

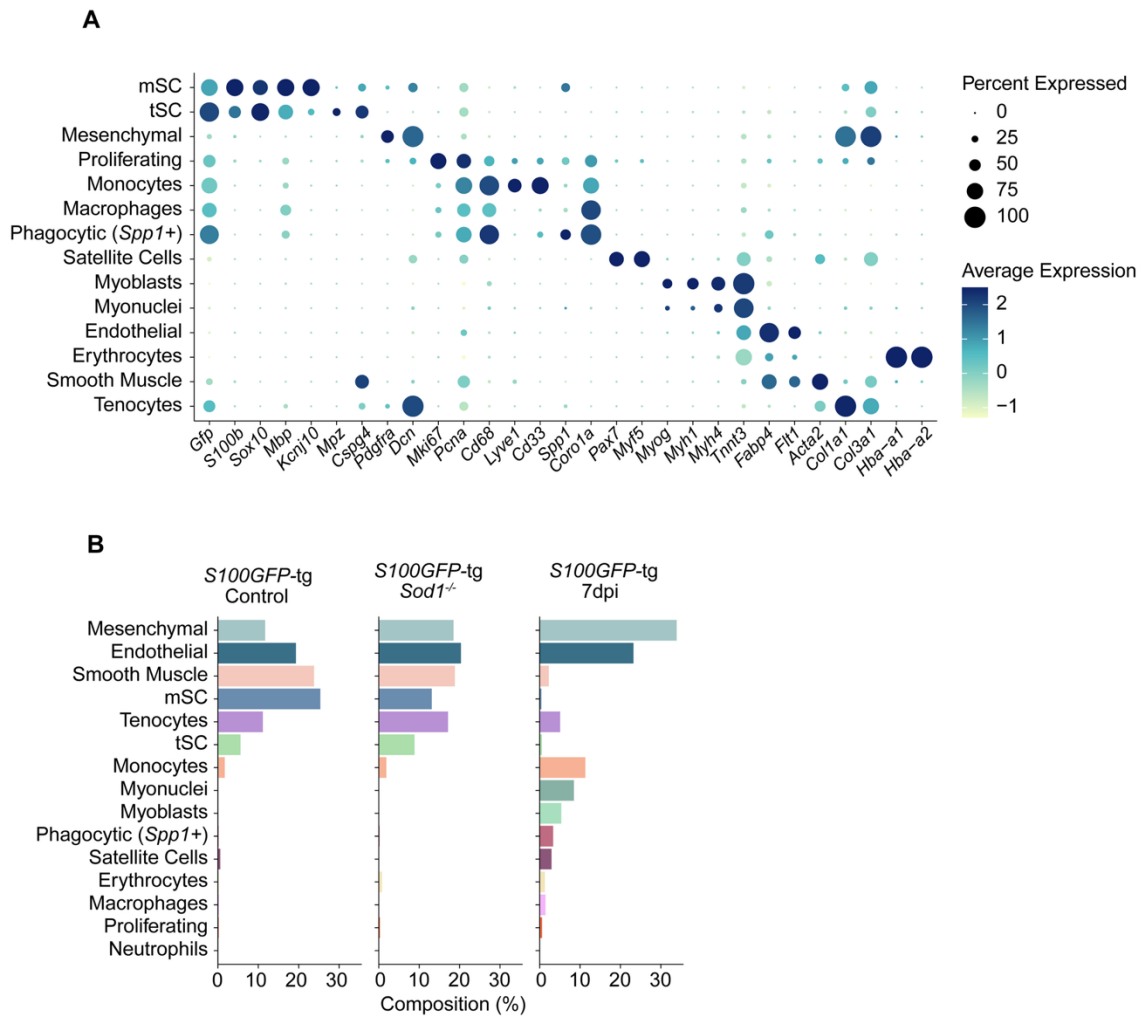


fig. S1. scRNA-Seq annotations and cell composition. (A) Dotplot showing the expression pattern of canonical markers, including *Gfp*, for each identified cell type cluster in the integrated dataset. (B) Bar plots showing the percentage (%) of each cell type cluster across all three groups.

