Electronic Supplementary Material (ESI) for Analyst.

Supplementary Information

Construction of novel near-infrared fluorescent Nile Blue@MOF nanoprobe for imaging mitochondrial ATP in living cells

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Reagents and instruments. Zinc acetate dihydrate ($Zn(CH_3COOH)_2 \cdot 2H_2O$), 2-ICA and NB were obtained from Aladdin (Shanghai, China). Apyrase were purchased from Sigma-Aldrich (St. Louis, MO). ATP, uridine triphosphate (UTP), cytidine triphosphate (CTP), and adenosine monophosphate (AMP) were obtained from Sangon Biotech Co., Ltd. (Shanghai, China). Cell Counting Kit-8 (CCK-8) and MitoTracker@Green were purchased from Beyotime Biotechnology Co. Ltd. (Shanghai, China). RPMI 1640 medium, penicillin-streptomycin, fetal bovine serum (FBS), and DPBS were ordered from Gibco. Unless mentioned, all the reagents were analytical grade and used without further purification. Millipore Milli-Q water (18.2 MΩ cm) was used in all experiments.

UV-vis spectra were performed using UV-2600 spectrophotometers (Shimadzu, Japan). Fluorescence were collected in a Cary Eclipse fluorescence spectrometer (Varian, USA). Scanning electron microscopy (SEM) images were performed using Zeiss Sigma-500 (Germany). Transmission electron microscopy (TEM) images were recorded on a JEM-2100F microscopy (JEOL, Japan). Power X-ray diffraction (PXRD) analysis was conducted using a Bruker D8 Advance X-ray diffractometer (Germany). Fourier-transform infrared (FTIR) spectra were recorded using a IRTracer-100 instrument (Janpan). Dynamic light scattering (DLS) measurements was collected using a Malvern Instruments Zetasizer Nano ZS (Worcester-shire, UK). Confocal laser scanning microscopy (CLSM) images were recorded with a LSM800 laser scanning confocal microscope (Carl Zeiss, Germany). Cell viability was assessed using a Varioskan LUX microplate reader (Thermo Fisher, USA).

Synthesis of ZIF-90. 0.022 g of $Zn(CH_3COOH)_2 \cdot 2H_2O$ and 0.0192 g 2-ICA were separately dissolved in 2 mL of DMF, followed by mixing them under vigorous stirring for 15 min at room temperature. Whereafter, the resulting ZIF-90 suspension was centrifuged, washed once with DMF and three times with ethanol. The ultimately obtained off-white ZIF-90 were collected and dried overnight at 60 °C.

Synthesis of NB@ZIF-90. For obtaining NB@ZIF-90, a solution of NB (1 mM) was poured into 2-ICA (2 mL, 0.2 M) dissolved in DMF and mixed vigorously until total

dissolution. Then, $Zn(CH_3COOH)_2 \cdot 2H_2O$ (2 mL, 0.1 M) dissolved in DMF was added to the mixture solution under vigorous stirring for 5 min at room temperature. After adding an additional 10 mL of DMF to stabilize the spherical structure, the mixture was subjected to centrifugation at 10,000 rpm and washed with DMF followed by multiple washes with ethanol. In the end, a blue powder was obtained and dried under vacuum overnight at 60 °C.

Release experiments. In a typical experiment, 0.4 mg/mL of NB@ZIF-90 were incubated with exceed ATP (20 mM). The suspension was then collected by centrifugation, and the free NB was monitored via the absorption band of the dye centered at 630 nm. The amount of NB inside ZIF-90 was calulated using the calibration curve of NB shown in Figure S3.

