SUPPLEMENTARY EXPERIMENTAL PROCEDURES

Expression Constructs - The bacterial expression constructs were cloned as follows: the coding region of human CHRAC-17 as *Nhe*I/*EcoR*I fragment into the respective sites of pET-28b(+) (Novagen); the coding regions of human p12 and CHRAC-15 as *EcoR*I/*Not*I fragments into the respective sites of pGEX 4T-1 (GE Healthcare).
The eukaryotic expression constructs were cloned as follows: the coding regions or gene fragments of human p12, CHRAC-15 and CHRAC-17 as *EcoR*I/*BamH*I fragments into the respective sites of pEGFP-C1 (Clontech) and pPW1 (modified pEGFP-C1 in which EGFP was replaced by RFP using the restriction sites *Nhe*I/*Bgl*II); the coding regions of human p12, CHRAC-15 and CHRAC-17 as *EcoR*I/*BamH*I fragments into the respective sites of pEGFP- N1 (Clontech) and pmRFP-N1 (modified pEGFP-N1 in which EGFP was replaced by mono RFP using the restriction sites *Age*I/*Not*I); the coding region and gene fragments of importin 13 as *EcoR*I/*Xho*I fragments into the respective sites of pCS2flag (modified pCS2plus in which a flag tag was inserted N-terminally of the multiple cloning site as *Nco*I/*EcoR*I fragment); the coding regions of human importinβ, *Xenopus* importin7, and murine importin9 as *Nru*I fragments into the *Stu*I sites of pCS2flag; the coding region of human importin5 as *Stu*I/*Xho*I fragment into the respective sites of pCS2flag; the coding region of rat HSP70 as *EcoR*I/*Xba*I fragment into the respective sites of pCS2flag; murine PGC7/Stella as *EcoR*I/*Xba*I fragment into the respective sites of pCS2flag. All constructs were verified by DNA sequencing. (Primer sequences are available upon request.)

Site-directed Mutagenesis - To generate the nucleotide exchanges K47A, K92A, R69A, R45A in p12; R25A, K70A, K47A, R23A in CHRAC-15; K100A, R92A, K62A, K86A in CHRAC-17; K18A, K63A, R40A, R19A in NC2α and R101A, R102A, K95A, K64A, K88A in NC2β site-directed mutagenesis was performed according to the Quick Change Site-directed Mutagenesis Kit protocol (Stratagene). The following oligonucleotides were used for mutagenesis: 5'-CTGCCTCTATCCCGCATCGCCGTCATCATGAAGAGCTCC- 3' (sense) and 5'-GGAGCTCTTCATGATGACGGCGATGCGGGATAGAGGCAG- 3' (antisense) for R25A, 5'-GGCAGTGGAAAGGAAAAGGCCGTACTGACTTACAGTGAT- 3' (sense) and 5'-ATCACTGTAAGTCAGTACGGCCTTTTCCTTTCACTGCC- 3' (antisense) for K70A, 5'-GCGTTGGTGCTCACGGCCGCCGCCACGGAGCTCTTTGTT- 3' (sense) and 5'-AACAAAGAGCTCCGTGGCGGCGGCCGTGAGCACCAACGC- 3' (antisense) for K47A and 5'-ATCTCGCTGCCTCTATCCGCCATCGCCGTCATCATGAAG- 3' (sense) and 5'-CTTCATGATGACGGCGATGGCGGATAGAGGCAGCGAGAT- 3' (antisense) for R23A in CHRAC-15.
5'- CGGGAGCAGAAAGGCAAGGCCGAGGCCTCAGAGCAAAAG- 3' (sense) and 5'-CTTTTGCTCTGAGGCCTCGGCCTTGCCTTTCTGCTCCCG- 3' (antisense) for K100A, 5'-GAAGCTCTGGAAGCATATGCCCGGGAGCAGAAAGGCAAG- 3' (sense) and 5'-CTTGCCTTTCTGCTCCCGGGCATATGCTTCCAGAGCTTC- 3' (antisense) for R93A, 5'-GCAATGAAAGGAAAGCGGGCCACGCTGAATGCCAGTGAT- 3' (sense) and 5'-ATCACTGGCATTCAGCGTGGCCCGCTTTCCTTTCATTGC- 3' (antisense) for K62A and 5'-CGGTTCGTTATCCCATTGGCCGAAGCTTGGAAGCATAT- 3' (sense) and 5'-ATATGCTTCCAGAGCTTCGGCCAATGGGATAACGAACCG- 3' (antisense) for K86A in CHRAC-17.
5'- TTGCCTCTGGCGCGAGTGGCAGCCTTGGTGAAGGCAGAT- 3' (sense) and 5'-ATCTGCCTTCACCAAGGCTGCCACTCGCGCCAGAGGCAA- 3' (antisense) for K47A, 5'-GCTCAGCAGGGAAAAAGGGCAACCCTTCAGAGGAGAGAC- 3' (sense) and 5'-GTCTCTCCTCTGAAGGGTTGCCCTTTTTCCCTGCTGAGC- 3' (antisense) for K92A, 5'-GCCATCTTCATTCTGGCAGCAGCCGCGGAACTGTTTGTG- 3' (sense) and 5'-CACAAACAGTTCCGCGGCTGCTGCCAGAATGAAGATGGC- 3' (antisense) for R69A and 5'-TCGAGGTTGCCTCTGGCGGCAGTGGCAGCCTTGGTGAAG- 3' (sense) and 5'-CTTCACCAAGGCTGCCACTGCCGCCAGAGGCAACCTCGA- 3' (antisense) for R45A in p12.
5'- TTCCCGCCGGCGCGGATCGCAAAGATCATGCAGACGGAC- 3' (sense) and 5'-GTCCGTCTGCATGATCTTTGCGATCCGCGCCGGCGGGAA- 3' (antisense) for K18A, 5'-ACCCAGTCGCGGAACGCGGCAACCATGACCACATCCCAC- 3' (sense) and 5'-