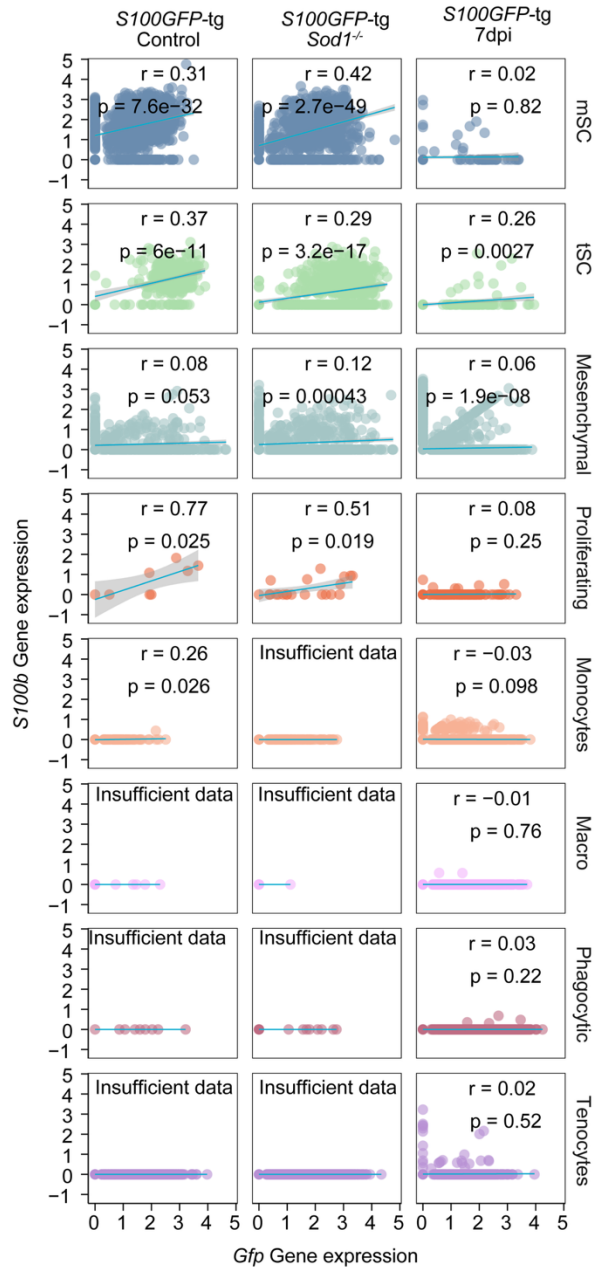


fig. S2. Correlation between *Gfp* and *S100b* expression across diverse cell types. Linear regression analysis of *Gfp* versus *S100b* expression in individual cells across various cell types within the integrated dataset that exhibited *Gfp* gene expression. Each subplot illustrates the regression line and includes the Pearson correlation coefficient, annotated where sufficient data points were available to perform the analysis.

