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Supplementary Materials for

Antibody blockade of IL-15 signaling has the potential to durably reverse vitiligo

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Other Supplementary Material for this manuscript includes the following:

(available at www.sciencetranslationalmedicine.org/cgi/content/full/10/450/eaam7710/DC1)

Table S3 (Microsoft Excel format). Primary data.

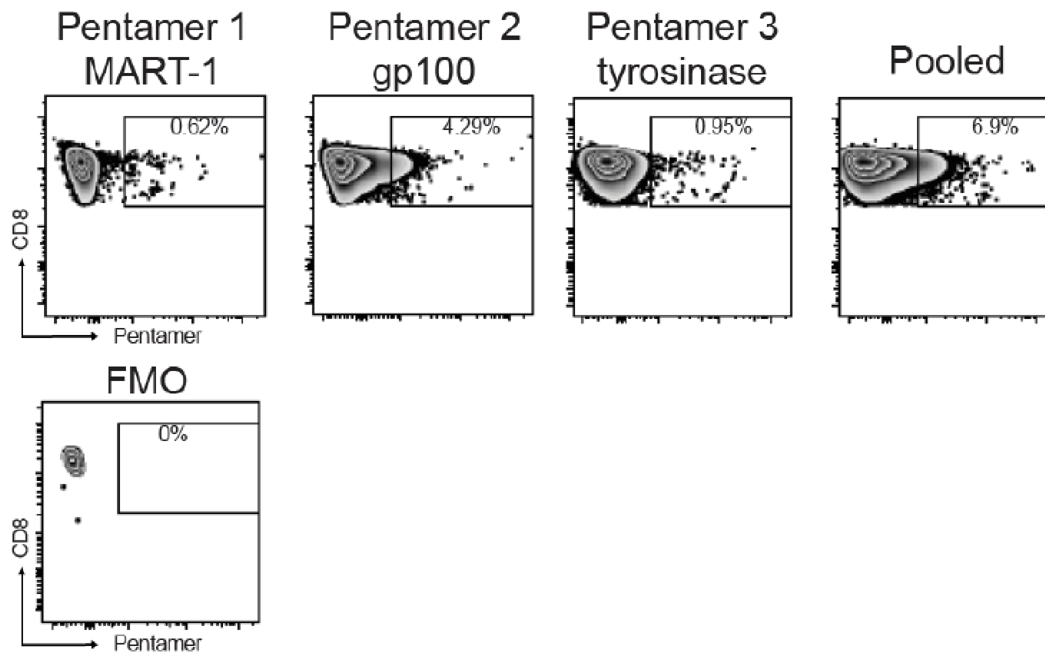

Fig. S1. Analysis of single versus pooled pentamer stains. PBMCs or blister fluid were stained with MART-1, gp100, or tyrosinase pentamers, and frequencies of antigen-specific cells were compared to those with staining with the same pentamers pooled together. The frequencies of the live, single, $CD8^{+}CD3^{+}pentamer^{+}$ T cells were summative (representative patient staining of 2 patients shown.)

