Proton-dependent zinc release from intracellular ligands

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Supporting Information

Supplementary Figures S1 – S4

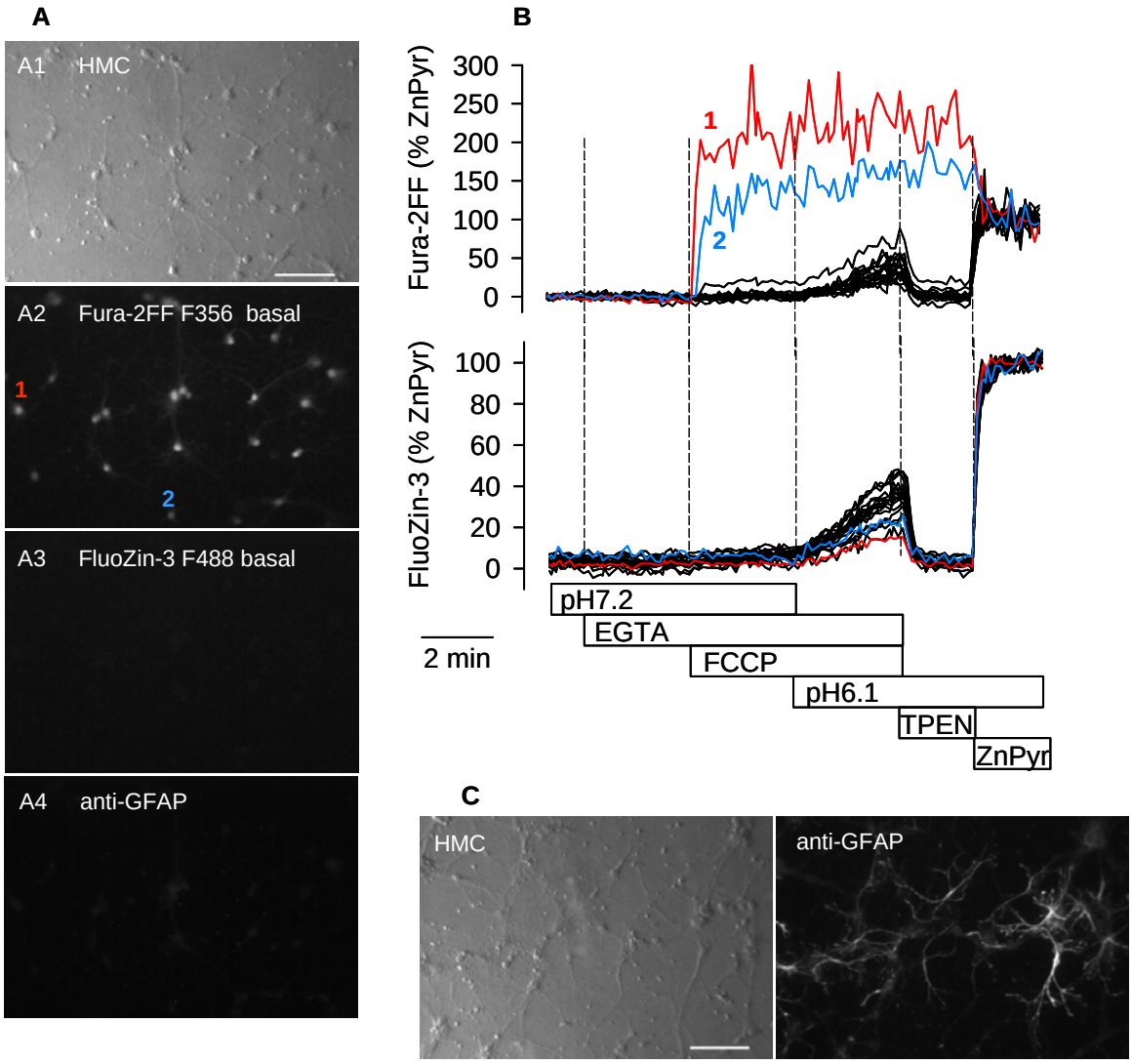

Fig. S1 Simultaneous monitoring of Fura-2FF and FluoZin-3 fluorescence in hippocampal cultures. To improve culture viability, glial proliferation was not prevented and, therefore, astrocytes were sometimes encountered in the field in which the Fura-2FF and FluoZin-3 fluorescence was monitored. The astrocytes were identified with anti-GFAP antibody after the experiments. **A,** Images from an experiment in which astrocytes were not detected: A1, Hoffman modulation contrast (HMC). A2, Basal Fura-2FF fluorescence excited at 356 nm (isosbestic point). A3, Basal FluoZin-3 fluorescence excited at 488 nm; no fluorescence is visible because the cells were loaded with very low amounts of FluoZin-3 such that the maximal fluorescence (when the probe was saturated with Zn^{2+}) did not exceed the dynamic range of the camera. A4, anti-GFAP fluorescence in the same field; the lack of fluorescence indicates that astrocytes were not present in