Claims

1. Nucleic acid sequence comprising or coding for

a) a coding region, encoding at least one peptide or protein;

b) at least one histone stem-loop, and

c) a poly(A) sequence or a polyadenylation signal;

wherein said peptide or protein comprises a therapeutic protein or a fragment, variant or derivative thereof, selected from

(i) therapeutic proteins for use in the treatment of metabolic or endocrine disorders,

(ii) therapeutic proteins for use in the treatment of blood disorders, diseases of the circulatory system, diseases of the respiratory system, cancer or tumour diseases, infectious diseases or immunedeficiencies,

(iii) therapeutic proteins used for hormone replacement therapy ,

(iv) therapeutic proteins used for reprogramming of somatic cells into pluri- or omnipotent stem cells,

(v) therapeutic proteins selected from adjuvant or immunostimulating proteins; and

(vi) antibodies.

2. The nucleic acid according to claim 1, wherein the at least one histone stem loop is heterologous to the coding region encoding the at least one peptide or protein, preferably, wherein the coding region does not encode a histone protein or fragment, derivate of variant thereof having histone or histone-like function.

3. The nucleic acid according to claim 1 or 2, wherein the peptide or protein encoded by the coding region comprises a therapeutic protein or a fragment, variant or derivative thereof, the fragment, variant or derivative of the therapeutic protein retaining at least 50% of the biological activity of the naturally occuring full-length therapeutic protein.

4. The nucleic acid of any of claims 1 to 3, wherein its coding region does not encode a reporter protein or a marker or selection protein.

5. The nucleic acid sequence according to claims 1 to 4, wherein said peptide or protein comprises a therapeutic protein or a fragment, variant or derivative thereof, selected from therapeutic proteins for use in the treatment of metabolic or endocrine disorders including Acid sphingomyelinase, Adipotide, Agalsidase-beta, Alglucosidase, alpha-galactosidase A, alpha-glucosidase, alpha-L-iduronidase, alpha-N-acetylglucosaminidase, Amphiregulin, Angiopoietins (Ang1, Ang2, Ang3, Ang4, ANGPTL2, ANGPTL3, ANGPTL4, ANGPTL5, ANGPTL6, ANGPTL7), Betacellulin, Beta-glucuronidase, Bone morphogenetic proteins BMPs (BMP1, BMP2, BMP3, BMP4, BMP5, BMP6, BMP7, BMP8a, BMP8b, BMP10, BMP15), CLN6 protein, Epidermal growth factor (EGF), Epigen, Epiregulin, Fibroblast Growth Factor (FGF, FGF-1, FGF-2, FGF-3, FGF-4, FGF-5, FGF-6, FGF-7, FGF-8, FGF-9, FGF-10, FGF-11, FGF-12, FGF-13, FGF-14, FGF-16, FGF-17, FGF-17, FGF-18, FGF-19, FGF-20, FGF-21, FGF-22, FGF-23), Galsulphase, Ghrelin, Glucocerebrosidase, GM-CSF, Heparin-binding EGF-like growth factor (HB-EGF), Hepatocyte growth factor HGF, Hepcidin, Human albumin, increased loss of albumin, Idursulphase (Iduronate-2-sulphatase), Integrins αVβ3, αVβ5 and α5β1, Iduronate sulfatase, Laronidase, N-acetylgalactosamine-4-sulfatase (rhASB; galsulfase, Arylsulfatase A (ARSA), Arylsulfatase B (ARSB)), N-acetylglucosamine-6-sulfatase, Nerve growth factor (NGF, Brain-Derived Neurotrophic Factor (BDNF), Neurotrophin-3 (NT-3), and Neurotrophin 4/5 (NT-4/5), Neuregulin (NRG1, NRG2, NRG3, NRG4), Neuropilin (NRP-1, NRP-2), Obestatin, Platelet Derived Growth factor (PDGF (PDFF-A, PDGF-B, PDGF-C, PDGF-D), TGF beta receptors (endoglin, TGF-beta 1 receptor, TGF-beta 2 receptor, TGF-beta 3 receptor), Thrombopoietin (THPO) (Megakaryocyte growth and development factor (MGDF)), Transforming Growth factor (TGF (TGF-a, TGF-beta (TGFbeta1, TGFbeta2, and TGFbeta3))), VEGF (VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, VEGF-F und PlGF), Nesiritide, Trypsin, adrenocorticotrophic hormone (ACTH), Atrial-natriuretic peptide (ANP), Cholecystokinin, Gastrin, Leptin, Oxytocin,

