Supplementary information for

Efficacy of a resveratrol nanoformulation prepared by a facile solvent-free method

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Supplementary images (Figure S1-S10)

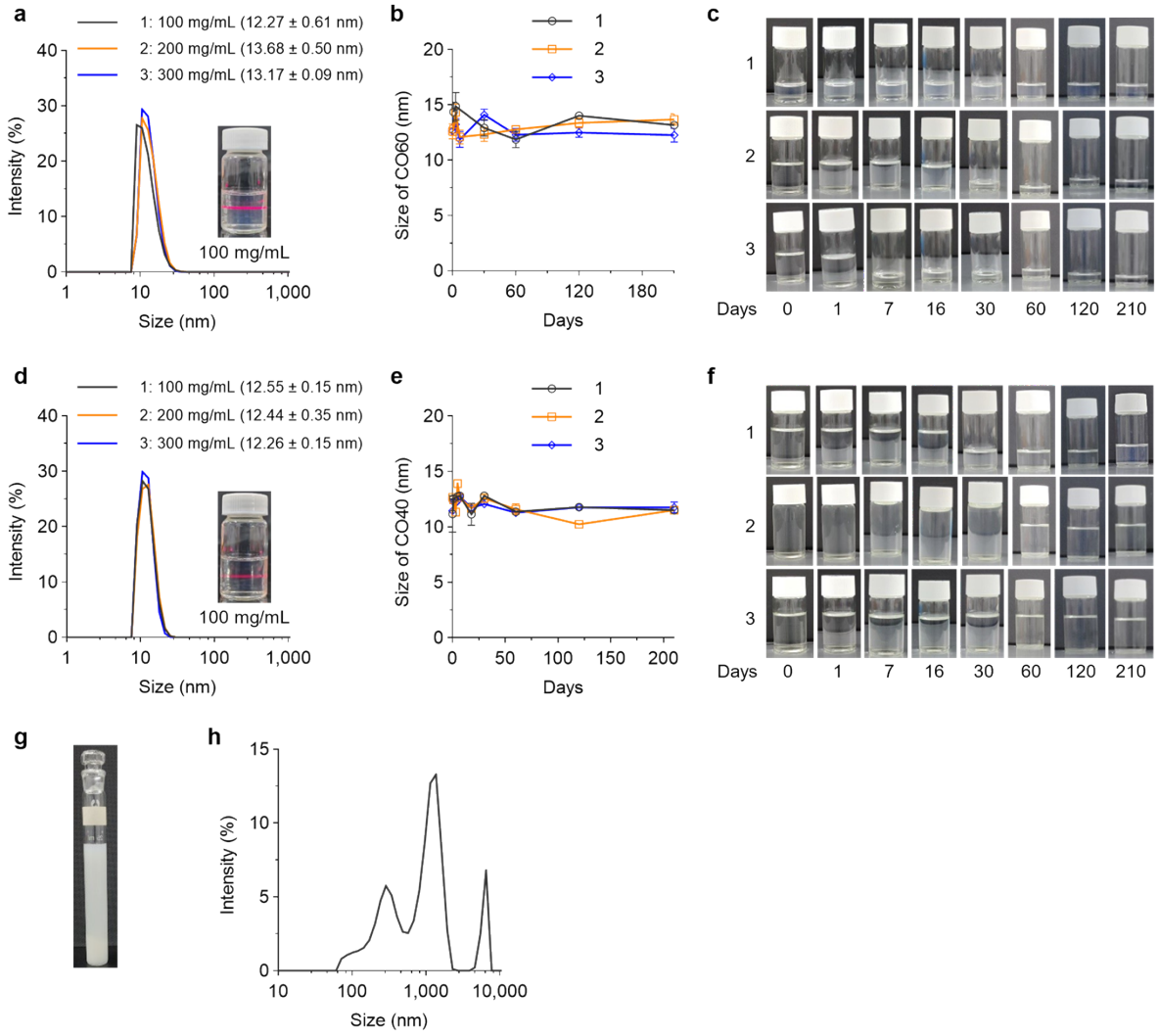

Figure S1. PEGylated hydrogenated castor oil dissolved in water.

