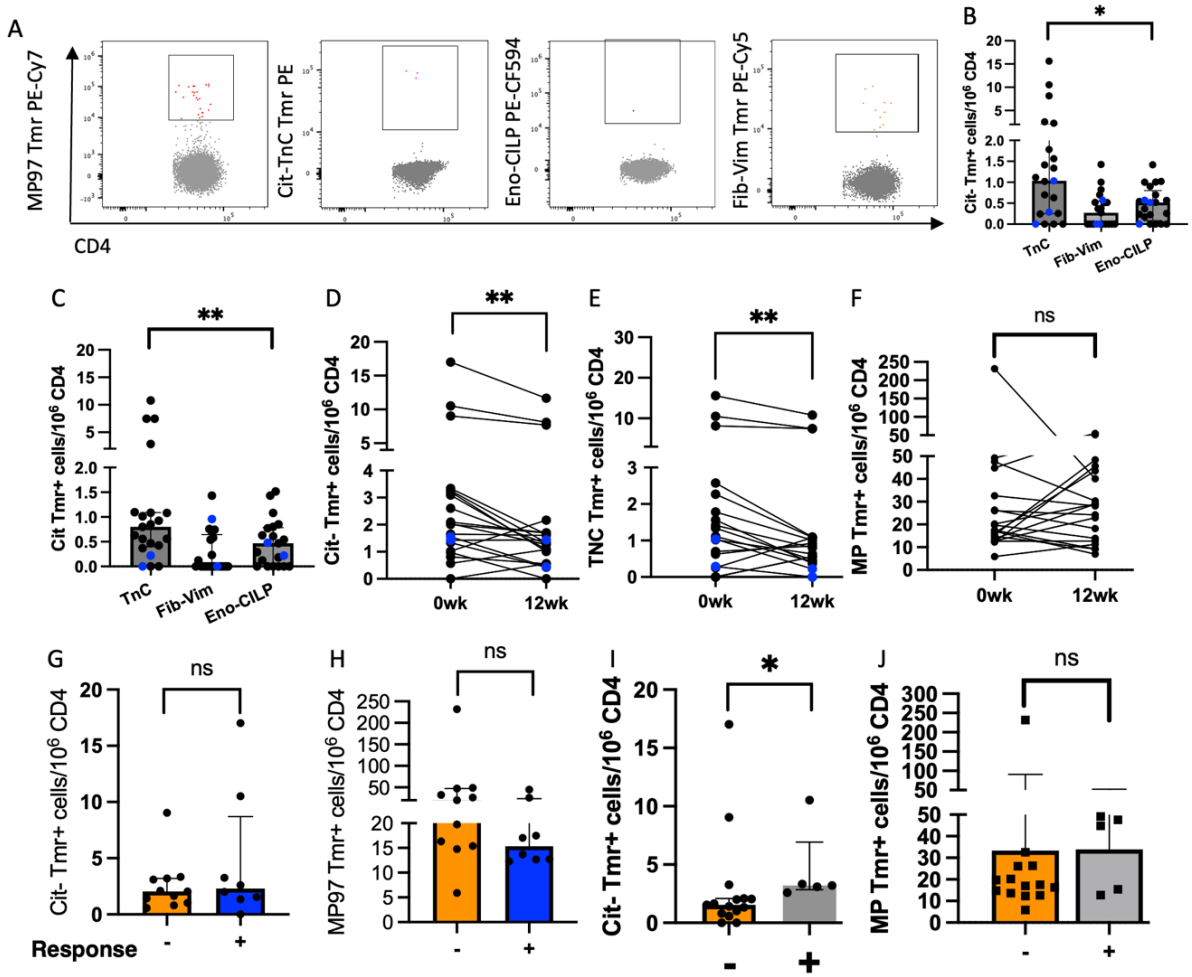
Citrulline specific T cells were gated in 3 fluorescence channels (PE, PE-CF594 and PE-Cy5), while influenza MP97 specific T cells were gated in PE-Cy7 channel (Figure 2A). Analysis of citrulline specific T cells at baseline showed significantly higher proportion of cit-Tenascin C specific T cells (2.51±4.1/106 CD4+) as compared to cit-Fibrinogen-Vimentin (0.34.51±0.41 /106 CD4+) and cit-CILP-enolase (0.48±0.42 /106 CD4+) cocktails (p = 0.01) (Figure 2B). We observed similar trend with dominance of cit-Tenascin C specific T cells at 12 weeks (p= 0.0045) acer abatacept treatment (Figure 2C). Abatacept treatment resulted in a significant decrease in proportion of citrulline specific T cells at week 12 (p = 0.002) (Figure 2D). Amongst the 3 fine-specificies of citrulline specific T cells, we only observed a significant reduction in proportion of cit-Tenascin C specific T cells upon treatment (p = 0.009) (Figure 2E). Analysis of influenza MP97 specific T cells showed no difference between baseline and 12 weeks (Figure 2F), however, there was a significant at 24 weeks compared to baseline (p= 0.018) (data not shown). To evaluate if baseline levels of citrulline specific T cells corresponded with response to treatment at week 12, subjects were defined as responders (+) and non-responders (-) based on whether they had a reduction of >1.2 in DAS28-ESR score at week 12 compared to baseline. This analysis revealed that the number of citrulline specific T cells at baseline did not significantly differ between responders and non-responders (Figure 2G). Notably, the number of influenza MP97 specific T cells did not significantly differ between responders and non-responders (Figure 2H). We also defined response to treatment at week 24 and assessed whether baseline levels in citrulline specific T cells corresponded with response to treatment. In this instance, responders had a significantly higher proportion of cit-specific T cells at baseline than non-responders (p = 0.024) (Figure 2I). Once again, no differences where observed in the influenza MP97 specific T cell population across responders and non-responders (p = 0.30) (Figure 2J).

