

Supplement Table 1

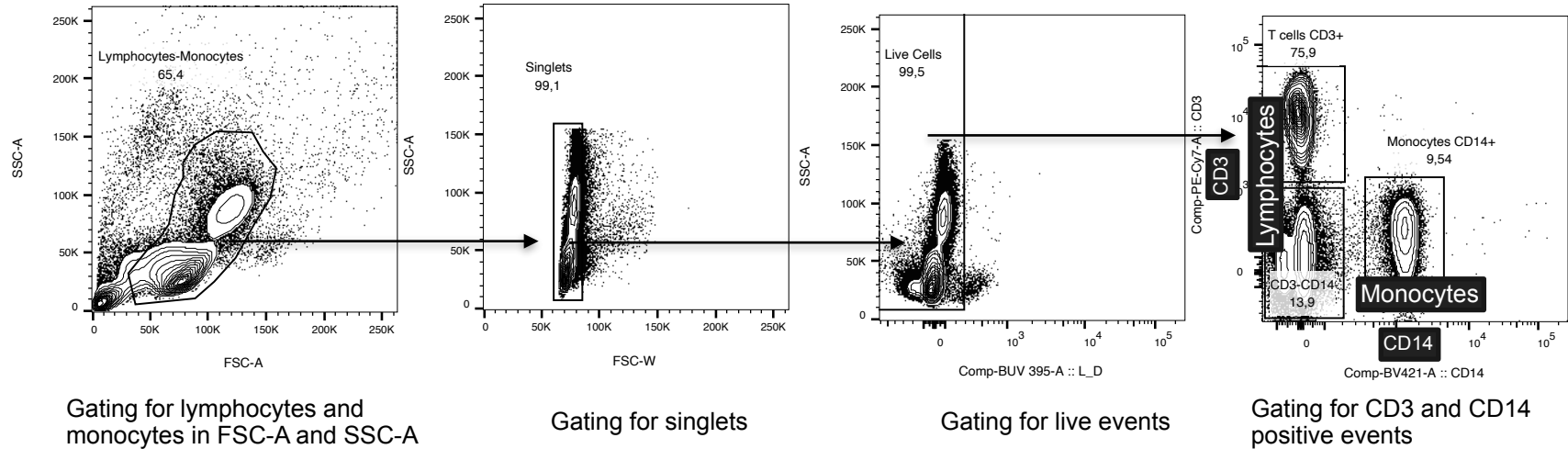
Staining at Fortessa→B cells in MS

target of antibody	clone	fluorochrome	manufacturer
CD3	UCHT1	PE-Cy7	BD Biosciences, Heidelberg, Germany
CD4	RPA-T4	APC-Cy7	BD Biosciences, Heidelberg, Germany
CD14	M5E2	BV421	Biolegend, San Diego, USA
CD19	SJ25C1	BV711	BD Biosciences, Heidelberg, Germany
CD20	2H7	BV510	Biolegend, San Diego, USA
CD25	M-A251	PE-CF594	BD Biosciences, Heidelberg, Germany
CD27	M-T271	APC-R700	BD Biosciences, Heidelberg, Germany
CD38	HIT2	PerCpCy5.5	BD Biosciences, Heidelberg, Germany
CD127	HIL-7R-M21	BV786	BD Biosciences, Heidelberg, Germany
CD138	MI15	BUV737	BD Biosciences, Heidelberg, Germany
IgD	IA6-2	PE	BD Biosciences, Heidelberg, Germany
HLA-DR	L243	FITC	Biolegend, San Diego, USA

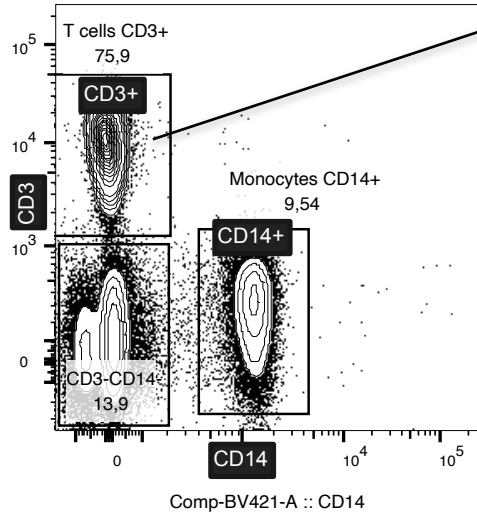
Alvarez C., Wiedemann A., Schroeder-Castagno M., *et al.* **Lymphocyte profiles after a first demyelinating event suggestive of multiple sclerosis reveal early monocyte and B cell alterations**

