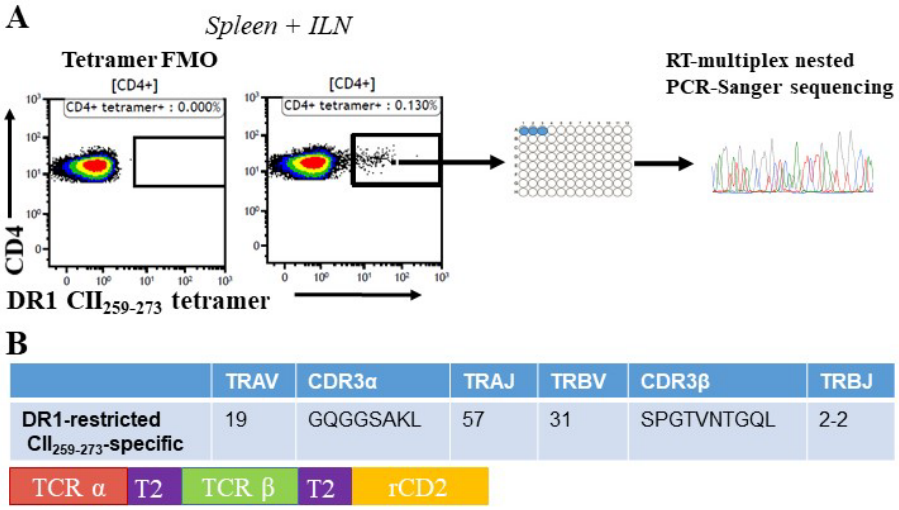
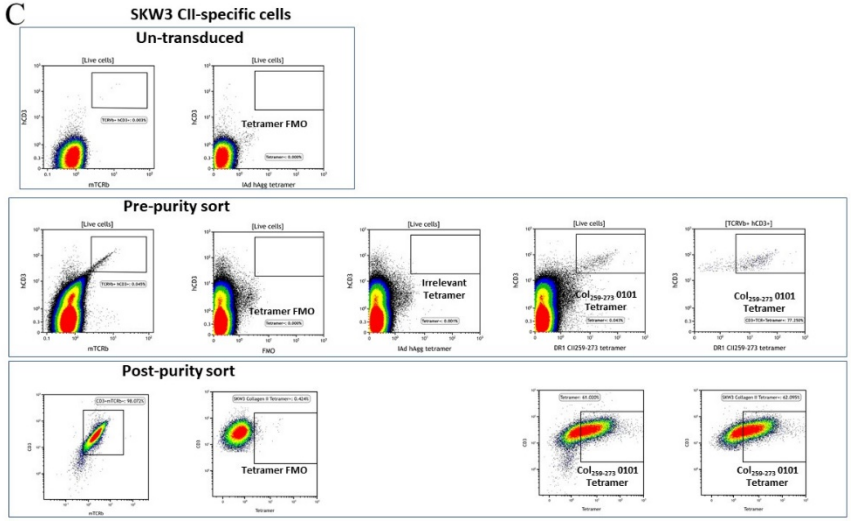
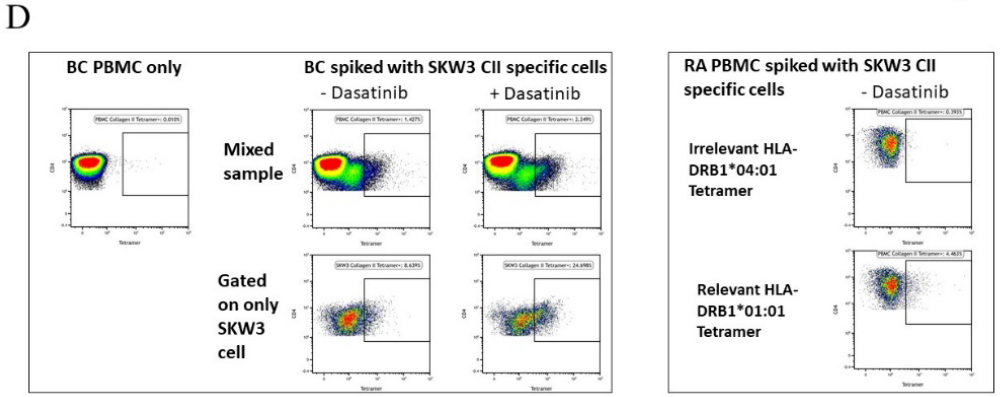
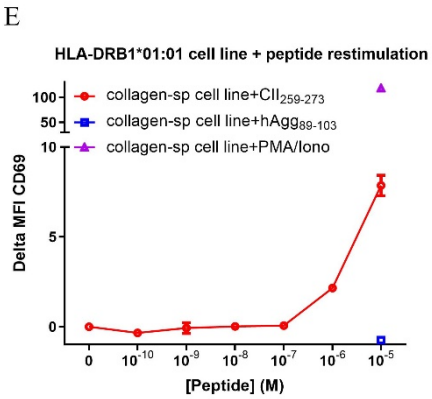
Supplementary Figure 1: Generation of human collagen II$_{259-273}$-specific CD4$^+$ T cell line. (A) Splenocytes and inguinal lymph node cells from HLA-DR1 transgenic mice immunized with bovine type II collagen/CFA, incubated with hCII$_{259-273}$ for 7 days, were stained with PE-conjugated-HLA-DRB1*01:01-CII$_{259-273}$ tetramer then sorted as single tetramer$^+$ and tetramer$^-$ cells. TCR gene usage and clonality was profiled. **(B)** The oligoclonal, CII$_{259-273}$ -specific paired TCRαβ sequence used for TCR cloning. The colored boxes represent the TCR plasmid schematic. **(C)** hCD3, mTCRβ and HLA-DRB1*01:01-CII$_{259-273}$ staining of untransduced, CII-TCR-transduced SKW3 cells before or after sorting for TCR/CD3 stained with FMO, irrelevant tetramer or HLA-DR1*01:01-CII$_{259-273}$ tetramer in presence of Dasatanib, gated on either live cells or CD3+TCR+ cells. **(D)** Healthy buffy coat (BC) PBMC without or spiked with CII-TCR-transduced SKW3 cells without or with Dasatanib. RA PBMC spiked 1:30 with CII-TCR-transduced SKW3 cells, stained with relevant or irrelevant tetramer **(E)** CD69 expression by CII-TCR-transduced SKW3 cells in response to varying concentrations of CII$_{259-273}$, aggrecan$_{89-103}$ or PMA/Ionomycin, in the presence of HLA-DRB1*01:01+ PBMCs. Each data point represents mean ± SEM of duplicates.







