Supporting Information.

The influence of charge on hemocompatibility and immunoreactivity of polymeric nanoparticles

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Synthesis of Polymer Materials.

Polymers were synthesized as previously reported by our group. In brief, the hyperbranched polymers consisting of poly(polyethyleneglycol methacrylate) (poly(PEGMA)), poly(dimethylaminoethylmethacrylate) (poly(DMAEMA)) or poly(methacrylic acid) (poly(MAA)) were synthesized via the RAFT process using prop-2-yn-1-yl 4-cyano-4 (((ethylthio)carbonothioyl)thio)pentanoate (PCEPA) as chain transfer agent. In a typical polymerization procedure, PEGMA (2.000 g, 4.21 mmol), DMAEMA (0.662 g, 4.21 mmol) or tBMA (0.599 g, 4.21 mmol) was mixed with ethyleneglycol dimethacrylate (0.042 g, 0.211 mmol), PCEPA (0.063 g, 0.221 mmol), initiator azobisisobutyronitrile (0.00693 g, 0.0422 mmol) and tetrahydrofuran (4 ml) in a round bottom flask. The mixture was purged with argon in an ice-water bath for 25 minutes to remove oxygen followed by heating to 70 °C in an oil bath. After 24 hours, the reaction was quenched by immersion in an ice-water bath and air was introduced. Excess THF was gently removed under nitrogen, and the residual mixture was precipitated three times in excess hexane to remove unreacted monomer. The precipitate was dried under vacuum at room temperature (RT) overnight. For further purification, the poly(PEGMA) and poly(DMAEMA) polymers were dialyzed in deionized water which was changed regularly over 72 hours prior to isolation and lyophilisation.

To produce HBPs with a negatively charged surface, poly(tBMA) was dissolved in THF, then trifluoroacetic acid (TFA) (five times equivalent to the tert-butyl ester) was added into the reaction flask to remove isobutene as the elimination product. After a period of 12 hours stirring at RT, excess THF was removed through gently bubbling nitrogen. For purification, the polymer was precipitated through hexane twice, followed by vacuum drying overnight. Then, the resulting residue was dissolved in water and dialyzed in deionized water which was changed regularly for 72 hours. The final product (poly(MAA)) was obtained by freeze

drying. All polymers were analysed by a variety of techniques, including GPC, NMR, UV-Vis and DLS as described in the discussion and supporting information.

Table S1. Physical and chemical properties of the three different hyperbranched polymers utilised in this study.

Polymer	Mnd 1H NMR / kDa	Mn GPC-MALLS / kDa (ÐM)	Arms/HBP	In Water		
				TEM Size/nm	Size/nm (number average)	Zeta Potential (mV)
Poly(PEGMA)a	10.7	47.1 (1.31)	4.3	4.4±0.3	4.8±0.2	3.5±0.2
Poly(DMAEMA)b	5.2	23.9 (1.33)	4.6	4.8±0.4	4.9±0.7	+37.6±0.4
Poly(tBMA)c,d	7.0	25.7 (1.26)	3.7	-	-	-
Poly(MAA)	-	-	-	4.9±0.3	5.3±0.2	-33.1±0.3

[a]dn/dc Poly(PEGMA) = 0.042. [b]dn/dc Poly(DMAEMA) = 0.052. [c]dn/dc Poly(tBMA) = 0.069. [d]Mn of each arm length in the HBP. [e]DLS was not conducted for poly(tBMA) due to its insolubility in water.

Table S2. Endotoxin Levels of Various-Charged HBPs

Spike	Spike Run Time	Sample Run Time	Endotoxin level

	Recovery / %	CV / %	CV / %	EU ml-1
Standard	50~200	<25	<25	0.005~0.5
Neutral HBP	104	6.4	6.3	<0.006
Cationic HBP	56	1.6	1.3	<0.005
Anionic HBP	89	3.3	3.2	<0.005

In order to control for potential nanoparticle interference with the assay, the cartridge had four wells and two of the channels contained an endotoxin spike. According to the United States, European and Japanese pharmacopeia, a test is considered valid if the CVs of both the sample were < 25% and spike recovery was between 50% and 200%. The values of the spike recovery for our three HBPs samples were in this range.

