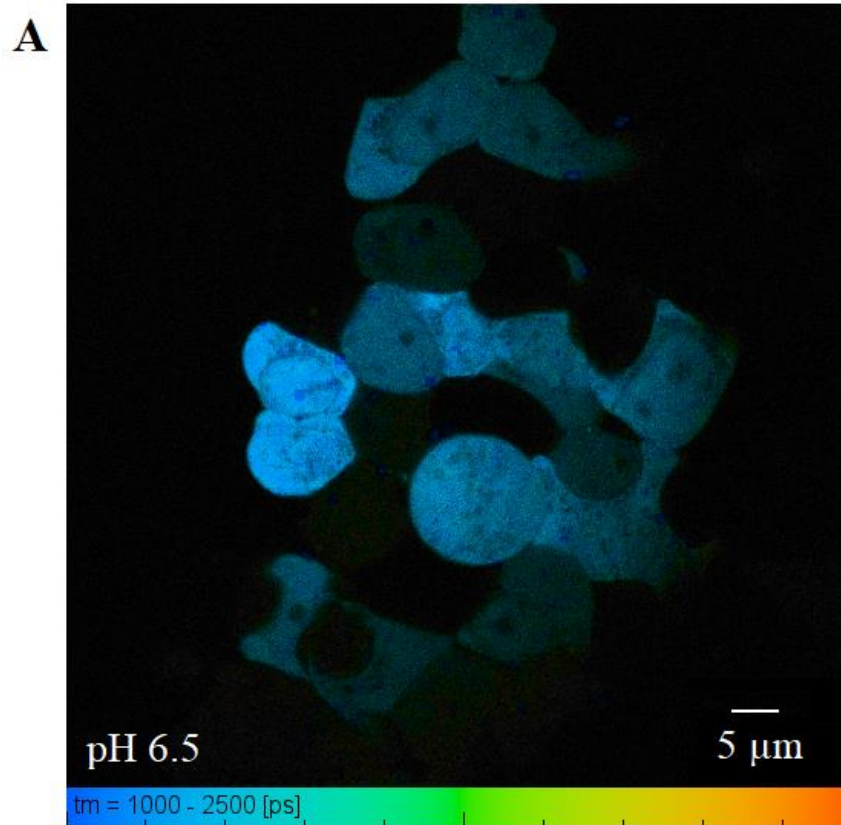
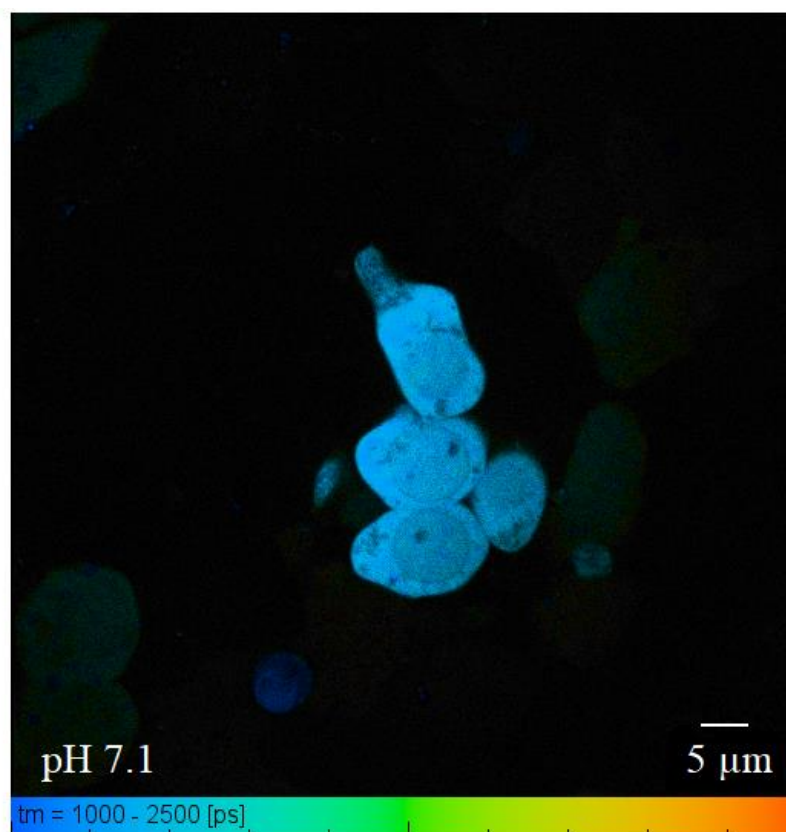