a, CO60 dissolved in water at 100–300 mg/mL. Insertion showed the red-light path of Tyndall effect for 100 mg/mL CO60 solution.

b–c, Stability of CO60 aqueous solution at room temperature for 7 months. The DLS size (**b**) and picture (**c**) at related time point are shown.

d, CO40 dissolved in water at 100–300 mg/mL. Insertion showed the red-light path of Tyndall effect for 100 mg/mL CO40 solution.

e–f, Stability of CO40 aqueous solution at room temperature for 7 months. The DLS size (**e**) and picture (**f**) at related time point are shown.

g, CO20 dispersed in water at 100 mg/mL. A turbid solution was obtained.

h, DLS size distribution of CO20 solution.

Data presented here are mean ± standard deviation (SD) (n = 4 **a–b** and **d–e**).

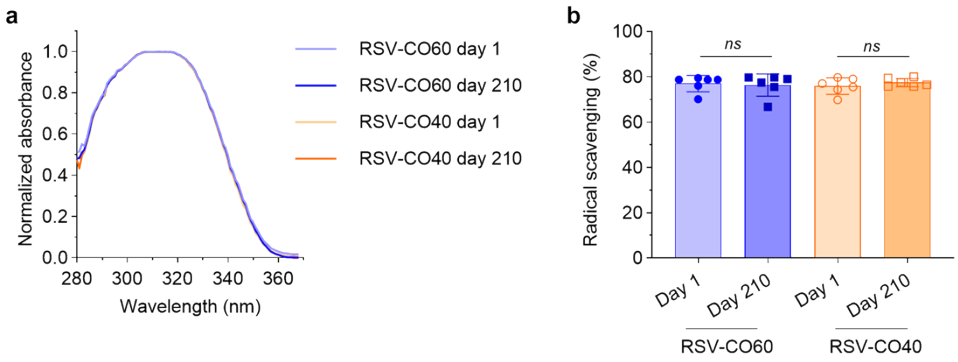

Figure S2. Properties of RSV after long-term storage.

RSV-CO60 and RSV-CO40 solutions were stored at room temperature for 210 days before the measurement of UV-vis spectra (**a**) and antioxidant activity (**b**) of RSV. Freshly prepared RSV-CO60 and RSV-CO40 solutions were also measured as controls. Data in **b** were analyzed using unpaired t test and are presented as mean ± SD (*n* = 6).

