Supplementary Information

Real-time imaging of oxidative and nitrosative stress in the liver of live animals for drug-toxicity testing

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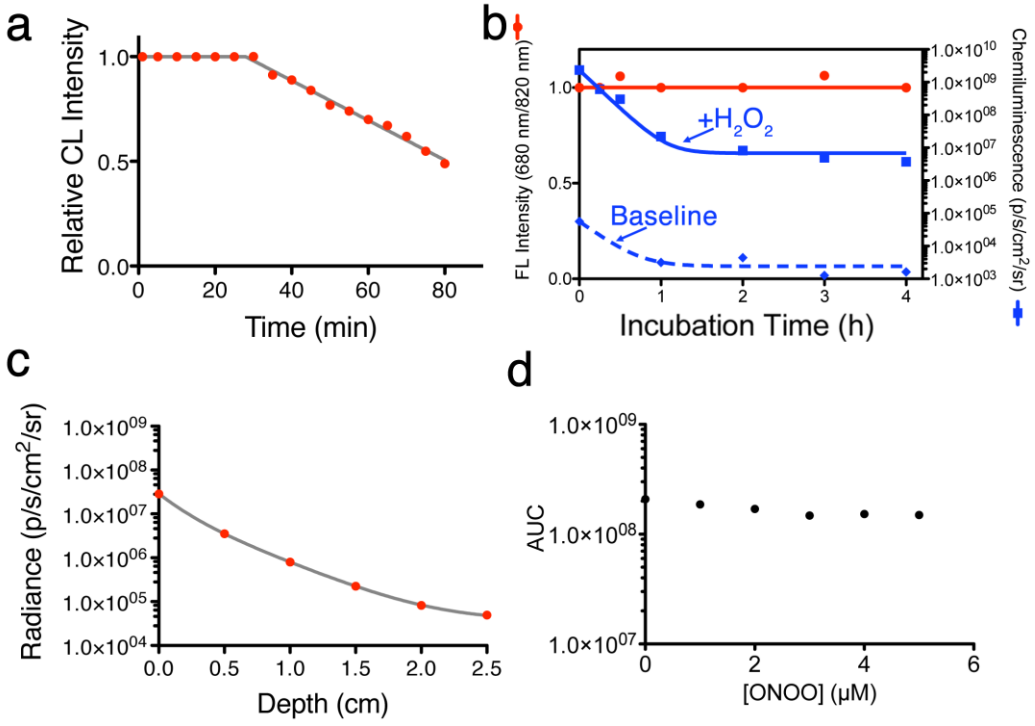

Supplementary Figure S1. Signal stability and imaging depth penetration for CF-SPN *in vitro*. (a) The lifetime of chemiluminescent signal production from CF-SPN (5 μg/mL) in 1x PBS incubated with H_2O_2 (50 mM). (b) Stability of the baseline fluorescence ratio (red) and baseline chemiluminescence emission (dashed blue) upon incubation of CF-SPN (5 μg/mL) in undiluted mouse serum at 37 °C. To demonstrate the prolonged capacity for ROS detection, H_2O_2 (6 μM) as added to CF-SPN incubations at indicated times (solid blue). (c) Chemiluminescence imaging depth of penetration of CF-SPN (5 μg/mL) through a gelatin-hemoglobin-intralipid imaging phantom. (d) The total chemiluminescence, as measured by area under the luminescence curve (AUC), from CF-SPN after incubation with different concentrations of $ONOO^-$.

