

5 Summary

In a first aspect, the disclosure relates to a modified non-human animal cell having a genetic modification in one or more genes associated with genome surveillance, cell cycle control and/or cell death control and wherein the animal is of an animal species suitable for human or animal consumption, optionally used in agriculture. In a second aspect, the disclosure relates to a modified animal cell as described herein, wherein the modification immortalises the cell, optionally wherein the animal is selected from a pig, bovine, poultry, sheep, goat, fish, crustaceans or mollusc, further optionally wherein the modified cell is a somatic cell, further optionally the modified cell is selected from one of the following cell types: myoblast, fibroblast, myofibroblast, adipose derived stem cell, epithelial cell, mesenchymal stem cell, satellite cell or hepatocyte.

In one example, the modification immortalises the cell.

In one example, the animal is selected from a pig, bovine, poultry, sheep, goat, fish, crustaceans or mollusc. In one example, the modified cell is a somatic cell.

In one example, the modified cell is selected from one of the following cell types: myoblast, fibroblast, myofibroblast, adipose derived stem cell, epithelial cell, mesenchymal stem cell, satellite cell or hepatocyte.

In one example, said cell does not express an exogenous nucleic acid to manipulate the genome surveillance, cell cycle control and/or cell death control pathway.

In one example, the animal cell has a genetic modification in one or more of the following genes: *RB1*, *TP53*, and/or a *RAS* gene.

In one example, the animal cell has a genetic modification in *RB1*.

In one example, the animal cell has a genetic modification in *TP53*.

In one example, the animal cell has a genetic modification in a *RAS* gene.

In one example, the animal cell has a genetic modification in *RB1* and in *TP53*.

In one example, the animal cell has a genetic modification in *RB1* and in a *RAS* gene.

In one example, the animal cell has a genetic modification in *TP53* and in a *RAS* gene.

In one example, the animal cell has a genetic modification in *RB1*, *TP53* and in a *RAS* gene.

In one example, the *RAS* gene is *HRAS*, *NRAS*, or *KRAS*.

In one example, the *RAS* gene is *HRAS*.

In one example, the modification is in the promoter region or coding region of the one of more genes, or is a regulatory element that regulates one or more of the genes.

In one example, the modification is introduced using targeted genome modification.

In one example, an endonuclease is used.

In one example, the endonuclease is selected from TALEN, ZFN or CRISPR/Cas9.

In one example, wherein the gene is selected from one or both of *RB1* and/or *TP53* and the modification is a loss of function modification.

In one example, the loss of function modification comprises a knock-out of the gene.

In one example, the gene is a *RAS* gene and the modification is a hyperactivation modification, optionally, wherein the *RAS* gene is *HRAS*, *NRAS*, or *KRAS*.

In one example, the hyperactivation modification comprises one or more amino acid substitutions.

In one example, the one or more amino acid substitutions comprises substituting the glycine at positions 12 or 13 or the Glutamine at position 61 of SEQ ID NO: 46, 47, or 48.

