Supplementary Materials 1

The pancreatic cancer secretome alters cells in the tumor microenvironment through the STAT3 pathway to induce a muscle atrophic phenotype.

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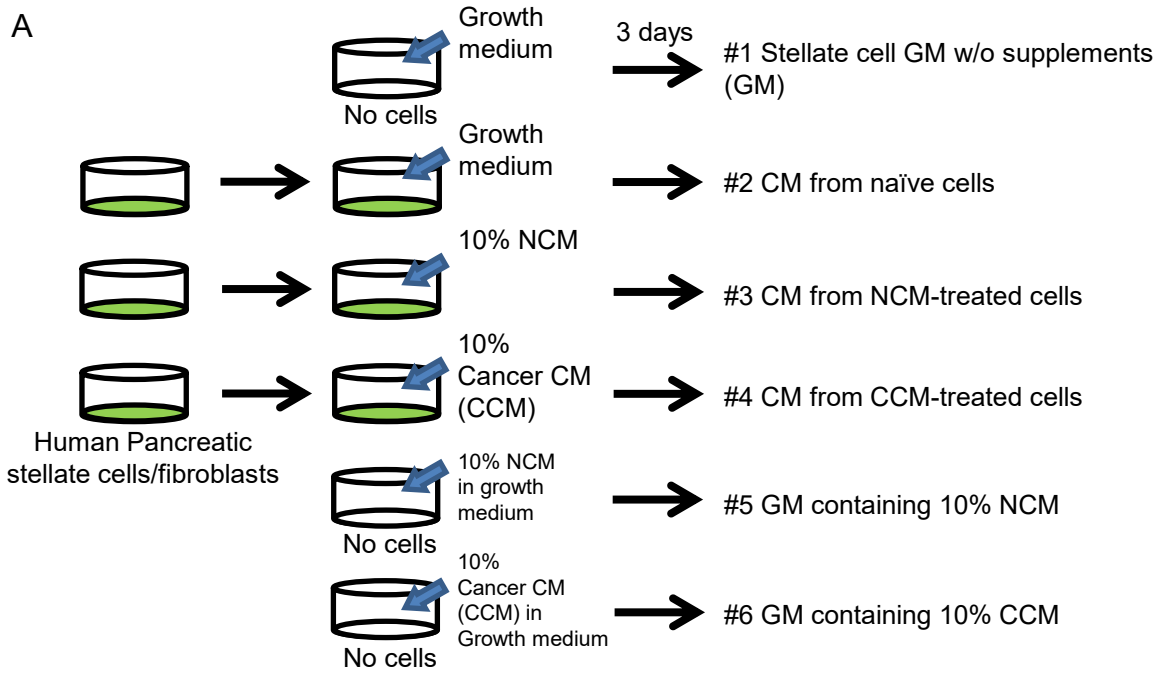
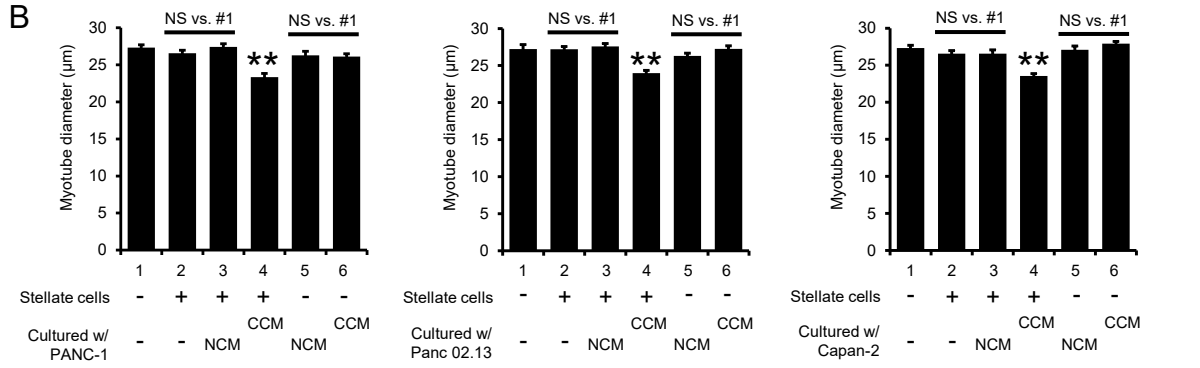
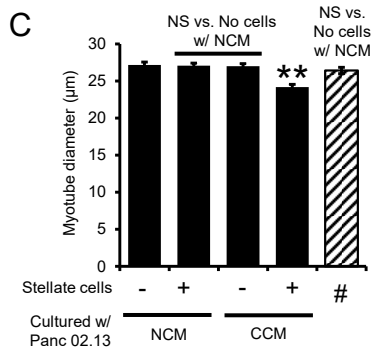
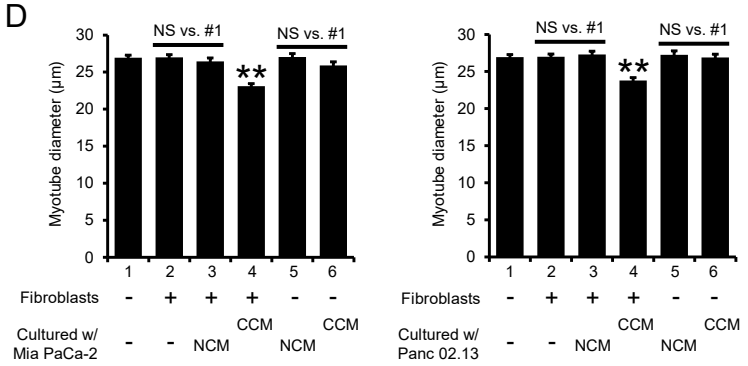
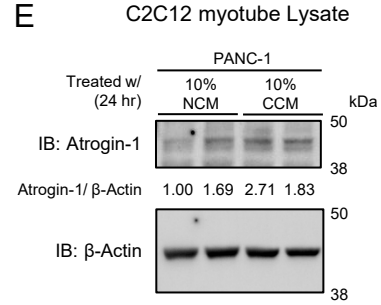






