



Supplementary information

Ribociclib plus letrozole alters the blood immune profile in older patients with HR⁺/HER2⁻ metastatic breast cancer

Yentl Lambrechts, Sigrid Hatse, Cindy Kenis, Lore Decoster, Evandro de Azambuja, Patrick Neven, Claire Quaghebeur, Frank Cornelis, Didier Verhoeven, Annelore Barbeaux, Jeroen Mebis, Julie Van Dievel, Vincent Verschaeve, Lionel Duck, Eveline De Cuypere, Giuseppe Floris, Christine Desmedt, Annouschka Laenen, Noam Pondé, Hans Wildiers

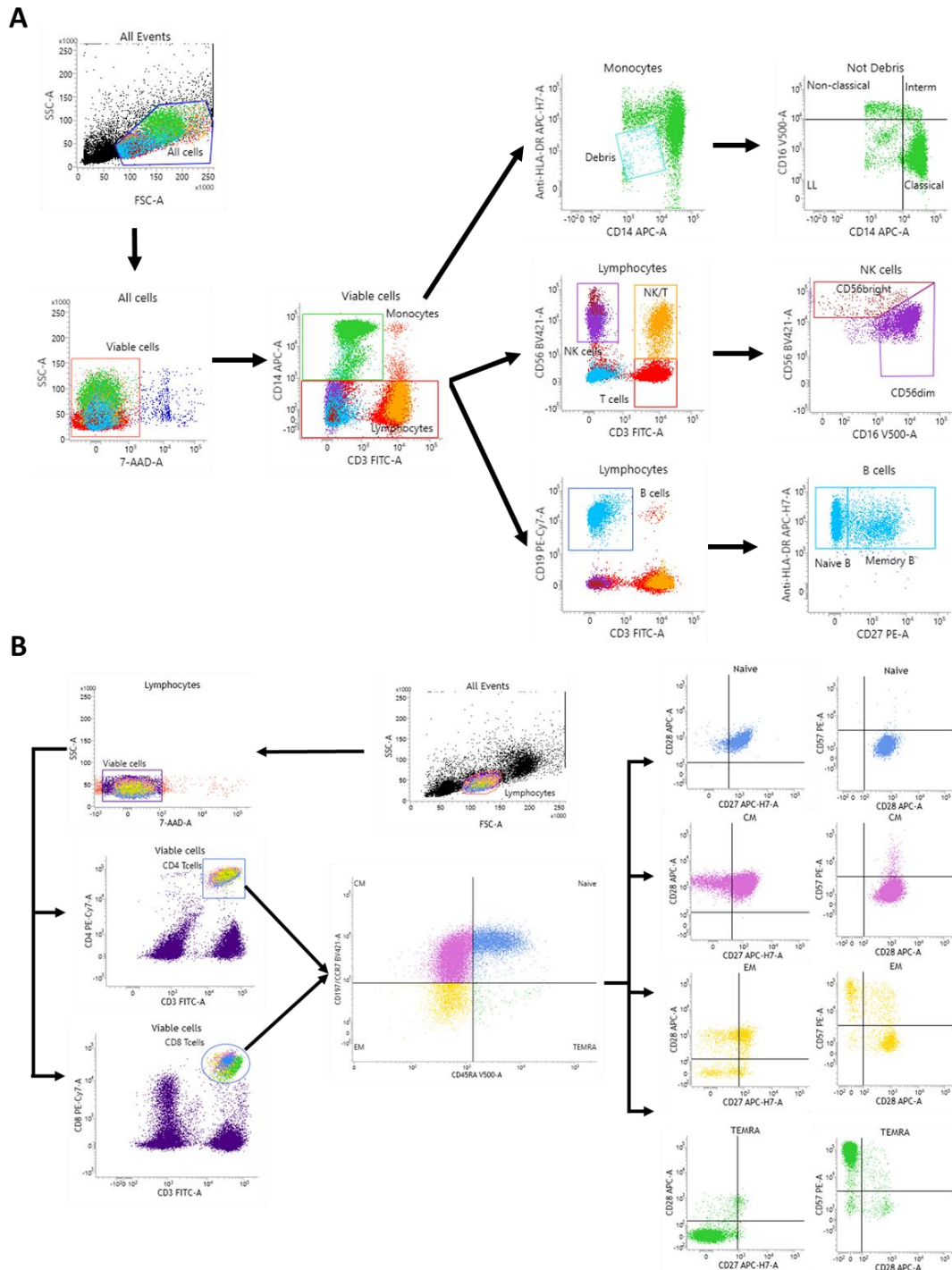


Figure S1. Schematic overview of the flow cytometry gating strategy applied for the (A) major PBMC subsets, containing monocytes, NK-cells, B-cells, NK/T-cells, and total T-cells and (B) CD4⁺ and CD8⁺ T-cell subset profiling using 8-color flow cytometry. FMO controls were used to discriminate between positive and negative signals and to aid gating of the different cell populations. (A) This staining panel included 7-AAD for dead cell exclusion (first gate). The major PBMC subsets (i.e., monocytes, NK-cells, B-cells, NK/T-cells, and total T-cells) were determined using a panel with the following antibody-fluorochrome combination: CD3-FITC, CD14-APC, CD16-V500, CD19-PE.Cy7, CD27-PE, CD56-BV421, and HLA-DR-APC.H7; (B) This staining panel included 7-AAD for dead cell exclusion (first gate). The CD4⁺ T-cell subsets naïve, CM, EM, and terminally differentiated effector memory (TEMRA) were determined using a panel with the following antibody-fluorochrome combination: CD3-FITC, CD4-PE.CY7, CD27-APC.H7, CD28-APC, CD45RA-V500, CD57-PE, and CD197/CCR7-BV421. The CD8⁺ T-cell subsets naïve, CM, EM, and terminally differentiated effector memory (TEMRA) were determined using a panel with the following antibody-fluorochrome combination: CD3-FITC, CD8-PE.CY7, CD27-APC.H7, CD28-APC, CD45RA-V500, CD57-PE, and CD197/CCR7-BV421. CD: cluster of differentiation; Class MO: classical monocytes; Int MO: intermediate monocytes; Non-class MO: non-classical monocytes; MO: monocytes; NK: natural killer; CM: central memory; EM: effector memory; FMO: fluorescence minus one.

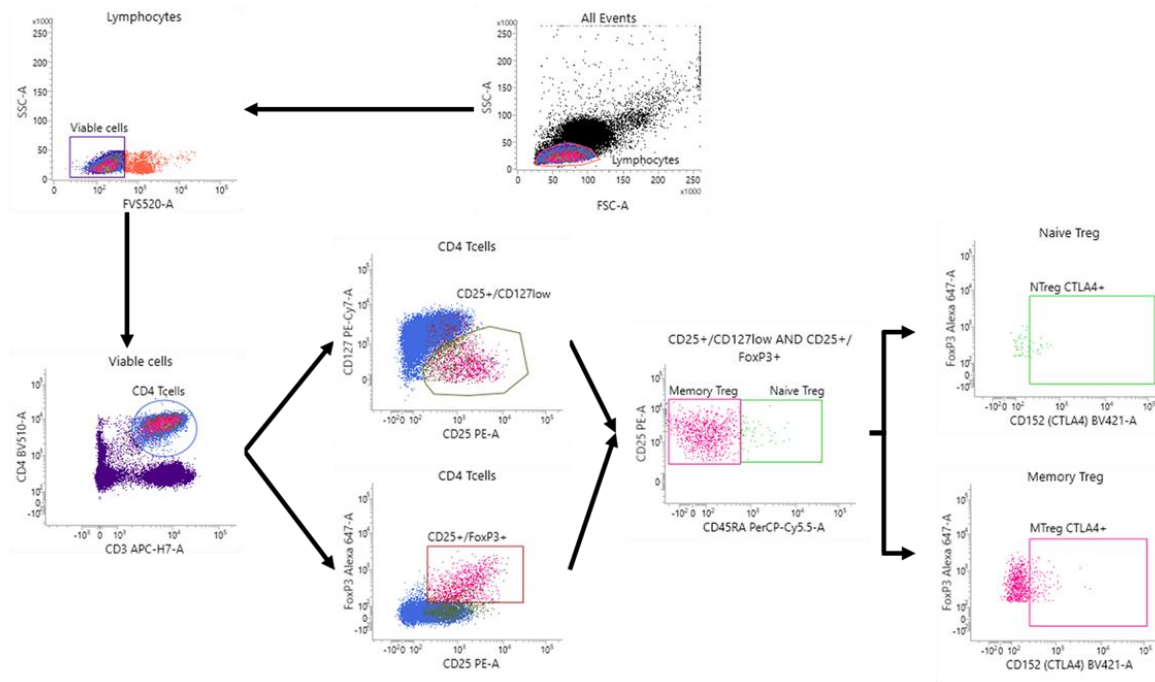
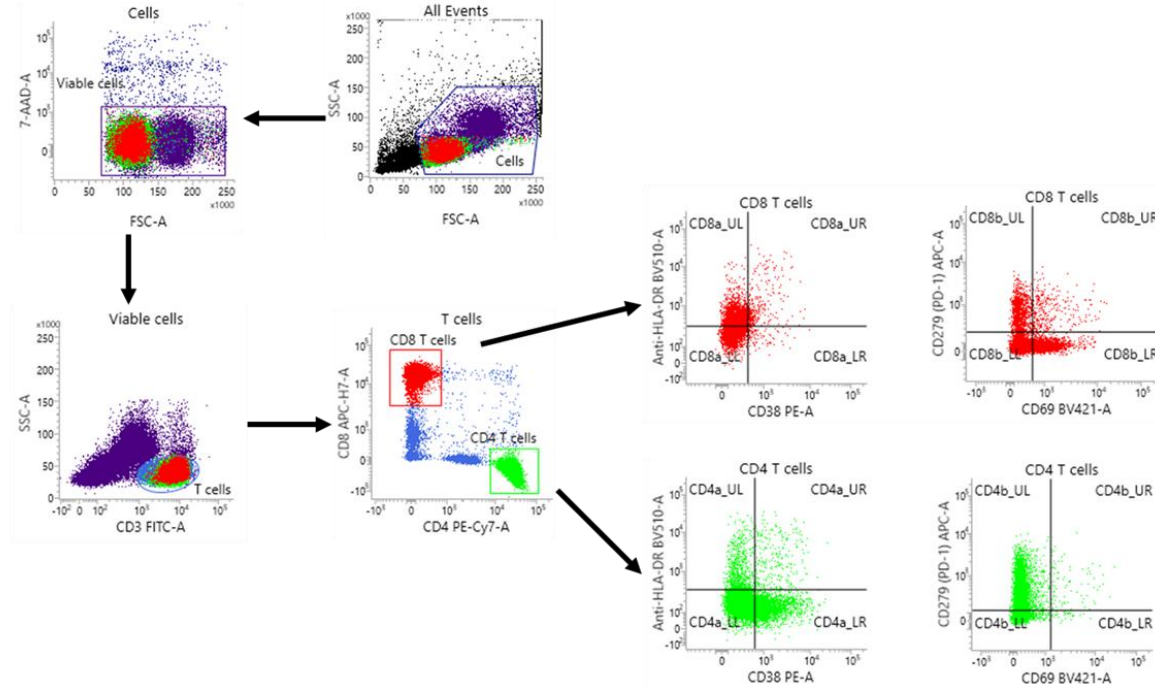
A**B**

Figure S2. Schematic overview of the flow cytometry gating strategy applied for the (A) T-regulatory cell subset and (B) CD4⁺ and CD8⁺ T-cell activation status profiling using 8-color flow cytometry. FMO controls were used to discriminate between positive and negative signals and to aid using of the different cell populations. (A) First gating was applied on the lymphocyte population. Thereafter, this staining panel included FVS520 for dead cell exclusion (second gate). The T-regulatory cells including the naïve and memory subset were determined using a panel with the following antibody-fluorochrome combination: CD3-APC-H7, CD4-BV510, CD25-PE, CD45RA-PerCP-Cy5.5, CD127-PE-Cy7, CD152 (CTLA-4)-BV421, and FoxP3-AF647; (B) This staining panel included 7-AAD for dead cell exclusion (first gate). The CD4⁺ and CD8⁺ T-cell activation status subsets were determined using a panel with the following antibody-fluorochrome combination: CD3-FITC, CD4-PE-Cy7, CD8-APC-H7, CD38-PE, CD69-BV421, CD279 (PD-1)-APC, and HLA-DR-BV510. CD: cluster of differentiation; CTLA-4: cytotoxic T-lymphocyte-associated protein 4; FVS: fixable viability stain; PD-1: programmed cell death protein 1; LL: lower left; LR: lower right; UL: upper left; UR: upper right; FMO: fluorescence minus one.

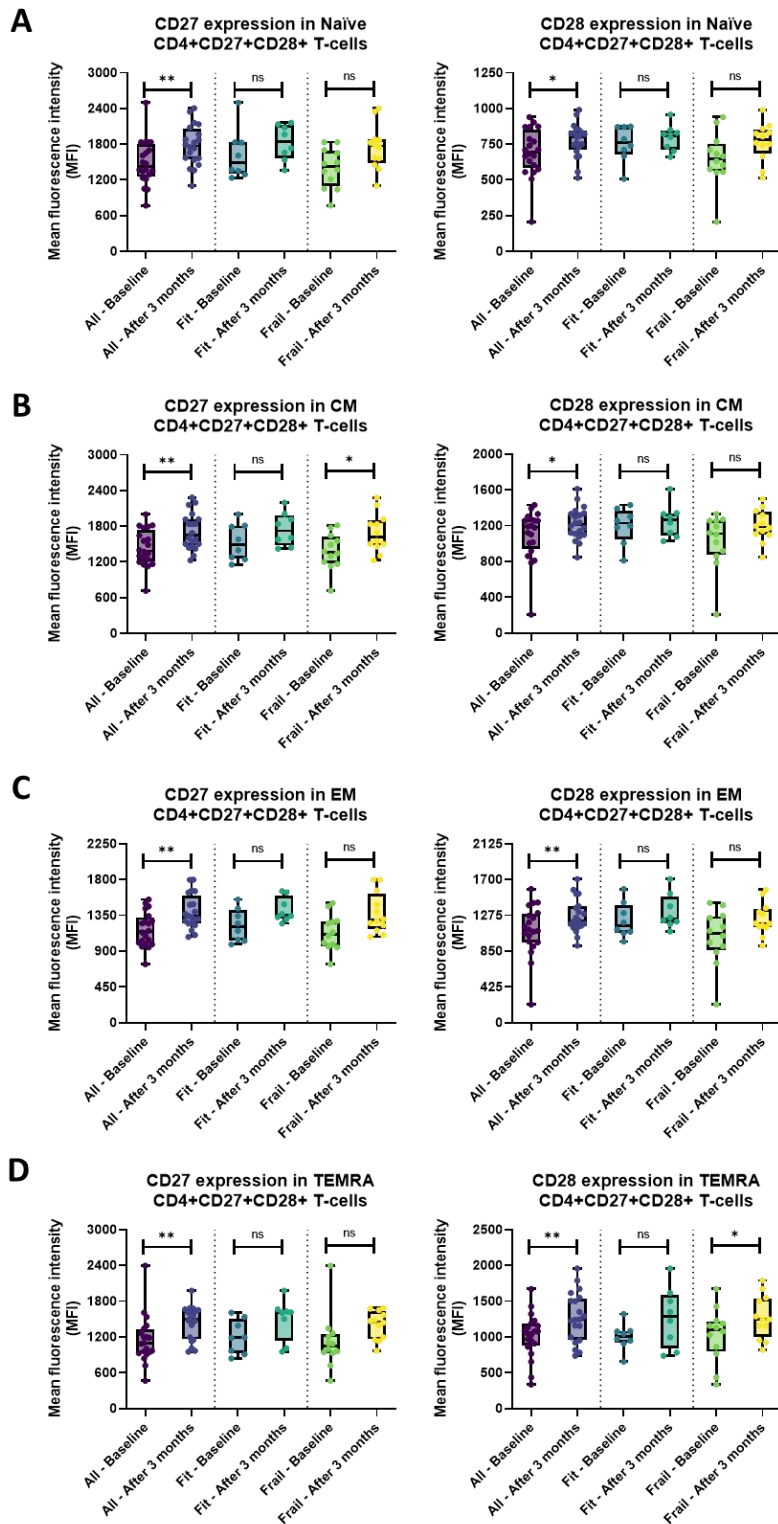


Figure S3. Overview of the CD4⁺ T-cell subsets in the whole patient cohort and the fit and frail patient cohort separately. (A) The CD27 and CD28 expression of the double positive naïve CD4⁺CD27⁺CD28⁺ T-cells was significantly increased after three months of combination therapy in the whole cohort. No significant results were demonstrated in the fit and frail patient population; (B) A significantly increased expression of CD27 and CD28 of the CM CD4⁺CD27⁺CD28⁺ T-cells was noticed after three months of treatment in the whole patient cohort. Within the fit and frail population, no significant differences were found; (C) Within the entire patient population, the expression of the costimulatory receptors CD27 and CD28 of the EM CD4⁺CD27⁺CD28⁺ T-cell population was significantly increased after three months of treatment. Within the frail and fit population, no significant difference was found after three months of combination treatment; (D) In the whole cohort, after three months of treatment, the expression of CD27 and CD28 of the TEMRA CD4⁺CD27⁺CD28⁺ T-cells was significantly increased. In the frail population, a significant increase was shown in the CD28 expression of the TEMRA CD4⁺CD27⁺CD28⁺ T-cells. Within the fit population, no significant results were demonstrated. The boxplots represent the IQR of the percentage of the specific CD4⁺ T-cell subsets or the mean fluorescence intensity (MFI) value. The level of significance is indicated with * for FDR-corrected $P \leq 0.05$, with ** for $P \leq 0.01$, and with ns: no statistical significance. CD: cluster of differentiation; CM: central memory; EM: effector memory; FDR: false discovery rate; IQR: interquartile range; TEMRA: terminally differentiated effector memory re-expressing CD45RA.

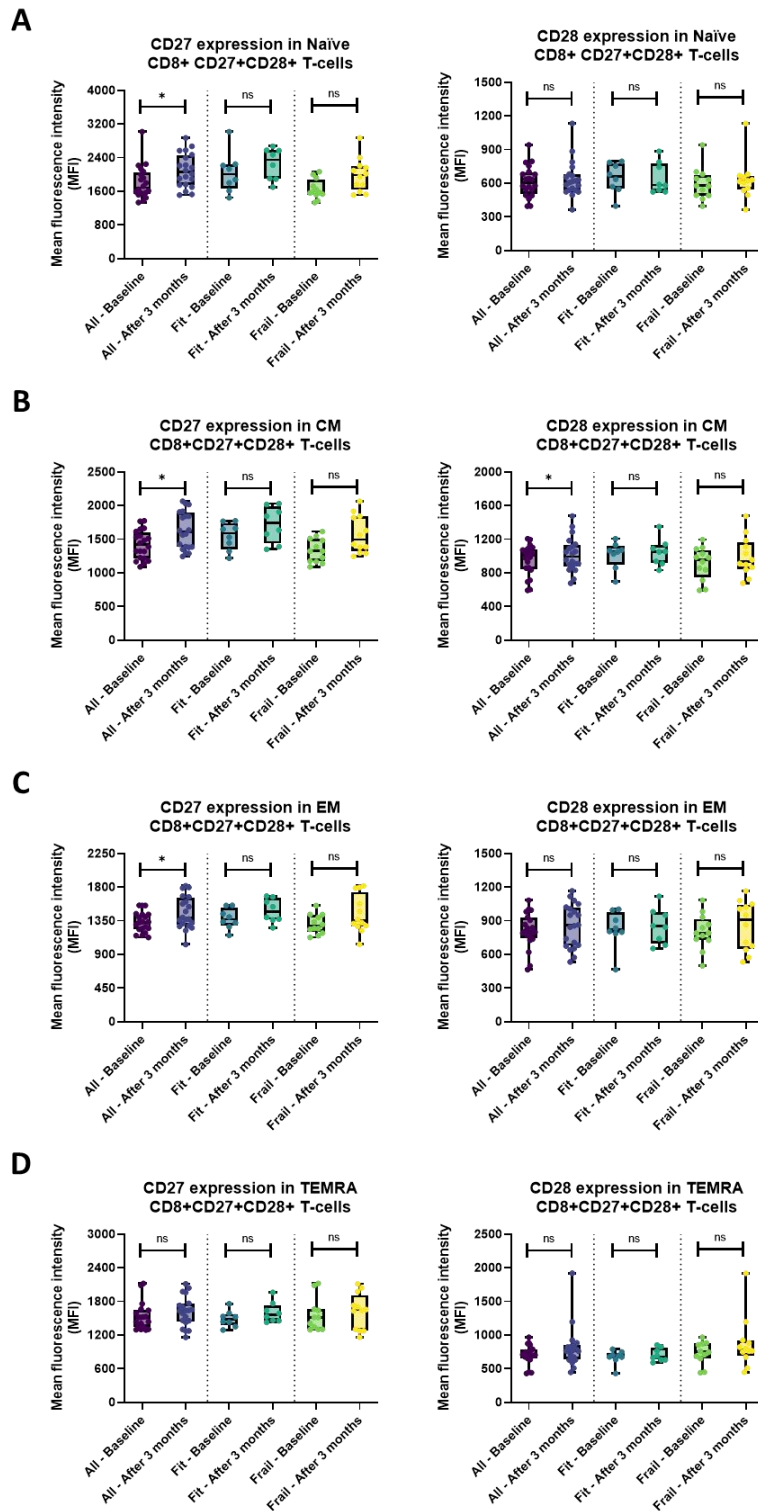


Figure S4. Overview of the CD8+ T-cell subsets in the whole patient cohort and the fit and frail patient cohort separately. (A) In the entire cohort, the CD27 expression in the naïve CD8+CD27+CD28+ T-cells was significantly elevated after three months of combination treatment. Within the frail and fit population, no significant differences were found; (B) In the CM CD8+ T-cell population, the CD27 and CD28 expression were significantly increased. No significant differences were demonstrated in the fit and frail patient population; (C) The expression of CD27 in the double positive EM CD8+CD27+CD28+ T-cells was significantly increased after three months of treatment. No significant differences were found within the fit and frail patient population; (D) No significance was found in the expression of CD27 or CD28 in the double positive TEMRA CD8+CD27+CD28+ T-cells in the entire patient population and the fit and frail population. The boxplots represent the IQR of the percentage of the specific CD8+ T-cell subsets or the mean fluorescence intensity (MFI) value. The level of significance is indicated with * for FDR-corrected $P \leq 0.05$ and with ns: no statistical significance. CD: cluster of differentiation; CM: central memory; EM: effector memory; FDR: false discovery rate; IQR: interquartile range; TEMRA: terminally differentiated effector memory re-expressing CD45RA.

