Inclusion	Exclusion
Diagnosis of Metastatic Melanoma (clinically or radiographically)	Special classes of patients: fetuses, pregnant women, prisoners, children, institutionalized individuals and other vulnerable individuals
18 years or older	Patients taking steroids
Able to consent and consented	Nursing and pregnant patients
BRAF mutation status at position 600 known, and if V600E present, have refused or failed treatment with BRAF inhibitor	Patients with prior malignancies except if in situ cervical cancer, adequately treated basal cell or squamous cell cancer, adequately treated Stage I or II cancer from which the patient is in complete remission or any other cancer from which the patient has been disease free for at least five years
If received anti-CTLA-4 therapy, must wait at least 3 months from last dose prior to enrollment	Patients with a history of metastatic melanoma involving the brain if they had active disease within the prior six months that was not controlled with surgery or radiotherapy
Melanoma must be positive for tyrosinase and HLA-A2 per pathologic review (Supplemental Figure 1)	Patients that have undergone prior immunotherapy combined with non-myeloablative chemotherapy
	Patients that have undergone prior immunotherapy targeting tyrosinase
	Patients with abnormal left ventricular function measured by screening echocardiogram
	Patients with active HIV, HBV, HCV, or HSV infections
	Patients with performance status of ≥ 2, absolute neutrophil counts <1500/µL, platelet counts < 100,000/µL, bilirubin > 1.5x the upper limit of normal, ALT[a] or AST[b] values > 2.5x upper limit of normal, albumin <2.5g/dL, INR[c] > 1.5, or creatinine clearance (Cockcroft and Gault) < 50mL/minute

Supplementary Table 1: Inclusion/Exclusion Criteria

[a]ALT: Alanine Aminotransferase

[b]AST: Aspartate Aminotransferase

[c]INR: International Normalized Ratio

Day		Patient 1	Patient 2	Patient 3
Transduction and Initial Phenotype				
0	Number of PBMCs collected in apheresis	1.62×10^{10}	1.00×10^{10}	7.23×10^{9}
0	Number of PBMCs stimulated	6.00×10^{8}	6.00×10^{8}	6.00×10^{8}
2	Number of PBMCs post-stimulation	2.33×10^{8}	5.95×10^{8}	1.10×10^{9}
2	Number of PBMCs transduced	1.92×10^{8}	1.92×10^{8}	1.92×10^{8}
3	Number of PBMCs after transduction	2.93×10^{8}	2.54×10^{8}	1.25×10^{8}
Enrichment of transduced ($CD34^{+}$) T cells				
6	Percent $CD34^{+}$ of $CD3^{+}$ post-enrichment (percent pre-enrichment)	94.9 (28)	98.6 (30.8)	97.3 (16.3)
6	Percent $CD34^{+}CD8^{+}$ of $CD3^{+}$ post-enrichment (percent pre-enrichment)	35.5 (8.5)	22.2 (10.0)	30.7 (7.4)
6	Percent $CD34^{+}CD4^{+}$ of $CD3^{+}$ post-enrichment (percent pre-enrichment)	61.5 (16.5)	77.1 (18.9)	67.0 (11.9)
6	Percent $CD34^{+}$ $v\beta12^{+}CD8^{+}$ of $CD3^{+}$ post-selection (percent pre-selection)	13.7 (2.7)	9.5 (1.4)	9.8 (0.7)
6	Percent $CD34^{+}v\beta12^{+}CD4^{+}$ of $CD3^{+}$ post-selection (percent pre-selection)	33.4 (7.5)	47.0 (5.5)	30.4 (2.2)
6	Number of cells enriched for CD34 (number pre-enrichment)	2.21×10^{8} (1.23×10^{9})	3.00×10^{7} (6.25×10^{8})	8.10×10^{6} (1.78×10^{8})
6[a]	Copy number of cells enriched for CD34 expression	3.3	2.3	2.6
Cell Growth				
	Doubling time for cells cultured day 3 to 6 (days/division)	1.45	2.31	5.90
10	Cell number expanded in Rapid Expansion Protocol (REP)	4.00×10^{6}	6.00×10^{6}	4.00×10^{6}
15	REP day 5 cell number	5.60×10^{8}	4.75×10^{8}	2.8×10^{8}
18	REP day 8 cell number	4.00×10^{9}	1.90×10^{9}	1.90×10^{9}
20	REP day 10 cell number	4.00×10^{9}	7.46×10^{9}	5.80×10^{9}
	Doubling time for cells in REP (days/division)	1.06	1.5	1.09
Final Product Phenotype and Function				
18[b]	IFNγ: T2+Tyrosinase (T2 alone) [pg/mL]	879 (32)	1492 (449)	2738 (176)
18[b]	IFNγ: 624-Mel (A2-negative 624-28) [pg/mL]	356 (10)	494 (18)	908 (77)
20[c]	Final Cell Number	7.43×10^{9}	7.06×10^{9}	5.38×10^{9}
20[c]	Final Product Percent $CD34^{+}$ of $CD3^{+}$	88.1	95.80	92.90
20[c]	Final Product Percent $v\beta12^{+}$ of $CD34^{+}$	27.7	46.5	43.7
20[c]	Final Product Percent $CD8^{+}CD34^{+}$ of $CD3^{+}$	50.5	44.4	36.8
20[c]	Final Product Percent $CD4^{+}CD34^{+}$ of $CD3^{+}$	40.1	51.4	57.3
20[c]	Transduced Cell Number Infused into Patient (Total Cells Infused into Patient)	2.00×10^{8} (2.27×10^{8})	2.72×10^{8} (2.87×10^{8})	2.06×10^{8} (2.22×10^{8})
20[c]	Cell Dose per kilogram patient weight	1.67×10^{6}	2.5×10^{6}	2.5×10^{6}
13[d]	Final viral copy number	7.5	2.4	2.1

Supplementary Table 2: Patient T cell Product Generation

[a] Day 12 was day of copy number testing for patient 1. After Patient 1 had a copy number higher than protocol allowed for, an earlier copy number test was implemented

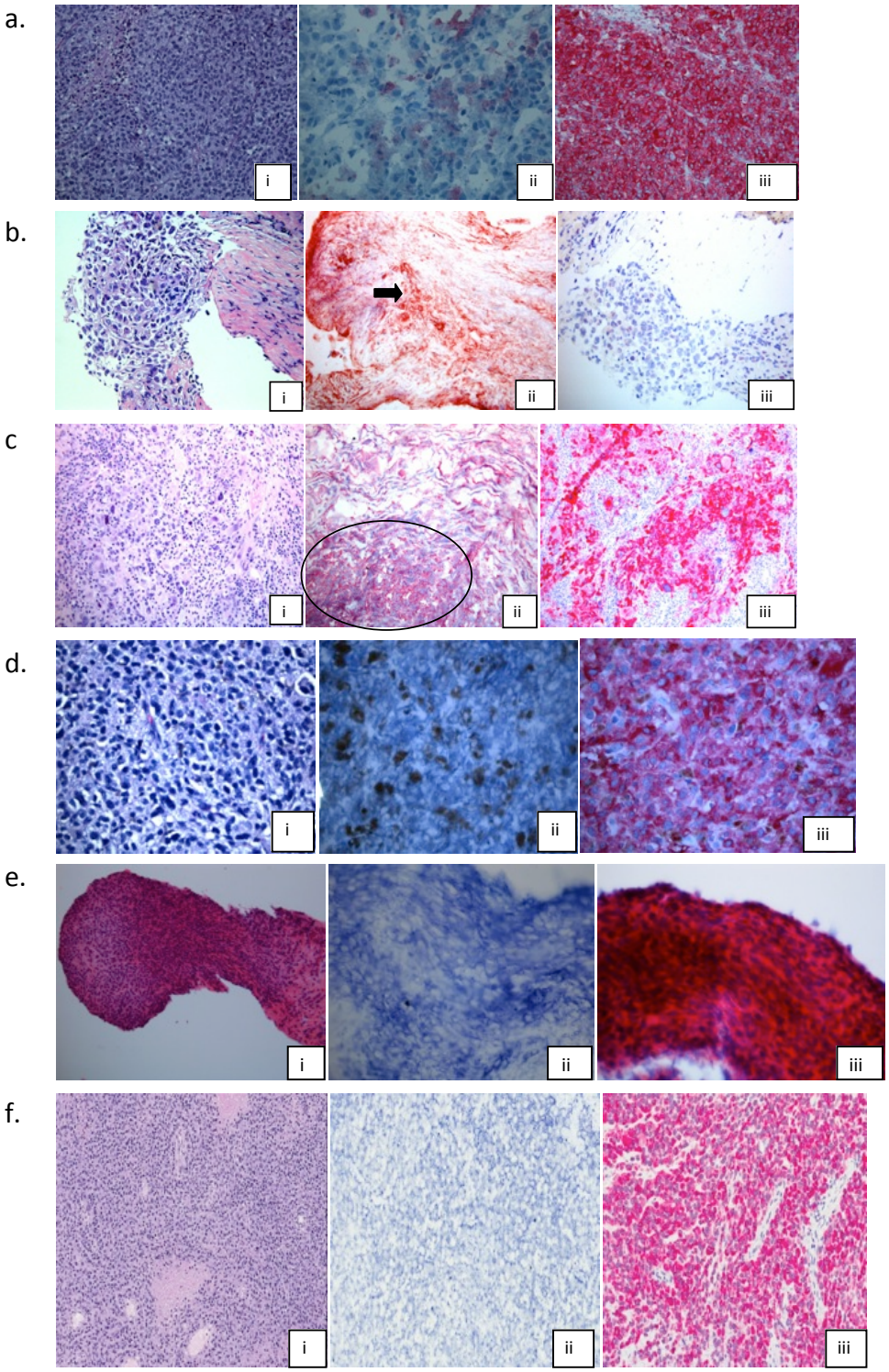
[b] Day 21 for Patient 1.

