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Supplementary Information for

Identification of a biologically active fragment of ALK and LTK-Ligand 2 (Augmentor-α)

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This PDF file includes:

Supplementary text
Figs. S1 to S3

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Supplementary Information Text

Protein sequences of Fc-AUG-α (mammalian expression construct cloned in pCEP4 vector):

*METDTLLLWVLLLWVPGSTGA*GSTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI
SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV
LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELT
KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV
DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKASGAGSTTGGGGSGGG
GSGGGGS**LEVLFQ/GP**GAEPREPADGQALLRLVVELVQELRKHHSAEHKGLQL
LGRDCALGRAEAAGLGPSPEQRVEIVPRDLRMKDKFLKHLTGPLYFSPKCSKH
FHRLYHNTRDCTIPAYYKRCARLLTRLAVSPVCMEDKQ

Signal peptide is in the italic; 3C protease cleavage site is in bold (/ is the actual cleavage site)

Protein sequences of Fc-ALK (mammalian expression construct cloned in pCEP4 vector):

*METDTLLLWVLLLWVPGSTGA*GSTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI
SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV
LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELT
KNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV
DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKTSLYKKAGF**ENLYFQ/G**L
NDIFEAQKIEWHETAPKSRNLFERNPNKELKPGENSPRQTPIFDPTVHWLFTTC
GASGPHGPTQAQCNNAYQNSNLSVEVGSEGPLKGIQIWKVPATDTYSISGYGA
AGGKGGKNTMMRSHGVSVLGIFNLEKDDMLYILVGQQGEDACPSTNQLIQKVC
IGENNVIEEEIRVNRSVHEWAGGGGGGGGATYVFKMKDGVPVPLIIAAGGGGR
AYGAKTDTFHPERLENNSSVLGLNGNSGAAGGGGGWNDNTSLLWAGKSLQE
GATGGHSCPQAMKKWGWETRGGFGGGGGGCSSGGGGGGYIGGNAASNND
PEMDGEDGVSFISPLGILYTPALKVMEGHGEVNIKHYLNCSHCEVDECHMDPE
SHKVICFCDHGTVLAEDGVSCIVSPTPEPHGLNDIFEAQKIEWHE

Signal peptide is italic; TEV protease cleavage site is in bold; Biotin Acceptor Peptide is underlined.

Protein sequences of Trx-AUG-α AD *E. coli* expression construct cloned in pET42 vector):

MHHHHHH**ENLYFQ/G**SDKIIHLTDDSFDTDVLKADGAILVDFWAEWCGPCKMIA
PILDEIADEYQGKLTVAKLNIDQNPGTAPKYGIRGIPTLLLFKNGEVAATKVGALS
KGQLKEFLDANLAGGGGS**LEVLFQ/GP**SPEQRVEIVPRDLRMKDKFLKHLTGPL
YFSPKCSKHFHRLYHNTRDCTIPAYYKRCARLLTRLAVSPVCMEDKQ

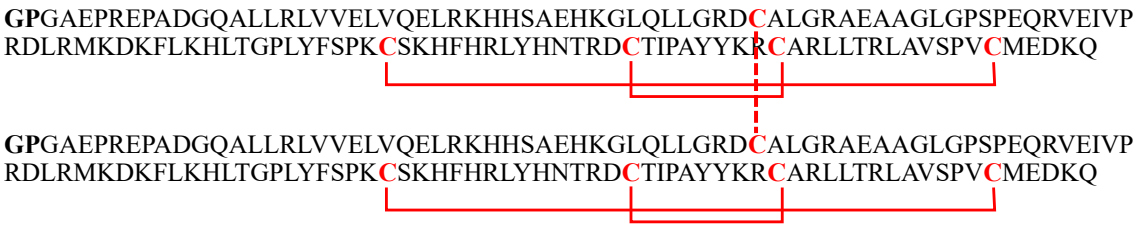
TEV protease and 3C protease cleavage sites are in bold (/ is the actual cleavage site);

Fig. S1 Multiple Sequence Alignment.