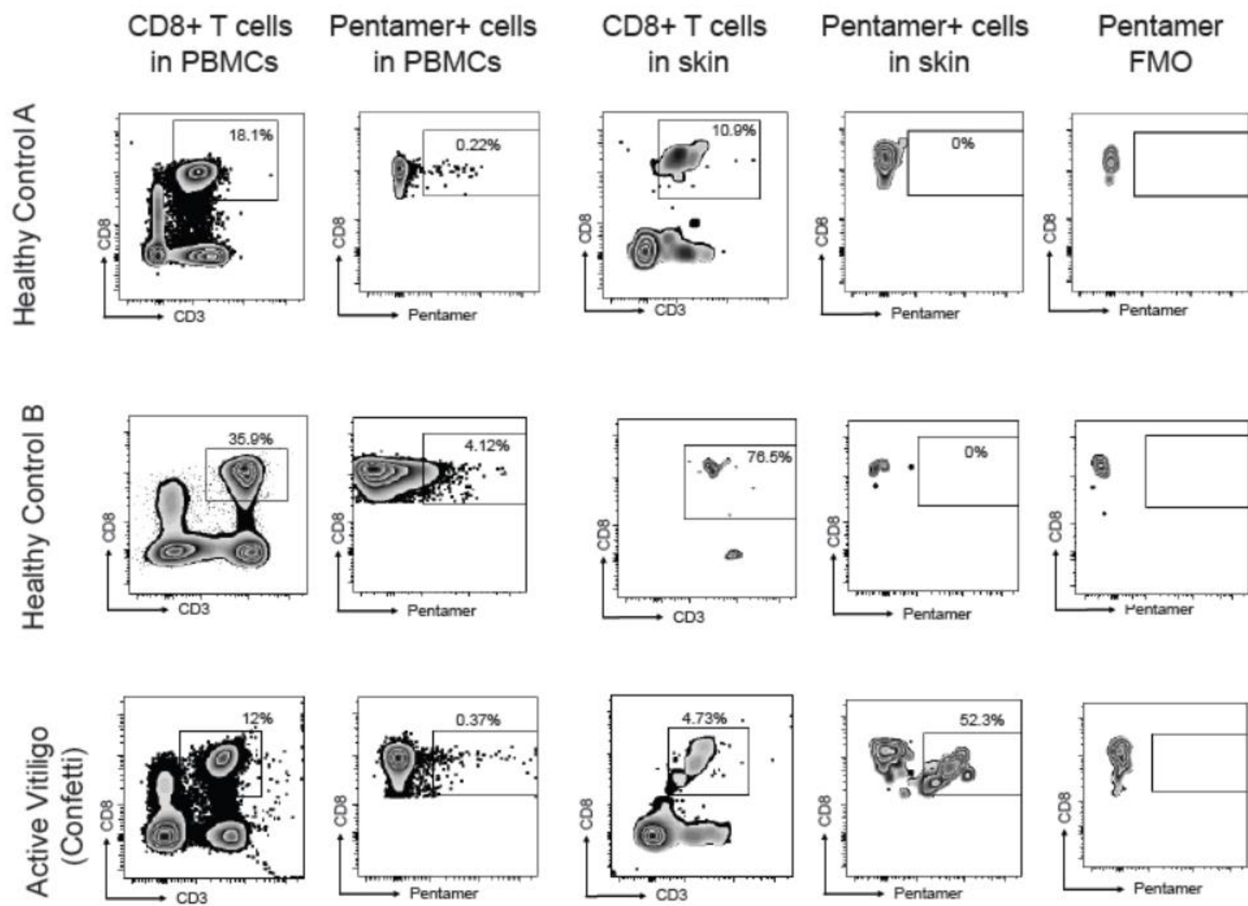

Fig. S2. Analysis of melanocyte-specific cells in blister biopsies from healthy controls. Melanocyte-specific T cells were analyzed using pooled HLA-A2*0201 pentamers in 2 healthy controls and compared to an active vitiligo patient. All cells were pregated on live, single, $CD45^{+}$ prior to $CD3^{+}CD8^{+}$ as shown in the first and third columns, followed by pentamer staining in the second and fourth columns. Gates were drawn using the pentamer FMO as shown in the last column.

