JCB

Supplemental material

Ben-Yishay et al., https://doi.org/10.1083/jcb.201901127

Provided online are three MATLAB scripts in a ZIP file. The Nup_dis MATLAB script interpolates the data obtained from Excel files representing the intensity of two fluorescent channels, and finds the distance between their maximum intensity positions. The NPC_diameter MATLAB script interpolates the data obtained from two Excel files representing fluorescent intensity along a line to find two maximum intensity positions in each file and calculates the distance between them and the average between the two calculated distances. The FRAP_fit MATLAB script obtains times and average intensities of FRAP recovery experiments and provides a fit (nonlinear least squares) of the data.

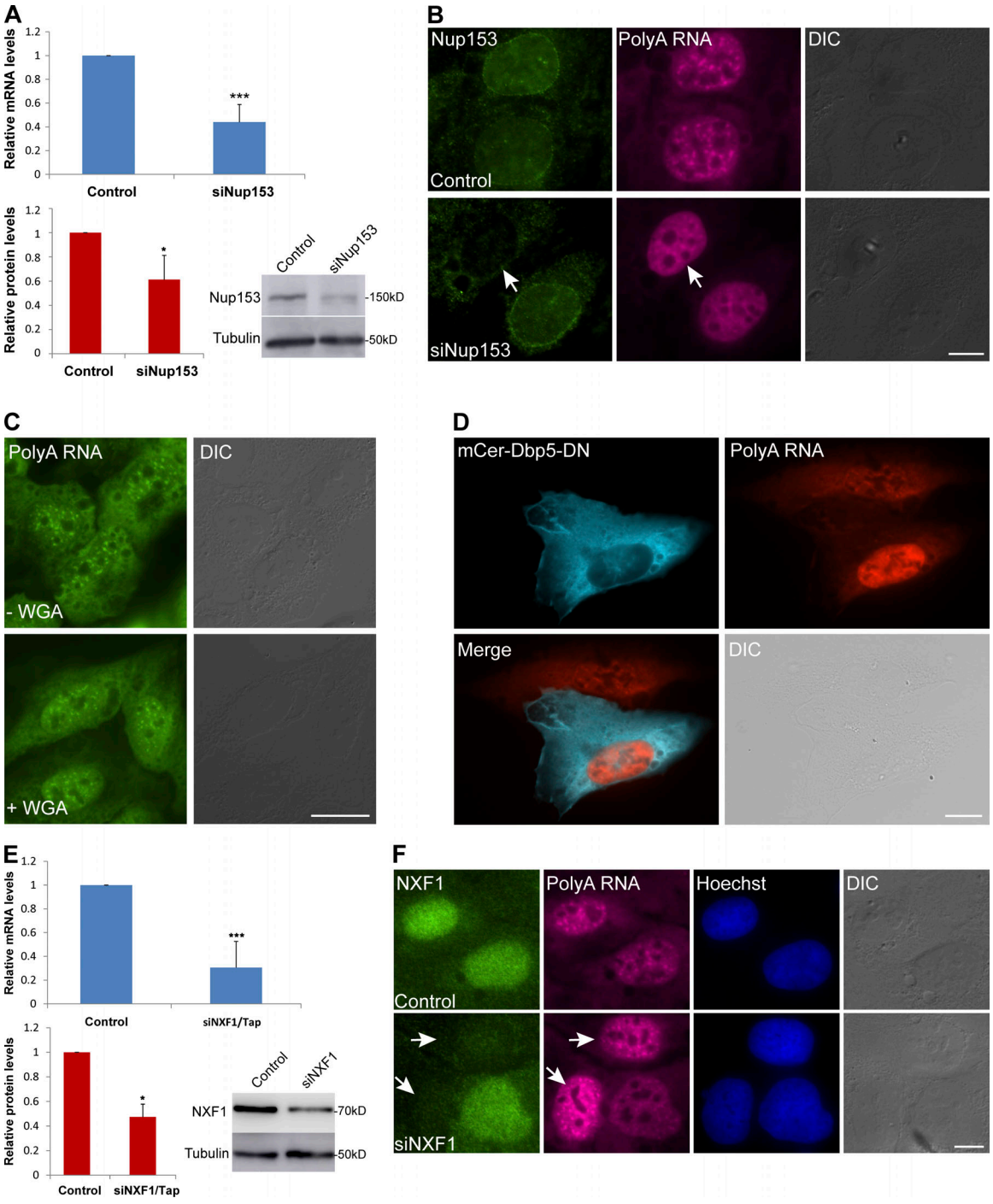


Figure S1. **mRNA export inhibition treatments. (A)** qRT-PCR (top) and Western blotting (bottom) showing Nup153 mRNA and protein levels in siNup153 treated cells relative to control (average ± SD). Molecular weight (MW) is marked on the right. Tubulin and 18S rRNA served as loading controls for qRT-PCR (n = 3, ***, P < 0.001; *, P < 0.05). **(B)** RNA-FISH staining of PolyA+ RNA (magenta) followed by immunostaining of Nup153 (green) in control and siNup153-treated cells. Arrows point at a Nup153-depleted cell. Scale bar, 10 µm. **(C)** RNA-FISH staining of PolyA+ RNA in control (digitonin without WGA) and WGA-treated cells. Differential interference contrast (DIC) is in gray. WGA was administered together with digitonin for 5 min. **(D)** RNA-FISH staining of PolyA+ RNA in cells transfected with Cer-Dbp5-DN. Scale bars, 20 µm. **(E)** qRT-PCR (top) and Western blotting showing NXF1 mRNA and protein levels in siNXF1-treated cells relative to control (average ± SD). Molecular weight is marked on the right. Tubulin and 18S rRNA served as loading controls for the qRT-PCR (n = 3, ***, P < 0.001; *, P < 0.05). **(F)** RNA-FISH staining of PolyA+ RNA (magenta) followed by immunostaining of NXF1 (green) in control and siNXF1-treated cells. Arrows point at NXF1-depleted cells. Hoechst staining is in blue. Scale bar, 10 µm.