this field. **B,** The Fura-2FF and FluoZin-3 data from the cells shown in A. Indicated in the legend are: extracellular pH (7.2 or 6.1), superfusion with Ca^{2+}- free and Zn^{2+}-free medium (EGTA), and applications of 3 µM FCCP and 10 µM TPEN. At the end of the experiment, 100 µM $ZnCl_2$ plus 20 µM pyrithione (ZnPyr) was applied to saturate Fura-2FF and FluoZin-3 with Zn^{2+}. Marked 1 (red) and 2 (blue) are two cells showing an FCCP-induced irreversible destabilization of Ca^{2+} homeostasis. Data from such cells were routinely excluded when typical data were averaged. **C,** Images from a culture in which astrocytes were detected. The cultures shown in A and C are sister cultures at day-in-vitro 16. Bar = 100 µm.

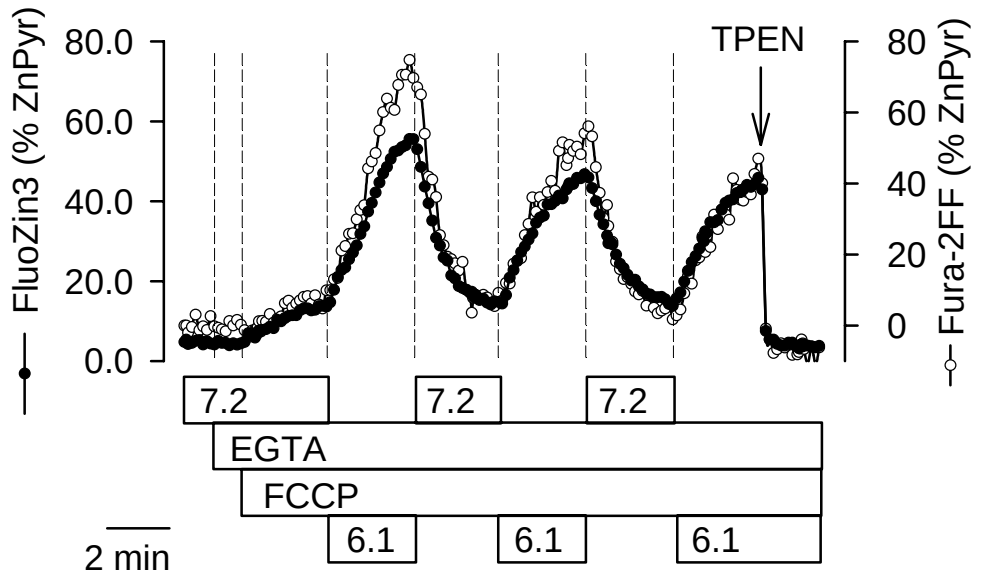

Fig. S2 Effects of acid pulses on the Fura-2FF and FluoZin-3 signals in hippocampal neurons. The acid pulses (pH 6.1) were applied using a Zn^{2+} and Ca^{2+}-free medium (100 µM EGTA) supplemented with 3 µM FCCP. Experimental details are the same as described in Fig. 1a. The data are means (24 neurons) from a single experiment that was repeated six times with similar results

