

Supporting Information

ROS-mediated liposomal dexamethasone: a new FA-targeted nanoformulation to combat rheumatoid arthritis *via* inhibiting iRhom2/TNF- α /BAFF pathways

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Supplementary Figures

Scheme S1. Synthetic route of di-S-PC lipids.

Figure S1. (a) ¹H NMR and (b) ¹³C NMR (600 MHz, CDCl₃) spectra of C₁₈-S-COOH intermediate.

Figure S2. ¹H NMR (600 MHz, CDCl₃) spectrum of ROS-responsive di-S-PC lipids.

Figure S3. ¹³C NMR (600 MHz, CH₃OH-*d*₄) spectrum of ROS-responsive di-S-PC lipids.

Figure S4. The *in vitro* cellular association of Dex@FA-ROS-Lips. Confluent LPS-activated RAW264.7 cells exposed to free Dex solution or liposomes containing Dex (50 μ M) at various time intervals.

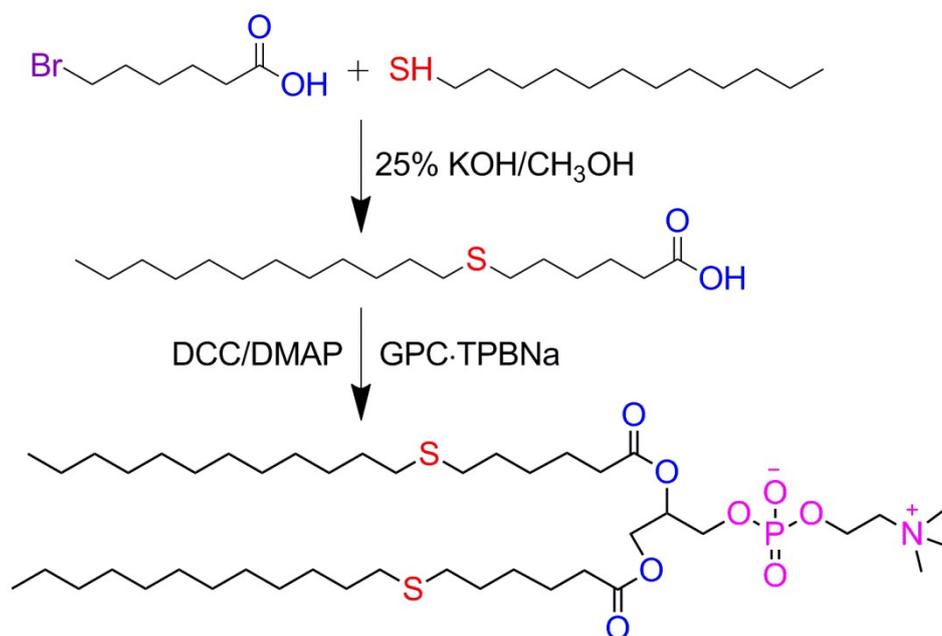
Figure S5. (a) Expression levels and (b) the quantified densitometric analysis of iRhom2, TNF- α and BAFF proteins detected by Western blotting ($n = 3$). β -actin antibody was used to ensure after incubation equal protein loading. Data are presented as Mean \pm S.D. and were statistically analyzed by one-way ANOVA. ** $P < 0.01$ vs the control group; * $P < 0.05$, ** $P < 0.01$ vs free Dex group.

Figure S6. (a) Schematic representation of AIA mice model protocol. 100 μ L of CFA with the heat-killed mycobacteria (10 mg/mL) was injected subcutaneously into SD males on day 0; (b) Arthritis first signs appeared 14 days after the first immunization step; (c) Hind paws and fore paws images according to the inflammation visual scores shown in table.