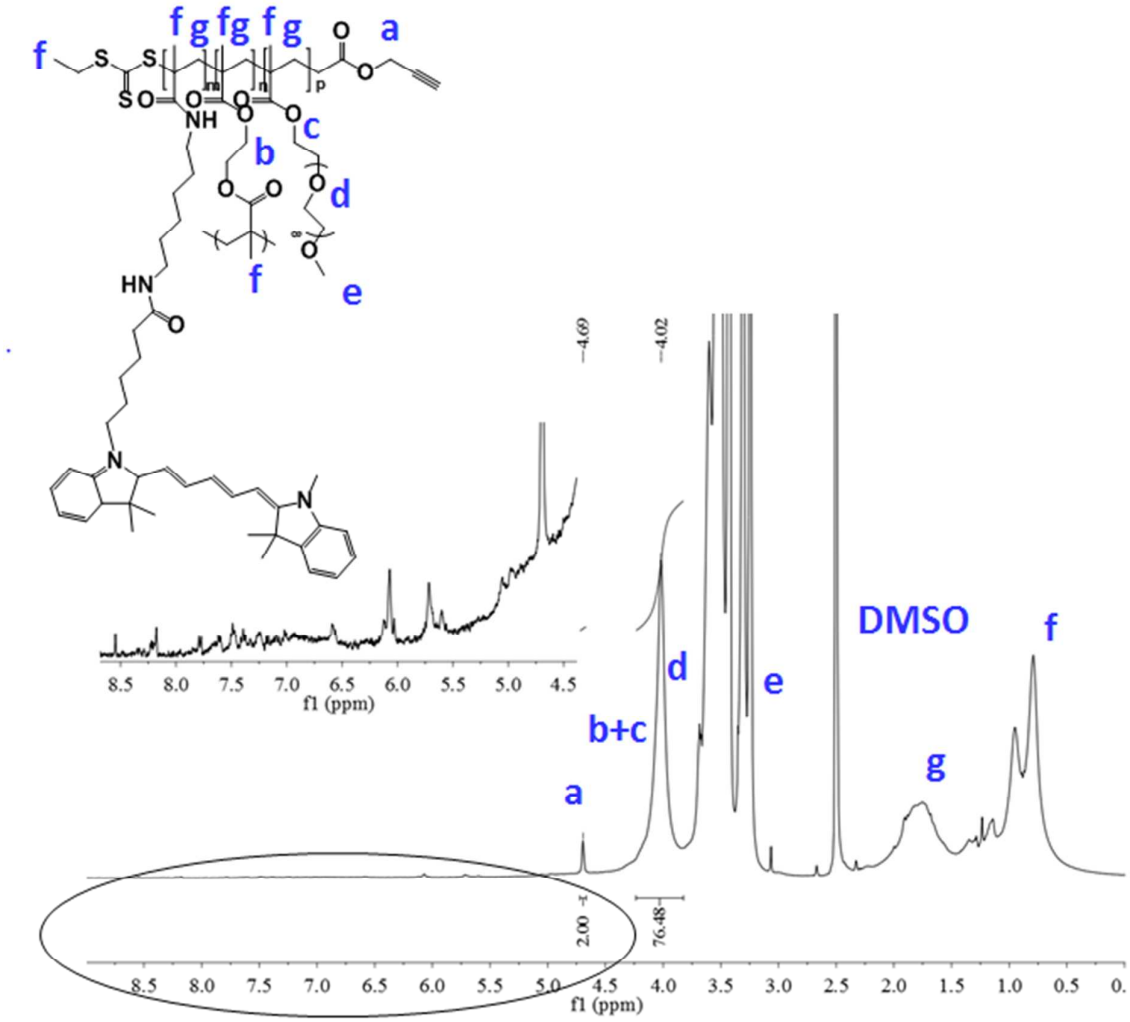

Figure S1. ^{1}H NMR spectra of Cy5-poly(PEGMA) with peaks assigned to Cy5 highlighted between 5 ~ 8.5 ppm.

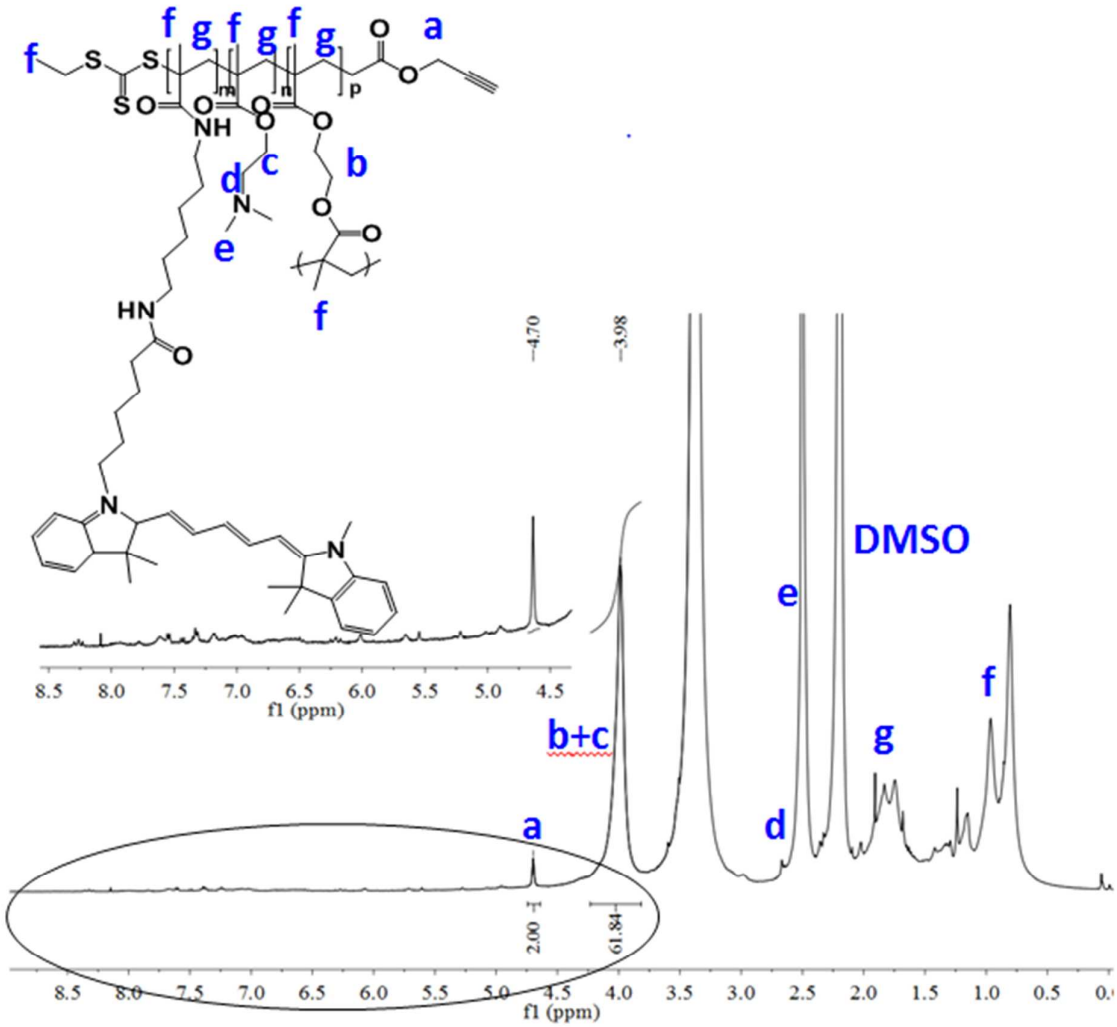

Figure S2. ^{1}H NMR spectra of Cy5-poly(DMAEMA) with peaks assigned to Cy5 highlighted between 5 ~ 8.5 ppm.

