Figure S1

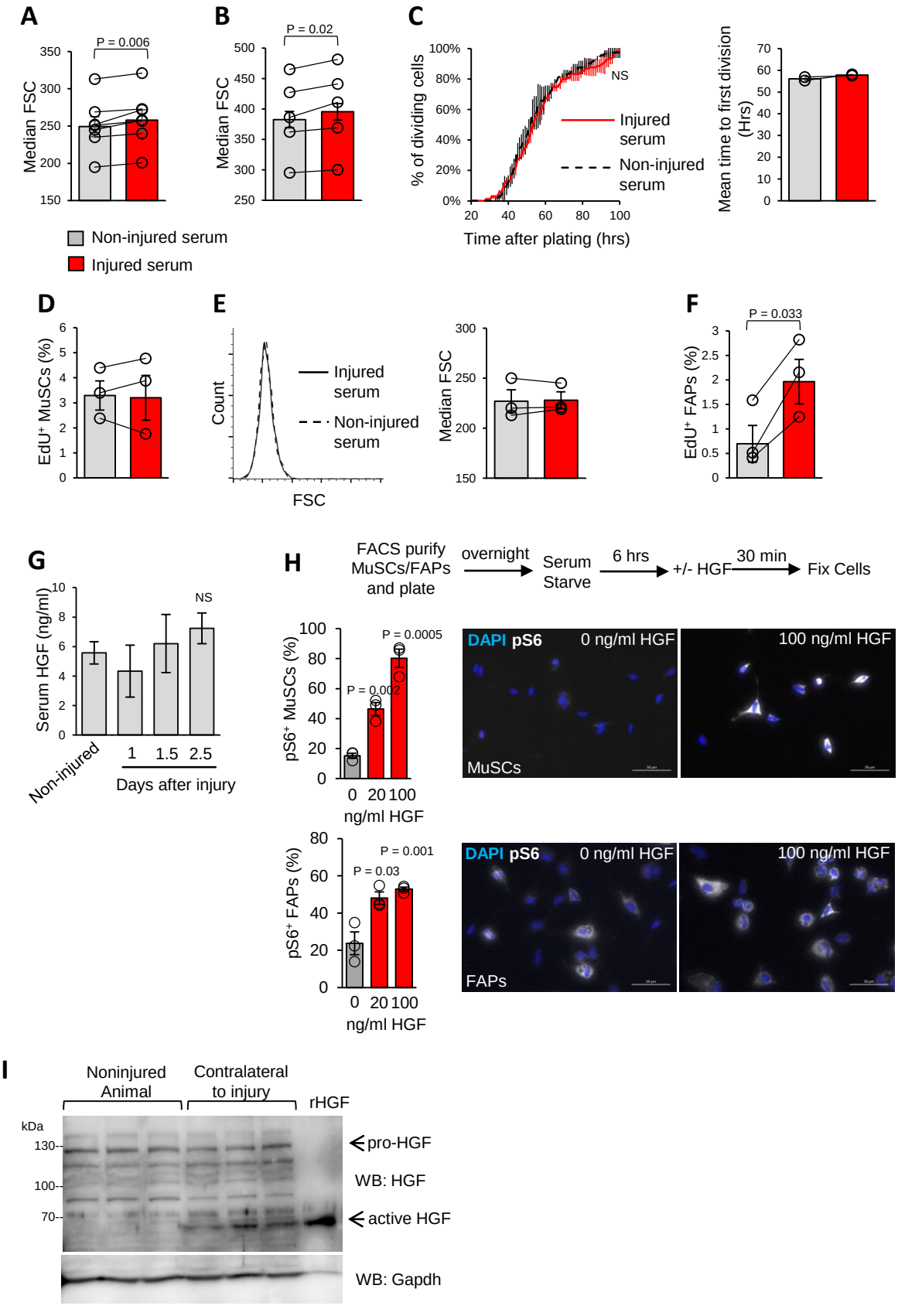

A
P = 0.006
Median FSC
Non-injured serum
Injured serum
B
P = 0.02
Median FSC
C
% of dividing cells
NS
Injured serum
Non-injured serum
Time after plating (hrs)
Mean time to first division (Hrs)
D
EdU+ MuSCs (%)
E
Count
FSC
Injured serum
Non-injured serum
Median FSC
F
P = 0.033
EdU+ FAPs (%)
G
Serum HGF (ng/ml)
NS
Non-injured
1
1.5
2.5
Days after injury
H
FACS purify MuSCs/FAPs and plate
overnight
Serum Starve
6 hrs
+/- HGF
30 min
Fix Cells
pS6+ MuSCs (%)
P = 0.0005
P = 0.002
0 20 100
ng/ml HGF
DAPI pS6
0 ng/ml HGF
100 ng/ml HGF
MuSCs
pS6+ FAPs (%)
P = 0.001
P = 0.03
0 20 100
ng/ml HGF
DAPI pS6
0 ng/ml HGF
100 ng/ml HGF
FAPs
I
Noninjured Animal
Contralateral to injury
rHGF
kDa
130
100
70
pro-HGF
WB: HGF
active HGF
WB: Gapdh

Figure S1. cMet is required for the MuSC response to injured serum. *Relates to Figure 1*

A) MuSCs in animals injected with injured serum are larger. Data from FSC measurements of MuSCs by FACS are presented as geometric mean ± s.e.m. (n = 7).

B) FAPs in animals injected with injured serum are larger. Data from FSC measurements of FAPs by FACS are presented as geometric mean ± s.e.m. (n = 5).

C) MuSCs from cMet cKO animals display no changes in time to first division in response to injured serum. A cumulative histogram of the time to first division is displayed on the left (n = 2) and a bar graph of the mean time to division on the right; NS denotes not statistically significant.

D) Injured serum does not improve the cell cycle entry kinetics of cMet cKO MuSCs. Data from EdU incorporation assays are presented as geometric mean ± s.e.m. of the percentage of cells which were EdU^+ 24 hours after isolation (n = 3).

