# Supplemental material

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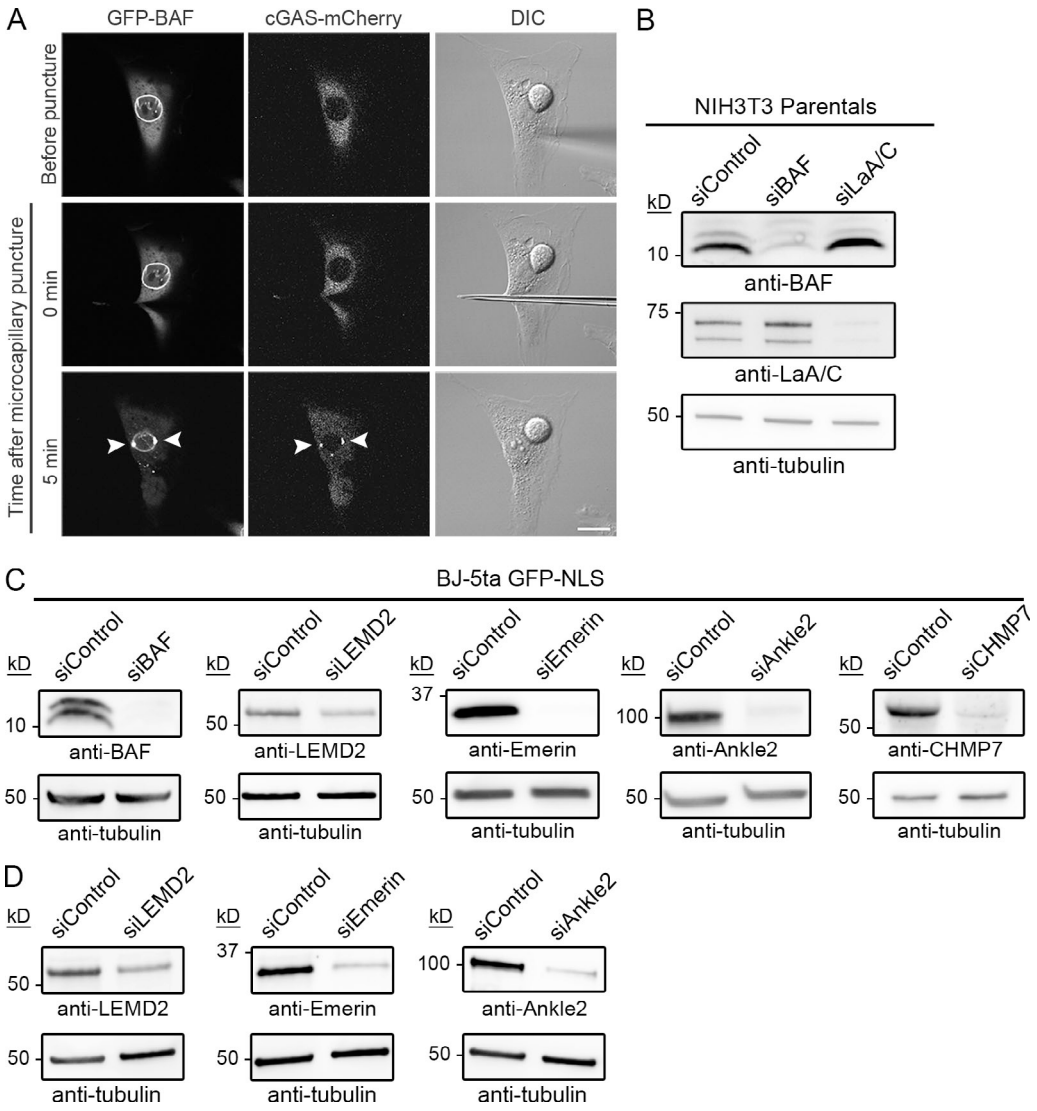



