Supplementary Information: Multidomain Peptide Hydrogel Adjuvants Elicit Strong Bias Towards Humoral Immunity

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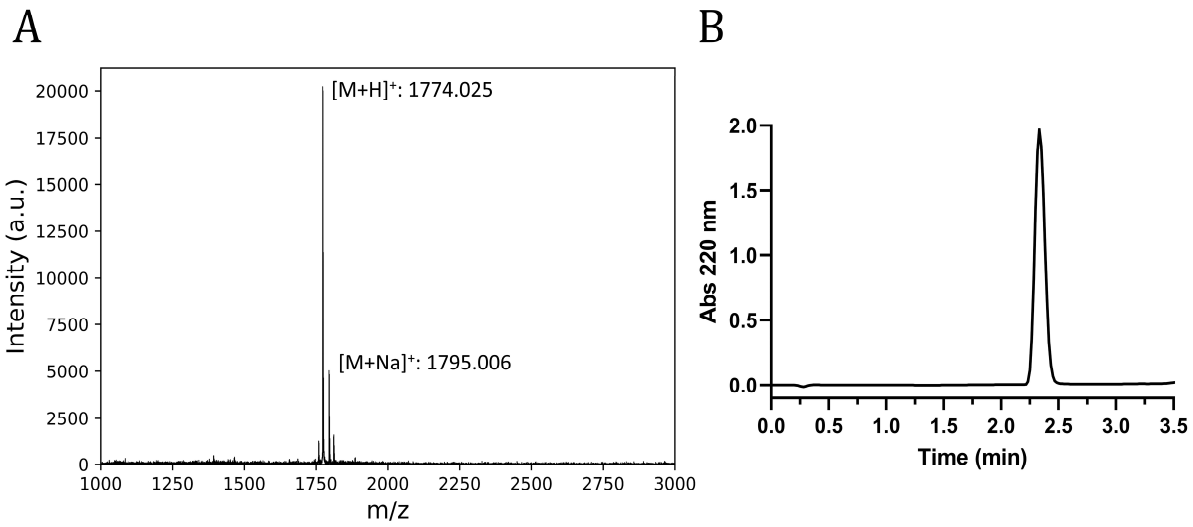


Figure S1. A) MALDI MS positive ionization of HPLC-purified K_2 observed $[M+H]^+$: 1774.025, expected $[M+H]^+$: 1774.29. B) UPLC trace of peptide. The presence of a single peak confirms purity.

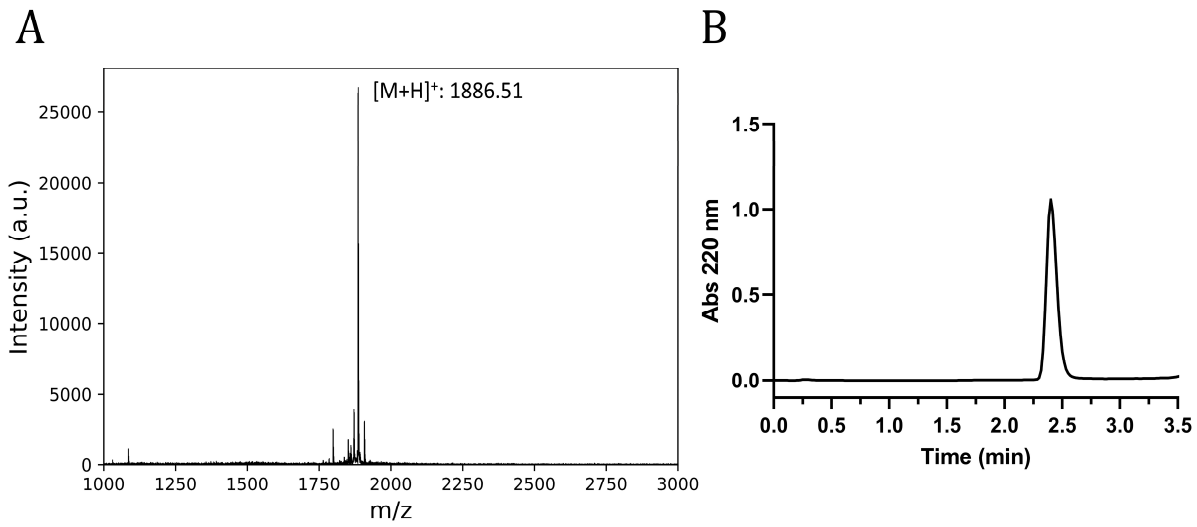


Figure S2. A) MALDI MS positive ionization of HPLC-purified R_2 observed $[M+H]^+$: 1886.51, expected $[M+H]^+$: 1886.33. B) UPLC trace of peptide. The presence of a single peak confirms purity.