Somatostatin, Vasopressin (antidiuretic hormone), Calcitonin, Exenatide, Growth hormone (GH), somatotropin, Insulin, Insulin-like growth factor 1 IGF-1, Mecasermin rinfabate, IGF-1 analog, Mecasermin, IGF-1 analog, Pegvisomant, Pramlintide, Teriparatide (human parathyroid hormone residues 1–34), Becaplermin, Dibotermin-alpha (Bone morphogenetic protein 2), Histrelin acetate (gonadotropin releasing hormone; GnRH), Octreotide, and Palifermin (keratinocyte growth factor; KGF);

therapeutic proteins for use in the treatment of blood disorders, diseases of the circulatory system, diseases of the respiratory system, cancer or tumour diseases, infectious diseases or immunedeficiencies including Alteplase (tissue plasminogen activator; tPA), Anistreplase, Antithrombin III (AT-III), Bivalirudin, Darbepoetin-alpha, Drotrecogin-alpha (activated protein C, Erythropoietin, Epoetin-alpha, erythropoetin, erthropoyetin, Factor IX, Factor VIIa, Factor VIII, Lepirudin, Protein C concentrate, Reteplase (deletion mutein of tPA), Streptokinase, Tenecteplase, Urokinase, Angiostatin, Anti-CD22 immunotoxin, Denileukin diftitox, Immunocyanin, MPS (Metallopanstimulin), Aflibercept, Endostatin, Collagenase, Human deoxy-ribonuclease I, dornase, Hyaluronidase, Papain, L-Asparaginase, Peg-asparaginase, Rasburicase, Human chorionic gonadotropin (HCG), Human follicle-stimulating hormone (FSH), Lutropin-alpha, Prolactin, alpha-1-Proteinase inhibitor, Lactase, Pancreatic enzymes (lipase, amylase, protease), Adenosine deaminase (pegademase bovine, PEG-ADA), Abatacept, Alefacept, Anakinra, Etanercept, Interleukin-1 (IL-1) receptor antagonist, Anakinra, Thymulin, TNF-alpha antagonist, Enfuvirtide, and Thymosin α1;

therapeutic proteins selected from adjuvant or immunostimulating proteins including

human adjuvant proteins, particularly pattern recognition receptors TLR1, TLR2, TLR3, TLR4, TLR5, TLR6, TLR7, TLR8, TLR9, TLR10, TLR11; NOD1, NOD2, NOD3, NOD4, NOD5, NALP1, NALP2, NALP3, NALP4, NALP5, NALP6, NALP6, NALP7, NALP7, NALP8, NALP9, NALP10, NALP11, NALP12, NALP13, NALP14,I IPAF, NAIP, CIITA, RIG-I, MDA5 and LGP2, the signal transducers of TLR signaling including adaptor proteins including e.g. Trif and Cardif; components of the Small-GTPases signalling (RhoA, Ras,