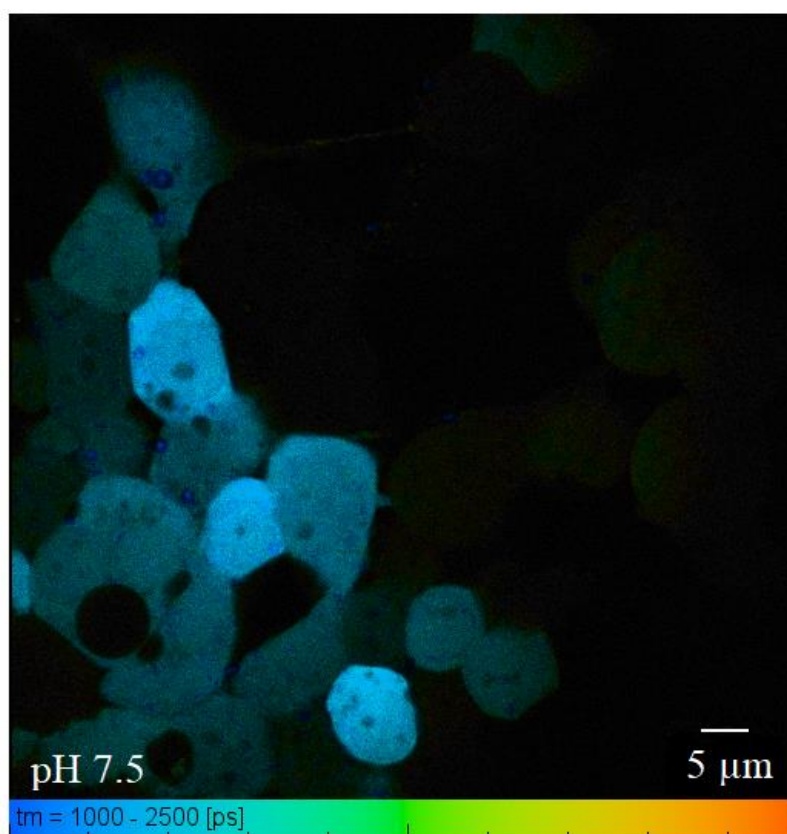
Figure S1. Fluorescent images of the fixed HeLa cells expressing SypHerExtra sensor.



