

past, CRISPR/Cas9 recently has been established for versatile and simple targeting of genomic sequences for knockout purposes. For example, miRNA-744 was targeted in CHO cells using CRISPR/Cas9 by using multiple gRNA enabling sequence excision (Raab, N., et al., Biotechnol. J. (2019) 1800477).

5 US 2007/160586 discloses methods for extending the replicative lifespan of cells.

EP 3 308 778 discloses arginine and its use as a T-cell modulator.

Fischer, S., et al. disclose enhanced protein production by microRNA-30 family in CHO cells is mediated by the modulation of the ubiquitin pathway (J. Biotechnol. 212 (2015) 32-43).

10 Knockouts of single endogenous genes that increase productivity are highly desired because of its simplicity to be introduced in host cell lines.

Summary of the Invention

~~One independent aspect according to the invention is~~ The present disclosure provides

15 a method for generating a recombinant mammalian cell expressing a heterologous polypeptide and a method for producing a heterologous polypeptide using said recombinant mammalian cell, wherein in the recombinant mammalian cell the activity or function or expression of one or more, i.e. at least one, endogenous gene(s) selected from the group consisting of MYC, STK11, SMAD4, PPP2CB, RBM38, NF1, CDK12, SIN3A, PARP-1, ATM, Hipk2, BARD1, HIF1AN, SMAD3, PALB2, 20 FUBP1, RBL2, RPS6KA3, GPS2, ETS1, E2F5, CDKN1A, RNF43, EEF2K, AKT1, BRCA1, ATR, SMAD2, BAD, FOXO1, PBRM1, NRAS, BRCA2, NOTCH1, CREBBP, and RBX1 has been reduced or eliminated or diminished or (completely) knocked-out.

25 The ~~invention~~disclosure is based, at least in part, on the finding that the functional knockout of at least one of the genes from the group of genes consisting of MYC, STK11, SMAD4, PPP2CB, RBM38, NF1, CDK12, SIN3A, PARP-1, ATM, Hipk2, BARD1, HIF1AN, SMAD3, PALB2, FUBP1, RBL2, RPS6KA3, GPS2, ETS1, E2F5, CDKN1A, RNF43, EEF2K, AKT1, BRCA1, ATR, SMAD2, BAD, FOXO1, PBRM1, NRAS, BRCA2, NOTCH1, CREBBP, and RBX1 in a mammalian cell, e.g. 30 such as a CHO cell, improves recombinant productivity, especially of complex antibody formats.

For the current ~~invention~~disclosure the sequence of steps to generate the recombinant mammalian cell is not decisive, i.e. if the transgene is introduced prior to the

functional knockout of at least one of the genes from the group consisting of MYC, STK11, SMAD4, PPP2CB, RBM38, NF1, CDK12, SIN3A, PARP-1, ATM, Hipk2, BARD1, HIF1AN, SMAD3, PALB2, FUBP1, RBL2, RPS6KA3, GPS2, ETS1, E2F5, CDKN1A, RNF43, EE2F2K, AKT1, BRCA1, ATR, SMAD2, BAD, FOXO1, PBRM1, NRAS, BRCA2, NOTCH1, CREBBP, and RBX1, of if first the functional knockout is effected and thereafter the cell is transfected with the transgene. In one preferred ~~embodiment~~example of all aspects and embodiments, the transgene, i.e. the nucleic acid encoding the heterologous polypeptide, is introduced prior to the functional knock out of the endogenous gene. In certain preferred ~~embodiment~~examples, the endogenous gene is the MYC gene.

~~One independent aspect of the current invention is~~The present disclosure provides a mammalian cell wherein the activity or/and function or/and expression of at least one endogenous gene selected from the group of genes consisting of MYC, STK11, SMAD4, PPP2CB, RBM38, NF1, CDK12, SIN3A, PARP-1, ATM, Hipk2, BARD1, HIF1AN, SMAD3, PALB2, FUBP1, RBL2, RPS6KA3, GPS2, ETS1, E2F5, CDKN1A, RNF43, EE2F2K, AKT1, BRCA1, ATR, SMAD2, BAD, FOXO1, PBRM1, NRAS, BRCA2, NOTCH1, CREBBP, and RBX1 has been reduced or eliminated or diminished or (completely) knocked-out. In certain preferred ~~embodiment~~examples, the endogenous gene is the MYC gene.

