Supplementary Materials

Table S1. Prediction matrix to identify peptides that contain an HLA DR0401 binding motif and at least one arginine residue

Fig. S1: Citrulline is preferentially accepted in multiple DR0401 binding pockets.

Fig. S2: Responses to novel citrullinated peptides are detected in PBMC of RA patients by in vitro tetramer assays.

Fig. S3: Overall frequencies of citrulline reactive CD4+ T cells are higher in RA patients and controls.

Fig S4: Ex vivo profiles of CCR7, CCR6, CD28, and CD25 are similar between citrulline specific T cells from RA patents and controls.

Fig. S5: No differences are seen between the frequencies of flu specific T cells from RA patents with disease <5 years, RA patients with disease > 5 years & healthy controls.

Fig. S6: RA patients on non-biologic therapies have significantly more CXCR3+CD45RO+ CD4+ T cells than RA patients on biologic therapies and healthy controls.

Fig S7: RA patents with disease <5 years have significantly more cit-specific T cells when on non-biologic treatment than when on biologics.

Table S1. Prediction matrix to identify peptides that contain an HLA DRB1*0401 binding motif and at least one arginine residue

Pocket 1*	C_{p1}#	Pocket 4*	C_{p4}#	Pocket 6*	C_{p6}#	Pocket 7*	C_{p7}#	Pocket 9*	C_{p9}#
G	0.00	G	0.31	G	0.43	G	1.80	G	1.00
A	0.08	A	0.86	A	0.93	A	1.82	A	1.00
V	0.24	V	0.67	V	1.56	V	0.60	V	0.50
L	0.24	L	1.32	L	0.65	L	0.42	L	0.40
I	0.30	I	1.16	I	0.91	I	0.42	I	0.35
M	0.18	M	2.08	M	0.54	M	1.08	M	1.12
P	0.00	P	0.07	P	0.90	P	0.10	P	0.17
F	1.50	F	1.97	F	0.30	F	0.51	F	0.10
W	0.93	W	0.47	W	0.39	W	0.60	W	0.10
S	0.00	S	1.14	S	1.36	S	0.57	S	3.48
T	0.00	T	0.91	T	1.83	T	0.57	T	0.85
N	0.00	N	3.65	N	1.21	N	1.50	N	0.72
Q	0.00	Q	1.04	Q	0.32	Q	0.90	Q	0.60
Y	0.97	Y	0.89	Y	0.27	Y	0.56	Y	0.18
C	0.00	C	1.36	C	0.72	C	0.12	C	0.40
K	0.00	K	0.06	K	0.02	K	0.03	K	0.05
R	0.00	R	0.03	R	0.02	R	0.09	R	0.07
H	0.00	H	1.27	H	0.30	H	0.39	H	2.64
D	0.00	D	1.17	D	0.84	D	0.12	D	0.70
E	0.00	E	1.93	E	0.87	E	0.12	E	0.40
X	0.00	X	1.66	X	0.00	X	0.77	X	0.07

* X indicates citrulline

predicted relative binding affinity = $C_{p1} \times C_{p4} \times C_{p6} \times C_{p7} \times C_{p9}$

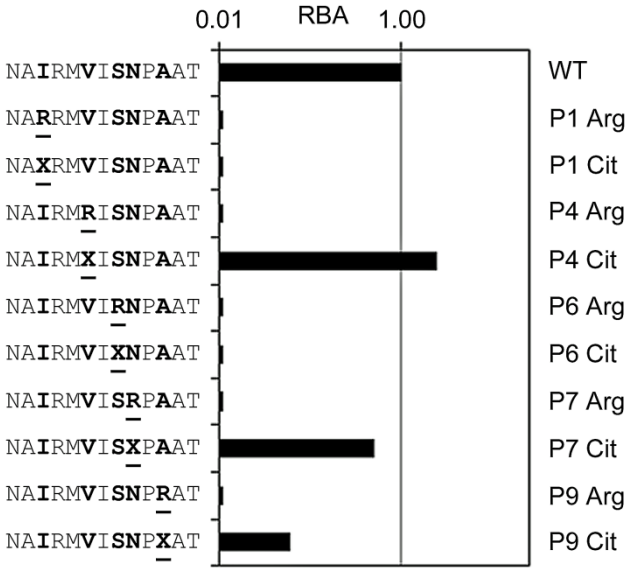

Fig. S1. Citrulline is preferentially accepted in multiple DR0401 binding pockets. Peptides with a single arginine or citrulline substitutions (substituted residues are underlined) at the P1, P4, P6, P7, or P9 position (anchor positions are in boldface) were bound to DR0401 protein in competition with a biotin labeled reference peptide. Each bar indicates the relative binding affinity (RBA) of the substituted peptide as compared to the reference peptide (1.0 indicates equivalent binding, 0.05 indicates no detectable binding). While no peptide with an arginine substitution was able to bind, peptides with citrulline at P4, P7, and P9 were able to bind to DR0401 protein.