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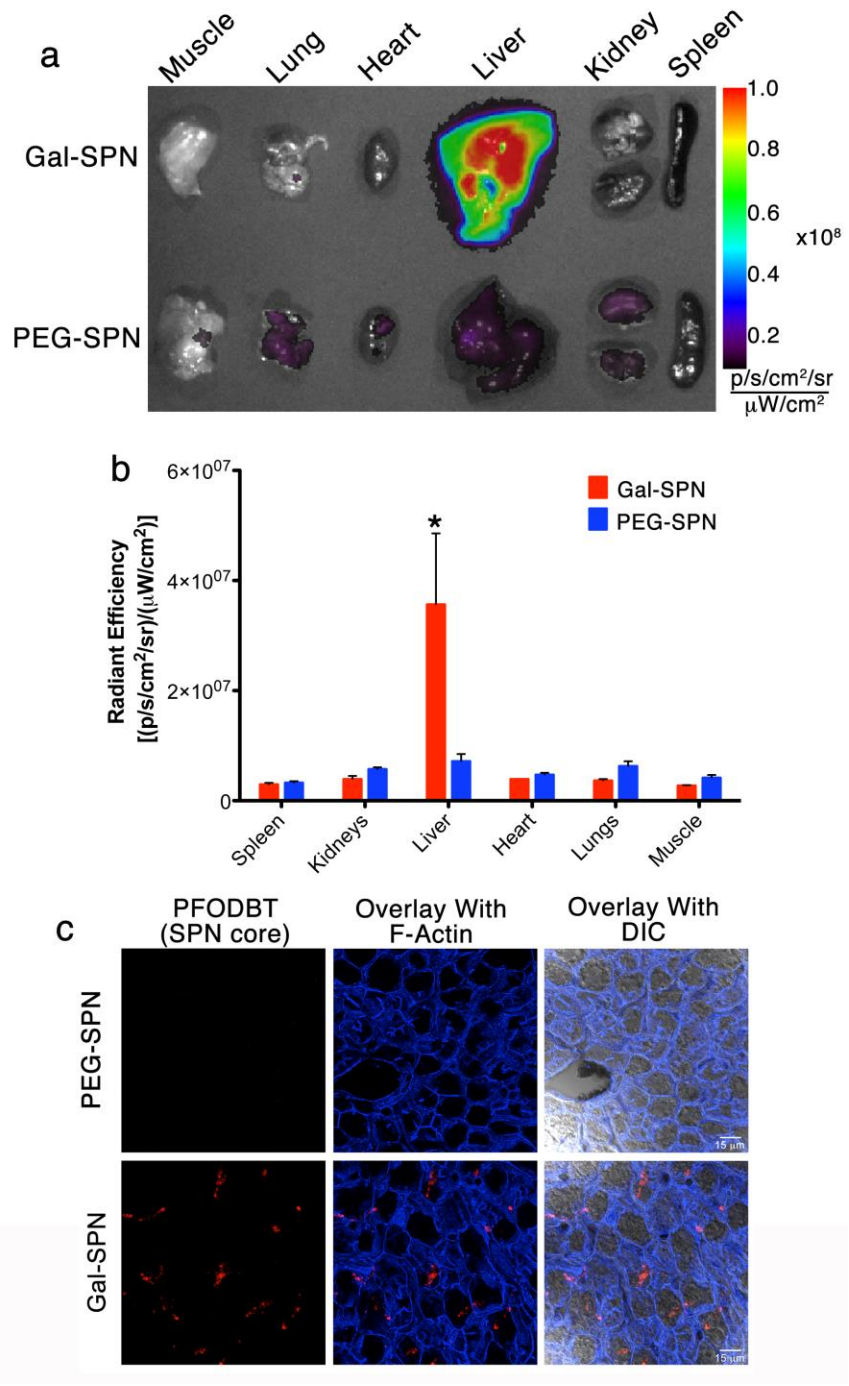

Supplementary Figure S2. Effective liver targeting through the conjugation of galactose to the SPN surface. Nanoparticles composed of PFODBT and PS-*g*-PEG-Galactose (Gal-SPN) or PS-*g*-PEG (PEG-SPN) were administered i.v. (0.8 mg each). Tissues were excised and imaged 45 min after nanoparticle administration. (a) Representative image of the biodistribution of Gal-SPN (top) and PEG-SPN (bottom). (b) Organ fluorescence (ex/em=580/680 nm) was quantified and represented as the mean±s.d. (n=3). * $p<0.05$ (Mann-Whitney U-test). (c) Uptake of untargeted SPN (top) and asialoglycoprotein