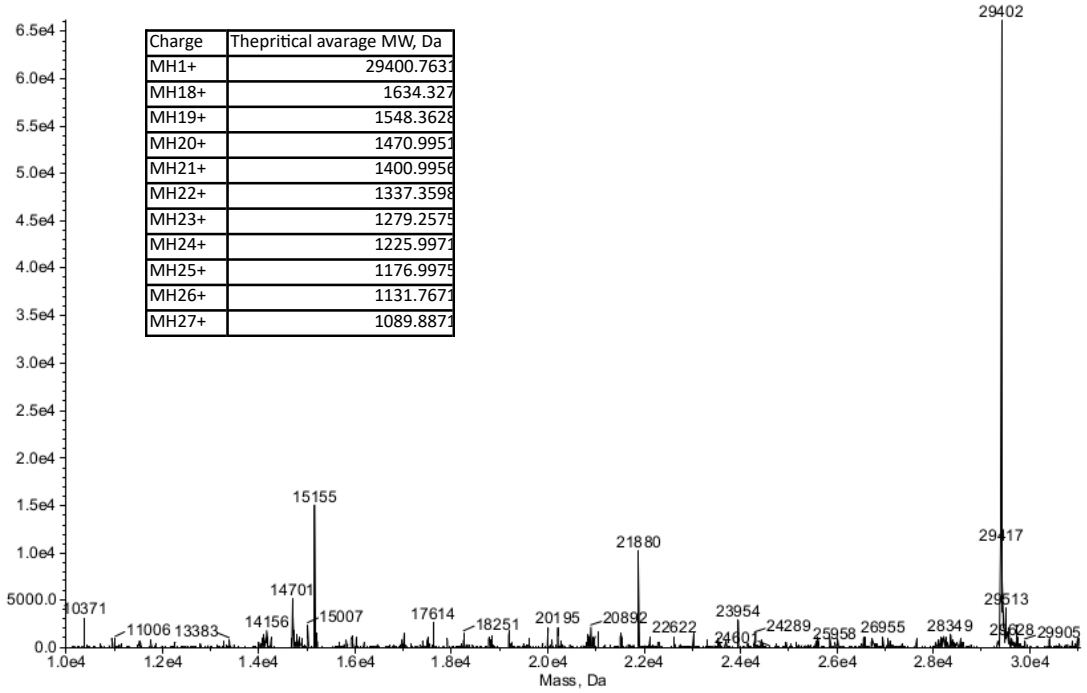
Multiple sequence alignment of AUG-α (A) and AUG-β (B) from different species. Signal peptide is highlighted in yellow, N-terminal variable region in green, and C-terminal conserved augmentor domain in blue. All cysteines are marked with red. (C) Sequence alignment of conserved augmentor domain of human AUG-α and human AUG-β.

Fig. S2 Mass Spectrometric Analysis of Dimeric AUG-α.