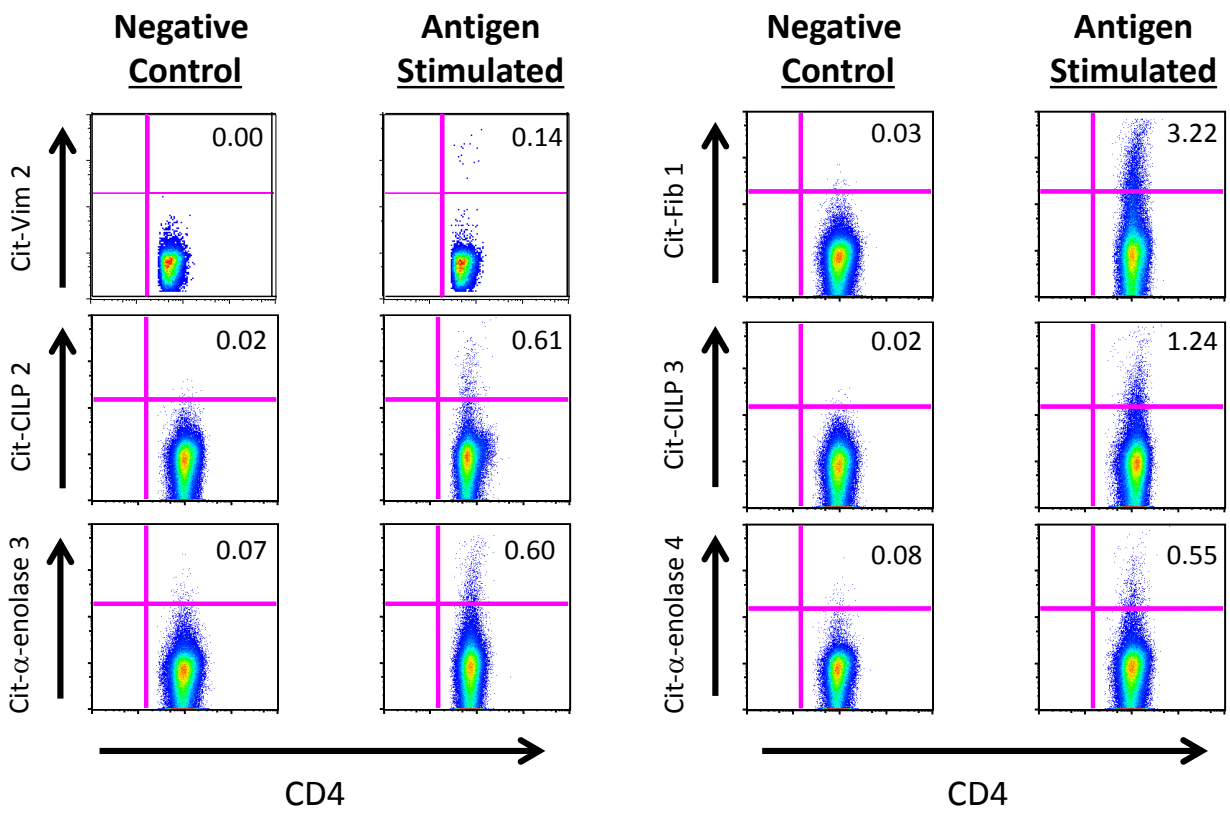

Fig. S2: Responses to novel citrullinated peptides are detected in PBMC of RA patients by in vitro tetramer assays. In vitro cultures were stimulated for 14 days with either the citrullinated peptide of interest or a control peptide before being stained with the DR0401 tetramers loaded with the corresponding citrullinated peptide. Shown are representative plots (data is from multiple subjects, since no subject was positive for every epitope) for cultures stained with DR0401 Cit-Vim 2, Cit-Fib 1, Cit-CILP 2, Cit-CILP 3, Cit-a-enolase 3, and Cit-a-enolase 4.

