Electronic Supplementary Material (ESI) for Biomaterials Science.


# "Spatial delivery of immune cues to lymph nodes to define therapeutic outcomes in cancer vaccination"

SUPPLEMENTARY INFORMATION

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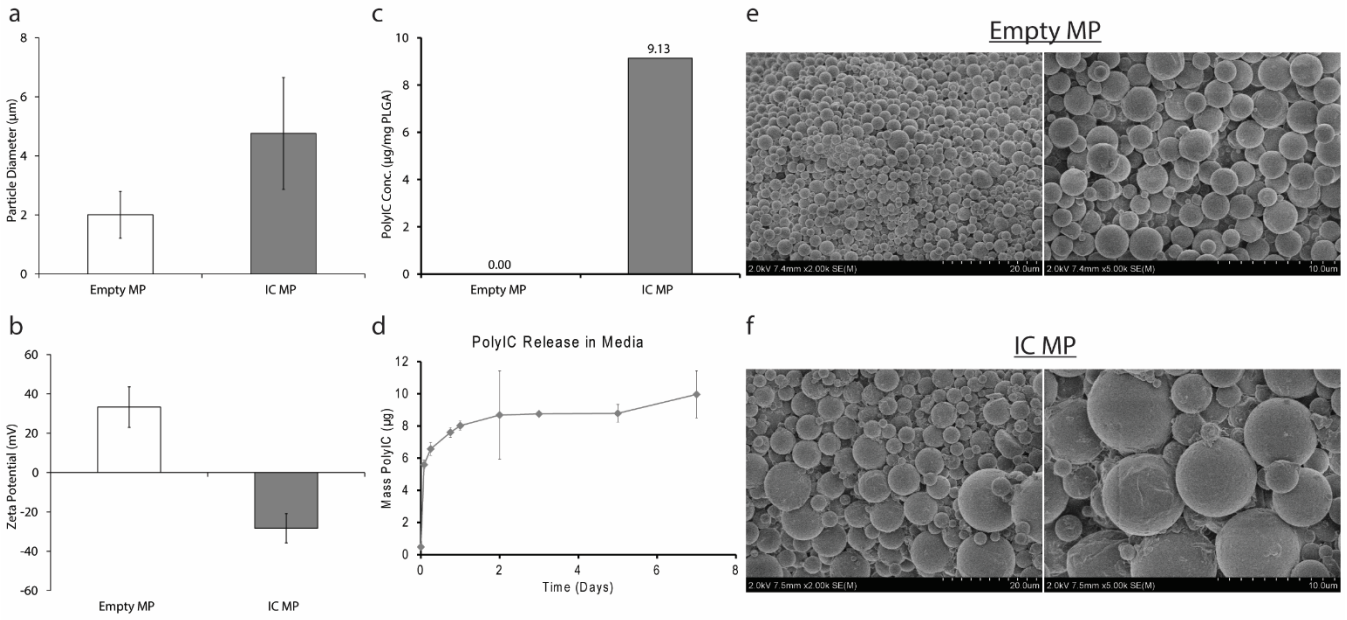
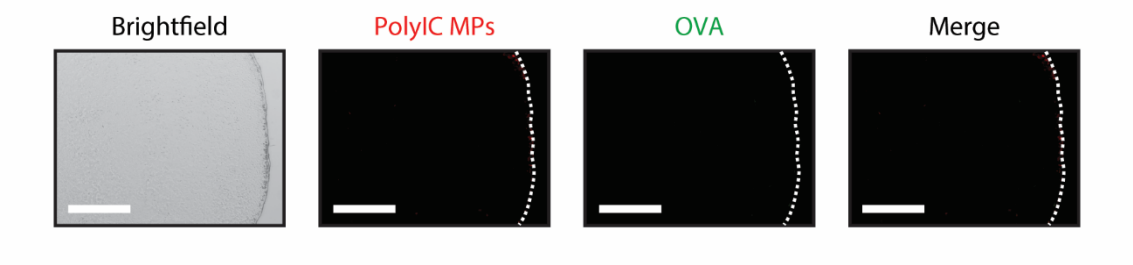
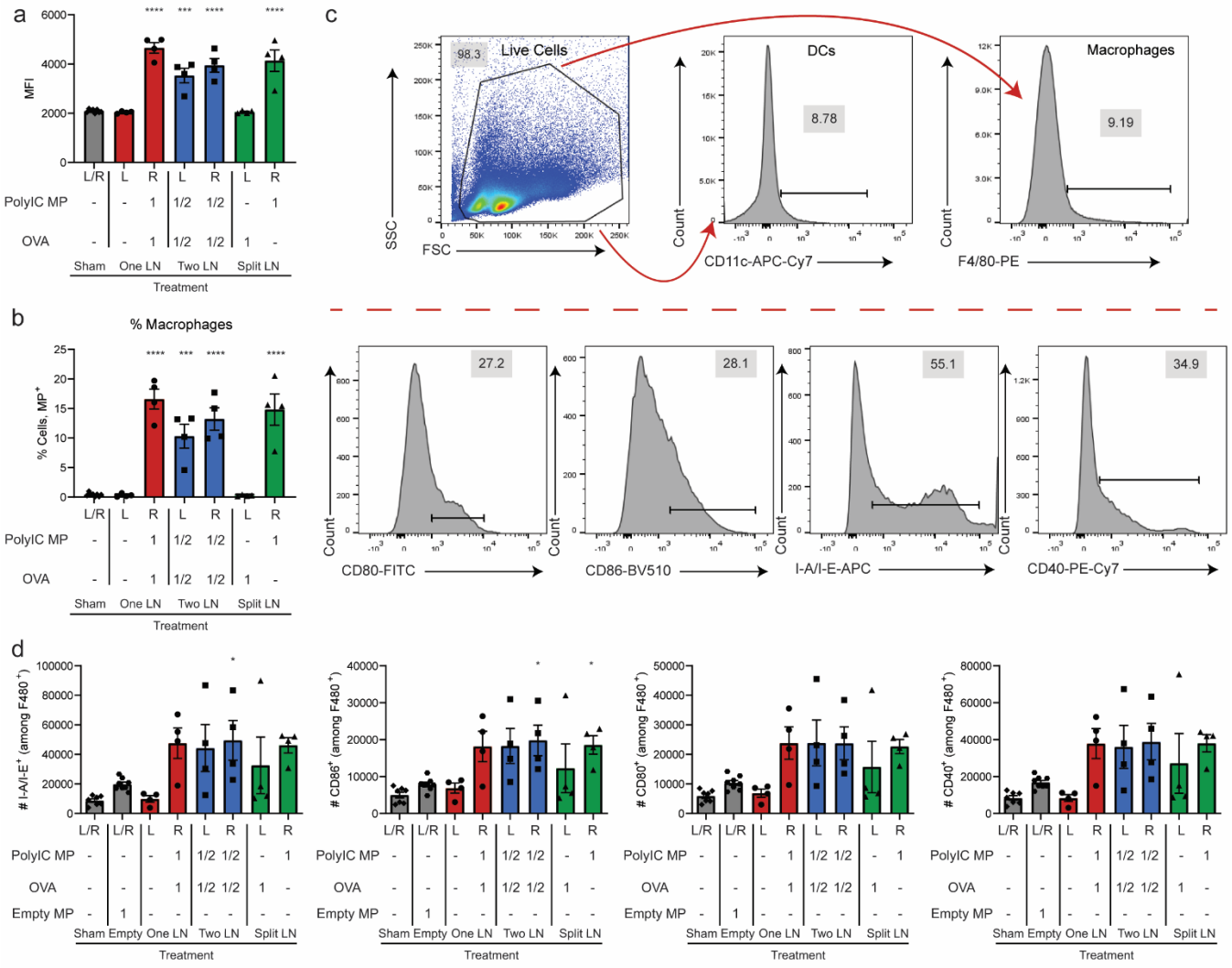


