Supplementary Data

table e-1. Description of immunological variables investigated.

Cell type	Gating strategy used for FlowJo	Expressed as
Lymph	Tot. lymphocytes	% Ungated
T	$CD3^+$	% Tot. lymphocytes
CD4	$CD4^+CD8^-$	% Tot. lymphocytes
CD4.RTE	$CD4^+CD8^-CD45RA^+CCR7^+CD31^+$	% $CD4^+CD8^-$
CD4.Naive	$CD4^+CD8^-CD45RA^+CCR7^+$	% $CD4^+CD8^-$
CD4.TEM	$CD4^+CD8^-CD45RA^-CCR7^-$	% $CD4^+CD8^-$
CD4.TEMRA	$CD4^+CD8^-CD45RA^+CCR7^-$	% $CD4^+CD8^-$
CD4.TCM	$CD4^+CD8^-CD45RA^-CCR7^+$	% $CD4^+CD8^-$
Treg	$CD4^+CD8^-CD25^+Foxp3^+$	% $CD4^+CD8^-$
Th1	$CD4^+CD8^-IFN\gamma^+$	% $CD4^+CD8^-$
Th2	$CD4^+CD8^-IL4^+$	% $CD4^+CD8^-$
Th1/Th2	-	Log(Ratio)
Th17	$CD4^+CD8^-IL17^+$	% $CD4^+CD8^-$
Tfh	$CD4^+CD8^-CXCR5^+$	% $CD4^+CD8^-$
Tfh.CD45RA-	$CD4^+CD8^-CXCR5^+CD45RA^-$	% $CD4^+CD8^-$
CD4.IL2	$CD4^+CD8^-IL2^+$	% $CD4^+CD8^-$
CD8	$CD4^-CD8^+$	%Tot. lymphocytes
CD8.RTE	$CD4^-CD8^+CD45RA^+CCR7^+CD31^+$	% $CD4^-CD8^+$
CD8.Naive	$CD4^-CD8^+CD45RA^+CCR7^+$	% $CD4^-CD8^+$
CD8.TEM	$CD4^-CD8^+CD45RA^-CCR7^-$	% $CD4^-CD8^+$
CD8.TEMRA	$CD4^-CD8^+CD45RA^+CCR7^-$	% $CD4^-CD8^+$
CD8.TCM	$CD4^-CD8^+CD45RA^-CCR7^+$	% $CD4^-CD8^+$
CD8.IFNG	$CD4^-CD8^+IFN\gamma^+$	% $CD4^-CD8^+$
CD8.IL2	$CD4^-CD8^+IL2^+$	% $CD4^-CD8^+$
CD4/CD8	-	Log(Ratio)
B	$CD4^-CD19^+$	% Tot. lymphocytes
B.Transitional	$CD19^+$CD24hiCD38hi	% B cells
B.Naive	$CD4^-CD19^+IgM^+CD27^-$	% B cells
B.Switched	$CD4^-CD19^+IgM^-CD27^+$	% B cells
Plasmablast	$CD19^+CD24^-$CD38hi	% B cells
B.Memory	$CD4^-CD19^+IgM^+CD27^+$	% B cells
T/B	-	Log(Ratio)
T.gd	$CD4^-CD8^-\gamma\delta TCR^+$	% Tot. lymphocytes
NK	$CD3^-CD14^-CD19^-CD11c^-CD56^+$	% FSC,SSC
NKT	$CD3^+CD56^+$	% $CD3^+$
mDC	$CD3^-CD14^-CD19^-CD56^-CD11c^+HLA^-DR^+$	% DCs
pDC	$CD3^-CD14^-CD19^-CD56^-CD11c^-CD123^+HLA^-DR^+$	% DCs
mDC/pDC	-	Log(Ratio)

