Electronic Supplementary Information

Inverting glucuronidation of hymecromone *in situ* by catalytic nanocompartments

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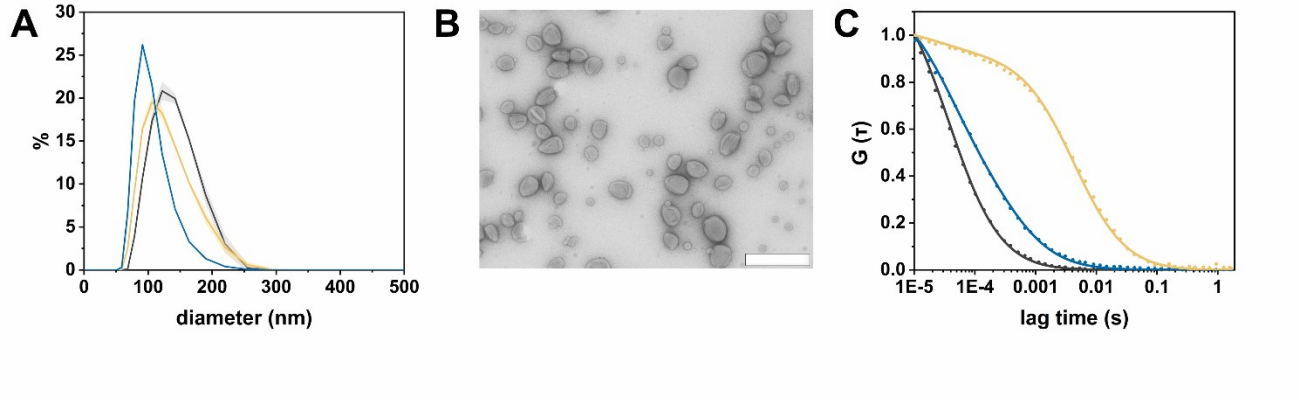

Fig. S1 Characterization of GUS-CNCs. (A) Size distribution of GUS-CNCs measured by DLS (black: intensity, yellow: volume, and blue: number, curves represent mean ± s.d. of 3 replications). (B) Transmission electron micrograph of GUS-CNCs showing the deflated structure typical for hollow spheres and their variation in size. (Scale bar: 500 nm) (C) Normalized FCS autocorrelation curves of

the free Atto488 dye (black), Atto488-labeled GUS (blue) and GUS-CNCs (yellow). Symbols represent raw data and solid lines represent fitted curves.

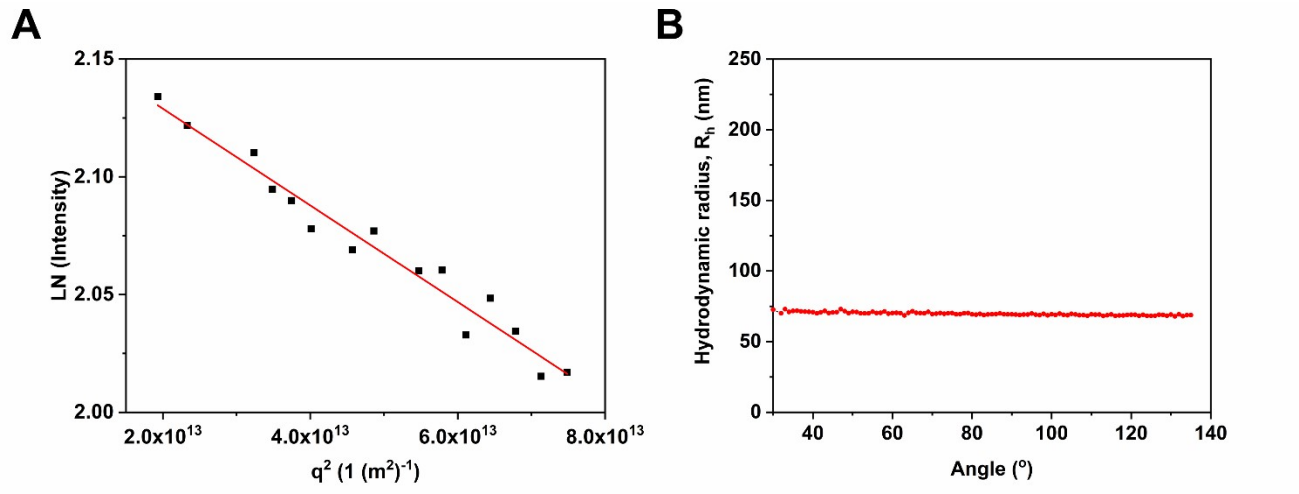

Fig. S2 (A) SLS data of GUS-melCNCs and linear fit to the Guinier equation, (B) DLS profile of GUS-melCNCs showing the mean hydrodynamic radius, R_h.

