Snow et al., http://www.jcb.org/cgi/content/full/jcb.201212117/DC1

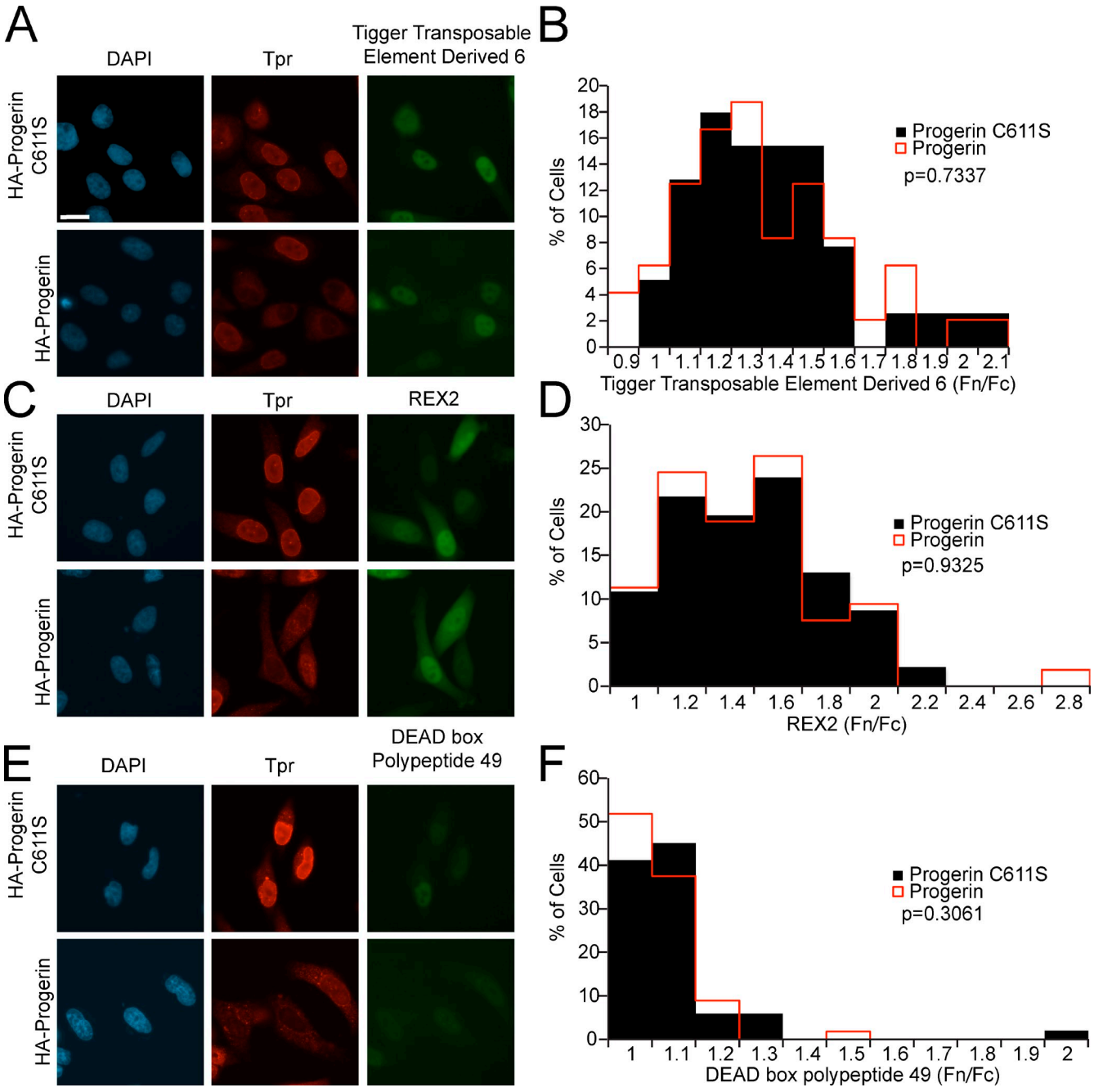

Figure S1. **Progerin does not inhibit nuclear localization of small cargoes.** IF microscopy and histograms of Fn/Fc levels of reporter proteins in HeLa cells transfected with Progerin. (A and B) YFP-tagged Tigger transposable element derived 6 cotransfected with HA-Progerin C611S (n = 39)– and HA-Progerin (n = 48)–transfected cells. P = 0.7337. (C and D) YFP-tagged REX2 cotransfected with HA-Progerin C611S (n = 46)– and HA-Progerin (n = 53)–transfected cells. P = 0.9325. (E and F) YFP-tagged DEAD box polypeptide 49 cotransfected with HA-Progerin C611S (n = 51)– and HA-Progerin (n = 56)–transfected cells. P = 0.3061. The IF microscopy in all panels is from a single experiment and is representative of at least two experiments. Bar, 20 µm. Bar applies to all IF images.

