Supplementary Material

Esculentin-1a-derived peptides promote clearance of *P. aeruginosa* internalized in cystic fibrosis bronchial cells as well as lung cells migration: Biochemical properties and a plausible mode of action

Floriana Cappiello[¥], Antonio Di Grazia[¥], Segev-Zarko Li-av[¶], Silvia Scali[ǁ], Loretta Ferrera*, Luis Galietta*, Alessandro Pini[ǁ], Yechiel Shai[¶], Y. Peter Di[Φ], Maria Luisa Mangoni[¥#]

[¥] Department of Biochemical Sciences, Sapienza University of Rome, Rome, 00185, Italy; [¶] Department of Biological Chemistry, The Weizmann Institute of Science, 76100 Rehovot, Israel; [ǁ] Department of Medical Biotechnology, University of Siena, Siena, 53100 Italy;* U.O.C. Genetica Medica, Giannina Gaslini Institute, Genoa, Genoa, 16148, Italy; [Φ] Department of Environmental and Occupational Health, University of Pittsburgh, PA 15260, USA

Corresponding author: Maria Luisa Mangoni, Department of Biochemical Sciences, Sapienza University of Rome. Via degli Apuli, 9-00185, Rome-ITALY

Phone: +39 06 49917693. Email: marialuisa.mangoni@uniroma1.it

Legends to Supplementary figures

Fig. S1. Peptides' effect on the viability of bronchial epithelial cells in Hank's buffer. About $4x10^4$ wt-CFBE (A) or ΔF508-CFBE (B) cells suspended in culture medium were plated in wells of a microtiter plate. After overnight incubation at 37 °C in a 5% CO_2 atmosphere, the medium was removed and replaced with 100 μl Hank's supplemented with the peptides at different concentrations. After 2h, cell viability was determined by the MTT reduction to insoluble formazan. Cell viability is expressed as percentage with respect to the control (cells not treated with the peptide). All data are the mean of a representative single experiment, performed in triplicate.

Fig. S2. Proteolytic activity of elastase from human neutrophils on both Esc(1-21) and Esc(1-21)-1c. Mass spectrometry profile of the two peptides immediately after enzyme addition (A, B) and after 5h (C, D) or 24h (E, F) incubation with the enzyme are reported. Molecular masses for intact peptides and proteolytic fragments produced by elastase treatment are indicated. Insets within each panel show HPLC profile of a peptide. The retention time of the intact peptide is also indicated (18 min). The tables show the mass spectra peaks and the corresponding cleavage fragments. Proteolytic sites of the enzyme on the two peptides are indicated by the arrows. D-amino acids are in italics and underlined.

Fig. S3. Proteolytic activity of elastase from *P. aeruginosa* on both Esc(1-21) and Esc(1-21)-1c. As indicated in the legend to Fig. S2, HPLC and mass spectra of each peptide immediately after enzyme addition (A, B) and after 5h (C, D) or 24h (E, F) incubation with the enzyme are reported. Molecular masses for intact peptides and proteolytic fragments produced by elastase treatment are indicated. The tables show the mass spectra peaks and the corresponding cleavage fragments. Proteolytic sites of the enzyme on the two peptides are indicated by the arrows. D-amino acids are in italics and underlined.

Fig. S4. Proteolytic activity of elastase from human neutrophils on LL-37. HPLC (A-C) and mass spectrometry profiles (D-F) of the peptide immediately after enzyme addition (A, D) and after 5h (B, E) or 24h (C, F) incubation with the enzyme are reported. Molecular masses for intact peptides and proteolytic fragments produced by elastase treatment are indicated. Retention time of intact peptide is also indicated (23,9 min). Proteolytic sites of the enzyme on LL-37 are indicated by the arrows.

Fig. S5. Proteolytic activity of elastase from *P. aeruginosa* on LL-37. HPLC (A-C) and mass spectrometry profiles (D-F) of the peptide immediately after enzyme addition (A, D) and after 5h (B, E) or 24h (C, F) incubation with the enzyme are reported. Molecular masses for intact peptides and proteolytic fragments produced by elastase treatment are indicated. Retention time of intact peptide is also indicated (23,9 min). Proteolytic sites of the enzyme on LL-37 are indicated by the arrows.

