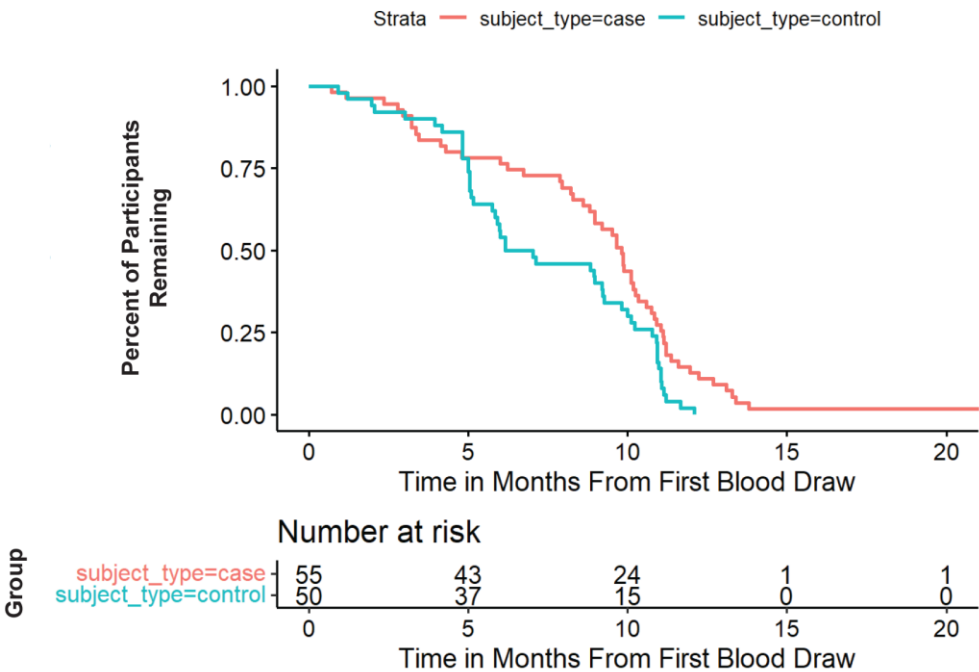


Supplemental Material

Supplementary Figure S1. The sample follow-up in ALS and control participants for the overall study, related to Table 1 and STAR Methods



Supplementary Figure S1. Sample follow-up in ALS and controls. Plot shows the number of monthly samples provided by each study participant group, ALS cases (red, N = 55 participants) or controls (blue, N = 50 participants). Participants had fewer than 12 sample visits for a variety of reasons: withdrawal from the study, death, or interruption of the study by COVID-19.

Supplementary Table S1. The initial immune measurements in ALS and control participants, related to Figure 1

Characteristic	Control, N = 50 ¹	ALS, N = 55 ¹	p-value ²	q-value ³
Neutrophils ⁴	2.00 (1.60, 2.53)	2.91 (2.33, 3.48)	<0.001	<0.001
Eosinophils ⁴	0.10 (0.06, 0.15)	0.09 (0.06, 0.14)	0.636	0.7
CD16+ Monocytes ⁴	0.034 (0.027, 0.047)	0.037 (0.024, 0.054)	0.637	0.7
CD16- Monocytes ⁴	0.19 (0.14, 0.22)	0.23 (0.17, 0.31)	0.011	0.048
NK Cells ⁴	0.10 (0.06, 0.15)	0.13 (0.09, 0.17)	0.069	0.218
CD4 T Cells ⁴	0.58 (0.42, 0.67)	0.55 (0.41, 0.79)	0.796	0.796
CD8 T cells ⁴	0.17 (0.13, 0.25)	0.20 (0.13, 0.33)	0.56	0.684
CD27+ NK Cells ⁴	0.004 (0.002, 0.008)	0.003 (0.002, 0.005)	0.288	0.423
CD57+ NK Cells ⁴	0.045 (0.026, 0.074)	0.065 (0.033, 0.087)	0.164	0.288
CD62L+ NK Cells ⁴	0.037 (0.024, 0.051)	0.039 (0.033, 0.068)	0.107	0.236
CD94+ NK Cells ⁴	0.058 (0.028, 0.071)	0.062 (0.041, 0.085)	0.098	0.236
CXCR3+ NK Cells ⁴	0.018 (0.009, 0.033)	0.027 (0.011, 0.043)	0.204	0.32
KIR2DL1+ NK Cells ⁴	0.027 (0.015, 0.049)	0.029 (0.020, 0.048)	0.404	0.542
KIR2DL2+ NK Cells ⁴	0.030 (0.018, 0.058)	0.041 (0.025, 0.055)	0.13	0.26
NK Cell CD11a MFI ⁵	28.02 (19.58, 37.39)	48.86 (30.80, 92.71)	<0.001	<0.001
NK Cell CD11b MFI ⁵	4.16 (3.72, 5.62)	5.82 (3.91, 8.42)	0.035	0.129
NK Cell CD38 MFI ⁵	58.93 (34.56, 78.10)	93.87 (54.24, 162.02)	<0.001	0.002
NK Cell CD45RA MFI ⁵	30.91 (22.67, 40.22)	34.13 (18.36, 49.53)	0.784	0.796
NK Cell CX3CR1 MFI ⁵	14.73 (9.50, 19.50)	22.03 (14.55, 37.97)	<0.001	<0.001
NK Cell NKG2D MFI ⁵	3.18 (2.72, 3.73)	3.66 (2.84, 4.49)	0.107	0.236
NK Cell NKp30 MFI ⁵	3.81 (2.34, 6.88)	3.44 (2.44, 4.42)	0.419	0.542
NK Cell NKp46 MFI ⁵	8.18 (6.60, 10.47)	10.12 (7.03, 12.34)	0.17	0.288

