Polymerized Ovalbumin Nanoparticles for Cancer Immunotherapy

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Table S1. Size data that were used for calculation of NP swelling. The SEM size (dry size) was measured using ImageJ (>500 particles/group were measured – size distribution graphs below. PEG/OVA=50%:blue; 30%:green, 10%:red, 5%:grey). The reported DLS size (swollen size) was obtained from Gaussian fits of raw DLS data.

	5% 20k	**10% 2k**	**30% 2k**	**50% 2k**
SEM size (nm)	176.6	86.6	85.2	78.4
DLS size (nm)	514.1	266.1	202.0	165.2

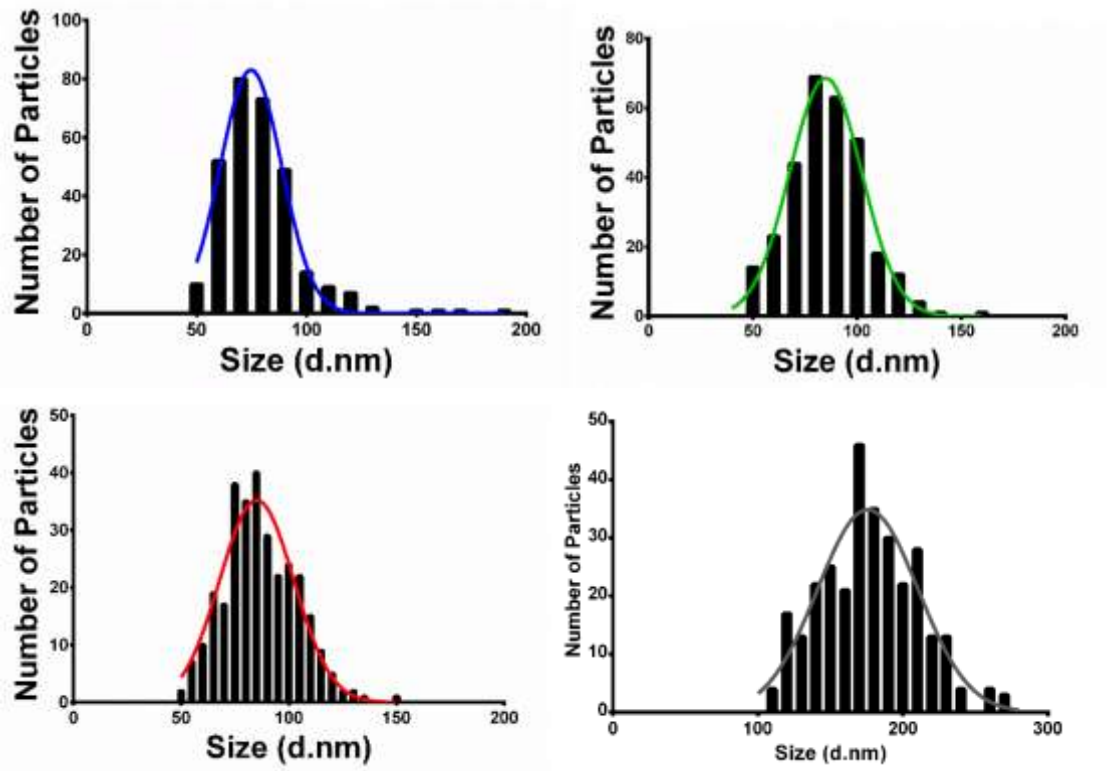

Table S2. MFI of AlexaFluor 647 labeled OVA pNPs measured by plate reader. Data were measured in duplicate and averaged.

	MFI
50% 2k	11291.5
10% 2k	11353
30% 2k	10930
5% 20k	10878

Table S3. Zeta potential of OVA pNPs (mV).

Particle Group	**Zeta Potential (mV)**	
	Average	**SD**
5% 20k	-6.3267	0.32294
10% 2k	-6.7733	0.49466
30% 2k	-6.64	0.72392
50% 2k	-5.9167	0.39203

Fabrication of samples for AFM measurements. AFM force-distance profiles were obtained by jetting the OVA nanoparticles onto an amine-reactive polymer coating, namely poly(4-Pentafluorophenyl-p-xylene)-coatings. This was done to ensure that the nanoparticles remain on the surface during the AFM measurements in PBS buffer. The amine-reactive coating was prepared on silicon substrates using CVD polymerization, as previously reported elsewhere [1]. Afterwards, the coatings were rinsed with acetone and dried under a stream of nitrogen. OVA NPs were directly jetted onto the coated substrates. They were left at room temperature for about two weeks before conducting the AFM measurements.

[1] *Adv. Mater.* **2016**, *28*, 3145–3151

Serial centrifugation protocol. OVA NPs were jetted onto a collector sheet and collected in PBS buffer containing 0.1% Tween20 using a razor blade.

1. **10%, 30%, 50% crosslinked OVA NPs:** The collected OVA NPs were tip-sonicated, passed through a 40μm filter and centrifuged at 4,000rpm for 5mins and the supernatant was saved. The pellet was re-dispersed in PBS containing 0.1% Tween20, tip-sonicated and centrifuged again at 4,000rpm for 5mins. The supernatant was combined with the supernatant from the first centrifugation and centrifuged for 60mins at 14,000rpm. The supernatant was discarded, and the

pellet was re-dispersed in 1mL PBS. The pellet was washed 3x with 1mL of PBS and stored in the fridge for subsequent analysis.

2. **5% (500nm size) OVA NPs:** The collected OVA NPs were tip-sonicated, passed through a 40μm filter and centrifuged at 1,000rpm for 1min and the supernatant was saved. The pellet was re-dispersed in PBS containing 0.1% Tween20, tip-sonicated and centrifuged again at 1,000rpm for 1min. The supernatant was combined with the supernatant from the first centrifugation and centrifuged for 1min at 10,000rpm. The supernatant was discarded, and the pellet was re-dispersed in 1mL PBS. The pellet was washed 3x with 1mL of PBS and stored in the fridge for subsequent analysis.

