SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Expression Constructs - The coding region of human NC2β as *Sal*I/*BamH*I fragment into the respective sites of pEGFP-EGFP-GST-C1 (modified pEGFP-C1 in which GST was inserted N-terminally of the MCS as *Bgl*II/*Xho*I fragment and in which a second EGFP was inserted at the N terminus as *Nhe*I fragment).

Site-directed Mutagenesis - To generate EGFP-EGFP-GST-NC2β-(S105/106E) site-directed mutagenesis was performed according to the Quick Change Site-directed Mutagenesis Kit protocol (Stratagene). The following oligonucleotides were used: 5'-TTAAAAAGAAGAAAGGCCGAGGAGCGTTTGGAAAAC CTTGGC-3' (sense) and 5'-GCCAAGGTTTTCCAAACGCTCCTCGGCCTTTCTTCTTTTTAA-3' (antisense).

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Fig. S1. **The subcellular localization of the individual NC2 subunits depends on the position of the fluorescent fusion protein in transfected HeLa P4 cells.** *A* and *B*, HeLa P4 cells were transiently transfected with plasmid DNA encoding NC2 subunits (i) C-terminally fused to EGFP-EGFP or RFP (*A*) and (ii) N-terminally fused to EGFP or RFP (*B*). The subcellular distribution of the gene products was examined 24 h post transfection by direct fluorescence. The DNA was counterstained with Hoechst. *A*, tandem EGFP or mono RFP fused to the C terminus caused a dominant cytoplasmic distribution of the individual NC2 subunits. *B*, the N-terminal fusion of EGFP to NC2α and of RFP to NC2β caused a nuclear localization and a fairly homogeneous distribution, respectively.

Supplemental Fig. S2. **Schematic representation of the location of import and export signals present in NC2 subunits.** NC2α and NC2β dimerize via histone-fold domains in their amino terminal regions. The histone-fold domain consists of three helices (H1, H2, and H3) that are separated by short loops. Each NC2 subunit, NC2α and NC2β, contains a monopartite classical nuclear localization signal (cNLS, shown as green triangles). In addition, NC2β also exhibits a leucine-rich nuclear export signal (NES, shown as red triangle). aa, amino acids.

Supplemental Fig. S3. **Binding of exportin1 to the NC2 complex is not RanGTP-dependent.** GST-NC2β/His-NC2α and GST-NC2α/His-NC2β complexes were incubated with recombinant exportin1 (exp1) in the absence or presence of RanGTP (for details see "Experimental Procedures"). For a negative control exp1 was omitted (mock). After binding to glutathione-Sepharose bound fractions were washed, analyzed by SDS-PAGE and Coomassie-stained. Input of exp1 corresponds to 10% of the protein that was used. Mw, molecular weight; aa, amino acids.

Supplemental Fig. S4. **Nuclear accumulation of the NC2 complex via importin α/β requires the cNLSs of both subunits.** *A*, HeLa P4 cells were transiently co-transfected with plasmid DNA encoding wild type (wt) and mutated EGFP-NC2α and RFP-NC2β, respectively. The subcellular distribution of the gene products was examined 24 h post transfection by direct fluorescence. The DNA was counterstained with Hoechst. Co-expression of wild type EGFP-NC2α and RFP-NC2β resulted in a nuclear co-localization shown in yellow (merge, *top*). Mutation of either the cNLS of NC2α (K5A) or the cNLS of NC2β (R101A) reduced the nuclear accumulation leading to a homogeneous localization of both subunits (*middle*). Nuclear import of the EGFP-NC2α/RFP-NC2β complex was efficiently blocked when the cNLSs of both subunits were mutated (*bottom*). *B*, for quantitative analysis, the mean green (EGFP) and red (RFP) fluorescence value in the nucleus and cytoplasm of 20 cells that co-expressed EGFP-NC2α and RFP-NC2β was measured using the ImageJ Software (NIH). After subtraction of the background value the percentage of nuclear localization of the different NC2 complexes was calculated. Bars indicate the standard deviation from the mean.

