Electronic Supplementary Material (ESI) for RSC Advances.

Supporting Information

Ratiometric fluorescent pH-sensitive polymers for high-throughput monitoring of extracellular pH†

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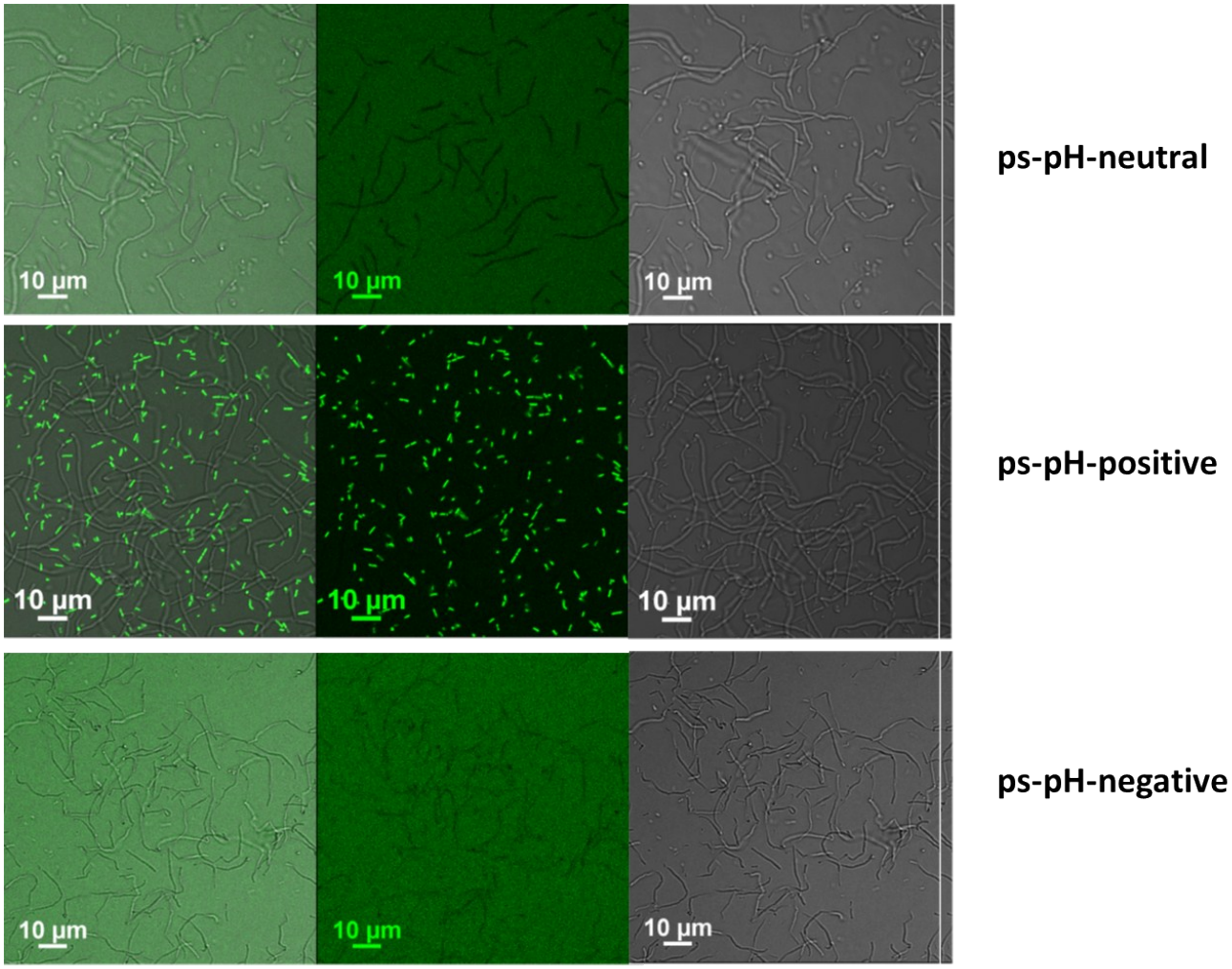
Contents:


10 µm
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ps-pH-neutral
10 µm
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10 µm
ps-pH-positive
10 µm
10 µm
10 µm
ps-pH-negative
overlay
pH sensor
DPI

Figure S1. Cellular distribution of pH sensors in *L. fermentum.* 10 µg/mL of sensors were incubated with *L. fermentum* in 96-well microplate for 24 hours. Fluorescent images were taken by confocal microscopy with 488 nm excitation.