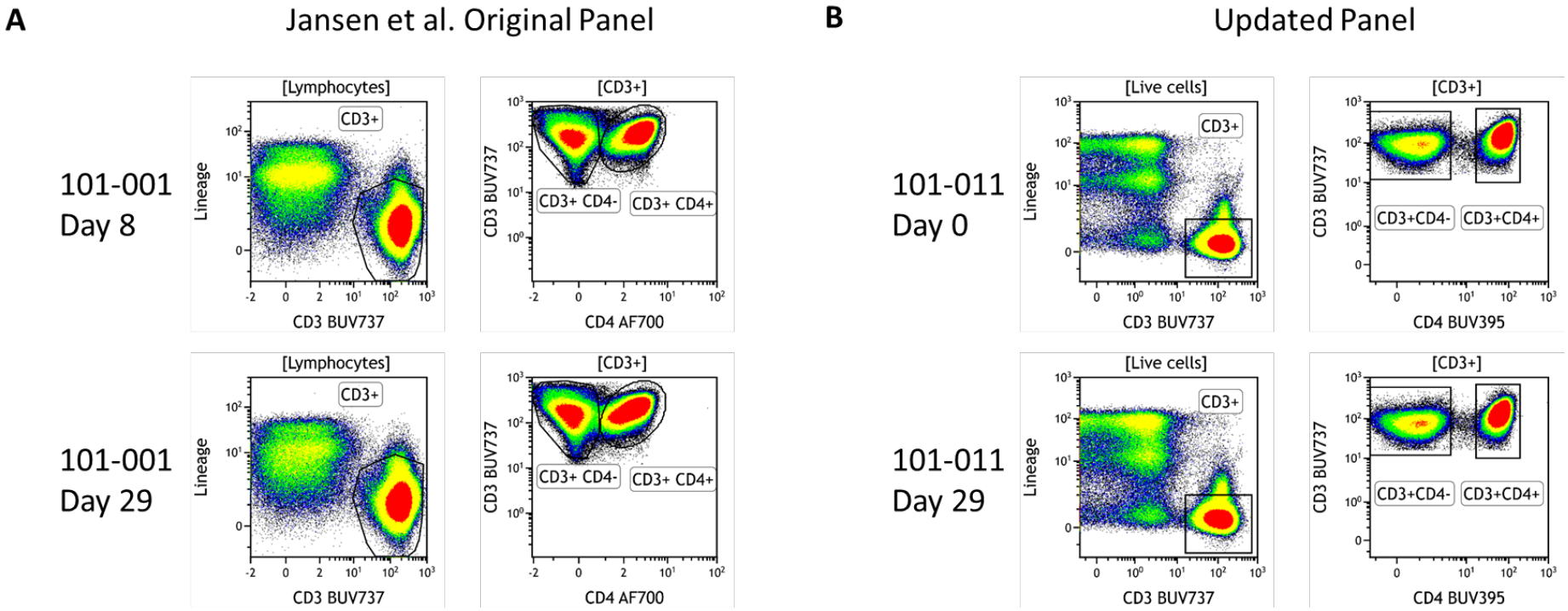


Supplementary Table 1: Nucleotide sequence of human HLA-DRB1*01:01 Collagen II (259-273) TCRα and TCRβ.