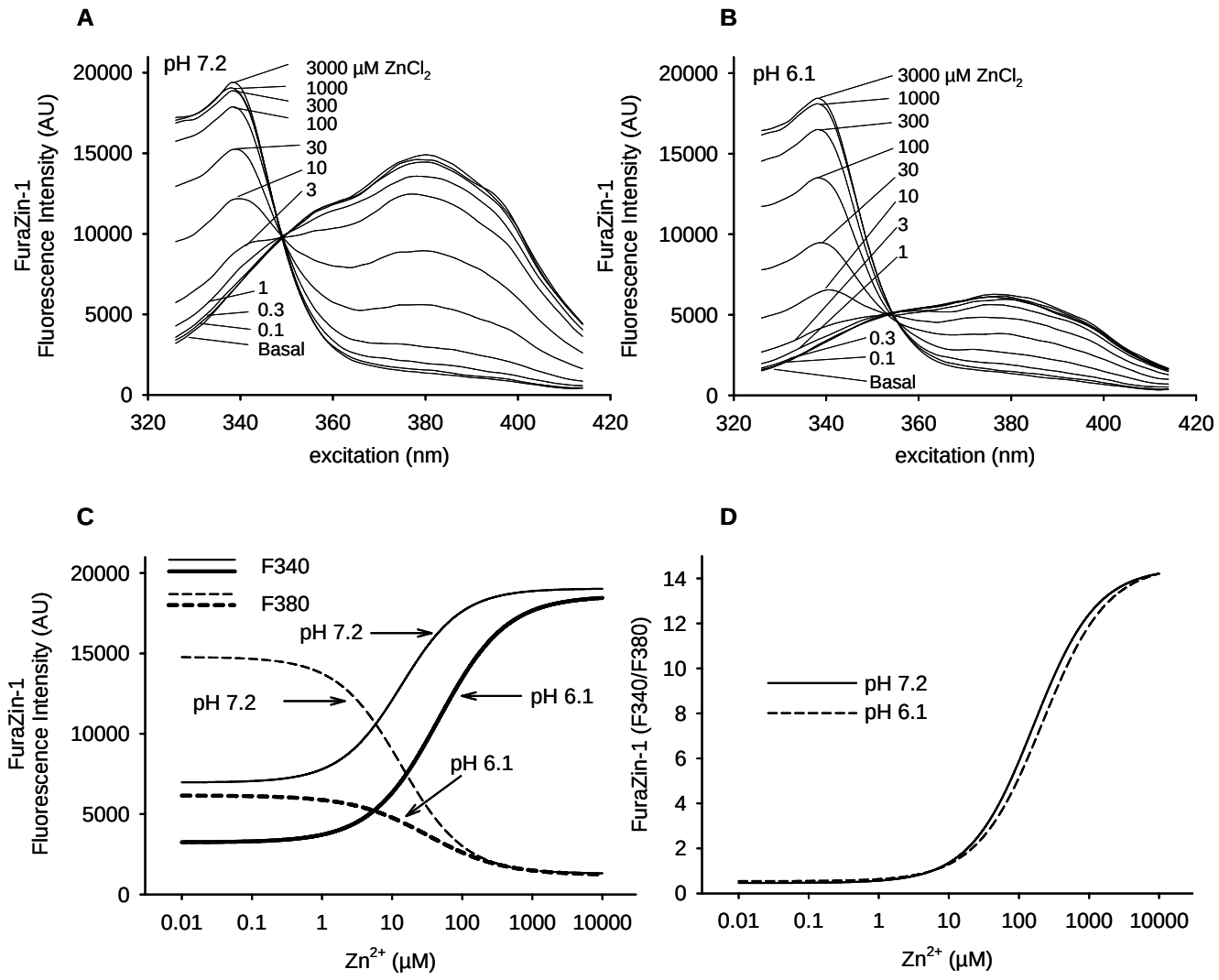

Fig. S3 Effects of [Zn^{2+}] and pH on FuraZin-1 fluorescence. **A,** Excitation spectra at pH 7.2. **B,** Excitation spectra at pH 6.1. The Basal in A and B refers to solutions not supplemented with Zn^{2+}; these solutions contained 100 nM FuraZin-1, 100 mM KCl, and 50 mM PIPES. **C,** The impact of [Zn^{2+}] and pH on the FuraZin-1 fluorescence excited at 340 nm (F340) and 380 nM (F380). **D,** FuraZin-1 F340/F380 ratios calculated from the data in C.

The FuraZin-1 Zn^{2+} K_d values determined from the F340 data at pH 7.2 and 6.1 shown in C were 13 µM and 39 µM, respectively; the dissociation constant K'_d values determined from the FuraZin-1 F340/F380 ratios at pH 7.2 and 6.1 shown in D were 174 and 216 µM, respectively. The difference between the K'_d and K_d stems from the fact that $K'_d = K_d \times S_{f2}/S_{b2}$, where S_{f2} and S_{b2} are the F380 values of Zn^{2+}-free and Zn^{2+}-saturated FuraZin-1, respectively (Grynkiewicz et al. 1985, J. Biol. Chem. 260, 3440-3450).

