

Supplemental information

**Peripheral T cell profiling reveals downregulated
exhaustion marker and increased diversity
in lymphedema post-lymphatic venous anastomosis**

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Supplemental information

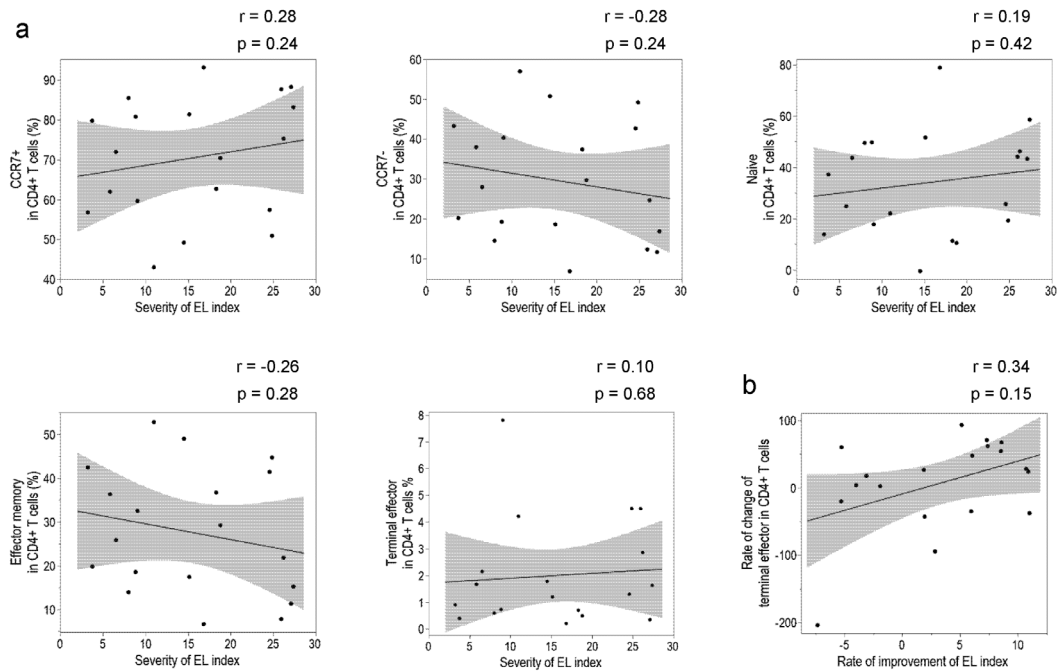


Figure S1. Correlation between Naïve memory phenotype in CD4⁺ T cells and clinical features; Related to Figure 5. **(a)** Correlation between Naïve memory phenotype in CD4⁺ T cells and severity of extremity lymphedema (EL) index. **(b)** Correlation between rate of change of terminal effector (TE) and rate of improvement of EL index.