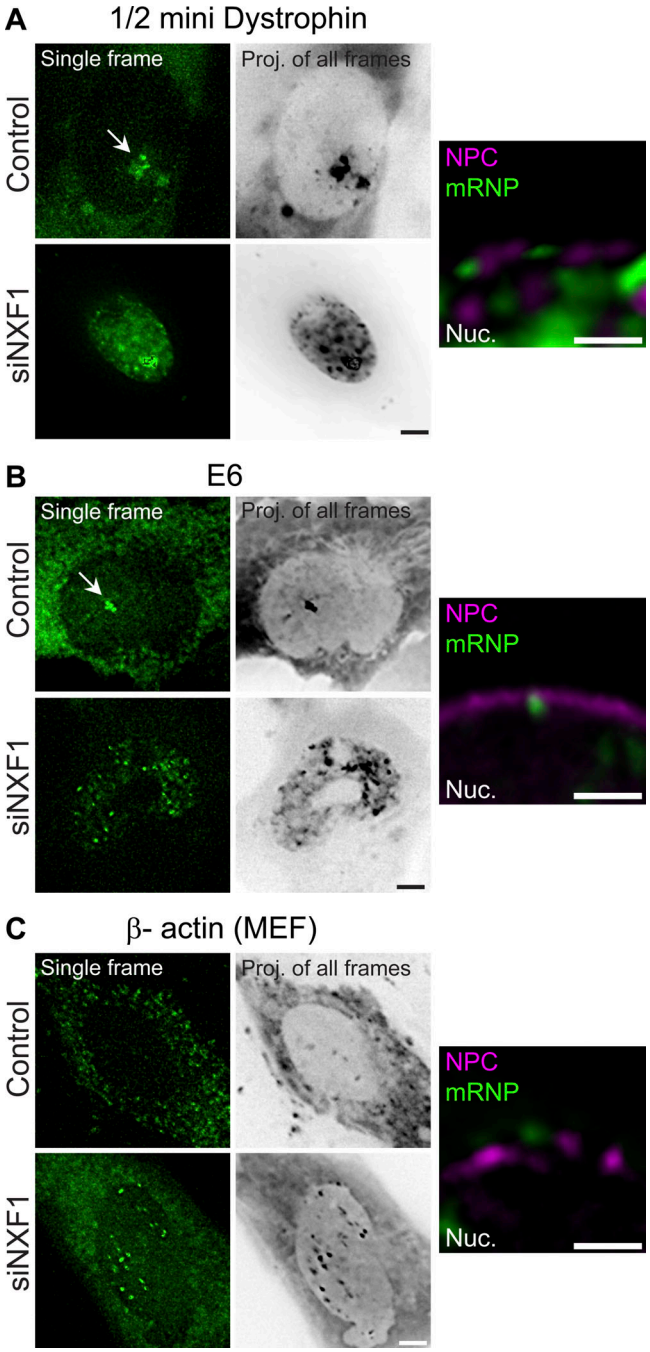


Figure S2. **Reduction in NXF1 levels causes an mRNA export block. (A–C)** Examples of YFP-MS2–tagged mRNPs in cells followed by live-cell imaging under untreated and NXF1 siRNA depletion conditions. Detection of cells that received the siRNA was performed by cotransfection with mCherry-POM121 and mainly by the export defect seen on the tagged mRNPs in the time-lapse videos. Left: Frames from videos showing single mRNPs (green dots; arrows point to sites of transcription). Middle: Average time projections (proj.). Right: Average time projections from Videos 5, 6, and 7 showing the interactions of single mRNPs (green) with POM121-Cherry–tagged NPCs (magenta) under the export blockage condition. The cell lines used were (A) U2OS cells stably expressing YFP-MS2–tagged Cerulean-1/2-minidystrophin-MS2 mRNPs (Mor et al., 2010) induced to transcribe with Ponasterone A for 4 h, (B) U2OS cells stably expressing YFP-MS2–tagged E6 mRNPs (Brody et al., 2011) induced to transcribe by doxycycline for 6 h, and (C) MEFs expressing endogenously tagged β-actin-MS2 mRNPs induced to transcribe by serum (Lionnet et al., 2011). Scale bars, 5 µm (left) and 2 µm (right).