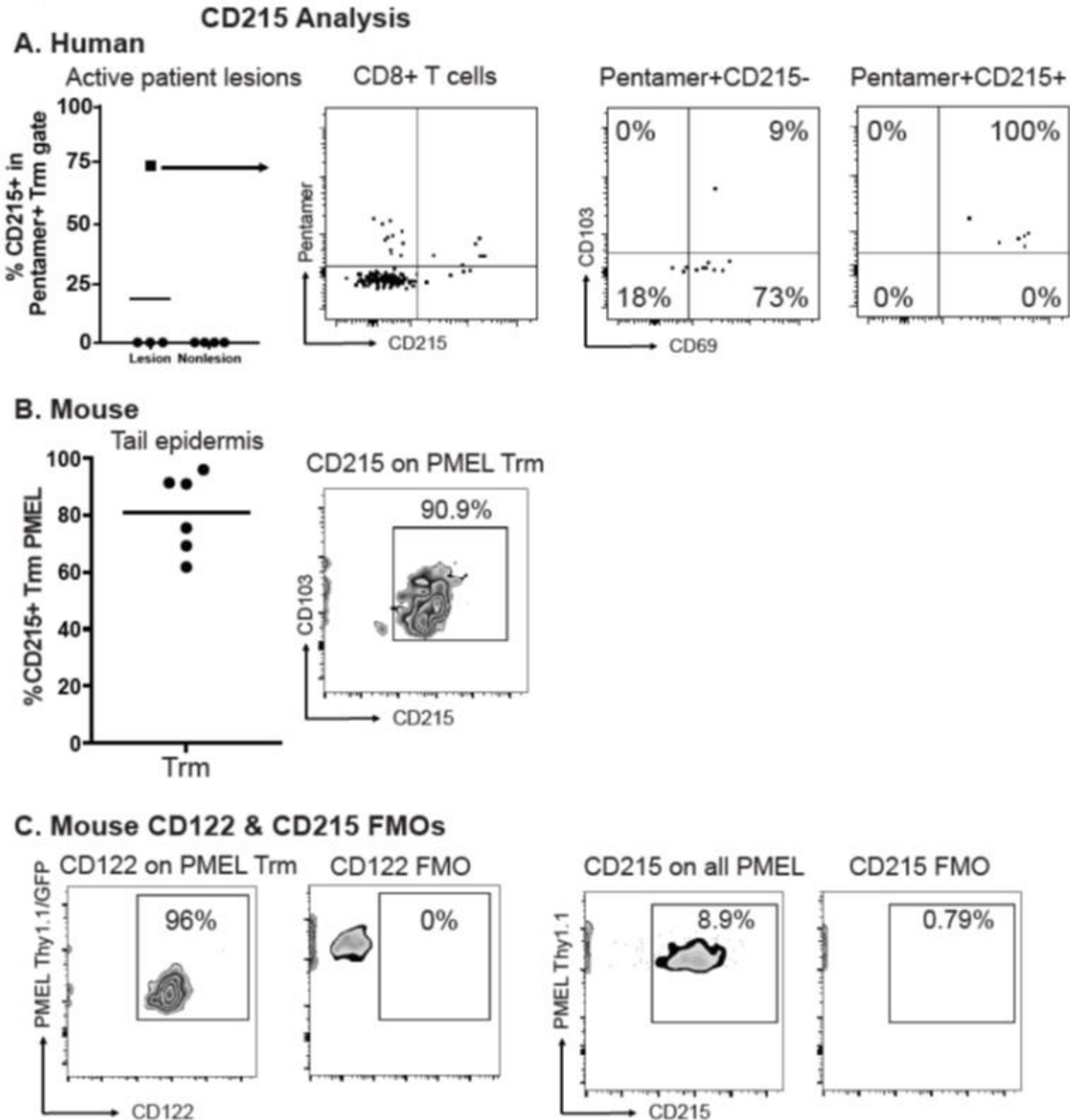

Fig. S3. CD215 staining on human and mouse melanocyte-specific T cells. (**A**) CD215 staining on melanocyte-specific Trm in vitiligo patient blister fluid. Only one patient was positive for surface CD215 staining, as shown in the sample flow plots. In this patient, 100% of CD215$^+$ melanocyte-specific T cells were CD69$^+$CD103$^+$, whereas CD215$^-$ melanocyte-specific T cells were CD69$^+$ only. All other patients' cells were CD215$^-$. (**B**) Quantification and sample flow cytometry staining of CD215 on PMEL in vitiligo mouse epidermis at week 8 (n=6 animals pooled from 2 separate experiments). (**C**) Representative flow plots for CD122 & CD215 gating based on FMOs in mice. Cells were pregated on live single CD45$^+$CD8$^+$Thy1.1 and/or GFP$^+$ PMELs or PMEL Trms as indicated prior to assessing CD122 (left panels) or CD215 (right panels).

