Supporting Information

Tailoring Nanocrystalline MOFs as Fluorescent Dye Carriers for Bioimaging

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Section S1. Materials and methods

Chemicals: Biphenyl-4,4'-dicarboxylic acid (97%, Aldrich), Zirconium (IV) chloride (≥99.5% trace metals basis, Aldrich), *N*,*N*-Dimethylformamide (99%, Aldrich), acetic acid (≥99.7%, Aldrich), Methanol (≥99.9%, Aldrich), Rhodamine-6G (dye content 99%, Aldrich), Resorufin (dye content 95%, Sigma-Aldrich). D-(+)-Galactosamine hydrochloride (≥99%, Aldrich), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide Hydrochloride (≥98.0%, Tokyo Chemical Industry), Dulbecco's Modified Eagle's Medium (HyClone), Fetal bovine serum (HyClone), 4',6-Diamidine-2'-phenylindole dihydrochloride (Molecular Probes).

Synthesis of nMOF-801: It need to prepare as ligand part and metal part separately. $ZrCl_4$ (33.4 mg, 0.14 mmol) was dissolved in DMF (5mL) with acetic acid (0.69 mL) in a 20 mL glass vial. Ligand part is prepared as solution of fumaric acid (18 mg, 0.15 mmol), TEA (0.03 mL) and DMF (5 mL). Well dispersed ligand solution poured into metal part and sealed vial placed in 100°C oven for 12 h. Then, it is cooled down to room temperature and separated solid part with a centrifuge (8000 rpm for 10 min). It was washed with DMF once and methanol three times. After washing, product was dried in a vacuum oven.

Synthesis of nUiO-67: $ZrCl_4$ (18.6 mg, 0.08 mmol) was dissolved in DMF (5 mL) with acetic acid (1.38 mL) in a 20 mL glass vial. Ligand part is prepared as solution of biphenyl-4,4'-dicarboxylic acid (19.4 mg, 0.08 mmol), TEA (0.03 mL) and DMF (5 mL). Well dispersed ligand solution poured into metal part and sealed vial placed in 100°C oven for 12 h. Then, it is cooled down to room temperature and separated solid part with a centrifuge (8000 rpm for 10 min). It was washed with DMF once and methanol three times. After washing, product was dried in a vacuum oven.

Synthesis of Rs⊂nMOF-801: $ZrCl_4$ was dissolved in Resorufin-DMF solution (0.48 mg/ml, 5 mL) with acetic acid (0.69 mL) in a 20 mL glass vial. Ligand part is prepared as solution of fumaric acid, TEA (0.03 mL) and Resorufin-DMF solution (0.48 mg/ml, 5 mL). Well dispersed ligand solution poured into metal part and sealed vial placed in 100°C oven for 12 h. Then, it is cooled down to room temperature and separated solid part with a centrifuge (8000 rpm for 10 min). It was washed with DMF once and methanol when the extra dye was disappeared. After washing, product was dried in a vacuum oven.

Synthesis of Rs×nUiO-67: $ZrCl_4$ was dissolved in Resorufin-DMF solution (0.48 mg/ml, 5 mL) with acetic acid (1.38 mL) in a 20 mL glass vial. Ligand part is prepared as solution of biphenyl-4,4'-dicarboxylic acid, TEA (0.03 mL) and Resorufin-DMF solution (0.48 mg/ml, 5 mL). Well dispersed ligand solution poured into metal part and sealed vial placed in 100°C oven for 12 h. Then, it is cooled down to room temperature and separated solid part with a centrifuge (8000 rpm for 10 min). It was washed with DMF once and methanol when the extra dye was disappeared. After washing, product was dried in a vacuum oven.

Synthesis of R6G×nMOF-801: $ZrCl_4$ was dissolved in Rhodamine-6G-DMF solution (0.21 mg/ml, 5 mL) with acetic acid (0.69 mL) in a 20 mL glass vial. Ligand part is prepared as solution of fumaric acid, TEA (0.03 mL) and Rhodamine-6G-DMF solution (0.21 mg/ml, 5 mL). Well dispersed ligand solution poured into metal part and sealed vial placed in 100°C oven for 12 h. Then, it is cooled down to room temperature and separated solid part with a centrifuge (8000 rpm

for 10 min). It was washed with DMF once and methanol when the extra dye was disappeared. After washing, product was dried in a vacuum oven.

Synthesis of R6G⊂nUiO-67: $ZrCl_4$ was dissolved in Rhodamine-6G-DMF solution (0.21 mg/ml, 5 mL) with acetic acid (1.38 mL) in a 20 mL glass vial. Ligand part is prepared as solution of biphenyl-4,4'-dicarboxylic acid, TEA (0.03 mL) and Rhodamine-6G-DMF solution (0.21 mg/ml, 5 mL). Well dispersed ligand solution poured into metal part and sealed vial placed in 100°C oven for 12 h. Then, it is cooled down to room temperature and separated solid part with a centrifuge (8000 rpm for 10 min). It was washed with DMF once and methanol when the extra dye was disappeared. After washing, product was dried in a vacuum oven.

Surface functionalization of Rs⊂nMOF-801 with galactosamine: Rs⊂nMOF-801 was dissolved in distilled water (5 mg/mL, 10 mL) and mixed with 5-fold weight excess of D-(+)-galactosamine hydrochloride. The pH of solution was adjusted to 4.8 by the addition of 0.1 N HCl, and then mixed with 20-fold molar excess of EDC. During the reaction, the pH of the solution was consistently maintained at 4.8 and stirred. After 2 h, the pH of the solution was raised to 7.0 by the addition of NaOH (0.1 N) to terminate the reaction. The resulting solution was dialyzed against NaCl aqueous solution (100 mM) for 2 days, 25% ethanol for a day and distilled water for 2 days. [Rs⊂nMOF-801]-GSs were obtained by freeze-drying.

Surface functionalization of R6G⊂nUiO-67 with galactosamine: R6G⊂nUiO-67 was dissolved in distilled water (5 mg/mL, 8 mL) and mixed with 5-fold weight excess of D-(+)-galactosamine hydrochloride. The pH of solution was adjusted to 4.8 by the addition of 0.1 N HCl, and then

mixed with 20-fold molar excess of EDC. During the reaction, the pH of the solution was consistently maintained at 4.8 and stirred. After 2 h, the pH of the solution was raised to 7.0 by the addition of NaOH (0.1 N) to terminate the reaction. The resulting solution was dialyzed against NaCl aqueous solution (100 mM) for 2 days, 25% ethanol for a day and distilled water for 2 days. [R6G⊂nUiO-67]-GSs were obtained by freeze-drying.

