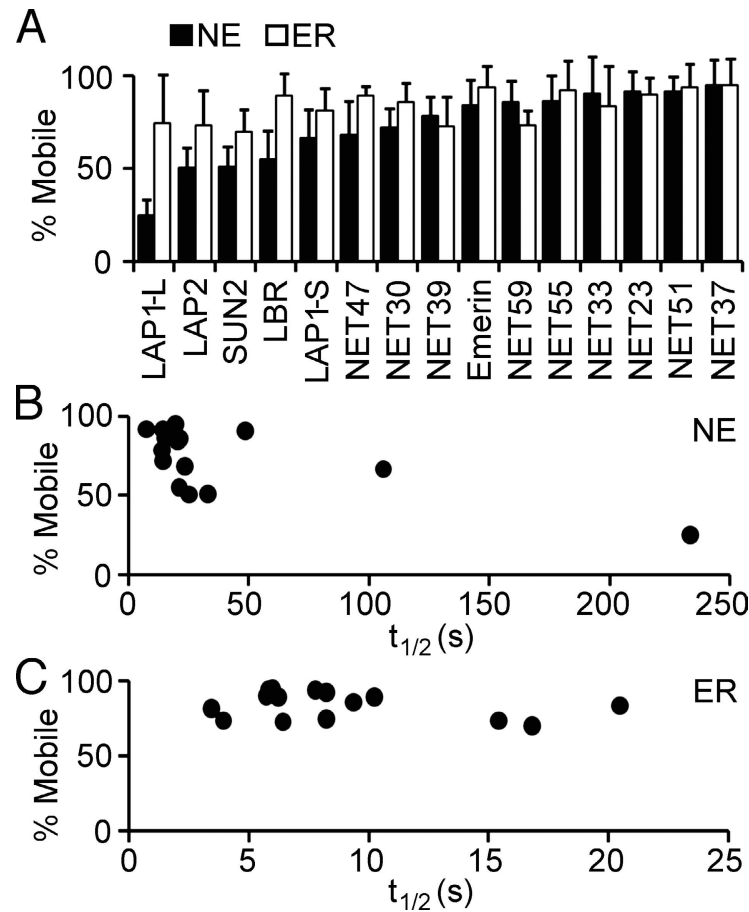
Figure S2. **Relationship between $t_{1/2}$s and mobile fractions.** The fluorescence recovery percentage compared with the prebleach intensity levels was measured for each NET in the FRAP studies shown in Fig. 2. (A) Percentage of each NET recovery (percentage mobile) in the NE (black bars) and ER (white bars) are plotted in increasing order for the NE. Although the mobile percentages covered a wide range for the NE, very little variation was observed in the ER and no correlation was observed between mobile/ immobile fractions in the two compartments. Error bars indicate SD. (B) Mobile percentages in the NE plotted against $t_{1/2}$s in the NE. A strong correlation is not observed, but some of the slower NETs also had low mobile fractions. (C) Mobile percentages in the ER plotted against $t_{1/2}$s in the ER.
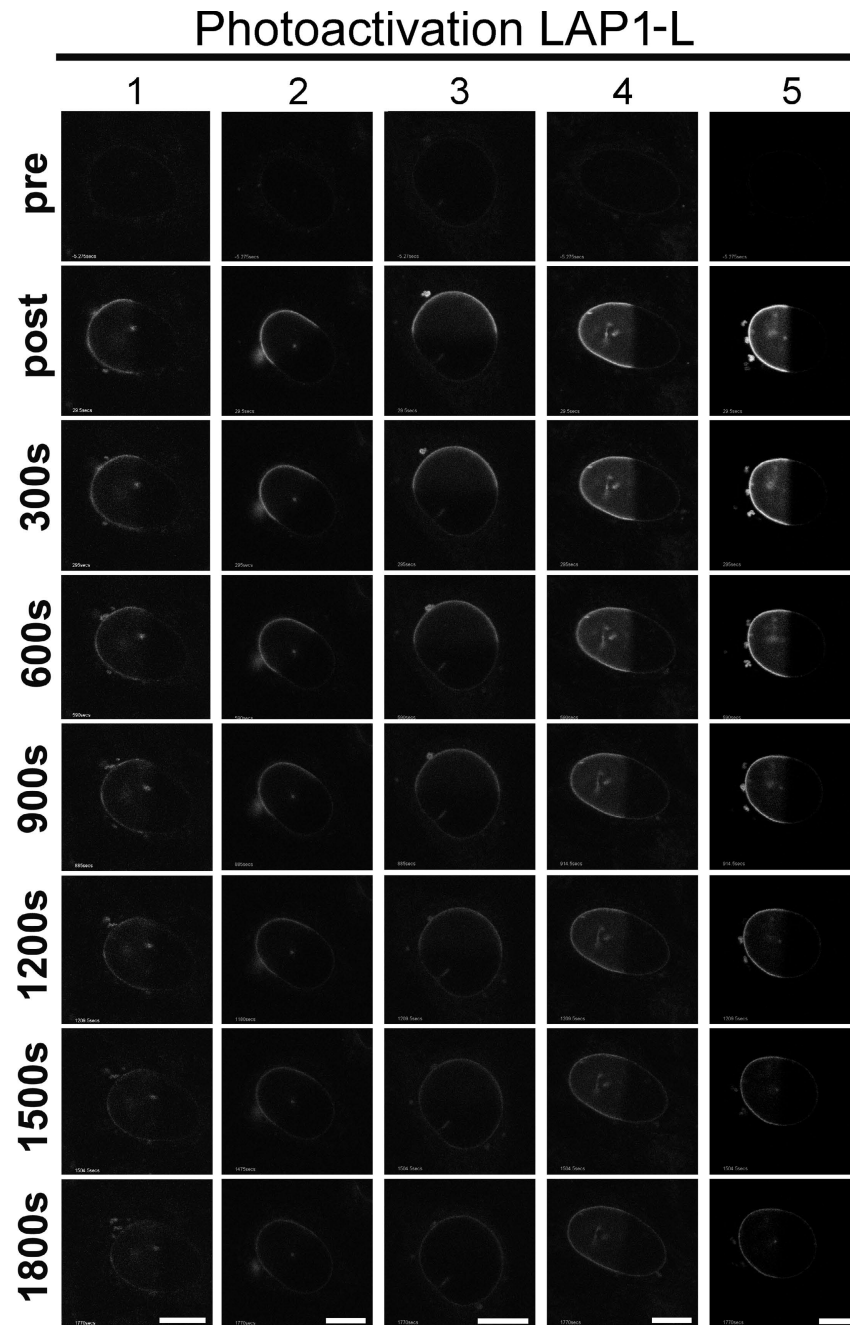
Photoactivation LAP1-L

