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Supplemental information

Irf5 siRNA-loaded biodegradable lipid nanoparticles ameliorate concanavalin A-induced liver injury

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Supplemental Figures

Figure S1

	AF488-labeled siRNA-LNP	siCtrl-LNP	si*Irf5*-LNP
Z-average (nm)	84	77	73
Polydispersity index	0.02	0.03	0.05
Encapsulation efficiency (%)	ND	> 90	> 90

Figure S1. Physical properties of the LNPs used in this study

Z-average indicates the mean value of LNP particle size. The polydispersity index is the width parameter of LNPs. Encapsulation efficiency indicates the percentage of siRNA entrapped into LNPs. ND, not determined.

Figure S2

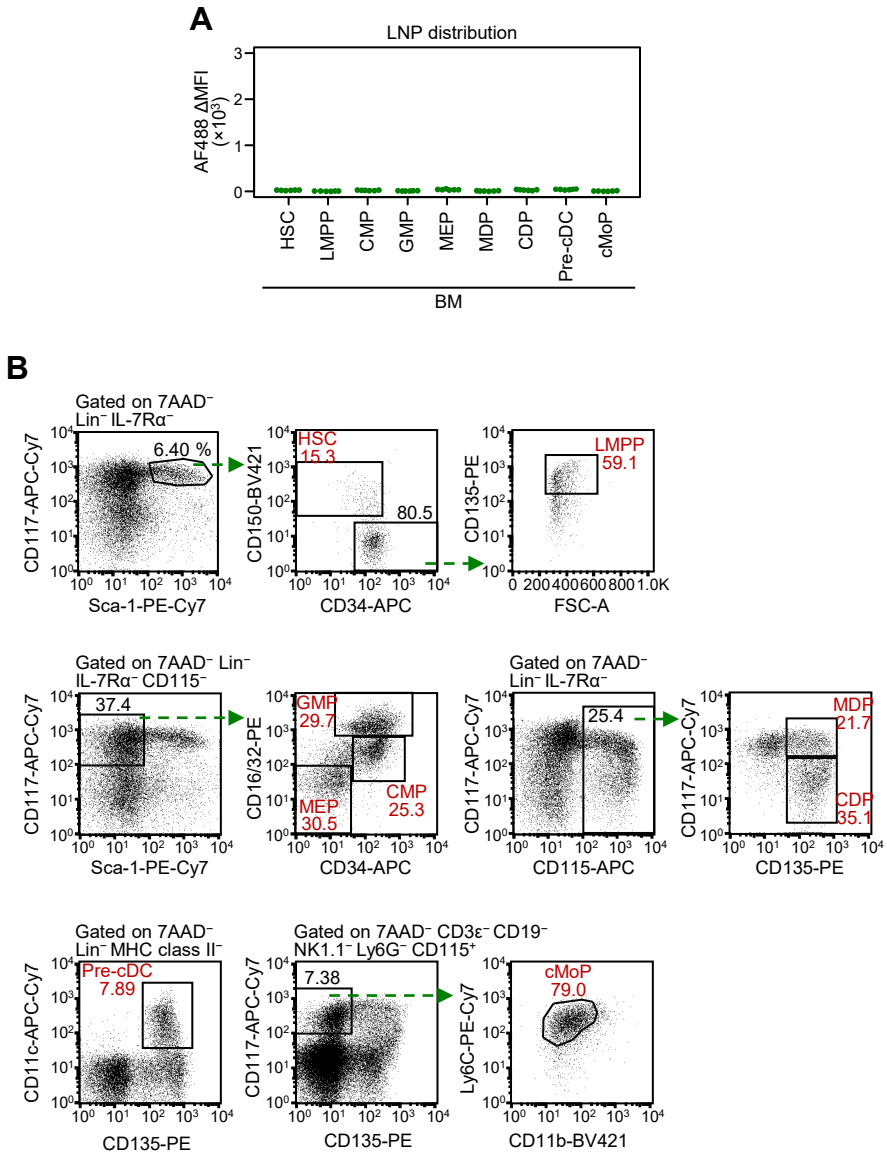

Figure S2. Analysis of biodegradable LNP uptake into hematopoietic stem and progenitor cells
Flow cytometry analysis of AF488-labeled siRNA distribution following intravenous injection of LNPs in mice. Hematopoietic stem and progenitor cells in the bone marrow were analyzed 3 h after the intravenous injection of LNPs-encapsulated AF488-labeled siRNA at a dose of 0.8 mg/kg. (A) The MFI of AF488 in each hematopoietic stem and progenitor cell population is shown in boxplots. Values from two independent experiments are shown. (B) Representative dot plots of hematopoietic stem and progenitor populations. HSC, hematopoietic stem cell; LMPP, lymphoid-primed multipotent progenitor; CMP, common myeloid progenitor; GMP, granulocyte monocyte progenitor; MEP, megakaryocyte erythroid progenitor; MDP, monocyte dendritic cell progenitor; CDP, common dendritic cell progenitor; pre-cDC, committed classical dendritic cell precursor; cMoP, common monocyte progenitor.