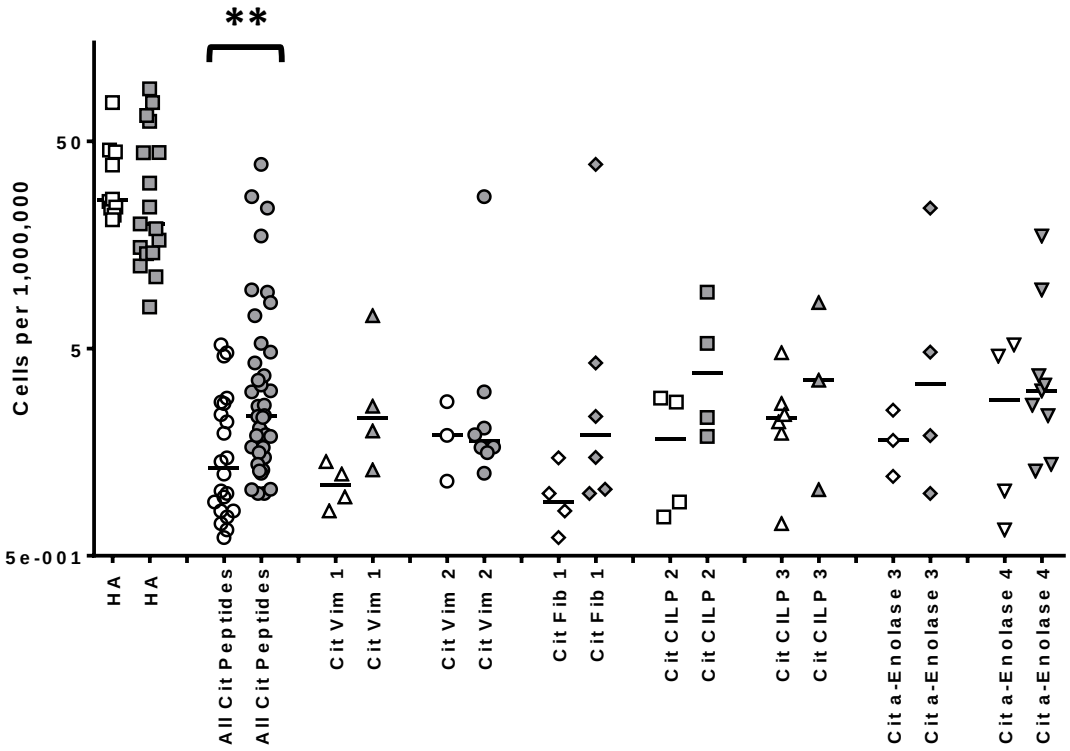

Fig. **S3: Overall frequencies of citrulline reactive CD4+ T cells are higher in RA patients and controls.** T cell frequencies were determined directly ex vivo for DR0401-tetramer+ antigen specific T cells. All frequencies are expressed as number of antigen specific cells/million T cells. Comparisons are made between healthy controls (white symbols) and RA patients (gray symbols) for T cells specific for flu ($HA_{306-318}$), all citrullinated antigens tested, and individual citrullinated peptides tested. Statistical significance was determined by an unpaired T test with Welch's correction after normalization for logarithmic distribution (**p=0.007).

