**Supplemental Methods**

**Mice**

This study used C57BL/6J (RRID:IMSR\_JAX: 000664), and DBA/2J mice (RRID: IMSR\_JAX: 000671) carrying a nonsense mutation in *Gpmnb* (D2*.Gpnmb-*), the coisogenic strain DBA/2J-Gpnmb+/SjJ with a functional *Gpnmb* allele (D2.*Gpnmb+*; RRID: IMSR\_JAX: 007048) and *mdx* mice on the DBA/2J background (D2.B10-Dmdmdx/J; RRID: IMSR\_JAX: 013141) carrying the dystrophin gene mutation (D2.*mdx*).Mice were obtained from the Jackson Laboratories, bred under specific-pathogen-free conditions, with *ad libitum* access to food and water, and a 12-hour light cycle. Prednisolone suspensions were prepared in a cherry syrup vehicle for weekly *per os* (PO) treatment of 5 mg/kg dose (equates to a dose of 0.41 mg/kg in humans when normalized to the body surface area). Euthanasia was performed by CO2 exposure per IACUC guidelines.

**Acute sterile muscle injury**

Mice (8-12 weeks-old) were anesthetized with isoflurane and 50 µl of 10 μM cardiotoxin (EMD Millipore, 217503-1MG) was injected into the TA muscle (1). Mice were monitored after recovery until they were euthanized. Muscles were recovered for flow cytometry analysis on days 1-4 post-injury or for histopathological assessments on days 4 to 8 post-injury.

**Histological analysis of muscle regeneration**

Muscles were removed, mounted on precut cork discs (EMS #63305) using tragacanth gum (MP Biomedicals, #104792), and snap-frozen in nitrogen-chilled isopentane (-160°C). 8 µm thick cryosections were cut (Leica CM1950) and stained with hematoxylin-eosin (H&E) as described previously (2). H&E-stained muscle sections were scanned with a Leica Aperio Versa digital slide scanner. For each histological analysis, at least six sections (per condition) were selected where the total regenerative region within the CTX injured TA muscle was at least 70%. For each TA, myofibers in the entire injured area were analyzed and quantified with the *Muscle Fiber* 2.0 module of the HALO Digital Pathology software (Indica Labs). The regeneration area is expressed as the number of centrally nucleated fibers over the total area. For the ST sample selection and histological annotations, two active/experienced clinical pathologists at the Johns Hopkins All Children’s Hospital specializing in muscle pathology performed and guided the assessments. Their evaluation of each injury/regeneration stage included a pool of 20 biological replicates per timepoint, from which we selected the samples that collectively incorporated most of the features the pathologists had identified to place on the Visium ST slides.

