

SUPPLEMENTAL INFORMATION

MyD88 in antigen-presenting cells is not required for CD4+ T-cell responses during peptide nanofiber vaccination

*Youhui Si,¹ *Yi Wen,^{1,2} Jianjun Chen,¹ Rebecca R. Pompano,^{1,3} Huifang Han,¹

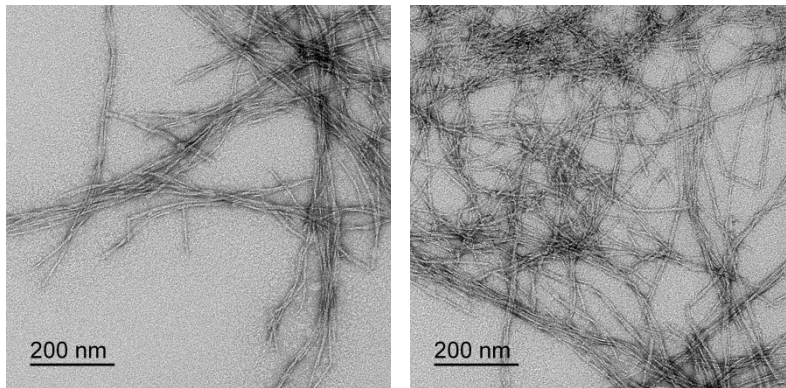
**Joel Collier^{1,2} and **Anita S. Chong¹

* contributed equally; ** co-corresponding authors

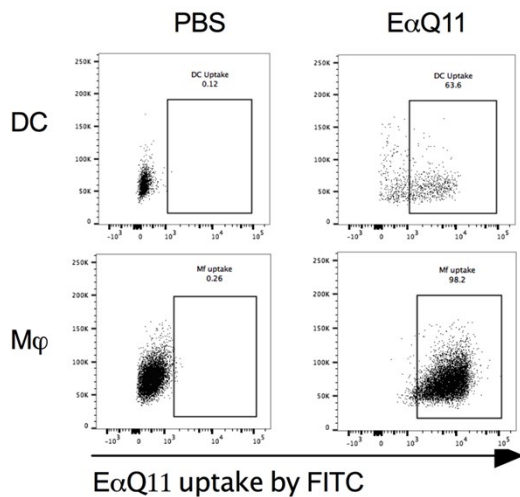
1. Department of Surgery, The University of Chicago, Chicago, IL 60637, USA
2. Department of Biomedical Engineering, Duke University, Durham, NC 27708, USA
3. Department of Chemistry, The University of Virginia, Charlottesville, VA 22904, USA