E) Injured serum does not increase the size of cMet cKO MuSCs. Data are presented as a representative FACS histogram of the FSC parameter (left) and a bar graph of replicate experiments (right) (geometric mean ± s.e.m.; n = 3).

F) MuSC-specific cKO of cMet does not alter the response of FAPs to injured serum. A higher percentage of FAPs isolated from cMet cKO mice ($cMet^{flox/flox}$;$Pax7^{CreER/+}$;$Rosa26^{EYFP/+}$) injected with injured serum incorporated EdU after 24 hours in culture. Data from EdU incorporation assays are presented as a bar graph of the geometric mean ± s.e.m. (n = 3).

G) Serum HGF levels do not change in response to injury. Serum samples were prepared from non-injured or injured mice at indicated days after injury and HGF levels were measured by ELISA. Bar graphs represent mean ± s.e.m. (non-injured, n = 26; 1 day, n = 5; 1.5 days, n = 4; 2.5 days, n = 15; NS denotes not statistically significant).

H) MuSCs and FAPs induce mTORC1 activity in response to *ex vivo* HGF stimulation. The day following isolation, serum starved MuSCs and FAPs were stimulated with indicated levels of recombinant, active HGF. Thirty minutes after stimulation, cells were fixed and mTORC1 activity was assayed by IF staining for pS6. Data are displayed as percentages of cells that are $pS6^+$ (mean ± s.e.m., n = 3). Displayed on the right are representative images of pS6 staining of MuSCs and FAPs treated with 0 or 100 ng/ml HGF. The scale bar is 50 μm.

I) Levels of active HGF are increased in muscles contralateral to injury. Western blotting of whole TA muscle tissue extracts showed a clear increase in active HGF in muscles that were contralateral to the site of muscle injury (2.5 days after injury). Purified recombinant active human HGF (rHGF) was run as control for the size of active HGF.