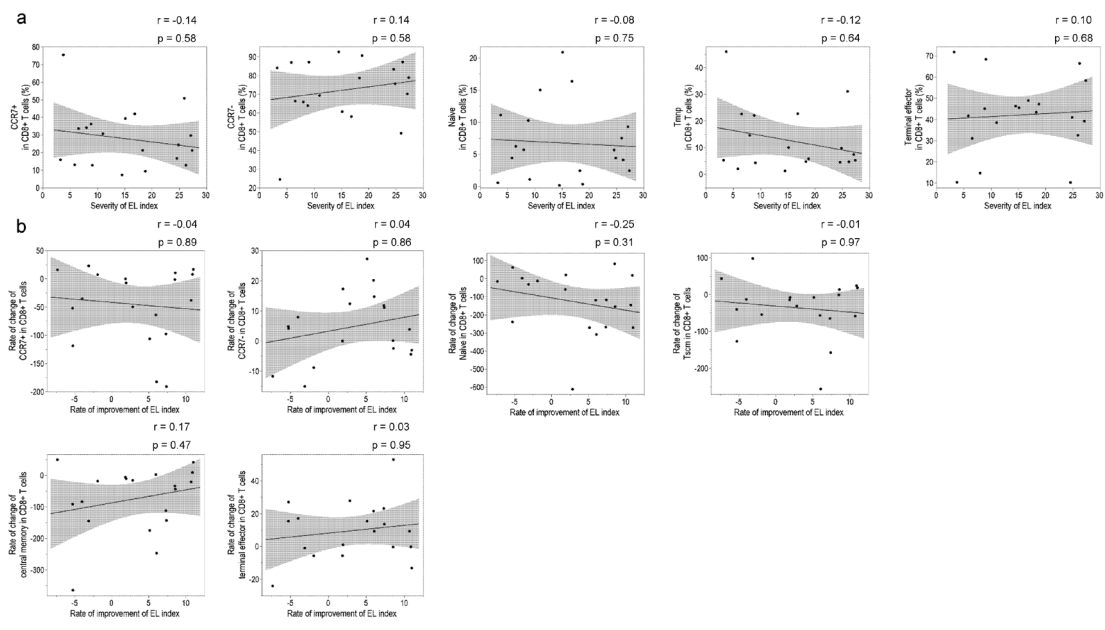


Figure S2. Correlation between naïve memory phenotype in CD8⁺ T cells and clinical features; Related to Figure 6. **(a)** Correlation between naïve memory phenotype in CD8⁺ T cells and severity of extremity lymphedema (EL) index. **(b)** Correlation between rate of change of Naïve memory phenotype and rate of improvement of EL index.

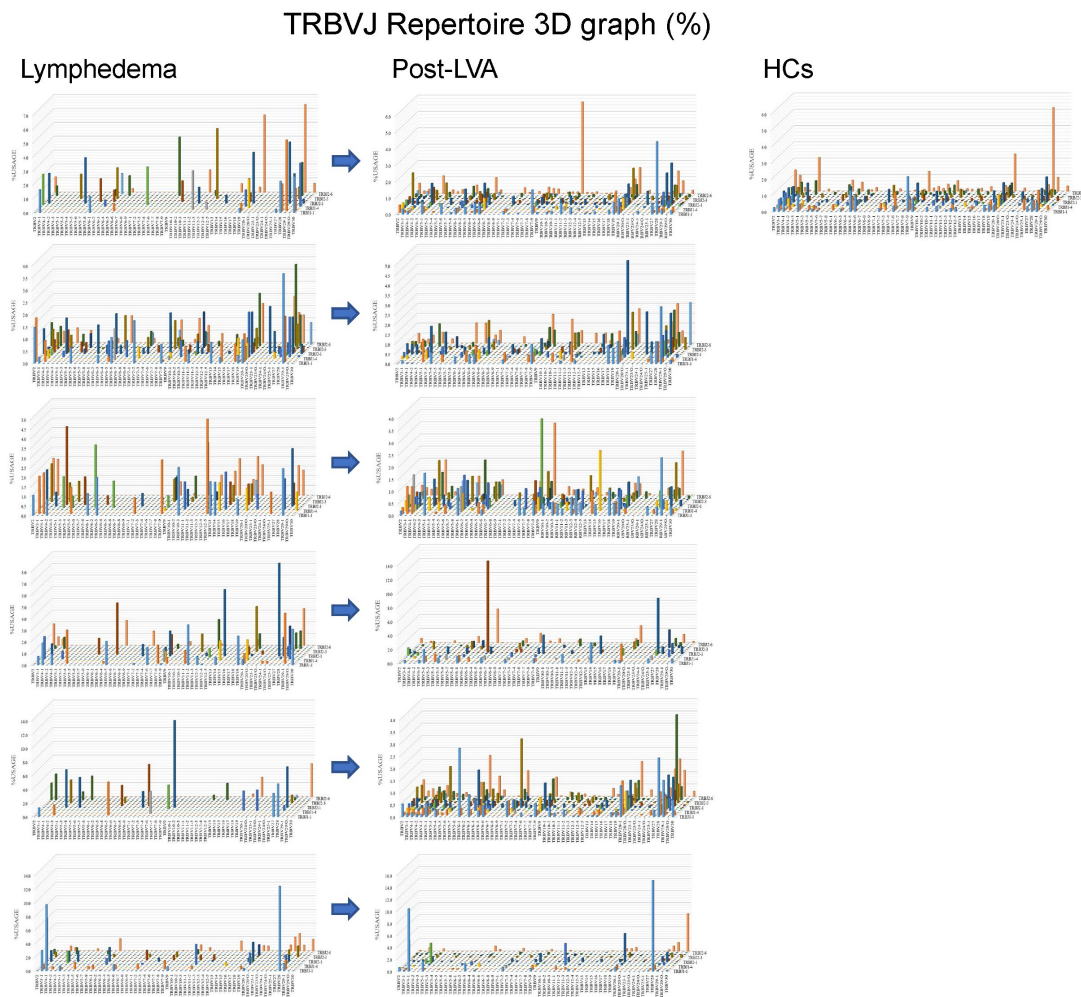


Figure S3. Variety of TRBV and J combination in lymphedema, post-lymphatic venous anastomosis (LVA), and healthy control (HC) samples; Related to Figure 9.

