CLAIMS

1. An extracellular vesicle (EV) comprising at least one polypeptide construct comprising:

(i) at least one polypeptide of interest (PoI);

(ii) at least one exosomal polypeptide; and,

(iii) a polypeptide-based release system,

in which the polypeptide-based release system releases the at least one PoI from the at least one exosomal polypeptide upon endogenous activation,

and in which the polypeptide-based release system is selected from a cis-cleaving release system or a nuclear localization signal (NLS)- nuclear localization signal-binding protein (NLSBP) (NLS- NLSBP) release system.

2. An EV according to Claim 1, in which the PoI is a therapeutic polypeptide selected from the group comprising antibodies, intrabodies, single chain variable fragments, affibodies, enzymes for e.g. enzyme replacement therapy or gene editing, tumor suppressors, viral or bacterial inhibitors, cell component proteins, DNA and/or RNA binding proteins, DNA repair inhibitors, nucleases, proteinases, integrases, transcription factors, growth factors, apoptosis inhibitors and inducers, toxins, structural proteins, neurotrophic factors, membrane transporters, nucleotide binding proteins, heat shock proteins, CRISPR-associated proteins, and any combination thereof.

3. An EV according to Claim 1 or 2, in which the exosomal polypeptide is selected from the group comprising CD9, CD53, CD63, CD81, CD54, CD50, FLOT1, FLOT2, CD49d, CD71, CD133, CD138, CD235a, ALIX, Syntenin-1, Syntenin-2, Lamp2b, TSPAN8, TSPAN14, CD37, CD82, CD151, CD231, CD102, NOTCH1, NOTCH2, NOTCH3, NOTCH4, DLL1, DLL4, JAG1, JAG2, CD49d/ITGA4, ITGB5, ITGB6, ITGB7, CD11a, CD11b, CD11c, CD18/ITGB2, CD41, CD49b, CD49c, CD49e, CD51, CD61, CD104, tetraspanins, Fc receptors, interleukin receptors, immunoglobulins, MHC-I or MHC-II components, CD2, CD3 epsilon, CD3 zeta, CD13, CD18, CD19, CD30, CD34, CD36, CD40, CD40L, CD44, CD45, CD45RA, CD47, CD86, CD110, CD111, CD115, CD117, CD125, CD135, CD184, CD200, CD279, CD273, CD274, CD362, COL6A1, AGRN, EGFR, GAPDH, GLUR2, GLUR3, HLA-DM, HSPG2, L1CAM, LAMB1, LAMC1, LFA-1,

LGALS3BP, Mac-1 alpha, Mac-1 beta, MFGE8, SLIT2, STX3, TCRA, TCRB, TCRD, TCRG, VTI1A, VTI1B, and other exosomal polypeptides.

4. An EV according to Claim 1, 2 or 3, in which the EV further comprises at least one polynucleotide of interest.

5. An EV according to Claim 4, in which the polynucleotide of interest is transported into the EV through interaction with an RNA-binding PoI or a DNA-binding PoI.

6. An EV comprising at least one PoI, in which the PoI has been released from at least one exosomal polypeptide through endogenous activation of a polypeptide-based release system.

7. An EV according to Claim 6, in which the EV further comprises a polynucleotide of interest.

8. An EV according to any preceding claim, in which the EV further comprises at least one targeting moiety.

9. A method for producing an EV according to any preceding claim, comprising:

(i) introducing into an EV-producing cell at least one polynucleotide construct encoding at least one polypeptide construct comprising at least one PoI, an endogenously activatable polypeptide-based release system, and at least one exosomal polypeptide;

(ii) expressing in the EV-producing cell at least one polypeptide construct encoded for by the at least one polynucleotide construct; and

(iii) collecting from the EV-producing cell EVs comprising a PoI.

10. A polynucleotide construct encoding for:

(i) at least one PoI;

(ii) at least one endogenously activatable polypeptide-based release system, in which the polypeptide-based release system is selected from a cis-cleaving release system or a nuclear localization signal (NLS) - nuclear localization signal-binding protein (NLSBP) (NLS-NLSBP) release system; and

(iii) at least one exosomal polypeptide.

11. A polypeptide construct comprising:

(i) at least one PoI;

(ii) at least one endogenously activatable polypeptide-based release system, in which the polypeptide-based release system is selected from a cis-cleaving release system or a nuclear localization signal (NLS) - nuclear localization signal-binding protein (NLSBP) (NLS-NLSBP) release system; and

(iii) at least one exosomal polypeptide.

12. A cell comprising:

(i) at least one polynucleotide construct according to Claim 10;

(ii) at least one polypeptide construct according to Claim 11; and/or

(iii) at least one EV according to any of Claims 1 to 8.

13. An *in vitro* method for intracellular delivery of at least one PoI, comprising contacting a target cell with at least one EV according to any of Claims 1 to 8.

14. A pharmaceutical composition comprising:

(i) at least one polynucleotide construct according to Claim 10;

(ii) at least one polypeptide construct according to Claim 11;

(iii) at least one EV according to any of Claims 1 to 8; and/or

(iv) a cell according to Claim 12.

15. An EV and/or a population of EVs according to any of Claims 1 to 8, for use in medicine.