

CLAIMS

1. A C-terminal CDFN fragment comprising or consisting of at least the consecutive amino acid residues at positions 38-70 or 25-57 of the sequence as set forth in SEQ ID NO:1:

5 MPAMKICEKL KKLDSQICEL KYEKTLDLAS VDLRKMRVAE LKQILHSWGE
ECRACAEKTD YVNLIQELAP KYAATHPKTE L

or a sequence which has at least 80 % homology or sequence identity with the sequence of positions 38-70 or 25-57 in SEQ ID NO:1, wherein said fragment is a cell membrane penetrating peptide and has a protective effect on neuronal cells, for use as a medicine.

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2. The fragment for use according to claim 1 in the treatment of a degenerative disease or disorder.

3. The fragment for use according to claim 2, wherein said degenerative disease is a
15 neurodegenerative disease.

4. The fragment for use according to claim 3, wherein said neurodegenerative disease is a central nervous system (CNS) disease selected from the group consisting of: Alzheimer's disease, Parkinson's disease, multiple system atrophy, amyotrophic lateral sclerosis,
20 frontotemporal lobar degeneration, dementia with Lewy bodies, mild cognitive impairment, Huntington's disease, traumatic brain injury, drug addiction and stroke.

5. The fragment for use according to claim 4, wherein said CNS disease is Parkinson's disease.

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6. The fragment for use according to claim 2 in the treatment of type I or type II diabetes.

7. The fragment for use according to claim 3 in the treatment of amyotrophic lateral sclerosis.

8. The fragment for use according to claim 2 in the treatment of retinal disorders such as
5 retinitis pigmentosa.

9. The fragment for use according to any of claims 1-8, wherein said fragment is administered by intravenous administration, intraperitoneal, subcutaneous, intrathecal, intracerebroventricular, intranasal, transdermal, intracerebral, intramuscular, intraocular, or
10 intraarterial administration or said fragment is administered via a viral expression vector.

10. The fragment for use according to claim 9, wherein said intravenous administration is peripheral administration.

11. The fragment for use according to any of the previous claims preferably with a length up to 33-81 amino acids, wherein said fragment comprises at least the consecutive amino acid residues at positions 38-70 or 25-57 of the sequence as set forth in SEQ ID NO:1 or a sequence which has at least 80 % homology or sequence identity with the sequence of positions 38-70 or 25-57 in SEQ ID NO:1, and, if present, the sequence flanking said
20 consecutive amino acid residues preferably has at least 80 % homology or sequence identity with the sequence at corresponding positions in SEQ ID NO:1.

12. The fragment for use according claim 11, wherein said fragment comprises or consists of at least the consecutive amino acid residues at positions 37-70, 36-70, 35-70, 34-70, 33-70, 32-70 or 31-70 of the sequence as set forth in SEQ ID NO:1 or a sequence which has at least
25 80 % homology or sequence identity with the sequence of positions 37-70, 36-70, 35-70, 34-70, 33-70, 32-70 or 31-70 in SEQ ID NO:1, and, if present, the sequence flanking said consecutive amino acid residues preferably has at least 80 % homology or sequence identity with the sequence at corresponding positions in SEQ ID NO:1.

13. The fragment for use according claim 11, wherein said fragment comprises or consists of at least the consecutive amino acid residues at positions 25-57, 25-58, 25-59, 25-60, 25-61, 25-62, 25-63, 25-64, 25-65, 25-66, 25-67, 25-68, 25-69 or 25-70 of the sequence as set forth in SEQ ID NO:1 or a sequence which has at least 80 % homology or sequence identity with the sequence of positions 25-57, 25-58, 25-59, 25-60, 25-61, 25-62, 25-63, 25-64, 25-65, 25-66, 25-67, 25-68, 25-69 or 25-70 in SEQ ID NO:1, and, if present, the sequence flanking said consecutive amino acid residues preferably has at least 80 % homology or sequence identity with the sequence at corresponding positions in SEQ ID NO:1.

