Table S1. Dissociation constants

Construct	Binding partner	Additional construct	K_D (nM)
importin α	GST-SV40T NLS	none	1100
		Npap60LN	3700
		Npap60SN	2.8

Supplemental Figure Legends

Figure S1. mRNA expression of the two Npap60 isoforms in tissues.

The mRNAs of the Npap60 isoforms were detected by RT-PCR using 100 ng of total RNA from the human brain, liver, lung, kidney and testis and the same primers as in Fig. 1B. Bands of approximately 350 bp and 540 bp corresponding to Npap60L mRNA and Npap60S mRNA, respectively, were detected in all of the samples.

Figure S2. The binding between importin α and GST-SV40 T NLS varies in the presence of Npap60LN or Npap60SN. (A) Importin α alone at concentrations of 50, 100, 200 or 500 nM or in combination with 3 μM of either Npap60LN or Npap60SN was incubated with 1 μM of GST-SV40T NLS immobilized on glutathione beads, and the bound proteins were analyzed by CBB stains. (B) To calculate the Kd accurately, importin α at concentrations of 5, 10 or 20 nM (lanes 1, 2 and 3, respectively) in combination with 3 μM of Npap60SN was incubated with 1μM of GST-SV40T NLS immobilized on glutathione beads, and the bound proteins were analyzed by CBB stains. Fourfold and twofold amount of samples were applied in lanes 1 and 2, respectively, compared to that in lane 3.

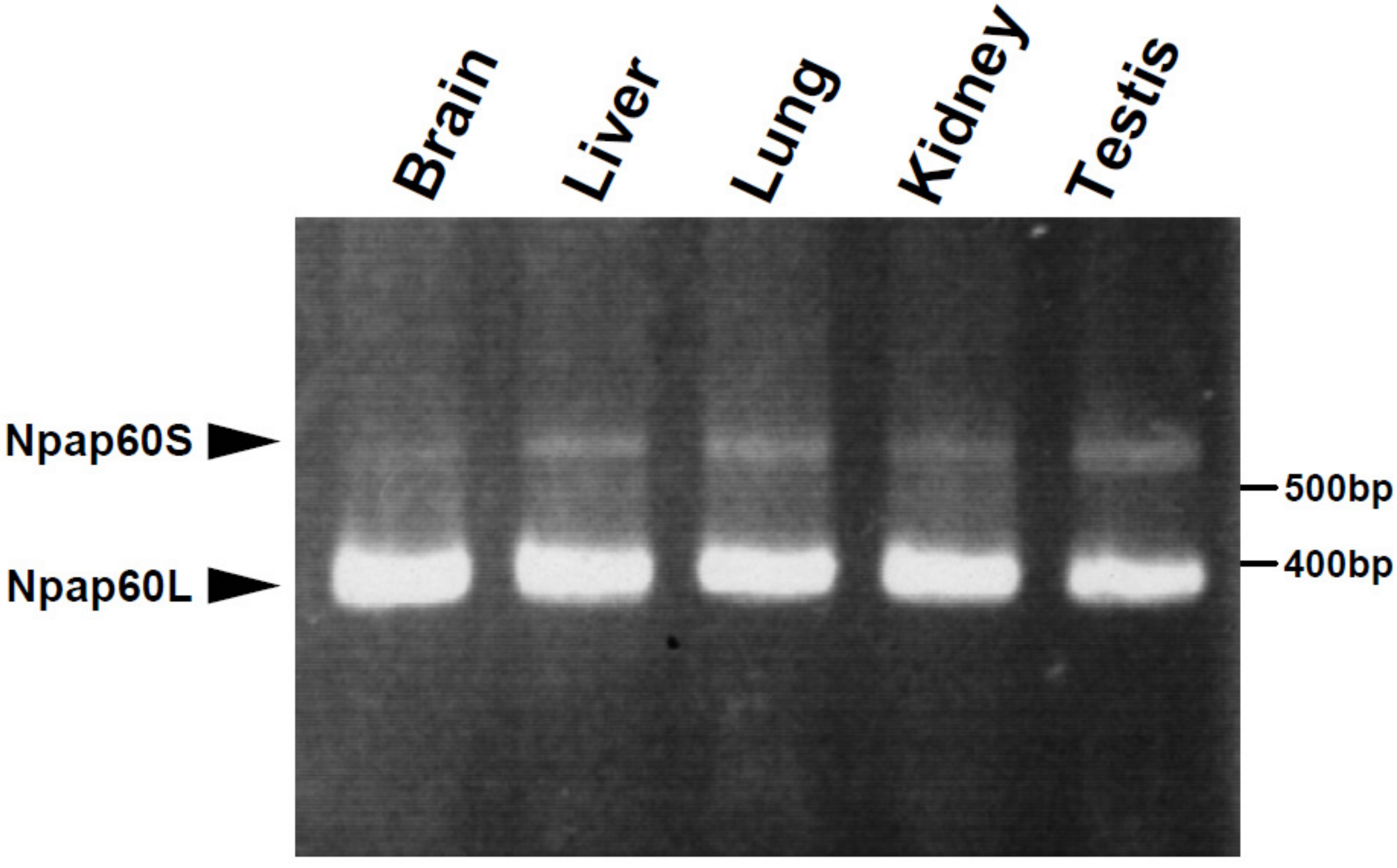
Figure S3. Over-expression of the untagged Npap60 isoforms induces the nuclear accumulation of endogenous importin α.

