SUPPLEMENTARY INFORMATION

Peripheral proinflammatory Th1/Th17 immune cell shift is linked to disease severity in amyotrophic lateral sclerosis

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Supplementary Tables:

Supplementary Table1: Correlation of immune cells to clinical parameters in ALS

Immune cells	ALSFRS-R score	ALSFRS-R slope	disease duration	ppFVC	Age	BMI
CD3+ T cells	r = -0.17 p = 0.188 N = 64	r = 0.23 p = 0.071 N = 64	r = -0.13 p = 0.312 N = 66	r = -0.04 p = 0.766 N = 61	r = 0.09 p = 0.449 N = 66	r = -0.14 p = 0.270 N = 65
CD4+ T cells	r = 0.07 p = 0.563 N = 64	r = 0.005 p = 0.966 N = 64	r = -0.10 p = 0.41 N = 66	r = 0.06 p = 0.638 N = 61	r = -0.14 p = 0.24 N = 66	r = 0.002 p = 0.987 N = 65
CD8+T cells	r = 0.07 p = 0.582 N = 64	r = -0.07 p = 0.567 N = 64	r = 0.02 p = 0.900 N = 66	r = 0.18 p = 0.161 N = 61	**r = -0.36** **p = 0.003** **N = 66**	r = 0.04 p = 0.759 N = 65
CD4/CD8	r = 0.04 p = 0.726 N = 64	r = 0.08 p = 0.518 N = 63	r = 0.08 p = 0.543 N = 64	r = -0.25 p = 0.062 N = 59	r = 0.14 p = 0.260 N = 66	r = -0.13 p = 0.312 N = 63
Th1 cells	**r = - 0.45** **p = 0.002** **N = 64**	**r = 0.29** **p = 0.021** **N = 64**	r = 0.16 p = 0.206 N = 66	**r = -0.26** **p = 0.045** **N = 61**	r = -0.21 p = 0.101 N = 66	r = 0.05 p = 0.708 N = 65
Th2 cells	r = 0.05 p = 0.722 N = 64	r = -0.03 p = 0.800 N = 64	r = 0.05 p = 0.690 N = 66	r = -0.12 p = 0.365 N = 61	r = -0.03 p = 0.820 N = 66	r = 0.02 p = 0.885 N = 65
Th1/Th2	r = -0.13 p = 0.291 N = 64	**r = 0.31** **p = 0.0114** **N = 64**	r = -0.16 p = 0.170 N = 63	r = -0.0045 p = 0.974 N = 57	r = -0.07 p = 0.581 N = 63	r = -0.04 p = 0.775 N = 62