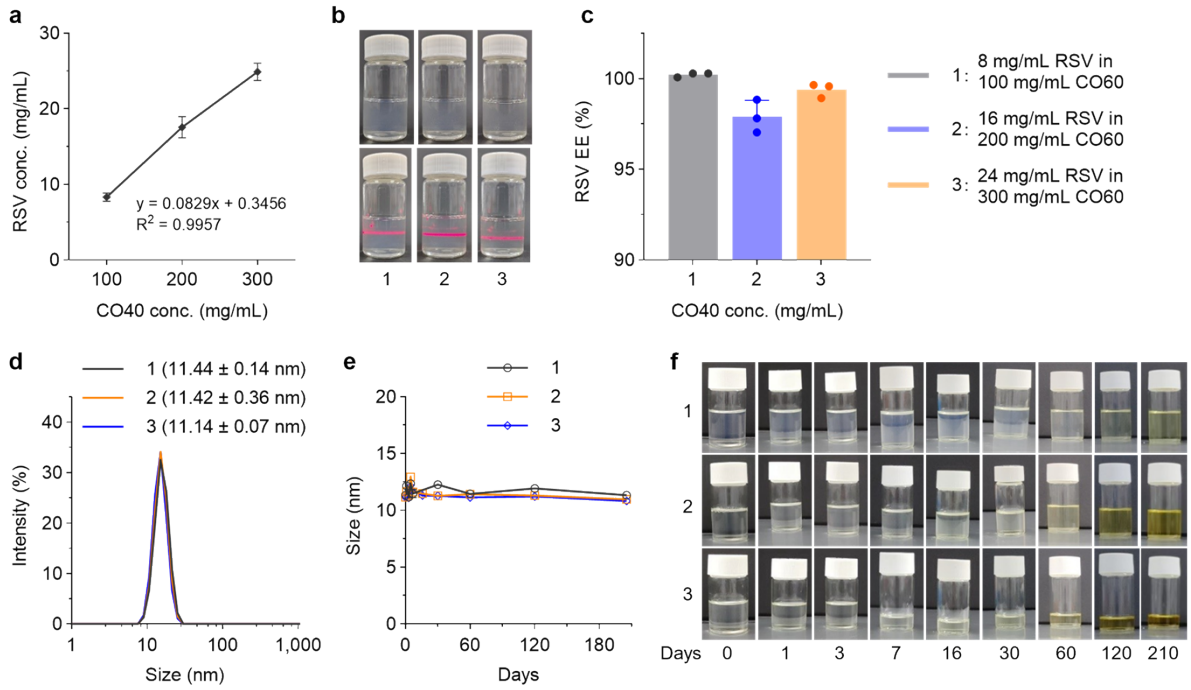

Figure S3. RSV dissolved in CO40 aqueous solution.

a, RSV solubility in CO40 solution with related concentration.

b, Pictures of RSV-CO40 solutions. The Tyndall effect of visible red-light path showed the formation of colloidal solutions by RSV-CO40.

c, RSV encapsulation efficiency in CO40 solution.

d, Size distribution of RSV-CO40.

e–f, Stability of RSV-CO40 at room temperature. The DLS size (**e**) and picture (**f**) of RSV-CO40 are shown.

Data presented here are mean ± SD (*n* = 3 for **a** and **c**, *n* = 4 for **d** and **e**).

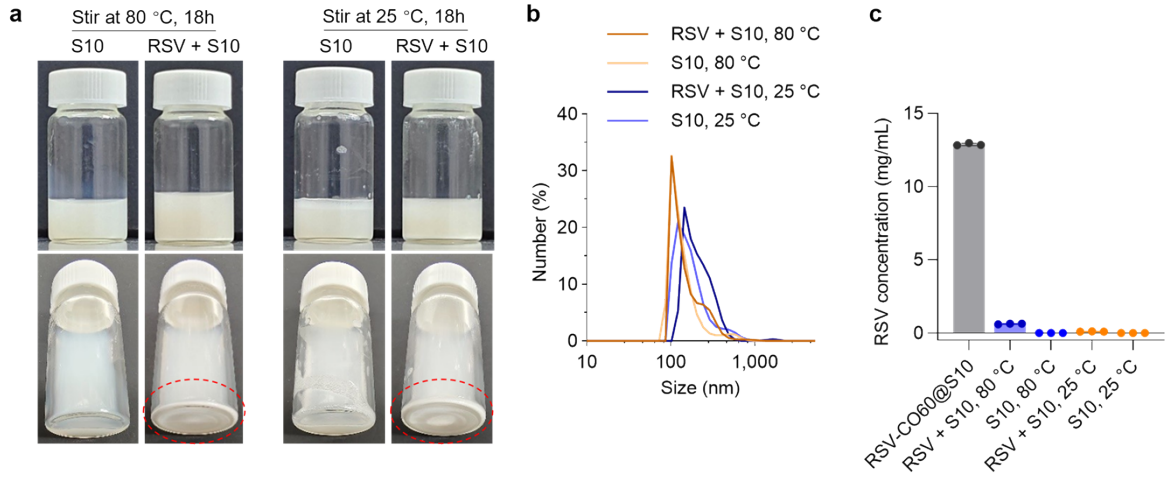

Figure S4. Solid RSV powder cooperated with S10 solution.

a, Solid RSV powder (10 mg/mL) was directly added to 50 mg/mL S10 solution and stirred at 25 °C and 80 °C overnight (~18 h). After sitting quietly for hours, undissolved RSV particles can be found in the bottom of S10 solution (Red circle). 50 mg/mL RSV aqueous solution was parallelly stirred as the control.

b, Size distribution of samples prepared in **a**.

c, RSV content of samples prepared in **a**. These solutions were centrifuged at 3000 g 10 min to pellet the insoluble residues, and the RSV concentration was measured as described in Methods. An RSV-CO60@S10 solution was used as the positive control. No or little RSV was detected in samples prepared by mixing solid RSV powder with S10 solution as did in **a**. Data shown are mean ± SD (n = 3).