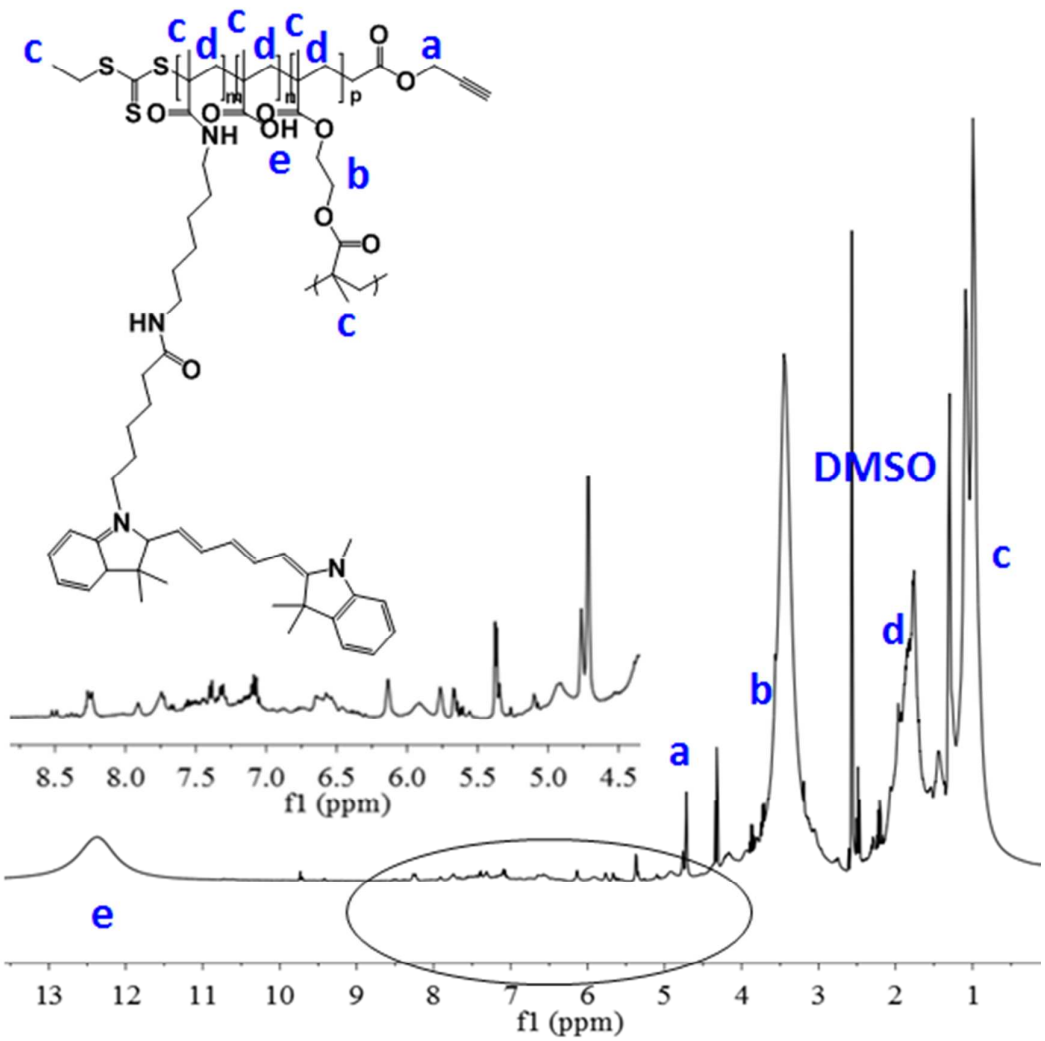

Figure S3. 1H NMR spectra of Cy5-poly(MMA) with peaks assigned to Cy5 highlighted between 5 ~ 8.5 ppm.

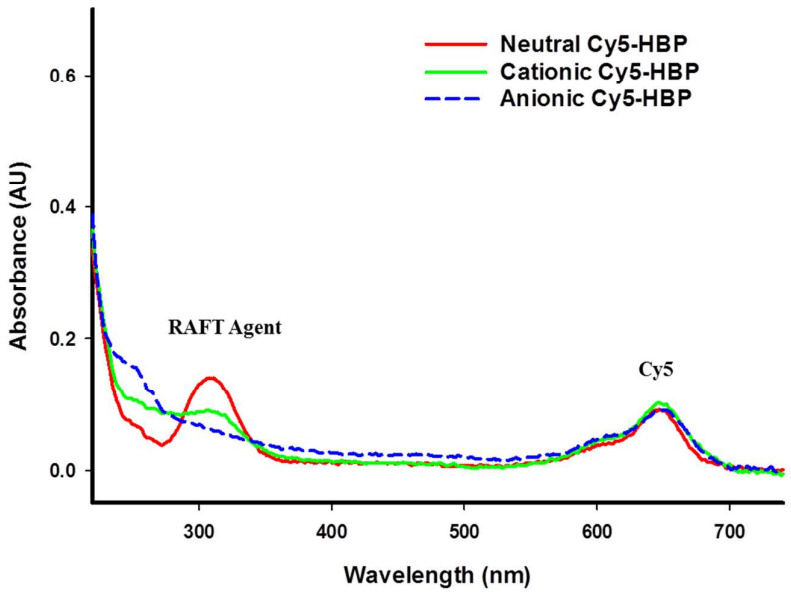

Figure S4. UV-Vis of Cy5-HBPs with the Cy5 absorbance at 647 nm.