Figure S1

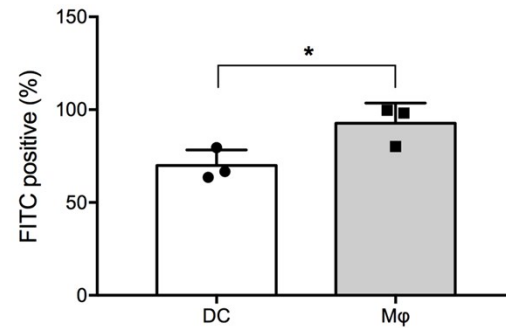
a



b

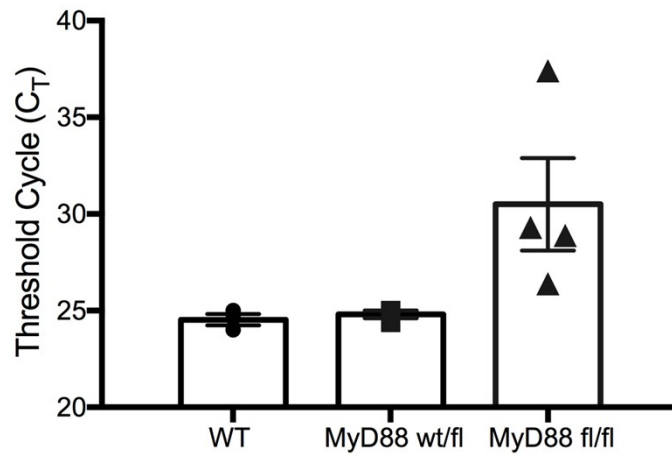


c



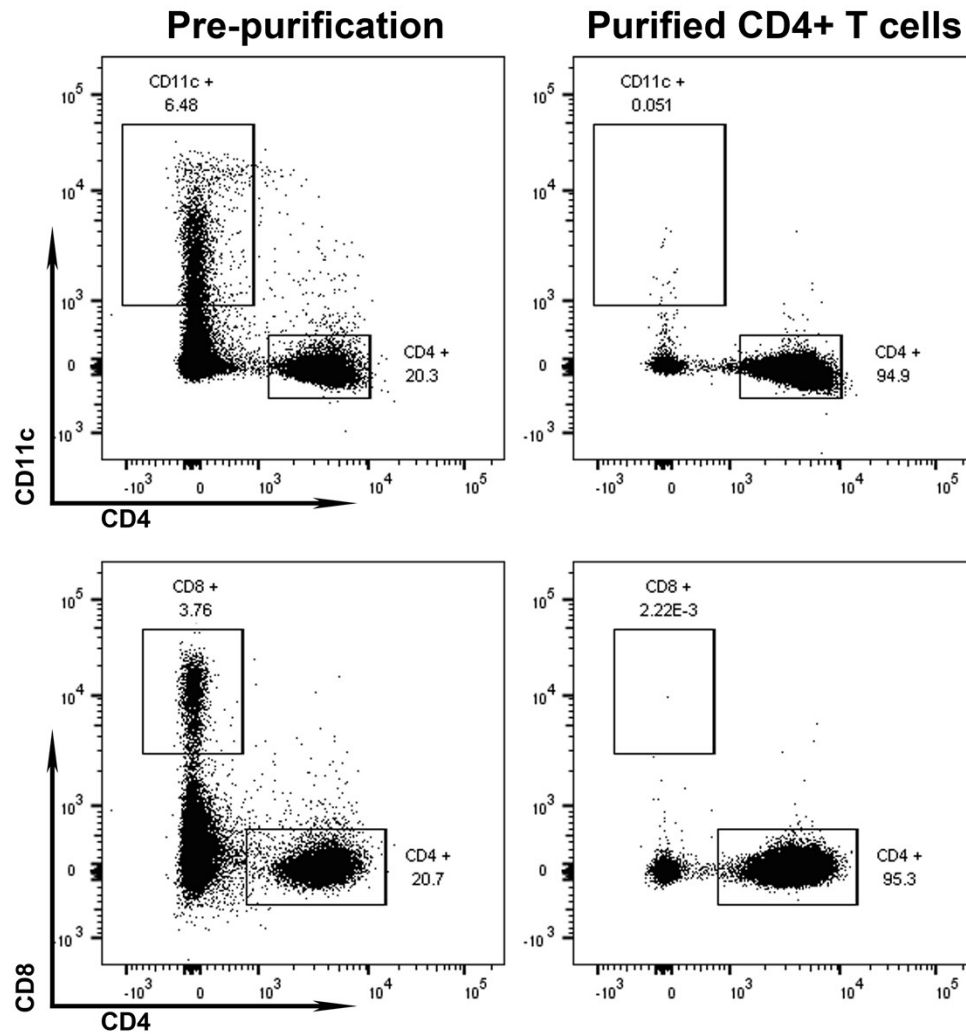
EαQ11 nanofibers and uptake by DCs and macrophages. Peptides (1.33 mM EαQ11 and 0.67 mM Q11) were allowed to assemble at a final concentration of 2 mM in PBS. Nanofibers were diluted in PBS to a final concentration of 0.2 mM for TEM (scale bar: 200 nm). Like its OVAQ11 counterpart, EαQ11 was able to form nanofibers with Q11 (a). When injected intraperitoneally, EαQ11 nanofibers were internalized by both DCs and macrophages (b and c) when analyzed 24 h post administration, comparable to OVAQ11 uptake (as shown in Figure 2).

Figure S2



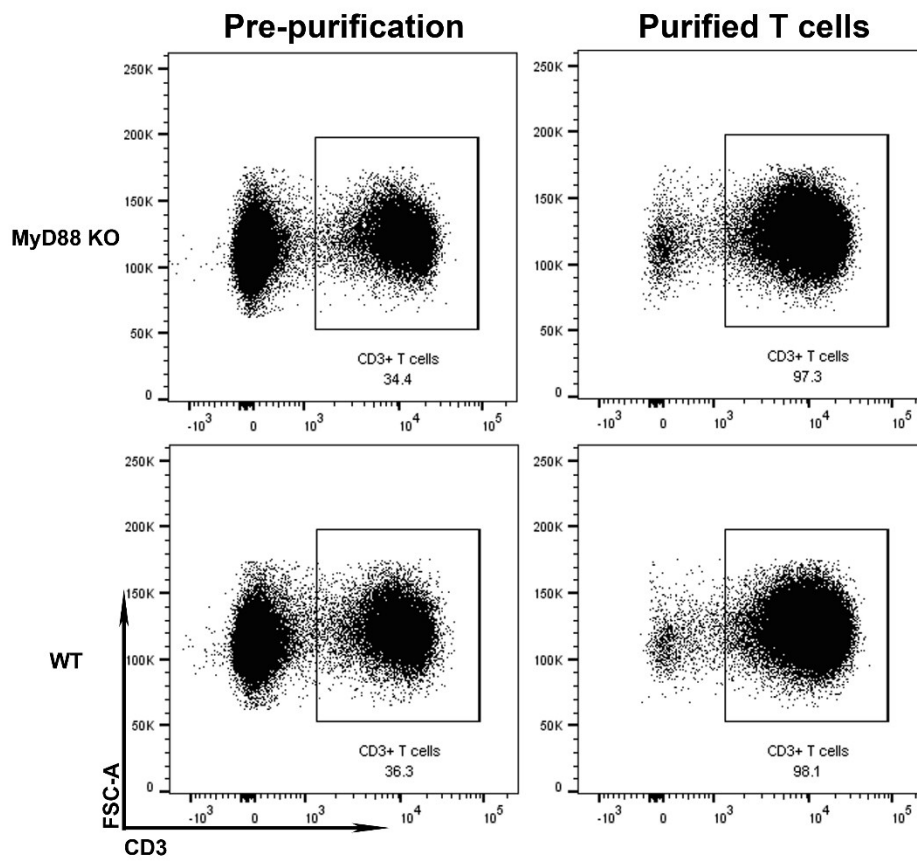
Deletion of *MyD88^{fl}* allele in CD11c⁺ DCs. DCs were sorted from CD11c-MyD88 KO mice based on CD11c expression, and MyD88 was measured by quantitative real-time (qRT) PCR on genomic DNA. Higher threshold cycle numbers indicate lower MyD88 levels.