Figure S1. **An NIH3T3 cell with GFP-BAF accumulation on the NE following mechanical disruption with a microcapillary, along with representative Western blots of proteins knocked down using siRNAs. (A)** A representative NIH3T3 cell coexpressing GFP-BAF and cGAS-mCherry was mechanically disrupted with a microcapillary needle, causing both proteins to accumulate on the NE (arrowheads). Bar, 20 µm. **(B)** Representative Western blots of siBAF and siLaA/C efficacy from NIH3T3 cell lysates 72 h after siRNA transfection. **(C)** Representative Western blots of siBAF, siLEMD2, siEmerin, siAnkle2, and siCHMP7 BJ-5ta GFP-NLS cell lysates 96 h after siRNA transfection. **(D)** Representative Western blots of BJ-5ta GFP-NLS cell lysates transfected with a combination of siLEMD2, siEmerin, and siAnkle2 for 96 h. Molecular masses (in kD) of proteins are shown.

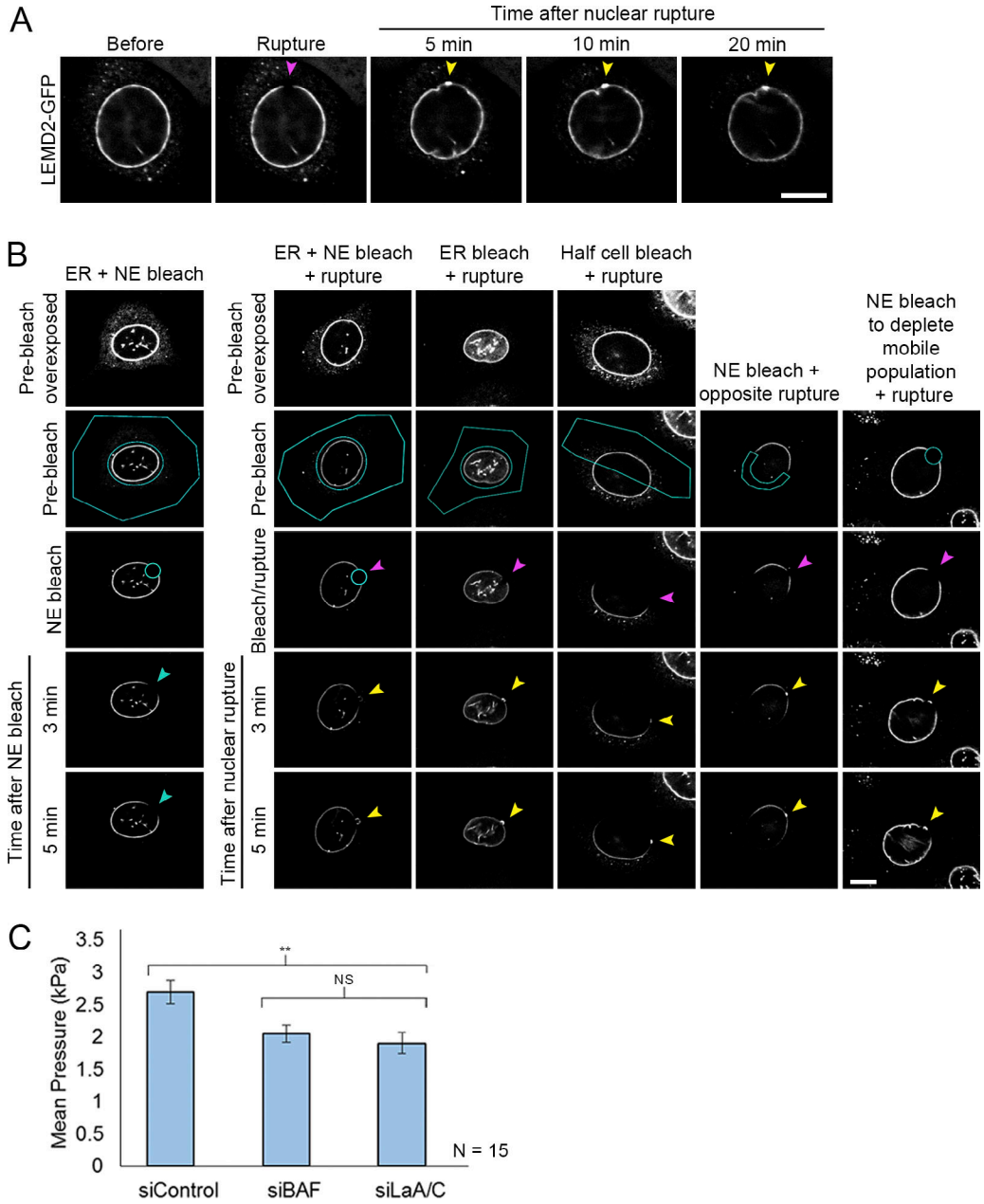



Figure S2. **LEMD2-GFP recruitment to a nuclear rupture and experiments to examine the source of LEMD2-GFP recruitment, as well as pressure needed to rupture the cell nucleus through micro-indentation. (A)** Representative images of a NIH3T3 cell expressing LEMD2-GFP showing LEMD2 retention at the rupture scar (yellow arrowheads) up to 20 min after nuclear rupture (purple arrowhead). **(B)** To