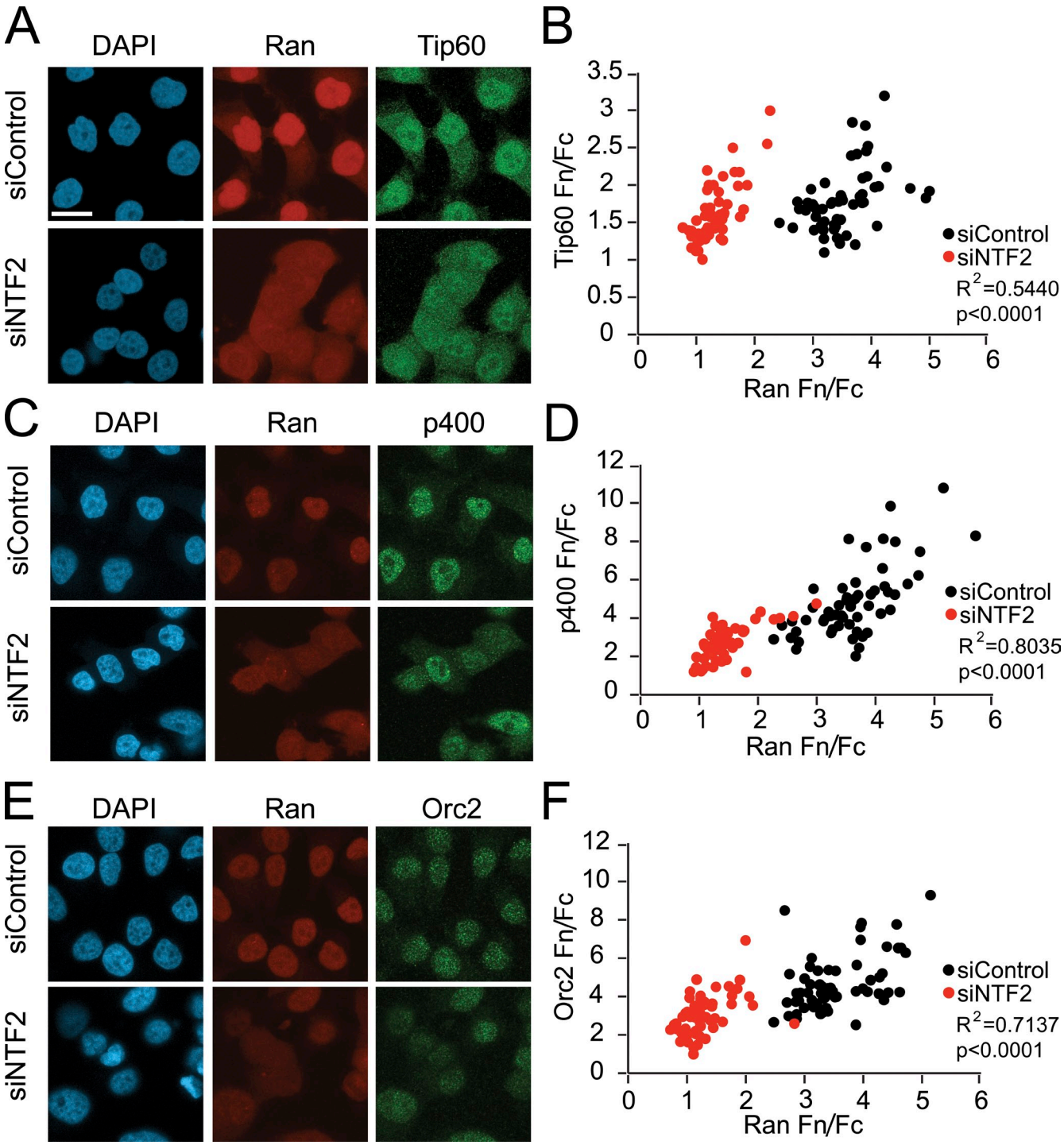

Figure S2. **Proteins that are part of large protein complexes depend on the Ran protein gradient for nuclear import.** HeLa cells were treated with NTF2 siRNA to disrupt the Ran gradient, and Fn/Fc values of the indicated proteins were plotted as a function of Ran Fn/Fc. (A and B) Tip60 in control siRNA (siControl; *n* = 59) and siNTF2 cells (*n* = 56). Spearman P < 0.0001 for collated control siRNA and siNTF2 data. (C and D) p400 in control siRNA (*n* = 58) and siNTF2 cells (*n* = 52). Spearman P < 0.0001 for collated control siRNA and siNTF2 data. (E and F) Orc2 in control siRNA (*n* = 67) and siNTF2 cells (*n* = 