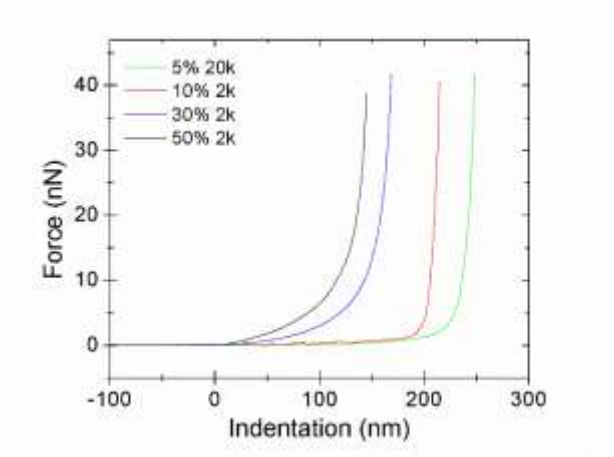

Figure S1. Force-indentation profiles from which Young's moduli were calculated using the Hertz model for a conical indenter.

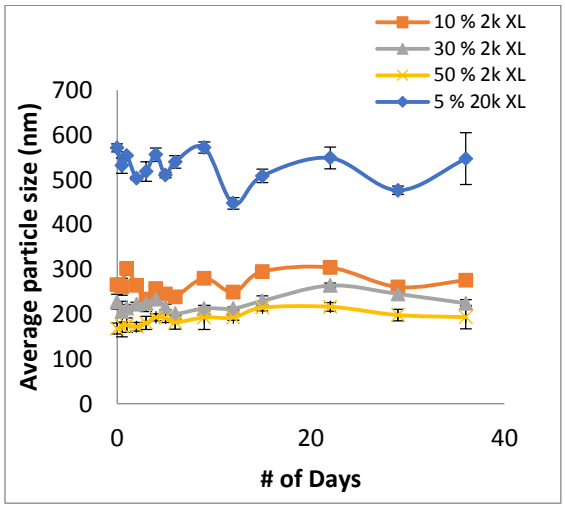

Figure S2. Stability of OVA NPs in PBS monitored by DLS over a period of 36 days.

CD8α+ T Cell Tetramer Staining: Blood samples were collected from the submandibular vein of mice, and red blood cells were lysed with Ammonium-Chloride-Potassium (ACK) lysis buffer. Tetramer staining assay was used to quantify the percentage of tumor antigen-specific CD8α+ T cells among PBMCs, as described previously [2]. Briefly, PBMCs were isolated, washed with FACS buffer, and incubated with anti-CD16/32 blocking antibody. Cells were then incubated with tetramer for 1 h on ice, then incubated with anti-mouse CD8α-APC for 20 min on ice. Cells were then washed 2× with FACS buffer, resuspended in DAPI solution (2 mg/ml), and analyzed by flow cytometry.

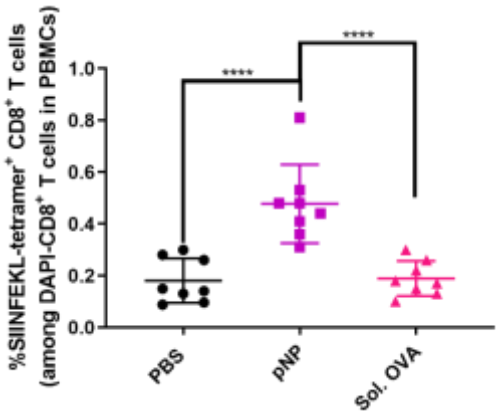

Figure S3. The percentage of SIINFEKL-tetramer+ CD8+ T cells among total CD8+ T cells in PBMCs on day 13.

[2] *Adv. Healthc. Mater*. **2017**, 6, 1601418.