Heparinized blood was collected and rested at 22˚C for four hours prior to isolation of peripheral blood mononuclear cells (PBMC) and serum using lymphocyte separation medium (LSM, MP Biomedicals). PBMCs were frozen in 10% dimethyl sulfoxide (Sigma) and stored at -80˚C prior to analysis. Thawed cells were stained with eBioscience antibodies against CD11c (3.9), CD3 (SK7), CD4 (RPA-T4), CD8a (RPA-T8), CD19 (HIB19), CD45Ra (HI100), CD56 (MEM188), HLA-DR (LN3), FOXP3 (206D, Biolegend), IFN-γ (4S.B3 IL-17 (eBio64DEC17), IL-2 (MQ1-17H12), CXCR5 (IgG23, R&D), CD31 (WM-59), CCR7 (3D12), IgM (MHM-88, Biolegend), CD27 (O323), IgE (IgE21), CD24 (eBioSN3, SN3 A5-2H10), CD38 (HIT2), γδTCR (B1.1), Vα24Jα18 (6B11), CD56 (MEM188), CD14 (61D3), CD123 (6H6) and IL-4 (8D4-8). Cytokine staining was performed using T cells stimulated *ex vivo* for five hours in 50 ng/ml phorbol 12-myristate 13-acetate (Sigma) and 500 ng/ml ionomycin (Sigma) in the presence of GolgiStop (BD Biosciences). Stimulated cells were surface stained, then fixed and permeabilized using Cytofix/cytoperm (BD), prior to staining for cytokines. Ki67 and Foxp3 staining was performed on thawed cells following treatment with fixation/permeabilization buffer (eBioscience). Cells were analysed in five flow cytometric panels using the following combinations of stains: CD4, CD8, CD45Ra, CD25, CXCR5 and Foxp3; CD4, CD8, CD45Ra, CCR7, CD31 and γδTCR; CD3, CD56, CD14/CD19, CD11c, HLA-DR and CD123; CD4, CD19, IgM, CD27, CD24 and CD38; CD4, CD8, IL2, IFNγ, IL4 and IL17. Data was acquired on a BD FACSCantoII and analyzed with FlowJo (Tree star).

table e-2. Associations with gender, age, disease duration and disease.
Linear regression of autoimmune disease (AITD N=55, untreated MS N=52, untreated MS and AITD: N=4) versus controls (N=36) with covariates age, gender and disease duration: [a]Regression coefficient, [b]*p* value. **A.** Measurements expressed as proportions of (grand-)parental cells as described in Supplementary table 1. **B.** Follow-up for significant findings as proportion of total lymphocytes.

