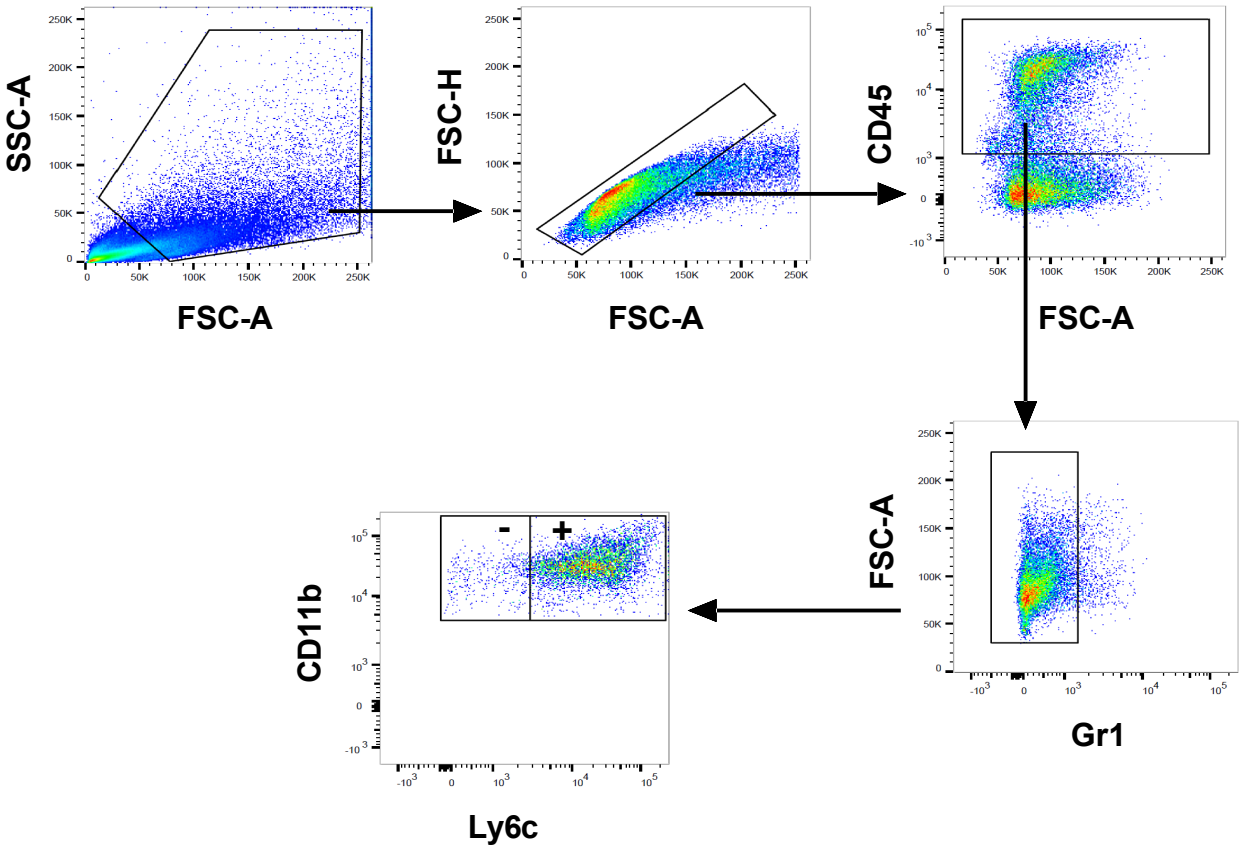
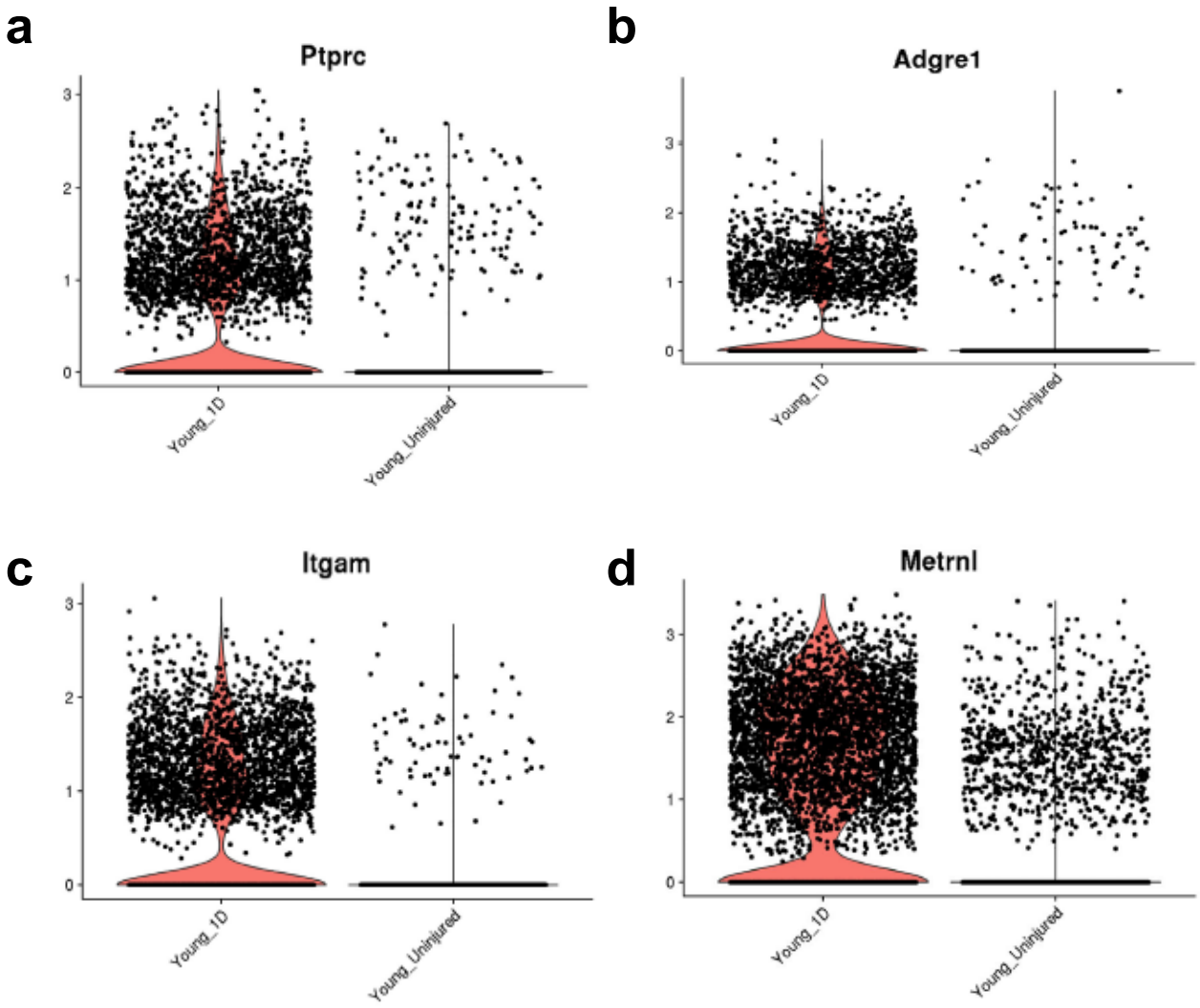
Supplementary Figure 1



