Supporting information

Integrated Portable Food Safety Testing Pipette Based on a Color-Switchable Fluorescence Probe for Rapid Visual Discrimination of Mild Food Deterioration

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1. Experimental Section

Materials and reagents. Fresh shrimps, chickens, and crabs were purchased from local Sam's CLUB in Nantong, Jiangsu Province, China. Cellulose acetate was purchased from Sinopharm Chemical Reagent Co., Ltd. 5-Fluorescein isothiocyanate was purchased from Dalian Meilun Biotechnology Co., Ltd. Rhodamine 101 Inner Salt was purchased from Sigma Aldrich (Shanghai) Trading Co., Ltd. Tert butyl-4-aminophenyl acetate, 1H-benzotriazol-1-acyloxy-tripyrrolidine-ammonium-hexafluorophosphate (PyBOP) were purchased from Shanghai Baide Pharmaceutical Technology Co., Ltd. N. N '- dimethylformamide (DMF), trifluoroacetic acid (TFA), polyvinyl alcohol (PVA) and dichloromethane (DCM) were purchased from Shanghai Macklin Biochemical Co., Ltd. Ammonia was purchased from Aladdin Reagent Co., Ltd. Sylgard 184 polydimethylsiloxane (PDMS) was purchased from the Dow Corning. The smart label supporting materials (Cotton pulp filter paper), switch, UV lamp beads, and button cells, etc. were purchased online. Ultraviolet-vis absorption and fluorescence spectra were obtained by Shimadzu UV-1900 ultraviolet-visible spectrophotometer (Japan) and fluorescence spectrophotometer FS5 (Edinburgh, UK), respectively. The morphology of the Rh101-M@PVA, CA-FITC, and the mixture was investigated by field emission scanning electron microscope (Gemini SEM 300). A synchronous thermal analyzer (STA 449 F5) from NETZSCH-Gerätebau GmbH was used to record the TGA curves and DSC curves of Rh101-M@PVA. The FTIR spectra were measured by NICOLET IS10 from Thermo-Fisher Scientific.

Synthesis of Cellulose acetate-FITC (CA-FITC). A mixture of fluorescein isothiocyanate (4.00 mg,0.01 mmol) and cellulose acetate (1 g, 0.0169 mol) was dissolved in DMF (20 mL) and after the addition of dibutyltin laurate (20 μL), the mixture was refluxed at 100 °C for 36 h under nitrogen protection. The mixture was then precipitated in a methanol/water (V_1:V_2=1:1) solution, filtered and the solution was freeze-dried to obtain the product (867.25 mg, 86.37% yield).

Modification of Rhodamine 101 Inner Salt. A mixture of rhodamine 101 inner salt (50 mg, 0.102 mmol) and benzotriazol-1-yl-oxotripyrrolidinyl phosphorus

hexafluorophosphate (156.12 mg, 0.3 mmol) was dissolved in DCM (10 mL), added with triethylamine (20 μL) and refluxed under nitrogen protection at 25 °C for 6 h. The intermediate product was obtained by column chromatography (silica gel, DCM: Methanol=40:1) to purify the residue. Finally, the purified intermediate was dissolved in DCM (5 mL), and TFA (600 μL) was added and the reaction was carried out under the ambient temperature for 6 h. The residual solvent was removed by rotary evaporation.

Synthesis of modified Rh101 inner salt PVA solution. PVA (1 g, 0.012 mol) was added to DI water (9 mL) and refluxed at 90 °C for 12 h to obtain a PVA gel. Modified rhodamine 101 inner salt (concentration:0.2 mmol/L) and glycerol (500 μL) were then added to the PVA gel and stirred for 20 min to obtain the final product.

Preparation of ratiometric fluorescent material membrane. CA-FITC solid (500 mg) was dissolved in DMF (10 mL) to obtain a homogeneous CA-FITC solution. PVA gels with a modified rhodamine 101 inner salt were then mixed with the CA-FITC solution in various proportions to obtain a mixed suspension. Finally, the mixed solution was added dropwise to cotton pulp filter paper to form a homogeneous film.

Preparation and detection of amine solutions. Different concentrations of histamine solutions were obtained by diluting known concentrations of histamine solutions with different amounts of deionized water. The fluorescent smart labels were then immersed in the histamine solution for 3 minutes. Immediately after removal from the solution, the fluorescence of the smart tags was tested and recorded. Additional solutions of biogenic amines and ammonia were prepared and tested using the same procedure.

Preparation of biogenic amine detection devices. The mold involved in this work was designed with Sketchup and prepared using a CR-10 max 3D printer. For chip device: The PDMS mixture (PDMS: catalyst = 10:1) was thoroughly stirred and then poured onto the mold. After degassing in the desiccator, the PDMS was cured at 72 °C for 2 h. After treatment with plasma, the PDMS was preset with the fluorescence label and finally bonded to a glass slide (plasma treatment). The filter cotton was then set into the chip inlet. For portable device: PDMS layer was prepared as described above and

finally sealed the porous membrane material with a fresh PDMS mixture. For integrated pipette device: The switch, UV lamp (365 nm, 3 W), and coin cell battery (3.7 V) were purchased online. The cavity of the device was prepared by a 3D printer. One side of the cavity was covered with a rubber layer (as an air chamber to drive the gas phase into the device), while the other side was connected to an adapter that can hold the detection tip. Finally, the ratiometric fluorescence sensing film was placed in the tip to set as a detection window. The structural details of the devices are shown in Scheme 1 (a).

