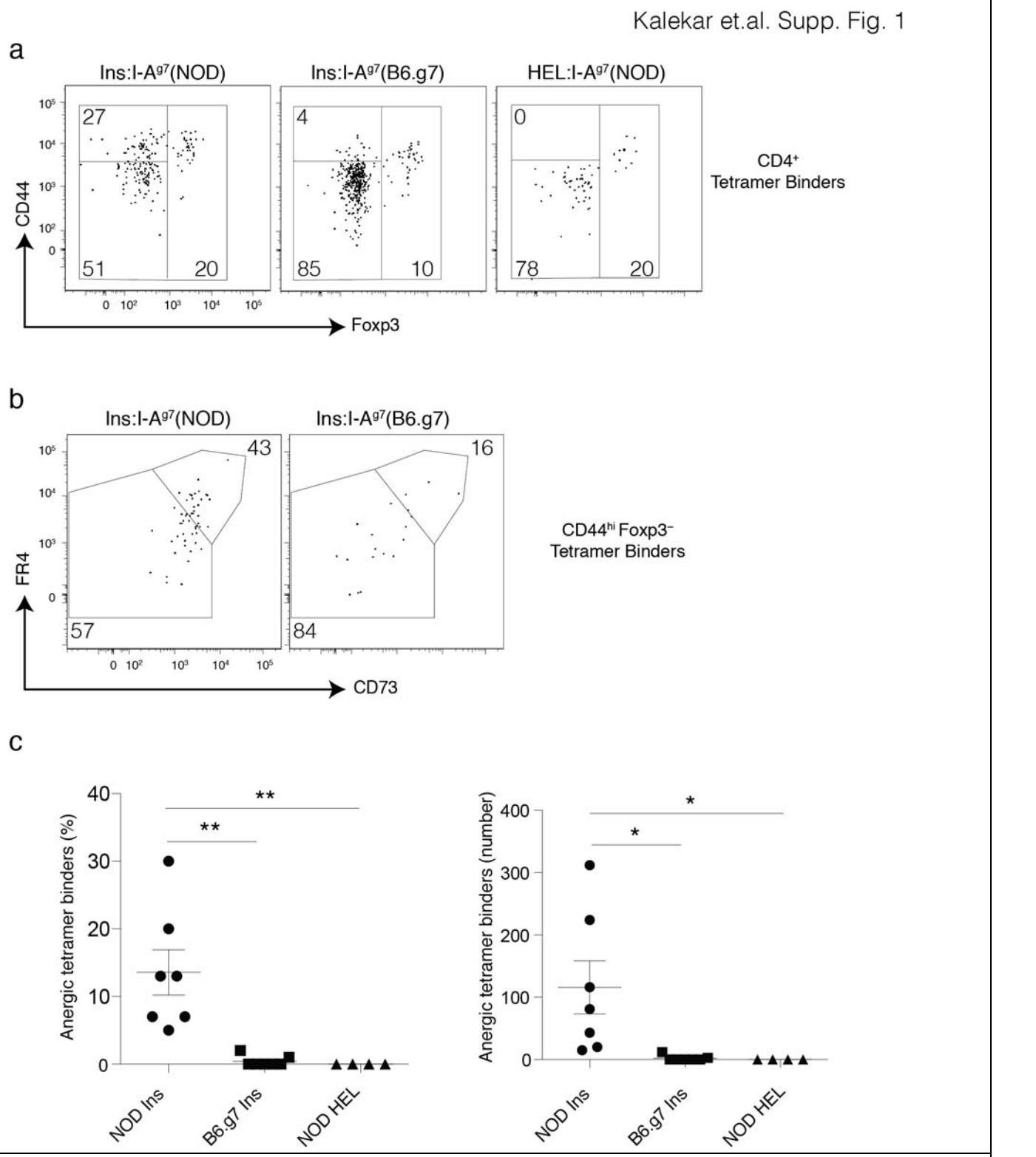
