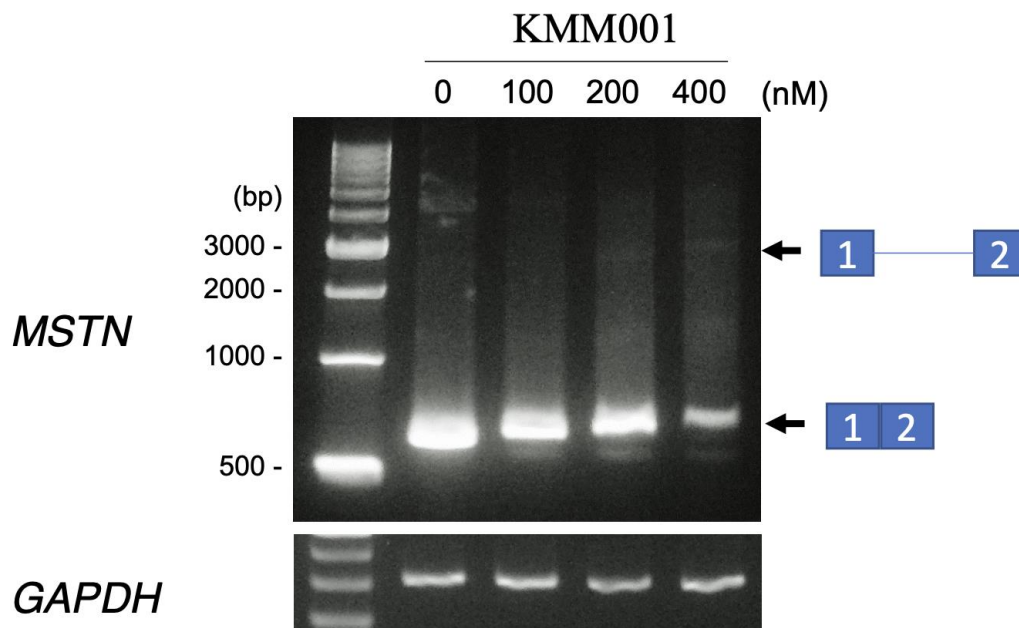


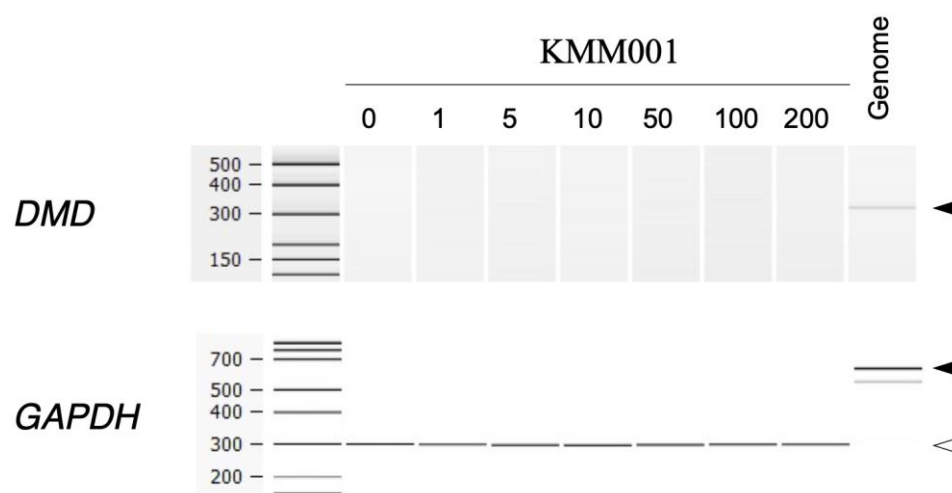
supplementary data

supplementary Figure S1 PCR amplification of the region spanning exon 1 to exon 2 of the *MSTN* gene



