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<120> Disrupting the LINC complex for treating Laminopathy

<130> SP100397WO

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<151> 2018-01-19

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gctcactcat taggcacccc aggctttaca ctttatgctt ccggctcgta tgttgtgtgg 5640

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<210> 4
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<222> (1459)..(1461)

<400> 4

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tctcgcctga agcagcctct gcagggtgac agtgaggctt ttccgtggca ttggatgagt 180

ggcgtggagc agcaggtggc ctctctgtct ggacagtgcc accaccatgg tgagaatctc 240

cgagagctga ccactttgct acagaagctg caggctcggg tggaccagat ggaaggcggc 300

gctgccgggc cgtcagcttc ggtcagagac gctgtgggac agcccccgag ggagactgac 360

tttatggcct ttcaccaaga acatgaagtg cgtatgtcac acttggaaga tattctggga 420

aaactgagag aaaaatctga ggccatccag aaggaactag aacagaccaa gcaaaaaaca 480

atcagtgcgg ttggtgagca gctcctgccc acagtcgagc acctccagct ggagctggat 540

cagctaaagt cagagctgtc cagctggcga cacgtgaaga ccggctgtga gacagtggat 600

gccgtacaag aaagagtgga cgtgcaagtc agagaaatgg tgaaactcct gttttccgaa 660

gatcagcaag gcggttctct ggaacagctg ctgcagaggt tctcatcaca gtttgtgagc 720

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gtcaagaaag atgagttgtg a 1461

<210> 5
<211> 1482
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<220>
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<220>
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<220>
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<220>
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<220>
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<222> (1480)..(1482)

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catttccagg ctgagcagcg tgttatgtcc cgggtacact ctctggagcg gcgtctggaa 180

gctcttgctg ctgaattttc ctccaactgg cagaaggagg ccatgcggct ggaacgtctg 240

gagctgcggc aaggggctcc tggccaggga ggtggtggtg gcctgagcca cgaggacacc 300

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agggaaactg ctgctcgcat ccaggaagaa ctgtctgccc tgagagcaga gcatcagcaa 420

gactcagaag acctcttcaa gaagatcgtc cgggcctccc aggagtccga ggctcgcatc 480

cagcagctga agtcagagtg gcaaagcatg acccaggagt ccttccagga gagctctgtg 540

aaggagctga ggcggctgga ggaccagctg gccggcctgc agcaggagct ggcggctctg 600

gcactgaagc agagctcggt ggcggaagaa gtgggcctgc tgccccagca gatccaggcc 660

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caggacggcg agcctattca gacgtttcac tttcaggccc ctacgatggc cacgtaccag 1380

gtggtggagc tgcggatcct gactaactgg ggccaccccg agtacacctg catctaccgc 1440

ttcagagtgc atggggagcc cgcccacaaa gatgagttgt ag 1482

<210> 6
<211> 180
<212> DNA
<213> Artificial Sequence

<220>
<223> KASH1, derived from Nesprin-1

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ctcatcgggc ttgcctgcct tgtaccaatg tcagaggaag actacagctg tgccctctcc 120

aacaactttg cccggtcatt ccaccccatg ctcagataca cgaatggccc tcctccactc 180

<210> 7
<211> 60
<212> PRT
<213> Artificial Sequence

<220>
<223> KASH1 amino acid sequence

<400> 7

Arg Gly Phe Leu Phe Arg Val Leu Arg Ala Ala Leu Pro Leu Gln Leu

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1               5                   10                  15

Leu Leu Leu Leu Leu Ile Gly Leu Ala Cys Leu Val Pro Met Ser Glu
            20                  25                  30

Glu Asp Tyr Ser Cys Ala Leu Ser Asn Asn Phe Ala Arg Ser Phe His
        35                  40                  45

Pro Met Leu Arg Tyr Thr Asn Gly Pro Pro Pro Leu
    50                  55                  60
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<210> 8
<211> 180
<212> DNA
<213> Artificial Sequence

<220>
<223> KASH2, derived from Nesprin-2

<400> 8

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ctgctgctcc tggcctgcct gctgccctcc tccgaagaag actacagctg cactcaggcc      120

aacaactttg cccggtcctt ttaccccatg ctgaggtaca ccaatgggcc acccccccaca      180
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<210> 9
<211> 60
<212> PRT
<213> Artificial Sequence

