SUPPLEMENTAL INFORMATION

Figure S1

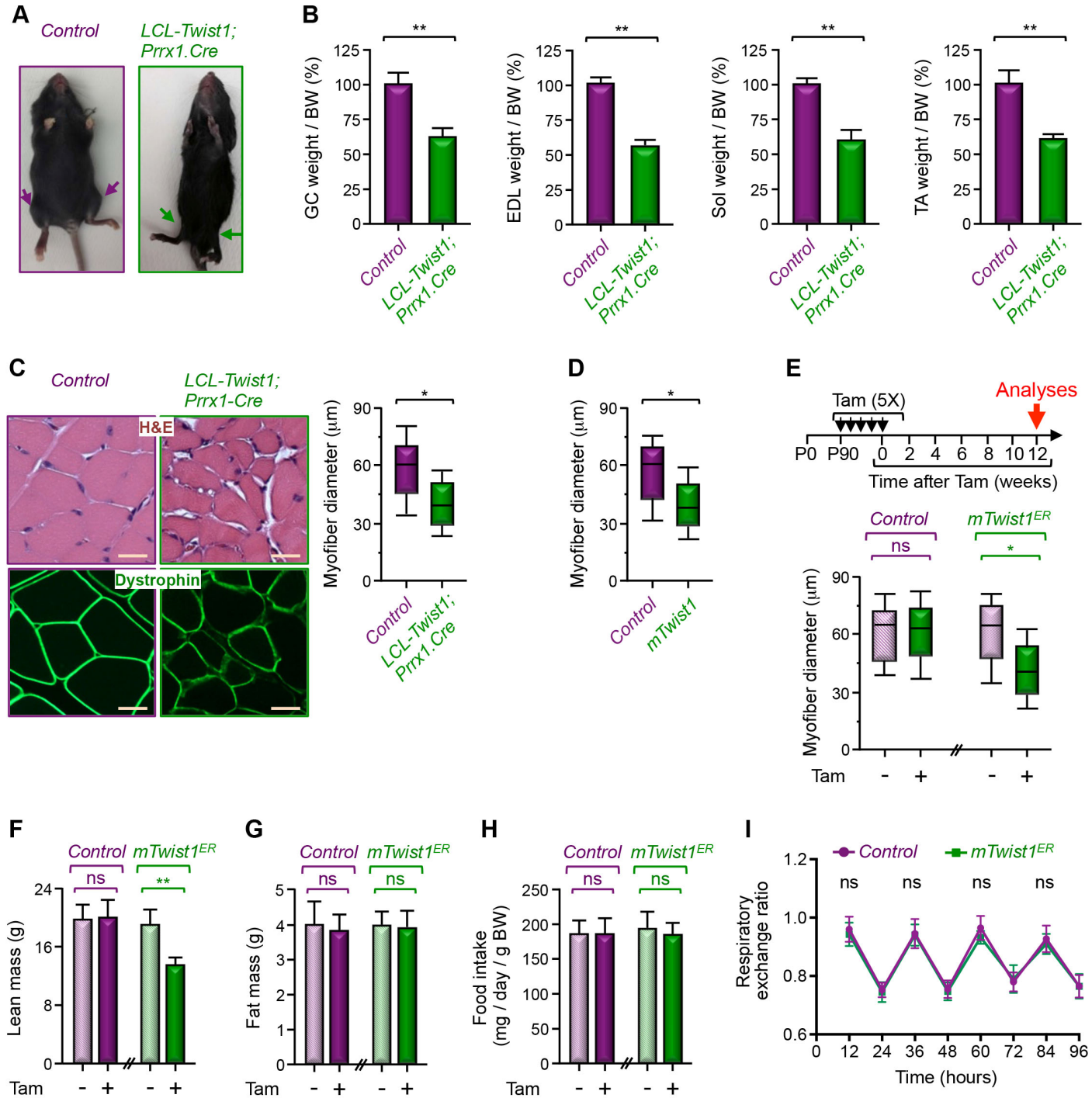

Figure S1: Twist1 Overexpression in Muscle Progenitor Cells Causes Muscle Atrophy. Related to Figure 1

(A) Representative pictures of 2-month-old *LCL-Twist1;Prrx1.Cre* and control mice.
(B) The weights of gastrocnemius (GC), extensor digitalis longus (EDL), soleus (Sol), and tibialis anterior (TA) muscles from 2-month-old *LCL-Twist1;Prrx1-Cre* and control mice were normalized by body weight (BW) (n= 6).
(C) GC muscle cross-sectional areas in 2-month-old *LCL-Twist1;Prrx1-Cre* and control mice were analyzed by staining with H&E or immunostaining with anti-dystrophin antibody. Myofiber diameters were measured and presented as boxplot (n= 200). Scale bars: 25 μM.
(D) GC muscle cross-sectional areas in 2-month-old *mTwist1* and control mice were analyzed by immunostaining with anti-dystrophin antibody. Myofiber diameters were measured and presented as boxplot (n= 200).
(E-I) Three-month-old *mTwist1*^{ER} or control mice were treated with vehicle or Tam and subjected to muscle cachexia analysis 12 weeks following treatment (n= 6). GC muscle cross-sectional areas were analyzed by immunostaining using anti-dystrophin antibody and myofiber diameters were measured and presented as boxplot (n= 200) (E). Lean (F) or fat (G) mass was determined by EchoMRI. Food intake (H) and respiratory exchange ration (I) were monitored by CLAMS (metabolic cages).
Data in B, F, G, and H are expressed as mean ± SEM. *p< 0.05; **p< 0.01; ns, not significant.

