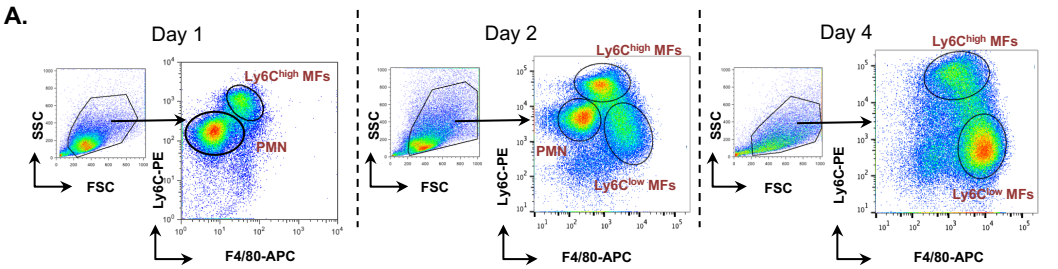
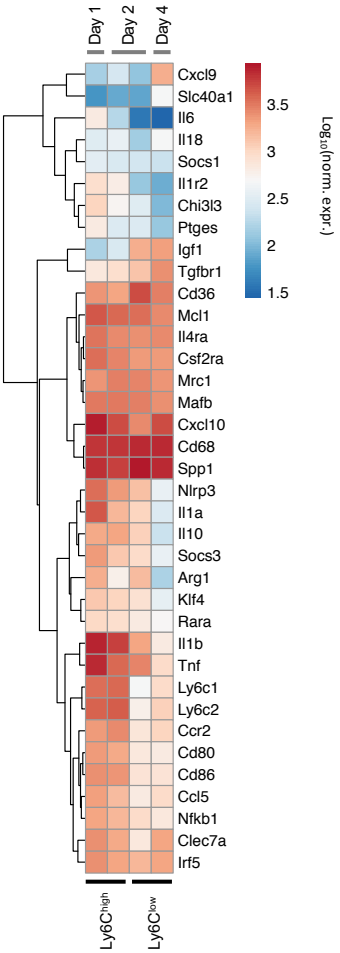
Supplementary Materials



B.



C.

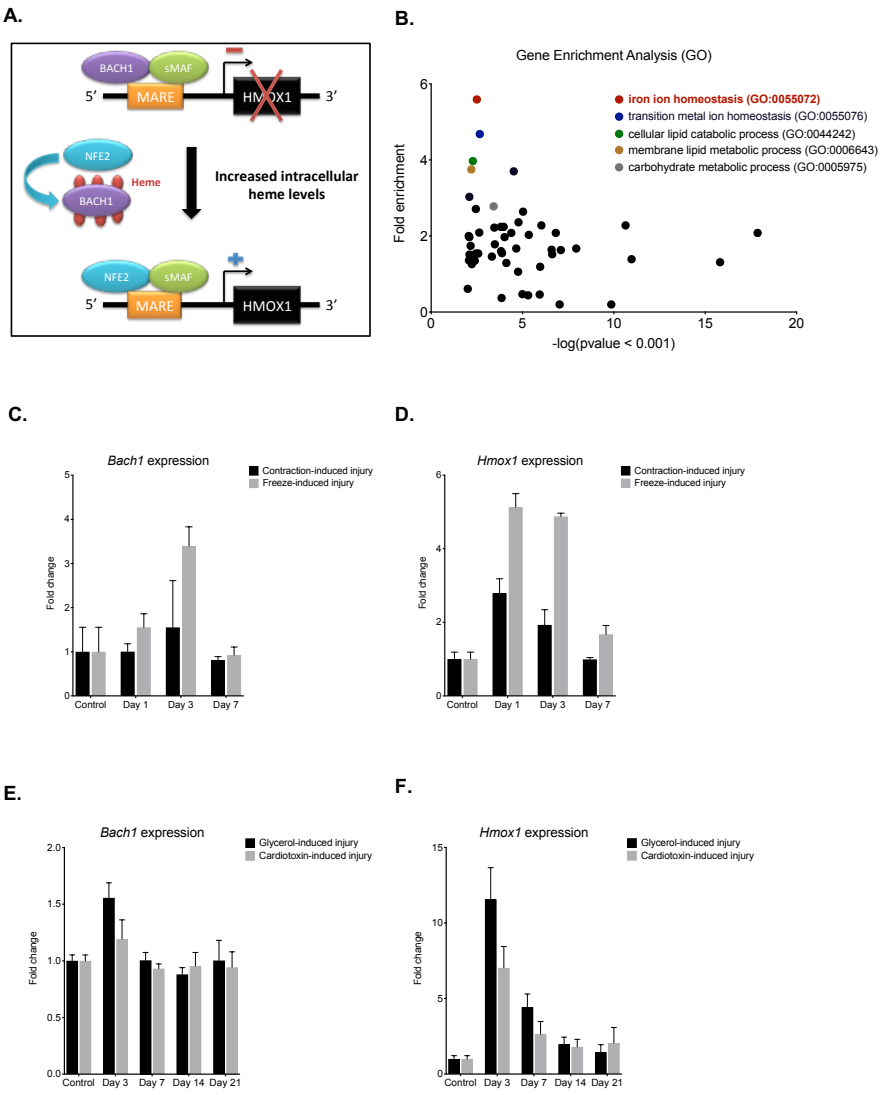
Logo	Motif Name	Score Threshold	Cluster 1 (%)	Cluster 2 (%)	Cluster 3 (%)	Cluster 4 (%)
	TRE (AP-1)	6.5	46.54	54.66	49.31	6.85
	MARE	7.5	10.59	12.85	13.38	3.60
	PU-box (PU.1)	7	19.21	17.41	15.97	38.20
	C/EBP	6	22.40	17.15	17.05	7.90
	GT-box (KLF)	8	12.57	10.98	10.29	2.06