<220>
<223> KASH2 amino acid sequence

<400> 9

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Arg Ser Phe Leu Ser Arg Val Val Arg Ala Ala Leu Pro Leu Gln Leu
1               5                   10                  15

Leu Leu Leu Leu Leu Leu Leu Leu Ala Cys Leu Leu Pro Ser Ser Glu
            20                  25                  30

Glu Asp Tyr Ser Cys Thr Gln Ala Asn Asn Phe Ala Arg Ser Phe Tyr
        35                  40                  45

Pro Met Leu Arg Tyr Thr Asn Gly Pro Pro Pro Thr
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50 55 60

<210> 10
<211> 177
<212> DNA
<213> Artificial Sequence

<220>
<223> KASH3, derived from Nesprin-3

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ttcctcctcc tgctgttcct gctcccaatc agggaagagg accgcagctg caccctggcc 120

aacaacttcg cccgctcctt cacgctcatg ctgcgctaca atggcccacc acccacc 177

<210> 11
<211> 59
<212> PRT
<213> Artificial Sequence

<220>
<223> KASH3 amino acid sequence

<400> 11

Gly Ser Leu Phe Arg Arg Ala Cys Cys Val Ala Leu Pro Leu Gln Leu
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Leu Leu Leu Leu Phe Leu Leu Leu Leu Phe Leu Leu Pro Ile Arg Glu
20 25 30

Glu Asp Arg Ser Cys Thr Leu Ala Asn Asn Phe Ala Arg Ser Phe Thr
35 40 45

Leu Met Leu Arg Tyr Asn Gly Pro Pro Pro Thr
50 55

<210> 12
<211> 174
<212> DNA
<213> Artificial Sequence

<220>
<223> KASH4, derived from Nesprin-4

<400> 12

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ctggtgggtg ccatgtttct cctgcccgcg tcaggaggcc cctgctgctc tcatgcccga 120

atacccagga caccctacct ggtgctcagc tatgtcaatg gtcttccccc agtc 174

<210> 13
<211> 58
<212> PRT
<213> Artificial Sequence

<220>
<223> KASH4 amino acid sequence

<400> 13

Asp Pro Ala Ser Arg Gln Pro Leu Thr Phe Leu Leu Ile Leu Phe Leu
1 5 10 15

Leu Phe Leu Leu Leu Val Gly Ala Met Phe Leu Leu Pro Ala Ser Gly
20 25 30

Gly Pro Cys Cys Ser His Ala Arg Ile Pro Arg Thr Pro Tyr Leu Val
35 40 45

Leu Ser Tyr Val Asn Gly Leu Pro Pro Val
50 55

<210> 14
<211> 144
<212> DNA
<213> Artificial Sequence

<220>
<223> KASH5, derived from CCDC155/KASH5

<400> 14
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ctgctctctg tcctgctgct tggcccgtcc ccacctccca cctggcccca cctccagctc 120

tgctacctcc agccccctcc agtg 144

<210> 15
<211> 48
<212> PRT
<213> Artificial Sequence

<220>
<223> KASH5 amino acid sequence

<400> 15

Leu Arg Val Thr Arg His Pro Leu Ile Pro Ala Pro Val Leu Gly Leu
1 5 10 15

Leu Leu Leu Leu Leu Leu Ser Val Leu Leu Leu Gly Pro Ser Pro Pro
20 25 30

Pro Thr Trp Pro His Leu Gln Leu Cys Tyr Leu Gln Pro Pro Pro Val
35 40 45

<210> 16
<211> 22
<212> DNA
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<220>
<223> FLX/FLX-F1 primer

<400> 16
ccagcttaca gagcaccgag ct 22

<210> 17
<211> 22
<212> DNA
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<220>
<223> FLX/FLX-F2 primer

<400> 17
tccttgcagt ccctcttgca tc 22

<210> 18
<211> 21
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<220>
<223> FLX/FLX-R1 primer

<400> 18
aggcaccatt gtcacagggt c 21

<210> 19

<211> 29
<212> DNA
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<220>
<223> Sun1-F primer