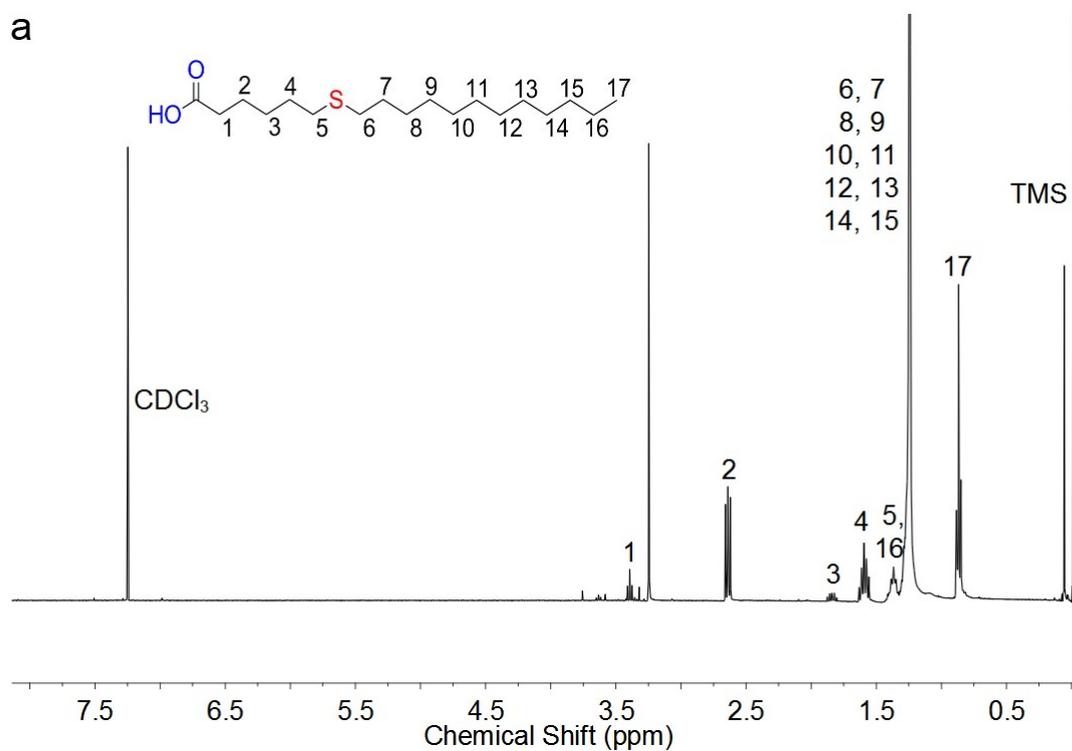
Figure S7. Hemolysis of Dex@FA-ROS-Lips: (a) Micrograph of RBCs exposed to 250 μ g/mL ROS-Lips and Dex@FA-ROS-Lips after incubated at 37 $^{\circ}$ C for 2 h. 0.9%

saline (0% hemolysis) and distilled water (100% hemolysis) was set as negative and positive controls; (b) Images of tubes containing red blood cells (RBCs) incubated with different groups (after $3000 \times$ centrifugation); (c) Hemolysis rate (%) of tested liposomes and the value less than 5% was considered safe for *in vivo* administration.

Scheme S1. Synthetic route of di-S-PC lipids.



As shown, ROS-responsive lipids (di-S-PC) were synthesized by a two-step reaction that C18-S-COOH intermediate was firstly prepared by conjugation between 6-Bromohexanoic acid and 1-Dodecanethiol in the presence of 25% KOH and further attached to GPC·TPBNa using DCC/DMAP condensation system.