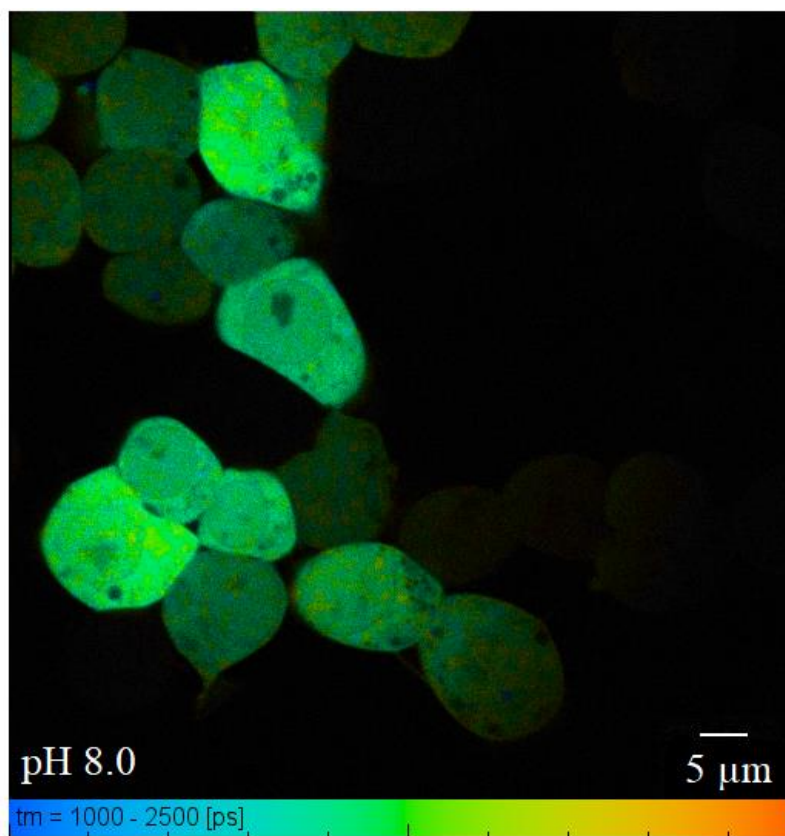
B



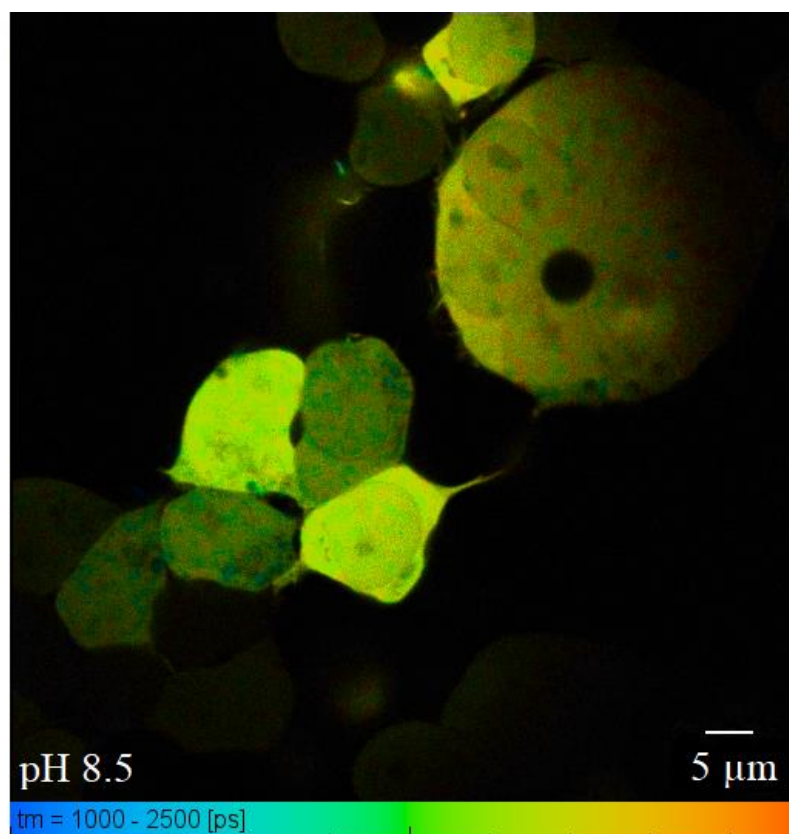