Supplementary Fig. 1. Gating strategy and ATAC-seq based motif prediction in muscle-infiltrating macrophages.

A. FACS gating strategy for the analysis and sorting of macrophage subsets and neutrophils (PMNs) from CTX-injured muscles. Leukocytes were gated on FSC/SSC to discriminate live cells, and then markers for Ly6G, F4/80 and Ly6C were used to isolate them.

B. Heatmap showing the expression pattern of key inflammation and regeneration-related genes by regenerating Ly6C^{high} and Ly6C^{low} muscle-derived macrophages at indicated timepoints post CTX injury. Expression values are visualized as $\log_{10}$(normalized expression).

C. Motif enrichment in each of the four ATAC-seq clusters from muscle-infiltrating macrophages. Top 5 motifs predicted are shown. The motif matrices used in the analysis are indicated along with percentages of regions having a given motif and the lowered score thresholds.



Supplementary Fig. 2. Gene enrichment analysis of muscle infiltrating macrophages reveals iron homeostasis related pathways.

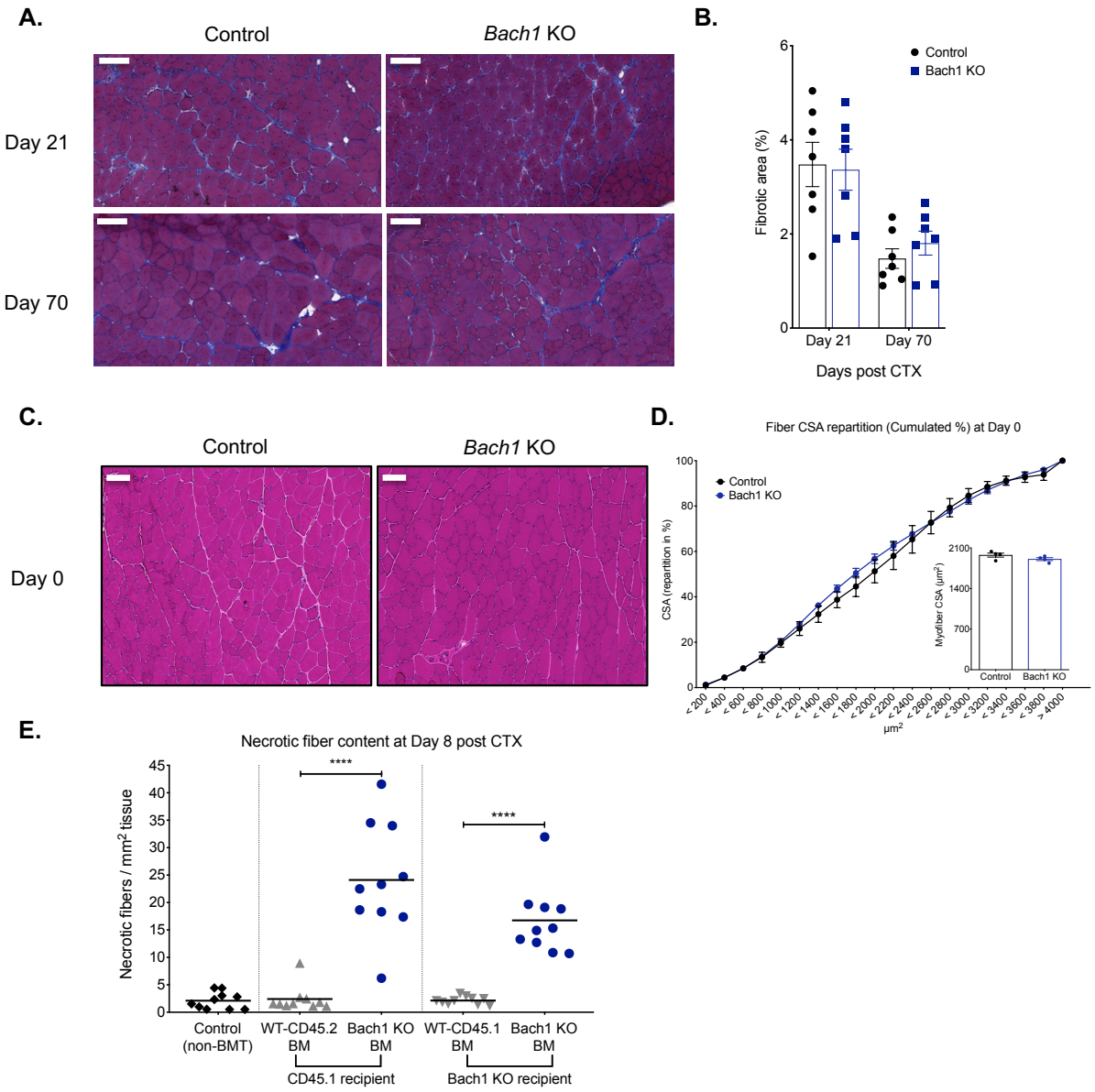
A. Model describing the regulation of *Hmox1* by BACH1 and heme. Maf-related factors may serve as partners for BACH1. BACH1 occupies MARE enhancers to repress transcription of *Hmox1* gene under normal conditions. An increase in heme levels alleviates BACH1-mediated repression through inhibition of its DNA-binding activity and subsequent nuclear export, making MAREs available for activating Maf complexes.

B. Gene enrichment/ontology (GO) analysis of the genes that are differentially expressed as inflammatory Day 1 Ly6C^{high} macrophages differentiate into reparatory Ly6C^{low} macrophages during muscle regeneration at day 4 post CTX injury. All terms shown have p value < 0.001.

C. and D. mRNA expression of *Bach1* and *Hmox1* from publicly available microarray data in contraction and freeze-induced injured muscles at timepoints indicated. Bars indicate fold changes over control.

E. and F. mRNA expression of *Bach1* and *Hmox1* from publicly available microarray data in glycerol and cardiotoxin-induced injured muscles at timepoints indicated. Bars indicate fold changes over control.