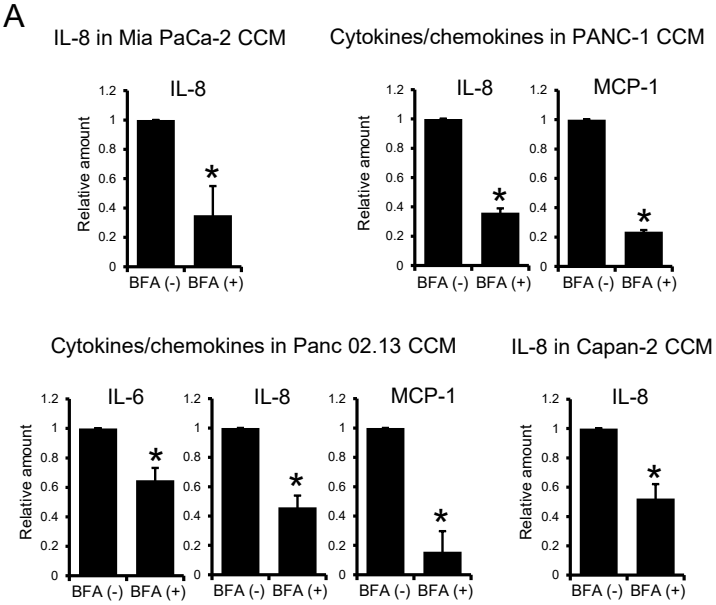
#: Mixture (10% Panc 02.13 CCM + 90% naïve hTERT-HPaSteC) was added to C2C12 myotubes.