Supplemental Fig. S5. **Importin13 also mediates nuclear transport of the NC2 complex.** *A*, HeLa P4 cells were transiently co-transfected with plasmid DNA encoding mutated EGFP-NC2α (K5A), RFP-NC2β (R101A), and flag-tagged import receptors. The subcellular distribution of the gene products was examined 24 h post transfection by direct fluorescence of the RFP and EGFP fusion proteins. The overlap between the green EGFP fusion protein and the red RFP fusion protein is shown in yellow (merge). The DNA was counterstained with Hoechst. EGFP-NC2α-(K5A) and RFP-NC2β-(R101A) both predominantly remained in the cytoplasm of co-transfected cells. While co-expression of importin13 led to a strong nuclear accumulation of the NC2 complex, co-expressed importinβ, importin5, importin7, and importin9 did not affect the subcellular distribution of the NC2 complex. *B*, quantification of nuclear import of the cNLS deficient NC2 complex. The mean fluorescence value of co-localized EGFP-NC2α-(K5A) and RFP-NC2β-(R101A) was measured in 15 cells using the ImageJ Software (NIH). The percentage of nuclear localization in the presence of co-expressed import receptors was calculated. Bars indicate the standard deviation from the mean.

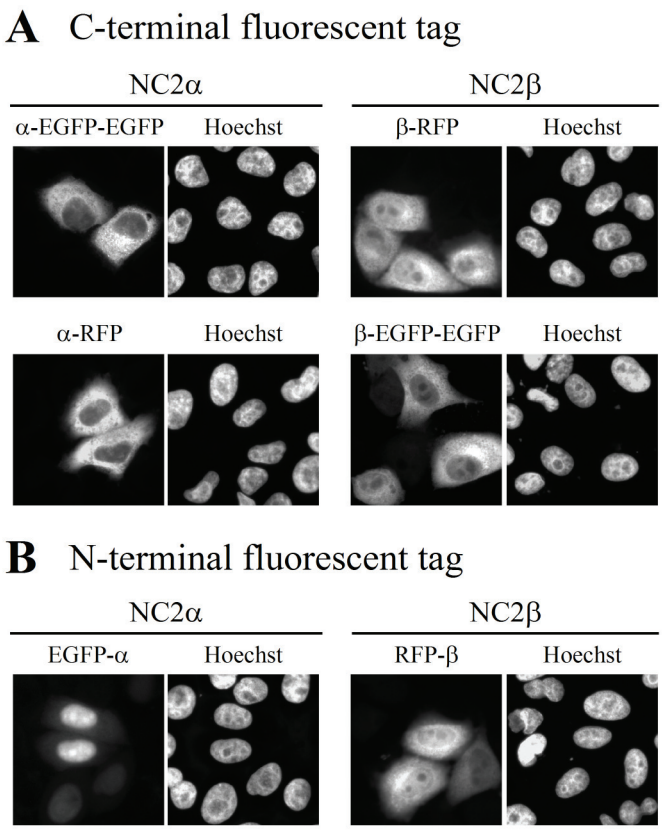
Supplemental Fig. S6. **Importin13 does not mediate nuclear import of the individual NC2 subunits.** *A* and *B*, HeLa P4 cells were transiently co-transfected with plasmid DNA encoding fluorescently-labeled NC2 subunits and flag-tagged importin13. The subcellular distribution of the gene products was examined 24 h post transfection by direct fluorescence of the RFP and EGFP fusion proteins and indirect immunofluorescence of flag-importin13. The DNA was counterstained with Hoechst. *A*, the subcellular distribution of wild type (wt) and mutated RFP-NC2α (K5A) was not influenced by co-expression of importin13, still remaining homogeneously and cytoplasmically localized, respectively (compare to Fig. 1*A*). *B*, the homogeneous localization of EGFP-NC2β and cytoplasmic localization of NC2β-RFP was only slightly changed upon co-expression of importin13 (compare to Fig. 1*B* and 2*B*). imp, importin.

Supplemental Fig. S7. **The C terminus of importin13 is dispensable for nuclear import of the NC2 complex.** *A*, HeLa P4 cells were transiently co-transfected with plasmid DNA encoding mutated RFP-NC2α (K5A), EGFP-NC2β (R101A), and different flag-importin13 fragments. The subcellular distribution of the gene products was examined 24 h post transfection by direct fluorescence. The overlap between the green EGFP fusion protein and the red RFP fusion protein is shown in yellow (merge). The DNA was counterstained with Hoechst. *A*, in the absence of exogenous importin13 (w/o) the RFP-NC2α-(K5A)/EGFP-NC2β-(R101A) complex remained largely in the cytoplasm while the co-expression of full length importin13 (1-963aa) led to a strong nuclear accumulation of the NC2 complex. Among the co-expressed importin13 fragments, only amino acids 1-784 and 1-669 were able to accumulate the cNLS deficient RFP-NC2α/EGFP-NC2β complex in the nucleus of transfected cells. *B*, for semi-quantitative analysis of the nuclear transport of the cNLS deficient RFP-NC2α/EGFP-NC2β complex mediated by importin13 fragments, 100 co-transfected cells (per condition) were scored into the following categories: N>C (more reporter proteins in the nucleus), N<C (more reporter proteins in the cytoplasm), and N=C (equal distribution of the reporter proteins between nucleus and cytoplasm). aa, amino acids.

