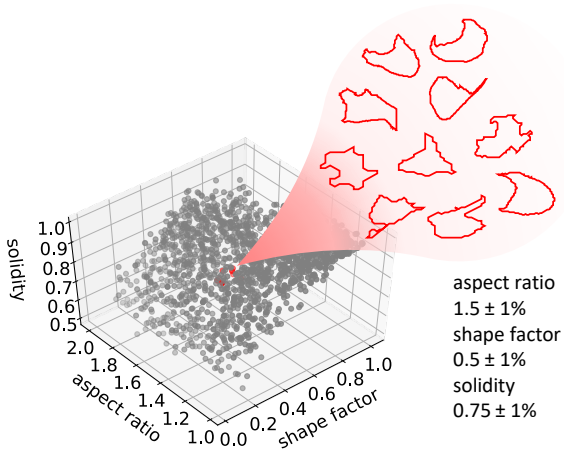


Figure 1. Cells confined to narrow ranges of traditional morphological parameters still exhibit highly variable shapes. Scatter plot showing the distributions of aspect ratio, shape factor, and solidity of 37,750 mouse embryonic fibroblast cells (MEF) grown on collagen-coated substrates. The subset of 10 cells highlighted in red display substantial morphological heterogeneity, despite highly similar aspect ratio, circularity and solidity.

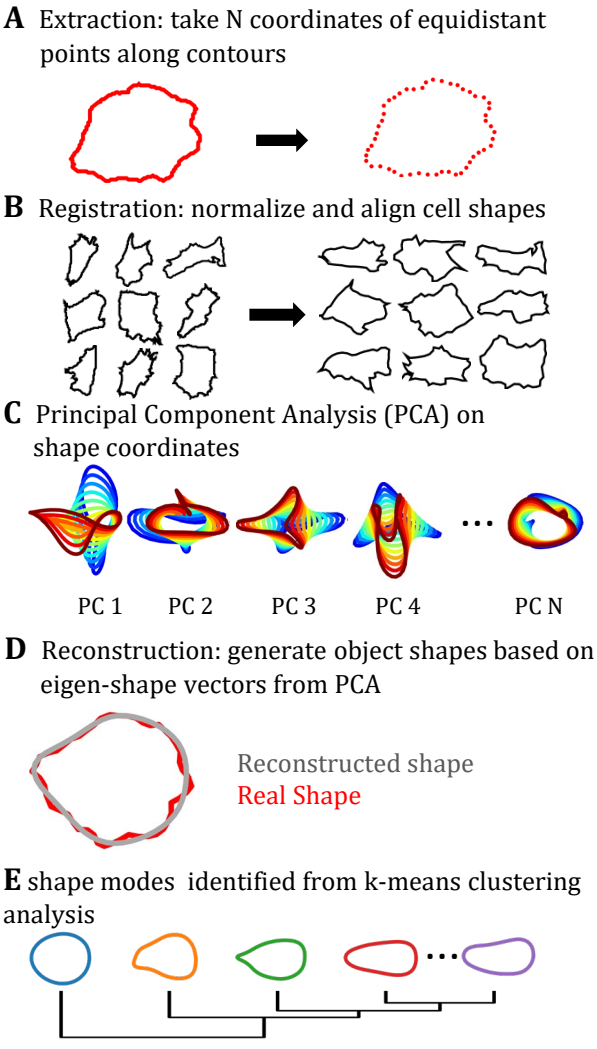


Figure 2. Overview of VAMPIRE analysis, from the extraction of contour coordinates to the automatic generation of shape modes A. The contour of a cell described by 50 equidistant points along its contour. **B.** Unaligned (left) shapes of a set of cells are pooled, normalized by size, and aligned (right). **C**. Eigen-shape vectors (i.e., principal components or PCs) are obtained from a principal component analysis (PCA) of the contour coordinates of aligned cells. **D**. Reconstructed cell shape from a reduced number of eigen-shape vectors. The reduced number of eigen-shape vectors was dafulted at the number of vectors that comprise 95% of the shape variations among all assessed cells. **E**. Representative cellular shape modes are obtained by applying a k-means clustering method to a set of cell morphology data described by the reduced number of eigen-shape vectors.

