Supporting Information

Engineered Polymer-siRNA Polyplexes Provide Effective Treatment of Lung Inflammation

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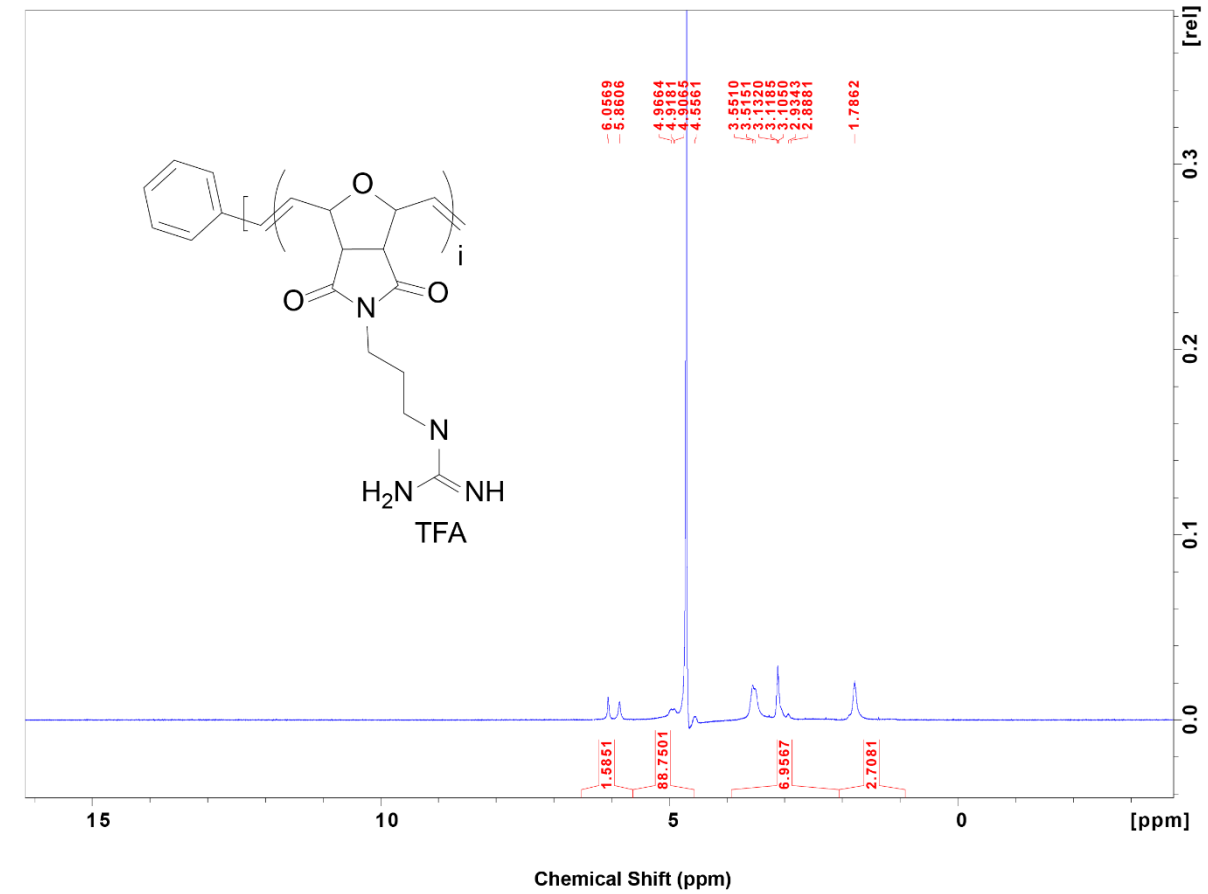

