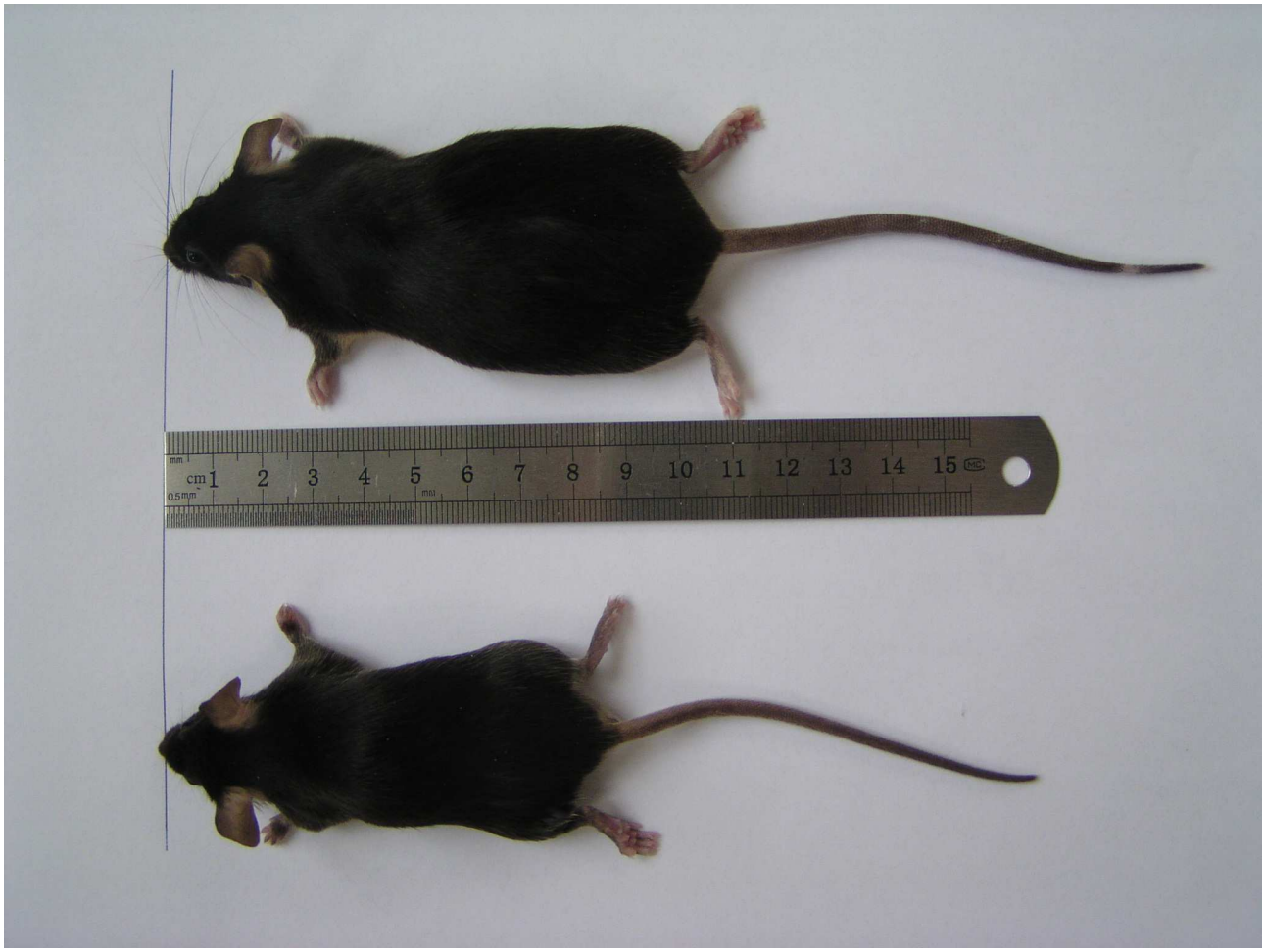
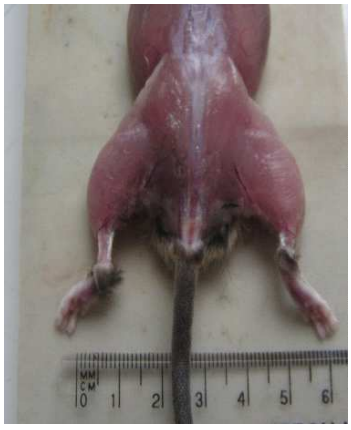
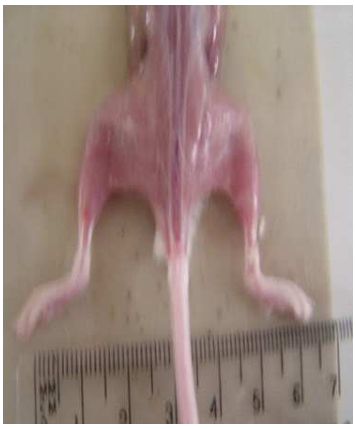
A