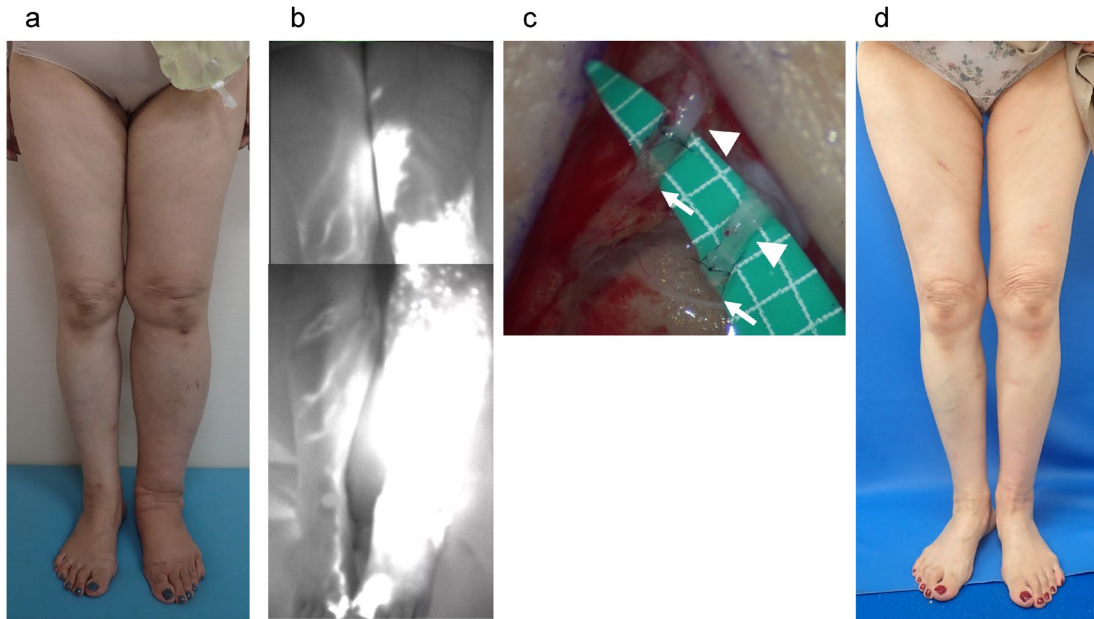


Figure S4. Edematous condition of patients with left lower limb lymphedema; Related to Figure 1. **(a)**. Compared with those on the right side, the circumferences are higher on the left side. **(b)**. The indocyanine green lymphology shows marked dermal backflow on the left side, while a linear pattern can be noted on the right side. **(c)** Lymphatic venous anastomosis (LVA) was performed for the patient. Arrow indicates the lymphatic channel, and the arrowhead indicates the vein. The scale of the background sheet is 1 mm. **(d)** The circumference in the left lower extremity was decreased at 1-year post-LVA.

Table S1. Clinical characteristics of patients; Related to Figure 1.

Demographic	Lymphedema (n = 21)	Healthy controls (n = 20)	p Value
Sex, n (%)			
Male	0 (0)	0 (0)	1.0
Female	21 (100)	20 (100)	
Age, y, median (IQR)	54 (45–59.8)	51 (44.5–63)	0.55
BMI, kg/m ² , median (IQR)	20.7 (19.2–25.1)	N/A	N/A
Upper or lower extremity lymphedema, n (%)		N/A	N/A
Upper extremity lymphedema	4 (19.0)		
Lower extremity lymphedema	17 (81.0)		
Etiology, n (%)		None	None
Uterine cancer	14 (66.7)		
Ovarian cancer	2 (9.5)		
Bladder cancer	1 (4.8)		
Breast cancer	4 (19.0)		
Duration of edema, y, median (IQR)	2.7 (0.5–7.5)	None	None
Chemotherapy, yes, n (%)	16 (76.2)	None	None
Radiotherapy, yes, n (%)	7 (33.3)	None	None
Cellulitis episode, yes, n (%)	7 (33.3)	None	None
ISL stage, n (%)		N/A	N/A
Stage 1	0 (0)		
Stage 2a	5 (23.8)		
Stage 2b	13 (61.9)		
Stage 3	3 (14.3)		
Dermal backflow stage, n (%)		N/A	N/A
Stage I	0		
Stage II	4 (19.0)		
Stage III	7 (33.3)		
Stage IV	4 (19.0)		
Stage V	4 (19.0)		

