**Supplementary Figure Legends**

**Supplementary Figure S1 (related to main Figure 1).**

1. Number of genes at spot-level resolution of TA muscles at day 4 post-CTX.
2. UMAP representation of all spots from the ST samples colored by the Leiden-guided clustering as in **Fig.** **1A**. The number of spots per cluster is indicated.
3. Spatial expression patterns at *BayesSpace* subspot resolution of canonical healthy (*Myl1*), regenerative (*Myh8, Bgn*), and inflammatory (*Stab1*) gene expression by Visium spot. The color scale shows the log-normalized counts for each subspot.
4. Differential expression analysis between the three Leiden/pathologist-predicted clusters highlighted spatial differences in the expression of immune genes, regeneration markers, and genes encoding extracellular matrix proteins. GPNMB, a recently identified GFEM marker, is highlighted (1). The average marker gene expression after z-score transformation is shown for each cluster.
5. Gene ontology pathway analysis of the three Leiden-predicted and pathologist-annotated clusters. Top enriched pathways with p<0.001 and fold enrichment > 2 are shown for each cluster.
6. Negative-log likelihood plot. The elbow at q=7 was selected as the number of clusters to analyze.
7. Gene ontology pathway analysis of the seven *BayesSpace*-predicted clusters in panel **1C**. Top enriched pathways with fold enrichment > 5 are shown, indicating their correlation to the other clusters (scaled by negative log of P value).
8. Spatial expression patterns at *BayesSpace* subspot resolution of marker genes defining the myeloid subsets previously characterized by scRNA-seq (1). The color scale shows the log-normalized counts for each subspot. The color of the gene label corresponds to the MF and DC subset classification shown in panel **1H**.
9. Upper panel: IF images of CD68 (green), GPNMB (red), GDF15 (yellow), and nuclei (grey) stained muscles in C57BL/6J animals at day 4 post-CTX. Insets indicate split channels. Scale bars: 100 μm. Lower panel: Co-localization and distribution map of total CD68+ MFs (green), CD68+GPNMB+ subset (gold), CD68+GDF15+ subset (red), and other CD68- cells (black) quantified by HALO’s *Cytonuclear* and *Spatial Proximity*/co-localization modules. The percent of co-localization (80.4%) between CD68+GPNMB+ and CD68+GDF15+ subsets is indicated.

**Supplementary Figure S2 (related to main Figure 2).**

1. Violin plots representing the number of unique molecular identifiers (UMIs; upper left panel), genes per cell (upper right panel), mitochondrial (lower left panel) and ribosomal genes percentage (lower right panel) of all cells from PBMC, day 1, 2 and 4 post-CTX and 2-mo D2.*mdx* CD45+ cells before QC filtering and clustering. Colored lines indicate the filtering parameter cutoff values. The cellular source is color-coded.
2. Cell type annotation confidence score matrix visualized as a heatmap. Each cell is a column, while each row is a label in the integrated scRNA-seq dataset (PBMCs, day 1, 2, and 4 post-CTX CD45+ cells). The final label (after fine-tuning) for each cell is shown in the top color bar.
3. Dot-plot showing top DE genes that distinguish the 10 major cell type clusters (integrated dataset from PBMCs, day 1, 2, and 4 post-CTX CD45+ cells). The dot size represents the percentage of cells expressing the respective marker gene within a cluster. Selected markers are color coded.
4. PaCMAP feature plots (expression z-score) of selected markers from panel **S2C** that define the various cell types from the regeneration time course. Selected markers are color coded as in panel **S2C**.
5. Silhouette distribution plot for the isolated monocyte/MF/DC scRNA-seq dataset in **Fig. 2D**. Each dot represents a cluster at a given clustering parameter value. Medians with 95% CI are shown for each parameter value. The vertical red line marks the optimal resolution.
6. t-SNE representation of cells, colored by silhouette score at the suggested optimal resolution value = 0.6.