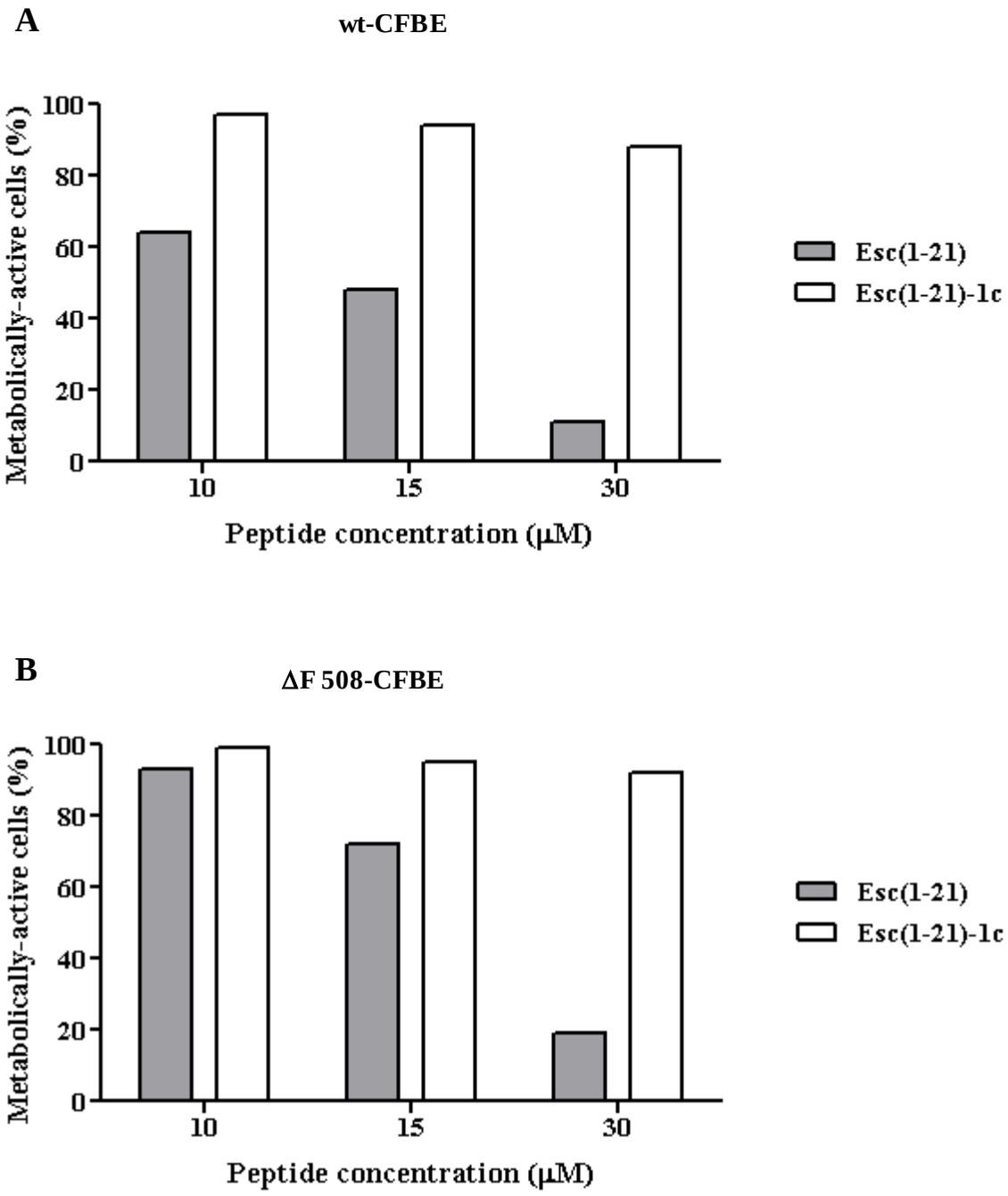
