# SUPPLEMENTARY MATERIALS

## Accelerated Blood Clearance of Lipid Nanoparticles Entails a Biphasic Humoral Response of B-1 Followed by B-2 Lymphocyte to Distinct Antigenic Moieties

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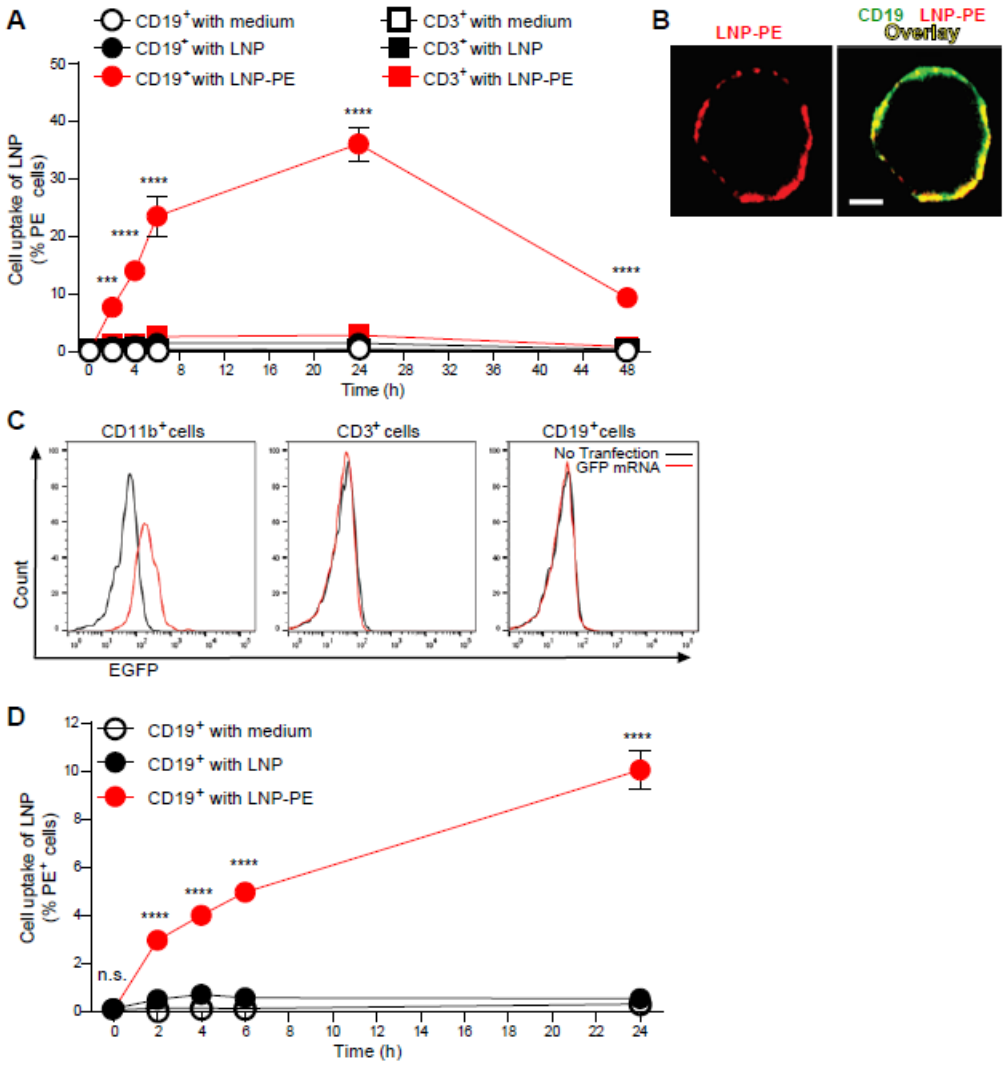
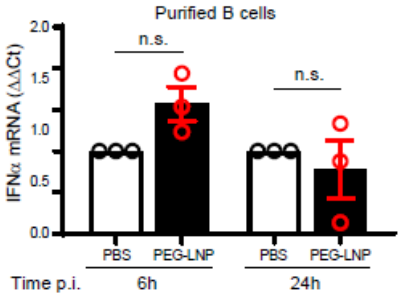
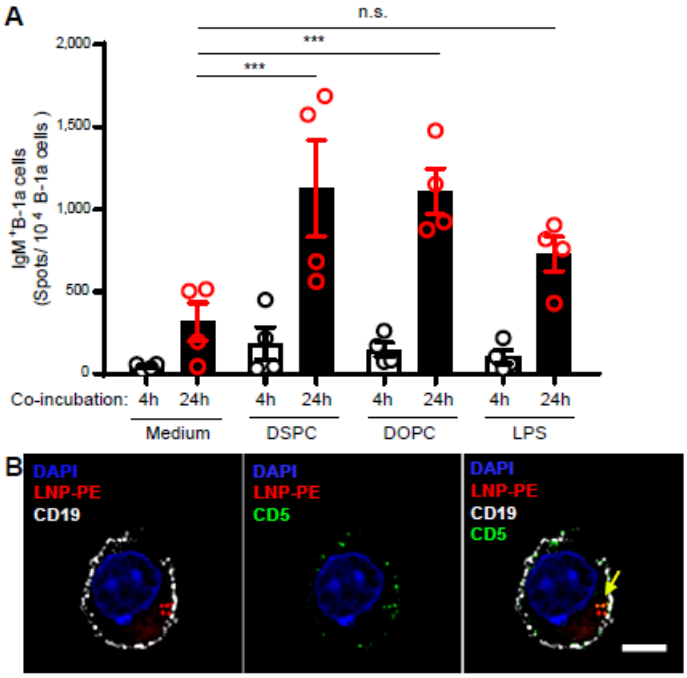