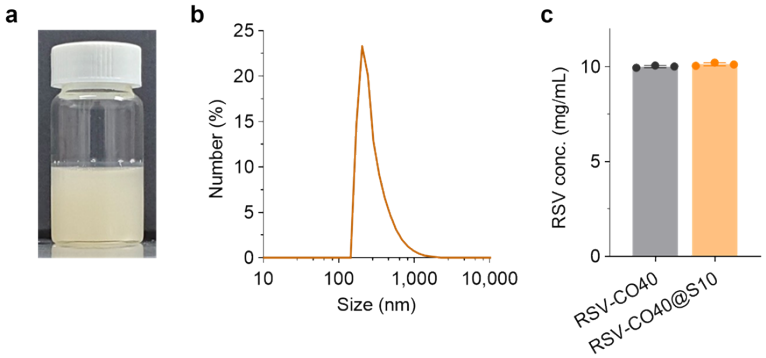

Figure S5. RSV-CO40 formulated with S10.

a, RSV-CO40 (final concentration 10 mg/mL of RSV) was mixed with S10 (final concentration of 25 mg/mL) solution and stirred overnight (~18 h) at 80 °C. Appearance of the resultant solution was shown.

b, Size distribution of RSV-CO40@S10.

c, RSV content in RSV-CO40@S10. RSV-CO40 was parallelly mixed with the same volume of water as S10 solution, and the RSV concentration was measured. Data shown are mean ± SD (*n* = 3).

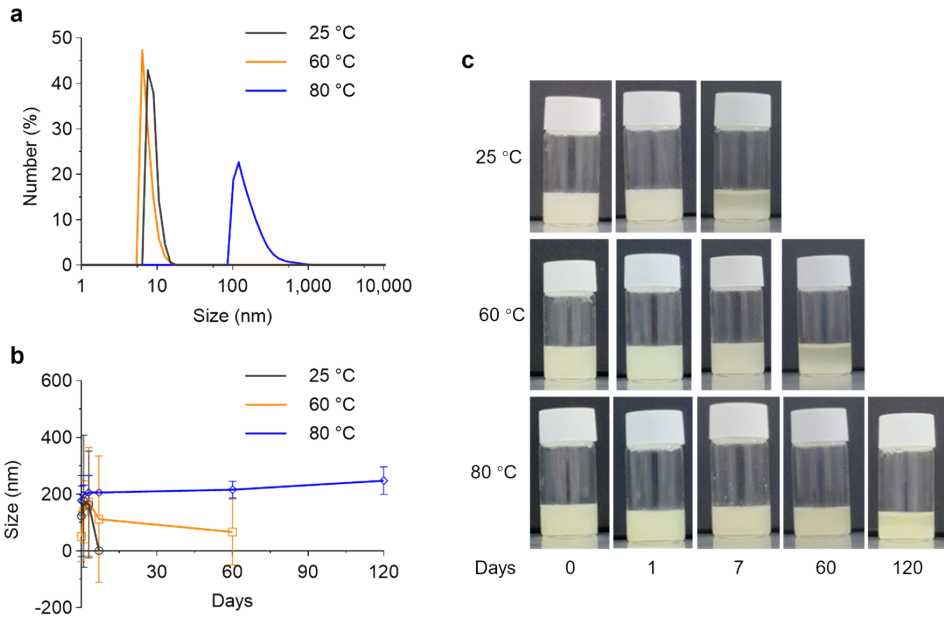

Figure S6. Stability of RSV-CO60@S10 made by cooperating solid S10 powder with RSV-CO60 solution.