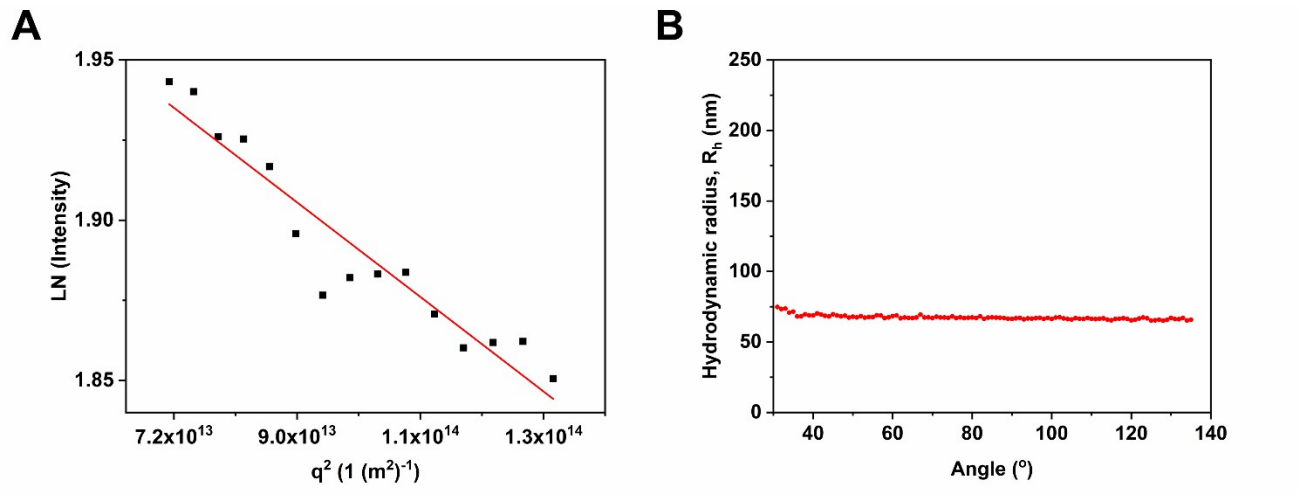

Fig. S3 (A) SLS data of GUS-CNCs and linear fit to the Guinier equation, (B) DLS profile of GUS-CNCs showing the mean hydrodynamic radius, R_h.

