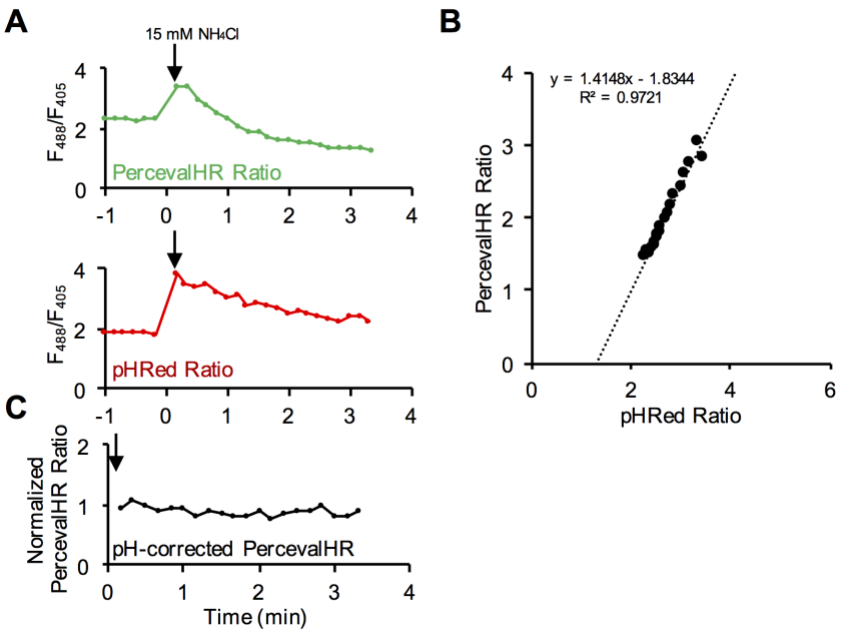
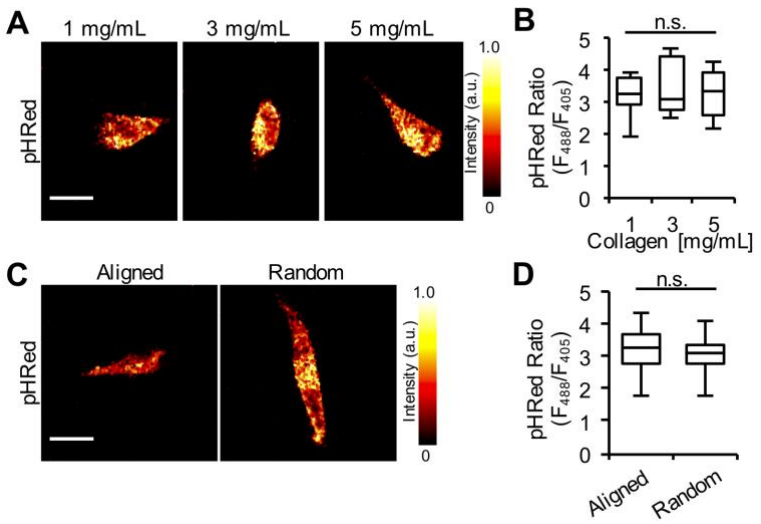
Supplemental Materials

Molecular Biology of the Cell

Zanotelli et al.



Supplemental Figure S1. pH calibration of PercevalHR signal. (A) Raw PercevalHR ratiometric signal and (B) raw pHRed ratiometric signal of a single cell after the addition of 15 mM NH_4Cl to induce intracellular alkalinization. (B) A relationship between the PercevalHR signal and pHRed signal shows a linear correlation demonstrating the pH sensitivity of PercevalHR, which can be used to correct of pH bias (n = 5 cells). (C) pH-corrected PercevalHR signal.



Supplemental Figure S2. Changes in intracellular pH across 3D matrix conditions. (A) Representative pHRed ratios of MDA-MB-231 cells co-expressing PercevalHR and pHRed cultured in a 1, 3, and 5 mg/mL collagen matrix, and (B) quantification of pHRed ratios (*n* = 30 cells from three independent experiments). (C) Representative pHRed ratios of MDA-MB-231 cells co-expressing PercevalHR and pHRed cultured in an aligned and randomly oriented 1.5 mg/mL three-dimensional collagen matrix, and (D) quantification of pHRed ratios (*n* = 40 cells from three independent experiments). Box and whisker plots show medians and $25^{th}/75^{th}$ and $5^{th}/95^{th}$ percentiles. n.s.=not significant for (B) one-way ANOVA with Tukey's HSD post-hoc test and (D) Wilcoxon rank test. Scale bars = 20 µm.