C



D



E



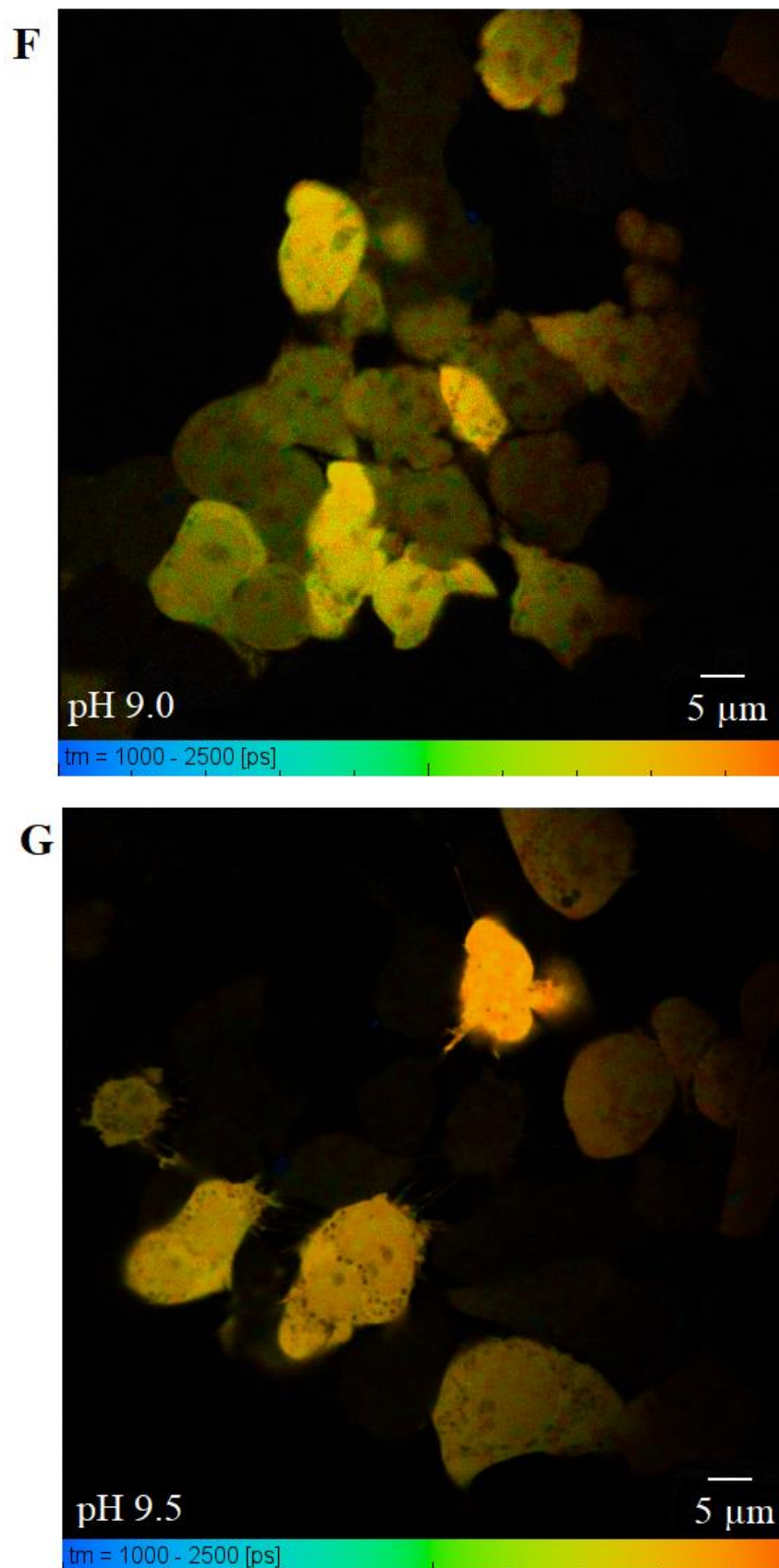
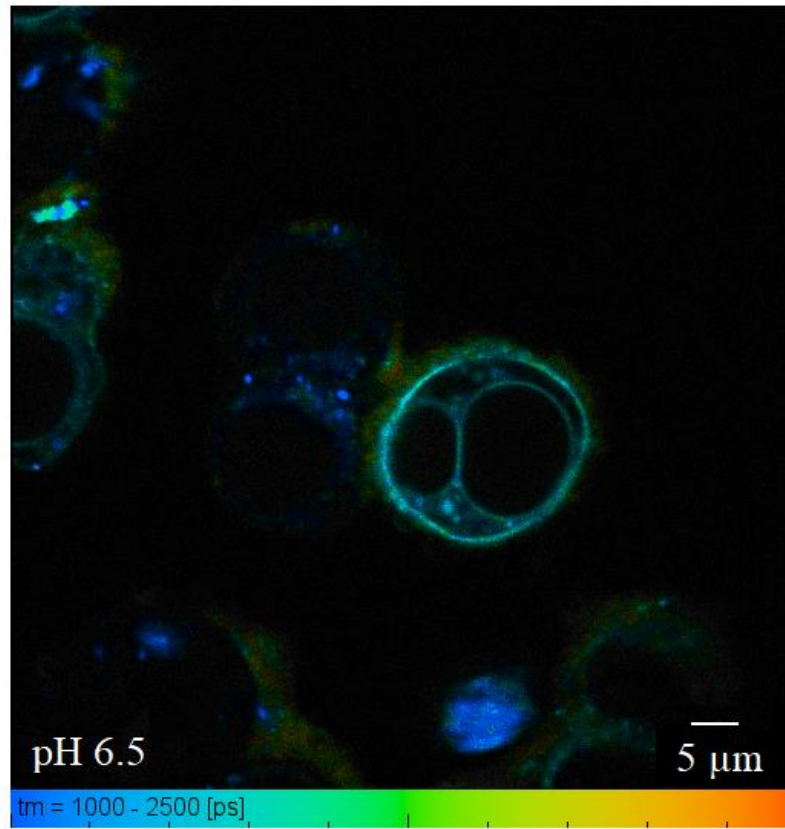