Figure S3



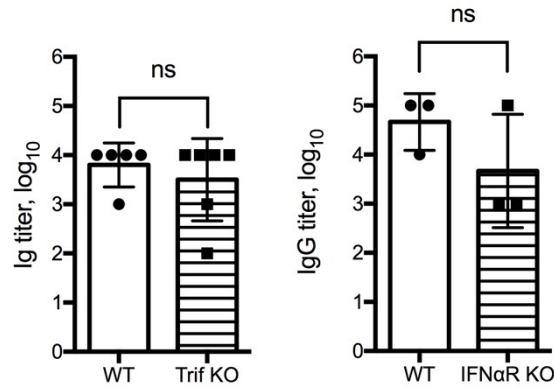
Enrichment of OTII cells. The percentage of CD4⁺ T cells from OTII mice was ~95%, with <1% CD11c⁺ DCs, following enrichment using a CD4⁺ T cell enrichment kit.

Figure S4



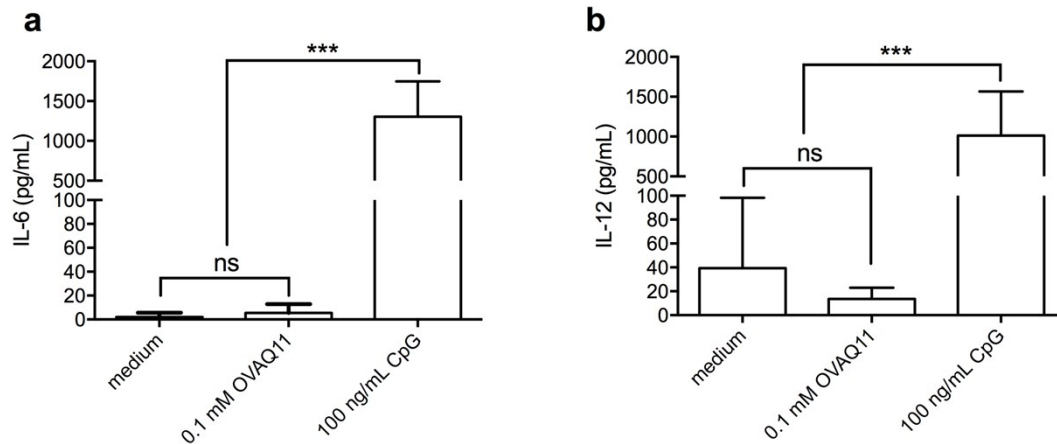
T cell enrichment for adoptive transfer. The percentage of CD3⁺ T cells from WT or MyD88 KO mice was ~98%, following enrichment using a Pan T Cell Isolation Kit II.

Figure S5



Trif and IFNαR are not required for antibody responses. Mice were immunized (day 0) and boosted (day 28) with OVAQ11 nanofibers subcutaneously. IgG titer in sera was analyzed by ELISA 1 week after boost (day 35). Data shown were from one representative study and analyzed by t test. ns: not significant.

Figure S6



OVAQ11 nanofibers do not induce the production of IL-6 and IL-12 by BMDCs in vitro. Flt-3L-induced BMDCs were treated with 0.1 mM OVAQ11 nanofibers overnight, and concentrations of IL-6 and IL-12 in the supernatant were measured by ELISA. CpG was used as a positive control. ***p>0.001 by one-way ANOVA with Tukey's post-hoc test.