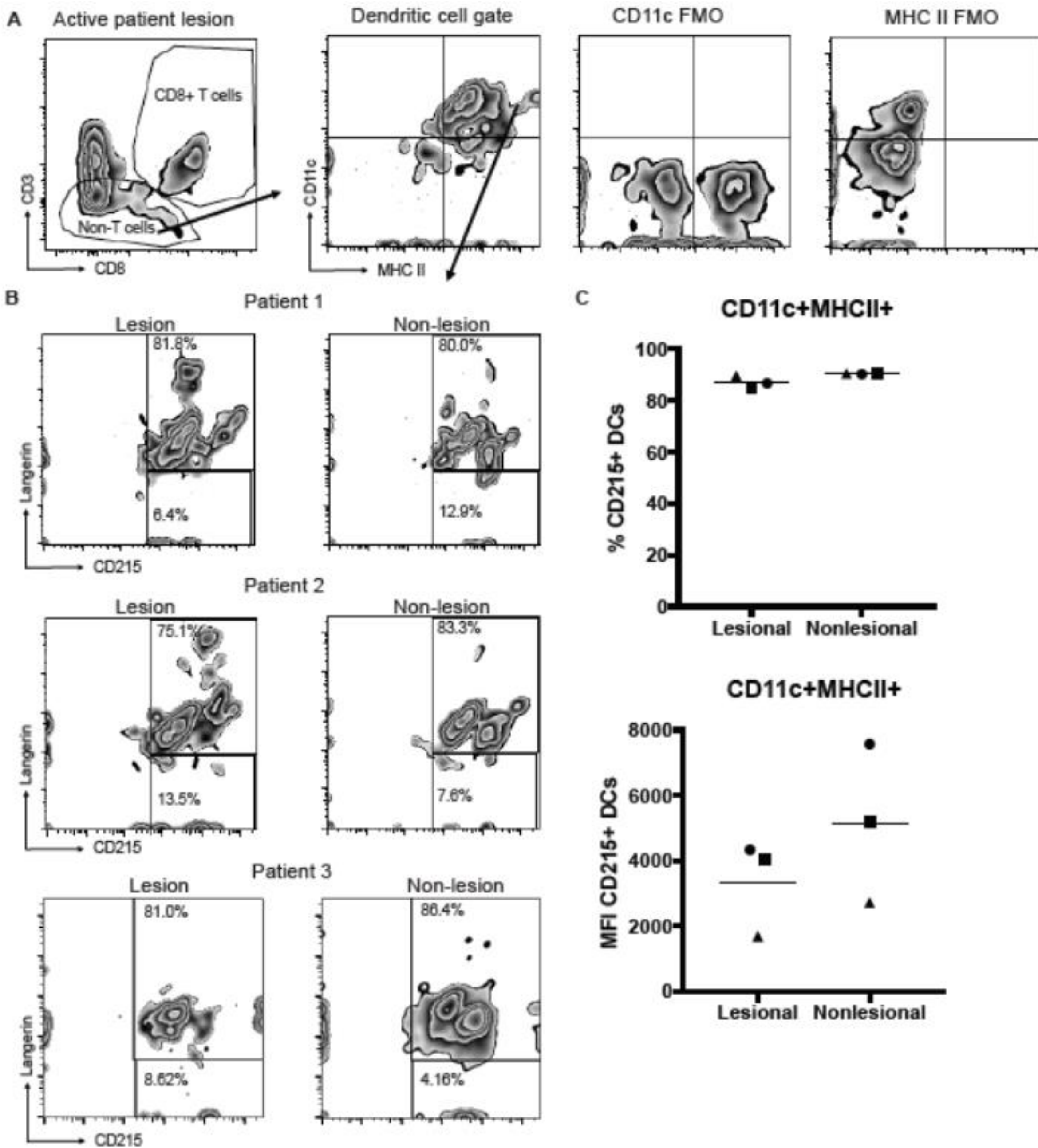

Fig. S4. Examining CD215 expression on dendritic cells in vitiligo patient blister fluid. (A) Gating strategy for analyzing dendritic cells. (**B**) Flow cytometry staining of CD215 on blister fluid dendritic cells in 3 vitiligo patients pregated on live single $CD45^+CD11c^+MHC\text{-}II^+$. There were no significant differences in lesional versus nonlesional CD215 expression. (Each symbol represents one patient).