[c] Day 24 for Patient 1. Due to the high copy number in Patient 1, delivery of final product was delayed (within the acceptable window as laid out by the protocol).

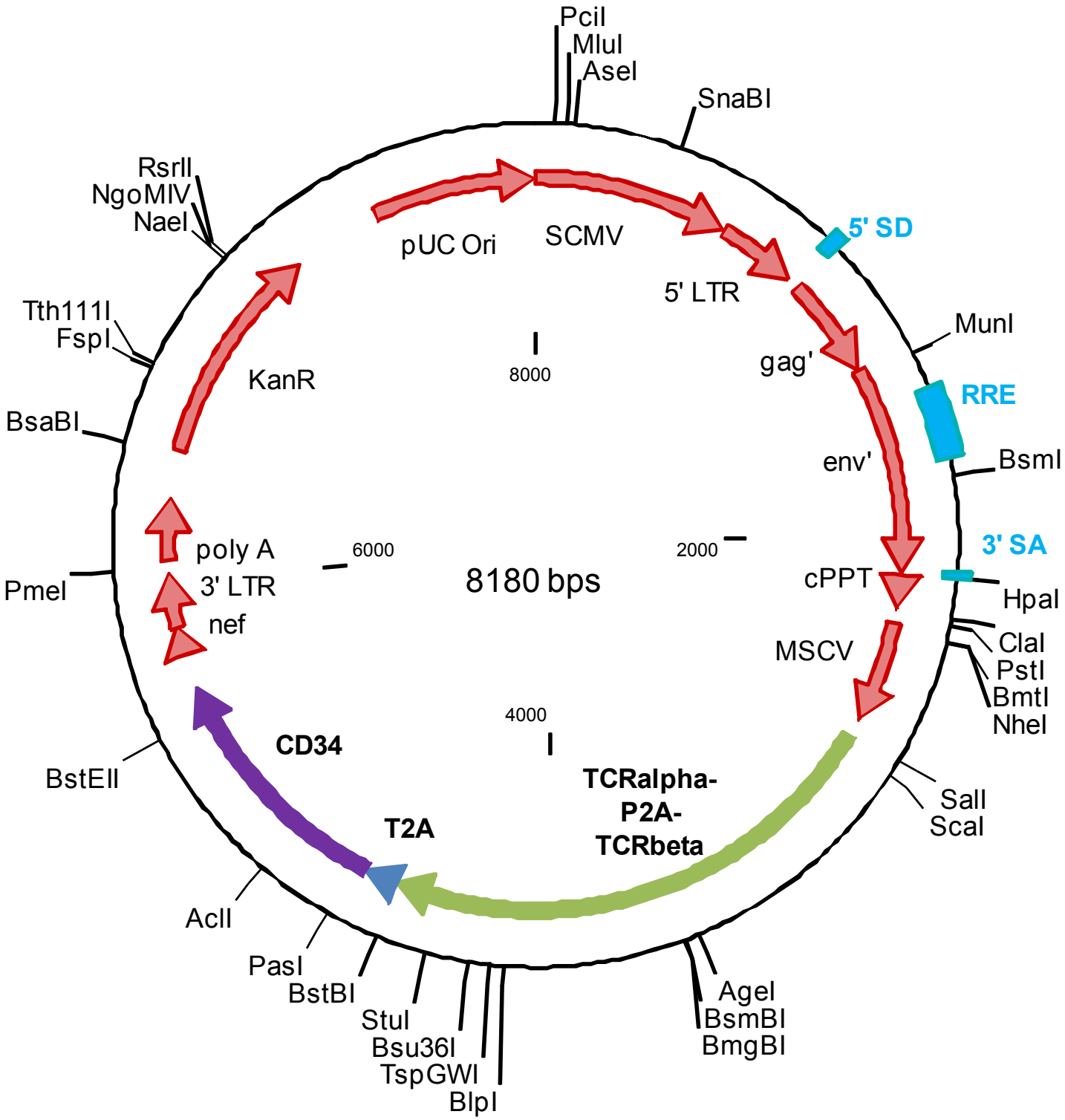
[d] REP day 13 for Patient 1, day 10 pre-REP for Patients 2, 3

	Day	Symptoms & Responses
Patient 1	-3	Found pulmonary emboli incidentally on imaging and started on anticoagulation.
	0	Developed melena with a subsequent hemoglobin decrease from 9.7 to 7.7 g/dL. An emergent upper endoscopy was performed, which demonstrated two large gastric ulcers with irregular borders concerning for metastatic disease. Anticoagulation was discontinued and the patient's symptoms resolved.
	1	Developed neutropenia
	3	Developed grade 4 lymphopenia, resolved after outpatient followup
	4	Neutropenia progressed to grade 4, recovered by discharge from hospital to 500 cells/ microliter
	7	Grade 4 thrombocytopenia, developed fevers—central line-associated bacteremia. Resolved after full course of antibiotics and removal of the line.
Patient 2	3	Developed neutropenia
	4	Developed grade 4 lymphopenia, resolved by time of outpatient follow-up. Neutropenia progressed to grade 4, resolved by time of discharge to over 500 cells per microliter Developed a maculopapular rash on his lower extremities along with erythematous area on his chest, symptoms were well controlled with diphenhydramine and topical ointments
	5	Developed fevers, given febrile neutropenia treatment, including fluconazole and meropenem
	6	Experienced a witnessed tonic-clonic seizure, despite therapeutic levels of usual anti-epileptic medication. No further seizure episodes after discontinuing fluconazole and meropenem used to treat febrile neutropenia
	7	Developed grade 3 thrombocytopenia, resolved by time of outpatient appointment No evidence of infections was found during the hospital course.
Patient 3	3	Developed grade 4 lymphopenia, resolved by time of outpatient follow-up
	4	Developed grade 4 neutropenia and fevers Developed developed pink rash on abdomen that later spread to thighs bilaterally. Noted to have erythematous areas on face, chest, and back
	6	Developed grade 4 thrombocytopenia, resolved by time of outpatient follow-up
	10	Neutrophil count recovered to above 500 cell/microliter and fevers resolved.