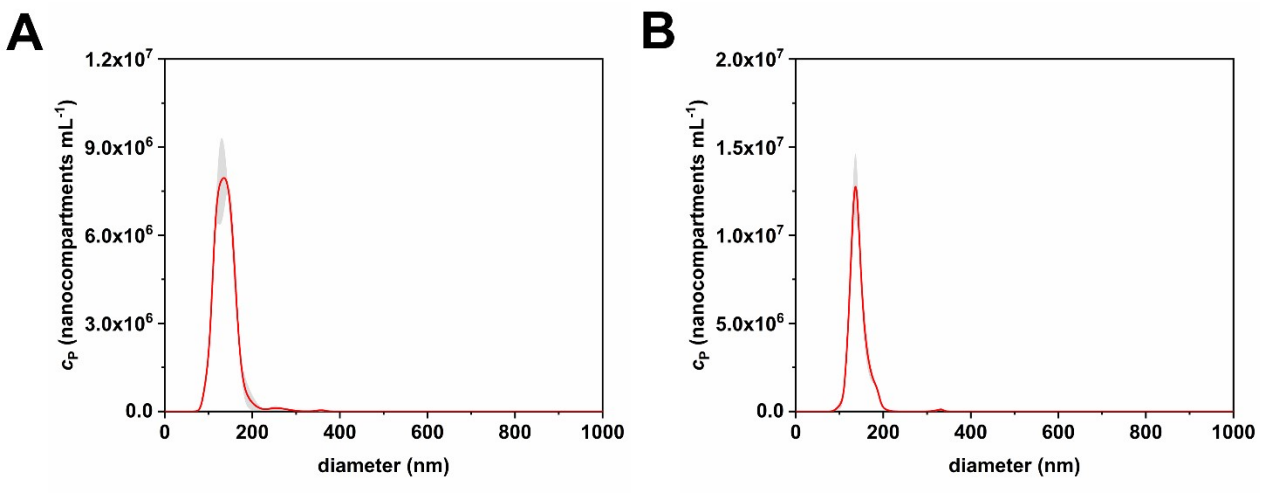

Fig. S4 Determination of the size and respective concentration of nanocompartments by NTA. Size distribution of (A) GUS-melCNCs (139.1 ± 29 nm) and (B) GUS-CNCs (143.1 ± 22.4 nm) in a 1:1000 dilution of the initial nanocompartment solution.

Table S1 Enzyme- and nanocompartment-related parameters quantified by FCS.

	Free dye	Dye-labeled GUS	GUS-melCNCs	GUS-CNCs
Diffusion time (τ_D) (µs)	35 ± 1.6	427 ± 40	3364 ± 705	3505 ± 1186
Hydrodynamic diameter (nm)	na	na	120 ± 19	120 ± 33

na, not applicable

Calculation of hydrodynamic radius (R_h) by Fluorescence Correlation Spectroscopy (FCS)

The hydrodynamic radius (R_h) of polymersomes, was calculated using Einstein-Stokes equation (1), where D is the diffusion coefficient, k_B – Boltzmann's constant, T – absolute temperature, and η – viscosity of the surrounding medium.

$$D = \frac{k_B T}{6\pi\eta R_h} \quad (1)$$

Estimation of number of melittin pores

The number of melittin pores per nanoreactor (NMP) was calculated by equation (2):

$$NMP = \frac{\frac{c\,N_A}{M_w\,c_{max}}}{12} \quad (2)$$

where c is the concentration of melittin in the total volume of polymersomes, N_A – the Avogadro number, M_W – the molecular weight of melittin, and c_{max} – the total concentration of GUS-melCNCs. This value was divided by 12, which is the average number of melittin monomers forming a pore.[1]

To estimate the number of melittin pores per nanoreactor, we prepared polymersomes with inserted melittin pores (50 μM) (melPSs) by film rehydration method. After SEC purification, the fraction containing non-inserted melittin was collected and its concentration was measured using a NanoDrop 2000c spectrophotometer (Thermofischer, U.S.A.). This value was multiplied by the volume of SEC fraction and subtracted by the initial concentration, resulting in the concentration of melittin in the total volume of polymersomes. The total concentration of polymersomes was determined by single nanoparticle tracking analysis (NTA) using a NanoSight NS300 device (Malvern, U.K.).

Estimation of β-glucuronidase encapsulation efficiency by BCA protein assay

The encapsulation efficiency of β-glucuronidase in polymersomes was calculated using the enhanced Pierce bicinchonic acid (BCA) protein assay according to the supplier's protocol with the following modifications: different GUS concentrations were used to generate a calibration curve for the quantification of the sample (Figure S5). Nanocompartments were first ruptured by sonication and then incubated with ethanol at a ratio of 3:1 (v/v) for 1 hour at 37 °C. The solution was filtered through 0.2 μm nylon membrane, 4 mm filter (Whatman™, General Electric, U.K.) and mixed at 1:2 (v/v) ratio with the BCA reagent. The mixtures of standards and samples were incubated for 2 hours at 37°C and the absorbance was measured at 562 nm using a SpectraMax id3 plate reader. The amount of encapsulated protein was multiplied by the volume of the sample after SEC purification and subtracted from the total amount that was used for rehydration, yielding the final GUS concentration in the nanocompartments solution. Accordingly, the encapsulation efficiency of GUS was found 15% ± 1.6%

