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Supplemental information

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Supplementary material

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The pH responses of fluorescence intensity ratio I_{488}/I_{405} and fluorescence lifetime of SypHer are shown in Figure S1.

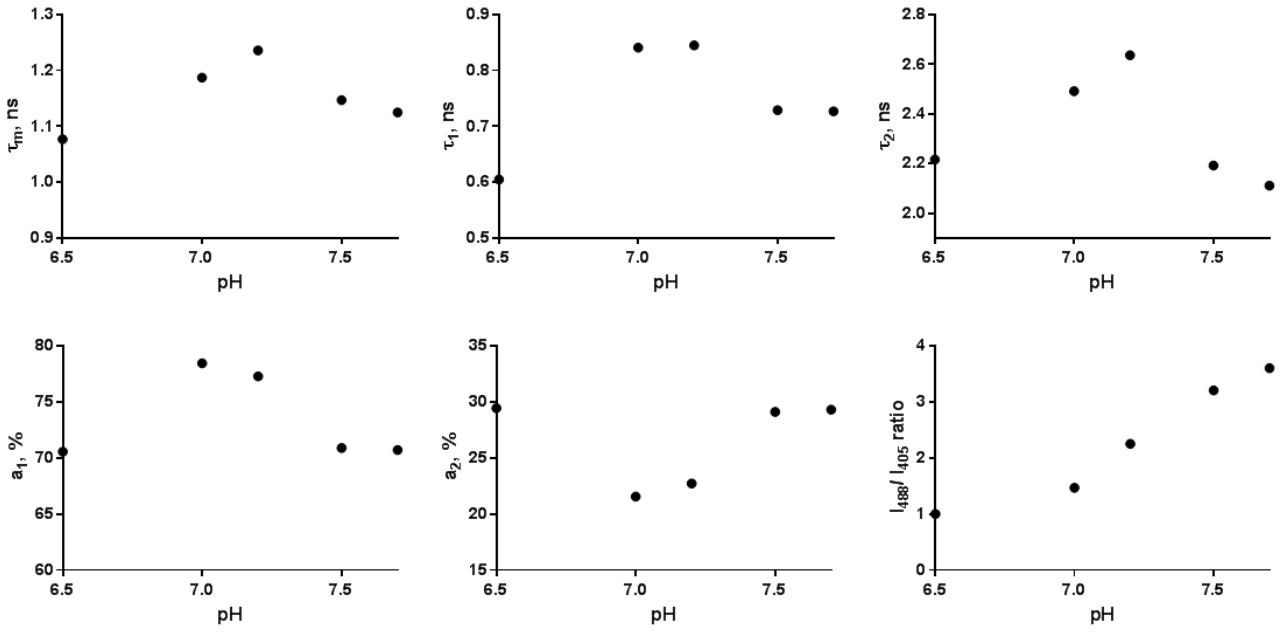

Figure S1. The fluorescence decay parameters τ_m, τ_1, τ_2, a_1, a_2 and fluorescence intensity ratio of SypHer plotted against pH. N = 20-30 cells for each pH value.

The pH responses of fluorescence intensity ratio I_{488}/I_{405} and fluorescence lifetime of SypHer-2 are shown in Figure S2.