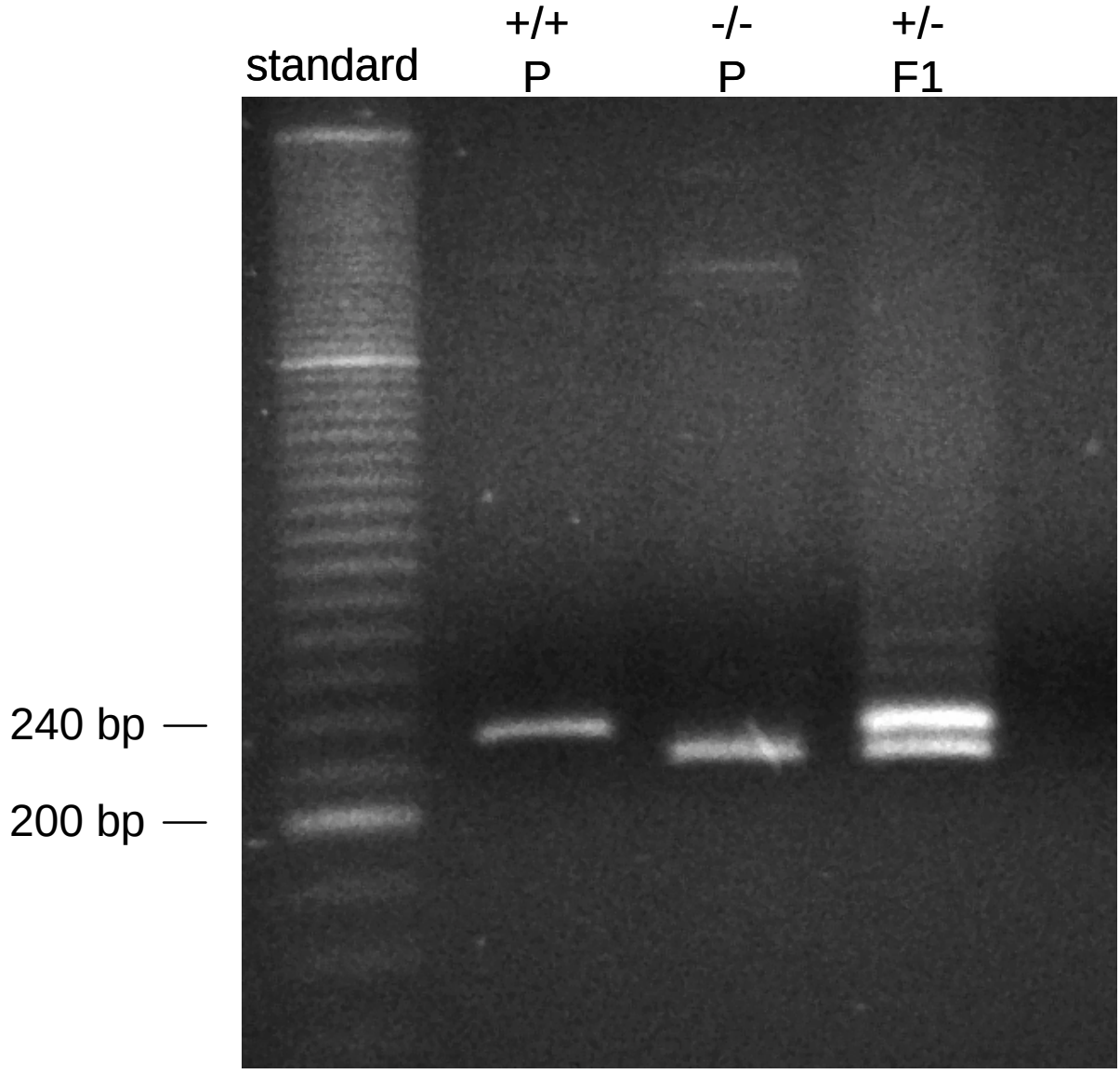
B



C



SP1. Fenotyping of the mice
Cmpt mice (A panel top, B) are significantly larger than their control (A panel bottom, C) counterparts. 25 week old mice.