Section S2. Supporting characterization of MOF, Dye⊂nMOF and Dye×nMOF

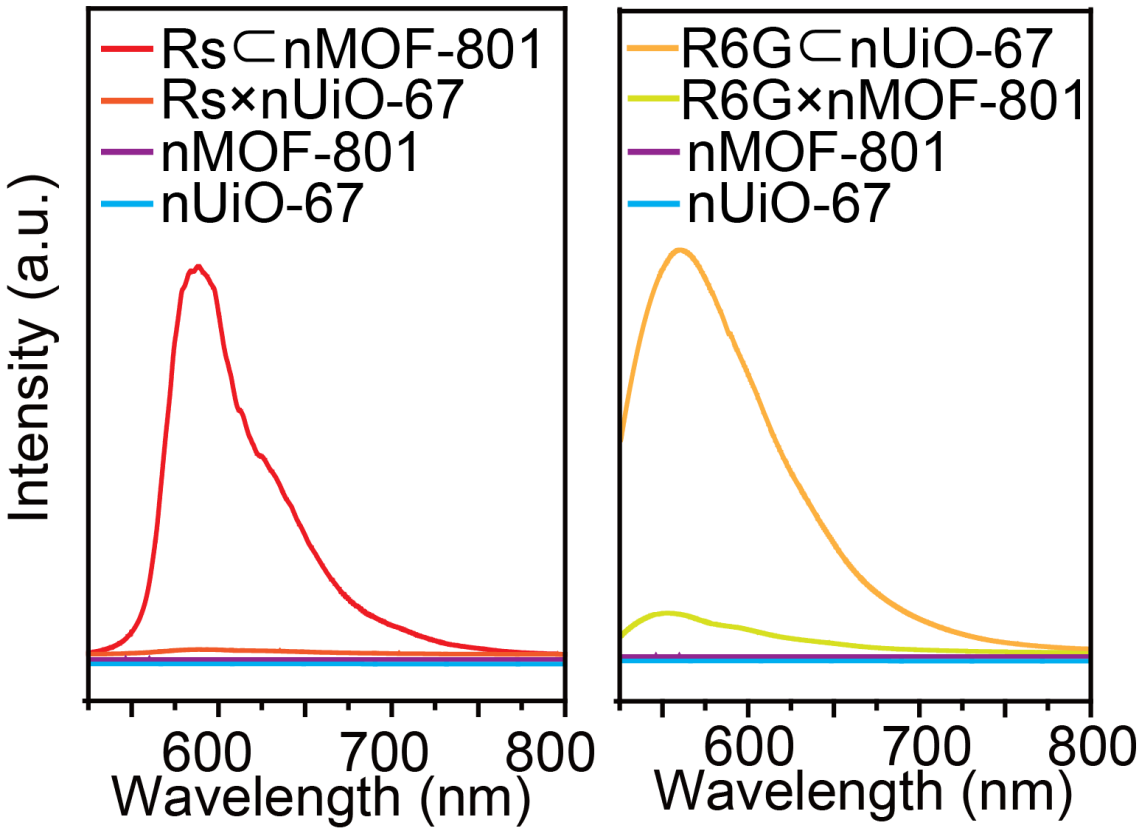

Figure S1. PL spectra of Dye⊂nMOF, Dye×nMOF and nMOF without Dye.

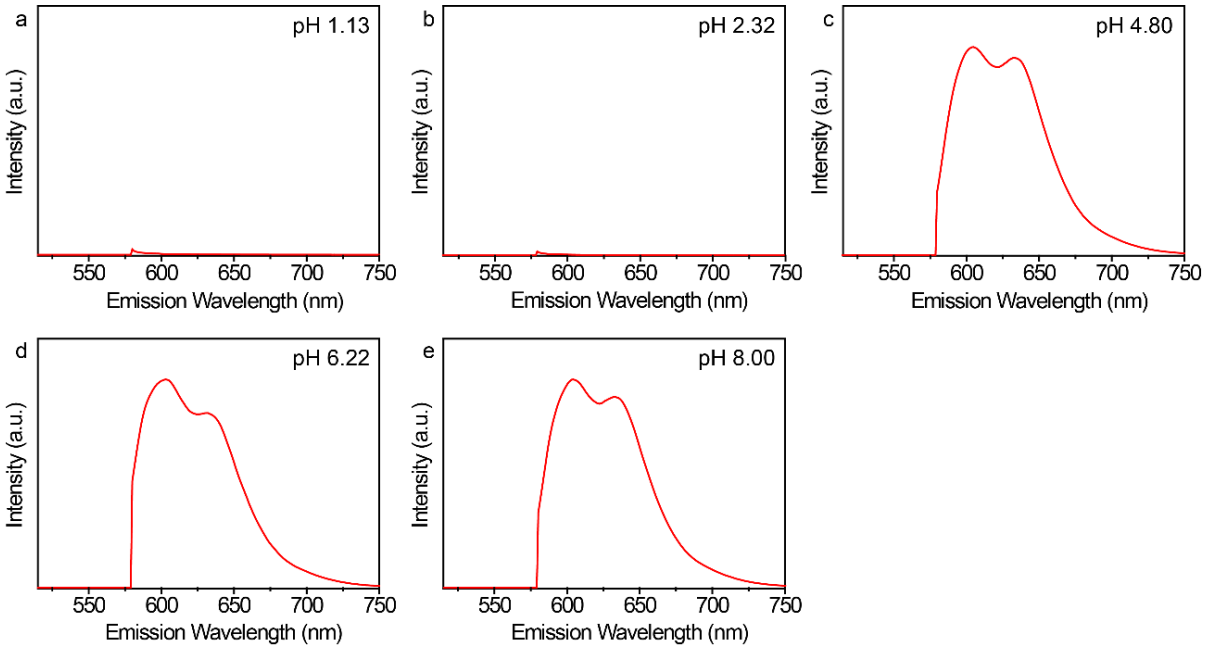

Figure S2. PL spectra of Resorufin at pH (a) 1.13, (b) 2.32, (c) 4.8, (d) 6.2, (e) 8.

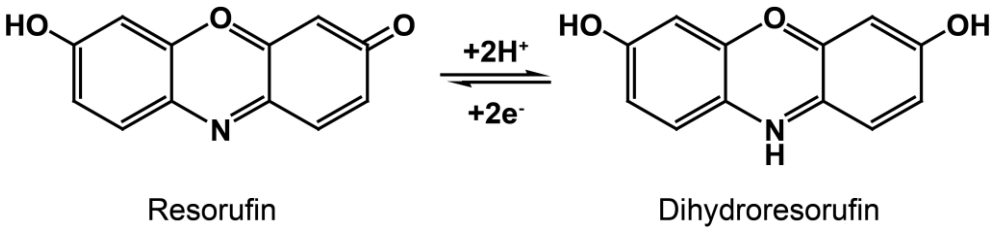

Figure S3. Conversion of Resorufin to Dihydroresorufin.