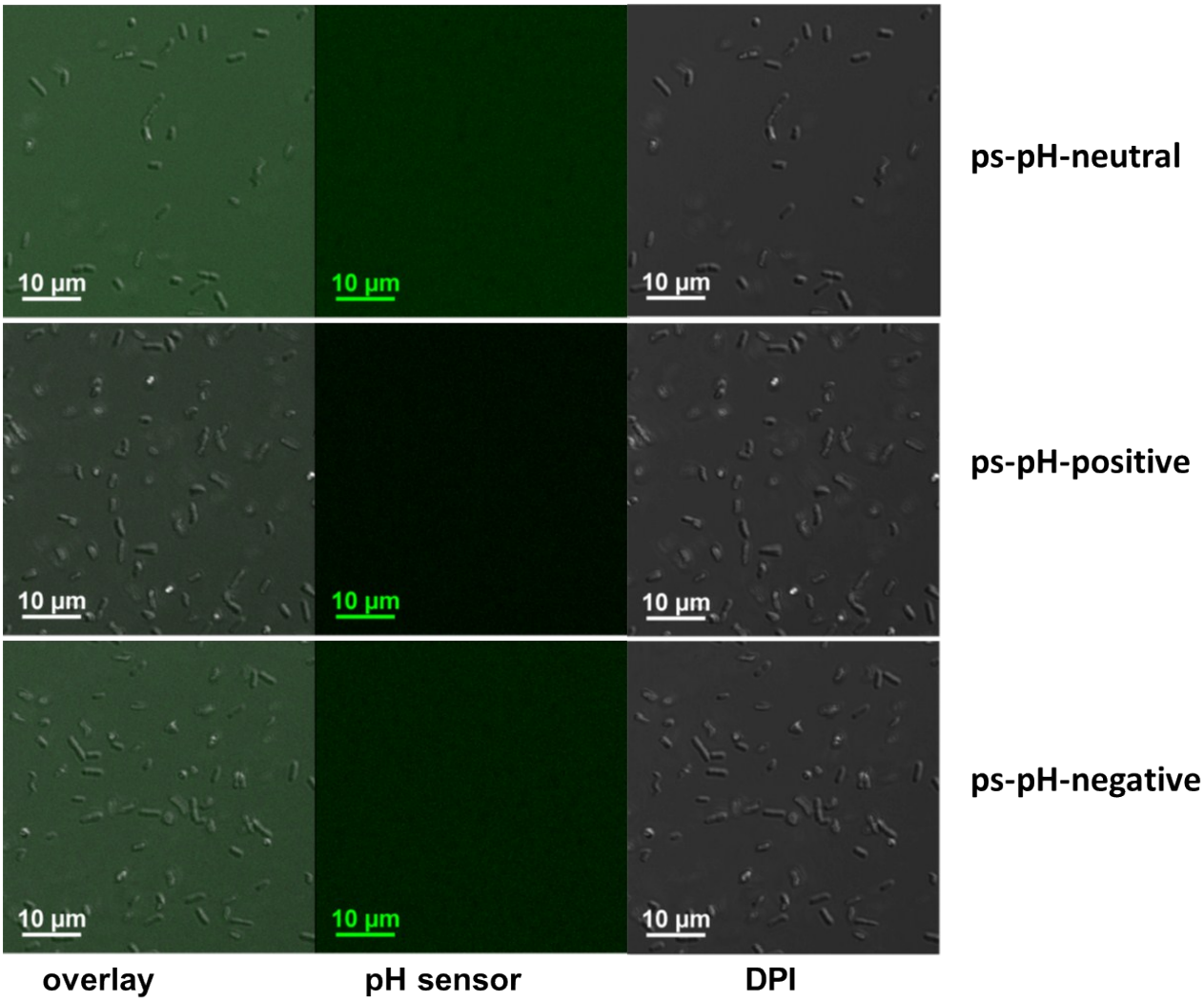