GTGGGATGTGGTCATGGTTGCCGCGTTCCGCGACTGGGT- 3' (antisense) for K63A, 5'-CCTGTCATCATCTCCGCAGCGCTCGAGCTCTTC- 3' (sense) and 5'-GAAGAGCTCGAGCGCTGCGGAGATGATGACAGG- 3' (antisense) for R40A and 5'-CGGTTCCCGCCGGCGGCAATCGCGAAGATCATG- 3' (sense) and 5'-CATGATCTTCGCGATTGCCGCCGGCGGGAACCG- 3' (antisense) for R16A in NC2α.
5'- ACAGTAGCATTAAAAGCAGCAAAGGCCAGTTCTCGTTTG- 3' (sense) and 5'-CAAACGAGAACTGGCCTTTGCTGCTTTTAATGCTACTGT- 3' (antisense) for R102A, 5'-GAAGTCTTGCAAGAGTGTGCAACAGTAGCATTAAAAGCA- 3' (sense) and 5'-TGCTTTTAATGCTACTGTTGCACACTCTTGCAAGACTTC- 3' (antisense) for K95A, 5'-TGTAACAAATCGGAAAAGGCGACCATCTCACCAGAGCAT- 3' (sense) and 5'-ATGCTCTGGTGAGATGGTCGCCTTTTCCGATTTGTTACA- 3' (antisense) for K64A and 5'-TCTTACATCAGTGAAGTAGCAGAAGTCTTGCAAGAGTGT- 3' (sense) and 5'-ACACTCTTGCAAGACTTCTGCTACTTCACTGATGTAAGA- 3' (antisense) for K88A in NC2β.

RNAi transfection procedure - Stealth™ RNAi (Invitrogen, Karlsruhe) duplex oligoribonucleotides were generated, using the BLOCK-IT™ RNAi Express design and ordering tool (https://rnaidesigner.invitrogen.com/rnaiexpress/rnaiExpress.jsp) from Invitrogen. Transfection of HeLa P4 cells with siRNA was performed using Oligofectamine™ Reagent. One day before transfection, cells with a density of 10^5 cells/well were plated in a 6 well plate with 3 ml DMEM containing 10% FBS and 2mM glutamine but without further addition of antibiotics. Transfection was carried out accounting to the manufacturer's instructions. For that, 10 µl of the 20 µM oligonucleotide stock were diluted in 175 µl Opti-MEM® I Reduced Serum Medium (Invitrogen, Karlsruhe) and mixed gently. Subsequently, 4 µl of Oligofectamine™ were diluted in Opti-MEM® I Reduced Serum Medium to a final volume of 15 µl, mixed gently and incubated at room temperature for 5-10 minutes. The diluted Oligonucleotides were combined with diluted Oligofectamine™ to a final volume of 200 µl and incubated for another 15-20 minutes at room temperature. While forming transfection complexes, HeLa P4 cells were washed once with FCS deficient DMEM medium. Prior to the Oligonucleotides/Oligofectamine mix, 800 µl of this medium was added to each well. Cells were incubated for 4 hours at 37°C. Afterwards, 500 µl of DMEM with 3x FCS were added,without removing the transfection mixture. HeLa P4 cells were assayed for gene knockdown by western blotting analysis 3 and 7 days post transfection.

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Fig. S1. **Importin 13 mediates the nuclear uptake of different histone fold heterodimers.** HeLa P4 cells were transiently cotransfected with DNA coding for the histone fold heterodimers CHRAC-15/CHRAC-17 or p12/CHRAC-17, with a RFP-tag at the CHRAC-15 (*A*) or p12 (*B*) subunit and an EGFP-tag at the CHRAC-17 subunit (*A-B*). In addition, plasmid DNA coding for different flag-tagged transport receptors was added as indicated. The subcellular localization of the gene products was examined 24h post transfection by direct fluorescence. Using Hoechst the DNA was counterstained. The merged picture results from the overlap between the green EGFP and red RFP fluorescence. For a semi-quantitative analysis, the mean distribution of the CHRAC-15/ CHRAC-17 heterodimer with or without the additional coexpression of flag-tagged import receptors was classified in the categories: N>C, N=C and N<C. *A*, coexpression of importin 13 leads to an exclusive nuclear accumulation of the CHRAC-15/17 complex. Neither importin β, importin 5, importin 7 nor importin 9 mediates the nuclear accumulation of the CHRAC-15/17 heterodimer when cotransfected. *B*, the p12/CHRAC-17 complex localizes in the cytoplasm of transfected cells. Coexpression of importin 13 mediates the nuclear entry of the p12/CHRAC-17 complex whereas the coexpression of other import receptors did not change the subcellular distribution. imp, importin.