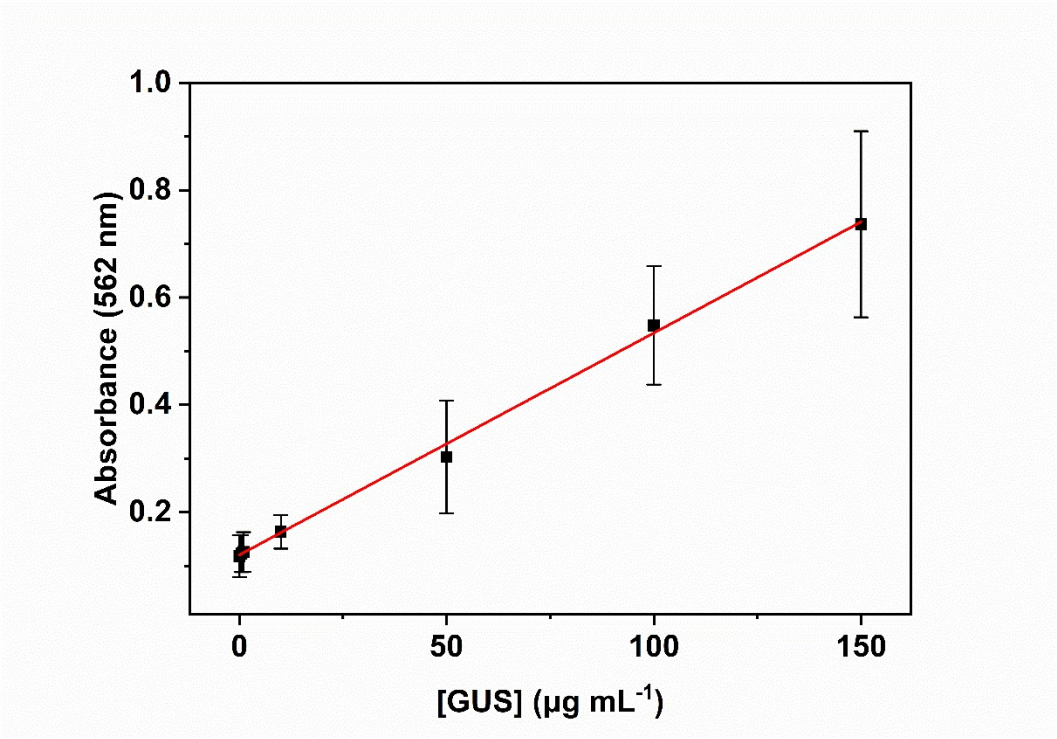

Fig. S5 GUS calibration curve for the BCA protein assay ($R^2 = 0.99$).

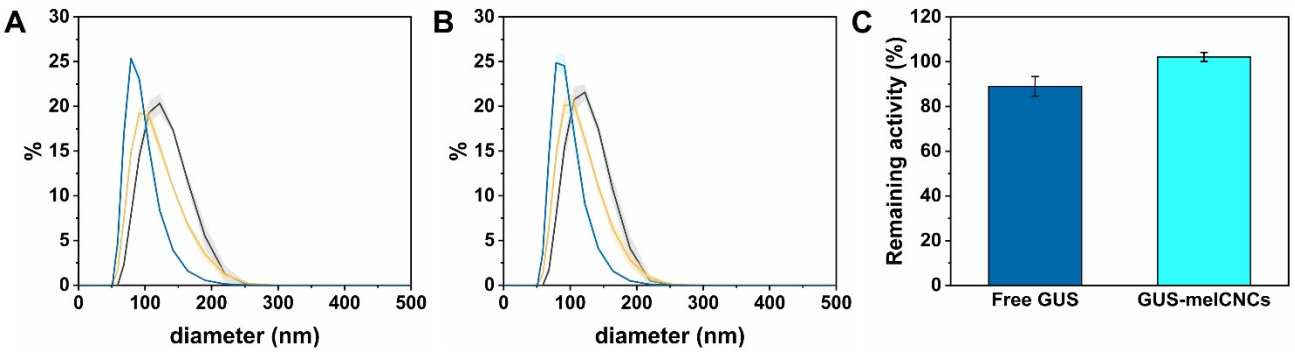

Fig. S6 Size distribution of (A) GUS-melCNCs and (B) GUS-CNCs, measured by DLS, after storage for 2 months at 4 °C. (black: intensity, yellow: volume, and blue: number). (C) Remaining percentages of activity of free (blue) and encapsulated (cyan) GUS upon storage at 4 °C for 2 months, Measurements represent the mean ± s.d.

