Supplemental Methods

Judge et al. ***Loss of Myoc via Mef2c mediates cancer cachexia***

Magnetic Resonance Imaging

Hindlimb muscle Imaging: Magnetic resonance imaging (MRI) was performed in a 4.7T, horizontal bore magnet (Agilent; VMJ version 3.1). Animals were anesthetized prior to and during scanning using isoflurane (3% induction, 1-1.5% maintenance) while their respiratory rate was monitored (Small Animal Instruments). Body temperature of animals was monitored using rectal temperature probes and maintained using heated flexible water pads. The lower hindlimbs of the mouse were inserted up to the knee into a 2.0 cm internal diameter, custom-built solenoid ^{1}H coil (200MHz). Three-dimensional gradient **(3D-GRE)**, **$^{1}H_2O$** spectroscopic relaxometry and **spin echo diffusion tensor image (DTI)** data was acquired using parameters described previously (1).

MR analysis

Hindlimb muscles: Images were converted to Digital Imaging and Communication in Medicine (DICOM) format using a custom written IDL code for Varian data (EXELIS; CO). Posterior compartment was outlined on axial images of the whole limb to determine maximum cross sectional area (CSA_{max}) as described previously (2,3). Muscle water-only ($^{1}H_2O$-T_2) data was analyzed using a custom written software in IDL (EXELIS, CO). Specifically, $^{1}H_2O$-T_2 was determined by a non-linear curve fitting the decay in water signal as a function of TE using a mono-exponential model and non-negative least squares (T_2-NNLS) (4). Finally, using a validated IDL based MRI analysis software (MAS;http://marecilab.mbi.ufl.edu/software/MAS/), mean diffusivity (MD), radial diffusivity (RD), total diffusivity (TD), fractional anisotropy (FA), and eigenvalues ($\lambda1$, $\lambda2$, $\lambda3$) were all determined within a region of interest in posterior (GAS-SOL complex) compartment muscles. MD, RD and TD were calculated using ($\lambda1+\lambda2+\lambda3/3$), ($\lambda2+ \lambda3/2$), and ($\lambda1+\lambda2+\lambda3$) respectively.

Statistical Analysis of MR Data

Statistical analyses of MR data were performed using GraphPad Prism 6 software (GraphPad Software, La Jolla, CA, USA) and included one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. All data are presented as mean ± standard deviation (SD), unless otherwise specified. Statistical significance was set at $p<0.05$.

Primers

Primer sets used in TaqMan qRT-PCR gene expression assays were purchased from Applied Biosystems: Eukaryotic 18S: Hs99999901 (housekeeping, normalizing gene); *Myoc* (mouse: Mm00447900_m1; rat: Rn00578382_m1), *Mef2c* (mouse: Mm01340842_m1; rat: Rn01494040_m1), MuRF1/Trim63 (Mm01185221_m1), Musa1/Fbxo30 (Mm01191299_m1) and atrogin-1/MAFbx/Fbxo32 (Mm00499523_m1).

Analysis of Microarray Data

Microarray data (Series GSE130563) generated from rectus abdominis muscle biopsies from non-cancer controls undergoing benign abdominal surgery (n = 16) and Stage I-III PDAC patients undergoing tumor-resection surgery (n = 20) (5) was utilized to examine correlative relationships between our specific transcript of interest (*MYOC*)

with its upstream regulators (*FOXO1* and *MEF2c*), and with clinical parameters and body composition, including CT-defined measurements of skeletal muscularity and muscle attenuation, and to survival time post-surgery. Detailed characteristics of non-cancer controls and PDAC patients were described previously (5), and can also be found in **Supplemental Tables S1 and S2**. Correlations between these variables in PDAC patients, or across PDAC patients and non-cancer control patients combined, can be found in the Correlation Matrices presented in **Supplemental Tables S3 and S4**.

Supplemental References

1. Vohra R, Batra A, Forbes SC, Vandenborne K, Walter GA. Magnetic Resonance Monitoring of Disease Progression in mdx Mice on Different Genetic Backgrounds. Am J Pathol **2017**;187:2060-70
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3. Ye F, Baligand C, Keener JE, Vohra R, Lim W, Ruhella A*, et al.* Hindlimb muscle morphology and function in a new atrophy model combining spinal cord injury and cast immobilization. J Neurotrauma **2013**;30:227-35
4. Forbes SC, Willcocks RJ, Triplett WT, Rooney WD, Lott DJ, Wang DJ*, et al.* Magnetic resonance imaging and spectroscopy assessment of lower extremity skeletal muscles in boys with Duchenne muscular dystrophy: a multicenter cross sectional study. PLoS One **2014**;9:e106435
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