Supplemental Fig. S2. **The CHRAC-15/17 heterodimer is not actively exported from the nucleus by exportin 1 and the localization is not affected by either HSP70 or ACF1.** HeLa P4 cells were transiently transfected with plasmid DNA encoding EGFP-CHRAC-15 and RFP-CHRAC-17 or RFP-CHRAC-15 and EGFP-CHRAC-17 under the influence of Leptomycin B (LMB) (*A*), or the additional

cotransfection of HSP70 (*B*) or ACF1 (*C*). The subcellular localization of the overexpressed CHRAC-15/17 heterodimer was determined by direct fluorescence of the individual subunits 24h post transfection, and colocalization is shown in yellow (merge). The DNA was counterstained using Hoechst. *A*, the subcellular localization of the histone fold heterodimer was not affected by blocking the Leptomycin B sensitive exportin 1 pathway. Thus, the cytoplasmic distribution of CHRAC-15/17 does not result from an active export from the nucleus mediated by exportin 1. *B*, to enhance the proper folding of the overexpressed gene products, HeLa cells were additionally cotransfected with HSP70. Despite the overexpression of the chaperone, the distribution of the histone fold heterodimer did not change and the subunits remained in the cytoplasm. *C*, CHRAC-15/17 were cotransfected with ACF1 to analyze the potential influence of ACF1 in the nuclear import via a piggyback mechanism. The overexpression of ACF1 did not affect the subcellular distribution of the heterodimer compared to transfection experiments of the CHRAC subunits alone (see Fig. 2*A*, upper row). The results are valid for both tag combinations. LMB, Leptomycin B.

Supplemental Fig. S3. **Influence of importin 5 on the endogenous distribution of CHRAC-17 and the known cargo protein PGC7/Stella.** *A*, RNAi experiments with siRNA against importin 5 were accomplished using Stealth™ RNAi duplex oligoribonucleotides (Primer 1: IPO5HSS105868 (RNAi) - UCG GAG ACU GCA GCU GCU AUG UUA A and Primer 2: IPO5HSS105868 (RNAi) - UUA ACA UAG CAG CUG CAG UCU CAG A; Invitrogen). HeLa P4 cells were transfected with Oligofectamine Reagent according to the manufacturer's instructions (for detail see supplemental "Experimental Procedures"). Cells were assayed for gene knockdown by western blotting analysis 3 and 7 days post transfection using a specific antibody against importin 5 (courtesy of Dirk Görlich). Mock treated HeLa P4 cells served as expression control. The software 1Dscan Ex (Scanalytics, Inc) was used to analyze band intensities. Mock treated cells were set 100% and an importin 5 depletion of 74% after 3 days and 90% after 7 days was calculated. An antibody against actin was used to control equal loading. *B*, Depleted HeLa P4 cells (3 and 7 days after siRNA treatment), cells treated with control siRNA (after 7 days) and mock treated cells were used to detect the subcellular distribution of endogenous CHRAC-17 using anti-CHRAC-17 antibody. Hoechst was used to counterstain DNA. Despite the depletion of importin 5, the localization of endogenous CHRAC-17 was not affected. Thus, importin 5 is no major import factor for CHRAC-17 containing heterodimers. Unfortunately, depletion of importin 13 by siRNA could not be achieved. *C*, HeLa P4 cells were transiently transfected with plasmid DNA encoding flag-tagged PGC7/Stella (referred to as PGC7), a known importin 5 cargo protein (41). In addition, importin 5 and importin 13 were cotransfected as indicated. The subcellular localization of PGC7/Stella was determined 24h post transfection using specific antibody (Santa Cruz). The DNA was counterstained with Hoechst. Since PGC7/Stella (accession No. AB072734) contains both a putative NLS and a putative NES it localizes diffusely within the cell when expressed alone. Analogous to Nakamura and colleagues (41), PGC7/Stella accumulates predominantly in the nucleus upon importin 5 overexpression. Overexpression of flag-tagged importin 13 had no effect. Localization of the PGC7/Stella fusion protein was additionally quantified by measuring the fluorescence intensity in 20 cells, followed by a calculation of the ratio between nuclear (nucl.) and cytoplasmic (cyto.) distribution. *D*. HeLa P4 cells were additionally transfected with EGFP-PGC7/Stella. The localization of the green fluorescent fusion protein was determined 24h post transfection. Strong overexpression of EGFP-tagged PGC7/Stella results in an aggregated cytoplasmic localization. This pattern was changed when importin 5 was coexpressed, leading to a mainly nuclear localization of the PGC7/Stella fusion protein. Coexpression of importin 13 had no influence on the subcellular distribution of PGC7/Stella. The percentage of cytoplasmic (cyto.) and nuclear (nucl.) localization of EGFP-PGC7/Stella was quantitative analyzed using the ImageJ software. WB, western blot; imp, importin.