ATP responsiveness of NB@ZIF-90 in aqueous solution. The following procedures were performed to explore the ATP-responsing behavior of NB@ZIF-90. Firstly, NB@ZIF-90 was dispersed into DPBS at a concentration of 4 mg/mL. Later, ATP with different concentrations (0, 1, 2, 3, 4, 5, 6, and 7 mM) were added to the dispersions of NB@ZIF-90. After incubation at room temperature for 5 min, the resulting mixtures were centrifuged at 13,000 rpm for 5 min. Finally, the fluorescence spectra of the supernatants were collected with excitation at 634 nm and emission in the range of 654-850 nm. For the specificity test, ATP and its analogues (GTP, UTP, and AMP) were added at a concentration of 5 mM, and the fluorescence intensity at 680 nm was measured after incubation at room temperature for 5 min.

The ATP responsiveness of NB@ZIF-90 was further characterized by observing the morphology change of NB@ZIF-90 upon the addition of ATP. In brief, NB@ZIF-90 (4 mg/mL) was incubated with 5 mM ATP at room temperature for 5 min. The resulting mixture was then analyzed using SEM and TEM image.

Cell cytotoxicity assay. The biocompatibility of NB@ZIF-90 was appraised using a CCK-8 assay. Briefly, HeLa cells were placed on 96-well plates at a density of 1×10^4 cells per well and allowed to attach overnight at 37°C under 5% CO_2. The cells were then treated with 100 μg/mL of NB@ZIF-90 for different durations ranging

from 0.5 to 2.5 h. After beening washed three times with DPBS, 10 μL of CCK-8 was added into the wells and cubated in RPMI 1640 medium for another 30 min. Whereafter, the absorbance of each well was measured at 450 nm using a microplate reader. Cell viability was calculated as the percentage of viable cells compared to the control group without NB@ZIF-90.

Monitoring ATP in living cells. To test the imaging capability of NB@ZIF-90 for ATP, HeLa cells were placed on a 25-mm glass-bottom confocal laser dishes and cultured in RPMI 1640 supplemented with 10 % FBS and 1% apenicillin-streptomycin at 37°C under 5% CO_2. After thoroughly washed with DPBS, the cells were separately incubated with NB (25 μg/mL) or NB@ZIF-90 (100 μg/mL) at 37 °C for 20 min. In order to regulat the ATP level, the cells were pretreated with 0.5 U/mL of apyrase at 37 °C for 30 min prior to the addition of NB@ZIF-90. After removing the free probes with DPBS, the cells were imaged in DPBS (500 μL) using a CLSM with excitation at 640 nm. The acquired images were analysed with ImageJ software.

For the colocalization experiment, Hela cells were cubated with 100 μg/mL of NB@ZIF-90 and a mercantile mitochondria tracker (MitoTracker@Green). After a 20 min incubation, the cells were washed three times with DPBS and imaged in DPBS (500 μL) using a CLSM. The red channel and green channel were excited at 640 nm and 488 nm, respectively.

Figure S1. Images of ZIF-90 (left) and NB@ZIF-90 (right) powders.

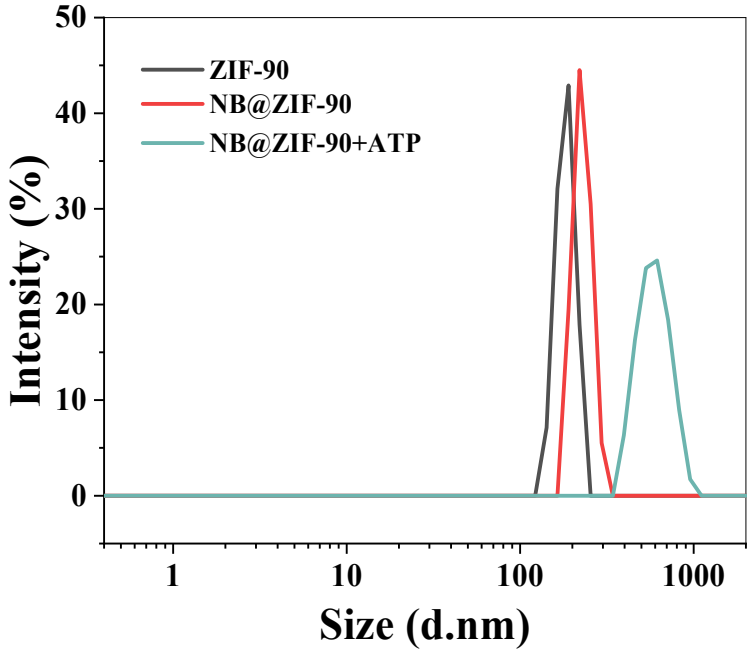

Figure S2. Size distribution of ZIF-90 and NB@ZIF-90 measured by DLS.