Figure S1. ^{1}H NMR characterization of the PONI-Guan polymer (60 kDa) in D_2O

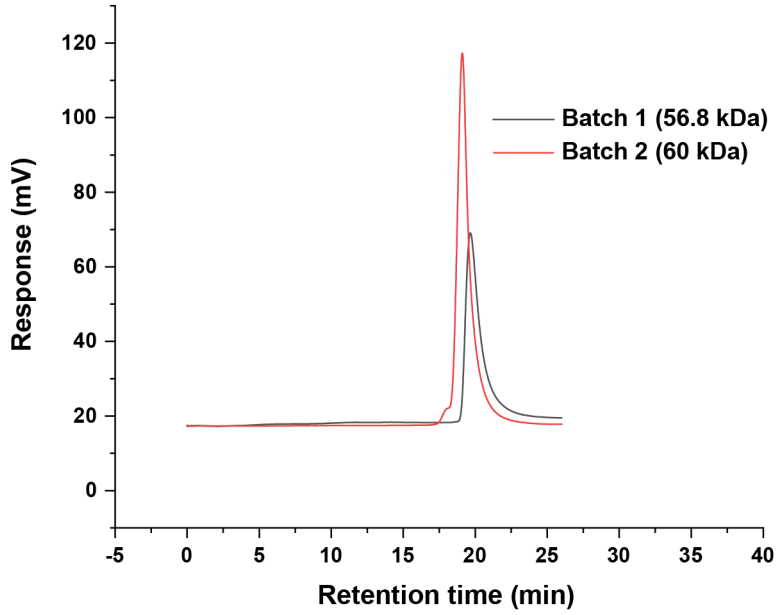

Figure S2. GPC characterization of the PONI-Guan polymers in different batches.

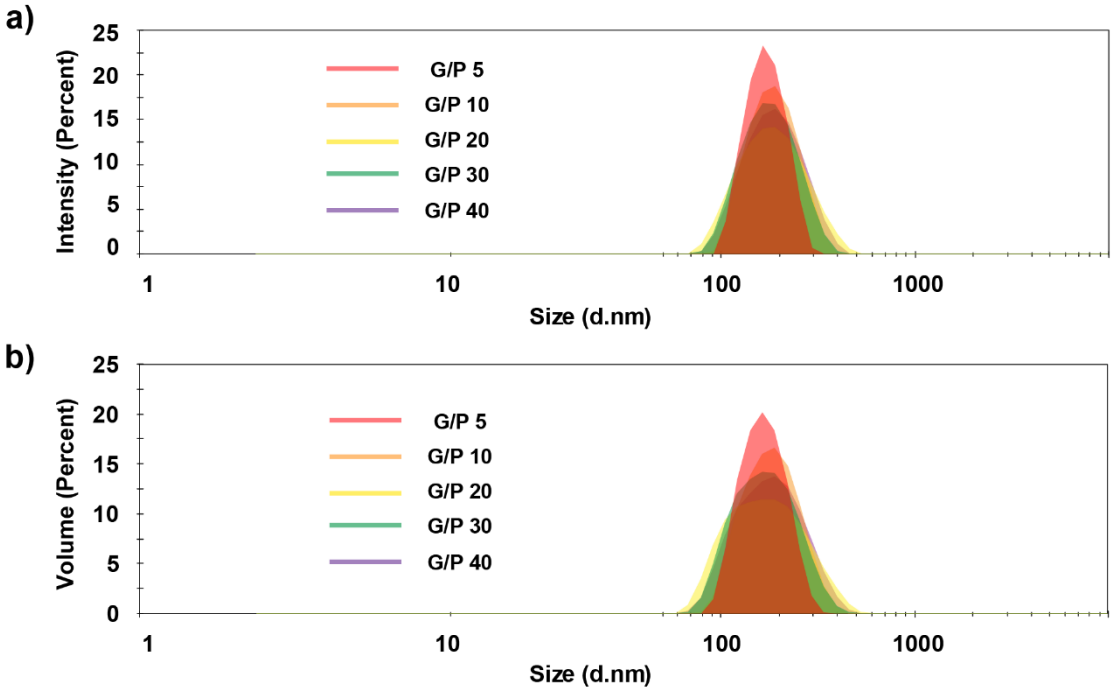

Figure S3. Representative DLS spectra of PONI/siRNA polyplexes at varied G/P ratios, represented by a) Intensity and b) Volume. Average of hydrodynamic diameter of PONI/siRNA polyplexes are relatively constant (~170 nm), with low PDI (all less than 0.1).

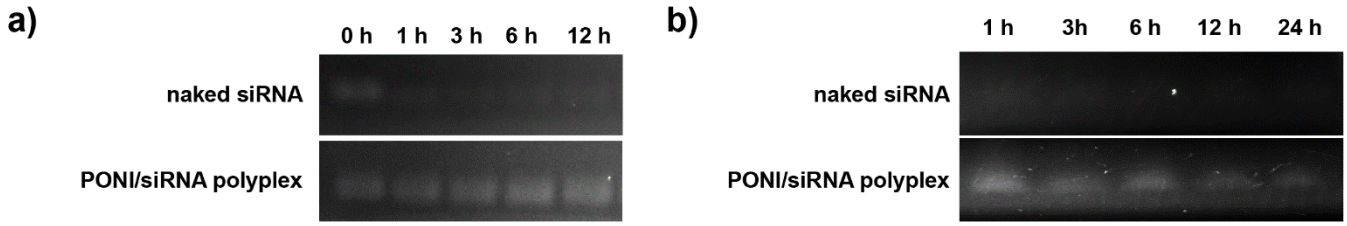

Figure S4. Stability of naked siRNA or PONI/siRNA polyplex in (a) 10% serum and (b) human whole blood.