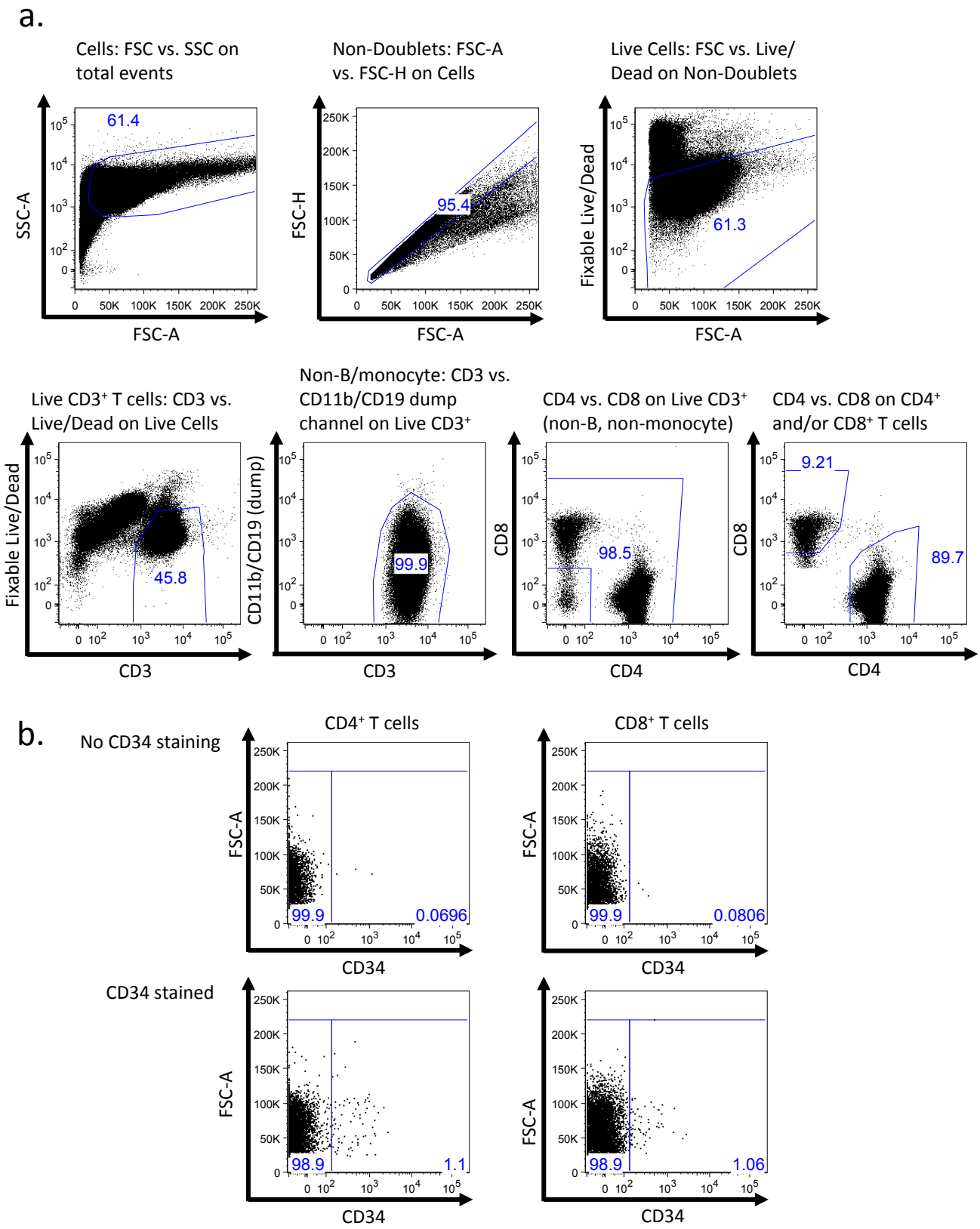
Supplementary Table 3: Patient Toxicities



Supplementary Figure 1: HLA-A2 and tyrosinase expression in patients. **a.** Patient 1 mesenteric lymph node (i) H&E of melanoma replacing lymph node. 200x. (ii) HLA-A2 staining (red chromogen) highlights cytoplasm in 20% of tumor cells. 400x. (iii) Tyrosinase staining (red chromogen) highlights cytoplasm of nearly 100% of tumor cells. 200x. **b.** Excluded patient pleural biopsy (i) H&E melanoma cells. (ii) HLA-A2 (red chromogen) highlights melanoma cells (black arrow). (iii) Tyrosinase was negative in the melanoma cells. 200x. **c.** Patient 2 subcutaneous nodule biopsy. (i) H&E stained melanoma cells. (ii) HLA-A2 (red chromogen) highlights melanoma cells (circle). (iii) Tyrosinase highlights the melanoma cells (red chromogen). 200x. **d.** Excluded patient arm mass biopsy (i) H&E stained melanoma with pigment deposition. (ii) HLA-A2 (blue chromogen) highlights melanoma cells. (iii) Tyrosinase highlights the melanoma cells (red chromogen). 200x. **e.** Patient 3 adrenal biopsy (i) H&E stained melanoma with spindle cell features. 100x. (ii) HLA-A2 (blue chromogen) highlights melanoma cells. 200x. (iii) Tyrosinase highlights the melanoma cells (red chromogen). 200x. **f.** Excluded patient subcutaneous thigh nodule (i) H&E melanoma tumor cells in the subcutis. (ii) HLA A2 is positive in the tumor cells (blue chromogen). (iii) Tyrosinase immunohistochemical stain (red chromogen) highlights melanoma cells. 200x.

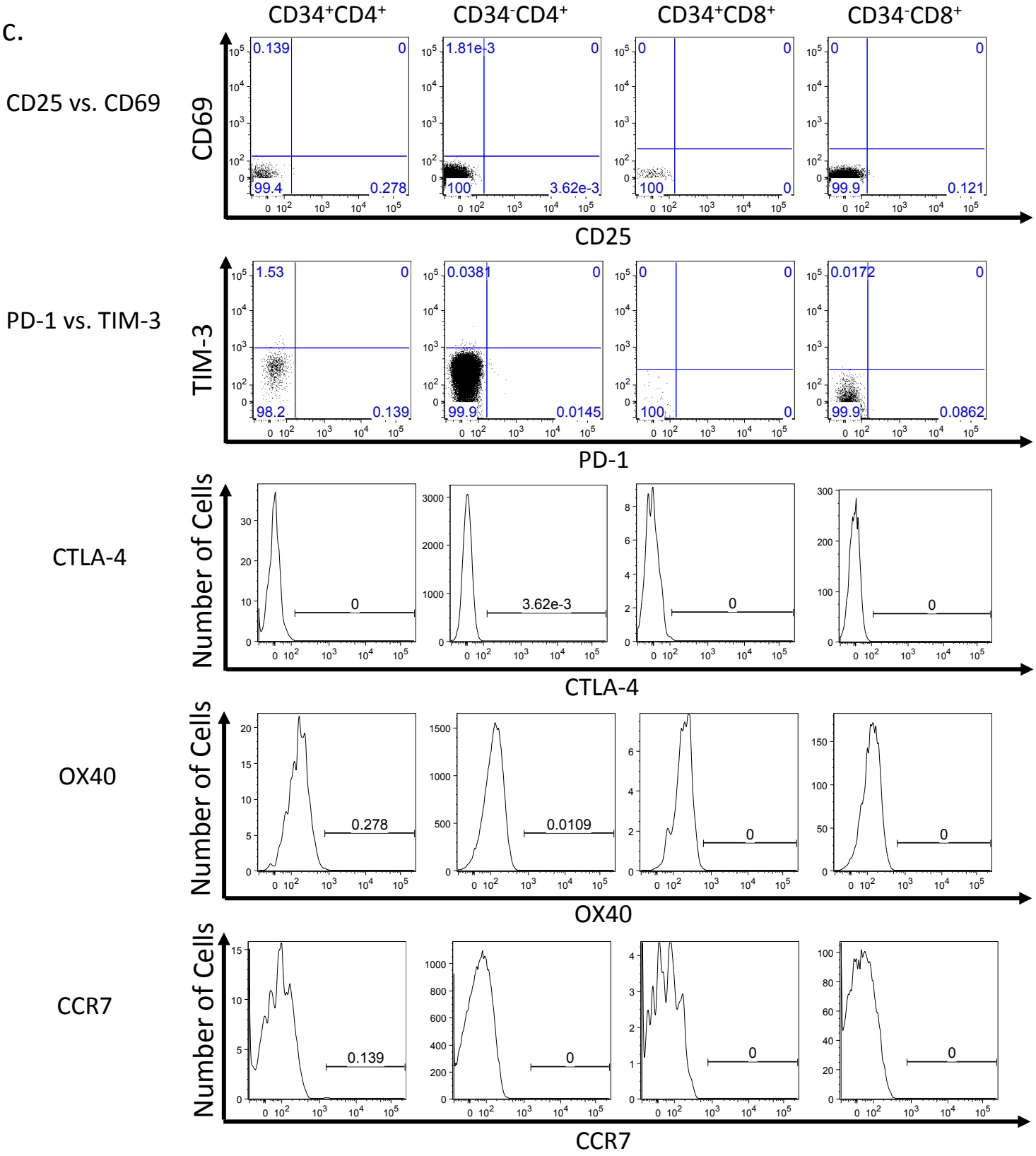


Supplementary Figure 2: Schematic of the lentiviral vector used to transduce patient T cells
The TCRalpha-P2A-TCRbeta region represents the TCR α chain cloned from the tyrosinase-reactive CD8-independent TIL 1383I T cell clone followed by a P2A ribosomal skipping site then the TCR β chain cloned from the TIL 1383I T cell clone.