<400> 19
ggcaagtgga tctcttgtga attcttgac 29

<210> 20
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<220>
<223> Sun1-R primer

<400> 20
gtagcaccca ccttggtgag ctggtac 27

<210> 21
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<212> DNA
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<220>
<223> Sun1-E8 primer

<400> 21
agccacataa ccacctggag 20

<210> 22
<211> 26
<212> DNA
<213> Artificial Sequence

<220>
<223> MyHC-tF primer

<400> 22
atgacagaca gatccctcct atctcc 26

<210> 23
<211> 24
<212> DNA
<213> Artificial Sequence

<220>
<223> MyHC-tR primer

<400> 23
ctcatcactc gttgcatcat cgac 24

<210> 24
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> MyHC-F primer

<400> 24
caaatgttgc ttgtctggtg 20

<210> 25
<211> 20
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<223> MyHC-R primer

<400> 25
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<210> 26
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<220>
<223> mcm-3798t primer

<400> 26
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<210> 27
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<220>
<223> mcm-8346t primer

<400> 27
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<210> 28

<211> 24
<212> DNA
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<220>
<223> mcm-7338 primer

<400> 28
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<210> 29
<211> 25
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<220>
<223> mcm-7339 primer

<400> 29
gtaggtggaa attctagcat catcc 25

<210> 30
<211> 44
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<220>
<223> aav Sun1 F primer

<400> 30
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<210> 31
<211> 46
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<220>
<223> aav Sun1 R primer

<400> 31
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<210> 32
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<210> 33
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<400> 33
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<210> 34
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<220>
<223> Syne1-stop guide RNA sequence

<400> 34
ccgttggtat atctgagcat 20

<210> 35
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<220>
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<400> 35
ggttatggcc gataggtgca t 21

<210> 36
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<220>
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<400> 36
aaaagcaccg actcggtgcc 20

<210> 37

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<220>
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<400> 37
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<210> 38
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<220>
<223> Syne1-stop gRNA-specific forward primer

<400> 38
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<210> 39
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<220>
<223> Tyrosinase4a gRNA-specific forward primer

<400> 39
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<210> 40
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<220>
<223> Syne1CT' del8 forward primer

<400> 40
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<210> 41
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<223> Syne1CT' del8 reverse primer

<400> 41
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<210> 42
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<220>
<223> Sun1 CRISPR forward primer

<400> 42
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<210> 43
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<220>
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<400> 43
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<223> Potential guide RNA sequence, SYNE1

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<210> 45
<211> 20
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<220>
<223> Potential guide RNA sequence, SYNE1

<400> 45
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<210> 46

<211> 20
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<220>
<223> Potential guide RNA sequence, SYNE1

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<210> 47
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<220>
<223> Potential guide RNA sequence, SYNE1

<400> 47
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<400> 60
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<213> Artificial Sequence

<220>
<223> Cas9 mRNA and gRNA targeting SUN1 domain

<400> 66
gcacaatagc ctcggatgtc g 21

<210> 67
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Cas9 mRNA and gRNA targeting the KASH1 domain

<400> 67
ccgttggtat atctgagcat 20

<210> 68
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> gRNA targeting tyrosinase

<400> 68
ggttatggcc gataggtgca t 21

<210> 69
<211> 176
<212> DNA
<213> Mus musculus

<400> 69
catgaccttg agctgaaact gctgcagaat atcacacacc acatcaccgt gacaggacag 60

gccccgacat ccgaggctat tgtgtctgcc gtgaatcagg cagggatttc aggaatcaca 120

gaagcgcaag cacatatcat tgtgaacaat gctctgaagc tgtactccca agacaa 176

<210> 70
<211> 180
<212> DNA
<213> Artificial Sequence

<220>
<223> Sun1_plus4

<400> 70
catgaccttg agctgaaact gctgcagaat atcacacacc acatcaccgt gacaggacag 60

gccccgagac acatccgagg ctattgtgtc tgccgtgaat caggcaggga tttcaggaat 120

cacagaagcg caagcacata tcattgtgaa caatgctctg aagctgtact cccaagacaa 180

<210> 71
<211> 169
<212> DNA
<213> Artificial Sequence

<220>
<223> Sun1_del7

<400> 71
catgaccttg agctgaaact gctgcagaat atcacacacc acatcaccgt gacaggacag 60

gccccgaggc tattgtgtct gccgtgaatc aggcagggat ttcaggaatc acagaagcgc 120

aagcacatat cattgtgaac aatgctctga agctgtactc ccaagacaa 169

<210> 72
<211> 253
<212> PRT
<213> Mus musculus

<400> 72

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Trp Leu Leu Glu Lys Leu Ser Ser Arg Phe Val Ser Lys Asp Glu Leu
1               5                  10                  15