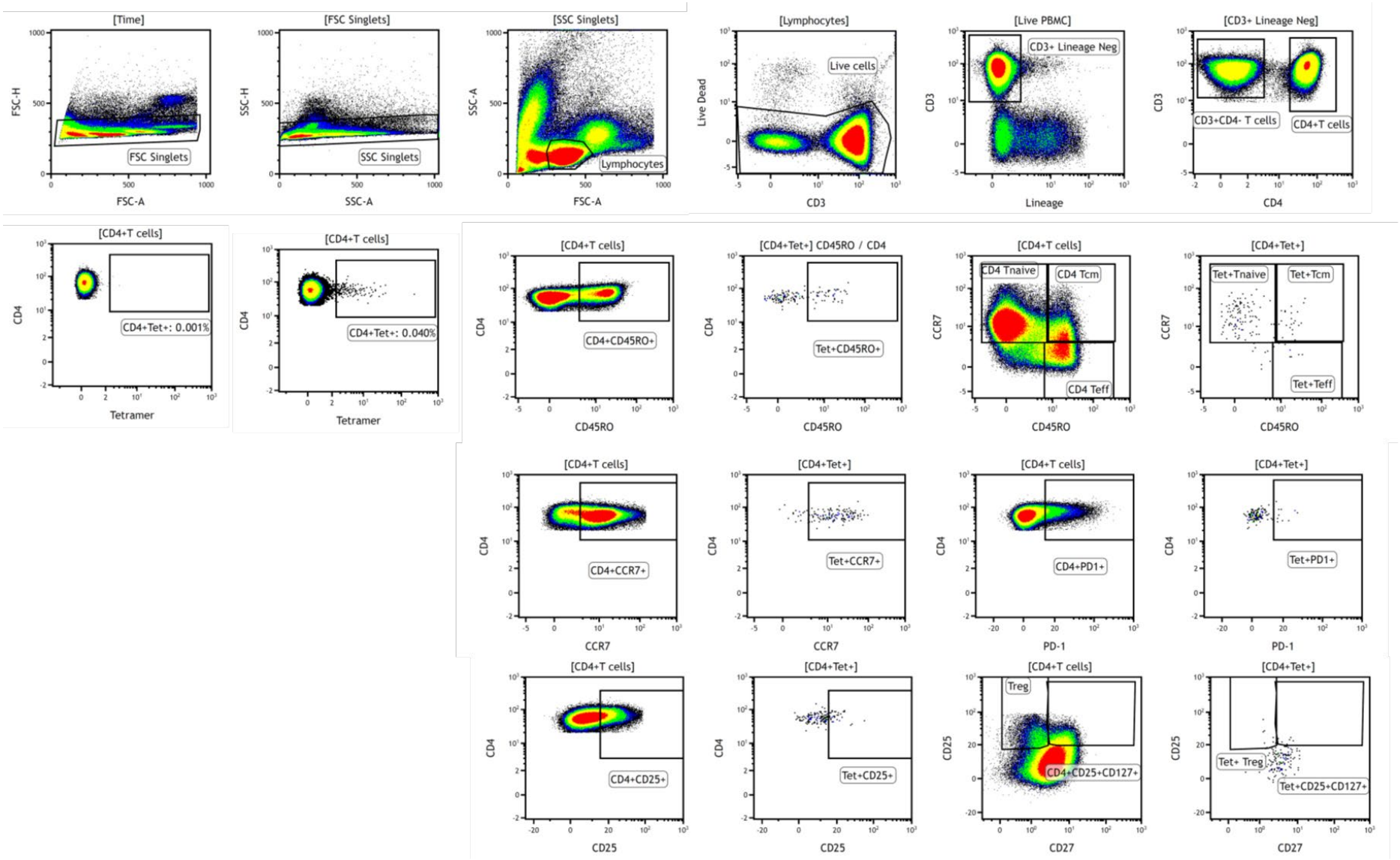
TRAV	Vα	CDR3α	Jα	TRAJ
19	YLCAA	GQGGSAKL	IFG	57
TGCTGTCCTGAGACCGAGGATCTTTTAACTGGTACACAGCAGGTTCTGGGTTCTGGATGTATGAGCTCACTGTCAGCTTTGTCCCC TCCCCAAAGATGAGCTTCGCAGACCCTCCTTGCCCTGCTGCGCAGAGGTACACTGCTGTGTCTTCAGGCTGGGAGGCTGTGATGTG CAGTGAGAAGTGTTTGCCGCTTTTGTTCAAGAAAACTGTGAGTCTTCCGTCTTCCCTCTTATCCACATTTGAGCGTACGGATATCA GGAGTGTGGGGCTGTTGTCAGGGTATTTTTTGTACCAGGCAAAATAGTCAAACATATCATTCTCGTAGTCACAATTCAGGATCGAT ATCCCTCCCTCTT				

TRBV	Vβ	CDR3β	Jβ	TRbJ
31	YLCAW	SPGTVNTGQL	YFG	2-2
TGGCCAGCACACGAGGGTAGCCTTTTGTTTGTTTGCAATCTCTGCTTTTGATGGCTCAAACAAGGAGACCTTGGGTGGAGTCACAT TTCTCAGATCCTCCAGCACTGTCAGCTTTGAGCCTTCACCAAAGTAGAGCTGCCCGGTGTTTACCGTCCCCGGACTCCAGGCACAG AGGTAGAAGCCAGAGTGGCTGAGAAGCAGCTTCTCCGTGCTTAGGATGAATTGGTCGTCCTTCGGCCTGGAAGCTGAGAGGTTCA GTTGCACCACCGACTCTACCTGGCCAACAGA				

