Chang et al., https://doi.org/10.1083/jcb.201705168

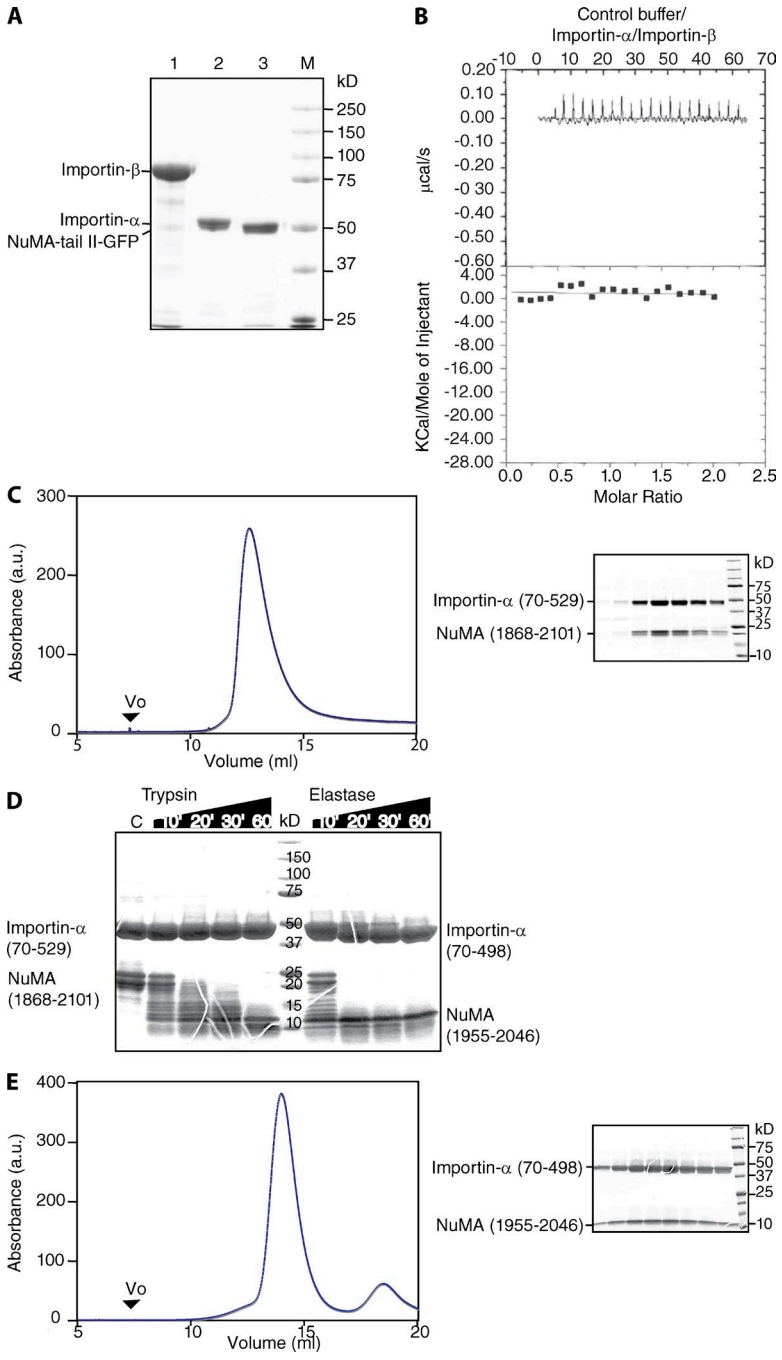

Figure S1. **Identification of suitable regions of the Importin-α–NuMA-tail II complex for crystallographic studies.** (A) Purified recombinant Importin-α, Importin-β, and NuMA-tail II-GFP shown in lanes 1, 2, and 3 were analyzed by SDS-PAGE analysis, followed by Coomassie blue staining. (B) ITC titration curves (upper) and binding isotherms (lower) of buffer control in the presence of full-length Importin-α/-β. (C) Size exclusion chromatography (Superdex 200) elution profile for the NuMA (1,868–2,101)–Importin-α (70–529) dimeric complex. The peak fraction of size exclusion chromatography was analyzed by SDS-PAGE and stained with Coomassie blue (lane 4). Void volume (*Vo*) and absorbance (a.u.) at 280 nm are shown. (D) The NuMA-tail II (aa 1868–2101)–Importin-α (aa 70–529) complex was subjected to limited proteolysis. Protein was incubated with trypsin and elastase over different time courses, and two stable protein fragments could be observed after protease treatment. Protease-digested samples were analyzed by SDS-PAGE analysis, followed by Coomassie blue staining. Different time points of protease treatment are indicated. N and C termini of protein peptides determined by Edman sequencing and mass spectrometry are indicated. (E) A 1-h elastase-treated sample was reanalyzed by size exclusion chromatography (Superdex 200 10/300). Peak fractions were analyzed by SDS-PAGE and stained with Coomassie blue. Void volume (*Vo*) and absorbance (a.u.) at 280 nm are shown.