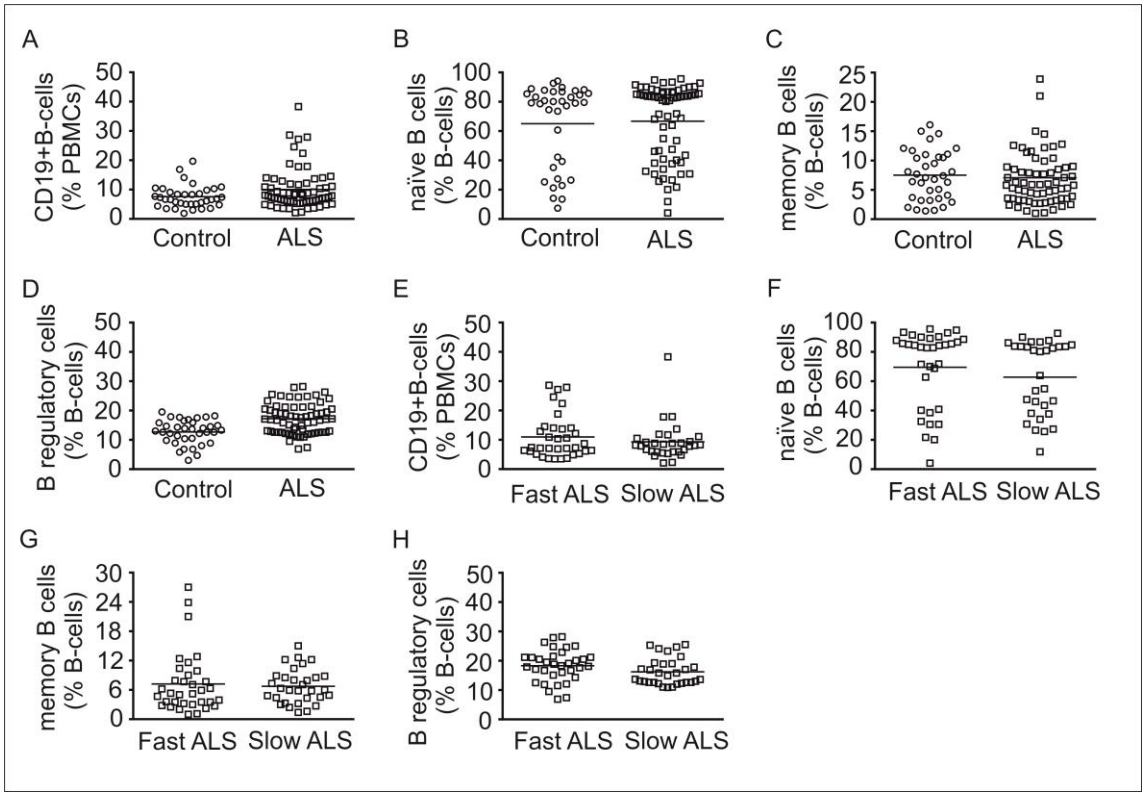
Treg cells	r = -0.03 p = 0.802 N = 65	r = 0.02 p = 0.860 N = 65	r = -0.07 p = 0.600 N = 67	r = -0.01 p = 0.365 N = 62	r = -0.07 p = 0.600 N = 67	r = 0.07 p = 0.583 N = 66
Th17 cells	**r = -0.41** **p = 0.00089** **N = 64**	r = 0.06 p = 0.660 N = 64	**r = 0.34** **p = 0.006** **N = 66**	**r = -0.30** **p = 0.018** **N = 61**	r = -0.06 p = 0.605 N = 66	r = -0.12 p = 0.345 N = 65
Treg/Th17	**r = 0.29** **p = 0.020** **N = 64**	r = -0.10 p = 0.434 N = 64	r = -0.03 p = 0.785 N = 65	r = 0.22 p = 0.097 N = 61	r = -0.11 p = 0.390 N = 65	r = -0.02 p = 0.886 N = 64
NK cells	r = 0.09 p = 0.464 N = 64	r = -0.03 p = 0.800 N = 64	r = -0.05 p = 0.700 N = 66	r = -0.04 p = 0.745 N = 61	r = 0.00071 p = 1.000 N = 66	r = 0.06 p = 0.626 N = 65
CD56^{bright} **CD16^{bright}** **NK cells**	r = -0.02 p = 0.890 N = 64	r = -0.01 p = 0.965 N = 64	r = 0.03 p = 0.812 N = 66	r = 0.07 p = 0.588 N = 61	r = 0.06 p = 0.660 N = 66	r = -0.15 p = 0.249 N = 65
CD56^{bright} **CD16^{dim}** **NK cells**	r = 0.05 p = 0.720 N = 64	r = -0.13 p = 0.298 N = 64	r = 0.10 p = 0.422 N = 66	r = 0.13 p = 0.305 N = 61	r = -0.22 p = 0.072 N = 66	r = 0.11 p = 0.400 N = 65
CD56^{bright} **CD16-** **NK cells**	r = 0.02 p = 0.900 N = 64	r = -0.09 p = 0.465 N = 64	r = 0.12 p = 0.333 N = 66	r = 0.09 p = 0.504 N = 61	r = -0.16 p = 0.189 N = 66	r = 0.01 p = 0.960 N = 65
CD56^{dim} **CD16^{dim}** **NK cells**	r = 0.07 p = 0.607 N = 64	r = 0.09 p = 0.472 N = 64	r = -0.17 p = 0.173 N = 66	r = -0.05 p = 0.703 N = 61	r = -0.08 p = 0.500 N = 66	r = 0.09 p = 0.498 N = 65
CD56^{dim} **CD16^{bright}** **NK cells**	r = -0.01 p = 0.925 N = 64	r = 0.05 p = 0.705 N = 64	r = -0.06 p = 0.650 N = 66	r = -0.12 p = 0.379 N = 61	**r = 0.39** **p = 0.001** **N = 66**	r = -0.12 p = 0.346 N = 65