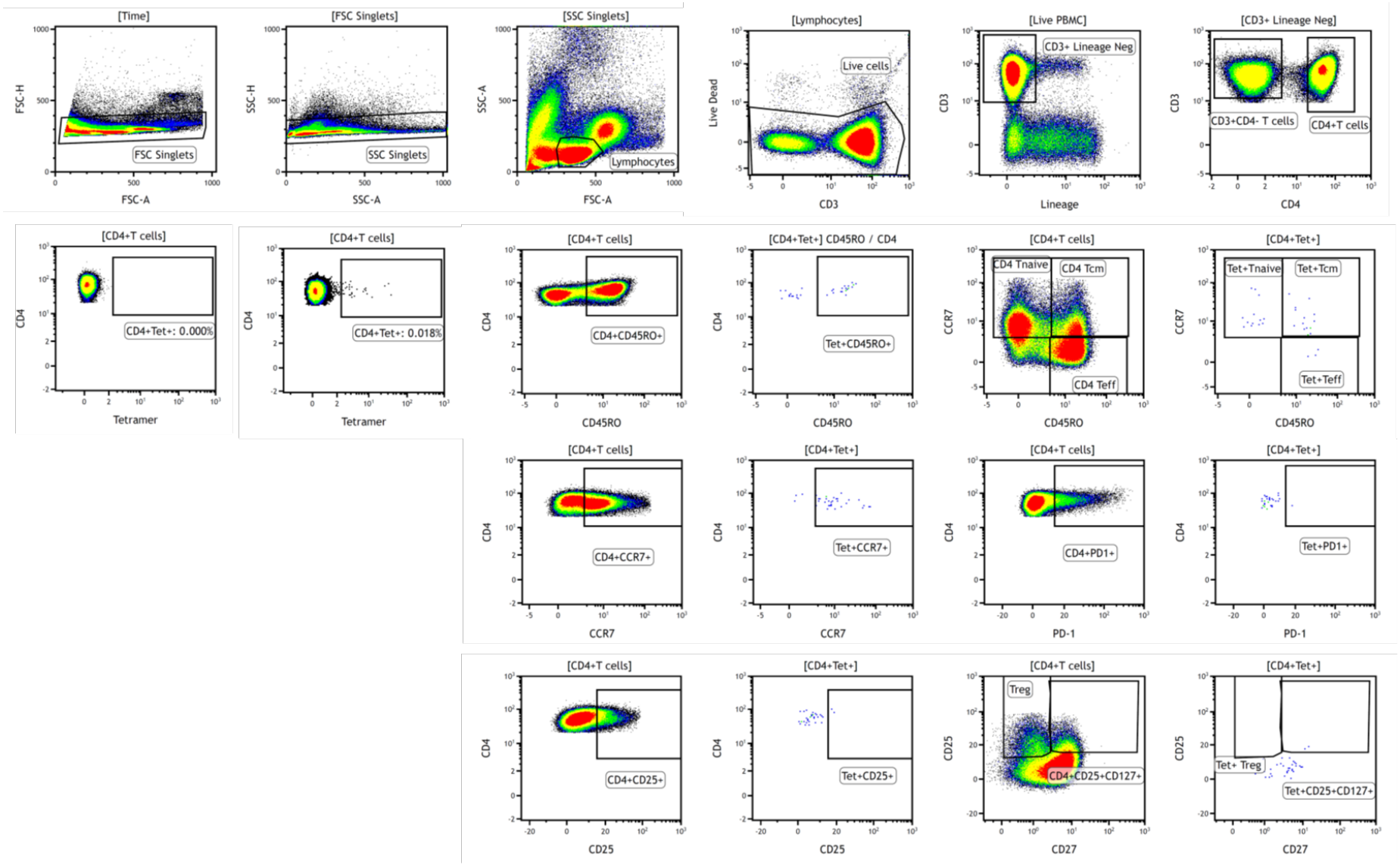
Supplementary Figure 2: Improving CD3+ and CD4+ T cell resolution. Frozen PBMC samples from two RA patient samples, taken at two different points in disease (Baseline and Day 29), were processed and stained with different combinations of fluorescently conjugated antibodies in order to clearly distinguish between CD4+ and CD4- T cells. Representative dot plots are depicted of cells gated using FSC and SSC, singlets, dead cell exclusion and lymphocyte gates. **(A)** Patient 101-001 samples stained according to Jansen et al. (20), where Lineage - CD3+ T cells were pre-gated and dot plots were displayed as CD3 BUV737 vs CD4 AF700. **(B)** Patient 101-011 samples stained according to the updated panel, where Lineage - CD3+ T cells were pregated and dot plots were displayed as CD3 BUV737 vs CD4 BUV395.



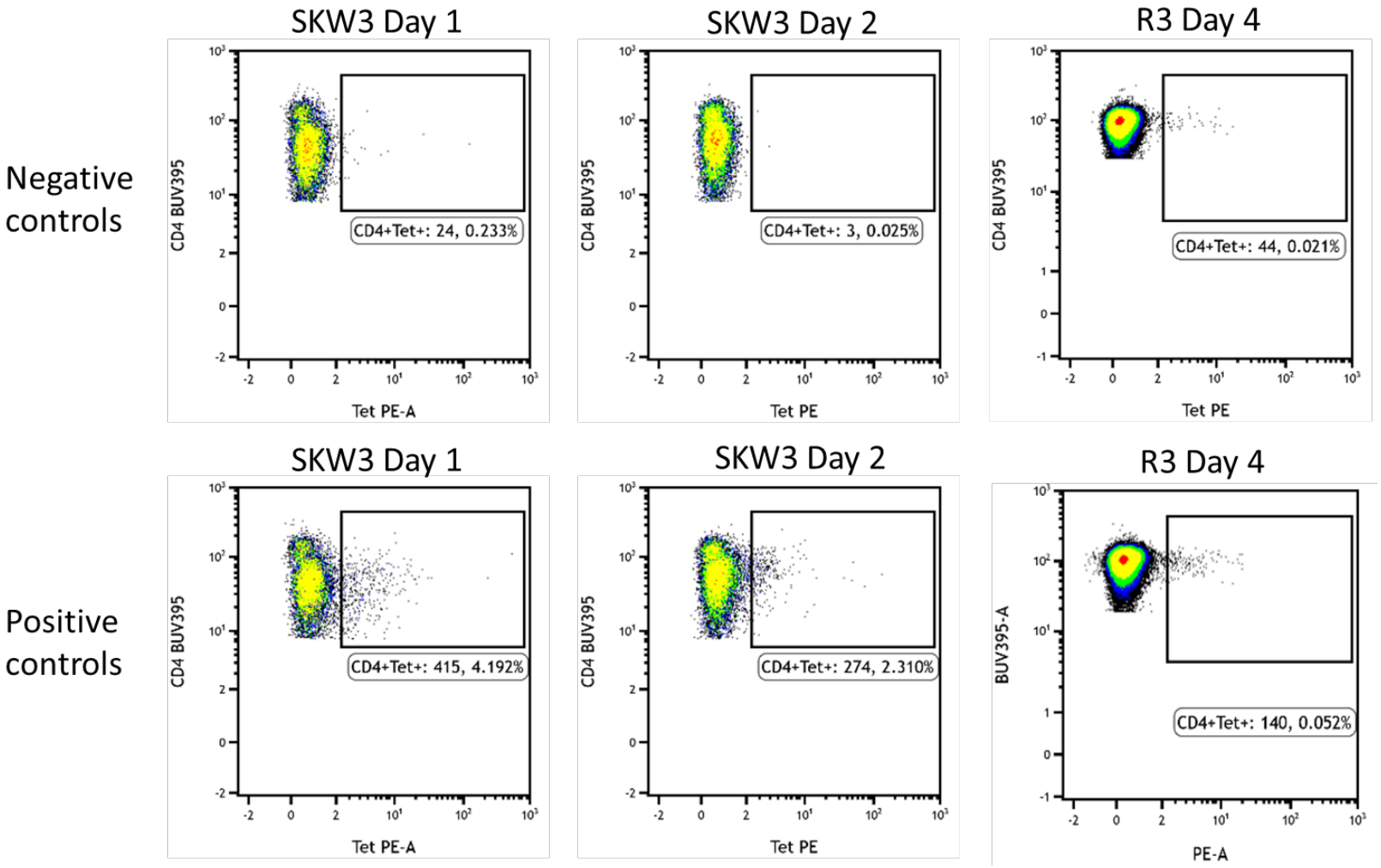
Supplementary Figure 3: Comparative gating strategy for tetramer analysis of patient R1.