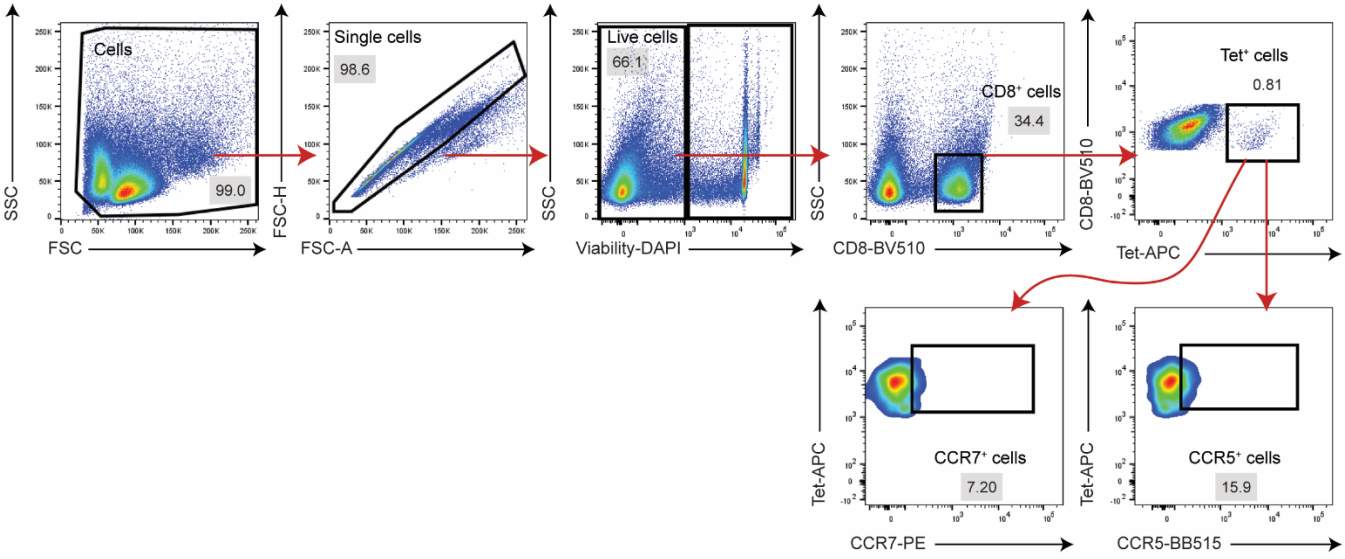

**Supplementary Figure 1: PolyIC MP characterization.** Representative data showing particle size (a), zeta potential (b), PolyIC loading (normalized to Empty MP) (c), and PolyIC release in media over time (d) following particle synthesis. (e) SEM micrographs of Empty and (f) PolyIC MPs. (panels a-d) Data represents three separate particle batches and errors bars represent standard deviation. (panels (e-f) SEM images display scale bars of 20µm (left) and 10µm (right), respectively.