- 5 In one example, the one or more amino acid substitutions comprises substituting the glycine at positions 12 or 13 of SEQ ID NO: 46, 47, or 48, wherein the one or more amino acid is selected from a list comprising alanine, cysteine, aspartic acid, arginine, serine, and valine.
- In one example, the one or more amino acid substitutions comprises substituting the glycine at positions 12 or 13 of SEQ ID NO: 46, 47, or 48 with valine.
- 10 In one example, the one or more amino acid substitutions comprises substituting the glycine at position 61 of SEQ ID NO: 46, 47, or 48, wherein the one or more amino acid is selected from a list comprising glutamic acid, histidine, lysine, proline, and arginine.
- In one example, the one or more amino acid substitutions comprises substituting the glycine at position 12 of SEQ ID NO: 1 with valine.
- 15 In a third aspect, the disclosure relates to a method of producing cultivated meat or a cultured meat product comprising culturing the modified animal cell as described herein.
- In one example, the method comprises continuous or batch culture of the modified cell.
- In one example, the method comprises the step of forming the cells into a tissue like structure.
- In one example, the cells are formed into a muscle tissue like structure.
- 20 In another aspect, the disclosure relates to a method of producing the modified animal cell as described herein, wherein the method comprises introducing a genetic modification in one or more genes associated with genome surveillance, cell cycle control and/or cell death control and wherein the animal is an animal suitable for human or animal consumption, optionally used in agriculture.
- In another aspect, the disclosure relates to a method of immortalising an animal cell, wherein the method
- 25 comprises introducing a genetic modification in one or more genes associated with genome surveillance, cell cycle control and/or cell death control and wherein the animal is an animal suitable for human or animal consumption, optionally used in agriculture.
- In one example, the genetic modification alters the expression or function of one or more genes associated with genome surveillance, cell cycle control and/or cell death control.
- 30 In one example, the animal cell is immortalised.
- In one example, the animal is selected from a pig, bovine, poultry, sheep, goat, fish, crustaceans or mollusc.
- In one example, the modified cell is a somatic cell.
- In one example, the modified cell is selected from one of the following cell types: myoblast, fibroblast, myofibroblast, adipose derived stem cell, epithelial cell, mesenchymal stem cell, satellite cell or hepatocyte.
- 35 In one example, the cell does not express an exogenous nucleic acid to manipulate the genome surveillance, cell cycle control and/or cell death control.
- In one example, the animal cell has a genetic modification in one or more of the following genes: *RB1*, *TP53*, and/or a *RAS* gene.
- In one example, the animal cell has a genetic modification in *RB1*.
- 40 In one example, the animal cell has a genetic modification in *TP53*.
- In one example, the animal cell has a genetic modification in a *RAS* gene.
- In one example, the animal cell has a genetic modification in *RB1* and in *TP53*.
- In one example, the animal cell has a genetic modification in *RB1* and in a *RAS* gene.

5 In one example, the animal cell has a genetic modification in *TP53* and in a *RAS* gene.
 In one example, the animal cell has a genetic modification in *RB1*, *TP53* and in a *RAS* gene.
 In one example, the *RAS* gene is *HRAS*, *NRAS*, or *KRAS*.
 In one example, the *RAS* gene is *HRAS*.
 In one example, the modification is made to the promoter region or coding region of the one of more genes.

10 In one example, the modification is introduced using targeted genome modification.
 In one example, an endonuclease is used.
 In one example, the endonuclease is selected from TALEN, ZFN or CRISPR/Cas9.
 In one example, the gene is selected from one or both of *RB1* and/or *TP53* and the modification is a loss of function modification.

15 In one example, the loss of function modification comprises a knock-out of the gene.
 In one example, the gene is a *RAS* gene and the modification is a hyperactivation modification, optionally, wherein the *RAS* gene is *HRAS*, *NRAS*, or *KRAS*.
 In one example, the hyperactivation modification comprises one or more amino acid substitutions.
 In one example, the one or more amino acid substitutions comprises substituting the glycine at positions 12

20 or 13 or the Glutamine at position 61 of SEQ ID NO: 46, 47, or 48.
 In one example, the one or more amino acid substitutions comprises substituting the glycine at positions 12 or 13 of SEQ ID NO: 46, 47, or 48, wherein the one or more amino acid is selected from a list comprising alanine, cysteine, aspartic acid, arginine, serine, and valine.
 In one example, the one or more amino acid substitutions comprises substituting the glycine at positions 12

25 or 13 of SEQ ID NO: 46, 47, or 48 with valine.
 In one example, the one or more amino acid substitutions comprises substituting the glycine at position 61 of SEQ ID NO: 46, 47, or 48, wherein the one or more amino acid is selected from a list comprising glutamic acid, histidine, lysine, proline, and arginine.
 In one example, the one or more amino acid substitutions comprises substituting the glycine at position 12

30 of SEQ ID NO: 1 with valine.
 In one example, the one or more amino acid substitutions comprises substituting the glycine at position 12 of SEQ ID NO: 1 with valine.
 In another aspect, the disclosure relates to a cultivated or cultured animal tissue or a cultivated or cultured meat product comprising a modified cell as described herein.

