

1 ***Targeting glutamine metabolism combined***
2 ***with radiotherapy in radioresistant***
3 ***hepatocellular carcinoma***

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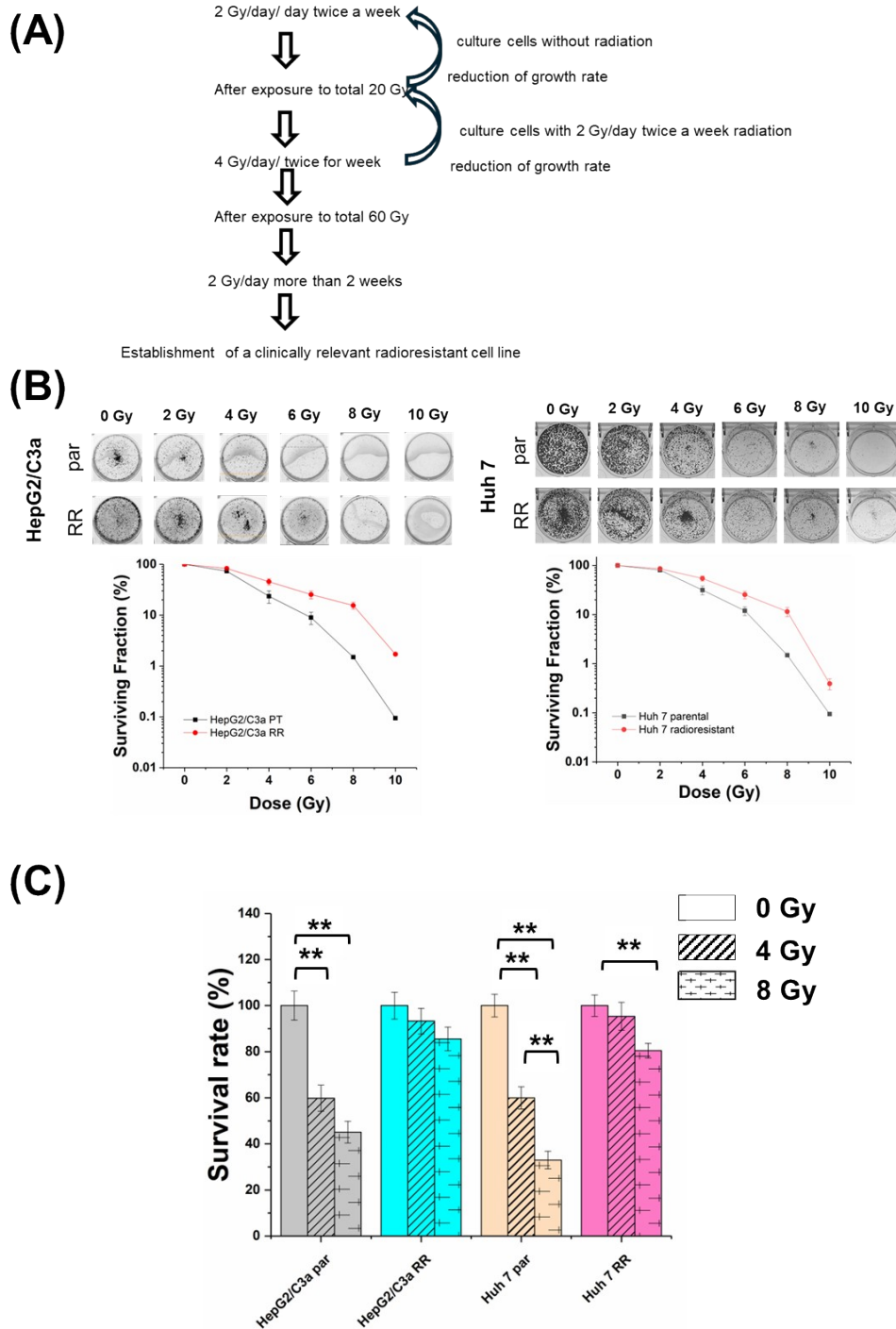
31 * *Author for equal contribution for corresponding author*