54). Spearman P < 0.0001 for collated control siRNA and siNTF2 data. All data are representative of two experiments. Bar, 20 µm. Bar applies to all IF images.

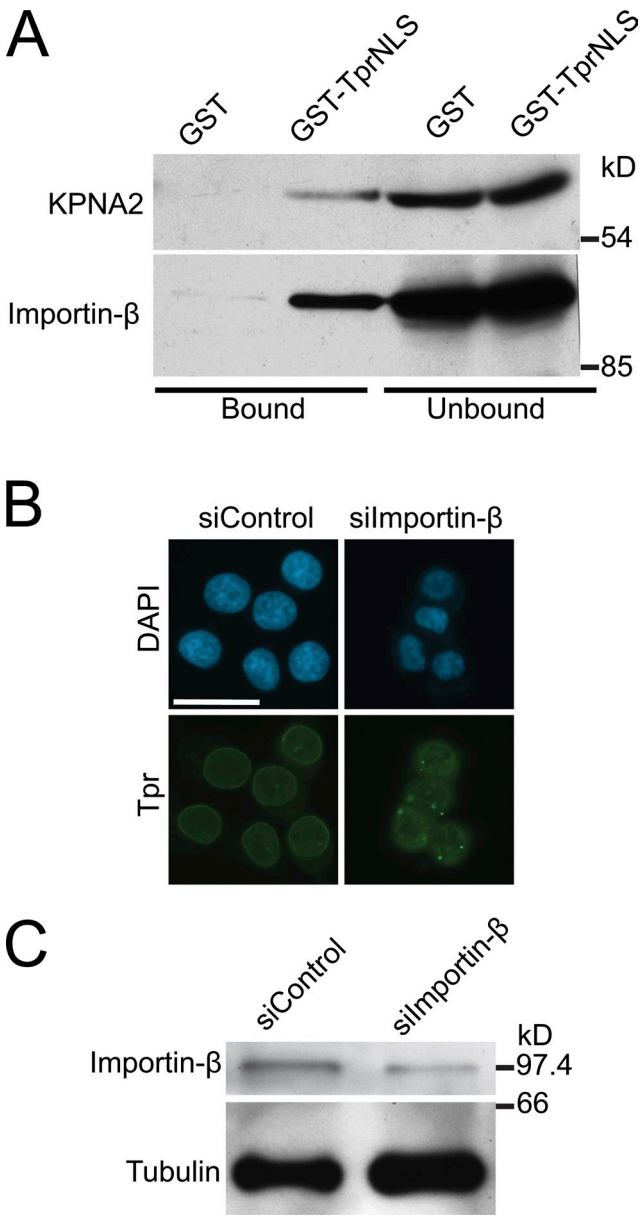

Figure S3. **Importin-β mediates Tpr import.** (A) GST pull-down assay with reticulocyte lysate using immobilized GST and GST-TprNLS. KPNA2 and Importin-β were detected by immunoblotting. (B) IF microscopy for endogenous Tpr in cells treated with siRNA to reduce Importin-β levels. Bar, 20 μm. (C) Immunoblotting of siRNA-treated cells. The gel sample from cells treated with siRNA to Importin-β was overloaded to help estimate knockdown levels. siControl, control siRNA.

