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Extracellular protonation modulates cell-cell interaction mechanics and tissue invasion in human melanoma cells

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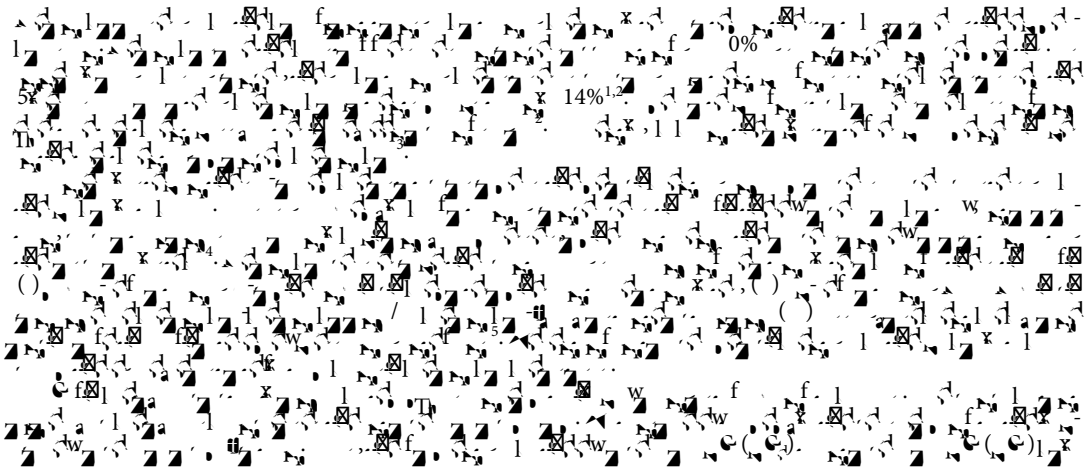
Detachment of cells from the primary tumour precedes metastatic progression by facilitating cell release into the tissue. Solid tumours exhibit altered pH homeostasis with extracellular acidification. In human melanoma, the Na^+/H^+ exchanger NHE1 is an important modifier of the tumour nanoenvironment. Here we tested the modulation of cell-cell-adhesion by extracellular pH and NHE1. MV3 tumour spheroids embedded in a collagen matrix unravelled the efficacy of cell-cell contact loosening and 3D emigration into an environment mimicking physiological confinement. Adhesive interaction strength between individual MV3 cells was quantified using atomic force microscopy and validated by multicellular aggregation assays. Extracellular acidification from pH_e 7.4 to 6.4 decreases cell migration and invasion but increases single cell detachment from the spheroids. Acidification and NHE1 overexpression both reduce cell-cell adhesion strength, indicated by reduced maximum pulling forces and adhesion energies. Multicellular aggregation and spheroid formation are strongly impaired under acidification or NHE1 overexpression. We show a clear dependence of melanoma cell-cell adhesion on pH_e and NHE1 as a modulator. These effects are opposite to cell-matrix interactions that are strengthened by protons extruded via NHE1. We conclude that these opposite effects of NHE1 act synergistically during the metastatic cascade.