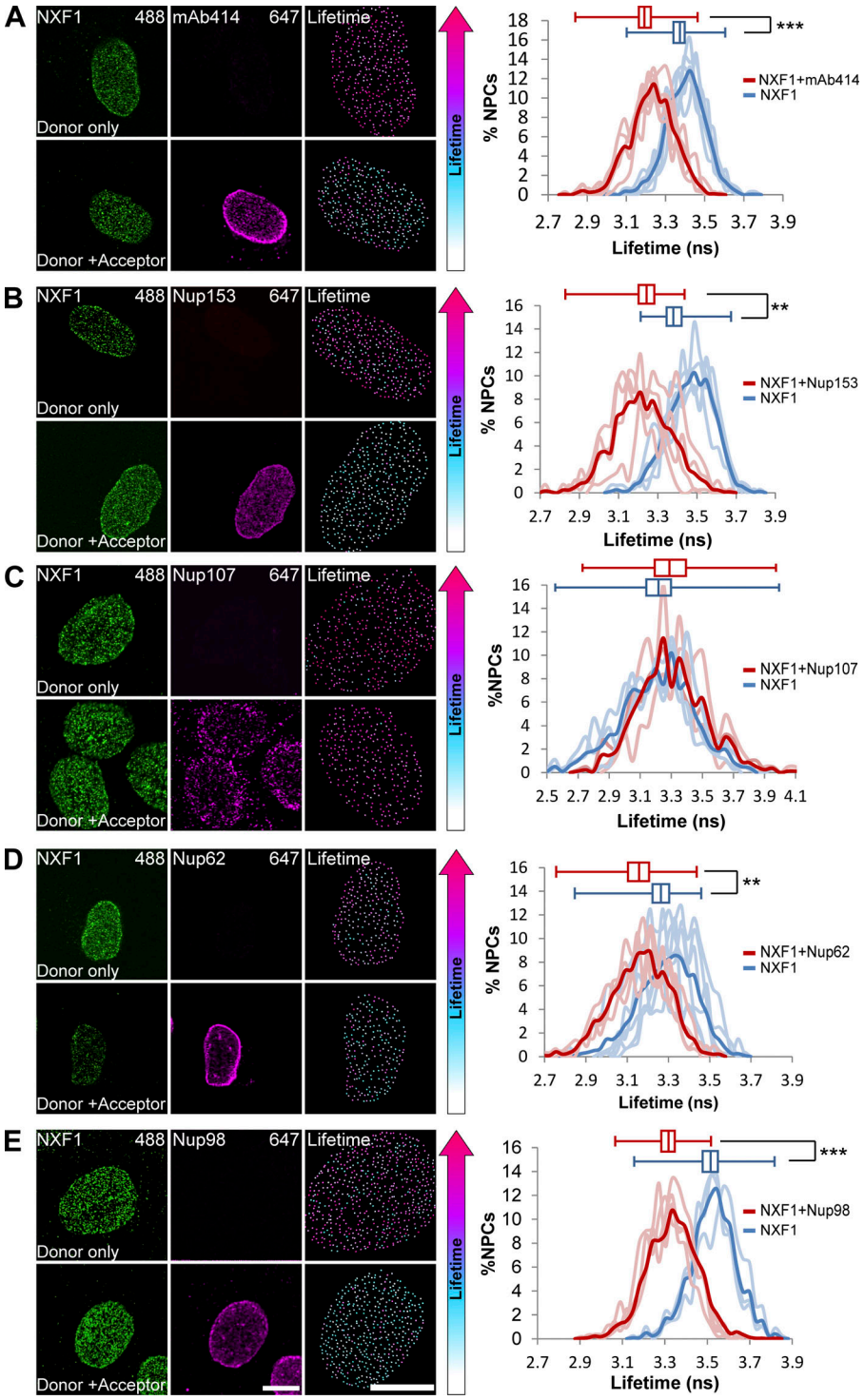


Figure S3. **Detecting interactions of NXF1 with Nups in single NPCs using FLIM-FRET. (A–E)** Immunostaining and LT analysis of NXF1 (donor) along with (A) mAb414, (B) Nup153, (C) Nup107, (D) Nup62, and (E) Nup98 (acceptor). Cells were immunostained with either donor-only or donor + acceptor primary antibodies, and with two secondary antibodies (Alexa Fluor 488 as donor and Alexa Fluor 647 as acceptor). Single NPCs were detected using Imaris, and the LT values for the pixels of each individual NPC were measured. All detected NPCs were pseudocolored according to the donor's LT values. Right-hand boxes show an enlarged image of the nucleus. The histograms show the LT values of the NXF1 donor in single NPCs in donor-only cells (blue) compared with donor + acceptor cells (red). Light lines represent data from single cells, and the bold plot contains data from all cells. Box plots: Center line, median; box limits, upper and lower quartiles; whiskers, minimum to maximum range. 1,977 NPCs, *n* = 6 cells, (NXF1-donor only); 1,864 NPCs, 6 cells (NXF1 + mAb414, donor + acceptor) ***, P < 0.001; 1,604 NPCs, 5 cells (NXF1-donor only); 1,245 NPCs, 4 cells (NXF1 + Nup153, donor + acceptor) **, P < 0.01; 970 NPCs, 3 cells (NXF1-donor only); 820 NPCs, 3 cells (NXF1 + Nup107, donor + acceptor); 1944 NPCs, 6 cells (NXF1-donor only); 991 NPCs, 4 cells (NXF1 + Nup62, donor + acceptor) **, P < 0.01; 1,193 NPCs, 3 cells (NXF1-donor only); 2,252 NPCs, 4 cells (NXF1 + Nup98, donor + acceptor). ***, P < 0.001. Scale bars, 10 µm.