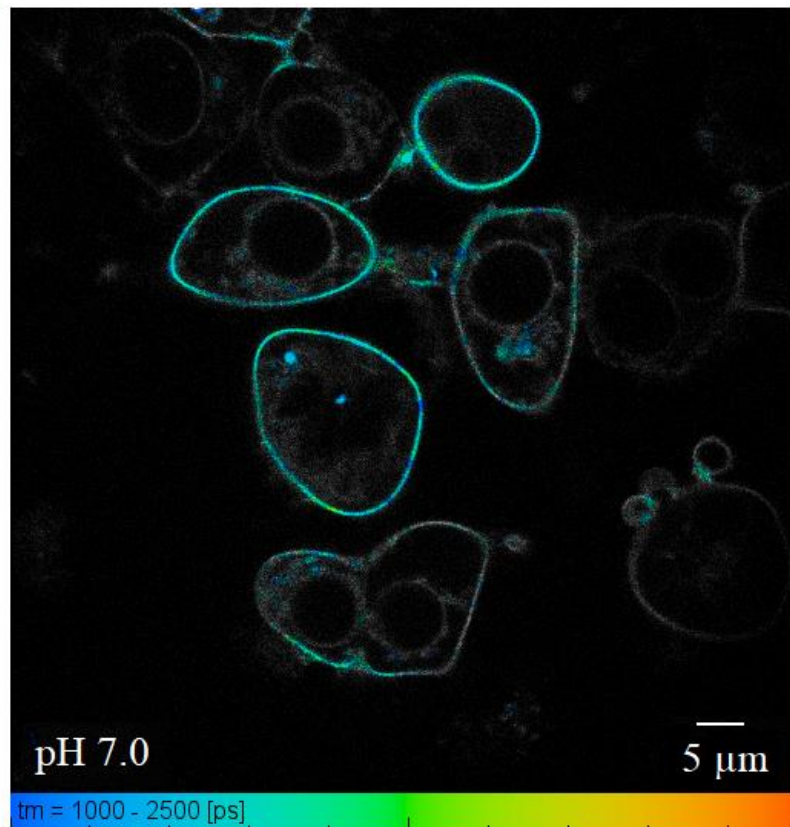
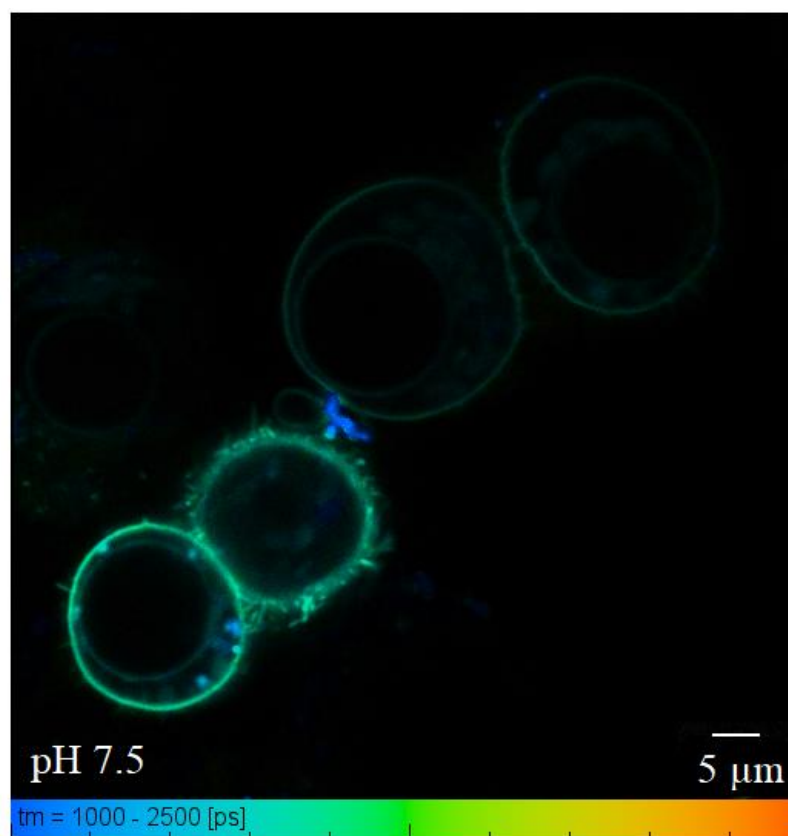