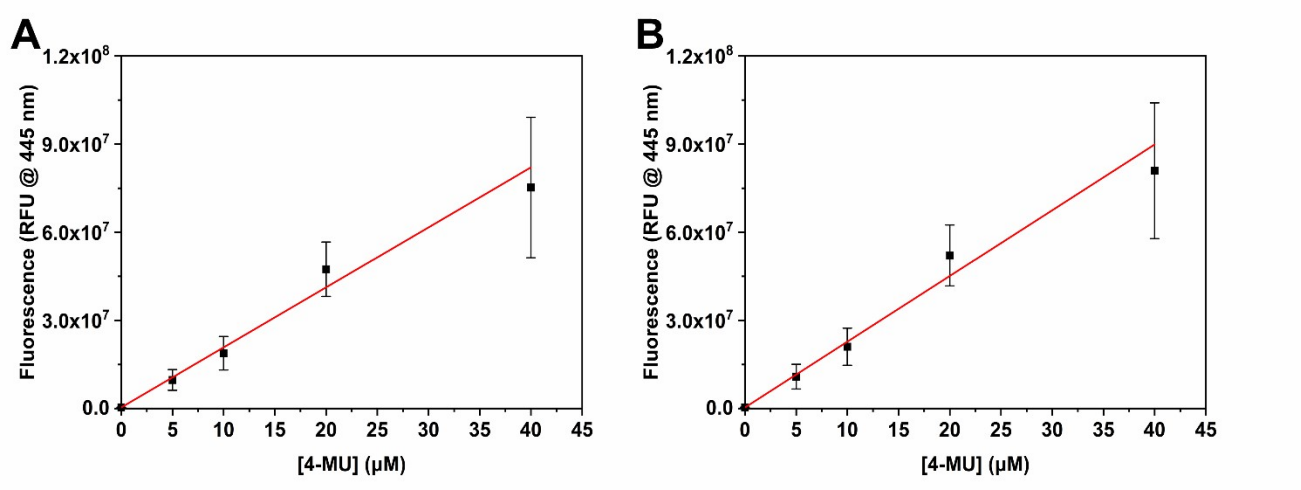

Fig. S7 Calibration curve using 4-MU standards in (A) PBS and (B) MEM ($R^2 = 0.98$).

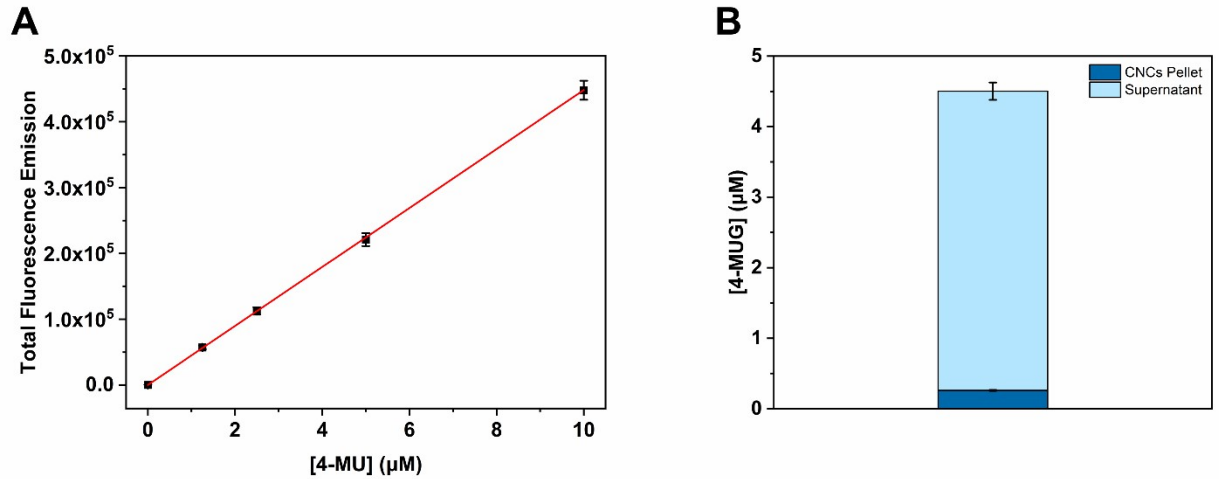

Fig. S8 (A) Calibration curve using 4-MU standards in ($R^2 = 0.99$), (B) Concentration of 4-MU found in GUS-melCNCs (blue) and in the supernatant (cyan) after incubation of GUS-melCNCs with 5 µM 4-MU for 1 hour.