Gln Val Leu Leu His Asp Leu Glu Leu Lys Leu Leu Gln Asn Ile Thr
            20                  25                  30

His His Ile Thr Val Thr Gly Gln Ala Pro Thr Ser Glu Ala Ile Val
        35                  40                  45

Ser Ala Val Asn Gln Ala Gly Ile Ser Gly Ile Thr Glu Ala Gln Ala
    50                  55                  60

His Ile Ile Val Asn Asn Ala Leu Lys Leu Tyr Ser Gln Asp Lys Thr
65                  70                  75                  80

Gly Met Val Asp Phe Ala Leu Glu Ser Gly Gly Gly Ser Ile Leu Ser
                85                  90                  95

Thr Arg Cys Ser Glu Thr Tyr Glu Thr Lys Thr Ala Leu Leu Ser Leu
            100                 105                 110

Phe Gly Val Pro Leu Trp Tyr Phe Ser Gln Ser Pro Arg Val Val Ile
        115                 120                 125

Gln Pro Asp Ile Tyr Pro Gly Asn Cys Trp Ala Phe Lys Gly Ser Gln
    130                 135                 140

Gly Tyr Leu Val Val Arg Leu Ser Met Lys Ile Tyr Pro Thr Thr Phe
145                 150                 155                 160

Thr Met Glu His Ile Pro Lys Thr Leu Ser Pro Thr Gly Asn Ile Ser
                165                 170                 175

Ser Ala Pro Lys Asp Phe Ala Val Tyr Gly Leu Glu Thr Glu Tyr Gln
            180                 185                 190

Glu Glu Gly Gln Pro Leu Gly Arg Phe Thr Tyr Asp Gln Glu Gly Asp
        195                 200                 205
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Ser Leu Gln Met Phe His Thr Leu Glu Arg Pro Asp Gln Ala Phe Gln
210 215 220

Ile Val Glu Leu Arg Val Leu Ser Asn Trp Gly His Pro Glu Tyr Thr
225 230 235 240

Cys Leu Tyr Arg Phe Arg Val His Gly Glu Pro Ile Gln
245 250

<210> 73
<211> 101
<212> PRT
<213> Artificial Sequence

<220>
<223> Sun1_plus4 protein

<400> 73

Trp Leu Leu Glu Lys Leu Ser Ser Arg Phe Val Ser Lys Asp Glu Leu
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Gln Val Leu Leu His Asp Leu Glu Leu Lys Leu Leu Gln Asn Ile Thr
20 25 30

His His Ile Thr Val Thr Gly Gln Ala Pro Arg His Ile Arg Gly Tyr
35 40 45

Cys Val Cys Arg Glu Ser Gly Arg Asp Phe Arg Asn His Arg Ser Ala
50 55 60

Ser Thr Tyr His Cys Glu Gln Cys Ser Glu Ala Val Leu Pro Arg Gln
65 70 75 80

Asp Gly Asp Gly Gly Leu Cys Ser Gly Val Trp Arg Trp Gln His Pro
85 90 95

Lys His Ser Val Leu
100

<210> 74
<211> 48
<212> PRT

<213> Artificial Sequence

<220>
<223> Sun1_del7 protein

<400> 74

Trp Leu Leu Glu Lys Leu Ser Ser Arg Phe Val Ser Lys Asp Glu Leu
1 5 10 15

Gln Val Leu Leu His Asp Leu Glu Leu Lys Leu Leu Gln Asn Ile Thr
20 25 30

His His Ile Thr Val Thr Gly Gln Ala Pro Arg Leu Leu Cys Leu Pro
35 40 45

<210> 75
<211> 180
<212> DNA
<213> Mus musculus

<400> 75
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caactttgcc cgatccttcc atccgatgct cagatatacc aacggtcctc ctccactctg 120