Supplement Figure 1: Gating Strategy

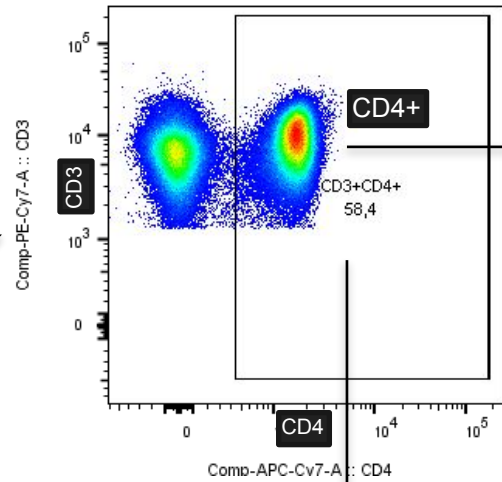
A) Lymphocytes and Monocytes



B) CD3+ T cells and subsets

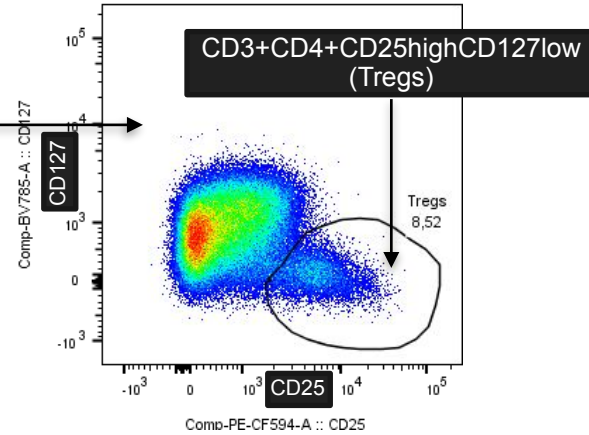


From CD3+ events gate CD4+ events

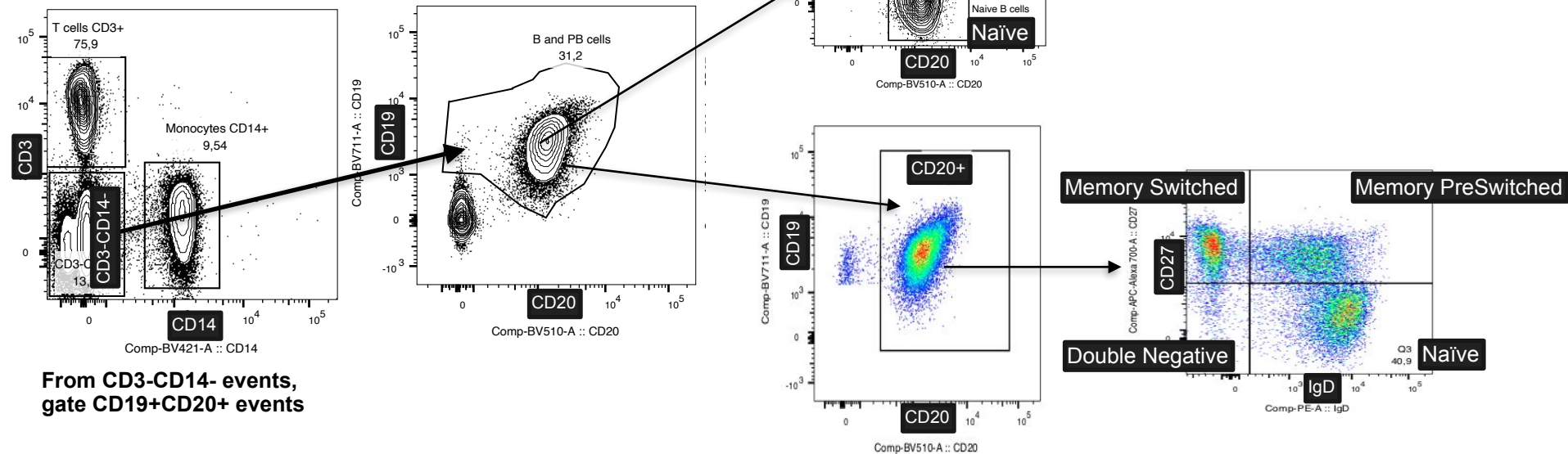


Gate for:

CD3+CD4+CD38+
CD3+CD4+HLADR+
CD3+CD4+CD38+HLADR+

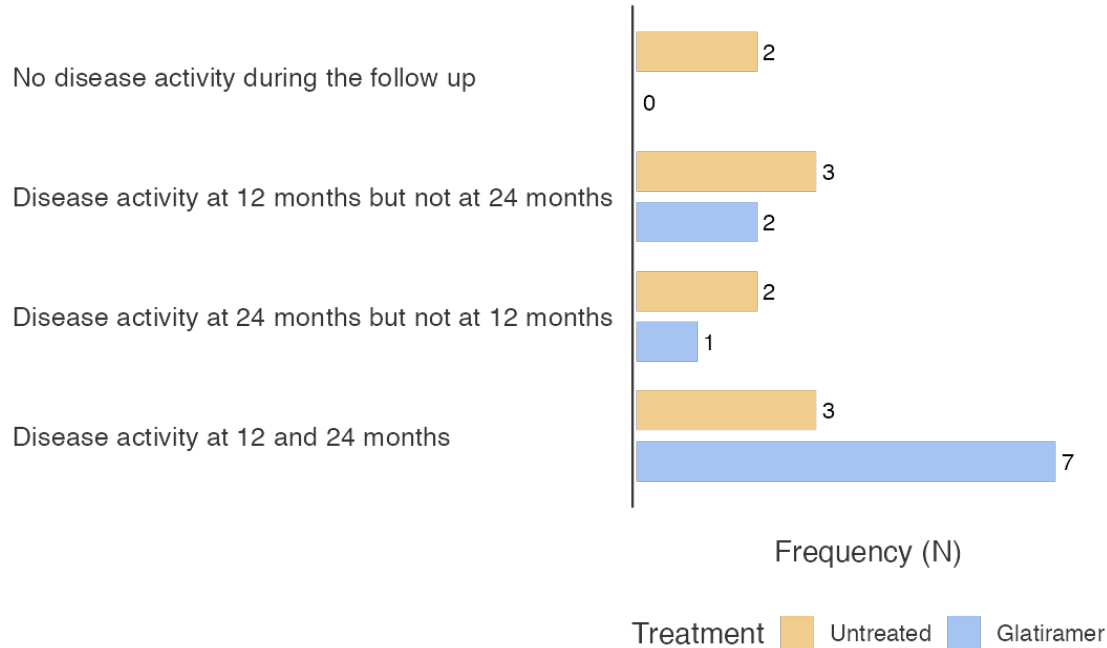


C) CD19+CD20+ B cells and subsets



Supplement Figure 2: Disease Activity

Activity Total

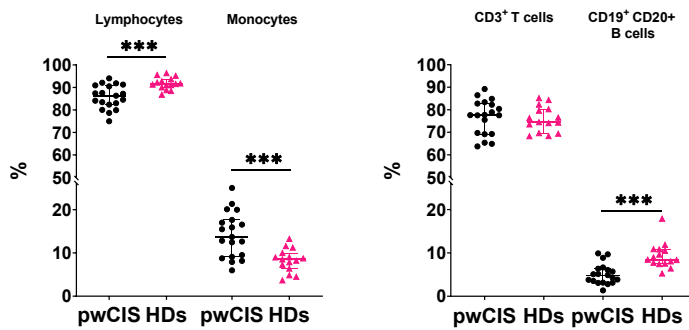


Supplement Table 2. Descriptive results of pwCIS and HDs at Baseline

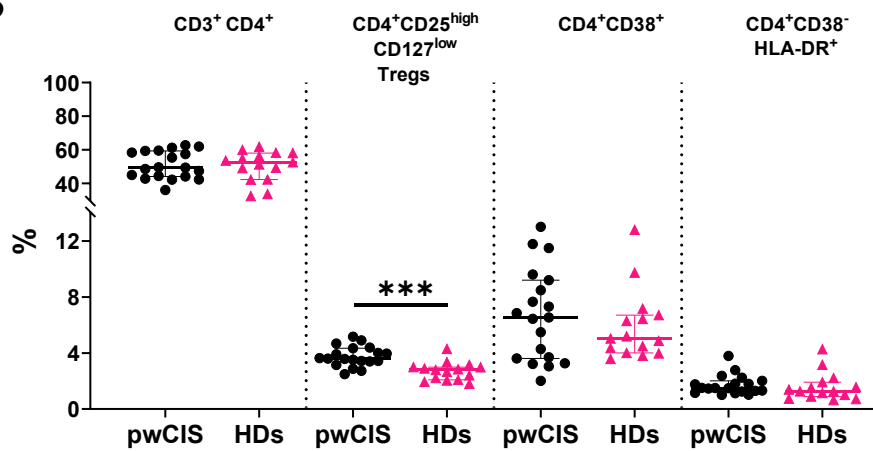
Cell Population (%)	Groups	N	Mean	SE	Median	SD
% of Lymphocytes	pwCIS	19	85.7934	1.20292	86.3150	5.2434
	HDs	15	91.7422	0.69271	91.5025	2.6829
% of Monocytes	pwCIS	19	14.2066	1.20292	13.6850	5.2434
	HDs	15	8.2578	0.69271	8.4975	2.6829
% of CD3+ (T cells)	pwCIS	19	76.6404	1.75204	77.6206	7.6370
	HDs	15	75.7543	1.45014	74.4667	5.6164
% of CD4+	pwCIS	19	50.9302	1.88247	49.3898	8.2055
	HDs	15	50.3401	2.33512	52.5042	9.0439
% of CD4+CD38+	pwCIS	19	6.6866	0.75167	6.5443	3.2765
	HDs	15	5.8940	0.65072	5.0397	2.5202
% of CD4+ CD38- HLA-DR+	pwCIS	19	1.7218	0.15900	1.4946	0.6931
	HDs	15	1.5770	0.25707	1.2590	0.9956
% of CD4+ CD38+ HLA-DR+	pwCIS	19	0.3359	0.02760	0.3484	0.1203
	HDs	15	0.2328	0.03627	0.1794	0.1405
% of CD4+ CD25high CD127low (Tregs)	pwCIS	19	3.7478	0.16471	3.5994	0.7180
	HDs	15	2.6959	0.16826	2.7913	0.6517
% of CD19+CD20+ (B cells)	pwCIS	19	5.1218	0.54334	4.8820	2.3684
	HDs	15	9.3180	0.76821	8.4420	2.9752
% of CD20+ CD27- Naïve	pwCIS	19	3.3571	0.46839	2.5883	2.0417
	HDs	15	6.6776	0.84367	6.3229	3.2675
% of CD20+ CD27+ Memory	pwCIS	19	1.6519	0.18176	1.4935	0.7923
	HDs	15	2.5450	0.25944	2.2394	1.0048
% of CD19+ CD20- CD27high Plasmablasts	pwCIS	19	0.0472	0.00822	0.0465	0.0358
	HDs	15	0.0326	0.00601	0.0225	0.0233
% of CD19+CD20+CD27-IgD+ Naïve	pwCIS	19	59.4501	3.17569	56.5424	13.8425
	HDs	15	64.2632	3.65156	62.0955	14.1424
% of CD19+CD20+CD27+IgD+ pre-switched memory	pwCIS	19	14.4304	1.65485	11.6766	7.2133
	HDs	15	13.2042	1.88216	8.9897	7.2896
% of CD19+CD20+CD27+IgD- switched memory	pwCIS	19	19.9121	1.81677	20.2467	7.9191
	HDs	15	17.1319	2.17346	15.9067	8.4178
% of CD19+CD20+CD27-IgD- double negative	pwCIS	19	6.2499	0.73292	4.8285	3.1947
	HDs	15	5.4006	0.93733	3.9471	3.6303