Supplemental Fig. S8. **Phosphorylation can modulate the nuclear activity of NC2β.** *A* and *B*, HeLa P4 cells were transiently (co-)transfected with plasmid DNA encoding wild type (wt) NC2α and mutated (S105/1006E) NC2β fused to either EGFP-EGFP and RFP (*A*) or EGFP-EGFP-GST (EEG) (*B*). The subcellular distribution of the gene products was examined 24 h post transfection by direct fluorescence. The DNA was counterstained with Hoechst. *A*, co-expression of NC2α and NC2β-(S105/1006E) resulted in nuclear co-localization shown in yellow (merge) comparable to the co-expression of wild type NC2 subunits (see Fig. 3*A*). *B*, substitution of serine residues 105 and 106 for glutamate to mimic the effect of phosphorylation did strongly reduce nuclear import of NC2β-(S105/106E) in the presence of 10ng/ml Leptomycin B (LMB). *C*, for semi-quantitative analysis of the nuclear transport of wild-type and mutated (S105/106E) NC2β fused to EGFP-EGFP-GST (EEG), 50 transfected cells (per condition) were scored into the following categories: N>C (more reporter proteins in the nucleus), N<C (more reporter proteins in the cytoplasm), and N=C (equal distribution of the reporter proteins between nucleus and cytoplasm).

SUPPLEMENTAL FIGURES

Supplemental Fig. S1



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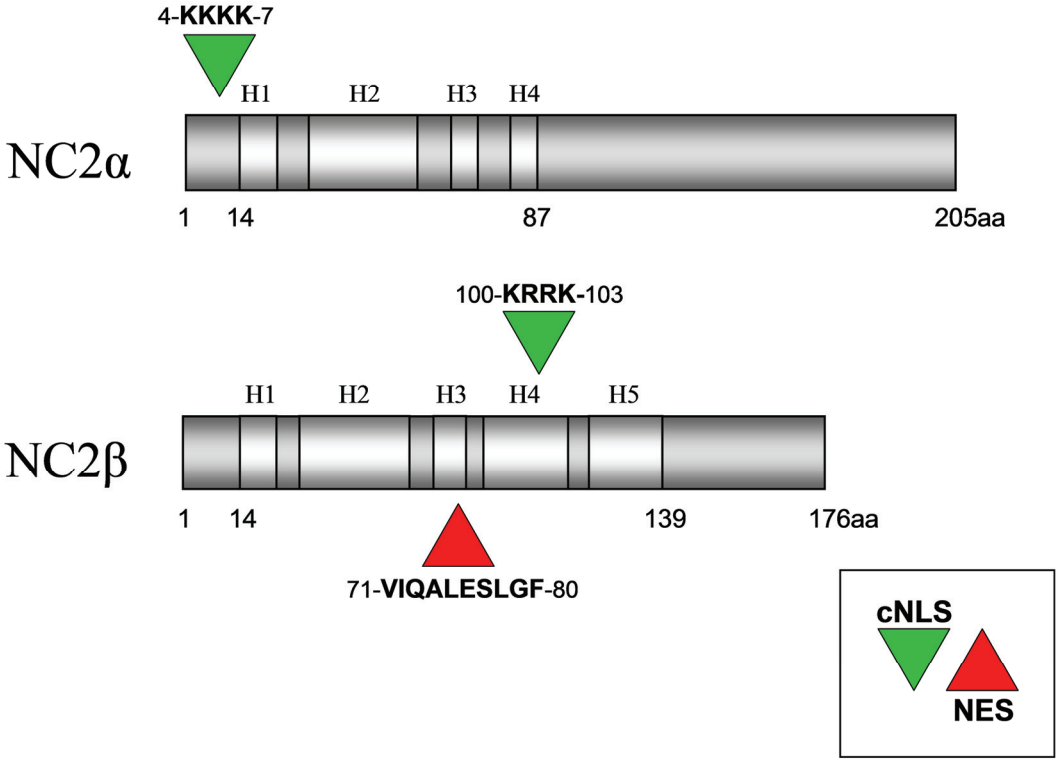