Rac1, Cdc42, Rab etc.), components of the PIP signalling (PI3K, Src-Kinases, etc.), components of the MyD88-dependent signalling (MyD88, IRAK1, IRAK2, IRAK4, TIRAP, TRAF6 etc.), components of the MyD88-independent signalling (TICAM1, TICAM2, TRAF6, TBK1, IRF3, TAK1, IRAK1 etc.); the activated kinases including e.g. Akt, MEKK1, MKK1, MKK3, MKK4, MKK6, MKK7, ERK1, ERK2, GSK3, PKC kinases, PKD kinases, GSK3 kinases, JNK, p38MAPK, TAK1, IKK, and TAK1; the activated transcription factors including e.g. NF-κB, c-Fos, c-Jun, c-Myc, CREB, AP-1, Elk-1, ATF2, IRF-3, IRF-7, heat shock proteins, such as HSP10, HSP60, HSP65, HSP70, HSP75 and HSP90, gp96, Fibrinogen, TypIII repeat extra domain A of fibronectin; or components of the complement system including C1q, MBL, C1r, C1s, C2b, Bb, D, MASP-1, MASP-2, C4b, C3b, C5a, C3a, C4a, C5b, C6, C7, C8, C9, CR1, CR2, CR3, CR4, C1qR, C1INH, C4bp, MCP, DAF, H, I, P and CD59, or induced target genes including e.g. Beta-Defensin, cell surface proteins; or human adjuvant proteins including trif, flt-3 ligand, Gp96 or fibronectin, cytokines which induce or enhance an innate immune response, including IL-1 alpha, IL1 beta, IL-2, IL-6, IL-7, IL-8, IL-9, IL-12, IL-13, IL-15, IL-16, IL-17, IL-18, IL-21, IL-23, TNFalpha, IFNalpha, IFNbeta, IFNgamma, GM-CSF, G-CSF, M-CSF; chemokines including IL-8, IP-10, MCP-1, MIP-1alpha, RANTES, Eotaxin, CCL21; cytokines which are released from macrophages, including IL-1, IL-6, IL-8, IL-12 and TNF-alpha; as well as IL-1R1 and IL-1 alpha;

bacterial (adjuvant) proteins particularly bacterial heat shock proteins or chaperons, including Hsp60, Hsp70, Hsp90, Hsp100; OmpA (Outer membrane protein) from gram-negative bacteria; bacterial porins, including OmpF; bacterial toxins, including pertussis toxin (PT) from *Bordetella pertussis*, pertussis adenylate cyclase toxin CyaA and CyaC from *Bordetella pertussis*, PT-9K/129G mutant from pertussis toxin, pertussis adenylate cyclase toxin CyaA and CyaC from *Bordetella pertussis*, tetanus toxin, cholera toxin (CT), cholera toxin B-subunit, CTK63 mutant from cholera toxin, CTE112K mutant from CT, *Escherichia coli* heat-labile enterotoxin (LT), B subunit from heat-labile enterotoxin (LTB) *Escherichia coli* heat-labile enterotoxin mutants with reduced toxicity, including LTK63, LTR72; phenol-

soluble modulin; neutrophil-activating protein (HP-NAP) from *Helicobacter pylori*; Surfactant protein D; Outer surface protein A lipoprotein from *Borrelia burgdorferi*; Ag38 (38 kDa antigen) from *Mycobacterium tuberculosis*; proteins from bacterial fimbriae; Enterotoxin CT of Vibrio cholerae, Pilin from pili from gram negative bacteria, and Surfactant protein A and bacterial flagellins,

protozoan (adjuvant) proteins particularly Tc52 from Trypanosoma cruzi, PFTG from Trypanosoma gondii, Protozoan heat shock proteins, LelF from Leishmania spp., profiling–like protein from Toxoplasma gondii,

viral (adjuvant) proteins particularly Respiratory Syncytial Virus fusion glycoprotein (F-protein), envelope protein from MMT virus, mouse leukemia virus protein, Hemagglutinin protein of wild-type measles virus,

fungal (adjuvant) proteins particularly fungal immunomodulatory protein (FIP; LZ-8);

and Keyhole limpet hemocyanin (KLH), OspA;

therapeutic proteins used for hormone replacement therapy, particularly oestrogens, progesterone or progestins, and testosterone;

therapeutic proteins used for reprogramming of somatic cells into pluri- or omnipotent stem cells, particularly Oct-3/4, Sox gene family (Sox1, Sox2, Sox3, and Sox15), Klf family (Klf1, Klf2, Klf4, and Klf5), Myc family (c-myc, L-myc, and N-myc), Nanog, and LIN28; and

therapeutic antibodies selected from antibodies used for the treatment of cancer or tumour diseases, particularly 131I-tositumomab, 3F8, 8H9, Abagovomab, Adecatumumab, Afutuzumab, Alacizumab pegol, Alemtuzumab, Amatuximab, AME-133v, AMG 102, Anatumomab mafenatox, Apolizumab, Bavituximab, Bectumomab, Belimumab, Bevacizumab, Bivatuzumab mertansine, Blinatumomab, Brentuximab vedotin, Cantuzumab, Cantuzumab mertansine, Cantuzumab ravtansine, Capromab pendetide, Carlumab, Catumaxomab, Cetuximab, Citatuzumab bogatox, Cixutumumab, Clivatuzumab tetraxetan, CNTO 328, CNTO 95, Conatumumab, Dacetuzumab, Dalotuzumab, Denosumab, Detumomab, Drozitumab, Ecromeximab, Edrecolomab, Elotuzumab, Elsilimomab, Enavatuzumab, Ensituximab, Epratuzumab, Ertumaxomab, Ertumaxomab,