¹Median (interquartile range)

²Wilcoxon rank sum test for all variables in this table

³Benjamini-Hochberg correction for multiple testing

⁴(10⁶ cells per ml of blood)

⁵(fold increase versus non-specific IgG control antibody)

N, number of participants

MFI, median fluorescence intensity

Supplementary Table S2. All of the immune measurements in ALS and control participants, related to Figure 1

Characteristic	Control, N = 306 ¹	ALS, N = 435 ¹	p-value ²	q-value ³
Neutrophils ⁴	1.96 (1.45, 2.53)	2.64 (1.91, 3.44)	<0.001	<0.001
Eosinophils ⁴	0.10 (0.06, 0.16)	0.08 (0.05, 0.14)	0.460	0.567
CD16+ Monocytes ⁴	0.041 (0.030, 0.058)	0.035 (0.025, 0.050)	0.008	0.025
CD16- Monocytes ⁴	0.20 (0.16, 0.27)	0.22 (0.16, 0.30)	0.396	0.552
NK Cells ⁴	0.13 (0.08, 0.19)	0.12 (0.08, 0.17)	0.402	0.552
CD4 T Cells ⁴	0.62 (0.47, 0.83)	0.49 (0.35, 0.70)	0.012	0.034
CD8 T cells ⁴	0.21 (0.14, 0.32)	0.17 (0.12, 0.26)	0.212	0.333
CD27+ NK Cells ⁴	0.005 (0.003, 0.009)	0.004 (0.002, 0.007)	0.003	0.012
CD57+ NK Cells ⁴	0.053 (0.030, 0.099)	0.058 (0.034, 0.090)	0.896	0.896
CD62L+ NK Cells ⁴	0.046 (0.029, 0.073)	0.042 (0.028, 0.061)	0.464	0.567
CD94+ NK Cells ⁴	0.068 (0.040, 0.100)	0.063 (0.042, 0.100)	0.731	0.777
CXCR3+ NK Cells ⁴	0.021 (0.010, 0.044)	0.026 (0.014, 0.043)	0.712	0.777
KIR2DL1+ NK Cells ⁴	0.035 (0.016, 0.058)	0.030 (0.019, 0.047)	0.076	0.152
KIR2DL2+ NK Cells ⁴	0.039 (0.024, 0.063)	0.044 (0.029, 0.066)	0.742	0.777
NK Cell CD11a MFI ⁵	27.53 (20.36, 35.48)	35.28 (25.60, 64.91)	<0.001	<0.001
NK Cell CD11b MFI ⁵	5.00 (3.77, 6.48)	5.71 (4.20, 7.53)	0.040	0.088
NK Cell CD38 MFI ⁵	49.01 (32.04, 68.27)	63.98 (41.90, 99.31)	<0.001	0.003
NK Cell CD45RA MFI ⁵	27.73 (18.91, 39.81)	36.40 (23.31, 50.81)	<0.001	0.003
NK Cell CX3CR1 MFI ⁵	13.59 (9.70, 17.44)	16.50 (11.93, 24.81)	<0.001	0.003
NK Cell NKG2D MFI ⁵	3.55 (2.88, 4.44)	3.83 (3.03, 4.99)	0.093	0.17
NK Cell NKp30 MFI ⁵	4.61 (2.67, 7.79)	3.39 (2.28, 5.69)	0.200	0.333
NK Cell NKp46 MFI ⁵	8.21 (6.63, 10.58)	9.77 (6.71, 13.14)	0.032	0.079