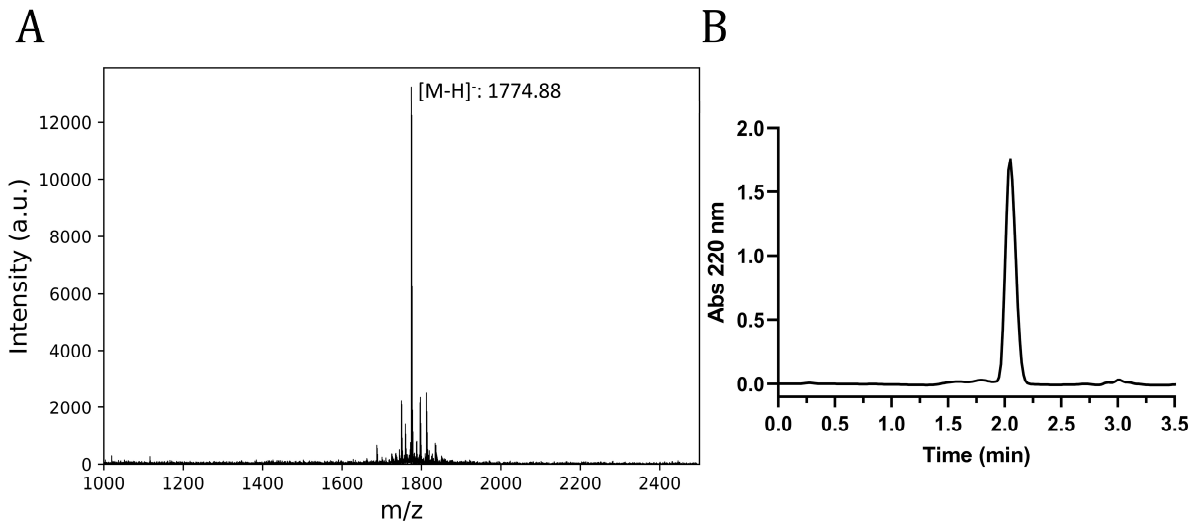
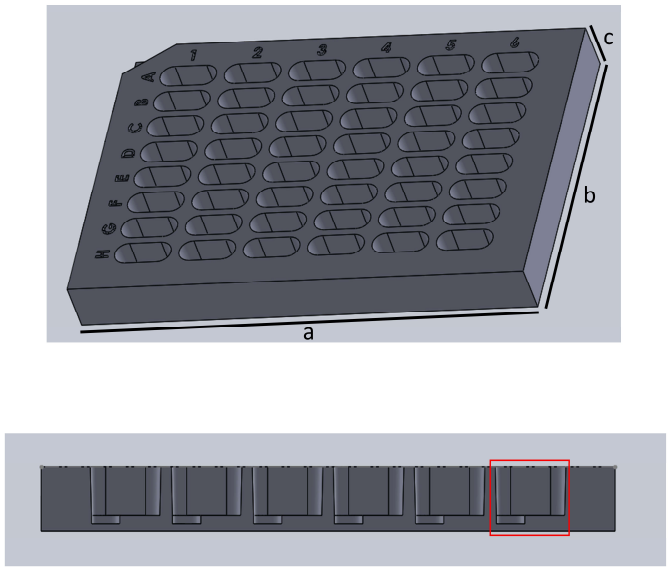
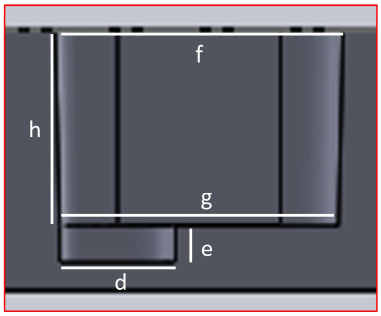


Figure S3. A) MALDI MS negative ionization of HPLC-purified E_2 observed $[M+H]^-$: 1774.88, expected $[M+H]^-$: 1774.90. B) UPLC trace of peptide. The presence of a single peak confirms purity.





a	127.76 mm
b	85.47 mm
c	14.22 mm
d	6.4 mm
e	2 mm
f	15.79 mm
g	15.29 mm
h	10.7 mm

Figure S4. Schematic of the custom 3D-printed microplate used in the *in vitro* release studies with corresponding dimensions. The gels were plated in divot on the left side of the well and fluorescence measurements were taken from the right side to isolate material that has been released from the gels.

