

## Supporting Information

### Polysaccharide Nanoparticles Can Efficiently Modulate the Immune Response Against an HIV Peptide Antigen

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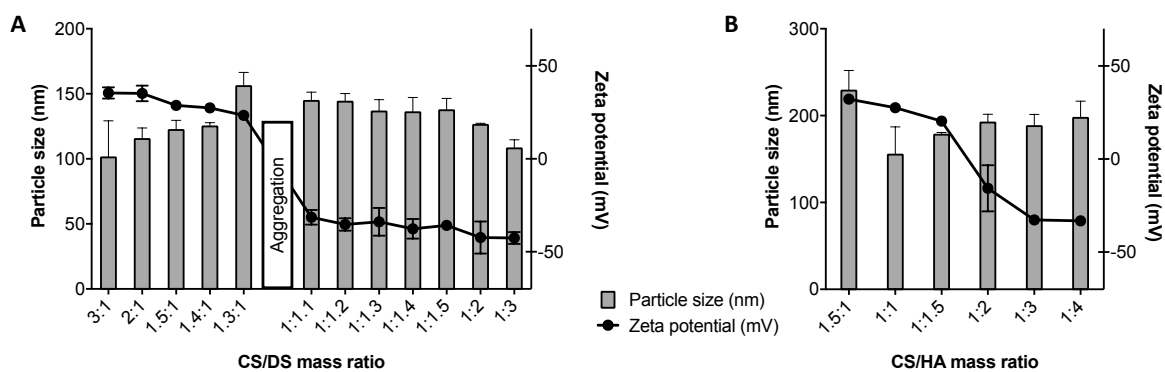
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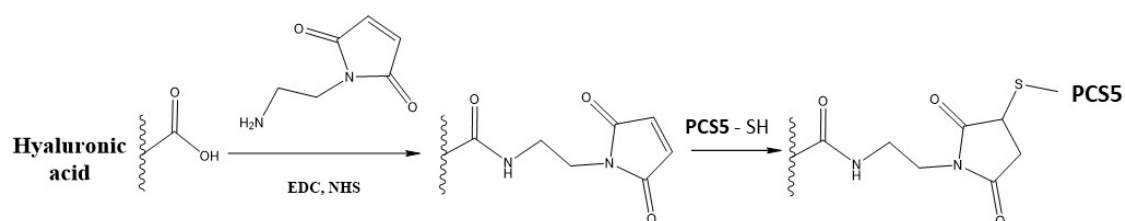
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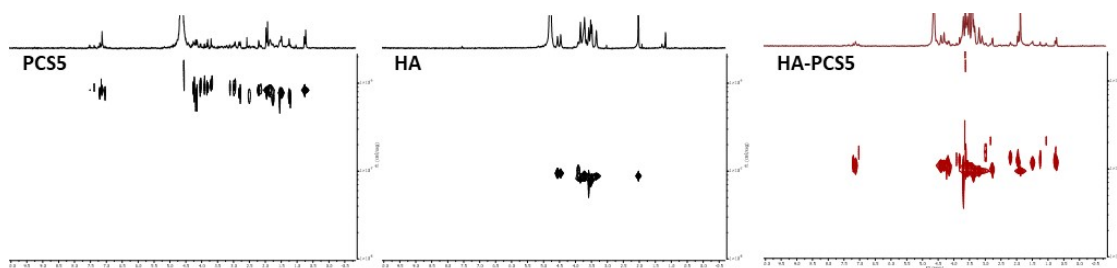
**Keywords:** HIV vaccine; peptide antigen; polysaccharide; nanovaccine; poly(I:C); nanoparticle; antigen encapsulation



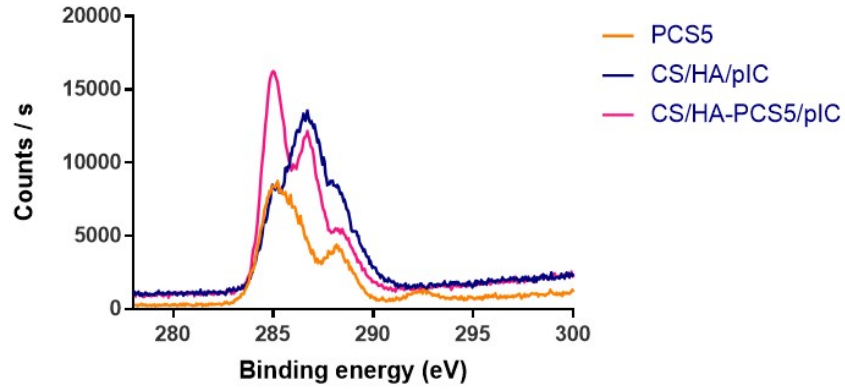
**Supporting Figure S1.** Evolution of particle size and zeta potential of blank (A) CS/DS NPs and (B) CS/HA NPs as the mass ratio of the negative polymer is increased. CS: chitosan, DS: dextran sulfate, NPs: nanoparticles, HA: hyaluronic acid.