Supplementary Figure 4: Comparative gating strategy for tetramer analysis of patient R2.



Supplementary Figure 5: The specificity of HLA-DRB1*04:01- or HLA-DRB1*01:01-CII$_{259–273}$ tetramers for CII-specific cells. Representative positive control samples stained with HLA-DRB1*04:01-CII$_{259-273}$ tetramer, conducted as part of precision (Table 2).



Supplementary Table 2: Results from R1. CD4 T cell subpopulations as percent gated from R1 (HLA-DRB1*04:01) samples performed on Day 1 and 2 with replicate values and calculations of mean, SD and intra-assay precision (%CV). Bold text and values marked with * are identified as outliers when replicates from four assays from the same sample are combined and analysed by outlier test ROUT (Q=5%). Values >35% in %CV are presented in red, as failing to meet for recommended %CV for flow cytometry methods.

Day 1 & 2 HLADRB*0401 R1																	
Day/ Assay No./ Analyst	**Tet+**	**Tet+ CD127- CD25+**	**CD127- CD25+**	**Tet+ CD127+ CD25+**	**CD127+ CD25+**	**Tet+ PD1+**	**PD1+**	**Tet+ CD45RO+**	**CD45RO+**	**Tet+ CCR7+**	**CCR7+**	**Tet+ CD4 Tcm**	**CD4 Tcm**	**Tet+ CD4 Teff**	**CD4 Teff**	**Tet+ CD4 Tnaive**	**CD4 Tnaive**
Day 1 Assay 1 Analyst 1	0.07 ND	8.20 ND	7.11 ND	18.58 ND	17.41 ND	12.77 ND	8.73 ND	27.66 ND	26.40 ND	90.43 ND	84.40 ND	12.23 ND	9.34 ND	6.92 ND	8.28 ND	77.13 ND	73.19 ND
Mean	0.07	8.20	7.11	18.58	17.41	12.77	8.73	27.66	26.40	90.43	84.40	12.23	9.34	6.92	8.28	77.13	73.19
Day 1 Assay 2 Analyst 2	0.05 ND	10.26 ND	7.17 ND	14.10 ND	13.09 ND	8.64 ND	7.24 ND	32.10 ND	29.66 ND	87.65 ND	81.74 ND	18.52 ND	10.29 ND	8.64 ND	12.07 ND	69.14 ND	69.86 ND
Mean	0.05	10.26	7.17	14.10	13.09	8.64	7.24	32.10	29.66	87.65	81.74	18.52	10.29	8.64	12.07	69.14	69.86
Day 2 Assay 3 Analyst 1	0.04 ND	4.26 ND	5.91 ND	4.26 ND	9.83 ND	10.64 ND	11.28 ND	**60.64*** ND	**56.81*** ND	**78.72*** ND	**66.68*** ND	40.43 ND	**24.25*** ND	18.09 ND	**27.32*** ND	36.17 ND	**40.66*** ND
Mean	0.04	4.26	5.91	4.26	9.83	10.64	11.28	60.64	56.81	78.72	66.68	40.43	24.25	18.09	27.32	36.17	40.66
Day 2 Assay 4 Analyst 2	0.04 0.06	10.22 11.48	6.19 7.69	13.14 19.67	11.21 14.90	10.79 8.07	6.82 7.54	30.22 33.87	26.40 26.45	89.93 88.17	81.08 82.82	20.14 19.89	9.80 10.77	3.60 8.07	11.56 10.71	67.63 66.13	70.04 70.79
Mean	0.05	10.85	6.94	16.41	13.06	9.43	7.18	32.04	26.42	89.05	81.95	20.02	10.29	5.83	11.13	66.88	70.41
SD	0.01	0.89	1.06	4.62	2.61	1.93	0.51	2.58	0.03	1.24	1.24	0.18	0.69	3.16	0.60	1.06	0.53
% CV	25.51	8.19	15.26	28.16	19.98	20.45	7.10	8.07	0.13	1.39	1.51	0.89	6.67	**54.18**	5.42	1.58	0.75

Supplementary Table 3: Results from R2. CD4 T cell subpopulations as percent gated from R2 (HLA-DRB1*01:01) samples performed on Day 1 and 2 with replicate values and calculations of mean, SD and %CV. Bold text and values marked with * are identified as outliers when replicates from four assays from the same sample are combined and analysed by outlier test ROUT (Q=5%). Values >35% in %CV are presented in red, as failing to meet for recommended %CV for flow cytometry methods.