~~One independent aspect of the current invention is~~The present disclosure provides a mammalian cell, wherein the expression of at least one endogenous gene selected from the group of genes consisting of MYC, STK11, SMAD4, PPP2CB, RBM38, NF1, CDK12, SIN3A, PARP-1, ATM, Hipk2, BARD1, HIF1AN, SMAD3, PALB2, FUBP1, RBL2, RPS6KA3, GPS2, ETS1, E2F5, CDKN1A, RNF43, EE2F2K, AKT1, BRCA1, ATR, SMAD2, BAD, FOXO1, PBRM1, NRAS, BRCA2, NOTCH1, CREBBP, and RBX1 has been reduced, and wherein said mammalian has increased productivity for heterologous polypeptides compared to a cell that has the identical genotype but the respective endogenous gene expression of said at least one endogenous gene selected from the group of genes consisting of MYC, STK11, SMAD4, PPP2CB, RBM38, NF1, CDK12, SIN3A, PARP-1, ATM, Hipk2, BARD1, HIF1AN, SMAD3, PALB2, FUBP1, RBL2, RPS6KA3, GPS2, ETS1, E2F5, CDKN1A, RNF43, EE2F2K, AKT1, BRCA1, ATR, SMAD2, BAD, FOXO1, PBRM1, NRAS, BRCA2, NOTCH1, CREBBP, and RBX1 and that is cultivated under the same conditions. In certain preferred ~~embodiment~~examples, the endogenous gene is the MYC gene.

~~One independent aspect of the current invention is~~The present disclosure provides a method for increasing heterologous polypeptide titer of a recombinant mammalian cell, which has reduced expression of at least one endogenous gene selected from the group of genes consisting of MYC, STK11, SMAD4, PPP2CB, RBM38, NF1, CDK12, SIN3A, PARP-1, ATM, Hipk2, BARD1, HIF1AN, SMAD3, PALB2, FUBP1, RBL2, RPS6KA3, GPS2, ETS1, E2F5, CDKN1A, RNF43, EE2K, AKT1, BRCA1, ATR, SMAD2, BAD, FOXO1, PBRM1, NRAS, BRCA2, NOTCH1, CREBBP, and RBX1, and which comprises an exogenous nucleic acid, i.e. a transgene, encoding said heterologous polypeptide compared to a cell cultivated under the same conditions that has the identical genotype but endogenous gene expression of said at least one endogenous gene selected from the group of genes consisting of MYC, STK11, SMAD4, PPP2CB, RBM38, NF1, CDK12, SIN3A, PARP-1, ATM, Hipk2, BARD1, HIF1AN, SMAD3, PALB2, FUBP1, RBL2, RPS6KA3, GPS2, ETS1, E2F5, CDKN1A, RNF43, EE2K, AKT1, BRCA1, ATR, SMAD2, BAD, FOXO1, PBRM1, NRAS, BRCA2, NOTCH1, CREBBP, and RBX1. In certain preferred ~~embodiments~~examples, the endogenous gene is the MYC gene.

In one aspect, the present disclosure provides a method for increasing heterologous polypeptide expression of a recombinant mammalian cell comprising an exogenous nucleic acid encoding a heterologous polypeptide by reducing expression of at least the endogenous gene MYC, compared to a mammalian cell cultivated under the same conditions that has the identical genotype but endogenous expression of said expression-reduced gene.

~~One independent aspect of the current invention is~~The present disclosure provides a method for producing a recombinant mammalian cell with improved recombinant productivity, wherein the method comprises the following steps:

- a) applying a nuclease-assisted and/or nucleic acid targeting at least one endogenous gene selected from the group of genes consisting of MYC, STK11, SMAD4, PPP2CB, RBM38, NF1, CDK12, SIN3A, PARP-1, ATM, Hipk2, BARD1, HIF1AN, SMAD3, PALB2, FUBP1, RBL2, RPS6KA3, GPS2, ETS1, E2F5, CDKN1A, RNF43, EE2K, AKT1,

BRCA1, ATR, SMAD2, BAD, FOXO1, PBRM1, NRAS, BRCA2, NOTCH1, CREBBP, and RBX1 in a mammalian cell to reduce the activity of said endogenous gene, and

5 b) selecting a mammalian cell wherein the activity of said endogenous gene has been reduced,

thereby producing a recombinant mammalian cell with increased recombinant productivity compared to a cell cultivated under the same conditions that has the identical genotype but endogenous gene expression of said at least one endogenous gene selected from the group of genes consisting of MYC, STK11, SMAD4,
10 PPP2CB, RBM38, NF1, CDK12, SIN3A, PARP-1, ATM, Hipk2, BARD1, HIF1AN,

SMAD3, PALB2, FUBP1, RBL2, RPS6KA3, GPS2, ETS1, E2F5, CDKN1A, RNF43, EEF2K, AKT1, BRCA1, ATR, SMAD2, BAD, FOXO1, PBRM1, NRAS, BRCA2, NOTCH1, CREBBP, and RBX1. In certain preferred ~~embodiment~~examples, the endogenous gene is the MYC gene.

