Supplementary Figure 1. NK cell depletion and CD49b+ NK cell levels in ALS mice. (A) A representative plot of NK cell NK1.1 and CD49b expression in control mice (Control), ALS mice receiving sham non-specific IgG control antibody (ALS IgG), and ALS mice receiving NK1.1-depleting antibody (Treatment group) is shown. (B) Quantification of peripheral NK1.1- CD49b+ NK cells in Control (black, n = 6), ALS IgG (red, n = 9), or Treatment (blue, n = 7) mice at end of life. Mean and SEM are displayed; ANOVA was used to assess significance.
Supplementary Figure 2. NK cell populations and surface marker expression in the peripheral blood of control and ALS study subjects. (A) Total NK cell numbers and the numbers of specific NK cell subpopulations was examined in control (n = 94) and ALS (n = 205) subjects using flow cytometry. (B) Expression levels (MFI) of NK cell-associated surface markers was analyzed using flow cytometry in control (n = 94) and ALS (n = 205) subjects. MFI for each marker was normalized to the MFI of a non-specific IgG control to generate a fold-increase in MFI. Data are presented in log2 for ease of interpretation. For ALS subjects who provided samples over multiple visits, data from all visits was averaged to generate a single value per subject. For both NK numbers and surface expression horizontal lines indicate mean and SEM. Control and ALS were compared using Mann-Whitney. *p < .05, **p < .01, ***p < .001, **** p < .0001
Supplementary Figure 3. NK cell depletion efficacy in C57BL/6 mice. C57BL/6 mice were treated for 4 and 8 weeks with NK1.1-specific depleting antibody and peripheral blood leukocyte expression of NK1.1+ and CD49b+ NK cells were assessed using flow cytometry. Non-specific control IgG expression is also shown. For each time point n = 3 mice; panels are representative for each time point.
Supplementary Figure 4. Gating strategy for leukocytes in peripheral blood in mice. Panels of fluorescently labeled antibodies were used to identify immune cell populations via flow cytometry. Following elimination of doublets, immune cells were identified using a combination of surface marker stains and forward scatter- and side-scatter profiles. CD45 was used to identify leukocyte populations and eliminate residual red blood cells. Neutrophils were then identified via side scatter profile and Ly6G expression. Forward scatter-low cells were used to identify other populations. After gating out CD3+ or CD19+ cells, monocyte populations were identified using CD11b and Ly6C. CD3+ cells were identified as either CD4 T cells or CD8 T cells. CD3- cells were examined for NK1.1 and CD49b expression to identify NK cells. A stain using control IgG was used to calculate and remove background counts.
Supplementary Figure 5. Gating strategy for leukocytes in spinal cord in mice. Panels of fluorescently labeled antibodies were used to identify immune cell populations via flow cytometry. Following elimination of doublets, immune cells were identified using a combination of surface marker stains and forward scatter- and side-scatter profiles. CD45 was used to identify leukocyte populations and eliminate residual red blood cells, astrocytes, and neuronal debris. Myeloid cells were identified by gating out CD3+ or CD19+ cells, monocyte populations were identified using CD11b and Ly6C. Neutrophils were then identified via side forward profile and Ly6G expression. Forward scatter-low cells were further analyzed for CD11b and CD45 expression to identify monocytes and microglia. Activated microglia were identified using CD11c. CD3+ cells were identified as either CD4 T cells or CD8 T cells. CD3- cells were examined for NK1.1 and CD49b expression to identify NK cells. A stain using control IgG was used to calculate and remove background counts.
Supplementary Figure 6. Gating strategy for NK cells in peripheral blood in human participants. Panels of fluorescently labeled antibodies were used to identify immune cell populations via flow cytometry. Following elimination of doublets, NK cells were identified as side scatter-low, CD3-. Within this population, the NK cell population was CD56-mid, CD16+. CD14 staining was also used to eliminate monocyte contamination.
| Marker         | Function/Expression Pattern                               | MFI | Subgroups | Ref.   | Antibody Catalogue # |
|---------------|-----------------------------------------------------------|-----|-----------|--------|----------------------|
| CCR4          | Trafficking; activation; subgroups                        | –   | –         | (1-3)  | 359407               |
| CD11a         | Cell adhesion; trafficking                               | +   | –         | (4)    | 301207               |
| CD11b         | Cell adhesion; trafficking; development                   | +   | –         | (4, 5) | 301323               |
| CD27          | Subgroups; inhibition receptor                            | -   | +         | (6, 7) | 124211               |
| CD38          | Activation receptor                                       | +   | –         | (8, 9) | 356603               |
| CD40L         | Cytotoxic activity                                        | –   | –         | (10)   | 310823               |
| CD45RA        | Activation/activation history; development                | +   | –         | (11, 12)| 304129              |
| CD57          | Maturation; NK cell subgroup                              | –   | +         | (13-15)| 322313               |
| CD62L         | Maturation; polyfunctionality; migration                  | –   | +         | (16)   | 304805               |
| CD69          | Activation                                                | –   | –         | (17, 18)| 310929              |
| CD94          | Inhibition receptor                                       | –   | +         | (18-20)| 305508               |
| CX3CR1        | Migration; adhesion                                       | +   | –         | (3, 21-23)| 341603          |
| CXCR3         | Migration; adhesion                                       | –   | +         | (24)   | 353715               |
| Fasl          | Cytotoxic activity                                        | –   | –         | (25)   | 306406               |
| KIR2D L1/S1/S3/S5 | Activation receptor                                      | –   | +         | (15, 26, 27)| 339505          |
| KIR2D L2/L3   | Activation receptor                                       | –   | +         | (15, 26, 27)| 312611          |
| NKG2D         | Cytotoxic activity                                        | +   | –         | (5, 28-30)| 320821          |
| Nkp30         | Activation receptor                                       | +   | –         | (31-33)| 325209               |
| Nkp44         | Activation receptor                                       | –   | –         | (5, 34) | 325107               |
| Nkp46         | Activation receptor                                       | +   | –         | (5, 33) | 331913               |
| TRAIL         | Cytotoxic activity                                        | –   | –         | (25)   | 308209               |
| APC IgG       | APC negative control                                      | N/A | N/A       | 400120 |                      |
| BV421 IgG     | Brilliant Violet 421 negative control                     | N/A | N/A       | 306721 |                      |
| PE IgG        | PE negative control                                       | N/A | N/A       | 400112 |                      |

N/A = not applicable
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