Electronic Supplementary Material (ESI) for Nanoscale.

Tuning innate immune function using microneedles containing multiple classes of toll-like receptor agonists

SUPPORTING INFORMATION

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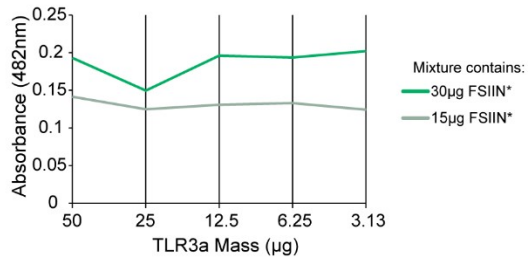
KEYWORDS. Microneedle, toll-like receptor agonists, biomaterials, immunology, polyelectrolyte multilayer, self-assembly

A



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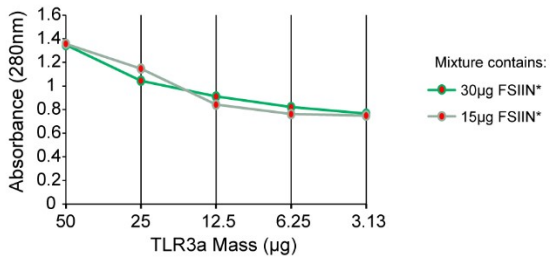

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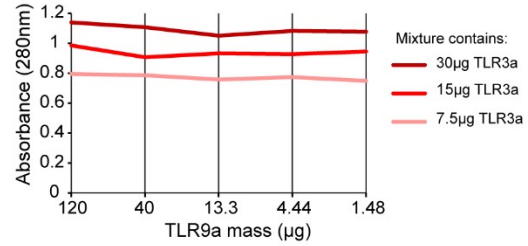

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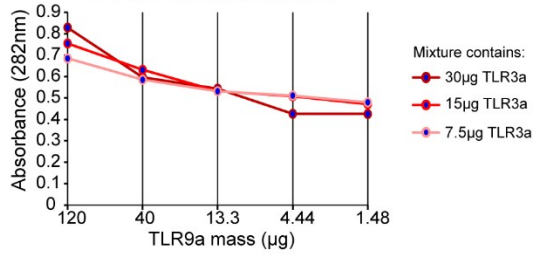

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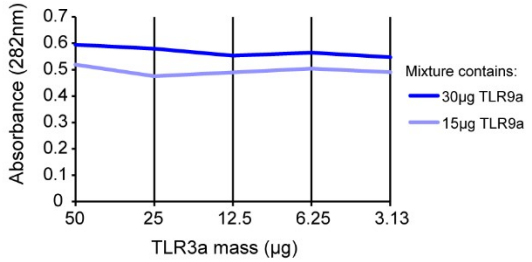

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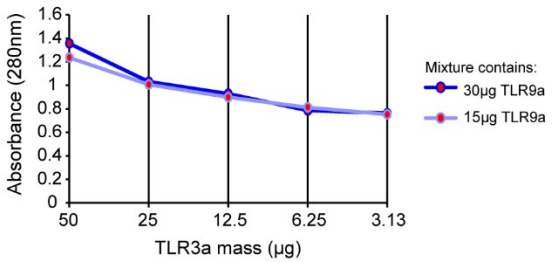
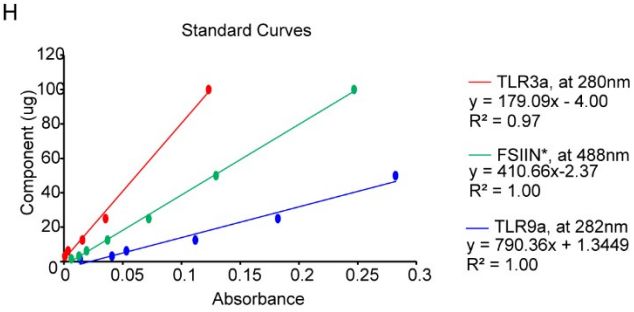


