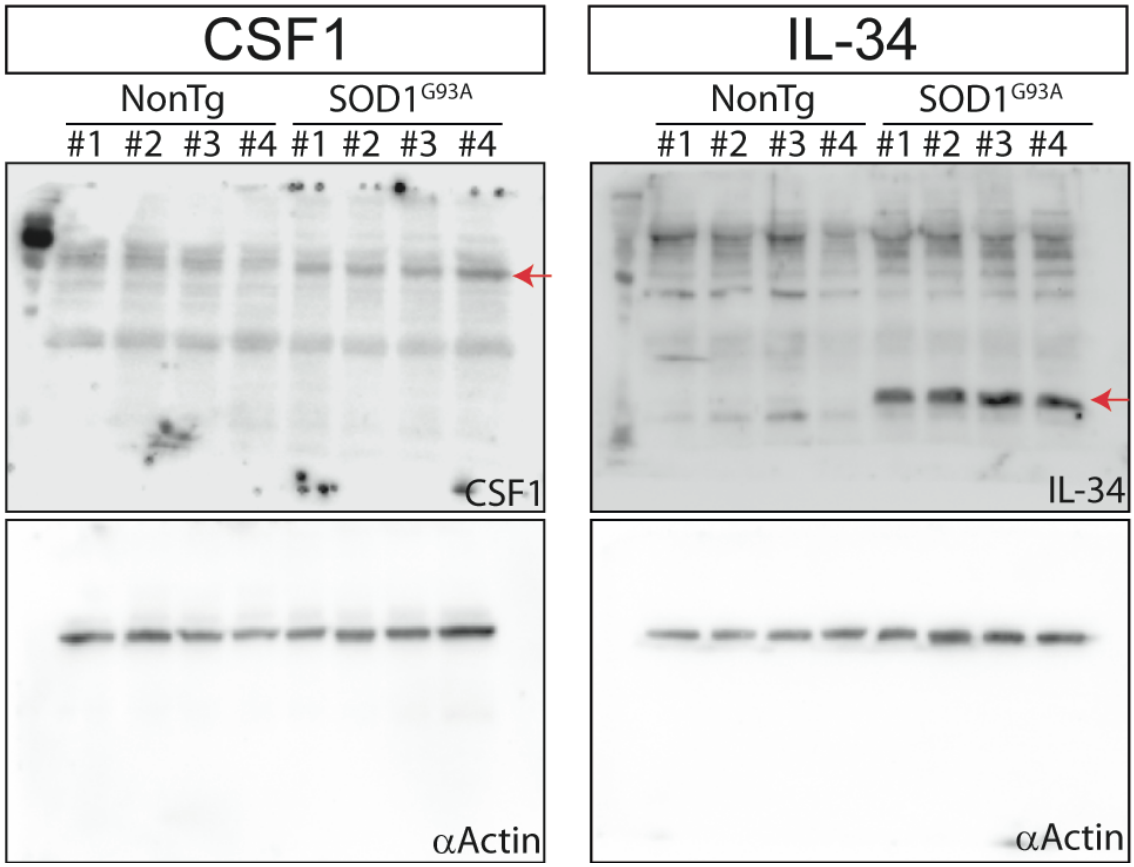
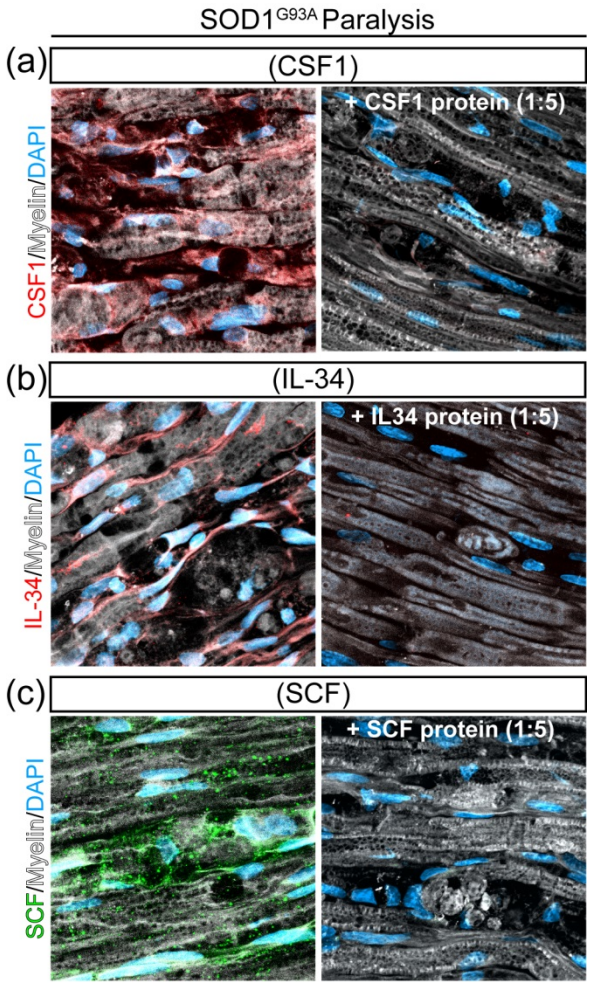
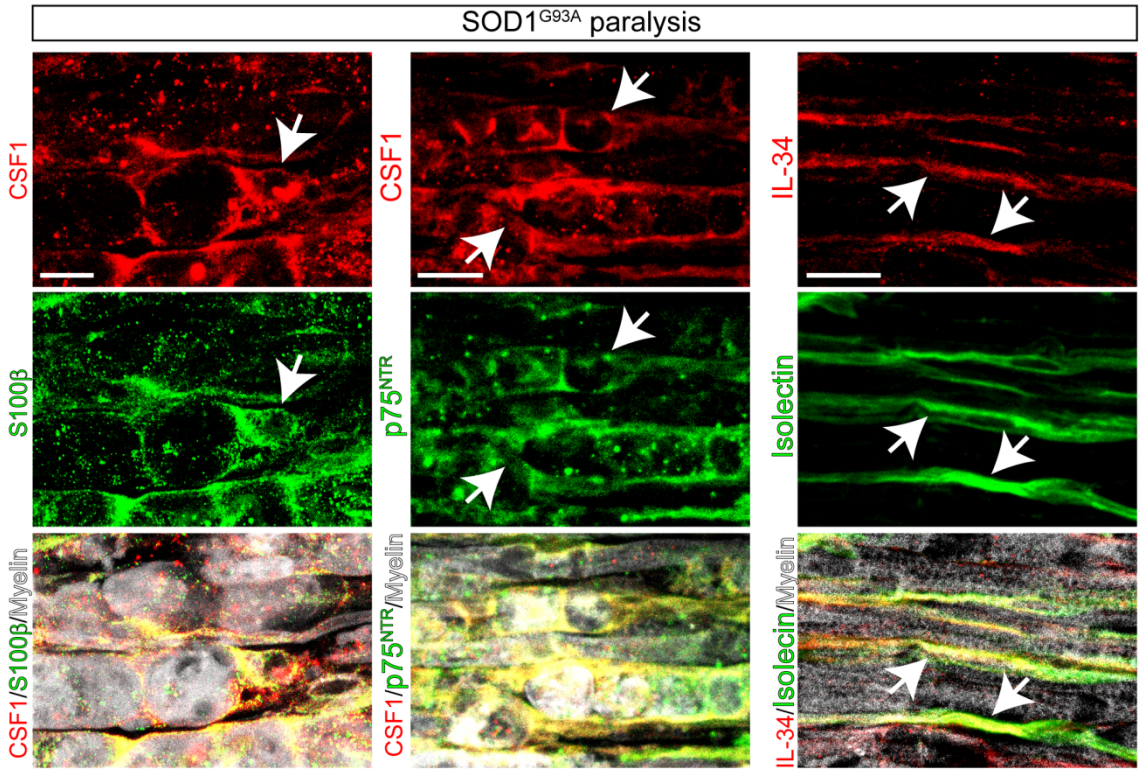
Supplementary Figures



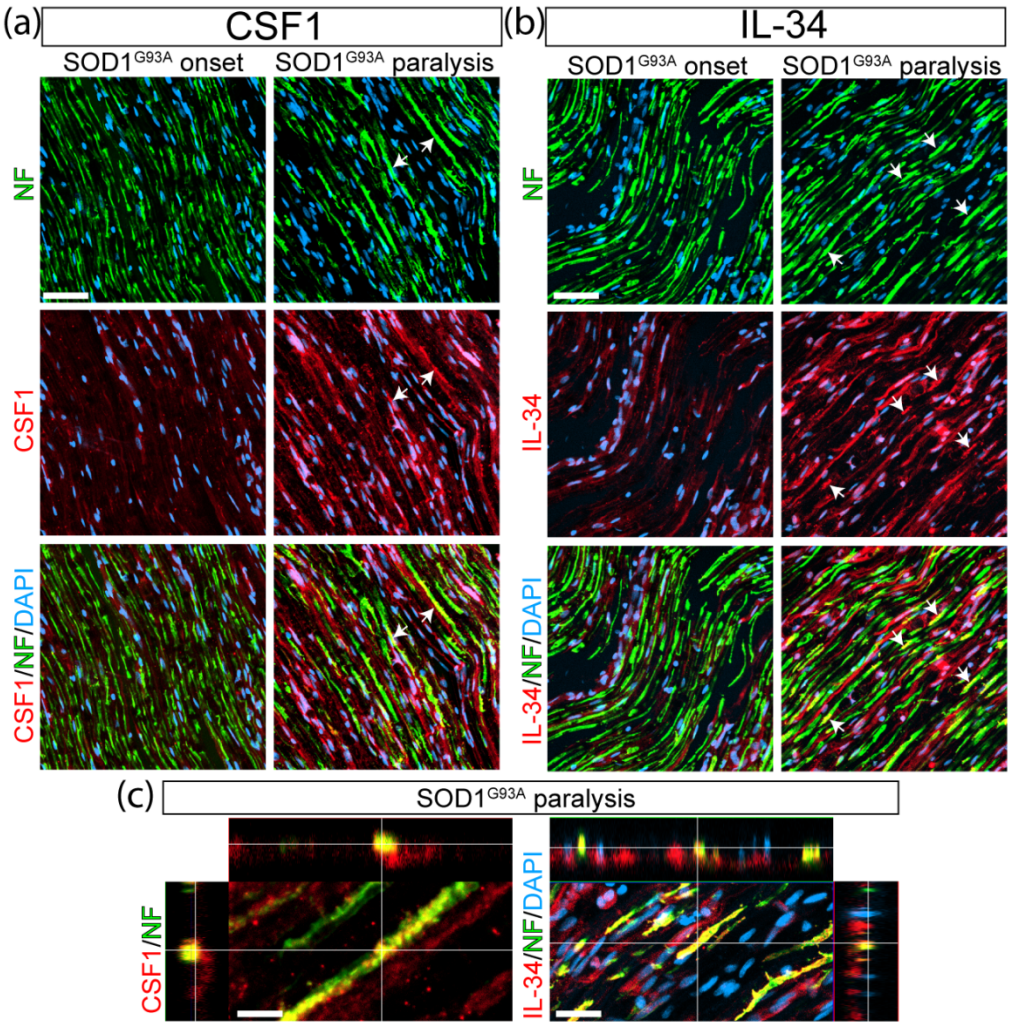
Supplementary Figure 1. Western blot analysis of CSF1 and IL-34 levels in the proximal sciatic nerve of non-transgenic and symptomatic SOD1^{G93A} rats. Upper panels show original western blot membranes where CSF1 and IL-34 were analyzed using specific antibodies (CSF 1:500 dilution; IL-34 1:500 dilution). Red arrows indicate the bands that were