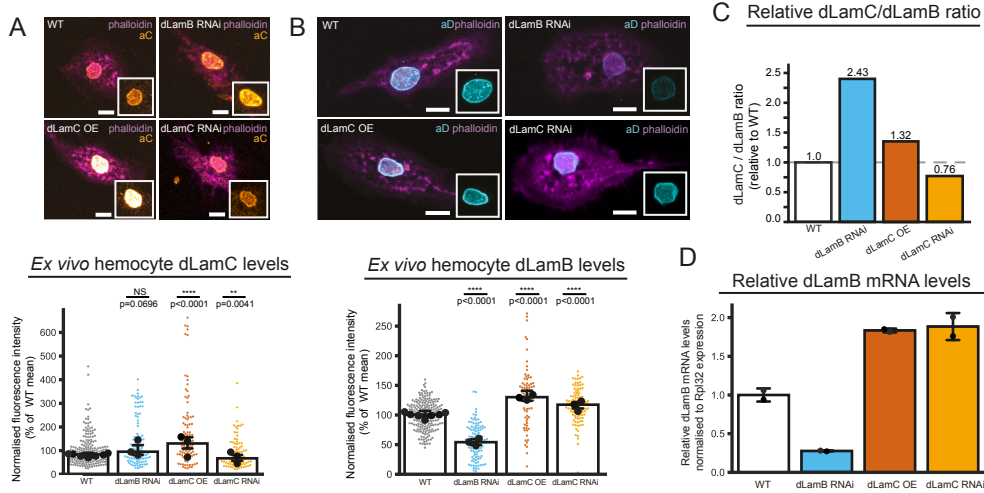


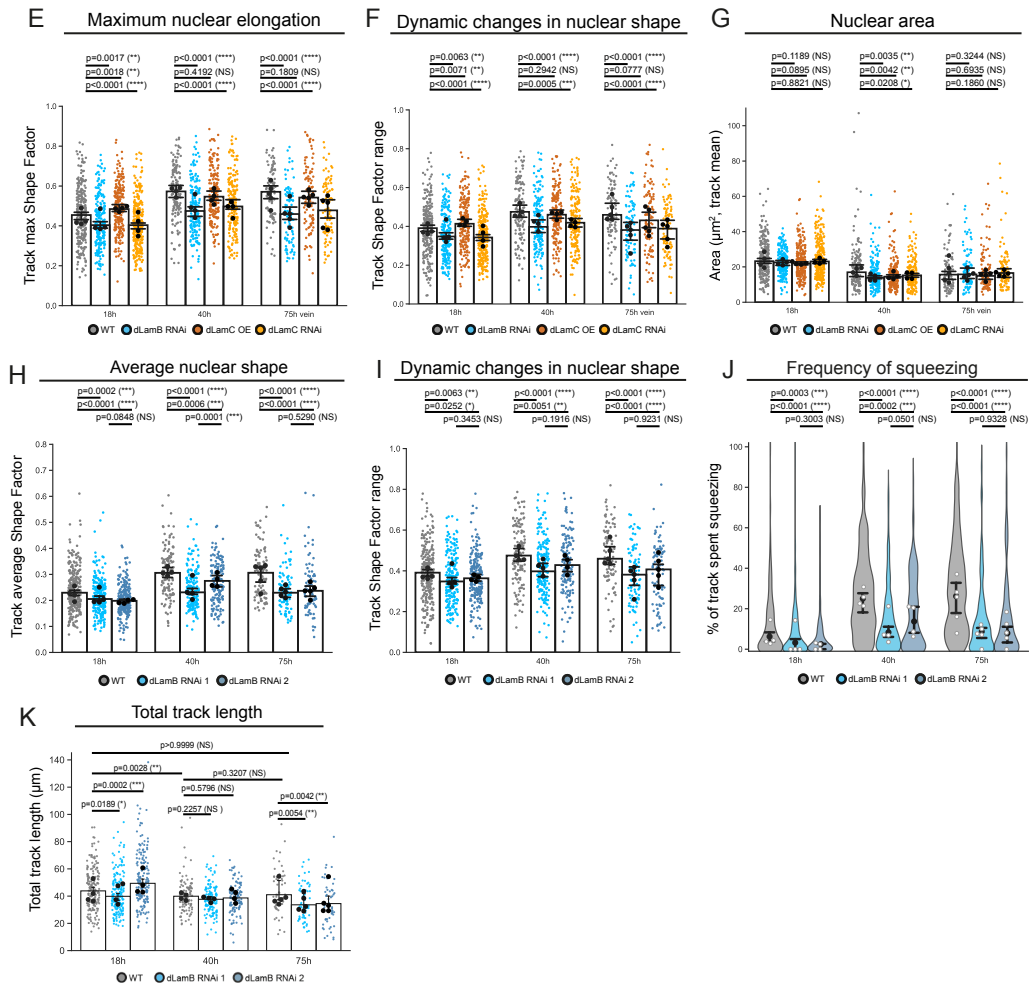
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Ex vivo imaging of lamina composition