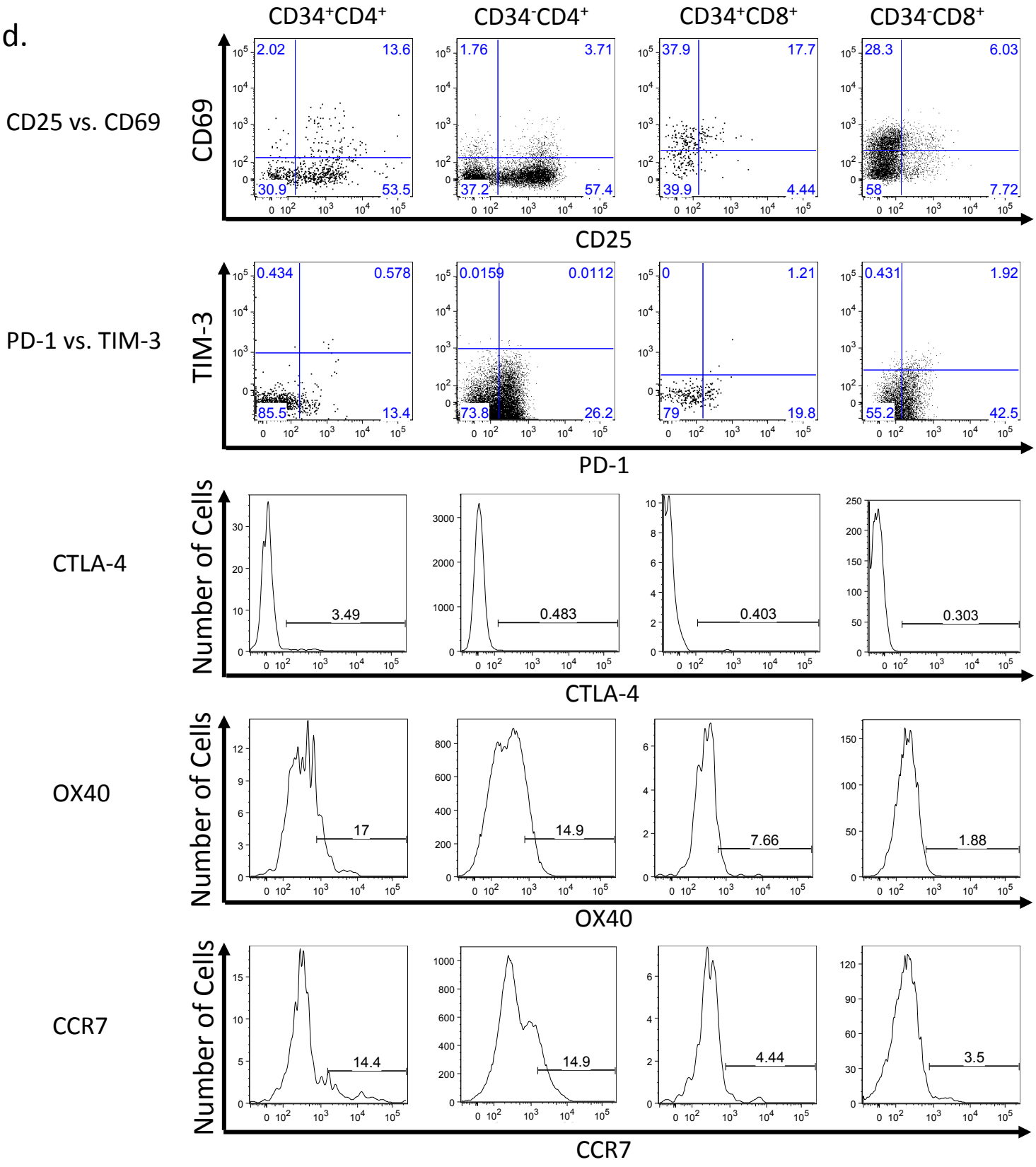


Supplementary Figure 3: Gating strategy to identify and characterize transduced T cells. **a.** Identification of live CD4⁺ and CD8⁺ T cells. **b.** Identification of CD34⁺ T cells in the CD4⁺ and CD8⁺ T cells**. c-e.** Representative gating for activated (CD25⁺CD69⁺), exhausted/ inhibited (PD-1⁺TIM-3⁺), and other (CTLA-4⁺, OX40⁺, and CCR7⁺) markers shown from Patient 3 day 14 sample blood. **c.** Representative gating on Patient 3 day 14 sample blood cells not stained for phenotype markers (negative control). **d.** Sample gating on fully stained Patient 3 day 14 blood sample. **e.** Sample gating on fully stained Patient 3 Final Product prior to transfer into the patient

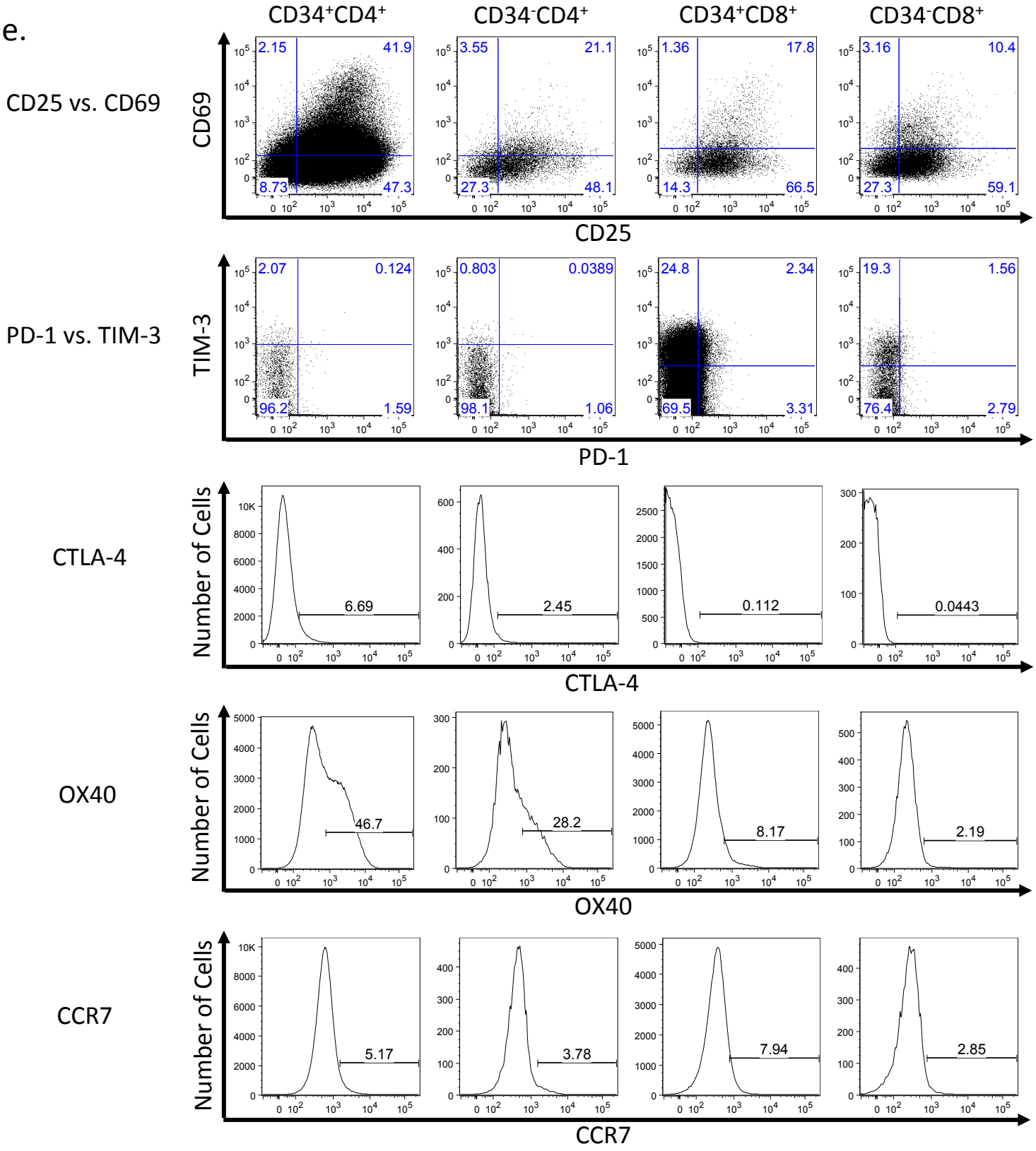
C.