35 In one example, the culture is a suspension culture.
 In another aspect, the disclosure relates to a use of the modified animal cell as described herein for cellular agriculture.
 In another aspect, the disclosure relates to a method for producing an immortalised animal cell line comprising the method as described herein.

40 In another aspect, the disclosure relates to a guide RNA for use in a method of producing the modified, immortalised cell, or immortalised animal cell line as described herein.
 In another aspect, the disclosure relates to a guide RNA comprising any sequence selected from SEQ ID NOs. 15, 16, 17, 18.

In one example, the guide RNA is for use in a method of producing the modified or immortalised cell as described herein.

In one example, the modified cell is a modified cell as described herein.

In another aspect, the disclosure relates to a kit of parts comprising the guide RNA as described herein.

Figures and tables

The invention is further described in the following non-limiting figures and tables.

Figure 1A is a schematic representation of the key cell cycle regulatory programmes, highlighting the contribution of the key CRISPR target genes (*RB1*, *TP53* and *Ras*) to the different phases of DNA replication along with the regulatory programmes targeted for CRISPR-Cas9 gene editing (*pRB/E2F*, *p53* and *Ras/MEK/ERK* pathways). **Figure 1B** is a workflow of CRISPR-Cas9 editing. Single cell suspensions were incubated with Cas9 and sgRNA ribonucleoprotein complexes and then subjected to nucleofection (Amaxa 4D, Lonza). Successful editing was assessed by amplicon sequencing and trace deconvolution. Extension of replicative capacity was determined by assessing proliferation (cell doublings) and transcriptional changes (qPCR).

Figure 2 is a table highlighting the CRISPR targets genes (single, double, and triple editing targets) in three isolated primary porcine cell lines, IF-p036-A, O and AD. The lines highlighted in red boxes are the tripled edited cell lines currently being evaluate for their doubling times and immortalisation status, relative non-edited controls.

Figure 3 is two bar graphs that represent the relative efficiency of H-RasG12V, *TP53* and *RB1* editing in two primary porcine cell lines, IF-p036-A and IF-p036-O. % editing was calculated using ICE analysis (Inference of CRISPR Edits).

Figure 4 is a bar graph that shows the efficient multiplex CRISPR editing of a primary porcine cell line. The bars represent the relative efficiency of H-*Ras*G12V, *TP53* and *RB1* editing in the serum free cell line, IF-p036-AD. Percentage (%) editing was calculated using ICE analysis (Inference of CRISPR Edits).

Figure 5 shows porcine muscle derived cell lines edited via CRISPR/cas9 for HrasG12V (-/+), *TP53* (-/-) and *RB1* (-/-) display a growth advantage, reduced dependence on extracellular matrices and growth factors when compared to cas9 controls. a) Light micrographs of IFp036-A-27-A and IFp036-O-31-A triple edit CRISPR cell lines and respective cas9 controls at P15 in cell culture. b) Cumulative generations and c) doubling times of parental (IFp036-A/O), CRISPR and cas9 cell lines. d) Doubling times of IFp036-A-27-A and cas9 cell lines following removal of the extracellular matrix Matrigel, evidencing retained growth in triple edited CRISPR lines compared to cas9 controls. e) Doubling times are reduced in both IFp036-A-27-A and cas9 cell lines following removal of bFGF, however, edited cell lines evidence a reduced dependence compared to cas9 controls. These doubling times are however significantly higher than when cultured with bFGF (d vs. inset of e). Data presented as mean +/- SD.