Figure S2

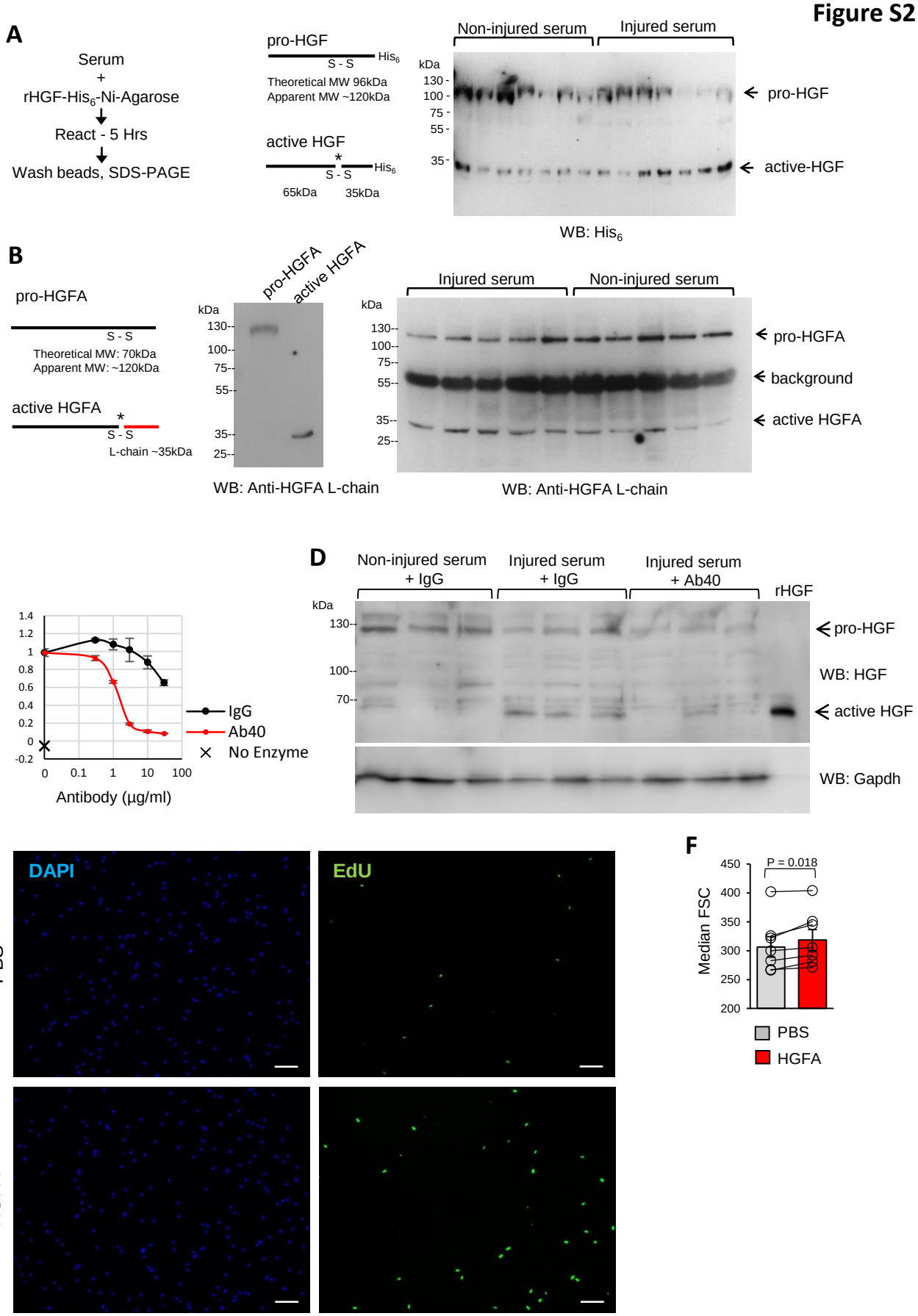

A
Serum
+
rHGF-His6-Ni-Agarose
React - 5 Hrs
Wash beads, SDS-PAGE
pro-HGF
His6
S - S
Theoretical MW 96kDa
Apparent MW ~120kDa
active HGF
*
S - S
His6
65kDa
35kDa
Non-injured serum
Injured serum
kDa
130 -
100 -
75 -
55 -
35 -
pro-HGF
active-HGF
WB: His6
B
pro-HGFA
S - S
Theoretical MW: 70kDa
Apparent MW: ~120kDa
active HGFA
*
S - S
L-chain ~35kDa
pro-HGFA
active HGFA
kDa
130--
100--
75--
55--
35--
25--
WB: Anti-HGFA L-chain
Injured serum
Non-injured serum
kDa
130--
100--
75--
55--
35--
25--
pro-HGFA
background
active HGFA
WB: Anti-HGFA L-chain
C
Reaction Rate (Δ fl.units/min)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-0.2
0
0.1
1
10
100
Antibody (µg/ml)
IgG
Ab40
No Enzyme
D
Non-injured serum + IgG
Injured serum + IgG
Injured serum + Ab40
rHGF
kDa
130--
100--
70--
pro-HGF
WB: HGF
active HGF
WB: Gapdh
E
PBS
HGFA
DAPI
EdU
F
P = 0.018
Median FSC
450
400
350
300
250
200
PBS
HGFA

Figure S2. The activity of serum HGFA increases following injury. *Relates to Figure 1.*

A) Injured serum has greater HGF-processing activity than non-injured serum. Displayed is a schematic depiction of HGF-processing assay (left) and of pro- and active HGF proteins (middle). On the right is Western blot analysis of the HGF-processing reactions.