¹Median (interquartile range)

²Calculated using linear mixed effect (LME) models

³Benjamini-Hochberg correction for multiple testing

⁴(10⁶ cells per ml of blood)

⁵(fold increase versus non-specific IgG control antibody)

N, number of participants

MFI, median fluorescence intensity

Supplementary Table S3. Demographics of ALS participants stratified by progression tertiles, related to Figure 2

Characteristic	Slow, N = 19 ¹	Medium, N = 18 ¹	Fast, N = 18 ¹	p-value ²
Rate of ALSFRS-R change	-0.33 (-0.47, -0.16)	-0.95 (-1.26, -0.85)	-1.90 (-2.43, -1.73)	<0.001
Sex				0.300
Female	7 (36.8%)	11 (61.1%)	10 (55.6%)	
Male	12 (63.2%)	7 (38.9%)	8 (44.4%)	
Age (years)	66.10 (58.90, 72.36)	58.40 (53.47, 70.37)	62.74 (60.28, 70.46)	0.382
Age at onset	64.53 (55.33, 69.70)	57.19 (51.27, 68.54)	61.91 (59.02, 69.35)	0.465
Number of measurements	8.00 (5.00, 11.00)	9.00 (7.00, 10.75)	5.00 (3.00, 9.75)	0.122
Ethnicity				NA
Not Hispanic or Latino	19 (100.0%)	18 (100.0%)	18 (100.0%)	
Race				>0.999
Asian	1 (5.3%)	0 (0.0%)	0 (0.0%)	
Black or African American	1 (5.3%)	0 (0.0%)	0 (0.0%)	
White or Caucasian	17 (89.5%)	18 (100.0%)	18 (100.0%)	
Family history of ALS	3 (15.8%)	4 (22.2%)	1 (5.9%)	0.451
Onset segment				0.023
Bulbar	2 (10.5%)	5 (27.8%)	10 (55.6%)	
Cervical	11 (57.9%)	5 (27.8%)	5 (27.8%)	
Lumbar	6 (31.6%)	8 (44.4%)	3 (16.7%)	
El Escorial initial diagnosis				0.229
Definite	5 (26.3%)	6 (33.3%)	9 (50.0%)	
Possible	6 (31.6%)	3 (16.7%)	0 (0.0%)	
Probable	4 (21.1%)	4 (22.2%)	3 (16.7%)	
Probable, lab supported	4 (21.1%)	5 (27.8%)	6 (33.3%)	
Riluzole use at study commencement	9 (47.4%)	7 (38.9%)	6 (33.3%)	0.680
Riluzole use at any point during study	12 (63.2%)	12 (66.7 %)	14 (77.8 %)	0.471
Force vital capacity	73% (68, 89)	76% (63, 92)	66% (51, 74)	0.110
ALSFRS-R at baseline	39.00 (31.50, 40.50)	36.00 (29.75, 38.00)	32.50 (29.50, 37.00)	0.286
Time from symptom onset to diagnosis (days)	435.00 (293.50, 864.50)	380.00 (263.75, 460.00)	331.00 (189.00, 503.25)	0.176
Time from diagnosis to 1st immune assessment (days)	178.00 (96.00, 249.00)	131.50 (56.75, 248.50)	99.00 (31.50, 217.00)	0.345

¹N (%); median (interquartile range)

²Pearson's Chi-squared test (Sex); Kruskal-Wallis rank sum test (Rate of ALSFRS-R change, Age, Age at onset, Number of measurements, Riluzole use, ALSFRS-R at baseline, Time from symptom onset to diagnosis, Time from symptom onset to 1st immune assessment); Fisher's exact test (Race, Ethnicity, Family history of ALS, Age Group)