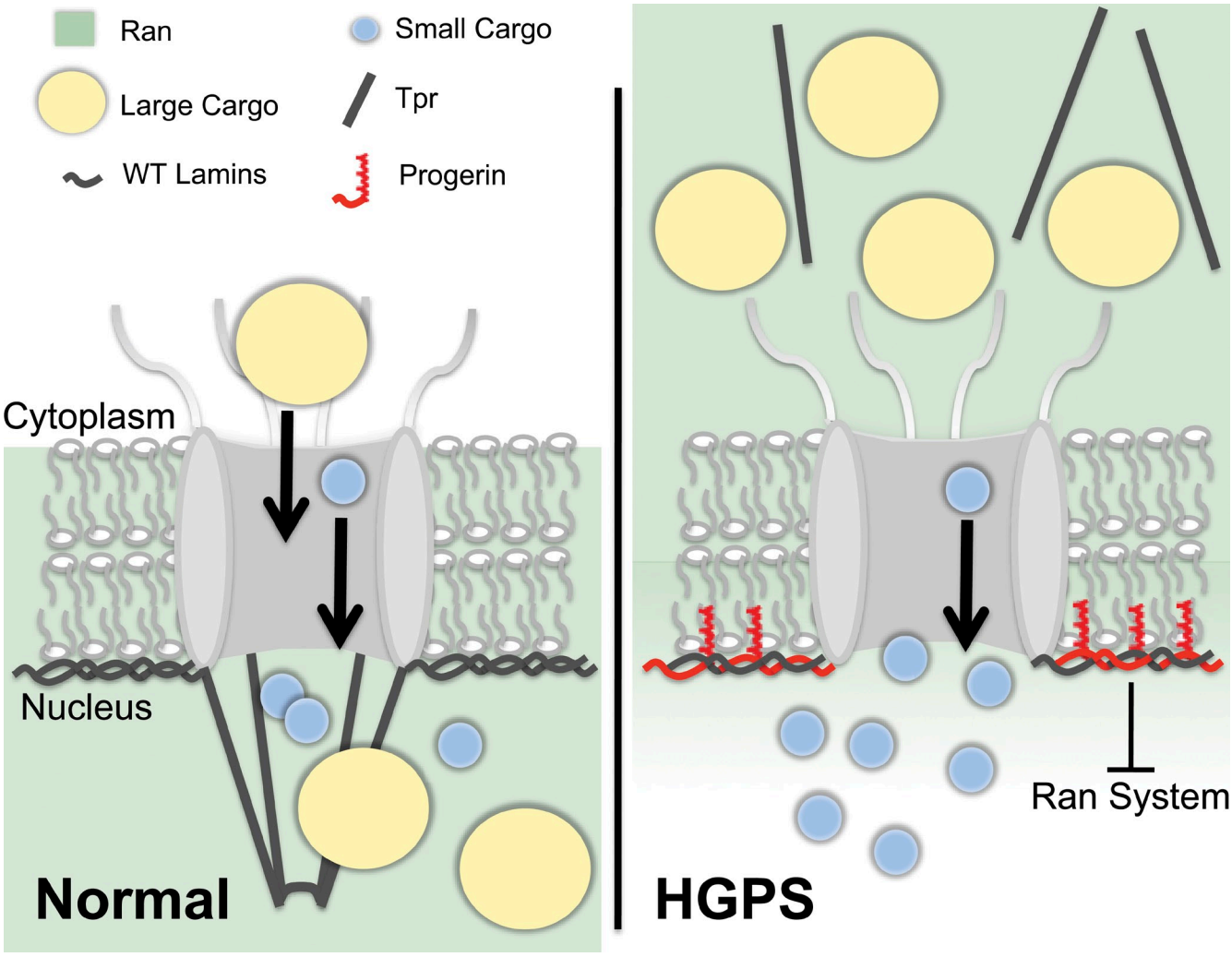

Figure S4. **Model of Progerin effects on nuclear import.** In normal cells (left), the Ran protein gradient (green) facilitates the nuclear import of Tpr and other large protein cargoes. In HGPS, Progerin that is constitutively anchored to the nuclear envelope is transduced as a dominant-negative effect on the Ran system, resulting in disruption of the Fn/Fc Ran protein gradient and defective import of Tpr and other large cargoes. Nuclear levels of Ran in HGPS cells are sufficient for small cargo import. WT, wild type.