**Immunofluorescence**

Tibialis anterior and gastrocnemius muscles embedded in tragacanth gum were cryosectioned at 8 μm, fixed in ice-cold acetone for 5 min, blocked for 1 hour at room temperature in PBS containing 5% BSA, and stained for immunofluorescent (IF) analysis using a combination of Phalloidin (CF568-conjugated; Biotium #00044; 1:100), rabbit anti-laminin (Sigma-Aldrich L9393; 1:200), mouse anti-eMyHC (DSHB F1.652; 1:20), rabbit anti-MYH3 (Proteintech 22287-1-AP; 1:250), rabbit anti-Desmin (Abcam 32362; 1:200), rat anti-F4/80 (Abcam 6640; 1:200), rat anti-CD68 (Bio-Rad MCA1957GA; 1:100), goat anti-GPNMB (R&D Systems AF2330; 1:250), rat anti-CCL2 (Bio-Techne MAB479-SP; 1:100), rabbit anti-MMP-12 (Proteintech 22989-1-AP; 1:400), rabbit anti-GDF15 (Abcam ab105738; 1:100), rat anti-CD18/ITGB2 (Thermo Scientific 14-0181-82, 1:200), rat anti-CD44 (BD 550538; 1:100), rat anti-CD206-PE (BioLegend #141705; 1:50), rat anti-CD163-PE (BioLegend #155307; 1:50), rabbit anti-ATF3 (Novus Biologicals NBP1-85816; 1:250), and mouse anti-Ki67 (BD Pharmingen 550609; 1:20) primary antibodies for 1 hour at RT. Unconjugated primary antibody staining was followed by three 5-minute PBST washes and stained with donkey anti-rabbit Cy3 (JIR 711-165-152; 1:200), donkey anti-rat FITC (JIR 712-095-153; 1:200), goat anti-rat Alexa Fluor 647 (Invitrogen A-21247; 1:200), donkey anti-goat Alexa Fluor 647 (Invitrogen A-21447; 1:200), donkey anti-mouse Alexa Fluor 488 (JIR 715-545-151; 1:200), and goat anti-mouse Alexa Fluor 488 (Invitrogen A-21121; 1:200) secondary antibodies for 45 min at RT. Following three PBST washes, the nuclei were counterstained with 1 µg/ml Hoechst 33342 for 1 minute, washed twice in PBS, and samples were mounted with Fluoromount (Sigma F4680). The IF slides were scanned at 20x and visualized with the dedicated fluorescent cameras of a Leica Aperio Versa digital slide scanner. Whole slide IF images were processed and analyzed for detection and co-localization of CD68+/Ki67+ cycling MFs, inflammation index (CD68+ or F4/80+ areas), as well as regenerating muscle fiber distribution (eMyHC+ fibers) by a blinded investigator using the *Object Colocalization FL* 1.0and *Area* Quantification *FL* 1.0 modules of the HALO software (Indica Labs). For quantifying the distance of the GFEMs (CD68+GPNMB+) and other MF subtypes (CD68+GPNMB-) to the regenerating fibers (eMyHC+), the *Cytonuclear FL* 1.0and *Spatial Analysis FL* 1.0 (Nearest Neighbor Analysis workflow) modules (HALO software; Indica Labs) were used. For calculating cell densities within and outside an interface layer (i.e., necrotic lesion), the *Cytonuclear FL 1.0* and *Infiltration Analysis* workflow (HALO software; Indica Labs) were used. Min-max normalization was then applied to scale the cell densities in each interface distance bin and plotted with *ggplot* as a 100 percent stacked bar. Representative high-resolution IF images were obtained under a confocal microscope with a resonant scanning disk (Nikon A1R, Nikon Instruments) with Z-sectioning (0.5 μm). The Nikon NIS-Elements AR Analysis 4.40 software was used to create the volume projection image (3-D reconstruction), and the final figures were assembled in Illustrator v27.2 (Adobe).

***In vivo* isolation of MFs from muscle**

Isolation of muscle-infiltrating MFs was performed as described previously (3, 4). Briefly, the fascia of the TA was removed, and muscles were dissociated in either RPMI containing 0.2% collagenase B (Roche Diagnostics GmbH) at 37°C for 1 hour or by using the MACS Skeletal Muscle Dissociation Kit (Miltenyi, 130-098-305) or gentleMACS Octo Dissociator, per kit instructions. Cell homogenate was filtered through a 100 µm and a 40 µm filter, and CD45+ cells were isolated using magnetic sorting (Miltenyi Biotec). For FACS, myeloid cells were treated with Fcγ receptor blocking antibodies and with 10% normal rat serum: normal mouse serum 1:1 mix, then stained with a combination of PE-conjugated anti-Ly6C antibody (HK1.4, eBioscience), APC-conjugated or FITC-conjugated F4/80 antibody (BM8, eBioscience), FITC-conjugated Ly6G antibody (1A8, Biolegend), Pacific Blue-conjugated MHCII antibody (M5/114.15.2, Biolegend) and eFluor660-conjugated GPNMB antibody (CTSREVL, eBioscience). Ly6Clow F4/80high Gpnmb- MFs, Ly6Clow F4/80high Gpnmb+ MFs, and Ly6Clow F4/80high MHCII+ MFs were quantified (gating strategy is shown in **Figs. S4A** and **S4G**). In each experiment, compared samples were processed in parallel to minimize experimental variation. Cells were analyzed on either a Cytoflex LX (Beckman Coulter), or MoFlo Astrios EQ (Beckman Coulter) sorter, and data analysis was performed using FlowJo V10 software.