b

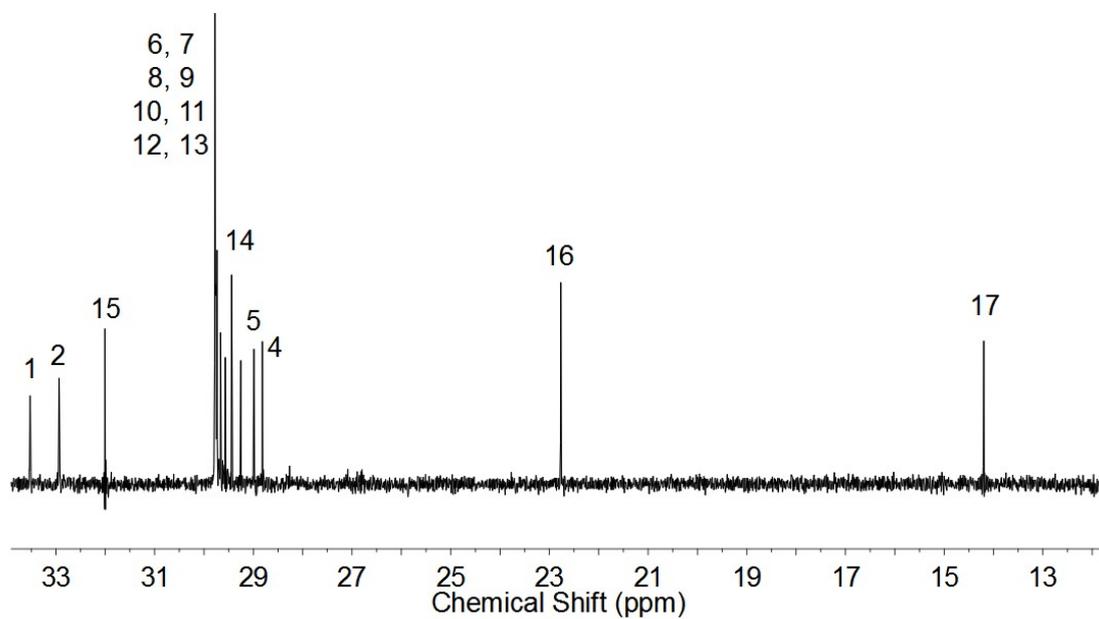


Figure S1. (a) ^1H NMR and (b) ^{13}C NMR (600 MHz, CDCl_3) spectra of $\text{C}_{18}\text{-S-COOH}$ intermediate.

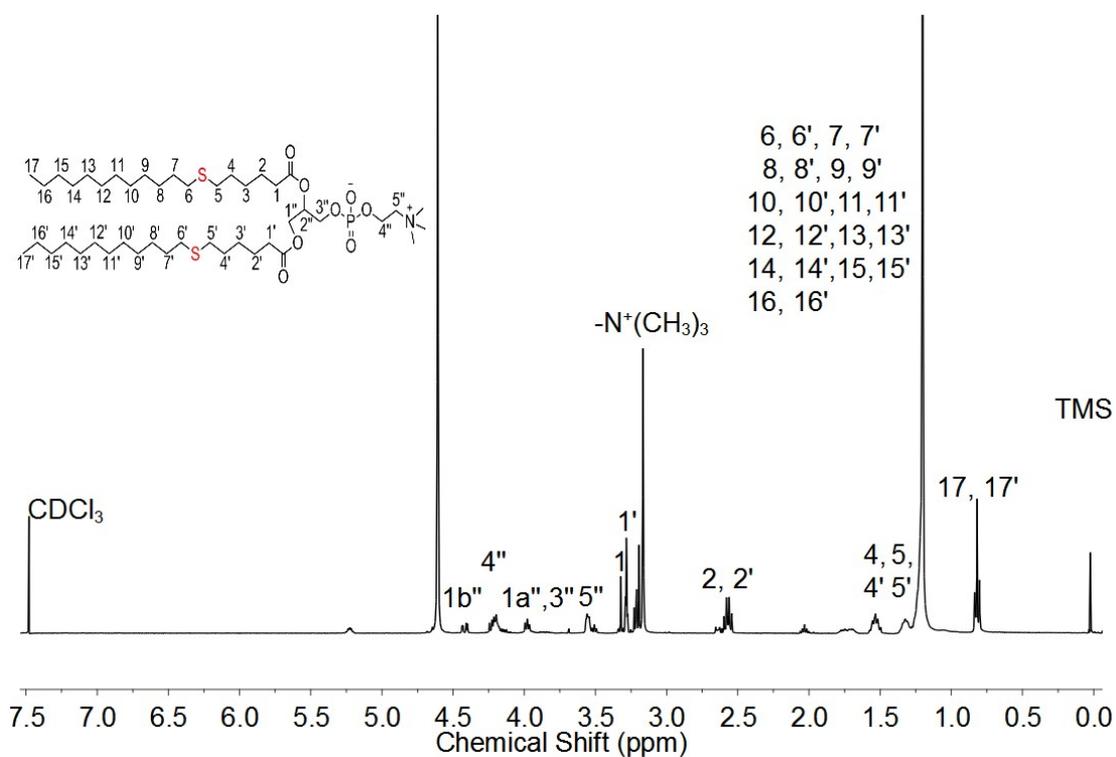


Figure S2. ^1H NMR (600 MHz, CDCl_3) spectrum of ROS-responsive di-S-PC lipids. ^1H NMR (600 MHz, CDCl_3): δ 5.40 (s, 1H, H-2''), 4.48 (d, $J=6.4$ Hz, 1H, H-1b''), 4.36

(m, 2H, H-4''), 4.14 (m, 1H, H-1a''), 3.53 (m, 4H, H-3'', 5''), 3.46 (s, 4H, H-1, 1'), 3.25 (s, 9H, -N+(CH₃)₃), 2.61 (m, 4H, H-2, 2'), 1.46-1.25 (m, -CH₂-), 0.92 (m, 6H, H-17, 17') ppm.