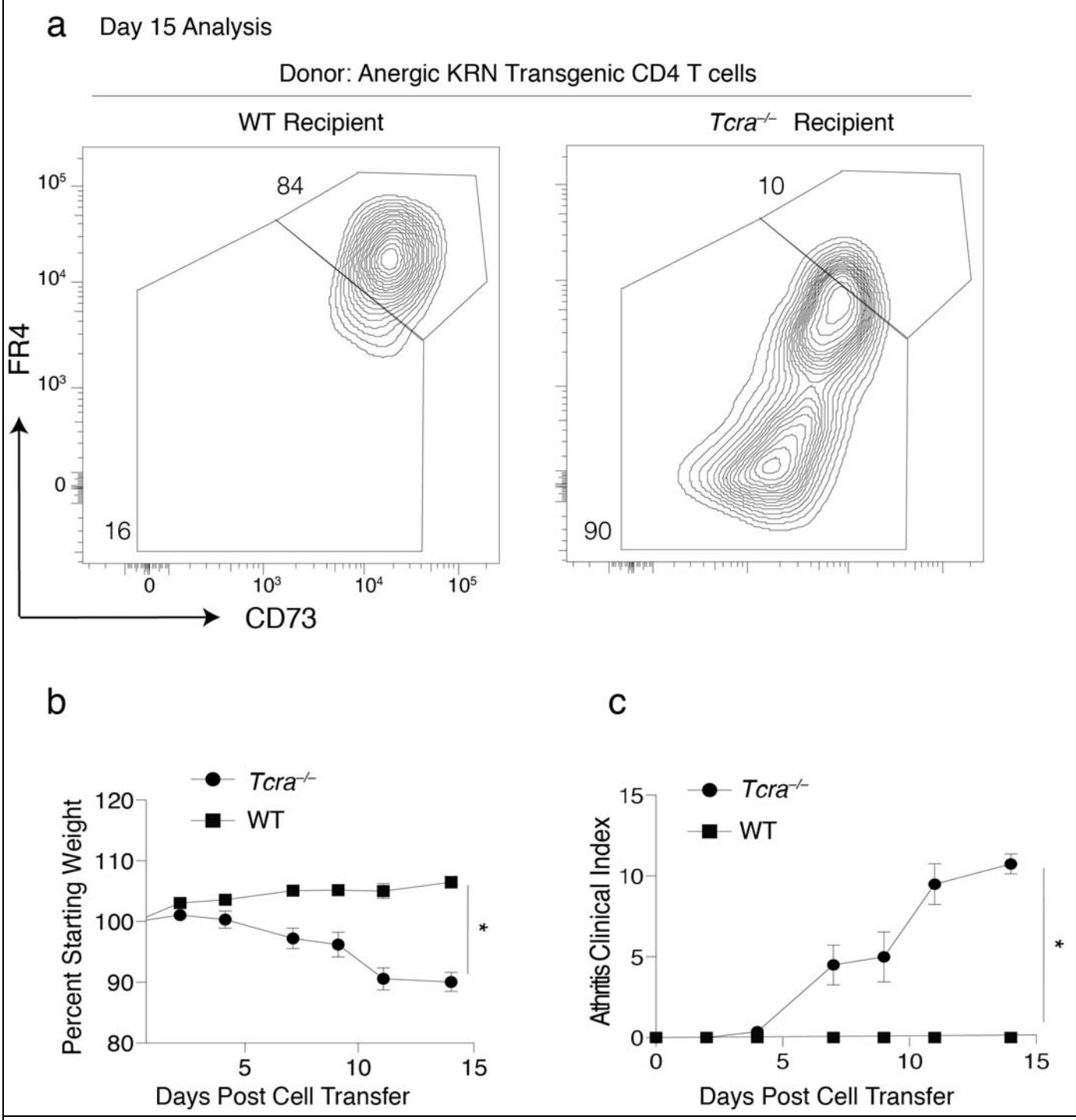