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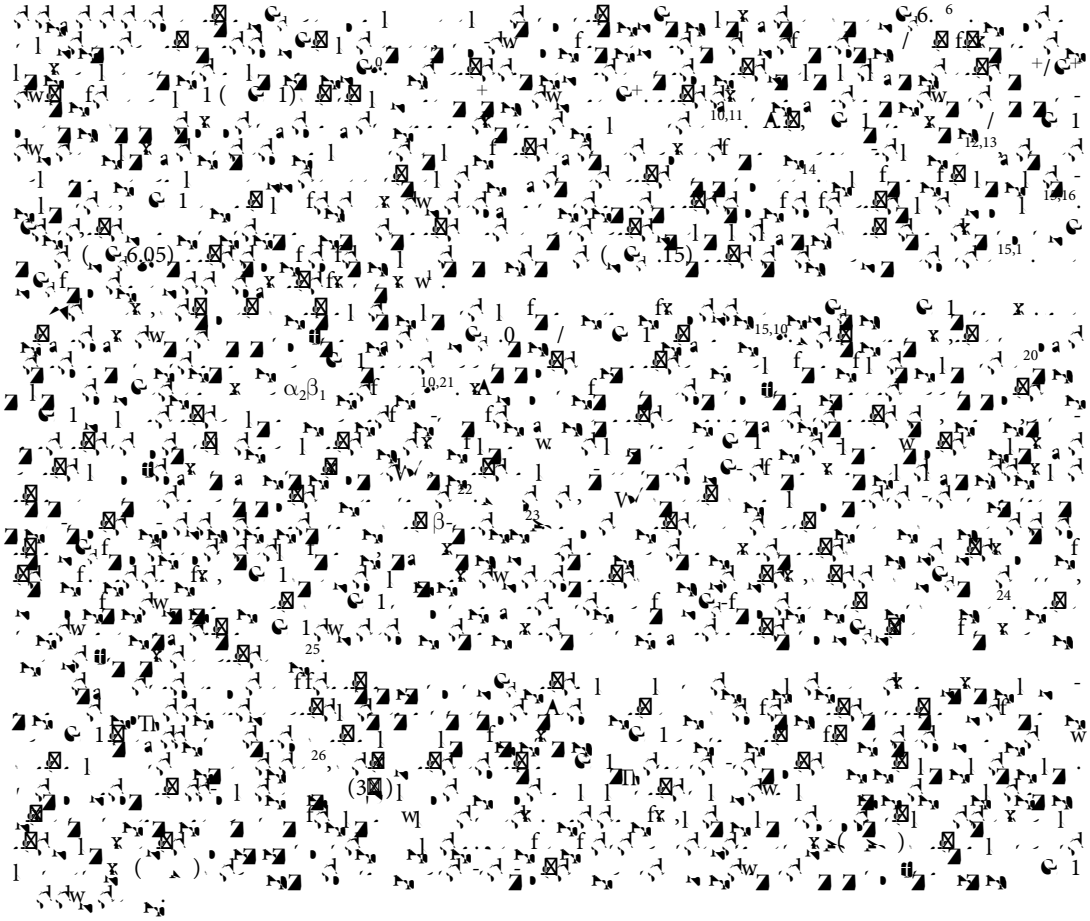


