ADDITIONAL METHODS

RFLP analysis
To confirm the allele-specificity of p57 expression, cDNAs from MyoD-infected polymorphic fibroblasts (C57BL/6 x SD7) were amplified by semiquantitative PCR with the following primers:

F: 5’-TTCAGATCTGACCTCAGACC-3’
R: 5’-AGTTCTCTTGGCGCTTGGC-3’.

Maternal and paternal products were distinguished by AVA I (Promega) digestion of a previously described polymorphic restriction site for this enzyme (Battistelli et al., 2014).

Methylated DNA Immunoprecipitation (MeDIP) assays
Genomic DNA was extracted, purified and sonicated in order to obtain fragments of a length in a range of 300-600 bp. 6 µg of sonicated DNA was re-suspended in 500 µL of Ip Buffer [Immunoprecipitation Buffer (NaCl 140mM, Triton X-100 0.05%, sodium phosphate 10mM pH=7)]. The denaturation of DNA was performed 5 minutes at 95 °C and the incubation with 5 µg of mouse anti 5-Methylcytidine antibody (Bi-Mecy 0100, Eurogentec) or with anti normal mouse IgG antibody (12-371, Merk Millipore) over-night at 4°C. 40 µL of protein G magnetic beads were added to samples and incubated 4 hours at 4 °C. The supernatant of the IgG sample was taken as Input and beads were washed 3 times with Ip Buffer. After washes samples were incubated for 2 hours at 56 °C and overnight at 37°C with proteinase K (Sigma-Aldrich). The extraction of DNA was performed with phenol-chloroform solution, precipitated with ethanol and the pellet of DNA was re-suspended in 50 µl of nuclease-free distilled water. qPCR analyses of immunoprecipitated maternal and paternal p57 intragenic region and, as negative control, for of the Translocase of inner mitochondrial membrane 17 (Timm) were performed each in triplicate using 5 ng of DNA, GoTaq qPCR Master Mix (Promega) using the termocycler “CFX Connect Real Time system” (Bio-Rad) and the following set of primers:

Maternal p57i F: 5’- CAGATCTGACCTCAGACCCG-3’
R: 5’- GACCTGTTCCTCGCCATCCT-3’;
Paternal p57i F: 5’-AACCTCCAGCGATGTGCC-3’
R: 5’-CATCCACTGCAGACGACCAG-3’;
Timm prom: F: 5’-ACGGATGTGGCCCTTCTGGC-3’
R: 5’-CCGTTCGAAACGCCACAA-3’. 