Comparison between the results obtained from the smart labels and those obtained using Nessler's reagent. The ammonia nitrogen content in shrimp meat was measured by Nessler's reagent. Technically, the ammonia nitrogen content was determined from the turbidity of the yellow-brown complex, that is, the higher absorbance value of the turbid phase signifies the higher ammonia nitrogen content in the food. The Nessler's reagent test revealed a slight change in color of the solution containing shrimp grinds at 25°C, indicating initial stages of decay on the first day. While the shrimp stored at 4 °C still relatively fresh observed by clear solution (Figure S12a-b). The amount of ammonia nitrogen generated from 5 g shrimp under room temperature increased obviously with the increasing storage days, from 3 μg to 40 μg within three days. The level of ammonia nitrogen, in contrast, increased from 3 μg to approximately 15 μg after a storage period of 5 days at a temperature of 4 °C. Meanwhile, no obvious changes were found at -20 °C (Figure S12 c-d). The results of the Nessler reagent test were consistent with the results obtained using smart labels. It confirmed that the smart labels designed here can be potentially used for fresh shrimp packaging to monitor freshness in a rapid, accurate, and visual way. To this end, the feasibility of integrating the labels into various package matrix and portable devices for biogenic amine detection were finally investigated. The structural details of the three devices have been shown in **Figure** S13-15. For the detection of liquid biogenic amines, a press-down chip was designed as shown in **Figure** S15a. The device allows for the suction of the detection target (in either liquid or gas phase) through negative pressure applied to the press-down area, resulting in a distinct fluorescence

signal. The intelligent labels could also function as a ventilation device and be integrated into the food packaging matrix to monitor the freshness of the food (**Figure** S15b-c). However, additional excitation light was required for the devices' testing.

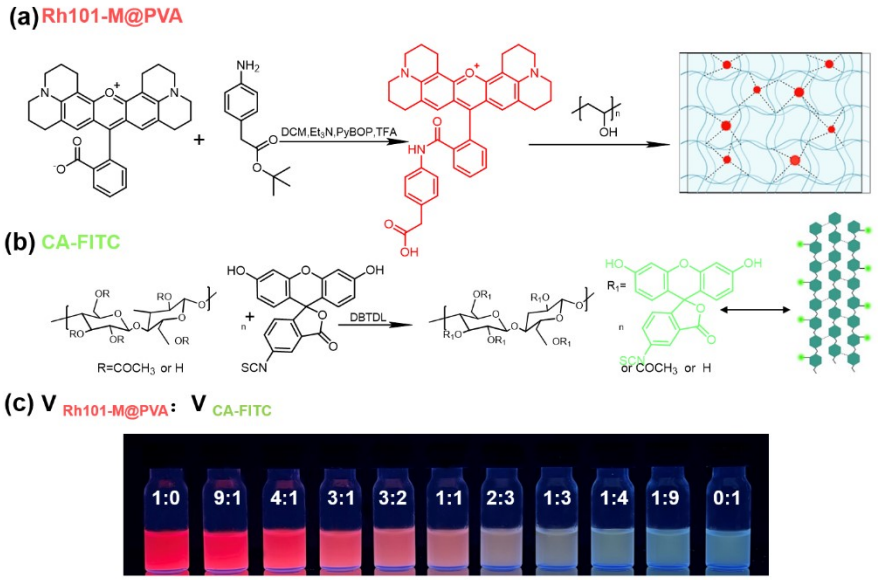

Figure S1. Synthetic routes of (a) Rh101-M@PVA and (b) CA-FITC. (c) Fluorescence image of Rh101-M@PVA and CA-FITC mixtures in different ratios (v/v=1:0, 9:1, 4:1, 3:1, 3:2, 1:1, 2:3, 1:3, 1:4, 1:9, 0:1, separately) under 365 nm excitation, respectively.

