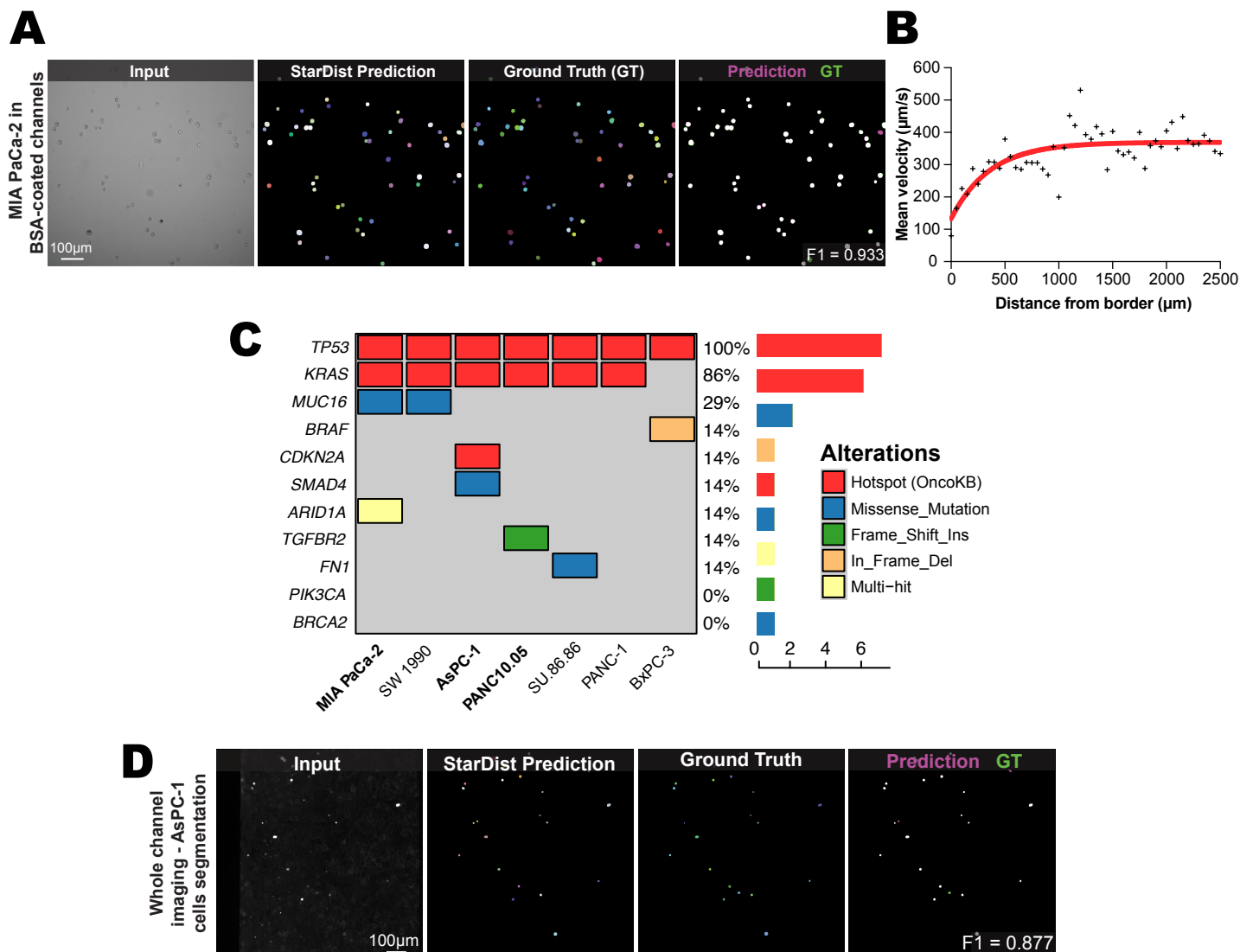


Appendix Figures

Table of contents

Appendix figures	page
Appendix Figure S1: Flow profiles, PDAC line characterization, and deep learning model used for detecting PDAC cells in microfluidic channels.	1
Appendix Figure S2: Deep learning model used to detect PDAC cells in microfluidic channels containing endothelial cells, and detailed track speed metrics.	2
Appendix Figure S3: Deep learning model used to detect Immune cells in microfluidic channels containing endothelial cells and detailed track speed metrics.	3
Appendix Figure S4: Deep learning-based segmentation of endothelial nuclei and cell-cell junctions.	4
Appendix Figure S5: Spatial analysis of cell arrest in relation to endothelial nuclei and junctions.	6
Appendix Figure S6: CD44 expression in PDAC cells.	7