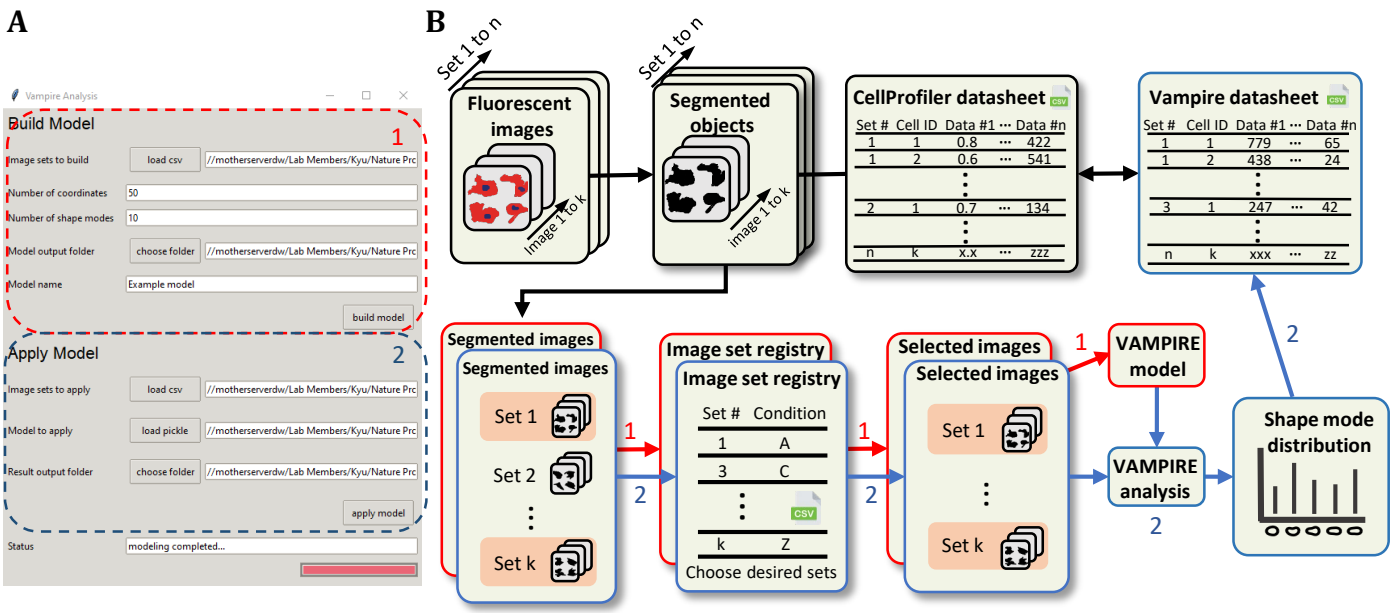


Figure 3. Overview of VAMPIRE implementation with the VAMPIRE GUI. A. The VAMPIRE Graphic User Interface (GUI). **B.** Flow diagram illustrating key steps in the implementation of VAMPIRE analysis with VAMPIRE GUI. Image of cells are first segmented into binary image that highlight cellular region and/or nuclear region. In VAMPIRE GUI top sections allows user to specify analysis parameters and (multi-)set of segmented images to be used to create a VAMPIRE analysis model (highlighted in red). Once the VAMPIRE analysis model is established, the user can specify (multi-)set of segmented images to be analyzed with the previous established model (highlighted in blue).

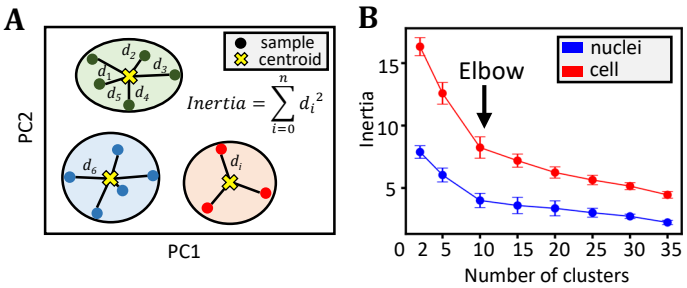


Figure 4. **Determinants of cluster coherence in the shape mode distributions. A.** Schematic illustrating the concept of inertia in K-means clustering. The inertia is measured by total distances of all data points to the centroids of their corresponding subtype. The lower the inertia value indicates better clustering result with more coherence. **B.** The inertia in principal decays with increasing number of clusters. The corresponding cluster number at the elbow point where the inertia decay rate starts to drop is the suggested cluster number to use in VAMPIRE for k-means clustering. The inertia is calculated as the distance from the cluster centroid to each of 17,093 mouse embryonic fibroblast cell and nucleus. VAMPIRE models were built using number of clusters = 2, 5, 10, 15, 20, 25, 30, and 35 with repeats of five (N=5). Because of the random initialization of K-means clustering, the cluster results (centroid and distance) change between repeats. The errorbars represent the standard deviation of inertia between repeats.