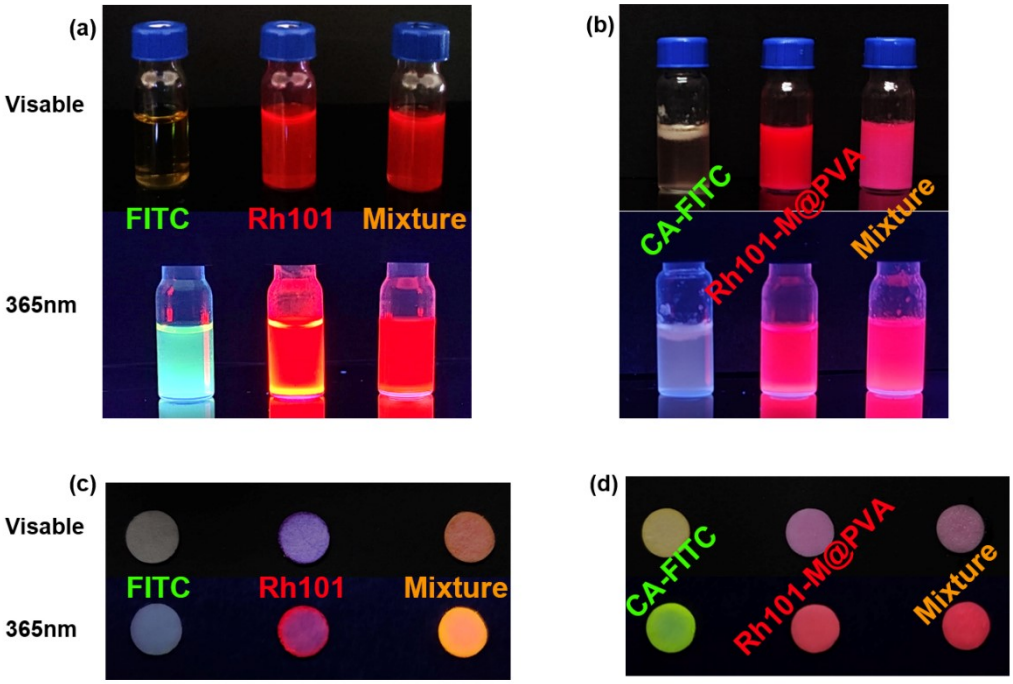

Figure S2. (a) Photographs of FITC solution, Rh101 solution, and their mixed solutions under visible light and 365 nm UV light. (b) Photographs of CA-FITC, Rh101-M@PVA solution, and their mixed solutions under visible light and 365 nm UV light. (c) Photographs of FITC solution, Rh101 solution, and their mixed solutions dropwise on a special filter membrane under visible light and 365 nm UV light. (d) Photographs of CA-FITC, Rh101-M@PVA solution, and their mixture dropwise on a special filter membrane under visible light and 365 nm UV light.

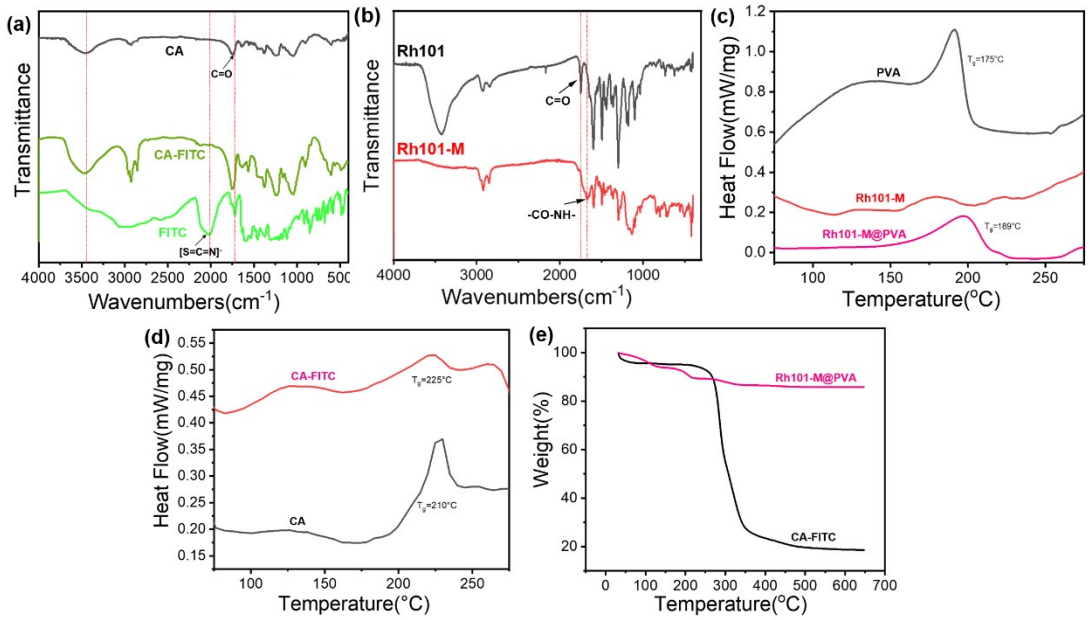

Figure S3. Characterization of CA-FITC and Rh 101-M@PVA. (a) FTIR spectra of CA, FITC, and CA-FITC. (b) FTIR spectra of Rh101 and Rh101-M. (c) DSC curves of PVA, Rh101-M, and Rh 101-M@PVA. The materials containing PVA have exothermic thermal decomposition in the temperature range of 170 °C to 190 °C. (d) DSC curves of CA and CA-FITC with exothermic charring above 200 °C for the material containing CA. (e) TGA curves of CA-FITC and Rh 101-M@PVA. The TGA curves indicate that both materials are thermally stable below 300 °C.