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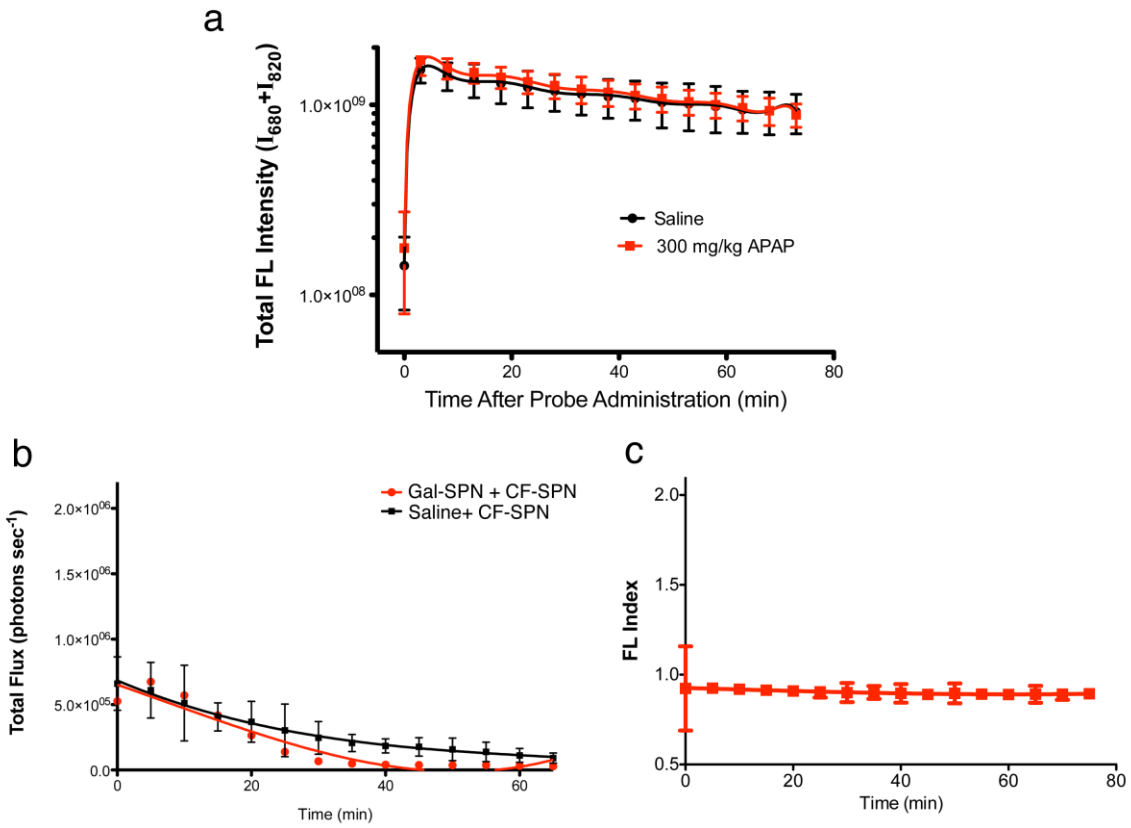
receptor-targeted Gal-SPN (bottom) 30 min following intravenous administration. Images are Z-projections averaged over 20 slices and a total z-depth of 7.7 μm. Nanoprobe uptake was marked by fluorescence from the conjugated polymer core composed of PFODBT (red), and cellular boundaries were marked by staining of F-actin (blue). Fluorescence images were overlaid with DIC of the liver sections. Scale bars = 15 μm.



Supplementary Figure S3. (a) The uptake and retention of CF-SPN in the liver as measured by tracking total fluorescence intensity over time. Total fluorescence intensity is the sum of emission at 680 nm and at 820 nm (I_{680}+I_{820}), which was measured for mice treated with saline (black curve) or with 300 mg/kg APAP (red curve). Data points represent the mean±s.d. of 3 mice. (b & c) *In vivo* assessment of hepatotoxic potential of administered nanoparticles. The galactose-targeted nanoparticles (Gal-SPN, red circles) or saline (black squares) were administered i.v. 15 min prior to CF-SPN, and the chemiluminescence (b) and fluorescence index (c) was recorded. Data represents the mean±s.d. of n=3 mice.