NKT cells	r = 0.14 p = 0.263 N = 64	r = 0.002 p = 0.987 N = 64	r = -0.18 p = 0.141 N = 66	r = 0.07 p = 0.612 N = 61	r = -0.18 p = 0.143 N = 66	r = 0.09 p = 0.497 N = 65
CD4+NKT cells	r = -0.05 p = 0.691 N = 64	r = 0.02 p = 0.893 N = 64	r = -0.04 p = 0.776 N = 66	r = -0.02 p = 0.890 N = 61	r = 0.02 p = 0.842 N = 66	r = -0.11 p = 0.387 N = 65
CD8+NKT cells	r = 0.01 p = 0.938 N = 64	r = 0.06 p = 0.628 N = 64	r = -0.16 p = 0.194 N = 66	r = -0.15 p = 0.256 N = 61	r = -0.07 p = 0.590 N = 66	r = -0.03 p = 0.801 N = 65
CD14+Mo	r = 0.14 p = 0.287 N = 63	r = -0.11 p = 0.403 N = 63	r = -0.13 p = 0.322 N = 65	r = 0.02 p = 0.868 N = 60	r = -0.14 p = 0.260 N = 65	r = -0.05 p = 0.711 N = 65
Classical Mo	r = 0.15 p = 0.256 N = 63	r = -0.01 p = 0.920 N = 63	r = -0.05 p = 0.670 N = 65	r = 0.03 p = 0.827 N = 60	r = 0.03 p = 0.810 N = 65	r = -0.02 p = 0.895 N = 64
Intermediate Mo	r = -0.01 p = 0.928 N = 63	r = -0.02 p = 0.890 N = 63	r = 0.04 p = 0.750 N = 65	r = 0.07 p = 0.582 N = 60	r = -0.04 p = 0.760 N = 65	r = -0.04 p = 0.744 N = 64
Non-classical Mo	r = -0.04 p = 0.762 N = 63	r = -0.07 p = 0.590 N = 63	r = -0.0024 p = 0.900 N = 65	r = 0.09 p = 0.496 N = 60	r = -0.05 p = 0.700 N = 65	r = 0.10 p = 0.443 N = 64

Supplementary Table 2: Correlation between cytokines in serum and clinical parameters in ALS

