

# Supplementary Information

## Cinerascetins, New Peptides from *Hypsiboas cinerascens*: MALDI LIFT-TOF-MS/MS *de novo* Sequence and Imaging Analysis

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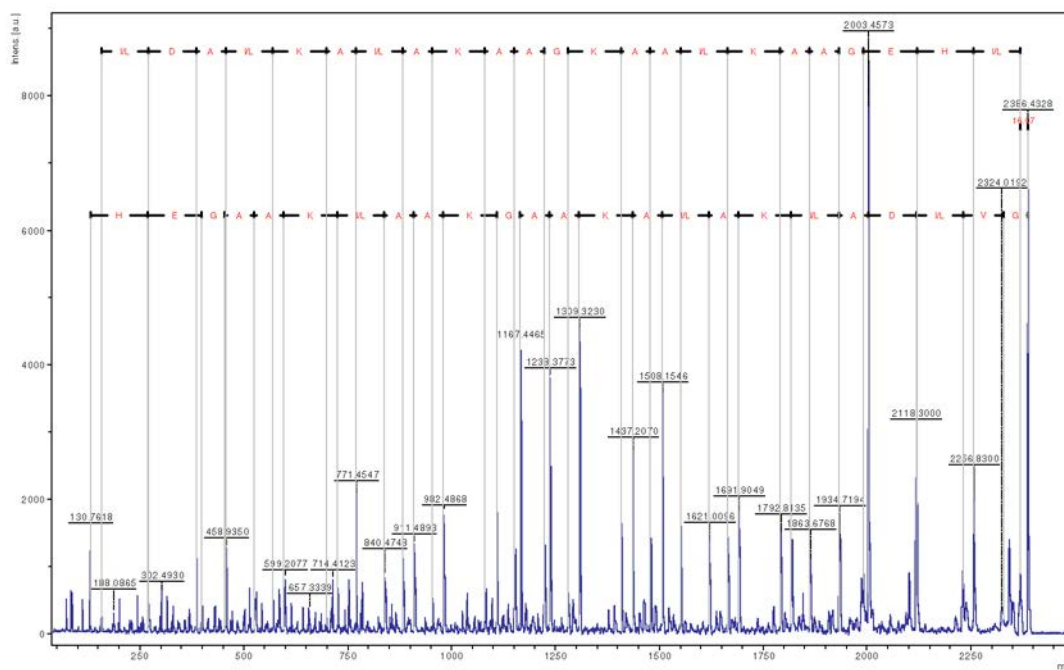