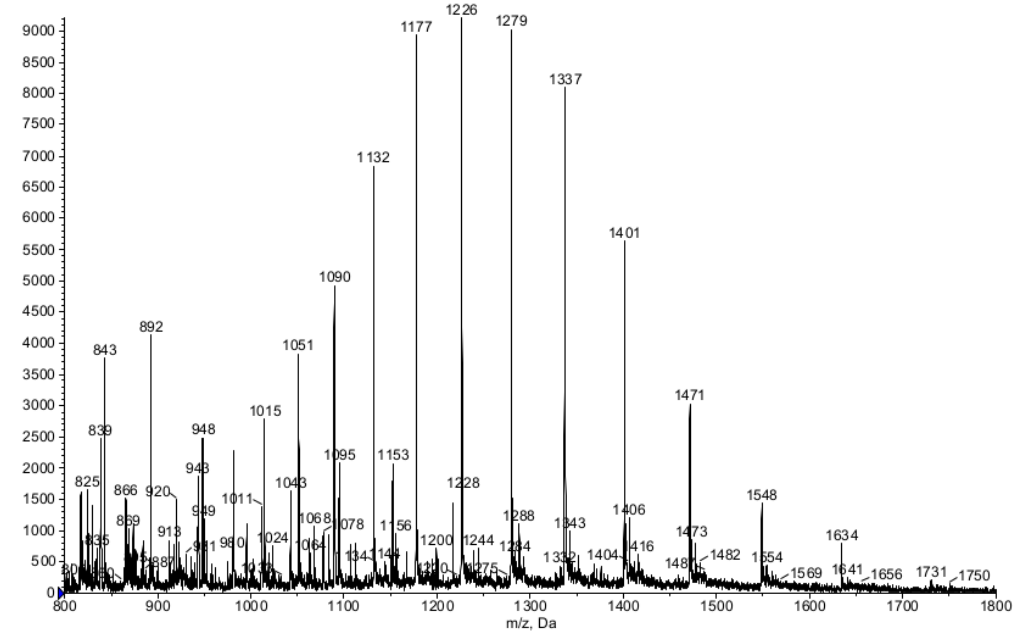
A



B

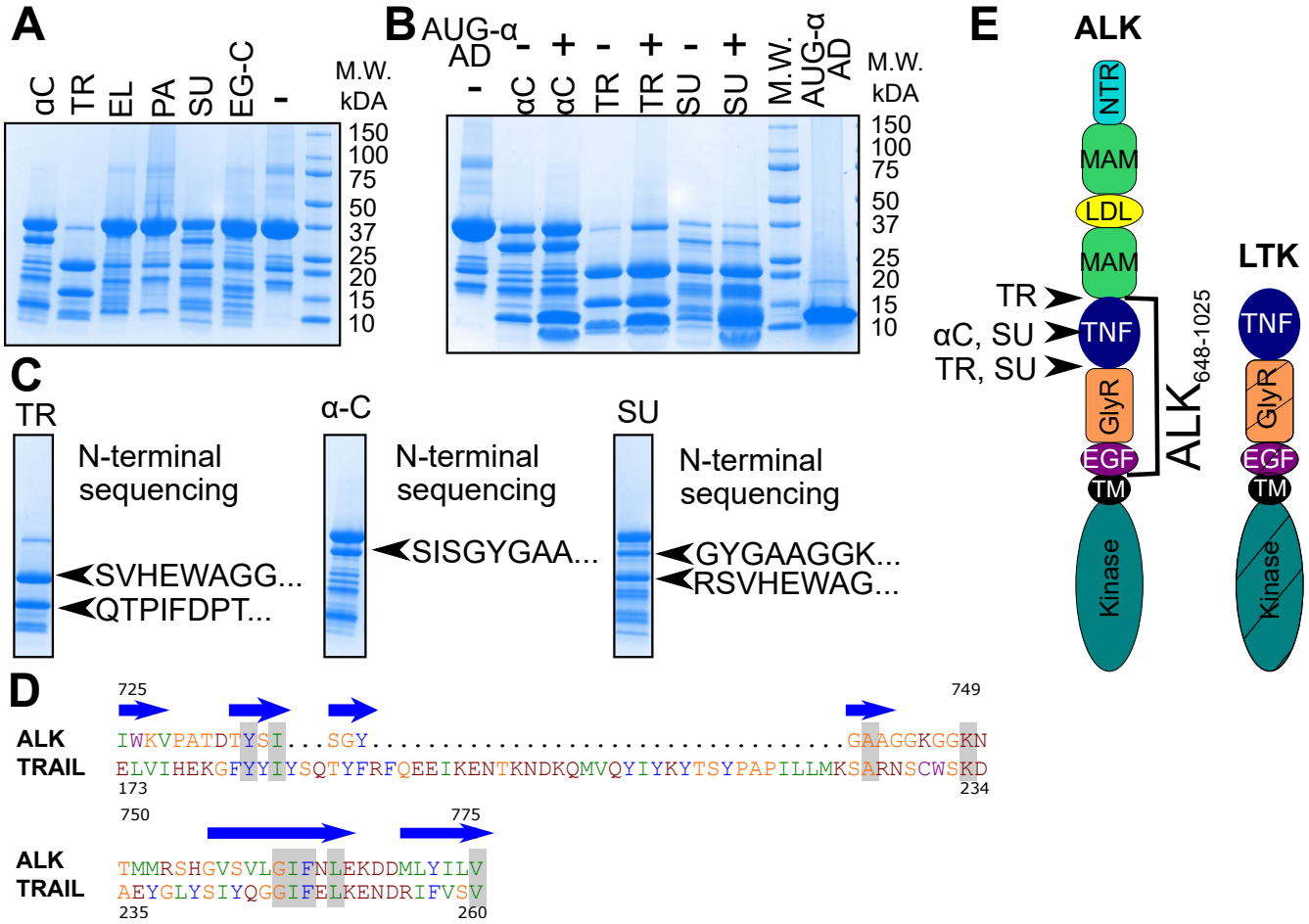


C



(A) Primary structure of dimeric AUG-α. First two residues highlighted in bold are artificial residues from 3C protease cleavage site. All cysteines are highlighted in red and bold. Intramolecular disulfide bridges are shown with solid red lines; intermolecular disulfide bridge is shown with dash red line.
(B and C) ESI-TOF high accuracy mass spectrometric analysis of dimeric AUG-α. Theoretically predicted average molecular mass is represented in the insert of (B).

Fig. S3 Limited Proteolysis of ALK Ligand Binding Region.



(A and B) SDS-PAGE analysis of ALK fragment (residues 648-1025) after limited proteolysis with a set of proteolytic enzymes. ALK fragment was incubated with different proteolytic enzymes at a ratio 1:100 protease:ALK at room temperature for 1 hour, and cleavage reaction was monitored by SDS-PAGE. Abbreviations are as follows: αC – α-Chymotrypsin, TR – trypsin, EL – elastase, PA – papain, SU – subtilisin and endoproteinase Glu-C. - is a negative control ALK fragment which was not incubated with any proteolytic enzymes. (B) Aug-α AD was added into limited proteolysis reaction where indicated (+). The ratio of protease:ALK was increased to 1:30, and ALK:Aug-α AD ratio was 1:5.

(C) Bands which were used for N-terminal sequencing are marked by arrows and corresponding sequences are shown for each band.

(D) Sequence alignment of putative ALK TNF-like motif with TRAIL motif. Alignment was performed using the Phyre2 server [1].

(E) Schematic representation of ALK and LTK domain organization. N-terminal domain (NTR) colored in cyan, MAM in green, LDL in yellow, TNF-like motif in dark blue, glycine-rich (Gly-Rich) in orange, EGF-like motif in purple, transmembrane (TM) in black, and kinase domain in blue. LTK domains are shown with striations.

References

1. Kelley LA et al. (2015) The Phyre2 web portal for protein modeling, prediction and analysis *Nature Protocols* 10: 845-858.