Table S1. Flow cytometry antibodies, volume, excitation lasers, and bandpass filters used in the current study.

Antibody	Fluorophore	BioLegend Catalog #	Dilution for 1 Million Splenocytes	Excitation Laser (nm)	Bandpass Filter/Bandwidth (nm)
CD45	BV421	103133	1 µL	405	450/50
CD4	PerCP-Cy5.5	100539	0.5 µL	488	695/40
CD8a	APC-Fire 750	100765	0.5 µL	633	780/60
CD3e	Alexa Fluor 700	100216	1 µL	633	695/40
CD86	PE/Cy7	105013	1 µL	561	780/60
CXCR4	BV421	146511	1 µL	405	450/50
CCR6	BV605	129819	2.5 µL	405	610/20
CXCR3	PE	155903	2.5 µL	561	582/15
CCR4	APC	131211	5 µL	633	660/20
PD-1	BV785	135225	0.75 µL	405	780/60
SIINFEKL Tetramer	APC	N/A (NIH Tetramer Core)	0.15 µg	633	660/20

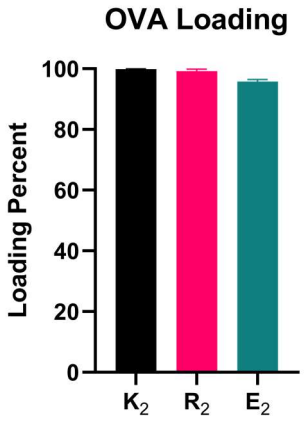


Figure S5. OVA loading efficiency into MDP hydrogels as calculated from the first time point from the in vitro release assay.