5 ~~One independent aspect of to the current invention is~~The present disclosure provides
a method for producing a heterologous polypeptide comprising the steps of

10 a) cultivating a recombinant mammalian cell comprising an exogenous deoxyribonucleic acid encoding the heterologous polypeptide optionally under conditions suitable for the expression of the heterologous polypeptide, and

b) recovering the heterologous polypeptide from the cell or the cultivation medium,

15 wherein the activity or/and function or/and expression of at least one endogenous gene selected from the group of genes consisting of MYC, STK11, SMAD4, PPP2CB, RBM38, NF1, CDK12, SIN3A, PARP-1, ATM, Hipk2, BARD1, HIF1AN, SMAD3, PALB2, FUBP1, RBL2, RPS6KA3, GPS2, ETS1, E2F5, CDKN1A, RNF43, EEF2K, AKT1, BRCA1, ATR, SMAD2, BAD, FOXO1, PBRM1, NRAS, BRCA2, NOTCH1, CREBBP, and RBX1 has been reduced or eliminated or diminished or (completely) knocked-out in said mammalian cell. In
20 certain preferred ~~embodiment~~examples, the endogenous gene is the MYC gene.

In another aspect, the present disclosure provides a method for producing a heterologous polypeptide comprising the steps of

a) cultivating a mammalian cell comprising a deoxyribonucleic acid encoding the heterologous polypeptide, and

25 b) recovering the heterologous polypeptide from the cell or the cultivation medium,

wherein the expression of at least the endogenous gene MYC has been reduced.

~~Another independent aspect of the current invention is~~The present disclosure provides a method for producing a recombinant mammalian cell having/with improved and/or increased recombinant productivity, wherein the method comprises the following steps:

- 5 a) applying a nucleic acid or an enzyme or a nuclease-assisted gene targeting system targeting at least one endogenous gene selected from the group of genes consisting of MYC, STK11, SMAD4, PPP2CB, RBM38, NF1, CDK12, SIN3A, PARP-1, ATM, Hipk2, BARD1, HIF1AN, SMAD3, PALB2, FUBP1, RBL2, RPS6KA3, GPS2, ETS1,
10 E2F5, CDKN1A, RNF43, EEF2K, AKT1, BRCA1, ATR, SMAD2, BAD, FOXO1, PBRM1, NRAS, BRCA2, NOTCH1, CREBBP, and RBX1 to a mammalian cell to reduce or eliminate or diminish or (completely) knockout the activity or/and function or/and expression of said endogenous gene, and

- b) selecting a mammalian cell wherein the activity or/and function or/and expression of said endogenous gene has been reduced or eliminated or diminished or (completely) knocked-out,

thereby producing a recombinant mammalian cell having/with improved and/or increased recombinant productivity.

In certain preferred ~~embodimentsexamples of all aspects and embodiments of the invention~~, the endogenous gene is the MYC gene.

In another aspect, the present disclosure provides a method for producing a recombinant mammalian cell with improved recombinant productivity, wherein the method comprises the following steps:

- a) applying a nuclease-assisted and/or nucleic acid targeting the endogenous gene MYC in a mammalian cell to reduce the activity of said endogenous gene, and
b) selecting a mammalian cell wherein the activity of said endogenous gene has been reduced,

thereby producing a recombinant mammalian cell with increased recombinant productivity compared to a compared to a mammalian cell cultivated under the same conditions that has the identical genotype but endogenous expression of said gene.

In certain ~~dependent embodimentsexamples of all aspects and embodiments of the current invention~~, the mammalian cell comprises a nucleic acid encoding a heterologous polypeptide.

In certain ~~dependent embodimentsexamples of all aspects and embodiments of the current invention~~, the nucleic acid encoding the heterologous polypeptide is operably linked to a promoter sequence functional in said mammalian cell and operably linked to a polyadenylation signal functional in said mammalian cell. In certain ~~embodimentsexamples~~, the mammalian cell secretes the heterologous polypeptide when cultivated under suitable cultivation conditions.