1 **Table S1. Characterization of the GNDs@gelatin NPs**

	GNDs@gelatin	GNDs@gelatin/DON
Particle mean size (nm)	125.4 ± 16.3 nm	120.6 ± 11.4 nm
PDI	0.098 ± 0.019	0.093 ± 0.012
Zeta potential (mV)	-11.37 ± 0.42 mV,	-2.52 ± 0.32 mV
Encapsulation efficiency (%)		66.5 %
Drug loading (%)		5.4 %

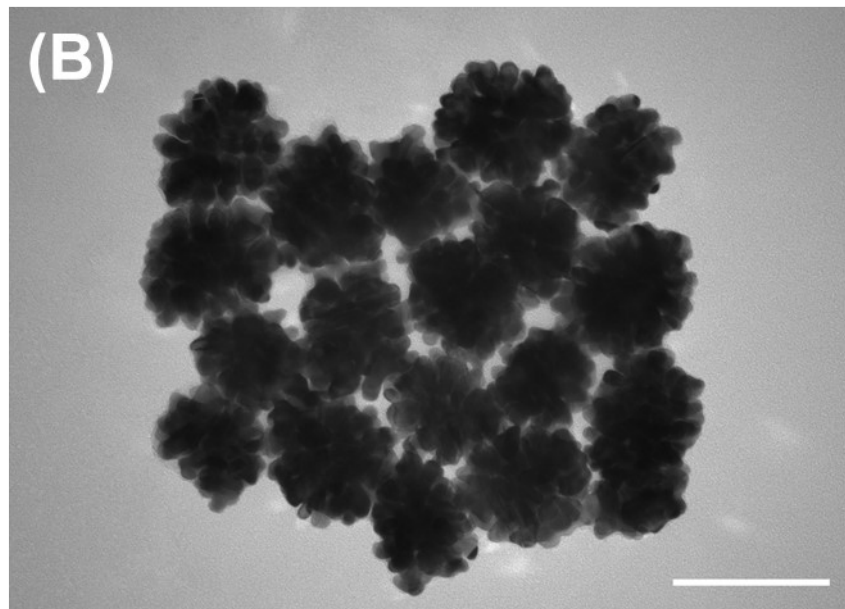
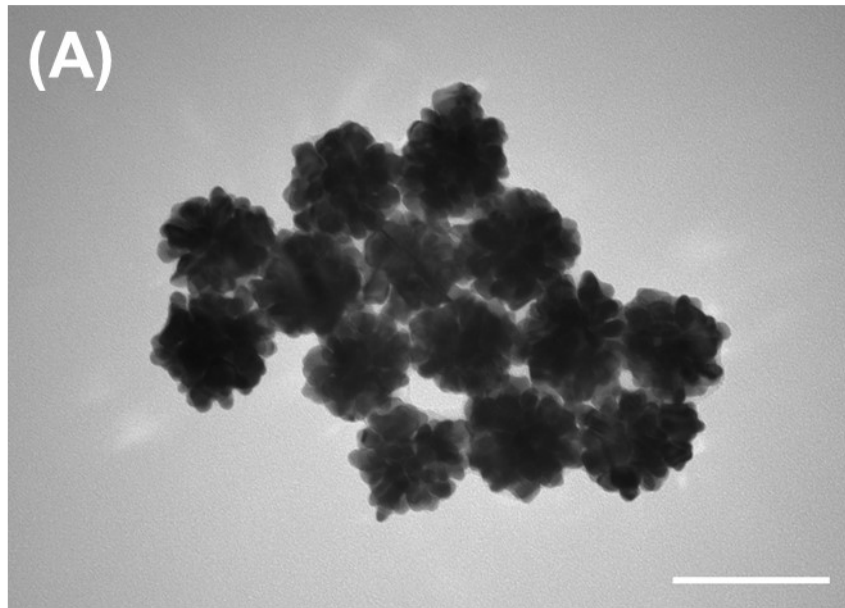
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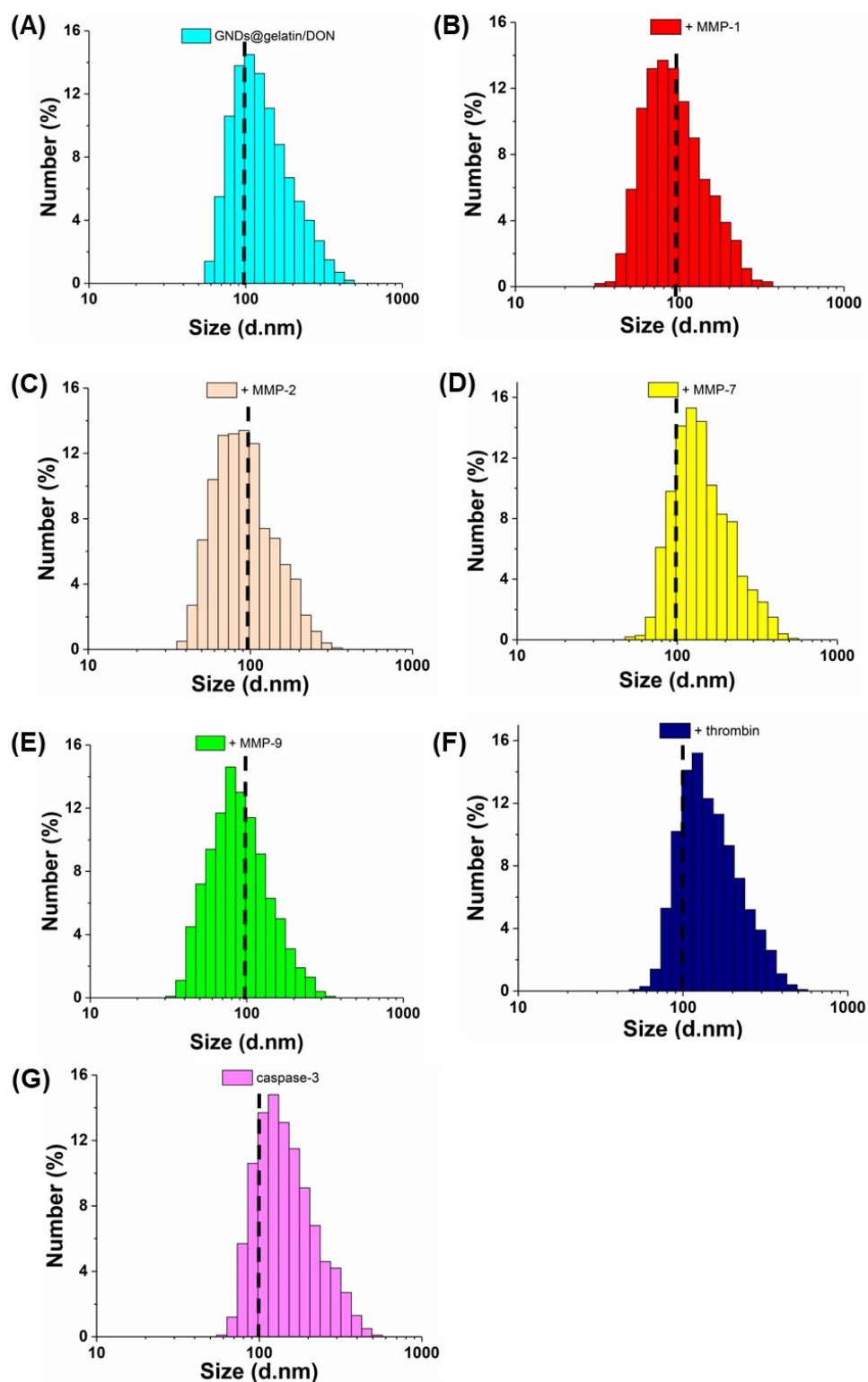
2 **Figure S1.** Establishment of clinically relevant radioresistant (CRR) hepatocellular
 3 carcinoma cell lines. (A) Stepwise induction of CRR cell lines through incremental
 4 increases in X-ray dose. (B) Clonogenic survival of parental HepG2/C3a and Huh7
 5 (PT) cells compared with their CRR counterparts (RR). (C) Survival assays of HCC PT
 6 and RR cells following 5 days of treatment with radiation and subsequent X-ray
 7 exposure. Statistical significance was determined using Student's t-test; * $p < 0.05$, ** p
 8 < 0.01 .



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2 **Figure S2.** Corresponding TEM images of GNDs (A) before, and (B) after DON
3 modification. TEM scale bars are 100 nm.