BMI: Body mass index. IQR: Interquartile range. ISL: International Society of Lymphology

Table S2. Comparison of extremity lymphedema index and leukocyte characteristics between lymphedema, post-lymphatic venous anastomosis (LVA), and healthy controls (HCs); Related to Figure 1.

Demographic, median (IQR)	Lymphedema (n = 21)	Post-LVA (n = 21)	HCs (n = 20)	p-value		
				Lymphedema vs. Post-LVA	HCs vs. Lymphedema	Post-LVA vs. HCs
UEL index	126.7 (106.0–159.8)	125.4 (108.9–143.2)	N/A	0.25	N/A	N/A
LEL index	265.5 (245.2–307.7)	251.7 (241.9–263.5)	N/A	0.01	N/A	N/A
Severity of EL index	16.8 (8.4–25.4)	12.3 (4.1–18.1)	N/A	< 0.01	N/A	N/A
Leukocyte count, n/ μ l	5300 (4535–6045)	4515 (3705–5298)	5110 (3885–5920)	0.27	0.48	0.57
Lymphocyte count, n/ μ l	1790 (1132–2010)	1410 (1184.5 – 1939)	1810 (1551–2229)	0.50	0.44	0.14
CD4/8 ration in CD3 ⁺ T cells	1.5 (0.9–2.5)	1.5 (0.9–2.0)	1.8 (1.4–2.5)	0.07	0.21	0.08

IQR: Interquartile range. UEL: Upper extremity lymphedema index. LEL: Lower extremity lymphedema index

Table S3. Comparison of PD-1, Tim-3, Lag-3, and PD-1⁺Tim3⁺ expression (%) on CD4⁺ and CD8⁺ T cells between lymphedema, post-lymphatic venous anastomosis (LVA), and healthy controls (HCs); Related to Figures 2 and 3.

Demographic %, median (IQR)	Lymphedema (n = 21)	Post-LVA (n = 21)	HCs (n = 20)	p-value		
				Lymphedema vs. Post-LVA	HCs vs. Lymphedema	Post-LVA vs. HCs
PD-1 ⁺ in CD3 ⁺ 4 ⁺ T cells	30.7 (19.8–39.1)	27.1 (17.6–35.4)	19.9 (13.9–24.7)	0.03	< 0.01	0.02
Tim-3 ⁺ in CD3 ⁺ 4 ⁺ T cells	1.4 (0.9–3.0)	1.0 (0.7–1.4)	1.3 (0.6–1.5)	< 0.01	0.29	0.51
Lag-3 ⁺ in CD3 ⁺ 4 ⁺ T cells	0.6 (0.4–0.8)	0.4 (0.3–0.7)	0.4 (0.3–0.6)	0.06	0.01	0.40
PD-1 ⁺ Tim-3 ⁺ in CD3 ⁺ 4 ⁺ T cells	0.7 (0.4–1.1)	0.3 (0.2–0.6)	0.3 (0.2–0.6)	< 0.01	0.04	0.69
PD-1 ⁺ in CD3 ⁺ 8 ⁺ T cells	17.1 (11.7–25)	15.9 (10.2–19.4)	17.5 (9.4–20.6)	0.01	0.26	0.66
Tim-3 ⁺ in CD3 ⁺ 8 ⁺ T cells	3.5 (1.8–6.5)	2.2 (1.3–4.0)	3.4 (2.5–4.2)	0.01	0.57	0.09
Lag-3 ⁺ in CD3 ⁺ 8 ⁺ T cells	1.2 (0.7–1.7)	0.7 (0.5–1.6)	0.8 (0.5–1.3)	0.15	0.09	0.75
PD-1 ⁺ Tim-3 ⁺ in CD3 ⁺ 8 ⁺ T cells	0.6 (0.3–1.0)	0.2 (0.1–0.5)	0.3 (0.2–0.7)	0.01	0.08	0.56

IQR: Interquartile range

Table S4. Comparison of percentage of Treg subsets among CD3⁺CD4⁺ T cells (%)

between lymphedema, post-lymphatic venous anastomosis (LVA), and healthy controls

(HCs); Related to Figure 4.

