SUPPLEMENTARY FIGURE LEGEND

Figure S1. Characterization of the ACS phenotype in mice bearing Pten\(^{\pm}\)E\(\mu\)-Myc or Tsc2\(^{\pm}\)E\(\mu\)-Myc tumors. (A) Schematic diagram illustrating generation of Pten\(^{\pm}\)E\(\mu\)-Myc and Tsc2\(^{\pm}\)E\(\mu\)-Myc tumors and subsequent transplantation assay. (B) Tumor burden of C57BL/6 mice harboring lymphomas of the indicated genotype. Tissues were harvested at the end of the experiment in Fig. 1B. Values are mean ± SEM; n=5 mice. (C) Percent of B220+ cells in mice harbouring tumors of the indicated genotype. Blood samples were taken six days following detection of palpable tumors. The number of B220+ cells are set relative to the total cell population remaining after ACK lysis of red blood cells relative to the levels obtained from control mice. Values are expressed as mean ± SEM; n=5 mice. (D) Spleen weights of C57BL/6 mice bearing tumors of the indicated genotypes. Values are mean ± SEM; n=5 mice. (E) Cross-sectional area measurements of H&E stained sections from the mid-point of the gastrocnemius muscles from E\(\mu\)-Myc or Tsc2\(^{\pm}\)E\(\mu\)-Myc-bearing mice six days following appearance of palpable tumors. Gastrocnemius muscle was dissected and placed in 10% neutral-buffered formalin overnight. The following day, samples were dehydrated and embedded in paraffin. Sections of 4 \(\mu\)m were prepared from the mid-point of the muscle and stained with Hematoxylin/Eosin. The surface area of each muscle slice was calculated using Photoshop image analysis software. Values are mean ± SEM; n=5 mice.

Figure S2. Tsc2\(^{\pm}\)E\(\mu\)-Myc tumor-bearing mice lose muscle mass and adipose fat coincidentally. (A) Body weight of C57BL/6 mice harboring tumors of the indicated genotypes at different days post tumor cell transplantation. Values are mean ± SEM; n=3 mice. (B) Changes in epididymal fat pad weight for the indicated genotypes. Values are mean ± SEM; n=3 mice. (C) Changes in gastrocnemius muscle mass for the indicated genotypes. Values are mean ± SEM; n=3 mice.

Figure S3. Mice bearing E\(\mu\)-Myc/Bcl2 tumors do not display features of ACS. (A) Changes in body weight of C57BL/6 mice harbouring tumors of the indicated genotypes. Values are mean ± SEM; n=5 mice. (B) Epididymal fat pad weights of E\(\mu\)-Myc or E\(\mu\)-Myc/Bcl2 lymphoma-bearing mice. Tissues were harvested at the end of the experiment in (A). Values are mean ± SEM; n=5 mice. (C) Weight of gastrocnemius muscle of C57BL/6 mice bearing tumors of the indicated genotypes. Tissues were harvested at the end of the experiment in (A). Values are mean ± SEM; n=5 mice.

Figure S4. Alterations in oxygen consumption, CO\(_2\) and heat production in mice bearing Tsc2\(^{\pm}\)E\(\mu\)-Myc or E\(\mu\)-myc tumors. (A) Oxygen consumption, (B) CO\(_2\) production, and (C) heat production were measured or calculated from Tsc2\(^{\pm}\)E\(\mu\)-myc and E\(\mu\)-myc mice. The downward arrow indicates the onset of palpable tumours. Values represent the mean ± SEM; n = 4 mice in each group.

Figure S5. Loss of Tsc1 in E\(\mu\)-Myc lymphomas leads to ACS. (A) Food consumption of E\(\mu\)-Myc and Tsc1\(^{\pm}\)E\(\mu\)-Myc tumor-bearing animals. Values are mean ± SEM; n=8 mice. (B) Western blot of extracts (50 \(\mu\)g) from multiple tumors of the indicated genotypes. Antibodies against ribosomal S6 protein (2212, 1:1,000, Cell Signalling; Beverly, MA), phospho-ribosomal S6 (2215, 1:1,000, Cell Signalling; Beverly, MA), \(\alpha\)-tubulin (B-5-1-2, 1:5,000, Sigma; St.-Louis;
MO), and 4E-BP1 (9452, 1:1,000, Cell Signalling; Beverly, MA) were used as probes and were detected using enhanced chemiluminescence (GE Healthcare; Piscataway, NJ).

**Figure S6.** IL-10 and leptin levels are deregulated in the gastrocnemius of mice bearing Tsc2^{+/−} Eµ-Myc tumors. (A) IL-10 levels in serum (left panel) or gastrocnemius extracts (right panel) prepared from mice of the indicated genotypes. (B) Leptin levels in serum (left panel) or gastrocnemius extracts (right panel) prepared from mice of the indicated genotypes.

**Figure S7.** Assessment of RNA quality from polysomes of Tsc2^{+/−} Eµ-Myc/Mcl-1 cells treated with vehicle or rapamycin. RNA was isolated from individual fractions in Fig. 3E and analyzed for the presence of 28S, 18S, and 5.8/5S rRNA by fractionation on a 0.8% agarose/formaldehyde gel and stained with SYBR gold (InVitrogen, Carlsbad, CA).

**Figure S8.** Monitoring Rapamycin and HHT efficacy in vivo. (A) Western blot of two tumors extracted from mice harbouring Tsc2^{+/−} Eµ-Myc/Mcl-1 lymphomas that had been treated with vehicle or Rap (4 mg/kg). Tumors were excised one hour after treatment and Western blots probed with antibodies directed to the indicated proteins. (B) Polysome analysis of tumors extracted from mice harboring Tsc2^{+/−} Eµ-Myc/Mcl-1 lymphomas and treated with HHT (0.25 mg/kg). Tumors were excised one hour after delivery of HTT.

**Figure S9.** Recovery of cytokine levels in Tsc2^{+/−} Eµ-Myc/Mcl-1 lymphoma bearing mice treated with Rap or HHT. Serum was harvested from wild-type and Tsc2^{+/−} Eµ-Myc/Mcl-1 lymphoma bearing mice treated with vehicle, Rap, or HHT at the endpoint of the experiment in Fig. 3C and probed for the indicated cytokines and metabolic markers.

**Figure S10.** Muscle-wasting induced by interferon γ/TNF-α is not Rap-responsive. (A) Changes in body weight of C57BL/6 mice injected I.P. with saline or Rap (4 mg/kg) (day 0) and subsequently injected intramuscularly the following day (day 1) and every day thereafter for 4 additional days with saline or 7500 units of IFNγ and 3 μg of TNFα. Mice continued to receive I.P. injections of saline or Rap during this five day period (days 0-4). (B) Weight of gastrocnemius muscle of C57BL/6 mice harvested on day 6 after treatments in (A). Values are mean ± SEM; n=4 mice.