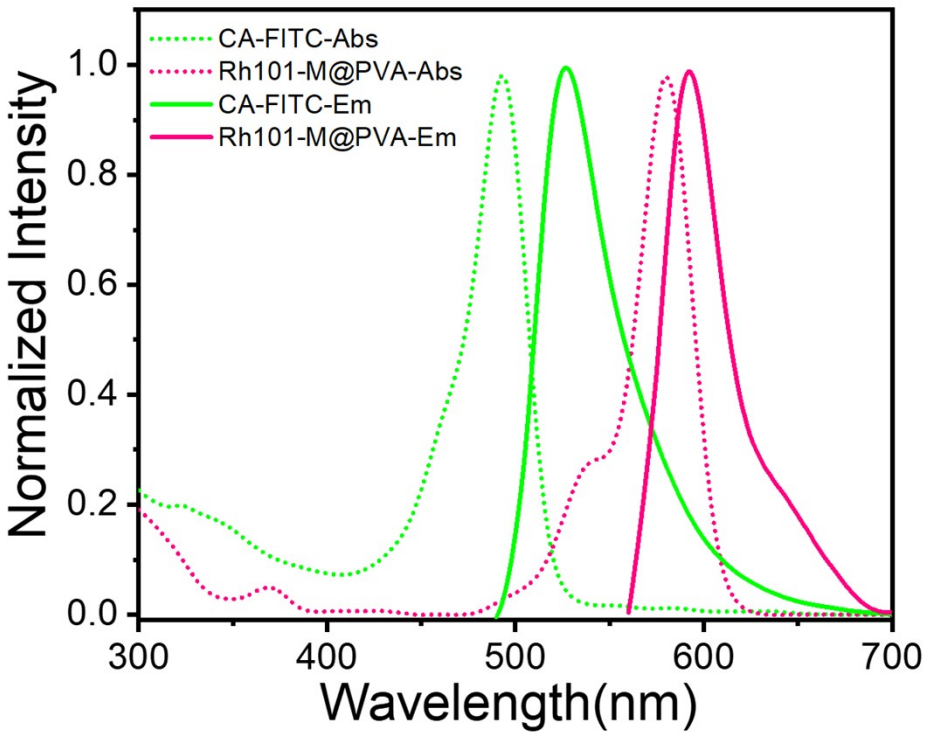

Figure S4. Normalized excitation and emission spectra of CA-FITC and Rh101-M@PVA.

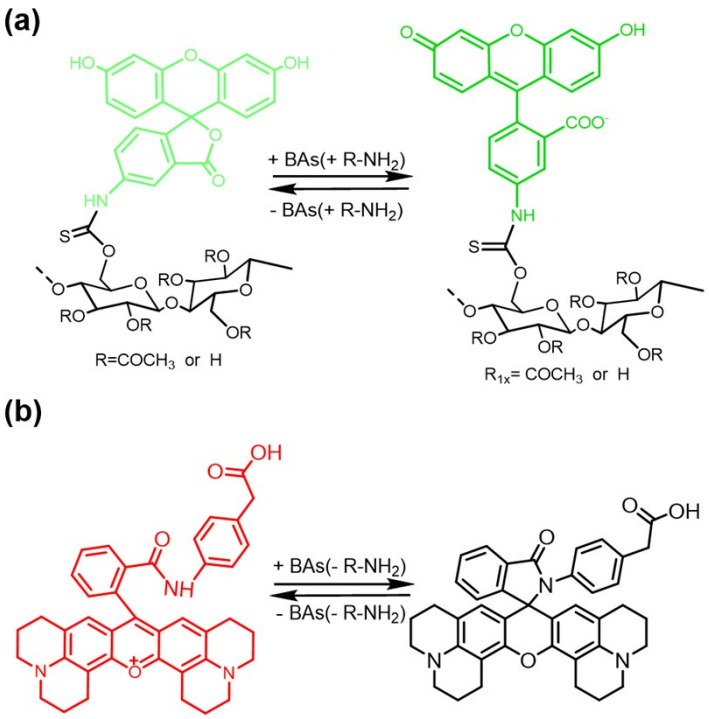

Figure S5. Mechanism of the amine reaction with CA-FITC and Rh101-M. (a) Schematic diagram of the molecular structure change of CA-FITC reacting with ammonia. (b) Schematic diagram of the molecular structure change of Rh101-M reaction with ammonia.