Demographic %, median (IQR)	Lymphedema (n = 21)	Post-LVA (n = 21)	HCs (n = 20)	p-value		
				Lymphedema vs. Post-LVA	HCs vs. Lymphedema	Post-LVA vs. HCs
Treg in CD3 ⁺ 4 ⁺ T cells	5.3 (3.0–7.4)	5.1 (2.9–6.1)	2.5 (1.4–4.6)	0.17	<0.01	0.03
Treg I in CD3 ⁺ 4 ⁺ T cells	0.6 (0.4–0.9)	0.5 (0.3–0.7)	0.4 (0.3–1.1)	0.09	0.26	0.67
Treg II in CD3 ⁺ 4 ⁺ T cells	1.1 (0.8–2.1)	1.4 (0.9–2.6)	0.5 (0.3–0.9)	0.25	< 0.001	< 0.0001
Treg III in CD3 ⁺ 4 ⁺ T cells	2.8 (1.8–4.5)	2.5 (1.7–3.3)	1.6 (0.9–3.0)	0.06	0.02	0.14

IQR: Interquartile range

Table S5. Comparison of naïve and memory phenotype frequencies in CD4⁺ and CD8⁺ T cells between lymphedema, post-lymphatic venous anastomosis (LVA), and healthy controls (HCs); Related to Figures 5, 6.

Demographic %, median (IQR)	Lymphedema (n = 21)	Post-LVA (n = 21)	HCs (n = 20)	p-value		
				Lymphedema vs. Post-LVA	HCs vs. Lymphedema	Post-LVA vs. HCs
CCR7 ⁺ in CD4 ⁺ T cells	72.0 (57.4–83.2)	74.1 (57.8–79.5)	83.6 (76.5–87.2)	0.31	0.02	< 0.01
CCR7 ⁻ in CD4 ⁺ T cells	28.0 (16.8–42.7)	26 (20.1–42.2)	16.4 (12.9–23.5)	0.32	0.02	< 0.01
Naive in CD4 ⁺ T cells	37.2 (17.8–49.5)	35.1 (17.6–40.0)	51.1 (39.6–57.0)	0.05	0.01	< 0.01
Tscm in CD4 ⁺ T cells	3.1 (2.1–4.8)	4.2 (2.7–5.4)	2.8 (2.1–3.9)	0.06	0.51	0.03
Tmnp in CD4 ⁺ T cells	3.4 (1.5–6.2)	8.1 (2.7–13.6)	3.1 (1.5–9.1)	0.12	0.78	0.17
Central memory in CD4 ⁺ T cells	27.8 (20.0–32.0)	29.3 (22.1–31.8)	24.8 (18.2–29.1)	0.25	0.31	0.19
Effector memory in CD4 ⁺ T cells	25.8 (15.2–41.4)	24.9 (18.5–38.2)	16.0 (12.2–21.8)	0.46	0.02	< 0.01
Terminal effector in CD4 ⁺ T cells	1.3 (0.6–2.9)	2.4 (0.9–4.5)	0.7 (0.4–1.3)	0.02	0.17	0.01
CCR7 ⁺ in CD8 ⁺ T cells	24.3 (13.1–36.1)	18.6 (9.2–36.5)	41.5 (35.9–54.6)	0.02	< 0.001	< 0.001
CCR7 ⁻ in CD8 ⁺ T cells	75.7 (63.9–87)	81.4 (63.6–90.8)	58.6 (45.4–64.2)	0.02	< 0.001	< 0.001
Naive in CD8 ⁺ T cells	5.7 (2.4–10.3)	2.2 (0.7–7.6)	10.8 (6.5–23.1)	< 0.01	< 0.01	< 0.001
Tscm in CD8 ⁺ T cells	3.4 (2.1–5.5)	3.0 (2.2–3.4)	3.5 (2.0–4.6)	0.04	1.0	0.16
Tmnp in CD8 ⁺ T cells	6.6 (4.7–22.2)	7.9 (3.3–23.0)	22.3 (11.5–29.3)	0.42	< 0.01	0.01
Central memory in CD8 ⁺ T cells	3.6 (2.4–6.3)	2.6 (1.8–3.6)	4.1 (3.0–5.2)	< 0.01	0.61	0.01
Effector memory in CD8 ⁺ T cells	30.9 (16.7–43.5)	26.1 (19.2–33.3)	31.2 (19.4–35.6)	0.22	0.95	0.82
Terminal effector in CD8 ⁺ T cells	43.4 (32.5–48.9)	49.3 (30.9–58.6)	23.5 (17.3–34.1)	0.02	< 0.01	< 0.01