14. The fragment for use according to claim 12 or 13, wherein said fragment comprises or consists of at least the consecutive amino acid residues at positions 31-73 (peptide 6; SEQ ID NO: 15), 25-73 (peptide 4; SEQ ID NO: 13), 21-73 (peptide 3; SEQ ID NO: 12), 21-70 (peptide 7; SEQ ID NO: 16), 31-81 (peptide 5; SEQ ID NO: 14), 25-81 (peptide 2; SEQ ID NO: 11), 25-57 (peptide 15, SEQ ID NO:24), or 37-73 (peptide 8, SEQ ID NO:17) of the sequence as set forth in SEQ ID NO:1 or a sequence which has at least 80 % homology or sequence identity with the sequence of positions 31-73, 25-73, 21-73, 21-70, 31-81, 25-81, 25-57, or 37-73 in SEQ ID NO:1, and, if present, the sequence flanking said consecutive amino acid residues preferably has at least 80 % homology or sequence identity with the sequence at corresponding positions in SEQ ID NO:1.

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15. The fragment for use according to claim 14, wherein said fragment comprises or consists of at least the consecutive amino acid residues at positions 31-73 or 25-57 of the sequence as set forth in SEQ ID NO:1 or a sequence which has at least 80 % homology or sequence identity with the sequence of positions 31-73 or 25-57 in SEQ ID NO:1 and, if present, the sequence flanking said consecutive amino acid residues preferably has at least 80 % homology or sequence identity with the sequence at corresponding positions in SEQ ID NO:1.

16. The fragment for use according to any one of the previous claim with a length up to 43-81, preferably 43-61 amino acids.

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17. The fragment for use according to any of the preceding claims, wherein the terminals of the fragment comprises further modifications protecting the fragment from enzymatic degradation.

- 5 18. The fragment for use according to any of the preceding claims, wherein said C-terminal CDNF fragment consisting of at least 50 consecutive amino acid residues of the sequence as set forth in SEQ ID NO:1:

MPAMKICEKL KKLDSQICEL KYEKTLDLAS VDLRKMRVAE LKQILHSWGE
ECRACA EKTD YVNLIQELAP KYAATHPKTE L

- 10 or a sequence which has at least 90 % homology or sequence identity with the sequence of SEQ ID NO:1.

19. The fragment for use according to claim 18, wherein said sequence which has at least 90 % homology or sequence identity with the sequence of SEQ ID NO:1 comprises sequence
15 CXXC in positions 52-55 of SEQ ID NO:1, wherein X is any amino acid.

20. The fragment for use according to claim 19, wherein said sequence which has at least 90 % homology or sequence identity with the sequence of SEQ ID NO:1 consists of at least 50 consecutive amino acid residues of the sequence of SEQ ID NO:3:

- 20 MPAMKICEKL KKLDSQICEL KYEKTLDLAS VDLRKMRVAE LKQILHSWGE
ECXXCAEKTD YVNLIQELAP KYAATHPKTE L

wherein X is any amino acid.

21. The fragment for use according to claim 19, wherein said sequence which has at least 90
25 % homology or sequence identity with the sequence of SEQ ID NO:1 comprises sequence CKGC in positions 52-55 of SEQ ID NO:1

22. The fragment for use according to any of claims 17-21, wherein said fragment comprises the C-terminal amino acid L in position 81 of SEQ ID NO:1.

23. The fragment for use according to any of claims 17, 18 or 20, wherein said fragment
5 lacks the ER retention signal KTEL corresponding to positions 78-81 of SEQ ID NO:1.

24. The fragment for use according to any of claims 17-23, wherein the fragment comprises amidation of the C-terminus and acetylation of the N-terminus as further modification.

10 25. The fragment for use according to any of the preceding claims further conjugated to a detectable chemical or biochemical moiety.

26. The fragment for use according to any of the preceding claims, wherein said fragment is a cell-penetrating peptide and is capable of penetrating human blood-brain barrier.

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27. The fragment for use according to any of the preceding claims, wherein said fragment is resistant to protease cleavage.

28. The fragment for use according to claim 27, wherein said fragment comprises a
20 elongation, deletion, insertion, substitution or modification such that said elongation, deletion, insertion, substitution or modification abolishes at least one protease cleavage site.

29. The fragment for use according to claim 27 or 28, wherein the protease is selected from the group consisting of a cysteine protease, a metalloprotease, and a serine protease.