N, number of participants

Supplementary Table S4. Initial immune marker values stratified by ALS progression tertiles, related to Figure 2

Characteristic	Slow, N = 19 ¹	Medium, N = 18 ¹	Fast, N = 18 ¹	p-value ²	q-value ³
Neutrophils ⁴	2.94 (2.28, 3.48)	2.95 (2.34, 3.67)	2.85 (2.39, 3.46)	0.996	0.996
Eosinophils ⁴	0.12 (0.08, 0.21)	0.07 (0.06, 0.12)	0.09 (0.05, 0.12)	0.075	0.706
CD16+ Monocytes ⁴	0.042 (0.026, 0.049)	0.036 (0.025, 0.050)	0.039 (0.022, 0.054)	0.915	0.977
CD16- Monocytes ⁴	0.24 (0.19, 0.32)	0.22 (0.17, 0.30)	0.23 (0.16, 0.30)	0.793	0.977
NK Cells ⁴	0.15 (0.11, 0.20)	0.12 (0.10, 0.16)	0.10 (0.08, 0.15)	0.289	0.706
CD4 T Cells ⁴	0.58 (0.44, 0.82)	0.67 (0.45, 0.79)	0.44 (0.38, 0.78)	0.466	0.977
CD8 T cells ⁴	0.18 (0.15, 0.40)	0.22 (0.14, 0.41)	0.18 (0.12, 0.25)	0.236	0.706
CD27+ NK Cells ⁴	0.004 (0.002, 0.005)	0.004 (0.002, 0.007)	0.003 (0.002, 0.005)	0.616	0.977
CD57+ NK Cells ⁴	0.078 (0.056, 0.111)	0.058 (0.031, 0.085)	0.049 (0.032, 0.080)	0.218	0.706
CD62L+ NK Cells ⁴	0.058 (0.036, 0.092)	0.045 (0.036, 0.071)	0.035 (0.026, 0.046)	0.061	0.706
CD94+ NK Cells ⁴	0.070 (0.053, 0.112)	0.061 (0.046, 0.085)	0.046 (0.038, 0.071)	0.238	0.706
CXCR3+ NK Cells ⁴	0.028 (0.008, 0.042)	0.029 (0.015, 0.044)	0.021 (0.011, 0.043)	0.872	0.977
KIR2DL1+ NK Cells ⁴	0.038 (0.020, 0.054)	0.031 (0.020, 0.054)	0.026 (0.020, 0.046)	0.897	0.977
KIR2DL2+ NK Cells ⁴	0.046 (0.038, 0.074)	0.041 (0.020, 0.045)	0.033 (0.026, 0.057)	0.261	0.706
NK Cell CD11a MFI ⁵	31.85 (26.30, 40.74)	29.73 (23.57, 37.78)	34.37 (21.68, 41.82)	0.933	0.977
NK Cell CD11b MFI ⁵	7.08 (3.91, 8.74)	5.03 (3.20, 8.11)	5.78 (4.27, 7.27)	0.641	0.977
NK Cell CD38 MFI ⁵	74.79 (54.72, 94.44)	59.10 (31.09, 96.96)	72.43 (40.73, 119.64)	0.576	0.977
NK Cell CD45RA MFI ⁵	34.39 (23.82, 55.80)	38.99 (14.16, 47.76)	23.76 (19.14, 46.40)	0.925	0.977
NK Cell CX3CR1 MFI ⁵	21.89 (15.52, 29.44)	20.61 (13.82, 24.62)	19.34 (13.72, 25.26)	0.704	0.977
NK Cell NKG2D MFI ⁵	3.55 (2.46, 4.68)	3.46 (2.62, 4.93)	4.03 (3.12, 4.13)	0.877	0.977
NK Cell NKp30 MFI ⁵	4.02 (3.07, 4.61)	2.62 (1.81, 3.92)	3.84 (2.87, 4.54)	0.145	0.706
NK Cell NKp46 MFI ⁵	8.83 (6.45, 10.89)	9.17 (7.86, 12.07)	10.91 (7.04, 13.89)	0.223	0.706

¹Median (interquartile range)

²Kruskal-Wallis rank sum test for all variables

³Benjamini-Hochberg correction for multiple testing

⁴(10⁶ cells per ml of blood)

⁵(fold increase versus non-specific IgG control antibody)

N, number of participants

MFI, median fluorescence intensity

Table S5. Differences in all immune markers stratified by ALS progression tertiles, related to Figure 2