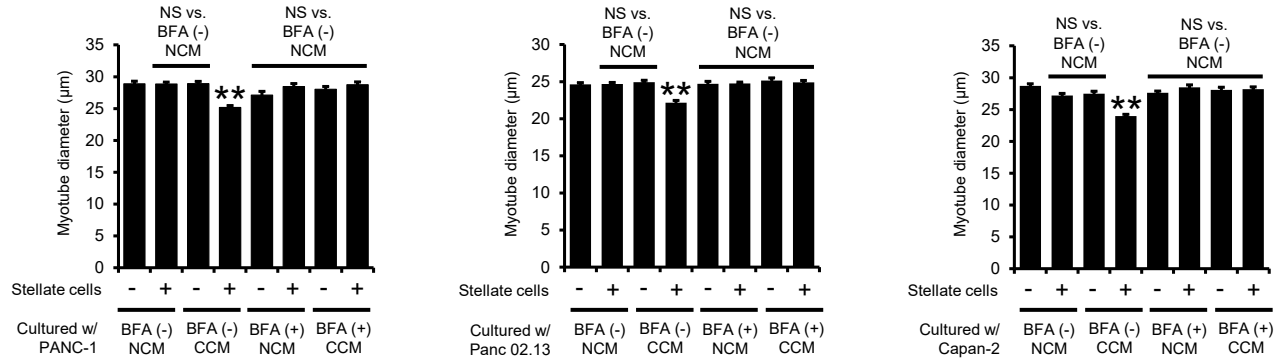


Supplemental Figure S1. Conditioned medium from pancreatic cancer cells induces a muscle atrophic phenotype in pancreatic stellate cells and fibroblasts. (A) A Schematic diagram on the treatment of stellate cells and fibroblasts with cancer conditioned medium (CCM). (B) hTERT-HPaSteC were treated with nonconditioned medium (NCM) or CCM from PANC-1, Panc 02.13, and Capan-2 cells as shown in (A), and CM from stellate cells was collected. Differentiated C2C12 myotubes were cultured with 10% stellate cell growth medium with FBS and without growth supplements (GM) or CM from hTERT-HPaSteC for 2 days. The myotubes were stained with MHC, and the diameter was measured. (C) CCM from Panc 02.13 cells and CM from naïve hTERT-HPaSteC were collected separately. One volume of Panc 02.13 CCM was mixed with 9 volumes of hTERT-HPaSteC CM (final concentration was 10% Panc 02.13 CCM in GM), and the mixture was added to C2C12 myotubes (denoted with #). After 2 days, the diameter was measured. (D) The myotubes were cultured with 10% fibroblast growth medium or CM from pancreatic fibroblasts (hTERT-HPaFB) treated with 10% CCM for 2 days, and the diameters were measured. (E) C2C12 myotubes were treated with 10% nonconditioned medium (NCM) or CCM from PANC-1 cells for 24 hours. Cells were lysed, and Atrogin-1 protein expression was measured. Full-length blots are presented in Supplementary Materials 2. The data shown for the diameter of the myotubes are representative of three independent experiments and shown as a mean ± S.E. (n=44 myotubes). **$p < 0.01$ vs. all other groups; NS: not significant.

