Supplemental Figures

Table S1: Mixed-effects model estimates of group means in the Control and Patient populations with p-values associated with the group difference (P-value* are Holm adjusted p-values). Western values are scaled ($\times 10^5$). Summary of analysis of **Figures 1, 3,** and **4**.

Table S2: Mean ± SE of 3 replicated observations from 2 Controls and 6 subjects for MDA, NOS2, and Myosin; 5 subjects for Jun-D; and 4 subjects for CKM. Summary of analysis of **Supplemental Figures 1 – 4** and **6**.

Figure S1: Increased oxidative stress by immunohistochemistry in the skeletal muscle of cachectic patients

A. Skeletal muscle samples from control (N=2; C1, C2; two rectus abdominus) and cachectic (N=6; A2, Ca1-Ca3, Ca6, and Ca8; four rectus abdominus and two vastus lateralis) subjects were processed as described in Methods. Scanning confocal laser microscopy was performed for nucleic acids (TO-PRO-3) (blue) and MDA (green). One representative control value is shown each for longitudinal or transverse sections. Expression of MDA was increased in skeletal muscles from cachectic patients. No immunofluorescence was detected when omitting the first antibody. The size bar represents 100 μm.

B. These findings by immunohistochemistry were quantified using the Metamorph Offline program, Universal Imaging Corp. Product version 6.1. The quantitative measurements of five random fields each (200 x) showed significant increases in MDA in the muscles of cachectic patients ($p = 0.001$). Two representative control values are shown. These are representative

data from six independent experiments. Mixed-effects model estimates of group means in the Control and Patient populations with p-values associated with the group difference [**ST2**]. The entire group of cachectic patients could not be analyzed by this methodology due to insufficient tissue. Age, gender, and muscle biopsy location did not have statistical effects. All samples were run in triplicate and replicate values were within an acceptable range of each other.

Figure S2: Increased NOS2 by immunohistochemistry in the skeletal muscle of cachectic patients

A. Skeletal muscle samples from control (N=2; C1, C2; two rectus abdominus) and cachectic (N=6; A2, Ca1-Ca3, Ca6, and Ca8; four rectus abdominus and two vastus lateralis) subjects were processed as described in Methods. We were not able to analyze the entire group of cachectic samples by this assay due to sample quantity limitations. Scanning confocal laser microscopy was performed for nucleic acids (TO-PRO-3) (blue) and NOS2 (green). One representative control value is shown each for longitudinal or transverse sections. Expression of NOS2 was increased in skeletal muscles from cachectic patients. No immunofluorescence was detected when omitting the first antibody. The size bar represents 100 μm.

B. These findings by immunohistochemistry were quantified using the Metamorph Offline program, Universal Imaging Corp. Product version 6.1. The quantitative measurements of five random fields each (200 x) showed significant increases in NOS2 in the muscles of cachectic patients ($p = 0.019$). Two representative control values are shown. These are representative data from six independent experiments. Mixed-effects model estimates of group means in the Control and Patient populations with p-values associated with the group difference [**ST2**]. The entire group of cachectic patients could not be analyzed by this methodology due to insufficient tissue. Age, gender, and muscle biopsy location did not have statistical effects. All samples were run in triplicate and replicate values were within an acceptable range of each other.

Figure S3: Decreased myosin by immunohistochemistry in the skeletal muscle of cachectic patients

A. Skeletal muscle samples from control (N=2; C1, C2; two rectus abdominus) and cachectic (N=6; A2, Ca1-Ca3, Ca6, and Ca8; four rectus abdominus and two vastus lateralis) subjects ~~4~~ were processed as described in Methods. Scanning confocal laser microscopy was performed for nucleic acids (TO-PRO-3) (blue) and myosin (green). One representative control value is shown each for longitudinal or transverse sections. We were not able to analyze the entire group of cachectic samples by this assay due to sample quantity limitations. Expression of myosin was decreased in skeletal muscles from cachectic patients. No immunofluorescence was detected when omitting the first antibody. The size bar represents 100 μm.

B. These findings by immunohistochemistry were quantified using the Metamorph Offline program, Universal Imaging Corp. Product version 6.1. The quantitative measurements of five random fields each (200 x) showed significant decreases in myosin in the muscles of cachectic patients ($p < 0.001$). Two representative control values are shown. These are representative data from three independent experiments. Mixed-effects model estimates of group means in the Control and Patient populations with p-values associated with the group difference [**ST2**]. The entire group of cachectic patients could not be analyzed by this methodology due to insufficient tissue. Age, gender, and muscle biopsy location did not have statistical effects. All samples were run in triplicate and replicate values were within an acceptable range of each other.