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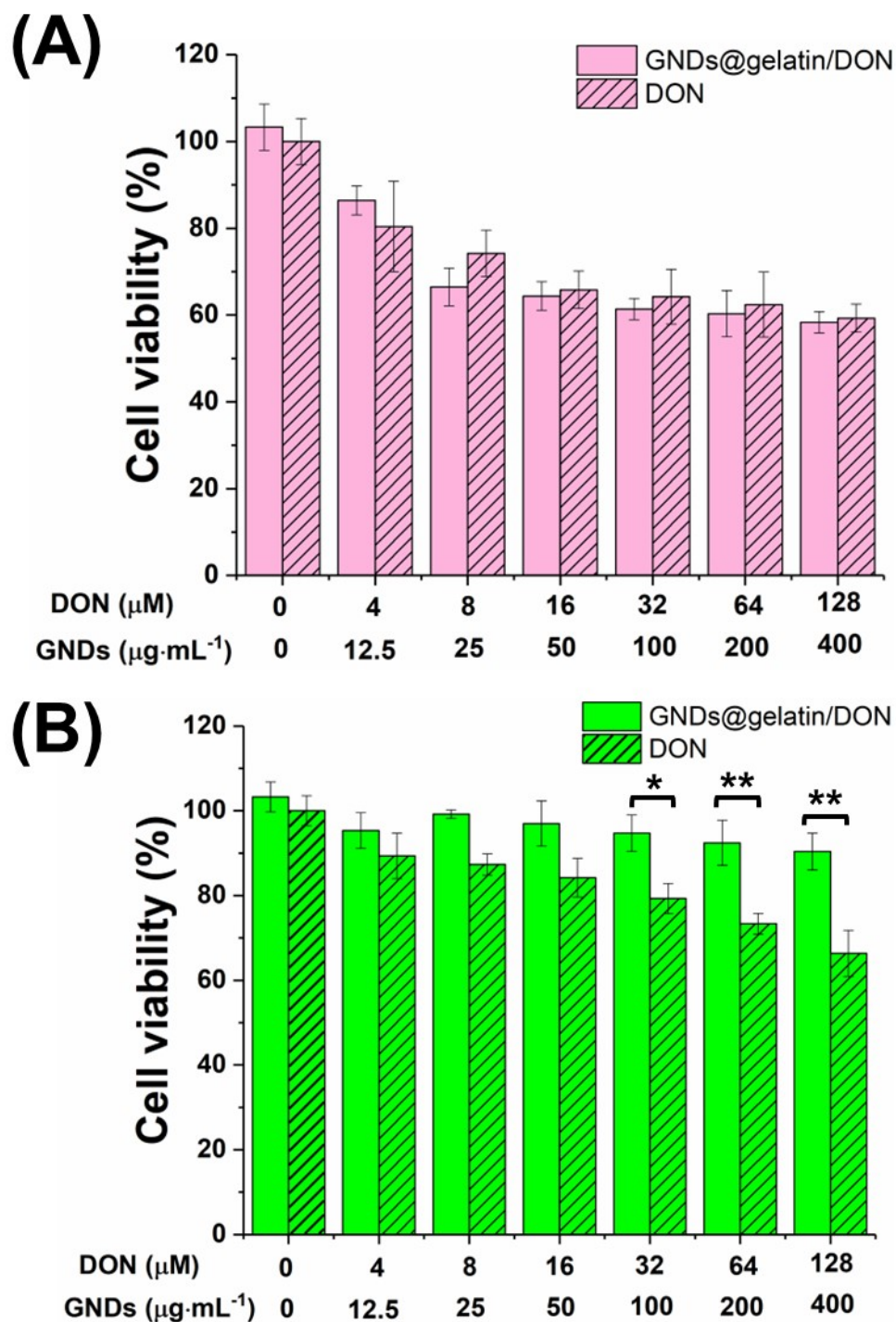
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2 **Figure S3.** Characterization of protease-triggered shrinkage of GNDs@gelatin/DON.