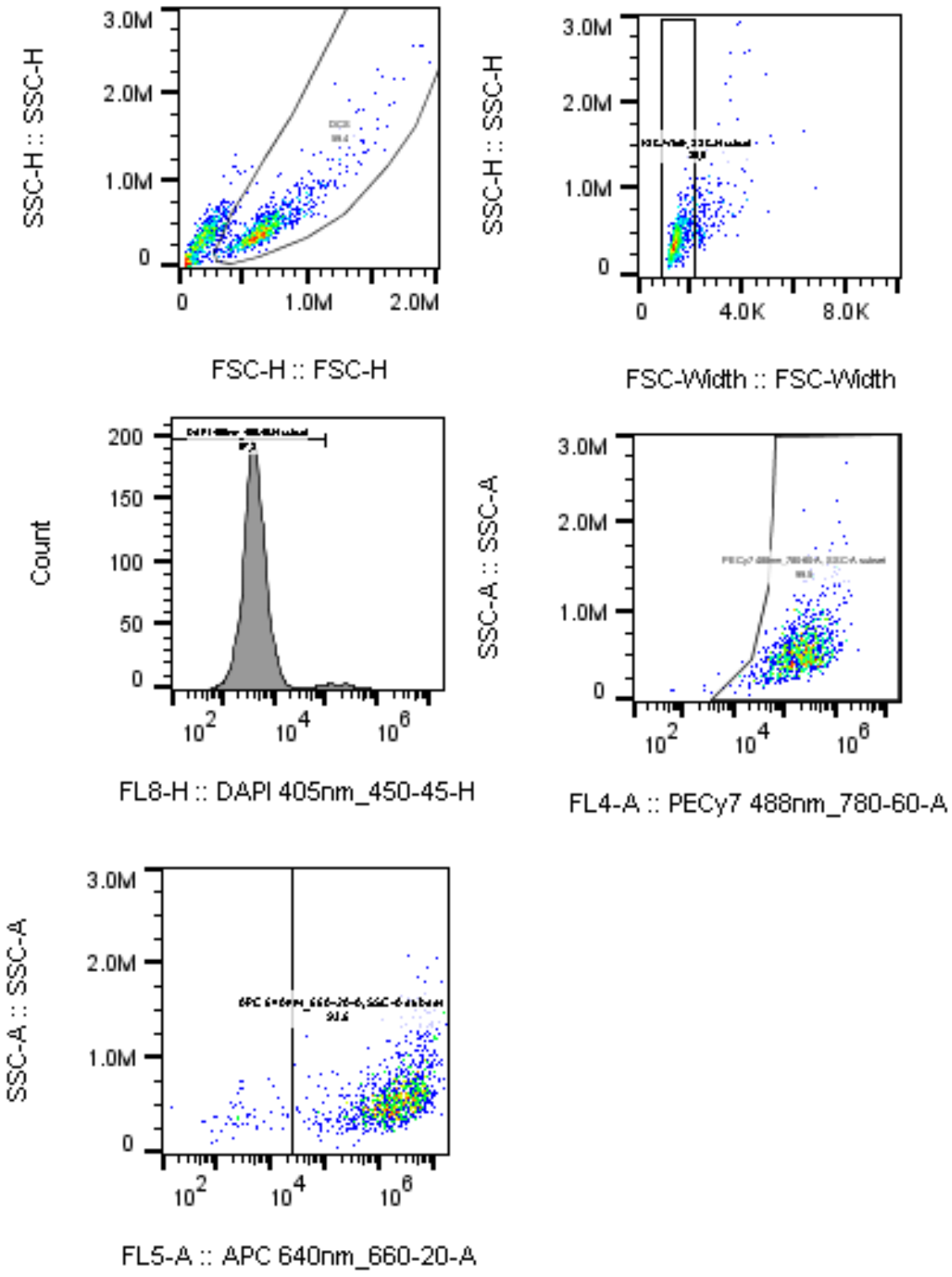

Figure S4. Flow cytometry gating for BMDC uptake: 5%AlexaFluor 647 (APC) labeled OVA NPs. Live/dead stain: DAPI; CD11c+ (DC stain): PECy7.

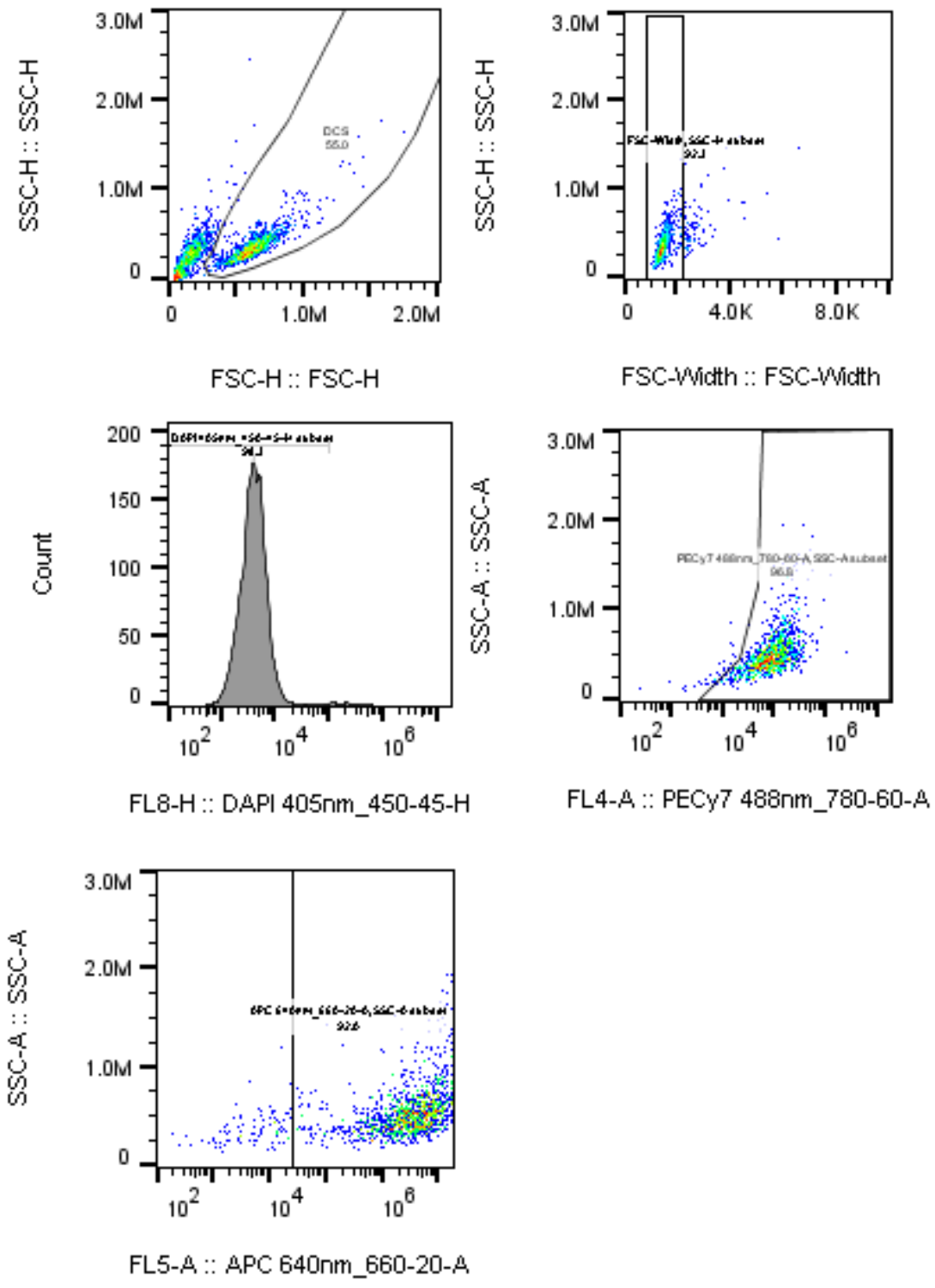

Figure S5. Flow cytometry gating for BMDC uptake: 10%AlexaFluor 647 (APC) labeled OVA NPs. Live/dead stain: DAPI; CD11c+ (DC stain): PECy7.

