Supplementary Table S1

Table S1. Drug effect on IL-2 production

		IL-2 concentration (pg/ml)[a]				
	Protein/peptide	HW6 scFv	HW6-$OVA_{250-264}$	3D8 scFv	3D8-$OVA_{250-264}$	SIINFEKL
	No drug	0	0	0	1379.3	3794.3
Drug	Heparin	0	0	0	257.4	3813.0
	MG-132	0	0	0	193.0	3797.4
	Chloroquine	0	0	0	1373.6	3773.6
	Pepstatin A	0	0	0	1386.1	3838.0
	Primaquine	0	0	0	1387.4	3725.5
	Brefeldin A	0	0	0	285.5	3668.6
	Cycloheximide	0	0	0	401.1	2534.9

[a]The levels of IL-2 secreted by CD8OVA1.3 T cells when co-cultured with DC2.4 cells pulsed with proteins/peptide in the presence of each drug.

Supplementary Figure S1

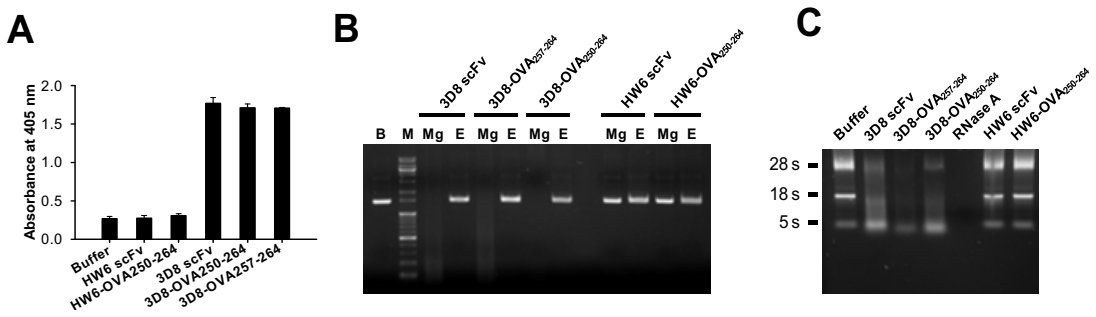

Fig. S1. Nucleic acid-binding and -hydrolyzing activity of proteins. (A) DNA binding activity measured by ELISA. The wells of a 96-well plate were coated with pUC19 plasmid DNA (200 ng/well), washed, and blocked with 5% skimmed milk for 1 h at room temperature (RT). Each protein (10 μg/ml) was added and incubated for 1 h at RT. The wells were incubated with anti-3D8 antibody followed by alkaline phosphatase-conjugated goat anti-rabbit IgG. Finally, *p*-nitrophenyl phosphate solution was added to each well and absorbance was read at 405 nm. The *error bars* represent the standard deviation for triplicate experiments. The results showed that 3D8-$OVA_{250-264}$ and 3D8-$OVA_{257-264}$ bound to pUC19 DNA to the same extent as 3D8 scFv. **(B)** DNA hydrolyzing activity. pUC19 plasmid (200 ng) was incubated with each protein (0.5 μM) for 2 h at 37°C in TBS buffer (50 mM Tris-Cl, 50 mM NaCl, pH 7.4) containing 2 mM $MgCl_2$ ("Mg") or 50 mM EDTA ("E"). The reaction mixtures were analyzed by electrophoresis on an agarose gel (0.8%), which was then stained with ethidium bromide. B: TBS buffer, M: molecular mass marker. The results showed that in 2 h, 3D8-$OVA_{250-264}$, 3D8-$OVA_{257-264}$ and 3D8 scFv completely hydrolyzed pUC19 in an Mg^{2+}-dependent manner. **(C)** RNA hydrolyzing activity. Each protein (0.5 μM) was incubated with total cellular RNA (1 μg) from HeLa cells in TBS buffer at 37°C for 2 h prior to agarose gel electrophoresis and ethidium bromide staining. RNase A (1 U) was used as a positive control in the buffers provided by the manufacturer (New England Biolabs). The results showed that RNA (28S and 18S) was degraded when incubated with 3D8 proteins but not with irrelevant scFv HW6 proteins prepared using the same procedures, confirming that 3D8-$OVA_{250-264}$ and 3D8-$OVA_{257-264}$ possessed the same RNase activity as 3D8 scFv.

Supplementary Figure S2

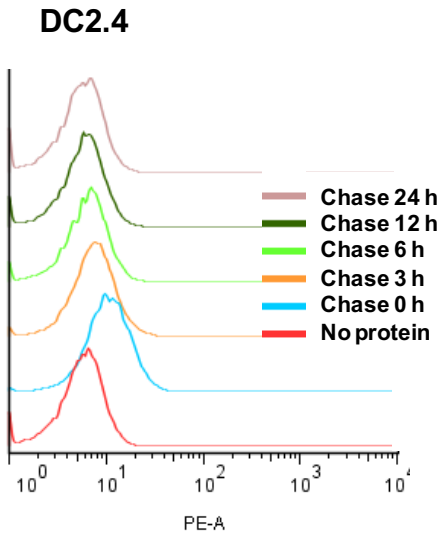

Fig. S2. The intracellular disappearance of internalized 3D8-OVA$_{250-264}$ in DC2.4 cells. DC2.4 cells (5×10^5/well in a 6-well plate) were incubated with 5 μM of 3D8-OVA$_{250-264}$ for 2 h at 37°C, and then chased for 3–24 h. Cells were washed twice with cold PBS, fixed with 2% paraformaldehyde-PBS for 10 min at RT, and then permeabilized with Perm-buffer (1% BSA/0.1% saponin/0.1% sodium azide in PBS) for 10 min at RT. After blocking with 2% BSA-PBS for 1 h, the cells were incubated with rabbit IgG (1 μg/ml), followed by TRITC-labeled anti-rabbit IgG antibody. Cells were analyzed by flow cytometry (FACSCanto II^{TM}). The results showed that most of 3D8-OVA$_{250-264}$ disappeared from DC2.4 cells within 6 h.