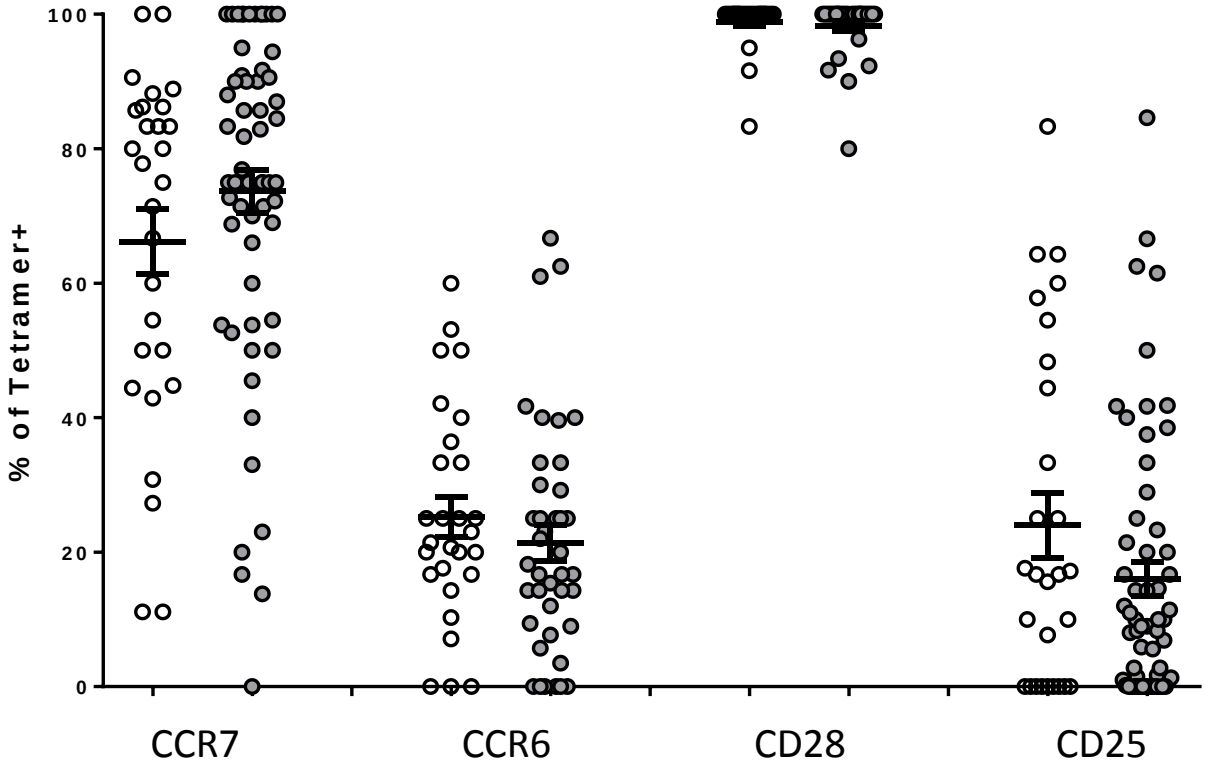

Fig. S4: Ex vivo profiles of CCR7, CCR6, CD28, and CD25 are similar between citrulline specific T cells from RA patents & controls. Antigen specific T cells detected directly ex vivo by DR0401 tetramer and specific for citrullinated peptides were phenotyped for their expression of CCR7, CCR6, CD28, and CD25. Comparisons were made between healthy controls (white circles) and RA patents (gray circles), but no differences were seen as determined by Mann-Whitney tests.

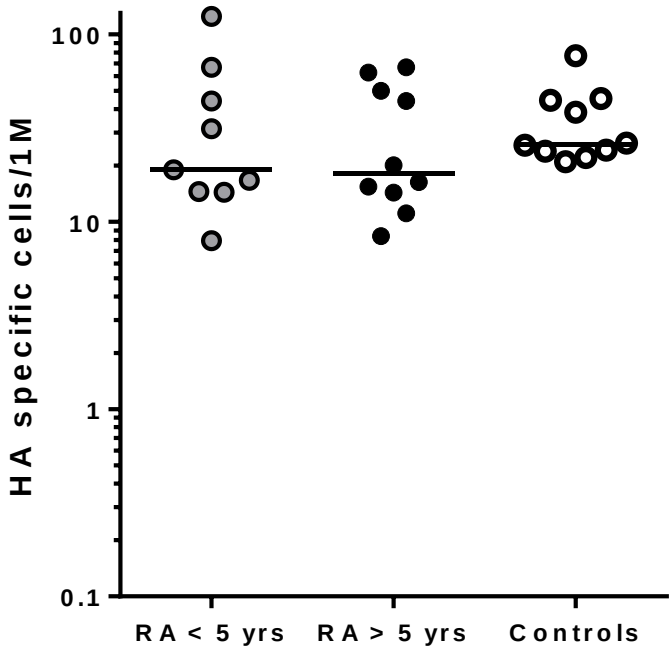

Fig. S5: No differences are seen between the frequency of flu specific T cells from RA patients with disease <5 years, RA patients with disease > 5 years and healthy controls. The frequency of influenza specific T cells detected directly ex vivo using DR0401-$HA_{306-318}$ tetramers in individuals with RA < 5 years (gray circles), RA patients with disease > 5 years (black circles), and healthy controls (white circles) was determined to not be significantly different as determined by an unpaired t test with Welch's correction after normalization for logarithmic distribution. The Y-axis shows frequency as the number of DR0401-HA tetramer+ T cells/million T cells.