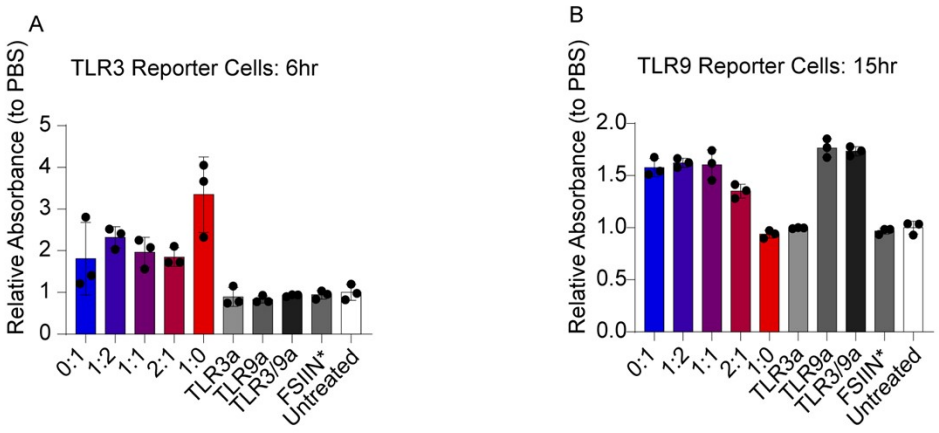
G

Wavelength	Uncoated MNA #1	Uncoated MNA #2
488nm (FSIIN*)	0	0
280nm (TLR3a)	0	0.002
282nm (TLR9a)	0	0.002

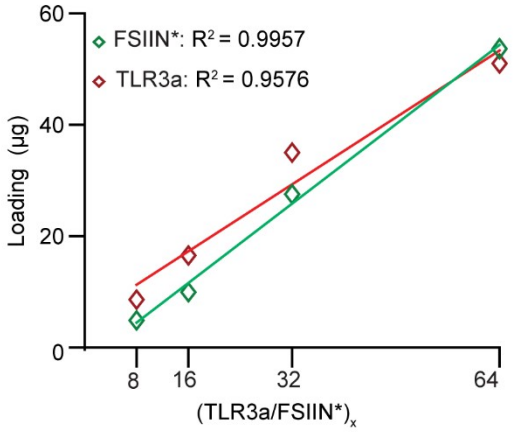
H



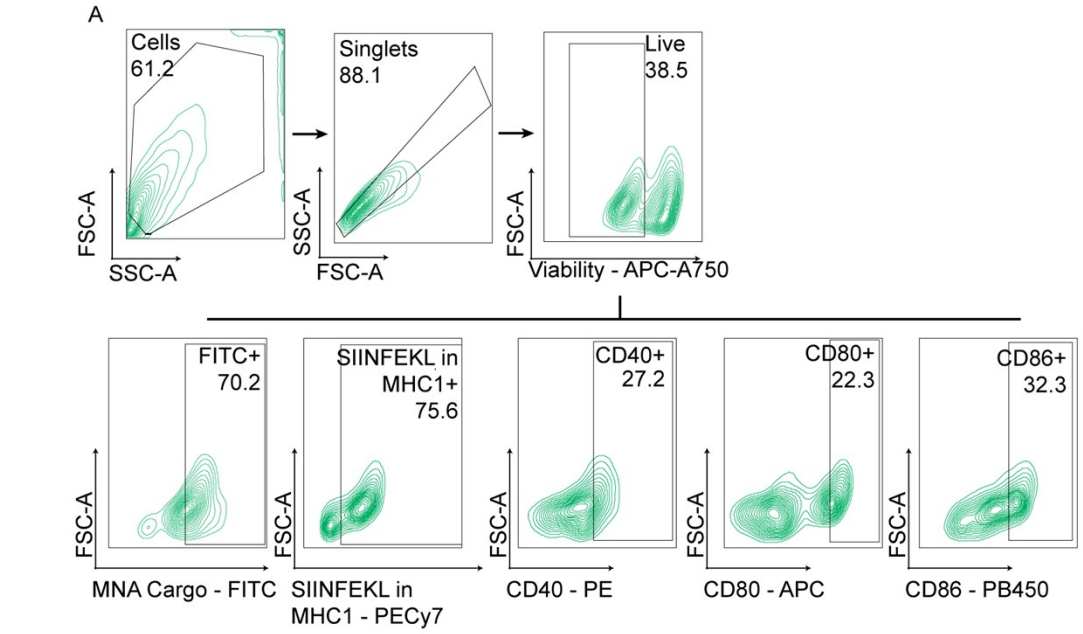
Supplemental Figure 1. Verification of direct loading measurement. Separate, constant masses of one component were mixed with varying masses of another component. After separation, **(A)** absorbance of SIIN* was measured in each mixture containing different amounts of PolyIC and **(B)** absorbance of PolyIC was measured in each mixture containing either 30μg or 15μg of SIIN*. After separation, **(C)** absorbance of PolyIC was measured in each mixture containing different amounts of CpG and **(D)** absorbance of CpG was measured in each mixture containing either 30μg, 15μg, or 7.5 μg of PolyIC. After separation, **(E)** absorbance of CpG was measured in each mixture containing different amounts of PolyIC and **(F)** absorbance of PolyIC was measured in each mixture containing either 30μg or 15μg of CpG. **(G)** Confirmation that PLLA MNA matrix has no impact on absorbance values of different components. **(H)** Mixtures of PolyIC, SIIN*, and CpG at 100, 50, 25, and 12.5 μg each of cargo were created, separated, and measured to create working standard curves. These standard curves were used in all direct loading measurements of MNA cargo.