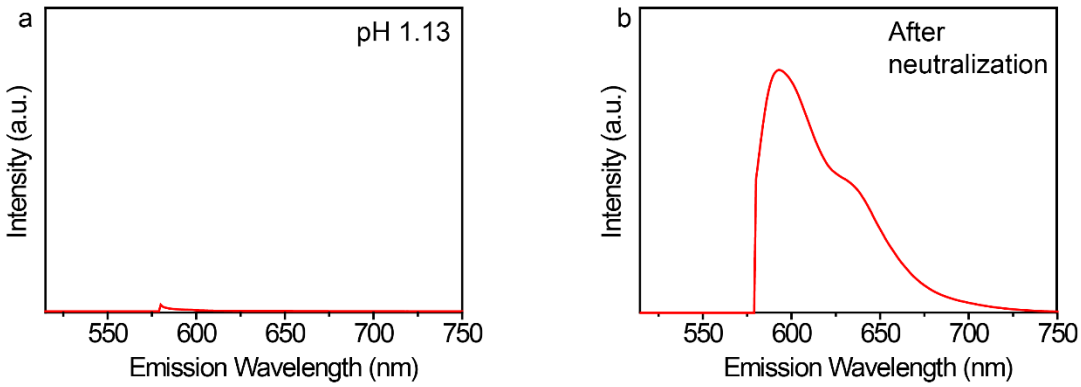

Figure S4. PL spectra of Resorufin at pH (a) 1.13, (b) 7.4 after neutralization.

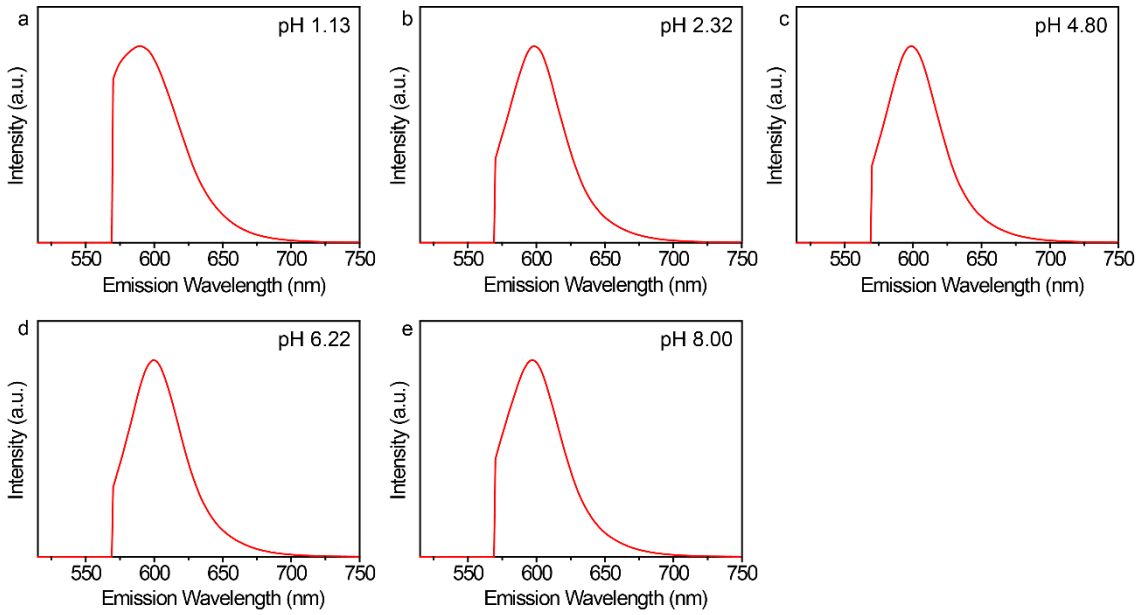

Figure S5. PL spectra of Rhodamine-6G at pH (a) 1.13, (b) 2.32, (c) 4.8, (d) 6.2, (e) 8.

We have measured the photoluminescence (PL) spectra of Resorufin and Rhodamine-6G in the pH range from 1.13 to 8, which covers the pH conditions of MOF synthesis, galactosylation, and cell imaging. It should be noted that the PL spectra of the dyes in aqueous solution cannot be perfectly same with those in MeOH due to the solvent effects (including electrophilic or nucleophilic solvation and solvent relaxation) that could lead to PL shifts resulting from interactions between dye and solvent molecules.

In the case of Resorufin, this dye preserves its PL in the pH range 4.8–8; however it would degrade at pH lower than 4 (Figure S2). The reason is that nitrogen of Resorufin can be protonated at a low pH condition to form Dihydroresorufin that has been known as a nonfluorescent molecule (Figure S3).[1] These Resorufin and Dihydroresorufin can be easily switched between each other depending on pH levels, so Dihydroresorufin can be readily deprotonated to return to Resorufin when pH levels > 4. To confirm the restoration of Resorufin, we added NaOH solution to neutralize the pH 1.13 Resorufin solution and recorded the PL spectra. As shown in Figure S4, the

neutralization of Dihydroresorufin gives Resorufin, where its original PL is fully recovered. Therefore, pH conditions in the MOF synthesis do not play a significant role in the synthesis of [Re⊂nMOF-801]-GS because the pH level of this final product is set to 7.4 before cell imaging. In the case of Rhodamine-6G, this dye preserves its PL regardless of pH conditions due to its intrinsically high resistance against protonation (Figure S5).

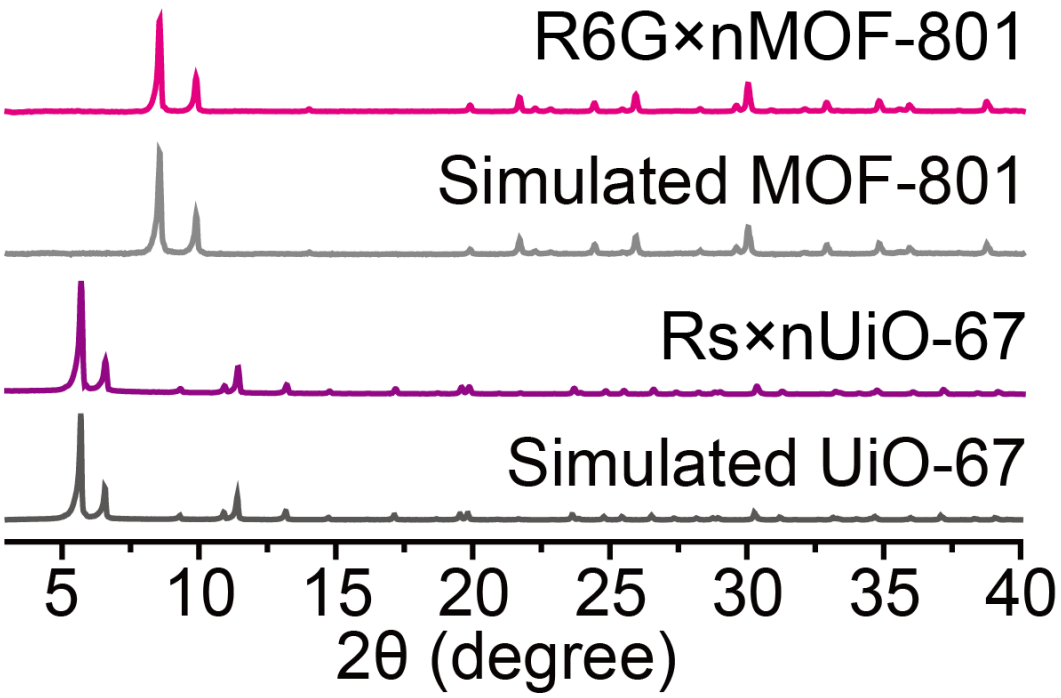

Figure S6. PXRD patterns of R6G×nMOF-801 and Rs×nUiO-67.

