

1 **SUPPLEMENTARY MATERIALS**

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3 **Accelerated Blood Clearance of Lipid Nanoparticles Entails a Biphasic Humoral Response**

4 **of B-1 Followed by B-2 Lymphocyte to Distinct Antigenic Moieties**

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10 **SUPPLEMENTARY FIGURE S1.** LNP associates with surface of B-cells

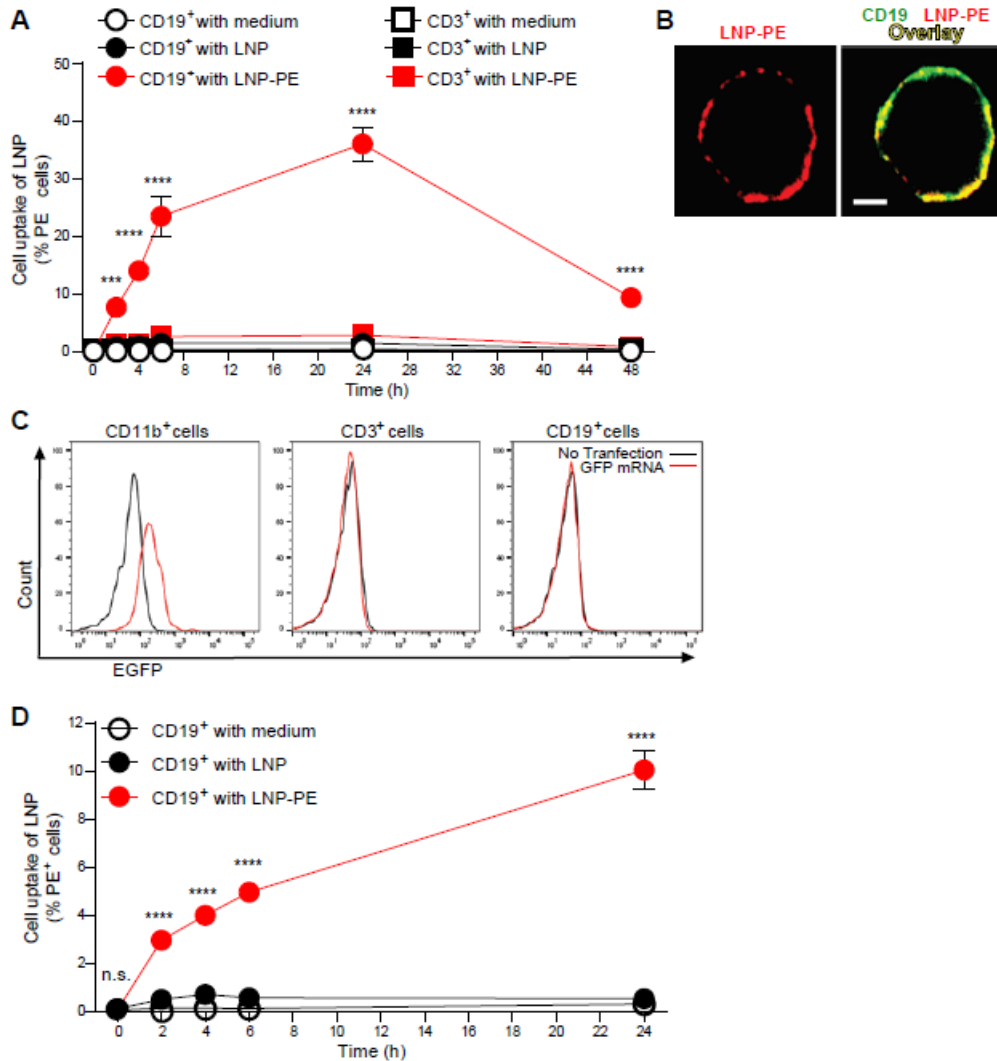
11 **SUPPLEMENTARY FIGURE S2.** LNP alter cytokine production by B cells directly

12 **SUPPLEMENTARY FIGURE S3.** LNP associate with surface of B-cells via both PEG-

13 dependent and PEG-independent mechanisms

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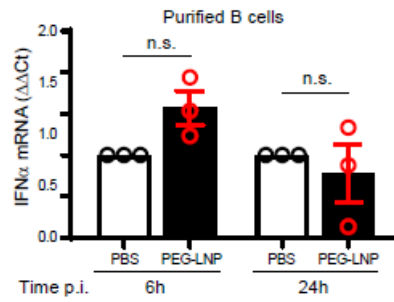


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17 **SUPPLEMENTARY FIGURE S1. LNP associates with surface of B-cells.** (A) Splenocytes
 18 were incubated in the presence of medium (white symbols), non-fluorescent LNP (LNP, black
 19 symbols) or rhodamine-labeled LNP (LNP-PE, red symbols). CD19⁺ B cells (circles) and CD3⁺
 20 T cells (squares) were analyzed by flow cytometry for PE incorporation at 2, 4, 6, 24, and 48h.
 21 Significance was calculated using two-way ANOVA with Dunnet's post-test vs medium control
 22 for each timepoint. (B) Splenocytes incubated with rhodamine-labeled LNP (LNP-PE, red) for
 23 24h, stained with FITC-conjugated anti-CD19 (green), and imaged by fluorescence microscopy
 24 to visualize fluorescent LNP colocalized with CD19 (yellow). Scale bar= 2µm. (C) Splenocytes
 25 were incubated with EGFP mRNA formulated with LNP-PE and EGFP expression was
 26 analyzed by flow cytometry in CD11b⁺, CD3⁺ or CD19⁺ cells. (D) Splenocytes were incubated
 27 with medium, (white symbols), empty non-fluorescent LNP (black symbols), or empty LNP-PE
 28 (red symbols) and then stained for CD19 and analyzed by flow cytometry for PE incorporation
 29 at 2, 4, 6 and 24 h. Significance was calculated using 2-way ANOVA with Dunnet's post-test vs

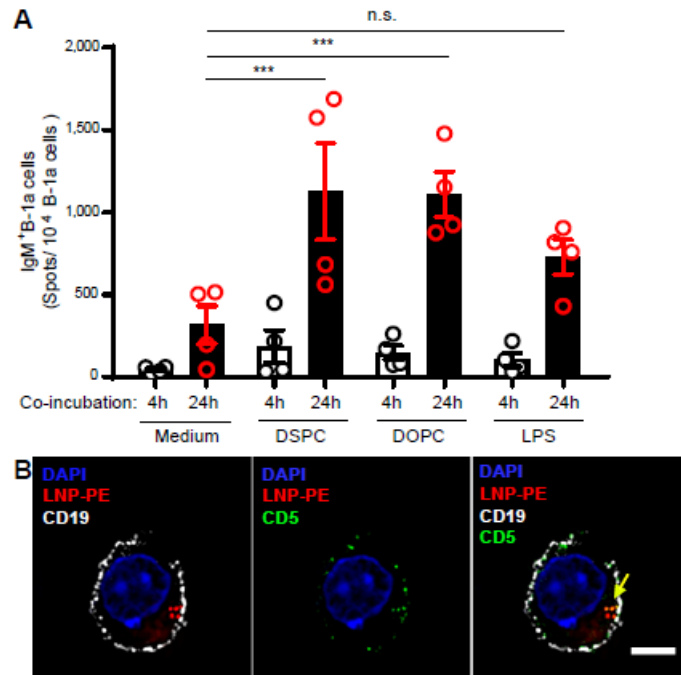
30 medium at each timepoint. In all graphs, * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; n.s.=
31 not significant.

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



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