Supplementary Material:

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Identification of a superagonist variant of the immunodominant Yellow fever virus epitope NS4b$_{214-222}$ by combinatorial peptide library screening

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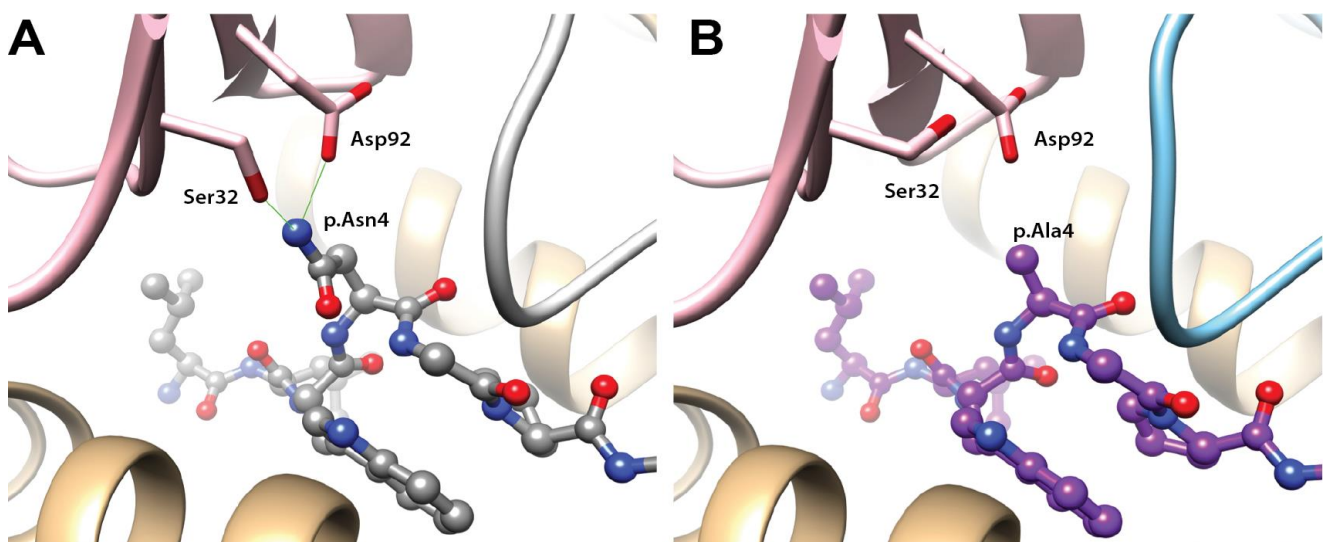
Fig S1. Molecular modeling of the YF5048 TCR bound to the 4A mutant peptide in complex with HLA-A2. Ribbons represent TCR β –chain in grey; the MHC molecule in tan ribbon, and peptides in ball and stick representation. WT peptide on the left (in grey) and 4A peptide on the right (in purple). Hydrogen bonds are shown as green thin lines.