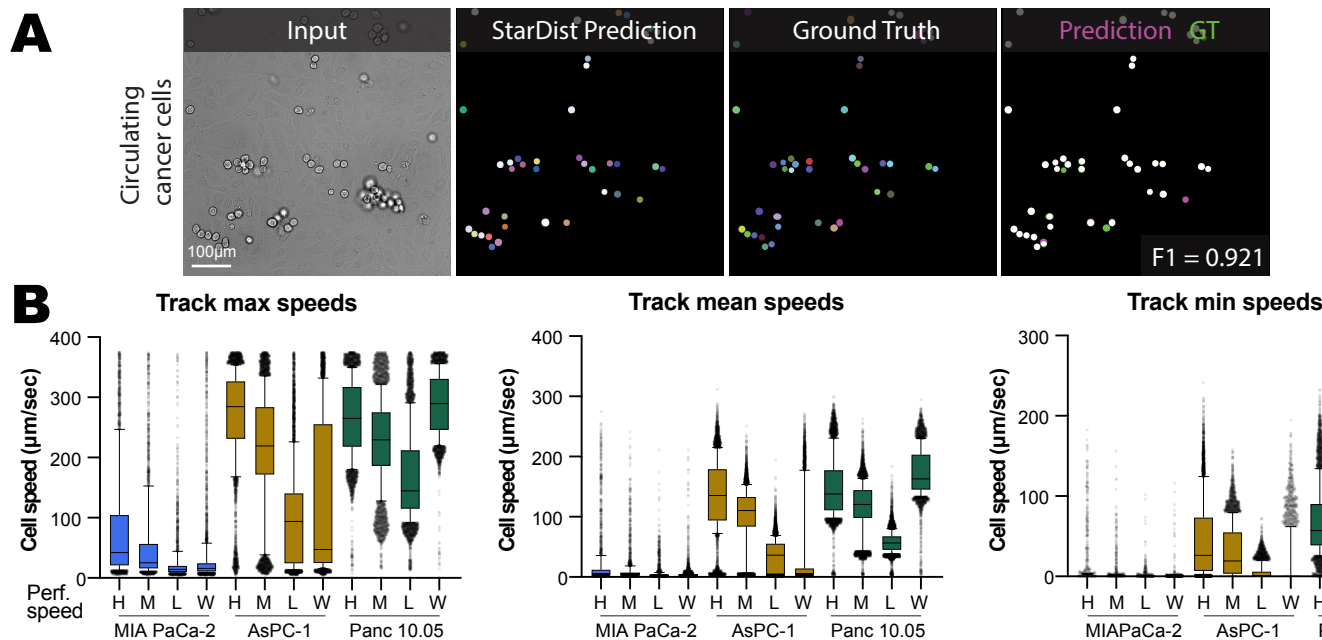


Appendix Figure S1: Flow profiles, PDAC line characterization and deep learning model used for detecting PDAC cells in microfluidic channels.

(A-B) Validation of flow profiles in microfluidic channels. PDAC cells were perfused through BSA-coated channels and imaged using brightfield microscopy just above the glass bottom of the channel. Cell detection was performed using a custom-trained StarDist model, and cell tracking was accomplished with TrackMate. (A) Validation of the StarDist model used to detect the perfused cancer cells and (B) quantification of mean cell velocity in the microfluidic channel. The Input, the StarDist prediction, and the ground truth (GT) image are displayed alongside the F1 score. (A) Scale bar = 100 μ m.