Day 1 & 2 HLADRB*0101 R2																	
Day/ Assay No./ Analyst	**Tet+**	**Tet+ CD127- CD25+**	**CD127- CD25+**	**Tet+ CD127+ CD25+**	**CD127+ CD25+**	**Tet+ PD1+**	**PD1+**	**Tet+ CD45RO+**	**CD45RO+**	**Tet+ CCR7+**	**CCR7+**	**Tet+ CD4 Tcm**	**CD4 Tcm**	**Tet+ CD4 Teff**	**CD4 Teff**	**Tet+ CD4 Tnaive**	**CD4 Tnaive**
Day 1 Assay 1 Analyst 1	0.01	13.33	**9.77***	20.00	22.64	**25.00***	12.44	75.00	56.97	68.75	66.62	43.75	22.85	18.75	25.50	18.75	41.53
	0.02	12.12	7.54	18.18	15.09	9.09	11.75	63.64	58.87	45.46	65.64	18.18	23.43	42.42	28.29	27.27	40.11
Mean	0.02	12.73	8.66	19.09	18.86	17.05	12.10	69.32	57.92	57.10	66.13	30.97	23.14	30.59	26.89	23.01	40.82
SD	0.01	0.86	1.58	1.29	5.34	11.25	0.49	8.04	1.35	16.47	0.69	18.08	0.41	16.74	1.98	6.03	1.00
% CV	**50.22**	6.73	18.25	6.73	28.32	**66.00**	4.03	11.59	2.33	28.85	1.04	**58.38**	1.76	**54.73**	7.35	26.19	2.46
Day 1 Assay 2 Analyst 2	0.04	5.95	6.65	11.91	10.05	15.29	9.50	55.29	57.49	65.88	59.01	23.53	17.90	27.06	31.67	42.35	39.22
	0.05	10.00	7.42	18.57	12.54	9.86	10.73	63.38	57.49	60.56	59.09	21.13	18.23	36.62	31.73	36.62	39.09
Mean	0.04	7.98	7.03	15.24	11.29	12.58	10.11	59.34	57.49	63.22	59.05	22.33	18.06	31.84	31.70	39.49	39.16
SD	0.00	2.86	0.54	4.71	1.76	3.84	0.87	5.72	0.00	3.76	0.06	1.70	0.23	6.76	0.04	4.05	0.09
% CV	7.70	**35.89**	7.67	30.93	15.59	30.56	8.61	9.64	0.01	5.95	0.10	7.61	1.28	21.23	0.12	10.27	0.23
Day 2 Assay 3 Analyst 1	0.03	6.25	7.51	15.63	16.76	12.31	10.38	61.54	56.28	75.39	63.43	38.46	21.48	21.54	29.19	36.92	40.21
	0.03	5.88	7.93	19.61	16.59	7.69	7.48	29.81	**28.02***	90.39	80.56	18.27	10.77	5.77	**12.27***	**71.15***	**68.54***
Mean	0.03	6.07	7.72	17.62	16.67	10.00	8.93	45.67	42.15	82.89	71.99	28.37	16.13	13.65	20.73	54.04	54.38
SD	0.00	0.26	0.30	2.82	0.12	3.26	2.05	22.44	19.98	10.61	12.11	14.28	7.57	11.15	11.97	24.20	20.04
% CV	7.26	4.29	3.87	15.99	0.73	32.64	22.93	**49.12**	**47.40**	12.80	16.82	**50.34**	**46.95**	**81.67**	**57.72**	**44.79**	**36.85**
Day 2 Assay 4 Analyst 2	0.02	0.00	7.07	9.68	11.06	6.45	7.77	64.52	53.09	90.32	53.71	48.39	14.50	9.68	31.51	35.48	37.63
	0.02	0.00	7.08	20.59	11.76	5.88	8.45	76.47	53.18	76.47	57.79	50.00	16.41	14.71	30.19	26.47	39.72
Mean	0.02	0.00	7.08	15.13	11.41	6.17	8.11	70.49	53.14	83.40	55.75	49.19	15.45	12.19	30.85	30.98	38.68
SD	0.00	0.00	0.00	7.72	0.50	0.40	0.48	8.45	0.06	9.79	2.89	1.14	1.35	3.56	0.94	6.37	1.48
% CV	5.68	0.00	0.07	**50.98**	4.39	6.54	5.88	11.99	0.12	11.74	5.18	2.32	8.76	29.17	3.04	20.57	3.82

Supplementary Table 4: Results from buffy coat sample. CD4 T cell subpopulations as percent gated from buffy coat sample performed on Day 3 with replicate values and calculations of mean, SD and %CV. Bold text and values marked with * are identified as outliers when replicates from four assays from the same sample are combined and analysed by outlier test ROUT (Q=5%). Values >35% in %CV are presented in red, as failing to meet for recommended %CV for flow cytometry methods.