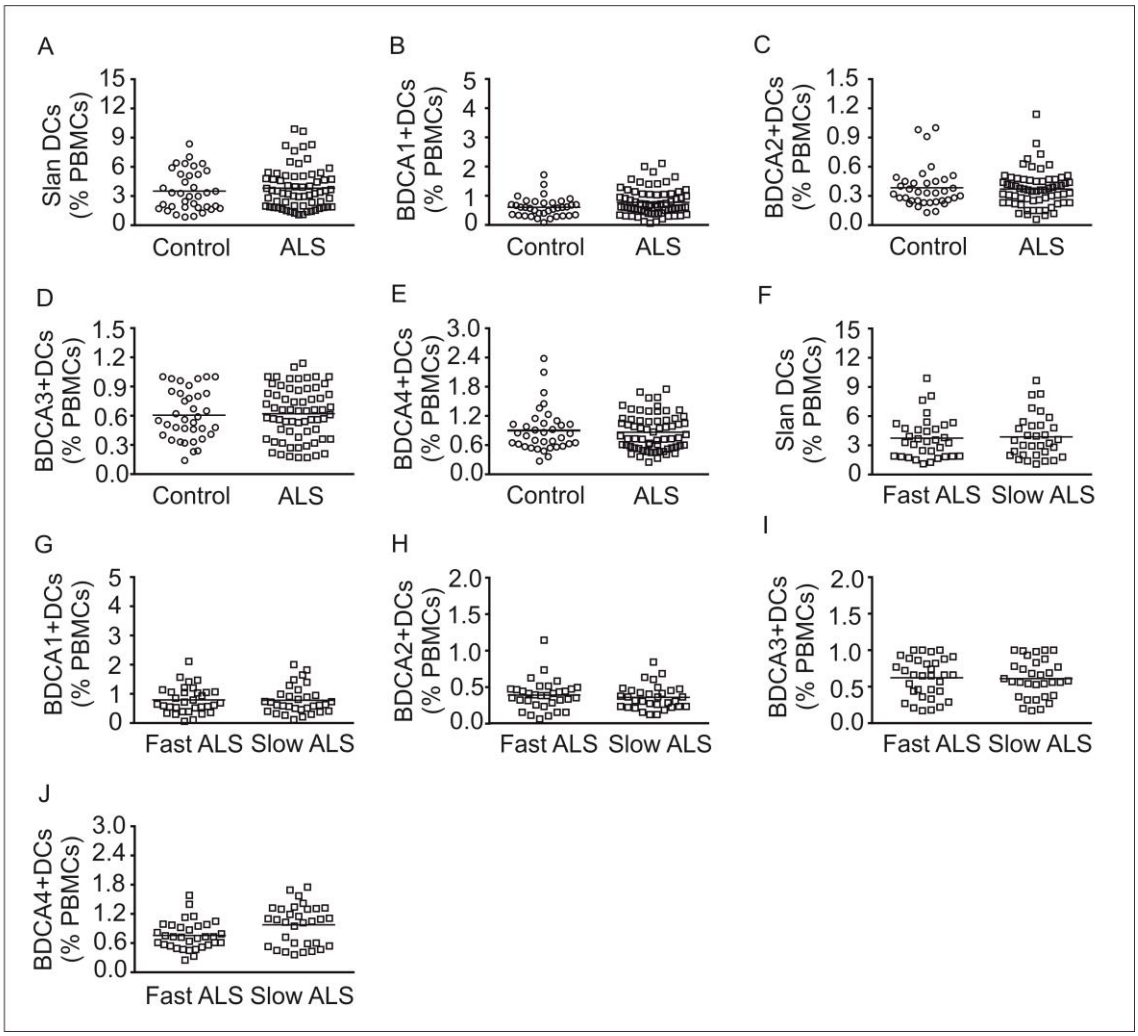
Cytokines in the Serum	ALSFRS-R score	ALSFRS-R slope	disease duration	ppFVC	Age	BMI
IL-6	r = 0.11 p = 0.349 N = 71	r = -0.13 p = 0.272 N = 70	**r = 0.52** **p < 0.0001** **N = 71**	r = 0.03 p = 0.821 N = 65	r = 0.07 p = 0.550 N = 71	r = 0.03 p = 0.789 N = 70
IL-1beta	r = -0.03 p = 0.79 N = 71	**r = 0.35** **p = 0.004** **N = 70**	r = 0.03 p = 0.831 N = 71	r = -0.16 p = 0.205 N = 65	r = -0.13 p = 0.278 N = 71	r = -0.01 p = 0.3 N = 70
IFN-gamma	r = 0.086 p = 0.476 N = 71	r = -0.0492 p = 0.686 N = 70	r = 0.16 p = 0.184 N = 71	r = -0.10 p = 0.446 N = 65	r = 0.003 p = 0.980 N = 71	r = -0.10 p = 0.417 N = 70