a, Size distribution of RSV formulation made at related temperatures. Solid S10 powder (final concentration at 25 mg/mL) was added to RSV-CO60 aqueous solution (10 mg/mL of RSV), and the mixture was stirred at room temperature for 30 min to let S10 dissolve. Then solutions were stirred at 25 °C, 60 °C and 80 °C for 18 h, respectively. DLS size was then analyzed and shown here.

b, Stability of RSV formulations prepared in **a**. Those samples were stored at 4 °C for 4 months, and the DLS size was measured at related time point. Data shown are mean ± SD (*n* = 4).

c, Corresponding picture to **b** at related storage time.

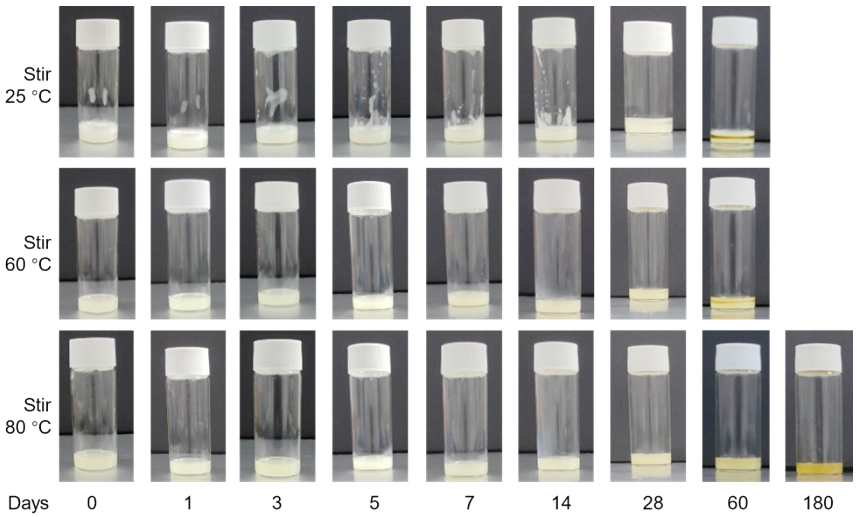

Figure S7. Stability of RSV-CO60@S10 stored at room temperature corresponding to Figure 3g.

RSV-CO60@S10 were prepared by mixing RSV-CO60 solution with S10 solution and stirred at 25 °C, 60 °C and 80 °C for 18 h, respectively. Samples were sealed and stored at room temperature (~ 25 °C) for 6 months, and pictures were taken at related time point.

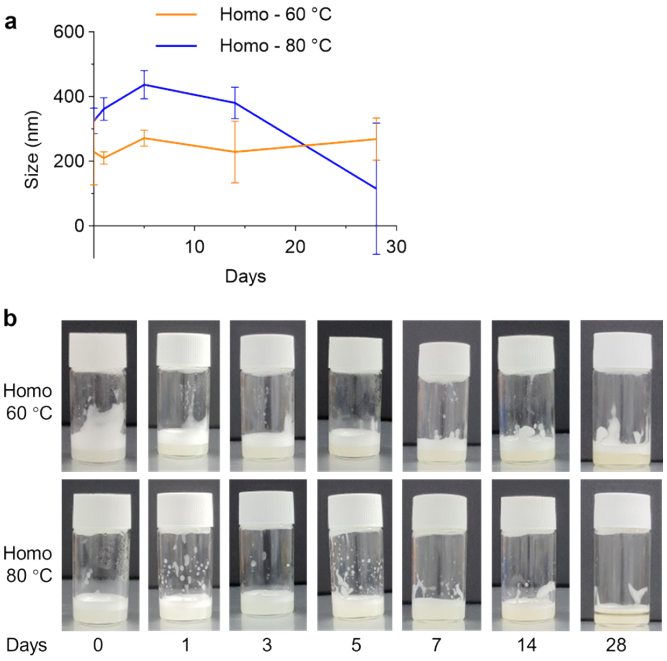

Figure S8. Stability of RSV-CO60@S10 made by homogenizer corresponding to Figure 2c.

a, Size of RSV-CO60@S10 made under homogenizer at related temperatures. The solutions were stored at room temperature. Data shown are mean ± SD (*n* = 4).

b, Corresponding picture to **a**. On 28 days of storage, we observed solution layering for both samples.