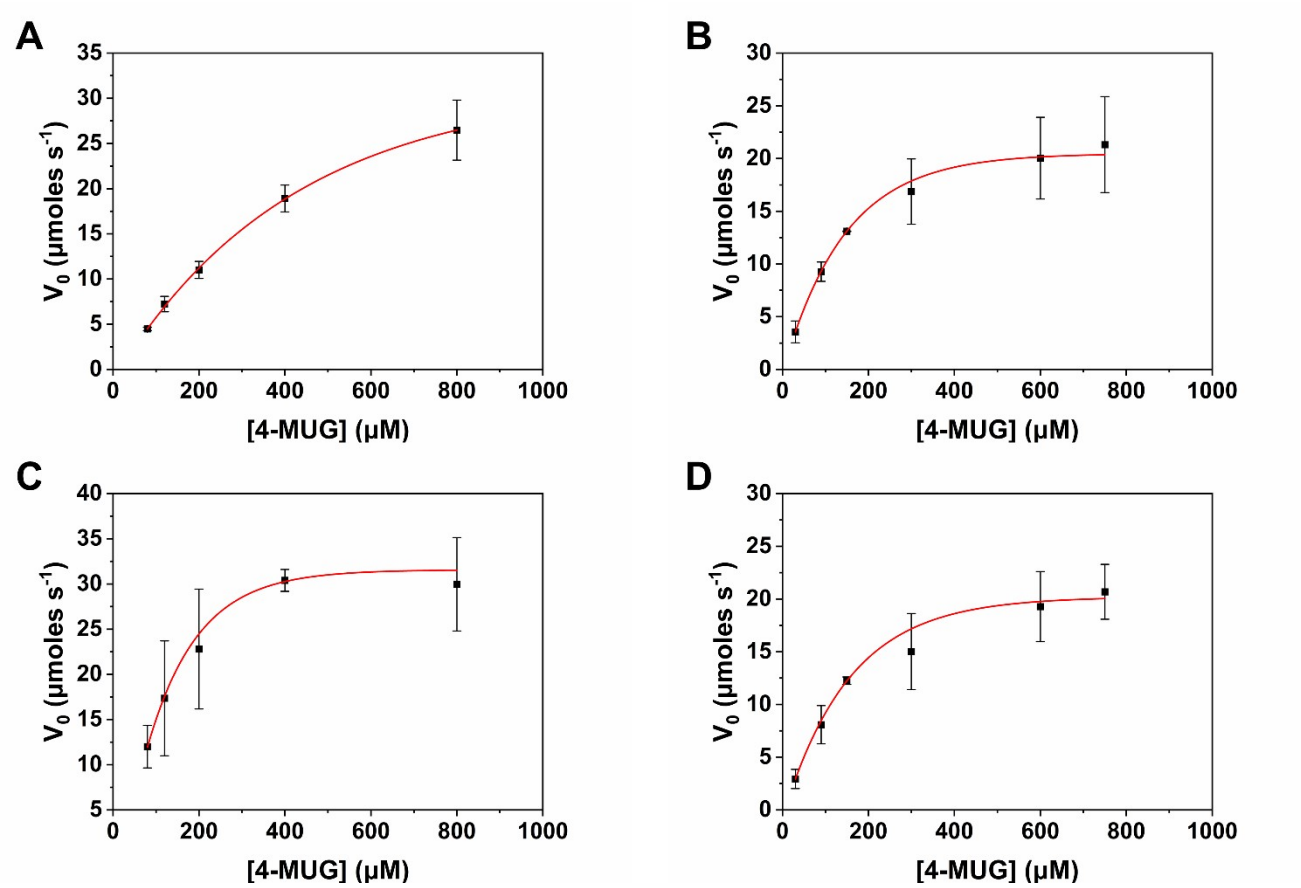

Fig. S9 Michaelis-Menten kinetics of (A) free GUS in PBS, (B) GUS-melCNCs in PBS, (C) free GUS in MEM and (D) GUS-melCNCs in MEM ($R^2 = 0.99$)