(C) Mutational burden of the PDAC lines included in this study. Source data: Cancer Cell Line Encyclopedia, queried for mutations in genes: KRAS, BRAF, CDKN2A, PIK3CA, SMAD4, ARID1A, TGFBR2, TP53, FN1, MUC16, and BRCA2.

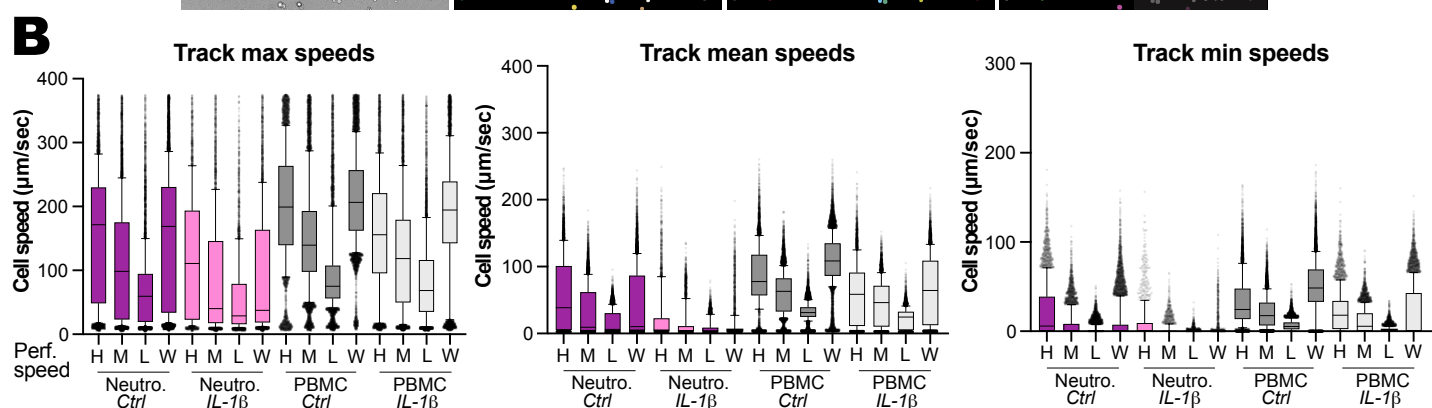
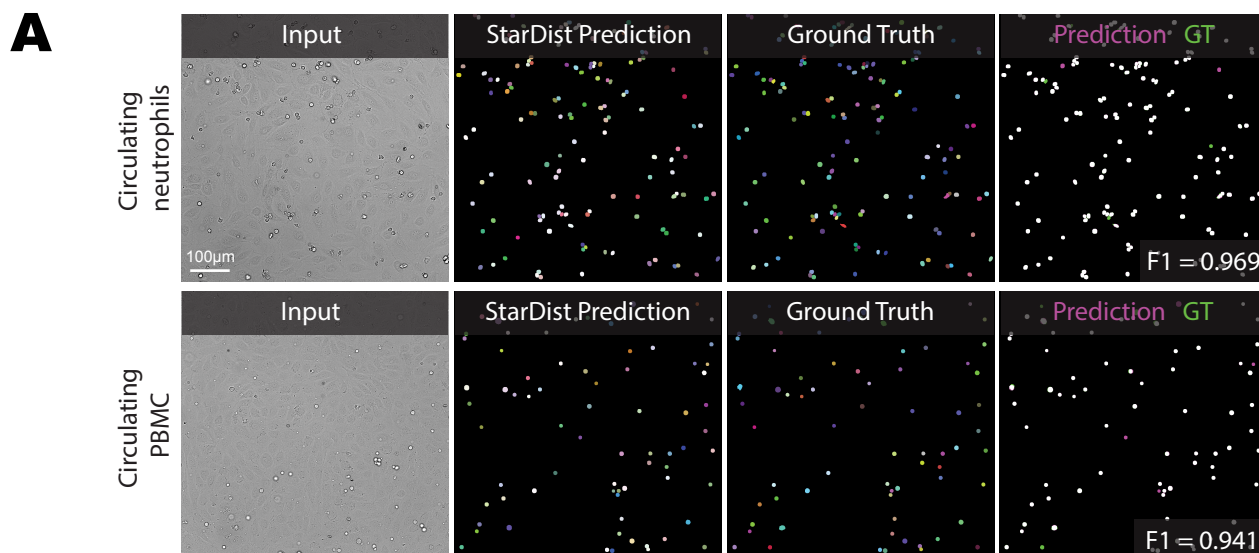
(D) Validation of the StarDist model used to detect the cancer cells attached to the channel, Related to Fig.EV2AB. Scale bar: 100 μ m. Adhesion of labeled PDAC cell lines to an endothelial monolayer without flow. Cells were fixed, stained with phalloidin and DAPI, and imaged using a spinning disk confocal microscope.