The region from exon 1 to exon 2 of the *MSTN* gene was PCR amplified. The electrophoretic patterns of the amplified products are shown. No amplified band of 2.4 kilobase pairs was obtained in the absence of KMM001 treatment, but an amplified band of the expected size was weakly observed in the presence of 200 nM KMM001 (→). A target size band was obtained for the *GAPDH* gene in both samples (GAPDH).

supplementary Figure S2 PCR amplification of the region spanning exon 45 of the *DMD* gene



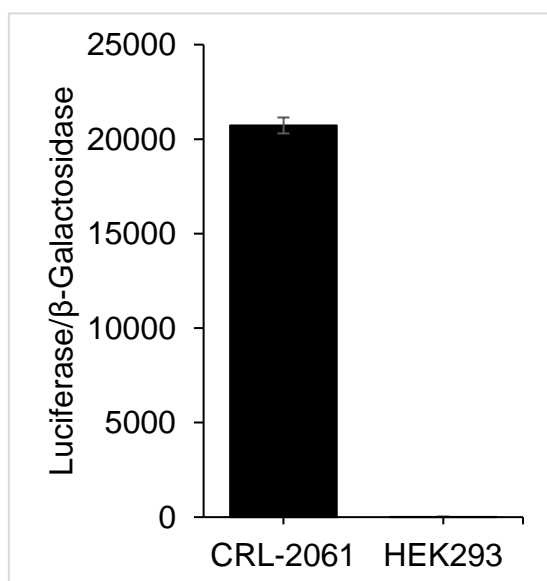
The exon 45 region of the *DMD* gene was PCR amplified. The electrophoretic patterns of the amplified products are shown. A target size band was obtained for the *GAPDH* gene in samples (GAPDH). On the other hand, the exon 45 region of the *DMD* gene was not amplified in any of the samples (DMD), except genomic DNA (genome).

supplementary Figure S3. Alignment of *MSTN* exon1 and *GDF11* exon 1 nucleotide sequences

MSTN_Ex1	1	AGATTCACTGGTGTGGCAAGTTGTCTCTCAGACTGTACATGATTAAAAATTTGCTTGGC	60
GDF11_Ex1	1	----TCCCCGCC--CCCAGTCTCCCTC--CCCTCCCCCTCCAG--CATGGTGCTCGGC	49
MSTN_Ex1	61	ATTACTCAAAAGCAAAAGAAAAGTAAAAGGAAGAAACAAGAAAGAAAAGATTATAT	120
GDF11_Ex1	50	GCCCCGCTGCTGCTGG-GCTTCCT-----GCTCCTCGCCCTGGA-----GCTCGGC	95
MSTN_Ex1	121	TGATTTTAAATCATGCAAAAGTCAAACTCTGTGTTTATATTACCTGTTTATGCTG-A	179
GDF11_Ex1	96	CCCGGGGGGAGGCGGCCGAGGGCCCCGCGGGCGGGCGGGCGGGCGGCG-GCGGCGGCGGCA	154
MSTN_Ex1	180	TTGTTGCTGGTCCAGTGGATCTAAATGAGAACAGTGAGCAAAAAGAAAATGTGGAAG	239
GDF11_Ex1	155	GCGGCGGGGGTCTGGGGGGG-----AGCGCTCCAGCCGCCAGCCCGTCCGTGGCGCCG	209
<div>KMM001</div>			
MSTN_Ex1	240	AGG-GGCTGTGTATGATGTAC--TTGGAGACAAACA--CTAAATCTTCAAGATAGA	294
GDF11_Ex1	210	AGCCGGACGGCTGCCCGTGTGCGTTTGGCGGACGACAGCCGCGAGCTGC--GCCTAGA	267
MSTN_Ex1	295	AGCATTAAAGATACAAATCCTCAGTAACTTCGTCTGGAACAGCTCCTAACATCAGCAA	354
GDF11_Ex1	268	GAGCATCAAGTCGCAGATCTTGAGCAAACTGCGGCTCAAGGAGGCGCCCAACATCAGCCG	327
MSTN_Ex1	355	AGATGTTATAAGACAACCTTTACCCAAAGCTCCTCCACTCCGGGAAGTATTGATCAGTA	414
GDF11_Ex1	328	CGAGGTGGTGAAGCAGCTGCTGCCAAGGCGCCGCGCTGCAGCAGATCCTGGACCTACA	387
MSTN_Ex1	415	TGATGTCCAGAGGGATGA-CAGCAGC--GATGGCTCTTTGGAAGATGACGATTATCAGCG	471
GDF11_Ex1	388	CGACTTCCAGGGCGACGCGCTGCAGCCGAGGACTTCCTGGAGGAGGACGAGTACCACGC	447
MSTN_Ex1	472	TACAACGGAACAATCATTACCATGCCTACAGAGT	506
GDF11_Ex1	448	CACCACGAGACCGTCATTAGCATGGCCAGGAGA	482