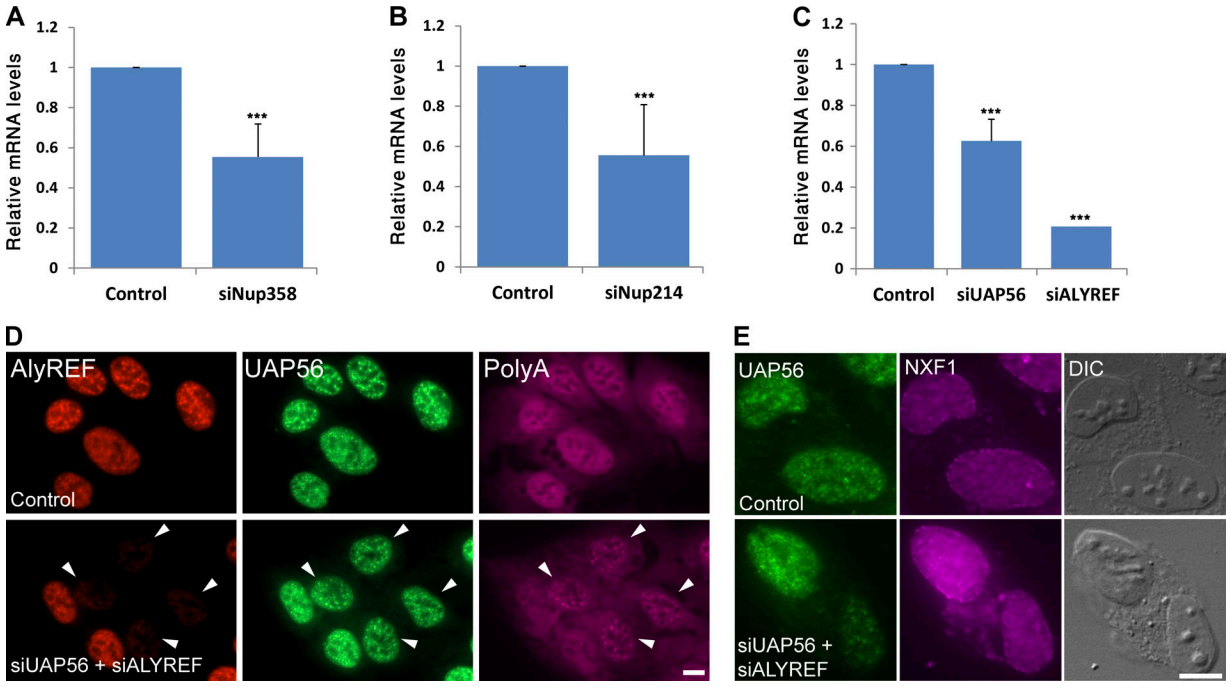


Figure S4. **mRNA export inhibition treatments. (A–C)** qRT-PCR showing (A) Nup358, (B) Nup214, and (C) AlyREF and UAP56 mRNA levels in siRNA-treated cells relative to control (average ± SD). Tubulin and 18S rRNA served as loading controls for the qRT-PCR (n = 3; ***, P < 0.001). **(D)** RNA-FISH staining of polyA+ RNA (magenta) followed by immunostaining of AlyREF (red) and UAP56 (green) in control and siUAP56 + siAlyREF–treated cells. Arrowheads point to cells in which an export block was detected. **(E)** NXF1 is still found in the nuclear periphery when the levels of UAP56 and siAlyREF are knocked down (since only two antibodies could be used, AlyREF could not be imaged as well). Scale bars, 10 µm.