examine the source of LEMD2-GFP recruited to the nuclear rupture site, several methods were used. Blue areas indicate photobleached areas, and purple arrowheads indicate laser-induced nuclear rupture. The blue arrowheads in the first column indicate the NE photobleaching in cells without induced rupture. Prerupture photobleaching of the ER and/or various regions of the NE did not prevent normal LEMD2 localization and enrichment at the rupture site (yellow arrowheads). Persistent photobleaching (10-s intervals for 2 min) of LEMD2-GFP before rupture to deplete any potential mobile fraction also failed to prevent LEMD2-GFP accumulation at rupture sites. **(C)** NIH3T3 cells expressing cGAS-mCherry were treated with siControl, siBAF, or siLA/C for 72 h. The pressure needed to rupture the cell nucleus through micro-indentation was measured for each condition. A significantly lower pressure was necessary to rupture the siBAF (pressure, 2.05 kPa; P = 0.008) and siLaA/C (pressure, 1.90 kPa; P = 0.003) compared with the siControl (pressure, 2.69 kPa). No statistically significance was seen in the pressure differences to rupture the siBAF and siLaA/C (P = 0.5021). Error bars represent SEM (*n* = 15 indentation measurements for each condition). **, P < 0.01 by unpaired student's *t* test. Bars, 10 µm.