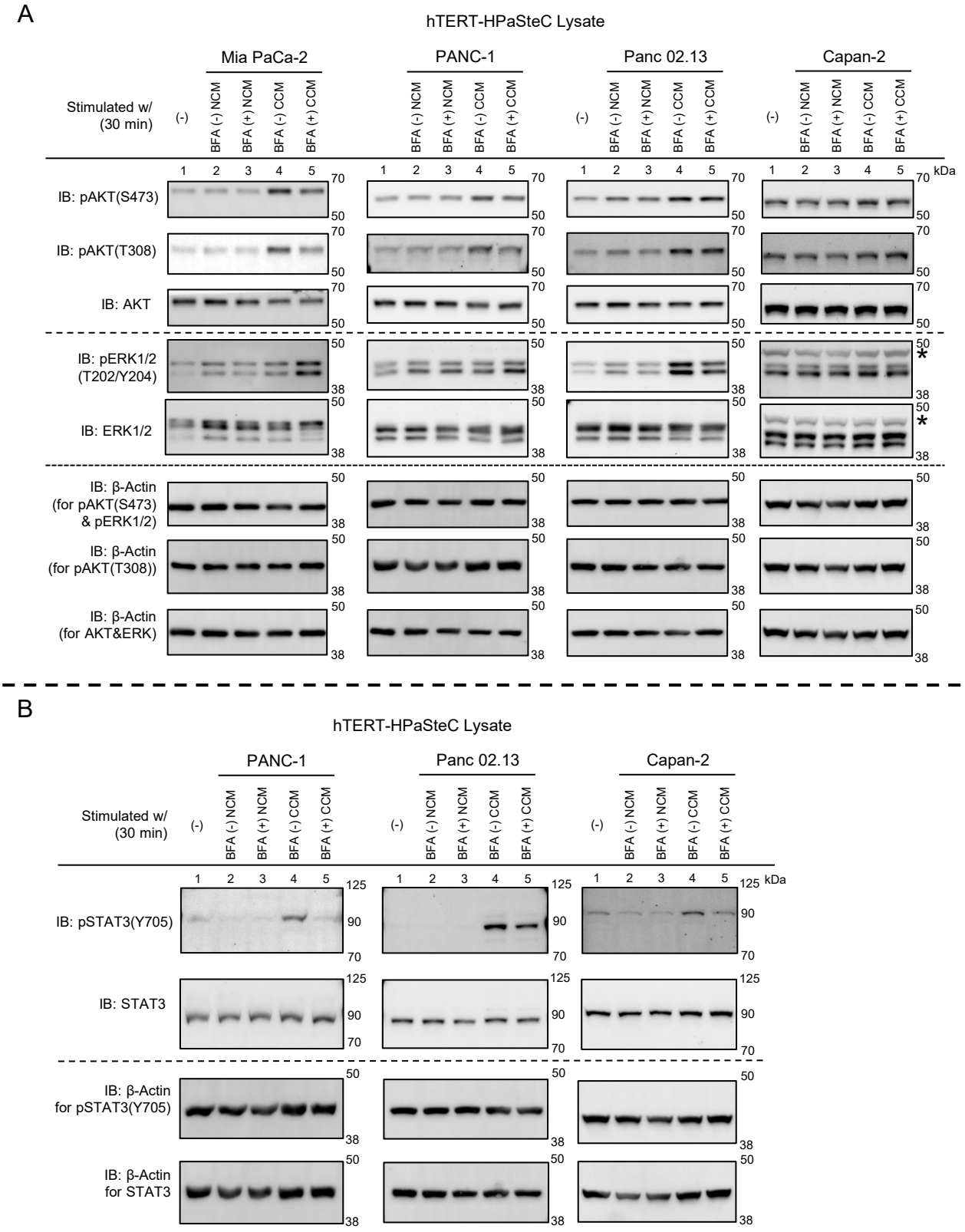
A


IL-8 in Mia PaCa-2 CCM
Cytokines/chemokines in PANC-1 CCM
IL-8
MCP-1
Relative amount
BFA (-) BFA (+)
Cytokines/chemokines in Panc 02.13 CCM
IL-8 in Capan-2 CCM
IL-6

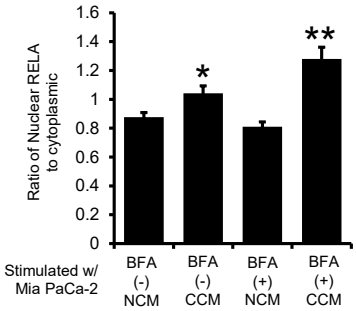
B


NS vs. BFA (-) NCM
Myotube diameter (μm)
Stellate cells
Cultured w/ PANC-1
Cultured w/ Panc 02.13
Cultured w/ Capan-2
BFA (-) NCM
BFA (-) CCM
BFA (+) NCM
BFA (+) CCM