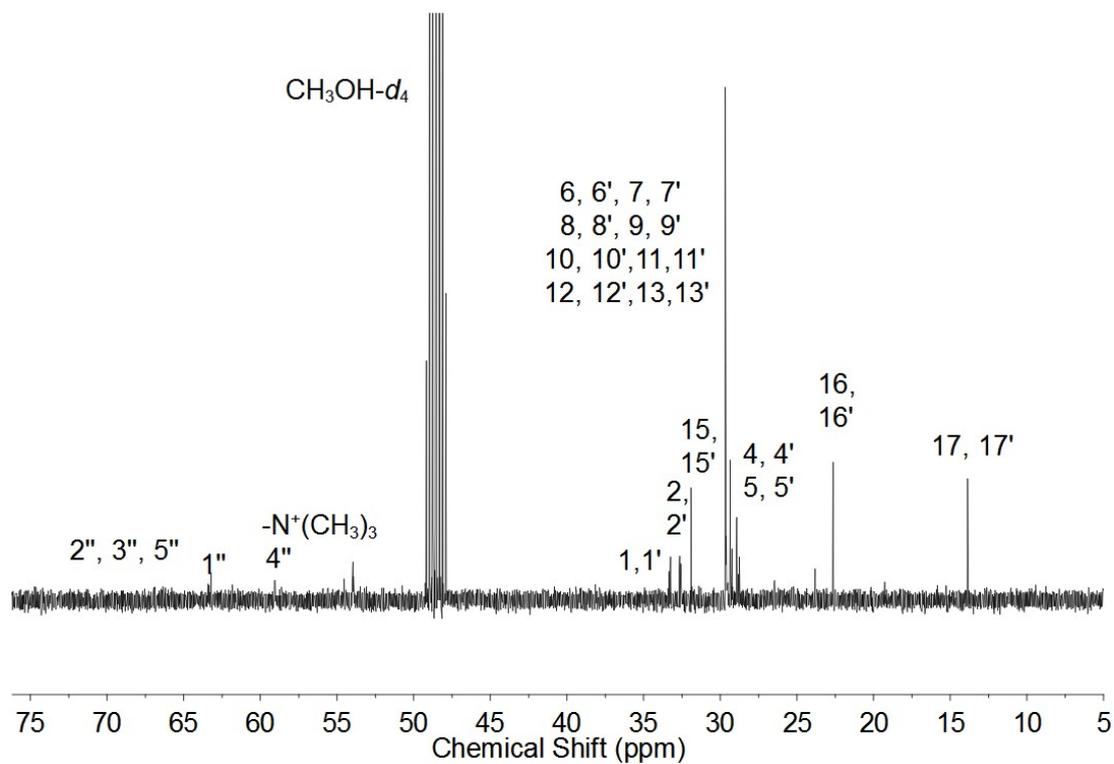


Figure S3. ¹³C NMR (600 MHz, CH₃OH-*d*₄) spectrum of ROS-responsive di-S-PC lipids. ¹³C NMR (500 MHz, CH₃OH-*d*₄): δ 77.82, 77.50, 77.17, 53.96, 49.18, 33.25, 32.65, 31.91, 29.77, 29.02, 22.64, 13.87 ppm.