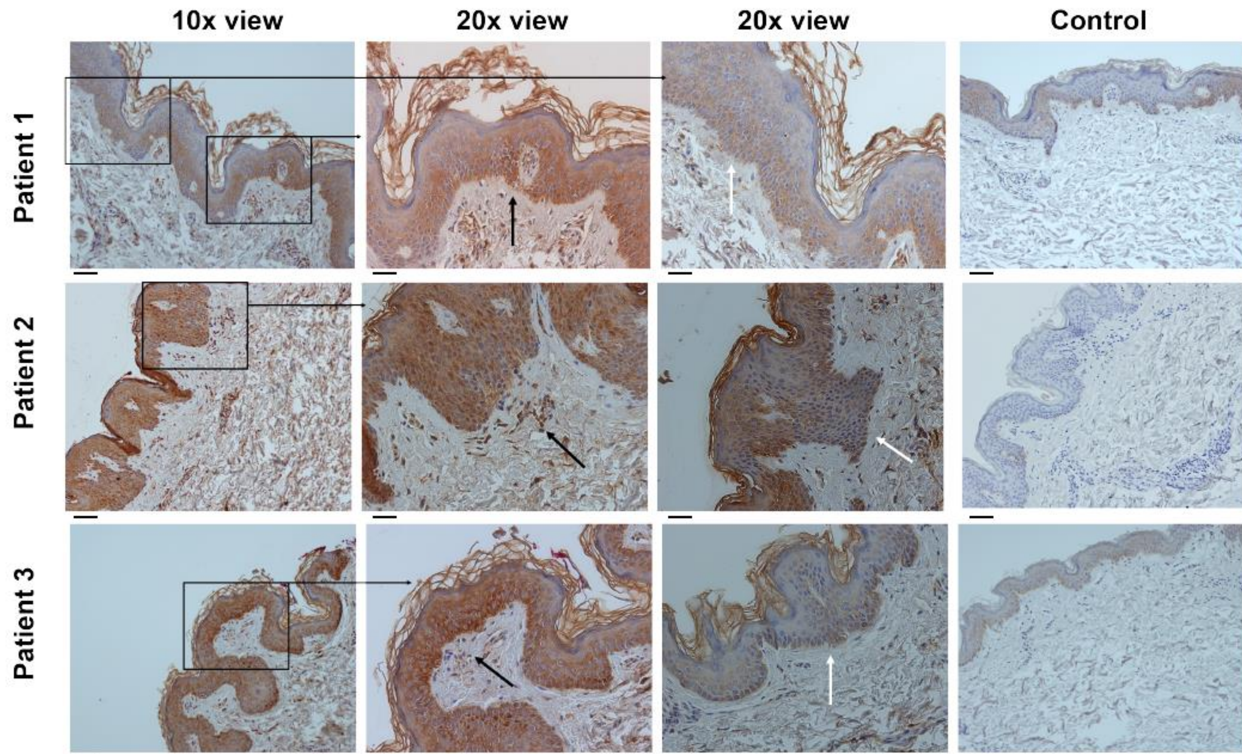

Fig. S5. Immunohistochemistry of CD215 in vitiligo skin biopsies. Ellipse biopsies with one end in depigmented skin and one end in pigmented skin were taken from three active confetti vitiligo patients and stained for CD215. Areas with mononuclear infiltrates characteristic of interface dermatitis exhibited darker CD215 staining in keratinocytes (black arrows) as compared to areas without infiltrates (white arrows). Scale bars = 100µm in 10x images and 50µm in 20x images.