Characteristic	Slow, N = 152 ¹	Medium, N = 153 ¹	Fast, N = 110 ¹	p-value ²	q-value ³
Neutrophils ⁴	2.91 (1.98, 3.54)	2.29 (1.66, 3.36)	2.71 (2.20, 3.53)	0.2	0.5
Eosinophils ⁴	0.14 (0.08, 0.22)	0.07 (0.04, 0.11)	0.07 (0.04, 0.11)	<0.001	0.011
CD16+ Monocytes ⁴	0.036 (0.026, 0.050)	0.035 (0.024, 0.046)	0.036 (0.025, 0.051)	>0.9	>0.9
CD16- Monocytes ⁴	0.25 (0.19, 0.32)	0.19 (0.13, 0.24)	0.23 (0.17, 0.30)	0.08	0.4
NK Cells ⁴	0.17 (0.12, 0.21)	0.09 (0.07, 0.13)	0.10 (0.08, 0.14)	0.11	0.4
CD4 T Cells ⁴	0.52 (0.37, 0.78)	0.49 (0.36, 0.66)	0.45 (0.32, 0.59)	0.5	0.7
CD8 T cells ⁴	0.17 (0.11, 0.28)	0.18 (0.12, 0.27)	0.17 (0.12, 0.23)	0.13	0.4
CD27+ NK Cells ⁴	0.004 (0.002, 0.007)	0.004 (0.002, 0.007)	0.003 (0.002, 0.005)	0.4	0.6
CD57+ NK Cells ⁴	0.083 (0.058, 0.110)	0.042 (0.023, 0.074)	0.054 (0.032, 0.074)	0.5	0.7
CD62L+ NK Cells ⁴	0.057 (0.041, 0.091)	0.036 (0.025, 0.052)	0.034 (0.022, 0.046)	<0.001	0.011
CD94+ NK Cells ⁴	0.094 (0.062, 0.129)	0.051 (0.036, 0.078)	0.055 (0.040, 0.079)	0.13	0.4
CXCR3+ NK Cells ⁴	0.031 (0.013, 0.047)	0.027 (0.016, 0.044)	0.023 (0.013, 0.040)	0.3	0.6
KIR2DL1+ NK Cells ⁴	0.042 (0.023, 0.067)	0.023 (0.016, 0.039)	0.028 (0.020, 0.042)	0.1	0.4
KIR2DL2+ NK Cells ⁴	0.055 (0.040, 0.080)	0.038 (0.023, 0.058)	0.038 (0.028, 0.055)	0.2	0.5
NK Cell CD11a MFI ⁵	28.73 (22.76, 37.42)	30.98 (21.50, 42.43)	26.38 (20.55, 38.58)	0.6	0.8
NK Cell CD11b MFI ⁵	5.69 (4.12, 8.32)	5.18 (3.85, 6.85)	5.98 (4.74, 7.08)	0.4	0.6
NK Cell CD38 MFI ⁵	62.81 (39.84, 90.77)	44.30 (28.14, 70.21)	52.08 (34.42, 82.88)	0.2	0.5
NK Cell CD45RA MFI ⁵	36.32 (24.65, 47.91)	34.82 (19.49, 53.10)	36.96 (25.76, 50.42)	0.8	0.9
NK Cell CX3CR1 MFI ⁵	15.92 (11.41, 22.61)	14.03 (10.66, 20.11)	16.15 (11.53, 22.71)	0.5	0.7
NK Cell NKG2D MFI ⁵	3.76 (2.85, 4.80)	3.82 (2.93, 5.36)	3.79 (3.12, 4.63)	0.8	0.9
NK Cell NKp30 MFI ⁵	3.19 (2.35, 5.07)	2.70 (1.82, 4.56)	4.79 (2.86, 7.72)	0.5	0.7
NK Cell NKp46 MFI ⁵	9.10 (6.83, 12.19)	8.66 (5.61, 13.88)	11.11 (7.81, 13.58)	0.8	0.9

¹Median (interquartile range), analysis utilizes all sample visits²Calculated using linear mixed effect (LME) models³Benjamini-Hochberg correction for multiple testing⁴(10⁶ cells per ml of blood)⁵(fold increase versus non-specific IgG control antibody)

N, number of participants

MFI, median fluorescence intensity