Supplemental Figure S1. The FG-repeat region of Nup98 does not grossly affect the importin alpha/beta-mediated nuclear import. NIH 3T3 cells were transfected with EGFP, EGFP-Nup98 or EGFP-Nup98 (A.A.1-480) expression vectors together with mRFP-NLS(SV40) expression vector. After 48 h, the cells were fixed, washed and mounted for microscopic observation. The nuclei were visualized by DAPI staining.

Supplemental Figure S2. Subcellular localization of Nup98FG-EGFP, Nup98-HoxA9-EGFP, or HoxA9HD-EGFP. HeLa cells were transfected with plasmids encoding Nup98FG-EGFP (A.A.1-480 of Nup98 fused to EGFP at C-terminus), Nup98-HoxA9-EGFP (Nup98FG fused to homeodomain of HoxA9 and EGFP at C-terminus), or HoxA9HD-EGFP (homeodomain of HoxA9 fused to EGFP at C-terminus). After 48 h, the cells were fixed, permeabilized and immunostained with anti-Crm1. DAPI staining was used to visualize nuclei. Bar, 5 μm.

Supplemental Figure S3. Nup98-HoxA9-EGFP is not targeted to NPC. HeLa cells transfected with Nup98-HoxA9-EGFP were immunostained with mAb414, an anti-NPC monoclonal antibody. DAPI staining was used to visualize nuclei. A merged image of Nup98-HoxA9-EGFP (green) and mAb414 staining (red) is shown. Bar, 5 μm.

Supplemental Figure S4. Nup98-HoxA9-EGFP partially colocalizes with Crm1. HeLa cells expressing Nup98-HoxA9-EGFP were immunostained with anti-Crm1. Endogenous Crm1 (red) partially colocalized with microdots of Nup98-HoxA9-EGFP (blue). Arrows indicate microdots exhibiting colocalization. Bar, 5 μm.

Supplemental Figure S5. The Nup98 FG-repeat domain binds to Crm1. Purified recombinant GST, GST-Nup98 (A.A. 1-360) or GST-Nup98 (A.A. 451-880) were incubated with the indicated combinations of Crm1 (200 nM), RanQ69L (1 μM), NES peptide (Rev) (6 μM) for 2 h at 4°C, and precipitated using glutathione-Sepharose 4B beads. The bound proteins were analyzed by immunoblotting with anti-Crm1.

Supplemental Figure S6. Purified recombinant proteins. Bacterially expressed recombinant proteins (GST, GST-Nup98, RanBP3, Crm1, Ran-Q69L) were purified and analyzed by SDS-PAGE and Coomassie staining.

Supplemental Figure S7. Microinjection of anti-Nup98 partially inhibits nuclear import of co-injected conventional NLS-substrate. HeLa cells were cytoplasmically microinjected with anti-Nup62, or anti-Nup98 together with GST-GFP-NLS and Alexa568-conjugated anti-sheep IgG

(injection marker). After 15 min of incubation at 37°C, the cells were fixed, washed and mounted for microscopic observation.