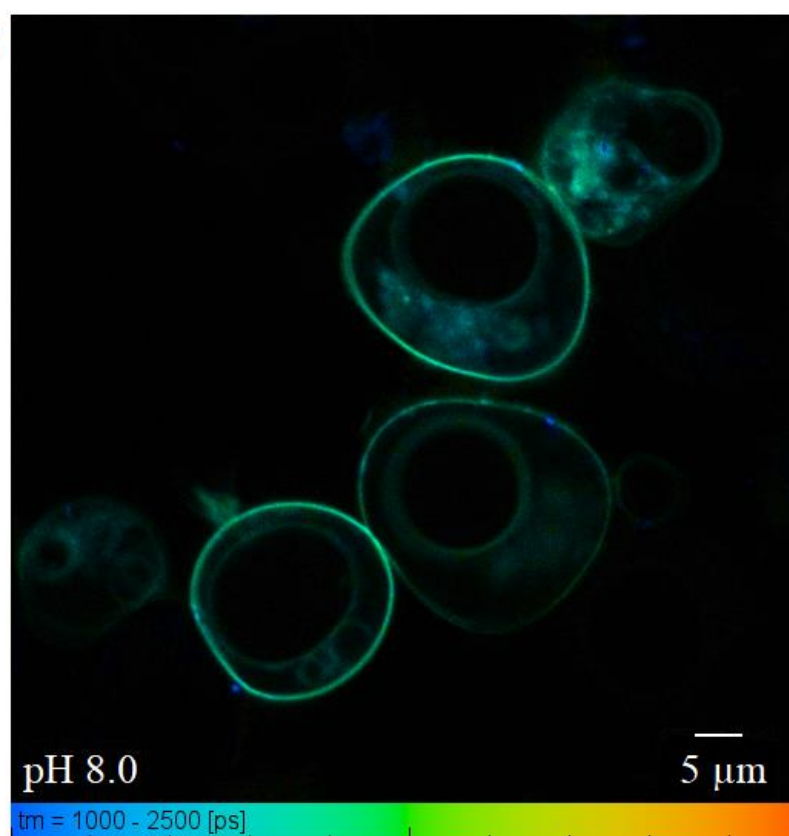
Figure S2. FLIM images of the nigericin-treated HEK293 cells expressing cytoplasmic SypHer3s sensor at different pH values: **(A)** pH 6.5; **(B)** pH 7.1; **(C)** pH 7.5; **(D)** pH 8.0; **(E)** pH 8.5; **(F)** pH 9.0; **(G)** pH 9.5;