Supplemental Fig. S4. **Full length importin 13 is required for efficient nuclear accumulation of the CHRAC-15/17 heterodimer.** *A*, plasmid DNA encoding EGFP-CHRAC-15 and RFP-CHRAC-17 were cotransfected in HeLa P4 cells. Different flag-tagged importin 13 fragments were additionally cotransfected as indicated. The subcellular distribution of the EGFP-CHRAC-15/RFP-CHRAC-17 complex was determined 24h post transfection and is shown in yellow (merge). The addition of full length importin 13 (1-963aa) results in a strict nuclear accumulation (see also Fig. 2). In the absence of exogenous importin 13 (w/o) the CHRAC-15/17 heterodimer localizes mainly in the cytoplasm.

Compared to wild type importin 13 (1-963), the C-terminally truncated 1-784 fragment and the N-terminally truncated 45-963 importin 13 fragment show a reduced potential to translocate the heterodimer into the nucleus of transfected cells. All other fragments were not able to import the complex in the nucleus. *B*, importin 13 constructs, previously used by Kahle et al.[2], are listed. The amino acids contained in the different importin 13 fragments are indicated and represented by grey bars, and lines indicate the deleted regions. Construct 1-963aa represents wild type importin 13. *C*, quantification of the nuclear import of EGFP-CHRAC-15/RFP-CHRAC-17 mediated by full length and truncated importin 13. 100 transfected cells were quantitatively analyzed and the nucleocytoplasmic colocalization of the CHRAC-15/17 heterodimer scored into the following three categories: N>C, N=C and N<C. imp13, importin 13; aa, amino acid.

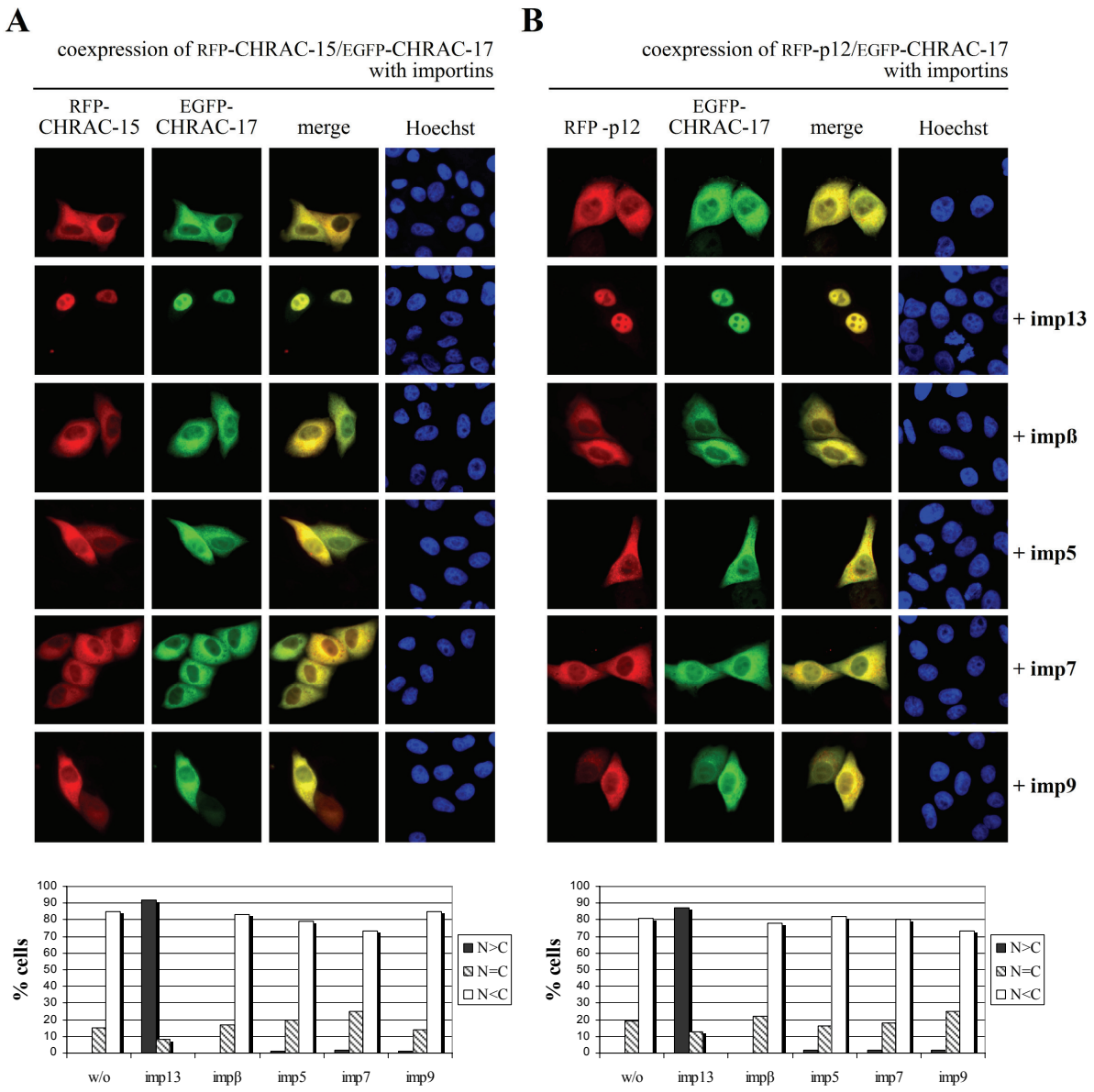
Supplemental Fig. S5. **Basic amino acids are conserved between related histone fold subunits.** *A* and *B*, partial sequence alignments of histone fold proteins of the H2B (*A*) and H2A (*B*) family. Identical amino acids are presented by a black background whereas related residues are shaded in grey. The histone fold motifs of CHRAC-17 and CHRAC-15 (taken from Poot *et al.* (2000)) are indicated by a black bar underneath the sequences. *C*, conserved basic amino acids responsible for DNA binding but presumably not involved in subunit heterodimerization were selected for progressive substitution with up to four basic amino acids against alanine. The substituted lysine or arginine residues in the aligned sequences are indicated by a number (*1-4*) and this also represents the order of consecutive mutations.