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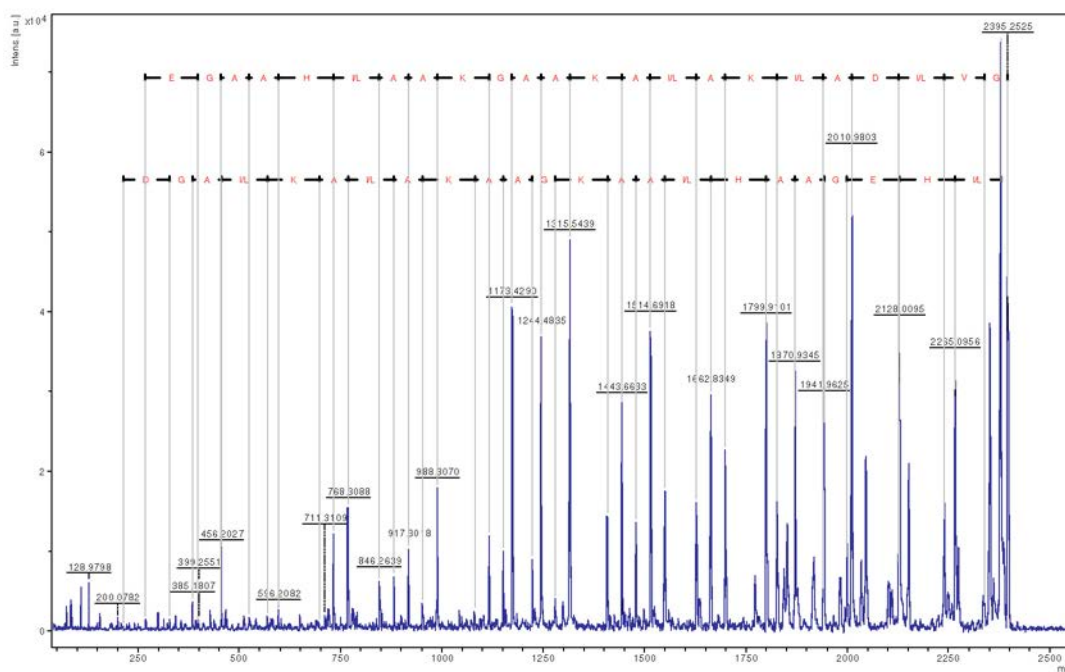
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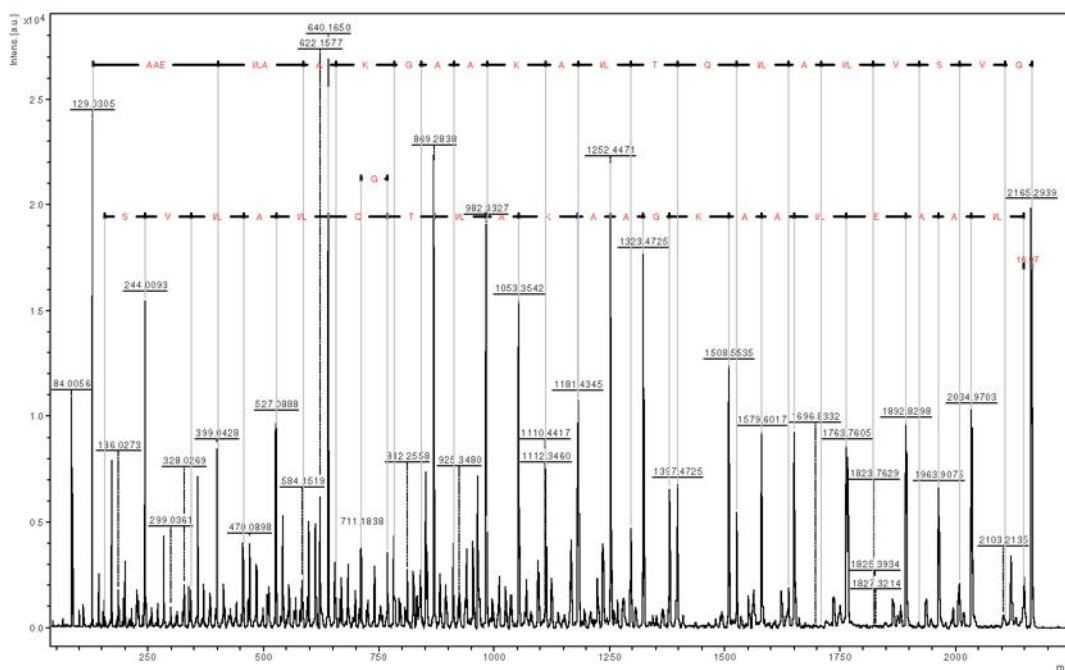