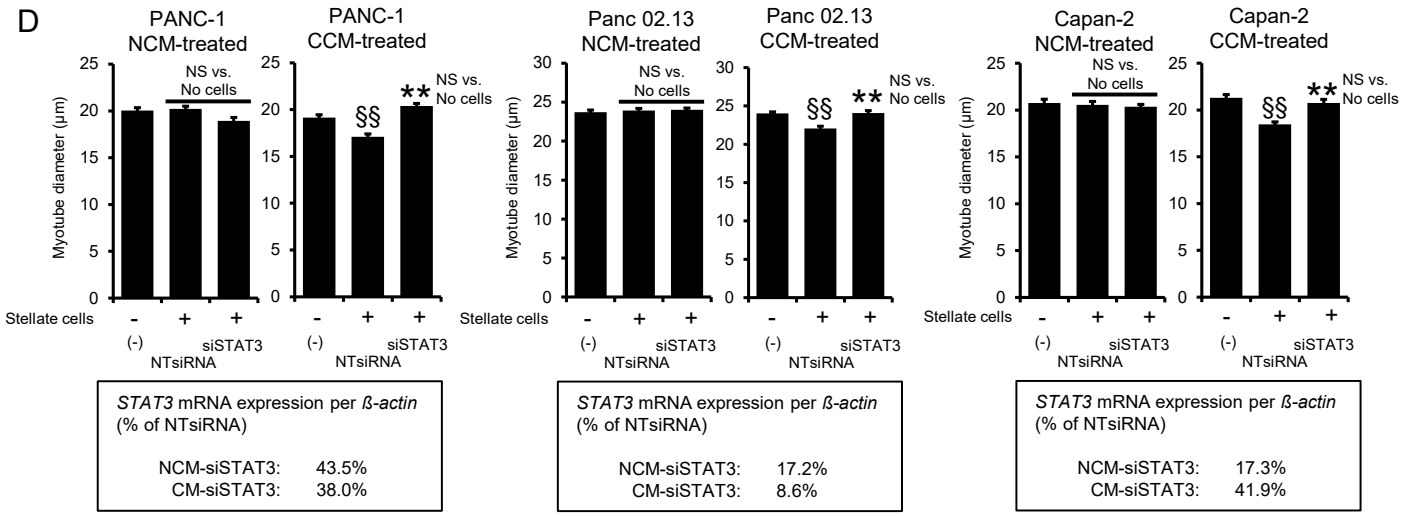
Supplemental Figure S2. CCM from brefeldin A (BFA)-treated cancer cells does not induce atrophy in pancreatic stellate cells. (A) The relative amounts of IL-6, IL-8, and MCP-1 in CCM from BFA-treated to nontreated cancer cells. (B) PANC-1, Panc 02.13, and Capan-2 cells were treated with or without BFA for 24 hours, after which the CCM was collected. hTERT-HPaSteC were cultured with CCM from 10% BFA-treated or nontreated cells for 3 days, and CM from hTERT-HPaSteC was subjected to myotube atrophy assay. The cytokine data are shown as the mean ± S.E. from three independent experiments. *$p < 0.05$ vs. CCM from nontreated cancer cells. The data shown for the diameter of the myotubes are representative of three independent experiments and shown as a mean ± S.E. (n=44 myotubes). **$p < 0.01$ vs. all other groups; NS: not significant.


A
hTERT-HPaSteC Lysate
Mia PaCa-2
PANC-1
Panc 02.13
Capan-2
Stimulated w/ (30 min)
(-)
BFA (-) NCM
BFA (+) NCM
BFA (-) CCM
BFA (+) CCM
kDa
IB: pAKT(S473)
IB: pAKT(T308)
IB: AKT
IB: pERK1/2 (T202/Y204)
IB: ERK1/2
IB: β-Actin (for pAKT(S473) & pERK1/2)
IB: β-Actin (for pAKT(T308))
IB: β-Actin (for AKT&ERK)
B
hTERT-HPaSteC Lysate
PANC-1
Panc 02.13
Capan-2
IB: pSTAT3(Y705)
IB: STAT3
IB: β-Actin for pSTAT3(Y705)
IB: β-Actin for STAT3

C



D



STAT3 mRNA expression per *ß-actin* (% of NTsiRNA)

NCM-siSTAT3:	43.5%
CM-siSTAT3:	38.0%

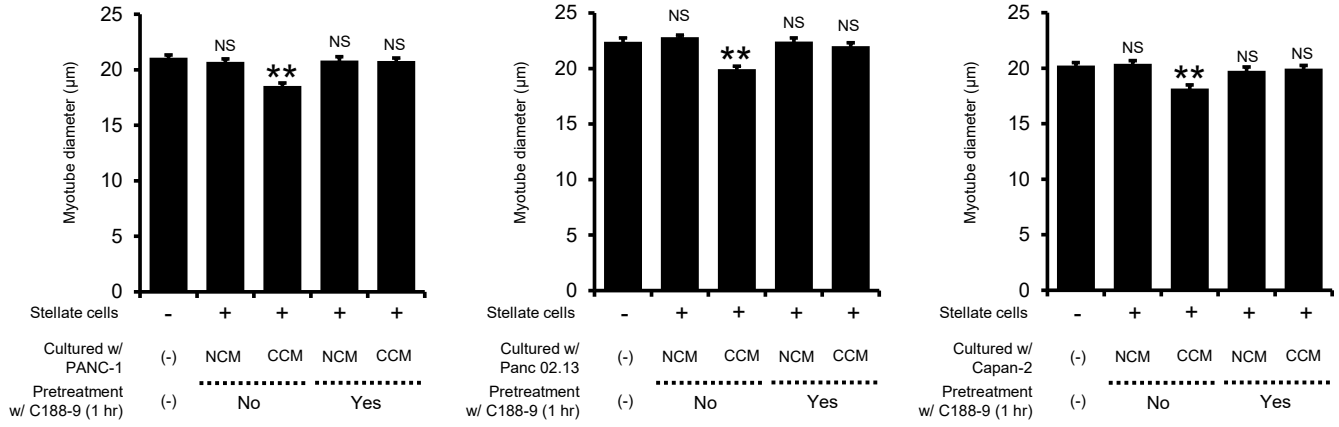
STAT3 mRNA expression per *ß-actin* (% of NTsiRNA)