Supplement Figure 3

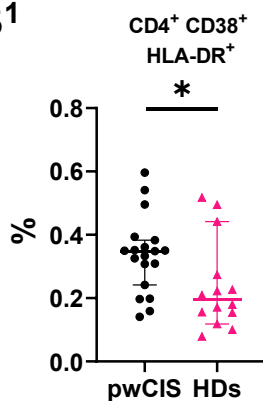
A



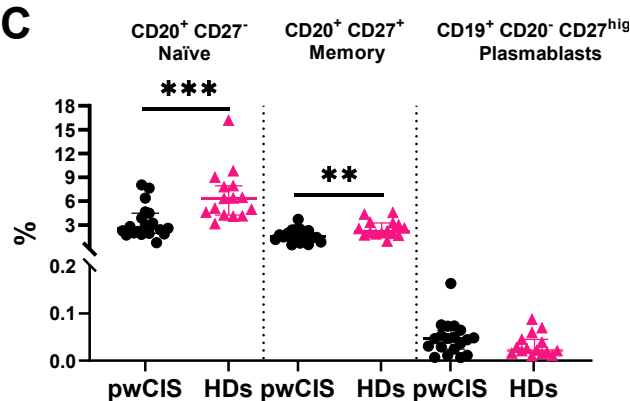
B



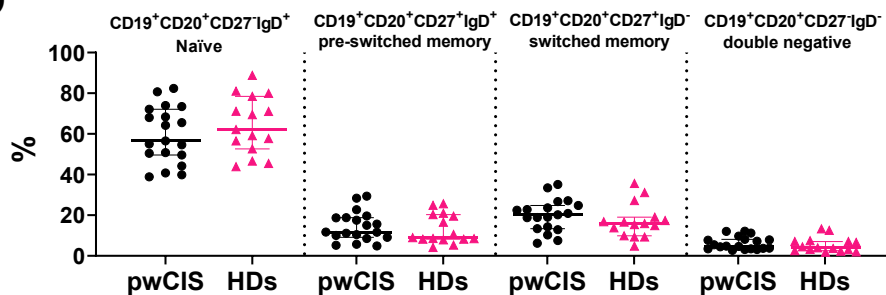
B¹



C



D



Supplement Figure 3. Differences between pwCIS and HDs at Baseline. A) Median percentage of lymphocytes and monocytes between isolated PBMCs. Median percentage of CD3⁺ T cells and

CD19⁺CD20⁺ B cells in the lymphocyte population. B and C) Median percentage of different T cell subpopulations in the lymphocyte population. D) Median percentage of different B cell subpopulations in the lymphocyte population. E) Median percentage of different B cell subpopulations in the CD20⁺ subpopulation. Data are presented as median percentage 95% CI. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Mann-Whitney test was utilised to compare the percentages between two unpaired groups.