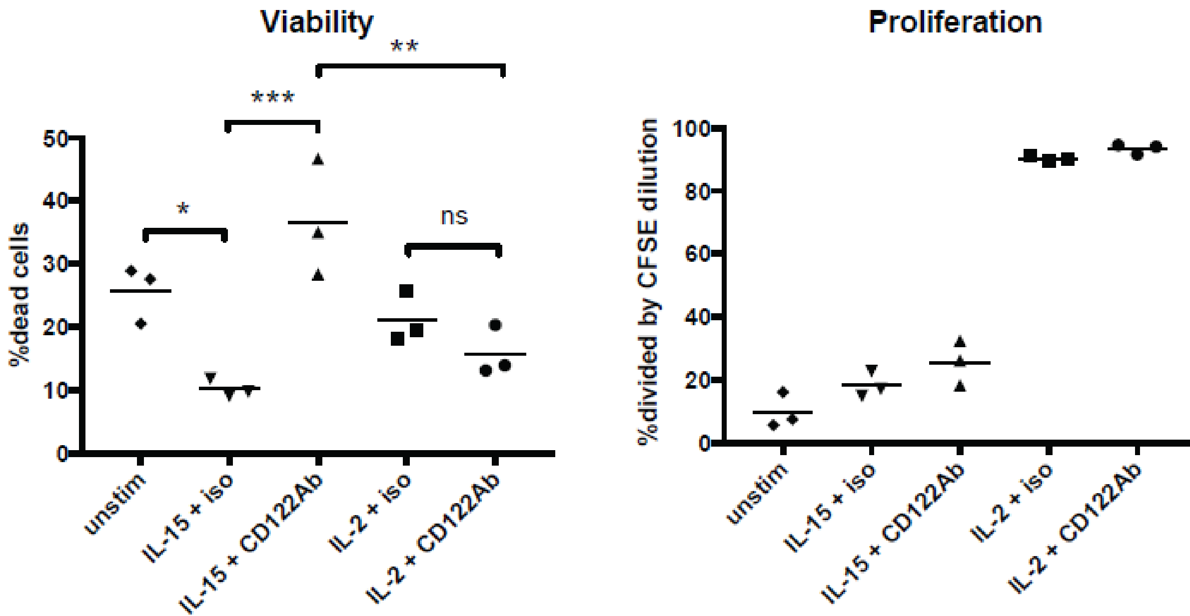

Fig. S6 CD122 antibody blocks IL-15 activity, but not IL-2 activity, in mouse T cell bioassays. PMEL T cells were isolated from donor mice, stimulated *in vitro* with anti-CD3/CD28 for 1 week, then labeled with CFSE and cultured in the conditions as indicated for an additional week (50ng/mL cytokine, with 100µg/mL antibody). (**A**) Cell death was assessed via flow cytometry, and CD122 antibody treatment significantly increased cell death as compared to isotype control in IL-15 treated wells. There was no significant difference in the amount of cell death in CD122 antibody treated versus isotype control in IL-2 treated wells. IL-15 treatment significantly reduced the number of dead cells compared to unstimulated controls, whereas IL-2 treatment did not. (**B**) Cell proliferation was assessed via flow cytometry analysis of CFSE dilution, and CD122 antibody treatment was not able to block IL-2 mediated proliferation. IL-15 did not induce a significant amount of PMEL proliferation, and antibody treatment did not have a significant effect as compared to unstimulated or isotype controls. (Each symbol represents 1 replicate; one representative experiment of two shown. One-way ANOVA with Tukey's post-tests, *p=0.0321, ***p=0.0008, **p=0.0048).

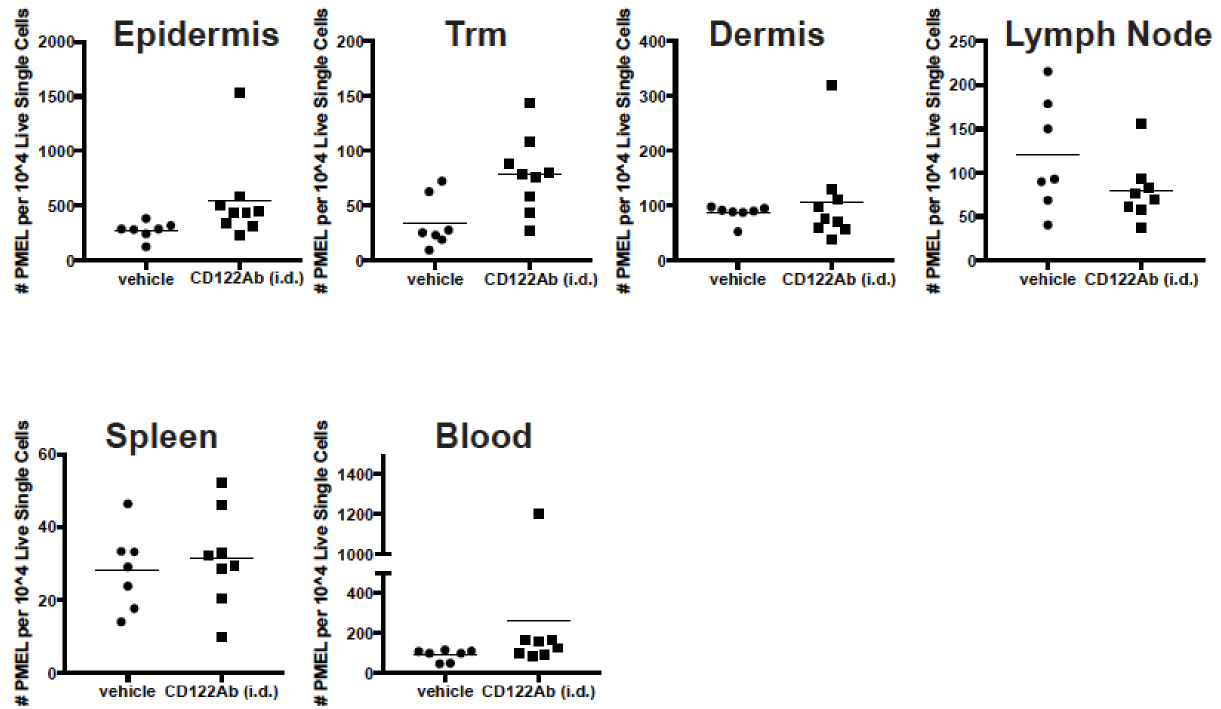

Fig. S7. Analysis of PMEL T cell populations in mice treated with intradermal CD122 antibody. Quantification of PMEL numbers in treated animals within the indicated tissues. (Each symbol represents 1 animal; pooled from 2 separate experiments; all t tests ns)