NCM-siSTAT3:	17.2%
CM-siSTAT3:	8.6%

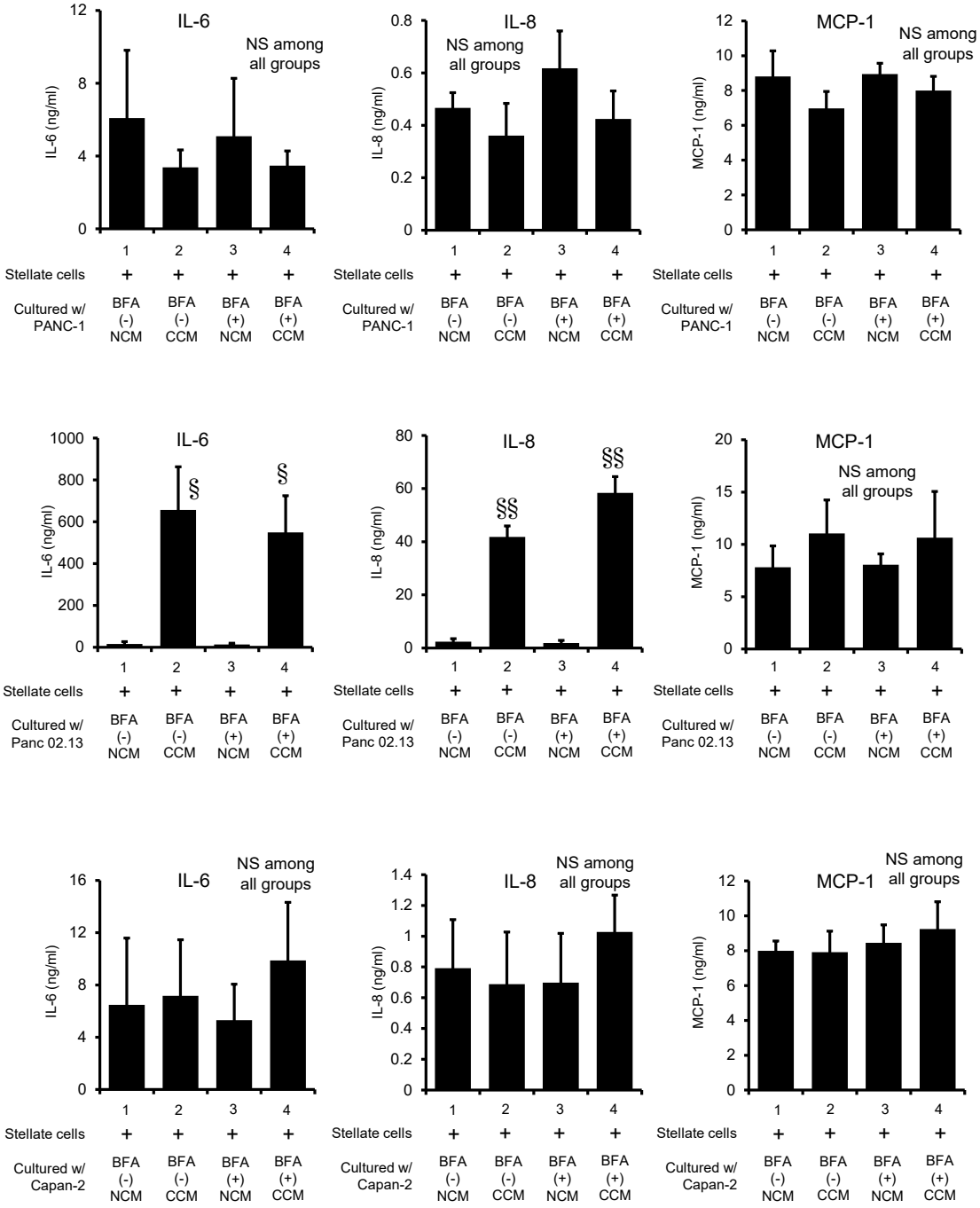
STAT3 mRNA expression per *ß-actin* (% of NTsiRNA)