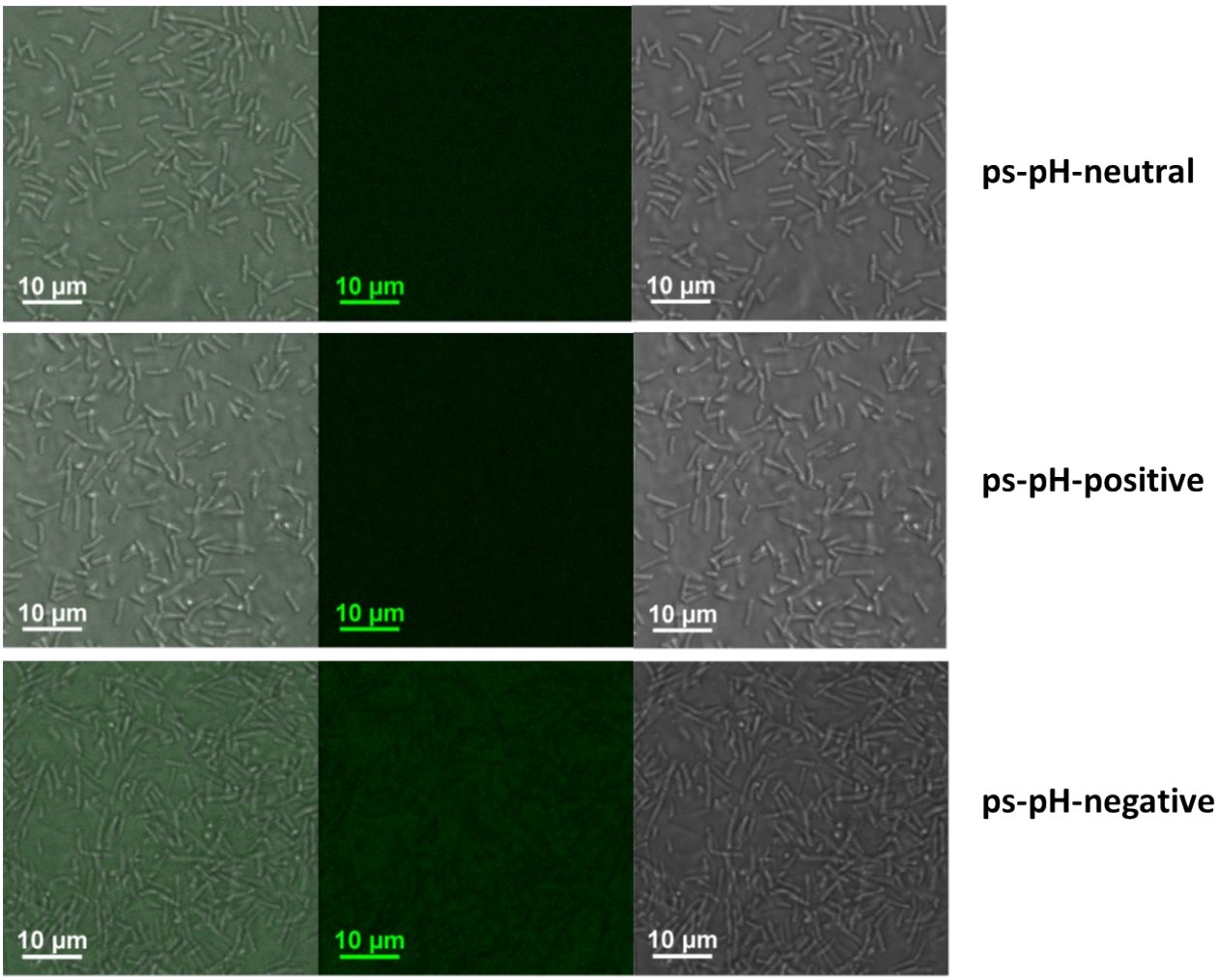