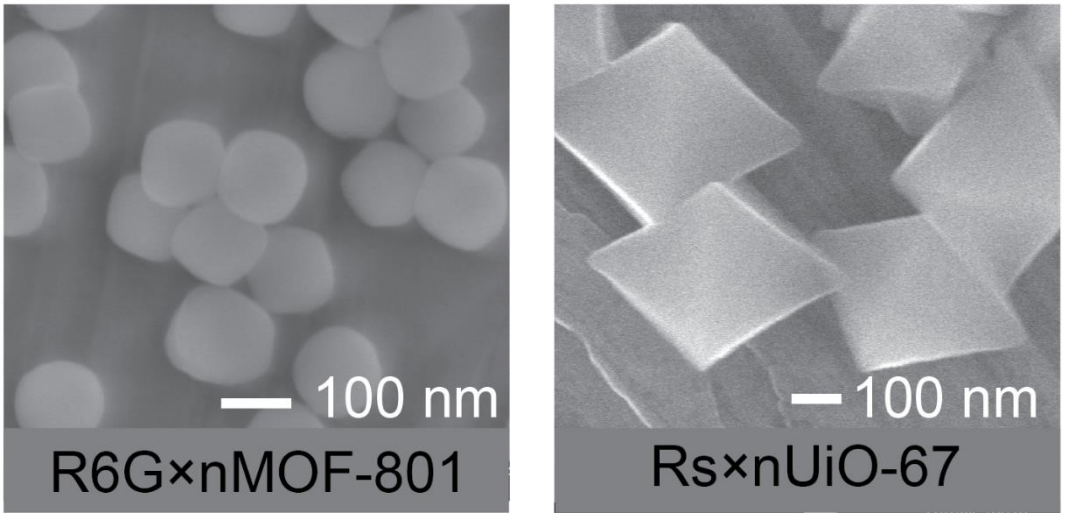

Figure S7. SEM images of R6G×nMOF-801 and Rs×nUiO-67.

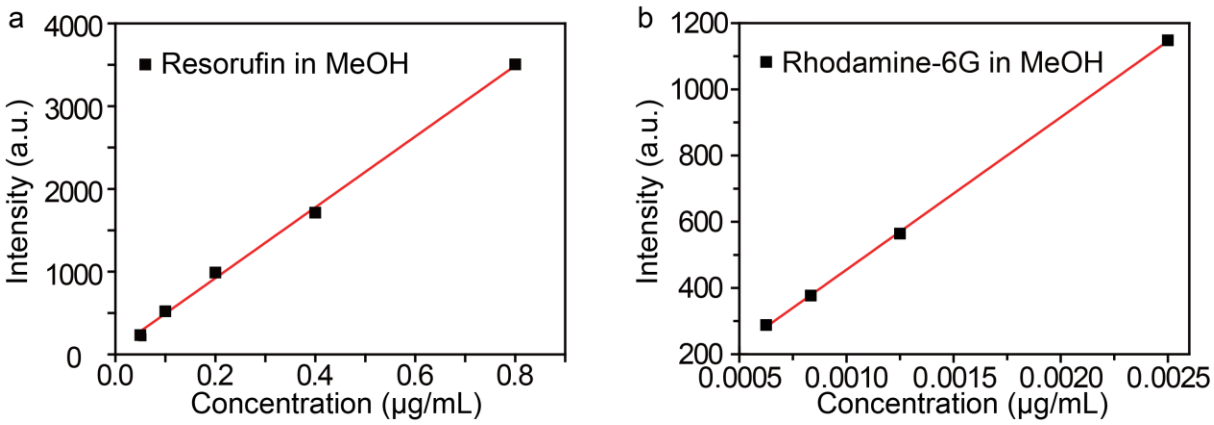

Figure S8. The PL intensity of the dye molecules (a) Resorufin excited at 525 nm and (b) Rhodamine-6G excited at 570 nm against their concentrations in methanol.

Sample	Slope	Intercept	R-square
Resorufin	4.820×10^3	65.137	0.99764
Rhodamine-6G	4.604×10^8	-5.217	0.99974

Table S1. The trend line of the dye molecules against their concentrations in methanol.

Sample	PL intensity of 1 mg/mL solution	Dye concentration (mg/mL)	The number of dyes per weight of MOFs (#/mg)
Rs⊂nMOF-801	2956.65	6.76×10^{-4}	1.91×10^{15}
R6G⊂nUiO-67	1021.41	2.23×10^{-6}	2.80×10^{12}

Table S2. Calculation of the amounts of dye molecules encapsulated within Dye⊂nMOFs.