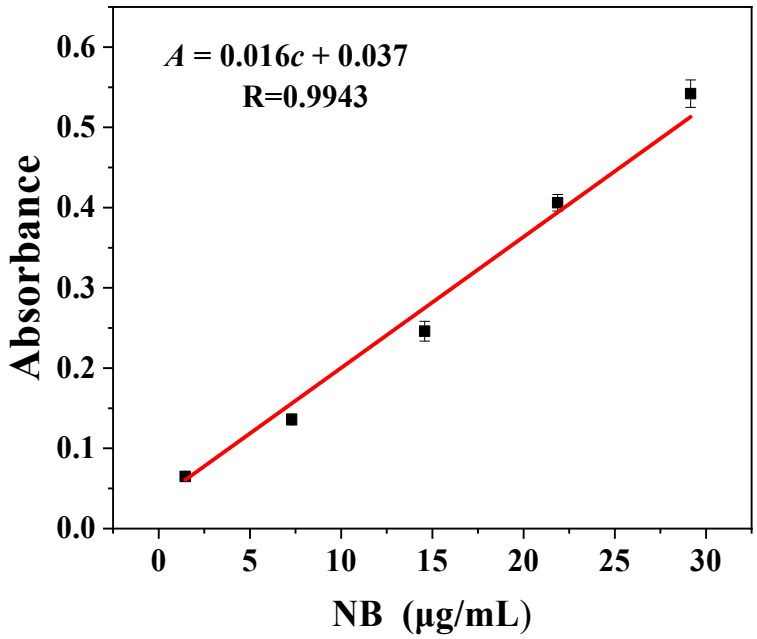

Figure S3. Absorption of NB at 634 nm *vs* the concentration of NB.

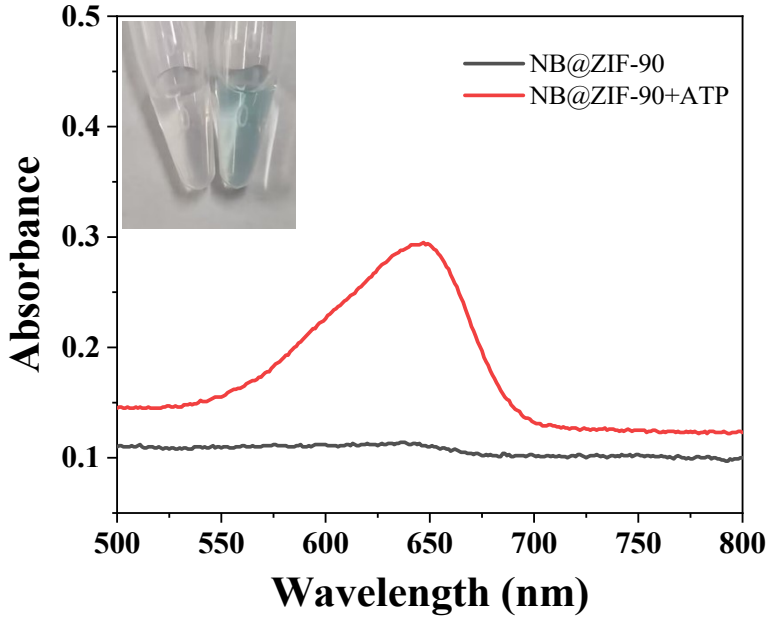

Figure S4. Absorption spectra of NB@ZIF-90 in the absence and presence of 5 mM ATP. Inset: the corresponding color image of the supernatant for NB@ZIF-90 before and after adding ATP.