Figure S2. Cellular distribution of pH sensors in *E. coli (JM109).* 10 μg/mL of sensors were incubated with *E. coli* in 96-well microplate for 24 hours. Fluorescent images were taken by confocal microscopy with 488 nm excitation.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ps-pH-neutral
ps-pH-positive
ps-pH-negative
overlay
pH sensor
DPI

Figure S3. Cellular distribution of pH sensors in *B. subtilis.* 10 µg/mL of sensors were incubated with *B. subtilis* in 96-well microplate for 24 hours. Fluorescent images were taken by confocal microscopy with 488 nm excitation.

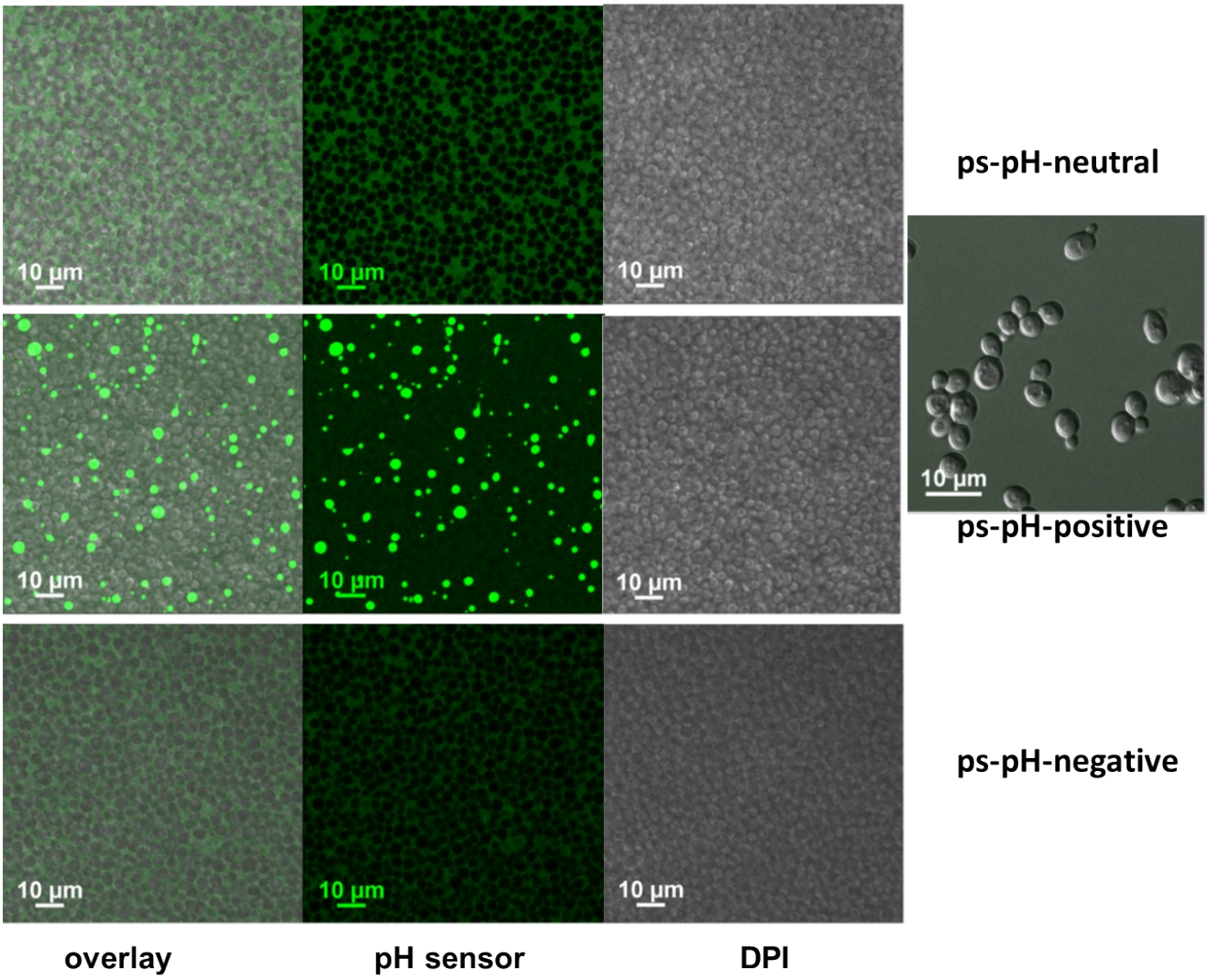

Figure S4. Cellular distribution of pH sensors in yeast cells *(S. cerevisiae).* 10 µg/mL of sensors were incubated with *S. cerevisiae* in 96-well microplate for 24 hours. Fluorescent images were taken by confocal microscopy with 488 nm excitation.

