## **Supplementary Information**

## Machine-Learning-Guided Quantitative Delineation of Cell Morphological Features and Responses to Nanomaterials

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## **Supporting Figures**



**Figure S1.** Morphological features of macrophages and epithelial cells: (a) mean radius, (b) median radius, (c) equivalent diameter, (d) perimeter, (e) minor axis length, (f) form factor, (g) eccentricity, (h) solidity, and (i) extent. n = 68 for RAW 264.7 macrophages, 24 for MCF-10A normal epithelial cells, and 34 for MDA-MB-231 cancerous epithelial cells. \* p < 0.05, \*\* p < 0.01, \*\*\*\* p < 0.001, \*\*\*\* p < 0.0001 based on either Brown-Forsythe and Welch ANOVA test coupled with Dunnett's T3 multiple comparisons test or Kruskal-Wallis test coupled with Dunn's multiple comparisons test.



**Figure S2.** Morphological features of the different macrophage phenotypes: (a) mean radius, (b) median radius, (c) equivalent diameter, (d) perimeter, (e) minor axis length, (f) form factor, (g) eccentricity, (h) solidity, and (i) extent. n = 68 for M0, 53 for M1-like, and 77 for M2-like macrophages. \* p < 0.05, \*\* p < 0.01, and \*\*\*\* p < 0.001 based on either Brown-Forsythe and Welch ANOVA test coupled with Dunnett's T3 multiple comparisons test or Kruskal-Wallis test coupled with Dunn's multiple comparisons test.



**Figure S3.** Semi-quantitative evaluation of the viability of macrophages treated with ethanol over time. \* p < 0.05 based on Brown-Forsythe and Welch ANOVA test coupled with Dunnett's T3 multiple comparisons test.



**Figure S4.** Morphological features of the different ethanol-treated macrophages with varying viability: (a) mean radius, (b) median radius, (c) equivalent diameter, (d) perimeter, (e) minor axis length, (f) form factor, (g) eccentricity, (h) solidity, and (i) extent. n = 68 for control 0 min, 101 for + EtOH 10 min, 74 for + EtOH 30 min, 97 for + EtOH 60 min, and 87 for + EtOH 120 min. \* p < 0.05, \*\* p < 0.01, \*\*\* p < 0.001, and \*\*\*\* p < 0.0001 based on Kruskal-Wallis test coupled with Dunn's multiple comparisons test.



**Figure S5.** Morphological features of the different nanoparticle-treated macrophages: (a) mean radius, (b) median radius, (c) equivalent diameter, (d) perimeter, (e) minor axis length, (f) form factor, (g) eccentricity, (h) solidity, and (i) extent. n = 45 for control 0 h, 44 for + Nanoparticles 1 h, 43 for + Nanoparticles 6 h, and 53 for + Nanoparticles 24 h. \* p < 0.05, \*\* p < 0.01, and \*\*\* p < 0.001 based on Kruskal-Wallis test coupled with Dunn's multiple comparisons test.



**Figure S6.** Gini index of various morphological features of (a) macrophages and normal and cancerous epithelial cells, (b) different macrophage phenotypes, and (c) ethanol-treated macrophages with varying viability.



**Figure S7.** Unsupervised *k*-means clustering of the different macrophage phenotypes: (a, b) M1like and (c, d) M2-like macrophages. (a, c) Silhouette plots and (b, d) scattering plots of the different macrophage clusters of the respective groups as identified through the unsupervised *k*means clustering.