A**B**

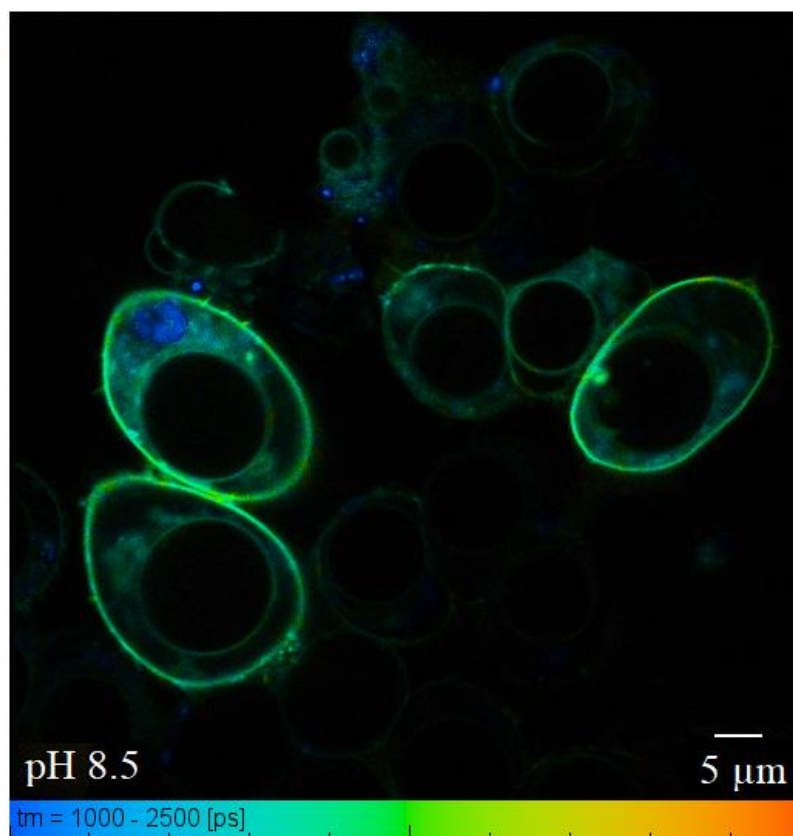
C



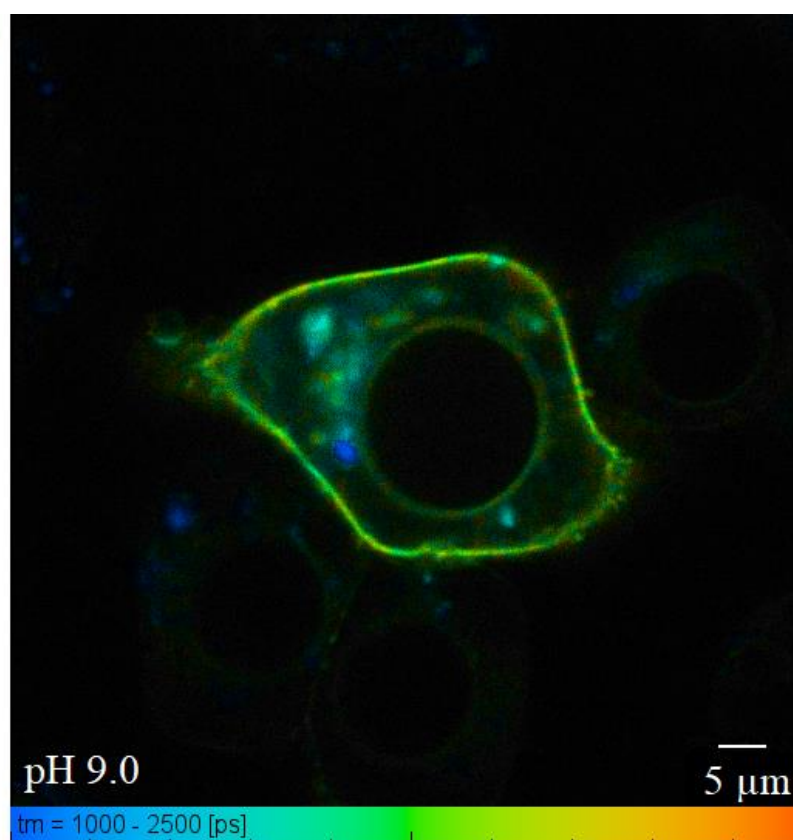
D



E



F



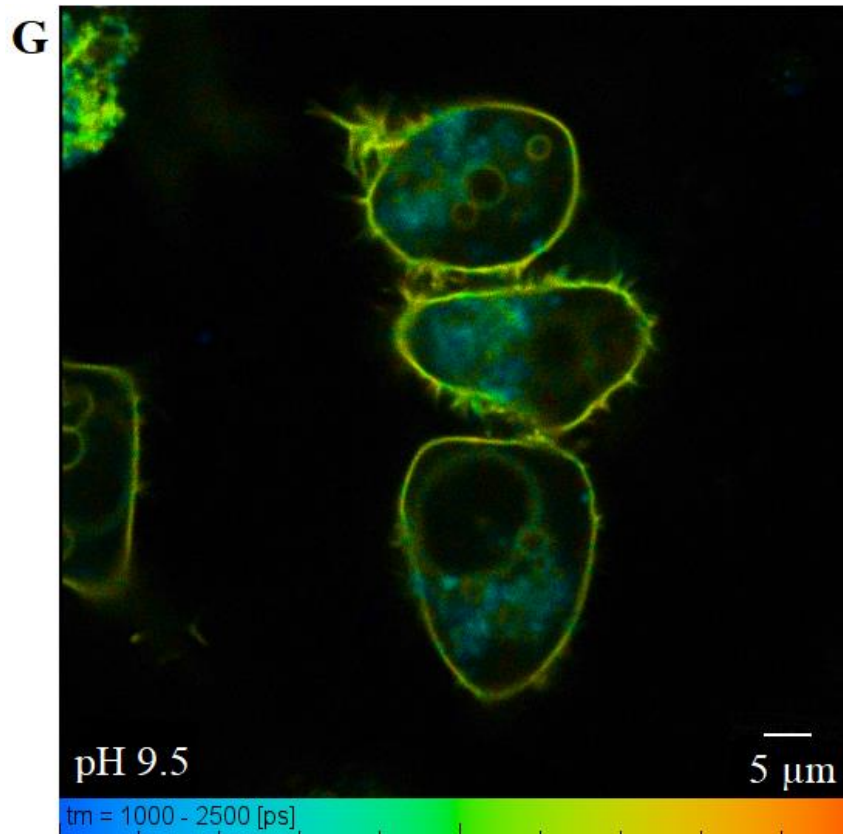


Figure S3. FLIM images of the HEK293 cells expressing SypHerExtra sensor at different pH values: **(A)** pH 6.5; **(B)** pH 