SP2. Genotyping of the mice
RT-PCR analysis of myostatin composition in different tail segments from a control C57/Bl6 (+/+,Lane 2), a *Cmpt* (-/-,Lane 3) and a heterozygote (+/-, Lane 4) mouse. Lane 1: standard. (P: parents; F1: first generation)

Extension to Materials and methods

RT-PCR procedure

The 12-bp deletion in the myostatin gene of the Cmpt mouse was detected with RT-PCR according to the genebank sequence for myostatin accession number: NM 010834. The following primer pairs were used: myostatin forward 5' ACTGGAATCCGATCTCTGAAACTT 3' and myostatin reverse 5' GACCTCTTGGGTGTGTCTGTCAC 3'. These primers amplified a 233 bp and a 221 bp fragment, as expected. PCR products were analysed on 2.5% agarose gel.
The mixture (20 µL) for reverse transcriptase (RT) reactions contained 500 ng total RNA, 0.25 µL RNase inhibitor, 0.25 µL oligo dT primer, 1 µL dNTP Mix (200 µM), Omniscript™ RT (1 µL) in 10× RT buffer (High Capacity RT kit; Qiagen) and complementary cDNA was transcribed at 37 °C for 1 hours. PCR reactions were carried out in a final volume of 50 µL containing 2–2 µL forward and reverse primers (10 µM), 1 µL cDNA, 1 µL dNTP Mix (200

μM), and Promega GoTaq® DNA polymerase (0.5 μL) in 5× GoTaq® Reaction Buffer in a programmable thermal cycler (C 1000 Gradient Thermal Cycler; BioRad) with the following settings: 2 min at 95 °C for initial denaturation followed by 35 repeated cycles of denaturation at 94 °C for 1 min, primer annealing for 1 min at an optimised temperature (58 °C), and extension at 72 °C for 90 s. After the final cycle, further extension was allowed to proceed for another 10 min at 72 °C.

Whole-cell intracellular Ca^{2+} concentration measurement

Fibres were equilibrated in Tyrode's solution for 30 minutes at room temperature. Cover slips with Fura-2 loaded fibres were placed on the stage of an inverted fluorescence microscope (Diaphot, Nikon, Tokyo, Japan). The excitation wavelength was altered between 340 and 380 nm by a microcomputer-controlled dual-wavelength monochromator (Deltascan, Photon Technology International, New Brunswick, NJ), whereas the emission was monitored at 510 nm using a photomultiplier at 10 Hz acquisition rate of the ratios at 22°C. Fibres were permanently washed with Tyrode's solution using a background perfusion system, whilst the depolarizing solution (120 mM NaCl was replaced by 120 mM KCl) was applied through a local perfusion system, which was positioned in close proximity of the measured fibre.

Detection of calcium release events

After enzymatical dissociation, FDB fibres were placed into culture dishes with relaxing solution (in mM, 150 K-glutamate, 2 $MgCl_2$, 10 HEPES and 1 EGTA). The fibres were permeabilized using 0.01% saponin for a few seconds. Fibres were monitored persistently during the permeabilization. Spontaneous calcium release events were visualised by 50 μM Fluo-3 using a confocal laser scanning microscope (Zeiss 5 META, Oberkochen, Germany) at 22 ^{o}C. Line-scan images (512 pixels/line) were used to monitor the fluorescence intensity changes at 1.53 ms/line scanning speed and using a 63x water immersion objective. Fluo-3 was excited with an argon ion laser at 488 nm.

Ca^{2+} sensitivity of the contractile proteins

After enzymatical isolation, permeabilization was performed with 0.5 % Triton-X 100 detergent. The pCa of the relaxing solution (in mM, 37.34 KCl, 10 BES, 6.24 $MgCl_2$, 7 EGTA, 6.99 Na_2ATP, and 15 sodium creatinin-phosphate, pH 7.2) was 9, while the fully activating solution (relaxing solution supplemented with 7 mM Ca) had a pCa of 4.75. Fibres were attached to two thin needles: one of them was connected to a force transducer and the other one to a electromagnetic motor. Isometric force measurements were performed during repeated activation–relaxation cycles at a sarcomere length (SL) of 2.3 μm at 15°C.