Supplemental Materials

Molecular Biology of the Cell

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Figure S1. Characterization of U2OS shLMNB1 cell line, BAF recruitment to clustered ruptures, and kinetics of GFP-BAF loss from rupture sites. (A) Representative immunoblot of lamin B1 protein levels in normal versus LMNB1 shRNA expressing U2OS cells. Mean lamin B1 expression in shLMNB1 cells relative to control was 0.25, n = 3 blots. (B) Representative images of U2OS and U2OS shLMNB1 cells labeled with antibodies to lamin B1 and lamin A/C. Images are a single slice from the center or bottom (“surface”) of the nucleus. Boxes indicate nuclei enlarged in surface slices, which show an increase in nuclear lamina gaps in cells depleted of lamin B1. (C) Quantification of RFP-NLS mean intensity during nucleus ruptures (extent rupture = 0.3 to 0.5) in U2OS and U2OS shLMNB1 cells (n values: U2OS, 15; U2OS shLMNB1, 6 ruptures). t0 = start of repair (first value of consistent RFP-NLS intensity increase). Mean and 95% CI plotted. (D) Still images of multiple ruptures on the same herniation in a U2OS GFP-BAF RFP-NLS shLMNB1 repair cell, 30 sec pass time. Each row depicts a separate rupture event and GFP-BAF accumulation. Right: GFP-BAF images from left panels are overlaid and color coded for time of rupture. (E) Top: RFP-NLS mean intensity and GFP-BAF integrated density traces for three ruptures in a single nucleus (R1-3) occurring within 11 min of each other, 30 sec pass time. Bottom: still images of rupture 1, with RFP-NLS intensity inverted and oversaturated to show RFP-NLS in cytoplasm concurrent with GFP-BAF accumulation. (F) RFP-NLS mean intensity and GFP-BAF integrated density traces aligned and normalized as in figure 1C, for ruptures where duration > 5 min, 30 sec pass time. Mean (thick line) and replicates (open circles) are plotted. Arrow = time of repair start, arrowhead = time of first GFP-BAF decrease. n = 2 ruptures. (G) RFP-NLS mean intensity and GFP-BAF integrated density traces from the primary nucleus (PN) and micronucleus (MN) of the cell in figure 1F. RFP-NLS is absent from the MN after membrane rupture. Scale bars = 10 μm. AU = arbitrary units.
Figure S2. BAF depletion controls. (A-B) Representative images of U2OS RFP-NLS shLMNB1 cells 48 hr after transfection with siControl (Ctl) or siBAF and labeled with antibodies to lamin A/C and emerin. Scale bars = 10 µm. (C) Quantification of the proportion of nuclei that ruptured at least once during 24 hr of imaging after transfection with siCtl or siBAF (n values: siCtl, 597; siBAF, 616 ruptures from 3 experiments. ns = p > 0.05, Fisher’s exact test. (D) Quantification of mean ruptures/nucleus (n values: siCtl, 61; siBAF, 64 nuclei from 5 and 4 experiments, respectively). (E) Representative immunoblot of BAF protein levels in U2OS cells 48 hr after siRNA transfection. (F) Quantification of nucleus rupture durations after transfection with siCtl or siBAF (n values: siCtl, 165; siBAF, 124 ruptures from 3 experiments). *p<0.05, K-S test. (G) Still images of RFP-NLS from U2OS RFP-NLS shLMNB1 cells transfected with indicated siRNAs after laser-induced rupture. Start repair = RFP-NLS regain start in figure 2A. (H) Quantification of nucleus rupture durations in cells transfected with indicated siRNAs after laser-induced rupture (n values: spontaneous, 12; laser-induced, 25). ***p<0.001, Mann-Whitney test. (I) Still images of RFP-NLS from cells transfected with siCtl showing maximum extent rupture during either spontaneous or laser-induced nucleus ruptures. Arrowheads in J-K indicate rupture sites and scale bars = 10 µm. (J) Quantification of nucleus rupture extent during either spontaneous (Spont.) or laser-induced nucleus ruptures (n values: spontaneous, 12; laser-induced, 25). ***p<0.001, Mann-Whitney test. (K) Quantification of nucleus rupture duration of spontaneous or laser-induced ruptures in cells transfected with siCtl (n values: spontaneous, 126; laser-induced, 32). **p<0.01, K-S test. (L) Histogram of proportion of ruptures shown in Figure 2C with indicated nucleus rupture durations. **p<0.01, *p<0.05, Fisher’s exact test. (M) Nucleus rupture duration, in order of rupture, for 3 representative cells undergoing at least 6 ruptures during imaging. (N) RFP-NLS intensity regain duration compared to rupture extent from siCtl and siBAF cells, both quantified from RFP-NLS intensity trace analysis (n values: siCtl, 10; siBAF, 9 ruptures). Best fit line for the combined data sets is shown.
Figure S3. BAF is required for lamin A recruitment to rupture sites and controls for NE protein knockdowns. (A) Still images of U2OS cells stably expressing mCherry-Lamin A (RFP-LmnA), 3xGFP-NLS, and shLMNB1 48 hr after transfection with siControl (siCtl) or siBAF. Cells were imaged at 40x every 3 min for 24 hr. RFP-lo are unsaturated versions of the indicated RFP-LmnA images. Arrowheads mark membrane rupture site. (B) Quantification of rupture sites with mCherry-LmnA protein accumulation. N values: siCtl, 63; siBAF, 53 ruptures from 3 experiments. ***p<0.001, Fisher’s exact test. (C) Representative immunoblot of NE protein levels after 48 hr siRNA treatment. Mean values of targeted protein expression compared to control are siLMNA: 0.24 (n = 2 blots), siEmerin: 0.18 (n = 4), siLEMD2: 0.49 (n = 3). (D-F) Representative images of protein depletions in cells transfected with indicated siRNAs and labeled with antibodies against the targeted protein. All FP-NLS images are gamma adjusted. All scale bars = 10 μm.