Figure S2

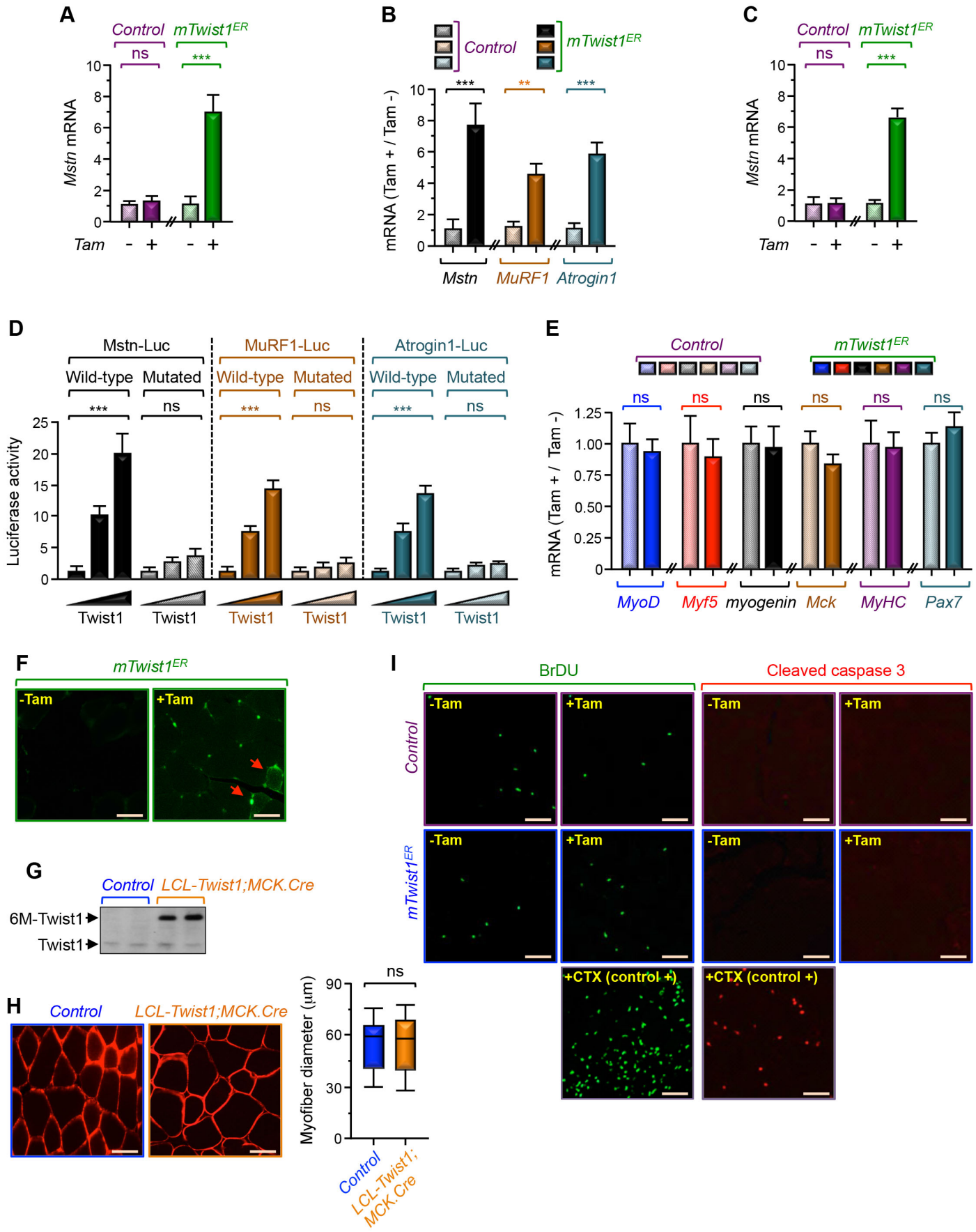
A
Control
mTwist1ER
ns
***
Mstn mRNA
Tam
B
Control
mTwist1ER
mRNA (Tam + / Tam -)
Mstn
MuRF1
Atrogin1
C
D
Mstn-Luc
MuRF1-Luc
Atrogin1-Luc
Wild-type
Mutated
Luciferase activity
Twist1
E
MyoD
Myf5
myogenin
Mck
MyHC
Pax7
F
-Tam
+Tam
G
LCL-Twist1;MCK.Cre
6M-Twist1
Twist1
H
Myofiber diameter (µm)
I
BrDU
Cleaved caspase 3
+CTX (control +)

Figure S2: Twist1 Induces Muscle Protein Degradation. Related to Figure 2

(A) Satellite cells were isolated from hindlimb muscle of 3-month-old *mTwist1*^{ER} and control mice treated with vehicle or Tam in vivo. Expression of *Mstn* mRNA was assessed by RT-PCR (n= 6).

(B) Three-month-old *mTwist1*^{ER} and control mice were treated with vehicle or Tam and analyzed for expression of *Mstn*, *MuRF1*, and *Atrogin1* in hindlimb muscle by RT-PCR 12 weeks following treatment (n= 6).