Claims

1. A modified animal cell having a genetic modification in one or more genes associated with genome surveillance, cell cycle control and/or cell death control and wherein the animal is of an animal species suitable for human or animal consumption, optionally used in agriculture.
2. A modified animal cell according to claim 1, wherein the modification immortalises the cell, optionally wherein the animal is selected from a pig, bovine, poultry, sheep, goat, fish, crustaceans or mollusc, further optionally wherein the modified cell is a somatic cell, further optionally the modified cell is selected from one of the following cell types: myoblast, fibroblast, myofibroblast, adipose derived stem cell, epithelial cell, mesenchymal stem cell, satellite cell or hepatocyte.
3. The modified animal cell according to any preceding claim, wherein said cell does not express an exogenous nucleic acid to manipulate the genome surveillance, cell cycle control and/or cell death control pathway, optionally wherein the animal cell has a genetic modification in one or more of the following genes: RB1, TP53, and/or a RAS gene, further optionally the animal cell has a genetic modification in RB1, further optionally the animal cell has a genetic modification in TP53, further optionally the animal cell has a genetic modification in a RAS gene, further optionally the animal cell has a genetic modification in RB1 and in TP53, further optionally the animal cell has a genetic modification in RB1 and in a RAS gene, further optionally the animal cell has a genetic modification in TP53 and in a RAS gene, further optionally the animal cell has a genetic modification in RB1, TP53 and in a RAS gene, further optionally the RAS gene is *HRAS*, *NRAS*, or *KRAS*, further optionally wherein the RAS gene is *HRAS*.
4. The modified animal cell according to any preceding claim, wherein the modification is in the promoter region or coding region of the one of more genes, optionally wherein the modification is introduced using targeted genome modification, optionally using an endonuclease, further optionally the endonuclease is selected from TALEN, ZFN or CRISPR/Cas9.
5. The modified animal cell according to any preceding claim, wherein the gene is selected from one or both of RB1 and/or TP53 and the modification is a loss of function modification, optionally wherein the loss of function modification comprises a knock-out of the gene, further optionally wherein the gene is a RAS gene and the modification is a hyperactivation modification, optionally, wherein the RAS gene is *HRAS*, *NRAS*, or *KRAS*, further optionally the hyperactivation modification comprises one or more amino acid substitutions, further optionally the one or more amino acid substitutions comprises substituting the glycine at positions 12 or 13 or the Glutamine at position 61 of SEQ ID NO: 46, 47, or 48, further optionally the one or more amino acid substitutions comprises substituting the glycine at positions 12 or 13 of SEQ ID NO: 46, 47, or 48, wherein the one or more amino acid is selected from a list comprising alanine, cysteine, aspartic acid, arginine, serine, and valine, further optionally the one or more amino acid substitutions comprises substituting the glycine at positions 12 or 13 of SEQ ID NO: 46, 47, or 48 with valine, further optionally the one or more amino acid substitutions comprises

substituting the glycine at position 61 of SEQ ID NO: 46, 47, or 48, wherein the one or more amino acid is selected from a list comprising glutamic acid, histidine, lysine, proline, and arginine.