**Supplementary Figure S3 (related to main Figures 2 and 4).**

1. t-SNE representation of Seurat clusters (integrated dataset from PBMCs, day 1, 2, and 4 post-CTX CD45+ cells) at resolution 0.6 results in nine clusters.
2. Clustering tree (2, 3) visualizing the relationships between resolution parameters from 0.1 to 1 (step 0.1). At a resolution of 0.6, we see the formation of 9 main branches, one of which (cluster 3) continues to split up to a resolution of 0.7, after which there are only minor changes.
3. Heatmap of silhouette score at the predicted optimal resolution value = 0.6, indicating cluster and myeloid cell type relations. Cluster 3 shows a large overlap with all three myeloid subtypes (also shown in **Fig. 2D** with celltype annotations), supporting our decision to split this cluster.
4. Arrows indicating the projection of the velocities derived from the *scVelo* dynamical model (4) of the monocyte/MF/DC subtypes are projected into a t-SNE-based embedding.
5. t-SNE visualization of the speed/rate of differentiation given by the length of the velocity vector (*scVelo* dynamic modeling) (4) in the isolated monocyte/MF/DC scRNA-seq dataset.
6. Study schematic and workflow for the analysis of single-nuclei RNA-sequencing of human (healthy and DMD patients) vastus lateralis biopsies (5).
7. t-SNE visualization of myeloid subsets extracted from the human muscle single-nuclei RNA-sequencing datasets. Left panel: Indicates the origin of the myeloid subsets (red indicates cells from the healthy muscle biopsies and green cells originating from the DMD patients). Right panel: SNN clustering resolved five distinct myeloid subsets that are color-coded.
8. 2D embeddings visualizing cell cycle phases of the five myeloid subsets in the human snRNA-seq datasets generated using *VeloViz* embeddings. Cycling MFs are a conserved feature of dystrophy-mediated chronic inflammation in human DMD pathology.
9. Single-nuclei expression levels for selected functional markers. These markers allowed the delineation of functionally distinct MF subtypes present in human DMD biopsies. PLK1 indicates the presence of cycling MFs.
10. Top marker genes for the five identified MF clusters in the human snRNA-seq datasets. The dot size represents the percentage of cells within a group with an expression level > 0 and color-scale represents the average expression level (row Z-score) across all cells within the cluster.