(C) Satellite cells were isolated from hindlimb muscle of 3-month-old *mTwist1*^{ER} and control mice and treated with vehicle or Tam in vitro. Expression of *Mstn* mRNA was assessed by RT-PCR (n= 3).

(D) C2C12 cells were transfected with wild-type or mutated MuRF1-Luc, Atrogin1-Luc, or Mstn-Luc reporters and increasing amounts of Twist1. Luciferase activity was measured 48 hr following transfection and normalized on the basis of co-transfected Renilla luciferase (n= 3).

(E) Three-month-old *mTwist1*^{ER} or control mice were treated with vehicle or Tam for 6 weeks and expression of *MyoD*, *Myf5*, *myogenin*, *Mck*, *MyHC*, or *Pax7* in hindlimb muscle was assessed by RT-PCR (n= 6).

(F) Three-month-old *mTwist1*^{ER} or control mice were treated with vehicle or Tam and sections from GS muscle were analyzed for exogenous Twist1 expression (6xMyc-Twist1, for tracing) by immunofluorescence. Scale bars: 50 μM.

(G and **H)** Muscles from 3-month-old *mTwist1;MCK.Cre* or control mice were analyzed by immunoblotting using anti-Twist1 antibody (G) or by immunofluorescence using anti-dystrophin antibody (H). Myofiber diameters were measured and presented as boxplot (n= 200). Scale bars: 50 μM.

(I) Three-month-old *mTwist1*^{ER} or control mice were treated with vehicle or Tam and analyzed for cell proliferation and apoptosis (n= 6). For cell proliferation, mice were injected with BrdU and analyzed by immunofluorescence using anti-BrdU antibody. Apoptosis was assessed by immunofluorescence using anti-cleaved caspase-3 antibody. Muscles from wild-type mice collected during recovery (4 days) from local injury with cardiotoxin (CTX) were used as positive controls. Representative images of BrdU or cleaved caspase-3 immunostaining are shown, Quantification of BrdU or cleaved caspase-3 showed no significant difference between *mTwist1*^{ER} and control mice in either the absence or presence of Tam. Scale bars: 50 μM.

Data in A, B, C, D, and E are expressed as mean ± SEM. **p< 0.01; ***p< 0.001; ns, not significant.