3 Hydrodynamic size distributions of GNDs@gelatin/DON (A) before treatment and

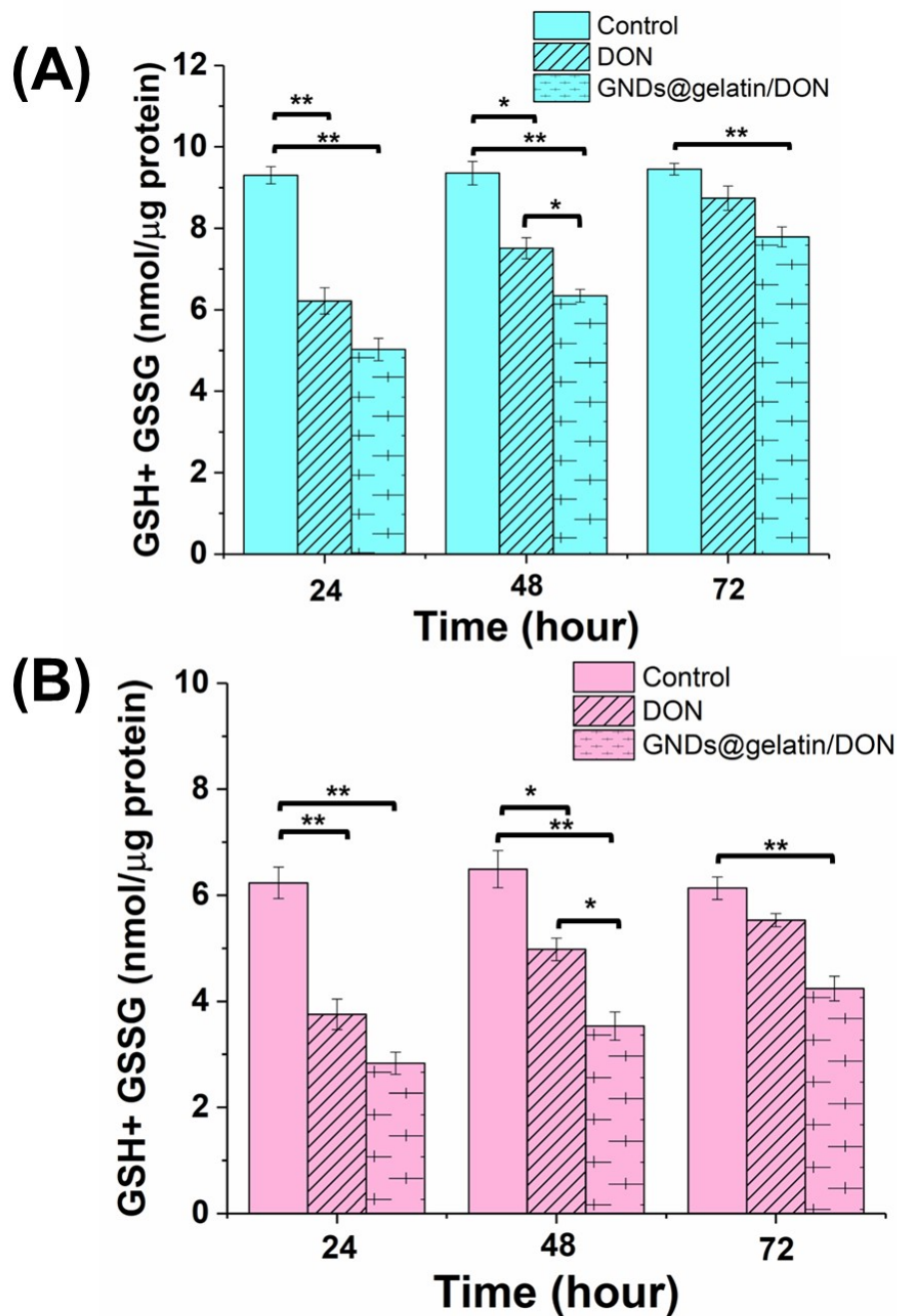
4 after 6 h incubation at 37 °C with (B) MMP-1, (C) MMP-2, (D) MMP-7, (E) MMP-9,

1 (F) thrombin, and (G) caspase-3.