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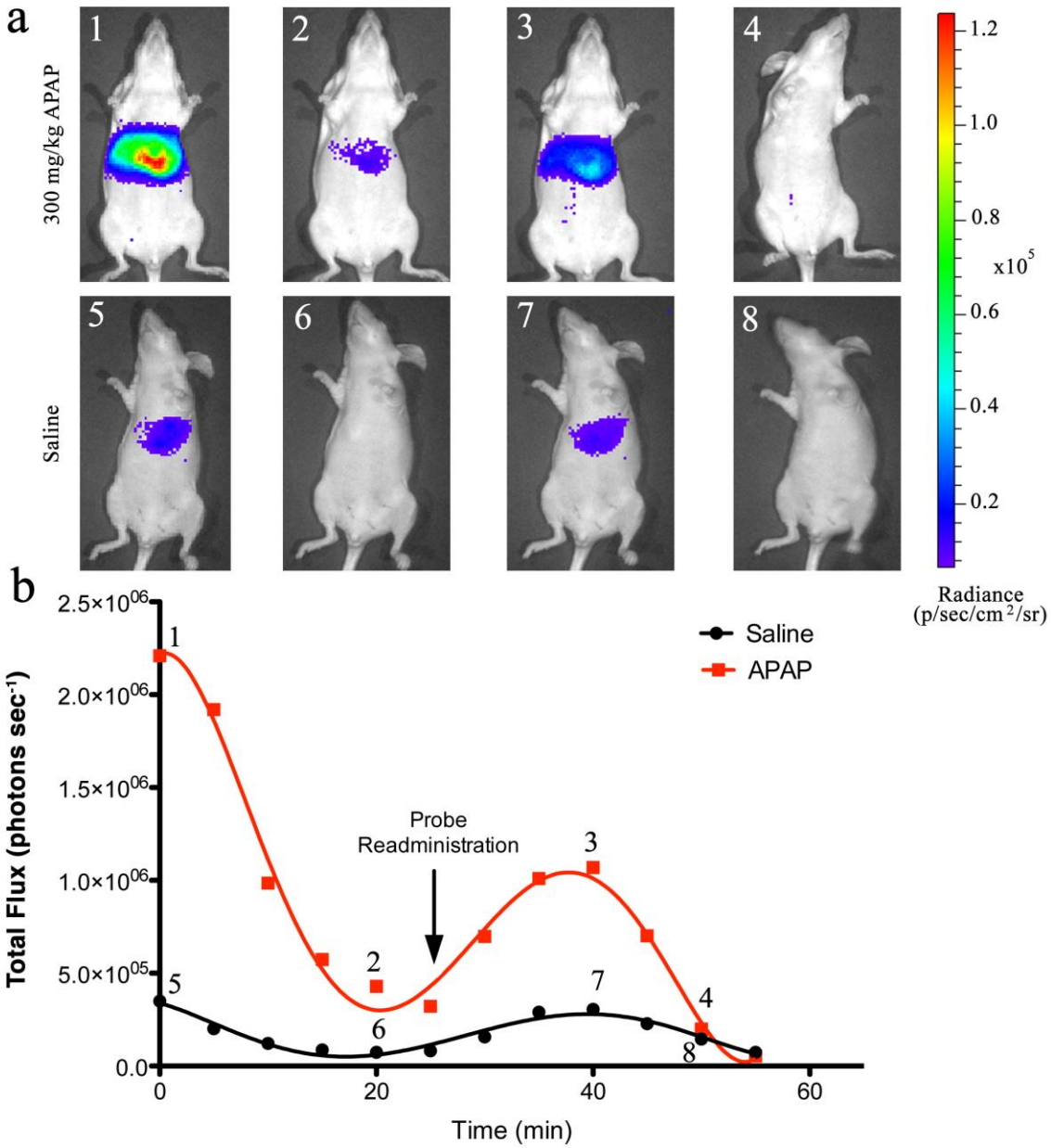

Supplementary Figure S4. Extension of the time course of H_2O_2 detection after drug challenge by re-administration of CF-SPN. Mice were administered 300 mg/kg APAP (top row, 1-4) or saline (bottom row, 5-8) i.p., followed by the administration of 0.8 mg CF-SPN i.v. After 25 min, CF-NP was re-administered i.v. (a) Luminescence images and (b) quantitation of liver luminescence are shown. Black arrow in (b) indicates re-administration of CF-SPN. Numbers on images correspond to time points indicated on plot (n=1 mouse per group).