**Supplementary Figure S4 (related to main Figure 3).**

1. FACS contour plots and gating strategy of CD45+ Ly6Clow F4/80hi GPNMB+ and CD45+ Ly6Clow F4/80hi GPNMB- muscle-infiltrating MFs in WT C57BL/6Janimals at day 4 post-CTX.
2. Number of centrally nucleated myofibers per mm2 of tissue in D2.*Gpnmb*+ and D2.*Gpnmb*- (DBA2/J) muscles at day 8 post-CTX injury (n=6 per group). Unpaired t-test was performed with P value 0.0022.
3. Percentage of regeneration area over the total injured area in D2.*Gpnmb*+ and D2.*Gpnmb*- (DBA2/J) muscles at day 8 post-CTX injury (n=6 per group). Unpaired t-test was performed with P value 0.0007.
4. Number of regenerating myofibers (assessed by embryonic MyHC positivity) per mm2 of tissue in D2.*Gpnmb*+ and D2.*Gpnmb*- (DBA2/J) muscles at day 8 post-CTX injury (n=5 per group). Unpaired t-test was performed with P value 0.0002.
5. Percentage of inflammation area (assessed by CD68 positivity) over the total injured area in D2.*Gpnmb*+ and D2.*Gpnmb*- (DBA2/J) muscles at day 8 post-CTX injury (n=6 per group). Unpaired t-test with Welch’s correction was performed with P value < 0.0001.
6. Upper panel: Number of CD45+ cells per gof tissue in C57BL/6J, D2.*Gpnmb*+ and D2.*Gpnmb*- (DBA2/J) muscles at indicated time points post-CTX injury (n=6 per group). Two-way ANOVA with Dunnett's multiple comparisons test was performed between genotypes and time points (Day 4: C57BL/6J vs D2.*Gpnmb*+ and C57BL/6J vs D2.*Gpnmb*- and Day 8: C57BL/6J vs D2.*Gpnmb*- and D2.*Gpnmb*+ vs D2.*Gpnmb*- comparisons have P < 0.0001). Lower panel: Number of DCs (CD45+CD11c+ F4/80- Ly6C-) and neutrophils (CD45+ Ly6G+F4/80-Ly6Cint) per gof tissue in D2.*Gpnmb*+ and D2.*Gpnmb*- (DBA2/J) muscles at indicated time points post-CTX injury (n=6 per group). Two-way ANOVA with Dunnett's multiple comparisons test was performed between genotypes and time points (Day 2 DCs: D2.*Gpnmb*+ vs D2.*Gpnmb*- comparison has P < 0.01).
7. FACS contour plots of CD45+ Ly6Clow F4/80hi MHCII+ and CD45+ Ly6Clow F4/80hi MHCII- muscle-infiltrating MFs in D2.*Gpnmb*+ and D2.*Gpnmb*- animals at day 4 post-CTX. Insets indicate the frequency and MHCII MFI for each population (n=6 biological replicates per group). The frequency of CD163 and CD206 in CD45+ Ly6Clow F4/80hi MHCII+ is also shown.
8. IF detection of CD163 (left panel; red) and CD206 (right panel; red) MFs (F4/80; green) in D2.*Gpnmb*+ and D2.*Gpnmb*- animals at day 8 post-CTX injury (F-actin/phalloidin is indicated in gray and nuclei in blue). Scale bars: 100 μm.
9. Quantification of CD163+ F4/80+ (upper panel) and CD163+ F4/80+ (lower panel) in D2.*Gpnmb*+ and D2.*Gpnmb*- animals at day 8 post-CTX injury. Unpaired t-test with Welch's correction was performed in each comparison with P values 0.0048 and 0.0185, respectively.
10. Number of genes at spot-level resolution in the ST day 8 post-CTX injured muscle samples from C57BL/6J and D2.*Gpnmb*- animals.
11. UMAP visualization of all spots in the *harmony*-integrated ST datasets from C57BL/6J and D2.*Gpnmb*- at day 8 post-CTX. The number of spots per condition is indicated.
12. Negative-log likelihood plot. The elbow at q=5 was selected as the number of clusters for downstream analysis.
13. Spatial expression of representative mature MFs marker genes (*Adgre1* and *S100a4*) in WT (C57BL/6J; upper) and D2.*Gpnmb*- (GPNMB KO; lower) samples from Day 8 post CTX. Note the increased and prolonged presence of MFs in the absence of GPNMB.
14. Volcano plot indicating the DE genes between C57BL/6J vs D2.*Gpnmb*- spatial spots (padj<0.05, logFC>0.25). The enriched gene set for each sample group is color-coded and the top gene labels are shown.
15. Dot plot of the estimated/relative NMF weights and cell abundance of ten subtypes (rows) described in **Fig. 2** across five predicted NMF components (columns) in the day 8 post-CTX spatial samples.
16. Identification of tissue compartments in the day 8 post-CTX samples (upper: C57BL/6J, lower: D2.*Gpnmb*-) using NMF-based decomposition (*Cell2location*) and reference immune subtype expression signatures from **Fig. 2**. Spatial plots show cell abundance (color intensity) for each subtype.

In all bar graphs, bars represent mean ± SD (\*\*p < 0.01, \*\*\*p < 0.001, \*\*\*\*p < 0.0001).