Supplementary Figures



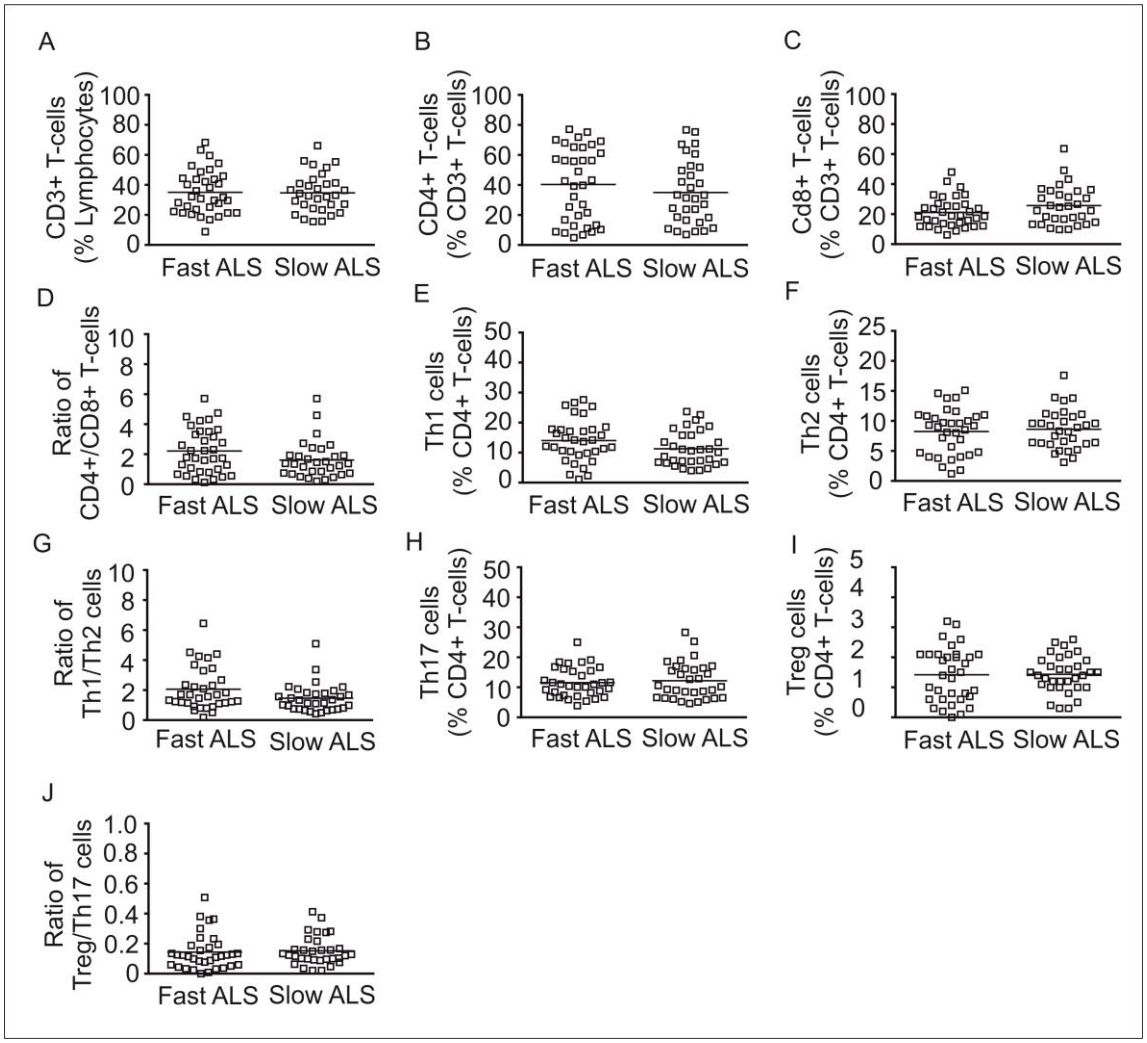
Supplementary Figure 1 Peripheral B cell profiles in ALS patients and healthy controls.

Depicted are relative counts of CD19+ B cells (**A**), naïve B cells (**B**), memory B cells (**C**) and B regulatory cells (**D**) of ALS versus controls as well as relative counts of CD19+ B cells (**E**), naïve B cells (**F**), memory B cells (**G**) and B regulatory cells (**H**) of fast ALS versus slow ALS. Results are presented as raw data, each circle represents one healthy control and each quad represents one ALS patient, horizontal lines indicate mean values. *p < 0.05, **p < 0.01, ***p < 0.001.