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30. The fragment for use according to claim 27-29, wherein the cysteine protease is cathepsin K.

31. The fragment for use according to claim 27-29, wherein the metalloprotease is MMP-9.

32. The fragment for use according to claim 27-29, wherein the metalloprotease is MMP-3.

5 33. The fragment for use according to claim 27-29, wherein the serine protease is chymotrypsin A or elastase-2.

34. The fragment for use according to claim 27-33, wherein any one amino acid within the region corresponding to residues 1-8, 16-23, 26-33, 32-39, 37-44, 38-45, 43-50, 46-53, 57-
10 64, 59-66, 60-67 or 68-75 of SEQ ID NO:1 is substituted, deleted or modified.

35. The fragment for use according to claim 27-34, wherein any amino acid at position corresponding to 46, 49, 35, 63, 4, 71, 19, 40, 41, 62, or 29 of SEQ ID NO:1 is substituted or modified.

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36. A pharmaceutical composition comprising the C-terminal CDFN fragment as defined in any of claims 1 and 11-35 and at least one of the following: physiologically acceptable carrier, buffer, excipient, preservative and stabilizer.

20 37. The pharmaceutical composition according to claim 36 for intravenous administration preferably peripheral administration, intraperitoneal, subcutaneous, intrathecal, intracerebroventricular, intranasal, transdermal, intracerebral, intramuscular, intraocular, or intraarterial administration or said composition is administered via a viral expression vector.

25 38. The pharmaceutical composition according to claim 36 or 37 for use as a medicine.

39. The pharmaceutical composition for use according to claim 38 in the treatment of a degenerative disease or disorder.

40. The pharmaceutical composition for use according to claim 39, wherein said degenerative disease or disorder is a central nervous system (CNS) disease selected from the group consisting of: Alzheimer's disease, Parkinson's disease, multiple system atrophy, amyotrophic lateral sclerosis, frontotemporal lobar degeneration, dementia with Lewy bodies, mild cognitive impairment, Huntington's disease, traumatic brain injury, drug addiction and stroke.

41. The pharmaceutical composition for use according to claim 40, wherein said CNS disease is Parkinson's disease.

42. The pharmaceutical composition for use according to claim 40, wherein said CNS disease is amyotrophic lateral sclerosis.

43. The pharmaceutical composition according to claim 40 for use in the treatment of type I or type II diabetes.

44. The pharmaceutical composition according to claim 40 for use in the treatment of retinal disorders such as retinitis pigmentosa.

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45. A C-terminal CDNF fragment comprising or consisting of at least the consecutive amino acid residues at positions 38-70 or 25-57 of the sequence as set forth in SEQ ID NO:1:

MPAMKICEKL KKLDSQICEL KYEKTLDLAS VDLRKMRVAE LKQILHSWGE
ECRACAEKTD YVNLIQELAP KYAATHPKTE L

25 or a sequence which has at least 80 % homology or sequence identity with the sequence of positions 38-70 or 25-57 in SEQ ID NO:1, wherein said fragment is a cell membrane penetrating peptide and has a protective effect on neuronal cells.

46. The fragment according to claim 45 with a length up to 57, 53, 51, 50, 49, 43, 37 or 33 amino acids, wherein said fragment comprises at least the consecutive amino acid residues at positions 31-70 or 25-57 of the sequence as set forth in SEQ ID NO:1 or a sequence which has at least 80 % homology or sequence identity with the sequence of positions 31-70 or 25-57 in SEQ ID NO:1 and the sequence flanking said consecutive amino acid residues has at least 80 % homology or sequence identity with the sequence at corresponding positions in SEQ ID NO:1.

47. The fragment according to claim 46, wherein said fragment comprises or consists of at least the consecutive amino acid residues at positions 37-70, 36-70, 35-70, 34-70, 33-70, 32-70 or 31-70 of the sequence as set forth in SEQ ID NO:1 or a sequence which has at least 80 % homology or sequence identity with the sequence of positions 37-70, 36-70, 35-70, 34-70, 33-70, 32-70 or 31-70 in SEQ ID NO:1.