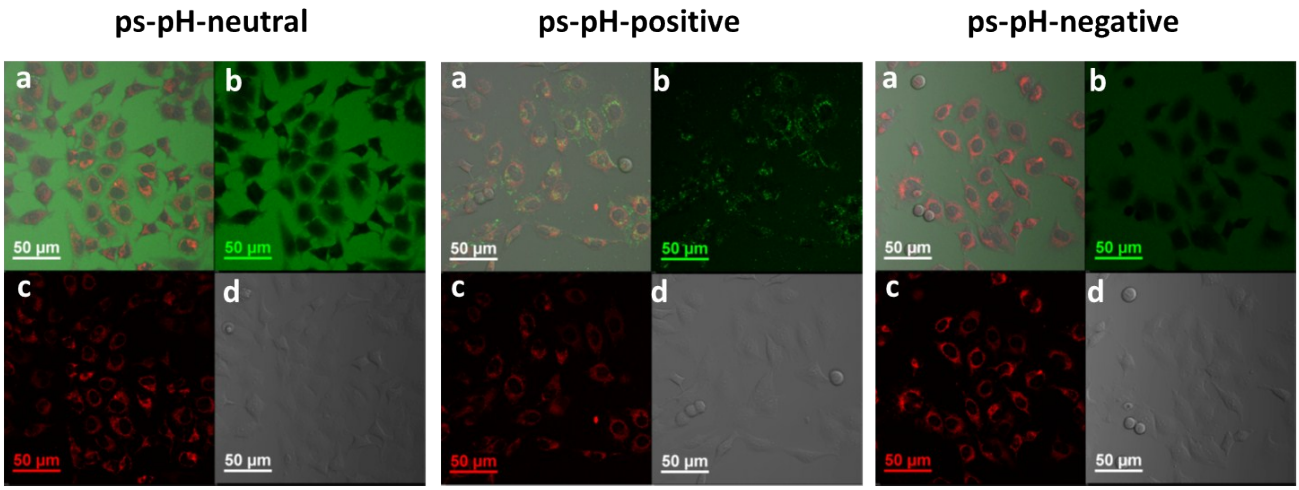

Figure S5. Sensors' distribution in HeLa cells. a, overly; b, pH sensors; c, MitoTracker Red FM; d, DPI. HeLa cells were incubated with 10 μg/mL of sensors in 96-well microplate for 24 hours. Fluorescent images were taken by confocal microscopy with 488 nm excitation.

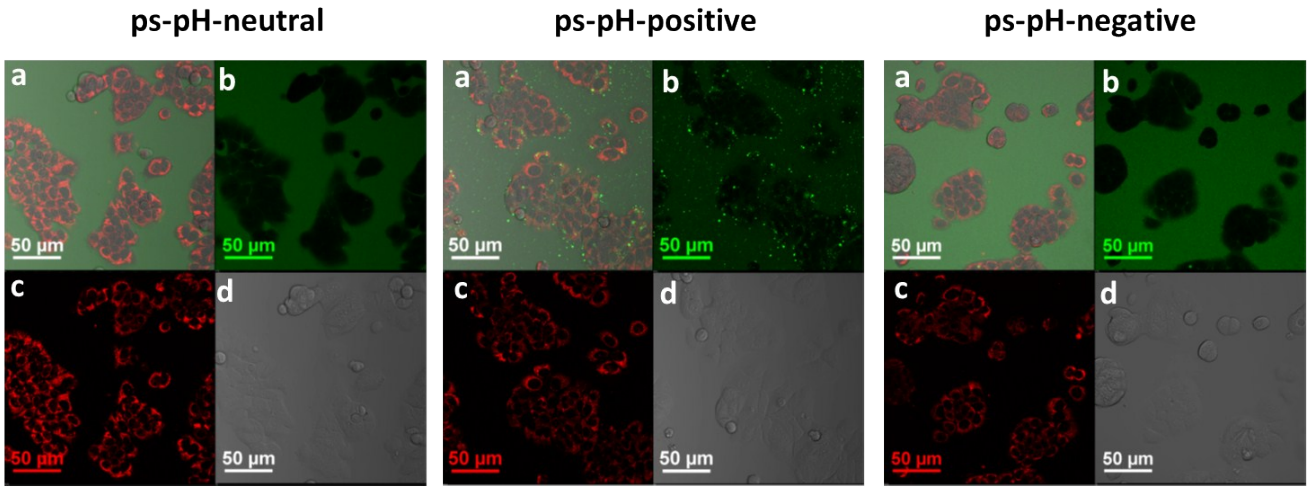

Figure S6. Sensor distribution in MCF-7 cells. **a**, overlay; **b**, pH sensors; **c**, MitoTracker Red FM; **d**, DPI. MCF-7 cells were incubated with 10 μg/mL of sensors in 96-well microplate for 24 hours. Fluorescent images were taken by confocal microscopy with 488 nm excitation.

