Supplementary data

Figure S1. (A) Depletion of endogenous nuclear transport receptors. Isotopically labeled ("light" (l), "medium" (m), "heavy" (h)) HeLa cell extracts were left untreated (uncleared) or incubated with phenyl-Sepharose for selective depletion of endogenous nuclear transport receptors and with the HZZ-tag immobilized on IgG-Sepharose to remove proteins binding unspecifically to the affinity matrix (cleared). Cleared and uncleared lysates were separated by SDS-PAGE, followed by immunoblotting for importin 13, importin β, transportin, Ran and eIF1A. Note that on the blot probed with the anti-eIF1A antibody, the band below 25 kDa results from a previous incubation of the blot with another antibody. (B, C) Importin 13 binds endogenous eIF1A (B) and Ubc9 (C) from a HeLa cytosolic extract. HZZ-tagged importin 13 was immobilized on IgG-Sepharose and incubated with cytosolic cell extracts in the absence or presence of 10 μM (B) or 0.6 μM (C) RanQ69L or 5 μM Ubc9 (B), as indicated. Bound proteins were analyzed by SDS-PAGE, followed by immunoblotting with anti-eIF1A (B) or anti-Ubc9 (C) antibodies. The input corresponds to 1% of the starting material.

Figure S2. Venn diagrams. (A) Overlap of importin 13 cargo candidates from SILAC 2-4 (proteins reduced by Ubc9 or enriched by Ran) and SILAC 1 (proteins binding to importin 13 but not the HZZ-matrix). (B) Comparison of importin 13 cargo candidates identified in this study (SILAC 2-4) and previous studies (29, 30). 2nd and 3rd Z-score candidates from Kimura et al. and proteins enriched with an adjusted Fisher P-value < 0.01 from Mackmull et al. were used for the analysis. (D) Comparison of putative importin 13 (this study, SILAC 2-4) and Crm1 cargoes (25, 26). Cargoes of the categories "A", "B" and "low abundant" from Kirli et al. were used for the analysis.

Figure S3. Endogenous importin 13 and importin β concentrations in HeLa cells. 25,000, 50,000 or 100,000 HeLa cells and His-tagged importin 13 or importin β were analyzed by

SDS-PAGE, followed by immunoblotting with anti-importin 13 (A) or anti-importin β (B) antibodies. (C) Bar graph showing the mean endogenous importin 13 (~80 nM) and importin β (~1,700 nM) levels from three independent experiments. Bars, standard error of the mean.

Figure S4. Importin 13 has different binding modes for cargo proteins. As Fig. 8A with staining for DNA (DAPI) and FLAG-importin 13 included.

Table S1. Identification of importin 13 cargo candidates

Table S2. Peptide identification and quantification

Table S3. Overview Perseus evaluation of SILAC 1 proteome data

Table S4. Overview Perseus analysis of SILAC 2-4 proteome data