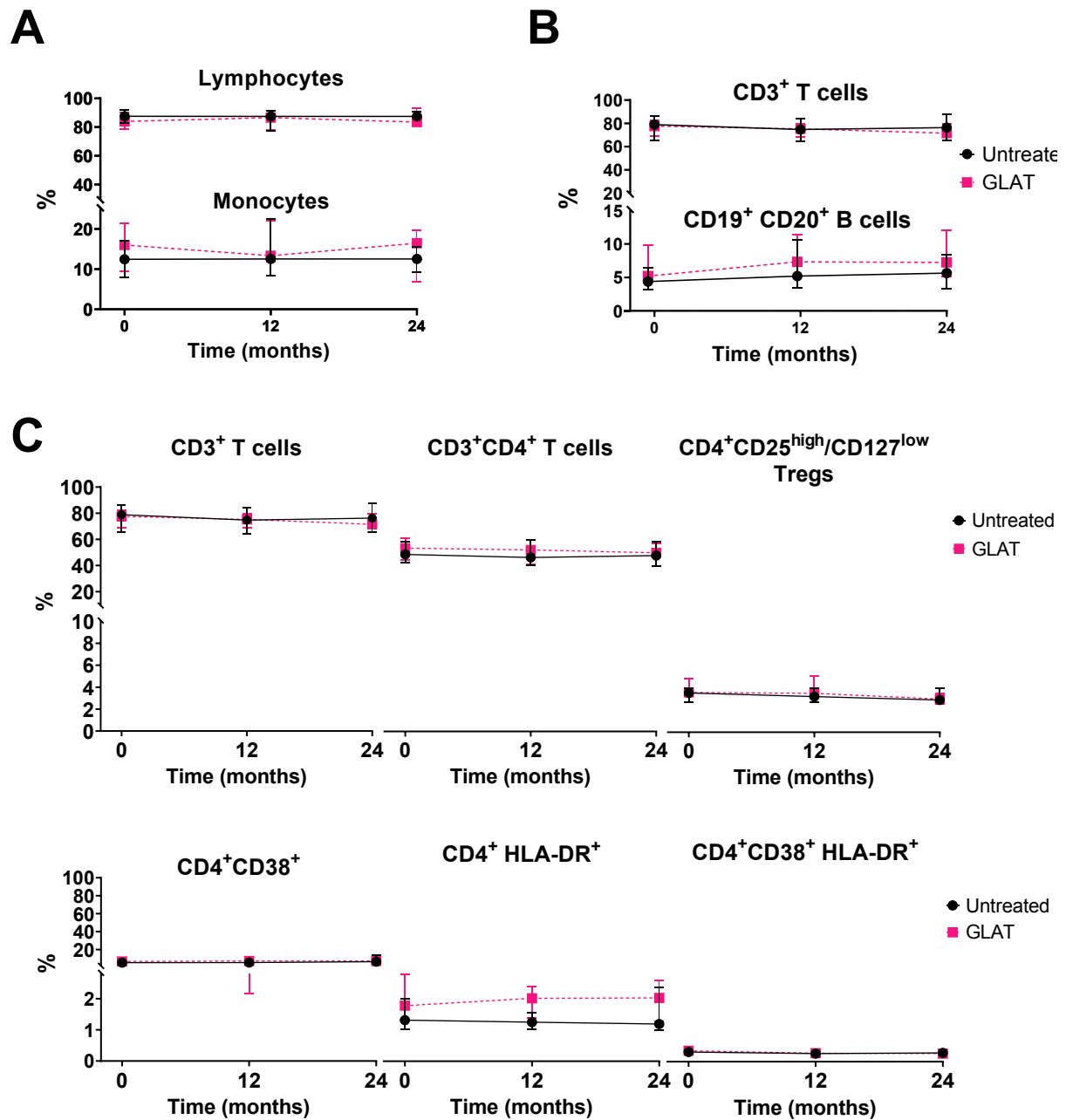
Supplement Table 3. Descriptive results of sub-analysis of lymphocytes in treated-GLAT and untreated pwCIS