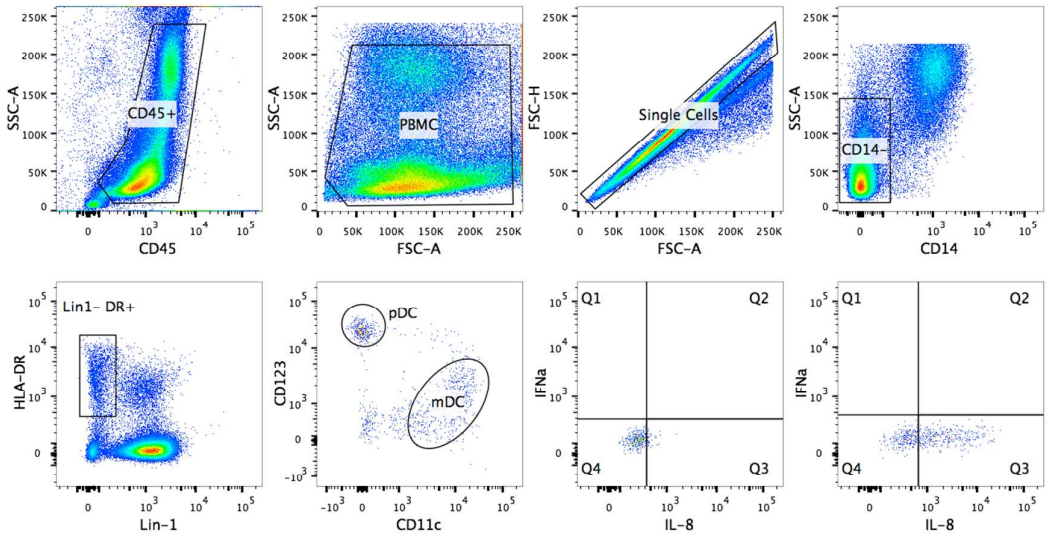

Figure S5. Identification of human DC subsets by flow cytometry. Leukocytes are identified as CD45+ events, before gating on PBMC by forward scatter (FSC) and side scatter (SSC). Single cells are determined by FSC-Area vs. FSC-Height, before removing CD14+ monocytes. DCs are identified as Lin-1-/HLA-DR+ cells, and divided into mDC (CD11c+ CD123low) and pDC (CD11c- CD123hi) subsets. The expression of cytokines IL-8 and IFNa can then be examined. The response of a representative donor to cationic HBPs (100 µg/ml) is displayed.

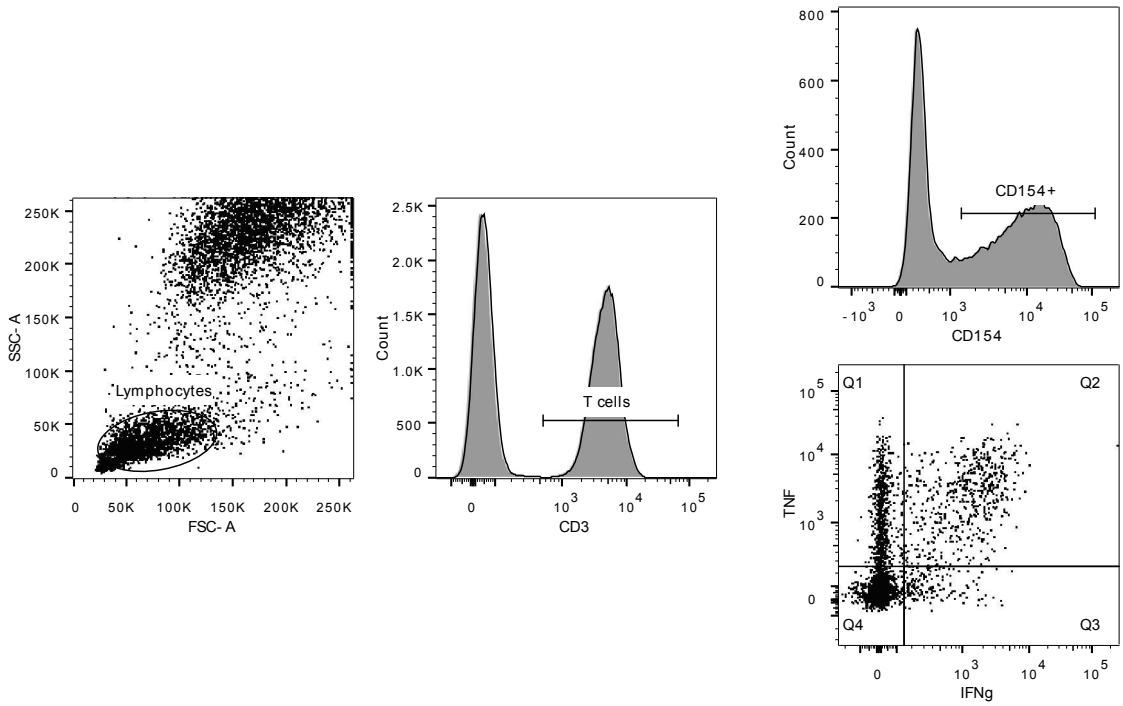

Figure S6. Identification of human T cells by flow cytometry. Lymphocytes are gated by FSC vs. SSC and T cells are defined as CD3+ lymphocytes, whose activation status is observed by IFNg, TNF and CD154 experesion. The response to PMA/Ionomycin stimulation by a representative donor is displayed.