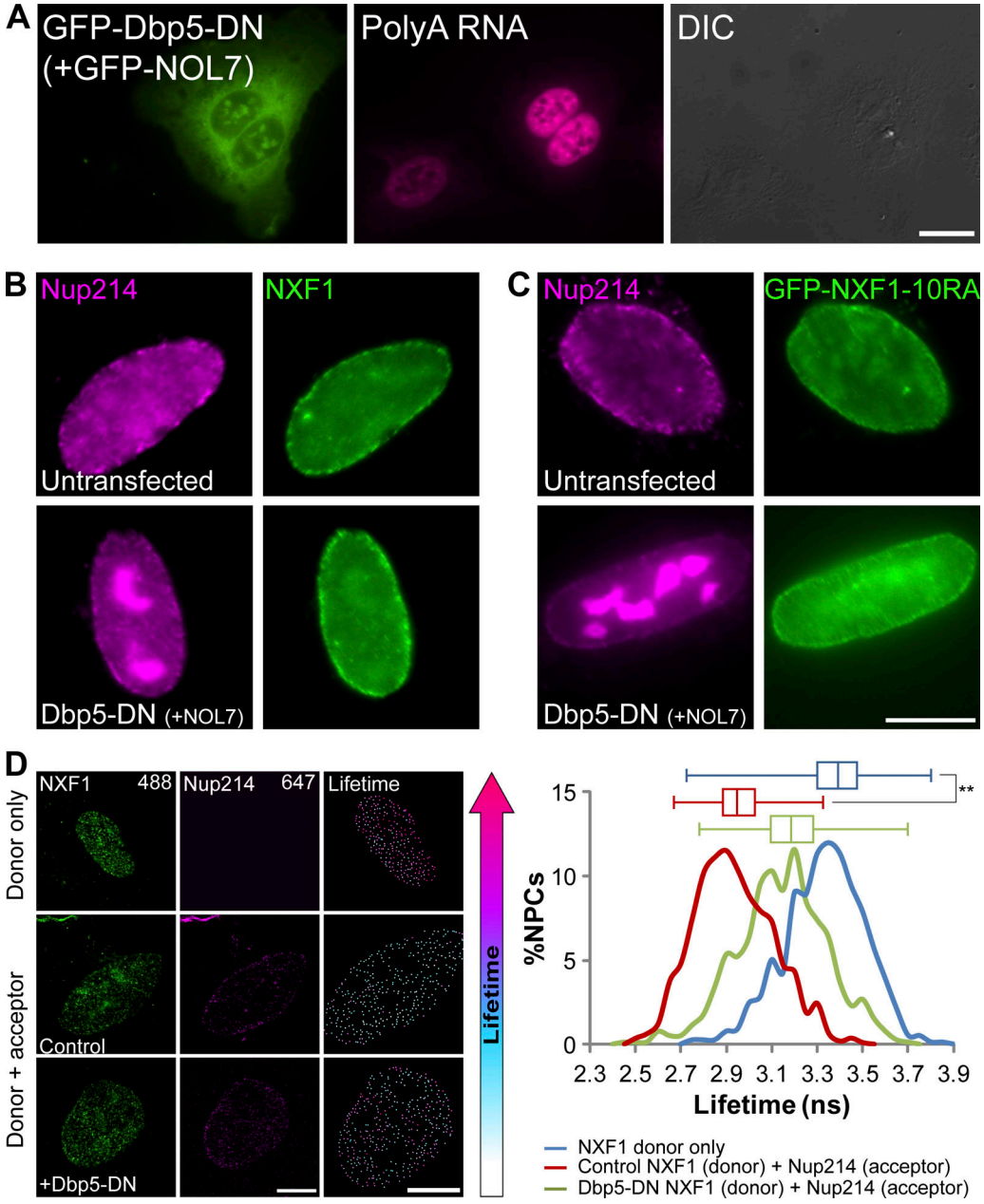
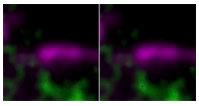
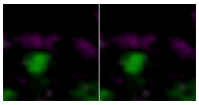