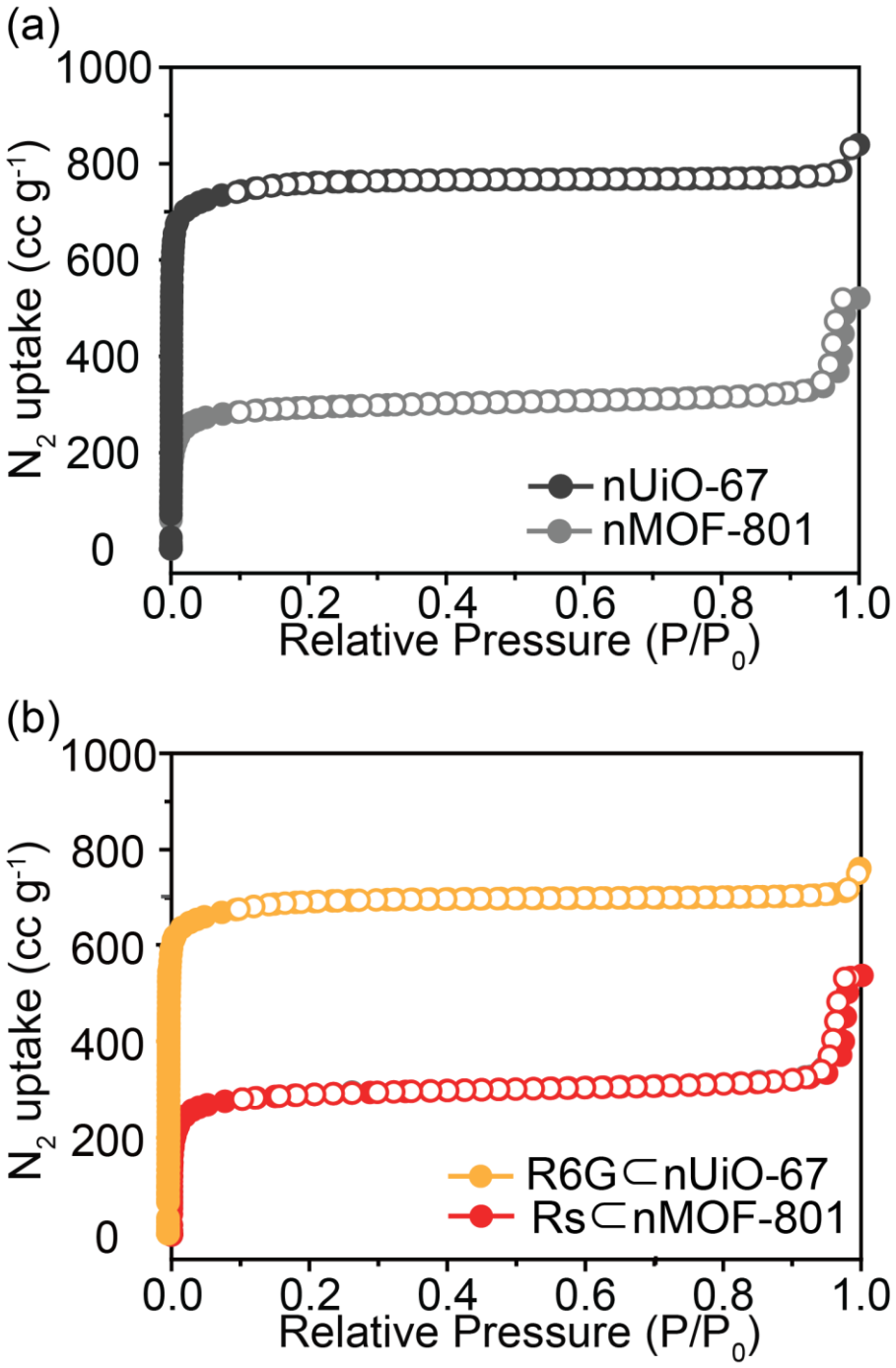

Figure S9. N_2 adsorption-desorption isotherms of (a) nMOF-801, nUiO-67 and (b) Rs⊂nMOF-801, R6G⊂nUiO-67

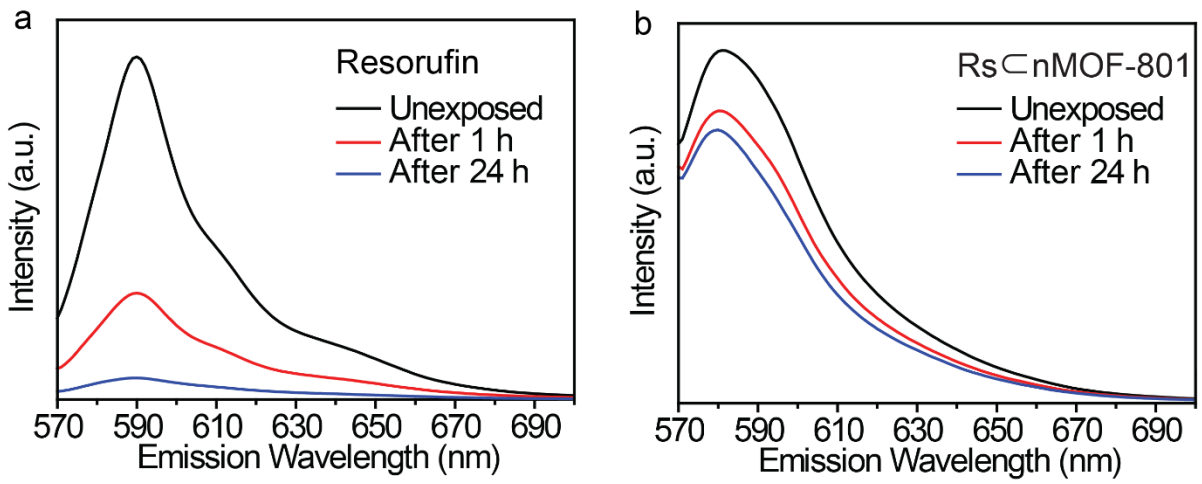

Figure S10. PL spectra of (a) Resorufin, (b) Rs⊂nMOF-801 excited at 560 nm in methanol under continuous irradiation of 365 nm UV light.

NOTE: Resorufin and Rs⊂nMOF-801 in methanol were exposed to 365 nm using a UV lamp (UVITEC, LF-106L) for increasing time (0-24 h) to test the photostability.

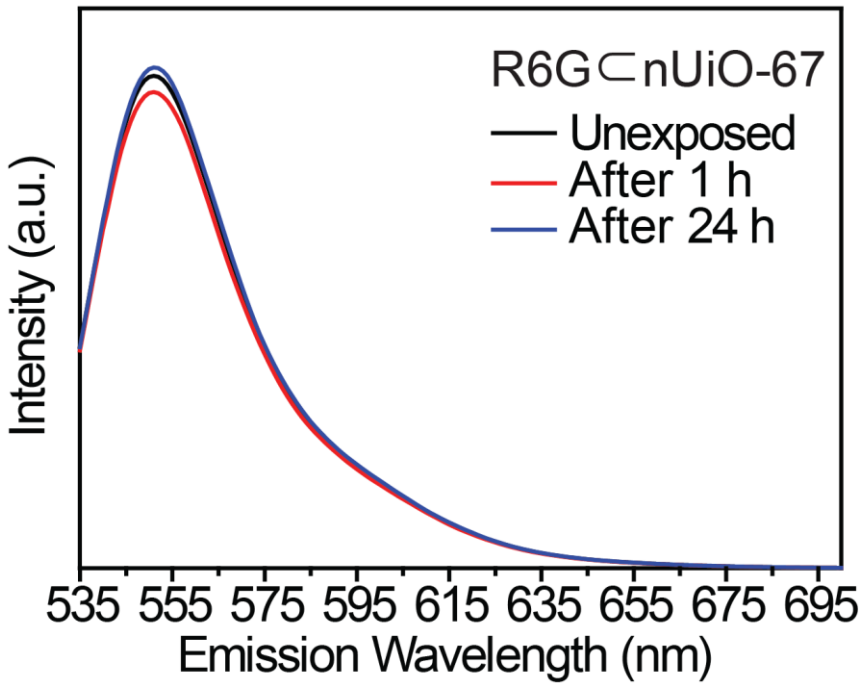

Figure S11. PL spectra of Rhodamine-6G excited at 525 nm in methanol under continuous irradiation of 365 nm UV light.

NOTE: Rhodamine-6G in methanol were exposed to 365 nm using a UV lamp (UVITEC, LF-106L) for increasing time (0-24 h) to test the photostability.