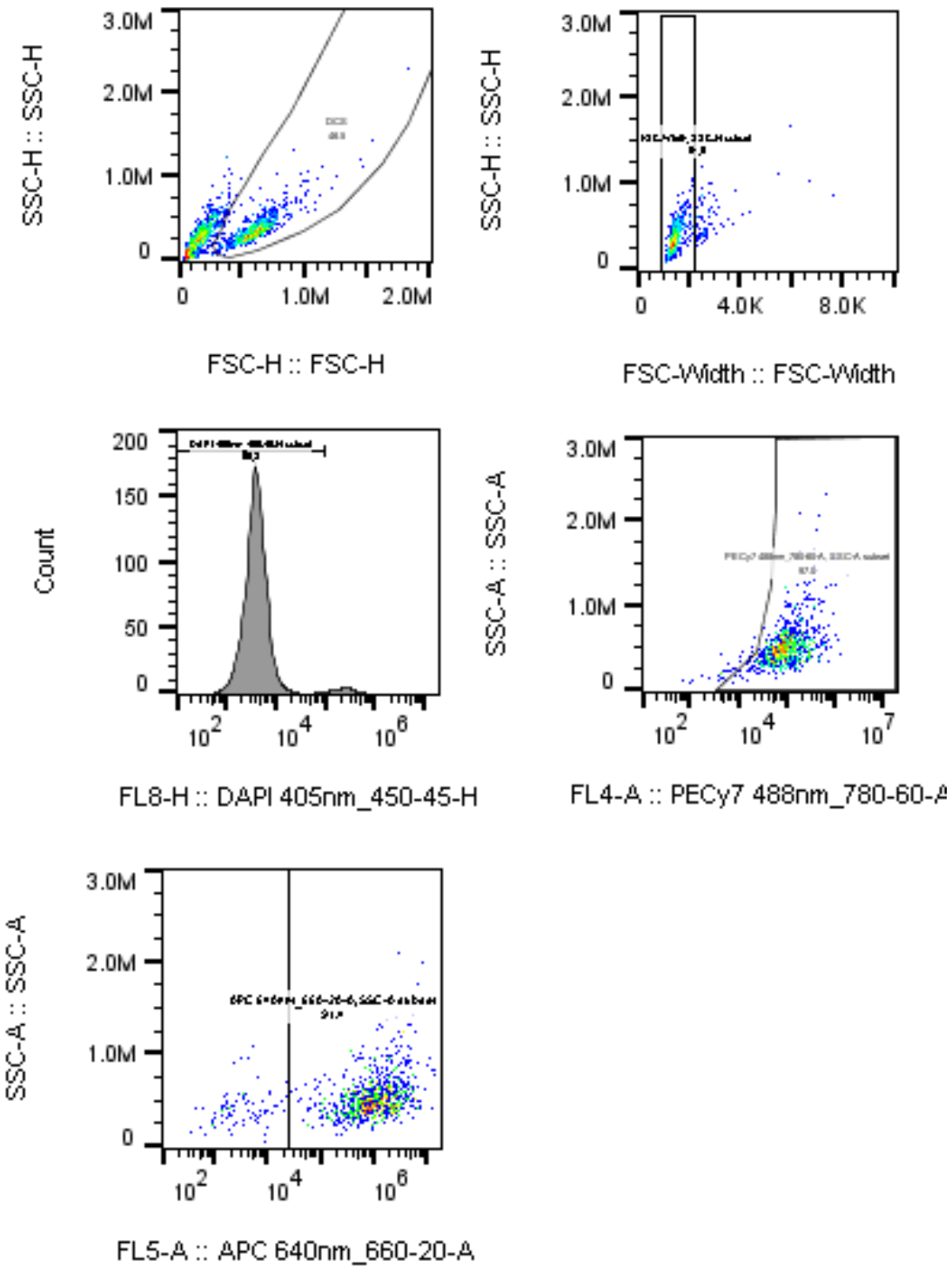

Figure S6. Flow cytometry gating for BMDC uptake: 30%AlexaFluor 647 (APC) labeled OVA NPs. Live/dead stain: DAPI; CD11c+ (DC stain): PECy7.

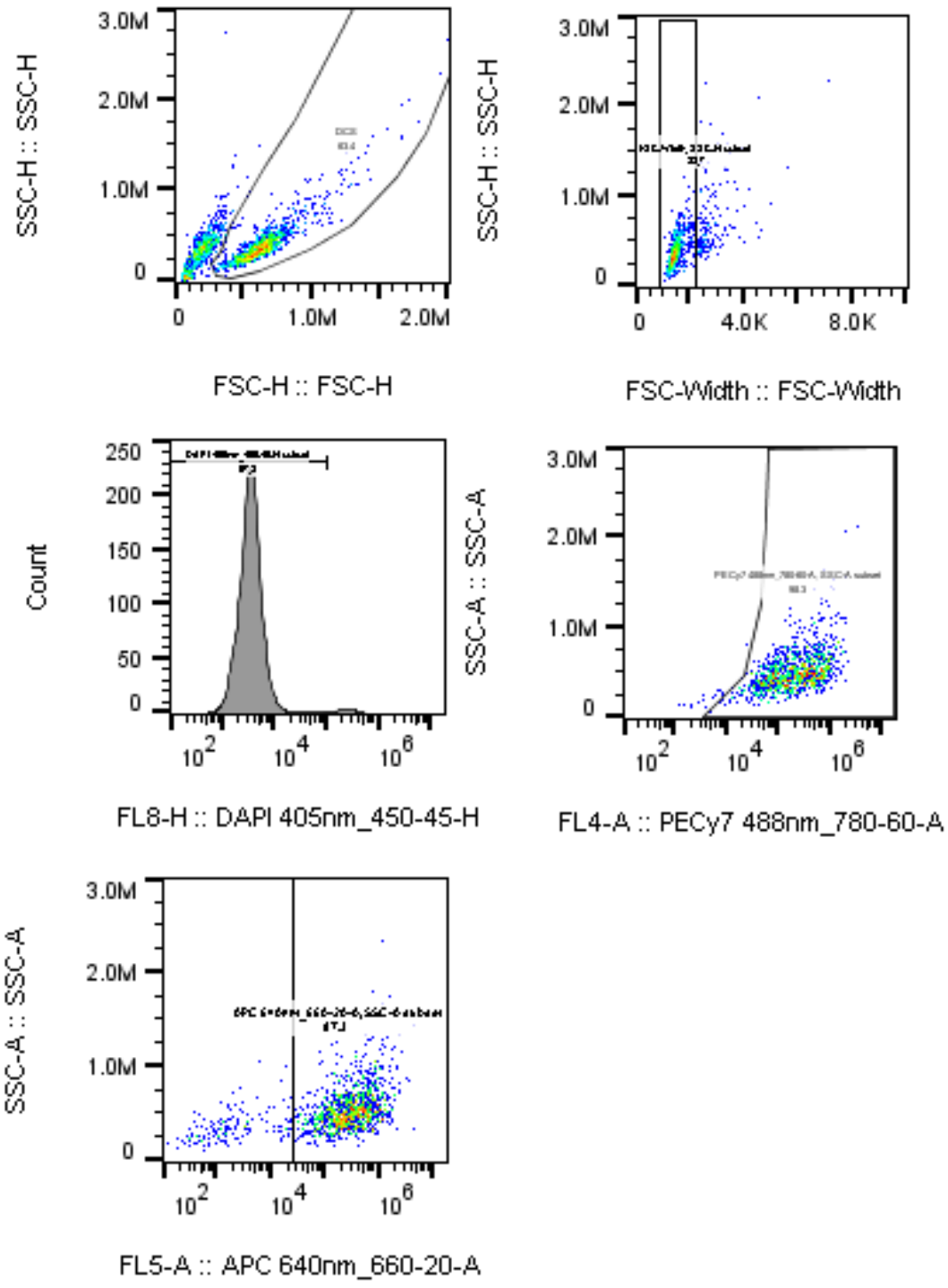

Figure S7. Flow cytometry gating for BMDC uptake: 50%AlexaFluor 647 (APC) labeled OVA NPs. Live/dead stain: DAPI; CD11c+ (DC stain): PECy7.