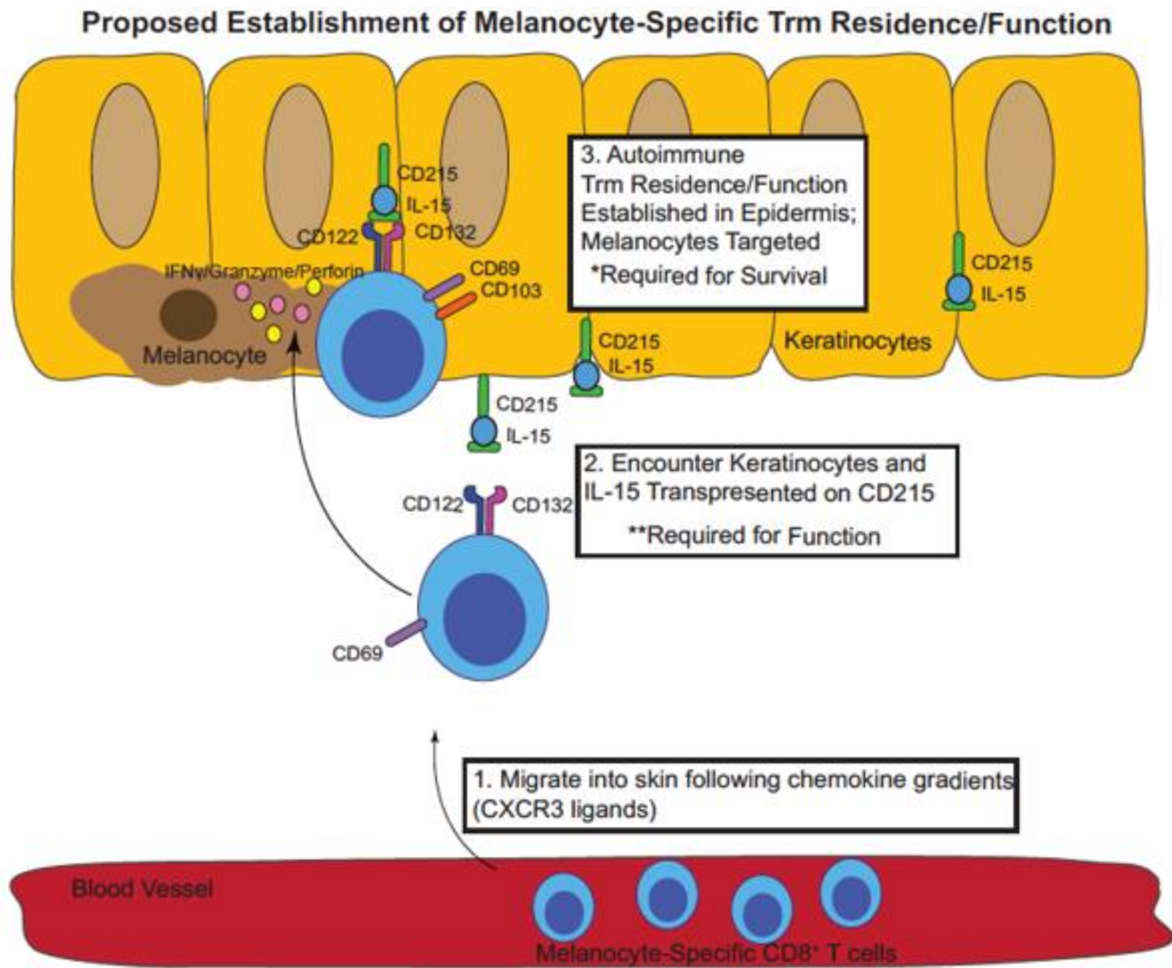

Fig. S8. Proposed model for CD122 and IL-15/CD215 biology in vitiligo. Melanocyte-specific $CD8^+$ T cells migrate into the skin following CXCR3 ligand gradients. They encounter keratinocytes presenting IL-15 on CD215 and establish residence in the epidermis and functional capacity via IFNγ/Granzyme/Perforin production.

Table S1. Characteristics of patients used in this study.