IQR: Interquartile range

Tscm: stem cell-like memory T cell

Tmnp: memory T cells with naïve phenotype

Table S6. Comparison of cytokine production in CD4⁺ and CD8⁺ T cells between

lymphedema, post-lymphatic venous anastomosis (LVA), and healthy controls (HCs);

Related to Figures 7 and 8.

Demographic %, median (IQR)	Lymphedema (n = 10)	Post-LVA (n = 10)	HCs (n = 10)	p-value		
				Lymphedema vs. Post-LVA	HCs vs. Lymphedema	Post-LVA vs. HCs
IFN- γ in CD3 ⁺ 4 ⁺ T cells	10.8 (7.1–20.3)	9.7 (6.1–20.1)	6.9 (4.6–9.7)	0.18	0.03	0.28
IFN- γ in CD3 ⁺ 4 ⁺ PD-1 ⁺ T cells	30.1 (19.7–40.9)	24.7 (19.0–26.1)	20.6 (16.0–25.1)	0.04	0.03	0.44
IFN- γ in CD3 ⁺ 4 ⁺ PD-1 ⁻ T cells	5.8 (3.6–11.1)	5.9 (2.8–12.5)	3.7 (3.0–5.0)	0.29	0.05	0.38
IL-4 in CD3 ⁺ 4 ⁺ T cells	2.1 (1.5–5.8)	2.3 (2.2–3.8)	1.9 (1.2–2.3)	0.47	0.28	0.12
IL-4 in CD3 ⁺ 4 ⁺ PD-1 ⁺ T cells	4.9 (4.3–11.1)	4.5 (3.0–5.5)	4.5 (3.5–5.5)	0.34	0.25	0.92
IL-4 in CD3 ⁺ 4 ⁺ PD-1 ⁻ T cells	1.4 (0.8–3.9)	1.8 (1.3–2.9)	1.1 (0.8–1.5)	0.29	0.37	0.10
IL-17A in CD3 ⁺ 4 ⁺ T cells	2.7 (1.8–3.3)	1.6 (0.9–2.3)	1.7 (1.5–2.2)	0.01	0.06	0.60
IL-17A in CD3 ⁺ 4 ⁺ PD-1 ⁺ T cells	5.5 (3.6–6.3)	4.0 (2.7–4.5)	6.2 (4.9–6.8)	0.04	0.29	< 0.01
IL-17A in CD3 ⁺ 4 ⁺ PD-1 ⁻ T cells	1.3 (0.8–2.5)	0.8 (0.4–1.6)	0.7 (0.5–1.1)	0.01	0.10	0.79
IFN- γ in CD3 ⁺ 8 ⁺ T cells	47.1 (38.1–56.6)	41.2 (40.3–49.8)	31.3 (19.5–44.8)	0.23	0.02	0.05
IFN- γ in CD3 ⁺ 8 ⁺ PD-1 ⁺ T cells	66.2 (59.5–70.5)	52.6 (43.0–61.1)	64.6 (42.5–75.3)	0.07	0.76	0.32
IFN- γ in CD3 ⁺ 8 ⁺ PD-1 ⁻ T cells	45.9 (34.5–54.8)	41.0 (39.0–47.9)	27.6 (17.4–40.3)	0.27	0.01	0.02
IL-4 in CD3 ⁺ 8 ⁺ T cells	1.8 (0.8–2.6)	1.6 (0.5–2.4)	1.0 (0.8–1.4)	0.34	0.16	0.38
IL-4 in CD3 ⁺ 8 ⁺ PD-1 ⁺ T cells	2.3 (0.9–2.7)	2.0 (1.3–2.2)	2.1 (1.2–2.7)	0.34	0.87	0.44
IL-4 in CD3 ⁺ 8 ⁺ PD-1 ⁻ T cells	1.7 (0.7–2.6)	1.5 (0.5–2.4)	1.0 (0.7–1.2)	0.40	0.20	0.24
IL-17A in CD3 ⁺ 8 ⁺ T cells	0.5 (0.2–0.6)	0.2 (0.1–0.4)	0.2 (0.1–0.5)	0.16	0.33	0.66
IL-17A in CD3 ⁺ 8 ⁺ PD-1 ⁺ T cells	0.7 (0.5–1.2)	0.4 (0.2–0.8)	0.7 (0.3–0.9)	0.10	0.50	0.37
IL-17A in CD3 ⁺ 8 ⁺ PD-1 ⁻ T cells	0.4 (0.1–0.6)	0.1 (0.1–0.4)	0.2 (0.1–0.4)	0.16	0.29	0.79