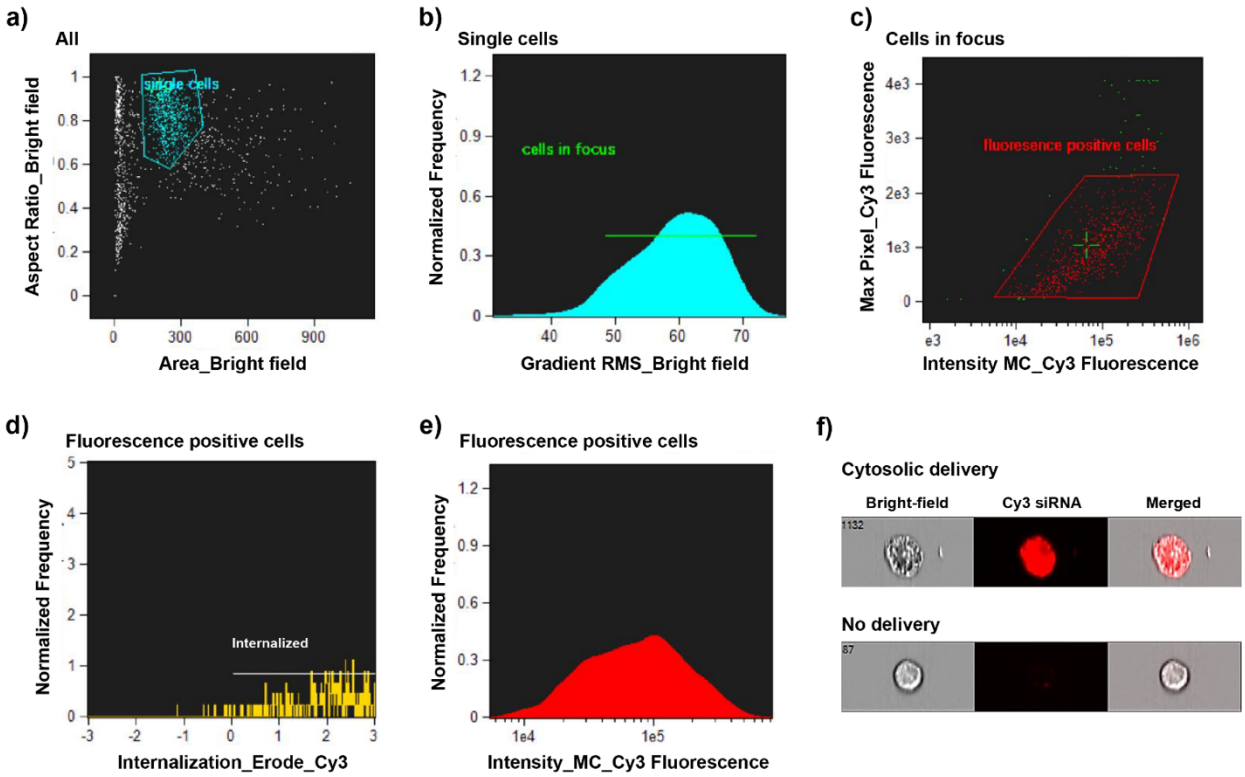

Figure S5. Representative gating strategies for analysis of the imaging flow cytometry data. a) Single cells and doublets including debris were separated from multicellular aggregates using areas and aspect ratios of brightfield images. b) To gate cells in focus, gradient root mean squared (RMS) of the brightfield images are plotted in a histogram. A higher gradient value indicates a more focused image. c) Cy3 fluorescence positive cells were selected by gating the cells with max pixel values and intensities in the fluorescence channel. d) Cells with internalized Cy3-siRNA were selected by choosing the cell populations with internalization scores greater than 0. Cells receiving a score less than 0 were considered to be surface bound or had no delivery. e) Representative histogram image of fluorescence positive cells with internalized agents. f) Representative imaging flow cytometry images of cytosolic delivery and those where it did not occur.