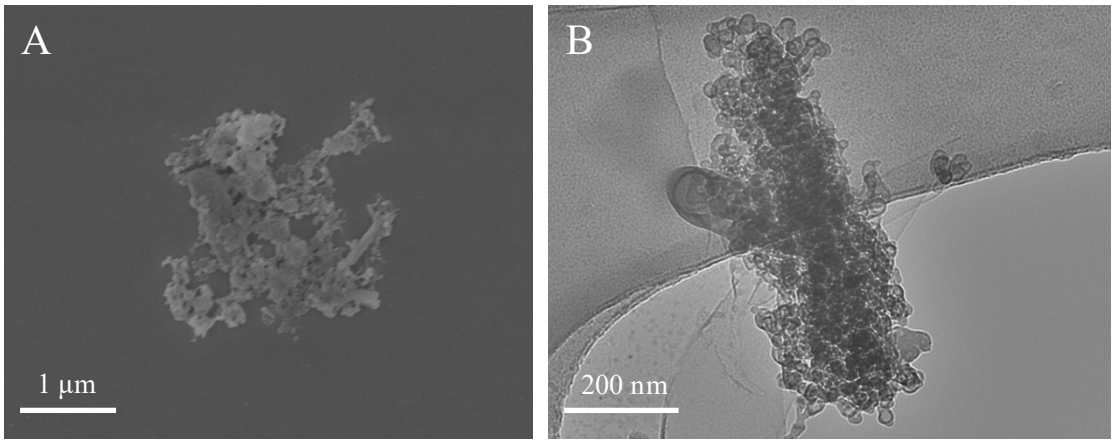

Figure S5. SEM images (A) and TEM (B) image of NB@ZIF-90 nanoprobe reacted with ATP (5 mM).

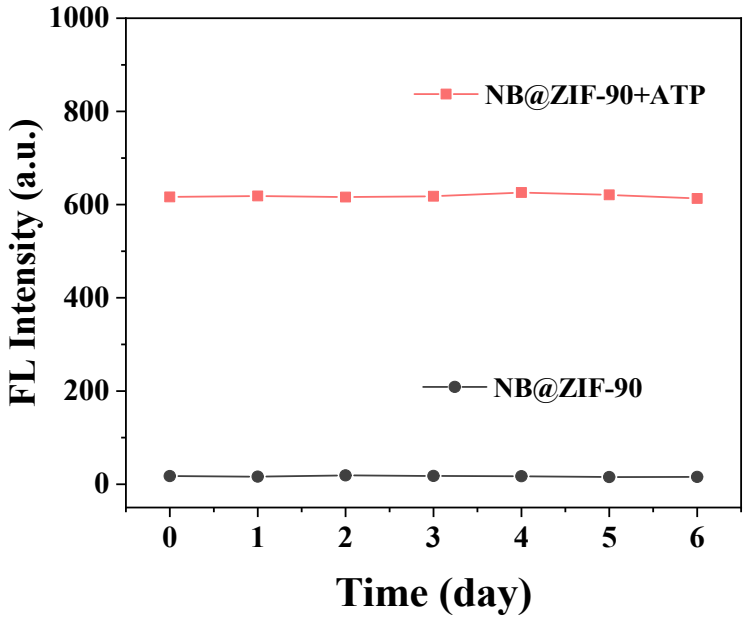

Figure S6. Effect of storage time on the fluorescence intensity of NB@ZIF-90 without or with ATP.

