Figure e-1

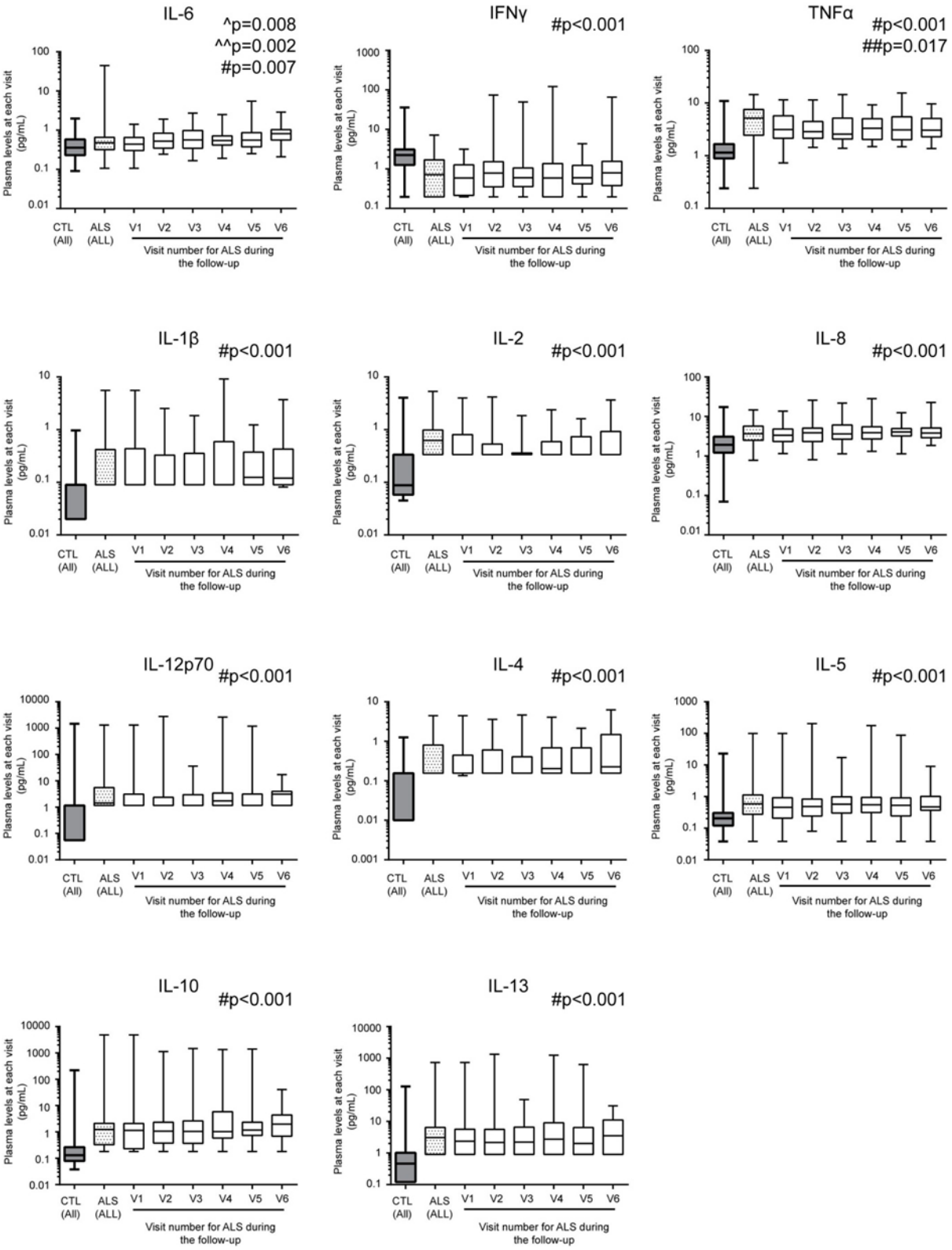
IL-6
^p=0.008
^^p=0.002
#p=0.007
IFNγ
#p<0.001
TNFα
#p<0.001
##p=0.017
IL-1β
#p<0.001
IL-2
#p<0.001
IL-8
#p<0.001
IL-12p70
#p<0.001
IL-4
#p<0.001
IL-5
#p<0.001
IL-10
#p<0.001
IL-13
#p<0.001
Plasma levels at each visit (pg/mL)
CTL (All)
ALS (ALL)
V1
V2
V3
V4
V5
V6
Visit number for ALS during the follow-up

Figure e-1. Plasma cytokine levels of ALS patients at each visit in the longitudinal and case-control studies.

The box-and-whisker plots show the expression of the 11 cytokines obtained at baseline (V1) and during the 15-month follow-up period (V2-V6) from 59 ALS cases (clear box) who underwent serial sampling. The boxes represent the Median (IQR) levels at each visit and the whiskers indicate the ranges. Only IL-6 showed a small but significant increase in expression between V1 and V6. Expression data from all the controls (filled box) and the ALS patients (dotted box) are also presented for reference. ^P-value: Kruskal-Wallis test examining changes of IL-6 expression between visits; ^^p-value adjusted for multiple comparison between V1 and V6; #p-value: Mann-Whitney test examining changes in IL-6 expression between all controls and all ALS; ##p-value: Mann-Whitney test examining changes of TNFα expression between all ALS and the 59 ALS included in the longitudinal cohort; not significant p-values are not reported.