48. The fragment according to claim 46, wherein said fragment comprises or consists of at least the consecutive amino acid residues at positions 25-57, 25-58, 25-59, 25-60, 25-61, 25-62, 25-63, 25-64, 25-65, 25-66, 25-67, 25-68, 25-69 or 25-70 of the sequence as set forth in SEQ ID NO:1 or a sequence which has at least 80 % homology or sequence identity with the sequence of positions 25-57, 25-58, 25-59, 25-60, 25-61, 25-62, 25-63, 25-64, 25-65, 25-66, 25-67, 25-68, 25-69 or 25-70 in SEQ ID NO:1, and, if present, the sequence flanking said consecutive amino acid residues preferably has at least 80 % homology or sequence identity with the sequence at corresponding positions in SEQ ID NO:1.

49. The fragment according to claim 47 or 48, wherein said fragment comprises or consists of at least the consecutive amino acid residues at positions 31-73 (peptide 6), 25-73 (peptide 4), 21-73 (peptide 3), 21-70 (peptide 7), 31-81 (peptide 5), 25-81 (peptide 2), 25-57 (peptide 15), or 37-73 (peptide 8) of the sequence as set forth in SEQ ID NO:1 or a sequence which has at least 80 % homology or sequence identity with the sequence of positions 31-73, 25-73, 21-73, 21-70, 31-81, 25-81, 25-57 or 37-73 in SEQ ID NO:1.

50. The fragment according to claim 49, wherein said fragment comprises or consists of at least the consecutive amino acid residues at positions 31-73 of the sequence as set forth in SEQ ID NO:1 or a sequence which has at least 80 % homology or sequence identity with the sequence of positions 31-73 in SEQ ID NO:1.

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51. The fragment according to any one of the claims 45-50 with a length up to 57, 53, 51, 50, 49, 43, 37 or 33 amino acids.

52. A C-terminal MANF fragment, preferably with the length of 36-78 amino acids,
10 comprising or consisting of at least the consecutive amino acid residues at positions 33-68 or 19-52 of the sequence as set forth in SEQ ID NO:2:

ICEKLKKKDS QICELKYDKQ IDLSTVDLKK LRVKELKKIL DDWGETCKGC
AEKSDYIRKI NELMPKYAPK AASARTDL

15 or a sequence which has at least 80 % homology or sequence identity with the sequence of positions 33-68 or 19-52 in SEQ ID NO:2 and the sequence flanking said consecutive amino acid residues preferably has at least 80 % homology or sequence identity with the sequence at corresponding positions in SEQ ID NO:2, wherein said fragment is a cell membrane penetrating peptide and has a protective effect on neuronal cells.

20 53. The fragment according to claim 52 with the length of 36-59 amino acids.

54. The fragment according to claim 52 or 53 for use as a medicine.

55. The fragment for use according to claim 54 in the treatment of a degenerative disease or
25 disorder, wherein said fragment is preferably administered by intravenous more preferably peripheral administration, intraperitoneal, subcutaneous, intranasal, transdermal, intramuscular, intraocular, or intraarterial administration.

56. The C-terminal MANF fragment for use according to claim 55 consisting of at least 50 consecutive amino acid residues of the sequence as set forth in SEQ ID NO:2:

ICEKLKKKDS QICELKYDKQ IDLSTVDLKK LRVKELKKIL DDWGETCKGC
AEKSDYIRKI NELMPKYAPK AASARTDL

- 5 or a sequence which has at least 90 % homology or sequence identity with the sequence of SEQ ID NO:2.

57. The fragment for use according to claim 56, wherein said fragment has the sequence of SEQ ID NO:5:

- 10 KYDKQ IDLSTVDLKK LRVKELKKIL DDWGETCKGC AEKSDYIRKI NELMPKYAPK
AASARTDL

or a sequence which has at least 90 % homology or sequence identity with the sequence of SEQ ID NO:5

- 15 58. The fragment for use according to claim 56 or 57, wherein said fragment comprises the C-terminal amino acid L in position 78 of SEQ ID NO:2.

59. The fragment for use according to claim 56 or 57, wherein said fragment lacks the ER retention signal RTDL corresponding to positions 75-78 of SEQ ID NO:2.