Appendix Figure S2: Deep learning model used to detect PDAC cells in microfluidic channels containing endothelial cells and detailed track speed metrics.

(A) Validation of the StarDist model used to detect circulating cancer cells perfused in the channels in the presence of endothelial cells. The Input, the StarDist prediction, and the ground truth image are displayed alongside the F1 score. Scale bar = 100 μm.

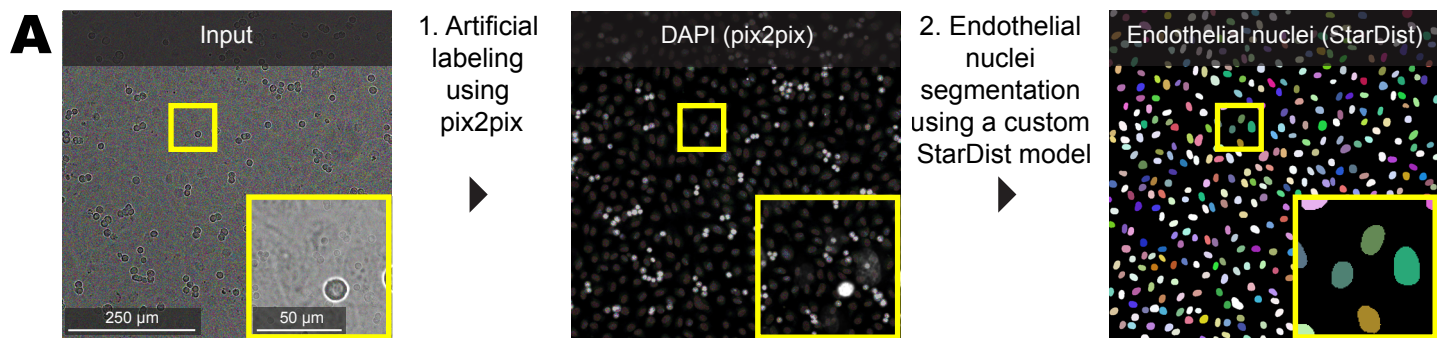
(B) Detailed analysis of track speed metrics for MIA PaCa-2, AsPC-1, and Panc 10.05 cells at different flow speeds. Plots display the maximum, mean, and minimum track speeds for each cell line. Lower speed indicates the presence of adhered cells. Results are presented as boxplots, where the whiskers extend from the 10th to the 90th percentiles. The boxes capture the interquartile range, with the median marked by a line within each box. Data points outside the whiskers are depicted as individual dots (n = 1522-12904 tracks, 2-8 biological repeats, see Methods).