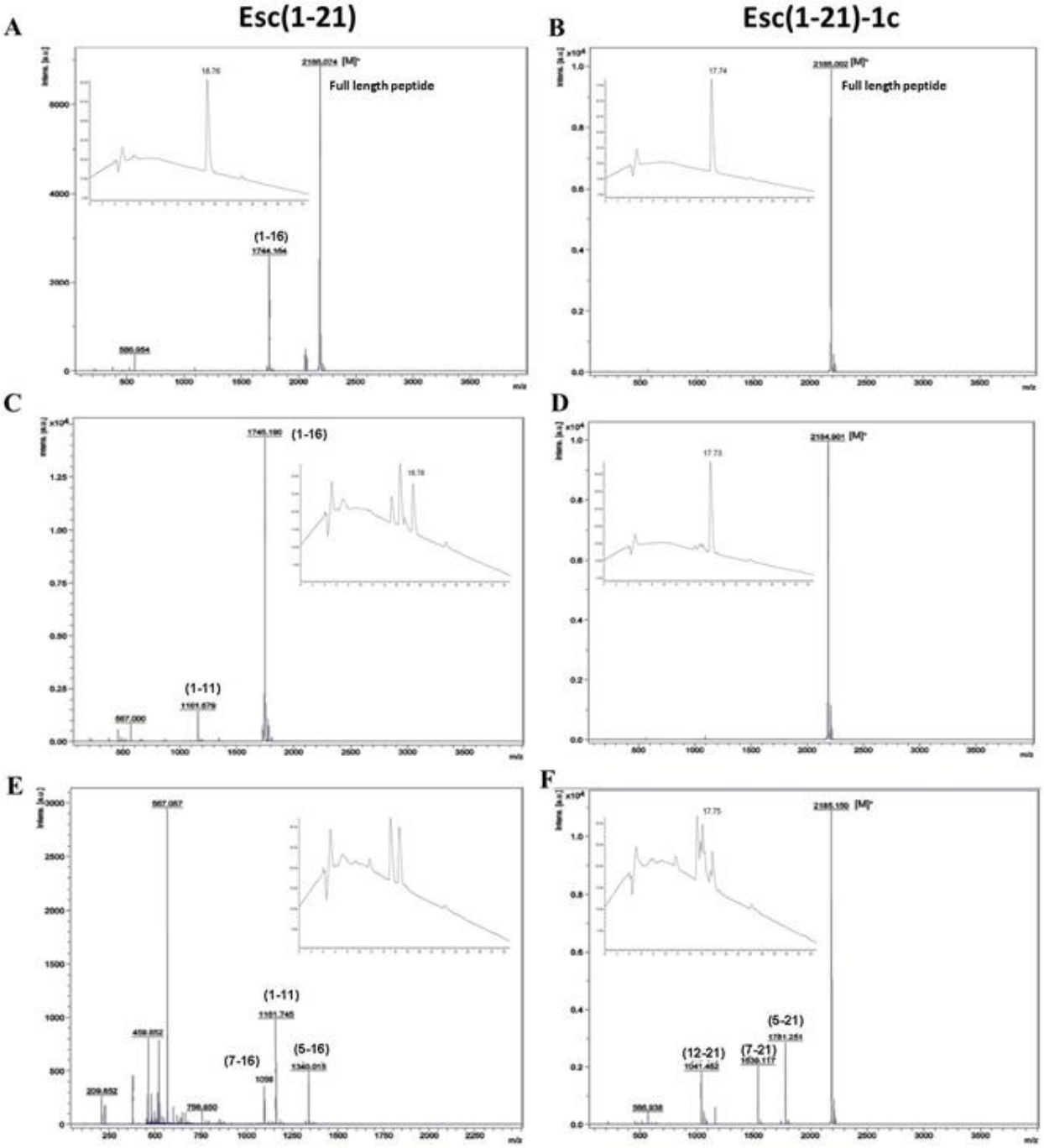