IQR: Interquartile range

Table S7. Comparison of diversity index of T cell receptor between lymphedema, post-lymphatic venous anastomosis (LVA), and healthy controls (HCs); Related to Figure 9.

Demographic, median (IQR)	Lymphedema (n = 6)	Post-LVA (n = 6)	HCs (n = 5)	P value		
				Lymphedema vs. Post-LVA	HCs vs. Lymphedema	Post-LVA vs. HCs
Total read, n	223228 (156631–333851)	235896 (154459–379691)	336850 (249651–431229)	0.22	0.12	0.24
Functional read, n	122939 (75361–184743)	135626 (75706–193075)	185662 (134533–226968)	0.28	0.17	0.32
In flame read, n	118995 (70013–181235)	130535 (72726–188194)	175301 (127220–214288)	0.16	0.24	0.41
Unique read, n	6596 (5515–9933)	8532 (7497–10447)	11358 (10681–20184)	0.28	0.03	0.04
Shannon Weaver index	4.7 (4.3–5.4)	6.1 (5.4–6.9)	6.1 (5.8–6.6)	0.02	0.01	0.86
Inverse Simpson's index	44.1 (30.6–95.1)	200.5 (47.2–287)	151.0 (132.0–299.5)	0.02	0.03	1.00
Pielou's evenness	0.56 (0.50–0.61)	0.67 (0.60–0.75)	0.67 (0.63–0.69)	0.02	< 0.01	1.00
Diversity Evenness 50	0.4 (0.2–0.6)	1.2 (0.4–2.7)	1.1 (0.7–1.2)	0.03	0.01	0.72

IQR: Interquartile range

Table S8. Top 50 increased TCR β clones in post-lymphatic venous anastomosis compared with that in lymphedema; Related to Figure 10.

TRBV	TRBJ	CDR3	Amount reads of change
9	2-1	CASSVGQFNEQFF	14897
7-3	2-2	CASIREYTGELFF	7204
28	2-1	CASSIQGNEQFF	7012
5-1	2-1	CASSLEPGGPQFF	6254
6-5	1-2	CASNYKGGNYGYTF	5768
30	2-7	CAWSVTDNEQYF	5723
12-4	2-3	CASSLGWADTQYF	5342
5-5	2-1	CASSPGDSHNEQFF	5182
30	1-1	CAWFADGQNTAEFF	5157
20-1	2-7	CSAREEVGGDEQYF	4957
5-4	1-2	CASSEWDSGSIYGYTF	4891
20-1	2-3	CSALRGSSGGRDTQYF	4748
28	1-1	CASSSLVGFMNTEAFF	4660
14	2-1	CASSPDRARQFF	4623
7-9	2-5	CASSLQGETQYF	4453
3-1	2-5	CASSRRLATQETQYF	4365
7-9	1-6	CASNGGSHSPLHF	4129
5-6	2-5	CASSAVTTQETQYF	4059
27	1-1	CASRRTGEPNTEAFF	4013
5-1	2-3	CASSFQGLSTDTQYF	3954
7-6	1-3	CASSLGTANSNGNTIYF	3918
29-1	2-7	CSAIASGSWEQYF	3885
29-1	1-1	CSVVTGSTEAFF	3661
23-1	1-5	CATGGSQGNQPQHF	3589
6-6	2-2	CASTQTATGELFF	3575
11-2	1-1	CASSLDRGKNTEAFF	3508
12-4	2-5	CASSLQARREETQYF	3448
12-5	1-2	CASGHGPGTGYYGYTF	3432
6-5	1-1	CASSYSIPGAGTEAFF	3399
19	1-5	CASSMTAVGGNQPQHF	3335
20-1	2-7	CSAFGGGLRSYEQYF	3296