used for quantitive analysis, according to manufacturer instructions. In lower panels, primary antibodies were stripped and membranes were reincubated with anti-αActin antibody (1:4000) as housekeeping protein for loading control. n= 4 animals/group.



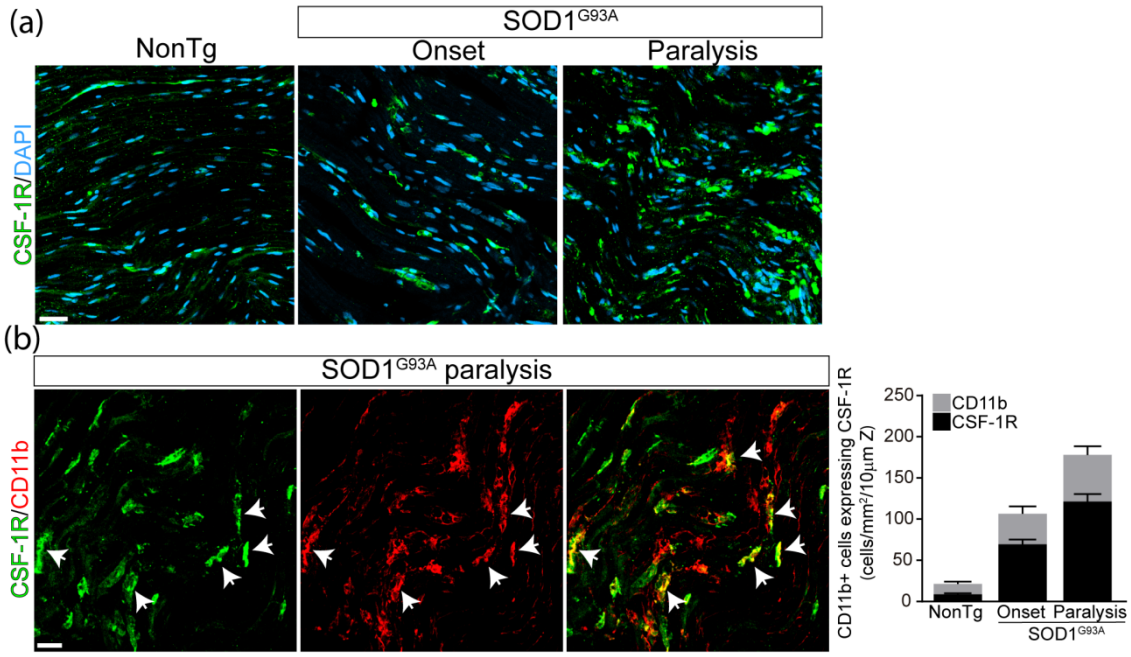
Supplementary Figure 2. Analysis of antibodies (CSF1, IL-34 and SCF) sepecificity using blocking peptides. Images show confocal immunohistochemistry using CSF1, IL-34 and SCF antibodies in longitudinal sections of proximal sciatic nerves from