	Gender		Age		AITD		MS		Disease Duration AITD		Disease Duration MS	
Cell Type	**Beta**	***p***	**Beta**	***p***	**Beta**	***p***	**Beta**	***p***	**Beta**	***p***	**Beta**	***p***
A. As % of (grand-)parental cells												
Lymph	0.240	0.918	-0.042	0.608	-0.263	0.925	-1.075	0.741	-0.037	0.923	-0.121	0.557
T	1.778	0.416	-0.191	0.026	-2.033	0.462	-3.727	0.232	0.309	0.435	0.136	0.451
CD4/CD8	0.494	0.0011	-0.006	0.260	-0.156	0.383	0.091	0.658	0.024	0.328	0.012	0.364
CD4	5.400	0.0069	-0.060	0.387	0.581	0.807	0.570	0.835	0.615	0.061	0.176	0.309
CD4.RTE	8.615	0.0010	-0.251	0.0055	6.105	0.047	-2.027	0.573	-0.117	0.779	0.030	0.895
CD4.Naive	5.784	0.137	-0.184	0.171	8.346	0.073	-1.123	0.837	0.284	0.653	-0.119	0.728
CD4.TEM	-5.899	0.137	0.022	0.874	-7.482	0.115	0.307	0.956	-0.322	0.618	0.101	0.771
CD4.TEMRA	-0.615	0.811	0.069	0.436	-0.853	0.781	-0.692	0.849	-0.391	0.353	-0.047	0.838
CD4.TCM	0.728	0.559	0.093	0.033	-0.012	0.994	1.507	0.392	0.429	0.037	0.064	0.560
CD4.IL2	0.795	0.202	0.069	0.0046	-0.865	0.262	0.458	0.606	0.012	0.900	-0.066	0.199
Treg	-1.861	0.0028	0.046	0.052	0.087	0.908	1.294	0.139	-0.013	0.889	-0.037	0.465
Th1	-1.532	0.173	0.001	0.980	-0.605	0.663	-0.125	0.937	-0.162	0.350	-0.078	0.390
Th2	-3.441	0.113	0.015	0.857	1.285	0.631	1.259	0.684	-0.397	0.228	-0.075	0.670
Th1/Th2	0.194	0.429	-0.001	0.934	-0.121	0.689	-0.128	0.711	0.013	0.720	-0.006	0.753
Th17	-0.039	0.479	0.001	0.572	0.137	0.046	-0.002	0.981	0.013	0.143	0.002	0.727
Tfh	0.025	0.967	0.014	0.633	0.711	0.334	-0.360	0.664	-0.010	0.935	-0.007	0.872
Tfh.CD45RA$^-$	0.026	0.964	0.012	0.671	0.513	0.456	-0.430	0.580	-0.008	0.947	-0.005	0.915
CD8	-4.881	0.036	0.061	0.453	4.546	0.105	-0.040	0.990	-0.074	0.846	-0.060	0.766
CD8.RTE	4.841	0.104	-0.442	3.84E-05	2.025	0.567	-6.807	0.106	0.190	0.695	0.137	0.601
CD8.Naive	3.794	0.264	-0.399	0.0010	1.200	0.767	-6.372	0.184	0.136	0.806	0.033	0.912
CD8.TEM	1.168	0.731	0.037	0.757	2.209	0.587	12.316	0.012	0.625	0.263	-0.123	0.684
CD8.TEMRA	-5.627	0.175	0.349	0.016	-3.472	0.483	-6.708	0.252	-0.834	0.219	0.118	0.747
CD8.TCM	0.665	0.125	0.014	0.364	0.057	0.912	0.769	0.207	0.074	0.293	-0.029	0.450
CD8.IFNG	-6.342	0.069	0.344	0.012	-2.078	0.629	5.436	0.268	-0.410	0.443	-0.467	0.099
CD8.IL2	-1.744	0.194	0.126	0.015	1.628	0.327	3.420	0.076	0.131	0.528	-0.029	0.795
B	1.308	0.440	0.111	0.088	0.680	0.747	6.043	0.013	-0.337	0.201	-0.247	0.079
B.Transitional	-0.530	0.819	0.214	0.066	5.789	0.046	-3.779	0.257	-1.078	0.089	-0.028	0.873
B.Naive	-2.508	0.227	0.045	0.569	4.636	0.073	-2.357	0.427	-0.270	0.396	0.049	0.773
B.Switched	1.167	0.281	-0.098	0.019	-4.244	0.0019	-0.784	0.612	0.382	0.023	0.161	0.073
Plasmablast	-0.112	0.626	-0.009	0.439	0.007	0.980	-0.574	0.086	-0.089	0.151	0.009	0.581
B.Memory	0.057	0.951	-0.030	0.408	-1.224	0.294	2.498	0.065	0.040	0.782	-0.089	0.251
T/B	-0.037	0.730	-0.008	0.065	-0.066	0.633	-0.326	0.036	0.021	0.278	0.012	0.167
gdT	0.059	0.871	-0.012	0.336	-0.563	0.207	-0.674	0.190	-0.046	0.446	-0.006	0.861