B) Injured serum contains a higher proportion of HGFA in the active form. Displayed on the left is a schematic depiction of pro- and active HGFA proteins. In the middle is detection and molecular weight validation of pro- and active HGFA using purified recombinant proteins by Western blotting. On the right is Western blotting of injured and non-injured serum samples with HGFA antibodies.

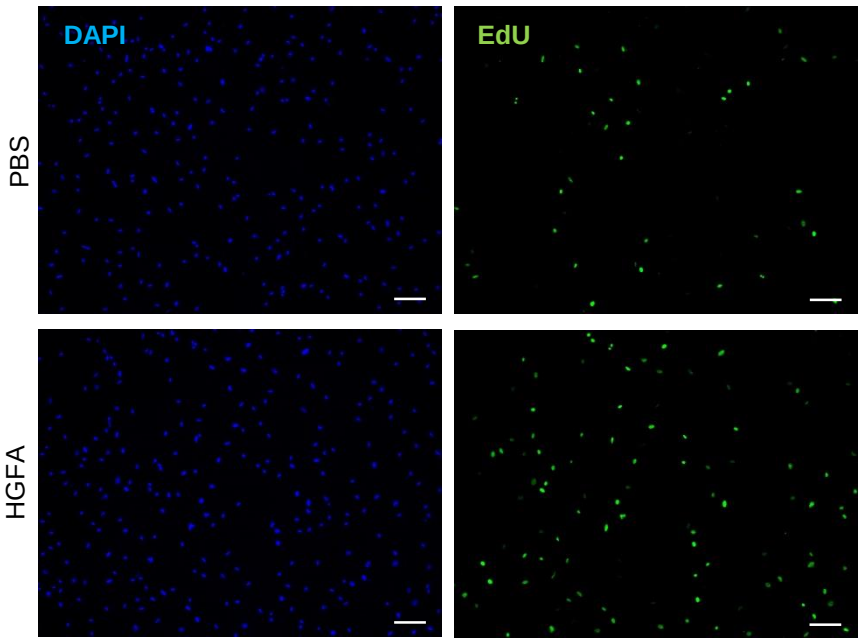
C) Ab40 inhibits HGFA enzymatic activity *in vitro*. Purified mouse recombinant HGFA was mixed with Ab40 or control IgG and HGFA enzymatic activity measured by cleavage of a fluorogenic substrate peptide. Data are displayed as mean ± s.e.m. of the reaction rate at each antibody concentration.

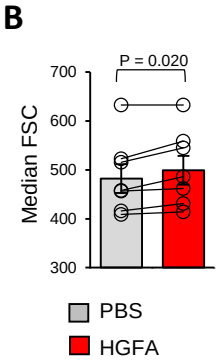
D) Ab40 blocks the injured serum-mediated processing of pro-HGF into active HGF *in vivo*. Western blotting of whole TA muscle extracts shows a clear increase in the amount of active HGF compared to animals injected with non-injured serum. Animals injected with injured serum that was incubated with Ab40 show a strong decrease in the amount of active-HGF. Purified recombinant active HGF was run as a control for the molecular weight of active HGF.

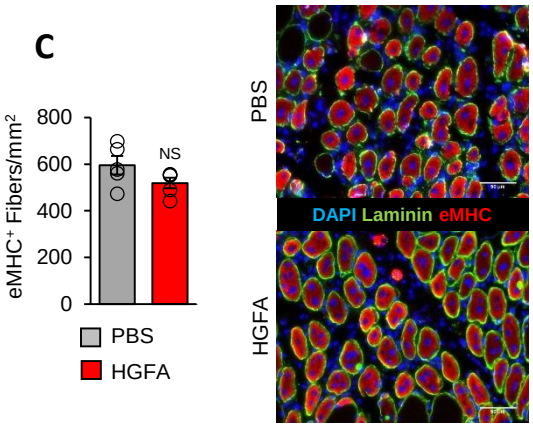
E) HGFA improves the cell cycle entry kinetics of MuSCs. Data presented are representative images of EdU incorporation assays of MuSCs isolated from PBS- and HGFA-injected mice after 24 hours in culture. The scale bar is 100 μm.