NCM-siSTAT3:	17.3%
CM-siSTAT3:	41.9%

E

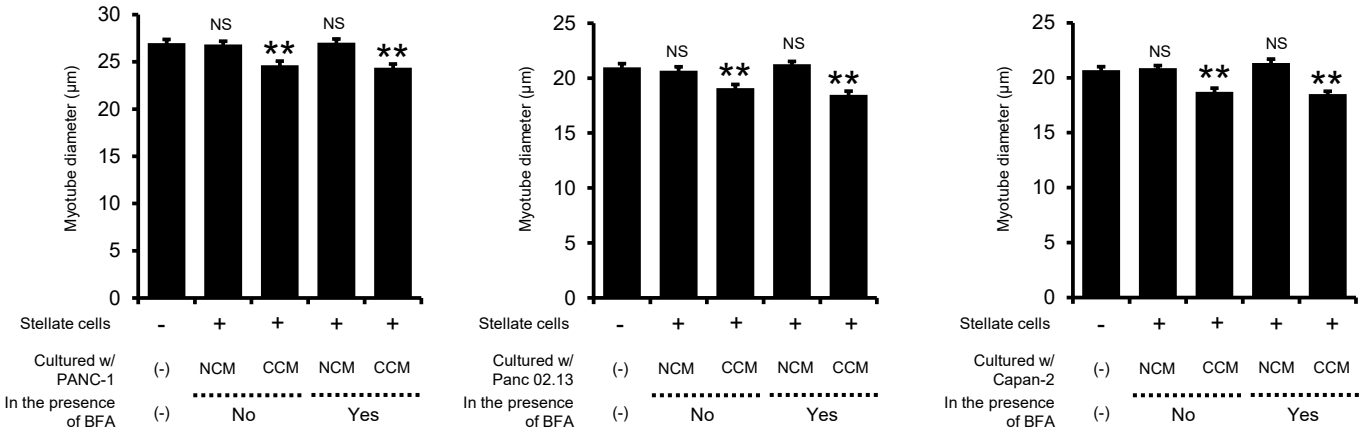


Supplemental Figure S3. CCM confers muscle atrophic phenotype via the STAT3 pathway in pancreatic stellate cells. (A) CCM from BFA-treated cancer cells does not consistently induce phosphorylation of AKT and ERK. (*) β-actin. (B) CCM from BFA-treated PANC-1, Panc 02.13, and Capan-2 cells induces decreased STAT3 phosphorylation in pancreatic stellate cells. (C) RELA (p65) nuclear translocation after stimulation of stellate cells with 10% NCM or CCM from BFA-treated Mia PaCa-2 cells. (D) hTERT-HPaSteC were transfected with either nontargeted siRNA (NTsiRNA) or siRNA targeting *STAT3* (siSTAT3). Then cells were cultured with 10% NCM or CCM for 3 days, and CM from hTERT-HPaSteC was subjected to a myotube atrophy assay. (E) hTERT-HPaSteC were treated with 10 μM C188-9 (STAT3 inhibitor) for 1 hour. hTERT-HPaSteC were washed and subsequently cultured with 10% NCM or CCM for 1 day. CM from these stellate cells was added to C2C12 myotubes, and the diameter of myotubes was measured. Full-length blots are presented in Supplementary Materials 2. The data shown for RELA nuclear translocation are shown as Mean ± S.E. (n=18-67 cells). *p<0.05, **p<0.01 vs. NCM stimulation. The data shown for the diameter of the myotubes are shown as a mean ± S.E. (n=44 myotubes). (D) **p<0.01 vs. NTsiRNA CM; §§ p<0.01 vs. no cells; NS: not significant. (E) **p < 0.01 vs. all other groups; NS: not significant vs. no cells.