Figure S3. PA of LAP1-L. Because, according to FRAP data, LAP1-L mobility was very slow in the NE, LAP1-L was also tested by PA to determine if FRAP was principally measuring ER-to-NE translocation or movement within the NE. Images from five videos are shown at the time points indicated on the left. NE FRAP yielded a $t_{1/2}$ of 233.5 s, whereas only a small fraction of the LAP1-L has moved at 300 s past PA. This suggests that translocation is still the dominant factor being measured by FRAP. Bars, 10 µm.
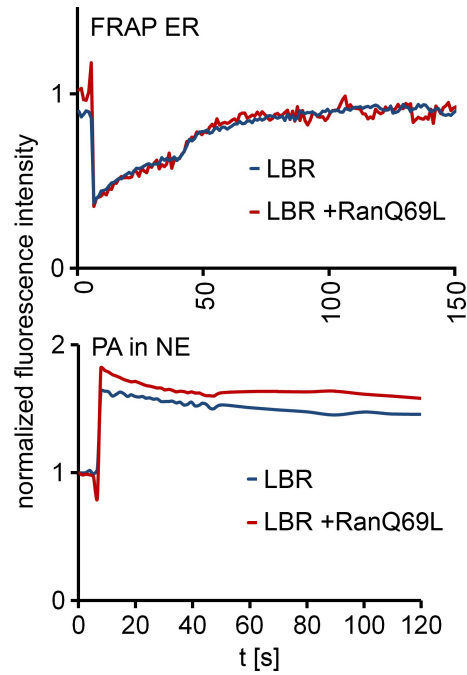
Figure S4. RanQ69L likely affects the translocation step of LBR. Although what is known of Ran function would argue that the effect of the RanQ69L mutant in doubling the $t_{1/2}$ occurs at the NPC, it was possible that Ran affected LBR binding to INM tethering points or LBR mobility in the ER. However, FRAP of LBR in the ER was unaffected by the Ran mutant, and likewise the shape of PA curves for LBR in the NE were very similar, which suggests that the effect of the Ran mutant occurs at the translocation step through the peripheral channels of the NPC.
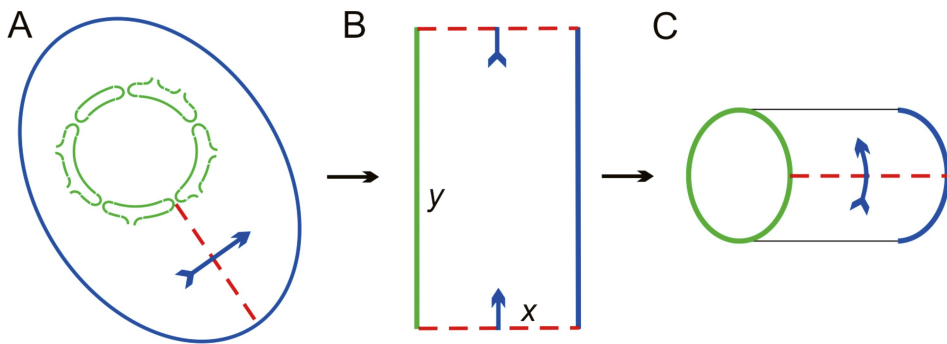