The Npap60 isoforms and Npap60ΔN (47-469a.a. of Npap60L), which lacks both BS1 and BS2, were expressed by transfecting pIRES-EGFP2-Npap60L, pIRES-EGFP2-Npap60S and pIRES-EGFP2-Npap60ΔN into HeLa cells. pIRES-EGFP2 is a mammalian expression vector that

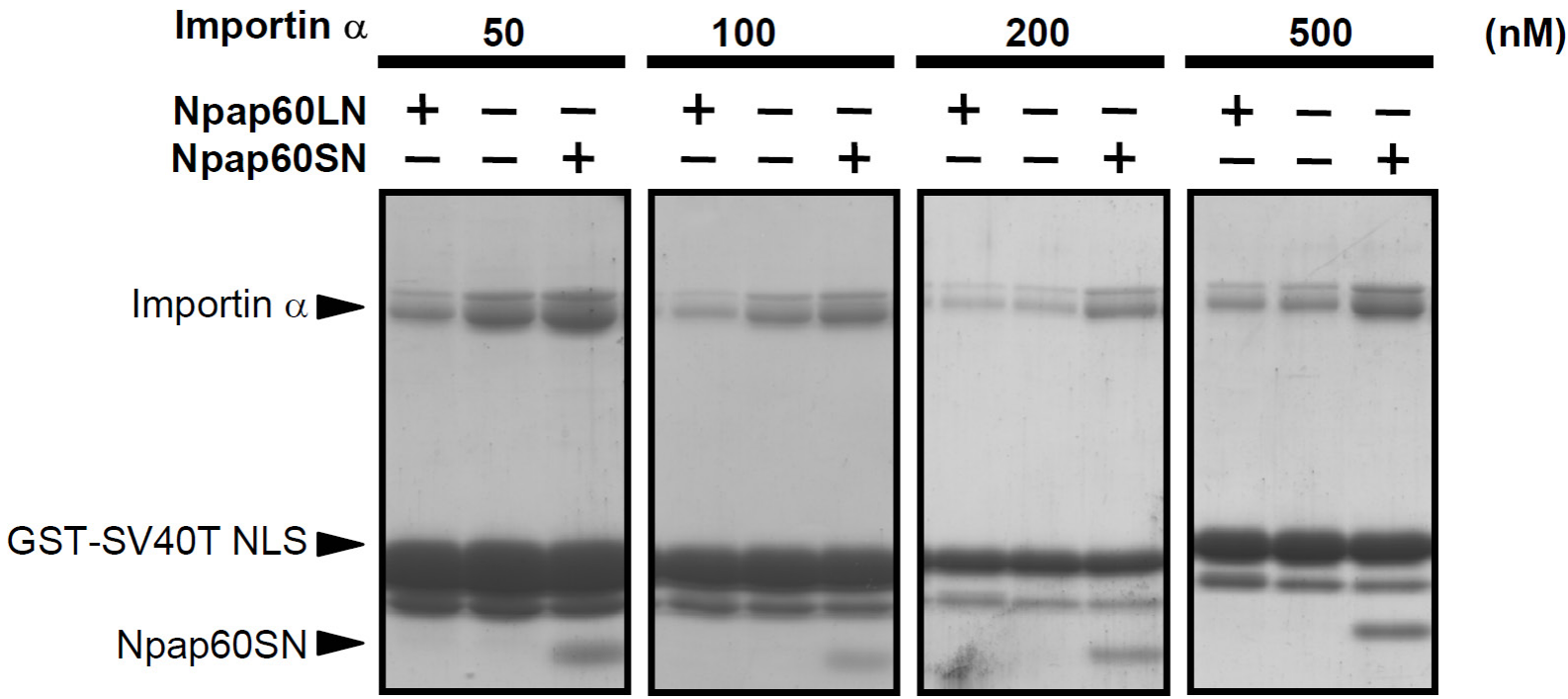
expresses both the target protein and GFP simultaneously. The cells were stained with an anti-Npap60 antibody and anti-karyopherin α/Rch-1 antibody, followed by Alexa 647- and Alexa 568-conjugated secondary antibodies, respectively. The Npap60 overexpressing cells are indicated by arrowheads.