In certain ~~dependent embodiments~~examples of all aspects and embodiments of the current invention, the knockout of at least one endogenous gene selected from the group of genes consisting of MYC, STK11, SMAD4, PPP2CB, RBM38, NF1, CDK12, SIN3A, PARP-1, ATM, Hipk2, BARD1, HIF1AN, SMAD3, PALB2, FUBP1, RBL2, RPS6KA3, GPS2, ETS1, E2F5, CDKN1A, RNF43, EEF2K, AKT1, BRCA1, ATR, SMAD2, BAD, FOXO1, PBRM1, NRAS, BRCA2, NOTCH1, CREBBP, and RBX1 is a heterozygous knockout or a homozygous knockout.

In certain ~~dependent embodiments~~examples of all aspects and embodiments of the current invention, the productivity of the knockout cell line is at least 5 %, preferably 10 % or more, most preferred 20 % or more increased compared to the respective mammalian cell with the same genotype but fully functional expression of said at least one endogenous gene selected from the group of genes consisting of MYC, STK11, SMAD4, PPP2CB, RBM38, NF1, CDK12, SIN3A, PARP-1, ATM, Hipk2, BARD1, HIF1AN, SMAD3, PALB2, FUBP1, RBL2, RPS6KA3, GPS2, ETS1, E2F5, CDKN1A, RNF43, EEF2K, AKT1, BRCA1, ATR, SMAD2, BAD, FOXO1, PBRM1, NRAS, BRCA2, NOTCH1, CREBBP, and RBX1.

In certain ~~dependent embodimentsexamples of all aspects and embodiments of the current invention~~, the reduction or elimination or diminishment or knockout of at least one endogenous gene selected from the group of genes consisting of MYC, STK11, SMAD4, PPP2CB, RBM38, NF1, CDK12, SIN3A, PARP-1, ATM, Hipk2, BARD1, HIF1AN, SMAD3, PALB2, FUBP1, RBL2, RPS6KA3, GPS2, ETS1, E2F5, CDKN1A, RNF43, EEF2K, AKT1, BRCA1, ATR, SMAD2, BAD, FOXO1, PBRM1, NRAS, BRCA2, NOTCH1, CREBBP, and RBX1 is mediated by a nuclease-assisted gene targeting system. In certain ~~embodimentsexamples~~, the nuclease-assisted gene targeting system is selected from the group consisting of CRISPR/Cas9, CRISPR/Cpf1, zinc-finger nuclease, TALEN and meganucleases.

In certain ~~dependent embodimentsexamples of all aspects and embodiments of the current invention~~, the reduction of the expression of at least one endogenous gene selected from the group of genes consisting of MYC, STK11, SMAD4, PPP2CB, RBM38, NF1, CDK12, SIN3A, PARP-1, ATM, Hipk2, BARD1, HIF1AN, SMAD3, PALB2, FUBP1, RBL2, RPS6KA3, GPS2, ETS1, E2F5, CDKN1A, RNF43, EEF2K, AKT1, BRCA1, ATR, SMAD2, BAD, FOXO1, PBRM1, NRAS, BRCA2, NOTCH1, CREBBP, and RBX1 is mediated by RNA silencing. In certain ~~embodimentsexamples~~, the RNA silencing is selected from the group consisting of siRNA gene targeting and knockdown, shRNA gene targeting and knockdown, and miRNA gene targeting and knockdown.

In certain ~~dependent embodimentsexamples of all aspects and embodiments of the current invention~~, the knockout of at least one endogenous gene selected from the group of genes consisting of MYC, STK11, SMAD4, PPP2CB, RBM38, NF1, CDK12, SIN3A, PARP-1, ATM, Hipk2, BARD1, HIF1AN, SMAD3, PALB2, FUBP1, RBL2, RPS6KA3, GPS2, ETS1, E2F5, CDKN1A, RNF43, EEF2K, AKT1, BRCA1, ATR, SMAD2, BAD, FOXO1, PBRM1, NRAS, BRCA2, NOTCH1, CREBBP, and RBX1 is performed i) before the introduction of the exogenous nucleic acid encoding the heterologous polypeptide, or ii) after the introduction of the exogenous nucleic acid encoding the heterologous polypeptide.

In certain ~~dependent embodimentsexamples of all aspects and embodiments of the current invention~~, the heterologous polypeptide is an antibody. In certain ~~embodimentsexamples~~, the antibody is an antibody comprising two or more different binding sites and optionally a domain exchange. In certain ~~embodimentsexamples~~, the antibody comprises three or more binding sites or VH/VL-pairs or Fab fragments and optionally a domain exchange. In certain ~~embodimentsexamples~~, the antibody is a multispecific antibody.