Results

In a 3D assay, acidificati

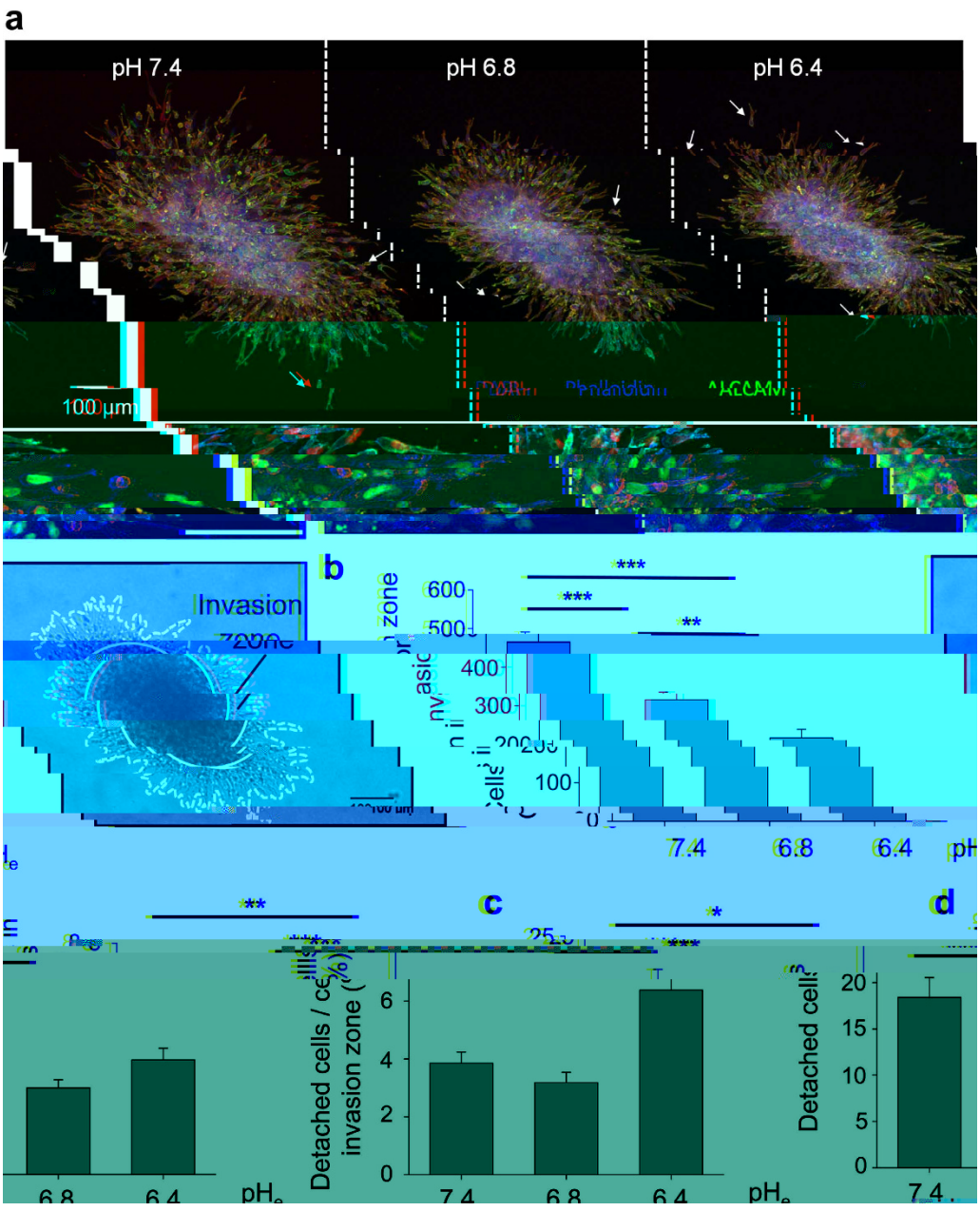


Figure 1. 3D emigration assays through a collagen I matrix. (a) [illegible] **(b)** [illegible] 464.6 ± 26.1 [illegible] 216.4 ± 21.0 [illegible] **(c)** [illegible] **(d)** [illegible] 3. 5 ± 0.30% [illegible] 3.1 ± 0.35% [illegible].

NHE1-overexpressing cells do not form stable tumour spheroids in multicellular cell aggregation assays. [illegible]

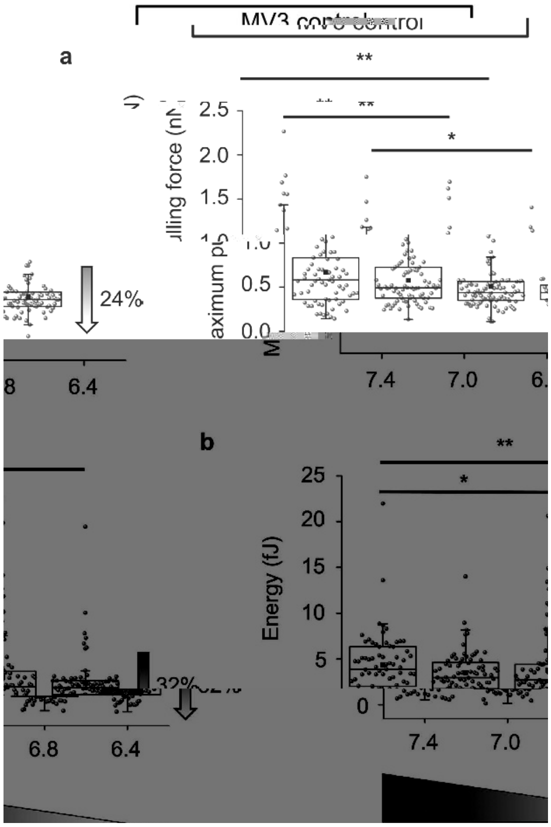


Figure 2. Single cell force spectroscopy at varying pH$_e$. [illegible] **(a)** [illegible] 0.5 (0.36/0. [illegible] 0.40 (0.3 [illegible] 0.44 (0.35/0.56 [illegible] 6.4 0.44 (0.36/0.53 [illegible] **(b)** [illegible] (2.0/6.32 [illegible] 2.05 (1. [illegible] 4/4.6 [illegible] (1.66/4.4 [illegible] 6.4 2.62 (1.01/3.3 [illegible]

[illegible] 36 [illegible] 16 [illegible] 466 μ [illegible] 0.16 [illegible] 6.4 [illegible]

Extracellular acidification increases adhesion between NHE1-overexpressing MV3 cells. [illegible] (0.22 (0.14/0.32 [illegible] (0.5/1.36 [illegible]

NHE1 expression correlates with the expression of melanoma cell adhesion molecule. [illegible]