Supplementary Figure S3

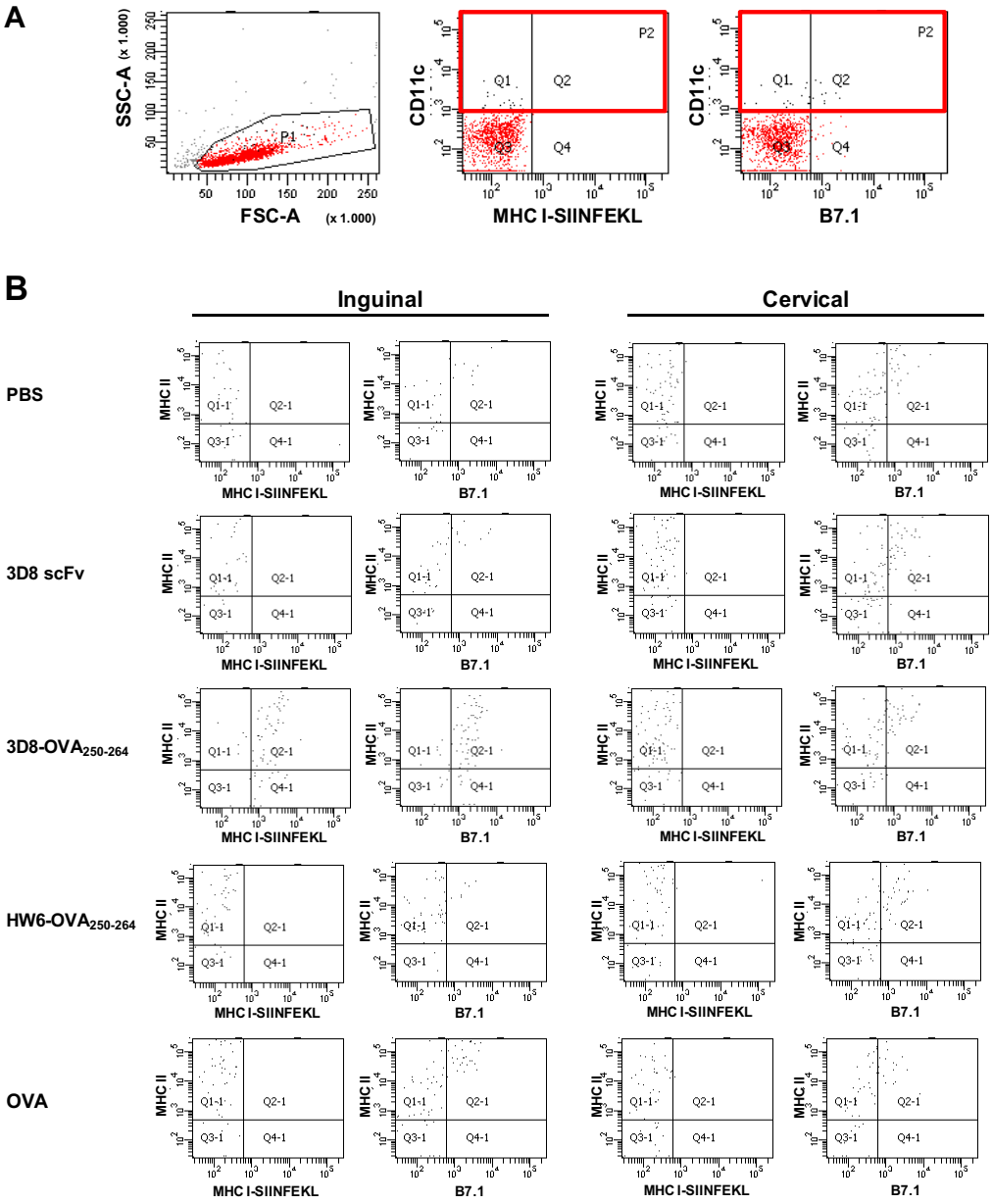

Fig. S3. Dot plots showing lymph node cells isolated from immunized mice. C57BL/6 mice were s.c-injected in the right flank with the indicated proteins (50 μg) or PBS. After 48 hr, lymph node cells isolated from the right inguinal and left cervical lymph nodes were labeled with Abs specific for CD11c and MHC class II molecules, followed by either an Ab specific for the H-2k^b-SIINFEKL complex or B7.1 for flow cytometric analysis. (A) Representative dot plots showing the gated CD11c+ cell population. (B) Expression of H-2k^b-SIINFEKL, MHC II, and B7.1 in the CD11c$^+$ population gated as in A. Data are representative of two independent experiments (n = 8 mice per group).