**Figure S1.** MS/MS of peptide C-01  $m/z$  2386.4328  $[M + H]^+$ . The peptide was fragmented by MALDI-TOF-MS/MS. *De novo* sequencing was realized manually using the FlexAnalysis 3.3 (Bruker Daltonics) program.

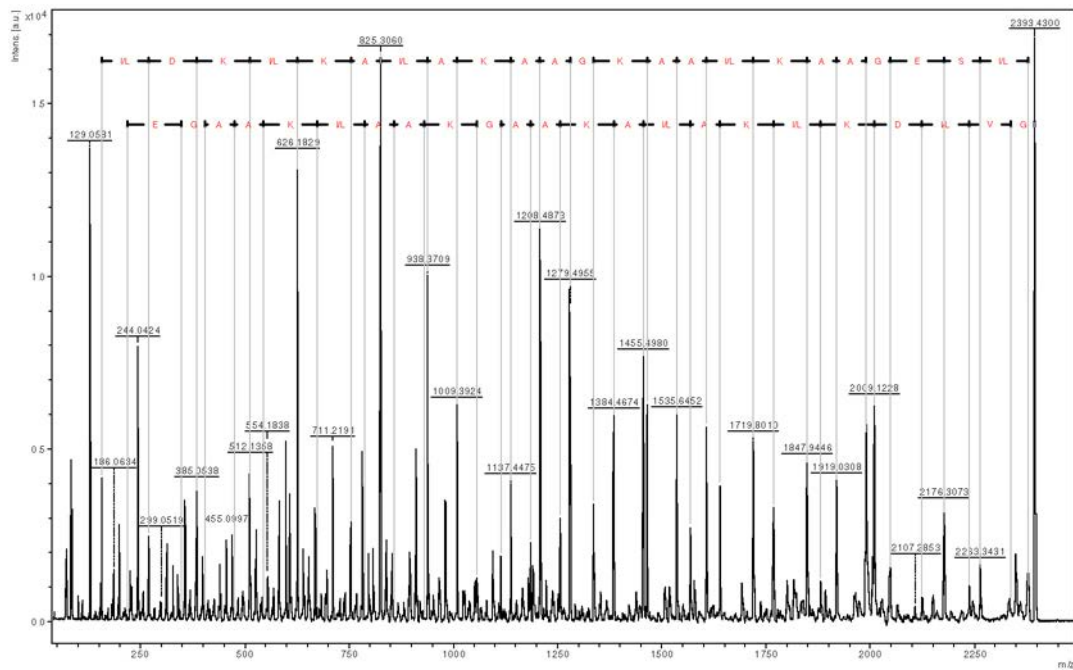
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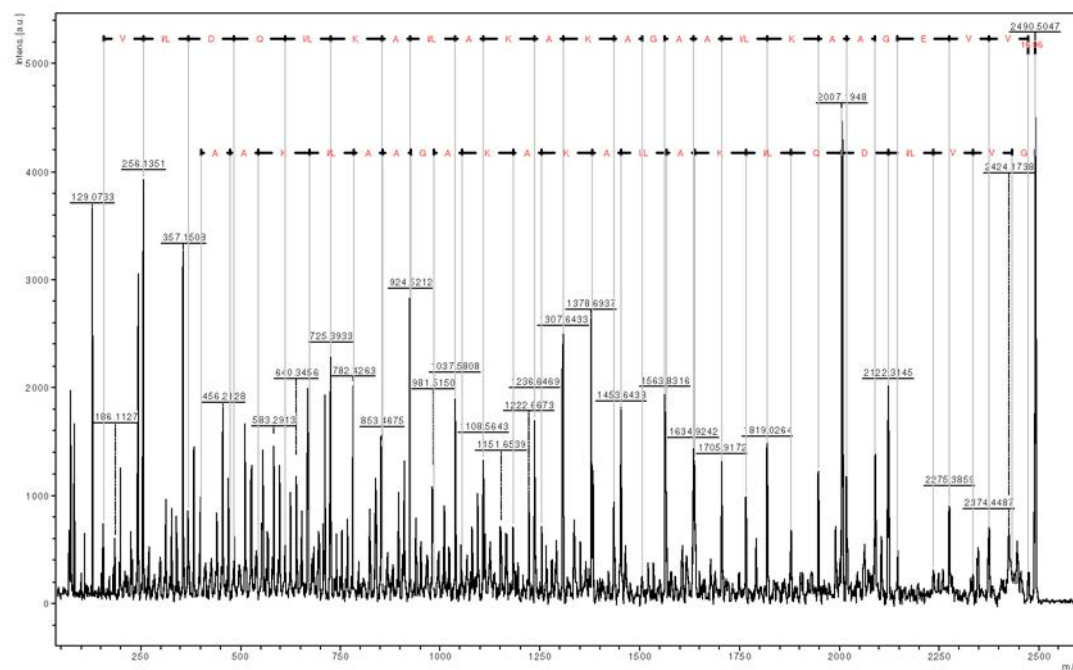
**Figure S2.** MS/MS of peptide C-02  $m/z$  2395.2525  $[M + H]^+$ . The peptide was fragmented by MALDI-TOF-MS/MS. *De novo* sequencing was realized manually using the FlexAnalysis 3.3 (Bruker Daltonics) program.



**Figure S3.** MS/MS of peptide C-03  $m/z$  2165.2939  $[M + H]^+$ . The peptide was fragmented by MALDI-TOF-MS/MS. *De novo* sequencing was realized manually using the FlexAnalysis 3.3 (Bruker Daltonics) program.



**Figure S4.** MS/MS of peptide C-04  $m/z$  2393.4300  $[M + H]^+$ . The peptide was fragmented by MALDI-TOF-MS/MS. *De novo* sequencing was realized manually using the FlexAnalysis 3.3 (Bruker Daltonics) program.



**Figure S5.** MS/MS of peptide C-05  $m/z$  2490.5047  $[M + H]^+$ . The peptide was fragmented by MALDI-TOF-MS/MS. *De novo* sequencing was realized manually using the FlexAnalysis 3.3 (Bruker Daltonics) program.