Figure S3

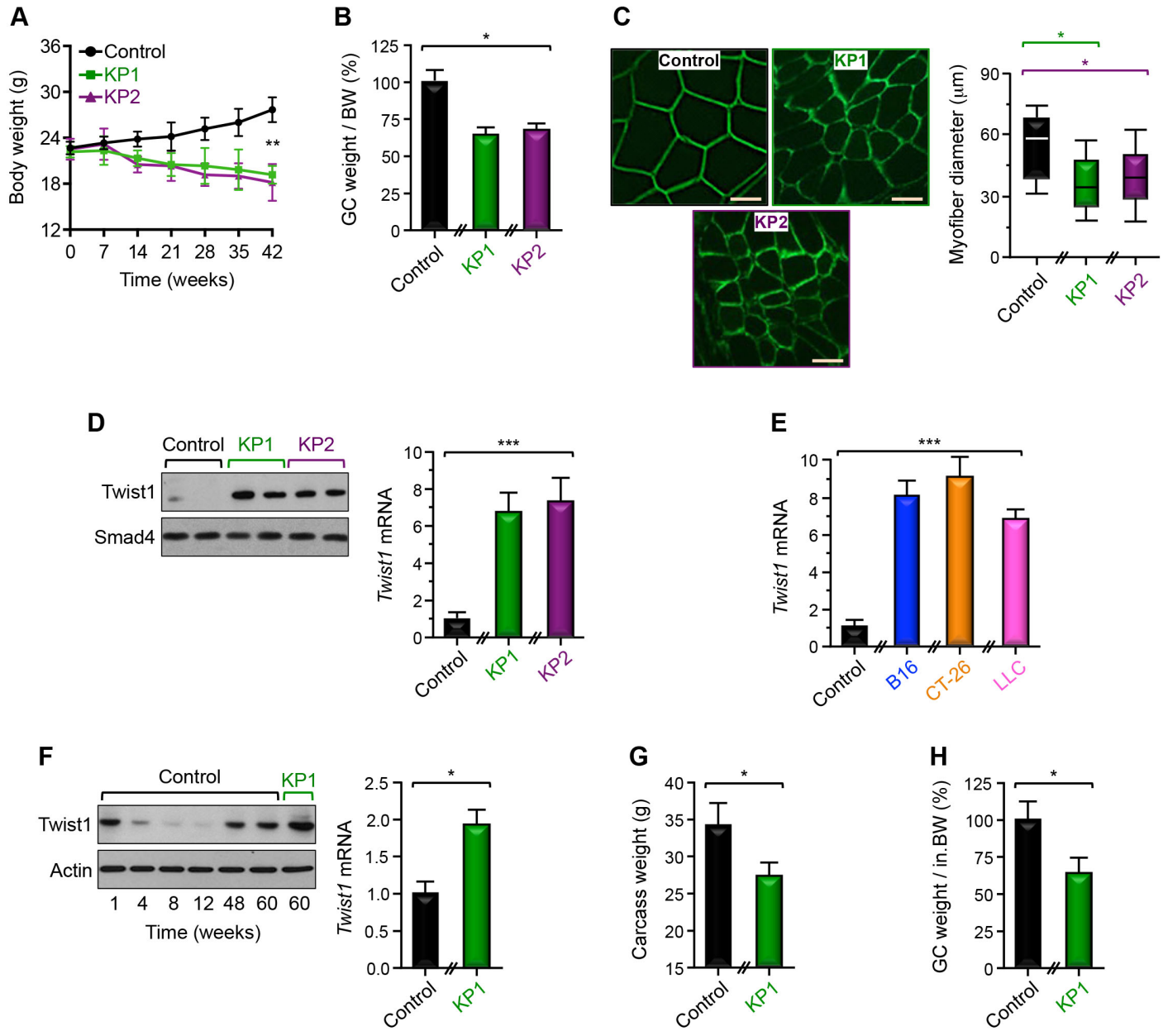

Figure S3: De-repression of *Twist1* in Muscle Undergoing Cachexia. Related to Figure 3

(A-D) Three-month-old mice were inoculated with KP1 or KP2 cells (n= 12) and subjected to muscle cachexia analysis up to 42 days following cell inoculation. Body weight was measured every 7 days (A). GC muscle weight per BW (B), GC muscle cross-sectional areas and myofiber diameters (C), and Twist1 protein and mRNA expression (D) were analyzed 30 days following cell inoculation. For Twist1 protein expression, extracts from two representative mice are shown (D). Scale bars: 50 μM.

(E) Three-month-old mice were inoculated with B16 (melanoma), CT-26 (colon adenocarcinoma), or LLC (Lewis lung carcinoma) cells, and muscle *Twist1* mRNA expression was determined by RT-PCR 20 days following cell inoculation (n= 6).
(F) Expression of Twist1 expression in mice at different ages was examined by immunoblotting. To study changes in Twist1 expression in cachectic old mice, 15-month-old mice were inoculated with KP1 cells and analyzed for Twist1 expression by immunoblotting and RT-PCR (n= 6).
(**G** and **H**) Fifteen-month-old mice were inoculated with KP1 cells and carcass weight (G) or GC muscle weight per BW (H) were examined 30 days following cell inoculation (n= 6).
Data in A, B, D, E, F, G, and H are expressed as mean ± SEM. *$p < 0.05$; ***$p< 0.001$.