**Supplementary Figure S5 (related to main Figure 4).**

1. Number of genes at spot-level resolution of two gastrocnemius muscles from 2-mo D2.*mdx* animals.
2. UMAP representation of the integrated spots from 2 biological replicates. The number of spots per biological replicate is indicated.
3. Negative-log likelihood plot. The elbow at q=7 was selected as the number of clusters to analyze.
4. t-SNE visualization of the monocyte/MF/DC cells from the D2.*mdx* scRNA-seq sample. *SingleR (ImmGen* database*)* cell type annotation prediction was used for the classification and cell type selection.
5. Arrows indicating the projection of the velocities derived from the *scVelo* dynamical model (4) of the monocyte/MF/DC subtypes in the D2.*mdx* scRNA-seq dataset are projected into a t-SNE-based embedding.
6. t-SNE visualization of the speed/rate of differentiation given by the length of the velocity vector (*scVelo* dynamic modeling) (4) in the 2-mo D2.*mdx* monocyte/MF/DC scRNA-seq dataset.
7. Gene expression dynamics of the 7 monocyte/MF/DC subpopulations in the scRNA-seq D2.*mdx* dataset resolved along latent time (*scVelo* dynamic modeling). The top likelihood-ranked genes predicted to drive cell differentiation are ordered within each branch.
8. Number of non-proliferating CD68+ Ki67- and CD68+ Ki67+ cycling MFs per mm2 of tissue in C57BL/6J and DBA2/J animals at day 4 post-CTX injury and in 2-mo D2.*mdx* animals (an unpaired t-test with Welch’s correction was performed for all indicated comparisons with P values < 0.0001).
9. Left panel: IF images (inverted and color-coded) of CD68 (red), Laminin (orange), Ki67 (green), and nuclei (blue) stained muscles from C57BL/6J and DBA2/J animals at day 4 post-CTX injury for the detection of cycling MFs. No cycling double-positive MFs were detected. Scale bars: 50 μm. Right panel: Co-localization and distribution map of CD68+ Ki67- non-proliferating MFs (blue), CD68+Ki67+ cycling MFs (red), and non-MF proliferating cells (CD68-Ki67+) quantified by HALO co-localization modules.
10. Left panel: IF images (inverted and color-coded) of CD68 (red), Laminin (grey), Ki67 (green), and nuclei (blue) stained muscles from 2-mo D2.*mdx* animals for the detection of cycling MFs. Scale bars: macroscopic views 1 mm; insets 50 and 100 μm. Right panel: Co-localization and distribution map of CD68+ MFs (blue) and CD68+Ki67+ cycling MFs (red) quantified by HALO co-localization modules.

**Supplementary Figure S6 (related to main Figures 6 and 7).**

1. Experimental schematic workflow. 4-week-old D2.*mdx* animals were treated weekly orally with 5 mg/kg Prednisolone for 4 weeks. Gastrocnemius muscles were then subjected to ST.
2. Number of genes at spot-level resolution of three gastrocnemius muscles from 2-mo D2.*mdx* + Q.W. Pred animals.
3. Negative-log likelihood plot. The elbow at *q*=7 was selected as the number of clusters to analyze.
4. UMAP representation of the integrated spots from D2.*mdx*-UNT and D2.*mdx* + Q.W. Pred. The number of spots per condition is indicated.
5. Top marker gene expression after z-score transformation is shown for each spatial cluster. The dot size represents the percentage of cells within a group.
6. Spatial feature plots of representative DE genes from the D2.*mdx* - UNT *vs.* D2.*mdx* + Q.W. Pred. comparison is shown.
7. Upper panel: A magnified IF image of an inflammatory lesion in 2-mo D2.mdx + Q.W. Pred GAST muscle (the lesion location label is indicated in the upper left corner; see also **Figs.** **7E** and **S7C** for different magnifications and more representative examples). The MF subtypes were visualized with CCL2 (red; Zone A) and MMP12 (yellow; Zone B) and regenerating fibers with eMyHC (green; Zone C). Scale bar: 100 μm. Lower panel: A stacked bar histogram reflecting CCL2+, MMP12+, eMyHC+, and Other Cells (CCL2- MMP12- eMyHC-) cell density inside (-1 to -50 μm) and outside the necrotic boundary (+1 to +300 μm). Note the near complete absence of MF subtypes (i.e., MMP12+ cells), regenerating fibers (eMyHC+ cells), and unstructured damage-clearing and RIZ tissue organization.