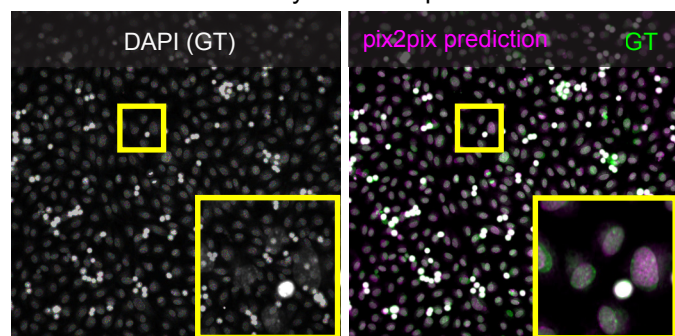
Appendix Figure S3: Deep learning model used to detect immune cells in microfluidic channels containing endothelial cells and detailed track speed metrics.

(A) Validation of the StarDist models used to detect neutrophils and PBMCs perfused in the channels. The Input, the StarDist prediction, and the ground truth image are displayed alongside the F1 score. Scale bar = 100 µm.

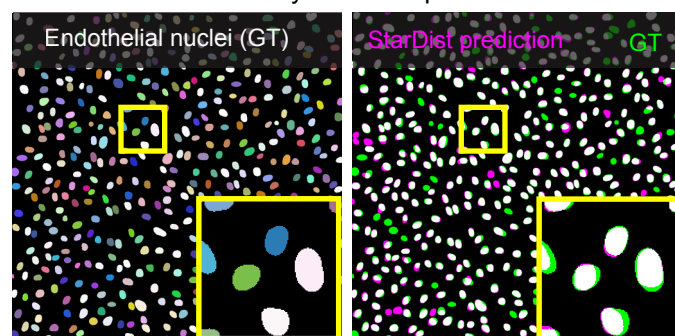
(B) Detailed analysis of track speed metrics for PBMCs and neutrophils in the presence or absence of IL-1β stimulation at different flow speeds. Plots display the mean, maximum, and minimum track speeds for each cell type. Results are presented as boxplots, where the whiskers extend from the 10th to the 90th percentiles. The boxes capture the interquartile range, with the median marked by a line within each box. Data points outside the whiskers are depicted as individual dots (n = 1522-12904 tracks, 2-8 biological repeats, see Methods).



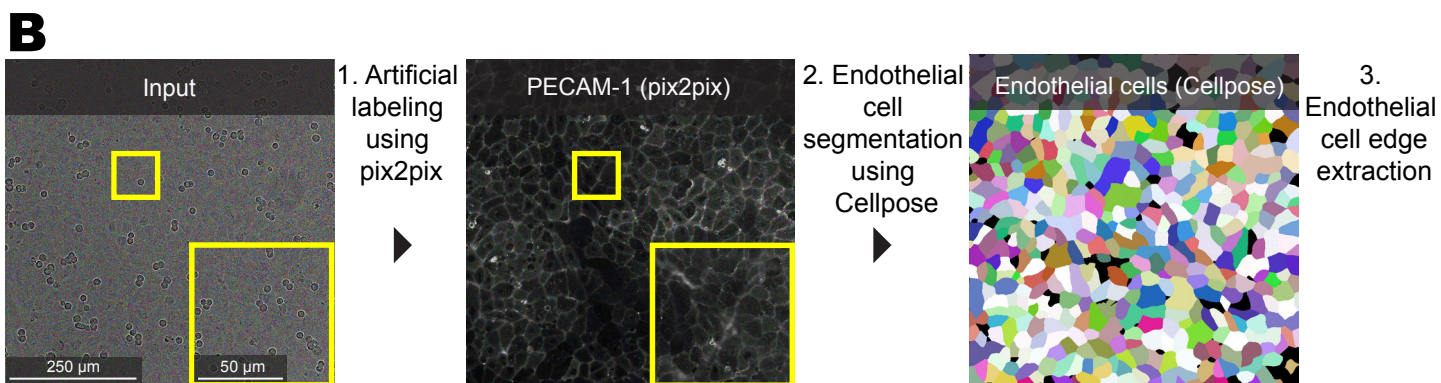
Quality check step 1.