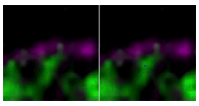
Figure S5. **NXF and NXF1-10RA remain positioned at the NPC after Dbp5-DN export inhibition as measured by FLIM-FRET. (A)** U2OS cells were transfected with GFP-Dbp5-DN and GFP-NOL7 (at a ratio of 10:1, respectively). RNA FISH with an oligo(dT) probe detected polyA+ RNA. Cells expressing GFP-NOL7+Dbp5-DN demonstrated an mRNA export defect (increase in nuclear polyA+ RNA signal), showing that cotransfection of GFP-NOL7 is a reliable indicator of Dbp5-DN expression and export inhibition. Scale bar, 20 µm. **(B)** U2OS cells were either untransfected or transfected with GFP-Dbp5-DN, together with RFP-NOL7, and immunostained with anti-NXF1 and anti-Nup214 antibodies. NXF1 was present in the NPCs under both conditions. **(C)** U2OS cells stably expressing GFP-NXF1-10RA were either untransfected or transfected with RFP-NOL7 together with GFP-Dbp5-DN and immunostained with an anti-Nup214 antibody. Scale bar, 10 µm. **(D)** FLIM-FRET analysis of NXF1 interactions with Nup214 in U2OS cells during an mRNA export block caused by Dbp5-DN. Since the cytoplasmic GFP-Dbp5 signal is lost during the fixation procedure, we used a nucleolar protein (GFP-NOL7) as a transfection marker (NOL7 signal was not included in the analysis; see Materials and methods). Cells transfected with GFP-NOL7 only (control) or GFP-Dbp5-DN along with GFP-NOL7 were immunostained with either donor-only (anti-NXF1) or donor + acceptor (anti-NXF1 + anti-Nup214) primary antibodies and two secondary antibodies (Alexa Fluor 488 as donor and Alexa Fluor 647 as acceptor). Analysis was performed only on single NPCs, detected by Imaris. All detected NPCs were pseudocolored according to the donors' lifetime values, measured by the Pico-Quant system. Right-hand boxes show an enlarged image of the nucleus. Scale bar, 10 µm. Histogram showing the lifetime values of the NXF1-donor in single NPCs in donor-only cells (blue, *n* = 774 NPCs, 3 cells) compared with donor + acceptor control cells (red, *n* = 1,115 NPCs, 3 cells) and donor + acceptor cells expressing Dbp5-DN (green, *n* = 783 NPCs, 3 cells). Box plots: center line, median; box limits, upper and lower quartiles; whiskers, minimum to maximum range. **, $P < 0.01$.



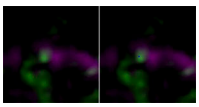
Video 1. **Nup153 is required for the interaction of mRNPs with the NPC.** Nuclear mRNPs (marked by YFP-MS2, green) in a Nup153-depleted Mini-Dys cell do not stall at the nuclear pores (marked by mCherry-POM121, magenta) when mRNA export is blocked. Top, cytoplasm; bottom, nucleus. Imaged every 0.8 s for 23.2 s, 7 frames per second (fps).



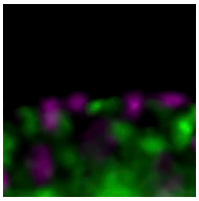
Video 2. **WGA export block prevents the entry of mRNPs into the NPC, resulting in static mRNPs anchored to the nuclear side of the pores.** Nuclear mRNP (marked by YFP-MS2, green) in a WGA-treated Mini-Dys cell is observed anchored to the nuclear side of the NPC (marked by mCherry-POM121, magenta) and is unable to enter into the pore. The right-hand image includes the track in blue. Top, cytoplasm; bottom, nucleus. Imaged every 0.8 s for 62.4 s, 7 fps.



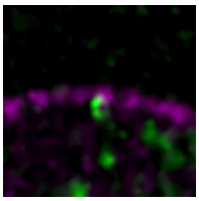
Video 3. **Dbp5-DN expression prevents the release of mRNPs into the cytoplasm.** Nuclear mRNP (marked by YFP-MS2, green) in a Dbp5-DN–expressing Mini-Dys cell is observed entering into the NPC (marked by mCherry-POM121, magenta) but is not released into the cytoplasm. Top, cytoplasm; bottom, nucleus. The right-hand image includes the track in blue. Imaged every 0.8 s for 46.4 s, 7 fps.