Supplemental Fig. S6. **Nuclear import of NC2α/NC2β is mediated by importin 13 and depends on basic amino acids.** *A*, conserved basic amino acids in NC2α and NC2β were progressively substituted. The numbers (1-4) represents the order of consecutive mutations against alanine (see also supplemental Fig. 5*A* and *B*). *B*, HeLa P4 cells were transiently cotransfected with plasmid DNA encoding flag-importin 13. Additionally EGFP-NC2α and RFP-NC2β deficient in cNLS (46) and consecutively mutated in conserved basic amino acids as indicated were cotransfected. The subcellular localization of the NC2α/NC2β complex was examined 24h post transfection by direct fluorescence. Using Hoechst the DNA was counterstained. Colocalization is shown in yellow (merge). Increasing amounts of mutated basic amino acids in cNLS-deficient NC2α and NC2β, result in an increased cytoplasmic accumulation of the complex in transfected cells. *C*. the subcellular distribution of colocalized wild type (wt) and mutated NC2α/NC2β complex was quantitatively analyzed using the program ImageJ (NIH). The fluorescence intensity of 20 cotransfected cells was measured and the ratio between nuclear (nucl.) and cytoplasmic (cyto.) localization was calculated. wt, wild type ; R, arginine; K, lysine; A, alanine.

Supplemental Fig. S7. **Progressive mutation of positively charged amino acids in CHRAC-15 and p12 reduces the nuclear accumulation of the corresponding heterodimer.** *A.* HeLa P4 cells were transiently transfected with plasmid DNA coding for stepwise mutated EGFP-CHRAC-15 and wild type (wt) RFP-CHRAC-17 as indicated. Flag-importin 13 DNA was coexpressed. The subcellular localization of the CHRAC-15/17 complex was determined 24h post transfection by direct fluorescence. The DNA was stained with Hoechst and colocalization of the heterodimer is shown in yellow (merge). The subcellular distribution of the heterodimerized CHRAC-15/17 complex was quantitatively analyzed with the ImageJ software (NIH). The percentage between nuclear (nucl.) and cytoplasmic (cyto.) localization of the heterodimer was analyzed by measuring the fluorescence intensity of 20 transfected cells. Stepwise mutation of basic amino acids in the CHRAC-15 subunit leads to an increased cytoplasmic retention of the CHRAC-15/17 heterodimer. *B.* HeLa P4 cells were transiently transfected with plasmid DNA encoding flag-tagged importin 13, wild type (wt) RFP-CHRAC-17 and EGFP-p12 with increasing numbers of mutated basic amino acids as indicated. The subcellular localization of the green EGFP and red RFP fusion proteins was determined by direct fluorescence 24h post transfection. The DNA was counterstained with Hoechst. The overlap is shown in yellow (merge). For quantitative analysis, the fluorescence intensity of colocalized wild-type CHRAC-17 and mutated p12 was measured in 20 cells using the ImageJ software (NIH). The percentage of nuclear (nucl.) and cytoplasmic (cyto.) localization of the heterodimer was calculated. The stepwise mutation of basic amino acids against alanine in the p12 subunit results in an increased

cytoplasmic localization of the p12/CHRAC-17 complex. wt, wild type; R, arginine; K, lysine; A, alanine.