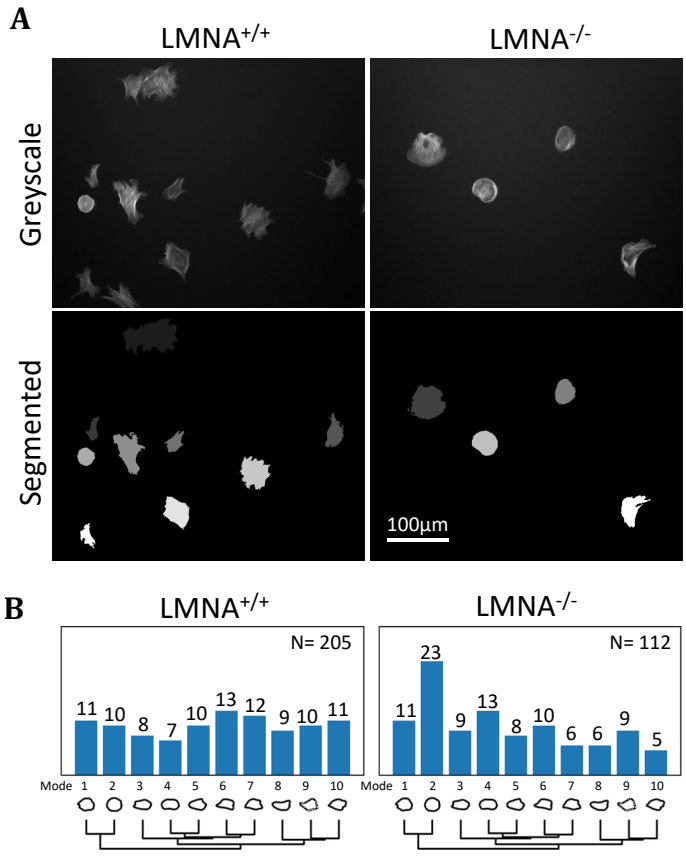


Figure 5. **VAMPIRE analysis of example *LMNA*+/+ and *LMNA*-/- mouse embryonic fibroblasts. A.** Images of phalloidin-stained (top) wild-type (*LMNA*+/+, left) and lamin-deficient (*LMNA*-/-, right) mouse embryonic fibroblasts are provided in Supplementary Data so that users can test the protocol and adapt it for their own application. Images of segmented cells (bottom) are also provided in the Supplementary Data. Segmentation is obtained through CellProfiler and the pipeline file used in CellProfiler is also provided so that users can adapt and test it. **B**. Bar graphs show the distribution of cell shape modes from the VAMPIRE analysis with the example images of MEFs. Numbers above the bars represent the abundances [%] of cells in each shape mode.