Figure S4. Permeabilization changes the localization of endogenous Npap60.

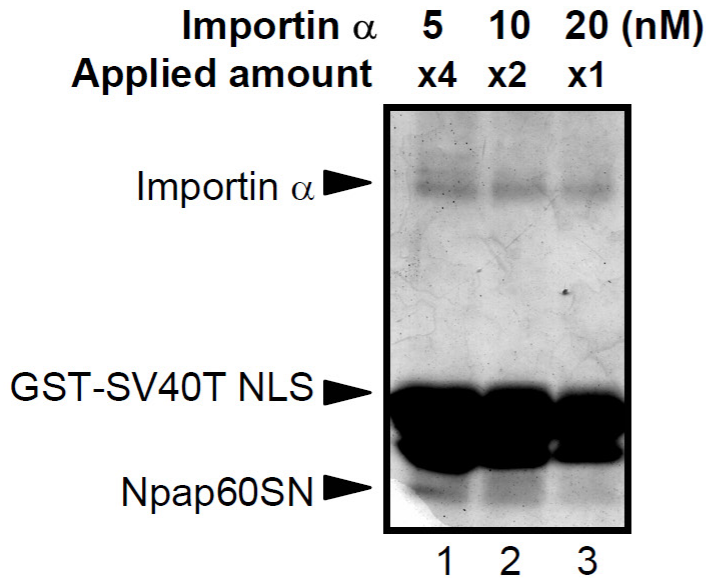
(A-1, B-1, and C-1) HeLa cells were fixed, and then both the plasma and nuclear membranes were permeabilized with 0.1% Triton-X 100. (A-2, B-2, and C-2) The plasma membranes were permeabilized with 40 μg/ml Digitonin, and then the cells were fixed. Next, the nuclear membranes were permeabilized with 0.1% Triton-X 100. The cells were triple stained with an anti-Npap60 antibody (1:100), anti-Nup153 antibody (1:10), and mAb414 (1:200), followed by Alexa 647-, Alexa 488-, and Alexa 568-conjugated secondary antibodies, respectively.


