

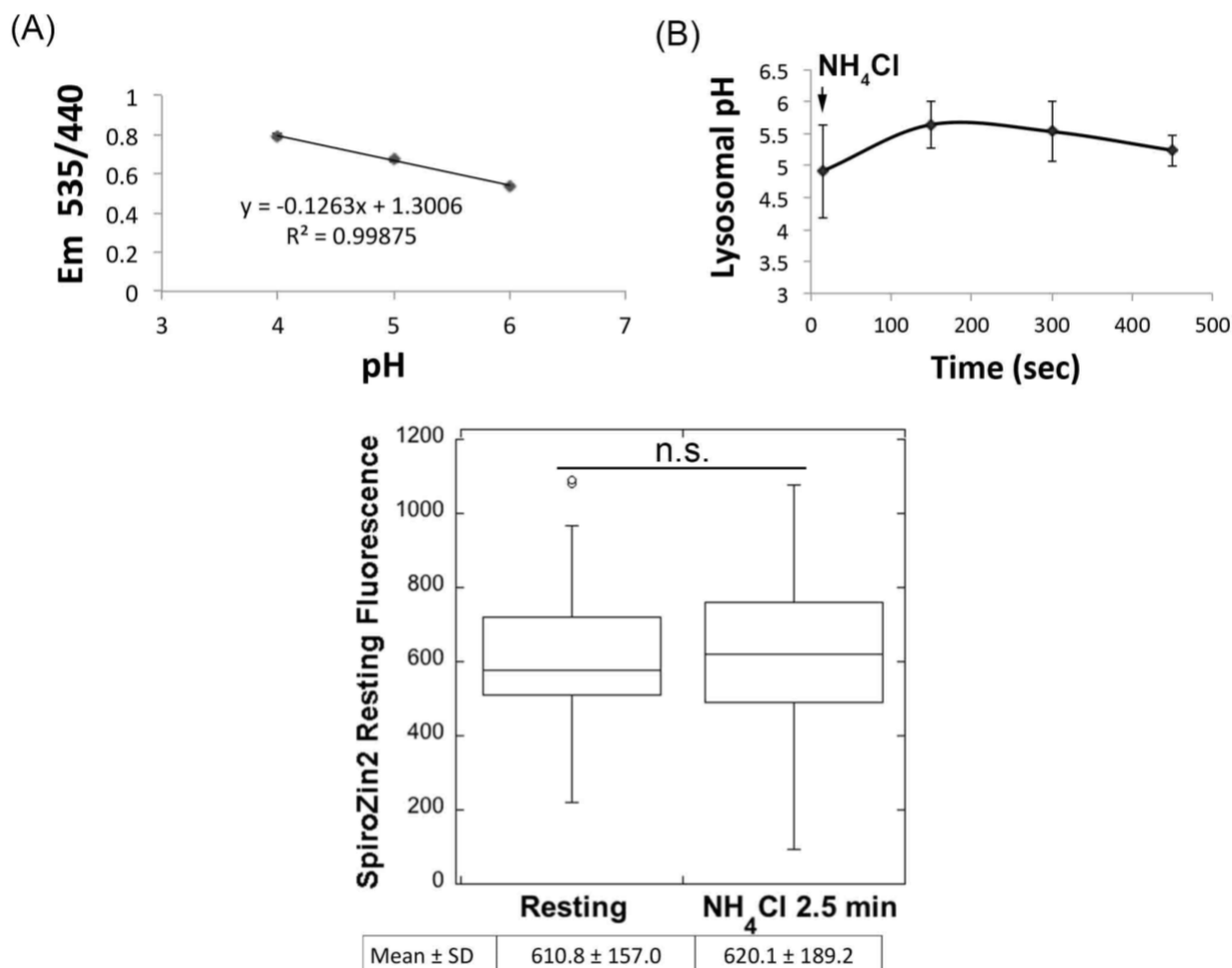
**Supplementary information for:**

**Superiority of SpiroZin2 Versus FluoZin-3 for monitoring vesicular Zn<sup>2+</sup> allows tracking of lysosomal Zn<sup>2+</sup> pools**

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Supplementary information contains 1 supplementary figure.



**Supplementary Figure 1.** SpiroZin2 is insensitive to rapid pH increase in HC11 cells. Lysosomal pH was measured as previously described<sup>1</sup>. Briefly, HC11 cells were grown in a 96-well plate in growth media supplemented with 0.5 mg / mL of LysoSensor™ Yellow / Blue Dextran (ThermoFisher Scientific, L22460) overnight. The standard curve (A) was generated by incubating cells in 10  $\mu\text{M}$  monensin and 10  $\mu\text{M}$  nigericin in MES buffer (5 mM NaCl, 115 mM KCl, 1.3 mM  $\text{MgSO}_4$ , 25 mM MES), with the pH adjusted to within the range of 4.0–7.0 for 10 min. After the incubation, fluorescence was quantified with a fluorescence microplate reader (BioTek Synergy H1 hybrid) at an emission wavelength of 535 and 440 nm with excitation at 340 nm. The ratio of emission 535nm / 440 nm was plotted against the pH value of MES buffer. To test the in situ pH sensitivity of SpiroZin2, first, the effect of  $\text{NH}_4\text{Cl}$  treatment on lysosomal pH was investigated. Briefly, HC11 cells were loaded with 0.5 mg / mL of the pH sensor overnight. Cells were washed with phosphate-free HHBSS buffer and then 10 mM of  $\text{NH}_4\text{Cl}$  was added to cell media. The ratio of emission 535 / 440 was recorded at 15 sec, 2.5 min, 5 min and 7.5 min post  $\text{NH}_4\text{Cl}$  treatment and then was converted to lysosomal pH using the standard curve (B). Figure 1 (B) showed that lysosomal pH peaked at 2.5 min post treatment (pH = 5.64). To test the pH sensitivity of SpiroZin2, HC11 cells were stained with SpiroZin2 and then treated with 10

mM of NH<sub>4</sub>Cl. Resting fluorescence of SpiroZin2 was recorded prior to (resting) and 2.5 min post treatment (NH<sub>4</sub>Cl 2.5 min), as shown in Figure 1 (C). Statistical analysis showed that the pH increase had no effect on SpiroZin2 signal (n.s., not significant, paired student's t-test, N = 175 cells).

## Reference

1. Yanagawa, M. *et al.* Cathepsin E Deficiency Induces a Novel Form of Lysosomal Storage Disorder Showing the Accumulation of Lysosomal Membrane Sialoglycoproteins and the Elevation of Lysosomal pH in Macrophages. *J. Biol. Chem.* **282**, 1851–1862 (2007).