Figure S4

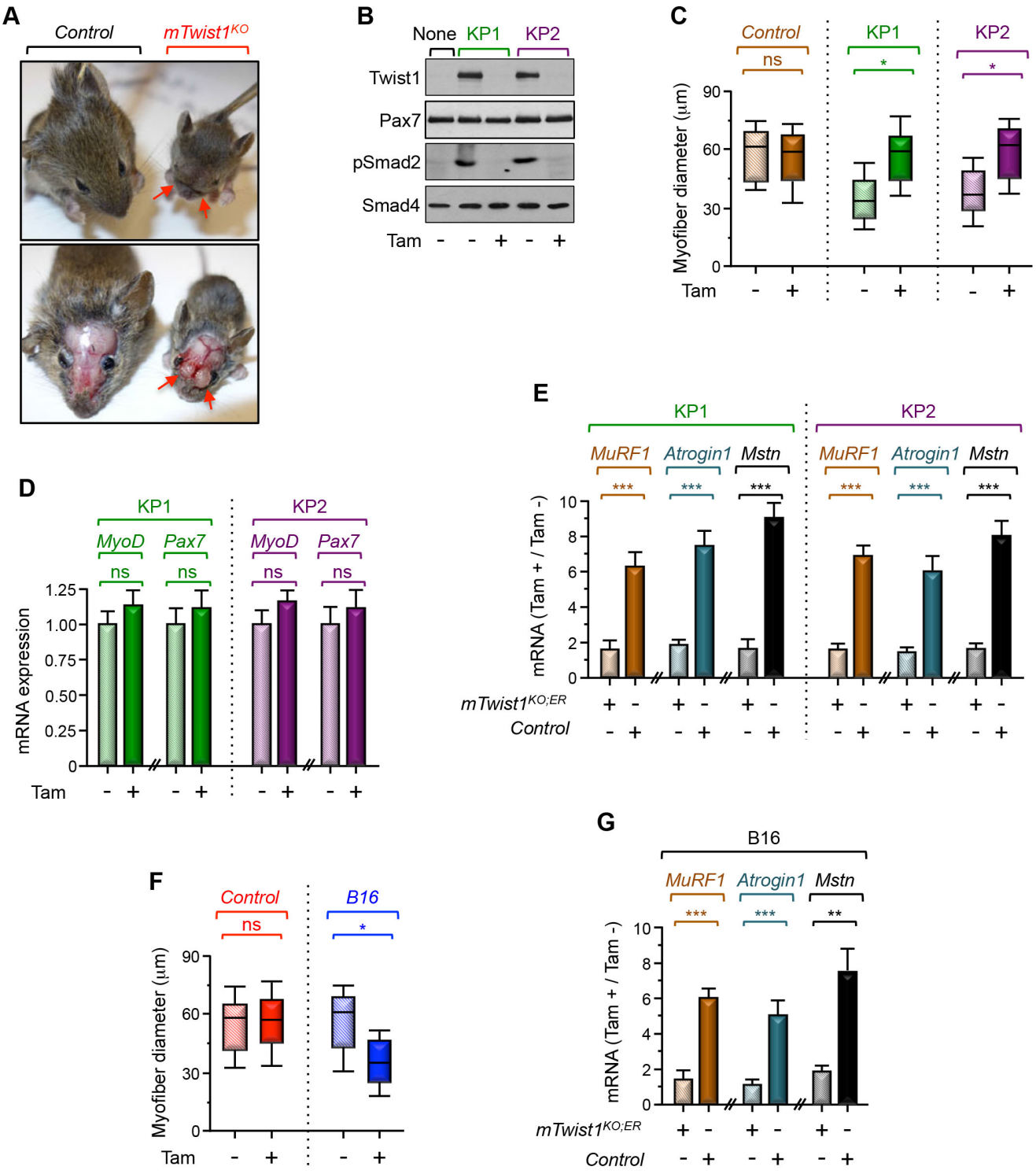

Figure S4: *Twist1* Deletion in Satellite Cells Blocks Cachexia. Related to Figure 4

(A) Representative pictures of 3-week-old control and *mTwist1*^{KO} mice (with constitutive conditional deletion of *Twist1* in muscle). Red arrows indicate hypertrophy of head muscles.

(B and **C)** Three-month-old *mTwist1*$^{KO;ER}$ mice were treated with vehicle or Tam, inoculated with KP1 or KP2 cells, and subjected to cachexia analyses 30 days after cell inoculation (n= 6). Expression of Twist1 and Pax7 and phosphorylation of Smad2 were determined by immunoblotting (B). GC muscle cross-sectional areas were analyzed by immunostaining with anti-dystrophin antibody and myofiber diameters (n= 200) were measured and presented as boxplot (C).

(D, E) Three-month-old *mTwist1*$^{KO;ER}$ or control mice were treated with vehicle or Tam and inoculated with KP1 or KP2 cells. Expression of *MyoD*, *Pax7*, *MuRF1*, *Atrogin1* or *Mstn* mRNA was determined by RT-PCR 30 days following cell inoculation (n= 6).

(F and **G)** Three-month-old *mTwist1*$^{KO;ER}$ mice were treated with vehicle or Tam, inoculated with B6 cells (n= 6), and analyzed for cachexia 24 days following cell inoculation. GC muscle cross-sectional areas were analyzed by immunostaining with anti-dystrophin antibody and myofiber diameters (n= 200) were measured and presented as boxplot (E). Expression of *MuRF1*, *Atrogin1*, or *Mstn* mRNA was determined by RT-PCR (F).

Data in D, E, and G are expressed as mean ± SEM. *p < 0.05; **p < 0.01; ***p< 0.001; ns, not significant.

Figure S5

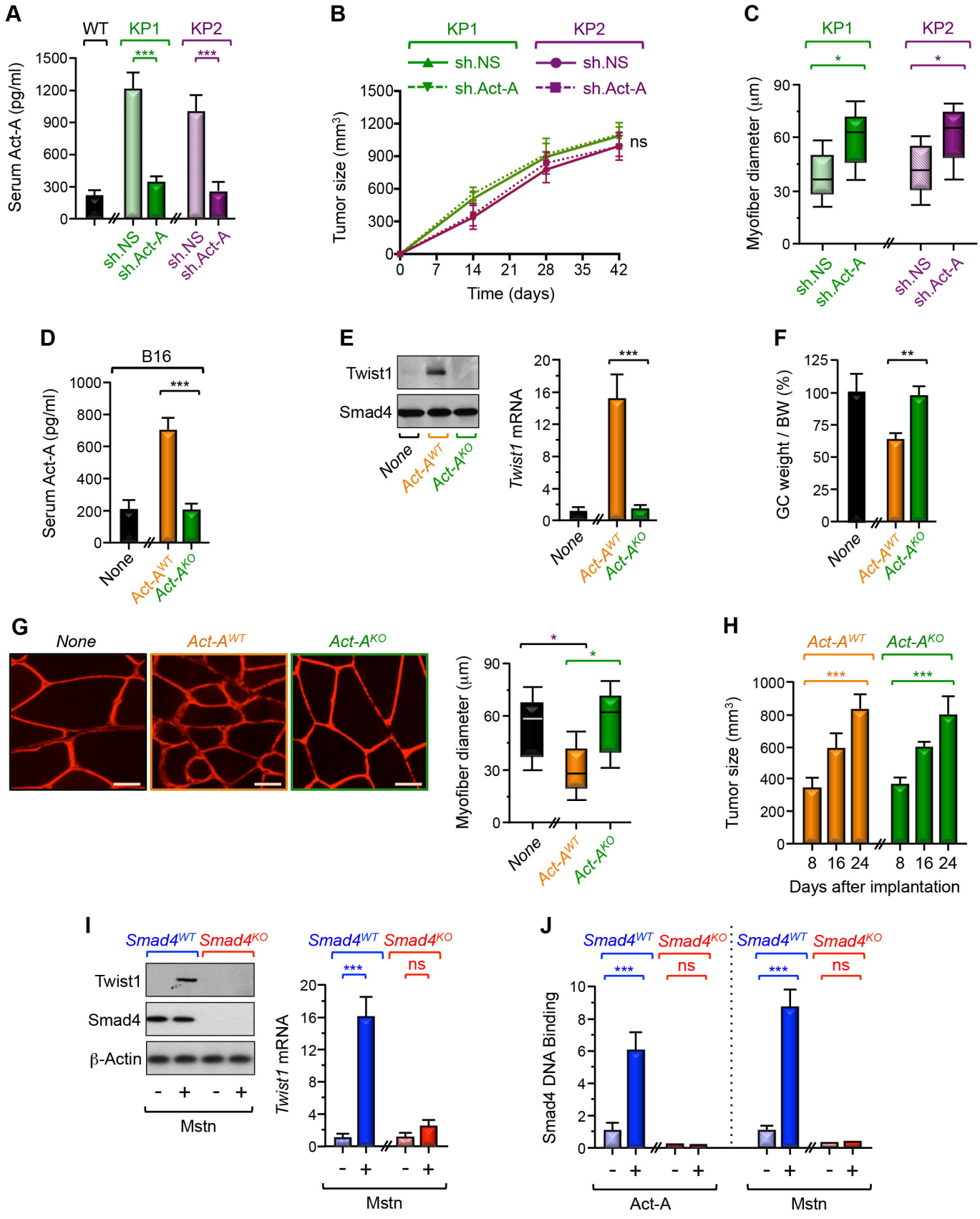
A
WT
KP1
KP2
Serum Act-A (pg/ml)
sh.NS
sh.Act-A
B
KP1
KP2
sh.NS
sh.Act-A
Tumor size (mm3)
Time (days)
ns
C
KP1
KP2
Myofiber diameter (μm)
D
B16
Serum Act-A (pg/ml)
None
Act-AWT
Act-AKO
E
Twist1
Smad4
Twist1 mRNA
F
GC weight / BW (%)
G
None
Act-AWT
Act-AKO
Myofiber diameter (μm)
H
Tumor size (mm3)
Days after implantation
I
Smad4WT
Smad4KO
Twist1
Smad4
β-Actin
Mstn
Twist1 mRNA
J
Smad4 DNA Binding
Act-A
Mstn