Section S3. Biological Experiment

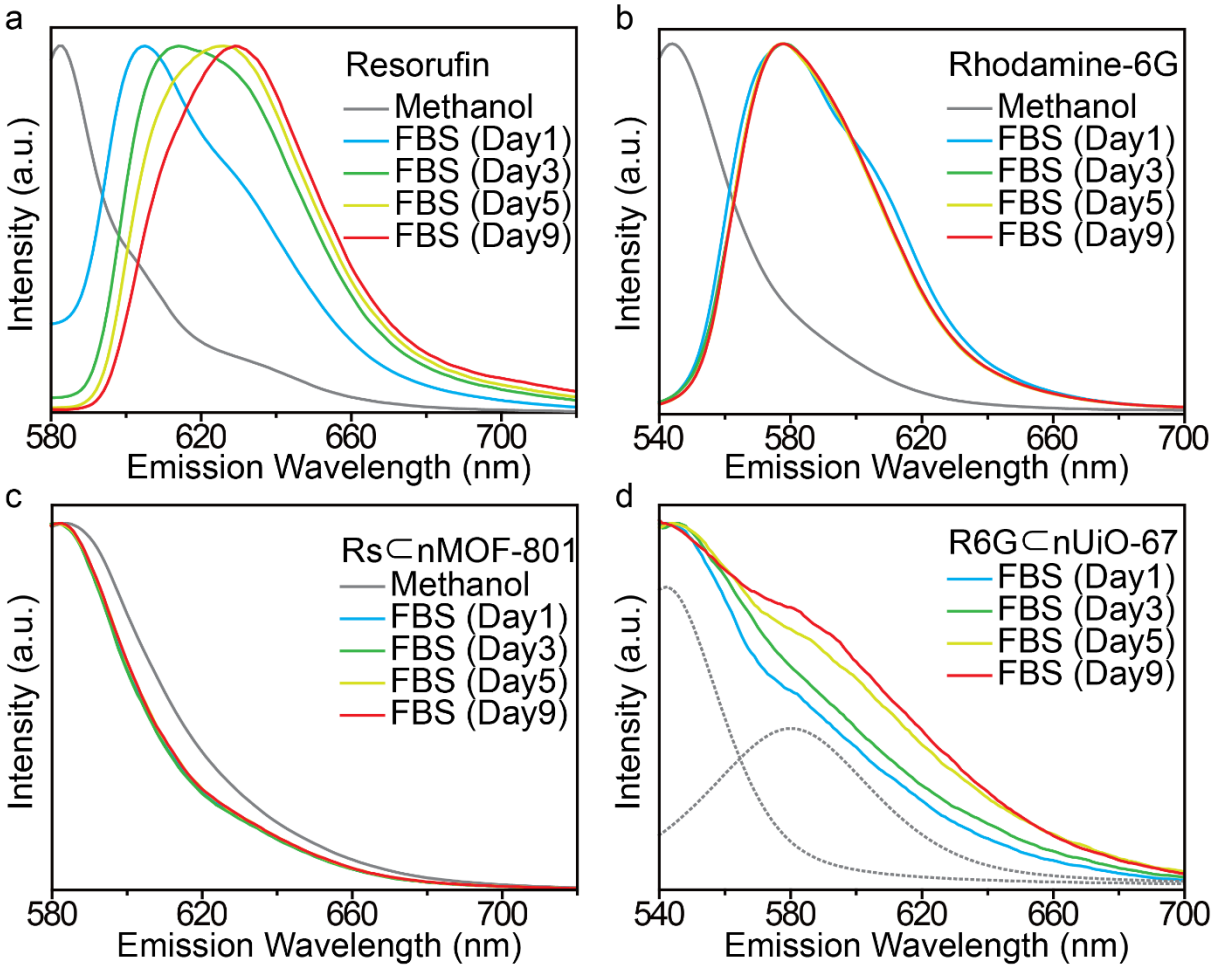

Figure S12. PL spectra of (a) Resorufin, (b) Rhodamine-6G, (c) Rs⊂nMOF-801, and (d) R6G⊂nUiO-67 excited at (a and c) 570 nm and (b and d) 525 nm in methanol and FBS. In FBS, the PL spectra are recorded on a daily basis for 9 days.

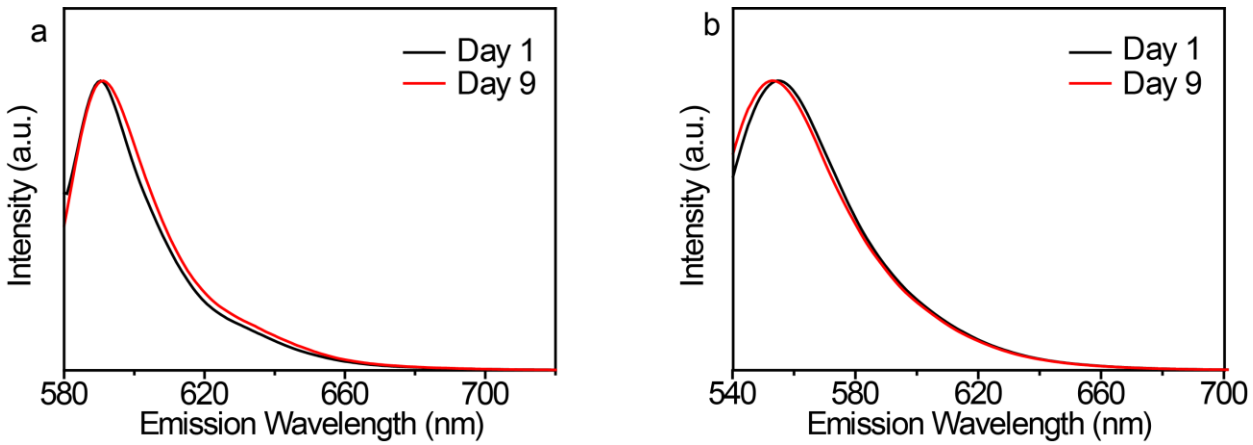

Figure S13. PL spectra of (a) Rs⊂nMOF-801 excited at 570 nm and (b) R6G⊂nUiO-67 excited at 525 nm in PBS. In PBS, the PL spectra are recorded on a daily basis for 9 days.