Supplementary Figure 1

Auto-reactive insulin-specific CD4 polyclonal T cells are enriched in a Foxp3^{-}CD44^{hi}CD73^{hi}FR4^{hi} anergic compartment.

(a) InsB10-23:I-A^{g7} and HEL:I-A^{g7} tetramer-binding CD4 polyclonal T cells (pulled-down and detected as previously reported) in NOD and B6.g7 mice were stained for Foxp3 and CD44. (b) CD73 and FR4 expression on the $Foxp3^{-}CD44^{hi}$ tetramer-binding $CD4^{+}$ polyclonal T cells. (c) Percent and number of $Foxp3^{-}CD44^{hi}CD73^{hi}FR4^{hi}$ anergic CD4 polyclonal T cells for each tetramer-binding specificity. Mean data shown are representative of 2 independent experiment, n = 5 to 7 animals per experiment. Error bars represent the SEM. Unpaired student's t-test (c); * $p < 0.05$, ** $p < 0.01$. Points denote individual mice.

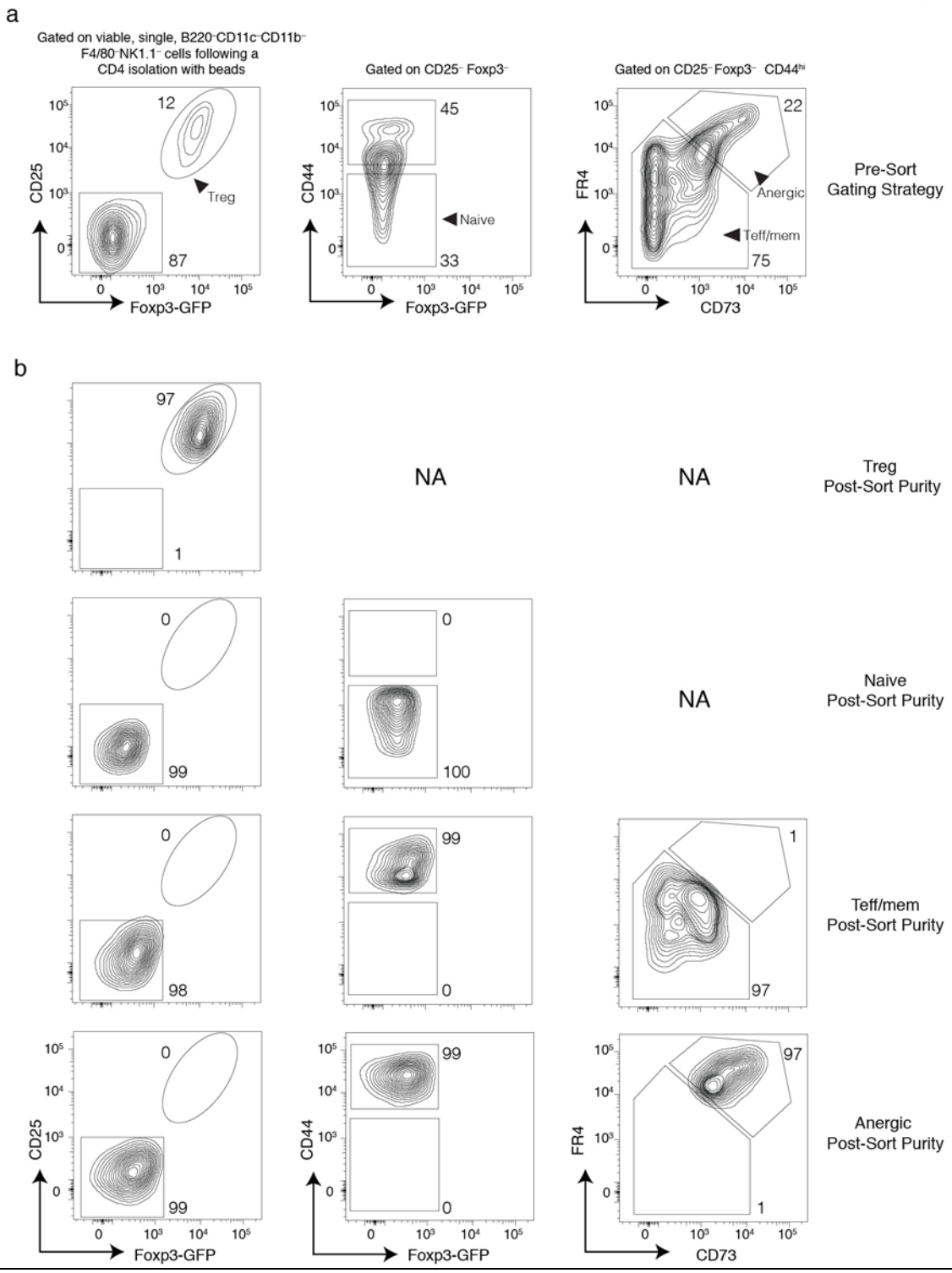


Supplementary Figure 2

KRN CD4 T cells cause arthritis in lymphopenic *Tcra*$^{-/-}$ mice following anergy reversal