Table S1. Overview of antibody marker information in the PBMC staining panels used in the study cohort. Per PBMC subset, the antibody marker, clone, fluorochrome, company, and catalog number information is provided.

PBMC Subset	Marker	Clone	Fluorochrome	Company	Catalog number
Major Subsets					
	7-AAD			BD Biosciences	555815
	CD3	SK7	FITC	BD Biosciences	345764
	CD14	M ϕ P9	APC	BD Biosciences	345787
	CD16	3G8	V500	BD Biosciences	561394
	CD19	H1B19	PE.Cy7	BioLegend	302216
	CD27	M-T271	PE	BioLegend	56406
	CD56	NCAM16.2	BV421	BD Biosciences	562751
	HLA-DR	G46-6	APC.H7	BD Biosciences	561358
	Brilliant Stain Buffer			BD Biosciences	563794
CD4 T-cells					
	7-AAD			BD Biosciences	555815
	CD3	SK7	FITC	BD Biosciences	345764
	CD4	SK3	PE.Cy7	BD Biosciences	557852
	CD27	MT271	APC.H7	BD Biosciences	560222
	CD28	CD28.2	APC	BioLegend	302912
	CD45RA	HI100	V500	BD Biosciences	561640
	CD57	HNK-1	PE	BioLegend	359612
	CD197/CCR7	G043H7	BV421	BioLegend	353208
	Brilliant Stain Buffer			BD Biosciences	563794
CD8 T-cells					
	7-AAD			BD Biosciences	555815
	CD3	SK7	FITC	BD Biosciences	345764
	CD8	SK1	PE.Cy7	BioLegend	344712
	CD27	MT271	APC.H7	BD Biosciences	560222
	CD28	CD28.2	APC	BioLegend	302912
	CD45RA	HI100	V500	BD Biosciences	561640
	CD57	HNK-1	PE	BioLegend	359612
	CD197/CCR7	G043H7	BV421	BioLegend	353208
	Brilliant Stain Buffer			BD Biosciences	563794
Tregs					
	FVS520			BD Biosciences	564407
	CD3	SK7	APC.H7	BD Biosciences	560275
	CD4	SK3	BV510	BD Biosciences	562971
	CD25	M-A251	PE	BD Biosciences	560989
	CD45RA	HI100	PerCP-Cy5.5	BD Biosciences	563429
	CD127	HIL-7R-M21	PE.Cy7	BD Biosciences	560822
	CD152 (CTLA-4)	BNI3	BV421	BD Biosciences	562743
	FoxP3	236A/E7	AF647	BD Biosciences	561184
	Brilliant Stain Buffer			BD Biosciences	563794
T-cell activation status					
	7-AAD			BD Biosciences	555815
	CD3	SK7	FITC	BD Biosciences	345764
	CD4	SK3	PE.Cy7	BD Biosciences	557852
	CD8	SK1	APC.H7	BD Biosciences	560179
	CD38	HIT2	PE	BD Biosciences	555460
	CD69	FN50	BV421	BD Biosciences	562884
	CD279 (PD-1)	EH12.2H7	APC	BioLegend	329908

HLA-DR	G46-6	BV510	BD Biosciences	563083
Brilliant Stain Buffer			BD Biosciences	563794

Table S2. Overview of the most common adverse events during the combination (ribociclib + letrozole) treatment in the patient cohort ($n = 20$).

Adverse event	Severity		
	Grade 1-2	Grade 3 <i>number of patients (percent)</i>	Grade 4
Any adverse event	20 (100)	12 (60)	2 (10)
Neutropenia	-	7 (35)	2 (10)
Hypertension	1 (5)	12 (60)	-
Pain	12 (60)	4 (20)	-
Fatigue	11 (55)	1 (5)	-
Infections	2 (10)	1 (5)	-
Dyspnea	11 (55)	-	-
Nausea	10 (50)	-	-
Anorexia	10 (50)	-	-
Constipation	9 (45)	-	-
Rash	9 (45)	-	-
Diarrhea	8 (40)	-	-
Dizziness	8 (40)	-	-
Edema	8 (40)	-	-
Dysgeusia	6 (30)	-	-
Cough	6 (30)	-	-
Alopecia	6 (30)	-	-
Vomiting	5 (25)	-	-
Itch	4 (20)	-	-
Hot flashes	4 (20)	-	-
Bloating	4 (20)	-	-
Palpitations	3 (15)	-	-
Myalgia	3 (15)	-	-
Epistaxis	3 (15)	-	-
Other AEs	10 (50)	8 (40)	-

Table S3. Comparison of PBMC subsets (major subsets, CD4 subsets, CD8 subsets, Tregs, and T-cell activation status) and plasma immune response and checkpoint markers in the whole patient population between baseline and after three months of combination therapy. The number of measurements (N), median, interquartile range (IQR), raw p-values, and FDR-corrected p-values are reported. The P-values were calculated using a paired Wilcoxon signed rank test. The p-values below the significance threshold of 5% are indicated in bold italics. FDR: false discovery rate; MFI: mean fluorescence intensity.

Biomarker	Baseline		After 3 months		P-values	
	N	Median [IQR]	N	Median [IQR]	Raw	FDR
PBMC - Major Subsets						
Viable cells (% of total cells)	20	98.99 [98.29;99.26]	20	99.19 [98.76;99.61]	0.182	0.327
Viable lymphocytes (% of total lymphocytes)	20	97.36 [93.46;98.17]	20	97.92 [96.94;98.59]	0.019	0.087
Total T-cells (% of viable lymphocytes)	20	62.16 [55.47;68.32]	20	61.97 [52.98;69.93]	0.841	0.946
Total T-cells (% of viable cells)	20	57.50 [45.84;62.30]	20	57.07 [46.77;64.40]	1.000	1.000
CD4+ T-cells (% of viable lymphocytes)	20	51.66 [39.48;56.80]	20	51.17 [41.65;56.88]	0.841	0.946
CD8+ T-cells (% of viable lymphocytes)	20	15.91 [11.36;18.93]	20	12.58 [9.09;18.00]	0.123	0.327
CD4/CD8 ratio (% CD4/CD8 ratio)	20	3.58 [2.42;4.65]	20	3.31 [2.62;6.73]	0.202	0.331
NK/T-cells (% of viable cells)	20	2.61 [1.49;5.78]	20	3.70 [1.78;5.62]	0.898	0.951
Total NK cells (% of viable cells)	20	7.86 [3.69;11.22]	20	9.63 [6.76;13.51]	0.154	0.327
CD56 bright CD16- (% of total NK cells)	20	4.66 [3.22;9.34]	20	5.90 [3.54;12.09]	0.414	0.622
CD56 dim CD16+ (% of total NK cells)	20	68.35 [47.60;82.71]	20	64.98 [50.43;88.52]	0.571	0.790
Total B-cells (% of viable cells)	20	8.65 [6.16;9.83]	20	8.28 [5.35;11.73]	0.622	0.799
Naïve B-cells (% of total B-cells)	20	60.80 [50.18;74.87]	20	74.80 [63.53;80.94]	0.001	0.012
Memory B-cells (% of total B-cells)	20	37.94 [24.10;47.79]	20	24.48 [18.23;35.21]	0.001	0.012
Total monocytes (% of viable cells)	20	14.81 [10.16;23.10]	20	14.40 [7.36;19.17]	0.177	0.327
Non-classical monocytes (% of total monocytes)	20	4.23 [1.98;6.55]	20	6.16 [3.42;11.02]	0.154	0.327
Intermediate monocytes (% of total monocytes)	20	6.17 [4.46;7.04]	20	8.41 [6.23;11.84]	0.002	0.012
Classical monocytes (% of total monocytes)	20	80.89 [72.55;85.10]	20	73.97 [63.39;81.42]	0.044	0.159
PBMC - CD4 Subsets						
Naïve CD4+ (% of CD4+ T-cells)	20	42.15 [19.56;50.27]	20	45.14 [24.22;58.97]	0.012	0.023
CD27 expression in Naïve CD4+ (MFI)	20	1334.50 [1162.00;1668.50]	20	1733.00 [1476.50;2001.00]	0.002	0.005
CD28 expression in Naïve CD4+ (MFI)	20	686.50 [569.00;825.00]	20	806.00 [712.00;844.50]	0.015	0.025
Naïve CD4+ CD27+ CD28+ (% of Naïve CD4+ T-cells)	20	91.71 [87.13;94.63]	20	96.29 [94.83;98.49]	<0.001	0.005
CD27 expression in Naïve CD4+ CD27+CD28+ (MFI)	20	1424.50 [1265.00;1765.50]	20	1770.50 [1552.00;2023.50]	0.005	0.010
CD28 expression in Naïve CD4+ CD27+CD28+ (MFI)	20	696.50 [590.00;829.00]	20	803.00 [717.50;841.50]	0.023	0.032
Naïve CD4+ CD57+ (% of Naïve CD4+ T-cells)	20	4.07 [1.48;11.63]	20	3.06 [0.78;8.97]	0.504	0.554
CM CD4+ (% of CD4+ T-cells)	20	53.77 [37.11;62.96]	20	46.21 [29.38;58.71]	0.017	0.026
CD27 expression in CM CD4+ (MFI)	19	1170.00 [885.00;1436.00]	19	1477.00 [1216.00;1758.00]	0.001	0.005
CD28 expression in CM CD4+ (MFI)	20	1180.00 [936.00;1290.50]	20	1224.00 [1098.50;1358.00]	0.019	0.028
CM CD4+ CD27+ CD28+ (% of CM CD4+ T-cells)	20	76.72 [65.75;82.10]	20	85.23 [77.53;88.89]	<0.001	0.004
CD27 expression in CM CD4+ CD27+CD28+ (MFI)	20	1386.50 [1222.00;1711.50]	20	1647.00 [1483.00;1917.00]	0.001	0.005
CD28 expression in CM CD4+ CD27+CD28+ (MFI)	20	1185.50 [961.50;1263.50]	20	1211.00 [1103.00;1335.50]	0.014	0.024
CM CD4+ CD57+ (% of CM CD4+ T-cells)	20	2.47 [1.61;3.44]	20	1.95 [1.53;3.06]	0.335	0.394
EM CD4+ (% of CD4+ T-cells)	20	10.51 [6.70;15.07]	20	9.15 [5.77;13.73]	0.231	0.293
CD27 expression in EM CD4+ (MFI)	19	380.00 [280.00;522.00]	19	502.00 [368.00;793.00]	0.001	0.005
CD28 expression in EM CD4+ (MFI)	20	964.00 [834.50;1242.50]	20	1131.00 [1023.00;1260.00]	0.012	0.023
EM CD4+ CD27+ CD28+ (% of EM CD4+ T-cells)	20	31.26 [21.65;41.04]	20	39.21 [32.27;52.50]	0.001	0.005

CD27 expression in EM CD4+ CD27+CD28+ (MFI)	20	1146.00 [995.50;1310.00]	20	1336.50 [1252.00;1563.50]	0.001	0.005
CD28 expression in EM CD4+ CD27+CD28+ (MFI)	20	1099.50 [960.00;1286.50]	20	1209.00 [1148.00;1374.50]	0.002	0.007
EM CD4+ CD27+CD28- (% of EM CD4+ T-cells)	20	0.13 [0.00;0.40]	20	0.11 [0.04;0.27]	0.106	0.140
EM CD4+ CD27-CD28- (% of EM CD4+ T-cells)	20	2.43 [1.23;8.52]	20	1.79 [0.69;5.37]	0.522	0.555
EM CD4+ CD27-CD28+ (% of EM CD4+ T-cells)	20	58.93 [53.20;67.31]	20	48.15 [44.20;64.09]	0.003	0.007
EM CD4+ CD57+ (% of EM CD4+ T-cells)	20	1.88 [1.13;8.33]	20	1.87 [1.20;4.17]	0.312	0.381
TEMRA CD4+(% of CD4+ T-cells)	20	0.48 [0.27;1.92]	20	0.49 [0.26;1.63]	0.735	0.758
CD27 expression in TEMRA CD4+ (MFI)	17	398.00 [154.00;545.00]	17	632.00 [346.00;891.00]	0.003	0.008
CD28 expression in TEMRA CD4+ (MFI)	20	692.50 [231.50;943.50]	20	963.00 [506.50;1171.00]	0.012	0.023
TEMRA CD4+ CD27+CD28+ (% of TERMA CD4+ T-cells)	20	26.93 [11.58;42.57]	20	32.74 [20.24;53.91]	0.002	0.005
CD27 expression in TEMRA CD4+ CD27+CD28+ (MFI)	20	1092.50 [937.00;1301.50]	20	1493.00 [1172.00;1639.50]	0.001	0.005
CD28 expression in TEMRA CD4+ CD27+CD28+ (MFI)	20	1061.00 [887.00;1173.00]	20	1245.00 [975.00;1525.50]	0.001	0.005
TEMRA CD4+ CD27+CD28- (% of TEMRA CD4+ T-cells)	20	1.63 [0.00;10.42]	20	3.00 [0.07;5.95]	0.782	0.782
TEMRA CD4+ CD27-CD28- (% of TEMRA CD4+ T-cells)	20	18.64 [8.39;38.84]	20	10.10 [3.57;31.62]	0.016	0.025
TEMRA CD4+ CD57+ (% of TEMRA CD4+ T-cells)	20	34.32 [17.33;67.51]	20	28.59 [10.78;61.93]	0.368	0.419
PBMC - CD8 Subsets						
Naïve CD8+ (% of CD8+ T-cells)	20	17.62 [8.89;44.02]	20	21.62 [14.09;41.89]	0.036	0.085
CD27 expression in Naïve CD8+ (MFI)	20	1396.00 [1188.00;1865.00]	20	1921.00 [1434.50;2255.00]	0.006	0.023
CD28 expression in Naïve CD8+ (MFI)	20	577.50 [466.00;662.00]	20	584.00 [510.00;663.00]	0.257	0.339
Naïve CD8+ CD27+ CD28+ (% of Naïve CD8+ T-cells)	20	72.14 [60.73;86.15]	20	88.64 [73.50;94.74]	0.006	0.023
CD27 expression in Naïve CD8+ CD27+CD28+ (MFI)	20	1683.50 [1548.50;2036.00]	20	2057.00 [1755.50;2437.00]	0.002	0.019
CD28 expression in Naïve CD8+ CD27+CD28+ (MFI)	20	605.50 [517.50;691.50]	20	610.50 [538.00;674.50]	0.231	0.331
Naïve CD8+ CD57+ (% of Naïve CD8+ T-cells)	20	4.13 [2.10;7.11]	20	2.40 [1.40;4.15]	0.316	0.373
CM CD8+ (% of CD8+ T-cells)	20	25.91 [17.36;33.04]	20	18.02 [15.82;28.72]	0.105	0.166
CD27 expression in CM CD8+ (MFI)	20	1059.00 [800.50;1284.00]	20	1313.00 [955.00;1638.00]	0.002	0.019
CD28 expression in CM CD8+ (MFI)	20	939.50 [769.50;1038.00]	20	947.00 [809.00;1111.00]	0.040	0.088
CM CD8+ CD27+ CD28+ (% of CM CD8+ T-cells)	20	66.45 [51.44;73.09]	20	75.44 [61.14;85.70]	0.011	0.035
CD27 expression in CM CD8+ CD27+CD28+ (MFI)	20	1416.50 [1221.00;1582.00]	20	1605.00 [1383.00;1874.50]	0.002	0.019
CD28 expression in CM CD8+ CD27+CD28+ (MFI)	20	1006.00 [847.00;1079.50]	20	993.00 [882.00;1127.00]	0.058	0.113
CM CD8+ CD57+ (% of CM CD8+ T-cells)	20	10.78 [6.78;13.82]	20	10.64 [7.35;14.80]	0.701	0.746
EM CD8+ (% of CD8+ T-cells)	20	25.14 [14.66;34.27]	20	26.06 [12.65;33.73]	0.083	0.136
CD27 expression in EM CD8+ (MFI)	20	498.50 [390.50;658.00]	20	690.00 [485.00;877.50]	0.003	0.020
CD28 expression in EM CD8+ (MFI)	20	482.00 [372.00;605.50]	20	583.00 [354.00;703.00]	0.071	0.127
EM CD8+ CD27+ CD28+(% of EM CD8+ T-cells)	20	17.57 [11.12;27.87]	20	29.98 [15.76;37.23]	0.006	0.023
CD27 expression in EM CD8+ CD27+CD28+ (MFI)	20	1329.00 [1241.50;1434.50]	20	1385.00 [1297.50;1636.50]	0.012	0.036
CD28 expression in EM CD8+ CD27+CD28+ (MFI)	20	802.00 [752.50;919.00]	20	859.00 [679.00;1014.50]	0.273	0.347
EM CD8+ CD27+CD28- (% of EM CD8+ T-cells)	20	3.01 [1.44;5.60]	20	3.48 [1.85;6.10]	0.058	0.113
EM CD8+ CD27-CD28- (% of EM CD8+ T-cells)	20	25.63 [18.46;40.40]	20	24.14 [15.89;38.55]	0.312	0.373
EM CD8+ CD27-CD28+ (% of EM CD8+ T-cells)	20	46.55 [35.80;59.77]	20	37.81 [28.56;49.84]	0.004	0.020
EM CD8+ CD57+ (% of EM CD8+ T-cells)	20	44.77 [28.23;57.00]	20	48.30 [31.43;54.52]	0.956	0.956
TEMRA CD8+(% of CD8+ T-cells)	20	23.61 [11.02;33.31]	20	24.01 [10.82;31.82]	0.898	0.926
CD27 expression in TEMRA CD8+ (MFI)	20	266.50 [205.00;438.50]	20	291.50 [218.50;578.50]	0.021	0.058
CD28 expression in TEMRA CD8+ (MFI)	20	141.50 [76.00;225.50]	20	141.00 [81.00;228.50]	0.430	0.490
TEMRA CD8+ CD27+CD28+ (% of TERMA CD8+ T-cells)	20	4.02 [1.95;5.575]	20	4.36 [2.34;10.53]	0.033	0.083
CD27 expression in TEMRA CD8+	19	1515.00 [1345.00;1642.00]	19	1638.00 [1455.00;1767.00]	0.073	0.127