Figure S4: Decreased CKM by immunohistochemistry in the skeletal muscle of cachectic patients

A. Skeletal muscle samples from control (N=2; C3, C4; two vastus lateralis) and cachectic (N=4; A1, Ca1, Ca4, and Ca7; two rectus abdominus and two vastus lateralis) subjects were

processed as described in Methods. Scanning confocal laser microscopy was performed for nucleic acids (TO-PRO-3) (blue) and CKM (green). One representative control value is shown each for longitudinal or transverse sections. Expression of CKM was decreased in skeletal muscles from cachectic patients. We were not able to analyze the entire group of cachectic samples by this assay due to sample quantity limitations. No immunofluorescence was detected when omitting the first antibody. The size bar represents 100 μm.

B. These findings by immunohistochemistry were quantified using the Metamorph Offline program, Universal Imaging Corp. Product version 6.1. The quantitative measurements of five random fields each (200 x) showed significant decreases in CKM in the muscles of cachectic patients ($p < 0.001$). Two representative control values are shown. These are representative data from five independent experiments. Mixed-effects model estimates of group means in the Control and Patient populations with p-values associated with the group difference [**ST2**]. The entire group of cachectic patients could not be analyzed by this methodology due to insufficient tissue. Age, gender, and muscle biopsy location did not have statistical effects. All samples were run in triplicate and replicate values were within an acceptable range of each other.

Figure S5: CKM DNA E-box binding activities are decreased in skeletal muscle from cachectic patients.

Upper Panel: Mobility shift analysis of skeletal muscle nuclear extracts incubated with ^{32}P-labeled CKM-E box oligonucleotide, and processed as described in Methods. We were not able to analyze the entire group of cachectic samples by this assay due to sample quantity limitations. The positions of the bound (b) and free (f) DNA are indicated. Representative samples of human skeletal muscle from control (lane 2; C2, rectus abdominus) and cancer cachexia (lane 3, Ca2; rectus abdominus); control + Jun-D antibodies (lane 4); control + myogenin antibodies (lane 5); cachexia + recombinant Jun-D (lane 6); cachexia + recombinant

Ref-1 (lane 7); and cachexia + DTT (lane 8). On lane 1, the probe was processed without nuclear extracts. Representative data from three independent experiments.

Lower Panel: Quantitation of these CKM E-box binding activities was obtained using the Alpha Ease FC program, Alpha Innotech Corp. Version 3.2.2. The binding to the CKM E-box was decreased in cachexia, and rescued by the addition of Jun-D or Ref-1 to cachectic nuclear extracts ($p < 0.05$). These are representative data from three independent experiments. The sample size was too small to support rigorous statistical analysis.

Figure S6: Decreased Jun-D by immunohistochemistry in the skeletal muscle of cachectic patients

A. Skeletal muscle from control (N=2;C1 and C2;two rectus abdominus) and cachectic (N=5; Ca1,Ca5-Ca6, Ca8, and Ca10; four rectus adbominus and one vastus lateralis) subjects were processed as described in Methods. Scanning confocal laser microscopy was performed for nucleic acids (TO-PRO-3) (blue) and Jun-D (green). One representative control value is shown each for longitudinal or transverse sections. We were not able to analyze the entire group of cachectic samples by this assay due to sample quantity limitations. Expression of Jun-D was decreased in skeletal muscles from cachectic patients. No immunofluorescence was detected when omitting the first antibody. The size bar represents 100 μm.

B. These findings by immunohistochemistry were quantified using the Metamorph Offline program, Universal Imaging Corp. Product version 6.1. The quantitative measurements of five random fields each (200 x) showed significant decreases in Jun-D in the muscles of cachectic patients ($p < 0.001$). Two representative control values are shown. These are representative data from five independent experiments. Mixed-effects model estimates of group means in the Control and Patient populations with p-values associated with the group difference [**ST2**]. The entire group of cachectic patients could not be analyzed by this methodology due to insufficient

tissue. Age, gender, and muscle biopsy location did not have statistical effects. All samples were run in triplicate and replicate values were within an acceptable range of each other.