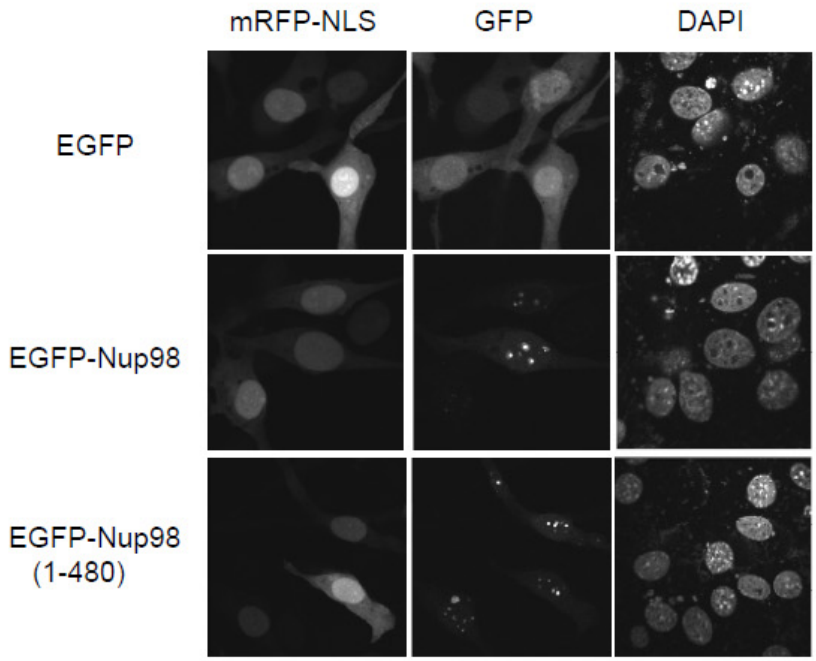

Figure S1

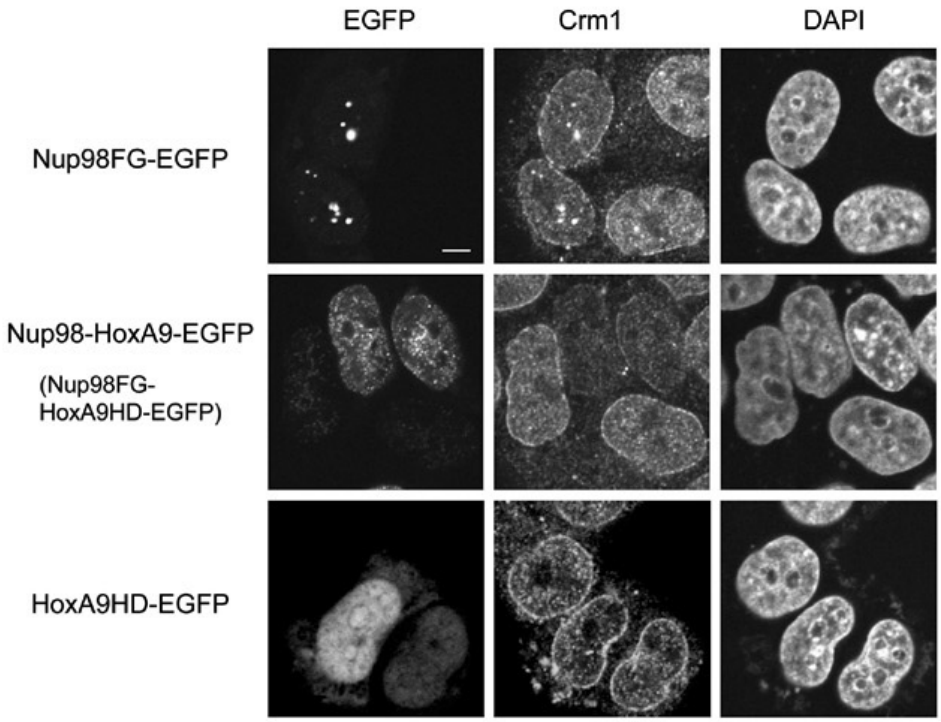
EGFP
Crm1
DAPI
Nup98FG-EGFP
Nup98-HoxA9-EGFP
(Nup98FG-
HoxA9HD-EGFP)
HoxA9HD-EGFP

