

Figure 1. Chromatogram of ajoene extract in high performance liquid chromatography (HPLC). HPLC conditions are as follows, column: Spherisorb silica, 5 μm , 4.6 \times 250 mm, eluent: n-hexane/10% iso-propyl alcohol in ethyl acetate (67/33) 1 ml/min, detection 254 nm UV.

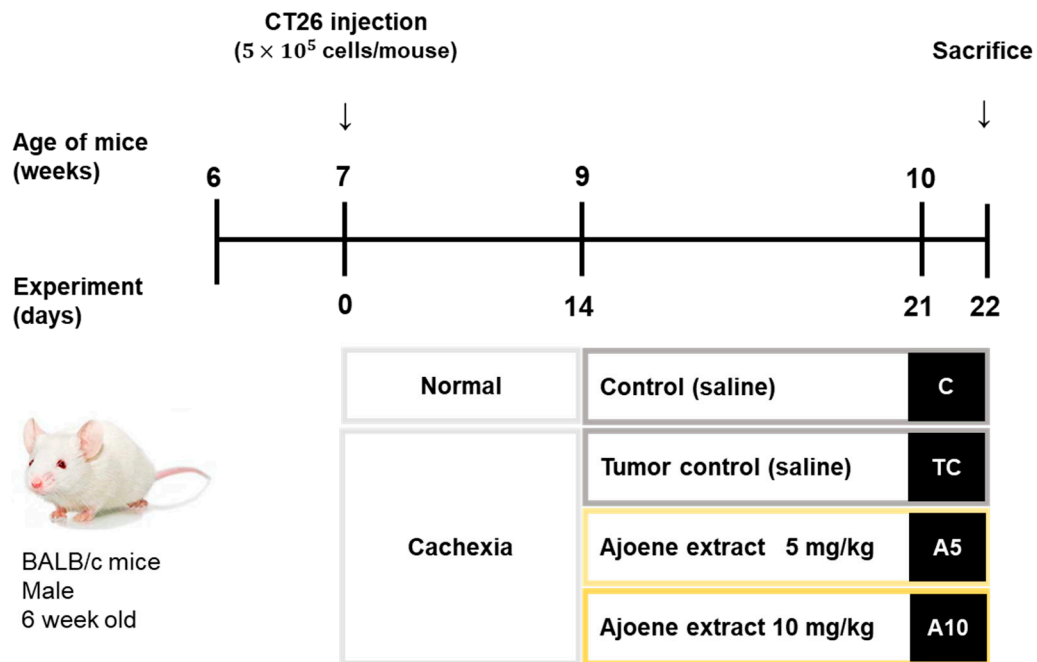


Figure 2. Experimental design of mouse model of cancer cachexia.