**Supplementary Figure S7 (related to main Figures 7 and 8).**

1. Six representative regions of RIZs in 2-mo D2.*mdx* GAST validated by IF. The MF subtypes and zones were visualized with IF for CCL2 (red; Zone A), MMP12 (yellow; Zone B), and eMyHC (green; Zone C). White rectangles indicate the region magnified in the middle panel. Scale bars: macroscopic view 1 mm; insets 100 μm. Right panel indicates the cell density and distribution of cells in regions 2 and 6. Note the consistent and expected organization and architecture of RIZs.
2. RIZ quantification in 2-mo D2.*mdx* muscles. Dotted lines indicate the zones and interface layer (red; necrotic lesion) selected for cell density quantification. The bottom panels show stacked bar histograms reflecting CCL2+, MMP12+, and eMyHC+ cell density inside (-1 to -50 μm) and outside (+1 to +200 μm) the necrotic lesion boundaries. Scale bars: 100 μm.
3. Five IF regions with inflammatory lesions in 2-mo D2.*mdx* + Q.W. Pred GAST samples. The MF subtypes and zones were visualized with IF for CCL2 (red; Zone A), MMP12 (yellow; Zone B), and eMyHC (green; Zone C). Note the unexpected collapse of RIZ organization in large inflammatory lesions with the overall diminished expression of MMP12 and the signal overlap of CCL2 with the remaining MMP12 (as observed previously by ST; **Fig. 7B-C**). Scale bars: macroscopic view 500 μm; insets 100 μm.
4. IF regions with inflammatory lesions in 2-mo D2.*mdx* samples. The presence of MF subtypes within the lesions was visualized with other ST predicted Zone B markers (**Figs. 7C-D**) CD18/ITGB2 (upper left panel; red), CD44 (upper right panel; red), and CD206 (middle panels; yellow). Mature MFs are marked with F4/80, pro-inflammatory monocytes/MFs with CCL2 and regenerating fibers with eMyHC. The middle right panel indicates the cell density quantification of F4/80+CD206+ and F4/80+CD206- cells within inflammatory lesions, placing the former closer to the center of the lesions and the latter closer to the periphery. Scale bars: 100 μm.
5. IF regions with inflammatory lesions enriched in damage-clearing zones but without advanced regeneration zones in 6-wks D2.*mdx* gastrocnemius muscles. These experiments are consistent and predictive of the architecture and organization of recently formed lesions, as revealed in the ST clustering analysis of age-matched samples (**Fig. S8E**). The MF subtypes and zones were visualized with IF for CCL2 (red; Zone A), MMP12 (yellow; Zone B), and eMyHC (green; Zone C). White rectangles indicate the region magnified in the middle panel. Scale bars: macroscopic view 500 μm; insets 100 μm.
6. IF regions with inflammatory lesions in 2-mo D2.*mdx* samples. The presence of MF subtypes within the lesions was visualized with another Zone B ST-predicted marker (ATF3; **Figs. 7C**). Pro-inflammatory monocytes/MFs were visualized with CCL2 and regenerating fibers with eMyHC. The right panel indicates the cell density quantification of ATF3+ cells within inflammatory lesions in regions 2 and 4 (occupying Zone B), placing them adjacent to CCL2+ cells (Zone A). Scale bars: 100 μm.

**Supplementary Figure S8 (related to main Figure 7).**

1. H&E images of mouse gastrocnemius from 6-wks D2*.mdx* animals used for ST (6, 7). Histopathological annotation areas are noted (yellow: regenerative muscle; green: necrotic/inflammatory lesions; blue: healthy muscle) and classified as described in **Fig. 1A**. Latin numbers indicate the reference sample number. Each section is derived from a different biological replicate, and all datasets were integrated to remove any batch effects.
2. Enhanced subspot resolution clustering (*BayesSpace*) for the five samples indicated in panel **S8A**. The seven spatial clusters are color-coded, as in **Fig. 4B**. The white rectangles highlight areas with inflamed and structured regeneration zones.
3. The spatial expression of representative genes coding for damage-clearing and regenerative inflammation markers is shown. *Pvalb* indicates the healthy muscle (cluster 7), *Csf1* and *Ccl7* indicate the LGCs (cluster 2*), Mmp12* and *Nckap1l* indicate the presence of resolution-related MFs and GFEMs (cluster 6), and *Myh8* and *Myl4* indicate the newly regenerating fibers (cluster 1). Note the differential spatial expression patterns between the indicated markers and the regenerative architecture, as observed previously in **Figs. 4B-D**.
4. Magnified histological and *BayesSpace* clustering view of lesions in regions IV and V from panel **S8B**. The spatial expression of indicated RIZ markers in each of these regions is provided at the bottom panel. Note the expected RIZ organization and architectureas described previously in **Fig. 7**.
5. Magnified histological and *BayesSpace* clustering view of lesions in regions II and III from panel **S8B**. The spatial expression of indicated RIZ markers in each of these regions is provided at the bottom panel. Note the unique spatial clustering, with Zone B (spatial cluster 6) and C (regenerating fibers; spatial cluster 1) being absent and Zone A (spatial cluster 2) strongly enriched in necrotic fibers, pointing towards recent lesion formation.

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