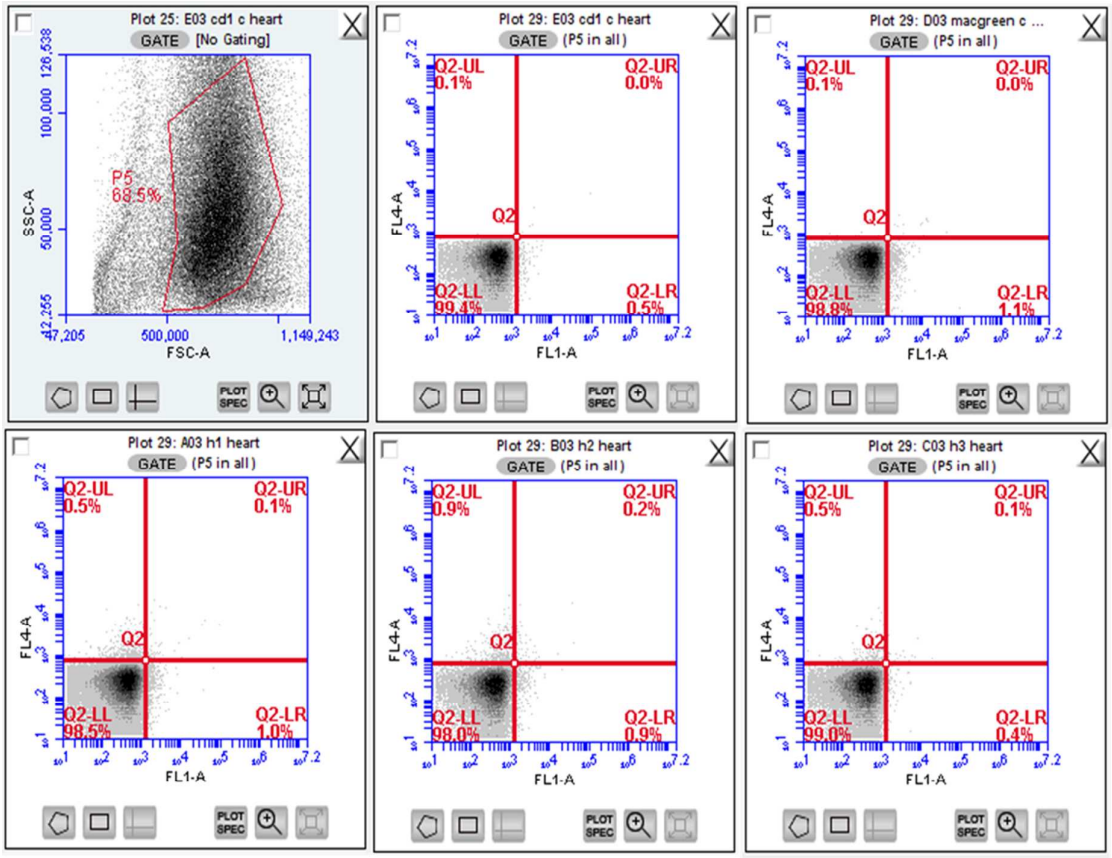

Figure S7. FACS Data for Single-Cell Suspension from Heart for Macgreen Mice, 6 hours Following Injection of Variou-Charged Cy5-HBPs (h1 represting neutral charged HBPs; h2 represting cationic HBPs; h3 representing anionic HBPs). The X-axis displayed the FL1 signal coming from the macrophages of heart while the Y-axis displayed the FL4 signal coming from Cy5-HBPs. Percentage of cells displayed both GFP signal and Cy5 signal was recorded here (the upper right percentage number), indicating the uptake of heart macrophage. CD 1 and Macgreen Mice without HBPs injection were used as control.