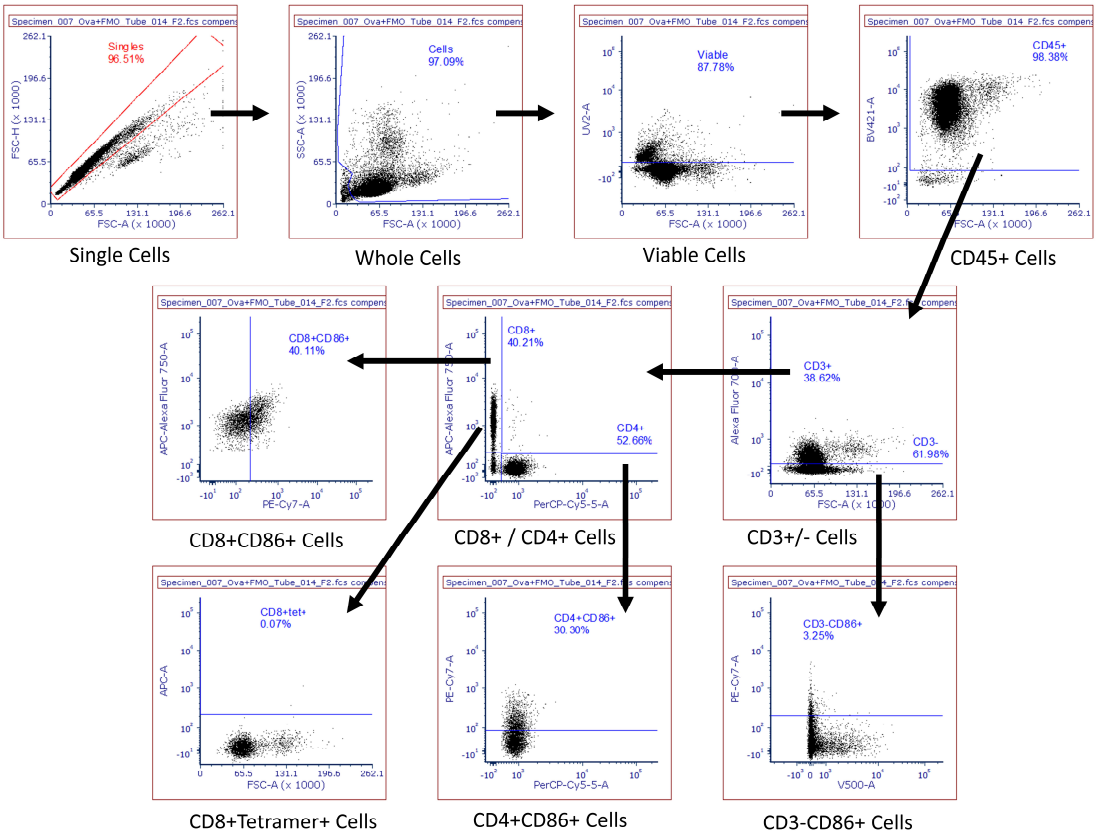


Figure S6. Flow cytometry gating strategy on a representative sample.

Table S2. HRP-conjugated secondary antibodies used for ELISA-based titer quantification via ELISA.

Antibody	Supplier (Catalog #)	Concentration used
Rabbit anti-mouse IgG	Jackson ImmunoResearch (315-035-045)	0.8 µg/mL
Goat anti-mouse IgG2c	Novus Biologicals (NB7566)	0.1 µg/mL
Goat anti-mouse IgM	Novus Biologicals (NB7497)	0.1 µg/mL

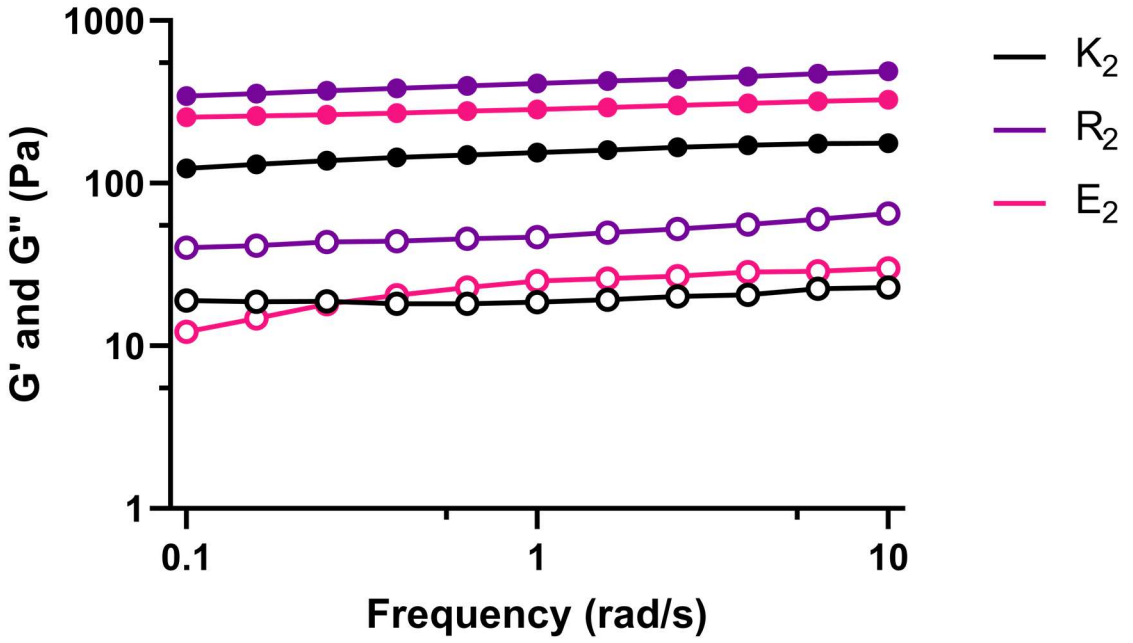


Figure S7. Frequency sweeps from 0.1-10 rad/s on a representative sample of each of the three MDP hydrogels loaded with 0.2 mg/mL OVA. Filled symbols represent G' and empty symbols represent G" values.