CD27+CD28+ (MFI)					
CD28 expression in TEMRA CD8+ CD27+CD28+ (MFI)	19	703.00 [653.00;791.00]	19	761.00 [633.00;827.00]	0.214 0.321
TEMRA CD8+ CD27+CD28- (% of TEMRA CD8+ T-cells)	20	1.54 [0.52;2.82]	20	3.71 [1.35;5.48]	0.001 0.019
TEMRA CD8+ CD27-CD28- (% of TEMRA CD8+ T-cells)	20	73.78 [59.53;84.14]	20	71.32 [57.63;84.78]	0.452 0.498
TEMRA CD8+ CD57+ (% of TEMRA CD8+ T-cells)	20	64.46 [56.19;77.01]	20	69.87 [59.59;79.18]	0.246 0.338
PBMC - Tregs					
Total Treg (% of CD4+ T-cells)	20	5.03 [3.88;6.89]	20	4.59 [3.34;5.36]	0.165 0.259
CD25 expression in Total Treg (MFI)	20	1606.00 [1185.00;2203.50]	20	2077.00 [1235.50;2947.50]	0.033 0.072
CTLA-4 expression in Total Treg (MFI)	20	250.50 [228.50;296.50]	20	284.00 [237.00;316.00]	0.153 0.259
Naïve Treg (% of total Tregs)	20	5.94 [3.67;9.51]	20	10.41 [5.20;17.12]	<0.001 0.001
CD25 expression of Naïve Treg (MFI)	20	1447.50 [835.00;1940.50]	20	2021.00 [1382.50;2454.50]	0.011 0.039
CTLA-4 expression of Naïve Treg (MFI)	20	229.00 [186.00;284.00]	20	268.50 [204.50;310.50]	0.245 0.337
Naïve Treg CTLA-4+ (% of Naïve Tregs)	20	23.25 [15.94;36.73]	20	27.44 [17.37;32.09]	0.953 0.953
Memory Treg (% of total Tregs)	20	93.77 [89.80;96.00]	20	88.90 [82.46;93.80]	<0.001 0.001
CD25 expression of Memory Treg (MFI)	20	1633.00 [1186.00;2178.00]	20	2057.00 [1255.50;2984.00]	0.024 0.066
CTLA-4 expression of Memory Treg (MFI)	20	255.00 [230.50;296.00]	20	273.00 [233.50;313.50]	0.316 0.384
Memory Treg CTLA-4+ (% of Memory Tregs)	20	18.10 [15.59;20.69]	20	18.50 [14.28;24.46]	0.349 0.384
PBMC - T-cell activation status					
CD4+ PD1+ (% of CD4+ T-cells)	20	1.55 [0.89;2.14]	20	1.32 [0.92;3.03]	0.738 0.876
PD1 expression in CD4+ (MFI)	20	201.50 [127.50;235.00]	20	196.00 [145.00;236.00]	0.335 0.669
CD4+ CD69+ (% of CD4+ T-cells)	20	31.94 [22.42;40.79]	20	28.54 [21.94;40.29]	0.546 0.819
CD69 expression in CD4+ (MFI)	20	253.50 [235.00;270.00]	20	254.50 [229.00;297.50]	0.373 0.689
CD4+ PD1+CD69+ (% of CD4+ T-cells)	20	0.93 [0.64;1.58]	20	0.90 [0.58;1.80]	0.985 0.985
CD4+ PD1-CD69- (% of CD4+ T-cells)	20	67.43 [58.65;76.96]	20	70.80 [58.65;77.06]	0.729 0.876
CD4+ HLADR+ (% of CD4+ T-cells)	20	14.41 [7.91;19.78]	20	15.46 [10.39;22.02]	0.114 0.455
HLADR expression in CD4+ (MFI)	20	401.00 [332.50;552.50]	20	421.50 [348.00;506.50]	0.674 0.876
CD4+ CD38+ (% of CD4+ T-cells)	20	31.90 [20.25;45.58]	20	34.62 [22.85;44.25]	0.177 0.472
CD38 expression in CD4+ (MFI)	20	653.50 [488.50;777.00]	20	655.00 [504.00;780.00]	0.877 0.957
CD4+ HLADR+CD38+ (% of CD4+ T-cells)	20	3.08 [1.80;4.02]	20	3.99 [1.94;5.94]	0.066 0.364
CD4+ HLADR-CD38- (% of CD4+ T-cells)	20	54.84 [48.00;67.32]	20	54.62 [41.38;59.53]	0.133 0.455
CD8+ PD1+ (% of CD8+ T-cells)	20	21.31 [12.77;29.14]	20	23.81 [11.97;36.39]	0.430 0.738
PD1 expression in CD8+ (MFI)	20	270.50 [223.00;357.00]	20	292.50 [222.50;380.00]	0.766 0.876
CD8+ CD69+ (% of CD8+ T-cells)	20	31.63 [21.01;47.02]	20	37.83 [19.23;45.60]	0.701 0.876
CD69 expression in CD8+ (MFI)	20	390.50 [319.00;522.50]	20	470.50 [371.00;645.50]	0.154 0.461
CD8+ PD1+CD69+ (% of CD8+ T-cells)	20	7.85 [5.28;12.60]	20	8.60 [4.92;18.08]	0.294 0.642
CD8+ PD1-CD69- (% of CD8+ T-cells)	20	50.24 [39.40;65.04]	20	49.07 [39.66;62.29]	0.927 0.968
CD8+ HLADR+ (% of CD8+ T-cells)	20	40.22 [26.35;46.38]	20	41.86 [32.36;51.53]	0.076 0.364
HLADR expression in CD8+ (MFI)	20	505.50 [449.00;615.50]	20	513.00 [405.00;629.00]	0.515 0.819
CD8+ CD38+ (% of CD8+ T-cells)	20	11.97 [6.20;18.90]	20	12.11 [7.00;24.92]	0.007 0.172
CD38 expression in CD8+ (MFI)	20	280.00 [203.00;350.00]	20	274.50 [201.00;435.50]	0.273 0.642
CD8+ HLADR+CD38+ (% of CD8+ T-cells)	20	5.13 [3.40;9.04]	20	6.51 [3.86;11.61]	0.064 0.364
CD8+ HLADR-CD38- (% of CD8+ T-cells)	20	53.63 [48.44;58.46]	20	48.05 [41.17;57.65]	0.036 0.364
Plasma - Essential Immune Response					
IL-4 (pg/mL)	20	0.06 [0.00;4.26]	20	0.33 [0.00;9.84]	0.890 0.898
IL-2 (pg/mL)	20	2.06 [0.96;4.02]	20	1.44 [0.08;6.75]	0.898 0.898
CXCL-10 (IP-10) (pg/mL)	20	218.23 [157.45;354.14]	20	391.26 [166.73;459.55]	0.036 0.217
IL-1 beta (pg/mL)	20	5.27 [0.77;14.48]	20	4.57 [1.16;13.83]	0.862 0.898
TNF alpha (pg/mL)	20	2.02 [1.53;3.43]	20	1.92 [0.86;3.38]	0.294 0.589

CCL2 (MCP-1) (pg/mL)	20	153.92 [115.28;207.57]	20	185.79 [148.30;248.67]	0.133	0.531
IL-17A (pg/mL)	20	2.55 [1.23;8.50]	20	2.33 [1.36;6.85]	0.196	0.587
IL-6 (pg/mL)	20	6.46 [2.88;9.01]	20	5.88 [2.73;10.83]	0.261	0.589
IL-10 (pg/mL)	20	1.85 [0.67;3.12]	20	0.61 [0.02;1.68]	0.009	0.101
IFN gamma (pg/mL)	20	1.29 [0.00;6.07]	20	2.33 [1.30;6.23]	0.799	0.898
IL-12p70 (pg/mL)	20	4.05 [1.81;6.93]	20	3.67 [2.08;12.22]	0.596	0.898
CXCL-8 (IL-8) (pg/mL)	20	4.30 [2.49;11.62]	20	5.47 [3.20;14.38]	0.648	0.898
Plasma - Immune Checkpoint						
sCD25 (IL-2Ra) (pg/mL)	20	1139.20 [837.97;1965.60]	20	1449.60 [994.13;2002.60]	0.024	0.096
4-1BB (pg/mL)	20	70.44 [54.40;156.36]	20	59.59 [43.26;128.39]	0.048	0.097
sCD27 (pg/mL)	20	55467.00 [48711.00;81131.00]	20	54618.00 [49390.00;71250.00]	0.048	0.097
B7.2 (CD86) (pg/mL)	20	543.30 [463.44;697.47]	20	368.31 [314.93;584.11]	0.001	0.017
Free Active TGF-B1 (pg/mL)	20	25.01 [18.60;35.43]	20	21.13 [8.38;35.87]	0.083	0.142
CTLA-4 (pg/mL)	20	8.84 [2.71;17.19]	20	7.90 [2.69;14.50]	0.032	0.097
PD-L1 (pg/mL)	20	200.04 [118.47;306.25]	20	195.22 [149.03;303.77]	0.956	0.956
PD-L2 (pg/mL)	20	12681.00 [10866.00;15195.00]	20	14435.00 [11462.00;16359.00]	0.123	0.185
PD-1 (pg/mL)	20	49.94 [16.51;151.25]	20	27.45 [16.80;98.56]	0.005	0.029
Tim-3 (pg/mL)	20	22351.00 [13890.00;33622.00]	20	19419.00 [14642.00;33057.00]	0.898	0.956
LAG-3 (pg/mL)	20	2905.30 [1775.20;7456.10]	20	2098.10 [1575.00;6071.20]	0.596	0.715
Gal-9 (pg/mL)	20	105359.00 [83066.00;150470.00]	20	118096.00 [93697.00;167533.00]	0.165	0.220

Table S4. Comparison of PBMC subsets (major subsets, CD4 subsets, CD8 subsets, Tregs, and T-cell activation status) and plasma immune response and checkpoint markers in the fit patient population between baseline and after three months of combination therapy. The number of measurements (N), median, interquartile range (IQR), raw p-values, and FDR-corrected p-values are reported. The P-values were calculated using a paired Wilcoxon signed rank test. The P-values below the significance threshold of 5% are indicated in bold italics. FDR: false discovery rate; MFI: mean fluorescence intensity.

Biomarker	Baseline		After 3 months		<i>P</i> values	
	N	Median [IQR]	N	Median [IQR]	Raw	FDR
PBMC - Major Subsets						
Viable cells (% of total cells)	8	99.20 [96.94;99.51]	8	99.29 [98.88;99.61]	0.688	0.773
Viable lymphocytes (% of total lymphocytes)	8	98.15 [96.27;98.48]	8	97.79 [96.94;98.59]	0.547	0.703
Total T-cells (% of viable lymphocytes)	8	61.50 [56.82;71.20]	8	65.62 [56.74;72.41]	0.461	0.691
Total T-cells (% of viable cells)	8	59.39 [42.18;64.59]	8	59.69 [43.29;67.63]	0.383	0.689
CD4+ T-cells (% of viable lymphocytes)	8	52.70 [29.20;56.34]	8	52.78 [38.14;60.31]	0.250	0.500
CD8+ T-cells (% of viable lymphocytes)	8	16.44 [14.48;20.99]	8	15.24 [10.03;19.46]	0.078	0.201
CD4/CD8 ratio (% CD4/CD8 ratio)	8	3.16 [1.60;4.12]	8	2.78 [2.62;5.98]	0.078	0.201
NK/T-cells (% of viable cells)	8	4.46 [2.65;10.06]	8	4.74 [2.69;7.44]	0.461	0.691
Total NK cells (% of viable cells)	8	9.16 [5.54;12.23]	8	8.74 [6.76;11.65]	0.641	0.769
CD56 bright CD16- (% of total NK cells)	8	3.96 [2.64;12.78]	8	3.82 [2.55;14.38]	1.000	1.000
CD56 dim CD16+ (% of total NK cells)	8	82.71 [68.17;91.11]	8	71.87 [50.83;90.52]	0.148	0.334
Total B-cells (% of viable cells)	8	9.00 [7.47;10.06]	8	7.61 [5.91;9.87]	0.844	0.893
Naïve B-cells (% of total B-cells)	8	58.95 [54.53;71.71]	8	71.81 [64.05;81.13]	0.023	0.084
Memory B-cells (% of total B-cells)	8	40.22 [27.47;44.21]	8	27.87 [17.79;35.21]	0.008	0.070
Total monocytes (% of viable cells)	8	13.77 [12.65;16.06]	8	12.89 [8.16;20.14]	0.547	0.703
Non-classical monocytes (% of total monocytes)	8	4.47 [3.04;5.03]	8	9.63 [7.70;12.98]	0.008	0.070
Intermediate monocytes (% of total monocytes)	8	5.76 [4.17;6.75]	8	8.63 [7.65;12.87]	0.016	0.070
Classical monocytes (% of total monocytes)	8	83.73 [80.18;86.12]	8	70.19 [64.80;78.07]	0.016	0.070
PBMC - CD4 Subsets						
Naïve CD4+ (% of CD4+ T-cells)	8	26.32 [18.89;49.10]	8	40.81 [18.93;47.80]	0.461	0.563
CD27 expression in Naïve CD4+ (MFI)	8	1458.50 [1248.50;1752.50]	8	1829.50 [1559.00;2084.50]	0.219	0.380
CD28 expression in Naïve CD4+ (MFI)	8	766.00 [666.00;864.00]	8	806.00 [712.00;838.50]	0.313	0.469
Naïve CD4+ CD27+ CD28+ (% of Naïve CD4+ T-cells)	8	96.01 [90.34;98.05]	8	98.49 [97.00;98.68]	0.078	0.287
CD27 expression in Naïve CD4+ CD27+CD28+ (MFI)	8	1491.00 [1323.50;1831.50]	8	1849.50 [1599.50;2109.50]	0.195	0.358
CD28 expression in Naïve CD4+ CD27+CD28+ (MFI)	8	764.00 [690.50;871.00]	8	810.50 [717.50;840.50]	0.383	0.486
Naïve CD4+ CD57+ (% of Naïve CD4+ T-cells)	8	4.70 [2.43;18.71]	8	6.98 [1.03;10.63]	0.844	0.870
CM CD4+ (% of CD4+ T-cells)	8	60.90 [39.22;66.37]	8	48.86 [44.26;61.18]	0.148	0.327
CD27 expression in CM CD4+ (MFI)	8	1279.00 [970.00;1521.00]	8	1502.00 [1233.00;1767.00]	0.078	0.287
CD28 expression in CM CD4+ (MFI)	8	1265.00 [1068.50;1379.00]	8	1271.50 [1119.50;1358.00]	0.383	0.486
CM CD4+ CD27+ CD28+ (% of CM CD4+ T-cells)	8	80.46 [74.87;83.76]	8	85.62 [82.75;90.89]	0.109	0.301
CD27 expression in CM CD4+ CD27+CD28+ (MFI)	8	1490.00 [1261.50;1803.00]	8	1722.50 [1510.50;1962.00]	0.109	0.301
CD28 expression in CM CD4+ CD27+CD28+ (MFI)	8	1227.50 [1092.50;1347.00]	8	1266.50 [1098.50;1335.50]	0.328	0.471
CM CD4+ CD57+ (% of CM CD4+ T-cells)	8	2.01 [1.53;3.10]	8	1.95 [1.64;2.49]	0.844	0.870
EM CD4+ (% of CD4+ T-cells)	8	10.51 [6.97;18.67]	8	11.79 [7.20;15.59]	0.844	0.870
CD27 expression in EM CD4+ (MFI)	8	456.00 [322.50;505.00]	8	691.00 [421.00;843.50]	0.039	0.287
CD28 expression in EM CD4+ (MFI)	8	1027.00 [890.00;1284.50]	8	1141.50 [996.00;1267.50]	0.148	0.327
EM CD4+ CD27+ CD28+ (% of EM CD4+ T-cells)	8	34.08 [26.25;42.46]	8	51.89 [34.75;54.52]	0.039	0.287
CD27 expression in EM CD4+ CD27+CD28+ (MFI)	8	1203.00 [1036.50;1389.00]	8	1355.50 [1316.50;1563.50]	0.055	0.287
CD28 expression in EM CD4+ CD27+CD28+ (MFI)	8	1149.50 [1072.50;1365.50]	8	1232.50 [1187.50;1459.00]	0.078	0.287

EM CD4+ CD27+CD28- (% of EM CD4+ T-cells)	8	0.18 [0.08;0.81]	8	0.13 [0.05;0.27]	0.148	0.327
EM CD4+ CD27-CD28- (% of EM CD4+ T-cells)	8	4.47 [0.92;13.05]	8	1.46 [0.69;13.98]	0.383	0.486
EM CD4+ CD27-CD28+ (% of EM CD4+ T-cells)	8	53.20 [50.65;61.83]	8	45.53 [42.46;46.81]	0.055	0.287
EM CD4+ CD57+ (% of EM CD4+ T-cells)	8	1.54 [0.74;3.57]	8	1.74 [1.00;2.51]	0.945	0.945
TEMRA CD4+(% of CD4+ T-cells)	8	0.41 [0.20;1.47]	8	0.35 [0.24;0.91]	0.719	0.845
CD27 expression in TEMRA CD4+ (MFI)	8	500.50 [95.50;697.50]	8	546.00 [107.50;1033.50]	0.297	0.467
CD28 expression in TEMRA CD4+ (MFI)	8	519.00 [230.50;733.50]	8	766.50 [228.00;1233.50]	0.109	0.301
TEMRA CD4+ CD27+CD28+ (% of TEMRA CD4+ T-cells)	8	26.93 [11.58;48.75]	8	27.19 [12.42;59.22]	0.195	0.358
CD27 expression in TEMRA CD4+ CD27+CD28+ (MFI)	8	1189.50 [939.50;1464.50]	8	1609.50 [1258.00;1646.50]	0.008	0.258
CD28 expression in TEMRA CD4+ CD27+CD28+ (MFI)	8	1007.00 [929.00;1082.00]	8	1289.00 [889.00;1559.00]	0.039	0.287
TEMRA CD4+ CD27+CD28- (% of TEMRA CD4+ T-cells)	8	10.42 [4.09;19.63]	8	5.21 [1.53;11.04]	0.250	0.413
TEMRA CD4+ CD27-CD28- (% of TEMRA CD4+ T-cells)	8	24.13 [11.51;53.79]	8	16.57 [6.87;54.53]	0.195	0.358
TEMRA CD4+ CD57+ (% of TEMRA CD4+ T-cells)	8	47.02 [28.37;76.66]	8	45.56 [22.38;77.28]	0.742	0.845
PBMC - CD8 Subsets						
Naïve CD8+ (% of CD8+ T-cells)	8	8.89 [6.98;26.00]	8	21.62 [13.01;34.47]	0.109	0.490
CD27 expression in Naïve CD8+ (MFI)	8	1873.50 [1517.00;2110.50]	8	2255.00 [1848.50;2482.50]	0.195	0.496
CD28 expression in Naïve CD8+ (MFI)	8	642.50 [541.50;737.50]	8	554.00 [514.00;758.00]	0.641	0.729
Naïve CD8+ CD27+ CD28+ (% of Naïve CD8+ T-cells)	8	88.32 [76.96;91.95]	8	94.74 [92.60;95.92]	0.148	0.490
CD27 expression in Naïve CD8+ CD27+CD28+ (MFI)	8	2005.00 [1706.50;2230.50]	8	2349.00 [1898.50;2572.50]	0.195	0.496
CD28 expression in Naïve CD8+ CD27+CD28+ (MFI)	8	657.00 [558.00;770.00]	8	584.50 [531.50;766.50]	0.844	0.898
Naïve CD8+ CD57+ (% of Naïve CD8+ T-cells)	8	3.16 [1.80;7.34]	8	2.39 [1.77;3.50]	0.969	0.969
CM CD8+ (% of CD8+ T-cells)	8	17.90 [16.32;25.91]	8	17.98 [16.38;28.72]	0.742	0.816
CD27 expression in CM CD8+ (MFI)	8	1335.50 [1083.50;1461.00]	8	1566.00 [1222.50;1757.50]	0.148	0.490
CD28 expression in CM CD8+ (MFI)	8	1015.50 [896.00;1078.50]	8	1043.00 [844.50;1111.00]	0.547	0.722
CM CD8+ CD27+ CD28+ (% of CM CD8+ T-cells)	8	74.34 [69.93;84.43]	8	86.13 [76.94;87.44]	0.195	0.496
CD27 expression in CM CD8+ CD27+CD28+ (MFI)	8	1589.50 [1382.00;1715.00]	8	1740.00 [1484.50;1961.50]	0.148	0.490
CD28 expression in CM CD8+ CD27+CD28+ (MFI)	8	1057.50 [942.50;1103.00]	8	1052.00 [924.50;1116.00]	0.641	0.729
CM CD8+ CD57+ (% of CM CD8+ T-cells)	8	10.78 [8.77;12.74]	8	10.64 [8.03;12.77]	0.945	0.969
EM CD8+ (% of CD8+ T-cells)	8	30.36 [24.22;41.33]	8	27.05 [21.24;33.77]	0.039	0.490
CD27 expression in EM CD8+ (MFI)	8	631.00 [481.00;708.00]	8	769.00 [534.50;953.00]	0.078	0.490
CD28 expression in EM CD8+ (MFI)	8	474.50 [327.00;622.50]	8	519.00 [349.00;677.50]	0.266	0.548
EM CD8+ CD27+ CD28+(% of EM CD8+ T-cells)	8	23.70 [15.16;33.40]	8	34.13 [19.64;44.19]	0.078	0.490
CD27 expression in EM CD8+ CD27+CD28+ (MFI)	8	1369.50 [1297.00;1500.00]	8	1467.00 [1367.00;1636.50]	0.078	0.490
CD28 expression in EM CD8+ CD27+CD28+ (MFI)	8	819.50 [793.00;950.50]	8	849.50 [711.50;965.50]	0.547	0.722
EM CD8+ CD27+CD28- (% of EM CD8+ T-cells)	8	4.95 [3.01;6.91]	8	4.96 [3.76;8.90]	0.250	0.548
EM CD8+ CD27-CD28- (% of EM CD8+ T-cells)	8	27.68 [22.95;43.91]	8	23.53 [15.96;47.75]	0.641	0.729
EM CD8+ CD27-CD28+ (% of EM CD8+ T-cells)	8	36.82 [31.41;44.71]	8	28.56 [23.77;37.50]	0.078	0.490
EM CD8+ CD57+ (% of EM CD8+ T-cells)	8	44.74 [43.34;53.29]	8	44.96 [37.21;53.60]	0.641	0.729
TEMRA CD8+(% of CD8+ T-cells)	8	27.01 [18.18;39.86]	8	27.24 [14.57;32.13]	0.383	0.632
CD27 expression in TEMRA CD8+ (MFI)	8	257.50 [205.00;438.50]	8	234.00 [174.50;672.00]	0.313	0.573
CD28 expression in TEMRA CD8+ (MFI)	8	86.00 [67.00;132.50]	8	95.00 [51.00;171.50]	0.313	0.573
TEMRA CD8+ CD27+CD28+ (% of TEMRA CD8+ T-cells)	8	4.33 [2.76;5.20]	8	3.04 [2.48;10.97]	0.383	0.632
CD27 expression in TEMRA CD8+ CD27+CD28+ (MFI)	8	1481.50 [1392.00;1560.50]	8	1558.00 [1459.00;1702.50]	0.148	0.490
CD28 expression in TEMRA CD8+ CD27+CD28+ (MFI)	8	685.50 [655.00;726.00]	8	677.50 [632.50;797.50]	0.547	0.722
TEMRA CD8+ CD27+CD28- (% of	8	2.82 [2.26;4.50]	8	4.92 [1.29;5.52]	0.250	0.548