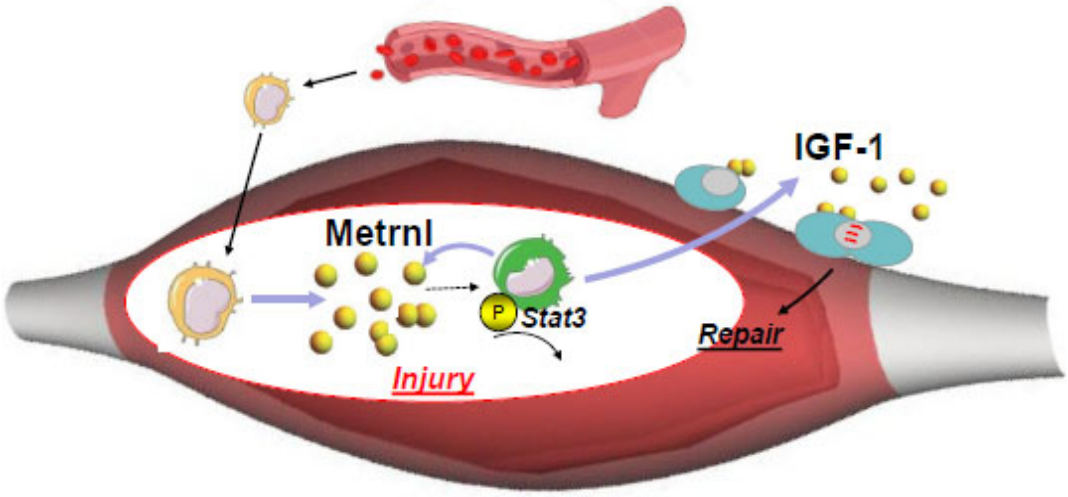
Supplementary Figure 1. Gating strategy for identifying immune cell inflammatory phenotype of the TA muscle. Single-cell suspensions were prepared from uninjured and injured TA muscles and were analyzed by flow cytometry for the indicated markers. Top row of plots: gating strategy to isolate non-granular myeloid cells ($CD45^+$, $Gr1^-$, $CD11b/Mac1^+$) and quantify Ly6c expression.