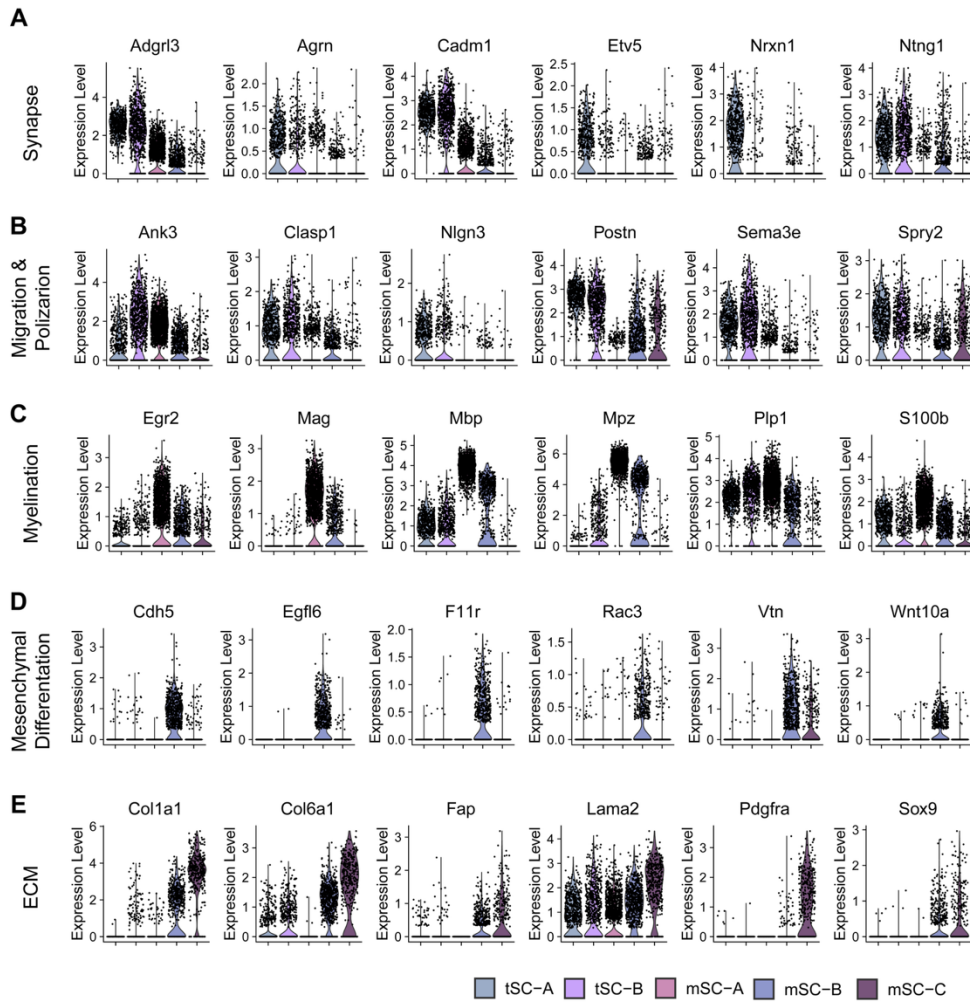


fig. S3. Differential expression of upregulated genes across pathway analysis categories in muscle-resident Schwann cells. Violin plots illustrate the expression patterns of enriched markers within the broad categories identified in Fig. 2—Synapse, Migration & Polarization, Myelination, Mesenchymal Differentiation, and ECM—across five muscle-resident Schwann cell subclusters.