CD73^{hi}FR4^{hi} KRN transgenic CD4^{+} T cells made anergic by adoptive transfer into WT B6G7F1 hosts for 6 days were subsequently recovered by flow cytometric cell sorting and transferred (10^4) into either WT or *Tcra*$^{-/-}$ B6G7F1 hosts. (**a**) CD73 and FR4 expression on donor KRN cells recovered from the WT and *Tcra*$^{-/-}$ hosts 15 days later. (**b**) Percent change in the body weight of WT and *Tcra*$^{-/-}$ mice

receiving anergic KRN T cells. (**c**) Arthritis Clinical Index score for WT and *Tcra*$^{-/-}$ mice receiving anergic KRN T cells. Data shown are representative of 2 independent experiments, n = 2 to 3 animals per experiment. Error bars represent the SEM. Unpaired student's t-test (b) or Mann-Whitney U-test (c) at day 15. * $p < 0.0001$

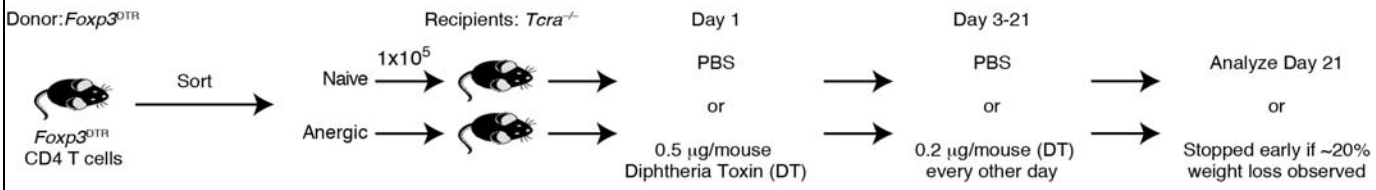


Supplementary Figure 3

Gating strategy and post-sort purity

(**a**) Polyclonal $CD4^+$ T cells from *Foxp3*^{DTR} mice were first isolated by MACS CD4 negative selection, and then the naive, Teff/mem, anergic and T_{reg} cell subsets were physically sorted by flow cytometry using the gating strategy shown. Arrowheads indicate the