Table e-1. Summary of cut-off used to determine tertiles in Logistic regression analysis and Cos regression analysis.

Table in the main text	**Table e-2A**		**Table e-2B**		**Table e-2C & Table e-2D**	
Defined by	All subjects		Case and Controls without any missing data		ALS only	
Cut-off for 1st and 2nd tertiles (A) & 2nd and 3rd tertiles (B)	**A**	**B**	**A**	**B**	**A**	**B**
CRP (mg/L)	2.000	5.000	2.000	4.500	2.000	6.000
CK (U/L)	98.655	165.017	91.662	169.000	127.988	273.006
Ferritin (µg/L)	95.000	177.339	97.324	159.676	118.652	235.670
IL-6 (pg/mL)	0.321	0.538	0.317	0.545	0.362	0.569
IFNγ (pg/mL)	0.915	2.175	0.843	2.218	0.397	1.304
TNFα (pg/mL)	1.405	5.041	1.185	2.544	3.138	6.496
IL-1β (pg/mL)	0.038	0.090	0.020	0.090	0.090	0.472
IL-2 (pg/mL)	0.189	0.356	0.101	0.335	0.335	0.920
IL-8 (pg/mL)	2.010	3.620	1.752	3.189	2.931	4.301
IL-12p70 (pg/mL)	0.110	1.451	0.069	1.150	1.150	5.083
IL-4 (pg/mL)	0.012	0.166	0.010	0.155	0.155	0.801
IL-5 (pg/mL)	0.197	0.579	0.165	0.406	0.373	0.946
IL-10 (pg/mL)	0.180	1.157	0.158	0.745	0.638	1.658
IL-13 (pg/mL)	0.900	2.945	0.595	1.253	1.039	4.853

Cut-off values used for tertile transformation in the logistic regression analysis and Cox regression analysis.

Table e-2. Summary of logistic regression analysis for each plasma marker.

Table e-2A	***ALS vs Controls:* OR and relative 95% CI of *individual* plasma markers, with and without further adjustment for clinical variables.**			
	Basic model[a]		**Multivariate model[b]**	
Markers	**OR (95%CI)**	**p-value**	**OR (95%CI)**	**p-value**
CK	2.41 (1.54, 3.78)	<0.001	3.03 (1.80, 5.10)	<0.001
Ferritin	2.10 (1.37, 3.23)	0.001	2.17 (1.35, 3.47)	0.001
CRP	1.26 (0.79, 2.01)	0.33	N/A	N/A
IL-6	1.34 (0.89, 2.02)	0.16	1.28 (0.80, 2.04)	0.31
TNFα	8.00 (4.33, 14.76)	<0.001	7.24 (3.81, 13.77)	<0.001
IL-1β	7.41 (4.01, 13.69)	<0.001	6.60 (3.51, 12.39)	<0.001
IL-8	3.31 (2.08, 5.26)	<0.001	3.42 (2.03, 5.74)	<0.001
IFNγ	0.20 (0.12, 0.34)	<0.001	0.21 (0.12, 0.37)	<0.001
IL-2	15.49 (7.12, 33.68)	<0.001	14.22 (6.32, 32.01)	<0.001
IL-12p70	12.51 (7.12, 33.68)	<0.001	11.41 (5.32, 24.48)	<0.001
IL-4	9.74 (5.02, 18.90)	<0.001	10.96 (5.19, 23.18)	<0.001
IL-5	3.49 (2.17, 5.61)	<0.001	3.59 (2.14, 6.03)	<0.001
IL-13	11.38 (5.52, 23.46)	<0.001	11.05 (5.15, 23.69)	<0.001
IL-10	10.67 (5.27, 21.59)	<0.001	10.19 (4.84, 21.48)	<0.001
Table e-2B	***ALS vs Controls* OR and relative 95% CI of *mutually-adjusted* plasma markers in a basic and a multivariate model.**			