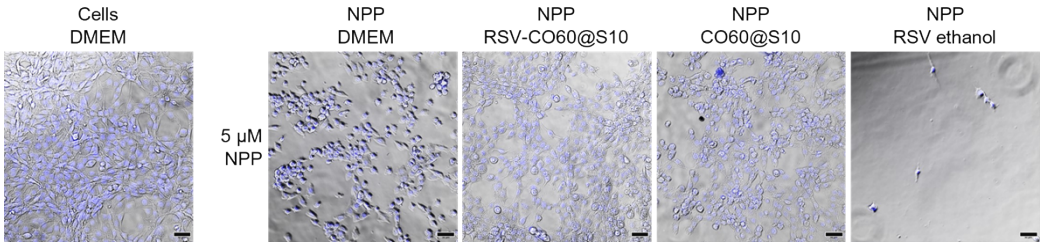

Figure S9. Images of Cells corresponding to Figure 5c.

Cells treated with 5 μM NPP and related vesicles, corresponding to figure 5c. 3T3 cells were incubated in FBS free DMEM containing 5 μM NPP and 10 μg/mL RSV vesicles. After 3 h of incubation, cells were washed, fixed, stained with DAPI and imaged under a SIM microscope. Bright field and blue fluorescence (DAPI for nuclei) images were merged and presented here. Scale bars, 50 μm.

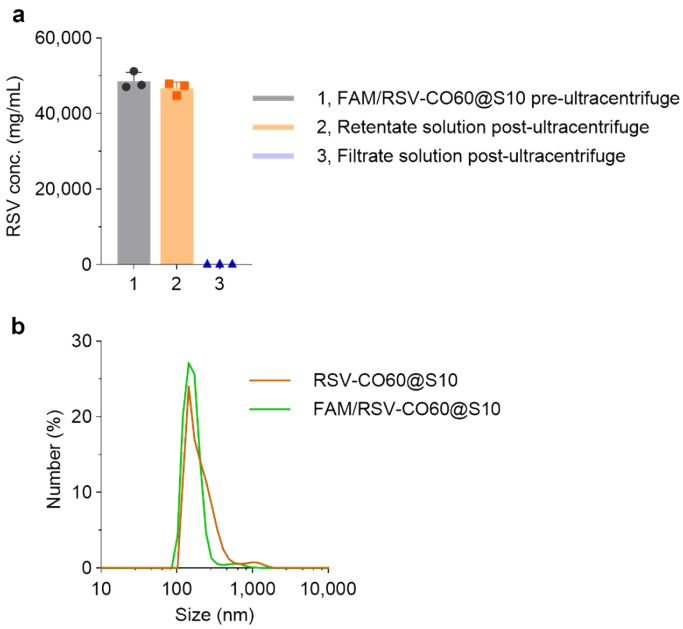

Figure S10. Characterization of FAM/RSV-CO60@S10.

a, FAM content in the vesicles. FAM/RSV-CO60@S10 was prepared and purified via dialysis. The obtained FAM/RSV-CO60@S10 was then ultracentrifuged (molecular weight cut-off, MWCO 3000 Da) to collect the retentate (FAM/RSV-CO60@S10 vesicles containing) and filtrate (aqueous solvent). The retentate solution was resuspended in ultrapure water to the original volume. Then the fluorescence intensity in the recovered retentate solution, filtrate solution and original FAM/RSV-CO60@S10 solution was measured by a plate reader (Ex. 485 nm; Em. 520 nm). Data shown are mean ± SD (n = 4).

b, Size distribution of RSV-CO60@S10 and FAM loaded RSV-CO60@S10.