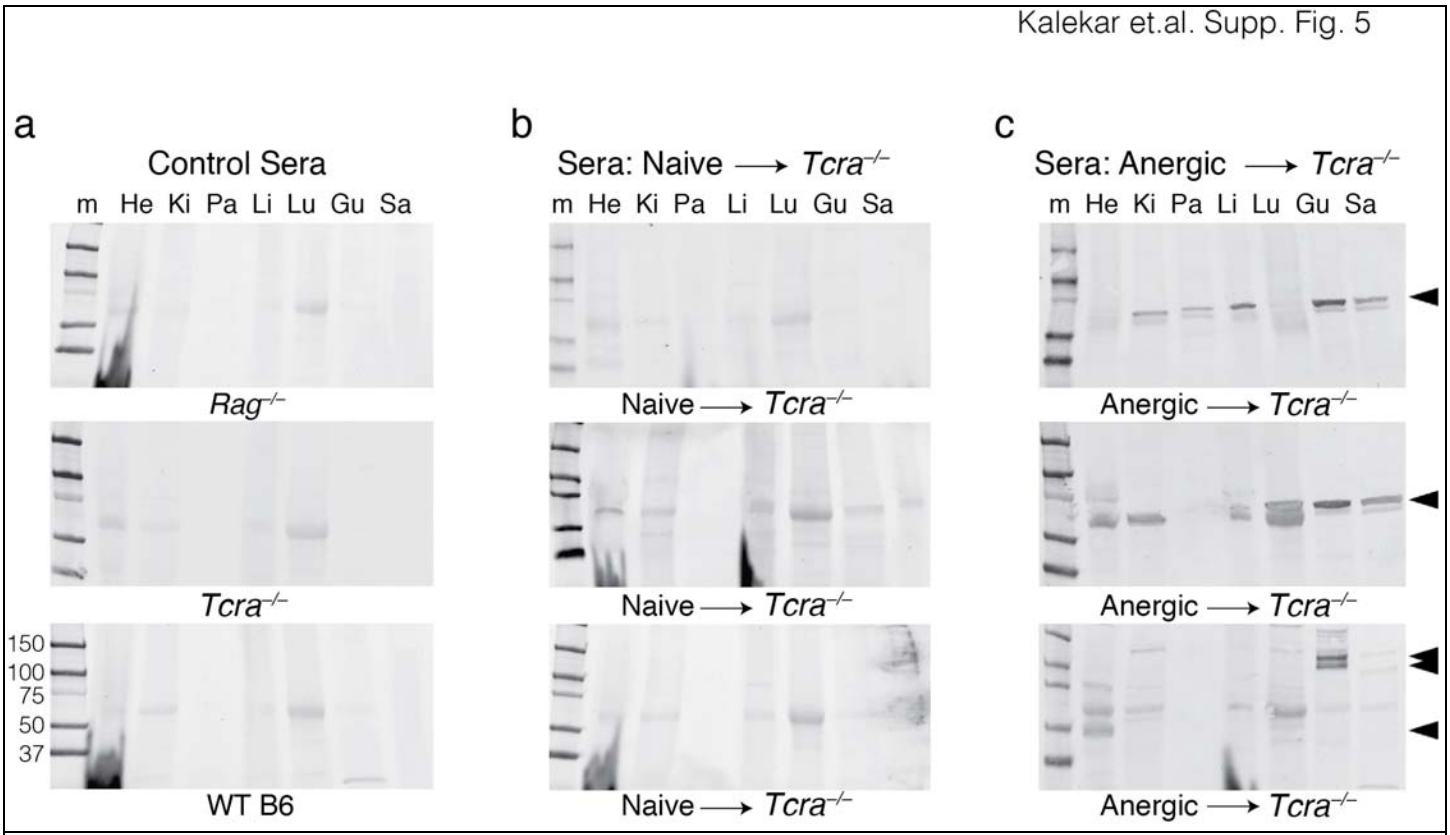
subpopulations collected. (**b**) Post-sort purity for naive, Teff/mem, anergic and T_{reg} cells.



Supplementary Figure 4

Experimental design for figure 5.

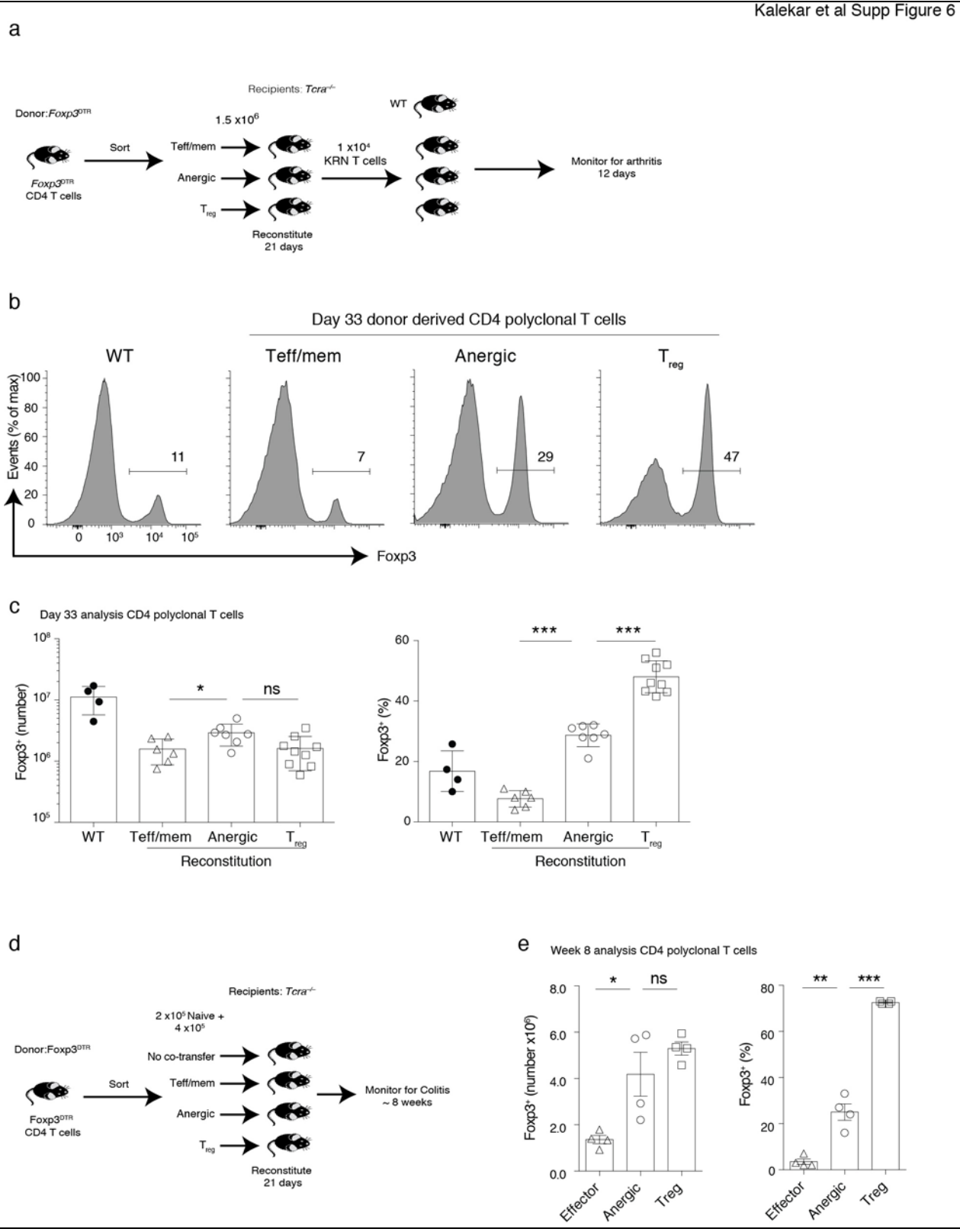
Sorted naive or anergic syngeneic *Foxp3*^{DTR} polyclonal CD4^{+} T cells were transferred (10^5) into syngeneic lymphopenic *Tcra*$^{-/-}$ hosts and treated with either PBS or diphtheria toxin (DT), as indicated. Mice were monitored for weight loss and the experiment stopped if ~20% weight loss was observed.