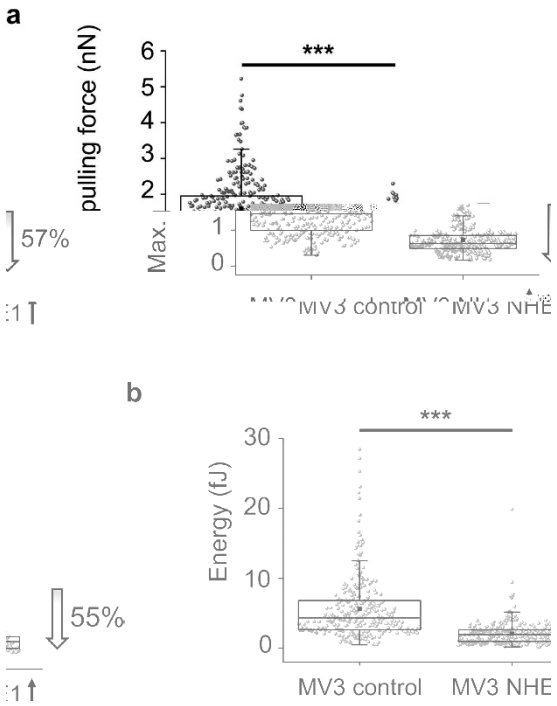


Figure 3. Single cell force spectroscopy using MV3 cells with different NHE1 expression levels.