IL-6
NS among all groups
IL-6 (ng/ml)
IL-8
NS among all groups
IL-8 (ng/ml)
MCP-1
NS among all groups
MCP-1 (ng/ml)
§
§§
Stellate cells
Cultured w/ PANC-1
Cultured w/ Panc 02.13
Cultured w/ Capan-2
BFA (-) NCM
BFA (-) CCM
BFA (+) NCM
BFA (+) CCM

Supplemental Figure S4. Cytokine/chemokine secretion from stellate cells cultured with BFA-treated or nontreated PANC-1, Panc 02.13, and Capan-2 cells. The levels of IL-6, IL-8, and MCP-1 in CM from hTERT-HPaSteC as prepared in Figure 2B and Supplemental Figure S2B were measured using a sandwich ELISA. The amount of IL-6, IL-8, and MCP1 secreted from stellate cells was calculated by subtracting the amount in GM containing 10% CCM from that in CM from stellate cells treated with 10% CCM. The data are shown as the mean ± S.E. from three independent experiments. § $p<0.05$, §§ $p<0.01$ vs. NCM; NS: not significant.

A



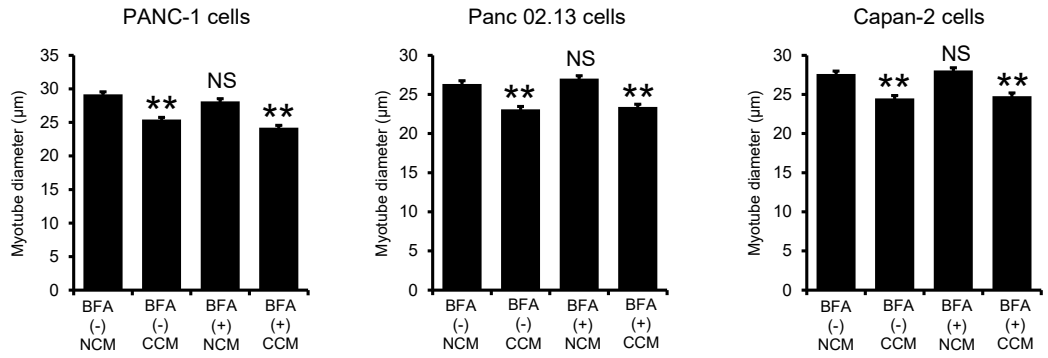
B

Heatmap of cytokine/chemokine secretion from stellate cells treated with CCM in the presence or absence of BFA

Stimulated w/	Mia PaCa-2		PANC-1		Panc 02.13		Capan-2	
Treatment	BFA (-)	BFA (+)	BFA (-)	BFA (+)	BFA (-)	BFA (+)	BFA (-)	BFA (+)
GRO								
GRO-alpha								
IL-6								
IL-8								
MCP-1								
M-CSF								
EGF								
IGF-1								
Angiogenin								
BDNF								
HGF								
IGFBP-2								
Osteoprotegerin								
TIMP-1								
TIMP-2								

High Low

C



Supplemental Figure S5. BFA does not inhibit the secretion of muscle atrophy-inducing factors from CCM-stimulated stellate cells as well as pancreatic cancer cells. (A) hTERT-HPaSteC were cultured with 10% NCM or CCM from PANC-1, Panc 02.13, and Capan-2 cells in the presence or absence of 0.5 μg/mL BFA for 1 day. CM from stellate cells was passed through a desalting column and subjected to a myotube atrophy assay. (B) Heatmap showing the amounts of cytokines and chemokines in CM prepared in (A). (C) PANC-1, Panc 02.13, and Capan-2 cells were cultured with or without 0.5 μg/mL BFA for 1 day. CCM was collected, the BFA was removed, and CM was subjected to an atrophy assay. The data shown for the diameter of the myotubes are representative of three independent experiments (mean ± S.E.; n = 44 myotubes). (A) **$p < 0.01$ vs. NCM and no cells; NS: not significant vs. no cells. (C) **$p < 0.01$ vs. NCM; NS: not significant vs. BFA (-) NCM.

Supplemental Table S1 Primers for real-time RT-qPCR

Targets	Sequence (5’ -> 3’)
Human STAT3 FWD	CTTTGAGACCGAGGTGTATCACC
Human STAT3 REV	GGTCAGCATGTTGTACCACAGG
Human ß-Actin FWD	CACCATTGGCAATGAGCGGTTC
Human ß-Actin REV	AGGTCTTTGCGGATGTCCACGT