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60. The fragment for use according to any of claims 52-59, wherein the fragment comprises further modifications protecting the fragment from enzymatic degradation, preferably selected from the group consisting of amidation of the C-terminus and acetylation of the N-terminus.

- 25 61. The fragment for use according to any of claims 52-60, wherein said fragment is resistant to protease cleavage.

62. The fragment for use according to claim 61, wherein said fragment comprises a elongation, deletion, insertion, substitution or modification such that said elongation, deletion, insertion, substitution or modification abolishes at least one protease cleavage site.

- 5 63. The fragment for use according to claim 61 or 62, wherein the protease is selected from the group consisting of a metalloprotease and a serine protease.

64. The fragment for use according to claim 63, wherein the metalloprotease is MMP-9 or MMP-2.

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65. The fragment for use according to claim 63, wherein the serine protease is chymotrypsin A or elastase-2.

- 15 66. The fragment for use according to claim 52-65, wherein any one amino acid within the region corresponding to residues 11-18, 18-25, 21-28, 24-31, 33-40, 52-59, 54-61, 59-66, or 63-70 of SEQ ID NO:2 is substituted, deleted or modified.

- 20 67. The fragment for use according to claim 52-66, wherein an amino acid at position corresponding to 62, 27, 14, 66, 36, 21, 55, 57, or 24 of SEQ ID NO:2 is substituted or modified.

68. The fragment for use according to any of claims 52-67 further conjugated to a detectable chemical or biochemical moiety.

- 25 69. The fragment for use according to any of claims 52-68, wherein said fragment is a cell-penetrating peptide and is capable of penetrating human blood-brain barrier.

70. The fragment for use according to any of claims 52-69, wherein said degenerative disease is a central nervous system (CNS) disease selected from the group consisting of: Alzheimer's disease, Parkinson's disease, multiple system atrophy, amyotrophic lateral sclerosis, frontotemporal lobar degeneration, dementia with Lewy bodies, mild cognitive impairment, Huntington's disease, traumatic brain injury, drug addiction and stroke.

71. A pharmaceutical composition comprising the C-terminal MANF fragment according to any of claims 52-69, and at least one of the following: physiologically acceptable carrier, buffer, excipient, preservative and stabilizer, for use in the treatment of a central nervous system (CNS) disease wherein said fragment is administered by intravenous preferably peripheral administration, intraperitoneal, subcutaneous, intranasal, transdermal, intramuscular, intraocular, or intraarterial administration.

72. A C-terminal MANF fragment, preferably with the length of 36-78 amino acids, comprising or consisting of at least the consecutive amino acid residues at positions 33-68 or 19-52 of the sequence as set forth in SEQ ID NO:2:

ICEKLKKKDS QICELKYDKQ IDLSTVDLKK LRVKELKKIL DDWGETCKGC
AEKSDYIRKI NELMPKYAPK AASARTDL

or a sequence which has at least 80 % homology or sequence identity with the sequence of positions 33-68 or 19-52 in SEQ ID NO:2 and the sequence flanking said consecutive amino acid residues preferably has at least 80 % homology or sequence identity with the sequence at corresponding positions in SEQ ID NO:2, wherein said fragment is a cell membrane penetrating peptide, for use in the treatment of a type 1 or type 2 diabetes or a retinal disease.

73. The fragment for use according to claim 72 with the length of 36-59 amino acids.

74. The fragment for use according claim 73 consisting of at least 50 consecutive amino acid residues of the sequence as set forth in SEQ ID NO:2:

ICEKLKKKDS QICELKYDKQ IDLSTVDLKK LRVKELKKIL DDWGETCKGC
AEKSDYIRKI NELMPKYAPK AASARTDL

or a sequence which has at least 90 % homology or sequence identity to the sequence of SEQ ID NO:2.

75. The fragment for use according to claim 74, wherein said fragment has the sequence of
5 SEQ ID NO:5:

KYDKQ IDLSTVDLKK LRVKELKKIL DDWGETCKGC AEKSDYIRKI NELMPKYAPK
AASARTDL

or a sequence which has at least 90 % homology or sequence identity to the sequence of
SEQ ID NO:5.

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76. The fragment for use according to any of claims 72-75, wherein the fragment comprises
further modifications protecting the fragment from enzymatic degradation preferably
selected from the group consisting of amidation of the C-terminus and acetylation of the N-
terminus.