Day 3 Buffy coat									
Day/ Assay No./Analyst	**CD127- CD25+**	**CD127+ CD25+**	**PD1+**	**CD45RO+**	**CCR7+**	**CD4 Tcm**	**CD4 Teff**	**CD4 Tnaive**	**CD4 T Cells**
Day 3	7.61	19.79	13.51	55.73	78.53	31.66	19.68	45.25	76.16
Assay 5	7.39	17.47	13.16	54.54	75.94	28.51	21.32	45.74	75.96
Analyst 1	7.97	20.30	14.25	55.59	81.99	34.28	16.89	46.03	76.23
Mean	7.66	19.19	13.64	55.28	78.82	31.48	19.30	45.67	76.12
SD	0.29	1.51	0.56	0.65	3.04	2.89	2.24	0.39	0.14
% CV	3.84	7.88	4.08	1.18	3.85	9.19	11.62	0.86	0.18
Day 3	5.72	10.40	11.80	53.26	77.13	27.96	20.55	47.49	75.88
Assay 6	5.77	10.37	11.55	53.56	77.48	28.49	20.37	47.30	75.81
Analyst 2	5.80	10.61	11.50	52.36	78.88	28.51	19.19	48.67	75.76
Mean	5.77	10.46	11.62	53.06	77.83	28.32	20.04	47.82	75.82
SD	0.04	0.13	0.16	0.62	0.93	0.31	0.74	0.74	0.06
% CV	0.73	1.26	1.39	1.18	1.19	1.10	3.69	1.56	0.08

Supplementary Table 5: Results from R3. CD4 T cell subpopulations as percent gated from buffy coat sample performed on Day 3 with replicate values and calculations of mean, SD and %CV. Bold text and values marked with * are identified as outliers when replicates from four assays from the same sample are combined and analysed by outlier test ROUT (Q=5%). Values >35% in %CV are presented in red, as failing to meet for recommended %CV for flow cytometry methods.

HLADRB*0401 R3																			
Day/ Assay No./ Analyst	**Tet+**	**Tet+ CD127 - CD25+**	**CD127 - CD25+**	**Tet+ CD127 + CD25+**	**CD127 + CD25+**	**Tet+ PD1+**	**PD1+**	**Tet+ CD45RO +**	**CD45RO+**	**Tet+ CCR7+**	**CCR7+**	**Tet+ CD4 Tcm**	**CD4 Tcm**	**Tet+CD4 Teff**	**CD4 Teff**	**Tet+CD4 Tnaive**	**CD4 Tnaive**	**Tet+ CD25+**	**CD25+**
Day 4 Assay 7 Analyst 1	0.05	6.58	7.12	35.53	27.39	54.25	41.54	73.86	66.25	75.82	67.23	56.21	43.49	17.65	20.43	24.84	30.67	56.58	48.83
	0.07	6.64	7.93	35.07	31.49	44.70	38.55	71.43	66.27	70.05	68.63	48.85	44.53	19.82	19.41	27.19	30.76	58.77	56.15
	0.05	9.09	7.85	44.70	32.52	53.24	42.25	71.22	66.22	73.38	68.65	49.64	44.46	20.86	19.52	28.06	30.88	68.18	58.52
	0.06	7.55	7.82	33.33	30.13	46.63	37.04	70.55	66.16	71.17	68.44	52.15	44.01	15.34	19.92	23.93	30.92	56.60	55.16
	0.06	8.64	7.34	29.01	27.19	43.64	33.63	66.06	63.86	70.30	64.39	47.27	39.55	16.36	21.72	27.88	32.43	53.70	51.97
Mean	0.06	7.70	7.61	35.53	29.74	48.49	38.60	70.62	65.75	72.14	67.47	50.82	43.21	18.01	20.20	26.38	31.13	58.77	54.13
SD	0.01	1.14	0.36	5.74	2.40	4.93	3.50	2.84	1.06	2.44	1.82	3.49	2.09	2.31	0.94	1.88	0.73	5.56	3.78
% CV	14.43	14.83	4.72	16.15	8.06	10.16	9.08	4.02	1.61	3.38	2.69	6.87	4.83	12.84	4.65	7.12	2.35	9.47	6.98
Day 4 Assay 8 Analyst 2	0.05	6.62	5.73	22.79	17.92	30.71	27.47	68.57	66.18	65.00	62.22	45.71	37.47	21.43	26.68	29.29	30.58	39.71	36.22
	0.07	5.20	5.81	26.01	17.85	40.68	26.25	68.36	66.21	72.32	60.77	48.59	36.42	17.51	27.77	29.94	30.36	42.77	36.29
	0.05	13.51	5.71	18.92	16.67	39.13	24.80	71.30	66.09	60.87	60.06	38.26	35.80	30.43	28.09	26.09	30.30	46.85	34.90
	0.05	8.33	5.68	19.17	17.81	37.60	27.58	71.20	66.14	64.80	61.97	43.20	37.21	25.60	26.85	25.60	30.52	41.67	36.17
	0.05	3.10	5.86	22.48	18.34	26.36	27.95	70.54	66.27	62.02	63.31	48.06	38.37	22.48	25.84	25.58	30.58	35.66	37.03
Mean	0.05	7.35	5.76	21.87	17.72	34.90	26.81	69.99	66.18	65.00	61.67	44.76	37.05	23.49	27.05	27.30	30.47	41.33	36.12
SD	0.01	3.94	0.07	2.93	0.62	6.10	1.29	1.43	0.07	4.46	1.27	4.22	0.99	4.84	0.90	2.14	0.13	4.11	0.77
% CV	16.56	**53.60**	1.30	13.40	3.52	17.49	4.82	2.04	0.10	6.86	2.06	9.42	2.67	20.60	3.33	7.82	0.43	9.93	2.13

Supplementary Table 6: Calculated required viability to achieve desired precision level. For correlation of precision and viability, from all subpopulations tested across all assays, five subpopulations - tet+ (n=7), tet+PD1+ (n=7), tet+CCR7+ (n=7), CD127-CD25+ (n=9) and CD127+CD25+ (n=9) cells – were significantly negatively correlated ($p \leq 0.05$), with Pearson r values of -0.8142, -0.9684, -0.8421, -0.794 and -0.8012 respectively. The linear regression equations generated from the correlation graphs of these significantly correlated subpopulations were used to extrapolate the minimum viability required to achieve %CV of 10%, 25% and 35% respectively.

