Explanation of Supplementary Figures

Supplementary Figure 1 Doubling time of primary cultured human myogenic cells Hu20 (filled square), Hu23 (triangle) and Hu26 (circle) was determined at passage 3, 7, and 10. Estimates of doubling time were based on cell numbers.

Supplementary Figure 2 Immortalized human myogenic cells did not grow in soft agar. E18, KD3, and rhabdomyosarcoma cell line RD were seeded at 5 x 10^4 pr 35-mm plate in an appropriate medium (pmGM or DMEM supplemented with 20% FBS alone) with 0.4% agarose and a 0.7% agarose underlay as described (Narisawa-Saito et al., Oncogene 26, 2988-96, 2007). Colonies over 150 μm in diameter were photographed (A) and counted (B) after 11 days. The experiments were performed in triplicate. Colony-forming efficiency represents [colony number]/[seeded cell number] (%).

Supplementary Figure 3 Growth properties of primary cultured human myogenic cells. Primary cultured human myogenic cells were obtained from abdominal muscles of 75-year-old man (Hu20, passage 2; A), 50-year-old man (Hu21, passage 2; B), 69-year-old man (Hu23, passage 2; C) and 65-year-old man (Hu26, passage 2; D), and then cultured in pmGM. Estimates of doubling time were based on cell numbers.

Supplementary Figure 4 Cell cycle exit of KD3 during terminal differentiation. Immortalized human myogenic cell clone KD3 was cultured for up to 78 h in pmDM. To detect DNA synthesis, cells were incubated with 10 μM BrdU for the last 6 h of a

78-h differentiation culture (A and B). Phase contrast images (A and C) and immunofluorescence analysis with anti-BrdU antibody (red in B), or Ki67 (red in D) of the same fields are shown in each row. Nuclei were stained with DAPI (blue in B and D). Scale bars, 50 μm.

Supplementary Figure 5 CDK4 and Cyclin D1 levels in cells exposed to doxycycline. KD3 cells were cultured for 2 d in medium containing 0.1% ethanol (vehicle) (lane 1) or medium containing 250 nM doxycycline (lane 2). TKD1 cells were cultured for 2 d in medium containing 0.1% ethanol (vehicle) (lane 3), and for 2 d (lane 4) or 5 d (lane 5) in medium containing 250 nM doxycycline. Fifteen μmicrograms of total proteins were subjected to immunobloting analysis with antibodies against CDK4, cyclin D1, and β-tubulin.

Supplementary Figure 6 Proliferation and differentiation capacity of immortalized myogenic cells derived from a Leigh disease patient. (A and B) Growth properties of mortal and immortal human myogenic cells derived from the biceps brachii muscle of a Leigh disease patient. Primary cultured human myogenic cell clone HM1-8 was isolated from cryopreserved primary cultured muscle cells of a Leigh disease patient (3-mo-old male). An immortalized multiclonal population, HM183, was established from HM1-8 by transduction with hTERT, cdk4R24C, and cyclin D1. Passage numbers and doubling times are shown in the panels. (C-E) Multipotentiality of immortalized human myogenic cells HM183. The cells were induced to undergo myogenic, osteogenic, and adipogenic differentiation. (C) Cells were cultured for 5 d in the primary myocyte differentiation medium. MyHC was detected by

immunostaining with a horseradish peroxidase reaction product. Nuclei were detected with staining with hematoxylin (blue). Passage numbers are shown in the panels. Scale bar, 50 μm. (D) The cells were cultured for 9 d in serum-containing medium supplemented with βGP (10 mM). The cells were then stained with Alizarin Red S. Whole 35 mm dishes are shown. Scale bar, 10 mm. (E) The cells were cultured for 5 d in serum-containing medium supplemented with γ-linolenic acid (100 μM). Numerous lipid droplets (red) were stained with Oil Red O. Nuclei were stained with hematoxylin (blue). Scale bar, 10 μm.