TEMRA CD8+ T-cells)					
TEMRA CD8+ CD27-CD28- (% of TEMRA CD8+ T-cells)	8	81.72 [72.29;85.97]	8	82.27 [62.76;86.50]	0.461 0.722
TEMRA CD8+ CD57+ (% of TEMRA CD8+ T-cells)	8	73.62 [64.18;77.01]	8	76.42 [62.60;82.14]	0.547 0.722
PBMC - Tregs					
Total Treg (% of CD4+ T-cells)	8	5.11 [4.28;6.19]	8	4.90 [3.90;6.14]	0.641 0.641
CD25 expression in Total Treg (MFI)	8	2203.50 [1610.00;2842.50]	8	2638.00 [2015.00;3325.00]	0.250 0.430
CTLA-4 expression in Total Treg (MFI)	8	228.50 [213.50;247.00]	8	264.00 [217.50;326.00]	0.039 0.143
Naïve Treg (% of total Tregs)	8	6.30 [4.76;8.05]	8	8.28 [4.24;15.33]	0.109 0.301
CD25 expression of Naïve Treg (MFI)	8	2048.50 [1173.50;2256.00]	8	2503.00 [1761.50;2730.50]	0.039 0.143
CTLA-4 expression of Naïve Treg (MFI)	8	186.00 [178.50;220.00]	8	204.50 [173.50;279.00]	0.297 0.430
Naïve Treg CTLA-4+ (% of Naïve Tregs)	8	15.94 [8.51;19.89]	8	17.37 [11.25;29.65]	0.383 0.468
Memory Treg (% of total Tregs)	8	93.51 [91.47;94.70]	8	91.09 [84.16;94.57]	0.039 0.143
CD25 expression of Memory Treg (MFI)	8	2178.00 [1627.50;2838.50]	8	2610.00 [1972.00;3329.00]	0.250 0.430
CTLA-4 expression of Memory Treg (MFI)	8	230.50 [215.50;256.50]	8	248.00 [205.00;293.50]	0.313 0.430
Memory Treg CTLA-4+ (% of Memory Tregs)	8	15.59 [8.43;17.68]	8	14.28 [6.42;21.34]	0.461 0.507
PBMC - T-cell activation status					
CD4+ PD1+ (% of CD4+ T-cells)	8	1.95 [1.69;2.89]	8	2.14 [1.32;2.78]	0.469 0.986
PD1 expression in CD4+ (MFI)	8	224.50 [160.50;297.00]	8	220.50 [180.00;289.50]	0.484 0.986
CD4+ CD69+ (% of CD4+ T-cells)	8	35.46 [25.93;42.24]	8	29.77 [24.87;40.96]	0.461 0.986
CD69 expression in CD4+ (MFI)	8	249.50 [236.00;290.50]	8	266.00 [243.00;289.50]	0.836 0.986
CD4+ PD1+CD69+ (% of CD4+ T-cells)	8	1.41 [0.98;2.13]	8	1.28 [0.90;1.47]	0.313 0.986
CD4+ PD1-CD69- (% of CD4+ T-cells)	8	64.00 [56.19;73.22]	8	69.58 [58.10;74.39]	0.641 0.986
CD4+ HLADR+ (% of CD4+ T-cells)	8	8.37 [6.88;14.41]	8	12.19 [3.66;20.04]	0.078 0.625
HLADR expression in CD4+ (MFI)	8	333.00 [297.50;381.00]	8	416.50 [204.00;501.50]	0.250 0.986
CD4+ CD38+ (% of CD4+ T-cells)	8	33.65 [18.60;46.64]	8	34.62 [26.71;37.29]	0.945 0.986
CD38 expression in CD4+ (MFI)	8	671.00 [500.00;874.00]	8	622.00 [547.00;795.50]	0.547 0.986
CD4+ HLADR+CD38+ (% of CD4+ T-cells)	8	2.41 [1.40;3.89]	8	3.91 [1.40;4.98]	0.688 0.986
CD4+ HLADR-CD38- (% of CD4+ T-cells)	8	59.02 [44.86;69.63]	8	54.98 [50.48;60.05]	0.641 0.986
CD8+ PD1+ (% of CD8+ T-cells)	8	29.14 [21.31;40.55]	8	26.38 [23.81;40.81]	1.000 1.000
PD1 expression in CD8+ (MFI)	8	325.00 [230.00;526.00]	8	310.50 [194.00;494.00]	0.031 0.469
CD8+ CD69+ (% of CD8+ T-cells)	8	36.86 [21.01;55.88]	8	31.47 [18.73;50.64]	0.039 0.469
CD69 expression in CD8+ (MFI)	8	513.00 [390.50;931.50]	8	565.00 [470.50;774.50]	0.844 0.986
CD8+ PD1+CD69+ (% of CD8+ T-cells)	8	11.68 [7.68;15.99]	8	11.39 [7.91;18.08]	0.742 0.986
CD8+ PD1-CD69- (% of CD8+ T-cells)	8	36.20 [30.95;53.39]	8	41.03 [36.80;57.28]	0.109 0.656
CD8+ HLADR+ (% of CD8+ T-cells)	8	31.78 [16.88;42.36]	8	38.80 [9.82;46.05]	0.945 0.986
HLADR expression in CD8+ (MFI)	8	474.50 [341.00;488.00]	8	497.00 [284.00;540.50]	0.742 0.986
CD8+ CD38+ (% of CD8+ T-cells)	8	14.06 [7.27;17.44]	8	11.40 [8.02;22.77]	0.383 0.986
CD38 expression in CD8+ (MFI)	8	293.00 [230.50;342.50]	8	274.00 [227.50;403.00]	0.547 0.986
CD8+ HLADR+CD38+ (% of CD8+ T-cells)	8	7.82 [1.71;10.11]	8	5.59 [2.36;11.15]	0.945 0.986
CD8+ HLADR-CD38- (% of CD8+ T-cells)	8	64.70 [38.71;77.09]	8	54.58 [45.98;78.05]	0.945 0.986
Plasma - Essential Immune Response					
IL-4 (pg/mL)	8	0.11 [0.00;28.48]	8	6.56 [0.31;17.75]	0.813 0.886
IL-2 (pg/mL)	8	3.34 [1.92;9.66]	8	2.35 [0.68;16.71]	0.461 0.820
CXCL-10 (IP-10) (pg/mL)	8	159.08 [125.71;355.70]	8	394.51 [100.25;495.51]	0.313 0.820
IL-1 beta (pg/mL)	8	6.06 [1.47;34.62]	8	5.96 [4.57;32.43]	0.547 0.820
TNF alpha (pg/mL)	8	1.81 [1.21;5.54]	8	1.14 [0.63;2.74]	0.109 0.594
CCL2 (MCP-1) (pg/mL)	8	193.48 [149.28;232.19]	8	202.58 [168.50;296.80]	0.461 0.820
IL-17A (pg/mL)	8	2.23 [1.18;22.16]	8	3.36 [1.45;11.39]	0.547 0.820

IL-6 (pg/mL)	8	8.40 [3.86;24.28]	8	5.80 [2.63;10.56]	0.641	0.854
IL-10 (pg/mL)	8	2.23 [1.54;3.82]	8	0.54 [0.00;1.69]	0.016	0.188
IFN gamma (pg/mL)	8	0.91 [0.00;35.63]	8	3.67 [1.90;7.15]	0.945	0.945
IL-12p70 (pg/mL)	8	6.93 [4.28;58.97]	8	12.22 [5.56;45.39]	0.742	0.886
CXCL-8 (IL-8) (pg/mL)	8	7.23 [3.55;25.42]	8	5.67 [2.23;22.07]	0.148	0.594
Plasma - Immune Checkpoint						
sCD25 (IL-2Ra) (pg/mL)	8	928.26 [798.87;1140.80]	8	1132.10 [955.26;1562.20]	0.195	0.335
4-1BB (pg/mL)	8	68.07 [50.57;119.55]	8	56.21 [38.70;88.13]	0.250	0.375
sCD27 (pg/mL)	8	52670.00 [45645.00;65276.00]	8	53535.00 [49080.00;62195.00]	0.383	0.459
B7.2 (CD86) (pg/mL)	8	524.80 [474.15;697.47]	8	411.43 [320.29;609.87]	0.109	0.263
Free Active TGF-B1 (pg/mL)	8	25.39 [22.12;32.09]	8	13.43 [7.00;28.59]	0.023	0.234
CTLA-4 (pg/mL)	8	10.14 [4.40;15.98]	8	7.36 [4.35;10.07]	0.195	0.335
PD-L1 (pg/mL)	8	279.06 [172.00;401.70]	8	265.37 [151.94;325.49]	0.313	0.417
PD-L2 (pg/mL)	8	11121.00 [10196.00;14318.00]	8	12081.00 [11067.00;16080.00]	0.078	0.234
PD-1 (pg/mL)	8	92.41 [16.51;201.92]	8	66.15 [19.51;101.02]	0.078	0.234
Tim-3 (pg/mL)	8	15345.00 [6568.40;26546.00]	8	18190.00 [8331.30;22119.00]	0.641	0.699
LAG-3 (pg/mL)	8	5456.70 [2198.80;16183.00]	8	3388.50 [2008.30;18530.00]	1.000	1.000
Gal-9 (pg/mL)	8	102158.00 [76361.00;127351.00]	8	113572.00 [87969.00;171239.00]	0.055	0.234

Table S5. Comparison of PBMC subsets (major subsets, CD4 subsets, CD8 subsets, Tregs, and T-cell activation status) and plasma immune response and checkpoint markers in the frail patient population between baseline and after three months of combination therapy. The number of measurements (N), median, interquartile range (IQR), raw p-values, and FDR-corrected P-values are reported. The P-values were calculated using a paired Wilcoxon signed rank test. The P-values below the significance threshold of 5% are indicated in bold italics. FDR: false discovery rate; MFI: mean fluorescence intensity.

Biomarker	Baseline		After 3 months		P-values	
	N	Median [IQR]	N	Median [IQR]	Raw	FDR
PBMC - Major Subsets						
Viable cells (% of total cells)	12	98.65 [98.29;99.18]	12	99.19 [98.76;99.61]	0.339	0.747
Viable lymphocytes (% of total lymphocytes)	12	96.73 [90.71;97.76]	12	97.92 [96.82;98.60]	0.034	0.235
Total T-cells (% of viable lymphocytes)	12	62.16 [53.54;64.67]	12	60.47 [49.61;66.66]	0.519	0.747
Total T-cells (% of viable cells)	12	54.68 [47.77;61.56]	12	56.93 [47.61;60.10]	0.622	0.747
CD4+ T-cells (% of viable lymphocytes)	12	50.22 [44.03;57.76]	12	49.89 [41.65;55.80]	0.569	0.747
CD8+ T-cells (% of viable lymphocytes)	12	13.96 [8.49;18.23]	12	10.72 [7.78;16.06]	0.569	0.747
CD4/CD8 ratio (% CD4/CD8 ratio)	12	3.99 [2.61;6.86]	12	4.13 [2.80;7.17]	0.733	0.777
NK/T-cells (% of viable cells)	12	1.81 [1.43;3.70]	12	2.82 [1.52;4.51]	0.850	0.850
Total NK cells (% of viable cells)	12	6.52 [3.69;10.64]	12	11.08 [6.38;15.27]	0.043	0.235
CD56 bright CD16- (% of total NK cells)	12	4.74 [4.00;8.47]	12	6.54 [4.76;10.49]	0.569	0.747
CD56 dim CD16+ (% of total NK cells)	12	58.52 [19.32;76.44]	12	64.98 [43.70;88.52]	0.110	0.330
Total B-cells (% of viable cells)	12	7.51 [5.58;9.54]	12	9.29 [4.52;12.36]	0.622	0.747
Naive B-cells (% of total B-cells)	12	62.99 [42.09;75.50]	12	74.80 [62.54;80.94]	0.021	0.235
Memory B-cells (% of total B-cells)	12	35.87 [23.20;56.53]	12	24.48 [18.23;36.41]	0.052	0.235
Total monocytes (% of viable cells)	12	18.11 [8.34;24.38]	12	15.94 [5.82;19.00]	0.266	0.684
Non-classical monocytes (% of total monocytes)	12	3.41 [1.19;9.72]	12	4.79 [2.77;7.91]	0.733	0.777
Intermediate monocytes (% of total monocytes)	12	6.17 [4.74;7.32]	12	8.36 [5.13;10.63]	0.077	0.278
Classical monocytes (% of total monocytes)	12	79.92 [68.76;83.23]	12	75.53 [59.36;82.19]	0.622	0.747
PBMC - CD4 Subsets						
Naïve CD4+ (% of CD4+ T-cells)	12	44.61 [22.85;50.60] 1312.00	12	54.15 [30.13;63.86] 1717.50	0.012	0.045
CD27 expression in Naïve CD4+ (MFI)	12	[914.50;1596.50] 614.00	12	[1410.50;1818.00]	0.007	0.038
CD28 expression in Naïve CD4+ (MFI)	12	[548.50;726.50]	12	791.00 [676.50;850.00]	0.033	0.063
Naïve CD4+ CD27+ CD28+ (% of Naïve CD4+ T-cells)	12	88.82 [71.31;92.93] 1424.50	12	95.25 [91.67;96.83] 1770.50	0.002	0.032
CD27 expression in Naïve CD4+ CD27+CD28+ (MFI)	12	[1134.00;1677.50] 649.50	12	[1509.50;1875.00]	0.021	0.053
CD28 expression in Naïve CD4+ CD27+CD28+ (MFI)	12	[565.50;739.50]	12	783.50 [705.00;845.00]	0.034	0.063
Naïve CD4+ CD57+ (% of Naïve CD4+ T-cells)	12	2.68 [1.35;11.63]	12	1.81 [0.47;5.06]	0.274	0.312
CM CD4+ (% of CD4+ T-cells)	12	47.86 [33.74;59.69] 1006.00	12	38.75 [27.56;57.95] 1477.00	0.052	0.078
CD27 expression in CM CD4+ (MFI)	11	[817.00;1326.00] 1089.50	11	[1135.00;1758.00] 1198.00	0.005	0.032
CD28 expression in CM CD4+ (MFI)	12	[868.00;1247.50]	12	[1098.50;1370.50]	0.027	0.059
CM CD4+ CD27+ CD28+ (% of CM CD4+ T-cells)	12	70.12 [59.29;79.39] 1359.50	12	85.03 [72.43;87.00] 1621.00	0.001	0.016
CD27 expression in CM CD4+ CD27+CD28+ (MFI)	12	[1183.50;1601.00] 1111.00	12	[1483.00;1878.50] 1186.00	0.009	0.038
CD28 expression in CM CD4+ CD27+CD28+ (MFI)	12	[888.50;1256.50]	12	[1103.00;1342.00]	0.016	0.053
CM CD4+ CD57+ (% of CM CD4+ T-cells)	12	2.87 [1.96;4.05]	12	2.17 [1.37;3.41]	0.151	0.192
EM CD4+ (% of CD4+ T-cells)	12	10.35 [5.42;14.76] 371.00	12	7.59 [4.40;10.94]	0.176	0.215
CD27 expression in EM CD4+ (MFI)	11	[134.00;579.00] 955.00	11	490.00 [333.00;793.00] 1103.50	0.042	0.070
CD28 expression in EM CD4+ (MFI)	12	[802.50;1201.50]	12	[1023.00;1260.00]	0.052	0.078
EM CD4+ CD27+ CD28+ (% of EM CD4+ T-cells)	12	31.17 [17.30;38.43] 1114.00	12	38.07 [30.48;44.77] 1292.00	0.021	0.053
CD27 expression in EM CD4+ CD27+CD28+ (MFI)	12	[971.00;1274.00]	12	[1192.50;1573.00]	0.027	0.059

CD28 expression in EM CD4+ CD27+CD28+ (MFI)	12	1062.00 [874.00;1257.50]	12	1179.50 [1135.50;1339.50]	0.021	0.053
EM CD4+ CD27+CD28- (% of EM CD4+ T-cells)	12	0.04 [0.00;0.32]	12	0.11 [0.00;0.40]	0.445	0.474
EM CD4+ CD27-CD28- (% of EM CD4+ T-cells)	12	2.19 [1.45;4.06]	12	2.16 [0.57;3.75]	1.000	1.000
EM CD4+ CD27-CD28+ (% of EM CD4+ T-cells)	12	62.95 [56.89;72.30]	12	57.76 [47.14;64.68]	0.043	0.070
EM CD4+ CD57+ (% of EM CD4+ T-cells)	12	2.14 [1.37;11.21]	12	2.33 [1.33;5.32]	0.204	0.240
TEMRA CD4+(% of CD4+ T-cells)	12	0.62 [0.33;2.60] 396.00	12	0.56 [0.38;2.25]	0.733	0.756
CD27 expression in TEMRA CD4+ (MFI)	9	[201.00;418.00] 799.50	9	632.00 [573.00;878.00] 1008.00	0.004	0.032
CD28 expression in TEMRA CD4+ (MFI)	12	[455.00;1068.50]	12	[629.00;1171.00]	0.110	0.151
TEMRA CD4+ CD27+CD28+ (% of TEMRA CD4+ T-cells)	12	27.59 [13.65;35.75] 1044.50	12	32.86 [25.25;53.91] 1454.50	0.005	0.032
CD27 expression in TEMRA CD4+ CD27+CD28+ (MFI)	12	[937.00;1229.00]	12	[1172.00;1609.00]	0.034	0.063
CD28 expression in TEMRA CD4+ CD27+CD28+ (MFI)	12	1104.00 [816.00;1208.00]	12	1245.00 [1055.00;1525.50]	0.009	0.038
TEMRA CD4+ CD27+CD28- (% of TEMRA CD4+ T-cells)	12	0.00 [0.00;1.88]	12	1.15 [0.00;5.68]	0.129	0.170
TEMRA CD4+ CD27-CD28- (% of TEMRA CD4+ T-cells)	12	12.95 [5.33;35.81]	12	4.82 [2.48;26.06]	0.067	0.097
TEMRA CD4+ CD57+ (% of TEMRA CD4+ T-cells)	12	27.99 [8.55;53.71]	12	22.99 [9.38;44.65]	0.424	0.466
PBMC - CD8 Subsets						
Naïve CD8+ (% of CD8+ T-cells)	12	20.00 [14.10;48.67] 1229.50	12	21.17 [14.13;52.44] 1676.00	0.266	0.398
CD27 expression in Naïve CD8+ (MFI)	12	[1014.00;1579.00] 538.00	12	[1227.50;2032.00]	0.016	0.099
CD28 expression in Naïve CD8+ (MFI)	12	[436.00;641.00]	12	599.50 [499.00;641.00]	0.301	0.398
Naïve CD8+ CD27+ CD28+ (% of Naïve CD8+ T-cells)	12	66.27 [46.33;77.72] 1579.00	12	81.19 [61.98;88.81] 1983.50	0.027	0.111
CD27 expression in Naïve CD8+ CD27+CD28+ (MFI)	12	[1526.50;1820.50]	12	[1671.00;2189.00]	0.003	0.056
CD28 expression in Naïve CD8+ CD27+CD28+ (MFI)	12	576.50 [491.50;661.50]	12	619.00 [546.50;661.00]	0.151	0.278
Naïve CD8+ CD57+ (% of Naïve CD8+ T-cells)	12	5.19 [2.72;6.86]	12	2.40 [1.33;4.85]	0.266	0.398
CM CD8+ (% of CD8+ T-cells)	12	29.27 [22.16;39.40] 905.50	12	18.44 [14.94;32.76] 1178.00	0.052	0.144
CD27 expression in CM CD8+ (MFI)	12	[694.50;1069.00] 878.50	12	[876.50;1488.50] 867.50	0.009	0.077
CD28 expression in CM CD8+ (MFI)	12	[691.00;1015.00]	12	[795.00;1126.00]	0.064	0.151
CM CD8+ CD27+ CD28+ (% of CM CD8+ T-cells)	12	56.80 [45.18;66.45] 1323.50	12	70.19 [53.41;75.44] 1495.00	0.021	0.099
CD27 expression in CM CD8+ CD27+CD28+ (MFI)	12	[1172.00;1497.50]	12	[1338.00;1832.00]	0.009	0.077
CD28 expression in CM CD8+ CD27+CD28+ (MFI)	12	956.50 [775.00;1057.00]	12	933.00 [851.50;1148.50]	0.052	0.144
CM CD8+ CD57+ (% of CM CD8+ T-cells)	12	9.92 [6.56;14.91]	12	10.62 [5.94;16.58]	0.910	0.938
EM CD8+ (% of CD8+ T-cells)	12	17.33 [10.72;28.47] 437.50	12	17.09 [10.91;31.89]	0.622	0.708
CD27 expression in EM CD8+ (MFI)	12	[357.00;578.50] 497.50	12	638.50 [478.00;822.00]	0.021	0.099
CD28 expression in EM CD8+ (MFI)	12	[396.50;591.00]	12	586.00 [354.00;722.50]	0.129	0.251
EM CD8+ CD27+ CD28+(% of EM CD8+ T-cells)	12	15.30 [8.64;23.33] 1274.50	12	26.96 [15.76;32.24] 1347.50	0.052	0.144
CD27 expression in EM CD8+ CD27+CD28+ (MFI)	12	[1211.00;1414.50]	12	[1279.50;1682.50]	0.129	0.251
CD28 expression in EM CD8+ CD27+CD28+ (MFI)	12	791.00 [732.00;899.50]	12	906.50 [658.50;1036.50]	0.258	0.398
EM CD8+ CD27+CD28- (% of EM CD8+ T-cells)	12	2.16 [0.85;3.14]	12	1.96 [1.65;4.51]	0.176	0.306
EM CD8+ CD27-CD28- (% of EM CD8+ T-cells)	12	24.00 [12.54;40.40]	12	24.14 [14.47;32.75]	0.470	0.554
EM CD8+ CD27-CD28+ (% of EM CD8+ T-cells)	12	56.06 [42.69;64.97]	12	49.03 [35.57;54.79]	0.043	0.144
EM CD8+ CD57+ (% of EM CD8+ T-cells)	12	49.35 [15.50;60.11]	12	49.91 [21.78;54.52]	0.970	0.970
TEMRA CD8+(% of CD8+ T-cells)	12	22.32 [6.91;26.87] 268.50	12	18.43 [7.98;30.10]	0.339	0.415
CD27 expression in TEMRA CD8+ (MFI)	12	[195.50;386.00] 188.00	12	318.50 [238.50;469.50]	0.064	0.151
CD28 expression in TEMRA CD8+ (MFI)	12	[105.00;354.00]	12	188.00 [113.00;330.00]	0.850	0.905
TEMRA CD8+ CD27+CD28+ (% of TEMRA CD8+ T-cells)	12	3.60 [1.80;6.91] 1515.00	12	6.09 [1.99;10.27] 1657.00	0.077	0.170
CD27 expression in TEMRA CD8+ CD27+CD28+ (MFI)	11	[1303.00;1664.00]	11	[1300.00;1977.00]	0.278	0.398
CD28 expression in TEMRA CD8+ CD27+CD28+ (MFI)	11	747.00 [653.00;873.00]	11	765.00 [674.00;925.00]	0.320	0.407