Discussion

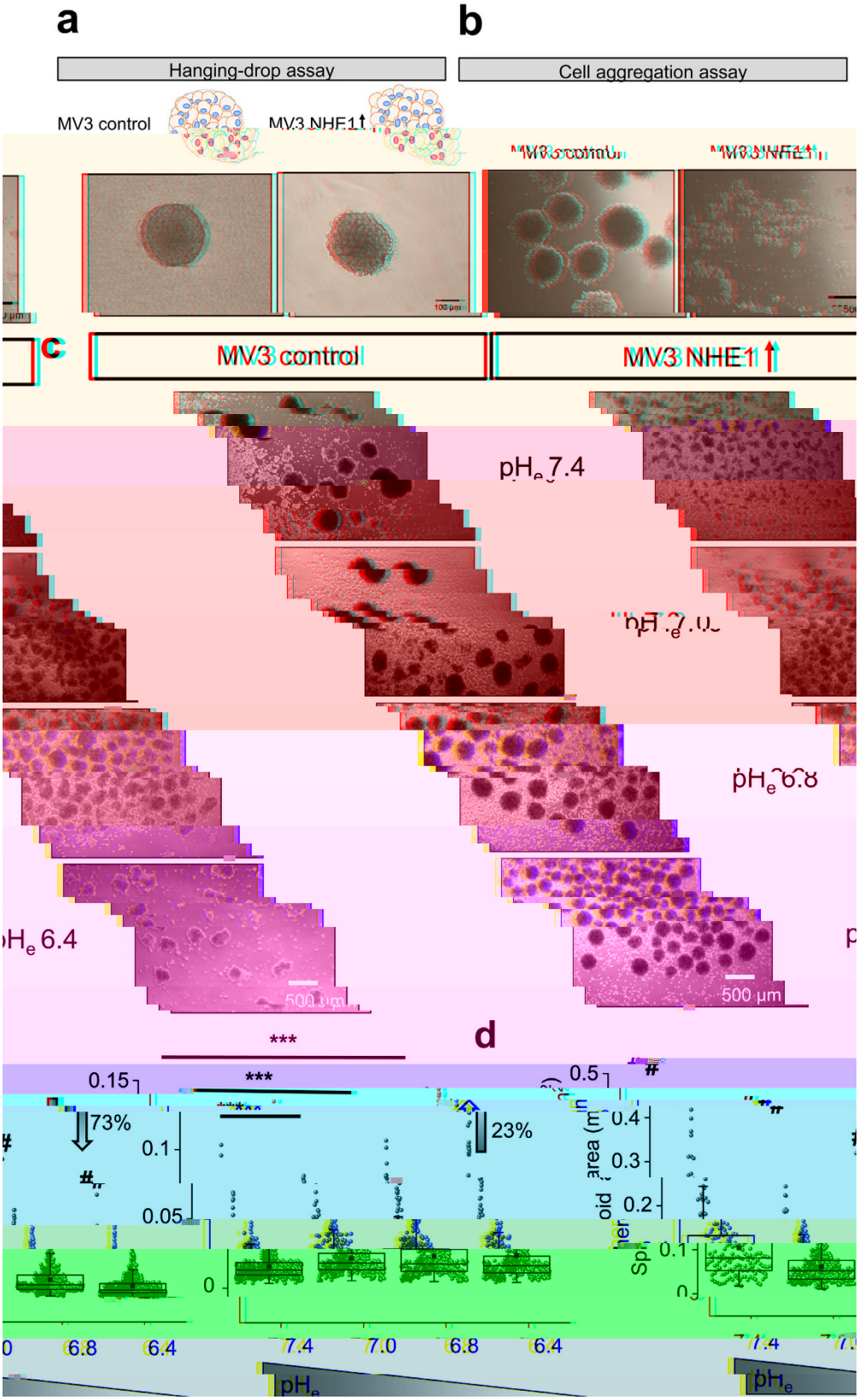
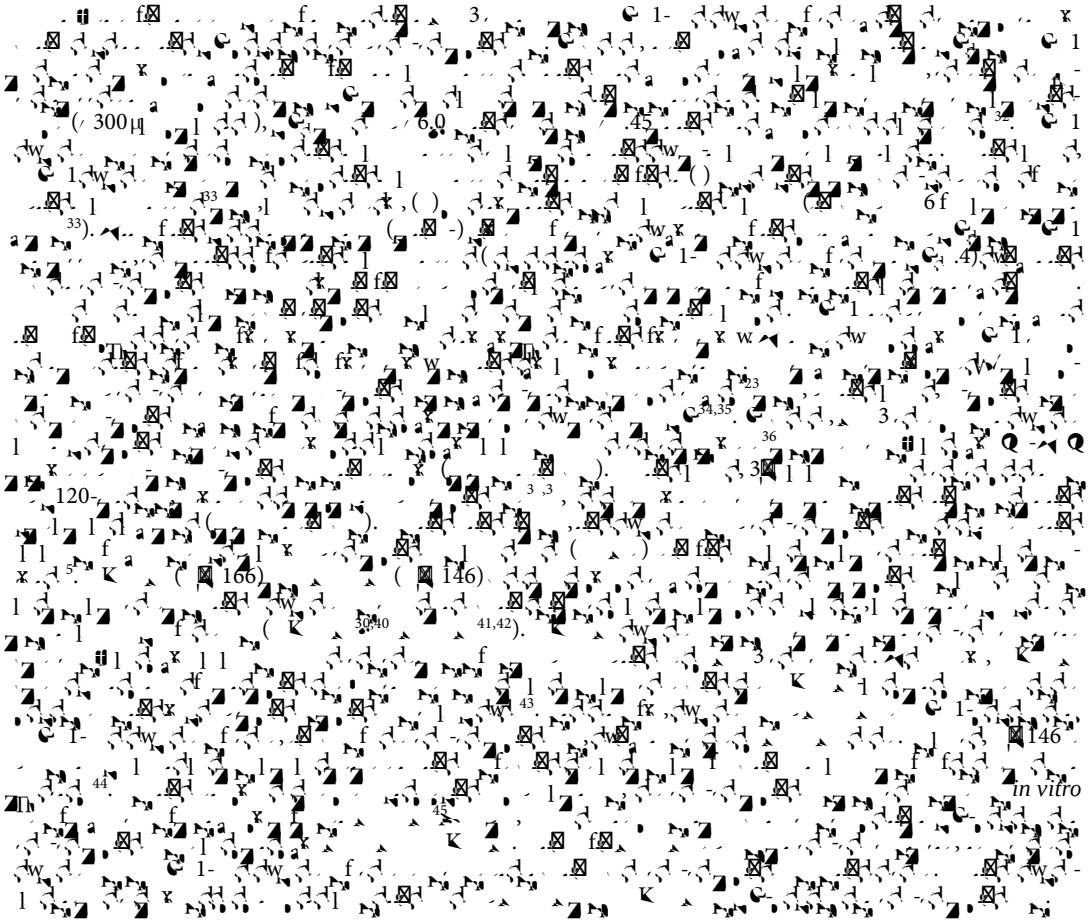
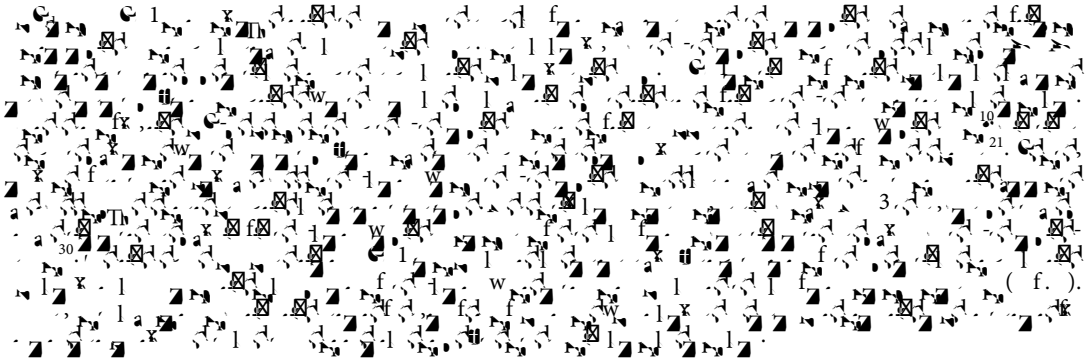