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77. The fragment for use according to claim 76, wherein said fragment is resistant to
protease cleavage.

78. The fragment for use according to claim 77, wherein said fragment comprises a
20 elongation, deletion, insertion, substitution or modification such that said elongation,
deletion, insertion, substitution or modification abolishes at least one protease cleavage site.

79. The fragment for use according to claim 77 or 78, wherein the protease is selected from
the group consisting of a metalloprotease and a serine protease.

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80. The fragment for use according to claim 79, wherein the metalloprotease is MMP-9 or
MMP-2.

81. The fragment for use according to claim 79, wherein the serine protease is chymotrypsin A or elastase-2.

82. The fragment for use according to claim 72-81, wherein any one amino acid within the
5 region corresponding to residues 11-18, 18-25, 21-28, 24-31, 33-40, 52-59, 54-61, 59-66, or 63-70 of SEQ ID NO:2 is substituted, deleted or modified.

83. The fragment for use according to claim 72-82, wherein an amino acid at position
10 corresponding to 62, 27, 14, 66, 36, 21, 55, 57, or 24 of SEQ ID NO:2 is substituted or modified.

84. The fragment for use according to any of claims 72-83, wherein said fragment is a cell-penetrating peptide and is capable of penetrating human blood-brain barrier.

15 85. A pharmaceutical composition comprising the C-terminal MANF fragment according to any of claims 72-84 and at least one of the following: physiologically acceptable carrier, buffer, excipient, preservative and stabilizer for use in the treatment of type 1 or type 2 diabetes or a retinal disease.

20 86. The pharmaceutical composition for use according to claim 85 for peripheral administration.

87. The pharmaceutical composition for use according to claim 86, wherein said composition is administered by intravenous, intraperitoneal, subcutaneous, intrathecal,
25 intracerebroventricular, intranasal, transdermal, intracerebral, intramuscular, intraocular, or intraarterial administration or said fragment is administered via a viral expression vector.

88. An isolated polynucleotide comprising a nucleotide sequence encoding the C-terminal CDNF fragment consisting of the consecutive amino acid residues at positions 31-73

(peptide 6), 25-73 (peptide 4), 21-73 (peptide 3), 21-70 (peptide 7), 31-81 (peptide 5), or 25-81 (peptide 2), 25-57 (peptide 15), or 37-73 (peptide 8) of the sequence as set forth in SEQ ID NO:1 or a sequence which has at least 80 % homology or sequence identity with the sequence of positions 31-73, 25-73, 21-73, 21-70, 31-81, 25-81, 25-57, or 37-73 in SEQ ID NO:1.

89. An expression vector encoding the isolated polynucleotide according to claim 88.

90. A host cell transformed with the vector according to claim 89.

91. A method of treating a degenerative disease or disorder comprising administering an effective amount of C-terminal CDNF fragment comprising or consisting of at least the consecutive amino acid residues at positions 38-70 or 25-57 of the sequence as set forth in SEQ ID NO:1:

MPAMKICEKL KKLDSQICEL KYEKTLDLAS VDLRKMRVAE LKQILHSWGE
ECRACA EKTD YVNL IQELAP KYAATHPKTE L

or a sequence which has at least 80 % homology or sequence identity with the sequence of positions 38-70 or 25-57 in SEQ ID NO:1.

92. A method of treating a degenerative disease, a type 1 or type 2 diabetes or a retinal disease comprising administering an effective amount of C-terminal MANF fragment, preferably with the length of 36-78 amino acids, comprising or consisting of at least the consecutive amino acid residues at positions 33-68 or 19-52 of the sequence as set forth in SEQ ID NO:2:

ICEKLKKKDS QICELKYDKQ IDLSTVDLKK LRVKELKKIL DDWGETCKGC
AEKSDYIRKI NELMPKYAPK AASARTDL

or a sequence which has at least 80 % homology or sequence identity with the sequence of positions 38-70 or 19-52 in SEQ ID NO:2 and, if present, the sequence flanking said consecutive amino acid residues preferably has at least 80 % homology or sequence identity with the sequence at corresponding positions in SEQ ID NO:2.