TEMRA CD8+ CD27+CD28- (% of TEMRA CD8+ T-cells)	12	1.30 [0.42;1.54]	12	3.28 [1.42;4.08]	0.001	0.032
TEMRA CD8+ CD27-CD28- (% of TEMRA CD8+ T-cells)	12	65.24 [43.68;81.82]	12	66.02 [50.96;78.16]	0.850	0.905
TEMRA CD8+ CD57+ (% of TEMRA CD8+ T-cells)	12	61.92 [39.21;76.18]	12	67.67 [46.39;78.59]	0.301	0.398
PBMC - Tregs						
Total Treg (% of CD4+ T-cells)	12	4.57 [3.88;6.90]	12	3.97 [3.34;5.32]	0.233	0.428
CD25 expression in Total Treg (MFI)	12	1293.50 [1096.50;1816.50]	12	1535.50 [1124.50;2303.00]	0.092	0.242
CTLA-4 expression in Total Treg (MFI)	12	272.50 [246.50;301.00]	12	292.50 [258.00;314.00]	0.807	0.807
Naïve Treg (% of total Tregs)	12	5.15 [3.04;13.94]	12	11.08 [7.28;23.51]	0.003	0.019
CD25 expression of Naïve Treg (MFI)	12	1279.50 [827.50;1556.50]	12	1773.50 [1169.50;2216.50]	0.110	0.242
CTLA-4 expression of Naïve Treg (MFI)	12	252.50 [216.50;318.50]	12	292.50 [242.50;313.50]	0.636	0.699
Naïve Treg CTLA-4+ (% of Naïve Tregs)	12	33.18 [23.25;40.31]	12	30.24 [24.04;32.85]	0.365	0.574
Memory Treg (% of total Tregs)	12	94.74 [85.54;96.79]	12	88.49 [76.07;92.20]	0.003	0.019
CD25 expression of Memory Treg (MFI)	12	1345.00 [1094.50;1845.50]	12	1555.00 [1173.00;2373.50]	0.064	0.235
CTLA-4 expression of Memory Treg (MFI)	12	271.00 [248.00;305.00]	12	287.50 [255.50;331.50]	0.583	0.699
Memory Treg CTLA-4+ (% of Memory Tregs)	12	19.74 [16.52;21.61]	12	19.98 [18.26;24.46]	0.622	0.699
PBMC - T-cell activation status						
CD4+ PD1+ (% of CD4+ T-cells)	12	1.26 [0.88;1.62]	12	1.12 [0.84;3.21]	0.380	0.593
PD1 expression in CD4+ (MFI)	12	195.50 [99.50;216.00]	12	165.50 [120.50;236.00]	0.424	0.593
CD4+ CD69+ (% of CD4+ T-cells)	12	31.54 [17.07;38.34]	12	27.01 [16.96;37.41]	0.910	0.949
CD69 expression in CD4+ (MFI)	12	262.00 [226.50;270.00]	12	245.50 [216.00;305.50]	0.183	0.487
CD4+ PD1+CD69+ (% of CD4+ T-cells)	12	0.74 [0.50;1.04]	12	0.66 [0.37;2.15]	0.470	0.593
CD4+ PD1-CD69- (% of CD4+ T-cells)	12	67.83 [61.28;82.28]	12	72.24 [61.23;82.71]	0.970	0.970
CD4+ HLADR+ (% of CD4+ T-cells)	12	16.98 [11.69;22.80]	12	17.99 [11.79;22.56]	0.424	0.593
HLADR expression in CD4+ (MFI)	12	459.00 [388.00;627.00]	12	442.50 [380.00;522.00]	0.622	0.711
CD4+ CD38+ (% of CD4+ T-cells)	12	28.78 [20.25;45.58]	12	34.99 [22.85;46.95]	0.077	0.309
CD38 expression in CD4+ (MFI)	12	644.50 [488.50;777.00]	12	686.00 [481.00;780.00]	0.692	0.755
CD4+ HLADR+CD38+ (% of CD4+ T-cells)	12	3.47 [2.14;4.28]	12	4.04 [2.57;7.23]	0.064	0.307
CD4+ HLADR-CD38- (% of CD4+ T-cells)	12	51.84 [48.00;63.98]	12	46.41 [38.73;59.53]	0.129	0.388
CD8+ PD1+ (% of CD8+ T-cells)	12	15.24 [9.90;23.11]	12	15.11 [8.96;32.54]	0.470	0.593
PD1 expression in CD8+ (MFI)	12	267.50 [203.50;323.50]	12	292.50 [233.00;361.00]	0.424	0.593
CD8+ CD69+ (% of CD8+ T-cells)	12	31.63 [22.80;42.70]	12	37.83 [24.52;41.97]	0.266	0.581
CD69 expression in CD8+ (MFI)	12	342.50 [279.50;437.00]	12	391.00 [292.00;541.50]	0.092	0.316
CD8+ PD1+CD69+ (% of CD8+ T-cells)	12	6.17 [5.13;9.99]	12	7.28 [4.22;16.06]	0.470	0.593
CD8+ PD1-CD69- (% of CD8+ T-cells)	12	58.50 [47.46;69.09]	12	52.81 [42.74;67.57]	0.233	0.560
CD8+ HLADR+ (% of CD8+ T-cells)	12	41.85 [33.45;47.17]	12	45.47 [36.28;60.72]	0.021	0.161
HLADR expression in CD8+ (MFI)	12	531.50 [489.50;709.00]	12	535.50 [439.00;806.00]	0.622	0.711
CD8+ CD38+ (% of CD8+ T-cells)	12	8.47 [6.20;21.45]	12	13.74 [6.99;29.79]	0.002	0.047
CD38 expression in CD8+ (MFI)	12	243.50 [192.50;355.00]	12	282.50 [180.00;456.50]	0.458	0.593
CD8+ HLADR+CD38+ (% of CD8+ T-cells)	12	4.98 [4.12;6.99]	12	6.96 [4.14;11.61]	0.027	0.161
CD8+ HLADR-CD38- (% of CD8+ T-cells)	12	52.45 [48.44;55.66]	12	45.36 [34.90;51.94]	0.009	0.111
Plasma - Essential Immune Response						
IL-4 (pg/mL)	12	0.06 [0.00;1.38]	12	0.00 [0.00;3.83]	0.844	0.922
IL-2 (pg/mL)	12	1.80 [0.67;2.24]	12	0.95 [0.00;4.18]	0.622	0.829
CXCL-10 (IP-10) (pg/mL)	12	311.58 [176.37;354.14]	12	370.47 [189.67;459.55]	0.092	0.369
IL-1 beta (pg/mL)	12	5.27 [0.77;11.90]	12	3.02 [0.75;9.23]	0.470	0.705
TNF alpha (pg/mL)	12	2.07 [1.63;3.43]	12	2.43 [1.38;3.60]	0.910	0.922
CCL2 (MCP-1) (pg/mL)	12	133.65 [97.65;174.79]	12	165.28 [140.36;248.67]	0.176	0.470
IL-17A (pg/mL)	12	2.78 [1.35;8.50]	12	2.31 [1.36;5.32]	0.240	0.470

IL-6 (pg/mL)	12	3.86 [2.88;8.14]	12	6.16 [3.75;10.83]	0.034	0.314
IL-10 (pg/mL)	12	1.37 [0.62;2.63]	12	0.70 [0.09;1.68]	0.274	0.470
IFN gamma (pg/mL)	12	1.32 [0.00;6.07]	12	1.94 [0.91;4.83]	0.922	0.922
IL-12p70 (pg/mL)	12	2.05 [0.71;4.19]	12	2.75 [1.30;5.44]	0.266	0.470
CXCL-8 (IL-8) (pg/mL)	12	3.77 [1.94;11.62]	12	5.28 [3.78;9.94]	0.052	0.314
Plasma - Immune Checkpoint						
sCD25 (IL-2Ra) (pg/mL)	12	1504.05 [978.83;2268.00]	12	1689.80 [1086.20;2303.20]	0.129	0.349
4-1BB (pg/mL)	12	80.31 [54.40;156.65]	12	72.05 [51.50;143.09]	0.204	0.349
sCD27 (pg/mL)	12	66274.00 [50880.00;87801.00]	12	62981.00 [49390.00;73961.00]	0.064	0.256
B7.2 (CD86) (pg/mL)	12	582.31 [452.28;700.38]	12	360.66 [300.49;565.35]	0.007	0.082
Free Active TGF-B1 (pg/mL)	12	23.19 [11.09;42.99]	12	25.66 [11.16;41.20]	0.677	0.813
CTLA-4 (pg/mL)	12	8.84 [2.25;27.96]	12	8.88 [1.04;23.83]	0.148	0.349
PD-L1 (pg/mL)	12	165.63 [113.50;213.15]	12	187.01 [149.03;223.63]	0.204	0.349
PD-L2 (pg/mL)	12	13370.00 [11972.00;16020.00]	12	14498.00 [12619.00;16398.00]	0.569	0.759
PD-1 (pg/mL)	12	33.25 [16.94;118.63]	12	24.74 [14.23;95.23]	0.052	0.256
Tim-3 (pg/mL)	12	26074.00 [18660.00;38595.00]	12	29643.00 [16492.00;37149.00]	0.791	0.850
LAG-3 (pg/mL)	12	2560.40 [1596.90;3621.40]	12	2042.20 [1398.00;4275.60]	0.569	0.759
Gal-9 (pg/mL)	12	105868.00 [90925.00;159163.00]	12	118096.00 [97476.00;167533.00]	0.850	0.850

Table S6. Interaction analysis between time and frailty status for PBMC subsets (major subsets, CD4 subsets, CD8 subsets, Tregs, and T-cell activation status) and plasma immune response and checkpoint markers. A change score was calculated between patients' values at three months and baseline (positive values indicating increase). The difference in change score was tested using a Mann-Whitney U test. The number of measurements (N), median, interquartile range (IQR), raw P-values, and FDR-corrected P-values are reported. The P-values below the significance threshold of 5% are indicated in bold italics. FDR: false discovery rate; MFI: mean fluorescence intensity.

Biomarker	Fit		Frail		<i>P</i> -values	
	N	Median [IQR]	N	Median [IQR]	Raw	FDR
PBMC - Major Subsets						
Viable cells (% of total cells)	8	-0.01 [-0.08;2.39]	12	0.35 [-0.04;1.02]	0.729	0.937
Viable lymphocytes (% of total lymphocytes)	8	0.23 [-0.45;0.91]	12	0.99 [0.30;2.01]	0.263	0.527
Total T-cells (% of viable lymphocytes)	8	3.81 [-2.26;6.06]	12	-0.85 [-9.24;1.74]	0.203	0.527
Total T-cells (% of viable cells)	8	2.28 [-0.87;5.90]	12	-3.07 [-5.87;4.56]	0.263	0.527
CD4 ⁺ T-cells (% of viable lymphocytes)	8	1.51 [-0.63;8.54]	12	-1.37 [-7.94;3.19]	0.298	0.536
CD8 ⁺ T-cells (% of viable lymphocytes)	8	-2.24 [-4.90;-0.20]	12	-0.67 [-2.97;0.63]	0.375	0.614
CD4/CD8 ratio (% CD4/CD8 ratio)	8	0.94 [0.01;1.93]	12	0.09 [-1.38;1.46]	0.263	0.527
NK/T-cells (% of viable cells)	8	-0.35 [-1.48;0.61]	12	-0.03 [-0.29;1.33]	0.418	0.627
Total NK cells (% of viable cells)	8	-1.02 [-2.55;2.26]	12	5.07 [0.37;7.44]	0.114	0.527
CD56 bright CD16 ⁻ (% of total NK cells)	8	-0.32 [-0.70;0.93]	12	1.73 [-2.36;5.06]	0.512	0.709
CD56 dim CD16 ⁺ (% of total NK cells)	8	-9.64 [-16.30;-2.25]	12	10.79 [2.18;22.49]	0.049	0.442
Total B-cells (% of viable cells)	8	-0.98 [-1.52;1.96]	12	0.76 [-2.47;4.11]	0.969	0.969
Naïve B-cells (% of total B-cells)	8	9.37 [2.97;11.87]	12	9.04 [2.03;14.08]	0.847	0.953
Memory B-cells (% of total B-cells)	8	-9.25 [-10.63;-3.24]	12	-8.87 [-13.01;-2.05]	0.908	0.961
Total monocytes (% of viable cells)	8	-2.59 [-5.57;2.52]	12	-2.22 [-10.92;2.83]	0.787	0.945
Non-classical monocytes (% of total monocytes)	8	5.39 [3.29;9.00]	12	-0.42 [-5.55;2.41]	0.010	0.176
Intermediate monocytes (% of total monocytes)	8	4.04 [2.26;5.38]	12	1.95 [-0.32;4.61]	0.203	0.527
Classical monocytes (% of total monocytes)	8	-10.08 [-16.99;-6.40]	12	-0.89 [-11.98;6.74]	0.203	0.527
PBMC - CD4 Subsets						
Naïve CD4 ⁺ (% of CD4 ⁺ T-cells)	8	2.82 [-1.65;14.32]	12	7.13 [3.12;11.87] 196.00	0.512	0.925
CD27 expression in Naïve CD4 ⁺ (MFI)	8	339.50 [94.00;481.00]	12	[34.00;888.00] 105.00 [-	0.787	0.932
CD28 expression in Naïve CD4 ⁺ (MFI)	8	60.00 [-34.00;114.00]	12	3.50;214.50]	0.487	0.925
Naïve CD4 ⁺ CD27 ⁺ CD28 ⁺ (% of Naïve CD4 ⁺ T-cells)	8	1.10 [0.71;8.12]	12	3.88 [1.58;13.85] 180.00	0.464	0.925
CD27 expression in Naïve CD4 ⁺ CD27 ⁺ CD28 ⁺ (MFI)	8	317.00 [88.00;397.50]	12	[27.00;729.50] 88.00 [-	1.000	1.000
CD28 expression in Naïve CD4 ⁺ CD27 ⁺ CD28 ⁺ (MFI)	8	48.50 [-29.00;102.50]	12	3.00;182.50]	0.375	0.925
Naïve CD4 ⁺ CD57 ⁺ (% of Naïve CD4 ⁺ T-cells)	8	0.10 [-1.40;3.47]	12	-0.34 [-1.16;0.27]	0.563	0.925
CM CD4 ⁺ (% of CD4 ⁺ T-cells)	8	-0.83 [-10.94;-0.16]	12	-5.08 [-10.21;-0.68] 176.00	0.671	0.925
CD27 expression in CM CD4 ⁺ (MFI)	8	322.00 [96.00;483.00]	11	[92.00;743.00] 134.00	0.837	0.932
CD28 expression in CM CD4 ⁺ (MFI)	8	91.50 [-74.50;177.00]	12	[12.50;280.00]	0.335	0.925
CM CD4 ⁺ CD27 ⁺ CD28 ⁺ (% of CM CD4 ⁺ T-cells)	8	4.08 [2.21;12.56]	12	10.13 [5.46;15.48] 145.50	0.154	0.925
CD27 expression in CM CD4 ⁺ CD27 ⁺ CD28 ⁺ (MFI)	8	262.50 [72.50;407.50]	12	[60.50;691.00] 95.50	0.847	0.932
CD28 expression in CM CD4 ⁺ CD27 ⁺ CD28 ⁺ (MFI)	8	70.00 [-60.00;180.00]	12	[13.00;249.00]	0.464	0.925
CM CD4 ⁺ CD57 ⁺ (% of CM CD4 ⁺ T-cells)	8	0.38 [-0.71;0.56]	12	-0.41 [-1.16;0.17]	0.335	0.925
EM CD4 ⁺ (% of CD4 ⁺ T-cells)	8	0.50 [-3.42;2.34]	12	-1.94 [-3.41;0.13] 172.00 [-	0.375	0.925
CD27 expression in EM CD4 ⁺ (MFI)	8	248.00 [86.50;359.50]	11	74.00;254.00] 159.50 [-	0.386	0.925
CD28 expression in EM CD4 ⁺ (MFI)	8	17.00;226.50]	12	32.00;226.00]	0.969	1.000
EM CD4 ⁺ CD27 ⁺ CD28 ⁺ (% of EM CD4 ⁺ T-cells)	8	9.65 [3.73;18.21] 252.00	12	7.90 [2.71;15.97] 329.00 [-	0.671	0.925
CD27 expression in EM CD4 ⁺ CD27 ⁺ CD28 ⁺ (MFI)	8	[100.00;347.50]	12	9.50;456.50]	0.512	0.925
CD28 expression in EM CD4 ⁺ CD27 ⁺ CD28 ⁺ (MFI)	8	115.00 [14.00;196.50]	12	189.50 [- 60.00;345.50]	0.418	0.925