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      M A F L K K S L F F V L F L G
1 ATGGCTTTC CTGAAAAA TCCCTTTT TTTGTA CTTCCTGG
  TACCGAAAG GACTTTTT AGGGAAAA AAACATGAA AAGGAACCT
      I V F L S I C Q D E K R E G D
51 ATAGTTTTT TTGTCCATC TGTCAAGAT GAGAAAAGA GAGGGGGAC
  TATCAAAAA AACAGGTAG ACAGTTCTA CTCTTTTCT CTCCCCCTG
      E Q Y E E E E N E E A S E E K
101 GAGCGTAT GAAGAGGAA GAAAATGAA GAGGCAAGT GAGGAAAAG
  CTCGTCATA CTTCTCCTT CTTTACTT CTCCGTTCA CTCCTTTTC
      R G V L D A I R A I A K A A G
151 AGAGGAGTA CTTGATGCA ATTAGAGCT ATAGCGAAA GCAGCTGGT
  TCTCCTCAT GAACTACGT TAATCTCGA TATCGCTTT CGTCGACCA
      K A A F Q A A G E H I G *
201 AAAGCCGCT TTTCAAGCT GCTGGTGAA CACATAGGT ATTCGTTAA
  TTTCCGCGA AAAGTTCGA CGACCACTT GTGTATCCA TAAGCAATT
251 TGATTTCATC TTTATGGAA CATAACTGT TAGTTGTGT CAGACATAT
  ACTAAGTAG AAATACCTT GTATTGACA ATCAACACA GTCTGTATA
301 AATAAAGCC TATTATTG AAAAAAAAAA AAAAAAAAAA GTCGACATC
  TTATTTCCG ATAATAAAC TTTTTTTTT TTTTTTTTT CAGCTGTAG
351 GATAAGGGT
  CTATTCCCA

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**Figure S6.** Nucleotide and translated amino acid sequences of cloned skin secretion-derived cDNA encoding the biosynthetic precursor of C-06. The signal peptide is shown double-underlined, mature peptide in single-line underline and stop codon is indicated with asterisk. Glycine amino acid indicated possible amidation.

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      M A F L K R N L F S L Y Y S L
1 ATGGCTTTC CTGAAGAAA TCGCTTTT CTTGTA CTTCCTGG
  TACCGAAAG GACTTCTTT AGCGAAAA GAACATGAT AAGGAACCT
      F L F P W Q P V K M E K D R E
51 ATAGTTTCT CTGTCCATC TGTCAAGAT GAGAAAAGA GAGGGAGAC
  TATCAAAGA GAGAGGTAG ACAGTTCTA CTCTTTTCT CTCCCTCTG
      T R S M K R N E E E N A S E E
101 GAGAGTAC GAAGAGGAA GAAAATGAA GAGAGCAAG TGAGGAAAA
  CTCGTCATG CTTCTCCTT CTTTACTT CTCTCGTTC ACTCCTTTT
      K R G V F D A I K A I A K A A
151 GAGAGGAGT ATTCGATGC AATAAAAAGC TATAGCGAA AGCAGCTGG
  CTCTCCTCA TAAGCTACG TTATTTTCG ATATCGCTT TCGATCGACC
      G K A A L H A A G D S I G *
201 TAAAGCCGC TCTTCATGC TGCTGGTGA CTCCATAGG AACACTTTA
  ATTTCCGCG AGAAGTACG ACGACCACT GAGGTATCC TTGTGAAAT
251 ATGATTCAT CTCTATGGA ACATAAATG TTAGTTGTG TCAGACATA
  TACTAAGTA GAGATACCT TGTATTTAC AATCAACAC AGTCTGTAT
301 TAATAAAGC AAATTATCT GAAAAAAAAA AAAAAAAAAA GTCGACATC
  ATTATTTTCG TTTAATAGA CTTTTTTTT TTTTTTTTT CAGCTGTAG
351 GATACGCGT
  CTATGCGCA