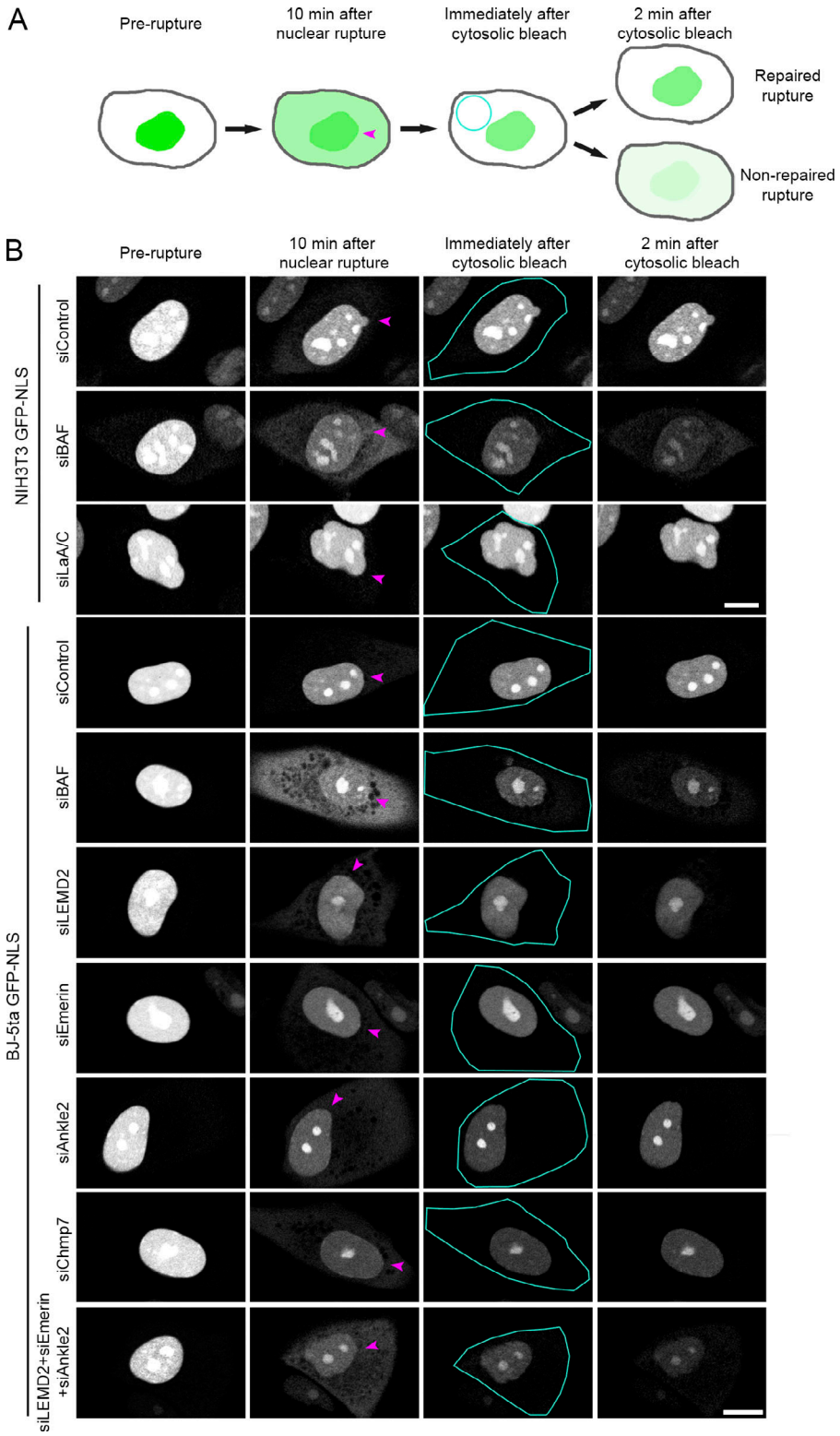


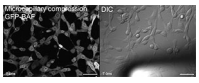

Figure S3. **NE repair verification in GFP-NLS cell lines via cytoplasmic photobleaching after rupture. (A)** Schematic representation of testing for re-sealing of nuclear ruptures. 10 min after laser-induced nuclear rupture (purple arrowhead), the cell's cytoplasmic GFP-NLS is photobleached for 40 s. Cells were allowed 2 min to re-equilibrate and were then imaged. Cells with repaired NE ruptures do not exhibit GFP-NLS leakage into the cytoplasm, whereas cells with nonrepaired NE ruptures will exhibit considerable cytoplasmic GFP-NLS at the expense of nuclear GPF-NLS intensity. **(B)** To assess if ruptured nuclei were repaired 10 min after laser rupture in NIH3T3 and BJ-5ta cells transfected with siControl, siBAF, siLEMD2, siEmerin, siANKLE2, siChmp7, or a combination of siLEMD2, siEmerin, and siANKLE2 for 96 h, we used the approach described in A. Cell boundaries are demarcated in the image acquired immediately after cytosolic bleach (blue lines). Experiments were performed on a minimum of two cells for each condition. Bars, 10 µm.

