SUPPLEMENTAL DATA

Supplemental Experimental Procedures

Cell culture and EGF treatment- All cells were maintained in Dulbecco's modified Eagle's medium (DMEM)/F12 supplemented with 10% fetal bovine serum and antibiotics. In this series of experiments, cells were treated with 50 ng/ml EGF under serum-starved conditions for 24 hr.

Antibodies and chemicals- The following antibodies and chemicals were purchased for our study: anti-EGFR antibodies (Santa Cruz; NeoMarker); anti-Importin β, anti-calregulin, and anti-calnexin antibodies (Santa Cruz); anti-CD44 and anti-emerin antibodies (NeoMarker); anti-tubulin antibodies, mouse IgG and recombinant human EGF (Sigma); anti-lamin B antibodies (Calbiochem); anti-lamin A+C antibodies (Chemicon); anti-Sec61α and anti-Sec61β antibodies (Upstate); anti-Nup62 antibodies (BD); and streptavidin-agarose beads (Fluka). For siRNA experiments, siRNA oligonucleotides targeting importin β, Nup62, Sec61β, and nonspecific siRNA control were purchased from Drarmacon. The siRNA sequences are importin β-siRNA (#1): 5'-GGACUUAUGUACAGCAUUU-3'; importin β-siRNA (#2): 5'-GGAAGGAUGUACUGAAAUU-3'; Sec61β-siRNA (#1): 5'-GCUCAAAGUUGGCCCUGUU-3'; Sec61β-siRNA (#2): 5'-GCAAGUACACUCGUUCGUA-3'; Sec61β-siRNA (#3): 5'-CUGUAAGCUUGCUGUUUUAUU-3'.

Confocal microscopy- Cultured cells were washed three times with PBS, fixed in 4% paraformaldehyde for 15 min, permeabilized with 0.5% Triton X-100 for 15 min, and incubated with 5% bovine serum albumin for 1 h. Cells were then incubated with the primary antibodies overnight at 4°C. Cells were washed with PBS and then further incubated with the appropriate secondary antibody diluted at 1:500 and tagged with fluorescein isothiocyanate, Texas red, or Alexa 647 (Molecular Probes) for 45 min at room temperature. Nuclei were stained with DAPI before mounting. Confocal fluorescence images were captured using a Zeiss LSM 710 laser microscope. In all cases, optical sections through the middle planes of the nuclei as determined using nuclear counterstaining were obtained.

Immuno-electron microscopy (Immuno-EM)- Cells were fixed in 2% paraformaldehyde containing 0.1% glutaraldehyde for 1 hr, permeabilized with 0.5% Triton X-100 for 15 min, and incubated with 5% bovine serum albumin for 15 min. Cells were then incubated with the primary antibodies overnight at 4°C. Cells were washed with PBS and then further incubated with a gold particle-labeled secondary antibody (Amersham Biosciences) overnight at 4°C for immunogold labeling. After post-fixation with 2% glutaraldehyde, cells were washed and stained with 1% Millipore-filtered uranyl acetate. The samples were dehydrated in ethanol at increasing concentrations, infiltrated, and embedded in Spurr's low-viscosity medium. The samples were then polymerized in a 70°C oven for 2 days. The glass coverslips were removed by dipping the blocks in liquid nitrogen. Ultrathin sections were cut using a Leica Ultracut microtome (Leica, Deerfield, IL), stained with uranyl acetate and lead citrate using a Leica EM Stainer, and examined using a JEM 1010 transmission electron microscope (JEOL Ltd., Peabody, MA) at an accelerating voltage of 80 kV. Digital images were obtained using an AMT Imaging System (Advanced Microscopy Techniques Corp, Danvers, MA).

Supplemental Figure Legends

Figure S1. EGF-dependent nuclear transport of EGFR. EGF induced nuclear translocation of EGFR using three-dimensionally reconstructed z-stack images. HeLa cells treated with or without EGF were immunostained with EGFR (green) and analyzed using confocal microscopy. All nuclear areas were

confirmed using DAPI staining (red). Images corresponding to *xz* sections reconstructed along white solid lines are displayed at the bottom of each *xy* section. Images corresponding to *yz* sections reconstructed along green solid lines are displayed at the right side of each *xy* section. Each dashed line indicates the vertical position of each displayed *xy* section. Bar, 5 μm.

Figure S2. Knockdown of Nup62 by Nup62 siRNA in HeLa cells reduced EGF-dependent EGFR translocation to the INM and NP. Cells were transfected with Nup62 siRNA (+) or a nonspecific control siRNA (-) using electroporation. The diagrams shown in the middle panel indicate the relative densities of the immunoblots as quantified using the ImageJ software program (1.38x).

Figure S3. Association of translocon Sec61β and EGFR in the INM in A431 cells. EGFR was associated with Sec61β in the INM in A431 cells in response to EGF in a time-dependent manner. The INM and NP portions of cells treated with EGF in a different time period were isolated using subnuclear fractionation, immunoprecipitated with anti-EGFR antibodies and subjected to immunoblotting with the indicated antibodies.

