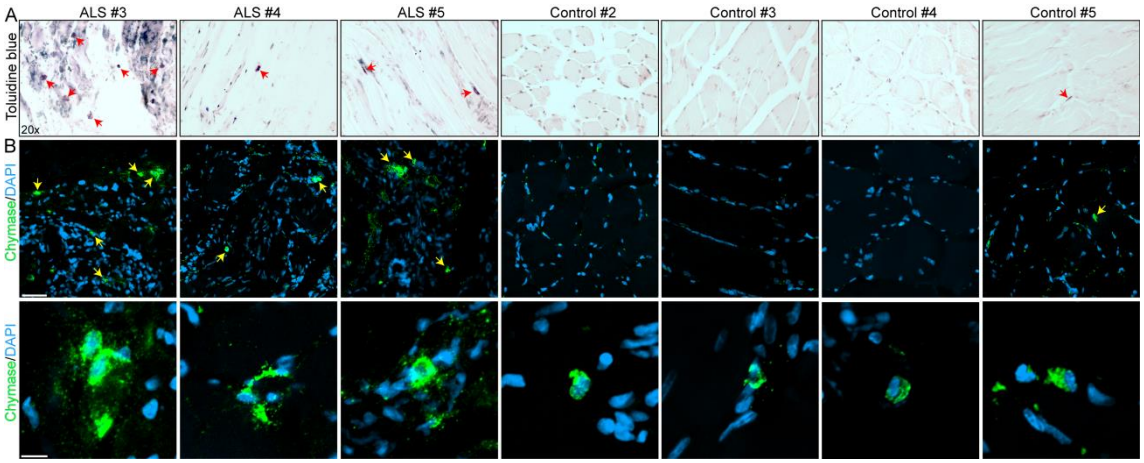
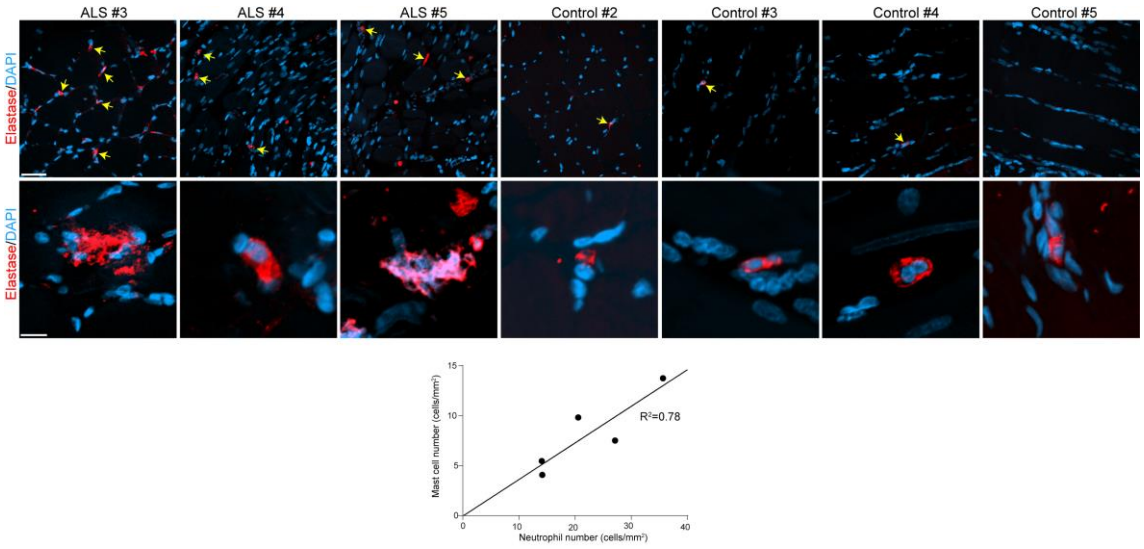
Supplementary material

Sup. Fig. 1



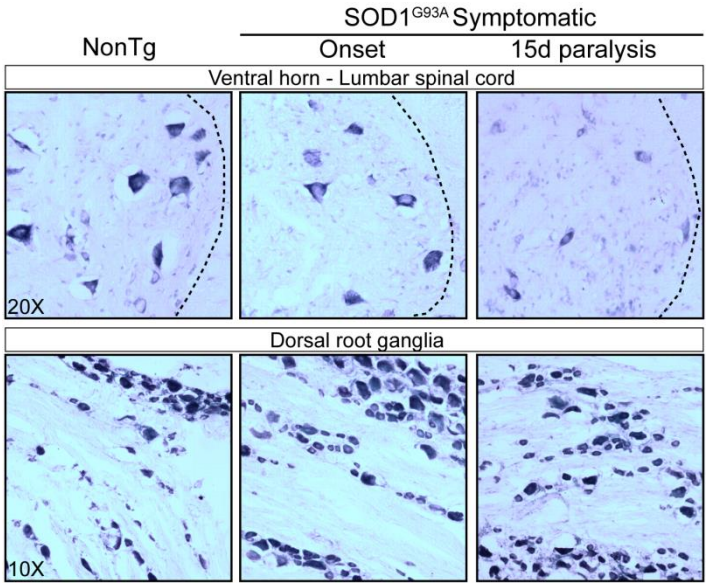
Supplementary Figure 1. Mast cells infiltrate and degranulate into skeletal muscle of ALS patients. **A)** Representative low magnification microphotographs of toluidine blue staining showing the infiltration of mast cells into quadriceps muscles of three ALS patients compared with four controls. Few mast cells are observed and mostly associated to blood vessels in control patients. **B)** Representative confocal images showing chymase-positive mast cells (green – arrows) infiltrating quadriceps muscles from three ALS and four control donors. It can be observed a significant infiltration of mast cells in ALS patients when compared to controls, where few smaller chymase-positive mast cells are observed associated mostly to blood vessels. Lower panels show high magnification pictures where degranulating chymase-positive mast cells are observed only in ALS patients. Scale bars: 50 μm in upper panels and 10 μm in lower panels.