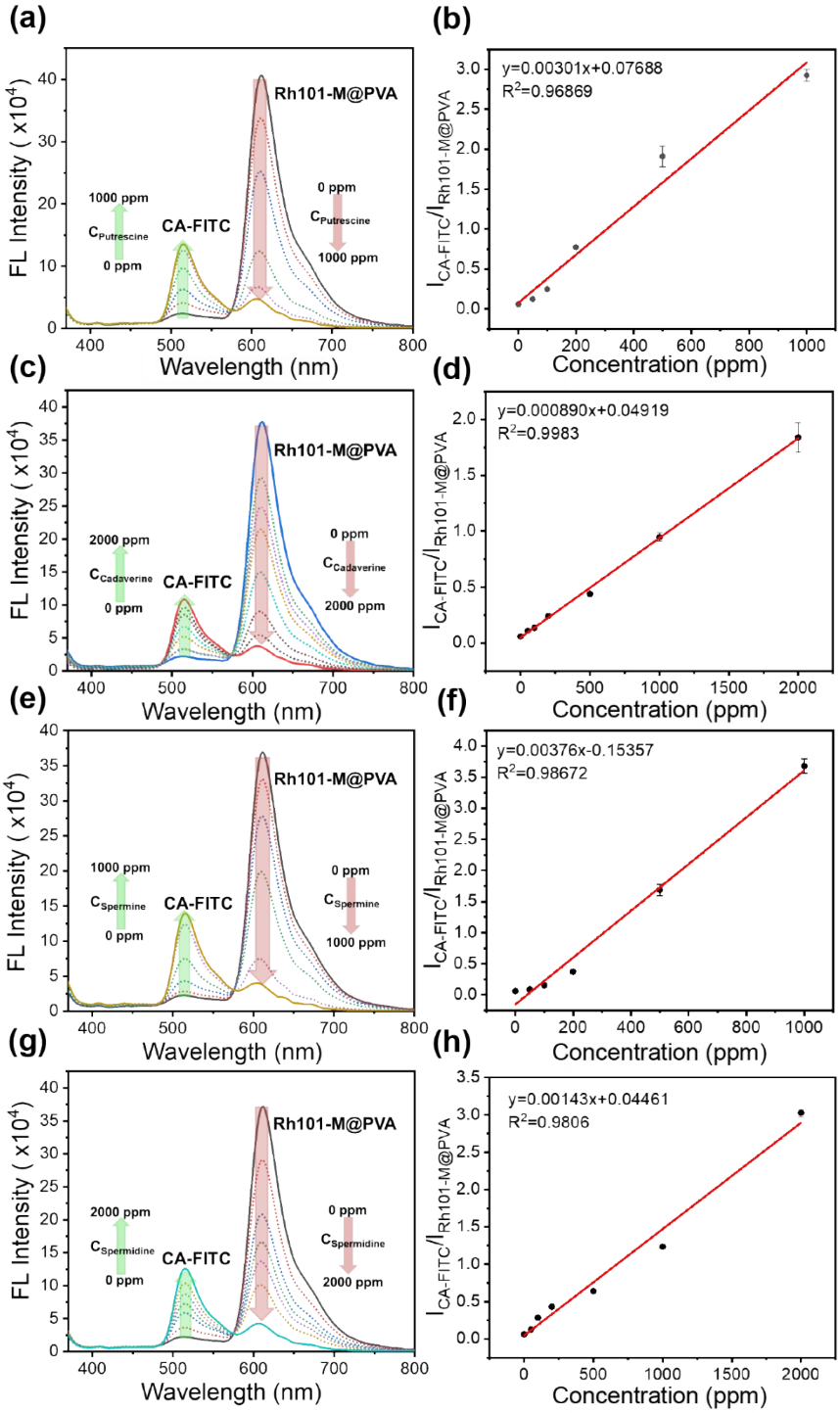

Figure S6. Fluorescence spectra of specific ratiometric fluorescent materials and the plot of the $I_{CA\text{-}FITC}/I_{Rh101\text{-}M@PVA}$ versus concentration in response to (a-b) putrescine, (c-d) cadaverine, (e-f) spermine and (g-h) spermidine.

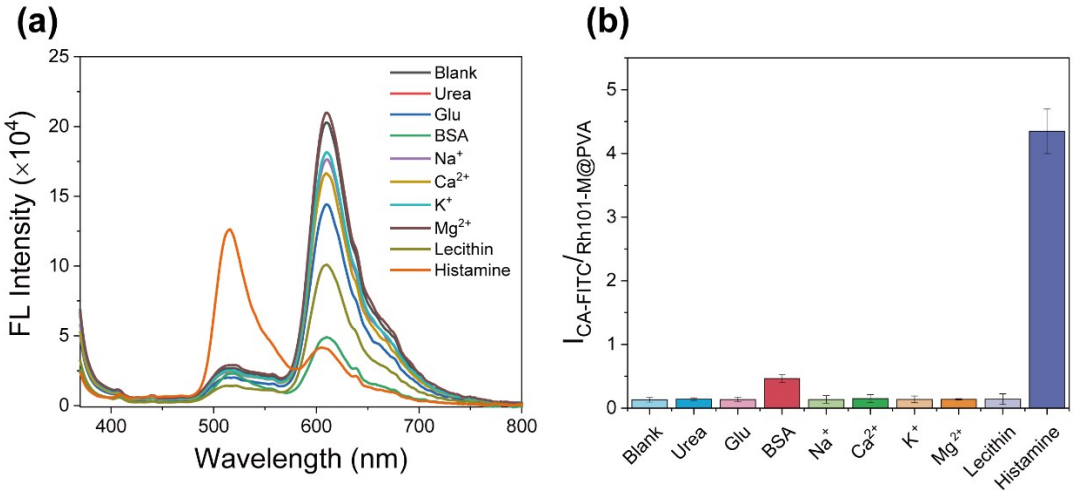

Figure S7. (a) The fluorescence spectra of the ratiometric fluorescent materials response to common substances. (b) The ratio changes of the ratiometric fluorescent materials response to common substances (1000 ppm).