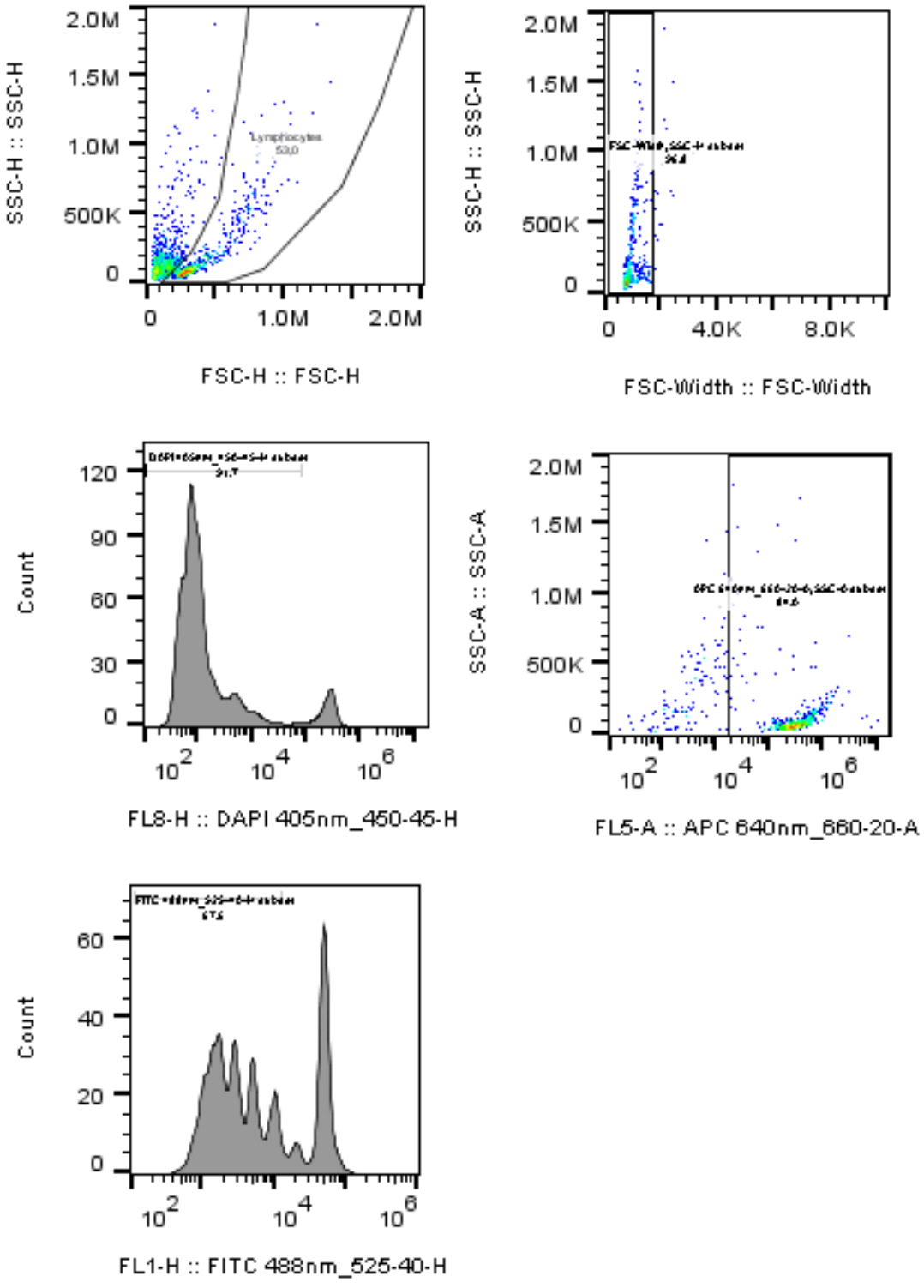

Figure S8. Flow cytometry gating for CFSE dilution assay: 5% XL OVA NPs. Live/dead stain: DAPI; CD8+ T cell stain: APC. CD8+ T cell proliferation marker: FITC.