A
wt-CFBE
Metabolically-active cells (%)
100
80
60
40
20
0
10
15
30
Peptide concentration (µM)
Esc(1-21)
Esc(1-21)-1c
B
ΔF 508-CFBE
Metabolically-active cells (%)
100
80
60
40
20
0
10
15
30
Peptide concentration (µM)
Esc(1-21)
Esc(1-21)-1c

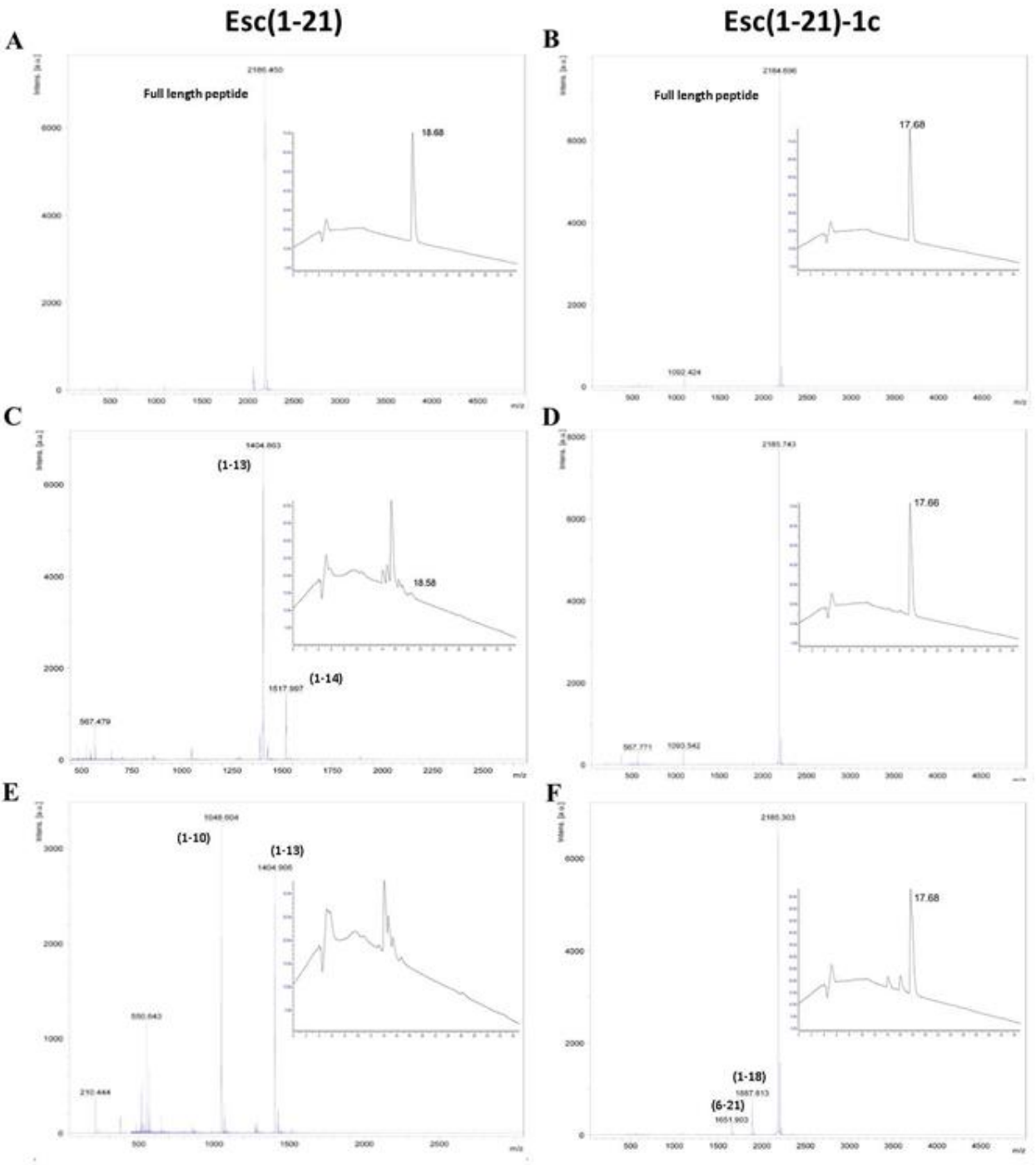
Fig.S1



Fragment	Sequence	MW (Da)
Esc(1-21)	GIFSKLAGKKIKNLLISGLKG	2,185
1-16	GIFSKLAGKKIKNLLI	1,743
5-16	KLAGKKIKNLLI	1,338
7-16	AGKKIKNLLI	1,096
1-11	GIFSKLAGKKI	1,160

Fragment	Sequence	MW (Da)
Esc(1-21)-1c	GIFSKLAGKKIKNLLISGLKG	2,185
5-21	KLAGKKIKNLLISGLKG	1,781
7-21	AGKKIKNLLISGLKG	1,539
12-21	KNLLISGLKG	1,041