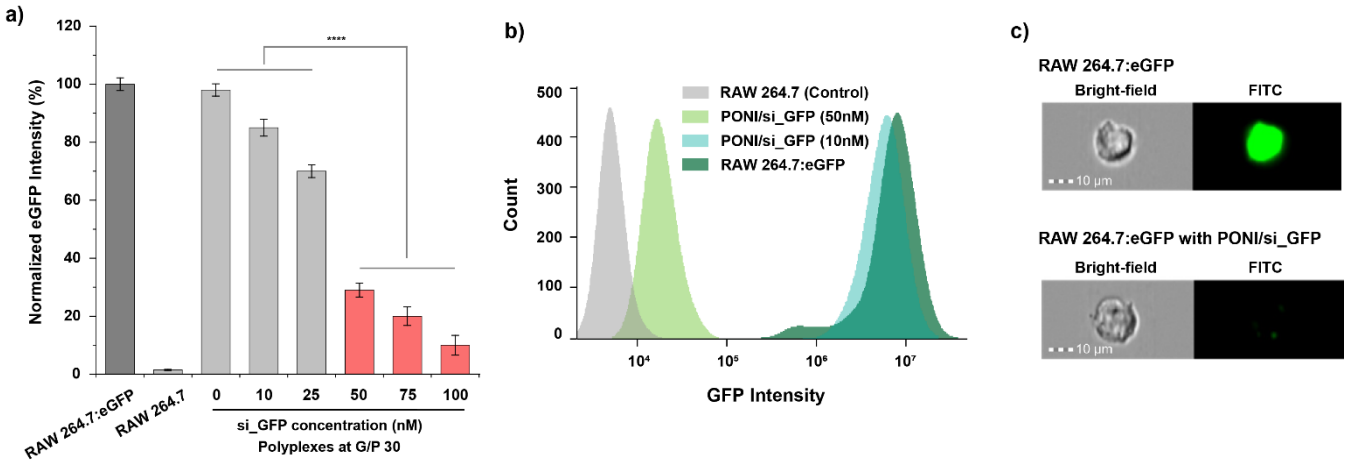

Figure S6. eGFP expression profiles of eGFP-expressing RAW 264.7 cells with different siRNA concentrations. a) Quantitative analysis of eGFP expression in RAW 264.7:eGFP cells treated with increasing concentrations of PONI/si_GFP polyplexes. Error bars represent standard deviation (SD) of three experimental replicates (Data are presented as mean ± SD, one-way Anova and Tukey multiple comparisons, ****$p < 0.001$). b) Representative flow cytometry histogram profiles of GFP knockdown in RAW 264.7:eGFP cells after 48 h incubation with different concentrations of PONI-Guan/si_GFP polyplexes. Control is non-treated RAW 264.7 cells. c) Representative imaging flow cytometry images of non-treated cells and GFP knockdown cells. All images at 60X magnification. Scale bar = 10 µm.

