Perivascular fibroblasts activity precedes the onset of ALS neurodegeneration with high plasma SPP1 associated with short patient survival.

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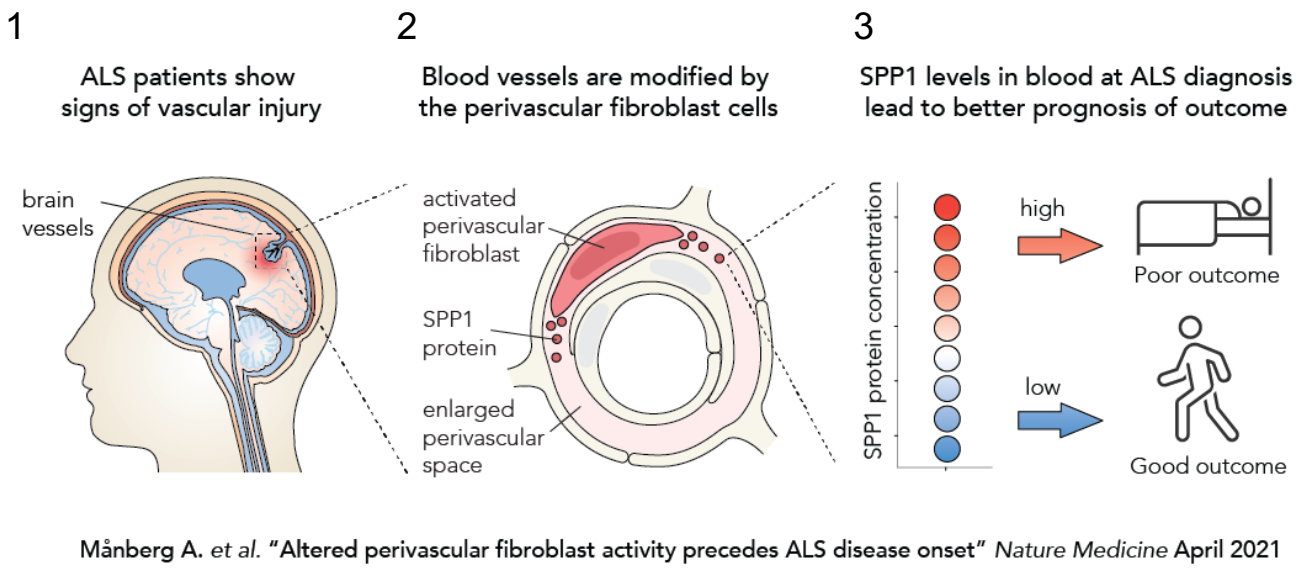

Project Summary

1. Perivascular fibroblasts (PVF) cells become active in early stages of ALS neurodegeneration (see Figure. 1).

2. Perivascular fibroblasts are located within vascular basement membranes, and secrete the SPP1 protein within the enlarged perivascular spaces (see Figure. 2).

3. Increase of fibroblast-specific protein SPP1 in ALS patient plasma can be a marker of short disease survival (see Figure. 3).