In certain ~~dependent embodimentsexamples of all aspects and embodiments of the current invention~~, the at least one endogenous gene is selected from the group of genes consisting of MYC, STK11, SMAD4, PPP2CB, RBM38, CDK12, PARP-1, ATM, Hipk2, BARD1, and SMAD3. In certain ~~embodimentsexamples~~, the at least one endogenous gene is selected from the group of genes consisting of MYC, STK11, SMAD4, PPP2CB, RBM38, and CDK12. In certain ~~embodimentsexamples~~, the at least one endogenous gene is selected from the group of genes consisting of MYC and STK11. In one preferred ~~embodimentexample~~, the at least one endogenous gene is MYC.

In certain ~~dependent embodimentsexamples of all aspects and embodiments of the current invention~~, in the recombinant mammalian cell the activity or function or expression of the endogenous SIRT-1 gene and one or more, i.e. at least one, further endogenous gene(s) selected from the group consisting of MYC, STK11, SMAD4, PPP2CB, RBM38, CDK12, PARP-1, ATM, Hipk2, BARD1, and SMAD3 has been reduced or eliminated or diminished or (completely) knocked-out. In certain ~~embodimentsexamples~~, the at least one further endogenous gene is selected from the group of genes consisting of MYC, STK11, SMAD4, PPP2CB, RBM38, and CDK12. In certain ~~embodimentsexamples~~, the at least one further endogenous gene is selected from the group of genes consisting of MYC and STK11. In one preferred ~~embodimentexample~~, the at least one further endogenous gene is MYC, i.e. the activity or function or expression of the endogenous SIRT-1 and the endogenous MYC gene have been reduced or eliminated or diminished or (completely) knocked-out.

In certain ~~dependent embodimentsexamples of all aspects and embodiments of the current invention~~, the heterologous polypeptide is selected from the group of heterologous polypeptides comprising multispecific antibodies and antibody-multimer-fusion polypeptide. In certain ~~embodimentsexamples~~, the heterologous polypeptide is selected from the group consisting of

- i) a full-length antibody with domain exchange comprising a first Fab fragment and a second Fab fragment,

wherein in the first Fab fragment

- a) the light chain of the first Fab fragment comprises a VL and a CH1 domain and the heavy chain of the first Fab fragment comprises a VH and a CL domain;

- b) the light chain of the first Fab fragment comprises a VH and a CL domain and the heavy chain of the first Fab fragment comprises a VL

second part of the non-antibody multimeric polypeptide and an antibody light chain constant domain if the first polypeptide comprises an antibody heavy chain CH1 domain or an antibody heavy chain CH1 domain if the first polypeptide comprises an antibody light chain constant domain,

wherein

(i) the antibody heavy chain of (a) and the first fusion polypeptide of (b), (ii) the antibody heavy chain of (a) and the antibody light chain of (a), and (iii) the first fusion polypeptide of (b) and the second fusion polypeptide of (b) are each independently of each other covalently linked to each other by at least one disulfide bond,

wherein

the variable domains of the antibody heavy chain and the antibody light chain form a binding site specifically binding to an antigen.

In certain preferred embodimentsexamples, the at least one endogenous gene is selected from the group of genes consisting of MYC, STK11, SMAD4, PPP2CB, RBM38, CDK12, PARP-1, ATM, Hipk2, BARD1, and SMAD3. In certain embodimentsexamples, the at least one endogenous gene is selected from the group of genes consisting of MYC, STK11, SMAD4, PPP2CB, RBM38, and CDK12. In certain embodimentsexamples, the at least one endogenous gene is selected from the group of genes consisting of MYC and STK11. In one preferred embodimentexample, the at least one endogenous gene is MYC. In one further preferred embodimentexample, further the activity or function or expression of the endogenous SIRT-1 gene has been reduced or eliminated or diminished or (completely) knocked-out.

In certain embodimentsexamples, in the recombinant mammalian cell the activity or function or expression of the endogenous SIRT-1 gene and one or more, i.e. at least one, further endogenous gene(s) selected from the group consisting of MYC, STK11, SMAD4, PPP2CB, RBM38, CDK12, PARP-1, ATM, Hipk2, BARD1, and SMAD3 has been reduced or eliminated or diminished or (completely) knocked-out. In certain embodimentsexamples, the at least one further endogenous gene is selected from the group of genes consisting of MYC, STK11, SMAD4, PPP2CB, RBM38, and CDK12. In certain embodimentsexamples, the at least one further endogenous gene is selected from the group of genes consisting of MYC and STK11. In one preferred embodimentexample, the at least one further endogenous gene is MYC, i.e. the activity or function or expression

of the endogenous SIRT-1 gene and the endogenous MYC gene has been reduced or eliminated or diminished or (completely) knocked-out.