Figure S2

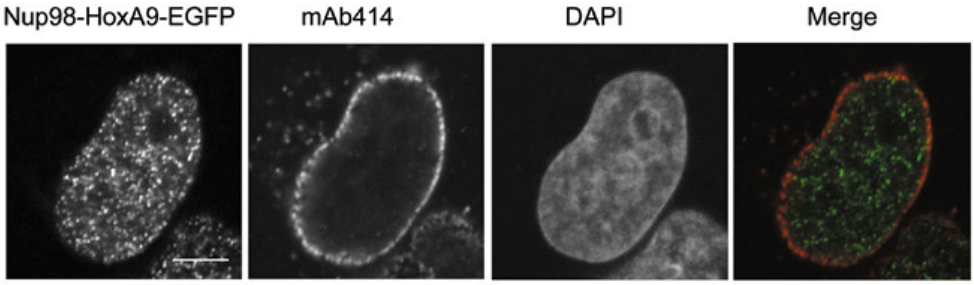

Figure S3

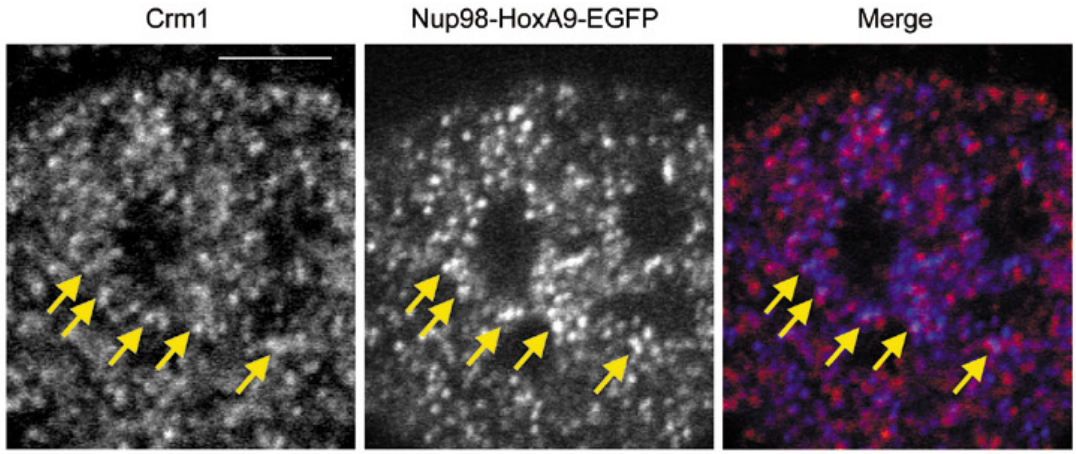

Figure S4

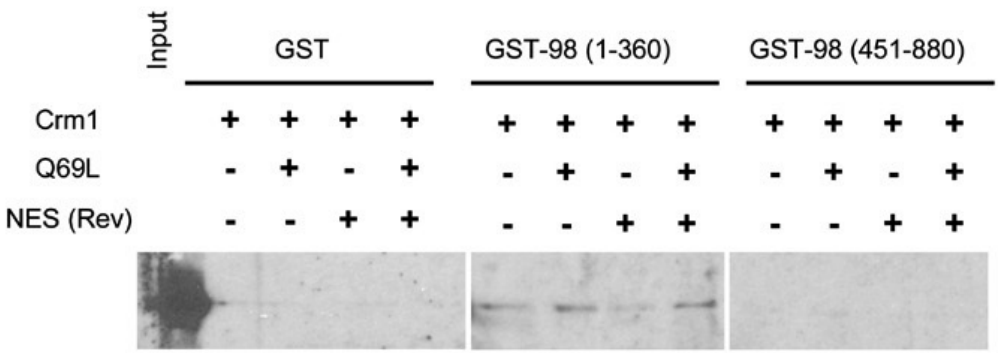

Figure S5

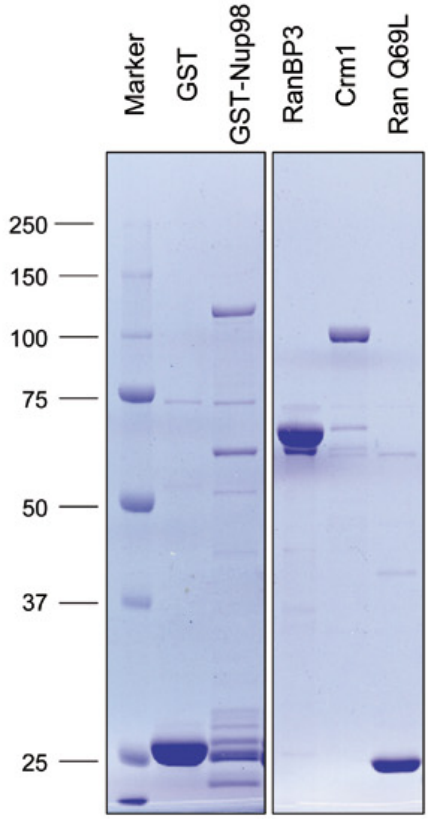
Marker
GST
GST-Nup98
RanBP3
Crm1
Ran Q69L
250
150
100
75
50
37
25

Figure S6

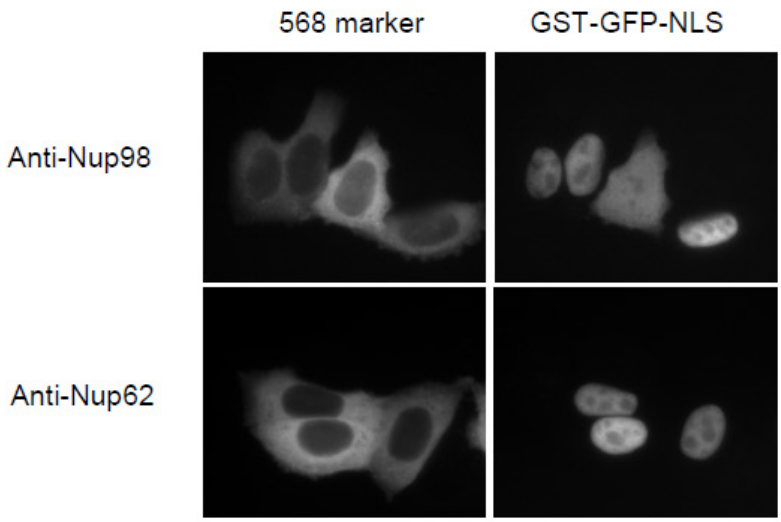

Figure S7