Supporting Information for

Lipid-mediated Delivery of CD47 siRNA aids JQ1 in ensuring simultaneous

downregulation of PD-L1 and CD47 and hence antitumor immunotherapy

efficacy

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Figure S1. The expression of CD47 and PD-L1 on the surface of 4T1 and B16 cells measured by Flow cytometry.



Figure S2. Flow cytometry showing the effect of JQ1 on the expression of PD-L1 and CD47 on the surface of tumor cells in vitro. The expression of PD-L1 and CD47 on the surface of tumor cells was detected by flow cytometry. (A) 4T1 tumor cells. (C) B16 tumor cells. The expression of C-MYC on the surface of tumor cells was detected by flow cytometry. (B) 4T1 tumor cells. (D) B16 tumor cells. Mean fluorescence intensity of PD-L1, CD47 and C-MYC expressed on the surface of tumor cells. (E) 4T1 tumor cells. (F) B16 tumor cells.



Figure S3. Determination of CD47siRNA encapsulation efficiency and JQ1 drug release determination. (A) RNA agarose gel electrophoresis. The unencapsulated siRNA during the preparation of the CLN/JQ-1/siCD47 and CLN/siCD47 was measured by RNA agarose gel electrophoresis. (B) The siRNA loading efficiencies of CLN/siCD47 and CLN/JQ-1/siCD47. (C) The cumulative release of JQ-1 from CLN/JQ-1/siCD47 and free JQ1. Data are presented as mean \pm SEM (n = 3). **p* < 0.05; ** *p* < 0.01; *** *p* < 0.001; **** *p* < 0.0001.



Figure S4. Bodyweight changes in 4T1 tumor-bearing BALB/c mice in the therapeutic experiment described in Figure 4B. (n = 4 per group)



Figure S5. Histological analysis of tissues from 4T1 tumor-bearing mice. Shown are representative H&E-stained sections of heart, lung, liver, spleen and kidney taken from the indicated groups after 23 days of PBS and CLN/ JQ1/ siCD47 treatment. Scale bar is 20 μ m. No detectable difference was seen in the tissues analyzed between groups.



Figure S6. The tumor immune microenvironment of 4T1 tumor-bearing mice after PBS and CLN/JQ1/siCD47 treatment was detected by flow cytometry.