Table S1. **Primers used in this study**

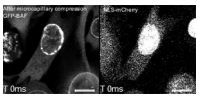
| Primer name | Sequence (5′ to 3′) |
|---|---|
| 1255 | TGTGGTGGTACGTAGGAATTCGCCACCATGCTGTGGCCGCGGCTGGCG |
| 1211 | AAACTTAAGCTACTCGAGCTTGTACAGCTCGTCCATGCC |
| 1369 | TGGTACGTAGGAATTCTAGCCACCATGGTGAGCA |
| 1370 | CCATTCCCGGAAGCGGTCTTCATC |
| 1371 | GATGAAGACCGCTTCCGGGAATGG |
| 1372 | CGGTCATGGTAAGCTTTTTGCAAAAGCCTAGGCC |
| 1374 | GTACAAGGACCTCGAGGAAGAGCAAAAGCACCGAG |
| 1375 | ATTCCACAGGGTCGACTCACAAGAAGGCGTCGCA |
| 1447 | GTACAAGGACCTCGAGACAACCTCCCAAGCCCACCGAGACTTCGTG |
| 1513 | TGGTACGTAGGAATTCGCCACCATGGTGAGCAAGGGC |
| 1517 | CCAGTGTGGTGGTACGTATGCCACCATGGAAGATCCGCGTAGAAGGAC |
| 1518 | CATGGTGGCGACCGGTGCAAGCTTGTCAAAAATTGGAAACCCATTATTTC |
| 1535 | ATTCCACAGGGTCGACTTATCTAGATCCGGTGGATCCTACCTTTCT |
| 1538 | GTACAAGGACCTCGAGGCGGCGGCAGCA |
| 1539 | ATTCCACAGGGTCGACTCAGGAACTTCCTTGAGAATTGGTTAGG |
| 1540 | GTACAAGGACCTCGAGGACAACTACGCAGATCTTTCGG |
| 1541 | ATTCCACAGGGTCGACCTAGAAGGGGTTGCCTTCTTCAG |
| 1545 | GAGGCACTGGTATGGTGAGCAAGGGCGAG |
| 1546 | CTTGCTCACCATACCAGTGCCTCC |
| 1547 | TGGTACGTAGGAATTCGCCACCATGGCCGGCCTGTCG |
| 1548 | ATTCCACAGGGTCGACTTACTTGTACAGCTCGTCCATGCC |
| 1663 | GATCCAAAAAAGAAGAGAAAGGTAGATCCAAAAAAGAAGAGAAAGGTAG |
| | ATCCAAAAAAGAAGAGAAAGGTAGGATCCACCGGATCTAGATAA |
| 1664 | TCGATTATCTAGATCCGGTGGATCCTACCTTTCTCTTCTTTTTTGGATCTAC |
| | CTTTCTCTTCTTTTTTGGATCTACCTTTCTCTTCTTTTTTG |
| 1735 | GGTACGTAGGAATTCTAGCCACCATGGTGAGCAAGGGC |
| 1736 | ATTCCACAGGGTCGACTCACAATGGCTTTAGAGTCGGTTCCAATTGC |
| 1737 | TGGTACGTAGGAATTGCCACCATGCCGGAGTTCCTGGAAG |
| 1738 | CCGCTCGACGACAGGGGCCCGTTGGATTTTCTAGGGTCAACATGAAGAAAATTAGAG |
| 1792 | TGGTACGTAGGAATTCGCCACCATGCCGGAGTTCCTGGAAG |
| 1793 | CCCCTCCGCCCTCGAGGTGTTTATTTCCACGCTTTTTAATTACTTTGCATAC |