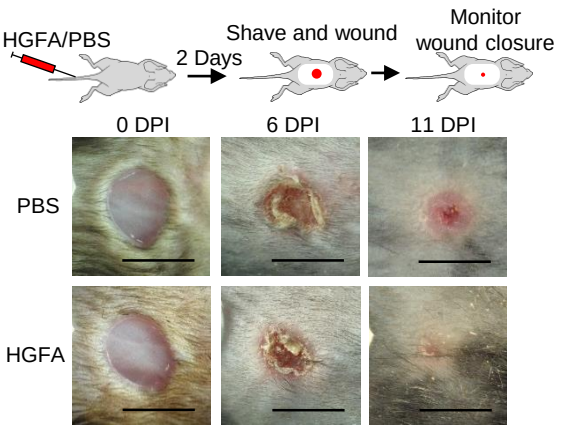
F) MuSCs in animals injected with HGFA are larger. Data from FSC measurements of MuSCs by FACS are presented as geometric ± s.e.m. of replicate experiments (n = 7).

Figure S3









E

Antibody	host	clone	company	catalog	Notes
Pax7	mouse	mAb	DSHB	Pax7	1:50 with M.O.M kit
eMHC	mouse	mAb	DSHB	F1.652	1:10 with M.O.M kit
PDGFR-alpha	goat	poly	R&D systems	AF1062	1:100, stained overnight
phospho-S6 (Ser235/236)	rabbit	D57.2.2E	Cell Signaling	4845	1:100, stained overnight
Laminin	rat	4H8-2	Abcam	ab11576	1:500
CD31-FITC	rat	MEC13.3	Biolegend	102506	1.5ug per muscle preparation
CD31-APC	rat	MEC13.3	Biolegend	102510	1.5ug per muscle preparation
CD45-FITC	rat	30-F11	Biolegend	103108	1.5ug per muscle preparation
CD45-APC	rat	30-F11	Biolegend	103112	1.5ug per muscle preparation
Sca-1-PacBlue	rat	D7	Biolegend	108120	2ug per muscle preparation
VCAM-Biotin	rat	429	BD Bioscience	553331	4ug per muscle preparation
HGF	rabbit	poly	SCBT	sc-7949	WB: 1:1000
HGF	goat	poly	R&D systems	AF2207	WB: 1:1000
HGFA (L-chain, catalytic domain)	goat	poly	SCBT	sc-1373	WB: 1:1000
His6	rabbit	poly	Cell Signaling	2365	WB: 1:1000
GAPDH-HRP	rabbit	poly	SCBT	sc-25778	WB: 1:5000
Streptavidin-PE-Cy7			Biolegend	405206	4ug per muscle preparation
Streptavidin-Alexa 594			ThermoFisher	S11227	1:500
anti-Rabbit-Alexa 647	donkey		ThermoFisher	A31573	1:500
anti-Rat-Alexa 488	donkey		ThermoFisher	A21208	1:500
anti-Goat-Alexa 594	donkey		ThermoFisher	A11058	1:500
Ab40	human	mAb	Genentech		50µg of Ab to 100µL of serum

Figure S3. HGFA improves stem cell activation and tissue repair. *Relates to Figure 3.*

A) HGFA improves the cell cycle entry kinetics of FAPs. Data presented are representative images of EdU incorporation assays of FAPs isolated from PBS- and HGFA-injected mice after 40 hours in culture. The scale bar is 100 µm.

B) FAPs in animals injected with HGFA are larger. Data from FSC measurements of FAPs by FACS are presented geometric ± s.e.m. of replicate experiments (n = 7).

C) HGFA does not affect the density of regenerating myofibers in injured muscle. Data are presented as mean ± s.e.m. of the number of $eMHC^+$ regenerating myofibers per mm^2 of injured muscle at 5 DPI (n = 5); NS denotes not statistically significant. Panels on the right are representative images of damaged muscles in PBS- and HGFA-injected mice. The scale bar is 50 µm.

D) Schematic depiction of skin wound healing experiments and representative images showing the HGFA-induced acceleration of skin wound closure. The scale bar is 5 mm.

E) Table detailing the antibodies used.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES (Relates to Experimental Procedures)

SDS-PAGE and Western Blotting
Serum samples were diluted 1:10 with PBS and 5 µL of diluted serum was subject to reducing SDS-PAGE.
Whole muscle tissue extracts were prepared by boiling 15 10 µm TA muscle cryosections in SDS sample buffer prior to reducing SDS-PAGE.