CD34+CD4+
CD34-CD4+
CD34+CD8+
CD34-CD8+
CD25 vs. CD69
CD69
CD25
0.139
0
99.4
0.278
1.81e-3
0
100
3.62e-3
0
0
100
0
0
0
99.9
0.121
PD-1 vs. TIM-3
TIM-3
PD-1
1.53
0
98.2
0.139
0.0381
0
99.9
0.0145
0
0
100
0
0.0172
0
99.9
0.0862
CTLA-4
Number of Cells
0
3.62e-3
0
0
CTLA-4
OX40
Number of Cells
0.278
0.0109
0
0
OX40
CCR7
Number of Cells
0.139
0
0
0
CCR7

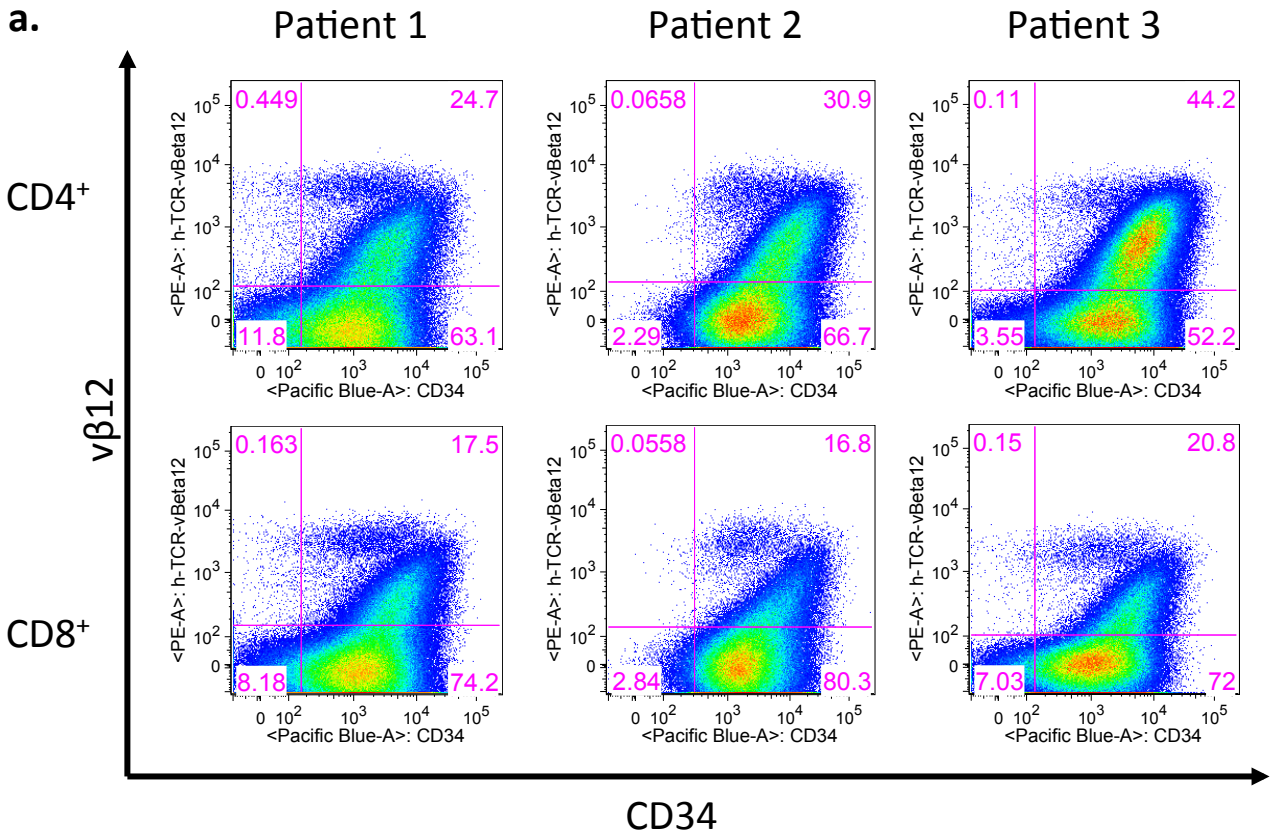
d.


CD34+CD4+
CD34-CD4+
CD34+CD8+
CD34-CD8+
CD25 vs. CD69
CD69
CD25
2.02
13.6
30.9
53.5
1.76
3.71
37.2
57.4
37.9
17.7
39.9
4.44
28.3
6.03
58
7.72
PD-1 vs. TIM-3
TIM-3
PD-1
0.434
0.578
85.5
13.4
0.0159
0.0112
73.8
26.2
0
1.21
79
19.8
0.431
1.92
55.2
42.5
CTLA-4
Number of Cells
3.49
0.483
0.403
0.303
OX40
17
14.9
7.66
1.88
CCR7
14.4
14.9
4.44
3.5

e.


CD34+CD4+
CD34-CD4+
CD34+CD8+
CD34-CD8+
CD25 vs. CD69
CD69
CD25
2.15
41.9
8.73
47.3
3.55
21.1
27.3
48.1
1.36
17.8
14.3
66.5
3.16
10.4
27.3
59.1
PD-1 vs. TIM-3
TIM-3
PD-1
2.07
0.124
96.2
1.59
0.803
0.0389
98.1
1.06
24.8
2.34
69.5
3.31
19.3
1.56
76.4
2.79
CTLA-4
Number of Cells
6.69
2.45
0.112
0.0443
OX40
46.7
28.2
8.17
2.19
CCR7
5.17
3.78
7.94
2.85