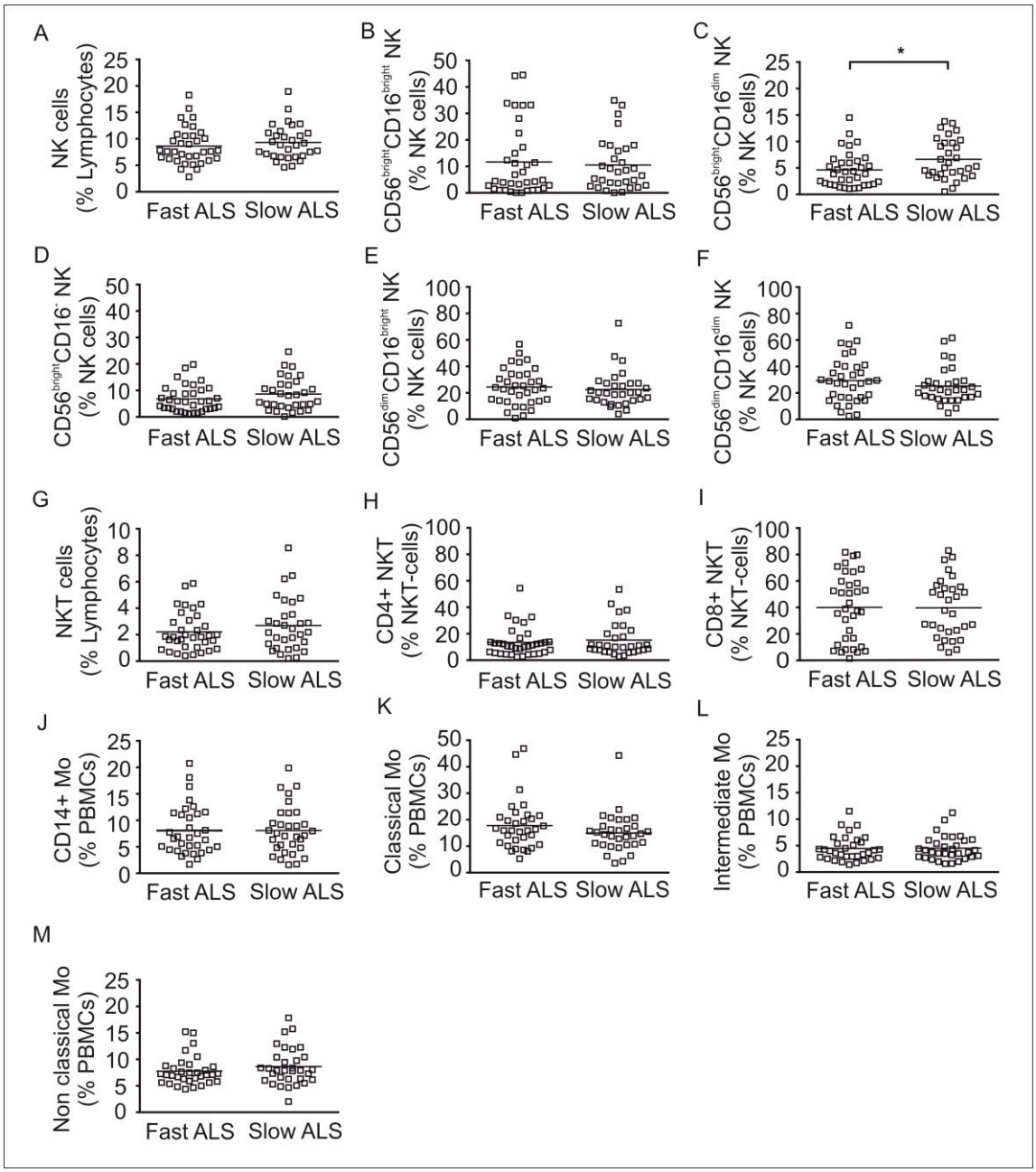


Supplementary Figure 2 Peripheral DC subsets in ALS patients and healthy controls.