Figure S5: Tumor-Derived Activin Induces Twist1 Expression in Muscle During Cancer Cachexia. Related to Figure 5

(A-C) Three-month-old mice were inoculated with KP1 or KP2 cells stably expressing control (sh.NS) or sh.RNA targeting Act-A (sh.Act-A) and subjected to cancer cachexia analysis up to 42 days following cell inoculation (n= 6). Circulating Act-A concentrations in control or tumor-bearing mice were assessed by ELISA (A). Tumor sizes were measured every 7 days (B). GC muscle cross-sectional areas were analyzed by immunostaining with anti-dystrophin antibody and myofiber diameters (n= 200) were measured and presented as boxplot (C).
(D-H) Three-month-old wild-type mice were inoculated with wild-type (WT) or Act-A knockout B16 (*Act-A*^{KO}) cells (n= 6). Circulating Act-A concentrations (D), muscle Twist1 protein and mRNA expression (E), GC muscle per BW (F), and GS muscle cross-sectional areas and myofiber diameters (G) were analyzed 24 days following cell inoculation. Tumor size was measured every 8 days (H). Scale bars: 25 μM.
(I and **J)** Primary satellite cells from 3-month-old wild-type (*Smad4*^{WT}) or *Smad4*$^{fl/fl}$ (*Smad4*^{KO}) mice were transduced with Ad.Cre (n= 6). Cells were then treated with Act-A or Mstn for 24 hr and analyzed for Twist1 protein and mRNA expression (I) or Smad4 binding to the *Twist1* promoter by ChIP using Smad4 antibody (J).
Data in A, B, D, E, F, H, I, and J are expressed as mean ± SEM. *p< 0.05; **p< 0.01; ***p< 0.001; ns, not significant.