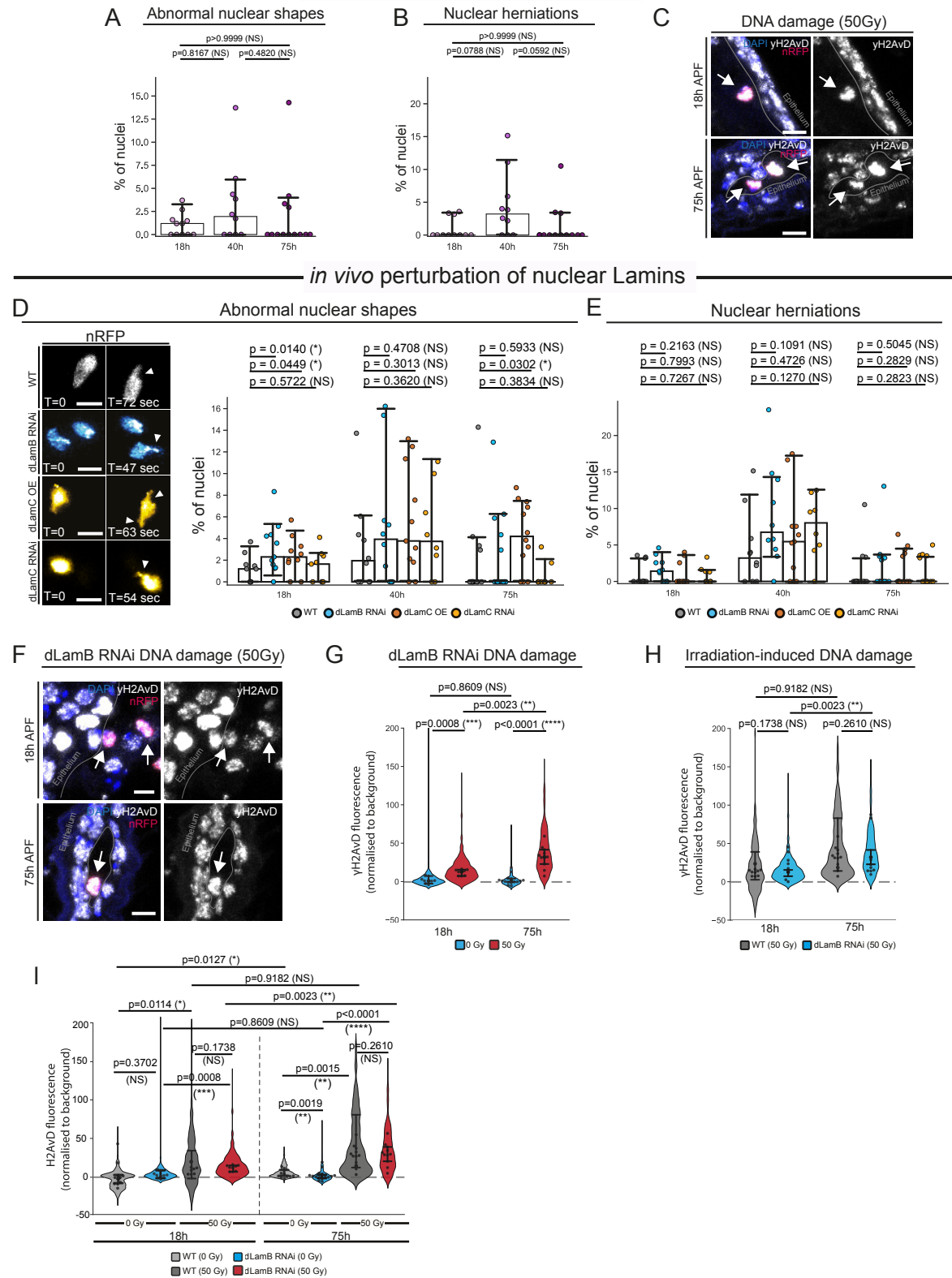


Live in vivo imaging of immune cell dynamics



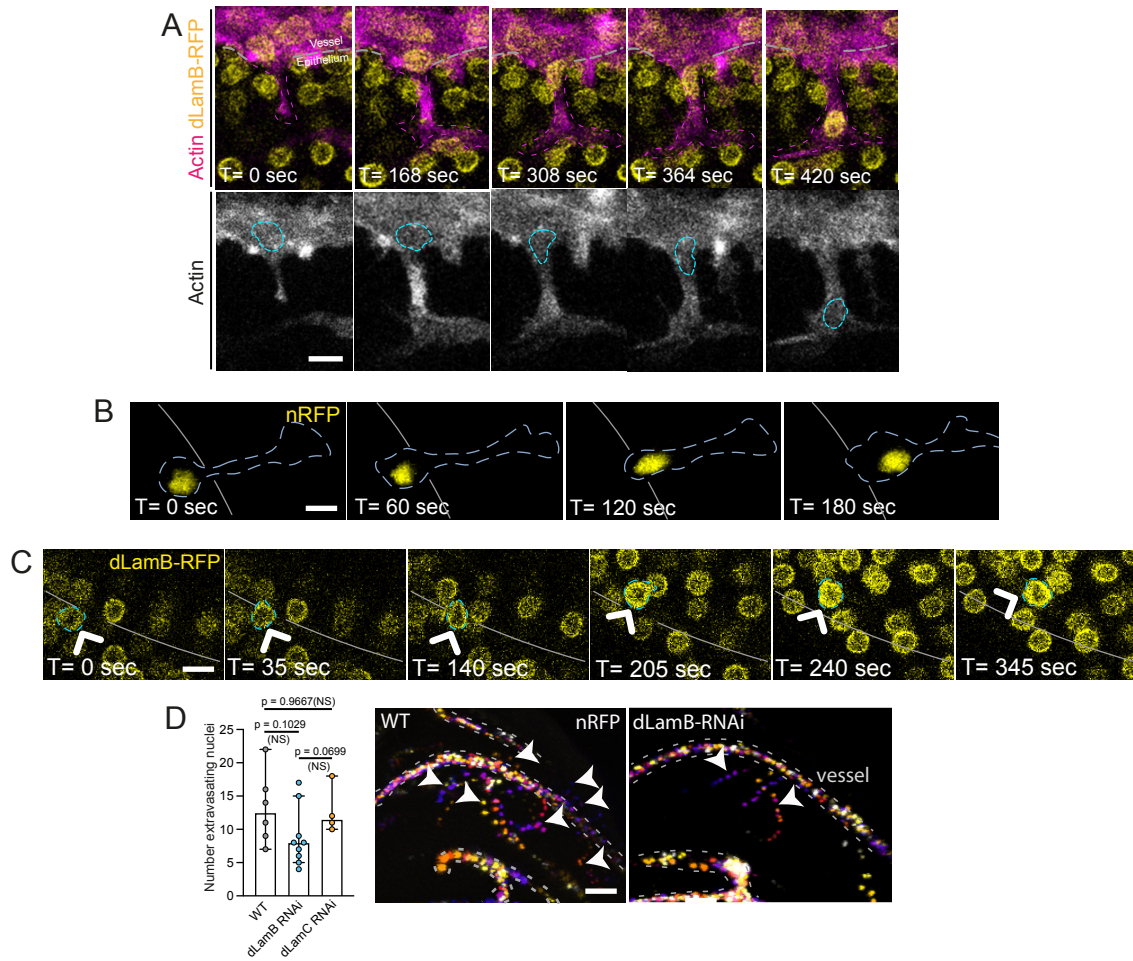
Appendix Figure S1. Immune cell finely-tuned nuclear lamina composition supports nuclear plasticity. A-B) *Ex vivo* hemocytes from WT, *dLamB-RNAi*, *dLamC-OE* and *dLamC-RNAi* pupal wings at 40h APF, stained for anti-dLamC (yellow, aC) (A) or anti-dLamB (cyan, aD) (B) and phalloidin (Actin, magenta). Scatter plots show small dots (hemocytes) and large dots (medians of technical replicates), where anti-dLamC levels (A) or anti-dLamB (B) are normalised to WT, from the technical replicate processed at the same time. Bar chart shows median and 95% CI. **C)** Relative dLamC/dLamB ratio of *ex vivo* hemocyte Lamin levels, normalised to WT ratio. **D)** Relative whole pupae 18h APF