In certain ~~dependent embodimentsexamples of all aspects and embodiments of the current invention~~, the at least one endogenous gene is selected from the group of genes consisting of MYC, STK11, SMAD4, PPP2CB, RBM38, NF1, CDK12, SIN3A, PARP-1, ATM, Hipk2, BARD1, HIF1AN, SMAD3, and CDKN1A. In certain ~~embodimentsexamples~~, the at least one endogenous gene is selected from the group of genes consisting of MYC, STK11, SMAD4, PPP2CB, RBM38, NF1, and CDK12. In certain ~~embodimentsexamples~~, the at least one endogenous gene is selected from the group of genes consisting of MYC, STK11, SMAD4, and PPP2CB. In one preferred ~~embodimentexample~~, the at least one endogenous gene is MYC.

In certain ~~dependent embodimentsexamples of all aspects and embodiments of the current invention~~, in the recombinant mammalian cell the activity or function or expression of the endogenous SIRT-1 gene and one or more, i.e. at least one, further endogenous gene(s) selected from the group consisting of MYC, STK11, SMAD4, PPP2CB, RBM38, NF1, CDK12, SIN3A, PARP-1, ATM, Hipk2, BARD1, HIF1AN, SMAD3, and CDKN1A has been reduced or eliminated or diminished or (completely) knocked-out. In certain ~~embodimentsexamples~~, the at least one further endogenous gene is selected from the group of genes consisting of MYC, STK11, SMAD4, PPP2CB, RBM38, NF1, and CDK12. In certain ~~embodimentsexamples~~, the at least one further endogenous gene is selected from the group of genes consisting of MYC, STK11, SMAD4, and PPP2CB. In one preferred ~~embodimentexample~~, the activity or function or expression of the endogenous SIRT-1 gene and of the endogenous MYC gene has been reduced or eliminated or diminished or (completely) knocked out.

In certain ~~dependent embodimentsexamples of all aspects and embodiments of the current invention~~, the heterologous polypeptide is an antibody-multimer-fusion polypeptide comprising

(a) an antibody heavy chain and an antibody light chain, and

(b) a first fusion polypeptide comprising in N- to C-terminal direction a first part of a non-antibody multimeric polypeptide, an antibody heavy chain CH1 domain or an antibody light chain constant domain, an antibody hinge region, an antibody heavy chain CH2 domain and an antibody heavy chain CH3 domain, and a second fusion polypeptide comprising in N- to C-terminal direction the

second part of the non-antibody multimeric polypeptide and an antibody light chain constant domain if the first polypeptide comprises an antibody heavy chain CH1 domain or an antibody heavy chain CH1 domain if the first polypeptide comprises an antibody light chain constant domain,

wherein

(i) the antibody heavy chain of (a) and the first fusion polypeptide of (b), (ii) the antibody heavy chain of (a) and the antibody light chain of (a), and (iii) the first fusion polypeptide of (b) and the second fusion polypeptide of (b) are each independently of each other covalently linked to each other by at least one disulfide bond,

wherein

the variable domains of the antibody heavy chain and the antibody light chain form a binding site specifically binding to an antigen.

In certain embodimentsexamples, the at least one endogenous gene is selected from the group of genes consisting of MYC, STK11, SMAD4, PPP2CB, RBM38, NF1, CDK12, SIN3A, PARP-1, ATM, Hipk2, BARD1, HIF1AN, SMAD3, and CDKN1A. In certain embodimentsexamples, the at least one endogenous gene is selected from the group of genes consisting of MYC, STK11, SMAD4, PPP2CB, RBM38, NF1, and CDK12. In certain embodimentsexamples, the at least one endogenous gene is selected from the group of genes consisting of MYC, STK11, SMAD4, and PPP2CB. In one preferred embodimentexample, the at least one endogenous gene is MYC. In one further preferred embodimentexample, in addition the activity or function or expression of the endogenous SIRT-1 gene has been reduced or eliminated or diminished or (completely) knocked-out.

In certain embodimentsexamples, in the recombinant mammalian cell the activity or function or expression of the endogenous SIRT-1 gene and one or more, i.e. at least one, further endogenous gene(s) selected from the group consisting of MYC, STK11, SMAD4, PPP2CB, RBM38, NF1, CDK12, SIN3A, PARP-1, ATM, Hipk2, BARD1, HIF1AN, SMAD3, and CDKN1A has been reduced or eliminated or diminished or (completely) knocked-out. In certain embodimentsexamples, the at least one further endogenous gene is selected from the group of genes consisting of MYC, STK11, SMAD4, PPP2CB, RBM38, NF1, and CDK12. In certain embodimentsexamples, the at least one further endogenous gene is selected from the group of genes consisting of MYC,

STK11, SMAD4, and PPP2CB. In one preferred embodimentexample, the at least one further endogenous gene is MYC.