Supplemental Fig. S1



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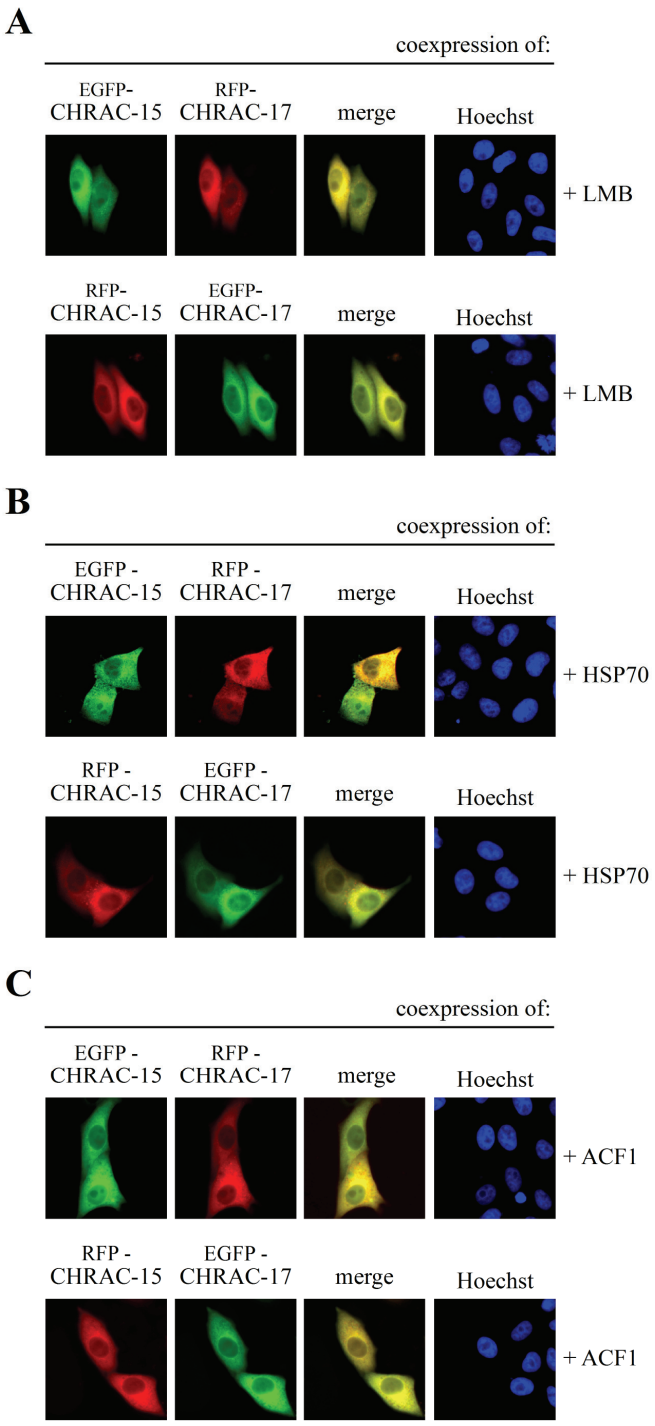

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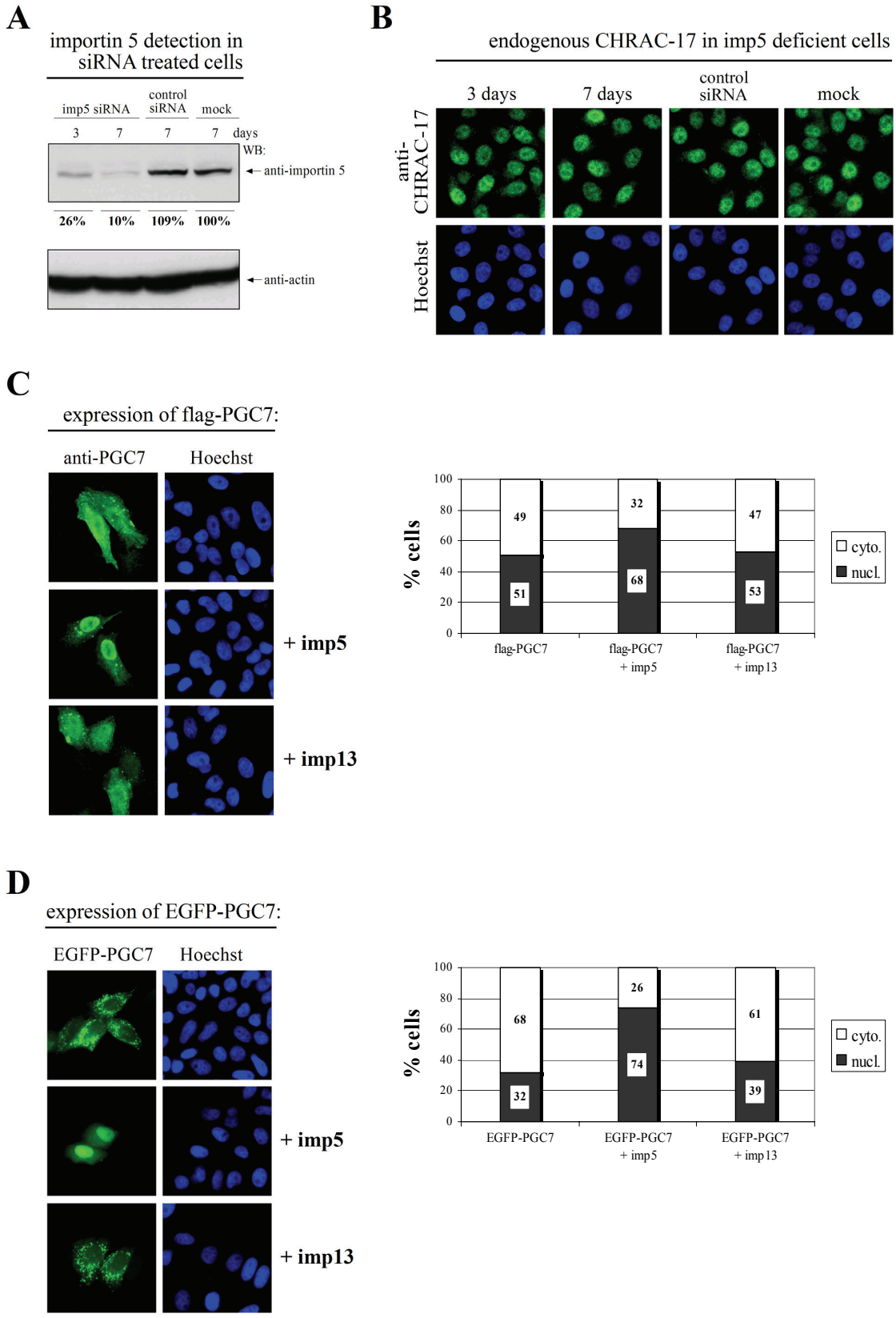