Supplemenatry Figure 2



Supplementary Figure 2. Metrnl gene expression after injury is associated with myeloid-based immune cell influx. Violin plots from single cell RNAseq analysis. Plots of (A). Ptprc, (B). Adgre1, (C). Itgam and (D). Metrnl. Each dot represents individual cells and their relative gene expression. (N=1/group).

Supplementary Figure 3



Supplementary Figure 3. Working model for Metrnl regulation on skeletal muscle regeneration.

Our working model showing the influx of pro-inflammatory macrophages (orange) into the injured muscle secreting Metrnl in an autocrine/paracrine manner to activate Stat3 and stimulate the transformation to an anti-inflammatory phenotype (green). The anti-inflammatory macrophages secrete Metrnl as well, acting in a paracrine manner to incoming macrophages. In addition, the Metrnl-induced induction of IGF-1 is secreted from the macrophage promoting local satellite cell proliferation and eventual myogenesis and repair of the injured tissue.

Supplementary Table 1: Primers sets used in this manuscript

Gene Name	Forward Primer	Reverse Primer	Species
Arg1	CTCCAAGCCAAAGTCCTTAGAG	GGAGCTGTCATTAGGGACATCA	mouse
Chi3l3	CAGGTCTGGCAATTCTTCTGAA	GTCTTGCTCATGTGTGTAAGTGA	mouse
Mrc1	CTCTGTTCAGCTATTGGACGC	TGGCACTCCCAAACATAATTTGA	mouse
IL-10	CTTACTGACTGGCATGAGGATCA	GCAGCTCTAGGAGCATGTGG	mouse
TNF-α	CAGGCGGTGCCTATGTCTC	CGATCACCCCGAAGTTCAGTAG	mouse
IL-1β	GAAATGCCACCTTTTGACAGTG	TGGATGCTCTCATCAGGACAG	mouse
Nos2	GTTCTCAGCCCAACAATACAAGA	GTGGACGGGTCGATGTCAC	mouse
IL-6	CTGCAAGAGACTTCCATCCAG	AGTGGTATAGACAGGTCTGTTGG	mouse
Metrnl	TCTGTGGAGTGGATGTACCCA	CCGCACCAACAGTCTTAGTTC	mouse
IGF-1	CACATCATGTCGTCTTCACACC	GGAAGCAACACTCATCCACAATG	mouse
SOCS3	TGCGCCTCAAGACCTTCAG	GCTCCAGTAGAATCCGCTCTC	mouse
Bcl3	CCGGAGGCCCTTTACTACCA	GGAGTAGGGGTGAGTAGGCAG	mouse
TBP	ATGATGCCTTACGGCACAGG	GTTGCTGAGATGTTGATTGCTG	mouse
Metrnl	AGTGGATGTACCCAACAGGTG	TACCAGCAGTCTCAGTTCTCC	human
TBP	CCACTCACAGACTCTCACAAC	CTGCGGTACAATCCCAGAACT	human

Supplementary Table 2: Antibodies used in this manuscript

Reagent	Cat #	Concentration	Company
BrdU-PE	12-5071-42	1:250	invitrogen/ebiosource
eMHC	BF-45/F1.652	1:40	Developmental Studies Hybridoma Bank, Iowa City, IA
Rat anti mouse Alexa Flour 570	41-4015-82	1:2000	Life Technologies
phosho Stat3 (tyr705)	9131	1:1000	Cell Signaling, Beverly, MA
mouse anti rabbit 2nd Ab	7074	1:2000	Cell Signaling, Beverly, MA
total Stat3	9139	1:1000	Cell Signaling, Beverly, MA
VCAM -PE	105703	1:200	Biolegend
CD45 -PE Cy7	25-0451-82	1:200	invitrogen/ebiosource
CDIIb -488	53-0112-82	1:200	invitrogen/ebiosource
Ly6c -APC	128015	1:200	Biolegend
Ly6g/Gr1 -702	67-966-880	1:200	eBioscience