NK	-1.763	0.122	0.076	0.086	-0.607	0.672	-2.018	0.214	0.102	0.619	0.022	0.818
NKT	-2.695	0.137	0.097	0.168	-0.423	0.853	0.640	0.804	-0.407	0.215	-0.096	0.520
mDC	-5.727	0.014	0.096	0.287	1.914	0.512	-2.824	0.391	0.070	0.866	0.126	0.509
pDC	-0.268	0.712	-0.018	0.534	-1.157	0.202	-0.649	0.533	0.039	0.760	-0.024	0.683
mDC/pDC	-0.119	0.412	0.011	0.051	0.196	0.279	-0.105	0.613	0.000	0.988	0.007	0.568

B. As % of total lymphocytes

CD4.RTE	2.230	0.030	-0.060	0.090	2.998	0.014	-0.180	0.901	-0.022	0.895	-0.020	0.823
Th17	-0.001	0.921	0.00024	0.426	0.023	0.021	-0.003	0.817	0.002	0.206	0.000	0.742
B.Transitional	-0.384	0.554	0.066	0.044	1.518	0.060	-0.896	0.335	-0.288	0.103	-0.006	0.900
B.Switched	0.486	0.112	-0.015	0.196	-1.062	0.0056	0.486	0.267	0.077	0.102	0.016	0.520
CD8.TEM	-0.096	0.877	-0.021	0.319	-0.990	0.180	0.675	0.442	0.083	0.405	-0.040	0.458
B.Memory	0.109	0.683	-0.004	0.676	-0.342	0.304	1.113	0.0042	0.000	0.994	-0.044	0.050

table e-3. Associations with four common immunomodulatory treatments in MS.
Linear regression of MS patients treated with four immunomodulatory treatments (IFNB: interferon-beta, GA: glatiramer acetate, NAT: natalizumab, FTY720: fingolimod) compared to grouped controls and untreated patients (N=92, except for *: untreated MS patients only) including age and gender as covariates. Multiple testing correction: $p < 0.00033$ (underlined), nominal significance: $p < 0.05$ (italics).