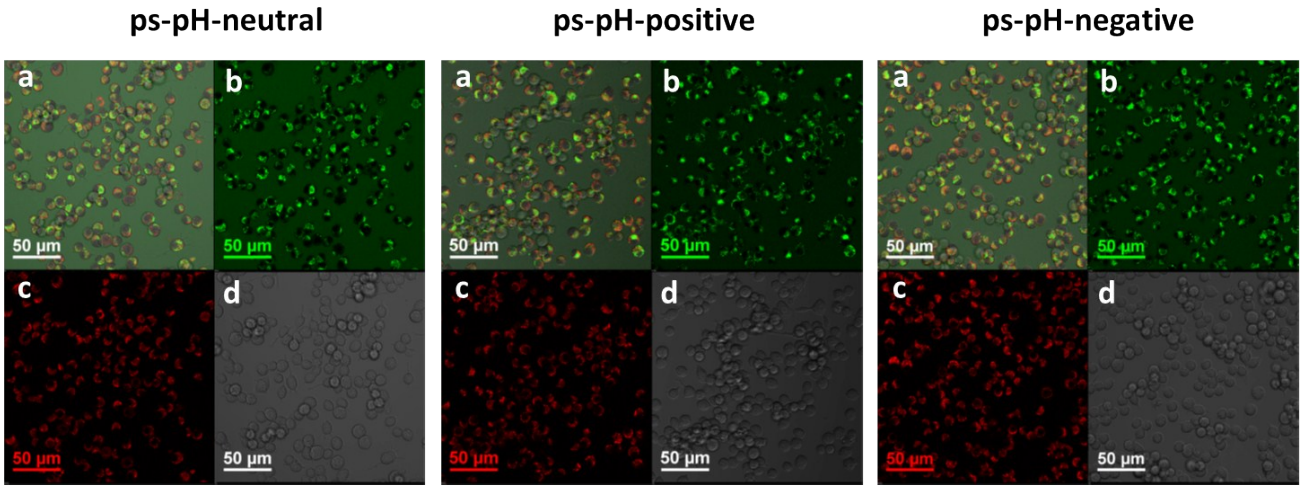

Figure S7. Sensor distribution in J774 cells. **a**, overlay; **b**, pH sensors; **c**, MitoTracker Red FM; **d**, DPI. J774 cells were incubated with 10 µg/mL of sensors in 96-well microplate for 24 hours. Fluorescent images were taken by confocal microscopy with 488 nm excitation.

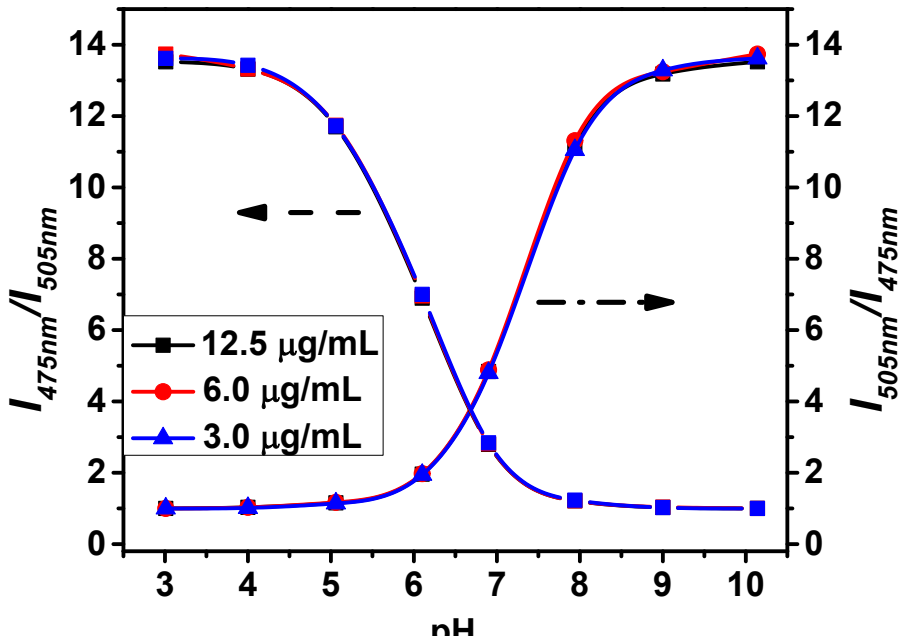
14
12
10
8
6
4
2
0
3
4
5
6
7
8
9
10
pH
I_{475nm}/I_{505nm}
I_{505nm}/I_{475nm}
12.5 μg/mL
6.0 μg/mL
3.0 μg/mL