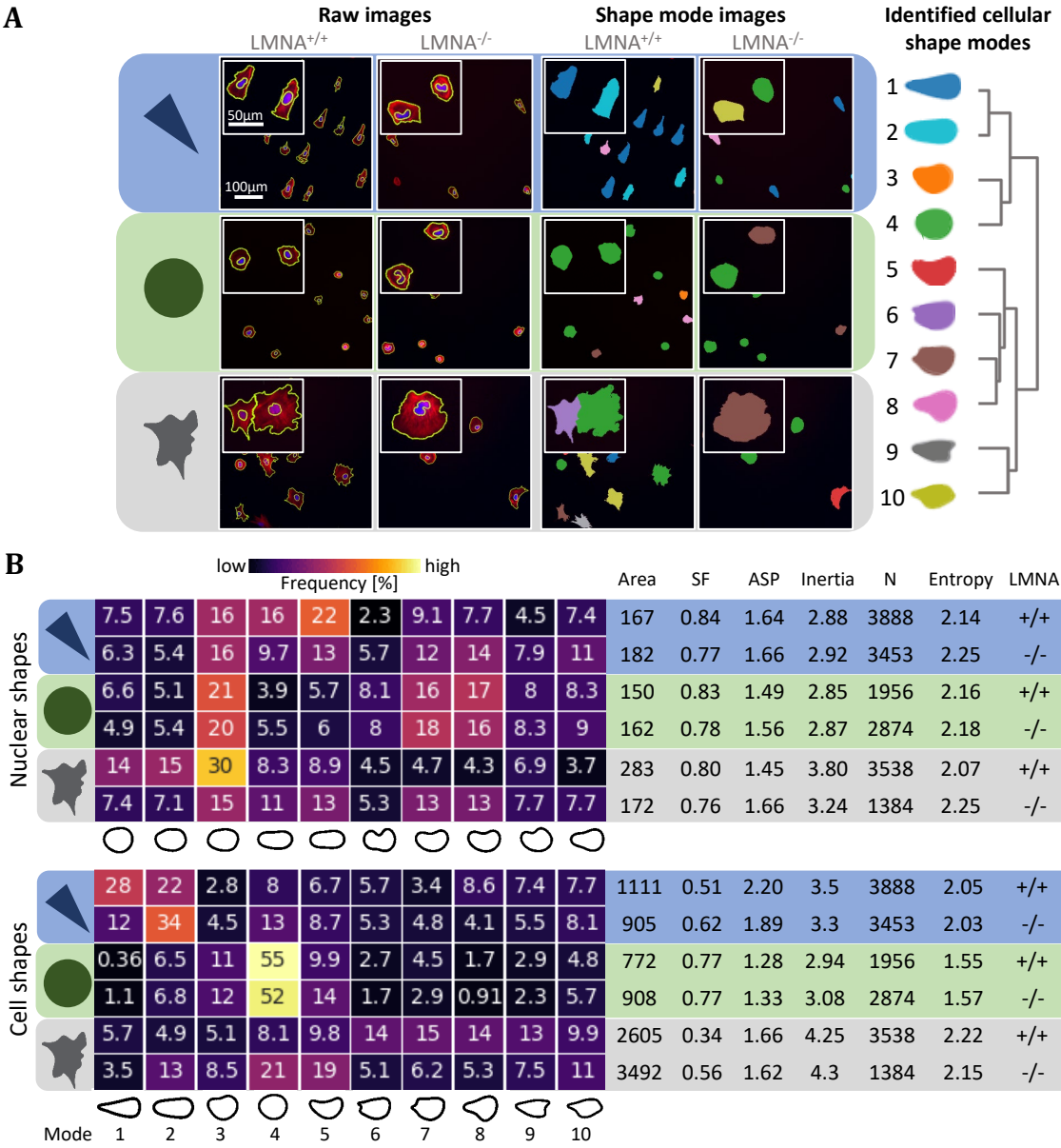
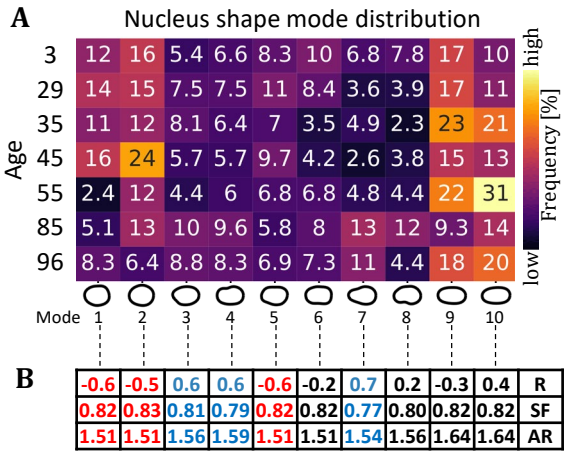


Figure 6. VAMPIRE analysis of mouse embryonic fibroblasts seeded on adhesive micro-patterned surfaces. A. Fluorescence microscopy images of wild-type (*LMNA*+/+) and lamin-deficient (*LMNA*-/-) mouse embryonic fibroblasts cultured on circular (top row) and triangular (middle row) adhesive fibronectin-coated micropatterns. Control cells (bottom row) are placed on the fibronectin-coated glass. Cells were fixed and stained for F-actin (red) and nuclear DNA (blue). Segmented fluorescence images (right). On the left are the raw images of cells and their nuclei with the segmented contours highlighted in yellow; on the left are the same cells color-coded according to the shape mode to which they belong. Inserts are magnified views of cells. The identified shape modes are located on the right of the panel. **B.** Shape mode distributions for MEF++ and MEF-- cultured on circular or triangular micropatterns (top and middle rows) and unpatterned surfaces (bottom row). The table on the right displays the values for traditional morphological parameters, including average area, shape factor, and aspect ratio of cells, as well as the number of cells analyzed (N), lamin A/C status and the Shannon entropy of the cells. The number of cells or nuclei analyzed for each condition are N= 3888, 3453, 1956, 2874, 3538, 1384 from the top row to bottom row. The measurements for each condition were calculated as the mean of all objects detected in two distinct samples. These results show that traditional morphological parameters cannot discriminate between the nuclear morphological responses of MEF++ and MEF-- on different adhesive micropatterns (right table). In contrast, the differential morphological response of these cells is readily revealed when measured via shape mode distributions (left color-coded table).



