

Supplemental Figure Legends

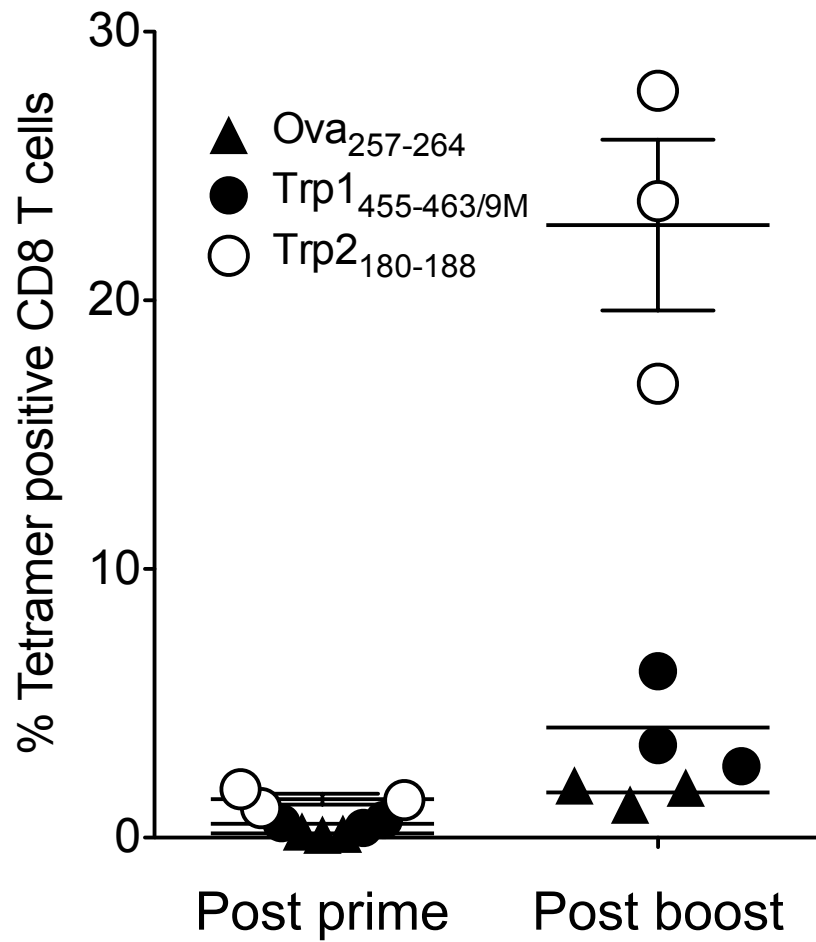
Supplemental Fig. 1 CD8 T cell responses induced by BiVax using three minimal peptide epitopes. Mice (3 per group) were vaccinated i.v. on days 0 and 12 with BiVax composed of 100 μg of indicated peptide and 50 μg of Poly-IC, 7 days after each immunization the presence of antigen-specific CD8 T cells in blood was evaluated by tetramer analysis. Results represent the percentage of tetramer positive CD8 T cells for each mouse per group.

Supplemental Fig. 2 Hydrophobicity and amphiphilicity analysis of CD8 T cell epitopes. For illustration purposes amino acids were grouped into three categories (High, Intermediate and low) depending on the degree of hydrophobicity according to Kyte-Doolittle (KD) scale (18). Four CD8 T cell epitopes were analyzed for their overall hydrophobicity by the sum (Σ) of the KD values for each residue. Peptides HPV16-E7₄₉₋₅₇ and Trp2₁₈₀₋₁₈₈ (which function in BiVax) were found to contain in each half a continuous segment of 4 or 5 mostly hydrophobic residues (framed in red) and a relatively hydrophilic portion on the other half, making them amphiphilic.

Supplemental Fig. 3 Extending peptide using hydrophilic residues did not improve the immunogenicity of BiVax. Mice (3 per group) were vaccinated i.v. on days 0 and 12 with BiVax composed of 150 μg of peptide and 50 μg of Poly-IC, 7 d post-booster administration, presence of antigen-specific CD8 T cells in blood was evaluated by tetramer analysis. Results represent the average percentage of tetramer positive CD8 T cells from 3 mice per group with SD (*bars*) of the means.

Supplemental Fig. 4 Increasing peptide hydrophobicity of Ova₂₅₇₋₂₆₄ enhances immunogenicity of BiVax. **(A)** Mice (4 per group) received identical BiVax immunizations (prime/boost) with either minimal peptide (SIINFEKL) or palmitilated peptide (Pam2KMFVSIINFEKL). Immune responses were measured in blood 7 d after the prime and boost as noted.