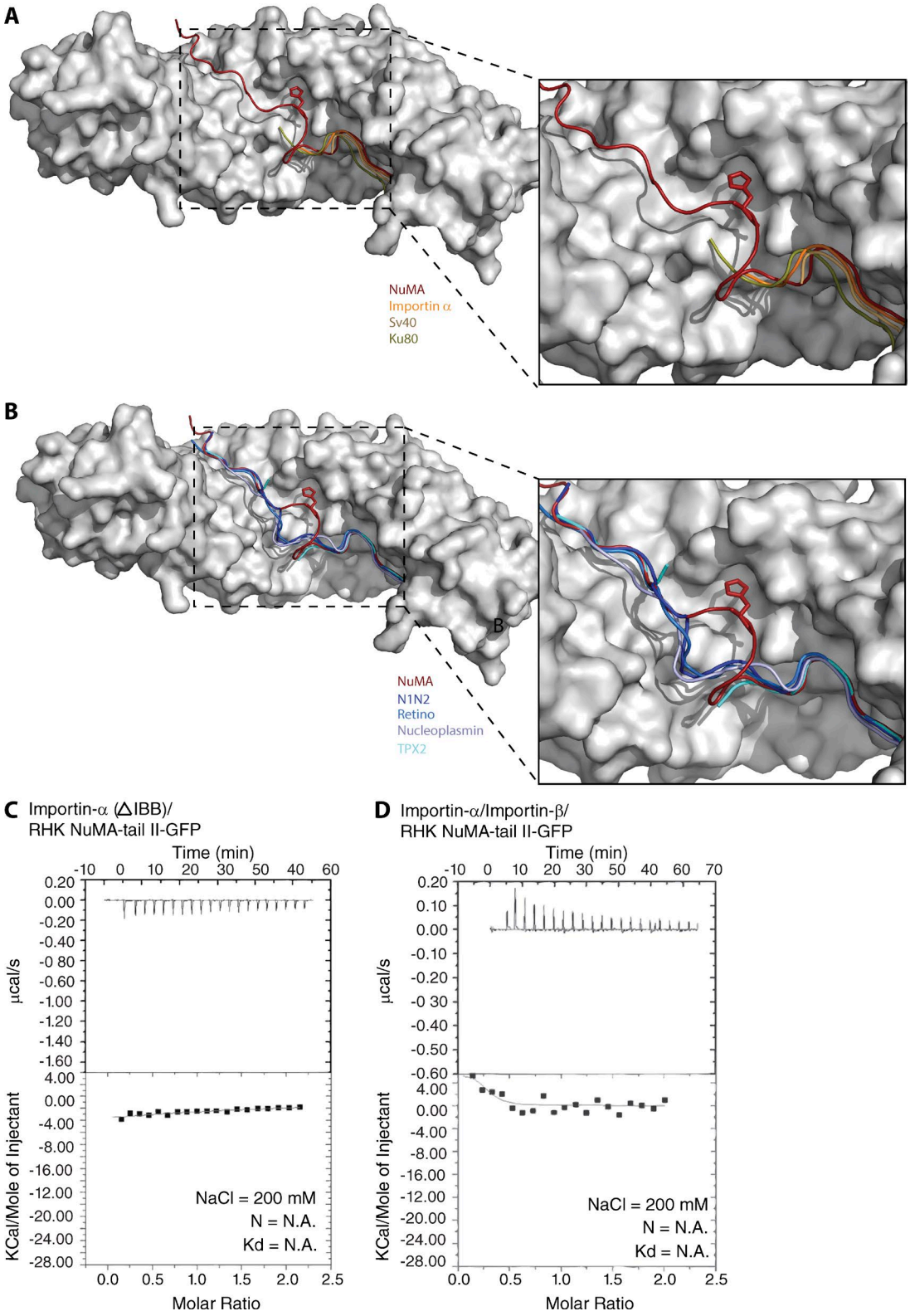

Figure S2. **Structural comparison of NuMA NLS with other classic NLS peptides.** Superimposition of the NuMA-NLS peptide (cyan) with classic monopartite NLS (A) and dipartite NLS (B) peptides. Importin-α (70–498) is shown in gray. Insets show an enhanced view of the overlaid NLS peptides. (C and D) ITC titration curves (top) and binding isotherms (bottom) of RHK-NuMA-tail II-GFP in the presence of Importin-α (ΔIBB; C) and full-length Importin-α/-β (D).