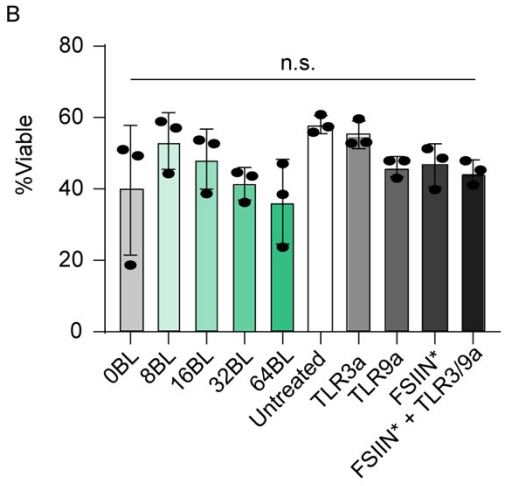


Supplemental Figure 2. TLR reporter cells have different kinetics; for that reason, absorbance of reporter cells were measured across a broad time frame per the manufacturers' instructions and optimum time points chosen for each. Here, we present **(A)** TLR3 reporter cell absorbent reporter protein at the time point presented in the main document for TLR9 reporter cells, and **(B)** TLR9 reporter cell absorbent reporter protein at the time point presented in the main document for TLR3 reporter cells.



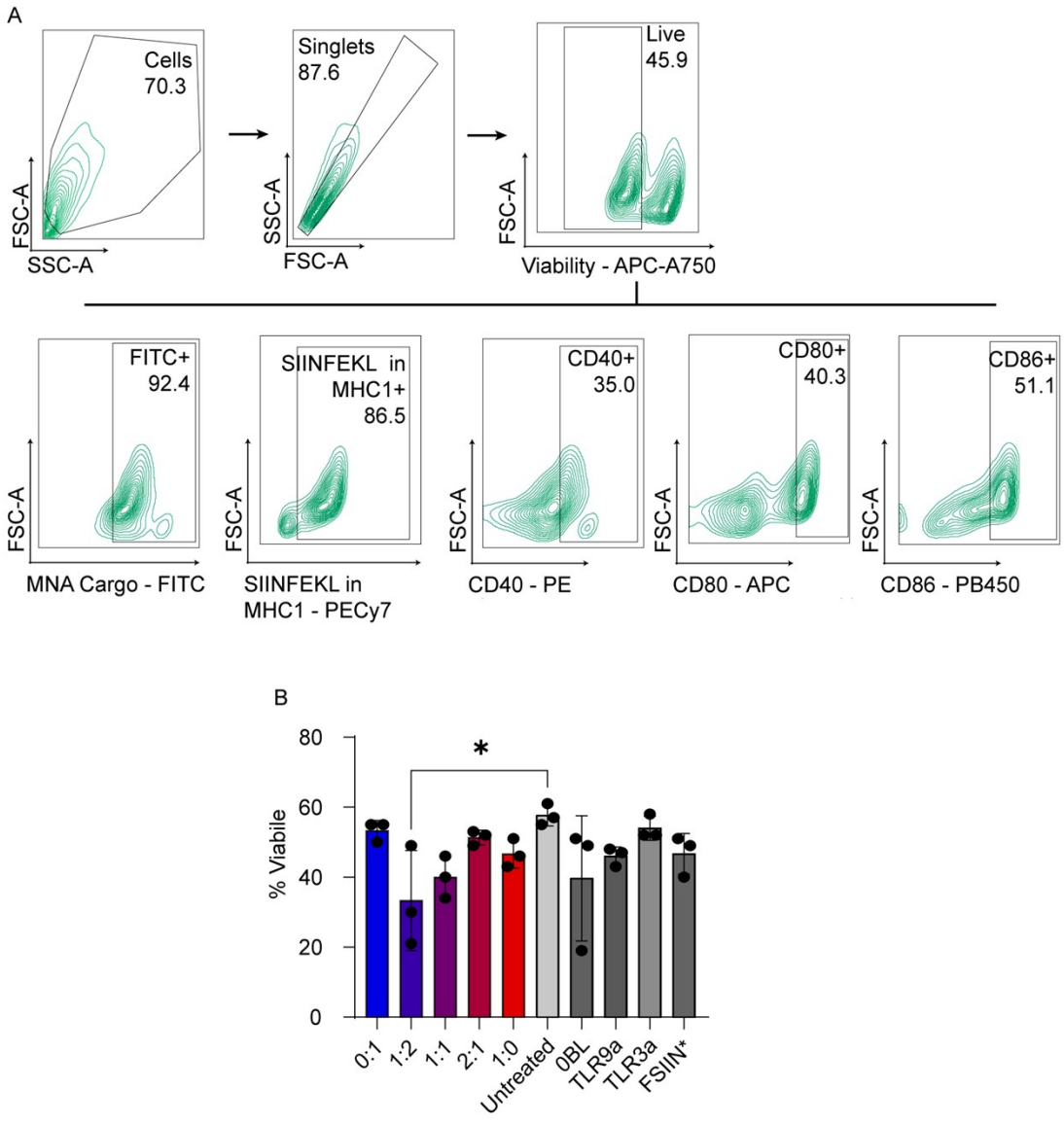
Supplemental Figure 3. Linearization of antigen (SIINFEKL) and TLR3a (PolyIC) loading as a function of number of BL deposited.



