

## Supplementary file

# Combination Treatment of Arazyme and Soy Leaf Extract Attenuates Hyperglycemia and Hepatic Steatosis in High-fat Diet-fed C57BL/6J Mice

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Primer sequences used in this article are described in Table S1.

The inguinal mRNA expression of browning-related factors, including *Ucp1*, *Pgc1a*, and *Adrb3*, were not changed in all groups (Figure S1).

Arazyme and ESL combination treatment significantly reduced lipid accumulation-related gene expression including sterol regulatory element-binding transcription factor 1 (*Srebf1*), MLX-interacting protein-like (*Mlxipl*), acetyl-CoA carboxylase 1 (*Acc1*), *Acc2*, fatty acid synthase (*Fas*), stearoyl-CoA desaturase 1 (*Scd1*), and *Scd2*, compared with the single treatment groups (Figure S2).

Table S1. Sequences of primers used for real-time qRT-PCR

| Gene name                      | Forward primer               | Reverse primer              |
|--------------------------------|------------------------------|-----------------------------|
| <i>Acc1</i> (NM_13360)         | AGTTTCCCAGCCAGCAGATT         | ATCCATCACCAACAGCCTTCA       |
| <i>Acc2</i> (NM_133904)        | CCCATCACCACTCCTCTGA          | GTCCGAGTCTCCACAGCAAT        |
| <i>Acox</i> (NM_015729)        | GCTGGGCTGAAGGCTTTACTA        | AATCCCAC TGCTGTGAGAATAGC    |
| <i>Adiponectin</i> (NM_009605) | CATGCCGAAGATGACGTTAC         | CGATACACATAAGCGGCTTC        |
| <i>Adrb3</i> (NM_013462)       | AGGCAACCTGCTGGTAATCA         | TCCACAGTTCGCAACCAGTT        |
| <i>Apob</i> (NM_009693)        | CCCGTGATACCTACCAGAAT         | CTGCCATGTGGTACTTCTGA        |
| <i>Cd36</i> (NM_007643)        | TGCTGGAGCTGTTATTGGTG         | CTCAAAGATGGCTCCATTGG        |
| <i>Cpt1a</i> (NM_013495)       | CTGCACTCCTGGAAGAAGAA         | GTTCTCGTCTGGCTTGACA         |
| <i>Cyp7a1</i> (NM_007824)      | GACATGGAGAAGGCTAACGAC        | TGAAGTCCTCCTTAGCTGTC        |
| <i>Cyp7b1</i> (NM_007825)      | TAGGAGCACATCATCTTGGC         | TCGGATGATGCTGGAGTATG        |
| <i>Cyp8b1</i> (NM_010012)      | TCTGGGT CCTCTTATTCCCTG       | GGTGAGGAACCGATCATAAC        |
| <i>Fas</i> (NM_007988)         | TGTGAGTGGTCAGAGGCAT          | TTCTGTAGTGCCAGCAAGCT        |
| <i>Gapdh</i> (NM_001001303)    | ACATCATCCCTGCATCCACT         | AGATCCACGACGGACACATT        |
| <i>Glut2</i> (NM_031197)       | TTTGT CATCGCCCTCTGCTT        | GCAGCGATTTCCTCAAAGACT       |
| <i>Hmgcr</i> (NM_008255)       | TAGGCTTGGTCCTTGTTCAC         | GCTTCTTGAGGTACGAC           |
| <i>Il1b</i> (NM_008361)        | ATGGCAACTGTTCTGA ACTCAACT    | ATATTCTGTCCATTGAGGTGGAGAGCT |
| <i>Ins1</i> (NM_008386)        | ACCCACCCAGGCTTTGTC           | CGGGACTTGGGTGTAGAAG         |
| <i>Ins2</i> (NM_001185083)     | CCCCACCCAGGCTTTGT            | CGGGGACATGGGTGTGAG          |
| <i>Insr</i> (NM_010568)        | CTGAACAAAGATGACAACGAGGAA     | CTTACAGATGGTGGCAAACCTT      |
| <i>Irs1</i> (NM_010570)        | GAGAAGAGACTGGCTCGGAAGA       | GCCTATTCTGCCAACTCAACT       |
| <i>Irs2</i> (NM_001081212)     | GGCCCGAACCTCAATAACAA         | CCGCGAACACGAAAAAG           |
| <i>Mlxipl</i> (NM_021455)      | CAGATGCCGGACATGTTGA          | AATAAAGGTGGATGAGGATGCT      |
| <i>Mttp</i> (NM_001355051)     | CGTCCCGCTGCTGTACATCTTA       | CCCCAATGGACAGCAGGAT         |
| <i>Nr1h4</i> (NM_009108)       | GACAGCGAAGGGCGTGACT          | TTTATTGTGATTCCCTGAGGCATT    |
| <i>Pcsk1</i> (NM_013628)       | CTGTTTCCGCCTTCTTGT           | CCGCCGCCATTCAATT            |
| <i>Pcsk2</i> (NM_008792)       | GAAGACCGAGCCTACACCATAA       | CTCTCTTTTACGGTCAAATCCTTCT   |
| <i>Pgc1a</i> (NM_008904)       | GTGCAGCCAAGACTCTGTAT         | GGTCGCTACACCACTCAAT         |
| <i>Pparα</i> (NM_011144)       | CCTGAACATCGAGTGTGAA          | GTACTGGCATTGTTCCGGT         |
| <i>Pparg1</i> (NM_001127330)   | ATGTCTCACAAATGCCATCAGGTT     | GGCGGAAGGACTTATGTATGAGT     |
| <i>Pparg2</i> (NM_011146)      | TGTCTCACAAATGCCATCAGGTT      | AGCGGGAAAGGACTTATGTATGAGT   |
| <i>Scd1</i> (NM_009127)        | ACGCCGACCCCTCACAAATCC        | AGTTTCCGCCCTCTCTTGT         |
| <i>Scd2</i> (NM_009128)        | CCGTGGCTTCTTTCTCTCA          | TTCCGCCCTCTCTTGTGACA        |
| <i>Srebf1</i> (NM_011480)      | GAGCGAGCGTTGA ACTGTAT        | ATGCTGGAGCTGACAGAGAA        |
| <i>Srebf2</i> (NM_033218)      | TCCTCCATCAACGACAAAATCA       | ACTTGTGCATCTGGCATCTGT       |
| <i>Tnfa</i> (NM_001278601)     | CTCAGATCATCTCTCAAAATTGAGTGAC | CTTCACAGAGCAATGACTCCAAAGT   |
| <i>Ucp1</i> (NM_009463)        | CCAGGCTCCAGTACCATTA          | GCCACACCTCCAGTCATTAA        |
| <i>Ucp2</i> (NM_011671)        | GCCTCTACGACTCTGTAAA          | CTTCGACAGTGTCTGGTAT         |

