

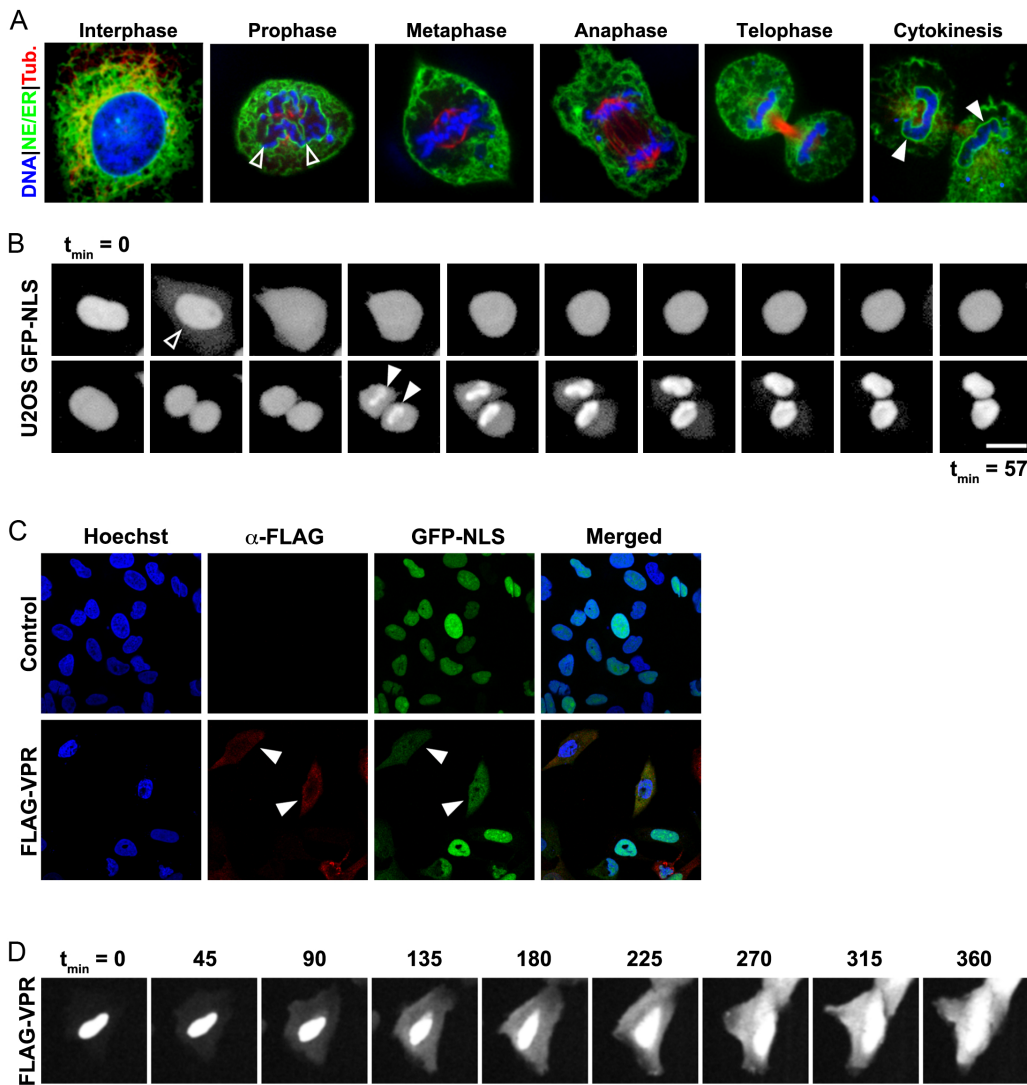
Supplementary Material to:

Transient nuclear envelope rupturing during interphase in human cancer cells

Jesse D. Vargas, Emily M. Hatch, Daniel J. Anderson and Martin W. Hetzer

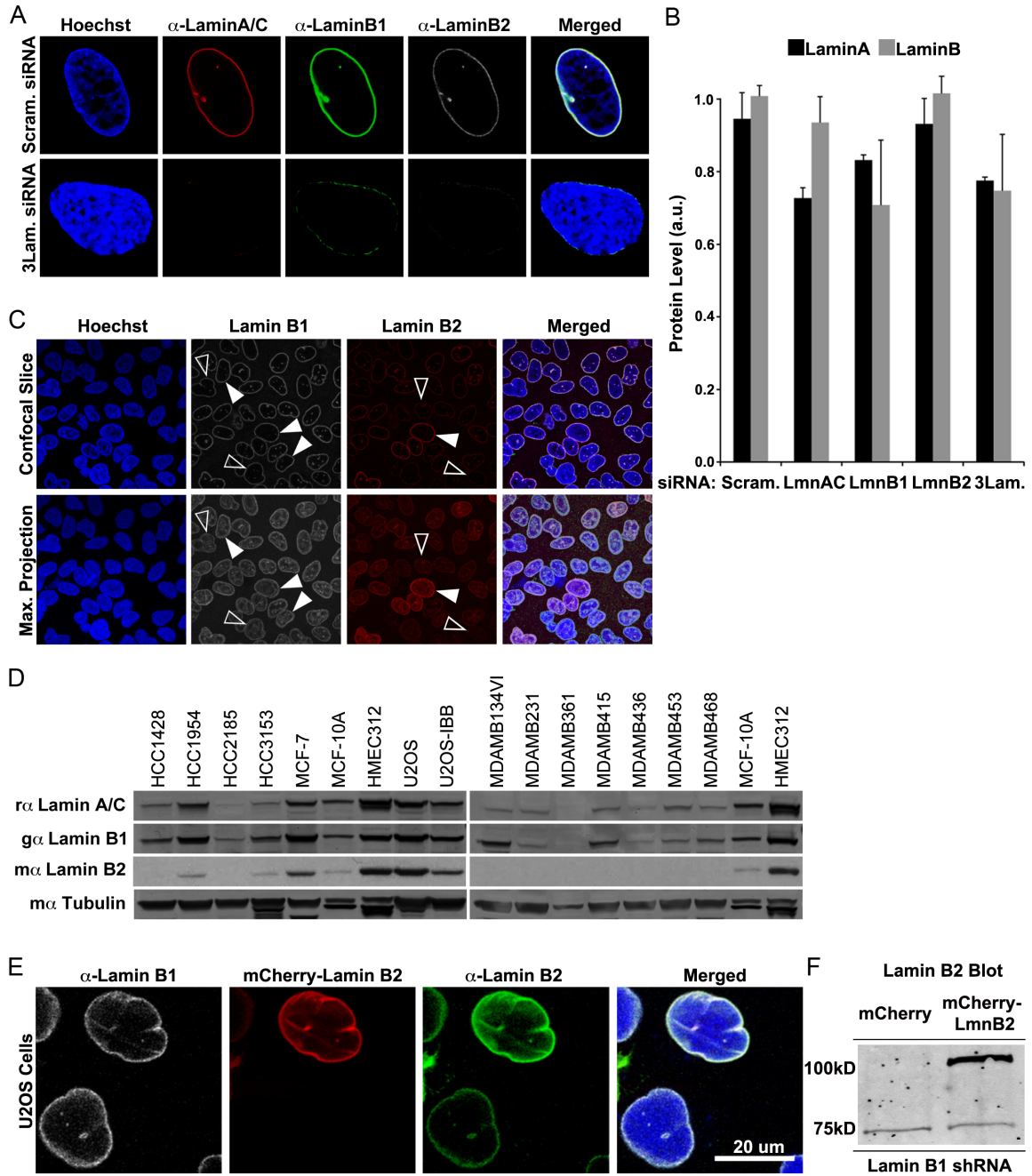
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Supplemental Figure 1: GFP₃-NLS Expression and Localization. A) Phases of the cell cycle. Freeze frame images from live imaging of U2OS cells expressing a fragment of Sec61b-GFP (green) to visualize the NE/ER, mCherry-tubulin (red), and stained for DNA with Hoechst (blue) show mitotic NEBD (open arrows) and nuclear envelope reformation (solid arrows). **B)** Freeze frame images from live imaging of U2OS cells expressing GFP₃-NLS showing mitotic NEBD (open arrows) and re-accumulation of GFP₃-NLS after nuclear envelope reformation (solid arrows). **C)** Immunofluorescence images of untransfected (top) and FLAG-VPR transiently transfected U2OS cells expressing GFP₃-NLS stained for the FLAG epitope (red) demonstrate cytoplasmic localization of GFP₃-NLS in cells expressing FLAG-VPR. **D)** Freeze frame images from live imaging of U2OS cells expressing GFP₃-NLS and transiently transfected with FLAG-VPR show NE rupture persisting for 300 minutes without recover of GFP₃-NLS into the nucleus.