Figure S3

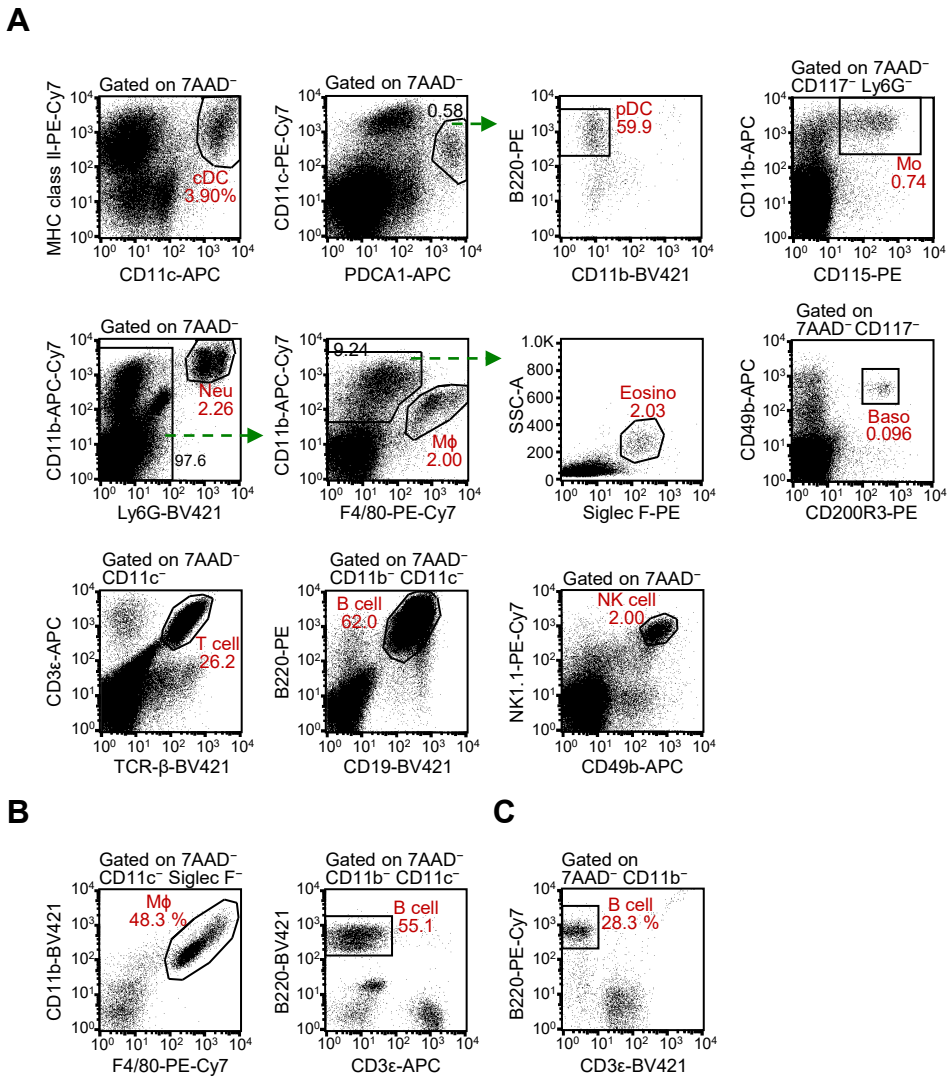

Figure S3. Incorporation of biodegradable LNPs in splenocytes
Flow cytometry gating strategy for immune cells in the spleen (A), peritoneal exudate cells (B), and the liver (C). cDC, conventional dendritic cell; pDC, plasmacytoid dendritic cell; Mo, monocyte; Mϕ, macrophage; Neu, neutrophil; Eosino, eosinophil; Baso, basophil.

Figure S4

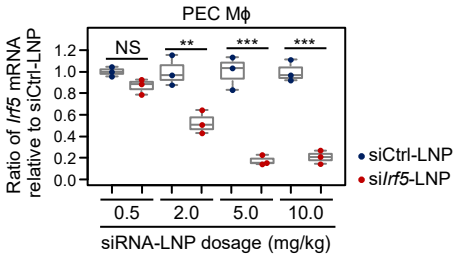

Figure S4. Dose-dependent *Irf5* silencing effect of biodegradable LNPs
Mice were injected with siCtrl-LNPs or si*Irf5*-LNPs at siRNA doses of 0.5, 2.0, 5.0, and 10.0 mg/kg. Twenty-four hours after LNP administration, *Irf5* mRNA expression in peritoneal Mϕs was analyzed by qRT-PCR. Values were normalized to the expression levels of *Gapdh* using the ΔΔCT method. The relative expression values to the mean of siCtrl-LNP values were calculated. Data from three independent experiments are presented. $^{**}p < 0.01$, $^{***}p < 0.001$ (Student's *t* test). NS, not significant.