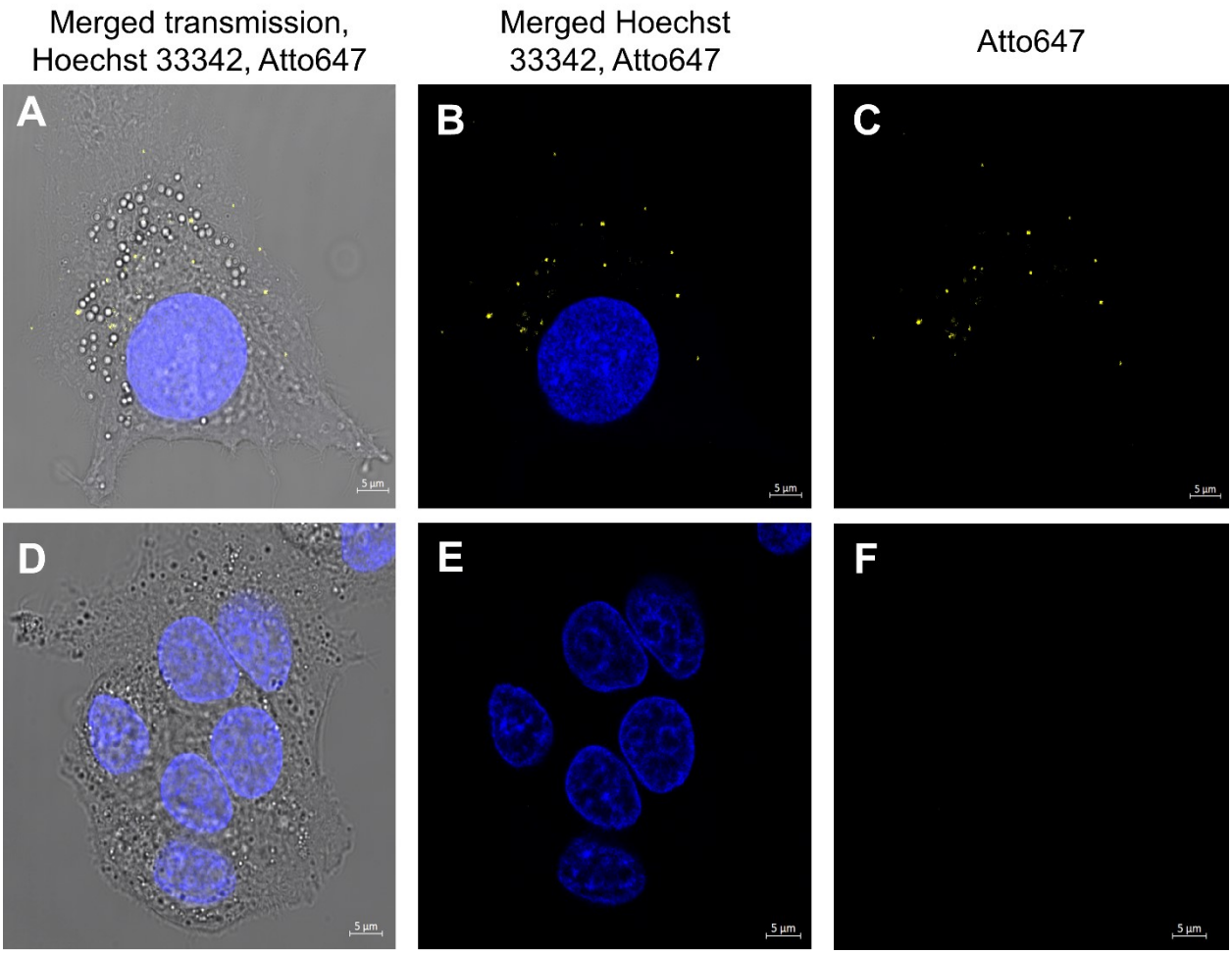

Fig. S10 (A - C) HepG2 cells incubated with Atto647-PSs (1.25 mg mL^{-1}) for 24 hours. (D - F) HepG2 control cells imaged under identical conditions.