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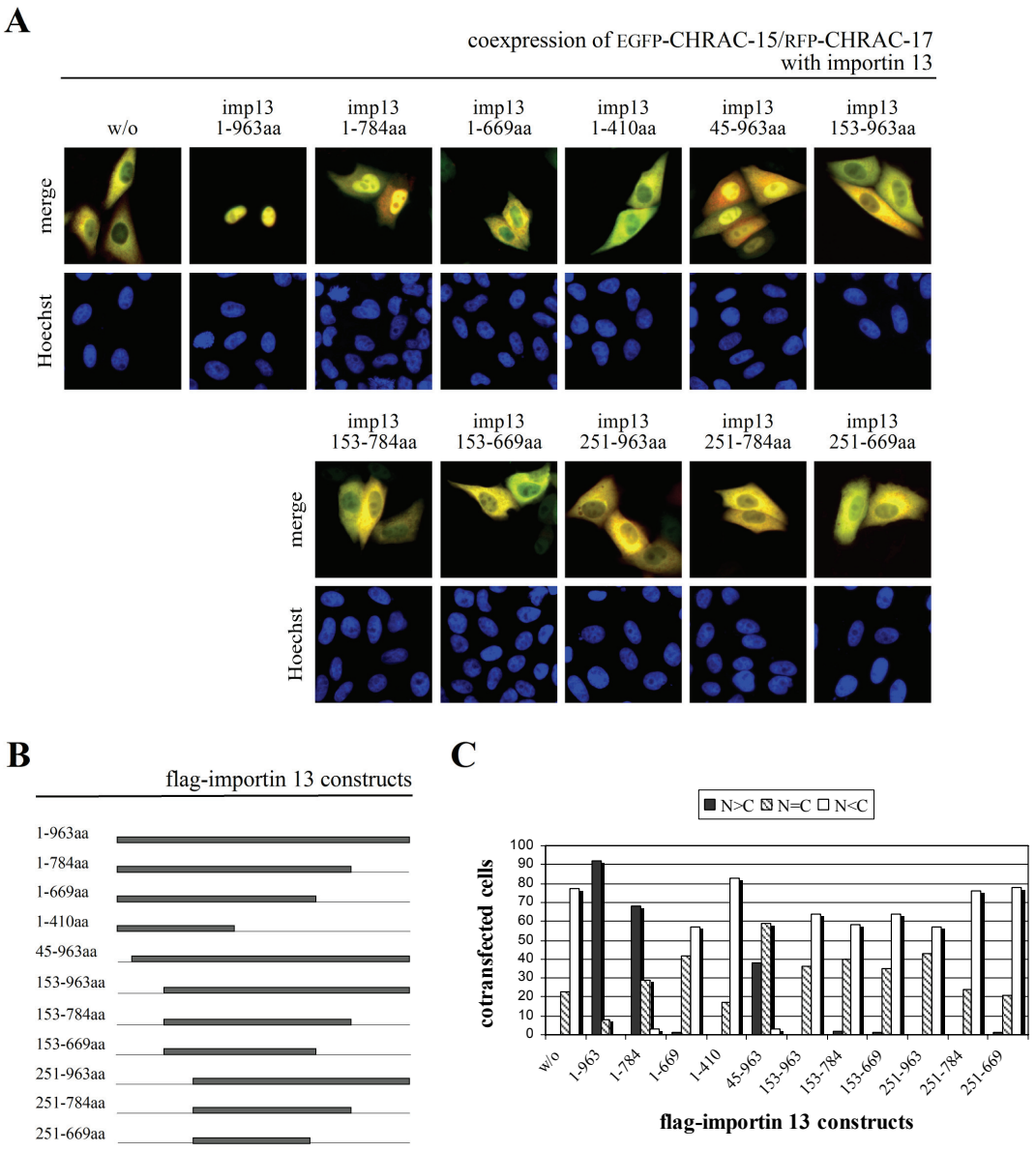
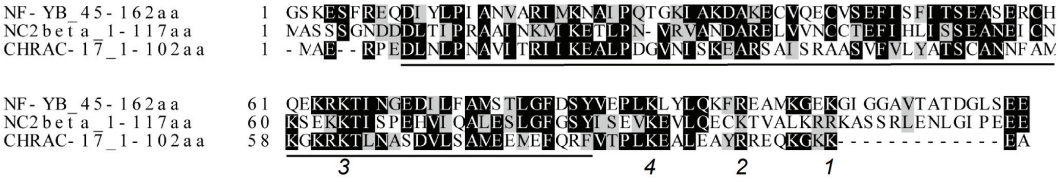