Subject	Group, Disease status	Age	Sex	Race/Ethnicity	Related Family History	Other Auto-immune diseases	Duration of Disease in years	HLA-A2*0201 Status	Pentamers used	Treatment status
1	Stable	39	M	White	None	None	15+	Positive	1	Untreated
2	Stable	47	F	White	None	None	30+	Positive	1	Untreated
3	Stable	31	F	White	None	None	25	Positive	1	Untreated
4	Active Non-Confetti & Stable Lesions	33	M	White	None	None	7	Positive	1	Untreated
5	Stable Lesional	58	M	Black	None	None	34+	Positive	1	Untreated
6	Confetti	47	F	White	None	Type I Diabetes	11	Positive	1, 2, 3	Untreated
7	Confetti	57	M	White	None	None	4+	Negative*	N/A	Untreated
8	Confetti	70	M	Indian	Vitiligo	None	1+	Positive	1, 2, 3	Untreated
9	Confetti	34	F	White	None	Hashimoto's Thyroiditis	12	Positive	1, 2, 3	1x Treatment 1 month prior to study, Tacrolimus
10	Confetti	25	F	White	None	None	9	Positive	3	Untreated
11	Confetti	52	F	Hispanic	Type 1 Diabetes	None	32+	Positive	1	Untreated
12	Confetti	45	F	Asian	Type 1 Diabetes, Thyroid Disease	None	38+	Positive	1, 2, 3	Untreated
13	Confetti	58	F	White	Vitiligo	None	30	Unknown*	N/A	Untreated
14	Inflammatory	18	F	White	Vitiligo, Type 1 Diabetes	None	7	Unknown*	N/A	Alternating protopic and clobetasol topical creams, stopped 2 months prior to study
15	Confetti	52	M	Asian	Vitiligo, Psoriasis	None	10	Unknown*	N/A	Untreated
16	Confetti, trichrome	52	F	Black	None	Hypothyroidism	17	Unknown**	N/A	Untreated
17	Confetti	34	F	White/Hispanic	None	None	21	Unknown**	N/A	Untreated
18	Confetti, trichrome	44	F	White	None	Hypothyroidism	17	Unknown**	N/A	Untreated
C1	Healthy Control	25	F	White	None	None	N/A	Positive	1	N/A
C2	Healthy Control	26	M	White	Vitiligo	None	N/A	Positive	1, 2, 3	N/A

*used for CD215 staining in blister roof

**used for immunohistochemistry of CD215

Table S2. PMEL analysis in CD122 antibody systemic durability studies.

CD122 antibody lot/experiment	PMEL population	Average # normalized to 10^4 live singlets ± SEM	P value summary (t test)	Significant depletion?
Lot A – experiment 1	Trm	CD122 Ab: 116.5±34.29 Veh: 172.5+55.98	0.4078	No
	Tcm spleen	CD122 Ab: 7.53±1.686 Veh: 34.31+10.31	*0.0293	Yes
	Tcm lymph node	CD122 Ab: 21.39±5.696 Veh: 79.83+19.99	*0.0229	Yes
Lot A – experiment 2	Trm	CD122 Ab: 64.73±14.8 Veh: 174+44.09	*0.0468	Yes
	Tcm spleen	CD122 Ab: 10.93±2.692 Veh: 10.35+4.019	0.9076	No
	Tcm lymph node	CD122 Ab: 15.89±6.939 Veh: 12.59+2.787	0.6702	No
Summary lot A experiments 1 & 2	Trm	CD122 Ab: 87.74±18.43 Veh: 173.4+32.1	*0.0309	Yes
	Tcm spleen	CD122 Ab: 9.419±1.686 Veh: 19.34+6.032	0.1158	No
	Tcm lymph node	CD122 Ab: 18.34±4.44 Veh: 37.8+14.03	0.1846	No
Lot B – experiment 3	Trm	CD122 Ab: 219.1±106.5 Veh: 54.19+21.81	0.1677	No
	Tcm spleen	CD122 Ab: 20.87±5.688 Veh: 30.88+6.873	0.3046	No
	Tcm lymph node	CD122 Ab: 31.16±5.736 Veh: 43.89+9.955	0.3101	No
Summary all 3 experiments	Trm	CD122 Ab: 134.7±41.05 Veh: 127.6+26.67	0.8878	No
	Tcm spleen	CD122 Ab: 12.94±2.475 Veh: 23.18+4.735	0.0621	No
	Tcm lymph node	CD122 Ab: 22.28±3.814 Veh: 39.83+9.66	0.0951	No

Table S3. Primary data. Please see attached Excel file.