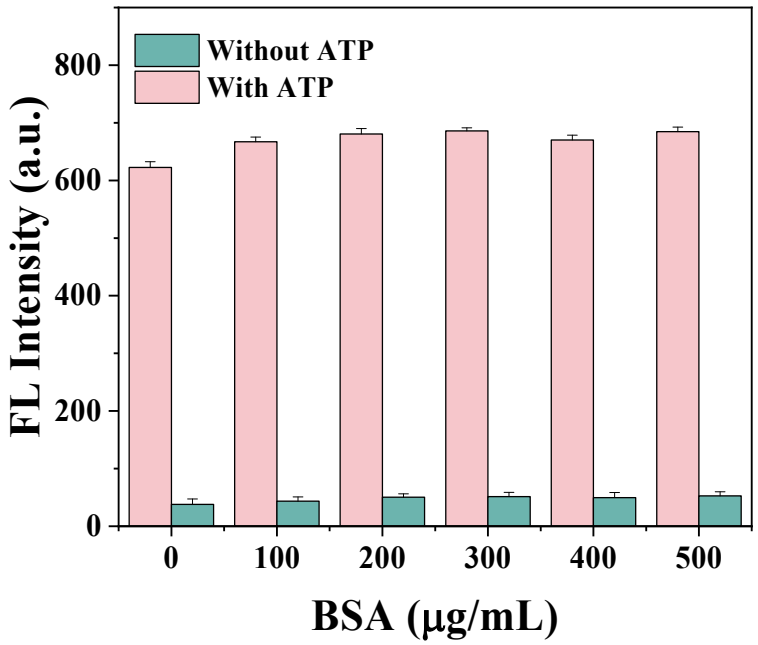

Figure S7. Fluorescence intensity of the NB@ZIF-90 without or with 5 mM ATP at 680 nm as a function of different BSA concentration.

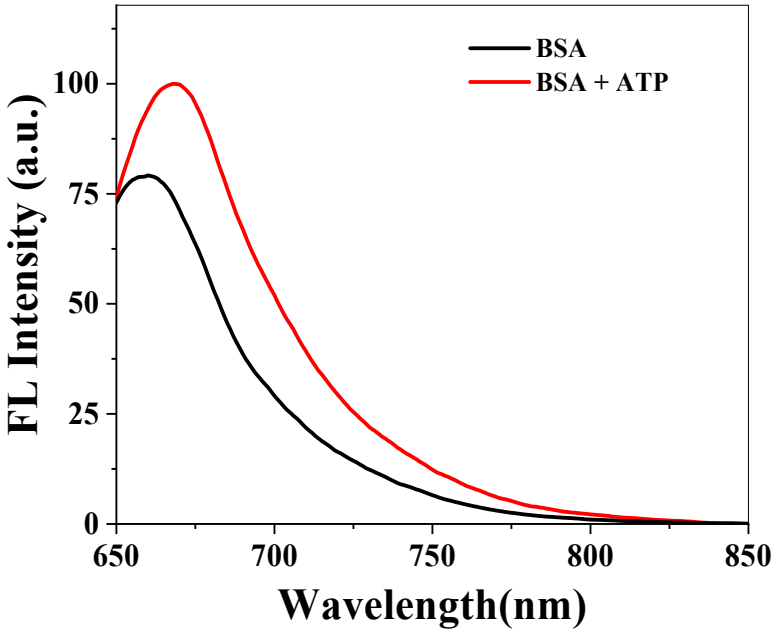

Figure S8. Flurerescence spectra of NB@ZIF-90 under different conditions. [BSA]= 40 mg/mL, [NB@ZIF-90]= 4 mg/mL, [ATP] = 7 mM.