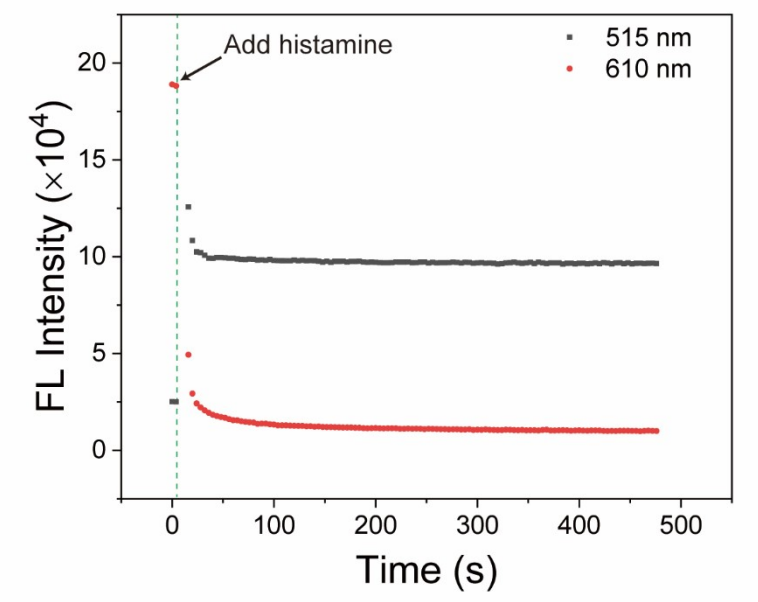

Figure S8. The detection time of ratiometric fluorescent materials response to histamine (1000 ppm).

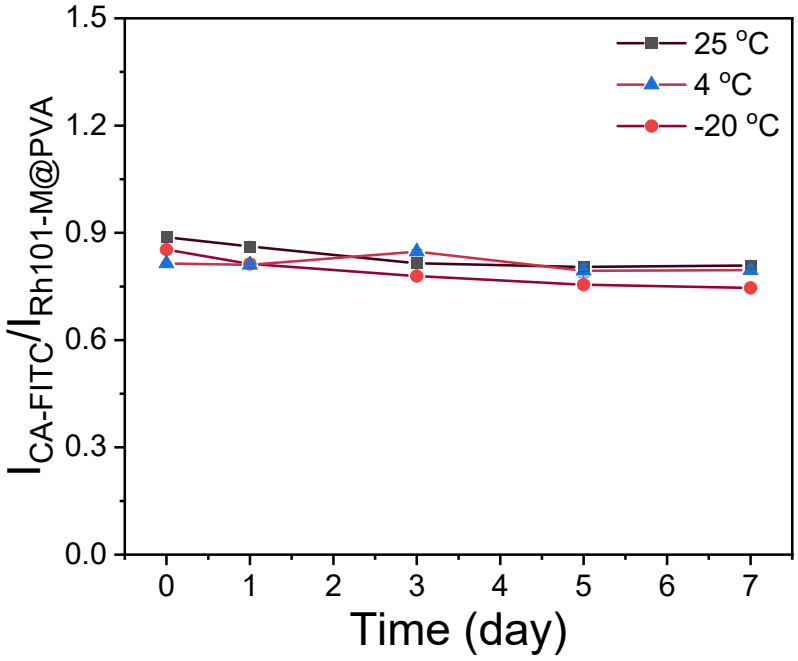

Figure S9. Changes in the fluorescence ratio of smart labels after storage at different temperatures and reaction with histamine (100 ppm).

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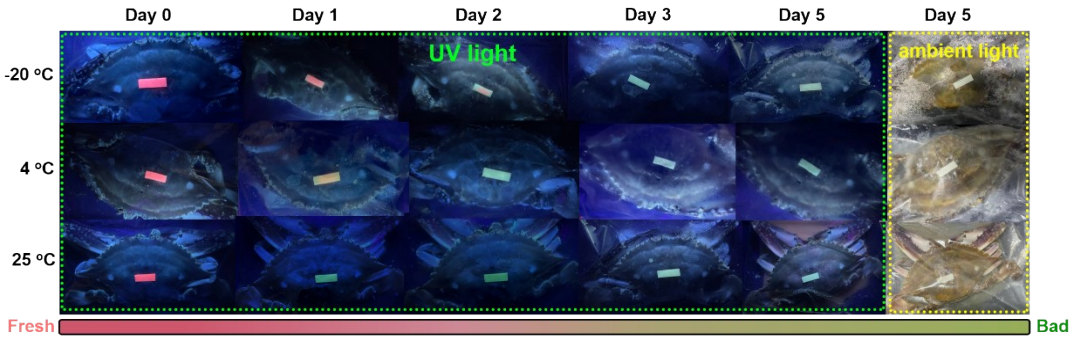

Figure S10. Photographs of ratiometric fluorescent labels for monitoring the freshness of crabs stored under different conditions.