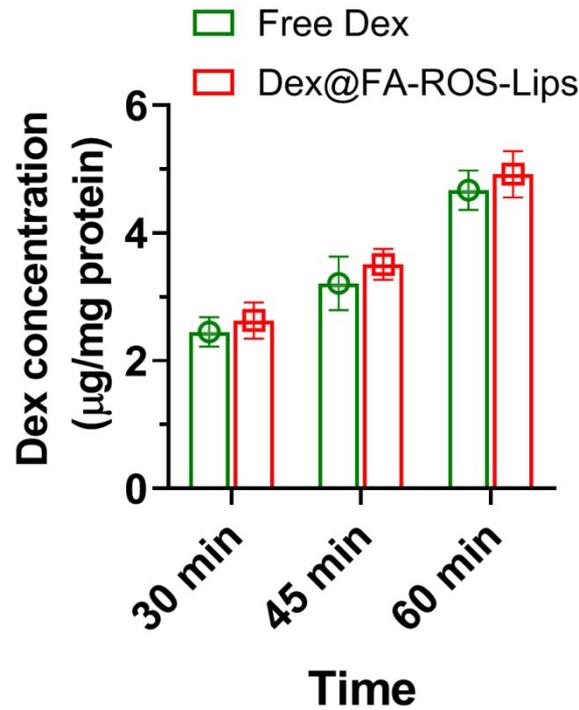


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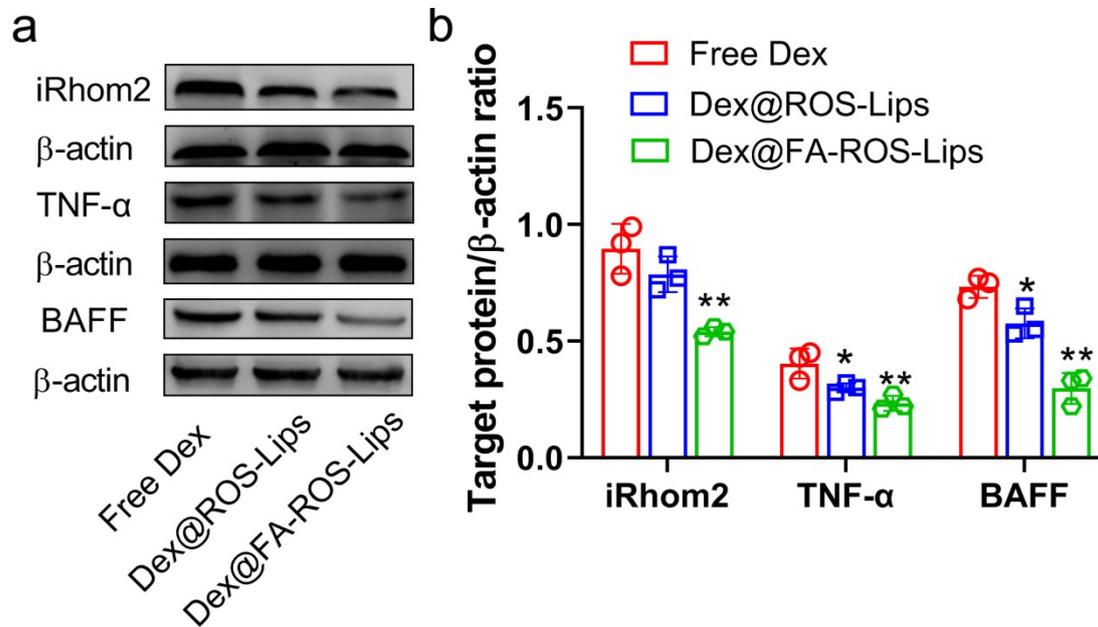


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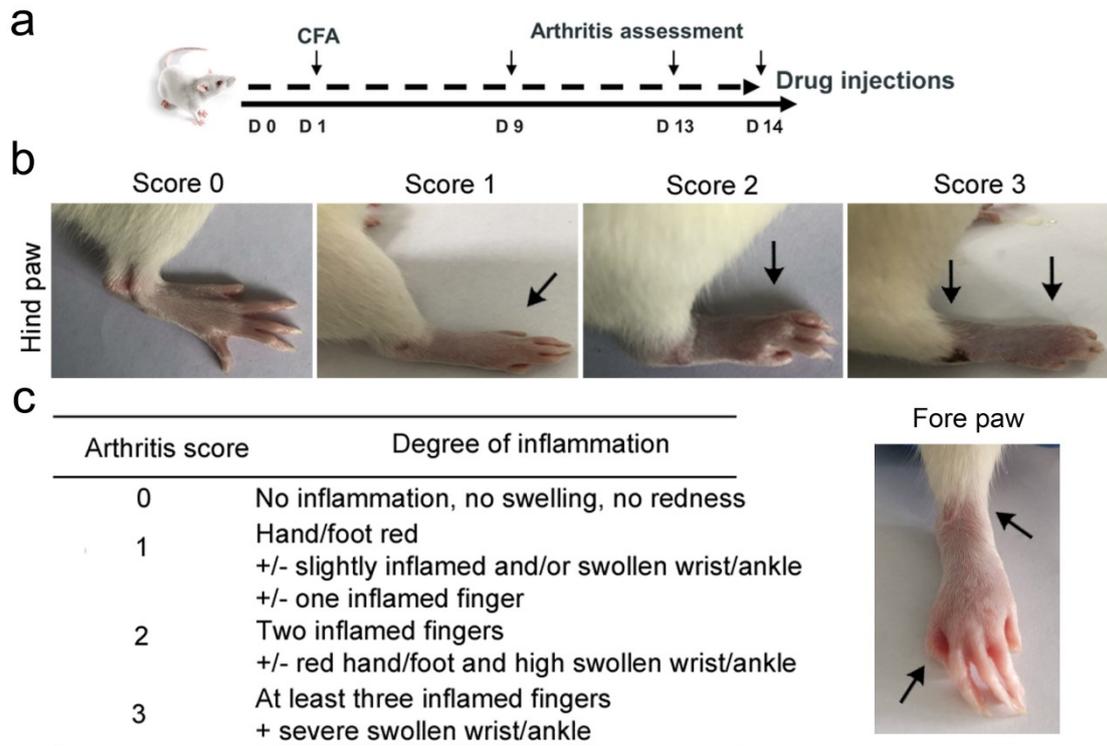