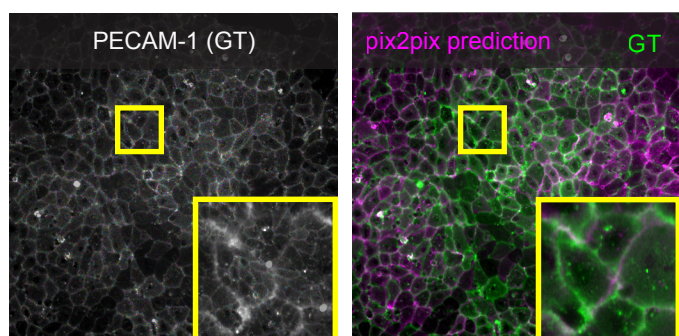
Quality check step 2.



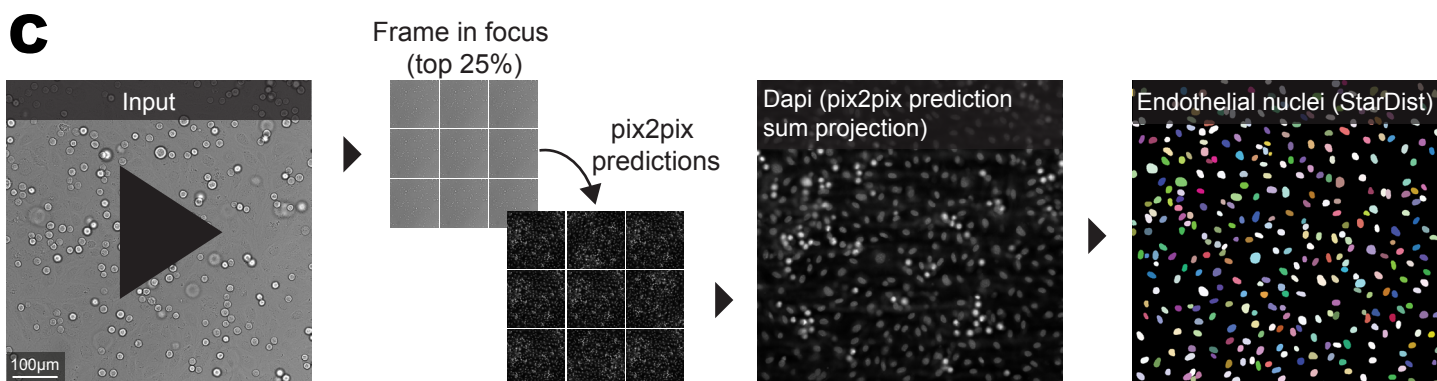
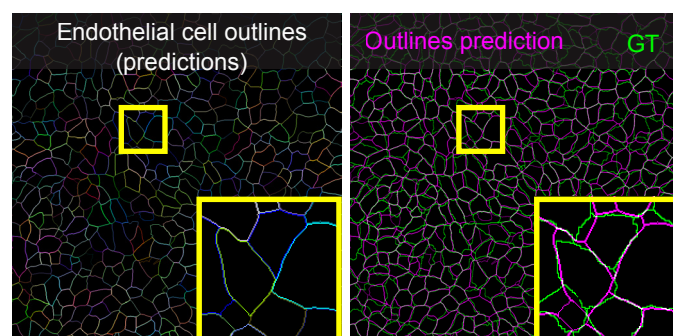
F1 score = 0.845



Quality check step 1.



Quality check step 2-3.