**Muscle-infiltrating MF cell culture for conditioned medium generation and apoptosis assay**

Wild-type (C57BL/6J) Ly6Clow F4/80high Gpnmb- MFs, and Ly6Clow F4/80high Gpnmb+ MFs were sorted from CTX-injured muscle at day 4. An equal number of sorted cells per population were seeded (1x106 cells per well) and cultured with DMEM containing 20% endotoxin-reduced fetal bovine serum (FBS) and 20% conditioned medium of L929 cell line (enriched in saturating levels of CSF-1; tested in a 5-day BMDM differentiation assay) for 12 hours (5, 6). The supernatant was then collected and centrifugated to obtain the MF-conditioned medium used in myoblast proliferation and differentiation assays (see below). For the apoptosis assay, sorted MFs were seeded in coverslips, fixed with 4% PFA, and immunostained with Cleaved Caspase 3 (Cell Signaling #9661; 1:200) for 1 hour at RT. Immunofluorescent and brightfield images were obtained using a Carl Zeiss Axio Imager Z2 microscope and analyzed for detection of Cleaved Caspase 3+ MFs by a blinded investigator using Fiji. Representative images and figures were then assembled in Illustrator v27.2 (Adobe).

**Myoblast proliferation and differentiation assay**

Murine myoblast C2C12 cells were obtained from American Type Culture Collection (CRL-1772) and were maintained according to the company’s instructions. In brief, cells were cultured in DMEM supplemented with 10% FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin (growth medium) at 37°C in 5% CO2 and 95% air at 100% humidity. For proliferation assays, C2C12 cells were seeded at 10,000 cells/cm2 on Matrigel (1:10) and incubated for 1 day with the MF-conditioned medium plus 2.5% FBS (7, 8). For differentiation assays, C2C12 cells were seeded at 30,000 cells/cm2 on Matrigel (1:10) and incubated for 3 days with the MF-conditioned medium plus 2% horse serum (7, 8). Cells were then fixed with 4% PFA, incubated with anti-Ki67 (Abcam #15580; 1:200), or anti-Myosin heavy chain 1E (DSHB MF 20; 1:20) for 1 h at room temperature, and were subsequently visualized using Cy3-conjugated secondary Abs (JIR; 1:200). The nuclei were counterstained with 0.1–1 μg/ml Hoechst. IF images were obtained using a Carl Zeiss Axio Imager Z2 microscope and analyzed for proliferation (% of Ki67+ myoblasts) and fusion (% of myotubes with > 3 myonuclei) index using Fiji.

**Spatial RNA sequencing library preparation**

Fresh frozen skeletal muscle samples were cryosectioned (Leica CM1950) at 10-µm section thickness and were placed on the pre-chilled Optimization slides (Visium, 10X Genomics, PN-1000191) to determine the optimal lysis time. The tissues were treated as recommended by 10X Genomics, and the optimization procedure showed an optimal permeabilization time of 30 min of digestion and release of RNA from the tissue slide. Spatial gene expression slides (Visium, 10X Genomics, PN-1000185) were used for ST following the Visium User Guides, and whole slide images were taken using a 20X objective of a Leica Aperio Versa scanner. Next-generation sequencing libraries were prepared according to the Visium user guide. Libraries were loaded at 300 pM and sequenced on a NovaSeq 6000 System (Illumina) as recommended by 10X Genomics. Each section is derived from a different biological replicate, and each library was obtained from a separate Visium experiment followed by bioinformatic integration to remove any batch effects.