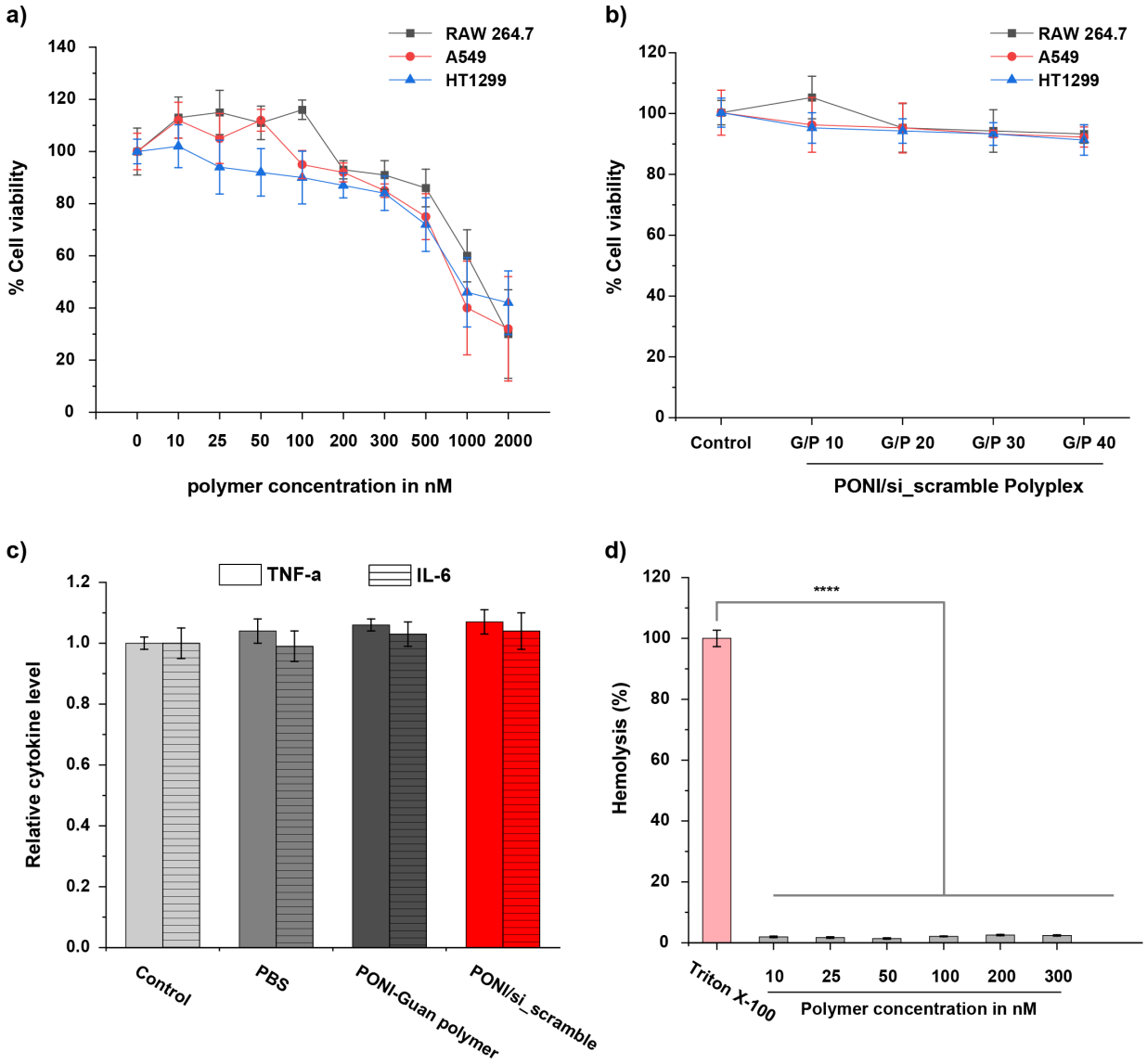

Figure S7. Biocompatibility of PONI-Guan/siRNA polyplexes. a-b) Viability of macrophage (RAW 264.7) and lung epithelial cells (A549 and HT1299) after 48 h exposure to PONI-Guan polymers at varied concentration or G/P ratios. c) Quantification of relative cytokine levels using the enzyme-linked immunosorbent assay (ELISA). No significant changes in any parameter were observed for treatment groups (G/P 30 ratio with 50 nM of siRNA) compared to untreated cells. d) Hemolytic activity of PONI-Guan/siRNA polyplexes at varied G/P ratios. Error bars represent standard deviation (SD) of three experimental replicates.