EM CD4+ CD27+CD28- (% of EM CD4+ T-cells)	8	-0.11 [-0.59;0.03]	12	-0.01 [-0.20;0.03]	0.374	0.925
EM CD4+ CD27-CD28- (% of EM CD4+ T-cells)	8	-0.29 [-2.52;0.54]	12	-0.37 [-0.81;1.22]	0.616	0.925
EM CD4+ CD27-CD28+ (% of EM CD4+ T-cells)	8	-8.04 [-11.64;-3.94]	12	-7.74 [-13.09;2.64]	1.000	1.000
EM CD4+ CD57+ (% of EM CD4+ T-cells)	8	0.04 [-0.30;0.37]	12	-0.30 [-4.79;0.44]	0.418	0.925
TEMRA CD4+(% of CD4+ T-cells)	8	0.02 [-0.06;0.04]	12	0.01 [-0.13;0.45]	0.847	0.932
CD27 expression in TEMRA CD4+ (MFI)	8	240.00 [-17.00;351.00]	9	316.00 [192.00;422.00]	0.361	0.925
CD28 expression in TEMRA CD4+ (MFI)	8	150.00 [-234.50;470.00]	12	150.00 [15.00;330.50]	0.563	0.925
TEMRA CD4+ CD27+CD28+ (% of TEMRA CD4+ T-cells)	8	3.68 [-0.08;11.75]	12	321.00 [11.93 [2.64;20.72]	0.232	0.925
CD27 expression in TEMRA CD4+ CD27+CD28+ (MFI)	8	278.00 [107.00;393.00]	12	95.50 [110.00;467.50]	0.729	0.925
CD28 expression in TEMRA CD4+ CD27+CD28+ (MFI)	8	182.00 [54.50;488.50]	12	46.50 [46.50;452.50]	0.729	0.925
TEMRA CD4+ CD27+CD28- (% of TEMRA CD4+ T-cells)	8	-1.46 [-4.93;0.50]	12	0.07 [-0.38;5.68]	0.082	0.925
TEMRA CD4+ CD27-CD28- (% of TEMRA CD4+ T-cells)	8	-2.13 [-5.89;0.05]	12	-3.71 [-12.99;-0.57]	0.464	0.925
TEMRA CD4+ CD57+ (% of TEMRA CD4+ T-cells)	8	-2.48 [-14.55;8.90]	12	-1.31 [-15.41;5.08]	0.729	0.925
PBMC - CD8 Subsets						
Naïve CD8+ (% of CD8+ T-cells)	8	4.66 [1.18;12.36]	12	2.50 [-2.32;8.18]	0.418	0.999
CD27 expression in Naïve CD8+ (MFI)	8	372.00 [70.50;785.00]	12	248.50 [45.50;881.00]	0.787	0.999
CD28 expression in Naïve CD8+ (MFI)	8	27.00 [-36.50;82.00]	12	48.00 [-26.00;112.00]	0.589	0.999
Naïve CD8+ CD27+ CD28+ (% of Naïve CD8+ T-cells)	8	3.48 [1.07;16.91]	12	9.99 [2.78;23.19]	0.375	0.999
CD27 expression in Naïve CD8+ CD27+CD28+ (MFI)	8	342.00 [59.00;657.50]	12	209.00 [64.00;672.50]	0.908	0.999
CD28 expression in Naïve CD8+ CD27+CD28+ (MFI)	8	13.50 [-49.50;76.50]	12	47.50 [-34.00;100.00]	0.563	0.999
Naïve CD8+ CD57+ (% of Naïve CD8+ T-cells)	8	0.00 [-2.94;0.98]	12	-0.93 [-2.90;0.26]	0.671	0.999
CM CD8+ (% of CD8+ T-cells)	8	0.74 [-1.55;2.29]	12	-5.30 [-9.86;-1.56]	0.049	0.999
CD27 expression in CM CD8+ (MFI)	8	263.50 [73.00;488.00]	12	228.50 [46.00;583.00]	0.908	0.999
CD28 expression in CM CD8+ (MFI)	8	40.50 [-43.00;136.00]	12	90.00 [3.50;283.00]	0.464	0.999
CM CD8+ CD27+ CD28+ (% of CM CD8+ T-cells)	8	4.51 [2.42;16.05]	12	13.63 [-0.02;21.95]	0.464	0.999
CD27 expression in CM CD8+ CD27+CD28+ (MFI)	8	234.00 [54.00;368.00]	12	183.00 [47.00;486.00]	0.729	0.999
CD28 expression in CM CD8+ CD27+CD28+ (MFI)	8	32.50 [-53.00;111.50]	12	63.00 [11.50;228.50]	0.464	0.999
CM CD8+ CD57+ (% of CM CD8+ T-cells)	8	1.15 [-3.86;2.83]	12	-0.15 [-2.13;2.59]	0.908	0.999
EM CD8+ (% of CD8+ T-cells)	8	-3.25 [-4.58;-0.88]	12	-0.77 [-2.57;1.39]	0.097	0.999
CD27 expression in EM CD8+ (MFI)	8	79.00 [41.50;265.50]	12	165.00 [20.50;370.00]	0.512	0.999
CD28 expression in EM CD8+ (MFI)	8	55.00 [-43.50;130.50]	12	61.50 [-45.50;227.50]	0.563	0.999
EM CD8+ CD27+ CD28+ (% of EM CD8+ T-cells)	8	3.24 [1.81;15.12]	12	8.36 [-0.11;19.98]	0.729	0.999
CD27 expression in EM CD8+ CD27+CD28+ (MFI)	8	157.00 [29.50;203.50]	12	56.00 [-21.50;331.00]	0.969	1.000
CD28 expression in EM CD8+ CD27+CD28+ (MFI)	8	30.00 [-34.50;83.50]	12	7.00 [-90.50;199.00]	0.969	1.000
EM CD8+ CD27+CD28- (% of EM CD8+ T-cells)	8	1.89 [-0.38;2.27]	12	0.66 [-0.20;2.34]	0.847	0.999
EM CD8+ CD27-CD28- (% of EM CD8+ T-cells)	8	-3.60 [-6.12;3.68]	12	-3.90 [-9.66;4.78]	0.847	0.999
EM CD8+ CD27-CD28+ (% of EM CD8+ T-cells)	8	-7.79 [-13.54;0.46]	12	-8.87 [-15.78;2.93]	0.787	0.999
EM CD8+ CD57+ (% of EM CD8+ T-cells)	8	1.18 [-1.91;3.53]	12	-0.40 [-7.18;4.76]	0.847	0.999
TEMRA CD8+(% of CD8+ T-cells)	8	-1.45 [-6.26;0.94]	12	1.61 [-1.30;6.37]	0.133	0.999
CD27 expression in TEMRA CD8+ (MFI)	8	17.50 [-23.50;176.00]	12	110.00 [-5.00;158.00]	0.487	0.999
CD28 expression in TEMRA CD8+ (MFI)	8	29.50 [-16.00;52.00]	12	-1.50 [-35.00;55.50]	0.787	0.999
TEMRA CD8+ CD27+CD28+ (% of TEMRA CD8+ T-cells)	8	0.94 [-1.30;6.69]	12	3.14 [-0.43;5.13]	0.847	0.999
CD27 expression in TEMRA CD8+ CD27+CD28+ (MFI)	8	125.50 [-32.50;204.00]	11	41.00 [-109.00;361.00]	0.710	0.999
CD28 expression in TEMRA CD8+ CD27+CD28+ (MFI)	8	29.00 [-6.50;84.50]	11	14.00 [-62.00;323.00]	1.000	1.000
TEMRA CD8+ CD27+CD28- (% of TEMRA CD8+ T-cells)	8	0.42 [-0.55;3.01]	12	1.80 [1.06;2.59]	0.298	0.999
TEMRA CD8+ CD27-CD28- (% of TEMRA CD8+ T-cells)	8	-2.15 [-8.27;2.86]	12	-1.20 [-6.26;4.30]	0.671	0.999
TEMRA CD8+ CD57+ (% of TEMRA CD8+ T-cells)	8	0.64 [-1.76;4.40]	12	1.52 [-3.25;9.60]	0.847	0.999

PBMC - Tregs					
Total Treg (% of CD4+ T-cells)	8	-0.10 [-0.50;0.14] 196.50 [-	12	-0.95 [-1.85;0.12] 229.50 [-	0.375 0.766
CD25 expression in Total Treg (MFI)	8	57.00;1125.50]	12	42.50;767.00] -6.00 [-	0.787 0.932
CTLA-4 expression in Total Treg (MFI)	8	35.50 [5.00;64.00]	12	32.50;43.00]	0.165 0.766
Naïve Treg (% of total Tregs)	8	3.12 [-0.51;3.84]	12	4.84 [1.84;8.89] 322.50 [-	0.298 0.766
CD25 expression of Naïve Treg (MFI)	8	355.50 [44.00;672.00]	12	174.00;951.50]	0.616 0.932
CTLA-4 expression of Naïve Treg (MFI)	8	20.00 [4.00;69.00]	12	3.00 [-15.50;34.50]	0.418 0.766
Naïve Treg CTLA-4+ (% of Naïve Tregs)	8	1.74 [-0.49;10.56]	12	-0.73 [-16.63;3.83]	0.232 0.766
Memory Treg (% of total Tregs)	8	-3.28 [-4.41;-0.12] 214.50 [-	12	-4.97 [-8.73;-1.76] 204.00	0.263 0.766
CD25 expression of Memory Treg (MFI)	8	95.50;1111.00]	12	[64.00;762.00]	0.969 0.969
CTLA-4 expression of Memory Treg (MFI)	8	18.50 [-7.50;48.00]	12	1.50 [-37.00;46.00]	0.700 0.932
Memory Treg CTLA-4+ (% of Memory Tregs)	8	0.81 [-0.71;5.38]	12	1.08 [-3.34;3.82]	0.847 0.932
PBMC - T-cell activation status					
CD4+ PD1+ (% of CD4+ T-cells)	8	-0.31 [-0.81;0.39]	12	0.15 [-0.20;1.47] 16.50 [-	0.298 0.794
PD1 expression in CD4+ (MFI)	8	15.00 [-6.00;22.50]	12	33.50;53.50]	0.847 0.884
CD4+ CD69+ (% of CD4+ T-cells)	8	-0.32 [-6.04;3.50]	12	0.03 [-4.91;3.84]	0.729 0.884
CD69 expression in CD4+ (MFI)	8	-8.50 [-18.50;33.00]	12	13.50 [-4.50;43.50]	0.375 0.836
CD4+ PD1+CD69+ (% of CD4+ T-cells)	8	-0.57 [-0.81;0.32]	12	0.03 [-0.09;0.80]	0.203 0.695
CD4+ PD1-CD69- (% of CD4+ T-cells)	8	-0.04 [-3.61;6.86]	12	-0.10 [-4.21;3.97]	0.616 0.884
CD4+ HLADR+ (% of CD4+ T-cells)	8	4.03 [-1.75;4.49]	12	0.90 [-1.75;5.80] -17.00 [-	0.847 0.884
HLADR expression in CD4+ (MFI)	8	35.50 [-36.50;171.50]	12	86.00;35.50]	0.177 0.695
CD4+ CD38+ (% of CD4+ T-cells)	8	-0.35 [-3.58;6.10]	12	1.95 [-0.22;8.70] 15.00 [-	0.418 0.836
CD38 expression in CD4+ (MFI)	8	-48.00 [-85.00;98.00]	12	52.00;109.00]	0.512 0.878
CD4+ HLADR+CD38+ (% of CD4+ T-cells)	8	0.31 [-0.51;1.98]	12	0.85 [0.31;1.81]	0.418 0.836
CD4+ HLADR-CD38- (% of CD4+ T-cells)	8	-1.07 [-5.49;2.06]	12	-2.68 [-12.19;1.44]	0.464 0.856
CD8+ PD1+ (% of CD8+ T-cells)	8	1.28 [-9.20;8.50]	12	0.07 [-0.99;8.24] -4.50 [-	0.729 0.884
PD1 expression in CD8+ (MFI)	8	-15.50 [-37.00;-5.00]	12	24.50;56.50]	0.153 0.695
CD8+ CD69+ (% of CD8+ T-cells)	8	-2.89 [-6.68;-0.49] 60.00 [-	12	0.94 [-2.55;8.64] 27.50 [-	0.070 0.695
CD69 expression in CD8+ (MFI)	8	209.50;133.50]	12	0.50;146.50]	0.729 0.884
CD8+ PD1+CD69+ (% of CD8+ T-cells)	8	1.15 [-2.99;2.37]	12	0.06 [-0.48;4.16]	0.969 0.969
CD8+ PD1-CD69- (% of CD8+ T-cells)	8	1.62 [0.36;13.59]	12	-1.51 [-10.33;3.14]	0.232 0.695
CD8+ HLADR+ (% of CD8+ T-cells)	8	-1.67 [-5.26;5.20]	12	4.14 [1.26;14.62] 36.50 [-	0.083 0.695
HLADR expression in CD8+ (MFI)	8	18.50 [-34.00;51.50]	12	66.50;208.00]	0.758 0.884
CD8+ CD38+ (% of CD8+ T-cells)	8	2.53 [-0.90;8.00]	12	1.83 [0.94;7.01]	0.787 0.884
CD38 expression in CD8+ (MFI)	8	39.00 [-20.00;95.00]	12	7.50 [-36.00;82.50]	0.787 0.884
CD8+ HLADR+CD38+ (% of CD8+ T-cells)	8	-0.07 [-1.08;3.42]	12	1.91 [0.28;4.75]	0.203 0.695
CD8+ HLADR-CD38- (% of CD8+ T-cells)	8	0.96 [-8.41;4.39]	12	-6.17 [-17.26;-3.46]	0.133 0.695
Plasma - Essential Immune Response					
IL-4 (pg/mL)	8	0.29 [-1.15;6.22]	12	0.00 [-0.44;0.50]	0.560 0.840
IL-2 (pg/mL)	8	0.31 [-1.48;7.05]	12	-0.15 [-1.83;0.83] 74.28	0.464 0.835
CXCL-10 (IP-10) (pg/mL)	8	99.44 [-26.50;259.46]	12	[11.17;197.84]	0.908 0.969
IL-1 beta (pg/mL)	8	3.19 [-10.07;18.89]	12	-3.19 [-8.79;3.59]	0.487 0.835
TNF alpha (pg/mL)	8	-1.11 [-3.32;0.15]	12	0.11 [-1.00;1.13] 38.48 [-	0.133 0.397
CCL2 (MCP-1) (pg/mL)	8	21.50 [-59.56;132.87]	12	13.15;95.82]	0.908 0.969
IL-17A (pg/mL)	8	-1.08 [-10.87;2.35]	12	-0.90 [-1.51;0.56]	0.787 0.969
IL-6 (pg/mL)	8	-0.57 [-9.71;2.34]	12	1.43 [-0.07;5.02]	0.097 0.397
IL-10 (pg/mL)	8	-1.87 [-2.28;-1.21]	12	-0.89 [-1.81;0.70]	0.114 0.397
IFN gamma (pg/mL)	8	0.47 [-24.19;2.61]	12	0.00 [-3.44;1.90]	0.969 0.969
IL-12p70 (pg/mL)	8	-1.61 [-14.13;4.29]	12	1.30 [-1.48;4.03]	0.298 0.714
CXCL-8 (IL-8) (pg/mL)	8	-1.56 [-8.51;-0.44]	12	1.47 [0.51;3.24]	0.023 0.274

Plasma - Immune Checkpoint						
sCD25 (IL-2Ra) (pg/mL)	8	142.73 [51.07;345.70]	12	65.99 [-40.75;226.39]	0.464	0.795
4-1BB (pg/mL)	8	-19.40 [-39.76;7.14]	12	-4.20 [-17.44;2.91]	0.464	0.795
sCD27 (pg/mL)	8	-599.80 [-3829.00;846.70]	12	-5006.00 [-17099.00;1198.10]	0.203	0.609
B7.2 (CD86) (pg/mL)	8	-143.10 [-203.50;-55.31]	12	-108.20 [-134.00;-35.32]	0.616	0.924
Free Active TGF-B1 (pg/mL)	8	-14.20 [-18.56;-5.11]	12	-8.73 [-11.22;9.84]	0.133	0.609
CTLA-4 (pg/mL)	8	-3.70 [-8.00;1.19]	12	-2.03 [-4.54;0.70]	0.787	0.990
PD-L1 (pg/mL)	8	-50.06 [-106.10;38.33]	12	15.21 [-13.97;40.45]	0.154	0.609
PD-L2 (pg/mL)	8	1297.00 [-126.30;1712.80]	12	495.34 [-276.10;2131.10]	0.847	0.990
PD-1 (pg/mL)	8	-26.26 [-100.90;0.81]	12	-9.40 [-42.33;2.62]	0.464	0.795
Tim-3 (pg/mL)	8	815.64 [-1557.00;2143.90]	12	93.20 [-4254.00;2849.00]	0.908	0.990
LAG-3 (pg/mL)	8	-408.80 [-1412.00;2921.60]	12	-347.70 [-593.80;448.45]	1.000	1.000
Gal-9 (pg/mL)	8	12127.00 [2463.40;35168.00]	12	3440.50 [-10789.00;18306.00]	0.203	0.609

Table S7. Comparison of PBMC subsets (major subsets, CD4 subsets, CD8 subsets, Tregs, and T-cell activation status) and plasma immune response and checkpoint markers between the fit and frail patients at baseline. The number of measurements (N), median, interquartile range (IQR), raw P-values, and FDR-corrected p-values are reported. The P-values were calculated using an unpaired Mann-Whitney U test. The P-values below the significance threshold of 5% are indicated in bold italics. FDR: false discovery rate; MFI: mean fluorescence intensity.

Biomarker	Fit		Frail		P-values	
	N	Median [IQR]	N	Median [IQR]	Raw	FDR
PBMC - Major Subsets						
Viable cells (% of total cells)	8	99.20 [96.94;99.51]	12	98.65 [98.29;99.18]	0.232	0.596
Viable lymphocytes (% of total lymphocytes)	8	98.12 [96.27;98.48]	12	96.73 [90.71;97.76]	0.154	0.531
Total T-cells (% of viable lymphocytes)	8	61.50 [56.82;71.20]	12	62.16 [53.54;64.67]	0.643	0.930
Total T-cells (% of viable cells)	8	59.39 [42.18;64.59]	12	54.68 [47.77;61.56]	0.908	0.969
CD4+ T-cells (% of viable lymphocytes)	8	52.70 [29.20;56.34]	12	50.22 [44.03;57.76]	0.671	0.930
CD8+ T-cells (% of viable lymphocytes)	8	16.44 [14.48;20.99]	12	13.96 [8.49;18.23]	0.177	0.531
CD4/CD8 ratio (% CD4/CD8 ratio)	8	3.16 [1.60;4.12]	12	3.99 [2.61;6.86]	0.154	0.531
NK/T-cells (% of viable cells)	8	4.46 [2.65;10.06]	12	1.81 [1.43;3.70]	0.049	0.442
Total NK cells (% of viable cells)	8	9.16 [5.54;12.23]	12	6.52 [3.69;10.64]	0.375	0.836
CD56 bright CD16- (% of total NK cells)	8	3.96 [2.64;12.78]	12	4.74 [4.00;8.47]	0.563	0.921
CD56 dim CD16+ (% of total NK cells)	8	82.71 [68.17;91.11]	12	58.52 [19.32;76.44]	0.015	0.272
Total B-cells (% of viable cells)	8	9.00 [7.47;10.06]	12	7.51 [5.58;9.54]	0.418	0.836
Naïve B-cells (% of total B-cells)	8	58.95 [54.53;71.71]	12	62.99 [42.09;75.50]	0.969	0.969
Memory B-cells (% of total B-cells)	8	40.22 [27.47;44.21]	12	35.87 [23.20;56.53]	0.969	0.969
Total monocytes (% of viable cells)	8	13.77 [12.65;16.06]	12	18.11 [8.34;24.38]	0.512	0.921
Non-classical monocytes (% of total monocytes)	8	4.47 [3.04;5.03]	12	3.41 [1.19;9.72]	0.969	0.969
Intermediate monocytes (% of total monocytes)	8	5.76 [4.17;6.75]	12	6.17 [4.74;7.32]	0.787	0.969
Classical monocytes (% of total monocytes)	8	83.73 [80.18;86.12]	12	79.92 [68.76;83.23]	0.154	0.531
PBMC - CD4 Subsets						
Naïve CD4+ (% of CD4+ T-cells)	8	26.32 [18.89;49.10]	12	44.61 [22.85;50.60]	0.335	0.567
CD27 expression in Naïve CD4+ (MFI)	8	1458.50 [1248.50;1752.50]	12	1312.00 [914.50;1596.50]	0.232	0.497
CD28 expression in Naïve CD4+ (MFI)	8	766.00 [666.00;864.00]	12	614.00 [548.50;726.50]	0.203	0.497
Naïve CD4+ CD27+ CD28+ (% of Naïve CD4+ T-cells)	8	96.01 [90.34;98.05]	12	88.82 [71.31;92.93]	0.019	0.307
CD27 expression in Naïve CD4+ CD27+CD28+ (MFI)	8	1491.00 [1323.50;1831.50]	12	1424.50 [1134.00;1677.50]	0.464	0.567
CD28 expression in Naïve CD4+ CD27+CD28+ (MFI)	8	764.00 [690.50;871.00]	12	649.50 [565.50;739.50]	0.177	0.497
Naïve CD4+ CD57+ (% of Naïve CD4+ T-cells)	8	4.70 [2.43;18.71]	12	2.68 [1.35;11.63]	0.563	0.641
CM CD4+ (% of CD4+ T-cells)	8	60.90 [39.22;66.37]	12	47.86 [33.74;59.69]	0.203	0.497
CD27 expression in CM CD4+ (MFI)	8	1279.00 [970.00;1521.00]	11	1006.00 [817.00;1326.00]	0.231	0.497
CD28 expression in CM CD4+ (MFI)	8	1265.00 [1068.50;1379.00]	12	1089.50 [868.00;1247.50]	0.133	0.497
CM CD4+ CD27+ CD28+ (% of CM CD4+ T-cells)	8	80.46 [74.87;83.76]	12	70.12 [59.29;79.39]	0.114	0.497
CD27 expression in CM CD4+ CD27+CD28+ (MFI)	8	1490.00 [1261.50;1803.00]	12	1359.50 [1183.50;1601.00]	0.375	0.567
CD28 expression in CM CD4+ CD27+CD28+ (MFI)	8	1227.50 [1092.50;1347.00]	12	1111.00 [888.50;1256.50]	0.217	0.497
CM CD4+ CD57+ (% of CM CD4+ T-cells)	8	2.01 [1.53;3.10]	12	2.87 [1.96;4.05]	0.232	0.497
EM CD4+ (% of CD4+ T-cells)	8	10.51 [6.97;18.67]	12	10.35 [5.42;14.76]	0.464	0.567
CD27 expression in EM CD4+ (MFI)	8	456.00 [322.50;505.00]	11	371.00 [134.00;579.00]	0.536	0.631
CD28 expression in EM CD4+ (MFI)	8	1027.00 [890.00;1284.50]	12	955.00 [802.50;1201.50]	0.464	0.567
EM CD4+ CD27+ CD28+ (% of EM CD4+ T-cells)	8	34.08 [26.25;42.46]	12	31.17 [17.30;38.43]	0.464	0.567
CD27 expression in EM CD4+ CD27+CD28+ (MFI)	8	1203.00 [1036.50;1389.00]	12	1114.00 [971.00;1274.00]	0.335	0.567
CD28 expression in EM CD4+ CD27+CD28+ (MFI)	8	1149.50 [1072.50;1365.50]	12	1062.00 [874.00;1257.50]	0.177	0.497
EM CD4+ CD27+CD28- (% of EM CD4+ T-cells)	8	0.18 [0.08;0.81]	12	0.04 [0.00;0.32]	0.241	0.497