Figure 2. Primary outcomes in context of citrulline specific T cell responses: (A) Representative flow cytogram showing an9gen-specific CD4+T cells including MP97 PE-Cy7, Cit-Tenascin C PE, Cit-Enolase CILP panel PE-CF594 and cit-Fibrinogen Vimen9n PE-Cy5 adjacent panels. (B,C) The comparison of different fine specificies of citrulline specific T cells at baseline and 12 weeks after abatacept treatment respectively. (D-F) The difference between total proportion (sum of all fine-specificies) of citrulline specific T cells, cit-Tenascin C specific T cells and influenza MP97 specific T cells respectively was tested between baseline and 12 weeks after abatacept treatment. The difference between groups was tested using Wilcoxon test. (G,H) The proportion of citrulline specific T cells and MP97 specific T cells was tested between responders and non-responders (as defined by DAS28-ESR reduction more than 1.2) using Mann-Whitney test. (I,J) The proportion of citrulline specific T cells and MP97 specific T cells was tested between responders and non-responders (as defined by DAS28-ESR reduction more than 1.2 between baseline and week 12) using Mann-Whitney test. Symbols – and + (Fig.G-J) indicate non-responders and responders respectively.


A
MP97 Tmr PE-Cy7
CD4
Cit-TnC Tmr PE
Eno-CILP PE-CF594
Fib-Vim Tmr PE-Cy5
B
Cit- Tmr+ cells/10^6 CD4
TnC
Fib-Vim
Eno-CILP
C
Cit Tmr+ cells/10^6 CD4
D
Cit- Tmr+ cells/10^6 CD4
0wk
12wk
E
TNC Tmr+ cells/10^6 CD4
F
MP Tmr+ cells/10^6 CD4
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Response
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MP97 Tmr+ cells/10^6 CD4
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Cit- Tmr+ cells/10^6 CD4
J
MP Tmr+ cells/10^6 CD4