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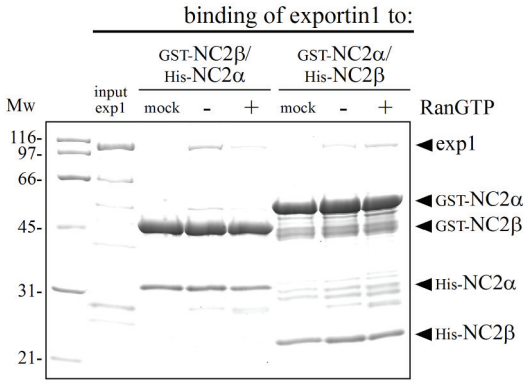

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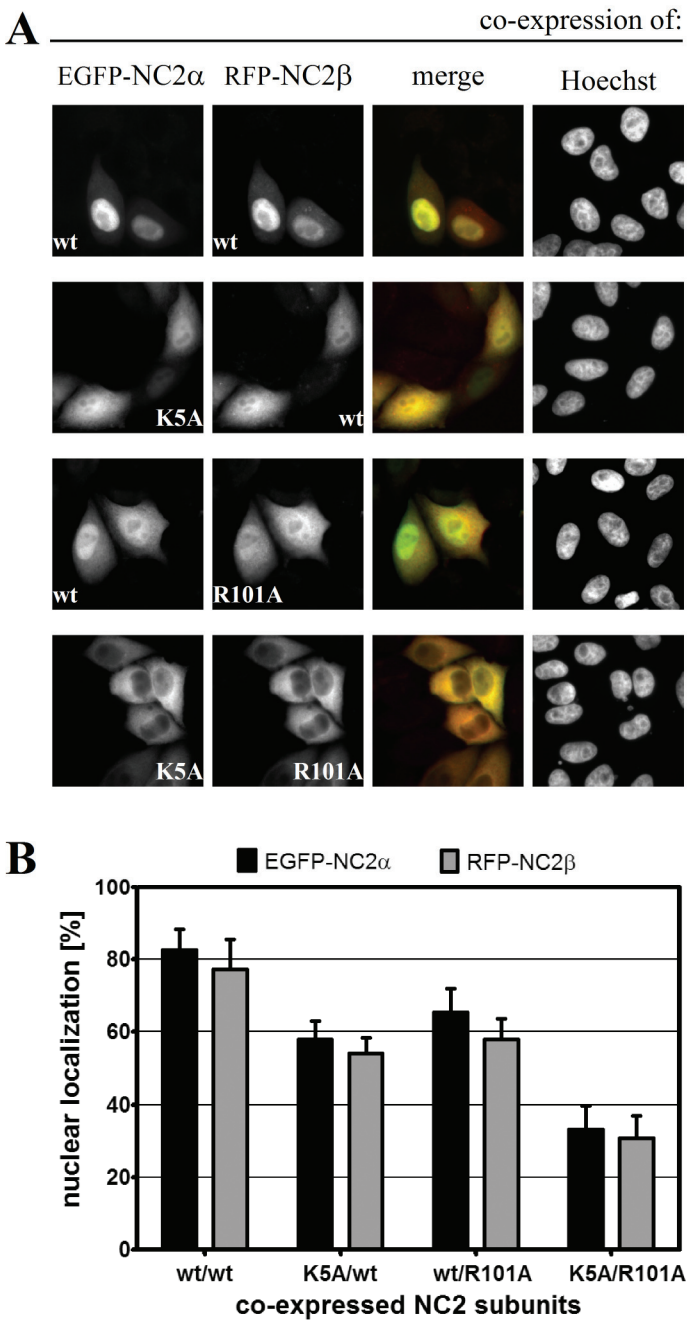