Significantly correlated subpopulations	Tet+	Tet+PD1+	Tet+CCR7+	CD127- CD25+	CD127+ CD25+
Linear regression equation used in extrapolating viability values	Y = -1.405*X + 139.3	Y = -2.125*X + 209.4	Y = -0.8525*X + 83.61	Y = -0.6134*X + 59.92	Y = -0.9026*X + 89.03
Extrapolated required % viability for %CV= 35%	74.23	**82.07**	57.02*	40.63*	59.86*
Extrapolated required % viability for %CV= 25%	81.35	**86.78**	68.75*	56.93*	70.94*
Extrapolated required % viability for %CV= 10%	92.03	93.84	**86.35**	**81.38**	**87.56**

Note: In equations, Y = %CV and X= % viability.

*The highest %CV observed in subpopulation tet+CCR7+, CD127-CD25+ and CD127+CD25+ are 28.85%, 28% and 18.25% so calculated viability for % CV 25% and 35% are from out-of-range calculations and hence less reliable than that of viability required for %CV =10%.

Supplementary Table 7: Results from additional RA samples R4 and R5 analysed after qualification exercise. Patient sample details and CD4 T cell subpopulations as percent gated from RA patients R4 and R5 samples performed after completion of the formal qualification exercise, with replicate values and calculations of mean, SD and %CV. Values marked with * are identified as outliers from the replicates of the same sample when analysed by Dixon's test for a single outlier for small data sets and were excluded from the calculations. One replicate from R5 is fully excluded as most of the marker values are identified as outliers. Values >35% in %CV are presented in red, as failing to meet recommended %CV for flow cytometry methods.

Patient sample details:

Patient No.	HLA Type	Cells per vial at the time of freezing (No of Vials thawed)	Frozen on	Cell count after thawing from all vials combined	Cell recovery after thawing (%)	Cell Viability (%)
R4	HLA DR * 0401/0404	20 x 10^6 (2 vials)	22/11/2019	25.7 x 10^6	68.75	98.40
R5	HLA DR * 0401	22 x 10^6 (2 vials)	15/06/2018	37.3 x 10^6	84.77	96.60

CD4 T cell subpopulations as percent gated:

Patient No.	Tet+	Tet+ CD127 - CD25+	CD127 - CD25+	Tet+ CD127 + CD25+	CD127 + CD25+	Tet+ PD1+	PD1+	Tet+ CD45RO+	CD45RO+	Tet+ CCR7+	CCR7+	Tet+ CD4 Tcm	CD4 Tcm	Tet+CD4 Teff	CD4 Teff	Tet+CD4 Tnaive	CD4 Tnaive
R4	283.00	2.12*	7.07	14.49	37.81	92.58	27.21	8.83	63.96	4.32	5.91	12.64	35.18	93.00	26.77	7.51	65.46
	252.00	5.95	10.32	17.86	40.08	91.67	29.76	9.52	60.71	4.28	6.39	13.06	35.04	93.55	27.24	6.95	65.61
	59.00*	5.09	5.09	11.86	32.20	93.22	25.42	6.78	67.80	4.36	6.44	11.81	35.03	92.92	26.61	7.55	65.57
Mean	215.33	6.38	8.32	16.46	39.23	92.57	29.38	8.76	61.86	4.29	6.22	12.52	35.04	93.24	26.92	7.25	65.60
SD	91.68	1.56	2.72	4.23	6.06	0.66	4.23	1.39	5.44	0.06	0.25	0.52	0.11	0.33	0.28	0.32	0.12
% CV	42.57	24.39	32.72	25.69	15.44	0.71	14.39	15.91	8.79	1.49	3.96	4.15	0.33	0.35	1.03	4.43	0.18
R5	52.00*	1.92*	57.69*	19.23*	67.31*	92.31*	55.77*	5.77*	36.54*	7.38*	41.33*	16.37*	51.38*	93.65*	40.82*	6.74*	52.09*
	146.00	34.25	27.40	30.82	73.97	76.71	47.95	23.97	27.40	7.36	40.98	16.28	51.03	93.50	40.39	6.87	52.35
	126.00	43.65	23.81	34.13	73.81	73.02	42.06	30.95	26.19	7.42	40.17	15.94	50.97	93.53	40.40	6.88	52.38
Mean	136.00	38.95	25.60	32.47	73.89	74.86	45.00	27.46	26.79	7.39	40.58	16.11	51.00	93.51	40.39	6.87	52.36
SD	14.14	6.65	2.54	2.34	0.12	2.61	4.16	4.93	0.85	0.05	0.58	0.24	0.04	0.02	0.01	0.01	0.02
% CV	10.40	17.07	9.91	7.20	0.16	3.49	9.24	17.97	3.19	0.61	1.42	1.51	0.08	0.02	0.02	0.08	0.04