6. A method of producing cultivated meat or a cultured meat product comprising culturing the modified animal cell according to any of claims 1 to 5.
7. The method according to claim 6, wherein the method comprises continuous or batch culture of the modified cell, optionally the method comprises the step of forming the cells into a tissue like structure, further optionally the cells are formed into a muscle tissue like structure.
8. A method of producing the modified animal cell according to any of claims 1 to 5, wherein the method comprises introducing a genetic modification in one or more genes associated with genome surveillance, cell cycle control and/or cell death control and wherein the animal is an animal suitable for human or animal consumption, optionally used in agriculture.
9. A method of immortalising an animal cell, wherein the method comprises introducing a genetic modification in one or more genes associated with genome surveillance, cell cycle control and/or cell death control and wherein the animal is an animal suitable for human or animal consumption, optionally used in agriculture.
10. The method according to claim 8 or 9, wherein the genetic modification alters the expression or function of one or more genes associated with genome surveillance, cell cycle control and/or cell death control, optionally the animal cell is immortalized, further optionally the animal is selected from a pig, bovine, poultry, sheep, goat, fish, crustaceans or mollusc, optionally the modified cell is a somatic cell, further optionally the modified cell is selected from one of the following cell types: myoblast, fibroblast, myofibroblast, adipose derived stem cell, epithelial cell, mesenchymal stem cell, satellite cell or hepatocyte, further optionally said cell does not express an exogenous nucleic acid to manipulate the genome surveillance, cell cycle control and/or cell death control.
11. The method according to any of claims 8 to 10, wherein the animal cell has a genetic modification in one or more of the following genes: RB1, TP53, and/or a RAS gene, optionally wherein the animal cell has a genetic modification in RB1, further optionally the animal cell has a genetic modification in TP53, further optionally the animal cell has a genetic modification in a RAS gene, further optionally the animal cell has a genetic modification in RB1 and in TP53, further optionally the animal cell has a genetic modification in RB1 and in a RAS gene, further optionally the animal cell has a genetic modification in TP53 and in a RAS gene, further optionally the animal cell has a genetic modification in RB1, TP53 and in a RAS gene, further optionally the RAS gene is *HRAS*, *NRAS*, or *KRAS*, further optionally the RAS gene is *HRAS*, further optionally wherein the modification is made to the promoter region or coding region of the one of more genes, further optionally wherein the modification is introduced using targeted genome modification, further optionally using an endonuclease, further optionally the endonuclease is selected from TALEN, ZFN or CRISPR/Cas9.
12. The method according to claims 8 to 11, wherein the gene is selected from one or both of RB1 and/or TP53 and the modification is a loss of function modification, optionally wherein the loss

of function modification comprises a knock-out of the gene, further optionally wherein the gene is a RAS gene and the modification is a hyperactivation modification, optionally, wherein the RAS gene is HRAS, NRAS, or KRAS, optionally wherein the hyperactivation modification comprises one or more amino acid substitutions, further optionally the one or more amino acid substitutions comprises substituting the glycine at positions 12 or 13 or the Glutamine at position 61 of SEQ ID NO: 46, 47, or 48, further optionally the one or more amino acid substitutions comprises substituting the glycine at positions 12 or 13 of SEQ ID NO: 46, 47, or 48, wherein the one or more amino acid is selected from a list comprising alanine, cysteine, aspartic acid, arginine, serine, and valine, further optionally the one or more amino acid substitutions comprises substituting the glycine at positions 12 or 13 of SEQ ID NO: 46, 47, or 48 with valine, further optionally the one or more amino acid substitutions comprises substituting the glycine at position 61 of SEQ ID NO: 46, 47, or 48, wherein the one or more amino acid is selected from a list comprising glutamic acid, histidine, lysine, proline, and arginine.

13. Cultivated or cultured animal tissue or a cultivated or cultured meat product comprising a modified cell according to any of claims 1 to 5.
14. Cultured animal tissue according to claim 13, wherein the culture is a suspension culture.
15. Use of the modified animal cell according to any of claims 1 to 5 for cellular agriculture.
16. A method for producing an immortalised animal cell line comprising the method according to any of claims 4 to 5.
17. A guide RNA for use in a method of producing the modified, immortalised cell, or immortalised animal cell line according to any of claims 8 to 12 or claim 16.
18. A guide RNA comprising any sequence selected from SEQ ID NOs. 15, 16, 17, 18.
19. A guide RNA according to claim 18 for use in a method of producing the modified or immortalised cell according to any of claims 8 to 12, optionally wherein the modified cell is a modified cell according to any of claims 1 to 5.
20. A kit of parts comprising the guide RNA according to any of claims 17 to 19.