	IFNB (N=54)		**GA (N=21)**		**NAT (N=9)**		**FTY720 (N=14)**	
Cell Type	**Beta**	***p***	**Beta**	***p***	**Beta**	***p***	**Beta**	***p***
Lymph	-2.08	0.42	-4.46	0.23	-1.76	0.69	-3.17	0.56
T	-2.59	0.24	3.46	0.27	-6.16	0.16	-6.77	0.24
CD4.RTE	6.00	*0.017*	7.44	*0.045*	-0.54	0.9	-14.14	*0.0078*
CD4.Naive	12.44	*0.0011*	9.49	0.087	5.00	0.46	-25.17	*0.0017*
CD4.TEM	-10.98	*0.0055*	-7.73	0.18	-2.24	0.75	12.97	0.12
CD4.TEMRA	-2.85	0.27	1.61	0.67	-1.65	0.72	19.87	*0.00039*
CD4.TCM	1.39	0.25	-3.37	0.062	-1.13	0.61	-7.68	*0.0031*
Treg	0.40	0.53	-1.31	0.15	NA	NA	2.75	*0.0098*
Th1	-3.39	*0.0014*	0.22	0.88	-0.72	0.73	4.62	*0.0095*
Th2	-2.20	0.3	-1.24	0.68	-6.13	0.14	25.37	3.91E-12
Th1/Th2	-0.42	0.078	0.22	0.51	0.43	0.36	-0.77	0.052
Th17	0.09	0.38	0.37	*0.013*	0.04	0.85	0.15	0.37
Tfh	0.58	0.15	0.30	0.60	NA	NA	0.48	0.45
Tfh.CD45Ra-	0.60	0.13	0.39	0.49	NA	NA	0.55	0.39
CD4.IL2	-0.53	0.35	-1.15	0.16	-1.41	0.21	-5.07	1.46E-07
CD8	-3.3	0.16	4.95	0.14	-6.64	0.1	14.22	*0.0047*
CD8.RTE	10.57	*0.00038*	-1.92	0.65	-5.14	0.33	-14.43	*0.019*
CD8.Naive	9.80	*0.0033*	-2.27	0.64	-6.30	0.29	-15.31	*0.028*
CD8.TEM*	-6.62	0.10	-5.33	0.34	6.80	0.31	-16.34	*0.037*
CD8.TEMRA	-6.86	0.064	5.94	0.28	-3.31	0.62	30.30	0.00016
CD8.TCM	0.48	0.24	-0.66	0.28	-0.12	0.87	-1.39	0.11
CD8.IFNG	-10.69	0.00032	0.070	0.99	-6.18	0.29	23.89	2.68E-06
CD8.IL2	2.64	*0.037*	-2.61	0.15	2.91	0.24	-8.63	4.88E-05
CD4	2.63	0.20	-2.42	0.42	2.85	0.42	-9.51	*0.032*
CD4/CD8	0.26	0.088	-0.38	0.086	0.51	0.055	-2.00	1.00E-08
B*	5.51	*0.0064*	-2.86	0.30	4.71	0.23	-22.38	1.16E-10
B.Transitional	8.79	0.00030	-0.58	0.87	NA	NA	13.44	*0.0012*
B.Naive	7.52	*0.00037*	3.64	0.23	-6.84	0.12	-21.62	6.44E-09
B.Switched	-4.2	0.000080	-2.12	0.16	5.05	*0.023*	-6.91	0.00014
Plasmablast	0.11	0.71	-0.14	0.76	NA	NA	3.64	3.50E-09
B.Memory	-1.46	0.10	-0.39	0.76	3.13	0.092	-2.20	0.14
T/B*	-0.22	0.075	0.20	0.24	-0.36	0.13	1.49	1.43E-06
gdT	-0.48	0.12	-0.45	0.32	-0.48	0.36	-0.38	0.56
NK	-2.28	*0.020*	-0.12	0.93	2.79	0.15	9.30	*0.00034*
NKT	-5.9	*0.00048*	0.25	0.91	-4.22	0.2	16.87	0.00014
mDC	-9.39	9.22E-06	4.37	0.13	-7.82	0.054	24.6	4.50E-05
pDC	-1.5	*0.038*	0.10	0.92	1.10	0.42	-2.75	0.23
mDC/pDC	-0.25	0.093	0.35	0.1	-0.4	0.17	1.52	*0.0018*

figure e-1. Overview of cell types included in the immunophenotyping platform.