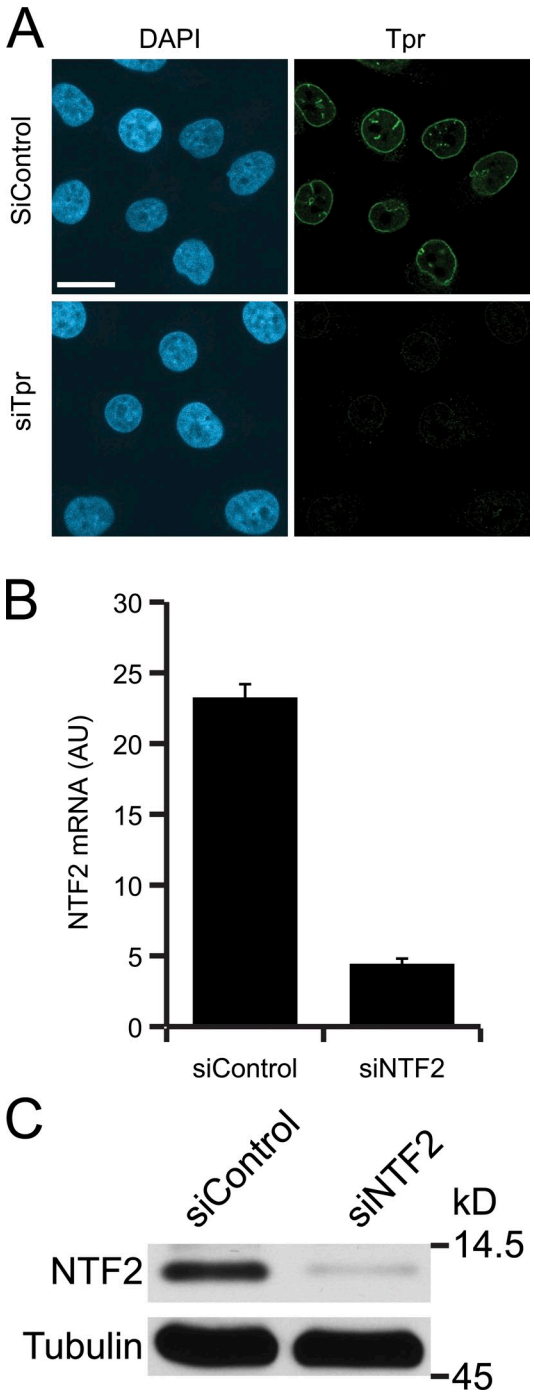

Figure S5. **Tpr-specific antibody and NTF2 knockdown.** (A) A recombinant fragment of Tpr (amino acids 1,649–1,912) was used as the immunogen and for affinity purification. Depletion of Tpr by siRNA reduces antibody signal by IF microscopy, indicating it is specific for Tpr. Bar, 20 µm. (B) NTF2 transcript levels in control and NTF2-depleted HeLa cells determined by RT-PCR. Error bars represent the standard deviation of duplicate wells. (C) NTF2 protein levels in control and NTF2-depleted HeLa cells determined by immunoblotting. AU, arbitrary unit; siControl, control siRNA.