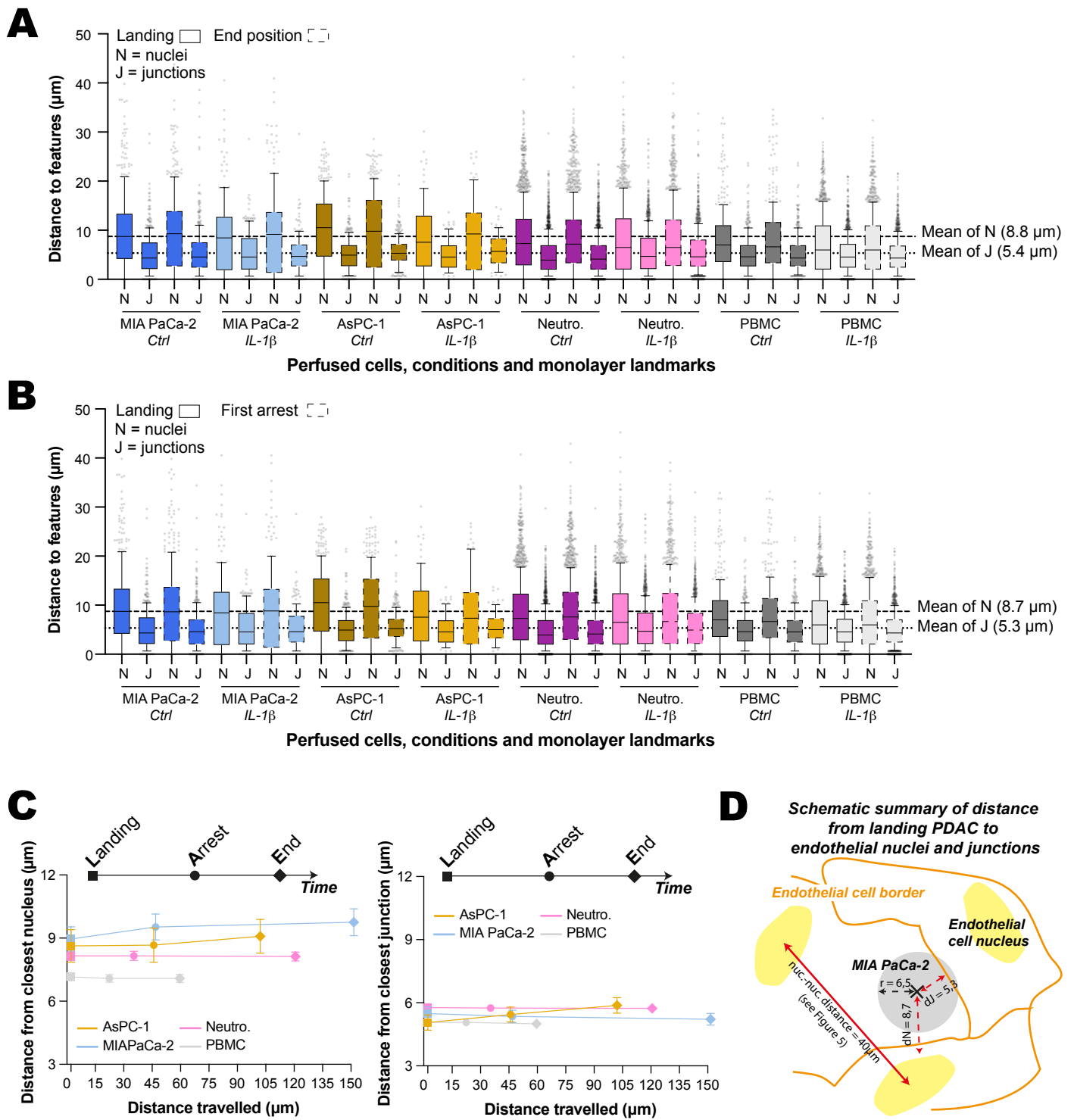
Appendix Figure S4: Deep learning-based segmentation of endothelial nuclei and cell-cell junctions

(A) Validation of our pipeline to segment endothelial nuclei from brightfield images. A pix2pix model was trained to artificially label nuclei (DAPI) from brightfield images. A StarDist model was then used to segment the nuclei from the predicted DAPI images. Representative images and their associated ground truths (GTs) are displayed. The nuclei GT was acquired using a widefield fluorescence microscope following staining, while the endothelial nuclei GT was derived from manual segmentation of endothelial cell nuclei from the acquired DAPI images.