Figure S8. pH titration of **ps-pH-neutral** at different concentrations, 3.0 μg/mL (blue), 6.0 μg/mL (red) and 12.5 μg/mL (black).

$$y = A_2 + \frac{A_1 - A_2}{1 + exp\frac{x - x_0}{dx}}$$

y		Value
I_{475nm}/I_{505nm}	A_1	1.0283
	A_2	13.51766
	x_0	7.27698
	dx	0.46213
	span	12.48936
	EC50	1446.619
I_{505nm}/I_{475nm}	A_1	13.52722
	A_2	0.94678
	x_0	6.02378
	dx	0.51926
	span	12.58044
	EC50	413.1382

Figure S9. Equation of fitting curves in Figure 4C and the values of related parameters.

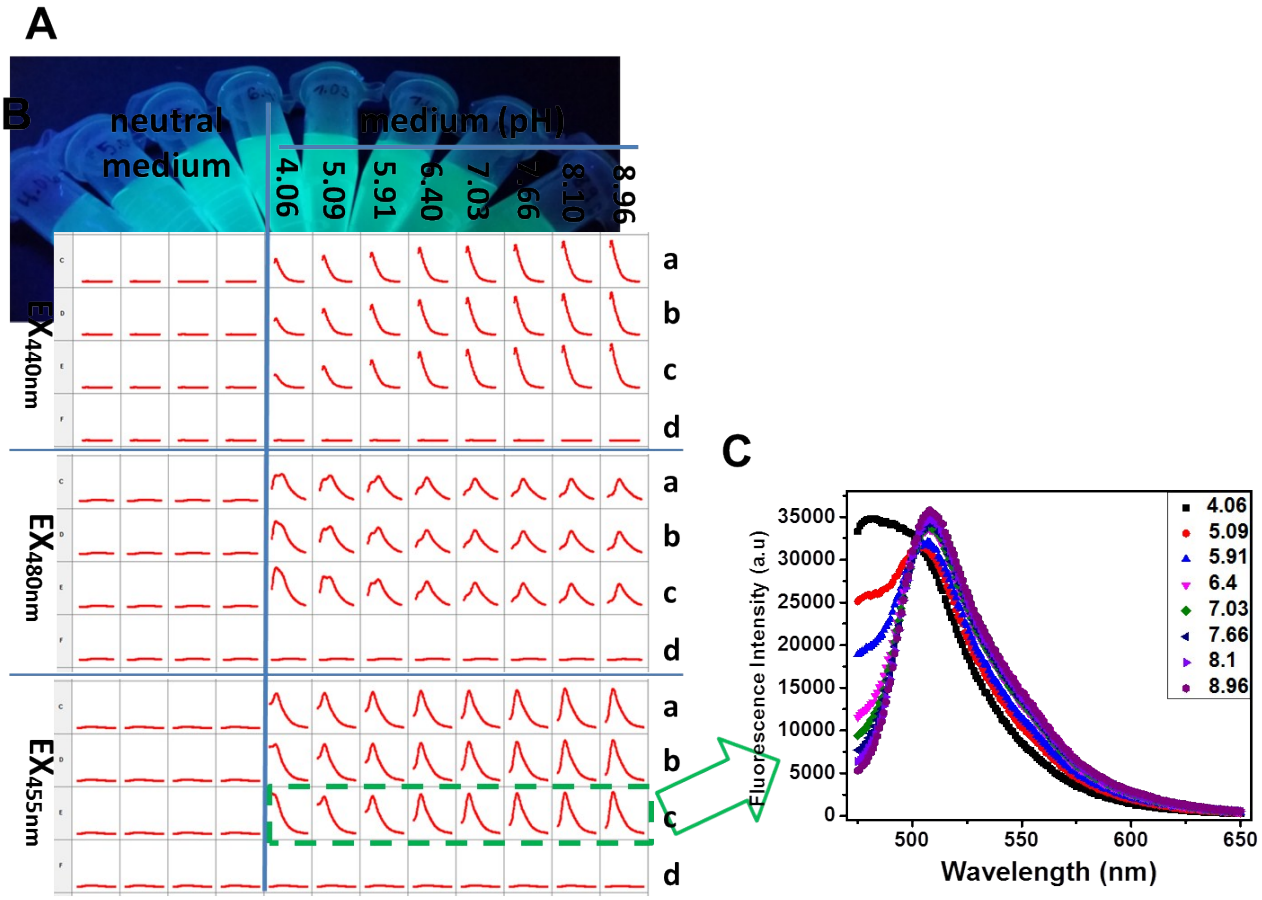

Figure S10. Application of ps-pH-neutral in mammalian cells culture. **A**, fluorescent imaging of medium (DMEM) containing **ps-pH-neutral**, from left to right are 4.06, 5.09, 5.91, 6.40, 7.03, 7.66, 8.10, and 