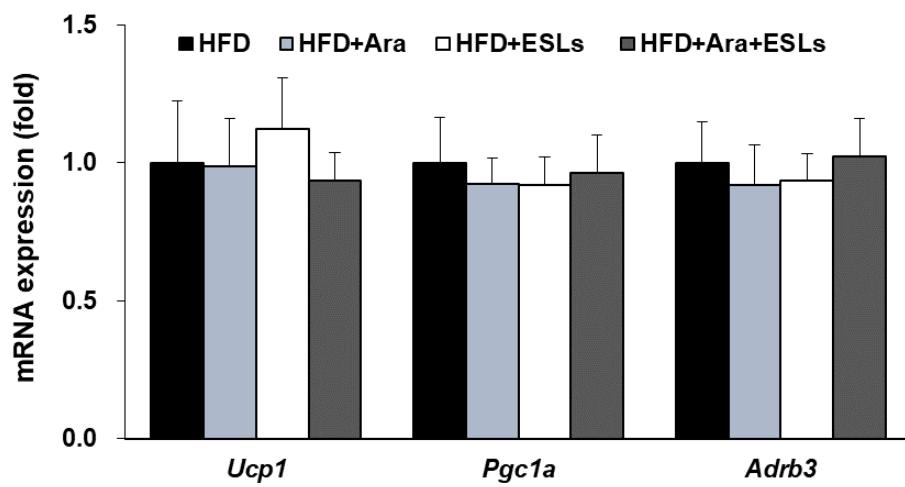


Figure S1. The browning-related gene expression in inguinal adipose tissue. Values are presented as means  $\pm$  SE (n = 6).