B

-0.6	-0.5	0.6	0.6	-0.6	-0.2	0.7	0.2	-0.3	0.4	R
0.82	0.83	0.81	0.79	0.82	0.82	0.77	0.80	0.82	0.82	SF
1.51	1.51	1.56	1.59	1.51	1.51	1.54	1.56	1.64	1.64	AR

Figure 7. VAMPIRE analysis of human dermal fibroblasts from donors of different ages. A. Distributions of nuclear shape modes for dermal fibroblasts from age 3 to 96. Each row is a distribution for each donor with sample number of nuclei N=643, 420, 407, 531, 373, 575, 637 respectively from age 3 to 96. Cells from younger donors populate the rounder shape mode (mode 1 and 2), while cells from older donors have nuclei classified that populate the non-rounded shape modes (mode 3, 4, and 7). **B.** Pearson's correlation (R), shape factor (SF), and aspect ratio (AR) of each nuclear shape mode. R is correlation between age and abundance of nuclei in a specific shape mode. SF and AR in red indicate shape modes that have negative correlations with chronological age, while those in blue indicate positive correlations. SF and AR of each shape mode are calculated as the mean of all nuclei identified as that shape mode across all ages.

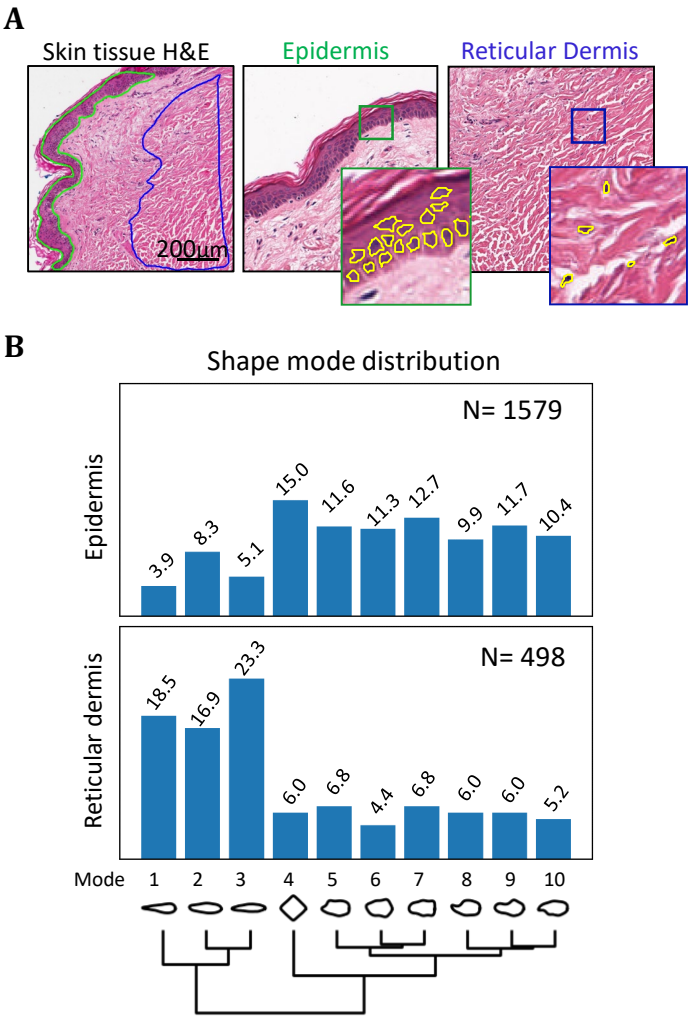


Figure 8. Analysis of nuclear shape in H&E stained tissue sections with VAMPIRE. A. Images of a skin tissue section stained with hematoxylin and eosin (H&E) and obtained from the cancer genome atlas (TCGA case ID: EE-A20I). Nuclei in the epidermis and the reticular dermis regions were segmented and analyzed with VAMPIRE. **B.** Bar graphs show the distribution of nuclei shape modes, comparing epidermal cells (N=1579) and dermal cells (N=498) using VAMPIRE analysis. Numbers above the bars represent the abundances [%] of nuclei in each shape mode.