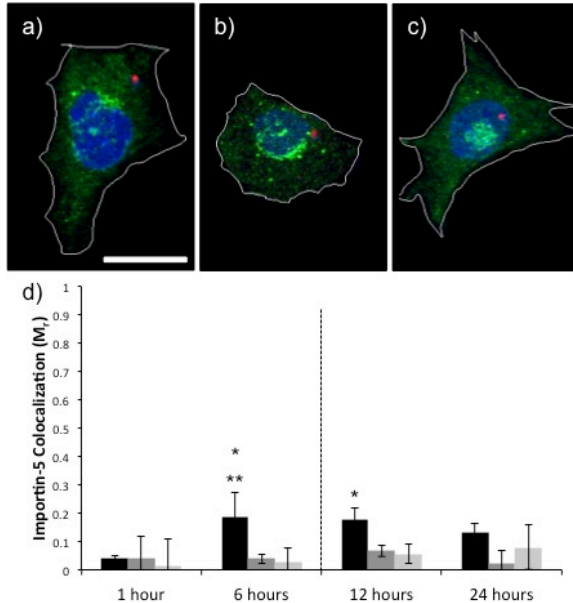
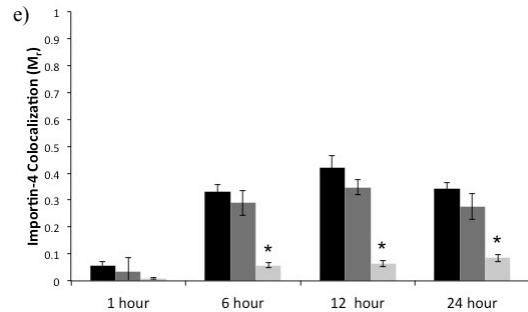
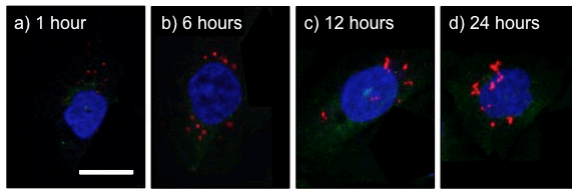


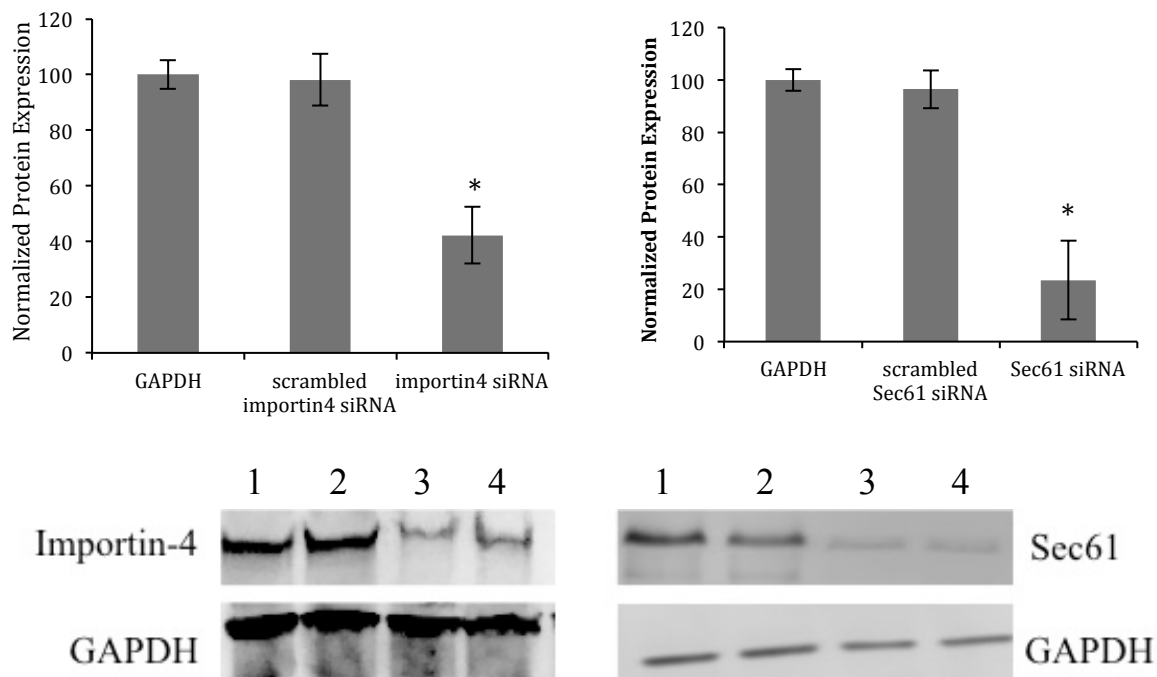
## Supplemental Information



**Figure S1:** (a-c) Representative confocal microscopy z-slice images of cells expressing importin-5 (green) with the nuclei stained with DAPI (blue) 12 hours after a pulse-transfection with PEI polyplexes (a), H3-targeted polyplexes (b), and sH3 polyplexes (c). Polyplexes are in red. The scale bar (shown in a) = 10  $\mu$ m. The cell borders were outlined in white by comparison with the corresponding phase images by using Zen software. (d) Mander's coefficients quantifying colocalization between polyplexes and importin-5 from confocal microscopy images taken at different times post-transfection, performed by Volocity Image Analysis Software. Untargeted PEI polyplexes (black), H3-targeted polyplexes (dark gray), and sH3 polyplexes (light gray) were transfected in CHO cells and colocalization was analyzed at various times post-transfection. Each data point represents the mean  $\pm$  SE with a minimum of 80-100 polyplexes analyzed. The dotted line indicates mitosis. \*Indicates a statistically significant difference from PEI polyplexes at the same time point ( $P < 0.05$ ). \*\*Indicates a statistically significant difference from the previous time point for the given polyplex ( $P < 0.05$ ).



**Figure S2:** (a-d) Representative confocal microscopy z-slice images of cells treated with importin-4 siRNA with the nuclei stained with DAPI (blue) and importin-4 stained by ICC (green) at 1 hour (a), 6 hours (b), 12 hours (c), and 24 hours (d) after a pulse-transfection with H3-targeted polyplexes (red). The scale bar (shown in a) = 10  $\mu$ m. The cell borders were outlined in white by comparison with the corresponding phase images by using Zen software. (e) Quantification of colocalization with importin-4 after treatment with importin-4 siRNA from confocal microscopy images at different times post-transfection, performed by Velocity Image Analysis Software. CHO cells with no siRNA treatment (black), treatment with scrambled importin-4 siRNA (dark gray), and treatment with importin-4 siRNA (light gray). Each data point represents the mean  $\pm$  SE with a minimum of 80-100 polyplexes analyzed. \*Indicates a statistically significant difference from cells without siRNA treatment ( $P < 0.05$ ).



**Figure S3:** Western blots and quantification for Importin-4 (left) and Sec61 (right). In both blots, lane 1 is a cell sample that has no treatment, lane 2 is a cell sample treated with scrambled siRNA, and lanes 3 and 4 are cell samples treated with siRNA. Each data point represents the mean  $\pm$  SE of at least 3 independent replicates. \*Indicates a statistically significant difference from cells without siRNA treatment ( $P < 0.05$ ).