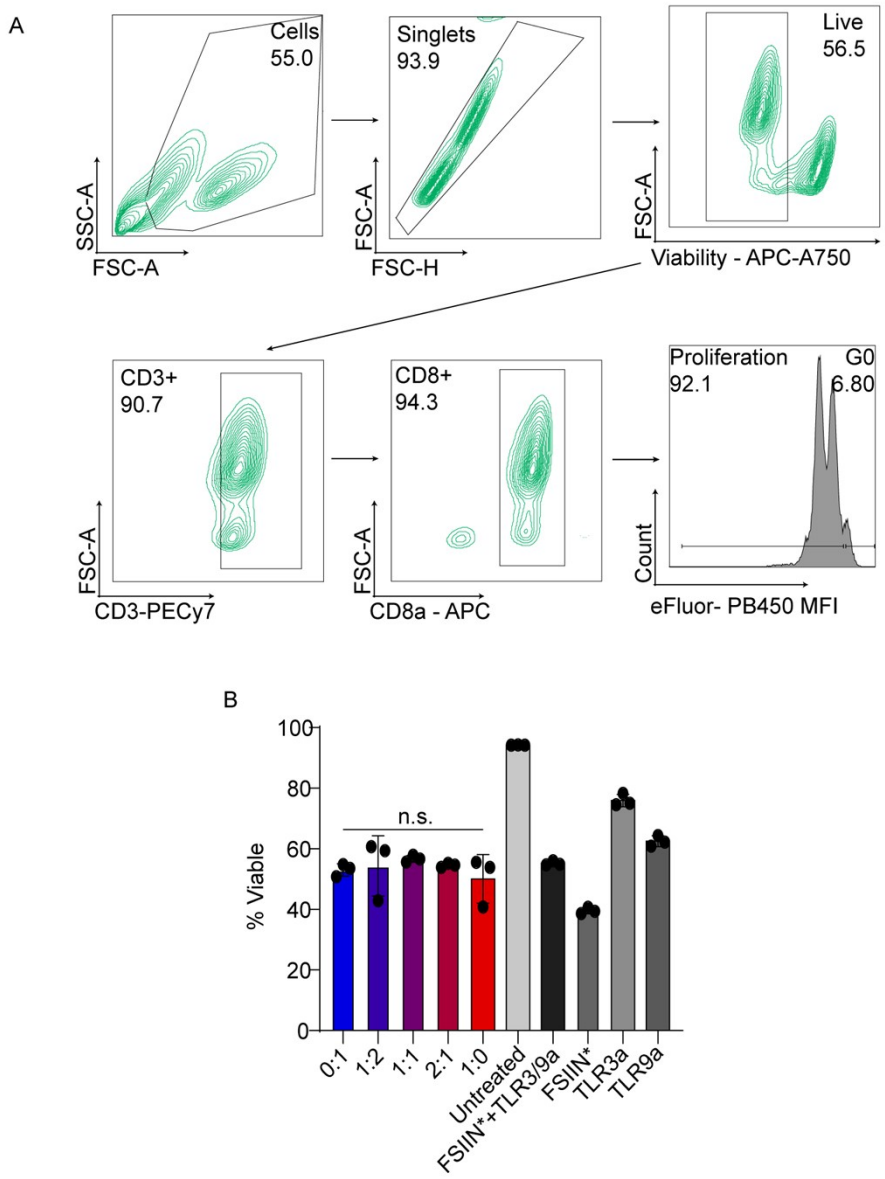


Suppleme

ntal Figure 4. (A) Gating scheme for varying bilayers on MNAs and **(B)** frequency of viable cells across different bilayers. Of importance is the equal viability of 0BL (uncoated) treatment to other treatment, indicating that the PLLA matrix had no impact on cell viability.



Supplemental Figure 5. (A) Gating scheme for varying compositions of MNAs and **(B)** frequency of viable cells after treatment with different MNA compositions.



Supplemental Figure 6. (A) Gating scheme and **(B)** viability for T cell coculture studies.