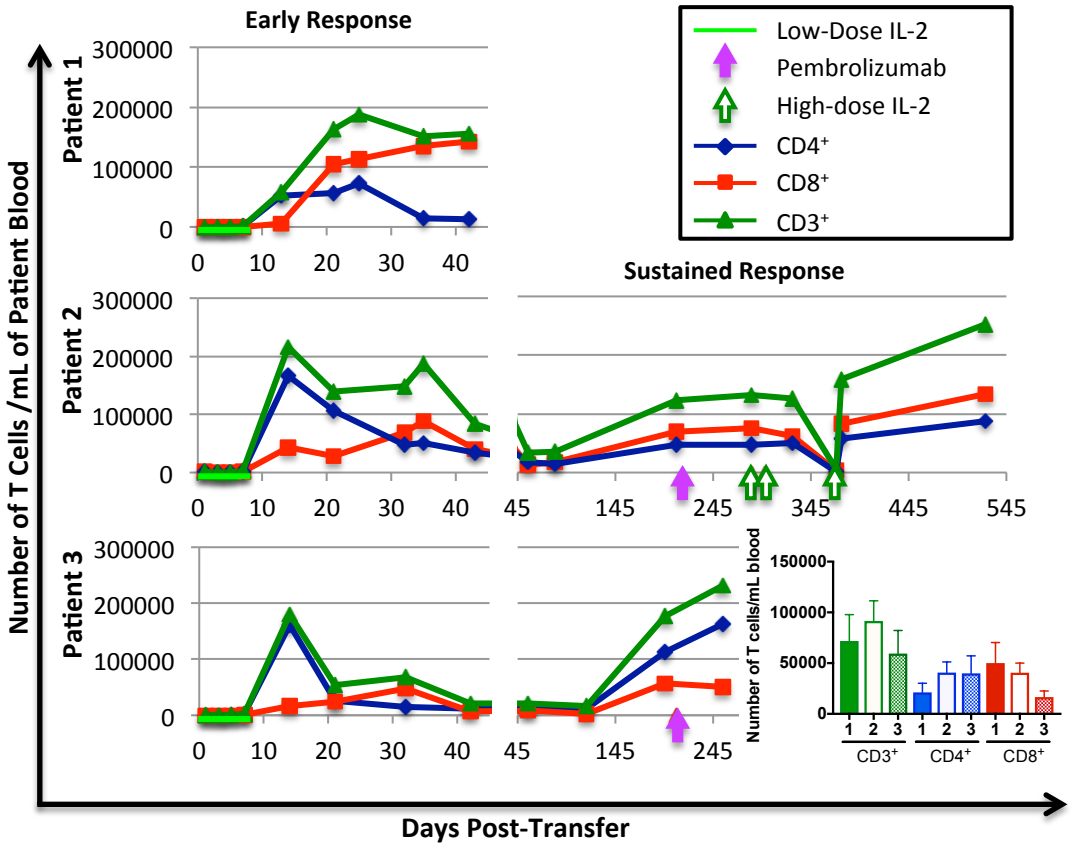
a.



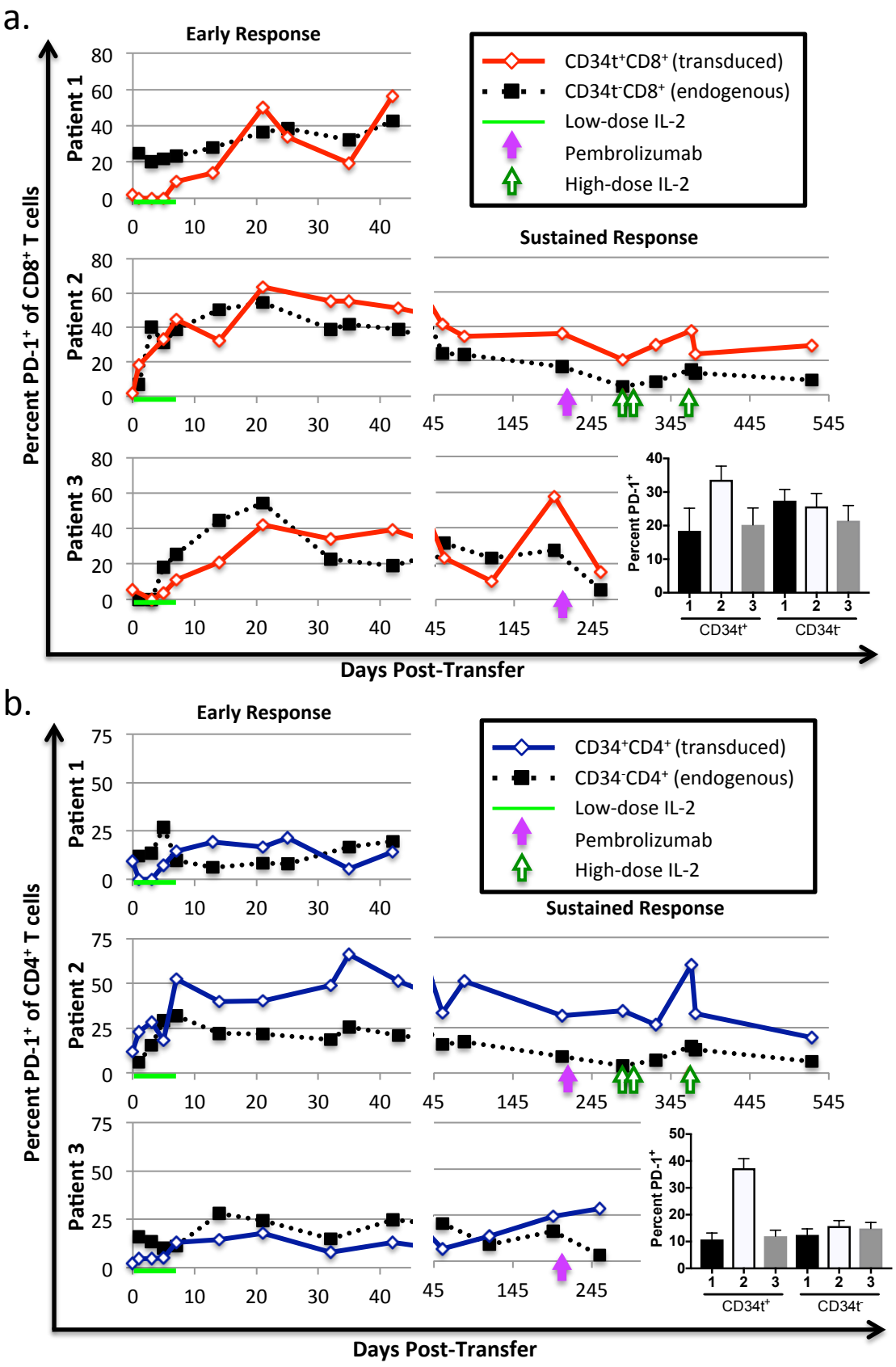
b.

Patient	MFI of CD34 on CD4⁺ T cells	MFI of CD34 on CD34⁺ CD4⁺ T cells	MFI of vβ12 on CD4⁺ T cells	MFI of vβ12 on CD34⁺ CD4⁺ T cells	MFI of CD34 on CD8⁺ T cells	MFI of CD34 on CD34⁺ CD8⁺ T cells	MFI of vβ12 on CD8⁺ T cells	MFI of vβ12 on CD34⁺ CD8⁺ T cells
1	2661	3025	186	208	3163	3445	112	122
2	6808	6967	268	274	4267	4408	104	107
3	5721	5937	317	329	2703	2909	89.6	96

