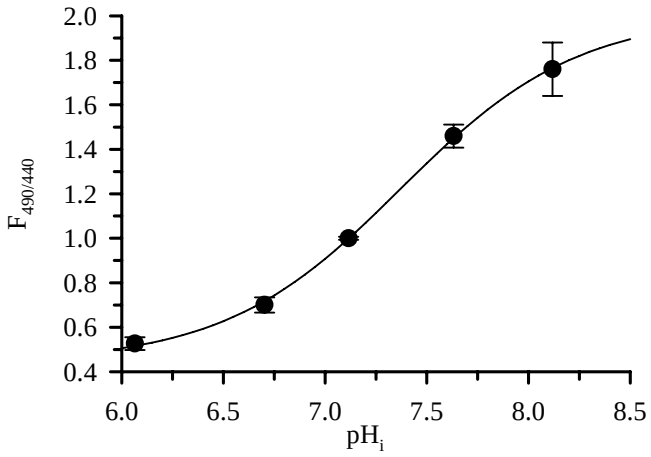

Supplemental Figure 1

To calculate the inherent variability in BCECF method, we plotted the means ± SD (standard deviation) from a typical in-situ calibration that is performed at the end of each experiment. Because the high K^+/nigericin technique equilibrates H^+ across the plasma membrane, the $[H^+]$ should be identical in all cells. The intracellular differences in the observed F490/F440 ratios would be a reflection of the inherent error in the BECEF method. The data was fit to a non-linear curve. At pH 7.116, SD of the F490/F440 ratios would give a calculated variability of +0.0075 and -0.0095 pH units. As seen in the figure, the variability in the BCECF method is considerably higher at alkaline pHs. However, this is outside the range of pH_i relevant to our study.