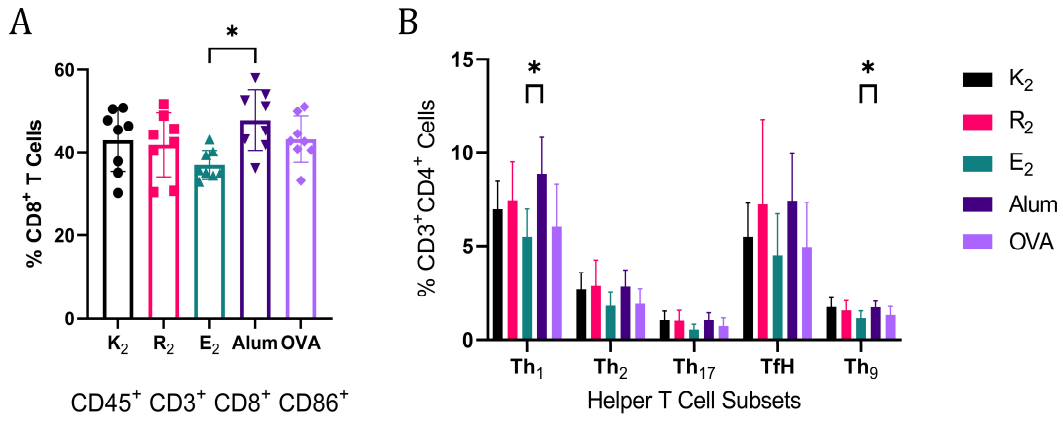


Figure S8. A) Percentage of activated $CD8^+$ $CD86^+$ in the total splenocyte CD8 T cell population in vaccinated mice (n=8). B) Helper T cell subsets characterized from the spleens of vaccinated mice (n=7-8) as a percent of the total $CD3^+$ $CD4^+$ helper T cell population. There were no significant differences between adjuvanted and unadjuvanted vaccine groups in the populations of Th_1 ($CXCR3^+$ $CCR4^-$), Th_2 ($CXCR4^+$ $CCR4^+$), Th_{17} ($CCR4^+$ $CCR6^+$), TfH ($CXCR4^+$ $PD\text{-}1^+$), and Th_9 ($CCR6^+$ $CCR4^-$) helper T cells.

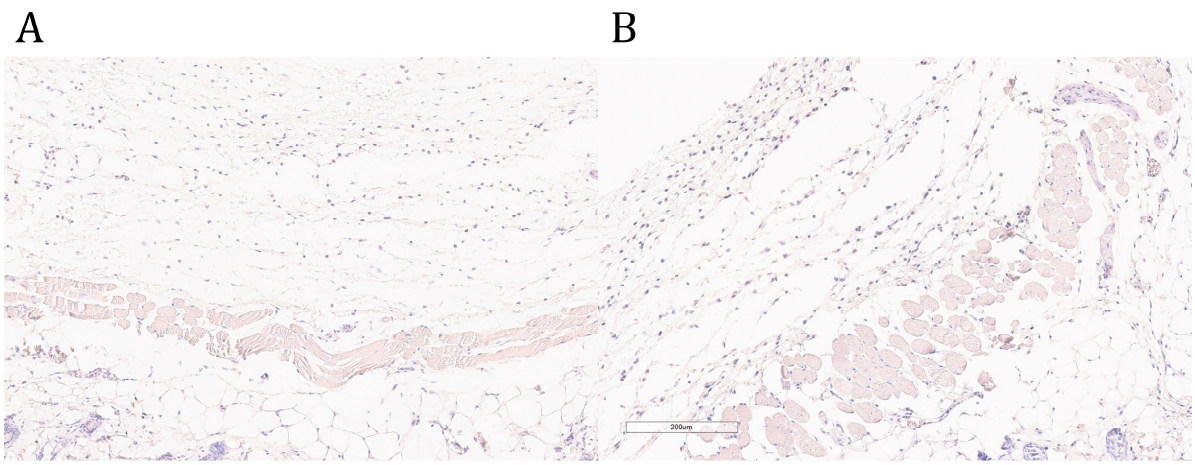


Figure S9. Representative caspase-3 staining of C57BL/6J mouse tissue sections 3 days post-injection with A) soluble OVA and B) OVA+E_2 where the scale bar represents 200 µm. Brown indicates the presence of caspase-3 (apoptosis), and blue is a nuclei counterstain.