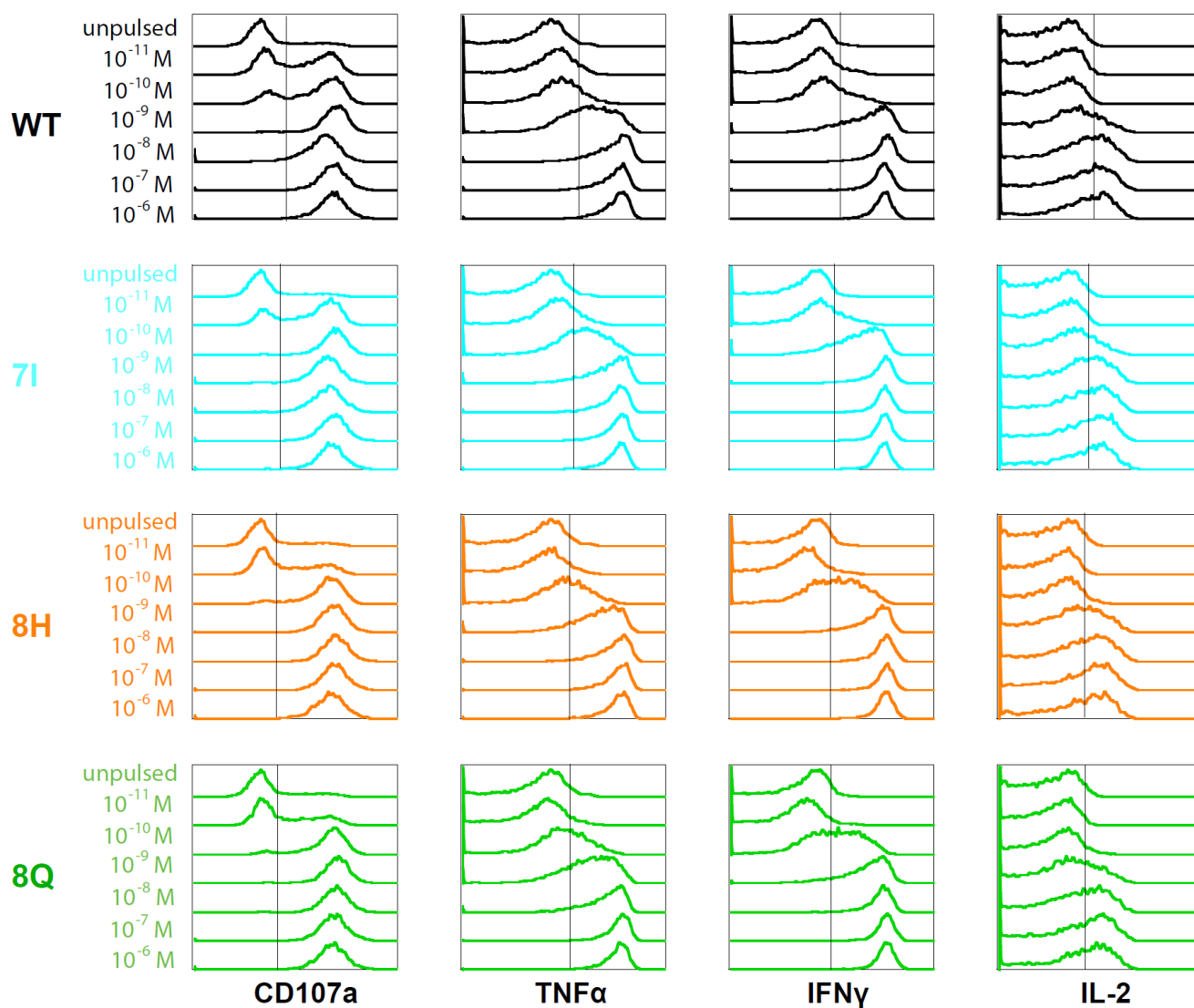
Fig S2. Histogram overlays of the intracellular cytokine staining. Staining was performed on the YF5048 clone following stimulation with T2 cells pulsed with the indicated peptides at concentrations ranging between 10^{-11} and 10^{-6}M. Unpulsed T cell clone was used as negative control. A representative flow cytometry experiment is shown.
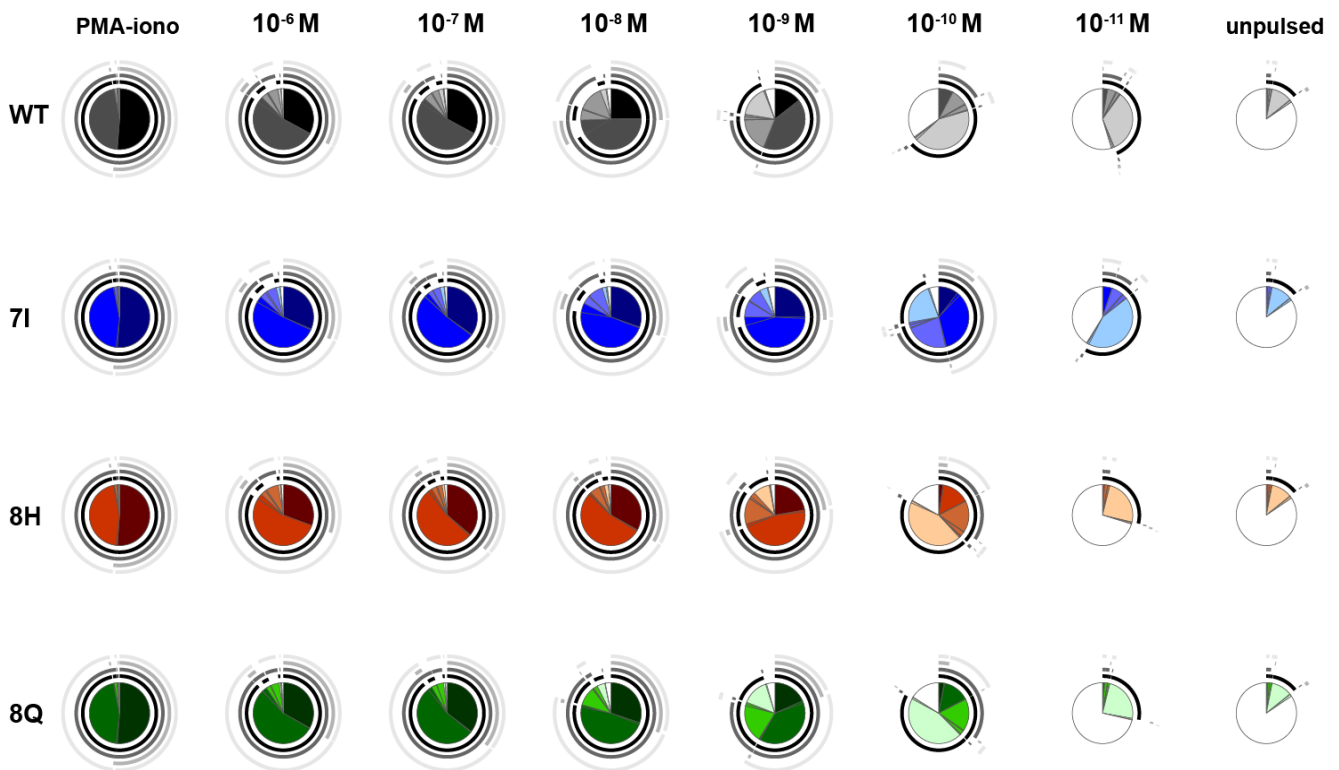
Fig S3. SPICE analysis of a representative intracellular cytokine staining. Pie charts showing frequencies