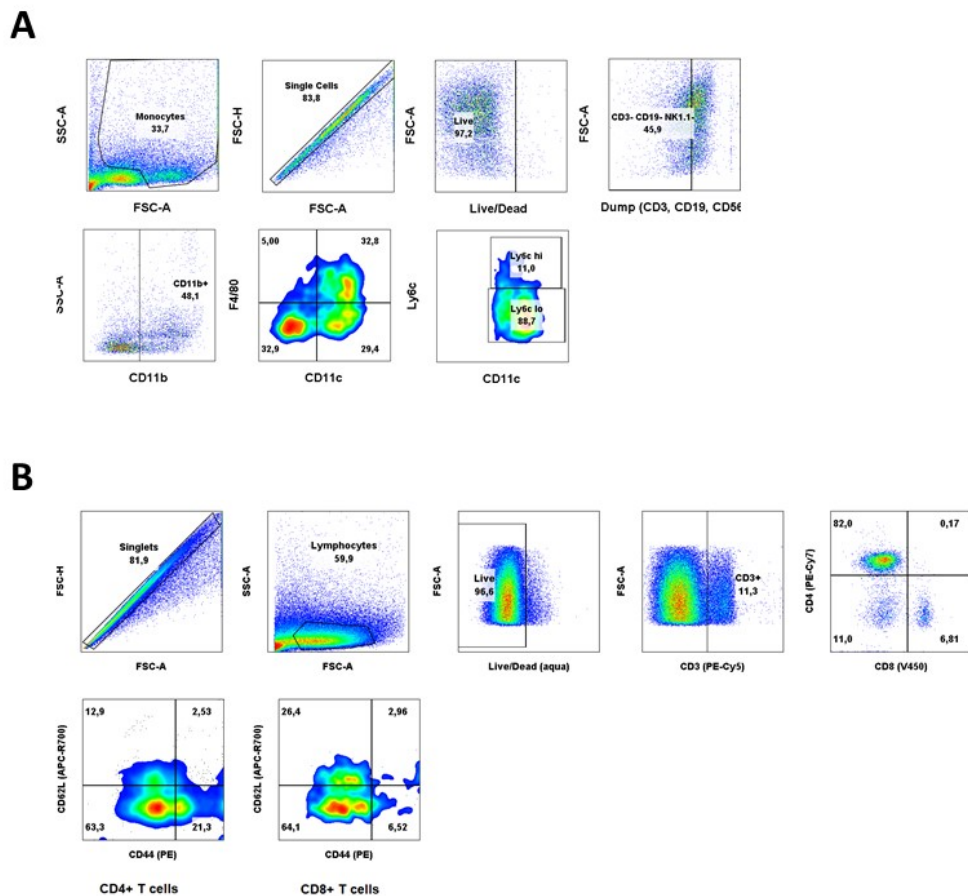
**Supporting Figure S2.** Preparation of the conjugate HA-PCS5 in a 2-step thiol-maleimide conjugation reaction.



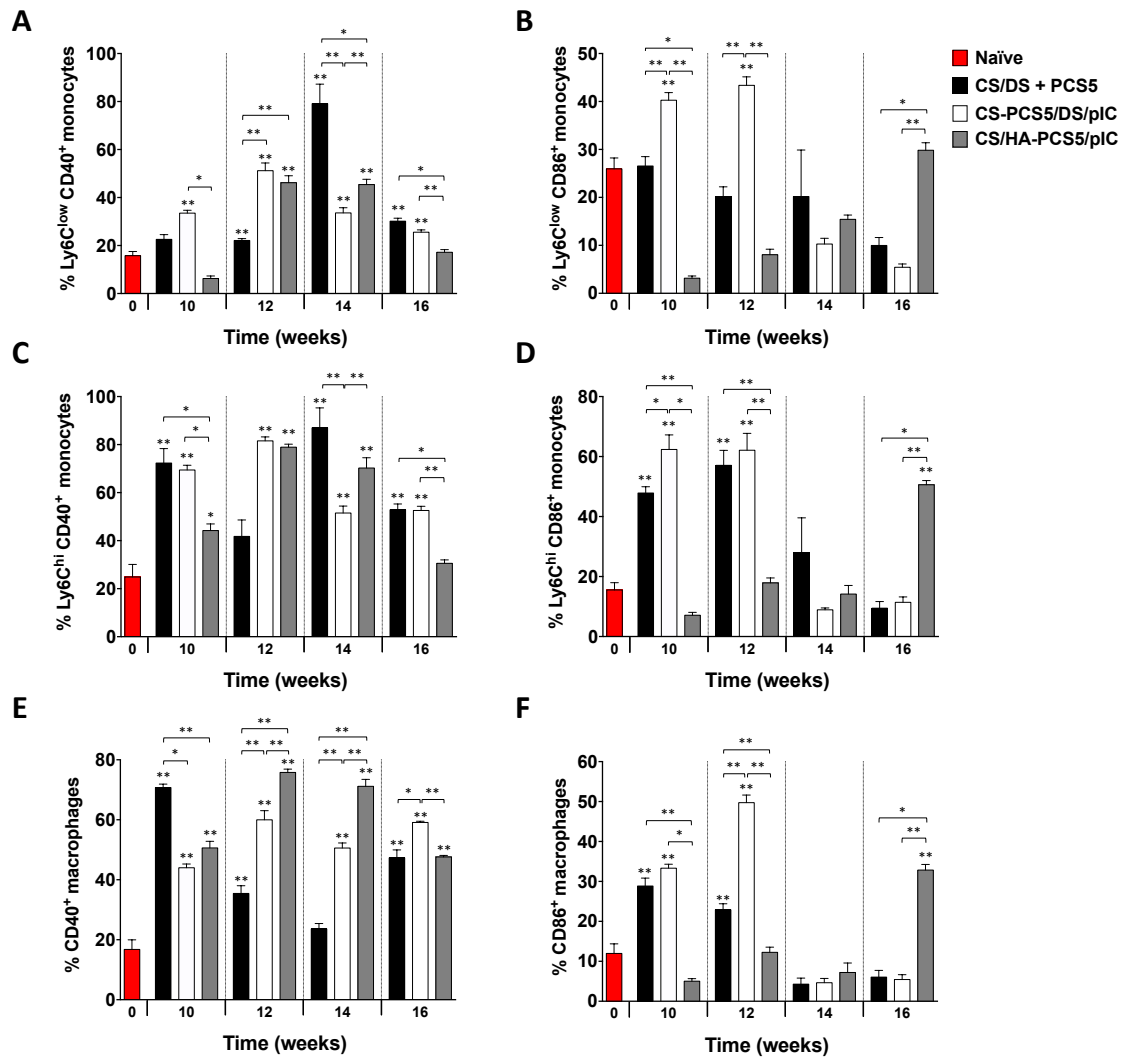
**Supporting Figure S3.** Diffusion-ordered spectroscopy (DOSY) spectra of PCS5, HA and HA-PCS5. PCS5: protease cleavage site 5, HA: hyaluronic acid.