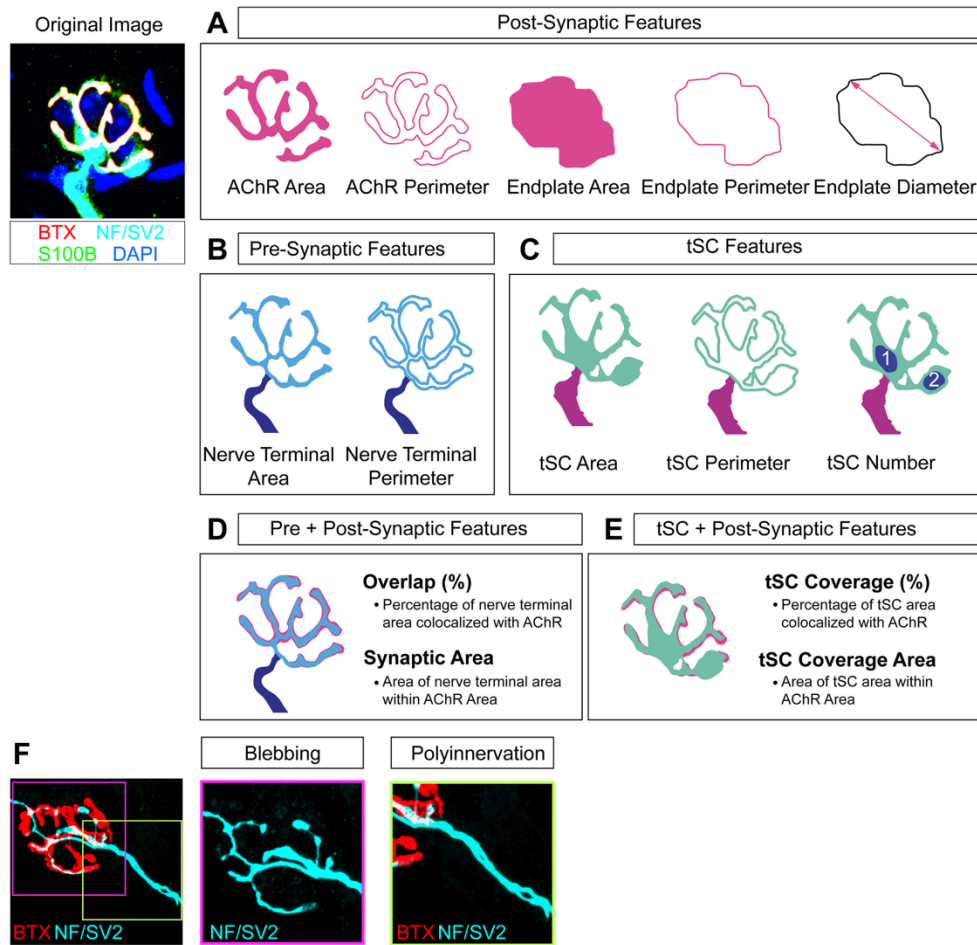


fig. S4. NMJ features analyzed. Binary masks were generated from the original image, and 16 en face NMJ morphological features were measured that represented the (A), post-synaptic (AChR and Endplate), (B), pre-synaptic (nerve terminal, axonal blebbing, polyinnervation), and (C), tSC structures. These pixel measurements are visualized by distinct colorations in each mask: red for post-synaptic features, light blue for pre-synaptic features, and green for tSC structures. (D), In addition, the percentage “Overlap” between axon terminal and AChR areas were measured. This overlap was further quantified in terms of synaptic area (μm^2), denoting the area where the nerve terminal co-localizes with the AChR region. (E), Similarly, the “tSC coverage” was determined representing the tSC area that overlaps with the AChR region, and measured the “tSC Coverage Area,” which is the expanse of tSC found within the AChR area. (F), The number of axon terminal blebs and the presence of polyinnervation were also assessed for each NMJ.