Figure S5. Construction of the spatial domain for the computational model. (A) Schematic representation of the HeLa cell cross section. The double-membrane system of NE is shown in green and the plasma membrane in blue. (B) After the collapse of the NE into a single membrane (see Materials and methods), the cell cross section in A has been cut along the red dashed line and deformed into a rectangular 2D domain. (C) Two boundaries with periodic boundary conditions shown in B as red dashed lines are equivalent to folding of the rectangle into a continuous cylinder.

Table S1. **Statistical results from the χ^2 test for Fig. 5 EM data**

Set A	Set B	P-value	χ^2
INM:ONM ratio Sec61β	INM:ONM ratio Sec61β-NLS	$P < 2.2 \times 10^{-16}$	115.509
INM:ONM ratio Sec61β-NLS	INM:ONM ratio Sec61β-NLS + RanQ69L	P = 0.315	1.0096
INM:ONM ratio LBR	INM:ONM ratio LBR + RanQ69L	P = 0.3086	1.0367

Table S2. **Statistical results of the Kolmogorov-Smirnov test against the hypothesis that any two datasets are different for Fig. 7 data**

Set A	Set B	P-value	D-value
Mitochondrial FGi	Mitochondrial FGo	$P = 0.1467$	0.1633
NE FGi	NE FGo	$P = 1.056 \times 10^{-13}$	0.392
NE FGt	Mitochondrial FGt	$P = 4.77 \times 10^{-5}$	0.2847
Mitochondrial AAi	Mitochondrial AAo	$P = 0.001176$	0.2755
NE AAi	NE AAo	$P < 2.2 \times 10^{-16}$	0.5377
NE AAi	Mitochondrial AAi	$P = 0.07058$	0.1596
NE AAo	Mitochondrial AAo	$P = 7.385 \times 10^{-10}$	0.4067

Mitochondrial indicates transmembrane proteins identified by proteomics (Mootha et al., 2003), whereas NE represent the 199 NETs identified by proteomics (Schirmer et al., 2003). FG indicates the number of FG pairings in the protein, whereas AA indicates number of amino acids; i, within nucleoplasmic/cytoplasmic face of protein according to membrane topology prediction; o, lumenal face of protein according to membrane topology prediction; t, total protein minus transmembrane segment.

References

Mootha, V.K., J. Bunkenborg, J.V. Olsen, M. Hjerrild, J.R. Wisniewski, E. Stahl, M.S. Bolouri, H.N. Ray, S. Sihag, M. Kamal, et al. 2003. Integrated analysis of protein composition, tissue diversity, and gene regulation in mouse mitochondria. *Cell*. 115:629–640. doi:10.1016/S0092-8674(03)00926-7

Schirmer, E.C., L. Florens, T. Guan, J.R. Yates III, and L. Gerace. 2003. Nuclear membrane proteins with potential disease links found by subtractive proteomics. *Science*. 301:1380–1382. doi:10.1126/science.1088176