Supporting Information

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SI Text

Detailed Serum ELISA Methods. High-binding ELISA plates (Costar cat 9018) were coated with 20 μg/ml solution of the immunizing peptide in PBS overnight at 4 °C. Washing steps were performed with 0.5% (v/v) Tween-20 in PBS (PBST), and blocking was performed with 1% BSA (Sigma cat A3803) in PBST (PBST-BSA) for 1 h at room temperature. Sera were serially diluted in PBST-BSA from $1:10^2$ to $1:10^9$ in PBST-BSA and added to the sample and control wells (100 μL per well). The primary antibody was peroxidase-conjugated goat anti-mouse IgG (H + L) (Jackson Immuno Research, cat 115035003, 1:5,000 dilution in PBST-BSA, 100 μL per well), and plates were developed for 2 min using TMB substrate (eBioscience CA, cat 00-4201-56, 100 μL per well). 50 μL of stop solution (1 M H_3PO_4) was then added and absorbance was measured at 450 nm on a Spectramax M5 plate reader (Molecular Devices CA). Antibody isotypes were analyzed similarly using goat anti-mouse IgG1 (Sigma cat M5532), IgG2a (M5657), IgG2b (M5782), IgG3 (M5907), and IgM (M6157) as the primary antibodies and HRP-conjugated rabbit anti-goat IgG (1:1000 in PBST-BSA) as the secondary antibody. Plates were developed for 5 min using TMB substrate and the reaction was stopped using 50 μL of stop solution, and absorbance was measured at 450 nm.

Detailed Cytokine ELISA Methods. After 24 h incubation with the challenging peptides, IFN-γ, IL-2, and IL-4 were measured in the cell culture supernatants using sandwich ELISA. Washing steps were performed with PBST, and blocking was performed with PBST-BSA. Capture antibodies for IFN-γ (eBioscience 14-6311-63), IL-2 (eBioscience 14-7022-81), and IL-4 (eBioscience 14-7041-81) were coated overnight (1 μg/mL in PBS at 4 °C). Cell culture supernatants (1:20 dilution in PBST-BSA, 100 μL per well) were then applied, and the plates were incubated in the dark for 2 h at room temperature. Biotin-conjugated detection antibodies (100 μL per well) for IFN-γ, IL-2, and IL-4 (eBioscience cat 13-7312-81, 13-7021-81, and 13-7042-81, respectively) were then applied for 1 h at room temperature (1μg/mL). Plates were developed using avidin-HRP (1:1000 in PBST-BSA, eBioscience cat 18-4100-94) and TMB substrate (eBioscience cat 00-4201-56). After 3 min of incubation 50 μL of stop solution (1 M H_3PO_4) was added and absorbance was measured at 450 nm. Standard curves were prepared using capture antibody and recombinant mouse IFN-γ, IL-2, and IL-4 (eBioscience 39-8311, 39-8021, and 39-8041 respectively). The absorbance values for each mouse were averaged, and cytokine levels were calculated from the standard curves.

Detailed Endotoxin Measurement Methods. Three batches of each peptide were synthesized independently to accomplish the reported work, and endotoxin levels of these peptides were measured two different ways using an LAL chromogenic endpoint assay (Lonza). For the first set of peptides (one batch each of Q11, $OVA_{323-339}$, and O-Q11), endotoxin levels were measured in 50 μL samples containing peptide concentrations of 1 mg/mL in water, and working endotoxin levels for the immunizations were calculated from these measurements. For the second and third batches of each peptide, endotoxin levels were measured in the exact peptide concentrations as those injected (2 mM peptide in water, 50 μL volume tested). No significant differences between these two methods of endotoxin measurement were observed, and the values listed in Table S1 represent the endotoxin levels either calculated to be present (for the first batch) or directly measured to be present (for the second and third batches) in the samples delivered to the animals. All endotoxin levels were within acceptable limits [Malyala P, Singh M (2008), Endotoxin limits in formulations for preclinical research, *J Pharm Sci* **97,** 2041-2044].

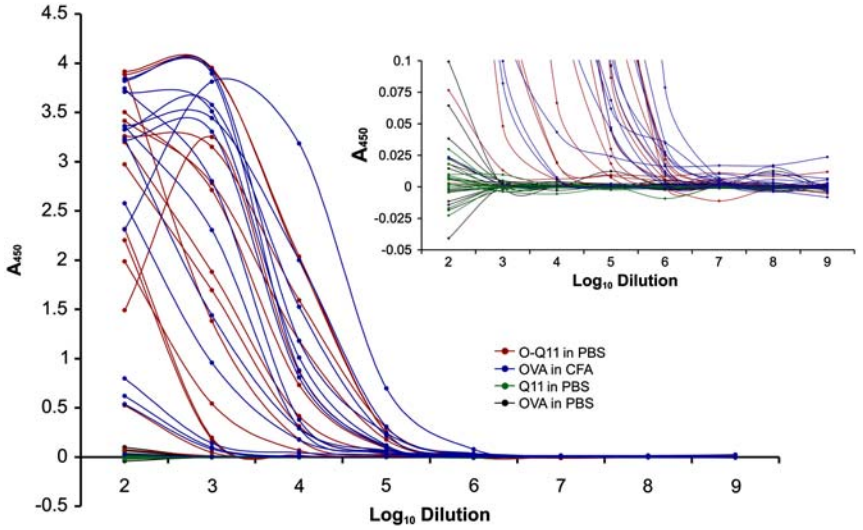

Fig. S1. Total IgG ELISA absorbance values for serially diluted serum from individual mice, corresponding to the titer calculations shown in Fig. 3*A* in the main text. Each line represents one mouse. Mice receiving O-Q11 in PBS are shown in red, OVA in CFA in blue, Q11 in PBS in green, and OVA in PBS in black. The inset shows an expanded scale, for visualizing the low absorbance values of the Q11 and negative control (OVA in PBS) mice.

Table S1. Purity, mass, and endotoxin levels of peptides

	Purity (%) by HPLC	Endotoxin (EU/mL)	(*m*/*z*) calc'd	(*m*/*z*) found
Q11	92.0–97.5%	0.024–0.098	1526.7	1527.2–1529.4
$OVA_{323-339}$	91.6–95%	bkgd–0.112	1773.9	1774.0–1774.3
O-Q11	96.2–98.0%	0.08–0.28	3528.8	3528.3–3530.1

Ranges represent high and low values among three batches for each peptide. See *SI Methods for Endotoxin Measurement Methods*.