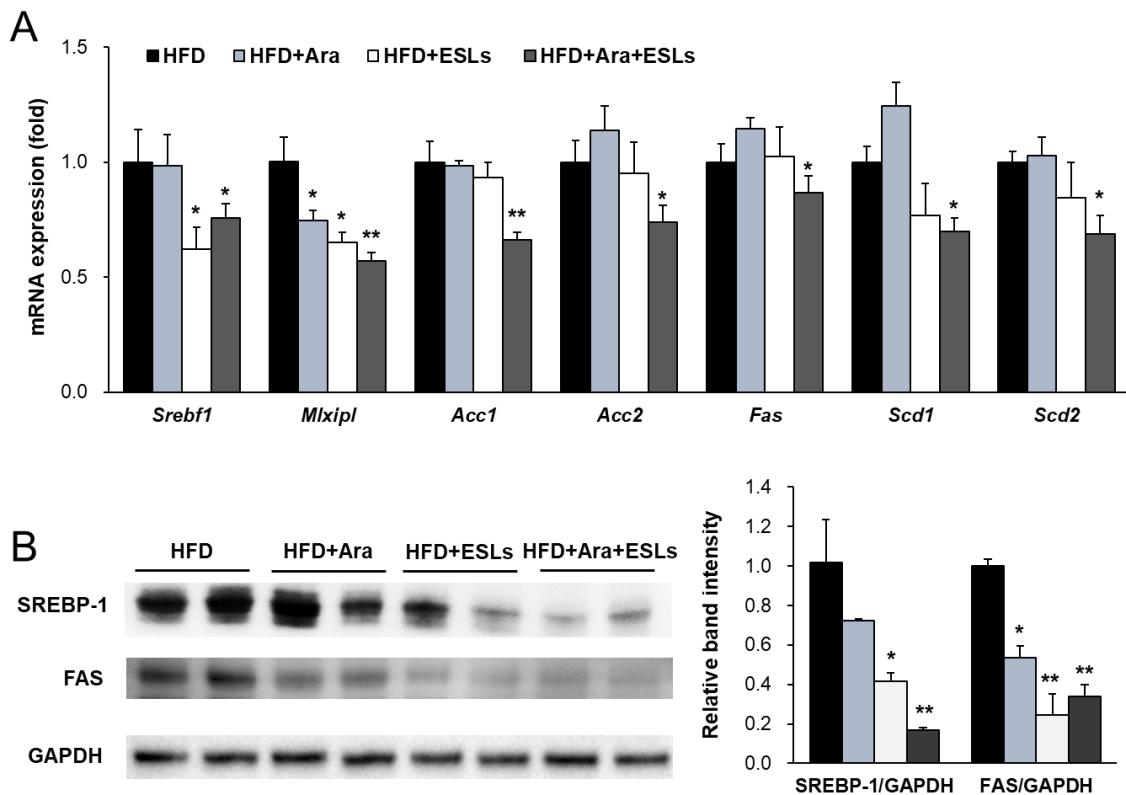


Figure S2. The combination treatment of Arazyme (Ara) and ESL suppressed hepatic lipogenesis-related gene and protein expression. (A) The hepatic mRNA expression levels were detected by real-time qRT-PCR and normalized using *Gapdh* as a reference gene. (B) The hepatic protein expression levels were detected by western blotting with anti-SREBP-1, anti-FAS, and anti-GAPDH antibodies. Values are presented as means  $\pm$  SE ( $n = 6$ ). \* $P < 0.05$ , \*\* $P < 0.01$  vs. HFD group.