hemocyte dLamB mRNA levels normalised to housekeeping (*Rpl32*) expression. Bar chart shows median and 95% CI, with replicates as black dots (N=1 RT-qPCR run and N=2 technical replicates after outlier removal). **E-G)** Maximum nuclear SF value (E), SF range (F) and average nuclear area (G) during a 20 minute imaging period. **H-I)** Average nuclear SF (H) and SF range (I) measured with two RNAi lines *dLamB-RNAi* 1 and *dLamB-RNAi* 2. Scatter plots with small dots (individual nuclear tracks) and large dots (wing medians). Bar charts show median and 95% CI. **J)** Violin plot depicting the percentage of nuclear tracks during which SF is >0.4 with two independent RNAi lines; wing medians (white dots) with sample median and 95% CI (black). **K)** Hemocyte migration track length measured over 15 minute periods; bar charts show median and 95% CI. Data information: Scale bars represent 5µm (A-B). Genotypes used were: *Srp>nRFP* (A-K), *Srp>nRFP*; *UAS-dLamB-RNAi* (A-K), *Srp>nRFP*; *UAS-dLamC-OE* (A-G) and *Srp>nRFP*; *UAS-dLamC-RNAi* (A-G). N=7 and 3 technical replicates for WT and Lamin manipulation, respectively, with >8 wings per replicate (A-B). N=4, 4 and 5 wings for all genotypes at 18h, 40h and 75h APF respectively (E-K). N = 205, 111, 101, 93 and 103 nuclei (A) or N = 202, 113, 80, 106 and 117 nuclei (B) measured for WT, *dLamB-RNAi*, *dLamC-OE* and *dLamC-RNAi*. N = 286, 139 and 102 total hemocytes for WT; 237, 183 and 96 for *dLamB-RNAi*; 238, 193 and 113 for *dLamC-OE*; 293, 199, 101 for *dLamC-RNAi* and 242, 152 and 90 for *dLamB-RNAi* 2 (E-K). Mann-Whitney U test used to calculate significance (A-B, E-K).