Etaracizumab, Farletuzumab, FBTA05, Ficlatuzumab, Figitumumab, Flanvotumab, Galiximab, Galiximab, Ganitumab, GC1008, Gemtuzumab, Gemtuzumab ozogamicin, Girentuximab, Glembatumumab vedotin, GS6624, HuC242-DM4, HuHMFG1, HuN901-DM1, Ibritumomab, Icrucumab, ID09C3, Indatuximab ravtansine, Inotuzumab ozogamicin, Intetumumab, Ipilimumab, Iratumumab, Labetuzumab, Lexatumumab, Lintuzumab, Lorvotuzumab mertansine, Lucatumumab, Lumiliximab, Mapatumumab, Matuzumab, MDX-060, MEDI 522, Mitumomab, Mogamulizumab, MORab-003, MORab-009, Moxetumomab pasudotox, MT103, Nacolomab tafenatox, Naptumomab estafenatox, Narnatumab, Necitumumab, Nimotuzumab, Nimotuzumab, Olaratumab, Onartuzumab, Oportuzumab monatox, Oregovomab, Oregovomab, PAM4, Panitumumab, Patritumab, Pemtumomab, Pertuzumab, Pritumumab, Racotumomab, Radretumab, Ramucirumab, Rilotumumab, Rituximab, Robatumumab, Samalizumab, SGN-30, SGN-40, Sibrotuzumab, Siltuximab, Tabalumab, Tacatuzumab tetraxetan, Taplitumomab paptox, Tenatumomab, Teprotumumab, TGN1412, Ticilimumab (= tremelimumab), Tigatuzumab, TNX-650, Tositumomab, Trastuzumab, TRBS07, Tremelimumab, TRU-016, TRU-016, Tucotuzumab celmoleukin, Ublituximab, Urelumab, Veltuzumab, Veltuzumab (IMMU-106), Volociximab, Votumumab, WX-G250, Zalutumumab, and Zanolimumab;

antibodies used for the treatment of immune disorders, particularly Efalizumab, Epratuzumab, Etrolizumab, Fontolizumab, Ixekizumab, Mepolizumab, Milatuzumab, Pooled immunoglobulins, Priliximab, Rituximab, Rontalizumab, Ruplizumab, Sarilumab, Vedolizumab, Visilizumab, Reslizumab, Adalimumab, Aselizumab, Atinumab, Atlizumab, Bertilimumab, Besilesomab, BMS-945429, Briakinumab, Brodalumab, Canakinumab, Canakinumab, Certolizumab pegol, Erlizumab, Fezakinumab, Golimumab, Gomiliximab, Infliximab, Mavrilimumab, Natalizumab, Ocrelizumab, Odulimomab, Ofatumumab, Ozoralizumab, Pexelizumab, Rovelizumab, SBI-087, SBI-087, Secukinumab, Sirukumab, Talizumab, Tocilizumab, Toralizumab, TRU-015, TRU-016, Ustekinumab, Ustekinumab,

Vepalimomab, Zolimomab aritox, Sifalimumab, Lumiliximab, and Rho(D) Immune Globulin;

antibodies used for the treatment of infectious diseases, particularly Afelimomab, CR6261, Edobacomab, Efungumab, Exbivirumab, Felvizumab, Foravirumab, Ibalizumab, Libivirumab, Motavizumab, Nebacumab, Tuvirumab, Urtoxazumab, Bavituximab, Pagibaximab, Palivizumab, Panobacumab, PRO 140, Rafivirumab, Raxibacumab, Regavirumab, Sevirumab, Suvizumab, and Tefibazumab;

antibodies used for the treatment of blood disorders, particularly Abciximab, Atorolimumab, Eculizumab, Mepolizumab, and Milatuzumab;