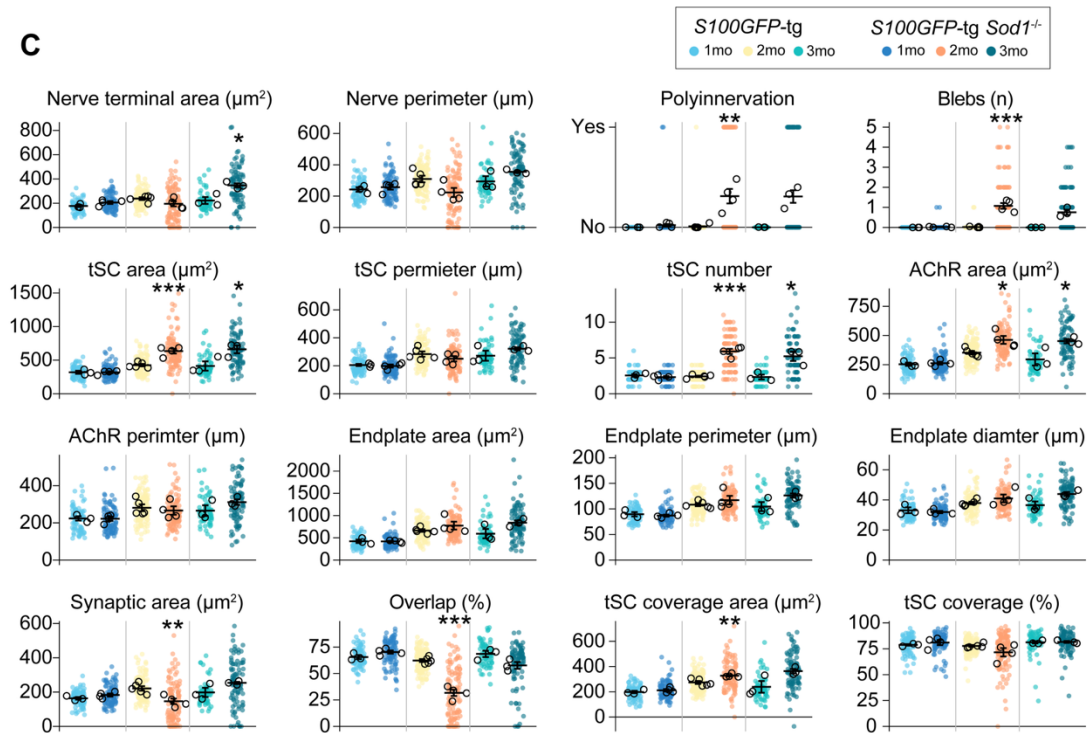
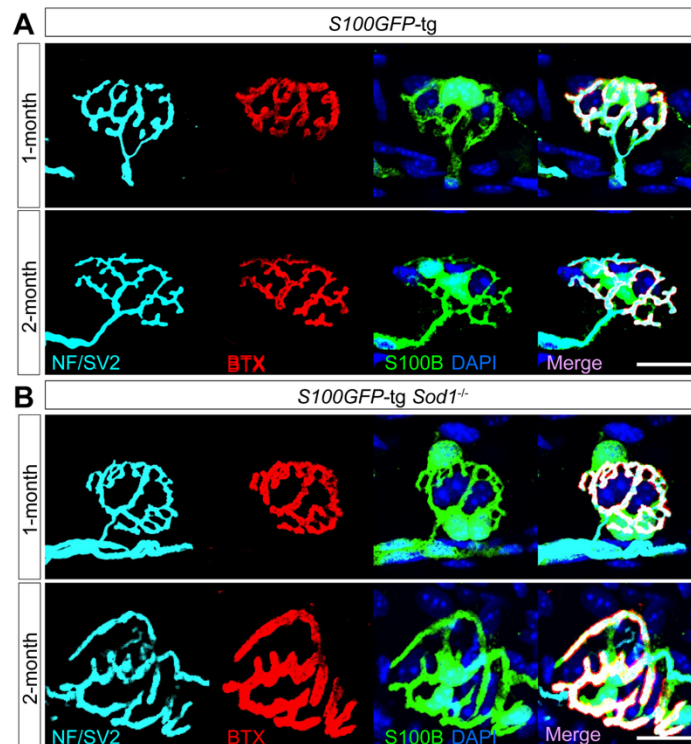


fig. S5. | Analysis of NMJ in *S100GFP-tg* and *S100GFP-tg Sod1^{-/-}* mice. (A and B) Images of representative NMJs from 1- and 3-month-old mice, stained for S100B (indicating Schwann cells in Green), NF/SV2 (Nerve in Cyan), bungarotoxin (AChR in Red), and DAPI (Nucleus in Blue). (A), Shows NMJs from *S100GFP-tg* mice, while (B) are NMJs from *S100GFP-tg Sod1^{-/-}* mice. (C), Graphical representation of NMJ features in *S100GFP-tg* and *S100GFP-tg Sod1^{-/-}* mice aged 1, 2, and 3 months. Each colored dot represents a single NMJ. Black circles denote the average for all NMJs in an individual animal. The horizontal bars show the overall mean for each group, while error bars illustrate the mean $\pm$ SEM. *Denotes $p < 0.05$ *S100GFP-tg* vs *S100GFP-tg Sod1^{-/-}* by two tailed unpaired t-test. Scale bar equals 25 μ m and images constrained to the same dimensions. C, N = 3-5/group.