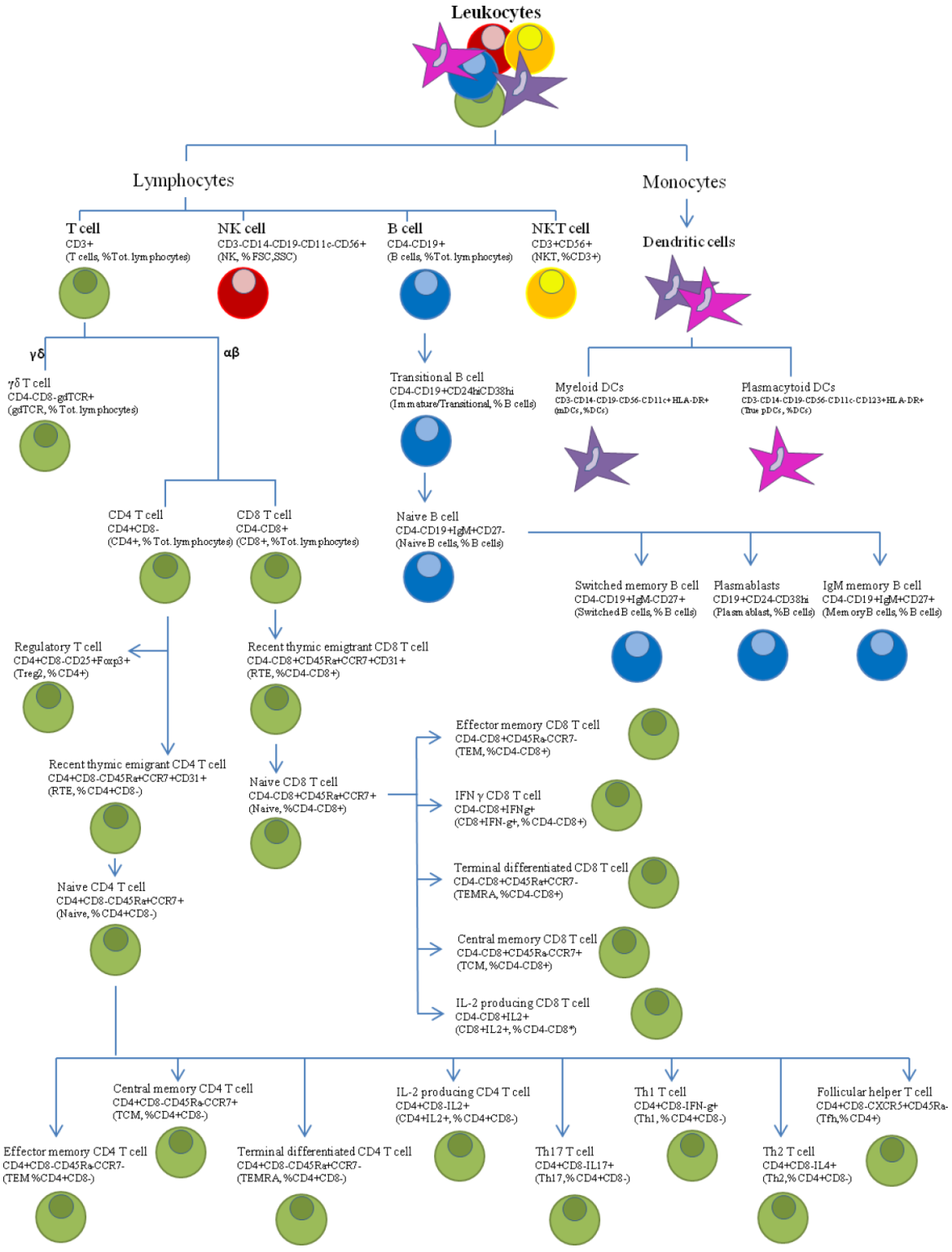

figure e-2. Effect of immunomodulatory multiple sclerosis treatments on T cell subsets as a proportion of total lymphocytes. Effect of four immunomodulatory treatments (IFNB: interferon-beta, GA: glatiramer acetate, NAT: natalizumab, FTY720: fingolimod) is compared with combined controls (CON) and untreated (UNT) multiple sclerosis patients, after establishing no significant difference between the latter two groups. A linear regression with covariates age and gender is applied. Data is displayed for **A.** total T cells, **B.** Th1 cells, **C.** Th2 cells, **D.** Th17 cells, **E.** follicular helper T cells. Median with boxes indicating 25th and 75th percentile and whiskers indicating range.