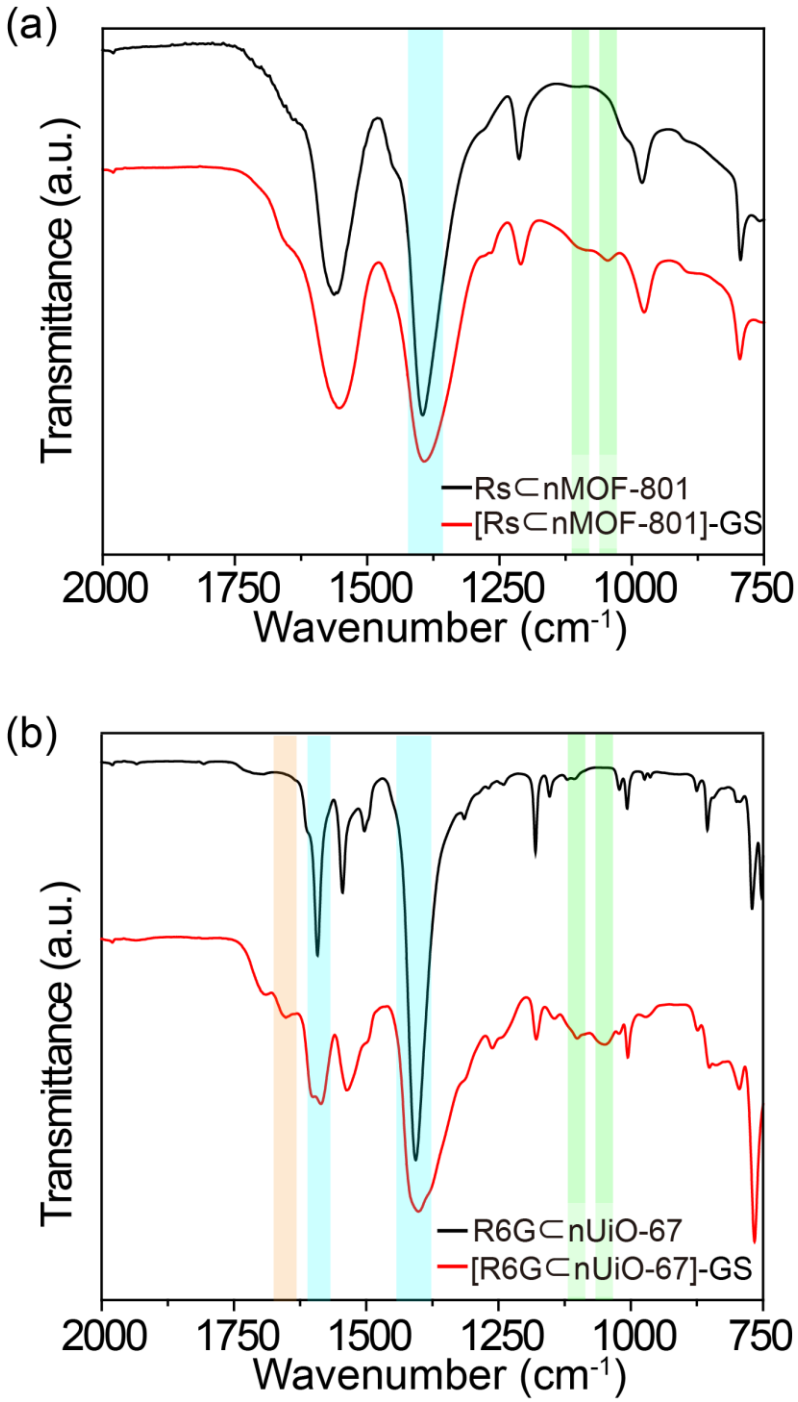

Figure S14. ATR-FTIR spectra of (a) Rs⊂nMOF-801 and [Rs⊂nMOF-801]-GS and (b) R6G⊂nUiO-67 and [R6G⊂nUiO-67]-GS.

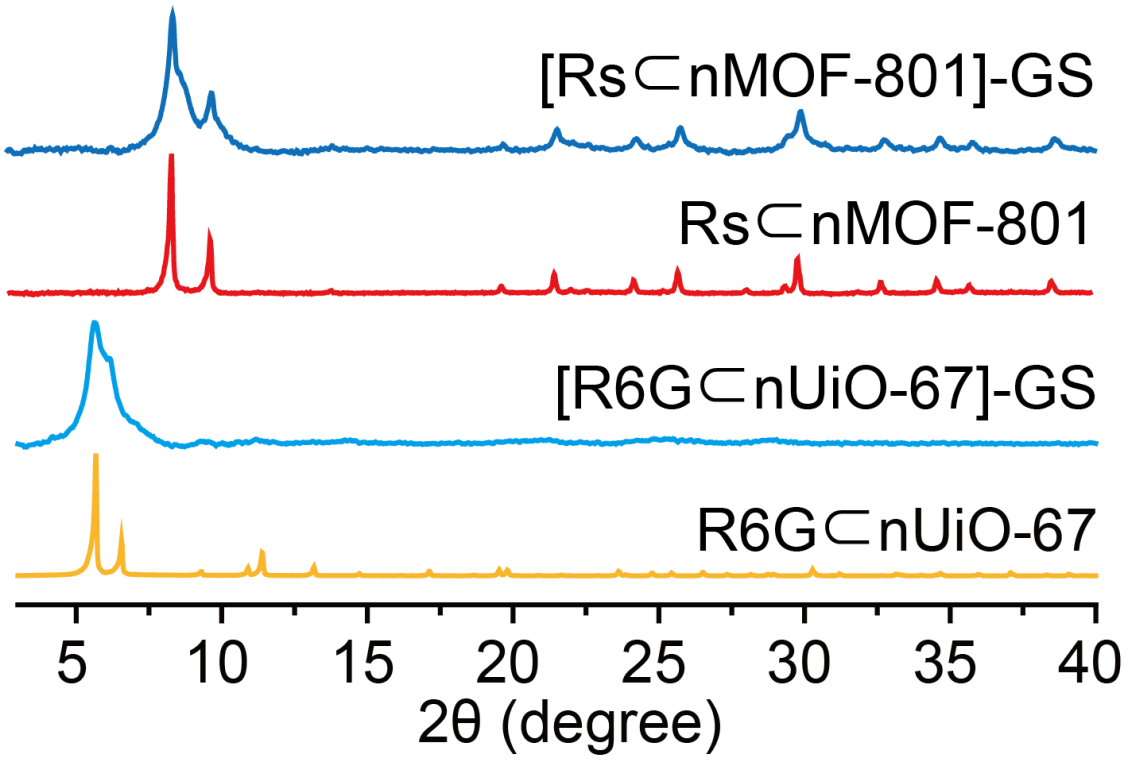

Figure S15. PXRD patterns of [Rs⊂nMOF-801]-GS and [R6G⊂nUiO-67]-GS.