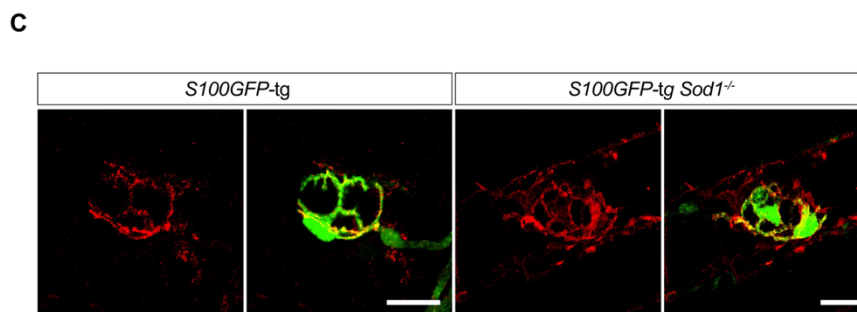
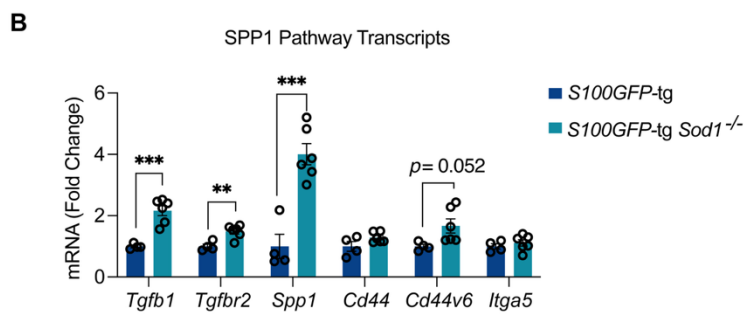
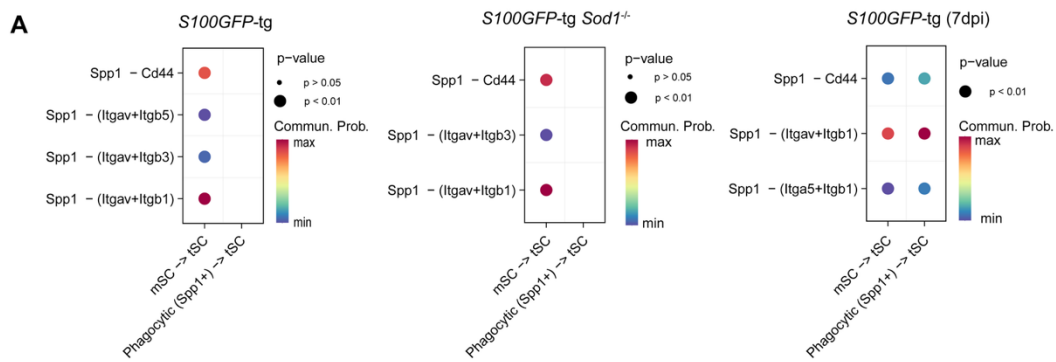
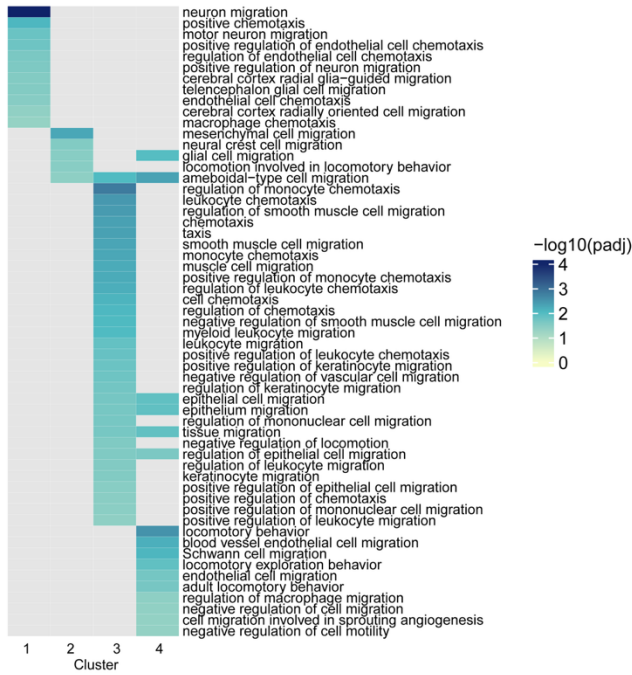