Brain
Liver
Lung
Kidney
Testis
Npap60S
Npap60L
500bp
400bp

A


Importin α
50
100
200
500
(nM)
Npap60LN
Npap60SN
Importin α
GST-SV40T NLS
Npap60SN

B


Importin α 5 10 20 (nM)
Applied amount x4 x2 x1
Importin α
GST-SV40T NLS
Npap60SN
1 2 3

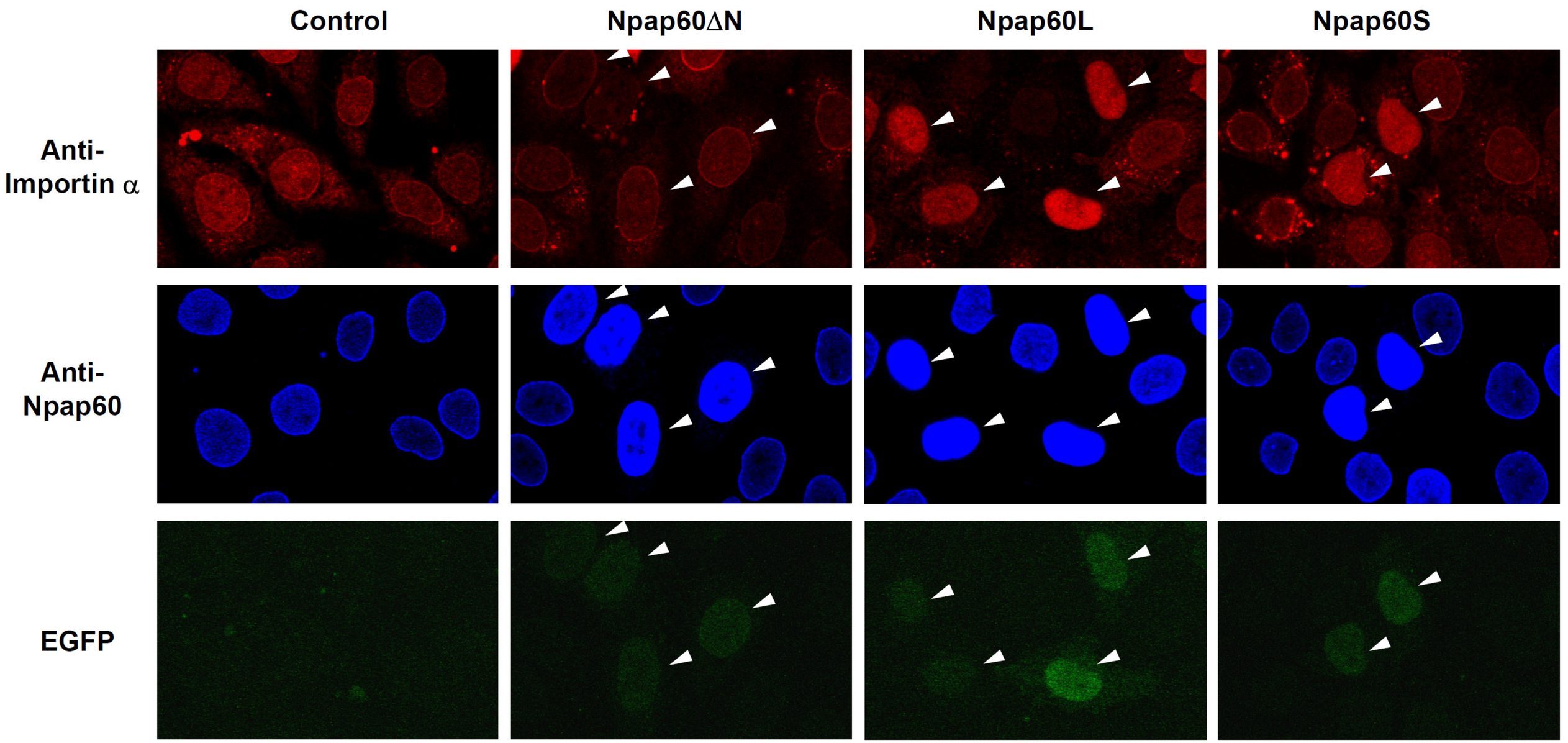
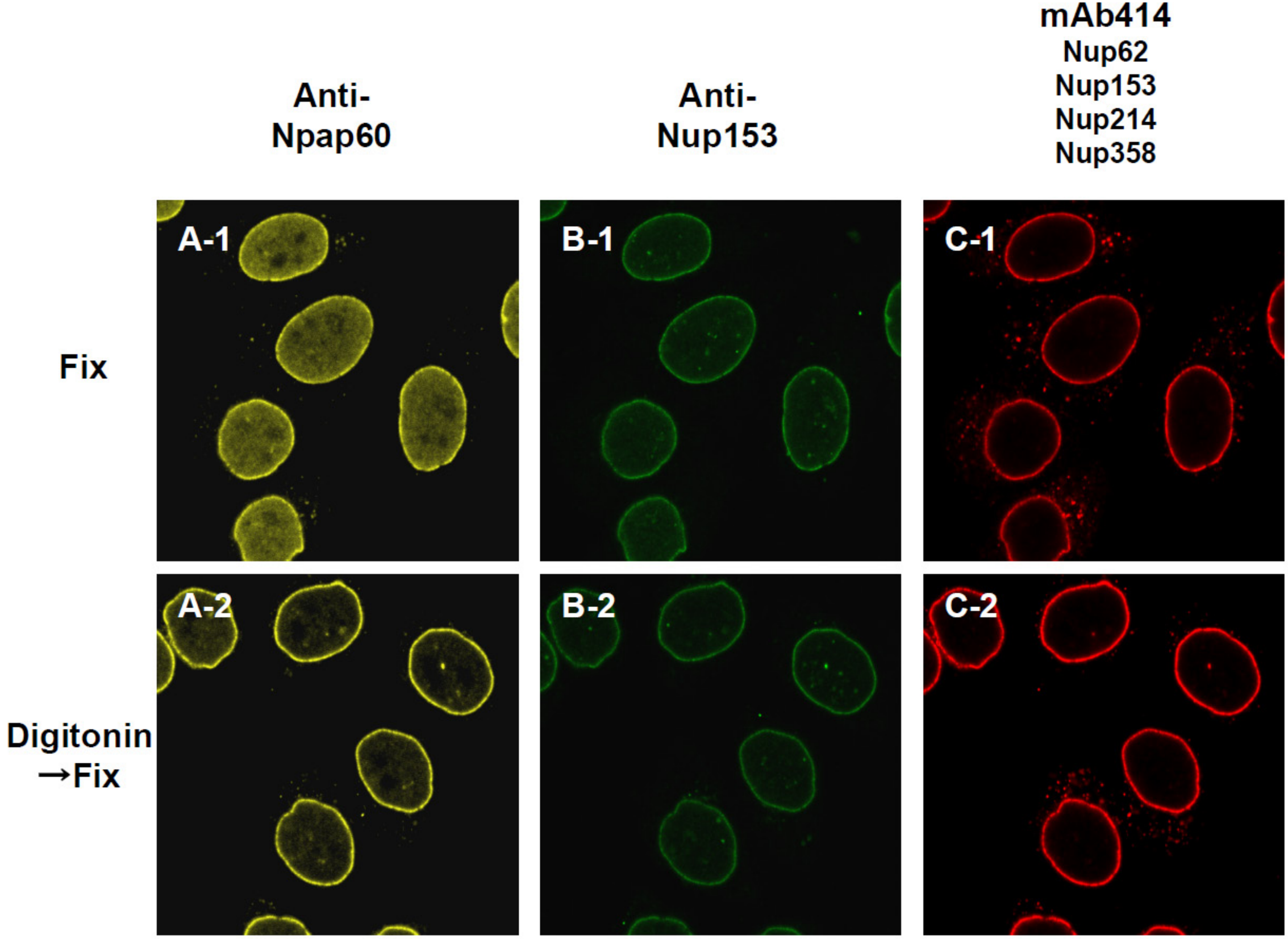

Control
Npap60ΔN
Npap60L
Npap60S
Anti-
Importin α
Anti-
Npap60
EGFP

Fig.S1


Anti-
Npap60
Anti-
Nup153
mAb414
Nup62
Nup153
Nup214
Nup358
Fix
A-1
B-1
C-1
Digitonin
→Fix
A-2
B-2
C-2