**Supporting Figure S4.** Carbon s<sub>1</sub> binding energies of PCS5 (orange), CS/HA/pIC NPs (blue) and CS/HA-PCS5/pIC NPs (pink). PCS5: protease cleavage site 5, CS: chitosan, HA: hyaluronic acid, pIC: poly(I:C) (polyinosinic:polycytidylic acid), NPs: nanoparticles.



**Supporting Figure S5.** Multicolor flow gating of (A) monocytes Ly6c<sup>hi</sup> and Ly6c<sup>low</sup> and macrophages CD11b<sup>+</sup> CD11c<sup>-</sup> F4/80<sup>+</sup> and (B) central memory and effector memory T cells (CD44<sup>+</sup> CD62L<sup>+</sup> and CD44<sup>+</sup> CD62L<sup>-</sup>).



**Supporting Figure S6.** Monocyte and macrophage expression of co-stimulatory factors at 10, 12, 14 and 16 weeks post prime. CD40<sup>+</sup> and CD86<sup>+</sup> expression in (A-B) Ly6C<sup>low</sup> monocytes; (C-D) Ly6C<sup>high</sup> monocytes and (E-F) macrophages was quantified by multicolor flow cytometry of splenocytes obtained from non-treated naïve (red bars) and NP-vaccinated mice: CS/DS + PCS5 (black bars), CS-PCS5/DS/pIC (white bars) or CS/HA-PCS5/pIC (gray bars). Values represent mean  $\pm$  SEM ( $n \geq 3$ ). Statistical comparison between groups was done using a Mann-Whitney test. Significant statistical differences are represented as \* ( $p < 0.05$ ) and \*\* ( $p < 0.01$ ) for comparison between groups and to naïve mice. NPs: nanoparticles, CS: chitosan, DS: dextran sulfate, PCS5: protease cleavage site 5, pIC: poly(I:C) (polyinosinic:polycytidylic acid), HA: hyaluronic acid.

**Supporting Table S1.** Elemental composition (%) by XPS of the surface of CS, HA, PCS5, blank CS/HA/pIC NPs and loaded CS/HA-PCS5/pIC NPs.

<b>Sample</b>	<b>C</b>	<b>O</b>	<b>N</b>	<b>Cl</b>	<b>Mg</b>	<b>F</b>	<b>Na</b>	<b>S</b>	<b>C/O</b>	<b>C/N</b>
<b>Chitosan</b>	50.53	32.78	7.46	6.18	2.75	0.30	-	-	1.54	6.77
<b>Hyaluronic acid</b>	51.73	34.87	3.95	0.45	0.71	5.42	5.88	-	1.48	13.10
<b>PCS5</b>	58.49	16.72	15.96	-	-	6.79	-	2.05	3.50	3.66
<b>CS/HA/pIC NPs</b>	55.19	29.38	4.29	2.05	-	-	4.56	0.28	1.87	12.86
<b>CS/HA-PCS5/pIC NPs</b>	65.51	20.52	7.04	3.67	-	0.39	1.72	1.15	3.19	9.31

Key: CS, chitosan; HA, hyaluronic acid; PCS5, protease cleavage site 5; pIC, poly(I:C); NPs, nanoparticles.