Figure S4. Co-localization of EGFR and Sec61β in the INM. *A*, EGFR and Sec61β were distributed to the INM. INM-Sucrose fractions were purified using sucrose gradient as described in *Fig. 2A* and subjected to immunoblotting with the indicated antibodies. The arrow above the panels indicates the direction of the gradient from top to bottom. *B*, MDA-MB-468 cells were immunostained with EGFR (green), Sec61β (red), and emerin (blue) and analyzed using confocal microscopy. Bar, 5 μm.

Figure S5. Localization of Sec61β in MDA-MB-468 cells. MDA-MB-468 cells were treated with EGF and subjected to immuno-EM using two different primary antibodies against Sec61β. PM, plasma membrane; Cy, cytoplasm; NP, nucleoplasm; NE, nuclear envelope; INM, inner nuclear membrane; ONM, outer nuclear membrane. Bar, 1 μm.

Figure S6. Knockdown of Sec61β prevented EGF-dependent transport of EGFR from the INM to the NP in A431 cells. Knockdown of Sec61β partially prevented EGF-dependent transport of EGFR from the INM to the NP. A431 cells were transfected with Sec61β siRNA (siRNApool-Sec61β) (+) or a nonspecific control siRNA (-) using electroporation.

Fig. S1

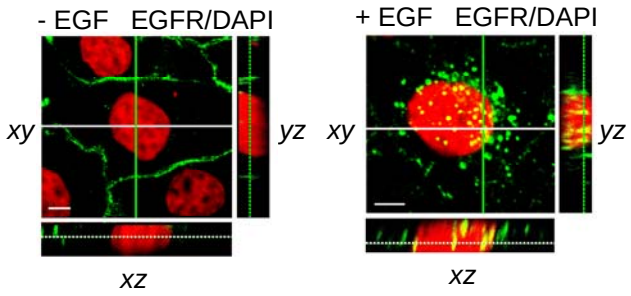


Fig. S2

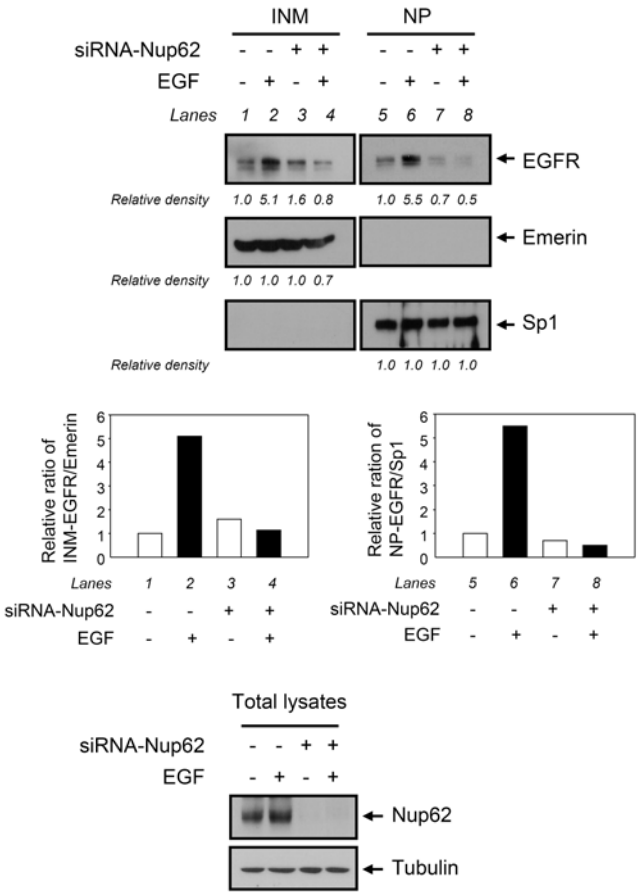

INM
NP
siRNA-Nup62 - - + + - - + +
EGF - + - + - + - +
Lanes 1 2 3 4 5 6 7 8
EGFR
Relative density 1.0 5.1 1.6 0.8 1.0 5.5 0.7 0.5
Emerin
Relative density 1.0 1.0 1.0 0.7
Sp1
Relative density 1.0 1.0 1.0 1.0
Relative ratio of INM-EGFR/Emerin
Relative ration of NP-EGFR/Sp1
0 1 2 3 4 5 6
Lanes 1 2 3 4
siRNA-Nup62 - - + +
EGF - + - +
Lanes 5 6 7 8
siRNA-Nup62 - - + +
EGF - + - +
Total lysates
siRNA-Nup62 - - + +
EGF - + - +
Nup62
Tubulin