of the combinatorial expression of the indicated cytokines and degranulation marker following stimulation of the YF5048 clone with various peptides at concentrations ranging between 10^{-11} and 10^{-6}M. Each peptide condition has one color (one titration line per peptide), and Color color graduation corresponds to the number of simultaneous functions (white = none of the functions; darkest graduation of the color = 4 functions). Grey-scaled pie arcs indicate the fraction that expresses a given function, according to the indicated legend.
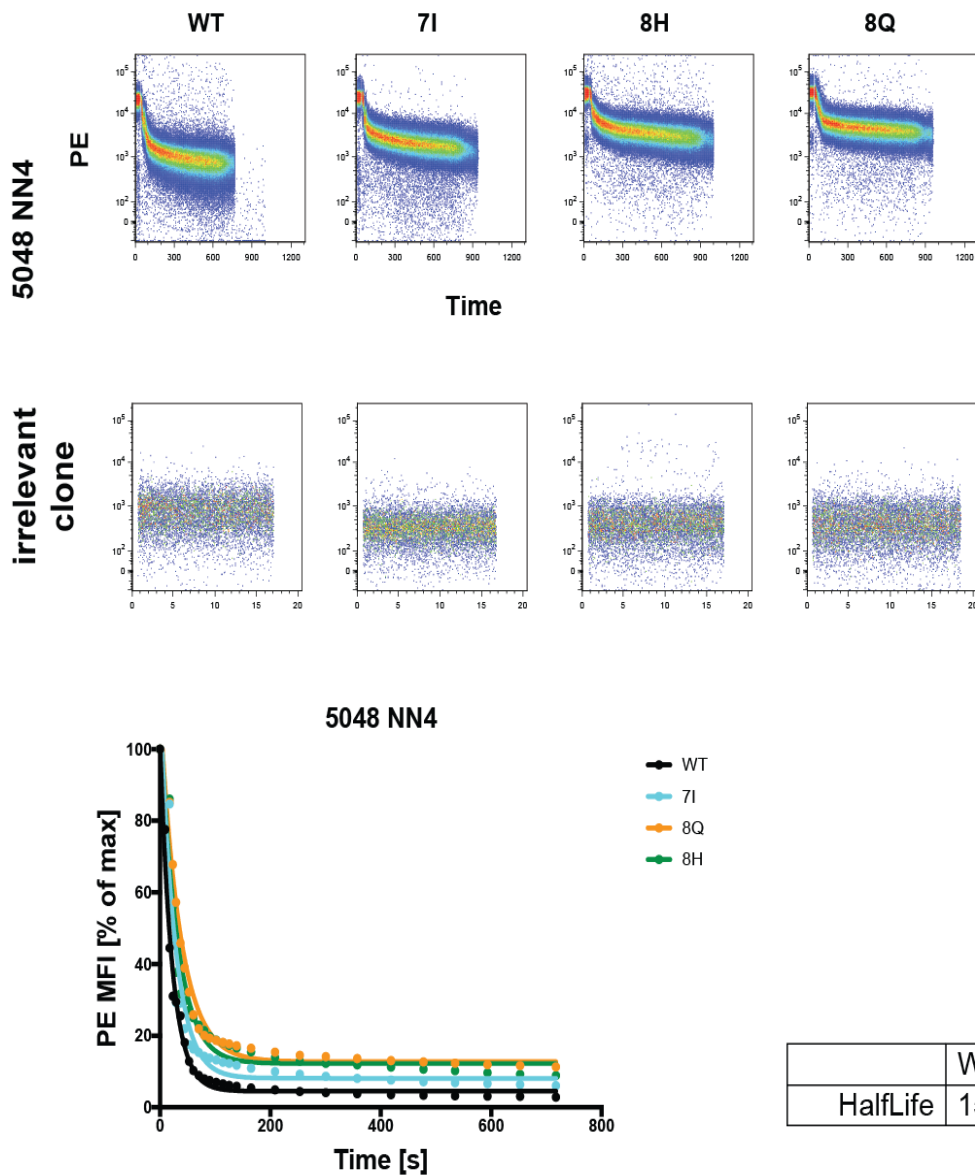
5048 NN4