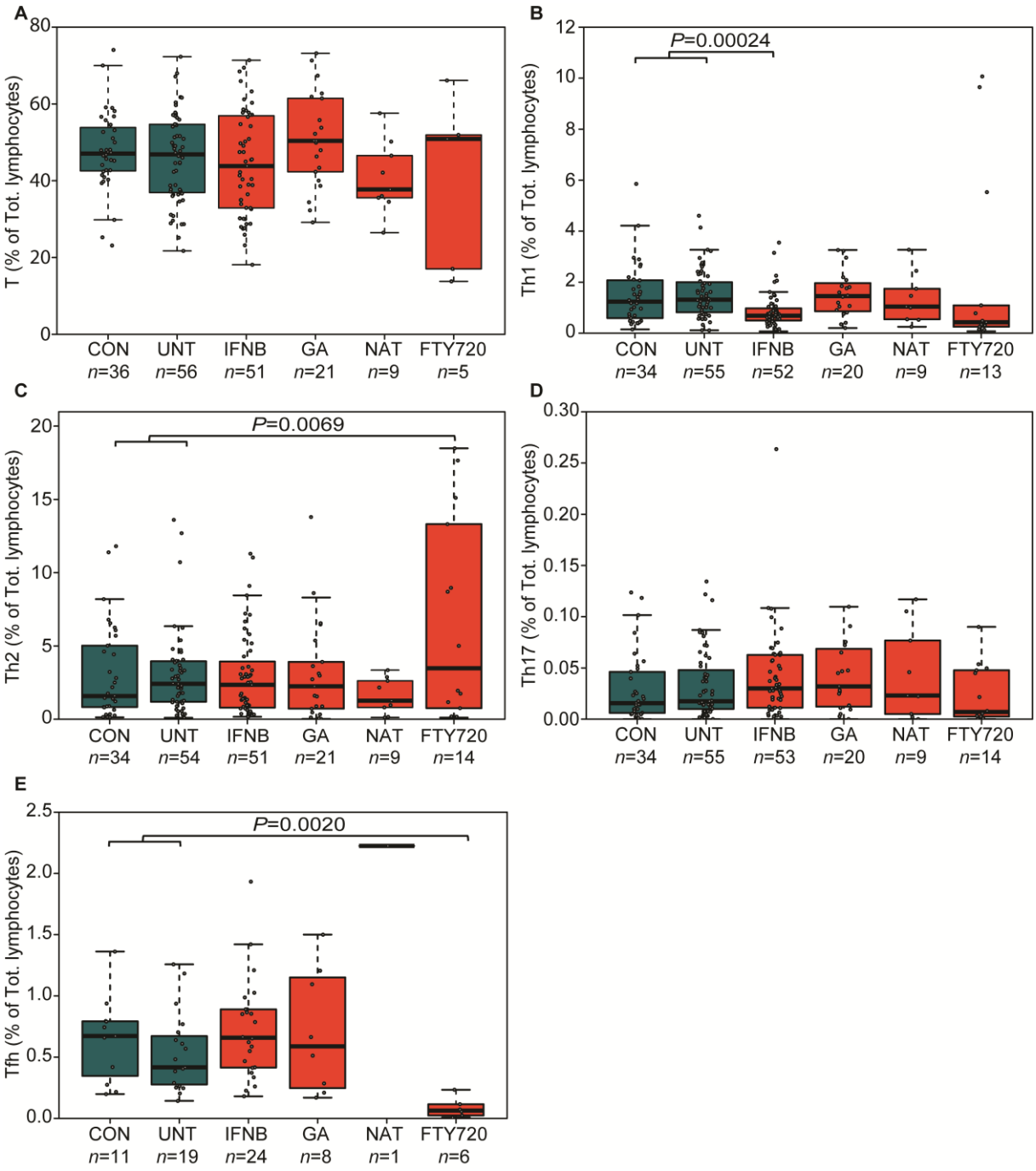

figure e-3. Effect of immunomodulatory multiple sclerosis treatments on B cell subsets as a proportion of total lymphocytes. Effect of four immunomodulatory treatments (IFNB: interferon-beta, GA: glatiramer acetate, NAT: natalizumab, FTY720: fingolimod) is compared with combined controls (CON) and untreated (UNT) multiple sclerosis patients, after establishing no significant difference between the latter two groups. A linear regression with covariates age and gender is applied. Data is displayed for **A.** total B cells, **B.** transitional B cells, **C.** naïve B cells, **D.** class-switched B cells, **E.** plasmablasts, and **F.** memory B cells. Median with boxes indicating 25th and 75th percentile and whiskers indicating range.