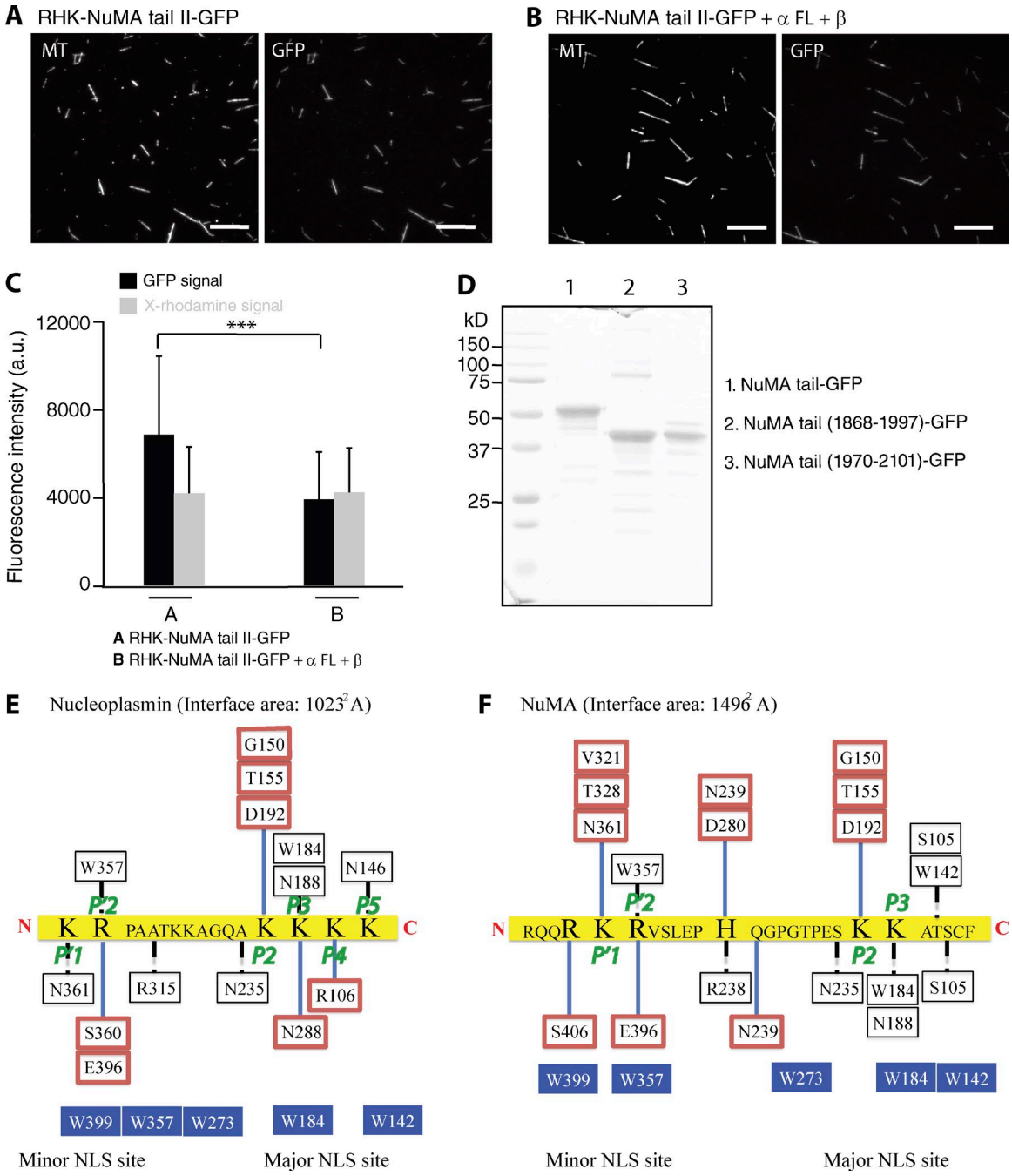

Figure S3. **The nonclassic NuMA-NLS and Importin-α interaction network crucial for microtubule binding.** RHK-NuMA-tail II-GFP in the absence (A) or presence (B) of full-length Importin-α and Importin-β. Bars, 10 µm. (C) Analysis of NuMA (GFP) and microtubule (rhodamine) fluorescence signals. Mean fluorescence signals under the different conditions shown in A and B were measured and plotted. SD was determined from data pooled from three independent experiments. Student *t* test; statistical differences: ***, $P < 0.001$. (D) 5 µg of NuMA-tail II-GFP, NuMA-tail (1868–1991), and NuMA-tail (1970–2091) were analyzed by SDS-PAGE, followed by Coomassie blue staining. (E and F) Schematic illustration of the interaction network of Importin-α with nucleoplasmin and NuMA, respectively. Salt bridges are indicated by blue lines. Hydrogen bonds are shown as black dotted lines. Residues in Importin-α involved in hydrophobic interaction are highlighted by blue boxes.

Table S1. **Crystallographic analysis of the orthorhombic crystals**

Space group	$P2_12_12_1$
Cell dimensions	
a, b, c (Å)	a = 78.1
	b = 89.7
	c = 101.2
α, β, γ (°)	α = β = γ =90°
Data collection	
Wavelength (Å)	1.5418
Resolution (Å)	20.0–2.4
Total reflections	161,007
Unique reflections	27,965
R_{sym} (%)[a]	9.2 (44.0)
CC1/2 (%)[a]	99.9 (83.4)
$\langle I/\sigma I \rangle$[a]	13.3 (2.9)
Completeness (%)[a]	98.3 (85.7)
Redundancy	5.8
Refinement	
R_{work}/R_{free} (%)	19.8/22.5
Bond length rmsd (Å)	0.006
Bond angle rmsd (°)	1.62
Ramachandran plot (%)	
Favored	95.0
Additional allowed	4.3
Generously allowed	0.8
Disallowed	0.0

rmsd, root mean square deviation.
[a]Highest resolution shell is shown in parentheses.