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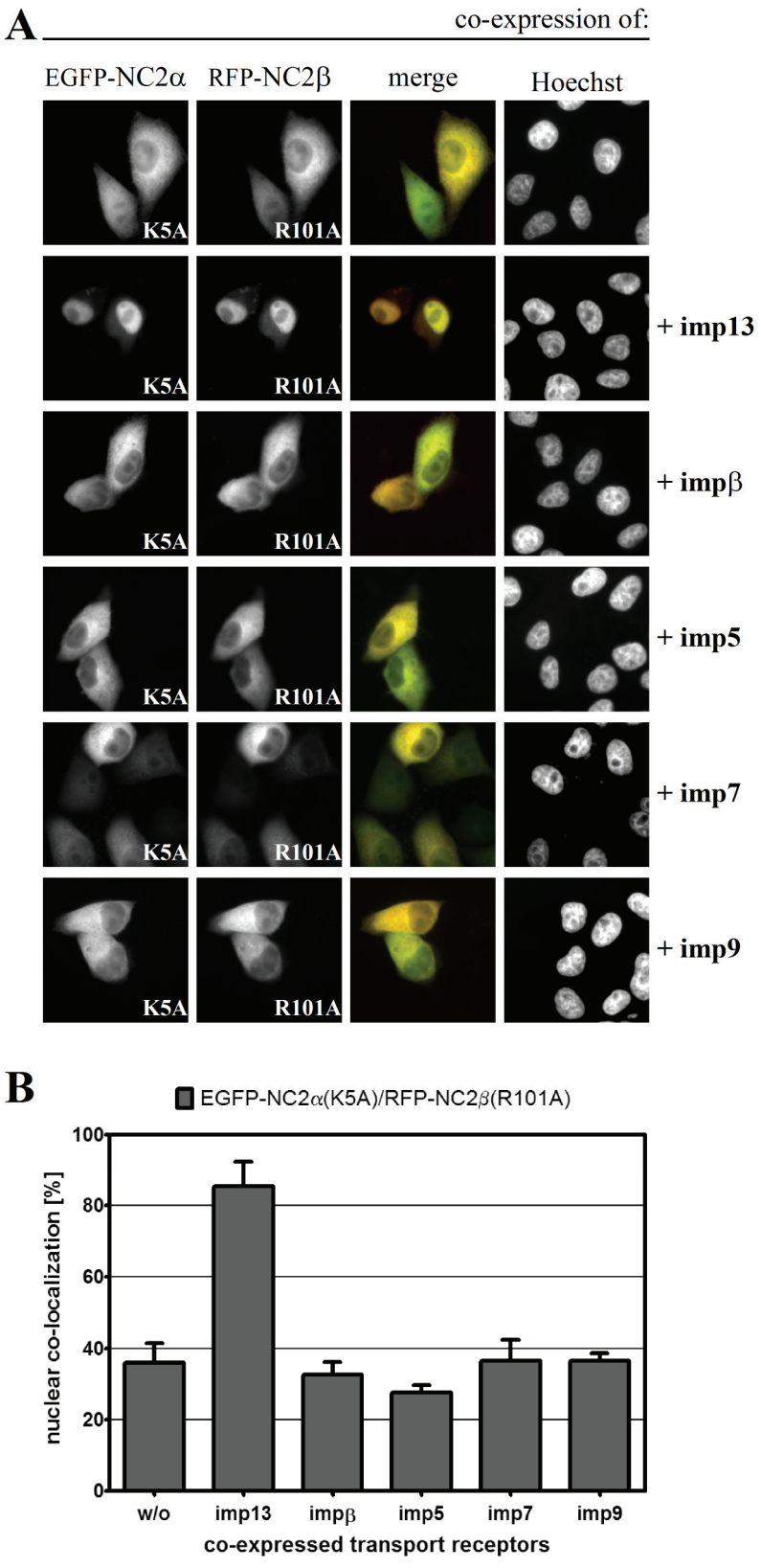