Table S2. **siRNAs used in this study**

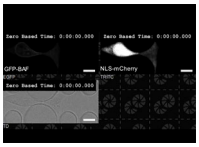
| Target | Species | Catalog number | Name | Sequence (5′ to 3′) |
|---|---|---|---|---|
| BAF | Mouse | L-062803-01 | J-062803-09 | GCACCGAGACUUCGUGGCA |
| | | | J-062803-10 | UUGGUGACGUCCUGAGCAA |
| | | | J-062803-11 | CGAGAAUGGCUGAAGGAUA |
| | | | J-062803-12 | CCUAUGUACUCCAGGGUCU |
| LaA/C | Mouse | L-040758-00 | J-040758-05 | UUAGGGUGAACUUCGGUGG |
| | | | J-040758-06 | UCAAACUCUCGCUGCUUCC |
| | | | J-040758-07 | UCUUCAUCGACUUCCUCUA |
| | | | J-040758-08 | UUCCUCGCUGUAAAUGUUC |
| BAF | Human | L-011536-02 | J-011536-10 | UACGACAAUAGCAAUCUUU |
| | | | J-011536-11 | UUCAUCUUUCUUUAGCACC |
| | | | J-011536-12 | UGCCACGAAGUCUCGGUGC |
| | | | J-011536-13 | UUGCCCAGGACUUCACCAA |
| LEMD2 | Human | L-017941-02 | J-017941-17 | CUAAAAUAUCGGUGGCGAA |
| | | | J-017941-18 | UCACAGAAGCUGCGACUCU |
| | | | J-017941-19 | CUGAAUUGGUGACGACUGU |
| | | | J-017941-20 | CGAGGAGCGGUUACGGGAA |
| Emerin | Human | L-011025-00 | J-011025-06 | GUAUCCGAAAGAUCUGCGU |
| | | | J-011025-07 | UACGAGUUGAUCCUACUAC |
| | | | J-011025-08 | UAGGAUAAUAGGACAGGUC |
| | | | J-011025-09 | UGAAACAGGGCGGUAGUGC |
| Ankle2 | Human | L-181819-00 | J-181819-12 | AUACUCUCUGAUCCGCUCC |
| | | | J-181819-13 | UUCCCGAGUUGGUCUGCGG |
| | | | J-181819-14 | AACGCUACGUCCUAUAUUU |
| | | | J-181819-15 | AUUCCGAACAACCCAAAUC |
| Chmp7 | Human | L-015514-01 | J-015514-09 | CGACCUUGGUAAACGGAAA |
| | | | J-015514-10 | GGGUUUAUCCUGUCGCUAA |
| | | | J-015514-11 | GGAGGUGUAUCGUCUGUAU |
| | | | J-015514-12 | GUAACAAAUGGCUUAGAUU |
| NT control | | D-001810-10-05 | D-001810-01 | UGGUUUACAUGUCGACUAA |
| | | | D-001810-02 | UGGUUUACAUGUUGUGUGA |
| | | | D-001810-03 | UGGUUUACAUGUUUUCUGA |
| | | | D-001810-04 | UGGUUUACAUGUUUUCCUA |



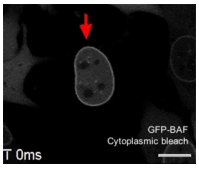
Video 1. **NIH3T3 GFP-BAF cells mechanically compressed using a blunted microcapillary and imaged using time-lapse confocal microscopy, with GFP (left) and differential interference contrast (right) channels represented.** Frames were collected every 2 s and are displayed at 15 frames/s. Bar, 50 µm.



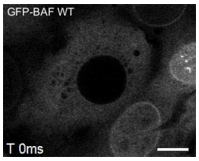
Video 2. **Videos of Fig. 1, A and B.** NIH3T3 cells coexpressing GFP-BAF (left) and mCherry-NLS (right) were compressed with a blunted microcapillary and immediately imaged using time-lapse confocal microscopy (first video). Frames were collected every 5 s and are displayed at 10 frames/s. NIH3T3 cells coexpressing GFP-BAF (left) and cGAS-mCherry (right) undergoing laser-induced nuclear rupture (second video) and imaged using time-lapse confocal microscopy. Frames were collected every 15 s and are displayed at 3 frames/s. Bar, 10 µm.