Figure S6

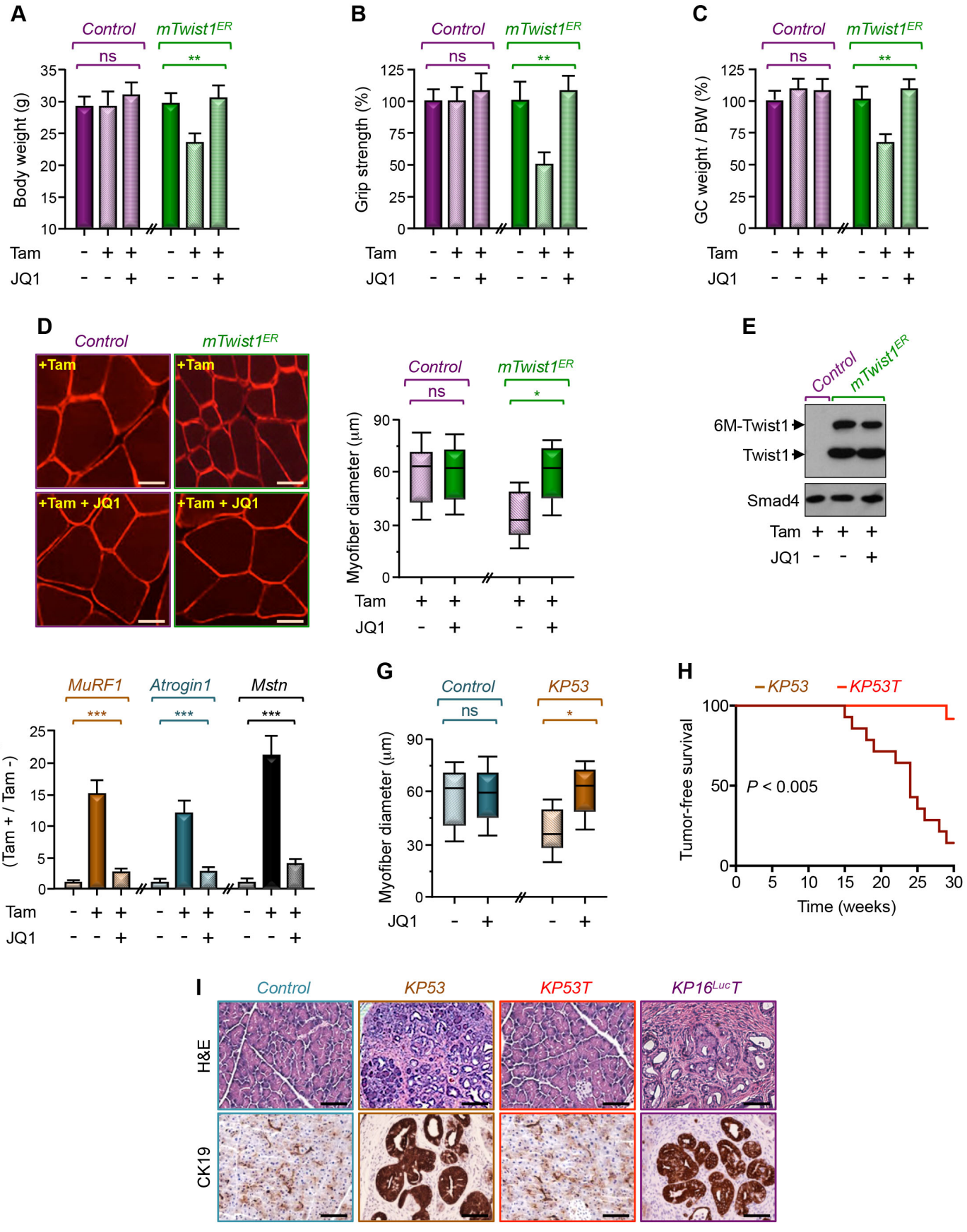
A
Control
mTwist1ER
ns
**
Body weight (g)
35
30
25
20
15
10
Tam - + + - + +
JQ1 - - + - - +
B
Control
mTwist1ER
ns
**
Grip strength (%)
125
100
75
50
25
0
Tam - + + - + +
JQ1 - - + - - +
C
Control
mTwist1ER
ns
**
GC weight / BW (%)
125
100
75
50
25
0
Tam - + + - + +
JQ1 - - + - - +
D
Control
mTwist1ER
+Tam
+Tam
+Tam + JQ1
+Tam + JQ1
Control
mTwist1ER
ns
*
Myofiber diameter (μm)
90
60
30
0
Tam + + + +
JQ1 - + - +
E
Control
mTwist1ER
6M-Twist1
Twist1
Smad4
Tam + + +
JQ1 - - +
F
MuRF1
Atrogin1
Mstn
***
***
***
Twist1 DNA binding
(Tam + / Tam -)
25
20
15
10
5
0
Tam - + + - + + - + +
JQ1 - - + - - + - - +
G
Control
KP53
ns
*
Myofiber diameter (μm)
90
60
30
0
JQ1 - + - +
H
KP53
KP53T
Tumor-free survival
100
50
0
P < 0.005
0 5 10 15 20 25 30
Time (weeks)
I
Control
KP53
KP53T
KP16LucT
H&E
CK19

Figure S6: JQ1 Inhibits Twist1-Induced Muscle Atrophy. Related to Figure 6

(A-F) Three-month-old *mTwist1*^{ER} or control mice were treated with vehicle or Tam before being injected with JQ1 (n= 6) and analyzed for muscle atrophy 6 weeks following treatment. Total body weight was measured (A). Muscle strength was analyzed by the grip strength method (B). GC muscle weight was measured and normalized by BW (C). GC muscle cross-sectional areas were visualized by immunofluorescence using anti-dystrophin antibody and myofiber diameters (n= 200) were measured and presented as boxplot (D). Expression of Twist1 protein in hindlimb muscles was analyzed by immunoblotting using anti-Twist1 antibody (E). Expression of *MuRF1*, *Atrogin1*, or *Mstn* mRNA in muscles was analyzed by RT-PCR (F). Scale bars: 25 μM.

(G) *KP53* or control mice were treated with JQ1 for 6 weeks (n= 10 or 12) and GC muscle cross-sectional areas were analyzed by immunofluorescence with anti-dystrophin antibody 3 weeks following treatment. Myofiber diameters were measured and presented as boxplot (n= 200).

(H) Kaplan-Meier survival analysis of *KP53* and *KP53T* mice (n= 14 for *KP53* and n= 12 for *KP53T*). p< 0.005 by log-rank test for significance.

(I) Pancreatic tissues from *KP53, KP53T*, *KP16*^{Luc}*T*, or control mice were analyzed by staining with H&E or immunostaining with anti-CK19 antibody. Scale bars: 50 μM.