In certain ~~dependent-embodimentexamples~~, the first fusion polypeptide comprises as first part of the non-antibody multimeric polypeptide two ectodomains of a TNF ligand family member or a fragment thereof that are connected to each other by a peptide linker, and the second fusion polypeptide comprises as second part of a non-antibody multimeric polypeptide only one ectodomain of said TNF ligand family member or a fragment thereof, or vice versa. In certain embodimentexamples, the first fusion polypeptide comprises in N- to C-terminal direction a first part of a non-antibody multimeric polypeptide, an antibody light chain constant domain, an antibody hinge region, an antibody heavy chain CH2 domain and an antibody heavy chain CH3 domain, and the second fusion polypeptide comprising in N- to C-terminal direction the second part of the non-antibody multimeric polypeptide and an antibody heavy chain CH1 domain. In certain embodimentexamples, in the CL domain adjacent to the part of a non-antibody multimeric polypeptide the amino acid at position 123 (Kabat EU numbering) has been replaced by arginine (R) and the amino acid at position 124 (Kabat EU numbering) has been substituted by lysine (K), and wherein in the CH1 domain adjacent to the part of a non-antibody multimeric polypeptide the amino acids at position 147 (Kabat EU numbering) and at position 213 (Kabat EU numbering) have been substituted by glutamic acid (E). In certain embodimentexamples, the variable domains of the antibody heavy chain and the antibody light chain form a binding site specifically binding to a cell surface antigen selected from the group consisting of Fibroblast Activation Protein (FAP), Melanoma-associated Chondroitin Sulfate Proteoglycan (MCSP), Epidermal Growth Factor Receptor (EGFR), Carcinoembryonic Antigen (CEA), CD19, CD20 and CD33. In certain embodimentexamples, TNF ligand family member co-stimulates human T-cell activation. In certain embodimentexamples, the TNF ligand family member is selected from 4-1BBL and OX40L. In one preferred embodimentexample, the TNF ligand family member is 4-1BBL and the cell surface antigen is FAP or CD19 or CEA.

In certain ~~dependent-embodimentexamples-of-all-aspects-and-embodiments-of-the-current-invention~~, the heterologous polypeptide is a fusion polypeptide comprising a bivalent, mono- or bispecific full-length antibody and a non-immunoglobulin moiety, wherein the antibody is conjugated to the non-immunoglobulin moiety at a single terminus of one of the heavy or light chains of the antibody optionally via a peptidic linker. In certain embodimentexamples, the at least one endogenous gene is selected from the group of genes consisting of MYC, STK11, SMAD4, PPP2CB, RBM38,

CDK12, PARP-1, ATM, Hipk2, BARD1, SMAD3, PALB2, FUBP1, RBL2, RPS6KA3, GPS2, ETS1, E2F5, CDKN1A, RNF43, EEF2K, AKT1, BRCA1, ATR, SMAD2, BAD, FOXO1, PBRM1, NRAS, BRCA2, NOTCH1, CREBBP, and RBX1. In certain [embodimentexamples](#), the at least one endogenous gene is selected from the group of genes consisting of MYC, STK11, SMAD4, PPP2CB, RBM38, CDK12, PARP-1, BARD1, ETS1, E2F5, RNF43, EEF2K, AKT1, BRCA1, BAD, FOXO1, PBRM1, BRCA2, NOTCH1, and CREBBP. In certain [embodimentexamples](#), the at least one endogenous gene is selected from the group of genes consisting of MYC, STK11, and CDK12. In one preferred [embodimentexample](#), the at least one endogenous gene is MYC. In one further preferred [embodimentexample](#), in addition the activity or function or expression of the endogenous SIRT-1 gene has been reduced or eliminated or diminished or (completely) knocked-out. In certain [embodimentexamples](#), the heterologous polypeptide is an anti-PD-1 antibody conjugated to interleukin-2. In certain [embodimentexamples](#), the interleukin-2 is an engineered IL2v moiety with abolished binding to IL-2Ra (CD25) to avoid undesired CD25-mediated toxicities and Treg expansion.