HGF quantification
HGF levels were measured using the Mouse/Rat HGF Quantikine Elisa kit (R&D Systems). Serum samples were diluted 1:10 with PBS for ELISA analysis. Muscle HGF quantifications were performed using whole muscle tissue extracts and measurements of HGF were normalized to the muscle tissue extract protein concentration.

***Ex vivo* HGF stimulation**
Twelve hours after isolation and maintenance in culture medium, MuSCs and FAPs were washed twice with 1X PBS (HyClone) and medium was replaced with serum starvation medium (Hams F10 (Cellgro), 0.2% FA-free BSA (Alfa Aesar J64949), and 1x Pen/Strep (Gibco)). Six hours later, the medium was aspirated and replaced with serum starvation medium with or without addition of purified recombinant active HGF (Life Technologies). Cells were fixed 30 minutes after HGF stimulation.

HGF Processing Assay
Briefly, purified recombinant pro-HGF was incubated with serum samples and the amount of HGF processing was determined by SDS-PAGE and Western blotting. Purified recombinant pro-HGF was prepared by transfecting HEK 293F cells (Invitrogen) with plasmids containing the entire cDNA sequence of human pro-HGF with a C-terminal myc-His6 tag (pcDNA4c-HGF-myc-His). Media from transfected cells was collected and pro-HGF was purified using Ni-agarose beads (Sigma). Serum samples were prepared as described above. Approximately 200 ng of pro-HGF, bound to Ni-Agarose beads, was mixed with 30 µL of serum, the volume was brought to 500 µL with reaction buffer (20 mM Tris pH 8.3, 150 mM NaCl, 2.5 mM $CaCl_2$), and samples were incubated, with agitation, for five hours at room temperature. Following the reactions, the samples were washed two times with reaction buffer, pelleting the Ni-Agarose beads between washes, and once with PBS 0.3% Triton. Samples were resolved by reducing SDS-PAGE and processing of HGF was determined by Western blotting using an anti-His_6 antibody to detect the proportion of HGF that was in the processed form (~35 kDa) versus total HGF (active HGF plus pro-HGF). Quantification of Western blots was performed using a BioRad Versa Doc system and quantification software.

HGFA enzyme assay
Briefly, HGFA was mixed with Ab40 or control IgG and enzymatic activity was determined by measuring the cleavage of a fluorogenic substrate peptide. In a final volume of 100 µL, 100 ng of purified, recombinant, active, mouse HGFA (R&D Systems) was mixed with differing amounts of Ab40 or control IgG antibodies and HGFA substrate peptide (R&D Systems #ES002) (10 µM final concentration) in reaction buffer (described above). Reactions were monitored in a fluorescence plate reader by taking measurements every minute for 20 minutes. Reaction rates were determined by calculating the slope of the fluorescence vs. time plot.

Wheel running experiments
Wheel running studies were performed by administering HGFA or PBS to animals one day before transferring and singly housing them in computer-monitored wheel running cages (Lafayette, model 80820). One day after the being placed in wheel running cages, animals were subject to $BaCl_2$-mediated muscle injury to the left gastrocnemius muscle and returned to wheel running cage. Data in wheel running experiments are presented as the distance the animal ran in the previous 24-hour period (i.e. day 0 represents running distance in the 24-hours prior to muscle injury).

HGFA blocking antibody
HGFA blocking antibody (Ab40) was obtained from Dr. Daniel Kirchhofer (Genentech) (Ganesan et al., 2009). Prior to injection into mice, 100 µL of non-injured or injured serum was mixed with 50 µg of Ab40 or control IgG, brought to a final volume of 200 µL with sterile PBS, and incubated for 30 minutes on ice. Serum-antibody mixtures were then administered to mice by intravenous tail vein injection.

Skin Wounding
Skin wounding was performed using a biopsy punch. Briefly, 12 week old male C56BL6 mice were anesthetized using isoflurane. The back fur was shaven and the skin was cleaned with isopropanol and Betadine. The back skin was pulled using forceps and two 5-mm full-thickness skin wounds were created along the midline using a sterile 5 mm circular biopsy punch by pressing through both layers of the skin pull. Animals were then administered buprenorphine and Baytril and allowed to recover. Skin wound healing was measured every 2-3 days by anesthetizing the animals and imaging the wounded area.