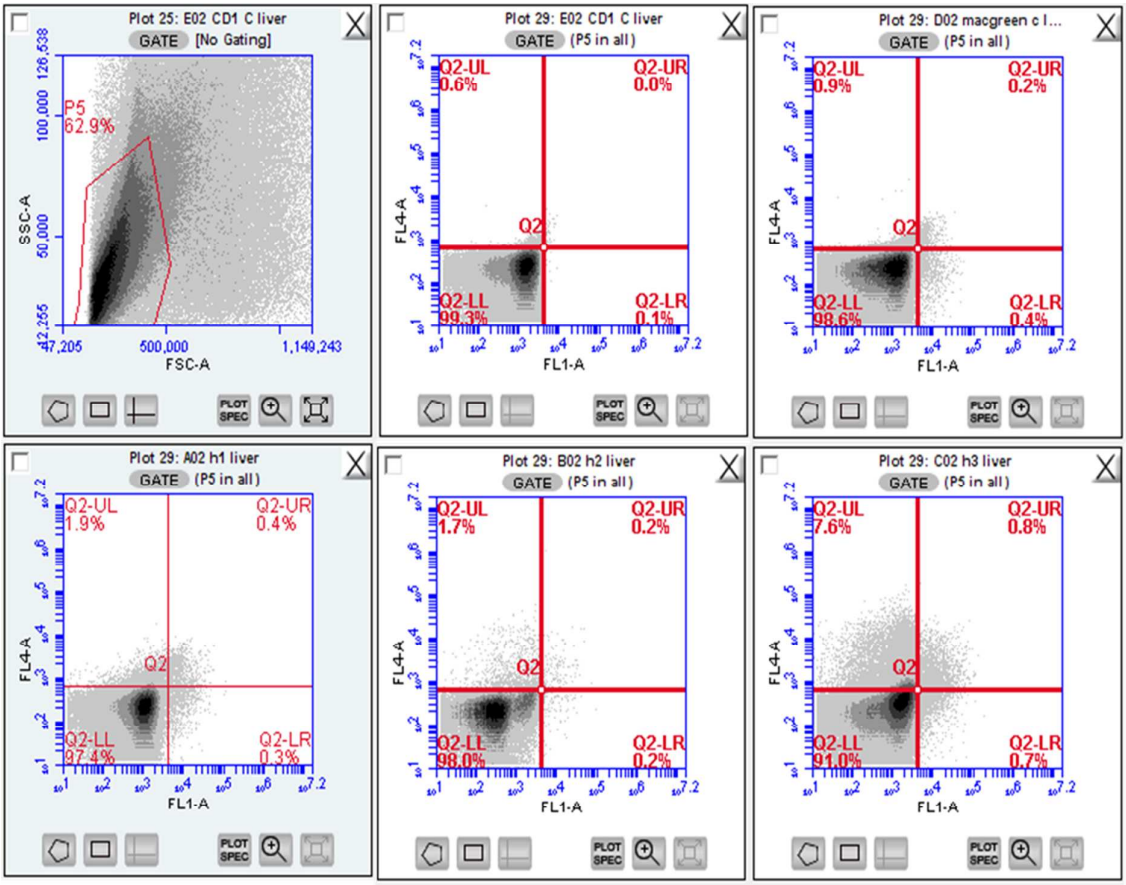

Figure S8. FACS Data for Signle-Cell Suspension from Liver for Macgreen Mice, 6 hours Following Injection of Variou-Charged Cy5-HBPs (h1 represting neutral charged HBPs; h2 represting cationic HBPs; h3 representing anionic HBPs). The X-axis displayed the FL1 signal coming from the macrophages of heart while the Y-axis displayed the FL4 signal coming from Cy5-HBPs. Percentage of cells displayed both GFP signal and Cy5 signal was recorded here (the upper right percentage number), indicating the uptake of liver macrophage. CD 1 and Macgreen Mice without HBPs injection were used as control.

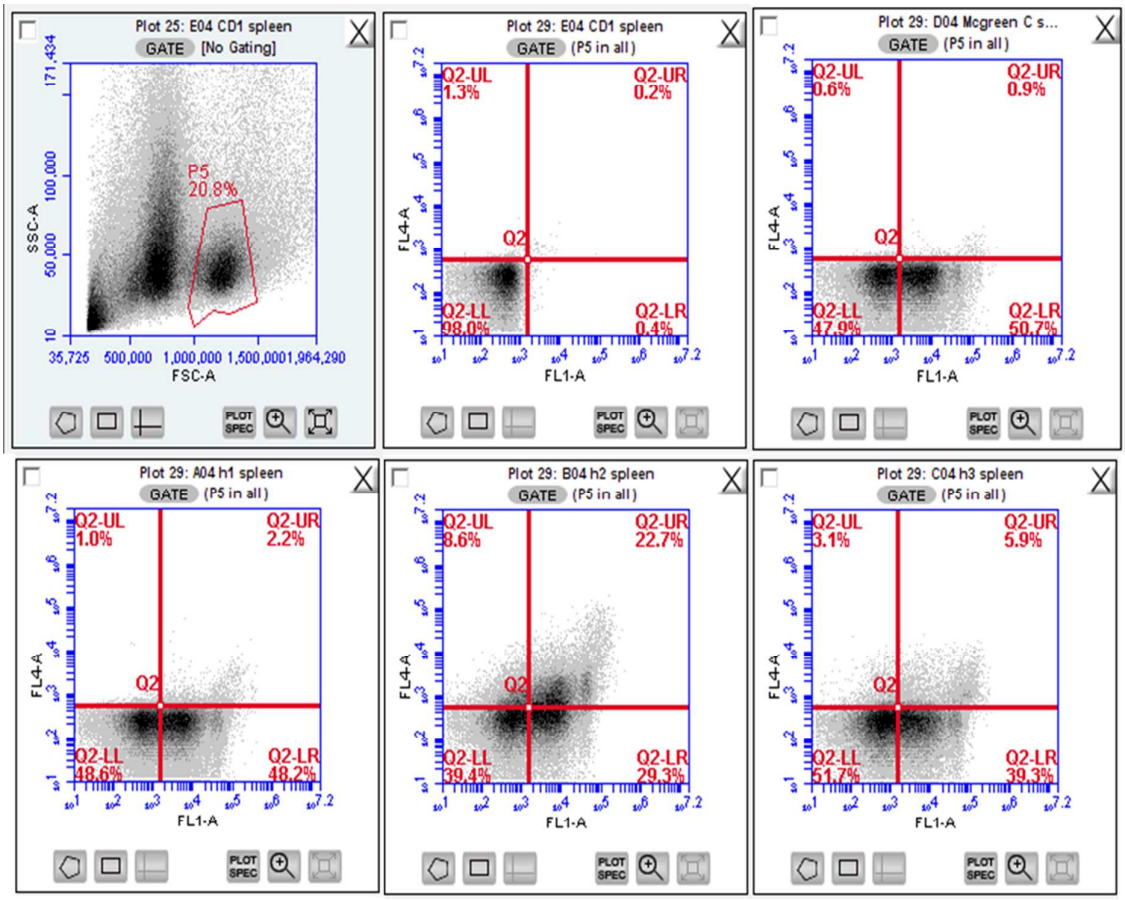

Figure S9. FACS Data for Signle-Cell Suspension from Spleen for Macgreen Mice, 6 hours Following Injection of Variou-Charged Cy5-HBPs (h1 represting neutral charged HBPs; h2 represting cationic HBPs; h3 representing anionic HBPs). The X-axis displayed the FL1 signal coming from the macrophages of liver while the Y-axis displayed the FL4 signal coming from Cy5-HBPs. Percentage of cells displayed both GFP signal and Cy5 signal was recorded here (the upper right percentage number), indicating the uptake of spleen mcrophage. CD 1 and Macgreen Mice without HBPs injection were used as control.