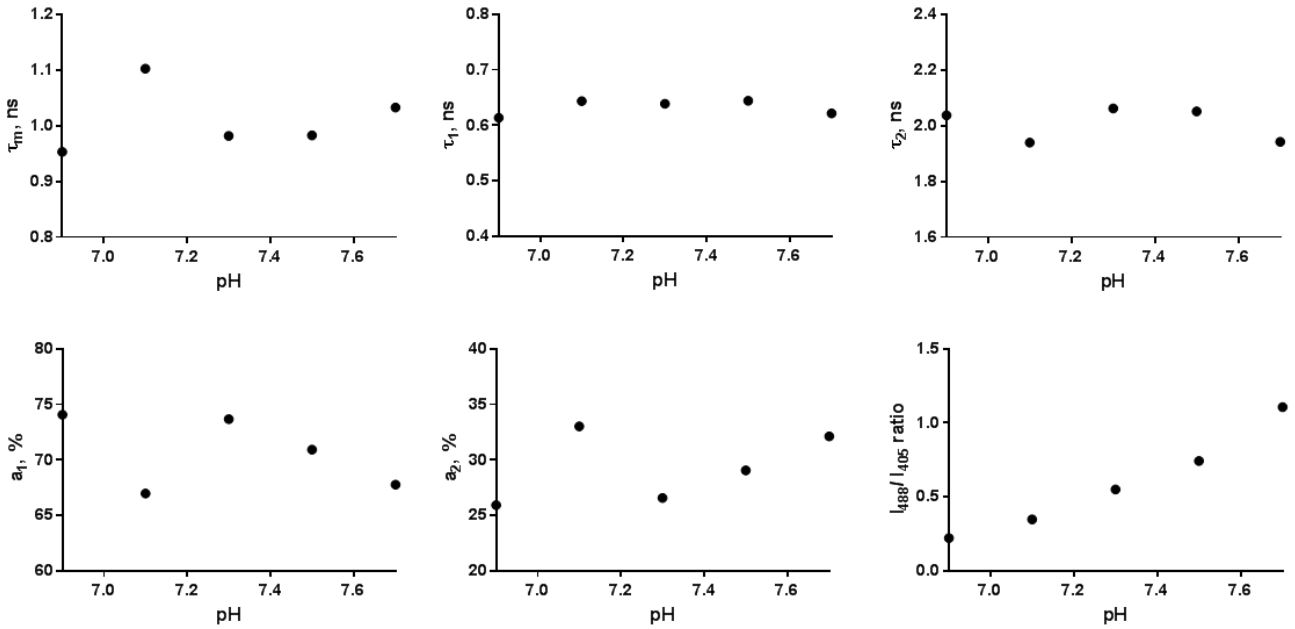

Figure S2. The fluorescence decay parameters τ_m, τ_1, τ_2, a_1, a_2 and fluorescence intensity ratio of SypHer-2 plotted against pH. N = 20-30 cells for each pH value.

In the case of SypHerRed, the short component of fluorescence lifetime τ_1 also demonstrated strong (non-linear) dependence on pH in the physiological range (Figure S3).

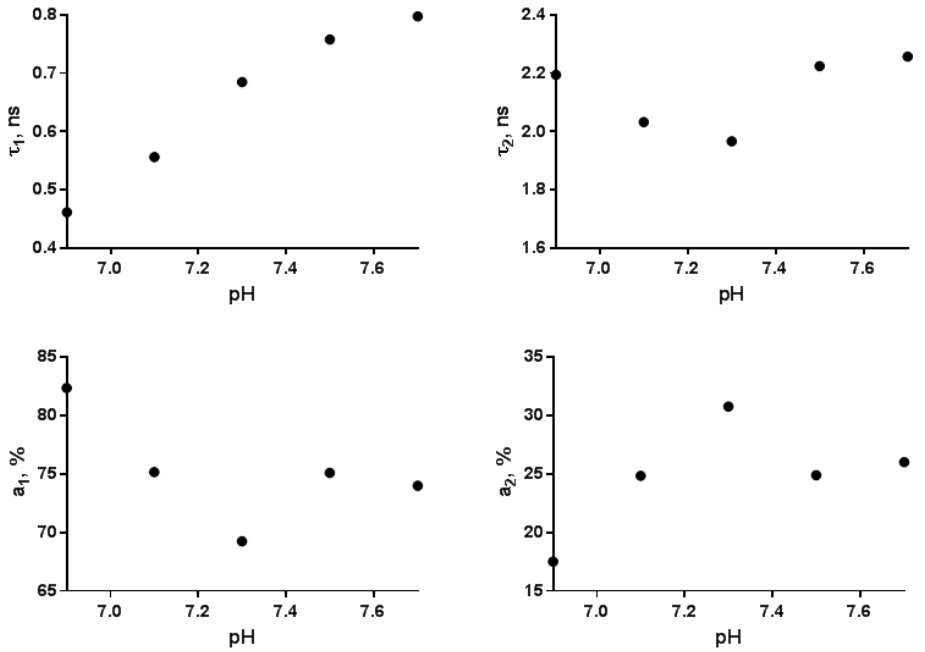

Figure S3. The fluorescence decay parameters τ_1, τ_2, a_1, and a_2 of SypHerRed plotted against pH. N = 20-30 cells for each pH value.

In SypHerRed-expressing cells, high inter-cellular variability of fluorescence intensity was observed at different pH due to different expression level of the protein (Figure S4).