(B) Validation of our pipeline to identify endothelial cell-cell junctions from brightfield images. A pix2pix model was trained to artificially label PECAM-1 from brightfield images. Using the predicted DAPI (**A**) and the predicted PECAM-1 image, endothelial cells were segmented with Cellpose (Cyto2 model). Endothelial cell-cell junctions were then determined using cell boundary delineation. Representative images and their associated GTs are displayed. The PECAM-1 GT was acquired using a widefield fluorescence microscope following PECAM-1 immunostaining, while the junction GT was manually labeled from the acquired PECAM-1 staining.

(C) Segmenting endothelial cell nuclei and cell-cell junctions from live brightfield imaging. The segmentation process involved normalizing brightness and contrast using adaptive histogram equalization and assessing sharpness with Sobel and LoG operators. The sharpest 25% of frames were artificially labeled using pix2pix models to predict DAPI and PECAM-1 staining (**A** and **B**). Consensus DAPI and PECAM-1 images were generated by sum-projection of the labeled frames. Endothelial nuclei were segmented as in A, while endothelial cell-cell junctions were segmented as in B. Scale bar: 100 μm .

The numerical data and images used for this figure, as well as statistical summaries including pairwise Cohen's d values and results from statistical tests, have been archived on Zenodo (<https://doi.org/10.5281/zenodo.13846276>).



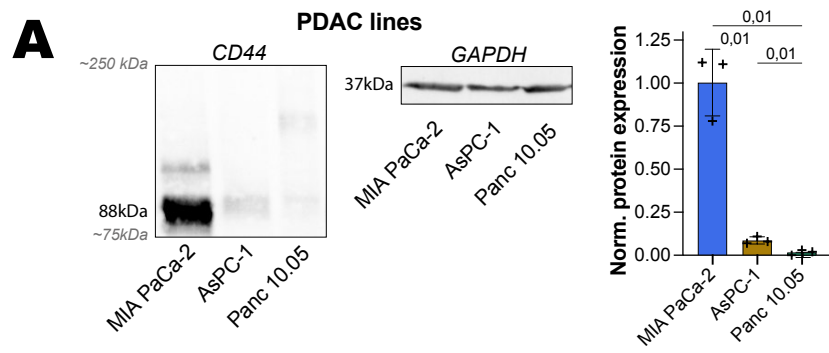
Appendix Figure S5: Spatial analysis of cell arrest in relation to endothelial nuclei and junctions.

(A-B) Proximity of arresting cells to endothelial nuclei and cell-cell junctions. The distance between a single cell and the nearest nucleus (N) or endothelial cell-cell junction (J) was measured at specific steps during the arrest process (in **A**: end position, in **B**: first arrest) for MIA PaCa-2, AsPC-1, neutrophils (Neutro.), and PBMCs. Data are summarized in figure 4B and figure EV5C (IL-1 β stimulation). The results are presented as boxplots, with whiskers representing the 10th to 90th percentiles and boxes indicating the interquartile range, with the median value marked. Outliers are displayed as individual data points ($n = 84$ -1710 tracks, 2-8 biological repeats, see methods).

(C) Effect of IL-1 β treatment on cell distance to endothelial structures. For each condition under IL-1 β treatment, the average distance from the cell center to the nearest nucleus and the nearest endothelial cell-cell junction is plotted as a function of the total distance travelled from the initial landing to the first arrest and the end of the track ($n = 334$ -1710 tracks, 2-8 biological repeats, see methods).

(D) Schematic representation of MIA PaCa-2 cell arrest. The diagram illustrates a MIA PaCa-2 cell arresting on the endothelial monolayer, with actual measurement means provided. This schematic illustrates how the in vitro model replicates in vivo observations, indicating that cells frequently interact with endothelial cell-cell junctions during arrest.

The numerical data and images used for this figure, as well as statistical summaries including pairwise Cohen's d values and results from statistical tests, have been archived on Zenodo (<https://doi.org/10.5281/zenodo.13846276>).



Appendix Figure S6: CD44 expression in PDAC cells

(A) CD44 protein levels in MIA PaCa-2, AsPC-1, and Panc 10.05 cells. A representative western blot and the quantification of the 88 kDa band (n=3 biological replicates) are displayed.