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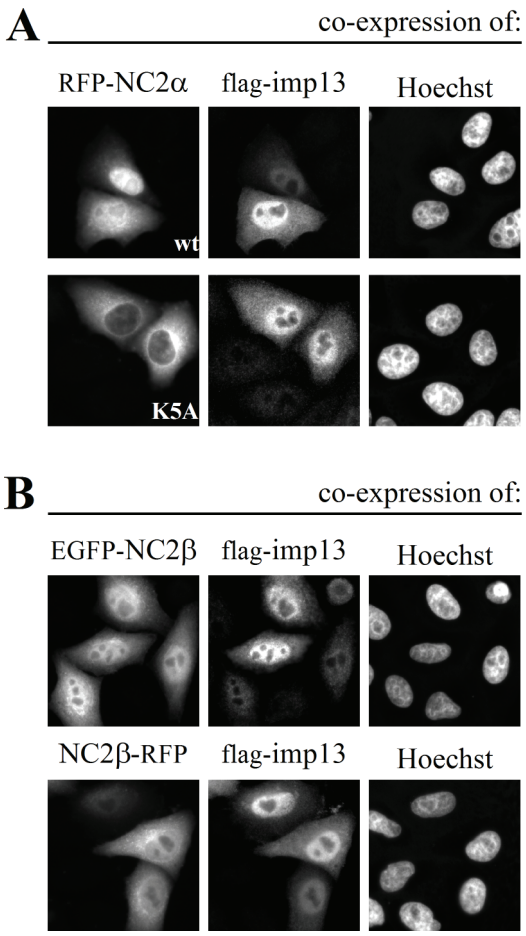
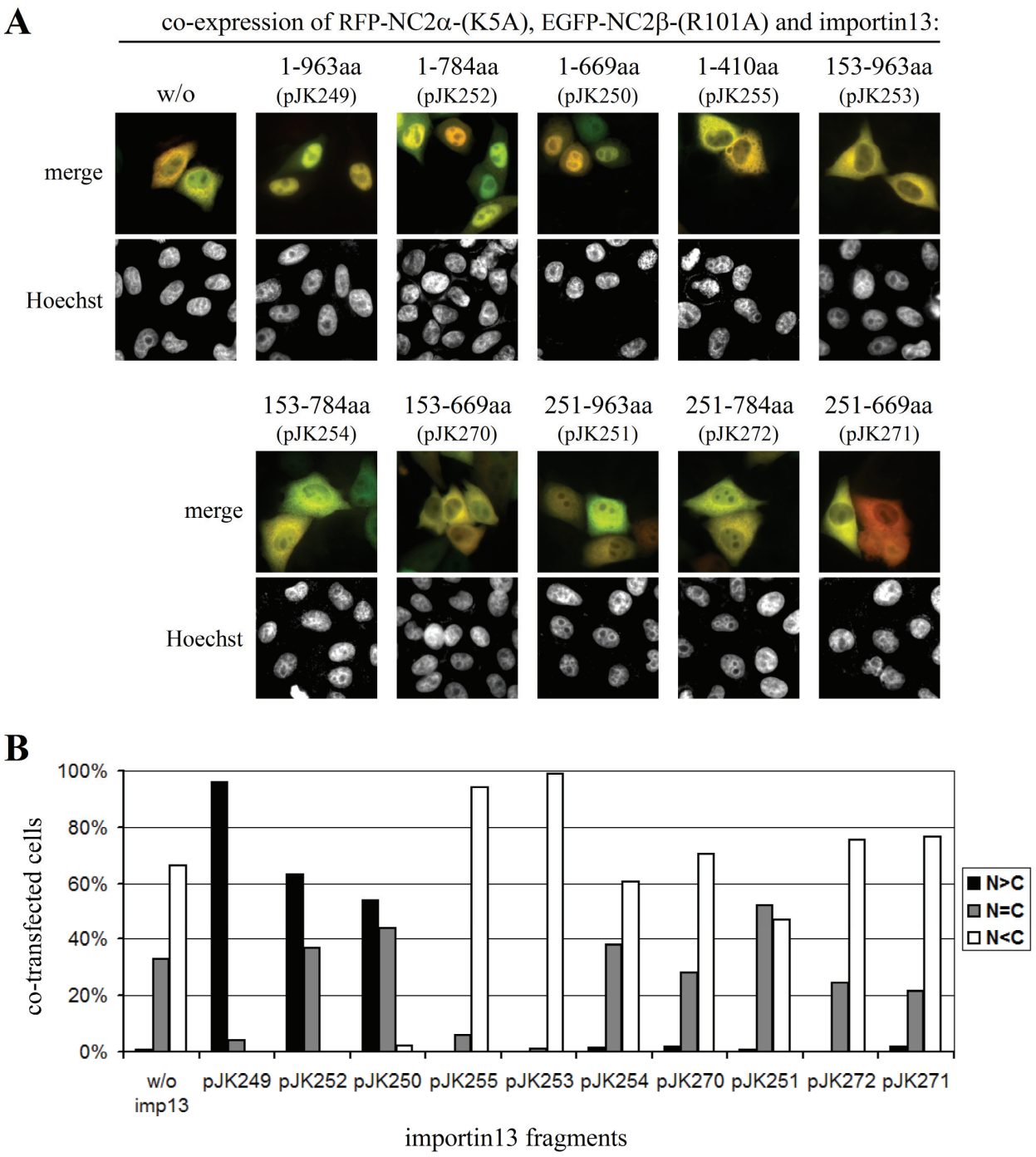


Supplemental Fig. S7



Supplemental Fig. S8