Figure 4. Multicellular adhesion assays. (a) A[illegible] 3[illegible] 3 [illegible] 1- [illegible] w [illegible] f [illegible] l [illegible] l [illegible] f [illegible] f [illegible] f- [illegible] x [illegible] f [illegible] 2 [illegible] 1- [illegible] w [illegible] f [illegible] f [illegible] f [illegible] z [illegible] **(b)** [illegible] f [illegible] f [illegible] x [illegible] 3 [illegible] w [illegible] l [illegible] l [illegible] f [illegible] c [illegible] 1- [illegible] w [illegible] l [illegible] l [illegible] l [illegible] f [illegible] 3 [illegible] l [illegible] 466 µl (300/526 µl [illegible] = 3 [illegible] w [illegible] l [illegible] = 4 [illegible]).



Conclusion



Methods

Cell culture.

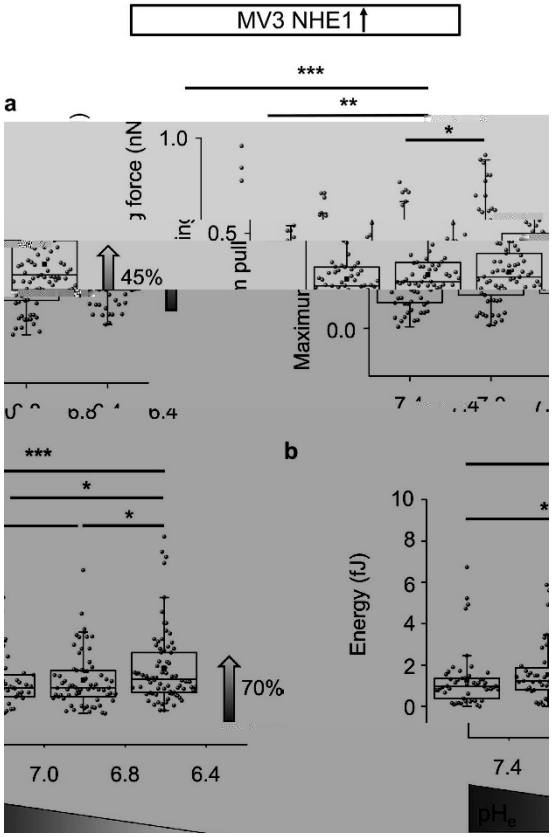


Figure 5. Single cell force spectroscopy using MV3 NHE1-overexpressing cells at varying pH_e. (a)

3D spheroid culture using the hanging-drop method.

Spheroid embedding in 3D matrix and fixation.