fig S6. SPP1 signaling across different innervation states and confirmation of enhanced SPP1 gene expression and protein staining in *Sod1*^{-/-} mice muscles. (A) Dotplot visualizing signaling of the Spp1 ligand to Cd44 and integrin receptors, denoting interactions between mSCs and tSCs in both *S100GFP*-tg and *S100GFP*-tg *Sod1*^{-/-}, *S100GFP*-tg (7 dpi) mice. (B) mRNA levels determined by qPCR for several presumed components of the Spp1 signaling pathway for muscles of *S100GFP*-tg (n = 4) and *S100GFP*-tg *Sod1*^{-/-} (n = 6) mice. (C) Representative immunofluorescent images of NMJs stained for CD44 (Red) and S100B (Green). Open circles indicate values for individual mice and bars represent the mean across animals $\pm$ SEM. Scale bars represent 20 μ m. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, by two tailed unpaired t-test (*S100GFP*-tg vs *S100GFP*-tg *Sod1*^{-/-}).

A

Significant Cell Migration Associated Terms



B

Significant Cell Proliferation Associated Terms

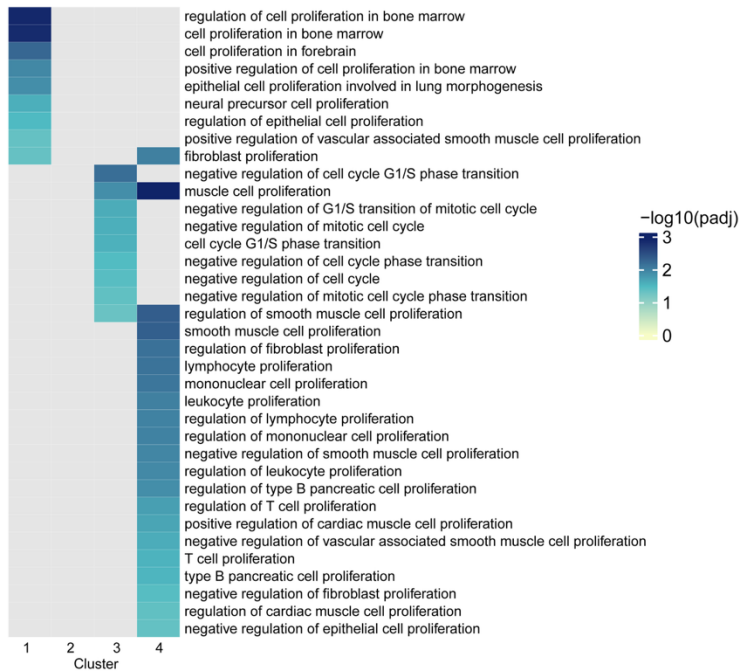


fig S7. SPP1 neutralization selectively impacts migration and proliferation programs in tSC clusters. (A) Heatmap of migration/motility-associated GO:Biological Process terms enriched within tSC Clusters 1–4. For each cluster, differentially expressed genes from IgG vs. SPP1-nAb were subjected to GO:BP enrichment; rows show curated migration/motility terms and cells display the raw $-\log_{10}(\text{p-adjusted})$ enrichment value for that cluster (higher values indicate stronger enrichment; gray denotes no significant term). Migration terms are most prominent in Cluster 3, with additional neural-crest/glial migration terms in Cluster 2. (B) Heatmap of proliferation/cell-cycle-associated GO:BP terms using the same analysis as in (A). Proliferation terms are largely confined to Cluster 4 and are minimal or absent in Cluster 2.