Cell Population (%)	Treatment	N	Mean	SE	Median	SD
Lymphocytes (Baseline)	Untreated	9	87.7862	1.61045	87.5507	4.8314
	Glatiramer	10	84.0000	1.63379	83.9972	5.1665
CD3+ (Baseline)	Untreated	9	77.6213	3.11314	78.9008	9.3394
	Glatiramer	10	75.7575	1.93094	77.5435	6.1062
CD4+ (Baseline)	Untreated	9	48.8100	2.81079	48.5799	8.4324
	Glatiramer	10	52.8384	2.50829	53.4231	7.9319
CD4+CD38+ (Baseline)	Untreated	9	6.3664	1.04954	5.4899	3.1486
	Glatiramer	10	6.9747	1.11639	6.6955	3.5303
CD4+HLADR+ (Baseline)	Untreated	9	1.4417	0.14277	1.3196	0.4283
	Glatiramer	10	1.9739	0.25466	1.7829	0.8053
CD4+CD38+HLARDR+ (Baseline)	Untreated	9	0.3014	0.03958	0.3092	0.1187
	Glatiramer	10	0.3670	0.03760	0.3505	0.1189
CD4+CD25highCD127low: Tregs (Baseline)	Untreated	9	3.5116	0.20725	3.5747	0.6218
	Glatiramer	10	3.9603	0.24111	3.6189	0.7625
Monocytes (Baseline)	Untreated	9	12.2138	1.61045	12.4493	4.8314
	Glatiramer	10	16.0000	1.63379	16.0028	5.1665
CD19+CD20+ (Baseline)	Untreated	9	4.4065	0.56453	4.3016	1.6936
	Glatiramer	10	5.7656	0.87730	5.1053	2.7743
CD19+CD20+CD27- Naïve (Baseline)	Untreated	9	2.5904	0.39768	2.4081	1.1930
	Glatiramer	10	4.0472	0.77167	2.7997	2.4402
CD19+CD20+CD27+ Memory (Baseline)	Untreated	9	1.7378	0.33722	1.6255	1.0117
	Glatiramer	10	1.5745	0.18212	1.4892	0.5759
CD19+CD20-CD27high Plasmablasts (Baseline)	Untreated	9	0.0335	0.00762	0.0292	0.0229
	Glatiramer	10	0.0596	0.01321	0.0499	0.0418
CD19+CD20+ (Baseline, No plasmablasts)	Untreated	9	4.3470	0.56354	4.2097	1.6906
	Glatiramer	10	5.6550	0.86954	4.9828	2.7497
CD20+IgD-CD27+ Memory Switched (Baseline)	Untreated	9	21.1792	2.71268	20.8879	8.1380
	Glatiramer	10	18.7718	2.52002	19.6569	7.9690
CD20+IgD+CD27+ Memory PreSwitched (Baseline)	Untreated	9	17.5969	2.76839	18.6758	8.3052
	Glatiramer	10	11.5806	1.53954	10.2769	4.8684
CD20+IgD+CD27- Naïve (Baseline)	Untreated	9	55.0889	4.95639	50.4192	14.8692
	Glatiramer	10	63.3752	3.88027	66.0922	12.2705
CD20+IgD-CD27- Double Negative (Baseline)	Untreated	9	6.1353	1.16227	4.8285	3.4868
	Glatiramer	10	6.3530	0.97882	5.8434	3.0953

Cell Population (%)	Treatment	N	Mean	SE	Median	SD
Lymphocytes (12m)	Untreated	10	85.8251	2.01614	87.4792	6.3756
	Glatiramer	10	84.7633	1.70465	86.6657	5.3906
CD3+ (12m)	Untreated	10	75.4878	3.16059	74.7352	9.9947
	Glatiramer	10	75.4105	1.50023	75.2325	4.7441
CD4+ (12m)	Untreated	10	47.6431	2.71158	46.2702	8.5748
	Glatiramer	10	52.2352	2.40317	52.0727	7.5995
CD4+CD38+ (12m)	Untreated	10	7.2508	1.17735	5.5658	3.7231
	Glatiramer	10	7.0554	1.13698	7.2854	3.5954
CD4+HLADR+ (12m)	Untreated	10	1.3384	0.12594	1.2536	0.3983
	Glatiramer	10	1.8958	0.16436	2.0189	0.5197
CD4+CD38+HLADR+ (12m)	Untreated	10	0.3113	0.05248	0.2596	0.1660
	Glatiramer	10	0.2984	0.04122	0.2719	0.1304
CD4+CD25highCD127low (12m)	Untreated	10	3.3967	0.24776	3.2465	0.7835
	Glatiramer	10	3.6589	0.30679	3.5444	0.9701
Monocytes (12m)	Untreated	10	14.1749	2.01614	12.5208	6.3756
	Glatiramer	10	15.2367	1.70465	13.3343	5.3906
CD19+CD20+ (12m)	Untreated	10	5.7591	0.91785	5.0998	2.9025
	Glatiramer	10	7.5630	0.79693	7.1895	2.5201
CD19+CD20+CD27- Naïve (12m)	Untreated	10	3.5575	0.70529	2.8592	2.2303
	Glatiramer	10	5.5806	0.76651	5.1331	2.4239
CD19+CD20+CD27+ Memory (12m)	Untreated	10	2.0879	0.38210	1.7902	1.2083
	Glatiramer	10	1.8316	0.21854	1.8507	0.6911
CD19+CD20-CD27high Plasmablasts (12m)	Untreated	10	0.0413	0.01094	0.0353	0.0346
	Glatiramer	10	0.0588	0.01559	0.0463	0.0493
CD19+CD20+ Non Plasmablasts (12m)	Untreated	10	5.6806	0.90611	5.0360	2.8654
	Glatiramer	10	7.4443	0.79652	6.9892	2.5188
CD20+IgD-CD27+ Memory Switched (12m)	Untreated	10	19.3871	2.56476	18.9367	8.1105
	Glatiramer	10	15.0433	2.01952	14.2399	6.3863
CD20+IgD+CD27+ Memory PreSwitched (12m)	Untreated	10	18.0298	2.73232	15.9306	8.6404
	Glatiramer	10	12.0758	2.08285	11.8274	6.5866
CD20+IgD+CD27- Naïve (12m)	Untreated	10	57.1868	4.79672	59.5811	15.1686
	Glatiramer	10	67.5421	3.76479	70.5143	11.9053
CD20+IgD-CD27- Double Negative (12m)	Untreated	10	5.3963	0.67378	5.0342	2.1307
	Glatiramer	10	5.3388	0.96283	4.3846	3.0447
Lymphocytes (24m)	Untreated	10	87.9137	1.11212	87.4664	3.5168
	Glatiramer	10	85.4291	1.54685	83.5509	4.8916
CD3+ (24m)	Untreated	10	76.7153	2.71842	76.2855	8.5964
	Glatiramer	10	71.9719	2.31649	71.4847	7.3254