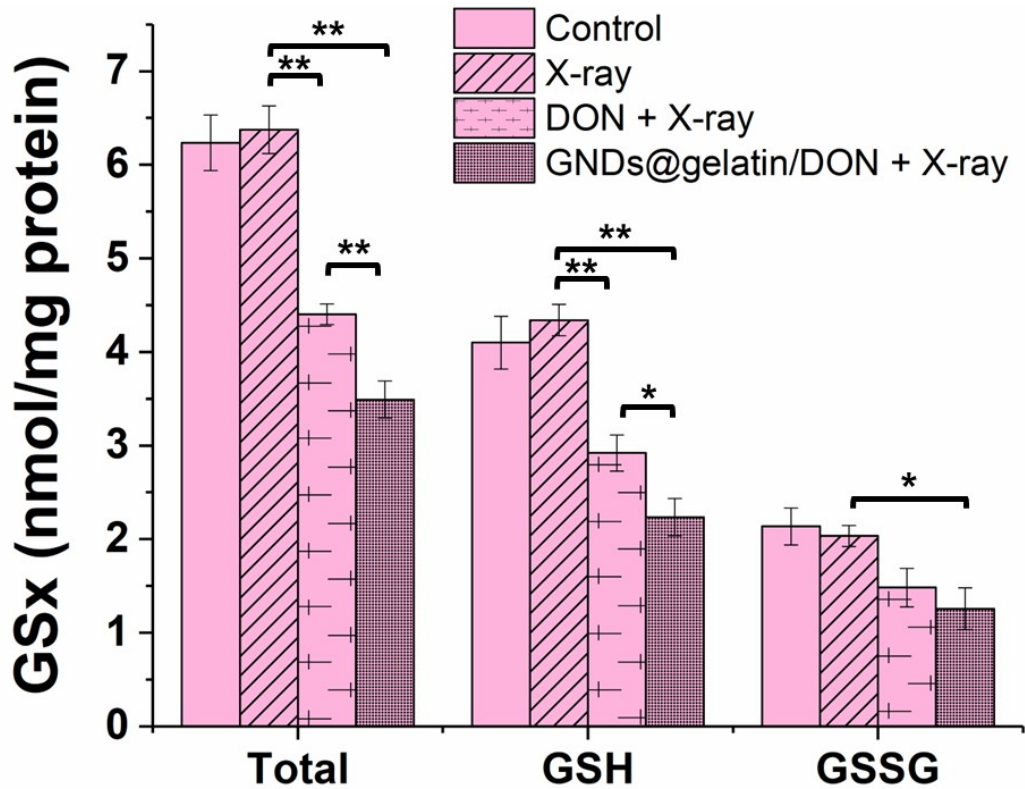
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3 **Figure S4.** Cell viability of (A) Huh7 RR and (B) H-4-II-E cells after 48 h incubation
4 with different concentrations of DON or GNDs@gelatin/DON. Data are expressed as
5 mean ± SEM (n = 3). Statistical significance was determined using Student's *t*-test; **p*
6 < 0.05, ***p* < 0.01.