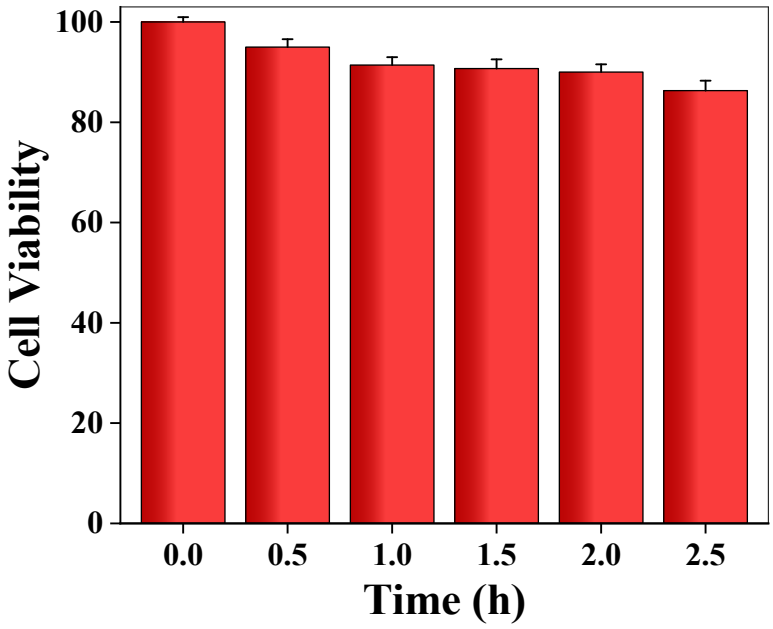

Figure S9. Cytotoxicity assay of NB@ZIF-90 against HeLa cells.

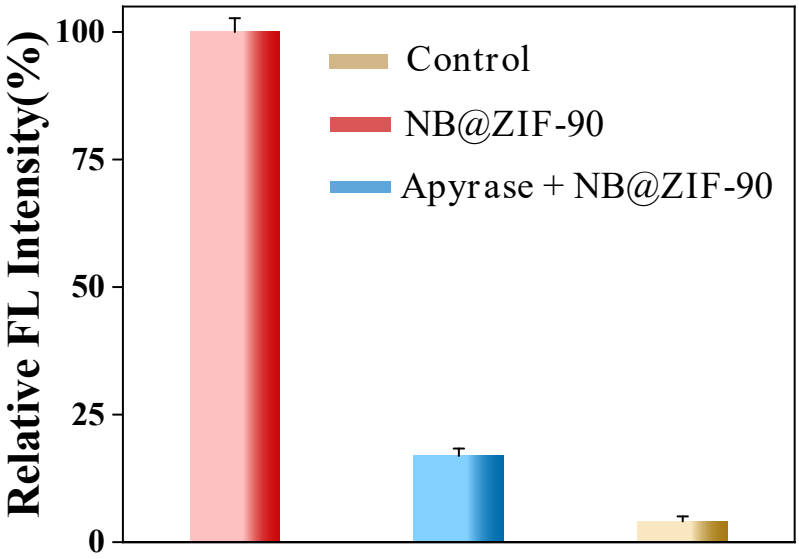

Figure S10. Normalized fluorescence signal from Figure 4A, where the signal of the cells treated with NB@ZIF-90 was set as 100%, and other samples were calculated accordingly. The data were recorded by mean value ± standard deviation.