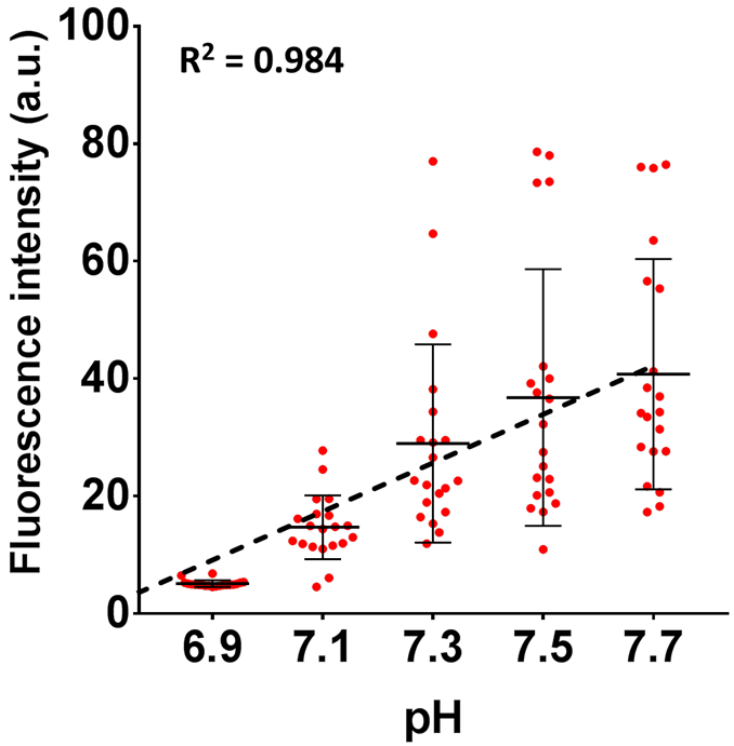

Figure S4. The fluorescence intensity of SypHerRed plotted against pH. Red dots are the experimental measurements for individual cells; dashed line is the approximation curve. N = 20-30 cells for each pH value. Mean ± SD.

Figure S5 shows calibration curve for intracellular pH assessment in the whole cells imaged using FLIM-microscopy.

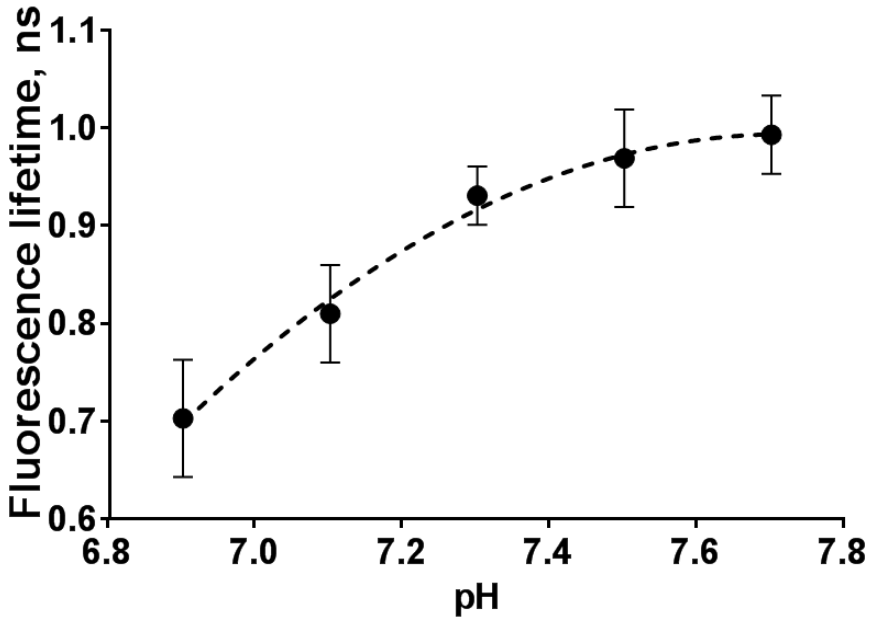

Figure S5. The fluorescence lifetime τ_m plotted against pH for the whole cells. Mean ± SD. N=20-30 cells for each pH value. Dashed line is the approximation curve.