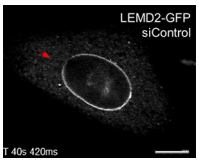

Video 3. **Videos of Fig. 1 C.** NIH3T3 cells coexpressing GFP-BAF (top left; eGFP) and mCherry-NLS (top right; TRITC) cells migrating through a constricted channel in a microfluidic device and imaged using time-lapse confocal microscopy. The differential interference contrast channel (bottom left) is also represented. Frames were collected every 10 min and are displayed at 3 frames/s. Bar, 10 µm.



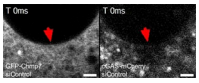

Video 4. **Videos of Fig. 2, C and D.** NIH3T3 cells expressing GFP-BAF were compartmentally photobleached in the cytoplasm (first video) or nucleoplasm (second video) using a 488-nm laser before laser-induced nuclear rupture (red arrow) and time-lapse confocal imaging. Frames were collected every 30 s are displayed at 4 frames/s. Bar, 10 µm.



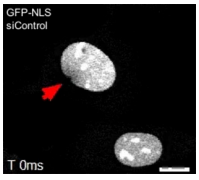

Video 5. **Videos of Fig. 2 E.** NIH3T3 cells expressing GFP-BAF WT (first video), K6A (second video), L58R (third video), K6A-L58R (fourth video), MEEEQ (fifth video), and MAAAQ (sixth video) were compartmentally photobleached in the nucleoplasm using a 488-nm laser before laser-induced nuclear rupture and time-lapse confocal imaging. Frames were collected every 15 s and are displayed at 4 frames/s. Bar, 10 µm.



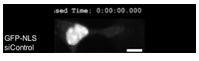

Video 6. **Videos of Fig. 3 A.** NIH3T3 cells expressing LEM-domain proteins were transfected with siBAF siRNAs for 72 h before laser-induced nuclear rupture (red arrow) and imaged using time-lapse confocal microscopy. Cells expressing GFP-Emerin and transfected with siLaA/C siRNAs for 72 h before laser-induced nuclear rupture. The order of each video segment is as follows: LEMD2-GFP siControl (segment 1) and siBAF (segment 2); GFP-Man1 siControl (segment 3) and siBAF (segment 4); GFP-Emerin siControl (segment 5), siBAF (segment 6), and siLaA/C (segment 7); Ankle2-GFP siControl (segment 8) and siBAF (segment 9); Lap2alpha-GFP siControl (segment 10) and siBAF (segment 11); and Lap2β-GFP siControl (segment 12) and siBAF (segment 13). Frames were collected every 10 s and are displayed at 4 frames/s. Bar, 10 µm.



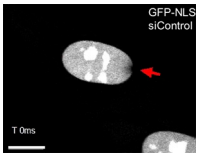

Video 7. **Videos of Fig. 3 B.** NIH3T3 cells expressing GFP-Chmp7 (left) and cGAS-mCherry (right) were transfected with siControl (first video), siBAF (second video), or siLEMD2 (third video) siRNAs for 72 h before laser-induced nuclear rupture (red arrow) and imaged using time-lapse confocal microscopy. Frames were collected every 30 s and are displayed at 4 frames/s. Bar, 2 µm.




Video 8. **Videos of Fig. 4 A.** NIH3T3 cells expressing GFP-NLS were transfected with siControl (first video), siBAF (second video), or siLaA/C (third video) siRNAs for 96 h before laser-induced nuclear rupture (red arrow). Frames were collected every 1 min and are displayed at 4 frames/s. Bar, 10 µm.




Video 9. **NIH3T3 GFP-NLS cells treated with siControl (first video) or siBAF (second video) for 72 h before nuclear rupture during migration through a constricted channel.** Frames were collected every 10 min and are displayed at 3 frames/s. Bar, 10 µm.




Video 10. **BJ-5ta GFP-NLS cells treated with siControl (first video), siBAF (second video), or a combination of siLEMD2, siEmerin, and siANKLE2 (third video) for 96 h before laser-induced nuclear rupture (red arrow).** Frames were collected every 1 min (first and second videos) and 2 min (third video) and are displayed at 4 frames/s. Bar, 10 µm.