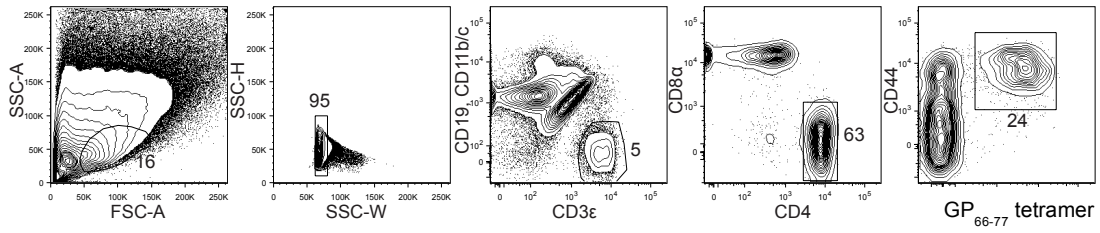Supplementary Figure 1. Identification of antigen-specific donor cells in WT LDA.
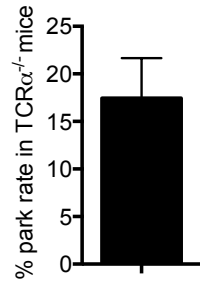
(A) Gating strategy for Thy1.2 CD4 T cell transfer into WT (Thy1.1) mice following tetramer+ LDA. (B) Gating strategy for Thy1.2 CD4 T cells into Thy1.1 mice for LDA low-affinity CD4 T cell calculations.

Supplementary Figure 2. Identification of antigen-specific CD4+ T cells by tetramer.

Supplementary Figure 3. Park rate of CD4 T cells 24 hours after IV transfer into TCRα$^{-/-}$ mice as a percent of input (n=4 mice, two experiments, data shown as mean +/- SEM).
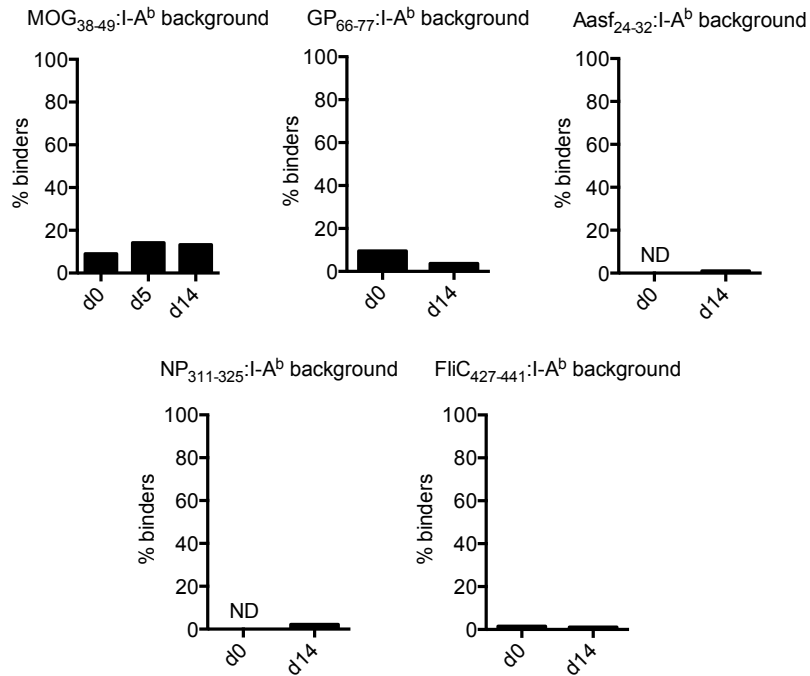
Supplementary Figure 4. MP background calculation (data shown as mean frequency of binders for all points, 2 mice per timepoint).

Target	Fluorochrome	Clone	Host	Isotype	Manufacturer	Cat#	Reference
CD3e	BV 605	145-2C11	Hamster	IgG1,κ	Biolegend	100351	1
CD4	BV 510	RM4-5	Rat	IgG$_{2a}$, κ	Biolegend	100553	2
CD8α	V450	53-6.7	Rat	IgG$_{2a}$, κ	Tonbo Biosciences	75-0081-U100	3
CD11b	PerCp/APC Cy7	M1/70	Rat	IgG$_{2b}$, κ	BD Pharmigen	561114 / 561039	4
CD11c	PerCp/ APC-Cy7	HL3	Hamster	IgG1, $\lambda2$	BD Pharmigen	560584 / 561241	5
CD19	PerCp	1D3	Rat	IgG$_{2a}$, κ	BD Pharmigen	551001	6
CD44	AF700	IM7	Rat	IgG$_{2b}$, κ	eBioscience	56-0441-82	7
CD69	BV605	H1.2F3	Hamster	IgG1, $\lambda3$	BioLegend	104529	8
F4/80	APC-Cy7	BM8	Rat	IgG$_{2a}$, κ	eBioscience	47-4801	9
Nur77	PE	12.14	Mouse	IgG1	eBioscience	12-5965	10
TCRβ	PE	H57-597	Armenian Hamster	IgG	eBioscience	12-5961	11
B220	APC-efluor780	RA3-6B2	Rat	IgG$_{2a}$, κ	eBioscience	47-0452	12
Thy1.1	PerCp	HIS51	Mouse	IgG$_{2a}$, κ	eBioscience	45-0900	13
Thy1.2	APC	53-2.1	Rat LOU	IgG$_{2a}$, κ	BD Pharmigen	561974	14
I-A/I-E	PE	M5/114.15.2	Rat BNxLEW	IgG$_{2b}$, κ	BD Pharmigen	557000	15
Bcl-6	PE-CF594	K112-91	Mouse	IgG$_1$, κ	BD Horizon	562401	16

Supplementary Table 1. Antibodies used in experiments.

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