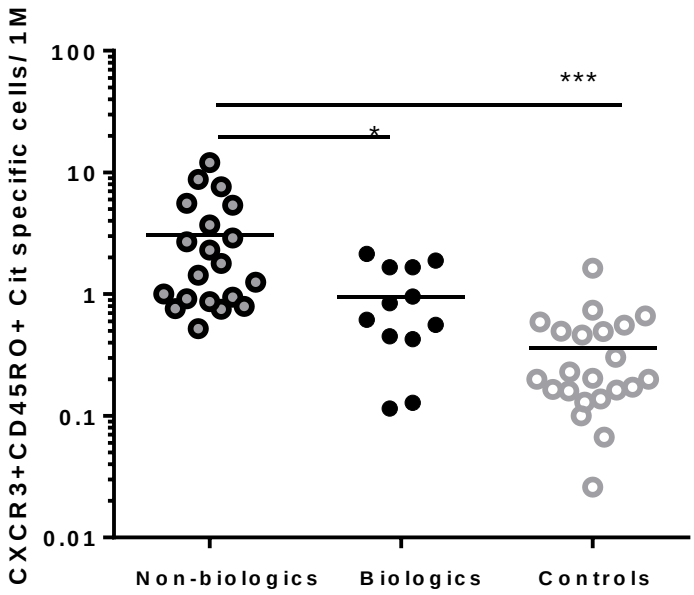

Fig S7: RA patients on non-biologic therapies have significantly more CXCR3+CD45RO+ CD4+ T cells than RA patients on biologic therapies and healthy controls. A comparison of ex vivo frequencies of cit-specific CXCR3+CD45RO+ CD4+ T cells in 8 RA patients on non-biologic therapies (gray circles), 8 RA patients on biologic therapies (black circles), and 11 healthy controls (white circles) shows that RA patients on non-biologic therapies have significantly more cit-specific CD4+T cells than RA patients on biologic therapies and healthy controls. Frequencies are shown as CXCR3+CD45RO+ tetramer+ antigen specific T cells/million CD4+ T cells. Statistical significance was determined by ANOVA using the Sidak multiple comparisons post-test after normalization for logarithmic distribution. *=$p<0.05$ and ***=$p<0.001$.

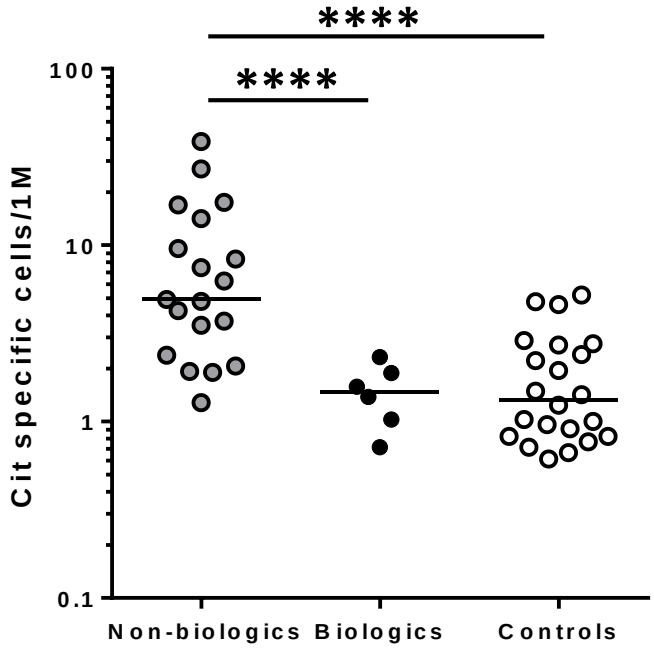

Fig S7: RA patents with disease <5 years have significantly more cit-specific T cells when on non-biologic treatment than when on biologics. A comparison of ex vivo frequencies of cit-specific CD4+ T cells in RA patients on non-biologic therapies (gray circles), RA patients on biologic therapies (black circles), and healthy controls (white circles), where all RA patients have disease for <5 years, shows that the RA patients on non-biologic therapies have significantly more cit-specific CD4+T cells than the RA patients on biologic therapies and healthy controls. Frequencies are shown as tetramer+ antigen specific T cells/million T cells. Statistical significance was determined by an unpaired T test with Welch's correction after normalization for logarithmic distribution (****p=<0.0001).