antibodies used for immunoregulation, particularly Antithymocyte globulin, Basiliximab, Cedelizumab, Daclizumab, Gavilimomab, Inolimomab, Muromonab-CD3, Muromonab-CD3, Odulimomab, and Siplizumab;

antibodies used for the treatment of diabetes, particularly Gevokizumab, Otelixizumab, and Teplizumab;

antibodies used for the treatment of the Alzheimer's disease, particularly Bapineuzumab, Crenezumab, Gantenerumab, Ponezumab, R1450, and Solanezumab;

antibodies used for the treatment of asthma, particularly Benralizumab, Enokizumab, Keliximab, Lebrikizumab, Omalizumab, Oxelumab, Pascolizumab, and Tralokinumab;

and antibodies for the treatment of diverse disorders, particularly Blosozumab, CaroRx, Fresolimumab, Fulranumab, Romosozumab, Stamulumab, Tanezumab, and Ranibizumab.

6. Nucleic acid sequence according to claims 1 oto 5, wherein the nucleic acid is an RNA, preferably an mRNA.

7. Nucleic acid sequence according to any of claims 1 to 6, wherein the at least one histone stem-loop is selected from following formulae (I) or (II):

formula (I) (stem-loop sequence without stem bordering elements):

$$\underbrace{[N_{0-2}GN_{3-5}]}_{\text{stem1}}\ \underbrace{[N_{0-4}(U/T)N_{0-4}]}_{\text{loop}}\ \underbrace{[N_{3-5}CN_{0-2}]}_{\text{stem2}}$$

formula (II) (stem-loop sequence with stem bordering elements):

$$\underbrace{N_{1-6}}_{\text{stem1 bordering element}}\ \underbrace{[N_{0-2}GN_{3-5}]}_{\text{stem1}}\ \underbrace{[N_{0-4}(U/T)N_{0-4}]}_{\text{loop}}\ \underbrace{[N_{3-5}CN_{0-2}]}_{\text{stem2}}\ \underbrace{N_{1-6}}_{\text{stem2 bordering element}}$$

wherein:

| | |
|---|---|
| stem1 or stem2 bordering elements $N_{1-6}$ | is a consecutive sequence of 1 to 6, preferably of 2 to 6, more preferably of 2 to 5, even more preferably of 3 to 5, most preferably of 4 to 5 or 5 N, wherein each N is independently from another selected from a nucleotide selected from A, U, T, G and C, or a nucleotide analogue thereof; |
| stem1 $[N_{0-2}GN_{3-5}]$ | is reverse complementary or partially reverse complementary with element stem2, and is a consecutive sequence between of 5 to 7 nucleotides; |
| | wherein $N_{0-2}$ is a consecutive sequence of 0 to 2, preferably of 0 to 1, more preferably of 1 N, wherein each N is independently from another selected from a |

nucleotide selected from A, U, T, G and C or a nucleotide analogue thereof;

wherein $N_{3-5}$ is a consecutive sequence of 3 to 5, preferably of 4 to 5, more preferably of 4 N, wherein each N is independently from another selected from a nucleotide selected from A, U, T, G and C or a nucleotide analogue thereof, and

wherein G is guanosine or an analogue thereof, and may be optionally replaced by a cytidine or an analogue thereof, provided that its complementary nucleotide cytidine in stem2 is replaced by guanosine;

loop sequence $[N_{0-4}(U/T)N_{0-4}]$ is located between elements stem1 and stem2, and is a consecutive sequence of 3 to 5 nucleotides, more preferably of 4 nucleotides;

wherein each $N_{0-4}$ is independent from another a consecutive sequence of 0 to 4, preferably of 1 to 3, more preferably of 1 to 2 N, wherein each N is independently from another selected from a nucleotide selected from A, U, T, G and C or a nucleotide analogue thereof; and

wherein U/T represents uridine, or optionally thymidine;

stem2 $[N_{3-5}CN_{0-2}]$ is reverse complementary or partially reverse complementary with element stem1, and is a consecutive sequence between of 5 to 7 nucleotides;

wherein $N_{3-5}$ is a consecutive sequence of 3 to 5, preferably of 4 to 5, more preferably of 4 N, wherein each N is independently from another selected from a

nucleotide selected from A, U, T, G and C or a nucleotide analogue thereof;