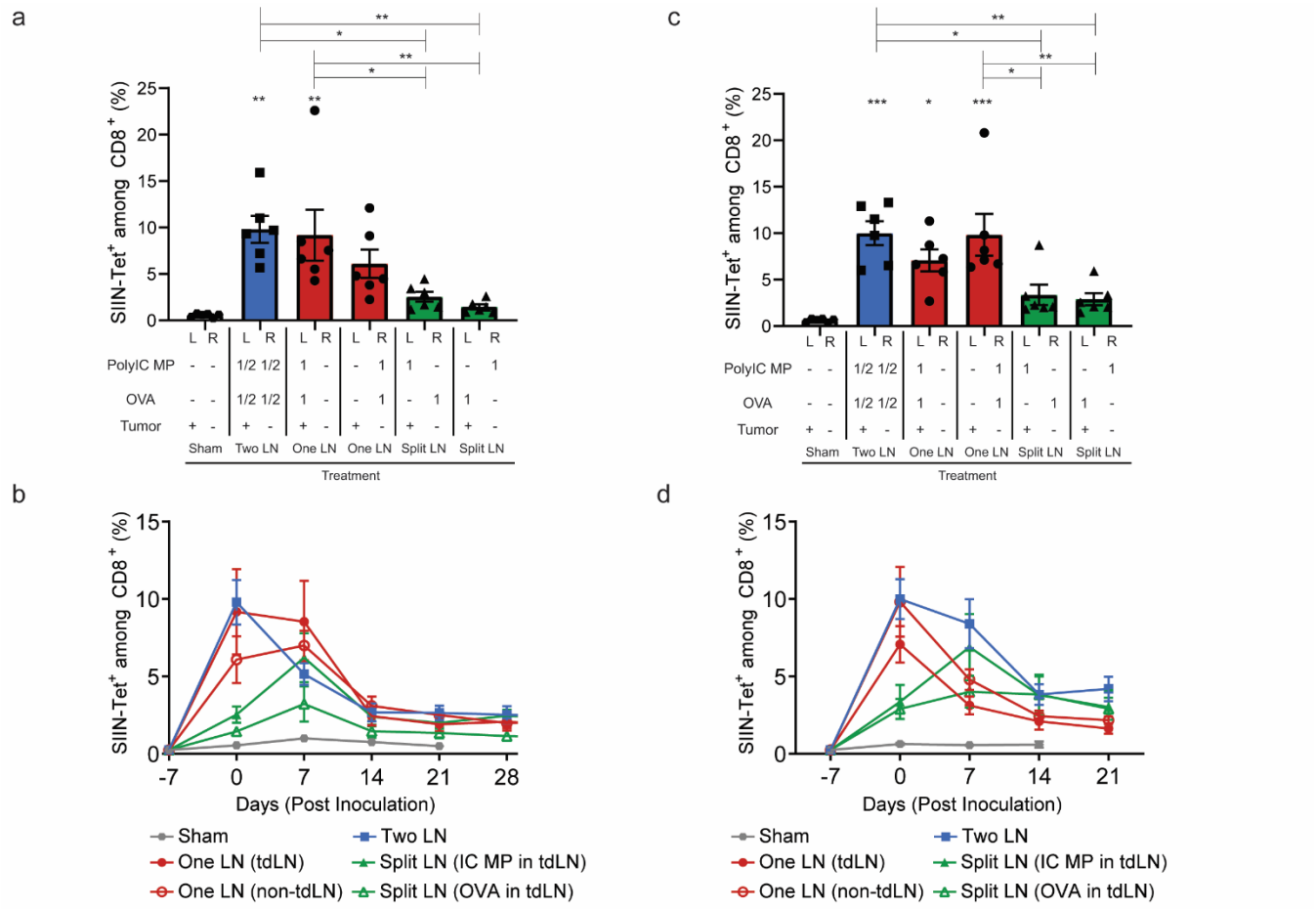

**Supplementary Figure 2: Sham treated LNs images.** Representative fluorescent micrographs of a Sham treated LN showing no colocalization of immune signals to LNs 24 hours after treatment. (PolyIC MP – red; FITC-OVA – green). Scale bar= 200µm.




**Supplementary Figure 3: Particle uptake and macrophage activation.** MFI (a) and percentage (b) of cells containing particles for each treatment, irrespective of cell subpopulations. (c) Representative gating scheme for innate cell activation; top shows identification of DCs and macrophages, bottom shows gating of activation markers. (d) Number of activated macrophages ($F4/80^+$) 7 days after treatment. N=4 mice per group and errors bars represent SEM. (Using a One-way ANOVA with a Tukey post-test, * $p<0.05$; ** $p<0.01$; *** $p<0.001$, ****$p<0.0001$ compared to Sham treatment).




**Supplementary Figure 4: Chemokine gating scheme and quantification.** Representative gating scheme for chemokine staining in treated LNs with CD8, Tet, CCR5, and CCR7.




**Supplementary Figure 5: Antigen specific T cell responses in blood during tumor models.** (a) Evaluation of SIINFEKL-specific $CD8^+$ T cells in the blood at day 7 and (b) over time following treatment as in **Figure 5b** with melanoma model. (c) Evaluation of SIINFEKL-specific $CD8^+$ T cells in the blood at day 7 and (d) over time following treatment as in **Figure 6a** with lymphoma model. (panels a-d) N=6 mice per group and errors bars represent SEM. (Using a One-way ANOVA with a Tukey post-test, * $p<0.05$; ** $p<0.01$; *** $p<0.001$, ****$p<0.0001$ compared to Sham treatment unless otherwise noted).

**Supplementary Table 1: B16-OVA multiple comparisons of survival curves.**

| Treatment Group | Survival Difference from Sham | Incidence Difference from Sham |
|---|---|---|
| Two LN | **, p<0.01 | **, p<0.01 |
| One LN (tdLN) | **, p<0.01 | **, p<0.01 |
| One LN (non-tdLN) | **, p<0.01 | **, p<0.01 |
| Split LN (IC MP in tdLN) | **, p<0.01 | **, p<0.01 |
| Split LN (OVA in tdLN) | **, p<0.01 | **, p<0.01 |

**Supplementary Table 2: Rechallenge of vaccinated mice surviving B16-OVA tumors.**

| Treatment Group | # Mice Surviving 1° Challenge | # Mice Surviving 2° Challenge | % Mice Surviving 2° Challenge |
|---|---|---|---|
| Sham | 6 | 4 | 67% |
| Two LN | 5 | 4 | 80% |
| One LN (tdLN) | 6 | 3 | 50% |
| One LN (non-tdLN) | 6 | 4 | 67% |
| Split LN (IC MP in tdLN) | 5 | 3 | 60% |
| Split LN (OVA in tdLN) | 1 | 0 | 0% |

**Supplementary Table 3: EG7-OVA multiple comparisons of survival curves.**

| Treatment Group | Survival Difference from Sham | Incidence Difference from Sham |
|---|---|---|
| Two LN | *, p<0.05 | **, p<0.01 |
| One LN (tdLN) | *, p<0.05 | **, p<0.01 |
| One LN (non-tdLN) | *, p<0.05 | **, p<0.01 |
| Split LN (IC MP in tdLN) | n.s. | n.s. |
| Split LN (OVA in tdLN) | *, p<0.05 | **, p<0.01 |