**Spatial transcriptomics data analysis**

Filtered feature-barcode expression matrices from *SpaceRanger* (v1.3.1) were used as initial input for the spot-level ST analysis using *Giotto (v2.0.0).* Spots with less than 25 measured genes were filtered out. Ribosomal, mitochondrial, *Rik*, and *Gm* genes were excluded from the analysis. Principal component analysis was run on the highly variable genes calculated by *calculateHVF* function. PCA coordinates were used for data integration with *harmony* (v0.1.1). UMAP dimension reduction was performed with harmony embeddings and visualized in R. Spot-level clustering was performed using the Leiden algorithm at resolution 0.3. Spatially variable genes were calculated with *binSpect* using log-normalized data (FDR < 0.001). Gene Ontology enrichment analysis was performed using PANTHER (9) and Metascape v3.5 (10). Filtered gene-spot matrix and metadata were further analyzed with *BayesSpace* (v1.6.0) using the top 10 PCs and the top 2000 HVGs. Clusters were then created at sub-spot resolution with the following parameters (d=10, platform=”Visium”, init.method=”mclust”, model=”t”, gamma=2). Optimal cluster numbers were determined by *qTune* function and manual curation of marker genes. Cluster marker genes were determined using *immunogenomics/presto* (v1.0.0) (padj<0.05, logFC>0.25). Cell-type compositions were calculated for each spot using *cell2location* (v0.1) (11). Reference expression signatures of major cell types were estimated using regularized negative binomial regressions and our integrated single-cell RNA-seq datasets. Each slide was later deconvoluted using hierarchical Bayesian models as implemented in *run\_cell2location* function. For cell-type-specific abundance estimations, we used 10 cells per location with a detection alpha of 20 as hyperparameters. The donut charts were created using the *ggpubr* package. To further investigate the spatial organization of the tissue zones and calculate the observed vs. expected ratio, we have developed an R shiny app (*SpatialZoneR*) to interactively define and analyze tissue zones in sub-spot resolution using Visium ST rds data. Gene set expression values representing different cell subtypes at each subspot were extracted and binarized (expression cutoff was selected at > q90 for each marker gene) to define tissue zone cell organization. *SpatialZoneR* is made available as a fully open-source tool at <https://github.com/hlszlaszlo/SpatialZoneR>.

**Single-cell RNA-sequencing library preparation**

After tissue digestion and bead selection, CD45+ single-cell sorted suspensions were washed and resuspended in 0.04% BSA in PBS at a concentration of at least 400 cells/μL. Cells were counted manually with a hemocytometer to determine their concentration. Single-cell RNA-sequencing libraries were then prepared using the Chromium Single-Cell 3’ reagent kit v3.1 (10X Genomics, Pleasanton, CA) in accordance with the manufacturer’s protocol. Briefly, the cells were diluted into the Chromium Single-Cell A Chip to yield recovery of ~10,000 single-cell transcriptomes with < 5% doublet rate. Following the library preparation, the libraries were sequenced on the NovaSeq 6000 sequencer (Illumina, San Diego, CA) to produce about 450 million reads per library and, on average, a minimum of 40,000 reads per single cell.