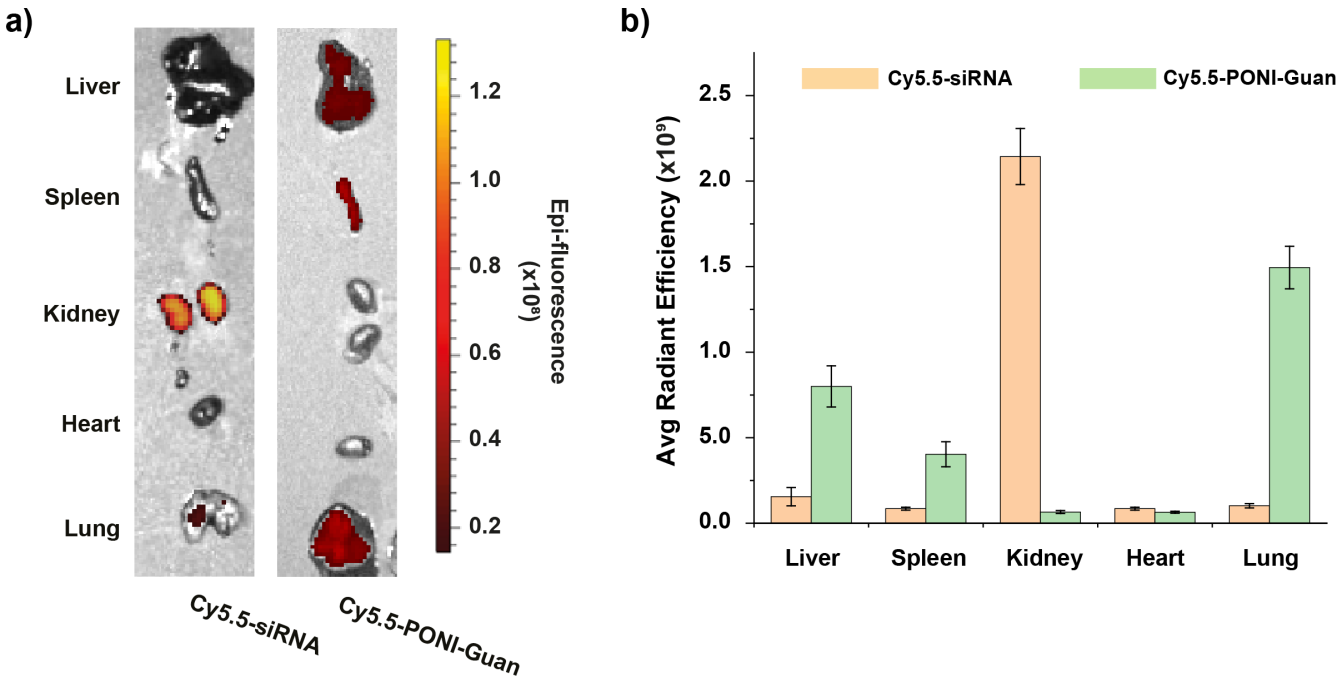

Figure S8. in vivo biodistribution of naked Cy5.5-siRNA or Cy5.5-PONI-Guan alone in BALB/c mice. a) Representative *ex vivo* imaging system (IVIS) organ images of Cy5.5 labeled naked siRNA (left) or PONI-Guan (right) 24 h after following systemic administration. b) Quantitative analysis of Cy5.5-siRNA or Cy5.5-PONI-Guan in major organs by the *ex vivo* fluorescence signals.

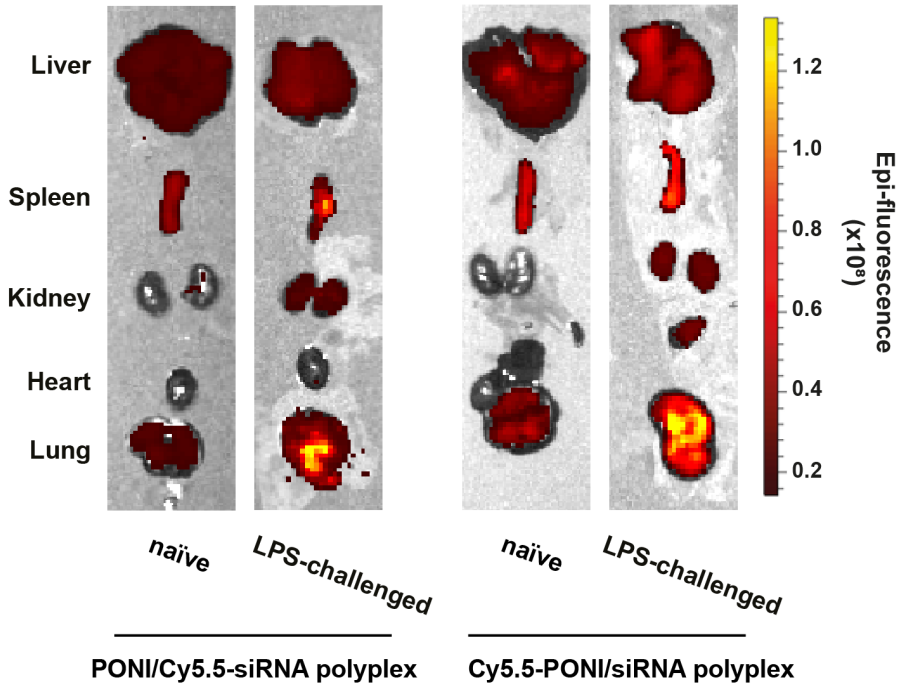

Figure S9. *in vivo* biodistribution of PONI/siRNA polyplexes in BALB/c or LPS-challenged BALB/c mice. Representative *ex vivo* imaging system (IVIS) organ images of Cy5.5-labeled polyplexes 24 h after following systemic administration.