wherein $N_{0-2}$ is a consecutive sequence of 0 to 2, preferably of 0 to 1, more preferably of 1 N, wherein each N is independently from another selected from a nucleotide selected from A, U, T, G and C or a nucleotide analogue thereof; and

wherein C is cytidine or an analogue thereof, and may be optionally replaced by a guanosine or an analogue thereof provided that its complementary nucleotide guanosine in stem1 is replaced by cytidine;

wherein

stem1 and stem2 are capable of base pairing with each other

forming a reverse complementary sequence, wherein base pairing may occur between stem1 and stem2, or

forming a partially reverse complementary sequence, wherein an incomplete base pairing may occur between stem1 and stem2.

8. Nucleic acid sequence according to claim 7, wherein the at least one histone stem-loop is selected from at least one of following formulae (Ia) or (IIa):

$$\underbrace{[N_{0-1}GN_{3-5}]}_{\text{stem1}}\ \underbrace{[N_{1-3}(U/T)N_{0-2}]}_{\text{loop}}\ \underbrace{[N_{3-5}CN_{0-1}]}_{\text{stem2}}$$

formula (Ia) (stem-loop sequence without stem bordering elements)

$$\underbrace{N_{2-5}}_{\text{stem1}}\ \underbrace{[N_{0-1}GN_{3-5}]}_{\text{stem1}}\ \underbrace{[N_{1-3}(U/T)N_{0-2}]}_{\text{loop}}\ \underbrace{[N_{3-5}CN_{0-1}]}_{\text{stem2}}\ \underbrace{N_{2-5}}_{\text{stem2}}$$

bordering element bordering element

formula (IIa) (stem-loop sequence with stem bordering elements).

9. Nucleic acid sequence according to any of claims 1 to 8, wherein the poly(A) sequence comprises a sequence of about 25 to about 400 adenosine nucleotides, preferably a sequence of about 50 to about 400 adenosine nucleotides, more preferably a sequence of about 50 to about 300 adenosine nucleotides, even more preferably a sequence of about 50 to about 250 adenosine nucleotides, most preferably a sequence of about 60 to about 250 adenosine nucleotides.

10. Nucleic acid sequence according to any of claims 1 to 9, wherein the polyadenylation signal comprises the consensus sequence NN(U/T)ANA, preferably AA(U/T)AAA or A(U/T)(U/T)AAA.

11. Nucleic acid sequence according to any of claims 1 to 10, wherein the nucleic acid sequence is a modified nucleic acid, in particular a stabilized nucleic acid.

12. Nucleic acid sequence according to claim 11, wherein the G/C content of the coding region encoding at least one peptide or protein of said modified nucleic acid is increased compared with the G/C content of the coding region of the wild-type nucleic acid, the coded amino acid sequence of said modified nucleic acid preferably not being modified compared with the coded amino acid sequence of the wild-type nucleic acid.

13. A composition or kit or kit of parts comprising a plurality or more than one of nucleic acid sequences each according to any of claims 1 to 12.

14. Nucleic acid sequence as defined according to any of claims 1 to 12 or composition or kit or kit of parts as defined according to claim 13 for use as a medicament.

15. Nucleic acid sequence as defined according to any of claims 1 to 12 or composition or kit or kit of parts as defined according to claim 13 for use in gene therapy.

16. Pharmaceutical composition comprising a nucleic acid sequence as defined according to any of claims 1 to 12 or a composition as defined according to claim 13 and optionally a pharmaceutically acceptable carrier.

17. Use of a nucleic acid sequence as defined according to any of claims 1 to 12 or a composition or kit or kit of parts as defined according to claim 13 for increasing the expression of said encoded peptide or protein.

18. Use of a nucleic acid sequence as defined according to any of claims 1 to 12 or composition or kit or kit of parts as defined according to claim 13 for increasing the expression of said encoded peptide or protein in gene therapy.

19. A method for increasing the expression of an encoded peptide or protein comprising the steps:

a) providing the nucleic acid sequence as defined according to any of claims 1 to 12 or the composition as defined according to claim 13,

b) applying or administering the nucleic acid sequence or the composition to a cell-free expression system, a cell, a tissue or an organism.

**What is claimed is:**

1. An invention not claimed in any divisional application.