Supplemental Fig. S5

A



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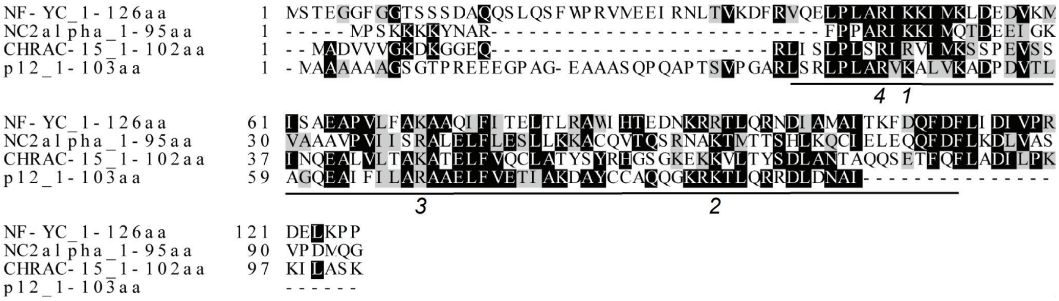


C

substitution of basic amino acids in the histone fold subunits

mutations in:	**CHRAC-17**	mutations in:	**CHRAC-15**	**p12**
1	K100A	*1*	R25A	K47A
2	K100A+R93A	*2*	R25A+K70A	K47A+K92A
3	K100A+R93A+K62A	*3*	R25A+K70A+K47A	K47A+K92A+R69A
4	K100A+R93A+K62A+K86A	*4*	R25A+K70A+K47A+R23A	K47A+K92A+R69A+R45A

Supplemental Fig. S6

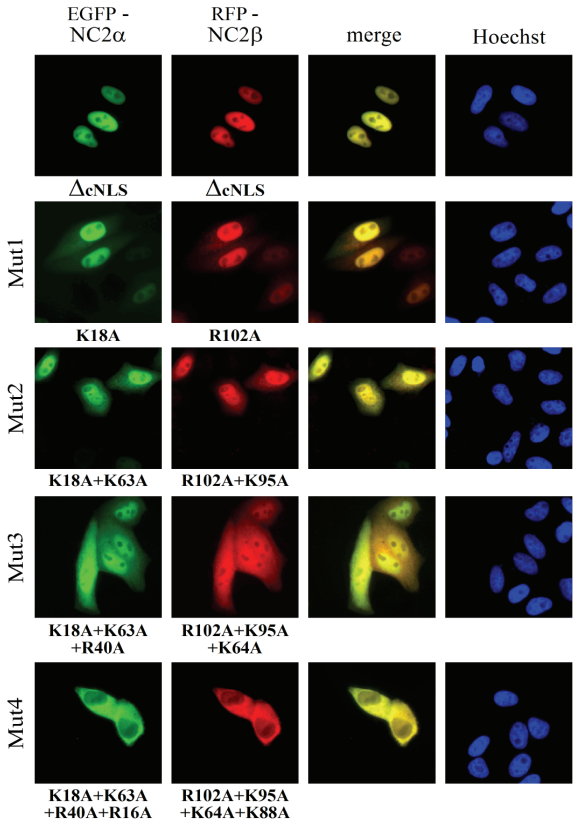
A

substitution of basic amino acids in the histone-fold subunits

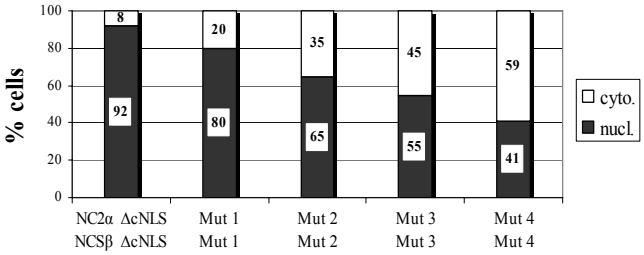
mutations in:	**NC2α**	mutations in:	**NC2β**
1	K18A	1	R102A
2	K63A	2	K95A
3	R40A	3	K64A
4	R16A	4	K88A

B

coexpression of EGFP-NC2α/RFP-NC2β with importin 13



C



Supplemental Fig. S7