7.0; **(C)** pH 7.5; **(D)** pH 8.0; **(E)** pH 8.5; **(F)** pH 9.0; **(G)** pH 9.5;

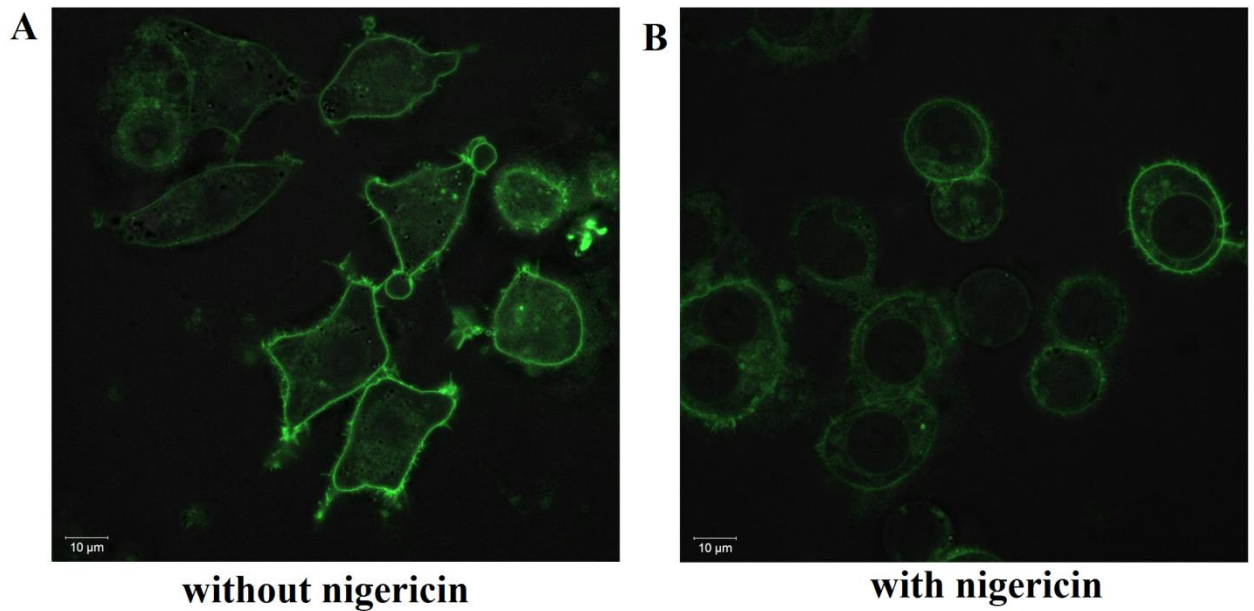


Figure S4. Fluorescent images of the HEK293 cells expressing SypHerExtra sensor at pH 8.5 **(A)** without nigericin and **(B)** after incubation with nigericin.