**Single-cell RNA-seq data analysis**

Single-cell sequencing reads were processed and aligned to the mouse reference transcriptome (*mm10*) with the *CellRanger* (v7.0.1) (10x Genomics, Pleasanton, CA). We used *CellBender* (v0.2.2) to eliminate technical artifacts. From the gene expression matrix, the downstream analysis was carried out in R (v4.2.1). Quality control, filtering, data clustering and visualization, and the differential expression analysis were carried out using *Seurat* (v4.1.3) R package (12) with custom modifications. Genes expressed in <10 cells and cells with <500 detected genes were removed from the gene expression matrix. Cells with high mitochondrial mapped read percentage (determined by MiQC) (13), as well as outliers with UMI counts in the lower and upper 2.5% (q97.5) were excluded from downstream analysis. Doublets identified using *scDblFinder* (v1.10.0) were also removed. Ribosomal, mitochondrial, Rik and Gm genes were excluded from the analysis. After log-normalizing the data, the expression of each gene was scaled, and PCA was performed on the top feature genes determined by *DUBStepR* (v1.2.0). Data integration was carried out using *harmony* (14). Harmony embeddings were used for dimension reduction, clustering, and visualization. Unsupervised shared nearest neighbor (SNN) clustering was performed with a k parameter of 20 using the Leiden algorithm, and visualization was done using PaCMAP (15) using the *ReductionWrappers* (v2.5.4) R package. Cell type automatic annotations were predicted using the *SingleR* (v1.10.0) package and the *ImmGen* database from *celldex* as reference (16, 17). Confidence scores were visualized using the *plotScoreHeatmap* function from SingleR. We also verified the accuracy of the assignment by exploring the expression of known cell-type gene markers and by evaluating the top differential genes between cell clusters on *PanglaoDB* (18). Cluster marker genes were identified using *presto* to perform a Wilcoxon rank-sum test and auROC analysis (logFC > 0.25, p-adj < 0.0001, AUC > 0.65, and ranked based on their exclusive presence in each cluster calculated as the difference between the percentage of inclusion vs. exclusion). Cells in the macro-clusters of interest (Monocytes, MFs, and DCs) were extracted and reanalyzed. Visualization was done using t-distributed stochastic neighbor embedding (t-SNE) to reveal local differences (19). To determine optimal cluster resolution, we first utilized a subsampling-based approach *chooseR* that guides parameter selection while characterizing cluster robustness (20) (**Figs. S2E-F**). We also independently performed the clustering workflow with the *FindClusters* function from resolution 0.1 to 1 in steps of 0.1 (**Fig. S3B**). The resolution 0.6 was evaluated as best by both approaches. However, one cluster emerged that expressed marker genes representing multiple cell populations (**Figs. 2D** and **S3A-C**), suggesting re-clustering/splitting into two clusters. This choice was evaluated and confirmed with the aid of the *Clustree* package (**Fig. S3B**) for a total of 10 clusters. To assign identities to these immune subclusters, we manually curated and cross-referenced their marker genes with known subtype markers data from the literature and relevant mouse models and studies (**Fig. 5**) (21-25). For computing the Spearman similarity correlation between the subpopulations in the different samples, the R package *corrplot* was used (logFC > 0.5, p-adj < 0.1, auc > 0.5, sig.level = 0.05, and AOE order) (26). Feature plots were generated using the *Nebulosa* package (27), heatmaps using the *pheatmap* (v1.0.12) package, *tricycle* (v1.4.0) for cell cycle stage scoring (28), and *ggalluvial* (v0.12.3)(29) for compositional plots. Combined feature expression module scores were calculated using *Seurat*'s *AddModuleScore* function. Lastly, the dynamic changes in gene expression were evaluated by performing a trajectory analysis using three packages (*Slingshot, Monocle v2,* and *scVelo*) (30-33). To give a finer definition of cell states and unknown myeloid subpopulations, the trajectory analyses were performed only on the MF, monocyte, and DC subsets. RNA velocity estimations and visualizations were performed using dynamic modeling by *scvelo (32, 34)*. Counts were filtered to a minimum of 20 shared counts across samples, and the top 2000 variable genes were selected for velocity analyses. Fifty nearest neighbors were used when calculating moments of velocity, followed by velocity estimation and embedding on dimensional reductions using scvelo’s *velocity\_embedding\_stream* function. Pseudotime analysis was performed using *Slingshot* and the *harmony*-corrected principal components to calculate cluster lineages with *getLineages* providing cluster 3 (circulating monocytes) as a start cluster. Pseudotime trajectories were then calculated using the t-SNE embeddings and visualized appropriately. RNA velocity-informed embedding was used to represent the proliferating MF cluster in isolation using the *veloviz* package (35). The cell cycle phase (G1, S, G2M) of individual cells was assigned using the calculated standardized z-scores of highly expressed stage-specific cell cycle marker genes using a previously defined list of cell cycle genes (36, 37). *tricycle* was then used to visualize the cell cycle stage scoring of the clusters as Cartesian coordinates by computing the kernel density of θ conditioned on a phenotype using the von Mises distribution (28). To generate the TF heatmap, the average log-normalized expression values for the list of TFs were calculated, ordered, and decile-filtered to select the top 75% for visualization.