	WT	7I	8H	8Q
HalfLife	15.01	18.35	20.85	24.93

Fig S4. PE-NTA fluorescence decay. Dot plot from a representative flow cytometry experiment is shown. Half lives (measured in seconds; Half-life = ln 2/k_{off}) of the YF5048 clone were analyzed in *Prism* with a first-order monomeric decay function after subtraction of non-specific background from an irrelevant clone (recognizing A2/NLVPMVATV).

	WT	7I	8H	8Q
HalfLife	12.03	22.96	173.1	130.2

Fig S5. Cy5-pMHC fluorescence decay. Dot plot from a representative flow cytometry experiment is shown. Half lives (measured in seconds; Half-life = ln $2/k_{off}$) of the YF5048 clone were analyzed in Prism with a first-order monomeric decay function after subtraction of non-specific background from an irrelevant clone (recognizing A2/NLVPMVATV).
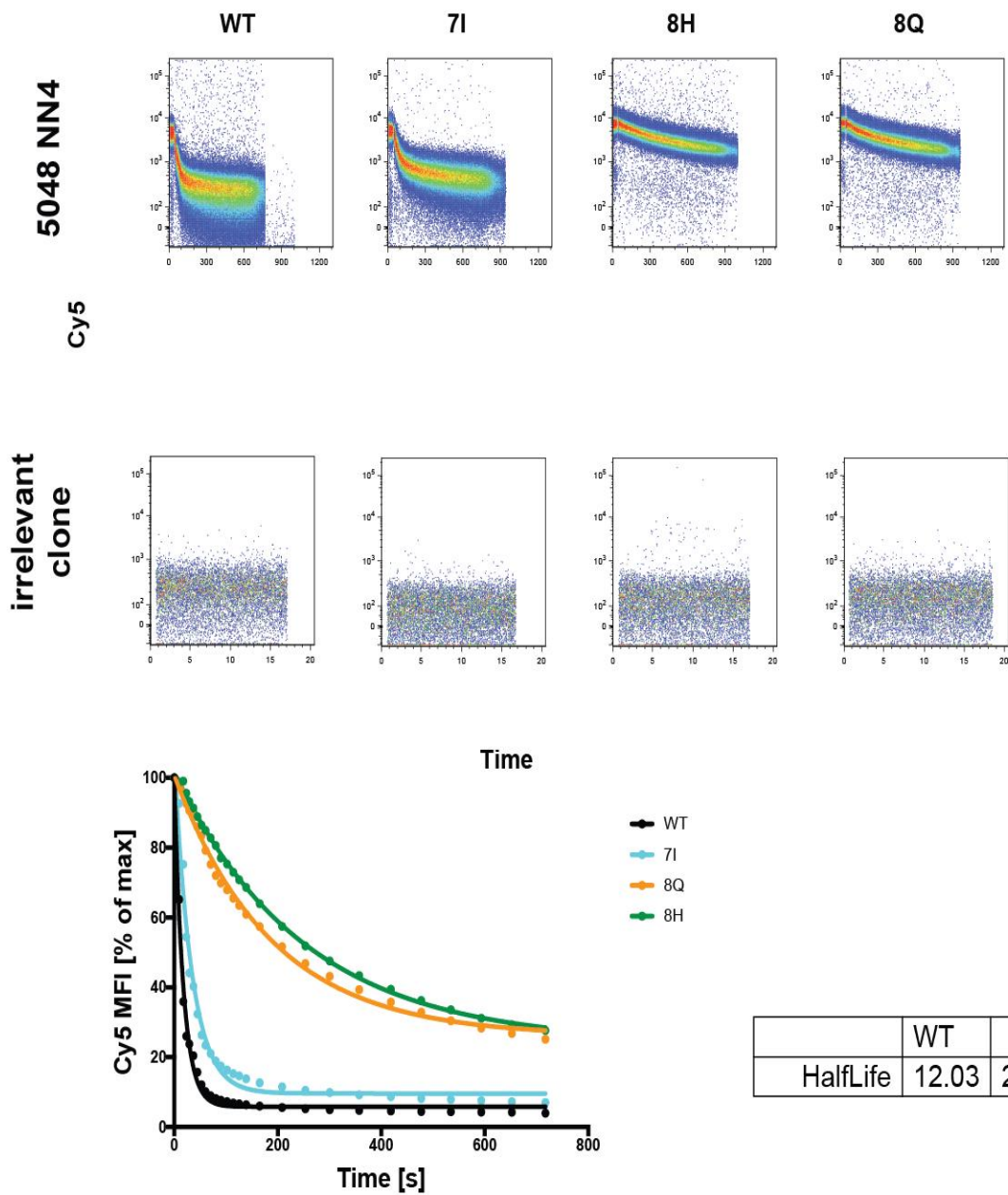
Table S1. Data reduction and refinement statistics (molecular replacement) of HLA Class-I Yellow-Fever Altered Epitopes