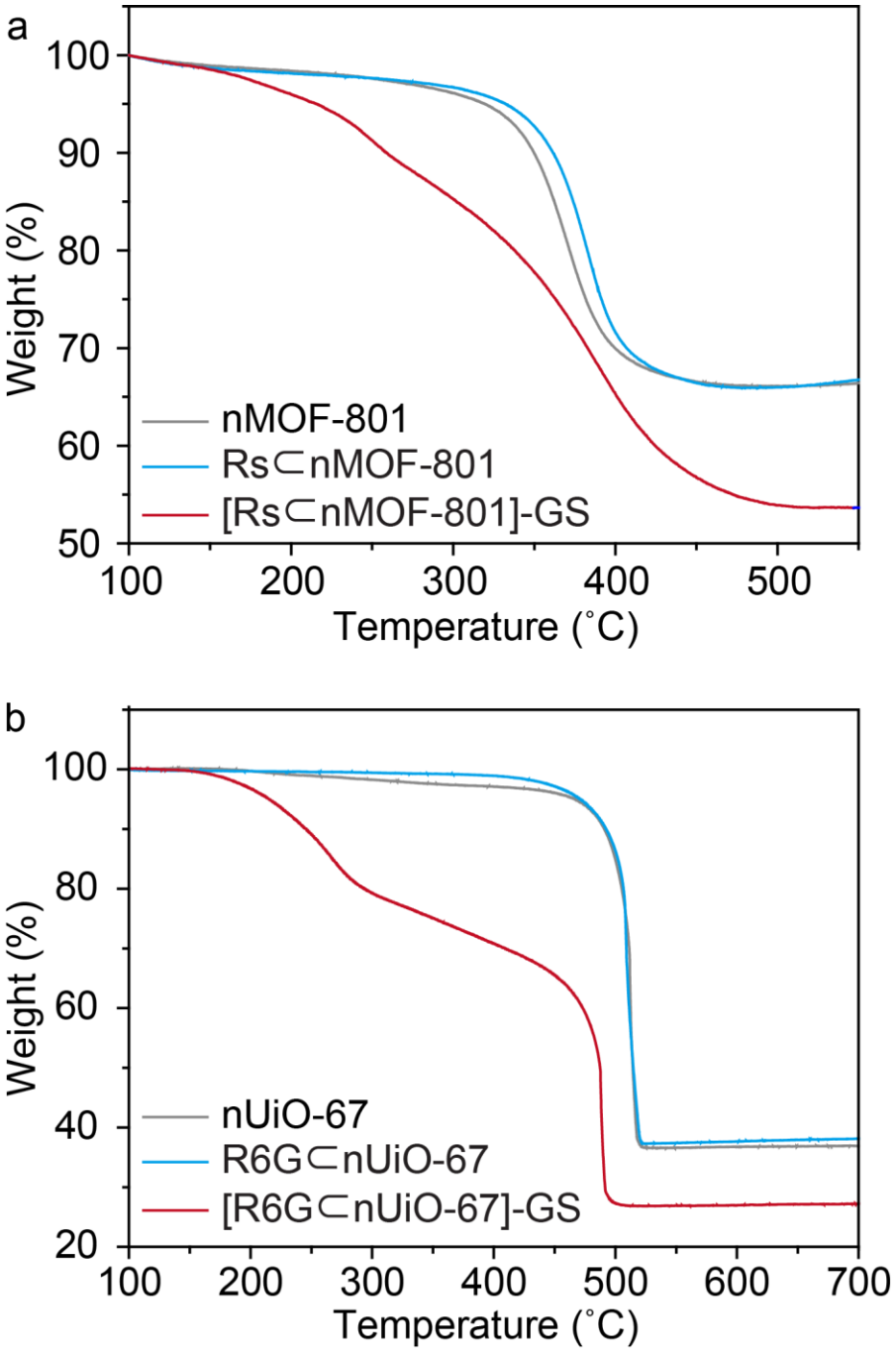

Figure S16. TGA profiles of (a) nMOF-801, Rs⊂nMOF-801 and [Rs⊂nMOF-801]-GS and (b) nUiO-67, R6G⊂nUiO-67 and [R6G⊂nUiO-67]-GS.

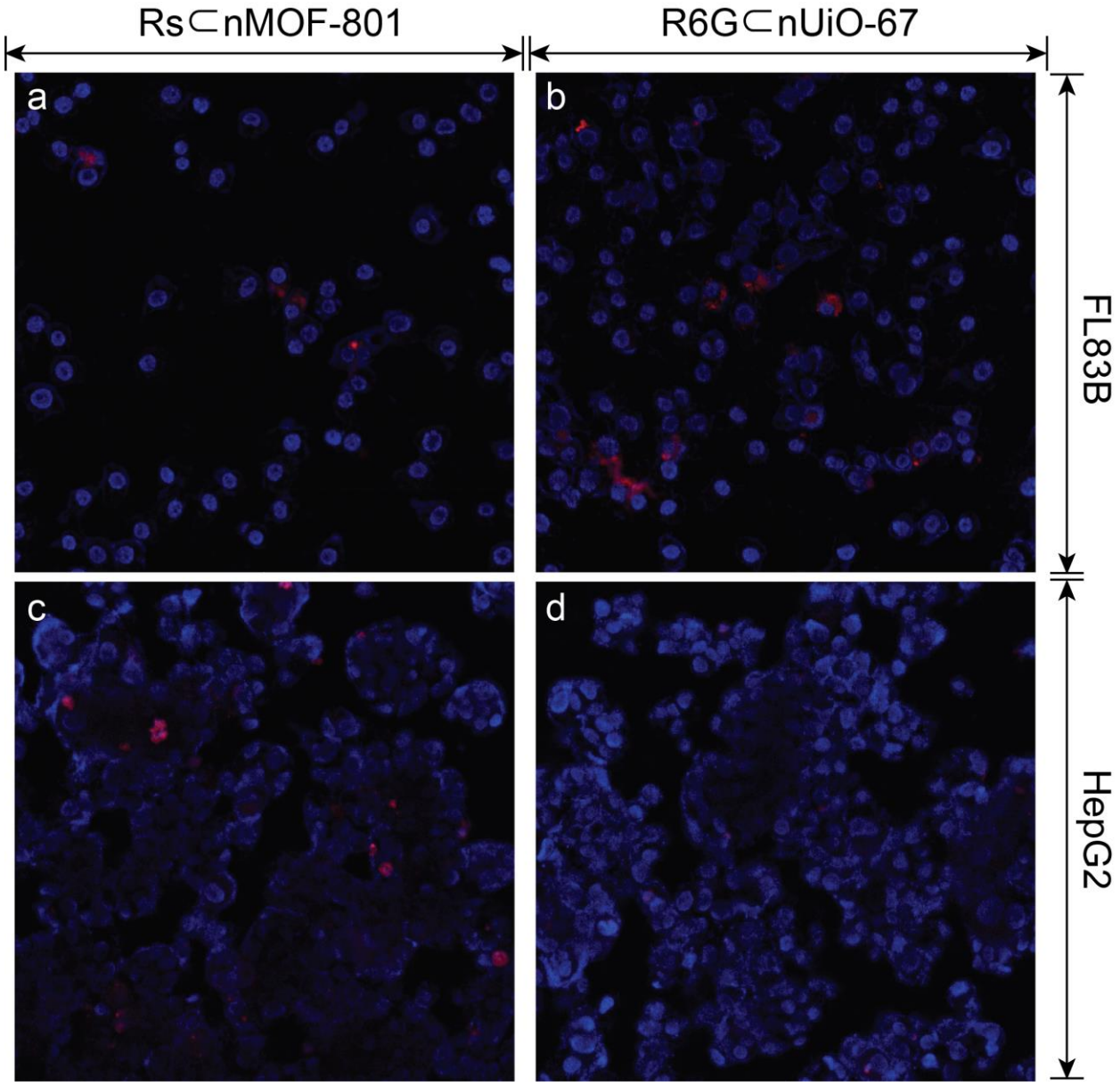

Figure S17. Images for (a and b) FL83B and (c and d) HepG2 using (a and c) Rs⊂nMOF-801 and (b and d) R6G⊂nUiO-67 (both without galactosylation).

REFERENCES

[1] Chen, J. L.; Ortiz, R.; Xiao, Y.; Steele, T. W. J.; Stuckey, D. C. Rapid fluorescence-based measurement of toxicity in anaerobic digestion. *Water Research* **2015**, *75*, 123.