Figure S5

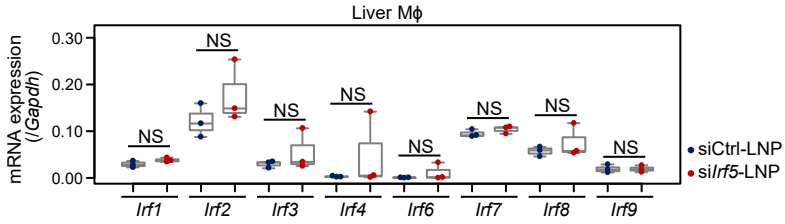

Figure S5. mRNA expression of IRF family transcription factors after injection of LNPs
Mice were injected with siCtrl-LNPs and si*Irf5*-LNPs at a siRNA dose of 5.0 mg/kg. Twenty-four hours after LNP administration, the mRNA expression of IRF family transcription factors in liver F4/80^{+}CD11b^{+} Mϕs was analyzed by qRT-PCR. Values were normalized to *Gapdh* levels using the ΔΔCT method. Data from three independent experiments are presented. NS, not significant.

Figure S6

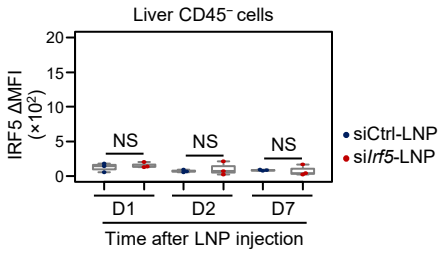

Figure S6. IRF5 protein expression of CD45⁻ cells in the liver
After LNP administration, IRF5 protein expression in liver CD45⁻ cells were analyzed by immunofluorescence staining at indicated time points. Boxplots indicate ΔMFI of IRF5 expression. Data from three independent experiments are shown. NS, not significant.