Data in A, B, C, and F are expressed as mean ± SEM. *p < 0.05; **p < 0.01; ***p < 0.001; ns, not significant.

Figure S7

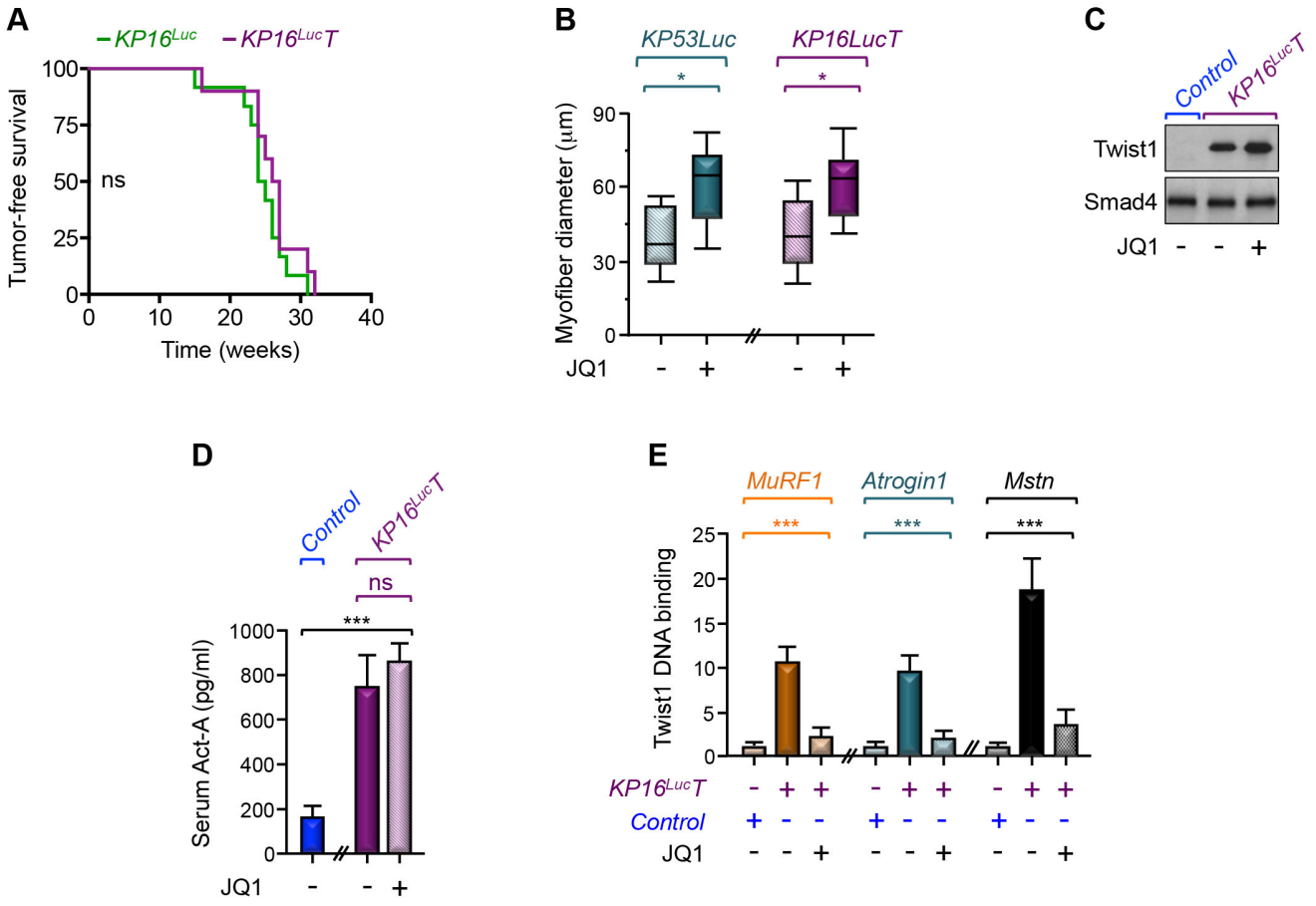

Figure S7: Pharmacological or Genetic Inactivation of Twist1 Does not Suppress PDAC in *KP16^{Luc}* Mice. Related to Figure 7

(A) Kaplan-Meier survival analysis of *KP16^{Luc}* or *KP16^{Luc}T* mice (n= 10 for *KP16^{Luc}* and n= 12 for *KP16^{Luc}T*).
(B) *KP53^{Luc}* or *KP16^{Luc}T* mice (n= 6) were treated with JQ1 for 6 weeks and GC muscle cross-sectional areas were analyzed by immunostaining with anti-dystrophin antibody and myofiber diameters were measured and presented as boxplot (n= 200).
(C-E) *KP16^{Luc}T* mice were treated with vehicle or Tam before being injected with JQ1 for 6 weeks (n= 6). Expression of Twist1 protein in GC muscle was analyzed by immunoblotting using anti-Twist1 antibody (C). Plasma Act-A concentrations were assessed by ELISA (D). Expression of *MuRF1*, *Atrogin1*, or *Mstn* mRNA in hindlimb muscle was analyzed by RT-PCR (E).
Data in D and E are expressed as mean ± SEM. *p < 0.05; ***p < 0.001; ns, not significant.