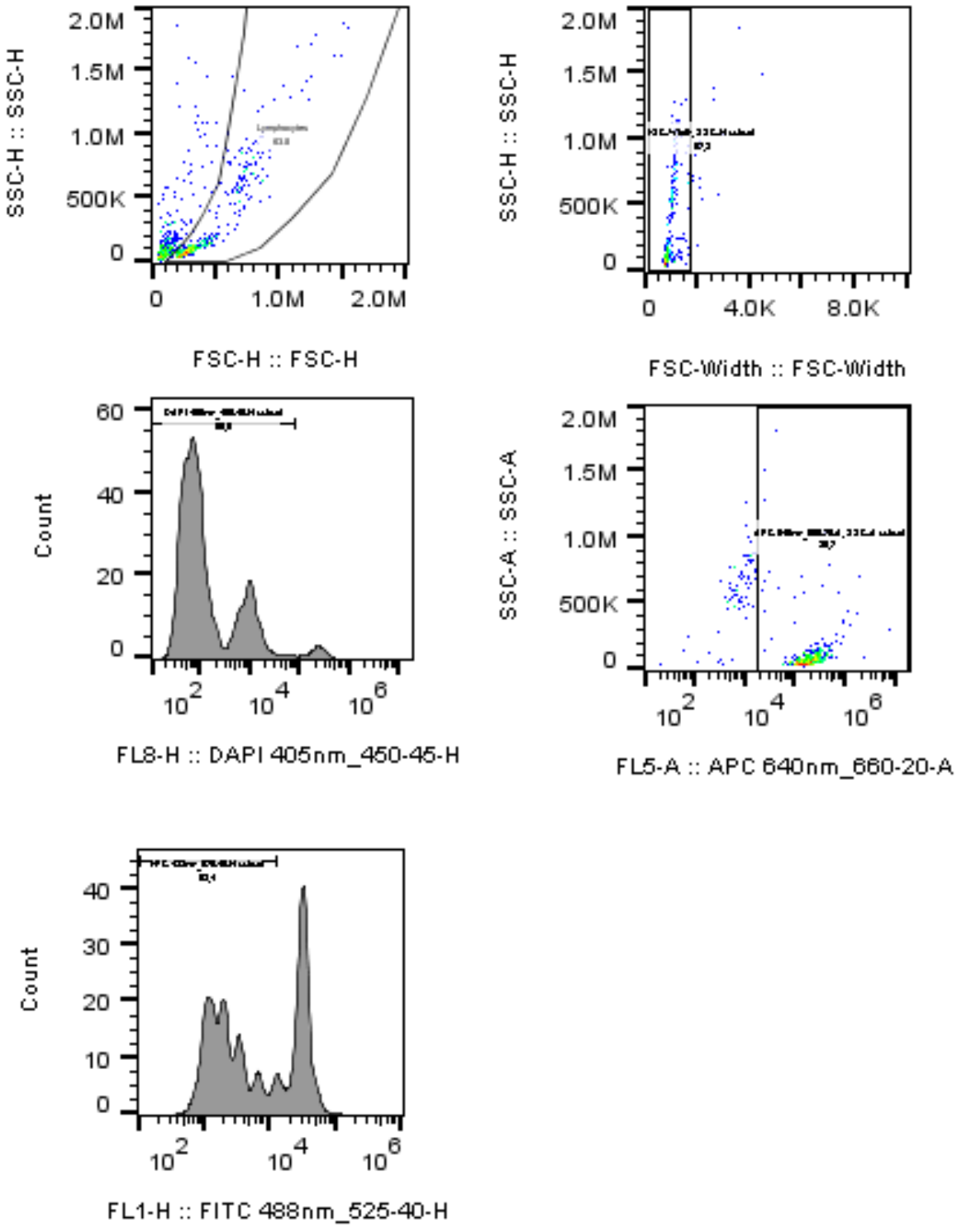

Figure S9. Flow cytometry gating for CFSE dilution assay: 10% XL OVA NPs. Live/dead stain: DAPI; CD8+ T cell stain: APC. CD8+ T cell proliferation marker: FITC.

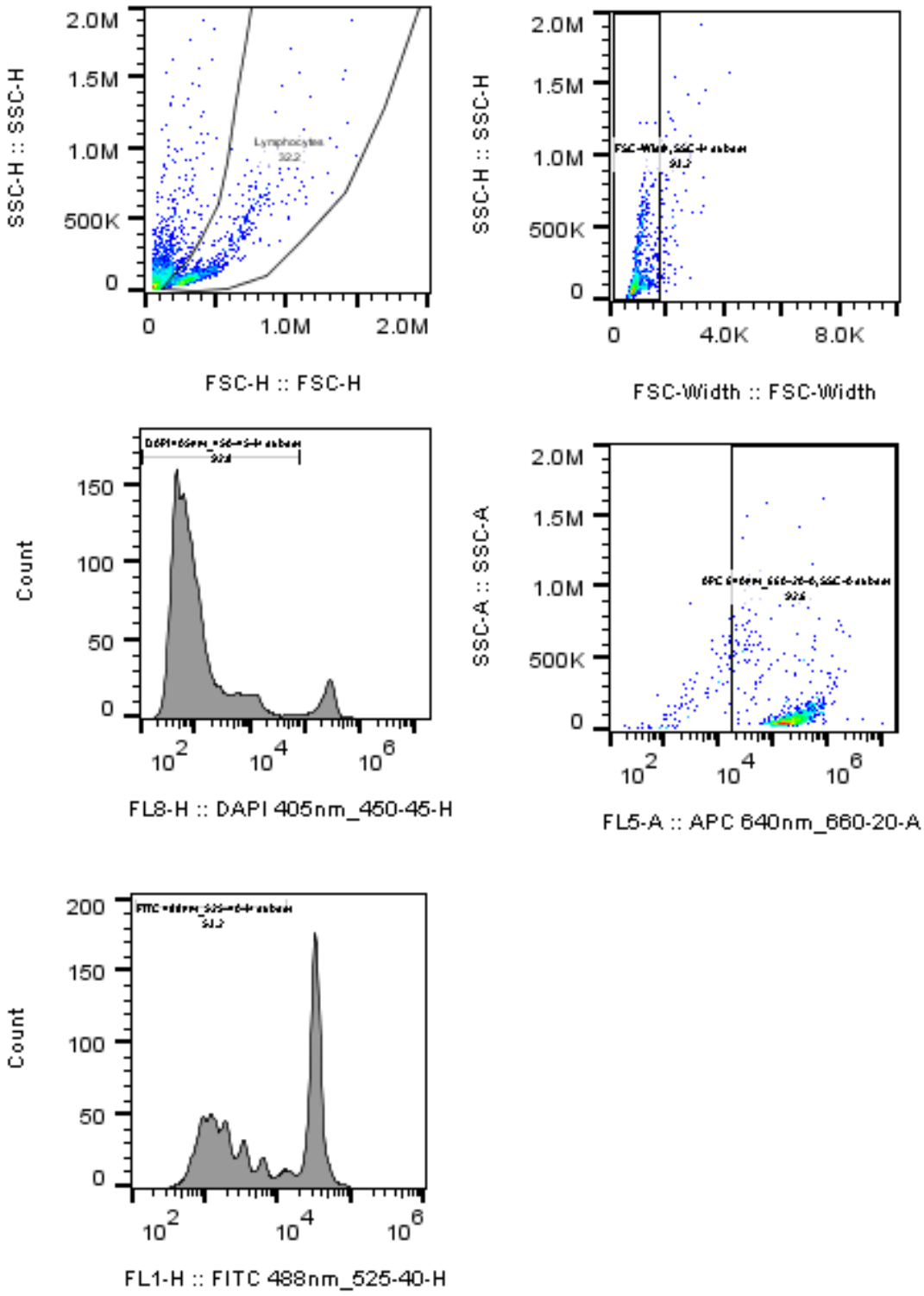

Figure S10. Flow cytometry gating for CFSE dilution assay: 30% XL OVA NPs. Live/dead stain: DAPI; CD8+ T cell stain: APC. CD8+ T cell proliferation marker: FITC.