**SUPPLEMENTARY FIGURE S1. LNP associates with surface of B-cells.** (**A**) Splenocytes were incubated in the presence of medium (white symbols), non-fluorescent LNP (LNP, black symbols) or rhodamine-labeled LNP (LNP-PE, red symbols). $CD19^+$ B cells (circles) and $CD3^+$ T cells (squares) were analyzed by flow cytometry for PE incorporation at 2, 4, 6, 24, and 48h. Significance was calculated using two-way ANOVA with Dunnet's post-test vs medium control for each timepoint. (**B**) Splenocytes incubated with rhodamine-labeled LNP (LNP-PE, red) for 24h, stained with FITC-conjugated anti-CD19 (green), and imaged by fluorescence microscopy to visualize fluorescent LNP colocalized with CD19 (yellow). Scale bar= 2µm. (**C**) Splenocytes were incubated with EGFP mRNA formulated with LNP-PE and EGFP expression was analyzed by flow cytometry in $CD11b^+$, $CD3^+$ or $CD19^+$ cells. (**D**) Splenocytes were incubated with medium, (white symbols), empty non-fluorescent LNP (black symbols), or empty LNP-PE (red symbols) and then stained for CD19 and analyzed by flow cytometry for PE incorporation at 2, 4, 6 and 24 h. Significance was calculated using 2-way ANOVA with Dunnet's post-test vs

medium at each timepoint. In all graphs, *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001; n.s.= not significant.




**SUPPLEMENTARY FIGURE S2. LNP alter cytokine production by B cells directly.** Purified B cells were incubated in the presence of PBS or PEG-containing LNP. Fold change in IFNα mRNA over PBS control was measured by qPCR at 6 and 24h. Significance was calculated using 2-way ANOVA with Sidak's post-test vs PBS for each timepoint. n.s.= not significant.




**SUPPLEMENTARY FIGURE S3. LNP associate with surface of B-cells via both PEG-dependent and PEG-independent mechanisms.** (**A**) Sorted splenic $CD19^+$ $CD5^+$ B-1a cells were evaluated for IgM secretion by ELISPOT. Cells were placed on the ELISPOT membrane for 4 or 24h in the presence of medium, PC-containing LNP (DSPC), PC-containing liposomes (DOPC) or LPS. After incubation, the spots were enumerated to determine the percent of IgM secreting cells B-1a cells. Significance was calculated using 2-way ANOVA with Dunnet's post-test vs medium control for each timepoint. (**B**) Splenocytes were incubated with PC-containing, rhodamine-fluorescent LNP (LNP-PE) for 24h, fixed, and stained with anti-CD5 and anti-CD19. The colocalization of LNP-PE (red), CD5 (green) CD19 (white) molecules, and the nuclear stain, DAPI (blue), were assessed by fluorescence microscopy. Scale bar= 2μm. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001; n.s.= not significant.