in vivo analysis of nuclear health



Appendix Figure S2. Finely-tuned leukocyte lamina limits nuclear instability during confined migration. **A-B** Percentage of nuclei with abnormal nuclear shapes (A) or herniations (B) over 20 minute imaging period. **C** Cryosections of WT 18h and 75h APF pupal wings stained with DAPI (blue) and anti- γ H2AvD (grey). Hemocyte nuclei in red (arrows). **D** Example images of abnormal nuclear morphologies (arrowheads) in WT, *dLamB-RNAi*, *dLamC-OE* and *dLamC-RNAi* hemocytes. Bar chart shows percentage of nuclei with abnormal nuclear morphology at 18h, 40h and 75h APF over 20 minutes. **E** Percentage of nuclei with herniations at 18h, 40h and 75h APF. **F** Cryosections of *dLamB-RNAi* 18h and 75h APF pupal wings stained with DAPI (blue) and anti- γ H2AvD (grey); hemocyte nuclei in red (arrows). **G** *dLamB-RNAi* hemocyte nuclear γ H2AvD fluorescence normalised to background without irradiation (0Gy) or after irradiation (50Gy). **H** Irradiated (50Gy) WT and *dLamB-RNAi* hemocyte nuclear γ H2AvD fluorescence normalised to background. **I** WT and *dLamB-RNAi* hemocyte nuclear γ H2AvD fluorescence normalised to background, without irradiation (0Gy) or after irradiation (50Gy). Genotypes used were: *Srp>nRFP* (A-I), *Srp>nRFP; UAS-dLamB-RNAi* (D-I), *Srp>nRFP; UAS-dLamC-OE* (D-E) and *Srp>nRFP; UAS-dLamC-RNAi* (D-E).

Data information: Scale bars represent 5 μ m (C, D and F). Large dots in scatter plots show percentages per wing, bar chart shows median and 95% CI of all wings (A-B, D-E). Violin plot shows all nuclei, slide medians are shown as dots and median and 95% CI shown in black (G-I). N = 11, 10 and 13 wings for WT; 11, 10 and 13 for *dLamB-RNAi*; 10, 11 and 12 for *dLamC-OE*; 9, 8 and 11 for *dLamC-RNAi* (A-B, D-E). N = 616, 382 and 209 total hemocytes for WT; 791, 444 and 343 for *dLamB-RNAi*; 540, 484 and 338 for *dLamC-OE*; 567, 402 and 307 for *dLamC-RNAi* (A-B, D-E). N = >450 nuclei per condition, 3 technical replicates that included >4 wings (G-I). One-way ANOVA (Kruskal-Wallis) with Dunn's multiple comparisons test (A-B) or Mann-Whitney U test (D-E, G-I) was used to calculate significance.



Appendix Figure S3. Nuclear plasticity during immune cell transmigration across vessel walls. A) Hemocyte (dLamB in yellow, actin in magenta or grey) extravasating from a vessel. Hemocyte outlined in magenta, nucleus in cyan. **B)** Nuclear deformation (yellow) during hemocyte extravasation; cytoplasm is outlined (dashed cyan). **C)** Dynamic changes in nuclear lamina (dLamB, yellow) during hemocyte extravasation. Hemocyte nucleus (arrowhead) is outlined in cyan. Grey line indicates approximate vessel wall location. **D)** Rate of hemocyte extravasation following Lamin perturbation, quantified as number of extravasation events over 2 hours of imaging; time series projections over 2 hours of imaging illustrate extravasating hemocyte nuclei (arrowheads).

Data information: Scale bars represent 5 μ m (A-C) and 30 μ m (D). Bar chart (D) shows median and 95% CI; N = 6, 9 and 4 wings for WT, *dLamB-RNAi* and *dLamC-RNAi*, respectively. Genotypes used were: *dLamB-tagRFP*; *SrpGMA* (A, C), *Srp>nRFP*; *SqhAX3*; *sqh-Sqh-GFP* (B), *Srp>nRFP* (D), *Srp>nRFP*; *UAS-dLamB-RNAi* (D) and *Srp>nRFP*; *UAS-dLamC-RNAi* (D).