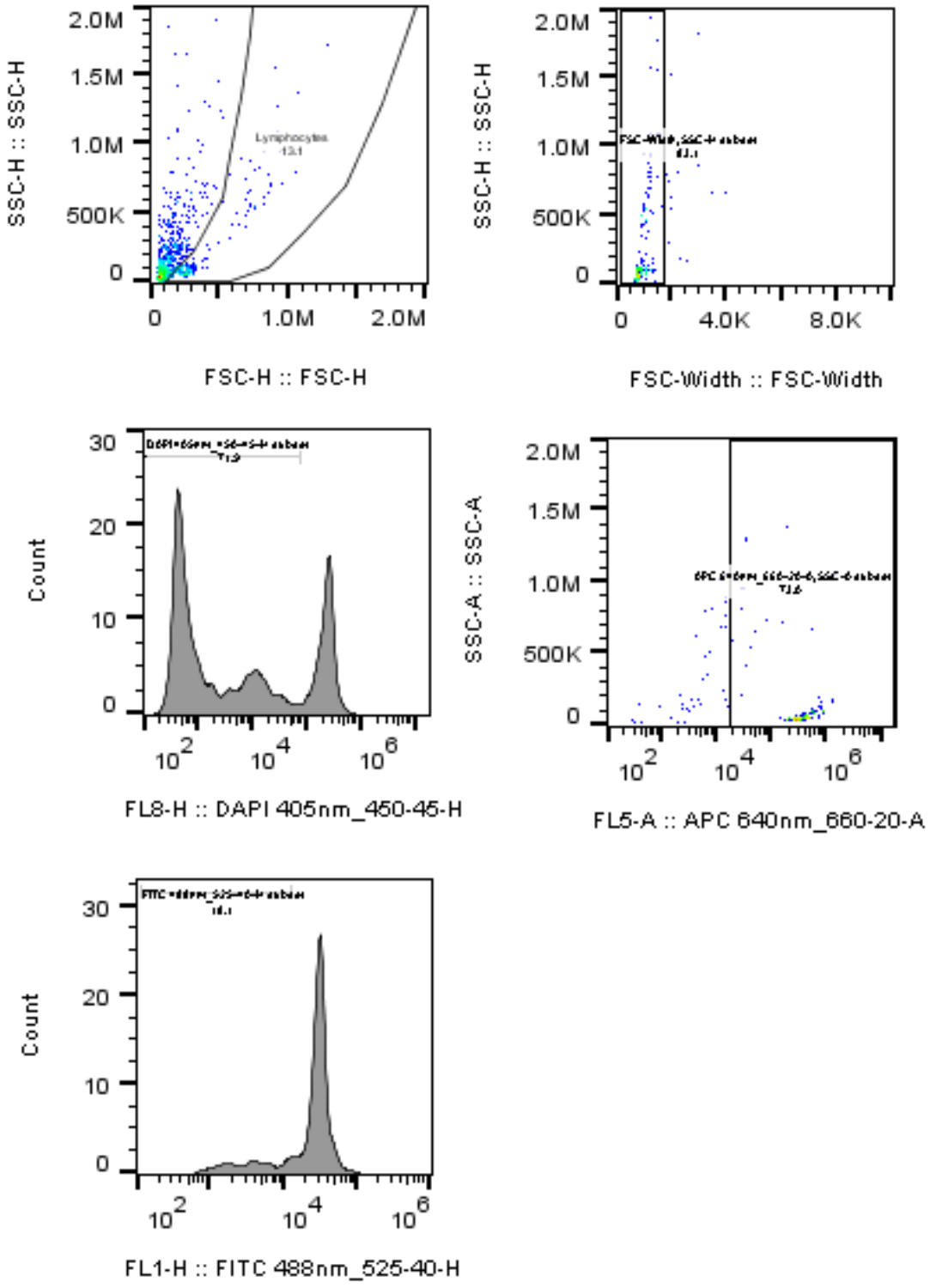

Figure S11. Flow cytometry gating for CFSE dilution assay: 50% XL OVA NPs. Live/dead stain: DAPI; CD8+ T cell stain: APC. CD8+ T cell proliferation marker: FITC.