Fig. S3

A431 cells

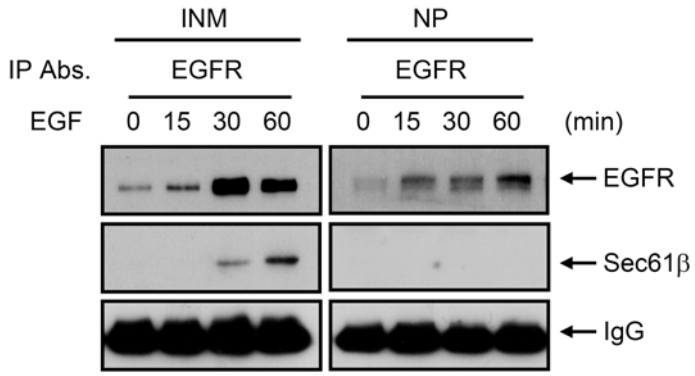
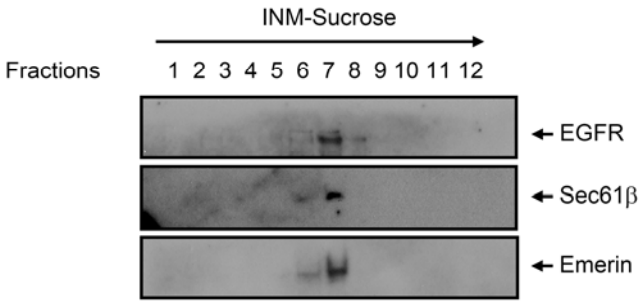


Fig. S4

A


INM-Sucrose
Fractions
1 2 3 4 5 6 7 8 9 10 11 12
EGFR
Sec61β
Emerin

B

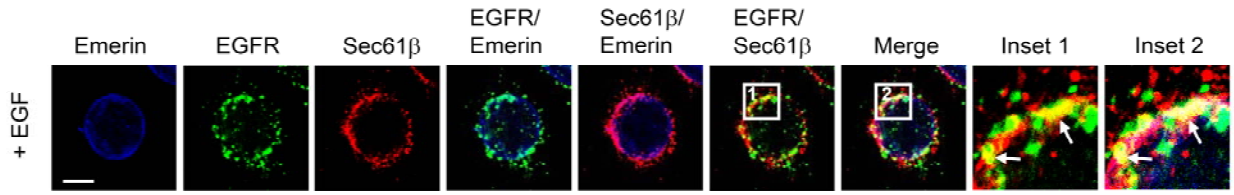

Emerin
EGFR
Sec61β
EGFR/ Emerin
Sec61β/ Emerin
EGFR/ Sec61β
Merge
Inset 1
Inset 2
+ EGF
1
2

Fig. S5

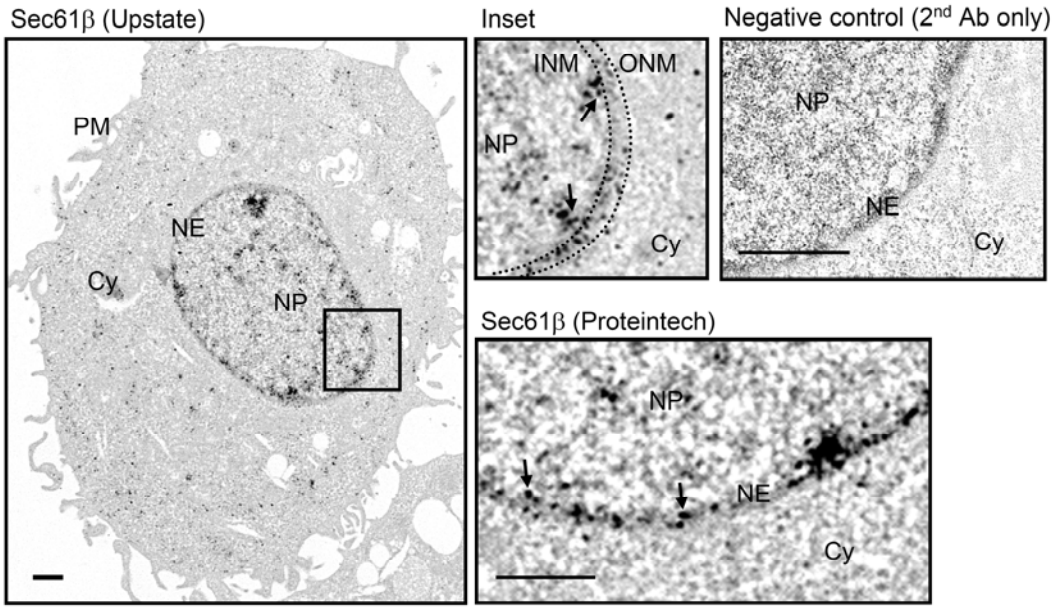

Sec61β (Upstate)
PM
NE
Cy
NP
Inset
INM
ONM
NP
Cy
Negative control (2nd Ab only)
NP
NE
Cy
Sec61β (Proteintech)
NP
NE
Cy

Fig. S6

A431 cells