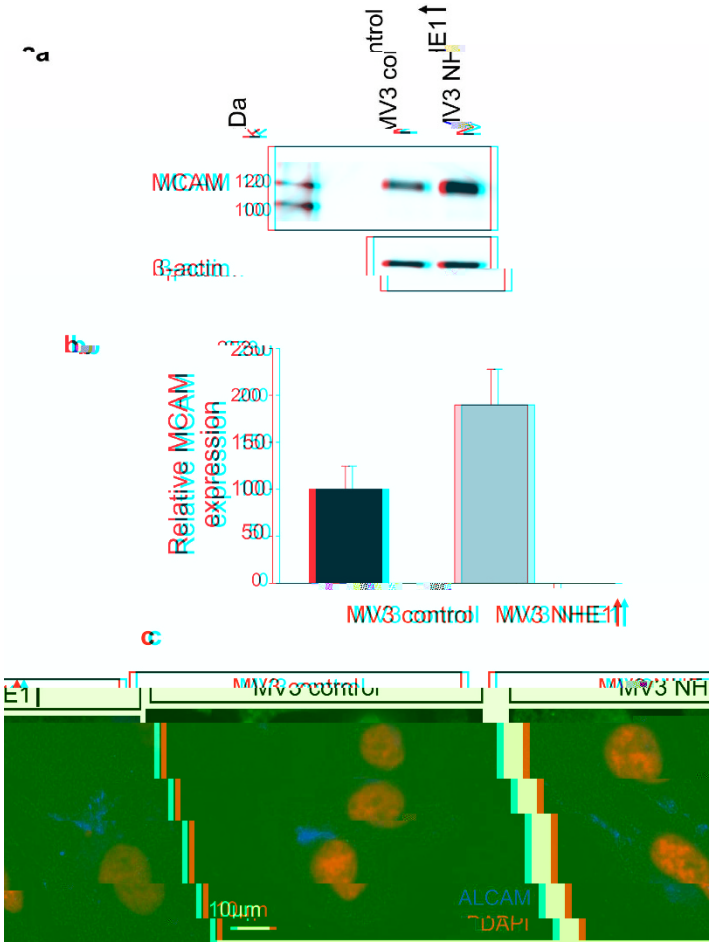


Figure 6. Expression of Cell Adhesion Molecules. (a,b) [illegible] (c) [illegible]

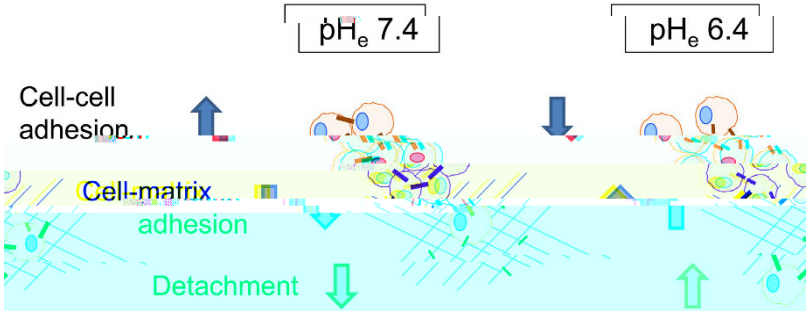


Figure 7. Summary. [illegible]

3D Immunofluorescence staining and confocal microscopy. [illegible]

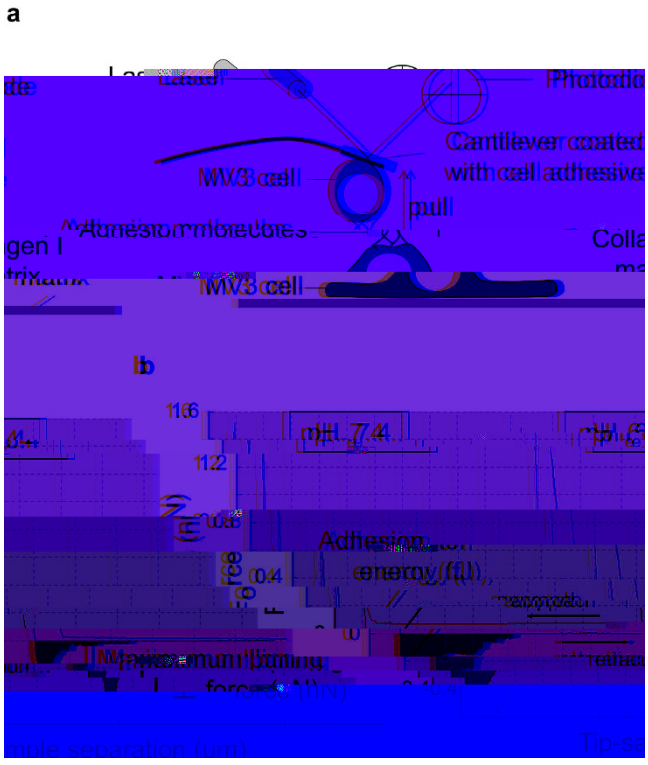


Figure 8. Schematic illustration of cell-cell adhesion analysis using AFM. (a)

Single cell force spectroscopy (SCFS).

Multicellular cell aggregation assay.

Immunofluorescence staining of ALCAM in 2D cultures.

Western Blot analysis of MCAM from spheroid cultures.

Analysis and statistics.

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