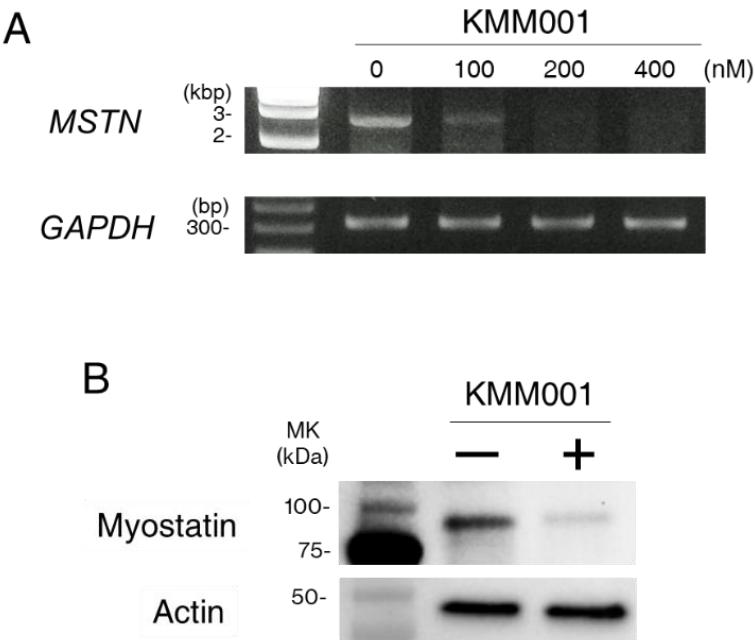
Alignment of *MSTN* exon 1 and *GDF11* exon 1 nucleotide sequences is shown (MSTNEx1 and GDF11-Ex1, respectively). Identity is 46.2%. A red bar indicates a sequence complementary to KMM001. Numbers of nucleotides indicate base number of exon 1. Nucleotide sequence alignment was analyzed by Pairwise Sequence Alignment with EMBOSS Needle (https://www.ebi.ac.uk/Tools/psa/emboss_stretcher/).

supplementary Figure S4 SMAD dependent luciferase activity in CRL-2061 cells



The relative luciferase activity was determined in CRL-2061 and HEK293 cells by transfecting the SMAD responsive reporter gene. The luciferase activity is shown by columns. It was detected in CRL-2061 cells (left) but not in HEK293 cells (right).

supplementary Figure S5. KMM001 decreased *MSTN* mRNA and myostatin protein in human myoblast.



A. Mature *MSTN* mRNA in human myoblasts was RT-PCR-amplified. Electrophoretograms of the RT-PCR-amplified products are shown. A target size band decreased by increase of KMM001 concentration (*MSTN*). The *GAPDH* gene was amplified as control (*GAPDH*). B. Myostatin protein in human myoblast transfected with KMM001 was assayed by Western blot analysis using an antibody against the N-terminal domain of human myostatin. Immunoblot result is shown. One clear band was identified in untreated human myostatin (-). In contrast, in KMM001-treated cells, a band corresponding to myostatin was weakly visualized (+). Mk refers to the size marker.