EM CD4+ CD27-CD28- (% of EM CD4+ T-cells)	8	4.47 [0.92;13.05]	12	2.19 [1.45;4.06]	0.700	0.721
EM CD4+ CD27-CD28+ (% of EM CD4+ T-cells)	8	53.20 [50.65;61.83]	12	62.95 [56.89;72.30]	0.083	0.497
EM CD4+ CD57+ (% of EM CD4+ T-cells)	8	1.54 [0.74;3.57]	12	2.14 [1.37;11.21]	0.203	0.497
TEMRA CD4+(% of CD4+ T-cells)	8	0.41 [0.20;1.47]	12	0.62 [0.33;2.60]	0.375	0.567
CD27 expression in TEMRA CD4+ (MFI)	8	500.50 [95.500;697.50]	9	396.00 [201.00;418.00] 799.50	0.597	0.656
CD28 expression in TEMRA CD4+ (MFI)	8	519.00 [230.50;733.50]	12	[455.00;1068.50]	0.177	0.497
TEMRA CD4+ CD27+CD28+ (% of TERMA CD4+ T-cells)	8	26.935[11.58;48.75] 1189.50	12	27.59 [13.65;35.75] 1044.50	0.728	0.728
CD27 expression in TEMRA CD4+ CD27+CD28+ (MFI)	8	[939.50;1464.50]	12	[937.00;1229.00]	0.464	0.567
CD28 expression in TEMRA CD4+ CD27+CD28+ (MFI)	8	1007.00 [929.00;1082.00]	12	1104.00 [816.00;1208.00]	0.616	0.656
TEMRA CD4+ CD27+CD28- (% of TEMRA CD4+ T-cells)	8	10.42 [4.09;19.63]	12	0.00 [0.00;1.88]	0.009	0.283
TEMRA CD4+ CD27-CD28- (% of TEMRA CD4+ T-cells)	8	24.13 [11.51;53.79]	12	12.95 [5.33;35.81]	0.418	0.567
TEMRA CD4+ CD57+ (% of TEMRA CD4+ T-cells)	8	47.02 [28.37;76.66]	12	27.99 [8.55;53.71]	0.263	0.511
PBMC - CD8 Subsets						
Naïve CD8+ (% of CD8+ T-cells)	8	8.89 [6.98;26.00] 1873.50	12	20.00 [14.10;48.67] 1229.50	0.083	0.195
CD27 expression in Naïve CD8+ (MFI)	8	[1517.00;2110.50]	12	[1014.00;1579.00]	0.010	0.064
CD28 expression in Naïve CD8+ (MFI)	8	642.50 [541.50;737.50]	12	538.00 [436.00;641.00]	0.177	0.278
Naïve CD8+ CD27+ CD28+ (% of Naïve CD8+ T-cells)	8	88.32 [76.96;91.95]	12	66.27 [46.33;77.72]	0.004	0.064
CD27 expression in Naïve CD8+ CD27+CD28+ (MFI)	8	2005.00 [1706.50;2230.50]	12	1579.00 [1526.50;1820.50]	0.041	0.148
CD28 expression in Naïve CD8+ CD27+CD28+ (MFI)	8	657.00 [558.00;770.00]	12	576.50 [491.50;661.50]	0.335	0.442
Naïve CD8+ CD57+ (% of Naïve CD8+ T-cells)	8	3.16 [1.80;7.34]	12	5.19 [2.72;6.86]	0.671	0.821
CM CD8+ (% of CD8+ T-cells)	8	17.90 [16.32;25.91] 1335.50	12	29.27 [22.16;39.40] 905.50	0.059	0.176
CD27 expression in CM CD8+ (MFI)	8	[1083.50;1461.00] 1015.50	12	[694.50;1069.00] 878.50	0.006	0.064
CD28 expression in CM CD8+ (MFI)	8	[896.00;1078.50]	12	[691.00;1015.00]	0.177	0.278
CM CD8+ CD27+ CD28+ (% of CM CD8+ T-cells)	8	74.34 [69.93;84.43] 1589.50	12	56.80 [45.18;66.45] 1323.50	0.002	0.064
CD27 expression in CM CD8+ CD27+CD28+ (MFI)	8	[1382.00;1715.00]	12	[1172.00;1497.50]	0.041	0.148
CD28 expression in CM CD8+ CD27+CD28+ (MFI)	8	1057.50 [942.50;1103.00]	12	956.50 [775.00;1057.00]	0.203	0.305
CM CD8+ CD57+ (% of CM CD8+ T-cells)	8	10.78 [8.77;12.74]	12	9.92 [6.56;14.91]	0.729	0.859
EM CD8+ (% of CD8+ T-cells)	8	30.36 [24.22;41.33]	12	17.33 [10.72;28.47]	0.070	0.192
CD27 expression in EM CD8+ (MFI)	8	631.00 [481.00;708.00]	12	437.50 [357.00;578.50]	0.097	0.214
CD28 expression in EM CD8+ (MFI)	8	474.50 [327.00;622.50]	12	497.50 [396.50;591.00]	0.847	0.932
EM CD8+ CD27+ CD28+(% of EM CD8+ T-cells)	8	23.70 [15.16;33.40] 1369.50	12	15.30 [8.64;23.33] 1274.50	0.083	0.195
CD27 expression in EM CD8+ CD27+CD28+ (MFI)	8	[1297.00;1500.00]	12	[1211.00;1414.50]	0.165	0.278
CD28 expression in EM CD8+ CD27+CD28+ (MFI)	8	819.50 [793.00;950.50]	12	791.00 [732.00;899.50]	0.512	0.650
EM CD8+ CD27+CD28- (% of EM CD8+ T-cells)	8	4.95 [3.01;6.91]	12	2.16 [0.85;3.14]	0.028	0.131
EM CD8+ CD27-CD28- (% of EM CD8+ T-cells)	8	27.68 [22.95;43.91]	12	24.00 [12.54;40.40]	0.335	0.442
EM CD8+ CD27-CD28+ (% of EM CD8+ T-cells)	8	36.82 [31.41;44.71]	12	56.06 [42.69;64.97]	0.012	0.067
EM CD8+ CD57+ (% of EM CD8+ T-cells)	8	44.74 [43.34;53.29]	12	49.35 [15.50;60.11]	0.847	0.932
TEMRA CD8+(% of CD8+ T-cells)	8	27.01 [18.18;39.86]	12	22.32 [6.91;26.87]	0.177	0.278
CD27 expression in TEMRA CD8+ (MFI)	8	257.50 [205.00;438.50]	12	268.50 [195.50;386.00]	0.877	0.934
CD28 expression in TEMRA CD8+ (MFI)	8	86.00 [67.00;132.50]	12	188.00 [105.00;354.00]	0.045	0.148
TEMRA CD8+ CD27+CD28+ (% of TERMA CD8+ T-cells)	8	4.33 [2.76;5.20] 1481.50	12	3.60 [1.80;6.91] 1515.00	0.908	0.936
CD27 expression in TEMRA CD8+ CD27+CD28+ (MFI)	8	[1392.00;1560.50]	11	[1303.00;1664.00]	1.000	1.000
CD28 expression in TEMRA CD8+ CD27+CD28+ (MFI)	8	685.50 [655.00;726.00]	11	747.00 [653.00;873.00]	0.231	0.332
TEMRA CD8+ CD27+CD28- (% of TEMRA CD8+ T-cells)	8	2.82 [2.26;4.50]	12	1.30 [0.42;1.54]	0.010	0.064
TEMRA CD8+ CD27-CD28- (% of TEMRA CD8+ T-cells)	8	81.72 [72.29;85.97]	12	65.24 [43.68;81.82]	0.133	0.273
TEMRA CD8+ CD57+ (% of TEMRA CD8+ T-cells)	8	73.62 [64.18;77.01]	12	61.92 [39.21;76.18]	0.154	0.278

PBMC - Tregs					
Total Treg (% of CD4+ T-cells)	8	5.11 [4.28;6.19] 2203.50	12	4.57 [3.88;6.90] 1293.50	0.787 0.787
CD25 expression in Total Treg (MFI)	8	[1610.00;2842.50]	12	[1096.50;1816.50]	0.041 0.075
CTLA-4 expression in Total Treg (MFI)	8	228.50 [213.50;247.00]	12	272.50 [246.50;301.00]	0.031 0.075
Naïve Treg (% of total Tregs)	8	6.30 [4.76;8.05] 2048.50	12	5.15 [3.04;13.94] 1279.50	0.787 0.787
CD25 expression of Naïve Treg (MFI)	8	[1173.50;2256.00]	12	[827.50;1556.50]	0.059 0.081
CTLA-4 expression of Naïve Treg (MFI)	8	186.00 [178.50;220.00]	12	252.50 [216.50;318.50]	0.025 0.075
Naïve Treg CTLA-4+ (% of Naïve Tregs)	8	15.94 [8.51;19.89]	12	33.18 [23.25;40.31]	0.025 0.075
Memory Treg (% of total Tregs)	8	93.51 [91.47;94.70] 2178.00	12	94.74 [85.54;96.79] 1345.00	0.787 0.787
CD25 expression of Memory Treg (MFI)	8	[1627.50;2838.50]	12	[1094.50;1845.50]	0.037 0.075
CTLA-4 expression of Memory Treg (MFI)	8	230.50 [215.50;256.50]	12	271.00 [248.00;305.00]	0.049 0.077
Memory Treg CTLA-4+ (% of Memory Tregs)	8	15.59 [8.43;17.68]	12	19.74 [16.52;21.61]	0.019 0.075
PBMC - T-cell activation status					
CD4+ PD1+ (% of CD4+ T-cells)	8	1.95 [1.69;2.89]	12	1.26 [0.88;1.62]	0.049 0.147
PD1 expression in CD4+ (MFI)	8	224.50 [160.50;297.00]	12	195.50 [99.50;216.00]	0.133 0.289
CD4+ CD69+ (% of CD4+ T-cells)	8	35.46 [25.93;42.24]	12	31.54 [17.07;38.34]	0.177 0.327
CD69 expression in CD4+ (MFI)	8	249.50 [236.00;290.50]	12	262.00 [226.50;270.00]	0.877 0.908
CD4+ PD1+CD69+ (% of CD4+ T-cells)	8	1.41 [0.98;2.13]	12	0.74 [0.50;1.04]	0.023 0.110
CD4+ PD1-CD69- (% of CD4+ T-cells)	8	64.00 [56.19;73.22]	12	67.83 [61.28;82.28]	0.177 0.327
CD4+ HLADR+ (% of CD4+ T-cells)	8	8.37 [6.88;14.41]	12	16.98 [11.69;22.80]	0.034 0.136
HLADR expression in CD4+ (MFI)	8	333.00 [297.50;381.00]	12	459.00 [388.00;627.00]	0.023 0.110
CD4+ CD38+ (% of CD4+ T-cells)	8	33.65 [18.60;46.64]	12	28.78 [20.25;45.58]	0.908 0.908
CD38 expression in CD4+ (MFI)	8	671.00 [500.00;874.00]	12	644.50 [488.50;777.00]	0.671 0.732
CD4+ HLADR+CD38+ (% of CD4+ T-cells)	8	2.41 [1.40;3.89]	12	3.47 [2.14;4.28]	0.354 0.532
CD4+ HLADR-CD38- (% of CD4+ T-cells)	8	59.02 [44.86;69.63]	12	51.84 [48.00;63.98]	0.563 0.711
CD8+ PD1+ (% of CD8+ T-cells)	8	29.14 [21.31;40.55]	12	15.24 [9.90;23.11]	0.015 0.110
PD1 expression in CD8+ (MFI)	8	325.00 [230.00;526.00]	12	267.50 [203.50;323.50]	0.263 0.421
CD8+ CD69+ (% of CD8+ T-cells)	8	36.86 [21.01;55.88]	12	31.63 [22.80;42.70]	0.464 0.655
CD69 expression in CD8+ (MFI)	8	513.00 [390.50;931.50]	12	342.50 [279.50;437.00]	0.015 0.110
CD8+ PD1+CD69+ (% of CD8+ T-cells)	8	11.68 [7.68;15.99]	12	6.17 [5.13;9.99]	0.076 0.202
CD8+ PD1-CD69- (% of CD8+ T-cells)	8	36.20 [30.95;53.39]	12	58.50 [47.46;69.09]	0.023 0.110
CD8+ HLADR+ (% of CD8+ T-cells)	8	31.78 [16.88;42.36]	12	41.85 [33.45;47.17]	0.133 0.289
HLADR expression in CD8+ (MFI)	8	474.50 [341.00;488.00]	12	531.50 [489.50;709.00]	0.041 0.140
CD8+ CD38+ (% of CD8+ T-cells)	8	14.06 [7.27;17.44]	12	8.47 [6.20;21.45]	0.671 0.732
CD38 expression in CD8+ (MFI)	8	293.00 [230.50;342.50]	12	243.50 [192.50;355.00]	0.563 0.711
CD8+ HLADR+CD38+ (% of CD8+ T-cells)	8	7.82 [1.71;10.11]	12	4.98 [4.12;6.99]	0.671 0.732
CD8+ HLADR-CD38- (% of CD8+ T-cells)	8	64.70 [38.71;77.09]	12	52.45 [48.44;55.66]	0.203 0.348
Plasma - Essential Immune Response					
IL-4 (pg/mL)	8	0.11 [0.00;28.48]	12	0.06 [0.00;1.38]	0.773 0.928
IL-2 (pg/mL)	8	3.34 [1.92;9.66]	12	1.80 [0.67;2.24]	0.154 0.423
CXCL-10 (IP-10) (pg/mL)	8	159.08 [125.71;355.70]	12	311.58 [176.37;354.14]	0.232 0.423
IL-1 beta (pg/mL)	8	6.06 [1.47;34.62]	12	5.27 [0.77;11.90]	0.616 0.858
TNF alpha (pg/mL)	8	1.81 [1.21;5.54]	12	2.07 [1.63;3.43]	0.643 0.858
CCL2 (MCP-1) (pg/mL)	8	193.48 [149.28;232.19]	12	133.65 [97.65;174.79]	0.070 0.419
IL-17A (pg/mL)	8	2.23 [1.18;22.16]	12	2.78 [1.35;8.50]	0.969 0.969
IL-6 (pg/mL)	8	8.40 [3.86;24.28]	12	3.86 [2.88;8.14]	0.203 0.423
IL-10 (pg/mL)	8	2.23 [1.54;3.82]	12	1.37 [0.62;2.63]	0.247 0.423
IFN gamma (pg/mL)	8	0.91 [0.00;35.63]	12	1.32 [0.00;6.07]	0.905 0.969
IL-12p70 (pg/mL)	8	6.93 [4.28;58.97]	12	2.05 [0.71;4.19]	0.008 0.093
CXCL-8 (IL-8) (pg/mL)	8	7.23 [3.55;25.42]	12	3.77 [1.94;11.62]	0.232 0.423
Plasma - Immune Checkpoint					

sCD25 (IL-2Ra) (pg/mL)	8	928.26 [798.87;1140.80]	12	1504.50 [978.83;2268.00]	0.133	0.531
4-1BB (pg/mL)	8	68.07 [50.57;119.55] 52670.00	12	80.31 [54.40;156.65] 66274.00	0.464	0.695
sCD27 (pg/mL)	8	[45645.00;65276.00]	12	[50880.00;87801.00]	0.298	0.595
B7.2 (CD86) (pg/mL)	8	524.80 [474.15;697.47]	12	582.31 [452.28;700.38]	0.969	0.969
Free Active TGF-B1 (pg/mL)	8	25.39 [22.12;32.09]	12	23.19 [11.09;42.99]	0.616	0.821
CTLA-4 (pg/mL)	8	10.14 [4.40;15.98]	12	8.84 [2.25;27.96]	0.847	0.924
PD-L1 (pg/mL)	8	279.06 [172.00;401.70] 11121.00	12	165.63 [113.50;213.15] 13370.00	0.154	0.531
PD-L2 (pg/mL)	8	[10196.00;14318.00]	12	[11972.00;16020.00]	0.177	0.531
PD-1 (pg/mL)	8	92.41 [16.51;201.92] 15345.00	12	33.25 [16.94;118.63] 26074.00	0.787	0.924
Tim-3 (pg/mL)	8	[6568.40;26546.00] 5456.70	12	[18660.00;38595.00] 2560.40	0.114	0.531
LAG-3 (pg/mL)	8	[2198.80;16183.00] 102158.00	12	[1596.90;3621.40] 1058680.00	0.263	0.595
Gal-9 (pg/mL)	8	[76361.00;127351.00]	12	[90925.00;159163.00]	0.375	0.643

Table S8. Comparison of PBMC subsets (major subsets, CD4 subsets, CD8 subsets, Tregs, and T-cell activation status) and plasma immune response and checkpoint markers between the fit and frail patients after three months of combination therapy. The number of measurements (N), median, interquartile range (IQR), raw p-values, and FDR-corrected P-values are reported. The P-values were calculated using an unpaired Mann-Whitney U test. The P-values below the significance threshold of 5% are indicated in bold italics. FDR: false discovery rate; MFI: mean fluorescence intensity.