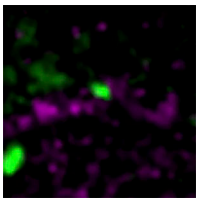
Video 4. **NXF1 in the NPC is not required for the initial interaction and translocation of mRNPs into the NPCs but is essential for their final release into the cytoplasm.** Nuclear mRNP (marked by YFP-MS2, green) in an NXF1-depleted Mini-Dys cell is observed entering into the NPC (marked by cotransfection with mCherry-POM121, magenta) but is not released into the cytoplasm. Top, cytoplasm; bottom, nucleus. The right-hand image includes the track in blue. Imaged every 0.8 s for 63.2 s, 7 fps.



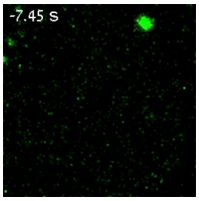
Video 5. **mRNPs stalled at the NPCs: 1.** Nuclear mRNP (marked by YFP-MS2, green) in an NXF1-depleted Cer-1/2-Mini-Dys U2OS cell is stalled at the nuclear periphery (marked by mCherry-POM121, magenta) but is not released into the cytoplasm. Top, cytoplasm; bottom, nucleus. Imaged every 1 s for 80 s, 7 fps.



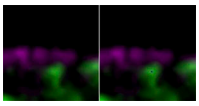
Video 6. **mRNPs stalled at the NPCs: 2.** Nuclear mRNP (marked by YFP-MS2, green) in an NXF1-depleted E6 mRNA expressing U2OS cell is stalled at the nuclear periphery (marked by mCherry-POM121, magenta) but is not released into the cytoplasm. Top, cytoplasm; bottom, nucleus. Imaged every 1 s for 80 s, 7 fps.



Video 7. **mRNPs stalled at the NPCs: 3.** Nuclear mRNP (marked by YFP-MS2, green) in an NXF1-depleted β-actin-MS2 mRNA–expressing MEF is stalled at the nuclear periphery (marked by mCherry-POM121, magenta) but is not released into the cytoplasm. Top, cytoplasm; bottom, nucleus. Imaged every 1 s for 80 s, 7 fps.



Video 8. **NXF1 reaches the NPC within seconds.** U2OS cells expressing photoactivatable PAGFP-NXF1 were photoactivated in the nucleoplasm, and the signal (pseudocolored black) was detected and followed over time. Imaged every 1.49 s for 58.11 s, 5 fps.



Video 9. **Removal of NXF1 from the NPC by FG domain overexpression does not disrupt the mRNP–NPC interaction.** Nuclear mRNP (green) in a Cerulean-2xGLFG-HoxA9 Mini-Dys cell is observed entering into the NPC (marked by mCherry-POM121, magenta) is not released into the cytoplasm and returns back into the nucleus. Top, cytoplasm; bottom, nucleus. The right-hand image includes the track in blue. Imaged every 0.8 s for 63.2 s, 7 fps.

References

Brody, Y., N. Neufeld, N. Bieberstein, S.Z. Causse, E.M. Böhnlein, K.M. Neugebauer, X. Darzacq, and Y. Shav-Tal. 2011. The in vivo kinetics of RNA polymerase II elongation during co-transcriptional splicing. *PLoS Biol.* 9:e1000573. https://doi.org/10.1371/journal.pbio.1000573

Lionnet, T., K. Czaplinski, X. Darzacq, Y. Shav-Tal, A.L. Wells, J.A. Chao, H.Y. Park, V. de Turris, M. Lopez-Jones, and R.H. Singer. 2011. A transgenic mouse for in vivo detection of endogenous labeled mRNA. *Nat. Methods.* 8:165–170. https://doi.org/10.1038/nmeth.1551

Mor, A., S. Suliman, R. Ben-Yishay, S. Yunger, Y. Brody, and Y. Shav-Tal. 2010. Dynamics of single mRNP nucleocytoplasmic transport and export through the nuclear pore in living cells. *Nat. Cell Biol.* 12:543–552. https://doi.org/10.1038/ncb2056