Table S1. **Reporter proteins used in this study**

Protein	ID	NCBI protein accession number	Tag	Localization	Affected by Progerin	Size	Citation
						kD	
pdEGFP-GST-NLS			2×GFP	N	No	76[a]	Obtained from S. Neimanis[b]
p(EGFP)3-RevNLS			3×GFP	N + nucleoli		79[a]	Obtained from S. Hutten[c]
$pGFP_2$-Jun			2×GFP	N > C, N = C	No	37	Obtained from I. Waldmann[d]
Rev-NES-GFP_2-cNLS			2×GFP	N > C, N = C	No	80[a]	Hutten et al., 2008
Negative cofactor 2 (NC2-β)		NP_001929.1		N = C, C > N	No	20	Kahle et al., 2009
Telomerase	NM198253.2	NP_937983.2	GFP	N > C	No	126	
SIRT	OMIM604479	NP_001135970.1	GFP	N	No	82	
Caspase	OMIM147678	NP_001214	GFP	N = C	No	60	
Nmd3	NM015938.3	NP_057022	GFP	N = C	No	55	
SRY (sex determining region Y)-box 6	DKFZp434N1217	AL136780	YFP	N + Dots	No	92	
Tigger transposable element derived 6	DKFZp761E2110	NP_112215	YFP	N > C	No	59	
Cyclin D1	IMAGp958K23187	AAH01501	YFP	N = C, C > N	No	32	
DNA polymerase-transactivated protein 6	IRAUp969E0476	AAH18736	YFP	C > N	No	61	
Zinc finger and BTB domain–containing 33	DKFZp686J23115	CAD98016	YFP	N > C	No	74	
MIS12 homolog (yeast)	DKFZp434I209	CAB66840	YFP	N>, N = C + Dots	No	24	
Deleted in breast cancer gene-1protein (DBC1)	NM199205	NP_954675	GFP	N > C, N = C	No	103	
DEAD (Asp-Glu-Ala-Asp) box polypeptide 43	DKFZp434H2114	AAH66938	CFP	N = C, C > N	No	73	
DEAD (Asp-Glu-Ala-Asp) box polypeptide 59	DKFZp564B1023	CAB66546	YFP	N > C	No	69	
Upstream transcription factor 1	DKFZp686K19103	CAL38711	YFP	N	No	34	
DNA methyltransferase 1–associated protein 1	DKFZp564C0464	CAB66592	CFP	N	No	53	
KIAA1967	DKFZp761I2217	CAD39016	CFP	N, N = C	No	103	
RAD17 homolog (*Schizosaccharomyces pombe*)	DKFZp434A1135	CAB59244	CFP	N = C	No	77	
NUAK family, SNF1-like kinase, 2	DKFZp434J037	CAB66825	CFP	N + Dots	No	74	
KIAA1387 protein	DKFZp761I0112	CAB66491	CFP	N	No	93	
REX2, RNA exonuclease 2 homolog (*Saccharomyces cerevisiae*)	DKFZp566E144	NP_056338	YFP	N > C, N = C	No	27	
mSin3A-associated protein 130	DKFZp434A112	NP_078821.2	YFP	N	No	130	
Zinc finger and BTB domain–containing 20	DKFZp566F123	CAB43377	YFP	N + Dots	No	81	
Chondroitin polymerizing factor	DKFZp434E0423	CAB66748	YFP	N = C, C > N + Dots	No	85	
Tuftelin-interacting protein 11	DKFZp434B194	CAB45740	YFP	N + Dots	No	96	
HSPC065 protein	DKFZp434N1418	CAB66719	YFP	N	No	44	
Cleavage- and polyadenylation-specific factor 3–like	DKFZp434A1923	CAB66747	YFP	N > C, N = C	No	68	
Unc-84 homolog B (*Caenorhabditis elegans*)	DKFZp686F10107	CAD97926	YFP	Rim of N	No	85	
Hypothetical protein DKFZp564O0523	DKFZp564O0523	CAG38580	YFP	N	No	42	

N, nucleus; C, cytoplasm.
[a]Sizes are exclusive of tags except where noted.
[b]Zentrum für Biochemie und Molekulare Zellbiologie, Göttingen, Germany.
[c]Zentrum für Biochemie und Molekulare Zellbiologie, Göttingen, Germany.
[d]Georg-August-University of Göttingen, Göttingen, Germany.

References

Hutten, S., A. Flotho, F. Melchior, and R.H. Kehlenbach. 2008. The Nup358-RanGAP complex is required for efficient importin alpha/beta-dependent nuclear import. *Mol. Biol. Cell*. 19:2300–2310. http://dx.doi.org/10.1091/mbc.E07-12-1279

Kahle, J., E. Piaia, S. Neimanis, M. Meisterernst, and D. Doenecke. 2009. Regulation of nuclear import and export of negative cofactor 2. *J. Biol. Chem.* 284:9382–9393. http://dx.doi.org/10.1074/jbc.M805694200