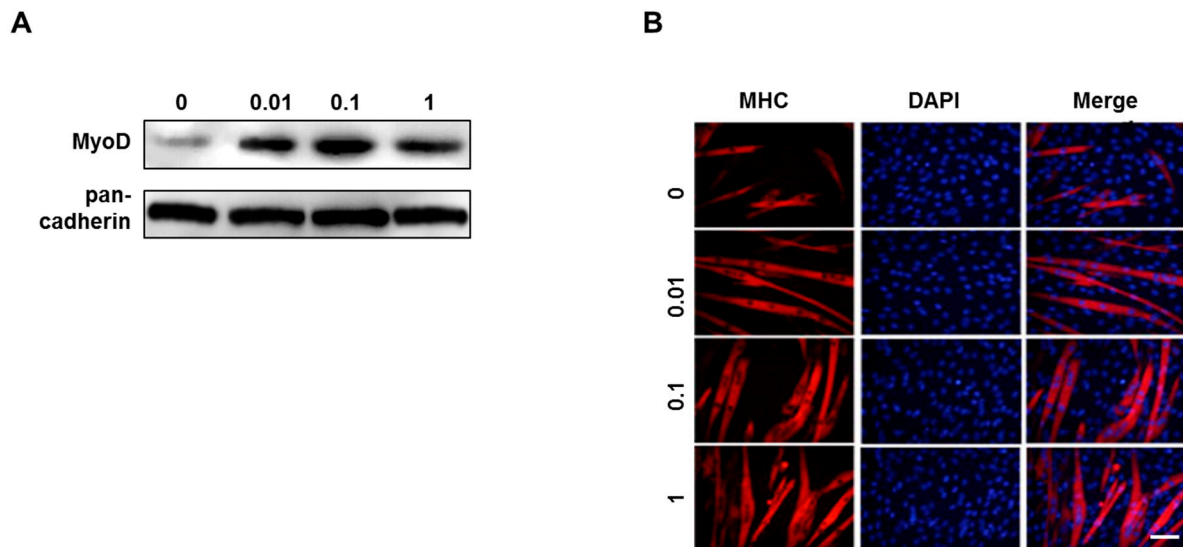


Figure 3. Effect of ajoene extract on myoblast differentiation. C2C12 cells were differentiated using differentiation medium (DM) supplemented with ajoene extract (0.01, 0.1, or 1 $\mu\text{g/mL}$) for 3 days, and then cells were collected to perform Western blotting analysis to determine the level of myoD expression (A) and immunostaining of MHC (red) and 4',6-diamidino-2-phenylindole (DAPI, blue) (B). Scale bar = 200 μm .

Table 1. Oligonucleotide primer sequences used for the real-time quantitative polymerase chain reaction (qRT-PCR) analysis.

| Gene | Forward Primer | Reverse Primer | Accession Number |
|-----------------|------------------------------|------------------------------|--------------------|
| <i>In vitro</i> | | | |
| <i>MAFbx</i> | CGACCTGCCTGTGTGCTTAC | CTTGCGAATCTGCCTCTCTG | BC027211 |
| <i>MuRF1</i> | GGTGCCTACTTGCTCCTTGT | CTGGTGGCTATTCTCCTTGG | NC_000070 |
| <i>GAPDH</i> | TGCACCACCAACTGCTTAG | GGCATGGACTGTGGTCATGA G | BC096042 |
| <i>In vivo</i> | | | |
| <i>FoxO1</i> | CAAAGTACACATACGGCCAA TCC | CGTAACTTGATTTGCTGTCCT GAA | NM_019739.3 |
| <i>IL-6</i> | TTTCCTCTGGTCTTCTGGAGT | GTGACTCCAGCTTATCTCTT G | NM_00131114 4.1 |
| <i>IL-6R</i> | ATGATGCCTTGCAGGAGTG | TGGGCTCTATCCAAGGAGTG | NM_00131067 6.1 |
| <i>Jak</i> | GGAGTACTACACAGTCAAGG ACGA | AAACATTCCGGAGCGTACC | NM_013567.1 |
| <i>MAFbx</i> | AGTGAGGACCGGCTACTGTG | GATCAAACGCTTGCGAATCT | NM_026346.3 |
| <i>Mstn</i> | CAGACCCGTCAAGACTCCTAC A | CAGTGCCTGGGCTCATGTCA AG | NM_010834.3 |
| <i>MuRF1</i> | TGACATCTACAAGCAGGAGT GC | TCGTCTTCGTGTTTCCTTGC | NM_00136924 5.1 |
| <i>Myh</i> | CTCAGGCTTCAAGATTTGGTG G | TTGTGCCTCTCTTCGGTCATT C | NM_00103954 5.2 |
| <i>MyoD</i> | AAACCCCAATGCGATTTATCA GG | TAAGCTTCATCTTTTGGGCGT GA | NM_010866 |
| <i>Smad2</i> | AAGCCATCACCCTCAGAATT G | CACTGATCTACCGTATTTGC TGT | XM_006525699 .2 |
| <i>Smad3</i> | GGGGAGCAGAGTACAGGAGA | CTGCAAGGGTCCATTCAGGT | NM_016769.4 |
| <i>Smad4</i> | AAGGTGGGGAAAGTAAAC | ATGCTTTAGTTCATTCTTGTG | NM_00136496 8.1 |
| <i>Stat3</i> | TTTCATCAGCAAGGAGCGGG | GGGGTAGAGGTAGACAAGT GGA | NM_011486.5 |
| <i>GAPDH</i> | CAACTTTGGCATTGTGGAAG | CTGCTTCACCACCTTCTTG | NM_00128972 6.1 |

FoxO1, forkhead box protein O1; *IL-6*, interleukin 6; *IL-6R*, interleukin 6 receptor; *Jak*, janus kinase; *MAFbx*, muscle atrophy F-box (MAFbx/atrogen-1); *Mstn*, myostatin; *MuRF1*, muscle-specific RING finger protein-1; *Myh*, myosin heavy chain; *MyoD*, myoblast determination protein 1; *Smad2*, SMAD family member 2; *Smad3*, SMAD family member 3; *Smad4*, SMAD family member 4; *GAPDH*, glyceraldehyde 3-phosphate dehydrogenase.