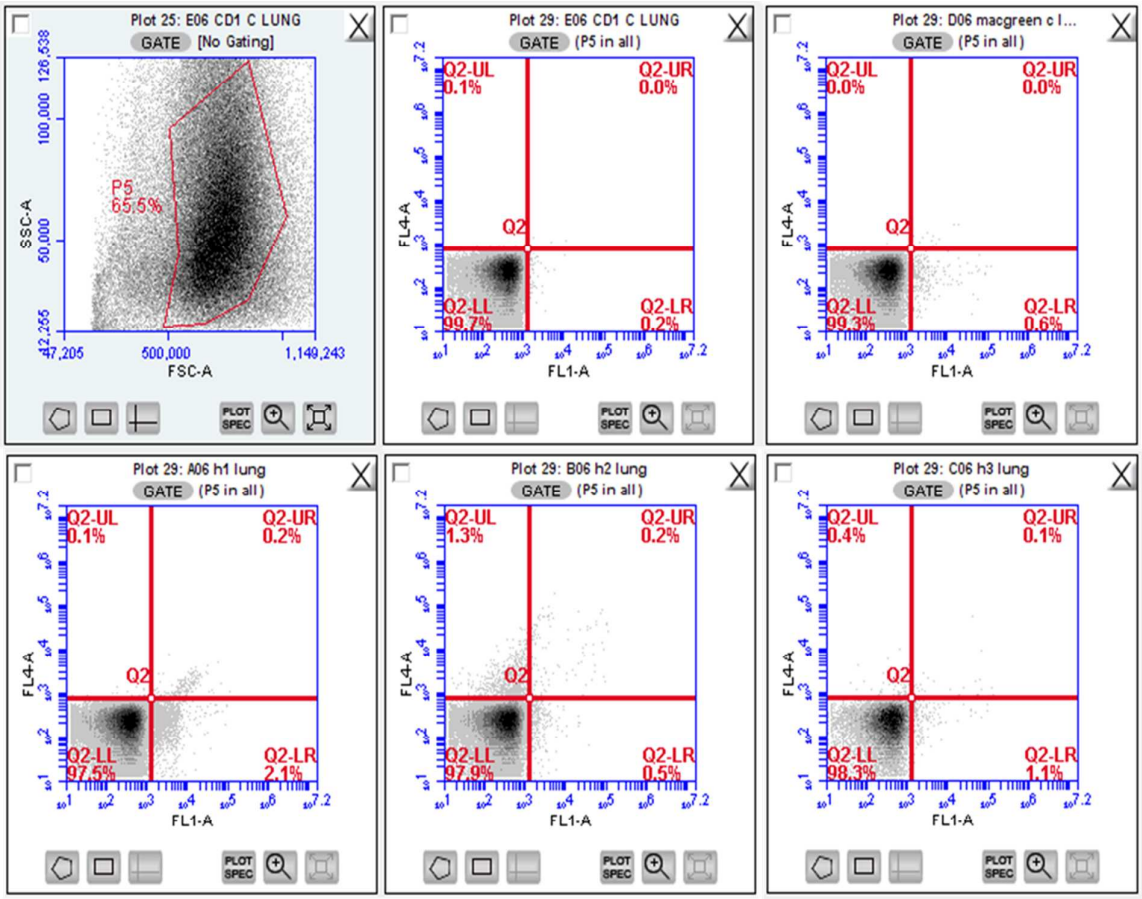

Figure S10. FACS Data for Signle-Cell Suspension from Lung for Macgreen Mice, 6 hours Following Injection of Variou-Charged Cy5-HBPs (h1 represting neutral charged HBPs; h2 represting cationic HBPs; h3 representing anionic HBPs). The X-axis displayed the FL1 signal coming from the macrophages of lung while the Y-axis displayed the FL4 signal coming from Cy5-HBPs. Percentage of cells displayed both GFP signal and Cy5 signal was recorded here (the upper right percentage number), indicating the uptake of lung mcrophage. CD 1 and Macgreen Mice without HBPs injection were used as control.