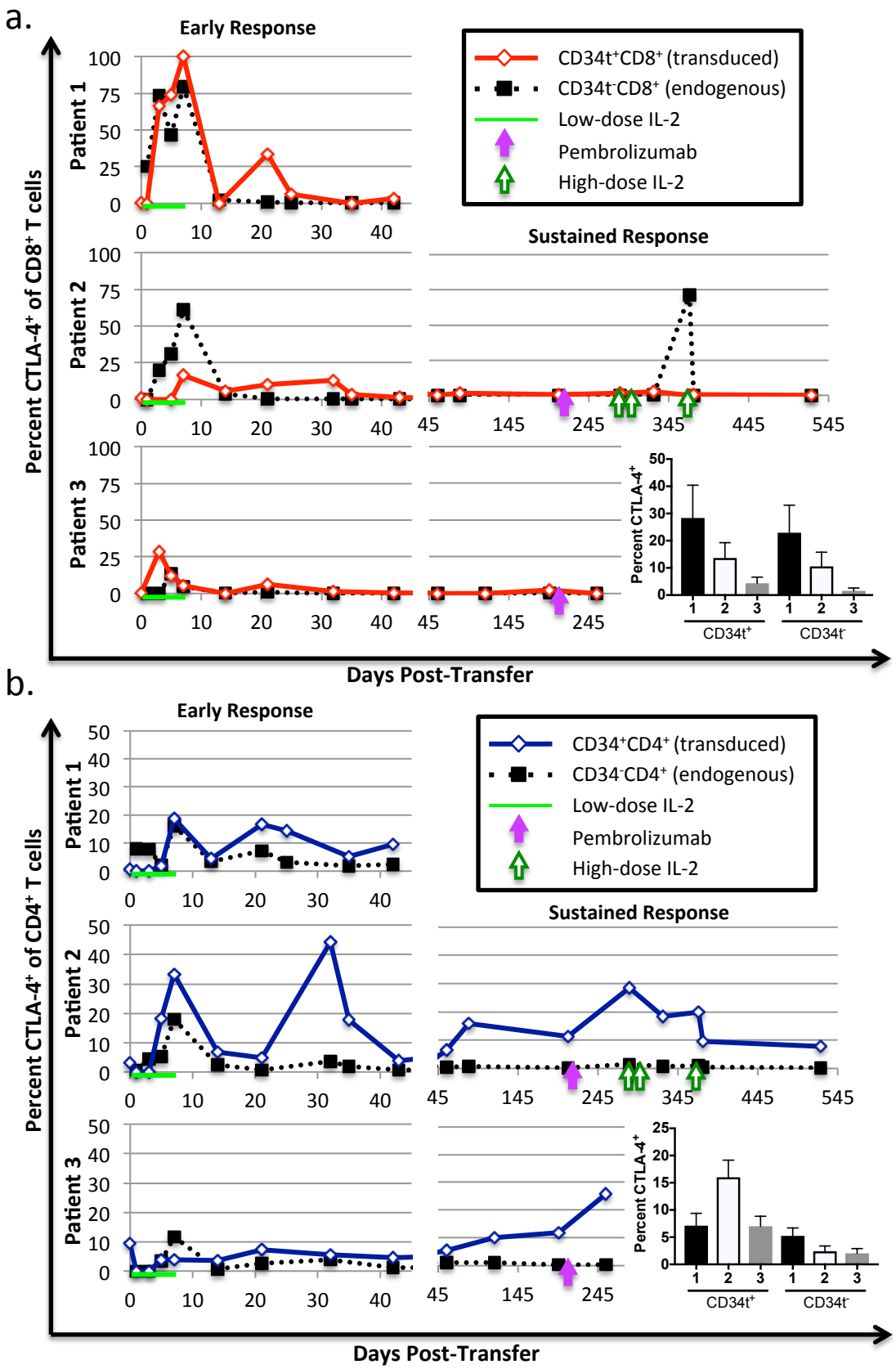
Supplementary Figure 4 : CD34 and vβ12 expression on patient final products
Frozen samples of final preparations of T cells were thawed and analyzed with each patients blood sample cohort. **a.** CD34 and vβ12 expression on CD4⁺ and CD8⁺ CD3⁺ T cells were analyzed by flow cytometry. **b.** Mean fluorescence intensities of CD34 and vβ12 were calculated on both total CD4⁺ and CD8⁺ CD3⁺ T cells and CD34⁺ CD4⁺ and CD8⁺ CD3⁺ T cells.



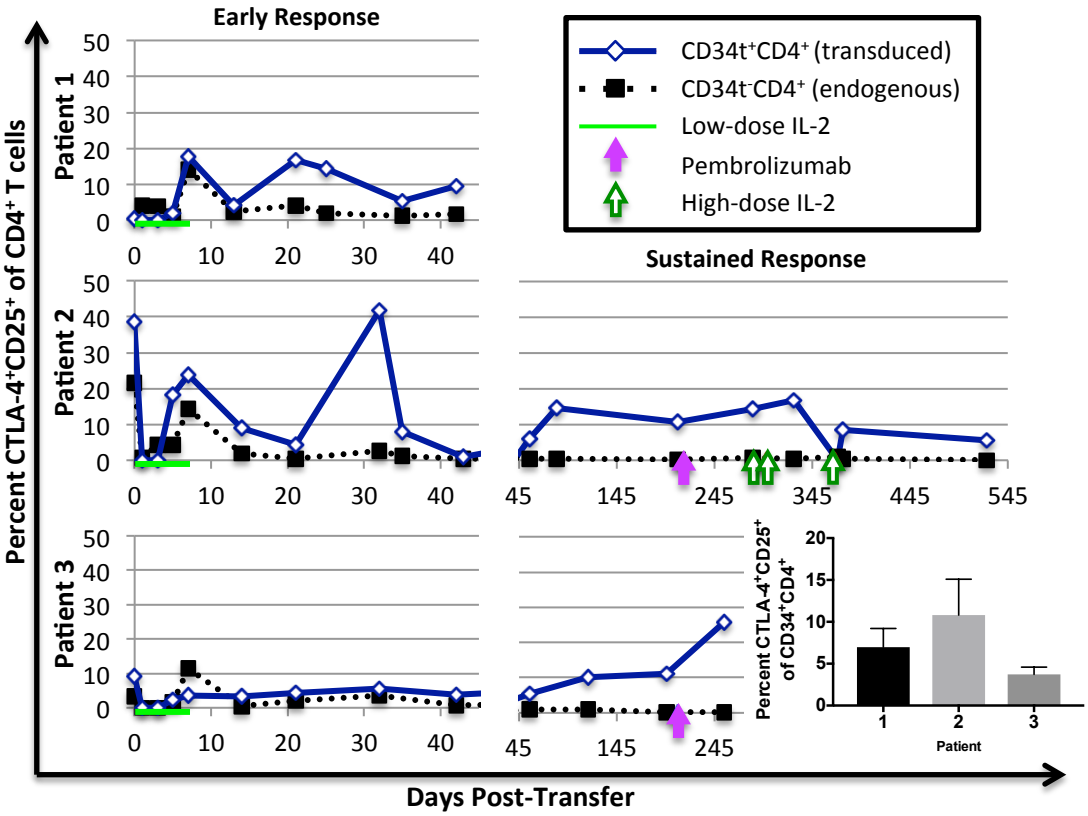
Supplementary Figure 5: Number of T cells per mL in patient blood samples. Patient blood samples were collected and PBMCs isolated as described in Fig. 2. Patients were treated with low-dose IL-2 (72,000IU/kg, IV, three times daily) for seven days after T cell infusion (green line). Two patients went on to receive pembrolizumab, each course (2mg/kg, given i.v. every 3 weeks) indicated by closed purple arrows, and one patient further received high-dose IL-2, with each course (600,000 IU/m2, given i.v. every 8 hours for a maximum of 14 doses on days 284, 298, and 368 post-T cell infusion) indicated by open green arrows. The number of total (green triangles), $CD4^+$ (blue diamonds), and $CD8^+$ (red squares) $CD3^+$ T cells per mL of patient blood are shown.



Supplementary Figure 6: PD-1 expression on transduced and endogenous T cells. Patient blood samples were collected and PBMCs isolated as described in Fig. 2. **a.** The percent of transduced ($CD34^+$, red open diamonds) and endogenous ($CD34^-$, black squares, dotted line) $CD8^+$ T cells expressing PD-1 are shown. **b.** The percent of transduced ($CD34^+$, blue open diamonds) and endogenous ($CD34^-$, black squares, dotted line) $CD4^+$ T cells expressing PD-1 are shown.