**Human single-nuclei RNA-seq data analysis**

Raw sequence data for human samples (vastus lateralis biopsies from healthy and DMD patients) from BioProject PRJNA772047 (25) were processed using *CellRanger (v7.0.1)* to generate gene expression matrices aligned to the *hg38* reference genome. Single nuclei data were logged normalized, and scaled with *Seurat*. Doublets identified using *scDblFinder*, nuclei with < 500 UMI, and high mitochondrial gene content (*miQC*) were removed before integrating datasets using *harmony*. Major cell-type populations were identified using the *SingleR* package and the Human Primary Cell Atlas, and only cells classified as “Monocyte,” and “Macrophage” were selected for downstream analysis (16). Clustering trees were used to identify the optimal number of clusters (cluster resolution = 0.9) and visualized with t-SNE. For each immune subset, marker genes were identified using *presto* using the same parameters as described above for the scRNA-seq datasets. Feature plots were generated using the *Nebulosa* package (27) and *tricycle* for cell cycle stage scoring of individual cells, as described previously (28).

**ATF3 KO microarray gene expression data analysis**

*GEOquery* and *limma* v 3.58.1 were used to perform differential expression analysis using the original submitter-supplied processed data tables as input and by applying multiple-testing corrections (Benjamini & Hochberg FDR) on P-values to correct for the occurrence of false positives (p-value cutoff of 0.05).

**Chromatin immunoprecipitation (ChIP)**

ChIP was performed as previously described (38) with minor modifications. Briefly, sorted cells were crosslinked with DSG (Sigma) for 30 minutes and then with formaldehyde (Sigma) for 10 minutes. After fixation, chromatin was sonicated with Diagenode Bioruptor to generate 200-1000 bp fragments. Chromatin was immunoprecipitated with an antibody against H3K27ac (ab4729). Chromatin antibody complexes were precipitated with Protein A-coated paramagnetic beads (Life Technologies). After six washing steps, complexes were eluted and reverse crosslinked. DNA fragments were column purified (Qiagen, MinElute). The amount of immunoprecipitated DNA was quantified with Qubit fluorometer (Invitrogen). Libraries were prepared by Ovation Ultralow Library Systems (Nugen) from two biological replicates according to the manufacturer’s instructions.

**ChIP-seq analysis**

The collection of TF ChIP-seq data and the primary analysis of raw sequence reads were carried out as described earlier (39, 40). In more detail, raw data were selected and downloaded from the SRA database of NCBI. Alignment to the *mm10* mouse reference genome assembly was performed by the *BWA* v0.7.17 tool (41). BAM files were created by *SAMtools* v1.7 (42). Genome coverage (bedgraph) files were generated by *makeUCSCfile.pl* (HOMER v4.9.1) (43) and visualized by Integrative Genomics Viewer (IGV v2.16.1) (44).

**Assay for Transposase-Accessible Chromatin with high-throughput sequencing (ATAC-seq)**

ATAC-seq was carried out as described earlier with minor modifications (45). 20,000 Ly6Chigh circulating cells from C57BL/6J were sorted in ice-cold PBS. Nuclei were isolated with ATAC-Lysis Buffer (10mM Tris-HCl pH7.4, 10mM NaCl, 3mM MgCl2, 0.1% IGEPAL) and were used for tagmentation using Nextera DNA Library Preparation Kit (Illumina). After tagmentation DNA was purified with MinElute PCR Purification Kit (Qiagen). Tagmented DNA was amplified with Kapa Hifi Hot Start Kit (Kapa Biosystems) using 9 PCR cycles. Amplified libraries were purified again with MinElute PCR Purification Kit. Fragment distribution of libraries was assessed with Agilent Bioanalyzer and libraries were sequenced on a HiSeq 2500 platform.