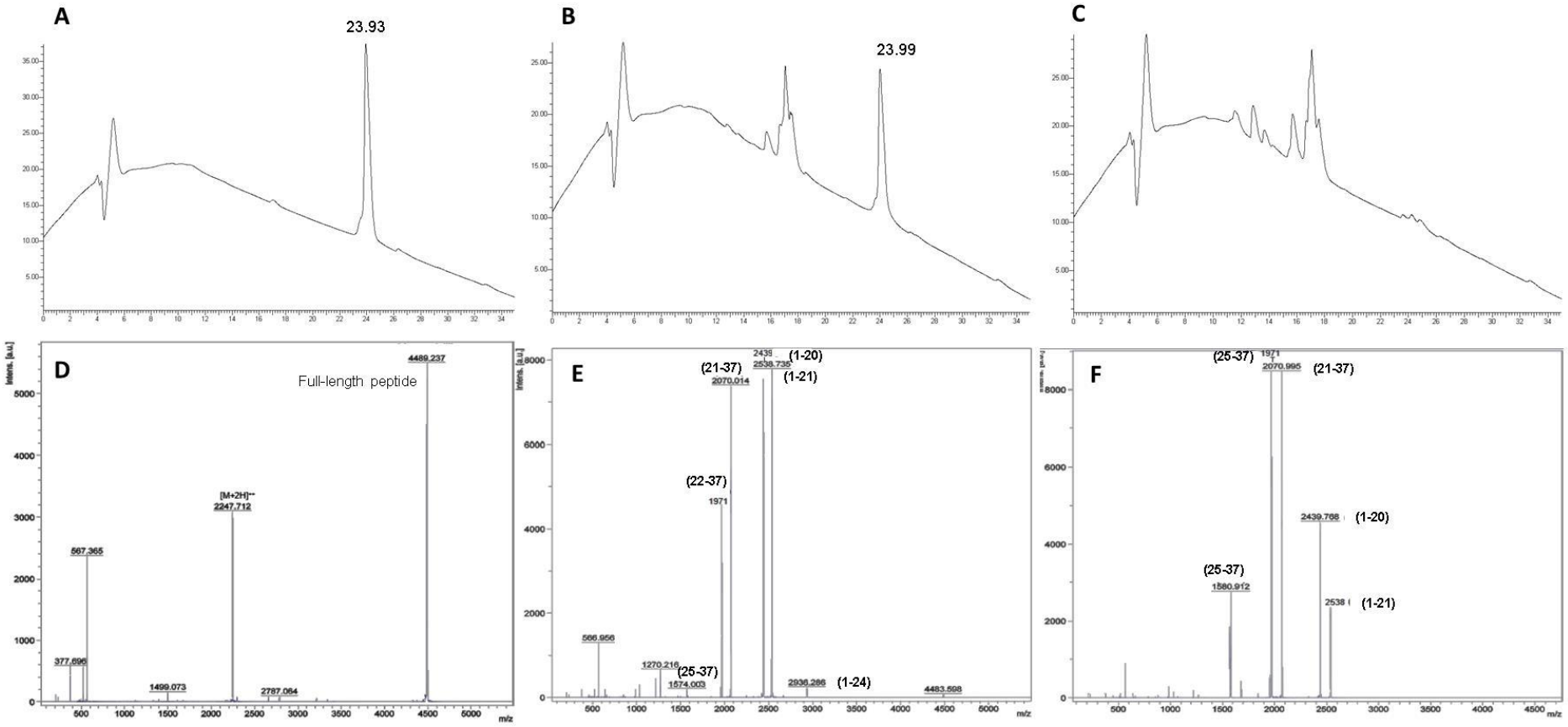
Fig. S2



Fragment	Sequence	MW (Da)
Esc(1-21)	GIFSKLAGKKIKNLLISGLKG	2,185
1-14	GIFSKLAGKKIKNL	1,516
1-13	GIFSKLAGKKIKN	1,403
1-10	GIFSKLAGKK	1,048

Fragment	Sequence	MW (Da)
Esc(1-21)-1c	GIFSKLAGKKIKNLLISGLKG	2,185
1-18	GIFSKLAGKKIKNLLISG	1,887
6-21	LAGKKIKNLLISGLKG	1,653