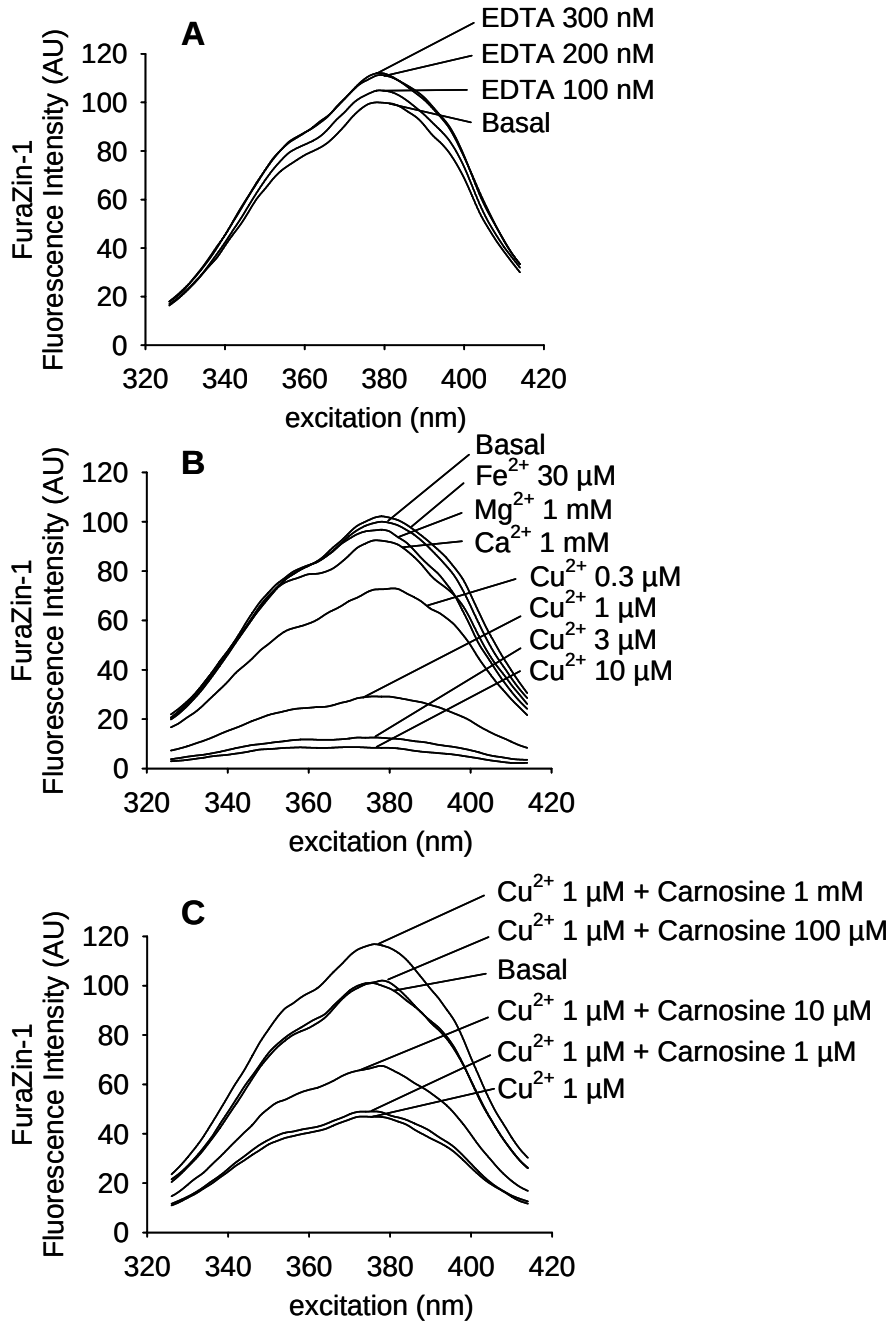

Fig. S4 Carnosine prevents FuraZin-1 fluorescence quenching by copper. **A,** EDTA chelates a contaminating ion that quenches FuraZin-1 fluorescence. Note that an addition of up to 200 nM EDTA boosts the fluorescence. **B,** Cu^{2+} (but not Ca^{2+}, Mg^{2+}, or Fe^{2+}) quenches FuraZin-1 fluorescence (in all cases, chloride salts were tested); effects of higher than 30 µM Fe^{2+} could not be tested because of precipitation. **C,** Carnosine dose-dependently removes the Cu^{2+}-induced quenching of FuraZin-1 fluorescence. In all panels, the Basal refers to a solution containing 100 nM FuraZin-1, 100 mM KCl, and 50 mM PIPES (pH 7.2) without supplements.