symptomatic SOD1G^{93A} rats **(a-c)** Representative confocal images showing immunohistochemistry analysis of the indicated antibodies before (left panels) and after (right panels) primary antibody preincubation with the respective proteins. Competition of each primary antibodies with their respective ligand (ratio= 1:5) completely abrogated the immunohistological staining. Scale bars: 10 μm in all panels.



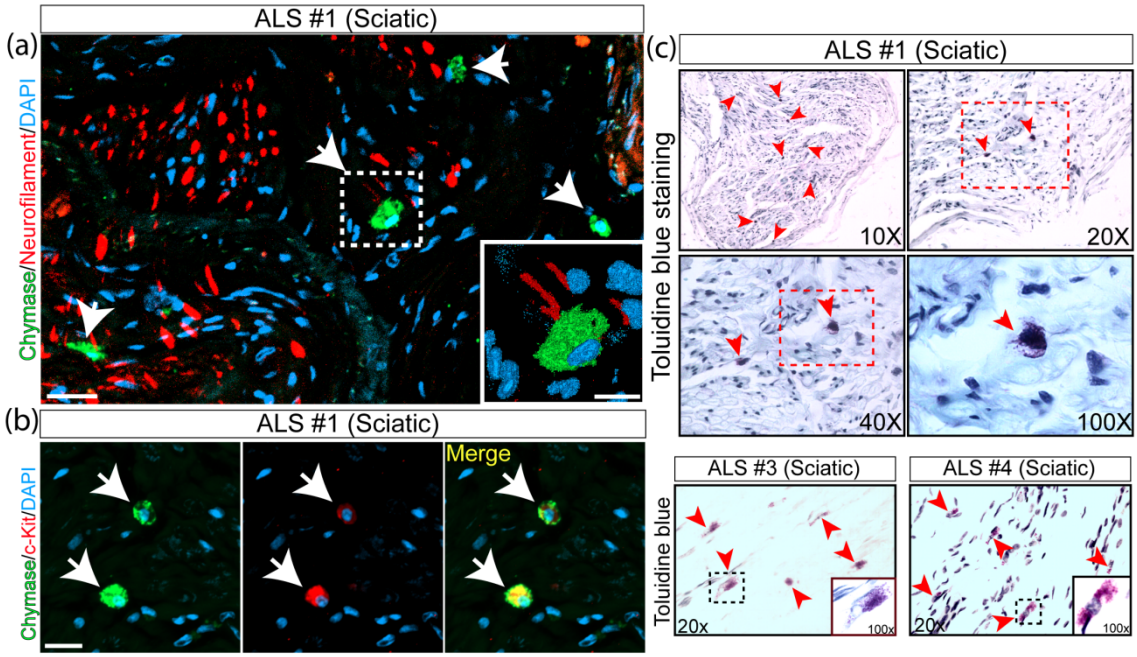
Supplementary Figure 3. Phenotypic characterization of SCs expressing CSF1 in SOD1^{G93A} proximal sciatic nerve. Immunohistochemical analysis of CSF1 (red, left and midle panel) and IL-34 (red, right panel) expression in proximal sciatic nerve longitudinal sections co-stained with SCs markers S100β (green), p75^{NTR} (green) and Isolectin (green). Note that CSF1 was clearly expressed in a subset of S100β+ (lef panels) and p75^{NTR}+ (right panels) SCs bearing phagocytic morphology (white arrows), while IL-34 was expressed by denervated SCs stained with Isolectin (right panel, white arrows). Scale bars: 20 μm.