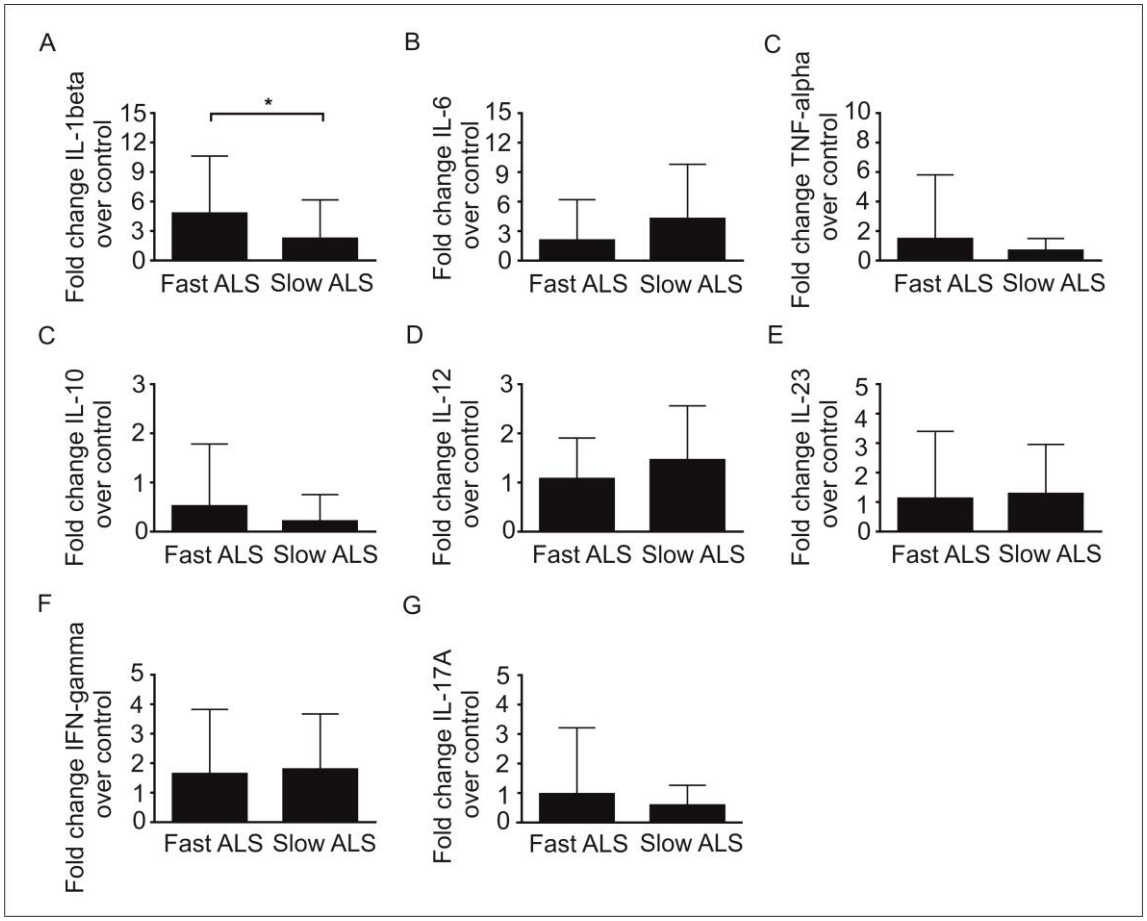
Depicted are relative counts of slanDCs (**A**), BDCA1+DCs (**B**), BDCA2+DCs (**C**), BDCA3+DCs (**D**) and BDCA4+DCs (**E**) of ALS versus controls as well as relative counts of SlanDCs (**F**), BDCA1+DCs (**G**), BDCA2+DCs (**H**), BDCA3+DCs (**I**) and BDCA4+DCs (**J**) of fast ALS versus slow ALS. Results are presented as raw data, each circle represents one healthy control and each quad represents one ALS patient, horizontal lines indicate mean values. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.