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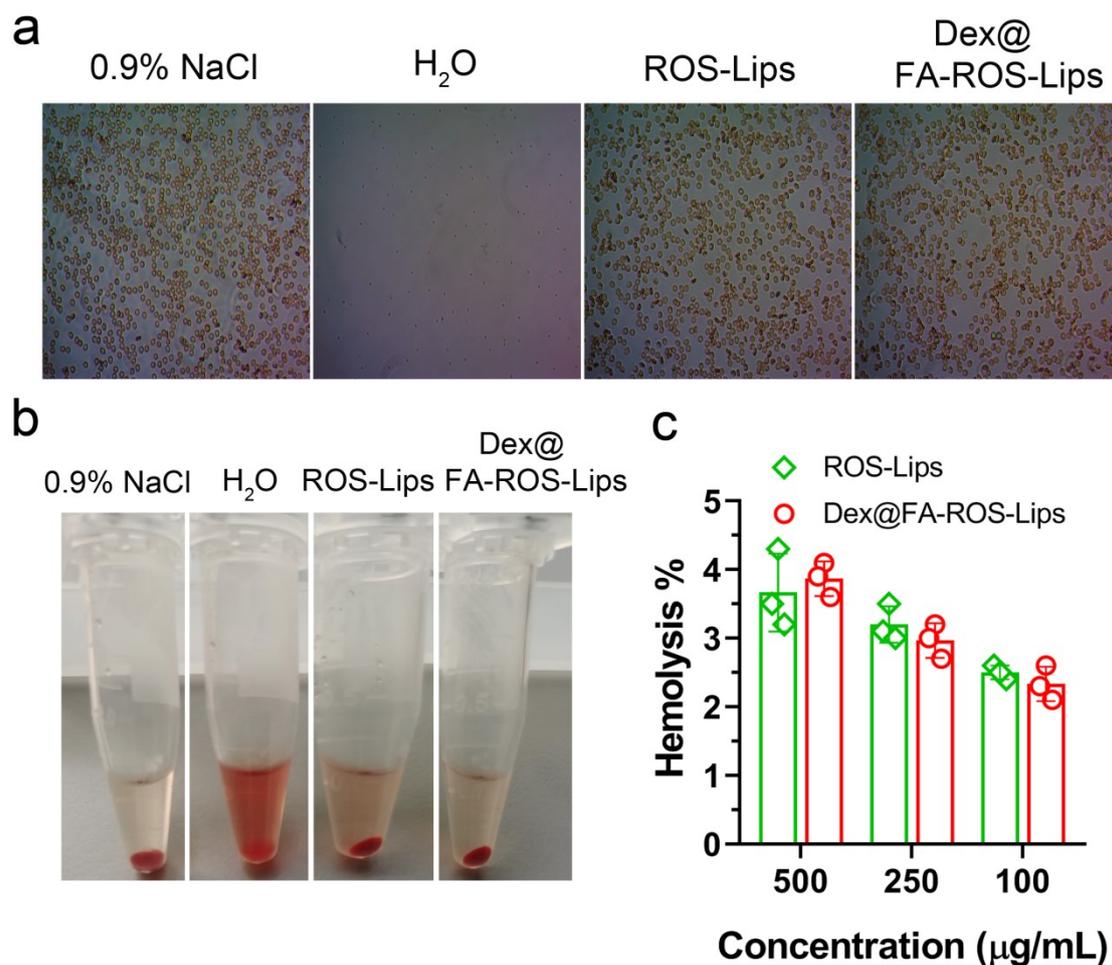


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