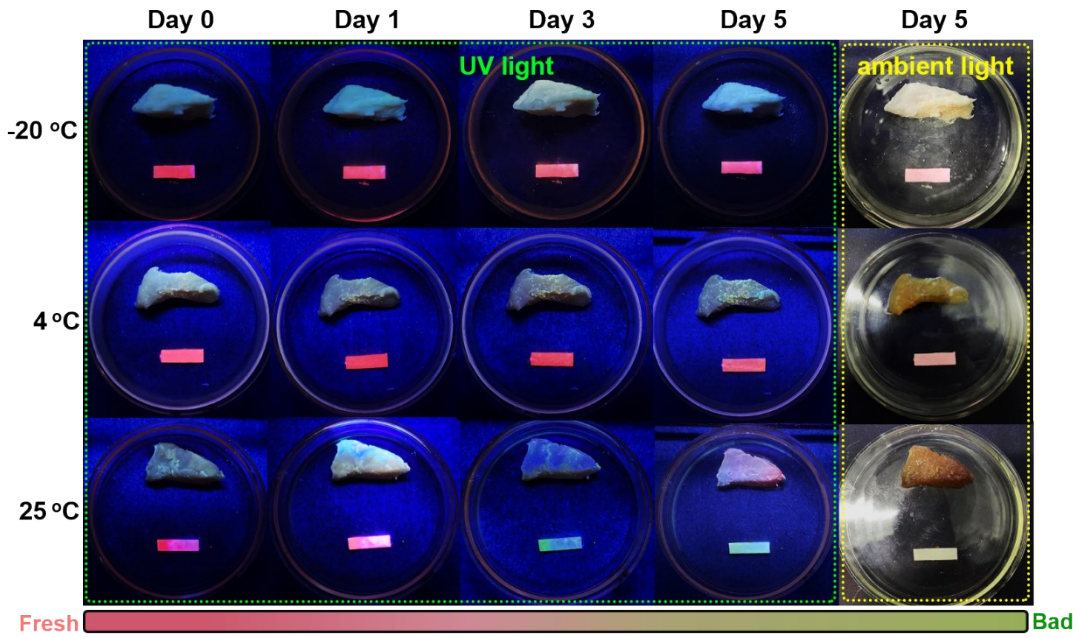

Figure S11. Photographs of ratiometric fluorescent labels for monitoring the freshness of chicken meat stored under different conditions.

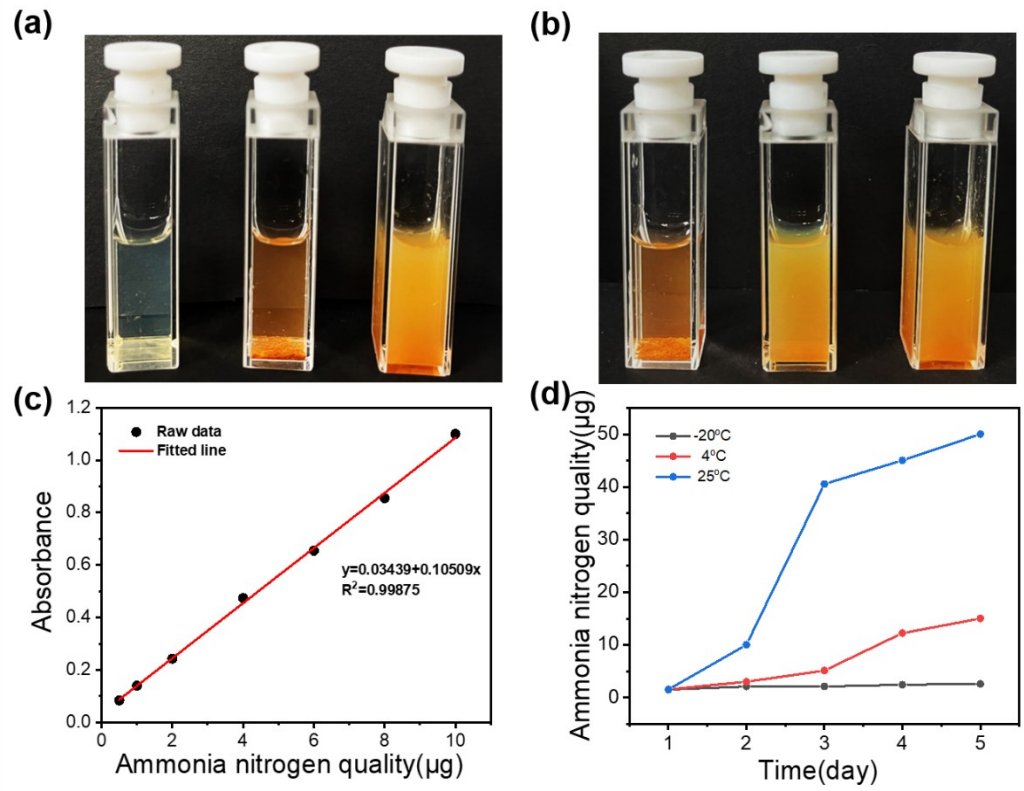

Figure S12. Ammonia nitrogen content in shrimp meat infusion monitored with Nessler's reagent. (a) The cuvette from left to right represent the samples stored at 4 °C for 1, 2, and 3 days, respectively. (b) The cuvette from left to right represent the samples stored at 25 °C for 1, 2, and 3 days, respectively. (c) Standard curve of Nessler's reagent for ammonia nitrogen detection. (d) The ammonia nitrogen content of 5 g fresh shrimp (stored at -20 °C, 4 °C, and room temperature for five days) was determined by Nessler's reagent method.