Parameters	A2/LLW-4A	A2/LLW-7I	A2/LLW-8H	A2/LLW-8Q
Protein Data Bank code	6SS7	6SS8	6SS9	6SSA
Data set statistics				
Space group	P 21 21 2	P 21 21 2	C 1 2 1	P 1 2 1
a,b,c (Å)	117.56 169.25 49.07	118.11 168.62 48.93	199.75 48.26 117.75	117.91 49.07 169.01
α,β,γ (°)	90.0 90.0 90.0	90.0 90.0 90.0	90.0 123.47 90.0	90.0 92.484 90.0
Radiation source	DIAMOND I03	P 21 21 2	C 1 2 1	P 1 2 1
Resolution (Å)	2.36 – 84.62	2.24 – 29.53	2.52 – 29.41	2.00 – 117.77
Outer shell (Å)	2.36 – 2.42	2.24 – 2.29	2.52 – 2.59	2.00 – 2.05
Total Measurements	297,709 (22,369)	325,685 (15,526)	114,887 (7,831)	476,741 (34,202)
Unique Reflections	41,105 (2,956)	47,512 (2,730)	31,568 (2,105)	131,906 (9,683)
Completeness (%)	99.6 (99.4)	98.3 (78.6)	98.6 (89.6)	99.9 (100)
Multiplicity	7.2 (7.6)	6.9 (5.7)	3.6 (3.7)	3.6 (3.5)
I / σ(I)	6.3 (1.2)	8.3 (1.4)	9.6 (1.6)	5.0 (1.7)
R-pim	14.5 (98.8)	12.2 (63.4)	6.3 (54.5)	8.8 (84.9)
R-merge (%)	24.8 (171.3)	19.8 (100.3)	7.7 (68.6)	13.9 (136.1)
Refinement Statistics				
Resolution Range Used	ᶦ2.50 – 84.62	2.24 – 29.53	ᶦ2.70 – 98.233	ᶦ2.20 – 70.07
Non-H Atoms	6,717	6,718	6,449	14,046
R-work reflections	32,915	45,016	24,732	94,051
R-free reflections	1,756	2,436	1,287	4,797
R-work/R-free (%)	22.5 / 30.4	22.8 / 27.7	23.3 / 30.1	22.3 / 27.1
Root mean square deviation from ideal geometry				
Bond lengths (Å)	0.018 (0.019)	0.014 (0.014)	0.013 (0.014)	0.011 (0.013)
Bond Angles (°)	1.888 (1.934)	1.660 (1.686)	1.691 (1.674)	1.655 (1.656)
[1]Coordinate error	0.191	0.227	0.388	0.156
Mean B value (Å^2)	35.3	39.0	69.1	25.2
Ramachandran Statistics				
Favoured	735 (97.4%)	731 (96.8%)	720 (95.4%)	1453 (98.6%)
Allowed	19 (2.5%)	21 (2.8%)	26 (3.4%)	21 (1.4%)
Outliers	1 (0.1%)	3 (0.4%)	9 (1.2%)	0 (0.0%)

* One crystal was used for determining each structure.

* Figures in brackets refer to outer resolution shell, where applicable.

[1] Coordinate Estimated Standard Uncertainty in (Å), calculated based on maximum likelihood statistics.

ᶦThe resolution range for this data set was truncated due to t-NCS and/or twinning