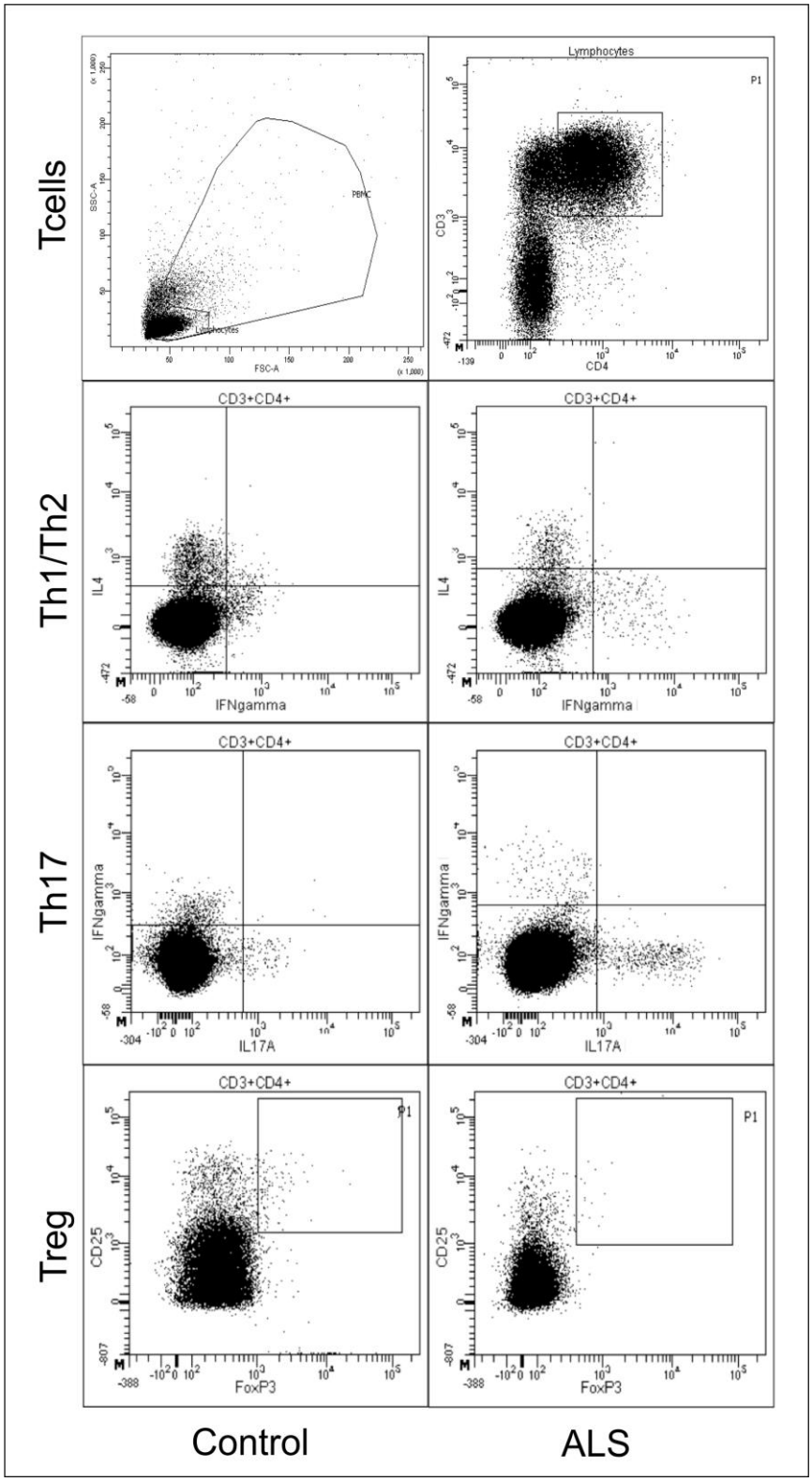
Supplementary Figure 3 Peripheral T cell and B cell subsets in comparison of fast versus slow ALS. Depicted are relative counts of CD3+T cells (**A**), CD4+T cells (**B**), CD8+T cells (**C**), ratio of CD4+/CD8+T cells (**D**), Th1 cells (**E**), Th2 cells (**F**), ratio of Th1/Th2 cells (**G**), Th17 cells (**H**) and Treg cells (**I**) and the ratio of Treg/Th17 cells (**J**) of fast ALS versus slow ALS. Results are presented as raw data, each circle represents one healthy control and each quad represents one ALS patient, horizontal lines indicate mean values. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.



Supplementary Figure 4 Peripheral NK cells and Mo subset of fast versus slow ALS. Depicted are relative counts of NK cells (**A**), $CD56^{bright}CD16^{bright}$NK cells (**B**), $CD56^{bright}CD16^{dim}$NK cells (**C**), $CD56^{bright}CD16^{-}$NK cells (**D**), $CD56^{dim}CD16^{bright}$NK cells (**E**), $CD56^{dim}CD16^{dim}$NK cells (**F**), NKT cells (**G**), CD4+NKT cells (**H**), CD8+NKT cells (**I**), CD14+Mo (**J**), classical Mo cells (**K**), intermediate Mo cells (**L**), and non-classical Mo cells (**M**) of fast versus slow ALS. Results are presented as raw data, each circle represents one healthy control and each quad represents one ALS patient, horizontal lines indicate mean values. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.



Supplementary Figure 5 Cytokines in the blood serum of fast versus slow ALS. Levels of IL-1beta (**A**), IL-6 (**B**), TNF-alpha (**C**), IL-10 (**D**), IL-12 (**E**), IL-23 (**F**), IFN-gamma (**G**) and IL-17A (**H**) in the serum of fast ALS and slow ALS were calculated as fold change over the mean of healthy controls. Data are depicted as histograms (means ± SD). *$p < 0.05$.



Supplementary Figure 6 Representative Facs plots of Tcells, Th1, Th17 and Treg cells in ALS patients and healthy controls.