**Mapping and normalization of ATAC-seq**

Circulating monocytes (Ly6Chigh) and muscle-derived Ly6Chigh MFs of day 1 and Ly6Clow MFs of day 4 upon muscle injury datasets were used. The primary analysis of ATAC-seq-derived raw sequence reads has been carried out using the newest version of the ChIP-seq analysis command line pipeline, including the following steps: Alignment to the *mm10* mouse genome assembly was done by the BWA tool (41), and BAM files were created by *SAMTools* (42). Signals (peaks) were predicted by MACS2 (46), artifacts were removed according to the blacklist of ENCODE (47), and filtered for further analysis by removing low mapping quality reads (MAPQ score < 10), duplicated reads and reads located in blacklisted regions. All regions derived from at least any two samples were united within 0.5kb and those summits having the highest MACS2 peak score in any sample were assigned to each region. Promoter-distal regions were selected, excluding the TSS+/-0.5kb regions according to the mouse GRCm38.p1 (*mm10*) annotation version. Tag directories used by HOMER in the following steps were generated with a 120-nucleotide fragment length with makeTagDirectory (43). Genome coverage (bedgraph and tdf) files were generated by makeUCSCfile.pl (HOMER) and igvtools, respectively, and used for visualization with IGV2 (44). Coverage values were further normalized by the upper decile value detected in the consensus regions for each sample to minimize the inter-sample variance.

**Motif enrichment analysis**

Peaks derived from day 4 Ly6Clow muscle MFs and closer than 50 kb to any TSS of *Gpnmb* and other GFEM marker genes showing an expression dynamic similar to *Gpnmb* (Pearson correlation > 0.8) were determined by *intersectBed* (*bedtools* v2.27.1) (48) and used as inputs for a *de novo* motif enrichment analysis. The central 200 bp of the peaks were used as target sequences, and the enrichment of 10-, 12-, and 14-mers was determined by *findMotifsGenome.pl* (HOMER). P-values were calculated by comparing the number of target and random (background) sequences carrying a certain motif. To generate the motif lolliplot, we used *trackViewer* (49) to visualize individual motif prediction scores for selected DNA sequences.

**Chromatin interactions of the -23kb enhancer of the Gpnmb gene locus**

We applied a capture Hi-C (cHi-C) approach (50) to map chromatin interactions in the murine *Gpnmb* gene locus (chr6:49007000-49060000, mm10). Briefly, differentiated mouse BMDMs were cross-linked with 1% formaldehyde and stored as cell pellets at -20oC. Cells were lysed and processed using the Arima Hi-C kit according to the manufacturer’s protocol. A capture enrichment step was applied to the generated Hi-C libraries using custom-designed probes (SureSelect, Agilent Tier 2 -2,9Mb) targeting a selective group of myeloid-specific genomic regulatory elements, including the -23kb enhancer of the *Gpnmb* gene locus shown in **Fig. 8C**. cHi-C libraries were prepared with the Arima Hi-C kit and Agilent SureSelect library systems according to the manufacturers’s protocol. cHi-C raw sequence output was processed with *Hicup* (v0.6.1) to produce a filtered set of mapped interaction pairs in the mm10 genome. Interactions between virtual restriction fragments were detected using *Chicago* (v1.6.0) and custom weights calculated from high-confidence interactions in the data. Bigwig files containing mapped normalized interaction pairs were uploaded to the IGV browser to visualize chromatin loops of the -23kb enhancer of the *Gpnmb* gene locus together with ChIP-seq tracks.

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