	Basic model[a]		Multivariate model[b]	
Markers	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
Age at sampling, y	1.05 (0.99, 1.12)	0.11	0.92 (0.80, 1.06)	0.26
Gender[c]	0.17 (0.03, 0.86)	0.03	0.29 (0.02, 4.39)	0.37
CK	1.36 (0.54, 3.43)	0.51	0.88 (0.23, 3.33)	0.85
Ferritin	1.74 (0.70, 4.33)	0.23	0.61 (0.13, 2.81)	0.53
CRP	0.54 (0.14, 2.13)	0.38	0.34 (0.05, 2.15)	0.25
IL-6	5.53 (1.31, 23.30)	0.02	18.55 (1.37, 251.95)	0.03
TNFα	5.05 (0.93, 27.59)	0.06	35.78 (1.86, 689.20)	0.02
IL-1β	1.37 (0.24, 7.78)	0.72	0.79 (0.06, 10.11)	0.86
IL-8	0.63 (0.28, 1.73)	0.37	0.34 (0.07, 1.78)	0.20
IFNγ	0.14 (0.04, 0.55)	0.005	0.09 (0.01, 0.62)	0.02
IL-2	1.96 (0.16, 24.74)	0.61	0.23 (0.01, 8.41)	0.43
IL-12p70	0.60 (0.04, 10.22)	0.72	0.06 (0.001, 2.79)	0.15
IL-4	2.05 (0.40, 10.52)	0.39	90.90 (1.25, 6603.62)	0.04
IL-5	1.27 (0.33, 4.84)	0.73	1.64 (0.31, 8.73)	0.57
IL-13	4.88 (0.52, 45.99)	0.17	20.69 (0.65, 656.10)	0.086
IL-10	1.001 (0.99, 1.02)	0.86	1.005 (0.98, 1.03)	0.74
Arthritis[d]	N/A		190.63 (0.27, 133073.73)	0.12

Autoimmune pathology[d]			7.61 (0.02, 2561.16)	0.49
Hypertension[d]			0.03 (0.001, 0.97)	0.048
Diabetes[d]			12.18 (0.04, 3510.54)	0.39
Hyperlipidaemia[d]			8.65 (0.24, 314.33)	0.24
Statin Usage[d]			0.24 (0.005, 10.76)	0.46
CVD Risk			68.82 (1.94, 2444.97)	0.02
Table e-2C	**HR and relative 95% CI of *individual* plasma markers with and without further adjustment for clinical variables to evaluate survival *among ALS patients.***			
	Basic model[a]		**Multivariate model**[b]	
Markers	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**
CK	0.95 (0.70, 1.29)	0.74	0.92 (0.67, 1.26)	0.59
Ferritin	1.74 (1.26, 2.41)	0.001	1.71 (1.20, 2.44)	0.003
CRP	1.19 (0.88, 1.61)	0.26	N/A	N/A
IL-6	1.04 (0.77, 1.40)	0.82	1.02 (0.73, 1.42)	0.92
TNFα	1.26 (0.94, 1.71)	0.13	1.31 (0.94, 1.81)	0.11
IL-1β	0.81 (0.60, 1.11)	0.18	0.70 (0.48, 1.03)	0.07
IL-8	1.07 (0.80, 1.45)	0.65	1.18 (0.84, 1.66)	0.35
IFNγ	1.05 (0.78, 1.42)	0.74	1.01 (0.73, 1.39)	0.97

IL-2	1.29 (0.96, 1.74)	0.09	1.43 (1.04, 1.98)	0.03
IL-12p70	1.07 (0.80, 1.42)	0.66	1.09 (0.80, 1.48)	0.60
IL-4	0.94 (0.69, 1.27)	0.68	0.98 (0.68, 1.40)	0.90
IL-5	0.95 (0.72, 1.25)	0.71	0.89 (0.65, 1.22)	0.47
IL-13	1.01 (0.75, 1.36)	0.96	1.00 (0.72, 1.38)	0.99
IL-10	1.06 (0.80, 1.41)	0.69	1.05 (0.77, 1.43)	0.77
Table e-2D	**HR and relative 95% CI of *mutually-adjusted* plasma markers in a basic and a multivariate model to evaluate survival *among ALS patients.***			
	Basic model[a]		**Multivariate model[b]**	
Markers	**HR (95% CI)**	**p-value**	**HR (95% CI)**	**p-value**
Age at sampling, y	1.05 (1.02, 1.08)	0.002	1.03 (0.97, 1.08)	0.37
Gender[c]	1.77 (1.01, 3.12)	0.047	2.42 (1.12, 4.88)	0.02
Ferritin	1.46 (1.04, 2.04)	0.03	1.38 (0.95, 1.99)	0.09
IL2	1.61 (1.05, 2.47)	0.03	1.77 (1.10, 2.84)	0.02
IL1β	0.55 (0.35, 0.85)	0.01	0.52 (0.32, 0.85)	0.009
TNFα	1.18 (0.77, 1.81)	0.45	1.20 (0.77, 1.87)	0.42
Hypertension[d]	N/A		1.47 (0.80, 2.70)	0.21
Diabetes[d]			0.09 (0.02, 0.49)	0.005
Hyperlipidaemia[d]			1.89 (0.64, 5.55)	0.25
Statin usage[d]			1.83 (0.76,4.43)	0.18

CVD risk		1.27 (0.61, 2.66)	0.52
CRP		1.13 (0.79, 1.60)	0.51
Arthritis[d]		0.40 (0.13, 1.22)	0.11
Autoimmune pathology[d]		2.13 (0.76, 6.03)	0.15

Abbreviations: OR, odds ratios; CI, confidence intervals; HR, hazard ratios; N/A, not applicable. [a], Basic model: adjusted for gender and age at sampling; [b], Multivariate model: adjusted for gender, age at sampling, CRP, hypertension, diabetes, hyperlipidaemia, statin usage, CVD risk, arthritis and autoimmune pathology; [c], reference group: male; [d], reference group: without the disease/treatment.