Quadriceps

- Creatine
  - Glutamate (State III)
  - Succinate (State III)
  - Pyruvate/Malate (State II)

+ Creatine
  - Glutamate (State III)
  - Succinate (State III)
  - Pyruvate/Malate (State II)
Quadriceps

Protein Density (AU)

AMPK
p-AMPK
p-AMPK/AMPK

PBS (2wk)
C26 (2wk)

2 Weeks

Protein Density (AU)

AMPK
p-AMPK
p-AMPK/AMPK

PBS (4wk)
C26 (4wk)

4 Weeks

P = 0.06

P = 0.08

Diaphragm

Protein Density (AU)

AMPK
p-AMPK
p-AMPK/AMPK

PBS
C26
PBS
C26
PBS
C26
PBS
C26

2 weeks
4 weeks
2 weeks
4 weeks

AMPK 62 KDa
p-AMPK 62 KDa

PBS
C26
PBS
C26
PBS
C26
PBS
C26

2 weeks
4 weeks
2 weeks
4 weeks

AMPK 62 KDa
p-AMPK 62 KDa
Succinate Stimulated $H_2O_2$

2 Weeks

Quadriiceps

2 wk

State II

+ ADP

- Creatine

+ Creatine

State II + ADP

2 Weeks

Diaphragm

4 Weeks

State II + ADP

4 Weeks
Supplemental Figure Legends

**Figure S1** Multiple substrate evaluation of oxygen consumption in quadriceps permeabilized muscle fibre bundles. Oxygen consumption was evaluated in the absence of creatine at 2 weeks and 4 weeks post C26 implantation or PBS injections in permeabilized muscle fibres when stimulated with glutamate (A, B), succinate (E, F) and pyruvate/malate (I, J). This was repeated in the presence of 20mM Creatine (C, D, G, H, K, L). Results represent mean ± SD; n=8-16; # P<0.05 PBS (2wk) vs C26 (2wk); * P<0.05 PBS (4wk) vs C26 (4wk).

**Figure S2** Multiple substrate evaluation of oxygen consumption in diaphragm permeabilized muscle fibre bundles. Oxygen consumption was evaluated in the absence of creatine at 2 weeks and 4 weeks post C26 implantation or PBS injections in permeabilized muscle fibres when stimulated with glutamate (A, B), succinate (E, F) and pyruvate/malate (I, J). This was repeated in the presence of 20mM Creatine (C, D, G, H, K, L). Results represent mean ± SD; n=8-16; # P<0.05 PBS (2wk) vs C26 (2wk); * P<0.05 PBS (4wk) vs C26 (4wk).

**Figure S3** Muscle-specific changes in markers of growth in C26 tumour-bearing skeletal muscle. Protein content of AMPKα and P-AMPKα were quantified at in the quadriceps at 2 weeks (A, n=8) and 4 weeks (B, n=12). Markers were also quantified at in the diaphragm at 2 weeks (C, n=8) and 4 weeks (D, n=12). E, representative image for quadriceps and F, representative image for diaphragm. Results represent mean ± SD.

**Figure S4** Succinate stimulated mH₂O₂ emission in quadriceps and diaphragm muscle of C26 tumour bearing mice. At 2 and 4 weeks, quadriceps mH₂O₂ emission supported by succinate
(10mM) (FADH$_2$) was assessed under maximal State II (no ADP) conditions in the absence of creatine (A, E) and in the presence of 20mM Creatine (C, G). State III (range of [ADP] to model metabolic demand) was also assessed in the absence of creatine (B, F) and in the presence of 20mM creatine (D and H). These measures were repeated in the diaphragm (I-P). Results represent mean ± SD; n=7-16; # P<0.05, PBS (2wk) vs C26 (2wk); * P<0.05, PBS (4wk) vs C26 (4wk).