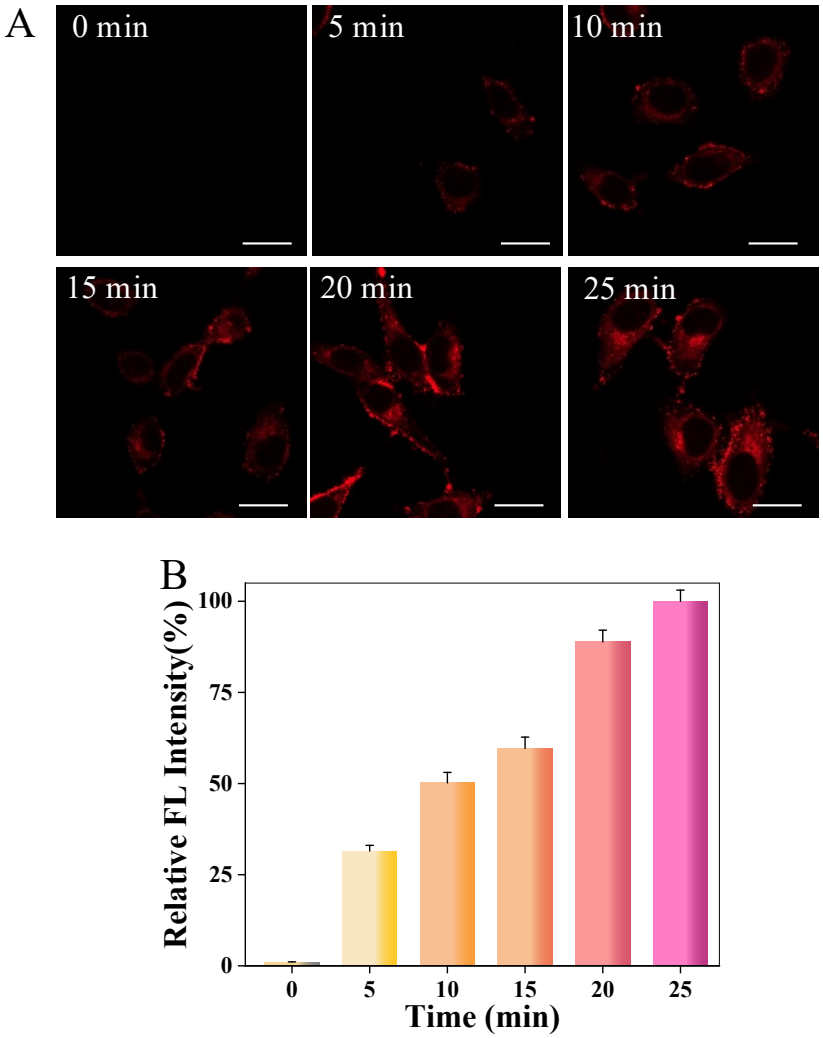

Figure S11. (A) CLSM images of Hela cells treated with NB@ZIF-90 for different times. (B) Normalized fluorescence signal from (C), where the signal of the cells treated with NB@ZIF-90 for 25 min was set as 100%, and other samples were calculated accordingly. The concentration of NB and NB@ZIF-90 was 25 μg/mL and 100 μg/mL, respectively. Excitation wavelength: 640 nm. Scale bars: 20 μm.

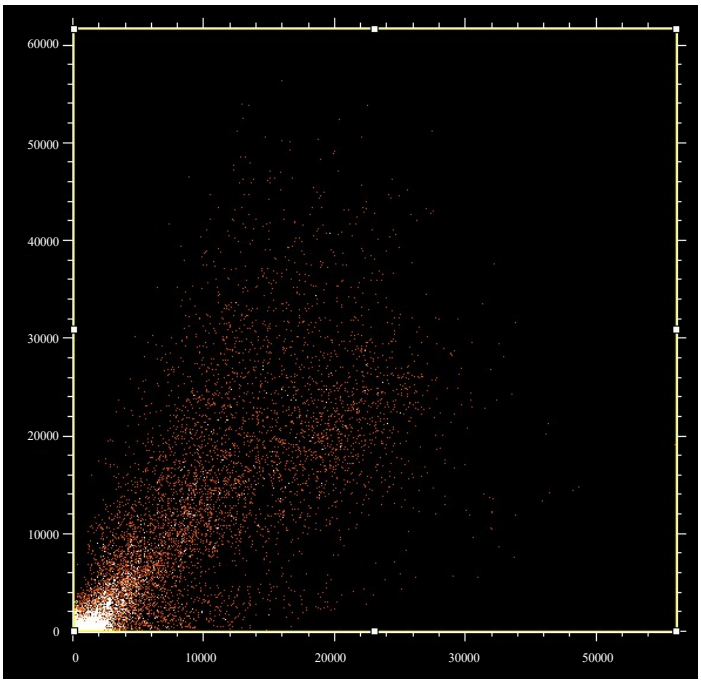

Figure S12. Intensity correlation plots from Figure 4B.