8.96. **B**, fluorescence spectra detected by microplate reader. **a**, MCF-7 cells + sensor (10 μg/mL); **b**, HeLa cells + sensor (10 μg/mL); **c**, medium (pH) + sensor (10 μg/ml); **d**, medium (pH) only. **C**, data transferred from **B-c**, medium (pH) + sensor.

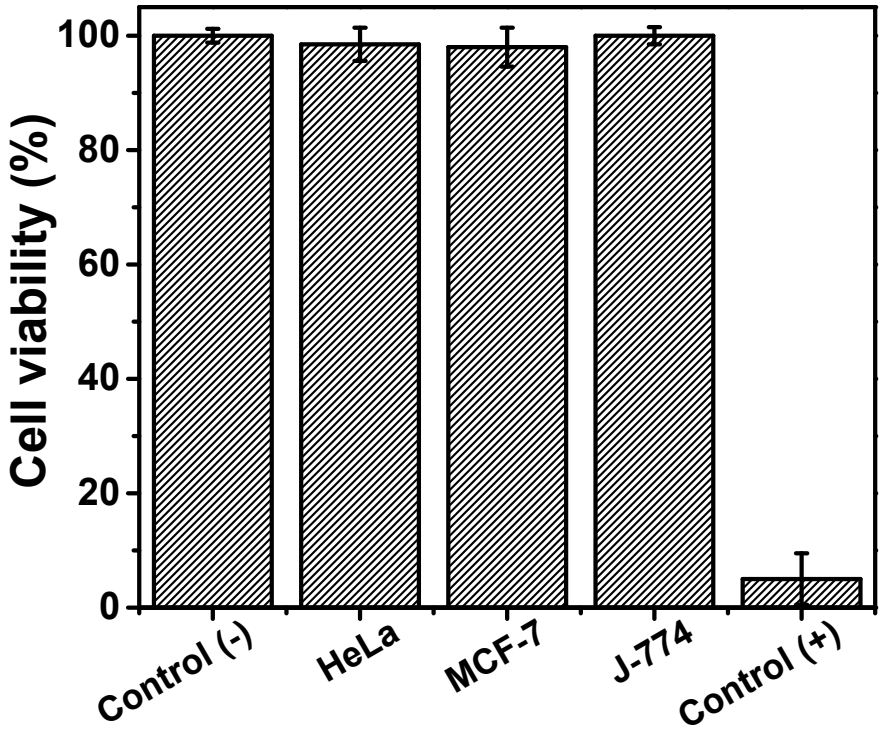

Figure S11: Cytotoxicity of ps-pH-neutral to mammalian cells. Cells (HeLa, MCF-7 and J-774) were incubated with culture medium containing 10 µg/mL pH sensors for 24h. The cell viability was assessed by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyltetrazoliumbromide) assay following the protocol from supplier (Promega Co. USA). Control (-), HeLa cells treated with medium + PBS; Control (+), HeLa cells treated with 70% ethanol.