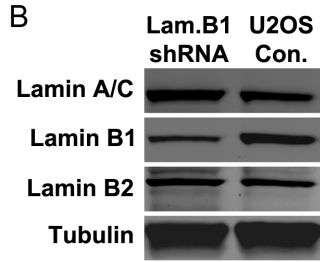
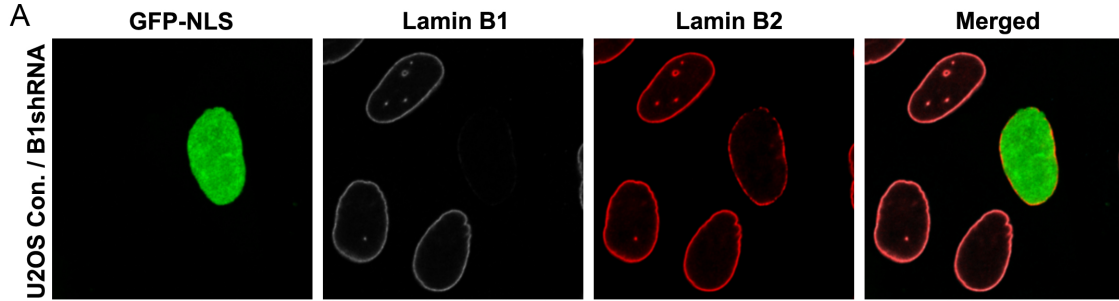


Supplemental Figure 2: Lamin Knock Down and Differential Expression. A) Immunofluorescence of U2OS cells treated with scrambled control or 3 lamin siRNA cocktail showing knock down efficiency of lamins A/C (red) B1 (green) and B2 (white) when siRNAs are delivered as a pool by transient transfection. **B)** Analysis of western blots of U2OS whole cell lysates from cells treated with individual siRNAs against lamin A/C, B1, or B2 and the combination of the 3 siRNAs delivered as a pool by transient transfection show an average reduction of 20% of lamin protein expression across a population of cells in culture. **C)** Differential expression of lamin protein in U2OS cells demonstrated by immunofluorescence of U2OS cells stained for lamins B1 (white) and B2 (red). Confocal slice (top) and maximum projections (bottom) show different intensities between cells for both b-type lamins, indicated by arrows. **D)** Western blot of whole cell lysates from 12 breast cancer cell lines (HCC, MCF-7 and MDAMB lines) compared to untransfected U2OS osteosarcoma lines, U2OS stable line expressing the integrity reporter GFP-IBB, MCF-10A spontaneously immortalized line, and HMEC312 primary cell line show diverse levels of expression of the three lamin proteins. **E)** Normal U2OS cells stained for endogenous lamin B1 (white) and B2 (green) expression and for DNA (blue) transiently transfected with an mCherry-lamin B2 expression construct (red) showing localization of the full length lamin B2 construct and the endogenous b-type lamin proteins. **F)** Western blot showing expression of mCherry-lamin B2 in U2OS cells stably reduced for lamin B1 expression by shRNA compared to controls transfected with mCherry.

See next page.



Supplemental Figure 3: shRNA Stable Lamin Knock Down. A) Immunofluorescence of U2OS cells stably expressing GFP₃-NLS (green) and an shRNA against lamin B1, showing lamin B1 (white) and lamin B2 (red) expression. **B)** Western blot of lamin B1 shRNA knock down efficiency showing lamin A/C, lamin B1, and lamin B2 protein expression with tubulin as a loading control.



Please see

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for Supplemental VIDEO files.

Supplemental Movie 1: Nuclear envelope rupture during interphase. U2OS cells expressing GFP₃-NLS were imaged over time and show the rapid loss of nuclear GFP intensity into the cytoplasm with a slower recovery of nuclear signal and concomitant clearing of cytoplasmic GFP.

Supplemental Movie 2: Rupturing appears heritable and recoverable. U2OS cells expressing GFP₃-NLS stably reduced for lamin A/C, B1, and B2 expression by triple siRNA spill frequently, recover, and go on to divide with progeny also exhibiting interphase NE rupturing.

Supplemental Movie 3: Rupturing of nuclei produces micronuclei-like bodies. U2OS cells from Movie 2 above shown through an interphase rupture event and cell division have associated extra-nuclear spheres containing GFP₃-NLS.

Supplemental Movie 4: Localized nuclear herniation precedes rupturing. U2OS cell expressing GFP₃-NLS is shown during a rupture event. The movie is slowed for facilitated visualization of the nuclear herniation.

Supplemental Movie 5: Nuclear mitochondria. 3D reconstruction of a U2OS cell stably reduced for lamin B1 expression by shRNA and expressing pTurboRFP-Mito show the presence of mitochondria within the DNA.