## Supplemental Materials and Methods

### Xenopus *nuclear transport assay*

*Xenopus* cytosol, membrane, and sperm chromatin were combined and nuclei were reconstituted at room temperature as in (73). MBP-M3, which contains the M9 NLS within its sequence [MBP-M3a(Y244C)] (100), in PBS/5% glycerol was labeled with Alexa Fluor 568 using Alexa Fluor 568 Monoclonal Antibody Labeling Kit (Invitrogen/Life Technologies) according to manufacturer's protocol. Forty μM GFP-M9 was added as a transport competitor 30 minutes after the start of a reaction. Import substrates TRITC-SV40-NLS-HSA (73) or Alexa568-MBP-M3 were added 30 minutes later. The reactions were then incubated for an additional 30 minutes before fixation with 3% paraformaldehyde. DNA was visualized with Hoechst.

### *Permeabilized HeLa cell assay*

A permeabilized HeLa import assay was performed as described in Materials and Methods. Controls where the ATP regeneration system was omitted ("no energy") or where the rabbit reticulocyte lysate was omitted (no lysate") were performed and compared to a control assay.

## Supplemental Figure Legends

**Supplemental Figure 1: Excess GFP-M9 inhibits transportin-mediated, but not importin β-mediated transport.** Excess GFP-M9 (40 μM) was added to a *Xenopus* nuclear reconstitution assay 30 minutes after the start of the reaction. **(A)** TRITC-labeled classical NLS import substrate SV40-NLS-HSA, or **(B)** Alexa-568-labeled transportin substrate MBP-M3 was added 30 minutes after the addition of the excess GFP-M9. The nuclei were incubated for another 30 minutes before fixation. Bar: 10 μm.

**Supplemental Figure 2: DNA import in permeabilized HeLa cells is dependent on energy and cytosolic factors.** Permeabilized cell assays were performed and analyzed as in Figure 4 and 5, except the transport mixture did not contain **(A)** an energy regenerating system, or **(B)** rabbit reticulocyte lysate. GFP-A1 was added at 0.7 μM. GFP is shown in green, DNA staining in blue, and CX-Rhodamine-labeled DNA import is shown in red. DNA import images were projected from five z-sections through the middle of the nuclei at 0.5 μm apart using ImageJ

software (http://rsb.info.nih.gov/ij/). Bar: 10 μm.