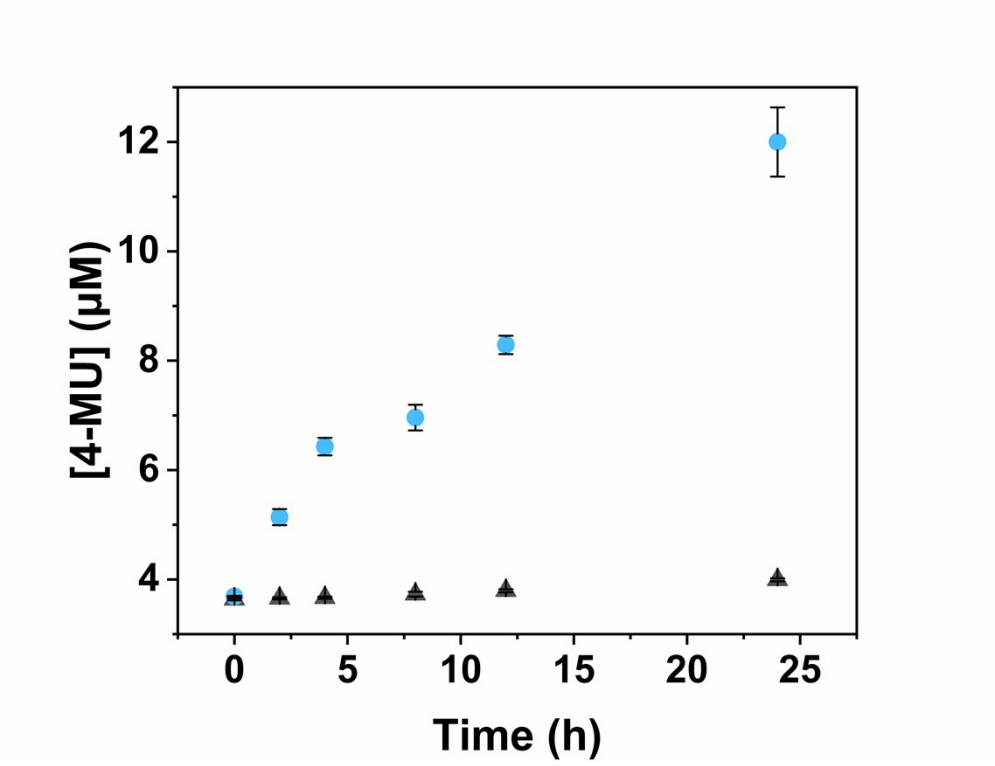

Fig. S11 Concentration of hymecromone produced by GUS-melCNCs (cyan circles) or PBS (black triangles) treated HepG2 cells over time. Relative fluorescence units from Figure 5 of the main manuscript were converted to 4-MU concentrations according to the calibration curve shown in Figure S7B. Graph shows mean ± s.d. of three independent experiments.

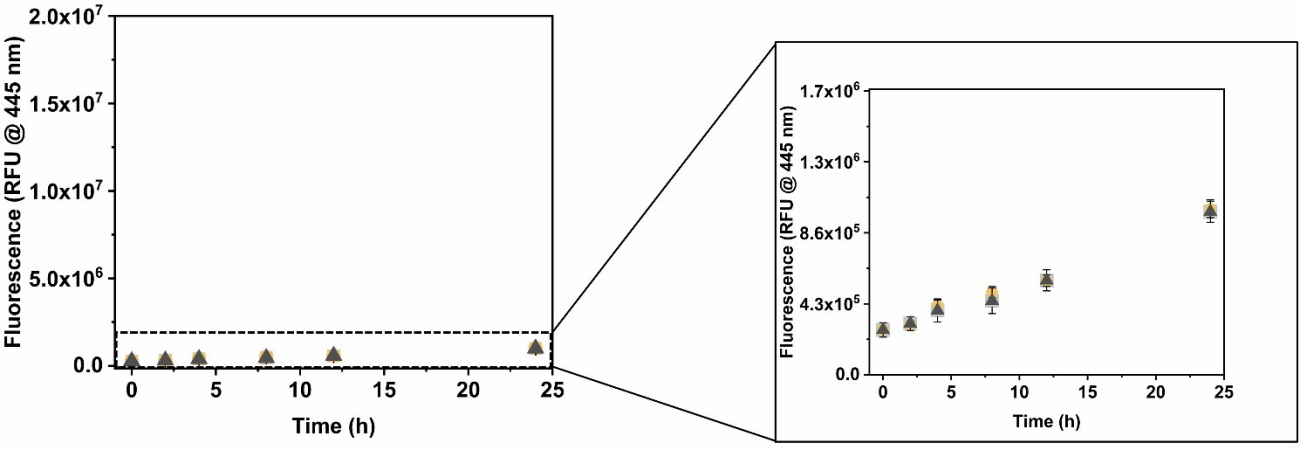

Fig. S12 Increase in fluorescence in HepG2 cells incubated for 24 hours with PBS (black triangles), PSs (grey squares), melPSs (yellow circles) and 400 µM 4-MUG, Graph shows mean ± s.d. of three independent experiments.

References

1 J. H. Lin and A. Baumgaertner, *Biophys. J.*, 2000, **78**, 1714–1724.