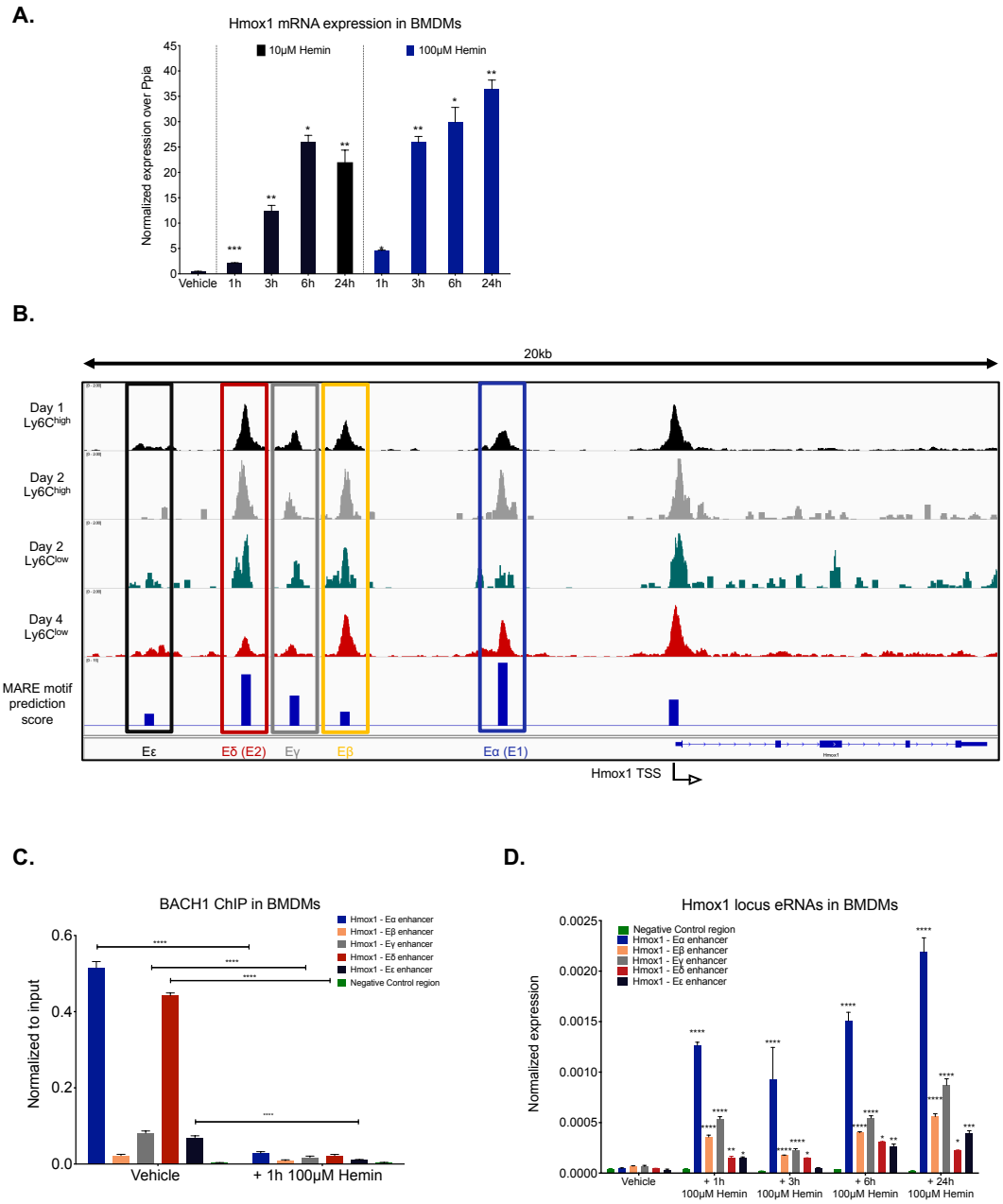
In all bar graphs, bars represent mean ± SEM.



Supplementary Fig. 3. BACH1 ablation doesn't affect development or muscle growth in uninjured animals.

A. Representative images of Masson's trichrome stained skeletal muscle from WT-control and *Bach1*-KO mice at day 21 and 70 post CTX injury. Scale bars in the upper left represent 100µm.
B. Fibrosis (connective tissue deposition) quantification in WT-control and *Bach1*-KO injured muscles expressed as the percentage of the area of fibrosis over total regeneration area (n=7 per group).
C. Representative images of H&E stained skeletal muscle from WT-control and *Bach1*-KO mice prior any injury (day 0) are shown. Scale bars in the upper left represent 100µm.
D. Fiber size repartition of uninjured muscle from WT-control and *Bach1*-KO animals. Mean fiber CSA is shown in the inset (n=6 per group).
E. The number of necrotic fibers relative to the regeneration area (in mm^2) at day 8 post CTX-injury in control (non-BMT) and BMT chimeric muscle sections are shown (n=10 per group).

In all graphs, bars and lines represent mean ± SEM.



Supplementary Fig. 4. Identification of novel regulatory elements around the *Hmox1* locus using ATAC-seq.

A. *Hmox1* mRNA expression in WT BMDMs treated with various doses of hemin at the indicated timepoints (n=6 per group).
B. Identification of possible enhancers upstream the *Hmox1* TSS (12kb) using muscle-derived macrophages ATAC-seq data. Putative enhancers are labeled by boxes and in the lower panel the motif prediction score for MARE is represented for each enhancer.
C. BACH1-ChIP on the putative enhancer regions in BMDMs reveal BACH1 binding in all marked enhancers around the *Hmox1* locus with Eα and Eδ being the strongest ones. Heme treatment for 1 hour alleviates BACH1 binding validating our results (n=6 per group).
D. *Hmox1* enhancer RNA measurements in BMDMs upon heme treatment at various timepoints (n=6 per group).

In all bar graphs, bars represent mean ± SEM.