20-1	2-1	CSAGSLSYNEQFF	3163
4-1	1-1	CASSYDRLLDTEAFF	3147
2	2-7	CASRGTVYEQYF	3111
29-1	2-7	CSVAVHTTYEQYF	3084
5-6	2-1	CASSPPGGGPHEQFF	3065
20-1	1-4	CSARASGGPNEKLFF	3004
2	2-5	CASSEERETQYF	2968
7-3	1-6	CASSLNAGANSPLHF	2935
11-2	1-3	CASSQGSMTGFTIYF	2916
14	1-4	CASSQRGTGINEKLFF	2874
7-9	2-2	CASAPGTLTGELFF	2859
7-2	2-1	CASSLYGGGGRNEQFF	2814
13	2-5	CASSPWTSQVQETQYF	2800
30	1-1	CAWVPGPSSTEAFF	2767
7-8	1-2	CASSRTGIYYGYTF	2713
7-2	1-1	CASSLRTGEAFF	2711
2	1-1	CASTSWTTGDAFF	2669
29-1	1-1	CSSRWGAEAFF	2615
29-1	1-2	CSVEAMDRGRNYGYTF	2613

Table S9. Top 50 reduced TCR β clones in post-lymphatic venous anastomosis compared with that in lymphedema; Related to Figure 10.

TRBV	TRBJ	CDR3	Amount reads of change
6-5	2-2	CASSYSGNTGELFF	17473
11-2	2-7	CASSLTDQGYEQYF	6265
6-5	2-7	CASSSDGFYEQYF	5097
28	1-1	CASMDRGNTEAFF	4438
14	1-1	CASSQDRIEAFF	4289
7-9	1-6	CASSSHESGGVSSPLHF	3324
28	1-1	CASSPPDRGAFF	3178
20-1	2-1	CSARDPPPSGRGNEQFF	2973
30	2-7	CAWSPLGLADLRDNEQYF	2601
7-6	2-7	CASSPGYEQYF	2545
5-1	1-1	CASSLAQGGKHTTEAFF	2458
11-2	1-1	CASSSDSKLNTEAFF	2385
7-9	2-1	CASSLIGVSSYNEQFF	2327
20-1	1-2	CSARGASRAYGYTF	2171
5-4	1-2	CASSRKLTYTF	2095
2	1-6	CASRTGQSYNSPLHF	2063
6-1	2-3	CASSVDHFTDTQYF	2043
30	2-6	CAGQGVLGANVLTF	2039
4-3	2-3	CASSQDLGGRSTDTQYF	2012
19	1-6	CASSQDRNSPLHF	1961
29-1	1-5	CSVAGVNQPQHF	1904
6-3	2-4	CASSYSSSGGTSVAKNIQYF	1851
6-1	1-1	CASSEKENTTEAFF	1828
29-1	1-1	CSVGTGNTEAFF	1827
5-5	2-1	CASSLGWSSGRVSYNEQFF	1810
6-6	2-1	CASRNNGPNNEQFF	1795
20-1	1-2	CSARGDVTDSGDLFNQYTF	1782
6-1	1-1	CASSVVGLNTEAFF	1730
6-1	1-2	CASSDGRELLKLYGYTF	1688
6-1	2-4	CASSVRQEAGKNIQYF	1619
29-1	2-7	CSVASDIHSYEQYF	1595

30	2-1	CACNELGNKGAEQFF	1584
20-1	2-5	CSAGPGLAQETQYF	1569
20-1	2-1	CSAPRAGIADNEQFF	1536
6-1	2-1	CASSDGEQGGKFF	1534
7-9	2-1	CASSPTGGARYNEQFF	1508
20-1	2-7	CSAIQGANEQYF	1503
28	2-5	CASWRLRQETQYF	1502
7-2	2-3	CASSFLPDSGMSTDTQYF	1501
6-6	1-1	CATDRIQWKTNTEAFF	1498
25-1	1-6	CASSESVDGDSPLHF	1481
6-1	2-7	CATGTLFSYEQYF	1478
25-1	2-1	CASSDRRQRSSYNEQFF	1478
5-1	1-2	CASSLEAAGGSGYTF	1460
5-6	1-3	CASSLGQAGGNTIYF	1432
4-1	1-1	CASSQEEQGIKNTEAFF	1408
7-2	2-5	CASSRTGTSGGLQETQYF	1402
5-1	1-6	CASRTGGYADSPLHF	1391
20-1	1-2	CSARAQDYGYTF	1371
5-5	1-1	CASSWGGEFAEAF	1366