In certain ~~[dependent embodimentexamples of all aspects and embodiments of the invention](#)~~, the mammalian cell is a CHO cell or a HEK cell. In one preferred [embodimentexample](#), the mammalian cell is a CHO-K1 cell. In certain [embodimentexamples](#), the mammalian cell is a suspension growing mammalian cell.

In certain ~~[dependent embodimentexamples of all aspects and embodiments of the invention](#)~~, the productivity for heterologous polypeptides is determined in a 4-day batch cultivation. In certain [embodimentexamples](#), the 4-day batch cultivation is inoculated/started with a cell density of at least 1×10^6 cells/ml ($10E6$ cells/ml). In certain [embodimentexamples](#), the 4-day batch cultivation is inoculated/started with a cell density of at least 2×10^6 cells/ml. In certain [embodimentexamples](#), the 4-day batch cultivation is inoculated/started with a cell density of at least 5×10^6 cells/ml. In certain [embodimentexamples](#), the 4-day batch cultivation is inoculated/started with a cell density of at least 10×10^6 cells/ml. In certain [embodimentexamples](#), the 4-day batch cultivation is in a chemically defined, serum-free medium.

In certain ~~[dependent embodimentexamples of all aspects and embodiments of the invention](#)~~, the reduction or elimination or diminishment or knockout of the one, two or more endogenous genes is by a CRISPR/Cas9 nuclease-assisted gene targeting system. In one preferred [embodimentexample](#), the endogenous gene is SIRT-1 and three guide RNAs of SEQ ID NO: 12, 13 and 14 are used. In one further preferred [embodimentexample](#), the endogenous gene is MYC and three guide RNAs of SEQ ID NO: 15, 16 and 17 are

used. In certain [embodimentsexamples](#), the endogenous gene is STK11 and three guide RNAs of SEQ ID NO: 18, 19 and 20 are used. In certain [embodimentsexamples](#), the endogenous gene is SMAD4 and three guide RNAs of SEQ ID NO: 21, 22 and 23 are used. In certain [embodimentsexamples](#), the endogenous gene is PPP2CB and three guide RNAs of SEQ ID NO: 24, 25 and 26 are used. In certain [embodimentsexamples](#), the endogenous gene is RBM38 and three guide RNAs of SEQ ID NO: 27, 28 and 29 are used. In certain [embodimentsexamples](#), the endogenous gene is NF1 and three guide RNAs of SEQ ID NO: 30, 31 and 32 are used. In certain [embodimentsexamples](#), the endogenous gene is CDK12 and three guide RNAs of SEQ ID NO: 33, 34 and 35 are used. In certain [embodimentsexamples](#), the endogenous gene is SIN3A and three guide RNAs of SEQ ID NO: 36, 37 and 38 are used. In certain [embodimentsexamples](#), the endogenous gene is PARP-1 and three guide RNAs of SEQ ID NO: 39, 40 and 41 are used. In certain [embodimentsexamples](#), the endogenous gene is ATM and three guide RNAs of SEQ ID NO: 42, 43 and 44 are used. In certain [embodimentsexamples](#), the endogenous gene is Hipk2 and three guide RNAs of SEQ ID NO: 45, 46 and 47 are used. In certain [embodimentsexamples](#), the endogenous gene is BARD1 and three guide RNAs of SEQ ID NO: 48, 49 and 50 are used. In certain [embodimentsexamples](#), the endogenous gene is HIF1AN and three guide RNAs of SEQ ID NO: 51, 52 and 53 are used. In certain [embodimentsexamples](#), the endogenous gene is SMAD3 and three guide RNAs of SEQ ID NO: 54, 55 and 56 are used. In certain [embodimentsexamples](#), the endogenous gene is CDKN1A and three guide RNAs of SEQ ID NO: 57, 58 and 59 are used.

In addition to the various [embodimentsexamples](#) depicted and claimed, the disclosed subject matter is also directed to other [embodimentsexamples](#) having other combinations of the features disclosed and claimed herein. As such, the particular features presented herein can be combined with each other in other manners within the scope of the disclosed subject matter such that the disclosed subject matter includes any suitable combination of the features disclosed herein. The description of specific [embodimentsexamples](#) of the disclosed subject matter has been presented for purposes of illustration and description. It is not intended to be exhaustive or to limit the disclosed subject matter to those [embodimentsexamples](#) disclosed.

Detailed Description of Embodiments of the Invention

Herein is reported a method for generating a recombinant mammalian cell expressing a heterologous polypeptide and a method for producing a heterologous polypeptide using said recombinant mammalian cell, wherein in the recombinant cell the activity/function/expression of at least one endogenous gene selected from the group