Sup. Fig. 2



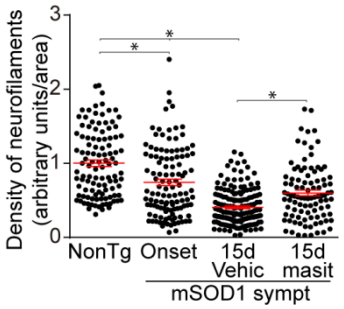
Supplementary Figure 2. Neutrophils infiltrate into the degenerating skeletal muscle of ALS patients. Representative confocal images showing the infiltration of elastase-positive (red) neutrophils into three quadriceps post-mortem samples from ALS patients and four control donor (upper panels). High magnification in panels of the lower row show how neutrophils from ALS patients form aggregate resembling neutrophil extracellular traps. Neutrophils from control donors were seen associated to blood vessels and constitute small single cells which do not aggregate. Scale bars: 50 μm in low magnification panels, 10 μm in high magnification panels. The graph below shows the correlation between mast cells and neutrophil number in ALS patients (R^2=0.78).

Sup. Fig. 3



Supplementary Figure 3. Lack of metachromatic mast cells in lumbar spinal cord and dorsal root ganglia. Representative images of the ventral horn of the spinal cord and DRG from NonTg and SOD1 onset and 15d paralysis rats. Toluidine blue staining was used to analyze metachromatic mast cell infiltration. No metachromatic cells were observed in the spinal cord. None or few mast cells associated to blood vessels were evidenced into DRG, but no differences were observed among groups.

Sup. Fig. 4



Supplementary Figure 4. Post-paralysis treatment with masitinib prevents axonal degeneration in sciatic nerve of SOD1^{G93A} rats. Density of axon (neurofilament) per area in longitudinal sciatic nerve cryostat sections was analyzed and quantify. Note how masitinib treatment significantly prevents axonal degeneration 15 days after onset. Data are expressed as mean ± SEM; data were analyzed by Kruskal-Wallis followed by Dunn's multiple comparison test, $p < 0.05$ was considered statistical significant. n=4 animals/condition

Sup. Table 1

Mast cells/mm^2	ALS #1 (13.7±1.3)	ALS #2 (9.8±1.0)	ALS #3 (7.5±1.1)	ALS #4 (4.1±0.5)	ALS #5 (5.5±0.8)
Control #1 (0.78±0.37)	∗ (p<0.0001)	∗ (p<0.0001)	∗ (p=0.0003)	ns (p=0.16)	∗ (p=0.025)
Control #2 (2.5±0.58)	∗ (p<0.0001)	∗ (p<0.0001)	∗ (p=0.036)	ns (p>0.99)	ns (p>0.99)
Control #3 (0.39±0.22)	∗ (p<0.0001)	∗ (p<0.0001)	∗ (p<0.0001)	∗ (p=0.008)	∗ (p=0.0008)
Control #4 (1.6±0.5)	∗ (p<0.0001)	∗ (p<0.0001)	∗ (p=0.0038)	ns (p=0.98)	ns (p=0.17)
Control #5 (3.5±0.7)	∗ (p<0.0001)	∗ (p=0.0005)	ns (p=0.910)	ns (p>0.99)	ns (p>0.99)

Supplementary Table 1. Statistical multivariate analysis of mast cell density among patients and controls. Toluidine-positive mast cells were counted as described in methods. Data are expressed as mean ± SEM; data were analyzed by Kruskal-Wallis followed by Dunn's multiple comparison test, $p < 0.05$ was considered statistically significant.

Sup. Table 2.

Neutrophils/mm^2	ALS #1 (35.6±5.2)	ALS #2 (20.6±2.4)	ALS #3 (27.1±2.8)	ALS #4 (14.2±1.9)	ALS #5 (14.1±2.2)
Control #1 (7.3±1.3)	✱ (p=0.0008)	✱ (p<0.0001)	✱ (p=0.0031)	ns (p=0.55)	ns (p>0.99)
Control #2 (9.9±1.7)	✱ (p=0.017)	✱ (p<0.0001)	ns (p=0.0568)	ns (p=0.998)	ns (p>0.99)
Control #3 (7.4±1.7)	✱ (p<0.0001)	✱ (p<0.0001)	✱ (p=0.0004)	ns (p=0.15)	ns (p=0.59)
Control #4 (5.2±1.1)	✱ (p<0.0001)	✱ (p<0.0001)	✱ (p=0.0001)	⁎ (p=0.0068)	⁎ (p=0.049)
Control #5 (3.4±0.9)	✱ (p<0.0001)	✱ (p<0.0001)	✱ (p=0.0001)	⁎ (p=0.0008)	⁎ (p=0.0078)

Supplementary table 2. Statistical multivariate analysis of neutrophil density among patients and controls. Elastase-positive cells were counted as described in methods Data are expressed as mean ± SEM; data were analyzed by Kruskal-Wallis followed by Dunn's multiple comparison test, $p < 0.05$ was considered statistically significant.