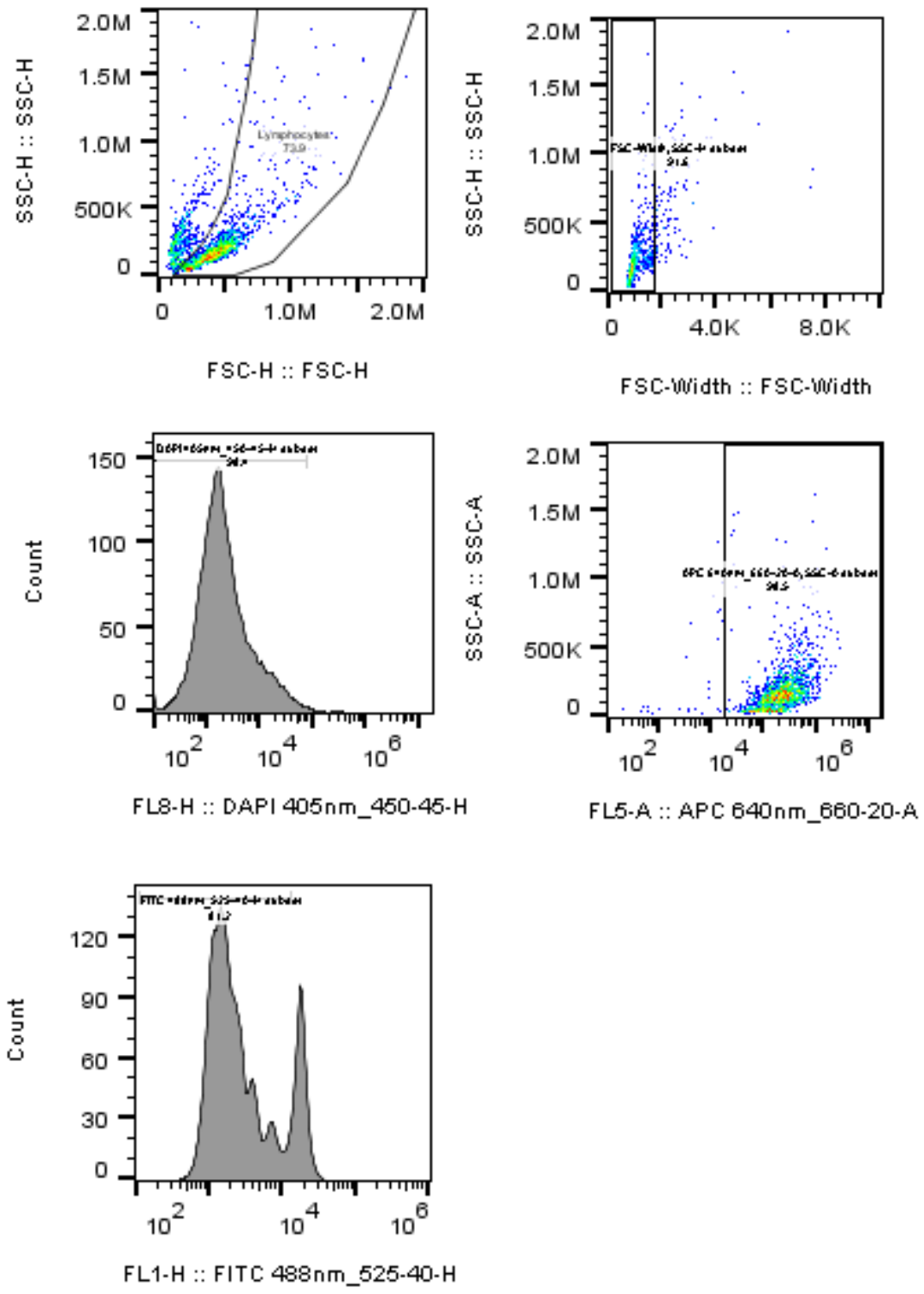

Figure S12. Flow cytometry gating for CFSE dilution assay: SIINFEKL. Live/dead stain: DAPI; CD8+ T cell stain: APC. CD8+ T cell proliferation marker: FITC.

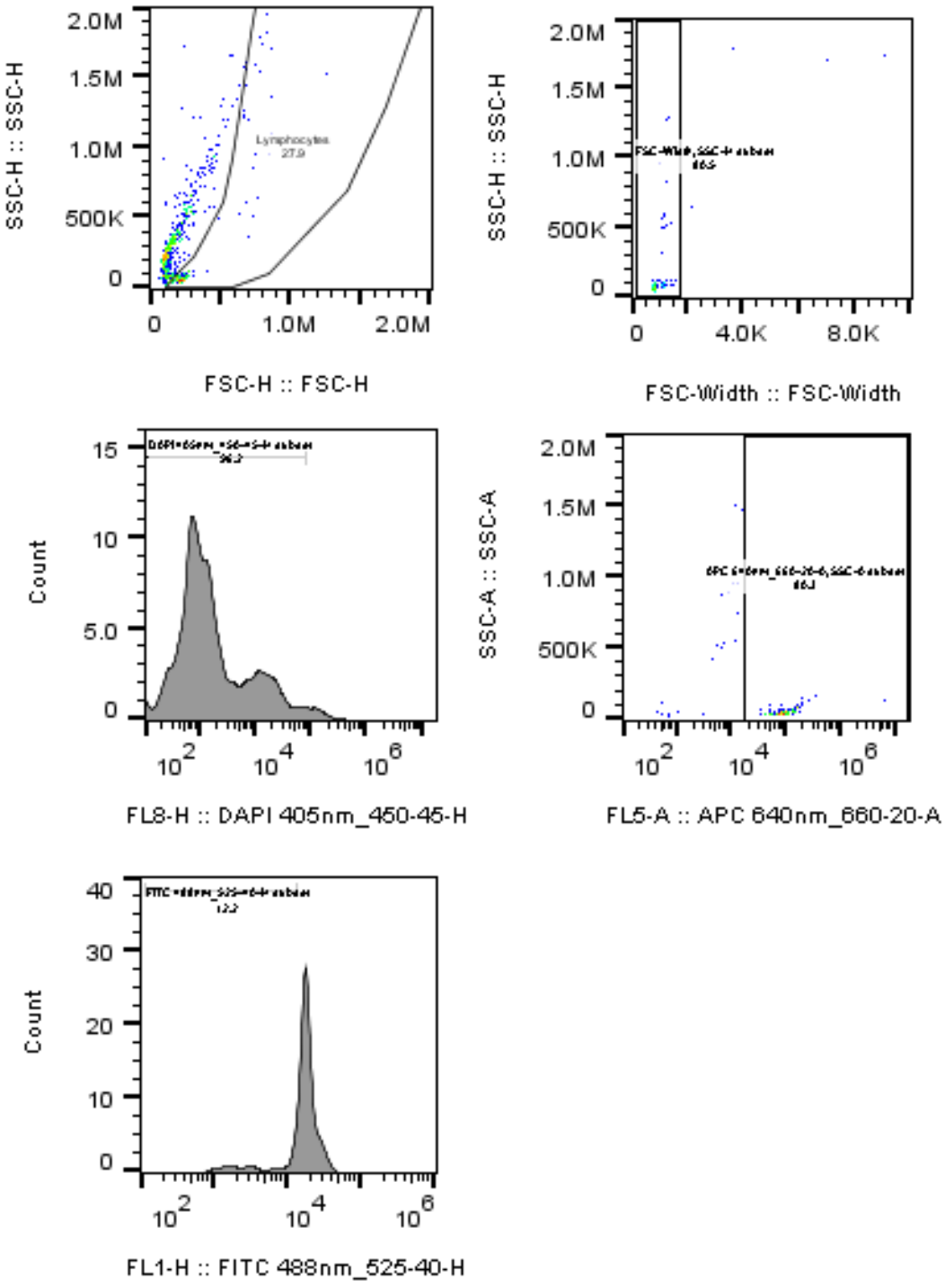

Figure S13. Flow cytometry gating for CFSE dilution assay: soluble ovalbumin. Live/dead stain: DAPI; CD8+ T cell stain: APC. CD8+ T cell proliferation marker: FITC.