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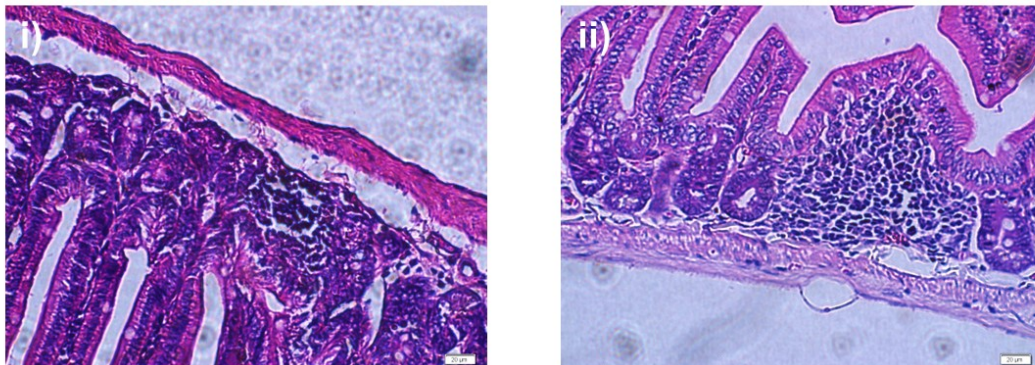
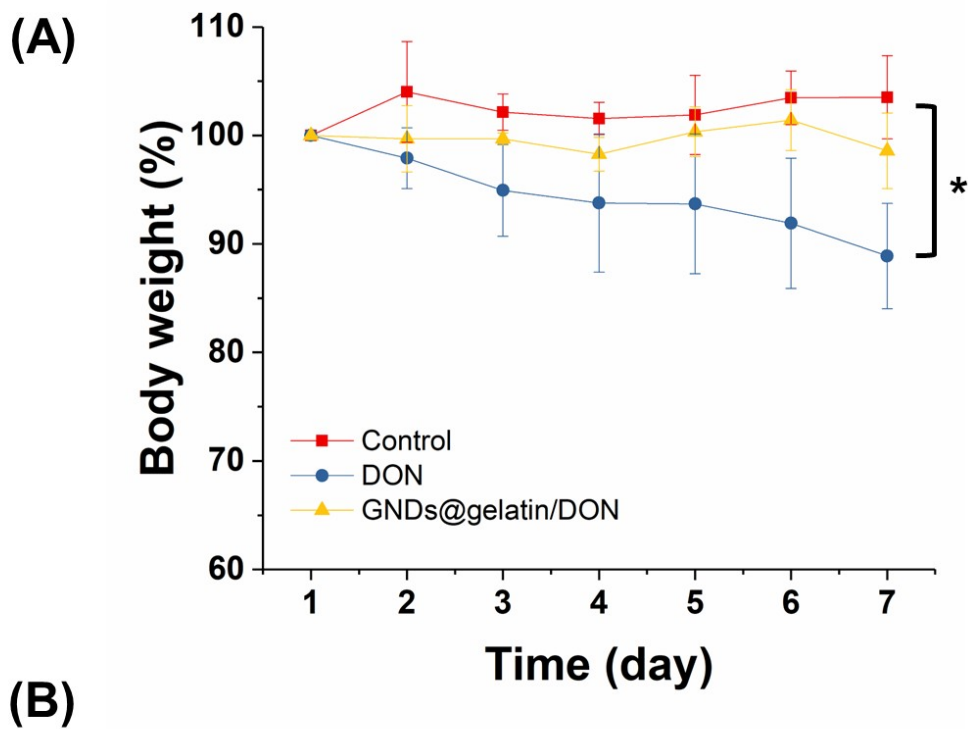
2 **Figure S5.** Effects of DON and GNDs@gelatin/DON on intracellular total glutathione.
 3 (A) HepG2/C3a RR and (B) Huh7 RR cells were treated with DON or
 4 GNDs@gelatin/DON, and total glutathione levels were quantified using the
 5 GSH/GSSG assay at 24, 48, and 72 h post-treatment. Data are presented as mean ±
 6 SEM (n = 3). Statistical significance was determined using Student's *t*-test; **p* < 0.05,
 7 ***p* < 0.01.



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2 **Figure S6.** Effects of X-ray, DON, or GNDs@gelatin/DON, alone or in combination,
 3 on total glutathione, GSH, and GSSG levels in Huh7 RR cells. Cells were treated with
 4 8 μM DON or 25 $\mu\text{g}\cdot\text{mL}^{-1}$ GNDs@gelatin/DON (equivalent to $\sim 8 \mu\text{M}$ DON) for 24 h,
 5 followed by 6 Gy 10 MV photon irradiation and an additional 24 h incubation at 37 °C.
 6 Treated cells were then harvested for glutathione analysis. Data are presented as mean
 7 \pm SEM (n = 5). Statistical significance was determined using Student's *t*-test; * $p < 0.05$,
 8 ** $p < 0.01$.

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2 **Figure S7.** Evaluation of body weight changes, side effects, and selective reduction *in*
 3 *vivo* following GNDs@gelatin/DON treatment. (A) Comparison of systemic toxicity
 4 and side effects between free DON and GNDs@gelatin/DON. Data are presented as
 5 mean \pm SEM (n = 4), and statistical significance was determined using Student's *t*-test.
 6 $*p < 0.05$ (B) Representative H&E staining of gastrointestinal tissues from mice treated
 7 for 7 days with (i) DON or (ii) GNDs@gelatin/DON.

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