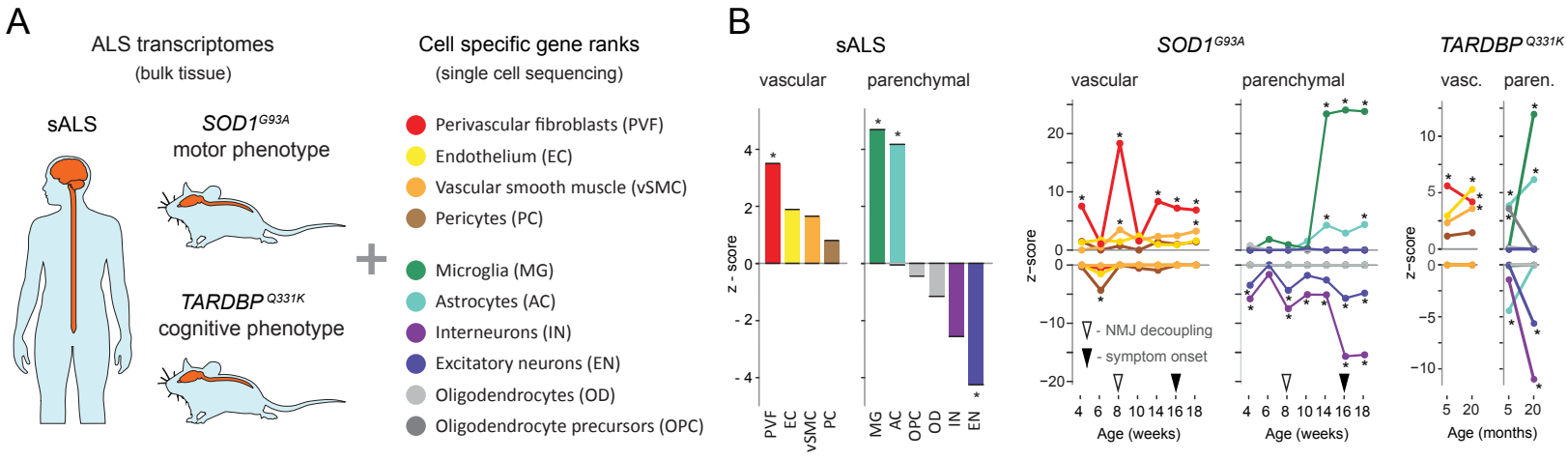

Figure 1. ALS patients and mouse models show early activity of perivascular fibroblast cell genes
(**A**) Schematic of expression weighted cell type enrichment (EWCE) analysis. Bulk tissue gene expression is interpreted with single cell seq data to infer cell type activity.
(**B**) Enrichment z - scores for ten major cell types within CNS in sALS patients, *SOD1*^{G93A} and *TARDBP*^{Q331K} mice.
(**C**) Perivascular fibroblasts have distinct mRNA expression markers.
(**D**) Schematic illustration represents reported PVF location between astrocytes and mural/endothelial cells.
(**E**) TEM of mouse spinal cord tissue points to location of perivascular fibroblasts cells (PVF - shaded red) between basement membrane layers from astrocyte endfeet (AC) and mural cells (MC), endothelial cell (EC), aBM - astrocyte basement membrane, mBM - mural basement membrane). Scale bar 2µm.

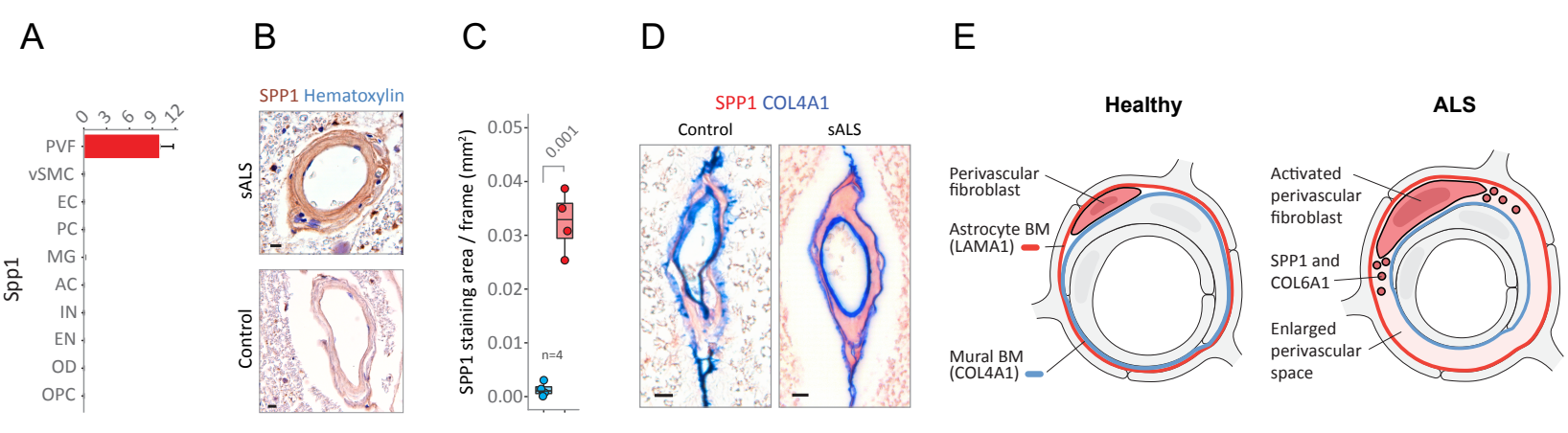

Figure 2. Perivascular fibroblast protein SPP1 accumulates during ALS progression. (**A**) Spp1 mRNA specificity within CNS cell types.
(**B**) SPP1 histochemistry in sALS spinal cords, bar: 10µm. (**C**) Quantifications of SPP1 histochemistry. sALS and Ctrl n=4. (**D**) SPP1 accumulate within increased perivascular spaces (outlined with COL4A1) in spinal cords of sALS patients, bar: 10µm. (**E**) Schematic graphic of perivascular fibroblast activity in ALS.