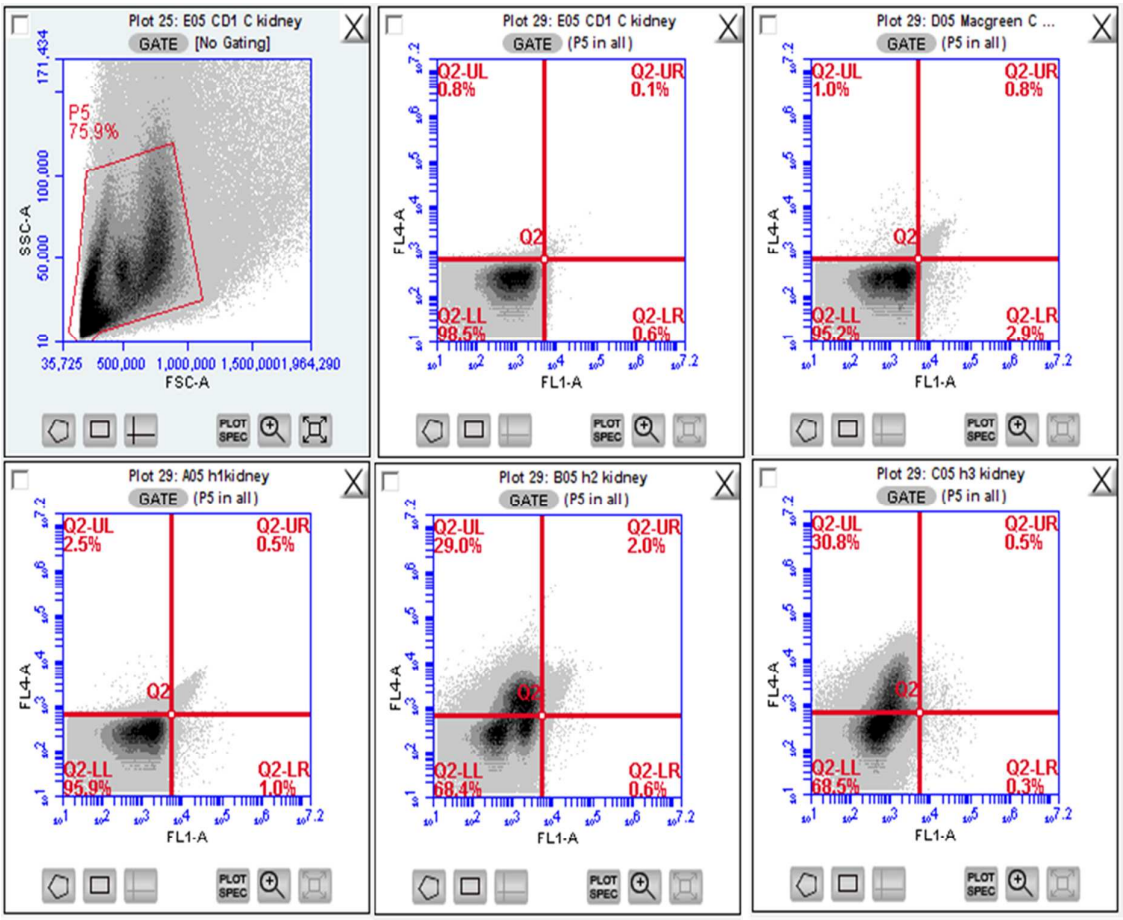

Figure S11. FACS data for single-cell suspension from kidneys for Macgreen Mice, 6 hours following injection of various-charged Cy5-HBPs (h1 representing neutral charged HBPs; h2 representing cationic HBPs; h3 representing anionic HBPs). The X-axis displayed the FL1 signal coming from the macrophages of kidney while the Y-axis displayed the FL4 signal coming from Cy5-HBPs. Percentage of cells displayed both GFP signal and Cy5 signal was recorded here (the upper right percentage number), indicating the uptake of kidney macrophage. CD 1 and Macgreen Mice without HBPs injection were used as control.

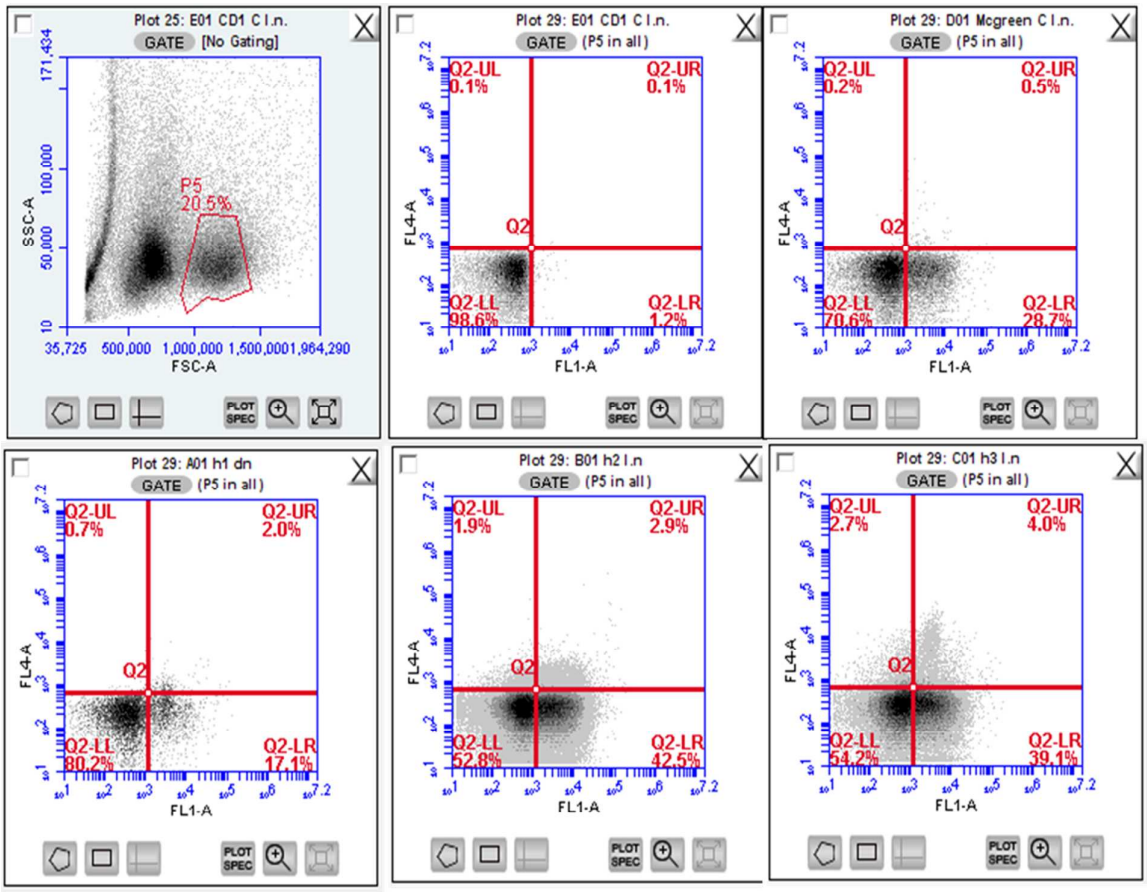

Figure S12. FACS Data for Single-Cell Suspension from Lymph Nodes for Macgreen Mice, 6 hours Following Injection of Variou-Charged Cy5-HBPs (h1 representing neutral charged HBPs; h2 representing cationic HBPs; h3 representing anionic HBPs). The X-axis displayed the FL1 signal coming from the macrophages of lymph nodes while the Y-axis displayed the FL4 signal coming from Cy5-HBPs. Percentage of cells displayed both GFP signal and Cy5 signal was recorded here (the upper right percentage number), indicating the uptake of lymph nodes macrophage. CD 1 and Macgreen Mice without HBPs injection were used as control.

References.

1. Chen, L.; Simpson, J. D.; Fuchs, A. V.; Rolfe, B. E.; Thurecht, K. J., Effects of Surface Charge of Hyperbranched Polymers on Cytotoxicity, Dynamic Cellular Uptake and Localization, Hemotoxicity, and Pharmacokinetics in Mice. *Mol Pharm* **2017**. 14(12), 4485-4497.