Biomarker	Fit		Frail		P-values	
	N	Median [IQR]	N	Median [IQR]	Raw	FDR
PBMC - Major Subsets						
Viable cells (% of total cells)	8	99.29 [98.88;99.61]	12	99.19 [98.76;99.61]	0.758	1.000
Viable lymphocytes (% of total lymphocytes)	8	97.79 [96.94;98.59]	12	97.92 [96.82;98.60]	0.969	1.000
Total T-cells (% of viable lymphocytes)	8	65.62 [56.74;72.41]	12	60.47 [49.61;66.66]	0.375	1.000
Total T-cells (% of viable cells)	8	59.69 [43.29;67.63]	12	56.93 [47.61;60.10]	0.908	1.000
CD4 ⁺ T-cells (% of viable lymphocytes)	8	52.78 [38.14;60.31]	12	49.89 [41.65;55.80]	0.671	1.000
CD8 ⁺ T-cells (% of viable lymphocytes)	8	15.24 [10.03;19.46]	12	10.72 [7.78;16.06]	0.203	1.000
CD4/CD8 ratio (% CD4/CD8 ratio)	8	2.78 [2.62;5.98]	12	4.13 [2.80;7.17]	0.418	1.000
NK/T-cells (% of viable cells)	8	4.74 [2.69;7.44]	12	2.82 [1.52;4.51]	0.132	1.000
Total NK cells (% of viable cells)	8	8.74 [6.76;11.65]	12	11.08 [6.38;15.27]	0.464	1.000
CD56 bright CD16 ⁻ (% of total NK cells)	8	3.82 [2.55;14.38]	12	6.54 [4.76;10.49]	0.616	1.000
CD56 dim CD16 ⁺ (% of total NK cells)	8	71.87 [50.83;90.52]	12	64.98 [43.70;88.52]	0.671	1.000
Total B-cells (% of viable cells)	8	7.61 [5.91;9.87]	12	9.29 [4.52;12.36]	0.671	1.000
Naïve B-cells (% of total B-cells)	8	71.81 [64.05;81.13]	12	74.80 [62.54;80.94]	0.787	1.000
Memory B-cells (% of total B-cells)	8	27.87 [17.79;35.21]	12	24.48 [18.23;36.41]	1.000	1.000
Total monocytes (% of viable cells)	8	12.89 [8.16;20.14]	12	15.94 [5.82;19.00]	0.908	1.000
Non-classical monocytes (% of total monocytes)	8	9.63 [7.70;12.98]	12	4.79 [2.77;7.91]	0.083	1.000
Intermediate monocytes (% of total monocytes)	8	8.63 [7.65;12.87]	12	8.36 [5.13;10.63]	0.512	1.000
Classical monocytes (% of total monocytes)	8	70.19 [64.80;78.07]	12	75.53 [59.36;82.19]	0.847	1.000
PBMC - CD4 Subsets						
Naïve CD4 ⁺ (% of CD4 ⁺ T-cells)	8	40.81 [18.93;47.80] 1829.50	12	54.15 [30.13;63.86]	0.177	0.845
CD27 expression in Naïve CD4 ⁺ (MFI)	8	[1559.00;2084.50]	12	1717.50 [1410.50;1818.00]	0.512	0.845
CD28 expression in Naïve CD4 ⁺ (MFI)	8	806.00 [712.00;838.50]	12	791.00 [676.50;850.00]	0.939	0.968
Naïve CD4 ⁺ CD27 ⁺ CD28 ⁺ (% of Naïve CD4 ⁺ T-cells)	8	98.490 [97.000;98.685] 1849.50	12	95.250 [91.675;96.830]	0.028	0.845
CD27 expression in Naïve CD4 ⁺ CD27 ⁺ CD28 ⁺ (MFI)	8	[1599.50;2109.50]	12	1770.50 [1509.50;1875.00]	0.671	0.886
CD28 expression in Naïve CD4 ⁺ CD27 ⁺ CD28 ⁺ (MFI)	8	810.50 [717.50;840.50]	12	783.50 [705.00;845.00]	0.969	0.969
Naïve CD4 ⁺ CD57 ⁺ (% of Naïve CD4 ⁺ T-cells)	8	6.98 [1.03;10.63]	12	1.81 [0.47;5.06]	0.232	0.845
CM CD4 ⁺ (% of CD4 ⁺ T-cells)	8	48.86 [44.26;61.18] 1502.00	12	38.75 [27.56;57.95]	0.203	0.845
CD27 expression in CM CD4 ⁺ (MFI)	8	[1233.00;1767.00] 1271.50	12	1404.00 [1117.00;1698.00]	0.616	0.884
CD28 expression in CM CD4 ⁺ (MFI)	8	[1119.50;1358.00]	12	1198.00 [1098.50;1370.50]	0.616	0.884
CM CD4 ⁺ CD27 ⁺ CD28 ⁺ (% of CM CD4 ⁺ T-cells)	8	85.62 [82.75;90.89] 1722.50	12	85.03 [72.43;87.00]	0.464	0.845
CD27 expression in CM CD4 ⁺ CD27 ⁺ CD28 ⁺ (MFI)	8	[1510.50;1962.00] 1266.50	12	1621.00 [1483.00;1878.50]	0.616	0.884
CD28 expression in CM CD4 ⁺ CD27 ⁺ CD28 ⁺ (MFI)	8	[1098.50;1335.50]	12	1186.00 [1103.0;1342.00]	0.671	0.886
CM CD4 ⁺ CD57 ⁺ (% of CM CD4 ⁺ T-cells)	8	1.95 [1.64;2.49]	12	2.17 [1.37;3.41]	0.908	0.966
EM CD4 ⁺ (% of CD4 ⁺ T-cells)	8	11.79 [7.20;15.59]	12	7.59 [4.40;10.94]	0.203	0.845
CD27 expression in EM CD4 ⁺ (MFI)	8	691.00 [421.00;843.50]	12	481.00 [379.50;733.50]	0.512	0.845
CD28 expression in EM CD4 ⁺ (MFI)	8	1141.50 [996.00;1267.50]	12	1103.05 [1023.00;1260.00]	0.729	0.890
EM CD4 ⁺ CD27 ⁺ CD28 ⁺ (% of EM CD4 ⁺ T-cells)	8	51.89 [34.75;54.52] 1355.50	12	38.07 [30.48;44.77]	0.335	0.845
CD27 expression in EM CD4 ⁺ CD27 ⁺ CD28 ⁺ (MFI)	8	[1316.50;1563.50] 1232.50	12	1292.00 [1192.50;1573.00]	0.418	0.845
CD28 expression in EM CD4 ⁺ CD27 ⁺ CD28 ⁺ (MFI)	8	[1187.50;1459.00]	12	1179.50 [1135.50;1339.50]	0.335	0.845
EM CD4 ⁺ CD27 ⁺ CD28 ⁻ (% of EM CD4 ⁺ T-cells)	8	0.13 [0.05;0.27]	12	0.11 [0.00;0.40]	0.510	0.845

EM CD4+ CD27-CD28- (% of EM CD4+ T-cells)	8	1.46 [0.69;13.98]	12	2.16 [0.57;3.75]	0.787	0.896
EM CD4+ CD27-CD28+ (% of EM CD4+ T-cells)	8	45.53 [42.46;46.81]	12	57.76 [47.14;64.68]	0.114	0.845
EM CD4+ CD57+ (% of EM CD4+ T-cells)	8	1.74 [1.00;2.51]	12	2.33 [1.33;5.32]	0.232	0.845
TEMRA CD4+(% of CD4+ T-cells)	8	0.35 [0.24;0.91]	12	0.56 [0.38;2.25]	0.335	0.845
CD27 expression in TEMRA CD4+ (MFI)	8	546.00 [107.50;1033.50]	11	576.00 [376.00;878.00]	0.773	0.896
CD28 expression in TEMRA CD4+ (MFI)	8	766.50 [228.00;1233.50]	12	1008.00 [629.00;1171.00]	0.729	0.890
TEMRA CD4+ CD27+CD28+ (% of TERMA CD4+ T-cells)	8	27.19 [12.42;59.22]	12	32.86 [25.25;53.91]	0.512	0.845
CD27 expression in TEMRA CD4+ CD27+CD28+ (MFI)	8	1609.50 [1258.00;1646.50]	12	1454.50 [1172.00;1609.00]	0.464	0.845
CD28 expression in TEMRA CD4+ CD27+CD28+ (MFI)	8	1289.00 [889.00;1559.00]	12	1245.00 [1055.00;1525.50]	0.908	0.966
TEMRA CD4+ CD27+CD28- (% of TEMRA CD4+ T-cells)	8	5.21 [1.53;11.04]	12	1.15 [0.00;5.68]	0.260	0.845
TEMRA CD4+ CD27-CD28- (% of TEMRA CD4+ T-cells)	8	16.57 [6.87;54.53]	12	4.82 [2.48;26.06]	0.232	0.845
TEMRA CD4+ CD57+ (% of TEMRA CD4+ T-cells)	8	45.56 [22.38;77.28]	12	22.99 [9.38;44.65]	0.154	0.845
PBMC - CD8 Subsets						
Naïve CD8+ (% of CD8+ T-cells)	8	21.62 [13.01;34.47]	12	21.17 [14.13;52.44]	0.671	0.886
CD27 expression in Naïve CD8+ (MFI)	8	2255.00 [1848.50;2482.50]	12	1676.00 [1227.50;2032.00]	0.070	0.461
CD28 expression in Naïve CD8+ (MFI)	8	554.00 [514.00;758.00]	12	599.50 [499.00;641.00]	0.616	0.884
Naïve CD8+ CD27+ CD28+ (% of Naïve CD8+ T-cells)	8	94.74 [92.60;95.92]	12	81.19 [61.98;88.81]	0.019	0.204
CD27 expression in Naïve CD8+ CD27+CD28+ (MFI)	8	2349.00 [1898.50;2572.50]	12	1983.50 [1671.00;2189.00]	0.154	0.633
CD28 expression in Naïve CD8+ CD27+CD28+ (MFI)	8	584.50 [531.50;766.50]	12	619.00 [546.50;661.00]	1.000	1.000
Naïve CD8+ CD57+ (% of Naïve CD8+ T-cells)	8	2.39 [1.77;3.50]	12	2.40 [1.33;4.85]	0.787	0.962
CM CD8+ (% of CD8+ T-cells)	8	17.98 [16.38;28.72]	12	18.44 [14.94;32.76]	0.969	1.000
CD27 expression in CM CD8+ (MFI)	8	1566.00 [1222.50;1757.50]	12	1178.00 [876.50;1488.50]	0.105	0.578
CD28 expression in CM CD8+ (MFI)	8	1043.00 [844.50;1111.00]	12	867.50 [795.00;1126.00]	0.512	0.805
CM CD8+ CD27+ CD28+ (% of CM CD8+ T-cells)	8	86.13 [76.94;87.44]	12	70.19 [53.41;75.44]	0.012	0.201
CD27 expression in CM CD8+ CD27+CD28+ (MFI)	8	1740.00 [1484.50;1961.50]	12	1495.00 [1338.00;1832.00]	0.217	0.789
CD28 expression in CM CD8+ CD27+CD28+ (MFI)	8	1052.00 [924.50;1116.00]	12	933.00 [851.50;1148.50]	0.464	0.805
CM CD8+ CD57+ (% of CM CD8+ T-cells)	8	10.64 [8.03;12.77]	12	10.62 [5.94;16.58]	0.847	0.998
EM CD8+ (% of CD8+ T-cells)	8	27.05 [21.24;33.77]	12	17.09 [10.91;31.89]	0.298	0.789
CD27 expression in EM CD8+ (MFI)	8	769.00 [534.50;953.00]	12	638.50 [478.00;822.00]	0.512	0.805
CD28 expression in EM CD8+ (MFI)	8	519.00 [349.00;677.50]	12	586.00 [354.00;722.50]	0.671	0.886
EM CD8+ CD27+ CD28+(% of EM CD8+ T-cells)	8	34.13 [19.64;44.19]	12	26.96 [15.76;32.24]	0.375	0.805
CD27 expression in EM CD8+ CD27+CD28+ (MFI)	8	1467.00 [1367.00;1636.50]	12	1347.50 [1279.50;1682.50]	0.512	0.805
CD28 expression in EM CD8+ CD27+CD28+ (MFI)	8	849.50 [711.50;965.50]	12	906.50 [658.50;1036.50]	0.939	1.000
EM CD8+ CD27+CD28- (% of EM CD8+ T-cells)	8	4.96 [3.76;8.90]	12	1.96 [1.65;4.51]	0.041	0.337
EM CD8+ CD27-CD28- (% of EM CD8+ T-cells)	8	23.53 [15.96;47.75]	12	24.14 [14.47;32.75]	0.563	0.844
EM CD8+ CD27-CD28+ (% of EM CD8+ T-cells)	8	28.56 [23.77;37.50]	12	49.03 [35.57;54.79]	0.010	0.201
EM CD8+ CD57+ (% of EM CD8+ T-cells)	8	44.96 [37.21;53.60]	12	49.91 [21.78;54.52]	0.969	1.000
TEMRA CD8+(% of CD8+ T-cells)	8	27.24 [14.57;32.13]	12	18.43 [7.98;30.10]	0.335	0.789
CD27 expression in TEMRA CD8+ (MFI)	8	234.00 [174.50;672.00]	12	318.50 [238.50;469.50]	0.463	0.805
CD28 expression in TEMRA CD8+ (MFI)	8	95.00 [51.00;171.50]	12	188.00 [113.00;330.00]	0.154	0.633
TEMRA CD8+ CD27+CD28+ (% of TERMA CD8+ T-cells)	8	3.04 [2.48;10.97]	12	6.09 [1.99;10.27]	0.969	1.000
CD27 expression in TEMRA CD8+ CD27+CD28+ (MFI)	8	1558.00 [1459.00;1702.50]	12	1649.50 [1287.50;1852.50]	0.729	0.925
CD28 expression in TEMRA CD8+ CD27+CD28+ (MFI)	8	677.50 [632.50;797.50]	12	781.50 [712.50;904.50]	0.263	0.789
TEMRA CD8+ CD27+CD28- (% of TEMRA CD8+ T-cells)	8	4.92 [1.29;5.52]	12	3.28 [1.42;4.08]	0.512	0.805
TEMRA CD8+ CD27-CD28- (% of TEMRA CD8+ T-cells)	8	82.27 [62.76;86.50]	12	66.02 [50.96;78.16]	0.263	0.789
TEMRA CD8+ CD57+ (% of TEMRA CD8+ T-cells)	8	76.42 [62.60;82.14]	12	67.67 [46.39;78.59]	0.335	0.789

PBMC - Tregs					
Total Treg (% of CD4+ T-cells)	8	4.90 [3.90;6.14] 2638.00	12	3.97 [3.34;5.32]	0.418 0.563
CD25 expression in Total Treg (MFI)	8	[2015.00;3325.00]	12	1535.50 [1124.50;2303.00]	0.041 0.151
CTLA-4 expression in Total Treg (MFI)	8	264.00 [217.50;326.00]	12	292.50 [258.00;314.00]	0.616 0.616
Naïve Treg (% of total Tregs)	8	8.28 [4.24;15.33] 2503.00	12	11.08 [7.28;23.51]	0.512 0.563
CD25 expression of Naïve Treg (MFI)	8	[1761.50;2730.50]	12	1773.50 [1169.50;2216.50]	0.059 0.151
CTLA-4 expression of Naïve Treg (MFI)	8	204.50 [173.50;279.00]	12	292.50 [242.50;313.50]	0.083 0.151
Naïve Treg CTLA-4+ (% of Naïve Tregs)	8	17.37 [11.25;29.65]	12	30.24 [24.04;32.85]	0.083 0.151
Memory Treg (% of total Tregs)	8	91.09 [84.16;94.57] 2610.00	12	88.49 [76.07;92.20]	0.512 0.563
CD25 expression of Memory Treg (MFI)	8	[1972.00;3329.00]	12	1555.00 [1173.00;2373.50]	0.059 0.151
CTLA-4 expression of Memory Treg (MFI)	8	248.00 [205.00;293.50]	12	287.50 [255.50;331.50]	0.177 0.278
Memory Treg CTLA-4+ (% of Memory Tregs)	8	14.28 [6.42;21.34]	12	19.98 [18.26;24.46]	0.070 0.151
PBMC - T-cell activation status					
CD4+ PD1+ (% of CD4+ T-cells)	8	2.14 [1.32;2.78]	12	1.12 [0.84;3.21]	0.354 0.607
PD1 expression in CD4+ (MFI)	8	220.50 [180.00;289.50]	12	165.50 [120.50;236.00]	0.298 0.595
CD4+ CD69+ (% of CD4+ T-cells)	8	29.77 [24.87;40.96]	12	27.01 [16.96;37.41]	0.263 0.595
CD69 expression in CD4+ (MFI)	8	266.00 [243.00;289.50]	12	245.50 [216.00;305.50]	0.643 0.848
CD4+ PD1+CD69+ (% of CD4+ T-cells)	8	1.28 [0.90;1.47]	12	0.66 [0.37;2.15]	0.298 0.595
CD4+ PD1-CD69- (% of CD4+ T-cells)	8	69.58 [58.10;74.39]	12	72.24 [61.23;82.71]	0.263 0.595
CD4+ HLADR+ (% of CD4+ T-cells)	8	12.19 [3.66;20.04]	12	17.99 [11.79;22.56]	0.232 0.595
HLADR expression in CD4+ (MFI)	8	416.50 [204.00;501.50]	12	442.50 [380.00;522.00]	0.418 0.669
CD4+ CD38+ (% of CD4+ T-cells)	8	34.62 [26.71;37.29]	12	34.99 [22.85;46.95]	0.847 0.968
CD38 expression in CD4+ (MFI)	8	622.00 [547.00;795.50]	12	686.00 [481.00;780.00]	0.908 0.969
CD4+ HLADR+CD38+ (% of CD4+ T-cells)	8	3.91 [1.40;4.98]	12	4.04 [2.57;7.23]	0.335 0.607
CD4+ HLADR-CD38- (% of CD4+ T-cells)	8	54.98 [50.48;60.05]	12	46.41 [38.73;59.53]	0.464 0.695
CD8+ PD1+ (% of CD8+ T-cells)	8	26.38 [23.81;40.81]	12	15.11 [8.96;32.54]	0.049 0.590
PD1 expression in CD8+ (MFI)	8	310.50 [194.00;494.00]	12	292.50 [233.00;361.00]	0.671 0.848
CD8+ CD69+ (% of CD8+ T-cells)	8	31.47 [18.73;50.64]	12	37.83 [24.52;41.97]	0.969 0.969
CD69 expression in CD8+ (MFI)	8	565.00 [470.50;774.50]	12	391.00 [292.00;541.50]	0.037 0.590
CD8+ PD1+CD69+ (% of CD8+ T-cells)	8	11.39 [7.91;18.08]	12	7.28 [4.22;16.06]	0.298 0.595
CD8+ PD1-CD69- (% of CD8+ T-cells)	8	41.03 [36.80;57.28]	12	52.81 [42.74;67.57]	0.203 0.595
CD8+ HLADR+ (% of CD8+ T-cells)	8	38.80 [9.82;46.05]	12	45.47 [36.28;60.72]	0.232 0.595
HLADR expression in CD8+ (MFI)	8	497.00 [284.00;540.50]	12	535.50 [439.00;806.00]	0.203 0.595
CD8+ CD38+ (% of CD8+ T-cells)	8	11.40 [8.02;22.77]	12	13.74 [6.99;29.79]	0.729 0.874
CD38 expression in CD8+ (MFI)	8	274.00 [227.50;403.00]	12	282.50 [180.00;456.50]	0.969 0.969
CD8+ HLADR+CD38+ (% of CD8+ T-cells)	8	5.59 [2.36;11.15]	12	6.96 [4.14;11.61]	0.512 0.723
CD8+ HLADR-CD38- (% of CD8+ T-cells)	8	54.58 [45.98;78.05]	12	45.36 [34.90;51.94]	0.097 0.595
Plasma - Essential Immune Response					
IL-4 (pg/mL)	8	6.56 [0.31;17.75]	12	0.00 [0.00;3.83]	0.080 0.330
IL-2 (pg/mL)	8	2.35 [0.68;16.71]	12	0.95 [0.00;4.18]	0.260 0.510
CXCL-10 (IP-10) (pg/mL)	8	394.51 [100.25;495.51]	12	370.47 [189.67;459.55]	0.616 0.824
IL-1 beta (pg/mL)	8	5.96 [4.57;32.43]	12	3.02 [0.75;9.23]	0.083 0.330
TNF alpha (pg/mL)	8	1.14 [0.63;2.74]	12	2.43 [1.38;3.60]	0.143 0.428
CCL2 (MCP-1) (pg/mL)	8	202.58 [168.50;296.80]	12	165.28 [140.36;248.67]	0.298 0.510
IL-17A (pg/mL)	8	3.36 [1.45;11.39]	12	2.31 [1.36;5.32]	0.729 0.824
IL-6 (pg/mL)	8	5.80 [2.63;10.56]	12	6.16 [3.75;10.83]	0.729 0.824
IL-10 (pg/mL)	8	0.54 [0.00;1.69]	12	0.70 [0.09;1.68]	0.756 0.824
IFN gamma (pg/mL)	8	3.67 [1.90;7.15]	12	1.94 [0.91;4.83]	0.297 0.510
IL-12p70 (pg/mL)	8	12.22 [5.56;45.39]	12	2.75 [1.30;5.44]	0.015 0.181
CXCL-8 (IL-8) (pg/mL)	8	5.67 [2.23;22.07]	12	5.28 [3.78;9.94]	1.000 1.000
Plasma - Immune Checkpoint					

sCD25 (IL-2Ra) (pg/mL)	8	1132.10 [955.26;1562.20]	12	1689.80 [1086.20;2303.20]	0.263	0.632
4-1BB (pg/mL)	8	56.21 [38.70;88.13] 53535.00	12	72.05 [51.50;143.09] 62981.00	0.263	0.632
sCD27 (pg/mL)	8	[49080.00;62195.00]	12	[49390.00;73961.00]	0.616	0.732
B7.2 (CD86) (pg/mL)	8	411.43 [320.29;609.87]	12	360.66 [300.49;565.35]	0.787	0.787
Free Active TGF-B1 (pg/mL)	8	13.43 [7.00;28.59]	12	25.66 [11.16;41.20]	0.263	0.632
CTLA-4 (pg/mL)	8	7.36 [4.35;10.07]	12	8.88 [1.04;23.83]	0.616	0.732
PD-L1 (pg/mL)	8	265.37 [151.94;325.49] 12081.00	12	187.01 [149.03;223.63] 14498.00	0.512	0.732
PD-L2 (pg/mL)	8	[11067.00;16080.00]	12	[12619.00;16398.00]	0.512	0.732
PD-1 (pg/mL)	8	66.15 [19.51;101.02] 18190.00	12	24.74 [14.23;95.23] 29643.00	0.512	0.732
Tim-3 (pg/mL)	8	[8331.30;22119.00] 3388.50	12	[16492.00;37149.00]	0.133	0.632
LAG-3 (pg/mL)	8	[2008.30;18530.00] 113572.00	12	2042.20 [1398.00;4275.60] 118096.00	0.154	0.632
Gal-9 (pg/mL)	8	[87969.00;171239.00]	12	[97476.00;167533.00]	0.671	0.732