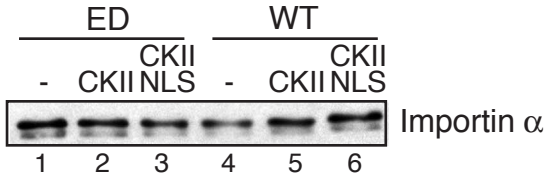
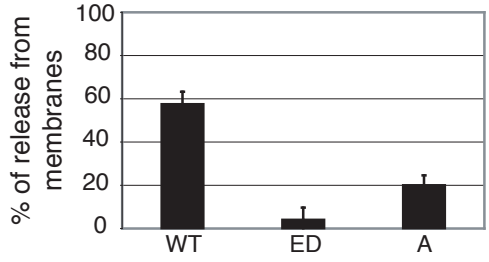
**Supplementary figure legends.**

**Supplementary figure 1. (A)** *In vitro* phosphorylation of recombinant Importin α WT and ED. Reactions were carried out in the absence (CKII) or presence (CKII/NLS) of BSA-NLS. (-) corresponds to the imput recombinant protein. A mobility shift is observed for the WT Importin α when incubated in the presence of CKII and NLS (compare lanes 5 and 6), but no mobility shift is seen for the ED mutant protein (compare lanes 2 and 3). **(B)** Cytosolic Importin α is recruited to salt-stripped membranes. Importin α depleted membranes were generated by high salt washes (NaCl M) and were incubated with cytosol for 60 min at 20°C , then washed and re-isolated (NaCl M + C). Untreated membranes (M) are also shown. The presence of Importin α on the membranes was analysed by Western blotting. **(C)** Exogenous Importin α ED and A pre-bound to stripped membranes are not efficiently re-released upon phosphorylation. Recombinant Importin α WT, ED or A (2 μM) were bound to stripped membranes as indicated. The membranes were re-purified by centrifugation and subjected to phosphorylation by addition of CKII kinase and BSA-NLS. Membrane pellets (P) and supernatants (S) were analysed by Western blotting with anti-Importin α antibodies. Western blotting was performed using an Alexa-680 secondary antibody and quantitation used a Li-cor fluoroimager with Odyssey software. The percentage of release was determined by the ration between S and P. **(D)** Addition of BSA-NLS or of Importin α to an NE assembly reaction does not affect membrane-bound Importin α. BSA-NLS (NLS), BSA-SLN (SLN) (20 μM) or recombinant Importin α (rec α) (10 μM) were pre-incubated with *Xenopus laevis* interphase egg extracts for 10 min on ice before addition of sperm and membranes. Reactions were then incubated at 20°C for 90 min. Membranes (M) were washed and recovered by centrifugation over a sucrose cushion (repurified M). (-) corresponds to buffer-treated membranes. The presence of Importin α on the membranes was analysed by Western blotting.

A.




C.

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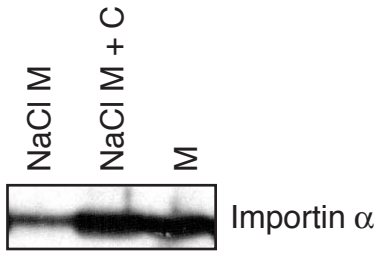
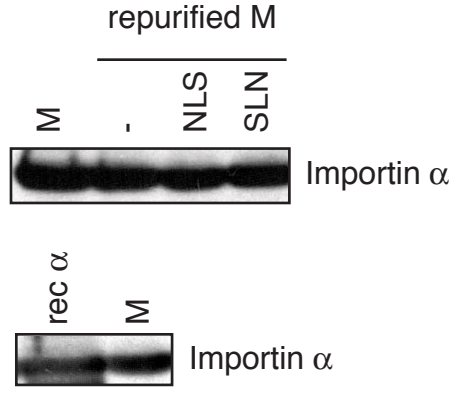



D.




**Supplementary figure 1**