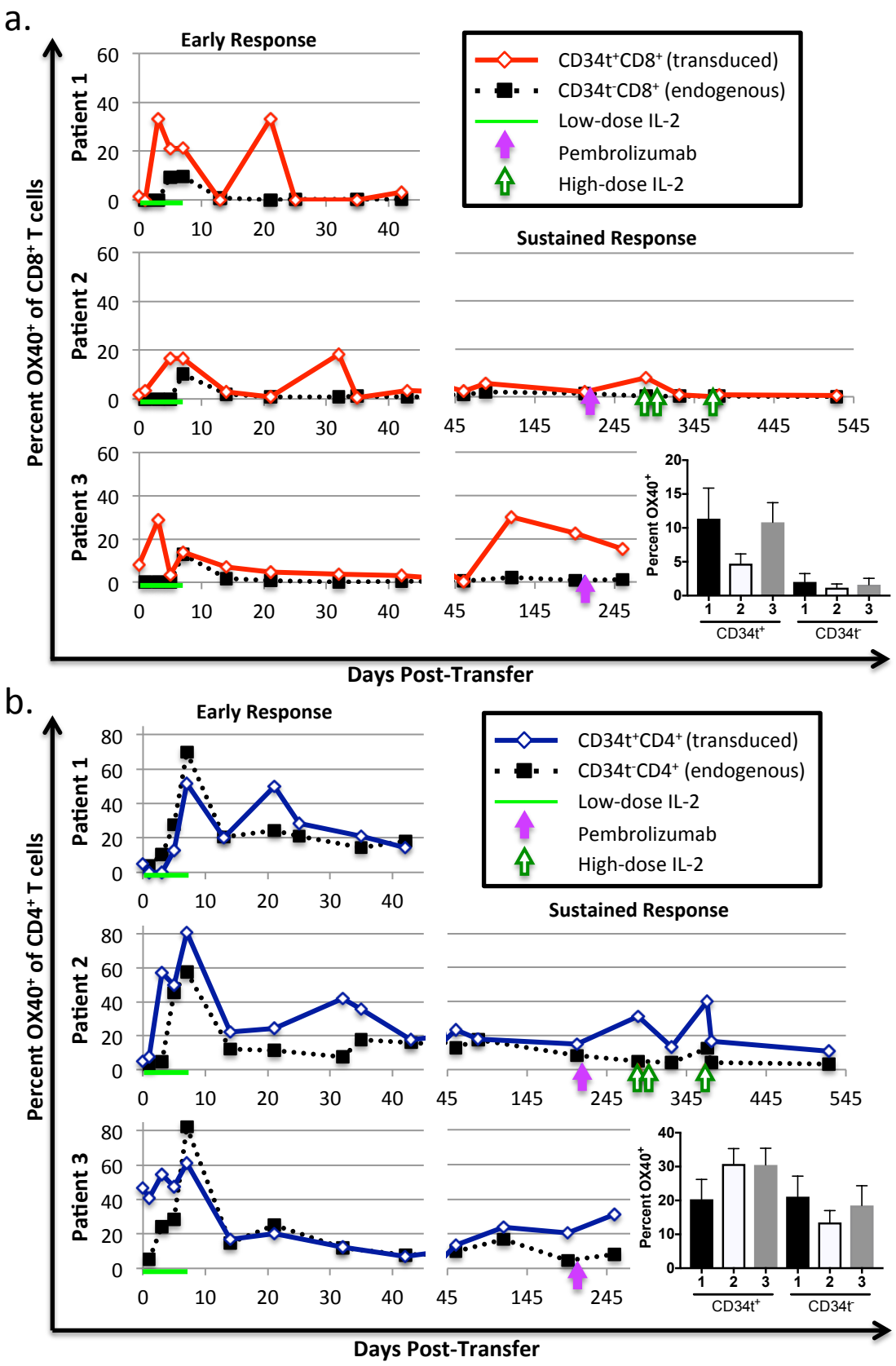


Supplementary Figure 7: CTLA-4 expression on transduced and endogenous T cells. Patient blood samples were collected and PBMCs isolated as described in Fig. 2. **a.** The percent of transduced ($CD34^+$, red open diamonds) and endogenous ($CD34^-$, black squares, dotted line) $CD8^+$ T cells expressing CTLA-4 are shown. **b.** The percent of transduced ($CD34^+$, blue open diamonds) and endogenous ($CD34^-$, black squares, dotted line) $CD4^+$ T cells expressing CTLA-4 are shown.

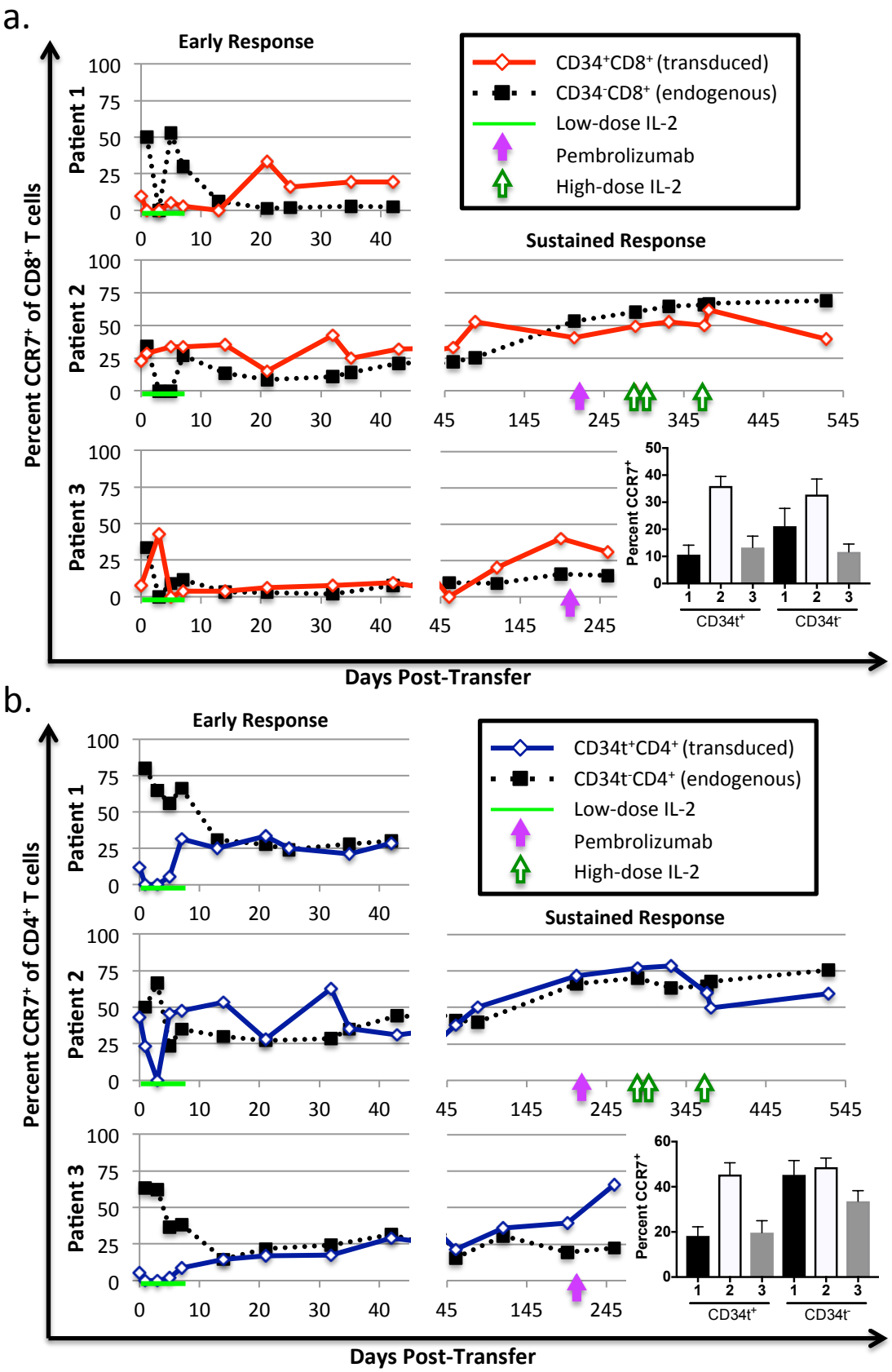


Supplementary Figure 8: CTLA-4 and CD25 co-expression on transduced and endogenous CD4$^+$ T cells.

Patient blood samples were collected and PBMCs isolated as described in Fig. 2. Shown are the percent of transduced (CD34t$^+$, blue open diamonds) and endogenous (CD34t$^-$, black squares, dotted line) CD4$^+$ T cells co-expressing CD25 and CTLA-4.



Supplementary Figure 9: OX40 expression on transduced and endogenous T cells. Patient blood samples were collected and PBMCs isolated as described in Fig. 2. **a.** The percent of transduced ($CD34^+$, red open diamonds) and endogenous ($CD34^-$, black squares, dotted line) $CD8^+$ T cells expressing OX40 are shown. **b.** The percent of transduced ($CD34^+$, blue open diamonds) and endogenous ($CD34^-$, black squares, dotted line) $CD4^+$ T cells expressing OX40 are shown.



Supplementary Figure 10: CCR7 expression on transduced and endogenous T cells. Patient blood samples were collected and PBMCs isolated as described in Fig. 2. **a.** The percent of transduced ($CD34^+$, red open diamonds) and endogenous ($CD34^-$, black squares, dotted line) $CD8^+$ T cells expressing CCR7 are shown. **b.** The percent of transduced ($CD34^+$, blue open diamonds) and endogenous ($CD34^-$, black squares, dotted line) $CD4^+$ T cells expressing CCR7 are shown.