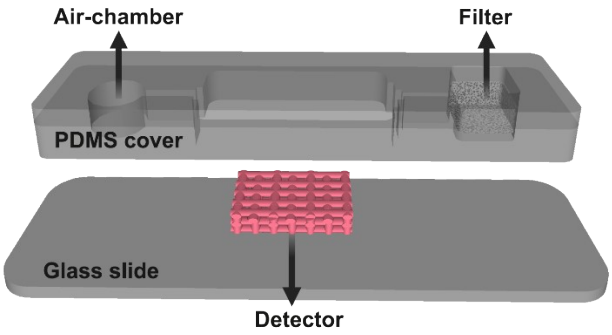

Figure S13. The split structure of the press-down chip.

The chip was composed of PDMS cover layer, glass slides, and the ratiometric fluorescent labels, while the filter and detector were preset in the chip. The filter was used to filter the impurities (in the liquid samples) to avoid blocking the channel and the detector. Pressing the air chamber to generate the negative pressure to drive the liquid samples into the chip and contact the detector.

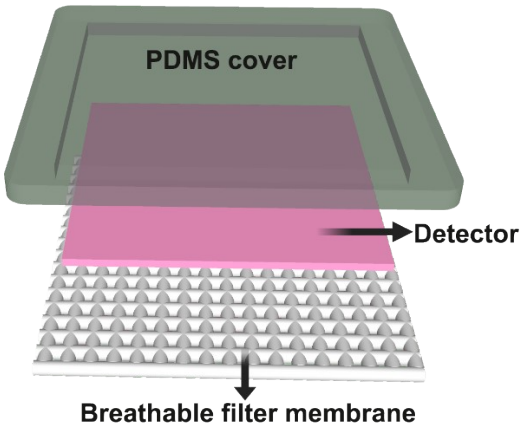

Figure S14. The split structure of ventilation device.

The device was prepared by bonding the PDMS cover layer and a breathable filter membrane. The membrane with porous was obtained from a commercial desiccant bag. The device can be co-stored in the bag with dry food. The porous structure allows the evaporative amine into the device spontaneously.

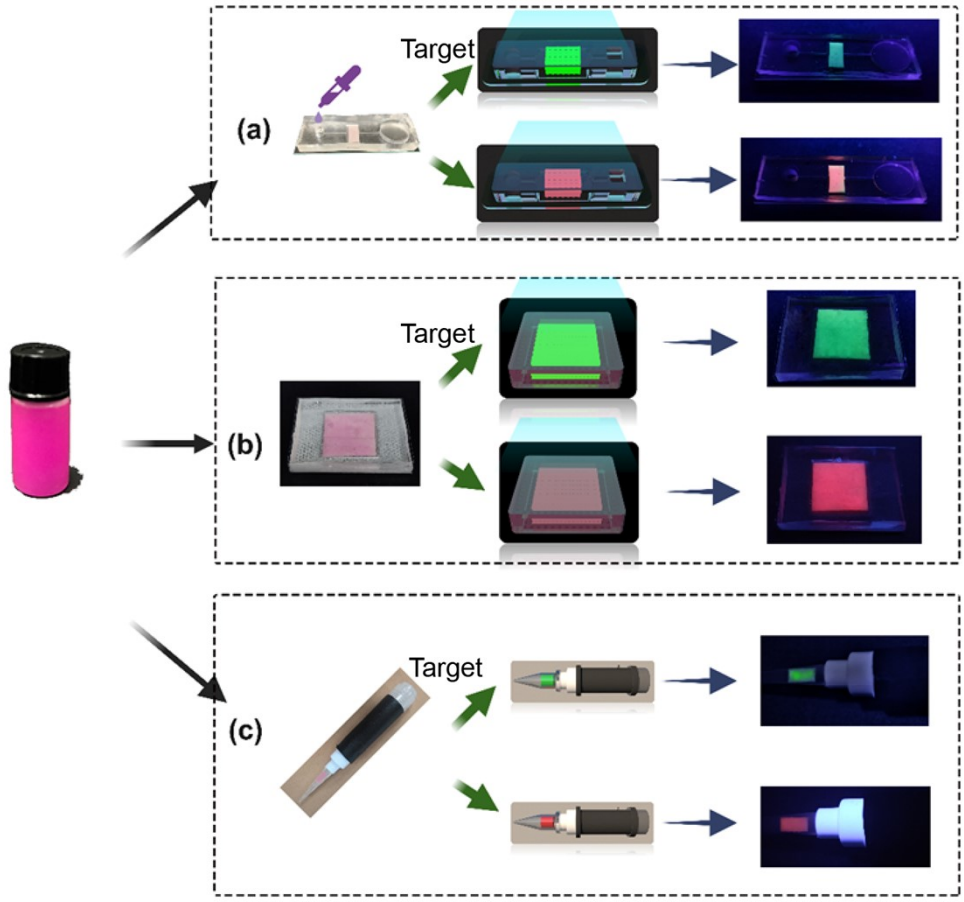

Figure S15. Smart labels-integrated device for BAs detection. (a) press-down chip; (b) ventilation device. (c) pipette type device (with built-in UV-light). In the dotted box, the left images represented hand-made devices, the middle images are drawn by Sketchup (with vary), the right images are the fluorescence results of the hand-made device.