Cell Population (%)	Treatment	N	Mean	SE	Median	SD
CD4+ (24m)	Untreated	10	48.2311	2.55333	47.7134	8.0743
	Glatiramer	10	48.9324	2.51619	49.8149	7.9569
CD4+CD38+ (24m)	Untreated	10	7.2268	1.25677	6.4953	3.9743
	Glatiramer	10	6.6929	1.16544	7.2463	3.6854
CD4+HLADR+ (24m)	Untreated	10	1.4577	0.18813	1.1937	0.5949
	Glatiramer	10	2.0291	0.18618	2.0337	0.5888
CD4+CD38+HLADR+ (24m)	Untreated	10	0.2981	0.04001	0.2810	0.1265
	Glatiramer	10	0.2601	0.02053	0.2599	0.0649
CD4+CD25highCD127low (24m)	Untreated	10	3.2104	0.22249	2.9355	0.7036
	Glatiramer	10	3.2429	0.36600	3.0308	1.1574
Monocytes (24m)	Untreated	10	12.0863	1.11212	12.5336	3.5168
	Glatiramer	10	14.5709	1.54685	16.4491	4.8916
CD19+CD20+ (24m)	Untreated	10	5.4659	0.70731	5.5267	2.2367
	Glatiramer	10	7.8512	0.84220	7.0958	2.6633
CD19+CD20+CD27- Naïve (24m)	Untreated	10	3.2494	0.47700	2.7588	1.5084
	Glatiramer	10	5.9870	0.79829	5.3351	2.5244
CD19+CD20+CD27+ Memory (24m)	Untreated	10	2.1005	0.38931	1.8149	1.2311
	Glatiramer	10	1.7009	0.15553	1.6599	0.4918
CD19+CD20-CD27high Plasmablasts (24m)	Untreated	10	0.0504	0.01268	0.0285	0.0401
	Glatiramer	10	0.0797	0.03909	0.0463	0.1236
CD19+CD20+ No Plasmablasts (24m)	Untreated	10	5.3894	0.71073	5.4833	2.2475
	Glatiramer	10	7.7199	0.85533	7.0126	2.7048
CD20+IgD-CD27+ Memory Switched (24m)	Untreated	10	19.9960	2.56953	21.0089	8.1256
	Glatiramer	10	13.3819	2.62634	12.1846	8.3052
CD20+IgD+CD27+ Memory PreSwitched (24m)	Untreated	10	19.5831	2.72557	16.3556	8.6190
	Glatiramer	10	10.4485	1.51220	9.6176	4.7820
CD20+IgD+CD27- Naïve (24m)	Untreated	10	55.4410	4.80968	55.1520	15.2095
	Glatiramer	10	71.1666	4.49084	75.0904	14.2013
CD20+IgD-CD27- Double Negative (24m)	Untreated	10	4.9366	0.64368	4.6223	2.0355
	Glatiramer	10	5.0030	0.79571	5.1136	2.5163