aagcaagcag acatccccac acaagtgcag gcagtaagag gagaaggaat atcaaatggc 180

<210> 76
<211> 172
<212> DNA
<213> Artificial Sequence

<220>
<223> Syne1_CTdel8

<400> 76
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caactttgcc cgatccttcc atccgatata ccaacggtcc tcctccactc tgaagcaagc 120

agacatcccc acacaagtgc aggcagtaag aggagaagga atatcaaatg gc 172

<210> 77
<211> 79
<212> PRT
<213> Mus musculus

<400> 77

Ser Thr Arg Asp Gly Ser Asp Ser Ser Arg Ser Asp Pro Arg Pro Glu
1   5   10   15

Arg Val Gly Arg Ala Phe Leu Phe Arg Ile Leu Arg Ala Ala Leu Pro
            20   25   30

Phe Gln Leu Leu Leu Leu Leu Leu Ile Gly Leu Thr Cys Leu Val Pro
        35   40   45

Met Ser Glu Lys Asp Tyr Ser Cys Ala Leu Ser Asn Asn Phe Ala Arg
    50   55   60

Ser Phe His Pro Met Leu Arg Tyr Thr Asn Gly Pro Pro Pro Leu
65   70   75

<210> 78
<211> 129
<212> PRT
<213> Artificial Sequence

<220>
<223> Nesprin1_CTdel8

<400> 78

Ser Thr Arg Asp Gly Ser Asp Ser Ser Arg Ser Asp Pro Arg Pro Glu
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Arg Val Gly Arg Ala Phe Leu Phe Arg Ile Leu Arg Ala Ala Leu Pro
            20   25   30

Phe Gln Leu Leu Leu Leu Leu Leu Ile Gly Leu Thr Cys Leu Val Pro
        35   40   45

Met Ser Glu Lys Asp Tyr Ser Cys Ala Leu Ser Asn Asn Phe Ala Arg
    50   55   60

Ser Phe His Pro Ile Tyr Gln Arg Ser Ser Ser Thr Leu Lys Gln Ala
65   70   75   80

Asp Ile Pro Thr Gln Val Gln Ala Val Arg Gly Glu Gly Ile Ser Asn
                85   90   95

Gly Arg Glu Ala Pro Lys Glu Lys Phe Asn Ile Leu Asn His Gln Gly
100 105 110

Asn Ala Asn Gln Asn Asn Pro Glu Met Glu Thr Asn Ala Pro Pro Cys
115 120 125

Ser

<210> 79
<211> 183
<212> DNA
<213> Homo sapiens

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ctcatcgggc ttgcctgcct tgtaccaatg tcagaggaag actacagctg tgccctctcc 120

aacaactttg cccggtcatt ccaccccatg ctcagataca cgaatggccc tcctccactc 180

tga 183

<210> 80
<211> 534
<212> DNA
<213> Homo sapiens

<400> 80
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cgctgttctg aaacttacga aaccaaaacg gcgctgatga gtctgtttgg gatcccgctg 120

tggtacttct cgcagtcccc gcgcgtggtc atccagcctg acatttaccc cggtaactgc 180

tgggcattta aaggctccca ggggtacctg gtggtgaggc tctccatgat gatccaccca 240

gccgccttca ctctggagca catccctaag acgctgtcgc caacaggcaa catcagcagc 300

gcccccaagg acttcgccgt ctatggatta gaaaatgagt atcaggaaga agggcagctt 360

ctgggacagt tcacgtatga tcaggatggg gagtcgctcc agatgttcca ggccctgaaa 420

agacccgacg acacagcttt ccaaatagtg gaacttcgga ttttttctaa ctggggccat 480

cctgagtata cctgtctgta tcggttcaga gttcatggcg aacctgtcaa gtga 534

<210> 81

<211> 117
<212> DNA
<213> Homo sapiens

<400> 81
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catcctgagt atacctgtct gtatcggttc agagttcatg gcgaacctgt caagtga 117