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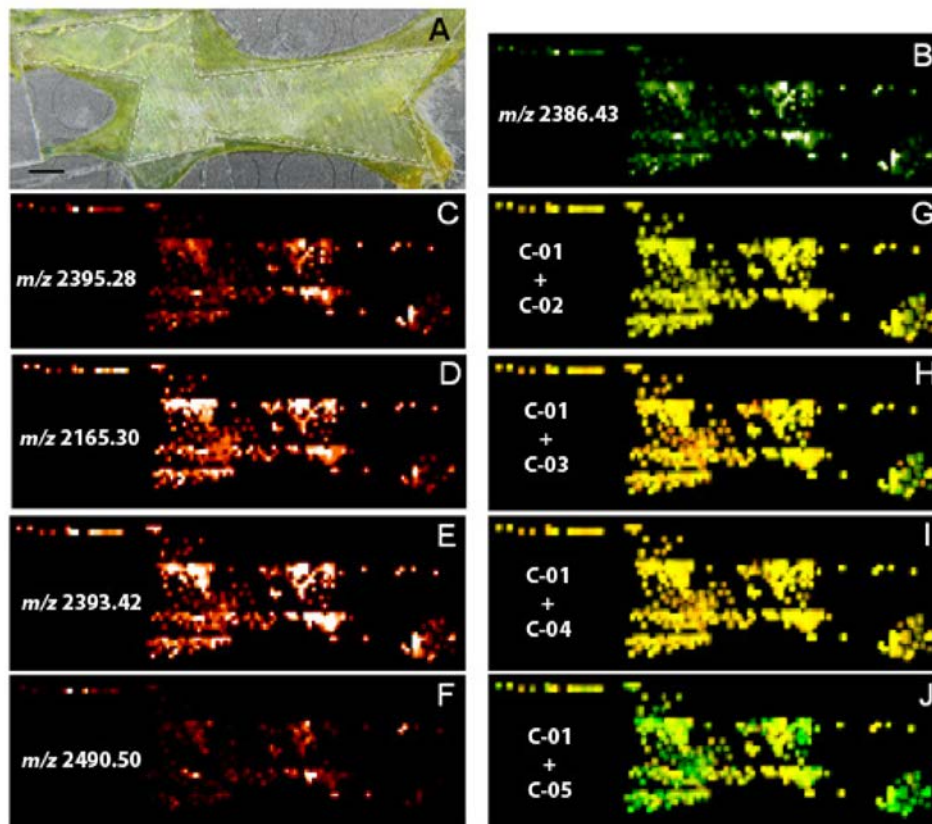
**Figure S7.** Nucleotide and translated amino acid sequences of cloned skin secretion-derived cDNA encoding the biosynthetic precursor of C-07. The signal peptide is shown double-underlined, mature peptide in single-line underline and stop codon is indicated with asterisk. Glycine amino acid indicated possible amidation.

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      M A S L K K F F F L V N F L G
1  ATGGCTTCC TTGAAGAAA TTTTTTTTC CTTGTATTA TTCCTTGGA
   TACCGAAGG AACTTCTTT AAAAAAAAAAG GAACATAAT AAGGAACCT
   I V F L S I C Q D E K R E G D
51 ATAGTTTTT CTGTCCATC TGTCAAGAT GAGAAAAGA GAGGGAGAC
   TATCAAAAA GACAGGTAG ACAGTTCTA CTCTTTTCT CTCCTCTG
   E Q Y E E E E N E E A S E E K
101 GAGCAGTAT GAAGAGGAA GAAAATGAA GAGGCAAGT GAGGAAAAG
   CTCGTCATA CTTCTCCTT CTTTACTT CTCCGTTCA CTCCTTTTC
   R G V L D A I K A I A K A A G
151 AGAGGAGTA CTCGATGCA ATTAAAGCT ATAGCGAAA GCAGCTGGT
   TCTCCTCAT GAGCTACGT TAATTTCTGA TATCGCTTT CGTCGACCA
   K A A F Q A A G E H I G *
201 AAAGCCGCT TTTCAAGCT GCTGGTGAA CACATAGGT TCACGTTAA
   TTTCGGCGA AAAGTTCGA CGACCACTT GTGTATCCA AGTGCAATT
251 TGATTCATC TCTATGGAA CATAACTGT TAGTTGTGT CAGACATAT
   ACTAAGTAG AGATACCTT GTATTGACA ATCAACACA GTCTGTATA
301 AATAAAGCA TATTATTTG AAAAAAAAAA AAAAAAAAAA AAAAAAAAAA
   TTATTTTCGT ATAATAAAC TTTTTTTTTT TTTTTTTTTT TTTTTTTTTT
351 AAAAAACTC
   TTTTTTTGAG

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**Figure S8.** Nucleotide and translated amino acid sequences of cloned skin secretion-derived cDNA encoding the biosynthetic precursor of C-08. The signal peptide is shown double-underlined, mature peptide in single-line underline and stop codon is indicated with asterisk. Glycine amino acid indicated possible amidation.



**Figure S9.** IMS analysis of *H. cinerascens* skin. (A) Analyzed dorsal skin total fragment. (B)-(F) Pictorial representation of C-01 to C-05. (G)-(J) Colocalization between C-01 and the other peptides.