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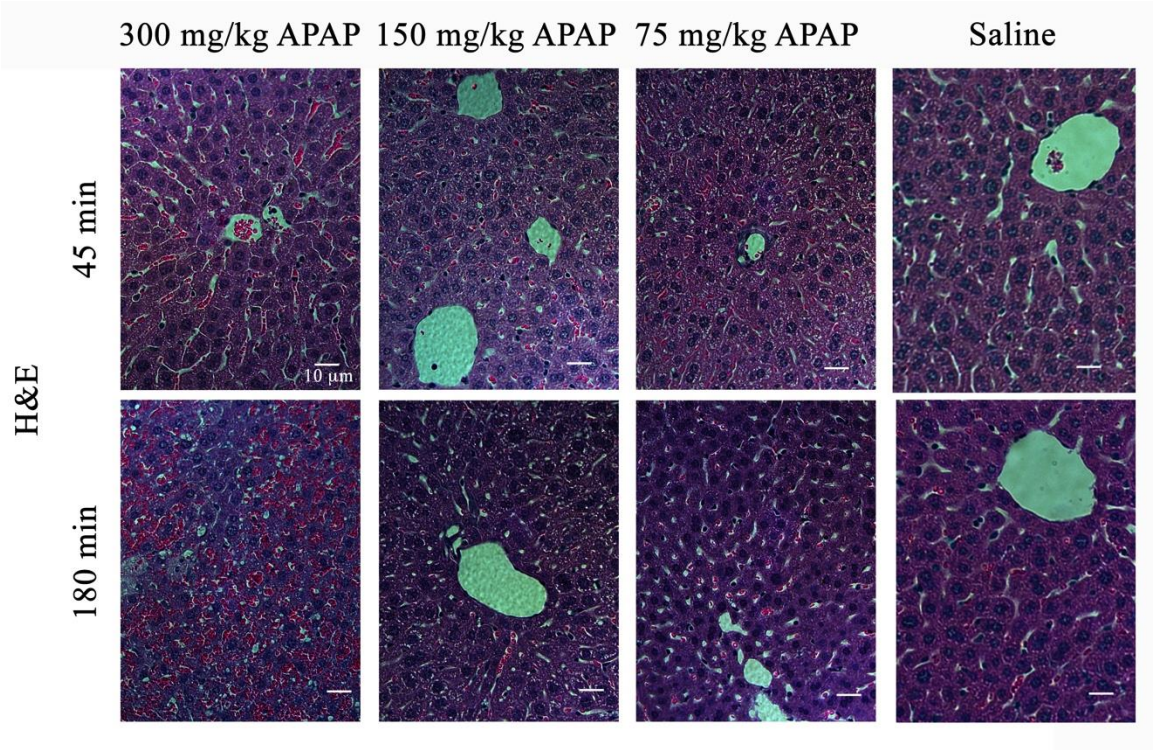

Supplementary Figure S5. Histological analysis of liver tissues. Mice were treated, from left to right, with 300, 150, 75 mg/kg APAP, or saline, and euthanized 45 min (top row) or 180 min (bottom row) after drug administration. Sections were stained with hematoxylin and eosin. Scale bar represents 10 µm.

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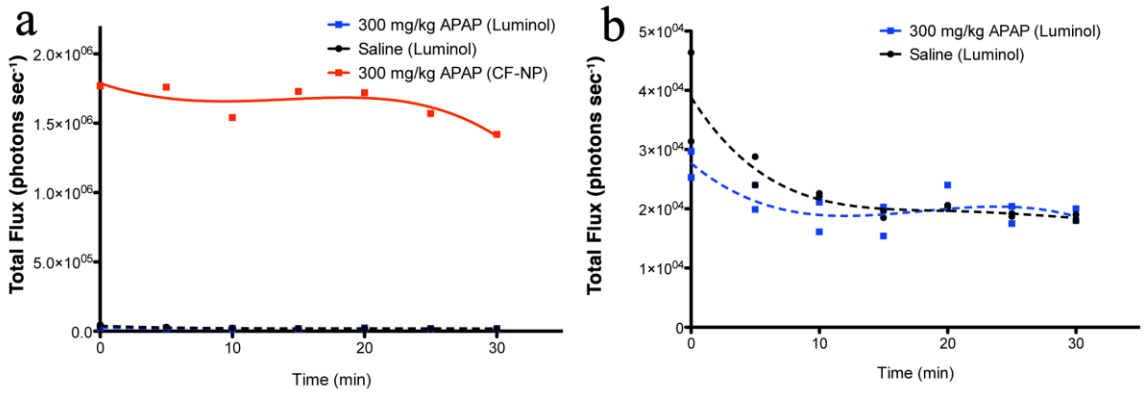

Supplementary Figure S6. Comparison between luminol and CF-SPN for their ability to detect drug-induced liver production of H_2O_2. Mice were administered 300 mg/kg APAP i.p., followed either by i.v. injection of 0.8 mg CF-SPN (containing 0.2 mg CPPO), or 0.2 mg luminol. (a) Chemiluminescent signals from CF-SPN (red) or luminol with (dashed blue) or without (dashed black) APAP treatment are shown. (b) Rescaled y-axis of (a) showing the lack of any signal generation from luminol. Each group, n=1 mouse.

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