Supplementary Figure 5

Reversal of anergy in CD4 polyclonal T cells results in autoantibody production when newly generated T_{reg} cells are ablated.

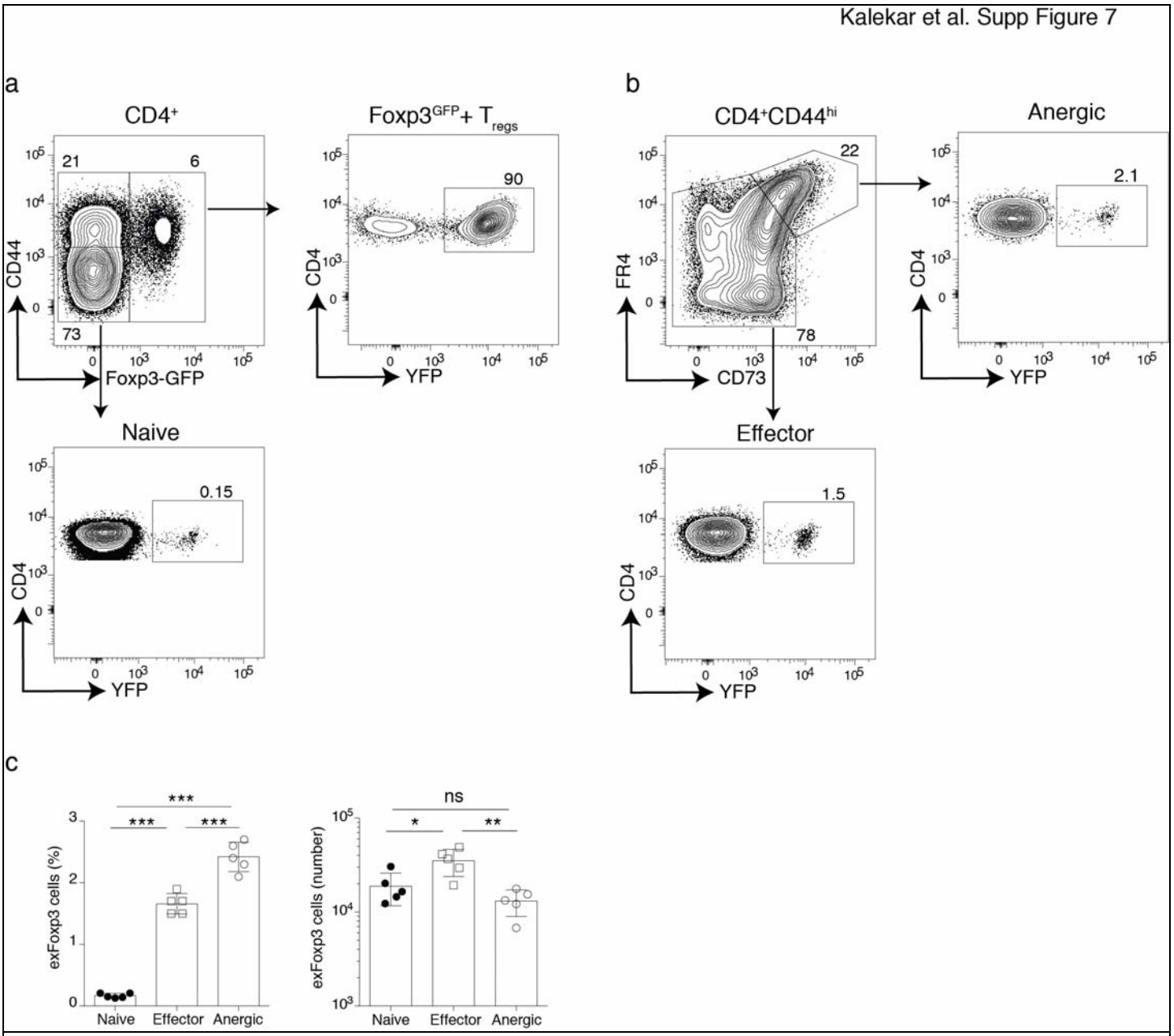
Lymphopenic *Tcra*$^{-/-}$ mice were given an adoptive transfer (10^5) of either naive or anergic syngeneic *Foxp3*^{DTR} polyclonal CD4$^+$ T cells, followed by every other day treatment with diphtheria toxin (DT). Sera were recovered from mice 21 days later and used to probe various tissue extracts (as indicted). (**a**) Sera taken from *Rag*$^{-/-}$ (top), *Tcra*$^{-/-}$ (middle), and WT B6 (bottom) mice were assayed as a negative control for autoantibody generation. (**b**) Sera obtained from three separate adoptive transfer recipients of naive T cells or (**c**) anergic T cells. Arrowheads indicate self antigen-binding by serum antibodies that are uniquely present with recipients of anergic CD4$^+$ T cells. m = marker, He = heart, Ki = kidney, Pa = pancreas, Li = liver, Lu = lung, Gu = gut, Sa = salivary gland. Summary data are shown in figure 5e. Note that an irrelevant 60 Kd background band (most prominent in lung extracts) was demonstrated in all blots even in the absence of serum antibody (not shown), and this band is disregarded.



Supplementary Figure 6

Number and percentage of T_{reg} cells recovered in models of arthritis and colitis from polyclonal anergic cells

(**a-c**) Experimental design for the KRN model of arthritis (**a**). The number and percentage of T_{reg} cells recovered on day 33 of reconstitution (b-c). 3 independent experiments. 1-3 mice per group. (**d-e**) Experimental design for colitis experiment (**d**). Percentage and number of T_{reg} cells recovered on week 8 (**e**). 2 independent experiments. 2 mice per group. Mean data shown. Error bars represent the SEM. One-Way ANOVA (c); * $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$, ns (non-significant). Points denote individual mice.



Supplementary Figure 7

exFoxp3 cells make up a very small fraction of anergic cells

(**a**) CD4$^+$ T cells from the spleen and all lymph nodes of Foxp3-Cre-GFP x R26-YFP were gated on CD44 and *Foxp3*^{GFP}, then the naive and T_{reg} cells are gated on YFP. (**b**) Foxp3$^-$CD44^{hi} anergic and Teff/mem cells were analyzed for exFoxp3 cells by YFP expression. (**c**) Percent and number of exFoxp3 cells in naive, Teff/mem and anergic cells is shown. Mean data shown. Error bars represent the SEM. One-Way ANOVA (c); * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$, ns (non-significant). Points denote individual mice.