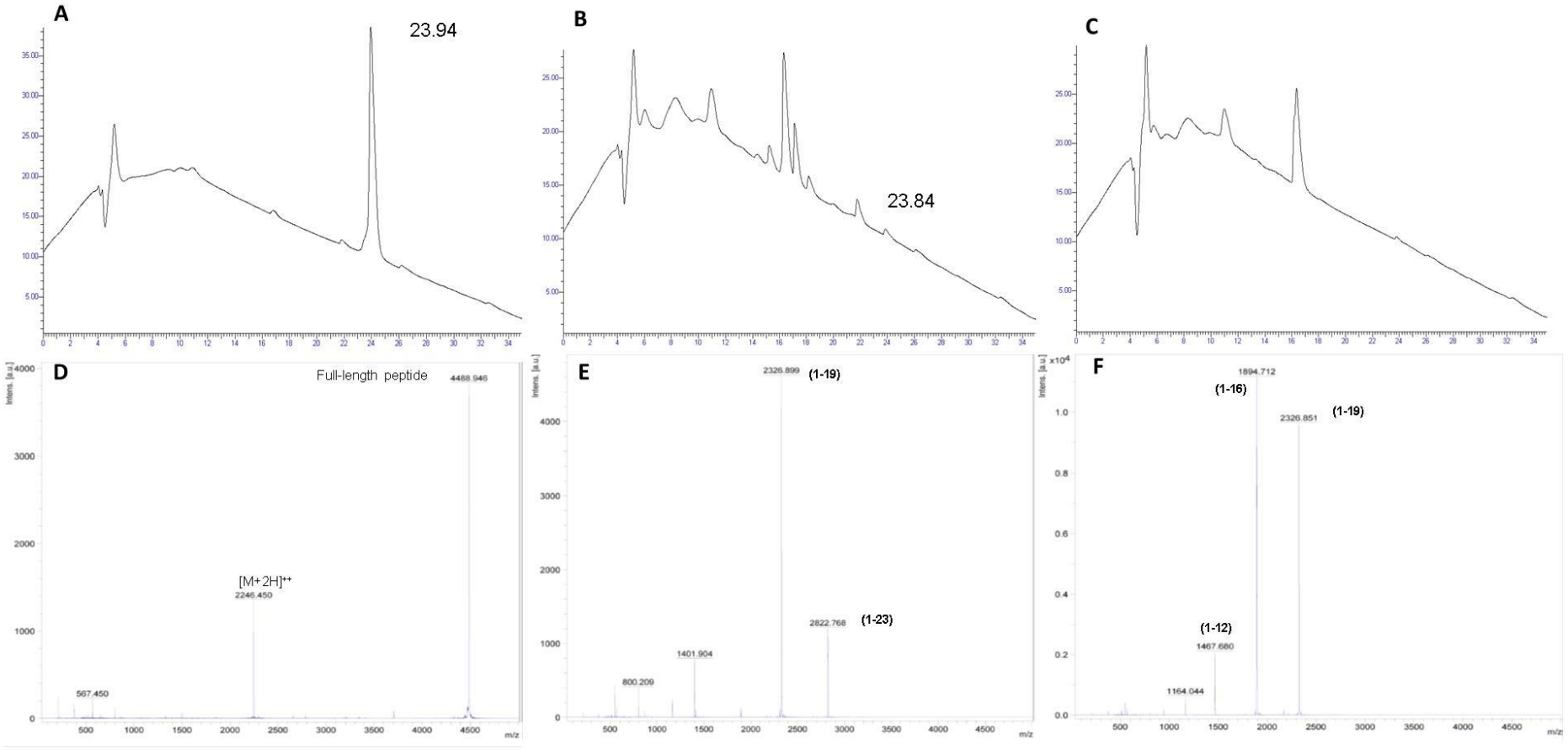
Fig. S3



LL37 [1] LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES [37] 4,493 Da

Fragment 1-24	[1] LLGDFFRKSKEKIGKEFKRIVQRI [24]	2,936 Da	Fragment 21-37	[21] VQRIKDFLRNLVPRTES [37]	2,070 Da
Fragment 1-21	[1] LLGDFFRKSKEKIGKEFKRIV [21]	2,538 Da	Fragment 22-37	[22] QRIKDFLRNLVPRTES [37]	1,971 Da
Fragment 1-20	[1] LLGDFFRKSKEKIGKEFKRI [20]	2,439 Da	Fragment 25-37	[25] KDFLRNLVPRTES [37]	1,574 Da

Fig. S4



LL37	[1] LLGDFFRKSKEKIGKEFKRIVQRIKDFLRNLVPRTES [37]	4,493 Da
Fragment 1-23	[1] LLGDFFRKSKEKIGKEFKRIVQR[23]	2,823 Da
Fragment 1-19	[1] LLGDFFRKSKEKIGKEFKR[19]	2,326 Da
Fragment 1-16	[1] LLGDFFRKSKEKIGKE[16]	1,895 Da
Fragment 1-12	[1] LLGDFFRKSKEK[12]	1,467 Da

Fig. S5