Supplemental Figure 1



Supplemental Figure 2

AA	KD	Hydrophobicity
I	4.5	High
V	4.2	
L	3.8	
F	2.8	
C	2.5	
M	1.9	Intermediate
A	1.8	
G	-0.4	
T	-0.7	
S	-0.8	
W	-0.9	
Y	-1.3	
P	-1.6	Low
H	-3.2	
E	-3.5	
Q	-3.5	
D	-3.5	
N	-3.5	
K	-3.9	
R	-4.5	

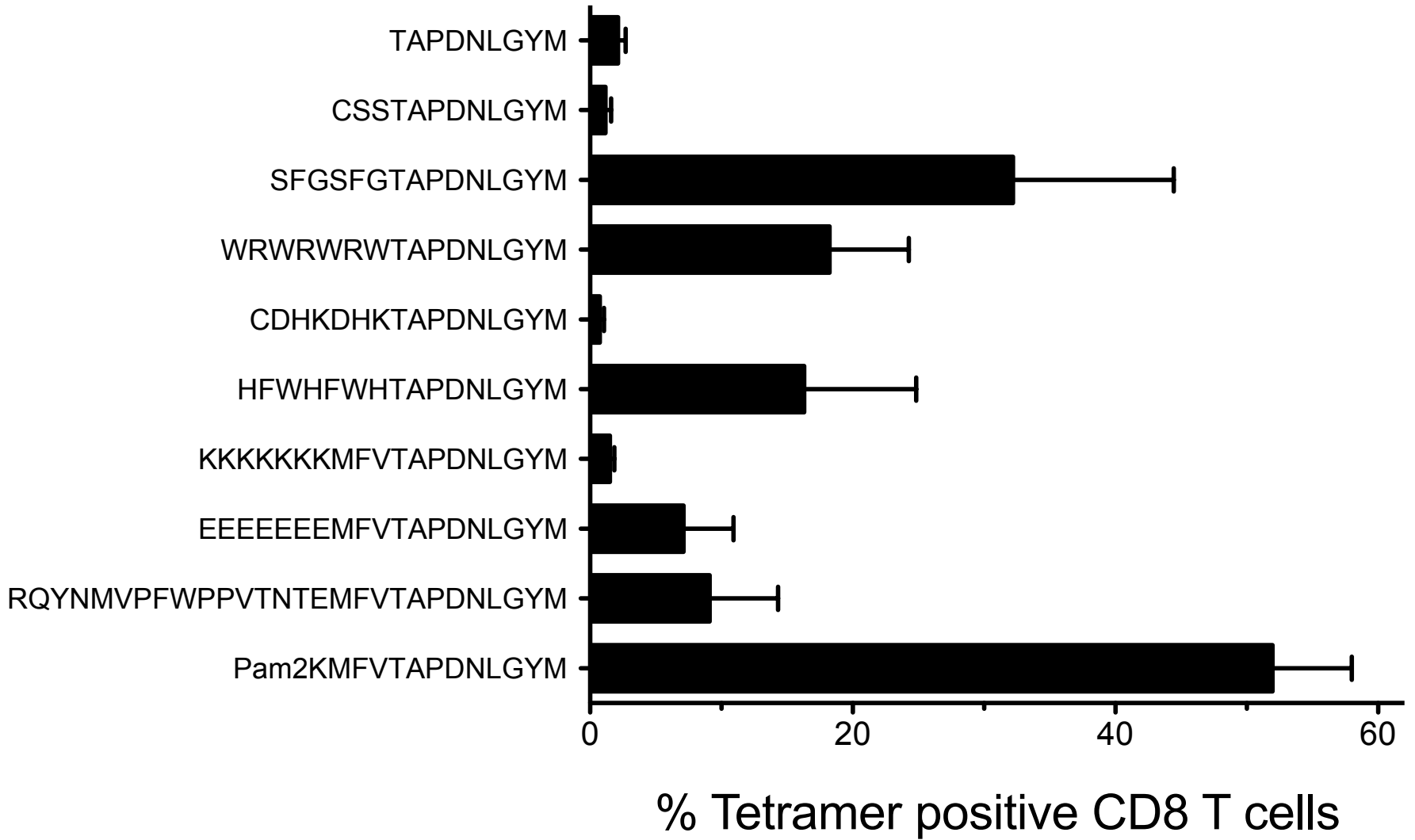
	aa	kd
HPV16-E7 ₄₉₋₅₇	R	-4.5
	A	1.8
	H	-3.2
	Y	-1.3
	N	-3.5
	I	4.5
	V	4.2
	T	-0.7
	F	2.8
	Σ	0.1

	aa	kd
Trp2 ₁₈₀₋₁₈₈	S	-0.8
	V	4.2
	Y	-1.3
	D	-3.5
	F	2.8
	F	2.8
	V	4.2
	W	-0.9
	L	3.8
	Σ	11.3

	aa	kd
Ova ₂₅₇₋₂₆₄	S	-0.8
	I	4.5
	I	4.5
	N	-3.5
	F	2.8
	E	-3.5
	K	-3.9
	L	3.8
	Σ	3.9

	aa	kd
Trp1 _{455-463/9M}	T	-0.7
	A	1.8
	P	-1.6
	D	-3.5
	N	-3.5
	L	3.8
	G	-0.4
	Y	-1.3
	M	1.9
	Σ	-3.5

Supplemental Figure 3



Supplemental Figure 4