Supplement Figure 4

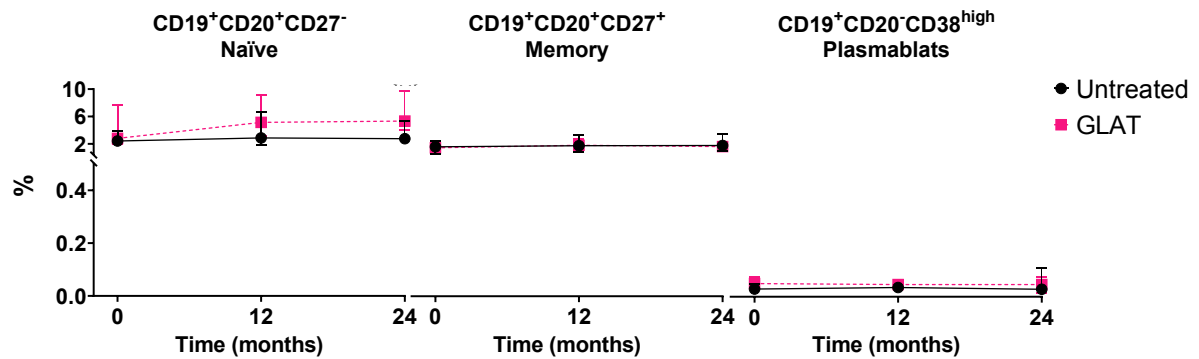


Supplement Figure 4. Glatiramer acetate in pwCIS's peripheral lymphocytes. A)

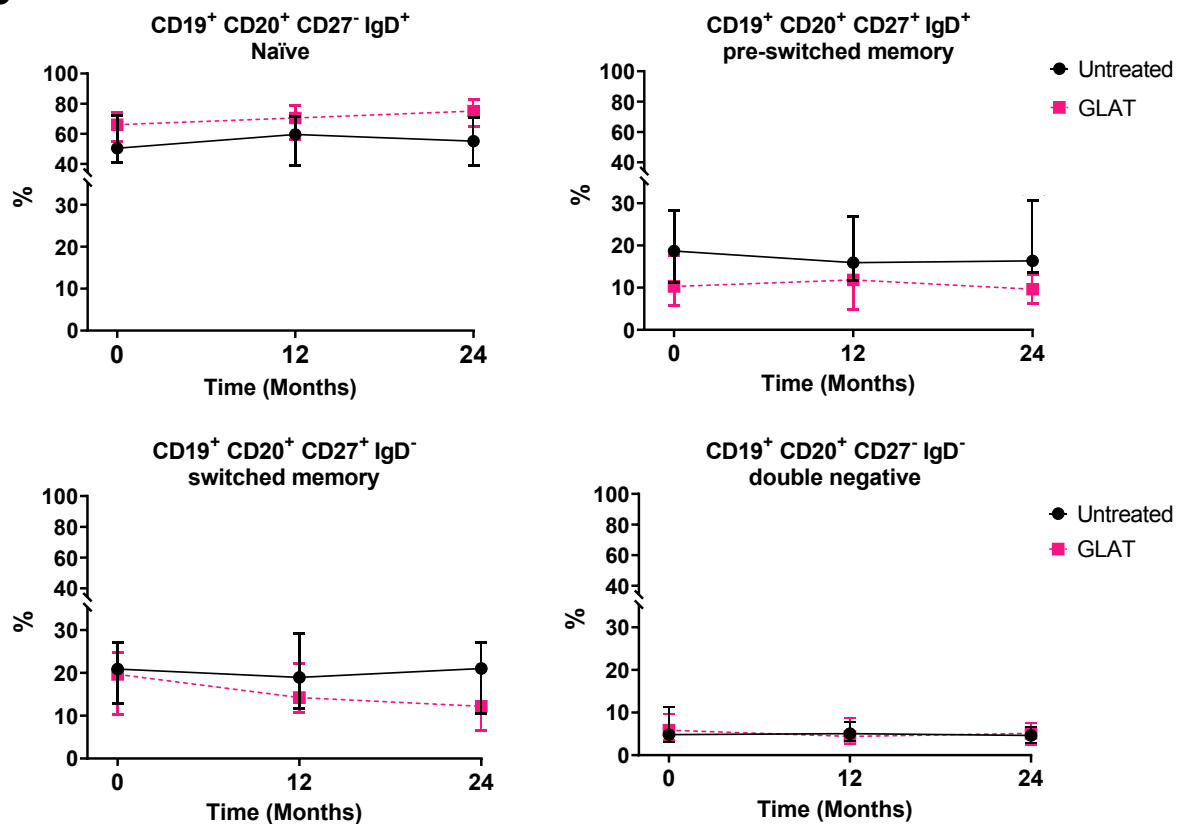
Median percentage of lymphocytes and monocytes B) Median percentage of CD3⁺ T cells and CD19⁺CD20⁺ B cells in the lymphocyte population. C) Median percentage of different T cell subpopulations in the lymphocyte population. Data are presented as median percentage 95% CI.

Supplement Figure 5

A



B



Supplement Figure 5. Glatiramer acetate effect in pwCIS's peripheral B cells.

A) Median percentage of different B cell subpopulations in the lymphocyte population. B)

Median percentage of different B cell subpopulations into the CD20⁺ subpopulation.

Data are presented as median percentage 95% CI.