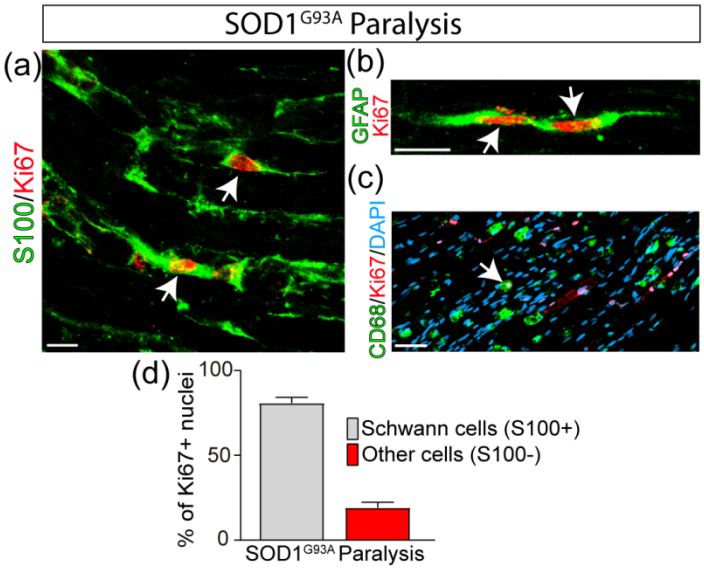
Supplementary Figure 4. A subset of axons express CSF1 and IL-34 in the sciatic nerve of symptomatic SOD11^{G93A} rats. **(a, b)** Longitudinal section of sciatic nerve showing the colocalization analysis between neurofilament (NF-200 heavy chain) with CSF1 or IL-34. Note CSF1 (left panels) and IL-34 (right panels) colocalize with a subset of NF-200+ axons (green) (white arrows). **(c)** Higher magnification images showing the orthogonal view of the colocalization. Scale bars: 50 μm in (a); 20 μm in (c).



Supplementary Figure 5. Accumulation of CSF-1R+ myeloid cells into the sciatic nerve of SOD1^{G93A} rats. (a) Representative confocal images showing the comparative infiltration of CSF-1R+ cells into the degenerating sciatic nerve among conditions. Note the significant increase of CSF-1R+ cells in rats developing overt paralysis. **(b)** Note that CSF-1R+ cells mostly correspond to myeloid cells expressing CD11b (white arrows). The graph to the right shows the quantitative analysis of CD11b+ myeloid cells expressing CSF-1R. Scale bars: 20 μm in (a) and (b).



Supplementary Figure 6. Accumulation of c-Kit+ mast cells into the sciatic nerve of ALS patients. **(a)** Representative confocal images showing Chymase+ mast cells infiltrating the degenerating sciatic nerve of an ALS patient (white arrows). The inset shows the interaction of mast cells (green for Chymase) with neurofilaments (red, NF-200). **(b)** High magnification images showing that Chymase+ mast cells (green) express c-Kit (red, white arrows). **(c)** Sections of three ALS sciatic nerves stained for toluidine blue, showing accumulation of mast cells displaying metachromasia (red arrows). Scale bars: 20 μm in (a) and (b).



Supplementary Figure 7. Analysis of cell proliferation in the degenerating sciatic nerve of symptomatic SOD1^{G93A} rats. Images show confocal immunohistochemical analysis of Ki67, SCs and infiltrating macrophages in longitudinal sections of proximal sciatic nerve during the symptomatic phase of SOD1^{G93A} rats. (a) Representantive confocal image showing S100+ SCs (green, white arrows) expressing Ki67 nuclei (red). (b) Representative image of GFAP+ small SCs expressing Ki67 (red, white arrows). (c) Confocal tile reconstruction showing CD68+ macrophages (green) and Ki67 expression (red). Note that most Ki67+ nuclei are not localized in infiltrating CD68+ cells. White arrow denote one small monocyte/macrophage expressing Ki67. (d) Quantitative analysis shows that most Ki67+ nuclei belongs to S100+ SCs (80%, grey bar), while 20% of the Ki67+ nuclei were localized in cells devoid of S100 staining (red bar).