Representative FLIM images (τ_m and χ^2) of HeLa cells expressing SypHerRed and fluorescence decay curve of SypHerRed in the specific spot of the cell are shown in Figure S6.

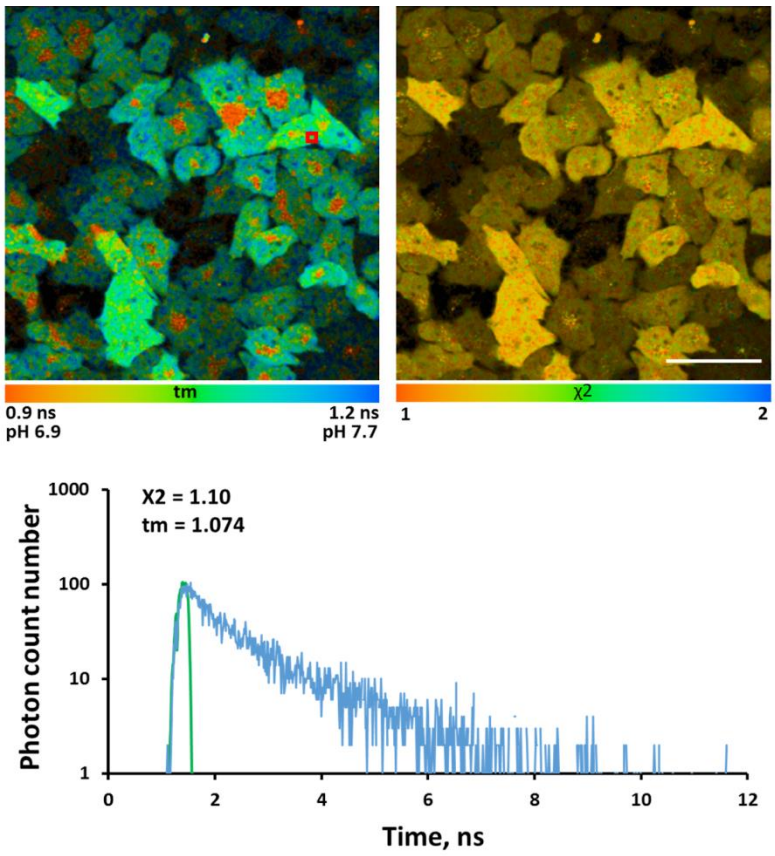

Figure S6. FLIM, τ_m and χ^2 images and fluorescence decay curve of SypHerRed at pH 7.3 . Bar: 40 μm. Nonlinear least squares algorithm and instrument response function IRF are used to obtain fluorescence lifetimes from the decay curves. IRF is green, decay curve is blue.

The study of tumor xenografts generated from SypHerRed-expressing HeLa cells on the macro-level, using IVIS-Spectrum system, showed a high degree of heterogeneity of fluorescence intensity in the tumor (Figure S7).

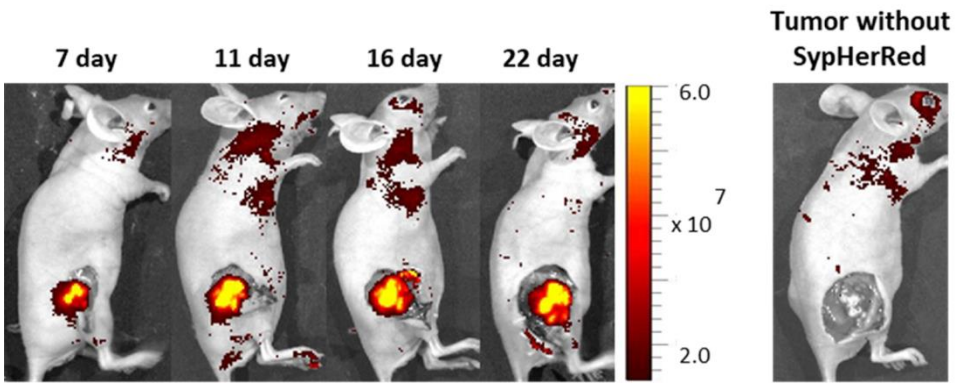

Figure S7. Fluorescence intensity of SypHerRed in mouse tumor *in vivo* during tumor growth (*left row*) and control image of the tumor without SypHerRed (*right*). Excitation at 540 nm, emission at 600 nm.