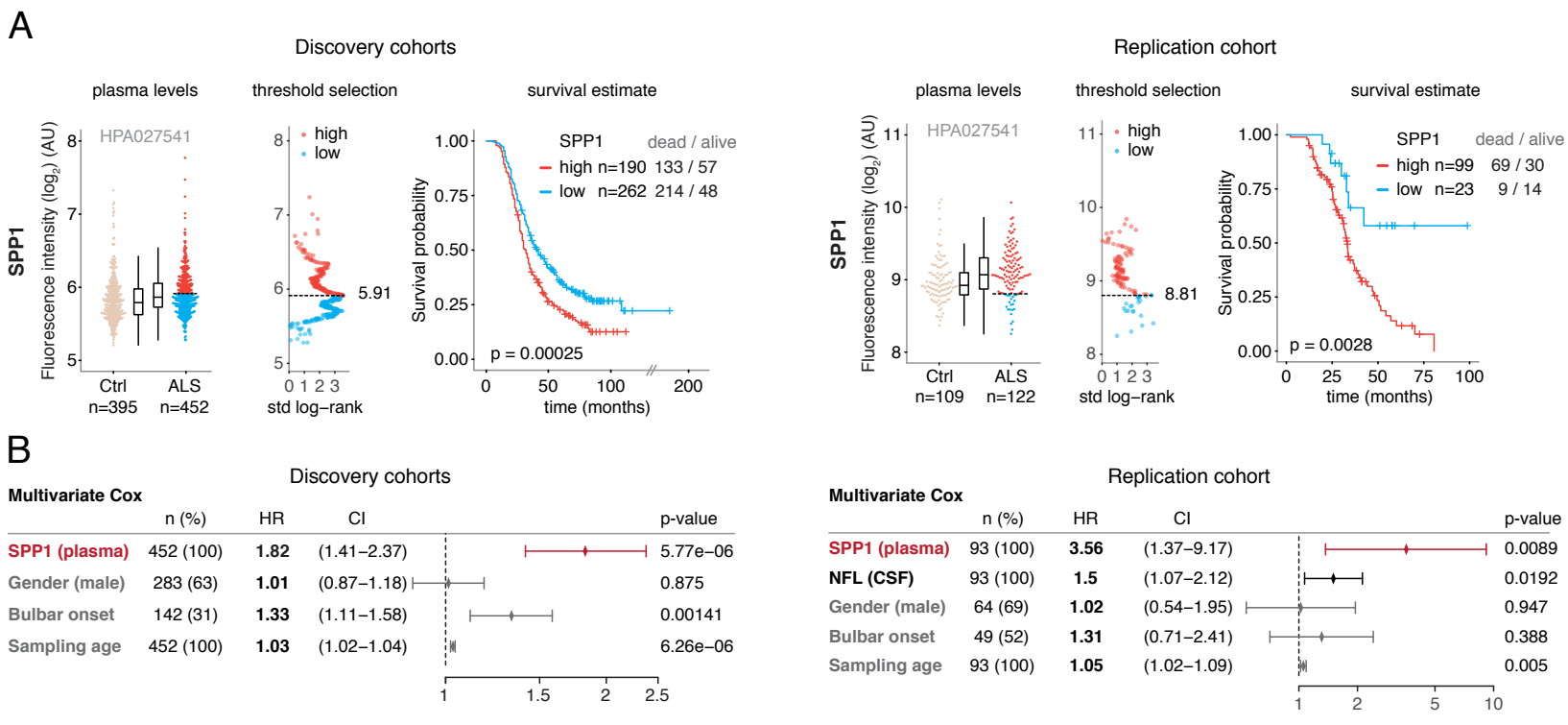

Figure 3. Prognostic value of SPP1 protein in plasma of ALS patients. (**A**) Relative levels of SPP1 protein in plasma as measured by the HPA027541 antibody. Threshold selection and Kaplan-Meier survival estimates of ALS patients in discovery cohorts (Netherlands, Germany and Belgium, n=452) and in the replication cohort (Sweden, n=122). Red color indicates thresholded protein level. Thresholds are established using maximally selected log rank statistics. Survival probability graphs show Kaplan-Meier logrank p-values. (**B**) Multivariate Cox proportional hazard models for continuous increase of plasma SPP1 relative to hazard ratios indicated by bulbar onset type, neurofilament light (NFL) in CSF (in the replication cohort), gender and plasma sampling age.

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